EDITORIAL

Academician General Victor Voicu at the anniversary

An anniversary is rather meant to reflect on the past, present and future, being at the same time, and a reason for celebration.

On Wednesday, June 26, 2019, the book of Academician Victor Voicu – "Under the sign of Hippocrates" was released in the Auditorium of the Romanian Academy – a book recently published by the prestigious European Idea Publishing House. It was a moment of great and wonderful celebration.

We will only remind you the essential things about Academician General Victor Voicu – a distinguished and emblematic personality of civil and military medicine (in the context in which the assertion of civilian physician – military physician is increasingly obsolete) from our country.

Born June 29, 1939 in the village of Bolovani, Dambovita county, student of "St. Sava" National College in Bucharest, transferred in the last year to "Ştefan cel Mare" Military Highschool in Iaşi (currently in Câmpulung Moldovenesc) – the moment when he put on the military coat, selected from the best for the Medical-Military Institute, the Faculty of Medicine – Medical Pharmace-utical Institute in Bucharest, which he graduated in 1962, apparently we would not have too much to say... and yet!

He chooses pharmacology and clinical pharmacology and the intuition of his master, Alfred Teitel, imposes the future creator of the School of Pharma-cology, Toxicology and Psychopharmacology from our country.

University Assistant, starting from 1966 Scientific Researcher at the Radiobiology and Molecular Biology Center, since 1972 Associate Professor and Head of the Pharmacology Department of the recently established Faculty of Medicine from Craiova, since 1990 Professor of Pharmacology, Toxicology and Psychopharmacology at the University of Medicine and Pharmacy "Carol Davila", Head of the Medical Department of the Ministry of National Defense between 1990-1995, Commander of the Medical-Military Scientific Research Center for 26 years (1987-2013), Corresponding Member (1991) and Full member (2001) of the Romanian Academy, Secretary-General in two legislatures and Vice-President of this High Authority from April 2018.

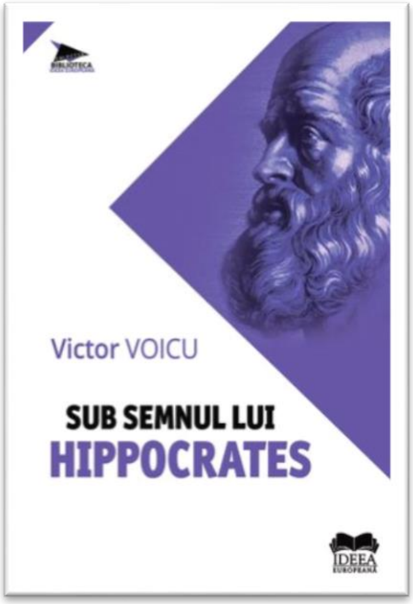

The life, work, efforts and achievements of a MAN cannot be reduced only to the previous sentence. Those who want to learn more about the frankness and the visionary spirit of Academician Victor Voicu, can do that by making use of "search engines", as they say nowadays.

Academician Nicolae Breban enlightened the thoughts of the audience when he pronounced the word "energetic", adding it to the joy felt by those present at the release of the book I previously mentioned.

General Academician Victor Voicu, a descendent in time of our common ancestor - General physician and pharmacist Academician p.m. (2003) Carol Davila is not only an "energetic" man, but also an "energetic" spirit for those around him, for all of us, when through his vision, calm and analytical spirit, he manages to clarify things and "indicate" the right direction to follow.

The release of the book, "Under the Sign of Hippocrates," was for all participants, as well as for future readers, a celebration of the spirit.

The editorial staff of the
Romanian Journal of Military Medicine
Wishes
Happy Birthday!
To

Academician Victor Voicu

Honorary Editor

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ORIGINAL ARTICLE

Health risk behaviors among Iranian university students: A cross-sectional study

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Abstract: *Background and Aim: The prevalence of high-risk behaviors is one of the most serious issues threatening the health of young people. The first step in preventing youth risk-taking is to recognize the problem and its dimensions. The purpose of this study was to investigate high-risk behaviors among students at Tehran's universities.*

Methods: This cross-sectional descriptive-analytical study was conducted from March to August 2019. The sample set was 4,000 students. Data was collected by multi-stage sampling at eight universities in Tehran. Demographic information and standard youth-risk questionnaires were used. SPSS 19 software was used to perform an independent t-test, Pearson ANOVA, and linear regression at a significance level of 0.05 on the questionnaire data collected.

Results: The mean of the total tendency to high-risk behavior was 130.34±40.37. Significant risks identified were driving (27.3±8.33), violence (18.86±6.4), smoking (18.86±7.68), drugs (22.47±8.83), alcohol (24.10±8.57), and high-risk sexual behavior (18.47±7.49). High-risk behaviors were more common in men, single people, and people living in dormitories (P<0.05). Age and gender were the best-correlated predictors of students' high-risk behaviors.

Conclusions: Results show that the tendency to high-risk behaviors in students is a worrying situation. as, at younger ages, the tendency to engage in high-risk behaviors is greater. Among young men and in dormitory life, students are more prone to high-risk behaviors. So, more monitoring in dormitories, especially male dormitories, and better planning and education to reduce high-risk behaviors among students, is necessary.

Keywords: *high-risk behaviors, students, Iran*

INTRODUCTION

The prevalence of high-risk behaviors is one of the most crucial health threats to young people. Health organizations, law enforcement agencies, and social policymakers have raised the issue. Due to rapid social change, some of these behaviors are the cause of death in adolescents and young people and have negative effects on communities [1,2]. High-risk behaviors include violence, arguing, smoking, verbal and non-verbal threats, neglect of studies, high-risk sexual behaviors, drug abuse, suicide attempts, and aggression [3,4]. Adolescents and young people are more at risk than others because they are more likely to engage in high-risk behaviors [5]. By 2030, tobacco-related deaths alone are projected to reach 10 million a year. If the physical, psychological, and social consequences of other high-risk behaviors, such as drug abuse, violence, and high-risk sexual behaviors, are taken into account, the harms are multiplied [6,7].

Among younger people, drug use and high-risk sexual behavior are at the top of the list of high-risk behaviors [8-10]. Also, adolescence is one of the periods in which the person is most affected by the environment and friends, so those around can play a significant role in creating high-risk behavior in this period [11]. In recent years, alcohol and drug use has been one of the biggest health concerns in Iran [8, 12,13]. Research has shown that the most common occurrence of these behaviors is in the university

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environment [6]. Iran has a relatively young population, with almost a quarter of the total population being 15 to 29 years old; and based on the latest census, 25% of the whole Iranian youth population attends universities [14]. The student period is one of the most significant periods for most young people. This period plays an important role in shaping future professional and social life due to the transition from adolescence to youth [1]. The behaviors that students acquire or reinforce on campus may shape their future health and the health of future generations through the potential role of students as parents and leaders [15].

The first step in preventing youth risk-taking is to recognize the problem and its dimensions [4]. However, few integrated studies have been conducted to examine the risk-taking of young people by smoking, psychotropic drugs, alcohol, violence, dangerous driving, and sexual risk in Iran. The purpose of this study is to investigate high-risk behaviors of university students in Tehran, in order to determine the current situation in the field of high-risk behaviors in students and to identify the necessary interventions to improve their health.

METHODS

Design

This descriptive-analytical cross-sectional study was conducted from March to August 2019 on 4000 students of eight universities in Tehran (the capital of Iran). Sampling was done in several stages. At the first stage, eight universities were randomly selected from the list of universities located in Tehran (including University of Tehran, Sharif, Shahid Beheshti, Tehran University of Medical Sciences, Iran University of Medical Sciences, Shahid Beheshti Medical Sciences, Payame Noor and Azad). At the next stage, each university was considered as a class and the list of students was prepared with the coordination and cooperation of the vice-chancellor of each university. The student numbers of the students were systematically selected and entered into the study. If the identified student did not want to participate in the study for sampling, the next person on the list replaced him/her.

The sample size for each university was determined by considering the alpha=0.05 and the accuracy=0.4, standard deviation=0.05 for 484 cases. To increase the validity of the study, the number of samples was determined to 500. A total of 4,000 subjects were examined in 8 state and private universities in Tehran. The inclusion criteria for this study were working in one of the studied universities, spending at least two semesters in the university, being Iranian, and willingness to participate in the study. The condition for leaving the study was the unwillingness to participate in the study.

Study Tools

Data were collected using a questionnaire in the form of self-reports. The first part of the questionnaire included demographic information such as age, gender, educational level, location. In the second part, the standard youth risk-taking questionnaire was used. The risk-taking scale of this study, which was standardized by Zadeh Mohammadi et al., has 48 items in six areas of drugs (8 questions), alcohol (9 questions), smoking (7 questions), violence (7 questions), sexual risk-taking behavior (8 questions) and driving (9 questions). This questionnaire has a five-point Likert scale (strongly disagree: 1, disagree: 2, have no opinion: 3, agree: 4, strongly agree: 5). The total score of the questionnaire is 240-48. This scale does not have a specific cut-off point for distinguishing normal from abnormal individuals and higher scores indicate a greater tendency toward high-risk behavior. Cronbach's alpha coefficient of this questionnaire is 0.93 [16].

This study was approved by the Research Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC 1396.32610). After obtaining permission from the educational deputies of the studied universities, the questioners were sent to the universities. The objectives of the study were explained to the participants by the questioners and informed consent was obtained from the individuals to participate in the study. It was ensured that the identity of the participants and all information would remain confidential.

Data Analysis

Data were analyzed using SPSS software (Version 19). First, the normality of the data was measured based on the Kolmogorov–Smirnov test and it was found that the data had a normal distribution. Therefore, independent t-test, ANOVA, Pearson correlation test, and linear regression were used. A confidence interval of 95٪ was used for the data analysis.

RESULTS

Descriptive statistics

The mean age of study participants was 22.95±4.16 years. 53.4% of the participants were male, and 84.8% were single; about half of the subjects (48.1%) were studying at the undergraduate level. More than half of the residents lived in dormitories. Other information can be seen in Table 1.

Findings of the independent t-test showed that the mean scores of high-risk behaviors and their dimensions, in terms of demographic data in men, had a significantly higher mean in total score and dimensions than women ($P<0.05$). The results showed the mean of the total tendency to high-risk behavior in single students compared to married students was higher. This significance was also observed in the dimensions of high-risk driving, alcohol, and drugs ($P<0.05$). Students living in dormitories had significantly higher mean scores on risk behavior and its dimensions, except for the line driving dimension and high-risk sexual behavior ($P<0.05$) (Table 2).

Table 1: Socio-demographic characteristics (N=4000).

Characteristic	Group	No. (%)
Gender	Male	2135 (53.4)
	Female	1865 (46.6)
Marital status	Unmarried	3394 (84.8)
	Married	606 (15.2)
Educational level	Bechelor	1925 (48.1)
	Master	1077 (26.9)
	MD	779 (19.5)
	PHD and uper	219 (5.5)
Place of residence	Students dormitory	2544 (63.6)
	Others	1456 (36.4)

Comparison of the mean using the ANOVA test showed that there is a significant difference in the score of high-risk behaviors and their dimensions according to different educational levels. As shown in the results of the Tukey post hoc test, the total score of high-risk behaviors in undergraduates was higher than master's, and also the general doctorate had a high average of high-risk behaviors compared to bachelor's and master's degrees. Under the driving scale, undergraduate students scored higher than others. The next grade of violence in the general doctoral program was higher than the bachelor's and master's degrees. Smoking was also observed in General Doctoral students more than in other courses. In terms of high-risk behavior, drug use was higher in undergraduates but less than in doctoral students. Alcohol consumption was also observed in undergraduates over college graduates. High-risk sexual behavior in undergraduate students had higher scores than postgraduate and lower than doctoral ($P<0.05$) (Table 2). The results showed that there were an inverse and significant relationship ($P<0.05$) between age and the total score of high-risk behavior. Therefore, with increasing age, the score obtained from the questionnaire decreased (Table 2).

Table 2: Distributions of total HRB a subscale by demographic characteristics (N=4000)

Characteristic	Total HRBy	High-risk drivers	Agressive	Smoking	Behaviors drug use	Alcohol consumption	High-risk sexual
Total	130.34 ± 40.34	27.56 ±8.33	18.86 ± 6.4	18.86± 6.68	22.47± 8.83	24.1 ±8.57	18.47±7.49
Gender							
Male	132.71 ±38.92	28.12± 8.33	19.12±6.47	19.32±7.59	22.92± 8.44	24.55 ± 8.42	18.67± 7.49
Female	127.62±41.81	26.91±8.3	18.56± 6.31	18.35 ± 7.75	21.95 ± 9.24	23.57 ± 8.71	18.25 ± 7.49
P-value	>0.001*	>0.001*	0.005*	>0.001*	0.001*	>0.001*	0.93
Marital status							
Unmarried	130.92± 40.42	27.71±8.38	18.86 ± 6.48	18.95± 7.71	22.64 ± 8.89	24.32 ± 8.54	18.41± 7.52
Married	127.05± 39.9	26.69 ± 8.02	18.86 ± 5.92	18.36 ± 7.49	21.51± 8.44	22.84± 8.66	18.76± 7.32
P-value	0.29	0.004*	1	0.089	0.004*	0.001*	0.29
Educational level							
Bechelor	131.7±39.56	28.54± 8.21	18.59±6.47	18.93± 7.66	22.53±9	24.43± 8.49	18.65± 7.39
Master	124.99±40.36	26.05±8.2	18.40±6.03	17.99± 7.54	21.6 ±8.67	23.14± 8.57	17.78± 7.53
MD	134.09±40.96	27.53±8.37	19.78±6.4	19.53± 7.62	23.46± 8.49	24.69 ± 8.54	19.07± 7.6
PHD and uper	131.3 ± 42.95	26.39±8.72	20.24± 6.97	20.16 ± 8.32	22.62 ± 8.94	23.74± 9.1	18.12± 7.58
P-value	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*	0.001*
Place of residence							
Students dormitory	131.47±40.33	27.48± 8.48	19.11 ± 6.41	19.15± 7.64	22.87± 8.78	24.35± 8.55	18.48 ± 7.62
Others	127.83 ±40.34	27.72 ± 8.01	18.31± 6.34	18.23 ± 7.72	21.57 ± 8.89	23.53 ± 8.59	18.43 ± 7.2
P-value	0.008*	0.421	>0.001*	>0.001*	>0.001*	0.005*	0.831
Age							
Correlation	-0.12	-0.098	-0.098	-0.096	-0.109	-0.105	-0.107
P-value	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*

**p<0.05*

The linear regression model for predictors of high-risk behaviors showed that age and gender were the main predictors. This, 18% of the total changes in scores predicted high-risk behaviors (Table 3). As can be seen, age inversely predicted high-risk behavior, as age decreased, the score of high-risk behaviors increased (-0,119). Also, the regression model showed that men had more high-risk behaviors than women (-0.06) (Table 3).

Table 3: High risk behaviors predictors in the linear regression model

Model	Unstandardized coefficients		Standardized coefficients	T	sig	95.0% Confidence interval for B	
	B	Standard error	Beta			Lower Bound	Upper bound
Constant	163.91	3.96		41.34	>0.001	156.13	171.68
Age	-1.153	0.152	-0.119	-7.58	>0.001	-1.45	-8.55
Sex	-4.874	1.269	-0.06	-3.84	>0.001	-7.36	-2.38
R2: 0.234 R: 0.18							

Dependent variable: HRB
Predictors: sex, age

DISCUSSION

This study aimed to investigate the tendency of high-risk behaviors and their relationship with demographic variables in students in Tehran. The average scores of high-risk behaviors in students in all dimensions were above average. These results have been reported in another Iranian study, including the an other study [17]. The results of the present study showed that male students had a significantly higher tendency to high-risk behavior than female students. This finding is consistent with the findings of other studies [18-20]. However, in terms of high-risk sexual behavior, the difference between girls and boys was not significant. This result contradicted the results of other studies [1,18], in which boys were more likely to engage in sexual behavior. Many studies showed that men are more likely to engage in more high-risk behaviors, such as driving [21], violence [20, 22], smoking [23], alcohol [23-25], and drug abuse [25], which is in line with the findings of the present study. In the present study, gender was a predictor of high-risk behavior, and this was confirmed by the findings of Deressa's research [26]. In the present study, high-risk behaviors, other than sexual behavior, were influenced by gender. In the student community, this can be considered as a sign of boys' freedom of action. On the contrary, girls try to avoid high-risk behavior due to the cultural context of the community. In their study, Warner et al. Considered that one of the main reasons for the difference in alcohol consumption among male and female students was because of negative societal pressures and severe stigma among women against these behaviors [27]. The results of this study showed that the tendency to high-risk behavior in single students in the dimensions of high-risk driving, alcohol, and drugs was significantly higher than married students. Numerous studies had shown that the risk of alcohol consumption was higher in those who were not married [28-31]. A longitudinal study by Kendler in 2016 showed that marriage had a protective effect against alcoholism. These results are consistent with the hypothesis that the psychological and social aspects of marriage, especially the spouse's relationship to health control, strongly protect against alcohol abuse [32]. Consistent with the results of the present study, it was observed in other studies that marriage reduced drug abuse [31,33,34]. Studies had shown that high-risk driving and road injuries were more common in single people than in married people [35,36]. Given the protective role of marriage in preventing high-risk behaviors in students, creating the necessary conditions and social support for young people to get married can be effective in reducing high-risk behaviors.

The findings showed that dormitory students were more prone to high-risk behaviors and more prone to high-risk driving, smoking, drug abuse, and alcohol consumption. This finding is consistent with the research of other authors [18,20].

Heydarabadi et al. and Rigi Kootesh et al. and Yoosefi Lebni et al. found that students living in dormitories were more likely to be addicted to drugs due to the lack of parental supervision and peer pressure [37-39]. It was also observed in this study that the tendency to high-risk behavior in all dimensions was significantly higher in general doctoral students than graduate students. Consistent with these findings, Fathian et al., in their study showed that medical students had higher-risk behaviors than other non-medical students [40]. Findings showed that age had a significant negative relationship with the overall score of high-risk behaviors, and, also with all dimensions of high-risk behaviors, i.e., with increasing age, the tendency to high-risk behaviors decreased. This finding is consistent with a study by Farmer et al. [17]. Given the characteristics of young people, these results seemed to be consistent with reality.

For preventing students from tending to high-risk behaviors, it is necessary to take extracurricular activities in universities seriously along with academic activities so that students can be entertained with healthier activities.

One of the limitations of the present study was the use of a questionnaire in the form of a self-report of high-risk behaviors that may have caused bias or incorrect responses. There are also direct questions about high-risk behaviors such as drug use or sexual behavior that are still taboo in society. These questions caused the resistance and dishonesty of the respondent, and as a result, the number of correct answers and accuracy of information collection was reduced. Also, the questionnaire used in this study did not measure economic and social factors and health determinants. Therefore, it is suggested that in future studies, economic and social factors and other health determinants be considered, and for more accurate information, an observation method next to the questionnaire be used.

CONCLUSION

The results of this study showed that the tendency to high-risk behaviors in students was not favorable, especially considering that single students at a younger age and in dormitory conditions were prone to high-risk behaviors. Improving this situation requires proper planning for education, various skills such as dormitory lifestyle, more supervision in universities and student dormitories, so it is recommended to hold the necessary workshops for students at the beginning of entering the university. Also, university officials and administrators need to provide healthy conditions and entertainment for students to be less prone to high-risk behaviors.

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Conflict of interest

We, the authors, declare that there is no computing interest.

Competing interests

The authors declare that they have no competing interests.

Consent to participate

Informed consent was obtained from all individual participants included in the study before conducting.

Ethics approval

This study was conducted with the financial support of the Iran University of Medical Sciences with grant number 96-04-27-32610.

Consent to publish

All participants consented verbally to the publication of the interview data.

Availability of data and materials

The datasets using in the study are available from the corresponding author on reasonable request.

Authors' Contributions

All authors were responsible for the study. RP, MM, and AZ were accountable for the study Conceptualization and led the writing of the paper. ZRKK conducted the Literature review and assisted in writing the paper. AZ and MM performed the Statistical analysis, assisted in interpreting the data and writing the paper. RP and NM assisted with the interpretation of the results and drafting programmatic Implications. OS and MM were responsible for data collection and coordination of the study. AZ co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

Relationship Between Psychological Resilience and Attitudes Towards A.I. in Emergency Workers

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Abstract: The impact of artificial intelligence (AI) on our lives can no longer be neglected, and thus, understanding this phenomenon and how humans interact with it is becoming more important than ever. The study emphasizes one of the psychological characteristics that helps us in accepting AI and using it to its fullest, namely the psychological resilience that each of us, and in particular emergency service workers, possesses in different proportions. The analysis of the relationship between the two dimensions (psychological resilience and AI) highlights, among other things, the importance of how organizations can use this interaction to achieve a framework for developing the psychological resources of their employees so that they are increasingly receptive to the use of AI in their work, pursuing the benefits it brings. The study is conducted on 89 emergency service workers and focuses on the positive and highly statistically significant relationship between psychological resilience and attitudes towards AI. The study also particularly emphasizes the relationship between tenacity and self-efficacy, self-confidence, ability to learn from life experiences, rapid recovery from negative events, social and family resources as dimensions of psychological resilience in relation to attitude towards IA of emergency workers.

Keywords: mental resilience, artificial intelligence (A.I.), attitude, emergency workers

INTRODUCTION

People everywhere have suffered from various life events, but these have also provided an open research background from which we can affirm that some psychological characteristics, including psychological resilience, can support a sense of well-being, the ability to quickly restore happiness, post-traumatic growth, and some positive coping strategies after experiencing these events. One of the occupations where increased resilience is needed is that of emergency workers (firefighters, paramedics, police, psychologists, search and rescue teams).

Intervening in emergencies places maximum demands on the cognitive and neurological resources of each person working in such situations. It is thus imperative to understand how the brain processes information, manages stress, and makes quick decisions, because this understanding will lead to optimizing performance and maintaining the long-term mental health of these workers [1, 2, 3, 4]. Also, if we take into account that repeated exposure to traumatic events and high levels of stress can negatively affect the hippocampus, leading to difficulties in concentration, altered prosexic function, memory errors, or even freezes, all this makes it even more important to study the effects of stress on these workers and how they build resilience. One answer to all this may be A.I. through the various ways it offers to stimulate understanding of situations, acceptance of differences in reaction, stimulate coping mechanisms, highlight stress levels (including its various health effects through wearable devices), etc. A.I. can be used in different simulations to understand how they learn, or the different reactions in interventional cases, or afterwards; monitoring with wearable devices of different data and then analyzing them with A.I. (early signs of deterioration of health or well-being can be identified and proactive interventions can be proposed), identification of training needs, alternative learning routes customized on different worker characteristics, personalized training scenarios etc.

AI can be used to create a resilient infrastructure, a resilient organization, and resilient ways of working, and thus contribute to increasing the resilience of the participants in the process. Health is an important component for all people and the systems in which they function [5, 6, 7, 8, 9, 10].

A.I. is very helpful to emergency workers by:

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- rapid data collection and analysis (huge volumes of information can be processed in a short time to provide a quick and accurate overview of the situation);
- predictability and anticipation (A.I. can predict the evolution of an emergency by analyzing existing data, movement patterns of fire, typhoon, etc.);
- optimization of resource allocation (A.I. can help to optimize the distribution of crews, vehicles, and equipment, taking into account factors such as distance, traffic, type of emergency, and availability of resources);
- decision support (through rapid data analysis, scenario generation, AI can provide emergency workers with critical information for decision-making in situations of stress, and uncertainty - e.g., rapid patient resuscitation, prioritizing serious cases, etc.);
- robotics, and automation (robots, drones can penetrate areas that are dangerous for humans, and collect data to streamline intervention);
- effective, and improved communication (A.I. systems can help with real-time translation, answering emergency calls, and relaying information efficiently to teams and the public).

All these provide emergency workers with increased safety, increased efficiency in situations they would not have coped with on their own (e.g. dangerous areas they could not enter), better decisions through access to more complete information or a large volume of information processed quickly, reduced cognitive load (A.I. can take over repetitive tasks or data analysis, allowing staff to focus on human, and operational aspects).

Over the years, researchers have defined resilience in various ways. For example, in some studies [11], psychological resilience is considered to be the ability of individuals or outcomes to adapt successfully to challenging or threatening circumstances. Resilience also reflects a person's ability to maintain stability and equilibrium in the face of difficulties caused by particular life situations, including the epidemics that we are increasingly facing, and people with resilience might also experience positive emotions. It also states that resilience is characterized by positive behaviour, and attitudes in the face of adversity, courage, and perseverance in coping with life's difficulties, possessing a positive attitude even in the face of adversity, enjoying intimate relationships, and expanding social circles.

Other studies [12,13] highlight some key components of resilience, including self-improvement, and particularly positive attitudes maintained during adverse situations, learning from negative and/or positive experiences, novelty seeking, emotional regulation, positive future orientation, and the pursuit of meaningful life goals. Research on mental resilience considers it to be a person's ability to adapt positively to adverse circumstances or to maintain adequate functioning even in the midst of disastrous situations.

Experts are more and more concerned about the effects of life events on the psychological apparatus, especially long-term psychological problems, such as a decrease in subjective well-being, a decrease in adaptability, the onset of psychological trauma, etc., and how to combat them [14]. When the adversity experienced in PTG (Posttraumatic Growth) is high, it can stimulate the person's ability to withstand hostile and traumatic environments, therefore also increases the ability to enhance or accelerate his or her experience of posttraumatic growth [15]. However, research over the last two decades indicates that not all people who face adversity will suffer from mental or emotional health problems. It is known that some people experience positive emotions and psychological growth/development, and this is known as positive mental health, and changes include post-traumatic growth and resilience [16,17]. Scientists' concerns for resilience are all the greater as the world is constantly changing, natural disasters have multiplied, and people are often in stressful situations due to these changes. All the more, emergency workers have more challenges in the current context of climate change and the presence of many natural disasters. Certainly, A.I. facilitates knowledge of data that predicts the occurrence and evolution of traumatic events, and through this, can be useful for building resilient cities, resilient communities, and resilient people in general [18].

Although A.I. can be of real help to emergency workers in building their resilience, there are also some challenges they face in relation to A.I.:

- acceptance of A.I. – the level of acceptance of A.I. is related to the age of people (digital immigrants or digital natives, for example), the degree to which they find it useful, and easy to use;

- dependence on technology – over-reliance on A.I. can lead to problems in the event of technical failures or cyber-attacks;

- data quality – the proper functioning of the A.I. depends on the quality and accuracy of the input data;

- privacy, and ethics – the use of A.I. raises questions about the privacy of personal data, and the ethical aspects of decisions made by algorithms, and this is not yet sufficiently regulated;

- training, and adaptation – emergency workers need to be trained to understand, and use A.I. systems effectively (and this depends on how they are implemented in each institution or system in which they work);

- cost – Implementing and maintaining advanced AI systems can be expensive.

AI is a powerful tool that can revolutionize emergency management by providing essential support to emergency workers and can actively contribute to building their resilience. Resilience has a positive impact on both mental health, and wellbeing, it can help maintain subjective wellbeing, it maintains people's optimism, and belief that things will get better when faced with various challenges, it includes positive adaptive patterns that are developed over time in response to adversity, and people with high resilience can recover effectively from the impact of everyday stressors, so it becomes all the more important that we can optimize it.

Based on some research in the international literature that has highlighted that positive interaction with technology provides a framework for the development of psychological resources [19, 20], and that artificial intelligence (AI) promotes more effective coping mechanisms by increasing resilience, especially in a professional setting, and during emergencies [21], the objective of the present research was to analyze the relationship between psychological resilience – as a resource that ensures effective adaptation to various situations (new or difficult) –, and the attitude towards AI of Romanian employees.

MATERIALS AND METHODS

The general objective of the research was investigated by means of a questionnaire-based online survey, through a Typeform link containing information about the subjects' informed consent, their socio-demographic data, and the items of the two questionnaires - one analyzing psychological resilience, and the other analyzing attitudes towards AI.

Research hypotheses

The following hypotheses have been formulated to fulfill the general research objective:

Hypothesis 1 - We assume that there are interdependent relationships between psychological resilience and some of its dimensions (tenacity, self-efficacy, self-confidence, ability to learn from life experiences, rapid recovery from negative events, social, and family resources), and the attitude towards IA of emergency workers.

Hypothesis no. 2 - We anticipate that the attitude towards IA can be predicted and explained in terms of the psychological resilience of emergency workers, and its dimensions.

Subjects

The study participants were 89 people, employed in emergency services, from the South, aged between 19 and 43 years (M=29.56, SD=14.26), 51 male, and 38 female. The research sample was a convenience sample, utilizing available subjects, and the only selection criterion was that they were employed. Subjects participated in the research voluntarily, being informed about the research topic, that the results will be confidential, anonymized, and used for research purposes only, and also that they had to fill in two questionnaires (and a set of socio-demographic data) sent via a Typeform link.

Instruments

Psychological resilience was investigated by applying the adapted ARES-i25 scale [22], which contains 25 items distributed on the following scales:

1. tenacity, and self-efficacy – the competence to evaluate the life situation, and the necessary steps to solve the various problematic contexts, and personal resources/limits, as well as the ability to organize resources to solve problems;

2. self-confidence – reflects a positive self-image obtained in objective relation to intuition, and capabilities;

3. ability to learn from life experiences (personal, and/or other people's);

4. rapid recovery from negative life events - tolerance of negative affect, and uncertainty, recovery from failure to adopt resolving means, identification of sources of support, focus on goal achievement, and resistance to disruptive factors;

5. social, and family resources – are the factors external to the person that play a supportive role in coping with various life problems, and in manifesting resilience.

Participants' responses are scored in five steps (1 = strongly disagree, 5 = strongly agree), and the results can be reported both overall and by dimension. ARES-i25 was applied and validated on 423 participants in Bucharest, aged between 19 and 42 years, 260 female, 163 male. Following the factorial analysis, it was found that the scale, at the general level, explains 72.1% of psychological resilience, with coefficients showing that it has a very good internal consistency (Cronbach's $\alpha = 0.83$) at the general level, but also by dimensions ($0.71< \alpha > 0.89$). ARES-i25 also has content/construct validity, which was also verified by the Delphi method (10 experts). For the present study, the internal consistency of the scale items that were applied to the research subjects (Cronbach's α coefficient = 0.78) demonstrated the fidelity of the ARES-i25 scale in the new context in order to draw valid conclusions.

Attitudes towards artificial intelligence were investigated using the scale constructed by [23], abbreviated ATTARI-12, the authors building on other studies [24, 25]. The 12 items of the scale represent the three facets of attitude (cognitive, affective, and behavioral), with the scale containing an equal number of items for each of these, and reflecting equally positive or negative attitudes towards AI. Participants' responses are scored in five steps (1 = strongly disagree, 5 = strongly agree), and results can be reported both overall and by dimension. The ATTARI-12 was administered and validated on 488 U.S. participants, aged 19-72 years, 211 female, 273 male, and 4 subjects who did not indicate gender. The factor analysis found that the scale, overall, explains 79% of attitudes towards AI, with coefficients showing that it is a unidimensional scale with very good internal consistency (Cronbach's $\alpha = 0.93$), and that it also has convergent validity, with a statistically significant positive correlation with the scale of attitudes towards specific AI applications (r = 0.93), and that it has convergent validity, with a statistically significant positive correlation with the scale of attitudes towards specific AI applications (r = 0. 60), which proves the psychometric characteristics of the ATTARI-12 scale [26]. For the present study, the scale was translated, and the internal consistency of the items (indicated by Cronbach's α = 0.76) demonstrated the fidelity of the ATTARI-12 scale to draw valid conclusions.

RESULTS

THE IBM SPSS Statistics v23 program was used to analyze and process the data from this research.

For analyzing the first hypothesis, which assumes that between psychological resilience, and some of its dimensions (tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources -, and employees' attitude towards AI there are interdependent relationships), and the employees' attitude towards AI, the Pearson correlation statistical procedure was used. The obtained results are presented in Tables 1 and 2.

Table 1. Descriptive statistics for the variables psychological resilience, its dimensions, and attitude towards IA

Research variables	Mean	Standard Deviation
Psychological resilience	64.19	22.35
Tenacity, and self-efficacy	13.22	5.39
Self-confidence	12.97	4.24
The ability to learn from life experiences	16.45	5.13
Quick recovery from negative life events	13.89	3.94
Social, and family resources	17.38	5.36
Employee attitudes towards AI	32.44	11.57

Table 2. Correlations between psychological resilience, its dimensions, and employee attitudes towards AI

Research variables	Employee attitudes towards AI
Psychological resilience	0.91**
Tenacity, and self-efficacy	0.79**
Self-confidence	0.74**
The ability to learn from life experiences	0.83**
Quick recovery from negative life events	0.82**
Social, and family resources	0.75**

*** Correlation is significant at the 0.01 level (2-tailed)*

These results demonstrate a positive and highly statistically significant correlation between psychological resilience and employees' attitudes towards artificial intelligence (AI). Specifically, the more psychologically resilient a person is, the more positive their attitudes towards AI. This strong relationship (r = 0.87) is also supported by the analysis of individual dimensions of resilience. All the dimensions of psychological resilience - including tenacity, self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative events, and social and family resources - show strong positive correlations with employees' attitudes towards IA (with correlation coefficient values ranging from 0.74 to 0.91). This suggests that each aspect of resilience contributes to a favorable perception of IA. In conclusion, psychological resilience is a key factor in shaping employee attitudes towards AI. These findings may have important implications for organizations in the context of the ever-accelerating adoption of AI technologies, suggesting that developing employees' psychological resilience could facilitate a smoother and more positive integration of AI in the workplace. There are also statistically significant correlations ($p < 0.05$) between psychological resilience and its dimensions, and between all dimensions of resilience, but these are not within the focus of the research and have not been analyzed or presented in this paper. The effect size of each statistically significant correlation between different variables was calculated using the r2 coefficient; the values of this coefficient ranged between 0.54 and 0.82, thus indicating a high level of association between variables [27], which, beyond the statistical level, adequately reflects reality.

The results of the second hypothesis, predicting that attitudes towards AI can be predicted and explained by the psychological resilience of employees and its dimensions, were obtained by multiple regression. The standard analysis method (Enter) was used, considering 6 predictor variables (psychological resilience, tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources) for employees' attitude towards AI (criterion variable).

Examination of the multiple correlation coefficient (with R-value = 0.81) indicates a high correlation between the predictor variables, and the criterion variable,, and its R2 value (0.65) shows that 65% of the variance in employees' attitudes towards AI is determined by the predictor variables (psychological resilience, tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources).

The analysis of variance tested the significance of R, and the value of F (68.22 at $p = 0.01$) denotes that the predictor variables jointly predict the variance of the criterion variable.

The significance of the individual regression coefficients was assessed using the t-test, and their values (significant at $p < 0.05$) reveal that all predictor variables are important for the estimation of the criterion variable (attitude towards AI). The results are summarized in Table 3.

Table 3. Degree of contribution of predictor variable values to the regression line, and significance of multiple regression

	Unstandardized coefficients		**Standardized coefficients**	**t**	**p**	**R**	**R^2**	**F**	**p**
Model	B	Std. error	Beta						
(Constant)	87.13	11.16		38.45	0.01	0.81	0.65	68.22	0.01
Psychological resilience	42.15	12.43	2.31	10.57					
Tenacity, and self-efficacy	28.52	10.24	1.98	9.38					
Self-confidence	22.14	8.28	0.88	10.22					
The ability to learn from life experiences	21.76	8.33	0.71	11.54					
Quick recovery from negative life events	18.33	5.66	0.56	10.87					
Social, and family resources	14.99	8.47	0.42	11.07					

Based on these data the regression equation can be written: ***Employees' attitude towards*** AI = 87.13 + (42.15) * *psychological resilience* + (28.52) * *tenacity, and self-efficacy* + (22.14) * *self-confidence* + (21.76) * *ability to learn from life experiences* + (18.33) * *quick recovery from negative life events* + (14.99) * *social, and family resources*.

The reliability of the regression equation prediction was verified by calculating the bivariate correlation between the employees' attitude towards AI and its estimation (pre_1), obtaining $r = 0.81$ (value identical to the multiple correlation coefficient).

The statistical data obtained in the present research indicate that there are 6 predictors of employees' attitude towards artificial intelligence, which both individually and jointly estimate this attitude.

The results show that psychological resilience is the strongest predictor of employees' attitude towards AI, with a regression coefficient of 42.15. This suggests that, independent of its specific dimensions, an overall high level of resilience contributes significantly to a positive attitude towards AI and is a fundamental factor shaping employees' perceptions. The equation shows that attitude towards AI is not only influenced by overall resilience but also by the independent contribution of each dimension. Even though there is a conceptual overlap between overall resilience and its dimensions, the regression equation allows us to see the added impact of each component.

At the same time, these results suggest that organizations wishing to improve employee attitudes towards AI should focus on developing psychological resilience through specific programs, which would lead to easier acceptance of AI and multiple possibilities of its use.

DISCUSSION

The present research started from a number of studies in the literature that have emphasized that positive interaction with technology provides a framework for the development of psychological resources, and that artificial intelligence promotes more effective coping mechanisms by increasing resilience, especially in a professional setting, and during emergencies.

The objective of this research was to analyze the relationship between psychological resilience – as a resource that ensures effective adaptation to various new or difficult situations –, and the attitude of Romanian employees towards artificial intelligence.

The research results (through the significant bivariate correlations identified) proved that people with a high level of psychological resilience have an open attitude towards AI, but also that this attitude is directly proportionally associated with psychological resilience, and its dimensions (tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events,, and employees' social, and family resources).

Deeper statistical analyses have shown that these variables associated with employees' attitudes towards AI are also predictors of AI.

The novelty of the research is represented by the association between the level of psychological resilience - seen as a resource that ensures adaptation (to uncertainty, difficulties in life) of people from a psychological perspective -, and the employees' reporting to artificial intelligence.

Implementing AI in the workplace often brings with it significant changes: new tasks, the need to learn new skills, redefinition of roles, and sometimes even the fear of replacement. In such a context, psychological resilience acts as a protective shield and an adaptive engine. Resilient employees are, by definition, better able to manage stress and uncertainty, to adapt quickly, to see opportunities rather than threats in AI. In terms of the contribution of specific dimensions of resilience in attitudes towards AI:

- tenacity, and self-efficacy - people who believe in their ability to complete tasks, and overcome obstacles (self-efficacy), and who persist in the face of difficulties (tenacity) will approach AI integration with a proactive mindset, seeing it as a challenge that can be overcome;

- self-confidence - a strong self-esteem helps employees not to feel threatened by AI, but to perceive it as a tool that can enhance their own capabilities, not diminish them;

- the ability to learn from life experiences - this dimension emphasizes the importance of cognitive flexibility and openness to new things. Employees who have demonstrated this ability in the past will be more likely to embrace the new knowledge needed to work with AI.

- rapid recovery from negative events - in the context of AI, this translates to the ability to bounce back quickly from initial frustrations or learning difficulties. They do not give up easily but find ways to move on;

- social, and family resources - a strong support system provides a sense of security and stability, reducing anxiety about change, and allowing employees to better focus on adapting and learning.

This deep underst, anding of the link between resilience, and attitudes towards AI has major practical implications for organizations, namely investing in resilience development programmes, transparent, and empathetic communication (an organizational culture that promotes open dialogue, and underst, anding of employees' concerns can strengthen collective, and individual resilience, facilitating acceptance of AI), .creating a supportive work environment (where difficulties can be discussed, and employees find support in resolving them). In essence, the success of integrating AI into organizational structures depends not only on the technology itself but also, to a large extent, on the psychological preparation of the workforce. By cultivating resilience, organizations not only improve attitudes towards AI but also build a more adaptable, motivated, and prepared workforce for future challenges.

These results cannot be considered as exhaustive conclusions, but in relation to other particularities that could be mediating factors - for example, certain personality traits, openness to novelty, resistance to change, adoption of certain coping strategies, etc. [28, 29, 30].

The results of the present research are in line with the existing literature, with the concept of the importance of socio-technical adaptation of employees,, and the experience, and type of organization play a crucial role in facilitating adaptation to AI; conversely, social, and psychological adaptation of employees can be facilitated with the help of AI-based gadgets, and can reflect on the level of well-being (Giudici, Bento & Falk, 2024). In other words, the differences between organizations in their digital readiness are reflected in the individual's ability to adapt, which supports the socio-technical adaptation framework. Resilience as a central coping mechanism enables individuals to maintain their psychological equilibrium in diverse contexts, including those involving adaptation to new technologies [31, 32, 33, 34, 35, 36, 37].

From the literature review and the data obtained in the present research, it can be considered that psychological resilience as a personal resource helps to adapt to job demands, including those related to AI, helps to understand how AI can be used to increase performance, and well-being at work.

To clarify the influential relationship of psychological resilience on attitudes towards AI research, several directions should be pursued:

- exp, anding the number of subjects (starting from the main limit of this study, consisting of N = 89 subjects);

- to equalize the sample by gender to increase the validity of the conclusions drawn;

- analyzing the role of personality, and other particularities (such as openness to new, resistance to change, stress management, etc.) to establish a psycho-socio-demographic profile of people who would have an open but rational, and cautious attitude towards AI (maintaining a balance between addiction, and fear), respecting ethical principles in the use of AI products;

- exploring the involvement of the organization in developing the psychological resilience of employees to adapt more easily to new technologies, and to ensure an open but rational attitude towards AI.

CONCLUSION

In conclusion, starting from the fact that the research sample contains only 89 subjects, the research has the characteristics of a pilot study,, and the conclusions cannot be extrapolated to the general population of employed people, this being the main limitation of the study. The results of this research are important, however, in that they help to identify trends in the relationship of the influence of psychological resilience on open, rational attitudes towards AI, and to draw directions for further studies to complete the picture of the analysis of this relationship.

Conflicts of interest, and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript,, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, R.M., A.P., and M.L; methodology, R.M; software, A.C.; validation, R.M., A.P., and M.L; formal analysis, R.M; investigation, M.L, and A.C.C.; resources, A.C.; data curation, A.P., and M.L.; writing—original draft preparation, R.M.; writing—review, and editing, R.M, and A.P.; visualization, M.L..; supervision, R.M; project administration, R.M.; funding acquisition, M.L., and A..C. All authors have read, and agreed to the published version of the manuscript.

Ethics approval, and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines, and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

An overview of the complications of acute and chronic mercury exposures. Past, present, and future

Genica Caragea[1,2], Andreea C. Vãrzaru[1], Oana Avram[2,3], Radu Macovei[2,3], Andreea Costea[4], Dragos M. Popescu[5], Andreea M. Smarandache[3]

Abstract: *Mercury is a chemical element that has been known since prehistoric times and has been used over time as a component of paints, in the treatment of various diseases, and later in various industrial compounds. Mercury enters the body transcutaneously, by inhaling or by ingesting food contaminated with mercury. The most common cause of mercury poisoning is eating seafood, but people can get mercury poisoning from industrial processing, thermometers, dental work, and old paints. The most vulnerable are people at occupational risk due to chronic exposure, as well as fetuses in the intrauterine period. Mercury poisoning produces extremely serious effects on the nervous, renal, and gastrointestinal systems. The nature and extent of mercury poisoning depend on factors such as the route of exposure, the rate of exposure, the distribution and biotransformation of mercury in the body, the chemical form of mercury or the mercury compound. Both mercury in the blood and the urine should not exceed 10 µg/L. The critical values of mercury in the blood are >150 µg/L and the lethal ones exceed 800 µg/L. New diagnostic methods allow the rapid and accurate identification of the concentration of mercury in biological products. Treatment is based on the use of chelating agents. However, the prognosis of mercury poisoning is extremely variable, due to the potentially irreversible nature of the lesions.*

Keywords: *mercury exposure, neurotoxicity, gastrointestinal toxicity, diagnosis, chelation therapy*

INTRODUCTION

Mercury or hydrargyrum (from the Greek language), is one of the 6 liquid elements at a temperature and pressure close to those of the room [1, 2]. It is found in nature in the form of sulfide, also known as cinnabar. Under the name of vermillion, it was used by people to paint the walls of caves in Spain and France in prehistoric times. Archaeological discoveries have shown that mercury has been known since antiquity by the Romans, Greeks, Chinese, and Hindus [3].

Beginning in the 16th century, new information regarding mercury emerged. Agricola detailed the use of mercury and analyzed the effects of inhalation of its vapors on human health [4]. Thanks to the writings of Paracelsus [5], mercury had become, along with antimony [6], an element used in the treatment of venereal diseases [7]. Its anti-inflammatory and antiseptic effects have also determined its use in the treatment of syphilis. It was used in the form of dimethylmercury, but its action on the central nervous system led to the cessation of these treatments [8]. Around

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1913, diuretics based on organic mercury compounds were used, which were released on the market for more than 30 years [8]. Mercury is also used in dental work (especially fillings), in some antiseptics, or vaccine preservatives. Mercurochrome was used to treat wounds, later being given up due to not being recognized as safe and effective.

SOURCES OF MERCURY

Mercury is a pollutant of global concern owing to its great toxicity even at very low concentrations [9]. Most of it is deposited in oceanic sediments and soil. The global mercury cycle involves its release from the earth's crust, the air transport of mercury vapor, and its storage in soil and water. Aquatic ecosystems are an essential component of the global biogeochemical cycle of mercury, as inorganic mercury can be converted to toxic methylmercury in these environments [10]. Through burning organic matter and industrial processes, volatile forms of mercury can be released into the atmosphere. The origin of mercury is determined by the type of fuel that was used, such as coal, oil, or municipal waste. Mercury also enters the environment due to the incineration of materials such as car components, batteries, fluorescent bulbs, medical products, thermometers, and thermostats [11]. Leather manufacturing companies or tanneries are highly polluting, generating enormous amounts of solid and liquid waste, including mercury compounds [12].

The most common cause of mercury poisoning is from eating seafood, but people can get mercury poisoning from industrial processing, thermometers, and blood pressure machines, dental work, and old paints [13]. Methylmercury can be absorbed from the water by all sea creatures, but it also continues through the food chain.

Small sea creatures, such as shrimps, often ingest methylmercury and are then eaten by other fish. These fish will now have more methylmercury in them than the original shrimp. This process continues up the food chain, so that a large fish may contain much more mercury than the fish it has eaten.

ROUTES OF PENETRATION

Mercury enters the body transcutaneously, by inhalation, or by ingesting food contaminated with mercury [14]. Transcutaneous absorption of mercury is low. The physical properties of elemental mercury limit its absorption through intact skin. Inhalation of toxic mercury vapors leads to their absorption into the circulatory system, then being distributed throughout the body [15]. The absorbed mercury circulates through the bloodstream, and about half of it is bound to plasma albumin (in association with sulfhydryl groups), while the other half enters the red blood cells and then is rapidly distributed to the tissues; in a few hours, the highest concentration is found in the kidneys. Mercury can build up in the bones, bone marrow, and liver for a short time. One week after exposure, 85-95% of the body's mercury is stored in the kidneys [16].

MERCURY TOXICITY

The primary mechanism of mercury toxicity involves its irreversible inhibition of selenoenzymes, such as thioredoxin reductase (IC50 = 9 nM) [17]. Thioredoxin reductase restores vitamins C and E, as well as several other important antioxidant molecules, back into their reduced forms, enabling them to counteract oxidative damage [18].

Mercury in its various forms is particularly harmful to fetuses as an environmental toxin in pregnancy, as well as to infants. Women who have been exposed to mercury in substantial excess of dietary selenium intakes during pregnancy are at risk of giving birth to children with serious birth defects [19].

The destruction of the proteins that form the glomerular basement membrane leads to the alteration of the renal filtration capacity, with the appearance of severe proteinuria. At the digestive level, mercury destroys the gastric mucosa, a process that favors its absorption in the body. Gastrointestinal symptoms are extremely polymorphic, including digestive hemorrhages, nausea and vomiting, diffuse abdominal pain, and transit disorders, such as constipation or diarrhea [20, 21]. The differential diagnosis should consider other causes of the acute surgical abdomen, such as acute cholecystitis, gastroenteritis, or intestinal occlusion [22-24]. Even less frequent causes such as Amyand hernia or pheochromocytoma may be taken into account [24-26]. Lately, during the COVID-19 pandemic, the SARS-Cov 2 infection has been another cause to be excluded, due to the tropism of the virus for angiotensin convertase (ACE) receptors at the level of the intestinal epithelium [27-29]. A carefully obtained history is necessary to evaluate the risk of mercury poisoning and to avoid unnecessary surgeries in suspected cases. Implementing the principle of safe surgery in the emergency ward is important for a comprehensive evaluation of the patient during admission and to minimize the anesthetic-surgical risk [30].

Mercury poisoning causes some neuropsychiatric, sensory, visual, cognitive, and motor disorders. Previous clinical trials have reported symptoms of tremor, emotional lability, insomnia [31, 32] memory disorders, muscle weakness, muscle atrophy and spasms, headaches, polyneuropathy (paresthesia, hyperactive tendon reflexes, decreased nerve conduction and motor speed), and cognitive impairment [32, 33].

Recent studies found a good correlation between exposure to mercury and other heavy metals and the risk of type 2 diabetes in young adults [34-36]. One possible mechanism is the direct toxic effect of mercury on the beta-pancreatic cells [37].

Toxicity of mercury compounds

There are three different forms of mercury that cause health problems. They are:
- Elemental mercury, also known as liquid mercury or quicksilver;
- Inorganic mercury salts;
- Organic mercury.

The nature and extent of mercury poisoning depend on factors such as the route of exposure, the rate of exposure, the distribution and biotransformation of mercury in the body, the chemical form of mercury or the mercury compound. Organic mercury compounds often produce severe and irreversible toxicity to the nervous system in overdoses, while inorganic compounds produce primary peripheral effects, such as gastroenteritis and renal failure [38-40] (Table 1).

Table 1: Manifestations due to the route of exposure, the rate of exposure, the distribution, and the biotransformation of mercury in the body [34].

	Elemental mercury	**Inorganic mercury**	**Organic mercury**
Primary route of exposure	Inhalation	Oral	Oral
Primary tissue distribution	Central Nervous System, Kidney	Kidney	Central Nervous System, Kidney, Liver
Clearance	Renal clearance GI clearance	Renal clearance GI clearance	Methyl: GI Aryl: GI, Renal
Clinical Effects			
Central Nervous System	Tremor	Tremor, Heresy	Paresthesias, ataxias, tremor, dysarthria, tunnel vision
Pulmonary	+++	-	-
GI	+	+++ (caustic)	+
Renal	+	+++ (ATN)	+
Acrodinia	+	++	-
Therapy			
	BAL, succimer	BAL, succimer	Succimer (Early)

GI - gastrointestinal

Quicksilver (liquid metallic mercury) is poorly absorbed by ingestion (Figure 1). Metallic mercury, due to its high volatility and low absorption, has no systemic toxicity. Mercury compounds tend to be much more toxic than the elemental form, and the organic forms are extremely toxic, being involved in kidney and brain destruction. Mercury cyanide can enter the body both by inhalation and by transcutaneous absorption and ingestion. Ingestion of mercury cyanide can cause clinical phenomena similar to both mercury ingestion and cyanide ingestion, leading to extremely severe clinical signs. The degree to which cyanide poisoning occurs is thought to be correlated with the cyanide ions released in the stomach, which depend on the amount ingested, gastric acidity, and volume of gastric contents [41].

Figure 1: Liquid metallic mercury in the large bowel. Source - ICU Toxicology Prof. Macovei collection

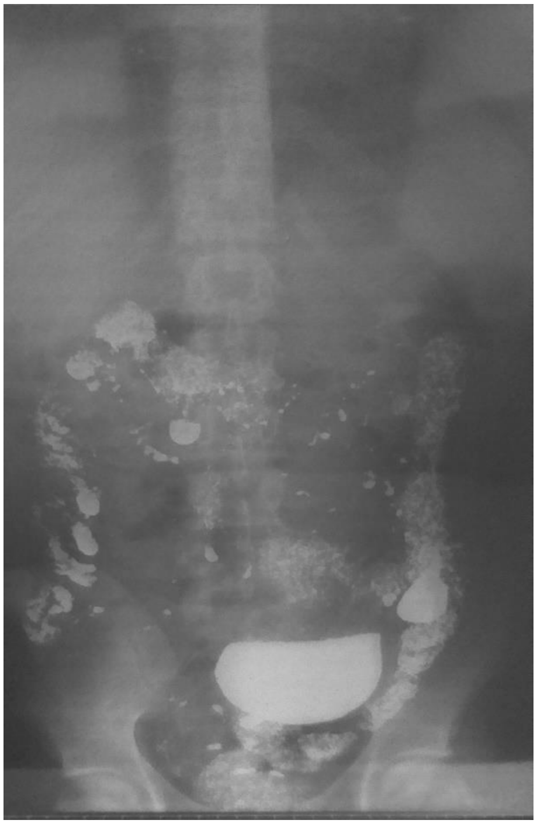

Mercury occurs as salts such as mercuric chloride ($HgCl_2$) and mercurous chloride (Hg_2Cl_2), the latter also known as calomel. Because they are more soluble in water, mercuric salts are usually more acutely toxic than mercurous salts. Their higher solubility allows them to be more readily absorbed from the gastrointestinal tract. Mercury salts primarily affect the gastrointestinal tract and the kidneys, and can cause severe kidney damage [20]; however, as they cannot cross the blood-brain barrier easily, these salts inflict little neurological damage without continuous or heavy exposure. Low-grade chronic inorganic mercury poisoning is the basis of acrodynia (pink disease) in children, who typically developed irritability after exposure to mercury-containing teething powder, ointments, and drugs [41-43].

In 1960, JA Freeman of Orange, New Jersey, described the Mad Hatter syndrome, characterized by Danbury-type tremor, found in workers in hat factories [44]. Mercury was inhaled by workers during the textile dyeing process with mercury nitrate. The characteristic symptoms are tooth loss, salivation, neurological deficits described by Ramazzine, the father of occupational medicine, in 1970. One of the most toxic mercury compounds is dimethylmercury. The toxicity is so high that, at doses of a few microliters in contact with the skin, they can cause death.

Methylmercury (MeHg) is the main source of organic mercury. Due to bioaccumulation, it achieves high concentrations found in some species, such as tuna or swordfish [45]. Clinical phenomena occur after a latency varying from a few weeks to a few months after a single exposure [46]. Paresthesias appear as the first manifestation [47], followed later by much more severe manifestations, such as coma. High-level exposure to methylmercury is known as Minamata disease [48, 49].

Another organic mercury compound responsible for human intoxications is ethylmercury, derived from the metabolism of thimerosal (o-carboxyphenyl-thio-ethyl-sodium salt), which is the most widely used form of organic mercury, still being used as a preservative in some human and animal vaccines [50]. The biologic profile is different from methylmercury, with a shorter blood half-life and increased renal elimination [50, 51]. The nervous system affected by intoxication with this substance has the same symptoms as intoxication with methylmercury, adding signs of kidney tissue damage, such as proteinuria, digestive disorders such as nausea, vomiting, and abdominal and dermatological pain [8, 52].

Under the 2013 Minamata Convention on Mercury, organized by the United Nations Environment Program (UNEP), 140 countries have committed themselves to end mercury emissions [53].

LABORATORY DIAGNOSTIC OF MERCURY INTOXICATION

Diagnosis of elemental or inorganic mercury poisoning involves determining the history of exposure, physical findings, and an elevated body burden of mercury. Although whole-blood mercury concentrations are typically less than 6 μg/L, diets rich in fish can result in blood mercury concentrations higher than 200 μg/L [54, 55]; it is not very useful to measure these levels for suspected cases of elemental or inorganic poisoning because of mercury's short half-life in the blood. Free mercury is not well absorbed through the gastrointestinal tract and is excreted almost completely in the feces after ingestion; however, when it persists, mercury volatilizes at room temperature into vapors that are well absorbed by the lungs.

The diagnosis of organic mercury poisoning differs in that whole-blood or hair analyses are more reliable than urinary mercury levels [56].

Both mercury in the blood and the urine should not exceed 10 μg/L. The critical values of mercury in the blood are more than 150 μg/L and the lethal ones exceed 800 μg/L.

Mercury levels in the blood and urine are normally very low. The result of the test, which does not show mercury, indicates that it is likely that the tested person was not exposed to excessive levels of mercury, at least not during the test. Elevated levels of mercury in the blood suggest a relatively recent exposure to mercury. Mercury levels in the blood or urine will not indicate the form or amount of mercury a person has been exposed to.

Blood and urine mercury concentrations are commonly used as biomarkers of mercury exposure. Hair has been used as a biomarker of exposure to methylmercury [57]. Occupational studies show that recent mercury exposure is reflected in blood and urine [54, 58]. A 24-hour urine sample is the most useful in determining the degree of exposure.

The preanalytical phase in determining the species of mercury or total mercury involves a succession of procedures such as sample collection, conservation, and storage, extraction, purification, preconcentration, and separation of species of interest (if applicable).

Exhaustive handling of samples but also the properties of mercury (especially mobility and high volatility) can lead to erroneous results due to either the loss of analyte or contamination of the sample. In addition, interconversion of the species can occur by manipulating the sample. Quantification is usually performed by atomic spectrometry techniques [59].

Some detection techniques that have been used for total Hg analysis are cold vapor atomic fluorescence spectrophotometry (CV-AFS), cold vapor atomic absorption spectroscopy (CV-AAS), graphite furnace atomic absorption spectrophotometry (GF-AAS), cold vapor inductively coupled plasma atomic emission spectrophotometry (CV-ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) [60]. Mercury determination through flame atomic absorption spectroscopy (FAAS) gives rise to poor sensitivity [61].

Of all these available methods, cold atomic absorption spectrometry is rapid, sensitive, and accurate, but through this method, the forms of mercury cannot be distinguished. Thin layer and technical gas chromatography are used in the determination of mercury species because with the help of these techniques, the forms of mercury can be distinguished.

Analytical parameters of reviewed research papers about the speciation and determination of mercury by spectrometric instruments (CV-AAS, AAS, AFS, GC-AFS, HPLC-CV-ICPMS, ICP-MS) are presented in Table 2 [60-69].

Table 2: Analytical parameters of reviewed research papers about the speciation and determination of mercury by spectrometric instruments (after Suvarapu LN et al)

No.	Analyte	Detection	Method	Limit of detection	Linearity range	Samples	Ref.
1	Total Hg	CV-AAS	Digestion	3.98×10^{-10} M	2.5–10.0 μg L^{-1}	Biological samples	[61]
2	Total Hg	AAS	Amalgamation	0.2 ng/g for hair 0.02 ng/g for blood	—	Hair Blood	[62]
3	Hg(II)	AFS	Fluorescence optical sensor	9.57×10^{-12} M	2.27×10^{-11} 1.13×10^{-3} M	Hair Urine	[63]
4	MeHg	GC-AFS	SPE	12 ng /g	> 1.5 ng/ mL	Biological samples	[64]
5	MeHg, Hg(II), EtHg	HPLC-CV-ICPMS	Extraction and separation	5.98×0^{-11} (Hg(II)) 2.17×10^{-11} (EtHg) 1.8×10^{-8} (MeHg) M	—	Plasma/serum	[65]
6	Total Hg	ICP-MS	Isotope dilution and UV-photochemical vapor generation	0.5 pg/ g	—	Biological tissues	[66]
7	Hg Total	ETAAS	Extraction of mercury species in toluene	2 mcg/dm^3	-	Blood	[67]
8	Total Hg	GC/MPD	Cleavage with HCl, toluene extraction, addition of Grignard reagent	0.4 mcg/L	-	Blood	[68]

The most powerful tool for the determination of trace elements in a variety of matrices is chemical vapor generation in conjunction with atomic absorption spectroscopy techniques. An absorption technique such as cold vapor atomic absorption spectroscopy (CV-AAS) is one of the most sensitive methods for mercury determination. A reducing agent, stannous chloride ($SnCl_2$), is used to convert ions of Hg_2^+ (aq) in solution to Hg0 (g) which produces a higher analytical absorbance signal. Mercury, having a high vapor pressure, is liberated and entrained in an inert gas of argon after passing through the liquid-gas separator. The Hg vapor is transferred into the optical path of the atomic absorption spectrophotometer where it absorbs light of a specific wavelength at room temperature [60].

Another method of determining mercury is atomic absorption spectrometry (AAS) with graphite furnace atomization, using palladium and magnesium nitrate as modifiers; their use makes it easier to separate the analyzed element from the matrix.

Due to its simplicity and low operating costs, AAS is widely applied in the single-elemental analysis [70, 71]. The samples are exposed to concentrated acids and extended heating, which increase analyte loss. Atomic absorption spectrometry is an analytical method for determining the number of metals or metalloids in a sample, based on the amount of UV or VIS electromagnetic radiation absorbed by the atoms brought into the atomizing state. To establish the atomization temperatures, it is necessary to highlight the interferences that can occur through reaction mechanisms in the graphite furnace. In the case of volatiles, the use of chemical modifiers aims to:

- Shift the value of the atomization temperature outside the non-atomic absorbance that may occur due to the complexity of the matrix, which is especially characteristic of biological samples.
- Obtaining a much higher calcination temperature due to the reduced volatility of the analyte, which allows the elimination of compounds that could give a non-atomic absorbance.
- Increasing the sensitivity of the method by simultaneously atomizing the different forms in which the analyte is present in the matrix.

When obtaining optimal operating parameters of the GF-

AAS system, it is desirable to reduce non-atomic absorbance by optimizing drying, calcination, and atomization temperatures and by using the deuterium lamp.

For example, a method for mercury analysis was established by the authors of this paper using a Graphite Furnace Atomic Absorption Spectrophotometer (GF- AAS) Varian SpectraAA-880, with a hollow cathode lamp (Agilent Technologies) and a deuterium lamp (Agilent Technologies) for background correction, coupled to a GTA-100 atomizer and a programmable sample dispenser (Varian, Australia).

The calibration curve was obtained by measuring 5 concentration levels of the standard mercury solution. The results are shown in Figure 2.

Figure 2: The calibration curve used for the determination of mercury

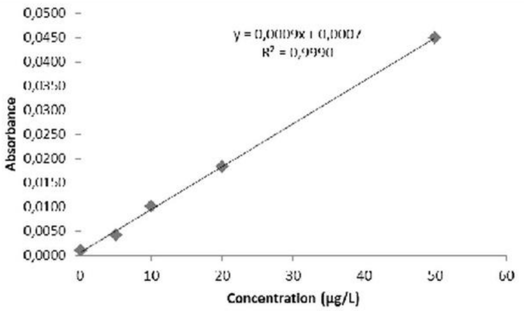

A linear relationship was found between the absorbance at 253.7 nm and the concentration of mercury in the range of 5.0 to 50 µg/L. The representative linear equation was y = 0.0009 x + 0.0007 where: y is the absorbance and x is the mercury concentration (µg/L) calculated by the least-squares method.

The coefficient of determination for the calibration curve was 0.9990 (Figure 2) indicating a good correlation between the absorbance read using the equipment and the concentration of mercury in the standard calibration solution.

The method was applied to the urinary determinations of mercury involved in acute intoxications and to monitor urinary mercury levels during chelation therapy.

However, according to several authors, mercury levels in urine less than 10 mcg/L and bloodless than 20 mcg/L are considered normal. Mercury concentrations in the blood and urine poorly support the neurological clinical findings on electrophysiological measurements. Organic forms of mercury, including phenylmercury, are metabolically transformed into inorganic mercury, compared to methyl- and ethylmercury, which do not undergo metabolic transformations but are eliminated in the bile and feces, so alkylmercury compounds cannot be detected in urine [72].

Urinary elemental mercury levels are useful in assessing acute or chronic mercury vapor poisoning. The concentration of mercury in urine samples in approximately 98% of all evaluated subjects without known mercury exposure is less than 5 µg/L [24].

So, in methyl mercury poisonings, for example, urinary mercury levels do not provide suggestive information, but because methylmercury is concentrated in red blood cells, the whole-blood level may reflect acute methylmercury exposure but does not correlate with the severity of the poisoning/exposure.

THERAPEUTIC OPTIONS

Acute ingestion of mercuric salts can be treated by inducing vomiting or gastric lavage. Resins with polythiol can be administered orally to bind mercury in the gastrointestinal tract. The most effective chelating agents are dimercaprol, succimer, and penicillamine, which have active mono- and dithiol groups.

Acute inorganic mercury poisoning can be treated with dimercaprol in doses not exceeding 24 mg/kg daily, administered intramuscularly in several doses [73]. The treatment is divided into 5-day courses, separated by a few days off. The N-acetylated form of penicillamine is also useful at doses of 30 mg/kg daily in divided doses. Peritoneal dialysis, hemodialysis, and regional extracorporeal hemodialysis with succimer complexation have been used with some success in the treatment of patients with renal impairment [73, 74].

Chelation therapy for acute inorganic mercury poisoning is based on DMSA, 2,3-dimercapto-1-propanesulfonicacid (DMPS), D-penicillamine (DPCN), or dimercaprol (BAL).

Children who ingest mercury can be treated with DMSA. DMSA is the most frequently used agent for severe methylmercury poisoning [75]. When administered orally, it is superior to BAL, DPCN, and DMPS. α-Lipoic acid (ALA) requires correct dosing, as inappropriate dosages increase toxicity. N-acetylcysteine (NAC) as a glutathione precursor is recommended by physicians but has been shown to increase mercury concentrations in the kidneys and the brain.

CONCLUSIONS

Generally, two population groups are more sensitive to the effects of mercury. One of these is fetuses who are most susceptible to developmental effects due to mercury. The second group consists of people who are regularly exposed (chronic exposure) to high levels of mercury (such as populations that rely on subsistence fishing or people who are occupationally exposed). Once the source of mercury poisoning has been identified, the exposure must be discontinued. Novel laboratory methods, such as GF-AAS, allow quick identification of mercury in biological products. However, the prognosis of mercury poisoning is highly variable, depending on the irreversible neurological and organ damage that is present at the moment of diagnosis.

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ORIGINAL ARTICLES

Crude venom of *Pseudocerastes persicus* snake: From the antibacterial to anticancer effects

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Abstract: *Antibiotic resistance has been reported as one of the world's most critical public health problems.*

Recent investigations have demonstrated that the venom of some species of snakes has antimicrobial and anticancer activities. The purpose of this study was to examine the antibacterial activity of Persian horned viper venom toward Escherichia coli, Bacillus subtilis, and Staphylococcus aureus bacteria and also investigate antitumor effects of concentrations of venom with higher antibacterial activity in a human hepatocellular liver carcinoma cell line (HepG2) to evaluate the potential use of its components as antimicrobial and anticancer agents. Bactericidal activity of crude venom in concentrations of 6.25-400 µg/ml was performed using MTT reduction, minimum inhibitory concentration (MIC), agar-well diffusion, and disc diffusion methods. Tetracycline (50 µg/ml) was used as a standard antibiotic. Cytotoxic effect in HepG2 cells was measured by MTT reduction assay and confirmed with neutral uptake assay following exposure of cells with different concentrations of venom (50-400 µg/ml). The apoptotic effect was investigated using the comet assay. Our findings demonstrated that venom displays higher inhibitory effects against Gram-positive bacteria as compared to Gram-negative. Furthermore, venom showed anticancer activity on the HepG2 cell line through induction of apoptosis and necrosis. The results of this study confirmed that the venom of Persian horned viper induces antibacterial and anticancer effects. These properties make the venom of this viper a potential source for isolation of effective molecule(s) having antibacterial and antitumor activity.

Keywords: *Pseudocerastes persicus, Antibacterial, Cytotoxicity, Snake venom, anti Cancer*

INTRODUCTION

In the world of pharmacology, along with the discovery and use of new antibiotics, pathogenic bacteria also have features that are gradually resistant to them. This has always been a problem for microbiologists, a constant research field for researchers to discover more effective and newer chemical antibiotics as well as antimicrobials derived from natural sources such as toxic plants and animals, including snakes (Park et al., 2012). Studies have shown that some of the active compounds in venomous animals are an important source for the separation of effective drug substances, some of which are naturally capable of destroying bacteria (Harvey and Robertson, 2004; Koh et al., 2006). Snake venom is a mixture of various biological substances, including various proteins with enzymatic and non-enzymatic properties (Chellapandi and Jebakumar,

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2008) which generally contain neurovenom, proteolytic, coagulant, phospholipase, cholinesterase, hyaluronidase, amino acid oxidase, and other enzymes (Sachidananda et al., 2007). Studies show that the effective ingredients in oral saliva or venomous snakes have long been considered by researchers and pharmacists for the treatment of some diseases, the production of various drugs, serums, and vaccines (Kuhn-Nentwig, 2003). The first reports of antibacterial activity in the venomous snakes of the family of Alpideh and Vipride in 1948 and 1968 were published (Ferreira, 1965) and Skarns also reported antimicrobial activity of some enzymes in Snake in 1970 for the first time (Aloof-Hirsch et al., 1968). Subsequently, many studies have been done about the presence of antibacterial agents in the venom of different species of snakes, which caused the isolation of some antibacterial peptides from them. Also, reports show that some of the molecules in the venoms of snakes have anti-tumor activity and prevent the growth and proliferation of cancer cells (Latifi, 2000). Chalmette's first study in 1933 examined the use of snake venom for the treatment of cancer in laboratory animals. It has introduced the results of snake venoms as an important natural source for the isolation and identification of effective molecules in the treatment of sudden illnesses like cancer in the world of pharmacology (Latifi, 2000). Pseudocerastes persicus snake is one of the most dangerous venomous snakes and a member of the Viperidae family. In addition to Iran, this Pseudocerastes persicus snake has been reported from Pakistan, Afghanistan, Oman, and Iraq, and in Iran, in the sandy, rocky and volcanic areas of Yazd, Khorasan, Kerman, Sistan and Baluchestan, Isfahan, Fars, Semnan, Central, Khuzestan, Zanjan, Tehran, Qom, and Hormozgan (Latifi, 2000). This study aimed to investigate the effect of Pseudocerastes persicus snake venom on the growth of gram-positive and negative bacteria and also the effect of probable concentrations of antibacterial effects of Pseudocerastes persicus snake venom on the growth of liver cancer cells in vitro conditions for evaluating the possibility of isolation of antibacterial and anti-cancer component.

MATERIALS AND METHODS

Venom Preparation and Protein content determination

To prepare the venom from the vein, while observing the safety considerations and placing its toxic teeth inside the glass of venom collection, and with brief pressurization to the toxic glands of the viper, the venom contained in them was injected into the glass (Lu et al., 2002). The lyophilized solution was stored and kept until it was used in experiments at -20°C. To carry out antibacterial tests, some of the venoms were pulverized in 250 µl of 50 mM sterile tris hydrochloride buffer, and a few of the venom was mixed in 150 µl water twice per sterilized distillation. The amount of protein in the homogenized toxic solution was determined using the Bradford method (Bradford 1976) at 595 nm (Bradford, 1976). To eliminate potential microbial contamination in the venom solution, the required 1% antibiotic-anti-smectic antibody (Antibiotic-Antimycotic: Invitrogen, USA) is required, and after one night at 4°C was used in experiments.

The bacteria and cell line used

Gram-positive bacteria Bacillus subtilis (nonpathogenic) (Bacillus subtilis subsp. spizizenii, ATCC 6633), gram-negative bacteria of Escherichia coli (ATCC 25922 Escherichia coli), and gram-positive strain of Staphylococcus aureus (pathogenic)(ATCC 25923) from the collection center of Persian Type Culture Collection (PTCC) and liver cancer cells (HepG2) were developed and used from the cell bank of Pasteur Institute of Tehran.

Antibacterial properties of crude venom

Culture media and antibiotics: For a culture of bacteria used from liquid and solid Mueller Hinton (MH) and for comparing the toxic effect of venom with a standard antibiotic used from tetracycline (Sigma, USA) at a concentration of 50 µg/ml.

MTT assay

MTT test is a colorimetric method based on the regeneration and breakdown of tetrazolium yellow crystals by the enzyme succinate dehydrogenase present in the cytoplasm and eventually the formation of insoluble blue crystals. These crystals are dissolved by adding DMSO (Wang et al., 2010). Test microorganisms were grown in MH broth for 5 h (exponential phase) and adjusted to 0.5 McFarland turbidity standard (A600 = 1.0), corresponding to 1.5×106 cfu/ml (Yalcın et al., 2014), 5µl was added in sterile plate 96-wells. Also, the final volume of each well was measured using a liquid culture medium (MH Broth) to 100µl using serial dilution. The bacteria were contacted at concentrations of 6.25, 12.5, 25, 50, 100, 200, and 400µg/ml of crude venom. In this experiment, tetracycline (50µg/ml) as a positive control, a culture medium containing bacteria as negative control, and a non-bacterial culture medium was used as blank. The plate was incubated for 23 hours at 37°C. Then, 5µl MTT (concentration 5µg/ml) was added to all wells and placed in dark conditions at 37°C for one hour. Then, 100µl of DMSO was added to each well. After 2 hours of incubation in dark conditions, the optical absorption of wells at 595 nm was measured using a plate reader (Biotek, USA). The above experiment was performed 3 times and at each time 3 wells (3 repetitions) were considered for each concentration. The percentage of bacterial survival after contact with different concentrations of the venom was calculated using the following formula (Yalcın et al., 2014).

Bacterial vital percent:

Viable Bacterial = (a/b) ×100
a = Optical Absorption Sample - Blank Optical Absorption
b = Negative optical absorption - Blank optical absorption

Minimum Inhibitory Concentration Test (MIC)

The steps of performing the minimum inhibitory test on plate 96-well were similar to the MTT assay but after placing the bacterium in a culture medium containing different concentrations of venom and incubating them for 24 hours at 37°C without adding any marker. Also, the absorbance of wells at 605 nm was measured using a plate reader (Biotek, USA). This experiment was repeated 3 times and at each concentration 3 wells (3 repetitions) were considered for each concentration. The inhibitory percentages of the effect of venom and standard antibiotics on the tested bacteria were calculated using the following formula (Shebl et al., 2012).

Percentage of inhibition of bacteria = [1-(a/b)]×100
a = Optical Absorption Sample - Blank Optical Absorption
b = Optical Absorption Control - Blank Optical Absorption

Disc diffusion assay

This experiment was carried out according to Bauer et al (Bauer et al., 1966).

The bacteria were first cultured in a culture medium Mueller–Hinton broth and incubated for 24 to 18 h at 37°C. A solid culture medium with a thickness of 4 mm was prepared in glass plates. 200µl of the 0.5 MacFarland suspension was placed on a plate surface and a cotton seedling was cultured using a sterile loop. Using sterile pin disks (prepared from antibacterial medicine) containing 40µl of venom in the concentration of 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml on the surface plate was placed. In this experiment, 50µg of tetracycline antibody was used as a positive control for 50 µM tetracycline, and 50 mM Tris hydrochloride was used as a negative control. Plates were incubated for 24-18 hours at 37°C, and then an inhibition zone was determined and its size was determined. This experiment was performed 3 times for each bacterial strain.

Well diffusion assay

This method is similar to the disk diffusion method, except that in this test, the venom solution was added to the well instead of being placed on the disk (Torres et al., 2010). In summary, after the cultivation of grass, using the end of the pasteurized pipette, the wells were transferred to the medium and cut into pieces of gel, and 20µl of liquid agar medium was used to block the bottom of the well and prevent leakage and solution leakage. The venom was poured into the well under the medium. After pouring 40µl of Crude venom in concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml, the plate was incubated for 24 to 18 hours in an incubator at 37°C. After the mentioned time, the presence of a halo around the wells was investigated and its diameter was determined. This experiment was performed 3 times for each bacterial strain in two replicates.

Evaluation of anti-cancer properties of crude venom

Cell culture

Liver cancer cell line (HepG2) was cultured using DMEM-F12 (Gibco, USA) containing 10% Fetal Bovine Serum (Gibco, USA). These cells were cultured in flasks of 25-50 ml cubic meters and stored at 37°C and 5% carbon dioxide. Replacing every 2-3 days of the culture medium and after providing the cell population required for each test, using a Trypsin-EDTA prepared from Sigma-Aldrich, the USA from a flask and after counting using a Neobar lam were placed.

MTT reduction assay

This experiment was carried out according to the method performed by Zargan et al. (Zargan et al., 2011). To perform this test, 3×104 cells/well were plated in 96 wells containing 100µl of non-serum culture media. After overnight incubation at 5% CO_2, moisture content of about 80% and temperature of 37°C were removed outside the culture medium and the cancerous cell was exposed to a new culture medium (lacking serum) containing different concentrations of venom (50, 100, 200 and 400µg/ml). After 24 hours, 5µl of MTT solution (concentration 5mg/ml) was added to each well. After the formation of crystalline (formalin) in living cells, the culture medium of each well was removed and after washing in the well with PBS, 100µl of DMSO was added. After 4 to 3 hours of incubation in dark conditions and dissolving purple crystals in DMSO, the optical absorption of wells at 570 nm was measured using a plate reader (Biotek, USA). In this essay, the culture media was used as a blank and control medium containing the cells as control. This experiment was repeated 3 times, and 3 wells (3 repetitions) were considered for each concentration. The percent survival of the cell after contact with different concentrations of the venom was calculated using the following formula (Shebl et al., 2012).

% Viable cell = (a/b) × 100
a = the absorbance of treated cells - the absorbance of the blank

b = the absorbance of the control - the absorbance of the blank

Cytotoxicity study by Neutral Red Uptake Assay

The steps of performing a neutral color red color test (Waheed et al., 2013) were similar to the MTT assay, but after incubating the cell with a toxic solution for 24 hours, a microplate of neutral red solution (5 mg/ml) instead of MTT was added to each well. After binding of neutral red matter to the lysosome surface of the living cell and the formation of red crystals in the living cytoplasm, the culture medium containing the neutral red of each well washed and after washing twice with each PBS, 100 µl of fixative buffer (37% formaldehyde, Calcium chloride (10%) was added. After one minute, the buffer was removed and 100µl of solvent buffer (0.5% acetic acid) was added. The plate was measured on a shaker for twenty minutes under microwave conditions and incubated at lab temperature and absorbed by wells at a wavelength of 540 nm using a plate reader (Biotek, USA). This experiment was repeated 3 times, and 3 wells (3 repetitions) were considered for each concentration at each concentration. The mortality rate due to different concentrations of venom on cell growth was calculated using the following formula.

Percentage of cell death = [1-(a/b)] ×100

a = the absorbance of treated cells - the absorbance of the blank

b = the absorbance of the control - the absorbance of the blank

Investigation of induction of apoptosis by venom using comet assay test

This test is one of the best methods for checking the damaged DNA in the cell (Sajad et al., 2013). To conduct this experiment, at first, in each well of the plate, 24 sterile wells containing 300µl of serum medium, cultured 12×104 cells. The plate was incubated overnight under conditions of 5% CO2, humidity 80%, and temperature 37 ° C. In this assay, the culture media was used as a blank and control medium containing the cells as control. After that, the initial culture medium of each well, evacuation, and cells was exposed to 300µl of a new culture medium (without serum) containing different concentrations of venom (50, 100, 200, and 400µg/ml).

After incubating the cell for 24 hours at 37 ° C, 80% moisture content, and 5% CO2, cells of each well were isolated using trypsin. Microtubes containing cells were evacuated for 5 minutes at 4 ° C with 1500 rpm of the centrifuge and aqueous solution. 400µl of PBS (pH = 7.4) was added to each of the tubes to wash the cells, and after centrifugation, the supernatant was evacuated. Then, 200µl of PBS was added to the tubes, and cells were separated from each other by the separate sampler and insulin syringe. Also, the slides needed for testing were coated with agarose with normal melting point (NMA% 1), test cell suspension and control with low melting point agarose (LMA% 1), one to two on the slides a flat layer was placed. To lyse the cell and nucleus membrane, all slides are placed in the refrigerator in a refrigerator buffer (NaCl 2.5M, EDTA 100mM, Tris 10mM, NaOH 0.2M, Triton X-100% 1 pH = 10) for 16-18 hours. After removing the buffer, the slides were washed for 20 minutes and 2 times with electrophoresis buffer (3M NaOH, EDTA 1mM, and pH>13), and then to open the DNA for 40 minutes in a cold electrophoresis buffer temperatures were maintained at 4°C. The slides were then extracted from the solution and placed in a buffer containing an electrophoresis tank and electrophoresed at 45°C for 45 minutes at a voltage of 25 and 300 mA. In the next step, to undo the playing environment, the slides were placed in a neutralizing buffer (Tris% 4 M, pH = 7.5) for 10 minutes. To staining cells, 100 µl of ethidium bromide solution (concentration 20 µg/ml) was added to each slide, and after 10 minutes incubated at the laboratory for 10 minutes, they were distilled twice with water. DNA of each slide cell was studied by fluorescence microscopy. For each sample, different slide positions and at least an image of hundreds of cells' DNA were prepared and the results were analyzed statistically.

Statistical analysis

The results of each test were reported as Mean±SD and the data were analyzed using software (GraphPad InStat). Different concentrations of venom were compared to the control group and compared to each other by one-way ANOVA and Tukey tests. P<0.05 was considered significant in all tests. Draw diagrams in the Microsoft Excel software (2013 version).

RESULTS

Evaluation of the Anti-Bacterial Properties of Crude venom

Test results MTT assay

The results showed that venom only at 400µg/ml concentration significantly inhibited the growth of Escherichia coli bacteria compared to the negative control (normal culture medium). The critical percentage of this bacterium in concentrations of 6.25-50 µg/ml was 100 and in 100, 200, 400 µg/ml concentrations respectively were 95.79, 96.29, and 83 (Figure 1).

In the case of the Bacillus subtilis strain, Crude venom in the concentration of 12.5 to 400µg/ml produced a significant inhibitory effect on bacterial growth compared to the negative control.

The percentage of vital bacteria in different concentrations of venom 6.25, 12.5, 25, 50, 100, 200 and 400 μg/ml respectively were 89.62, 62. 49, 13.73, 6.56, 2.14, 1.63 and 4.6.

Figure 1: Antibacterial effect of different concentrations of Pseudocerastes persicus snake crude venom on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus using MTT assay. The effect of concentrations was evaluated in comparison with the negative control group.

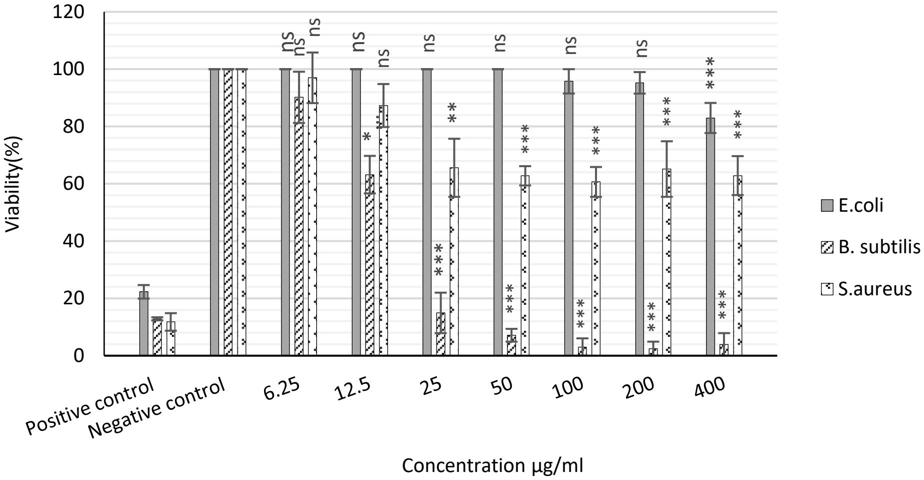

(: p<0. 05), (**: p<0. 01), (***: p<0.001), (ns: non-significant).*

Venom hurt the growth of this bacterium at a concentration of 6.25μg/ml, but this effect was not significant in comparison with negative control. In the range of 25-400, the inhibitory effect of the venom was greater than the positive control (tetracycline). Statistical analysis showed that the effect of venom in a concentration of 50-400 μg/ml was significant for this bacterium but it was not significant compared to each other. According to the results, IC50 of the venom for Bacillus subtilis was determined to be 15.32μg/ml. The results also showed that the vital percentage of Staphylococcus aureus bacteria in the concentration of 6.25, 12.5, 25, 50, 100, 200, and 400μg/ml of crude venom were 95.11, 85.29, 72.48, 62.23, 59.98, 63.31, and 63.96. Crude venom at a concentration of 25-400 μg/ml produced a significant inhibitory effect on the bacteria compared with the negative control. Venom in the concentration of 12.5μg/ml hurt the growth of this bacterium, but this effect was not significant compared to negative control. Statistical analysis showed that the effect of venom in concentrations of 25-400 micrograms per ml was significantly less than the negative control, but compared with each other, it is not significant (Figure 1).

Minimum Inhibitory Concentration Test results

Pseudocerastes persicus snake venom at concentrations of 6.25-100 did not inhibit the growth of Escherichia coli, but induced inhibitory effects at concentrations of 200 and 400 μg/ml, respectively were 12.15 % and 24.99 % which, compared to the intolerance of tetracycline, and the difference in their effect is significant (Figure 2).

The inhibitory effect of venom on Bacillus subtilis bacteria in the concentrations of 6.25, 12.5, 25, 50, 100, 200, and 400 μg/ml was 23.22, 58.92, 92.80, 99.48, 99.65, 99.71, and 99.3, respectively. According to the results, the IC50 of the venom for Bacillus subtilis was determined by 10.81μg/ml. The statistical analysis showed that Crude venom at a concentration of 6.25-400 μg/ml had a significant inhibitory effect on Bacillus subtilis bacteria growth compared to the negative control. The inhibitory effect of venom was 25-400 μg/ml in comparison to the negative control, but compared with each other and in comparison with standard antibiotics was not significant (Figure 2).

Crude venom has an inhibitory effect on the growth of Staphylococcus aureus, and the inhibitory effect induced in it was 6.25, 12.5, 25, 50, 100, 200, and 400 were 13.95, 25.28, 29.13, 27.30, 30.52, 35.91, and 31.69 μg/ml, respectively. Statistical analysis showed that the toxic venom has a significant inhibitory effect on the growth of this bacterium at concentrations of 6.25-400 μg/ml, compared to the negative control. The losses caused by the effect of venom in different concentrations ranged from 25-400 μg/ml compared to the negative control but were not significant in comparison with each other (Figure 2).

Figure 2: Antibacterial effect of various concentrations of Pseudocerastes persicus snake crude venom on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus using MIC assay. The effect of concentrations was evaluated in comparison with the negative control group.

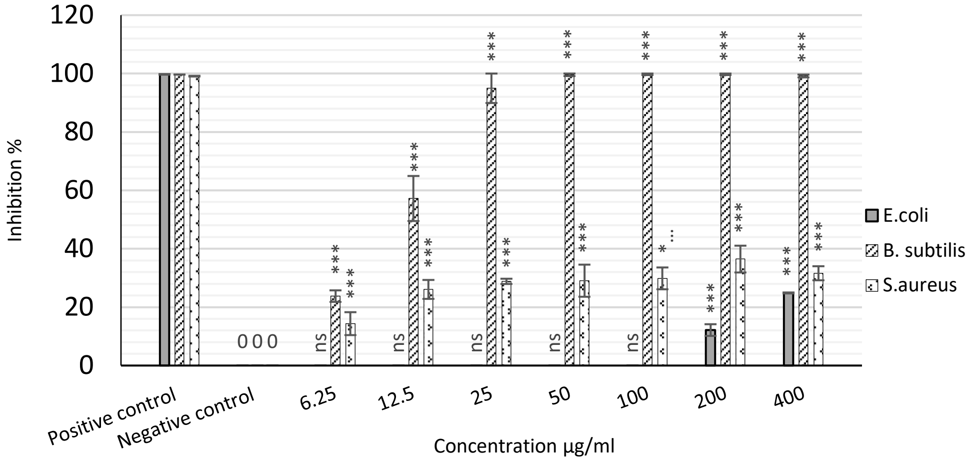

(: p<0. 05), (**: p<0. 01), (***: p<0.001), (ns: non-significant)*

Results of diffusion tests on disk and wells

The results of the effect of various concentrations of venom in the diffusion test on the disk and wells indicate that the results of these two tests, especially at low concentrations of venom, are not repeatable. However, according to the results of this study, it was found that the venom in these concentrations did not inhibit the growth of Escherichia coli (Figure 3).

Figure 3: Antibacterial effect of various concentrations of Pseudocerastes persicus snake crude venom on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus using MIC assay. The effect of concentrations was evaluated in comparison with the negative control group.

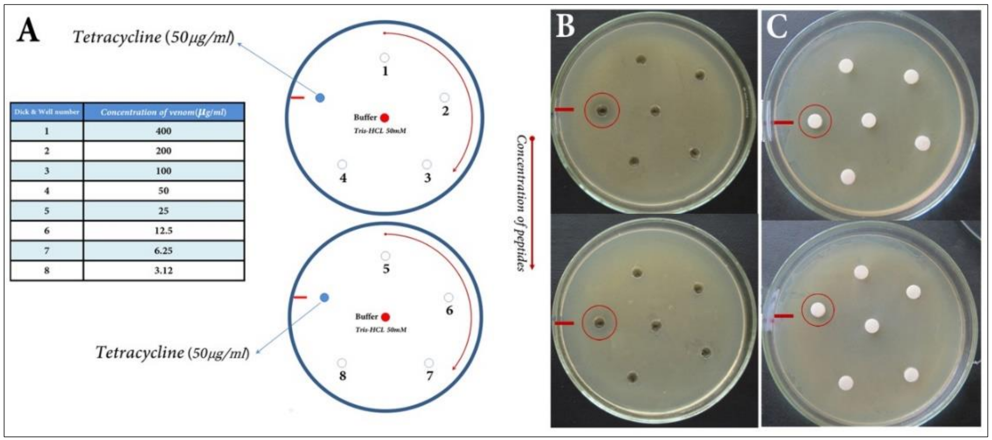

Disk & Well number	Concentration of venom(μg/ml)
1	400
2	200
3	100
4	50
5	25
6	12.5
7	6.25
8	3.12

The results of these experiments on Bacillus subtilis bacteria showed that the venom had an inhibitory effect on bacterial growth at a concentration of 50-400 µg/ml, but did not have an inhibitory effect on the growth of the bacteria in the concentration of 6.5- 25 µg/ml (Figure 4).

Also, venom only prevented the growth of Staphylococcus aureus at concentrations of 200 and 400 µg/ml and did not inhibit the effects of lower concentrations (Figure 5).

Evaluation of anti-cancer properties of Crude venom

Results of cytotoxicity testing by MTT method

Venom at concentrations of 50, 100, 200, and 400 µg/ml causes high mortality in liver cancer cells, respectively, in which the vital percent of the cell at the above concentrations were 14.12, 10.97, 10.43, and 9.96% has dropped. The cells losses caused by the different concentrations of venom are not significant (Figure 6).

Cytotoxicity test results with neutral red colorimetric method

The mortality rate of liver cancer cells in the concentration of 50, 100, 200, and 400 µg/ml of crude venom was 60.13, 72.58, 81.61, and 77.48 %, respectively. Venom has a significant inhibitory effect at concentrations of 100-400 µg/ml in comparison with the control (containing cell and culture media), but the mortality rate is compared between all concentrations is not significant (Figure 7).

Figure 4: Antimicrobial effect of crude venom on diffusion and disk diffusion methods against growth Bacillus subtilis bacteria. A) map and concentrations used; b) well distribution; c) disk diffusion test.

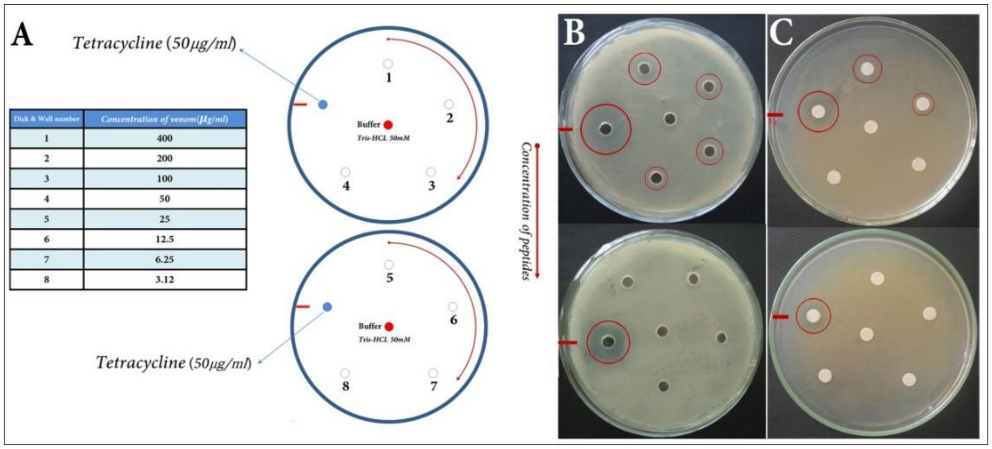
A
Tetracycline (50µg/ml)
Buffer
Tris-HCL 50mM
1
2
3
4
5
6
7
8
Tetracycline (50µg/ml)
B
C
Concentration of peptides

Dick & Well number	Concentration of venom(µg/ml)
1	400
2	200
3	100
4	50
5	25
6	12.5
7	6.25
8	3.12

Figure 5: Antibacterial effect of crude venom on diffusion and disk diffusion methods against growth Staphylococcus aureus bacteria. A) map and concentrations used; b) well distribution; c) disk diffusion test.

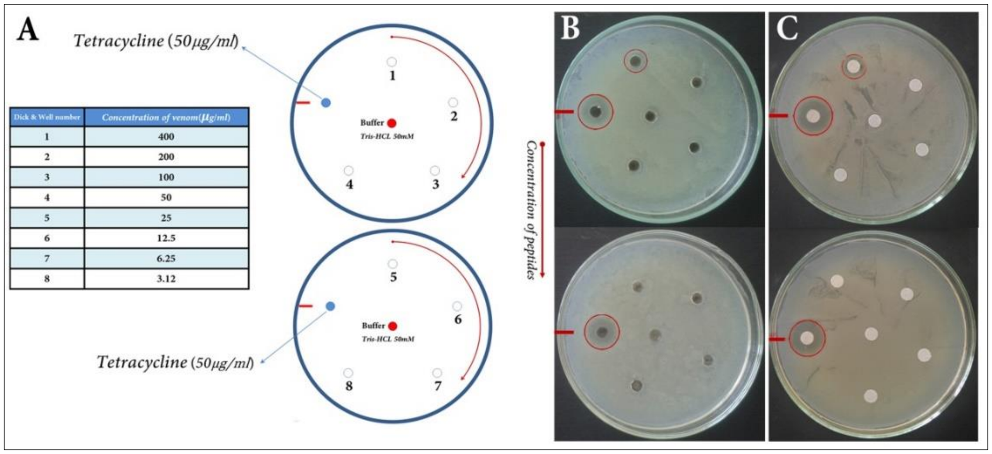
A
Tetracycline (50µg/ml)
Buffer
Tris-HCL 50mM
1
2
3
4
5
6
7
8
Tetracycline (50µg/ml)
B
C
Concentration of peptides

Dick & Well number	Concentration of venom(µg/ml)
1	400
2	200
3	100
4	50
5	25
6	12.5
7	6.25
8	3.12

Figure 6: Percentage of vital liver cancer cells after 24-hour contact with different concentrations of the crude venom according to the MTT assay method. The effect of concentrations was evaluated in comparison with the control group. (***: p<0.001).

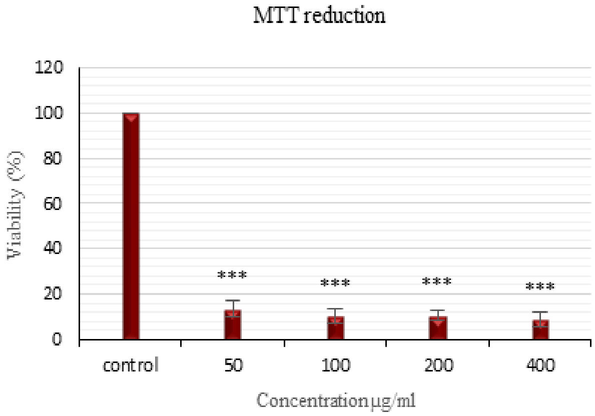

Figure 7: Percentage of mortality of liver cancer cells after exposure to different concentrations of crude venom Pseudocerastes persicus snake based on Neutral Red Color. The mortality due to the effect of each concentration is compared with that of the control group. (***: p<0.001)

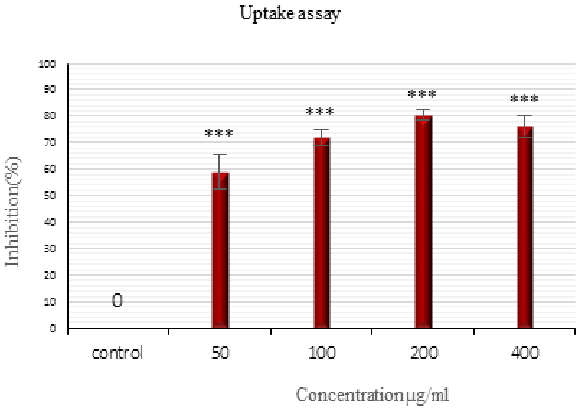

Investigation of venom apoptosis induction using comet assay test

The results of this study showed that Pseudocerastes persicus snake venom induces apoptosis and causes mortality in liver cancer cells. Venom at concentrations of 50, 100, 200, and 400 were 36.6, 43.28, 51.5, and 47.13%, respectively, induced apoptosis in the cell. The morphological study of venomous cells also showed that the venom of this aphid, in addition to apoptosis, induces necrosis in liver cancer cells, and this effect has an upward trend of more than 200 µg/ml in comparison with lower concentrations. These results indicate that mortality in cancer cells is due to the common effects of apoptosis and venous necrosis (Figure 8).

Figure 8: Rate of apoptosis induced by Pseudocerastes persicus snake crude venom in liver cancer cells based on the alkaline test.
A: The morphological changes in DNA from the concentration of each concentration compared to the control group.
B: Morphological changes in DNA of proximal cells with Pseudocerastes persicus snake crude venom compared to control cells
[1-3]: DNA image of the normal cell(×40), [4-6]: DNA image of apoptosis cell(×40).

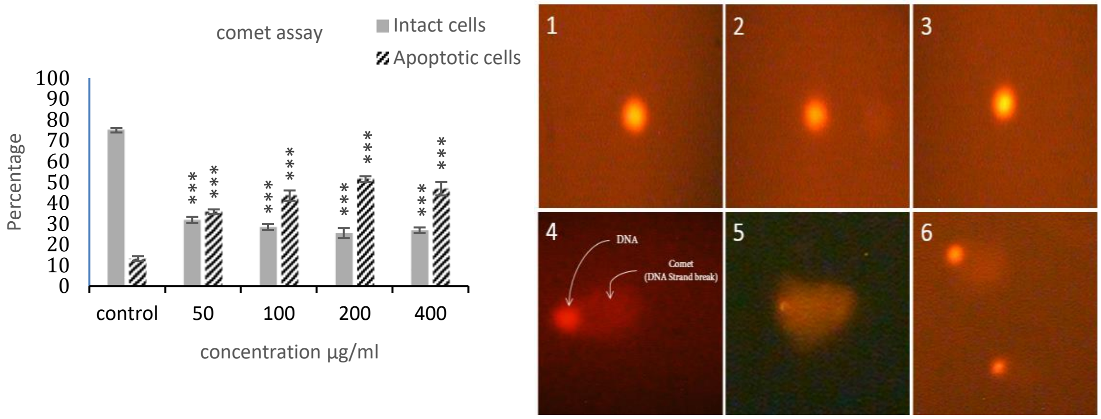

DISCUSSION AND CONCLUSIONS

According to the World Health Organization, 40% of the 50 million annual human mortality is due to bacterial contamination and microbial diseases (Ivnitski et al., 1999). Also, bacterial infections caused by resistant strains and problems due to the lack of treatment of people with them have become an important health problem. This has led researchers to investigate more in living organisms, including various venomous snakes, to discover more effective and new antibiotics. According to studies conducted in recent years, venomous snakes are a source of unknown molecules of active substances (25) that can be considered as an appropriate candidate for the production of antimicrobial and anticancer drugs. In this study, for the first time, the antibacterial and cytotoxic effects of Pseudocerastes persicus snake were studied in vitro conditions to evaluate the possibility of isolating molecules with antibacterial and toxic effects for cancer cells. MTT reduction, MIC assay, Disc diffusion assay, and Well diffusion test were used to study the antibacterial effects of crude venom. The results obtained from MTT reduction and MIC tests showed that Pseudocerastes persicus snake venom has an inhibitory effect on Escherichia coli bacteria as a representative of gram-negative bacteria only at the highest concentration (400 µg/ml). However, bacteria Bacillus subtilis and Staphylococcus aureus have been prevented from bacterial growth at concentrations of 12.5-400 and 25-400 µg/ml, respectively. The reason for the difference in the results of MTT and MIC is due to the difference in the calculation of the inhibitory effect of the venom in two methods, in the MIC method, the opacity is due to the presence of bacteria, which includes dead and living bacteria, is measured at a wavelength of 605 nm. While in the MTT method, the optical absorption of formazone particles formed in the cytoplasm of the living bacteria is determined after dissolution in DMSO at 570 nm. MTT testing seems to provide more accurate results than the MIC method. Also, the results of the study showed the antibacterial effects of crude venom using two methods of diffusion in wells and discs. In this study, snake venom has no inhibitory effect on gram-negative bacteria but has an antibacterial effect on two-gram positive bacteria. The results showed that these two methods qualitatively confirm the effects of MTT and MIC, but did not quantitatively produce repeatable results. One of the reasons for this issue, it seems, is that the proteins and toxic peptides are deposited at the bottom of the well, and the walls and around the wells act as a filter and penetrate the entire contents of the venom into the well prevents solid culture. In the case of a disk, it is also possible that the disk itself acts as a filter and prevents the outflow and full penetration of the components of the venom into the medium. The rapid absorption of water in the upper surface of the medium using a paper disk and the lack of complete absorption of toxic compounds that allow heterogeneous release in the medium is another disadvantage of this method. In this study, it was determined that Pseudocerastes persicus snake crude venom is more effective than gram-positive bacteria. This selective antibacterial activity may be due to several factors such as the difference between bacterial species such as density and lipopolysaccharide structure present in the wall of gram-negative bacteria or fat composition in the cytoplasmic membrane and electrostatic potential across the membrane in gram-positive bacteria Be (San et al., 2010). The results of studies by Husniye et al. (2012), Shebell et al. (2012), Stabley et al. (2004), Steels et al. (1991), and similar studies show that venomous affect gram-positive bacteria (Stiles et al., 1991; Lu et al., 2002; Yalcın et al., 2014; de Lima et al., 2005; Stábeli et al., 2004). Also, the results of the antibacterial effect of Jafri's venom (Jami et al., 2011) showed that snake venom has a better effect on Staphylococcus aureus bacteria than Escherichia coli bacteria. The results obtained in this study are consistent with previously reported results regarding the antibacterial effects of snake venom. On the other hand, the inhibitory effect of Pseudocerastes persicus snake crude venom in the highest concentrations studied in this study on E. coli is an effective component of this bacterium at very low concentrations in venom. The results of studies by Chalapandi et al. (2008), Bostley et al. (2008), Starker and Trierworth in 1986, and similar studies have reported the inhibitory effects of venomous organisms on gram-negative bacteria (Chellapandi and Jebakumar, 2008; Stiles et al., 1991; Shebl et al., 2012; Bustillo et al., 2008). Snakes venom are mainly composed of proteins and peptides that have diverse biological activities. Some of these molecules have been shown to have inhibitory effects on cancer cell growth in clinical trials and may find their way toward developing anticancer drugs in the future (Jain and Kumar, 2012). In this research, the effects of toxicity of venomous snake, which has antimicrobial properties induced by the use of MTT assay, Neutral red uptake assay, and Single cell gel electrophoresis (Comet assay) on liver cancer cells (HepG2 cell line) was taken into consideration in vitro conditions. Reports on the presence of cytotoxic effects in the venom snake have led to the use of HepG2 cells in this study (Latifi, 2000). The results of the MTT assay and uptake assay showed that crude venom at concentrations of 50, 100, 200, and 400 µg/ml has cytotoxic effects and causes the death of liver cancer cells. These results are consistent with findings from Bozemans et al. (2005), Yang et al. (2006), Depeca et al. (2012), Sang et al. (2012), to induce cytotoxic properties by venoms of a snake (Jain and Kumar, 2012; Bosmans et al., 2005; Cancer Control Office, 2005; Song et al., 2012; Yang et al., 2006). As well as the results of Shebel, et al suggesting that mortality in the cell would be increased by increasing the concentration of venom (Shebl et al., 2012). To investigate the possibility of inducing cell death through crude venom through apoptosis in HepG2 cells, this study was conducted using Comet assay. The results showed that crude venom at concentrations of 50, 100, 200, and 400 µg/ml caused apoptosis in cancer cells the liver causes it to die. It was also found that with an increase in concentrations of 50 to 200 µg/ml of crude venom, apoptosis has a tendency to increase, which is consistent with previous reports (Sajad et al., 2013). Generally, the results of this study showed that the crude venom of snakes contains molecules that can have anti-bacterial and anti-cancer activity. These results suggest that the venom of a snake is a suitable candidate for the isolation, purification, and introduction of valuable drug molecules to combat antibiotic-resistant bacteria as well as anti-cancer agents.

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ORIGINAL ARTICLES

Treatment persistence of biologics in psoriatic disease

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Abstract: *Psoriasis is a chronic, immune-mediated inflammatory disease that significantly affects a patient's quality of life. Several systemic complications can influence disease progression and response to treatment. Biological therapy is considered the most effective therapeutic option in treating moderate to severe forms of psoriasis. The success of therapy may depend on its persistence, also known as drug survival. The dermatologist needs to be aware of comorbidities and factors that influence the persistence or discontinuation of biological treatment to make appropriate treatment decisions. We have identified several studies on the persistence of biologic therapy or drug survival in patients with psoriasis and summarized some of the most important issues known to date. In several recently published studies, the survival of ustekinumab is better than TNF-α inhibitors but lower than ixekizumab. Notably, the studies were performed on a limited number of patients and follow-up time. Moreover, secukinumab appears to have a shorter drug survival than other biological agents, especially in patients with biological experience.*

A real indicator of therapeutic success could be a high quality of life. Female gender and obesity have been consistently highlighted among the predictors of treatment discontinuation, while psoriatic arthritis could be a predictor of persistence and maintenance of biological therapy. We are currently aiming to improve the therapy for patients treated with biological agents to reduce the pressure on the national public health system.

Keywords: *psoriasis, psoriatic disease, biological therapy, persistence, predictive factors, drug survival*

INTRODUCTION

Psoriasis is a chronic, immune-mediated disease with high genetic susceptibility, of still unknown etiology which may be associated with abnormal T-lymphocyte function [1]. Lately, there has been a lot of talk about the concept of psoriatic disease. It is no longer considered an exclusive skin condition and the management of this complex disease has been simplified by the recent understanding of the pathophysiological mechanisms.

Understanding comorbidities and factors that can easily destabilize the psoriasis patient guides the physician in choosing the proper therapy and leads to improved treatment effects [2, 3, 4]. Conventional therapies have not fully met the needs of psoriatic patients due to limited efficacy, side effects with cumulative use, and patient inconvenience [5].

Recently, the development of new biologic therapies has revolutionized the management of patients with moderate to severe psoriasis vulgaris, patients with multiple comorbidities who require long-term therapy [6, 7]. Currently, in our country, the biological therapies used to treat psoriasis are TNF inhibitors (TNFi): adalimumab,

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etanercept, infliximab, and their biosimilars [8, 9], anti-IL12/23 (ustekinumab), anti-IL-17A (secukinumab, ixekizumab), and anti-IL-23 (risankizumab, guselkumab). These therapies could lead to a long-term remission of psoriasis, however, the effectiveness may decrease over time, the loss of response to the treatment being the main cause of its discontinuation [2]. As a result, the trend of long-term use of biological substances has led to an attempt to understand the persistence of treatment over time, also known as drug survival.

What Is Drug Survival?

Recently, the term “drug survival” also known as “drug retention”, “drug persistence” or “drug adherence” has made its way through the field of dermatology. “Drug adherence” can be defined as the time between the initiation of treatment with a particular drug and its discontinuation for various reasons [10, 11]. This is a marker of therapeutic success, fully encompassing the elements of treatment efficacy and safety because ineffective or poorly tolerated therapy will most likely be discontinued [12, 13]. Furthermore, the continuation of treatment can be directly influenced by country, patient characteristics, and specific drugs used for treatment, cost, flexibility, quality of life, dosage range, or accessibility [6].

The first meta-analysis [2] that included 16 cohort studies on predictive factors of persistence of biological therapies was published in 2019. The main purpose was to identify the predictive factors of persistence or discontinuation of therapy, regardless of the therapeutic option (choice of biological therapy). It was pointed out that female patients who benefit from biological therapy are more likely to discontinue treatment, especially if they associate obesity compared to men, especially if they are normal weight. The reasons why women are more affected seem to be the side effects associated with therapy on the one hand and insufficient therapeutic satisfaction on the other. Possible arguments to explain why discontinuation of medication is more common in obese patients would be lower levels of drugs in the blood, large body surface area, or increased proinflammatory cytokines in adipose tissue that are thought to interfere with therapy. The results of the study emphasize the importance of weight loss in patients with psoriasis vulgaris to maintain the therapeutic effect. Moreover, according to this meta-analysis patients who suffer from psoriatic arthritis are more likely to continue therapy compared to patients who have only skin lesions. Other factors previously identified as predictors of biological discontinuation included smoking, metabolic syndrome, age, dyslipidemia, high socioeconomic status, DLQI, and biological naivety, but there were insufficient data for the meta-analysis of these factors [2].

Another meta-analysis [14] based on no less than 109 studies compared the efficacy and safety of known systemic therapeutic classes: conventional systemic agents (acitretin, cyclosporine, fumaric acid esters, methotrexate), small molecules (apremilast, tofacitinib, ponesimod), TNFi (etanercept, infliximab, adalimumab, certolizumab), anti-IL12/23 (ustekinumab), anti-IL17 (secukinumab, ixekizumab, brodalumab), anti-IL23 (guselkumab, tildrakizumab), and other biologics (alefacept, itolizumab) for patients with moderate to severe psoriasis. All studies included in this meta-analysis were limited to the induction phase (assessment at less than 24 weeks after randomization). At the drug level, in terms of reaching PASI 90, all anti-IL17 agents and guselkumab (an anti-IL23 drug) were significantly more effective than TNFi agents such as infliximab, adalimumab, and etanercept, excepting certolizumab. Another conclusion was that ustekinumab was superior to etanercept. No clear difference was shown between infliximab, adalimumab, and etanercept. Tofacitinib was significantly superior to methotrexate, and no clear difference was shown between any of the other small molecules compared to conventional treatments. Cochrane review has found that biologics are more effective than conventional, systemically administered drugs (such as methotrexate or cyclosporine) in a selected population of patients with moderate-to-severe psoriasis. However, the evidence is limited to the induction phase (12 to 16 weeks) and is not sufficiently relevant for chronic diseases [14].

In 2016, an article published in a prestigious journal [6] highlighted that lack of response and efficacy was the most commonly observed reason for discontinuation of biological treatment. There was also a better persistence of ustekinumab compared to TNFi (infliximab, adalimumab, etanercept). Notably, patients who are receiving methotrexate simultaneously with biologic therapy are more likely to discontinue treatment [6]. In this article, males were associated with persistent treatment, as shown in other studies [15, 16, 17].

Ustekinumab appears to be administered for a longer period, with lower discontinuation rates compared to anti-TNFα, information documented in the last 5 years [12, 13, 18-23]. A systematic review and comparative meta-analysis published in 2021 highlighted that the IL-12/23 inhibitor (ustekinumab) demonstrated superior biologic persistence when compared to TNFi. The same study makes a comparison between TNF-α inhibitors and reveals that adalimumab was superior to etanercept and infliximab at year 5. Of note, there was no difference between the pooled drug survival for adalimumab versus etanercept and etanercept versus infliximab at year 2. At year 1, secukinumab was superior to ixekizumab; however, there was no difference when secukinumab was compared to TNF-α inhibitors. Interestingly, the survival of ustekinumab remained superior when compared to the pooled secukinumab drug survival [24].

Although there are few recent studies, they have shown increased interest in assessing persistence in patients treated with anti-IL-17 agents: secukinumab and ixekizumab. In a nationwide cohort of patients with psoriasis in Denmark, drug survival was longer for ixekizumab, however, the number of patients and follow-up time were limited [25]. Moreover, in an observational study conducted in Sweden [26], the median persistence of biologic therapies in patients with psoriasis vulgaris was approximately 23.8 months. One year after treatment, the percentage of persistent patients was higher for ixekizumab (81.3%), followed by ustekinumab (79.9%), secukinumab (75.9%), adalimumab, and etanercept. Biologic-naive patients appear to have had a longer persistence than those with biologic experience, except for certolizumab pegol and ixekizumab, both of which were limited by the small number of patients in this study. There is sufficient information to show that for bio-naive or bio-experienced patients on anti-interleukin therapies there is no difference in terms of achieving efficacy or rapid onset of efficacy.

Another observational study in the Swedish population, this time patients with biological treatment associating psoriatic arthritis, showed a persistence profile of therapy in patients treated with ustekinumab, both naive and biologically experienced, while secukinumab has a favorable persistence only in biologically naive patients, compared to adalimumab, which also had resulted in patients with biological experience. This study also highlighted factors that may predict treatment discontinuation, namely female gender, axial involvement, recent onset of the disease, and increased experience in the use of biological treatment [27]. In a study in Germany, a group of patients with psoriatic arthritis showed that the persistence rates of biological disease-modifying biologic antirheumatic drugs (bDMARDs) were higher for ustekinumab compared to infliximab or golimumab. Moreover, mean persistence was higher for ustekinumab and lower for the other two molecules. The causes of non-persistence were switching from one bDMARD to another and discontinuation of therapy. Lower risk of discontinuation of treatment was significantly correlated with the male sex. [28].

Additional measures are important to improve outcomes in medication persistence such as increasing access to rheumatologists and implementing a pharmaceutical care strategy. In Brazil, medication persistence was lower than in other countries. A total of 161 patients were included in a study published in 2019 and the results demonstrate that anti-TNF agents are important therapeutic alternatives and present lower discontinuation as compared to conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) after 6 and 12 months of follow-up. Adalimumab and etanercept presented similar persistence results in the anti-TNF group and leflunomide and methotrexate in the csDMARDs group [29].

Regarding the administration of biological therapy in other forms of psoriasis, this is effective and well-tolerated for the treatment of hyperkeratotic pustular psoriasis (PP), but fewer data are available on the treatment of pustular psoriasis or palmoplantar psoriasis (PPP). Adalimumab, guselkumab, ixekizumab, secukinumab, and ustekinumab showed more than 80% efficacy for the treatment of hyperkeratotic PP, while infliximab and ustekinumab showed moderate efficacy for the treatment of pustular PP, and infliximab was the most efficacious treatment for PPP [30].

In randomized controlled trials, secukinumab, an interleukin 17A antagonist, is highly effective in treating psoriasis. However, there has been observed a tendency of reduced adherence to treatment in females, smokers, obese, and biologically experienced patients [31, 32].

IMPLICATIONS IN MEDICAL PRACTICE

Current evidence suggests that ustekinumab is associated with the best drug survival in all biologic-naive subjects. Ustekinumab is also the most common biological therapy least associated with discontinuation due to loss of efficacy.

A meta-analysis published in 2018 shows that the drug survival rate for etanercept, adalimumab, infliximab, and ustekinumab decreased year by year and overall, etanercept was associated with the lowest drug survival rate, and ustekinumab was associated with the highest one [33]. Ustekinumab has been shown to have a higher treatment rate, with effects lasting up to one year and up to five years of treatment, unlike TNFi [18, 19, 34].

Among the predictive factors for discontinuation of biological therapy were female gender, smoking, and obesity (BMI>35 kg/m^2) [35, 36]. However, the association of psoriatic arthritis may predict long-term maintenance of treatment (a predictor for drug survival) [2, 37, 38].

Drug survival rates differed among biologic therapies and decreased over time; second-line discontinuation due to adverse events was more common among those who discontinued first-line treatment for this reason [39].

These data are useful in guiding therapeutic management, as well as in optimizing the treatment of patients suffering from psoriasis.

CONCLUSION

The progress of personalized therapy and the introduction of new biological molecules in the last decade have facilitated a significant change in the treatment paradigm in psoriasis and psoriatic arthritis. Maintaining the long-term clinical efficacy of different classes of biological agents is vital in such chronic conditions, and drug survival is a marker for the sustainability of treatment.

Long-term therapy for psoriasis is affected by the gradual loss of effectiveness and discontinuation of treatment. The overall effectiveness of biological agents is reduced over time. Identifying therapy with long survival rates, as well as predictive factors of treatment discontinuation or persistence, may help individualize biological therapy and improve treatment outcomes and satisfaction. Psoriatic arthritis in parallel with psoriasis is associated with improved persistence.

Authors' contributions

SB, MDC, TC contributed to all stages of the article, designed the article, and revised the manuscript for important intellectual content. MMC and FS contributed to the conception of the manuscript, to the revision of the language, and contributed to the drafting of the work. SB, MMC, and FS critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

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ORIGINAL ARTICLES

The impact of website quality on customer loyalty with the intermediate role of social capital: A case study of the Bamilo website

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Abstract: *The main objective of this study is to investigate the impact of Bamillo website quality on customer loyalty through the mediating role of social capital. This research is applied in terms of purpose and it is a field study in terms of data collection. The population of the study included all users of the Bamilo website who had at least one purchase order from this site. Accordingly, 384 people were selected through available sampling. The instrument used in the research was the standard questionnaire of China et al. (2016), which confirmed the validity and reliability of the questionnaire. Structural equation modeling with the partial least squares method was used to analyze the data. The results showed that the quality of information on-site information quality on cognitive capital was rejected and its impact on structural capital was confirmed. The quality of the site system on cognitive capital is also rejected and its impact on structural capital is confirmed. On the other hand, the quality of site services has an impact on cognitive capital and structural capital. Also, cognitive capital and communication capital affect communication capital, and cognitive capital and communication capital affect customer loyalty. However, the impact of the capitalist mediation mediated the relationship between information quality and system quality with customer loyalty. Cognitive capital also mediates the relationship between service quality and customer loyalty, and structural capital mediates the relationship between information quality and customer loyalty. On the other hand, the mediating role of structural capital in the relationship between system quality and customer loyalty was confirmed and structural capital mediated the relationship between service quality and customer loyalty.*

Keywords: *website quality, customer loyalty, social capital, Bamillo*

INTRODUCTION

Influence and interpersonal influences of people in online environments affect customers' evaluations and decisions about buying a product or brand. The increasing growth of the Internet and its high penetration rate in our country has provided the necessary basis for doing business in this environment [1]. In recent years, many organizations and companies have attracted customers and sold their products using the Internet and creating websites. The special requirements of business activity in cyberspace, the special behavior of consumers in this environment, and the fierce competition between actual and potential actors in attracting customers for their online stores indicate the importance of examining activities in this field as closely as possible [2]. Online shopping can be considered as one of the major developments in retail sales in the business world. An Internet-based e-commerce environment allows customers to search for information and purchase goods and services through direct contact with online stores. Shopping online and doing so largely depends on consumer communication

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and how people interact with the computer. Processes such as providing information, guidance, and delivery, as well as timely delivery of orders, have been considered as an important factor in building trust in e-commerce, rather than traditional sales [3]. Customer loyalty in today's competitive and complex market is the main factor in gaining the competitive advantage of any organization; as retaining current customers is much less expensive than attracting new customers. Attracting customers on the Internet is very costly, and if customers are not loyal and their purchases are not frequent and frequent, profitability will be out of reach. Competitors are only a few clicks away from customers [4]. Therefore, customer loyalty has become a very complex process for managers. Increasing loyalty leads to predicting revenue streams from customers as well as a gradual increase in revenue [5]. A 5% increase in loyal customers, a 25% to 95% increase in the profitability of various industries. Increased loyalty will lead to the prediction of revenue streams by customers as well as a gradual increase in revenue [6]. Organizations lose a large number of their customers each year due to a lack of attention to them. One of the most effective and common strategies is to strive to provide quality, flawless and easy service. High quality and continuous customer service create a competitive advantage for the company [7]. Given the problems and obstacles in Internet businesses, which are mostly related to the lack of resources, both financial and human. The importance of using social capital and networking for the founders and entrepreneurs of these companies is evident [8]. Social capital plays a role within the organization in various ways such as facilitating and accelerating the flow of information and knowledge (whether tacit or codified knowledge) and if it is not addressed, it hinders the facilitation of human capital formation and reduces costs such as control costs. And monitoring, as well as facilitating access to people inside and outside the organization, prevents the organization from achieving the goals of the organization better and faster and reducing the obstacles ahead [9]. Social capital is an organizational attribute, not the property of individual members, that is, the organization and members jointly own it, and the result can affect customer behavior and loyalty [10].

Today, the web is one of the most important tools that countless people use as the main tool for accessing information. Websites play a key role in disseminating information. Websites are great tools for connecting organizations, users, and citizens. With the growing access of people to the Internet, the number of visitors to websites has also increased and the issue of their quality has become more and more important. The success and efficiency of websites depend to a large extent on the quality of the website. A high-quality website will grow over time due to the loyal readers as well as the search engine trust, which is more organic compared to the visitor [11]. Creating customer loyalty, especially in online environments and online shopping can affect the quality of websites. Creating lasting loyalty in e-commerce customers is very difficult because, on the Internet, the customer can easily leave one website and visit another website with the lowest transfer costs and with just one click. There is a consensus that enduring customer loyalty in electronic environments, especially online retail, is more sensitive and complex than traditional business loyalty [12]. In this regard, social capital can also play a decisive role. Perceived equity of social capital will increase customer loyalty to the company. Social capital will also increase the value of loyalty to corporate brands [13]. According to the above, the purpose of this study is to investigate the effect of Bamilo website quality on customer loyalty with the mediating role of social capital.

STUDY METHOD

The purpose of this research is to apply. It is applied research that is done using the results of basic research to improve and perfect the behaviors, methods, tools, tools, products, structures, and patterns used by human societies. The thematic area of the present study includes the study of the effect of Bamilo website quality on customer loyalty with the mediating role of social capital, which was implemented in the summer and autumn of 1398, and the location of Tehran. Given that in descriptive research, the researcher seeks how the subject is and wants to know what the phenomenon, variable, object, or subject is. In other words, this study examines the current situation and systematically and systematically describes its current situation and studies its features and characteristics and, if necessary, examines the relationship between variables, so this research is a descriptive study. Also, in this research, a survey method is used to collect data. In this type of research, the purpose is to study the distribution of characteristics of a society and most management research is of this type. In survey research, community parameters are examined. Here, the researcher examines the research variables by selecting a sample that represents the community. The statistical population refers to a set of people, objects, or phenomena in general that the researcher can generalize the result of his study to all of them. The statistical population of the research is identified by one or more common attributes. The statistical population included all users of Bemilo website who had purchased from this site at least once. A statistical sample is a set of indicators that are selected from a larger section, group or community so that this set represents the quality and characteristics of that larger section, group, or community, and sampling is the sample selection process. Based on this, 384 people have been selected by the available sampling method. Based on the research title, the research variables were determined as independent variables including website quality (information quality, system quality, service quality), dependent variable including customer loyalty, and mediator = social capital (capitalist, structural capital, communication capital). The research hypotheses were organized as follows:

1. The quality of site information affects capitalism
2. The quality of site information affects the structural capital
3. The quality of the site system affects the capitalism
4. The quality of the site system affects the structural capital
5. The quality of Bamilo site services has an impact on capitalism
6. The quality of site services affects the structural capital
7. Investigating the impact of capitalism on communication capital
8. Structural capital affects communication capital
9. Capitalism affects customer loyalty
10. Structural capital affects customer loyalty
11. Communication capital affects customer loyalty
12. Capitalism mediates the relationship between information quality and customer loyalty
13. Capitalism mediates the relationship between system quality and customer loyalty
14. Capitalism mediates the relationship between service quality and customer loyalty
15. Structural capital mediates the relationship between information quality and customer loyalty
16. Structural capital mediates the relationship between system quality and customer loyalty
17. Structural capital mediates the relationship between service quality and customer loyalty.

In this research, to collect information, the library and documentary methods were used in line with research literature and theoretical foundations. In this section, based on past studies, the theoretical foundations of research are stated. The theoretical basis of the definitions and concepts used in the research is clear and should be based on previous valid studies. The second part of the research literature presents similar studies previously done by other researchers. Data collection to test the research hypotheses, the field method was collected with a questionnaire. The instrument used in the research was the standard questionnaire of China et al. (2016) in which all the questions of the questionnaire were designed based on a five-point Likert scale (strongly agree to strongly disagree).

Table 1: Research questionnaire items

Variables	Items
Information quality	1-2-3-4
System quality	5-6-7-8
Service quality	9-10-11-12
Capitalism	13-14-15
Structural capital	16-17-18-19
Communication capital	20-21-22-23
Customer Loyalty	24-25-26-27-28

Validity refers to the logical connection between test questions and the material being assessed. The validity of the questionnaire means that the test questions accurately measure what is intended. Credibility has different aspects and the relationship between the question and the subject is achieved according to all its aspects. If this connection does not exist, no credit will be created. It is the most important feature of any test and shows the extent to which the test measures what it is designed to measure. In other words, validity shows the relationship of a test to its goals. Validity means the extent to which the method or tool used can accurately measure the desired characteristic determined by the research objectives. Experts' opinions can be a good help to improve the validity of measuring instruments. Accordingly, in this study, the validity of the present questionnaire was studied and confirmed by professors and experts including 12 people, especially the supervisor. One way to calculate reliability is to use Cronbach's formula. This method is used to calculate the internal consistency of measuring instruments such as questionnaires or tests that measure different characteristics. In such tools, the answer to each question can take different numerical values. In this study, Cronbach's alpha value was performed using SPSS 22 software. The alpha value for the questionnaire variables was obtained, which indicates the reliability of the questionnaire used in this research.

Table 2: Reliability of research variables

Variables	Cronbach's alpha values
Information quality	0.801512
System quality	0.735864
Service quality	0.847528
Capitalism	0.734281
Structural capital	0.814755
Communication capital	0.796902
Customer Loyalty	0.793798

Cronbach's alpha value for the questionnaire was more than 0.7, which indicates the reliability of the questionnaire used in this study. Two levels of descriptive and inferential statistics were used to analyze the data. In descriptive statistics, the demographic data of the questionnaire were analyzed using descriptive statistical methods such as methods of frequency, frequency percentage, mean, fashion, standard deviation, etc. using SPSS software. In inferential statistics of research hypotheses, using the research default and Kolmogorov-Smirnov test, the normality and abnormality of the data distribution of research variables are investigated. Finally, the structural equation model using the partial least squares method is used to test the hypotheses.

RESULTS

384 people participated in this study. 179 (46.6%) had a diploma, 44 (11.5%) had a postgraduate degree, 90 (23.4%) had a bachelor's degree and 71 (18.5%) had a master's degree or higher. Findings show that 76.6% of the participants were male and 23.4% were female. Other descriptive findings of the study sample include how people are distributed based on age groups. The age range of 21 to 30 years, which includes 39.1% of the total sample, has the highest frequency and the age range of 10 to 20 years has the lowest frequency. Findings indicate that 181 (47.1%) of those present are single and 203 (52.9%) are married. The questionnaire was designed with a Likert scale with five options (1 to 5) and there were several questions for each variable. Likerts ranged from 1 to 5; the numerical range of all variables must also be variable in the range (5, 1). The results of Table 4-7 show that the range of scores of all variables is calculated between 1 and 5, which means that the data was collected correctly in the soft environment The software has been coded, logged, and computerized. The total score of information quality was evaluated using an average of 4 items. The average score of the respondents in this variable (perceived benefits) is 3.56 and its standard deviation is 0.73 so that the lower limit (minimum) is 1.5 and the upper limit (maximum) is 5. Also, the coefficient of skewness and elongation of the mentioned variable is estimated to be -0.43 and -0.08, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level. The total quality score of the system was evaluated using an average of 4 items. The average score of the respondents in this variable (cognitive social identity) is 3.24 and its standard deviation is 0.86 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the coefficient of skewness and elongation of the mentioned variable is estimated to be -0.40 and 0.12, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level. The total score of service quality was examined using an average of 4 items. The average score of the respondents in this variable (local economic situation) is 3.46 and its standard deviation is 0.83 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the skewness and elongation coefficients of the mentioned variable are estimated to be -0.41 and -0.32, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level.

Table 3: Descriptive indicators of the studied variables

Variables	Sample size	Average	Standard deviation	Variance	Lowest score	Highest score	Cholegi	Elongation
Information quality	384	3.56	0.73	0.54	1	5	-0.43	-0.08
System quality	384	3.24	0.84	0.71	1	5	-0.40	0.12
Service quality	384	3.46	0.83	0.70	1	5	-0.41	-0.32
Capitalism	384	2.18	0.86	0.74	1	5	0.92	0.46
Structural capital	384	3.19	0.86	0.74	1	5	-0.09	-0.58
Communication capital	384	3.69	0.73	0.54	1	5	-0.64	0.96
Customer Loyalty	384	3.56	0.82	0.67	1	5	-0.67	-0.22

The total capitalist score is examined using an average of 4 items. The average score of the respondents in this variable (perceived costs) is 2.18 and its standard deviation is 0.86 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the skewness and elongation coefficients of the mentioned variable are estimated to be 0.92 and 0.46, respectively, and in the safe range (2+ and -2), which is at a desirable and appropriate level. The total score of structural capital is examined using an average of 6 items. The average score of the respondents in this variable is 3.19 and its standard deviation is 0.86 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the skewness and elongation coefficients of the mentioned variable are estimated to be -0.09 and -0.58, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level. The total score of communication capital is examined using an average of 3 items. The average score of the respondents in this variable (importance to the development of local tourism) is 3.69 and its standard deviation is 0.73 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the coefficient of skewness and elongation of the mentioned variable is estimated to be -0.64 and 0.96, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level. The total customer loyalty score was evaluated using an average of 5 items. The average score of the respondents in this variable is 3.56 and its standard deviation is 0.82 so that the lower limit (minimum) is 1.4 and the upper limit (maximum) is 5. Also, the coefficient of skewness and elongation of the mentioned variable is estimated to be -0.67 and -0.22, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level.

Table 4: Matrix of correlation coefficients between research variables

Variables	Information quality	System quality	Service quality	Capitalism	Structural capital	Communication capital	Customer Loyalty	Variables
Information quality	correlation	1						
	Significance	0	0					
System quality	correlation	0.56	1					
	Significance	0.00	0					
Service quality	correlation	0.46	0.44	1				
	Significance	0.00	0.00	0				
Capitalism	correlation	- 0.46	- 0.40	- 0.43	1			
	Significance	0.00	0.00	0.00	0			
Structural capital	correlation	0.53	0.47	0.45	- 0.47	1		
	Significance	0.00	0.00	0.00	0.00	0		
Communication capital	correlation	0.51	0.45	0.43	- 0.42	0.52	1	
	Significance	0.00	0.00	0.00	0.00	0.00	0	
Customer Loyalty	correlation	0.65	0.55	0.55	- 0.50	0.55	0.52	1
	Significance	0.00	0.00	0.00	0.00	0.00	0.00	0

Due to the abnormality of data distribution, the Pearson correlation test was used. Correlation coefficients are used to calculate the degree and degree of the linear relationship between two variables. The correlation coefficient range varies from -1 to +1. The closer this value is to +1, the stronger and more positive the relationship between the two variables. In other words, with the increase of each of the variables, the other also increases, and vice versa, with the decrease of each of the variables, the other also decreases. Also, the closer the value of this coefficient is to 1, the stronger and negative the relationship between the two variables is. In other words, with the increase of each of the variables, the other decreases, and with the decrease of each of the variables, the other increases.

Table 5: Summary of results from Hypotheses 1 to 11

Hypothesis number	Independent variable	Dependent variable	Path coefficient	Significance number	Result
1	Information quality	Capitalism	0.145	1.762	Reject
2	Information quality	Structural capital	0.186	2.904	Confirmation
3	System quality	Capitalism	0.097	1.380	Reject
4	System quality	Structural capital	0.389	5.598	Confirmation
5	Service quality	Capitalism	0.629	9.215	Confirmation
6	Service quality	Structural capital	0.291	4.408	Confirmation
7	Capitalism	Communication capital	0.3	3.289	Confirmation
8	Structural capital	Communication capital	0.428	5.264	Confirmation
9	Capitalism	Customer Loyalty	0.350	3.842	Confirmation
10	Structural capital	Customer Loyalty	0.278	3.984	Confirmation
11	Communication capital	Customer Loyalty	0.327	4.071	Confirmation

This test examines the following hypothesis:

Assumption Zero: The correlation coefficient between the two variables is zero (the two variables are independent of each other).

Opposite assumption: The correlation coefficient between the two variables is not zero (the two variables are related to each other).

Considering that the significance level of the correlation coefficient test between the two variables is less than the 0.05 error level, it can be stated that there is a significant correlation and relationship between all variables. A better and more detailed analysis of the above table is given in the section on hypotheses. The results of Hypotheses 1 to 11 are summarized according to Table 5.

The results of Hypotheses 12 to 19 are summarized according to Table 6.

Table 6: Summary of results from Hypotheses 12 to 17

Hypothesis number	Independent variable	Mediator	Dependent variable	Path coefficient	Significance number	Result
12	Information quality	Capitalism	Customer Loyalty	0.05	1.60	Reject
13	System quality	Capitalism	Customer Loyalty	0.03	1.29	Reject
14	Service quality	Capitalism	Customer Loyalty	0.22	3.54	Reject
15	Information quality	Structural capital	Customer Loyalty	0.05	2.34	Confirmation
16	System quality	Structural capital	Customer Loyalty	0.10	3.24	Confirmation
17	Service quality	Structural capital	Customer Loyalty	0.08	2.95	Confirmation

DISCUSSION AND CONCLUSION

One of the content differences between the chapter on data analysis and the chapter on summarizing and concluding in research works is that unlike the chapter on analysis where the reader is left alone among a multitude of numbers and statistical techniques, in the chapter on summarizing and concluding most of this ambiguity is omitted.

The reader understands the essence of the word as a simple and non-technical Babiani. In writing this chapter, an attempt has been made to write Babiani as simple as possible on the findings and research suggestions so that readers and respected managers can make the most of these findings. This study sought to investigate the effect of website quality on customer loyalty with the mediating role of social capital. Statistical analysis provides a framework for the researcher to make judgments about society. For this purpose, in this study, effective variables were extracted by studying the research literature, and then the explained hypotheses were proved using statistical methods.

In the present study, an attempt has been made to identify the effect of website quality by considering the variables of information quality, system quality, and service quality with the mediating role of social capital and by considering the variables of capitalist, communication capital, and structural capital on customer loyalty; According to this purpose, the present research method is applied based on the purpose and in terms of data collection method is descriptive-correlation from the branch of structural equation model.

In this research, 17 hypotheses were explained in the form of a conceptual model, which is followed by the analysis of the results obtained from testing these hypotheses.

Hypothesis 1: The quality of site information has an impact on capitalism.

Given that the t-statistic obtained from the hypothesis test (1.762) is inside the critical area (-1.96 and 1.96), this hypothesis is not confirmed; Therefore, based on the response of the subjects in the statistical population, the quality of site information has no significant effect on capitalism, although this meaningless effect is positive based on what the coefficient (0.145) shows. Based on the conceptual definition of system quality, "a system that is well designed, developed and implemented and provides the conditions for organizational benefits" can be said that the organizational benefits perceived by the customer promote his capitalism "such as trust, "Norms, and values that affect interactions between people." This finding is consistent with the results of research by Chen et al. (2016) [14] because they also concluded that all three dimensions of website quality (information quality, system quality, and service quality) are significantly capitalist and structural they are not related.

Hypothesis 2: The quality of site information affects the structural capital.

Given that the t-statistic obtained from the hypothesis test (2.904) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the response of the subjects in the statistical population, the quality of information has a significant effect on structural capital. This significant effect is positive and desirable based on what the obtained coefficient (0.186) shows. This means that as the quality of website information improves, the structural capital of the organization's customers increases, so that; by improving one unit in the level of information quality, the structural capital of customers is improved by

0.186 units. This finding contradicts the findings of Chen et al. (2016) [14].

Hypothesis 3: The quality of the site system has an impact on capitalism

Given that the t-statistic obtained from the hypothesis test (1.380) is inside the critical region (-1.96 and 1.96), this hypothesis is rejected; Therefore, based on the subjects' answers in the statistical population, the quality of the site has no significant effect on the capitalist life and savings customers. This finding is consistent with research by Chen et al. (2016).

Hypothesis 4: The quality of the site system affects the structural capital.

Given that the t-statistic obtained from the hypothesis test (5.598) is outside the critical area (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' answers in the statistical population, the quality of the system has a significant effect on structural capital. This significant effect is positive and desirable based on what the obtained coefficient (0.389) shows. This means that as the quality of the website system improves, the structural capital of the organization's customers increases, so that; by improving one unit in the level of information quality, the structural capital of customers is improved by 0.389 units. This finding contradicts the findings of Chen et al. (2016) [14].

Hypothesis 5: The quality of Bamilo site services has an impact on capitalism.

Given that the t-statistic obtained from the hypothesis test (9.219) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the answers of the subjects in the statistical population, the quality of services has a significant effect on capitalism. This significant effect is positive and desirable based on what the obtained coefficient (0.629) shows. This means that as the quality of website services improves, the capital of the organization's customers increases, so that; by improving one unit in the level of service quality, customers' capital is improved by 0.629 units. This finding contradicts the findings of Chen et al. (2016) [14].

Hypothesis 6: The quality of site services has an impact on structural capital.

Given that the t-statistic obtained from the hypothesis test (4.408) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' answers in the statistical population, the quality of services has a significant effect on structural capital. This significant effect is positive and desirable based on what the obtained coefficient (0.291) shows. This means that as the quality of website services improves, the structural capital of the organization's customers increases, so that; by improving one unit in the level of information quality, the structural capital of customers is improved by 0.291 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 7: Capitalism affects communication capital.

Given that the t-statistic obtained from the hypothesis test (3.298) is outside the critical range (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' responses in the statistical population of the study, capitalist capitalism has a significant effect on communication capital. This significant effect is positive and desirable based on what the obtained coefficient (0.3) shows. This means that as capitalism improves, the communication capital of the organization's customers increases, so that; by improving a unit at the capitalist level, customers' communication capital improves by 0.3 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 8: Structural capital affects communication capital.

Given that the t-statistic obtained from the hypothesis test (5.264) is outside the critical range (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' answers in the statistical population, structural capital has a significant effect on communication capital. This significant effect is positive and desirable based on what the obtained coefficient (0.428) shows. This means that as structural capital improves, the communication capital of the organization's customers increases, so that; by improving one unit at the level of structural capital, customers' communication capital is improved by 0.428 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 9: Capitalism affects customer loyalty.

Given that the t-statistic obtained from the hypothesis test (3.842) is outside the critical range (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' responses in the statistical population studied, the capitalist has a significant effect on customer loyalty. This significant effect is positive and desirable based on what the obtained coefficient (0.350) shows. This means that as the quality of capitalism improves, the loyalty of the organization's customers increases, so that; by improving one unit at the capitalist level, customer loyalty is improved by 0.35 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 10: Structural capital affects customer loyalty.

Given that the t-statistic obtained from the hypothesis test (3.984) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' responses in the statistical population, structural capital has a significant effect on customer loyalty. This significant effect is positive and desirable based on what the obtained coefficient (0.278) shows. This means that as structural capital improves, the loyalty of the organization's customers increases, so that; by improving one unit at the level of structural capital, customer loyalty is improved by 0.278 units. This finding contradicts the findings of Chen et al. (2016) [14].

Hypothesis 11: Communication capital affects customer loyalty.

Given that the t-statistic obtained from the hypothesis test (4.071) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the answers of the subjects in the statistical population, communication capital has a significant effect on customer loyalty. This significant effect is positive and desirable based on what the obtained coefficient (0.327) shows. This means that as communication capital improves, the loyalty capital of the organization increases, so that; by improving one unit at the level of communication capital, customer loyalty improves by 0.327 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 12: Capitalism mediates the relationship between information quality and customer loyalty.

Due to the non-confirmation of the first hypothesis that there is a significant effect between information quality and capitalism, this hypothesis is also rejected. On the other hand, according to the level of significance calculated by the Sobel test, it can be seen that this hypothesis is not confirmed again.

Hypothesis 13: Capitalism mediates the relationship between system quality and customer loyalty.

Due to the non-confirmation of the third hypothesis that there is a significant effect between system quality and capitalism, this hypothesis is also rejected. On the other hand, according to the level of significance calculated by the Sobel test, it can be seen that this hypothesis is not confirmed again.

Hypothesis 14: Capitalism mediates the relationship between service quality and customer loyalty.

Considering the significance of the existing paths between the independent, mediating, and dependent variables, we have calculated the significance value using the Subel test. As observed in the data analysis chapter, the calculated value is equal to 3.54 and outside the absolute value range is 1.96. Therefore, this hypothesis was also confirmed. On the other hand, the coefficient of effectiveness of customer loyalty through the capitalist mediator variable has been reported equal to 0.22.

Hypothesis 15: Structural capital mediates the relationship between information quality and customer loyalty.

Considering the significance of the existing paths between the independent, mediating, and dependent variables, we have calculated the significance value using the Subel test. As observed in the data analysis chapter, the calculated value is equal to 2.34 and outside the absolute value range is 1.96. Therefore, this hypothesis was also confirmed. On the other hand, the coefficient of effectiveness of customer loyalty through the mediating variable of structural capital is reported to be 0.05.

Hypothesis 16: Structural capital mediates the relationship between system quality and customer loyalty.

Considering the significance of the existing paths between the independent, mediating, and dependent variables, we have calculated the significance value using the Subel test. As observed in the data analysis chapter, the calculated value is equal to 3.24 and outside the absolute value range is 1.96. Therefore, this hypothesis was also confirmed. On the other hand, the coefficient of influence of customer loyalty through the mediating variable of structural capital is equal to 0.10.

Hypothesis 17: Structural capital mediates the relationship between service quality and customer loyalty.

Considering the significance of the existing paths between the independent, mediating, and dependent variables, we have calculated the significance value using the Subel test. As observed in the data analysis chapter, the calculated value is equal to 2.95 and outside the absolute value range is 1.96. Therefore, this hypothesis was also confirmed. On the other hand, the coefficient of effectiveness of customer loyalty through the mediating variable of structural capital is equal to 0.08.

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Contribution

All authors participated in the presentation of the original idea and design, search for sources and review of articles, initial writing or revision of the article, and all with the final approval of the present article, take responsibility for the accuracy of the content.

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ORIGINAL ARTICLES

The Covid-19 pandemic – A statistical interpretation updated in early 2021

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Abstract: *One year after the beginning of the Covid-19 pandemic, on the world graph of daily morbidity can be seen that we are in the downturn of the third epidemic wave, consistent with the downturn of the third wave of daily deaths from/with Covid-19. However, the written press and the audiovisual media continue to present alarming balance sheets several times a day that lead the public to the idea of public danger.*

Doubts about the adequacy of declaring the pandemic were interpreted by some "naysayers" as doubts about the existence of the disease and the virus, and some loudly expressed their opposition against the prophylactic measures. Increasingly, public opinion, the press, and the judiciary system are shedding light on the causes of these approaches, which claim to be based on scientific criteria but sometimes prove to be pseudo-scientific, causing an „infodemic".

The best objective orientation in this informational chaos is based on the mathematical-statistical processing of official data that is centralized daily from all countries of the world and published on the Internet. The statistical calculation suggests that in addition to the inequalities inherent in different populations, there are also cases of over-reporting, over-diagnosis, over-treatment, and/or over-media coverage of the Covid-19 case practice.

As a general conclusion, the virus is real, the disease is real, but the risk to public health is not particularly serious except in certain geographical areas. If scientifically established prophylaxis and treatment measures are followed, the pandemic may end this year.

Keywords: *epidemiology, pandemic, Covid-19, statistics, infodemic, „fake news"*

INTRODUCTION

One year (the first?) after the formalization of the Covid-19 viral pneumonia epidemic/pandemic caused by the novel SARS-CoV-2 coronavirus, it can be seen on the world daily morbidity chart (www.worldometercoronavirus) that we are in the downturn of the third epidemic wave, consistent with the downturn of the third wave of daily deaths from/with Covid-19. If viral mutations or adverse events do not occur, the pandemic may enter the downturn phase and come to an end this year.

However, the written press and the audiovisual media continue to present alarming balance sheets several times a day that lead the public to the idea of public danger. This emotional approach, although mainly good because it maintains the vigilance of the population, in some cases, provokes exaggerated reactions leading to suicides for fear of the disease or on the contrary unjustified denial of the disease and/or of the virus, preventing the implementation of prophylactic measures.

Doubts about the adequacy of declaring the pandemic were interpreted by some „naysayers" as doubts about the existence of the disease and the virus, and some loudly expressed their opposition against the prophylactic

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measures, even going so far as „conspiracy theories”. Psychologists believe that this attitude is mainly influenced by mental and/or intellectual deficiencies and is enhanced by „fake news” and „conspiracy”.

Increasingly, public opinion, the press, and the judiciary system are shedding light on the causes of extreme approaches: political, economic, financial, etc. Although they all claim to be based on scientific criteria and many are published, they sometimes prove to be pseudo-scientific, causing an „infodemic”. The best objective orientation in this informational chaos is based on the mathematical-statistical processing of official data that is centralized daily from all countries of the world and published on the Internet (www.worldometercoronavirus) [1, 2].

State of the art

The statistical processing of official and public data allows an objective view of the pandemic now on the anniversary of its first year and a summing-up of realistic conclusions about the Covid-19 disease caused by the novel SARS-CoV-2 coronavirus. This potentially fatal viral pneumonia was first reported in the Republic of China in December 2019; in January 2020 an epidemic was declared in China, and in March the World Health Organization (WHO) declared it a pandemic. But the origin of the virus has not yet been officially established.

Like any other new viral infectious disease, it was initially compared to other similar viruses. But soon, the peculiarities of the disease triggered fears about the virulence and transmissibility of the virus. As a new disease, there were no specific reagents for virological diagnosis, no antiviral or other specific drugs, and no vaccines.

The year 2020 was characterized as the “Covid year” not only because of the new pandemic but also because of the unprecedented involvement and international technical-scientific competition to obtain specific means of diagnosis, prevention, treatment, and anti-infective protection equipment. As a result, more or less interested, the press and scientific publications launched information, misinformation, and anti-information campaigns in which they claimed, with or without arguments, the danger of the disease and the virus or on the contrary the lack of real danger. Former US President Donald Trump did not implement the anti-epidemic measures recommended by the WHO, in order not to favor the economic crisis and caused the worst evolution of the current epidemic, both in health and economic terms. Sweden has not applied any restrictions and is in line with the European health and economic trend.

In this multitude of facts and opinions were blurred the informed opinions of independent scientists, who have no conflicts of interest with the pharmaceutical or medical industry and no partisan political involvement. Big Pharma and some politicians have exaggerated the risks to ultimately make a maximum profit. The political opposition has minimized the risks and unwittingly favored the spread of the pandemic. Each considered that the other side launches „fake news”. International censorship (the Internet and the press) has been imposed on dissenting opinions, including on the publications of US President D. Trump. Some countries, including Romania (to exemplify with our country of origin), have instituted censorship (without naming it so) against everything that contradicts the official statements on Covid-19, against the "Spread of disinformation online". Romania, through the ministries and designated agencies, has proactive acts to counter disinformation through official statements. The State through the Ministry of Internal Affairs based on the declaration of the State of Emergency decides to suspend the access or the online functioning license of the channels that were promoting disinformation. (Report about COVID-19 Strategic Communication Group – A proposal to disable, Ministry of Internal Affairs, România, 18th of March, 2020, URL: https://stiridemoment.ro)[3]

Scientists and their sponsors have published scientific papers on this subject more than any other, but many are invalidated or even misleading. Scandals and article retractions followed, even from prestigious magazines, and investigations were launched. The proposal to change the criteria for publication in scientific journals has been reached. Many politicians, businessmen, doctors, and others have given or will give an account for their opinions (crimes of opinion) and/or their (criminal) facts related to the current pandemic.

Prophylactic measures have gone beyond the medical sphere and have sometimes been so harmful that they have favored the spread of the disease or affected the health of the population (as a whole or some social or risk groups) from a physical, mental, intellectual, and social point of view. All this has led to an economic and financial crisis and ultimately a political one. Currently, public health and living standards in most countries are at the lowest level, confidence in science is affected, medical authority is questioned and part of the population is agitated. The health crisis is doubled by the social, economic, financial crisis, the „crisis of anything”. [4]

All this triggered the current „infodemic” a widespread information pandemic, with „fake news” vertically and horizontally. Part of the population no longer trusts that the authorities and governments try to „discipline” the population through „soft" (communications, centralized information) and/or „hard" (ordinances, laws, fines, and enforcement) measures. The existence of a possible „global governance" was discussed at the World Economic Forum in Davos 2021. [5,6] In reality, it would comprise much less than a quarter of the world's population, and only a part of Europe, North America, South America, and Oceania. But it is known that in biology and sociology the general rule „20:80" is applied for the cause/effect relationship, respectively about 20% of the population creates about 80% of the effects, so this fraction would be the one that matters worldwide. [7]

MATERIALS AND METHODS

In the chaos created by the current „infodemic" regarding the Covid-19 pandemic, the facts must be interpreted objectively, from a medical point of view, starting from the serious statistics that are constantly updated by the World Health Organization, Geneva, Switzerland (WHO), Center for Diseases Control, Atlanta, USA (CDC) and worldometer, US. Simple mathematical statistics, based on arithmetic means and percentages, are the easiest for anyone to apply to this metadata. [8]

If official data are viewed in the light of classical knowledge and terms of epidemiology, the statistical calculation clearly shows that the world situation and the evolution of the disease are not very serious. But there are discrepancies between the populations of the continents, some civilized regions (in the sense proposed by Huntingdon), compared to the world arithmetic mean are better and others less well. All comparisons were made as a percentage to mitigate the influence of very different numbers of populations studied. (Table 1)

Table 1. World population, numerical ranking, and percentage by continents (www.worldometer accessed on 08.02.2021)

No.	Continent	Population	Percentage	Comments
1	Asia	4.629.797.188	59,35%	predominantly non-European civilizations
2	Africa	1.363.619.464	17,5%	predominantly non-European civilizations
3	Europe *of which Romania*	747.902.271 *19.160.208*	9,6% *2,6%*	European civilization
4	North America	591.862.172	7,6%	European civilization
5	South America	432.886.432	5,5%	European civilization
6	Oceania	42.589.860	0,5%	European civilization
7	Antarctica	0	0,0%	uninhabited
	WORLD TOTAL	7.844.139.370	100,0%	

It is observed that the vast majority of the world's population (76.8%) includes populations with predominantly non-European civilization, and a minority (23.2%) has a predominantly European civilization.

RESULTS AND DISCUSSION

The statistical processing of data reported by all countries of the world, centralized and published on www.worldometercoronavirus on 08.02.2021 shows interesting and sometimes unexpected aspects, from which useful conclusions can be drawn for public health. Mark Twain said, „The facts are stubborn. It's easier to deal with the statistics. But we love the facts".

Mathematical statistics suggest that, first of all, the disease is real but not very dangerous, 95.5% of cases being mild or asymptomatic (according to the WHO). This means that they do not need specific treatment or that treatment can be done at home in self-isolation. [9]

The regions most affected by Covid-19 are in the area of predominantly European civilization, including the Euro-Atlantic.

We calculated the values for prevalence, incidence, and mortality, compared for continents and compared to the world average but also as dynamics over time. It is interesting to note that the daily morbidity of Covid-19 was reduced from the peak in wave 3 to approx. half, which suggests that the pandemic would have entered the downward trend and will end with or without vaccination unless other disruptive factors intervene.

The average percentages on continents are presented, compared to the world average (Figures 1-8). It is observed that the prevalence of morbidity with Covid-19 is well above the world average in North America, Europe, and South America (which totals 22.7% of the world population). Romania falls within European limits (well above the world average), by 3.88%.

Figure 1: Percentage average by continent for the prevalence of Covid-19 cases reported over the general population, compared to the global prevalence of the disease.

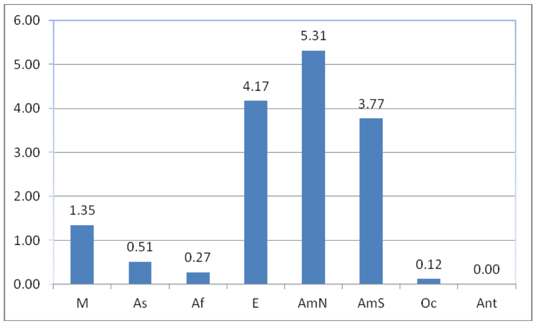

M = world average, As = Asia, Af = Africa, E = Europe, AmN = North America, AmS = South America, Oc = Oceania, Ant = Antarctica

Figure 2: The percentage by continent of the prevalence of Covid-19 mortality in the general population, compared to the global prevalence of Covid-19 deaths.

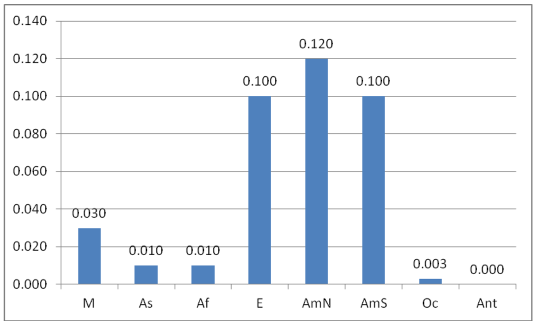

M = world average, As = Asia, Af = Africa, E = Europe, AmN = North America, AmS = South America, Oc = Oceania, Ant = Antarctica

It is observed that the prevalence of Covid-19 mortality in the general population is well above the world average in North America, Europe, and South America (which totals 22.7% of the world population), in line with morbidity. Romania falls within the European limits (well above the world average), by 0.10%.

Figure 3: The average percentage on continents of the current prevalence of active cases of Covid-19 in the general population, compared to the world prevalence.

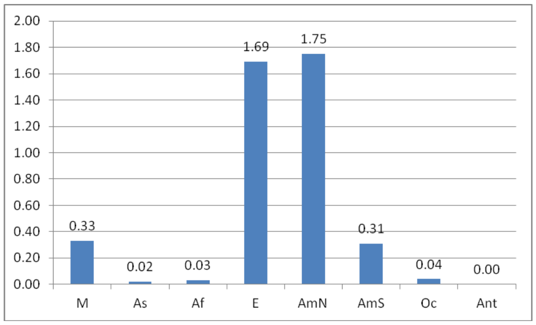

M = world average, As = Asia, Af = Africa, E = Europe, AmN = North America, AmS = South America, Oc = Oceania, Ant = Antarctica

It is observed that the prevalence of active cases of Covid-19 is well above average in North America and Europe (about 17.2% of the world's population). Romania falls within the European limits, with values below the world average, by 0.18%.

Figure 4: Incidence of mortality in Covid-19 patients on continents, compared to the global incidence in 2020.

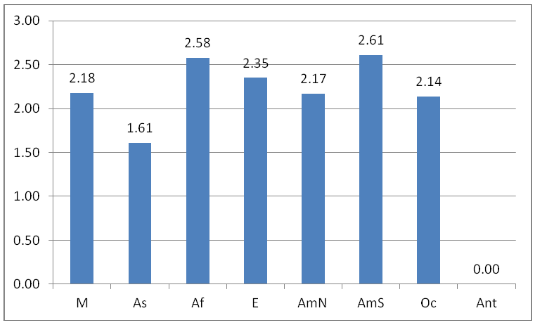

M = world average, As = Asia, Af = Africa, E = Europe, AmN = North America, AmS = South America, Oc = Oceania, Ant = Antarctica

It is observed that the incidence of mortality is slightly higher than the average in South America, Africa, and Europe. In Romania, it is 2.53%, higher than the European average and than the world average, so either the therapy is deficient or there is a possible overreporting of mortality from/with Covid-19.

Figure 5: Incidence of mortality in patients with Covid-19 with new cases, on continents, compared to the global incidence in early 2021.

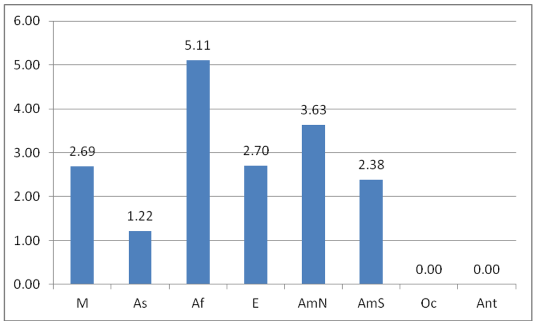

M = world average, As = Asia, Af = Africa, E = Europe, AmN = North America, AmS = South America, Oc = Oceania, Ant = Antarctica

It is observed that mortality in new cases of Covid-19 is well above the world average in Africa and North America (25.1% of the world's population). Romania falls within European limits, close to the world average, by 2.34%.

Figure 6: Incidence of serious active cases (serious, critical) out of the total Covid-19 reported cases, by continents, compared to the global incidence at the end of 2020 and the beginning of 2021.

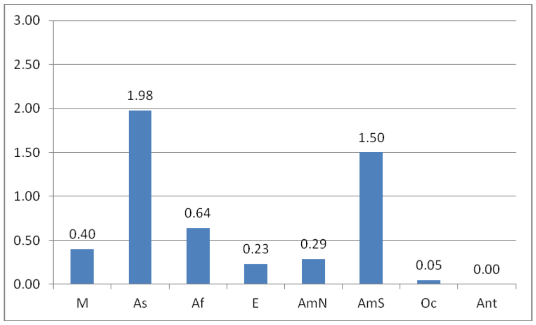

M = world average, As = Asia, Af = Africa, E = Europe, AmN = North America, AmS = South America, Oc = Oceania, Ant = Antarctica

It is observed that the incidence of severe active cases of Covid-19 is higher in Asia, South America, and Africa (82.3% of the world's population) but with a low prevalence. In Romania, it is 2.75% so, over 10 times higher than the European average, there would be a possible overreporting by overdiagnosis and/or overtreatment (in ICU) of the usual cases of Covid-19.

Figure 7: Prevalence of active cases from the total number of Covid-19 reported cases, by continent, compared to world prevalence.

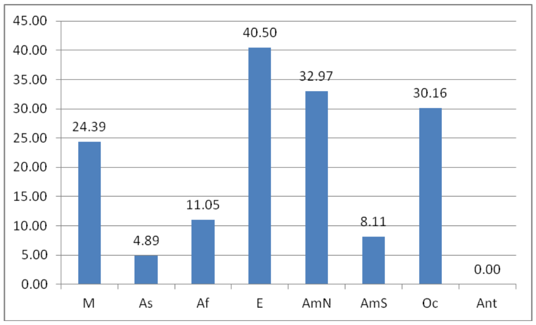

M = world average, As = Asia, Af = Africa, E = Europe, AmN = North America, AmS = South America, Oc = Oceania, Ant = Antarctica

It is observed that the prevalence of active cases compared to the total number of covid-19 cases is higher in Europe, North America, and Oceania (respectively 17.7% of the world's population). Romania falls within European limits, with very low values of active cases, with 4.72%.

It is observed that the incidence of active Covid-19 cases with severe forms compared to the number of premature deaths of/with Covid-19 is higher in Asia and South America (64.8% of the world's population). In Romania, the incidence of severe active cases (by 5.12%) far exceeds the European average, suggesting an overreporting through overdiagnosis and/or overtreatment of Covid-19 cases.

Figure 8: Incidence of serious active cases in the Covid-19 reported cases, compared to the total deaths registered with/by Covid-19.

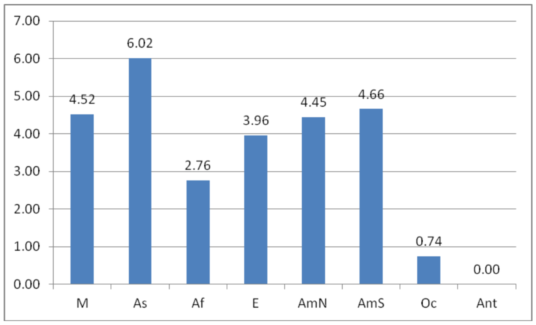

M = world average, As = Asia, Af = Africa, E = Europe, AmN = North America, AmS = South America, Oc = Oceania, Ant = Antarctica

Observation

The results obtained by simple statistical calculation are sometimes unexpected, even surprising, the world situation is not as serious as it is generally believed, and in the "Third world" (underdeveloped and developing countries) the situation seems paradoxically better than in "First World" (industrialized countries). In general, the situation in warm areas is better than in cold areas, although they are less economically developed. But in the case of infectious-contagious diseases, the influence of environmental conditions is decisive, in this case, high temperatures prevent infection with respiratory viruses. Thus, compared to a total global average index (conventionally established by the arithmetic mean of the percentage sums from all analyzed parameters) of 5.12%, an average index of 7.61% is obtained for predominantly temperate areas and 3.49% for predominantly hot areas.

Several possible causes or the association of some of them can be discussed, which lead to the observed statistical inequalities, which will be briefly presented below.

1. In the areas with material and financial possibilities and therefore the most advanced medicine in the world, most cases of Covid-19 were diagnosed and treated, so they reported a higher number of cases.

2. The higher standard of living in these areas, which also led to a longer average life expectancy, favored the existence of an aging population with comorbidities, as well as many patients who survive due to permanent medical care, so important risk groups for the Covid-19 infection.

3. The financial interests of the pharmaceutical, medical, and health insurance industries have probably led to over-reporting, over-diagnosis, over-treatment, and over-media coverage of Covid-19 cases.

4. The health insurance system (state and/or private) which

is more efficient for top modern medicine and those with a good financial situation, seems to be less efficient for public health, where the risk of illness is higher for the poor.

5. The classic sanitary territorialization systems ensure better evidence and traceability of cases, isolation, and treatment for the entire rounded population, and no one is left out of the system.

6. The spread of the disease has also been favored by the system of national and international relations, transport, trade, and relatively free travel, existing worldwide.

7. some politicians and the multinationals that support them may have found the opportunity to blame the pandemic for the economic and financial crisis that was already in its infancy in the Western world.

8. Undifferentiated and inconsistent implementation of WHO recommendations resulting from China's experience, which cannot be transposed to all countries and/or cannot be accepted by the population.

9. Implementation of the recommendations of the World Economic Forum in Davos, 2020 and 2021, for the Great Reset (zeroing) aimed at maintaining the New World Order, which would favor multinationals against state authority and small businesses in general, to counteract the possible rise of China towards even newer world order.

10. The intention of some politicians to keep the population under an authoritarian leadership (foreshadowed in the social-fiction novel „1984”) to ensure the dominant position of those already in power

11. The WHO has not yet established the origin of the novel coronavirus, so one could also discuss "genetic engineering" and/or intentional or unintentional dissemination of this new virus, more or less natural.

12. According to the evolution of statistical data, it seems that the „white race” and especially the „Caucasians” would be more susceptible to this disease, in which case a „biological agent of bioterrorism” with racial characteristics could be discussed. (a possible ethnic weapon?).

13. Any other causes, alone or associated, which we cannot yet intuit or afford to discuss.

Now, one year after the formalization of the Covid-19 epidemic/pandemic, it can be seen on the world daily morbidity chart that we are in the downturn of the third epidemic wave, consistent with the downturn of the third wave of daily deaths. The disease is less contagious and less serious, if no adverse mutations occur, the pandemic may enter a downturn phase and end this year. But the novel SARS-CoV-2 coronavirus is likely to remain endemic, with some outbreaks or even minor outbreaks. In principle, no infectious-contagious disease with an animal microbial reservoir can be eradicated. [11]

The arithmetic means of the Covid-19 reported cases to show a somewhat paradoxical reality, with possible over-reporting: false-positive cases in PCR and immunological tests, the number of patients hospitalized and in the ICU, the number of deaths with/by Covid-19 with possible overdiagnosis due to the same tests (PCR with too many multiplication cycles, antibodies to other coronaviruses or from previous infections) and their repetition as well as overtreatment at home, in hospital, intensive care or resuscitation. A large part of the press also voluntarily, willingly, or unwillingly contributed to this situation, which led to the artificial amplification of the pandemic. “You can fool all the people some of the time and some of the people all the time, but you cannot fool all the people all the time” (Abraham Lincoln).

CONCLUSIONS

The vast majority of the world's population (76.8%) includes populations with predominantly non-European civilization, and a minority (23.2%) has a predominantly European civilization, which according to statistics is more affected by the Covid-19 pandemic.

The global prevalence of morbidity is 1.35%, of mortality below 0.03% and the incidence of serious cases is 0.5% of the total Covid-19 reported cases.

The prevalence of morbidity with Covid-19 is well above the world average in North America, Europe, and South America (totaling 22.7% of the world's population). Romania falls within European limits (well above the world average), by 3.88%.

The prevalence of Covid-19 mortality in the general population is well above the world average in North America, Europe, and South America (totaling 22.7% of the world population), consistent with morbidity. Romania falls within the European limits (well above the world average), by 0.10%.

The prevalence of active cases of Covid-19 is well above average in North America and Europe (about 17.2% of the world's population). Romania falls within the European limits, with values below the world average, by 0.18%.

The incidence of mortality is slightly higher than the average in South America, Africa, and Europe. In Romania, it is 2.53%, higher than the European average and than the world average, so either the therapy is deficient or there is a possible overreporting of mortality from/with Covid-19.

Mortality in new cases of Covid-19 is well above the world average in Africa and North America (25.1% of the world's population). Romania falls within the European limits, close to the world average, by 2.34%.

The incidence of severe active cases of Covid-19 is higher in Asia, South America, and Africa (82.3% of the world's population) but with a low prevalence. In Romania, it is over 10 times higher than the European average, so there is a possible overreporting by overdiagnosis and/or overtreatment (in ICU) of the usual cases of Covid-19, by 2.75%.

The prevalence of active cases compared to the total number of cases of Covid-19 is higher in Europe, North America, and Oceania (respectively 17.7% of the world's population). Romania falls within European limits, with very low values of active cases, with 4.72%.

The incidence of severe active Covid-19 cases relative to the number of premature deaths from/with Covid-19 is higher in Asia and South America (64.8% of the world's population). In Romania, the incidence of severe active cases (by 5.12%) far exceeds the European average, suggesting an overreportation probably through overdiagnosis and/or overtreatment of Covid-19 cases.

The statistical calculation suggests that in addition to the inequalities inherent in different populations, there would also be cases of over-reporting, over-diagnosis, over-treatment, and/or over-mediation of the Covid-19 case practice. [12, 13]

As a general conclusion, the virus is real, the disease is real, but the risk to public health is serious only in certain geographical areas. If scientifically established prophylaxis and treatment measures are followed, the pandemic may end this year.

The lessons learned from this pandemic in general and the statistical analysis of the world situation, in particular, are multiple and useful for managing an epidemic, pandemic, or epizootic. But as Socrates said (5th century BC): „You can't teach anyone anything, you can only make them think”.

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ORIGINAL ARTICLES

Survey of the patients' satisfaction with services provided in an Iranian Naval Hospital in 2019: A cross-sectional study

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Abstract: *Background and Aim: All hospital services are carried out to serve the patient, which is the main axis in the hospital. Patient satisfaction can be indicative of the right performance service delivery and is considered a reliable source for evaluating the quality of health care. The present study was conducted to evaluate the satisfaction rate of discharged patients in a Navy hospital.*

Methods: The descriptive cross-sectional study was performed on 220 discharged patients selected by convenience sampling in a Navy hospital during 2019. The data gathering tool was the Patient Satisfaction Questionnaire designed by Amerioon in 2009 and its validity and reliability were confirmed in 2016. The questionnaire assessed patient satisfaction in two healthcare sections with 21 questions and three dimensions of hygiene, nursing and physician services, and non-health care with 27 questions and six dimensions of acceptance, staff attitudes, nutrition, welfare, facilities, religious issues, and refines evaluation. Data were analyzed by SPSS software version 18.

Results: The patient satisfaction in the medical and non-medical wards was 88.1% and 86.57% respectively. Satisfaction in the medical and non-medical wards was excellent at all dimensions. There was a significant relationship between satisfaction with the cause of referral, and level of education (P <0.05). Other demographic variables such as age, marital status, patient gender, frequency of hospitalization, type of ward, and type of insurance were not significantly correlated with satisfaction(P>0.05).

Conclusion: Considering the high level of patients 'satisfaction in medical and non-medical services, patient satisfaction in medical services was slightly higher than that of non-medical services, this highlights the need for independent research in this area and the actions taken by hospital managers to improve satisfaction in these sectors. It is also suggested to consider patient satisfaction as part of the discharge process in all hospitals

Keywords: *patient, satisfaction, hospital, Navy*

INTRODUCTION

Satisfaction as an indicator of the quality of services provided over the past few decades has found a special place. The quality of services provided to patients and the level of satisfaction with the services provided show the general situation of providing services in that hospital [1]. Evaluating patient satisfaction is an important source of information to identify problems and desirable programs in the field of health care services and is an indicator of the

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quality of service delivery in various interpersonal, organizational and technical areas [2]. At present, satisfaction is an important issue in health care, and patient satisfaction plays an important role in increasing accountability among health care providers in terms of advancing the organization's goals [3]. Particular attention is paid to the patient's needs as an intrinsic goal, as well as the fact that this is an important component in improving the quality of care, and should be considered by policymakers. Also, patient satisfaction as a basic system should play an important role in managers' decisions to make that organization successful [4]. Therefore, patient satisfaction is one of the main factors and important indicators in evaluating the quality of health organizations [5].

In the past, to improve the level of health services, the opinion of experts in this field was the most important determinant of how to change the service process. However, recent studies have paid special attention to the views of patients on the provision of health services as an important indicator of the quality of service delivery. Comments that may even differ from or even contradict the views of health professionals and are recognized as a determining indicator for evaluating and improving the quality of services provided by services [6]. The importance of this issue is such that patients' satisfaction with the way health services are provided from the perspective of the World Health Organization, as one of the five indicators of quality of services [7] and according to some researchers, this factor is one of the most important determinants. The quality of health services is important [8].

Patients' views on how to provide health services can include important information about the quality of various aspects of health care delivery. The findings of various studies have shown that various factors affect the patient's satisfaction with the way the medical services are provided in the hospital, such as the way doctors, nurses, staff, and even the physical environment of the medical centers' function; also the patient's satisfaction with the hospital complex and its staff. The predictor of future behaviors and behaviors of patients during treatment and even seek discharge from the hospital [9, 10].

Patients' greater satisfaction with care makes them more willing to follow doctor's instructions, follow up on care and treatment, follow more dietary regimens and treatment recommendations, respect health care guidelines, and communicate more deeply with providers. Health services, as a result, reduce recovery time and reduce the number of patient complaints and achieve better health outcomes [11].

Patient satisfaction is also essential for marketing purposes. It provides patient satisfaction information, useful information about the structure, process, and outcome of nursing care, and more, such as staff adequacy, treatment needs, and patient behaviors [12]. Also, a patient who is satisfied with the health care provider often tends to go to the same hospital when he or she needs re-health care. This will increase productivity and profitability for the collection [13].

In the field of patient satisfaction assessment, several studies have been conducted in Iran, but since most studies have measured how to provide medical services or factors affecting satisfaction, and also because satisfaction is affected by various underlying factors such as individual, cultural, social, economic and religious factors of patients [14, 15] and considering that most studies conducted in Iran, except for hospitals and special urban areas and the views of residents of those geographical areas, on how to provide medical services and found It has led to many different ones [16-18], and since the studies are limited In hospitals under the auspices of the Armed Forces, patients have been treated with satisfaction, so due to the importance of these hospitals due to a large number of clients, to achieve patients' views on how to provide medical and non-therapeutic services, it is necessary to do so. The study is necessary in this case. Therefore, the present study was conducted to investigate patients' satisfaction with the services provided in one of the naval hospitals of the Revolutionary Guards in the city of Chalous.

ANALYZATION METHOD

Type of study

The present cross-sectional study was performed on patients discharged from one of the naval hospitals in 1398.

Sampling and determining sample size

Sampling was performed by the available method. To determine the sample size, the sample size estimation formula was used in the prevalence studies in terms of the inferential test. In terms of the first type of error of five percent, the second type of error of twenty percent and the probability of 50% satisfaction with the maximum sample size estimate, the required number of samples was calculated using the formula below 196; It was calculated that 185 usable questionnaires were returned in the present study, which was more than the minimum acceptable number of calculated questionnaires.

Input and output criteria

The criteria for entering the study were those over 18 years of age and the desire to participate in the study and not hospitalization in the psychiatric wards of the hospital. The criteria for leaving the study were the reluctance to continue participating in the study.

How to complete research questionnaires?

After obtaining a license from the research of Baqiyatallah University of Medical Sciences, he was referred to the selected hospital. After presenting the permit to enter the environment, the researcher entered the research environment and the research questionnaires were completed according to the entry and exit criteria. In cases where the clients were illiterate, the questionnaire questions were read by the researcher to the client and their answers were recorded.

Questionnaire for satisfaction with non-therapeutic medical services

The data of this study were confirmed by the standard patient satisfaction questionnaire which was designed by Amerioon et al. In 2009 and in 2016 its localization, validity, and reliability were confirmed. The questionnaire included two sections of general information such as age, gender, literacy level, marital status, section, type of insurance, and hospital history. The second part of the questionnaire included 48 questions, the level of patients' satisfaction with the way of providing services in two therapeutic sections with 3 dimensions and 21 questions, including services provided by the nurse (9 questions), physician (8 questions), and hospital health and cleaning services (4 questions) and the non-therapeutic section with 6 dimensions and 27 questions including services of admission section (2 questions), staff approach (5 questions), nutrition (2 questions), welfare and facilities (9 questions), clearance (4 questions) and observance of issues Shari'a-doctrinal (5 questions) evaluated. Responses were graded based on the Likert 5 spectrum (completely satisfied, satisfied, no comment, dissatisfied, completely dissatisfied). At the end of the questions, two open questions were asked. The content of the first question was related to the person's return to the current hospital in case of illness and the second question was about the current hospital's recommendation to friends and acquaintances. To calculate the satisfaction score, the respondents were given completely dissatisfied and completely satisfied with the score of zero to 100, respectively. Thus, the total scores of each field separately and the overall satisfaction score was determined from a score of 100, with a higher score indicating more patient satisfaction in the context of how services were provided during hospital stay (19). The criteria for classifying satisfaction were based on four categories: low (scores up to 25 percent), medium (26 to 50 percent), good (51 to 75 percent), and excellent (76 to 100 percent) [15].

Reliability and Validity

The validity and reliability of this questionnaire were evaluated in the country in 2016 by Amerioon and colleagues in the same community and its validity and reliability were confirmed. The overall alpha coefficient of the treatment questionnaire was 0.975 and the non-therapeutic questionnaire was 0.936 [19].

Moral considerations

This study was approved by the University Research Council with the code 98000238, which was approved by the ethics committee of Baqiyatallah University of Medical Sciences at IR.BMSU.REC.1398.269. Adequate information about the objectives of the study and its importance, maintaining the anonymity and voluntary participation, and confidentiality of the information obtained was given to the participants and was completed after obtaining the written consent of the questionnaires.

Statistical analysis

SPSS software version 18 was used to analyze the data. The level of significance in all cases was considered less than 0.05. To describe the qualitative variables, frequency and frequency percentages were used, and to describe quantitative information, mean and standard deviation were used. To investigate the research hypotheses, a test of a Smirnov-Kolmogorov sample was first used. The results showed that the distribution of the studied data was normal, so to investigate the research hypotheses, parametric t-test, Pearson, and ANOVA tests were used.

FINDINGS

Description of population-cognitive information

220 questionnaires were completed and returned, which was more than the minimum acceptable number of calculated questionnaires (215 questionnaires). The average age of the participants in the study was 44/17 ± 13/53 years. Most of the male research samples (62.2%) and most of the participants in the study were married (67%) (Table 1).

Information in medical departments

The mean and standard deviation was the overall score of patients' satisfaction with the 95 ±9/12 treatment sections. The mean score of satisfaction in terms of health and cleanliness was 18/54±1/92 in the nursing dimension 26/94±3/11 and the medical dimension 49/52±5/23.

The percentage of patients 'satisfaction in the health and cleaning sector was 90/87 in the nursing ward, 87/25, and in the physicians' ward, 87/55, and the overall satisfaction with the treatment ward was 88/1 (Table 2).

Table 1: Distribution of frequency characteristics of patients participating in research

Variable		Abundance	Percentage
Gender	Female	70	37/8
	Man	115	62/2
Marital status	Married	124	67
	Single	61	33
Education	High school	62	33/6
	Diploma and postgraduate	85	45/9
	Bachelor's degree and higher	38	20/5
Type of insurance	Treatment services	47	25/4
	Armed Forces	54	29/2
	Social Security	46	24/9
	Other insurances	17	9/1
	Uninsured	21	11/4
Inpatient ward	Men	105	56/8
	CCU	3	1/6
	Maternity hospital	3	1/6
	Obstetrics and Gynecology	59	31/9
	Children	1	0/5
	Emergency	14	7/6
Hospital history	First time	120	64/9
	Second time	22	11/9
	Third time	8	4/2
	Fourth time	4	2/2
	More than four times	31	16/8
The cause of hospitalization	Contract with the hospital	89	48/1
	Being close to home	34	4/18
	Hospital facilities	29	15/7
	Observance of religious values	6	3/2
	Satisfaction in previous visits	9	4/9
	Offer others	12	6/4
	References from other centers	2	1/1
	Doctor's advice	4	2/2

Table 2: Distribution of patient satisfaction in the study participants

Satisfaction of medical departments	Minimum score	Maximum	Percentage	Standard deviation	Average
Cleanliness and hygiene	11	20	18/54	1/92	90/87
Nursing services	18	30	26/94	3/11	87/25
Medical services	28	55	49/52	5/23	87/55
The total score of the dimensions of the questionnaire	65	105	95	9/12	88/1

Information on non-therapeutic sections

In the non-therapeutic part, the mean and standard deviation of the overall score of patients' satisfaction was 120/50 ± 12. The mean and standard deviation of the satisfaction score in the admission dimension was 8/84 ± 1/34, in the employee attitude dimension it was 22/36 ±2/52, in the nutrition dimension it was 9/02 ± 1/24, in the welfare dimension, and the facility 39/99 ± 4/42. 39, in the dimension of clearance 18/08±2/13, and the dimension of Observance of religious values, it was 22/20±2/85. The mean and standard deviation of the total score of the clients' satisfaction with the non-therapeutic sections was 120/50±12.

The percentage of patients' overall satisfaction with non-treatment services was 86/57%, which was 85/33% according to the satisfaction level of the admission department, 86/14 employees, 87/83 nutrition, 86/09 welfare facilities, clearance and subsequent observance of religious issues - Belief was 86/02 (Table 3).

Table 3: Distribution of patient satisfaction in the study participants

Satisfaction with non-therapeutic areas	Minimum score	Maximum	Percentage	Standard deviation	Average
The reception	3	10	8/82	1/34	85/33
Staff	14	25	22/36	2/52	86/14
Nutrition	4	10	9/02	1/24	87/83
Facilities-welfare	26	45	39/99	4/42	86/09
Clearance	12	20	18/08	2/13	88
Observance of religious issues	12	25	22/20	2/85	86/02
The total score of the dimensions of the questionnaire	81	135	120/50	12	86/57

Pearson correlation coefficient showed that there was no significant relationship between age with the overall score of satisfaction with medical services (P = 720 and r = 0.026) and the overall score of satisfaction with non-therapeutic services (P = 0.897 and r = 0.010). Independent statistical t-test between sex with an overall score of satisfaction with health services (P = 0.581) an overall score of satisfaction with non-therapeutic services (P = 0.922) and between marriages with an overall score of satisfaction with health services (P = 0.653) an overall score of satisfaction with Non-therapeutic services (P2 = 0.238) did not show a significant difference.

The one-way analysis of variance test did not show a significant difference between the type of insurance with the overall score of satisfaction with medical services (P22 = 0.222) and the overall score of satisfaction with non-medical services (P = 0.251); However, there was a significant difference between the reason for visiting the hospital with the overall score of satisfaction with medical services and the overall score of satisfaction with non-medical services (P <0.0001). Regarding the difference between the overall score of medical services satisfaction and the reason for visiting the hospital, Tuki's follow-up test showed that this difference was related to having a contract with the hospital (P = 0.005), the hospital's proximity to the place of residence (P00 = 0.009), and others' suggestions. 0.0001> P) and the doctor's recommendation (P = 0.026), although the difference between satisfaction and hospital facilities and facilities (P = 0.062) and referral from other centers (P = 0.095) was not significant; this difference was close to the level of significance. You had. The one-way analysis of variance test did not show a significant difference between the number of visits to the hospital with the overall score of satisfaction with medical services (P = 0.225) and the overall score of satisfaction with non-medical services (P = 0.961).

The one-way analysis of variance test showed a significant difference between the variable of education and the overall score of satisfaction with medical services (P = 0.002) and the overall score of satisfaction with non-therapeutic services (P = 0.006). Regarding the overall score of satisfaction with medical services, Tuki's follow-up test showed that this difference was related to the level of diploma with post-diploma (P = 0.005) and diploma with bachelor's degree and above (P = 0.011). Regarding the overall score of dissatisfaction with non-medical services, Tuki's follow-up test showed that this difference was related to the level of diploma with post-diploma (P = 0.012) and diploma with bachelor's degree and higher (P = 0.025). Sideways showed that there was no significant difference between the variable type of hospital ward with the overall score of satisfaction with medical services (P = 0.161) and the overall score of satisfaction with non-therapeutic services (P2 = 0.230).

In general, the findings showed that there was no significant statistical relationship between age, marriage, patient gender, type of insurance, type of hospital ward, number of hospital visits, and an overall score of satisfaction with medical and non-therapeutic services. However, there was a statistically significant difference between the reasons for visiting this center and the satisfaction with medical and non-therapeutic services (P <0.05), so that people who had a contract with the hospital or the hospital was close to their place of residence, as well as people who Others suggested a doctor's recommendation and had a higher percentage of satisfaction. Also, the statistical test of one-way analysis of variance showed that there is a significant statistical relationship between education and an overall score of satisfaction with medical and non-therapeutic services so that the percentage of satisfaction decreases with increasing education.

The percentage of satisfaction scores was divided into four categories: low (up to 25%), medium (26 to 50%), good (51 to 75), and excellent (76 to 100). There were no scores in the lower category. The highest satisfaction scores were in the top category. Regarding the two open questions at the end of the questionnaire, the results showed that 74.1% of people choose this hospital again for treatment in case of illness, and 72.4% recommend this hospital to their friends and acquaintances.

DISCUSSION

This study aimed to examine patients' satisfaction with the services provided in one of the naval hospitals. According to the results, the general satisfaction of patients in the medical and non-therapeutic sectors in all dimensions, including health and hygiene, nursing, medicine, admission, discharge, staff services, facilities, observance of religious and religious issues, and discharge at the highest level.

Patient satisfaction is one of the most important tools in evaluating the quality of treatment and planning to improve the health system [20]. Despite a large number of clients in hospitals under the auspices of the Armed Forces, especially in cities where only one hospital may provide services to the military and their families, a limited number of studies have been conducted on patient satisfaction in these centers [15, 21, 22, 23] While examining satisfaction in hospitals of the Armed Forces due to their important role in providing services, can improve the quality of services and reflect the performance of these centers to the community and public acceptance of the services of these hospitals. Play a huge role. Therefore, the present study is new in terms of measuring the satisfaction of patients discharged from the Marine Corps Hospital of the Islamic Revolutionary Guard Corps.

The level of satisfaction with nursing services in this study was excellent, similar to this finding in most studies that assessed patients' satisfaction with optimal nursing services [24, 25]. In the Riskuz study in Spain, the level of patients' satisfaction with the desired nursing care was also reported [26]. In the Al-Asad study, the mean score of patients' satisfaction with nursing care was 90.67% and very high [27]. In the Jannati study, the percentage of patient satisfaction was lower than in our study, so that 77% of patients were dissatisfied with nursing services [28].

According to research, nurses' satisfaction is an important factor in patient satisfaction. In various studies, it has been emphasized that there is a direct relationship between nurses' job satisfaction and patients' satisfaction with their services and any issue that leads to a decrease in nurses' job satisfaction. Including unfavorable work environments, lack of manpower in the field of nursing, inappropriate care, moral, social, and organizational values can make efforts to improve patient satisfaction by nurses ineffective. Satisfaction with nursing services plays an important role in the overall satisfaction of the patient, and other studies emphasize the satisfaction of nursing services as one of the determinants of overall patient satisfaction [29, 30]. Akbari said in his study that because nursing medical care plays a pivotal role in restoring patients' health, it has the greatest impact on patient satisfaction [31], so since most care providers in hospitals are nurses and This group is in the first line of patient care, the quality of their care has a great impact on patient satisfaction, and therefore it is necessary to draw the attention of officials to improve the conditions for improving the quality of nursing and medical services. The difference in the results of various studies can be justified by the fact that the cultural and social context can determine the expectations and expectations of people in society from services. For this reason, health policymakers should pay special attention to the job satisfaction of nurses and, consequently, patient satisfaction with medical services. Conversely, in studies that were highly satisfied with nursing services, appropriate organizational culture, including employment standards, financial benefits, and retention of qualified and committed nursing workers, were cited as reasons that may have led to high patient satisfaction [27].

The high satisfaction of medical services in this study was similar to the results of Heydari and Ansari studies, which reported patients' satisfaction with medical services at an excellent level [32]. Similar to this finding, the overall satisfaction of patients with medical services in the study of Azizi [33], 29.5%, in the study of Noor Al-Sana, 50% [34] and in the study of Alidosti was equal to 56.4% [35], which is the result of the present study. It's not true. Considering the 6 to an 8-year interval between these studies and the recent study, it can be concluded that due to the holding of medical ethics workshops in recent years and the importance of medical ethics, and the impact of these workshops, physicians 'treatment of patients has improved and patients' satisfaction has increased. Therefore, in older studies, the rate of physician satisfaction was lower than in a recent study.

In the present study, satisfaction with non-therapeutic services in all dimensions was excellent (85.33%). In a study conducted in another hospital under the auspices of the Navy, the satisfaction of the admission department was excellent and similar to this study [15]. In studies conducted by ZafarGhandi, patients' satisfaction with the admission of hospitals under the auspices of Tehran University of Medical Sciences is 44% [36] and in the study of Rudbari in Zahedan 64% [37] is reported. In all these studies, satisfaction with admission services is less than Our study has found that the reason for this difference is the better location of admission to the hospital under study, as well as the lower number of clients than educational hospitals covered by the university and therefore more speed of admission and better treatment processes and proper communication with clients by staff.

Also, in this study, the satisfaction of the clearance section was at the highest level (88%), and dissimilar to this finding in the Ansari study, the clearance section had the lowest level of satisfaction (38.6) [38]. Excellent satisfaction with nutrition services in this study was another finding that was obtained similar to this finding in a large study. 90% of patients were satisfied with the nutrition service [39] Satisfaction with nutrition services in Ansari's study [38] and Mousavi [40] and Gholi Zadeh [41] were average and less than our study. In his study, Gholizadeh stated that the nutrition sector has been ignored as one of the pillars of patients' health in hospitals or has played a small role in the treatment process. Therefore, hospital managers need to take steps to increase the awareness of foodservice personnel about health and food quality, increase the number of nutrition experts and their more active presence in patients' beds, and give patients the right to choose.

Patients' satisfaction with high-level and non-similar welfare services and facilities was studied by Abbasi [15], Karimi [42], and Zol Havariyeh [43]. He noted the cold in some areas, the poor health of the health services, the deteriorating condition of some of the patients' boards, and the deterioration of the elevator. It should be noted that the use of patient feedback to increase the level of medical services and improve hospital hotels is very important to increase patient satisfaction [43]. Similar to this finding, patients' satisfaction with facility welfare services in a cross-sectional descriptive study on 131 hospitalized patients in 6 military hospitals across the country reported patient satisfaction with 98.2% of hospital services [23].

In this study, the level of satisfaction with observing religious issues was excellent. However, in the satisfaction studies, this case was not considered and one of the advantages of the questionnaire used in this study is to consider this item because according to Hassan Tehrani's qualitative study, it was found that privacy protection is one of the basic human needs and the most important concepts of nursing ethics. One of the dimensions of privacy is the patient's spiritual and religious privacy, which should be taken into account when caring for the patient, which was excellent in this study [44].

Another finding of this study was a significant relationship between education level and patient satisfaction so that with increasing level of education, the level of satisfaction decreased. Similar to this finding, in July and Farahani and Farzianpour's studies, the patient's level of education was an effective factor in the level of satisfaction [25, 29, 45]. Thus, people with higher education are less satisfied for reasons such as higher expectations of the treatment system, greater social communication, and greater access to information resources, and greater ability to identify system deficiencies and defects [45]. In Quintana and Findick's research, patients with higher levels of education were less satisfied with nursing care [46, 47]. Contrary to this finding, in the study of Mozaffari [48] Khezri [49], with increasing patient education, his satisfaction with nursing care services increased. In Abbasi's study, there was no relationship between education and satisfaction.

In terms of the reason for the visit, there was a significant relationship between satisfaction and the reason for the visit, so that having a contract with the hospital, the doctor's suggestion and the advice of acquaintances, as well as the hospital's welfare facilities, had a significant relationship with satisfaction. The results of the study in one of the military hospitals are the most important reasons for patients to choose the hospital in terms of the ability to use the appropriate insurance benefits of the hospital, the presence of experienced physicians, and the suggestion of others. Eventually, the center was easily accessible to patients [22].

Another finding of the recent study was the significant lack of relationship between the patient's gender and his satisfaction. This finding is consistent with the studies of Hajjian [17] and Rudbari [37] and can indicate that the quality of service is determined by satisfaction and not the sex of the patient [28]. Also in Johnson's [13] and Elnager's [51] research, this relationship was significant and male patients had a more positive experience and satisfaction with nursing care than female patients. Differences in satisfaction between men and women may be due to behavioral differences between men and women, or men may receive more information from nursing staff than women. The relationship between marriage and satisfaction was not significant in the present study, but the study of Goodarzian [52] and Akbari [22] showed that there is a significant relationship between marital status and satisfaction. In the present study, the relationship between insurance type and satisfaction was not significantly similar to this finding, Study of Farzanpour [45] and Moin [53]

Health insurance was reported as one of the factors affecting patient satisfaction. In this study, there was no relationship between the type of section and the level of satisfaction, but in Akbari's study, this relationship was significant [35].

The provision of the same services and facilities to the wards and the existence of optimal medical and nursing services have been effective in creating patient satisfaction regardless of the type of ward so that in hospitals that have a significant relationship between satisfaction and type of ward, the percentage of satisfaction with medical and nursing services is less.

Also, in the study of Akbari [35] and Salehian, the type of insurance showed a significant relationship with satisfaction. The reason for the difference in the results of studies is that in this medical center, no insurance or organ has a special priority and all patients benefit from the same amount of facilities and medical services. The association between hospitalization and patient satisfaction was not statistically significant; similar to the results of the present study, satisfaction increased in a study with increasing hospitalization [54].

In general, the reason for the difference in satisfaction results in recent studies and other studies can be considered the difference in the type of staff, hospital environment, and how to provide services in different centers, which greatly affects the quality of staff work and subsequent satisfaction. But, factors such as differences in the characteristics of the study population and, most importantly, the different methods of assessing satisfaction and overcrowding in metropolitan hospitals should also be considered. Also, people's cultural conditions, such as proper etiquette and communication, prevent the reflection of real opinions and their criticisms. In addition, dissatisfied patients usually do not cooperate in completing the questionnaires and do not want to participate in the study, which increases participation. Satisfied people are satisfied and as a result, the percentage of people's satisfaction increases falsely. Another important difference between patients' satisfaction in this study and other studies is that other hospitals may be educational due to the prevailing atmosphere in university hospitals, such as the centrality of education, research, and training of human resources, as well as the multiplicity of decisions and crowded sections. Various consequences have negative consequences, such as the lack of justice for patients in terms of access to medical facilities; while in the hospital under study, the focus has been on patient management by experienced staff, not students, and there is no overcrowding in university hospitals, and therefore patient satisfaction has been further ensured.

In the present study, a high percentage of patients announced that they would choose this hospital for their next visit and would also recommend this center to friends and acquaintances. This finding is consistent with the results of a genetic study that found that 85% of patients' satisfaction could lead to a recurrence of about 92% of them and the hospital's recommendation to others at 89% [28].

Strengths

One of the advantages of this research is that it provides a sufficient questionnaire to the patient and there is no need for the questioner to be present with the patient to check the level of satisfaction and also its compliance with the conditions, rules, and culture of the hospital. As much as possible, an attempt was made to use the patient's comments during the discharge and when the discharge was performed by the companion so that the patient could respond calmly. In this study, we have tried to consider all the factors affecting patient satisfaction in compiling the questionnaire and therefore the results of satisfaction with this questionnaire can identify key factors affecting the management of the hospital in improving hospital conditions to obtain more patient satisfaction.

Limitations

One of the limitations of the research is the use of only one military hospital to measure satisfaction and non-comparison with other centers, so the results of this study cannot be generalized to the entire health care system in Iran. Also, the reluctance of dissatisfied people with the services to complete the questionnaire may have created a measurement bias in the results. To eliminate this limitation, an attempt was made to provide complete explanations and the purpose of the research and to mention the importance of the dissatisfied patients' point of view to improve the performance of the hospital. As much as possible, participants should be encouraged to participate in the research to complete the questionnaire.

CONCLUSION

Given that patient satisfaction is of great importance as a measure of the quality of care, the results can provide a wealth of information about the success rate of programs in achieving the values and expectations of patients. It can also help with higher levels of management in planning, policy-making, and program evaluation. The results of the study showed that the level of client satisfaction with the hospital is excellent and the level of satisfaction in the treatment department is slightly higher than in non-therapeutic departments. In this regard, to improve the performance of the hospital and improve patient satisfaction, it is necessary to conduct an independent study to investigate the causes of dissatisfaction. Use more advanced statistical methods such as structural equations to determine the contribution of each of the measured areas to patient satisfaction. Finally, it can be stated that by using the results of this study and determining the prioritization of executive measures to provide better services, more patient satisfaction can be achieved.

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Conflict of interest

The authors state that there is no conflict of interest in the present study.

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ORIGINAL ARTICLES

Study on the chest radiographic results in patients with pulmonary tuberculosis referred to Zabol Tuberculosis Center: A descriptive study

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Abstract: *Introduction and Purpose: Tuberculosis is the most common cause of death from infectious diseases in the world. This study aimed to evaluate the findings of chest radiography in patients with pulmonary tuberculosis referred to the Zabol tuberculosis center.*

Materials and Methods: This descriptive-analytical study was performed on 101 patients with positive tuberculosis from January to December 2020. Data were collected using an information form and finally analyzed by SPSS software version 22.

Results: In this study, 101 patients were studied, of which 71 were female and 30 were male, and the mean age of patients was 62.68±13.61 years. The frequency of turbidity in patients with smear grades 1, 2, and 3 was 71.4%, 78.5%, and 76.5%, respectively. The frequency of cavitation in patients with grades 1, 2, and 3 was 11.5%, 28.5%, and 52.9%, respectively. The frequency of nodular view in patients with grades 1, 2, and 3 was 18.6, 42.8, and 35.3%, respectively.

Conclusion: In general, the results of this study showed that with increasing smear grading, the frequency of cavitation cases increases significantly and the frequency of reticulonodular view cases decreases significantly.

Keywords: *tuberculosis, chest radiography, smear grade*

INTRODUCTION

Tuberculosis is a bacterial disease caused by Mycobacterium tuberculosis and sometimes Mycobacterium bovis. About one-third of the world's population (2 billion people) is infected with the tuberculosis virus and is at risk of contracting tuberculosis, and each year about 9 million people become infected with active tuberculosis (1 to 2 million people) and die from the disease [1].

It is the leading cause of death from single-agent infectious diseases (even more so than AIDS, malaria, and measles) and ranks tenth in the global burden of disease, and is projected to maintain its current status by 2020 and even reaches to rank seventh [2]. The basis of the diagnosis of pulmonary tuberculosis is a direct and simple test of patients' sputum is suspected. In the best case, the sensitivity of the sputum test in the diagnosis of pulmonary tuberculosis is fifty to sixty percent [3, 4]. In pulmonary form, children often get sputum or gastric juice test results are usually negative even in culture, so the diagnosis is mainly based on clinical history, history of contact of a child with pulmonary tuberculosis with a positive sputum smear (especially in the family), chest radiography and tuberculin test [5]. According to standard definitions of a patient who has at least two positive sputum smear tests for fast acid bacilli. Or a patient who has only one sputum test positive for fast acid bacilli with radiographic changes in the chest confirming pulmonary tuberculosis. Or a case of positive smear in terms of acid-fast bacilli

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accompanied by sputum culture is considered positive smear tuberculosis [6]. The degree of positive smear is determined based on the number of bacilli in each microscopic field. Some studies have considered the degree of positivity of the primary smear to be effective in determining the outcome of treatment and have named it as a predictor that if the degree of positivity is more, the probability of treatment failure and death is higher [7, 8]. In some studies, the relationship between the degree of positivity of the primary smear and clinical manifestations has been suggested [9].

Chest radiography is also a suitable and sensitive tool for diagnosing lung lesions, including tuberculosis so that if the chest picture is normal, the diagnosis of tuberculosis is partially removed [10]. On the other hand, in cases where we are actively looking for this disease and when it is diagnosed in its early stages, pulmonary involvement can indicate our success in the early detection of these patients, which is achieved by radiographic findings [11]. No study is performed about chest radiographic results in patients with pulmonary tuberculosis in Iran and chest radiographic findings of patients, and in the world, few studies have been done in this regard, and due to the importance of this issue in early diagnosis and reduction of transmission of infection and treatment, this study was performed to determine the radiographic findings of the chest in patients with pulmonary tuberculosis.

METHODS

This descriptive-analytical study was performed on all patients with pulmonary tuberculosis referred to Zabol Tuberculosis Center from January to December 2020, except for patients with an immunodeficiency – hepatitis, and diabetes. In this study, 101 patients were studied, of which 71 were female and 30 were male. Samples were recorded according to a checklist that included demographic information, sputum smear grade, and radiographic findings of the chest. Demographic information of patients such as age, sex, and place of residence as well as smear information and its degree and chest radiographic findings were collected. Data were collected using SPSS software in the form of frequency, percentage, mean and standard deviation and analyzed the data using chi-square test.

RESULTS

Of the 101 participants, 71 (70.3%) were male and the rest were female. The mean age of patients was 62.68 years with a standard deviation of 13.61. The youngest and oldest patients were 18 and 86 years old, respectively. There was no significant difference between the two sexes in terms of grade smear (p= 0.192).

Table 1: Gender distribution of patients

Smear degree Gender	Grade 1	Grade 2	Grade 3	P value
men	5 29.4%	7 50%	18 25.7%	0.192
female	12 70.6٪	7 50%	52 73.3%	

The table below shows that the frequency of turbidity in patients with smear grades 1, 2, and 3 was 71.4%, 78.5%, and 76.5%, respectively. This difference in turbidity in patients with different grades was not statistically significant (p = 0.833).

Table 2: Frequency of turbidity cases by the degree of smear

		Grade 1	Grade 2	Grade 3	P value
Consolidation	Yes	13 76.5%	11 78.5%	50 71.4%	0.833
	No	4 23.5%	3 21.4%	20 28.5%	

The table below shows that the frequency of cavitation in patients with grades 1, 2, and 3 was 11.5%, 28.5%, and 52.9%, respectively. This difference in the frequency of cavities in the three groups was statistically significant (P = 0.001).

Table 3: Frequency of cavities by the degree of smear

		Grade 1	Grade 2	Grade 3	P value
Cavitation	Yes	9 52.9%	4 28.5%	8 11.5%	0.001
	No	8 47.05%	10 71.4%	62 88.5%	

The table below shows that the frequency of nodular view in patients with grades 1, 2, and 3 was 18.6%, 42.8%, and 35.3%, respectively. This difference in the frequency of nodular view in the three groups was not statistically significant (p = 0.086).

Table 4: Frequency of nodular cases by the degree of smear

		Grade 1	Grade 2	Grade 3	P value
Nodular	Yes	6 35.3%	6 42.8%	13 18.6%	0.086
	No	11 64.7%	8 57.1%	57 81.4%	

The table below shows that the frequency of reticulonodular involvement in patients with grades 1, 2, and 3 was 24.2%, 7.1%, and 0.0%, respectively. This difference in the frequency of reticulonodular involvement in the three groups was statistically significant (p = 0.022). On the other hand, patients with reticulonodular involvement were significantly more likely to have grade 1 smear. The lowest frequency of reticulonodular view belonged to the grade 3 smear.

Table 5: Frequency of reticulonodular cases by the degree of smear

		Grade 1	Grade 2	Grade 3	P value
Reticulonodular	Yes	0 0%	1 7.1%	17 24.2%	0.022
	No	17 100%	13 92.8%	53 75.7%	

DISCUSSION

In this study, 101 patients with tuberculosis were studied. Of these, 71 (70.3%) were female and the rest were male. In the studied patients, 70 cases had grade 1 (74.3% female and 25.7% male), 14 cases had grade 2 (male = female) and 17 cases had grade 3 (70.6% female and 35.7% male). As a result, there was no significant difference between the two sexes in terms of grade smear (p = 0.192). Due to the lack of similar studies that have examined the relationship between sexes and smear grading, the findings of this study cannot be compared. The mean age of patients was 62.68 years with a standard deviation of 13.61. The youngest and oldest patients were 18 and 86 years old, respectively. The mean age of patients with grades 1, 2, and 3 was 64.47, 62.07, and 55.82 years, respectively. The age difference between patients in different grades was not statistically significant (p = 0.122). There was also no relationship between increasing or decreasing age and smear rating. In other studies such as the study of Ores (2007) and Mohammad Blursaz et al. (2014) children under 15 years of age were in the study group and therefore due to differences in the study population, it is not possible to compare the results. Most of the patients were from Zabol city (52.5%) and the least of them were from Hamoon and Helmand cities (2%). The frequency of turbidity in patients with 1, 2, and 3 smear degrees was 71.4, 78.5, and 76.5%, respectively. This difference in the degree of turbidity in patients with different grades was not statistically significant. (P = 0.833) Although patients with grade 2 had more turbidity than patients with grade 1 and 3, but the difference between the three groups was not significant. The opacity was the same, so there does not appear to be a correlation between the degree of smear and the opacity in the graph. This finding is not consistent with other studies. In Minko's (2015) study, with increasing smear degree (+1, +2, +3, or +4), the frequency of turbidity cases increased significantly and its frequency increased to +1, +2, +3, and +4 degrees. It was 81%, 95% and 100%, respectively [12]. In the study of Ores (2007), with increasing the degree of smear, the number of turbidity cases also increased significantly [13]. Matsuka et al. (2004) also showed that the frequency of turbidity increased with an increasing number of fast acid bacilli [14]. The reason for the difference between the results of this study and other studies can be attributed to the fact that we have studied in this study Chest radiographs were based on smear grading, while other studies examined the relationship between CT scan and HRCT findings and smear grading. The frequency of cavitation in patients with grades 1, 2, and 3 was 11.5%, 28.5%, and 52.9%, respectively. This difference in the frequency of cavities in the three groups was statistically significant (P = 0.001). Patients with different grades were different in terms of cavitation, i.e. in patients with grade 3 cavitation was significantly higher than patients with grades 1 and 2, so it seems to be between smear degrees. And there is a connection hole. In Minko (2015) study, with increasing smear degree (+1, +2, +3, or +4), the frequency of CT scan findings including cavitation also increased significantly, so that the frequency of Cavitation cases in degrees +1, +2, +3, and +4 were 33%, 68%, 94%, and 100%, respectively [15]. In Ores (2007) study, cavitation increased significantly with increasing smear degree. Matsuka (2004) also showed that the frequency of cavitation increased with an increasing number of fast acid bacilli [16]. In the study of Mohammad Reza Bloursaz et al. (2014), cavitation had a significant relationship with smear rating [17]. This study showed that the frequency of nodular view in patients with grades 1, 2, and 3 was 18.6, 42.8, and 35.3%, respectively. The difference between the frequency of nodular view in the three groups was not statistically significant (P = 0.086). Although patients with grade 2 had more nodular view than patients with grade 1 and 3, the difference between the three groups of smears was not significant. There is no correlation between nodules and graphs. Matsuka (2004) also showed that the frequency of nodules increased with increasing smear degree but their difference was not statistically significant (P = 0.16) [18].

In the study of Ores (2007), with increasing the degree of smear, the score of nodules also increased significantly (P = 0.0001) [19]. The frequency of reticulonodular involvement in patients with grades 1, 2, and 3 were 24.2%, 7.1%, and 0%, respectively. This difference in the frequency of reticulonodular involvement in the three groups was statistically significant (p = 0.022). On the other hand, patients with reticulonodular involvement had significantly more grade 1 smear. And the lowest frequency of reticulonodular lesions belonged to grade 3 smears (0.0%). These results showed that between the degree of smear and involvement, there is a reticulonodular involvement in the connection graph so that with increasing smear rating (+1, +2, and +3) the frequency of reticulonodular view decreases.

Due to the lack of similar studies examining CXR findings based on smear rating, the findings cannot be compared with this study.

CONCLUSION

Despite the lack of relevance in the present study, the results of the present study can be used as a basis for other future studies.

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ORIGINAL ARTICLES

The outcome of Covid-19 in patients receiving biologics for the treatment of psoriasis

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Abstract: *Psoriasis is a chronic inflammatory disease, with multisystemic implications and genetic predisposition, characterized by the development of erythematous, scaling plaques on the skin. Management of moderate to severe psoriasis vulgaris may involve a biological treatment that suppresses the immune system. Because of this, the patients with psoriasis on an immunosuppressive treatment are theoretically at an increased risk of infections, including SARS-CoV-2 infection. SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is responsible for the current pandemic. In Romania, COVID-19 (Coronavirus Disease 19) was first reported on February 26, 2020, by confirming the first patient with SARS-CoV-2 by RT-PCR method. COVID-19 progression was divided into three phases: early infection, pulmonary phase, and hyperinflammatory phase. Although the inhibition of pro-inflammatory cytokines by biologic therapy is detrimental in the viral phase, it seems to be beneficial in the hyperinflammatory phase, by protecting the patients with psoriasis in progressing towards extra-pulmonary manifestations and death. Analyzing the evolution of the 8 cases of patients from our dermatology department – Colentina Clinical Hospital, with generalized psoriasis vulgaris on biologic immunosuppressive treatment and SARS-CoV-2 infection, we observed that they did not develop a more severe form of COVID-19 than the general population. Patients on biologic immunosuppressive treatment are more susceptible to the development of infections, including by SARS-CoV-2, but it was found that the biologic therapy can protect against a more severe type of COVID-19.*

Keywords: *psoriasis, biologics, SARS-CoV-2, COVID-19, hospitalization*

INTRODUCTION

Psoriasis is a chronic inflammatory disease, immune-mediated, with multisystemic implications and genetic predisposition, characterized by the development of erythematous, scaling plaques on the skin [1]. The psoriatic disease spectrum includes the psoriasis vulgaris, as well as psoriatic arthropathy and the concept of this unique disease was proved its validity over time [2]. The comorbidities associated with psoriasis are numerous and of multidisciplinary interest, wherefrom the most important being the cardiovascular effects, the psycho-emotional impact, and metabolic syndrome [1]. The presence of various comorbidities represents an important risk factor for an unfavorable evolution [1].

The patients with generalized psoriasis vulgaris are included into an increased risk of developing the infection with Coronavirus because of the pathogenic mechanism of the disease which incurs a dysfunction of the immune system with a multisystem effect, as well as the frequency of

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treatments administered to control the disease [3, 4].

The current therapy with biologic agents induces long-term remissions in patients with moderate or severe psoriasis vulgaris, who are not responding to the conventional systemic therapy [5]. The biologic therapies available in Romania for the treatment of moderate to severe psoriasis vulgaris are tumor necrosis factor (TNF) alpha inhibitors (Etanercept, Infliximab, Adalimumab, Certolizumab) and their biosimilars, IL-12/23 inhibitor (Ustekinumab), IL-17 inhibitors (Ixekizumab, Secukinumab) and IL-23 inhibitors (Risankizumab, Guselkumab) [6, 7].

The anti-TNF-α agents are antibodies obtained by genetic engineering that target the TNF-α, a cytokine with a central role in the pathogenesis of the psoriasis vulgaris, having a pro-inflammatory pleiotropic action, with multiple effects on a large number of cells [5, 8].

The anti-IL-12/23 agents links specifically on the p40 protein subunit of the human cytokines IL-12 and IL-23, preventing the p40 to connect to the protein receptor IL12Rβ1 expressed on the surface of the immune cells, thus inhibiting the bioactivity of IL-12 and IL-23, regulatory cytokines involved in the pathogenesis of psoriasis [5, 8, 9].

IL-17 inhibitors selectively aim and neutralize the pro-inflammatory cytokine IL17A, either within the homodimers 17AA or heterodimer 17AF, thus inhibiting the release of pro-inflammatory cytokines, chemokine, and mediators responsible for the tissue deterioration in psoriasis [5, 8].

The last release on the "biologic market" active in psoriasis are the IL-23 inhibitors, which are selectively blocking the interleukin-23 p19, (but not the IL-12), inhibiting the cellular signaling dependent of IL-23 and the release of pro-inflammatory cytokines, acting like a true "master-switch" in the etiopathogenic cascade of psoriasis [5, 8].

In the context of the SARS-CoV-2 pandemic, studies and clinical experience revealed that the hospitalization rate in patients with psoriasis vulgaris and COVID-19 was lower among those receiving biological therapy, concerning those receiving non-biologic systemic therapy or no systemic therapy, which was somewhat unexpected considering the immunosuppression potential of the biologic agents [10, 11, 12, 13].

Also, no significant difference was observed concerning the hospital admission risk between different biologic therapies [10].

On one hand, patients on an immunosuppressive treatment are more susceptible to developing infections, including by SARS-CoV-2, yet on the other hand, the same biologic therapy may protect against a more severe development in COVID-19 [4, 14].

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a positive-stranded RNA virus, similar to other coronaviruses, from the Coronaviridae family [15,16]. In Romania, COVID-19 (Coronavirus Disease 19) was first reported on February 26, 2020, by confirming the first patient with SARS-CoV-2 by RT-PCR method. Although most patients with COVID-19 are displaying more mild symptoms, approximately 15% are developing pneumonia and 5% are progressing towards systemic hyper-inflammation and acute respiratory distress syndrome, with an increased risk of septic shock, multiple organ failure, and death [10].

On patients with SARS-CoV-2, were identified three stages in the development of the disease [4]:

Stage I: recent infection or the stage of the viral response, wherein the viral ARN stimulates the cells of the host to express genes involved in the way of interferon, a critical part of the immune response. A reduced or delayed IFN type 1 response was associated with mild COVID-19 effects [4, 17].

Stage II: the pulmonary phase, clinically characterized by viral pneumonia with or without hypoxia [4].

Stage III: hyper-inflammatory phase, the so-called "cytokine storm" which incurs an exaggerated immune response to triggers, such as viral infections, observed in patients with severe SARS-CoV-2 infection. Clinically it is characterized by difficulty in managing the fever and multisystem involvement, including acute respiratory distress syndrome, heart failure, and renal failure. From the biological standpoint, it is found an increase in the pro-inflammatory cytokines also involved in psoriasis vulgaris, including TNF-α, IL1β, IL6, IL8, IFN-γ, and IL17 [4, 18, 19, 20].

Although the inhibition of pro-inflammatory cytokines is unfavorable in the viral phase, it seems to be beneficial in the hyperinflammatory phase, by protecting the patients with psoriasis in progressing towards extra-pulmonary manifestations and death [4, 19].

The angiotensin-converting enzyme 2 (ACE2) is considered the main receptor of SARS-CoV-2. Linking the viral spike protein to ACE2 facilitates the penetration of the virus into the cell, viral replication, and intercellular transmission [14]. The connection of the virus to the ACE2 determines a decrease of the angiotensin 1, by the increase of the angiotensin 2, responsible for the occurrence of the immune-mediated pulmonary inflammation and the injury of the pulmonary parenchyma [14]. Interestingly, the mediated inflammation IL-17 in patients with psoriasis was associated with the increase of the expression of the ACE2 receptor on the injured skin. The treatment with IL-17 inhibitors decreased the expression of the ACE2 to the levels observed in the non-injured skin and this can be a possible explanation for the favorable response of the psoriatic patients regarding COVID-19 [14, 19].

CLINICAL CASES

Moving forward we will present the development of 8 cases of patients from our dermatology department – Colentina Clinical Hospital, with generalized psoriasis, vulgaris, on biologic immunosuppressive treatment who contracted the SARS-CoV-2 infection.

Case 1: We are presenting the case of a 53-year-old female patient, known with arterial hypertension, type II diabetes, and generalized psoriasis vulgaris for 17 years, on biologic immunosuppressive treatment with etanercept since August 2018, who developed COVID-19 in August 2020 displaying as a clinical manifestation of the SARS-CoV-2 infection – hyposmia and hypogeusia for several days. The patient continued the biologic immunosuppressive treatment, and for COVID-19 she followed a treatment with acetaminophen, acetylsalicylic acid, vitamin D, vitamin C, and zinc, the evolution of the disease being favorable although the lesions incurred by psoriasis extended, with psoriasis area and severity index (PASI) 15.8.

Case 2: A 51-year-old male patient, without a significant pathological history, known with generalized psoriasis vulgaris since 2000, on biologic immunosuppressive treatment with Ixekizumab since October 2019, displayed an asymptomatic infection with SARS-CoV-2, but the administration of the treatment was postponed by two weeks (patient’s decision). The applied treatment consisted of vitamin D, vitamin C, zinc. The evolution of the disease was favorable, lasting approximately one week, and psoriasis did not worsen.

Case 3: A 41-year-old female patient, former smoker abstinent for approximately 14 years, known with arterial hypertension, bronchial asthma, treated pulmonary TB, generalized psoriasis vulgaris since the age of 7, experienced multiple biological treatments, currently on treatment with Etanercept. In February 2020 the patient was admitted to the hospital for pneumonia of unknown etiology, but no RT-PCR was performed for SARS-CoV-2. On August 12, 2020, the patient was referred to our Clinic for the occurrence of an eruption with erythematous-squamous plaques and placards disseminated at the level of the whole body, most lesions presenting pustules. With topic and antibiotic treatment, the lesions had a favorable evolution. During the admission increased IgG anti-SARS-CoV-2 antibodies were detected with a negative RT-PCR.

Case 4: A 72-year-old female patient, smoker, known since 2000 with generalized psoriasis vulgaris, on immunosuppressive biologic treatment with Etanercept since September 2017, following a SARS-CoV-2 infection, developed a severe broncho-pneumonia with acute respiratory failure which required admission into the hospital where she followed a treatment with anticoagulant, broad-spectrum antibiotic, systemic corticosteroids, antifungals, bronchodilators, proton pump inhibitors, non-steroidal anti-inflammatory drugs and oxygen therapy. The patient associates numerous comorbidities, namely essential arterial hypertension, ischemic stroke, chronic obstructive pulmonary disease, obesity. The evolution was favorable with the mitigation of the suffering, biologic and radiologic relief, but with residual pericarditis. We are mentioning that the patient interrupted the biological treatment during the illness, but psoriasis did not worsen [21].

Case 5: A 25-year-old male patient, obese, known with generalized psoriasis vulgaris since November 2016, on immunosuppressive treatment with Ixekizumab since January 2018, presented fever, cough, ageusia, and anosmia following the infection with the new coronavirus, wherefore the administration of the treatment was postponed for a week. The duration of the symptoms was two weeks and the followed treatment was symptomatic with anti-pyretic, cough suppressing medicine, vitamin C, vitamin D, and zinc. The PASI score before the SARS-CoV-2 was 0, and 2 months upon healing was 0.8.

Case 6: A 63-year-old female patient, without a significant pathological history, known with generalized psoriasis vulgaris since 2010, on immunosuppressive treatment with Ixekizumab since March 2018, presented cough, headache, ageusia, and anosmia following a SARS-CoV-2 infection. In this case, the biological treatment was also interrupted during the illness, and the evolution of psoriasis was favorable. The patient was treated with non-steroidal anti-inflammatory drugs, cough suppressant medicine, and vitamins, and the duration of the symptoms was approximately two weeks.

Case 7: We are presenting the case of a 67-year-old male patient, with no significant family or personal history of diseases, known with generalized psoriasis vulgaris since 2018, on biologic immunosuppressive treatment with Ixekizumab since March 2020. He presented an asymptomatic infection with SARS-CoV-2 wherefore he followed only a multivitamins-based treatment. The biologic therapy was not interrupted and the evolution of the psoriasis was favorable with PASI and DLQI 0.

Case 8: We are presenting the case of a 38-year-old female patient, no significant family or personal history of diseases, known with generalized psoriasis vulgaris since 2018, on biologic immunosuppressive treatment with Secukinumab since September 2019 with favorable evolution, the PASI and DLQI being 0, who developed a SARS-CoV-2 infection in December 2020. In terms of symptoms, she had a fever, chills, dry cough, myalgia, articular pain, fatigue, ageusia, and anosmia, with evolution of approximately two weeks. The biologic therapy was not interrupted and for the infection with SARS-CoV-2, the patient followed a treatment with azithromycin for 3 days, paracetamol, and non-steroidal anti-inflammatory drugs when needed and vitamins. The evolution was favorable, with full remission, no after effects, and the underlying disease did not worsen.

DISCUSSIONS

Although patients with generalized psoriasis vulgaris on biologic immunosuppressive treatment fall within the high-risk category of the coronavirus infection, no hospital admission or higher death rates were observed.

Patients on biologic immunosuppressive treatment are more susceptible to the development of infections, including by SARS-CoV-2, but it was found that the biologic therapy can protect against a more severe type of COVID-19, with extra-pulmonary manifestations and increased death risk. The explanation may consist in the fact that the immunomodulation was also the target for other biologic like remdesivir or tocilizumab.

The presence of some risk factors and comorbidities of generalized psoriasis vulgaris could additionally increase the risk of immunosuppression and unfavorable development of COVID-19.

The most frequent symptoms, associated with the SARS-CoV-2 infection were ageusia, anosmia, dry cough, and fever (as well reported on free from psoriasis and biologic treatment population).

Among the reported patients, seven recovered completely upon COVID-19, only one patient with multiple associated comorbidities who required hospital admission, developed residual pericarditis.

Out of the 8 presented patients, 4 patients interrupted the administration of the treatment during the illness, whilst 4 continued the treatment.

The evolution of the patients who continued the administration of the biologic therapy was no more severe than that of the patients who interrupted the treatment.

According to the PsoProtect registry, upon the editing time of this article, there were 952 reported cases of COVID-19 patients with psoriasis vulgaris and 11 deaths [22, 23].

The average duration of the symptomatology was 12 days [23].

Hospital admission due to the SARS-CoV-2 infection:
- 84% did not require hospital admission;
- 16% required hospital admission;
- <1% unknown.

In terms of biological therapy, 46% interrupted the treatment during the infection, 51% continued with the treatment and in 3% of the cases, this detail is unknown [23].

CONCLUSION

Upon analyzing the 8 cases, we observed that our patients with generalized psoriasis vulgaris on biologic immunosuppressive treatment and SARS-CoV-2 infection did not develop a more severe form of COVID-19 than the general population. One patient out of eight required hospital admission, presenting a more severe form of the SARS-CoV-2 infection and multiple associated comorbidities. A possible explanation for the favorable evolution in patients with psoriasis and immunosuppressive treatment, consists in the inhibition of the pro-inflammatory cytokines by the biologic immunosuppressive treatment, protecting such patients to develop extra pulmonary manifestations and death. The increase in the pro-inflammatory cytokines is recorded in the pathogenesis of psoriasis vulgaris, as well as in the hyperinflammatory phase of the SARS-CoV-2 infection, so their inhibition is beneficial in both cases.

Moreover, another explanation would be the decrease in the expression of the ACE2, the main receptor of the SARS-CoV-2, by the immunosuppressive treatment with IL-17 inhibitors.

Author Contributions

RO, TC contributed to all the stages of the article, they designed the article and revised the manuscript for important intellectual content. AMC, MMC, and SB contributed to the conception of the work, revision of the language, and contributed to drafting the work. RO, AMC, TC, DOC, and SB revised the work critically. The final manuscript was approved by all authors.

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ORIGINAL ARTICLES

Military psychological distress-virtues model

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Abstract: *Background and Aim: Military personnel, parallel to appropriate personality traits to the military environment, need virtues and character strengths(CS) that contain values appropriate to the environment. We designed a model that examines the impact of comprehensive virtues and CS in reducing psychological distress.*

Methods: The present study is an SEM study. The sample community is an available example of 410 persons of military men in Tehran. We used a demographic information questionnaire, Kessler Psychological Distress Scale (K-10), and Virtues Inventory in Action (VIA-120).

Results: findings showed virtues and CS of wisdom, justice, humanity, and transcendence (except for spirituality) are significantly related to psychological distress. With increasing CS, psychological distress in employees decreased, but with increasing virtues, psychological distress in employees increased. The virtues and CS of temperance and courage were not significantly associated with psychological distress.

Conclusions: According to the results of this study, military services should pay more attention to the virtues and CS effective in reducing staff distress and strengthening these traits in them.

Keywords: *Military personnel, Psychological distress, Virtues, Character Strengths*

INTRODUCTION

Psychological distress is a mental health outcome embodied by psycho-physiological and behavioral symptoms that are not specific to a given mental pathology. It includes symptoms of anxiety, depressive reactions, irritability, declining intellectual capacity, tiredness, sleepiness, and may lead to premature morbidity and mortality and Low-efficiency organizations [1]. A review of texts related to the psychological distress in military personnel showed that studies conducted in this area are more focused on personality traits and behavior. Such as hardiness and adjustment [2], sleep, resilience [3], perceived support, substance use, suicidal ideation [4], self-compassion and morale injury [5], negative life events [6], and like that. In military psychology, analytical and practical intelligence, motivational factors, and personality (for example that low conscientiousness and high extraversion in the big 5-personality model) are considered a predictor of the outcomes of performance in military colleges. Personality is about differences between individuals and how one reacts to circumstances, but these characters do not address values and have limitations in predicting successful military personnel [7]. So, other variables have been incorporated into the network of predictive variables because of theoretical advances in military psychology and the evolution of the institutional function of the military [8]. One of that is positive military psychology with the assumption that the military being a positive institution consists of

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pathology-free mentally and physically fit soldiers who share positive traits, positive emotions, and positive relations. Therefore, it is imperative to identify, nurture and optimize all those positive aspects of a soldier which can strengthen him for better operational effectiveness, adjustment, and optimal functioning [9]. According to positive military psychology, Virtues and strengths can be more suitable predictors for successful military personnel and institution [7]. Military doctrine has affirmed that character and values are critical for successful military environments [10]. An individual expresses his or her values through one's CS. This has been found to play an important role in leadership, adaptability, and achievement [11]. Therefore, CS is an important factor for being resilient applicants to high-risk organizations [12].

A soldier must not only have the courage and aggressiveness for combat operations but also cultivate the empathy and compassion necessary to treat noncombatants and enemy prisoners humanely [13]. In addition, virtue ethics as being the best way of enhancing the chances of soldiers behaving morally because the rules or codes of conduct imposed from above, lack flexibility, and leave less room for personal integrity. changes in the military's environment have led to a shift from traditional tasks to more complex tasks, and especially in today's missions, one could expect that the proper virtues are not solely the martial ones [14].

Gordon Alport's theory argued that character belonged to philosophy and not psychology, and denied any connection between the character's strengths and the personality concept. Later, Peterson and Seligman´s research on positive psychology from 2004 sparked a renewed interest in the concepts of character and CS (15). Peterson and Seligman formulated a taxonomy of 24 "CS within six different virtues" based on Confucian, Taoist, Buddhist, Hindu, Greek, Judeo-Christian, and Muslim writings concerning virtue [16].

Wisdom and knowledge – cognitive CS that entail the acquisition and use of knowledge (creativity, curiosity, open-mindedness, love of learning, perspective), Courage - emotional CS that involve the exercise of will to accomplish goals in the face of opposition, external or internal (authenticity, bravery, persistence, zest), humanity – interpersonal CS that involve tending and befriending others (kindness, love, and social intelligence), justice – civic CS that underlie healthy community life (fairness, leadership, citizenship), temperance-CS that protect against excess (forgiveness, modesty, prudence, self-regulation) and Transcendence – CS that forge connections to the larger universe and provide meaning (appreciation of beauty, gratitude, hope, humor, spirituality) [17].

CS are personality traits that are positively valued, and represent different routes to the superordinate virtues [18]. The relationship between virtues and PTG in different countries demonstrated these three virtues (Interpersonal, Vitality, and Cautiousness) were positively related to life with satisfaction and flourishing, as well as negatively related to depression, anxiety, psychological distress, and pathology Internet use [19].

A review of the literature on virtues and CS showed that many studies have been done on the relationship between psychological well-being and virtues. We found no study to examine the role of a complete list of virtues and character strength in causing psychological distress. More subjective/ psychological well-being can be achieved by applying CS [20].

Studies explored a significant positive relationship between the three virtues (Interpersonal, Vitality, and Cautiousness) and life satisfaction [21] and psychological well-being and enhanced mental health among diverse populations in western and eastern cultures. In a study, CS use is a unique predictor of subjective well-being, but not the health-related quality of life. The VIA strengths of hope and zest were significant positive predictors of life satisfaction [22].

A Structural equation modeling study that specifies the predictive effects of some CS (Seven CS of critical thinking, emotional intelligence, gratitude, forgiveness, hope, spirituality, and zest) in a depression on individuals with major depression, demonstrated an indirect effect on depression through the mediation of dysfunctional attitudes, negative affect, and happiness. CS had negative effects on dysfunctional attitudes and positive effects on happiness [23].

According to Leontopoulou & Triliva (2012), Wisdom, courage, and transcendence were each highly and positively correlated with the wellbeing subscales of environmental mastery, purpose in life, and self-acceptance. Good physical and mental health were positively predicted different aspects of wellbeing and CS; nevertheless, mental health was a stronger predictor of wellbeing and CS than physical health.

Positive psychology interventions that involve the enhancement of strengths are found to increase well-being and decrease depression effectively, as suggested by a meta-analysis of 51 studies (Sin and Lyubomirsky, 2009). Indeed, empirical findings suggest that having a good character through the use of strengths is inversely related to depression, suicidal ideation, and substance abuse, whilst the use of strengths can have a buffering effect against stress, reduce the disability caused by mental disorders and contribute to their prevention. Wright and Lopez (2005) proposed that strengths constitute important resources for mental health improvement and as such their enhancement should be incorporated into the health and well-being interventions [24].

Based on an international sample of 238 adults, general CS underuse and overuse were related to negative outcomes, while optimal use was related to positive outcomes. The overuse of social intelligence and modesty, and underuse of zest, humor, self-regulation, and social intelligence were associated with social anxiety. Using discriminant analysis, this combination successfully resorted 87.3% of the participants into those that do and do not have clinical levels of social anxiety. These findings suggest that strengths are multifaceted, providing novel insight into the role that sub-optimal-use facets play in undesirable outcomes, providing a glimpse of psychopathology through the lens of positive psychology [25].

Exploring psychological distress through the lens of virtues and CS allows for understanding individual differences and kinds of virtues and characteristics that help decrease psychological distress in people. As mentioned, we did not find a comprehensive study that examines the wide range of negative emotions (distress) based on all the virtues and strengths. Studies have focused more on the role of some of the strengths and ignored the role of the six virtues of Seligman and Patterson. The concept of psychological distress without any diagnostic label can help identify and assess people at risk for psychological problems. Therefore, the authors decided to examine the role of each of the virtues and characteristics in reducing psychological distress. The purpose of this study is to investigate the model of psychological distress based on virtues and CS.

METHOD

The present study is a cross-sectional study. The sample community is an example of all the military men in Tehran. Sample in the present study, the military organizations of Tehran have been selected by available sampling. The sample size in this study is 384 people, which has been done by available sampling based on the Cochran sample size formula:

$$n=\frac{Z^2\,pq}{d^2}$$

In this formula: z= 1.96; p = q = 0.5; and d (error value) = 0.05. To compensate for possible errors and increase the test power, the sample size was increased by 410 units [26].

Demographic information questionnaire: This questionnaire will be designed by the researcher and includes information such as age, education, work experience, marital status.

Kessler Psychological Distress Scale (K-10): The Kessler Psychological Distress Scale (2002), identifies mental disorders in the general population. Questions on this scale are scored as a Likert scale from always (0) to never (4). This scale doesn’t target a specific psychological disorder, but generally, identify the level of anxiety and depressive symptoms that a person has experienced over the past few weeks. Various studies have shown that the K-10 questionnaire has good validity and reliability. Studies on K-10 show a strong correlation between high scores on the Kessler Psychological Distress Questionnaire and the diagnosis of mood and anxiety disorders by the International Composite Diagnostic Interview Form (CIDI). There is also a less but significant relationship between questionnaire scores and other psychiatric disorders [27].

Virtues Inventory in Action (VIA-120): Seligman and Patterson prepared a short form of 120 that includes 6 virtues of wisdom, courage, humanity and love, justice, temperance, and transcendence. Each of the moral virtues contains several CS that is measured by 5 questions, and are complemented on a Likert scale from very similar to me (5) to opposite to me (1), and dimensionally each point Show the strength of character. The 120-item scale is fully consistent with the original English form VIA-240 in terms of reliability, validity, and factor structure [28]. The reliability and validity of this scale were assessed and confirmed by Namdari [29].

RESEARCH PROCESS

To implement the questionnaires, first, the necessary permits were obtained from the relevant centers and then the command of each unit was coordinated. Questionnaires were distributed among the staff and the necessary explanations were given on how to answer and confidentiality of the answers. After completing the answer, questionnaires were collected.

Result

Descriptive findings of demographic variables showed the mean and standard deviation of the age and work experience (years of work in the organization) of the participants, respectively are 36.72± 7.02 and 17.35±6.19. In addition, 20% were married and 30% were single. 32% had one child, 20% had two children and the rest had more than two children. Almost 14% had a diploma, 56% had a bachelor's degree, 20 % had a master's degree, and 10% had a doctorate. 60% worked in the morning shift, 30% in variable work shifts, and 10% in the evening shift.

Measurement Model

In measurement model a): Internal consistency (Cronbach's alpha and Composite reliability), convergent validity (Indicator reliability, Average Variance Extracted) (in Table 1), and discriminant validity were suitable. According to Table 1, all values CR and AVE are in the appropriate ranges. Discriminant validity showed that all variables had appropriate HTMT. Also, according to the Fornell-Larcker scale, each variable had more variance in common with its variable than the other. In Figure 1 the model of psychological distress of military personnel based on the virtues and CS is shown.

Table 1: Cronbach's alpha, Composite reliability and Average Variance Extracted for virtues and CS

Virtues (second order variables)	CS (First order variables)	Cronbach's alpha	CR	AVE
Wisdom	----		0.893	0.736
Wisdom	Creativity	0.618	0.796	0.566
	Curiosity	0.706	0.819	0.532
	Open-mindedness	0.700	0.816	0.529
	Love of learning	0.762	0.841	0.516
	Perspective	0.618	0.796	0.566
Courage	----		0.893	0.672
	Orginity	0.731	0.832	0.553
	Persistence	0.786	0.854	0.541
	Bravery	0.579	0.778	0.541
	Vitality	0.764	0.841	0.515
Humanity	----		0.900	0.695
	Kindness	0.719	0.826	0.543
	Love	0.629	0.777	0.563
	Social intelligence	0.728	0.831	0.55
Justice	----		0.901	0.770
	Fairness	0.615	0.795	0.564
	Leadership	0.515	0.755	0.507
	Teamwork	0.705	0.819	0.531
Temperance	----		0.893	0.736
	Forgiveness	0.742	0.836	0.561
	Modesty	0.534	0.763	0.517
	Prudence	0.716	0.824	0.54
	Self-regulation	0.401	0.765	0.621
Transcendence	----		0.955	0.782
	Appreciation of beauty	0.668	0.818	0.601
	Gratitude	0.753	0.835	
	Hope	0.769	0.839	0.511
	Humor	0.755	0.835	0.506
	Spirituality	0.821	0.873	0.579

Table 2 shows path coefficients of virtues and CS with the psychological distress of military personnel. According to this table, virtues, and CS of wisdom, justice, humanity, and transcendence (except for spirituality) are significantly related to psychological distress. With increasing CS, psychological distress in employees decreased, but with increasing virtues, psychological distress in employees increased. The virtues and CS of temperance and courage were not significantly associated with psychological distress.

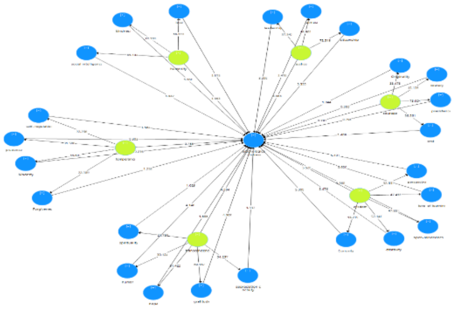

Figure1: Military psychological distress-virtues model

Table 2: The path coefficients of virtues and CS with the psychological distress

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	T Statistics (|O/STDEV|)	P Values
originality -> psychological distress	-0.287	-0.389	0.275	1.044	0.297
curiosity -> psychological distress	-5.938	-0.579	1.100	5.399	0.000
forgiveness -> psychological distress	-0.160	-0.136	0.127	1.258	0.209
modesty -> psychological distress	-0.028	0.014	0.128	0.216	0.829
appreciation of beauty -> psychological distress	-3.357	-0.812	0.774	4.337	0.000
bravery -> psychological distress	-0.091	-0.158	0.232	0.393	0.694
citizenship -> psychological distress	-5.834	-0.789	1.750	3.333	0.001
courage -> psychological distress	0.774	1.072	0.970	0.797	0.426
creativity -> psychological distress	-6.579	-0.796	1.201	5.476	0.000
fairness -> psychological distress	-4.621	-0.742	1.352	3.418	0.001
gratitude -> psychological distress	-5.333	-1.328	1.221	4.367	0.000
hope -> psychological distress	-5.516	-1.622	1.200	4.595	0.000
humanity -> psychological distress	10.598	2.516	3.123	3.393	0.001
humor -> psychological distress	-4.111	-0.885	0.991	4.148	0.000
justice -> psychological distress	12.501	1.852	3.717	3.363	0.001
kindness -> psychological distress	-3.981	-0.826	1.219	3.266	0.001
leadership -> psychological distress	-3.935	-0.527	1.193	3.299	0.001
love -> psychological distress	-3.887	-1.081	1.075	3.615	0.000
love of learning -> psychological distress	-7.009	-0.987	1.247	5.620	0.000
open-mindedness -> psychological distress	-6.131	-0.775	1.101	5.568	0.000
perspective -> psychological distress	-4.680	-0.527	0.866	5.401	0.000
persistence -> psychological distress	-0.268	-0.359	0.337	0.796	0.426
prudence -> psychological distress	-0.064	-0.011	0.163	0.393	0.695
self- regulation -> psychological distress	-0.170	-0.181	0.109	1.562	0.119
social intelligence -> psychological distress	-4.270	-0.979	1.276	3.347	0.001
spirituality -> psychological distress	-0.073	-0.069	0.071	1.029	0.304
temperance -> psychological distress	0.274	0.146	0.363	0.755	0.451
transcendence -> psychological distress	14.920	3.529	3.483	4.284	0.000
wisdom -> psychological distress	25.036	3.165	4.535	5.520	0.000
zest -> psychological distress	-0.421	-0.531	0.283	1.489	0.137

The most common measure used to evaluate the structural model is value R2 and Stone-Geisser's Q². The value of R2 equal to 0.75, 0.50, or 0.25 for the endogenous latent variables can be described as a general rule as strong, moderate, and weak, respectively. For Stone-Geisser's Q² if the value of Q2 for an endogenous structure achieves three values of 0.02, 0.15, and 0.35, it indicates the weak, medium, and strong predictive power of the structure with its related exogenous structures [30]. Table 3 shows these values for virtues and CS.

Table 3: Values of R² and Q² for virtues and CS

Virtues (second-order variables)	CS (First order variables)	R Square	R Square Adjusted	Q²
Wisdom	----	0.78	0.78	0.27
	Creativity	0.74	0.74	0.34
	Curiosity	0.67	0.67	0.39
	Open-mindedness	0.67	0.67	0.33
	Love of learning	0.66	0.66	0.31
	Perspective	0.59	0.59	0.32
Courage	----	0.84	0.84	0.33
	Originality	0.65	0.65	0.38
	Persistence	0.77	0.77	0.35
	Bravery	0.68	0.68	0.36
	Zest	0.67	0.67	0.33
Humanity	----	0.79	0.79	0.31
	Kindness	0.78	0.78	0.39
	Love	0.73	0.73	0.35
	Social intelligence	0.77	0.77	0.39
Justice	----	0.76	0.76	0.26
	Fairness	0.72	0.72	0.39
	Leadership	0.64	0.64	0.31
	Citizenship	0.83	0.83	0.41
Temperance	----	0.74	0.74	0.23
	Forgiveness	0.62	0.62	0.27
	Modesty	0.63	0.63	0.31
	Prudence	0.58	0.58	0.30
	Self-regulation	0.48	0.48	0.28
Transcendence	----	0.85	0.85	0.29
	Appreciation of beauty	0.59	0.59	0.34
	Gratitude	0.77	0.77	0.36
	Hope	0.76	0.76	0.36
	Humor	0.58	0.58	0.26
	Spirituality	0.47	0.47	0.24

DISCUSSION

Findings of this study showed, the CS of wisdom, including, creativity, curiosity, love of learning, open-mindedness, perspective, played a mediating role in reducing psychological distress. The research literature is consistent with our findings. Creativity led to looking at problems from different perspectives, establishing new solutions and connections, flexibility in the thought process, increasing their connection to the unknown [31], brainstorming phenomenon, and reducing workplace stresses [32]. Studies show that curiosity and open-mindedness with the tendency to tolerate potentially different opinions [33], new knowledge and information, the choice of diverse and different perspectives, the ability to use a balanced and objective mentality when participating in problem-solving [32]. Open-minded people think logically because they can accept all possible logical perspectives and come up with a better solution [34]. Curiosity with openness to the experience is associated with self-regulation, cognitive development, school and university learning, job performance, interpersonal communication, personal growth, and well-being [35].

In this study, justice's CS, including citizenship, fairness, and leadership had a significant role in reducing distress. "good citizens" have higher levels of social trust and a more positive view of human nature, score low on alienation and ethnicity [36], organizational citizenship behavior is likely to be formally associated with organizational rewards and helps the organization promote a positive social and psychological climate. Beyond their duties, employees find it more attractive to engage and inform about the organization, to talk and encourage others, to volunteer to take on additional responsibilities in the workplace, with the help and guidance of co-workers [37]. Also, supervisor-nurse relationships, teamwork, and wellbeing explain almost half of the nurses' commitment to the hospital, as well as half of the intention to leave the hospital [38]. About fairness, people with higher fairness in career advancement show more parental role, respect, and commitment to others, care, and understanding in relationships [36]. Employees' perceptions of fairness lead to more positive variables such as job satisfaction, organizational commitment, productivity, and organizational citizenship behavior, work results, or attitudes and work behaviors [39]. Also, according to Seligman and Peterson (2004) people with leadership power are more likely to be promoted in their jobs and feel more well-being [36].

In addition, findings of this study indicated a significant role of transcendence CS (except spirituality) including appreciation of beauty, hope, humor, and gratitude with psychological distress. The use of these CS led to reducing psychological distress. This finding is consistent with the research literature. Research has shown that appreciation of beauty is positively correlated with openness to experience, extraversion, and agreement [40], life satisfaction, positive impact, and post-traumatic growth [41].

Depression was negative and gratitude was a positive predictor of psychological well-being [42], and negatively related to several negative emotional [43]. Also, expressed higher mood gratitude with fewer depressive symptoms, and PTSD. About humor, higher levels of humor were associated with fewer depressive symptoms [44] low anxiety and depression, and anti-production behaviors in the workplace, and increased organizational citizenship behaviors, moderating role between organizational stressors and organizational citizenship behaviors. The use of humor leads to mental flexibility and intellectual stimulation and reduces the threat by restoring the situation against the destructive effects of stressors [45]. Our findings showed spirituality had not been significantly associated with psychological distress. Spirituality is positively associated with happiness and meaningful living. Many studies have shown that spirituality and religiosity are more closely linked to greater physical and mental health. For example, a meta-analysis concluded that more religiosity was associated with fewer depressive symptoms, with no evidence that age, gender, or ethnicity influenced the relationship. According to Einsliecht et al., belief in religion modulates anxiety [8].

Also, our study showed there is a positive and significant relationship between CS of love and humanity including kindness, love, and social intelligence, and psychological distress. This result is consistent with the literature. In the study of the effect of parental attachment and the language of love on resilience, it was found that secure attachment and receiving love from parents lead to more resilience. Parents' loving language, affirmative words, and supportive actions significantly build resilience by creating emotional, motivational, and practical resources [46]. The results of a study showed that spiritual care with the faith-hope-love model has had positive results for the patient [47]. Also confirmed that there is a significant relationship between health and love and faith. Multidisciplinary research evidence argues that different religions have one thing in common, at least in terms of one main message: that life means loving one another. The definition of love, in its essence, is an expression of generosity and altruism. Likewise, love can influence not only positive psychology and existentialism but also the fanatical and fundamentalist views and practices that still exist in the world today [48]. Regarding social intelligence, the results of a study showed that social intelligence can protect young people from the psychological damage of being victimized by peers [49]. Findings [50] showed that there is a significant relationship between social intelligence and depression. Also, a significant relationship was found between social skills and social awareness, but no statistically significant relationship was observed between social information processing and the level of depression. Also, important relationships between social desirability and social intelligence in smoking status have been found [51].

Other CS in the virtues of courage (courage, originality, perseverance, and vitality) and temperance (forgiveness, modesty, prudence, and self-regulation) were not significantly associated with psychological distress. The results of the most relevant research showed that each of these positive characteristics can affect psychological well-being and positive emotions and results. Research in these studies has shown, for example, that high self-control leads to thought control, emotion regulation, psychological well-being, greater academic achievement, better interpersonal relationships, and ultimately greater adaptation and low self-control, in contrast to many social problems, obesity, and Drug use, crime, shopping, and procrastination [52]. The constructive relationship between forgiveness and health through direct mechanisms may be accomplished by controlling the cognitive process of rumination, and the resulting negative emotions, such as anger or resentment or indirect effect, may be mediated through distinct variables such as health behavior, performance between Individuals develop social support and mental health and are associated with low suicidal behavior and depressive symptoms [53]. We reason that the virtues of temperance and courage and the CS of them, can logically and intuitively be incorporated into the nature of the military job and be considered as an inseparable feature of this job. Therefore, increasing it cannot affect reducing psychological distress.

In the present study, all six virtues (significant including wisdom, justice, and transcendence) and non-significant (humanity, courage, and temperance) were positively associated with psychological distress. That is, with the increase of these virtues, the level of psychological distress of the employees increased. The positive association of virtues with psychological distress is a strange consequence, given that according to Seligman and Peterson, virtues lead to well-being and ultimately self-fulfillment [36]. Based on our hypotheses, the relationship between virtues and psychological distress was expected to be negative. According to these findings, while the results of the first-order variables of CS were negative with psychological distress, but the increase in the virtues of wisdom, humanity, justice, and excellence in the sample led to more distress and suffering. According to Rosch et al. (1976), CS has a "natural classification" that manifests itself in practice conveniently and clearly. Experimental studies support this idea and show that cognitive processes also have a more specific efficiency when they have a primary and natural surface content. While CS at the abstract levels is called virtues and their performance is more complex [36]. According to early texts in this field, if we consider distress or suffering as opposed to happiness, any person who has a virtuous life will be happy. But according to Bloomfield, virtue does not guarantee happiness, and the ethics of virtue involves worries about oneself and others [54]. Suffering is included in the theoretical framework of virtue and is an inseparable part of virtue. Virtues have the appropriate structure to maintain different types of values. The connection between suffering and virtue already exists in the history of philosophy and theology, albeit sporadically. Suffering plays a key role in the proper functioning of groups or groups and their wisdom. In addition, suffering is very important in a group or social virtues such as justice and love and individual virtues such as faith [28].

Limitation

Our sample in this study included only men. These people mostly had administrative work and were somewhat away from the hardships of military life, such as being sent to war zones. Of course, they experience some limitations and difficulties of working in military services.

CONCLUSION

According to the results of this study, military organizations should pay more attention to the ethical virtues effective in reducing staff distress and strengthening these traits in individuals.

Ethical considerations

This article is a part of a doctoral dissertation approved with IR.BMSU.REC.1399.341 Ethics Code. The online version of the application is available at the following address and is open to the public: https://ethics.research.ac.ir/IR.BMSU.REC.1399.341. Target individuals were informed about the purpose of the study and their right to withdraw from the study at any point. Participants were free to withdraw from the study at any point. All interviews were kept confidential.

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ORIGINAL ARTICLES

The medical and social importance of mental and behavior disorders among military personnel in peacetime and warfare

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Abstract: *Introduction. The considerable numbers of servicemen who have applied for psychiatric help and who have been discharged for mental health from the Armed Forces of Ukraine testify the need to revise the whole system of providing psychiatric, psychomedical, and social aid to servicemen in warfare.*

Purpose. To identify medical and social aspects of mental and behavioral disorders among servicemen of the Armed Forces of Ukraine in peacetime and warfare for justification the developing and implementing the system of psychomedical care in Ukrainian Defense Forces with NATO standards adaptation.

Methods. A retrospective analysis of statistical data on the level, structure, dynamics of the overall incidence rate of mental disorders as well as hospitalized morbidity among the military personnel of Ukrainian Armed Forces and health status dismissal based on long-term observation data (2008-2017) as well as comparison of indicators of the period 2008-2013 and 2014-2017 based on official statistical reporting has been performed. The statistical processing of the sample results was carried out using descriptive statistics and analysis of alternative characteristics.

Results. It was discovered that in the structure of the total incidence of mental disorders among the military personnel of Ukrainian Armed Forces on class F (ICD-10) for the period of 2014-2017 years there was a significant redistribution of all categories of military personnel in comparison with 2008-2013 (among officers as well as contract servicemen, the overall incidence rate of mental disorders decreased dependably). The proportion of disorders of adult personality and behavior with other syndromes (F50-F99) significantly increased due to a significant decrease of neurotic, stress-related, and somatoform complaints cases.

Conclusions. The overall incidence rate of mental disorders, as well as hospitalized morbidity within the structure of overall incidence rate of diseases in 2008-2017 for officers and contract servicemen, significantly increased with peak values in 2015 ($p \leq 0.001$) with further decreasing in 2016-2017 years, but among conscripts - remained unchanged. It has been established that starting from 2014, the indicators of discharges among officers have tended to increase with the maximum rates in 2015 (compared to 2013 by 7 times higher) and contract servicemen in 2016 (compared to 2013 by 5.3 times higher) with a further decrease. The identified features of mental health disorder in servicemen and their consequences according to long-term observation (both in peacetime and in warfare) are the keys to the development and implementation of the proposed psychomedical care system in defense forces readapted to NATO standards.

Keywords: *military personnel of the Armed Forces of Ukraine, overall incidence rate, hospitalized morbidity, dismissal due to health state, mental and behavior disorders*

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INTRODUCTION

In the last decade in Ukraine, as in many countries around the world, under the influence of economic, social, psychological, and environmental factors, there has been increasing the incidence of mental illness, mainly due to non-psychotic mental disorders, which include neuroses, stress reactions, personality disorders and other violations [1]. In the conditions of a long socio-economic crisis, socio-psychological factors of mental disorders acquire a significant segment, which leads to the increasing internal conflict of people and contributes to their greater prevalence [2].

Mental health professionals from many countries around the world are very concerned about the scale of mental pathology, the cost to society and the state due to its high prevalence, medical & social consequences of mental disorders (comorbidity, suicide, and social dysfunction). Significant economic burden because of temporary and permanent disability due to mental disorders was manifested by losses of gross domestic product [3, 4, 5, 6].

The risk group for mental disorders is certain occupational groups, which primarily include the military, in which acute and prolonged stress can be layered on individual-typological and personal characteristics. It has been established that servicemen, especially conscripts, were affected by numerous factors of mental trauma, both non-specific (illness or death of relatives, financial difficulties of the family, family conflicts) and specific (workloads, restrictions, and denials due to military service, material, and domestic inconvenience). Modern military service characterized by a high level of requirements, rising prices for erroneous actions of the military specialist, and rapidly changing, intensely saturated daily rhythms do not allow individuals to sufficiently recover and adapt to the service rules [2, 7].

Moreover, powerful prerequisites for the formation of mental pathology in servicemen arise in the context of hostilities. The need to survive in a situation of death and destruction, negative impressions, moral and psychological unpreparedness, fear of not coping with responsibilities, guilt for the dead, uncertainty and lack of information, sleep disorders, rest and nutrition, illness, injury, lack of experience of the stock of possible appropriate reactions were the main factors influencing the mind of servicemen in modern warfare [8]. During combat operations, 75% of servicemen may develop short-term psychological stress reactions, accompanied by partial or complete loss of combat capability [9].

A significant number of servicemen who have applied for psychiatric care and have been discharged from the Armed Forces (AF) of Ukraine for mental health reasons indicate the need to review the entire system of providing psychiatric, medical, psychological, and social assistance to servicemen during crisis time [9]. All of the above makes the problem of mental and behavioral disorders one of the most pressing medical and social problems in the modern army.

THE OBJECTIVE OF THIS STUDY

The purpose of this study is to identify medical and social aspects of mental and behavioral disorders among servicemen of the Armed Forces of Ukraine in peacetime and warfare for justification for the developing and implementing the system of psychomedical care in Ukrainian Defense Forces, adapted to NATO standards.

MATERIALS AND METHODS

A retrospective analysis of statistical data on the level, structure, dynamics of the overall incidence rate of mental & behavioral disorders as well as hospitalized morbidity among the military personnel of Ukrainian Armed Forces and health status dismissal based on long-term observation data (2008-2017) as well as comparison of indicators of the period 2008-2013 and 2014-2017 based on official statistical reporting has been performed.

The material sources were the data of official sectorial statistical reporting on form 2/MED. Statistical data for filling in the reporting form 2/MED were entered into the automated information system of medical treatment facilities reports "Contingent". These medical and statistical indicators were studied for the period 2008-2013 and 2014-2017 separately for each category of servicemen (officers, contract servicemen (privates, sergeants, and non-commissioned officers) and conscripts). Disease groups (psychiatric disorders (F00-F39); neurotic, stress-related and somatoform disorders (F40-F48); disorders of adult personality and behavior with other syndromes (F50-F99)) and individual nosological forms were evaluated by ICD-10.

Statistical processing of the sample results was performed using Microsoft Excel and STATISTICA 8.0 [10]. The arithmetic mean (x) and the error of the mean (m) were calculated. When comparing the indicators for different periods (2008-2013 from 2014-2017), the significant difference in relative values was assessed by Student's t-test.

RESULTS

The analysis of general morbidity indicators of mental and behavioral disorders among servicemen of the Armed Forces of Ukraine in the dynamics of long-term observation for

2008-2017 showed that for 2008-2013 (during the peaceful functioning of the Armed Forces of Ukraine) the most significant value of total morbidity was observed in 2008 with a further downward trend (Figure 1).

Figure 1. Dynamics of the overall incidence rate of mental & behavioral disorders among servicemen of the Armed Forces of Ukraine for 2008-2017.

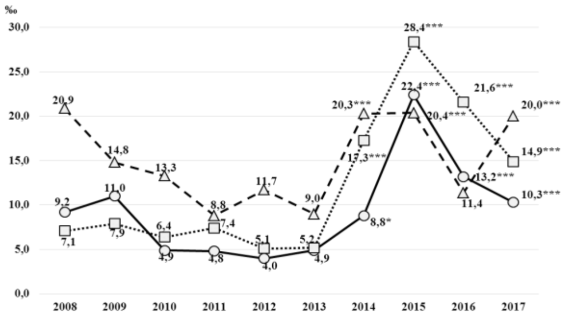

–o– officers; --□-- contract servicemen; –Δ–conscripts

*Note: *, **, *** - significant difference of exemptions of the overall incidence rate of mental & behavioral disorders between 2013 and 2014, 2015, 2016, 2017 years by Student's test corresponds to levels of p <0.05, p <0.01 and p < 0.001.*

It was established that in the structure of the overall incidence rate of mental and behavioral disorders for the period 2008-2013of servicemen of the Armed Forces of Ukraine by class the number of psychiatric disorders (F00-F39) in conscripts was significantly higher ($p \le 0{,}001$) compared to the officers and significantly higher ($p \le 0{,}01$) compared to the contract servicemen.

Also in this period, the share of the population with disorders of adult personality and behavior including other syndromes (F50-F99) in conscripts was significantly higher ($p \le 0.01$) compared to the indicators of the relevant nosology in officers, and significantly higher ($p \le 0.05$) compared to the contract servicemen. The proportion of neurotic, stress-related, and somatoform disorders (F40-F48) was significantly higher ($p \le 0.001$) in officers compared to conscripts, and no significant difference was found in contract servicemen compared to the officers.

In the period 2014-2017, the incidence of mental and behavioral disorders in all categories of servicemen was represented mainly by neurotic, stress-related, and somatoform disorders. This nosology was most prevalent in officers - 79.9% (10.93 ± 1.1 ‰), in contract servicemen it was - 77.6% (15.95 ± 3.03‰), in conscripts – 36.9% (6.63 ± 2.07 ‰).

The redistribution of the structure in all categories of servicemen was noteworthy. Thus, in 2014-2017, compared to 2008-2013, the incidence of disorders of adult personality and behavior including other syndromes among officers and contract servicemen significantly decreased ($p \le 0.001$) due to a significant increase ($p \le 0.001$) in psychiatric disorders (F00-F39). The share of the population with psychiatric disorders in conscripts increased significantly ($p \le 0.001$) due to a significant decrease ($p \le 0.001$) in the proportion of neurotic, stress-related, and somatoform disorders.

The overall incidence rate of mental disorders (ICD-10 class V) in the structure of overall incidence rate of diseases among servicemen from 2008 to 2017 is presented in Figure 2. It was established that the trend of the overall incidence rate of mental & behavioral disorders in the structure of the overall incidence rate diseases among all servicemen categories for 2008-2013 was almost the same in the range from 0.5% to 0.9%, namely: officers - from 0.5% up to 0.9%; for contract servicemen - from 0.6% to 0.8%; for conscripts - from 0.5% to 0.7%. There was no significant difference between the average incidence rates for class F diseases (ICD-10) in all categories of servicemen. During the period 2014-2017, compared to 2013, the occurrence of mental & behavioral disorders in the overall structure of diseases among all servicemen significantly increased with peak values in 2015 (officers – 2.21%, contract servicemen – 3.8%, conscripts – 1.11%, which were significantly higher ($p \le 0.001$ compared to 2013) with a further decrease in 2016-2017.

Figure 2. Dynamics of the overall incidence rate of mental & behavioral disorders in the structure of the overall incidence rate diseases among servicemen in 2008 - 2017, %.

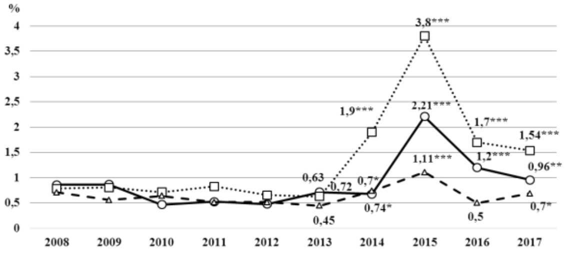

–o– officers; --□-- contract servicemen; –Δ–conscripts

*Note: *, **, *** - significant difference of exemptions of the overall incidence rate of mental & behavioral disorders in the structure of overall incidence rate diseases between 2013 and 2014, 2015, 2016, 2017 years by Student's criterion corresponds to the level of p <0,05, p <0.01 and p <0.001.*

In a further study, we analyzed the hospitalized morbidity of class F diseases among officers, contract servicemen, and conscripts in the dynamics for 2008-2017 (shown in Figure 3).

It was found that the highest rates of hospitalized morbidity of class F diseases ($p \le 0.001$) compared to previous years for contract servicemen and officers for the studied period were observed in 2015 (19.31 ‰ and 15.42 ‰, respectively).

Figure 3. Dynamics of hospitalized morbidity of mental & behavioral disorders among servicemen of the Armed Forces of Ukraine in 2008-2017.

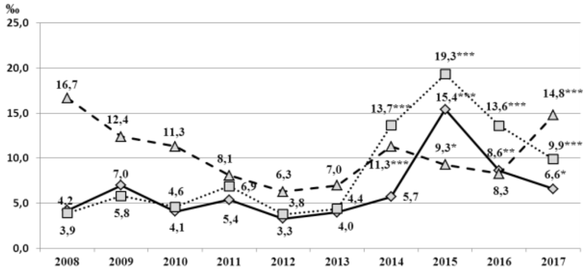

–o– officers; --□-- contract servicemen; –Δ–conscripts

*Note: *, **, *** - significant difference of exemptions of hospitalized morbidity of class F diseases between 2013 and 2014, 2015, 2016, 2017 years according to the Student's criterion corresponds to the levels of p <0.05, p <0.01, and p <0.001.*

It was noteworthy the increase in hospitalized morbidity due to mental and behavioral disorders among conscripts in 2014 with a further decrease in 2015-2016 and a sharp increase (p≤0.001) in 2017 (in 1.8 times compared to 2016).

During the observed period, the hospitalization due to mental and behavioral disorders was studied in all categories of servicemen, it was represented mainly by neurotic, stress-related, and somatoform disorders.

Between 2014 and 2017, the change in the structure of hospitalized morbidity in all categories of servicemen was noteworthy. Thus, in comparison with 2008-2013, the occurrence of psychiatric disorders (F00-F39) among officers and contract servicemen significantly increased (p≤0.001) (in 4.5 and 1.7 times higher, respectively) and the occurrence of disorders of adult personality and behavior including other syndromes (F50-F99) significantly decreased (p≤0.001), (in 8.0 and 3.0 times higher, respectively). Conscripts had a 2-fold (p≤0.001) increase in the proportion of F50-F99, and a 1.5-fold decrease in the proportion (p≤0.01) of stress-related and somatoform disorders.

Also, the occurrence of disorders of adult personality and behavior including other syndromes among conscripts for the period 2014-2017 was significantly higher (p≤0.001) and significantly lower (p≤0.001) in the proportion of neurotic, stress-related, and somatoform disorders compared to indicators on the relevant nosology of officers and contract servicemen.

It was established that the occurrence of hospitalized morbidity of class F diseases in the structure of overall incidence rate diseases among all categories of servicemen for 2008-2013 was almost the same. During the period 2014-2017, compared to 2013, this indicator significantly (p≤0.001) increased in 2015 for contract servicemen and conscripts (in 3.0 and 3.6 times higher, respectively) with a sharp decrease in 2016-2017, moreover, for officers, this indicator was significantly (p≤0.001) in 2.5 times lower compared to 2013.

The occurrence of hospitalized morbidity and mental illness in the structure of major diseases remained unchanged among conscripts.

The next stage of the research was to study the data of servicemen retirements from military service due to mental and behavioral disorders for the 2013-2017 years (Figure 4).

Figure 4. Dynamics of retirements due to mental and behavioral disorders among servicemen of the Armed Forces of Ukraine in 2013-2017.

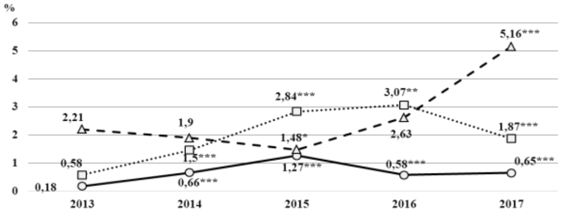

–o– officers; --□-- contract servicemen; –Δ–conscripts

*Note: *, **, *** - - a significant difference of exemptions of retirements due to mental and behavioral disorders between 2013 and 2014, 2015, 2016, 2017 years according to the Student's criterion corresponds to the levels p <0.05, p <0.01, and p <0.001.*

It was established that starting from 2014, the indicators of retirements due to mental and behavioral disorders among officers tended to increase from the maximum in 2015 (compared to 2013 by 7 times higher) with a further decrease in 2016 and 2017 years, however, compared to 2013 remained significantly higher (p≤0.001).

For contract servicemen, this rate increased every year with the maximum value in 2016 (compared to 2013 by 5.3 times higher). It was interesting that the indicators of hospital morbidity and overall incidence rate of mental & behavioral disorders in 2016 tended to decrease. In 2017, the indicator decreased almost 2 times, but compared to 2013 it remained significantly higher (p≤0.001).

The indicators of conscripts' discharges were noteworthy, which were decreasing from 2013 to 2015, while the indicators of hospital morbidity and overall incidence rate of mental & behavioral disorders were increasing. Since 2016, this rate has been increased with a decrease in-hospital morbidity and overall incidence rate of mental & behavioral disorders. In 2017, with a sharp increase in the incidence of mental & behavioral disorders, the rate of dismissals due to mental and behavioral disorders also increased sharply.

It should be noted, while the incidence rate of mental & behavioral disorders among servicemen of all categories was on the 9-13 range places, the indicators of discharges due to mental diseases took the first place (for contract servicemen in 2015-2017 – the 1st place, for officers – I-II, for conscripts – II-III places).

Thus, 2015 year was the most significant in terms of the growth of mental and behavioral disorders among servicemen as a negative consequence of the armed conflict in Eastern Ukraine. This was also confirmed by the rapid increase in suicides level in the Armed Forces of Ukraine (the number of suicides in 2015 increased almost 3 times compared to 2014) [11]. In the structure of expert decisions performed by the Medical Examination Board based on the results of treatment of psychiatric patients after combat conditions at the National Military Medical Clinical Center (for the period 2014-2017), 30.0% of patients were declared unfit for military service in peacetime or had complete unfitness for military service.

DISCUSSION

The experience of the armed conflict in Eastern Ukraine shows that it had a significant negative impact on the mental health disorders in servicemen with corresponding medical and social consequences, which require the development of effective preventive measures and appropriate medical and psychological assistance to servicemen. The highest incidence of mental and behavioral disorders in 2015 can be explained by several factors, such as the performance of combat missions in the anti-terrorist operation zone mostly by officers and contract servicemen who have acute or delayed effects of the severe stress response; conscription of a significant number of servicemen for mobilization and low quality of their selection. In addition, according to our scientists [11] during the first period of formation of the psychiatric care system to servicemen (2014-2015), a specific system of providing psycho-medical care to servicemen was absent. Such assistance was provided unsystematically, mostly by temporarily seconded psychiatrists and psychologists to military mobile hospitals, which successively replaced each other throughout the anti-terrorist operation period.

The analysis of own research and NATO's experience has become the basis for the development of focused measures to preserve the mental disorders in servicemen at combat zone and during the combat capability restoration of military units. “AmedP 8.6 Forward mental health care” and “AmedP-63 Management of post-deployment somatoform complaints” have been readopted, take into account the requirements of the US FM 4-02-51 "Combat and operational stress control" and the US Military First Aid Manual FM 4-25.11 section "Combat and Operational Stress Reaction", WHO recommendations “Psychological first aid: Guide for field workers”, other US and Canadian guidelines for suicide prevention in the military have been implemented in our previous work [13].

The developed psychomedical care model was based on the Mental Health Continuum Model accepted by NATO countries. In the first stages of mental disorders progression, the most important role in preserving mental health belongs to the commanders (Figure 5) [8].

Figure 5. Psychomedical care model in Ukrainian Armed Forces

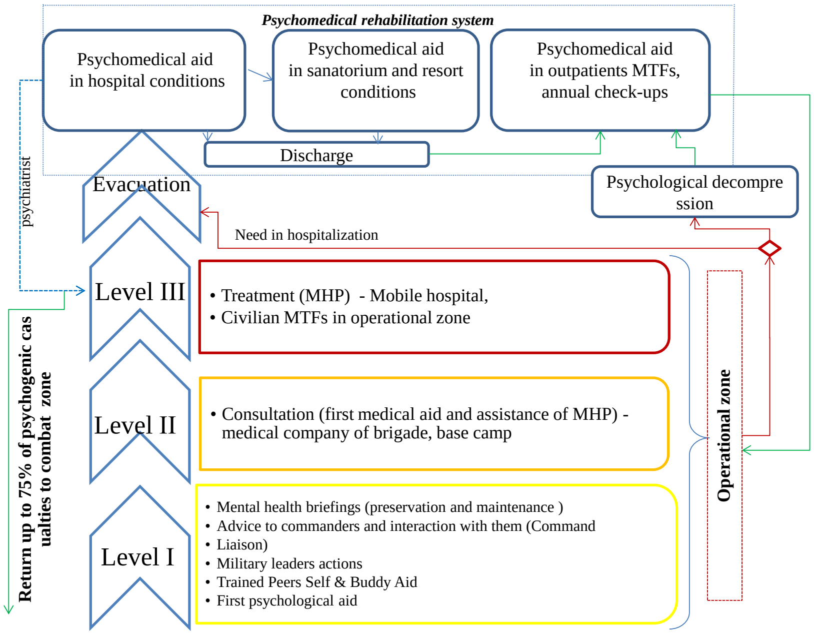
Psychomedical rehabilitation system
Psychomedical aid in hospital conditions
Psychomedical aid in sanatorium and resort conditions
Psychomedical aid in outpatients MTFs, annual check-ups
Discharge
Psychological decompre ssion
Evacuation
psychiatrist
Need in hospitalization
Level III
• Treatment (MHP) - Mobile hospital,
• Civilian MTFs in operational zone
Level II
• Consultation (first medical aid and assistance of MHP) - medical company of brigade, base camp
Level I
• Mental health briefings (preservation and maintenance)
• Advice to commanders and interaction with them (Command
• Liaison)
• Military leaders actions
• Trained Peers Self & Buddy Aid
• First psychological aid
Operational zone
Return up to 75% of psychogenic cas ualties to combat zone

Psychomedical aid should be carried out on all levels of medical evacuation (levels of medical care) following the main measures of care for the acute stress reaction. When moving from one level to another, the capacity of care increases, while the number of individuals requiring psychomedical aid decreases.

Level 1 support is the first line of support and includes leadership actions, self-help, and “buddy” support. This level normally occurs immediately following a potentially traumatic event and represents the most frequently in-demand level of support.

Level 2 support consists of a combination of medical and psychological assistance (provided by medical personnel of the military unit and mental health professionals). And Level 3 support involves assessment and treatment services by mental health professionals.

The organization of psychomedical aid was based on the principles of timeliness and accessibility, following the current level of scientific knowledge; necessity and adequacy of medical measures with minimal social and legal restrictions; consistency (implementation of medical and psychological assistance at the following levels with increasing capacity); continuity (the relationship between the stages of medical care with the capacity of medical and psychological aid, the maintenance of medical documentation); legality, humanity.

Psychomedical aid should not be performed only in a military conflict context, it must be ensured in peacetime daily activities, which will ultimately contribute to maintaining the combat capability of the troops.

After units’ departure, it is desirable to carry out measures of psychological decompression and the allocation of a group who need hospital medical care. We would like to note that due to the deployment of psychiatrists as reinforcement groups to the mobile hospitals, it was possible to significantly reduce the movement of patients with mental and behavioral disorders to the higher level MTFs and in 75% of cases return them to their duties [8].

Further research prospects are advance development of effective preventive measures to maintain the mental health of servicemen, which, from the one hand, will be aimed at improving the system of professional selection to the defense forces, progressing modern methods of training human resilience, and on the other hand - objectifying the process assessment of stressors during the daily activities of troops (forces), risk management of mental health by developing modern measures for maladaptation prevention in military personnel (neurotic and mental disorders, pre-suicidal behaviors), as well as providing training on medical and psychological care, psychological counseling by mental health professionals according to the NATO standards.

CONCLUSIONS

1. The overall incidence rate of mental disorders, as well as hospitalized morbidity within the structure of overall incidence rate of diseases in 2008-2017 for officers and contract servicemen, significantly increased with peak values in 2015 ($p \leq 0.001$) with further decreasing in 2016-2017 years, but among conscripts – remained unchanged.

2. It was established that in the structure of mental and behavioral disorders among Ukrainian servicemen there was a redistribution in all servicemen categories in 2014-2017 compared to 2008-2013 years. Thus, among officers and contract servicemen, the occurrence of disorders of adult personality and behavior with other syndromes (F50-F99) significantly decreased ($p \leq 0,001$ and $p \leq 0.01$, respectively) due to a significant increase ($p \leq 0.001$) of psychiatric disorders (F00-F39) cases. The quantity of F50-F99 nosology among conscripts increased significantly ($p \leq 0.001$) due to a significant decrease ($p \leq 0.001$) of neurotic, stress-related, and somatoform disorders.

3. It has been established that starting from 2014, the indicators of discharges among officers have tended to increase with the maximum rates in 2015 (compared to 2013 by 7 times higher) and contract servicemen in 2016 (compared to 2013 by 5.3 times higher) with a further decrease.

4. The identified features of mental health disorders among servicemen and their consequences according to long-term observation (both in peacetime and in warfare) are the keys to the development and implementation of the proposed psychomedical care system in defense forces readapted to NATO standards.

Conflict of interest
Nothing to declare.

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ORIGINAL ARTICLES

Introducing two effective techniques for the detection of adenovirus respiratory infection in human sample

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Abstract: *Recently, respiratory viral infections have witnessed substantial diagnostic evolution, with the advent of emergent pathogenic agents and the improvement of new diagnostic approaches. Acute viral respiratory infection (ARVI) commonly causes illness and fatality, particularly in pediatric patients. Adenovirus has the highest prevalence in upper and lower respiratory tract infections (RTIs) amongst respiratory pathogens of importance. The purpose of this research was to introduce two effective techniques for the detection of adenovirus respiratory infection in Human samples, cell culture, and real-time PCR. Samples from patients aged less than ten years were obtained from Baqiyatallah (as) Hospital and cultured on HEK cells after preparation. After observing the cytopathic effects of the virus, a molecular test (Real-time PCR) with the help of specific primers was done on samples taken from the cell culture. Both assays detected viruses in clinical samples, and results of cell culture and real-time PCR confirmed infection with adenovirus in samples. According to our findings, simultaneous use of tests (cell culture and PCR) based test real-time PCR is a somewhat reliable method for detecting adenovirus in clinical specimens.*

Keywords: *Adenovirus, detection, cell culture, real-time PCR*

INTRODUCTION

Adenoviruses were first discovered and characterized in the 1950s by two groups who searched for the etiologic causes of acute respiratory disease (ARD) [1]. Adenoviruses as icosahedral viruses that have no enveloped with an approximate size of 70-90 nm. The viruses were found epidemiologically to be the main causative agent of ARD in recruited militarians [2]. Human adenoviruses are causing merely a minor percentage of ARD in the general population and for almost 5% to 10% of pediatric respiratory disease. Respiratory tract infections by viruses affect children [3]. Adenoviruses are responsible for epidemic outbreaks in soldiers [4]. In addition to respiratory infection, diseases such as conjunctivitis and infantile gastroenteritis caused by adenoviruses can induce fulminant fatal pneumonia, hepatitis, and/or encephalitis in mankind [5]. The respiratory diseases consist of both upper respiratory (pharyngitis and colds) and lower respiratory (bronchiolitis and pneumonia) syndromes [5, 6].

Around 7% of upper RTIs in youngsters aged below five years result from an adenovirus [3]. The respiratory indications are often associated with systemic demonstrations (e.g., generalized malaise, fever, chills, myalgia, and headache). The usual serovars are Ad 1, 2, 5, and 6 (species C or HA

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group III) and, sometimes, Ad3 and Ad7 (species B or HA group I), being indigenous in the majority of populations [7]. Sporadic cases might be indistinguishable from other viral respiratory contagions (e.g., influenza, parainfluenza, and respiratory syncytial virus) [8]. If conjunctivitis comes with the signs and symptoms denoted above, the illness is known as pharyngoconjunctival fever. Ad3 is the adenovirus serovar with the uppermost involvement, but Ad7 and Ad14 isolates among the same HA group have been reported in these patients [9]. Besides, adenoviruses induce lower respiratory tract contagions in youngsters and are a possible cause of nearly 10% of pediatric pneumonia cases [10]. The majority of patients recover from such lower respiratory contagions. However, some pandemics have led to a remarkable fatality, respiratory symptoms duration of almost one week, ocular symptoms for 2–8 weeks, and gastrointestinal symptoms up to 10 days [11]. Adenoviruses are detectable through isolating in nasopharyngeal and conjunctival discharges and throat washes and are visualized via electron microscopic assay of feces in the course of the acute disease phase [12]. Respiratory infections typically represent an elevation in CF antibodies to a common antigen. Virus-related antigens are also detectable in samples clinically through various immunologic methods and PCR [13]. The current research aimed to evaluate the detectability of adenovirus respiratory infections by two techniques, cell culture, and real-time PCR.

MATERIALS AND METHODS

Origin of samples: In this study, samples from patients less than ten years old were received from the Baqiyatallah (as) Hospital and prepared as oral swabs from patients with the common cold and pharyngitis symptoms. In most cases, NP swabs are obtained from elder children and adults, whereas newborns and youngsters are the common sources of NPAs. Since most patients cannot tolerate NP sampling well, other samples were obtained as well. Clinical specimens were transferred to the laboratory on wet ice to preserve viral vitality. These samples were placed in a transport media.

Cell culture: Human embryonic kidney cells (HEK) were cultured in three T25 flasks grown in Dulbecco's altered Eagle medium (DMEM (1X)) complemented with 10% fetal bovine serum (FBS). After inoculation, cell culture tubes underwent incubation at 37 °C and 5% CO2. Samples of patients in the two flasks were inoculated, followed by adding the maintenance medium (DMEM (1X) supplemented with 2% FBS) under the above conditions. Up to a week daily, flasks were examined microscopically to record the viral cytopathic effect (CPE) [14].

Nucleic Acid Purification and Real-Time PCR: A pair of primers were designed for the hexone locus, with forward (5-ACCTGAGTCCGGGTCTGGTGC-3) and reverse (5-GTCAACGGGCACGAAGCGCA-3 primer sequences, as well as a probe sequence of GATGTGACCACCGACCGTAGCCAGCG. Extraction of nucleic acids from respiratory specimens was done by a DNA kit (High Pure Viral Nucleic Acid Kit - Roche) following the manufacturer's instructions. In the next step, a pair of primers was designed for the hexon gene of the adenovirus for specific detection of the virus according to the melt curve. Each reaction had a total volume of 20 µL, including 10 µL of Mastermix PCR probe base, 5 µL of template, 1 µL of primer (0.5 µM), 1 probe (0.5 µM), and 3H2O. The cycling conditions included a primary denaturation step at 95 °C (2 min), followed by 60 cycles (each for 20 sec) at 95 °C, and at 57 and 72 °C for 40 and 30 sec respectively. The annealing temperature was 57°C. A melting curve analysis program was used to identify specific PCR products. After the last cycle, the temperature was increased to 95 °C, then decreased to 75 °C, and slowly elevated to 95 °C at a rate of 0.1 °C per second. Positive and negative controls were added in each set [15, 16].

RESULTS

The basis of this study was to show that the involvement of viruses can be an important factor in the upper respiratory system of patients. In this study, adenovirus was studied as one of the causes of conflict in the upper respiratory tract of children.

The samples used in our study were taken in Baqiyatallah (as) Hospital from patients with ages of fewer than ten years who had symptoms such as common cold, pharyngitis, and fever. As permissive cells for adenovirus, HEK cells infected with the virus show typical CPE that resembles grape-like clusters. HEK cells were cultured in three T25 flasks grown in Dulbecco's altered Eagle medium (DMEM (1X)) enriched with 10% FBS and 1% Pen Strep (10000 Unit/ml penicillin (P-4687) + 10000 µg/ml streptomycin (S-1277) Bio-Idea made from SIGMA powder).

When the cell confluence in the flask was approximately 100%, the patient samples were inoculated by the cells in an incubator with CO2 for one hour, after which samples were washed with PBS and added to each flask containing maintenance medium (DMEM (1X) enriched with 2% FBS and 1% Pen Strep).

Flasks were incubated in an incubator at 37 °C and 5% CO2. Flasks were checked daily to observe CPE. On the third day, CPE started formation in the two flasks infected with the virus, which included rounding of cells to clustering. Almost on the seventh day, grape-like clusters, which are typical CPE for adenovirus, were visible (Figure 1).

Figure 1: The cytopathic effects in cells after inoculation. A) Control cells that had not been inoculated with the virus. B & C) Patient samples 1 and 2 in the two inoculated flasks. Cytopathic effect was evaluated daily. The result was observed associated with adenovirus infection including grape-like clusters by separating the cells from the bottom of the flask

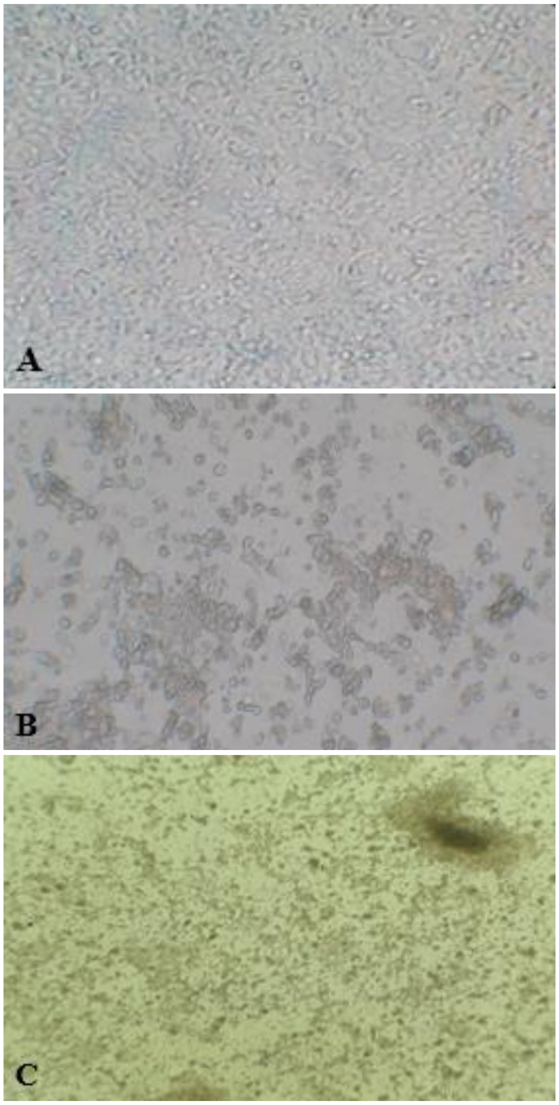

According to the figure of real-time PCR, clinical samples were positive for adenovirus in the form of double. The diagram shows that the amount of virus in sample 1 is greater than that of sample 2 (Figure 2A). Figure 2B shows real-time PCR results and related explanations.

DISCUSSION

Virus-related respiratory infections are prevalent during the year in all age clusters and all populations worldwide. Respiratory viruses can induce moderate ailments, including the common cold, or acute sickness (e.g., pneumonia), which affect both immunocompetent and immunocompromised hosts [17]. Despite the infection of both the upper and lower respiratory tract by every respiratory virus, special syndromes (e.g., croup or laryngotracheobronchitis) happen clinically by particular viruses. Both DNA and RNA viruses are reported to induce respiratory infection [18]. Researchers have described several novel viruses that have relationships with respiratory illnesses [19]. Pandemics of adenovirus respiratory infection are prevalent during winter, spring, and early summer, with protracted (a length of 1–3 years) durations [20]. Almost 80% of upper RTIs occur by viruses etiologically and can exacerbate asthma and acute otitis media [21].

Figure 2. A) Adenovirus molecular test results (real-time PCR) confirming the presence of adenovirus. In this test, a sequence-specific primer pair and a probe were used for the viral hexon sequence. B) Shows real-time PCR results and related explanations. In this test, other viruses were used as a negative control, the results of which are obvious.

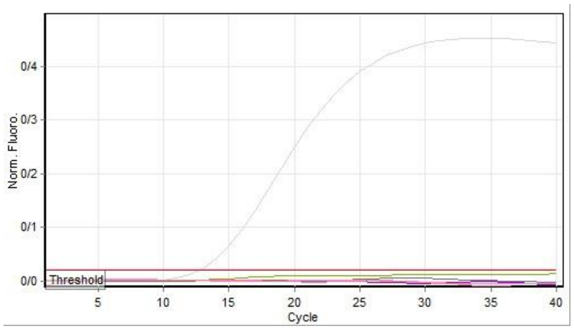

No.	Colour	Name	Type	Ct
22	🟩	HSV1	Negative Control	
26	🟪	NTC	NTC	
28	⬜	Adenovirus	Positive Control	12/69
31	⬛	HSV2	Negative Control	
37	🟪	CMV	Negative Control	
40	🟥	BKV	Negative Control	

Adenoviruses are the leading respiratory pathogenic agents and account for 5-8% of all RTIs in newborns and youngsters aged two years [22]. HAdV infections have clinical similarities to those of other respiratory viruses, and it is difficult to decisively detect and swiftly diagnose the virus type, which is not achievable clinically [23]. Due to the persistency and protracted casting of adenovirus, methods of diagnosis should have sufficient sensitivity for detecting small amounts of virus in clinical samples. Several laboratory methods are available to detect adenoviruses, including nested PCR, real-time PCR, PCR, IF, and cell culture, of which molecular and cell culture techniques are sensitive enough to detect the virus [24]. The viral antigen is swiftly detected by IF but is mostly not sensitive to detect some viruses;

hence it is occasionally required to be confirmed by viral culture [25]. Molecular analyses based on polymerase chain reaction (PCR) are gaining ground for diagnosing virus-related respiratory infections and conducting epidemiologic examinations [26]. As a benchmark, viral culture was well-known to test viral respiratory pathogens. Even so, the technique suffers from slowness in general, which usually requires over 14 days to deliver results [27]. Although the number of positive results can be increased by combining these two procedures, existing reports indicate that a considerable number of samples remain negative despite suspicious viral infections both clinically and epidemiologically [28]. During the last years, a growing body of evidence has been presented on the development of molecular biology diagnostic methods for viral infections, which will undoubtedly be the benchmark approaches presently applied in ordinary laboratory assays [29]. Molecular procedures significantly contributed to the fast detection of pathogenic viruses accompanying acute respiratory infections, which allow for rapid implementation of therapeutical actions and preemptive policies to prevent disease dissemination with high transmission ability [30]. Studies in Iran, Kenya, Brazil, China, Australia, and Hong Kong showed that 5-22% of samples were affected by adenoviruses [31].

Given the importance of cell culture and real-time PCR methods in recognition of respiratory adenovirus, cell culture was confirmed by real-time PCR in this study. Their grape-like clusters identified the CPE of the adenovirus in the cell culture. From the results of real-time PCR, it is concluded that the upper respiratory tract symptoms in children of our study might have arisen by adenoviruses. Suppose a large number of patients could be studied epidemiologically on this subject and in case of obtaining statically significant results. In that case, the season of adenovirus infection frequency can be verified in the studied district to demonstrate possible differences in the season frequency of infection.

CONCLUSIONS

This study showed that simultaneous use of tests such as cell culture and PCR-based tests, including the Real-time PCR, are somewhat reliable methods for detecting adenovirus in clinical specimens.

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Conflict of interest:

The authors report no conflicts of interest.

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ORIGINAL ARTICLES

In-silico evaluation of the potential of a viral protein (Apoptin) as an anticancer agent

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Abstract: *As a significant immunosuppressive virus, chicken anemia virus (CAV) is common among natural poultry hosts and newborn chickens. It contains a tiny single-stranded negative circular DNA genome, encoding three proteins vp1, vp2, and vp3. Apoptin, encoded by the vp3 gene of the CAV virus, is a small protein whose subcellular localization appears to be crucial for tumor-selective activity. Residing in the cytoplasm of normal cells, it translocates into the nucleus in cancerous cells. Apoptin has attracted much attention because of causes specific death in transformed and cancer cells and has toxic effects on cancer cells. In-silico analysis of apoptin revealed the unknown structural and functional features. Physicochemical features and sequence analysis and secondary structure prediction were also conducted using Expasy server tools. Furthermore, the global structure of apoptin was modeled using the I-TASSER server and antigenic features were obtained via the application of the IEBD web tools. The results of in-silico analysis indicated that this protein had a stable structure, was a suitable choice for therapeutic goals, capable of acting as a good anti-cancer agent.*

Keywords: *apoptin; in-silico analysis, chicken anemia virus; cancer*

INTRODUCTION

Cancer is defined as the Irregularity, growth, and proliferation of the cell cycle and sometimes the abnormal proliferation of cells in the body (metastasis). Since the onset of cancer is associated with mutations in some of the natural cell genes, it is considered a genetic disease. Tumor cell mass which is different in appearance and function from normal cells can be either benign (progressive and invasion free with slow growth) or malignant (with rapid growth, metastatic potential), ultimately leading to patient death [1]. Due to the high cost of the medications and cancer treatment facilities, numerous studies have concentrated on finding methods to prevent the disease or effectively treat it [2]. In previous studies, different viruses and their proteins have been used to find a treatment for cancer [3].

Viruses' ability to attach to the cancer cells has attracted the researchers' attention as a novel strategy comparable with conventional therapies, including surgery, radiation therapy, and chemotherapy [4]. The application of recombinant proteins has recently given rise to an interest in understanding and recognizing the physiology of tumor cells. In particular, the identification of these proteins as a new drug and therapeutic cancer vaccine which can use different mechanisms to defeat cancerous cells is crucial for cancer researchers. Unlike prophylactic vaccines, which are generally prescribed to healthy people, cancer vaccines are mostly prescribed to cancer patients. These vaccines are designed to kill cancer cells by boosting the patient's immune response. They kill different immune effects or

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mechanisms created by therapeutic vaccination, especially cancer cells and normal cells. The apoptin protein is one of these proteins encoded by the chicken anemia virus (CAV) genome [5, 6].

Infectious anemia virus is one of the most significant immunosuppressive viruses in newborn chicks which are regarded as the natural host of this virus. The virus has three protein sequences as follows: The 50kDa viral protein (VP1) which is the only protein isolated from virus components the 30kDa non-structural VP2 protein functioning as a scaffold protein during the vision accumulations, and the third viral protein VP3 weighing 14kDa, which is located the nucleus of infected cells, but not found in the purified virus components [7]. VP3 is called apoptin, a potent stimulant of apoptosis in and lymphoblastoid cell lines and chicken thymocytes [8]. VP3 gene product due to its apoptotic effect was renamed apoptin [9]. This protein causes apoptosis to be independent of the p53Bcl-2-dependent tumor inhibitor. Apoptosis-induced apoptosis included the induction of caspase, leading to mitochondrial changes and dispersion of cytochrome C [10]. The C-terminal region of apoptin has a bilateral nuclear localization sequence (NLS). NLS1 amino acids from 82 to 88 and NLS2 amino acids remain in the range 111 and 121 [11]. In addition, in the range of 75-107 amino acids, apoptin has a putative nuclear export sequence that transfers the apoptin through the nucleus. Apoptin proteins cause apoptosis in the altered cells (transformed cells), while they leave no effect on normal cells [12, 13].

Bioinformatics refers to the information technology used in molecular biology. Bioinformatics tools can easily identify and predict the primary and secondary structures in a new sequence. This diagnostic tool is applied to find patterns, detect several protein families and show the oligonucleotide primers for a polymerase chain reaction. It also serves as an essential prelude to molecular evolutionary analysis [14]. To the best of the author's knowledge, not much is known about the structural characteristics of Apoptin as an anticancer agent. The main objective in the current study is to evaluate the physicochemical features and determine the secondary and tertiary structure and antigenic features of the apoptin protein of CAV, using bioinformatics tools and databases.

MATERIALS AND METHODS

Sequence analysis

The primary sequence of a given protein (Apoptin) was retrieved from GenBank (ACC. No. AEB91668.1). The first structure of the protein in terms of aliphatic index, extinction coefficient, molecular weight, instability index, isoelectric point, amino acid composition, and half-life in vitro and in vivo are considered as physicochemical features which can be evaluated using various bioinformatics tools.

Physicochemical Features of Apoptin were evaluated using ProtParam, Predict protein, Compute PI/MW tools, and ExPASy server (http://expasy.org). ProtParam database calculated the parameters, such as extinction coefficient, estimated half-life, amino acid composition, theoretical PI, instability index, molecular weight, atomic composition, aliphatic index, and hydropathic mean (GRAVY).

Predict Protein tool can integrate feature prediction for globular regions, B-values, disorder regions, subcellular localization, transmembrane helices secondary structure, intra-residue contacts, structural switch regions, coiled-coil regions, solvent accessibility protein-protein, and protein-DNA binding sites, disulfide bridges, protein-protein, and protein-DNA binding sites, metal-binding sites, domain boundaries, beta-barrels, cysteine bonds.

Structural analysis

The secondary structure components include Alpha Helix, B-Sheet, Turn, Loop, and Coil. The tertiary structure is also formed by the twist of the protein chain caused by the bonding between amino acids which gives rise to functional domains.

Secondary structure was performed to identify Alpha Helix, B- strands, etc, using the Gor, Jpred tools from ExPASy server (http://expasy.org/cgi-bin). According to its amino acid composition and similarity to the sequences of the known secondary structure, the possible secondary structure of a protein can be predicted. RAMPAGE software (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) was used to determine the validity, stability, and quality of the spatial structure of the protein. Then, the stability of protein structure was evaluated based on the PDB model.

A pattern of comparative modeling was used by the I-TASSER server predicting the third structure based on Homology modeling. I-TASSER provides the biological function of protein molecules from amino acid sequences and the prediction of high-quality models of 3D structure. Energy minimization of the tree-dimensional structure obtained from I-TASSER was performed by the Swiss-Pdb Viewer 4.1.0 software.

Antigenicity

The http://www.iedb.org server was employed to identify antigenic fragments of the protein. IEDB contains experimental data on antibodies and T cell epitopes studied in the fields of infectious diseases, allergies, autoimmunity, and organ transplants in humans and other animal species. Predictions of B cell space epitopes were determined with ElliPro and Discotope servers.

We employed vaxijen server to estimate the antigenicity of the chimeric protein.

RESULTS

Primary sequence analysis

Table 1 shows the results obtained from the Prot param database, the number, and type of amino acids, and atomic components of Apoptin. This protein has 121 amino acids, most of which are Arg and the least are Met and Tyr. The molecular formula of the protein is C566H945N179O180S5. The protein's extinction coefficient is assumed to be 1740 if the number of cys is reduced to 1490. The protein's extinction coefficient is calculated using the amino acid composition (Table 1) of the protein. The estimated half-life span in mammals is 30 hours, in yeast 20 hours, and E. coli 10 hours. The protein instability index was calculated at 68.45.

The aliphatic index of protein was 65/37. The hydropathic mean (GRAVY) was also estimated to be -0.748

Table 1: Proteins parameters database

Number of amino acids	**121**
Molecular weight	13298/12
Theoretical pI	10/05
Formula	$C_{566}H_{945}N_{179}O_{180}S_5$
Total number of atoms	1875
Estimated half-life	30 hours (mammalian reticulocytes, in vitro)
	>20 hours (yeast, in vivo)
	>10 hours (Escherichia coli, in vivo)
Instability index	The instability index (II) is computed to be 68/45
Aliphatic index	65/37
Grand average of hydropathicity (GRAVY)	-0/748

The mean pI/Mw was calculated based on the Compute PI/MW database (PI=10/05, MW=13298/12)

According to the Ramachandran map obtained from RAMPAGE software, the protein is the apoptotic protein (Figure 1).

Figure 1: A (Image of 3D forms of the apoptin protein obtained from the i-Tasser database). B (prosaweb result). C (Image depicts the Ramachandran map of apoptin protein). D (Indicates the composition of the secondary structure of the protein)

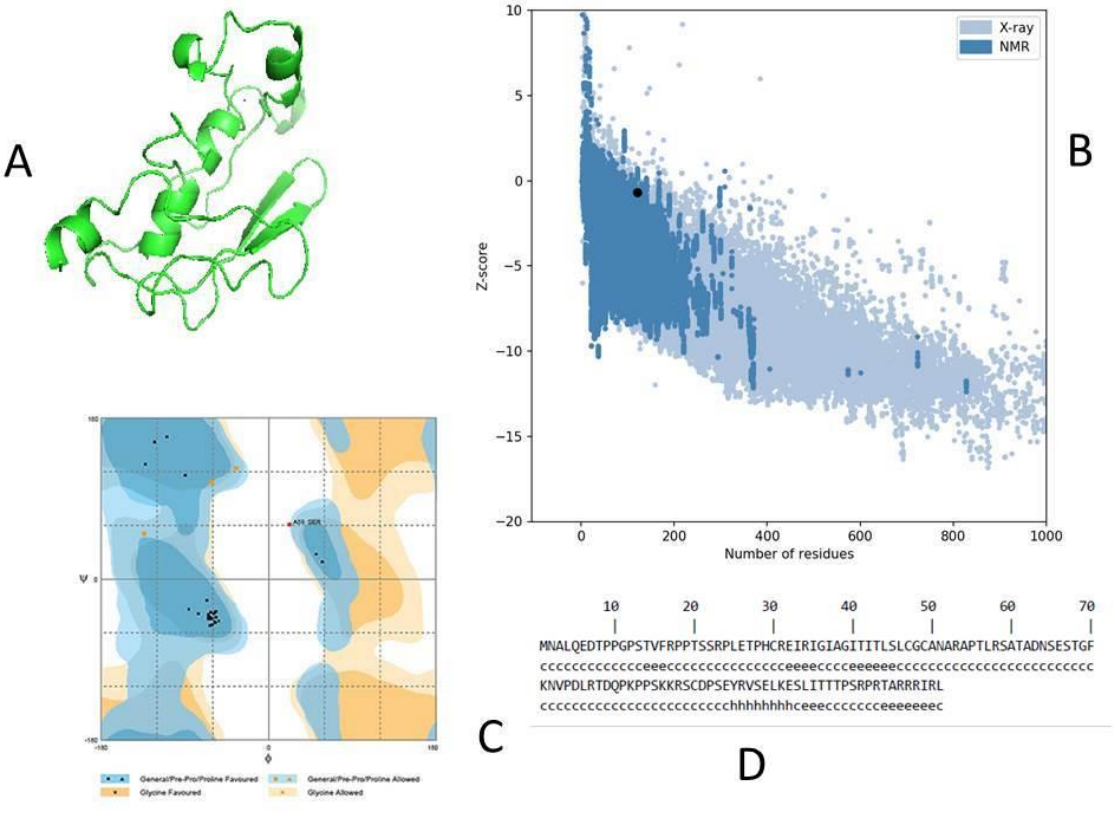

```
         10        20        30        40        50        60        70
          |         |         |         |         |         |         |
MNALQEDTPPGPSTVFRPPTSSRPLETPHCREIRIGIAGITITLSLCGCANARAPTLRSATADNSESTGF
ccccccccccccceeecccccccccccccceeeecccceeeeeeeccccccccccccccccccccccccc
KNVPDLRTDQPKPPSKKRSCDPSEYRVSELKESLITTTPSRPRTARRRIRL
cccccccccccccccccccccccchhhhhhhhhceeeccccccceeeeeeec
```

Prediction of tertiary structure

As shown in Figure 1, i-Tasser protein simulated models were calculated quantitatively by a C score based on the importance of pattern rules and convergence parameters of the structure assembly simulation. The energy minimizer of apoptin protein was obtained by using simulated models by the i-Tasser database in spdbv software indicating that apoptin is a stable protein.

Prediction of linear and discontinuous epitopes

According to Figure 2, the spatial structure of apoptin was obtained from the IEDB database, predicting an antigenically active peptide in the apoptin protein.

Figure 2. A (The Linear Epitopes spatial structure obtained from Ellipro). B (Discontinuous Epitope)

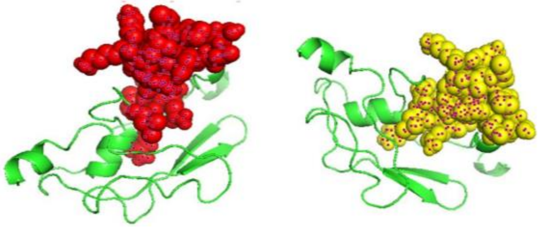

DISCUSSION

CAV is a small non-enveloping virus that has a single-stranded circular DNA genome measuring 2.3 KB. The major structural protein (VP1) of viruses is encoded by the genomic strand, while at least two other proteins (VP2 and VP3) are encoded by the antigenomic strand. VP3, called apoptin, is a non-structural protein made up of 121 amino acids. Apoptin contains some residues of proline, serine, and threonine and has a positive charge at the C- terminus [15].

Apoptin protein induces apoptosis just in altered cells (cancer cells) but does not affect normal cells. The specificity of the effect of Apoptin depends on its location in the cell. Its transfer from the outside to the nucleus of the cell in the attachment process to the cancer cells increases its specific effect compared to other normal cells. This protein, in cancer cells, is located in the nucleus and regulated by phosphorylation. Apoptin can induce specific apoptosis of tumor cells in a p53-independent pathway. Apoptosis is moderated by intracellular cysteine proteases (caspases) as the initiator and executor of the apoptosis process [16].

Zhuang et al. indicated that apoptin is capable of inducing apoptosis in human osteosarcoma cells. Subsequent studies found evidence that apoptin induces apoptosis in various human hematologic tumor cells. Also, another study on the effect of apoptin protein by bacteriophage carriers on human breast tumor cell line (BT-474) showed that it influences the cancer cells, while it has no inhibitory effect on normal cells [8].

Traditional laboratory-based cancer research consists of costly experimental trial and error strategies applied to humans, animals, and their harvested tissues. “In-silico experimentation,” combines computational technologies with the mathematical or theoretical properties of cancer bio cells and presents a new approach to advancing cancer treatment [17].

This study showed a simulated 3D model of apoptin generated by using homology modeling as a backup alternative to the conventional, crystallography- or NMR-based methods. In addition, bioinformatics tools for different purposes such as primary and secondary structures and computational modeling of protein structures can help predict new choices for cancer therapy [18, 19].

The results of physicochemical features of apoptin protein including instability index, GRAVY index, solubility index, and aliphatic index evaluated by Prot param database help predict desirable apoptin protein. Since no 3D structure has been identified to date, the present study aimed to evaluate the structure of apoptin using the I-TASSER server. The results stored in pdb were assessed for stable and appropriate energy conditions by Swiss-Pdb Viewer 4.1.0 software, and then energy minimization was analyzed. The image obtained from the server along with the prediction results compared to the Ramachandran graph prediction generally showed structural stability as a fusion protein. Lee et al. (2012) demonstrated that direct engineering of apoptin protein was a suitable and cost-effective strategy to enhance the expression of TAT-Apoptin protein in E. coli [20].

Despite having drawbacks targeting only one epitope or several epitopes of tumor-associated antigen (TAA), protein/ peptide-based vaccines are more cost-effective than autologous or personal vaccines. It is believed that the induction of antigen-specific CTLs and antigen-specific helper CD4 + T cells is essential for the optimal efficacy of the cancer vaccine. Some polypeptide vaccines (eg, Stimovax) potentially contain the CD4 and CD8 epitopes. Altering the peptide sequence of TAAs to introduce agonist-boosting epitopes is another method to increase the immunity of its antigen, which enhances the binding of the peptide to the MHC molecule or T cell receptor, resulting in a higher T cell response and/or T cells with higher or higher power [21]. In the present study, epitope mapping apoptin showed that this protein can be an appropriate candidate for therapeutic cancer vaccines.

To the best of the authors' knowledge, not much is known about the structural characteristics of apoptin. The main objective of the current study was to assess the potency and specificity of small apoptin protein in cancer therapy. However, more studies are needed for designing new small apoptin-like peptides to improve the anti-tumor activity of apoptin and avoid their undesirable side effects.

CONCLUSION

As the in-silico results indicate, apoptin has a stable structure and can be regarded as a suitable candidate for cancer treatment. Furthermore, the appropriate structure of apoptin may lead to its selection as a therapeutic cancer vaccine capable of stimulating both the humoral and cellular immune systems.

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Ethical Statement

Ethical approval was granted by the Medical Ethics Committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1397.360).

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ORIGINAL ARTICLES

Gene therapy by using stem cells in the treatment of hepatitis

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Abstract: *Chronic hepatitis can resolve spontaneously or progress to more advanced stages, i.e. fibrosis, cirrhosis, and liver cancer, respectively. Two million deaths each year are attributed to liver diseases globally. Chronic liver disease and cirrhosis comprise 11th position in leading causes of death worldwide. Viral infections account for the etiologic factor of most cases of hepatitis. Almost the only available way for the treatment of end stages of hepatitis is liver transplantation. The biggest problem with this therapeutic approach is the number of liver transplant donors is less than the number of recipients. Therefore, more advanced methods must investigate. Recently, gene therapy by the utilization of stem cells yielded promising results in clinical and preclinical investigations to the treatment of liver diseases. Novel genome editing tools such as TALENs and CRISPR/Cas9 consider as powerful genome correction approaches and can employ for iPSCs gene editing. This paper highlights the various aspects of gene therapy through stem cells for liver diseases*

Keywords: *gene therapy, stem cell, hepatitis, CRISPR/Cas9*

INTRODUCTION

The liver is the real organ in the body complicated in the bile production, metabolism, toxins elimination, and immune responses, and several disorders of metabolism are of hepatic basis. There are many different types of liver diseases, comprising hepatitis, alcoholic liver disease, fatty liver disease, hereditary disease, cirrhosis, fibrosis, etc. These may result in liver failure and lead to irreparable liver impairment. Even though the liver is capable to repair itself, while the injury is beyond repair it is hazardous and lethal to patients [1]. Hepatitis consider as a condition of the presence of inflammation in the liver that divides into two categories, i.e. acute hepatitis and chronic hepatitis. The latter is defined as lasting for more than six months. This inflammation can resolve spontaneously or progress to more advanced stages, i.e. fibrosis, cirrhosis, and liver cancer, respectively [2-4]. Liver diseases are responsible for nearly 2 million deaths whole the world yearly. Chronic liver disease and cirrhosis ranked as 11th and 5th leading causes of death worldwide and for the non-Hispanic AIAN population of the United States, respectively [5, 6]. Most cases of hepatitis attribute to viral infections as an etiologic factor. Other important leading causes include infectious agents except for viruses, alcohol, drugs, and autoimmune reactions. The only available way for the treatment of end stages of hepatitis is liver transplantation. One of the main problems of this way is the discrepancy in the number of liver transplant donors and recipients. Furthermore, the recipient's immune system could reject the grafted organ [7]. Consequently, different methods must be considered and explored. Recent progress in gene therapy opens a promising new window to the treatment of a broad range of diseases. In the procedure of gene therapy, to reduce the symptoms or completely cure a disease, genetic material is delivered to a patient [8]. This approach tries to sustain express a transgene, as well as minimizes adverse side

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effects. One of the best profits of gene therapy is that a single treatment is sufficient, therefore is no need for repeated administration [9, 10]. Gene therapy utilizes in various fields such as the treatment of cancers, monogenic diseases, infectious diseases, heart diseases, neurological diseases, eye diseases, etc. [11, 12]. By 2017, about 2600 clinical trials initiate that some of which approves. The most common types of gene therapy include gene replacement therapy, CAR (Chimeric antigen receptor) T-cell therapy, and gene editing. In recent years, stem cells, as one of the top topics in the broad range of advanced scientific research, have attracted the attention of many researchers and become a good tool for gene therapy [13]. In fact, in vitro stem cell culture is straightforward and they can differentiate into hepatocytes and new liver cell sorts. The results of novel scientific investigations in stem cell gene therapy open new horizons in the treatment of hepatitis [14, 15]. It hopes that shortly, these approaches will enter clinical practice. There are several sources for stem cell therapy of liver diseases that are summarized in Table 1 [16-22].

Table 1: Sources for stem cell therapy

Liver-Derived Stem Cells	
	• Adult liver stem cells (oval cells) • Fetal liver stem cells (hepatoblasts)
Bone Marrow-Derived Stem Cells (BM-DSCs)	
	• Hematopoietic stem cells (HSCs) • Mesenchymal stem cells (MSCs)
Annex Stem Cells	
Embryonic Stem Cells (ESCs)	
Induced Pluripotent Stem Cells (iPSCs)	

In this review, we discuss gene therapy via manipulated stem cells for the treatment of hepatitis.

ANIMAL MODELS AND THERAPEUTIC GENE TRANSFER BY USING STEM CELLS

Today, animal models are considered a key tool in research fields. They have played a key role in the advancement of medical sciences, especially in cell and gene therapy for hepatitis. Although many efforts make in this regard, however, there are still differences between animal models and humans [23-26]. Among animal models, mice can be a good choice for the study of hepatitis pathophysiology as well as the development of novel, safe, and efficient drugs. A big problem is the mice cannot be infected with hepatitis viruses. Several strategies were developed to solve this problem: Humanized chimeric, transfection, and transgenic mice [27]. Gene therapy by using stem cells is a new version of previous gene therapy approaches. In this procedure, therapeutic genes are inserted into stem cells, and then the genetically modified stem cells are delivered to the patient's body [28, 29].

Currently, outstanding achievements in the development of stem cell therapy approaches have brought new hopes in treating liver diseases. Many studies have been carried out in this field and emerging evidence suggests that the utilization of stem cell-based-gene therapy is a good way for the treatment of liver diseases, especially hepatitis [30-32]. For example, a study conducted by Zhu et al. on a mouse model showed that the use of bone marrow-derived mesenchymal stem cells (BM-MSCs) led to the immigration of these cells to the liver that boosts immune system performance, and improves liver damage caused by (concanavalin A) ConA [33]. Gilsanz et al. in their experiment, examined the effect of human/rat adipose-derived mesenchymal stem cells (AD-MSCs) injection to the liver parenchyma of rats and observed that the biochemical serum analytes of the liver, hepatomegaly, splenomegaly, and ascites have remarkably improved [34]. Masalova et al. used modified mouse mesenchymal stem cells (mMSCs) of DBA/2J (H-2d) mice that expressed 5 non-structural proteins of the hepatitis C virus (HCV), including NS3, NS4A, NS4B, NS5A, and NS5B. Then the analysis of innate and adaptive immune responses showed this approach drives high phagocytosis, lymphocyte proliferation, and type I/II interferon production, as well as, IFN-γ secretion, Immunoglobulin G (IgG) production [35].

ADVANTAGES AND DISADVANTAGES OF STEM CELL-BASED THERAPY

The aim of stem cell-based therapy for liver disease is to substitute disease cells with healthy ones and let the healthy cells be efficient. Around the world, several research groups are conducting the expansion of stem cell-based therapy for liver diseases. The objectives are to recognize stem cell varieties that are appropriate for the applications and transplantation. To attain these aims, scientists should tackle significant challenges. Because adult tissues compose of many diverse cell sorts, the identification and trying to pinpoint the stem cells are the tasks for experts. Afterward, the cell isolation from the tissues, inducing cells to differentiate into favorite cell types is an additional impediment. As well as, cells might be rejected by patients' bodies after grafting, which is an additional concern [36].

One of the most significant advantages of Induced Pluripotent Stem Cells (iPSCs) over other stem cells is its application as autologous stem cell therapy with the minimum risk of rejection induced by the immune system of the host (allogeneic immune rejection). In addition, these cells can differentiate from hepatic cells and have the capability of self-regeneration and pluripotency [37, 38]. Observed that using Yamanaka factors including Oct3/4, Sox2, c-Myc, and Klf-4 can reprogram mouse fibroblasts into iPSCs [22]. On the other hand, iPSCs can be differentiated to hepatocyte-like cells (HLCs) and then delivered to the diseased liver, ultimately recovering their proper function [39].

Recent work demonstrates that gene transferring, genome editing, three-dimensional (3D) culture, and extracellular matrix approaches improved hepatic differentiation of human iPS cell-derived hepatocyte-like cells with more than 90% purity and a satisfactory level of liver functions that are comparable to primary human hepatocytes [40].

INDUCED PLURIPOTENT STEM CELLS AS PATIENT-SPECIFIC MODELS TO ASSESS EXOGENOUS GENE DELIVERY

Human iPSCs are a possible basis for the treatment of end-stage liver disease. Some research has stated the improvement procedure for differentiation of human iPSCs into the hepatocytes. Hannan et al. defined a procedure that organized the differentiation of human iPSCs into a close-homogenous population of HLCs by 25 days [41].

One of the critical aspects of gene therapy is selecting an appropriate method for transgene delivery to the target. A variety of vector systems are available for this purpose. For the genetic material transfer to the target cells, both viral and non-viral vectors can be utilized. The transduction efficiency of non-viral vectors is lower than viral vectors that confer a shorter duration of transgene expression. Properties of an ideal vector are high capacity, low antigenic potential, high transduction efficiency, providing conditions for targeted and controlled transgene expression, and being safe. Both ex vivo and in vivo gene delivery approaches are used for the liver.

The ex vivo process involves the removal of single cells from a host and transfers to the outside the body, gene transduction in vitro (gene modification), and then transmission back again to the body. The ex vivo has more aggression for the patient and more cell-specificity than the in vivo method [42, 43]. Interferon-alpha (IFN-α) gene transferring to the liver by using a sustained expression shuttle vector may lead to long-term production of IFN- α in the liver. In a study, researchers examined the effect of an adenovirus expressing IFN-α in preventing viral hepatitis in mice [44]. It has been demonstrated the anti-HBV ZFNs can disturb viral DNA in cell culture without any cell toxicity and off-target cleavage [45]. The TALENs are similar in arrangement to the ZFNs. Bloom and colleagues showed that the Anti-HBV TALENs have nearly 87% targeted mutagenesis and 80% HBV gene expression reduction in a murine model [46].

GENOME EDITING STEM CELLS

Genome editing in iPSCs generates a population of differentiated cells that are valuable for disease modeling, drug improvement, genetic editing, and finally, for autologous cell therapy. New advancements in the genetic engineering area introduce several strategies such as transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated system (Cas9) that are powerful genome editing tools for iPSCs gene correction. When the gene-editing process is complete, the iPSCs can differentiate into HLCs and, consequently, engrafted to the target [47, 48].

As noted above, viral and non-viral vectors are available for the delivery and expression of a transgene to the liver. Viral vectors showed the best results in the previous investigations. The viral vectors divide into two groups: integrating (adeno-associated viruses or AAVs, lentivirus, retrovirus) and non-integrating vectors (adenovirus or Ad, poxvirus, herpes simplex virus, or HSV). Transgene expression stability and infection transmission rate of integrating viral delivery systems is satisfactory, but there is a risk of insertional mutagenesis. If an integrating viral vector inserts in an aberrant location on the host genome, leading to high expression of a proto-oncogene or inactivation of a functional and essential gene, which, in turn, it may have unintended consequences like malignant cell transformation. For example, 5 patients in clinical trials performed to treat severe combined immunodeficiency (SCID), developed leukemia that the cause of this phenomenon consider to retroviral-mediated gene transfer [49]. Collectively, researchers should be careful about integrating vectors because there is a risk of mutation in these vectors. Unlike the integrating viral vectors, the non-integrating viral vectors as adenoviral, poxviral, and herpes simplex viral vectors cannot integrate into the chromosomal DNA and remain in the nucleus as an episomal form [50, 51].

TALENs and ZFNs strategies have a severe defect: off-targets side effects that lead to low specificity; additionally, the massive size of TALENs and epigenetic variations damaging access to the preferred chromosomal site are substantial challenges for use in a clinical setting, but recently discovered CRISPR/Cas9 technology show higher efficiency [52]. CRISPR/Cas9 genome editing is a regular RNA-mediated acquired protection mechanism found in some prokaryotic organisms [53]. It has quickly become the most hopeful genome editing tool with the pronounced potential to modernize biomedical treatment [54].

CONCLUSION

The treatment of liver diseases has been complicated, which needs and requests for novel and improved knowledge. Liver inflammation can resolve spontaneously or progress to more advanced stages, i.e. fibrosis, cirrhosis, and liver cancer, respectively. These three late stages were considered irreversible phenomenon. Still, recent advancements in gene therapy, stem cell therapy, and genome editing open a good road to treating liver diseases.

Regarding the other types of liver diseases, it is possible to provide potentially effective treatments. Although there are many obstacles, especially in the preparation of different systems in terms of safety and efficiency, different gene delivery pathways, the immunogenicity of vectors used, and improving cells type specificity, still, the potential to correct genetic defects and increase the quality of life in patients is very promising. It was merging advanced gene-editing techniques with methods for using viral and non-viral vectors that can revolutionize medical sciences and cure many disorders. Improving these methods in the future could target many polygenic conditions, and the ability of these methods to regulate gene expression could provide more effective treatments for a wide kind of disease.

Among stem cell types, IPSCs technology has provided unique chances to study, inhibit and treat a variety of human diseases, especially liver diseases. The ability of these cells to regenerate themselves and differentiate has led to the total production of hepatocyte-like cells for transplantation and research. For example, these differentiated cells can be used in cell therapy to repair, replace, and regenerate liver tissue efficiently. In addition, IPSCs consider valuable resources for evaluating drugs interactions, metabolism, and toxicity of the drug and biological factors [55-57].

However, there are still barriers and limitations to using these cells that need to address. For instance, evidence on IPSC cell lines and reprogramming procedures and their differentiation into hepatocyte-like cells should be enhanced to increase their effectiveness.

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Immunopathogenesis and treatment of ulcerative colitis: from modern medicine to herbal medicines

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Abstract: *Ulcerative colitis is one of the idiopathic inflammatory diseases of the large intestine with an unknown cause, which occurs in recurrent attacks of inflammation in the lining of the mucosa and below the intestinal mucosa. Impaired mucosal immune responses, the involvement of inflammatory cytokines such as IL-1, TNF-α, and IL-6, and other inflammatory mediators such as arachidonic acid metabolites play an important role in the immunopathogenesis of ulcerative colitis. Based on medical findings on the pathogenesis of the disease, new therapies have focused on the responses of the intestinal immune system and the inflammatory process. However, the widespread use of treatments such as aminosalicylates and corticosteroids continues. Most of these drugs have severe side effects. Some patients do not respond well to these treatments and sometimes have to undergo surgery and colectomy. Therefore, extensive studies are being conducted to control and prevent disease to use natural treatment methods such as the use of antioxidants with the approach of removing free radicals that prevent the destruction of cells and tissues in the body. Today, various studies have pointed to the effects of herbal medicines as a complementary treatment in inflammatory diseases. Their low cost and high effectiveness have attracted the attention of researchers in this field. The aim of the present study is a comprehensive review of modern and traditional treatments based on herbal medicines in the field of ulcerative colitis.*

Keywords: *inflammatory bowel disease, ulcerative colitis, immunopathogenesis, modern medicine, herbal medicine*

INTRODUCTION

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) characterized by uncontrolled inflammation of the large intestine and rectum. The prevalence of UC has increased in the last two decades [1]. Due to the unknown causes of the disease, the high risk of recurrence, and the poor prognosis, UC has become a clinical challenge in terms of treatment. One of the prominent features of this disease is excretion in the form of bloody diarrhea. The disease is characterized by periods of recovery and relapse that may occur spontaneously or in response to treatment [2]. However, conventional therapies for UC treatment, in addition to being successful and preventing recurrence, may lead to a variety of side effects [3]. UC is characterized by innate immune responses. Neutrophils, the first line of innate immune cells, are responsible for intestinal tissue damage by releasing large amounts of toxic components and free radicals during stimulation during UC progression [4, 5]. Meanwhile, the response of type 2 T (Th) helper cells, including over-activity of normal T cells and non-classical killer cells and Th2 cells, and significant production of cytokines, e.g. IL-5 and IL-3, have been reported in the pathogenesis of UC. Higher cytokine levels have been reported in patients with UC, including IL-5, IL-13, and other

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proinflammatory cytokines such as tumor necrosis factor (TNF) (Figure 1).

Figure 1: Cytokines involved in ulcerative colitis.

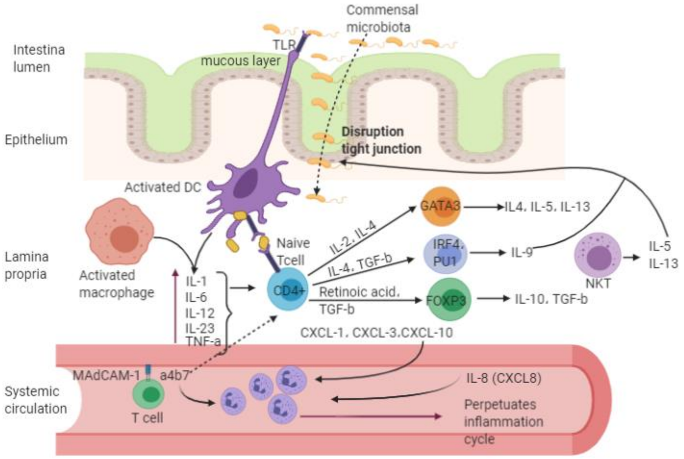

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After the release of immune cells, cytokines act to stimulate immune responses further, induce epithelial cell apoptosis, and regulate expression (claudin-2), here as damage to the epithelial barrier leading to disruption of the epithelial barrier. They become tightly connected to the intestinal epithelial cells [6-8]. Nuclear factor-kappa B (NF-κB) is a transcription factor responsible for the expression of a variety of genres, for example, TNF-α, in response to extracellular inflammatory stimuli [9]. Because high levels of TNF have been reported in the blood [10], fecal samples [11], and mucosa [12] of patients with active UC, it is widely accepted that NF-κB plays a pivotal role in UC progression. The association of NF-κB inhibition in IBD has been further demonstrated by the experimental treatment of colitis with an anti-NF-κB oligonucleotide that improves inflammation in the colon [13]. Infiltration of neutrophils and macrophages into the mucosal tissue of the colon is an important feature of ulcerative colitis. Activated neutrophils in the intestinal mucosa produce and secrete reactive oxygen species, including superoxide ions, hydroxide radicals, and hydrogen peroxide [14]. These factors cause lipid peroxidation, increase the permeability of mucosa and blood vessels, increase the entry of neutrophils into the mucosal tissue, and the development of inflammation [15]. Reactive oxygen species damage the intestinal wall, cause ulceration, bleeding, and diarrhea by affecting cytokine genes and enzymes involved in the inflammatory response. Oxidative stress also reduces antioxidant factors as a result of increasing disease severity [16, 17]. On the other hand, patients with ulcerative colitis are at risk for colon cancer for two reasons: lack of inflammation control and drugs such as anti-tumor necrosis alpha. The risk of cancer in these patients begins to increase after 8-10 years and increases by half to one percent per year [18-21]. For this reason, research is ongoing to find effective treatment with few side effects. The use of antioxidants can be effective in controlling the disease and preventing its recurrence. Antioxidant compounds can prevent cell damage by neutralizing free radicals [22]. Herbal medicine has been used for centuries as one of the most traditional medicine methods and clinical treatment of many diseases such as infections and functional disorders. Recently, the potential effect of herbs has been suggested as a UC treatment. But drugs such as aminosalicylates, corticosteroids, and immunosuppressants continue to be widely used. Amino salicylates are administered orally or topically (suppository and enema) and are safe and effective drugs and are always one of the first treatment options in the treatment of patients with mild to moderate forms of the disease and also for persistence. They are used to relieve symptoms. Corticosteroids are one of the most common therapy inactive diseases. These drugs can be given topically, orally, or intravenously. They have a great effect on active disease control but are not suitable for reducing symptoms in the long run (or maintenance treatment). Immune-suppressing drugs or immunosuppressants are also used to treat colitis. These drugs reduce inflammation but do so by suppressing the immune system response (which initiates inflammation) [23]. However, there is a constant effort to find better drugs. The aim of this study is a comprehensive review of modern and traditional therapies based on medicinal plants in the field of ulcerative colitis.

DISCUSSIONS

Modern medicine in the treatment of ulcerative colitis

As mentioned, the etiology of inflammatory bowel disease is unknown. Still, the influence of genetic predisposition, environmental factors, and immune system on each other has been evaluated, and the use of corticosteroid anti-inflammatory drugs, aminosalicylates, antibiotics, immunomodulators, and biological materials. They are effective methods in the treatment of ulcerative colitis [24]. Most of these drugs have severe side effects, including diarrhea, vomiting, fever, rash, growth retardation, anemia, and osteopenia, increased risk of infection, liver disorders, kidney disorders, pancreatitis, and gastrointestinal ulcers. Also, besides not all patients respond ideally to these therapies, and sometimes there is no choice but surgery and colectomy [25]. Prevention of colectomy, prevention of disease complications and death; and controlling inflammation with the least side effects of drugs are some of the important goals in treating ulcerative colitis [26]. In this part of the article, we describe modern medicine in the control and treatment of ulcerative colitis.

Amino salicylates

Amino salicylates have always been one of the first drug choices in treating patients with mild to moderate disease activity.

Sulfasalazine

Sulfasalazine is a combination of an antibacterial agent (sulfapyridine) and a salicylate (5-aminosalicylic acid). It was originally designed to treat rheumatoid arthritis and was first introduced in 1942 as a drug to treat inflammatory bowel disease (IBD). The effective therapeutic agent in treating IBD is 5-aminosalicylic acid and sulfapyridine is the only inactive carrier that allows this effective substance to be released in the colon. Despite the relative absorption of this drug in the small intestine, approximately 90% of the drug reaches the large intestine. After absorption in the large intestine, 20% of 5-aminosalicylic acid undergoes hepatic acetylation and is excreted in the urine. Approximately 15% of patients treated with sulfasalazine experience significant side effects that necessitate discontinuation of the drug [27-29].

Pentasa

One of the most common products is mesalazine, which contains micro granules coated with ethyl cellulose. The release of mesalazine in this product starts from the duodenum it continues throughout the small intestine and colon so that 50% of the 5-aminosalicylic acid of this product is released in the small intestine [28, 30]. Pentasa is a type of mesalazine released at the end of the ileum and colon, in a pH-dependent manner. The release of 5-aminosalicylic acid in this product, which occurs in the small intestine (release at pH above 7), is about 15-30% [28-32].

Closal

Used to treat active disease, it contains the 5-aminosalicylic acid molecule, which is attached to a benzoic acid derivative by azo. In the colon environment, 5-aminosalicylic acid is released by the bacterial enzyme reaction. Like sulfasalazine, almost the entire drug is transported intact to the colon, and its metabolites are rapidly excreted in the urine [33, 34].

Corticosteroids

Corticosteroids are one of the most common therapy inactive diseases. Prednisolone at a daily dose of 40 to 60 mg effectively treats severe to moderate disease the use of such systemic steroids should be limited to severe and refractory cases. Studies have shown that long-term use of corticosteroids does not affect the treatment of this disease. Although no study has directly compared the therapeutic effect of oral corticosteroids with injectable therapy, the injectable form is commonly used in more severe cases. Importantly, corticosteroids are not effective in maintaining improvement in ulcerative colitis, in addition to the side effects associated with corticosteroid use [35, 36].

Budesonide

Initially used to treat asthma and allergic rhinitis, it is structurally different from prednisolone. This structural difference has resulted in more local anti-inflammatory activity and a higher affinity for receptors than prednisolone. Budesonide has a metabolism of about 90% in the first hepatic passage. This low systemic bioavailability reduces the toxicity of the drug. The effect of oral budesonide in treating endoscopic findings similar to prednisolone has been reported [37, 38].

Antibiotics

Bacterial flora plays an important role in the pathogenesis of inflammatory bowel disease. In the case of infectious complications of inflammatory bowel disease, antibiotics have a therapeutic role in cases where the disease is active. But unlike Crohn's disease, these drugs are not very effective in treating an active disease or maintaining recovery in patients with ulcerative colitis [39, 40].

Immune-regulating drugs

The use of these drugs in the clinic for patients with inflammatory bowel disease is mainly due to the following reasons: 1 - Chronic active disease; 2 - Steroid-dependent disease; 3 - Long-term maintenance of disease recovery after treatment with biological agents; 4 - Prevention of the immune response to biological agents. Patients treated episodically with infliximab show a greater immune response to these agents, and concomitant use of immunomodulatory drugs reduces the immune response to Infliximab [41, 42].

Methotrexate

It is a folic acid antagonist that has anti-metabolic and anti-inflammatory activity. It is used in patients with Crohn's disease. Intramuscular injection of MTX at a weekly dose of 25 mg for 16 weeks is effective in patients with active Crohn's disease. A controlled study reported that a low oral dose of MTX of 12.5 mg per week was not effective in treating active ulcerative colitis. However, no controlled studies have been performed on the role of subcutaneous or intramuscular MTX in the active disease of ulcerative colitis [43, 44].

Cyclosporine A

This drug has been considered due to its immunomodulatory properties and therapeutic effect in patients with inflammatory bowel disease. Cyclosporine A may be appropriate in severe, fulminant, as well as steroid-resistant cases. For example, continuous infusion of cyclosporine can be effective in patients with severe colitis who have not responded to steroid therapy after 7 to 10 days. In one study, 82% of patients with active disease who did not respond to steroid therapy responded to intravenous cyclosporine-4 therapy. After six months of follow-up, 44% of respondents eventually underwent colectomy despite continuing maintenance treatment with oral CYA (45, 40). It seems that immunomodulators' use is effective in avoiding colectomy after induction of recovery by cyclosporine [45].

Immunosuppressive

Immunosuppressive drugs are commonly used when treatment with aminosalicylates and steroids is not effective or when steroids cannot be stopped to reduce inflammation without reactivating the symptoms and relapse. If the patient is dependent on treatment with steroid drugs, this method can be considered to receive immunosuppressive drugs, and then, slowly reduce or even stop using steroids without recurrence of inflammation. Slowly, for this reason, immunosuppressive drugs are sometimes called "steroid-sparing" drugs. In this group, azathioprine (Imuran, 2 to 2.5 mg/kg per day) and its active metabolite (6-mercaptopurine 6-MP); (1 to 1.5 mg/kg per day) as well as methotrexate and cyclosporine. Azathioprine and 6-mercaptopurine are effective treatments for persistent remission in both Crohn's disease and ulcerative colitis. Although their onset is slow (months), they are generally uncomplicated and well-tolerated. Other treatment regimens include intramuscular methotrexate to induce remission (15-25 mg weekly) and persistence remission (15-25 mg per week) in active Crohn's disease, as well as intravenous cyclosporine (2-4 mg/kg daily). Title Facilitators for severe ulcerative colitis are steroid-resistant. Due to the possibility of short-term or long-term side effects, and the need for careful follow-up, patients who need these drugs should be treated by a gastroenterologist [46].

Biological therapies

Recent advances in medical knowledge regarding the pathogenesis of inflammatory bowel disease have led to the introduction of new therapies that target specific aspects of the immune system and inflammatory processes in inflammatory bowel disease. One of these included in clinical therapy is treatment with anti-TNF-α. This cytokine drug is a key factor in the pathogenesis of inflammatory bowel disease. To date, only Infliximab (Remicade®), Certolizumab (Cimzia®), and Adalimumab have been shown to have a significant effect on inflammatory bowel disease. Recent data have shown that Infliximab is effective in inducing and maintaining recovery and the gradual reduction of steroids in patients with ulcerative colitis It should be noted that due to the increased risk of infection in patients using these drugs, examination of infectious foci such as chest mammography test before starting treatment is necessary [47-49].

Traditional medicine with a focus on herbs in the treatment of ulcerative colitis

Different types of herbs are used to control inflammation or reduce the symptoms of colitis. The herbal medicine includes a wide range of therapies within and outside the realm of modern medicine. However, the effectiveness of medicinal plants has limited evidence. Potential benefits of medicinal plants include high acceptance of these drugs by patients, good efficacy, relative safety, ease of use, and relatively low cost.

The effect of herbs on hundreds of people has been tested in ulcerative colitis. However, it seems that all over the world, herbal medicine has been widely used to treat and control the disease. In this part of the article, we will discuss some studies on medicinal plants concerning the control and treatment of ulcerative colitis.

Aloe vera

Aloe vera is a tropical plant used in traditional medicine around the world. Aloe vera has anti-inflammatory activity and has been used by some doctors for patients with UC. Aloe vera gel is widely used orally by patients with inflammatory bowel disease and is undergoing therapeutic evaluations for this disease [50]. This study aimed to investigate the effects of aloe vera in vitro on the production of active oxygen metabolites, eicosanoids, and interleukin-8, all of which can be pathogenic in inflammatory bowel disease. The antioxidant activity of aloe vera in free cell systems, radical generator, and luminescence was evaluated in a colorectal mucosal biopsy sample. The production of eicosanoids was measured by sampling and measuring interleukin eight by Caco-2 epithelial cells in the presence of Aloe vera by enzyme-linked ELISA. The study's findings showed that aloe vera gel had a dose-dependent inhibitory effect on the production of reactive oxygen metabolites. Aloe vera inhibited the production of prostaglandin E2 but so far had no effect on the production of B2 thrombosis and also reduced the release of interleukin-8 by Caco-2 cells. The results of this study show the anti-inflammatory effects of aloe vera gel in vitro and confirm that aloe vera gel can have favorable therapeutic effects in the treatment of inflammatory bowel disease [51]. In another study aimed at investigating the effect of aqueous extract of aloe vera gel on gastric acid secretion and colonic histopathology in the model of induction of acute ulcerative colitis by acetic acid enema on 32 Wistar rats (200-250 g); The animals were randomly divided into four groups: healthy group, acetic acid-induced colitis group, aloe vera treatment group (for eight days) and sulfasalazine treatment group. After ether anesthesia, the animals were induced with acute colitis using acetic acid. After treatment, colon biopsies were removed from the distal 10 cm section for histopathological studies. The scoring system (Bita 2012) was used to compare histopathological features quantitatively. Stomach acid concentration was measured by the titration method. The results were considered as the mean and standard deviation of expression, and $P < 0.05$ was significant. The results showed that treatment with an aqueous extract of aloe vera did not reduce gastric acid. Histological results showed an inflammatory response in the ulcerative colitis group. Aloe vera extract reduced the severity of histopathological symptoms of ulcerative colitis, although it was less effective than sulfasalazine. Thus, it can be concluded that according to the improving effects of this extract, it seems that this plant can be used to eliminate colon disorders. However, further studies are needed to determine the effective dose of this plant in reducing gastric acid secretion [52].

Jujube

Jujube (Ziziphus Vulgaris Lam) has been known as a medicinal plant in East Asian countries since ancient times. It is used in traditional medicine in these areas to treat diseases such as liver disorders, anemia, shortness of breath, vomiting, and cardiovascular diseases. Hypertension and gastrointestinal disorders are used [53, 54]. Phytochemical studies on different jujube species have shown the presence of compounds such as cyclopeptide alkaloids, flavonoids, sterols, tannins, and Triterpenoid saponins, and the key role of these compounds in scavenging radicals and proven, free. Numerous effects, such as antioxidant and anti-inflammatory effects have been attributed to this plant [55]. In a study by Yue et al. (2015) on the protective effects of jujube on inflammatory bowel disease, they induced colitis by injecting TNBS (Trinitrobenzene sulfonic acid) into the rat colon and found that jujube polysaccharides They significantly reduced the severity of colitis, which was characterized by less weight loss, decreased score of disease activity index, and improved mucosal damage in rat colitis. Jujube polysaccharides also inhibit inflammatory responses and reduce tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 IL-6), and myeloperoxidase (MPO) activity in rat colitis. They also found that by examining tight junction proteins, jujube improves intestinal wall activity, thus protecting against inflammatory bowel disease by enhancing intestinal wall function [56]. In a study conducted by Karawya et al. (2016), regarding the effects of jujube on acetic acid-induced colitis, they concluded that increased levels of myeloperoxidase and caspase-3 are associated with changes in the crypts and destruction of goblet cells. By administering jujube extract, a significant decrease in myeloperoxidase and caspase three was observed compared to the control group. Jujube extract was also found to act as a treatment for acetic-acid-induced colitis in rats [57].

Turmeric

Turmeric is known by its scientific name (Curcuma longa). Curcumin is the active ingredient in the rhizome of the turmeric plant with the chemical name (diferuloylmethane). In addition to Curcumin, it contains several chemical compounds, including volatile oils, zinc, aqueous acids, and aqueous acids, oats, oats, and oranges in the yolk sac [58-60]. In traditional medicine, turmeric is known as anti-flatulence, and its consumption with food improves digestion and reduces bloating. Also, its effect on stimulating and producing bile and thus digesting fats improves digestion. Therefore, this substance in people with chronic gastrointestinal disorders and people who feel tired and bloated after eating is recommended. In laboratory studies, oral administration of aqueous and methanolic turmeric extract to rabbits significantly reduces gastric acid secretion and Ingredients of gastric juice, Oral administration of turmeric methanolic extract reduces gastric juice secretion. It prevents damage caused by histamine, indomethacin, rasping, mercaptamine, methanol, hydrochloric acid, sodium hydroxide, and sodium chloride on the stomach and duodenum [61-63]. In one study, administration of 300 mg of turmeric twice daily to patients with duodenal ulcers showed that after four weeks, about 50% of patients recovered. After 8 and 12 weeks, all patients achieved complete recovery without any side effects [64]. Also, in a study performed on 98 patients with ulcerative colitis, the effect of this herbal product at a dose of 2 grams per day in the treatment and prevention of recurrence of the disease was significantly better than placebo. In another clinical study performed on ten patients with gastric ulcers, administration of 1 gram of turmeric per day accelerated wound healing and reduced gastric pain [65].

Curcumin

In a study to determine the therapeutic effect of Curcumin on ulcerative colitis caused by sodium dextran sulfate (DSS) and discover the relevant mechanism, 60 mice were randomly divided into 6 groups. A was the normal control group, B was the model group, and group C was the 1.5 mg/kg dexamethasone group. Groups D, E, and F received 15, 30, and 60 mg/kg of Curcumin, respectively. Mice were killed seven days after treatment. The expression of TNF-α and MPO in colon tissue was determined by ELISA and the expression of P38MAPK mRNA and P.P38MAPK was evaluated by immunohistochemistry and RT-PCR. The results obtained in groups F, D, C, E, TNF-a, and MPO levels decreased significantly. P38MAPK mRNA expression was decreased in groups F, E, C, D, and there was a significant difference between E and F. As a result, Curcumin has therapeutic effects, which play an important role in the treatment of UC by inhibiting the P38MAPK pathway and thus reduce TNF-α. Because there is a risk of recurrence and cancer in UC patients and Curcumin has beneficial effects in preventing cancer, Curcumin can prevent cancer. It should also be noted that Curcumin is inexpensive and has no specific side effects, and can lead to a new generation of anticancer drugs [66].

Silymarin

Silybum is a flavonoid compound extracted from the sage plant. This plant is an annual or biennial of the chicory family distributed in Europe and some parts of the United States and Iran [67, 68]. The substance in the seed extract of this plant includes silybin A and B, silibinin, apigenin, dihydrosilibin, dioxycylcysteine, silycrestin, dioxylcydianine, etc. Dried seed extract contains 1 and 4% of silymarin, which contains flavonoids such as silybin A and B, silybinine, silycristine, and dihydroxycylin [69]. This plant has antioxidant and anti-inflammatory properties [70]. Silymarin also acts as a cell membrane stabilizer and cell glutathione enhancer, responsible for detoxification and free radical scavenging in the body. Laboratory data indicate that silymarin stimulates neutrophils and chemo toxic and phagocytic activity. It does not affect. But when neutrophils are stimulated, silymarin prevents the release of myeloperoxidase. Inoculation of neutrophils with silybin prevents the movement of leukocytes (FLMP) [71]. In a double-blind, placebo-controlled trial in patients with alcoholic cirrhosis, treatment with silymarin increased leptin secretion, which induced lymphoblast deformity. Also, the number of T8 cells was reduced, and lymphocytosis was significantly suppressed compared to the control group [72]. In a study to evaluate the effect of silymarin on acetic acid-induced colonic ulcers in mice, 32 adult, Blab/C mice, weighing approximately 36 4 4 g were divided into four groups of 8. The face was intact and without induction of disease, and the control group had an intestinal ulcer (colitis) and was not treated. The wound treatment groups were treated with 40 and 80 mg/kg silymarin. Rats in the treatment group received silymarin daily for 14 days before the onset of the disease and then for one week after the disease´s onset. The drugs were given orally. To make a wound, 1 ml of 4% acetic acid was injected intrarectally. One week after the wound was diagnosed, part of the colon was removed for histological studies, and colonic lesions were examined by the Morty method. Alkaline phosphatase concentration was measured by ELISA. The extent of tissue edema was also assessed. Based on the results, the concentration of alkaline phosphatase enzyme in the wound group showed a significant increase ($P <0.05$) compared to the control group. In contrast, in the silymarin groups, this increase was significant (0.05). P <0) was controlled compared to the colitis group. As a result, it can be said that the use of silymarin can be considered as a proposed method in the treatment of disease [73].

Chamomile

Chamomile, with the scientific name of Matricaria chamomilla, is an annual, fragrant plant with a height of 20 to 40 cm that goes by car on farms and roadsides. The plant is now widely distributed in Europe, West Asia, North Africa, North and South America, and Australia. Chamomile is one of the oldest medicinal plants known to man, and its use dates back to ancient Greece [74]. In to investigate the effect of aqueous this plant in the animal model of colitis induction by acetic acid, five groups of 7 adult male NMRI rats in the weight range of 280-230 g were tested. Three groups received different doses of aqueous extract of chamomile (30 mg/kg, 20, 10), and the fourth group received the solvent orally. The last group was considered the control group. To induce colitis, rats were kept on an empty stomach for 36 hours and then subjected to mild anesthesia with ether.

2 ml of 4% acetic acid was injected into the colon. Twenty-four hours later, macroscopic observations of the stool showed signs of colitis. The results showed that in this study an aqueous extract of chamomile at doses of 30 and 20 mg/kg significantly reduced the ratio of weight to colon length. Also, the highest dose of the extract (30 mg/kg) reduced the severity and extent of inflammation. Histopathological studies of the colon showed that the therapeutic effect of chamomile aqueous extract at doses of 30 mg/kg and 20, 10 is more complete with increasing dose. As a result, it can be concluded that aqueous chamomile extract is effective in the treatment of ulcerative colitis in this model and has significantly reduced inflammatory and ulcerative parameters of ulcerative colitis [75].

Ginger

Ginger, which is used as a spice in various parts of the world, is a complementary treatment for some diseases, especially gastrointestinal diseases such as nausea and vomiting, and its anti-inflammatory, antioxidant and anti-ulcer properties have already been proven. It is classified in the Emonographs Valley as a plant with its properties and drug interactions [76]. A study aimed at investigating the effect of ginger as an anti-inflammatory and antioxidant food on quality of life, disease activity and serum levels of some inflammatory factors and oxidative stress in patients with mild to moderately active ulcerative colitis 46 patients with colitis Mild to moderate active took four capsules daily containing 500mg of dried ginger root powder or similar placebo for six weeks with meals. Use of accurate and valid questionnaires and serum levels of total antioxidant capacity (TAC), malondialdehyde (MDA), Tumor necrosis factor α (TNF-α), high sensitivity C-reactive protein, and nuclear factor kappa B (NF-kB) were evaluated before and after the intervention. The results showed that ginger consumption reduced the level of serum MDA factor in the experimental group compared to the placebo group ($P = 0.04$). Also, Besides TNF-α and disease activity in the ginger group decreased after six weeks of intervention compared to the beginning of the study. There was an increase in urinary incontinence from 1% to 3% ($P > 0/05$). It was concluded that consumption of 2 g of dried ginger root powder for six weeks reduces oxidative stress in patients with mild to moderately active ulcerative colitis [77].

Turpentine (Pistacia atlantica)

One of the plants used in cancer treatment prescriptions is turpentine. It is known as Bane and grows in the cities of central and western Iran. The scientific name is turpentine (Pistacia atlantica) and from the family Pistachio or Anacardiaceous [78, 79]. This study aimed to evaluate the therapeutic and protective effects of turpentine oleoresin in acetic acid-induced colitis in rats. Turpentine oleoresin dissolved in twine 80 (1% v/v) at doses of 100, 200, and 400 mg Kg was administered orally to animals. In this treatment, three days after induction of colitis, mice were treated for six days. After determining the most effective dose in the treatment, in this method, the animals were treated for six days with the optimal dose of gum. Finally, for microscopic and macroscopic examinations, the animals were anesthetized with ether, and the colon was removed. Based on the results obtained in turpentine oleoresin treatment in a dose-dependent manner, it showed significant effects in inducing recovery in acetic acid-induced colitis in the rats ($P < 0.05$). But in the protective method, no significant difference was observed between the treatment and negative control groups ($P > 0.05$). The results of this study showed the beneficial anti-inflammatory and wound healing properties of turpentine oleoresin in the treatment of acetic acid-induced colitis in rats [80].

Frankincense (Boswell serrate)

Frankincense extract has been used for many years to treat arthritis, heal wounds and strengthen the female hormonal system. According to previous studies, frankincense can positively affect brain development and possibly the formation of dendrites and axons and improve the relationship between them [81]. Frankincense has been used as an anti-inflammatory, anti-arthritis، analgesic in treating diseases related to chronic intestinal diseases, asthma, cerebral edema, and other diseases. Several clinical studies have confirmed their biological activity, and studies that have specifically investigated the mechanism of action of this plant have confirmed their anti-inflammatory and anti-tumor activity. Bosolic-acids are considered the main active ingredient in frankincense and are responsible for its therapeutic effects. In a study aimed at investigating the therapeutic effects of frankincense, based on the results of studies reviewed in this article, frankincense with anti-inflammatory, anti-arthritis, antimicrobial, and analgesic effects and with the least side effects in traditional and modern medicine in the recommended therapeutic doses can be effective in treating many diseases [74]. In vitro, animal studies and models have shown that boswellic acid can inhibit selective 5-lipoxygenase with the effects of inflammation and arthritis [83]. Studies show that Bosolicacid derivatives have strong anti-inflammatory properties and do not have the known side effects of steroids. Among these derivatives, AKBA and KBA are the strongest inhibitors of the 5-LOX enzyme with IC IC502.8 and M1.5 In different clinical studies, the efficacy of frankincense extract at a dose of 300 mg 3 times a day has been comparable with sulfasalazine and mesalazine to treat Crohn's disease and colitis [84].In a study of 30 patients with chronic UC, 20 patients were given 900 mg of frankincense gum daily in 3 doses for six weeks, and ten patients were given 3 grams of sulfasalazine daily in 3 doses for six weeks. This study showed that frankincense has beneficial effects on the disease because 14 patients out of 20 patients were directed to recovery and 18 patients out of 20 patients showed improvement in one or more parameters. In the sulfasalazine group, 4 out of 10 patients showed improvement, and 6 out of 10 patients showed improvement in one or more parameters [85]. In animal models, studies have shown that frankincense is effective in the treatment of inflammatory bowel diseases, including colitis and Crohn's disease [86].

Licorice

Licorice (Glycyrrhiza glabra L) is a perennial herb [87]. Licorice root contains various compounds such as flavonoids, sterols, amino acids, starches, essential oils, and saponins. Its major saponin is glycerin or glycyrrhizin [88, 89]. In addition to the above compounds, licorice root contains other compounds such as beta-glucuronosyl-glucuronide, asparagine, goose, sucrose, resin, volatile oils, flavonoid compounds as well as Coumarin compounds [90, 91].

One of the most well-known therapeutic properties of licorice rhizome has been its use in gastric ulcers [92], and its flavonoids are anti-Helicobacter pylori [93]. Licorice derivatives increase the prostaglandins concentration, inhibit gastrin secretion, and increase the lifespan of gastric epithelial cells, thereby healing gastric ulcers [94]. Some studies indicate the effects of some compounds of licorice extract on the gastrointestinal tract.

Evidence has also reported that glycerin in licorice can improve intestinal mucosal inflammation in mice by reducing the expression of NF-κB, TNF-α, and ICAM-1 in inflamed mucosa [95]. Clinical studies on licorice combined with other herbs have shown that licorice can be effective in controlling UC [96]. The anti-estrogenic function of glycerin has been proven to be high in concentration. Glycerin binds to estrogen receptors, thus acting as an antagonist for estrogen. However, estrogenic activity has also been reported for licorice and attributed to its isoflavone compounds [97]. Studies show that glycyrrhizin may inhibit the effect of mineralocorticoids by acting on 11b-hydroxysteroid dehydrogenase. Evidence has shown that glycerin can also suppress plasma renin activity and aldosterone secretion. Also, besides studies have shown that licorice has chemoprotective effects through its effect on Bcl-2 /Bax and is also effective in inhibiting cancer [98, 99].

Ulmus fulva

Ulmus fulva is a plant that can naturally prevent inflammation and help heal damaged and injured gastrointestinal tissue. It also helps to facilitate excretion naturally. Ulmus fulva extract has a beneficial role in maintaining the health of the gastrointestinal tract, especially the stomach. It should also be noted that this plant plays an important role in eliminating nutritional deficiencies and electrolyte imbalances in the body. This effective herbal substance can help control possible anemia by stopping bleeding. This herbal substance is a supplement that is made from the bark of the Ulmus fulva tree and is in powder form. This plant has long been used by Native Americans to treat coughs, diarrhea, and other gastrointestinal ailments. A recent study on slippery elm as a supplement for the treatment of IBD confirmed the antioxidant effects of slippery elm for use in patients with IBD. Research so far has been promising, but the available evidence is insufficient for the widespread use of slippery elm in the treatment of IBD [100,101].

Triticum aestivum

Triticum aestivum is used to treat various gastrointestinal problems. In a study in which Triticum aestivum was used for one month in patients with UC with conventional colitis medications, clinical outcomes improved in 78% of patients. Those who received a placebo improved 30% of clinical outcomes. The dose of Triticum aestivum is initially increased to 20 ml per day and 20 ml per day to a maximum of 100 ml per day. Triticum autism appears to be effective and safe as an adjunct therapy in the treatment of UC [102].

Bromelain

One of these supplements has long been used as a wound healer in traditional medicine, one of which is pineapple [103]. Pineapple contains phytonutrients, vitamin C and Bromelain. Bromelain is derived from the root of the pineapple fruit, and according to laboratory and clinical studies, several properties have been mentioned for it [104, 105]. Human and animal studies demonstrate the beneficial effects of Bromelain in reducing pain, wound healing and burn debridement, as well as its anti-inflammatory, anti-edematous, anticoagulant and cumulative platelet inhibitory properties. The major pharmacological effects of Bromelain are related to its proteolytic properties [104-106]. Studies have shown that oral Bromelain decreases dose-dependently levels of bradykinin, plasmacins, prostaglandin E2, and thromboxane B2 in patients with inflammation [105, 107]. Bromelain, Bromelain selectively inhibits thromboxane and changes the ratio of thromboxane to prostacyclin in favor of anti-inflammatory prostacyclin [108]. Also, besides it has been shown that Bromelain stimulates leukocytes to increase interleukin-1, 6, 8 levels and alpha tumor necrosis factor in the inflammatory region. Increased cytokines, especially interleukin 8, lead to the uptake of granulocytes and monocytes into the inflammatory region and increase phagocytic and chemotactic, chemotaxis activity [104, 105, 109, 110]. The anti-edematous properties of Bromelain are due to increased vascular permeability following fibrinolysis and increased circulating edema fluid reabsorption. Fibrinolysis activity of bromelain has been attributed to increased conversion of plasminogen to plasmin. Plasmin also breaks down fibrin, thereby increasing vascular permeability [106]. Other studies have shown that nutritional supplements, including Bromelain, trypsin, and papain, are effective in wound healing [103]. Other experimental results also show that the combination of bromelain and trypsin is effective in wound healing and reduction of edema and inflammation in those who have undergone major abdominal laparotomy [111]. Bromelain has anti-inflammatory properties and is used as a facilitator of gastrointestinal excretion and blood thinner. It is also used to treat sports injuries, sinusitis, osteoarthritis, and swelling from bromelain. Bromelain has been studied for use as an adjunct in the treatment of IBD, especially treating ulcerative colitis. Emerging research on pineapple shows that bromelain is an active ingredient in pineapple that may help treat UC by relieving inflammation. The mechanism that is primarily responsible for its anti-inflammatory effects is still unknown. However, proteolytic activity has been identified for the anti-inflammatory effects of bromelain on T cell activation and cytokine secretion in vitro and mouse models of inflammatory diseases [112, 113]. The major anti-inflammatory mechanism of action of bromelain appears to be proteolytic activity. However, there is also evidence of hormonal activity, such as acting through the intracellular signaling pathway. Bromelain has been reported to help reduce cell surface receptors, such as the hyaluronic acid receptor CD4, which is involved in leukocyte migration and the induction of inflammatory mediators [114, 115]. Also, besides bromelain has been reported to significantly reduce CD4 T cells, which in animal models are the primary inflammatory factors in the gut [116]. In a study performed on a clinical trial of bromelain in the treatment of mild ulcerative colitis, clinical and endoscopic evidence showed improvement [117].

Qing Dai

Qing Dai herbal medicine is traditionally used in Chinese medicine to treat patients with UC, but there is a lack of published information about the effects of Qing Dai in the treatment of UC. In a study of the mechanisms of action of Qingdai, nine patients with active UC who received common medications but were willing to take Chinghai as an alternative drug were studied. UC severity was determined based on the clinical activity index (CAI). Also, besides 5 out of 9 patients were evaluated endoscopically based on the Mats grading system. Each patient received 2 grams daily of Qing Dai orally and continued with other UC medications as prescribed. Electron rotation resonance was used to discover the working mechanisms of Qingdao. After four months of treatment with Qingdai, the CAI score decreased. Similarly, the degree of endoscopic mats decreased. Six of the seven patients treated with prednisolone at the time of enrollment were able to discontinue the corticosteroids. Electron rotation resonance showed that Qingdai has a strong hydroxyl radical scavenging activity. Qingdai showed significant clinical and endoscopic efficacy in patients who failed to respond to conventional medications. Modification of the activity of hydroxyl radicals seems to be a potential mechanism by which this drug acts. Still, the final decision on the ability to produce therapeutic effects of Qingdai in the treatment of colitis is due to the anti-inflammatory effect of this drug in the treatment of UC patients has shown that further research is needed on the therapeutic effects of this herbal medicine [118].

Tea grass

Tea grass (Hypericum perforatum), a perennial plant with yellow flowers belonging to the Clusiaceae family, is used to treat anxiety, depression, ulcers, and as an antioxidant, analgesic, and nervous system protector [119]. This plant has antibacterial and antiviral effects [120], which acts by inhibiting the transcription factor NF-Kb [121] and with serine/threonine kinase) which belongs to the protein kinase C family (PKC) [122]. Tea grass has a significant effect on wound healing and an anti-inflammatory effect [123]. The beneficial effect of tea grass on the healing of oral mucosa in the golden hamster has been investigated [119]. In a study, the effects of tea grass on histopathological changes and Malondialdehyde (MDA) levels obtained from colonic tissue of rats with colitis were evaluated. Seventy male Sprague Dawley rats were divided into seven equal groups. Colitis was caused by acetic acid in them. Groups 1 and 2 received one cc of oral solution at 600 and 300 mg, respectively. In groups 3 and 4, 1 cc of the gel was enema 20% and 10%, respectively. In group 5, 1 cc of Asacol was injected as a positive control group, and one cc of normal saline was injected as a negative control group in group 6. Group 7 received only the base gel via enema. Rat colon samples were evaluated for histopathological changes and tissue MDA after seven days of treatment. Based on the results, tea grass extract in both oral and enema forms had reduced therapeutic effects on the rats' tissues in which colitis had developed by reducing MDA activity. In the enema form, 20% gel showed a better effect than 10% gel. In the oral form with a dose of 600 mg/kg, a better therapeutic effect was observed than 300 mg/kg. Therefore, it can be concluded that tea grass can be used as a treatment of choice, especially in the form of enema in colitis [124].

Other treatments under consideration

As medical knowledge of the pathogenesis of inflammatory bowel disease progresses, the search for more effective treatments with fewer side effects continues. Some of these new therapies for Crohn's disease include Certolizumab and Adalimumab.

The treatments under consideration for the treatment of ulcerative colitis include peroxisome proliferator-activated receptor gamma (PPAR-anti-visilizumab, gamma) integrin therapy [32].

CONCLUSIONS

At present, medical treatment for ulcerative colitis is not entirely desirable, and none of the drugs that are now available and commonly used to treat this disease completely cure the disease. Continuous advances in the basic sciences related to ulcerative colitis provide the basis for further understanding of the cause of this disorder. Studying herbal medicines and using them in combination with other medicines used in the treatment of ulcerative colitis can open bright horizons for doctors to control this disease, which of course for the use of herbal medicines along with modern medicine. Extensive studies are needed.

We hope that these studies will lead to more specific and effective treatments for the complete cure of these diseases.

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Conflict of interest

No conflict of interest is reported by the authors.

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Can peritoneal carcinomatosis of colorectal origin be treated with oral everolimus alone or in combination with capecitabine? Preliminary results

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Abstract: *Background: We wanted to evaluate the effectiveness of oral everolimus alone and in combination with capecitabine treatment on colorectal cancer-induced peritoneal carcinomatosis animal model.*

Methods: Caco-2 colon adenocarcinoma cells were injected intraperitoneally to BALB/cOlaHsd-Foxn1nu mice to establish peritoneal carcinomatosis. Mice were divided into four groups (everolimus, everolimus plus capecitabine, capecitabine, control group) with 2 mice in each group. Treatment was initiated 5 days after inoculation of Caco-2 cells. 7.5 mg/kg everolimus was administered orally every 2 days. 2.1 mmol/kg capecitabine was administered orally every 5 days a week. 22 days after inoculation of Caco-2 cells, mice were sacrificed.

Results: The mean weight of the tumor was 25 ± 7.07 mg in the control group (n=2); 0.7 ± 0.7 mg in the everolimus group (n=2); 0.28 ± 0.36 mg in the capecitabine group; 0.48 ± 0.07 mg in the everolimus plus capecitabine group.

Conclusion: Tumor samples excised from BALB/cOlaHsd-Foxn1nu mice revealed that everolimus alone, and in combination with capecitabine suppressed tumor growth. However, a comparison of tumor weights revealed no statistically significant difference within the four groups (p=0.227). To reach statistically significant data, the sample size should be increased.

Keywords: *colon cancer, everolimus, peritoneal carcinomatosis*

INTRODUCTION

Peritoneal carcinomatosis secondary to colorectal cancer progression is characterized by the formation of solid tumor deposits on peritoneal surfaces [1]. Even today, chemotherapy protocols plus cytoreductive surgery and hyperthermic intraperitoneal chemotherapy applied for the treatment of metastatic colorectal cancer have been able to increase the median survival up to between 20-62 months at most [2-4]. Moreover, chemotherapy protocols used for the treatment of advanced colorectal cancer contain intravenous (iv) interventions and hours of chemotherapeutic agent infusions, which reduce both patient compliance and comfort.

In search of novel treatment options for peritoneal carcinomatosis of colorectal origin, an article published by Wagner et al. in 2009 drew our attention, in which they claimed that effective treatment of advanced colorectal cancer by rapamycin and 5-fluorouracil (5-FU)/oxaliplatin was achieved [5]. Rapamycin is both an antibacterial macrolide group antibiotic and a mammalian target of rapamycin (mTOR) inhibitor [6, 7]. Phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR signaling pathway is a central regulator in cell proliferation, cell growth, and angiogenesis [8-10]. Inhibition of mTOR by rapamycin prevents tumor proliferation and growth [7, 11]. Everolimus is the derivative of rapamycin that can be used orally and it has a higher

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bioavailability than rapamycin [12, 13]. Moreover, oral capecitabine treatment in metastatic colorectal cancer is equivalent to iv 5-FU plus leucovorin treatment has given the time to progression of the disease and overall survival [14]. Wagner et al. revealed that when oral rapamycin was administered in combination with iv 5-FU, colorectal cancer-induced peritoneal carcinomatosis was suppressed in a mouse model. However, 5-FU can only be used intravenously. Therefore, we thought that the treatment of peritoneal carcinomatosis with chemotherapeutic agents that could be used orally would both facilitate the compliance of the patient with the treatment and increase the patient's comfort. Thus, this study aimed to administer everolimus and/or capecitabine orally to BALB/cOlaHsd-Foxn1nu mice with colon cancer-induced peritoneal carcinomatosis to examine whether tumor regression was achieved.

MATERIALS AND METHODS

Local ethics committee approval was obtained before the study (Approval number: 2013/60).

Cell Lines and Chemical Substances

Caco-2 human epithelial colorectal adenocarcinoma cells were purchased from American Type Culture Collection (ATCC®, Rockville, MD, USA). Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), Dulbecco's Phosphate Buffered Saline (D-PBS), 0.05% trypsin-EDTA, and trypan blue stain 0.4% from Gibco® was purchased. Everolimus (Certican 0.75 mg, Novartis) and capecitabine (Xeloda 500 mg, Roche) were supplied from the market.

Animal Model

Eight BALB/cOlaHsd-Foxn1nu mice, 14-18 week-old female, weighing 14.1-24.2 g were used within this study. BALB/cOlaHsd-Foxn1nu mice were purchased from Harlan (Israel). BALB/cOlaHsd-Foxn1nu mice have housed in individually ventilated cages at 12/12 hours light/dark cycle at 23° C, and mice were fed with Altromin brand 1414 TPF catalog feed.

Preparation of Caco-2 Cell Culture

Caco-2 human epithelial colorectal adenocarcinoma cells were kept and propagated in DMEM medium containing 10% FBS within a 5% CO_2 incubator at 37°C. Cells were trypsinized when the cells completely covered the culture flask base. Afterward, these cells were counted and the viability of the cells was checked. The trypsinization procedure was performed as follows: The medium in the flask was poured out and washed twice with 10 ml D-PBS. 3 ml of 0.05% trypsin-EDTA was added to the cells and the cells were kept in an incubator containing 5% CO_2 at 37°C for 5 minutes. Cells were collected in a 50 ml centrifuge tube with the help of a Pasteur pipette. Cells were centrifuged at a 1200 rate per minute (rpm) for 5 minutes. After centrifugation, the supernatant was discarded. The pellet was mixed with 1 ml medium. To evaluate the viability of Caco-2 cells, 10 µl of the pellet which was mixed with 1 ml medium was taken. In a 1.5 ml Eppendorf tube, 10 µl pellet and 190 µl, trypan blue stain 0.4% (dilution factor 20, 10 µl pellet in 200 µl mixture) were mixed. 10 µl of this mixture (10 µl pellet and 190 µl trypan blue) were taken and placed on Thoma slide. The viability and number of cells on Thoma slide were determined with a 5x magnification light microscope. (There are 64 squares in four different areas on Thoma slide, and cells in each frame were counted). The total number of cells counted was divided by four to find out the mean value. The mean number of cells x 104 x dilution factor x 1 (1 ml of medium mixed with pellet after centrifugation) formula was applied to determine the number of cells in 1 µl.

Intraperitoneal injection of Caco-2 cells

1,500,000 Caco-2 human epithelial colorectal adenocarcinoma cells were injected intraperitoneally into each BALB/cOlaHsd-Foxn1nu mouse.

Treatment Protocol

Mice were randomly divided into 4 groups (everolimus, everolimus + capecitabine, capecitabine, control group). There were two mice in each group. The mice were taken to laboratory conditions one week before the experiment.

Treatment was started 5 days after intraperitoneal injection of Caco-2 cells into mice. Everolimus was diluted with saline (0.9% NaCl) and administered to two mice orally by gavage every two days at a dose of 7.5 mg/kg [15]. Capecitabine was diluted with saline and the maximum tolerated dose of capecitabine was administered to 2 mice 5/7 days per week by oral gavage at a dose of 2.1 mmol/kg/day (1 mmol capecitabine is 359 mg) [16]. Only PBS was given to 2 mice in the control group by gavage (Table 1).

Mice were daily evaluated. 22 days after the injection of Caco-2 cells into the intraperitoneal cavity, 17 days after starting treatment, all mice were anesthetized with intraperitoneal 50 mg/kg ketamine HCl and 5 mg/kg xylazine, and the mice were sacrificed by cervical dislocation method.

Surgical Procedure

Abdominal skin was opened by protecting the peritoneum with a midline incision. Saline solution was injected into the intraabdominal cavity and aspirated. Intraabdominal peritoneal washing fluid samples were reserved for cytological examination (Figure 1). Afterward, the peritoneum was excised without damaging other organs and the tumor developed at the intraperitoneal injection site was excised. The dimensions of the total tumor tissue removed were measured and the tumor was weighed. Tumor tissue formed in the peritoneum, mesenteric lymph nodes, liver, spleen, kidney, small intestine, colon, stomach, and intraperitoneal injection site were placed in formaldehyde solution for pathological examination.

Table 1: Treatment protocol

	Everolimus	Everolimus + Capecitabine	Capecitabine	Control Group
Number of Mice	2	2	2	2
Dose	7.5 mg/kg	7.5 mg/kg + 2.1 mmol/kg/day	2.1 mmol/kg/day	PBS*

**PBS: Phosphate-buffered saline*

Figure 1: (A) 0.9% NaCl was given into the abdominal cavity intraperitoneally and aspirated. Cytological examination of the aspirated fluid was investigated for malignant cells. (B) Excision of the peritoneum.

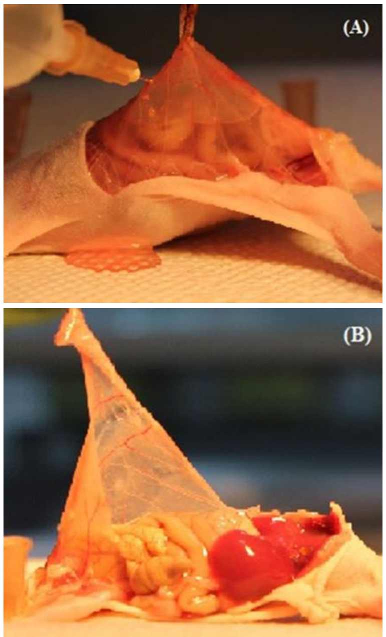

Statistical Analysis

Statistical analysis was performed using SPSS version 15.0. Kruskal-Wallis analysis of variance was used to evaluate the differences between continuous variables. $P<0.05$ was considered statistically significant.

RESULTS

Control Group

In the first mouse within the control group, a 1.5 cm lesion was observed where Caco-2 cells were injected in the left lower quadrant of the abdomen. The tumor which was located intraperitoneally was excised. The tumor size was 2x1.5x1.5 cm weighing 20 mg (Figure 2). No macroscopic tumor was found in other intraabdominal organs. Poorly differentiated adenocarcinoma was detected in the pathological examination of the tumoral lesion excised from BALB/cOlaHsd-Foxn1nu mouse.

Figure 2: (A) Tumor in the left lower quadrant of the BALB/cOlaHsd-Foxn1nu mouse within the control group. (B) The excised intraperitoneal tumor was 2x1.5x1.5 cm.

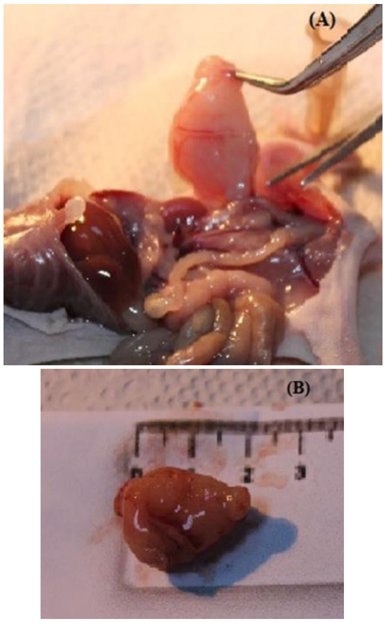

In the second mouse within the control group, the size of the lesion was 2.5x1.8x1.5 cm weighing 30 mg. The pathological examination of the excised tumor revealed poorly differentiated adenocarcinoma. No tumor, metastasis, or infiltration was detected in the peritoneum, mesenteric lymph nodes, liver, spleen, kidney, small intestine, colon, and stomach. No tumor cell was detected in the cytological examination of the peritoneal washing fluid.

Everolimus Group

During exploration, a tumoral lesion 0.2x0.4x0.4 cm in size, weighing 0.2 mg was detected in the first mouse. The cytological examination of intraabdominal fluid revealed no tumor cells. Tumor size was 1x0.6x0.4 cm weighing 1.2 mg in the second mouse. In addition, metastatic mesenteric, peripancreatic, and perisplenic lymph nodes were detected in the pathological examination. At the same time, two tumor deposits were detected on the small intestine surface. However, no tumor cell was detected in the cytological examination of the peritoneal washing fluid.

Capecitabine Group

In the first mouse given capecitabine, the tumor was associated with peritoneal tissue in exploration. Tumor size was 0.2x0.1x0.3 cm (Figure 3). No other macroscopic tumor tissue was detected. In the second mouse treated with capecitabine, a tumor 0.4x0.5x0.6 cm in size was detected. Apart from the other mice, poorly differentiated adenocarcinoma infiltrating diaphragm striated muscle was detected.

Figure 3: (A) Tumoral lesion detected on the peritoneum. (B) Poorly differentiated adenocarcinoma metastasis within the mesenteric lymph node (Hematoxylin&eosin; x40 magnification).

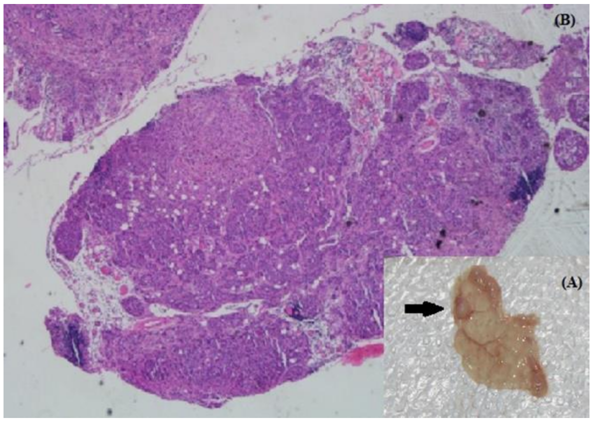

Everolimus plus Capecitabine Group

Tumor size was 1.2x1x0.8 cm in the first mouse which was treated with both everolimus and capecitabine. However, pathological examination revealed poorly differentiated adenocarcinoma metastases in mesenteric lymph nodes. Tumor size within the second mouse was 0.5x0.6x0.4 cm (Figure 4). No malignant cells were detected in other intraabdominal organs, peritoneum, and peritoneal lavage fluid.

Comparison of Tumor Weights

In the control group, tumor weights were 20 mg and 30 mg. In the group treated with everolimus tumor weights were 0.2 mg and 1.2 mg. Within the group treated with capecitabine, tumor weights were 0.03 mg and 0.54 mg. The tumor weights were 0.43 mg and 0.54 mg within the group treated with both everolimus and capecitabine (Figure 5).

Figure 4: (A) Significant tumor growth was not observed in the localization, where Caco-2 cells were injected. (B) Tumor size was 0.5x0.6x0.4 cm.

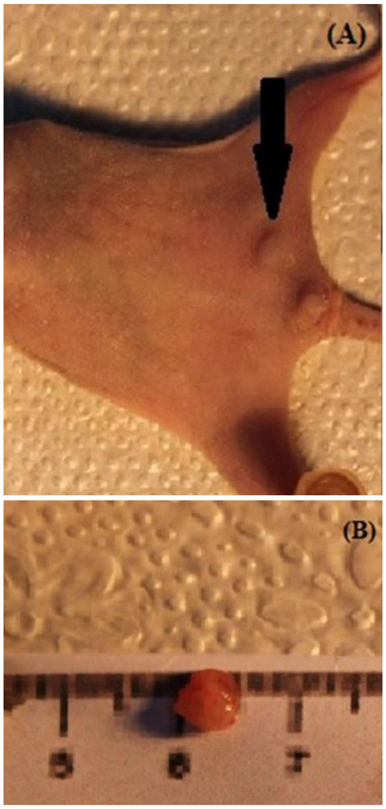

Figure 5: Weights of tumor samples excised from BALB/cOlaHsd-Foxn1nu mice. In mice treated with everolimus and/or capecitabine, tumor growth was suppressed compared to the control group. However, there was not a statistically significant difference between the four groups (p=0.227)

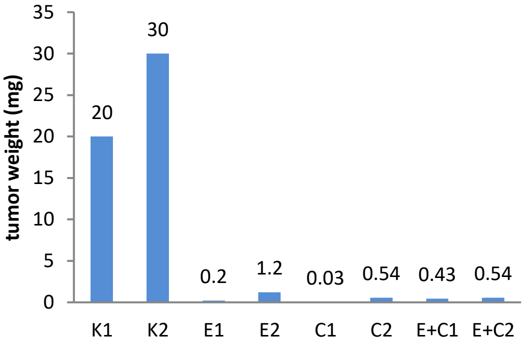

C: Capecitabine; E: Everolimus; K: Control

DISCUSSION

The mTOR (mammalian target of rapamycin) is a serine/threonine-protein kinase [17]. There are two mTOR complexes, mTORC1 and mTORC2 [18, 19]. mTORC1 includes mTOR, Raptor (regulatory-associated protein of mTOR), and mLST8. mTORC2 contains mTOR, Rictor (rapamycin-insensitive companion of mTOR), mLST8, and mSIN1 (mammalian stress-activated protein kinase interacting protein). The mTORC1 eukaryotic translation initiation factor controls protein synthesis through the ED (elF4E) binding protein (4E-BP1) and p70S6 ribosomal kinase (S6K) [20]. mTORC2 is involved in cell growth. Rapamycin combines with FK506 binding protein (FKBP) 12 to block mTOR activity. The mTORC1 is sensitive to rapamycin, while mTORC2 becomes susceptible to rapamycin only if it is exposed to the rapamycin effect for a long time [21]. Rapamycin-like drugs such as everolimus, temsirolimus, and ridaforolimus which are called rapalogs, have been produced after the anti-tumoral properties of rapamycin were discovered [22].

Nowadays, everolimus is used because of its tumor suppression feature in patients with subependymal large cell astrocytoma, advanced breast cancer, progressive pancreatic neuroendocrine tumor, subependymal giant cell astrocytoma, and advanced renal cell carcinoma [22]. In addition, capecitabine is used for the treatment of colorectal cancer and metastatic breast cancer [12, 23-25]. The side effects of both everolimus and capecitabine are very well known in detail because they are included within the current treatment protocols [26-28].

The positive effect of everolimus on peritoneal carcinomatosis has been demonstrated in several experimental models. Taguchi et al. used 58As1 human stomach cancer cell series, which were prone to peritoneal metastasis, to demonstrate the effectiveness of everolimus in advanced gastric cancers with peritoneal spread, and showed that everolimus significantly reduced peritoneal spread [29]. Chu et al. tested everolimus on a human colorectal carcinoma cell line with KRAS mutation in a mouse model and revealed that everolimus alone had significant antitumor activity [30]. Mabuchi et al. reported that everolimus delayed tumor onset and progression in the transgenic mouse model in ovarian cancer [31]. Moreover, studies on humans investigated the effects of everolimus on tumor growth when used as a single agent. Okamoto et al. published the results of phase 1 studies on daily everolimus treatment for patients with advanced solid tumors and reported significant tumor shrinkage in patients with esophageal and gastric cancer with 10 mg/day everolimus treatment [32]. However, Yoon et al. stated that 10 mg/day of everolimus treatment had only a limited impact on advanced gastric cancer [33]. There have been also phase studies on human beings with everolimus and capecitabine. Deenen et al. published the results of the phase I study of oral capecitabine and everolimus treatment in patients with advanced solid cancers [34]. In this study, 18 patients with the primary pancreas, major duodenal papilla, esophageal, gallbladder, brain, osteosarcoma, and hepatocellular cancer were treated with capecitabine 1.000 mg/m^2 bid and everolimus 10 mg/day every 3 weeks for 14 days, and prolonged clinical efficacy was observed in 39% of patients with advanced solid malignancies. Lim et al. published the results of a phase I study of capecitabine and everolimus treatment for patients with previously treated metastatic gastric cancer [35]. Fifteen patients with metastatic stomach cancer who had progression after chemotherapy were included in the study. With 650 mg/m^2 capecitabine twice daily and 5 mg everolimus twice daily, the median time without progression of the disease was 1.8 months (range: 0.8–2.8 months). At the same time, the reduction in the longest diameter of the tumor was 28.7%.

In our study, tumors formed in mice within the control group were approximately x20 times larger than tumors excised from other groups, however, tumor growth in the control group was limited only to the inoculation site of Caco-2 cells. Everolimus and capecitabine treatment suppressed primary tumor growth in the region where Caco-2 adenocarcinoma cells were injected. No liver toxicity or mortality were observed during treatment with everolimus and/or capecitabine.

In light of these findings, the mTOR inhibitor everolimus suppressed tumor growth in the mouse model of colon cancer-induced peritoneal carcinomatosis when administered orally or in combination with capecitabine. However, the limitation of this study was the small sample size. Our study is unique because it has investigated whether peritoneal carcinomatosis could be treated with oral chemotherapeutic agents. However, to reach a definitive conclusion, it is necessary to work with a larger experimental group.

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Conflict of interests

It is not declared by the authors.

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VARIA

Evaluation of fasting impact during Ramadan month on patients with a history of kidney stones in a military hospital

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Abstract: *Introduction: One of the cornerstones and common causes of kidney stone formation is the volume of fluid consumed during a 24- hour that influences on forming that by several mechanisms. Since fluid intake decreases during Ramadan, one of the common questions posed is whether Ramadan fasting is associated with the precipitating of stone formation. The present study was aimed to investigate the effect of Ramadan fasting on precipitating and Inhibitors of stone formation in patients with a history of renal kidney disease.*

Methods: The study population included all of the patients with kidney stone diseases who were seen at the Clinic of Urology and Nephrology affiliated with Baqiyatallah hospital during the month of Ramadan in 2014 and 2015. The number of 66 patients was chosen and included in the study through randomized simple sampling. All subjects underwent blood tests like serum uric acid, serum phosphate, serum calcium, and serum sodium. Samples were tested in terms of volume, magnesium, uric acid, Calcium, Phosphate, Sodium, citrate, oxalate, Calcium oxalate supersaturation, Calcium Phosphate supersaturation, Uric acid supersaturation.

Results: Sixty-six eligible patients were included that the mean age was 48.35±12.10 years. Fourteen men and 52 women were included. There were no significant differences in the mean pH of urine, urinary sodium, urinary oxalate, super-saturated levels of calcium oxalate in the urine, urinary phosphate levels, and super-saturated levels of urinary calcium phosphate between the two groups ($P>0.05$). The average volume of urine, urinary magnesium, urinary calcium, urinary citrate, the uric acid level in the non-fasting group was higher than the fasting group ($P<0.05$). There were no differences in the average blood calcium levels, blood sodium, blood phosphate levels, blood acidic levels between the two groups ($P>0.05$). There was no difference in the mean super-saturated level of uric acid between the two groups ($P=0.035$).

Conclusion: There was controversial evidence on the effect of Ramadan fasting on kidney stone formation and there is not enough evidence in the present study that Ramadan fasting precipitates risk factors associated with calculus formation

Keywords: *fasting, kidney calculi, inhibitor factors, and precipitating factors*

INTRODUCTION

Ramadan month is related to the Islamic calendar and is a holy month for Muslims. Healthy adult Muslims should be fasting during this month and the times spent on fasting maybe change from 12 to 18 hours depending on the seasonal and regional specialties. Any food and fluid eating are allowed only from sunset to sunrise in this month. Islam rules permit the break of fasting for particular people including unhealthy cases [1-5].

However, many Muslim patients reveal their willingness to keep the fast during Ramadan month to regard religious cultures. There are concerns regarding the influence of

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dehydration and the consequent renal hypoperfusion in Ramadan fasting for patients with renal illnesses [6]. This problem especially arises when Ramadan month happens during hot and dry summers with a long daytime span.

In the Ramadan month, fasting Muslims tend to alter their dietary habits. This involves changes in eating times and the content of food, in addition to fluid deprivation. It is fairly well known that fluid deprivation causes volume con-traction and renal hypoperfusion. The latter may cause renal dysfunction and aggravate existing renal impairment [7-9].

There is scarce scientific data about the safety of Ramadan fasting for patients with different renal diseases. The existing published reports are mainly focused on the impact of Ramadan fasting on patients with renal transplantation, patients with chronic kidney disease, and patients with the renal stone disease [10-12].

One of the cornerstones and common causes of kidney stone formation is the volume of fluid consumed during a 24- hour that influences on forming that by several mechanisms [13-15]. Since fluid intake decreases during Ramadan, one of the common questions posed is whether Ramadan fasting is associated with the precipitating of stone formation. The present study was aimed to investigate the effect of Ramadan fasting on precipitating and Inhibitors of stone formation in patients with a history of renal kidney disease.

METHODS

The study population included all of the patients with kidney stone diseases who were seen at the Clinic of Urology and Nephrology affiliated with Baqiyatallah hospital during the month of Ramadan in May 2016 and May 2017. The number of 66 patients was chosen and included in the study through randomized simple sampling. The sample size of the study consisted of 66 kidney stone patients (30 patients in the fasting and 36 patients in the nonfasting group). The case group was examined for three weeks after one week from the beginning of the holy month of Ramadan and their laboratory factors were recorded.

Inclusion criteria were included the presence of kidney stones at the time of referral or a history of kidney stones proven by ultrasound or CT scan, did not use drugs that affect the aggravation or treat of the kidney stones, did not use a diet that affects stone formation (Including frequent consumption of nuts such as nuts or frequent consumption of chocolate containing cocoa. Cola drinks, a high-salt or meaty diet, and plenty of citrus fruits). Also, patients with chronic liver disease, advanced cardiac disease, acute infection, or diabetes insipidus were excluded. All cases were fulfilled the consent form and they have consented to fast in Ramadan.

Collecting data was done by a Researcher-built Form. All subjects underwent blood tests like serum uric acid, serum phosphate, serum calcium, and serum sodium. Samples were asked to urinate for the first time at 9 a.m. and to be expelled. The urine is then poured into a special container until 9 pm and the entire urine collected within 12 hours is delivered the next day. Clinical evaluation of the patients included meticulous current and past medical history, and physical examination. Urine samples were tested in terms of volume, magnesium, uric acid, Calcium, Phosphate, Sodium, citrate, oxalate, Calcium oxalate supersaturation, Calcium Phosphate supersaturation, Uric acid supersaturation. Uric acid was calculated using the Tiselius formula in both groups. The evaluation of the patients was conducted near the end of the month of Ramadan.

After being collected, the data was inserted into SPSS software (Statistical Package for Social Science Version 20) and analyzed by using descriptive statistics and statistical tests such as t-Tests and the Mann-Whitney. A P-value of less than 0.05 was considered significant.

RESULTS

Sixty-six eligible patients were included. The mean age was 48.35±12.10 years. Fourteen men and 52 women were included. There were no differences between the two groups regarding age and gender (P=0.12 and 0.14 respectively).

Table 1: Demographic characteristics of all participants in the two groups

Groups	Non-fasting	Fasting	P-value
Age	48.3±13.37	43.4±11.06	0.118
Gender, M/F	8/28	6/24	0.452

There were no significant differences in the mean pH of urine, urinary sodium, urinary oxalate, super-saturated levels of calcium oxalate in the urine, urinary phosphate levels, and super-saturated levels of urinary calcium phosphate between the two groups (P>0.05) (Table 2).

The average volume of urine, urinary magnesium, urinary calcium, urinary citrate, the uric acid level in the non-fasting group was higher than the fasting group (P<0.05) (Table 2).

There were no differences in the average blood calcium levels, blood sodium, blood phosphate levels, blood acidic levels between the two groups (P>0.05) (Table 2).

There was no difference in the mean super-saturated level of uric acid between the two groups (P=0.035) (Table 2).

Table 2: Biochemical characteristics of all participants in the two groups

Groups	Non-fasting	Fasting	p-value
Blood Ca level	9.4±0.52	9.3±0.83	0.567
Blood Na level	141.80±2.01	140.80±1.91	0.101
Urine volume	1030.60±541.60	604.00±260.35	0.001
Urine PH level	5.60±0.67	5.80±0.86	0.317
Urine Mg level	105.70±27.14	43.20±14.86	<0.001
Urine Ca level	97.50±54.55	72.40±41.51	0.039
Urine Na level	97.30±43.46	77.10±40.66	0.066
Urine Sitrat level	1.90±1.02	1.40±0.88	0.034
Urine Exalarat level	0.31±0.81	0.30±0.80	0.36
Urine Extra ca Exalarat level	1.50±1.01	1.60±0.87	0.575
Blood Pa level	4.00±0.62	4.20±0.54	0.461
Urine Pa level	422.50±219.28	367.20±176.17	0.275
Urine Extra ca Pa level	477.90±433.29	2055.10±5856.15	0.159
Blood acid uric level	6.20±1.71	5.80±1.58	0.576
Urine acid uric level	208.59±73.49	154.90±66.57	0.004
Extra acid uric level	0.50±0.23	0.80±0.72	0.35

DISCUSSION

In this study, the effect of fasting during Ramadan month on the improvement of the urinary system was examined. The concept of ultra-stable saturation is due to the excessive increase in the constituent salts of the stone, such as calcium, phosphorus, oxalate, and uric acid in the urine, and the solubility of these minerals in the urine. It seems that the lack of saturation in non-stone individuals is due to the activity of crystallization inhibitors. These inhibitors include magnesium, citrate, phosphate ions, and coarse molecules such as Tamm-Horsfall protein. In the case of low inhibitors and the ability to form stone, the constituent salts of the stone adhere to the rock crystals and from these crystals, nodules or stone nuclei and finally from the enlargement of these nodules, one-millimeter pebbles and the enlargement and the accumulation of these pebbles form larger stones. Also, other conditions such as urinary saturation, increased deposition of kidney stones such as calcium, oxalate, uric acid, and phosphate, urine pH, decreased urine volume, the presence of crystallization facilitators such as uric acid, infection, acidity, and pointed to insoluble ions. The results of the present study showed that the level of uric acid in the blood, the level of calcium in the blood, the level of sodium in the blood, the level of urinary phosphate, the level of urinary sodium in non-fasting people is higher than in fasting people. On the other hand, urine volume, urinary magnesium, uric acid, calcium urine, total urine volume, and urinary citrate in non-fasting individuals were higher than in fasting individuals, which was statistically significant. Ultrasonic levels of calcium phosphate in the urine of fasting patients are higher than those of non-fasting patients but were not statistically significant.

In general, among the blood parameters involved in the formation of kidney stones, only the level of blood phosphate in the fasting group is higher than the fasting group, but statistically, this difference was not significant. According to the findings of this study, the average level of urinary citrate, urinary magnesium, urine volume in the fasting group is lower than the fasting group. The high levels of these three act as crystallization inhibitors and the low levels in fasting people provide the basis for the formation of kidney stones. But statistically, only urine volume and urine citrate among the above inhibitors were significantly lower in the fasting group than in the fasting group. They reduce the growth of crystals and their accumulations and prevent the formation of secondary nuclei. Facilitators refer to molecules that reduce the supersaturated surface needed to initiate the crystallization process. The imbalance between these two sets of factors is more important than other factors in the formation of kidney stones. These substances include organic compounds, proteins, and glycosaminoglycans.

In this study, the urine volume of fasting people was lower than that of non-fasting people. Excessive fluid intake has been a traditional treatment in recent decades, and doctors have been prescribed to prevent kidney stones in patients with kidney stones. The results of studies on the effect of urine volume on kidney stone formation are: Low volume of urine is considered as a risk factor for the onset of kidney stone formation and re-formation of kidney stone, an increase in urine volume due to excessive fluid consumption. It has a positive effect on the prevention of kidney stones and does not interfere with the normal activity of inhibitors. Mass consumption of liquids such as water, coffee, tea, beer has a deterrent effect on reversibility and kidney stone formation. Everything is in dispute and is always controversial. Therefore, high fluid intake, especially water, is considered an effective and economical method in preventing the formation of kidney stones, which is often ignored by people who have kidney stones [16]. In a study by Cheh et al., Which was conducted to investigate the effect of fasting on urinary excretion, the results showed that the volume of urine was reduced by 24 hours in resinous individuals, but this decrease was not significant. In this study, 24-hour urine volume was divided into three time periods, urine volume in the morning, evening, and night. In this study, the volume of nocturnal urine in the previous periods and the length of Ramadan have remained unchanged, and Ramadan does not affect the volume of nocturnal urine. But the volume of urine in the evening was less than in the pre-Ramadan period, and this decrease was significant. Which is consistent with the results of this study. Also, a decrease in urine in the second and third weeks was significant in fasting people, but in the last week of Ramadan, it was not significant, which is largely consistent with the findings of this study. Decreased urinary volume, indicating low fluid intake or loss of excess water, directly increases the risk of kidney stones by increasing the saturation of stone-forming salts in the urine. Fasting may limit fluid intake and, by affecting urine concentration and chemical composition of the blood, prepare the ground for the formation of kidney stones [17]. Magnesium is the fourth most abundant mineral in the body and is intentionally located in the bones. The magnesium in the diet is absorbed in the small intestine and excreted by the kidneys.

The normal amount of 12-hour urine magnesium is between 0.15 and 0.5. Only 0.1% of all magnesium in the body is mobile. Magnesium can combine with oxalate to reduce supersaturation. Oral magnesium intake reduces the absorption of oxalate and urinary excretion. Magnesium can also combine with calcium in the gut. Magnesium supplements increase the excretion of citrate in the urine during magnesium deficiency [18]. According to the findings of this study, the mean level of urinary magnesium in fasting people is significantly different from fasting people, so that its level is lower than in fasting people. In the study of Miladipour and colleagues, the level of urinary magnesium in fasting people Dar was higher than non-fasting people, but it was statistically insignificant, which differs from the findings of this study [19]. Zegal et al. investigated the effect of fluid and dietary restriction restrictions on fasting on the biochemical factors of kidney stone formation. In this study, 90 patients were divided into three groups of healthy fasting people (those without kidney stones), healthy people without fasting, and people with kidney stones without fasting. The results of this study showed that the mean inhibitory level of urinary magnesium among fasting groups without kidney stone disease is lower than non-fasting individuals, which is quite consistent with the results obtained in this study [19]. Another inhibitor of kidney stone formation is citrate, which is present in the form of trichloroxyl acid in the blood and combined with calcium, magnesium, and sodium at a pH of 7.4. Most citrate is derived from exogenous aerobic metabolism, which is freely cleared in the glomerulus. Approximately 75% of the filtered citrate is absorbed in the complex proximal open tubule. Hypothyroidism (low levels of citrate in the urine of 320 mg per day) is seen in more than 50% of people with kidney stones. The normal 12-hour urinary titrate is 0.6 to 4.8. Hypothyroidism is caused by a variety of factors, including medications such as acetazolamide and thiazides, renal acidosis, urinary tract infection, hypokalemia, low magnesium, and inflammatory bowel disease [20]. In the study of Zegal et al. Mentioned above, the level of citrate in fasting people was lower than in non-fasting people, which is consistent with the findings obtained in this field [19].

In this study, the mean urinary citrate level in the non-fasting group was higher than the fasting person, which was statistically significant. In the study, Miladipour et al. [19] in which 57 people participated, 37 had recurrent kidney stones and 20 had no kidney stones. Urine readings in three 24-hour urination periods before Ramadan (period 3), urination during Ramadan, and hours 19 to 15 (period 1), and urine was collected during Ramadan from 15 to 19 (4 hours urine as period 2). In this study, the amount of urinary citrate in people in the pre-Ramadan period (group 3) was lower compared to fasting people (group 1 and group 2), which contradicts the findings of this study. One of the facilitators of kidney stone formation in this study was uric acid. To form uric acid-containing stones, uric acid must be highly saturated in the urine. There are usually three main factors involved in the formation of uric acid-containing stones, including acidic urine, hyperuricemia, and low urine volume. Hyperuricemia is the excretion of more than 600 mg of uric acid per day in 24-hour urine. The degree of hyperuricemia is directly related to kidney stones [21]. High uric acid levels may be due to overproduction of uric acid in the body or reduced urinary excretion. In particular, the factors that increase the concentration of uric acid in the blood are diuretics, hypothyroidism, obesity, excessive alcohol and coffee consumption, genetic factors, etc. One of the findings of this study is The average uric acid level in the urine of fasting people was significantly higher than in fasting people. In the study of Miladipour et al. [19]. The mean uric acid level for 24-hour urine in non-fasting individuals was higher than the 24-hour uric acid level during Ramadan, but statistically, there was no significant difference following the findings of this study. Findings on uric acid and blood uric acid measurements show three major facts. 1. High concentrations of acid in the blood are due to decreased uric acid excretion in the urine, and low concentrations of urine are associated with increased uric acid. During fasting, a small portion of the uric acid that enters the nephrons from the blood is reabsorbed (reabsorbed.) There is a significant exchange of uric acid between the tissues and the blood for 24 hours a day.

12-hour normal urinary calcium is less than 250 mg. The results of this study showed that urinary calcium levels in fasting people were significantly lower than those without fasting. A study by Miladipour et al. [19] showed that the level of calcium in the urine of fasting people was significantly higher than that of fasting people, which is quite consistent with the results of this study. The concentration of calcium in the urine also indicates the concentration of calcium in the blood. The difference between urinary calcium levels in the two study groups appears to be due to the type of food and activity level, age, geographical location, and source of drinking water. Hyperoxaluria is an excessive increase in urinary oxalate (normal 12-hour urinary oxalate is between 0.23 and 0.68). In this study, urinary oxalate levels in the fasting and non-fasting groups did not differ significantly from each other. In the study of Miladipour et al. [19] the level of urinary oxalate in non-fasting and fasting people was not significantly different from each other, which is consistent with this study. Increased levels of oxalate in the urine of people are caused by factors such as diet or inflammatory bowel disease [22]. One of the major studies on the combination of calcium and oxalate is the cohort study conducted by Korhan et al. On 45619 men. After 4 years, 8.7% of the samples developed kidney stones. In this study, an inverse relationship was found between calcium intake and the formation of calcium-oxalate (CaOx) compounds [23]. Robertson's study found that there was a linear relationship between urinary oxalate levels and the formation of kidney stones, as well as the prevalence of kidney stones in different countries and the excretion of oxalate in the urine [24]. In general, 80% of calcium-based stones are oxalate calcium stones. In one study, randomized clinical trials of people with hypercalciuria were divided into two groups with low calcium and oxalate diets and one group with high calcium diets but oxalate, salt, and low protein. After five years, the renal stone recurrence rate in the second high-calcium group was reduced to 51% [25]. The results showed that the super-saturated levels of calcium oxalate in the urine were slightly lower in the fasting person than in the fasting person, but this difference was not significant. The amount of calcium oxalate in the urine may be altered by factors such as diet and fluid intake. Similar to the results of this study, in the study of Miladipour et al. [19] there was no significant difference between the mean calcium oxalate levels in fasting and non-fasting individuals, and also the desired variable was not significantly different in people with kidney stones during Ramadan and before Ramadan. In addition, there was no significant difference between healthy individuals during Ramadan and before Ramadan in terms of the average level of oxalate. Brochite ($CaHPO_{42}H_2O$) is the main cause of kidney stones of calcium phosphate origin. Urine in people who have kidney stones (which contain calcium) is saturated with super-high brushes due to the high concentration of calcium. Calcium is a type of calcium phosphate, but at least supersaturation is required for the formation and survival of rock containing calcium phosphate, because, in the lower levels of supersaturation, the primary nucleus of rock formation usually dissolves. All phosphates are calcium, containing calcium and phosphate ions. It is believed that the primary nucleus of calcium-containing rocks is calcium phosphate. Although calcium phosphate rocks may form in a supersaturated gaming solution, their formation in PH Urine is not known to be normal (26). In this study, the mean saturated level of urinary calcium phosphate in the fasting group was higher than the fasting population and this difference was statistically significant. In the study of Miladipour et al. [19], urinary phosphate is lower during fasting than before fasting, but it is statistically meaningless. The variability of calcium phosphate levels in the urine is influenced by factors such as diet and especially fluid intake, which is different for fasting and non-fasting days, but during Ramadan, there is not much difference for urine during different periods. In the study of rust, the amount of bruschetta was the same for healthy people fasting and people with kidney stones and without fasting, but for healthy people without fasting, this amount was less, which is consistent with our study. The excretion of uric acid in adults is 500 to 600 mg in 24 hours (the normal amount of uric acid in normal urine for 12 hours is less than 480).

The concentration of solution and pH plays an important role in the solubility of urate uric acid. However, the formation of uric acid stones is not common. Most people with an abnormal PH6 in their urine have urine saturated with uric acid, but they do not form a stone. More acidity (such as a pH of 3.5) or dehydration is needed to make a stone. Uric acid, which is not highly soluble, crystallizes at low pH and forms rock. The normal amount of uric acid in the blood is very important for the proper functioning of the body. The normal level of uric acid in the blood is between 0.3 and 0.7 mg / dL. Uric acid is formed in the human body system as a byproduct of purine metabolism and then excreted in the urine.

This means that the difference seems to be small due to various factors such as diet, urine volume, and dehydration, but this difference was not statistically significant. In the study of Miladipour et al. [19] the mean uric acid saturation level in the 4-hour evening urine (maximum concentrated urine) was higher than the pre-Ramadan uric acid saturation level and was statistically significant, which is consistent with our study. However, this rate is lower in the urine sample from 19:00 to 15:00 the next day than in the pre-Ramadan period. A small amount of sodium is absorbed from the stomach, but most are absorbed quickly through the small intestine. The absorbed sodium is carried by the bloodstream to the kidneys, where the sodium is purified and returned to the blood in the amount needed by the body. When the need for sodium increases, the action of some hormones stimulates the reabsorption of sodium by the kidneys. Conversely, when sodium intake increases, the secretion of these hormones decreases to reduce sodium retention. The amount of sodium in the urine depends on the daily diet, increases when consumption is high, and decreases when consumption is low. The increase or decrease in sodium in the blood should not exceed 0.5 to 1 mg/l. In the present study, the mean blood sodium level in the group of non-fasting people was higher than the fasting people, but this difference was not statistically significant. Also, the urinary sodium level of non-fasting people was higher than the fasting person, but this difference was not statistically significant.

In Miladipour's study, the amount of 24-hour sodium in non-fasting people was higher, but it is statistically meaningless, which is consistent with the present study. A study by Sakhai et al. showed that increasing the intake of sodium chloride from 140 to 310 mmol/L increases the amount of urinary calcium by 34% and reduces the amount of urinary citrate by 10%. Therefore, hyponitrite and calcification are among the main factors in the formation of kidney stones, which are caused by increased sodium chloride consumption [27]. In a study by Brutis et al., The results showed that sodium chloride salt consumption played a stronger role in increasing urinary calcium and the risk of stone formation compared to calcium and protein intake [28]. Consumption of sodium chloride salt is considered an indicator of the ratio of sodium to potassium in the urine [29]. The amount of normal 12-hour urinary phosphorus is between 1300-300. The average level of urinary phosphate in the non-fasting group was higher than in the fasting person, but this difference was not statistically significant. In Miladipour's study [19], the rate of 24-hour urinary phosphate in non-fasting individuals was higher and statistically significant, which is consistent with the present study. Decreased urinary phosphate levels in fasting people occur due to factors such as prolonged starvation during Ramadan.

The amount of phosphate reference in the adult blood is 2.7 to 4.5 milligrams per deciliter (mg/dL) or 0.87 to 1.45 milliliters per liter (mmol/L), which helps the kidneys control the amount of phosphorus in the body and excess phosphorus. It is filtered through the kidneys and excreted in the urine [30]. One of the serum factors studied in this study is the level of uric acid in the blood. The average level of uric acid in the blood of fasting people was higher than fasting people, but this difference was not statistically significant. In the study of Goma and colleagues, the serum uric acid level was compared with the serum uric acid level in the pre-Ramadan period at 6 am, 6 pm, and after iftar. Serum uric acid levels did not change at 6 a.m. during the first two weeks of Ramadan, but serum uric acid levels increased significantly during the second two weeks of Ramadan. Serum uric acid levels were significantly reduced at 6 p.m., then increased, and almost reached the pre-fasting period limit, which is consistent with the results of this study.

One study found that the average serum levels of calcium in fasting people were lower than in non-fasting people, but the difference was not significant. Also, the total amount of urinary calcium in the fasting group was lower than in the non-fasting group. This difference is statistically significant and is fully consistent with Miladipour's study [19]. Calcium balance is mainly achieved by the absorption of calcium in the intestine, its reabsorption in the kidneys, and the exchange and transfer between bone and other tissues. Serum calcium consists of ionic and protein compounds. Calcium reacts with compounds such as phosphate and citrate. In gaming environments, the amount of free ions in calcium serum decreases. 99.99% of the calcium ingested into the kidneys is reabsorbed, thereby regulating serum and urinary calcium levels [31]. No study found a link between fasting and serum calcium levels.

CONCLUSIONS

In recent years, it has been determined that the factors involved in the formation of kidney stones include urinary saturation levels with stone-forming components such as calcium, oxalate, sodium, etc., which were studied in this study. Fasting is important to prevent stone formation in the future. In general, by examining the results of this study and other studies, it seems that fasting during Ramadan has contradictory effects on urinary stone formation factors and there is not enough evidence about the effect of Ramadan on exacerbating exposure to stone-forming factors in this study.

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VARIA

Diagnostic challenges in late presenting diaphragmatic hernia

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Abstract: *Background: The incidence of congenital diaphragmatic hernia (CDH) has been reported to be 1 in 2000-5000 newborns. The late presentation can occur in 5-30% of all cases and it can be harder to diagnose, as symptoms are usually vague and non-specific.*

Methods: We performed a retrospective review of chart files of all patients admitted to our hospital with the diagnosis of congenital diaphragmatic hernia between 2010 and 2021.

Result: Of the ninety-two cases of CDH treated in our hospital between 2010 and 2021, twenty-six (28%) were late presenting. In the late-presenting CDH group, we found a slight female preponderance, with fifteen female patients (58%) and eleven male patients. A posterolateral left defect in the diaphragm was noted in fifteen cases (58%), a right defect was noted in just one case (4%) and ten patients (38%) were diagnosed with an anterior diaphragmatic hernia. The age at diagnosis varied from 2 months to 14 years. Associated anomalies were noted in just four cases. In all cases, a thoracoabdominal radiograph was performed and ten patients also had a CT scan. Primary repair was performed in 25 of the 26 patients. The mortality rate in our study population was 11%, we reported 3 cases with unfavorable evolution.

Conclusion: CDH presenting beyond the neonatal period is a usual finding and it represents a true diagnostic challenge.

Keywords: *congenital diaphragmatic hernia, late presentation, pediatric*

INTRODUCTION

One of the greatest challenges we face when diagnosing a pediatric patient is the constant concern that an unusual emergent condition, with non-specific symptoms, can easily masquerade as a common condition, and as such, they can be initially misdiagnosed.

Congenital diaphragmatic hernia is a congenital defect that occurs when the pleuroperitoneal canal does not close during the 8th week of fetal development, which allows the herniation of the bowel loops, stomach, solid abdominal organs, or the mesentery into the thoracic cavity. The incidence of CDH has been reported to be 1 in 2000-5000 newborns, accounting for 8% of all major congenital anomalies, with most cases being diagnosed prenatally or soon after birth (approximately 90%) [1–7].

CDH is a common cause of respiratory distress in the neonatal population and is associated with pulmonary hypoplasia, patients presenting with vomiting, tachypnoea, cyanosis, with diminished breath sound on auscultation after birth, but in some cases, it can go undiagnosed until later in life [3]. If CDHs are diagnosed later than 30 days they are considered late-onset CDHs [3, 8]. Late presentation in patients with CDH occurs in 5-30% of all cases, with patient age ranging between 2 months to 12.5 years [1, 3, 5].

Cases of CDH with delayed presentation are usually more subtle and because of that, they can be harder to diagnose [9]. Symptoms, diagnosis, and management of late-

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presenting CDH differ from those of neonatal CDH and are usually more diverse and non-specific. This usually leads to both clinical and radiological misdiagnosis, which can cause significant morbidity [4, 5, 10]. Massive pneumothorax and pleural effusion are the most common initial diagnosis on a chest radiograph in these cases and often, distinguishing between a CDH or a tension pneumothorax can be difficult and crucial, as the management of these two pathologies differs greatly [1, 3, 8]. Late-presenting CDH can cause acute or chronic respiratory or gastrointestinal symptoms such as dyspnoea, recurrent pulmonary infections, chest pain, postprandial fullness, or abdominal pain [2, 10].

This study aims to emphasize the fact that diagnosing CDH outside de neonatal period is often difficult and to help familiarize healthcare professionals with the clinical presentation that can occur in these cases to help facilitate early management and avoid misdiagnosis

MATERIALS AND METHODS

We performed a retrospective review of chart files of all patients admitted to our hospital with the diagnosis of congenital diaphragmatic hernia between 2010 and 2021. Patients who developed symptoms and were diagnosed in the first month of life were classified as having early presenting CDH, while those who were diagnosed after one month of age were considered to have a late-onset CDH. Only patients with late-presenting CDH age were included in our study. Patients with diaphragmatic eventration, hiatal hernia, or paraesophageal hernias were excluded from the study. Patient demographics, treatment, and outcome were then reviewed.

RESULTS

Of the ninety-two cases of CDH treated in our hospital between 2010 and 2021, twenty-six (28%) were late presenting. In the late-presenting CDH group, we found a slight female preponderance, with fifteen female patients (58%) and eleven male patients. A posterolateral left defect in the diaphragm was noted in fifteen cases (58%), a right defect was noted in just one case (4%) and ten patients (38%) were diagnosed with an anterior diaphragmatic hernia. The age at diagnosis varied from 2 months to 14 years. The patients were divided into 3 age groups: under 1 year, between 1 and 5 years, and over 5 years. We found that patients under the age of one presented with either left-sided defects or with an anterior hernia. In the case of patients with ages between 1 and 5 years, left-sided defects were more frequent than right-sided defects or anterior hernias, the same being the case for patients older than 5.

Associated anomalies were noted in just four cases: two patients had a history of esophageal atresia repair in the neonatal period, one patient presented with Down syndrome, and one patient presented with cardiac anomalies that did not require surgical correction.

Table 1 illustrated the relationship between age and location of the defect.

Table 1: The relationship between age and location of the defect

Age	Anatomical variant CDH		
	Left	**Right**	**Anterior**
< 1 year	2	0	5
1-5 years	9	1	3
> 5 years	4	0	2
Total number of patients	15	1	10

Clinical presentation in late-presenting CDH was mixed and various as can be seen in Table 2. For that reason, we dived the patients into four main categories as follows: 8 patients (31%) with respiratory symptoms, 6 (23%) with digestive symptoms, 6 (23%) with both, and 6 (23%) were asymptomatic.

Table 2: Classification of symptoms

Symptoms	Number of cases	Details
Respiratory	8	
Acute	6	Dyspnea 4 Cough 3 Febrile URTI 3
Chronic	2	Chronic respiratory distress
Gastrointestinal	6	
Acute	6	Vomiting 5 Abdominal pain 3 Loose stools 1
Chronic	0	
Both	6	
Acute	4	Dyspnea Vomiting Abdominal pain
Chronic	2	Failure to thrive + abdominal pain + chronic respiratory distress
Asymptomatic	6	

URTI – upper respiratory tract infection

Table 3 shows the site of the hernia concerning the presenting symptoms. Of the six patients presenting with gastrointestinal symptoms, five had a left-sided defect, and just one patient presented with an anterior hernia, while of the eight patients presenting with respiratory symptoms, four were found to have a left-sided defect, and four presented with an anterior hernia. The patient who was diagnosed with a right-sided defect presented with both respiratory and digestive symptoms. We found three patients with a left-sided defect and four patients with an anterior defect that were asymptomatic and were diagnosed incidentally when a chest radiograph was performed when monitoring another pathology.

Table 3: The relationship between symptoms and location of the defect

Symptoms	Right	Left	Anterior
Respiratory	0	4	4
Gastrointestinal	0	5	1
Both	1	4	1
Asymptomatic	0	2	4

In all cases, a thoracoabdominal radiograph was performed and it should be mentioned that almost half of our patients had previous chest radiographs performed routinely for other conditions that were normal at that time. (Figure 1, Figure 2) Ten patients also had a CT scan that confirmed the diagnosis of diaphragmatic hernia and four patients had upper gastrointestinal contrast studies performed. It bears mentioning that all the patients with upper GI contrast studies were admitted to our hospital at the beginning of the study period. In the last 7 years, upper GI contrast studies have not been performed to diagnose a late-presenting diaphragmatic hernia.

Figure 1: Chest radiograph showing left-sided diaphragmatic hernia

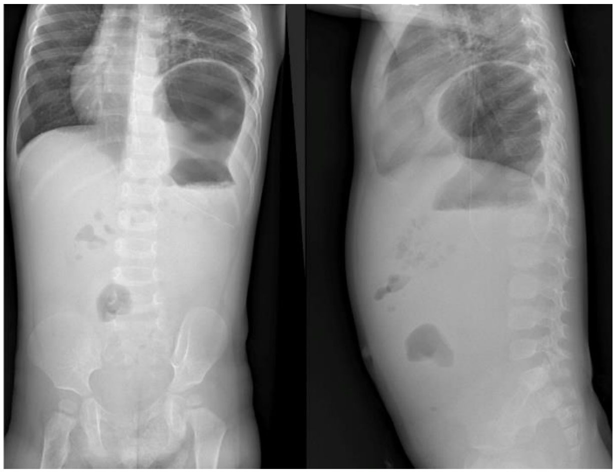

Primary repair was performed in 25 of the 26 patients. The open abdominal approach was favored in most cases (76%), while only six patients were operated on by minimally invasive techniques. Out of the six, four patients underwent laparoscopic approach, and two patients underwent thoracoscopic approach.

Figure 2: Chest radiograph showing anterior diaphragmatic hernia

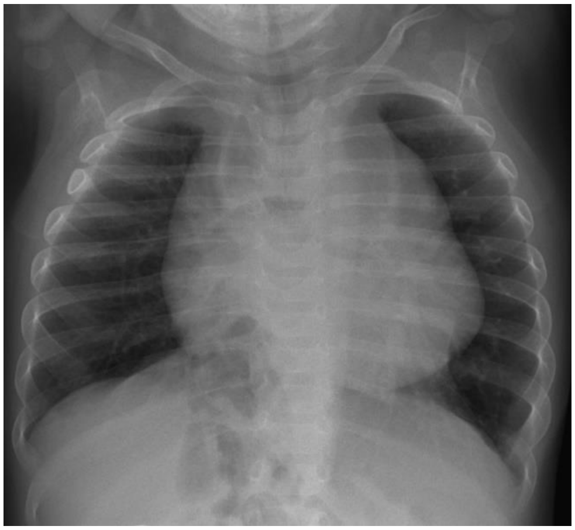

The mortality rate in our study population was 11%, with all three patients had presented with severe complications such as gastric volvulus. In the remaining patients, we identified just one case of pleural effusion after thoracoscopic approach that required drain placement, with no further complications being observed later on.

We report the cases of the three patients that had an unfavorable outcome.

The first case was that of a 14-year-old girl with a history of 3 days of diffuse abdominal pain and vomiting. On admission, clinical examination showed respiratory distress with diminished breath sounds on the left side. The patient went into cardiocirculatory arrest soon after admission that was successfully managed by external chest compressions and resuscitation drugs. Chest and the abdominal radiograph showed a hydroaeric image and the tip of the nasogastric tube situated in the left hemithorax, with no stomach bubble present in the abdominal area. A thoracoabdominal CT scan was performed to confirm the diagnosis of the left diaphragmatic hernia (Figure 3). Emergency surgery by laparotomy revealed a large left diaphragmatic defect with a herniated stomach. After stomach reduction into the abdominal cavity, a large quantity of blood was evacuated from the left thoracic cavity. The stomach was almost completely necrotic, perforated, and presented with thrombosis of the gastric vessels. Subtotal gastrectomy with oesophageal-gastric anastomosis and diaphragmatic defect suture was performed, but the postoperative outcome was unfavorable, the patient dying on postoperative day 10 due to multiple organ dysfunction syndrome and cardiac arrest.

Figure 3: CT scan showing a left-sided defect in the diaphragm with herniation of the bowel and stomach

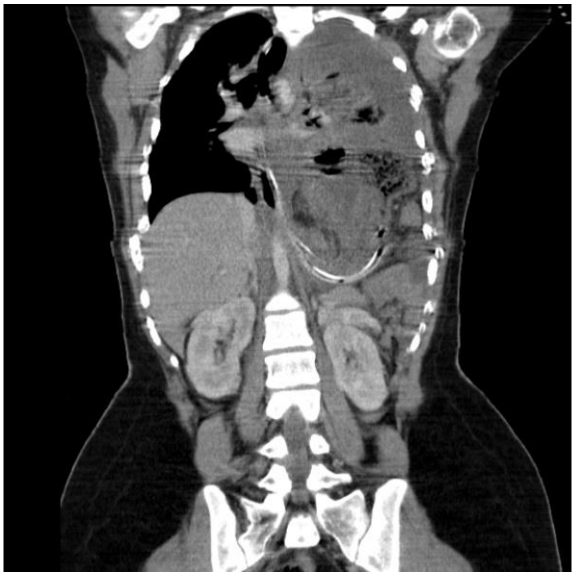

The second case was that of a 6-year-old girl with a history of abdominal distension, diffuse pain, and vomiting. On clinical examination, the patient presented with abdominal distension and diffuse abdominal pain with tenderness on palpation, dyspnoea, and paleness.

An abdominal radiograph showed a tension pneumoperitoneum, with an elevation of the left hemidiaphragm and collapsed bowel loops in the hypogastric region (Figure 4).

The patient was immediately taken into the operating room. She presented with severe bradycardia and went into cardiac arrest, which was managed by orotracheal intubation, external chest compressions, and resuscitation drugs. After 10 minutes, cardiac activity reappeared, and emergency laparotomy was performed. Intraoperatively, a large quantity of gastric liquid and chyme was evacuated from the peritoneal cavity. A large left diaphragmatic defect (10/6 cm) was found, with herniated stomach, spleen, and left hepatic lobe. After the reduction of the herniated content, a large perforation of the stomach was noted. A partial gastrectomy with diaphragmatic defect sutures was performed.

After surgery, the patient developed intravascular disseminated coagulation and acute renal failure, and 24h after admission she went into cardiac arrest that did not respond to resuscitation maneuvers.

Figure 4: Tension pneumoperitoneum with a left diaphragmatic hernia and gastric perforation

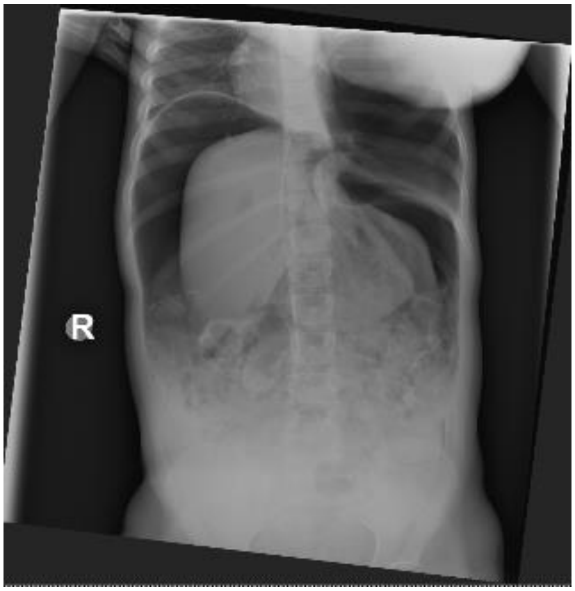

The third case was that of a 4-years-old patient who presented to the emergency room for diffuse abdominal pain and vomiting. An ultrasound was performed in the emergency room than normal. Due to the latest COVID 19 guidelines, the patient was admitted to the COVID ward, while awaiting her PCR test results. Six hours after admission, she went into cardiac arrest and the resuscitation protocol was started with external chest compressions and iv drugs administration. The patient was intubated, and a chest radiograph was performed that showed large hyper transparency in the left hemithorax with an important mediastinal shift to the right, with an imprecise contour of the left diaphragm (Figure 5).

Since the patient could not be ventilated after intubation and because of the image on the chest radiograph, the decision was made to insert pleural drainage. After the tube was inserted, the patient was stabilized.

A contrast substance was given through the nasogastric tube and showed extravasation of the contrast substance into the abdominal cavity, with an imprecise left diaphragm and a smaller hyper transparency in the left hemithorax. The diagnosis of gastric perforation was made and the patient was prepared for surgery. Before she could be taken into the operating room the patient went into cardiac arrest, but after multiple attempts of stabilization, she did not respond to resuscitation maneuvers.

Post mortem examination revealed a left diaphragmatic defect with a perforated stomach due to gastric volvulus.

Figure 5: Tension gastrothorax

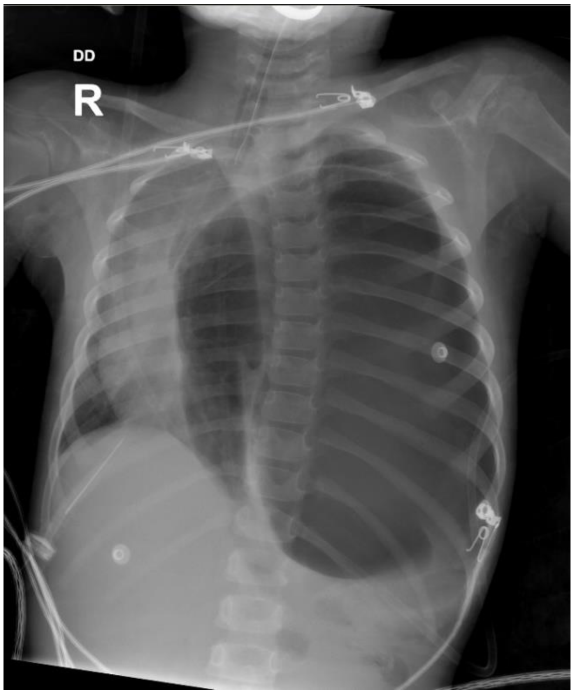

DISCUSSION

A congenital diaphragmatic hernia occurs because of the incomplete closure of the pleuroperitoneal canal during fetal development [11]. Diaphragmatic closure is usually complete by the ninth week of gestation, with the left side closing one week after the right side [12].

During the neonatal period, CDH is the most common cause of respiratory distress, and it can result in high mortality rates in this age group. In most cases, the diagnosis of CDH is established during fetal development or early in the neonatal period [11].

The classification of CDH includes 4 types of patients as follows:

- Type 1 – when the herniation occurs in the early period of fetal development, resulting in pulmonary hyperplasia and increased risk of perinatal death

- Type 2 – when the herniation occurs in the late period of rapid bronchial division, resulting in unilateral pulmonary hypoplasia and pulmonary hypertension

- Type 3 – when the herniation occurs during the late stages of gestation or early in the neonatal period; some patients can present with mild respiratory symptoms at birth, others may not present problems during the neonatal period

- Type 4 – when the herniation occurs after the neonatal period; these patients have no pulmonary hypoplasia [11, 13]

The incidence of late-onset CDH varies from 5 up to 45.5% [8, 9, 11]. There is a 2:1 male to female ratio in both left and right-sided hernias, with 32.7% of cases presenting with hernia sacs [11, 14, 15]. In our case series, we found a slight female preponderance.

Localization on the right side of the defect is more frequently encountered in patients with late-presenting CDH. Patients with right-sided CDH become symptomatic at a younger age compared to patients with left-sided defects [14]. In our study, most of the patients presented with left-sided defects (15 cases) and anterior defects (10 cases). There was just one case of right-sided hernia, in a 9-year-old patient.

Late-onset CDH is difficult and intriguing to diagnose due to various, nonspecific, vague, and misleading clinical presentations, with an array of symptoms that are not pathognomonic for this defect, making it a difficult diagnosis [4, 16–20]. Misdiagnosis of these cases is not uncommon (according to some reported occurring in 62% of cases) and can result in increased morbidity with herniated bowel necrosis, peritonitis, respiratory arrest, and, in some cases, even death [3]. A high index of clinical suspicion and a chest radiograph is essential in establishing a diagnosis [6]. Initial symptoms in patients with CDH can be respiratory (coughing, wheezing, frequent upper tract infections, dyspnea), gastrointestinal (abdominal pain, vomiting, nausea, constipation, failure to thrive), or a combination of the two [5, 21]. Symptoms of late presenting cases are often misleading, and patients are often treated for other conditions with similar symptoms [21]. There have been studies reporting a higher incidence of respiratory symptoms in younger children and right-sided hernias and gastrointestinal symptoms in older children as well as in those presenting with left side CDH [4, 18, 22–24]. 10-11% of patients may be asymptomatic at the moment of diagnosis [21, 25].

Our study population is too small to obtain statistical significance in this regard. Patients with left-sided defects presented with either respiratory symptoms, digestive symptoms, or both. Most patients with anterior defects presented with respiratory symptoms and, in the single case of right-sided diaphragmatic hernia we encountered, our patient presented with both respiratory and digestive symptoms. Four of the youngest patients in our study were asymptomatic, while one presented with respiratory symptoms and two presented with both respiratory and digestive symptoms.

Late-onset CDH presenting with gastrointestinal symptoms have a smaller hernia defect and the symptoms are usually caused by different degrees of obstruction secondary to incarceration [4].

A smaller defect can prevent herniation of the viscera, either due to the existence of a hernia sac that confines the viscera or secondary to occlusion of the defect by solid organs (such as the liver or the spleen), but in some cases, when the abdominal pressure increases (during coughing, sneezing or other strenuous activities) bowel may herniate into the thoracic cavity [1, 24, 26]. The patient may become symptomatic when the actual herniation occurs either after a hernia sac rupture or due to complications of the herniated content such as volvulus or strangulation [1]. The hernia may be intermittent and as such, a normal radiograph does not necessarily exclude the diagnosis [5, 6, 27]. In our cases series, more than half of our patients had normal previous chest radiographs.

Kim et al. reported that 23% of patients with late-onset CDH present with associated congenital malformations such as intestinal malrotation, cardiac defects, pulmonary hypoplasia, chromosomal anomalies, and urogenital anomalies, while others reported an increased prevalence between 8-80% [16, 24, 28-31]. Studies have shown that there is a lower incidence of associated anomalies in late-onset CDH when compared to early-onset CDH, diagnosed in the neonatal period [13, 31]. In our case, just two patients presented with a history of esophageal atresia repair.

Chest radiograph supplemented by additional imaging such as CT scan can be a useful diagnostic tool in the differential diagnosis of thoracic lesions such as lower lobe pneumonia, pneumothorax, pleural effusion, or other lung lesions [11, 22, 32].

There have been reports when CDH was misdiagnosed as pneumothorax and chest tubes were inserted into the thoracic cavity, which lead to morbidity in some cases. [6] Distinguishing between these two entities is extremely important as the management of these two conditions is different [3, 18, 24, 33]. Baglaj et al. reported that chest radiographs diagnose only 49% of cases, with almost 25% of them being diagnosed with other conditions [21, 34, 35].

If a chest radiograph fails to establish the diagnosis, upper gastrointestinal series or contrast-enhanced CT scans can be used to diagnose CDH [4]. Upper gastrointestinal studies are considered unnecessary in the neonatal period but have been recommended in older children [14]. Performing a CT scan means a high radiation burden for the patient, but this is a useful imaging modality that can bring important information regarding the anatomy of the defect, which helps in preoperative planning [3, 13]. Chest radiographs were performed in all cases and CT scans were performed in 10 cases to confirm the diagnosis. Contrast studies were not performed in four cases, but these patients were patients who had been diagnosed in the early years of our study period.

Once the correct diagnosis has been established, patients with late-onset CDH have a more favorable prognosis due to the less severe or the absence of pulmonary hypoplasia [8, 11, 18, 36]. In the case of late-onset CDH, a 3.6% postoperative complication rate was reported, with a 1.9% recurrence rate and a 1.95 mortality rate [37]. In our study, the mortality rate was higher, with patients having had an unfavorable outcome. Just one patient in our series presented with postoperative complications (pleural effusion) that was resolved by placing pleural drainage.

Late-presenting CDH is prone to rare complications such as tension gastrothorax, gastric perforation, splenic torsion or rupture, intestinal obstruction, or bowel necrosis [4, 27].

When the stomach herniates in the thoracic cavity, it can become distended resulting in tension gastrothorax. Acute gastric volvulus is a possible complication of patients with late-presenting CDH. Such cases are rare but can have important, life-threatening complications. The distended stomach leads to severe respiratory distress. Okuda et al. reported a case of a patient with acute tension gastrothorax with cardiopulmonary arrest and failed resuscitation. An acute gastric volvulus is a surgical emergency, with non-operative management being associated with an 80% mortality rate. There is also a risk of mediastinal spillage, with possible rupture of major blood vessels [13]. The first step in managing such patients is the immediate decompression of the stomach, reduction of the volvulus, and treatment of the underlying cause. The association of gastric volvulus and CDH is extremely rare, with only 27 pediatric cases having been reported thus far. This association can occur due to the absence of elongation of 2 of the 4 ligaments supporting the stomach (gastrophrenic and gastrosplenic) [13]. Two of our patients presented with this complication, with unfavorable outcomes [11, 27, 38, 39].

Surgical management is necessary to correct the defect and prevent further complications [20]. The type of approach varies in different centers and even in the same center among different surgeons. No consensus has been reached regarding the best approach for late-onset CDH. The current recommendation is for each surgeon to decide on the type of surgery based on one's experience and the patient's history [23, 40]. Thoracotomy allows for safer surgery when dissecting adherences between the abdominal viscera and the mediastinal contents. This approach is particularly useful in recurrent hernias, with some cases even requiring a combined thoracoabdominal approach [12].

For elective repair, the indication is to perform minimally invasive surgery [40]. Some studies have shown an increase in recurrence rate in minimally invasive surgery for the repair of CDH of 5-23.1%, with a higher rate in cases of thoracoscopic repair [9, 26, 41]. In their study, Obata et al. reported a higher recurrence rate after the laparoscopic approach, most likely due to difficult visualization of the posterolateral defect [23, 42].

Twenty-five of the twenty-six patients in our series underwent surgical repair of the diaphragmatic defect, nineteen by open abdominal approach, four by laparoscopic approach, and two by thoracoscopic approach. None of our patients presented with postoperative recurrence. There are some controversies when using the thoracoscopic approach regarding chest drainage placement, with some studies reporting good results with tube placement, while other authors suggest that the residual pneumothorax will spontaneously be resorbed, and the placement of the chest tube can cause negative intrathoracic pressure that can increase the risk of recurrence [9, 11]. In the two cases that were resolved by the thoracoscopic approach in our series, there was no tube placement, but one patient presented with postoperative pleural effusion that required a second surgery with fluid drainage and tube placement. After tube placement, the patient presented with no further complications

CONCLUSIONS

CDH presenting beyond the neonatal period is a usual finding and it represents a true diagnostic challenge, as the clinical presentation is non-specific, and misdiagnosis is a frequent occurrence and should always be considered as a differential diagnosis in different clinical settings. Raising awareness of late presenting diaphragmatic hernia may be key to improving prognosis and avoiding medical errors.

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VARIA

Desert malaria "Curse of the Thar Desert" – a unique geographical endemicity of malaria and combating it with modern technology

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Abstract: *Introduction: This study focuses on the Malaria endemic in the Thar Desert- The most populated desert in the world. Malarial endemicity in a desert environment is a unique and rare geographical presentation, as malaria is usually common in areas where there is rainfall and water stagnation - like ponds, fields, etc. In this study, we want to highlight the unique clinical presentation, lab findings, and reason behind the existence of malaria in the desert. Most importantly we also would like to share our innovative idea to eliminate breeding sites using Quadcopters and drones.*

Materials and methods: Study type – Prospective observation study, Study duration – October 2020 to December 2020, Sample size-103 cases, Inclusion criteria – all smear-positive Malaria cases, Exclusion criteria – Cases Referred to higher centers because of complications.Tools used for breeding site identification – Quadcopters and Drones with HD cameras. Since the area to be surveyed was vast (>30km), it was very difficult to do a manual survey in the harsh desert climate. The patients' demographics, Clinical Presentations, and laboratory parameters were collected systematically in a predesigned format and entered into an excel sheet. The finding of our study was compared with the malaria cases from different parts of the country.

Results: All malaria was identified as Plasmodium vivax. The mean age group was 36 years (min-21 and max-56). Clinical features- out of 103 cases, 78% of the patients presented with all 4 symptoms of fever, chills, headache, and bodyache.22% presented with atypical isolated symptoms of abdominal pain, vomiting, myalgia, severe joint pain. 77% of patients had mild hepatosplenomegaly, 7 patients presented with hypotension, and 2 were in sepsis. Lab parameters – 88% of the patients had anemia, of which 5% had severe anemia, 40% had leucopenia while 6% had leucocytosis.87%had thrombocytopenia, out of which 56% had a platelet count less than 50,000/ml. 2% had mild AKI, bilirubin levels were elevated in 53%, and 36% had transaminitis. Using technology, the survey of the entire area was done in 1 week and all the breeding sites were eliminated on the same day of identification and a drastic drop in malaria cases was noted.

Conclusion: We conclude that desert malarial presentation of Plasmodium vivax is unique in clinical presentation and lab derangements compared to other malarial cases. Usage of technology like quadcopters and drones for breeding site identification played a major role in curtailing cases drastically in a very short period in an effective way.

Keywords: *desert malaria, Plasmodium vivax, drone surveillance for breeding site*

INTRODUCTION

Desert Malaria, yes you have read it right! As commonsense dictates, malaria is common in tropical countries and areas, where the mosquito breeding site is more commonly seen in places where water gets stagnated during the rainy season, e.g., ponds, ditches, and marshy areas which serve as a

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temporary breeding ground for mosquitoes. Malarial endemicity in a desert environment is a unique and rare geographical presentation of this disease.

The Great Desert of Thar is the most widely populated desert in the world, with a population density of 83 people per km^2, which is located in the northwestern part of India [1]. Desert ecosystem, which has its peculiarities to offer, is yet another operating system for malaria.

Malaria is one of the oldest diseases known to mankind. In ancient India, malaria was described in The Compendium of Sushruta, a Sanskrit medical treatise. The World malaria report 2019 estimates that globally, there were an estimated 228 million cases of malaria in 89 countries. The estimated number of malaria deaths in 2018 and 2019 stood at 411,000 lives in 2018 and 409,000 lives in 2019 respectively [2]. In 2012, it was estimated that the total economic burden of malaria in India was around US$ 1940 million, with 75% from lost earnings and 25% from treatment costs borne by households. Besides saving lives, eliminating malaria in India would also avert these socioeconomic losses [3].

Malaria is caused by a protozoan parasite, Plasmodium, and transmitted by the vector anopheline mosquitoes. Malaria parasite in India has two major human malaria species, Plasmodium falciparum, and P. vivax. Approximately 65% of those at risk for becoming infected with malaria in Southeast Asia are individuals residing in India (WHO 2010). The central and eastern regions of India report the most malaria. An. culicifacies is responsible for 60–65% of the malaria burden [4]. The three most important challenges for malaria control in India are difficulties in control implementation and logistics, insecticide resistance, and antimalarial drug resistance. Being the world's second-most populous country, and having a faster rate of urbanization, adds to the challenges. India contributes 77% of total malaria in Southeast Asia. Multi-organ involvement/dysfunction is reported in both Plasmodium falciparum and P. vivax cases [4]. In this study, we did a detailed analysis of the unique presentation of desert malaria and compared it with other studies of malaria presentation in a tropical ecosystem.

MATERIALS AND METHODS

This is a prospective study done from Oct to Dec 2020, where 103 cases of malaria cases were studied.

Inclusion criteria – all smear-positive malarial cases

Exclusion criteria – cases referred to higher centers because of complications.

Patients' demographics, clinical presentations, and laboratory parameters were collected in a systematic method and the collected data was analyzed in windows excel sheet.

For malarial surveillance, we had used quadcopters and drones with HD cameras for regular and periodic surveillance, as it was very difficult to do breeding site surveillance manually due to the extreme temperatures and scattered sites.

The photos from the drone and quadcopters were collected and given to the respective head of units in the areas and necessary action was taken within 24 hours of target identification to eliminate the breeding sites.

RESULTS

103 diagnosed cases of plasmodium vivax malaria were admitted, out of which all were males. The minimum and maximum age of the patients reported was 21 and 56 respectively, with a mean age group of 36 years. Duration of the hospital stay ranged from a minimum of 2 to a maximum of 18 days.

The average temperature of the patients was 101.5 F, maximum temp was 103 F. 78% of the patients presented with all 4 symptoms of fever, chills, headache, and body ache. 22% presented with atypical isolated symptoms of abdominal pain, vomiting, myalgia, severe joint pain. 77% of patients had mild hepatosplenomegaly, 7 patients presented with hypotension, and 2 were in sepsis.

Lab parameters – 88% of the patients had anemia (Hb 9-11 g%), of which 5% had severe anemia (6.5-8.5 g%), 40% had leucopenia while 6% had leucocytosis. 87% had thrombocytopenia, out of which 56% had a platelet count less than 50,000/ml. 2% had mild AKI, bilirubin levels were elevated in 53%, and 36% had transaminitis.

Using technology, the survey of the entire area was done in 1 week and all the breeding sites were eliminated on the same day of identification and a drastic drop in malaria cases was noted. It was a very effective and time-saving modality.

Figures 2 and 3 show open tanks on the roofs of apartment buildings, as well as abandoned open manholes behind these buildings where people don't frequent and were therefore unnoticed by the residents.

One of the main breeding sites of Anopheles could also be the long canals that run for kilometers through the desert, providing a haven for the larvae, as shown in Figure 4.

DISCUSSION

Our results were comparable to that of a study done in south India by Madhu Muddaiahet al. [5], in which males (81%)

outnumbered females (19%) and Plasmodium vivax was the major parasite type (52.54%). In their study incidence of malaria increased from June onwards coinciding with monsoon season, contrary to our study, where caseload was more in October-December. A study by Pankajala Amritha et al. [6], also had 94.71% of P. Vivax cases, and the mean duration of hospital stay was 5.17±3.31 days.

Figure 1: The area identified using a quadcopter. The area seen in the photo was highly inaccessible; Red arrow marks show the sites of waterlogging and the Yellow outlines show the breeding sites of Anopheles mosquito where the larva develops, and the shady environment gives mosquito cover from the extremely hot weather

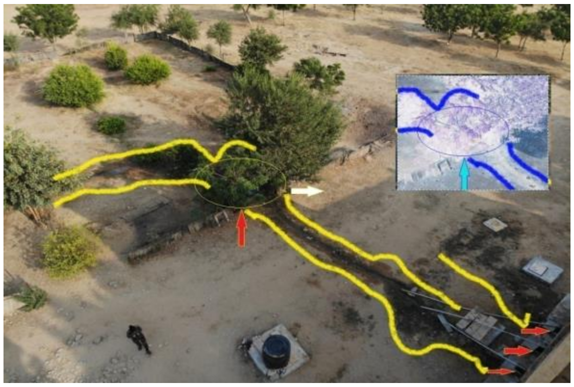

Figure 2

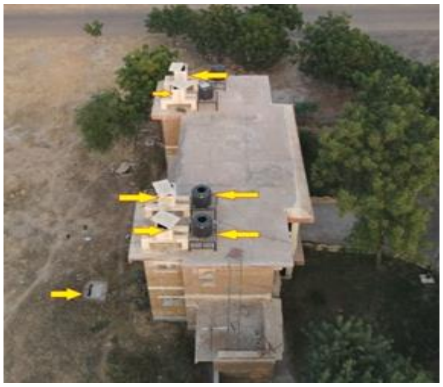

Figure 3

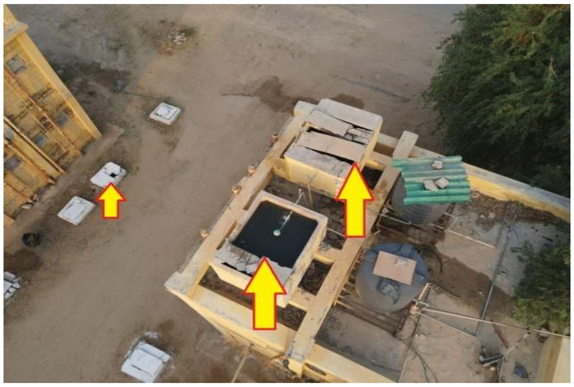

Figure 4

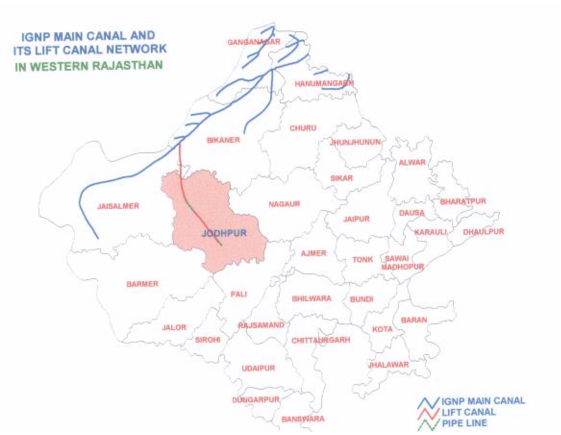
IGNP MAIN CANAL AND
ITS LIFT CANAL NETWORK
IN WESTERN RAJASTHAN
GANGANAGAR
HANUMANGARH
BIKANER
CHURU
JHUNJHUNUN
ALWAR
SIKAR
JAISALMER
NAGAUR
JAIPUR
BHARATPUR
DAUSA
KARAULI
DHAULPUR
JODHPUR
AJMER
TONK
SAWAI MADHOPUR
BARMER
PALI
BHILWARA
BUNDI
JALOR
SIROHI
RAJSAMAND
CHITTAURGARH
KOTA
BARAN
UDAIPUR
JHALAWAR
DUNGARPUR
BANSWARA
IGNP MAIN CANAL
LIFT CANAL
PIPE LINE

In a multicentre study done in Columbia [7], 92.5% of patients presented with fever, chills, headache, sweating, myalgia/ arthralgia, similar to our study. Mild hepatomegaly and splenomegaly as well as abnormal transaminases were mostly observed in P. vivax malaria patients.

A meta-analysis of malarial cases in Malaysia and Australia by Cho Naing et al. [8] provided evidence of the clinical relevance of severe thrombocytopaenia in P. vivax malaria which was in line with our result. In our study thrombocytopenia, anemia, and altered liver function were seen, contrary to study results found by Wajihullah Khan et al. [9] where anemia, jaundice, convulsions, and acute renal failure were significantly high (3-4 times) in subjects infected with P. falciparum than with vivax.

But a in study done in AIIMS Rishikesh by Jagdish Prasad Goyal et al. [10], there was no statistically significant difference found in complications such as anemia and thrombocytopenia between P. vivax and P. falciparum. Similarly in a Columbian study [11], anemia and thrombocytopenia were common in both.

A study was done in Aligarh Muslim University [12] in central India also showed that in P. falciparum infection, the incidences of anemia, splenomegaly, renal and liver dysfunction, and jaundice were higher compared to those in P. vivax infection. Several complications such as acute kidney injury, jaundice, severe anemia, metabolic acidosis, shock, hyperpyrexia, hypoglycemia, generalized tonic-clonic convulsions, etc. were found to be more prevalent in patients with P. vivax infection. These symptoms were until recently known to be associated with falciparum malaria [13].

Malarial surveillance programs by the government are adversely affected by an insufficient number of sanctioned posts of health workers and other program staff in different parts of the country. For instance, there are about 40000 multipurpose health workers (MPWs) against approximately 80 000 sanctioned posts for nearly 150000 sub-centers (SCs) across the country [14]. Over the past fifteen years, the fight against malaria improved dramatically. Insecticide-treated bed nets and indoor residual spraying resulted in an enormous reduction in the number of children dying of the disease [15]. Unfortunately, it is not enough.

For the first time, drones were tested to help fight malaria in Tanzania, on the island of Zanzibar, off the coast of Mozambique. High-tech 'Anti-Malaria Drones' drones were used to spray a silicone-based liquid gel (Aquatain) in endemic areas where malaria-carrying mosquitoes lay eggs, and where there are large concentrations of stagnant water with the hope that the new substance will stop mosquitoes' eggs from hatching. It is part of a program by the Zanzibar government, looking towards eliminating malaria by 2030 [16].

CONCLUSION

We conclude that desert malarial presentation of Plasmodium vivax is unique in its clinical presentation and lab derangements compared to other malarial cases. Doing manual surveillance for breeding sites is very difficult because of the large areas to be covered, the time involved, and the requirement of a lot of manpower. Using drones and quadcopters is a cost and time-effective method which helps in the very early identification and eradication of breeding sites.

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VARIA

Cloning and surface expression of engineered enzyme of organophosphorus hydrolase in E. coli and evaluation of its function

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Abstract: *Nowadays, the chemical compounds of organophosphate cause biological contamination due to their presence in the environment and production of metabolites, which endanger the health of humans and organisms. Thus, it is crucial to use a safe method to eliminate the contamination of these compounds. The objective of the present study was cloning and surface expression of the engineered enzyme of organophosphorus hydrolase in E. coli and comparing its function with the non-engineered enzyme. The present study aimed at improving the function of the organophosphorus hydrolase enzyme by expressing this enzyme along with Ice nucleation protein (INP) on the surface of bacterial cells. For this purpose, the OPH enzyme coding sequence was cloned in the pET28a-InaV-N plasmid and sent into E. coli, the strain of BL21 (DE3) and protein expression was evaluated at 37° C after 16 hours of induction. To examine the site of expression of the recombinant protein, cell components (cytoplasm, inner membrane, and outer membrane) were separated and the results were analyzed by using SDS-PAGE. The results revealed that OPH enzyme protein was presented as a protein fusion with a molecular weight of 58 KDa next to the INP anchor on the surface of the outer membrane of the cell. The specific activity of the resulting protein was calculated at 3828.8 U/mg after incubation of this enzyme in the vicinity of the substrate at 37° C. based on the results of the present study, it seems that the expression of engineered enzyme and its presentation on the cell surface is an achievement in using the cell as a degrading agent of organophosphate toxins and eliminating the costs of downstream processes such as purification and refolding of protein.*

Keywords: *clone, expression, engineered enzyme of organophosphorus hydrolase, E. coli, function*

INTRODUCTION

Following developments made in a recent century in the chemical synthesis of materials, new compounds have been made, some of which are toxic. Releasing of toxic compounds into the environment such as pesticides, insecticides, solvents, explosives, and dyes in industrial and agricultural applications has increased over the last decade [1]. One of these synthetic compounds is organophosphate compounds that are used in pesticides, insecticides, and neurochemical agents. These compounds have three phosphoester bonds, so they are also called phosphotriester. Phosphorus is attached to oxygen P = O or sulfur P = S by a double bond. Paraoxon, parathion, and diazinon are considered important pesticides and insecticides made from organophosphate compounds [2]. War nerve agents include G-type and V-type. It should be noted that V agents are much more toxic than G agents. The sudden release or poor management of pesticide and insecticide residues has contaminated the soil and groundwater in the region. Also, residues of nerve agents after wars can have devastating effects on the environment

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and people. These compounds inactivate a large family of serine hydrolase enzymes (lipases and esterases) [3].

Acetylcholinesterase is one of the most important enzymes inactivated by these compounds, leading to the accumulation of acetylcholine in the nerve synapse and the continuation of nerve stimulation, and muscle dysfunction and impairment in the respiratory system respiratory failure, which results in coma and eventually death. Thus, it is essential to develop bioremediation technology to facilitate the degradation of organophosphate contaminants [4].

At present, most chemical and physical methods such as heat, superoxidase, carbon dioxide, etc. are used to remove contamination from organophosphate compounds, but they are associated with many shortcomings and disadvantages since they are often toxic, allergenic, corrosive, non-proprietary, and harmful to the environment [5]. Thus, safe technology is required to hydrolyze toxic compounds [5]. One of the useful methods for degrading the organophosphate compounds is the use of bacterial strains with enzymes that degrade these compounds with a wide substrate range.

Enzymes that as biocatalysts can degrade organophosphate compounds include organophosphorus hydrolase (OPH) derived from Pseudomonas diminuta and species of Flavobacterium, organophosphorus acid anhydrolase (OPAA) obtained from Alteromonas, and diisopropyl-phosphatase obtained from fish called Loligo vulgaris [6]. Expression of these enzymes in other hosts, including E. coli as recombinant, is one of the suitable methods for its production. OPH enzyme is present in Flavobacterium ATCC 75512 and P. diminuta.

The natural substrate for organophosphorus hydrolase is not known. This enzyme is a homodimer with a molecular weight of 72 kDa [7]. OPH can hydrolyze phosphotriester P-O bond in paraoxon, phosphonofluoride (P-F) bonds in DFP, sarin, and soman, phosphorothioate bond (P-S) in VX, and phosphoro amido cyanide (P-CN) bond in Tabun. The speed of OPH enzyme function is very high for a substrate with P-O and P-S bonds.

OPH is the only enzyme known for the degradation of both paraoxon and V-type war agents [8]. In a previous study, an engineered sample of this enzyme (creating a disulfide bridge to increase stability) was produced in the cytoplasm of E. coli. Despite changes in expression conditions, the enzyme was produced as an air inclusion and underwent difficult stages of unfolding and refolding [9].

In the present study, to solve this problem and express the enzyme in solution, by designing and synthesizing the anchor, the production of the desired enzyme was done as surface expression.

For this purpose, bioinformatics studies to design the best anchor were first considered, and then cloning, expressing, and finally measuring enzyme activity were performed.

MATERIALS AND METHODS

Preparation of materials required

Ecoli BL21 (DE3 (Novagen), pET32a (Biomatik), pET28-InaV-(Laboratory of Baqiyatallah University of Medical Sciences), LB-Agar and LB-Broth (Quelab), Yeast and Tryptone (Merck), Ampicillin, Kanamycin, Calcium chloride, XhoI, SacI (Restriction endonuclease), Agarose, DNA Safe Stain, Boric acid, Bromophenol Blue, 2-Mercaptoethanol, Taq DNA Polymerase, PFU DNA Polymerase, DNA Ladder-1Kb (# DM3200), T_4 DNA Ligase, Loading Dye, Ethanol 96%, Plasmid Extraction Kit, Fragment DNA Purification, Sodium dodecyl sulfate, Tris Base, Glycine, Glycerol, Coomassie Brilliant Blue G250, Glacial Acetic Acid, Tetramethylethylene-diamine, Isopropyl-β-thiogalactopyranoside, Acidleryed, B acrylamide Ethylenediaminetetraacetic acid, Triton X-100, Phenylmethanesulfonylfluoride, ammonium persulfate, Lysozyme, NaCl, Sucrose, and Urea (Sigma) were purchased.

pET28a-InaV-N plasmid

the pET system is one of the most powerful systems available for cloning and expression of recombinant proteins by E. coli. One of these systems is the pET-28a (+) expression vector, which includes the T7 promoter.

The gene can be cloned in the pET expression system and E. coli BL21 (DE3) can be transformed by the recombinant vector and expressed as a result of inducing by isopropyl β-D-1-thiogalactopyranoside (IPTG). pET28a includes an antibiotic marker of kanamycin. This plasmid has been used in previous studies and contains an anchor called InaV-N.

Figure 1 illustrates an image of this plasmid [10].

pET32a-OPH Plasmid

This plasmid has a T7 promoter for high protein expression and due to having a Thioredoxin tag sequence; it leads to secretion of the produced protein and has the antibiotic marker ampicillin.

This plasmid was used in a previous study conducted by Farnoush et al. The sequence of this plasmid can be seen in Appendix 1.

Figure 2 provides an image of the pET32a plasmid [11].

Figure 1: Plasmid pET28

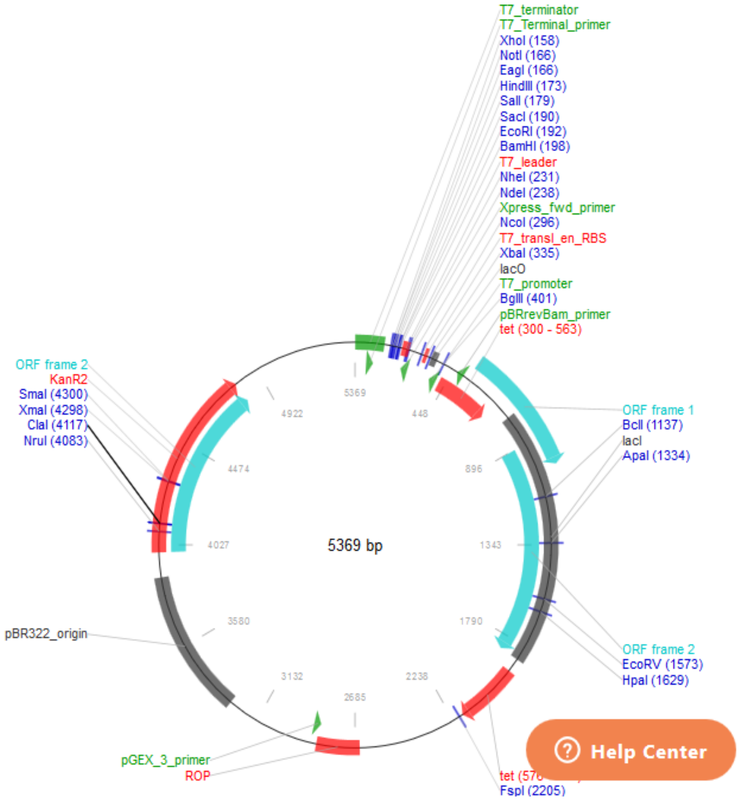
T7_terminator
T7_Terminal_primer
XhoI (158)
NotI (166)
EagI (166)
HindIII (173)
SalI (179)
SacI (190)
EcoRI (192)
BamHI (198)
T7_leader
NheI (231)
NdeI (238)
Xpress_fwd_primer
NcoI (296)
T7_transl_en_RBS
XbaI (335)
lacO
T7_promoter
BglII (401)
pBRrevBam_primer
tet (300 - 563)
ORF frame 2
KanR2
SmaI (4300)
XmaI (4298)
ClaI (4117)
NruI (4083)
ORF frame 1
BclI (1137)
lacI
ApaI (1334)
5369 bp
5369
448
896
1343
1790
2238
2685
3132
3580
4027
4474
4922
pBR322_origin
ORF frame 2
EcoRV (1573)
HpaI (1629)
pGEX_3_primer
ROP
tet (570
FspI (2205)
Help Center

Figure 2: Plasmid image containing OPH gene, The OPH gene is cloned between the two enzyme sites of BamHI and HindIII

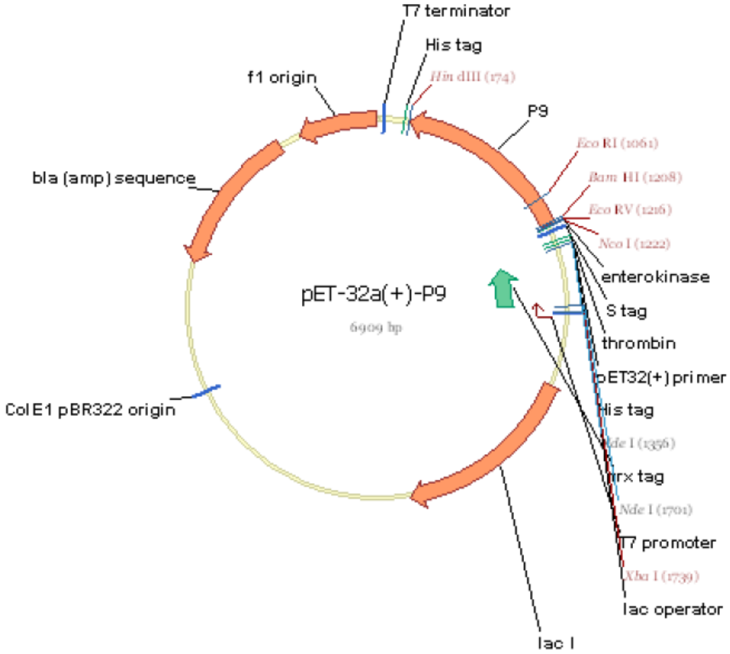
T7 terminator
His tag
Hin dIII (174)
f1 origin
P9
Eco RI (1061)
Bam HI (1208)
Eco RV (1216)
Nco I (1222)
bla (amp) sequence
enterokinase
S tag
thrombin
pET-32a(+)-P9
6909 bp
pET32(+) primer
His tag
Nde I (1356)
Col E1 pBR322 origin
trx tag
Nde I (1701)
T7 promoter
Xba I (1739)
lac operator
lac I

Plasmid purification

Plasmid purification was performed from strains containing plasmid pET28a and pET32a based on the standard protocol of the kit manufacturer. In short, 3 ml of the 18-hour culture of the strains was centrifuged and the supernatant medium culture was completely removed. The precipitate of cells was dissolved in 200 µl of FAPD1 buffer, 200 µl of FAPD2 buffer, and 300 µl of FAPD3 buffer and centrifuged at 250,000 rpm for 5 minutes. The transparent supernatant was poured on the columns for the kit and the columns were washed twice with 400 and 700 µl of W1 and Wash buffer and 50 µl of EB buffer was poured on each column and the desired plasmids were collected by centrifugation after 5 minutes [12].

Evaluation of purified plasmids by observing on agarose gel

Agarose gel electrophoresis is a standard method for isolating and identifying DNA fragments. A certain percentage of agarose is dissolved in the TBE buffer and, when cooled, forms a substrate that acts as a molecular sieve to separate DNA fragments based on their size in an electric field.

Polymerase chain reaction

For OPH gene amplification, two direct and inverse primers were required. These two primers were designed based on the initial and final sequences of the OPH gene. The direct primer is the complement at the beginning of the gene and the reverse primer is the reverse complement of the end of the gene. To insert this gene into the pET28a plasmid between SacI and XhoI sites, the cutting site of these enzymes was designed on direct and inverse primers and the synthesis order was given to the desired company (Table 1) [13].

Table 1: Primers of gene encoding OPH enzyme

Primer name	Primer sequence
Forward (p263)	5'_ CATGAGCTCTCTATCGGCACTGGTGAC_3'
Reverse (p264)	5'_ CATCTCGAGGCTTGCACGCAGAGTC_3'

Amplification of gene encoding OPH

To amplify the gene encoding OPH enzyme, the polymerase chain reaction was performed using designed primers according to the values presented in Table 2. PCR solution was prepared in 0.2 ml microtubes. In this reaction, the used model is pET32a, which contains the OPH gene, and a negative control sample was prepared according to previous values without the pET32a plasmid.

The microtubes were placed inside a thermocycler and reaction was done according to the temperature program presented in Tables 3-5 (Table 2) [14].

Confirming plasmid transfer into bacteria

To confirm the process of transfer of recombinant pET28-InaV-N/OPH plasmid into bacteria, PCR was performed for colonies grown on a solid culture medium. For each colony, some amounts were inoculated in 30 µl of sterile distilled water. Then, 15 µl of it was taken in 1 ml of liquid culture medium.

The remaining 15 µl was placed at 94° C for 15 minutes and then centrifuged at 13000 rpm for 2 minutes so that the broken cells precipitate. Supernatant media were used as a template in the PCR reaction.

The PCR reaction was prepared according to the values presented in Table 3 and performed according to the program of Table 4 [15].

Table 2: Values used in the PCR reaction to amplify the OPH gene

Material	Amount (µl)
Template (1 µg/µl)	10
Forward Primer (10µM)	2.5
Reverse Primer (10µM)	2.5
PFU SuperMasterMix 2x	25
Sterile deionized water	10
Total volume	50

Table 3: Values used in the PCR reaction for amplification of OPH gene

Material	Amount (µl)
Template (1 µg/µl)	2
Forward Primer (10µM)	0.25
Reverse Primer (10µM)	0.25
Taq 2x Master Mix RED	2.5
Total volume	5

Table 4: Values used in the PCR reaction to amplify the OPH gene

Cycle	Temperature (°C)	Time	Number of cycles
Primary denaturation	94	5 min	1
Denaturation Primer connection To lengthen	94 55 72	45 s 60 s 45 s	35
The final elongation	72	5 min	1

Enzymatic digestion

For cloning the OPH gene within the pET28a plasmid, both the amplified gene and the purified plasmid need to be cut using similar enzymes. It causes a complementary adhesive end on the gene and the plasmid and facilitates the binding of the gene to the plasmid.

Cutting both sides of the amplified gene was done using XhoI and SacI enzymes according to the values presented in Table 5.

The mentioned values were poured into 0.5 ml microtubes and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme [16].

Table 5: Values used for enzymatic digestion of gene amplified by PCR

Material	Amount (µl)
The amplified gene	50
Enzyme 10 Unit/µl XhoI	2
Enzyme 10 Unit/µl SacI	2
Universal buffer (10X)	7
Sterile water	9
Total volume	70

Cutting of plasmid pET28a using two enzymes of XhoI and SacI

The plasmid was cut according to the values of Table 3-10 by two enzymes of XhoI and SacI to linearize the pET28a-InaV-N plasmid and remove the previous fragment. The mentioned values were poured into a 0.5 ml microtube and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme (Table 6) [17].

Table 6: Values used in enzymatic digestion of plasmid pET28a

Material	Amount (µl)
Plasmid pET28a-InaV-N/OPH	5
Enzyme 10 Unit/µl XhoI	0.25
Enzyme 10 Unit/µl Sac I	0.25
Universal buffer (10X)	1
Sterile water	3.5
Total volume	10

Confirming entry of OPH gene into plasmid pET28-InaV-N by enzymatic digestion

To confirm the performed process and ensure the presence of the gene in the plasmid pET28-InaV-N, the plasmid was cut using two enzymes of XhoI and SacI with the values listed in Table 7. The mentioned values were poured into a 0.5 ml microtube and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme [18].

Table 7: Values used in enzymatic digestion of OPH

Material	Amount (µl)
Plasmid pET28a-InaV-N/OPH	5
Enzyme 10 Unit/µl XhoI	0.25
Enzyme 10 Unit/µl Sac I	0.25
Universal buffer (10X)	1
Sterile water	3.5
Total volume	10

Extraction of cut fragments from agarose gel using Kiagen kit

After cutting the gene and plasmid, it is necessary to separate these fragments from other extra fragments in the solution and then bind them together in a binding reaction. For this purpose, the product obtained from enzymatic digestion of plasmid was first taken on agarose gel and then extracted from the gel according to the kit protocol. The cut gene fragment was separated from the small cut fragments with the same kit without being placed on the gel. The gene was then purified by the clean-up method [19].

OPH enzyme protein expression

Plasmid gene-binding product was transferred into susceptible bacterial cells by the electroporation method. The culture was performed from colonies containing OPH gene plasmid in 5 ml of 2XY culture medium. When the optical absorption of the culture medium at 600 nm reached 0.7, after removing 1 ml of culture medium as an induction-free control, the promoter was induced by adding IPTG with a final concentration of 1 mM, and with the control sample, it was placed in an incubator shaker at 37° C for 18 hours. After the mentioned time, 1 ml of induced and control bacterial culture was precipitated and after adding 60 µl of 8 M urea, 16 µl of them were combined with 4 µl of sample buffer and heated for 5 minutes loaded on polyacrylamide gel [20, 21].

Primary evaluation of enzyme activity expressed on the surface

To evaluate the activity of the enzyme present on the surface, a precipitate of enzyme-expressing cells was collected and after twice washing with Tris buffer, 980 µl of cells with OD = 1 was poured into the microtube and 10 µl of cobalt chloride ion was added and 10 µl of parathion toxin was incubated for 1 h at 37° C [22].

Confirming surface expression of the enzyme

To locate the protein expression in the recombinant strain, it is necessary to first separate the cell components and then examine the fractions.

For this purpose, 20 ml of an overnight culture of control and test samples after protein expression, and their centrifugation and precipitate were dissolved in the lubricating buffer after washing twice in Tris buffer, and after half an hour of incubation at room temperature, the cell wall was broken with the sonicator device (4 30-second cycles with 80 power) was broken.

Then, centrifugation was performed at 2000 rpm for 3 minutes to separate the unbroken cells, and their supernatant was re-centrifuged at 14000 rpm for one hour. After emptying the supernatant into separate falcons, the precipitates were dissolved in Tris buffer containing Triton X-100 and 0.01 mM $MgCl_2$ and incubated at ambient temperature for 30 minutes to separate the inner and outer membranes.

Then, centrifugation was n performed at 13000 rpm for one hour. At this stage, the supernatant contains the inner membrane and the precipitate contains the outer membrane and the proteins attached to it. 500 µl of Tris buffer was added to both precipitates and dissolved.

Sampling was performed from each stage and examined using SDS-PAGE [23].

Evaluation of the specific activity of the enzyme in cell fractions

To evaluate the enzyme activity of the expressed OPH protein, a 50 mM Tris buffer was prepared.

The reaction medium containing 970 µl of Tris buffer, 20 µl of the sample (cytoplasmic samples, inner membrane, and outer membrane), 10 µl of cobalt ion with a concentration of 0.1 mM and 10 µl of parathion toxin with a concentration of 5 mg/ml were prepared.

After incubation at 37° C, the optical absorption of the samples was read at 405 nm and the specific activity of the enzyme was calculated using the following formula [24]:

$$\text{U/ml enzyme activity} = \frac{\text{dilution coefficient}\times\text{reaction volume}\times\text{absorption at 405nm}}{\text{time}\times\text{enzyme value}\times\text{PNP degradation coefficient}} \times 10^6$$

Reaction volume: 1 ml; Reaction time: 60 minutes; Dilution coefficient: 1; PNP degradation coefficient: 18000
Enzyme specific activity (U/mg) = normal activity (U/ml) / enzyme value (mg)

RESULTS

Amplification of OPH gene using PCR

In the first step, the OPH gene was amplified using p263 and p264 primers, and the PCR product was placed on agarose gel for confirmation. An observing band with a size of 1025 bp confirmed the amplification of the OPH gene. The OPH enzyme encoding gene is a 1008 bp sequence that, along with an enzyme site designed on primers, shows a length of about 1025 bp on the agarose gel (Figure 3).

Figure 3: OPH gene amplified by PCR.1- Marker # DM3200, DNA 1kb. 2. Negative control. 2. OPH amplified gene

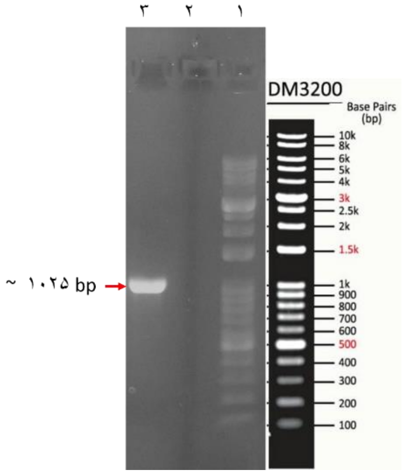

Evaluation of fragments obtained from PET28a-InaV-N plasmid cutting and PCR product on agarose gel

Plasmid pET28a-InaV-N and OPH gene PCR products were cut using XhoI and SacI enzymes and some of each reaction was loaded on an agarose gel to confirm the enzymatic digestion process. The 1017 bp band observed on the gel displaying the OPH gene and the 5978 bp band relate to the cut plasmid pET-InaV-N (Figure 4).

Figure 4: Fragments obtained from enzyme cutting of plasmid pET28a-InaV-N and PCR product. 1 and 2-fragments obtained from cutting OPH gene amplified by PCR.3-marker # DM3200, DNA 1kb. 4,5-plasmid pET28a- cut InaV-N

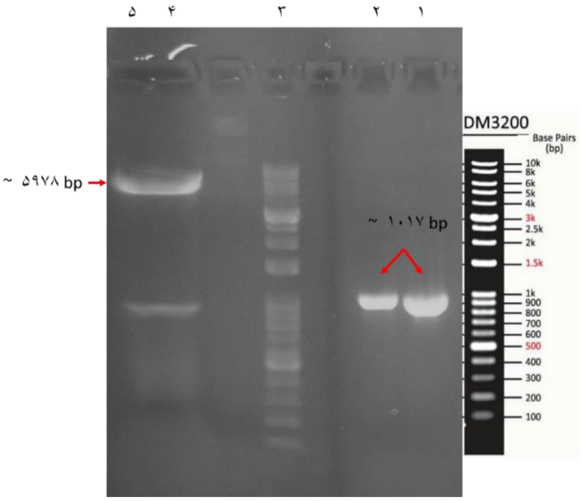

Extraction of plasmid and gene from agarose gel using Kiagen kit

After performing the enzymatic digestion process and examining the accuracy of cuts made with agarose gel, the area related to the desired fragment was cut and removed from the gel with the help of a special kit. The results of this purification were evaluated on a gel (Figure 5) and its concentration was measured with a nanodrop. The concentration of purified gene and plasmid was determined to be 144 ng and 82 ng, respectively.

Figure 5: Extraction of cut fragments from agarose gel. 1-marker # DM3200, DNA 1kb. 2. cut OPH gene

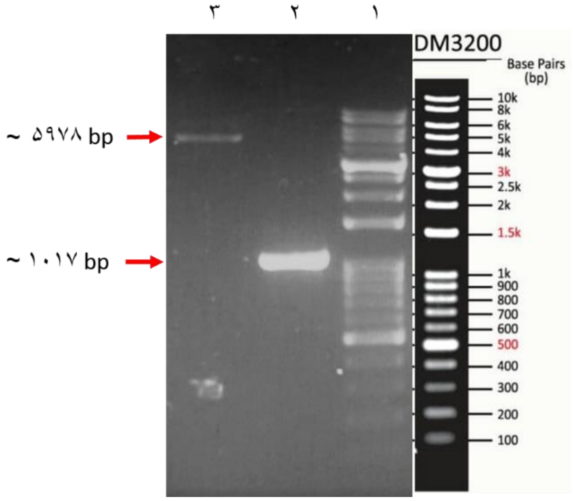
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DM3200
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Transfer of recombinant pET28a-InaV-N / OPH plasmid into E.coli BL21 strain (DE3)

After proximity of the OPH gene and pET28a-InaV-N at the presence of the enzyme ligase, the recombinant plasmid was transferred into a bacterium by electroporation E. coli BL21 (DE3) and cultured on an agar medium containing the antibiotic kanamycin.

As shown in Figure 6, plasmid-containing colonies grew overnight.

Figure 6: Colonies obtained from pET28a-InaV-N / OPH transforming into E. coli BL21 strain (DE3).

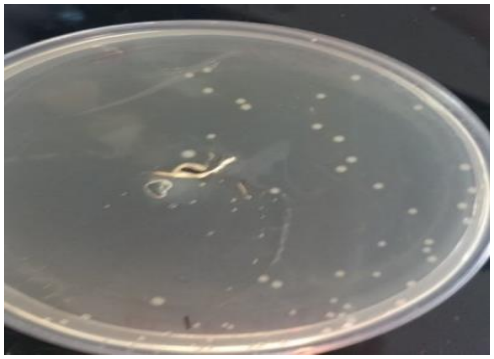

Confirming of colonies containing recombinant plasmid pET28a-InaV-N/OPH by direct PCR method from colonies

To evaluate the accuracy of transfer of the recombinant plasmid within the bacterial strain, 12 colonies grown on the medium were used for PCR. Among the tested colonies, the 1025 bp band, which is amplified by the OPH gene, was observed only in colonies 10 and 14.

The gene-free sample was considered as negative control and the sample containing plasmid pET32a-OPH was considered as a positive control (Figure 7).

Figure 7: Results of direct PCR of colonies grown on agar. Samples 1-10, 14, and 15 of PCR products of colonies. 11- DNA marker 1kb # DM3200. 12-Positive control. 13-Negative control

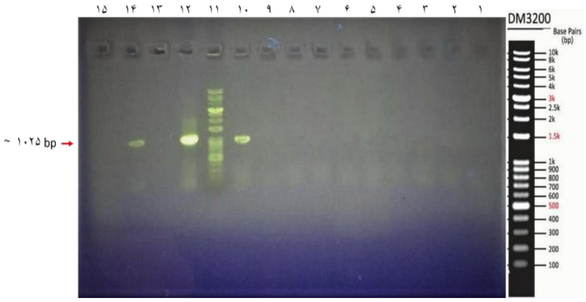
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Two-enzyme digestion of plasmid pET28a-InaV-N/OPH

The final confirmation of the presence of recombinant plasmids in BL21 strain (DE3) was performed by digestion with two enzymes of XhoI and SacI. The 1017 bp band obtained from the plasmid cut in Figure 8 indicates the presence of the gene.

Figure 8: Two-enzyme digestion of plasmid pET28a-InaV-N / OPH. 1. Uncut plasmid. 2-DNA1kb marker. 3. Cut plasmid.

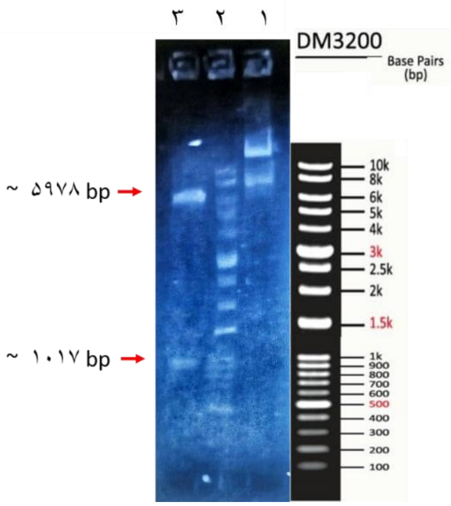
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Induction of expression for two confirmed colonies

Two confirmed colonies were inoculated in 5 ml of culture medium and expression was induced after reaching OD600 of culture medium to 0.7. The results obtained on polyacrylamide gel are as follows. As shown in Figure 9, the

58 kDa band is observed only in one of the test samples and is not observed in-band control sample.

Figure 9: Expression of OPH enzyme in BL21 strain (DE3) at 37° C. 1 and 5 - samples of the negative control. 2 and 4 - Induced samples after 5 hours of incubation at the presence of IPTG inductor. 3 - Protein marker # PM1700

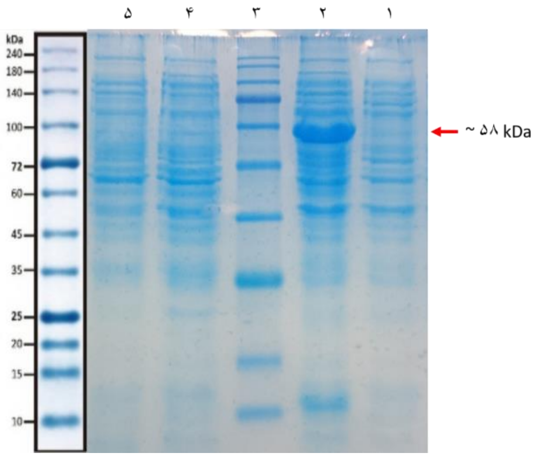

Evaluation of enzyme activity expressed on the surface

To evaluate the activity of the enzyme present on the surface at 37° C, 18 hours after adding IPTG, the precipitate of cells of test and control samples was collected and entered into the reaction medium. After one hour, the absorption of the reaction medium was read at 405 wavelengths and the enzyme activity was calculated. The samples include bacteria without the OPH gene and cytoplasmic enzyme-producing bacteria. As shown in Table 8, the activity of the enzyme expressed at the surface is lower than that of the enzyme produced as a solution in the cytoplasm.

Table 8: Comparison of enzyme activity expressed on the surface and sample expressed in the cytoplasm

Enzyme Activity (U/ml)	37^0 C
Gene without genes	0.74
Cytoplasmic expression	2.77
Superficial expression	7.67

Evaluation of the specific activity of OPH enzyme

After separating the cell components, the fractions related to each fraction of the cell were first examined by SDS-PAGE. Then, their activity was measured based on mentioned conditions, and the obtained data are shown in Figure 10. As shown in the figure, most of the activity is related to the outer membrane. It means the expression of enzymes on the surface of the outer membrane of the bacterium.

The specific activity of the OPH enzyme was estimated at 3828.8 U/mg using the formula mentioned in Figure 10 and Table 9.

Figure 10: Separation of cell fractions after protein expression at 37° C

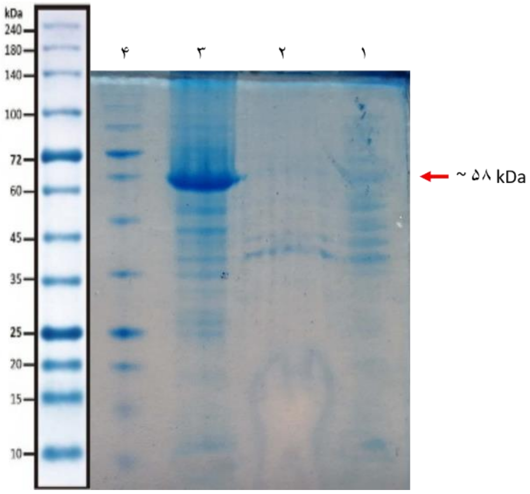

2. Cytoplasmic fraction. 2. Inner membrane fraction. 3. Outer membrane fraction. 4-Protein marker # PM1700

Table 9: Examining the enzymatic activity of each cell fraction

The activity of each cell segment (U/ml)	37^0 C
Cytoplasmic section	3.6936
Internal membrane section	1.2162
Outer membrane section	7.6576

DISCUSSIONS

Organophosphate compounds, including pesticides and neurochemicals, are very dangerous and insoluble in aqueous systems. Despite their high toxicity, organophosphate pesticides are still widely used to protect plants in agriculture. Due to the accumulation of these compounds in food products and water resources and its irreversible effects on human health and other organisms, it is essential to develop an advanced, appropriate, and cost-effective method to eliminate these compounds from the environment. Among the various physical, chemical, and biological methods, the use of natural strains as biodegraders has high importance. Strains such as Pseudomonas diminuta MG and Flavobacterium sp. ATCC 21881, which uses organophosphate molecules as food sources, can degrade a wide range of these compounds, including pesticides and neurochemical agents [25]. The degrading enzyme identified in these strains, called organophosphorus hydrolase (OPH), contains 336 amino acids and has a molecular weight of 36 kDa. The gene encoding this enzyme was used for expression in the cytoplasmic space of BL21 (DE3) bacterium after two mutations at sites 99 and 124 and altering two amino acids of threonine and glutamic acid to cysteine to create a disulfide bond, but its expression was mainly accumulated as protein masses in the cytoplasm and was dysfunctional and without proper structure [26, 27]. Thus, the bacterial cell must first be lysed and after extracting the accumulated proteins, they must be solubilized with a strong denaturator and the refolding process must be continued in the presence of appropriate stabilizers. All of these steps require much time and money, and finally, with the loss of a part of the protein, a protein is obtained that often does not regain its structure 100% and does not have good stability [28]. Another form of OPH enzyme expression is performed as periplasmic expression. In research carried out in 2015 by Latifi et al, the OPH enzyme encoding sequence in E. coli was synthesized and the OPH enzyme was expressed in the form of air inclusion using pET21a-opd and pET26b-opd in periplasmic space. Periplasmic expression of this enzyme also leads to limited production of this enzyme and requires complementary processes such as bacterial cell breakdown and protein extraction from the cell [25]. Besides the various methods proposed for the expression of recombinant proteins as a solution, including reducing the temperature, reducing the concentration of inducer, and using sequences that help solubilize the protein, the use of membrane anchors to provide protein on the bacterial cell surface is one of the most effective strategies used to express a wide range of proteins and has yielded promising results since molecule when attached to a matrix-like the cell surface is more accessible and much more stable than the free molecule and does not require long and costly purification steps [26, 27]. The use of natural integral proteins of the outer membrane as carriers is necessary for the goals of presentation on the surface. A variety of surface anchor motifs such as Lpp-OmpA, INP, and auto-transporters are used in this system due to their properties [31]. OPH enzyme expression in E.coli has been performed as a surface expression by various researchers. For example, in a study conducted by Richins et al in 1997, OPH enzyme expressed the OPH protein on the surface of E. coli using an Lpp-OmpA anchor system [32]. Shimazu et al in 2001 examined the expression of OPH protein by three anchors, including Inak-NC, InaV-NC, and Lpp-OmpA. The Inak anchor motif was derived from P. syringae KCTC 1832 and the InaV motif was derived from P. syringae INA [33]. Dong Kang et al in 2008 used only the second InaK amine to provide OPH on the surface [34]. In each of these studies, the OPH enzyme was expressed by different anchors on the cell surface. As a surface expression of OPH enzyme in previous studies in E. coli has resulted in the production of functional and highly stable proteins, and since the engineered OPH enzyme used in this study has been previously expressed in the cytoplasm due to its disulfide bond in the air inclusion and non-functional form, this sequence was re-expressed differently in E. coli in the form of surface expression using the InaV-N anchor. The results of investigating the activity of different parts of cell fractions showed that the fraction related to the outer membrane has the highest activity and has an activity more than twice as much as other cell fractions. Inav-N anchor not only can transfer OPH protein on the surface of bacteria but also has a positive effect on its activity.

CONCLUSIONS

The main objective of the present study was a surface expression and improving the efficiency of the engineered enzyme OPH (containing disulfide bridge) which was expressed in the previous study as air inclusion. The use of InaV-N anchors, which is one of the known anchors for surface expression, responded well to the desired enzyme and the enzyme could maintain its activity after surface expression. In a conclusion, this study could solve the problem of the insolubility of the protein, maintain its structure and its desirable activity, while surface expressing the engineered protein using the InaV-N anchor on E. coli bacteria.

Acknowledgment

The authors wish to thank all staff of Department of Biochemistry, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran; and Applied Biotechnology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran for their cooperation in implementing experimental procedures and analysis of data.

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VARIA

Clinical profile of Mediterranean fever – Study from an Indian tertiary care center

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Abstract: *Introduction: Brucellosis is a zoonotic disease which is also known as Mediterranean fever. It is acquired from the handling of infected animals or consuming contaminated milk or milk products. The incubation period varies from 1-3 weeks to several months before clinical disease appearance and it shows multiorgan involvement. This study focuses on the varied presentation of this disease and laboratory findings. Materials and Methods: This is a retrospective study, where the past 15 years data of cases diagnosed with brucellosis was studied in a tertiary care center in South India. Patient demographics, clinical presentation, duration of stay in the hospital, hematological and biochemistry profiles were collected and analyzed in SPSS. Results: More number cases were found among the male gender (70%), mostly in the 20-40 years age group (40%). 95% of cases reported fever, 40% with arthralgia (29% generalized in nature), and 32% with a headache. A Positive Pearson correlation between CRP and NLR, and CRP and PLR were found, which was statistically significant. Mean laboratory values were documented. 37% of patients were found to have hepatosplenomegaly on ultrasound Conclusion: Most of the patients were admitted as cases with pyrexia of unknown origin. Our study focused on the different clinical presentations and laboratory findings of brucellosis and will help give a better understanding and outline during brucellosis case diagnosis and management in a cost-efficient manner.*

Keywords: *brucellosis, undulant fever, Mediterranean fever*

INTRODUCTION

Brucellosis is a zoonotic disease caused by bacteria of the genus Brucella. It is also known as undulant fever or Mediterranean fever [1]. Caused by Gram-negative coccobacilli, non-spore-forming and non-motile and aerobic; B. melitensis, B. abortus, B. suisare the important human pathogens. It is acquired from the handling of infected animals or consuming contaminated milk or milk products. Exposure is frequently occupational. Human brucellosis is either an acute febrile disease or a persistent disease with a wide variety of symptoms like night sweats, asthenia, insomnia, anorexia, and headache.

The disease is controlled by the routine practice of pasteurizing milk and milk products, as well as by comprehensive campaigns to eradicate the disease by destroying domestic animals which exhibit positive serologic reactions to brucellae. Vaccines providing some protection to cattle, sheep, and goats are available [2]. The incubation period of this infection is between 1-3 weeks but can take several months before clinical disease appearance [1]. The disease shows multiorgan involvement including musculoskeletal, digestive, urogenital, hematological, cardiovascular, respiratory, and central nervous systems [3]. According to the duration of symptoms, it can be classified

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as acute (<8 weeks), sub-acute (8 to 52 weeks), and chronic (> 52 weeks).

Subacute brucellosis, typically presenting with undulant fever, described in endemic areas with localized infections such as epididymitis, orchitis, and osteoarticular complications, is most commonly seen. Osteoarticular involvement is the most common focal complication of brucellosis (10-85%) [4]. Sacroiliac joints are the most common site involved in younger patients; spondylitis and peripheral arthritis usually occur in older patients. Cardiovascular complications of brucellosis (endocarditis, myocarditis, and pericarditis) are rare (1% of cases) [5].

In humans, the most common route of entry of Brucella bacteria is through the gastrointestinal tract and it then localizes in the reticuloendothelial system. Hepatic enlargement is the most frequent gastrointestinal manifestation of brucellosis (32-63% of cases) [6]. Neurobrucellosis is the most serious complication of brucellosis with neither a typical clinical manifestation nor a specific cerebrospinal fluid (CSF) picture and mimics other neurological disorders leading to diagnostic dilemmas. The prevalence of Neurobrucellosis ranges from 1.7 to 10% of brucellosis worldwide [7].

This study aims to record the clinical features and cut off of certain laboratory values of human brucellosis cases in South India, for prompt diagnosis of the same.

MATERIALS AND METHODS

This is a retrospective observational study, where 15 years of data of patients diagnosed with brucellosis is collected in a pre-designed format. Details including demographics, clinical presentation, hematological and biochemistry profiles are collected and analyzed in SPSS. Inclusion criteria are patients diagnosed with brucellosis in our hospital, with positive IgM ELISA. Exclusion criteria include cases previously diagnosed from an outside hospital, patients who had taken the alternative form of treatment, etc to avoid bias in lab reports. Study design, data collection, and write-up were done in Amrita Medical College. Study analysis and review were done by V.S. Srikanth, using SPSS, where Pearson correlation and values of central tendency of the findings were recorded.

Data collection, analysis, and article preparation are done in accordance and with the permission of Amrita Institute's ethics review committee.

RESULTS

In our study, we found that 70% (54) were male and 30% (23) females, out of the total 77 cases. The age group of presentations were most in the 20-30 years group (22%); 30-40 years were 18% and remaining presentations were scattered across all other age groups (Table 1).

Table 1: Age distribution

Age	< 17 years	20-30 years	30-40 years	40-50 years	50-60 years	> 60 years
Number	13	17	14	10	11	12
% distribution	17%	22%	18%	13%	14%	16%

5 cases were reported outside Kerala. The hospital stay duration was a minimum of 3 days and a maximum of 30 days, with a mean duration of 10 days. Clinical presentation – Fever - 73 (95%) patients presented with fever, out of which 50 (68%) had a high-grade fever (102-104). The fever duration was a minimum of 5 days to a maximum of 120 days and a mean duration of 21 days. Arthralgia – 31 (40%) patients presented with severe arthralgia, rest had no features of arthralgia (Figure 1).

Figure 1: Arthralgia

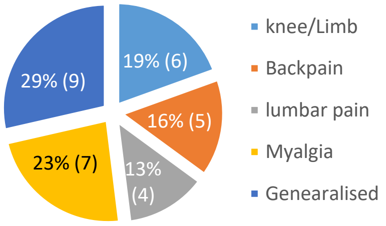

The most common GIT presentation was Constipation and vague abdominal pain and headache were present in 32% (25) of patients (Figure 2).

Figure 2: CNS symptoms

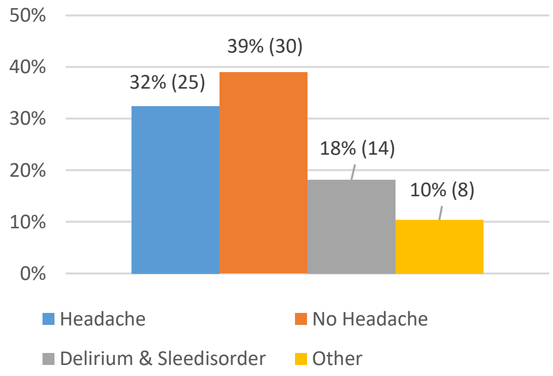

Mean values of neutrophil, lymphocyte, platelet counts, red cell distribution width, mean platelet volume, C reactive protein (CRP), procalcitonin, hemoglobin, SGOT, SGPT, serum protein, albumin, sodium, potassium, creatinine, and IgM Elisa values were found (Tables 2 and 3).

Table 2: Laboratory findings

Statistical Values	N (%)	L (%)	P (KU/ml)	RDW (%)	MPV (fL)	CRP (mg/L)	Procal (ng/mL)
Mean	56.32	31.79	303.01	14.95	8.39	61.58	0.78
Min Value	7.8	4.9	27.1	7	5	0.3	0.018
Max Value	90	72	687	27	25.8	306	5.3
Median	56	29.6	284.5	14.2	7.99	29.32	0.2
Std Deviation	17.33	16.12	147.01	3.09	2.99	72.46	1.42

Table 3: Laboratory findings – cont.

Statistical Values	Hb (g/dL)	SGOT (IU/L)	SGPT (IU/L)	Protein (g/dL)	Albumin (g/dL)	Na (mEq/l)	K (mEq/l)	Creat (mg/dL)	IgM ELISA (U/ml)
Mean	12.01	93.33	99.70	7.17	9.57	134.30	8.80	0.92	14.99
Min Value	6	9	9	5.33	1.77	120	2.7	0.05	0.8
Max Value	17.4	2142	2563	9.5	178.9	141.5	309	1.66	80.622
Median	12	36.35	51.5	7	3.7	134.55	4	1	5.5665
Std Deviation	2.31	253.51	293.64	0.84	27.77	4.12	37.82	0.32	20.65

Pearson correlation between CRP and neutrophil-lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) was found to be positive and statistically significant (Table 4, Figures 3 and 4 respectively). 37% (29) of patients were found to have hepatosplenomegaly on ultrasound.

Table 4: Pearson Correlation

		NLR	PLR
CRP	Pearson Correlation	0.476	0.579
	P value	0.001	0.001

Figure 3: Scatter Plot of Pearson Correlation between CRP and NLR

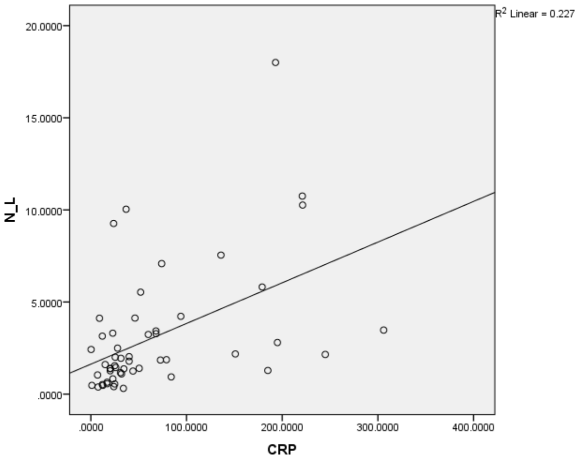

Figure 4: Scatter Plot of Pearson Correlation between CRP and PLR

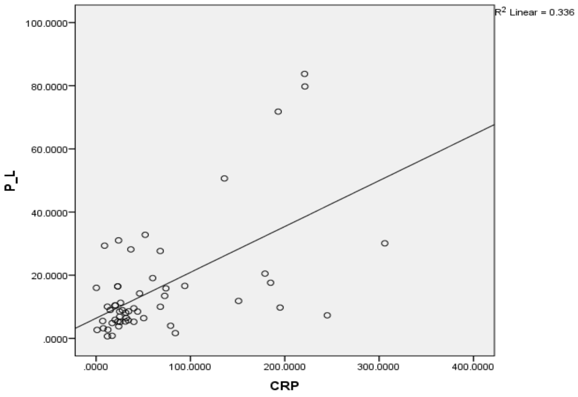

DISCUSSION

A higher incidence of cases was seen among males in our study (54, 70.1%), comparable to similar studies [9, 10, 18].

The mean age of brucellosis patients in our study was 36.95+19.22 years, similar to the findings of Huseyin S et al. [19]. In another similar study done by Mahmood Nabavi et al. [7], the mean age was 39.59 ± 17.28 years, and the most prevalent age groups was 31–50 (42.1%), while in another study done by Akhvlediani T et al. [9], mean age was 39.9 (standard deviation = 15.1) years and 45% of participants were 21–40 years old, which is comparable to our result.

Common clinical presentations in our study were fever (73, 94.8%), arthralgia (31, 40.26%), and generalized pain and swelling with myalgia (33, 43%), consistent with other studies [18]. Abdominal pain, loss of appetite, loss of weight, vomiting, etc were seen in 34 (44.1%) cases, while other symptoms like headache, altered sensorium, and fatigue were seen in 28 (36.3%) cases. In a similar study done by Akhvlediani T et al. [9], the most frequent complaints were sweats (92%), joint pain (91%), aches (90%), malaise (90%), fatigue (87%), and rigors (86%), arthralgia (72%). Loss of appetite, weight loss, and arthritis was also observed in 46%, 44%, and 24% of participants, respectively. The most prevalent clinical manifestations of brucellosis were fever, anorexia, weight loss, low back, and muscle pains in the study by Mahmood Nabavi et al. [7] and another similar study done by Kazanasmaz H [11], arthralgia (45.8%), fever (≥38.5 C0) (28.6%), and malaise and fatigue (11.5%) were the most common complaints at the time of presentation to the hospital [4].

29% of the patients with arthralgia presented with back pain, consistent with the findings of the study by Tasova Y et al. [15] and Turan H [16].

Mean neutrophil, lymphocyte, NLR, platelets, PLR, RDW, MPV, and CRP was found to be 56.3, 31.8, 3.22, 303, 15.67, 14.95, 8.39, and 61.58 respectively in our study. In a similar study done by Aktar F et al. {12], Neu – 71, Lym – 16, Plt – 282, NLR – 4.1, PLR – 154, RDW – 16.2, MPV – 8.2, and CRP-28.1. NLR can be determined from routine blood differentials at no additional cost, it increases in inflammatory conditions and this increase is considered as an indicator of systemic inflammation [13]. Studies showed that platelets also play an active role in inflammation while having regulatory effects on the immune system [14].

A similar MPV of 8.6 and a mean platelet count of 246 were also seen in the study done by Okan DH et al. [14]. Thrombocytopenia and lower MPV values found in our study are consistent with findings of other similar studies [12, 14]. Considering the same, MPV values in combination with platelet counts might be useful in patients with brucellosis [14].

C-reactive protein assay is a helpful adjunct in the diagnosis of patients with brucellosis [17]. High levels of CRP (61.58), SGPT (93.3), SGOT (99.7) was seen in our study, similar to the high CRP (39%), high ALT (33%), and high AST (20%) found in the study done by Shi et al. [18]. A positive correlation between standard agglutination titer and SGOT and SGPT was found by Huseyin S et al. [19].

AST, ALT, and CRP levels were found to be elevated in 27.6%, 21.6%, and 69.6% of the patients, respectively, and leukopenia (21.4%), thrombocytopenia (23%), and anemia (70%) in the study by Guler et al. [20]. 37% of patients were found to have hepatosplenomegaly on ultrasound, consistent with findings from a similar study done by Shi et al. [18].

Limitations

Only 77 cases could be included in the study. Not all the patients had all the laboratory tests done. Correlations were only weakly positive.

CONCLUSION

The most common clinical presentation of brucellosis was fever, arthralgia, abdominal pain, and headache. Elevated levels of CRP, procalcitonin, NLR, SGOT, and SGPT with thrombocytopenia and decreased levels of MPV were seen. A positive and significant correlation of CRP with NLR and PLR was also seen. Our study focused on the different clinical presentations and laboratory findings of brucellosis and will help give a better understanding and outline during brucellosis case diagnosis and management, in a more prompt manner.

Contribution of authors

Varsha Somakumar, V S Srikanth, Cibi Manoharan – concepts, design, the definition of intellectual content, literature search, data acquisition, data analysis, manuscript preparation, editing, and review; Vishal Marwaha, VR Mujeeb, M.G.K.Pillai – concepts, design, the definition of intellectual content, Manuscript editing and review; Anna Maria, Mehana Ashraf – data acquisition, data analysis, manuscript preparation, editing.

The manuscript has been read and approved by all the authors and each author believes the manuscript represents honest work. There is no conflict of interest among the authors.

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VARIA

Clinical characteristics and management evolution of cholelithiasis in a pediatric population: 123 cases experience

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Abstract: *Introduction: The incidence of cholelithiasis has recently increased in the pediatric population, although it remains a rare disease among children. The purpose of this study is to highlight the management and the appropriate time to perform surgery.*

Materials and Methods: We performed a retrospective study over 10 years (2010-2010) and we found 123 patients who presented with biliary lithiasis and who underwent surgery in our department. We divided the study population into two groups depending on the time of admission and we found that in the 2010-2015 period we had a number of 33 patients, while in the last 5 years we admitted 90 patients with this diagnosis. Patients with other conditions such as bile duct atresia, choledochal cyst, hepatoblastoma that required a cholecystectomy were excluded from our study.

Results: After analyzing the two groups we concluded that the number of surgical procedures increased from 33 to 90, which represents a 2.6 times increase. There was no significant difference between the two groups regarding the average age on admission (12.6 +/- 3.37 in the first group vs. 13.91 +/-3.34 in the second group). Surgical management of lithiasis cholecystitis registered an important difference in the two groups. In the first group, 9 patients (27.3%) underwent classic cholecystectomy while 24 (72.7%) – patients benefited from the laparoscopic approach, while in the second group just one patient underwent open surgery with 89 (98.9%) patients undergoing laparoscopic surgery. The percentage of patients who benefited from surgery in the same hospitalization remained high in both groups studied with a statistically significant p-value (p = 0.0417).

Conclusion: The number of cases with cholecystitis and lithiasis is increasing in the pediatric population. Surgical management for these patients has become similar to that of adult patients, with minimally invasive surgery becoming the gold standard approach.

Keywords: *lithiasis, cholecystitis, cholecystectomy, pediatric*

INTRODUCTION

Gallbladder disease is one of the most common and costly digestive diseases that require hospitalization. Gallstones are more common in the adult population and remain relatively uncommon in children. However, the incidence of gallbladder disease in children has markedly increased over the last several decades. Although uncommon during the first years of life, it is being diagnosed with increasing frequency in neonates and infants, probably because of the widespread use of ultrasonography [1, 2].

There are a few well-known risk factors for pediatric cholelithiasis such as hemolytic conditions, prematurity,

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prolonged parenteral nutrition, obesity, and puberty (for girls) [1]. Although in the past, biliary lithiasis was most often associated with benign hematological disease, nowadays the prevalence of non-hemolytic gallstone disease has been shown to increase as the prevalence of obesity also increases [3].

Even if for adults cholecystectomy is a routine procedure, the same cannot be stated for pediatric patients. According to early reports over recent years, there has been a significant increase in pediatric cholecystectomies due to the rising incidence of lithiasis cholecystitis [4].

The primary pediatric indication for cholecystectomy is biliary dyskinesia or symptomatic cholelithiasis. One reason for the increase in the frequency of pediatric cholecystectomy may be that laparoscopic cholecystectomy is commonly accessible. Laparoscopic cholecystectomy has become the gold standard in the treatment of symptomatic gallstones. The major advantages of laparoscopic cholecystectomy include less postoperative pain, less time required for hospitalization and recovery, and better cosmetic results [5].

This study is a retrospective analysis of patients under 18 years old, following the characteristics, evolution, and surgical management of the patients diagnosed with lithiasis cholecystitis who underwent cholecystectomy at Grigore Alexandrescu Emergency Pediatric Hospital of Bucharest in the pediatric surgery department.

MATERIALS AND METHODS

Patients who were admitted to our department for cholecystectomy between January 2010 and December 2020 were taken into consideration and included in this retrospective study. We took into account patients who presented in our service either through the emergency room, as acute cholecystitis, or in a scheduled manner.

The main inclusion criteria for patient selection from our database were patients under 18 years old, who underwent cholecystectomy for lithiasis cholecystitis. Patients with hepatoblastoma, choledochal cyst, congenital biliary atresia, and other conditions that required cholecystectomies but the cause was not lithiasis were excluded.

Data for this review was obtained from the Pediatric Surgery Department's database. One hundred and forty-one cholecystectomies were performed during this period but after we applied the exclusion criteria the total amount of cases remained 123.

After we have considered the variables for all the patients, we divided them into 2 groups of interest depending on the period in which they presented in our department (group 1: 2010-2015 and group 2: 2016-2020) to investigate the trend over time.

Afterward, patients were divided according to their diagnosis into symptomatic and asymptomatic. Those symptomatic were further divided into uncomplicated (diffuse abdominal pain, biliary colic, nausea, and vomiting, without any inflammatory signs) and complicated (which associated choledocholithiasis, biliary pancreatitis, or acute abdominal pain in the upper quadrant in association with inflammatory signs like fever, leukocytosis, and high CRP). Patients who were referred to the emergency department with acute cholecystitis due to lithiasis have benefited from surgical treatment either during the same hospital stay (if the liver enzymes and amylase decreased) or less than a month after the acute event in most cases. For those who were diagnosed with gallstones accidentally by ultrasonography, the conservative treatment (ursodeoxycholic acid-dose 10 mg/kg/day) was the first choice and if during subsequent evaluation still presented lithiasis, cholecystectomy was taken into consideration.

Abdominal ultrasound was performed for all patients. If an impacted calculus in the common bile duct was found during an ultrasound, magnetic resonance cholangiography (MRCP) or endoscopic retrograde cholangiopancreatography (ERCP) were then performed.

We also took into consideration anatomical abnormalities as a risk factor for lithiasis and found 3 cases with abnormal anatomy of the gallbladder. Criteria like surgical approach, intraoperative cholangiography, postoperative complication, and days of hospitalization and rehospitalization were also taken into account. Postoperative follow-up ranged from 6 months to 2 years.

For data collection, analysis, processing, and filtering we used Microsoft Excel. Comparisons between both 1st and 2nd periods were made using a two-tailed Student t-test for continuous variables and a one-sample t-test for categorical variables with a p-value ≤0.05 defined as significant.

RESULTS

This study was conducted on 123 patients with lithiasis cholecystitis who underwent either open or laparoscopic cholecystectomy. Before dividing the cohort into two main groups, we can easily observe the ascending trend of lithiasis cholecystitis cases with cholecystectomy over the 10 years (Figure 1).

Figure 1: Number of lithiasis cholecystectomies

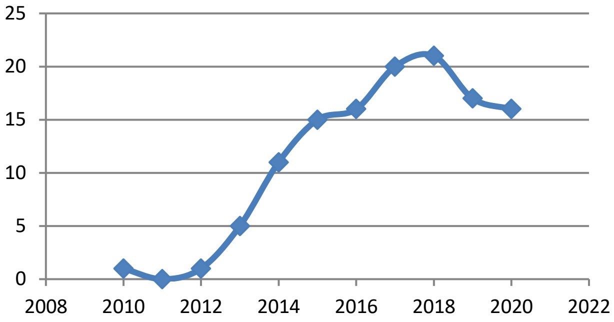

Figure 2: Mean age at the time of diagnosis in both groups

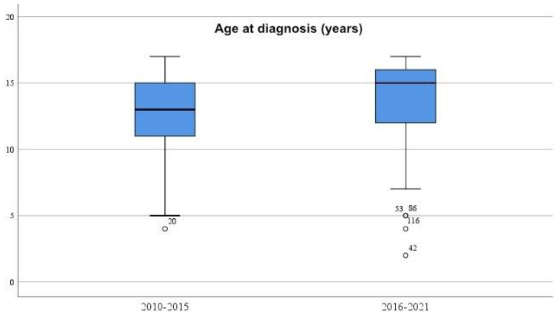

After the cohort was divided into two main groups according to the period, the median age registered a 2-year increase in the average age in group 2 compared to group 1 (p=0.2029) (range, 1 year to 17 years and 11 months) (Figure 2).

The vast majority of children were females in both groups (group 1 – 63.63%; group 2 – 66.66%). Taking into account the background, there is a slight increase in rural patients in group 2, but patients from the urban background are still the majority.

Table 1: Preoperative variables

Variable	**Category**	**Group 1 (G1) n=33 (100%)**	**Group 2 (G2) n=90 (100%)**	**P value <0.05** (One sample t-Test)	**P value <0.05** (Tow sample paired t-Test)
Gender	Male	12 (36.36%)	30 (33.33%)	0.0393	0.1266
	Female	21 (63.63%)	60 (66.66%)	0.0756	
Age (years)	Mean age (SD; Median)	12.6 (3.37; 13)	13.91 (3.34; 15)	-	0.2029
Background	Urban	20 (60.6%)	47 (52.22%)	0.0262	0.0005
	Rural	13 (39.4%)	43 (47.77%)	0.1239	
Comorbidities	Obesity	13 (39.4%)	37 (41.11%)	0.0744	0.002
	Hematologic diseases	1 (3%)	9 (10%)	0.4226	
	Neurologic diseases	0 (0%)	6 (6.66%)	0.6346	
	None	19 (57.6%)	38 (42.22%)	0.0054	
Clinical presentation	Simptomatic	23 (69.7%)	46 (51.11%)	0.0054	0.3454
	Asimptomatic	10 (30.3%)	44 (48.88%)	0.2245	
Simptomatic	Complicated	2 (8.7%)	10 (21.73%)	0.2672	0
	Uncomplicated	21 (91.3%)	36 (78.26%)	0.0003	
Pre- or intraoperative investigation	Ultrasonography	33 (100 %)	90 (100)	0.0619	-
	MRCP	1 (3%)	5 (5.55%)	0.2672	
	ERCP	0 (0%)	6 (6.66%)	0.6346	
	Colangiography	2 (6 %)	1 (1.11%)	0.0054	

Obesity is the most common comorbidity in both groups (p=0.0744), also the number of patients presenting with cholelithiasis associating comorbidities is higher, this being statistically significant (p=0.0054). In our study group, we took into consideration comorbidities like obesity (51), hematological (10), and neurological diseases (6). The main hematological disease was hereditary spherocytosis (10) and in the case of neurologic diseases were epilepsy (3) and spastic tetraparesis (3). One patient presented both epilepsy and spastic tetraparesis. A 20% increase in the number of patients diagnosed during a routine ultrasound examination can be observed, but the number of symptomatic patients is still high (group 2 - 51.11%; p = 0.0054). Even though the ultrasonographic examination is widely performed and asymptomatic patients are diagnosed early, the percentage of patients with complicated cholelithiasis has doubled (group 1 – 8.7% vs group 2 – 21.7%).

Ultrasonography is the main method of diagnosis of this pathology. However, there is a doubling of the percentage of patients who received MRCP (group 1 - 3% vs group 2 - 5.55%) and an increase in the percentage for ERCP (group 1 - 0% vs group 2 – 6.66%) in detriment of cholangiography which was preferred in the first period of our study (group 1 - 6% vs group 2 – 1.1%). Just the patients who associated choledocholithiasis symptomatology required the magnetic resonance cholangiopancreatography exam. Three patients with choledocholithiasis underwent ERCP before cholecystectomy and the other 3 has a cholangiography performed during cholecystectomy surgery.

Regarding the moment of surgery, the results are almost similar in both groups as can be seen in Table 2. There is an increase in the number of cholecystectomies (the difference between those two groups is about 2.6) taking into consideration the availability of radiological examination, especially ultrasonography and laparoscopic approach accessibility. Laparoscopic surgery was performed in 98.8% of patients in group 2 and 72.7% of patients in group 1 (Table 2).

Table 2: Surgical management

Variable	Category	Group 1 (G1) n=33 (100%)	Group 2 (G2) n=90 (100%)	P value <0.05 (One sample t-Test)	P value <0.05 (Tow sample paired t-Test)
Moment of surgery	Same hospitalization	19 (57.5)	48 (53.3)	0.0417	0.0004
	Less than a month	7 (21.2)	23 (25.5)	0.1216	
	More than a month	7 (21.2)	19 (21.1)	0.0605	
Surgical approach	Laparoscopy	24 (72.7)	89 (98.8)	0.1643	0.8698
	Clasic	9 (27.7)	1 (1.11)	0.4226	
	Converted laparoscopy	0 (0)	0 (0)	-	-
Priority	Urgent	19 (57.5)	48 (53.3)	0.0417	0
	Elective	14 (42.4)	42 (46.6)	0.091	
Postoperative complications	Bleeding or Local hematoma	1 (3.03)	4 (4.4)	0.1912	0.5294
	None	32 (96.9)	87 (96.6)	0.061	
Reintervention		1	2	0.0054	
Hospital stay (days)	Mean/Median	8.21/8	7.04/5		
Readmission	Yes	1 (3.03)	5 (5.5)	0.2672	0.4894
	No	32 (96.9)	85 (94.4)	0.0546	

The conversion was not considered in any patients. One patient underwent laparoscopic emergency surgery and a subtotal-cholecystectomy was performed due to the inflammatory process which did not allow safe dissection of the elements of the hilum. This patient underwent a second open surgery after 5 months during which the remaining gallbladder was removed.

In both groups, the number of emergency surgeries remains higher than the number of electively operated patients (Table 2). Postoperative complications were recorded in almost 7% of cases and they were only hemorrhagic complications. Three patients required a second surgery and in two cases the source of the active hemorrhage was the gallbladder bed, while in the third case a large hematoma was found without signs of active bleeding. Ultrasonography revealed hematoma in the gallbladder bed in 2 other patients, but they were treated conservatively. No complications such as bile leakage or bile duct injury, infection, or postoperative wound dehiscence were highlighted. No deaths were recorded in our study.

The median of hospitalization days in group 1 was 8 days, significantly higher when compared to group 2 where the median was 5 days (Table 2, Figure 3).

Figure 3: Mean hospital stay in both groups

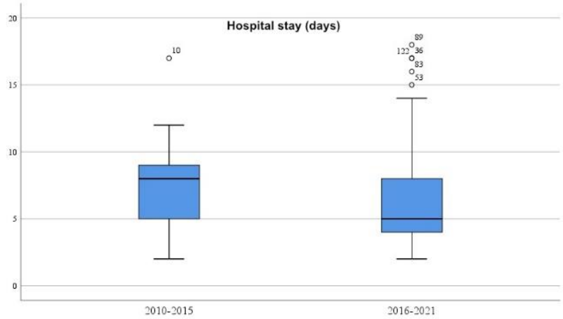

There is a slight increase in the percentage of patients readmitted in group 2 compared to group 1 (group 2-5.5%; group 1-3.03%), with the mention that from the patients readmitted in group 2 one needed surgical reintervention while the rest received symptomatic treatment. An overview of the time of intervention, depending on the patient's condition at presentation (acute or chronic), shows that most acute patients required surgery during the first presentation, compared to chronic patients who initially benefited from conservative treatment (Table 3).

Table 3: Moment of intervention depending on the general condition of the patient

	Same hospitalization	Less than a month	More than a month
Acute	39	10	4
Chronic	-	20	23

**27 of the chronic patients were previously diagnosed in other medical centers without being able to specify exactly the time of diagnosis, for this reason, they were excluded from this analysis.*

Considering the number of hospitalization days, we divided the patients into 2 groups, acute and chronic, and the results showed an average of 8.86 days for acute patients, compared to 5.58 days for chronic ones (Table 4).

Table 4: Mean of the days of hospitalization depending on the general condition of the patient

	Acute	Chronic
Days of hospitalization (mean)	8.86	5.58

Also, depending on the number of days of hospitalization, patients were divided into two groups regarding the surgical approach (classic and laparoscopic) resulting in a decrease of 2.55 of the average number of days of hospitalization for those operated laparoscopically (Table 5).

Table 5: Mean of the days of hospitalization depending on the surgery approach

	Classic	Laparoscopic
Days of hospitalization (mean)	9.7	7.15

DISCUSSION

Compared to adults, the prevalence of lithiasis cholecystitis is lower in children, so cholecystectomy is less common in this age group. Recently, several studies reported that cholecystitis and cholelithiasis are being diagnosed with increasing frequency in infancy, childhood, and adolescence and the incidence is reported to be between 0.15 & 0.22% [1]. As most cases of cholelithiasis are asymptomatic, the prevalence of this pathology cannot be correctly assessed. However, the symptoms that may occur are diffuse abdominal pain, biliary colic, nausea, and vomiting, without any inflammatory signs or by complications like choledocholithiasis, biliary pancreatitis, or acute abdominal pain in the upper quadrant in association with inflammatory signs like fever, leukocytosis, and high CRP. Comparing the two groups from our study, the number of cholecystectomies increased from 33 to 90, which represents an increase of 2.6 times higher in group 2, a result that is comparable to other studies [3]. This may be due to the frequent use of abdominal ultrasound in children with abdominal disorders which recently has resulted in the diagnosis of gallstones in a large number of asymptomatic children [1]. Also, the high number of cholecystectomies can be the result of the widespread use of laparoscopy.

As in the literature, there was a higher incidence of cholelithiasis in females (group 1 – 63.63%; group 2 – 66.66%). This can be explained by the hormonal changes that occur during puberty, with an increase in the saturation of cholesterol in bile [6, 7]. What strengthens our assumption is that the average age of cholelithiasis in both groups is around puberty (group 1 – 12.6 - group 2 – 13.9).

In contrast to other studies in which the incidence of cases of cholecystitis in patients with hematological diseases seems to decrease over time, in our study, there is a 7% increase in group 2 compared to group 1. However, the percentage of patients who have a hematological disease as a risk factor remains low compared to those presenting with obesity. Similar to other series, most of our patients were obese (group 1 – 39.4%, group 2 – 41.1%).

In general, the percentage of pediatric patients with symptomatic cholelithiasis is around 60% [2], similar to our data. However, even though the number of symptomatic patients is higher, our data showed a decrease of approximately 18% of symptomatic patients in group 2 compared to group 1, and an increase of 18% in asymptomatic patients. This is probably also the result of the widespread use of ultrasonography so that many patients with gallstones end up being diagnosed before the onset of symptoms.

The percentage of complicated cases was almost 3 times higher in the second group of our study, while the percentage of uncomplicated cases decreased. One theory that would explain this trend is that the diagnosis is established mainly in the asymptomatic phases of the disease, and in an attempt to treat them conservatively with ursodeoxycholic acid, the stones reach a diameter that allows them to migrate along the bile ducts and obstruct them.

In the adult population, ERCP has gained ground versus open exploration of the common bile duct, thus leading to a decrease in their risk of injury [8] and the same can be seen in our cohort, where in the last 5 years the number of pediatric patients undergoing ERCP has also increased. Gee et al. propose performing cholecystectomy and ERCP at the same time, arguing that this method is a safe way to treat choledocholithiasis, without increasing the rate of postoperative complications, and additionally it is a cost-effective method [9].

Acute cholecystitis due to lithiasis was considered a relative contraindication to laparoscopic cholecystectomy in the early years of the development of this technique but was later shown to be safe in the treatment of acute cholecystitis [10, 11]. Nowadays, the management of lithiasis cholecystitis in the pediatric population, as in the case of adults, consists in using laparoscopy as the main method of surgical treatment. This method is preferred to open cholecystectomy because it has many benefits such as reduced costs by reducing hospitalization days and the use of painkillers, as has been shown in other studies [12, 13, 14]. The fact that in the last decade laparoscopic treatment is used as the main approach in the case of cholelithiasis is also shown in our study.

The right time to perform the surgery in the case of patients presenting to the emergency room remains to be debated. While some studies suggest performing surgery after 6-9 weeks of conservative treatment in acute patients to reduce the acute inflammatory process, Lo et al. and Casillas et al. observed that conservative treatment of lithic cholecystitis for a long period leads to the formation of fibrotic tissue adhesions between the gallbladder and the surrounding structures due to inflammation and edema, which makes dissection difficult. They advocate that early laparoscopic cholecystectomy performed with appropriate timing will facilitate the dissection and reduce the possibility of late complications [10, 15, 16]. The indication for early cholecystectomy is supported by various publications, which report a 5% increase in complication risk for every 10 days surgical treatment is postponed [17-21]. Regarding postoperative complications in our cohort, no complications other than hemorrhagic ones were identified and even these were present in a small number of patients. Postoperative hospital stay was long (mean 8.21 days in group 1 and 7.04 days in group 2), although no major complications were noted.

CONCLUSION

The number of cases with cholecystitis and lithiasis is increasing in the pediatric population. Surgical management for these patients has become similar to that of adult patients, with minimally invasive surgery becoming the gold standard approach.

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VARIA

Common artifacts in myocardial perfusion imaging

Raluca Mititelu[1], Silviu Stanciu[2,3], Catalin Mazilu[1], Teodora Mititelu[4], Letitia Mititelu[4], Alina M. Gherman[1]

Abstract: *Myocardial perfusion imaging (MPI) is an important non-invasive imaging method, which allows the assessment of perfusion in the cells of the myocardial wall. The images are obtained after administration of a radiopharmaceutical at rest and/or after maximal stress (physical effort or pharmaceutical stress). Infarction areas or scars will not show any uptake of the radiopharmaceutical, neither in rest nor at stress (fixed defects), while the ischemic area will show reduced uptake at stress with recovery to normal perfusion at rest (reversible defects).*

MPI is a complex technique and it requires an experienced team to foresee, avoid, correct or minimize the artifacts that might appear in all the phases of the study

In this paper, we will present the most important categories of artifacts, based on the category of problem where they were generated. We will also present some solutions for limiting these artifacts or for recognizing and minimizing their effect for those which cannot be avoided.

Keywords: *myocardial perfusion imaging, artifacts, a non-invasive imaging method*

Myocardial perfusion imaging (MPI) is an important non-invasive imaging method, which allows the assessment of perfusion in the cells of the myocardial wall, after administration of a small quantity of radioactive material – a radiopharmaceutical. MPI is an imaging modality that helps identify the differences between myocardial perfusion at rest and stress (Figure 1).

The most used radiopharmaceuticals in MPI are 99mTc-MIBI and 99mTc-tetrofosmin as they distribute to the myocardial tissue proportional to the blood flow thus to the cell perfusion. The radiopharmaceutical is intravenously administered to the patient at rest and/or after a maximal effort – either physical effort or pharmaceutic stress. The pharmaceutical stress is achieved by administering substances that enhance blood flow in the healthy myocardium; this will lead to consequently decrease in variable degrees in ischemic territories. Therefore, the infarction areas or scars will not show any uptake of the radiopharmaceutical, neither in rest nor at stress (fixed defects), while the ischemic area will show reduced uptake at stress with recovery to normal perfusion at rest (reversible defects).

After the administration of the radiopharmaceutical, the patient is placed inside the gamma camera (SPECT scanner). With the rotation of the system detector around the patient, a series of planar images are obtained; these images (acquisition raw data) will later be used to obtain reconstructed views in the three-section plans of the heart: short axis, long horizontal axis, and long vertical axis. Two data sets of images are obtained in this way, illustrating

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myocardial perfusion at rest and stress.

Figure 1: MPI, 99mTc-tetrofosmin, one day protocol (stress-rest). Reversible defect in inferior wall

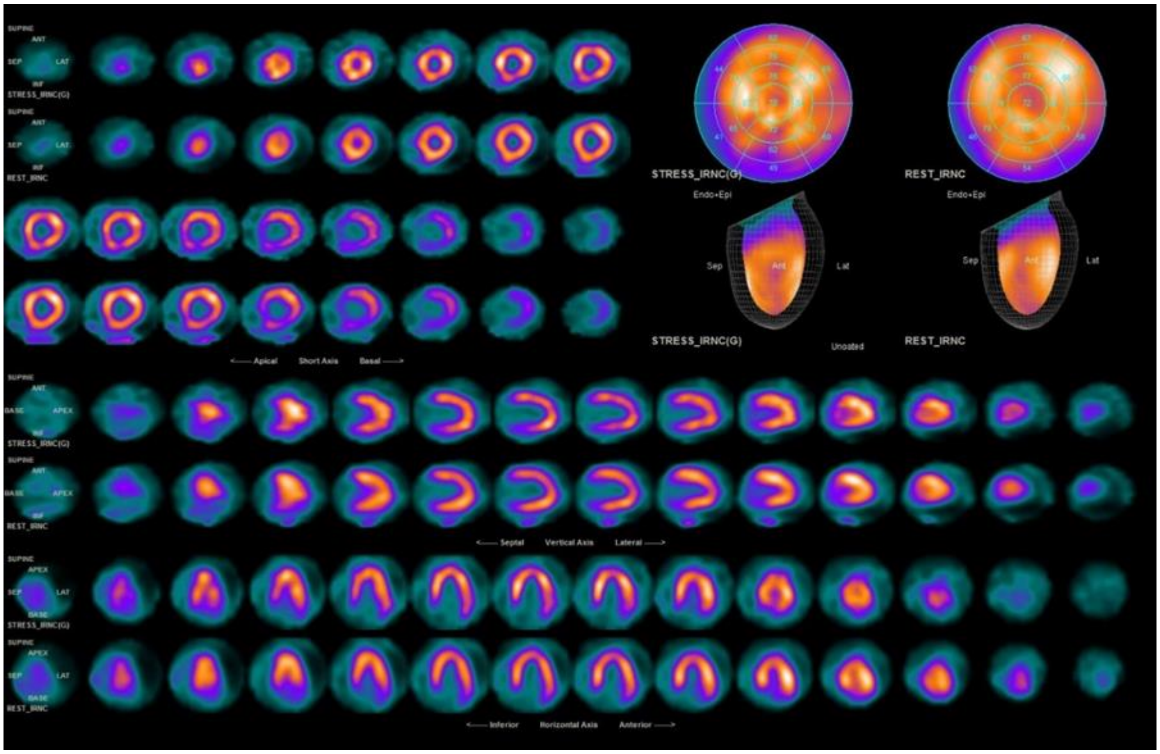

Modern systems allow image acquisition, synchronous with the ECG, resulting in a reconstruction that will provide information about the myocardial dynamic parameters like volumes (telesystolic and telediastolic), the systolic function, and systolic thickening.

In normal conditions, the myocardium of the right ventricle will not be seen on MPI. It can however show some degree of uptake in conditions that associate its hypertrophy.

MPI is a complex technique and it requires an experienced team to foresee, avoid, correct or minimize the artifacts that might appear in all the phases of the study.

Many types of artifacts can interfere with the MPI/ Most important of these artifacts can be classified according to the category of problem where they were generated (Table 1):

- problems related to the preparation of the investigation
- technical aspects involving scanning, processing, and the work protocol
- patient-related aspects: moving during the scan, attenuation of photons within the body, associated pathologies, intestinal radioactivity.

Problems within the preparation of the investigation

The investigation must be well prepared because neglecting some of the steps might lead to major artifacts or even compromise the study.

For the stress study, the patient is advised to stop medication containing beta-blocker or any other treatment that might prevent reaching the maximum heart rate [1]. Normally, the use of pharmacological stress is preferred for patients who can't perform physical effort that would lead to reaching a minimum of 80% of the maximum heart rate.

During the preparation of the radiopharmaceutical, it is necessary to follow a strict protocol to fulfill the radioprotection standards but also to avoid a higher percentage of free 99mTc (pertechnetate – $99mTcO_4$). Free 99mTc - $99mTcO_4$ has another physiological distribution compared to the 99m-Tc-MIBI/tetrofosmin. Furthermore, a higher free fraction will lead to a considerable shrinkage of the active doses.

The administered doses have to be sufficient and adjusted according to the patient's weight to obtain the awaited distribution, This is particularly important for one-day stress-rest studies that involve two injections. In this case for the second investigation is required a sufficient dose, because photons from the first administration might still be detected.

Table 1: Most common artifacts in MPI

Artifacts related to the investigation preparation)	Administering medication that limits the maximal heart rate at stress	
	Preparation of the radiopharmaceutical	
	Incorrect dose of administered radiopharmaceutical	
	The extravasation of substance during intravenous administration	
Artifacts due to technical issues	Equipment errors	Uniformity
		Rotation center
		Detector alignment
		Attenuation correction system
	Processing and postprocessing errors	Incorrect choice of section plans
		Choice of non-adapted filters
	Protocol errors	Non-compliance with the optimal post-injection acquisition time
		The short time between rest and stress studies (one-day protocol)
Patient-related artifacts	Movements during the scan	
	Photon attenuation within the body	Diaphragmatic attenuation
		Breast-induced attenuation
		Adipose-induced lateral defect in obese patients
	Associated pathologies	Left bundle branch block
		Arrhythmias
	Subdiaphragmatic Activity	
	Anatomical variations (apical thinness, shorter septum)	

The radiopharmaceutical is administered intravenously by trained medical personnel, avoiding extravasation.

Technical aspects involving scanning, processing, and the work protocol

Technical issues can be related to the scanning equipment (components of the scanner), image processing, or acquisition protocols.

The equipment requires daily check-up – both the gamma camera and the equipment required for measuring de radioactivity dose. There might be certain errors concerning the scanner's components that would lead to the appearance of artifacts and therefore to make the image interpretation more difficult.

Among the most frequent technical issues that can lead to image artifacts, we can name uniformity, misalignment of the rotation center, detector alignment, and desynchronized function of the attenuation correction system. All of these errors can be avoided or, at least, reduced by following a strict, periodical quality control protocol with systematic checking of the medical devices [1-3].

Processing of the acquisition raw data might be an important source of artifacts in the final images. Luckily, if the acquisition data were correctly obtained in a standard protocol – the processing procedure can be restarted and the artifacts corrected. The most frequent causes of artifact due to processing are related to incorrect filter application or mistakes in the reconstruction – heart orientation, choice of the section plans [4]. Processing errors can generate either false-positive or false-negative results.

Imaging acquisition at a shorter timespan after the administration of radiopharmaceutical than recommended might generate weaker statistics and faulty images. Additionally, for the one-day stress-rest protocol – the second investigation in a shorter timespan might considerably affect the quality of the study.

A good positioning within the scanner is required to get a full heart image.

Patient-related aspects

Artifacts resulting from patient movement during image acquisition might considerably influence the interpretation and in some cases even mimic a perfusion defect (false positive study).

The phenomenon of photon attenuation within the body is a common cause of artifacts. These are generally fixed defects visible both in rest and on stress images. Specifically, if there are no possibility of correcting the attenuation (e.g., through simultaneously transmission scanning as in CT) attenuation defects can be differentiated from real defects through gated SPECT. The territories which show attenuation defects will always present normal parietal kinetics [2, 5, 6]. Generally, there are three sources of soft tissues attenuation defects: the presence of big breast in women (anterior wall defects), diaphragm (especially in men – it determines false inferior wall defects), or in obese patients with important lateral adipose tissue (defects of the left ventricular lateral wall)

Some associated nonischemic pathologies can influence the appearance of the perfusion study. They are most commonly represented by arrhythmias and by the left bundle branch block.

Left bundle branch block can determine anteroseptal defect, either fixed or reversible, leading to a decrease in the specificity of MPI in this category of patients [4, 7, 8].

Other groups of pathologies that can influence and lead to artifacts are represented by arrhythmias, which might significantly influence ECG-gated studies.

Important subdiaphragmatic activity (hepatic or intestinal) is due to the excretion of the radiopharmaceutical through the hepatobiliary system. These phenomena can interfere with the radiopharmaceutical uptake on the inferior wall in two ways: (a) high uptake on the inferior wall (Compton effect) with the cover-up of a possible perfusion defect and (b) relative decrease of uptake on the inferior wall, next to the intestinal area of high uptake, a tributary to the use of reconstruction algorithms (false-positive results).

The subdiaphragmatic activity can be reduced through a low-intensity physical effort (resulting in a lower splenic activity), large quantities of fluids or even eating high-fat foods that might bias the radioactivity in the distal GI tract [5, 9].

Some anatomical variations might of course impact the visualization – e.g., the presence of a thin myocardial tissue layer at the apex might mimic a perfusion defect, as does a shorter sept (Figure 2).

Figure 2: MPI, 99mTc-tetrofosmin, one day protocole (stress-rest). Fix defect in apex (apexian thinning) and short septum

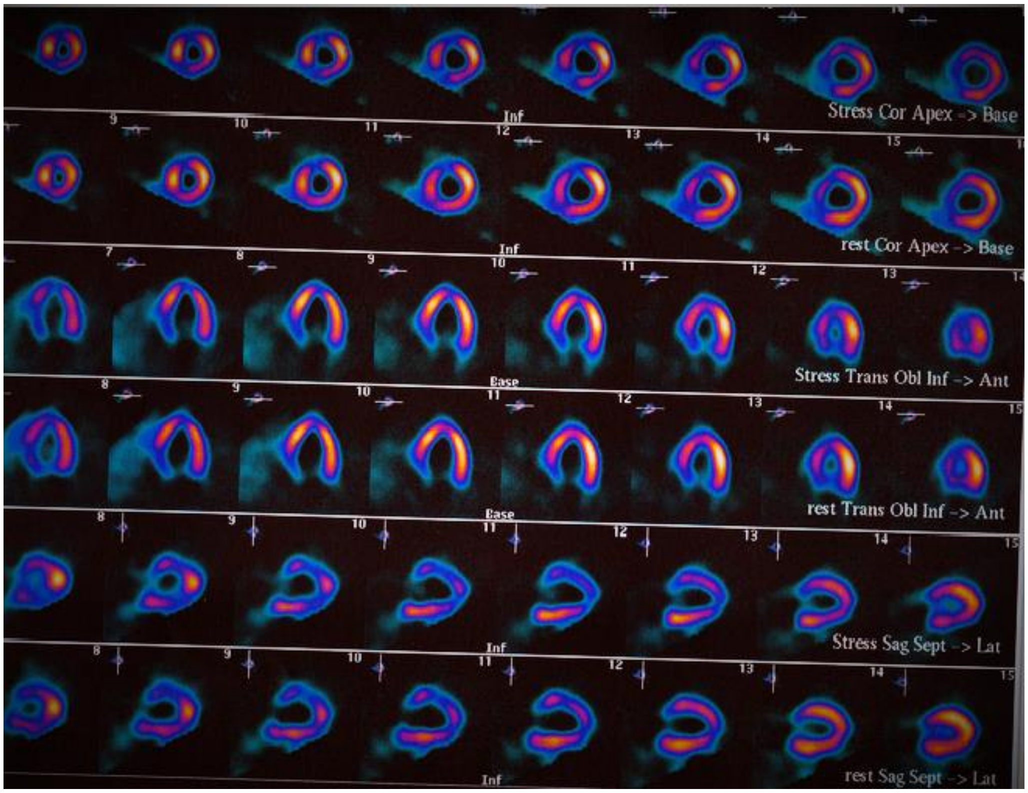

CONCLUSION

MPI represents a high-performance imaging modality that relates to advanced knowledge in pharmacology, pathology, and clinical cardiology combined with high-level technical aspects in medical devices and image processing systems. The role of a well-trained multidisciplinary team is mandatory for performing and interpreting the study. With the correct implementation of working protocols and the use of standardized methods, the presence of artifacts can be limited. However, there is several pitfall or artifacts that cannot be avoided due to particular conditions. In this case, it is important to recognize artifact changes with result in better accuracy of the procedure while maximizing the benefit of this imaging technique in routine clinical practice.

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SYSTEMATIC REVIEW

A systematic review of the various treatment options regarding the effectiveness of IVIG for nephropathy due to BK virus

Hasan Nikoeenejad[1], Behzad Einollahi[1], Mehrdad Ebrahimi[1]

Abstract: *Introduction: BK virus is an opportunistic infectious disease that causes disease and serious problems when the immune system is suppressed. One of the treatments used against this virus is intravenous immunoglobulin (IVIG). We aimed to review the major relevant articles in case of the efficacy of IVIG and determine its usefulness.*

Methods: We searched online databases such as PubMed, MEDLINE, Wiley, EMBASE, ProQuest Dissertations and Thesis, ISI Web of Knowledge, Scopus, and Google scholar. Two reviewers have independently assessed and extracted the titles and abstracts. Disagreements were being fixed by discussion. Where resolve was not feasible, a third review author was discussed.

Results: We screened a total of 6 full texts. Three studies evaluated the effectiveness of IVIG in the Treatment of BK Infection in Renal Transplant Patients. Also, three studies assessed the various treatment options for Nephropathy due to the BK virus. Results showed that mean peak BK reduced with IVIG therapy after a one-year follow-up. Also, a high percentage of patients have functioning grafts after IVIG therapy.

Conclusion: A review of studies shown powerful follow-up and early decrease of immunosuppression leading detection of BK viremia, with qualitative monitoring, can avoid the progress of clinically notable BK nephropathy. Combination treatment IVIG is more successful in removing viral load in patients with BKVAN, compared with traditional standard-of-care therapy.

Keywords: *BK virus, nephropathy, treatment*

INTRODUCTION

BK virus is an opportunistic infectious disease that causes disease and serious problems when the immune system is suppressed [1-5]. The BK virus is from the papovavirus class and it`s a double-stranded non-enveloped DNA virus [6, 7]. The prevalence of this virus in the normal population is 60-80%, but this virus has no symptoms at the beginning of an infection in the normal body [5-8]. The pathogenicity of the virus occurs when the immune system fails. Kidney transplantation is the most important cause of immunosuppression [9]. Transplant recipients take immunosuppressive drugs to reduce the risk of rejection. It is the most common cause of neutropenia or lymphopenia and ultimately lacks the immune system [10-12] At this risky period, the BK virus quickly replicates and develops in the

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body. The virus decreases the renal function and causes ureteral stenosis, hemorrhagic cyst, and ultimately transplant rejection in the patient [6-8].

There are different methods for identifying the virus and detecting its pathogenicity in the body. Serologic and traditional viral culture methods are not specific [12-14]. These methods don`t have enough accuracy and their required time to answer is too long. Now, polymerase chain reaction (PCR) is the best method for evaluating the virus in the urine and blood of patients. There is a controversy about the proper time to take a biopsy in kidney transplant patients [8]. According to new articles, we should take a biopsy from a transplanted kidney when there is viremia with increasing creatinine. It is the best time to diagnose BKVN (BK virus nephropathy) and take action as soon as possible. There are many different drugs and protocols to treat BK viremia and resolve their symptoms [9-11]. These include leflunomide, cidofovir, ciprofloxacin, etc. Dose reduction and changing the immunosuppressive drugs are other protocols too.

One of the treatments used against this virus is IVIG. IVIG has potent immunomodulatory effects in inflammatory and autoimmune diseases. IVIG increases the immunity against the virus and reduces the amount of virus in the blood and tissues [11-14].

There are various studies about the efficacy of IVIG and its combination with other therapeutic protocols. Immunoglobulin activity against the BK virus has been proven in vitro, but there is a controversy about its usefulness in the body (in vivo). Due to the high cost of this drug, it is necessary to ensure its efficacy for transplant patients [10-14].

We try to review the major relevant articles in case of the efficacy of IVIG and determine its usefulness; eventually, our goal is to choose the best option for patients to help clinicians and patients to have a better understanding of these technologies and choosing the better therapeutic option.

MATERIAL AND METHODS

In October 2018 we searched the following libraries and electronic databases for potentially relevant studies: PubMed, MEDLINE, Wiley, EMBASE, ProQuest Dissertations and Thesis, ISI Web of Knowledge, Scopus, and Google scholar. Also, Google and Google Scholar have been checked for more informative articles (Gray literature) that may not be listed in the previous resources. To cover other studies, booklets on Congress, abstracted articles, and seminars have been studied too. It is attempting to evaluate all the related articles by 2017 so that we do not miss out on the latest information.

The terminologies that were used to identify these articles included: Immunoglobulins, Intravenous, Immune Globulin, Intravenous, Intravenous Immune Globulin, IVIG, Immune Globulin, Intravenous, Intravenous Immune Globulin, Intravenous Immunoglobulins, IV Immunoglobulins, and BK Virus. Also, we used the suitable combination of terminologies as mentioned above for searching.

Two reviewers have independently assessed the titles and abstracts. Disagreements were being fixed by discussion. Where resolve was not feasible, a third review author was discussed. Two reviewers independently extracted data via a tested extraction sheet, and disagreements were being resolved by a meeting with a third reviewer.

RESULTS

Our search initially retrieved 184 studies published in 2018. However, 152 papers were eliminated because of duplication between databases. Then, 28 studies were included for primary screening. Upon screening titles and abstracts, 6 studies were identified for full-text review. We screened a total of 6 full texts (Table 1). Three studies evaluated the effectiveness of IVIG in the Treatment of BK Infection in Renal Transplant Patients. Also, three studies assessed the various treatment options for Nephropathy due to the BK virus.

Sener et al (2006) studied the effects of renal transplant patients with BK virus-associated nephropathy treated with IVIG. They reported that 8 renal allograft recipients identified with BKVAN after 11.4 months after transplantation. All of the patients received a reduction in immunosuppressive therapy; also they obtained 2 g/kg IVIG. All of the patients except one were off dialysis after a follow-up of 15 months. They reported that 88% of patients still have functioning grafts after IVIG therapy. Ultimately, they noted that further research including randomized, multicentered trials should be done about the advantages of concomitant reduction of immunosuppressive therapy and IVIG for BKVAN [9].

Kable et al (2017) did a retrospective, single-center cohort study to evaluate the efficiency of adjuvant IVIG in removing the virus from tissue and blood, against the standard of care controls. They evaluated the effectiveness of adjuvant IVIG to eliminate the virus from blood and tissue, in a retrospective, single-center cohort study, against standard-of-care controls in 50 BKVAN cases. The immunosuppression reduced in both groups underwent.

Table 1: Characterization of the studies included

Author's name	Participants	Outcomes	Details
Review the Effectiveness of IVIG in the Treatment of BK Infection in Renal Transplant Patients			
Sener et al (2006) [9]	Eight renal allograft recipients identified with BKVAN included the following 11.4 months after transplantation treated with IVIG.	They reported that 88% of patients still have functioning grafts after IVIG therapy.	they noted that further research including randomized, multicentered trials should be done about the advantages of concomitant reduction of immunosuppressive therapy and IVIG for BKVAN.
Kable et al (2017) [10]	They evaluated the effectiveness of adjuvant IVIG to eliminate the virus from blood and tissue, in a retrospective, single-center cohort study, against standard-of-care controls in 50 BKVAN cases	They reported that 92% as histological stage B, 46% as dysfunction, 20% as viremia. The mean viral loads reduced after treatment ($P < 0.001$); the viremia ($P = 0.003$), BK immune-histochemistry ($P = 0.028$) effectively cleared in IVIG group. The graft losses fewer occurred in IVIG group ($P = 0.06$).	The results of the study demonstrated that combination treatment with IVIG compared with conventional therapy is a more helpful and valuable method for eliminating virus from BKVAN. Although they recommended that a multicenter randomized trial is necessary for validation.
Vu et al (2015) [11]	The BKVN patients remained after anti-polyomavirus treatment (using leflunomide therapy with a reduction of immunosuppression). They gave IVIG to patients that did not respond to anti-polyomavirus treatment after 8 weeks. The 30 patients included in the study had persistent BKV and BKVN.	They showed that mean peak BK reduced (205,314 copies/mL to 697 copies/mL viruses) after 1-year follow-up. The viremia virus was cleared in 23 patients (90%) in response to treatment.	They concluded that cure with IVIG is safe and successful for the treatment of BKV viremia and BKVN, also cure with IVIG can inhibit graft loss in patients that did not respond to anti-polyomavirus treatment.
Review of the various treatment option for Nephropathy due to BK virus			
Brennan et al. (2005) [12]	They included 200 adult renal transplant recipients to CyA ($n = 66$) or FK506 ($n = 134$).	Analysis for BK did by blood and urine weekly for 16 weeks and at months 5, 6, 9, and 12 and through polymerase chain reaction (PCR). They showed that viruria was high with FK506-MMF (46%) and minimum with CyA-MMF (13%). The viremia was resolved by 95% after the reduction of immunosuppression without raised allograft dysfunction, graft loss, or acute rejection. They no observed any BK nephropathy	They concluded that the type of adjuvant immunosuppression did not influence BK viruria or viremia.
Halim et al. [13]	Group 1 ($n = 19$) was composed of kidney transplant recipients with twice positive BK virus-polymerase chain reaction in urine and blood who underwent graft biopsy to confirm BK virus-associated nephropathy. Once BK virus-associated nephropathy was diagnosed, an antimetabolite (mycophenolate mofetil or azathioprine) was changed to leflunomide, and intravenous immunoglobulin and oral ciprofloxacin were given. Group 2 ($n = 14$) was composed of BK virus-associated nephropathy patients treated conventionally with reduced immunosuppressive medications.	Maintenance immunosuppression was prednisolone and mycophenolate mofetil (2 g/d) in 31 patients (94%), and tacrolimus in 13 (39.4%). Tacrolimus was given to 12 patients in group 1 (63.1%), while sirolimus was given to 7 patients in group 2 (50%). One graft was lost in each group by the end of the study, and 1 patient died with a functioning graft in group 2.	No significant difference existed in 1-year graft outcomes between the treatment of BK virus-associated nephropathy by reduction of immunosuppressive medications or actively by leflunomide, intravenous immunoglobulin, and ciprofloxacin.
Halim et al. (2009) [14]	Renal transplant patients with two BK virus polymerase chain response analyses of urine and blood experienced graft biopsy to establish BKVN.	In the beginning, mean±SD creatinine clearance was 35.6±11.5 mL/min/1.732, which was reduced to 29.3±17.3 mL/min/ 1.732. Cases were distributed into two groups of nine each according to creatinine clearance values. In group one, baseline rate was 44.5+-6.6 mL/min/1.732, compared with 25.36±7.8 mL/min/1.732 in group two, which reduced to 42.66 ±12.8 mL/min/1.732 and 16.76 (9.0) mL/min/1.732. Three grafts (16.7%) were lost by the end of the study, all in group two.	They concluded late diagnosis and intense immunosuppression predispose to BKVN. Initial active therapy of BKVN may develop graft results at one year after posttransplantation.

The patients received IVIG at 1.01 ± 0.18 g/kg. 50 kidneys at 7 months after transplantation evaluated and the biopsy-proven BKVAN occurred in them; they reported that 92% as histological stage B, 46% as dysfunction, 20% as viremia. The mean viral loads reduced after treatment ($P < 0.001$); the viremia ($P = 0.003$), BK immunohistochemistry ($P = 0.028$) effectively cleared in IVIG group. The graft losses fewer occurred in the IVIG group ($P = 0.06$). The results of the study demonstrated that combination treatment with IVIG compared with conventional therapy is a more helpful and valuable method for eliminating the virus from BKVAN. Although they recommended that a multicenter randomized trial is necessary for validation [10].

Vu et al (2015) evaluated the influence of cure with IVIG on the result of BKVN in renal transplant recipients. The BKVN patients remained after anti-polyomavirus treatment (using leflunomide therapy with a reduction of immune-suppression). They gave IVIG to patients that did not respond to anti-polyomavirus treatment after 8 weeks. The 30 patients included studying that had persistent BKV and BKVN. They showed that mean peak BK reduced (205,314 copies/mL to 697 copies/mL viruses) after a 1-year follow-up. The viremia virus was cleared in 23 patients (90%) in response to treatment. They concluded that cure with IVIG is safe and successful for treatment BKV viremia and BKVN, also cure with IVIG can inhibit graft loss in patients that did not respond to anti-polyomavirus treatment [11].

Brennan et al. (2005) determined the frequency of nephropathy, viremia, or BK viruria with tacrolimus (FK506) versus cyclosporine (CyA). They also evaluated whether severe examination and discontinuation of azathioprine (AZA) or mycophenolate (MMF) upon recognition of BK viremia, can be inhibited BK nephropathy. They included 200 adult renal transplant recipients to CyA (n = 66) or FK506 (n = 134). Analysis for BK done by blood and urine weekly for 16 weeks and at months 5, 6, 9, and 12 and through polymerase chain reaction (PCR). They showed that viruria was high with FK506-MMF (46%) and minimum with CyA-MMF (13%). The viremia was resolved in 95% after a reduction of immunosuppression without raised allograft dysfunction, graft loss, or acute rejection. They no observed any BK nephropathy; also they concluded that the type of adjuvant immunosuppression did not influence BK viruria or viremia [12].

Halim et al. evaluated the impact of therapy with leflunomide, intravenous immunoglobulin, and ciprofloxacin on graft result following one year compared with a historical group treated with reduced immunosuppressive medications strategy. Group 1 (n = 19) was composed of kidney transplant recipients with twice positive BK virus-polymerase chain reaction in urine and blood who underwent graft biopsy to confirm BK virus-associated nephropathy. Once BK virus-associated nephropathy was diagnosed, an antimetabolite (mycophenolate mofetil or azathioprine) was changed to leflunomide, and intravenous immunoglobulin and oral ciprofloxacin were given. Group 2 (n = 14) was composed of BK virus-associated nephropathy patients treated conventionally with reduced immune-suppressive medications.

Thirty-three patients were treated, 23 were males (70%), there were 15 were deceased donors (45.5%), 15 were diabetics (45.5%), mean human leukocyte antigen mismatches were 3.76, seven had a zero DR mismatch (21.2%), and 8 were CW7 negative (24.2%). All patients received induction therapy (thymoglobulin in 22 [66.6%]), 7 had delayed graft function (21.2%) and 18 received antirejection therapy before receiving BK virus-associated nephropathy diagnosis (52.9%). Maintenance immune-suppression was prednisolone and mycophenolate mofetil (2 g/d) in 31 patients (94%), and tacrolimus in 13 (39.4%). Tacrolimus was given to 12 patients in group 1 (63.1%), while sirolimus was given to 7 patients in group 2 (50%). One graft was lost in each group by the end of the study, and 1 patient died with a functioning graft in group 2. They concluded no significant difference existed in 1-year graft outcomes between the treatment of BK virus-associated nephropathy by reduction of immunosuppressive medications or actively by leflunomide, intravenous immunoglobulin, and ciprofloxacin [13].

Halim et al. (2009) evaluated the effectiveness of leflunomide, intravenous immunoglobulins, and ciprofloxacin as active therapy of postrenal transplant BK virus nephropathy (BKVN) in graft result at one year. They included renal transplant patients with two BK virus polymerase chain response analyses of urine and blood experienced graft biopsy to establish BKVN. For patients with BKVN, antimetabolite treatment (mycophenolate mofetil or azathioprine) was modified to leflunomide therapy accompanied by a plan of immunoglobulin and oral ciprofloxacin. They assessed eighteen patients that 72% of them were men. Nine patients underwent cadaveric organs, with an average of 3.6 HLA mismatches. They administered to all patients induction treatment (61% thymoglobulin), and 61% antirejection treatment before BKVN was detected. In the beginning, mean±SD creatinine clearance was 35.6±11.5 mL/min/1.732, which was reduced to 29.3±17.3 mL/min/1.732. Cases were distributed into two groups of nine each according to creatinine clearance values. In group one, baseline rate was 44.5+-6.6 mL/min/1.732, compared with 25.36±7.8 mL/min/1.732 in group two, which reduced to 42.66 ±12.8 mL/min/1.732 and 16.76 (9.0) mL/min/1.732. Three grafts (16.7%) were lost by the end of the study, all in group two. They concluded late diagnosis and intense immunosuppression predispose to BKVN. Initial active therapy of BKVN may develop graft results at one-year post-transplantation [14].

DISCUSSION

A significant reason for renal dysfunction and allograft loss is BK nephropathy in renal transplant recipients. Using various agents such as MMF compared to AZA or FK506 compared to CyA, for preservation immunosuppression to determine BK viremia, viruria, and nephropathy has been interested as an important issue for research [15–18, 19–21]. Also, BK viremia has been demonstrated as a precondition for progression to BK nephropathy. Therefore, BK viremia can be a marker of extreme immunosuppression. Brennana et. al reported that BK viremia could be removed in safety by the decrease of immunosuppression and especially by removal of the antimetabolite modules of the immunosuppressive treatment, consequently inhibiting raise to BK nephropathy without acute rejection, graft loss, or renal dysfunction.

Brennana et. al demonstrated that powerful associations among the beginning, interval, and titer of virus in the urine, thus viremia indicates the severity of infection in the allograft. They presented an early; severe viral infection follows, with a 1000-fold raise in the level of urinary virus in a comparatively short time enclose of 2–3 weeks. In these patients, the rise in viral reproduction resulted in detectable viremia. They recommended that it can be attractive to focus on potential control efforts on this serious period and viral level. Although, period and viral level of viremia show to be significant, the positive prognostic value is low, because it may be particularly in recipients whose immunosuppression is raised at a later time point [12].

Other investigators have established a significant association between transplant nephropathy and BK viremia. For example, Hirsch et al., have reported that a plasma viral titer more than 10,000 copies in 1mL be characterized as ‘presumptive’ BKV nephropathy, despite the biochemical and histological data of nephropathy did not show [22]. They reported that BKV viremia presented 88% specificity and 100% sensitivity for BK nephropathy, and plasma titer more than 7700 copies/mL reveal in all recipients with BKV nephropathy. Although, in Brennana et. al study, 61% of all recipients with viremia present plasma titers more than 100000 copies/mL while any evidence of BK nephropathy or deterioration of renal function not be seen. Therefore, Brennana results propose that powerful follow-up and early decrease of immunosuppression leading detection of BK viremia, with qualitative monitoring, can avoid the progress of clinically notable BK nephropathy. Generally, Brennana et. al saw no changes in the frequency of BK viruria or viremia between those getting MMF or AZA, FK506 or CyA, separately [12].

Purighalla et. al (1995), reported a case report about a 34-year-old man with polycystic kidney disease who underwent renal transplantation. 12 and 22 days after transplantation occurred reversible episodes of acute rejection. 38 weeks after transplantation, the biopsy demonstrated changes dependable with rejection also demonstrated BK virus inclusions. All follow-up biopsies showed a mixture of rejection and BK virus infection. Ultimately, the graft loosed 56 weeks after transplantation [23].

Decreasing of immunosuppression is the most common curative interference for the cure of BKVN in renal transplant recipients; however, it is not constantly satisfactory to stabilize renal function [24- 27]. In recent years, using IVIG in the cure and management of the BKVN has improved [28, 9, 29, 30, 31]. The IVIG can decrease the infection of BKV through the straight neutralization of BKV via virus-specific antibodies, consequently sopping viral activation and infection. Recently, the constructive combination method of IVIG management and immunosuppression reduction was presented to be successful in the treatment of BKVN. Sener et al (2006) studied the effects of renal transplant patients with BK virus-associated nephropathy treated with IVIG [9]. They reported that 8 renal allograft recipients identified with BKVAN after 11.4 months after transplantation. All of the patients received a reduction of immunosuppressive therapy; also they obtained 2 g/kg IVIG. All of the patients except one were off dialysis after a follow-up of 15 months. Concluding, they reported that 88% of patients still have functioning grafts after IVIG therapy. Ultimately, they noted that further research including randomized, multicentered trials should be done about the advantages of concomitant reduction of immunosuppressive therapy and IVIG for BKVAN [28]. Only 1 patient lost the graft after 1 year of follow-up, while the 7 patients still had practical grafts. Sharma et al [31] illustrated utilizing IVIG in a pediatric patient identified as constant BKVN in a case report. Scr level raised and BK viremia reactivated after a month of achievement of cidofovir therapy. Followed by IVIG was given in a 7-dose regimen of 600 mg/kg. Viral load decreased after the fifth dose, from 20,800 to 2540 DNA copies/mL. Finally, Scr level becomes constant during 6 months and viral load reduced [31]. Dheir et al [29] demonstrated which IVIG treatment was to help inhibit acute rejection and delay graft endurance. Although, Dheir et. al did not show how the viral load was altered after IVIG treatment [29]. Vu et al (2015) evaluated the influence of cure with IVIG on the result of BKVN in renal transplant recipients [11]. The BKVN patients remained after anti-polyomavirus treatment (using leflunomide therapy with a reduction of immune-suppression). They have given IVIG to patients that did not respond to anti-polyomavirus treatment after 8 weeks. The 30 patients included in the study had persistent BKV and BKVN. They showed that mean peak BK reduced (205, 314 copies/mL to 697 copies/mL viruses) after a 1-year follow-up. The viremia virus was cleared in 23 patients (90%) in response to treatment. The allograft survival rates were 97.4%, and the 12-month patient was 100%. A loss of an allograft reported by they had a harsh rejection episode subsequent no feedback in removing BKV after treatment. They concluded that cure with IVIG is safe and successful for the treatment of BKV viremia and BKVN, also cure with IVIG can inhibit graft loss in patients that did not respond to anti-polyomavirus treatment. However IVIG therapy is expensive, but this price perhaps acceptable in selected patients because BKVN has appeared as a critical reason for the loss of renal graft and renal allograft dysfunction in the transplant recipient. In conclusion, IVIG seems to be effective and safe in the treatment of BKVN and inhibits graft loss in BKVN patients with combination therapy of leflunomide therapy and immunosuppression reduction.

CONCLUSIONS

A review of studies shown powerful follow-up and early decrease of immunosuppression leading detection of BK viremia, with qualitative monitoring, can avoid the progress of clinically notable BK nephropathy. Generally, there is no change in the frequency of BK viruria or viremia between those getting MMF or AZA, FK506, or CyA, separately.

Combination treatment IVIG is more successful in removing viral load in patients with BKVAN, compared with traditional standard-of-care therapy. Ultimately, further research including randomized, multicentered trials should be done about the advantages of concomitant reduction of immunosuppressive therapy, and IVIG for BKVAN IVIG seems to be effective and safe in the treatment of BKVN and inhibits graft loss in BKVN patients with combination therapy of leflunomide therapy and immunosuppression reduction.

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SYSTEMATIC REVIEW

A systematic review of the various treatment options regarding the effectiveness of IVIG for nephropathy due to BK virus

Hasan Nikoeenejad[1], Behzad Einollahi[1], Mehrdad Ebrahimi[1]

Abstract: *Introduction: BK virus is an opportunistic infectious disease that causes disease and serious problems when the immune system is suppressed. One of the treatments used against this virus is intravenous immunoglobulin (IVIG). We aimed to review the major relevant articles in case of the efficacy of IVIG and determine its usefulness.*

Methods: We searched online databases such as PubMed, MEDLINE, Wiley, EMBASE, ProQuest Dissertations and Thesis, ISI Web of Knowledge, Scopus, and Google scholar. Two reviewers have independently assessed and extracted the titles and abstracts. Disagreements were being fixed by discussion. Where resolve was not feasible, a third review author was discussed.

Results: We screened a total of 6 full texts. Three studies evaluated the effectiveness of IVIG in the Treatment of BK Infection in Renal Transplant Patients. Also, three studies assessed the various treatment options for Nephropathy due to the BK virus. Results showed that mean peak BK reduced with IVIG therapy after a one-year follow-up. Also, a high percentage of patients have functioning grafts after IVIG therapy.

Conclusion: A review of studies shown powerful follow-up and early decrease of immunosuppression leading detection of BK viremia, with qualitative monitoring, can avoid the progress of clinically notable BK nephropathy. Combination treatment IVIG is more successful in removing viral load in patients with BKVAN, compared with traditional standard-of-care therapy.

Keywords: *BK virus, nephropathy, treatment*

INTRODUCTION

BK virus is an opportunistic infectious disease that causes disease and serious problems when the immune system is suppressed [1-5]. The BK virus is from the papovavirus class and it`s a double-stranded non-enveloped DNA virus [6, 7]. The prevalence of this virus in the normal population is 60-80%, but this virus has no symptoms at the beginning of an infection in the normal body [5-8]. The pathogenicity of the virus occurs when the immune system fails. Kidney transplantation is the most important cause of immunosuppression [9]. Transplant recipients take immunosuppressive drugs to reduce the risk of rejection. It is the most common cause of neutropenia or lymphopenia and ultimately lacks the immune system [10-12] At this risky period, the BK virus quickly replicates and develops in the

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body. The virus decreases the renal function and causes ureteral stenosis, hemorrhagic cyst, and ultimately transplant rejection in the patient [6-8].

There are different methods for identifying the virus and detecting its pathogenicity in the body. Serologic and traditional viral culture methods are not specific [12-14]. These methods don`t have enough accuracy and their required time to answer is too long. Now, polymerase chain reaction (PCR) is the best method for evaluating the virus in the urine and blood of patients. There is a controversy about the proper time to take a biopsy in kidney transplant patients [8]. According to new articles, we should take a biopsy from a transplanted kidney when there is viremia with increasing creatinine. It is the best time to diagnose BKVN (BK virus nephropathy) and take action as soon as possible. There are many different drugs and protocols to treat BK viremia and resolve their symptoms [9-11]. These include leflunomide, cidofovir, ciprofloxacin, etc. Dose reduction and changing the immunosuppressive drugs are other protocols too.

One of the treatments used against this virus is IVIG. IVIG has potent immunomodulatory effects in inflammatory and autoimmune diseases. IVIG increases the immunity against the virus and reduces the amount of virus in the blood and tissues [11-14].

There are various studies about the efficacy of IVIG and its combination with other therapeutic protocols. Immunoglobulin activity against the BK virus has been proven in vitro, but there is a controversy about its usefulness in the body (in vivo). Due to the high cost of this drug, it is necessary to ensure its efficacy for transplant patients [10-14].

We try to review the major relevant articles in case of the efficacy of IVIG and determine its usefulness; eventually, our goal is to choose the best option for patients to help clinicians and patients to have a better understanding of these technologies and choosing the better therapeutic option.

MATERIAL AND METHODS

In October 2018 we searched the following libraries and electronic databases for potentially relevant studies: PubMed, MEDLINE, Wiley, EMBASE, ProQuest Dissertations and Thesis, ISI Web of Knowledge, Scopus, and Google scholar. Also, Google and Google Scholar have been checked for more informative articles (Gray literature) that may not be listed in the previous resources. To cover other studies, booklets on Congress, abstracted articles, and seminars have been studied too. It is attempting to evaluate all the related articles by 2017 so that we do not miss out on the latest information.

The terminologies that were used to identify these articles included: Immunoglobulins, Intravenous, Immune Globulin, Intravenous, Intravenous Immune Globulin, IVIG, Immune Globulin, Intravenous, Intravenous Immune Globulin, Intravenous Immunoglobulins, IV Immunoglobulins, and BK Virus. Also, we used the suitable combination of terminologies as mentioned above for searching.

Two reviewers have independently assessed the titles and abstracts. Disagreements were being fixed by discussion. Where resolve was not feasible, a third review author was discussed. Two reviewers independently extracted data via a tested extraction sheet, and disagreements were being resolved by a meeting with a third reviewer.

RESULTS

Our search initially retrieved 184 studies published in 2018. However, 152 papers were eliminated because of duplication between databases. Then, 28 studies were included for primary screening. Upon screening titles and abstracts, 6 studies were identified for full-text review. We screened a total of 6 full texts (Table 1). Three studies evaluated the effectiveness of IVIG in the Treatment of BK Infection in Renal Transplant Patients. Also, three studies assessed the various treatment options for Nephropathy due to the BK virus.

Sener et al (2006) studied the effects of renal transplant patients with BK virus-associated nephropathy treated with IVIG. They reported that 8 renal allograft recipients identified with BKVAN after 11.4 months after transplantation. All of the patients received a reduction in immunosuppressive therapy; also they obtained 2 g/kg IVIG. All of the patients except one were off dialysis after a follow-up of 15 months. They reported that 88% of patients still have functioning grafts after IVIG therapy. Ultimately, they noted that further research including randomized, multicentered trials should be done about the advantages of concomitant reduction of immunosuppressive therapy and IVIG for BKVAN [9].

Kable et al (2017) did a retrospective, single-center cohort study to evaluate the efficiency of adjuvant IVIG in removing the virus from tissue and blood, against the standard of care controls. They evaluated the effectiveness of adjuvant IVIG to eliminate the virus from blood and tissue, in a retrospective, single-center cohort study, against standard-of-care controls in 50 BKVAN cases. The immunosuppression reduced in both groups underwent.

Table 1: Characterization of the studies included

Author's name	Participants	Outcomes	Details
Review the Effectiveness of IVIG in the Treatment of BK Infection in Renal Transplant Patients			
Sener et al (2006) [9]	Eight renal allograft recipients identified with BKVAN included the following 11.4 months after transplantation treated with IVIG.	They reported that 88% of patients still have functioning grafts after IVIG therapy.	they noted that further research including randomized, multicentered trials should be done about the advantages of concomitant reduction of immunosuppressive therapy and IVIG for BKVAN.
Kable et al (2017) [10]	They evaluated the effectiveness of adjuvant IVIG to eliminate the virus from blood and tissue, in a retrospective, single-center cohort study, against standard-of-care controls in 50 BKVAN cases	They reported that 92% as histological stage B, 46% as dysfunction, 20% as viremia. The mean viral loads reduced after treatment ($P < 0.001$); the viremia ($P = 0.003$), BK immune-histochemistry ($P = 0.028$) effectively cleared in IVIG group. The graft losses fewer occurred in IVIG group ($P = 0.06$).	The results of the study demonstrated that combination treatment with IVIG compared with conventional therapy is a more helpful and valuable method for eliminating virus from BKVAN. Although they recommended that a multicenter randomized trial is necessary for validation.
Vu et al (2015) [11]	The BKVN patients remained after anti-polyomavirus treatment (using leflunomide therapy with a reduction of immunosuppression). They gave IVIG to patients that did not respond to anti-polyomavirus treatment after 8 weeks. The 30 patients included in the study had persistent BKV and BKVN.	They showed that mean peak BK reduced (205,314 copies/mL to 697 copies/mL viruses) after 1-year follow-up. The viremia virus was cleared in 23 patients (90%) in response to treatment.	They concluded that cure with IVIG is safe and successful for the treatment of BKV viremia and BKVN, also cure with IVIG can inhibit graft loss in patients that did not respond to anti-polyomavirus treatment.
Review of the various treatment option for Nephropathy due to BK virus			
Brennan et al. (2005) [12]	They included 200 adult renal transplant recipients to CyA (n = 66) or FK506 (n = 134).	Analysis for BK did by blood and urine weekly for 16 weeks and at months 5, 6, 9, and 12 and through polymerase chain reaction (PCR). They showed that viruria was high with FK506-MMF (46%) and minimum with CyA-MMF (13%). The viremia was resolved by 95% after the reduction of immunosuppression without raised allograft dysfunction, graft loss, or acute rejection. They no observed any BK nephropathy	They concluded that the type of adjuvant immunosuppression did not influence BK viruria or viremia.
Halim et al. [13]	Group 1 (n = 19) was composed of kidney transplant recipients with twice positive BK virus-polymerase chain reaction in urine and blood who underwent graft biopsy to confirm BK virus-associated nephropathy. Once BK virus-associated nephropathy was diagnosed, an antimetabolite (mycophenolate mofetil or azathioprine) was changed to leflunomide, and intravenous immunoglobulin and oral ciprofloxacin were given. Group 2 (n = 14) was composed of BK virus-associated nephropathy patients treated conventionally with reduced immunosuppressive medications.	Maintenance immunosuppression was prednisolone and mycophenolate mofetil (2 g/d) in 31 patients (94%), and tacrolimus in 13 (39.4%). Tacrolimus was given to 12 patients in group 1 (63.1%), while sirolimus was given to 7 patients in group 2 (50%). One graft was lost in each group by the end of the study, and 1 patient died with a functioning graft in group 2.	No significant difference existed in 1-year graft outcomes between the treatment of BK virus-associated nephropathy by reduction of immunosuppressive medications or actively by leflunomide, intravenous immunoglobulin, and ciprofloxacin.
Halim et al. (2009) [14]	Renal transplant patients with two BK virus polymerase chain response analyses of urine and blood experienced graft biopsy to establish BKVN.	In the beginning, mean±SD creatinine clearance was 35.6±11.5 mL/min/1.732, which was reduced to 29.3±17.3 mL/min/ 1.732. Cases were distributed into two groups of nine each according to creatinine clearance values. In group one, baseline rate was 44.5+-6.6 mL/min/1.732, compared with 25.36±7.8 mL/min/1.732 in group two, which reduced to 42.66 ±12.8 mL/min/1.732 and 16.76 (9.0) mL/min/1.732. Three grafts (16.7%) were lost by the end of the study, all in group two.	They concluded late diagnosis and intense immunosuppression predispose to BKVN. Initial active therapy of BKVN may develop graft results at one year after posttransplantation.

The patients received IVIG at 1.01 ± 0.18 g/kg. 50 kidneys at 7 months after transplantation evaluated and the biopsy-proven BKVAN occurred in them; they reported that 92% as histological stage B, 46% as dysfunction, 20% as viremia. The mean viral loads reduced after treatment ($P < 0.001$); the viremia ($P = 0.003$), BK immunohistochemistry ($P = 0.028$) effectively cleared in IVIG group. The graft losses fewer occurred in the IVIG group ($P = 0.06$). The results of the study demonstrated that combination treatment with IVIG compared with conventional therapy is a more helpful and valuable method for eliminating the virus from BKVAN. Although they recommended that a multicenter randomized trial is necessary for validation [10].

Vu et al (2015) evaluated the influence of cure with IVIG on the result of BKVN in renal transplant recipients. The BKVN patients remained after anti-polyomavirus treatment (using leflunomide therapy with a reduction of immune-suppression). They gave IVIG to patients that did not respond to anti-polyomavirus treatment after 8 weeks. The 30 patients included studying that had persistent BKV and BKVN. They showed that mean peak BK reduced (205,314 copies/mL to 697 copies/mL viruses) after a 1-year follow-up. The viremia virus was cleared in 23 patients (90%) in response to treatment. They concluded that cure with IVIG is safe and successful for treatment BKV viremia and BKVN, also cure with IVIG can inhibit graft loss in patients that did not respond to anti-polyomavirus treatment [11].

Brennan et al. (2005) determined the frequency of nephropathy, viremia, or BK viruria with tacrolimus (FK506) versus cyclosporine (CyA). They also evaluated whether severe examination and discontinuation of azathioprine (AZA) or mycophenolate (MMF) upon recognition of BK viremia, can be inhibited BK nephropathy. They included 200 adult renal transplant recipients to CyA (n = 66) or FK506 (n = 134). Analysis for BK done by blood and urine weekly for 16 weeks and at months 5, 6, 9, and 12 and through polymerase chain reaction (PCR). They showed that viruria was high with FK506-MMF (46%) and minimum with CyA-MMF (13%). The viremia was resolved in 95% after a reduction of immunosuppression without raised allograft dysfunction, graft loss, or acute rejection. They no observed any BK nephropathy; also they concluded that the type of adjuvant immunosuppression did not influence BK viruria or viremia [12].

Halim et al. evaluated the impact of therapy with leflunomide, intravenous immunoglobulin, and ciprofloxacin on graft result following one year compared with a historical group treated with reduced immunosuppressive medications strategy. Group 1 (n = 19) was composed of kidney transplant recipients with twice positive BK virus-polymerase chain reaction in urine and blood who underwent graft biopsy to confirm BK virus-associated nephropathy. Once BK virus-associated nephropathy was diagnosed, an antimetabolite (mycophenolate mofetil or azathioprine) was changed to leflunomide, and intravenous immunoglobulin and oral ciprofloxacin were given. Group 2 (n = 14) was composed of BK virus-associated nephropathy patients treated conventionally with reduced immune-suppressive medications.

Thirty-three patients were treated, 23 were males (70%), there were 15 were deceased donors (45.5%), 15 were diabetics (45.5%), mean human leukocyte antigen mismatches were 3.76, seven had a zero DR mismatch (21.2%), and 8 were CW7 negative (24.2%). All patients received induction therapy (thymoglobulin in 22 [66.6%]), 7 had delayed graft function (21.2%) and 18 received antirejection therapy before receiving BK virus-associated nephropathy diagnosis (52.9%). Maintenance immune-suppression was prednisolone and mycophenolate mofetil (2 g/d) in 31 patients (94%), and tacrolimus in 13 (39.4%). Tacrolimus was given to 12 patients in group 1 (63.1%), while sirolimus was given to 7 patients in group 2 (50%). One graft was lost in each group by the end of the study, and 1 patient died with a functioning graft in group 2. They concluded no significant difference existed in 1-year graft outcomes between the treatment of BK virus-associated nephropathy by reduction of immunosuppressive medications or actively by leflunomide, intravenous immunoglobulin, and ciprofloxacin [13].

Halim et al. (2009) evaluated the effectiveness of leflunomide, intravenous immunoglobulins, and ciprofloxacin as active therapy of postrenal transplant BK virus nephropathy (BKVN) in graft result at one year. They included renal transplant patients with two BK virus polymerase chain response analyses of urine and blood experienced graft biopsy to establish BKVN. For patients with BKVN, antimetabolite treatment (mycophenolate mofetil or azathioprine) was modified to leflunomide therapy accompanied by a plan of immunoglobulin and oral ciprofloxacin. They assessed eighteen patients that 72% of them were men. Nine patients underwent cadaveric organs, with an average of 3.6 HLA mismatches. They administered to all patients induction treatment (61% thymoglobulin), and 61% antirejection treatment before BKVN was detected. In the beginning, mean±SD creatinine clearance was 35.6±11.5 mL/min/1.732, which was reduced to 29.3±17.3 mL/min/1.732. Cases were distributed into two groups of nine each according to creatinine clearance values. In group one, baseline rate was 44.5+-6.6 mL/min/1.732, compared with 25.36±7.8 mL/min/1.732 in group two, which reduced to 42.66 ±12.8 mL/min/1.732 and 16.76 (9.0) mL/min/1.732. Three grafts (16.7%) were lost by the end of the study, all in group two. They concluded late diagnosis and intense immunosuppression predispose to BKVN. Initial active therapy of BKVN may develop graft results at one-year post-transplantation [14].

DISCUSSION

A significant reason for renal dysfunction and allograft loss is BK nephropathy in renal transplant recipients. Using various agents such as MMF compared to AZA or FK506 compared to CyA, for preservation immunosuppression to determine BK viremia, viruria, and nephropathy has been interested as an important issue for research [15–18, 19–21]. Also, BK viremia has been demonstrated as a precondition for progression to BK nephropathy. Therefore, BK viremia can be a marker of extreme immunosuppression. Brennana et. al reported that BK viremia could be removed in safety by the decrease of immunosuppression and especially by removal of the antimetabolite modules of the immunosuppressive treatment, consequently inhibiting raise to BK nephropathy without acute rejection, graft loss, or renal dysfunction.

Brennana et. al demonstrated that powerful associations among the beginning, interval, and titer of virus in the urine, thus viremia indicates the severity of infection in the allograft. They presented an early; severe viral infection follows, with a 1000-fold raise in the level of urinary virus in a comparatively short time enclose of 2–3 weeks. In these patients, the rise in viral reproduction resulted in detectable viremia. They recommended that it can be attractive to focus on potential control efforts on this serious period and viral level. Although, period and viral level of viremia show to be significant, the positive prognostic value is low, because it may be particularly in recipients whose immunosuppression is raised at a later time point [12].

Other investigators have established a significant association between transplant nephropathy and BK viremia. For example, Hirsch et al., have reported that a plasma viral titer more than 10,000 copies in 1mL be characterized as 'presumptive' BKV nephropathy, despite the biochemical and histological data of nephropathy did not show [22]. They reported that BKV viremia presented 88% specificity and 100% sensitivity for BK nephropathy, and plasma titer more than 7700 copies/mL reveal in all recipients with BKV nephropathy. Although, in Brennana et. al study, 61% of all recipients with viremia present plasma titers more than 100000 copies/mL while any evidence of BK nephropathy or deterioration of renal function not be seen. Therefore, Brennana results propose that powerful follow-up and early decrease of immunosuppression leading detection of BK viremia, with qualitative monitoring, can avoid the progress of clinically notable BK nephropathy. Generally, Brennana et. al saw no changes in the frequency of BK viruria or viremia between those getting MMF or AZA, FK506 or CyA, separately [12].

Purighalla et. al (1995), reported a case report about a 34-year-old man with polycystic kidney disease who underwent renal transplantation. 12 and 22 days after transplantation occurred reversible episodes of acute rejection. 38 weeks after transplantation, the biopsy demonstrated changes dependable with rejection also demonstrated BK virus inclusions. All follow-up biopsies showed a mixture of rejection and BK virus infection. Ultimately, the graft loosed 56 weeks after transplantation [23].

Decreasing of immunosuppression is the most common curative interference for the cure of BKVN in renal transplant recipients; however, it is not constantly satisfactory to stabilize renal function [24- 27]. In recent years, using IVIG in the cure and management of the BKVN has improved [28, 9, 29, 30, 31]. The IVIG can decrease the infection of BKV through the straight neutralization of BKV via virus-specific antibodies, consequently sopping viral activation and infection. Recently, the constructive combination method of IVIG management and immunosuppression reduction was presented to be successful in the treatment of BKVN. Sener et al (2006) studied the effects of renal transplant patients with BK virus-associated nephropathy treated with IVIG [9]. They reported that 8 renal allograft recipients identified with BKVAN after 11.4 months after transplantation. All of the patients received a reduction of immunosuppressive therapy; also they obtained 2 g/kg IVIG. All of the patients except one were off dialysis after a follow-up of 15 months. Concluding, they reported that 88% of patients still have functioning grafts after IVIG therapy. Ultimately, they noted that further research including randomized, multicentered trials should be done about the advantages of concomitant reduction of immunosuppressive therapy and IVIG for BKVAN [28]. Only 1 patient lost the graft after 1 year of follow-up, while the 7 patients still had practical grafts. Sharma et al [31] illustrated utilizing IVIG in a pediatric patient identified as constant BKVN in a case report. Scr level raised and BK viremia reactivated after a month of achievement of cidofovir therapy. Followed by IVIG was given in a 7-dose regimen of 600 mg/kg. Viral load decreased after the fifth dose, from 20,800 to 2540 DNA copies/mL. Finally, Scr level becomes constant during 6 months and viral load reduced [31]. Dheir et al [29] demonstrated which IVIG treatment was to help inhibit acute rejection and delay graft endurance. Although, Dheir et. al did not show how the viral load was altered after IVIG treatment [29]. Vu et al (2015) evaluated the influence of cure with IVIG on the result of BKVN in renal transplant recipients [11]. The BKVN patients remained after anti-polyomavirus treatment (using leflunomide therapy with a reduction of immune-suppression). They have given IVIG to patients that did not respond to anti-polyomavirus treatment after 8 weeks. The 30 patients included in the study had persistent BKV and BKVN. They showed that mean peak BK reduced (205, 314 copies/mL to 697 copies/mL viruses) after a 1-year follow-up. The viremia virus was cleared in 23 patients (90%) in response to treatment. The allograft survival rates were 97.4%, and the 12-month patient was 100%. A loss of an allograft reported by they had a harsh rejection episode subsequent no feedback in removing BKV after treatment. They concluded that cure with IVIG is safe and successful for the treatment of BKV viremia and BKVN, also cure with IVIG can inhibit graft loss in patients that did not respond to anti-polyomavirus treatment. However IVIG therapy is expensive, but this price perhaps acceptable in selected patients because BKVN has appeared as a critical reason for the loss of renal graft and renal allograft dysfunction in the transplant recipient. In conclusion, IVIG seems to be effective and safe in the treatment of BKVN and inhibits graft loss in BKVN patients with combination therapy of leflunomide therapy and immunosuppression reduction.

CONCLUSIONS

A review of studies shown powerful follow-up and early decrease of immunosuppression leading detection of BK viremia, with qualitative monitoring, can avoid the progress of clinically notable BK nephropathy. Generally, there is no change in the frequency of BK viruria or viremia between those getting MMF or AZA, FK506, or CyA, separately.

Combination treatment IVIG is more successful in removing viral load in patients with BKVAN, compared with traditional standard-of-care therapy. Ultimately, further research including randomized, multicentered trials should be done about the advantages of concomitant reduction of immunosuppressive therapy, and IVIG for BKVAN IVIG seems to be effective and safe in the treatment of BKVN and inhibits graft loss in BKVN patients with combination therapy of leflunomide therapy and immunosuppression reduction.

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ORIGINAL ARTICLES

Quality of life impairments and stress coping strategies during the Covid-19 pandemic isolation and quarantine – A Web-based survey

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Abstract: *Isolation and quarantine during the Covid-19 pandemic affected the lifestyle and daily functioning of the population around the world, leading to social, psychological, and economic changes which further multiplied the stress related to the threat of coronavirus contagion by adding financial, relational, academic, professional and mental health vulnerabilities. To assess the impact of isolation and quarantine over the quality of life in the Romanian population, we conducted a Web-based survey focused on the evaluation of stress level, perception of lifestyle changes, communication patterns, mental health, major concerns, perception of one's future, but also on the preferred coping strategies that people have used to deal with the isolation stress. The answers were collected during one month and the results for the first 2 weeks of quarantine/isolation were compared with the results after one month of such regimen. Several recommendations based on the survey results analysis were formulated regarding possible strategies for decreasing the impact of stress factors over the general population and specific, vulnerable groups.*

Keywords: *Covid-19, pandemic, quality of life, stress coping strategies, functional impairment, depressive disorders, anxiety disorders, behavioral addictions*

QUALITY OF LIFE AND COPING STRATEGIES DURING QUARANTINE AND ISOLATION IN THE CONTEXT OF COVID-19 PANDEMIC

Quarantine is defined as a separation of people potentially exposed to contagious disease from other members of the society until the results of their analyses turn negative or until further medical interventions are needed [1]. Regarding the Covid-19 pandemic, this procedure has been considered necessary for people who traveled in the so-called ”red zones”, where high rates of coronavirus disease have been reported, and they were screened for infection initially and after two weeks of quarantine [2]. Also, the quarantine regimen involved special places for monitoring these people who were considered at risk for developing Covid-19.

Isolation is conceptualized as a separation of people who have been in contact with infected others, but who do not have any symptoms yet or have only mild symptoms, depending on national healthcare services' operational procedures [2]. This concept involves the isolation of people in their own homes for two weeks and active monitoring from their GP or Public Healthcare Services [2]. Self-isolation or lockdown is defined as a method to maintain social distancing by reducing the time spent out of the house for each asymptomatic person, and it was enforced during the Covid-19 pandemics by the law. Self-isolation is a broader

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concept but it is fundamentally a population-large method of prevention, applied in cases of contagious diseases, with a duration defined by national laws during the state of emergency. It does not involve relocation to a special quarantine-designed institution, each self-isolated person remained at his or her home for a duration specified by the law, and maintains the right to leave his/her home for a pre-defined set of specific purposes.

According to a recent review (n=24 papers), the main psychological negative effects of quarantine were post-traumatic stress symptoms, confusion, and anger, with the most important stressors being long quarantine duration, fear of contamination, frustration, boredom, lack of sufficient supplies, inadequate information, financial loss, and stigma [1]. During a SARS outbreak in Singapore, the contagious infection-related psychiatric and posttraumatic morbidity rates reached 22.9% and 25.8%, respectively [3]. According to this research, the most significant factors associated with psychiatric disorders, in general, were being seen at fever stations, younger age, increased self-blame, less substance use, while posttraumatic disorder was associated with the increased use of denial and planning, as coping mechanisms [3]. Another Web-based survey that examined the psychological impact in a quarantined Canadian population during a SARS outbreak (n=129) revealed a high prevalence of psychological distress, with symptoms of posttraumatic stress disorder and depression observed in 28.9% and 31.2% of responders [4]. A longer duration of quarantine was associated in this paper also with an increased prevalence of posttraumatic stress disorder, and acquaintance with or direct exposure to someone with a SARS diagnosis was also a risk factor for this pathology [4].

In the context of the Covid-19 pandemic complete social isolation during many consecutive weeks was considered in Italy similar to a large-scale, absolutely new social experiment [5]. Therefore, activation of healthy coping strategies could be of major importance in the prevention of psychiatric disorders onset in this population. Separation, isolation, boredom, loneliness, feelings of uncertainty are challenges for many quarantined or isolated people [6]. Healthcare workers suffered from quarantine as well, as they accused exhaustion, alienation, anxiety, irritability, insomnia, indecisiveness, decreasing work performance, etc [6, 7]. Also, healthcare personnel had more severe symptoms of posttraumatic stress disorder than the quarantined general population, experienced greater stigma, more avoidance after quarantine, were more likely to believe they had been contaminated and were more preoccupied with the risk of infecting others [7, 8].

Treatment adherence may be negatively affected by denial, anxiety, depression, feelings of despair, and also the risk of suicide or aggression should be taken into account by physicians who take care of patients diagnosed with Covid-19 [9]. Obsessive-compulsive symptoms may appear as a consequence of repeated washing and temperature checking [8]. Financial losses, societal rejection, disappointment, and discrimination are other factors that should be addressed by targeted interventions [1, 10].

Regarding the main keypoints for the psychological crisis intervention in Covid-19- diagnosed patients in China, a group of authors suggested understanding of the mental health status in different categories of population, identifying people who are at high risk of suicide and aggression, and targeted interventions for those in need [9]. Chinese researchers have established four levels of populations to design specific interventions for each one: level 1 population includes the most vulnerable people to mental health problems, e.g. hospitalized patients with severe organic diseases, frontline health care professionals, level 2 includes isolated patients with minimal symptoms and patients at fever clinics, level 3 includes people with close contacts, family members, colleagues, friends, etc, and level 4 people who are affected by the epidemic preventative measures, susceptible people and general population [9].

OBJECTIVES AND METHODOLOGY

Although stress is considered a normal response to the coronavirus pandemic, quarantine and isolation are periods of high risk for the development of stress-related disorders [11]. This risk is high especially in vulnerable populations, e.g. people with a known history of mental disorders, currently remitted or controlled by treatment, and professionals who are on the frontlines of the coronavirus pandemic (physicians, police officers, military personnel, etc), but since the lockdown was enforced by the law to the general population as a preventative measure against spreading the Covid-19, many other populations were exposed to the risk of developing stress-related disorders (e.g., elderly people, patients relying on the support of others due to physical or mental impairments, patients immobilized in their own homes, people with chronic treatments which involved regular visits to the hospital, people who were unable to work from home and who have lost their financial support, owners of small and medium enterprises that could not run their businesses during the state of emergency, etc).

The main objective of this research was to evaluate the impact of the isolation stress over the quality of life in people from Romania during the first period of the Covid-19 pandemic. The secondary objective was to find out how people have been coping with isolation-induced stress and related factors during the same period.

Data on the effect of isolation and quarantine in terms of quality of life and coping strategies were collected through an online survey that could be completed by the respondents between the 21st of March and 20th of April 2020. During one month, 941 respondents answered the online survey, and their answers were analyzed after the first two weeks (n=103) and at the end (n=941) of the before-mentioned period. A comparative analysis of the responses from the two stages was also performed.

The survey was entitled "The psychological impact of the Covid-19 pandemic in the population subjected to self-isolation/quarantine" and consisted of 34 questions (Q1-Q34), with a completion time of 10-15 minutes. The answers of the people who completed 100% of the survey questions were analyzed (n=941).

QUALITY OF LIFE IMPAIRMENTS AND COPING STRATEGIES TO STRESS DURING THE COVID-19 PANDEMIC

Respondents were adults (81.5%), elderly (17.5%) and adolescents (1%), coming from urban (86%) or rural (14%) environment, both citizens from Romania (97.3%) and Romanian citizens residing in other countries (Great Britain, Germany, Austria, Canada, the Republic of Moldova, Tunisia, Belgium, the Czech Republic, Spain, Cyprus, Portugal, Italy and the USA, 2.7%). The respondents were female (81%) or male (19%), and they were married 54.7%, unmarried 23.7%, widowed 8.6%, or divorced 13%.

People who completed the questionnaire were living mostly in self-isolation for prophylactic purposes (68%), in quarantine (6.5%) or isolation (symptomatic) (3%), for one week (11, 5%), two weeks (8.5%) or more than 14 days (80%) (Figures 1 and 2).

The respondents were professionals from the medical staff (10.5%), police officers and auxiliary personnel (1.2%), workers in trade business with direct contact with potentially-infected people (5.3%), or were professionals involved in other high-risk categories of contracting Covid-19 (12.3%). Answers provided by respondents who did not fall in any of the previously mentioned professional categories represented 70.7%.

The educational background of the respondents was university (43.4%), postgraduate (24.6%), or high school (19.2%).

Survey respondents lived in the same home with children up to 7 years old (9%), children aged 8-14 (6.7%), adolescents (11%), elderly (over 65 years old) (12.7%), people with disabilities or other severe illnesses (2%), people undergoing treatment for various organic diseases (5.6%), people diagnosed with mental disorders and currently on specialized treatment (1.1%), or people who took care of other vulnerable individuals (3%). Among the respondents, 3% had mental disorders and were currently receiving treatment, while 13% had somatic disorders.

Figure 1: In which situation are you now? (Q2)

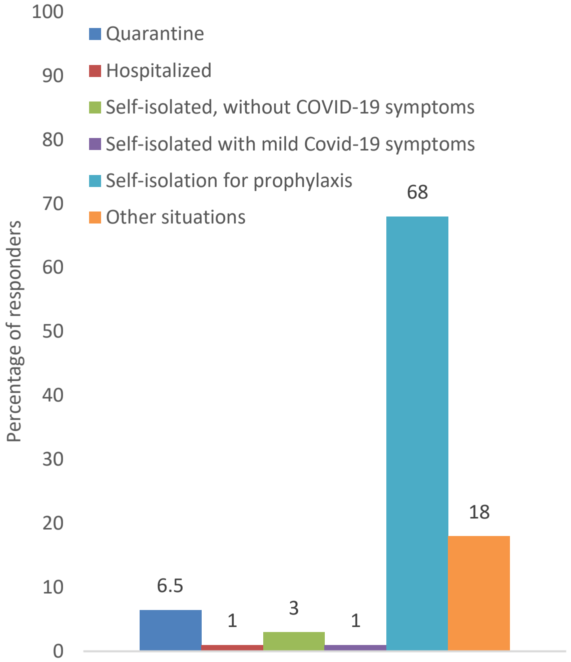

Figure 2: How long have you been in isolation or quarantine? (Q3)

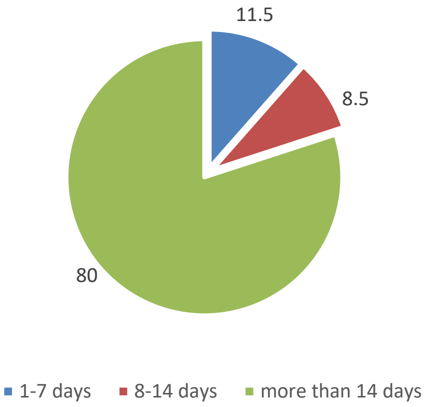

QUALITY OF LIFE IMPAIRMENTS DURING COVID-19 PANDEMIC- ASSOCIATED QUARANTINE AND ISOLATION

The level of stress induced by isolation or quarantine was felt differently by respondents, ranging from absent (10.6%) to extreme (7%) (Figure 3). Most of the respondents considered the level of stress associated with the quarantine or self-isolation regimen to be moderate (35.6%).

Communication is an important aspect of the quality of life in isolated populations, and changes in the communication patterns may have significant repercussions over people's social and psychological health. Our respondents continued to communicate with their relatives and friends by telephone calls (44.5%), through social networks using their smartphones (43.5%) or other electronic devices (laptop/ tablet/desktop) (10.1%) (Figure 4).

Figure 3: How do you evaluate the severity of the quarantine/ isolation-induced stress, from 1(absent) to 10 (extreme)? (Q11)

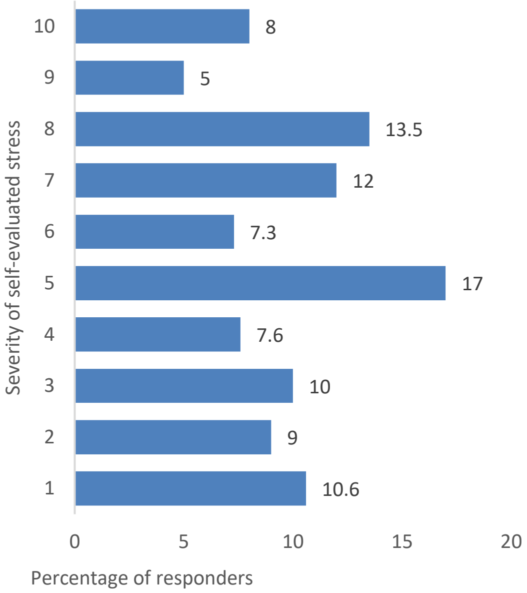

Inadequate information may negatively impact both mental health and quality of life, by raising the level of stress and fostering cognitive distortions. Most of the respondents collected data about the Covid-19 pandemic from the Internet (official sites) (57.5%), television (31.3%), Internet blogs and unofficial sites (5.3%), online or printed press (3.8%), and other sources (1.1%) (Figure 5). The majority of respondents stated they spent a maximum of one hour daily to find out news about the Covid-19 pandemic (71%), while 25% spent between one and 5 hours each day for the same purpose.

Regarding the most important concerns during the lockdown/quarantine that may influence the quality of life, our respondents mentioned primarily the concern about the health of those close to them (43.7%), then the concern related to their health (15.8%), followed by worries triggered by the family's financial security (12%), her or his financial future (7%), and his or her professional perspectives (4%). Other concerns reported by respondents were: the difficulties in running their own business, the current socio-political situation, the obligation to be vaccinated if a vaccine is to be discovered, the inability to continue daily routines they had before the pandemic, the restrictions applied to the freedom of movement, the restrictions applied to the access to religious services within the church, limitations to the professional/school activities, the collective mental health, the global economic crisis, the loneliness, the possible shortcomings in food production. About 13% of respondents did not mention any significant current concerns related to the Covid-19 pandemic that may relate to their quality of life.

Figure 4: How did you continue to communicate with your family, friends, neighbors during the isolation/quarantine? (Q13)

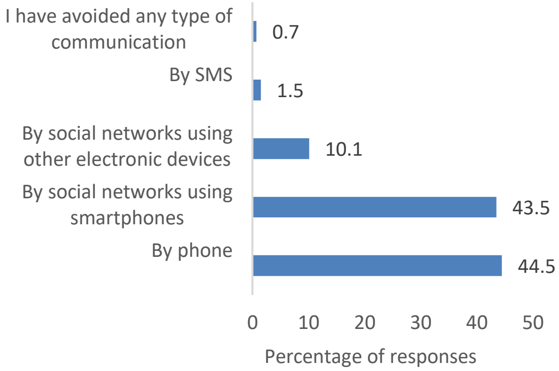

Figure 5: Where did you get your information about the CoViD-19 pandemic? (Q14)

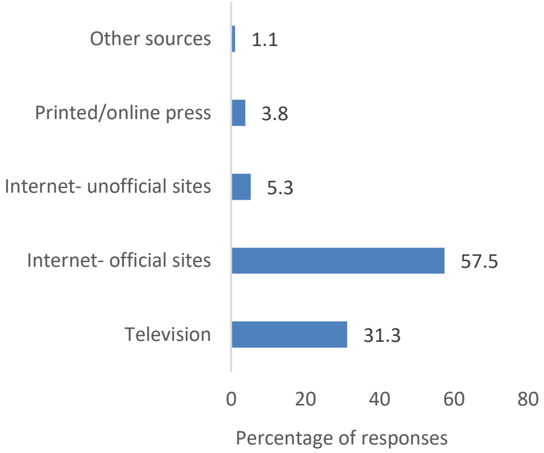

Financial aspects of quarantine and isolation have a significant potential to trigger changes in one's quality of life. During the period of self-isolation, 38.2% of people stated that they could work from home, without financial differences (20.8%), or with financial losses (17.4%), while

11% were in technical unemployment. It should be mentioned that 15.1% of the people stated they cannot work from home or receive financial support for this period, and a significant percentage of the respondents cannot work from home at all, because they have professions that cannot be done from distance.

Regarding the degree of optimism, respondents admitted a low (43%) or very low (35%) level, with only 20% recognizing a moderate level and 22% a high level of confidence in the possibility of solving the epidemiological crisis in the short-term (2-4 weeks) (Figure 6).

Figure 6: How do you rate your level of optimism regarding the possibility of solving the problem of the pandemic in a short time? (Q23)

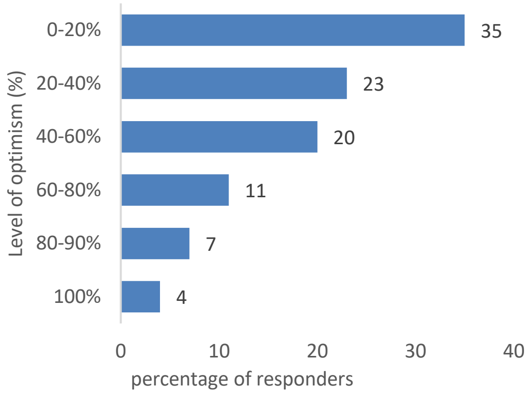

The way people look at their future is important for evaluating the quality of life in any population. Asked how they estimate their life will change as a consequence of the Covid-19-related (self)isolation/quarantine, the survey respondents said they expect to emerge stronger from this experience (47.7%), or that they will not suffer any medium or long-term negative impact of isolation (29.3%) (Figure 7). Only 10.1% of respondents consider that this experience will have a significant negative and permanent impact on their lives.

Regarding the appearance of psychopathological symptoms with functional impact during isolation, respondents mentioned insomnia (11.5%), nervousness/irritability (9%), unmotivated physical/mental fatigue (6.4%), panic (6%), catastrophic expectations related to pandemic (6%), increased appetite (4.5%), depressed mood (4%), decreased appetite (2%), crying spells (2.5%), ideas of uselessness or lack of self-worth (2.5%), concentration problems (2.2%), anhedonia (2.2%), feelings that life is meaningless (1.7%), drowsiness (1.7%), numbness in the hands or feet (1.6%), palpitations (1.6%), de-realization (1.3%), physical pain without an organic cause (1%), and many respondents admitted the presence of at least two of the listed symptoms. About 30% of respondents stated they did not experience any symptoms from the list presented.

Figure 7: What do you think that will change in your life as a result of isolation/quarantine? (Q29)

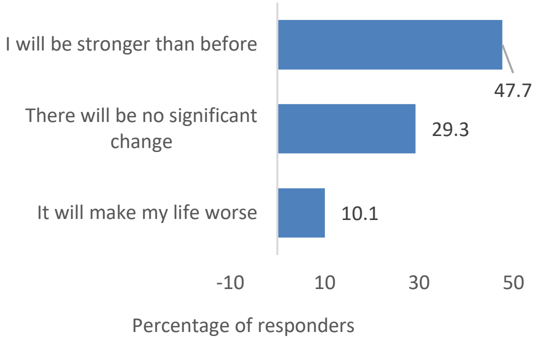

The communication with health services is another variable that may be correlated with the quality of life, especially during a world-scale epidemiological crisis. It should be noted that only 3.8% of the respondents sought the help of a mental health specialist during lockdown and quarantine. Of the people who went to the psychiatrist, 1% were already in treatment and adjustments of the current therapy were recommended, 1.2% were prescribed treatment for the first time, 0.5% did not receive any psychotropic treatment, being scheduled for a reassessment after the period of self-isolation/quarantine, 2.7% received a recommendation for online counseling/psychotherapy, and 40.1% did not receive a recommendation for any drug treatment or psychotherapy.

Of the patients who had medication recommended for any condition before self-isolation/quarantine, most of them stated that they were able to obtain the prescribed medications, but some people reported difficulties in procuring their usual medications (for hypothyroidism and diabetes, for example). Many respondents mentioned that they have appreciated the newly-launched system of electronic prescriptions sent by the physicians, without having them to travel to the doctors' office.

In terms of harmful behaviors with onset during the Covid-19-related self-isolation/quarantine, 23% of respondents ate more than before, 16% increased cigarette daily consumption, 9.6% resorted to excessive online shopping, which they later regretted, 5% took by themselves medication for sleep, and 4% increased alcohol use compared to their reference level. Other significant changes were abusive use of the Internet and changing in the sleep-wake rhythm, by advancing bedtime and waking up later than usual, and increased consumption of sweets. Changes in the physician-prescribed diet have also been reported, because people with dietary restrictions found harder on the market certain foods during this period. However, some respondents mentioned the appearance of certain favorable changes, stating that "I appreciate life more now", "I appreciate much more everything beautiful around me", "I treasure my health more", "I appreciate the freedom more", “I have managed to quit smoking”, “I realized that I can adapt to more difficult situations without repercussions on my psychological status”.

COPING STRATEGIES DURING COVID-19- RELATED QUARANTINE AND ISOLATION

Interaction with other family members in self-isolation was the preferred method of coping with the situation (17%), followed in descending order by use of the Internet - social networks (13.4%), television – entertainment programs (12%), reading (11%), gardening (9%), Internet – entertainment (6%), Internet – online games (3.8%), television – news editions (3.6%), smoking (3.5%), sportive activities at home (3%), religious activities performed at home (3%), crosswords/sudoku/puzzles (2.6%), DIY (2.3%), Internet – educational programs (2%), board games with other self-isolated family members (1.6 %), Internet – shopping other than those of vital necessity (1%), television – cultural programs (1%) and alcohol consumption (0.75%).

When asked what they would advise other people placed in isolation to do while they are inside their homes, respondents said the best coping methods would be: structuring the daily program, in which to include activities as diverse as possible (e.g., "to arrange for the next day at least two activities that he/she likes"); to exploit the available time they have for doing things they have not been able to do so far because of the procrastination; to be patient, to keep calm and to follow the rules, to think about the fact that it is only a period that will pass; to do gymnastics or any kind of sport, in the house or outside; to adopt a pet; to get involved in religious activities that can be carried out at home; to avoid continuous contact with news programs (e.g., "to filter the information they receive very well", "to be informed about the current situation from the TV or other sources only if necessary"; "to watch the news only once daily”); to listen to cultural programs on the radio; to continue their professional activity at home, if possible; to discover new hobbies ("to discover something they will enjoy", "to do something creative"); gardening (e.g., "those who can move to a house with a garden should do it", "even if you are living in a flat you can take care of flowers, because flowerpots can be placed on the balcony"); to take advantage of free time and spend it with loved ones, to communicate with the family by phone/social networks (e.g., “those who have children should enjoy these moments, going to work usually robs us a lot of time that we should spent with our children”, “to communicate daily with the children and with the family by phone/Internet”); to reflect on the self (e.g., "to explore the self as much as possible using all the means at their disposal"), meditation, yoga; to clean the house in an organized way; to watch new movies with his/her family, but also to watch old movies, which he/she likes; crosswords, sudoku; Internet- games, social networks, entertainment (e.g., shows broadcasted online), personal development trainings, parenting, etc.; sewing, tapestry, embroidery, crocheting, knitting, tailoring; DIY; cooking (e.g., "to try to prepare new things in the kitchen, to explore..."); drawing, painting; music (e.g., "to listen to music daily, especially relaxation music"); reading (e.g., "to read some of the books we have long wanted to read"); development of skills that could be useful after the end of the pandemic- language courses, acquisition of professional qualifications or overspecializations, computer skills trainings (e.g., "to look for information related to a specialization in a new field"); to study what they wanted to study but failed due to lack of time; board games with other family members in the house; to keep a diary of this period; to avoid food excesses and to focus on a healthy diet; to build plans for the future (e.g., “to plan their activities in the post-isolation period, to take into account what they want to do, who they want to see”).

To the question "What do you not recommend to other self-isolated people to do?" the most common responses were: to avoid prolonged lying in bed during the day; to avoid binge-watching TV (e.g., "do not watch news programs for more than an hour daily") and to avoid data collected from unauthorized sources; avoid alcohol use and excessive smoking; do not do excessive shopping; do not isolate themselves emotionally; do not fall prey to monotony; do not consume excessively sweets and rich-calories foods in general; do not take medication without a doctor's recommendation; to avoid leaving the house if it is not necessary; to avoid excessive discussions on social networks about coronavirus; to avoid self-victimization and panic; do not abuse the Internet; to avoid online gambling; to avoid alcoholic drinks and to not increase the number of daily cigarettes.

Communication with people living in the same home was not significantly affected, according to the majority of respondents placed in isolation/quarantine. They stated that they enjoyed the time spent together, this time is an opportunity for mutual discovery or closeness. Such responses were, for example, “This isolation strengthened the connection between us, divergences faded, we mobilized to face a general threat together”, “We found, after a long time, the pleasure of time spent together; we have different stages of life but we have re-discovered common interests/opinions”, “I think this period has approached us”, “We had enough time to discuss everything we had not discussed so far”,“ Self-isolation facilitated communication between me and other family members, we had more time to spend together, a time we would not have allocated to ourselves if it were not for these extreme circumstances", "If it were not for the crisis, everyone would have had other things to do and we would have inadvertently avoided this approach".

There were however signals about monotony or increased nervousness - "Stress level is quite high, not everyone understands self-isolation measures", "This period increased our stress and made us feel trapped", "We became more irritable towards each other, because we spent too much time together”, "Because of the stress and anxiety there were more and more discussions and sometimes quarrels". There have also been situations when ignoring others has been reported as a method of coping with interpersonal stress.

The following reactions were reported during the communication with people (family, friends) outside the respondents' home: frustration due to lack of intimacy (“Emotionally, lack of contact with loved ones generates frustration”, “It is difficult not to have meetings, we can't enjoy the outdoors together", "I'm sorry I can't physically express my love for them", "I’m afraid we will become estranged"), worries about changing one's self and one’s relationships ("We became more lonely, more suspicious, more indifferent to each other”), concerns about the impact of one’s professional status over the family members (“I have to go to the hospital every day, being a doctor, and the family is worried about my health”), concerns about lower income that can affect family members, lack of food supplies on the markets, lack of enough anti-Covid-19 protective materials), reassessment of previous social experiences (“I miss my work colleagues, even those I did not value enough”). Some people tried to focus on the positive aspects ("We have more time to discuss, to talk about anything... For the first time I talked to my child for an hour without arguing", "It's hard not to see each other, but I know that's how we protect ourselves and others”,“ We communicate better”, “I think now I have time to talk to more people than before”).

Religion is seen as an important coping method by 47% of those in isolation or quarantine, even if participation in religious rituals was not allowed within the churches during the emergency state.

For people living with children in self-isolation/quarantine, the most commonly used methods of organizing children's time were encouraging online communication with classmates (7%), continuing school training (6.2%), board games (3.9%), encouraging participation in physical exercises (3.1%), unrestricted use of the Internet (“let them stay on the Internet as long as they want”) (2.8%), watching TV programs (“we do not limit access to watching TV anymore to a certain amount of time”) (0.8%).

Regarding the communication with children about the Covid-19 pandemic data, 32% of parents responded that they tell them what is happening and maintain constant communication with them about the pandemic, while 16% prefer to distract their attention from what happens through the use of games or other means. Almost 7% of the parents prefer to tell them that nothing bad is happening and that it is just a form of holiday, and only 2% said they forbid their children to come into contact with information sources about pandemic or do not answer children’s questions about the pandemic openly.

DYNAMIC INTERPRETATION OF THE QUALITY OF LIFE AND COPING STRATEGIES DURING THE QUARANTINE OR ISOLATION

Between March 21 and April 7, 2020, 103 respondents completed the online survey, and between April 8 and 20, 2020, the number of respondents reached 838. The interpretation of the answers must take into account the significant change in the demographic distribution of the participants, respectively an increase of the percentage of elderly people from 2% to 17.5%, with the corresponding decrease in the number of adults aged between 18 and 64 (95% vs. 81.5%), while the number of adolescents remained relatively constant (1% vs. 3%). Also, the marital status of the respondents was different, with an increase of the proportion of married (40% vs. 54.7%) and divorced people (3% vs. 13%), in parallel with a decrease in the number of unmarried people (55% vs. 23.7%). No major differences were observed in the geographical distribution of respondents (urban vs. rural, people located in Romania vs. Romanian citizens located in other countries). From the point of view of the educational background, a higher number of people with high school answered the questionnaire in the second stage of the survey, so that their proportion increased to 19.2% vs. to 9% in the first stage. Respondents who were not in those categories involving a professional risk of contracting Covid-19 increased from 56.5% to 70.7%, a difference that may be correlated with the change in the age of the respondents.

The people who completed the questionnaire were mostly self-isolating for prophylactic purposes both in the first half of the month and at the end of the one month, but the duration of the isolation regimen increased, as expected - the proportion of those who stayed in the house for more than 14 days was at the end 80% vs.11.7% at the half of the evaluation duration. The proportion of those who cared for children, adolescents, or the elderly was not significantly different in the two situations, as was the proportion of those who had mental or organic disorders in treatment.

The level of stress induced by isolation/quarantine did not change much over time, the proportion of those who consider this parameter to be moderate was 33% at two weeks and 35.6% after 4 weeks, those who considered it not significant were more numerous (6% vs. 10.6%), and the percentage of those who considered it unbearable also increased slightly (5% vs. 7%).

Interaction with other family members in self-isolation was the preferred method of dealing with the situation after two weeks and remained so at week 4 (23.5% and 17%, respectively).

Several coping strategies have increased their frequency of use – for example, use of the social networks (12% vs. 13.4%), watching entertainment programs, music, movies, television documentaries (10% vs. 13%), interest in gardening (4% vs. 9%), DIY (1% vs. 2.3%), smoking (3% vs. 3.5%), home sports/gymnastics (less than 1% vs. 3%), crosswords/sudoku (1% vs. 2.6%) and religious activities at home (less than 1% vs. 3%).

From the category of methods that have remained at the same level throughout the 4 weeks of the survey was the interest in reading (10% vs. 11%), sportive activities at home (8%), TV news (3% vs. 3.6%), Internet- educational programs (2%) and Internet- online shopping (1%). There was a slight decrease in the interest in Internet-entertainment and online games (13.5% vs. 9.8%), inboard games with other family members (3% vs. 1.6%), but also in the interest for alcoholic drinks (2% vs. 0.75%).

Respondents continued to communicate with those close to them by phone, through social networks on mobile phones or other electronic devices (laptop/tablet/desktop) in similar proportions in the two stages. Most respondents informed themselves about the Covid-19 pandemic from the Internet (official sites) (69.6% vs. 57.5%), but also unofficial sites or blogs (less than 1% vs. 5.3%). More respondents preferred to inform themselves from television at week 4 (22.5% vs. 31.3%), while the press remained at a constant level of interest (5% vs. 3.8%). Most of the respondents stated they have spent a maximum of one hour daily to find out data about the Covid-19 pandemic in a slightly increasing percentage (64.7% vs. 71%), while the number of those who spend between one and 5 hours each day for the same purpose decreased slightly (30.6% vs. 25%).

Asked what they would advise other people situated in isolation to do while they are inside their homes, the respondents offered the same categories of answers, namely those focused on the accuracy of information (using only official sources), spending more time with hobby activities, structuring of the daily time (including a list with various daily activities), caring for other family members with whom they live, communicating with friends/relatives outside the home by electronic devices, building plans for the future, developing new skills, adhering to the principles of healthy eating, practicing activities they have postponed due to lack of time, rediscovering themselves through specific activities (online personal development workshops, meditation, yoga). New recommendations have emerged regarding religious activities practiced at home, in the context of the Easter holidays.

To the question "What do you not recommend to other people in self-isolation to do?" the most common responses were similar in the two-time intervals assessed, namely avoiding long-term exposure to news programs, avoiding excessive eating, avoiding lack of activities during the day, avoiding leaving the house if it was not necessary, avoiding excessive discussions on social networks about coronavirus, avoid self-victimization, panic, and monotony, avoid alcohol use and excessive smoking. There were also recommendations to avoid prolonged bedtime during the day, excessive shopping, abuse of sweets, and high saturated fat foods, online gambling, and abuse of the Internet.

Communication with people situated in the same house was not significantly affected, according to the majority of respondents in self-isolation after two and 4 weeks, with a predominance of people who showed that they enjoyed the time spent together, this interval being considered an opportunity for mutual discovery or closeness.

During the communication with other people (family, friends) outside the respondents' homes, the following reactions were reported: frustration due to lack of privacy, concerns about the impact of professional status on the family’s general wellbeing, concerns about the lack of finance that may affect family members, reframing of the previous social experiences, but also equilibrium or even focusing on the positive aspects of the situation. Besides, concerns were raised about changing one's person and one’s relationships, which could be long-lasting, according to the respondents.

Regarding the most important concerns of this period, respondents mentioned especially the anxiety related to the potential health issues of the loved ones (67% vs. 43.1%, decreasing trend), then those related to their health (11.6% vs. 15.8%, increasing trend), followed by those fears triggered by financial uncertainty, professional or school prospects and the global political context.

During the period of self-isolation, 54% of people stated that they can work from home without financial differences (35% vs. 38.2%, slight increase), or with financial losses (14.5% vs. 17.4%, slight increase), while technical unemployment affected an increasing proportion of people (4% vs. 11%). It should be noted that 12% (after two weeks) and 15.1% (after 4 weeks) stated that they could not work from home or receive financial support for this period, and a significant percentage of respondents were not able to work from home at all due to the specifics of their jobs.

Regarding the level of optimism, respondents admitted a reduced level after 4 weeks comparative to the 2-week level (26% vs. 43%), only 20% admitted a moderate level, and 9% vs. 22% (significant increase) a high level of confidence in the short-term resolution (2-4 weeks) of the epidemiological crisis.

Religion was seen as an important resource for a growing proportion of people (33% vs. 47%) in self-isolation/quarantine after 4 weeks comparative to the 2-week level.

For people living with children in self-isolation/quarantine, the most commonly used methods of organizing children's time were online communication with colleagues/teachers, board games, continuing school training program, then using the Internet, physical exercises, and watching TV. Regarding communication with children about Covid-19 pandemic data, a similar percentage of parents responded that they tell them what is happening and maintain constant communication with them about the pandemic (35% vs. 32%), while 17% vs. 16% prefer to distract them from what is happening outside by using games or other means.

Asked how they estimate they will be affected by self-isolation/quarantine, respondents said they expect to emerge stronger from this experience (55% vs. 47.7%, decreasing trend), or that they will not suffer any medium- or long-term negative impact of isolation (31% vs. 29.3%, relatively similar percentage).

Regarding the onset of psychopathological symptoms with functional impact during isolation, respondents mentioned insomnia (10% and 11%, respectively), followed by nervousness/irritability (10% vs. 9%) and anxiety/panic (5% vs. 6%, increasing trend), catastrophic expectations related to the pandemic (6%, increasing trend), unmotivated physical or mental fatigue (7% vs. 6.4%). Other reported symptoms were depressive mood (8% vs. 4%, decreasing trend), increased appetite (7% vs. 4.5%, decreasing trend), concentration problems (7% vs. 2.2%, decreasing trend), and several people have reported clusters of symptoms.

However, only 4.8% of the respondents sought the help of a mental health specialist during isolation/quarantine after two weeks and 3.8% after 4 weeks. Of the patients who had medication recommended for any condition before isolation/quarantine, most of them said they were able to purchase their prescribed medications.

During isolation/quarantine, harmful behaviors such as excessive online shopping decreased in time (13% vs. 9.6%), alcohol consumption compared to the previous level also decreased (7% vs. 4%), excessive smoking was increasingly reported (13.5% vs. 16%), and self-administered sedatives significantly increased (1% vs. 5%). Other important changes were the more intense use of the Internet and change in the sleep-wake rhythm by advancing the bedtime and waking up later than usual, and also an increase in the sweets consumption. After 4 weeks, changes in the structure of the diet were reported, people who had a diet recommended by the doctor stating that they can no longer buy those specific foods. After 4 weeks, some favorable changes were mentioned, such as raising awareness of the importance of life, health, and loved ones.

CONCLUSIONS AND RECOMMENDATIONS

The dynamic self-assessment of the stress level induced by isolation or quarantine in the context of the Covid-19 pandemic did not show significant changes between the analysis performed after two weeks and the one performed after 4 weeks. This stress level was very different from one respondent to another, being quite evenly distributed from absent to extreme, most often being chosen the average values. It should be noted that almost 50% of the respondents were caregivers for minors, elderly people, patients with organic or mental illnesses so that they were confronted with multiple stressors.

The most commonly reported coping methods used to deal with the stress of self-isolation were (1) direct communication with other family members with whom the respondents live, or by phone/Internet with loved ones who live outside their current place of isolation; (2) online entertainment (network games, movies, documentaries) or TV (movies, entertainment programs, sports); (3) reading,

(4) sports activities; (5) other – gardening, board games with other family members in isolation, TV news programs and educational programs (either TV or online); (6) keeping a diary, or planning daily activities for efficient time management; (7) personal development (online courses, meditation, yoga); (8) development of any professional skills that may offer an advantage on the labor market in the post-pandemic period (online language courses, computer programming workshops, overspecializations in one's field of activity, etc.).

It should be noted that the frequency of excessive smoking has increased after more than two weeks of isolation or quarantine, and this can be a dysfunctional coping method to deal with the isolation stress. Adequate measures of psychoeducation could be useful at the populational level for the prevention of excessive smoking, as well as dissemination of recommendations for adequate time management so that smoking can be replaced by relaxation methods free of harmful effects. Other dysfunctional coping methods were reported during self-isolation, namely, excessive online shopping, which was later regretted by the people concerned; compulsive eating, especially of sweets or other high-calorie foods; self-medication for sleep disorders; Internet abuse. In this regard, it should be noted that lockdown is a period during which behavioral addictions can develop unhindered if they are not actively fighting against - for example, the diet should be balanced to avoid the abusive use of sweets, between meals high-calorie foods are prohibited, prolonged sitting in front of the computer or TV should be considered harmful by people in isolation, and it is recommended to introduce active relaxation breaks between sessions of Internet or TV use; abusive online shopping or online gambling must be perceived as dangers and campaigned against; sleep medication should be taken only on prescriptions from a physician, strictly for the recommended duration.

The use of social networks and phone callings were used by respondents in relatively equal proportions to keep in touch with those close to them. The Internet was preferred as a source of information over television, but this is most likely the result of how respondents were selected (online platforms); the time spent daily informing about the Covid-19 pandemic has been reduced (over 70% estimated this time to be one hour), which indicates a realistic approach and avoidance of intoxication with data about the pandemic.

The main recommendations offered by the respondents for other people in isolation or quarantine were: (1) sports and relaxation activities (Pilates, yoga, gymnastics); (2) hobby activities (cooking, gardening, tapestry, drawing, dancing); (3) communication with loved ones; (4) caring for loved ones; (5) information only from official sources; (6) reframing of the current situation („you may consider that it is a holiday, a period of self-evaluation, of rediscovering your partner, of making plans for the future"); (7) developing new professional skills that may be useful in the future; (8) religious activities performed at home.

Contraindications that respondents considered important during self-isolation: (1) prolonged exposure to news programs; (2) self-blame and panic (it must be taken into account that the situation is limited in time anyway); (3) avoid excessive eating, smoking, and alcohol abuse; (4) avoid monotony by diversifying the daily program; (5) avoid unnecessarily leaving of one's own house; (6) avoiding lying in bed during the entire day; (7) avoid excessive consumption of sweets, online gambling and excessive use of the Internet.

Most of the people stated that there were no changes in communication with their loved ones who lived in the same house. However, from the category of those who felt such changes, most of them showed that they managed to enjoy the time spent together, using this period constructively and finding common interests and activities with their partner/children/other relatives in the house. The appearance of nervousness installed on the background of isolation-induced monotony was also reported, which leads to the need of implementing coping strategies based on the detection of common interests, on the practice of relaxation exercises, etc; ignoring one's partner or spouse was also signaled as a dysfunctional coping strategy, and in this case, we consider as optimal strategies the resignification of the situation, finding hobbies that several people from the same house may share, establish common plans for the future.

Communication with those outside the home was marked by (1) the feeling of lack of privacy which is commonly associated with physical, direct communication; (2) concerns about how the economic situation (technical unemployment, working from home with declining income, or even lack financial support) and social situation (separation from the group of colleagues, neighbors, friends) will affect oneself and his/her family; (3) emotional balance (acceptance of the current situation and associated changes of the communication paradigm); (4) capitalizing on current resources (using the time constructively, communication targeting the reconnection with old friends and relatives with whom they have not spoken for a long time, etc).

The respondents' main concerns were (1) worries related to the health of people close to them and their health; (2) financial security; (3) professional or academic future. Therefore, the officials should communicate more with the population about these issues and offer them adequate information about the pandemic-imposed crisis changes in the economy and academic activities.

The level of optimism regarding the resolution of the situation within a maximum of one month was reduced to 43% of the respondents, which shows a high level of awareness of the importance of the epidemiological situation.

Parents who live with their children at home have found as a means of organizing the children's time using board games within the home, the continuation of the school training program from home, then using the Internet for entertainment, online communication with colleagues and teachers, physical exercises and watching TV. The communication with the children regarding the Covid-19 pandemic data was good, in the sense that it was based on respecting the facts, with the concepts being formulated inappropriate terms.

The respondents did not expect self-isolation to change their long-term lifestyle, or even had favorable expectations, respectively to come out stronger from this experience, which shows a high level of confidence in their resources to cope and an effort to integrate this experience into the continuum of their life.

Psychopathological manifestations have been reported during self-isolation, in particular insomnia, nervousness/ irritability, anxiety/panic, unmotivated fatigue, depressed mood, appetite changes, concentration problems, and many people have shown symptomatic clusters, not just isolated symptoms. These elements had a recent onset and they can be largely attributed to the stress-induced by isolation or quarantine, given that only 3% of the survey respondents admitted to pre-existing mental disorders.

There was reported a reduced addressability to psychological or psychiatric services, while the use of one's methods of coping with symptoms increased in time (self-medication for sleep, coffee for drowsiness, alcohol for anxiety). Therefore, less than 4% of the respondents used the help of a mental health specialist during their isolation/ quarantine. These phenomena show not only that there is a risk of developing mental disorders in this period, especially anxiety and depressive disorders, which are to be expected in this period of isolation/quarantine, but also the risk that these disorders worsen rapidly in the absence of mental health-focused interventions. In this context, the possibility of accessing online medical platforms and psychological helplines dedicated to people going through difficult times should be disseminated into the population as an important means to prevent the worsening of stress-related disorders. Pharmacological treatment and psychotherapy should be used for anxiety, stress-related, and depressive-spectrum disorders, whenever needed [12]. Vulnerable populations to the isolation or quarantine-associated stress, represented by elderly patients, people with a psychiatric history or physical impairments should benefit from special attention from the mental health specialists, and special programs designed for the awareness of anxiety and depressive disorders should reach them through the media, general practitioners, or any official vector of information possible [13]. Depression is an important factor for lowering the quality of life and harms life expectancy so that it should be treated actively whenever detected [14, 15].

Disclaimer

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ORIGINAL ARTICLES

Burnout syndrome in the Emergency Department of the Central Military Emergency Hospital before and during the COVID-19 pandemic

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Abstract: *Introduction: Public interest in burnout has grown recently in developed countries, as has media coverage. Burnout has been a topic of scientific research in recent years for psychologists and sociologists alike. They have published numerous articles on the identification and classification of burnout syndrome.*

The medical staff is quite exposed to burnout because they often experience strong emotions such as the desire to treat or save their patients, fear of failure, occasional failures in the treatment of diseases.

Materials and methods: This study aims to highlight the impact of the burnout syndrome in the Emergency Department of the Central Military Emergency Hospital during the COVID-19 pandemic, by conducting a comparative study based on the results of a questionnaire applied to the medical staff before and during the COVID-19 pandemic.

Results: The study interviewed 65 participants aged between 20 and 60 years. They had to anonymously answer 16 questions marked with scores from 1 to 4.

Comparing the results highlighted by the questionnaire, applied before the COVID-19 period, with those obtained in the questionnaire applied between May and June 2020, we noticed an increase in the level of burnout in all categories interviewed during the pandemic.

Conclusions: In conclusion, the high level of contagiousness and the lack of a vaccine or treatment against SARS-CoV2 infection are additional concerns for burnout syndrome among healthcare professionals.

Keywords: *COVID-19, burnout syndrome, healthcare professionals*

INTRODUCTION

What is burnout?

The term "burnout" appeared 25 years ago in the United States. One of the first scientific descriptions of burnout syndrome was made by the psychoanalyst Freudenberger who described it as manifesting polymorphic symptomatology that fluctuates in degree from person to person [1]. In 1981, Maslach introduced a far-reaching definition and psychometric tool for assessing burnout syndrome, which is still the most commonly, used today, The Maslach Burnout Inventory [2].

An alternative to assessing the burnout syndrome has become "The Oldenburg Burnout Inventory: A Good Alternative to Measure Burnout (and Engagement),"

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published by Prof. Dr. Evangelia Demerouti in 2007 at the Utrecht University in the Netherlands. She is well-known for her research in the field of occupational burnout and introduced this method to quantify the burnout syndrome based on two proportions: exhaustion and disengagement. Her annotations in the 2008 article define exhaustion as being a repercussion of intense and prolonged physical, affective, and cognitive pressure such as the long-term effects of protracted tedious job demands. On the other hand, she noted that disengagement can be identified as a breakaway, chiefly from work, the activity, or the object of the said workplace, for example, becoming uninteresting, challenging, or even disgusting [3].

Burnout has been a topic of scientific research in recent years for psychologists and sociologists alike. They have published numerous articles on the identification and classification of burnout syndrome, but the major problem that remains is that there is no generally accepted definition of burnout. Identifying potential causal factors and separating them from other health disorders is difficult, which represents the main reason why it is difficult to identify a generally valid definition. Therefore, an interdisciplinary approach would probably help a broader understanding of burnout syndrome, which has become a common pathology in modern occupational medicine (Figure 1).

Although hard to systemize and cut down to a single phrase definition, studying the medical literature, we found it is widely accepted that burnout is a syndrome defined by emotional debilitation, a loss of the sense of self, and personal accomplishment. Symptoms that may be identified in medical staff are anxiety, impatience, mood swings, and depression. Moreover, physical health may be impaired, with manifestations such as disseminated aches and pains, digestive problems, and increased cardiovascular risk. All in all, these symptoms are not specific enough to guide a person in seeking help [4].

Burnout syndrome was found more widely in careers that involve personal contacts with other people, specifically those that imply a high level of demand and pressure on the worker. Even though the medical staff represents the majority of those affected by burnout, it is important to note that occupational groups such as advocates, teachers, or human resources have been reported to also be prone to this syndrome [5].

Specific psychiatric literature has not yet classified burnout, for example in the "Diagnostic and Statistical Mental Disorder, 5th edition (DSM-5) [6]. In some European countries, such as Sweden, symptoms of burnout represent a justification for sick leave. Proof of the developing awareness of this syndrome is its inclusion as health altering and contact with medical services in the 10th revision of the "International Classification of Diseases" [7] (ICD-10) where burnout is coded Z73.0 and characterized as a state of vital exhaustion [8].

Figure 1: The burnout syndrome evolution ("Burnout Waterfall") [9]

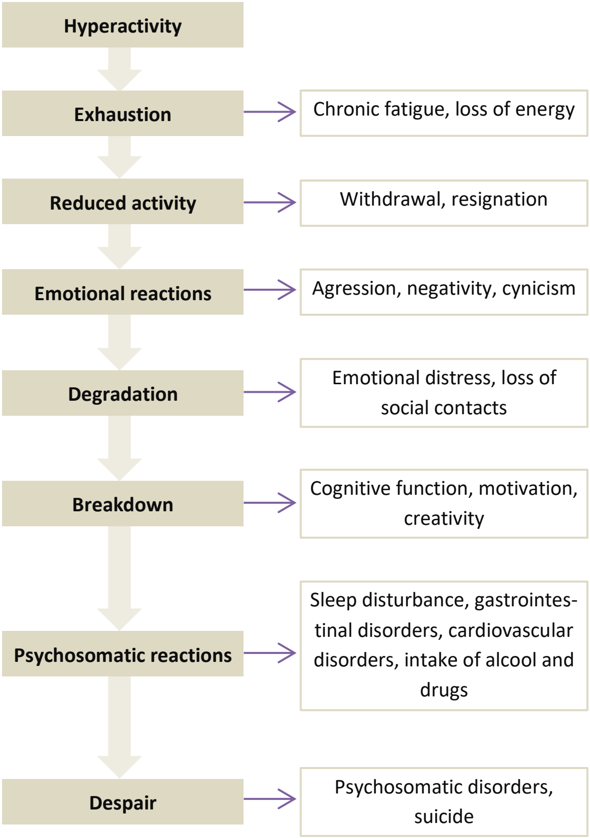
Hyperactivity
Exhaustion
Chronic fatigue, loss of energy
Reduced activity
Withdrawal, resignation
Emotional reactions
Agression, negativity, cynicism
Degradation
Emotional distress, loss of social contacts
Breakdown
Cognitive function, motivation, creativity
Psychosomatic reactions
Sleep disturbance, gastrointestinal disorders, cardiovascular disorders, intake of alcool and drugs
Despair
Psychosomatic disorders, suicide

Burnout on medical staff

In the last few years, there has been an increasing emphasis on the impact of professional activity regarding the health of medical staff, with numerous studies showing that medical staff is more affected by burnout syndrome than staff in other fields.

Healthcare workers have always had an active, routine life and have assumed from the beginning of their careers that they will have to make personal sacrifices for the good of patients. Besides, the daily decisions on which patients' chances of healing or their chance at life depend on them.

The factors that manifest a strong emotional impact on the medical staff have recently multiplied: there has been a risk of malpractice, the swift evolution of technology that requires continuous professional training (in detriment of free time), increased administrative burdens correlated with reduced consultation time for each patient.

Table 1: Factors that may influence a doctor's well-being [11]

Categories of factors	Examples
Chronic fatigue	Excessive workload Sleep deprivation Constant access demands (eg. Electronic availability) Decresed personal time
Perceived threats	Malpractice lawsuits Medical error Reduce compensation Research funding climate
Loss of autonomy	Practice environment Time to interact with patient constrained
Inefficiencies	Administrative requirements Lack of support staff Practice organization
Balancing needs	Suboptimal integration of work and life responsibilities Clinical service requirements and additional demands (teaching or administration)
Chronic stress	Work pace Practice setting
New technologies	Electronic medical health records Keeping up with technological advances in the practice
Physician factors	Perfectionism Internal drive and ambition Negligence regarding personal health and well being

All the aforementioned factors create an environment in which the practitioner is exposed to multiple high-stakes decisions, rendering the situation into a physically, psychologically, and emotionally stressful experience. As a consequence, medical professionals may show signs of emotional distress and avoidance behavior. Furthermore, long-term absence may be seen, with an alarming level of skill drain, leading to economical strain. The quality of medical services may also be affected [10].

All these factors have led to an increase in the number of medical staff exposed to burnout syndrome (Table 1).

The global effects of COVID-19

Coronavirus is a family of viruses that can cause diseases such as respiratory viruses, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS). In the winter of 2019, a new member of this family, SARS-CoV-2, was identified as originating in China, more precisely the source of the outbreak was the municipality of Wuhan. The disease caused by this virus is called COVID-19. In January of 2020 isolated cases were reported in some European Union states. By the end of February 2020, Italy reported an important increase in COVID-19 cases. [12] Following the accelerated evolution and spread of SARS-CoV2, the World Health Organization (WHO) declared COVID-19 as a global pandemic.

The rapid spread of SARS-CoV-2 globally had a strong psycho-emotional impact among the population, developing an increased level of stress and anxiety. The most affected by the psychological effects were the vulnerable groups directly involved in this infection, the elderly, the people from the placement centers, the staff in the front line. At the same time, this increase in the level of stress and anxiety was accentuated by the strict measures applied to prevent and combat the disease (quarantine, isolation, change of daily routine). Adding up to the ambiguity experienced by the population was a storm of catastrophic and sometimes sensational bits of information that was spread through various forms of media consumption platforms, rendering the citizens uncertain and helpless while sustaining a sense of distrust towards official information [13]. All these factors have led to an increase in the number of people affected by depression, an increase in alcohol and drug use, and an increase in the number of suicides, while in the long run post-traumatic stress disorder (PTSD) is anticipated (Figure 2) [11, 14].

Effects of COVID-19 on medical staff

The COVID-19 pandemic generated a major health crisis globally, which led to the reorganization of health services, with an emphasis on emergency services, intensive care units, and infectious disease and epidemiology departments.

At the national level, several support hospitals have been declared, for example in Bucharest, support hospitals – phase II – such units are the Central Military Emergency University Hospital-ROL2-COVID-19 Military Camp Hospital, Marius Nasta Institute of Pneumophtisiology.

Medical personnel is constantly exposed to the risk of becoming infected with viruses, bacteria, or parasites. This risk is accentuated during a global pandemic, thus increasing the predisposition for the development of burnout syndrome in the case of the health personnel. The fast pace of work, special conditions (requiring special protective equipment), the multitude of uncertainties since the beginning of the pandemic regarding the mode of transmission, the treatments, and prevention methods have created an additional level of stress and anxiety for the medical staff.

Figure 2: The psychological and emotional impact of COVID-19 [15]

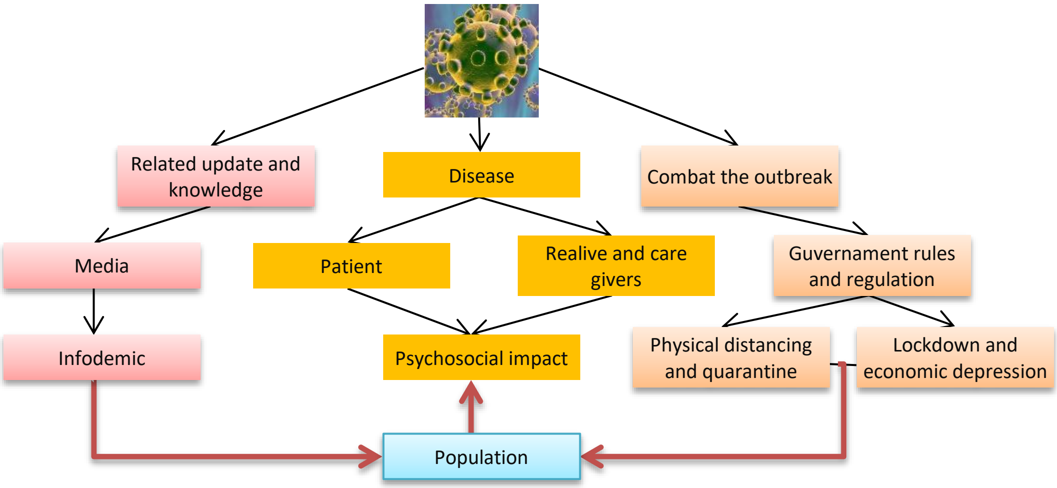
Related update and knowledge
Disease
Combat the outbreak
Media
Patient
Realive and care givers
Guvernament rules and regulation
Infodemic
Psychosocial impact
Physical distancing and quarantine
Lockdown and economic depression
Population

Medical teams in the emergency department were also at increased risk of contamination because at the time a patient was presented to the emergency department; health care staff could not tell if he or she was infected with COVID-19 until the PCR test was performed. This situation together with spending an increasing number of hours in the hospital to provide the best care to patients has led to a strong social and psychological impact on the medical staff.

Thus, many of those working in the health system during this period experienced conditions such as anxiety, fear, social marginalization, depression, and post-traumatic stress. Therefore, if we take into account the definition of burnout which involves long-term exposure to stress and exhaustion at the workplace, we can explain the increase in the intensity and impact of burnout syndrome during this period of medical staff.

OBJECTIVES

Healthcare workers are extremely vulnerable to stress and exhaustion due to the characteristics of the work performed. Numerous studies conducted so far highlight the presence of psychosocial impairment of health professionals in many countries, thus becoming a global problem. Furthermore, they may be exposed to additional factors in the work environment: that is physical, biological, chemical factors. The COVID-19 pandemic is an additional element that has amplified the risk of burnout syndrome among medical staff.

This study aims to highlight the impact of the burnout syndrome in the Emergency Department of the "Dr. Carol Davila" University Central Military Emergency Hospital during the COVID-19 pandemic, by conducting a comparative study based on the results of a questionnaire applied to medical staff before and during the COVID-19 pandemic.

MATERIALS AND METHODS

While writing the current study we have used the “Oldenburg burnout inventory scale” and the statistical data which have been obtained through a survey carried on the medical staff within the ER unit of the Central Military Emergency University Hospital "Dr. Carol Davila” in the May-June 2020 timespan.

The psychometric tool used in this situation – to assess the syndrome’s impact on the ER medical staff – has consisted of the “Oldenburg burnout inventory”. This involves a set of 16 queries (8 with a direct answer, the other 8 including indirect answers) which aim at measuring the burnout and professional involvement levels.

The clinical method was based on the application of a questionnaire to the medical staff from the Emergency Unit of the Central Military Emergency University Hospital „Dr. Carol Davila ”between May and June 2020.

The analytical method was used to interpret the results thus obtained. The data thus obtained were analyzed and shared with those resulting from the application of the same questionnaire in the period before the COVID-19 pandemic. For the statistic, we used the EpiInfo program and Excel.

RESULTS

The study interviewed 65 participants aged between 20 and 60 years. They had an anonymous answer to 16 questions marked with scores from 1 to 4 (1- Totally agree, 2- Agree, 3- Disagree, 4- Total disagree) (Table 2).

Table 2: Oldenburg Burnout Inventory in ER unit of the Central Military Emergency

	Questions	**Totally agree**	**Agree**	**Disagree**	**Totally disagree**
1	I always find new and interesting aspects in my work.				
2	There are days when I feel tired before I get to work.				
3	It happens more and more often that I speak badly to people.				
4	After the program, I need more time than before to relax.				
5	I can easily cope with the pressure at work.				
6	Lately, I tend to think less about work and do things more automatically.				
7	I find the service a positive challenge.				
8	During work, I often feel powerless.				
9	Over time, I may become disinterested in what I am currently working on.				
10	After work, I have enough energy left for my favorite leisure activities.				
11	Sometimes I feel disgusted with my tasks at work.				
12	After the program, I usually feel worn out and tired.				
13	I wouldn't see myself working on anything other than what I'm doing now.				
14	Usually, I can dose my work well.				
15	I feel more and more captivated by my work.				
16	When I work, I usually feel full of energy.				

In the questionnaire applied before the COVID-19 pandemic, 65 participants aged between 21 and 60 years were interviewed, of which 25 women and 40 men, distributed on the following personnel categories: 12 doctors, 31 nurses, 5 people belonging to other categories, 6 ambulances, and 8 stretchers.

At the same time, in the questionnaire applied between May and June 2020, 66 medical staff aged between 21-60 years participated, of which 32 women and 34 men, distributed as follows: 22 doctors, 19 nurses, 11 stretchers, 3 ambulances, and 5 people belonging to other categories.

We observe that in both questionnaires the doctors obtained the highest average scores of the burnout level, both in terms of the component of emotional exhaustion and the component of professional involvement. At the same time, we observe an increase in the level of burnout during the COVID-19 pandemic (in the study conducted before the pandemic, doctors obtained average scores of 40.66 out of 64 possible), while following the questionnaire applied during the pandemic the average scores were 43 of 64) (Figure 1).

Figure 1: Results obtained broken down by personnel categories

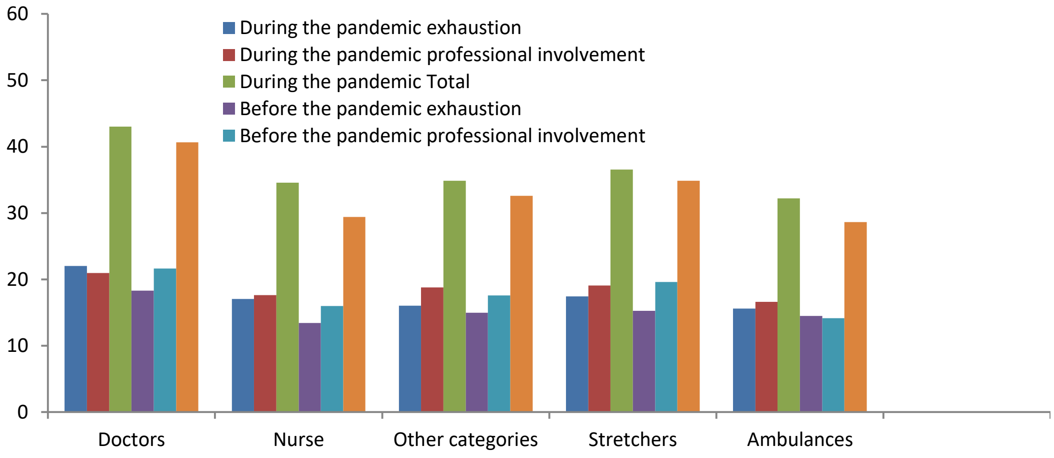

Also, we notice the biggest difference between the two periods among the nurses, before the pandemic the scores obtained were on average 29.43, unlike the current ones, which had an average of 34.57, the difference is due mainly to the marked increase of the exhaustion component. At the opposite pole, with the lowest level of burnout remain the outpatients, although comparing the results obtained in the two questionnaires, we notice among them a sharp increase in burnout.

If we talk about age, we observe the increase of the burnout level concerning the advancing age (Figure 2). This can be explained by the accumulation of fatigue due to the extended work schedule and the accumulation of problems at work with those in private life.

Figure 2: Results according to age

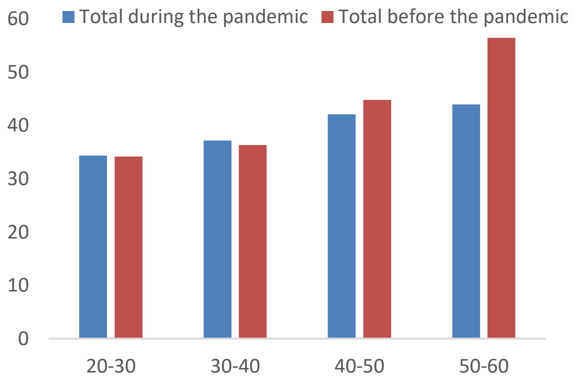

A perhaps surprising aspect was the fact that there were no notable differences in the degree of burnout between the men and women interviewed in any of the questionnaires. In the first questionnaire, the average values varied around 32 points, while in the second questionnaire the average values were 36.5 points (Figure 3).

Figure 3: Results according to sex

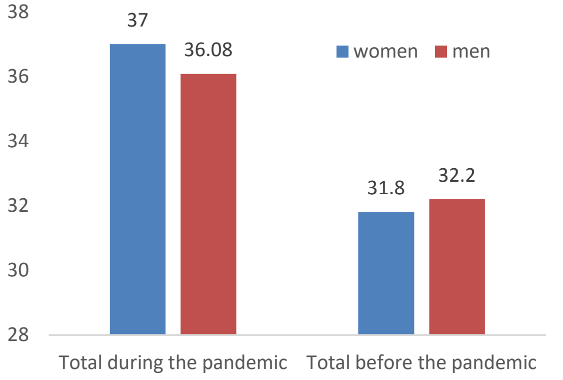

CONCLUSIONS

In recent years, interest in burnout has increased, being a syndrome more and more common in developing countries and with an increased impact in both personal and professional life.

Healthcare personnel represents one of the most exposed professional categories to this syndrome, due to overtime, desire to treat all patients, fear of failure, inability to suffer from certain pathologies, and exposure to additional factors in the workplace: physical, biological, chemical factors.

The COVID-19 pandemic was an additional stress factor for medical staff, on the one hand, due to the numerous unknowns related to treatment, prevention, disease evolution and on the other hand, due to the intensive work regime in special conditions, being forced to comply to a set of additional rules and the period in which they had to be separated from their loved ones to protect them.

According to the study conducted between May and June 2020, there is an increased level of burnout of health care personnel, the highest score being obtained by doctors.

Comparing the results highlighted by the questionnaire applied before the COVID-19 period with those obtained in the questionnaire applied between May and June 2020, we notice an increase in the level of burnout in all categories interviewed during the pandemic.

There is also a gradual increase in the level of burnout proportional to aging, but slower than before the pandemic.

The percentage of burnout reported by sex groups is close, with no significant differences between men and women. But even in this case, there is an increase in the incidence of burnout in the last 4 months.

Therefore, burnout syndrome remains a common problem in the health field, especially affecting doctors, probably because they represent a complex interface with patients, relatives, the rest of the ER staff, doctors from other specialties; have the responsibility of the performed medical act; working hours over time due to the need for continuous training.

To decrease the incidence of this syndrome, but also its effects, it is important to apply various means of prevention such as identifying and combating the triggering factors, observing and early intervention on the disease, and treating the effects produced by it.

On this basis, we conclude that stress, exhaustion and distress lead to the development of the burnout syndrome, which, in this period is conspicuously increased by the

COVID-19 pandemic, essentially breaking what should be the first and most important rule of any medical practitioner: the safety of the rescuer.

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ORIGINAL ARTICLES

Chest CT-scan findings in COVID-19 patients: the relationship between the duration of symptoms and correlation with the oxygen saturation level

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Abstract: *Purpose: This study is carried out to evaluate the diagnostic value of using common features of computed tomography (CT) imaging in COVID-19 disease, and to assess the relationship between blood oxygen saturation level and severity of CT findings.*

Materials and Methods: In this retrospective study, the chest CT of 173 test-confirmed COVID-19 patients have been evaluated to determine the patterns of involvement in multiple phases of illness. Then, the correlation between the severity of lung involvement and oxygen saturation levels has been assessed.

Results: The chest CT results show that 87.6% of patients had GGO, which was the most common pattern in our findings. 83.8% of patients had bilateral lung involvement with the dominant multifocal and peripheral distribution. peribronchovascular involvement was also a common finding in our study (47.2 %). we found predominantly peribronchovascular view in 3 patients (1.7%), pleural effusion in 4 patients (2.3%), lymphadenopathy in 10 patients (5.8%), the tree in the bud in one patient (0.6%), and nodules in 4 patients (2.3%). We also found that GGO is the most common pattern during the early phase of the disease (97.4% of early phase cases). However, in the intermediate and late phases, consolidation and crazy paving patterns are more common. Moreover, our findings indicate that there is a significant relationship between oxygen saturation level and Total Severity Score, with the exclusion of the young adult patients (20-40 years).

Conclusion: Relying on chest CT-scan findings apart from the oxygen saturation level is sufficient for the diagnosis and management of patients with COVID-19 pneumonia.

Keywords: *COVID-19, pneumonia, spiral CT, diagnosis, treatment, Real-Time RTPCR*

INTRODUCTION

Coronaviruses as part of the coronaviridae family are nonsegmented, enveloped, positive-sense, and single-strand ribonucleic acid viruses [1]. This is the seventh known coronavirus to infect humans [2]. Two other notable examples of this coronaviridae family include severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). SARS began spreading in southern China

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and resulted in 774 deaths out of 8,098 infected individuals in 29 countries from November 2002 through July 2003 while MERS originated in Saudi Arabia and caused 848 deaths among 2,458 individuals in 27 countries through July 2019 [3, 4].

The number of confirmed COVID-19 cases continues to grow rapidly in the world. The recent outbreak of the novel coronavirus (COVID-19) began in December 2019 in Wuhan, the capital of central China's Hubei province, and has led to a major pandemic [2, 5]. Widespread human-to-human transmission has resulted in 5,240,900 cases worldwide with 338,700 deaths as of May 23, 2020 [6].

Patients infected with this novel COVID-19 virus manifested with symptoms of severe pneumonia, including fever, fatigue, dry cough, and acute respiratory distress. 2019-nCoV caused clusters of fatal pneumonia with clinical presentation greatly resembling SARS-CoV. Patients infected with 2019-nCoV might develop acute respiratory distress syndrome, have a high likelihood of admission to intensive care, and might die. The cytokine storm could be associated with disease severity. More efforts should be made to know the whole spectrum and pathophysiology of the new disease [7].

According to available guidelines, the diagnosis of COVID-19 should be confirmed by reverse-transcription polymerase chain reaction (RT-PCR) or gene sequencing of respiratory or blood specimens. However, the RT-PCR has shown detection rates as low as 30% to 60% and also false-negative results at the initial presentation while this technique requires specific sample collection protocol, preparation, and particular facilities that have prevented accurate and fast results being available for further diagnosis [8]. These limitations in diagnosis and treatment subsequently result in a higher rate of infection demanding an effective strategy for the early diagnosis of COVID-19.

Radiological imaging is known as a critical assessment technique for the evaluation of severity and disease progression in upper and lower bronchial disorders such as COVID-19 infection. Among variable imaging techniques, Computed Tomography (CT) scans have shown promising potential in COVID-19 case ascertainment [9, 10]. Chest spiral CT is a crucial diagnosis test for COVID-19 disease, which is used to assess the severity of lung involvement in COVID-19 pneumonia. [10, 11]. A variety of imaging patterns in chest CT-Scan have been described in other studies such as bilateral lung opacities in infected patients and described lobular and subsegmental areas of consolidation as the most typical findings. Other investigators examined chest CTs in 21 infected patients and found high rates of ground-glass opacities and consolidation, sometimes with a rounded morphology and peripheral lung distribution [12, 13]. CT abnormalities might predate rtRT-PCR positivity in symptomatic and asymptomatic patients who were subsequently tested positive according to their rtRT-PCR results [14, 15, 16].

In this study, we characterize chest CT findings in 173 patients infected with COVID-19 and conformed with rt-RT-PCR in relationship to the time between symptom onset and the initial CT scan. Also, we evaluate the correlation between O_2 saturation and the severity of the lung involvement in chest CT scans.

MATERIAL AND METHODS

In this retrospective study from March 4, 2020, until March 10, 2020, 173 adult patients admitted to Baqyatollah hospital in Tehran province in Iran with confirmed COVID-19 infection and undergone chest CT scans were enrolled in our study. There have been no exclusion criteria considered in this research.

Confirmation of the disease was performed through laboratory testing for COVID-19 with real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) of respiratory secretions obtained by bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal swab, or oropharyngeal swab which were positive for all patients. The rRT-PCR test kit used in this study was provided by SinaPure TM viral (Tehran-Iran).

All patients were imaged with a 3.5-mm slice thickness CT on a Siemens Emotion 16 scanner (Siemens Healthineers; Erlangen, Germany). All scans were performed without intravenous contrast injections in the supine position during end inspiration.

In addition to the age [17] and gender parameters, other information such as the number of days between the onset of the symptoms and date of first chest CT-scan were recorded for further analysis. This disease changed rapidly at the early stages [18], so in this study, if the time interval between the first clinical symptom and CT was two days or less, the patient was considered to have been imaged in the "early" phase of the illness. If the time interval was between three and five days, the patient was considered to have been imaged in the "intermediate" phase of the illness. If the time interval was between six and 12 days, the patient was considered to have been imaged in the "late" phase of the illness.

Oxygen saturation of patients was also measured in the pressure of the air room once they were admitted to the hospital.

All CT images were reviewed by two trained and experienced radiologists with 10 years of experience. Imaging was reviewed independently and reported after the final decisions were made in consensus. Negative control cases were also examined.

For each patient, the chest CT scan was evaluated for the following characteristics:

(1) Presence of ground-glass opacities,
(2) Presence of consolidation,
(3) Laterality of ground-glass opacities and consolidation (unilateral or bilateral),
(4) Number of lobes affected where either ground-glass or consolidative opacities were present,
(5) Degree of involvement of each lung lobe in addition to the overall extent of lung involvement measured through a "total severity score",
(6) Presence of nodules,
(7) Presence of a pleural effusion,
(8) Presence of thoracic lymphadenopathy (defined as lymph node size of ≥10 mm in short-axis dimension),
(9) Distribution of the disease (categorized as a unifocal disease, multifocal disease, peribronchovascular disease, peribronchovascular predominant disease, or peripheral disease),
(10) Other abnormalities including opacities with a crazy-paving pattern, opacities with intralesional cavitation, the tree in bud appearance, and vascular enlargement were noted.

The ground-glass opacification was defined as hazy increased lung attenuation with preservation of bronchial and vascular margins, whereas consolidation was defined as opacification with the obscuration of margins of vessels and airway walls [19].

Regarding the total severity score, each of the five lung lobes was assessed for degree of involvement and classified as none (0%), minimal (1 - 25%), mild (26 - 50%), moderate (51 - 75%), or severe (76 - 100%). No involvement corresponded to a lobe score of 0, minimal to a lobe score of 1, mild to a lobe score of 2, moderate to a lobe score of 3, and severe to a lobe score of 4. An overall lung total severity score was reached by the sum of the five lobe scores (range of possible scores, 0 - 20). Then the correlation between "Total Severity Score "and O_2 saturation was calculated.

RESULTS

This study was conducted based on a CT-scan data set consists of 110 male and 63 female patients with an average age of 63± 3.94 years and the age range between 6 to 86 (Table 1).

Table 1: Patient characteristics

Gender	All patients N= 173	Early* (0-2 days) N= 39	Intermediate (3- 5 days) N=113	Late (6-12 days) N=21
Men	110 (63.5)	24 (61.5)	71 (62.8)	15 (71.4)
Women	63 (36.5)	15 (38.5)	42 (37.2)	5 (28.6)
Age (years)				
Mean	53.6	47.28	53.96	58.95
Range	7 - 86	21 – 85	7 – 86	36 – 85
SD	3.94	15.29	13.55	11.28
O_2 saturation				
Mean	89.44	93.05	89.27	83.67
Range	67 – 98	89 – 98	74 – 97	67 – 94
SD	4.76	2.82	3.58	6.88

**Early, intermediate, and late refer to time from symptom onset to time of the chest CT scan;*
SD – standard deviation.
Note: Numbers in parentheses are percentages.

According to the results, one-hundred and forty-four patients have shown multifocal bilateral lung involvement. Among these patients, 78 patients (54.2%) had only GGO (without consolidation), 14 patients (9.7%) had the only consolidation (without GGO) and 51 patients (35.4%) had both of them.

Figure 1: An axial CT image obtained without intravenous contrast in an 85-year-old male shows a "crazy-paving" pattern as manifested by bilateral ground-glass opacification with interlobular septal thickening with intralobular lines.

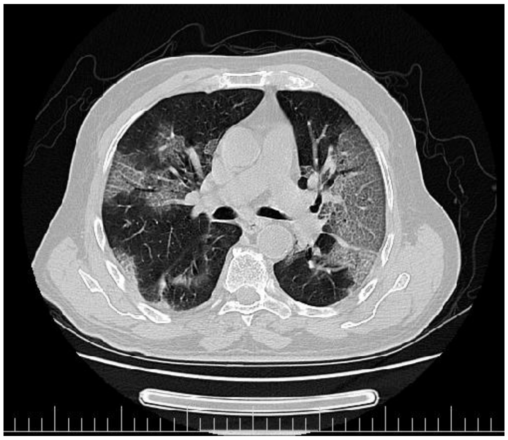

In this study, different types of lesions have been observed in the 173 patients with confirmed COVID-19 disease. These lesions are GGO (Figure 1, Figure 2, Figure 3), consolidation (Figure 3), crazy paving (Figure 1) and vascular enlargement (Figure 4), which are observed in 151 patients (87.6%), 77 patients (44.5%), 23 patients (13.3%) and 106 patients (61.3%), respectively.

Figure 2: An axial CT image obtained without intravenous contrast in a 52-year-old male shows bilateral peripheral and peribronchovascular ground-glass opacities with round morphology.

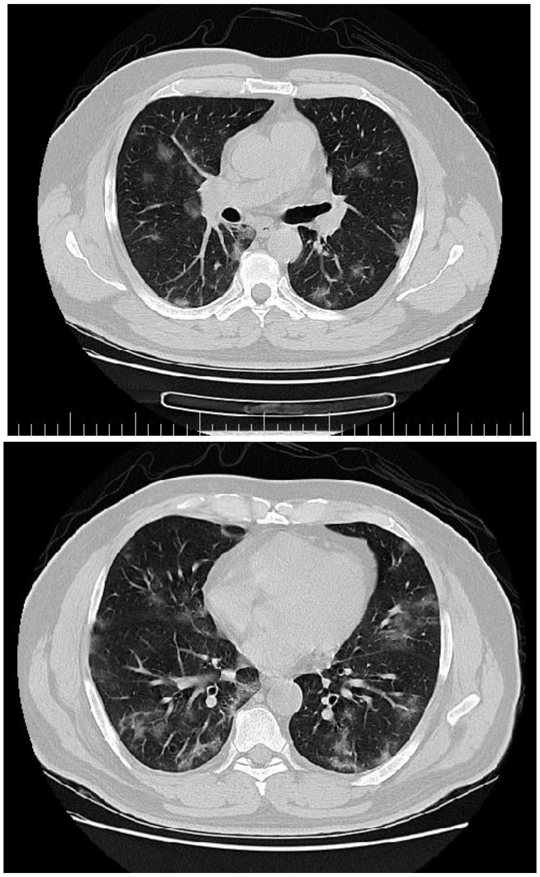

The number of affected lobes can vary in different patients. Among the 173 patients, 23 patients (13.3%) showed lung opacities in one lobe, 14 patients (8.1%) in two lobes, 13 patients (7.5%) in three lobes, 23 patients (13.3%) in four lobes, and 99 patients (57.2%) in all five lobes. Moreover, the right upper lobe of 133 (76.9%) patients, the right lower lobe of 151 patients (87.3%), the left upper lobe of 133 patients (76.9%), and the left lower lobe of 153 patients (88.4%) were involved.

145 patients out of 173 total patients (83.8%) had bilateral and 145 patients (16.2) had unilateral lung involvement as shown in Table 2. The average total severity score (ranged from 1 to 20) was 5.75±3.87. This study revealed that the relationship between the patient's age and the total severity score is statistically significant ($p<0.001$ and the correlation coefficient is 0.29).

Also, Table 2 shows the lesion distribution. According to this table, 21 patients (12.1%) had shown unifocal involvement, 153 patients (88.4%) had multifocal involvement, 170 patients (98.3%) had peripheral involvement and 82 patients (47.2%) had peribronchovascular involvement.

Figure 3: An axial CT image obtained without intravenous contrast in a 56-year-old male shows bilateral and peripheral ground-glass opacities with dominant consolidation.

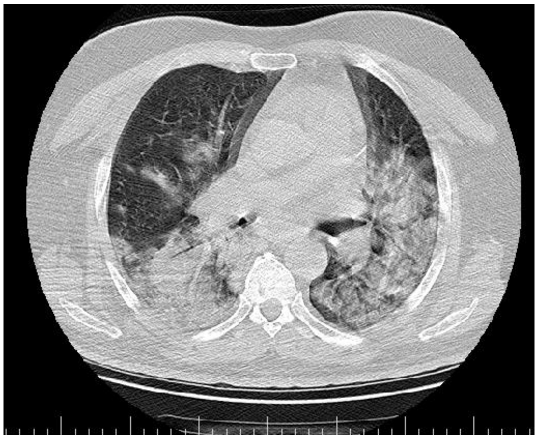

Figure 4: An axial CT image obtained without intravenous contrast in a 60-year-old female shows unilateral opacification with the tree in bud appearance which is a very rare presentation of COVID-19 pneumonia. Vascular enlargement is also noted which is a common finding.

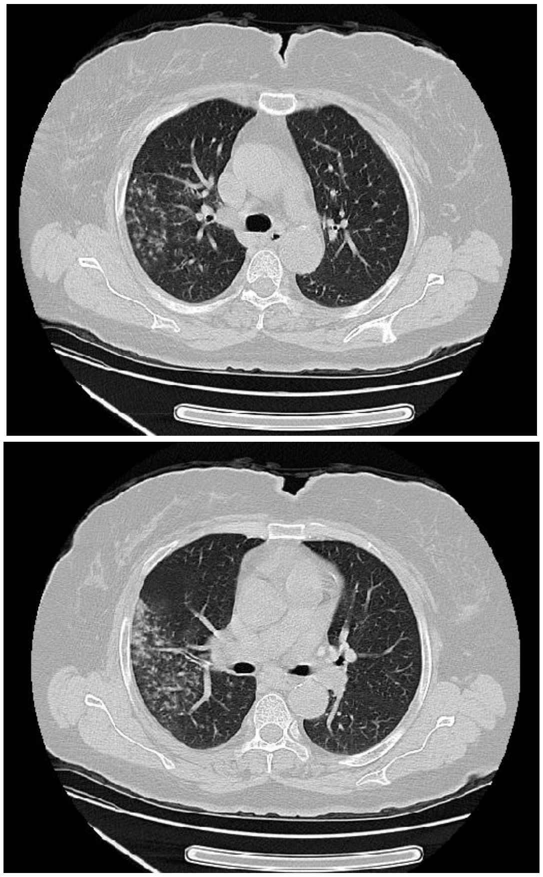

Table 2: Findings on chest CT in 173 patients

Ground-glass opacities and consolidation	
absence of both ground-glass opacities and consolidation	0 (0)
presence of either ground-glass opacities or consolidation	173 (100)
presence of ground-glass opacities without consolidation	96 (55.5)
presence of ground-glass opacities with consolidation	55 (31.8)
presence of consolidation without ground-glass opacities	22 (12.7)
Number of affected lobes	
0	0 (0)
1	23 (13.3)
2	14 (8.1)
3	13 (7.5)
4	23 (13.3)
5	99 (57.2)
more than 2 lobes affected	149 (86.6)
bilateral lung disease	145 (83.8)
Frequency of lobe involvement	
Right Upper Lobe	133 (76.9)
Right Middle Lobe	108 (62.4)
Right Lower Lobe	151 (87.3)
Left Upper Lobe	133 (76.9)
Left Lower Lobe	153 (88.4)

Note: Numbers in parentheses are percentages.

We also examined all patients to find any lesions unrelated to the COVID-19.

According to this examination, we found predominantly peribronchovascular view in 3 patients (1.7%), pleural effusion in 4 patients (2.3%), lymphadenopathy in 10 patients (5.8%), the tree in the bud in one patient (0.6%), and nodules in 4 patients (2.3%).

The cavity was not observed in any evaluated cases.

The results from the classification of patients based on the period between the early symptoms' observation and the CT-scan imaging showed that the number of ground-glass's opacity is significantly different within these three groups (p = 0.003), i.e., the GGO is observed much more in the early phase patient's group compared to the two other groups.

The GGO has been found in 97.4% of the early phase patient group, 87.6% of the intermediate phase patient group, and 66.7% of the late-phase patient group (Table 3, Figure 5).

The numbers of consolidation and crazy paving appearance are significantly different within these three groups (p <0.001). The number of consolidation and crazy paving appearance is increased by increasing the time elapsed from early symptom onset to CT-scan imaging.

Table 3: Findings on chest CT in patients categorized by infection time course

	Early (0-2 days)	**Intermediate (3- 5 days)**	**Late (6-12 days)**
ground-glass opacities	38 (97.4)	99 (87.6)	14 (66.7)
consolidation	3 (7.7)	60 (53.1)	14 (66.7)
lung opacities in 1 lobe	16 (41.0)	7 (6.3)	0 (0)
lung opacities in 2 lobes	8 (20.5)	6 (5.4)	0 (0)
lung opacities in 3 lobes	2 (5.1)	9 (8.0)	2 (9.5)
lung opacities in 4 lobes	7 (17.9)	16 (14.3)	0 (0)
lung opacities in 5 lobes	6 (15.4)	74 (66.1)	19 (90.5)
bilateral lung involvement	22 (56.4)	102 (90.3)	21 (100)
mean total severity score	2.59	5.87	10.95
"crazy-paving" pattern	1 (2.6)	12 (10.6)	10 (47.6)
peripheral distribution	37 (94.9)	112 (99.1)	21 (100)
pulmonary nodules	2 (5.1)	2 (1.8)	0 (0)
pleural effusion	0 (0)	3 (2.7)	1 (4.8)
lymphadenopathy	0 (0)	10 (8.8)	0 (0)

Note: Numbers in parentheses are percentages.

Figure 5: Frequency of selected chest CT findings as a function of time course from symptom onset

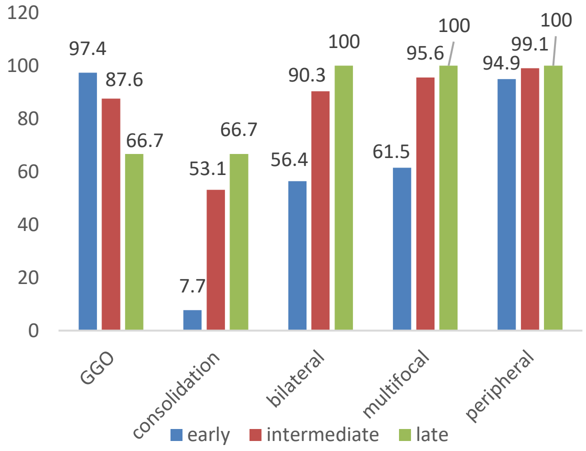

The observation within the early phase patient's group shows that consolidation and crazy paving appearance have been observed among 7.7% and 2.6% of the group, respectively. Besides, observation of the intermediate phase patient's group indicates that 53.1% and 10.6% of the patients showed consolidation and crazy paving appearance, respectively. Finally, in the late phase patient's group, 66.7% of consolidation, and 46.6% of crazy paving appearance have been observed (Table 3, Figure 5). Generally, there is a significant relevance between the time elapsed from early symptom onset to CT-scan imaging and the severity of lung involvement based on SST (p-value<0.001). Moreover, the severity of lung involvement is higher in the cases with the longest elapsed time from symptom onset to CT-scan imaging.

The level of oxygen (O_2) saturation has been measured for all patients before the CT-scan imaging. The average level of oxygen saturation was 89.01% (with a range of 67 to 97 and a standard deviation of 4.16). It is observed that there is a significant relevance between the oxygen saturation level and TSS ($p<0.001$ and correlation coefficient is -0.587); but there isn't any significant relevance between O_2 saturation and TSS within the age group of 20-40 years old.

DISCUSSIONS

COVID-19 has turned into a global challenge and a historic pandemic resulting in 2,019,088 cases worldwide with 131,886 deaths as of April 16, 2020 [6]. Although the rtRT-PCR testing of respiratory secretions or blood samples is the definitive diagnosis of COVID-19, the likelihood of false-negative cases has made chest CT-scan one of the important tests for relatively early diagnosis of COVID-19 pneumonia. Various imaging patterns have been reported in previous studies conducted on COVID-19 pneumonia, which varied in their importance.

In this study, we examined and classified the chest CT-scan imaging features of 173 patients with a definitive diagnosis of COVID-19 based on the rtRT-PCR test. The most common findings were ground-glass opacity (87.3%), vascular enlargement (61.3%), consolidation (44.5%) and crazy paving pattern (13.7 %). The majority of cases had bilateral (83.7%) and multifocal (88.4%) involvements. These findings are largely consistent with previous studies [12, 13], which indicates that the ground-glass view with or without pulmonary consolidation and in the forms of the bilateral peripheral and multifocal pattern are the diagnostic hallmarks of COVID-19 pneumonia.

Although the majority of patients had peripheral involvement, many of them had concomitant central or peribronchovascular involvement (47.7%). This distribution pattern was more common in the present study than in previous studies [13, 20], since the majority of our patients were in either the intermediate or the late-phase of the onset of the symptoms. It is worth noting that the public access to diagnostic-medical equipment, such as CT-scan, is lower in Iran than in the developed countries. Therefore, many infected individuals remained undetected in the early phases of the COVID-19 pandemic and thus had a late visit, which justifies more severe pulmonary involvement in the present study. Based on our findings, although the bilateral peripheral and multifocal involvements are the hallmarks of this disease, nevertheless, a portion of our patients had unilateral and even unifocal involvement, specifically in the early days of the onset of the symptoms. Besides, three patients and one patient have mildly and moderately pleural effusion, respectively. Previous studies highlighted that pleural effusion is detrimental to COVID-19 diagnosis. The present study confirms this finding since all of these four patients had congestive heart failure (CHF). Therefore, the authors recommend that in addition to examining CT-scan results for COVID-19, the underlying diseases of the patients should also be considered.

In total, 5.8% of the patients had mediastinal lymphadenopathy, which was higher than other studies [13, 21, 22]. It should be pointed out that lymphadenopathy can be seen in patients with heart failure, mediastinal infections, and/or cancer. In the present study, all 10 patients with lymphadenopathy had concomitant heart failure, based on their medical history and imaging results. Therefore, further studies with a more accurate design are required on a larger population for examining the underlying diseases to establish whether this type of involvement can be a marker of COVID-19.

In our study, the relationship between the imaging features and the elapsed time from early symptoms onset to CT-scan imaging shows that at the "early phase" of the disease the ground-glass has been the dominant imaging view, while by moving towards the intermediate and late phases of the disease, the intensity of the ground-glass opacity reduces, and instead the appearance of consolidation and crazy paving pattern increases. It is also observed that the consolidation view alone, without ground-glass opacities, is more common to have appeared at the late-phase of the disease. Moreover, pulmonary involvement increases with increasing the elapsed time. In total, these findings are consistent with the majority of previous studies [23, 24, 25].

Because it is very crucial to identify the O_2 saturation level at which a patient is needed to undergo a CT-scan test for measuring the severity of pulmonary involvement, we also considered the relationship between O_2 saturation level and the imaging's finding in our study. Our study showed a direct relationship between the severity of pulmonary involvement and decreased oxygen saturation, but this direct relationship was not observed among individuals aged between 20-40 years. This finding is important because it shows that younger patients with almost normal oxygen saturation can have severe pulmonary involvement. According to some studies in literature, patients with oxygen saturation < 93% should undergo imaging procedures [26, 27]. However, we argue this finding, because considering the recommend-dations provided in these works [26,27] may delay the proper treatment and makes the COVID-19 patients more vulnerable to the adverse events of the disease. Therefore, it is proposed that the oxygen saturation level alone is not an exclusive nor a decisive factor to decide on considering a chest CT-scan imaging, and yet patient's overall clinical conditions and all symptoms together should be considered more carefully to decide on offering chest CT-Scan and other diagnostic procedures.

CONCLUSIONS

This study aims to remind doctors involved in COVID-19 diagnosis and treatment around the world to start the treatments immediately and uninterruptedly for patients with chest CT-scan results similar to those highlighted in the present study and several other studies to prevent more losses. We believe that implementing these suggestive CT-scan patterns of COVID-19 patients, can accelerate the proper treatment and prevent more mortality and morbidity.

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Conflicts of Interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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ORIGINAL ARTICLES

Indian experience of tetanus – A study from south India

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Abstract: *Introduction: Tetanus is an old world disease where 2 centuries ago people had realized the link between wound leading to muscle spasm and fatality, Even today there are many cases of fatalities of tetanus being reported from different parts of the country even after viability of a tetanus toxoid and Immunoglobulin injections. This one of very few recent studies done in India on tetanus, as there is very little data available on tetanus so we are trying to share our experience on tetanus, so it will help the physicians to get a better understanding.*

Materials and Methods: This retrospective study has done collecting the patients' detail from 2017- 2019, detailed case sheet review was done and the patients' clinical presentation and the prognosis were noted in predesigned format. Inclusion criteria – all diagnosed cases of tetanus, exclusion criteria – patient already received treatment from the local hospital. The patient details were kept confidential during all times.

Results: The total of 58 cases – 35 males and 23 females, The average duration of hospital stay was 15 days. The most common occupation were farmers (barefoot workers). The site of injury was the foot in 65% cases followed by injuries to the fingers or the hand in 30% and 5% cases due to injury while tooth picking with a pin, splinter removal using pins. Clinical symptoms – trismus "lockjaw" (41), difficulty in walking (2), limb pain/stiffness (17), back muscle pain/stiffness (12), dysphagia (7), 72% autonomic dysfunction. Opisthotonus position and risus sardonicus developed after 7-8 days of infection. 20% of cases were vaccinated still developed diseases. 18% mortality was noted most cases were unvaccinated cases.

Conclusion: Tetanus is preventable diseases if TT vaccination and IMMUNOGLOBIN are administered on time. In all primary health care levels, the cold chain should be maintained for vaccines. The patients should be made sensitized about the consequence of the disease process.

Keywords: *tetanus, bacterial infection*

INTRODUCTION

Tetanus is an old world disease, where 2 centuries ago people had realized the link between wound leading to muscle spasm and fatality. It was a disease that the ancient physicians of Egypt and Greece dealt with often, however, now its prevalence in developed countries has decreased significantly due to improvements in wound care and hygienic practices [1]. Arthur Nicolire isolated tetanus toxin from the soil in 1884 [2]. Even today it's a major health problem in developing countries and developed countries

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(Anuradha 2006; Ogunrin 2009) [2, 3].

In 1884 the etiology was further understood. The first transmissibility was demonstrated by Antonio Carle and Giorgio Rattone who were pathologists in Turin They produced tetanus in rabbits by injecting pus from a person with fatal tetanus into their sciatic nerves [4].

Tetanus is a non-communicable, potentially fatal disease contracted by exposure to the spores of Clostridium tetani. It is a gram-positive, anaerobic spore-forming bacteria that produces an exotoxin called tetanospasmin which is responsible for the lethal effects of the disease. It is more commonly seen in developing countries as a result of low vaccination coverage, poorer medical care, and more risk of exposure [5].

Tetanus spores are present in the environment irrespective of specific geographical locations. Once the disease is contracted, it is difficult to manage despite even intensive advanced medical care if timely medical intervention is absent. The high infection and fatality rates in India could be a result of incomplete vaccination coverage, lack of awareness of the protocols to be followed after sustaining a wound, lack of resources in hard reach areas, lack of medical facilities in such areas [6].

Spores are present everywhere in the environment, more commonly in the soil of warm and humid areas. The dormant spores develop into active toxin-producing bacteria in the presence of a favorable environment, ie devitalized, dead or necrotic tissue. They enter the human system via open or infected wounds, or even through unclean delivery practices, burns, dental procedures, or surgeries. The toxin, tetanospasmin thus produced by the active bacteria results in widespread sustained contraction and spasm of the muscles in the body, i.e dystonia. This is via the prevention of the release of the inhibitory neurotransmitter Gamma-aminobutyric acid (GABA) into the synaptic cleft. This results in manifestations such as pain, headache, trismus, stridor, laryngeal spasm, rigidity, opisthotonus, and stiffness [7].

Dystonia can manifest in various ways, such as with tortipelvic, torticollis oculogyric, buccolingual, or opisthotonic [8].

The prognosis of a case can be assessed via the Phillips scoring system (Table 1).

The spasms are most in the first 2 weeks, the autonomic disturbances following them by a few days and peaking in the second week. The muscle spasms and convulsions are often precipitated by even minor stimuli, such as light, touch, or noise. The severe spasms and muscle rigidity often necessitate paralysis in cases of tetanus. Autonomic disturbances occur in the form of labile hypertension, sweating, tachycardia in severe cases.

Table 1: Prognostic Scoring Systems in Tetanus Phillips Score

Factor	Score
Incubation Time	
< 48 hours	5
2-5 days	4
5-10 days	3
10-14 days	2
> 14 days	1
Site of infection	
Internal and umbilical	5
Head, neck, and body wall	4
Peripheral proximal	3
Peripheral distal	2
Unknown	1
State of protection	
None	10
Possibly some or maternal immunization in neonatal patients	8
Protected > 10 years ago	4
Protected < 10 years ago	2
Complete protection	0
Complicating factors	
Injury or life-threatening illness	10
Severe injury or illness not immediately life-threatening	8
Injury or non-life-threatening illness	4
Minor injury or illness	2
ASA Grade 1	0
Total Score	

The diagnosis of tetanus is clinical for the most part. The WHO definition of adult tetanus requires at least one of the following signs: trismus or rhisus sardonicus; or painful muscular contractions [6].

To manage this condition: isolation in a dark and quiet room, with heavy sedation and muscle relaxant administration to prevent spasms. Benzodiazepines are most commonly used; it's favored for its combination of antispasmodic, muscle relaxant, anxiolytic and sedative effects, which are particularly useful for tetanus patients. Diazepam modulates GABA-A transmission and increases presynaptic inhibition. Dantrolene is a muscle relaxant that is effectively used in the treatment of malignant hyperthermia and neuroleptic malignant syndrome [9]. Studies have proven that magnesium sulfate has significant efficacy in the management of autonomic symptoms [10].

Antibiotics are also administered in tetanus, penicillin, and metronidazole being favored. It reduces the proliferation of the bacteria at the inoculation site.

For neutralization of circulating toxin, tetanus immunoglobulin is used. There is no certified fixed-dose; for prophylaxis in susceptible wounds, it is 250 IU of TIG administered intramuscularly For active tetanus case a dose ranging from 3000 to 60000 IU may be administered, 500 IU at a time [11].

Recurrent tetanus is a possibility as infection does not provide immunity to subsequent infections [12].

Tetanus being a potentially fatal disease, the medical fraternity must emphasize its prevention rather than its treatment. Despite it being an “old world” disease, it is surprisingly prevalent in India, along with the other developing nations. This could be attributable to many factors, such as inadequate knowledge of wound hygiene, irregular immunization, lack of awareness on what the disease is, and how fatal it can be, unequipped health centers, unavailability of vaccine, etc. However, the annual mortality rate per 100,000 people from tetanus in India has decreased by 86.3% from 1990 to 2017 [13]. We are doing this study to understand the clinical profile of tetanus in our region.

MATERIALS AND METHOD

This is a retrospective hospital-based study done during the year 2017-2019 in Epidemic Diseases Hospital, Bangalore. All the cases presenting to the hospital and which were diagnosed with tetanus were included in the study. The patients who were started on treatment and then referred to the hospital were excluded from the study.

The patient’s demographics and clinical data were collected and compiled in an excel sheet and analyzed. The patient data which was collected maintained patient confidentiality throughout.

RESULTS

The total number of patients who meet the inclusion criteria were 58 cases. The majority around 87% of the cases presented from the rural area and 13% were urban areas. The sex distributions were 35 males and 23 females. The majority of the patients were in the age group of 55-60 years; the minimum and maximum age being 5 years and 89 years respectively.

The average duration of hospital stay was 15 days, the minimum duration being 1 day and the maximum is 45 days. Out of these cases, Discharge against medical advice was done in 11 cases due to patients stating personal reasons.

The site of injury was an injury to the foot in 65% of the patients, followed by injuries to the fingers or the hand in 30% and 5% were cases of injury due to tooth picking with a pin, splinter removal using pins. Out of the injuries which occurred, the majority of them happened in agriculture fields summing up to 70%; and 10 % were from carpentry works; 10% due to fall/trauma/accidents.

In 25 cases the wound was noted and in the others, it was a healed wound.

The mean incubation period was around 14.5 days from the time of injury to the development of symptoms in the patients who survived. Totally 14 deaths noted, the cause of death was a late presentation to the hospital, taking alternative medicine treatment; ignorance also contributed to delayed presentation to the hospital. The fatal cases also had multiple comorbidities like type 2 diabetes mellitus, hypertension, almost all the cases were in the age group of 65-75 years. The progression of illness was rapid and they passed away within 5 days. The patients with nil comorbidities have a better chance to recover. Overall 36 patients had NIL co-morbidities. 95% of death cases hadn’t received tetanus immunoglobulin.

During the initial presentation of symptoms:

- lockjaw/inability to open mouth: 41
- neck stiffness/pain: 25
- difficulty in walking: 2
- limb pain/stiffness: 17
- back muscle pain/stiffness: 12
- dysphagia: 7

Towards the peak of the diseases, most of the symptoms were present for all the patients.

72% of the cases were noted to have autonomic dysfunction like fluctuation in pulse, blood pressures, sweating, and altered respiratory pattern on and off, postural fall of BP.

DISCUSSION

After compiling the epidemiological and clinical profiles of these patients we concluded that in a majority of the cases, the disease was introduced via injuries sustained in agricultural fields. This is in accordance with a paper published by Arijit Sinha, Bikash Chandra Seth et. In this study, they found that 81.34% of their patients hailed from rural areas [14]. The agricultural population constitutes a major part of the country’s society, therefore we must focus on preventative and awareness promoting methods to significantly reduce the risk as well as improving prognosis.

Another inference reached was the predisposition of tetanus to develop in males; according to our study 35 of the 58 cases were male which comes up to 60.34%. This is in line with a study conducted by Anuradha et. al [2].

Both of these inferences can be explained by the fact that the male population of the lower socio-economic strata who spend most of their day in the fields will inadvertently be at much higher risk of acquiring tetanus. Also, the female population whose attire covers more body surface, are additionally protected by the antenatal dose of tetanus vaccine that they receive as part of the routine immunization schedule prescribed by the government. These factors, along with the generally lower frequency of going into the fields, put them at lesser risk compared to men.

In the study conducted by us, it was also found that the majority of the patients were in the age group of 55-60 years. This is in accordance with the studies done by V G Marulappa [15] and Chalya et al. [16] which concluded that the majority of patients were above 40 years old.

Another finding of the study we conducted was that the average duration of hospital stay was 15 days, which is almost in accordance with to study done by S Chaudhary in which the mean duration of hospital stay was 12 days, ranging from 1-32 days [17].

In the collected data of patients presenting to our hospital, the site of injury the foot in 65% of the patients. This could be because field workers and people in rural areas do not use adequate foot protection when working in the fields or elsewhere and thus are more susceptible to sustaining tetanus prone wounds. This is in accordance with the findings of other studies [14, 17, 18], one being a paper by K.V.L. Sudha Rani [18] in which 79% of the cases included in their studied involved wounds on the lower extremities.

In 25 cases the wound was noted and in the others, it was a healed wound. In other studies however it was noted that commonly they presented with acute forms of injury rather than old ones [18,19]; in a study by V G Marulappa, they recorded that 47.9% of their patients presented with acute trauma, with 14.6% presenting with older wounds [15].

The mean incubation period was around 14.5 days from the time of injury to the development of symptoms in the patients who survived, which is in accordance with other studies conducted, the study conducted by AHM Feroz et al reported it to be 10.8±2.1 days with a range of 3-28 days [18].

According to the compiled data of the clinical presentations of the cases that came to the hospital, the most common presenting feature is lockjaw, i.e. inability to open the mouth, which was present in 70.68% of the patients. The others were neck stiffness and pain (43.1%), limb pain (29.3%), back pain (20.68%), dysphagia (12.06%), and difficulty in walking (3.4%) in decreasing order of frequency. This is in line with the findings of a study by Pornchai S in Thailand, in which 87.2% of the cases had presented with trismus [20]. Other studies also infer trismus to be the most common presenting feature [14, 21]. In a study conducted by Muhammad Saleh Khakheli et al., they reported patients to be clinically diagnosed with tetanus if they had the following symptoms- trismus, neck or abdomen rigidity, and reflex spasms; and were accordingly classified as generalized or cephalic tetanus [22]. According to a study carried out in Pakistan by Mahsud I U et.al, the most common symptoms tetanus patients presented with were lockjaw, dysphagia, and trismus [23].

Out of the 58 cases, 14 deaths were noted, which sums up a case fatality rate of 24.1%. Other studies yielded similar values, for example in a study conducted in Ethiopia by Amare A. they had a case fatality rate of 27% [24]. In a study conducted in Solapur by A B Pawar el at., they found that in 26.3% of the cases complications such as respiratory failure, cardiac arrest, septicemia, etc arose, and in those with complications, there was a 75% fatality [25]. Autonomous complications have been reported in various other studies which significantly worsened the prognosis [1, 26]. Case fatality can be attributed to several factors, such as the presence of comorbidities, advanced age, the degree and mechanism of the wound, the time of presentation, lack of awareness of preventative measures, and immunization. 95% of the fatalities had never received immunoglobulin. Also, mortality would seem to be inversely proportional to the duration of the incubation period according to various studies [27, 28, 29].

72% of the cases were noted to have autonomic dysfunction like fluctuation in pulse, blood pressures, sweating, and altered respiratory pattern on and off, postural fall of BP. In other studies also autonomic dysfunction was found to be a frequent complication [18, 30]. In a paper in which the study was carried out in Mysore, it is reported that most of their cases too died as a result of cardiorespiratory arrest [15].

Out of the total number of patients, 11 of them went DAMA, i.e. had to be discharged against medical advice. This could be due to several reasons. A simple lack of awareness as to the seriousness of the condition, financial constraints, customs and beliefs, faith in alternate methods of non-medical treatment, etc, etc. The only way to minimize such cases is to make admission financially more feasible, increase the availability of immunoglobulin, ensure the adequate counseling of patient and attenders, and to spread awareness in the community as to how this disease must be treated at a fully equipped hospital, as well as the potential fatality of the disease.

CONCLUSION

Tetanus is a treatable disease if there are timely precautions and interventions, such as taking tetanus toxoid injection, followed by Tetanus immunoglobulin and wound care. Through our study, we want to promote awareness among the doctors about the current situation of tetanus and emphasize that it’s a still ongoing and ever-prevalent problem and we have to address it by working together. We need to create awareness among the general public regarding basic wound treatment, the prescribed immunization schedule. We must also emphasize the symptoms and signs of the disease to them so that they may identify it early and allow for timely intervention, which significantly improves the prognosis. Most of all, it needs to be made known to the public that this is an ailment, that if left untreated, has a very high chance of fatality and must not be underestimated.

Disclosure statement

This is a retrospective study, where there was no involvement of any human subject or any intervention, it was just an observational study, and only data collected from the case sheet was done and patient details were kept confidential at all times.

The institutional ethics committees approved was this research complied with acceptable international standards (such as the Declaration of Helsinki)

Acknowledgments

Author contribution: *VSS was responsible for the idea, and conduct of the study; SN was responsible for the organization and fieldwork; AA was responsible for data collection; VRM was responsible for paper writing and coordination.*

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ORIGINAL ARTICLES

Mass shooting incidents: evolution of preventive procedures, preparation, treatment, and medical care supply

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Abstract: *Mass shootings incidents occur with increasing frequency over time. Studying these cases proved that, despite their diversity, several common features could be taken into account in the early detection and possible prevention of certain future cases. Accepting that such incidents may occur anywhere and anytime, societies need to be prepared for their more effective response. Informing citizens about the best way to react to a mass shooting event is considered crucial and essential. The "Run-Hide-Fight" guideline/directive seems to be the most appropriate guideline given to the public. Proper training, of both the Suppression Forces and the emergency medical care providers, is considered of utmost importance. The role of the Incident Commander, regarding the incident management, as well as the external bleeding control of the injured people, are factors of paramount importance in trying to mitigate the casualties from such an incident. The alertness of both citizens and organizations/structures may lead to early detection of potential perpetrators and thereby averting a mass shooting incident. To achieve increased survival and a reduced number of casualties from a mass shooting event it is vital proper education be present at all levels. The response to a mass shooting event should be imprinted in an Emergency plan. Such a plan should have been decided and made, by the Security and Suppression Forces, the Healthcare Institutions, and the Public Safety Answering Point.*

Keywords: *mass shooting incident, first responder, active bleeding, tourniquet, Incident Commander*

INTRODUCTION

Although mass shootings incidents have taken place throughout recent world history, the literature has been relatively limited, as such incidents were more sporadic before the 21st century [1]. A mass shooting incident is usually the result of one or more people killing or attempting to kill people within a limited and inhabited area. In most cases, the perpetrators use firearms/guns and there are no specific models for victim selection [2]. The immediate response as well as the perpetrators' neutralization are required to mitigate the consequences and casualties. However, because it will be some time before specialized forces arrive at the scene and take action, the way surrounding people react to such an event is of equal importance.

The purpose of this review is to present both the most appropriate ways of prevention and preparation, as well as the optimal procedures for responding and dealing with possible mass shooting incidents. Informing, preparing, and training citizens and stakeholders such as the Suppression Forces and emergency medical care providers, is crucial in reducing the casualties in a mass shooting incident in an

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urban environment.

DEFINITIONS

A mass shooting event involves multiple incidents of violence with a firearm/gun. However, there is not a widely accepted definition of the term "mass shooting incident". The US Federal Bureau of Investigation (FBI), for instance, does not use the term "mass shooting incident", but refers to these incidents as "active shooter incidents" [2]. FBI, therefore, defines as an active shooter "a person who is actively involved in a murder or attempting to kill people in a residential area", while it also defines as mass murder "four or more murdered during an event without time distinction (cooling-off period) between murders" [3]. The United States Congressional Research Service (CRS) acknowledges that there is not a universally accepted definition and describes as a mass shooting incident "an event in which a perpetrator selects four or more individuals and kills them indiscriminately", adding to the FBI's definition the word "indiscriminately" [4]. The Gun Violence Archive (GVA), a non-profit organization that has been monitoring gun violence in the United States since 2013, describes as a "mass shooting event" four or more people killed in a single incident at the same time and location, not including perpetrator [2,3]. Moreover, this organization does not exclude situations such as domestic violence, gang activities, etc., nor does it differentiate victims depending on the circumstances under which they were shot, which means that its definition is broader than of other organizations and therefore includes incidents that are not recorded in other databases. Meanwhile, in 2013, the United States Congress defined mass murder as "three or more murders in a single incident" as part of a bill to enable the Department of Justice to assist local authorities during the investigations [2].

STATISTICAL DATA

The vast majority of mass shooting incidents have taken place in the United States of America [5]. According to GVA, from 1st August 1996 until December 2019, a total of 172 cases have been reported [6]. In just a few of these cases, the perpetrators were two, while no incidents involving robberies or domestic violence were reported. A total of 1228 people were killed, coming from almost all races, religions, socioeconomic status, and age. Of these, 196 were children and adolescents. Also, thousands of survivors with permanent disabilities and serious physical and mental health problems should not be omitted [6]. Perpetrators often used or carried more than one weapon, while in a case one perpetrator was found carrying 24 weapons. Of the total 326 weapons used in the above-mentioned incidents, 179 were legally acquired (55%), 61 were illegally acquired (19%), while it is not clear how weapons were acquired in 86 cases (26%). Of a total of 179 perpetrators, some were known to have violent tendencies or criminal history records, but there were also several who had not presented any criminal behavior at the time of the incident. The vast majority (99%) were men and most were 20-50 years old. More than half (55%) lost their lives on or near the scene of the incident, often by committing suicide. Lastly, incidents in schools and places of worship tend to come first in the public’s memory but are a relatively small part of mass shooting incidents. The most common are those in offices, shops, and restaurants with the State of California having the most such attacks (29) of any State [6].

According to the FBI, after 2011 the frequency of mass shooting incidents has increased, with the period 2011-2014 occurring almost three times as many as in previous years [7]. During 2000-2013, a total of 160 cases (according to the wider definition than the GVA’s definition) were recorded, with an average of 11.4 cases per year. According to the same report, 16.9% of attacks took place in schools and 45.6% in shops and markets. 90 of the above mentioned 160 incidents, were completed before the Suppression Forces even intervened, most often with the perpetrator's suicide. Of the 64 cases in which the duration of the incident was ascertained, at 44 (69%) the incident was completed in less than five minutes, while at 23 (36%) in less than two.

Mass shooting events are expected to occur worldwide. Two of these incidents received worldwide publicity, evolving many victims: on November 13th, 2015, a total of nine perpetrators launched an attack in Paris, killing a total of 130 people and injuring more than 350 [8], while on New Year's Eve 2017 in Istanbul, a perpetrator stormed a nightclub, killing a total of 39 people and injuring another 79 [9]. In both cases, there was a religious or racist motive.

PERPETRATORS’ CHARACTERISTICS

To increase the chances of an early potential perpetrators’ detection, both mass shooting events and perpetrator’s physiometric characteristics have been studied [10]. According to an FBI study, regarding the pre-attack behavior of perpetrators in the United States for incidents that happened in 2000-2013 [11], the majority of perpetrators had exhibited 4 to 5 worrying-suspicious behaviors before the attacks. For perpetrators under the age of 18, these behaviors were more likely to have been noticed by classmates or teachers than by the family environment. Each perpetrator was exposed to an average of 3.6 stressors during the year before the attack. Of the cases in which some kind of suspicious behavior was observed, 54% did not take any action, while 41% of them were reported to authorities. According to the same study, in most cases (64%) at least one of the victims was pre-selected by the perpetrator [11].

As far as the age of the perpetrators is concerned, the youngest reported to be 12 years old and the oldest 88, with the average age of 37.8 years (94% were men and 6% women). Of the adult perpetrators, 20% were high school graduates, while 34% had completed at least an Institute of Technology or University. Of the latter, 5% were Master's Degree or Ph.D. holders. However, there was a significant percentage (36%) where the level of education could not be ascertained. 38% of the adult perpetrators were unemployed while 44% were workers. The remaining 19% included students, retirees, people with disabilities, etc. The 24% of adult perpetrators, at the time of the attack or in the past, were involved in some way with the Armed Forces or Security Forces, while 14% of perpetrators had one or more convictions before the attack [12].

The above-mentioned study also investigated two related but distinct time variables: a) the time the perpetrators spent planning the attack and b) the time they spent preparing it. The first category concerns the time from when the perpetrator began to think about a possible attack until its implementation. Because it is often difficult to determine its onset, in many cases this period could not be determined by the researchers. The second category is more specific, usually less than the first, and concerns the time from taking any kind of action by the perpetrator (eg gathering of relevant material, a supply of weapons and other equipment, etc.) to the implementation of the attack. In terms of design, 26% of the perpetrators took 1-2 months, 18% 3-5 months while 24% less than a week, with half of them less than just twenty-four hours. In terms of preparation, 28% needed a maximum of one day, 26% 1-7 days and only 4% consumed 6-12 months. The study also explored how perpetrators supplied the weapons they used. In 40% of cases, the perpetrator had obtained the weapons of the attack legally, 2% bought the weapons illegally, in 6% of cases the weapon was stolen, while in 11% it was borrowed. Besides, it seems that the majority of the perpetrators acted in a place known, if not familiar, to them.

This study also investigated the presence of stressors in perpetrators' lives. These included financial problems, physical and mental health issues, interpersonal conflicts with family, friends, colleagues, as well as alcohol abuse or drug use. The study acknowledges that many people occasionally face similar issues, but most have mechanisms (personality, psychological flexibility, interpersonal relationships) to cope effectively. Although the issue is multifactorial, it appears that the perpetrators usually experienced multiple stressors during the period before the attack (an average of 3.6 within the last year). The most common stressor detected was a mental health disorder in 62% of perpetrators. About half (49%) had financial problems, while 35% had work-related problems. In 34% of cases, serious family issues were reported, while 22% of the perpetrators were addicted to alcohol or drugs. Only 2% of the operators did not detect any of the studied stressors. Given that mental health disorder was the most common stressor, as well as the fact that it is a fairly broad and varied term, it is worth mentioning that of all cases in which mental health disorder was detected, only in 41% of cases, perpetrators had a previous official diagnosis by a health professional, with the 75% being diagnosed as a “mood disorder”.

Another interesting aspect of the study was the research for cases in which people from the perpetrator’s environment had found some kind of worrying behavior during the period before the attack. Thus, in 62% of the cases, deviant behaviors were observed from a psychological standpoint, in 57% interpersonal disorders, and in 54% of cases a problematic way of communication. Moreover, in 46% of perpetrators who were workers, there was a decline in performance at work and in 42% of those who were students, there was a corresponding decline in school performance. However, it is worth noting that, contrary to the common belief that the perpetrators of such attacks tend to be isolated, the study found that 68% of adult perpetrators lived with other people immediately before the attack. Additionally, 86% of the perpetrators were found to have a significant social relationship and contact with at least one person in the year before the attack.

So that research data be used for prevention, not only was it investigated when some deviant behavior began to be observed, but also by whom. Thus, it appeared that in 56% of cases the initial observation was made at least 2 years before the attack and in 29% between 1-2 years. Only in 2%, it was observed the week before the attack. Also in 87% of the cases, the deviant behavior was observed by the perpetrator's partner, in 68% by another family member, while among the perpetrators who were students in 92% of the cases the person who perceived some kind of behavior change was a classmate and in 75% a teacher.

Another particularly interesting finding was the fact that the majority of perpetrators (79%) appear to have been exposed to a mass shooting event in response to an injustice they experienced or believed they were experiencing. The reason for this feeling (injustice) varies from case to case, with the most common causes being injustice at the interpersonal level (33%) and in the work environment (16%). The fact that many people experience similar situations every day, the vast majority of whom choose to react in a non-violent way, proves that in the case of perpetrators of such attacks there is a synergy between various motives. Although in almost 4/5 of the cases the perpetrator was affected by some kind of injustice, the manifestation of the attack did not happen with the same percentages against the "target" people. This, according to the study, accounted for about 64% of cases (in 27% the victims that got murdered were pre-selected and in the rest 37% there were both pre-selected and random victims), while in about 37% of cases the victims were either completely unknown to the perpetrator, or known, without, however, being linked to his "complaints". This is explained by the fact that many times the perpetrator tries to act against a specific organization, such as a company, a school, etc. and not against specific people.

Another element of the study that could also be used in the field of prevention, is the fact that about half of the perpetrators of such attacks had committed suicide, several of whom had even attempted suicide within the year before the attack. If we exclude the cases in which, due to lack of sufficient evidence, the perpetrators were not registered in either of the two categories (suicidal ideation or not) then the percentage of those who had such behaviors is 85%.

In trying to outline the profile of a future perpetrator, it is also worth noting that more than half of the perpetrators (55%) had, to some extent, made their intentions known by making threats, mostly in person or, more rarely, in other ways such as in writing or by electronic means.

CITIZENS' RESPOND TO MASS SHOOTING INCIDENTS

a) Before Suppression Forces intervention

It is a fact that the reaction of citizens who have been present in a mass shooting incident varies and depends on multiple factors, including the individual judgment regarding which action will better protect their lives, their characteristics, any previous education and information (or not), as well as the available means and space where the event happens [13].

However, although there is no appropriate response to all possible scenarios, specific actions have been defined to reduce casualties, the consequences of a mass shooting event, and increase survival [14].

Citizens should be aware of these guidelines to evaluate the situation and be able to react in the best possible way [15].

These instructions/guidelines, to be quite memorable, have been coded as "Run - Hide - Fight" [16, 17].

- Run

The initial instruction given to citizens is that in a mass shooting incident their first action should be to run away from the perpetrator. The move should be made to a predetermined meeting point if they are aware of it-or moving as far away from the firing point as possible until they are in a safe place. Despite the complexity of the situation, those who can move safely are advised to do so [12].

Citizens are advised to run-move, leaving behind their personal belongings so that they do not spend time and be more flexible. They should also raise their hands, making a signal to security forces that they are unarmed. Rolling stairs and elevators should be avoided. During their run-move, citizens are advised to help their fellow citizens to leave together, but not to stay behind. Finally, when citizens feel safe, it is recommended to call and inform the authorities, giving as much information as possible. The information that would be useful concerns the exact location of the incident, the number of perpetrators as well as their description, type and number of weapons or the possible use of explosives, possible number of victims, and, finally, whether shots continue to occur at the time of the call.

- Hide

If quick removal is not considered safe, the next option should be to try hiding. If possible, the place/building with the thickest walls and the fewest windows should be chosen. Doors should be locked and windows closed where possible. It would also be wise to place heavy objects/furniture behind doors. The instructions also include maintaining absolute silence from citizens who remain in hiding. For this reason, electronic devices, as well as mobile phones, should be silenced and lights should be off. As long as citizens remain hidden, they will have to consider ways to escape, looking for possible escape routes, as well as ways to deal with the perpetrators, if necessary. Attempts to communicate with authorities or with Suppression Forces should only be made when this is considered safe.

- Fight

When neither citizens' safe removal nor their hiding is an alternative, it is recommended that citizens fight the perpetrators. According to an FBI investigation, in 17 of the 51 shooting events, the attack was stopped by civilians who were at the scene. Citizens may try to deal with the perpetrators mainly by throwing objects at them or using improvised weapons. In these cases, the energy must be as aggressive as possible. Naturally, trained individuals, such as security personnel, military, self-defense technicians, or professionals who may carry a weapon (eg security guards) are more likely to be able to deal with the perpetrator.

b) After Suppression Forces interference

A mass shooting event can take place long after the Suppression Forces have reached the scene. At this time, citizens must, on the one hand, remain as safe as possible and, on the other hand, not obstruct the work of authorities [13]. For this reason, citizens should try to stay calm and follow the instructions of the security forces. They should have their hands up and avoid screaming. This will help them to be easily distinguished from the suspects/perpetrators. Citizens should also move in the direction indicated, lying on the ground and generally being as cooperative and obedient as possible. Finally, it is useful that citizens provide relevant information that will facilitate the work of the Suppression Forces. Citizens will have to show similar behavior, remaining calm and following the instructions they receive, even after the attack has passed. This will help to better deal with the injured, identify/record the victims and not to alter the information required to investigate the incident and locate the perpetrators.

Apart from the widely used "Run - Hide - Fight" guideline, there are many other similar guidelines/instructions, also standardized in a way that is easy to remember that are proposed. One such is the "Avoid - Deny - Defend" [17-19]. This has to do with the directive, a) avoid danger (either by acknowledging the threat before the attack occurs, or moving away, b) deny access (preventing the perpetrator from accessing a safe place, if possible), c) take action (as a last resort, defending your life in any way possible by dealing with the perpetrator).

A similar process of four steps coded as "4As" also aims to reduce casualties in a mass shooting incident [20]. These steps are:
Accept: Accept that an emergency occurs
Assess: Evaluate what you need to do to increase your chances of survival
Act: take action following the "Run - Hide - Fight" direction
Alert: alert the Suppression Forces

Finally, the “ALICE” direction [21-23] is a five-step acronym that also aims to increase survival in such events:
Alert: be alert
Lockdown: if you decide not to try to escape, stay in a locked-secure area
Inform: if possible, forward the information to the authorities
Counter: If there is no other option, deal with the perpetrator
Evacuate: get away from the danger zone as soon as possible

It seems that the latest direction may be confusing, as the range of actions proposed is not in line with the rest and especially with the "Run - Hide - Fight" guideline, which has now been established as the optimal instruction for citizens to mass shooting events.

SUPPRESSION FORCES: FIRST RESPONDERS

The actions of the scene first responders are of utmost importance in a mass shooting incident [24, 25]. The first respondents are involved in both managing the incident and providing care to the injured [26]. The term "first responder" does not imply an official certification, restriction, or ability. If the attending citizens who may attempt to be involved are excluded, the first respondents are the first officials arrived at the scene and could be the police, military, firefighters, or even emergency medical personnel.

Moving on to the incident area, the first responder should ask for as much information as possible from the agency available (usually police). This information will allow access to the incident area from the safest and most convenient route, as well as the closest possible approach to perpetrators. In case that the first respondent does not have the authority to enforce order, he/she must remain safe and make an immediate request to the competent authority. Upon arrival at the scene, from the information already collected and the first scene assessment, first officials will identify the danger zone and install a point as an administration area. Depending on his training and the characteristics of the case, such as the number of perpetrators or the time needed to receive advanced help, the first responder will decide whether to operate on his own (or with the existing forces) or will wait for reinforcements to deal with the perpetrators. The first correspondent automatically undertakes the management of the incident (Incident Commander-IC) [27,28]. IC’s purpose is to develop a plan to deal with the incident based on the personnel and resources available, as well as to seek resources for the best response to the demands of the situation [29]. The first responder’s primary purpose is to stop the perpetrator, so even though he/she may assist in removing survivors outside the danger zone, IC should not be involved in helping injured people at this stage. Finally, the first responder should transmit, as soon as possible, all the information gathered to be used by the Forces that will be called to assist.

When additional Forces arrive in the incident area, they must contact the first correspondent who will have taken on the role of IC. As long as the mass shooting incident persists and the perpetrator has not been neutralized, all forces will work together IC’s common plan and guidelines to deal with [30].

As soon as the danger passes, all forces should assist the injured by applying the techniques and instructions mentioned below.

It should be noted that both IC and Command Post (the point where the administration is exercised) may change as the incident progresses. Once Suppression Forces have arrived and have started operating at the scene, the IC is usually the superior authorized officer. However, IC may be the most experienced or may be determined by other criteria, but in any case, IC should always be clearly defined to the forces involved.

Accordingly, the Command Post should be installed in a place known to all, which can be relocated, if necessary.

So that the main purpose of managing a mass shooting incident (which is minimizing casualties) be served, the incident scene is divided into three zones [31]. Within each of them, there is a different regime of security, accessibility, and actions that can take place. The direct intervention Forces must be aware of the characteristics of these three zones, while the determination of the zones should be clear to all the people involved. As a mass shooting incident is a dynamic situation, the boundaries of these zones may change over time.

- Hot zone

It is the area where there is still a risk of gunfire. Neither medical personnel nor Fire Service personnel (except for specially trained units) are not allowed to enter this zone because their entry would endanger their lives or the lives of others. Also, they may obstruct Suppression Forces' efforts, whose personnel are the only ones authorized to enter and operate in this zone, following IC's plan and instruction.

- Warm zone

It is the area where there is no immediate risk of gunfire, however, it cannot be characterized as a safe area. This implies specific restrictions on both entering the area and operating actions. Firefighters and medical/paramedic personnel may enter under IC's permission for only critical and extremely necessary actions. Medical/paramedic personnel, for example, will only participate in vital operation (life-threatening situations) to save injured people's lives [32]. The point of entry and exit as well as the possible assistance by Security Forces will also be determined by the IC. If allowed, there must be certification and registration of the people entering the warm zone. The time spent in the zone should be the minimum necessary.

- Cold Zone

This area is considered safe, without the risk of a shooting. Access is free for all emergency forces as well as their length of stay. Healthcare personnel can provide any kind of medical care required, operating based on purely medical criteria, having ensured their safety [33].

NOT ADVANCED MEDICAL CARE PROVISION

The experience of dealing with the injured during the US military operations, formerly in Vietnam and later in Iraq and Afghanistan [33], combined with similar medical research on wound care, has led to a large percentage in the establishment of modern procedures – instructions, regarding gunshot wound management. Particular emphasis has been given to tackling life-threatening active external bleeding.

The experience of war injuries in the late 1990s led to the standardization of a series of wound care procedures on the battlefield, called Tactical Combat Casualty Care (TCCC), focusing on the most common causes of death that could be treated infield, even by medical staff with no special skills [34].

Recognizing how this military experience could be useful and applicable to non-military operations, an independent group of citizens set up a Committee in 2011 (Committee for Tactical Emergency Casualty Care, C-TECC) [35]. The purpose of the Commission was to develop guidelines that would adapt the battlefield military experience to similar situations in an urban environment, taking into account the differences and peculiarities between the two operational applications [36].

In 2013, a group of Public Institutions, including police, Fire Department, the Army, Medical Institutions, pre-hospital care, etc. was convened (known as Hanford Consensus) [37,38] and the result was a consensus regarding strategies to increase survival in mass shooting events. The Committee developed the following acronym "THREAT", to standardize the process:

- **T**reat suppression
- **H**emorrhage control
- **R**apid **E**xtrication to safety
- **A**ssessment by medical providers
- **T**ransport to definitive care

The Committee argues that survivors of such an incident or the slightly injured may act as potential rescuers by following the instructions above. The Committee also considers that the control of active external bleeding should be a key skill of all the personnel of the Security and Emergency Forces. This training concerns, on the one hand, the use of a tourniquet [39] and, on the other hand, the placement of hemostatic gauzes and hemostatic agents in places when it is not possible to place a tourniquet [40].

Some of the TCCC-TECC skills that apply to mass shooting injuries in urban environments and that could also be applied by trained personnel of the Security Forces [41] to increase survival rates are:

- Use of a nasopharyngeal airway for injuries without maxillofacial trauma
- Placing the unconscious injured person in a prone position, in case of facial injury or airway bleeding
- Spinal immobilization for people with blunt trauma
- Ensuring an intravenous or intraosseous line for fluid management

ADVANCED MEDICAL CARE PROVISION

Gunshot wounds [10] can be quite deadly and therefore the most immediate medical care possible is vital to increasing victims' survival. The usefulness of an immediate intervention of specialized health care providers comes into contrast with the necessity for non-exposure of such personnel to danger. However, weighing the situation data each time, the general rule is to provide as much care as possible to the injured, while ensuring health personnel safety. At this point, the determination of action zones in the event scene is proved quite useful and crucial, since the provision of specialized health care begins before the perpetrators are even neutralized and the threat is completely stopped [42].

Healthcare providers should also follow the guidelines set out in Harvard Consensus [36, 38] and are concentrated in the THREAT algorithm. The medical care system can be divided into:

- Direct threat care: providing care while shooting or under adverse conditions
- Indirect threat care: providing care while the threat has been suppressed, but may reappear at any time
- Evacuation: providing care during the evacuation from the incident scene

The first line of care takes place within the hot zone. However, the entry of health personnel into this zone is only allowed under certain conditions. Providing care in the hot zone is restricted to only limited manipulations such as airway management and controlling active external bleeding by placing a tourniquet or applying pressure to the appropriate points. It also includes verbal instructions to victims to take care of themselves or others, if possible.

The second line of care is evolving in the warm zone [43], where the immediate threat has been suppressed for the time being and consequently, the health personnel may proceed with more invasive operations, without worrying about their personal safety, but also without complacency. In this zone, health care providers will provide advanced medical support aimed at maintaining life and stabilizing the injured before being transferred. Operations that take place at the warm zone may be: ensuring the airway access even in an invasive way, controlling bleeding using (except for tourniquet) hemostatic gauzes and agents, stabilizing fractures, as well providing fluids or blood factors (usually not at pre-hospital level), treating pneumothorax and, finally, preventing or treating hypothermia [44].

The final line of care takes place in the cold zone. This area is considered safe and has access to all health personnel involved in the incident. The priorities regarding the treatment of the injured remain the same, but there is the possibility of using additional means to achieve them. In this zone, the network for transporting the injured to the appropriate structures for the advanced medical care provider will also be established.

The limited resources available concerning the number of people that need medical support is a quite likely scenario in mass shooting incidents [45]. This may create the need for a screening system for the medical care process (triage) [42, 46]. "Triage" is defined as the process of separating the injured based on the need for immediate medical support, taking into account the possibility of benefiting from it. The triage process is applied when the needs for medical care exceed the available resources and aims to maximize the number of survivors. In other words, it aims to offer the greatest benefit to the largest number of victims with the resources available.

From time to time, various triage systems have been proposed, none has been universally accepted so far. In the case of gunshot wounds, the challenges for an effective triage system are even greater. These have to do with the nature of these injuries, where superficial injuries with minimal obvious bleeding may obscure extensive internal bleeding, and conversely, severe superficial injuries may not always indicate internal organ damage of similar severity [45].

Despite limitations, a widely used triage system, which has occasionally been used in mass shooting events, is the START system (Simple Triage And Rapid Treatment). It is a system developed in 1983 by the Fire Services and the Hoag Hospital personnel in California, and modified in 1996 [47]. Using a simple algorithm (RPM: 30-2-Can Do) evaluates respiration (Respiration, respiratory rate), circulation (Perfusion, capillary refilling time - in the presence of a radial pulse), and the level of consciousness (Mental status, execution of simple commands) [48]. Depending on the abovementioned criteria, the injured are classified into four categories:

- Immediate (marked in red): they are the injured who require immediate medical support at the scene
- Delayed (marked in yellow): refers to less serious trauma patients whose lives are not directly threatened and who require medical treatment, but not immediately
- Minor (marked in green): they are the least wounded with injuries that are not life-threatening and may be treated when possible
- Deceased/Expectant (marked in black): refers to the dead and those who have fatal injuries whose condition is considered irreversible

The triage process with the START system allows during sorting, the intervention only with simple maneuvers to open airway (jaw thrust, Tilt-chin lift) and applying pressure at the site of an active external hemorrhage [49]. The great advantage of this system is that it is relatively simple so that it can be used by properly trained non-medical staff. On the contrary, one of its disadvantages is that it usually leads to “over triage” [50].

The triage system and the injured classification will determine the priority of transfer. A key factor in the injured person transfer is time and the avoidance of unnecessary delays in assisting in the field [51]. However, at the same time, it is important to choose the destination (hospital-trauma center) that the injured should be transferred, a decision that should be made by the field healthcare providers. In cases where there is more than one possible option, the best one should be decided, taking into account both the health condition of the injured and the distance (and therefore the time) of the possible destinations, as well as the facilities of the various Health Institutions.

TRANSFER TO HOSPITAL

When a hospital’s emergency department is notified to receive victims of a mass shooting incident, appropriate preparatory actions must be taken immediately [52-54]. These actions would best be included in a pre-existing protocol for such cases [55]. The key to the best response to such events is the mobilization and recall of staff (especially doctors of surgical specialties, staff from the emergency department, operating room (OR) and laboratory personnel), informing the blood bank, providing the emergency department with crucial materials from hospital’s warehouses, as well as, decongesting the emergency department and OR, as possible [56].

Regarding the care of the wounded, initially, if needed (e.g. non-sufficient resources available) a triage procedure is also applied. At this point, there are again various triage systems, without any common acceptance. The Triage Revised Trauma Score [57, 58], even though is used mainly in a pre-hospital environment to determine the priority of injured that should be transported to a hospital/trauma center, may also be used when the injured arrive at the hospital in order to be reassessed. The parameters evaluated are the Glasgow Coma Scale, systolic blood pressure, and the respiratory rate. Depending on the score, each injured person can be categorized into one of four categories (again marked in the same colors as in the START system) which indicates the priority in terms of the need for medical care [59]

As for the injury types, the most common life-threatening situation is blood loss (active bleeding) [60]. For this reason, in case of trauma, the classic ABC life support algorithm (Airway - Breathing - Circulation) is converted to CBA, giving priority to hypovolemic shock treatment [61,62]. The need for blood, (or blood derivatives/substitutes) are expected to be high in a mass shooting incident with several injured. In the emergency department, regular assessment of the wounded should be made in order to adjust their priority. Finally, analgesia should not be omitted [63], including the trauma patients with low priority due to prior control of their bleeding by placing a tourniquet, as a significant degree of pain is expected approximately 15-20 minutes after its application [64].

In the hospital, the need for psychological assistance should not be skipped [65]. Psychological support will also be needed both to the relatives of the victims and the staff who will be in charge of dealing with a very difficult, demanding, and particularly stressful situation [66].

CONCLUSIONS

Although a mass shooting incident can occur anywhere and anytime, its prevention and deterrence must be pursued. Analyzing the perpetrator’s characteristics, it seems that, despite their great diversity, in many cases, there are precursors that can be detected. The alertness of both citizens and organizations/structures may lead to early detection of potential perpetrators and thereby averting a mass shooting incident. It turns out that it is essential citizens be informed about the best way to react to a mass shooting event. All citizens should be aware of the steps they need to take to improve their chances of survival. The "Run - Hide - Fight" directive is the most appropriate.

To achieve increased survival and a reduced number of casualties from a mass shooting event it is vital proper education be present at all levels. Security personnel must be trained in how to act in such an event. Security personnel should be trained in basic life support (first aid) and trauma skills, neutralizing the threat, and ensuring the staff's safety.

Healthcare personnel, especially those serving in crucial positions, such as pre-hospital life support and Emergency Department personnel, should also receive appropriate training. This training should include trauma management skills, knowledge about the recent guidelines, as well as familiarity with the relevant equipment. It would be useful that the critical healthcare personnel would be appropriately certificated, obtaining the required training/education to be considered suitable for involvement in a mass shooting incident.

Ordinary citizens should not be excluded from training/education. Educational programs can take place both in workplaces and in structures such as schools, places of worship, municipalities, public organizations.

The administration of a mass shooting event by the Incident Commander seems to be the most effective way to manage such an incident. For this reason, all those involved in a mass shooting incident should be aware of this management model and act accordingly.

In a mass shooting incident, the need for medical care may exceed the available resources. In this case, a triage process is needed. All emergency medical personnel should be trained in the triage process, as well as, a specific triage system has been pre-determined to achieve the best possible coordination and communication among all medical care providers involved.

The response to a mass shooting event should be imprinted in an Emergency plan. Such a plan should have been decided and made, by the Security and Suppression Forces, the Healthcare Institutions, and the Public Safety Answering Point (e.g 9-1-1). These operational plans must be updated regularly and adapted to the new data of each organization involved, by the time they are changed. All personnel involved in such an event should be aware of these plans. Ideally, the various stakeholders involved in a mass shooting event should have already worked together to draw up the plans, so that there is a clear division of roles and responsibilities, as well as common terminology and language of communication. Finally, at regular intervals, exercises should be carried out on these plans all the stakeholders become familiar with their implementation, as well as possible shortcomings/weaknesses may be identified and improve their completeness and effectiveness.

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ORIGINAL ARTICLES

Peptide nucleic acid (PNA) as a novel tool in the detection and treatment of biological threatening diseases

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Abstract: *Peptide Nucleic Acids (PNAs) are nanostructures similar to nucleic acid molecules (synthetic DNA/RNA analogs) wherein the negatively charged backbone (sugar-phosphate) present in DNA/RNA molecules is replaced by a backbone without polyamide or peptide charge. Later, it was found that PNAs containing both purine and pyrimidine bases form highly stable duplexes with DNA and RNA. Although it is not as stable as 2PNA/DNA triplexes containing a homopyrimidine strand, it is still more stable than DNA/DNA and/or DNA/RNA duplexes. The unique characteristics of PNAs add new aspects to these nanostructures relative to conventional analogs to make them appropriate for molecular biology studies. The most important applications include the use of these nanostructures in the detection and treatment of diseases caused by threatening biological agents using the antisense/antigen technology and as genetic regulator drugs.*

Keywords: *Peptide Nucleic Acids (PNAs), synthetic DNA analog, genetic regulator drugs, antisense-antigen technology*

INTRODUCTION

Nielsen et al. (1991) were the first who reported the synthesis of a molecule, called Peptide Nucleic Acid (PNA), as a novel, completely synthetic analog of nucleic acids, which was commercialized in 1993 [1]. PNA molecules are considered as DNA/RNA synthetic analogs where the negatively charged backbone (sugar-phosphate) present in DNA/RNA molecules is replaced by a backbone without polyamide or peptide charge [2].

Biochemically, the main backbone of PNA structure forms by repetitive units of N-(2-aminoethyl) glycine connected by peptide bonds, and different nucleobases (purine and pyrimidine) are attached to the main backbone through methyl carbonyl linkers [3]. The same peptides are also drawn as the C-terminus and N-terminus, a state corresponding to 3′ and 5′ termini in DNA [4]. These characteristics render PNA to be stable biologically, making it usable in therapeutic applications [5].

IMPORTANT CHARACTERISTICS OF PNA MOLECULES

1) PNAs have extremely high affinity, specificity, and sensitivity to interact with nucleic acids (RNA/DNA), which is 50-100 times stronger than a typical DNA/DNA or DNA/RNA hybridization.

2) They possess a very high hybridization ability.

3) The occurrence of a mismatch or single nucleotide polymorphism (SNP) in their hybridization with DNA or RNA leads to a very strong destabilizing effect in the hybrid

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formation, which strongly reduces the annealing temperature.

4) PNAs are very stable molecules both chemically and biologically.

- Extremely high chemical stability to temperature and pH.

- Extremely high biological stability to nuclease and protease enzymes, meaning that they are resistant to enzymatic digestion and hydrolytic processes.

5) Their hybridization with such nucleic acid molecules as DNA is independent of environmental salt concentrations.

6) They attach to DNA or RNA strands the same as common Watson-Crick attachments.

7) The formation of a triplex with DNA is possible when PNA containing two homopyrimidine oligomers interlinked by a flexible linker reacts with the DNA homopyrimidine fragment. In such a case, a PNA strand through Watson-Crick pairing and the other via Hoogsteen pairing interact with DNA, with a DNA strand extruding the triplex structure, which will have many applications in gene expression regulation.

8) They have an asymmetric, peptide-like, and uncharged structure, which is hydrophilic and water-soluble.

- Although the uncharged nature of PNA facilitates its delivery through the cell membrane, this transfer into the cell is a difficult process requires using a variety of carriers.

- The uncharged nature of PNA molecules results in their much stronger interconnection (than DNA/DNA and/or DNA/RNA) due to the lack of electrostatic deterrence with DNA and RNA molecules.

9) Tm is much higher for PNA hybrids than other hybrids (DNA/DNA and/or DNA/RNA), with Tm values of 50 and 70 °C for PNA hybrids of 10mer and 15mer, respectively.

It is noteworthy that triplex structures with DNA are better formed in acidic pH (4.5-6.5) in vitro, but the main prerequisite is the presence of PNA pyrimidine.

As mentioned above, the PNA-PNA hybrid is extremely thermostable and is independent of environmental ionic potential, such that PNA-PNA > PNA-RNA > PNA-DNA > DNA-DNA. This stability, however, is strongly affected by basic compositions in the PNA sequence, such that Purine PNA >> pyrimidine PNA; among pyrimidines, on the other hand, homopyrimidines show very high stability.

Due to the very high binding ability of PNA molecules with DNA, it is not necessary to design long PNA oligomers hence fragments of 20-25 nucleobases are appropriate [6-10].

PREPARATION AND STORAGE OF PNA SUSPENSION

PNA can be easily dissolved in 0.1% trifluoroacetic (TFA) and the prepared stock can be stored frozen for a long time [7].

PNA hybridization and the salt effect

As denoted previously, the Tm of the PNA molecule does not change significantly with changes in environmental ionic potential. As such, the Tm of a 15mer PNA duplex only decreases 5 °C with an increase in NaCl concentration from 10 mM to 1 M, but the altered concentration has a very strong effect on the Tm of DNA/DNA hybridization. As a result, PNA can serve as a very suitable probe for the identification of target sequences at low salt concentrations.

Tm drops significantly due to a mutation in the allele of a gene and the incidence of a mismatch with PNA. In mismatch detection, therefore, DNA probes are the winners in competition with PNA probes, and this hallmark can be used in the detection of allele type [8].

Advantages of PNA molecules with very stronger pairing characteristics

PNA fragments with very shorter lengths can be used as probes, with lengths of 20-25 meres for DNA probes, but those of 13-17 meres long also operate well as PNA probes.

2) This hybridization is over 100 times faster than DNA/DNA and/or DNA/RNA hybridization. For example, if DNA hybridization lasts about 3 h to overnight, PNA hybridization will last 30-45 min.

3) As mentioned above, the incidence of a mismatch in this hybridization will result in more destabilization than typical DNA/DNA duplexes. This will significantly reduce Tm, leading to a decrease in the specificity because of a reduction in the annealing temperature as well [9-13].

Some disadvantages of using PNA

1) It takes a high cost for mass production as it is produced synthetically.

2) In the PCR process, the polymerase primer cannot detect its C-terminus and only detects the 3’ terminus.

3) It cannot be amplified by the cloning process [14].

SOME PROBLEMS OF PNA IN CLINICAL APPLICATIONS

1) The dose and toxicity problem

A dose that responds properly in vitro is toxic to the body (both PNA itself and the associated molecules such as cationic peptides); hence, dosimetry should inevitably be done to determine the toxicity level.

2) The delivery problem: It is difficult to deliver it into target tissue cells and has a low efficiency [15].

PCR clamping

This feature can be used in the detection of point mutations or SNPs in which an allele-specific primer competes with an SNP-containing PNA primer. If the SNP occurs in the relevant allele, PNA will win the competition (due to its higher Tm in normal conditions) and PCR will cease. If SNP does not occur, PNA will have a mismatch, Tm drops, and consequently, the primer will be the actual winner, leading to the occurrence of PCR (due to a higher Tm), which will be visible on the PCR agarose gel (Figure 1) [16].

Figure 1: The use of PNA in the PCR process

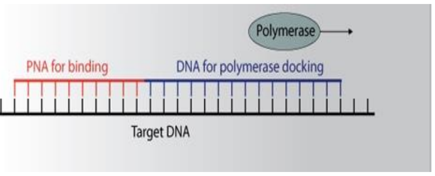

VARIOUS APPLICATIONS OF PNA MOLECULE

PNA molecule can be applied:

1. As a probe for the diagnosis and detection of biological threatening diseases, including:

1.1 Fluorescent In Situ Hybridization (FISH) probe

In this method, PNA labeled probes are utilized to detect certain telomeric reigns of the human chromosome or other specific sequences in various organisms. An example of this application in the detection of a conserved sequence on the rRNA of Staphylococcus aureus is depicted in Figure 2.

Figure 2: The use of PNA molecule as a specific probe in FISH

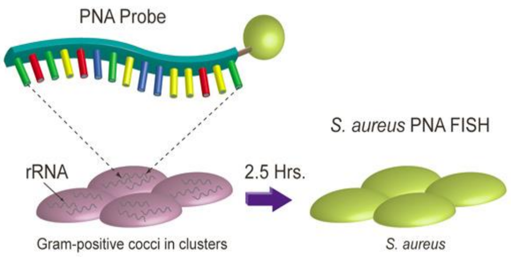

1.2 Detection of SNPs as described above
1.3 Light UP probe (or Q-PNA probe)

PNA is used as a probe in real-time PCR (RT-PCR) so that fluorescein and a quencher can be placed on each terminus of the molecule. A molecular beacon model of RT-PCR is shown in Figure 3.

Figure 3: The use of PNA as a probe in RT-PCR

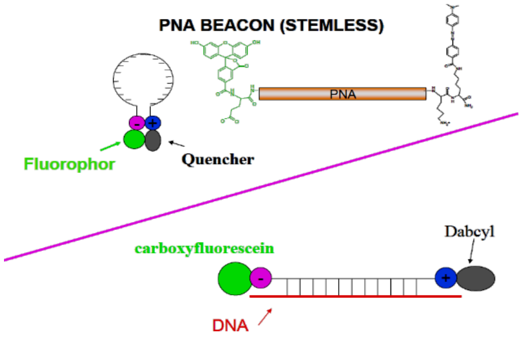

2. As a molecular tool in functional genomics research, including:
2.1 A probe for the northern and southern blot processes
2.2 PCR Clamping
2.3 In the separation and purification of the target DNA molecule
2.4 PNA array, which can be used in the PNA-based microarray technique.

3. The use of PNA as regulator drugs, including the antisense and antigene effects and so on, is one of the most important applications of PNA that has been the focus of worldwide research [17], which is discussed further in the following.

In the antisense application, PNA is designed against cellular RNAs, and in the antigene application, PNA is designed against the gene fragment (DNA). This indicates that many genetic diseases can be treated that are caused by defects in a gene function and/or overexpression; it can also be effective in cancer treatment, which often results from disrupted gene expression. In other groups of diseases, PNA is designed against cellular RNAs, such as in AIDS, where cDNA is synthesized from cellular RNAs through the action of reverse transcriptase. If PNA can react with RNA, it will inhibit the action of this enzyme on RNA and ceases the above reaction, thereby controlling the disease [18].

GENETIC REGULATOR DRUGS

1. The antigene technology

In this application, PNA is used as a gene inhibitor that needs to form a triplex with a double-strand DNA. Based on nucleobase combinations in DNA, this PNA is designed against external gene enhancer fragments that play a key role in the onset of transcription to inhibit the transcription process by RNA polymerase. This will occur when the designed PNA is attached to the non-coding strand of the gene enhancer as it plays the role of a template. PNA can be designed against the promotor region (−10 and −35) to further inhibit the non-coding strand and is not accessible to the enzyme. PNA can also be designed against the internal gene enhancers or other internal gene sequences containing homopurine sequences and their strong hybridization with DNA results in incomplete transcription of the gene and no expression of the target gene; it can also be very effective in cancer treatment as mentioned above [19].

2. Direct activation of gene

The gene is activated in this application unlike the antigene effect aiming at inhibition of gene expression as described above. The triplex forms in such a way that will extrude the non-coding strand. PNA is designed against the coding strand concerning nucleobase combinations and will be used in many genetic diseases caused by a defect in the functional expression of a gene. The target gene, which has become part of the heterochromatin, will occasionally return to the euchromatin by the effect of PNA and become accessible to transcription enzymes [20].

3. The antisense technology

This application uses synthetic PNAs against the transcribed gene product (sense-RNA) such that the PNA oligomers are started against the Shine-Dalgarno sequence, the codon region, or the internal mRNA sequences; in the latter case, it results in the inhibition of protein elongation process by the ribosome. In most cases, antisense PNA is simultaneously designed against the Shine-Dalgarno sequence and the codon region, as these two are not much apart. In such cases, the strong PNA/RNA hybrid becomes a stable duplex and is prevented from the translation process [21].

4. Inhibition of 16S rRNA

In this case, PNA is designed against rRNAs in the ribonucleoprotein (RNP) structure, the most important of which in bacteria is the 16s as it plays a role in the attachment of ribosome to the Shine-Dalgarno sequence and inhibition of 16S rRNA, i.e. inhibition of ribosome attachment to mRNA, thereby inhibiting protein synthesis. Existing evidence indicates that PNAs designed against 16S rRNA and 23S rRNA segments play a bacteriostatic role (cessation of bacterial growth and protein synthesis). These PNAs are considered effective drugs and genetic antibiotics (instead of chemical antibiotics) [22].

5. Inhibition of microRNAs

These RNAs play a regulatory role in gene expression. A PNA oligomer can be designed to inhibit the function of a given mi-RNA in which mi-RNA, as an inhibitor of certain gene expression, is bounced out to resume the expression of such genes. This application can be utilized in many diseases caused by the aberrant presence of mi-RNAs (where they must not be present) [23].

6. Inhibition of telomerase function

A reason for cancer cell immortality can be the continuous activity of this enzyme and its re-expression due to the activity of a series of oncogenes. If the PNA fragment is designed against the RNA part of this enzyme, it inhibits the enzyme function and cures cancer [24].

7. Alternative splicing

Here, a specific PNA is designed against splicing-specific sequences in pre-mRNA to change the mRNA product and ultimately achieve target gene expression. Accordingly, PNAs can play a role as regulator drugs in the treatment of bacterial and viral infections, as well as many other diseases [25].

SOME IMPORTANT CLINICAL APPLICATIONS OF PNA

1. Cancer therapy

As described above, this application can be achieved by the suppression of telomerase. Thus, PNA can also be considered as an anti-cancer drug, or exert antisense and antigene effects on such genes as Bcl2. This begins by designing a PNA probe against the codon region, the Shine-Dalgarno sequence, or homopurine sequences on the coding DNA strand to reduce the expression level of Bcl2 and prevent cancer cells from using this mechanism for their survival [26].

2. Treatment of biological threatening diseases

Research has shown that the PNA molecule can effectively inhibit the function of reverse transcriptase that has a contribution to disease development. Indeed, this enzyme synthesizes a cDNA by the detection and transcription of template RNA. An appropriate and specific PNA oligomers can be designed to effectively improve these viral infections (27).

PNA DELIVERY STRATEGIES TO THE CELL

As denoted before, it is very difficult to deliver PNA to eukaryotic and prokaryotic cells, with low efficiency. For this reason, strategies are employed to facilitate the delivery process, some of which are described below.

1. Making some modifications in their main backbone structure

2. Pairing them with peptide delivery groups

3. Paring them with cationic peptides (lysine and arginine

residues)

4. Coupling them with some DNA oligomers

5. Coupling them with specific ligands (e.g., antibodies) linking to cell surface receptors

6. Delivery using liposomes for which typical liposomes should be used instead of cationic liposomes due to their toxicity despite higher efficiency.

7. By the use of cationic polymers

Figure 4 illustrates a schematic of the delivery of a PNA molecule using liposomes.

Figure 4: Delivery of PNA molecule to a eukaryotic cell using liposomes

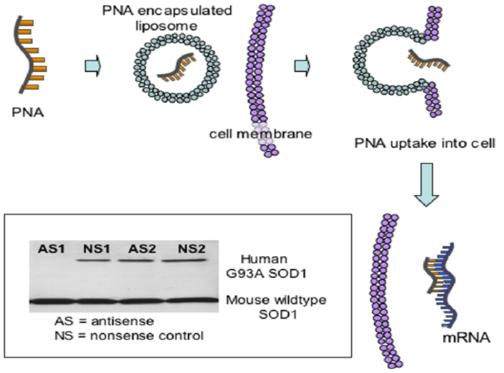
PNA
PNA encapsulated liposome
cell membrane
PNA uptake into cell
AS1 NS1 AS2 NS2
Human G93A SOD1
Mouse wildtype SOD1
AS = antisense
NS = nonsense control
mRNA

RESULTS AND DISCUSSION

As noted above, the main backbone of PNA structure forms by repetitive units of N-(2-aminoethyl) glycine connected by peptide bonds. As with peptides, these are drawn by C and N terminus that correspond to the 3′ and 5′ terminus in DNA (Figure 5).

Figure 5. Comparison of PNA, DNA, and protein structures

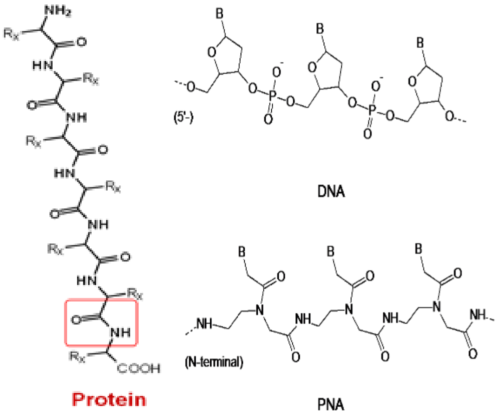
NH2
Rx
O
HN
NH
COOH
Protein
B
O
(5'-)
P
DNA
NH
N
(N-terminal)
PNA

Tm changes are insignificant for PNA with fluctuations in environmental ionic potential in comparison to DNA and RNA. Thus, PNA can serve as a very appropriate probe for the detection of target sequences at different salt concentrations (Figure 6) [28].

Figure 6: Ionic and thermal changes of PNA/DNA and DNA/DNA

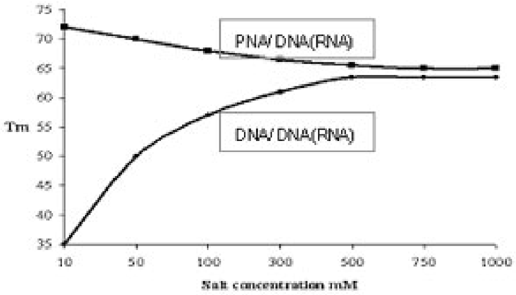

As mentioned above, PNA is designed against the gene fragment (DNA) and cellular RNAs in the antigene and antisense techniques, respectively (Figures 7 and 8).

Figure 7: Inhibition by the antigene technique

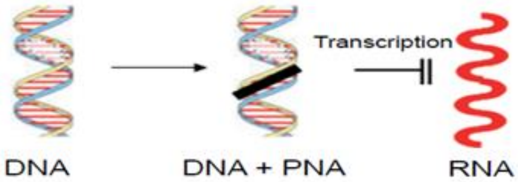
Transcription
DNA
DNA + PNA
RNA

Figure 8: Inhibition by the antisense technique

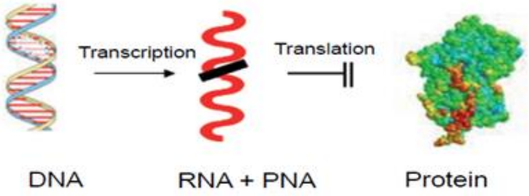
Transcription
Translation
DNA
RNA + PNA
Protein

Figure 9: Changes in β-galactosidase activity with rising inhibitory PNA concentrations

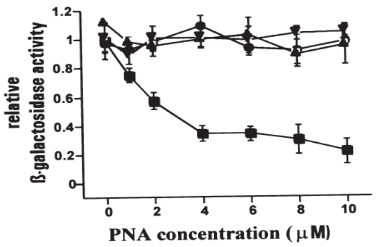

Anti-β-galactosidase PNA was reported to inhibit the expression of the β-galactosidase reporter gene in E. coli AS19. As shown in Figure 9, the activity of this enzyme decreases with an increase in PNA concentrations, indicating the decreased expression of this gene by a specific PNA. Control samples are presented in the diagram (Figure 9) showing a change in the enzyme activity.

Therefore, these two critical technologies can help to effectively improve diagnostic and therapeutic goals.

CONCLUSION

Overall, it can be concluded that PNA nanostructures are currently considered by scientists to have a wide array of applications.

Accordingly, further investigations on these nanostructures will provide an effective tool to achieve the above diagnostic and therapeutic goals.

Medical and molecular biotechnology has been revolutionized with the synthesis of PNA.

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ORIGINAL ARTICLES

Concepts for the implementation of a technological platform for the production of specific antidotes for CBRN medical protection

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Abstract: *All large armies (EU and/or NATO) have pharmaceutical production facilities to provide the necessary antidotes for the troops and the population: The French Army Medical Directorate produces many military-specific pharmaceutical products in its own laboratory, the Turkish Army owns its own medicines factory, including CBRN antidotes, the US Army, in addition to a sustained drug purchase program in the pharmaceutical industry has launched a new concept: Pharmacy on demand.*

Providing the armed forces with antidotes is a necessity, the concept for their endowement in this sense can be based on imports (sometimes impossible to achieve) or on the national development of a specialized production structure.

The design or construction of a specific production capacity for antidotes can be accomplished on multiple variants, with a complexity proportional to the identified need. The total costs are high, but the objective and implementation of effective antidote supply mechanisms is a security guarantee for the armed forces and the civilian population (through commercialization to allied forces), given the risks of terrorist threats and hybrid warfare.

Keywords: *medical protection, antidote, production, technological platform, medical countermeasures, pharmaceutical technique, orphan drug production*

INTRODUCTION

The creation of a technological platform for the production of antidotes for CBRN medical protection is presented in synthesis, as an initial medical approach to a complex problem of the pharmaceutical industry, resulting from a practical need for therapeutic countermeasures in the field of CBRN protection and a strengthening the capacity of action.

The strategic need to create a technological platform for the production of specific antidotes for CBRN medical protection has led to the concept of a technological platform for the production of specific antidotes, on technical, constructive and product volume variants, depending on needs and possibilities [1].

1. Existing solutions in other countries

Worldwide, large armies (EU and/or NATO) have pharmaceutical production facilities: The French Army Medical Directorate produces many military-specific pharmaceutical products in its own laboratory. The Turkish Army owns its own medicines factory, including CBRN antidotes, the US Army, has a sustained antidotes purchase

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program in the national pharmaceutical industry [2].

The US has begun research for a new concept: Pharmacy on demand. Recent advances within the Defense Advanced Research Projects Agency (DARPA) show that it is possible to achieve complete, miniaturized and flexible platforms for the manufacture of pharmaceuticals. Current progress related to continuous flow synthesis, chemistry, biological engineering coupled with online analysis, automation and improvement of control measures may be elements that enhance the pharmaceutical supply chain and drug production. These new technologies, together with scientific advances, can be the prerequisites for authorizing the "on demand" manufacture of drugs on the battlefield and in other austere environments, increasing the readiness for CBRN threats, increasing the ability of medical authorities to respond to natural disasters and other catastrophic events, minimizing drug shortages, addressing gaps in the orphan drug market, supporting ongoing efforts towards personalized medicine, and improving access to needed medication in disadvantaged areas around the world. The modular platforms being developed through DARPA programs can in the future improve the safety, efficiency and timeliness of drug manufacturing [3].

2. General considerations regarding constructive variants of technological platforms for the production of antidotes for CBRN medical protection

In order to create an image regarding the international context, the Military-Medical Research Center through the Army Information General Directorate has obtained information on the antidote portfolio and how to provide them to different NATO armies [4].

The concept of providing specific antidotes for the protection of the armede forces and the population must meet the Security needs of Romania, and must take into account complex economic, social and legislative realities. Although many states have their own production facilities, they do not provide their own antidote portfolio, being approved for use in that state, based on special legislative regulations. If there are entities willing to sell antidote products (excluding the top ones), over time the company may modify the sales policy or may go bankrupt, the Romanian state remaining out in the open in regars to their provision.

Some CBRN Antidotes have a special regime of use, being necessary to be used in a very short time from the action of the causative agent, having to be in stock and distributed to the armed forces. Also, the preventive antidotes for counteracting the effects in the event of exposure must be in stock in sufficient quantities. It is therefore necessary to modify the regulations regarding the establishment and maintenance of the stocks of these products, assuming from the beginning a periodic, bearable financial loss, in exchange for obtaining the medical security of the troops and maintaining the action capacity.

In the same way that the Air Force provides Security and not revenues, the costs for the endowement with technological means, personnel training and operation of the technological means being important, with much lower costs the security of the personnel can be ensured by creating a specialized structure in the production of antidotes.

The concept of developing the Technological Platform for the production of antidotes for CBRN medical protection is presented in synthesis, as an initial medical approach to a complex problem of the pharmaceutical industry, resulting from a practical need for CBRN medical countermeasures in the field of CBRN protection. It should be noted that worldwide, the pharmaceutical industry, which is one of the most profitable in the world, was removed from the Health field and was included in the Industry field. This denotes the fact that the economic-financial function (the profit of the investors) prevails the social function, which ended up being considered secondary. Large producers bypass antidotes as orphan drugs, having a small retail market, due to the fact that the profits would not cover the investment. Thus, antidotes are difficult or impossible to obtain in crisis situations. Therefore, it is up to the State to guarantee the operability of the armed forces under the conditions of CBRN events, even with slight financial losses [5, 6, 7].

Depending on the need established by the competent bodies, several variants of CBRN antidote production facilities can be designed. These variants must have flexibility, depending on the situation, and their production will adapt immediately to the estimated volume for the given context. Whatever the risk, direct or indirect consequences with CBRN classification will be projected on the population requiring the adoption of specific and appropriate medical countermeasures, cascading with the management of epidemics or epizootics. It shall suffice to recall the aftermath of the earthquake in Haiti that led to the worst epidemic of cholera in recent history, as well as the civil war in Yemen that caused a catastrophic cholera epidemic. In both situations, multiple systems were affected that facilitated the outbreak of epidemics, the national health services were not properly prepared, and the international aid appeared late and in a volume that failed to cover the necessary.

Depending on the identified needs and the allocation of funds by the competent bodies, the solutions for the manufacture of specific CBRN and related antidotes extend over a wide spectrum. In the following are provided three variants: minimum level (galenic laboratories for antidote production), medium level (pharmaceutical laboratory for antidote production) or maximum level (drug factory or specialized department for antidote production).

The ideal solution is a new drug factory that complies with European Union GMP and GLP regulations. In principle, the Integrated Technology Platform for the production of specific antidotes for CBRN medical protection should include:

- Pharmaceutical laboratories for the production of antidotes, by categories;

- Laboratory for pharmaceutical control and quality assurance;

- Production laboratory for chemical synthesis of pharmaceutical substances;

- Microbiology laboratory for in vitro analyzes and tests of pharmaceutical microbiology, required by the regulations in force, such as the Romanian Pharmacopoeia X and the European Pharmacopoeia 10/2016 [8, 9].

- Biobase for in vivo analyzes and tests on experimental animals, required by the regulations in force, according to the Biosafety Guide for medical laboratories, MS 2006;

- Research and development laboratories for applying own research in pharmacology and/or pharmacy;

- Annex facilities and utilities.

The costs with the initial investment and the exploitation for such a production facility of the antidotes (orphan drugs) cannot be covered by sales, which is an impediment in making a political decision to implement such a solution. Given the major risk for public health in crisis situations and the possible catastrophic implications at national and international level, it is necessary to establish directions of action, depending on needs and possibilities, on variants. This pharmaceutical industry facility must be subordinated to a governmental structure for rapid coordination between central structures in order to limit the effects of the crisis situation.

The technological platform for the production of antidotes for CBRN medical protection, depending on needs and possibilities, can be developed in three variants: minimum, average or maximum.

For the medical protection against CBRN agents, the minimum necessary antidotes for the peacekeeping units belonging to the Ministry of National Defence, for the Ministry of Internal Affairs and for the secret services involved in response and the specific risk groups (personalities, medical service, etc.) must be calculated on the basis of a political-military decision, which we estimate at over 100,000 individual doses for each assortment.

3. Minimal variant: adaptation of existing spaces

If the risk and threat become imminent and there is not yet a facility in the country to produce CBRN antidotes, it would be possible based on a government approval with the partial suspension of the provisions of the Medicines Law, to prepare by micro-production (daily galenic lots) the antidotes necessary, for the possible emergency use of exposed, contaminated or ill personnel, as a result of the effect of CBRN attacks. Moreover, at the end of the Medicines Law there is a paragraph that provides this exception for crisis situations [10], respecting the basic rules for the operation of a pharmacy with its own laboratory (galenic), under the coordination of the College of Pharmacists of Romania and with the approval of the Pharmaceutical Inspection. The antidotes produced in such manner, would be from the spectrum of medical prototypes against chemical warfare agents (CWA): neuroparalytics, vesicants, asphyxiants, etc., against radiological warfare agents (RWA): radioactive isotopes and irradiation, as well as biological warfare agents (BWA): living biological agents, toxins [11]. In the event of force majeur, the necessary specialized personnel can be provided from the competent personnel belonging to the military scientific research structures, seconded for technical assistance to the facility that ensures the first phase microproduction. The benefits are represented by the minimization of costs and the deficiencies are represented by latency with which the affected personnel gets the antidotes, sometimes with disastrous effects and the creation of stocks of raw materials.

4. Medium variant: modernization of existing spaces

Since 2002, in the context in which Romania was to become a member of NATO and the EU, the idea of creating an infrastructure for the production of antidotes had been proposed, by modernizing some areas in the heritage of the Ministry of National Defence.

Thus, with the support of a specialized company from Austria, the necessity of using an industrial type construction or pavilions on reconfigurable reinforced concrete structure was proposed.

In the case of pavilions, on the ground floor could be arranged the department of self-injecting syringes for antidotes (manufacture, filling and storage), with the creation of clean room areas for an automatic line of Austrian origin. Upstairs could be created a department of solutions and powders as well as pharmaceutical control laboratories. Also, related spaces of the building (eg attic / bridge) could be adapted for storing raw materials, finished products or laboratory technique. The connections between the departments and the storage spaces, in order to comply with all the necessary flows, would be made wherever necessary and from light constructions outside the existing spaces.

The estimation made by the Austrian specialists for the plant for the manufacture of self-injecting syringes exceeded the value of 10 million euros, and for the whole "turnkey" project, with spaces, equipment, consumables, commissioning and training of the personnel amounted to about 19 million euros. It was suggested to include a financial reserve of 1 million euros for operation (raw materials, utilities, etc.) the general total amounting to 20 million euros. At the level of that period the investment seemed very high, but it was considered to be bearable by equipping the army with the avoidance of imports, ensuring the necessary for the protection of the population and recovering a part of the investment by the possibility that the surplus of products could be exported to the allied armies, following that Romania would become a supplier of antidotes (manufactured according to European Union standards) for the eastern flank of the alliance. The investment was likely to become profitable for the country.

Another explored work option was the creation of a facility in collaboration with the Turkish army’s medicines factory, which would have turned out much cheaper, but which did not have approvals in line with European norms. This meant that after the accession to the EU the antidotes would no longer be used in Romania and, therefore, they could not have been exported to other EU countries. This option was eventually discarded.

The average variant could cover the need for specific antidotes for medical countermeasures against CBRN weapons/agents for the general mobilization military personnel (the Ministry of National Defence, The Ministry of Internal Affairs, the Secret Services), including for the risk groups and for the vulnerable population, with the capacity to produce over 1 million doses from each assortment.

5. The maximum variant: de novo construction of specific facilities

The facilities for the production of CBRN antidotes must comply with the CBRN protection requirements, as the manufacturing conditions for the drugs are more severe, according to the Medicines Law and must comply with the European norms in force.

Under these conditions, the old variants are no longer sufficient, except as a breakthrough formula in case of force majeure and therefore an approval for the establishment of a new building shall be needed, whose design, construction and equipment must comply with the minimum requirements for the pharmaceutical industry. According to the Medicines Law, it would result in a medicines factory, which should include: adequate space, unique flows, equipment, specialized personnel and procedures, according to the current requirements in the field.

This facility may be the only one able to ensure the complete protection of Romanian troops and of the civilian population, including of the military forces on the national territory and, in addition, an eventual export within the NATO or EU alliance. Depending on the political-military decision, this production could reach over 20 million doses from each assortment.

The space should in principle be presented as a miniature medicines factory or at least as a pharmaceutical manufacturing laboratory, in accordance with the WHO GMP and GLP standards [12, 13, 14]. The construction should be based on the concept of clean rooms in a separate or independent pavilion, resistant to an earthquake of 8 degrees on the Richter scale, with walls, ceiling and floor watertight and washable, with a height of approximately 5 m (for false ceiling and high floor for masking the pipes system), watertight doors and windows, divided into spaces for the main activities. The result is a total of at least 500 square meters, of which a minimum of 400 square meters inside the pavilion and a minimum of 100 square meters outside for the assembly of the technical installations. The clean rooms must comply with the provisions of the Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) guidelines, which are updated by the National Agency for Medicines and Medical Devices, depending on the biosafety level of the area: clean, aseptic, sterile.

The division of space and the access ways must allow the creation and the compliance of unique flows for the: personnel, equipment, materials and raw materials, for semi-finished products and finished products, solid and liquid waste, air (with a cascade of negative or positive pressure gradients, as applicable). This aspect is essential for the protection of the product and the operator. If they are to be protected from natural or intentional contamination of the environment, the enclosure will have positive pressure on the outside. If on the premises they are working with hazardous materials (biological, chemical, radioactive, etc.), a negative pressure must be maintained on a permanent basis, in order to avoid leaks that contaminate the outside. This is also one of the operating principles of highly secured laboratories (P4 or BSL4) [15].

The pavilion must have permanently complex systems of protection and alarm for the perimeter, for the building and for the workrooms. The construction and equipment works can only be performed by specialized and authorized companies in the field, that have the necessary experience, because the new objective must obtain manufacturing authorization for medicines, including injectables.

The endowment consists mainly of the purchase of a new plant for the manufacture and the filling of self-injecting syringes (about 10 million euros) and equipment for conditioning and filling the vials with powders, tablets, solutions, ointments, etc., of pharmaceutical control, packaging and of the mentioned technical installations, of the current pharmaceutical equipments: apparatus, inventory objects, consumables, protective equipment, raw materials, packaging, furniture, office supplies etc. (approx. 10 million euros) which are added to the construction costs, estimated at 23 million euros, of authorization, accreditation and training. Therefore, we are speaking of a total investment of about 50 million euros.

The necessary specialized personnel shall consist of a minimum of 23 specialists.

The procedures must describe each operation and must comply with the recommendations of the European Union’s Good Manufacturing Practices (GMP), Good Laboratory Practice (GLP), Good Clinical Practice (GCP) and must be endorsed by the National Agency for Medicines and Medical Devices of Romania (NADMR).

OBSERVATION

The need and the opportunity to design, build and operate this specific objective of pharmaceutical production is based on the realization of a prefeasibility study, which must be done with the multidisciplinary consultation of the specialists from qualified institutions, future operators and/or beneficiaries of this investment.

The need is conditioned by:

- The spectrum of threats in the current international political-military context, doubled by the threat of international terrorism, including with ADM CBRN;

- In order to ensure survival in CBRN events, endowment with antidotes is mandatory, in medical practice and especially in military medicine;

- In some crisis situations, the provision of antidotes may be deficient due to the lack of sufficient quantities and the impossibility of provision from import

- The first necessity is represented by the endowment with self-injection syringes of the active military staff, followed by the de-commisioned staff and the risk population, according to NATO norms.

Implementation costs: the total costs are important, but the objective can be amortized in a short time and the social benefit is very high; the average variant (proposed by the Austrian specialists) is estimated at 20 million euros; the optimal variant (the antidote medicines factory, in accordance with WHO, GMP norms) exceeds 50 million Euro, and the minimum variant (the adaptation of the existing one in case of force majeure) without self-injection syringes, under 1 million euro. Operating costs: depending on the variant and the production requirement, up to 1 million euros/year.

Conditions and legislation for certification/accreditation/ operation: Medicines Law, GLP, GMP, ISO 9001 norms.

It is possible to organize, within the military-medical system or in cooperation with the civilian system, a virtual structure for the production of specific CBRN antidotes, preventive and curative, and specific procedures that, in case of force majeure, can start producing small quantities of antidotes simple, necessary to the affected people.

Military history shows that, unfortunately, most of the time it is preferred to invest in offensive equipment and means at the expense of the defensive ones, ignoring the importance of protecting the troops and the civilian population. The human being is the most precious asset, both socially and militarily, since modern warfare uses sophisticated equipment whose use is only operated by highly qualified personnel, sometimes impossible to replace. Neglecting the protection of human capital can constitute the "Achilles heel" within a strategy of defense or army endowment, complex problems of the pharmaceutical industry, resulting from a practical need for CBRN medical countermeasures in the field of CBRN protection. This vulnerability is common to all armies, from all times.

CONCLUSIONS

The article presents brief elements regarding the development of a production/ microproduction facility of antidotes for CBRN medical protection, as an initial medical approach to a complex problem of the pharmaceutical industry, resulting from a practical need for CBRN medical countermeasures in the field of CBRN protection.

The problem is extremely complex, for choosing the optimum solution being involved many aspects (the doctrine on defense, the need for the military and population) established by the decision-makers taking into account the short and medium term threats to the security of Romania, the provision of funds and human capital etc.

It is possible to set up or build a specific production/ microproduction capacity for antidotes, depending on the complexity. The total costs may be high, but given the risk of terrorist actions of hybrid warfare in our geographical area, the military benefit of strengthening the capacity for action and the social one are very high.

The purpose of this article is to bring into discussion elements on issues of medical-military interest that can generate a constructive exchange of ideas between the decision makers that can lead to the identification of an optimal solution.

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ORIGINAL ARTICLES

Elastofibroma dorsi: clinical experiences of 19 cases

Hacer B. Yesilcay[1], Sencan Akdag[1]

Abstract: *In this study, symptoms, functions, and outcomes of patients who underwent surgery with the diagnosis of Elastofibroma dorsi between 2007-2019 in our clinic were discussed retrospectively.*

A total of 19 patients were operated on with the diagnosis of Elastofibroma dorsi in our clinic. The demographic characteristics of patients such as age and gender, symptoms, clinical findings, diagnostic and radiological features, surgical procedures, results of surgical treatments, and postoperative follow-up results were evaluated based on the records.

The mean age of patients who underwent surgery was 55.7 and there were 13 females and 6 males. The most common clinical complaint was swelling (61%). Seven of ED were located on the right side, 3 of them were located on the left side and 7 of ED were located bilaterally. The mass in all cases was over 5 cm in diameter, complete surgical excision was done via muscle-sparing technique. All patients were followed up postoperatively and there was no recurrence.

ED should be considered in terms of differential diagnosis when middle-aged patients present with a mass in the scapular region and shoulder pain. Total excision is surgically sufficient in symptomatic patients.

Keywords: *elastofibroma dorsi, shoulder pain, chronic back pain*

INTRODUCTION

Elastofibroma dorsi is a benign, rarely seen, slow-growing soft tissue lesion. First described by Jarvi and Saxen in 1961. The lesion is usually located at the inferior angle of the scapula., deep to the serratus anterior, and may be attached to the periosteum of the ribs [1]. The etiology is not known clearly [2]. Symptoms are usually chronic back pain, stiffness, swelling, snapping of the scapula, and increased pain during shoulder movement. Noninvasive imaging methods such as ultrasonography, computed tomography, and magnetic resonance imaging are used in the diagnosis of elastofibroma dorsi [3]. Total excision is the best treatment to prevent relapses and relieve symptoms [4]. In this study, we describe our experience of diagnosing and treating elastofibroma dorsi patients between 2007-2019.

METHODS

Between 2007 and 2019, 19 patients operated with the diagnosis of elastofibroma dorsi were retrospectively reviewed. The cases were evaluated in terms of age, gender, complaints, clinical findings, diagnostic and radiological features, surgical applications, surgical treatment results, and postoperative follow-ups. The radiological examination was applied by ultrasonography, thorax computed tomography (CT), and magnetic resonance imaging (MRI).

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The mass diameters of all cases were measured by radiological evaluation. None of the cases were directly diagnosed by biopsy. Excision was undertaken in all cases after pre-operative consent was obtained. A transverse or parabolic incision at the inferior pole of the scapula was used. All operative samples were sent for histopathological evaluation. The cases were followed up in six-month periods after surgery.

RESULTS

13 of the cases were female (69%), 6 were male (31%) and the mean age was 55.7 (31-68) years. The most common clinical complaint was swelling (61%). Other complaints were chronic back pain (31%), snapping of the scapula (23%), and increased pain with shoulder movement (19%). In our series, 27% of cases were found to be asymptomatic. The mean duration of symptoms was 20 months (3 to 96). The lesion was located in the subscapular region in all of the patients, being unilateral in ten and bilateral in nine. Of the unilateral tumors, three lesions occurred on the left side and seven on the right. The radiological examination was applied by ultrasonography and thorax computed tomography (CT) for all patients (Figure 1). Magnetic resonance imaging was applied in 7 cases that could not be evaluated adequately by thorax computed tomography.

Figure 1: CT scan image of a patient

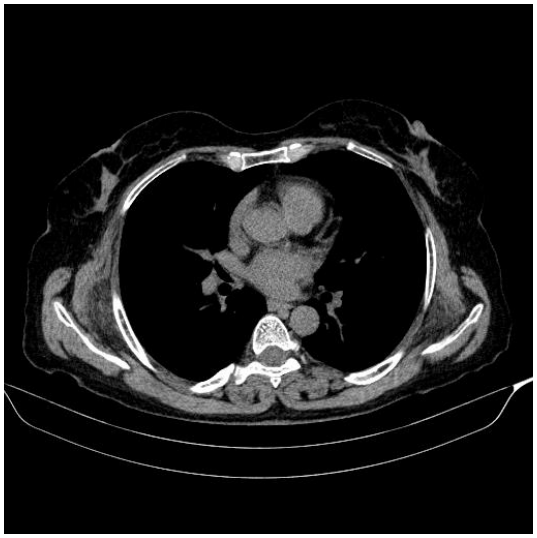

In all cases, total excision of the lesion was provided via muscle-sparing technique then, hemovac drain which was removed 24-72 hours after the operation was placed in the subscapular area and the layers were closed following the anatomy. All excised samples were sent for pathological examination. In the pathological examination macroscopically, solid lesions were observed with a yellow and white cross-section surface covered with fibrous capsule and containing adipose tissue (Figure 2). In the microscopic examination of hematoxylin-eosin sections, mature adipocytes and elastic fibrillar stained positive with Von Gieson were observed. Postoperative complications developed in four (21%) of operated cases; in one (5%) case antibiotic allergy was observed and in three (16%) cases seroma requiring needle aspiration was observed. The mean length of hospital stay was 4 days (2 to 8) and the mean follow-up was 5 months (3 months to 2 years). No recurrence was observed during the follow-up period.

Figure 2: Solid lesions with a yellow and white cross-section surface covered with a fibrous capsule and containing adipose tissue

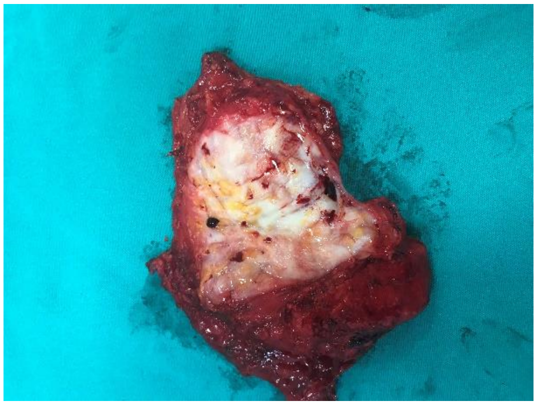

DISCUSSIONS

Elastofibroma dorsi is a benign, unencapsulated, slow-growing soft tissue tumor, is usually seen in women, in the 5th and 6th decade of life. First described by Jarvi and Saxen in 1961 [1]. Despite it is traditionally considered to be rare, Jarvi and Lansimies showed, in a series of 235 postmortems in patients older than 55 years, changes in the subscapular thoracic fascia similar to elastofibroma in 24.4% of women (29 of 119) and 11.2% in men (10 of 89) [5]. The age and gender of patients in our series were consistent with the literature.

ED is often localized in the subscapular region which is between the rhomboid and latissimus dorsi muscles and the sixth and eighth ribs. However, other localizations such as deltoid muscle, foot, greater trochanter, olecranon, cornea, stomach, greater omentum, ischial tuberosity, intraspinal space, and chest wall have been reported in the literature [2, 6, 7].

ED is often unilateral and right-sided. The number of bilateral cases is around 10-60%. Bilateral cases can be developed synchronously or asynchronously [8]. In our study, the lesion was located in the subscapular region in all of the patients, being unilateral in ten (52,7%) and bilateral in nine (47,3%). 16 of the 28 ED lesions were localized on the right side (57,1%). Lesions were synchronously detected in patients with bilaterally located elastofibroma dorsi.

The pathogenesis of ED is not clear, several hypotheses have been put forward. Repeated micro-injuries between the chest wall and the scapula, the source of excess elastin production, and collagen degeneration could play a physiopathological role in this rare lesion [9].

This view has been supported by the higher ED prevalence particularly among individuals who work at hard manual labors. However, patients who have never been involved in hard manual work, as well as those with elastofibromas in different locations, have undermined this view. In our study 3 patients (15,7%) were manual laborers. The rest of the cases did not have heavy labor history or heavy sporting activities in their anamnesis records.

In a study investigating genetic anomalies in ED cases, changes in DNA copy number were observed in tumor tissue, mainly in the chromosome Xq12-q22 and 19 regions [10]. In a cytogenetic study by Mc Comb et al., they detected genetic instability in chromosome number 1 and translocation in number 8-12, stated that they may be neoplastic, not reactive, due to these clonal abnormalities. In the largest reported series of 170 patients with the lesion, a familial predisposition was suggested with 32% having a positive family history for elastofibroma [8]. In our study, none of the patients had a family history of ED.

There are also opinions such as reactive fibromatosis, degeneration due to vascular insufficiency, elastotic degeneration, and enzyme defect [8].

Clinical findings are mostly related to the size of the lesion. They often grow slowly and are asymptomatic. As the lesion grows, there is swelling in the back, increased pain with shoulder movements, snapping of the scapula, and chronic back pain [11]. In our series, the most common clinical complaint was swelling (61%), 27% of cases were found to be asymptomatic.

Imaging modalities for diagnosis include ultrasonography, CT, and MRI. Solivetti et al. reported that the use of diagnostic USG is an adequate and inexpensive method [12]. Kransdorf et al. reported that radiological evaluation with MRI or CT is compatible with histopathological evaluation [13]. The most important imaging modality is accepted as an MRI. Malghem et al. reported that fibrous tissues within the mass have similar signal characteristics with the surrounding muscle tissues in the MRI examination, while fat tissue has higher signal characteristics than the mass, and these findings are pathognomic for the mass [14]. In our study, the radiological examination was applied by ultrasonography and thorax CT for all patients. MRI was applied in 7 cases that could not be evaluated adequately by thorax computed tomography. In our series, radiological findings were compatible with the literature.

Lipoma, neurofibromas, metastatic lesion, primary or metastatic sarcoma, fibrosarcoma, synovial sarcoma, the desmoid tumor should be considered in the differential diagnosis. Needle aspiration or incisional biopsy may be performed to eliminate the possibility of malignancy. However excisional biopsy is often preferred due to diagnostic radiological evaluation's sufficiency [15, 16]. In our series, a needle biopsy wasn't performed.

Macroscopically, ED is in the form of a fibrous lesion of dirty white color, is non-encapsulated, and contains streaks of fat tissue. Some elastofibromas may have cystic degeneration. The histological appearance of the lesion is typical. In large areas, it contains hyalinized collagenous stroma and little amount of fat tissue in between. In hypocellular collagenous stroma, fibrils [6] and globules that show eosinophilic staining are striking. In sections of hemotoxylin-eosin, the presence of fibrils and globules is important for determining the location of the lesion and its diagnosis [4, 9].

Elastofibroma dorsi is treated with total excision, but surgery is not recommended for asymptomatic lesions smaller than 5 cm [17]. In our series, the mass in all cases was over 5 cm in diameter, complete surgical excision was done via muscle-sparing technique, which requires preparation of latissimus dorsi and serratus anterior muscle flaps.

The most common complications after surgical excision are hematoma or seroma. Therefore, after excision of the mass, the bleeding control should be performed cautiously [11]. In this study, we used hemovac drainage and a compression bandage to reduce these complications. Postoperative complications developed in four (21%) of operated cases; in one (5%) case antibiotic allergy was observed and in three (16%) cases seroma requiring needle aspiration was observed.

Local recurrence after total excision is rare and malignant transformation has not been reported. In the first recurrence, total surgical excision can provide a cure, but in subsequent recurrence, total excision may not be performed [9]. In our cases, no recurrence was detected during the follow-up period.

As a result, ED should be considered in terms of differential diagnosis when middle-aged patients present with a mass in the scapular region and shoulder pain. Total excision is surgically sufficient in symptomatic patients.

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VARIA

The conduct lists of military physicians Ion Arsenie and Bucur (Hilarius) Mitrea during the Mexican campaign (1864-1866)

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Abstract: *The study aims at complementing the historical knowledge regarding the participation of Dr. Bucur (Hilarius) Mitrea and Dr. Ioan Arsenie within the Austrian volunteer corps in Mexico, using as sources the copies of their conduct lists preserved in the military archives of Vienna. The new data concern the battles they took part in, together with characterizations of their behavior and personality. The latter consolidate the image built by former biographers in the case of Dr. Mitrea and help to sketch a less known portrait in the case of Dr. Arsenie.*

Keywords: *military physicians, 19th century, Transylvania, Mexico, Österreichisches Freiwilligenkorps in Mexiko*

INTRODUCTION

Ion Arsenie (Arseniu) and Bucur (Hilarius, Ilarie) Mitrea are among the most frequently mentioned Romanian military physicians in the Austrian Monarchy, even though their service for the House of Habsburg was of short duration and took place entirely within the Austrian volunteer corps in Mexico (Das Österreichische Freiwilligenkorps in Mexiko, henceforth ÖFM). Their later careers evolved differently, and so did the historians' interest in their lives and activity. However, the period they have spent in Mexico remains a biographical landmark to be taken into account. The present study will complement previously known data on the military activity of the two physicians during the "Mexican adventure". The sources to be used are copies of their lists of conduct, discovered at the War Archive in Vienna (Österreichisches Staatsarchiv, Kriegsarchiv), unknown to specialists to this day.

Dr. Ion Arsenie (Arseniu) (1838–1883) was born in Gura Râului (Sibiu County). Son of a priest, he studied medicine in Vienna between 1857 and 1862 [1]. He joined ÖFM in the autumn of 1864, with the rank of first lieutenant senior physician (Oberlieutenant – Oberarzt) in the first Austrian volunteer group that left for Mexico to support emperor Maximilian. Shortly after his return in Europe (1866), he settled in Romania, where he practiced medicine in Brăila, up to his death in 1883. Little is known about his professional activity, both as a civilian and as a military physician. Throughout the campaign in Mexico, he sent financial donations to the Transylvanian Association for Romanian Literature and the Culture of the Romanian People (ASTRA), as well as travel stories, published in the press of those times. He was also involved in other Romanian literary and scientific societies of the time, such as "România Jună" from Vienna, and remained a member and financial supporter of ASTRA up until the end of his life. There are no biographical studies dedicated to him exclusively, only fragments spread

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out in different papers, most of the times associated with Dr. Mitrea [2].

The life and activity of Dr. Bucur (Hilarius, Ilarie) Mitrea (1842–1904) were the subjects of numerous works of research, covering a great part of his biography. The future physician and explorer was born in 1842 and was the only son of a wealthy shepherd in Rășinari. He studied medicine in Cluj and later in Würzburg (where he defended his doctoral thesis), Berlin, and Vienna [3]. After failing in his attempt to occupy the position of a physician in his commune of birth (1864), he was initially employed physician on board of an immigrant ship to Australia (1865–1866) and afterward enrolled in ÖFM (March 1866), where he was active until the unit was disbanded by emperor Maximilian (December 6th, 1866). He returned to Europe in April 1867, attended further specialty studies at the University of Berlin, and then worked again as a ship physician on board at least two transatlantic voyages [4]. In March 1869, his enrolment application in the Dutch colonial army was accepted, as a 3rd class "Officer of Health" (officier van gezondheid). Dr. Mitrea worked as an army doctor in the Dutch army in Indonesia from 1869 until his retirement in 1894. His last advancement in rank took place in May 1890, from 1st class Officer of Health to 2nd class Chief-Officer of Health [5]. He was married to a local woman and had two children, both educated from small ages in Europe. He died in 1904, in Vienna, probably taking his own life, on the background of unsettled family issues [3]. He donated to the National Museum in Bucharest the most of his rich natural sciences collection, that he had gathered throughout his activity in Indonesia. The National Museum of Natural History "Grigore Antipa" now hosts what remains of the collection [6-7].

General knowledge on the life and activity of the two physicians differs fundamentally: Dr. Arsenie remains to this day almost anonymous, whereas Dr. Mitrea naturally benefitted from consistent historiographic attention. Yet, regarding their military activity in Mexico little is known in both cases, and this will be the focus of our research in what follows.

METHODS

Some of the most important biographical sources for the officers of the Habsburg army (and other European armies) of the 19th century are the conduct lists. This type of document was periodically drafted within each military unit, had a tabular structure, and contained a rigorous description of the career, knowledge, behavior, and personality of the officer. An excerpt of the conduct lists drafted at Puebla, under the supervision of Major Dr. Michael Kubicza, ÖFM's chief-physician, on November 30th, 1866 – just one week before the disbandment of the volunteer corps – was kept for both Dr. Arsenie and Dr. Mitrea [AT-OeStA/KA AdT Mexiko, A42 & A43][1] [8].

Both conduct lists contain the following categories of information:

a) biographical (name, place and year of birth, confession, civil status, and whether they have children or not);

b) concerning the military career (current rank and previous ranks, the date when the first rank was obtained and the period covered by every rank starting with the date of enrollment, medals, military actions they were involved in, and whether the officer is suitable for advancement);

c) regarding their studies, linguistic knowledge, and the level of their professional knowledge (medical, military, and other fields, if such was the case);

d) characterizations of their general state of health, personality, behavior towards their superiors, comrades, or against the enemy, as well as the opinion of the author of the characterization on the respective army doctor.

Using the information provided by these documents, we will attempt to complete the currently known data regarding their activity and career as military physicians, through the method of historical reconstruction, comparing the bibliographical mentions with those in the archive documents.

RESULTS

Biographical data

Concerning biographical information, the conduct lists bring nothing new. In the case of Dr. Arsenie, there is no mention of the year or place of birth, confession, or civil status. In the case of Dr. Mitrea, it mentions the year and place of birth (1842, Reschinar in Siebenbürgen), the confession (Orthodox, griechisch nicht uniert), and the civil status (not married, ledig) [AT-OeStA/KA AdT Mexiko A43]. The lack of biographical information regarding Dr. Arsenie lead us to believe that he was not present in Puebla at the moment when the conduct lists were drafted, and the officer in charge did not have at his disposal any additional documents concerning him.

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[1] *Austrian State Archives. War Archive, Archives of Army Troops – Austrian-Belgian volunteer corps in Mexico – Administrative documents – Conduct lists and descriptions of individuals, Akten 42 (Arsenie) and Akten 43 (Mitrea).*

Military Career

The data regarding the military career of the two army doctors are much more rigorously recorded and allows for an exact reconstruction of the duration of their service, their advancements in rank, and the military actions they took part in. The data is also very valuable since up to this date, no details were known regarding the military activities that the two army doctors were involved in.

On November 30th, 1866, Dr. Arsenie had completed exactly two years and one month of service in ÖFM, meaning that he was on the payroll starting with November 1st, 1864, more than one month before the first ship of Austrian expeditionary troops left the port of Trieste (December 6th, 1864) [4]. Although we have no information about the ship he traveled on, nor on the exact moment of his arrival in Mexico, we know that he was there on February 6th, 1865, when he is mentioned as a participant in the skirmishes in Tesuitlan. He was enrolled with the rank of first lieutenant senior physician (Oberlieutenant-Oberarzt), which he kept for one year, four months and twenty-nine days, until March 30th, 1866, when he was promoted to the rank of captain senior physician, 2nd class (Hauptmann Oberarzt 2. Classe) [AT-OeStA/KA AdT Mexiko A42]. He held this rank at the date when the conduct list was drafted, and probably one week later, as well, at the date of ÖFM's disbandment (December 6th, 1866).

The same document mentions that he was decorated with the Mexican Imperial Order of Guadalupe, in the rank of knight (limited to some 500 decorated people), a fact already known from the photos and press of the time [2]. The order was created in 1822, during the first Mexican empire, annulled and reinstated twice since then, depending on the political changes in the country. Emperor Maximilian wished to consolidate the basis of his reign through symbolic gestures as well, making use of Catholic spirituality, i.e. the recognition of the importance of the Virgin of Guadalupe cult and reinstating the Order of Honor that carried her name, in 1865. During its time, the order was split into Grandes Cruces (30), Grandes Oficiales (100), Comendadores (200), and Caballeros (500) [9-11]. From the conduct list, we also learn the circumstances that led to him being decorated: for the deeds of merit performed in Sierra del Norte, and especially in Agua Dulce (für seine verdienstvollen Leistungen in der Sierra del Norte namentlich bei Agua Dulce) [AT-OeStA/KA AdT Mexiko A42]. There is, however, a photograph taken in Vienna in 1869, showing him in the volunteer corps' uniform, and wearing two medals [2]. Despite the poor quality of the image, the second medal can be identified, through visual comparison with the similar one received by Dr. Mitrea, and thanks to I. Petrescu's historiographical contribution to this particular topic [12]. It was the Commemorative medal of the Mexico expedition (Médaille commémorative de l'expédition du Mexique), issued by the French emperor Napoleon III, of which the black eagle on the ribbon is visible in the image.

Regarding the battles that Dr. Arsenie took part in, these are listed in a dedicated column: at Tesuitlan [Teziutlán] on February 6th and 10th 1865, at Zacapoaxtla on March 1st and 2nd 1865, at Xilotepec [Jilotepec] and Xochiapulco on April 13th, at Tlapacoyan on July 9th, 11th, 13th and 19th 1865, at Agua Dulce on January 11th, 1866, at Tres Cruces on July 13th and Pahuathlan on July 14th, 1866 [AT-OeStA/KA AdT Mexiko A42]. The battles in 1865 took place in a very small area, about 140 km North-West of Puebla, most probably where his garrison was stationed. The battles from 1866 took place about 100 km North and North-West of the previous ones (Sierra del Norte). For his bravery here, at Agua Dulce, he was awarded the Order of Guadalupe.

Dr. Mitrea enrolled in Vienna, on March 26th, 1866. Based on some documents from the family's archive, issued in Mexico, his main biographer, E. Pop, also mentions the possibility of him having received a three-month bonus to his pay (January 1st – March 31st, 1866) [3]. However, the conduct list only registers a service of eight months and five days on November 30th (starting with April 1st, 1866, probably the first day of his payroll) with the rank of first lieutenant senior physician (Oberlieutenant-Oberarzt). No medals are being recorded. The Commemorative Medal of the Expedition in Mexico, issued by emperor Napoleon III, was awarded at a later date. Dr. Mitrea took part in the battle at Cozautlan [Cosautlán de Carvajal] on September 23rd, 1866, the one in Banderilla on October 24th, 1866, and the defense of the city of Zalapa [Xalapa] between November 4th and November 11th, 1866 [AT-OeStA/KA AdT Mexiko A43].

Specialty training, abilities, and competencies

Data regarding their studies, linguistic knowledge, and the level of professional knowledge (medical, military, or in other fields) are brief, but it does bring up new data.

In both cases, it is mentioned that they had completed the secondary and university studies required by their current position. Based on their biographical and educational background, we know they were both speakers of Romanian and German, and we also know Dr. Mitrea was a speaker of Hungarian (given the fact that he had studied medicine in Cluj), but the conduct lists add new information. In the case of Dr. Arsenie, it mentions that he could speak and write in

Romanian, Hungarian, German, but also rather well in French, Italian, and Spanish. In the case of Dr. Mitrea, the languages recorded are Romanian, Hungarian, German, Latin, and a bit of Spanish (etwas spanisch). In both cases, they probably learned Spanish during the campaign.

The military knowledge of Dr. Arsenie is characterized as "low level" ("some", einige), and that of Dr. Mitrea as being "little" (wenige), which is only natural, given the lack of prior military experience and the fact that they were trained as civilian physicians. It is also mentioned that neither of them had any knowledge in other fields of activity.[AT-OeStA/KA AdT Mexiko A42, A43]

Personality traits and conduct

The general health state mentioned in the conduct lists is "very good" in the case of Dr. Arsenie and only "good" in the case of Dr. Mitrea. The former is characterized as being "silent and secretive" (still u. verschlossen), and the latter as "silent, calm, docile" (still, ruhig, willig). In official relations, Dr. Arsenie was "calm, as expected" (ruhig, den Anforderungen entsprechend), and Dr. Mitrea was "as expected". Relations with their comrades seemed to follow the same pattern: Dr. Arsenie was "friendly" and Dr. Mitrea was "very friendly". Small differences also appear when it comes to the effort they put in: Dr. Arsenie was considered to be "quite diligent, with results as expected" (ziemlich fleißig mit entsprechendem Erfolg), while Dr. Mitrea was "very diligent, with good results" (sehr fleißig mit gutem Erfolg). Both are considered able for military service, and their behavior against the enemy is considered to be "quite respectful" (recht brav). The conclusion of the superior officer who drafted the conduct lists is that each of them was a "really useful army doctor" (ist ein recht gut verwendbarer Truppenarzt) [AT-OeStA/KA AdT Mexiko A42, A43].

Whereas the personality of Dr. Mitrea fits the historiographic portrait created based on his contemporaries' testimonies [3], Dr. Arsenie's personality contradicts the assumptions of E. Pop. Starting from the fact that Dr. Arsenie sent a series of letters from Mexico to some Romanian journals in Transylvania, and analyzing the light style of his penmanship, scattered with humorous nuances (but probably also taking into account his financial donations to Astra), E. Pop concluded that, as opposed to Dr. Mitrea, he would have been more of an extrovert, who liked making public appearances [2]. However, the conduct lists indicate the opposite: out of the two, Dr. Mitrea was the one who had a more open personality in his direct social relations, though he was just as calm and silent as his colleague. E. Pop managed to very well perceive the difference between the two army doctors at the level of inner personality, as surely Dr. Arsenie was much more inclined than Dr. Mitrea to written, indirect, social communication and interaction. However, given the lack of more explicit documentation, such as the ones we have used, he could not have known that both of them had similar distant and reserved personas.

CONCLUSION

The information preserved by the excerpts of the conduct lists of the two Romanian army doctors from the Austrian volunteer corps in Mexico is extremely valuable, both in terms of new data and by nuancing pre-existing historiographic assumptions. The documents helped with the exact reconstruction of their short military career, and especially with the battles, they took part in. Moreover, they helped with the reconstruction of their personality (which, in the case of Dr. Arsenie, complements the image proposed half a century ago, by E. Pop) and of how their superior officers regarded and appreciated them.

Starting from these documents, a more in-depth research plan on the activity of the two army doctors (and the other few Romanians enrolled as volunteers in the same campaign) can be drafted. By knowing the battles they took part in, one can identify new sources, both in the Austrian and Mexican archives (the latter being up to this day completely unexplored about this subject).

Future plans

The current research should also be regarded as a signal, aiming at drawing attention to both the documentary potential of the conduct lists of officers in the Habsburg army, as well as to the particular subject of Romanian participants to the "Mexican adventure", about which very little is yet known. Regarding the first aspect, further research shall be focused on the area of the former border regiments in Transylvania, which is covered by rich and almost completely unexplored documentation preserved in the Viennese war archive. This includes, inter alia, a multitude of data on the organization of the sanitary service in military border areas, medical staff, medical and health practices, etc. Regarding the second aspect, the search for new sources related to the activity of the Romanian volunteers in Mexico will continue, in the hope of further detailing their activity on the battlefield and in the field hospitals.

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The use of Laser Doppler vibrometry (Doppler principle) for middle ear research and diagnosis

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Abstract: *The middle ear represents the middle component of the human ear. Its function is to transmit sound-waves from the external auditory canal (EAC) to the inner ear via the Tympanic Membrane (TM), the Ossicular Chain (OC), and Oval Window (OW) and at the same time to act as a transformer that produces a pressure gain in the sound wave, usually specified in literature as 27-30 dB. Although a very efficient biomechanical system, the OC has, within the human hearing range, minute vibration amplitudes of only a few nm which brings forward the problem of a reliable measuring technique for such movements*

The Laser Doppler Vibrometry (LDV) also known as Laser Doppler Interferonometry (LDI) is a method of measuring such minute vibrations without contact with the anatomical structures. The laser beam can be aimed at chosen points of a structure and the movement will be recorded as a graphical representation. As such, the method has been studied and applied for numerous purposes and experiments over the last decades. The present work aims to present a comprehensive review of these experiments to define LDV as a reliable method for middle ear research and diagnostics.

Keywords: *laser Doppler vibrometry, middle ear*

INTRODUCTION

The middle ear represents the middle part of the ear, located between the external ear (pavilion and external ear canal - EAC) and the inner ear (Cochlea). It is comprised of the tympanic membrane (TM) and the ossicular chain (OC) with their tendons and muscles. The OC is represented by the three smallest bones in the human body, the hammer (malleus), the incus, and the stapes.

The physiological function of the human middle ear is to transmit sound-waves from the external auditory canal (EAC) to the oval window via the Tympanic Membrane (TM) and the Ossicular Chain (OC) and at the same time to act as a transformer that produces a pressure gain in the sound wave, usually specified in literature as 27-30 dB. Although the structures are extremely small, the dynamic range of the system is essential. A tympanic membrane only 100 µm thick will be able to transform a 20 µPa sound wave into a perceivable sound for humans. The same membrane will be able to compensate for a variation in atmospheric pressure of up to 120 kPa [1].

The acoustic transformer that is the middle ear structure, matches the low impedance of air in the EAC to the relatively high impedance of fluid within the inner ear [2]. The acoustic gain is obtained through two structure-related factors: the aria ratio (the TM area divided by the stapes footplate area)

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and the ossicular lever (the length of the manubrium of the malleus divided by the length of the long process of the incus). Sometimes a third mechanism called the catenary lever (ratio of force acting on the TM to that acting on the malleus) is described, which could bring a total middle ear gain of about 34dB [3].

“The pathology of the auditory system is one of the main reasons for improper development of oral language and a person suffering from hypoacusis is more likely to have poor social and professional integration, lower competitiveness on the labor market and will have smaller chances to complete higher education” [4, 5].

The very efficient biomechanical system comprised of the OC has, within the human hearing range, vibration amplitudes within the mm and nm range which brings forward the problem of a reliable measuring technique for such movements. Many authors also mention the variable rotational axis of the ossicles and pluridimensional vibratory patterns for the OC [6] which can prove a challenge for demonstration and recording.

Although etiologically heterogeneous, at least 50% of all hearing losses can be explained by a genetic background while the rest is directly linked to the presence of external factors (environmental and clinical perinatal) [7].

The Laser Doppler Vibrometry (LDV) also known as Laser Doppler Interferonometry (LDI) is a method of measuring the minute vibrations of the TM and OC without contact with the anatomical structures. The laser beam can be aimed at specifically chosen points of a vibrating structure and the movement will be recorded as a graphical representation and can also provide acoustic results over headphones. This can also be used for measuring the transfer function of the middle ear (METF) in different situations such as intact middle ear, disrupted OC, reconstructed middle ear, etc.

THE PRINCIPLE OF LDV

Based on the Doppler shift principle, a Laser-Doppler Vibrometer compares the frequency of a laser’s emitted light to the frequency of the light reflected from a moving object and is capable to determine the instantaneous velocity of that object. We should consider the physical principle as medical research usually requires a high degree of abstraction [8].

The principle of the method is aiming the laser beam at an object that moves with the velocity v (for example TM). By reflecting the beam, a frequency change results between the incoming and the reflected beam (fd). This frequency change is proportional to the object’s velocity. The physical principle is usually presented using the following formulas:

$$fd = \frac{2v}{\boldsymbol{\lambda}} \qquad f' = \frac{v + v_0}{v - v_s}f \qquad \frac{\Delta\boldsymbol{\lambda}}{\boldsymbol{\lambda}_0} = \frac{v}{c}$$

Where:

fd – Doppler frequency; λ – wavelength of the beam; v – velocity of an object; f – actual frequency of soundwaves; f’– observed frequency; v – the speed of sound waves; v_0 *– velocity of the observer;* v_s *– velocity of the source; Δλ – wavelength shift;* λ_0 *– wavelength of source not moving; v – velocity of source; c – the speed of light*

The Laser-Doppler Vibrometer is a very sensitive, non-contacting optical displacement system capable of making displacement measurements in the ear to < 1 × 10-4 µm at frequencies from 100 Hz to above 10,000 Hz [9]. A Helium-Neon laser beam is aimed through the ear canal or the mastoid process at any vibrating site on the tympanic membrane, the malleus, or the stapes footplate. The laser beam is modulated at 40 MHz and focused on a reflective target using a lens. The reflected beam from the target site is analyzed in the detector portion of the system by using the Doppler principle, producing an output voltage proportional to the velocity of the vibrating target. Velocity is usually converted to displacement, the most commonly used measurement parameter. “The diameter of the target can be less than 1 mm. since the laser beam has a width of approximately 10 µm.” [9] The LDV is connected to an operating microscope to rapidly focus the beam on the target. “Measurements of incus and stapes vibrations can be performed as well if a TM perforation is present or during surgery.” [9] A sound-generating system delivers a constant sound pressure level (SPL) at the TM of 80-100 dB at representative frequencies within the 200-15,000 Hz range.

LDV IN EXPERIMENTAL MIDDLE EAR RESEARCH AND DIAGNOSIS

“Contact-free methods of measurement for analysis of middle ear vibrations became more and more sophisticated and allow highly accurate evaluations.” [10] The use of LDV attached to a microscope was first described by Nuttall et al. in a 1991 study of basilar membrane vibration in the guinea pig. [11] Although frequently employed for experimental use, measurements in live humans during surgery are not yet available [10].

Numerous authors have concerned themselves with the study of LDV and its different possible uses (See Table 1).

As early as 1993, Goode et al. use the LDV to measure the displacement of the umbo at SPL of 60, 70, and 80 dB in 6 live subjects and compare the results to 15 measurements of fresh temporal bones (TBs). They conclude that the measurement of umbo displacement or velocity in the intact middle ear at physiologic sound pressure over the auditory frequency range is the best way to characterize the function of the tympanic membrane [12].

Table 1: Studies that present the experimental use of LDV

Study	Status	Type of material	The target of LDV spot	Purpose of the study
Goode 1993	In vivo/In vitro	Live subjects/Fresh TBs	Umbo	Evaluation of displacement of umbo at SPL of 60,70 and 80dB
Goode 1996	In vivo/In vitro	Live subjects/Fresh TBs	TM, Malleus, Prosthesis head	Evaluation of displacement of TM, malleus, and prosthesis head
Voss 2000	In vitro	Fresh TBs	Different locations on the stapes	Describing sound transmission through the middle ear
Huber 2001a	In vivo	Live subject	Stapes footplate	Assessment of displacement amplitudes of human stapes
Huber 2001b	In vivo	Live subject	TM	Evaluation of LDV as a diagnostic tool
Rosowski 2002	In vivo	Live gerbils	Umbo, pars tensa	Assessment of the effects of the immobilized pars flaccida
Stenfelt 2002	In vitro	Fresh TBs	Umbo, stapes footplate	Study of malleus and stapes footplate motion during BC
Willi 2002	In vitro	Fresh TBs	TM, IMJ	Dynamics of IMJ
Rosowski 2003	In vivo	Live subjects	Umbo	Correlation between pre-op TM mobility and intra-op diagnosis
Stenfelt 2003	In vitro	Frozen human heads	Umbo	Investigation of sound radiated from the TM into the EAC
Huber 2003a	In vitro	Fresh human TBs	Stapes footplate, Incus	Quality control of stapes surgery (quality of prosthesis crimping)
Huber 2003b	In vivo/In vitro	Live subjects/Fresh TBs	Umbo, stapes footplate	Assessment of AML fixation effects
Rosowski 2004	In vivo	Chincillas	Umbo	Diagnosis of superior semicircular canal dehiscence
Whittemore 2004	In vivo	Live subjects	Umbo	Study of the sound-induced TM velocity at umbo
Zenner 2004	Artificial	Mechanical middle ear models	Different sites on the prosthesis head plate	Investigating the various types of prosthesis
Nakajima 2005a	In vitro	Fresh TBs	TM	Effects of AML fixation
Nakajima 2005b	In vitro	Fresh TBs	Umbo, stapes footplate	Effects of malleus, stapes or malleus+stapes fixation
Zhao 2005	In vitro	Frozen TBs	Umbo, stapes footplate	Determining the optimal length of titanium prosthesis
Chien 2006	In vitro	Fresh TBs	Stapes footplate	Study of the effects of methodological differences in sound-induced stapes velocity in live and cadaver ears
Huber 2006	In vitro	Fresh TBs	Stapes footplate	Feasibility of an implantable hearing aid
Chien 2007	In vivo	Live subjects	Three locations on the TM graft	Identifying causes for poor results of type III tympanoplasty
Dai 2007	In vitro	Fresh TBs	TM and stapes footplate	Investigation of the function of SML and AML
Turcanu 2007	In vivo	Live subjects	Umbo	Measuring the growth function of DP-OAE as the vibration of umbo
Eeg-Olofsson 2008	In vitro	Embalmed human heads	Promontory	Investigation for an ideal spot for BAHA implantation
Hüttenbrink 2008	In vivo/In vitro	Live subjects/Fresh TBs	RW membrane, footplate, promontory	Assessing the efficiency of TORP-Vibroplasty
Huber 2008a	In vivo	Guinea pigs	Stapes head	Study of motion of stapes footplate
Huber 2008b	In vivo	Live subjects	A long process of incus, Prosthesis loop	Evaluation of crimping quality of stapes prostheses

Study	Status	Type of material	The target of LDV spot	Purpose of the study
Neudert 2009	In vivo/In vitro	Live subjects/Fresh TBs	Footplate	Comparison of three types of prosthesis
Offergeld 2010	In vitro	Fresh TBs	Three sites on the footplate	Evaluation of rotational tomography for diagnosis of OCR malfunction.
Neudert 2018	In vitro	Fresh and thawed TBs	Footplate	Live feed-back for PORP and TORP implantations via LDV (graphic and acoustic)

Another report from Goode et al. in 1996 uses 95 human ears and 2 TBs for clinical LDV experiments and measures the TM, malleus, and ossicular prosthesis head displacement and suggest the potential for intra-operative use:

- "Afflicted TM function in hypoacusis cases with middle ear component suspicion but no or only small ABG (<20dB)",
- "Selection of best surgical options for TM and malleus displacement",
- "Analysis of postoperative results of reconstruction by evaluating prosthesis head displacement or TM displacement on different sites",
- "Live, intraoperative measurement of stapes and prosthesis displacement during surgery." [9]

In 2000 Voss et al. reported on measurements made on human fresh cadaver ears to describe sound transmission through the middle ear. The stapes velocity (VS) was determined by the use of LDV and concluded it is a reasonable method to describe sound transmission through the middle ear for frequencies up to 2000 Hz [13].

A 2001 article by Alexander Huber studies the diagnostic possibilities of the LDV in patients with both conductive and sensorineural hearing loss. He finds that LDV "can differentiate normal subjects from those with conductive hearing loss and also may distinguish between various middle ear pathology." [14]

Concerning the intraoperative use of LDV, the same Alexander Huber published an article on intra-operative assessment of stapes movement. The study was comprised of 7 patients with profound bilateral hearing loss who were undergoing cochlear implantation. The laser beam was aimed through the posterior tympanotomy onto the stapes and the angle between the beam and the footplate was estimated. The author concludes that amidst the future applications of the LDV we can also count intra-operative quality control of ossiculoplasties and active middle ear implants [10].

In 2002 Rosowski uses LDV to assess the effect of immobilized pars flaccida on the middle ear's response to static pressure. His experiments on gerbils test this hypothesis by comparing the effect of middle-ear static pressure on measurements of the sound-induced pars tensa velocity before and after immobilization of the pars flaccida with acrylic cement [15].

The use of LDV was extended by Stenfelt in 2002 to the study of the malleus and stapes footplate motion during bone conduction by in vitro stimulation of 26 TBs using an LDV over the frequency range of 0.1-10 kHz. "For lower frequencies, the ossicular sites of measurement followed the motion of the temporal bone. The differential motion between the malleus and the surrounding bone was greater than the differential motion of the stapes footplate; both resonated near 1.5 kHz." [16]

Willi et al. (2002) investigate the dynamics of the incudo-malleolar joint (IMJ) in 9 temporal bones using LDV scanning which helps to understand the dynamics of both ossicles by three degrees of freedom. Transfer functions (TFs) are shown for each of these degrees [17].

Rosowski et al. publish another work in 2003 in which they report preoperative LDV measurements from 17 patients with conductive hearing loss and a normal, intact tympanic membrane. "The velocity of the TM was measured by LDV near the umbo and showed a direct relation between preoperative TM mobility and the intra-operative diagnosis of ossicular interruption or fixation." [18]

In 2003 Stenfelt et al. investigate the sound radiated from the TM into the ear canal in 4 TB specimens and conclude that it is significantly lower than the sound pressure in an intact EAC with bone conduction (BC) stimulation."[19] The umbo velocity with air conduction stimulation was investigated in 9 TBs and compared with the umbo velocity obtained with BC stimulation in 5 cadaveric ears.

In 2003 Huber et al. extend the use of LDV on quality control of stapes surgery. They aimed to define the more frequent causes of stapes surgery failure and to discover a required crimping loop (attachment pattern) to obtain the best sound transmission results. The experiments were conducted on temporal bone models and measurements of the sound transmission properties between incus and prosthesis on 17 fresh human TBs were performed. LDV scanning, endoscopic photography, micro grinding technique, and scanning electron microscopy were used to assess three possible situations for attaching a titanium stapes piston: without crimping, loose crimping, and tight fixation to the incus [20].

Rosowski et al. 2004 induce umbo displacement with sound on chinchilla models with CT scan–confirmed superior semicircular canal dehiscence and perform LDV measurements; the sound-induced motions of the vestibular lymph (either perilymph or endolymph) extends the use of LDV for the study of inner ear conditions [21].

In 2004 Whittemore et al. use LDV to measure the sound-induced tympanic membrane (TM) velocity on 56 normal-hearing human subjects at nine sound frequencies. The second series of experiments was performed on 47 subjects with sensorineural hearing loss (SNHL). The authors conclude that “LDV can provide quick, safe and repeatable measurements of the sound-induced velocity of the umbo in awake patients using the natural reflectance of the TM” and that “the best uses for the LDV system would be the diagnosis of ossicular chain reconstruction (OCR) pathology in patients with significant conductive hearing loss, an intact TM and an aerated middle-ear cavity.” [22]

Zenner et al. (2004) use mechanical middle-ear models (MMM) made of plastics, carbon, ceramics, and various metals to evaluate the acoustical properties of various middle ear prostheses by use of LDV [23].

Nakajima et al. simulated the fixation of the anterior mallear ligament in temporal bones and measured the effects of this reaction on umbo and footplate displacement by comparing their findings with clinical data [24, 25].

A second study by Nakajima et al. aims to determine the effects of various types of malleus fixation using 18 cadaveric TB preparation and to “evaluate the clinical use for umbo velocity measurements in the preoperative differential diagnosis of OCR (malleus and stapes) fixation”. Malleus fixations were mimicked by controlled applications of adhesives and the effects on middle ear transmission were measured. The fixation of the stapes and combined malleus-stapes fixation were also investigated. Measurements were made of umbo velocity (VU) and stapes velocity (VS) by LDV before and after fixation [26].

Zhao et al. (2005) perform OCR on 7 human TBs before and after removal of the incus and insertion of the prosthesis and use LDV to determine the optimal length of a titanium prosthesis [27].

Chien et al. (2006) study methodological differences in sound-induced stapes velocity (Vs) measurements in live and cadaveric ears [28].

In 2006, Alexander Huber extends the use of LDV for the assessment of an implantable hearing device in humans. Three experimental situations were used: normal ear, PORP ossiculoplasty, and a VSB implant. The vibratory properties of the stapes in all situations were assessed by aiming the laser beam at the stapes footplate [29].

In 2007 Chien et al. aim to refine the uses of LDV for investigation of the middle ear mechanics in a Type III stapes columella tympanoplasty and defining the structural factors responsible for poor functional results. LDV measurements were performed in 22 patients (23 ears) at three locations on the TM graft: over the stapes head, over the round window, and on the anterior TM. The experimental results were correlated with clinical and audiology data. The conclusion is that LDV is found useful in the diagnosis of non-aeration of the middle ear but “does not readily diagnose stapes fixation.” [30]

Dai et al. (2007) follow the work of Nakajima et al. and investigate ligament fixation (superior malleolar ligament – SML and anterior malleolar ligament – AML) through simulations of fixation and detachment in 9 fresh frozen human TBs and a finite element model (FEM) of the human ear. Two LDVs were used to measure the vibrations of the TM and stapes footplate. A 3-D FEM predicted the transfer function of the middle ear (METF) in all cases of ligament fixation and/or detachment. The results of this study show that “either SML or AML fixation caused a reduction of umbo and footplate mobility at low frequencies [25].

Turcanu et al. (2007) bring a new approach to LDV use by combining it to objective functional tests of the inner era. The growth functions of the distortion products of otoacoustic emissions (DPOAE) are measured as vibrations of the umbo in 20 subjects. For comparison, DPOAE growth functions were also measured conventionally with an acoustic probe in the closed EAC. The authors conclude that the smaller standard deviation for the LDV data could be a result of the fact that the measurements are made in an open sound field and are therefore less susceptible to pressure calibration errors [31].

Eeg-Olofsson et al. (2008) investigate if BC sound transmission improves when the stimulation approaches the cochlea to obtain an ideal spot for BAHA implantation. Heads from seven human cadavers were used and vibrational stimulation was applied at eight positions on each side of the head. An LDV was used to measure the resulting velocity of the cochlear promontory. The study demonstrates that “the closer to the cochlea the stimuli are placed, the higher the velocity of the promontory, especially for distances < 2.5 cm from the EAC opening and when the stimulation position is placed in the opened mastoid bone.” [32]

A 2008 study by Hüttenbrink & Zahnert uses the LDV for temporal bone studies to assess the efficiency of a TORP-Vibroplasty. LDV measurements were performed at 3 locations: the round window (RW) membrane, the footplate, and at a drilled-out third window on the promontory (for comparison of the stimulation of both windows). This study is another fine example of the flexibility of LDV as a useful tool for the otologic surgeon [33].

Huber et al use a 3D LDV that has the capability of simultaneously measuring spatial vibrations, to monitor stapes vibration. This was done by using 3 separate laser beams at an angle of 12 degrees relative to the optical axis of the lens. The focus point was adjusted to the stapes head to prove that the piston-like motion of the stapes footplate is the only effective stimulus to the cochlea, and rocking-like stapes motions have no effect on hearing. The study was performed on 4 female guinea pigs [34].

Also, Huber et al. evaluate the crimping quality of a stapes prostheses in 23 patients by comparing intra-operative LDV results and postoperative pure tone thresholds. The study demonstrates the feasibility of intra-operative LDV and uses a method of mechanical stimulation of the middle ear that was developed and calibrated in TB preparations [35]. The method was applicable not only under general but also under local anesthesia. It is therefore practical for quality control in routine stapes surgery.

Andre Jakob uses LDV for the clinical diagnosis of otosclerosis. In this study, he aimed the LDV at the umbo and measured its movement in healthy and conductive hearing loss affected ears. He concluded that “all ossicular conditions represent increased umbo displacement” and that the method is reliable for detecting a discontinuity of the ossicular chain. However, LDV does not suffice as a single diagnostic tool for otosclerosis [36].

In 2009, Neudert et.al. use LDV to compare the results of three different middle ear prostheses (TAP, AII, TCP) on human temporal bones. In this study the LDV was also used only for TB specimens experiments (18 TBs), all in vivo implantations (66 patients) were retrospectively assessed up to 5 years after surgery [37].

In 2010, Offergeld employs an LDV experimental setup very similar to our own to make functional measurements of middle ear sound transmission on PORP/TORP implanted human TBs and to correlate the results to anatomical data obtained by rotational tomography. The goal of his study was to prove that RT is a reliable method for assessing the postoperative position of ossicular implants [38].

In 2018, Neudert et al. uses the LDV to obtain live feed-back for ossicular prosthesis positioning and concludes the method to be feasible for improving ossiculoplasty results [39].

CONCLUSIONS

Middle ear vibrations in response to acoustic stimulation are very difficult to record since they are measured within the micrometer and nanometer range. For this reason, devising a method to analyze these vibrations is extremely important and doing it without any contact with the structures of the middle ear is essential. Without LDV the surgeon must manipulate the ossicles with instruments, under visual control and therefore only perform a qualitative estimate as to the mobility of the ossicular chain.

After reviewing all the experiments above, there can only be one conclusion drawn from all the studies so far: the LDV method has great potential for preoperative diagnostic of patients with conductive hearing loss, for differential diagnostic between the types of pathology that determine the conductive hearing loss and especially for assessing the postoperative results of ossicular middle ear prosthesis. All that is needed now is more studies that can confirm this potential and hopefully state guidelines for clinical use.

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The authors declare that they have no competing interests.

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VARIA

Demons-Meigs syndrome – Diagnosis and therapeutic conduct

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Abstract: *Demons-Meigs syndrome is a rare benign pathology that can be confused with other benign and malignant pathologies. The clinical picture is marked by an increase in abdominal volume, pelvic-abdominal pain, but it can also be associated with breathing difficulties due to excess ascites fluid and pleurisy. Imaging paraclinical examination easily reveals the three components: ascites, pleurisy, and ovarian mass.*

Surgical treatment helps to relieve symptoms in a relatively short time.

Keywords: *Meigs, ovarian tumor, imaging examinations*

INTRODUCTION

Demons-Meigs syndrome is represented by three elements: benign ovarian tumor – fibroids, ascites, and pleural effusion. Most often, it is a diagnosis of exclusion. Sometimes, the ovarian tumor can also be represented by thecoma, fibrothecoma, granular cell tumor, less often Brenner tumor. The prevalence and incidence of this syndrome are not fully known; an increase proportional to age was observed, the average of this pathology being around 50 years of age. At the same time, cases of pseudo-Demons-Meigs syndrome were reported in prepuberty in which the benign tumor was represented by another type of ovarian tumor formation (teratoma or cystadenoma). Of the ovarian masses surgically excluded, a percentage between 2 and 5% is represented by ovarian fibroids, and only 1-2% of them meet the criteria necessary for Demons-Meigs syndrome [1].

Demons-Meigs syndrome was first described in 1937 by gynecologist Joe Vicent Meigs, professor at Harvard Medical School, and by the pulmonologist John Class, in the American Journal of Obstetrics and Gynecology. The name Demons associated with the syndrome is given by Albert Jean Octave Demons, in 1887, when he communicates 9 cases of ovarian tumors that associate ascites fluid and hydrothorax [1].

Demons-Meigs syndrome involves the remission of ascites and pleural fluid when a benign ovarian tumor is surgically excluded – this fact is noticed and established by Albert Jean Octave Demons in 1903. Life expectancy after surgical treatment is the same as that of the general population [1].

Ascites was found in 10-15% of patients with ovarian fibromatous formations, while pleural fluid was identified in 1% of them. Pleural effusion is frequently located on the right side – in about 70%, while in 15% it is located on the left or bilateral side [1, 2].

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In addition to this syndrome, the pseudo-Demons-Meigs syndrome has been described, in which, along with ascites and hydrothorax, there is another benign ovarian tumor such as struma ovarii tumor, a teratoma, or a mucinous cystadenoma. The associated tumor may also belong to the salpinx, uterus, or may be a distant metastasis or a gastrointestinal tumor.

Ovarian tumors can be classified into germ cell tumors, gonadal germ cell tumors, and surface epithelial tumors [2].

Germ cell tumors account for 10-15% of all ovarian tumors and can be classified: (mature and immature) teratoma, dysgerminoma, gonadoblastoma, embryonal carcinoma, non-gestational choriocarcinoma. Only mature teratoma and gonadoblastoma are benign tumors [2].

The group of sex cord-stromal tumors includes granular cell tumors, fibroids-thecoma group (95% benign), Sertoli-Leydig cell tumors (5% malignant), gynandroblastoma (always malignant), and steroid cell tumors (70% benign).

Surface epithelial-stromal tumors come from the coelomic epithelium and account for 80-85% of all ovarian tumors. They include serous and mucinous cystadenoma (80-85 % benign), endometrioid tumors, clear cell tumors (which are 95-98% malignant), and Brenner tumor (benign in 98% of cases) [2].

PHYSIOPATHOLOGY

The etiology of ascitic fluid is poorly understood. A possible explanation for its appearance is due to the phenomenon of peritoneal irritation caused by ovarian fibroids. Also, the ovarian tumor could favor the appearance of ascites by the effect on the surrounding vessels represented by both the lymphatic vessels and capillaries. Another explanation would be the discharge of certain cytokines and interleukins that would increase capillary permeability, the result being ascites. Other hypotheses suggest that ovarian tumor torsion or hormonal stimulation may cause ascites. Many authors claim that tumors with an increased diameter of more than 10 cm could stimulate the secretion of ascitic fluid [2, 3].

The etiology of hydrothorax is unclear. The accumulation of pleural fluid is frequently on the right side, but can also be on the left or bilateral side. It is speculated that it was due to the transfer through the transdiaphragmatic lymphatics from the ascitic fluid. The amount of pleural fluid is directly proportional to that of ascitic fluid. The blockage of transdiaphragmatic lymphatics has been found to prevent pleural fluid from accumulating, while the amount of ascitic fluid increases [3].

It has been shown that both ascitic and pleural fluid can be exudative (rich in protein, caused by increased membrane permeability due to inflammation) and transudative (low in protein, by the increase of the hydrostatic pressure and decrease of the colloid osmotic pressure). However, pleural fluid is often exudative. Electrophoresis has shown that pleural fluid and ascitic fluid are of the same nature [3].

Figure 1: Giant ovarian fibroids. A 69-year-old woman who was brought to the Emergency Room of the Central Military Emergency University Hospital for altered general condition, dyspnea with orthopnea, loss of appetite, and enlarged abdominal volume, CA 125 is dosed – it's the value being 2317.7 U/mL

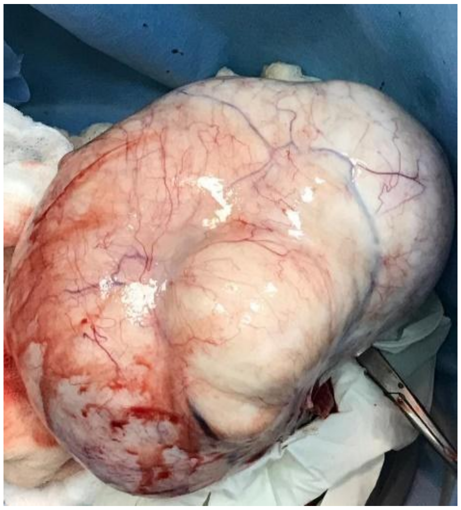

The signs and symptoms of this etiology include altered general condition, physical asthenia, fatigue, dry cough, weight gain, or loss. Among the gynecological signs and symptoms found in Meigs syndrome is amenorrhea, menstrual disorders (menometrorrhagia for women in the perimenopause period or metrorrhagia in the climax), iron deficiency anemia. A unilateral abdominal ovarian mass may be found, more often on the left or bilateral side, solid, asymptomatic, or symptomatic due to the presence of abdominal-pelvic pain, mass effect on surrounding organs [4].

The appearance of ascitic fluid generates abdominal distension, dyspeptic disorders (nausea and vomiting) due to increased intra-abdominal pressure, and constipation.

The appearance of pleural fluid leads to tachycardia, tachypnea, dyspnea with orthopnea, the abolition of the transmission of vocal vibrations, decrease or reduction of vesicular murmur, declivitous dullness to percussion. Pleural effusion is more common on the right side, may be exudative, protein-rich, or transudative. In the case of diagnosis or evacuation paracentesis or thoracentesis, it is found that the fluid does not contain malignant cells even if it is exudative or transudative [5].

The differential diagnosis of Demons-Meigs syndrome is broad and includes benign and malignant pathologies, and most often it is a diagnosis of exclusion.

The main differential diagnosis is with ovarian neoplasm, metastases from a breast neoplasm, colorectal, liver failure, nephrotic syndrome, Milroy's disease, tuberculosis, lung cancer, gastrointestinal tumors.

LABORATORY TESTS

The blood count shows information about hemoglobin, platelet count, and hematocrit. If the hemoglobin level is low, further investigations may be performed including syderemia, ferritin, total iron-binding capacity, and reticulocyte count. Iron deficiency anemia may require iron therapy before surgery or in more severe cases may be corrected by blood transfusion. Treatment with iron-containing supplements is continued postoperatively.

Carrying out clotting time is imperative before surgery. The ionogram is determined by checking and correcting any anomalies [1, 4].

Thoracentesis and paracentesis indicate whether the nature of the fluid is exudative or transudative type, and the cytological examination refutes the existence of malignant cells.

One blood biomarker used is CA 125. It is the antigenic representative of a 220-kD glycoprotein, and the physiological sources are represented by the coelomic epithelium and derivatives: pleura, pericardium, peritoneum as well as the epithelium of the fallopian tubes, endometrium, endocervix. Elevated values of this biomarker can be found in pregnancy, ovarian neoplasm, uterine fibromatosis, endometriosis, pelvic inflammatory disease, cancer of the fallopian tubes, but also in other non-gynecological pathologies (liver failure, kidney failure, autoimmune diseases) [5, 6].

In Demons-Meigs syndrome, there is an increase in the value of CA 125 which does not correlate in this case with the malignancy and which remits after the surgical exclusion of ovarian fibroids.

IMAGING EXPLORATIONS

Ultrasound is an investigation that detects tumor mass. The ultrasound appearance depends on the degree of compromise of vascularity, ovarian formations, and the presence or absence of adnexal hemorrhages. Ultrasound is the method of choice for symptomatic women but is limited in differentiating an ovarian lesion from a para ovarian one.

The radiography confirms and quantifies the existence of pleural effusion.

Figure 2: CT sections in the coronal plane. Tumor formation with probably left ovarian starting point, which includes tissue areas and areas of necrosis; ascitic fluid in increased amounts.

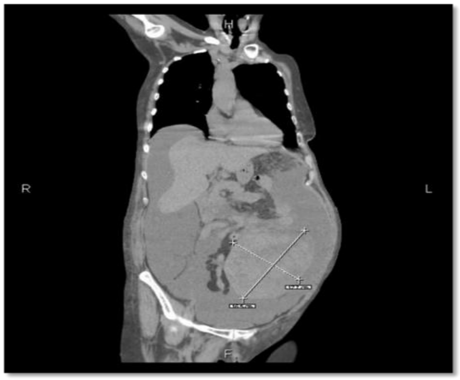

Figure 3: CT sections in the axial plane. Tumor formation with probably left ovarian starting point, which includes tissue areas and areas of necrosis; ascitic fluid in increased amounts.

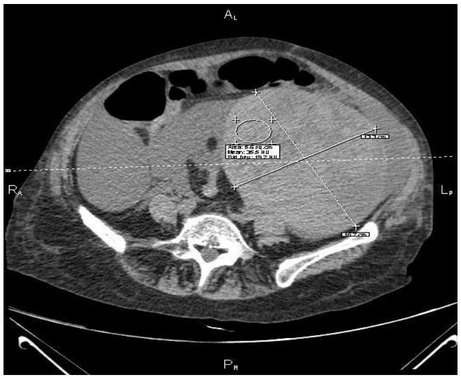

Figure 4: CT section of the thoracic region in the axial plane, parenchymal window. Accentuation of the interstitial lung disease and left pleural effusion.

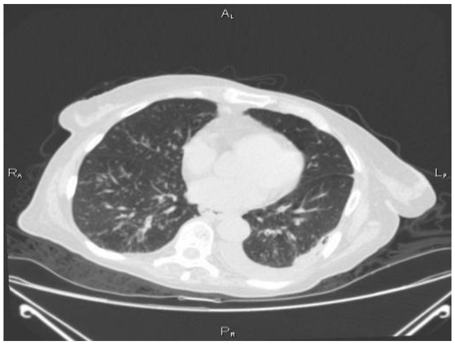

CT exploration of the abdomen and pelvis is a quick way to explore with great accuracy. Serial images formed during the exploration can be viewed on a screen, stored, and with them, three-dimensional reconstructions can be obtained, that provide precise details about the location of the tumor, vascular intake, but also useful information for the surgical approach. It can be done natively or after injecting an intravenous contrast-enhancing agent. CT examination may also be helpful when attempting tumor punch biopsy. The risks associated with this investigation are the cumulative effect of radiation. CT examination is contraindicated in pregnancy. Contrast enhancing agent allergy allows only the performance of native CT. Kidney failure is also a contraindication to the administration of the contrast-enhancing agent. In Demons-Meigs syndrome, CT examination confirms the existence of pleural fluid, ascitic fluid, ovarian tumor mass, and excludes distant metastases or the existence of other neoplasms [7, 8].

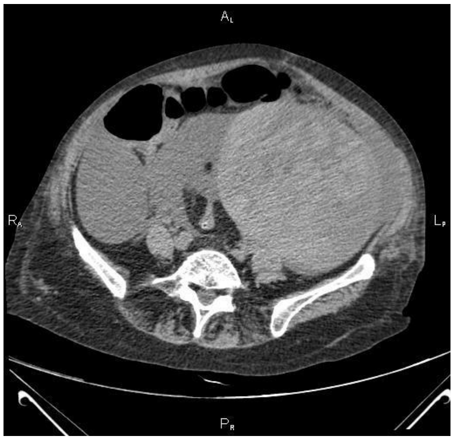

Figure 5: CT sections in the axial plane. Heterodense tumor formation with tissue structures and areas of necrosis included, clearly delimited, developed ilio-pelvic on the left side.

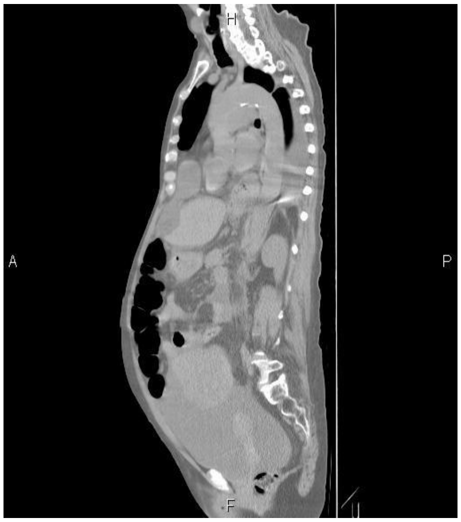

Figure 6: CT sections in the axial and sagittal plane. Heterodense tumor formation with tissue structures and areas of necrosis included, clearly delimited, developed ilio-pelvic on the left side.

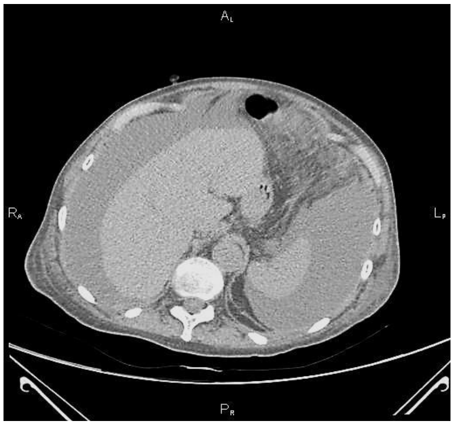

Figure 7: CT section of the upper abdomen in the axial plane. Fat densifications in the supramesocolic space.

Magnetic resonance imaging characterizes suspicious adnexal masses, establishes ovarian affiliation in inconclusive ultrasound cases, argues in favor of benignity or malignancy of ovarian tumor mass, and specifies other associated abnormalities – peritoneal carcinomatosis, adenopathies.

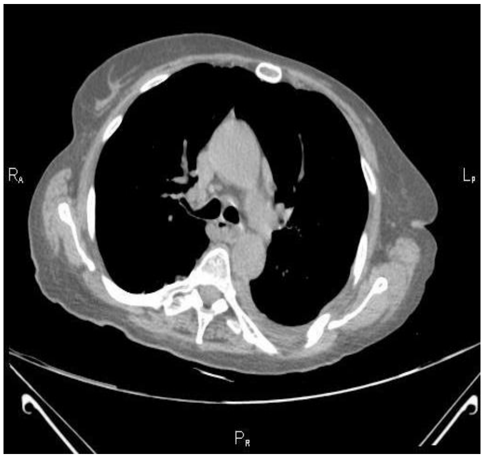

Figure 8: CT section in the axial plane in the mediastinum. No mediastinal tumor adenomegalies; left pleurisy.

TREATMENT

The treatment of Demons-Meigs syndrome is surgical and is represented by exploratory laparotomy with surgical staging. Biopsy of ovarian mass, omentum, and lymph nodes that reveal benign nature can be performed [7, 9].

If the patient is in the prepubertal period, tumorectomy, partial oophorectomy, and unilateral salpingectomy are used. In women of childbearing potential, surgical treatment involves unilateral adnexectomy. Surgical treatment of menopausal patients may include total hysterectomy with bilateral adnexectomy [7, 8].

Figure 9: Woman 69-year-old – intraoperative: ascitic fluid is found (approximately 500 ml which is collected and sent for histopathological examination); fibroid uterus; right adnexa modified according to age, and at the level of the left adnexa a tumor formation with dimensions of 18/14/10 cm, firm consistency, white-yellowish color, irregular, pedunculated shape. Total hysterectomy with bilateral adnexectomy is performed (with the sending of the samples to anatomopathological examination).

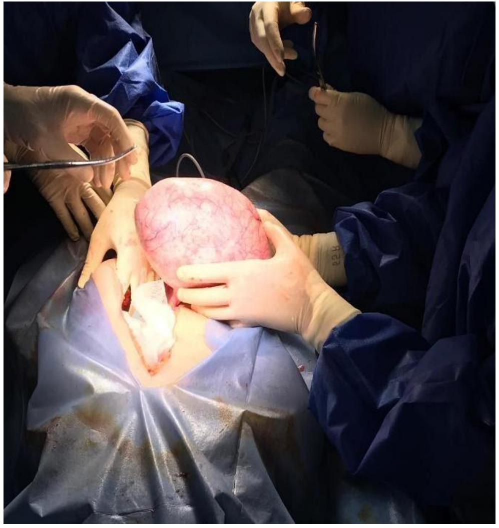

If the patient is in the prepubertal period, tumorectomy, partial oophorectomy, and unilateral salpingectomy are used. In women of childbearing potential, surgical treatment involves unilateral adnexectomy. Surgical treatment of menopausal patients may include total hysterectomy with bilateral adnexectomy.

The cure rate is high and no recurrences have been reported after the disappearance of the ovarian tumor. Ascites and pleural effusion disappear within a few weeks after surgical exclusion of the pelvic mass [7, 8].

Figure 10: Intraoperative image after total hysterectomy with bilateral abdominal adnexectomy for giant ovarian fibroids. The postoperative evolution was favorable under antibiotic, anticoagulant, anti-inflammatory treatment, and the treatment of the underlying pathology. Gradual remission of postoperative ascites and pleurisy was noticed.

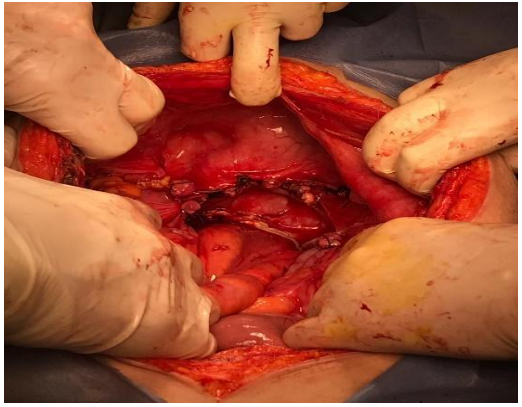

CONCLUSIONS

Demons-Meigs syndrome is defined by ovarian tumor with pleural effusion and ascites. Ovarian tumor is benign and is usually ovarian fibroids. After excluding the ovarian tumor, ascites, and pleural effusion remit. CA 125 tumor marker has elevated values similar to ovarian cancers, but values return to normal after surgical treatment. Even though this syndrome has been described for more than 100 years, it still poses problems in diagnosis, having an uncharacteristic picture. Molecular, hormonal, genetic, and mechanical factors are involved in its etiopathogenic mechanism.

The only treatment available for the remission of symptoms is surgery.

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VARIA

Anatomical study of the anterolateral ligament in Romanian population

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Abstract: *The purpose of this paper was to evaluate the existence of the anterolateral ligament of the knee in the Romanian population. Multiple studies have investigated the anterolateral structures of the knee, but there is some inconsistency regarding the existence of the anterolateral ligament.*

Materials and methods: A cadaver dissection study was performed on 10 knees (5 left and 5 right), 3 males and 2 females

Results: The anterolateral ligament was observed in all 10 knees. The mean length was 32±6mm, the width was 5.1±2 mm, and the thickness at the articular line was 1.1±5 mm.

Conclusions: A 100% presence was found, with a slight difference from the length, width, and thickness in other countries, race not being a decisional factor in differences.

Keywords: *anterolateral ligament, anatomy, lateral collateral ligament, dissection*

INTRODUCTION

In the year 1879, the French surgeon Paul Segond [1] described the existence of a fibrous band laying on the anterolateral facet of the knee. Until this moment in the specialty literature, we find ambiguous naming of this band, like lateral capsular ligament [2], the osseous-capsular layer of the iliotibial band [3, 4], or anterolateral ligament [5]. In this study made on 10 knees from the cadavers from The "Carol Davila" University of Medicine and Pharmacy Bucharest, there had been studied the femoral origin and tibial insertion, the trajectory and anatomical rapport with neighboring structures. So the anterolateral ligament was found like being a distinct ligamentous structure on the lateral facet of the knee, anteriorly its origin is on the lateral femoral epicondyle anterior of the collateral ligament and insertion on the anterolateral facet of the tibia at the half distance between the tibial tubercle and Gerdy's tubercle, being distinct of the iliotibial tract, with an oblique trajectory from its origin towards the insertion.

The knee joint is the largest joint in the body, and the following structures that are contributing to its stability are anterior and posterior cruciate ligament, medial and lateral collateral ligaments, quadriceps tendon, patellar tendon, popliteus tendon, and the anterolateral ligament [6].

MATERIALS AND METHODS

In this study were used 5 cadavers (10 knees), 3 males and 2 females. The mean height was 170 cm, the mean weight was

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62 kg, and the mean age was 75.1. For the dissection, three incisions were made: two circumferential in the medial 1/3 of the thigh and the medial 1/3 of the calf and 1 sagittal incision on the anterior aspect of the knee. The skin and subcutaneous tissue were removed using these incisions. The lateral vastus and iliotibial tract were detached. Once the lateral collateral ligament was shown, the tibia was internally rotated and the knee flexed to highlight the anterolateral ligament. Once shown, the knee was extended, and the origin and insertion of the ALL were marked, its length, width, and thickness were measured.

Figure 1: Superficial layer of the knee

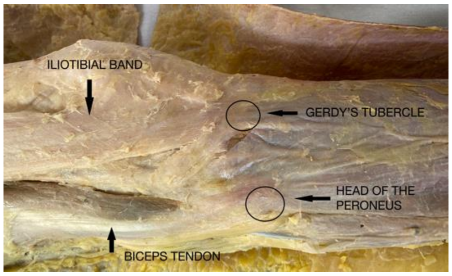
ILIOTIBIAL BAND
GERDY'S TUBERCLE
HEAD OF THE PERONEUS
BICEPS TENDON

Figure 2: Dissection of the superficial layer

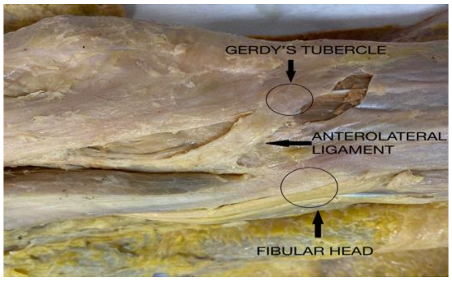
GERDY'S TUBERCLE
ANTEROLATERAL LIGAMENT
FIBULAR HEAD

RESULTS

The anterolateral ligament was observed on the anterolateral aspect of the intraarticular capsule of the knee after the detachment of the iliotibial tract. The ALL appeared as a white thickening that could be seen without a microscope and can be palpated. ALL was shown in all the 10 knees (5 right knees and 5 left knees).

Table 1: Prevalence of anterolateral ligament in the Romanian population

Total	Right	Left	Female	Male
100 % (5/5)	100 % (5/5)	100 % (5/5)	100 % (2/2)	100 % (3/3)

The origin of the ALL is at the lateral femoral epicondyle, posteriorly from the popliteus muscle-tendon, and has an oblique trajectory towards its insertion on the tibial plateau, and the half distance between de fibula head and Gerdy Tubercle. Fibers from the ALL insert on the lateral meniscus. ALL insertion is at 16±4mm anterior of the fibula head. Once the knee is extended the length was 32±6 mm, the width was 5.1±2 mm, and the thickness at the articular line was 1.1±5 mm.

Figure 3: Dissection of the deep layer

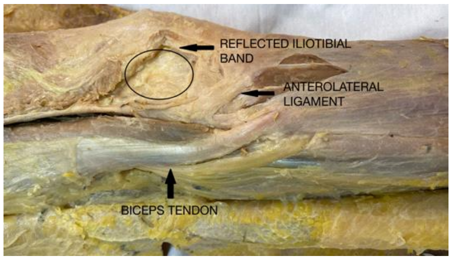
REFLECTED ILIOTIBIAL BAND
ANTEROLATERAL LIGAMENT
BICEPS TENDON

Figure 4: Expose anterolateral ligament

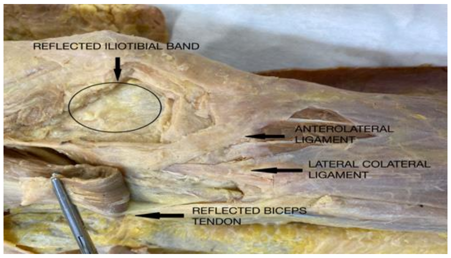
REFLECTED ILIOTIBIAL BAND
ANTEROLATERAL LIGAMENT
LATERAL COLATERAL LIGAMENT
REFLECTED BICEPS TENDON

Table 2: Prevalence of anterolateral ligament in the Romanian population

	Female	Male	Total	P-value
Length (knee extended)	33 ± 5.9 mm	31.6 ± 5.8 mm	32 ± 6mm	0.56
Width (articular line)	5.2 ± 2.2 mm	5 ± 1.8 mm	5.1 ± 2mm	0.78
Thickness (articular line)	1.2 ± 5.3 mm	1 ± 4.8 mm	1.1 ± 0.5 mm	0.16

There were multiple researches conducted regarding the presence and anatomical features of the anterolateral ligament in various populations. We choose a few studies that we considered more relevant for our ethnicity and we compared them with our study. Our results were similar with some of them (Kennedy et al (2015), Vincent et al (2012)), but others described the presence of the ALL in a minority of cases – Potu et al (2016), Roessler et al (2016)) – as it is seen in Table 3.

Table 3: Anterolateral ligament prevalence in the world

Autor (year)	Subject (nr)	Population (mean age)	Prevalence (%)	Lenght (mm)	Width (mm)	Thickness (mm)
This study	Cadaver (5)	Romania (75.1)	100	32 ± 6	5.1 ± 2	1.1 ± 0.5
Dodds et al (2014)	Cadaver (40)	UK (75)	83	59 ± 4	6 ± 1	
Kennedy et al (2015)	Cadaver (15)	USA (58.2)	100	36.8		
Vincent et al (2012)	Cadaver (10)	France (85.3)	100	34.1 ± 3.4	8.2 ± 1.5	2-3
Runer et al (2016)	Cadaver (44)	Austria (78.1)	45.5	42.2 ± 6.2	5.6 ± 1.3	1.2 ± 0.3
Claes et al (2013)	Cadaver (41)	Belgium (79)	97	38.5 ± 6.1	6.7 ± 3.0	1.6 ± 0.6
Helito et al (2013)	Cadaver (20)	Brazil (61.5)	100	37.3 ± 4.0	7.4 ± 1.7	2.7 ± 0.6
Caterine et al (2015)	Cadaver (19)	Canada (70)	100	40.3 ± 6.2	5.1 ± 1.8	1.4 ± 0.6
Potu et al (2016)	Cadaver (24)	Caucasian	4.16	34.23	4.04	1.78
Roessler et al (2016)	Cadaver (20)	Germany (79.4)	60	39.63 ± 0.78	5.28 ± 0.33	1.52 ± 0.31

DISCUSSION

Although the number of cadavers selected in this study was low (five cadavers), one of the most important observations was that the anterolateral ligament was highlighted on all 10 knees. In the specialty literature until now there was shown inconsistency in discovering the ALL [7-9]. After Claes et al. [5] conducted the study, the anterolateral ligament began to get high importance and from this more studies had been made, but had uncertain results. While some authors redact that they have a 100% prevalence rate for ALL [10-13], others describe its presence in a minority of cases [7-9]. Another important detail is that the anterolateral ligament is a capsular-ligamentous structure in the lateral aspect of the knee, and although many studies were made, some of them discovered the absence of this structure [14-16]. Seebascher described that the lateral facet of the knee has three layers [17], and Getwood said that the ALL is situated in the Seebacher layer [18]. Through the dissection of the cadavers, I had concluded to agree with Getwood and that the anterolateral ligament is situated proximal attached to the lateral epicondyle and distally to the lateral plateau, having fibers that unite with the lateral meniscus. Regarding other studies, in our dissection, we found the presence of the anterolateral ligament in all the 10 knees (5 right and 5 left knees). The study also demonstrated a shorter, thinner, and narrow ligament comparative with others. The mean length was 32±6 mm, 33±5.9 mm in females, 31.6±5.8 mm in males. The mean width was 5.1±2 mm, 5.2±2.2 mm in females, 5±1.8 mm in males. The mean thickness was 1.1±5 mm, 1.2±5.3 mm in females, 1±5.3 mm in males. It couldn't be demonstrated a significant difference between sexes.

CONCLUSIONS

The study was conducted to confirm the presence of the ALL in the anterolateral region of the knee and to analyze its anatomical characteristics using Romanian cadavers. A 100% presence was found, with a slight difference from the length, width, and thickness in other countries, race not being a decisional factor in differences. Therefore, the capsular thickening thas has been controversial with its naming can be called the anterolateral ligament.

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VARIA

Bladder injury – A team challenge

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Abstract: *Bladder trauma is caused by a direct blow to the distended bladder, severe injury fracturing the pelvis, iatrogenic, or penetrating wounds. Early detection and diagnosis are key to successful management of cases, but omission or late reveal of bladder damage increase mortality and create long-term problems. A bladder injury may not always present with immediate obvious signs and symptoms, especially in a multi-trauma patient, who is also more difficult to investigate. Moreover, during the COVID-19 pandemic, delayed patients' access to tertiary, multidisciplinary hospitals, increases the risk for delayed diagnosis and also increases the need for more specialists from different surgical and non-surgical areas to raise their awareness of less common manifestations of bladder trauma. We present a review of literature and cases of less common bladder damage from the perspective of a multi-disciplinary team in the University Emergency Hospital in Bucharest.*

Keywords: *bladder injury, pelvic fracture, urinary fistula*

BACKGROUND

Bladder injuries

Based on etiology, bladder rupture and injury can be blunt, penetrating, iatrogenic, or spontaneous. Anatomically, bladder injury can be extraperitoneal (40-60%), intraperitoneal (15-30%) or mixed (10-25%) [1].

Most of these injuries are caused by road traffic accidents, work-related accidents, falls, crashes, violent crimes, military conflicts, and iatrogenic maneuvers, such as obstetric, gynecologic, and urologic procedures [1, 2]. Common signs and symptoms of blunt bladder trauma are gross hematuria, suprapubic pain, abdominal bruising, extravasation of urine into the perineum and genital organs and thighs [1]. Delayed diagnosis may lead to peritonitis, fistula, and septicemia [1, 2]. In patients with other associated pathology or other associated trauma, undetected bladder injury can even be fatal [1].

Bladder fistula

A less common complication of delayed bladder injury

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diagnosis is bladder fistula [1, 3]. Fistulas are abnormal communications within the genitourinary system or may involve other structures as the gastrointestinal tract, skin, vascular and lymphatic systems [1-3]. Bladder fistulas may involve the urethra, skin, gastrointestinal organs, female reproductive tract, and even pelvic joints [1, 2].

MATERIALS AND METHODS

Because bladder trauma and genitourinary fistulas are so diverse in their anatomy and clinical appearance, we present a review of literature about the less common occurrence of bladder trauma and our experience with this pathology.

Patients' data and imaging were collected from the University Emergency Hospital in Bucharest database.

RESULTS

Tertiary unit hospitals usually confront with multiple trauma patients who need a complex evaluation. Some of these patients come from isolated areas or after being initially partially evaluated in other healthcare facilities.

As described in the literature, most patients with bladder injuries present after obstetrics and gynecology procedures, intra-abdominal injuries (44-68.5%), or pelvic fractures [1-3]. The principal sign of bladder injury is visible haematuria, suprapubic pain, and urinary leakage, but according to the type of injury and the associated complications, bladder trauma diagnosis can be a real challenge for the specialists confronting with it. Usually, urologists and general surgeons are those who operatively manage the cases, but the nature and timing of intervention depend on additional complications and may require a differential diagnosis.

A complication of bladder rupture is a fistula, but rare types of fistulas, such as vesical-cutaneous fistulas associated with pelvic trauma have been sporadically described and no guidelines exist for their management. A particular type of posttraumatic bladder fistula with no immediate clinical manifestations is a vesical-vulvar fistula. As illustrated in the case below, these fistulas can often be mistaken for labial tumors or abscesses.

Posttraumatic bladder injury with vesical-labial fistula

A gynecological and urological consultation was requested for a non-functioning urethral-vesical catheter of a 60 years-old female patient admitted in the Orthopedic Department that underwent surgery with external fixation 2 days prior for a complex pelvic fracture (transverse fracture of the right acetabulum, left obturator ring fracture, left sacral fracture, fifth lumbar transverse apophysis fracture with paralysis of the external left sciatic nerve) (Figure 1).

Figure 1: Preoperative x-ray scan showing a complex pelvic fracture type Tyle C2.

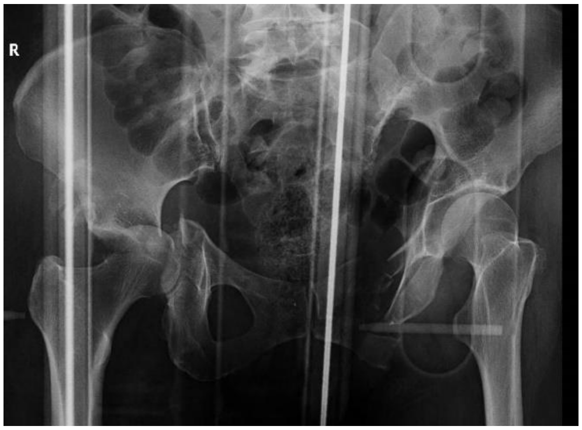

The patient presented with a left labial abscess with spontaneous exteriorization of content. Upon examination, the labial abscess raised suspicions due to the secretions exteriorized that were abundant and liquid, milky at first then clear (Figure 2).

Figure 2: Vesico-labial fistula in a patient with pelvic fracture and suspected bladder injury. The aspect could be easily misdiagnosed as a vulvar abscess.

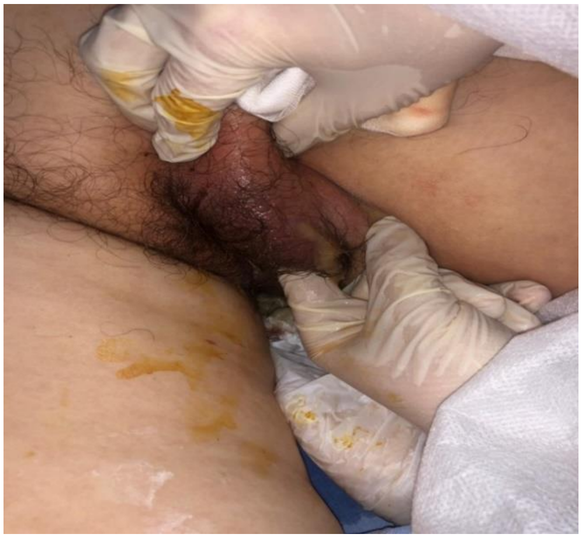

The suspicion of the vesical-cutaneous fistula was confirmed after administering methylene blue in the bladder via the functioning Foley catheter and observing the dye exteriorizing at the site of the labial orifice, therefore adding the diagnosis of extraperitoneal bladder injury complicated with a cutaneous fistula. The existing 14 CH Foley catheter was replaced with a 22 CH one and the external orifice of the fistula tract was drained with a draining tube.

The patient's recent history revealed the above-mentioned fractures with the left bladder wall tractioned laterally with no contrast extravasation 15 days before the consultation with surgical treatment delayed due to a positive RT-PCR COVID 19 test, the patient being transferred to a SARS-Cov 19 support Hospital. The onset or presence of hematuria in this time interval is not specified. The patient did benefit from bladder drainage, but with multiple clogging and rather unfunctional drainage before our consultation.

A second CT-scan was requested and confirmed the suspicion of vesical fistula (Figure 3) and revealed fluid accumulation (10/5.5/3cm) in the left external obturator space.

Figures 3: CT-scan showing complex pelvic fracture and the communication between the bladder and the left labia.

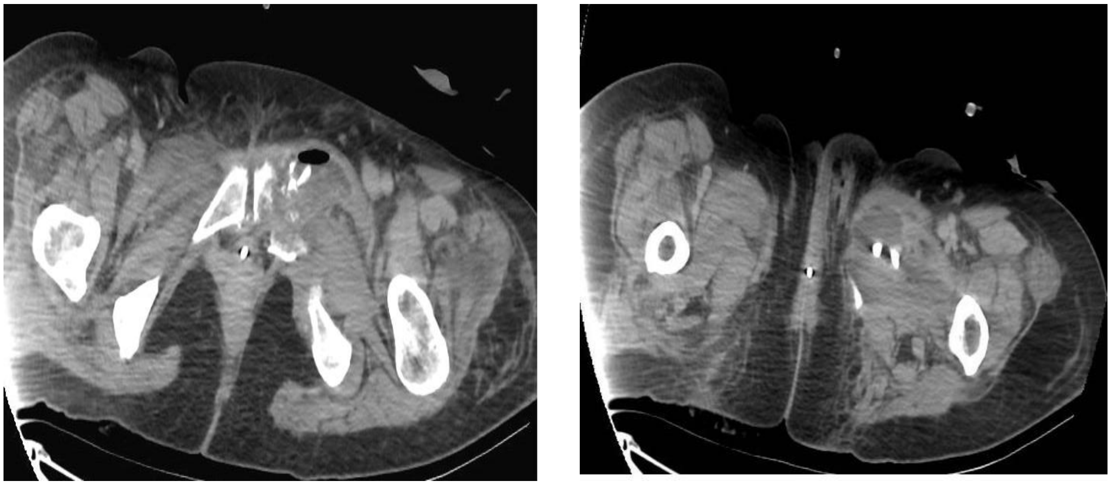

A second draining tube was inserted in this space with ultrasonic guidance to drain intramuscular collections (Figure 4).

Figure 4: Ultra-sonographic aspect of fluid and pus collection on the fistulous trajectory of the vesical-labial fistula.

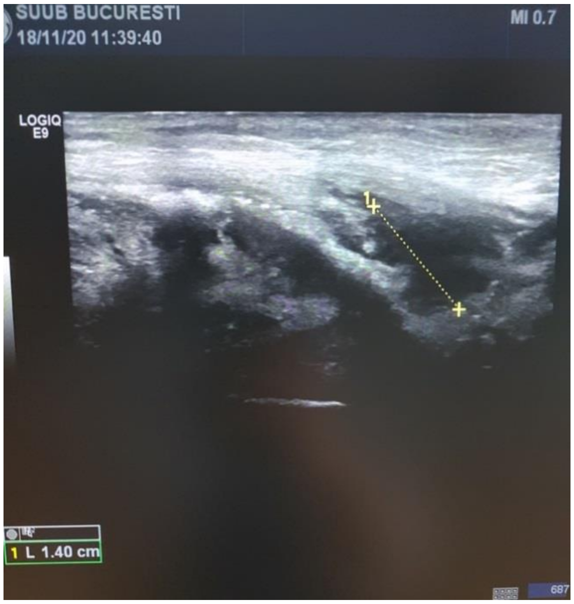

Since the urological consultation and the diagnosis of the fistula took place 2 days after the patient underwent external fixation surgery, the possibility for concomitant primary bladder repair was excluded, leading to the decision for conservative management, with antibiotic treatment, monitored diuresis, and drainage. In case of a favorable outcome, the conservatively treated bladder injury will be followed by cystography to rule out extravasation. On the other hand, if the conservative treatment fails, the patient will undergo abdominal surgery for bladder repair.

The particularity of this case is that the bladder rupture presented with unspecific signs and symptoms, with a rather unusual fistula. The nature of the pelvic fracture, the malfunctioning catheter drainage, and the postoperative status of the patient leads to a more difficult and delayed diagnosis and management of the bladder injury.

DISCUSSION

Researchers found that 85% to 100% of bladder injuries are associated with concomitant pelvic fractures [1-3]. The bony spicules of a fractured pelvis and the shearing forces are the underlying mechanisms of extraperitoneal bladder rupture. Extraperitoneal injury is almost always associated with pelvic fractures [2]. The highest risk of bladder injury was found in disruptions of the pelvic circle with displacement > 1 cm, diastasis of the pubic symphysis > 1 cm, and pubic rami fractures [3, 4].

Intraperitoneal bladder rupture most commonly occurs on the dome of the bladder because it is the least protected area. Direct blow and deceleration forces are the underlying mechanisms. Urine leaking into the abdomen can be absorbed by the peritoneum, causing increased urea and creatinine, metabolic alterations, and low urine output. Infected urinomas can cause pelvic abscesses and peritonitis. Mixed intra- and extraperitoneal bladder rupture are less common, occurring in 8% of bladder injuries associated with pelvic fractures [5].

Diagnosis of bladder injuries can be challenging due to the often vague and non-specific presentation. Literature studies found that up to 23% of all bladder and urethral injuries associated with a pelvic fracture are missed at initial evaluation [6]. Moreover, other studies highlight the delay in diagnosis since the patients were from isolated areas, with poor access to a tertiary trauma center [7].

Iatrogenic bladder injuries are not uncommon. The bladder is the most frequently injured organ during obstetric and gynecologic interventions, such as hysterectomies and cesarean sections. Frankman et al. reported an incidence of 13.8 bladder injuries per 1000 procedures in 2010, but as the incidence of cesarean sections has increased worldwide, the incidence of urologic injuries is expected to rise as well [8].

Vesicovaginal fistula is the oldest described type of fistula. It may occur as a complication of childbirth, hysterectomy, irradiation, and trauma. Patients with neoplasms of the cervix and bladder, especially those who underwent irradiation, are more prone to develop vesicovaginal fistulas [1,4]. Vesicocutaneous fistulas occur after surgical procedures or trauma. Those occurring at suprapubic cystostomy sites after catheter removal usually close spontaneously.

A rare complication of pelvic trauma with bladder injury is a vesical-vulvar fistula, which can be misdiagnosed as a labial tumor or abscess. Also, a vesical-articular fistula can involve the hip joint, causing septic arthritis [9].

Imaging diagnostic methods of bladder trauma and fistula

According to literature studies, conventional intravenous abdominal/pelvic CT will miss 40% of bladder ruptures [1, 10]. Absolute indications for bladder imaging include visible haematuria and a pelvic fracture or non-visible haematuria combined with high-risk pelvic fracture (disruption of the pelvic circle with displacement > 1 cm or diastasis of the pubic symphysis > 1 cm) or posterior urethral injury [4]. Cystography is the preferred diagnostic modality for non-iatrogenic bladder injury and a suspected iatrogenic bladder trauma in the postoperative setting [11]. CT cystography has a higher sensitivity and specificity (99-100%). Both the American Urological Association (AUA) and the European Association of Urology (EAU) recommend either plain-film or CT retrograde cystography for detecting bladder injuries in all suspect patients. In critically ill patients, when cystography cannot be initially performed, a Focused assessment with sonography for trauma (FAST) exam can be used. Bedside ultrasonography with intravesical saline instillation may assist in the early diagnosis of bladder rupture, but it depends on the ultrasonographer's experience [12, 13]. Ultrasonography can also detect intra or extraperitoneal fluid. In the case of pelvic fracture, CT-cystography is superior in detecting bony fragments within the bladder [2, 13]. Cystoscopy is the preferred method for the detection of intraoperative bladder injuries as it directly visualizes the laceration and can localize the lesion concerning the position of the trigone and ureteral orifices [14]. Large perforations are suggested by the lack of bladder distention during cystoscopy.

Imaging diagnostic methods of bladder fistula also include fistulography, cystoscopy, and voiding cystourethrography.

In large vesicovaginal fistulas, urine leaking into the vagina can be easily seen, or methylene blue dye can be used to mark the fistulous path [15].

Management options from international guidelines

The EAU states that the majority of uncomplicated extraperitoneal bladder injuries can be managed with catheter drainage alone and will heal in about 14 days. Conservative management, which comprises of clinical observation, continuous bladder drainage, and antibiotic prophylaxis is the standard recommended treatment in all uncomplicated extraperitoneal injury due to blunt or iatrogenic trauma [16, 17]. In any patient with penetrating or intraperitoneal injury, emergency exploration and repair are recommended even in the absence of pelvic fracture. Bladder neck involvement, bone fragments in the bladder wall, concomitant rectal, or vaginal injury, or entrapment of the bladder wall necessitate surgical intervention [5].

Orthopedic management of pelvic fractures includes internal fixation or temporary external fixation. Given the increased infection risk associated with prosthetic material used in orthopedic surgery, urologists are advised to consider primary repair of uncomplicated bladder injuries to reduce the risk of colonized urine infecting osteo-synthetic material (EAU guideline, Grade B recommendation). Recent studies comparing conservative management to concomitant bladder repair in the setting of internal fixation found an increased risk of infection in patients that did not benefit from concomitant bladder repair [18, 19]. Therefore, bladder repair is recommended at the same time with the internal fixation and exploration for other injuries to decrease the risk for complications and to reduce recovery time [20-22].

Bladder closure is performed with absorbable sutures. There is no evidence that a two-layer is superior to watertight single-layer closure [2, 23].

Follow-up of patients with bladder injury must always include bladder drainage to prevent elevated intravesical pressure and to allow the bladder to heal. Conservatively treated bladder injuries must be followed up by cystography to rule out extravasation and ensure proper bladder healing [4]. The first cystography is planned approximately ten days after injury. In case of ongoing leakage, cystoscopy should be performed to rule out bony fragments in the bladder, and a second cystography is warranted one week later [24, 25]. Cystography is always advised in cases with complex injury (trigone involvement, ureteric reimplantation) or with risk factors of impaired wound healing [13, 15]. In healthy patients with a surgical repair of a simple bladder injury, the catheter can be removed after 10 days without a cystography. Some authors recommend drainage catheter removal after 5 days in conservatively treated internal iatrogenic extraperitoneal bladder perforations and after 7 days for extraperitoneal perforations [11, 22].

Management options for vesico-vulvar and vesicovaginal fistula

The treatment of choice for vesicovaginal fistulas is surgical, although spontaneous closure of small fistulas with simultaneous permanent catheterization for 19-54 days is mentioned in the literature. Approximately 10% of vesicovaginal fistulas from hysterectomy resolve conservatively if the fistula is small (<1cm, <1.5cm), is recent and the Foley catheter is inserted as soon as possible [26-29].

Thus, if a fistula does not close spontaneously in 4 weeks, surgery is needed. Hillary et al. described high percentages of success for the vaginal approach of fistula repair (90.8%) as compared to the abdominal approach (83.9%) [30]. Studies about fistula approach and follow-up are generally scarce and report small numbers of patients. Kapoor et al., in a study on 52 cases, for which he used vaginal and abdominal approach, found that vaginal approach is superior in uncomplicated cases, with small fistulas, but an intraabdominal repair is mandatory for extragenital fistula, for highly situated fistula, when reimplantation of ureters is needed, or when the vaginal approach is impossible [31].

The best timing to proceed to surgical repair is between 3 to 6 months from the appearance of the fistula. Ideally, the best time would be in the first 2 weeks, before bladder inflammation and fibrosis appear, but unfortunately, it takes more than 2 weeks for the fistulas to be detected.

The surgical approach may be transvaginal, transabdominal, or transvesical. Bilateral ureteral catheterization before intervention is recommended, as illustrated in Figure 5. A Foley catheter will also be kept in place for 14 days and removed after a prior retrograde cystography [32].

Figure 5: Intraoperative image of a transabdominal repair of a vesical-vaginal fistula in a post-hysterectomy patient. Ureteral catheterization was also performed and the bladder was sutured.

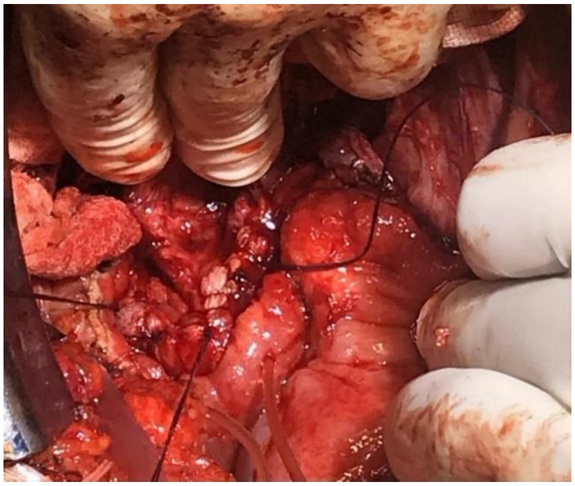

Vesico-labial fistulas are rare and have been scarcely described by literature. Their initial appearance which can lead to the misdiagnosis of vulvar abscesses and the need to primarily address the associated lesions makes this type of vesical-cutaneous fistula to have a later diagnosis and lead to a later approach by transabdominal repair.

CONCLUSIONS

Bladder rupture must be suspected in multi-traumatized patients with complex pelvic fractures, even in the absence of immediate onset of common symptoms like gross hematuria.

Because 40% of bladder injuries are missed at first emergency evaluation, repeating CT scans and using additional imaging techniques can reveal the bladder injury and other posttraumatic lesions.

Vesico-labial fistula is a rare complication of a delayed diagnosis of posttraumatic bladder rupture that can be easily misdiagnosed as a vulvar tumor or labial abscess, requiring a different approach to fistula repair.

Conservative management is the treatment of choice in a patient who already underwent surgical external fixation for pelvic fracture as long as the bladder injury is not complicated by infection and the bladder fistula is small. Bladder drainage with a large Foley catheter is mandatory, with permanent surveillance of catheter functionality and antibiotic prophylaxis. Concomitant bladder repair with internal pelvic fixation or other abdominal wound exploration is recommended to decrease complications and healing time.

The correct diagnosis and management of such cases always require a multidisciplinary team, including urologists, general surgeons, orthopedists, Ob&Gyn specialists, and radiologists.

Medical teams must raise their awareness of potential insidious complications of bladder injuries as some diagnosis can be delayed or incomplete due to the challenges that come with the COVID19 pandemic.

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Facial skin cancer: our surgical experience

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Abstract: *Skin cancer represents an important part of the plastic surgeon's practice, and surgical excision followed by reconstruction is the most frequently used procedure. The main objective of this paper is to report and evaluate our experience in the treatment of facial nonmelanoma skin cancer.*

Method. The study is based on 303 patients who were diagnosed with facial malignant tumors, between 2004 and 2015, in the Plastic and Reconstructive Surgery Clinic, Emergency University Hospital Bucharest Romania. We statistically analyzed the distribution by age, gender, facial location, the time from onset until the presentation, the type of tumor, the size of the tumor, the margin status, and the recurrence. Results were as follows: median age at 70 years, with an even gender distribution. The most affected areas were the cheek, followed by the nose, forehead, and eyelids. We calculated the dimensions of the tumors between 1.57 mm^2 and 1,846 mm^2, with a median value at 235 mm^2, and a mean value at 421.23 mm^2. Patients in whom safe margins were not obtained had a 4.15 times higher relapse rate versus the ones with safe margins at the first intervention, with a high statistical significance – p=0.002 (15% recurrence rate if safe margins were not obtained, versus 4.1% if margins were safe).

Conclusion. When discussing tumor excisions, one of the most controversial topics is that of safety edges. Currently, even if there are recommendations, a unified protocol is not formulated, which is why we found it useful to research this topic with extensive medical associations.

Keywords: *skin cancer, tumor excision, safety edge*

INTRODUCTION

Tumors represent an important part of the plastic surgeon's practice, surgical excision followed by reconstruction is the most frequently used procedure [1].

The current paper intends to present evidence-based information for such procedures, to establish the current status of cutaneous tumors approach in our clinic, in Romania, compared international guidelines, and to gather a set of recommendations for skin tumors surgery.

MATERIAL AND METHODS

The study is analytical observational and prospective – based on 303 patients who were diagnosed with facial malignant tumors, between 2004 and 2015, in the Plastic and Reconstructive Surgery Clinic, Emergency University Hospital Bucharest Romania.

The inclusion criteria were chronic malignant lesions of the face, with the complete histopathological result, which mentioned the surface and depth, the exclusion criteria

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were psychiatric disorders, epilepsy, patients who do not cooperate, incomplete histopathological result, tumors that affect other areas that the skin (ENT, ophthalmological, BMF).

Tumor surgical protocol, for malignant tumors, the excision aimed to encompass a safety margin of 3-6 mm. The excised pieces were sent for histopathological examination and the final and complete diagnosis was set after the result was available (7-21 days). The first postoperative evaluation was performed at a median of 3 weeks, and the patients were followed up every 3 months for 2 years.

The collected data was uniformly analyzed; statistical analysis was performed using Microsoft Office Excel for Windows, SPSS version 21.0 (Statistical Program for Social Sciences), and EpiInfo version 7. Non-parametric tests were used, as the studied population did not have a normal distribution. To establish risk rates, we computed the odds ratio (OR) and 95% confidence intervals using EpiInfo. Statistical significance was established for $p < 0.05$. To correctly interpret certain numerical variables and especially how these values may influence the safety margins, as well as the reconstructive procedures, we used ROC curves (receiver operating characteristic) – SPSS 21.0.

RESULTS

In the selected study group the distribution:
- by age was between 35-97 years, with the average age at approximately 71;
- by sex was 50,16% (152) vs 49,84% (151)-male vs female cases.

Analyzing the time since the first detection of the lesion and the moment of presentation to the doctor, we observed an average of 58 months and a median at 24 months; also, 19.3% of the patients had received different treatments for the lesions before presentation in our clinic.

The types of cutaneous malignant tumors were: 215 (70.95%) basal cell carcinomas, 56 (18.48%) squamous cell carcinomas, 5 (1.65%) malignant melanoma, 20 (6.6%) metatypical and mixed carcinomas, non-melanoma tumor relapses 5 cases (1.9%), and 2 cases of metastasis.

Depending on the location related to the face segments, we recorded: 94 lesions (31.03%) on the cheek, 52 lesions (17.16%) on the nose, 47 lesions (15.51%) on the forehead, 35 lesions (11.51%) on the eyelids, 17 lesions (5.61%) on the lip, 17 lesions (5.61%) on the ear, 2 (0.6%) chin lesions, 32 (10.56%) tumors with mixed locations and 7 (2.31%) tumors with an extended location.

Basal cell carcinomas were most commonly located on the cheek – 32.1%, nose – 18.1%, forehead – 14.9%, eyelids – 14%, ear – 4.7%, lip – 2.8%, and the squamous cell carcinoma were more commonly in cheek – 28%, lips – 22%, nose – 20%, forehead – 18%, eyelid – 6%.

We calculated the dimensions of the tumors between 1.57 mm^2 and 1,846 mm^2, with a median value of 235 mm^2, and a mean value at 421.23 mm^2.

Analyzing the tumor margins, we calculated that the safe margin (tumor-free) was obtained at 75.6% of the patients.

When the dimensions of the tumors were analyzed depending on the safe margin rate, we observed that the patients where safe margins were obtained had a median tumor dimension of 197.82 mm^2, significantly lower than the median dimension in patients where the margins were invaded (405.84 mm^2), p=0.0011 (Figure 1). Similar differences were recorded when comparing median depth of tumoral excisions for safe margins (5 mm), versus invaded margins (6 mm), p>0.05.

Figure 1: Free margin

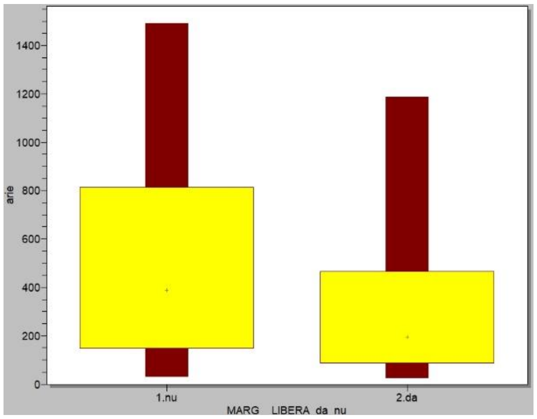

The rate of obtaining free margins depending on tumor location was as follows: for lips and chin were completely removed in all cases (100% rate of free margins) eyelid – 71.42%, nose – 73.06%, cheek – 72.33%, forehead – 72.34%, chin – 86.4%, ear – 64.70%, extended location – 57.1%, mixed location – 100%. For tumors with mixed locations, free margins were obtained in all patients (rate 100%), whereas for tumors with a unique location, the rate was 89.4% (p<0.05).

This apparent paradox is related to the lower medium dimensions of tumors with mixed location – approx. 146 mm^2 versus 247 mm^2 (for tumors with strict location), p>0,05. For tumors with extended location, on more than one esthetic unit, usually with a long history of evolution, free margins were obtained in only 62.5% of cases, presenting a 1.9 times higher odds ratio for lack of safe margin versus limited location tumors (p>0.05). The median dimensions of these tumors (extended location) was also significantly higher (1,041 mm^2 versus 219 mm^2, p=0.0007) for tumors limited to one esthetic unit.

Figure 2: Extensive location

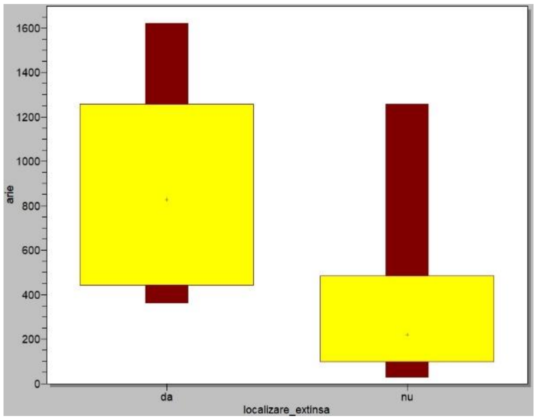

For malignant tumors, free margins were obtained in 64.20% of squamous cell carcinomas, 79.06% of basal cell carcinomas, 100% for melanomas, and 75% for metatypical and mixed carcinomas.

When analyzing the rate of safe margins depending on tumor dimensions for malignant tumors, we observed that the median dimension for basal cell carcinomas with free margins was 152.29 mm^2, versus 235.5 mm^2 for invaded margins, p=0.017.

For squamous cell carcinomas, median dimensions for free margins was 447.45 mm^2 compared to 706.50 mm^2 for non-tumor free.

For the most aggressive tumor, malignant melanoma, free margins were obtained for all patients (100% rate), due to the strict surgical protocol in these cases regarding macroscopic safety margins.

Figure 3: Free margin

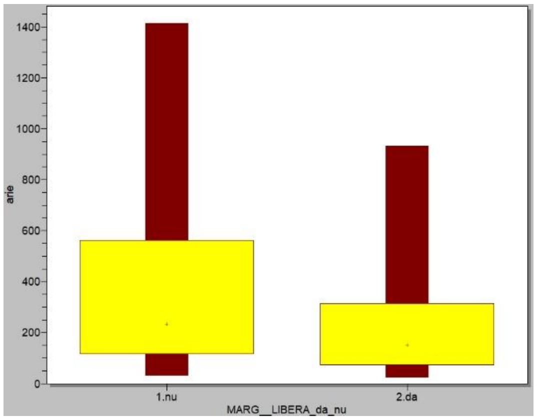

For more rare tumors (metatypical and mixed carcinomas) we obtained free safe margins in tumors with a median dimension of 663.53 mm^2 compared to 710.89 mm^2 for invaded margins.

We analyzed the recurrence rate for patients with malignant tumors and we observed that it appeared in 6.3% of the cases.

The recurrence rates differed on the type of tumor: 5.0% for basal cell carcinoma, 10% for squamous cell carcinomas, 0% for malignant melanomas, and 9.1% for mixed and metatypical carcinomas (Figure 4).

Figure 4: Extensive location (recurrence yes ●, recurrence no ●)

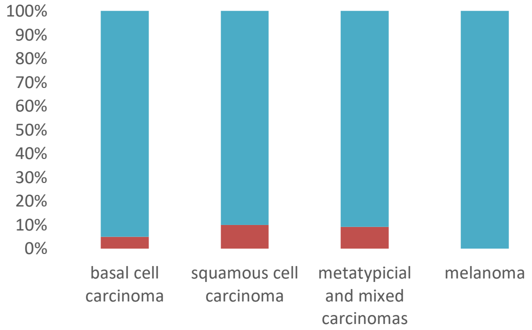

We made a separate analysis of the most frequent malignant tumors, respectively basal cell, and squamous cell carcinomas. Basal cell carcinomas had a very low relapse rate, with a relative risk of 0.48 (p>0.05) versus all other malignant tumors. The squamous cell carcinomas had a relapse risk of 1.90 (p>0.05) versus other malignant tumors.

Patients in whom safe margins were not obtained had a 4.15 times higher relapse rate versus the ones with safe margins at the first surgery, with a high statistical significance – p=0.002 (15% recurrence rate if safe margins were not obtained, versus 4.1% if margins were safe).

We did not observe any significant difference when we analyzed the recurrence rate by tumor dimensions.

Also, although the depth of the recurring tumors was higher than the depth of non-recurring ones (6 mm versus 5 mm), the difference was not statistically significant.

DISCUSSION

Skin tumors are the most common tumors, which is why reconstruction after tumor ablation is the most frequently performed procedure by the plastic surgeon and one of the most active areas of expertise [1]. More than in any other surgical area, the approach for facial tumors has to include a correct and complete tumor resection with a minimum area of excised tissue to preserve the function and shape of the reconstructed area. Our main interest in this study was represented by malignant facial tumors.

In our group, the median age was around 70 years and we observed an almost even gender distribution, consistent with the data reported for basal cell and squamous cell carcinomas [2-5]. Regarding the distribution by aesthetic areas, the most affected ones were the cheek, followed by the nose, forehead, and eyelids.

Regarding malignant tumor pathology, we found that basal cell carcinoma was most frequent, followed by squamous cell carcinoma; metatypical carcinomas – aggressive variants of basal cell carcinoma (they have squamous cell carcinomas behavior). This data overlaps the numerous reports that basal cell and squamous cell carcinomas are most common in the face [6-9, 21].

Analyzing the tumor size, we obtained a median size for the entire group of 303 patients, of 428.53 mm^2. The sizes of the tumors observed in our study are quite high, much larger than those reported by other studies. This was correlated with an increased positive margin rate, which in turn increases the risk of subclinical extension and recurrence [11].

When analyzing separately patients with basal cell carcinomas, we observed that free margin was obtained in 79.06% of cases. A meta-analysis of the literature led by Gulleth et al. on 16,066 cases, encompassing 106 articles on basal cell carcinomas, revealed an average diameter of basal cell carcinomas of 11.7 mm with an average percentage of free margins of 86% ± 12% [12]. Malik et al. in their analysis of 1,832 basal cell carcinoma obtained 86% negative margins excisions [13]. In a study on 2,016 cases for BCC with a diameter smaller than 10 mm, Breuninger obtained a probability of negative margin between 70%-95% depending on the size of the safety margin taken, 2, 3, or 5 mm [14]. In our group, the median dimension for basal cell carcinomas with positive margins was 235.50 mm^2, corresponding to lesions of approximately 23 mm/10 mm. The majority of available data on basal cell carcinomas report small lesions, respectively under 20 mm largest diameter [4, 5], whereas our series comprises patients with much larger lesions, as described above. Therefore, this could explain the lower rate of safe margins in our group, which is related to bigger lesions with a more prolonged evolution.

For squamous cell carcinomas, the median size for excisions with free margins was 447.45 mm^2, versus 706.50 mm^2 for invaded one, with 64.2% of negative margins. Studies on squamous cell carcinomas are much smaller, but we note the results of the prospective study by Peed-Yau Tan et al. in which although the authors do not mention the size of the lesions, they achieved a percentage of only 7.6% excisions with invaded edges [15]. Talbot S. and Hitchcock B. report an invaded edge of 14% in a study on basal cell and squamous cell carcinomas [16]. Again, the tumor size in our patients is quite high, much higher than the limits considered significant (respectively 20 mm largest diameter)[17], thus accounting for the lower rate of free margins.

Another variable tracked in our study was the depth of tumor invasion. There is no clear recommendation in the literature regarding how deep the excision should go; however, for basal cell carcinomas, tumor depth is correlated with the risk of recurrence, and respectively for squamous cell carcinoma, with the rate of metastases [10, 18]. Breuninger reported that, for over 6 mm depth, the metastases rate in squamous cell carcinoma is 15%, and Motley emphasized the depth of 4 mm as associated with recurrence [19]. In practice, deep excision is performed according to what is found intraoperatively and depending on the surgeon's experience. The average excision depth in our free margins lot was 5 mm, while for invaded edges it was 6 mm.

For basal cell carcinomas, recurrence occurred in 5% of patients with negative margins and 10% for positive margins, and for squamous cell carcinoma, respectively in 10% for negative margins and 15% for positive margins. In our study, we found no statistical relevance for recurrence concerning dimensions and tumor depth. In the literature, the BCC recurrence rate varies between 0-10.1% for complete excision, and for incomplete excisions, it is estimated at 27% but can reach as high as 35.5% [12, 20, 21, 23]. For SCC the local recurrence rate varies between 1.7-7% in some studies [24], but it can reach even 53.6% depending on location, the grade of differentiation, perineural involvement, and surgical excision method (standard or Mohs surgery) [25].

The first rule in nonmelanoma skin cancer surgery is to obtain a complete excision with negative margins. In our study, most of the cases had a long evolution period before the presentation, which explains why approximatively 57% were large tumors, above 2 cm. In the cases with positive margins, a re-excision was performed where it was possible, or the patient was referred to the oncology service.

CONCLUSIONS

When discussing tumor excisions, one of the most controversial topics is that of safety edges. What is the size of the safety edges required for complete excision, with free tumor margins, thus ensuring the lowest recurrence rates, and at the same time with a minimal defect for a successful reconstruction?

Currently, even if there are recommendations, a unified protocol is not formulated, which is why we found it useful to research this topic with extensive medical associations.

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VARIA

Updates in teenage acute intentional self-poisonings

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Abstract: *Acute intentional poisonings represent an important part of emergency pediatric pathology, as well as a psychiatric one, with an escalation tendency over the past years. The current paper consists of a descriptive prospective study, which took place over a period of 12 months, and included 342 children within the age range 6-18 years, that presented in the Emergency Unit, being subsequently treated as in-patients of the Pediatric Poisoning Center of "Grigore Alexandrescu" Emergency Clinical Hospital for Children. The number of pediatric acute intentional poisonings hospitalized within the time frame of the study was 819 cases. Of these, 342 were intentional, which constitutes a percentage of 41.75%, and 477 were accidental, i.e. 58.24%. The etiology of the acute intentional poisonings is varied; however, medication (54.38%), ethanol (28.65%) and drugs of abuse (12.28%) were predominant. A higher frequency of acute intentional poisonings was noticed in girls (65.49%) as compared to boys (34.50%), probably due to the particularities the psychological profile of this sex shown at this age, i.e. higher emotional instability and display tendency. Out of the total number of acute intentional poisonings, we identified 20 cases of suicide attempts, which represent 5.83% of the total acute voluntary poisonings. Moreover, some of these cases constitute a repeated suicide attempt. The studied group included 30 cases of chronic substance abuse and 6 chronic alcohol abuse cases, with ages within the 13-17 years range, 12 of which were females and 24 males. Out of the 36 chronic substance/alcohol abuse patients, 4 were social cases. Pediatric acute intentional self-poisonings are an important public health issue, alarming through its consequences and through its hidden neuropsychiatric and behavioral substrate. This is due to the fact that adolescence is a period of marked emotional fragility, sensitive to all sorts of influences.*

Keywords: *self-poisoning, teenager, psychiatric disorders, alcohol, substances of abuse*

INTRODUCTION

The prevalence of teenage acute intentional self-poisoning is high, due to the fact that adolescence is a period of profound changes with visible effects on an individual's physical aspect, behavior and relationships with the surrounding world. It features a personality focus on acquisitions and social statuses related to school life, family life, friend circle.

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Adolescence constitutes a time frame of intense development, marked by a series of profound changes with visible effects on physical appearance, behavior and relationships with the surrounding environment. Resorting to an extreme gesture, such as acute intentional self-poisoning, which puts one's life at risk, is often a potential exacerbation of a chronic psycho-social disorder [1, 2].

Teenage years are marked by intense developmental processes, on a sexual level included. Affectivity, relationships of all kind begin to include the sexual identity and its integration in the self-image.

Drug abuse, juvenile delinquency, poor learning achievements, teenage pregnancy, sexually transmitted diseases, feelings of despair are frequent causes that lead teenagers to resort more and more often to suicide attempts through various acute self-poisonings. Between the ages of 13 and 16 years, adolescents are confronted with frequent and unexpected mood swings. Both the regression and the progression of the psychological age represent explanations regarding the unpredictability of teenagers, as well as the difficulty to sometimes understand them. Behavioral disorders occur, the greater part of which is induced by disturbances in the emotional development caused by family issues, parental conflicts and splitting [3].

The lack in affection does not refer strictly to its absence in relationship to the adolescent, but also to its misguidance in a direction that ignores the affective needs of the child. The lack in education prevents the adolescent from acquiring necessary social life skills. All these family issues are the source of teenager disorders that lead to powerful social inadequacy behaviors and antisocial acts (4). The adolescent becomes introverted and develops inferiority complexes. It is important that parents combine proofs of affection with exigencies and demand respect, as well as teach their children the affinity towards imitating behaviors of individuals who distinguish themselves through moral and intellectual qualities [5].

The acute voluntary self-poisoning is a dynamic medical pathology that is often the result of a potential exacerbation of a chronic psychosocial disorder; frequently, patients have a personal history of various psychiatric disorders, such as emotional, behavioral and personality disorders, depression, mental retardation [6].

The purpose of the study is to determine the frequency of acute intentional self-poisoning cases, in order to identify the most frequently used substances as the etiology of the self-poisoning, to plot the distribution according to sex, age, month of the year, the presence of suicide-associated psychiatric pathology and to identify the age interval associated with the highest risk for this pathology.

Acute intentional self-poisonings are often associated with school dropouts or the patients are social cases. However, there are cases when the child is left unsupervised or cases when a teenager will abuse certain substances in a moment of emotional turmoil triggered by a conflict with her/his parents, guardians or life partner [7].

A particular intentional self-poisoning in the 12-13 year age group (adolescents) is due to ethanol. Ethanol is one of the most frequent causes of acute intentional self-poisonings in teenagers. The abuse is usually acute and within a social context, unlike adult cases, where the most common causes of acute intentional poisoning are psychotrope medication and medication acting on the cardiovascular system. In addition, another objective of our study was the identification of the particularities of alcohol consumption in teenagers living in our country compared to European teenagers [3].

PATIENTS AND METHODS

The personal study was undertaken in the Pediatric Poisoning Center of "Grigore Alexandrescu" Emergency Clinical Hospital for Children, in Bucharest.

The present research is a descriptive, prospective study that took place over a period of 12 months (1st of January 2017 - 31st December 2017), and included 342 children with ages in the 6-18 years range, who presented in the Emergency Unit and were subsequently treated as in-patients in the Pediatric Poisoning Center of "Grigore Alexandrescu" Emergency Clinical Hospital for Children.

The clinical cases selected for the study were treated as in-patients in the Pediatric Poisoning Center for acute voluntary self-poisoning with various substances. Out of the 2457 hospital admissions in the clinic, 342 cases fulfilled the inclusion criteria for the study.

Inclusion criteria for the study group:
- patients with acute intentional self-poisonings;
- under 18 years of age, or younger than 19, but still students.

Exclusion criteria:
- patients with accidental acute self-poisonings;

Studied parameters:
- patient identification data (age, sex);
- substances used as the etiology of acute intentional self-poisonings;
- motive for the acute intentional self-poisoning;
- number of suicide attempts;

- various chronic substances abuse;
- clinical picture;
- associated psychiatric disorders.

RESULTS

The number of acute poisonings in children treated as in-patients in the studied time frame was 819 cases. Out of these, 342 were intentional, representing 41.75%, and 477 were accidental, i.e. 58.24%.

In the studied group, which contained 342 patients, the etiology of acute voluntary self-poisonings is varied; however, medication (54.38%), ethanol (28.65%) and substances of abuse (12.28%) were predominant (Table 1). Statistical data withstand comparison to literature data - a study performed between 2003 and 2013 in the same clinic - the most common cause remains acute voluntary self-poisoning with medication [8].

Table 1: Case distribution as a function of the etiology of the acute intentional self-poisoning

Etiology	Cases	%
Medication	186	54.38%
Alcohol	98	28.65%
Coffein, energy drinks	4	1.16%
Tabacco	1	0.29%
Substances of abuse	42	12.28%
Household toxic substances	7	2.04%
Pesticides/Insecticides	2	0.58%
Caustic substances	1	0.29%
Plants/mushrooms	1	0.29%

As far as the distribution regarding the months of the year, the study pointed out a higher frequency of cases of acute intentional self-poisonings in the months of January (11.69%), June (11.98%), November (10.88%), but also February (9.35%), March (8.47%), December (9.64%). A lower frequency was noted in April (4.97%), July (6.14%), August (5.55%) and September (6.14%). Most cases of acute intentional self-poisonings were noted in June, which coincides with the end of the school year, and the least cases were noted in the months which correspond to the summer school-break.

A higher frequency of acute intentional self-poisonings was noted for females (224 cases, which represents 65.49%), as compared to males (118 cases - 34.50%), probably due to the particularities of the psychological profile of the gender shown at this age, i.e. higher emotional instability and display tendency [9, 10].

In the study group, we noticed that for female cases, the most frequent etiology for acute intentional self-poisonings was medication (70.5% of intentional self-poisonings in females), while for males, the most common etiology was alcohol (49.1% of the total self-poisonings in males), while the second most common one consisted of substances of abuse (23.7%) (Table 2).

Table 2: Case distribution according to etiology and sex

Etiology	Female	%	Male	%
Medication	158	46.19%	28	8.18%
Alcohol	40	11.69%	58	16.95%
Coffein, energy drinks	4	1.16%	0	0%
Tabacco	0	0%	1	0.29%
Substances of abuse	14	4.09%	28	8.18%
Household toxic substances	7	2.04%	0	0%
Pesticides/insecticides	2	0.58%	0	0%
Caustic substances	0	0%	1	0.29%
Plants/mushrooms	1	0.29%	0	0%

A predominance of acute intentional self-poisonings in children was noticed for the age interval 15-16 years (50.58%), which corresponds to adolescence, a period of marked emotional fragility towards all kind of influences (Table 3).

Table 3: Case distribution with respect to age

Age	No. Cases	%
<10 years	2	0.58%
10-14 years	100	29.23%
15-16 years	173	50.58%
17-18 years	67	19.59%

The study group (342 cases) comprised patients with ages within the range 6 to 18 years. For both sexes, a predominance in the age interval 15-16 years was noted: females (32.74%), males (17.83%) (Table 4).

Table 4: Case distribution according to age and sex

Age	Females	%	Males	%
<10 ani	0	0%	2	0.58%
10-14 ani	75	21.92%	25	7.30%
15-16 ani	112	32.74%	61	17.83%
17-18 ani	37	10.81%	30	8.77%

There were 100 patients in the 10-14 years age group. 75%

were girls.

There were 173 patients in the 15-16 years age group. 65.73% were girls.

There were 67 patients in the 17-18 years age group. 55.22% were girls.

The highest incidence in girls was noted in the 10-14 years age group and we noticed that as adult age is approached, the sex distribution tends to be equal for girls and boys.

In the study group, 79 children (42.47%) presented medication used in the treatment of CNS disorders as etiology of the acute intentional self-poisoning. 49 children ingested Acetaminophen (26.34%) and 19 children (10.21%) used medication acting on the cardiovascular system for the intentional self-poisoning (Table 5). Out of the medication acting on the CNS, patients used carbamazepine, benzodiazepine, valproic acid, levetiracetam, sertraline, risperidone, extraveral, cipralex (escitalopram).

Table 5: The distribution of cases according to medication types as etiology for acute intentional poisonings

Medication class	Cases	%
Medication used in the treatment of cardiovascular disorders	19	10.21%
Medication used in the treatment of central nervous system disorders	79	42.47%
Aspirin	11	5.91%
Acetaminophen	49	26.34%
Antibiotics	10	5.37%
Oral antidiabetics	4	2.15%
Opioids (Morphine)	1	0.53%
Antiemetics	5	2.68%
Vitamins	4	2.15%
Antidiarrheals	3	1.61%
Anti-acne medication (Roaccutane)	1	0.53%

In our study, we noted that, concerning medication self-poisonings, single-medication poisonings were the most frequent type: 63.44%, while multiple-medication poisonings represented 36.55%. Among the combinations encountered, we mention acetaminophen + CNS-acting medication, aspirin + cardiovascular acting medication or blood pressure-lowering medication, carbamazepine + euthyrox + omeprazol, aspirin + acyclovir.

Pediatric acute intentional self-poisonings of alcohol-related etiology were caused by the consumption of vodka (20 cases), wine (17 cases), whiskey (9 cases). Distilled drinks are predominant in the etiology of alcohol ingestion in the adolescents included in the study (Table 6). Moreover, the study showed a number of 26 cases of unknown etiology (the substance of abuse was either unknown or undeclared). Our results are different from those reported by other European countries [11].

Table 6: Case distribution according to the etiology of acute intentional alcohol self-poisonings

Medication class	Cases	%
Wine	17	17.34%
Vodka	20	20.40%
Whiskey	9	9.18%
Beer	6	6.12%
Champagne	2	2.04%
Distilled drinks	7	7.14%
Fruit-flavored distilled drinks	4	4.08%
Tequila	2	2.04%
Medical-use alcohol	1	1.02%
Ethylenglycol-antifreeze	4	4.08%
Unspecified etiology	26	26.53%

Most of the cases of alcohol ingestion took place in a social context, most often in the company of friends, but also with family.

As far as the context in which the acute intentional poisoning took place, most often socializing in a public space or at home with friends determined the occurrence of the poisoning, according to literature as well [12, 13]. In 46 cases, the reason for the ingestion/abuse remained unknown or could not be classified in one of the previously mentioned categories. In 7 of the cases, the etiology of the acute intentional self-poisoning was the combination of medication and alcohol (4 girls/3 boys), and in other 7 cases, the poisoning occurred through the consumption of both alcohol and substances of abuse (3 girls/4boys).

In the studied group: 98 of the cases of acute intentional self-poisonings presented alcohol as etiology; 60 cases showed no coma; 17 cases were complicated by 2nd degree coma; 12 cases showed 1st degree coma and only 9 cases showed 3rd degree coma.

Out of 38 cases that presented with coma, 15 were females (39.47%) and 23 were males (60.52%).

Out of 98 cases of acute intentional poisoning with alcohol, most cases ingested a quantity of alcohol below 500 ml, 8 cases ingested between 500 and 1000 ml (Table 7). Only one case ingested 3000 ml of alcohol (wine).

Table 7: Case distribution according to the quantity of alcohol ingested

Quantity of alcohol ingested	No. of cases	%
<500 ml	10	10.2%
500-1000 ml	8	8.16%
1000-3000 ml	1	1.02%
Not specified	79	80.61%

Coma occurred most often with the consumption of wine or vodka. In 7 cases, the type of alcohol ingested could not be precisely determined (Table 8).

Table 8: Case distribution according to the type of alcohol consumed that precipitated the occurrence of coma

Substance	No. of cases	%
Distilled drinks	4	10.52%
Wine	10	26.31%
Vodka	8	21.05%
Whiskey	6	15.78%
Beer	2	5.26%
Fruit-flavored distilled drinks	1	2.63%
Unspecified	7	18.42%

In the case of acute intentional poisonings with substances of abuse, the study showed that the most frequently used substances were cannabis and the new psychoactive substances (Table 9). The consumers of substances of abuse had ages in the 11-18 years range, the ratio of girls over boys being 1/2.

Table 9: Case distribution according to the abuse-substances used as etiology in acute intentional self-poisonings

Substance of abuse	No. of cases	%
Canabis/Marijuana	24	57.14%
Glue	4	9.52%
Heroin	1	2.38%
New psychoactive substances	13	30.95%

Acute intentional self-poisonings, especially in the case of adolescents, occur most often with display purposes (parasuicide) or suicide purposes in the context of family disputes. Most are meant to attract attention; few are actual suicide attempts [14, 15].

In this study, we have noted 20 cases of suicide attempts. We noted 10 cases at the 2nd suicide attempt, 6 cases at the 1st suicide attempt, 2 cases at the 5th attempt (Table 10).

Table 10: Case distribution according to the number of suicide attempts, as a function of the total number of patients

Number of suicide attempts	No of cases	%
1	6	1.75%
2	10	2.92%
3	1	0.29%
4	1	0.29%
5	2	0.58%

Out of the total number of acute intentional self-poisonings, we pointed out 20 cases of suicide attempts in our study, which represent 5.83% of the total cases. Moreover, we noticed a part of these were not the 1st attempt. The age of patients with a suicide attempt was in the 14-18 years interval; the ratio female/male was 3/1 (15 girls/5 boys). The majority of patients suffered from psychiatric disorders such as: depressive disorder (4 cases), behavior disorders (5 cases), personality disorders (2 cases), conversion disorders (1 case), emotional disorder (5 cases).

The etiology of suicide attempts consisted of multiple drugs in 9 cases, single-drug in 10 cases (CNS-acting drugs, acetaminophen, ibuprofen, blood pressure-lowering drugs) and in one case the etiology was chlorine ingestion.

54.39% of the patients with acute intentional poisonings could be placed in a category of psychiatric disorders, with the help of the extended team of a psychologist and a psychiatrist, after stabilizing the patient. The rest, 45.61% of the patients, were cases of acute intentional poisonings with no psychiatric involvement (Table 11).

Table 11: Case distribution according to associated psychiatric disorders

Psychiatric disorder	No. of cases	%
Behaviour disorder	30	8.77%
Depression	40	11.69%
Conversion disorder	1	0.29%
Emotional disorder	101	29.53%
Mental retardation	3	0.87%
Personality disorder	10	2.92%
Tourette syndrome	1	0.29%
No psychiatric disorder	156	45.61%

Out of the 342 acute intentional poisonings, 36 cases of chronic consumers of substances of abuse/alcohol were noted, representing 10.5% (Table 12). This is a worrisome percentage, bearing in mind the age group, i.e. less than 18 years. It is an alarm signal, which imposes the application of urgent measures, on a national level.

Table 12: Case distribution according to the chronic consumption of different substances

Chronic consumption	No. of cases	%
Alcohol	6	1.75%
Substances of abuse	30	8.77%

The study group contained 30 cases of chronic consumers of substances of abuse and 6 cases of chronic alcohol consumption, with ages in the interval 13-17 years, 12 female patients and 24 male patients. Out of the 36 cases of chronic substances of abuse/alcohol consumption, 4 were social cases.

The clinical study showed 30 social cases (low economical status, neglected child, disorganized families, domestic violence, children left in the care of relatives or institutionalized) and 11 patients who dropped out of school.

In 2 cases, patients aged 16, presented with evolving pregnancy. The etiology of the poisoning was medication-related: vitamins and ketoprofen were the culprits.

DISCUSSION

In the studied group, consisting of 342 patients, the etiology of acute voluntary self-poisonings varied, but medication, ethanol and substances of abuse were predominant. Statistical data withstand comparison to literature data - a study performed between 2003 and 2013 in the same clinic; the most common cause remains acute voluntary self-poisonings with medication.

Out of the 342 children with acute intentional self-poisoning, the ratio girls/boys is 1.89/1. A higher frequency of acute intentional self-poisonings was noted in females (65.49%), as compared to males (34.50%), probably due to the psychological profile of this gender at this age, i.e. higher emotional instability and display tendency. The psycho-behavioral features of the adolescents of the new generation make them vulnerable to the life problems.

In the study group, we noticed that for female cases, the most frequent etiology for acute intentional self-poisonings was medication (70.5% of intentional self-poisonings in females), while for males, the most common etiology was alcohol (49.1% of the total self-poisonings in males) and the second most common one consisted of substances of abuse (23.7%).

A predominance of acute intentional self-poisonings in children was noticed for the age interval 15-16 years (50.58%), which corresponds to adolescence, a period in which the changes produced by growth, biological function changes; not only does it affect the behavior, but also the inner feelings of the adolescent. Poisoning is a common reason for presentation to hospital and one of the top five acute medical presentations. The peak age groups are teenagers and young adults.

The highest incidence in girls was noted in the 10-14 years age group and we noticed that as adult age approaches, the sex distribution tends to be equal for girls and boys. This can be explained through the emotional particularities with respect to age in girls. In order to lower the incidence of acute poisonings, educational-prophylactic measures must be applied in schools, beginning at the age of 10, an age that corresponds to the fifth year within the gymnasium educational period.

In the study group, 42.47% of cases presented medication used in the treatment of CNS disorders as etiology of the acute intentional self-poisoning. 26.34% of children ingested Acetaminophen and 10.21% used medication acting on the cardiovascular system for the intentional self-poisoning. Out of the medication acting on the CNS, patients used carbamazepine, benzodiazepine, valproic acid, levetiracetam, sertraline, risperidone, extraveral, cipralex (escitalopram). Medication self-poisonings were the most frequent type of acute intentional self-poisonings (63.44%).

Pediatric acute intentional self-poisonings of alcohol-related etiology were caused by the consumption of vodka (20.4%), wine (17.3%), whiskey (9.1%). Distilled drinks are predominant in the etiology of alcohol ingestion in the adolescents included in the study. The results of the study performed in our country are different from the data obtained in other European adolescent populations [10]. According to literature, the drink preferred by European adolescents is beer (47%), followed by wine and distilled drinks (37-38%), cocktail drinks (32%) and cider (27%). As far as the context in which the acute intentional poisoning took place, most often socializing in a public space or at home with friends determined the occurrence of the poisoning.

In 7 of the cases, the etiology of the acute intentional self-poisoning was the combination of medication and alcohol (4 girls/3 boys), and in 7 other cases, the poisoning occurred through the consumption of both alcohol and substances of abuse (3 girls/4 boys). In the study group, acute intentional poisonings using substances of abuse were most often determined by the consumption of cannabis (57.14%) and new psychoactive drugs (30.95%), less often heroin (2.38%) or glue (9.52%). Out of the 42 cases, 14 were females and 28 were males, with ages within the 11-18 years interval, the ratio girls/boys was 1/2.

Acute intentional self-poisonings, especially in the case of adolescents, occur most often with display purposes (parasuicide) or suicide purposes in the context of family disputes. Most are meant to attract attention; few are actual suicide attempts. Out of the total number of acute intentional self-poisonings, we pointed out 20 cases of suicide attempts in our study, representing 5.83% of the total acute intentional poisonings. Moreover, we noticed a part of these were not the first attempt. The age of patients with a suicide attempt was in the 14-18 years interval; the ratio female/male was 3/1 (15 girls/5 boys). The majority of patients suffered from psychiatric disorders such as: depressive disorder, behavior disorders, personality disorders, conversion disorders, emotional disorder. Adolescents and young adults are the age group at highest risk for the first onset of commonly occurring mental disorders. This life stage has also been identified as a critical period for the onset of the first suicide attempt [16].

54.39% of the patients with acute intentional poisonings could be placed in a category of psychiatric disorders, with the help of the extended team of a psychologist and a psychiatrist, after stabilizing the patient. The fact that there are patients with recurrent suicide attempts proves the inefficacy of the psychiatric treatment and the lack of psychologic support after the period of hospitalization.

CONCLUSIONS

The clinical study showed a lot of social cases (low economic status, neglected child, disorganized families, domestic violence, children left in the care of relatives or institutionalized) and 11 patients who dropped out of school, 30 cases of chronic consumers of substances of abuse, with ages within the 11-18 years interval and 6 cases of chronic alcohol consumption. In addition, for two of the female patients a diagnosis of evolving pregnancy was established. Reducing socio-economic deprivation and its associated problems may be an important strategy in the prevention of suicidal behavior, especially in young people.

Out of the 342 acute intentional poisonings, 36 cases of chronic consumers of substances of abuse/alcohol were noted, representing 10.5%. This is a worrisome percentage, bearing in mind the age group, i.e. less than 18 years. It is an alarm signal, which imposes the application of urgent measures, on a national level.

Pediatric acute intentional self-poisonings are an important public health issue, alarming through its consequences and through its hidden neuropsychiatric and behavioral substrate. This is due to the fact that adolescence is a period of marked emotional fragility, sensitive to all sort of influences [17, 18].

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

SS contributed to the conception and design of the study, the acquisition, analysis and interpretation of data of the study. She also contributed to the drafting of the work and its critical revision for important intellectual content. IB contributed to the acquisition, the analysis and interpretation of data of the study, to the drafting of the work and its critical revision for important intellectual content. HTS contributed to the conception and design of the study, the acquisition, analysis and interpretation of data of the study, contributed to the drafting of the work and its critical revision for important intellectual content. CD contributed to the conception and design of the study, the acquisition, analysis and interpretation of data of the study, contributed to the drafting of the work and its critical revision for important intellectual content. MM contributed to the design of the study and to the drafting of the study and its critical revision for important intellectual content. ACT contributed to the analysis and interpretation of data of the study, to the drafting of the work and its critical revision for important intellectual content. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital, "Grigore Alexandrescu" Clinical Emergency Hospital for Children (Bucharest, Romania).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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VARIA

Economic analysis of hospital/healthcare costs in patients with colorectal digestive anastomosis

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Abstract: *Intestinal anastomoses have always been a major problem in digestive surgery. With the use of mechanical suture devices, intestinal anastomosis techniques have improved, allowing standardization of methods and shorter duration of surgical procedures. In addition to shortening the operative time, reduced operating room utilization and length of stay (LOS), health financial strain is also reduced by a lower rate of postoperative complications, quicker recovery and socioprofessional reintegration of patients, and by lowering costs related to treating permanent disabilities such as permanent intestinal stoma. The aim of this study was to estimate the costs of surgical patients with colorectal anastomosis (mechanical or manual suturing) and to identify economically efficient surgical techniques. Material and method: Unicentric 10-year retrospective study in the Bucharest "Prof. Dr. D. Gerota" Emergency Hospital. The costs associated with manually constructed and stapled anastomoses in colorectal surgery were compared. Results: In the group with manual colorectal anastomoses (363 patients) the costs per patient were on average 5190 RON and the average hospital stay 14.95 days. In the group with mechanical colorectal anastomoses (97 patients) the average cost per patient was 5037 RON with an average hospital stay of 11.5 days. Conclusions: The use of mechanical colorectal suture devices resulted in a cost reduction of approximately 150 RON per case and a shortening of average hospital stay by 3.45 days. In addition to these direct benefits, we also list the increase in surgical volume (including the diversification of the range of operations) or indirect effects such as faster socioprofessional reintegration of patients.*

Keywords: *intestinal anastomosis, mechanical sutures, costs*

INTRODUCTION

The first gastrointestinal anastomosis was performed over 200 years ago. Since then, the continuous development of medical technologies was accompanied by a continuous improvement of anastomotic techniques with the goal of reducing the complication rate, standardizing the methods, and shortening the operative time. These goals have led to the emergence of mechanical suture devices and the improvement of surgical techniques. Until the use of circular staplers, most patients with rectal tumors underwent rectal resections with removal of the sphincter (rectal amputation). Mechanical sutures made possible low colorectal anastomoses to be performed, significantly

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improving patients' quality of life [1]. The use of mechanical sutures has become routine and has a major impact on intestinal sutures. In terms of efficacy, applicability and safety, numerous studies have shown that the use of mechanical suture tools is comparable to manual sutures [2]. Mechanical sutures tends to become indispensable in gastrointestinal tract reconstruction - mainly at the extremities of the gastrointestinal tract (esophagus and rectum) [3,4]. For elective gastrointestinal tract surgery studies that compared mechanical with manual anastomoses found no significant differences between the two methods [5-9].

MATERIAL AND METHOD

We conducted a unicentric retrospective study at the Bucharest "Prof. Dr. D. Gerota" Emergency Hospital between January 1, 2010 and December 31. 2019. All patients admitted to the General Surgery Department who were operated for a colorectal disease were included in the study. Inclusion criteria: age over 18 years, surgery performed during the hospitalization for a benign or malignant colorectal disease which involved performing an intestinal anastomosis to the colon or rectum. Exclusion criteria: intestinal anastomoses performed to resolve a surgical complication from a previous hospitalization. Medical expenses were obtained from medical records, patient discharge form, patient expense account from the HYPOCRATE software, and data from the hospital Accounting Department. The following expenses have been taken into account: accommodation, drugs and medical supply costs, medical tests, investigations from the patient's account, and the average cost of the mechanical suture devices used. The costs related to the training of medical staff regarding the use of the medical equipment used in colorectal surgery (courses, training, etc.) were not taken into account.

RESULTS

At the Surgery Department of the „Prof. Dr. D. Gerota" Emergency Hospital we started using mechanical sutures in colorectal surgery since the 2000s. As with any procedure, there is a learning curve - which is why since 2007 the technique has been used more frequently. Several types of staplers have been used over time - the most frequently used devices and agreed by the operating teams being the GIA two row circular stapler of 60 mm/80 mm/100 mm length, EEA two row circular suture devices 29 mm/32 mm, and Ta Linear Stapler 60 mm/90 mm. There was no experience in the ward (during the study period) regarding the "three-layer" suture or the use of mechanical sutures in laparoscopic interventions. Data on the patients with colorectal diseases who underwent surgery were collected from the Hippocrates database. The collected data were divided into two groups – manual anastomosis and mechanical anastomosis cases (each for malignant and benign diseases), and the following were compared: average LOS, the average cost for each disease, postoperative complications and their costs. Mechanical suture devices were used in the following interventions: colo-colonic anastomosis, ileotransverse anastomosis, colorectal anastomoses, ileal or colonic reservoirs, and appendectomy. Four hundred fifteen patients who underwent surgery for colorectal cancer and respectively 47 patients operated for benign colorectal disease were identified. Among patients with malignant conditions, a predominance of left colon and rectal cancers was observed (approximately 38% and 27%, respectively), and among benign diseases, a relatively similar frequency was found for colorectal inflammatory disease and ischemic intestinal disease (about 36% and 32%, respectively). Average LOS was 14.3 days for cancer and t 15.68 days for benign diseases. The longer hospital stay in patients with benign diseases is accounted for by more days of investigations required before surgery.

Table 1: Malignant cases distribution

Malignant diseases	Number of cases	Average LOS	Average cost/ diagnosis
Malignant tumors of cecum and right colon	46	15.32	5267.95
Malignant neoplasm of hepatic flexure of colon	15	13.23	5372.79
Malignant tumors of the transverse colon	14	15.22	7134.25
Malignant tumors of splenic flexure of colon	21	16.93	6544.93
Malignant tumors of the left and sigmoid colon	158	12.15	4033.84
Malignant rectosigmoid tumor	46	13.13	4284.48
Malignant rectal tumor	115	14.16	4092.12
	415	**14.30**	**4464.63**

In the case of malignant diseases, the cost of surgical therapy ranged between 4033.84 RON and 7134.25 RON, with an average cost per case of about 4464.63 RON. In the case of benign pathology, the cost per procedure ranged between 2723.75 RON and 10314.33 RON, with an average cost of about 5916.91 RON – which supports the above-mentioned hypothesis that these categories of patients required more investigations before surgery.

Table 2: Benign cases distribution

Benign diseases	Number of cases	Average LOS	Average cost/ diagnosis
Benign colon tumors	6	14.33	4569.68
Colorectal inflammatory disease	17	12	2723.75
Intestinal volvulus	9	22.89	10314.33
Ischemic bowel disease	15	13.53	6059.91
	47	**15.6875**	**5916.9175**

Figure 1: Average cost of benign diseases

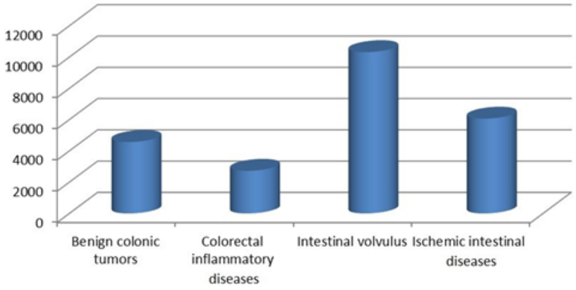

It should be noted that LOS in case of volvulus reaches about 22.9 days, which is associated with an increased cost per hospital stay. This results from an increased share in the medical care costs of accomodation expenses.

Figure 2: Number of cases

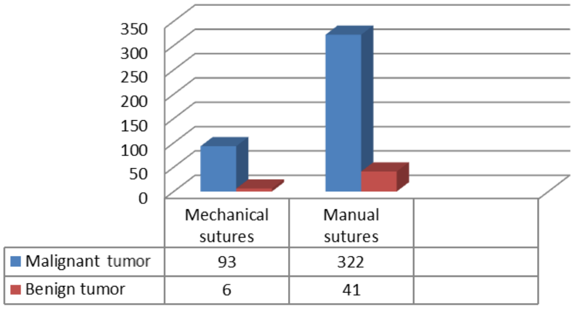

The 2 groups were divided as follows: 363 patients were treated with manual colorectal anastomoses of which 322 were operated for malignant tumors and 41 for benign tumors and a second group of 99 patients who underwent mechanical colorectal anastomoses of which 93 for malignant tumors and 6 for benign tumors (chart 2). As to the average LOS the following differences were found: in the group with mechanical sutures LOS was between 10.1-12.2 days, and in the group with manual sutures it ranged between 14.3 days for malignant tumors and 15.6 days for benign tumors.

Figure 3: Average lenght of stay

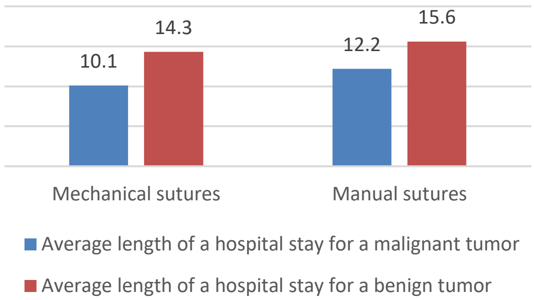

In the group with mechanical suture, the costs ranged between 4240 RON for malignant colorectal diseases and 5834.87 RON for benign colorectal diseases. In the group with manual suturing, the costs per case ranged between 4463 RON for malignant tumors and 5916.91 for benign colorectal diseases.

Figure 4: Average costs per pacient

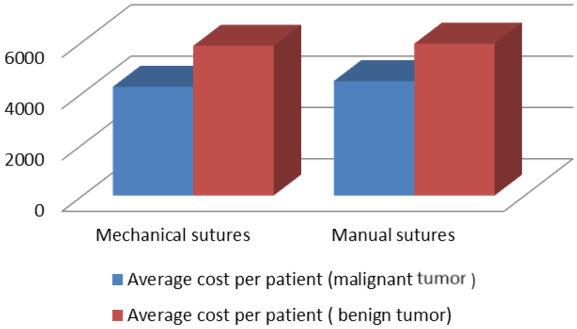

Of the total 462 patients operated for malignant and benign colorectal cinditions, 56 developed surgical complications that required surgical treatment in about half of the cases. The most common complications were intestinal fistulas (about 6%) - a common complication of both mechanical and manual anastomoses. There was no significant difference between the incidences of fistulas in mechanically sutured anastomoses compared to manual sutures.

Table 3: Case distribution according to age and sex

Postoperative complication	Number of cases	Average LOS (days)	Average cost/ diagnosis
Rectal stenosis	2	11,5	3089,1
Intestinal fistula	27	22,9	5532,49
Acute peritonitis	26	19	8930,32
Stoma stenosis	1	15	4447,09
	56	**17,1**	**5499,75**

In the case of anastomotic fistulas, LOS was longer, but the total cost was lower compared to the one identified in the case of septic complications which required broad-spectrum antibiotic therapy that increased expenses. There have also been several technical incidents associated with the use of mechanical suture devices (without them having been ratified or reported) – such as misfiriring or complete device failure during the procedure. Mac Rae et al. reported these incidents as common and associated them with significant morbidity [8]. In our study, no associations were found between intraoperative technical incidents and post-operative complications.

DISCUSSION

The mechanical suturing technique associates a series of benefits such as:

- faster healing of the anastomosis due to better vascularization, less inflammatory reactions of the anastomosis partners, less tissue manipulation, and better tissue alignment [10];
- reduction of septic intervals;
- reduction of operative time;
- technical advantages: can be used in low rectal anastomoses thus allowing the preservation of anal sphincter and significantly improving the patient quality of life
- shorter learning curve than in the case of manual sutures [11, 12];

Despite the above-mentioned advantages, the technique is not perfect and associates anatomical complications such as dehiscence, anastomotic fistulas, and anastomotic stenosis [13, 14]. The advantages and disadvantages of the 2 techniques should be discussed with the patient before signed informed consent is obtained [15]. On the other hand, it is important for health-care providers to report adverse events to manufacturers, to provide feedback helping the manufacturere to improve the design of these devices [13, 16]. The most commonly encountered complication remains the anastomotic fistulas. Reintervention and complications such as anastomotic fistulas are considered quality indicators in colorectal surgery. Anastomotic fistulas are among the most feared complications, especially after colorectal resections and are associated with increased morbidity and mortality, with a high reintervention rate, and increased LOS [17-21]. The prevalence of anastomotic fistulas after colon or rectal resections varies depending on anatomical location, with a lower frequency in the case of right-sided colon anastomoses. The incidence of radiologically identified anastomotic fistulas ranges between 0.5% and 21%, but of these only 1-12% are clinically significant anastomotic leaks after colorectal surgeries and up to 10-14% in low colorectal resections [10]. Overall, patients with anastomotic fistulas developed after colorectal surgery have a significantly higher risk of morbidity (56%) and mortality (32%) [10]. It is also associated with a significant economic burden because of multiple reinterventions, radiological procedures, the stomas needed to control fistulas and because of the increasing length of hospital stay [22]. Anastomotic fistulas are documented as a strong indicator that is associated with high costs in colorectal surgery and also with significant long-term costs for the patient and the health system due to the high rehospitalization rate (risk that is 1.3 times higher in the first 30 postoperative days in patients who have anastomotic fistula compared to those who do not have fistula after colorectal surgery). Also, high reintervention rates, increased incidence of postoperative infections, and longer LOS per admission increase the cost (in the literature was estimated on average at about 7 additional days) [23-26]. Factors of increased peritoneal aggression are known to cause adhesions, one of he most frequent causes of long term morbidity after abdominal surgeries [27-29]. The use of mechanical suture devices shortens the average surgical procedure by 15 minutes on average – from which benefits the patient by reducing anesthesia surgical stress and administrative costs, especially in hospitals with high workload. These have direct implications on the cost associated with anesthesia procedures and on increasing the utilization rate of the operating room, thus allowing more surgeries to be completed. The evaluation of the benefits related to shorter LOS and less medical care days due to a reduction in morbidity, in terms of the costs of using mechanical sutures, must be addressed to each situation, these being more obvious in clinics performing numerous esophageal and colorectal surgeries [12]. Reducing the costs for surgical patients and in particular for a patient with gastrointestinal surgery is a goal in the management of surgical patients.

CONCLUSIONS

Mechanical suturing technique must be mastered by each surgeon and the necessary devices must be found in each surgery department. Although the cost of these devices is high, overall, it seems that the cost/effectiveness ratio is in their favor. In addition to shortening the duration of colorectal surgeries, increasing the use of operating rooms, decreasing the LOS, the financial effort is reduced by a lower rate of postoperative complications, faster socioprofessional reintegration of patients, and lower costs related to treating permanent disabilities such as permanent intestinal stoma, directly influencing the decrease in costs associated with surgical patient care. An important component of surgical patient management is cost reduction, which can be achieved by reducing LOS and careful management of the case by selecting an appropriate surgical technique.

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VARIA

The interactions between risk factors for ischemic stroke

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Abstract: *Stroke has a significant prevalence in Romania. The predisposition for this multifactorial disease is partially known. The aim of this study is to investigate the predisposition for stroke in Romanian population. Material and methods. We selected cases with recent ischemic stroke (n=100) and healthy control subjects (n=100). The AGTR1 A1166C (rs5186) polymorphism was genotyped by restriction of amplicons with Dde I endonuclease. Results. Active cigarette smoking (O.R. =6.92, p=0.0001) or presence of the AT1R C variant (O.R. =6.69, p=0.0006) in overweight or obese women significantly increase the risk for ischemic stroke. The diagnosis of stroke (71.5 vs 68 years old) or T2DM (63.39 vs 60.77 years old) was recorded at an older age in women compared to men (p<0.05). Obesity considered independent (O.R. =4.22, p<0.05) or in association with T2DM (O.R. = 10.16, p=0.0002) confers the highest risk of stroke when compared to women. Conclusions. Obesity in association with T2DM confers the highest risk of stroke for men when compared to women. Active cigarette smoking or AT1R C variant significantly increase the risk for stroke in women with a high BMI compared with controls.*

Keywords: *stroke, AT1R, BMI, cigarette smoking*

INTRODUCTION

Stroke is an important cause of worldwide mortality and of acquired disability in adults [1]. The prevalence of disease in Romania seems to be several times higher than the average world-wide prevalence [2, 3].

Investigation of positional and functional candidate genes and their interactions with different non-genetic factors is important for understanding pathogenesis of stroke [4, 5]. One of them is angiotensin II type I receptor (AT1R). This receptor, widely expressed in the body, is responsible for the most important effect of angiotensin II. In pathological condition signals from AT1R are involved in oxidative stress, hypertrophy, fibrosis, and inflammation [6]. Consequently, it is an important functional candidate gene for different vascular diseases (including stroke), in human and animal models [7, 8]. In the 3' untranslated region of the AT1R gene was mapped the rs5186 (+1166A/C or A1166C) polymorphism that was associated with stroke in some cohorts but not in Romanian population.

MATERIAL AND METHOD

The aim of this study is to investigate the predisposition for stroke in Romanian population.
We selected cases with ischemic stroke in the last weeks before selection for this study. Cases with a prior stroke or those with stroke onset in young adult (≤45 years old) were

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not selected. Control subjects were considered clinically and paraclinically healthy. All subjects selected are Caucasians who lived in Bucharest or Ilfov County. Subjects who drank >50g alcohol/day or smoked >25 cigarettes/day were not selected for this research.

AGTR1 A1166C polymorphism was genotyped by restriction of amplicons with endonuclease Dde I [9].

Statistical analysis was performed using the StatsDirect software.

RESULTS

The main characteristics of patients and control individuals selected for this study are presented in Table 1 and Table 2. The diagnosis of stroke or T2DM was recorded at an older age in women compared to men (p<0.05).

Table 1: The characteristics of the subjects investigated in this study (p< *0.05; p<** 0.0001)

Gender	Men		Women	
Characteristics	Stroke	Healthy	Stroke	Healthy
Men / Women	52	52	48	48
Age at inclusion	66.92±6.21 (53-83)	68.02±5.14 (58-82)	71.44±4.90 (59-83)	70.33±7.01 (55-83)
Weight at inclusion	85.9±8.65 (69-101)	78.17±5.37 (67-92)**	79.25±7.65 (66-99)	69.92±3.89 (59-80) **
Hight at inclusion	1.71±0.04 (1.66-1.84)	1.72±0.04 (1.66- 1.86)	1.69±0.02 (1.66-1.77)	1.7±0.03 (1.66-1.78)
BMI at inclusion	29.35±2.99 (23.51-35.50)	26.39± 1.72 (23.30-29.76) **	27.44±2.59 (22.31-33.86)	24.21±1.23 (20.90-27.68) **
Normal weight/Overweight/ Obesity at inclusion	7/22/23	13/39/0	9/32/7	38/10/0**
Children (yes/no)	32/ 20	41/11	32/16	40/8
Number of children (1, >1)	13/19	18/ 23	20/11	19/21
Living environment (urban/ rural)	35/17	34/18	28/20	35/13
Alcohol (yes/no)	18/34	14/38	0/48	2/46
Cigarette smokers (current or former) (yes/no)	33/19	26/26	28/20	22/26
Average number of cigarettes/days	18.76±2.31 (13-22)	17.19±3.30 (10-20) *	13.46±3.65 (8-20)	14.18±4.17 (10-23)
The age at which this addiction was acquired	21.21±4.23 (13-29)	21.65±4.12 (15-32) *	22.75±4.71 (14-32)	20.5±5.02 (13-31)
Active smoking (years)	42.73±6.55 (19-52)	45.04±9.43 (27-70)	41.36±6.91 (30-58)	46.32±10.06 (32-70)
Former smokers (yes/no)	20/19	7/26 *	22/20	7/26 *
The age until they smoked	60.75±6.53 (44-70)	53.71±3.59 (50-60)	62.82±4.67 (50-70)	59.43±5.35 (50-66)
AGTR1 AA/AC/CC	34/14/4	27/21/4	24/18/6	27/17/4
ATR1 C and Smokers (current or former)	12/40	13/39	14/38	15/37
ATR1 C and BMI> 25 kg/m^2	16/36	20/32	21/27	5/43**
Smokers (current or former) and BMI> 25 kg/m^2	28/24	18/34*	26/22	7/41**

The BMI was higher in patients than in control, regardless of gender (p<0.0001). Supraponderal or obese women (BMI > 25 kg/m^2) diagnosed with stroke were more frequently active smokers (O.R. =6.92, 95% CI: 2.59-18.49, p=0.0001) or carriers of the AT1R C variant (O.R. =6.69, 95% CI: 2.25-19.84, p=0.0006) compared to healthy women. A less significant difference was observed between the percent of smokers who had increased BMI between male patients and healthy controls (O.R. =2.2, 95% CI: 1.0 -4.85, $p<0.05$).

The percentage of subjects who quit smoking is higher in the subset of men (O.R. =3.91, 95% CI: 1.38-11.11, p=0.02) or women (O.R. =4.09, 95% CI: 1.46-11.46, p=0.006) with stroke compared to the healthy control subjects.

We noticed that obesity considered independent (O.R. = 4.22, 95% CI: 1.15-15.50, $p<0.05$) or in association with active smoking (O.R. = 3.82, 95% CI: 1.28-11.45, $p<0.05$) increase the risk for stroke in men compared to women (Table 2). The highest risk of stroke was estimated for obese men with T2DM (O.R. = 10.16, 95% CI: 2.79-37.04, p = 0.0002).

Table 2: Comparison of risk factors in stroke patients stratified by gender (p< *0.05; p<** 0.0001)

Comorbidities	Men with stroke	Women with stroke
Age at stroke diagnosis	68 (48-69)	71.5 (56-69)*
Normal weight/Overweight/Obesity at inclusion	7/22/23	9/32/7 *
Obesity vs normoponderal	23/7	7/9*
Obesity and ATR1 C	9/43	4/44
Obesity and active cigarette smoking	16/36	5/43*
Obesity and T2DM	21/31	3/45*
Obesity and HBP	12/40	4/42
T2DM present	31/21	23/25
Age at T2DM diagnosis	60.77±4.03 (53-68)	63.39±3.38 (58-70) *
Weight at diagnosis	94.9±9.52 (77-110)	80.26±8.34 (63-97) **
BMI at T2DM diagnosis	32.51±3.08 (26.33-36.93)	27.77±2.60 (22.06-32.15) **
T2DM and ATR1 C	13/39	10/38
T2DM and BMI> 25 kg/m^2	31/21	21/27
T2DM and active cigarette smoking	19/33	16/32
HBP	28/24	23/25
Age at HBP diagnosis	60.11±5.09 (48-69)	62.52±3.81 (56-69)
HBP and ATR1 C	13/39	14/34
HBP and BMI> 25 kg/m^2	24/28	21/27
HBP and active cigarette smoking	16/36	16/32
Treatment for HBP	27/1	23/0
Adequate control of blood pressure (Yes/No)	18/10	20/3
HBP and T2DM	17/35	16/32
HBP, active smokers and ATR1 C carriers	9/43	9/39

DISCUSSION

Stroke is a heterogenous disease with respect to the etiology, contribution of risk and protective factors in different populations and baseline status at the time of the stroke. A series of characteristics for the ischemic or hemorrhagic forms and for those with onset in young adults or in elderly have been described [10]. Thus, we selected only the cases of ischemic stroke that begins in adults (> 45 years) in order to ensure the homogeneity of the lots investigated in this retrospective study. The relationship between gender and stroke may be influenced by age of investigated subjects [11]. The mean age of patients with stroke selected in our study (69.09 years old) was similar with the values reported in other publications [12, 13]. Women tend to be older than men at stroke onset in different populations. In our lot a similar tendency was identified, the median age at stroke onset was significantly higher in women than in men (71.5 vs 68 years old, $p<0.05$).

Different lifestyle factors can influence the risk for stroke [14, 15]. In this study cigarette smoking and alcohol consumption were found not to be risk factors for stroke, regardless these factors were analyzed independently or in association.

We found that a significant percent of patients with stroke had T2DM (54%), obesity (30%) or have both diseases (24%, 87.5% of these cases was found in men). The high presence of booth comorbidities is concordant with previous data which suggested that obesity or T2DM are significantly associated with stroke [16]. These associations can be influenced by different factors [17]. In our study obesity, considered independent (O.R. =4.22) or in association with active cigarette smoking (O.R. =3.82), increase the risk for stroke in men compared to women. Women (O.R. =6.92) and men (O.R. =2.2) with a BMI > 25 kg/m^2 who were active smokers had a significant risk of stroke.

High blood pressure (HBP) is a major risk factor for stroke. The risk for HBP in Caucasians is increased by the AT1R C variant [18]. More than half of the patients with stroke from this study also had HBP (51%) and ~53% of them were carrier of AT1R C variant. AT1R A1166C was associated with stroke in patients from Italy [19], the Nederlands [20], Sweden [21], and Japan [22]. In some population AT1R C was found to increase the risk for ischemic stroke only in hypertensive smokers (OR= 22.3, p<0.001) [23] or in subjects who carry other risk polymorphism [24, 25]. In other studies [26], including two meta-analysis, AT1R A1166C was not associated with susceptibility to ischemic stroke [27, 28]. In the present study univariate analysis did not reveal an association between AT1R A1166C polymorphism and stroke. However, we identified that women with BMI > 25 kg/m^2 diagnosed with stroke were more frequent carriers of the AT1R C variant compared to healthy control women (O.R.=6.69, p=0.0006).

CONCLUSIONS

The diagnosis of stroke was recorded at an older age in women compared to men. Women with a high BMI who are active smokers or who are carrier of AT1R C variant have a higher risk of stroke. The highest risk of stroke for men was estimated for obese T2DM subjects.

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VARIA

The conduct lists of military physicians Ion Arsenie and Bucur (Hilarius) Mitrea during the Mexican campaign (1864-1866)

Sandra Hirsch[1], Vlad Popovici[1]

Abstract: *The study aims at complementing the historical knowledge regarding the participation of Dr. Bucur (Hilarius) Mitrea and Dr. Ioan Arsenie within the Austrian volunteer corps in Mexico, using as sources the copies of their conduct lists preserved in the military archives of Vienna. The new data concern the battles they took part in, together with characterizations of their behavior and personality. The latter consolidate the image built by former biographers in the case of Dr. Mitrea and help to sketch a less known portrait in the case of Dr. Arsenie.*

Keywords: *military physicians, 19th century, Transylvania, Mexico, Österreichisches Freiwilligenkorps in Mexiko*

INTRODUCTION

Ion Arsenie (Arseniu) and Bucur (Hilarius, Ilarie) Mitrea are among the most frequently mentioned Romanian military physicians in the Austrian Monarchy, even though their service for the House of Habsburg was of short duration and took place entirely within the Austrian volunteer corps in Mexico (Das Österreichische Freiwilligenkorps in Mexiko, henceforth ÖFM). Their later careers evolved differently, and so did the historians' interest in their lives and activity. However, the period they have spent in Mexico remains a biographical landmark to be taken into account. The present study will complement previously known data on the military activity of the two physicians during the "Mexican adventure". The sources to be used are copies of their lists of conduct, discovered at the War Archive in Vienna (Österreichisches Staatsarchiv, Kriegsarchiv), unknown to specialists to this day.

Dr. Ion Arsenie (Arseniu) (1838–1883) was born in Gura Râului (Sibiu County). Son of a priest, he studied medicine in Vienna between 1857 and 1862 [1]. He joined ÖFM in the autumn of 1864, with the rank of first lieutenant senior physician (Oberlieutenant – Oberarzt) in the first Austrian volunteer group that left for Mexico to support emperor Maximilian. Shortly after his return in Europe (1866), he settled in Romania, where he practiced medicine in Brăila, up to his death in 1883. Little is known about his professional activity, both as a civilian and as a military physician. Throughout the campaign in Mexico, he sent financial donations to the Transylvanian Association for Romanian Literature and the Culture of the Romanian People (ASTRA), as well as travel stories, published in the press of those times. He was also involved in other Romanian literary and scientific societies of the time, such as "România Jună" from Vienna, and remained a member and financial supporter of ASTRA up until the end of his life. There are no biographical studies dedicated to him exclusively, only fragments spread

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out in different papers, most of the times associated with Dr. Mitrea [2].

The life and activity of Dr. Bucur (Hilarius, Ilarie) Mitrea (1842–1904) were the subjects of numerous works of research, covering a great part of his biography. The future physician and explorer was born in 1842 and was the only son of a wealthy shepherd in Rășinari. He studied medicine in Cluj and later in Würzburg (where he defended his doctoral thesis), Berlin, and Vienna [3]. After failing in his attempt to occupy the position of a physician in his commune of birth (1864), he was initially employed physician on board of an immigrant ship to Australia (1865–1866) and afterward enrolled in ÖFM (March 1866), where he was active until the unit was disbanded by emperor Maximilian (December 6th, 1866). He returned to Europe in April 1867, attended further specialty studies at the University of Berlin, and then worked again as a ship physician on board at least two transatlantic voyages [4]. In March 1869, his enrolment application in the Dutch colonial army was accepted, as a 3rd class "Officer of Health" (officier van gezondheid). Dr. Mitrea worked as an army doctor in the Dutch army in Indonesia from 1869 until his retirement in 1894. His last advancement in rank took place in May 1890, from 1st class Officer of Health to 2nd class Chief-Officer of Health [5]. He was married to a local woman and had two children, both educated from small ages in Europe. He died in 1904, in Vienna, probably taking his own life, on the background of unsettled family issues [3]. He donated to the National Museum in Bucharest the most of his rich natural sciences collection, that he had gathered throughout his activity in Indonesia. The National Museum of Natural History "Grigore Antipa" now hosts what remains of the collection [6-7].

General knowledge on the life and activity of the two physicians differs fundamentally: Dr. Arsenie remains to this day almost anonymous, whereas Dr. Mitrea naturally benefitted from consistent historiographic attention. Yet, regarding their military activity in Mexico little is known in both cases, and this will be the focus of our research in what follows.

METHODS

Some of the most important biographical sources for the officers of the Habsburg army (and other European armies) of the 19th century are the conduct lists. This type of document was periodically drafted within each military unit, had a tabular structure, and contained a rigorous description of the career, knowledge, behavior, and personality of the officer. An excerpt of the conduct lists drafted at Puebla, under the supervision of Major Dr. Michael Kubicza, ÖFM's chief-physician, on November 30th, 1866 – just one week before the disbandment of the volunteer corps – was kept for both Dr. Arsenie and Dr. Mitrea [AT-OeStA/KA AdT Mexiko, A42 & A43][1] [8].

Both conduct lists contain the following categories of information:

a) biographical (name, place and year of birth, confession, civil status, and whether they have children or not);

b) concerning the military career (current rank and previous ranks, the date when the first rank was obtained and the period covered by every rank starting with the date of enrollment, medals, military actions they were involved in, and whether the officer is suitable for advancement);

c) regarding their studies, linguistic knowledge, and the level of their professional knowledge (medical, military, and other fields, if such was the case);

d) characterizations of their general state of health, personality, behavior towards their superiors, comrades, or against the enemy, as well as the opinion of the author of the characterization on the respective army doctor.

Using the information provided by these documents, we will attempt to complete the currently known data regarding their activity and career as military physicians, through the method of historical reconstruction, comparing the bibliographical mentions with those in the archive documents.

RESULTS

Biographical data

Concerning biographical information, the conduct lists bring nothing new. In the case of Dr. Arsenie, there is no mention of the year or place of birth, confession, or civil status. In the case of Dr. Mitrea, it mentions the year and place of birth (1842, Reschinar in Siebenbürgen), the confession (Orthodox, griechisch nicht uniert), and the civil status (not married, ledig) [AT-OeStA/KA AdT Mexiko A43]. The lack of biographical information regarding Dr. Arsenie lead us to believe that he was not present in Puebla at the moment when the conduct lists were drafted, and the officer in charge did not have at his disposal any additional documents concerning him.

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[1] *Austrian State Archives. War Archive, Archives of Army Troops – Austrian-Belgian volunteer corps in Mexico – Administrative documents – Conduct lists and descriptions of individuals, Akten 42 (Arsenie) and Akten 43 (Mitrea).*

Military Career

The data regarding the military career of the two army doctors are much more rigorously recorded and allows for an exact reconstruction of the duration of their service, their advancements in rank, and the military actions they took part in. The data is also very valuable since up to this date, no details were known regarding the military activities that the two army doctors were involved in.

On November 30th, 1866, Dr. Arsenie had completed exactly two years and one month of service in ÖFM, meaning that he was on the payroll starting with November 1st, 1864, more than one month before the first ship of Austrian expeditionary troops left the port of Trieste (December 6th, 1864) [4]. Although we have no information about the ship he traveled on, nor on the exact moment of his arrival in Mexico, we know that he was there on February 6th, 1865, when he is mentioned as a participant in the skirmishes in Tesuitlan. He was enrolled with the rank of first lieutenant senior physician (Oberlieutenant-Oberarzt), which he kept for one year, four months and twenty-nine days, until March 30th, 1866, when he was promoted to the rank of captain senior physician, 2nd class (Hauptmann Oberarzt 2. Classe) [AT-OeStA/KA AdT Mexiko A42]. He held this rank at the date when the conduct list was drafted, and probably one week later, as well, at the date of ÖFM's disbandment (December 6th, 1866).

The same document mentions that he was decorated with the Mexican Imperial Order of Guadalupe, in the rank of knight (limited to some 500 decorated people), a fact already known from the photos and press of the time [2]. The order was created in 1822, during the first Mexican empire, annulled and reinstated twice since then, depending on the political changes in the country. Emperor Maximilian wished to consolidate the basis of his reign through symbolic gestures as well, making use of Catholic spirituality, i.e. the recognition of the importance of the Virgin of Guadalupe cult and reinstating the Order of Honor that carried her name, in 1865. During its time, the order was split into Grandes Cruces (30), Grandes Oficiales (100), Comendadores (200), and Caballeros (500) [9-11]. From the conduct list, we also learn the circumstances that led to him being decorated: for the deeds of merit performed in Sierra del Norte, and especially in Agua Dulce (für seine verdienstvollen Leistungen in der Sierra del Norte namentlich bei Agua Dulce) [AT-OeStA/KA AdT Mexiko A42]. There is, however, a photograph taken in Vienna in 1869, showing him in the volunteer corps' uniform, and wearing two medals [2]. Despite the poor quality of the image, the second medal can be identified, through visual comparison with the similar one received by Dr. Mitrea, and thanks to I. Petrescu's historiographical contribution to this particular topic [12]. It was the Commemorative medal of the Mexico expedition (Médaille commémorative de l'expédition du Mexique), issued by the French emperor Napoleon III, of which the black eagle on the ribbon is visible in the image.

Regarding the battles that Dr. Arsenie took part in, these are listed in a dedicated column: at Tesuitlan [Teziutlán] on February 6th and 10th 1865, at Zacapoaxtla on March 1st and 2nd 1865, at Xilotepec [Jilotepec] and Xochiapulco on April 13th, at Tlapacoyan on July 9th, 11th, 13th and 19th 1865, at Agua Dulce on January 11th, 1866, at Tres Cruces on July 13th and Pahuathlan on July 14th, 1866 [AT-OeStA/KA AdT Mexiko A42]. The battles in 1865 took place in a very small area, about 140 km North-West of Puebla, most probably where his garrison was stationed. The battles from 1866 took place about 100 km North and North-West of the previous ones (Sierra del Norte). For his bravery here, at Agua Dulce, he was awarded the Order of Guadalupe.

Dr. Mitrea enrolled in Vienna, on March 26th, 1866. Based on some documents from the family's archive, issued in Mexico, his main biographer, E. Pop, also mentions the possibility of him having received a three-month bonus to his pay (January 1st – March 31st, 1866) [3]. However, the conduct list only registers a service of eight months and five days on November 30th (starting with April 1st, 1866, probably the first day of his payroll) with the rank of first lieutenant senior physician (Oberlieutenant-Oberarzt). No medals are being recorded. The Commemorative Medal of the Expedition in Mexico, issued by emperor Napoleon III, was awarded at a later date. Dr. Mitrea took part in the battle at Cozautlan [Cosautlán de Carvajal] on September 23rd, 1866, the one in Banderilla on October 24th, 1866, and the defense of the city of Zalapa [Xalapa] between November 4th and November 11th, 1866 [AT-OeStA/KA AdT Mexiko A43].

Specialty training, abilities, and competencies

Data regarding their studies, linguistic knowledge, and the level of professional knowledge (medical, military, or in other fields) are brief, but it does bring up new data.

In both cases, it is mentioned that they had completed the secondary and university studies required by their current position. Based on their biographical and educational background, we know they were both speakers of Romanian and German, and we also know Dr. Mitrea was a speaker of Hungarian (given the fact that he had studied medicine in Cluj), but the conduct lists add new information. In the case of Dr. Arsenie, it mentions that he could speak and write in

Romanian, Hungarian, German, but also rather well in French, Italian, and Spanish. In the case of Dr. Mitrea, the languages recorded are Romanian, Hungarian, German, Latin, and a bit of Spanish (etwas spanisch). In both cases, they probably learned Spanish during the campaign.

The military knowledge of Dr. Arsenie is characterized as "low level" ("some", einige), and that of Dr. Mitrea as being "little" (wenige), which is only natural, given the lack of prior military experience and the fact that they were trained as civilian physicians. It is also mentioned that neither of them had any knowledge in other fields of activity.[AT-OeStA/KA AdT Mexiko A42, A43]

Personality traits and conduct

The general health state mentioned in the conduct lists is "very good" in the case of Dr. Arsenie and only "good" in the case of Dr. Mitrea. The former is characterized as being "silent and secretive" (still u. verschlossen), and the latter as "silent, calm, docile" (still, ruhig, willig). In official relations, Dr. Arsenie was "calm, as expected" (ruhig, den Anforderungen entsprechend), and Dr. Mitrea was "as expected". Relations with their comrades seemed to follow the same pattern: Dr. Arsenie was "friendly" and Dr. Mitrea was "very friendly". Small differences also appear when it comes to the effort they put in: Dr. Arsenie was considered to be "quite diligent, with results as expected" (ziemlich fleißig mit entsprechendem Erfolg), while Dr. Mitrea was "very diligent, with good results" (sehr fleißig mit gutem Erfolg). Both are considered able for military service, and their behavior against the enemy is considered to be "quite respectful" (recht brav). The conclusion of the superior officer who drafted the conduct lists is that each of them was a "really useful army doctor" (ist ein recht gut verwendbarer Truppenarzt) [AT-OeStA/KA AdT Mexiko A42, A43].

Whereas the personality of Dr. Mitrea fits the historiographic portrait created based on his contemporaries' testimonies [3], Dr. Arsenie's personality contradicts the assumptions of E. Pop. Starting from the fact that Dr. Arsenie sent a series of letters from Mexico to some Romanian journals in Transylvania, and analyzing the light style of his penmanship, scattered with humorous nuances (but probably also taking into account his financial donations to Astra), E. Pop concluded that, as opposed to Dr. Mitrea, he would have been more of an extrovert, who liked making public appearances [2]. However, the conduct lists indicate the opposite: out of the two, Dr. Mitrea was the one who had a more open personality in his direct social relations, though he was just as calm and silent as his colleague. E. Pop managed to very well perceive the difference between the two army doctors at the level of inner personality, as surely Dr. Arsenie was much more inclined than Dr. Mitrea to written, indirect, social communication and interaction. However, given the lack of more explicit documentation, such as the ones we have used, he could not have known that both of them had similar distant and reserved personas.

CONCLUSION

The information preserved by the excerpts of the conduct lists of the two Romanian army doctors from the Austrian volunteer corps in Mexico is extremely valuable, both in terms of new data and by nuancing pre-existing historiographic assumptions. The documents helped with the exact reconstruction of their short military career, and especially with the battles, they took part in. Moreover, they helped with the reconstruction of their personality (which, in the case of Dr. Arsenie, complements the image proposed half a century ago, by E. Pop) and of how their superior officers regarded and appreciated them.

Starting from these documents, a more in-depth research plan on the activity of the two army doctors (and the other few Romanians enrolled as volunteers in the same campaign) can be drafted. By knowing the battles they took part in, one can identify new sources, both in the Austrian and Mexican archives (the latter being up to this day completely unexplored about this subject).

Future plans

The current research should also be regarded as a signal, aiming at drawing attention to both the documentary potential of the conduct lists of officers in the Habsburg army, as well as to the particular subject of Romanian participants to the "Mexican adventure", about which very little is yet known. Regarding the first aspect, further research shall be focused on the area of the former border regiments in Transylvania, which is covered by rich and almost completely unexplored documentation preserved in the Viennese war archive. This includes, inter alia, a multitude of data on the organization of the sanitary service in military border areas, medical staff, medical and health practices, etc. Regarding the second aspect, the search for new sources related to the activity of the Romanian volunteers in Mexico will continue, in the hope of further detailing their activity on the battlefield and in the field hospitals.

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ORIGINAL ARTICLE

Assessment of the Sensitivity and Specificity of CT Investigations in Characterizing and Evaluating the Post-treatment Evolution of Ovarian Tumor Lesions

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Abstract: This study analyzes the contribution of Computed Tomography (CT) in the detection and characterization of ovarian tumors, particularly malignant ones, to enhance the accuracy of preoperative diagnosis and postoperative follow-up, thereby optimizing therapeutic planning. This paper presents a retrospective study conducted on a group of 63 patients with ovarian tumors who were operated on at the Department of Gynecology of the Central University Emergency Military Hospital "Dr. Carol Davila" in Bucharest. The patients were investigated preoperatively using Computed Tomography to characterize the ovarian tumors and assess the sensitivity and specificity of the imaging diagnosis. Computed Tomography utilizing new multidetector technologies (MDCT) provides superior characterization of adnexal masses, with a sensitivity of at least 90% and a specificity of 80%. This imaging modality is recommended for the preoperative staging of ovarian cancer.

Keywords: CT, MDCT, ovarian tumor, postoperative follow-up, postoperative evolution, sensitivity, specificity, accuracy

INTRODUCTION

Ovarian cancer is a heterogeneous disease that is the eighth most common cancer in women and the leading cause of gynecologic-related cancer death [1-3].

In relation to the postoperative histopathological/immunohistochemical diagnosis for each operated patient, the contribution of Computer Tomography was evaluated for the preoperative characterization of ovarian tumors and assessment of the post-interventional evolution through monitoring at 6 and 12 months, respectively [4-6].

The Computer Tomography (CT) examination is an imaging method that uses ionizing radiation, but demonstrates good sensitivity and specificity, thanks to advancements in multidetector technology. Computed tomography using new multidetector technologies (MDCT) achieves a better characterization of adnexal masses, with a sensitivity of at least 90% and a specificity of 80%. This type of imaging is recommended for preoperative staging of ovarian cancer [4]. This is especially recommended for the preoperative staging of ovarian tumors [7,8,9]. The CT examination is usually used in addition to an inconclusive abdominopelvic ultrasound for the differential diagnosis of pelvic pain syndrome [8]. Although the accuracy of CT is generally lower than that of magnetic resonance imaging (MRI) or transvaginal ultrasound for assessing ovarian pathology, its multiplanar reconstructions enable effective evaluation of the extent of ovarian lesions and identification of coexisting pathologies [4,10,11].

The ovaries are visualized near the iliac vascular axes as oval structures with low densities due to the presence of peripheral follicles. Ovarian ligaments may appear as dense linear structures, especially when free intraperitoneal fluid outlines them. The corpus luteum is thick-walled and shows peripheral contrast uptake [5,7,10,11].

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This study aimed to evaluate the performance of diagnostic imaging concerning postoperative histopathological results and the features that may be useful for the classification of tumors. The diagnostic imaging introduction should briefly place the study in a broad context and highlight its importance. It should define the purpose of the work and its significance.

MATERIALS AND METHODS

This retrospective study investigated a group of patients with ovarian tumors (n = 63), operated in the Gynecology Department of the Central University Emergency Military Hospital, who underwent preoperative CT examinations. The studied group included patients with benign ovarian tumors, histopathologically diagnosed postoperatively (n=30), and with malignant ovarian tumors (n = 33); 3 patients with malignant ovarian tumors were considered borderline.

The main imaging characteristics that were considered suspicious for malignancy were the following: solid components; endo/exocystic vegetations; wall with irregular thickness and intramural nodules; irregular intratumoral septa with thicknesses over 3mm; contrast uptake of the solid components (intratumoral, parietal or septal) with enhancement curve type II/III appearance (early contrast uptake of lower intensity/equal to the myometrium, persistent in the plateau, respectively intense early contrast uptake, more pronounced than the myometrial and dynamic "wash-out"); dimensions (>3 cm); secondary lesions involving the peritoneal lining, mesenteric or omental fat; ascites; pelvic extension; locoregional nodal disease; distant metastases (extraperitoneal) [5,8].

Fisher's test and Mann Mann-Whitney (Wilcoxon) test were used to determine the significance between groups. The correlation between age at diagnosis and CA125 values was performed using Pearson’s correlation coefficient.

The Sensitivity [TPR =TP/(TP+FN)], False negative ratio [FNR=FN/(TP+FN)]; False positive ratio [FPR=FP/(FP+TN)], Specificity [TNR =TN/(FP+TN)], Predictability of positive values [Precision PPV=TP/(TP+FP)]; Omission rate [Omission rate = FN/(FN+TN)] and Accuracy [Accuracy = (TP+TN)/Total] were estimated. For this estimation, the values for True positive (TP); True negative (TN); False positive (FP) and False negative (FN) were used.

RESULTS

This retrospective study analyzed 63 patients with ovarian tumors who were preoperatively CT investigated. Nine patients with malignant tumors and eight with benign tumors had bilateral tumors. Histopathological diagnosis established the presence of epithelial (n = 40), germ cell (n = 17), or stromal and sex cord tumors (n = 6). Epithelial tumors are more common in patients with malignant tumors compared to benign tumors. (31 vs 9, p<0.0001). Multilocular tumors were identified in 29 patients with malignant tumors and 4 patients with benign tumors (p<0.001). Endo or exocytic vegetations were identified in 29 of the patients with malignant or borderline tumors, but were not present in those with benign tumors.

Of the patients analyzed, 23 resided in rural areas (9 with benign tumors) and 36 (21 with benign tumors) in urban areas. In this sample, the distribution of malignant and benign tumors was not associated with place of residence (p>0.05). The age at diagnosis of patients from urban and rural areas was similar (44.58±13.72 vs 50.44±10.50, p>0.05).

The median age of these patients was 46 years (23-72 years old). The age of patients with benign tumors (average: 41.40±12.17 years; 23-67 years old) was significantly lower (p=0.0007) than that of those with malignant tumors (average: 51.88±10.61; 35-72 years old). The age at diagnosis in the subplot of women with tumors over 4 cm was lower in subjects with benign tumors when compared with carriers of malignant tumors (40.12±12.88 vs 51.88±10.61 vs p=0.003). The age of patients with malignant tumors, stratified according to the presence or absence of metastases, was similar (52.44±10.77 vs 51.35±10.76, p>0.05).

CA125 was elevated more frequently found to be elevated in carriers of benign tumors (15 patients with benign tumors, all 3 patients with borderline tumors, in 5 patients with malignant tumors, p<0.05). The values of this parameter were not significantly correlated with the age of the patients in the entire group of subjects (r = 0.25) or the groups of patients with benign tumors (r = 0.16) or malignant tumors (r = -0.06).

The ROMA score had increased values in all patients with malignant tumors and 53.33% of patients with benign tumors. The size of the tumors over 4 cm was recorded in all 33 malignant and borderline tumors and 17 of the benign lesions. Analysis of the intratumoral solid component. A solid intratumoral component was detected in 52 of the studied ovarian tumors (30 malignant tumors and 22 of benign tumors, p<0.05). The presence of irregular intratumoral septa with a thickness of over 3mm was found in 28 malignant and borderline tumors and only in 4 benign tumors (p<0.0001).

Contrast uptake analysis of the solid/parietal/septal tumor components. Of the benign tumors, 80% showed contrast uptake (80% with a progressive loading curve type I), and in the remaining 20%, contrast uptake was absent. Bilateral ovarian serous cystadenocarcinoma, with extensive peritoneal disease (especially at the level of the great omentum - “omental cake”) and a small amount of ascites near the liver, spleen and in the pouch of Douglas. Ascites were detected in 22 of 30 malignant tumors, in none of the borderline tumors, and in one case of a benign tumor (p<0.0001).

Analysis of locoregional lymph node disease. Several 32 tumors from the 63 cases presented with enlarged locoregional lymph nodes. Of these, most were malignant and borderline tumors (78% of this group), confirmed histopathologically with positive lymph node disease. Out of the total of benign tumors, only 20 % of benign tumors had locoregional ganglionic hypertrophies, confirmed histopathologically as reactive lymph nodes (inflammatory). These differences between malignant and benign tumors are statistically significant (p<0.0001).

Analysis of cases with macroscopic peritoneal disease. Macroscopic peritoneal disease was highlighted only in malignant tumors, 78% of malignant tumors presented this aspect. The age of women with or without macroscopic peritoneal disease was similar (51.80±10.89 vs 52.13±10.40, p>0.05).

Analysis of pelvic parietal extension/to the neighboring organs. Most of the malignant tumors (70%) and 2 of the borderline ones showed pelvic parietal extension/to the neighboring organs. Among the benign tumors, only one presented this aspect, which represents 3%.

Figure 1: Contrast-enhanced CT examination

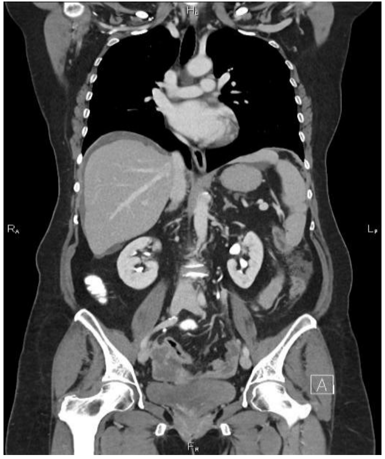

Analysis of cases with distant metastasis. This category included cases in which distant (non-peritoneal) metastases occurred. Among the malignant tumors, 53% showed distant metastases, while benign or borderline tumors did not show such metastases.

Evaluation of the sensitivity and specificity of CT examination. From the sample of 63 cases investigated by CT examination (the groups of patients evaluated by ultrasound and CT or ultrasound, CT, and MRI), the number of cases identified as positive for malignant tumors was 36, in relation to the post-interventional histopathological examination that established the diagnosis of malignancy in 33 of the analyzed cases.

There were 27 cases evaluated by CT with a benign appearance, and the post-interventional histopathological examination established the diagnosis of benignity in 30 of them.

The confusion matrix for assessing the sensitivity and specificity of CT evaluation of malignant ovarian tumors showed a sensitivity of 91%, with a specificity of 80% and an accuracy of 85%.

Table 1: Confusion matrix for sensitivity and specificity of CT examination

Results	Final Positive	Final Negative	
CT positive	TP=30	FP=6	Predictability of positive values, =0.83
CT negative	FN=3	TN=24	Omission rate =0.11
	Sensitivity = 0.91	False positive ratio =0.2	Accuracy=0.85
	False negative ratio=0.09	Specificity =0.80	

CT evaluation was particularly beneficial in cases of large tumors with distant extension, allowing for the assessment of secondary lesions in other organs and the presence of associated comorbidities, in order to optimize therapeutic management.

From the group of 63 cases investigated by CT examination, the number of cases identified as positive for malignant tumors was 36, compared to the post-interventional histopathological examination, which confirmed the diagnosis of malignancy in 33 of the analyzed cases.

The method remains useful in assessing nodal extension and distant metastatic disease and allows a sensitivity of 90%, a specificity of 89%, a positive predictive value of 78%, and a negative predictive value of 95%, with an overall accuracy in diagnosing malignancy of about 89% [2,7,10].

DISCUSSION

This retrospective study analyzed 63 patients with ovarian tumors who were preoperatively CT investigated. Histopathological analysis reveals that epithelial tumors, multilocular tumors, and cystic vegetations were more common in malignant tumors. These differences highlight the heterogeneity and differences between ovarian tumors.

The median age of patients investigated in this study was 46 years (23-72 years old). This result is concordant with data previously published [15], the majority of women diagnosed with ovarian cancer are in the perimenopausal state.

The size of the tumors over 4 cm was recorded in all 33 malignant and borderline tumors and 17 of the benign lesions. The large sizes found in patients diagnosed with malignant tumors may be due to the non-specific symptoms at the onset and the late presentation at the gynecological examination, which determines a long time until diagnosis, ovarian tumors being most frequently discovered incidentally during a routine examination.

A solid intratumoral component was detected in 52 of the studied ovarian tumors (30 malignant tumors and 22 benign tumors, $p<0.05$). The presence of a solid intratumoral component with enhancement curve type II/III is correlated with a higher risk of malignancy, as the Ovarian-Adnexal Reporting and Data System shows [16].

The presence of irregular intratumoral septa with a thickness of over 3mm was significantly more frequent in malignant and borderline tumors than in benign tumors ($p<0.0001$). The irregular septa with a thickness of over 3mm in any ovarian tumor seen on imaging studies should raise suspicion of malignancy, requiring further investigations.

Ascites was detected only in 22 of 30 malignant tumors. In most cases, ascites is present in ovarian cancer because neoplastic cells in the peritoneal lining affect the physiological drainage of peritoneal fluid [17]. Very rarely, ascites can be associated with a benign ovarian tumor, as we found in our patient who had Demos-Meigs syndrome [18].

Most of the malignant tumors (70%), 2 of the borderline ones, and one benign tumor showed pelvic parietal extension/to the neighboring organs. Parietal extension/invasion to the neighboring organs is an imaging feature of malignancy until proven otherwise. Sometimes, in imaging studies, it can be misinterpreted as malignancy in cases of chronic infections or previous surgeries [4].

The ROMA score had increased values in all patients with malignant tumors and 53.33% of patients with benign tumors. In some studies, high ROMA scores were correlated with advanced ovarian cancer before surgery [19,20].

Different imagistic methods can be used to differentiate between physiologic, benign, and malignant ovarian conditions in women [21,22]. Computer Tomography has increased value, especially for the preoperative staging of ovarian cancer, considering the increased spatial resolution of the method, the increased accuracy in the detection and characterization of masses through the new multi-detector technologies (MDCT) implemented, and the reduced radiation doses due to low-dose protocols optimization [6,23].

Computed tomography (CT) is the imaging modality of choice for staging ovarian cancer. Multidetector computed tomography (MDCT) provides clinically relevant information about these tumors (e.g., size of the primary tumor, size and location of peritoneal implants, and lymph nodes). These data can be used to help evaluate surgical options. The overall accuracy of MDCT in diagnosing malignant adnexal masses is as high as 89%. MDCT signs predictive of malignancy include the presence of papillary projections in a cystic lesion, necrosis in a solid mass, and peritoneal metastases. CT is frequently used to detect ovarian carcinoma and to assess response to therapy. CT has limitations in detecting small peritoneal metastases. CT can detect tumors larger than 1 cm with a sensitivity of 85–93% and a specificity of 91–96% [24].

CONCLUSION

Computed Tomography has significant value in the management of ovarian cancer patients, especially in the preoperative staging of ovarian cancer, given its high spatial resolution and improved accuracy in detecting and characterizing ovarian tumor masses and metastatic lesions. This is achieved through newly implemented multi-detector tomography (MDCT) technologies and significantly reduced radiation doses using optimized low-dose protocols. The method remains useful in assessing lymph node extension and distant metastatic lesions, offering high sensitivity and specificity, as demonstrated in this article.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, N.I.A. and N.M.F.; methodology N.N, S.N.M.E.D, A.F.C, A.D.S and D.L.M; software, N.F. and S.N.M.E.D.; validation, N.I.A. ,C.I.M, A.D.S, A.F.C. and N.M.F.; formal analysis, N.M.F.; investigation N.I.A.; resources A.D.S, S.N.M.E.D.; data curation N.F.; writing—original draft preparation, N.I.A.; writing—review and editing, N.I.A, N.M.F, D.L.M; visualization, N.I.A.; supervision, N.M.F; project administration, N.I.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the hospital's ethics committee Nr. 601/ 24.05.2023 and is performed under the ethical standards laid down by the Declaration of Helsinki.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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SYSTEMATIC REVIEW

Effect of the selective serotonin reuptake inhibitors over coagulation in patients with depressive disorders – a systematic review and retrospective analysis

Octavian Vasiliu[1]

Abstract: *Several problems related to coagulation dysfunctions induced by selective serotonin reuptake inhibitors (SSRIs) were reported in literature, as serotonin is widely distributed in the human organism and it has significant contribution in various vascular and hematologic regulatory mechanisms. First, a systematic review has integrated main results from the field of SSRIs and anticoagulants pharmacologic interactions. Secondly, a retrospective analysis was performed, based on the medical charts of hospitalized patients diagnosed with SSRI-treated depressive disorders, who also received concomitant anticoagulant treatment with acenocoumarol. Platelet count, prothrombin time, activated partial thromboplastin time, and bleeding time were monitored during hospitalization and their values before and after SSRI initiation treatment were compared.*

Keywords: *major depressive disorder, antidepressants, anticoagulants, serotonin*

CURRENT STAGE OF RESEARCH IN THE FIELD OF SSRIs-ANTICOAGULANTS INTERACTIONS AND SSRIs-INDUCED COAGULATION DYSFUNCTIONS

Data in the literature regarding selective serotonin reuptake inhibitors (SSRI) treatment in patients diagnosed with depressive disorders and cardiovascular pathology for which they receive anticoagulants contain contradictions regarding the magnitude of antidepressants' effect over coagulation parameters.

Currently available informations suggest the existence of a decrease in platelets serotonin due to serotoninergic antidepressants action, and this phenomenon appears to be explained by a pharmacogenetic mechanism, through serotonin transporter promotor gene (5-HTTLPR) polymorphism [1, 2]. Also, it has been observed that serotoninergic antidepressants could mitigate the depression- and anxiety-related procoagulant action [3], while yet another studies reveal the lack of significant effect of sertraline over coagulation in patients after an acute myocardial infarction [4].

An independent analysis of clinical trials published in the main electronic databases (PubMed, Cochrane, Medscape, and EMBASE), using keywords "anti-depressant", "serotonin selective reuptake inhibitors", "major depressive disorder (MDD)", "coagulation", "anticoagulants", "paroxetine", "sertraline", "escitalopram", "citalopram", "fluoxetine", "fluvoxamine", "warfarine", and "acenocoumarol" was performed. Only articles published between 1996 and 2018 have been selected.

Specific inclusion and exclusion criteria had been formulated according to Table 1.

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Table 1. Selection criteria for literature research

Operationalized criteria	Inclusion criteria	Exclusion criteria
Population	Inferior age limit is 18, no superior limit had been established. Diagnoses of MDD, dysthymia, adjustment disorder with depressive features, bipolar depression, mixed depressive and anxious disorder. Diagnoses according to DSM, ICD criteria, or compatible with these classifications. Lack of substance related comorbidities.	Children and adolescents. Heterogenous populations, where statistical procedures didn't allow a separate conclusion for patients aged over 18. Other disorders than those with a significant depressive component
Intervention	Any agent from the SSRI class. Monitored anticoagulant treatment.	Other, non-SSRIs-antidepressants. SSRIs in combination with other antidepressants. Concomitant use of CYP450 iso-enzymes inductors or inhibitors. Supra-therapeutic doses of SSRI agents.
Environment	In-patient or out-patient. In vitro or in vivo analysis of coagulation variables.	Unspecified environment.
Primary and secondary variables	Any coagulation-related indicator, like platelet count, bleeding time, prothrombin time etc. Discontinuation of treatment due to severe adverse events from the hematological domain, like gastro-intestinal bleeding, hemorrhagic stroke etc.	Any trial without coagulation related parameters as primary variables was excluded
Design	Randomized clinical trials, open-label, single-blind or double-blind, placebo-controlled or not, prospective or retrospective trials, case control studies, case reports or case series.	Unspecified design. Lack of a specific method for coagulation monitoring
Language	English, French, German, Romanian	Other language except for those mentioned

A number of 9 trials corresponded to these criteria and were included in analysis. A synthetic overview of the selected studies is presented in Table 2.

Table 2. Synthetic indicators for selected trials

Overall number of subjects	Mean duration of interaction	Age at inclusion
n=391 43.4 SD=70.9 Minimum=1 Maximum=225	n=2 7.5 weeks SD=6.3 Minimum=3 Maximum=12	n=3 68 years old SD=19.9 Minimum=45 Maximum=80

Due to the fact that not all of the papers published contain complete data, synthetic indicators were calculated using only those studies which included specified variables, like the number of participants, mean duration of treatment or the patients' age at inclusion.

The most relevant research data are presented in Table 3.

SSRIs as a pharmacologic class induced lower serotonin levels in the patients' platelets [1] and had a significant effect over several indicators of coagulation [3].

Fluoxetine increased significantly the bleeding time, although not above the normal values [5]. Another trial showed no significant influence of fluoxetine over mean prothrombine time during warfarin treatment [6].

Escitalopram had no effect on coagulation according to one trial [5].

Fluvoxamine could induced an over-anticoagulation status during acenocoumarol maintenance treatment, unlike other SSRIs used as controls [7]. Fluvoxamine effect over coagulation is supported also by two cases treated with warfarine [8, 9].

Sertraline is relatively safe in patients after acute MI, as it induced no changes in bleeding time [4]. Sertraline could interfere with warfarine at circulating binding proteins and displaced the anticoagulant, increasing its free fraction [10].

In conclusion, escitalopram, sertraline and fluoxetine seem to be quite safe during anticoagulant treatment, while fluvoxamine has a tendency for inducing an over-anticoagulation status. Not enough data have been found to formulate a conclusion regarding paroxetine and citalopram, but these agents seem safer than fluvoxamine, in trials who compared various serotoninergic agents. SSRIs, as a pharmacological class, doesn't induce significant effects over coagulation from a clinical point of view.

Table 3. Studies included in the systematic review

AUTHORS	DESIGN	RESULTS AND OBSERVATIONS
Reikvam AG, Hustad S, Reikvam H, et al., 2012 [1]	Case-control pilot study. In vitro measurements of platelet function. N=18. Blood donors using SSRIs vs. blood donors without treatment	Donors with SSRIs had significantly lower serotonin in their thrombocytes. Coagulation could be altered in patients using SSRIs based on lower serotonin platelet concentration.
Siddiqui R, Gawande S, Shende T, et al., 2011 [5]	Prospective, open-label, diagnosis of MDD. Bleeding time, clotting time, platelet count, prothrombin time, partial thromboplastin kaolin time – all were monitored for 12 weeks. N=40. Treatment with fluoxetine or escitalopram	At week 12 a significant increase in bleeding time was detected for fluoxetine, while escitalopram had no effect on coagulation variables. Fluoxetine is more powerful inhibitor of SERT than escitalopram. Fluoxetine increased significantly the bleeding time, but not beyond the normal values
Geiser F, Conrad R, Imbierowicz K, et al., 2011 [3]	Case-control study. Anxiety and comorbid depression. APTT, fibrinogen, factor VII, factor VIII, von Willebrand factor, von Willebrand ristocetin cofactor activity, prothrombin fragment 1 and 2, thrombin-antithrombin complex, d-dimer, alpha2-antiplasmin, PAP, tissue plasminogen activator and plasminogen activator inhibitor. N=62.	Fibrinogen, plasminogen activator inhibitor, PAP differentiated SSRIs treated patients from their matched controls. After controlling for smoke status and BMI, differences between groups were significant for PAP, von Willebrand ristocetin cofactor activity and APTT. Several coagulation indicators may be affected at significant levels ($p<0.05$) in anxious-depressive patients treated with SSRIs
Teichert M, Visser LE, Uitterlinden AG, et al., 2011 [7]	Prospective, populational-based cohort study. INR≥6 was the event monitored in patients treated with SSRIs during acenocumarol maintenance treatment. N=225 Age ≥ 45	The risk for over-anticoagulation during acenocoumarol maintenance treatment was increased in patients treated with fluvoxamine, but not with other SSRIs. Prothrombine time in users of acenocoumarol was increased by fluvoxamine above a critical value associated with bleeding risk. Number of exposed patients to other SSRIs except for fluvoxamine was low.
Limke KK, Shelton AR, Elliott ES, 2002 [8]	Case report. Warfarin+fluvoxamine 79 year old woman	Increased values of INR that persisted for 7 days. Fluvoxamine inhibits CYP1A2, 2C9, 2C19 and 3A4, while the metabolism of warfarine involves the same isoenzimes of CYP450.
Yap KB, Low ST, 1999 [9]	Case report. Warfarin + fluvoxamine. 80 year old woman	The interaction between fluvoxamine and warfarin could persist for up to 2 weeks after stopping the antidepressant
Shapiro PA, Lesperance F, Frasure-Smith N et al., 1999 [4]	Multicenter, open-label, pilot study. MDD patients identified 5 to 30 days after admission for acute MI. Serial bleeding time determinations. N=26	Bleeding time increased in 12 patients, decreased in 4 patients, was unchanged in 2 patients, 3 patients withdraw prematurely. No significant changes in coagulation measures. Sertraline seems to be relatively safe in patients after acute MI.
Ford MA, Anderson ML, Rindone JP, Jaskar DW, 1997 [6]	Open label. Stable dose of warfarin + fluoxetine. Prothrombine time was measured during the 22 days of the trial. N=6	No significant differences in mean prothrombine time before and during fluoxetine administration were detected. Fluoxetine at 20 mg/day doesn't influence the hypoprothrombinemic response of warfarin.
Apseloff G, Wilner KD, Gerber N, Tremaine LM, 1997 [10]	Non-blinded, randomised, placebo-controlled. Healthy male volunteer. Warfarin+sertraline or placebo. N=12	Increased prothrombine time significantly during sertraline versus placebo ($p=0.02$). After 22 days a significant increase ($p=0.02$) in unbound warfarine was observed in sertraline vs. placebo group. Differences between groups were statistically, but not clinically significant. Sertraline has minimal effect on the CYP2C9/10 isoenzyme, while warfarin is principally mediated by this enzyme.

SERT = serotonin transporter, BMI = body mass index, APTT = activated partial thromboplastin time, PAP = plasmin-alpha2-antiplasmin complex, INR = International Normalised Ratio, MI = myocardial infarction

Nevertheless, pharmacokinetic interactions between SSRIs and anticoagulants are possible, at the plasma protein binding level and at the CYP450 isoenzymes level, therefore monitoring of the main coagulation parameters is recommended in patients who undergo serotoninergic antidepressant and anticoagulant treatment.

RETROSPECTIVE ANALYSIS IN PATIENTS USING SSRIs AND ANTICOAGULANTS

Patients diagnosed with depressive disorders admitted in the hospital who received an SSRI agent, and who also were diagnosed with various cardio-vascular or hematologic diseases for which they received anticoagulant treatment, were analysed from coagulation parameters perspective.

Objective

To detect if significant statistical and/or clinical variation of coagulation variables are detected during combined, SSRIs and anticoagulant treatment.

Methods

We analyzed retrospectively charts of all patients evaluated for depressive disorders during one year in our department (01 January 2017 – 31 December 2017), which had anti-coagulant treatment with acenocoumarol and also received treatment with escitalopram, citalopram, fluoxetine, paroxetine, fluvoxamine or sertraline. Platelet count, prothrombin time as reflected in the INR values, APTT, and bleeding time were analyzed in all cases which were monitored during hospitalization and their values before and after SSRI initiation treatment were compared.

All included patients were over 18 years old, without previous treatment with an SSRI agent and were stabilized on acenocoumarol at time of the admission. Depressive disorders diagnoses were formulated according to ICD-10 criteria and included MDD, either first episode or recurrent, bipolar depression, dysthymia, adjustment disorder with depressive manifestations, mixed anxious-depressive disorder.

Patients diagnosed with comorbid substance related disorders were excluded from this analysis, and also those with mentioned history of non-adherence to their anticoagulation treatment.

Results

A number of 42 patients, mean age 56.6, 30 female and 12 male, diagnosed with MDD (n=23), mixed anxious-depressive disorder (n=10), bipolar depression (n=4), dysthymia and MDD (n=2) and adjustment disorder with depressive manifestations (n=3) were included in the statistical analysis. These patients had at least two determinations of INR and platelet count in their charts and most of them (62%) had at least 3 coagulation indicators recorded.

Figure 1: Treatment used during anticoagulation therapy

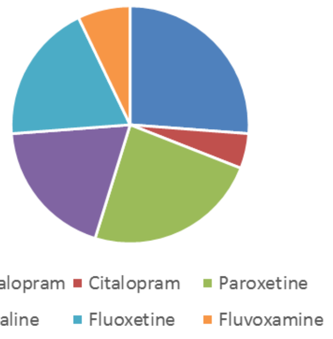

Patients received escitalopram (n=11), citalopram (n=2), paroxetine (n=10), sertraline (n=8), fluoxetine (n=8) or fluvoxamine (n=3).

Figure 2: SSRIs treatment influence over coagulation indicators (% of score variation)

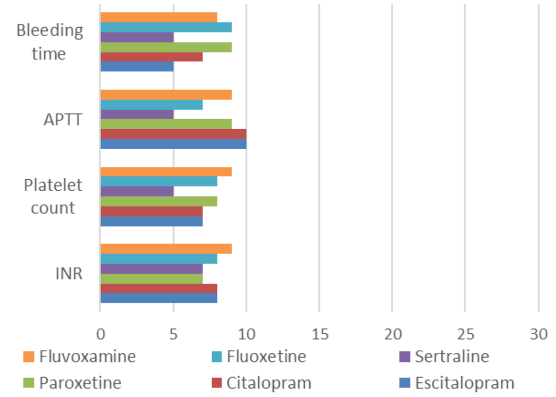

No significant differences (at $p<0.05$) were detected in any coagulation indicator for SSRIs as a pharmacologic class, as reflected by t test for dependent samples, when platelet count ($p=0.166$), INR ($p=0.098$), APTT ($p=0.110$), and bleeding time ($p=0.102$) were analyzed pre-SSRIs administration and after at least 7 days (mean duration of SSRIs treatment before hospital discharge was 8.4 days). When translated in percentage of absolute values variation, all SSRIs changes ranged from 5 to 10% in all the monitored parameters.

ANOVA univariate test resulted in F values ranging between 1.67 and 1.28, lower than Fcrit, at $p<0.05$, and $\eta 2$ varied between 0.025 and 0.069, which signifies an influence of 2.5-6.9% of the SSRIs agent over INR, APTT, bleeding time, and platelet count variation during acenocoumarol treatment. A post-hoc analysis showed a superior effect over bleeding time for fluvoxamine over escitalopram and sertraline (p=0.043 and 0.46), although the clinical impact of this difference wasn't relevant.

No significant adverse event in the hematologic area was recorded during hospitalization period and mixed (SSRI and anticoagulant) treatment.

Two cases (one treated with fluvoxamine 150 mg/day and one who received paroxetine 40 mg/day) registered values of INR above therapeutic value (5.2 and 6.1, respectively) after 8 days of treatment, and acenocumarol doses were adjusted.

CONCLUSIONS

SSRIs had a minor influence over main coagulation parameters (INR, APTT, platelet count, and bleeding time) during acenocoumarol treatment in patients with depressive disorder and comorbid cardio-vascular or hematologic diseases.

Fluvoxamine has been associated statistically with a lesser safe profile than escitalopram and sertraline, although differences in the clinical domain are not detectable.

Disclaimer

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Ethical considerations

All the analyzed medical charts included patients' informed consent for processing of their personal data for research and educational purposes.

List of abbreviations

5-HTTLPR= Serotonin-transporter-linked polymorphic region
APTT= Activated partial thromboplastin time
APTT= activated partial thromboplastin time,
BMI= body mass index
DSM= Diagnostic and Statistical Manual of mental Disorders
ICD= International Classification of Diseases
INR= International normalized ratio
MDD= major depressive disorder
MI= myocardial infarction
PAP= plasmin-alpha2-antiplasmin complex
SERT= serotonin transporter
SSRI= Selective serotonin reuptake inhibitor

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REVIEW

Nuclear Medicine Techniques in the Diagnosis of Cardiac Amyloidosis

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Abstract: Cardiac amyloidosis (CA) represents the accumulation and deposition of misfolded protein fibrils in the myocardium, resulting in progressive restrictive cardiomyopathy. Light chain (AL) and transthyretin (TTR) amyloidosis are the most common types of CA. While endomyocardial biopsy remains the gold standard for diagnosing cardiac amyloidosis, its invasive nature and associated risk of complications have led to increased reliance on clinical suspicion and noninvasive imaging modalities as alternative diagnostic tools. Over the last decade, radionuclide imaging studies have become a widely accepted tool in diagnosing ATTR CA, whereas its diagnostic utility in AL CA detection remains limited. Recent advances in PET-CT radiopharmaceuticals have further expanded the potential of nuclear imaging as a comprehensive tool for diagnosis, prognostication, and therapy monitoring. This literature review appraises the current applications of nuclear imaging in the clinical management of cardiac amyloidosis.

Keywords: Cardiac amyloidosis; Transthyretin amyloidosis; Light-chain amyloidosis; Radiolabeled bisphosphonates imaging; Amyloid-specific PET tracers; Non-invasive cardiac imaging

INTRODUCTION

Amyloidosis is a heterogeneous systemic disease characterized by the aggregation and extracellular deposition of misfolded protein fibrils in various tissues and organs [1]. The term ‘amyloid’, derived from the Greek άmylon and Latin amylum – both meaning ‘starch’, in reference to its iodine staining properties – was first introduced into the scientific literature in the 19th century to describe iodine-positive deposits observed in the brains of patients with dementia [2]. Cardiac amyloidosis (CA) refers to myocardial infiltration by amyloid fibrils, leading to progressive restrictive cardiomyopathy and associated cardiovascular manifestations. Although the type of CA cannot be differentiated based on patterns of deposition, there seems to be a prevalence of diffuse, pericellular, endocardial, and arterial or arteriolar deposits in AL amyloidosis, with nodular deposits in TTR amyloidosis [3].

The most frequent clinical forms – accounting for more than 95% of cases – are light-chain (AL) and transthyretin (TTR) amyloidosis [3]. AL (or primary) amyloidosis results from the deposition of misfolded immunoglobulin light chains, aggregated as amyloid fibril precursors. These last can damage the tissues directly – through their

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proteotoxicity – or form stable fibrils – causing organ damage through impaired histological architecture [2]. TTR amyloidosis is characterized by pathological deposits of transthyretin, a normally occurring protein used in transportation of thyroid hormones and retinol-binding [4]. Wild-type transthyretin amyloidosis (ATTRwt) – formerly known as senile systemic amyloidosis – is an age-related form of cardiac amyloidosis, most often presenting in the seventh decade of life, with a marked male predominance [2,5]. In contrast, variant transthyretin amyloidosis (ATTRv), which is inherited in a dominant autonomic fashion with incomplete dominance, may present as early as the third decade of life, depending on the specific mutation [2,3,5].

With divergent prognoses, treatment strategies, and therapeutic responses, early and precise diagnosis of the distinct forms of cardiac amyloidosis is therefore essential to guide appropriate and timely management. While endomyocardial biopsy remains the gold standard for diagnosing cardiac amyloidosis, its invasive nature and associated risk of complications have led to increased reliance on clinical suspicion and noninvasive imaging modalities as alternative diagnostic tools [4,6].

This paper aims to present current nuclear imaging modalities of diagnosing cardiac amyloidosis and to synthesize recent evidence regarding their prognostic significance and role in evaluating therapeutic response in patients with CA.

CLINICAL SPECTRUM, BIOMARKERS, AND CONVENTIONAL IMAGING

Clinical manifestations

Cardiac amyloidosis is associated with a spectrum of clinical manifestations reflecting the gradual myocardial infiltration and consequent restrictive physiology. Amyloid infiltration leads to progressive thickening of the atrial and ventricular walls, culminating in diastolic dysfunction and heart failure (HF) – often with preserved ejection fraction. Systolic dysfunction typically occurs in the later stages of the disease. While atrioventricular block and tachyarrhythmias also become increasingly clinically apparent as the disease advances, common cardiac symptoms include fatigue, shortness of breath, and syncope [2,3].

AL amyloidosis, caused by clonal plasma cell dyscrasias, typically presents as a multisystemic disease, most frequently involving the heart and kidneys (60-70%), but also the liver (15%) and peripheral nerves (10%) [3]. The amyloidotic heart shows symmetrical increase in biventricular wall thickness (pseudohypertrophy) with markedly increase LV stiffness and filling pressures – in the presence of nondilated or small ventricles [2]. Due to restrictive cardiomyopathy and fibril proteotoxicity, patients with AL amyloidosis can present with dyspnea, fatigue, arrhythmias, severe fluid retention, hepatomegaly and ascites [1,2]. Circulating light chains exert direct cardiotoxic effects, contributing to rapid disease progression and disproportionately elevated cardiac biomarkers relative to amyloid burden [3]. Late-stage diagnosis, when cardiac and renal damage is advanced, correlates with a poor survival – measured in weeks [1,3].

ATTR amyloidosis also presents with a cardiac-dominant phenotype – manifesting as left ventricular hypertrophy (LVH) and HF [5] – particularly in the wild-type form (ATTRwt), which affects elderly men and follows a slow progression. Early extracardiac manifestations, such as bilateral carpal tunnel syndrome, lumbar stenosis or biceps tendon rupture, may precede cardiac symptoms by several years [1,3]. Severe aortic stenosis (SA) has been associated with cardiac amyloidosis, especially in the ATTR variant, as amyloid fibril deposition within valvular tissue contributes to leaflet thickening and progressive stenosis. The coexistence of CA and SA has been reported to have a poorer prognosis; therefore, concomitant diagnosis is essential in guiding appropriate therapeutic management [7]. ATTRv manifestations vary by mutation: Val122Ile leads to predominant cardiac involvement, while Val30Met causes early-onset neuropathy and Thr60Ala produces mixed features [3]. Late-onset ATTRv and ATTRwt share similar cardiac presentations and fibril compositions, often involving both full-length and fragmented transthyretin [2].

Serum biomarkers

When cardiac amyloidosis is suspected, initial evaluation should include serum measurements of N-terminal pro-B-type natriuretic peptide (NT-proBNP), troponin, creatinine and free light chains, along with calculation of estimated glomerular filtration rate (eGFR). A study by Ionescu et al. [8] found the burden of cardiac amyloidosis on eGFR to be more pronounced in ATTR patients compared to AL, regardless of the method used (either by MDRD formula or the Cockcroft-Gault equation).

Assessment of serum and urinary free light chains should be performed to aid the differentiation between ATTR and AL amyloidosis. An abnormal kappa-to-lambda free light chain ratio may be indicative of AL amyloidosis. For further evaluation of the differential diagnosis, bone marrow biopsy is recommended to distinguish between AL amyloidosis and multiple myeloma [2].

Electrocardiography

As one of the simplest yet highly informative diagnostic tools, electrocardiography (ECG) can reveal several 'red flags' – particularly when interpreted in conjunction with imaging findings – allowing for a refined prognosis and stratification. ECG appearances suggestive of cardiac amyloidosis are defined by a pseudoinfarction pattern and disparity between the LV wall thickness and low QRS voltage. Due to myocardial infiltration of non-conductive material, the direct proportionality correlation between QRS voltages and LV mass is therefore abolished. The pseudoinfarction pattern consists of the presence of Q waves measuring at least 1mV in at least 2 contiguous leads, in the absence of ischemic heart disease and/or regional kinetic abnormalities of the LV [2,5]. In AL CA, low peripheral voltage is more common due to proteotoxicity of misfolded light chains, whereas ATTR CA presents more frequently with conduction disease (incomplete or complete bundle branch block), arrhythmias (atrial flutter or fibrillation) and bradycardia [2,3].

Echocardiography

Echocardiography signs suggestive for cardiac amyloidosis consist of increased LV wall thickness (>12 mm) with relative apical sparing ("cherry on top"), low tissue doppler velocities, restrictive filling pattern, increased biatrial volume, atrial septal thickening (>6 mm) and pericardial effusion. LV wall thickness is usually more pronounced in ATTR CA (>15 mm), which also presents more commonly with concomitant aortic stenosis [3,9]. Combining ECG and echocardiographic findings has been shown to provide higher diagnostic accuracy, with 91% sensitivity and 89% specificity in cases with a ratio<0.4 between R-wave amplitude in lead I and posterior wall thickness [2].

Cardiac magnetic resonance (CMR)

Cardiac magnetic resonance imaging, particularly late gadolinium enhancement (LGE) and T1 mapping techniques, allows for noninvasive tissue characterization, therefore aiding the differential diagnosis of cardiomyopathies with hypertrophic phenotypes by detecting amyloid deposits in the myocardium [9]. Morphofunctional quantification reveals concentric and symmetric left ventricular hypertrophy with preserved ejection fraction but reduced indexed stroke volume – often in the context of diastolic dysfunction. Associated findings include thickened valves, left atrial appendage thrombi, atrial enlargement, interatrial septum hypertrophy and mild pericardial and pleural effusions. Increased native T1 mapping is particularly sensitive for early myocardial involvement, often detecting abnormalities before late LGE becomes apparent. Especially during early to intermediate contrast phases, LGE typically demonstrates diffuse subendocardial enhancement in a non-coronary distribution with relative apical sparing. In advanced stages of CA, transmural or intramyocardial, patchy enhancement may be observed, extending into the right ventricle or atria. Characteristic features include myocardial nulling preceding blood pool nulling and early blood pool darkening. Additionally, elevated extracellular volume (commonly>40%) serves as an indirect marker of amyloid burden and correlates with disease severity and prognosis [2,10].

NUCLEAR MEDICINE IMAGING IN CARDIAC AMYLOIDOSIS

Radiopharmaceuticals

Non-amyloid specific radiopharmaceuticals currently used in cardiac scintigraphy are bone-avid radiotracers. These radiopharmaceuticals are labelled with 99mTechnetium: 99mTc-diphosphono-1,2-propanodicarboxylic acid ([99mTc]Tc-DPD), 99mTc-pyrophosphate ([99mTc]Tc-PYP) or 99mTc-hydroxymethylene diphosphonate ([99mTc]Tc-HMDP) [11]. A comprehensive study by Gilmore et al. [12] has shown high specificity and sensitivity of 99mTc radiotracers for TTR amyloidosis, which correlates with negative monoclonal proteins in blood and/or urine samples. It is not yet fully understood why phosphonate compounds accumulate in cardiac tissue at the sites of amyloid deposition. It is hypothesised that this is because amyloid fibril deposits have a higher concentration of microcalcifications in the cardiac TTR, together with slow disease progression and therefore the potential for greater accumulation of deposits in the myocardium and/or other systemic structures. [4,13,14]

A study conducted by Park et al. [15] comparing 99mTc-PYP and 99mTc-DPD showed that the latter may be more useful in evaluating the severity of the disease. They found a more significant relationship between uptake and visual grading, as well as with biological parameters in [99mTc]Tc-DPD scans. Further studies are necessary in order to formulate a proper conclusion.

Quarta et al. [16], in their study of [99mTc]Tc-DPD uptake in AL CA, found a high percentage of patients with bone tracer uptake, almost a third of them with moderate cardiac uptake. This emphasizes the necessity of serological exclusion of plasma cell dyscrasia in case of bone tracer uptake in the myocardial tissue.

There has been a growing interest in recent years for the applicability of amyloid specific radiotracers, used in positron emission tomography – computed tomography (PET/PET-CT) studies in the diagnosis of cardiac amyloidosis [17]. Their primary use is for detection of Alzheimer disease [18]. These radiotracers bind directly to the beta amyloid, thus identifying any other systemic sites of amyloid aggregates. They are not yet approved for clinical use in diagnosis of CA but have shown promising results [19]. Rosengren et al. [20] were able to show high accuracy of 11C-labeled Pittsburgh compound B ([11C]PiB) in the detection of AL CA. Patients with documented systemic amyloidosis and only cardiac wall thickness also had [11C]PiB uptake on PET-CT scans, suggesting a prospective use of it in preliminary stages of the disease. Furthermore, Choi et al. [21] showed that PET-CT with [11C]PiB is a reliable independent factor for 1 year survival in patients with AL CA, alongside other established bio-indicators. The survival rate after 1 year in their 35 patients with [11C]PiB uptake was a little over 50%. Lee et al. [22] determined that PET scans with [11C]PiB are useful in quantifying the degree of amyloid burden by grade of uptake. Their prospective study reflected the usefulness of [11C]PiB in predicting overall outcome. A reduction in the uptake after treatment was observed in a small number of patients, raising the possibility of [11C]PiB PET as a non-invasive tool of assessing treatment response.

Similarly, Genovesi et al. [23] tested the feasibility of [18F]Florbetaben PET-CT for detection of AL amyloidosis. Their results showed that it may be able to detect light chain amyloid burden and to discriminate between forms of CA and other restrictive cardiomyopathies. Along with a previous pilot study by Law et al. [24], both may endorse the use of [18F]Florbetaben as a non-invasive imaging technique to diagnose AL amyloidosis, yet further studies are necessary. It may represent an aid to scintigraphy, which cannot discriminate from other than TTR cardiac amyloidosis.

Apart from the amyloid-specific radiotracers, Wang et al. [25] showed the potential use of 68Ga-labeled Fibroblast Activation Protein Inhibitor ([68Ga]FAPI) PET-CT imaging for the diagnosis of AL CA, due to fibroblastic activity – a possible new instrument in heart failure and its cardiac implications [26].

Radionuclide imaging techniques

Planar acquisition

Interpretation of the cardiac planar images is based on a relatively simple visual scoring and a semiquantitative analysis. The visual grade consists of the Perugini score (Table 1), comparing the intensity of the cardiac uptake with rib cage uptake, and is more useful in case of [99mTc]Tc-DPD or [99mTc]Tc-HMDP [13, 27]. Grade 0 represents no cardiac uptake of the 99mTc radiotracer – and normal uptake of the ribs – being considered negative for CA. Grade 1 – equivocal for the diagnosis of ATTR amyloidosis – consists of mild cardiac uptake, less than rib uptake. Grade 2 represents cardiac uptake of the radiotracers equal to the rib uptake, while Grade 3 translates to a cardiac uptake greater than that of the rib cage. Grades 2 and 3 on planar studies are considered ATTR positive – after the exclusion of plasmatic dyscrasia [28].

Table 1: Visual grading system of myocardial radiotracer uptake on bone scintigraphy according to the Perugini score

Perugini score	Cardiac uptake
Grade 0	absent
Grade 1	cardiac uptake < rib uptake
Grade 2	cardiac uptake = rib uptake
Grade 3	cardiac uptake > rib uptake

Semiquantitative indicators consist of several methods of evaluation, including the heart-to-contralateral ratio (H/CL), most valuable in the case of bone scintigraphy with [99mTc]Tc-PYP [13]. The H/CL measures the ratio between the counts in the two regions of interest (ROI) – the heart and the contralateral lung. With a value greater than 1.5 on one-hour acquisitions, in correlation with a Perugini score of 2 or 3, the cardiac study is highly suggestive of TTR cardiac amyloidosis [28]. However, in cases where the Perugini score is classified as grade 1, histological confirmation of amyloid deposits (potentially located in the extracardiac tissues) is required to establish the diagnosis and determine the amyloid type [29].

The current standard time between injection and acquisition is between one (1h) and three hours (3h) [30]. Images at one hour have a higher sensitivity due to peak myocardial uptake, yet it is of higher specificity in the case of delayed images [17]. Saitou et al. [31] confirmed this remark by a comparison between planar acquisitions at 1h and 3h post-injection on radiolabeled bisphosphonate scans with [99mTc]Tc-PYP. Their study showed a higher specificity in planar 3h studies – results corroborated with single photon emission computed tomography with computed tomography (SPECT-CT). Moreover, a 3h scan can provide a more accurate diagnosis, as proven by their differences in positive predictive values (almost 80% after 3h versus 57% after 1h). Similarly, Nichols et al. [32] emphasized the advantages of using 3h planar and SPECT-CT images for diagnosis. Three-hour acquisitions are advantageous due to lesser blood pool activity and peak bone uptake [11].

SPECT and SPECT-CT

SPECT-CT, a hybrid imaging technique that evaluates function through SPECT and anatomy through CT, provides a useful three-dimensional assessment with the possibility of segmental evaluation and attenuation correction [13].

The visual scoring on planar images is a well-established and reliable method of analysis. Nevertheless, it is prone to misinterpretation because of blood pool activity, osseous uptake that overlaps the cardiac region or inexperience of the interpreter [30]. SPECT-CT images are able to confirm the diffuse left ventricular, biventricular, interventricular septum and/or atrial myocardial uptake of the radiotracers, highly suggestive of cardiac amyloidosis.

Quantification of cardiac uptake on SPECT-CT using standardized uptake values (SUV) was shown to have greater accuracy than visual assessment on planar-only acquisitions. SUV is a semiquantitative index that reflects the uptake of radiotracer in the tissue, but it has yet to be included in standard practice. It is the ratio between radioactivity in a region of interest (ROI) and the dose administered per the body weight of the patient. Kessler et al. [33] compared quantitative SPECT and SPECT-CT results with those obtained through Perugini score in suspected CA patients that underwent [99mTc]Tc-DPD scintigraphy. A value of SUVmax greater than 6.1 showed a higher specificity for CA and particularly for ATTR type – where a 99% specificity value has been reported. Furthermore, in a study by Taha et al. [34], the use of SPECT-CT fusion was able to enhance diagnostic accuracy by adding the anatomical element of the low-dose CT in order to differentiate between myocardial and ventricular diffuse uptake (blood pool uptake). Scully et al. [35] showed that the use of SPECT-CT and SUV values offers a more accurate means of distinguishing between Perugini scores of 2 and 3.

PET-CT

PET-CT imaging can be a more precise tool in describing anatomical and functional characteristics of the disease. PET-CT scans offer more accurate quantifying methods of the biological and physiological processes through SUV values, retention index, target-to-background ratio, as well as visual grading [30]. Amyloid-binding PET radiotracers have accumulated a substantial amount of evidence; however, they are not yet licensed for clinical application in CA. Further investigations are needed to optimize acquisition procedures and validate results in broader groups. In contrast to bone-avid scintigraphy and SPECT radiotracers, PET radiopharmaceuticals have been explicitly designed to attach to the amyloid fibrils. Consequently, PET is the sole imaging modality that is unequivocally selective for amyloid deposits and highly selective for amyloid variants. Moreover, PET is capable of detecting ATTR CA, AL CA, as well as uncommon hereditary variants. Advancements in the quantification of myocardial uptake in both SPECT and PET have produced more detailed and consistent results, facilitating improvements in early diagnosis, risk stratification, and treatment response assessment [36].

DISCUSSION AND FUTURE PERSPECTIVES

Atrial and right ventricular uptake: a new perspective in radionuclide cardiac imaging

The diagnostic role of scintigraphy with bone-avid radiotracers in the diagnosis of CA – specifically of TTR form – is almost in all cases unequivocal [12,37]. However, their role in the prognosis of CA remains still unclear. Recent years' studies have focused on this perspective.

The amyloid burden can be easily identified in the left ventricle alone – yet in most cases, biventricular and/or atrial strain is present, leading to restrictive myopathy [3]. Two promising fields of research regarding the role of radionuclides in prevention and prognosis assessment are the increased uptake in the atrium and in the left ventricle on scintigraphy. Porcari et al. [38] studied the implications of right ventricular uptake of bone radiotracers on scintigraphy. Their results showed a biventricular uptake in 100% of their total of 1422 patients with TTR amyloidosis, identified on SPECT and inconsistently on planar “whole-body” images. It is known that right ventricle involvement in heart failure is linked to an unfavorable disease evolution [5]. This study [38] identified a diffuse right ventricle uptake in more than half of their patients, which was an independent mortality predictor.

Atrial involvement is a part of the onset of cardiac alterations [37]. It appears as part of global amyloidosis (AL or TTR) or as regional impairment of the atrial function due to pathological natriuretic peptide accumulation age related [39]. Hussain et al. [40] reported a high percentage of patients with TTR amyloidosis and atrial fibrillation (AF) to have atrial uptake on [99mTc]Tc-PYP planar images and SPECT-CT. Scintigraphy with 99mTc derivatives is unequivocally useful in assessing the ventricular uptake. Nonetheless, this study raised awareness for atrial implications – with or independent of that of ventricular – related to prevalent high-risk cardiovascular manifestations (AF, thrombi, pulmonary embolism).

National and International Guidelines

Adopting a uniform, standardized protocol, locally and internationally, for nuclear medicine studies for CA, would ensure the best accuracy of disease detection, thus improving the therapeutic approach and prognosis of the patient.

A recent study done by Stan et al. [41] showed the necessity of implementing national standardized protocols – including acquisition times and interpretation algorithms – for accurate reporting of CA scintigraphy studies.

CONCLUSION

As of today, nuclear medicine imaging studies represent a viable and reliable non-invasive imagistic method to diagnose cardiac amyloidosis. Furthermore, what was once an incurable disease with scarcely any treatment prospects is now more easily and promptly diagnosed with the courtesy of vast improvements in the field of radiopharmaceuticals used in cardiac nuclear imaging. While the diagnostic value of bone-avid radionuclides is unequivocal in TTR cardiac amyloidosis, novel indicators of their use in earlier stages or cardiac particularities (atrial or right ventricle uptake associated with unfavorable outcomes) are highlighted through recent studies, persuading specialists to study more this field. Furthermore, with promising results in cardiac imaging, PET may be considered and approved as a viable means of diagnosis, assessment of prognosis, and response to therapy.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization G.S.P.,M.M.M. and S.M.S..; methodology, G.S.P., L.E.M., M.G.; validation, D.M. and S.M.S.; data curation, D.M., G.S.P., M.M.M.; writing—original draft preparation, G.S.P, M.M.M, B.C.; writing—review and editing, L.E.M.,C.M., V.C.M..; supervision, S.M.S.; project administration, S.M.S..; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Not applicable .

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ORIGINAL ARTICLE

Off-Label Percutaneous Solution for Primary Degeneration of a Bioprosthetic Valve in the Tricuspid Position: Case Report and Literature Review on Transcatheter Valve-in-Valve Insertion

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Abstract: Transcatheter valve-in-valve implantation in failing bioprosthesis is an emerging field in cardio-vascular surgery and cardiology. If a valve plasty is not possible, a biological valve is implanted with limited durability. Due to valve degeneration repeated valve exchanges are necessary in some patients. To expand the lifetime of a bioprosthesis in the tricuspid position percutaneous transcatheter valve-in-valve implantation was introduced recently. This is a promising new catheter interventional technology. There are no published outcomes of repeat surgical, but overall early mortality after tricuspid valve replacement (TVR) was 37% in patients undergoing TVR after prior tricuspid valve (TV) repair. The off-label use of transcatheter aortic valve prostheses for tri-cuspid valve–in–valve implantation within dysfunctional surgical tricuspid valve bioprosthesis has been described in small reports but has become of interest nowadays.

Keywords: valve-in-valve tricuspid, redo-surgery, endovascular surgery, off-label valve implantation

INTRODUCTION

A severely diseased tricuspid valve (TV) is classically treated by tricuspid valve repair (TVr) or, when the valve is too damaged, by tricuspid valve replacement (TVR) in classical open-heart surgery. In the case of TVR usually, a biological valve is used considering the thrombo-embolic risk, however, the life expectancy of this valve is limited due to degeneration [1]. Tricuspid stenosis is not an infrequent late complication of TVR. The 8-year follow-up of 31 patients showed that 1 out of 3 patients developed degeneration of the prosthesis with an average gradient above 5 mmHg, and 21% of patients needed surgical redo [2]. Redo surgery is associated with very high risk, with reported early mortality after TVR being 37% in patients undergoing TVR after prior TV repair [3,4]. The off-label use of transcatheter aortic valve prostheses for tricuspid valve–in–valve implantation within dysfunctional surgical tricuspid valve bioprosthetics has been described in small reports and has become of interest nowadays [5,6] especially in patients with high surgical risk or contraindications for surgery [7,8].

Case report

We describe the case of a 64-year-old female patient who presented in our department for fatigability and dyspnea with mild exertion. She had a past medical history of surgical excision of a right ventricle myxoma in 2008, followed by TVR with a biological prosthesis for residual severe tricuspid regurgitation in 2011, VVI pacemaker for iatrogenic grade III atrioventricular (AV) block in 2011 followed by lead fracture and DDD pacemaker upgrade with one lead in the right atrium and second lead in the coronary sinus in 2016. Between 2016 and 2021 the patient had several admissions for heart failure symptoms and degeneration of the tricuspid

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valve bioprothesis was diagnosed with moderate-severe regurgitation and moderate stenosis. The patient developed progressive signs of cardiac failure despite the administration of high doses of diuretics.

At the time of admission, the patient had signs of peripheral congestion and hepatomegaly. Lab tests showed hepatic failure, low albumin, thrombocytopenia, prolonged prothrombin time, and a BNP of 144 pg/mL. Repeated echocardiography showed severe stenosis of the bioprosthesis (max/medium gradient of 20/10 mmHg) and moderate-severe regurgitation. After a careful evaluation of the case by our Heart Team (which includes cardiac surgeons, a cardiologist, an interventional cardiologist, and an anesthesiologist), the patient was contraindicated for open heart cardiac surgery due to an evaluated very high surgical risk. An off-label transcatheter valve–in–valve procedure was considered and an Angio-CT was performed to confirm the feasibility. After careful clinical, biological, and anatomical evaluation, the procedure was scheduled, with the patient's consent.

The procedure was performed by transfemoral right vein approach with a percutaneous Proglide suture implanted before sheath insertion. The 16 F system delivery sheath was inserted without events. A long 0.035 regular wire was advanced with the aid of a JR catheter, passing the wire through the stenotic TV, and positioning a Pigtail catheter in the right ventricle (RV). An exchange was performed with the p of a Safari wire in the apex of the RV. A 26 mm balloon valvuloplasty catheter was then advanced and pre-dilatation was performed with good expansion of the balloon. Then the valve system was introduced into the sheath with a 180-degree counterclockwise turn by inserting the valve system with the Edwards E logo facing downward. The passage through the valve proved to be very difficult with prolapse of the guidewire in the superior vena cava (SVC) even with the use of the flex features of the system.

After several maneuvers, a 90-degree clockwise turn allowed us to obtain a loop into the RV as seen in Figure 1 (a), that stabilized the system and proved to be useful to cross with adequate alignment the degenerated valve, 3 mm below the ventricular edge of the surgical valve frame. A very slow inflation during implantation was performed. This gave us the possibility in the last part of the implant, when the valve tended to protrusion into the RV, to control the position and obtain a good final result as observed in Figure 1 (b, c). The procedure was finalized with the closure of the implanted Proglide suture. There was a good postprocedural result with no para-valvular leak and a mean gradient of 2 mmHg as observed in Figure 2. No vascular complications were encountered during and after the procedure.

Figure 1: Angiographic images during intervention: a – Loop of the system with valve; b – Valve implantation crossing the degenerated prosthesis; c – Final position of the valve-in-valve prosthesis

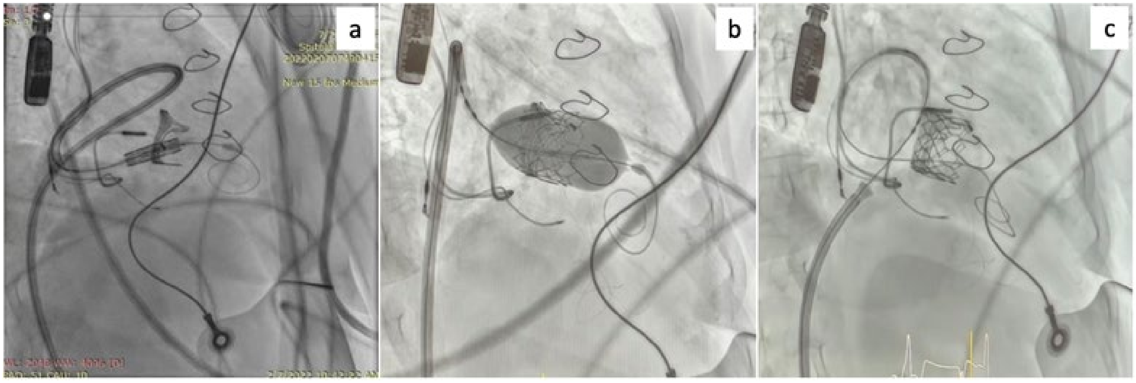

Figure 2: Echocardiographic aspect

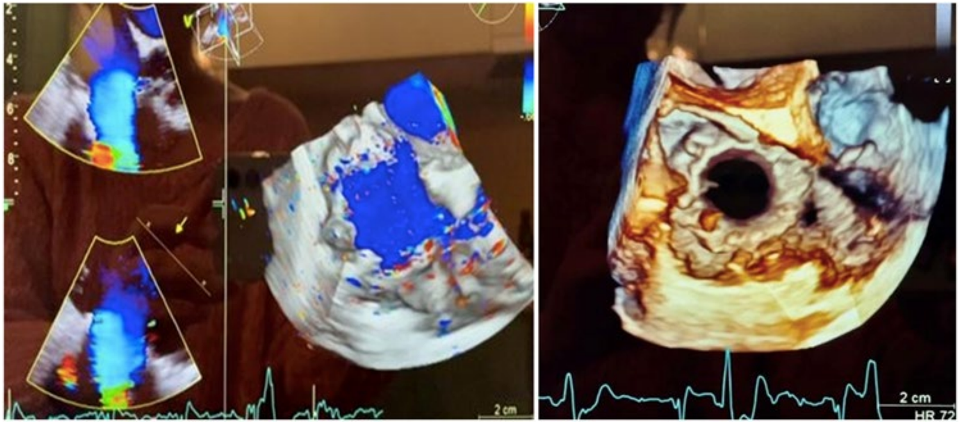

The patient had a favorable postprocedural evolution with symptom improvement. She was discharged on the 4th postprocedural day. At 1 month follow-up the patient had good clinical status (NYHA functional class II). Biologically, there was a significant decrease in natriuretic peptides and normalization of the hepatic markers. On echocardiography, there was no para-valvular leak, with a stationary mean gradient of 2 mmHg.

DISCUSSION

With the technical evolution of percutaneous valvular systems and clinically proven real benefits of transcatheter aortic valve implantation, a new treatment option has become available for patients with inoperable tricuspid valve stenosis [9]. The calcified degenerated native valves have a rough inner surface that acts as an effective anchor for the prosthesis [10]. The irregular inner surface and metal frame of a degenerated bio-prosthetic have similar characteristics and form an ideal docking station for a transcatheter valve. This has allowed valve-in-valve implants, mostly in the aortic position [11-13]. The first case of transcatheter valve-in-valve tricuspid valve replacement was described by Hon et al [14]. Their approach was through a thoracotomy and direct puncture of the right atrium [14]. This has definite advantages regarding the delivery of the device but remains more invasive. The first transcatheter tricuspid valve-in-valve procedure was performed by Leen van Garsse, aided by an extracorporeal circulation machine [15]. Today, with the aid Edwards Sapien valve system, the procedure result is similar and offers real benefits in severe clinical cases.

A successful procedure is highly dependent on the preprocedural evaluation of the patient. A good selection is mandatory in out-of-label cases. Confirmation of the tricuspid stenosis can be made by echocardiography with additional data from the trans-esophageal evaluation. A transvalvular gradient across the tricuspid valve of more than 5-10 mmHg at a heart rate of 70 beats per minute suggests severe stenosis. Gradients are affected by the patient's current hemodynamic status and evaluation needs to be performed with the patient under maximal medical therapy. Additional parameters may be evaluated: a pressure half-time of more than 190 msec, right atrial enlargement, and plethoric inferior vena cava suggest severe TS. Right ventricular performance is exceedingly difficult to assess after surgical tricuspid valvular replacement. Published data suggests that TAPSE and S' wave may be inaccurate parameters when we need to evaluate right ventricular function, at least in the first year after surgery, being highly dependent on the load volume [16]. However free wall longitudinal strain is a valid parameter even after immediate surgery. The mechanism of the prosthesis failure can be analyzed by echo and additional data on the stability of the surgical implanted prosthesis and paravalvular leak are mandatory to evaluate the procedural success. Also, clinical and echocardiographic assessment needs to eliminate the presence of acute endocarditis which is often caused by bacteria entering the bloodstream, adhering to damaged or abnormal heart tissue, and forming colonies [17-19].

The sizing of the valve is made considering three aspects: the dimensions and type of the surgically implanted valve with the aid of the ViV software developed by the Minneapolis Heart Institute Foundation, the Angio-CT sizing, and the echocardiographic findings.

As we demonstrated in this case the presence of hepatic failure appears to regress after the treatment, and this preprocedural condition should not be a contraindication.

The presence of cardiac stimulation leads may need judgment before the procedure, but several case reports suggest that the presence of a transvalvular lead in a valve-in-valve tricuspid procedure is safe and does not affect the functionality of the pacemaker. In our case, however, the ventricular lead was positioned in the coronary sinus, a position that reduced the risk of electric disturbances and paravalvular leak after the implant.

The procedure is done by general anesthesia considering the high dependency of the atrioventricular plane during positioning and implant of the valve with the respiratory activity, general anesthesia allows the control of respiratory movements with apnea if needed during the implant. Conscious sedation and local anesthesia may be considered in selected cases when good anatomy indicates a straightforward procedure.

There are three ways for valve delivery: the transfemoral, transjugular, and direct surgical trans-atrial approach. The decision is made considering the anatomy of the tri-cuspid plane: the valve is vertical, and the transfemoral approach is indicated. Nowadays, with the complex features of the systems that allow us flex movements, the trans-atrial and transjugular approaches are almost abandoned, transfemoral approach allowing good crossing and alignment in the valve [20]. The transjugular approach may remain a good option in case of inferior vena cava thrombosis. The trans-atrial approach is nowadays considered too invasive and is almost abandoned.

The transfemoral approach that we perform is a gradual one, with a first 7F approach, one Proglide suture implanted, and an 8F sheath insertion. As demonstrated in MitraClip studies [21], pre-closure of the femoral vein with Proglide suture is feasible and safe for the 14-16 F access needed for a tricuspid valve-in-valve procedure, considering the pressure and volume vein overload status before the valve treatment.

The insertion of the 16 F large system sheath is performed by gentle movements on a stiff wire, with the positioning of the sheath 5 to 10 cm below the entrance into the right atrium to allow free movement without tension of the system.

The crossing of the valve may be done with the aid of an angulated catheter (JR or vertebral catheter) with a regular J wire, a strait wire, or a hydrophilic wire depending on the degree and anatomy of the valvular complex. After the passage into the right ventricle, two final positions of the wire can be chosen: one in the apex of the RV and another one in the pulmonary artery. There are significant differences between the two positions: the RV position has a higher risk of complications by ventricular rupture and pericardial effusion but allows a better coaxially of the system in the valve with better control during the implant; the pulmonary position may give better support with the minus of poor coaxially and a higher risk of distal pulmonary perforation with pulmonary hemorrhage.

The more vertical the tricuspid plane is, the more the RV position is indicated. The positioning of the stiff wire should be made on a pigtail catheter to diminish the possible trauma on the RV muscle.

If many studies proved the benefit of a direct implant in the aortic position without pre-dilatation, in a tricuspid position during a valve-in-valve procedure we consider that pre-dilatation with a similar-sized balloon is mandatory to anticipate the behavior of the system in the valve, to facilitate the passage through the valve with the system and to anticipate the movement of the system during the gentle inflation.

As we already anticipated for the Edwards systems the insertion should be made opposite to an aortic implant so that the flex movement is possible on the left and not on the right side. This allows typical anatomies to align and cross into the valve without major difficulties. As we presented in our case prolapse of the wire into the right atrium (RA), especially in enlarged anatomies, is possible and in such case, there are two strategies to be tried: one is using a stiffer wire such as Lunderquist Extra-stiff wire by Cook Medical, eventually positioned into the PA- this strategy needs to be prepared in advance because once the system is inserted repositioning of another wire is virtually impossible. If this doesn't allow the positioning of the system in the TV the technique that we used was a gentle two-people synchronized maneuver with the return of the system in an aortic position, developing a loop with the wire in the RA and crossing the valve from the lateral wall of the RA using the loop as a stabilizer to increase pushability (a maneuver similar for the right heart catheterization by femoral approach in severe tricuspid regurgitation (TR) with the loop-technique with the Swan-Ganz catheter).

Once the system is positioned there is no need for rapid pacing during inflation, but several aspects may be considered: the inflation needs to be very slow to allow small adjustments, especially in the last part of the inflation when the rigidity of the full inflated balloon may change the position of the valve in the AV plane. Small movements of the wire and the system in slow inflations can achieve perpendicular lines on the valvular plane with a perfect result. Also, during implantation, apnea may help in maintaining the position.

We consider that transesophageal echocardiography (TEE) during the implant is mandatory to evaluate the result of the procedure, and the presence of the paravalvular leak, and evaluate complications such as pericardial effusion. There is no consensus about an antithrombotic treatment after a valve-in-valve procedure in the tricuspid position. We believe that the low regimen pressure of the right heart indicates us to consider oral anticoagulant treatment (OAC) treatment (without consideration of the presence or absence of sinus rhythm) associated with at least 6 months of clopidogrel. The regimen should be adapted to the patient, evaluating the bleeding risk. This strategy is highly debatable and further studies are needed to define the correct antithrombotic treatment.

CONCLUSION

In conclusion, tricuspid valve-in-valve implantation using the Edwards SAPIEN valve system is a feasible and effective procedure for selected patients with failing bio-prosthesis where redo open heart surgery presents considerable risk. The procedure proved a good clinical improvement for the patients in NYHA III or IV functional class at baseline, with consistent results at 1-year follow-up. Further studies are needed to analyze patient selection, implant technique, results, and antithrombotic treatment following a valve-in-valve procedure in the tricuspid position.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, H.M. and M.R.; methodology, H.M., V.A.I., A.G., E.N., G.G. and M.R.; software, A.G., E.N., A.B., A.Z., M.S.S. and A.B.; validation H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; formal analysis, H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; investigation, H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; resources, H.M., A.B., M.R. and A.B.; data curation, H.M., V.A.I., A.G., E.N., G.G. and M.R.; writing—original draft preparation, H.M., A.G., A.B., A.Z., M.S.S., M.R. and A.B.; writing—review and editing, H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; visualization, H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; supervision, H.M., V.A.I. and G.G.; project administration, H.M., G.G. and M.R; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Positive Experience and Patient Satisfaction – Determinants of Paradigm Shifts in Healthcare

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Abstract: Today's recognition of health as a fundamental value of humanity and the spectacular digital evolution of the environment dominated by permanent and dynamic changes has brought healthcare marketing to the forefront. The trends continuously affecting medical services have generated the need for increased involvement of marketing, with all current developments in healthcare having an indispensable marketing dimension. The successful implementation of a health marketing strategy aimed at enhancing patient satisfaction and fostering a positive care recipient experience, coupled with the cost-effectiveness of the health services offered, has necessitated the development of an adaptive marketing function that harmonizes the set objectives, addresses capability gaps, accommodates cultural realities, and considers individual mentalities and situational circumstances. The direct interaction with patients has made it possible to understand the need for a new paradigm of healthcare services, the demand to comprehend patient satisfaction and positive experience, the call for creating innovative models to address the patient experience, and, above all, the requirement to communicate with the patient continuously and effectively throughout the continuum of healthcare. The challenge for the management of future health systems and organizations is encapsulated in the words of Professor Drucker, who stated that the best way to predict the future is to create it. An exploratory research on the state-of-the-art of the literature was conducted with key references to the most important aspects captured by specialists on positive patient experience and satisfaction, which contributes by highlighting the determining factors that have led to the paradigm shift in healthcare.

Keywords: patient satisfaction, positive experience, new healthcare paradigm, adaptive healthcare marketing, management of healthcare, digital evolution

INTRODUCTION

Given the crucial role of a positive patient experience and satisfaction in enabling individuals who require medical care to make informed treatment choices and prioritize their own needs, the demand for innovative models to address this aspect is evident. These models must reflect the patient-centeredness of healthcare, rather than focusing on the doctor, procedure, or healthcare institution. Also, the models have to consider the medical condition which needs to be seen over the full cycle of care, reflecting the empathy and respect given to patients, the way communication with staff is carried out, the methods by which the information-communication binomial works during the hospitalization period and, of course, confirm the moral values of the hospital unit.

Experience is a core strategy in modern marketing, defined as "the perceptions, emotions and thoughts that consumers have when they come into contact with market services and engage in consumption activities" [1,2]; therefore, the experience is deeply personal and subjective in nature, requires learning and is based on human senses and is persistent over time [3].

Experience is used to influence consumers and is directly related to their level of satisfaction [4]. Four possible strategies are used for its planning:

- designing compelling experiences that stimulate the consumer's interest, attention, and desire to retain information about the service;
- engagement, which is achieved when the consumer feels that they resonate with the service, which they find relevant and necessary;
- emotion induction, which stimulates attention and learning,

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- creating temporary memories, which over time become permanent memories and define new experiences.

The ultimate strategy is to interconnect all these experiences in a holistic, intensive approach, designed to improve the life of the healthcare consumer significantly.

One innovative model for approaching the patient experience is the seven-step model, which includes the following processes: define, research, conceptualize, prototype, choose, implement, and learn. At Stanford University, students took a two-day course offered by the Hasso Plattner Institute of Design, seeking ways to enhance the patient experience in the Stanford Hospital Emergency Department. For the exercise, they role-played patients and their family members to get a sense of what it's actually like to be in the often chaotic atmosphere of an emergency department [5]. The following, among others, were highlighted in this context:

- empathy is a key element of design thinking, a step-by-step approach to problem solving that involves observing and interviewing patients as they go through an experience and then using this information to realize both a prototype and reliable ways to improve the product or process.

- patients wanted a regular flow of information to help them better understand what was happening and how their care providers communicated with each other;

- coordinated and clear communication, in the patients' view, is most helpful in calming anxiety and natural feelings of fear [5].

As is well known, the patient experience, one of the three activities that drive outcomes, along with diagnosis and safe, error-free execution, can be improved by coordinating the patient journey, reducing handoff time, focusing on the audiences of interest, the Disney management approach (every team member is trained to be an effective communicator), and building on traditional health systems (such as the holistic Ayurveda, which emphasizes the unbreakable connections between body, mind and spirit), progressing touchpoints and reforming interactions.

The HealthLeaders Media Intelligence Unit is a division of HealthLeaders Media and has for many years been considered a premier source for health care management research, providing analysis and forecasts through digital platforms, customized reports, customized reports, printed books, conferences, roundtables, peer-to-peer networking technology opportunities (where each computer has equal rights and responsibilities, with network devices sharing resources and communicating directly with each other), and various presentations for senior management [6].

The measurement of patient satisfaction is the application in health care of the concept of customer satisfaction, a concept that comes from both quality management and marketing [6].

As specialists argue, patient satisfaction has an impact on: retaining existing patients and gaining potential ones; the perceived quality of healthcare services by patients as a result of both the interaction with medical staff and the communication through which care recipients are informed about solutions to the medical problems they face, solutions offered by the healthcare facility in comparison to its competitors; compliance with doctors' recommendations; patients seeking a second medical opinion; health outcomes achieved by the health facility in question; satisfaction of the health facility's medical staff; patients not resorting to malpractice lawsuits; patients becoming promoters of the health facility's brand [7].

Several different types of satisfaction questionnaires exist in the medical literature, an established benchmark being, for example, the PSQ-III Patient Satisfaction Questionnaire (a more recent version of the PSQ), a survey of global satisfaction with health care, as well as satisfaction with six aspects of care: technical quality, interpersonal manner, communication, financial aspects of care, time spent with the doctor and accessibility of care [8].

On the other hand, as a short-form version of a hospital patient satisfaction questionnaire, we can consider a combination of a myriad of variants, including the one used by St. Joseph's General Hospital, Elliot Lake [9].

The constant concern about the satisfaction of patients receiving health care services in one of the health care organizations allows resorting to a questionnaire that would allow to objectively assess the degree of patient satisfaction (a significant indicator of health care) treated in a health care facility, and based on the interpretation of the results, to recommend to practitioners solutions to improve this experience, with the effect of optimizing health outcomes and increasing brand awareness in the relevant market.

OBJECTIVES AND METHODS

The main objectives of this study are twofold. First, it aims to examine the key determinants of the emerging patient-centered paradigm in healthcare, with particular emphasis on how positive patient experiences and high levels of patient satisfaction catalyze this paradigm shift. Second, the study evaluates the role of adaptive and innovative marketing strategies in healthcare, including experiential marketing approaches to enhance patient satisfaction and align healthcare delivery with patient needs and expectations. Taken together, these objectives underscore that patient satisfaction and positive patient experiences are central pillars of contemporary healthcare marketing, highlighting the necessity of adopting patient-centered strategies and illustrating how such approaches can drive the transformation of healthcare systems toward more empathetic, responsive, and digitally integrated services.

To accomplish these objectives, an exploratory review of relevant literature was conducted, complemented by a theoretical analysis of key sources. This bibliographic research focused on existing studies addressing the patient-provider service paradigm, experiential marketing in healthcare, and established tools for measuring patient satisfaction, thereby grounding the discussion in well-founded concepts and evidence. Through the synthesis of these sources, the study provides a comprehensive theoretical perspective on the factors driving the patient-centric shift and on the evolution of marketing practices tailored to patient expectations.

RESULTS AND DISCUSSIONS

Paradigm shift in healthcare: patient as healthcare provider

It is well known that paradigms are fundamental frameworks around which disciplines and schools of thought are generated, and most of the paradigms associated with service management have, according to numerous studies, originated from the marketing discipline and perspective. Inevitably, given the generous perspectives that marketing offers, many paradigms associated with services have emerged, with a particular focus on the management sphere.

Given that certain paradigms within service marketing may diverge from traditional operations management principles, leading scholars in both domains have advocated for the development of a renewed and integrative service paradigm.

The review of paradigms in the literature led to the proposal of a customer-supplier service paradigm superior to alternative service paradigms. Thus, the client-provider paradigm (patient-provider of health care services, in the case of health care services) is considered a useful and justified basis for the present and future study of (health care, in our case) services.

The service operations paradigm considered to be prominent is the customer contact model, introduced in the late eighties by R.B. Chase, whose fundamental argument is that the potential efficiency of service operations is limited by the amount of customer contact with the service provider's employees [9].

Furthermore, Chase recommended the famous equation below [10]:

$$\text{Operational potential efficiency} = {}^{\int}(1-)\frac{Client\ time\ contact}{Service\ time\ creation}$$

To understand the customer-supplier paradigm, it is necessary to realize that the customer-supplier paradigm does not focus on what customers do but on what they provide as inputs to service processes. This paradigm shift is that customer-provided inputs are the defining characteristic of services, as long as the customer is identified and the process being analyzed is specified. Also, processes rarely occur in isolation; rather, they exist within supply (value) chains, where one process feeds into another process. Thus, the customer-supplier service paradigm clarifies service supply chains, showing how they involve customers both as providers of inputs and as consumers of outputs [10].

For a correct understanding of this paradigm in the sphere of health services, two essential aspects must be taken into account, according to the rich literature [10]:

- Supply Chain Management: the patient-provider paradigm of health care services identifies the complexity of the supply of health care services and shows how to involve patients in their dual roles, those of input providers and output consumers.

- The universal input-output model: the patient-provider paradigm focuses on the patient's needs and wants, which is the defining characteristic of health services.

The primary paradigm in health services has been naturally generated by the interaction between the patient and the healthcare provider and has proven superior to alternative service paradigms, thus providing a viable basis for studying health services.

Examples of adaptive marketing in healthcare

The exponential developments in healthcare necessitate a continuous search for innovative solutions to meet the needs of existing patients, retain them, and attract new ones, while providing high-quality services that take into account the diverse perceptions of all patients. This requires dynamic and creative management strategies, as well as specific patient-centered marketing actions.

The service revolution continues, always presenting new challenges for marketing management, new rules of competition, new methods of organization, new commitments regarding the communication process and interpersonal relationships, as well as new approaches to economy and intelligence [11]. Dynamism, innovation, and technology are now considered essential tools in a scientific approach [11].

Professor Richard K. Thomas argued that "health professionals, especially physicians, fall into a special category, and the fact that they make most of the decisions about patients creates a unique dynamic to health care." [12]

Kotler recommended the development of stronger marketing (i.e., making it more important than production), replacing old marketing with new marketing, the latter of which should be holistic, strategic, technologically oriented, and financially focused [13].

The strong development and affirmation of marketing in the health sector are due to the rapid and diverse evolution of marketing tools, the marketing mix, and especially the various types of marketing that have adapted quickly and effectively in the health sector. One of these is experience marketing, also known as experiential marketing, a specific strategy through which organizations target different categories of consumers with varying cultural values, satisfying their wants and needs by offering emotionally triggering experiences that stimulate their senses and facilitate the formation of personalized bonds [14].

Experiential marketing can include five types of experiences [4]:

1. *Sensory experiences*: occur when human senses are stimulated:

- sight, through attractive colors and shapes, which are part of the brand identity and intended to be memorized by consumers;

- hearing, through music and sounds used as positioning elements;

- sense of touch, to which men are more sensitive than women;

- the sense of smell, which can trigger certain emotions.

Marketing strategies aim to differentiate an organization and its services from other similar organizations and to add value to services through their sensory appeal.

2. *Affective experiences*: These are determined by the inner feelings of the consumer, to whom the use of the service triggers certain emotions, feelings and moods. Marketing strategies are geared towards increasing the intensity of emotions and developing a sense of anticipation of the pleasant feelings that will follow after using the service.

3. *Experiences through thinking*: stimulates the consumer's creativity and challenges them to solve problems, actually encouraging them to get involved in evaluating the service received and the organization. The marketing strategy works in several directions, designed to surprise the consumer, arouse their curiosity, and create controversy by breaking conventional boundaries - actions that address both convergent, rigorous, and systematic problem analysis thinking and divergent, free thinking.

4. *Experiences through action*: involves the consumer in direct interaction with the health care service, towards which he or she takes an attitude and changes some of his or her lifestyle habits. Strategic directions refer to either physical reactions (such as reflex actions or body signals) or changes in mindset and lifestyle.

5. *Relational experiences*: They provide the consumer with the opportunity to learn about entities as a result of the buying and consuming process.

Marketing strategies also have multiple directions:

- public awareness of a particular social category and its advantages;

- identification of the consumer with an established reference group, so that he/she is convinced that he/she has the same needs as the members of the reference group;

- highlighting, whereby membership of the reference group is perceived as beneficial.

The evolution of holistic organizational ecosystems in the context of the broad and ongoing process of digital transformation, as well as the importance of aligning external trends with internal strategic priorities in addressing the new digital transformation model - digital marketing and digital health- are evident [15].

The potential of digital technologies and data to enrich understanding and marketing as a cultural architect is huge. Digital transformation plays a crucial role in advancing healthcare ecosystems, particularly through the strategic use of digital marketing [16]. This has underscored the importance of adopting a strong human-centered approach, effectively managing digital marketing efforts, and integrating artificial intelligence (AI) with the Internet of Things (IoT). Additionally, leveraging AI-driven insights for diagnostics and other applications is becoming increasingly essential.

Nowadays digital technology has become more and more used in everyday activities, and its intensive use and in most areas of activity has brought changes in the structure of society, and even in the psychology of individuals, who are gradually changing their conceptions about life, work, health and education, the ideal way of spending leisure time and the attributes of an optimal work climate. In this general context, it is clear that conceptions of the healthcare process are also changing, and, naturally, they should keep pace with technological progress and incorporate new tools, new treatments, new procedures, and new medicines, in an attempt to simplify and streamline it. Some of the recent spectacular developments in digital marketing worldwide:

- Digital transformation is enabling a closer connection with customers, responding to the new demands of supply chain management (few business functions are as profoundly disrupted by digitization as marketing).

- In the context of an organization's greater interactivity with more empowered customers, amidst facing particular challenges, marketing is now obliged to offer something for each and every customer [17].

- We live, today, in a much more complex world of digitally enabled smart customers, digital products, digital business operations, and digital competitors [18].
- It is important to identify the most useful insights by understanding what is happening at the confluence of people and technology, taking into account the ability of marketing technologies to bridge different technologies [19].
- Marketers need to enhance the customer experience by getting a complete picture of their customers and delivering real-time experiences [20].
- Marketers now have a critical role in the process of continuously identifying digital opportunities along the entire value chain.
- Marketers must first, and best, understand the difference between change and transformation.
- Marketers must integrate people, technology, and best practices, given the accelerated social interaction of social media.
- There are already new digital tools and new ways to drive change [9].

CONCLUSION

The beneficiaries of healthcare services, the patients, are people with fundamentally different lifestyles, varying ages, diverse social backgrounds, differing levels of education, diverse health conditions, and, of course, different expectations and conceptions.

To resonate with these characteristics, the portrait of the ideal doctor has also changed significantly: first and foremost, he must be an excellent professional, a good communicator, approachable and friendly, transparent, enthusiastic, passionate, able to draw realistic conclusions and objectives and provide immediate and effective feedback on health issues, willing to offer professional and emotional support to his patients.

In the midst of continuously identifying digital opportunities along the entire value chain, marketers need to understand the difference between change and transformation better, integrating people, technology and best practices, taking into account the accelerated social interaction of social media, enhancing the patient experience by improving knowledge of patients' needs, wants and attitudes, gaining a complete picture of them and delivering real-time experiences [20].

The transformation of economies into service economies - i.e., economies in which the production and consumption of services predominate - fundamentally changes the structure of society. This economic transformation is being significantly driven by rapid advances in technology, particularly in information and digital technology [21].

This new, highly topical paradigm in healthcare requires not only innovative models of approaching the patient experience and especially patient satisfaction, but also the obligation of permanent, accurate, and clear communication with the patient.

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The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization MCMS, ITS, ADS, DLDM, AGS, and VLP; methodology MCMS and ITS; software, MCMS and ITS; validation MCMS, ITS, ADS, DLDM, and VLP; formal analysis, MCMS; investigation, MCMS, and ITS; resources, ADS, DLDM, and MCMS; data curation, MCMS, and ITS; writing-original draft preparation, MCMS; writing-review and editing, MCMS; visualization, MCMS; supervision, ADS and DLDM; project administration, MCMS, and ITS. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Not applicable.

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REVIEW

Asymptomatic Bacteriuria – A Permanent Challenge in Clinical Practice: When Should We Treat It?

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Abstract: Asymptomatic bacteriuria (ABU) is a common finding in everyday clinical practice. Many patients, many of them with significant comorbidities, will present, when tested, with bacterial colonization of the urine even if they have no lower urinary tract symptoms at all. Therefore, the clinician is having a dilemma: Should I prescribe antibiotics to sterilize the urine or not? This article aims to update and eventually support physicians in the difficult decision of whether to treat or not to treat patients with ABS and when.

Keywords: asymptomatic bacteriuria, urinary tract infections, antibiotics, pregnant women, elderly patients

INTRODUCTION

Bacterial infections represent a serious health problem worldwide [1]. The growth of bacteria in the urine of a patient who has no symptoms is named as asymptomatic bacteriuria (ABU). ABU is quite a common finding in clinical practice and corresponds to a commensal colonization of the urinary tract [2]. Enterobacterales, B Streptococcus group, and gram-negative bacilli are often met in the urine of ABU patients [3]. Among these species, Escherichia coli, followed by Klebsiella sp, were frequently detected in ABU-urine patients [3-5]. Studies have reported ABU may potentially protect against superinfecting organisms that can determine urinary tract infections (UTIs), therefore the treatment of ABU should be offered only to selected patients. Avoiding unnecessary antibiotic treatment in a significant number of patients with ABU is highly likely to avoid the potential risk of acquired antimicrobial resistance and the risk of removing a potentially effective defense mechanism [4,5]. On the other hand, in some categories of patients, the treatment of ABU proved to be beneficial, avoiding possible serious complications, some of them life-threatening like urosepsis. ABU is quite common in everyday practice. It can be identified in healthy pre-menopausal women(1-5%), with the incidence increasing to 4-19% in postmenopausal women. The ABU varies between 0.7 and 27% in diabetic patients, up to 10% in pregnant patients, and impressive rates varying between 15 and 50% are found in patients living in care homes, and in those who have had spinal cord injuries between 23 and 89% [6]. The bacteria identified in patients with ABU are not different than those found in patients with symptomatic uncomplicated or complicated UTIs.

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DISCUSSIONS

ABU diagnosis

By definition, ABU is characterized by a mid-stream urine sample (MSU) culture with a bacterial growth of more than 105 colony-forming units/mL in a patient without any urinary tract symptoms. Two consecutive samples should be positive in female patients and only one in males [7,8].

No further investigations are required if the history is unremarkable. However, if the urine cultures confirm the presence of urease-producing bacteria, an ultrasound of the kidneys and urinary tract might be performed to rule out the urinary tract stones [8,9]. Digital rectal examination (DRE) needs to be performed in men to exclude the risk of prostate pathology, either infectious or benign/malignant. In selected cases, cystoscopy, either flexible or rigid, can offer a significant diagnostic or sometimes even treatment benefit when bladder wash-out, biopsies, or bladder stone laser disintegration is needed.

ABU management in patients with no identified risk factors

Studies have shown that ABU by itself in patients without any risk factors does not cause any urinary tract infections, or renal disease and does not determine acute or chronic pyelonephritis that can potentially alter the kidney function [10].

One study investigated the value of antibiotic treatment in adult, non-diabetic, non-pregnant women with ABU the incidence of symptomatic UTI was similar in both treated and not treated groups[4]. It was concluded that screening for ABU and the antibiotic treatment of ABU are not routinely recommended in patients with no risk factors and no lower urinary tract symptoms.

A study published in 2012 reviewed the ABU treatment in women with recurrent symptomatic UTIs otherwise healthy and demonstrated an increased risk of symptomatic UTIs in treated patients, compared to non-treated ones. This observation proves that ABU can play a prophylactic role in female patients with recurrent UTIs. Hence the treatment of ABU is not recommended in patients with no risk factors[5].

ABU management in pregnant women

ABU is present in pregnant women in between 2 to 10% of cases. Many studies were dedicated to this category of complex patients when potentially both the mother and the fetus can be affected.

A couple of RCTs [9,10] have been performed on pregnant women trying to identify if ASB treatment is needed or not. Those RCTs compared the groups with ABU treated with antibiotics versus placebo and versus no-treatment patients, also using various antibiotics and regimens. Therefore, these trials reported that the antibiotic treatment has significantly reduced the number of symptomatic UTIs in this group, versus placebo or no treatment ones. Another conclusion of these trials was that the ABU treatment has significantly decreased the rates of low birth weight and the rates of preterm delivery.

Other authors have remarked that the ABU treatment in pregnant women induces decreased incidence of symptomatic UTIs, pyelonephritis, and low birthweight or preterm delivery [11-14]. In conclusion, it seems that it is beneficial for both mother and fetus if the screening for ABU is used in pregnant women and, if bacterial growth is found in the urine, the patients should be subsequently treated appropriately. On the other hand, considering the variety and complexity of pregnant women as patients, it is always advisable to consult national guidelines and recommendations but also to consider each patient on a case-by-case basis.

As it seems that the ABU treatment in pregnant women is generally accepted, another question is which antibiotics and for how long should be used in pregnancy. A significant number of randomized clinical trials have been performed trying to answer these questions. [11-21]. These trials essentially compared different antibiotics, different regimens, or the same antibiotic prescribed in different doses or various durations. Many antibiotics have been used including mainly Nitrofurantoin, Sulphamethopirazine, Pivmecillinam, Amoxicillin, Amoxicillin associated with Clavulanic Acid, Cephalexin, Co-trimoxazole, Fosfomycin [13-15, 17, 18]. Different treatment groups were identified and used for comparison when the treatment length was considered: single dose; short course varying from 2 days to 7 days); long course considered between 8 and 14 days; and long-term course until delivery [17-20]. The data showed that single-dose treatment determined fewer side effects but more babies with low birth weight compared with the short course of treatment.

The value of Fosfomycin single dose was reviewed in a meta-analysis which compared the use of it in pregnant women with uncomplicated UTIs or ABU versus short-term courses of treatment with various antibiotics. The successful treatment of ABU was found to be not significantly different between the patients who received short-term courses of antibiotics, whatever the antibiotic used was. It was also apparent that both standard short-course treatment with various antibiotics and single dose Fosfomycin could be effective options in treating ABU in pregnant patients; however, further studies are needed [21,22].

ABU management in patients with identified risk factors- Diabetes Mellitus

If in 2019, worldwide 463 million people were diagnosed with diabetes mellitus (DM), in 2045 the number can increase to 700 million [23]. Long-term hyperglycemia induces several side effects affecting the eyes, nervous system, kidneys, cardiovascular disorders, [24], and periodontal disease [25]. Therefore, the diabetic patient has an increased risk of developing ABU. Even if diabetes is well controlled with the appropriate medication and diet, a significant proportion of cases with ABU [26]. A randomized control trial was addressed to these patients and found that the ABU treatment, with consecutive negative mid-stream urine, did not reduce the risk of symptomatic UTIs or pyelonephritis. On the other hand, the lack of ABU antibiotic treatment did not correlate with an increased risk of developing diabetic nephropathy [27]. It is now accepted that the ABU screening and treatment in well-controlled DM patients is not routinely recommended.

However, poorly controlled diabetes represents a significant risk factor for various infectious complications. In these patients, the doctors and nurses should make every effort to get the diabetes controlled using both medication and diet. If ABU and infectious complications are present, sounds sensible to not only treat the ABU but also further investigate these patients to rule out urological causes.

ABU management in patients with identified risk factors – Postmenopausal women

Another category of patients who have an increased incidence of ABU is represented by postmenopausal women. Randomized control trials were addressed to these patients and compared the groups with ABU antibiotic treatment vs the placebo or no treatment groups, using different antibiotics and regimens [28]. There were no reported benefits of antibiotic treatment regarding both the incidence rate of symptomatic UTIs and the incidence of bacteriuria resolution [29, 30]. Therefore, as currently there is no demonstrated benefit of ABU antibiotic treatment in post-menopausal women, ASB should be managed the same in both pre-menopausal and post-menopausal women and the screening and treatment of ABU are not recommended.

ABU management in patients with identified risk factors –Elderly/institutionalized patients

Studies have revealed that in elderly institutionalized patients, the rate of ABU is significantly elevated, varying between 15% to 50% [4,5]. Considering that the differential diagnosis between ABU and UTIs is extremely difficult or even impossible in bed-bound, poor mobility patients who also have multiple comorbidities and are potentially mentally deteriorated, it seems that unfortunately unnecessary antibiotic treatments are prescribed in a significant number of patients [30, 31].

An important number of randomized control trials were addressed to the elderly patient population (either institutionalized or not). They were divided into three groups and the group in whom they were treated with antibiotics (different antibiotics, different doses, and regimens were prescribed), was compared with placebo controls or no-treatment groups [30-34]. These RCTs reported that the ABU treatment did not show any significant benefit, compared to placebo or no treatment groups, in reducing the rate of symptomatic UTIs. Also, there was no reported benefit of the antibiotic treatment versus placebo in obtaining a persistently negative MSU culture and sensitivity. One randomized control study compared the incidence of urinary incontinence before and after the resolution of ABU and found no effect in the group of patients who were treated with antibiotics. Even more, other studies reported that the antibiotic treatment of ABU determined significant side effects without any clinical benefit [34]. Therefore, based on the current scientific literature, we can conclude that the screening and treatment of ASB are not recommended in this group.

ABU management in patients with identified risk factors - Renal Transplant Patients

Renal transplant patients represent a very important category, as they are complex patients with associated pathology and sophisticated medication frequently [35-38]. A meta-analysis including randomized control trials and retrospective studies compared the effect of antibiotic treatment to no treatment in renal transplant patients and did not find any benefit in decreasing the risk of symptomatic UTIs between 12 and 22 months after transplant. No benefit was reported in ABU resolution, graft loss, or renal function preservation for up to 24 months [38].

Other publications reported that the incidence of UTIs and pyelonephritis (15% vs. 2.5%) was higher in patients receiving antibiotic treatment for ABU compared to no treatment group [37]. In another RCT, in the first year after transplantation, no difference in the acute graft pyelonephritis was found, but, not surprisingly, the incidence of antimicrobial resistance was higher in the treatment group [36]. Therefore, routine screening and treatment are not routinely recommended in patients who had renal transplantation.

ABU management in patients with identified risk factors – Patients with dysfunctional or reconstructed lower urinary tract

Patients with dysfunctional or reconstructed lower urinary tracts become frequently colonized having ABU [39, 40]. A study reported that the incidence of ABU varies from 25 to 86% for intestinal urinary conduits and from 9.1 to 85% for orthotopic neobladders. It has demonstrated no long-term benefit of ABU treatment in this group [40]. Interestingly, the deliberate colonization with an ABU strain (Escherichia coli 83972) has shown a protective effect against symptomatic UTI recurrences [41]. ABU screening and treatment in these patients is therefore generally not recommended.

ABU management in patients with identified risk factors –Patients with urethral/suprapubic catheters, nephrectomies, or ureteral stents

Patients with urethral or suprapubic catheters and nephrostomy tubes routinely become colonized with bacteria and is generally accepted nowadays that antibiotic treatment is not needed as determines no benefit. No screening for ABU or antibiotic treatment is recommended for patients with indwelling ureteral stents. Hence routine antibiotics for ABU are not recommended, as offer no benefit and determine a significant risk of acquired antibiotic resistance.

In patients who need routine urethral/suprapubic catheter changes, there is no need to be screened for ABU or to have antibiotics. On the other hand, in patients subjected to nephrostomy tube replacement or ureteral stent change, ABU is a risk factor, and therefore prophylactic antibiotics or treatment of ABU are needed [42,43].

ABU management in patients with identified risk factors – Chronic Kidney Disease (CKD)

Worldwide, kidney failure represents another serious complication, especially among diabetic patients [44]. Studies reported an incidence between 9-20% of UTI among female DM patients, while for male DM patients, the incidence is between 3-11% [45].

Patients with CKD are another group in whom ABU can be present. There is no study to investigate if ABU is identified in patients with CKD needs to be treated or not. As it seems that in this group there is an increased risk of antimicrobial resistance, highly likely no routine screening and treatment is needed [46]. This physician should not forget that, if treatment is needed, then it will be necessary to prescribe the antibiotics in renal dose, considering the patients' kidney function test.

ABU Treatment options

If the clinician decides to treat ABU, the same antibiotics, regimens, and duration as in uncomplicated UTIs can be offered, depending on multiple characteristics like gender, co-morbidities, risk factors, allergy status, other medication the patient is taking, potential medication interactions, etc. Treatment should be always tailored on a case-by-case approach based on mid-stream urine culture and sensitivity and should not be empirical.

CONCLUSION

ABU is a clinical entity frequently found in everyday practice. Despite this, there are only a few indications to treat it. Unfortunately, even though the guidelines are clear, inappropriate antibiotic treatment is still offered and determines antimicrobial resistance and selection of extremely aggressive uropathogens. As we presented above, the screening and the antibiotic treatment of ABU are recommended only in pregnant women and in selected individuals who will have endourological procedures that can potentially determine the urothelial mucosa trauma. It remains that future studies could identify alternative methods and treatments, to better manage the patients with asymptomatic bacteriuria.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, F.L.T.M and I.A.V., methodology, A-R.M., D.E.G., M.R.M., and A.E.B-S., software, A.M.S., M.R.M.., I.A.V., and F.L.T., validation, I.A.V., and M.R.M.., formal analysis, F.L.T., A-R.M., D.E.G., A.M.S., and A.E.B-S., investigation, F.L.T., I.A.V., and M.R.M., resources, F.L.T., and I.A.V., data curation, A-R.M., D.E.G., and A.M.S writing—original draft preparation F.L.T.M and I.A.V., writing—review and editing, F.L.T.M, D.M., A.E.B-S., and I.A.V., visualization, F.L.T.M and I.A.V.; supervision, I.A.V., and D.M.; project administration F.L.T.M and I.A.V.; funding acquisition, F.L.T.M. All authors have read and agreed to the published version of the manuscript.

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REVIEW

Detection of Acupuncture Points and Meridians Based on their Electrical Properties: Current Evidence and Future Research Perspectives

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Abstract: Acupuncture is one of the main components of Traditional Chinese Medicine and has been applied as a therapeutical method for centuries. As a therapeutical system, it is based on the use of specific lines in the human body, called meridians, and specific acupuncture points, for choosing the locations where the needles will be applied, depending on the specific problem. In the last few decades, there have been increasing efforts to detect and document the existence of such meridians and points based on at least some of their electrical properties being different when compared to the surrounding skin. A number of researchers have designed experiments, which have had varying degrees of success. Since different researchers have used different experimental setups, and the sample size is usually low, there are issues of validity and comparison between different research results. On the other hand, the majority of available evidence is in favor of there being points and lines characterised by distinct electrical properties, corresponding to acupuncture points and meridians respectively. At the same time, several other theories have been proposed to explain the biological basis of acupuncture points and meridians, while their connection to specific cellular components or neural sequences is an intriguing possibility. While comprehensive research is required to fully document the electrical properties of acupuncture points and meridians, a connection between such properties and their biological basis must also be established.

Keywords: acupuncture points; meridians; electrical properties; TCM; impedance; resistances

INTRODUCTION

Acupuncture is an integrative part of Traditional Chinese Medicine, and has been practised in China [1] and Japan and also in Korea [2, 3] for centuries. It is certain that acupuncture appeared in China although it is difficult to trace its exact historical era of emergence [4]; much later it would be disseminated in Japan, during the 5th century AD [5]. The practises of acupuncture would later become known to Europe by the end of the 17th century, through the works of Willem Ten Rhijne and Engelbert Kaempfer [6]. Following a hiatus of almost a century, after 1850, it would be again be advocated fervently by George Soulié de Morant in 1940s France, and gradually spread to Europe [7].

In the West, there is an ongoing disagreement regarding the efficacy of acupuncture in particular and of alternative therapies in general, both within and outside of the medical community, despite there being numerous studies which are in favour of acupuncture’s effectiveness [8-16]; iatrogenic complications and side-effects are also very rare [17]. Even veterinary acupuncture has been gaining traction as of late [18-21], although we will not be dealing with that in the present article; notably, research on acupuncture points has already began in earnest since the 1990s [22,

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23] – there have even been some reports of the effects of acupuncture in plants [24-29]. It has long been accepted by numerous physicians and researchers that acupuncture can be integrated with modern Western medicine [11,30].

In contrast to the methods of Western medicine, also known as "evidence-based medicine", the exact definition of acupuncture points and meridians and the associated "flow of qi", which constitute the principal theory behind acupuncture therapy, is a concept that cannot be quantified and determined accurately by available technical means, at least for now. There are numerous research efforts which have attempted to describe the different electrical properties of acupuncture points.

The different electrical properties of acupuncture points compared to non-acupuncture points in the human body form the basis for the development of special acupuncture point locators, both for somatic and for auricular acupuncture points. It is important to note here that the existence of differences, at some physiological level, between random points and acupuncture points, lends credence to acupuncture being a legitimate therapeutic method more so than mere psychological suggestion [31] or some type of a placebo effect. A research by Vickland et al. [32] suggested that acupuncture changes the electrical properties of the acupuncture points themselves. Even so, the general difficulty in obtaining consistent and reproducible readings in certain points in the skin and the absence of any known physiological mechanism does not allow for a definite determination of the electrical characteristics of acupuncture points [33]. Recently, the advances in machine learning and artificial intelligence [34-39] may allow for different approaches and yield more conclusive results, although nothing definitive is available as of yet. It is possible that a part of the difficulties in establishing a clear-cut model for detecting TCM meridians and acupuncture points has to do with the biological structure of the skin.

The human skin comprises four successive layers, from the exterior to the interior: stratum corneum, living epidermis, dermis, and hypodermis. Detailed investigations into the structure and specific properties of the skin have been made by a number of authors [40-42]. Each of these layers has different conductive, capacitive and dielectric properties; predominantly these depend on the applied voltage although both age [43] and the relative health condition affect them [44]. In essence, parameters such as resistance, impedance and capacitance are a reflexion of the physical properties of the skin [45].

There exist a number of studies, detailing the different skin dielectric properties, depending on the applied voltage, at different frequencies, both for the entirety of the skin layer [46] and for each layer in particular [47-49]. In general, the living epidermis has higher resistivity than the dermis, while the resistivity of the dermis is not as variable. The higher the number of available ions the lower the resistivity values [50]; since the dermis is more hydrated it is reasonable that it has a higher ion content and therefore a lower resistivity [51]. The main component of impedance is the stratum corneum, the most hydrophobic of the skin layers [45]. Another important factor is the relevant permittivity, which reflects the amount of bound charge displacement or polarisation, in a given area, under the influence of an applied electric field [52]. This property is frequency-dependent and is influenced by the existing conditions at a cellular and molecular level [53-55].

In this review, we will present the most important research regarding the determination of acupuncture points and meridians, mostly based on the measurements of electrical parameters, in an effort to interpret their different and sometimes contradictory results. We will also try to interpret these results in the context of the general biological properties of the skin and underlying tissues. As it will become evident, there are a multitude of different studies and methods and we allude to as many of them as reasonably possible, but we have chosen to present in tables only those researches which have focused in measuring electrical properties using electrodes, as this is the focus of our paper. Finally, we will offer possible explanations for the observed phenomena, based on recent discoveries, and directions for future research.

TCM MERIDIANS AND RESEARCH ON THEIR EXISTENCE

There are numerous types of meridian groups, but here, for research purposes we will focus on the 12 principal meridians and the 2 most important extraordinary meridians (Table 1) – to our knowledge no research on locating any other type of meridian, such as collateral or connecting meridians, has been performed using modern methods.

As limb localisation, based on TCM, we define the limb on which most of a meridian's points can be located; most often it also contains the point of origin of the meridian. The time of maximum activity for each meridian indicates when its most active for therapy, and may correspond to the modern concept of circadian rhythms, which has been discussed in many clinical contexts [59-62].

The 12 principal meridians are associated with organs of the human body, although the properties of these organs do not always correspond to those attributed to them by Western Medicine, and medical acupuncture research. For example, in TCM, the Pericardium Meridian corresponds to the "organ" of pericardium, which is however recognised only as an anatomical structure surrounding the heart, and not an organ proper [63,64]. Moreover, Du Mai and Ren Mai do not correspond to any organ but are associated with the regulation of other processes and functions [65,66].

In another example, the Lung meridian is associated with skin health in TCM, a seemingly paradoxical connection by Western medicine standards; however, according to a number of studies, pulmonary pathologies have skin-related manifestations, as is the case, for example of chronic obstructive pulmonary disease [67,68], and of recurring pulmonary capillaritis [69]. In a reverse example, some skin disorders in children have pulmonary manifestations [70]. While these remarks are interesting and point to a corroboration of many of the principal tenets of TCM, the mainstay of research on verifying the existence of meridians is concerned with identifying them based on their different electrical properties.

Table 1: Principal TCM meridians and their principal characteristics
(based on Soulié de Morant [56], Liangyue et al. [57], Tilikidis [58], and Periferakis [6]).

Western Meridian Name	Chinese Meridian Name	Limb Localisation	Point of Origin	Acupuncture Points in Meridian	Time of Maximum Activity
Lung	Taiyin	Hand	Upper lateral chest, approximately at the level of the 1st intercostal space	11	0300-0500
Large intestine	Yangming	Hand	Radial end of the distal phalanx of the index finger, near the corner of the nail	20	0500-0700
Stomach	Yangming	Foot	Under the eye, directly on the line traversing the pupil, between the eyeball itself and the infraorbital ridge	45	0700-0900
Spleen	Taiyin	Foot	Medial side of the big toe, near the corner of the nail	21	0900-1100
Heart	Shaoyin	Hand	Apex of the axillary fossa	9	1100-1300
Small Intestine	Taiyang	Hand	Ulnar side of the distal phalanx of the little finger, near the corner of the nail	19	1300-1500
Urinary Bladder	Taiyang	Foot	Near the eye in the depression near the inner canthus	67	1500-1700
Kidney	Shaoyin	Foot	Sole of the foot, in the depression former with the foot in plantar flexion	27	1700-1900
Pericardium	Jueyin	Hand	In the 4th intercostal space, by the nipple laterally	9	1900-2100
Triple Boiler	Shaoyang	Hand	Ulnar side of the distal phalanx of the ring finger near the corner of the nail	23	2100-2300
Gall Bladder	Shaoyang	Foot	Lateral to the outer canthus, on the lateral side of the orbit	44	2300-0100
Liver	Jueyin	Foot	Lateral side of the big toe near the corner of the nail	14	0100-0300
Governor Vessel	Du Mai	-	Under the tip of the coccyx	28	-
Conception Vessel	Ren Mai	-	Perineum	24	-

Previous studies on the identification of meridians based on their electrical properties

In general, and compared to the studies on individual acupuncture points, which will be presented on the next section, meridian studies are characterised by a larger sample size and are thus of a higher statistical significance. A number of different measuring techniques were used, and this raises the issues of the comparability and reproducibility of results (Table 2). Probably the earliest research on the different electrical properties of meridians was conducted by Nakatani [71], in the 1950s.

The research of Reichmanis et al. [72] calculated electrical parameters between two different acupuncture points of the same meridian, and determined, using Laplace analysis, that the electrical resistance values were lower. Between the line connecting the two acupuncture points studied, the 14th and 12th of the Large Intestine meridian, there was no other acupuncture point, which could potentially alter the measurements. In the same year, the same authors used the same experimental setup to calculate electrical resistance between the 3th and the 4th acupuncture point of the Heart meridian, with similar positive results [73].

The research of Reichmanis et al. [74] this time measured the resistance between two non-acupuncture points on the Heart meridian; again skin impedance was significantly lower, compared to the control area. This indicates that the presence or absence of acupuncture points in a given meridian area does not affect the clearly different electrical properties of the meridians.

The study of Ogata et al. [75], while not focused on finding the location of meridians based on their electrical properties – indeed it took for granted that the meridians are located on the trajectories described by Traditional Chinese Medicine – detected that there were significant differences in the electrical resistance values of meridians during the different stages of general anaesthesia. We consider the fact that all 12 of the studied meridians had such variations is an, at least indirect, corroboration of meridian location based on different electrical properties, and this is why we included this research. Moreover, we have also included this research in the acupuncture points section of this paper, given that the meridian resistance values were calculated as averages from a number of distinct points in each meridian. Similar results, albeit of a lesser magnitude, were noticed by Ogata et al. [76] in patients under local anaesthesia. Due to the smaller extend of the electrical resistance fluctuation we classify the findings of this research as ambiguous.

It is important to note that the electrical parameters of the skin during post-surgical resuscitation are known to vary, based on the research of Bubnova et al. [77], but it is uncertain if this phenomenon has any bearing on the two aforementioned researches.

Following electrical stimulation at the Jing point of the Stomach (45th of the meridian), 98% of the patients, from a sample of 51 patients tested in an acupuncture clinic, were found to exhibit altered impedance values across the Stomach meridian [78]. The research of Hu et al. [79], which used a larger sample, compared to most of the research efforts examined in this paper, determined that the majority of low impedance points, in the areas examined, where found within a distance of less than 5 mm from acupuncture meridians. Interestingly, they performed this test for all 14 main meridians. A year later, Hu et al. [80] used an electrode moving at an even velocity over the forearm, and computer plotting to demonstrated that most of the detected low skin impedance points were located within the traditional courses of TCM meridians. Similar results were produced by Huang et al. [81], although few details exist on their experiments.

During the research of Zhang et al. [82], impedance was found to be lower in meridians, compared to other skin regions, and to change during needling, a process potentially associated with interstitial fluid fluctuation caused by neuronal reflexes. The research of Martinsen et al. [83], performed using a sample of 20 healthy subjects, did not discover any low resistance trajectories on the volar aspect of the forearm. However, the accuracy of the measuring method of this research has come into question [84]. A subsequent research by Johng et al. [85] yielded positive results, while the research of Ahn et al. [86] had ambiguous findings, in that it demonstrated that the impedance along the Pericardium meridian was decreased, but this was not the case for the Spleen meridian.

The research of Spaulding & Chamberlin [87], who performed acupuncture in order to measure the conductivity across the Large Intestine meridian, yielded statistically significant differences in favour of the meridian, but these were not pronounced enough; thus, we regard these results as being ambiguous.

Table 2: Basic characteristics and outcomes of research of the location of meridians based on their electrical properties

Authors & Reference	Year of Study	Sample Size	Healthy Individuals	Control	Current Used	Experimental Setup	Results	Evaluation
Reichmanis et al. (73)	1977	10	Yes	1-1.5 cm parallel lines medially and parallelly	DC, 1 V	1 cm carbon-impregnated conducting rubber electrodes (modified LIDC electrodes by Ritter Co.)	The electrical resistance values in the line connecting the two acupuncture points were lower compared to the control	Positive
Reichmanis et al. (72)	1977	10	Yes	1-1.5 cm parallel lines medially and parallelly	DC, 1 V	1 cm carbon-impregnated conducting rubber electrodes (modified LIDC electrodes by Ritter Co.)	The electrical resistance values in the line connecting the two acupuncture points were lower compared to the control	Positive
Reichmanis et al. (74)	1979	10	Yes	1.5 cm parallel lines medially and parallelly	DC, 1 V	1 cm carbon-impregnated conducting rubber electrodes (modified LIDC electrodes by Ritter Co.)	The electrical resistance values in the line connecting the two non-acupuncture points were lower compared to the control	Positive
Ogata et al. (75)	1983	10	No	None	AC*	Standard ECG electrodes taped over the chosen acupuncture points in each meridian; values measured during various intervals from preanaesthesia to postanaesthesia	There was a decrease of up to 36 % in skin resistance in 10 of the 12 studied meridians, and by a maximum of 100 % and 73 % on the kidney and bladder meridians respectively	Positive
Ogata et al. (76)	1983	6	No	None	AC*	Standard ECG electrodes taped over the chosen acupuncture points in each meridian; the values were measured before and after the application of local anaesthesia	There were fluctuations of a rather small scale in all of the meridians studied	Ambiguous

Xiang et al. (78)	1984	51	No	None	AC, 0-100 V	Stimulation of the Stomach Jing point using a bipolar electrode and measurements based on the process of Zhu et al. (88)	A distinctly localised meridian line for the Stomach was located in the overwhelming majority of subjects after the initial stimulation	Positive
Hu et al. (79)	1992	68	Yes	Areas laterally removed from the meridians	DC, 0-50 V	1 mm pure Ag tip electrode	The majority of the studied points within less than 5 mm from all 14 main meridians was found to have low impedance	Positive
Hu et al. (80)	1993	12	Yes	Areas not belonging to established meridians	AC[†]	Exploratory electrode moving at a standard velocity over the forearm – computer system used for plotting	Low skin impedance points (LSIPs) were discovered and were generally distributed along known meridian courses	Positive
Huang et al. (81)	1993	25	Yes	Areas not belonging to established meridians	AC[†]	n/a (perhaps similar to the setup of Hu et al. (80))	Low skin impedance points (LSIPs) were discovered to lie mostly within or very near to known meridian courses	Positive
Zhang et al. (82)	1999	12	Yes	Non-acupuncture points in surrounding skin	AC	4 electrode technique – 4 mm diameter pipes with saline cotton	There was a decrease in impedance in the meridian area studied, which fluctuated during needling	Positive
Martinsen et al. (83)	2001	20	Yes	None	AC	16 electrode (0.5 mm diameter) linear array	No linear low resistance trajectories were detected	Negative
Johng et al. (85)	2002	30	Yes	Lateral parallel control at a distance of 6-7 cm from the tested meridian	AC	4 electrode technique similar to that of Zhang et al. (82)	Higher capacitive effects at acupuncture meridians, compared to the control	Positive
Ahn et al. (86)	2005	23	Yes	Control segments 0.8 cm distant, medially and parallelly to the tested meridians	AC	4 electrode method (0.25 mm Au plated needles serving as the electrodes) supplemented by ultrasound imaging of the connective tissue planes along the tested meridians	While impedance in the case of the Pericardium meridians was indeed lower, the same cannot be said for the impedance of the Spleen meridian	Ambiguous
Spaulding & Chamberlin (87)	2011	20	Yes	Non-meridian tissue	AC[†]	A test configuration involving a data acquisition hardware, while processing was performed in Matlab; acupuncture was performed during the measurement	There is a somewhat better conduction along the Large Intestine meridian, compared to non-meridian tissue; while there is a statistical significance of the values, these are not dramatic	Ambiguous

** For these researches the exact data on the type of current used are not available; therefore, based on their use of ECG electrodes and apparatuses we have assumed that it is AC current (this being the more common modality in such machines)*

[†] Similarly, no data on the current type were mentioned and we have assumed it to be AC, which is the most common setting

The particular research parameters of the aforementioned studies on meridian locations are presented in the table below (Table 3).

As can be seen, meridian studies have a higher mean sample size, compared to the acupuncture point studies, which will be presented in the following chapter. In general, there is a greater homogeneity of in research methods and subject selection compared to the following studies on acupuncture points presented in the next section.

The studies with control have also used healthy subjects and there is about an equal number which used AC and DC; most of them have results which can be evaluated as "successful''. The fewer and more diverse studies with no control have also had a relatively positive total outcome. Of course, it is easier to locate meridians compared to acupuncture points, as acupuncture point location is a more difficult and precise process.

In the next subsection, a number of studies on locating meridians using other methods were presented. The association between meridians and interstitial fluid has also been explored by a number of authors along with a number of other proposed alternatives

Table 3: Data on the presented research on meridians

Study Type	N	Mean Sample Size	Subjects' Health			Type of Current Used			ReportedOutcome		
			Healthy	Not Healthy	Mixed	AC	DC	Both	Positive	Ambig.	Negative
Studies with control	10	22.00	10 (100%)	0 (0%)	0 (0%)	6 (60%)	4 (40%)	0 (0%)	8 (80%)	2 (20%)	0 (0%)
Studies with no control	4	21.75	2 (50%)	2 (50%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)	2 (50%)	1 (25%)	1 (25%)
Total studies number	14	21.90	12 (85.7%)	2 (14.3%)	0 (0%)	10 (71.4%)	4 (28.6%)	0 (0%)	10 (71.4%)	3 (21.4%)	1 (7.2%)

For example, Li & Zhao [89] proposed that meridians may coincide with the courses of some blood vessels, while Zhu [90] proposed that the meridians as explained by the TCM are, in fact, an erroneous representation of the nervous system. Several hypotheses were proposed by Zhang [91] while the current research consensus focuses on the meridians representing low hydraulic resistance channels associated with the movement of interstitial fluid and specific metabolites [92]. The association of meridians with stem cells was also proposed by Pawitan [93]; bearing in mind that the stem cell potential varies with a number of factors [94-100], this could have a bearing on the differences in meridian studies across individuals.

Other Studies and Theories on the Properties and Location of Meridians

Apart from the more traditional method of detecting and/or identifying meridians based solely on their different electrical properties, a number of authors have tried a variety of different approaches (Table 2). The most probable explanation is that the different electrical properties of meridians are based on a neural electrical field [101].

The tentative research of Jiang et al. [102] indicates that along meridians, the microcirculatory response to moxibustion therapy is different; thus it may be inferred that there may be well be some level of difference in the microvasculature of meridians, compared to other locations on the body. Research suggests that, in rat experiments, moxibustion therapy seems to modulate gene expression [103]. A clinical trial protocol on the same principle was proposed by Hu et al. [104]. On a different note, the research of Lin et al. [105], indicated that the electrical flow across meridians changes depending on acupuncture needle manipulation during therapy.

Crucially, as is also the case for certain acupuncture points, the electrical properties across meridians seem to differ between pathological and healthy conditions [106,107]. On a related note, a potential association between electrical resistance changes and pain after orthopaedic surgery, which however warrants further investigation, was proposed by Rezvani et al. [108].

The most currently prominent avenue of research on defining the morphofunctional characteristics of meridians centres on them being supposedly channels where a higher amount of interstitial fluid is found. A gel model based on this concept seems to suggest that there is some merit in this theory [91]. An earlier research by Zhang et al. [109] in rats, had not produced any corroborating results.

Finally, the different electrical properties of meridians may be associated by an ion drift theory, as proposed by Liu et al. [110]; notably the research of Lee et al. [111] also mentioned that the special properties of acupuncture points may be associated with different ion concentrations.

VARIATION OF SKIN ELECTRICAL PROPERTIES AT ACUPUNCTURE POINTS

The classical Chinese theory postulates that there exist over 300 acupuncture points, distributed across the 12 principal and 2 extraordinary meridians mentioned above, all over the human body.

The stimulation of these points, based on the view of traditional acupuncturists, adjusts the "qi flow" along the human body; the most frequent proposed interpretation for this notion of "qi energy" is electrical energy [112] and this has been the focus of the majority of meridian detection-associated research.

Electrical properties of acupuncture points – Research history

While, based on the traditional theory, the location of points is determined based on the anatomical parameters and locations of the human body, it was though also possible that these points exhibit distinct electrical properties; based on this speculation, relevant research was performed in the 1950s ([33,113]; and references therein), documenting that at least some acupuncture points where characterised by specific electrical properties.

Table 4: Basic characteristics and outcomes of research of the location of acupuncture points based on their electrical properties

Authors & Reference	Year of Study	Sample Size	Healthy Individuals	Control	Current Used	Experimental Setup	Results	Evaluation
Reichmanis *et al.* (122)	1975	7	Yes	Anatomically similar location within 1 cm^2	DC, 8 V	1.5 mm measuring electrode and a specially designed wheel electrode; both made from stainless steel	Most of the acupuncture points measured were identified based on their electrical parameters in all subjects	Positive
Reichmanis *et al.* (123)	1976	10	Yes	Suitable nearby anatomical location	DC, 2 V	36 stainless steel rods in a grid pattern over the acupuncture point	Over 75% of the acupuncture points were electrically different compared to the control areas	Positive
Hyvärinen, & Karlsson (124)	1977	5	Yes	Surrounding skin	DC, 6.7 V	0.5 mm circular electrode and 2.5 mm reference electrode	Points of consistently low electrical resistance, resembling acupuncture points, were identified	Positive
		5	Yes		DC & AC	0.5 mm silver wire	Massively lower, about 50-100 times, electrical resistance was identified in the same points as above	Positive
McCarroll & Rowley (125)	1979	8	Yes	None	AC (V is a measured variable)	5 x 5 grid with the acupoint in the centre	The decreased electrical impedance detected at a few points was attributed to temporal variations and the pressure of the probe on the stratum corneum	Negative
Poon et al. (127)	1980	9	Yes	Surrounding skin	AC, 0-18 V	Special epiductive tape electrode	Numerous spots of increased conductance were identified, many lying across the meridians, but a lot differing significantly from conventional acupuncture locations	Ambiguous
Jakoubek & Rohlícek (129)	1982	n/a	n/a	n/a	DC & AC	Multiple electrodes	Many points with increased electrical conductance, corresponding to acupuncture points, were detected	Positive
Ogata et al. (75)	1983	10	No	None	AC†	Standard ECG electrodes taped over the chosen acupuncture points in each meridian; results measured during various intervals from preanaesthesia to postanaesthesia	There were marked electrical resistance variations during the different stages of anaesthesia, in most of the acupuncture points studied	Positive
Ogata et al. (76)	1983	6	No	None	AC†	Standard ECG electrodes taped over the chosen acupuncture points in each meridian; the values were measured	There were fluctuations of a rather small scale in all of the individual points measured	Ambiguous

						before and after the application of local anaesthesia		
Margolin et al. (131)	1996	34	No	Auricular control zones not comprising any acupuncture points	DC	Specially developed device with a 1 mm Ag/AgCl electrode composition; use of biogel as a conducting medium – the measurements were performed using auricular acupuncture points; subsequent acupuncture of both control and active points was performed	The therapeutic effects of acupuncture in low impedance points were not very much different from the effects on non-acupuncture points	Ambiguous
Zhang et al. (82)	1999	12	Yes	Non-acupuncture points in surrounding skin	AC	4 electrode technique – 4 mm diameter pipes with saline cotton	There was a decrease in impedance in the meridian area studied, which fluctuated during needling	Positive
Falk et al. (130)	2000	34	No	Control zones on the helix of the ear	AC	An Ag/AgCl probe of 1 mm diameter in a 2 x 2 grid	The sites of lowest impedance were identified as being within active zones	Positive
Zhang et al. (138)	2004	12	Yes	Two points on either side of the low impedance points at a distance of 0.5 cm	AC	Four-electrode impedance instrument (Korean SPAC instrument) based on the methodology described by Zhang et al. (82)	It was found that 71 % of the acupuncture points tested had a lower impedance compared to the control points	Positive
Lee et al. (139)	2005	20	Yes	One non-acupuncture point	DC, 1.28 V	Electrodes affixed to two acupuncture points and one non-acupuncture point; constant pressure applied in the 4th point of the Large Intestine Meridian	Meridian points exhibit a greater conductivity compared to non-meridian points	Positive
Pearson et al. (141)	2007	20	Yes	Nearby control sites no more than 5 cm away from the acupuncture points	DC & AC	Prognos instrument (4.57 mm probe tip) along with a specially designed PT probe	None of the acupuncture points measured exhibited lower impedance values compared to the control points	Negative
Kramer et al. (142)	2008	43	Yes	Surrounding skin	DC, 4.8 V (mean)	Specially designed array comprising 64 electrodes covering an area of 36 cm^2	Only about 1/3 of the measured acupuncture points exhibited significantly decreased electrical resistance values	Negative
Kramer et al. (143)	2009	53	Yes	Surrounding skin	DC, 4.8 V (mean)	Specially designed array comprising 64 electrodes covering an area of 36 cm^2	Some acupuncture points displayed elevated and some reduced impedance values compared to the surrounding areas; it is therefore considered possible that acupuncture points possess different transient electrical properties compared to the surrounding skin	Ambiguous
Rezaei et al. (144)	2012	18	Yes	Parallel series of four control needles placed 0.8 cm	AC	4 electrode technique specifically designed for the study in question	The point studied (PC4) was found to be electrically distinct from other non-meridian points and similar	Positive

				medial to the meridian needles			to other points of the same meridian	
Wong (145)	2014	65	Yes	A neighbouring non-acupuncture point, 2 cm distant	DC, 0.5 V	Bipolar electrode (3 mm in-between electrodes) and a sensing probe	The point studied (LI14) was not found to be electrically distinct from the control point	Negative

** For these researches the exact data on the type of current used are not available; therefore, based on their use of ECG electrodes and apparatuses we have assumed that it is AC current (this being the more common modality in such machines)*

† Similarly, no data on the current type were mentioned and we have assumed it to be AC, which is the most common setting

Some decades earlier, in the 19th century, August Weihe (1840-1896), a medical doctor and homeopathy practitioner came to the conclusion that specific points in the human body were found to be more painful compared to the others, following the ingestion of a toxic quantity of certain homeopathic remedies [114]; it has been mentioned that Weihe knew the notion of specific therapeutic points from a relative in China [115]. It is also notable that Soulié de Morant mentioned that these points coincided with Chinese acupuncture points [116]. The theory of homeosiniatry was proposed by Roger de la Fuye (1890-1961), in 1932, to account for the physiological similarities between homeopathic remedies and acupuncture points; notably, he impregnated some acupuncture needles with homeopathic remedies in a number of his acupuncture therapies [116]. Today, both Weihe and de la Fuye are considered as the ancestors of the theory of electroacupuncture [117]. In 1953, probably the first electroacupuncture apparatus was designed and built by R. Voll (1909-1989) and F. Werner [113].

Despite many studies having taken place on this subject (Table 4), some of them are deemed as being of poor quality, due to a number of factors [118]. A particular point in researching the electrical properties of acupuncture points is the standard to which the data obtained are compared with; more precisely, while acupuncture points may not present minimal electrical resistance at an absolute level, the issue is if they exhibit a different electrical resistance compared to their surrounding tissues. Dedicated research to for the particular, if any, electrical properties of acupuncture points began in the 1970s; notable research efforts from this era are those of Frost & Orkin [119] and Kaslow [120]. Contemporary views on the existence and function of acupuncture points in particular and acupuncture in general can be found in McCarroll [121].

The research of Reichmanis et al. [122] was based on the principle that acupuncture points exhibit a reduced electrical resistance [30]; accordingly, they aimed to prove that acupuncture points exhibited a distinct resistance minimum compared to the surrounding tissue. They employed direct current and used some of the most easily accessible points of the Large Intestine (LI) and Pericardium (Per) meridians; they concluded that all of the studied points exhibited an increased electrical conductance, relevant to the surrounding tissues and most could be located in the majority of subjects. In a subsequent research [123], using a 36 rod grid, on skin disinfected with 90 % alcohol and moistened with tap water, over selected acupuncture loci; suitable control areas were also selected. The points selected were again easily accessible, from the Triple Boiler (TB) and Lung (Lu) meridians. Most, but not all, points were detectable based on different electrical parameters. Further promising results were obtained by Hyvärinen & Karlsson [124], who detected a number of low electrical resistance points on the forearm, hands and ears, which resembled acupuncture points, albeit in a small sample of five healthy individuals. Similarly, in these points the electrical impedance values were found to be massively lower compared to the surrounding skin [124].

Despite these positive results, McCarroll & Rowley [125], who measured the electrical conductance in certain acupuncture points in the hands and arms, came to an unfavourable conclusion. It is noteworthy that this study considered extensively the probable consequences of the pressure applied by the electrode on the skin, to the measurements; in addition, it considered whether any break in the stratum corneum may influence the results. The authors mentioned that, based on their measurements, the time-dependent nature of skin conductance, as asserted by Geddes & Valentinuzzi [126], applied to their measurements as well. They concluded that any detected variations were the result of differing electrode pressure on the stratum corneum and the temporal variability of skin conductance [125].

In an effort to minimise the effect of the electrode itself, Poon et al. (127) used a special epiductive tape electrode, based on the method of Burton & Maurer (128). They determined that a number of points which had different electrical properties corresponded with acupuncture points, but other such points did not; moreover, no statistical analysis demonstrated that across all subjects no significant coincidence between electrically "special" points and acupuncture points was revealed. Even more strikingly, the points of good conductance differed between the two arms of the same subject (127). In addition, a number of low resistance points coinciding with acupuncture points were detected in both humans and rats, and also some such points – not corresponding to acupuncture points – were found in areas of referred pain (129). The research of Ogata et al. (75), to which we have already referred to in the meridians section, also noted that electrical properties of acupuncture points changed during different stages of anaesthesia. For the same reason, the other research of Ogata et al. (76) is included in this chapter too.

A four-electrode method was employed by Zhang et al. (82) to evaluate a single acupuncture point, the 3rd of the Pericardium meridian, during, before and after needling, compared to a number of control points. It was noted that electrical impedance changed in all points, but the impedance reduction in acupuncture points was greater; it was theorised by the researchers that the impedance decrease was owed to a transient increase of interstitial fluid during needling, which is most probably associated with an axonal reflex and/or blood capillary expansion (82). We have already included this research in the section on meridians, but we also place it here because of its focus on the area around a single point.

Meanwhile, Falk et al. (130), using as test subjects patients under treatment for cocaine addiction, detected significantly lower impedance values in the active zones of the ear, i.e. those corresponding to auricular acupuncture points. A similar research had already been conducted by Margolin et al. (131), who tested the differential efficacy of acupuncture treatment in high and low impedance points in the ear; the results in this clinical trial had been ambiguous, in that while needling in low impedance points – corresponding to acupuncture points – had somewhat better effects, this difference was assessed as not being significant enough. Again, this therapeutic approach concerned auricular acupuncture in cocaine addicts. Interestingly, during auricular acupuncture it proved impossible for the subjects to identify, based on pain perception, when acupuncture points were needled, or when needle placement was random (132). Incidentally, there is as of yet no consensus regarding the efficacy of auricular acupuncture in treating cocaine addiction; the results range from mildly encouraging (133, 134) to inconclusive (135, 136) or even negative (137).

Using the four-electrode method, Zhang et al. (138) determined that in their research on a small number of heathy subjects, 71 % of the acupuncture points had a significantly lower impedance compared to the control points on either side. The research of Lee et al. (139) suggested that between two acupuncture points the conductance was greater than between a non-acupuncture and an acupuncture point. Since the second pathway did not belong to an established meridian, they concluded that their experiment perhaps corroborated the theory of Chen (140), who proposed that the dielectric constant along meridians is lower compared to other non-meridian trajectories. Contrary to this, the research of Pearson et al. (141), which was one of the most well-controlled studies, did not detect any significant differences in electrical impedance between acupuncture and non-acupuncture sites.

The research of Kramer et al. (142), who studied the variations of electrical skin resistance in the 34th point of the Gall Bladder meridian in 43 healthy volunteers concluded that, compared to the surrounding skin, only about 33% of the studied points exhibited statistically significant differences; they rightly considered such a small percentage to not be in agreement with most other performed research. A subsequent clinical trial (143) yielded ambiguous results.

The research of Rezaei et al. (144) aimed to determine if a certain acupuncture point, the 4th of the Pericardium meridian, had any different properties compared to its surrounding ones and other points in the same meridian; it was found that it was electrically distinct compared to non-meridian points, but not so much compared to meridian ones. The four-electrode method employed was based on the research of Zhang et al. (82).

The research of Wong (145) is particularly important, as it strived to do stringent lab-controlled measurement of skin resistance of an acupuncture point (14th point of Large Intestine Meridian) on a larger sample (65 participants) than other studies. In their larger sample, they found no statistically significant difference in the electrical resistance between acupuncture and non-acupuncture points.

The results from all the studies in Table 4 are presented in the following table (Table 5), in order to enable a more complete view of the current research consensus.

Table 5: Data on the presented research on acupuncture points

Study Type	**N**	**Mean Sample Size**	**Subjects' Health**			**Type of Current Used**			**ReportedOutcome**		
			Healthy	**Not Healthy**	**Mixed**	**AC**	**DC**	**Both**	**Positive**	**Ambig.**	**Negative**
Studies with control	15	23.1	13 (86.7%)	2 (13.3%)	0 (0%)	5 (33.4%)	8 (53.3%)	2 (13.3%)	9 (60%)	3 (20%)	3 (20%)
Studies with no control	3	8	1 (33%)	2 (66%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)	1 (33%)	1 (33%)	1 (33%)
Total studies number	18	20.6	14 (77.8%)	4 (21.2%)	0 (0%)	8 (44.5%)	8 (44.5%)	2 (11%)	10 (55.6%)	4 (22.2%)	4 (22.2%)

As it can be easily seen from the aforementioned concise table on research results, the studies with control have a considerably larger sample size compared to the studies with no control, where its absence along with the limited sample size further undermines their statistical significance.

In assessing the more significant first group of studies, the overwhelming majority used healthy subjects, while half of them used direct current. Only a small percentage were negative, while the majority was positive or ambiguous. The results were equally distributed in the second group of studies; overall, about half of the studies presented in this section were unambiguously positive, although we would suggest that this statistic has been negatively skewed due to results from the studies with no controls. The majority of studies selected healthy subjects only, while the most frequent current used was alternating current by a small margin, followed by direct current; very few studies opted for both currents.

The relative discrepancy in results can be partly, in our opinion, explained by the fact that the majority of studies have limited their measurements in the upper limbs. The most recent research indicates that acupuncture point resistance values are non-linear [146] and this may influence the results, because on one hand some researches may have focused in non-optimal areas, and on the other few data on the lower parts of the body, and the head exist, if any at all. It is, however, a fact that electrical resistance measurements exhibit great variations depending on anatomical locations [118].

A curious phenomenon, termed the “drift” effect, was discovered by Mayer-Gindner et al. [147], where, if alternating current (AC) is used by the electrode, in the same point, gradually the electrode-skin resistance will decrease. They proposed that at least 20-30 min, if not 40-50 min should pass between two consecutive measurements, even if the point measured is not the same but an adjacent one. They suggested a new method for locating acupuncture points using alternating current. However, this does not seem to be the case, at least to our knowledge, as 5 out of 6 control-group AC studies yielded a positive result.

Presumably, the same influence of the circadian rhythm on meridians, if confirmed, should apply also to acupuncture point measurements. It is known for certain that the circadian rhythm affects skin physiology [148]. The circadian rhythms are also known to affect skin hydration [149], a factor which for sure influences the electrical resistance of the skin and potentially the detectability of acupuncture points.

In addition, there are evidence which suggest that the partial oxygen pressure values in the tissues underlying the meridians are higher than the tissues under random anatomical sites [150]. We mention this research here, instead of the previous meridian chapter, since it was performed on acupuncture points but in essence concerns the whole meridians trajectories. The extensive scientific literature in matters other than electrical properties per se, as outlined in the previous section, seems to corroborate the existence of acupuncture points as distinct morphofunctional entities.

We may conclude that regardless of whether the loci of low electrical resistance can be considered as being the same as the acupuncture points of TCM or not, it is by far more probable that such distinct loci do exist. Their diameter ranges from 1 mm to about 4 mm [120,151,152]. A factor which complicates matters is the relative uncertainty in locating acupuncture points [153], as based on textbooks there may be slight variations [154]; taking into account the tiny diameter of the loci of decreased resistance, it is conceivable that some negative research outcomes were influenced by a slightly erroneous choice of measurement sites. Research on whether needling of the exact location is necessary for a successful treatment outcome is still ongoing [155]; perhaps a general location with a diameter of some mm with the locus of minimum impedance at the centre may be considered as an acupuncture point. What is more certain is that the stimulation of acupuncture points, or of areas around them at least, is by far more effective that the stimulation of random, non-acupuncture points, based on a number of studies [156-160].

Other studies and theories on the properties of acupuncture points

There are a number of other studies, which are associated with the particular properties of acupuncture points, but which do not adhere to the pattern thus described, i.e., the comparison between acupuncture and non-acupuncture points in terms of their electrical properties. The effort of morphologically defining acupuncture points has begun since the late 1970s [161] and it is still an ongoing field of research, where many suggestions and research results must be taken into account. From a structural standpoint, it has been proposed that the microvasculature is capable of providing a larger blood supply in acupuncture points [162].

For example, Liang et al. [163] have asserted that the NO (nitric oxide) concentration in the skin, in acupuncture points, is higher compared to non-meridian and non-acupuncture points. According to their experiment, a NO-noradrenaline system is associated with the different electrical resistance at acupuncture points. Their experiment was based on the previous findings of Chen & Ma [164]. In the same year, Ma [165] asserted that, in addition to the NO-noradrenaline system, the reduction of nitrates by bacteria at acupuncture points was also contributing to the different electrical properties. Other relevant studies, with quite favourable results also exist [166-169].

A limited research effort by Khorsand et al. [170] used an entirely different setup, in that it evaluated the changes of electrical properties of acupuncture points before and after acupuncture was performed – none were detected. A study by Turner et al. [171] aimed to ascertain whether, between arthritis patients which experienced pain and pain-free arthritis patients, there would be any difference between the electrical properties of certain acupuncture points. They concluded that patients which felt pain had different resistance values at acupuncture points, and ventured to suggest that the different electrical energy flow in these patients might be seen as a connection between Western and Chinese medicine concepts.

Ambiguous results, regarding menstrual pain, were yielded by She et al. [172]; the authors do remark, however, that it is a promising field for further investigation. Indeed, following a recent thermographic analysis, Xisheng et al. [173] suggested that a number of acupuncture points is sensitised in patients with primary dysmenorrhea. Interestingly, based on Xu et al. [174], the electrical resistance of specific acupuncture points changes with the variations of blood flow in the uterus, during the menstrual cycle, a result also previously yielded by the research of Wei et al. [175]. At any rate, a nerve-related model to explain acupuncture analgesia was put forward by Silberstein [176]. The values of the electrical properties of a number of acupuncture points were also found to be different in encephaloma patients undergoing surgery [177].

It is also compelling to consider the implications of the research of Weng et al. [178] who suggested that electrical conductance was different between healthy and obese patients, and in fact changed as those obese patients started losing weight.

Recently, an emerging theory suggested that at least some acupuncture points may be thermically distinct between healthy and unhealthy states [179]; this is also a promising field for future research. An earlier review on the subject [180] was more assertive in acupuncture points having a distinct thermal profile, but this is not a unanimously supported conclusion. The infrared profile of acupuncture points may also be of interest in determining the best course of acupuncture therapy [181]. Another study, focusing on asthma patients, suggests that it may be possible to use the values of electrical impedance at certain acupuncture points for screening and treatment effectiveness purposes [182].

The individuality, so to speak, of acupuncture points maybe also be attributable to their differing, compared to the surrounding tissue, ion concentrations, as suggested by the research of Lee et al. [111]. A number of inorganic ions, such as Ca2+ and Mg2+ are distributed prominently on the upper delimitation of the stratum granulosum [183-185], while the distribution of other ions varies in the other layers [184-187]. We might thus suggest, that from a morphological point of view, the distinct acupuncture points represent areas where, for some reasons, there is a differential horizontal and lateral ion distribution across the skin layers. Interestingly, given that the upper stratum corneum is permeable to external substances [188-191], its ionic profile can theoretically be externally modified; thus, by modifying the electrical resistance of acupuncture points it may be possible to modify treatment efficacy.

It must also be noted that, according to the review of Colbert et al. [192], there is an important clinical utility in assessing the variations of electrical properties in acupuncture points, given that they might reveal latent pathologies or be associated with a state of physical exhaustion. The stimulation of acupuncture points with chemical means, also has therapeutical effects as suggested by a number of researchers [193-196]. Regarding this aspect, it is interesting to remark that drug administration in acupuncture sites seems to be more effective compared to non-acupuncture sites [197]. Furthermore, the research of Periferakis & Periferakis [198], in a sample of 40 patients, both healthy and not, using the Pointo Select DT device, for detection of auricular acupuncture points, determined that in the majority of cases, most, but not all points are easily detectable.

An interesting concept is that of neuronal acupuncture [199], which is based on the premise that in acupuncture points, specific neuronal and neuroactive components are stimulated; these loci could comprise the 361 WHO-accepted acupuncture points [200]. The concept of neuronal acupuncture could perhaps be used to explain how or why acupuncture points exhibit different electrical properties, compared to the surrounding skin.

Finally, Cho et al. [201] measured biopotential differences between acupuncture and non-acupuncture points during acupuncture treatment; acupuncture points seemed to have greater variability which seems to be related to the classical concept of the “flow of Qi”. Even the practicing of Qigong, which is theorised to affect Qi circulation, results in significant changes of the electrical potential of acupuncture points [202]. It has also been documented that with needle placement in certain acupuncture points it is possible to observe electrocardiogram and electroencephalogram changes [203,204]; changes where affected during stimulation of non-acupuncture points also, but needle placement in acupuncture points produced more pronounced changes [204].

DISCUSSION

Research on the properties of acupuncture points and meridians has been one of the most prominent topics amongst relevant scholars [205]. It is obvious from the presented studies, that many different researchers have evaluated the identification of acupuncture points based on their electrical parameters, but many of these methods differ significantly, and it is little wonder that their results are not comparable. Of particular interest, however, is the fact that the voltage selected each time seems not to affect the results, i.e., a different voltage will not turn a low impedance point into a high impedance one [206], so at least this offers a modicum of result comparability even if the experimental parameters differ.

Nonetheless, it must be stressed that a host of factors from the parameters of the electrodes, to the physiological and psychological status of the subjects, to the frequencies used, affect the detection of low impedance points [85,207]; therefore, many research results may be rendered inaccurate, and this may also explain the discrepancies in-between the values even of positive-outcome research efforts. Another research by Prokhorov et al. (208) suggest that between racially different populations, the DC measurements of skin resistance are not comparable, a factor which must also be taken into account in future studies. A useful summary of confounding factors in acupuncture point detection research is provided by Luo et al. [209]. It should be noted here that considerable variations in the localisations of acupuncture points is a fairly common occurrence even amongst experienced acupuncturists [210], i.e., the discrepancies are not limited to apparatus-based location methods.

Current research efforts and their significance

Based on the cumulative research results on both the meridian and acupuncture studies (Table 6) we can see that the majority of them where positive, in that it was possible to identify electrically distinct locations and lines on the surface of the human body. Most of the studies used DC, although an almost equal number used AC, and only 2 used both types of current. Finally, the mean sample size was 21.2 subjects. It is difficult to conclude here what the optimal sample size should be for such a research, as there are quite a number of factors which influence its size [211]; moreover, the statistical significance of a sample does not directly in itself validate the experimental results [212]. However, since meridians and acupuncture points, should, in principle, be detectable in any individual, for this particular type of studies, even a small sample should suffice to determine the outcome. However, as mentioned by Reichmanis et al. [123], not all acupuncture points are detectable at all times, and this may be important for the therapeutic efficacy of acupuncture in different patients, or for different protocols used on the same patient. Anomalies in the detectability of acupuncture points, specifically for auricular acupuncture, have been reported by Periferakis & Periferakis [198].

Table 6: Combined and comparative data on the detection of acupuncture points and meridians based on their electrical properties

Study Type	N	Mean Sample Size	Subjects' Health			Type of Current Used			ReportedOutcome		
			Healthy	Not Healthy	Mixed	AC	DC	Both	Positive	Ambig.	Negative
Studies with control	25	21.9	23 (92%)	2 (8%)	0 (0%)	11 (44%)	12 (48%)	2 (8%)	17 (68%)	5 (20%)	3 (12%)
Studies with no control	7	20.6	3 (42.9%)	4 (57.1%)	0 (0%)	7 (100%)	0 (0%)	0 (0%)	3 (42.8%)	2 (28.6%)	2 (28.6%)
Total studies number	32	21.2	26 (81.3%)	6 (18.7%)	0 (0%)	18 (56.3%)	12 (37.5%)	2 (6.2%)	20 (62.5%)	7 (21.9%)	5 (15.6%)

A number of meridian and point locator devices have entered the market after the 1970s [138]. Given the differences in approaches and the fact that even with complicated experimental setups results cannot always be obtained, it is no wonder that the efficacy of early acupuncture point detectors, based on the properties of the points themselves, was called into question [213]. Newer and more sophisticated devices and sensor arrays, such as those proposed by a number of authors [214-216] may improve detection results and eliminate ambiguity.

Regarding research purposes, the delayed luminescence method [217] and the low energy laser (LEL) coherent light interaction with skin [218] may be of future use in evaluating the dielectrical properties of acupuncture points. The more modern method of electrodermal mapping may prove even more useful [219]. Indeed, in the recent years measuring reliability seems to have improved considerably [220].

Finally, the variations and importance of electrical resistance will prove useful even in considering the physical composition of the needles themselves; recent evidence suggests that by some specific material treatment methods it is possible to affect electrical resistance and therefore the therapeutic potential of the treatment [65]. At any rate, as discussed elsewhere, the electrosensitivity of acupuncture points may be a useful clinical and treatment marker [221]. Bearing in mind that there are a number of proposed mechanisms for the action of acupuncture [222], there exists a host of potential modifying factors in assessing the function and potential of acupuncture points and meridians.

Potential Future Directions of Research

Regarding attempts at a structural and biological interpretation for the existence of acupuncture points and meridians, there exists a number of recent research attempts regarding evidence on a cellular level. The most recently discussed theory is that telocytes may be the underlying biological bases of meridians. This is a specialised type of interstitial cells [223], with cytoplasmic extensions; such a structural particularity enables the communication with other distant cells [224]. Telocytes have been found to reside in a number of different tissues [225-232]. Although appearing similar to some other specialised cells types, both telocytes themselves and their extensions, the so-called telopodes are structurally different [233]; the telopodes are expressed both in the body and in cell cultures [234]. There are many theories on what their exact function may be [235-238], but as of yet there is no consensus. A detailed investigation into the potential association between telocytes and TCM meridians was performed by Yonghong et al. [239], on tissues of different animals. Indeed, it was proved that there exist both homocellular and heterocellular connection between telocytes and telocytes and other cell types respectively [239]. Since telocytes interconnect smooth muscle cells, glandular cells and cells for different organs, along with nerves and other structures, this could be the long-elusive network enabling the stimulation of superficial skin structures to have an effect on distant and apparently unrelated morphofunctional units. Notably, as Yonghong et al. [239] assert, the existence of telocytes could be a basis for the interpretation of all the current meridian-associated theories proposed by numerous authors [240-244]. Despite the promising aspects of telocyte research, it must be noted that, as of yet, and to the best of our knowledge, it has proven impossible to interpret all the physiological and pathological connections mentioned in TCM using the notion of telocytes. This of course might be traced down to the fact that telocytes are dynamic cells, so that static imaging techniques may not be always able to trace the full extent of their network. Alternatively, there might be telocytes that are sometimes overlooked in research for a number of reasons [245]. Or, apart from the two aforementioned constraints, it may just so happen that telocytes are not the only answer to this conundrum. Interestingly, a telocyte-based mechanism for the cure of ulcerative colitis by acupuncture was recently proposed by Bai et al. [246].

Another theory on what is the biological basis for meridians and acupuncture points involves keratinocytes, the most dominant cell type of the epidermis [247]. Regarding their electrical activity, it is known that they possess physiologically, a variety of transient receptor potential channels [248-253], along with a number of other channels and receptors [254, 255]. Based on this aspect, and on the experimental research of Denda & Tsutsumi [256], it is hypothesised that it is possible for keratinocytes to generate spatially restricted signalling patterns, which they can then transmit to the unmyelinated nerve fibres of the epidermis [256]. While this may not explain the exact localisation of the points and meridians as proposed by TCM, it does however propose a mechanism by which the stimuli by the needle puncture can be transmitted to the unmyelinated nerves present in the skin [257] and hence affect even spatially distant areas (256). In addition, other researchers have linked acupuncture to the concept of neuroimmunology (e.g., [258-262]].

One additional research avenue is the study of the location of acupuncture points or meridians, based on the effects of phytochemicals when applied at such locations. The most prominent such efforts concern the application of capsaicin, a phytochemical with numerous beneficial properties [263-267], in acupuncture points which was found to have favourable results [268-270]. Based on the past history of use of certain compounds at acupuncture points [116], other phytochemicals, with demonstrated health-related properties, such as kaempferol [271,272], curcumin [273,274], piperine [275], pinosylvin [276], and a host of natural compounds used in the field of oncology [277], could be used experimentally, to verify or dismiss such claims. Furthermore, it is known that acupuncture can measurably influence certain hormone levels in body fluids [278,279]; therefore, we propose that clinical trials could be designed, especially in cases where hormones or their analogues could be of therapeutical benefits, such as in the case of somatostatin [280, 281].

CONCLUSION

Studies on the detection of TCM meridians and acupuncture points have been ongoing, intermittently, during the last decades, by numerous different research teams, using different experimental setups. Whereas the majority of results is positive, i.e., TCM meridians and acupuncture points can be distinguished from the surrounding skin, due to theirs having different electrical properties, there exist a number of shortcomings in most experimental efforts. To be more precise, the different parameters used in most experiments do not permit an easy comparison of results, while the small sample size oftentimes used raises questions of statistical validity; in a number of cases there is also a lack of control factors. Even though we can conclude that it is possible to detect TCM meridians and acupuncture points using electrical properties, more research efforts are needed in order to standardise the experimental approaches and find a structural or functional basis for their existence.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, A.P., K.P.; methodology, A.P., K.P., A.I.; validation, A.P., and D.O.C.; formal analysis, A.P.; investigation, A.P., K.P., A.I., L.T., C.M.; resources, C.M.; writing—original draft preparation, , A.P., K.P., A.I., L.T., A.-T.P., C.M., D.O.C.; writing—review and editing, A.P., K.P., A.I., L.T., A.-T.P., D.O.C.; visualization, A.P.; supervision, A.P., K.P., D.O.C.; All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Exploring Gliosarcoma by Light Microscopy, Two-Photon Excitation Microscopy, and Radiological Imaging Techniques

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Abstract: Gliosarcoma is a rare and aggressive variant of glioblastoma, characterized by both a malignant glial component and a mesenchymal sarcomatous component. Gliosarcomas have genetic alterations with glioblastomas, including TP53, PTEN mutations, and EGFR amplification, but may also exhibit additional changes related to epithelial-mesenchymal transition pathways. Management is similar to glioblastoma, involving safe, maximal surgical resection followed by radiotherapy and chemotherapy with temozolomide, but the prognosis remains poor, with a median survival of 6-14 months. Both Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) play crucial roles in the detection, characterization, and treatment planning of gliosarcoma. While MRI is the gold standard, CT remains valuable in certain situations. Light microscopy is essential for definitive diagnosis, allowing pathologists to examine cellular morphology and tissue architecture. It is essential for distinguishing gliosarcoma from other high-grade gliomas and for guiding treatment decisions. In addition, two-photon excited fluorescence (TPEF) microscopy is an advanced optical imaging technique that enables real-time, high-resolution visualization of tumor tissue without the need for staining or contrast agents and enhances visualization of collagen structure and vascularization, key factors in gliosarcoma assessment. The study of radiological and histopathological (light microscopy) features in primary gliosarcomas of the brain is a priority to achieve an early diagnosis that can be translated into better outcomes. Here, we describe the radiological and histopathological features observed in multiple cases of gliosarcoma in current practice.

Keywords: gliosarcoma; histopathology; light microscopy; two-photon excited fluorescence; radiology; magnetic resonance imaging; neurosurgery

INTRODUCTION

Gliosarcoma is a rare and highly malignant primary neoplasm of the central nervous system (CNS), classified as WHO grade 4. It is characterized by a biphasic histopathological pattern, comprising both glial and mesenchymal components. The peak incidence occurs around the sixth decade of life, with a male-to-female ratio of 1.8:1 [1,2,3]. While most cases are primary, secondary gliosarcomas may develop in patients with previously resected glioblastomas or as a consequence of cranial irradiation. Clinically, gliosarcoma often presents with symptoms such as headache, seizures, and hemiparesis. Due to their aggressive nature, tumors are typically large at diagnosis and frequently cause midline shift [4,5], particularly in the temporal lobe, as revealed by computed tomography (CT) and magnetic resonance imaging (MRI).

Brain and other nervous system malignancies constitute a significant proportion of cancer-related deaths, particularly among children and young adults [6]. Gliomas, the most common primary brain tumors in adults, originate from glial cells and are highly invasive, diffusely infiltrating the surrounding brain parenchyma. Glioblastoma multiforme (GBM), the most aggressive glioma subtype, has a particularly poor prognosis, with a median survival of less than one year and a five-

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year survival rate below 5% [7,8]. The dismal survival outcomes are attributed to the tumor's extensive infiltration, limiting the effectiveness of surgical resection, and leading to inevitable recurrence.

Preoperative imaging modalities such as MRI, CT, single-photon emission computed tomography (SPECT), and positron emission tomography (PET) provide critical insights into tumor localization and characteristics [9]. However, intraoperative identification of residual tumor tissue remains a major challenge. The extent of surgical resection is a key determinant of overall survival and progression-free survival in glioma patients. Achieving maximal safe resection necessitates advanced intraoperative imaging techniques [10] to differentiate tumor tissue from normal brain structures with high specificity and sensitivity.

Recent advances in molecular biology have significantly improved the classification and diagnosis of brain tumors. The integration of molecular markers has refined tumor characterization, enabling more precise prognostic assessments [11]. However, the routine implementation of molecular testing is limited by accessibility constraints, particularly in resource-limited settings [12,13,14]. Furthermore, molecular data interpretation can be complex, and in certain cases, tumors are categorized as "not otherwise specified" (NOS) when genetic features are ambiguous or unavailable.

Histopathological evaluation remains a cornerstone in brain tumor diagnosis [15], with light microscopy providing critical insights into cellular morphology, mitotic activity, necrosis, and vascular proliferation. Hematoxylin and eosin (H&E) staining, alongside immunohistochemical markers, enhances tumor characterization. Despite its essential role, conventional light microscopy alone may not always provide sufficient contrast for distinguishing malignant from benign or reactive tissues. Consequently, there is growing interest in complementary optical imaging techniques for improved tumor assessment.

Emerging optical imaging modalities, including confocal microscopy and nonlinear optical (NLO) microscopy [16,17,18], offer promising non-invasive diagnostic capabilities. Among these, two-photon excited fluorescence (TPEF) microscopy [19] has gained attention due to its ability to generate high-resolution images of biological tissues with minimal photodamage [20,21,22]. TPEF operates by simultaneously absorbing two lower-energy photons, exciting endogenous or exogenous fluorophores, and producing a fluorescence signal. This technique enables detailed visualization of cellular and extracellular structures, making it a valuable tool for characterizing brain tumors, including gliosarcomas.

Most studies utilizing TPEF microscopy in brain tumor research have relied on exogenous fluorescent dyes or genetically expressed fluorescent proteins [23] to achieve image contrast for visualizing tumor cells, neurons, and blood vessels [24,25,26]. However, intrinsic fluorescence signals from endogenous biomolecules [27], such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), can also be leveraged for label-free tumor imaging. TPEF microscopy has demonstrated significant potential in various biomedical applications, including neurobiology [28], tissue engineering [29], and in vivo optical biopsies [30], highlighting its versatility in tumor diagnostics.

In this study, we explore the potential of TPEF microscopy as a complementary imaging tool for gliosarcoma diagnosis, alongside conventional modalities such as CT, MRI, and histopathology. By leveraging TPEF's high-resolution and label-free imaging capabilities, we aim to enhance the visualization of gliosarcoma-specific features, ultimately contributing to improved tumor identification and diagnostic precision.

MATERIALS AND METHODS

These cases were retrieved from the files of our department. In the past 9 years (2016-2024), the department's archive contained and diagnosed 10 cases of gliosarcoma. From these cases, we obtained MRI and CT images for illustration, which we compared with images acquired from tissue sections embedded in paraffin.

The tissue was fixed in a 10% buffered formalin and embedded in paraffin, with 4-5 microns-thick paraffin sections. On hematoxylin-eosin (HE) stained sections, the glial component showed malignant astrocytes, with associated microvascular proliferation and geographic necrosis, while the sarcomatous components showed pleomorphic spindle cells. Features identified on CT and MRI scans were partial uptake of contrast agent with a ring-enhancement pattern, peripheral edema, and central calcification. The tumor displayed a specific pattern in TPEF imaging. Selected regions of interest were examined using a Leica TCS SP laser scanning confocal microscope optimized for nonlinear imaging. The excitation source was a Ti: Sapphire laser (Coherent Chameleon Ultra II) tuned to 860 nm, with a pulse width of 140 fs and a repetition rate of 80 MHz. During scanning, the laser power at the objective focus was kept below 10 mW. A 10× objective (NA 0.3) was used to focus the excitation beam onto the samples and collect the TPEF signal in epi-detection. The resulting field of view was 1 × 1 mm^2. Spectrally resolved detection, inherent to the Leica TCS SP system, was used to capture TPEF signals in the 450–700 nm range. Also, the distinction between tumor and peritumoral brain zone was easy in TPEF analysis, allowing a clear visualization of the brain/tumor interface. TPEF imaging is a fast and efficient method allowing analysis of brain tumor tissue.

RESULTS

MRI and CT analysis

The images were obtained in our hospital, and the MRI characteristics were evaluated. Gliosarcomas can be very similar to glioblastomas in appearance. These are usually widely localized peripheral lesions with possible direct dural invasion or only reactive dural thickening (dural tail). On MRI, gliosarcomas typically present as heterogeneously enhancing lesions with irregular margins, often associated with significant peritumoral edema and central necrosis. They may also demonstrate areas of hemorrhage and variable diffusion restriction. The tumor frequently involves the cerebral hemispheres, with a predilection for the temporal and frontal lobes.

Figure 1: MRI images demonstrating a gliosarcoma with typical localization in the left temporal lobe – A) Sagittal section in T1-weighted imaging; B) Axial section in T2-weighted imaging; C) Coronal section in Fluid Attenuated Inversion Recovery (FLAIR) sequence; D) Sagittal section in post-contrast T1-weighted imaging; E-F) Maximum Intensity Projection (MIP) reconstruction; G) Diffusion-weighted imaging in axial plane; H) Apparent Diffusion Coefficient (ADC) in axial plane; I) Axial section in Susceptibility Weighted Imaging (DWI) sequence

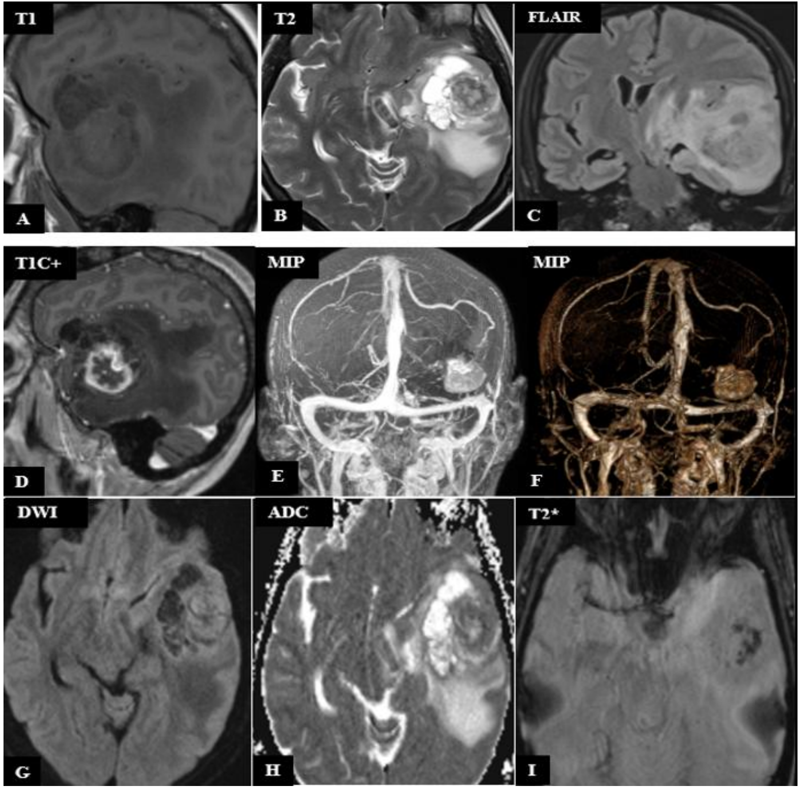

Figure 2: MRI image with postoperative appearance 4 days after surgery - No tumor debris - A) Sagittal section in T1-weighted imaging; B) Axial section in T2-weighted imaging; C) Sagittal section in post-contrast T1-weighted imaging; D) Axial section in subtraction imaging between native T1 and post-contrast T1; E) Diffusion-weighted imaging in axial plane; F) Apparent Diffusion Coefficient (ADC) in axial plane

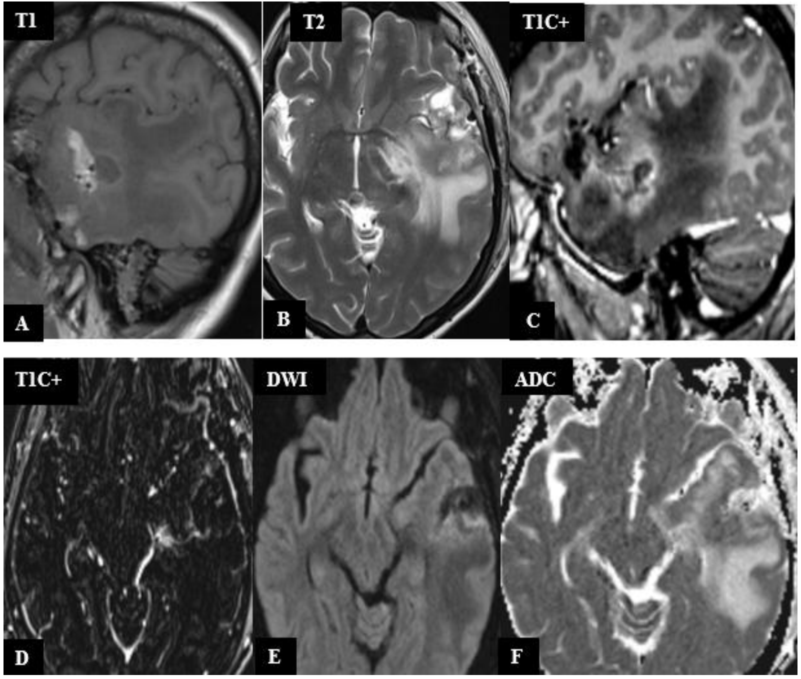

As can be observed in Figure 1, MRI confirms an intra-axial mass in the left temporal lobe. The lesion demonstrates a heterogeneous pattern of central hypersignal and marginal hyposignal on T1-weighted images (Figure 1A) and peripheral hypersignal with central hyposignal on T2-weighted images (Figure 1B), which is consistent with a solid-cystic mass. We can see the ring-enhancement pattern on T1-weighted Gadolinium images (Figure 1D) with central necrosis, as seen in DWI and ADC sequences (Figures 1G-H). A great amount of perilesional edema can be noticed on FLAIR images (Figure 1C). The high vascularity and intratumoral neo-vascularization can be observed on angio-MRI reconstructed images (Figures 1E-F). Small areas of intratumoral hemorrhage can be noted on T2*-weighted images (Figure 1).

In Figure 2, we have MRI images with postoperative appearance 4 days after surgery, with the presence of a marginal contrast-enhanced (Figure 2C, D), left temporal post-interventional porencephalic cavity (Figure 2A, B). No tumor debris present or diffusion restrictions in the surgical area were identified (Figure 2E, F).

MRI images of postoperative appearance 6 days after surgery showed postoperative left temporal changes with a slight decrease in the porencephalic cavity compared to the previous examination (Figure 3). Significant reduction of cerebral edema. On the MIP reconstructions performed from the T1 subtraction sequence with contrast, the disappearance of the tumor seen in Figure 1 is observed (Figure 3F).

Figure 3: MRI images of postoperative appearance 6 days after surgery - No tumor debris present - A) Sagittal section in T1-weighted imaging; B) Axial section in T2-weighted imaging; C) Coronal section in Fluid Attenuated Inversion Recovery (FLAIR) sequence; D) Axial section in post-contrast T1-weighted imaging; E) Coronal section in post-contrast T1-weighted imaging; F) Maximum Intensity Projection (MIP) reconstruction; G) Diffusion-weighted imaging in axial plane; H) Apparent Diffusion Coefficient (ADC) in axial plane; I) Axial section in Susceptibility Weighted Imaging (SWI) sequence

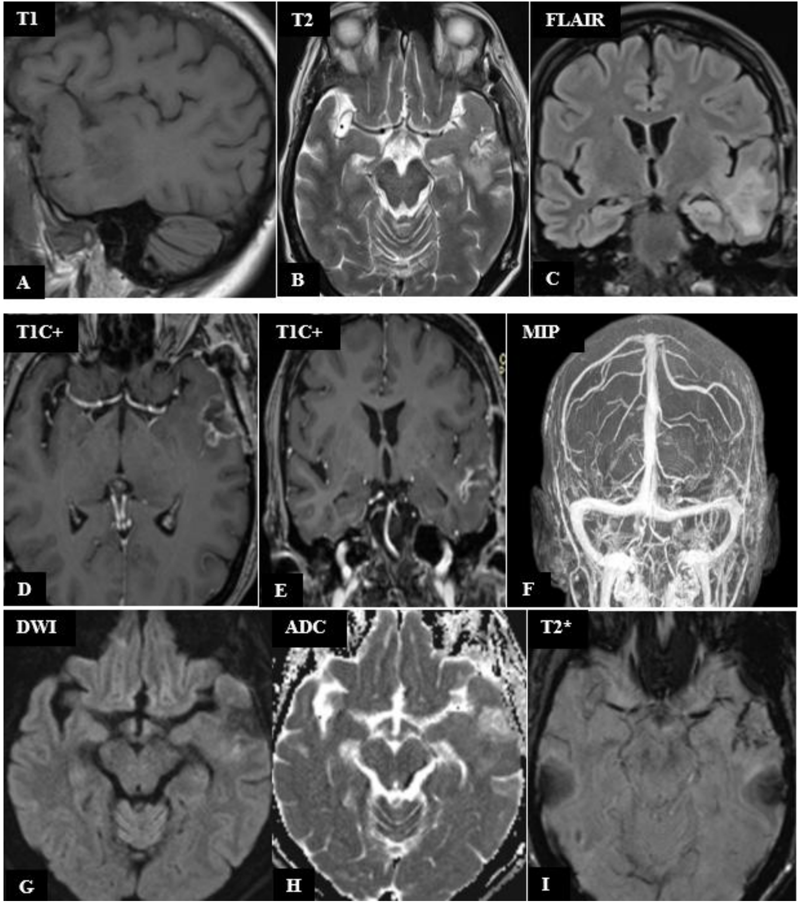

Unfortunately, after 4 months postoperatively, the patient presented again to the emergency department, and he had a head CT scan (Figure 4). The diagnosis was tumor recurrence, which causes mass effect (Figure 4A, C), peritumoral edema (Figure 4A, B, C), displacement of midline shift due to tumor and edema (Figure 4A, C) and herniation of the left contralateral cerebral parenchyma with a subfalcine herniation appearance (Figure 4C).

Gliosarcomas, like glioblastomas, exhibit a high recurrence rate, with most recurrences occurring within the first year following initial treatment. Recurrence often presents with rapid growth and can be more aggressive than the initial tumor, due to the tumor's inherent heterogeneity and resistance to therapy. The prognosis for patients with recurrent gliosarcoma is poor, with limited survival rates. The overall survival after recurrence is typically shorter than for primary tumors.

Figure 4: CT images were obtained 4 months postoperatively - tumor recurrence - A) Axial section (native); B) Sagittal section (native); C) Coronal section (native).

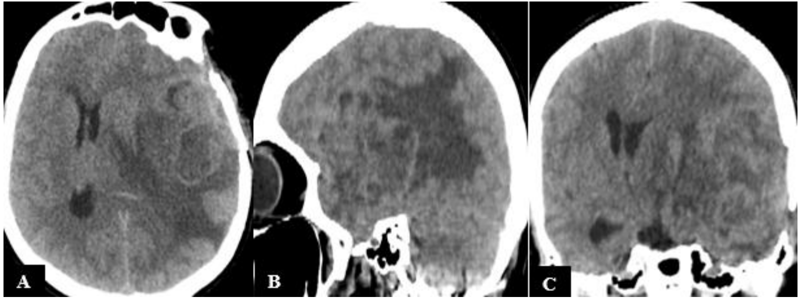

Light microscopy

Images acquired with bright-field microscopy on H&E-stained tissue sections highlight the aspects of a gliosarcoma, which exhibits a biphasic histological pattern, with alternating regions of glial and mesenchymal differentiation. The glial component resembles glioblastoma, characterized by atypical astrocytic cells with pleomorphic nuclei, increased mitotic activity, and areas of necrosis with pseudopalisading (Figure 5A; Figure 6B). The mesenchymal component consists of spindle-shaped cells arranged in fascicles, resembling fibrosarcoma (Figure 5B). Optical microscopy is essential for detecting vascular proliferation, a hallmark of gliosarcoma, including endothelial hyperplasia and abnormal vascular structures. This feature is significant in distinguishing gliosarcoma from other tumors (Figure 6A).

Figure 5: (A) Glial component showing large, pleomorphic, fibrillary astrocytic cells with atypical mitosis in the center (marked with an arrow). (B) Sarcomatous component showing spindle cells arranged in a pattern of densely packed, long bundles

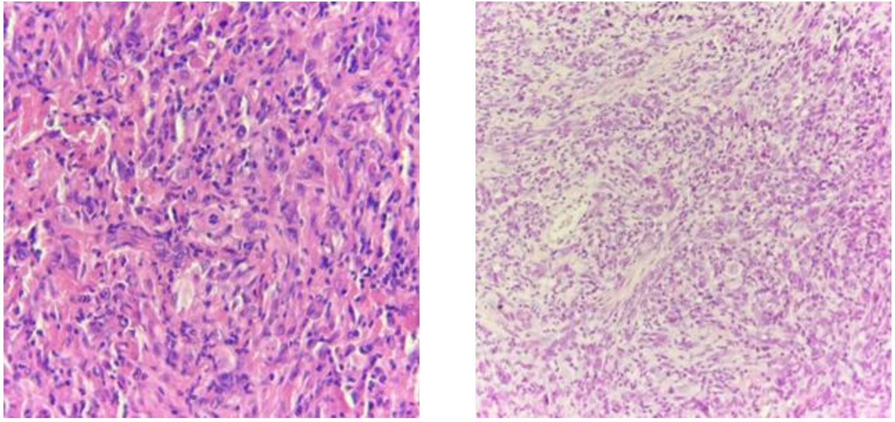

The mitotic index and cellular density observed through light microscopy are useful for grading the tumor. High mitotic activity correlates with a higher grade and a more aggressive tumor. Light microscopy can help assess the peritumoral edema and the extent of surrounding tissue invasion, giving insights into how the tumor interacts with the surrounding brain structures.

Figure 6: (A) Microvascular proliferation is represented by multilayered, small-caliber blood vessels with a glomeruloid appearance (several blood vessels marked with arrows). (B) Geographic necrosis (pseudopalisading neoplastic cells surrounding a central area of tumor necrosis), marked with an arrow

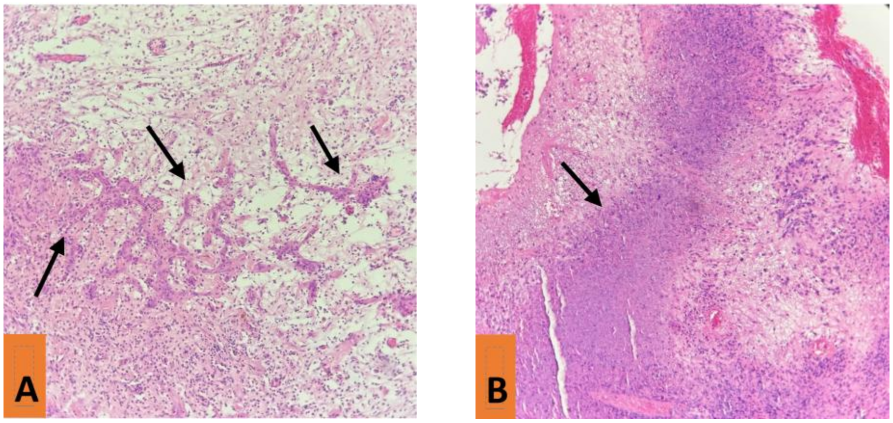

Recurrences often show similar histological features to the original tumor but may have increased cellularity, more prominent necrosis, or increased vascular proliferation.

Understanding the exact histopathological features through light microscopy assists clinicians in treatment planning, as the tumor's aggressiveness, heterogeneity, and vascular properties influence the choice of therapy (e.g., surgical resection, radiotherapy, chemotherapy).

Light microscopy compared to two-photon excited fluorescence (TPEF)

By integrating optical microscopy and TPEF, this study aims to enhance the understanding of gliosarcoma's complex microenvironment, exploring the advantages and limitations of each method in tumor characterization. Our findings provide insights into the potential of TPEF as a complementary diagnostic tool, bridging the gap between traditional histopathology and advanced optical imaging techniques.

Figure 7: (A) Light microscopy image of large, highly pleomorphic glial cells, with bizarre nuclei, mitotic activity in the center (arrow), and microvascular proliferation on the right (asterisk) with corresponding TPEF image – Figure 7 (B).

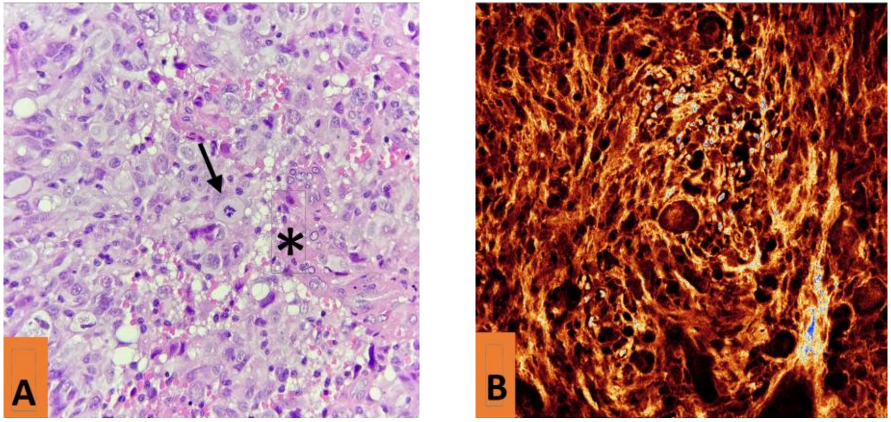

Figure 8: (A) Light microscopy image showing a biphasic pattern, represented by atypical, glial cells intermingled with spindle, sarcomatous cells. (B) Corresponding TPEF microscopy showing the corresponding image

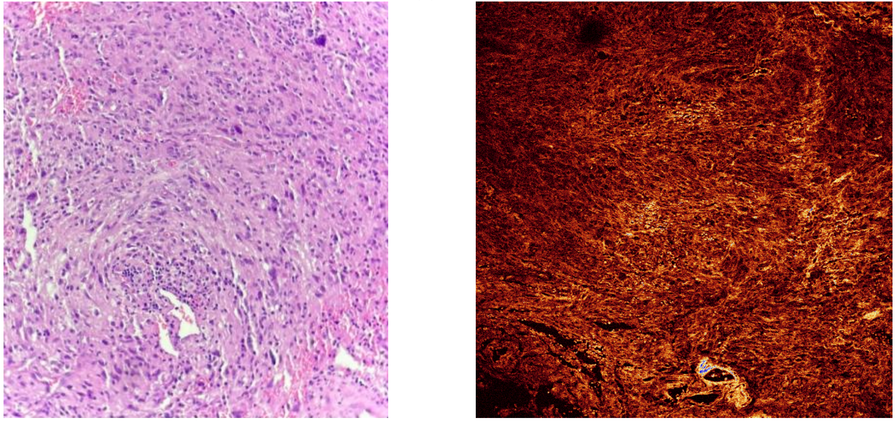

As can be observed in Figures 7 and 8, the pleomorphic nature of cells in gliosarcoma is one of the most striking features observed under light microscopy and TPEF. The glial component of the tumor typically consists of atypical astrocytic cells with varying sizes, shapes, and nuclear characteristics, which include prominent nucleoli, irregular nuclear contours, and increased mitotic figures (Figure 7A). This cellular pleomorphism reflects the aggressive nature of the tumor and contributes to its histopathological diagnosis as a high-grade glioma. In contrast, the mesenchymal component of gliosarcoma exhibits spindle-shaped cells with less defined borders, further adding to the pleomorphic architecture of the tumor (Figure 8A).

In Two-Photon Excitation Fluorescence (TPEF) imaging, the pleomorphic cellular structure is also identifiable, but through label-free imaging that highlights differences in tissue architecture based on endogenous fluorescence. While TPEF cannot provide the same cellular details as optical microscopy, it offers insights into the variations in cellular and extracellular matrix composition that correspond to the pleomorphic nature of gliosarcoma cells. The increased autofluorescence in areas with high cellularity and metabolic activity, as seen in the glial component (Figure 7B), contrasts with the more fibrous and structured mesenchymal regions, providing a complementary visualization of the tumor's heterogeneity (Figure 8B). TPEF's ability to image deep tissue also offers a broader view of how pleomorphic regions interact with surrounding structures, complementing the histopathological observations made under optical microscopy.

Figure 9: (A) Light microscopy image showing a vessel-rich, fibrosarcomatous area of the tumor, composed of spindle cells, with (B) the corresponding TPEF microscopy image

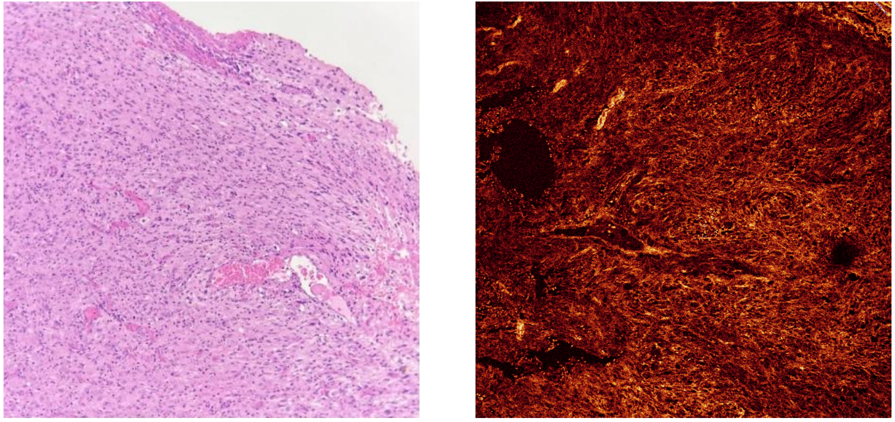

Under light microscopy, these vascular changes are readily observed, particularly the presence of abnormal, hyperplastic blood vessels that often exhibit endothelial cell proliferation and vascular lumen formation. These blood vessels may appear as tortuous and irregular in shape, contributing to the high degree of neovascularization characteristic of high-grade gliomas (Figure 9A and Figure 10A). In TPEF imaging, the vascularization in gliosarcoma is also detectable, albeit in a more indirect manner. While TPEF does not specifically stain blood vessels, the increased autofluorescence in regions of high vascularity can reveal the areas where blood vessels are more concentrated, especially in regions of new blood vessel formation and vascular leakage. TPEF imaging can also visualize the extracellular matrix surrounding the vessels, allowing for the assessment of the tumor's interaction with its vascular network (Figure 9B and Figure 10B). Furthermore, the deep-tissue penetration of TPEF offers a more comprehensive view of the tumor's vasculature, providing insights into both the microvascular architecture and the surrounding tissue environment that may not be fully captured with optical microscopy alone.

Figure 10: (A) Light microscopy image showing the gliomatous component, with foci of microvascular proliferation. (B) TPEF microscopy showing the corresponding image

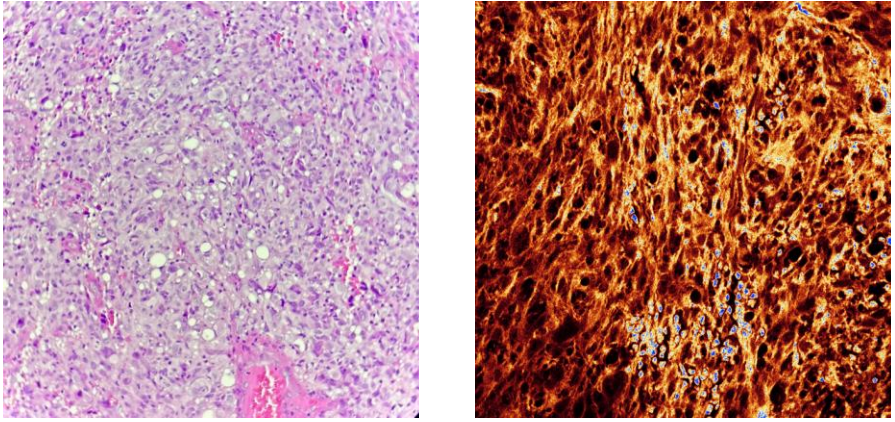

DISCUSSION

Early detection of brain tumors, including gliosarcoma, is crucial for improving treatment outcomes and patient survival. Delayed diagnosis can lead to uncontrolled tumor growth, reducing the effectiveness of therapeutic interventions. Conventional diagnostic approaches, including MRI, CT, and histopathological analysis using light microscopy, remain the gold standard for tumor identification and classification. However, these methods have inherent limitations, such as variability in manual interpretation and challenges in distinguishing tumor margins with high precision.

Light microscopy provided valuable histopathological insights into gliosarcoma, enabling the identification of distinct tumor cell characteristics and assessment of tissue architecture. However, conventional light microscopy is often complemented by immunohistochemical staining, which can introduce variability and require additional processing time. In contrast, TPEF microscopy offers a label-free, rapid, and high-resolution imaging approach for characterizing brain tumors. Our study demonstrates that TPEF microscopy effectively differentiates individual tumor cells and delineates their distribution in gliosarcoma samples.

Recent studies have explored the application of multiphoton imaging techniques in oncology. For example, TPEF microscopy has been successfully employed for brain tumor visualization, leveraging the endogenous fluorescence of metabolic coenzymes such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) to assess cellular metabolism [31,32]. Several studies have demonstrated the utility of metabolic imaging for distinguishing between normal and cancerous tissues [33,34]. These findings align with our results, where TPEF microscopy provided high-contrast visualization of gliosarcoma cells based on fluorescence.

Furthermore, recent advances in label-free optical imaging techniques, such as second-harmonic generation (SHG), have also been explored for brain tumor detection [35]. Compared to SHG, which primarily targets fibrillar collagen, TPEF provides direct cellular imaging, making it particularly useful for distinguishing tumor cells from surrounding neural tissue. Additionally, while confocal microscopy has been employed for intraoperative brain tumor imaging, it lacks the penetration depth and reduced photodamage advantages offered by TPEF microscopy [36].

This study introduces a novel application of TPEF microscopy for the analysis of gliosarcoma, showcasing several key advantages. Firstly, TPEF provides high-resolution with potential label-free imaging, eliminating the need for exogenous stains and thus reducing sample preparation time. The potential for intraoperative use is another strength, as real-time, high-contrast imaging of tumor margins could assist neurosurgeons in achieving maximal safe resection.

Despite these strengths, the study does have certain limitations. The analysis was performed on a limited number of gliosarcoma samples, and further validation on a larger dataset is necessary to establish robustness. Additionally, the ex vivo imaging of paraffin-embedded tissue sections, rather than fresh, in vivo samples, limits the immediate application of TPEF in clinical settings. Future studies should focus on exploring real-time, intraoperative applications of TPEF.

Looking ahead, several avenues for future research are proposed to expand the clinical applicability of TPEF microscopy. These include the integration with multimodal imaging, such as combining TPEF with SHG or Raman spectroscopy for more comprehensive tumor characterization. Further development of in vivo applications is also critical, including the creation of fiber-optic or handheld TPEF imaging systems for intraoperative brain tumor resection. Additionally, the incorporation of artificial intelligence-assisted analysis could help automate the classification of TPEF images, improving diagnostic accuracy. Finally, validation on diverse brain tumors, including glioblastoma and metastases, is essential to assess the broader diagnostic potential of TPEF microscopy.

CONCLUSION

Our study highlights the complementary roles of various imaging modalities in the comprehensive analysis of gliosarcomas. Light microscopy provides detailed insights into tissue morphology and cellular architecture, essential for initial tumor identification and classification. Two-photon excitation microscopy offers deeper tissue imaging with reduced photodamage, enabling visualization of tumor structures in their native environment. Radiological imaging techniques, such as MRI and CT scans, facilitate non-invasive assessment of tumor location, size, and progression within the brain. Integrating these methodologies enhances diagnostic accuracy and informs effective therapeutic strategies for gliosarcoma patients. When used as an additional technique with practical application in clinical settings, it may help to prevent under or overdiagnosis of certain lesions, whose accurate diagnosis is necessary for sufficient and effective treatment. Additionally, we will prevent the abuse of other currently available, somewhat expensive supplemental diagnostic methods (molecular biology techniques, genetic tests, IHC tests, electron microscopy, etc.).

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization A.E. and L.G.E.; methodology A.E., L.G.E and R.R.G..; software R.H.; validation L.G.E., R.H., O.M.S, M.S. and G.A.S.; formal analysis M.B. and O.C.V; investigation A.E.; resources L.G.E. and O.C.V.; data curation R.R.G.; writing—original draft preparation A.E.; writing—review and editing L.G.E, R.H., O.M.S. and G.A.S.; visualization R.R.G, M.B. and O.C.V.; supervision O.M.S., M.S. and G.A.S.; project administration A.E..; funding acquisition L.G.E, and R.H. All authors have read and agreed to the published version of the manuscript elaboration

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The approval of the Local Ethical Committee was obtained under the number 392/9G /2025. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols. Before the study, the committee reviewed and approved the methodology, including data collection and analysis procedures, to guarantee ethical standards were met. Additionally, all patient data used in the study were anonymized to maintain confidentiality and adhere to ethical principles.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW ARTICLE

Improved methodology of using simulators develops better practical skills in laparoscopy of future residents

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Abstract: *Background and aim: Minimally invasive surgery represents the actual tendency in many medical domains including urology. Gaining practical skills for these procedures becomes essential when preparing the future resident physicians in urology. The purpose of this study is to demonstrate that using medical simulation as an education tool improves the practical skills of the urology house officers in laparoscopy by using an accessible tool such as a box trainer.*

Methods: The study includes the objective and subjective evaluation of the practical skills of 54 students with no experience in laparoscopy. Each participant was evaluated before doing the practical activity on the simulator both in a subjective manner by filling a self-evaluation form and also objectively by timing the duration of doing laparoscopic basic tasks, knot tying and realizing a continuous suture. The participants were divided in two groups (A-28 participants, B-26 participants). Each group executed the same procedures with the same instruments, but with a different teaching technique.

Results and conclusions: Medical education with the help of a laparoscopic box trainer simulator is a useful tool for improving practical abilities and the time of execution of general procedures.

Keywords: *medical education, laparoscopy, box trainer simulator, students, methodology*

INTRODUCTION

On a continuous way to become an overall gold standard laparascopy and minimally invasive surgery in general shows better results in terms of patient recovery, hospitalization period, and fewer complications such as blood loss or side effects due to less required analgesia when compared to large open incisions [1]. Minimally invasive surgery also gives the advantage of smaller wound infection rates [2], fewer dehiscence rate and incisional hernia [1]. Key whole surgery requires more time, energy and financial resources. For example, the learning curve for laparoscopic radical prostatectomy was slower than the previously reported learning curves for open surgery [3]. All of these are due to less tactile real feel, loss of depth understanding, fulcrum effect and also due to manipulation: hand-eye coordination. Young urologists, especially residents, should be better prepared before performing their first interventions and this can be done in a safe, repeatable environment with the help of medical simulation.

Basic and essential skills such as camera manipulation, moving objects, manipulating intracorporeal materials (i.e. gauzes, tissue), knotting and suturing, tissue manipulation can be trained using medical simulation. In laparoscopy medical simulation can be trained with the help of basic pelvic trainers, virtual simulators which can also help improve procedural steps and live surgery on animal tissue such as porcine models.

The most reproducible, accessible and economic way of

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teaching laparoscopy to beginners is with pelvic trainers/box trainers. Unlike open surgery where one can encounter more learning possibilities, laparoscopic training for young surgeons is a challenge in their formation.

Training with the help of medical simulation has the purpose to transfer the acquired skills in the operating room [4]. In order to establish a minimum standard of training the European Society of Urology established a model called E-BLUS European Basic Laparoscopic Urologic Skills.

This model has a set of validated exercises which are applicable in urologic laparoscopic procedures. In addition to repeating the video showed skills one can improve the learning curves by identifying possible factors without taking into account the native ability of the surgeon or his previous surgical experience. [5] “And therefore I find that being a surgeon is a vocational profession, such as the athletes and airplane pilots” [6] thus meaning that the surgeon should also exercise before passing to real life scenarios, exactly how pilots train on advanced simulators in order to perfect their skills.

OBJECTIVE

To assess the better methodology of learning basic urologic laparoscopic skills by medical students using a box trainer.

METHODS

A study was realized with 54 medical students from all years of study from the University of Medicine and Pharmacy “Carol Davila” from Bucharest through from January 2017 to November 2018. The participants signed an informed consent and completed forms requiring basic demographic data such as age, year of study, gender, will to pursuit surgical careers and dominant hand.

The participants were divided in two groups. Group A had 28 students who were trained within 3 separate workshops (10, 9, 9) following one simple metho-dology; group B contained 26 participants who were divided in 3 separate workshops (10, 8, 8) and followed a more explicit and didactic technique.

All of the participants had to do the E-BLUS exercises. The first time they realized each exercise they were timed and after 15 minutes of practice they repeated the same measurement. The selected time was 15 minutes in order to have enough time, energy and concentration for the students to perform the tasks.

The instructors were represented by either urologists or general surgeons with laparoscopic experience. Pedagogical skills of the instructors were a criteria for inclusion in the study. All of the instructors who were part of the project had to train other senior students or residents with the aim of forming bench side instructors who would facilitate the communication between participants and main instructor and also give technical and logistic aid to the participants.

Tasks

The European training in Basic Laparoscopic Urological Skills consists of an online theoretical course which is followed by realizing 4 tasks in a specific amount of time. The tasks and their demo version can be found on the uroweb.org website. [7]

The tasks that were shown to GROUP A were similar to the ones from the E-BLUS. Peg transfer and circle cutting were identical. Needle guidance was observed in previous workshops to discourage students due to its difficulty and high energy consumption so we decided to take a thicker thread/suture without a needle so it would be easier to manipulate, but maintain the same rotation, piston like and hand eye coordination requirement. The laparoscopic suturing exercise was divided in two sub-tasks. The first one was to make a laparoscopic intracorporeal surgical knot, while the second one required for the volunteers to realize a surgical knot, make 3 sutures and then close with a second knot on a silicon model [8].

The methodology for group A was the following: instructor delivered two 15 minutes presentations on laparoscopy in general and laparoscopic instruments, after these all of the procedures were shown one by one by the main instructor at the demo pelvic trainer and immediately after the participants repeated each procedure with measuring the initial time and the after 15 minutes training timing.

Group B on the other hand repeated the same exercises, received the same live demonstration and video support. In addition to these, each of the exercise except circle cutting had a 3 minute training session with different exercises. Peg transfer exercise was preceded by lifting 6 pin board pins one by one, passing them from one instrument to the other and placing them in a plastic recipient. The thread passing exercise was preceded by a 3 minutes exercise which required passing of an articulated metal piece through a fixed orifice.

The breakthrough of this project besides improving the laparoscopic skills of all students was the significant improvement in time for the knot and suturing part of the workshop for Group B. The participants from Group B were made to repeat the intra-corporeal knot with the laparoscopic instruments under direct vision after the initial timing was measured.

This was done by disconnecting the monitor and changing the "abdominal wall" from the pelvic trainer with a transparent plastic sheet. In this way the movements were maintained restrained by the trocars, but the conversion from 2D to 3D natural view was present.

As a result the students at a cognitive level saw the procedure under 3D circumstances and when conversion to conventional 2D laparoscopic conditions was made the overall final improvement was significant as it will be shown in the results section.

RESULTS

The average year of study was 3.58, the male-female ratio was 1:1.4, and none of them had previous laparscopic surgery experience. All of the presented times are median scores over the same sector. All of the 28 scores of Peg Transfer Before section were summed and then divided by 28. The same principle was applied to all sections. The Time Difference column shows the performance in time before the Group B and Group A.

Figure 1: Comparison between groups

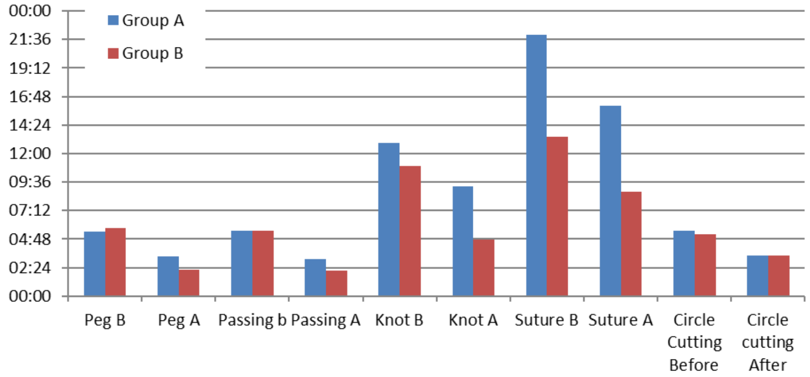

As one can see in Table 1 there is no significant difference in the before timing of the majority of exercises besides knotting and suturing which was enhanced in Group B probably due to the technique of showing the tasks under natural view and than reproduce the gestures and steps combining 3D cognitive perception with 2D laparoscopic vision.

Table 1: Overall timing in both groups A and B

Task		Group A	Group B	Time Diference	Significant[3]
1.	Peg Transfer Before	5:24	5:45	-0:21	No
	Peg Transfer After	3:22	2:11	1:11	Yes
2.	Thread Passing B[1]	5:31	5:32	-0:01	No
	Thread Passing A[2]	3:07	2:07	1:00	Yes
3.	Knot B	12:55	10:58	1:57	Yes
	Knot A	9:14	4:47	4:27	Yes
4	Suture B	21:59	13:26	8:33	Yes
	Suture A	16:03	8:49	7:14	Yes
5	Circle Cutting B	5:28	5:14	0:14	No
	Circle Cutting A	3:26	3:24	0:02	No

[1]B – before [2]A – after [3]Significant – more than 59 seconds difference

There was no significant difference in the Circle cutting group due to the fact that no extra exercise or technique was used in the Group B in order to enhance the performance.

In group B there was significant difference even in the "before" timing of knots and sutures probably due to the enhanced abilities given by the two extra exercises.

The overall extension of the Group B training time given by the three extra exercises of three minutes each was of nine minutes in practical extra activity and 21 minutes required to change the materials within the pelvic trainer.

Figure 2: Comparison – linear interpretation

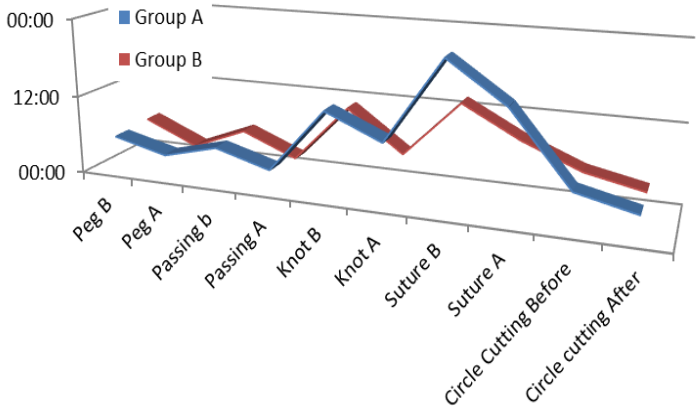

DISCUSSION

The timings could have been improved if the participants would have had more ergonomic instruments. All of the instruments had finger rings – Mayo Hegar laparoscopic needle holder.

Due to technical possibilities the table on which we placed the pelvic trainers had the same height which was inappropriate for short or tall students. Step stools were given to short students, but not offering them the best conditions. Tall students had the same problem.

The three speakers who took part in this project had two presences- one for group A and one for Group B. The sub-instructors were the same along the entire project.

CONCLUSION

It is well known that medical simulation in laparoscopy has its role in reducing the necessary time to acquire basic urologic skills in a safe and repeatable environment without the stress and pressure felt in operating room. This study demonstrates that even simple methodology can enhance the beginner's basic laparoscopic skills. In addition, due to experience, medical professional who teach these type of procedures can accelerate the process with tips and tricks which precede the proposed measuring tools. In this way the time from transferring the skills from laboratory to the operating room can be shortened and overall confidence of the surgeon can be enhanced.

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REVIEW

Psychotherapeutic Interventions for Combat Soldiers in Complex Clinical Settings

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Abstract: This narrative review examines psychotherapeutic interventions for combat soldiers who deal with post-traumatic distress and face a complex therapeutic setting where the relationships between clients and therapy providers are often conflictual. Despite available emotional aid, gaps between traumatized soldiers' emotional needs and institutional solutions are inevitable and are challenging for clients and therapists alike. These issues are discussed through Israeli soldiers' experiences, a population at high risk for trauma-related distress, due to this country's ongoing security threats. Following a narrative literature review, four key themes emerged: mutual difficulties in admitting mental injury, intergenerational trauma, preserving the soldier identity upon discharge, and choosing among therapeutic opportunities. The study highlights the need and rationale for diverse biopsychosocial interventions for veterans and proposes considerations for enhancing treatment success. Results emphasize the importance of tailored approaches that address both individual and systemic factors affecting veterans' mental health. Guidance for psychosocial interventions is provided, considering the multifaceted nature of combat-related trauma.

Keywords: combat, Israeli soldiers, military, psychotherapy, soldier identity, posttraumatic stress disorder, psychological trauma, veterans

INTRODUCTION

Military service and exposure to war zones affect millions worldwide, putting soldiers and veterans at high risk of physical, emotional, and psychological responses, from resilience to stress-related psychopathologies [1,2]. Posttraumatic stress disorder (PTSD) rates are higher among veterans than in the general population, estimated at 15% of combat veterans [3]. In Israel, military service is mandatory for Jewish and Druze men and Jewish women; many other citizens voluntarily enlist to promote social integration [4].

Active service begins at age 18 for 2–3 years, followed by reserve duty until age 40 [5]. Despite widespread exposure to potentially traumatic combat events, PTSD rates among Israeli Defense Forces (IDF) personnel remain unclear. Recent studies reported a trauma exposure rate of 96% among combat veterans [6], with PTSD estimates ranging from 2% to 16.5% [7–9]. However, the current ongoing conflict has likely increased these rates [10].

Official recognition of mental injuries in Israel, similar to that in other countries, is more complex than physical harm. Providing psychological aid to traumatized soldiers involves dilemmas about the nature, timing, and recipients of interventions, as well as the personal and social consequences [11,12].

The current narrative review [13,14] examines the psychological treatment of military personnel in conflict settings, a perspective that is rarely discussed [15]. It considers the diverse landscape of mental health opportunities offered for Israeli veterans, including official services, the private sector, and non-profit organizations. It aims to contribute to the international discourse on mental health care for military personnel in complex, conflict-prone environments by illuminating considerations for clinicians and policymakers dealing with emotionally injured soldiers or veterans.

This review explores two main research questions:

- What obstacles and dilemmas do professionals face when providing veterans with therapeutic solutions?
- What lessons can be learned from the Israeli experience to help clinicians worldwide better plan therapeutic interventions for military populations?

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MATERIALS AND METHODS

This paper presents a narrative review [13,14] of the literature on psychological interventions for Israeli combat veterans, supplemented by clinical case descriptions. This approach was chosen to provide a comprehensive overview of the topic while allowing for the integration of diverse perspectives and critique, and deepening the understanding of clinical insights through the inclusion of case materials.

A thorough literature search was conducted using PsycINFO and Medline (PubMed), supplemented with manual searches on Google Scholar. The search was restricted to peer-reviewed articles published in English and Hebrew between 1990 and May 2025. Additionally, clinical papers and book chapters that present the treatment of combat soldiers and veterans were also included. Non–peer–reviewed sources were intentionally sought to describe the study's socio-political context, and they included major local and international press. Papers and unofficial media or press publications were excluded if they were not initially published in Hebrew or English. No private or social media forums were included in the current narrative review. Search terms included psychological intervention-related keywords such as Psychotherapy, Group Therapy, Public health system, Private Sector; trauma-related keywords such as Trauma, Post-traumatic stress symptoms, PTSD, Suicidality; and military-related keywords such as Veterans, Soldiers, and Israeli Defense Forces.

The narrative synthesis of the literature focused on identifying key themes and issues relevant to the psychotherapeutic treatment of Israeli combat veterans, in light of global psychotherapeutic trends. These themes were organized into four major categories:

1. Admitting the Injury: Psychological Help for Mentally Injured Israeli Soldiers

2. Intergenerational Traumatic Consequences

3. Preservation of the "Soldier Identity"

4. Making Sense among Therapeutic Opportunities

To illustrate the clinical implications of these themes, case examples were drawn from the author's professional experience as a therapist and clinical supervisor. These case descriptions reflect patterns seen frequently in clinical practice and exemplify complex clinical situations. These composites offer a bridge between research findings and clinical practice, providing concrete examples of how theoretical concepts manifest in real-world therapeutic settings. All presented cases are composites of multiple real cases, with names and identifying details altered to protect client confidentiality.

The integration of literature review findings with clinical case descriptions allows for a rich, nuanced exploration of the psychological and psychosocial interventions that Israeli soldiers receive after developing traumatic distress during exposure to military-related trauma [14]. This approach aligns with the narrative review format, enabling a comprehensive discussion of complex clinical scenarios that may not be fully captured by more structured review methodologies. By combining a broad overview of the literature with detailed clinical examples, this study provides valuable insights for researchers and practitioners working with combat veterans, highlighting the unique challenges and opportunities in this field of psychotherapy. Since the majority of IDF combat soldiers are men, the examples presented describe treatment cases involving male soldiers only.

However, the ideas they illustrate are equally applicable to the experiences of female soldiers. Still, further research is needed to examine the unique challenges faced by female combat soldiers in coping with the emotional post-service impact on their lives, as well as how they utilize therapeutic services.

RESULTS

Admitting the Injury: Psychological Help for Mentally Injured Soldiers

Due to mandatory IDF service, Israel provides various treatment options for military-related distress [16]. IDF mental health services involve about 260 professionals, mostly psychotherapists and psychiatrists [17]. According to the press, these numbers have tripled since October 2023 due to increased demand [18]. Mental health professionals can be accessed across military units, and soldiers can seek help during or after service through their unit's psychologist or the Unit for the Treatment of Combat-Related PTSD. Professionals offer evidence-based psychotherapies in various settings. However, many veterans complain of insufficient or inadequate treatment, according to media reports [19].

The case of Sergeant Y.S., a 26-year-old Israeli veteran who self-immolated in 2021 to protest inadequate mental health support, highlights the challenges that IDF veterans face in seeking help. Unofficial reports indicate that this incident led to increased public awareness and the implementation of the "Nefesh Achat" (One Soul) reform to improve care for traumatized veterans [20]. However, the media keeps raising the voice of many veterans who still report insufficient mental health care [19,21]. Such reports highlight the risk of suicidality and suicide attempts, which have increased among Israeli soldiers in recent years [22], raising questions about the reform's effectiveness. According to these sources, many soldiers and veterans avoid seeking help due to stigma, fear of self-exposure, bureaucracy, or underestimation of their condition [23,24].

These barriers persist despite efforts to improve mental health services.

Intergenerational Traumatic Consequences

In Israel, most soldiers are second- or third-generation veterans, creating a unique context in which combat experience is viewed as a "civilian duty." The country's small size means combat often occurs near where soldiers live. Negative world views among parents can increase trauma-related stress in children through behavioral imitation and cognitive interpretation [25]. Indeed, mothers can play a crucial role in transmitting or buffering intergenerational trauma. These conditions make the effects of military service tangible for other family members and can lead to intergenerational and bidirectional traumatic stress, frequent shared traumatic reality, and secondary stress reactions in families of veterans [26]. Hence, therapists should consider the broader context of trauma, including its connections to a veteran's personal and interpersonal world.

Exemplifying this condition is the case of Josh, 27, who sought therapy 6 years after he finished his mandatory service. Growing up in a village known for its military sacrifices, he served in the armored corps, like his father did 30 years before him. During his service, Josh experienced traumatic events that triggered anxiety, doubt, and PTSD symptoms. Despite this, he described initially feeling undeserving of support and believing physical injuries were necessary to qualify for help. Only years later, when he experienced the horrific sights and fear of the October 7th, 2023, surprising attack by Hamas in the south of the country, he realized how his life followed a reliving of his father's traumatic experiences. As he joined his combat comrades on that day, he understood his emotional reaction was a transgenerational family burden, one he did not choose for himself. Encouraged by his girlfriend, Josh sought treatment for his military-related distress. Although emotionally clear to him, proving the connection between his current condition and past events was complicated, requiring careful navigation of bureaucracy and emotional sensitivities by both Josh and the professionals involved.

Preservation of the "Soldier Identity"

After service, soldiers face stress while readjusting to civilian life due to the stark contrast between military and civilian cultures [5]. Military service emphasizes power, aggression, obedience, and dichotomous thinking, whereas civilian life requires autonomous thinking, contextual understanding, and restraint. Transitioning between these worlds involves a challenging identity shift [27], affecting veterans in terms of personal, interpersonal, familial, and professional aspects of life. Due to their long-term reserve commitments, Israeli combat soldiers must maintain these conflicting identities for years and often never fully relinquish their military ways [28]. Combat experiences can lead to moral injury that exacerbates emotional burdens [29]. Facing longitudinal emotional stress and feeling trapped between ingrained military responses and unfamiliar, emotionally regulated approaches to problem-solving, veterans may resort to substance use [30].

David, aged 34, exemplifies these challenges. As a former infantry soldier, he struggled to adapt his combat instincts to civilian life. His aggressive responses, once valued in the military, became problematic in everyday situations. A turning point occurred when he reacted violently to his 5-year-old son's playful behavior that triggered memories of past military operations. In therapy, David realized how automatic responses to stress that he developed during service were now maladaptive. He grappled with guilt over his current actions as a father and past experiences as a soldier. This led to anger toward the Ministry of Defense, and David questioned the lack of support for veterans transitioning to civilian life and described feeling "used" and "disposable." This example illustrates the complex and long-term impact of military service on personal and family life.

Trauma exposure can interact with established traits among combat veterans, potentially leading to violent behavior [31]. Israeli therapists who are usually veterans may face challenges in helping other veterans process these complex situations, as they often struggle with their own identification or attempts to restore emotional balance in the face of military-related stress. This aspect often becomes a central transferential issue in treatment [32]. Helping veterans process such complicated situations and acknowledging their various emotional origins, as well as their current implications, is a challenging therapeutic task. Clinicians who deal with such conditions often find themselves either identifying with their patients or trying to help them reach a more balanced state, in which they can once again find order in their inner world. These reactions by clinicians are reflected in their hardships related to bearing witness to these violent acts [33]. Press and media reports indicate this situation is especially complicated for minority soldiers, who may face racism during service [33–35]. These individuals can experience both peer criticism and the stigma of seeking help [36]. Providing therapy in such contexts with multiple conflicts requires significant effort to build trust and emotional safety, along with a mature clinical attitude [37].

Choosing among Therapeutic Opportunities

Israeli veterans often struggle with the emotional toll of their service and rely on functional coping methods that can generate emotional avoidance and loneliness [5,38]. Like in other countries, many veterans hope time will heal their wounds or perceive their PTSD symptoms as minor, leading to underutilization of official support systems [40,41]. A recent study found 21% of Israeli combat veterans dealt with subthreshold PTSD, while 14% had clinical levels [39]. To address this increasing need, nongovernmental organizations have developed group intervention programs focusing on raising awareness, adjusting to civilian life, and enhancing emotional resilience [42,43]. These programs combine being in nature and outdoor activities, stress reduction techniques, and group processing of service-related events. They aim to enhance veterans' emotional coping, reduce guilt, and promote social recognition in the army service's psychological impact, helping veterans resolve unfinished business from their service in a supportive environment.

An example of these group interventions is the case of Daniel, 32, who grew up in an orthodox, traditional, low-income family. He described army service as a chance to achieve social mobility and volunteered for an elite commando unit, where he served as a sniper. After his service, he experienced emotional and physical stress, depression, and life dissatisfaction. He didn't connect these issues to his military service until participating in a group intervention program with his unit. There, he realized his depression stemmed from his role in killing others while in the military. The program helped him process a particularly conflictual incident during which he shot a young attacker. This shared experience with comrades helped him feel legit for his distress, relieve the big parts of it which were related to social demands and expectations, and seek further future therapy.

As described, army service often provides the first opportunity for minority youth to integrate into broader Israeli society [44]. However, minority soldiers often cope with both inner stress and social criticism when facing traumatic events and their consequences. Their race and socioeconomic background can impair resilience, increasing emotional vulnerability [37]. In such conditions, acknowledging combat-related emotions in therapy requires confronting difficult feelings, leading many young soldiers and veterans to avoid emotional processing and dwell in emotional numbing [45].

Sharon's case, a 23-year-old soldier who participated in a "friendly fire" incident where an Israeli soldier was killed, demonstrates this state of affairs. Right after this deadly incident, a group session with the unit's psychologist was provided, but all the squad's members remained silent throughout this whole session. In a later individual session, Sharon gave mostly rational answers to the therapist's attempts to reach his emotions, detaching from any emotional connection. Recognizing this as a defense mechanism, the therapist acknowledged the normalcy of stress and emotional detachment in such situations. He validated the ongoing challenges of combat service and encouraged Sharon to seek support, including after service completion. This message of partnership and hope proved crucial for Sharon's present coping and future development.

DISCUSSION

Balancing the Opposite Ends

Israeli society, like many others, often overlooks veterans' needs, focusing primarily on major psychopathologies while missing cases of long-term subthreshold PTSD [40,42,43]. Awareness has grown in recent years that combat soldiers need time to integrate into civilian life after service, with volunteer and "third-sector" organizations supporting this transition [5]. Providing therapy in this context is challenging due to unresolved questions about optimal timing and format [46]. Even though early and tailored interventions that leverage established social cohesion are considered beneficial [47], the effectiveness of psychological aid for young, cynical soldiers or veterans remains uncertain. The multicultural nature of Israeli society makes this tough clinical situation even more complex. Although army units are cultural melting pots, veterans often return to their original communities after service [44]. Balancing soldier and civilian identities is complicated, especially for people from traditional, patriarchal backgrounds in which admitting emotional hardships and seeking help through individual therapy can be intimidating [28,48]. Social workers must recognize both general and culture-specific aspects of these situations to support the integration of veterans into civilian life effectively [38,49]. This requires addressing controversies and the unique cultural needs of diverse populations when providing beneficial psychological aid. According to the press, these challenges have become even more difficult during the past 2 years, because Israeli reservists have been required to undertake numerous extended service rotations amid an atmosphere of extreme social division in Israeli society [49].

Resilience and social support are critical resources that can help combat veterans cope with PTSD and other stress-related symptoms. Although resilience levels among veterans are generally high, their impact often depends on individual coping skills [6]. Prior research and clinical experience have suggested that interventions should encourage authentic emotional expression, strengthen social relationships, and provide stress-management strategies, thereby reducing distress following military service without compromising resilience. Social support can play a particularly important role in mitigating symptoms of depression, like social withdrawal and emotional restriction [50]. Concurrently, identity integration remains a central challenge for many veterans during the transition from military to civilian life, particularly as they reconcile traumatic experiences and navigate societal expectations [27]. These processes differ based on cultural, social, and political contexts, highlighting the need for culturally competent practice. Group-based processing with peers can facilitate the integration of traumatic experiences into veterans' personal and social identities. Prior research [51] and clinical practice provide evidence of veterans who benefit from such interventions across a wide range of ages, depending on their emotional preparedness to utilize psychological help. Hence, practitioners must remain attentive to local cultural and familial attitudes toward military service and norms around emotional expression, which shape how veterans manage post-service adjustment and distress. These issues may be even more complex when dealing with female veterans' post-service emotional distress, which requires further study.

This study has limitations related to its narrative review methodology, which may have introduced author bias in the selection and interpretation of materials. Unlike systematic reviews, this approach does not reflect objectivity, reproducibility, or an exhaustive evidence search. However, it allows for holistic analysis, interpretation, and critique of the focal topic. This review synthesized current knowledge on treatment for Israeli veterans and highlighted knowledge gaps. It emphasizes the need for carefully considered solutions to treating traumatized veterans and encourages therapists to address their service-related emotional wounds, which may surface during therapy. Future research should explore the complex professional and relational aspects of this clinical area.

CONCLUSION

Both professionals and lay people who deal with PTSD symptoms often lack knowledge about the complexity of this condition and its effective treatments. Social workers and psychotherapists should provide traumatized veterans with comprehensive bio-psycho-social solutions. Professional coordination across sectors is crucial for optimal therapeutic outcomes, and public education is needed to help veterans recognize when and where to seek care. Although support for PTSD among veterans is a global need, recent developments in Israel and other countries demonstrate that progress in this field is achievable and should be prioritized by professionals and policymakers.

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The author declares no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

As the only author, the author is responsible for the design, data collection, reviewing, interpreting the results, and revision of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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REVIEW

Conventional and Modern Methods in the Diagnosis of Sepsis and Septic Shock: A Narrative Review

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Abstract: Background/Objectives: Sepsis and septic shock are critical conditions associated with high mortality rates and substantial impacts on healthcare systems. Accurate and rapid diagnosis is essential for the management of these conditions. The objective of this study is to assess the accuracy of contemporary and traditional methods for diagnosing sepsis and to determine whether improvements have been made concerning the integration of novel diagnostic approaches, to facilitate a prompt diagnosis, taking into account the rapid progression of complications associated with this disease. For this purpose, studies published between 2014 and 2024 were examined to highlight the benefits and limitations of each approach. Methods: A systematic literature review was conducted, including randomized clinical trials, observational studies, and retrospective studies assessing both conventional diagnostic methods (blood cultures and clinical scoring systems) and modern methods (rapid molecular tests, specific biomarkers, and machine learning algorithms). The studies included were selected based on strict design and methodology criteria to ensure a rigorous comparative evaluation of the interventions and technologies used in diagnosing and monitoring patients with sepsis. Results: A total of 23,822 patients were reviewed across the studies included in this systematic analysis. Modern methods, such as continuous monitoring through integrated biosensors and the use of molecular panels for pathogen detection, demonstrated high potential for the early and accurate diagnosis of sepsis. The reviewed studies suggest that these methods can significantly reduce diagnostic time and improve the ability to stratify mortality risk compared to conventional methods. Conclusions: Integrating modern diagnostic technologies, such as rapid pathogen identification tests and specific biomarkers, may complement traditional methods and bring significant benefits in the management of sepsis.

Keywords: sepsis; septic shock; diagnostic methods; biomarkers; rapid pathogen detection; machine learning; clinical outcomes; intensive care; advanced monitoring; mortality prediction

INTRODUCTION

Sepsis and septic shock are critical medical conditions that occur as a systemic inflammatory response to severe infections and have a devastating impact on global public health [1,2]. These conditions are considered medical emergencies, as they can quickly lead to multiple organ dysfunction and death, with mortality rates reaching up to 40% in severe cases [1,3].

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Sepsis is triggered by the presence of pathogenic microorganisms, such as bacteria, viruses, or fungi, which activate a complex and often excessive immune response [4]. The initial immune response to infection involves the activation of macrophages and other immune cells, which release cytokines and other inflammatory substances [5]. These proinflammatory molecules play a crucial role in defending the body; however, in sepsis, this response becomes disproportionate, damaging tissues and organs [6]. This excessive activation leads to vasodilation, increased vascular permeability, microvascular dysfunction, and, ultimately, multiple organ failure [7].

In essence, sepsis represents an overreaction of the immune system to an infection which, rather than protecting the body, causes extensive tissue and organ damage [8]. When this reaction escalates, resulting in a severe drop in blood pressure and inadequate perfusion of vital organs, septic shock occurs—an extreme form of sepsis that requires urgent and complex therapeutic interventions to prevent patient death [9].

The challenge of sepsis is compounded by the variability of symptoms, which can differ significantly from one patient to another, and the difficulty in quickly identifying the responsible infection [10]. This clinical heterogeneity makes timely diagnosis of sepsis challenging, contributing to its high mortality rate [11]. The fact that sepsis presents with symptoms common to other conditions—fever [12], tachycardia [13], rapid breathing [14], and low blood pressure [15]—can delay intervention.

In light of the aforementioned evidence, it can be surmised that the specific manifestations of sepsis fall within the differential diagnosis of other pathologies. In such cases, the therapeutic approach to sepsis (volemic repletion) may precipitate a worsening of the patient's condition if the underlying diagnosis is not correctly identified. This could potentially lead to delays in establishing an accurate diagnosis and initiating appropriate treatment. It is therefore imperative to identify more rapid and efficient diagnostic methods, to save lives and reduce the significant costs associated with the care of patients with sepsis and septic shock.

MATERIALS AND METHODS

Given the significant impact of sepsis and septic shock on public health and the limitations of current diagnostic methods, this study aims to identify the efficacy and clinical relevance of conventional diagnostic methods and modern approaches. This comparison will be conducted through a systematic review of the literature from 2014 to 2024, providing insights into the benefits, limitations, and potential of each clinical approach.

The study will synthesize evidence regarding the accuracy of diagnoses obtained through conventional methods, such as blood cultures and modern methods, including advanced molecular tests and specific biomarkers associated with sepsis. Additionally, it will examine the practical aspects of integrating these methods into clinical practice, with a focus on their potential to reduce diagnostic time and improve patient prognosis.

By highlighting how recent innovations can complement or even replace traditional methods, the study will provide a solid evidence base to support informed clinical decision-making. Ultimately, this review aims to contribute to the discussion on the broader integration of modern diagnostic methods within healthcare systems and to support the development of standardized practices that incorporate these advanced technologies in the management of sepsis and septic shock.

The methodology for this systematic review has been designed in accordance with the PRISMA guidelines to ensure transparency and rigor in the search, selection, and analysis process. This approach facilitates a detailed and comparative assessment of conventional and modern methods used in the diagnosis of sepsis and septic shock [16].

Article Identification Strategy

In our search strategy, we explored renowned international databases to obtain a representative and up-to-date selection of relevant scientific articles. The primary databases included MDPI and PubMed, as these provide access to a wide range of clinical studies, research articles, and other academic sources addressing the diagnosis of sepsis and septic shock.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were rigorously established to maintain the quality and relevance of selected articles. Only studies that include specific diagnostic methods for sepsis, whether conventional or modern, were included. Additionally, only articles published in peer-reviewed journals with complete data available were selected for analysis to ensure a robust data foundation and minimize publication bias.

Regarding exclusion criteria, review articles, opinion pieces, case reports, and studies lacking clinical validation were excluded. These types of publications do not provide direct comparative data or are not based on systematic evaluations of diagnostic methods, and their inclusion would have distorted the analysis results.

To ensure transparency and traceability in the selection process, we included a PRISMA flow diagram in the study, detailing the number of studies identified and filtered at each stage. The diagram specifically illustrates the initial number of articles obtained from the search, the number excluded after reviewing titles and abstracts, and finally, the number included in the detailed analysis. The reasons for excluding articles are also specified to clarify why certain studies were not included in the final analysis.

The PRISMA diagram is an essential tool for transparency, as it enables readers to follow the decision-making process behind article selection, ensuring that the criteria established at the beginning of the review are adhered to [16,17].

Keywords and Boolean operators were used in the search process to refine the results and select only articles relevant to the study objectives. The main search terms included phrases such as “sepsis diagnosis” (29,700 results), “septic shock” (21,800 results), “conventional methods sepsis and septic shock” (17,300 results), and “modern methods sepsis and septic shock” (17,200 results). These terms were combined using the AND, OR, and NOT operators to ensure a highly precise selection.

The timeframe set for the included articles was 2010–2024 to reflect recent developments in sepsis diagnosis and ensure the relevance and currency of the data analyzed.

Figure 1: PRISMA Flow Diagram of Articles Related to the Diagnosis of Sepsis and Septic Shock

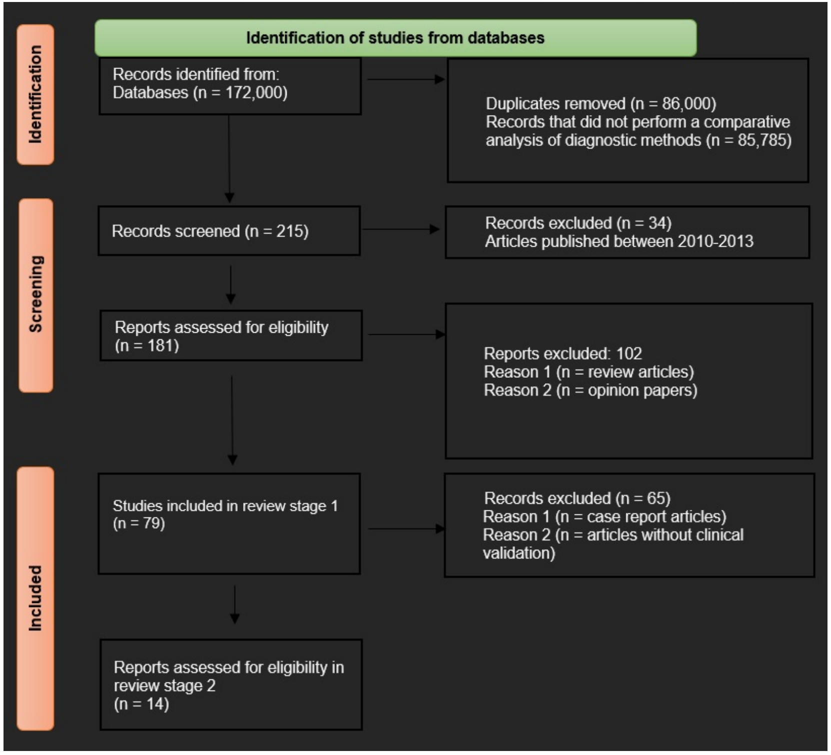
Identification of studies from databases
Identification
Records identified from: Databases (n = 172,000)
Duplicates removed (n = 86,000)
Records that did not perform a comparative analysis of diagnostic methods (n = 85,785)
Screening
Records screened (n = 215)
Records excluded (n = 34)
Articles published between 2010-2013
Reports assessed for eligibility (n = 181)
Reports excluded: 102
Reason 1 (n = review articles)
Reason 2 (n = opinion papers)
Included
Studies included in review stage 1 (n = 79)
Records excluded (n = 65)
Reason 1 (n = case report articles)
Reason 2 (n = articles without clinical validation)
Reports assessed for eligibility in review stage 2 (n = 14)

In Figure 1, we can see that the study identification process from the databases began with the selection of two main databases. In the course of this process, a total of 172,000 studies were identified. Of these, 86,000 records were eliminated as duplicates, as well as a further 85,785 records that did not perform a comparative analysis of diagnostic methods. Following these initial exclusions, 215 records remained for screening.

Of these, 34 were excluded after the selection process. These were articles published before 2014. We chose a period of 10 years rather than 14 because the literature on sepsis is constantly changing and the period 2010-2013 seemed outdated for today's requirements. A total of 181 reports were assessed for eligibility. At this stage, reports were excluded for several reasons, including review articles, and opinion pieces.

In the end, 79 were included in the phase 1 review, with studies without clinical validation and case reports being discarded. 14 studies were selected for inclusion in stage 2 of the review.

RESULTS

Two distinct groups of modern and conventional methods were generated. The conventional methods included: basic immunophenotyping, cardiac function monitoring and histopathology, manual assessment of CRT, serum lactate, MAP, vasopressor use, standard intravenous fluid administration, ICD-9 coding for sepsis, clinical scores (e.g., SOFA, APACHE II, SAPS II/III), standard biomarkers (CRP, PCT, lactate), and blood clutches and antibiogram. Modern methods included: PAR2 receptor inhibition with 1-PPA, proteomic analysis (2D-nanoUPLC-UDMSE), restrictive intravenous fluid administration, SOFA score and albumin combination, NLR, PLR, MLR, PLT/MPV ratio, AR stimulation (PediSepsisAR), remote photoplethysmography (rPPG) and aCRT, T2Bacteria® panel and T2MR®, continuous monitoring with biosensors (PO, NIRS, ST), advanced biomarkers (e. (e.g. IL-6, suPAR, irisin), ML (machine learning) algorithms.

In Table 1, we present a detailed analysis of 14 studies, each examining innovative interventions, biomarkers, and technologies used in the diagnosis and management of sepsis and septic shock. Data were extracted from recently published articles and structured according to study design, type of intervention, methodology, and key results.

Table 1: Comparison of Conventional and Modern Diagnostic Methods for Sepsis and Septic Shock

Author(s) and Year	Study Design	Sample	Intervention	Intervention Duration	Conventional Methods	Modern Methods	Key Results	Conclusions
Meyhoff et al., 2022 [18]	International, randomized, open-label study (CLASSIC trial)	1554 ICU patients with septic shock	Restriction of IV fluids vs. standard fluids	Until ICU discharge (max. 90 days)	Standard IV fluid administration according to guidelines	Restrictive IV fluid administration	Similar mortality between groups: 42.3% (restrictive) vs. 42.1% (standard). Similar incidence of adverse events: 29.4% (restrictive) vs. 30.8% (standard). Similar life support-free days and out-of-hospital days between groups	Fluid restriction did not improve survival rates compared to standard therapy. Fluid restriction did not reduce the incidence of severe adverse events. Restrictive intervention showed no significant benefits over standard intervention in the ICU.
Liberski, Szewczyk, & Krzych, 2020 [19]	Retrospective case-control study	138 ICU patients	Evaluation of NLR, PLR, MLR, PLT/MPV ratios vs. CRP, PCT, and lactate	On ICU admission	CRP, PCT, lactate	NLR, PLR, MLR, PLT/MPV	High NLR has predictive value for septic shock (AUROC = 0.66); combining NLR + CRP improves septic shock prediction (AUROC = 0.88)	NLR can identify patients with septic shock but must be interpreted alongside CRP/PCT; no index predicts ICU mortality
Toto et al., 2021 [20]	Randomized controlled feasibility study	50 paediatric participants	Simulation of septic shock with and without AR (PediSepsisAR)	Short-term simulation (per exercise)	Traditional mannequin simulation	AR simulation (PediSepsisAR)	Fluid administration time and volume were measurable in both groups; no significant differences in patient status recognition or fluid administration.	PediSepsisAR is feasible for simulation; improved perception of perfusion in most participants, but no impact on response times.

Wang et al., 2023 [21]	Retrospective bioinformatics study	98 sepsis patients (GSE26440) and 32 control; external test: 51 sepsis patients, 22 control	Diagnostic model using PCD-associated genes	Retrospective, pre-existing data	SOFA score and traditional inflammatory biomarkers	ML algorithms (RF, SVM, DNN) for gene-based diagnosis	ML models based on 10 genes (e.g., ITGAM, KIF1B) achieved AUC=0.7951 (internal) and AUC=0.9627 (external); analysis of immune cell infiltration in sepsis	Gene-based ML models show high diagnostic capacity for sepsis; potential to improve diagnosis and identify therapeutic targets
Lungu et al., 2024 [22]	Longitudinal observational study	121 neonates (39 late-onset sepsis, 35 early-onset sepsis, 47 control)	Multimodal monitoring (pulse oximetry - PO, near-infrared spectroscopy - NIRS, skin temperature - ST) with biosensor signal integration through ML algorithm	Max 72 hours of continuous monitoring	Standard biomarkers (CRP, PCT), cultures	Continuous monitoring with PO, NIRS, ST, ML algorithm	The model had 87.67% accuracy and detected sepsis 6-48 hours before clinical diagnosis; NIRS and ST had the highest predictive accuracy (0.90 and 0.85)	Non-invasive monitoring methods (NIRS, PO, ST) significantly improve early neonatal sepsis detection, complementing conventional tests and reducing the potential for clinical deterioration; require validation in varied clinical settings
Pipitò et al., 2024 [23]	Retrospective study	15,373 sepsis patients admitted to hospitals in Sicily between 2016-2020	Retrospective data collection from standard discharge forms for all sepsis cases in hospitals	5 years	ICD-9 for coding and identifying sepsis and septic shock	Temporal trend analysis through regression	Overall in-hospital mortality was 36.3%, higher in septic shock cases (66.7%) and ICU patients (74%). Rates increased over the analyzed years.	Sepsis and septic shock significantly contribute to in-hospital mortality; and require a focus on infection prevention, antimicrobial stewardship, and improved diagnostic coding.

Luka et al., 2024 [24]	Prospective observational study	67 sepsis and septic shock patients, ED, Cluj-Napoca, Romania	Evaluation of 6 biomarkers (IL-6, suPAR, AZU1, hsCRP, sTREM-1, PCT) combined with severity	28 days	Severity scores: SOFA, APACHE II, SAPS II/III, NEWS, CCI, GCS, qSOFA, SIRS, MEDS	Advanced biomarkers (IL-6, suPAR, AZU1) combined with clinical scores	Mortality was 49.25%; IL-6 was the most accurate biomarker, and SAPS II/III were the most predictive scores for mortality	IL-6 and SAPS II/III are the best predictors of 28-day mortality in ED sepsis cases; using biomarkers with clinical scores improves prognostic accuracy
Klibus et al., 2024 [25]	Prospective observational study	20 patients with bacterial septic shock or COVID-19-associated sepsis, ICU, Latvia	Evaluation of microcirculation via remote photoplethysmography (rPPG) and automated capillary refill time (aCRT) analysis in infusion	Various stages during the protocol	MAP, manual CRT, serum lactate levels, skin temperature	rPPG, aCRT	rPPG and aCRT detected perfusion changes more sensitively than manual CRT, highlighting significant differences between bacterial and COVID-19-associated sepsis	rPPG and aCRT are promising tools for microcirculation monitoring in intensive care, providing more precise assessments than conventional methods, useful for sepsis patient management
Bonura et al., 2024 [26]	Retrospective observational study	85 patients with septic shock or sepsis-induced hypotension, ICU	Evaluation of T2 Magnetic Resonance (T2MR®) technology for early ESKAPE pathogen detection using T2Bacteria®	First 6 hours after sepsis onset	Blood cultures and antibiogram	T2Bacteria® Panel for rapid bacterial identification	81% concordance rate between T2Bacteria® and blood cultures; T2Bacteria® detects pathogens in 5.15 hours vs. 94.62 hours for traditional cultures	T2Bacteria® Panel provides rapid and concordant ESKAPE pathogen detection, allowing faster decisions on antibiotic escalation or de-escalation in severe sepsis cases

Ayenew Mekuria et al., 2024 [27]	Retrospective cohort study	386 ICU septic shock patients	Septic shock management in ICU	2019-2023	Vasopressors (adrenaline, dopamine)	ICU surveillance, continuous monitoring	Mortality rate of 58.29%; predictive factors: age >60 years, delayed ICU admission, low MAP, comorbidities, unidentified pathogen	ICU mortality for septic shock remains high; need for early recognition and treatment protocols, in line with sepsis survival guidelines
Karampela et al., 2024 [28]	Prospective observational study	102 critical patients with sepsis or septic shock, 102 healthy control	No specific intervention, comparative analysis	28 days	CRP, procalcitonin as standard biomarkers	Serum irisin determination via ELISA	Patients with low circulating irisin had significantly higher 28-day mortality; irisin was negatively associated with sepsis severity and APACHE II and SOFA scores.	Circulating irisin decreases at sepsis onset and could be a promising biomarker for prognosis and diagnosis; needs confirmation in larger studies.
Alves et al., 2024 [29]	Observational proteomic study	5 septic shock patients, 10 healthy control	No specific intervention	N/A (not applicable)	Monocyte immunophenotyping	Proteomic analysis via 2D-nanoUPLC-UDMSE	Identified 67 proteins with altered expression in septic shock patients' monocytes, linked to immune dysfunction, hypotension, vasodilation, and endothelial damage	Identified proteins could serve as biomarkers for diagnosis, prognosis, and treatment of septic shock, offering new insights into this condition's pathophysiology.
Kim et al., 2024 [30]	Retrospective observational study	5805 septic shock patients from 12 EDs in South Korea	No specific intervention	N/A (not applicable)	Initial SOFA assessment	Combination of SOFA with serum albumin level	28-day mortality inversely proportional to albumin level; SOFA with albumin improved mortality prediction (AUC 0.71 vs. 0.68 SOFA alone)	Combining initial SOFA score with albumin level may enhance prognostic accuracy, providing a risk stratification tool in septic shock

Luisetto, Scarpa, Villano, Martini, et al., 2024 [31]	Experimental study, in vitro and in vivo	Mouse model of LPS-induced endotoxemia	Administration of 1-PPA molecule to inhibit PAR2 receptor	1 and 3 hours post-LPS injection (early and delayed administration)	Survival monitoring, cardiac function analysis, histopathological assessments	Real-time PCR, Western blot, cardiac ultrasound, flow cytometry	1-PPA administration reduced mortality and sepsis symptoms, improved cardiac function, and decreased inflammation via PAR2 inhibition	1-PPA demonstrates efficacy in reducing sepsis effects by blocking PAR2, preventing pro-inflammatory pathway activation, and reducing multiorgan dysfunction

*ICU: Intensive Care Unit; CRP: C-Reactive Protein, an inflammatory marker; PCT: Procalcitonin – Procalcitonin, a specific biomarker for bacterial infections; NLR: Neutrophil-to-Lymphocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; MLR: Monocyte-to-Lymphocyte Ratio; PLT/MPV: Platelet to Mean Platelet Volume Ratio; AR: Augmented Reality; PediSepsisAR: AR simulation for paediatric septic shock recognition; PCD: Programmed Cell Death; ML: Machine Learning; RF: Random Forest classification algorithm; SVM: Support Vector Machine classification algorithm; DNN: Deep Neural Network; AUC: Area Under the Curve; PO: Pulse Oximetry; NIRS: Near-Infrared Spectroscopy; ST: Skin Temperature; ICD-9: International Classification of Diseases, 9th Revision; AUROC: Area Under the Receiver Operating Characteristic; IL-6: Interleukin-6, an inflammatory biomarker; suPAR: Soluble Urokinase Plasminogen Activator Receptor; AZU1: Azurocidin 1 – Protein associated with inflammation and immune response; sTREM-1: Soluble Triggering Receptor Expressed on Myeloid Cells-1 – Triggering Receptor on Myeloid Cells-1; MAP: Mean Arterial Pressure – Mean Arterial Pressure; CRT: Capillary Refill Time; rPPG: Remote Photoplethysmography; aCRT: Automated Capillary Refill Time; T2MR®: T2 Magnetic Resonance; CCI: Charlson Comorbidity Index; GCS: Glasgow Coma Scale; NEWS: National Early Warning Score; qSOFA: Quick Sequential Organ Failure Assessment; SIRS: Systemic Inflammatory Response Syndrome; MEDS: Mortality in Emergency Department Sepsis; 2D-nanoUPLC-UDMSE: Two-dimensional Ultra Performance Liquid Chromatography-Ultimate Data-Independent Mass Spectrometry Enhancer – Advanced proteomic technology for monocyte analysis; ELISA: Enzyme-Linked Immunosorbent Assay – Enzyme-Linked Immunosorbent Assay, used for irisin determination; PAR2: Protease-Activated Receptor 2 – Protease-Activated Receptor 2, associated with inflammation.

The analyzed studies included ICU patients who were assessed and monitored for sepsis and septic shock using both conventional and modern methods. These studies comprised randomized, observational, retrospective, and prospective designs, with data covering clinical variables such as mortality, incidence of adverse events, length of hospital stay, and response to various interventions.

The interventions analyzed included techniques for intravenous fluid administration, the use of inflammatory biomarkers (NLR, PLR, MLR, CRP, PCT, lactate), machine learning algorithms for continuous monitoring (e.g., photoplethysmography, capillary refill time analysis), and gene expression-based models for diagnostics (e.g., using a panel of PCD genes in machine learning algorithms). Each study employed specific statistical analysis methods and regression models to evaluate the effect of these interventions on mortality and clinical outcomes of patients.

For biomarkers and continuous monitoring, predictive analysis methods were used, combining clinical scores (SOFA, APACHE II, SAPS II/III, qSOFA) with advanced biomarkers (IL-6, suPAR, sTREM-1). Additionally, rapid molecular technologies for pathogen identification, such as the T2Bacteria® panel using magnetic resonance, were utilized to enable quick identification of infectious agents.

The analysis also included proteomic diagnostic technologies that identified altered proteins in septic shock, used as potential biomarkers for diagnosis and prognosis. Experimental studies also involved animal models to assess the efficacy of interventions such as blocking inflammatory receptors (e.g., PAR2) with specific molecules aimed at reducing sepsis effects by preventing organ dysfunction.

In Table 1, we also observe that recent studies emphasize the importance of personalized approaches and advanced monitoring in sepsis and septic shock, incorporating both conventional clinical interventions and innovative methods. Although fluid restriction and standard biomarkers have shown limitations in predicting and managing sepsis effectively, emerging technologies like monitoring through machine learning algorithms, rapid diagnostics with molecular panels, and the use of specific biomarkers, such as irisin, offer promising perspectives for improving diagnostic and prognostic accuracy. However, further studies are recommended to validate these methods across various clinical settings and to implement them into intensive care protocols.

In terms of conventional methods, the most frequently used are clinical scores (e.g. SOFA, APACHE II, SAPS II/III) and standard biomarkers (CRP, PCT, lactate). These are each mentioned four times across studies, which serves to underscore their central role in traditional clinical practice.

Other conventional methods, such as blood cultures and antibiograms, appear twice, while standard intravenous fluid administration, ICD-9 coding for sepsis, vasopressor use, and cardiac function monitoring are each mentioned only once.

The high frequency of clinical scores (e.g., SOFA, APACHE II, SAPS II/III) and standard biomarkers (CRP, PCT, lactate) in traditional clinical practice is because these methods are well-validated, accessible, and easy to apply across various clinical settings. These tools are an integral part of international guidelines for the diagnosis and monitoring of sepsis and septic shock, with widely recognized utility in assessing patient severity and risk stratification [32,33].

Conversely, less frequently used conventional methods, such as blood cultures and antibiograms, standard intravenous fluid administration, ICD-9 coding for sepsis, vasopressor use, and cardiac function monitoring, are employed less often for various reasons. Blood cultures and antibiograms are useful for identifying pathogens but require a long time to yield results, making them less practical in emergencies. ICD-9 coding is important for epidemiological data collection but has a limited impact on immediate patient care.

Therefore, the more frequent use of clinical score assessment and standard biomarkers is due to their ability to provide rapid and reliable information about the patient's condition, making them suitable for initial assessment and real-time monitoring of patient progress.

On the other hand, among modern methods, the use of advanced biomarkers (e.g., IL-6, suPAR, irisin) has the highest frequency (3 mentions), followed by machine learning algorithms and continuous monitoring with biosensors (e.g., PO, NIRS, ST), each with two mentions. Other modern methods, such as molecular diagnostic panels (T2Bacteria® and T2MR®), remote photoplethysmography, AR simulations, and PAR2 receptor inhibition, were used less frequently, each mentioned only once, suggesting that they are either emerging technologies or applied only in specific contexts.

The high frequency of advanced biomarkers (e.g., IL-6, suPAR, irisin), machine learning algorithms, and continuous monitoring with biosensors (e.g., PO, NIRS, ST) as modern methods in the diagnosis and monitoring of sepsis and septic shock are due to their enhanced capacity for early detection and precise patient assessment. Advanced biomarkers are valued for their increased sensitivity in identifying inflammation and organ dysfunction associated with sepsis, and their inclusion in clinical practice is supported by growing evidence showing their predictive value.

Machine learning algorithms and continuous monitoring with biosensors are also highly attractive to researchers and clinicians, as they can analyze complex data and provide real-time alerts, enabling faster and more personalized interventions. In particular, machine learning algorithms can integrate information from multiple sources (biomarkers, vital signs) to improve risk stratification and allow continuous, proactive monitoring of patients.

On the other hand, modern methods with lower usage frequency, such as molecular diagnostic panels (T2Bacteria® and T2MR®), remote photoplethysmography, AR simulations, and PAR2 receptor inhibition, are relatively new and are typically used only in specific contexts or experimental studies. These technologies are still in the evaluation phase for widespread implementation and require additional studies to validate their effectiveness and cost-efficiency across various clinical settings. Additionally, the higher costs and infrastructure requirements for some of these emerging technologies limit their broad use in everyday clinical practice.

Thus, there is a continued preference for conventional methods in clinical practice, alongside a gradual trend towards the integration of more advanced modern methods, which have the potential to provide faster and more accurate detection of sepsis and septic shock.

DISCUSSIONS

A total of 23,822 patients were reviewed across the studies included in this systematic analysis. These patients come from various observational, randomized, and retrospective studies, each with different designs and intervention methods aimed at evaluating and monitoring sepsis and septic shock.

The results of this systematic analysis demonstrate that the treatment of sepsis and septic shock can be enhanced by combining conventional interventions with modern methods, including advanced biomarkers, digital monitoring, and artificial intelligence (AI) techniques. The studies reviewed cover a wide range of therapeutic approaches, from fluid therapy strategies and inflammatory biomarkers to continuous monitoring technologies and predictive genetic analyses.

A central aspect of the analysis is the impact of conventional and modern interventions. For example, the study by Meyhoff et al. (2022) shows that intravenous fluid restriction does not offer significant advantages over standard therapy in terms of mortality or reduction of severe adverse events [18]. Although fluid restriction may reduce the risk of volume overload, the results of this study indicate that there are no significant differences between restrictive intervention and the conventional approach regarding the survival of patients with septic shock. This suggests that the optimization of fluid volume should be evaluated based on the individual characteristics of each patient.

Similarly, the study by Liberski et al. (2020) highlights the role of biomarkers such as NLR (neutrophil-to-lymphocyte ratio) in assessing the risk of septic shock [19]. NLR has shown predictive value for identifying patients prone to septic shock, but its combination with conventional biomarkers like CRP and PCT significantly increases predictive accuracy. This suggests that advanced biomarkers may contribute to the early identification of high-risk patients, facilitating faster interventions. However, since no single biomarker directly predicted mortality, their optimal use remains as a complement to standard clinical measures, for more effective risk stratification.

Another essential aspect is the inclusion of modern monitoring technologies, such as near-infrared spectroscopy (NIRS) and remote photoplethysmography (rPPG). The study by Lungu et al. (2024) indicates that continuous monitoring using NIRS, PO, and skin temperature can detect sepsis 6–48 hours before clinical diagnosis, providing an important advantage in preventing the rapid progression of the disease [22]. NIRS and PO demonstrated the highest predictive accuracy, highlighting the potential of these non-invasive methods for continuous monitoring and early detection of neonatal sepsis. Microcirculation monitoring via photoplethysmography (rPPG) showed greater sensitivity than conventional methods for assessing perfusion, which could have important implications for managing critically ill patients with septic shock, according to the study by Klibus et al. (2024) [25]. The use of these technologies offers a more sensitive way to monitor subtle changes in tissue perfusion, which could precede overt clinical deterioration.

AI-based methods also bring innovative contributions to the diagnosis and prognosis of sepsis. The study by Wang et al. (2023) highlights the capability of machine learning (ML) algorithms to identify sepsis-associated genes, such as ITGAM and KIF1B, with significant accuracy [21]. These models achieved high-performance scores (AUC), indicating that ML-based approaches can support diagnosis and risk stratification by identifying specific genetic profiles. Using AI to analyze genetic profiles paves the way for personalized treatments and improved identification of therapeutic targets.

Additionally, the administration of the molecule 1-PPA, investigated by Luisetto et al. (2024), demonstrated positive effects by reducing mortality and sepsis symptoms in animal models [31]. This molecule inhibits the PAR2 receptor, reducing systemic inflammation and protecting cardiac function, suggesting that interventions targeting specific molecular pathways could offer new therapeutic solutions for patients with septic shock. Although these results are preliminary and obtained from in vitro and in vivo animal studies, they show promising potential for developing targeted therapies capable of blocking inflammatory cascades at the molecular level.

Overall, the results of this analysis underscore the need for an integrated approach that combines conventional interventions, such as fluid administration and vasopressors, with modern diagnostic and monitoring methods. Continuous monitoring, advanced biomarkers, and AI algorithms have the potential to improve prognosis and reduce mortality in sepsis, although their widespread applicability depends on validation in extensive clinical studies. While some methods, such as fluid restriction, have shown limited results, others, like biomarkers or digital monitoring, have significant potential in risk stratification and early identification of patient deterioration.

Thus, the combination of conventional and modern methods allows for better adaptation of treatment to the individual specificities of each patient, while also offering opportunities to improve survival and quality of care in intensive care units.

CONCLUSION

This comparative study of conventional and modern methods for diagnosing and monitoring sepsis and septic shock highlights the importance of adopting advanced technologies in the care of critically ill patients. While traditional methods, such as blood cultures and standard biomarkers, remain fundamental, the results of the reviewed studies suggest that modern technologies – including rapid molecular tests, continuous monitoring through biosensors, and machine learning algorithms – offer greater accuracy in early diagnosis and superior risk stratification capabilities. These advanced tools enable faster and more informed interventions, thereby contributing to improved prognosis and reduced mortality in sepsis and septic shock.

The comparative analysis between conventional and modern methods of diagnosis and monitoring for sepsis and septic shock reveals several practical implications for clinical practice. Conventional methods, such as the use of standard biomarkers (CRP, PCT, lactate) and clinical scores (SOFA, APACHE II, SAPS II/III), remain essential due to their accessibility and the familiarity healthcare professionals have with them. These methods provide a solid foundation for the initial assessment of patients and risk stratification in intensive clinical settings.

However, integrating modern methods, such as machine learning algorithms, continuous monitoring with biosensors, and the use of molecular diagnostic panels (e.g., T2Bacteria®), has the potential to significantly transform clinical approaches. These advanced technologies can improve diagnostic accuracy, enable early detection of sepsis, and facilitate faster and more informed decision-making. For instance, rapid molecular panels can identify pathogens within hours, thereby reducing the time needed to initiate appropriate antibiotic therapy, which is crucial for improving patient prognosis.

Implementing these modern methods in hospitals requires initial investments in infrastructure and medical staff training, but the potential benefits are substantial, including reduced mortality, shortened hospital stays, and optimized resource utilization in intensive care units. The use of advanced biomarkers and machine learning algorithms could also facilitate continuous monitoring of patients with sepsis and septic shock, providing real-time alerts for patient deterioration and supporting early intervention.

In the long term, these innovations may contribute to the standardization of more effective clinical protocols and the personalization of treatments for sepsis patients, tailoring interventions to the specifics of each patient. Furthermore, the gradual integration of these modern technologies could help raise the quality of medical care and improve the overall efficiency of the healthcare system.

To fully integrate these technologies into clinical practice, further clinical studies are needed to validate their effectiveness across a variety of clinical settings. It is essential to develop standardized protocols that include rapid molecular tests and machine learning algorithms for early detection and personalized monitoring of patients. Additionally, future research should explore the potential of new biomarkers and non-invasive monitoring technologies to support rapid clinical decision-making and facilitate integrated sepsis management in intensive care units.

This systematic analysis included studies with varied designs (randomized, observational, and retrospective studies), which may impact the direct comparability of results. Differences in study design, patient populations, and clinical settings (pediatrics, adults, ICU) make generalizing conclusions challenging, as each type of study may have its methodological limitations and biases. The present study encompasses a diverse range of patient populations, including neonates, children, adults, and the elderly. It is therefore evident that the newborn with sepsis has almost no defense mechanisms, whereas the middle-aged population demonstrates the most effective response to sepsis through early secretion of various mediators, a robust response to systemic inflammation, and a high capacity for regeneration. The elderly are included in the category of individuals who are considered to be frail, and as a result, may demonstrate a reduction in their ability to respond effectively to an encounter with a pathogen. Consequently, sepsis can be identified even in its most advanced stages in these patients. Furthermore, the capacity for regeneration is diminished in this population, resulting in more pronounced manifestations and consequences of sepsis and septic shock. Furthermore, patients with at least one additional health condition (such as autoimmune, cardiovascular, respiratory, or renal disease) are at significantly elevated risk of developing more severe forms of sepsis, including septic shock. Consequently, developing a diagnostic methodology for sepsis in the general population is challenging, necessitating the use of specific diagnostic techniques and combinations of traditional and modern methods adapted to the individual's age, associated diseases, and other factors.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, G.P.G. and A.N.D.; methodology, R.M.S and L.P; software, A.B. and I.S.C.; validation, V.T.G and D.R.T.; formal analysis, D.C, D.O.C. and E.F.L.; investigation, G.P.G, A.N.D and A.B.; data curation, V.T.G. and E.F.L ; writing—original draft preparation, A.B and R.M.S.; writing—review and editing G.P.G, D.O.C. and D.C.; visualization, L.P. and I.S.C.; supervision, G.P.G and D.R.T.; project administration, G.P.G and D.C. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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REVIEW ARTICLE

Current review of surgical management options for rotational alignment of the femoral and tibial component in total knee replacement

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Abstract: *Rotational malalignment complication following TKA, is common but can be avoided with proper surgical technique. This paper reviews the literature regarding rotational alignment during TKA, femoral and tibial rotation, and highlights the techniques prior in obtaining proper rotational positioning, nevertheless correct positioning in all three planes is important.*

Proper femoral component positioning in the axial plane is done using as landmarks the posterior condylar line (PCL), surgical transepicondylar axis (sTEA), anatomical transepicondylar axis and the trochlear anteroposterior (AP) axis. The paper describes the angular relationships between these landmarks and the distal femur. Axial tibial positioning is done when using intraarticular landmarks, the combination of more than one landmark could be a solution for solving this problem.

The consensus is that femoral component should be positioned according to TEA but the interobserver variability of this land mark is very high. The rotation of the tibial component remains an open subject, most studies suggesting a point between half of the distance of patellar ligament and 1/3 of the internal tuberosity as optimal landmark.

Keywords: *total knee arthroplasty; femoral component rotation; tibial component rotation*

INTRODUCTION

Total knee arthroplasty (TKA) is an intervention whose efficacy in the treatment of gonarthrosis is well known and documented [1]. The results of this intervention depend on the correct positioning of the prosthetic components in all three planes: frontal, sagittal, and axial [2]. The axial plane within TKA is represented by the alignment of the prosthetic components in the rotational plane. In 1979, Mochizuki and Schurman, who have shown that a lateral force produces the sprain of the patella when the tibial component is positioned according to the posterior plateau, described the importance of rotational positioning. They recommended that the tibial component should be aligned with the tibial tuberosity and the femoral component in a relative external rotation to the tibia at the time of a complete extension [3].

Femoral component positioning errors in the axial plane lead to complications in the femuropatellar joint, ligament instability and changes in normal kinematics [4]. The positioning of the tibial component from a rotational point of view has not been as studied, but its importance is equally large. The malposition of the tibial component is indirectly responsible for the femuropatellar complications that are often the cause of postoperative knee pain, reduced mobility and early revision [5]. Although current instrumentation methods have greatly reduced the malposition rate of

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components, the subject of rotation is not a closed one, and there is no consensus for the perfect positioning.

In this paper, we will try to present a review of recent literature on the correct rotational positioning of the femoral and tibial component, to describe the different axes guiding the rotational alignment, and to compare the known techniques.

ROTATION OF FEMORAL COMPONENT

The correct positioning of the femoral component from a rotational point of view is an important step for the success of TKA. The positioning of the femoral component in axial plane has repercussions on stability in flexion, knee kinematics, flexion alignment and femuropatellar joint [6, 7]. Berger demonstrated the relationship between the internal rotation of the femoral component and the patellar maltracking [8]. He was the first to use the CT to evaluate the rotation of prosthetic components. He showed that a low internal rotation, between 1 and 4o would lead to patellar maltracking and tilting, an average internal rotation, between 5 and 8o would lead to subluxation and a severe internal rotation of 7-17o, would lead to the sprain of the patella. For the analysis of both femoral and tibial rotation, Berger used the transepicondylar axis (TEA) and tibial tuberosity as anatomical landmark.

The external rotation of the femoral component also has negative repercussions on the TKA. Olcott et al. have shown that with the increase of external rotation of the femoral component, the medial flexion space increases, the result being a flexion instability symptomatology [9]. Another study led by Hanada et al. has shown that the excessive external rotation of the femur leads to varus flexion, the result being an excessive loading of the external compartment [10].

The consequences of external femoral rotation are well known; the best landmark for a good rotation is a topic yet debated. Many landmarks and axes have been described at the level of distal femur: posterior condylar line (PCL), transepicondylar axis (TEA), surgical transepicondylar axis (sTEA), anteroposterior axis (AP) or Whiteside line anterior femoral axis [11, 12].

“Tension gap” technique

The purpose of this technique is that after creating the gaps, the flexion space should be rectangular and equal to the extension space [13]. The soft parts of the knee are first balanced in complete extension by ligament rebalancing and subsequently at 90o flexion, either manually or with a laminar spreader. The aim is to obtain equal ligament tension both internally and externally and, after creating the posterior femoral cuts, the posterior femur should be parallel to the tibial surface after proximal tibial resection so that this space is rectangular. To support this theory, Laskin compared two groups of patients, in one group, the size of posterior femoral cuts was equal, and in the second group, the resection plane of posterior condyles was externally rotated to obtain a rectangular space. He noted that for this, it is necessary that the posterior medial femoral cut is larger than the lateral one, with a relative increase of this difference in the valgus knees due to lateral femoral condylar hypoplasia. The average external rotation for flexion space rectangulation was 3.20 to the posterior condylar line [14].

Correct valgus or varus extension positioning is a problem solved by current instrumentation systems but varus or valgus flexion alignment is often forgotten. This problem was investigated in a cadaver study, in which they compared the “tension gap” technique with the anteroposterior axis (Whiteside’s Line). Using the “tension gap” technique, they noted that they had good stability in extension and flexion, but the knees had varus deviations at flexion with an average of 8.2⍰, increasing pressure in the internal compartment. This phenomenon is largely due to the lateral collateral ligament, which in a normal knee is slightly laxer than the medial, this allowing the tibia to be pushed into varus (allowing the internal rotation of the femur against the tibia) when using the tensioning methods of equalizing the tensions between the two ligaments [15]. In the group in which the antero-posterior axis was used, the results were better in terms of stability and alignment.

Transepicondylar axis (TEA)

It is defined as a line crossing the two epicondyles, medial and lateral. This approximates the flexion-extension axis of the knee [16]. The positioning of the femoral component in the axial plane according to this line leads to an optimal patellar tracking, decreases the shearing forces at the beginning of flexion, and decreases the use of polyethylene insert. Internal or external rotations to this axis will result in changes in the patellar tracking [17]. The defect of this axis is that it cannot be located accurately intraoperatively. Berger et al. examined the knees of 75 cadavers and came to the following conclusions: surgical TEA is the axis between the lateral epicondyle and the medial sulcus, a channel below the medial epicondyle [18]. They concluded that the medial sulcus was an easily identifiable anatomical landmark and measured the difference between surgical TEA and the posterior condylar line as being 3.5° in males and 0.3° in females. After studying 32 cadavers, Yoshioka et al. concluded that there is a “condylar twist angle” of 5° in males and of 6° in females [19]. Although TEA is an axis difficult to highlight during surgery, especially in cases of significant destructive changes or in revisions, it could be the most accurate landmark for determining the rotation of the femoral component.

Posterior condylar line (PCL)

Using the posterior condylar line as a landmark for determining the femoral rotation is a well-known and generally accepted technique. PCL is on average 3-5° in internal rotation than TEA [20]. Most of the TKA instruments kits have additional pieces for external rotation attached to the instrument that palpates the posterior condyles, which try to position the two pins of the femoral cutting block parallel to TEA. In examination, PCL is identified by digitization and the desired external rotation is added to it.

Different studies highlighted that optimal patellar tracking was obtained with external rotation of the femoral component. Patients with the femoral component in external rotation do not need a rebalance of the extensor retinaculum and have better postoperative patellar tracking. This classic technique of positioning the femoral component in external rotation is easy to use but has the disadvantage that posterior condyles have to be perfectly palpated. This is not always possible due to the size differences of posterior condyles especially in cases of genu valgus with lateral femoral condylar hypoplasia in which there is a risk of internal rotation of the femoral component.

Trochlear anteroposterior (AP) axis

Also called the Whiteside line, the trochlear anteroposterior (AP) axis was originally described as a landmark in unicompartmental arthroplasty [21]. It was later used as a landmark for the positioning of the femoral component in axial plane in valgus deviation knees. It is defined as a line that joins the deepest point of femoral trochlea with the center of the deep lateral femoral notch. The perpendicular line on the AP axis is about 4° of external rotation compared to PCL. TEA has an average of 4.4° of external rotation compared with AP axis. The AP axis has the advantage of being easy to use and it can be identified during basic TKA.

TIBIAL COMPONENT ROTATION

The tibial component rotation is at least as important as the rotation of femoral component. The positioning of the tibial component in axial plane is responsible for optimal patellar tracking. An internal rotation will lead to a lateralization of tibial tuberosity and an increase of Q angle that will predispose to the subluxation of the patella [22]. The optimal tibial component position is not known exactly but we know that higher revision rates occur, and the clinical results are weaker at the time of malposition of the tibial component [23]. Many intra- and extra-articular landmarks are known. Intra-articular landmarks are tibial tuberosity, patellar ligament, and posterior tibial axis. There is also an option of alignment with the femoral component implanted according to TEA in extension [24]. Another option is to place the tibial component so that we have maximum tibial coverage [25]. The extra-articular landmarks are the transmalleolar axis of the ankle and the metatarsal II axis. The decision regarding what type of landmark to use for the axial alignment of the tibia is a difficult one and is influenced by other intraoperative factors. The external rotation of the plateau increases when an external parapatellar approach is used and an incomplete tibial plateau exposure resulting in internal rotation of the plateau [26].

Finding the optimal rotation of the tibial plateau requires a deep understanding of knee kinematics. In a normal knee, the tibia makes an internal rotation motion at the time the knee is in flexion and an external rotation motion at the end of the extension. This mechanism of external rotation at the end of the extension is called “screw home mechanism”, due to the geometry of the joint surfaces combined with the cruciate ligaments [27].

This physiological movement occurs between 0 and 15° of flexion. Often, this mechanism sometimes disappears, is low or paradoxically appears after TKA [28]. To reduce polyethylene use, we need to obtain a better axing of the two components in axial plane after TKA. The aim is to have a neutral rotation between the two components during the entire flexion, the rotational gap leading to femorotibial subluxation and early destruction of the polyethylene insertion.

Extra-articular landmarks

The most known extra-articular anatomic landmarks for rotation positioning of the tibial component are the transmalleolar axis of the ankle and the metatarsal II axis. Both axes have a significant variability in their knee orientation in different individuals [29]. In conclusion, these landmarks should not be the only ones used to determine the rotation. Extra-articular deformities secondary to trauma or arthrosis may result in alteration of these landmarks relative to the knee [30].

Intra-articular landmarks

Anterior tibial tuberosity is the most commonly used anatomical landmark for the proper determination of tibial plateau rotation. It is a landmark easy to identify and is not modified by gonarthrosis. This anatomical structure was split to increase the efficiency of its identification and its use. To determine the rotation, the internal edge of the tuberosity and 1/3 of the internal tuberosity are used [30].

In a study using the CT, the medial edge of the tuberosity was used in a lot of patients and in the second lot of patients, 1/3 of the internal tuberosity was used for the positioning of the tibial plateau in axial plane. The femoral component in all patients was positioned according to TEA. In the lot of patients in which 1/3 of the internal tuberosity was used as landmark, the femorotibial gap was lower, 67.5% of the patients between ±5°, 85% between ±10° and 97.5% between ±20°, compared to the lot of patients in which the medial edge of tuberosity was used, 3.8% of the patients between ±5°, 15% between ±10° and 68.8% between ±20°. In conclusion, the authors support the use of 1/3 of the internal tuberosity as landmark for the positioning of the tibial plateau [31].

Another study evaluated the relationship between TEA, the patellar ligament, and the posterior tibial axis in 30 healthy patients by MRI [32]. They found that the perpendicular line on TEA intersects the middle of the patellar ligament, the intersection being at about 41% of the width of the tendon measured from its internal edge. In order to have a smaller gap between the femur and the tibia, the anteroposterior axis of the tibial component must intersect the patellar ligament and the femoral component aligned with the TEA. This technique will finally result in a lack of coverage of the posteromedial tibia of about 5 mm with a variation between 2 and 10 mm. The patellar ligament is not modified by gonarthrosis, but its width is variable depending on the surgical approach. When the tibial plateau is positioned depending on the posterior cortex, it will enlarge the tibia coverage, but will also greatly raise the rate of the internal rotation of the tibial plateau. The authors of the study concluded that the optimal landmark is an immediate point located medial to the patellar ligament. The normal anteroposterior diameter of the tibial plateaus is different, and this will lead to the highlighting of the posteromedial bone at the time the tibial plateau is positioned correctly.

“Self-seeking” method

This method involves introducing trial components and performing full flexion and complete extension. The trial tibial plateau will be rotationally positioned according to the femoral component. The center of the trial tibial component is marked with the electrocautery and the final implantation is made using this point as landmark. This technique depends on the correct positioning of the femoral component, in femoral malrotation, the tibial component will also be affected. There is a risk that, when the femoral component is positioned in internal rotation and the tibial plateau is internally rotated, major problems of patellar tracking occur together with an increased instability. In order to overcome this problem and at the same time to use it, it is recommended to use a mobile plateau that will rebalance the tibiofemoral gap.

The mobile tibial plateau or “mobile-bearing” was built so that the polyethylene insertion follows the femoral component in rotation and the tibial component in flexion-extension. “Mobile-bearing” TKA reduces the need for tibial component placing in a perfect rotation and reduces the gap between the tibia and the femur. The disadvantage is that a second joint surface is created between the insertion and the tibial plateau and the destruction of polyethylene can occur at both interfaces.

CONCLUSIONS

TKA function and survival depend on an optimal rotation of the femoral component but also of the tibial component. The positioning of the femoral and tibial component is well known in the sagittal and frontal plane, but it is not very clear in the axial plane. There is a consensus that the femur should be positioned according to TEA but the methods this would be possible are not optimal. The combination of different techniques and the use of many landmarks could be a solution. The rotation of the tibial component remains an open subject, most studies suggesting a point between half of the distance of patellar ligament and 1/3 of the internal tuberosity as optimal landmark.

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ORIGINAL ARTICLES

3D echo in everyday life: Could it reset our threshold for interventions?

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Abstract: *Background Left ventricular ejection fraction (LVEF), the single most important metric in cardiology, is the cornerstone on which prognosis is estimated and costly decisions such as whether to implant an ICD are based. LVEF is most often assessed by 2D echocardiography (2DE), although 3D echocardiography (3DE) has been shown repeatedly to be more accurate.*

Aim of the study. We set out to assess whether using 3DE would reclassify the severity of LV impairment in patients with LVEF < 35% by 2DE.

Setting. Tertiary cardiac centre serving a population of one million, and performing approximately 100 ICD implants/year.

Methods. Successive patients in sinus rhythm, with good endocardial border definition and LVEF<35% by 2D Simpson's method, had scans according to the BSE protocol. 3D loops were acquired from the apical 4-chamber view and were analysed off-line using for regional wall motion abnormalities (RWMAs) and LVEF. The patients were classified in subgroups according to EF value: less than 20%, 20-25%, 25-30%, 35% and more than 35%. Moderate LV systolic was defined as LVEF between 35% and 45% and severe as LVEF ≤35%.

Results. We studied 100 patients (78 M, mean age (SD) 69.94 (13.54) years). 2DE had been requested for decision-making regarding ICD implantation in 86 patients (86%) and for LVEF measurement after acute coronary syndromes in 14 patients (14%). Regional wall motion abnormalities (RWMAs) were present in half of patients (55%, 51 pts – 56% in LAD territory, 31% in RCA/CX territory and 12% multiple territories).

3D LVEF regrouped 67% of patients: 10% to a lower EF and 57% to a higher EF subgroup. Twenty nine patients (29%) were reclassified from severe LV systolic dysfunction by to 2DE to moderate LV systolic dysfunction by 3DE. Patients with RWMAs were more often reclassified than patients without RWMAs (p=0.006). The LV dimensions were lower for the reclassified patients. The image quality had no effect on reclassification.

Conclusion Measuring LVEF by 3DE reclassifies the severity of LV systolic impairment in a substantial proportion of patients with 2D LVEF<35% and RWMAs, which may have important clinical and financial implications by resetting thresholds for costly interventions such as ICD implants.

Keywords: *biological attack, biological warfare, biological agents, international legislation, medical protection*

BACKGROUND

Left ventricular ejection fraction (LVEF), the single most important metric in cardiology, is the cornerstone on which prognosis is estimated and costly decisions (defibrillator implantation, resynchronisation therapy) [1] are based.

The clinical importance of echocardiography in ejection fraction measurement is undoubtedly, left ventricle systolic function evaluation being the most common reason for referring a patient for an echocardiogram [2]. In our department, from 117 patients scanned during one week, 60% were referred for LV systolic function evaluation. The main advantages of echocardiography comparing to more

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accurate methods (like MRI or CT (3)) are availability, inexpensive character, rapidity of performance, portability and radiation freedom.

Two-dimensional echocardiography (2DE) remains the most commonly used modality for EF estimation in clinical practice. The current guidelines recommend Simpson's discs method for LVEF calculation. However this method is subject to errors due to foreshortening and assumptions about LV shape (stacked disks with varying diameters).

Three-dimensional echocardiography (3DE) overcomes some of this limitation by capturing entire volumes, excluding foreshortening and geometrical assumption (very important in deformed ventricle). In addition, the endocardial position is measured at hundreds of points over the LV surface comparing to only two planes in conventional 2DE. Therefore, the calculation of LV volume is more accurate and reproducible.

OBJECTIVE

The primary aim of this study is to evaluate if LV EF assessment by 3DE in routine clinical practice can change the clinical decision making for patients with LV systolic dysfunction and if routine 3DE is useful and worthy versus largely used 2DE.

METHODS

We prospectively analyzed successive patients in sinus rhythm with good endocardial border referred to our echo department for left ventricle systolic function evaluation. The study was developed in a tertiary cardiac centre serving a population of one million, and performing approximately 100 ICD implants/year.

All patients had transthoracic 2D echocardiography obtained with harmonic imaging (Philips iE33, Philips Medical System, Holland). Left ventricle end-diastolic volume (LVEDV), left ventricle end-systolic volume (LVESV) and LVEF was measured on-line using Simpson's method of disks (using four and two chamber apical views). Patients with LVEF ≤35% were included in further analyse. The value of 35% was arbitrary selected because it is the cut-off limit for important and expensive treatments: ICD implant or cardiac resynchronisation therapy (1). LV systolic dysfunction was defined as severe for LVEF <35% and as moderate for LVEF 35-45%.

3D echocardiography was performed in the same time, using a matrix array ultrasonic transducer (X3-1 transducer, Philips iE33, Philips Medical System, Holland). An apical full volume image focused on LV, over four cardiac cycles was acquired. Measurement of 3DE LV volumes and ejection fraction was performed offline, using the 'mesh method' (Q- Lab 9, Philips Medical Systems) blinded to the 2D measurements. The semi-automated LV border detection was used for contour tracing, after first identifying the apex and the four mitral annular points (septal, lateral, anterior and inferior) (Figure 1A). A 3D endocardial shell was than produced and used for LV volumes and LVEF calculation (Figure 1B).

Figure 1. Full volume 3DE, 4 beats image of the left ventricle in a four chamber equivalent (apical probe position) and the corresponding end-diastolic four chamber (), two chamber () and short axis () view (A). The resulting end-diastolic volume (mesh), end-systolic volume (shell) and calculated 3DE after sequences analysis (B).

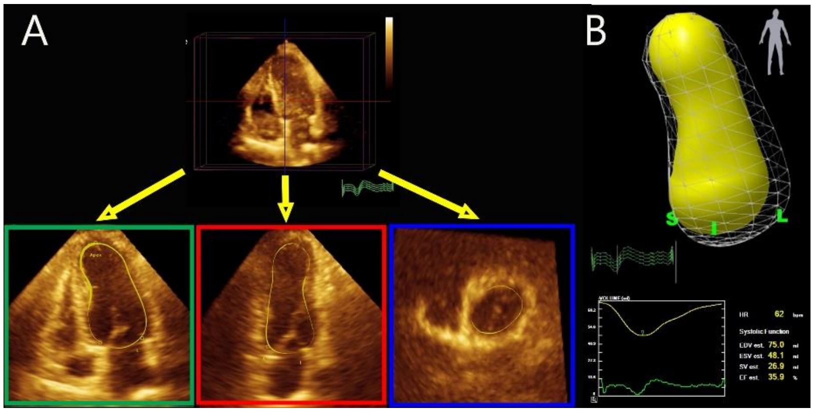

Statistical analysis: Agreement between 2DE and 3DE measurements was assessed by performing Bland-Altman analisys (MedCalc Software, version 12. 7.0, Acacialaan 22, B-8400 Ostend, Belgium). Means and standard deviations (SD) were calculated for continuous variables. Comparisons were performed using χ2 test for categorical data and Independent/Paired-Samples T test for continuous data with a two-tailed p value < 0.05 for statistical significance (IBM SPSS Statistics v.21, Armonk, NY).

RESULTS

During the enrollment period 106 patients were eligible. 6 patients were excluded due to inadequate quality of 3D acquisition (4 patients presented stitch artifacts and 2 patients had severely dilated LV, impossible to be included in 3D volume). The analyzed group was composed by 100 patients, 78 male, mean age (SD) 66.94 (13.54) years.

Transthoracic echocardiography had been requested for decision-making regarding ICD implantation in 86 patients (86%) and for LVEF measurement after acute coronary syndromes in 14 patients (14%). Regional wall motion abnormalities (RWMAs) were present in half of the cases: 51 patients (51%). The affected territory was corresponding to anterior descending artery in 29 patients (56%), to right coronary artery/circumflex artery in 16 patients (31%) ant to multiple vessels in 6 patients (12%). The QRS complex was narrow in 52% of patients and large in 48 patients (75% presented bundle branch block and 25% paced rhythm). Thirty two patients (32%) had left ventricle dilated (LVED volume >155ml).

Comparing 2DE and 3DE data

The mean value (SD) for LVEF calculated by 2DE (Simpson's method) was 27.36 (5.8%) and for LVEF calculated by 3DE was 30.53 (6.9%), p<0.001. The correlation between the two methods was good (Spearman's coefficient 0.79). On Bland-Altman analysis the bias was -3.2% and the agreement interval -4.1, -2.4 (Figure 2).

Figure 2. Bland-Altman plot representing the agreement between two methods for EF estimation: 2DE and 3DE.

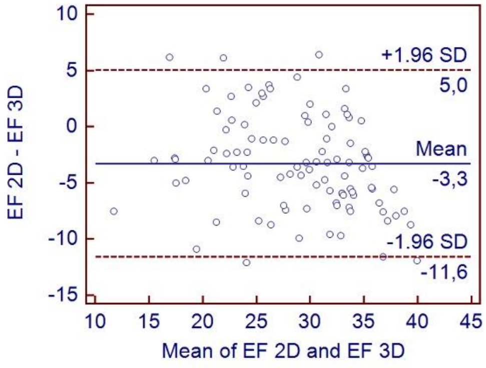

The mean value (SD) for LVEDV was 144.37 (69.2) ml by 2DE and 143.6 (67.2) ml by 3DE, p=0.8, Spearman's coefficient 0.86. For LVESV the mean value (SD) was 106.26 (59.53) ml by 2DE, 103.24 (55.02) ml by 3DE, p=0.051, Spearman's coefficient 0.85 (Figure 3).

Figure 3. Graphic representation of LVEDV and LVEDS distribution by 2DE vs 3DE.

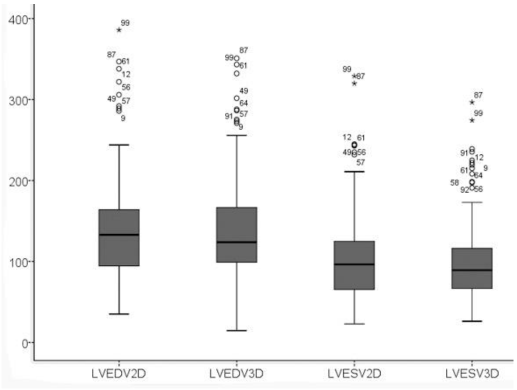

Reclassification by 3DE analysis

According to EF value, the patients were classified in four subgroups: <20%, ≥20% and <25%, ≥25% and <30%, ≥30% and ≤35%. Only for EF calculated by 3DE there was a new subgroup formed by patients with EF >35%. The 2DE and 3DE LVEF value for all patients is presented in Figure 4. The distribution of patients among subgroups comparing 2D to 3DE is illustrated in Figure 5. Considering the patients classification in these subgroups, 67% were reclassified by 3DE comparing to 2DE: 57% in a group with higher EF and 10% in a group with lower EF.

Twenty-nine patients (29%) were reclassified by 3DE from severe LV systolic dysfunction (EF ≤35%) to moderate LV systolic dysfunction (EF>35%). Eight patients reclassified from severe to moderate LV systolic dysfunction had 2DE EF 25-30% and 21 had EF 30-35% (Figure 4).

Figure 4. Graphic representation of 2DE EF (blue dots) vs 3DE EF (green dots) for all analyzed patients, grouped by 2DE in four subgroups (as previously mentioned).

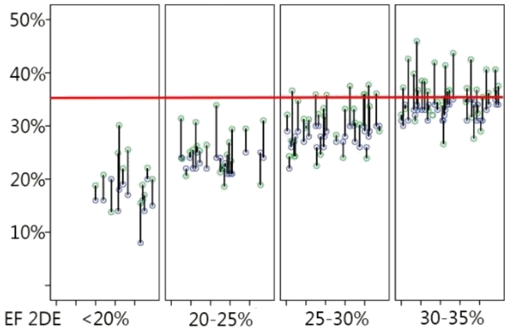

The difference between LVEF evaluated by 2DE and 3DE was more than 5% in 40 patients (40%). The mean (SD) difference EF was 3.2 (4.2) %. The difference between EF by 3DE and by 2DE was not significantly different among the 2DE defined four subgroups (mean value was 5.73% for EF <20%, 4.04% for EF 20-25%, 4.3% for EF 25-30% and 4.53% for EF 30-35%, p =0.4).

Characteristics of reclassified patients

a) Patients regrouped among subgroups by 3DE

The ejection fraction was not different between patients who were regrouped among the previously described subgroups and patients who remained in the same subgroup after 3DE. The end-diastolic and end-systolic LV volume was significantly lower for patients who were regrouped. The RWMAs and the QRS width did not influenced significantly the regrouping among subgroups (Table 1).

Figure 5. Graphic representation of number of patients from each subgroup by 2DE and 3DE.

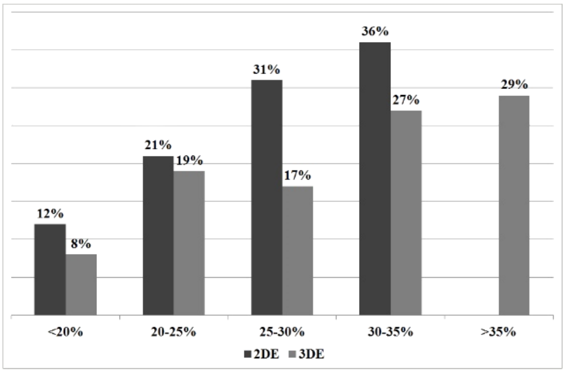

Table 1.

		Regrouped patients (subgroups)	Non-regrouped patients (subgroups)	p value
EF (SD)%		28.03 (5.09)	25.69 (7.0)	0.006
LVEDV (SD) ml		133.56 (57.58)	166.30 (85.22)	0.026
LVESV (SD) ml		97.25 (47.17)	127.60 (75.77)	0.016
RWMA	Yes	72.5%	27.5%	0.02
	No	61.2%	38.8%	
QRS	narrow	65.4%	34.6%	0.7
	large	68.8%	31.3%	

Table 2.

		Reclassified patients (LVEF by 3DE>35%)	Non-reclassified patients (LVEF by 3DE≤35%)	p value
LVEDV (SD) ml		106.27 (37.39)	159.93 (73.34)	0.000
Dilated LV (LVEDV>155ml)		6.3%	93.8%	0.001
Normal LV(LVEDV <155ml)		39.7%	60.3%	
LVESV (SD) ml		71.14 (26.43)	122.02 (63.00)	0.000
Image quality	average	30%	70%	0.82
	good	28%	72%	
RWMA	Yes	41.2%	58.8%	0.006
	No	16.3%	83.7%	
QRS	narrow	40.4%	59.6%	0.009
	large	16.7%	83.3%	

b) Patients reclassified from severe to moderate LV systolic dysfunction by 3DE

The end-diastolic and end-systolic LV volume was significantly lower in patients with severe LV systolic dysfunction who were reclassified as moderate systolic dysfunction after 3DE. The reclassified patients had more often regional wall motion abnormalities comparing to non-reclassified patients (Table 2). The number of reclassified patients was 16.3% form subjects with no regional wall motion abnormality, 48.3% for subjects with regional wall abnormality in LAD territory, 37.5% for RCA/Cx territory and 16.7% for multiple territory (p=0.018).

DISCUSSIONS

Can we trust 3DE? What do we know from cardiac magnetic resonance comparing studies?

A large number of studies during the last years have shown that 3DE is superior to 2DE for left ventricle quantification (LV volumes and EF) comparing to MRI. The superiority of the method is related to a better accuracy and a better reproducibility. The accuracy and the repeatability of 3DE are comparable with cardiac MRI for LV volumes and EF for unselected patients [4] or for patients with regional wall motion abnormalities [5, 6]. In a follow up study of patients with previous myocardial infarction the 3DE LV parameters changes were better correlated with MRI results comparing to 2DE [7]. Moreover, the 3DE estimation of LV volumes and EF was superior to 2DE measurement in predicting major cardiovascular events [8]. A recent meta-analysis showed that in studies comparing 2DE, 3DE and MRI LV measurements the 2DE/MRI pooled Bland Altman biases +/-SD were -48.2+/-55.9ml, -27.7+/-45.7ml and 0.1+/-13.9% for LVEDV, LVESV and EF. The 3DE/MRI pooled Bland Altman biases +/-SD were -15.7+/-31ml, -9.6+/-25.8ml and 0.0+/-9.2% for LVEDV, LVESV and EF. The differences in biases was not statistically significant for EF (p=0.42) [9]. However, most of the previous studies included only patients with good quality of echo pictures. In our study, we included all patients for which the EF calculation by Simpson's method was feasible, including patients with average quality of echo images.

Is 3DE evaluation a feasible method in clinical practice?

The feasibility of 3DE for LV parameters analysis was 89% in a large study, including unselected normal subjects [10]. In routine clinical practice 3DE was technically feasible in 83% of unselected patients [11]. The percentage of patients properly examined by 2DE that could not be analyzed by 3DE was not reported previously [12]. In our study, from 106 patients with adequate echo images quality for Simpson's evaluation, 6 patients (0.056%) could not be analyzed by 3DE.

Do we really need 3DE in clinical practice?

In nowadays clinical cardiology, when expensive decision makings are relied on ejection fraction value, its accurate determination becomes mandatory. The recent studies showed repeatedly that 3DE is superior to 2DE for LV quantification. However the EF estimation 2DE vs 3DE were not significantly different comparing to MRI in some studies [9]. Therefore the advantage of 3DE on 2DE in clinical echocardiography is uncertain.

Our data showed that routine 3DE use for LVEF estimation in every day practice can change the decision making in a significant number of patients. Twenty nine patients (29%) were reclassified from EF≤35% by 2DE to EF>35% by 3DE. Therefore, the clinical management of these patients (regarding ICD implant or CRT) should be reanalyzed in the view of the new data obtained by 3DE. The patients with 2DE severe LV systolic dysfunction and nondilated LV and RWMAs were often reclassified to 3DE moderate LV systolic dysfunction.

LIMITATIONS

The main limitation of the study is the lack of 'gold standard' measure. However, the superiority of 3DE over 2DE for LV assessment was previously demonstrated and the purpose of our study was to evaluate the possible clinical impact of 3DE in clinical practice.

CONCLUSIONS

Measuring LVEF by 3D echo reclassifies the severity of LV systolic impairment in a substantial proportion of patients with 2D LVEF<35% and RWMAs, which may have important clinical and financial implications by resetting thresholds for costly interventions such as ICD implants.

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3D reconstruction protocol in complex acetabular defects

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Abstract: *The current paper describes the protocol used in our clinic for 3D imaging, reconstruction and printing of complex acetabular defects that are used for surgical planning of complex acetabular defects. Surgical guides have also been developed to aid optimal acetabular reamer placement in order to recreate the optimal hip rotation centre for each patient undergoing revision hip arthroplasty that has impaired acetabular bone stock.*

Keywords: *3D printing, 3D reconstruction, acetabular defects, surgical guides, revision, convergent modelling*

INTRODUCTION

Acetabular bone defects are the main sources of prosthesis instability after total hip arthroplasty (THA) revision surgery if not addressed correspondingly. There are several areas important from a biomechanics point of view like the posterior and posterior-superior rims that are high stress areas that cannot be overlooked during surgery in comparison with the anterior and superior rim defects that can be ignored given that they do not generate instability [1].

Medialisation of the prosthesis is of key interest since this process minimizes prosthesis loading while excessive lateralization due to insufficient medialization or large medial wall defect increases joint reactive forces (JRF) that will eventually destabilize the cup starting from its inferior pole and then if the problem is not addressed the destabilized cup causes bone lysis at its upper pole creating a superior rim defect [1–3]. Other types of intraoperative prosthesis implantation difficulty require different surgical solutions [4]. As the acetabular defect complexity increases the required surgery becomes increasingly complicated requiring extensive soft tissue dissection with a higher risk of complications [3, 5, 6]. With these in mind the focus is oriented towards methods of increasing surgical success rate one such method represents the shift from conventional roentgenographic studies towards computed tomography (CT) that enables a better bone stock evaluation while at the same time offering the opportunity of 3D defect reconstruction, augmenting surgical planning and decision making [7–9]. In the following section we will describe our protocol for 3D reconstruction and printing of patient specific concept-guides for complex acetabular defects.

MATERIALS AND METHODS

CT image acquisition and later processing of acetabular bone stock prior to revision surgery has been performed according to ISO standard 12052 [10]. In the following section we will provide a detailed walk-through of each step employed from image processing to 3D printing the personalized surgical block [11, 12].

Image acquisition protocol

While CT offers arguably more information on acetabular bone stock in comparison to radiographic studiesit does possess the inconvenience of metal-related artifacts generated by competing mechanisms like photon starvation, beam hardening, scattering, partial volume effects, under-sampling and patient motion [13]. Our protocol focuses on the major mechanisms namely photon starvation and beam hardening. We have used Computed Tomography X-Ray machine (SIEMENS, SOMATON, serial no. 111382). The acquisition parameters that we use are exposure time of 1 second, nominal single collimation width of 0.7 mm and mean CT dose index (CTDI) 4.72mGy. CT X-ray source parameters are kVp 111 kV and exposure 273 mAs. Metal-artifact reduction (MAR) protocol used was a image based metal segmentation method named iterative MAR (iMAR).

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Image processing

After successful acquisition the images were processed using the picture archiving and communication system (PACS).

After this step, image storage and transfer was performed by converting the data to Digital Imaging and Communications in Medicine format (DICOM).

3D Reconstruction using DICOM data

The first step in the 3D reconstruction is the processing of DICOM data into stereolithography (STL) format. This process is done automatically during the 3D reconstruction and for this purpose we have used 3D Slicer. DICOM data was imported in the 3D Slicer database and opened in the segment editor module. Two different approaches were used:

The first technique called the two layers technique two segments were created, one named bone and the another one named other. With the bone layer selected the paint tool can be activated. Using the sphere brush the zones of interest for the bone were worked through each slice.

This technique can be extended to other layers for different bones in the same scan. With the other layer active and the sphere brush selected, zones that do not contain bone that are intended for 3D reconstruction are marked through some of the DICOM slices.

After proper marking, the grow from seeds tool is used. After processing, the DICOM slices should be marked accordingly (Figure 1). If the marking is not correct in some slices, it can be corrected by marking the correct layer in the DICOM slices and checking the live update box in the grow from seeds tool. The marked results can be observed in the 3D view (Figure 2). After proper marking the 3D we recommend to use the smoothing tool and generate the final 3D model.

Figure 1: Coronal view with marked bone segment (green) and other marked segment (yellow), processed in 3D Slicer with the “grow from seed” function (*this image is from the author’s personal database*)

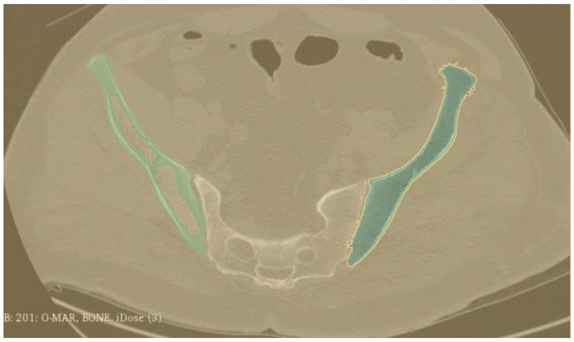

Figure 2: 3D view of bone segments processed in 3D Slicer (*this image is from the author’s personal database*)

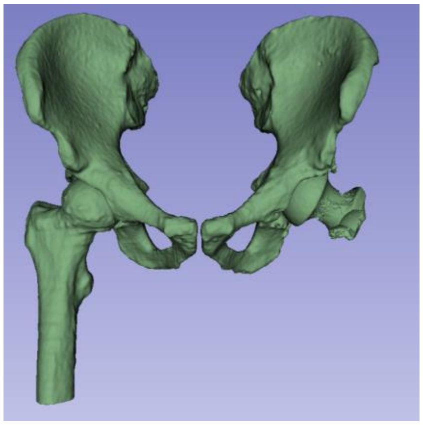

The second technique is simpler and faster but the 3D model quality can be inferior to the previous presented method due to noise in the CT scan or different bone interactions. The main tool for this method is the threshold tool. By opening this tool, an interval can be chosen for the intensity of the pixels. The program will select all the pixels with the intensity in the selected interval and will mark them. In order to edit specific zones or to add and delete pixels based on a threshold value the brush tool can be combined with the threshold function in order to do zone marking or subtraction based on the selected threshold interval. After proper marking were commend the use of the smoothing tool and to generate the final 3D model.

3D Reconstruction post-processing

After the desired 3D model is obtained post-processing of the triangle meshes is required. For the post-processing of the 3D model we recommend Autodesk Meshmixer (Autodesk Meshmixer, RRID:SCR_015736) software. The 3D model is imported in Meshmixer and using the Select tool all the surfaces are selected by double clicking on the model. Using the Separate command, the model is separated by connected surfaces. Using the object browser, bodies that are not required are deleted. After this preliminary clean-up the model is checked for errors using the inspect tool from the analyse tab. If errors are detected the geometry is repaired with the help of the Inspect tool.

For easy and fast handling of the 3D model we recommend to reduce the complexity of the model using the reduce tool from the Edit tab (the model needs to be selected). The reduce variables need to be adjusted until a result like the one in Figures 3 and 4.

The goal is to reduce the geometry of the model as much as possible but also maintain the shape. This model is saved and exported in STL.

Figure 3: Wireframe view of acetabulum before reduced geometry, processed in Autodesk Meshmixer (*this image is from the author's personal database*)

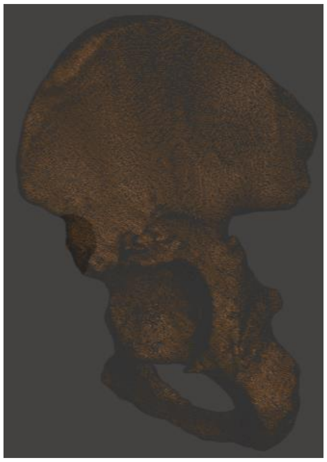

Figure 4: Wireframe view of acetabulum after reduced geometry, processed in Autodesk Meshmixer (*this image is from the author's personal database*)

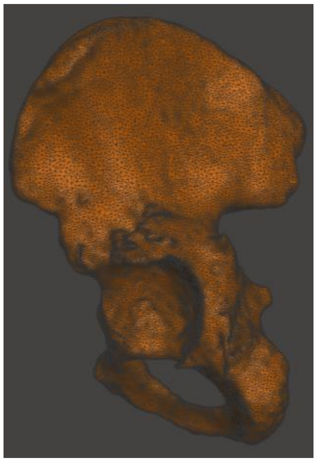

Convergent modellingTM

After post-processing we further edited the STL file into Solid Edge (Siemens AG).In this step we recommend doing a remeshing by using the re-mesh tool from the Reverse Engineering tab and align the model. After remeshing the identify regions tool is used. By selecting the automatic tool and choosing the body of the femur from the model, the program will automatically identify the surfaces from the model. Alternatively, in some cases is faster to use the manual tool from the Identify Regions section and paint the zones of interest on the model. By using the Fit tool, choosing the sphere entity and selecting the surfaces that describe the femural head, a sphere will be created on the femural head.

Using the 3D line command, 3 axes are placed between the 2 acetabula in the sagittal (Figure 5), coronal and axial planes. These axes will be later used to properly define the acetabular cup placement during surgery (Figure 6).

Figure 5: Lines placement for sagital plane construction, image processed in Solid Edge (*this image is from the author's personal database*)

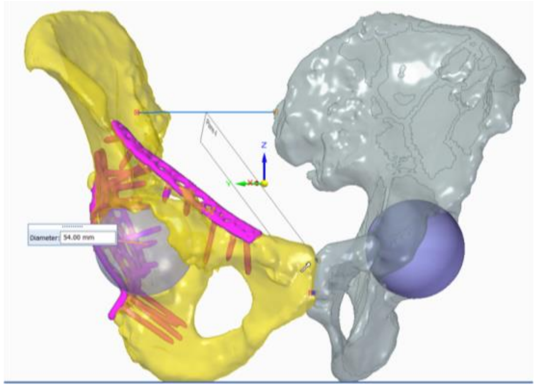

Figure 6: Abduction angle, image processed in Solid Edge (*this image is from the author's personal database*)

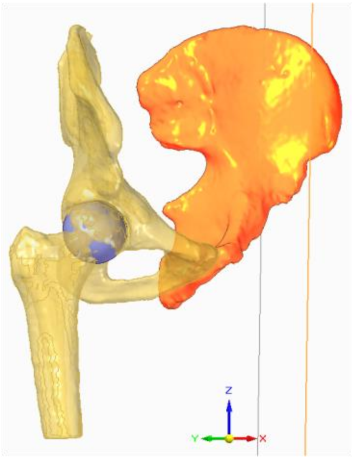

By creating a plane parallel to the median plane and rotate it 90 degree we built a plane parallel to the coronal axis. This plane can be moved in the centre of the constructed spheres using the steering wheel. Two more planes were created and placed parallel to the sagittal and axial axes. After all three planes were created, specific angles were defined to determine physiologic anteversion and abduction angles. In this procedure by taking the healthy acetabulum as reference we have defined 45 degrees cup abduction and 29 degrees cup anteversion (Figure 7).

After completion the model can be added into an assembly together with the pre-designed guide. The guide is constrained by using the Axial Align tool. The guide can be placed and rotated on the acetabulum by using the acetabular rim as support (Figure 8).

Figure 7: Anteversion planes, image processed in Solid Edge (*this image is from the author's personal database*)

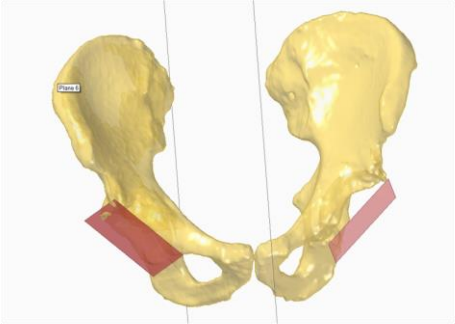

Figure 8: The pre-designed guide, image processed in Solid Edge (*this image is from the author's personal database*)

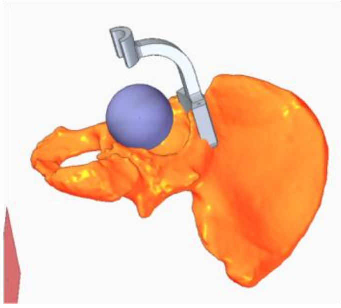

After placement the acetabulum can be subtracted from the guide by using the boolean tool, or the model can be exported in STL and the boolean operation can be made in Meshmixer. In this case we have employed (which one?)…

After the boolean operation the guide is prepared for printing. The STL model of the guide is imported into the printer slicer. In this case we have used PrusaSlicer.

In order to print the segmented acetabulum, we have used a Prusa i3 MK3 printer using a default profile of 0.15 mm with a 60% rectangular infill. The filament used for printing was made of poly-lactic acid (PLA).

RESULTS

After all the processing steps we have constructed a personalized guide for acetabular reaming and cup placement. The obtained guide construct is based on the local acetabular anatomy in order to allow the best obtainable stability. In order to obtain intra-operative stability, we have designed a locking mechanism that makes use of the superior acetabular rim anatomy. After proper acetabular exposure we can place the reaming guide on the superior acetabular rim and rotate it clock-wise in order to lock it into the bony landmarks that have been identified during the CT processing steps. This locking mechanism will make the acetabular reaming independent of patient positioning because of its improved stability which is an important problem to deal with especially in patients with higher body mass indexes (BMIs).

DISCUSSION

Several authors have published mixed results on patient specific guide preparation and outcomes [7–9]. Most relevant variables in direct connection with surgical utility are represented by blood loss, surgical time, dislocation rate, infection rate and overall patient reported outcome [14–16]. On the other hand, there are several factors that need to be considered when trying to implement new technologies into the operating room. Based on our current research experience, we are inclined to evaluate such a technology based on 1) tangible and 2) intangible results. We refer to tangible results when talking about implementing 3D printing in avoiding unnecessary surgical steps and evaluating relevant surgical variables like the ones mentioned above. On the other hand, intangible results are best evaluated based on patient reported outcomes.

Other researchers in the orthopaedic field have reached similar results in different areas where preoperative planning augmented through the use of modern technology has lead to a superior outcome for the patient, be it in surgical time, recovery time or overall patient satisfaction [17–19].

CONCLUSION

3D printing of patient-specific guides offers a new opportunity to innovate surgical practice by offering an augmented surgical planning with an enhanced control over surgical instrument placement. At this time the literature on the matter reports mixed results regarding the actual improvements, but we are confident that as the technology comes closer to the surgical needs we will see an increased use of patient-specific 3D printed guides especially in orthopaedic surgery practice. The current paper offers a comprehensive approach to personalized surgical guide creation using CT scans of complex acetabular defects.

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ORIGINAL ARTICLE

The Career Decision of Medical Students: A Tool for Organizational Psychology in the Healthcare System

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Abstract: Background: The deficit of medical staff in various health care systems points out the need for a more detailed strategic management toward sustainability in human resources. The purpose of this study is to explore the relationships linking self-esteem, self-efficacy, general attitudes, and career preferences among medical students, aiming to provide insights that could support sustainable career development. Methods: We performed a monocentric observational study that included 157 students. The participants completed four standardized questionnaires evaluating the self-efficacy, the self-esteem, the general attitudes and beliefs, and the perception of medical career specialty. We assessed the relationships between the psychological variables and career preferences. Results: Significant correlations emerged between the students' year of study and the need for achievement, the importance of mentorship, and career decision-making. Clinical students, in particular, placed greater emphasis on gaining practical experience in their chosen field. Conclusions: Our results reaffirm the impact of occupational psychology in the health care system, providing more sustainability in career decisions. Further research involving larger and more diverse samples is recommended to gain a more comprehensive understanding of how the study of various psychological factors could impact human resources management in the field.

Keywords: human resources, sustainability, health care, self-esteem, self-efficacy, medical students, career perception, occupational psychology

INTRODUCTION

The healthcare systems experience, nowadays, an important deficit of human resources, achieving health care quality very challenging, especially in developing countries. Thus, the strategic management of human resources should use various psychological tools in order to reach stability in various medical specialties, considering the psychological characteristics of the medical students [1].

Medical students often experience chronic stress during their academic development. Thus, self-esteem, self-efficacy, and general attitudes and beliefs are

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of paramount importance in the process of psychosocial growth and have a marked effect on thoughts, feelings, values, and professional goals [2]. Furthermore, future career decisions should rely on occupational psychology as a guarantee for the sustainability of the human resources in health care systems worldwide [3]. In this regard, the perception of self-esteem, self-efficacy, and general attitudes and beliefs among medical students serve as tools for occupational psychology in the field [4].

The integration of organizational psychology into the training of medical students is essential for the sustainable development of human resources in the health field. This approach contributes to preparing the future doctors for professional challenges and promoting an efficient work environment.

Self-esteem refers to the totality of the value judgments of an individual regarding their person. Both very low self-esteem and very high self-esteem can lead to psychological problems. Thus, the self-esteem should be at an average level. Self-efficacy refers to the beliefs of an individual about his or her ability to mobilize his or her cognitive, behavioral, and motivational resources to complete a task, respectively, to his/her ability to organize and execute purposeful actions. People with a high level of self-efficacy optimally allocate their resources to solve tasks successfully, whereas people with a low level of self-efficacy avoid initiating actions, withdraw from tasks, fail, and reinforce their beliefs about their incompetence in the respective tasks.

The general attitudes and beliefs refer to four dimensions: achievement, approval, comfort, and justice. With respect to cognition, it measures the global assessment of one's own person and the global assessment of the value of others, rationality, and preferences, considering the choice of students with regard to the medical specialty.

Given that medical students experience high levels of stress and are at increased risk of psychological disturbances during the learning process, self-esteem and self-efficacy may be important variables in their well-being and performance [5].

In the literature, self-esteem and self-efficacy have been associated with academic performance and job satisfaction. Recent studies have shown that these variables are interconnected and influence the perceptions and career choices of medical students [6,7].

Social perfectionism, defined by the need to meet the expectations of others, as a part of general attitudes and beliefs, can negatively affect academic self-efficacy and lead to academic burnout [8,9]. Educational interventions that support competence, learning from mistakes, and self-efficacy can reduce these negative effects [10].

Using four well-established questionnaires, which quantify the self-efficacy [11], the self-esteem [12], the general attitudes and beliefs [13], and the perception of medical career specialty [14], this study aimed to: (1) compare differences in self-esteem, self-efficacy, general attitudes and belief scores with respect to the career preferences of medical stu-dents; (2) correlate self-esteem, self-efficacy, general beliefs and attitudes scores, and ca-reer-related perceptions among medical students; and (3) identify how these variables influence the career preparation and orientation of the students at different stages of their academic training.

MATERIALS AND METHODS

This research consists of a monocentric observational study that includes medical students from Carol Davila University of Medicine and Pharmacy, Bucharest-Romania, who were interviewed between March and July 2024. We enrolled 157 participants (118 females, 39 males). The mean age was 21.6 years (+/- 2.16). They were asked to answer a survey consisting of 4 scales: the Self-Efficacy Scale (SES) (10 items) [11], the Self-Esteem Scale (SS) (10 items) [12], the General Attitudes and Beliefs Scale (GABS-SV) (26 items) [13], and the Perception of Medical Career Specialty Questionnaire (PMCS) (24 items) [14]. Table 1 reveals the main items included in each scale.

Table 1: The main items included in each scale

Name of the Scale	Items included in each Scale
Self-Efficacy Scale	I always manage to solve difficult problems if I try hard enough/Even if someone opposes me, I manage to identify ways and means to get what I want/It is easy for me to be consistent with my goals and achieve my goals/I have confidence that I could successfully cope with unforeseen events/Thanks to my resources I know how to deal with unexpected situations/I can solve most problems if I put in the necessary effort/I can stay calm when faced with difficulties because I can rely on my coping skills/When faced with a problem I can usually identify several solutions/Usually, when I have a problem, I can think of a solution/I can usually handle any situation and any kind of problem I am faced with
Self-Esteem Scale	I am generally satisfied with myself/Sometimes I think I'm good for nothing/I feel that I have many qualities/I am able to do things as well as other people/I feel like I do not have much reason to be proud of myself/Sometimes I feel useless /I feel that I am as valuable a person as anyone else/I wish I had more respect for myself /I generally tend to think of myself as a loser/I have a positive attitude toward my person
General Attitudes and Beliefs Scale	The need for achievement, the need for approval, the need for comfort, the absolutist demand for justice, the overall assessment of others, overall assessment of self-worth, rationality, irrationality

Perception of Medical Career Specialty questionnaire	Financial importance, perception of opportunities for further specialization, challenging domain perception, perception of less stressful field, the importance of flexible schedule, important establishment in an urban environment, important research opportunity, didactic opportunity, short working hours, combination of hobby and job, comfortable lifestyle, the importance of the sense of vocation

The data were analyzed via GraphPad Prism 6.00 (GraphPad Software Inc.). For the comparative analysis, we used one-way ANOVA followed by post hoc Tukey's test for interactions between groups. With respect to career perception, we divided the students into 4 groups: surgery (students who might choose a surgical field after graduation), non-surgery (students who might choose a medical field, nonsurgical field after graduation), paraclinical (students who might choose a medical field with no patient contact after graduation), and N/A (students who did not decide about a specific field after graduation) and compared the main scores among them.

Considering the study year, we divided the students into two groups, namely, the preclinical group (students in the first two years of medical study) and the clinical group (students in the last four years of medical study), in accordance with the curricula of the general medicine studies, and compared various items between them. The data are expressed as the mean ± SEM. A two-sided P value <0.05 was considered statistically significant. For binary data, we used the chi-square test.

For the correlational data, we used a correlation matrix that included all the items independently. The Spearman correlation coefficient, r, was determined, considering the non-normal distribution of the group. We considered a two-sided P value <0.05 to indicate statistical significance for each correlation. We assigned r values under 0.4 to have negligible correlative effects. Finally, we excluded nonsignificant correlations.

RESULTS

Figure 1 shows the comparative analyses of self-esteem, self-efficacy and general attitudes and beliefs scores with respect to career perception. We observed relatively homogenous score values among the groups regardless of the career perception.

Considering the self-efficacy score, we observed the following mean values among the groups: surgery group vs. non-surgery group, 31.58±0.67 vs. 29.05±0.52 (p=0.018); paraclinical group, 30.48±0.82; and N/A group, 28.25±1.48, without any other statistically significant changes (Figure 1A). The mean self-esteem score with respect to career perception did not differ significantly among the groups: 28.6±0.79 in the surgery group, 27.16±0.56 in the non-surgery group, 27.9±1.08 in the paraclinical group, and 25±2.67 in the N/A group (Figure 1B). Furthermore, we revealed relative homogeneity among the groups in terms of the general attitudes and beliefs score: 9±0.49 in the surgery group, 9.77±0.33 in the non-surgery group, 10.29±0.65 in the paraclinical group, and 12±1.08 in the N/A group (Figure 1C).

Figure 1: Comparative analysis of self-efficacy (A), self-esteem (B), and general attitudes and beliefs (C) scores between students who choose one of the following medical fields after graduation: surgical, non-surgical, paraclinical, or no option yet. The values are expressed as the means ± SEMs. * represents a p-value between 0.05 and 0.01

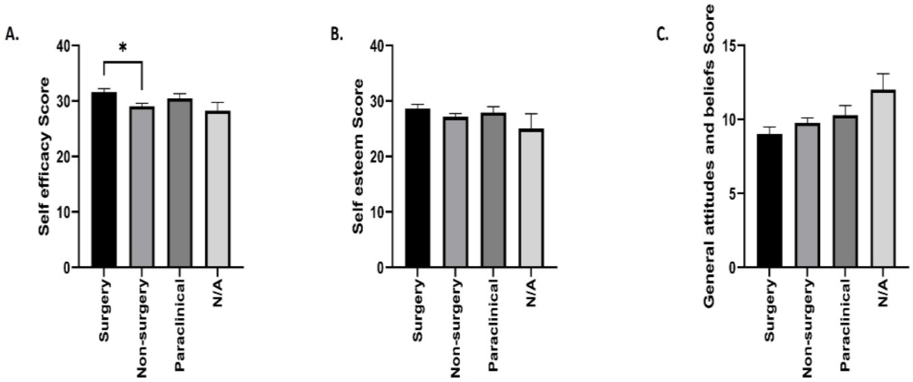

The correlations between self-esteem, self-efficacy, individual general beliefs and attitudes, and career-related perceptions among medical students are shown in Figure 2. The positive correlations are marked in blue, whereas the negative correlations are red.

Self-esteem correlated positively with rationality (r=0.51, p<0.001) and negatively with the overall assessment of self-worth (r=-0.56, p<0.001) and the need for comfort (r=-0.48, p<0.001). The rationality correlates negatively with the need for approval (r=-0.44, p<0.001). Additionally, the link between the overall assessment of self-worth and the need for comfort suggests a strong positive correlation (r=0.71, p<0.001). Moreover, the need for achievement correlates positively with the need for approval (r=0.53, p<0.001), comfort (r=0.51, p<0.001) and absolutist demand for justice (r=0.54, p<0.001). We also observed a positive correlation between the need for approval in student perception and the need for comfort (r=0.48, p<0.001) and absolutist demand for justice (r=0.51, p<0.001). The need for comfort also positively correlates with the absolutist demand for justice (r=0.42, p<0.001), which, further, correlates positively with the overall assessment of others (r=0.54, p<0.001). We revealed a weak positive correlation between financial importance and lifestyle (r=0.41, p<0.001) and between perceptions of opportunity for further specialization and perceptions of the challenging domain (r=0.41, p<0.001).

Figure 2: The main correlations between various items included in the 4 scores

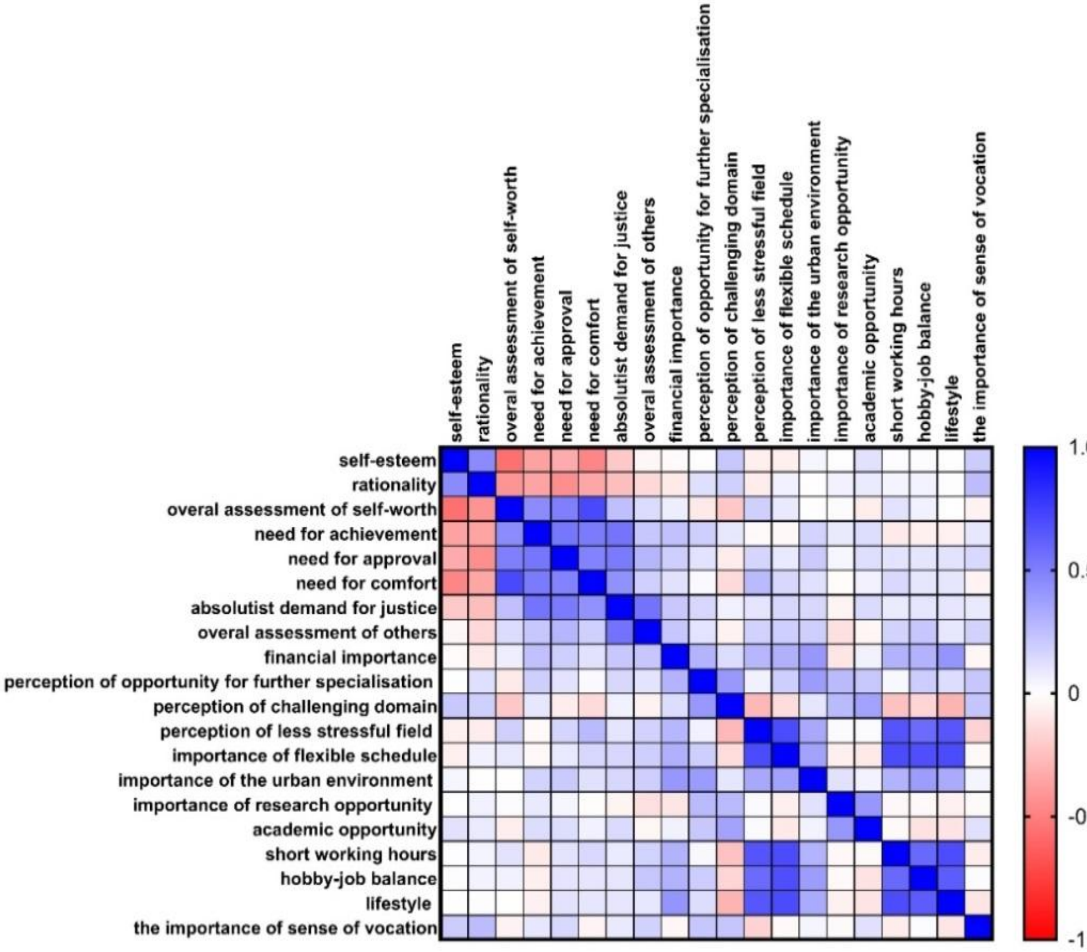

The perception of a less stressful field strongly correlates with the importance of a flexible schedule (r=0.7, p<0.001), short working hours (r=0.66, p<0.001), hobby-job balance (r=0.57, p<0.001), and lifestyle (r=0.65, p<0.001). Furthermore, we observed positive correlations between the importance of a flexible schedule and short working hours (r=0.7, p<0.001), hobby-job balance (0.68, p<0.001), and lifestyle (r=0.69, p<0.001), between short working hours and hobby-job balance (r=0.58, p<0.001) and lifestyle (r=0.69, p<0.001), and between hobby-job balance and lifestyle (r=0.63, p<0.001).

The comparative analyses between the years of study and the need for achievement, academic opportunity, opportunity for patient care, and importance of the models are shown in Figure 3.

Our study revealed a significantly greater score for need for achievement in the preclinical years than in the clinical years: 15.14±0.49 vs. 13.67±0.38 (p=0.03) (Fig. 3A). With respect to the academic opportunity, we observed a statistically significant increase in the perception score among the students from the clinical compared with those from the preclinical years: 3.29±0.14 vs. 2.69±0.17 (p=0.008) (Fig. 3B). Furthermore, the opportunity for patient care was better highlighted in the clinical group than in the preclinical group: 3.29±0.11 vs. 2.72±0.15 (p=0.008) (Fig. 3C). Finally, the importance of the models was greater for the students in the clinical years than for the students in the preclinical years: 3.8±0.12 vs. 3.38±0.15 (p=0.03) (Fig. 3D).

Figure 3: Comparative analysis between clinical and preclinical years of study and mean values of need for achievement (A), academic opportunity (B), opportunity for patient care (C), and importance of models (D). The values are expressed as the means ± SEM. The bars represent the SEM. * represents a p value between 0.05 and 0.01, ** represents a p value between 0.01 and 0.001

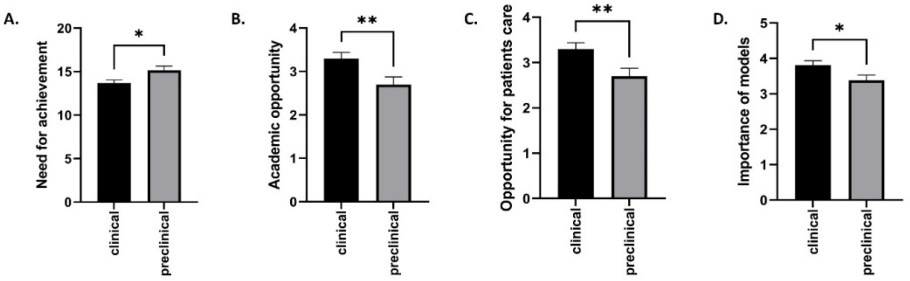

DISCUSSION

Modern management strategies in human resources in health care organizations should consider the sustainability potential of the providers, based on their self-efficacy, self-esteem, and general attitudes and beliefs perception. Thus, further organizational stability could be achieved and qualitative health care procedures guaranteed [15].

Our study reveals the role of occupational psychology among medical students, which better tailors the decisional tree for future medical subspecialties. Among the groups of students in different specializations, the analyzed variables, such as self-esteem, self-efficacy, general attitudes and beliefs, and career perceptions, are relatively homogeneous. Our results are in accordance with the literature. A recent study highlighted how self-esteem and self-efficacy are critical factors in career decision-making among medical students. Research indicated that medical students across various fields (surgery, general medicine, etc.) tend to show relatively uniform levels of self-efficacy and self-esteem, which are essential for their career maturity and decision-making abilities [16]. This is consistent with studies focusing on specialty identity and self-efficacy, where students from various medical disciplines demonstrated similar levels of career maturity and engagement [17].

Moreover, the importance of psychological factors such as self-esteem and career perception has been highlighted by a study examining flourishing and well-being. These studies revealed that regardless of their specialization, medical students benefit similarly from positive psychological constructs, which mediate their career decisions [18].

According to our results, the self-esteem of the medical students is closely related to rationality, suggesting that those with high self-esteem tend to think logically. Positive self-evaluations are associated with the need for justice, support, and approval, whereas irrationality is correlated with a desire for comfort and a critical evaluation of others. The need for absolute justice is linked to the importance of social status and a comfortable lifestyle, especially in the urban environment. Current research underscores the importance of self-esteem in rational decision-making and professional development in young adults, including medical students. For example, Harris and Orth highlighted in 2020 that high self-esteem is associated with rational behavior and an increased ability to face academic and professional challenges, which can positively influence students' career paths [19].

In terms of beliefs and attitudes, various data from the literature suggest that cognitive rigidity and irrational thinking can negatively affect professional adaptability. A study from David et al. discussed the negative impact of irrational beliefs on academic and professional performance, confirming observations that such beliefs can limit career success [20]. Career motivations, such as the need for specialization and the preference for a comfortable lifestyle, are critical to job satisfaction. Another study revealed that aligning career goals with personal values can increase motivation and career satisfaction [21].

Self-efficacy, defined as confidence in one's ability to achieve goals, plays a critical role in academic and professional success. Zajacova et al. identified that academic self-efficacy is a significant predictor of academic performance and the adjustment to university demands among young adults, highlighting the importance of this skill for medical students [22]. The results of our study show a significant correlation between interest in the specialty and the importance of a sense of duty. This suggests that students who show a strong interest in a particular medical specialty are motivated by a strong sense of duty to the profession and patients. Moreover, the importance of vocation and responsibility is related to the choice of medical specialties [23].

Global self-evaluation is a central construct in cognitive theory and is related to various forms of irrational demands, such as the need for absolutist justice and the need for approval, and research. Dryden and David suggest that absolutist demands and emotional needs are predictive factors for negative global self-evaluations [24]. Specifically, in our study, irrationality was correlated with the need for comfort, the absolutist demand for justice, and the global evaluation of others. Our results are in accordance with those of Ellis et al., in which irrationality might reflect a cognitive tendency to adopt extreme or unrealistic perspectives on the world and others [25]. This correlation suggests that when students face uncomfortable situations or perceive that justice is not respected, they may exhibit irrational tendencies, thus influencing their learning process and interpersonal relationships.

A statistically significant positive correlation between specialty interest and the importance of sense of duty suggests that students who are strongly interested in a particular specialty also have a well-developed sense of duty to the profession in that type of specialty and to their own patients, and these results are in accordance with the literature [26]. They noted that the perception of difficulty and complexity of a field can stimulate interest in in-depth specialization, as students seek to push their limits and acquire advanced skills. The importance of vocation and responsibility in choosing medical specialties integrates this psychosocial context [23].

Our study revealed that students who value teaching opportunities also appreciate the possibility of engaging in research activities. Our results are in accordance with those of Taylor et al., who revealed a link between the involvement of students in didactic activities and the research interest, considering it a natural extension of the academic learning [27].

Significant correlations between the year of study and the variables such as need for achievement, didactic opportunity, and opportunity for patient care, in our study, suggest a progressive development of the priorities of students and motivations as they progress through the degree program. These findings are consistent with another research showing that as students progress through medical education, they become more achievement-oriented and increasingly value role models of career success [28]. The importance of model perception with the progress through medical education could help further develop new sustainable management strategies in human resources.

Moreover, preclinical students often focus more on academic achievement and didactic learning. Some studies have demonstrated that role models significantly influence students' self-efficacy, particularly when these role models are seen as relatable and their success attainable [29,30]. Additionally, expectancy-value theory suggests that students who find mentors similar to themselves are more likely to value the associated career path, reinforcing the importance of mentorship in clinical education. This aligns with more recent research emphasizing the critical part that role models play in shaping the students' perceptions of their future careers [31]. Furthermore, achievement motivation and self-efficacy have been found to fluctuate on the basis of the type of mentorship and patient exposure students receive, which further highlights the transitional priorities from the preclinical to the clinical years [32]. These studies underscore the evolving nature of the motivations of the medical students and the influence of practical exposure and mentorship as they progress through their education.

The findings of this study emphasize the substantial influence of lifestyle factors on medical students' career preferences, which aligns with the literature. Many medical students today prioritize work–life balance, flexible schedules, and reduced working hours when selecting their specialties. A survey by Dorsey et al. revealed that the increasing importance of a controllable lifestyle has led to a shift in specialty preferences among U.S. medical students, with fewer students opting for time-demanding fields such as surgery and more favorable fields that offer a more predictable schedule [33]. Thus, in order to achieve stability of the human resource in the health care area, regardless of the medical sub-specialty, the medical organization should adhere to a predictable work program [34].

Furthermore, a study by Lambert and Holmboe revealed that medical students are increasingly focused on selecting specialties that allow them to maintain a balance between their professional and personal lives, contributing to their overall career satisfaction [35]. These findings mirror those of our study, where a flexible schedule and hobby-job balance were key factors for students aiming to pursue less stressful specialties.

Another study by Dyrbye et al. demonstrated that burnout among medical students and physicians is closely linked to lifestyle factors, with specialties offering better work–life balance associated with lower burnout rates [36]. These findings support our results, highlighting the need to consider lifestyle when guiding students in their career decision-making.

Finally, we observed an absence of a significant correlation between the importance of the sense of vocation and the other analyzed variables. This suggests that a sense of vocation, although considered an essential element in choosing a medical career, may function independently of other factors, such as teaching or research opportunities. This result is supported by the research of Newton and Grayson, who highlighted that vocation can be a strong intrinsic factor but is not necessarily related to external circumstances or other professional motivations [37].

Study limitations

Our research presents some limitations. Firstly, the lack of significant correlations does not necessarily indicate the absence of differences between groups but may be influenced by sample size. Secondly, the study was monocentric and did not consider the importance of intercultural variability among different societies. Finally, we did not perform a separate analysis of the enrolled international students.

CONCLUSION

Our study highlights the need for applying various occupational psychology tools for a more sustainable human resources in the health care system, considering the medical students as future providers. Further studies that explore other aspects or use alternative measurement methods are needed to confirm our results.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, E.N and S.I.; methodology, S.A.C., A.C.C., and E.N. ; software, S.I., and M.L.; validation, A.C.C., M.M.M, and T.I.; formal analysis, C.V.A., and S.I.; investigation, E.N., and G.D.; resources, T.I., and S.I.; data curation, M.C.P., and M.L.; writing—original draft preparation, M.C.P., S.A.C., and E.N.; writing—review and editing, T.I., S.I., and C.V.A; visualization, G.D., and M.M.M.; supervision, S.I.; project administration, G.D.; funding acquisition, E.N. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Fundeni Clinical Institute (10337/07.03.2024).

Participant consent for publication

The informed consent for publication was included in the questionnaires and signed by each participant.

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REVIEW

Traditional Ethnomedical and Ethnobotanical Applications and Uses of Piper Nigrum

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Abstract: Ethnomedicine and ethnobotany have been at the forefront of current scientific focus. The focus of this review is Piper nigrum, for which there exists a wide host of traditional ethnomedical and ethnobotanical applications, recorded mostly from India and the surrounding regions where black pepper is native. These applications cover a wide range of pathologies, including cardiovascular, pulmonary, gastrointestinal, gynaecological, endocrine, integumentary and neurological, and viral diseases and colds. While some of these applications are confirmed by modern research, the majority have not been tested using modern scientific methods, and perhaps many more applications remain to be documented. Finally, it must be noted that some interesting non-medical applications comprise the application of black pepper, as an insecticide/larvicide, are worth further consideration, especially given the newer policies at a European and international level.

Keywords: *Piper nigrum*; black pepper; ethnomedicine, traditional uses

INTRODUCTION

Herbs and spices have been used for medicinal, preservative, and culinary purposes since antiquity in numerous traditional medicine systems [1-4]. In recent years, there has been an increase in research efforts, dealing both with recording ethnomedical and ethnobotanical practices across different regions, and verifying the reported uses by employing modern analytical and experimental methods [5-8]. The focus of this review is black pepper (*P. nigrum*), which has a long history of culinary history and use.

In Indian culinary tradition, black pepper has been an integral part since about the 2nd millennium BC, and numerous traditional herbal recipes are mentioned by Venkatachalapathi et al. [9]. Many ethnozoological uses of *P. nigrum*, combined with other components, also exist in India [10]. Given that *Piper nigrum* was originally cultivated in India [11], it makes sense that the record of relevant ethnomedicinal and ethnobotanical applications will be rich in this country and in the surrounding regions. Traditional uses of *P. nigrum* are reported from nearby regions as well.

TRADITIONAL ETHNOBOTANICAL AND ETHNOMEDICINAL APPLICATIONS OF BLACK PEPPER

Applications in Cardiovascular System Pathologies

Regarding cardiovascular pathologies, the fruit of *P. nigrum* is mixed with other ingredients in a powder in the area of Odisha, India, to combat hypertension [17]. Based on in vivo experiments in rats, it was proven that piperine could normalise

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hypertension and aortic ring reactivity [62]; therefore, there is at least experimental confirmation of the previous usage. An additional use for cardiovascular pathologies is recorded in the area of Tiruvallur where *P. nigrum* is used in different formulations, along with other plants and herbs, in these uses [16].

Table 1: Traditional ethnomedical and ethnobotanical applications of P. nigrum.

Part of the Plant Used	Country/Region	Type of Ailment	Year	Reference
Cardiovascular Pathologies				
Seeds (used to make a decoction with *Z. officinale* leaves)	Maharashtra, India	Generalised oedema	2006	[12]
Seeds (used to make a paste with leaves of *C. ternatea* L.)	Tamil Nadu, India	Leg swelling	2012	[13]
Seeds (made into paste with other plant parts, and used to make tablets)	Odisha, India	Haemorrhoids	2012	[14]
Fruits	Tamil Nadu, India	Good circulation promotion	2015	[15]
Different parts in various formulations	Tamil Nadu, India	Cardiovascular pathologies	2016	[16]
Fruits (powdered with other ingredients)	Odisha, India	Hypertension	2018	[17]
Respiratory Pathologies				
Seeds (powder for internal administration)	Tamil Nadu, India	Cough	2007	[18]
		Bronchial disorders		
Seeds, fruits	Orissa, India	Cough	2010	[19]
Different parts of the plant (combined with *E. cardamomum*, *A. galanga*, *Z. officinale*, and sugar)	Western Ghats, India	Asthma	2011	[20]
Seeds (powdered added in *V. negundo L.* leaf decoction with honey)	Odisha, India	Cough	2012	[14]
Raw fruits	Kerala, India	Sinusitis	2015	[21]
Seed (powder combined with dried leaf powder of *G. bosvallia*)	Madhya Pradesh, India	Whooping cough	2018	[22]
Gastrointestinal Pathologies				
Leaves (leaf juice is mixed with leaf and root bark powder of *K. grandiflora*, and leaf powder of *C. halicacabum*)	Tamil Nadu, India	General gastric problems	2005	[23]
Seeds (dried and swallowed)	Tamil Nadu, India	Stomach ache	2006	[24]
Seeds (powdered, mixed with root of *P. margaritiferum* Scott)	Odissa, India	Dysentery	2010	[25]
Seeds (powdered, with bark decoction of *P. xylocarpum*)		Infantile diarrhoea		
Fruits (powdered, mixed with *T. dioica* leaf decoction)		Diarrhoea		
Different parts of the plant (combined with different plants)	Western Ghats, India	General gastric problems	2011	[20]
Different parts of the plant	Tamil Nadu, India	General gastric problems	2012	[26]
Fruits (combined with numerous different plants and made into a decoction)	Karnataka, India	Intestinal ulcers	2012	[27]
		Aphthae		
Seeds [combined with *T. asthmatica* (L. f.) Wight &Arn roots, garlic and fruits of *S. cumini*, made into paste]	Karnataka, India	Jaundice	2012	[28]
Fruits (in powder form – oral adm.)	Assam, India			
Seeds (mixed with seeds of *B. roxburghii* and jaggery, made into tablets)	Maharashtra, India			
Fruits (combined with *C. dactylon* and *P. amarus* leaves – subsequently an extract is produced)	Tamil Nadu, India			
Seeds	Assam, India	Indigestion	2013	[29]
Leaves (used to make an infusion)	Misiones, Argentina	Digestive complaints	2014	[30]
Fruits (macerated with other plants to make pills)	Jhenaidah, Bangladesh	Ulcer	2014	[31]
Fruits (powder for oral administration)	Kerala, India	Stomach ache,	2014	[32]
		Indigestion		
Raw fruit	Kerala, India	Vomiting	2015	[21]
Seeds (crushed and mixed with different ingredients)	Phnom Penh, Cambodia	Liver pathologies	2017	[33]

Different parts (combined with *C. diococcum*, *C. aromatica*, *P. nigrum*, and *P. betle* and salt)	South Western Ghats, India	Stomachache	2018	[9]
Fever, General Anti-Inflammatory Actions, Musculoskeletal Complaints and Stomatological Pathologies				
Fruit (powdered with *A. vasica* Nees)	Karnataka, India	Fever	2005	[34]
Seeds (mixed with powder of *H. isora* L., *A. sativum* rhizome, and gingelly oil, and boiled)	Tamil Nadu, India	Earache	2007	[18]
Different parts of the plant (combined with *A. vasica* and honey to form a paste)	Western Ghats, India	Headache, earache	2011	[20]
Different parts of the plant (combined with *E. cardamomum*, *A. galanga*, *Z. officinale*, and sugar)		Headache		
Fruits (mixed and macerated with other plant parts and then made into a paste)	Jessore & Narail, Bangladesh	Bone fractures	2011	[35]
Seeds (paste with *Caesalpinia bonduc* L. and honey)	Odisha, India	Fever	2012	[14]
Seeds (powdered and applied with salt)	Tamil Nadu, India	Toothache	2012	[26]
Fruits (combined with different plant parts, ground and boiled in water)	Karnataka, India	Gingival wounds	2012	[27]
Seeds [with macerated leaves of *E. indica* (L.) Gaertn]	Rajshahi & Nawabganj, Bangladesh	Spinal cord pain	2013	[36]
Seeds [powdered with macerated roots of *L. aspera* (Willd.) Link]		Headache due to fever or dog bite		
Seeds (powdered with crushed bark of *D. peregrina* L.)		Chest pain (various causes)		
Seeds (powdered with macerated whole *S. cordata* to create a paste)		Abscess		
Fruit (decoction or infusion)	Batan, Philippines	Toothache	2013	[37]
Seeds	Assam, India	Pain (of various causes)	2013	[29]
Fruit (powdered in *C. serratum* decoction/root extract)	Bengal & Andhra Pradesh, India	Fever	2014	[38]
Fruits	Tamil Nadu, India	Throat pain	2015	[15]
Seeds (paste)	Kerala, India	Fever, cuts, wounds	2015	[21]
Seeds/fruits (as part of a multi-plant mixture)	High Atlas, Morocco	Musculoskeletal complaints	2016	[39]
Seeds/fruit (powdered)	Khushab, Pakistan	Malarial fever and body pain	2017	[40]
Seeds (combined with animal and plant parts)	Chota Nagpur Plateau, India	Headache	2017	[41]
Fruit (powdered in *O. sanctum* L. leaf decoction with palmgur)	India (different regions)	Fever	2018	[42]
Different parts (combined with *C. caudata*, *O. sanctum*, *C. monophyla*, and *P. nigrum*, combined with milk and honey)	South Western Ghats	Fever	2018	[9]
Gynaecological and Reproductive System Pathologies				
Fruits (mixed with *C. pareira* L.)	Assam, India	Post-partum birth control	1982	[43]
Fruits	Assam, India	Post-partum pain	2008	[44]
		Lactation difficulties		
Seeds [combined with a paste of *T. cordifolia* (Willd.)]	Eastern Rajasthan, India	Leucorrhoea	2010	[45]
Fruit (powdered mixed with the root juice of *A. aspera*)	Narail & Jessore, Bangladesh	Menstrual pain	2011	[35]
Fruits (powdered and added to a macerated mixture of different components to make pills)		Prolapse of uterus		
Seeds (macerated with roots of *P. daemia* Forsk., and made into pills)	Rajshahi district, Bangladesh	Menstrual irregularities	2012	[46]
Seeds	Assam, India	Post-labour ailments	2013	[29]
Fruit (powdered, mixed with *P. hydropiper* leaf paste and made into pills)	Jhenaidah, Bangladesh	Menstrual pain	2014	[31]
Seeds (ground with newly-sprouted *N. arbor-tristis* leaves to make pills)	Jammu, India	Menorrhagia	2014	[47]

Seeds (powdered with leaves of *P. granatum* and mixed with water; a filtrate is then produced)		Leucorrhoea		
Seeds (powdered with eight spoons of *S. indicum* seeds and jaggery and boiled in water)		Oligomenorrhea		
Seeds (powdered with two spoons of *S. indicum* seeds and jaggery and boiled in water)		Hypomenorrhoea		
Fruit (macerated)	Andaman & Nicobar islands, India	Lactation difficulties	2015	[48]
Seeds/fruits (as part of a multi-plant mixture)	High Atlas, Morocco	General gynaecological complaints	2016	[39]
Different parts (combined with *A. excelsa*, *C. aromatica*, *P. nigrum* and *P. betle,* and coconut oil)	South Western Ghats, India	Menstrual irregularities	2018	[9]
Neurological Pathologies				
Seeds [powdered and added into the flower extract of *Calotropis procera* (Aiton)]	Uttarakhand, India	Epilepsy	2013	[49]
Leave extract	Thailand	Insomnia	2014	[50]
Seeds (made into pills combined with *L. interrupta* roots)	Chota Nagpur Plateau, India	Epilepsy	2017	[41]
Seeds (made into pills combined with other plants)				
Seeds (mixed with honey)		Insomnia		
Endocrine Pathologies				
Fruits (crashed with fruits of *C. nymphaeifolia* Vent.)	Jessore, Bangladesh	Goiter	2011	[51]
Seeds (added to a juice of three leaves of *M. charantia* L.)	Mauritius	Type 2 diabetes mellitus	2014	[52]
Fruits (used to make a decoction)	Songkhla, Thailand	Diabetic neuropathy	2015	[53]
Different parts (with *A. marmelos*, *C. aromatica*, *P. betle*, coconut oil and honey to make a herbal preparation)	South Western Ghats, India	Diabetes mellitus	2018	[9]
Ailments of Viral Cause and Common Cold				
Different parts of the plant (combined with other plants into a paste)	Shimoga, India	Common cold	2010	[54]
Seeds, fruits	Orissa, India	Common cold	2010	[19]
Different parts of the plant (combined with *A. vasica* and honey to form a paste)	Western Ghats, India	Common cold	2011	[20]
Seeds (powdered, with dried ginger and palm sugar made into a coffee-like preparation)	Tamil Nadu, India	Common cold	2012	[26]
Seeds (powdered, added with salt in an omelette)				
Fruits (mixed with multiple plant components in a complex preparation)	Rajshahi, Bangladesh	Influenza	2012	[55]
Seed (powder with roots of *S. virginianum* L.)	Rajshahi & Nawabganj, Bangladesh	Chickenpox	2013	[36]
Leaves (mixed with ginger and honey)	Kodagu, India	Common cold	2013	[56]
Fruits (powdered for oral administration)	Tamil Nadu, India	Viral hepatitis	2014	[57]
Seeds (used to make a decoction)	South Western Ghats, India	Common cold	2018	[9]
Different parts (combined with *E. variegata*, *O. tenuiflorum*, *P. nigrum* and *P. betle*, and honey)				
Treatment of Parasitic Infections				
Fruits (crushed and mixed with ghee)	Assam, India	Scabies	2006	[58]
Seeds (boiled in milk and water)	Eastern Rajasthan, India	Malaria	2010	[45]
Leaves (crushed, applied either alone or in combination with other components)		Ringworm		
Fruits	Karnataka, India	Scabies	2014	[59]
Treatment of Bites				
Seeds (powder for internal administration)	Tamil Nadu, India	Snake bite	2007	[18]
Seeds (powdered and mixed with butter)	Eastern Rajasthan, India	Snake bite	2010	[45]
Fruits (macerated with leaves of *A. indica*)	Narail & Jessore, Bangladesh	Snake bite	2011	[35]

Fruits (made into a paste with lime juice and the roots of *L. indica*, and *C. medica*)	Karnataka, India	Snake bite	2012	[27]
Fruits (ground with root of *D. metel*)	Jhenaidah, Bangladesh	Dog bite	2014	[31]
Flowers (mixed with ghee)	Various regions, India	Snake bite	2017	[60]
Different parts (combined with *C. diococcum*, *C. aromatica*, *P. nigrum*, and *P. betle* and salt)	South Western Ghats, India	Dog bite	2018	[9]
Different parts (combined with *T. indica*, C. *aromatica*, *P. betle* and coconut oil)		Scorpion bite		
Treatment of Skin Diseases				
Seeds (powdered and added to crushed, soaked and filtered *P. murex* L., mixed with sugar candy)	Eastern Rajasthan, India	Various skin diseases	2010	[45]
Seeds (macerated with part plants and honey to make pills)	Jessore, Bangladesh	Various skin diseases	2011	[51]
Fruits (ground with minerals, camphor and other plant parts)	Karnataka, India	Foot xeroderma	2012	[27]
Fruits (made into a paste with other plant parts and coconut oil, and boiled)		Gangrene		
Seeds (powder mixed with betel leaf paste)	Hyderabad, India	Eczema	2013	[61]
Fruits	Karnataka, India	Dandruff	2014	[59]
		Pruritus		
		Eczema		
		Bed sores		
		Boils		

Another application regarding the cardiovascular system concerns the use of seeds of *P. nigrum*, combined with roots of *Rawolfia serpentina*, dried rhizome of *Zingiber officinale*, and seeds of *Piper longum*, all in paste form, which are used to make tablets; these are then administered orally for 10 to 15 days to treat haemorrhoids [14]. In Tamil Nadu, India, the fruit of *P. nigrum* is used to promote a state of overall health and good circulation [15].

Many ethnomedical and ethnobotanical applications are concerned with swelling and the presence of oedema, either localised or generalised. In many cases, a pathology of cardiovascular nature is the cause of oedema formation [63]. In the district of Amravati, India, a decoction made from the leaves of *Zingiber officinale* and P*. nigrum* seeds is taken once daily for 5 days to treat generalised swelling [12]. A paste made from leaves of *Clitoria ternatea* L. and *P. nigrum* seeds is applied on swollen legs in Sivagangai district, India [13].

Applications in Respiratory System Pathologies

In the region of Western Ghats, the Kani healers combine *Elettaria cardamomum*, *Alpinia galanga*, and *Zingiber officinale*, combined with sugar, to treat headache and asthma [20]. In the region of Kerala, the raw fruit of black pepper is used to treat sinusitis [21], while the seed and fruit are used to treat cough [19], in Orissa, India. In Odisha, a decoction of black pepper seeds and *Vitex negundo* L. leaves mixed with honey is taken orally to relieve cough [14].

In Madhya Pradesh, India, a preparation consisting of the dried leaf powder of *Glossocardia bosvallia* and the powder of *P. nigrum* seeds is administered to patients with whooping cough [22]. This pathology is caused by *Bordetella pertussis*, a bacterial pathogen whose presence has been recorded in human populations for centuries. Its importance is currently considered to be of endemic proportions despite the existence of an appropriate vaccine [64].

Applications in Gastrointestinal System Pathologies

For general gastric problems, in the region of Western Ghats, India, *P. nigrum* is combined with *Tephrosia purpurea*, *Carmona retusa*, and *Piper hymenophyllum* and used by the Kani healers [20]. In the Nadar community of the Atoor village, in Tamil Nadu, black pepper is used to treat gastric complaints of various causes [26]. In another area of Tamil Nadu, gastric complaints are treated with a mixture of *P. nigrum* leaf juice, leaf powder, and root bark powder of *Kleinia grandiflora*, and powder from *Cardiospermum halicacabum* leaves [23].

The powder of *Piper nigrum* fruits is administered in the Silent Valley of Tamil Nadu, to treat stomach ache and indigestion [32]. In other parts of India, the dried seeds are taken orally [24]. An infusion made from *P. nigrum* leaves is used to treat digestive problems by Polish migrants in Misiones, Argentina [30].

In Assam, the seeds of the plant are given to treat indigestion [29]. The raw fruit of *P. nigrum* is given to treat vomiting in regions of Kerala [21]. In the case of a stomach ache, the same preparation as for dog bites is used in some regions of the SW Ghats, India [9]. In Bargarh, the powder of seeds and fruits is combined with different parts of different parts, in various formulations, to treat infantile diarrhoea, in infants and adults, and dysentery [65].

Quite a number of formulation exist regarding the treatment of jaundice; for example, in the region of Karnataka, *P. nigrum* seeds are mixed with roots of *Tylophora asthmatica* L., garlic and fruits of *Syzygium cumini*; in the region of Assam, the fruits of black pepper are used in powder form; in the region of Maharashtra, its seeds are mixed with jaggery and *Balanotis roxburghii* seeds; in the region of and Tamil Nadu, its fruits are is combined with ground leaves of Cynodon dactylon and Phyllanthus amarus and an extract is produced [28]. This last use is also mentioned by [66], based on the earlier research of [67]. Regarding liver pathologies, a paste containing crushed black pepper seeds and four other ingredients is used by the Khmer traditional healers in Cambodia [33].

For the treatment of ulcers, in the Jhenaidah district of Bangladesh, leaves of *Polygonum hydropiper*, nutmeg, a fruit of *Terminalia chebula*, *Carum carvi*, and young leaves of *Cynodon dactylon* are mixed with *Piper nigrum*, and macerated to prepare a pill mixture [31]. Another therapy, this time specifically for intestinal ulcers, comes from the Uttara Kannada district of India, where the fruits of *P. nigrum* are used in combination with about 10 different plant parts and made into a water decoction with jaggery to be orally administered; the same preparation is used in the case of aphthae.

Antipyretic and General Anti-Inflammatory Applications and Musculoskeletal Complaints

In the village of the Shimoga district, Karnataka, India, black pepper powder is mixed into the leaf paste of *Adhatoda vasica* Nees; this formulation is then made into pills for oral administration [34]. In other parts of India, black pepper powder is added to *Clerodendrum serratum* decoctions or aqueous root extracts, which are then administered once per day orally, for three days [38]. The Kani healers of Western Ghats have a preparation containing *P. nigrum*, honey, and parts of *Alpinia vasica*, which they use to cure headaches and earaches; the same tribe uses another formulation to treat headaches as well as asthma [20]. In the region of Assam, seeds of black pepper are used to treat pains in different parts of the body, from different causes [29]. The fruits of the plant are used to treat throat pain in the area of Tamil Nadu [15].

In different regions of India, *P. nigrum* and palmgur are added to the leaf decoction of *Ocimum sanctum* L. [42]. A similar preparation is used in the SW Ghats [9]. In the region of Kerala, the paste of the seeds is known to have an anti-inflammatory effect in cases of fever, cuts, and wounds [21]. In the district of Odisha, *Caesalpinia bonduc* L. is made into a paste along with seeds of *P. nigrum*, and taken with honey to cure fever [14]. A complex mixture of plant and animal parts, containing black pepper seeds, is made into pills and administered orally once per day, for 10 days, to treat headache in the Chota Nagpur Plateau [41].

For the treatment of earache, in the area of Tamil Nadu, the fruit powder of *Helicteres isora* L. is boiled with *P. nigrum* seeds, *A. sativum* rhizome, and gingelly oil; the resulting fluid is applied locally in the affected area [18].

In Pakistan, black pepper seeds are also known for their antipyretic properties, and also for their action against fever-induced body pain, when the fever is of malarial origin [40].

In Bangladesh, there are many different anti-inflammatory uses of black pepper reported, such as a paste made from black pepper seeds and macerated leaves of *Eleusine indica* (L.) Gaertn applied once daily for 21 days, in cases of pain in the spinal cord. In the case of severe headache due to fever or dog bites, the macerated roots of non-flowering *Leucas aspera* (Willd.) Link is mixed with 10-15 powdered seeds of *Piper nigrum* L., and prepared for oral administration. As a form of abscess treatment, a paste made from macerated *Sida cordata* (Burm.f.) Borss.Waalk. is mixed with black pepper seed powder and applied around the abscess. Finally, in the case of chest pain of various causes, seed powder is mixed with crushed bark of *Diospyros peregrina* L., and applied locally, particularly in the cases where the chest pain is caused by gout [36].

In other regions of Bangladesh, in Jessore and Narail, some of the leaves of *Morus indica* are mixed with rhizomes of *Zingiber officinale*, 20 fruits of *Piper nigrum*, and 25-30 drops of oil prepared from seeds of *Brassica campestris*; this mixture is then macerated thoroughly and applied as a thick paste over the affected area. A bandage is then placed over the applied paste and changed, along with the mixture, every 7 days [35]. Another preparation, containing *P. nigrum* as part of a multi-plant mixture called Msahan, is used in the High Atlas region of Morocco [39], to treat general musculoskeletal complaints.

In the Philippines, a decoction or infusion of black pepper is used to cure a toothache [37]. In India, again, to treat toothache, in the Atoor village, powdered pepper and salt are applied in dental cavities [26]. Still on the subject of oral-related pathologies, a complex infusion is made, containing many different plant parts along with *P. nigrum fruits*, which is then used, when lukewarm, to gargle, when there are gingival complaints [27].

Applications in Gynaecological and Reproductive System Pathologies

One ethnomedicinal preparation containing *P. nigrum*, as part of a multi-plant mix, comes from the High Atlas region of Morocco, and is named Msahan; it is used generally to treat various types of gynaecological complaints [39]. For post-labour ailments, the seeds of *P. nigrum* are used in Assam, India [29]; specifically for post-partum pain, the fruit of *P. nigrum* is administered in Tinsukia district, India [44]. In the same regions, there also exist some ethnomedical recipes for post-partum birth control [68]; for example, in some cases, the fruits of *P. nigrum* are used, along with pieces of *Cissampelos pareira* L., and the duration of administration depends on the desired length of contraception [43].

Menorrhagia, i.e., heavy menstrual bleeding, is defined as a bleeding of over 80 mL per menstrual cycle, and usually occurs as a result of uterine abnormalities, endometrial abnormalities, ovulation, or coagulation disorders; the treatments and their effectiveness vary depending on the primary cause [69]. In Udhampur, India, the newly sprouted leaves of *Nyctanthes arbor-tristis* are ground along with Piper nigrum seeds to make 2 g tablets, which are administered twice per day for two weeks [47].

Oligomenorrhoea is another type of menstrual cycle disorder, where the menstrual cycle lasts more than 35 days and less than 90 days [70], or the total menstrual cycles per year are between 5 and 7 [71]. It is a problem of increasing importance in recent years [72]. The usual causes are associated with endocrine disorders [73]. In Udhampur, India, eight spoons of *Sesamum indicum* seeds and the powder of *Piper nigrum* seeds and jaggery are boiled in water until the volume is reduced by half; the produced liquid is taken twice a day, for 15 days, before the due date of each successive period [47]. A similar treatment is used in cases of hypomenorrhea [47], which is defined as a menstrual bleeding of 1-2 days or when the amount of blood is too low, even in the context of a normal duration of menstruation [74].

Leucorrhoea, or fluor albus, may be physiological in women, during menstruation, or pathological; in this latter case, a foul smell, colour changes, and other symptoms such as itching and a burning sensation are present. Usually, pathological leucorrhoea is associated with a urogenital infection [75]. There are two recorded ethnobotanical treatments for leucorrhoea containing *P. nigrum* in India. The first one is from the region where a paste of *Tinospora cordifolia* (Willd.) is combined with five seeds of black pepper and administered once in the morning [45]. In the district of Udhampur, India, the leaves of *Punica granatum* are crushed, the powder from *P. nigrum* seeds is added, and then both are mixed with water; a filtrate is then produced, which is consumed twice a day [47].

Another common gynaecological problem is menstrual pain, i.e., primary dysmenorrhoea, which affects both young and adult females, especially from a quality-of-life perspective; treatment remains mostly symptomatologic [76]. The Kavirajes tribe in Bangladesh mixes one fruit of *P. nigrum* with the juice of one root of *Achyranthes aspera*, and gives it to affected females on an empty stomach once only [35]. In the same country, in the district of Jhenaidah, a paste obtained from the leaves of *Polygonum hydropiper* is mixed with 1 powdered fruit of *Piper nigrum*, to make pills that are taken thrice per day for 2-3 days [31].

Menstrual cycle irregularities are a relatively common problem in many populations, affecting between 5% and over 30% of females of reproductive age [77]. There exists several modifiable and non-modifiable risk factors associated with irregular menstruation [78,79]; regardless, irregular menstruation is associated with some pathologies and a decrease in quality of life (80). In the SW Ghats, India, *Ailanthus excelsa*, *Curcuma aromatica*, *Piper nigrum*, and *Piper betle* are combined with coconut oil and made into a herbal remedy for the cure of menstrual problems [9]. In the Rajshahi district, Bangladesh, the roots of *Pergularia daemia* Forsk. are macerated with 2-3 seeds of *Piper nigrum L.*, and then pills are made, which are taken thrice daily [46].

Uterine prolapse, one of the manifestations of pelvic organ prolapse in women, affects over 5% of most female population worldwide [81-83]. In Bangladesh, in the Jessore and Narail districts, the roots of *Abutilon indicum*, *Glycosmis pentaphylla*, *Eleusine indica*, and *Amaranthus spinosus*, are mixed together and macerated, and then sugar and 21 powdered black pepper fruits are added and 42 pills are prepared from this mixture; 1 pill is taken daily for 14 days [35].

In the case of lactation difficulties, the macerated fruit of *P. nigrum* is orally administered by the healers of the tribes of Andaman and Nicobar islands, India [48]. In Tinsukia district, the fruit of *Piper nigrum* is used to increase breast milk production [44].

Applications in Neurological Problems

Of particular note are the mentions of *P. nigrum* in the context of traditional Indian medicinal approaches to epilepsy. The seed of the plant, when combined with either *Laportea interrupta* roots or the stem of *Cissus repanda*, and the roots of *Leea macrophylla*, and *Mucuna pruriens*, is used to treat epilepsy in the Chota Nagpur Plateau, in India. Also in the same area, for the treatment of insomnia, *P. nigrum* is mixed with fresh honey from the Indian honey bee (*Apis cerana indica*), and applied inside the nose [41]. In the region of Uttarakhand, powdered seeds of *P. nigrum* are mixed with the flower extract of *Calotropis procera* (Aiton) [49]. Meanwhile, in Thailand, the leaf extract from *P. nigrum*, which has a demonstrably high melatonin content, is traditionally used to treat insomnia [50].

Applications in Endocrine Pathologies

In Jessore, Bangladesh, the crushed fruits of P. nigrum are combined with crushed fruits of *Colocasia nymphaeifolia* Vent. and taken twice daily for 7 days, to treat goiter [51]. Goiter can be attributed to a number of causes, the most common of them being iodine deficiency; the worldwide prevalence of goiter is quite high [84].

For the treatment of diabetes mellitus, in the Walayar valley, India, different parts of *Aegle marmelos*, *Curcuma aromatica*, *Piper nigrum*, and *Piper betle* are combined with coconut oil and honey to make the relevant herbal medicine [9]. Specifically for type 2 diabetes mellitus, a juice from three leaves of *Momordica charantia* L. and *Piper nigrum*, to be consumed once a week, is administered [52]. For the treatment of one of the complications of diabetes, diabetic neuropathy-associated numbness, a decoction made from the fruits of black pepper in *Songkhla province*, Thailand, its administration has a proven hypoglycaemic effect in rats [85].

Applications in Ailments of Viral Cause and Colds

In Bangladesh, the roots of *Solanum virginianum* L. are powdered and mixed with black pepper seed powder to cure chickenpox [36], an extremely contagious viral disease caused by the Varicella-Zoster virus [86]. In Tamil Nadu, India, the powder from *Piper nigrum* fruits is administered orally in cases of viral hepatitis [57]; viral hepatitis is attributable in most cases to the five different hepatitis viruses [87].

In the district of Shimoga, India, to treat the common cold, *P. nigrum* Linn. and *Allium sativum* Linn. are combined with plants of the Caesalpiniaceae spp. and made into a paste, which is taken orally for 3-4 days [54]. In Tirunelveli hills of Western Ghats, India, *P.*

nigrum is combined with *Alpinia vasica* and honey, and the resulting paste is used by Kani traditional healers against the common cold, but also against headache, and earache [20]. In the Karnataka region of India, the leaves of *P. nigrum* are pounded and mixed with ginger and honey; they are taken for 3-5 days orally to treat the common cold and associated cough [56]. The fruits and seeds of *Piper nigrum* are used to treat the common cold in Orissa, India [19]. Seed powder is swallowed to treat cough and bronchial disorders in Coimbatore district, India [18].

In the Kanyakumari district, Tamil Nadu, India, black pepper is used in a number of formulations for different ailments. Regarding the treatment of the common cold, black pepper, dried ginger, and palm sugar are mixed along with water to prepare a coffee-like drink, which is then administered to patients; omelettes are also made with powdered pepper and salt and given to such patients [26].

In the Walayar valley of India, a decoction made from seeds of *P. nigrum* is used as a treatment for cough and cold; another more complex herbal preparation containing black pepper, from the same area, also exists [9].

For the treatment of influenza, caused by the viruses of the Orthomyxoviridae family [88] in Rajshahi district, Bangladesh, a complex preparation comprising 12 fruits of black pepper, along with a number of different plant parts, is used [55].

Applications in the Treatment of Parasitic Infections

There are reports that if black pepper seeds are boiled in milk and water and administered orally, once in the morning for a period of 4 to 5 days, the multiplication of malarial parasites is decreased and splenomegaly regresses [45]. In the case of ringworm infection, the crushed leaves of the plant are rubbed directly onto the parasite; in other cases, the leaves are crushed and mixed with cow milk or mustard oil or crushed with bulblets of garlic and mixed with lemon juice is applied on ringworm to affect a complete cure [45]. Both of these antiparasitic applications are common in the region of Eastern Rajasthan, India.

Scabies is increasingly being diagnosed, at least in certain countries [89]; it is caused by the host-specific human scabies mite (Sarcoptes scabiei varietas hominis). While the infection is not life-threatening on its own, bacterial superinfections are common [90]. In the Central Western Ghats region, India, the fruits of black pepper are used to treat scabies [59]. In Assam, the crushed fruits of *P. nigrum* are mixed with ghee and the resulting product is orally administered [58].

Applications in the Treatment of Bites

In Eastern Rajasthan, seed powder mixed with butter is given orally against snake bite [45]. A similar preparation exists for snake-bitten animals in Uttaranchal, India [91]. The same recipe is also known in other areas of India, while the paste of *P. nigrum* flowers with ghee is administered orally for the same purpose [60]. In the region of Karnataka, the fruits of *P. nigrum* along with the root of *Citrus medica* and *Leea indica*, are used to make a paste, mixed with lime juice, which can be both applied externally and swallowed, in cases of snake bite [27]. The powder of black pepper seeds is administered internally to counteract the effects of snake poison, in the *Coimbatore district*, India [18]. In Bangladesh, several leaves of *Aristolochia indica* are mixed with 2-3 fruits of *Piper nigrum*, and are then macerated; finally, a juice is obtained which is administered immediately [35].

Finally, for the treatment of dog bites, in the district of Jhenaidah, fruits of *P. nigrum* are mixed with roots of Datura metel and ground. The resulting ground mass is administered orally as soon as possible [31]. In the SW Ghats, India, *Canthium diacocum* is combined with *Curcuma aromatica*, *Piper nigrum*, and *Piper betle*, and salt, to make a preparation used in the case of dog bites [9]. In the same region, *P. nigrum* is combined with *Tamarindus indica*, *Curcuma aromatica*, *Piper betle*, and coconut oil to treat scorpion bites [9].

Applications in the Treatment of Skin Diseases

Crushed *Pedalium murex* L. is soaked in water overnight, filtered the next morning, mixed with sugar candy and seed powder of black pepper, and then administered orally as a treatment for various skin diseases in Eastern Rajasthan, India [45]. Again, for skin diseases generically, seeds of *P. nigrum* are macerated with roots of *Rauwolfia serpentina* (L.) Benth. ex Kurz and rhizomes of *Zingiber officinale* Roscoe, with honey added afterwards; in this way, pills are made which are administered daily for 3 weeks, for prophylaxis, in Jessore, Bangladesh [51]. In Central Western Ghats, India, the fruits of *P. nigrum* are used to treat dandruff, pruritus, eczema, bed sores, and boils [59]. Regarding eczema, a different traditional cure, comprising the powder of black pepper seeds and betel leaf paste, applied daily, is used in Hyderabad, India [61].

For foot xeroderma, the fruit of *P. nigrum* along with the seed and bark of *Semecarpus anacardium*, the fruit of *Vernonia anthelmintica*, the flower bud of *Syzygium aromaticum*, and the rhizome of *Curcuma longa* are ground with copper sulphate, camphor tablets, and boiled in cow's ghee, grease, and paraffin; they are then applied externally [27]. A rather more complex preparation with more than 10 plant parts, comprising also the fruits of *P. nigrum*, is made into a paste with coconut oil and boiled; it is applied externally after it has cooled [27].

DISCUSSION

Research in phytochemistry has revealed the remarkable therapeutic properties of black pepper. While its antimicrobial, antiparasitic, and antiviral effects are well-known, many other beneficial properties of black pepper extracts have been researched thoroughly, including its anti-inflammatory, anticonvulsant, and analgesic properties [92-94]. It seems that it may be possible to use it, in an adjuvant role, in a mode similar to that of capsaicin [95,96]; perhaps its integration in 3D-printed biomaterials [97-99] as a potent antimicrobial would represent another promising application. Related to inflammation, based on a review of available data, concluded that the terpenes present in black pepper, in combination with taurine, can have an anti-atherogenic effect, with parallel antimicrobial and metabolic benefits [100]. Given the extensive number of ethnomedical and ethnobotanical data, as presented in this text, and also discussed by various authors [11,101,102], it can be observed that a number of them are corroborated by recent experimental data, while the effectiveness of others is still to be determined. For example, the addition of piperine in the treatment of jaundice or of various gynecological problems warrants further research. Cutaneous applications may show effectiveness due to the potential of piperine to modulate cellular and humoral components in the skin, as well as interact with inflammatory pathways, in a similar manner as other phytochemicals [103-106]. Other traditional applications of piperine in neurological problems, namely epilepsy, may be of importance, given the prevalence and severity of certain epileptic syndromes [107-109]. In addition to testing the effectiveness and applicability of reported uses, a sustained effort should be made to complete the recording of such uses across different cultures, where black pepper has been used extensively for centuries.

Combination of Black Pepper with Acupuncture

Given that capsaicin has been proven an effective anti-bacterial [110] anti-inflammatory agent when applied at acupuncture points [95], a promising research direction seems to be the application of black pepper cream in similar points, alone or combined with capsaicin and other phytochemicals. Currently, there exist protocols for acupuncture in diabetic patients, with the acupuncture points Zusanli (ST36), Shenshu (BL23), Sanyinjiao (SP6), Yishu (EX-B3), and Zhongwan (CV12) being the most studied; protocols for diabetic neuropathy, one of the main complications, also exist and are presented in detail by Ma et al. [111]. The available evidence indicates that acupuncture is useful as an adjuvant therapy in diabetic patients [112], although the design and completion of more long-term trials is considered necessary.

Continuing on the potential combinations of acupuncture with the traditional applications of black pepper, it is noted that based on the research from the area of Bangladesh, black pepper is applied as a paste in case of gout-related chest pain [36]. Regarding gout and gouty arthritis, there are currently available acupuncture protocols [113-115], while based on a recent case study, it is possible to use acupuncture and moxibustion in the context of renal insufficiency [116]. Notably, recent research has demonstrated the renoprotective potential of piperine in the case of hyperuricemic nephropathy [117].

Black Pepper in Metabolic Diseases

Another interesting research direction concerns the effect of black pepper on glucose and lipid regulation. Regarding glycaemic control, in rats, black pepper extract was found capable of reducing blood glucose [85,118]. Inhibition of aldose reductase was noted by [119]; aldose reductase is the enzyme most associated with the complications of diabetes [120,121]. More recent research results in vitro are also in favour of the hypoglycaemic activity of black pepper [122,123].

These findings are important in the context of increasing incidence and prevalence and complications of diabetes mellitus [124-132], the associated mortality [133-135], the adverse effects of antidiabetic drugs [136-138], and the burden of disease [139-141]. Moreover, intestinal dysbiosis, specifically with Enterobacteriaceae and Candida species, was found to be a risk factor for diabetes mellitus type 1 [131]; piperine is effective against such pathogens and thus it could, hopefully, be used in a dual antiglycemic-antimicrobial role.

In rats, the addition of black pepper to their diet had a hypolipidemic effect [142,143]. A more recent successful experiment, performed by administering the methanolic leaf extract of *Piper guineense*, a species of pepper native to West Africa, in female diabetic rats, indicates that it can be used in the management of diabetic patients, with no adverse biochemical effects [144]. A leaf extract from the same plant species has demonstrated properties beneficial for glycaemic control in vitro [145]. In rats, immunohistochemical and histological data support the antidiabetic potential of black pepper in combination with other compounds [146], although further research is required on the matter. Such experiments are in accordance with a recent trend in evaluating the potential beneficial effects of spices in diabetic patients [147].

Hyperlipidaemia is associated with diabetes mellitus [148,149] and is, in all contexts, a risk factor for morbidity and mortality [150-152]. Therefore, the administration of black pepper extracts in general and piperine in particular, in high enough – but tolerable – concentrations, will benefit glycaemic and lipidaemic control both in diabetic and non-diabetic patients. Based on evidence on the interplay between obesity and inflammation [153], and taking into account the evidence in favour of piperine application in cases of obesity and inflammation, it is reasonable to consider the potential of a two-pronged effect of piperine administration in such cases.

Anticarcinogenic Effect of Piperine

It appears that there is a significant corpus of data in favour of anticarcinogenic effects of piperine [11,154-157]. When taking into account the different pathways, like molecular and metabolic, implicated in carcinogenesis (e.g., [158-169]), it is clear that there are numerous potential pathways upon which piperine may act to exert its anticarcinogenic potential. We would like to highlight the potential effectiveness of piperine in the treatment of hepatocellular carcinoma [170], the most prevalent form of liver cancer [171], and other phytochemicals were already shown to be good candidates [172,173]; this raises the possibility of combining piperine and the already used somatostatin analogues [174,175], to increase their effectiveness, in this and other forms of cancers. Piperine could be combined with a number of other natural compounds, which have a known anticarcinogenic potential [168,176] to maximize their efficiency; furthermore, taking into account the potential associations of certain pathogens with cancer [177,178], it could function both in an anticarcinogenic and anti-inflammatory role. The association of diabetes and colorectal cancer [179] also opens new avenues for piperine applications with a dual effect.

Non-medical properties of P. nigrum

Black pepper also has some notable ethnomedical veterinary uses, with the fruit being included in pastes or creams or infusions, to treat blotting [180], constipation, skin infections [181], various ailments [55], and helminthic infections [182] in animals. Other uses are also reported in the literature [183-187].

Perhaps more important are the applications of *P. nigrum* as a component of insecticides and larvicides. Early evidence of the insecticidal and larvicidal potential of black pepper, piperine, and its derivatives was provided by a number of experiments [188-190]. A later experiment by [191], documented the toxicity of black pepper extract on the rice weevil, *Sitophilus oryzae* (L.). It was previously observed that when exposed to white pepper, all the *Anopheles larvae* died [192], and it was then that the potential of this plant as an anti-malarial agent was first realised. A mix of ground black pepper and piperine was found to be very effective against a number of larvae of the same species [193]. Crucially, the mode of action of black pepper extracts is different from that of pyrethroids, meaning that potential resistance to the latter will not affect the action of the former; they both exhibit a marked potential for synergistic action between them [194]. Even more importantly, black pepper extracts and piperine are not toxic to other organisms and do not persist in the environment [195,196]. Piperine itself has been shown to be effective against the larvae of *Aedes aegypti*, the vector of the yellow fever and Dengue fever viruses [197,198]. Regarding a less dangerous species of mosquito, *Culex pipiens*, the common house mosquito, the study of [199] demonstrated that piperine-based dienehydrazide derivatives were more effective than piperine itself or Deltamethrin. Another study investigated the potency of *P. nigrum* against ant species, along with that of other plant extracts; the results were promising, revealing a viable alternative [200].

Given the adverse impacts of larvicides and insecticides in both the environment and human health [201-203], and existing threats to the biodiversity of different environments [204-207], efforts to incorporate natural compounds into insecticides and larvicides should intensify. The increased effectiveness of such compounds is important both in the context of agriculture [208-210] and, perhaps more importantly, in the context of the insect-borne diseases [211-213].

In response to the aforementioned health and safety concerns, both the European Union (EU) and the United Nations (UN) (https://sdgs.un.org/goals) have implemented measures to regulate and reduce reliance on hazardous chemicals, while exploring natural alternatives. The EU's regulatory framework (e.g., Regulation (EC) No 1107/2009 [214], Regulation (EU) 2022/1438 [215], Directive 2009/128/EC [216]), multilateral treaties like the Rotterdam Convention [217,218] and the Stockholm Convention [219,220], and research and development (R&D) programs strongly emphasize promoting directly or indirectly innovation in plant-based substances. The EU also provides funding opportunities through Horizon Europe [221], the largest research and innovation program supporting projects in sustainable agriculture, biodiversity, and green technologies.

Additionally, global initiatives such as the International Plant Protection Convention (IPPC) [222], the intergovernmental treaty that aims to protect the world's plants, agricultural products and natural resources from plant pests and the FAO (Food and Agriculture Organization of the United Nations) Guidelines on Pesticide Management [223,224] provide the necessary foundation for the establishment of national legislation and policies for pest and pesticides management, with the potential inclusion of more favourable conditions for biopesticides. Other organizations, such as OECD (https://www.oecd.org/), have dealt with the topic of biopesticides while there are countries worldwide with dedicated frameworks on the matter, like the US EPA (www.epa.gov/pesticides/biopesticides) and China's Plan for Green and Sustainable Agricultural Development [225], encouraging biological control methods to reduce synthetic pesticide use.

CONCLUSION

Piperine and black pepper extracts exhibit ethnomedical and ethnobotanical applications, which should be explored further, with the aim of developing novel therapeutic solutions for a number of different pathologies. Future research should concentrate on elucidating the effectiveness of such applications in in vivo settings, using modern methods, and exploring the potential of increasing the active concentration via the application of novel delivery methods.

Finally, the non-medical applications of piperine and black pepper in general are promising in the field of insecticides/larvicide development, in accordance with the new directives of the EU and of other nations.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization AP, ATP, AFG, DOC and CC; methodology AP, AFG, DOC and CC; validation AP, MP, DOC, and CC; formal analysis AP, LT, KP, ATP, GMA, AFG and CC; investigation AP, LT, KP, ATP, GMA, SPG, AFG and DLC; resources AFG and DLC; data curation DOC; writing—original draft preparation AP, ATP, SPG, AFG; writing—review and editing AP, ATP, DOC, and CC; supervision AP, MP, DOC and CC; project administration AP, AFG, and DLC. All authors have read and agreed to the published version of the manuscript elaboration

Ethics approval and consent to participate

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REVIEW

The Impact of miRNAs in Diabetes Mellitus

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Abstract: Diabetes mellitus refers to metabolic disorders whose main characteristic is chronic hyperglycaemia. The cause is either disturbed insulin secretion, insulin resistance, or usually both. MicroRNAs represent a subclass of non-coding RNA molecules that are short in length, about 17-26 nucleotides. Since they are circulant and can be tissue-specific, their use as diagnostic biomarkers or screening for different diseases is currently undergoing deeper studies. It was found that 22 miRNAs were associated with the pathophysiology of T1DM, 34 with T2DM, and 16 miRNAs were identified to be common amongst T1DM and T2DM. All of them were reconfirmed in at least two separate studies.

Keywords: microRNA; diabetes mellitus; insulin resistance; pancreatic β cells; gene silencing

INTRODUCTION

Diabetes mellitus (DM) encompasses a group of heterogeneous diseases characterized by hyperglycemia. The most prevalent forms of disease are Type 1 DM (T1DM) and Type 2 DM (T2DM). IDF has also just confirmed type 5 diabetes. It is reportedly characterized by severe insulin deficiency, mainly caused by undernutrition during childhood or adolescent years [1]. T1DM affects approximately 8.75 million individuals globally, whereas T2DM currently impacts about 6.28% of the world's population, accounting for 80% of all diabetes cases. DM represents a significant health issue both in terms of prevalence and the impact of its chronic complications, which can lead to organ failure (e.g., kidneys, retina, heart) [2-4]. Projections indicate that these values are expected to increase significantly in the coming years [5].

T1DM and T2DM are multifactorial diseases. T1DM is typically diagnosed following extensive destruction of insulin-secreting pancreatic β cells (70-80%) by T lymphocytes, resulting in the presence of self-reactive autoantibodies (T1A-DM). Non-immune mechanisms account for 10-30% of T1DM cases (type 1B - T1B-DM subclass), which are classified as idiopathic T1DM [6-8]. Idiopathic T1DM does not have an autoimmune background. Although it can present with insulin deficiency, this subclass of DM does not have a clear trigger. Fulminant T1DM represents another subclass that is also non-immune-mediated. It manifests by fast β-cell death, which may be caused by environmental and genetic backgrounds [9]. Altered insulin secretion, often paired with insulin resistance, represents the main mechanism involved in T2DM [10].

The predisposition for DM remains only partially understood. With some exceptions, a complex network of genetic and epigenetic factors can predispose individuals to T1DM and T2DM [11-15]. Since 2004-2005, exosomal microRNAs (miRNAs) have been included in the list of factors associated with DM and its chronic complications [16-17].

miRNA biosynthesis

Small RNA molecules (e.g., microRNAs -miRNAs; small interfering RNAs -siRNAs) are synthesized in the nucleus and subsequently transported to the cytoplasm. The origin of the miRNA molecules is a primary transcript that contains hairpin-shaped

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structures [18]. The RNase Drosha recognizes and cuts these hairpin structures in RNA, resulting in a miRNA precursor (pre-miRNA) of about 70 nucleotides in length. These molecules exit the nucleus (by means of exportin-5 complex) and in the cytoplasm are recognized and cut at the loop part by the enzyme Dicer [19]. The double-stranded miRNA is then loaded into the Argonaute (Ago) protein complex, and a complete silencing complex is formed, and the miRNA is used as a template for target recognition [18].

These double-stranded RNA molecules are utilized by the RNA-Induced Silencing Complex (RISC) to regulate gene expression [19]. It has been reported that the structural similarity of these miRNA hairpins and anti-Tn-C aptamers indicates miRNA functionality beyond RISC, making them even more sophisticated regulators [20]. The RISC complex acts as a translational repressor or as an mRNA breaker, depending on the number of base pairs that form between base pairs. One miRNA can target multiple genes, and they often return to silence the primary transcript from which they were synthesized.

The silencing process has critical roles in the physiological processes (e.g., cell differentiation, organism development) [21]. The concentration of miRNA was estimated between 1000 copies/ 1000 mm3 cells and around 200 copies/cell (or 166pM). It has been reported in previous studies that the median half-life of miRNA is approximately 5 days, with some exceptions (e.g., miR-155 was reported to have lower stability) [20]. The temporal and spatial deregulation of miRNA expression and the silencing process can result in pathological consequences (e.g., by modulating the expression of pro-inflammatory and pro-apoptotic effectors), which impact disease onset, progression, and severity [18,22]. The possibility of repeated sampling has increased scientists' interest in assessing the potential of miRNAs as biomarkers for malignant (e.g., lung adenocarcinoma, gastric or breast cancers) and non-malignant diseases (e.g., including DM) [23-27]. It should be taken into consideration that the multi-role of these molecules when comprising a panel for clinical usage. Thus, miR-21, miR-326 (e.g., glioblastoma), miR-34a, miR-2a (e.g., hepatocellular carcinoma), miR-155 (e.g., pancreatic ductal adenocarcinoma), miR-23b (e.g., melanoma, prostate cancer), miR-98 (e.g., squamous cell carcinoma; head and neck carcinoma), miR-186 (e.g., Hodgkin's lymphoma), miR-223 (e.g., chronic myeloid leukemia) were associated with different malignant diseases. But some of these miRNAs were also associated with different nonmalignant diseases (e.g., miR-21- cardiomyocyte hypertrophy; immune response in sepsis; miR-326 and miR-186 - multiple sclerosis; miR-590- extrinsic cardiomyopathy, autoimmune myocarditis; miR-223 - hereditary neutrophilia) [28].

STUDY OBJECTIVES

The study aims to perform a bibliometric analysis to evaluate research trends in the field and to identify and characterize miRNAs implicated in DM using curated bioinformatic resources.

MATERIALS AND METHODS

This study was conducted in two main phases: an analysis of the scientific literature and the identification of miRNAs altered in DM based on records in specialized databases.

Bibliometric Analysis. A comprehensive literature search was performed using the Scopus database [29]. The search strategy targeted the Title and Abstract fields with the following keywords: ("hsa-microRNA" OR "hsa-miRNA" OR "microRNA" OR "miRNA") AND ("type 1 diabetes" OR "type 2 diabetes" OR "T1D" OR "T2D") AND ("human" OR "humans" OR "Homo sapiens") NOT ("mouse" OR "mice" OR "rat" OR "rats" OR "murine" OR "animal model" OR "in vivo" OR "non-human" OR "rodent"). The initial search yielded 1730 articles. After excluding irrelevant subject areas (e.g., Engineering, Mathematics, Materials Science, Arts and Humanities, Social Sciences, Physics and Astronomy), 1,707 articles remained. Further filtering by document type (research articles only) reduced the number to 1085. Applying language (English) and publication stage filters resulted in a final dataset of 1051 articles. The literature analysis was conducted using the Bibliometrix R-package (version 4.4.0) and Biblioshiny (version 5.0), which enabled descriptive statistics, thematic mapping, and trend analysis [30]. All analyses were performed in R (version 2025.05.0, Build 496).

MiRBase [31] and TargetScanHuman 8.0 [32] were consulted to check for predicted target transcripts and conservation status of each miRNA. Diseases associated with miRNA were extracted from the Human ncRNA Gene Database [28].

RESULTS

Literature data analysis

The timespan of the analyzed publications was 2008 to 2025. The dataset comprised 1051 documents published across 440 sources, involving a total of 6409 authors and citing 49120 references. The annual growth rate of scientific output was 18.34%. The average age of the documents was 5.34 years, and the mean number of citations per document was 28.12.

Interest in the publication of this data began between 2008 and 2012 (Figure 1). A high increase was noticed from then on until 2015, with a spike from 2016 to 2022.

As for the average number of citations per year, it aligned with the wave of scientific production (Figure 2). The first spike was observed in 2009, followed by a second spike in 2012. A slight rise was noticed again, from 2018 to 2020.

Figure 1: Annual scientific production of data regarding miRNA and DM.

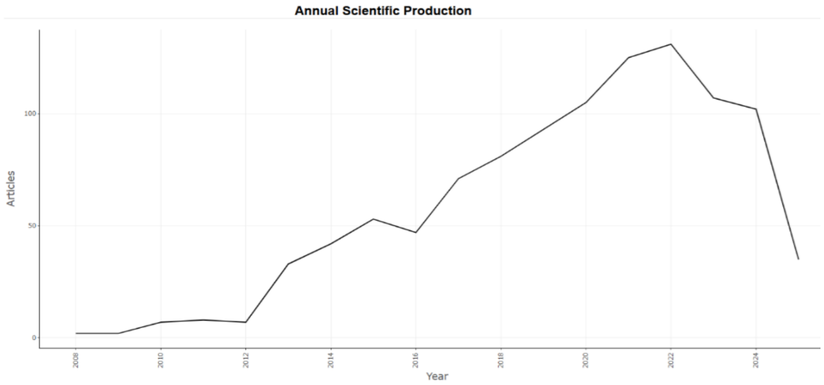

Figure 2: Annual scientific production of data regarding miRNA and DM.

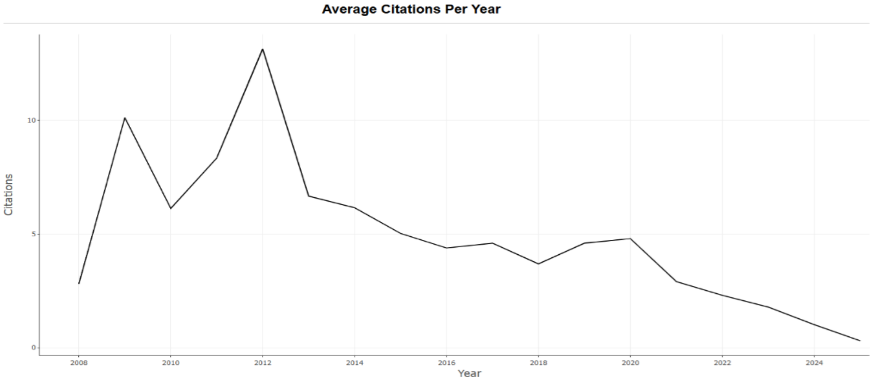

The 1051 articles were published in various sources. The majority of them can be found in PLOS One, Frontiers in Endocrinology, International Journal of Molecular Sciences, and Scientific Reports. Other sources included: Acta Diabetologica, Journal of Clinical Endocrinology and Metabolism, and Molecular Medicine Reports (Figure 3).

Figure 3: Most Relevant Sources containing the included articles.

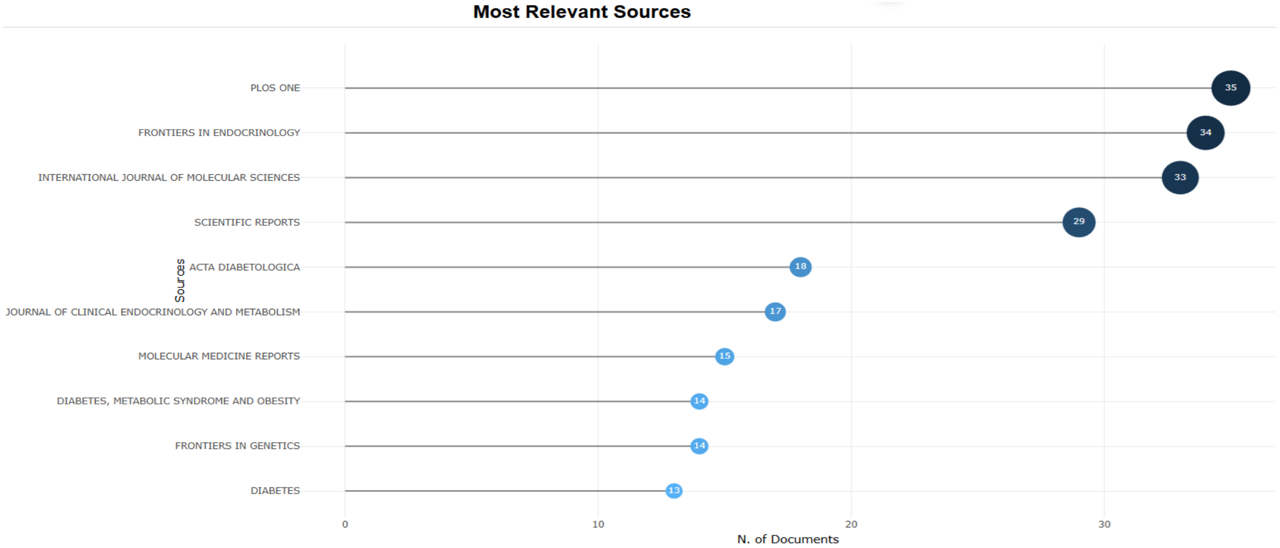

Figure 4: Most relevant authors of articles included in this study.

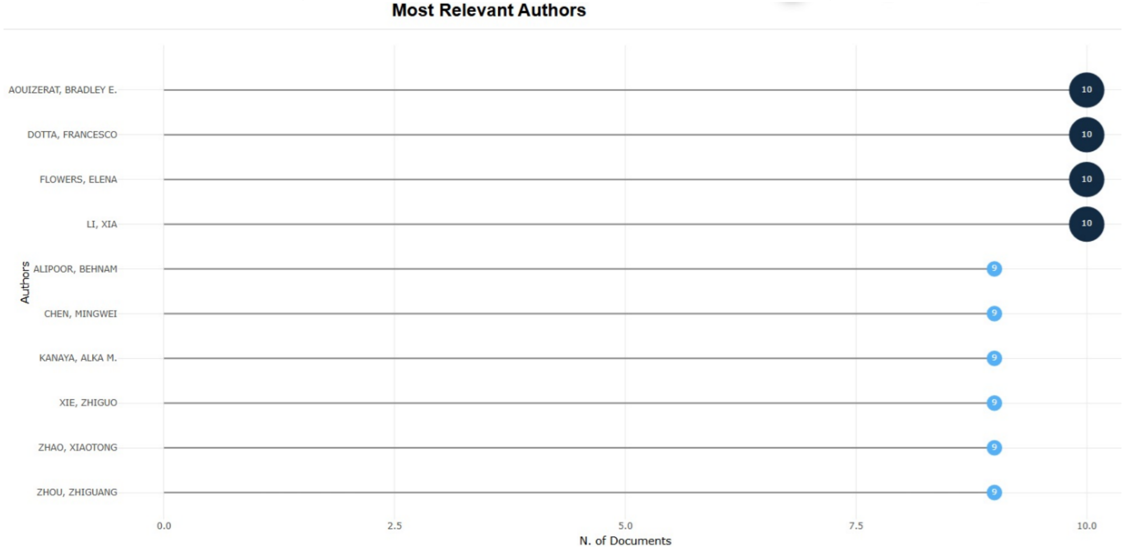

Figure 5: Most relevant affiliations of authors whose papers were cited in this article.

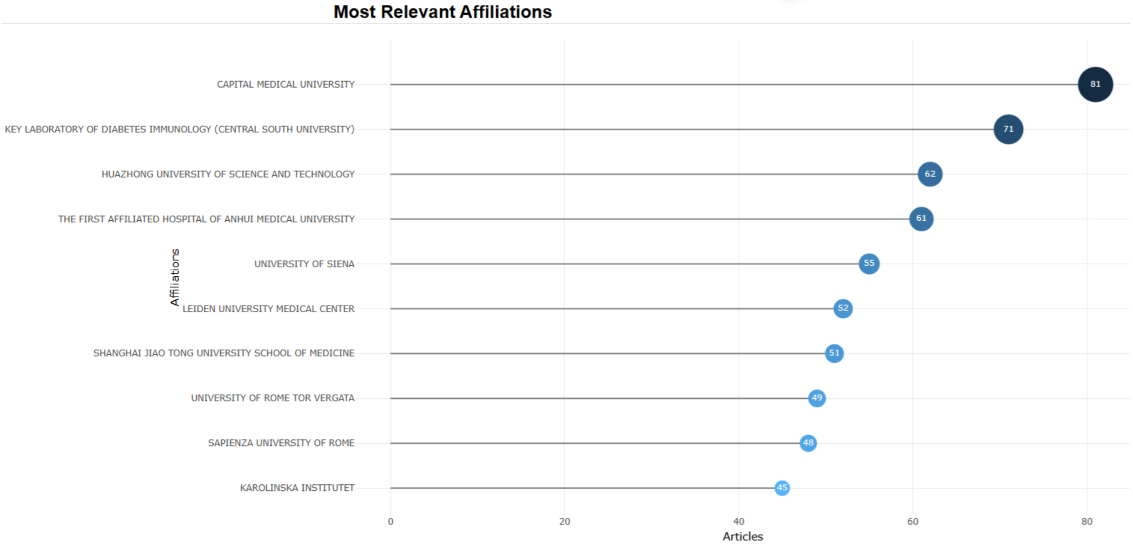

From the 6049 authors who participated in the research included in this paper, some of the most relevant are Dotta F., Flowers E., Li X., Chen M., and Zhao X. (Figure 4). Their affiliations include: Capital Medical University, Key Laboratory of Diabetes Immunology, University of Siena, and Huazhong University of Science and Technology (Figure 5).

Speaking at a global level, the papers with the most citations included in this study were [2,33-36]. The sources in which the works of the aforementioned authors were published include, in order: Oxidative Medicine and Cellular Longevity, Acta Diabetologica, The FASEB Journal, Journal of the American College of Cardiology, and The Journal of Clinical Endocrinology & Metabolism (Figure 6).

The trending topics identified in the reviewed articles highlight the evolving focus areas and emerging themes within the research field over time (Figure 7). Authors' keywords pinpointed in articles that constitute the database change over the years. First time span consisted of keywords such as: “cytokines”, “miR-375”, “single nucleotide polymorphism”. In recent years, keywords such as “diagnosis”, “molecular mechanisms”, “biomarker”, and “type 2 diabetes mellitus” were employed.

The co-occurrence network of authors’ keywords and the microRNA species miR-21, miR-126, and miR-146a highlights the relationship and thematic connections within the literature contained in the database of this paper (Figure 8).

The distribution of corresponding authors by country mirrors the ranking of the most cited countries (Figure 9; Figure 10). Leading the list are countries such as China, the United States, and Italy, which also represent the most active contributors to DM research. In terms of citation impact, Italy is followed by the United Kingdom, Iran, Spain, and Japan.

Figure 6: Most Globally Cited Documents cited in this paper.

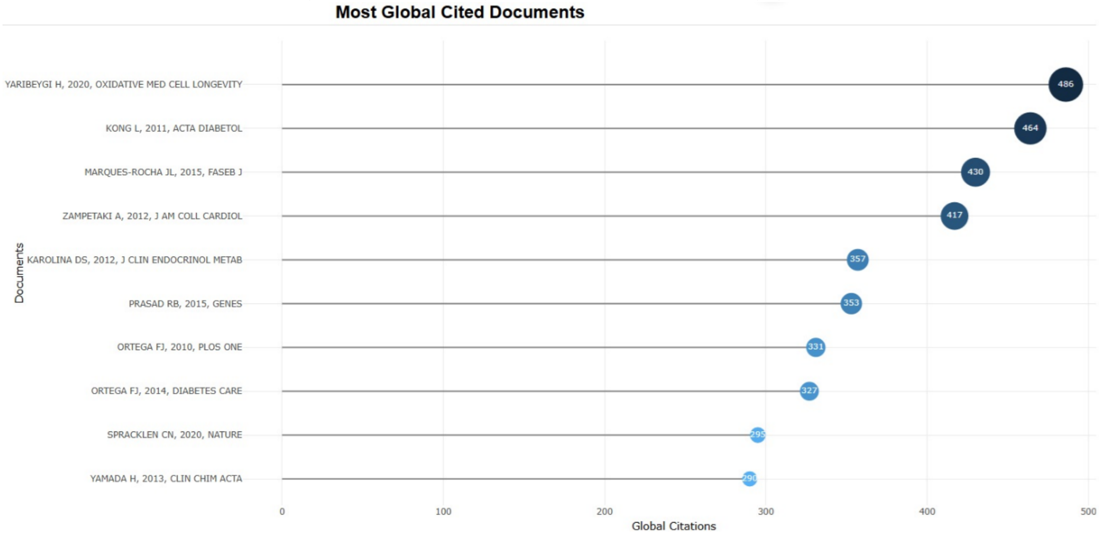

Figure 7: Trend topics identified in articles used in this systematic study.

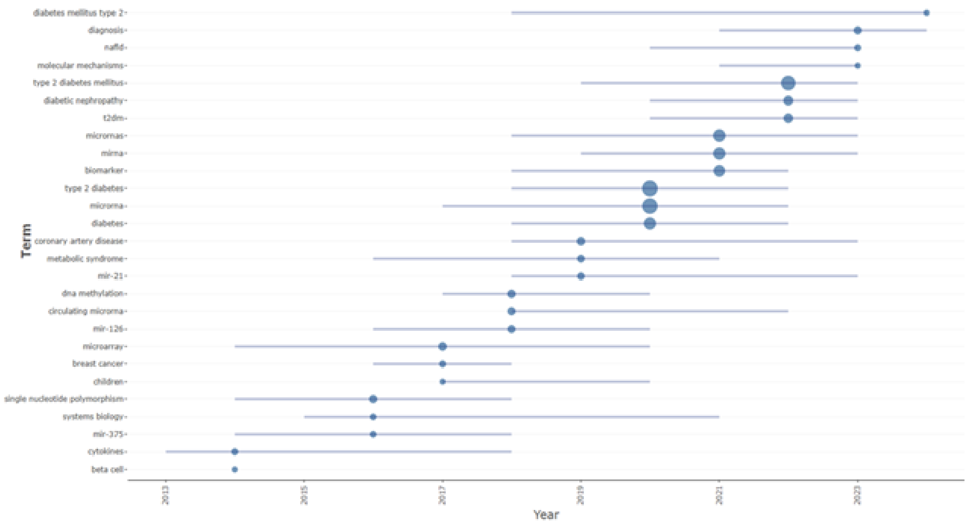

Figure 8: Co-occurrence network of authors' keywords (miRNAs: miR-21, miR-126, and miR-146a are represented in a green triangle).

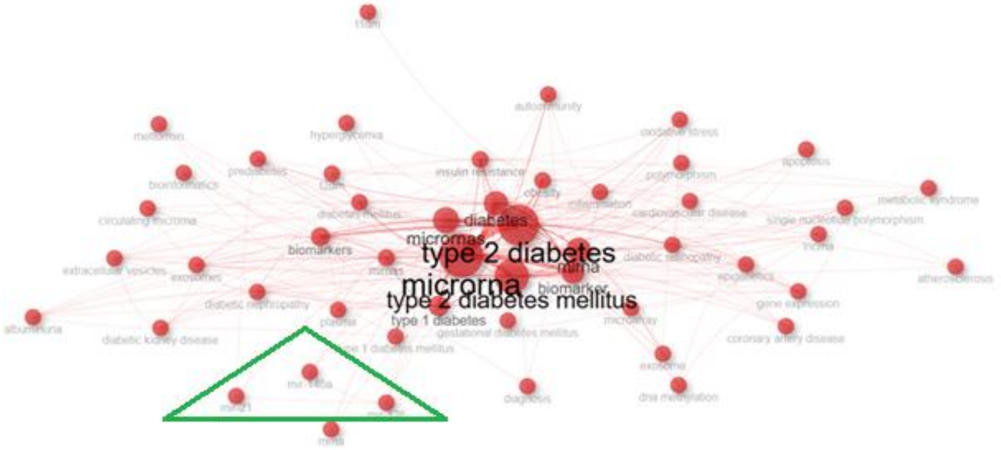
t1dm
autoimmunity
metformin
hyperglycemia
oxidative stress
apoptosis
prediabetes
insulin resistance
polymorphism
bioinformatics
t2dm
obesity
inflammation
cardiovascular disease
metabolic syndrome
circulating microrna
diabetes mellitus
diabetes
single nucleotide polymorphism
lncrna
micrornas
biomarkers
type 2 diabetes
mirna
extracellular vesicles
exosomes
mirnas
microrna
biomarker
epigenetics
atherosclerosis
diabetic nephropathy
type 2 diabetes mellitus
gene expression
albuminuria
plasma
type 1 diabetes
microarray
diabetic kidney disease
gestational diabetes mellitus
coronary artery disease
type 1 diabetes mellitus
exosome
mir-146a
mir-21
mir-126
diagnosis
dna methylation
mirna

Figure 9: Authors' corresponding countries that were included in this study.

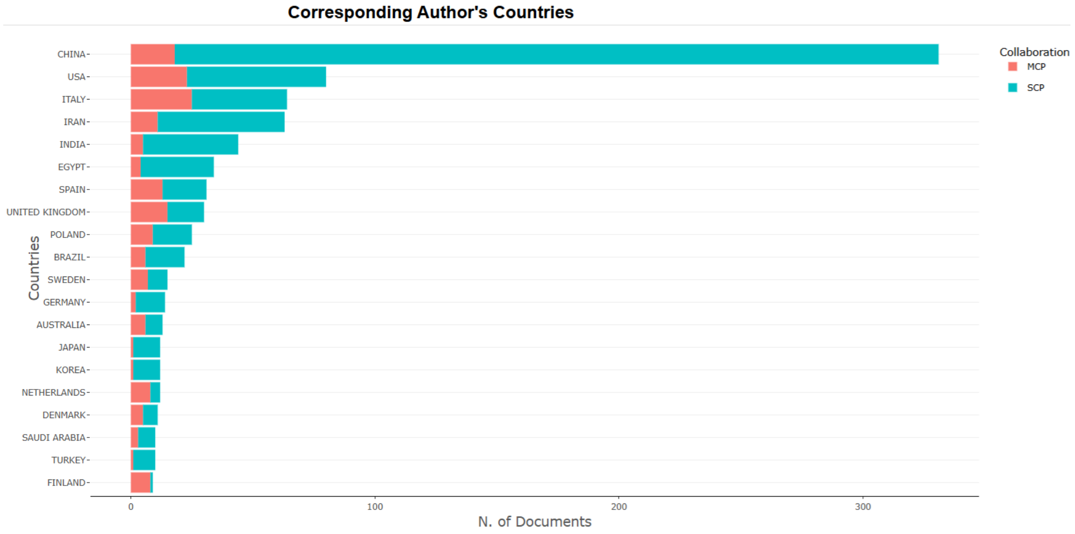

Figure 10: Most cited countries on subjects referring to miRNA and DM.

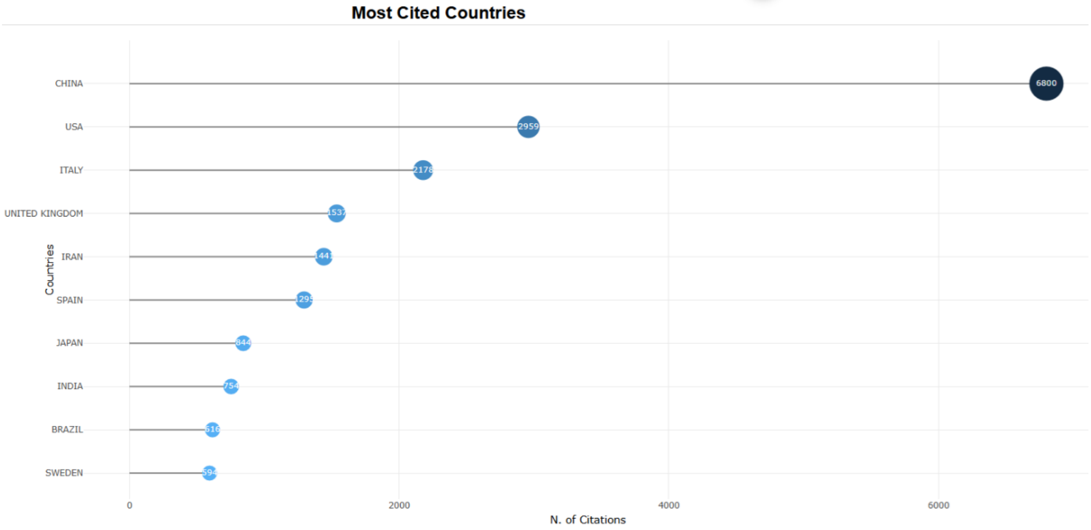

Figure 11: World map of countries collaborating on research on miRNA and DM

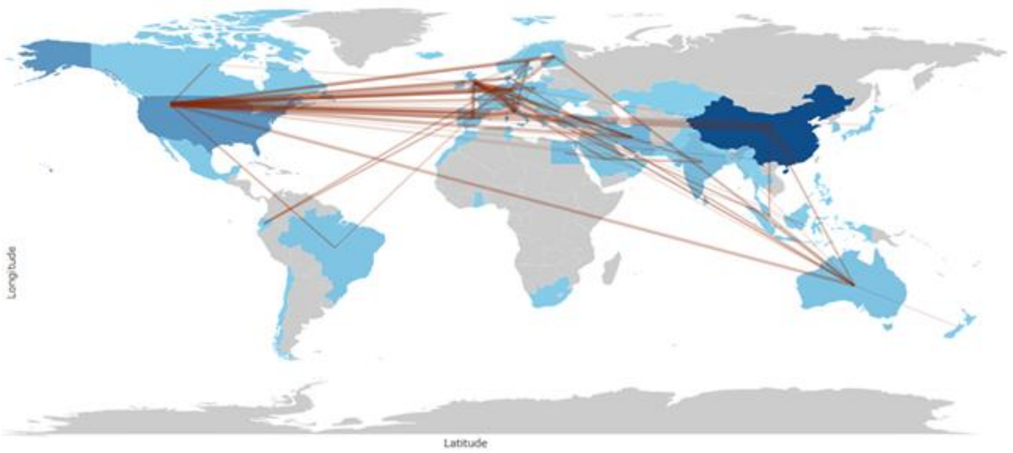

Visualization of international research collaborations by country is seen in Figure 11. Highlighting the global networks formed through co-authorship and cross-border scientific partnerships, its scope encompasses every continent.

miRNAs expression and DM

Bioinformatic analysis can be conducted in order to predict possible miRNA targets. Examples of databases constructed for this purpose are miRBase [31], TargetScan [32], and miRecords [37]. For this research, miRbase [31] and TargetScanHuman v8.0 [32] were used for consulting target transcripts and conservation status for each miRNA. Target gene IDs can be found in Tables 1, 2, and 3. MiR ID was normalized according to at least two articles and two miR databases, according to that miR's sequence.

Table 1: microRNAs downregulated (↓) or upregulated (↑) in T1DM analyzed in at least two studies.

miR in T1DM	Target Gene/Mechanism in which they are involved	Referenes
miR-326 ↑	TNFSF14, 15; CEP85	Zheng et. al., 2017
miR-23 ↑; miR-98 ↑; miR-590-5p ↑	Trail, Faslg	
miR-186 ↑	CXCL13; IRF8; STAT4	[38]
miR-223 ↑	HSP90B1; TP53; MCL1	
miR-142-5p ↑	HERPUD1	[39]
miR-155 ↑ / ↓	CASP3	[39]; Katsarou et. al., 2017
miR-100-5p ↓	RAVER2; AP1AR	Ferraz et. al., 2022
miR-181a-5p ↑	ZNF; TNFSRF11B	Liu et. al., 2019
miR-1275 ↑	KCNC3; KIR2DL4	[22]
miR-30b-3p ↑	CCL28, 22; TAF8; NCR3	Nizam et. al., 2024
miR-25-3p ↑	HIPK3; SNAPC1; RBM47	Liu et. al., 2019
miR-10a-5p ↑	CRLF2; HOXA3	Santos et. al., 2022
miR-98 ↑	IGDCC3; IGF2BP2; CCL3	Khan et. al., 2020
miR-22-3p ↓	H3F3C; GADD45A	Kaur et. al., 2015
miR-16-5p ↑	TNFSF13B; BTLA	Gao et. al., 2020
miR-574-3p ↓	NKG7; IL6; ATPIF1; IFNLR1; SRF	Garcia-Contreras et. al., 2017
miR-103a-3p ↑	SPI1 1; AGO4	Assmann et. al., 2018
miR-454-3p ↑	IRF1; IGF	Erener et. al., 2017
miR-450a-2-3p ↑	IFG2; IL6ST; NOX5; IL17A	Takahashi et. al., 2014
miR-100-5p ↓	INSM1; AGO2	Hezova et. al., 2010

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

Some factors involved in the pathophysiology of T1DM (e.g., ILs, tumor necrosis factor) can interfere with the expression of these miRNAs and thereby modulate the efficiency of silencing processes. These observations complicated the relationship between T1DM and miRNAs.

ABERRANT MICRORNA EXPRESSION IN TYPE II DIABETES

Database analysis

MiRNAs can interfere with T2DM physiopathological mechanisms [40] (Table 3). Some of these molecules were associated with both T1DM and T2DM (Table 2).

Although there is significant interest in developing new biomarkers for DM, only a few miRNAs have been consistently associated with T2DM in at least two studies (Table 3).

Table 2: microRNAs downregulated (↓) or upregulated in (↑) T1DM & T2DM analyzed in at least two studies.

miR in T1DM & T2DM	Target Gene/Mechanism in which they are involved	Referenes
miR-21 ↑	IL12A; FASLG; CCL1	[41]
miR-34a ↑	CDIP1; MSR1; PDCD6	
miR-146a ↓	INSR; ZNF; GRIN2B	
miR-29 ↑	NFIA; ATAD2B	Massart et. al., 2017
miR-142-3p ↑	CASP3	[39]; Zhu & Leung, 2015
miR-24-3p ↑	FASLG; BCL2L14	Blanco et. al., 2023; Garavelli et. al., 2020
miR-148a-3p ↑	ATP6AP2; GADD45A	Grieco et. al., 2018; Ghoreishi et. al., 2022

miR-150-5p ↓	MYB; TADA1; AIFM2	Kim et. al., 2019; Qiu et. al., 2021
miR-210-5p ↑	ERP29; GDF11	Patra et. al., 2023
miR-342-3p ↓	OSER1; SPI	Jiang et. al., 2020
miR-375 ↑	INS; HOXA5; ATP1B1	Higuchi et. al., 2015; Marchand et. al., 2016
miR-101 ↑	UBE2D1; APP	Santos et. al., 2019; Higuchi et. al., 2015
miR-199a ↑	PAWR; ACOX1	Wang, et. al., 2018; Yang et. al., 2020
miR-335-5p ↓	CASP; IL17RD	Li & Zhang, 2021; Hezova et. al., 2010
miR-20b-5p ↓	PDCD; IRF9	Katayama et. al., 2019; Hezova et. al., 2010
miR-15b ↑	CYP7A1; CASP	[42]
miR-186	CRP	
miR-155 ↑ / ↓	BCL2	Corral-Fernández et. al., 2013; Katsarou et. al., 2017

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

Table 3: microRNAs downregulated (↓) or upregulated (↑) in T1DM analyzed in at least two studies.

miR in T2DM	Target Gene/Mechanism in which they are involved	Authors & Year
miR-143	IGFBP; FADS6; ITM2B	[43]
miR-142-3p	BCL2	Zhu & Leung, 2015
miR-126	INSR; SCL7A5	Zhu & Leung, 2015
miR-375	INS; VEGFA; HOXA5; ATP1B1	Poy et. al., 2009; Chakraborty et. al., 2014
miR-26a-5p	POLR3G; INS; BAX	Xu et. al., 2020;
miR-146a-5p	IGSF1; IRAK1; PSMA4	Li et. al., 2012
miR-1 ↓	CYP7A1; XCR1; PRPF39	Chakraborty et. al., 2014
miR-16 ↓	RRM2B; MRPS22; C8B; PDX1	Bork-Jensen et. al., 2015
miR-30e ↓	GABRB1; IL1R1APL2; SOCS1	Dieter et. al., 2019
miR-9 ↑	DSCC1; GLT1D1	Al-Muhtaresh et. al., 2018
miR-33a ↑	TEX38; TLR4; PTPLAD1	Saeidi et. al., 2023
miR-320a ↑	ETFA; TRIAP1; RGS9BP	Du et. al., 2021
miR-148b ↑	GADD45A; MMD; TGFA	Liang et. al., 2020
miR-21 ↓	IL12A; FASLG; GPR64	[44]
miR-29a ↑	MANEA; HOXA10; RTP3	Massart et. al., 2017
miR-34a ↑	MSR1; PDCD6; GORAB	Shen et. al., 2017
miR-103	RNF38; TRIAP1; AGO4	Rafiee et. al., 2024
miR-15a ↓	POLR3K; SLC22A9; RAVER2;	Al-Kafaji et. al., 2015
miR-155 ↓	H3F3A; ZNF652; FGF7	Katsarou et. al., 2017
miR-221 ↑	GABRA1; KCNQ3; SOCS5	Liu et. al., 2018
miR-145 ↓	ATP6V0B; ITGB8; CDC37L1	He et. al., 2020
miR-24 ↓	SNN; BCL2L11; KCNK2	Xiang, 2015
miR-182 ↑	TMEM50B; CHIC1; VLDLR	Krause et. al., 2024
miR-192 ↑	insulin-like growth factor 2; SLC44A1	Jaeger et. al., 2018
miR-194 ↑	PRKAR1A; SLC10A7; BNIP2	Jaeger et. al., 2018
miR-223 ↑	FBXW7; IL6ST; HSP90B1	Sánchez-Ceinos et. al., 2021
miR-130a ↑	IGF1; TGFBR2; HIVEP2	Ofori et. al., 2017
miR-19a ↑	QKI;	Yan et. al., 2020
miR-26b ↑	ID2; IGFBP3; SOCS1	Xu et al., 2015
miR-27a	FOXO1, FOXA2, STAT3	[45]
miR-27b ↓	GOLM1; GPAM	Ghoreishi et. al., 2022
miR-28 ↑ / ↓	TRAF3	[45;46]

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

DISCUSSION

Literature data analysis

The utility of miRNAs as biomarkers for screening or diagnosis of T1DM is of high interest. There have been some attempts to propose a panel of miRNAs for elaborating these tests, although normalization of miRNA levels is still necessary [47].

A systematic review published in 2017 mentioned 48 miRNAs that were investigate in tissues relevant for T1DM pathogenesis (e.g. serum, plasma, pancreatic tissue, blood cells); for 11 of them the expression level differ significantly in T1DM patients and control subjects (e.g. miR-21-5p, miR-24-3p, miR-100-5p, miR-146a-5p, miR-148a-3p, miR-150-5p, miR-181a-5p, miR-210-5p, miR-342-3p, miR-375 and miR-1275) [22]. There are certain miRNAs (e.g., miR-375) that directly influence specific pathways leading to diabetogenesis. These molecules target genes that modulate immune T cell activity. Human and mouse T lymphocytes have been shown to release exosomal miRNAs (e.g., miR-142-3p, miR-142-5p, and miR-155), which can predispose or support the apoptosis of pancreatic β cells [39]. Additionally, overexpression of some miRNAs can reduce insulin synthesis, insulin secretion, or their biological effects [48].

Cytotoxic attack in T1DM is mediated by proinflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α). These cytokines have been found to induce the synthesis of miR-21, miR-34a, and miR-146a in vitro, in human and mouse pancreatic cells [41].

miR-21 is predicted to target multiple transcripts (e.g., IL12- natural killer cell stimulatory factor and cytotoxic lymphocyte maturation factor, CXCL1- chemokine ligand 1). Increased levels of IL-12 could be predictors of T1DM. IL-12 plays a part in determining the levels of CD4+T cells and increasing the CD8+ T cells' toxicity in DM [49]. It also has a role in activating the apoptotic pathway of the β cells. Existing literature proved the implication of the CXCR1/2 axis, CCL4-CCR5 axis, and CXCL10-CXCR3 axis in the inflammatory background of DM. These pathways were proven to be engaging more aggressive immune T-cells. This furthermore accentuates the β cell death [50].

A decrease in miR-155 expression has been shown to impair the function of regulatory T cells (Tregs), which exhibit an upregulated expression of suppressor of cytokine signaling 1 (SOCS1) upon maturation. Treg cells have a primary role in alleviating the aggressivity of CD4+ and CD8+ T cells. Therefore, their underdevelopment has been linked with one of the causes of T1DM, implicating the STAT3 mutations. The signal transducer was reported to recognize and enhance the miR-155 promoter. The development of Treg cells is also threatened by mutations in the FoxP3 gene, and its altered transcriptional activity has been linked with the presence of miR-155 [51].

The apoptotic genes (Trail, Faslg) are predicted to be targeted by miR-23, miR-98, and miR-590-5p. These miRNAs are overexpressed in CD8+ T cells of T1DM patients [52]. MiR-326 targets different genes (e.g., the gene coding the vitamin D receptor), which modulate the function of the immune system [44]. Expression levels of miR-326 were found to be increased in lymphocytes of T1DM patients who tested positive for the islet-specific autoantibodies IA-2A and GADA [53].

MiR-34a is known to inhibit macrophage polarization, thus impairing tissue homeostasis and repair. This miRNA is involved in the regulation of insulin signaling and glucose metabolism through the targeting of specific mRNAs, including those of sirtuin 1 (SIRT1) and vesicle-associated membrane protein 2 (VAMP2) [54]. Dysregulated miR-34a in cancer patients has been proven to lead to low survival chances. It targets multiple oncogenes and plays a part in apoptosis mediated by the p53 complex, the tumor suppressor gene [55].

In a cross-sectional study, Januszewski and his coworkers demonstrated increased levels of miR-186 and miR-223 in T1DM patients. MiR-186 is known for its role in apoptosis and chemokine and insulin signaling pathways [42]. MiR-186 is also known for being a tumor suppressor in prostate cancer. There was an increase in granulocytes in model mice with miR-223 deficiency, and its expression was highly decreased in patients with active nephritis. This miR-223, along with others, has been associated with multiple cellular processes: p53 pathway regulation, cell cycle regulation, tumor metabolism, and apoptosis [38].

The cumulative roles of miRNAs in T1DM are various, implicating many cellular and molecular pathways and mechanisms. Extensive research is needed to further validate the expression levels in vivo model organisms [47].

miRNAs expression and DM

Certain miRNAs found in scientific publications or databases are associated with the pathogenesis of diabetes or with particular features of diabetic patients. Subjects with impaired glucose tolerance presented abnormal levels of miR-18b [45]. miR-143 has a role in precursor adipocyte differentiation and in predisposition for T2DM (e.g., by targeting the insulin-AKT pathway) [43]. MiR-21 targets mainly FOXO1, FOXA2, and STAT3 transcripts. Its levels were considered informative for gluconeogenesis [56], insulin resistance [57], and altered glucose tolerance [45]. Markers of low-grade inflammation in T2DM (e.g., IL6, TNFα) are also associated with changes in miRNAs. TNFa enhances the intracellular accumulation of miR-223, which targets genes involved in lipid and glucose metabolism, inflammation, and T2DM (e.g., IGF-IR, Hsp90, FOXO1) [58;59].

A randomized community-based study analyzed a sample of 1000 individuals. It was identified several downregulated miRNAs (e.g. miR-126, miR-15a, miR-29b, miR-223, miR-20b, miR-197) in the plasma samples from T2DM patients [46]. Upregulated (e.g., miR-140, miR-142, miR-222) or downregulated (e.g., miR-125b, miR-126, miR-130, miR-192, miR-195, miR-423-5p, miR-532-5p) miRNAs in T2DM patients were also identified in a cross-sectional, double-blinded study. It was observed that the expression levels of four miRNAs (e.g., miR-126, miR-140-5p, miR-195, miR-423-5p) demonstrated a strong ability to distinguish subjects with T2DM from those with normal fasting glucose levels [60]. MiR-126 has been reported by a considerable number of research groups to be a suitable biomarker for the assessment of T2DM progression [44, 46, 61]. MiR-186 expression levels were found to be statistically significantly associated with HbA1C levels in T2DM patients and with the risk of diabetic neuropathy [62].

There are numerous factors to consider when evaluating the potential of miRNAs as biomarkers for DM. For instance, the dynamics of miRNA concentration may be correlated with causes of dysregulated expression (e.g., number of cells releasing these small non-coding molecules or with miRNA overexpression in individual cells), miRNA half-life, and factors influencing their stability. Other factors can also significantly impact the significance of results. For example, an increase in adipose tissue volume can increase the levels of some miRNAs; these associations may influence the results, particularly in studies involving obese patients with T2DM [45]. The timing of miRNA testing poses a challenge, especially in presymptomatic stages of diabetes [63]. It has been hypothesized that dysregulated miRNAs may be present only in specific cells or tissues. Comprehensive research involving paired analyses of body fluids and biopsy tissues, along with validation studies, could be beneficial for future investigations [64]. There also remains a critical need for the standardization of miRNA analysis across different research platforms, tissues, and conditions to facilitate the development of clinically useful biomarkers [65].

The size of the sample pool influences the study's power and the reliability of results. Studies with small sample sizes (e.g., fewer than 10 patients and 10 control subjects) may yield biased outcomes [66]. Consequently, findings from such studies may not be corroborated by research involving larger sample sizes. Therefore, the statistical significance and clinical relevance of some miRNA studies may be limited. Reliable statistics are needed against large sample pools and of different categories.

CONCLUSION

MiRNAs can target a large number of transcripts, and their levels in specific tissues change during the natural history of the disease. But these factors also increase the difficulty of identifying the relevant genes for diabetogenesis and validating the clinical use of specific miRNAs. Standardization in profiling techniques and validation of these biomarkers across large, diverse populations remain essential steps toward their clinical application.

This review underscores the emerging role of miRNA as one of the key molecular players in the pathogenesis, diagnosis, and potential treatment of DM. Numerous studies have identified distinct expression patterns of specific miRNAs (e.g., miR-21, miR-126, miR-143, miR-155, miR-223, and miR-375) that correlate with immune dysregulation, β-cell apoptosis, insulin resistance, and metabolic imbalance.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, X.I.P. and DC.; methodology, X.I.P., RN and DC; software, D.C. and X.P; validation, X.I.P., O.A.A.T, I.R, L.B., I.A.E. and S.N.; formal analysis, D.C.; investigation, D.C, X.I.P. and R.N; resources, D.C.; data curation, X.I.P., O.A.A.T, I.A.E, S.N, and R.N.; writing- X.I.P. and D.C; writing-review and editing, O.A.A.T, I.R, L.B., I.A.E, S.N. and R.N.; visualization, D.C.; supervision, D.C.; project administration, L.B.; funding acquisition, L.B. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Assessing the Effectiveness of an Ethical Communication Course for Romanian Medical Students: A Pretest-Posttest Study

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Abstract: Effective communication is essential in healthcare, prompting the inclusion of communication courses in medical education. However, the effectiveness of such courses for Romanian medical students has not been evaluated. This pilot study investigates the impact of an ethical communication course on Romanian medical students' communication self-efficacy, communication skills, and the perceived importance of communication. Students attended a 3-month course on ethical communication, covering active listening, assertive communication, conflict management, non-verbal communication, patient-centered care, and ethical considerations in various medical fields. The course was developed based on the European Psychiatric Association guide regarding ethical aspects of communication with patients and their families. The data were analyzed on a sample of 24 students who completed the questionnaires at pretest and posttest. Students reported higher levels of medical communication self-efficacy and perceived importance of medical communication after the course. However, no statistically significant differences were found in health professionals' communication skills. Future courses should include more patient interactions and varied teaching methods. Further research with larger samples and control groups is needed.

Keywords: communication self-efficacy; healthcare communication skills; ethical communication course; healthcare students

INTRODUCTION

Ethical issues are a daily reality in healthcare, affecting both patient care and professionals' well-being. Studies indicate that nurses and physicians frequently encounter dilemmas related to patient autonomy, informed consent, resource allocation, and end-of-life care [1,2,3]. Ethical conflicts often arise when professional values clash with institutional constraints, leading to moral distress and demoralization [4]. The World Medical Association (WMA) International Code of Medical Ethics (2022) and the International Council of Nurses (ICN) Code of Ethics [5] emphasize that healthcare professionals must uphold patient rights, confidentiality, and informed decision-making, yet the ability to navigate these obligations depends on effective communication skills. Nurses, in particular, report high levels of stress and burnout due to ethical conflicts, with staffing shortages and inadequate support being major contributors [3]. Younger and less experienced professionals struggle more with ethical dilemmas, highlighting the need for early training in ethical reasoning and communication during medical education [2].

Without structured guidance, healthcare professionals may feel powerless or unprepared to address ethical concerns, negatively impacting patient trust and care quality. The increasing ethical complexity of medical practice demands that future healthcare professionals receive formal training in ethical communication. Research demonstrates that ethical challenges are frequent and stressful, particularly for younger and less experienced clinicians [2,3]. Demoralization, moral distress, and institutional constraints further complicate decision-making, highlighting the need for early intervention through ethics education [4]. A structured ethical communication course for medical students would provide the necessary skills to navigate ethical dilemmas, improve patient relationships, and support professional integrity in healthcare practice.

Effective communication is a critical tool for resolving ethical dilemmas, reducing stress, and fostering patient-centered care [3,4]. The professionalization of healthcare ethics has gained momentum, emphasizing the need for structured training in ethical decision-making [6]. Ethical communication courses can serve as a preventive strategy against demoralization, equipping students with tools to

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navigate ethical tensions proactively. Current medical education often provides limited exposure to ethics beyond theoretical frameworks, leaving students unprepared for real-world ethical dilemmas [4]. Communication skills are considered essential in the healthcare professions, prompting the inclusion of courses aimed at developing these competencies within many medical education programs [7,8]. These courses generally aim to improve students' attitudes towards patient communication (i.e., their perception of the importance of communication skills), to increase students' self-efficacy regarding communication with patients (i.e., their confidence in their ability to communicate effectively), and to improve their actual communication skills [8]. Furthermore, the majority of medical education programs addressing communication skills encompass an ethical dimension related to patient communication [9]. Communication with patients is also a topic of interest for the Romanian academic medical community, with experts in the Romanian healthcare system highlighting the necessity of cultivating students' communication skills [10]. However, the efficacy of such courses for Romanian medical students has yet to be evaluated. To cover this gap, the purpose of the current study is to investigate the effectiveness of a course on ethical considerations in communication with patients for Romanian medical students. Specifically, we examined the course's impact on increasing students' medical communication self-efficacy, health professionals' communication skills, and perceived importance of medical communication.

Previous studies support the effectiveness of courses designed to enhance communication skills with patients. In one study [11], nursing students were trained in communication skills using complex care scenarios, with professional actors portraying patients. The training effectively enhanced self-efficacy and communication skills, with sustained benefits observed 6 months post-intervention. In another study [12], a communication skills training program that included experiential learning methods (e.g., role-playing) and a medical psychology course significantly improved medical students' positive attitudes toward communication skills. A recent research [13] evaluated the acceptability and efficacy of a communication skills training program tailored for nursing students. The program focused on building empathy and discussing complex situations such as end-of-life care. Results indicated that the training significantly improved nursing students' confidence and usage of essential communication skills in complex clinical scenarios. One study found that three experiential teaching methods (practicing patient interviews with peers, followed by debriefing and feedback; conducting interviews with trained actors simulating patients, followed by feedback sessions; watching actors playing a theatrical performance that included communication challenges between doctor and patient) are equally effective in teaching interpersonal communication skills to medical students [14].

In addition to effective communication, recent medical literature emphasizes the importance of ethical competence in protecting patients and promoting high-quality care [15]. It requires a combination of personal virtues, professional ethics, reflective practice, and a commitment to confidentiality and honesty [15,16]. Ethical competence enhances the quality of care and supports healthcare professionals in making ethical decisions [16]. In a systematic review that explored the literature on ethics education aimed at fostering ethical competence among healthcare professionals and students [17], based on the thematic analysis of 40 articles, the review highlights that ethics education is crucial for developing the ability to recognize and manage ethical issues in healthcare settings. Key themes identified include the importance of creating supportive conditions for ethical learning, designing effective educational strategies, and fostering interaction among learners. The studies reviewed indicate that ethics education can help healthcare professionals and students become more aware of ethical problems, enhance their ethical reasoning skills, and ultimately improve their ability to act in the best interests of patients. Indeed, another literature review based on 23 empirical studies [18] revealed that ethics interventions enhanced ethical knowledge, moral sensitivity, and the ability to identify ethical problems among healthcare workers and students. Interventions also improved ethical empowerment, including moral courage and the ability to cope with moral distress.

These findings underscore the importance of integrating comprehensive ethical communication skills training into medical students' education programs. Such training can significantly enhance medical students' confidence and skills, particularly in handling complex and emotionally charged interactions with patients and families, the ability to recognize and manage ethical issues, and ethical reasoning skills. Following this general trend of integrating communication and ethics courses in medical educational programs, the purpose of the current study is to evaluate the effectiveness of an ethical communication course for Romanian medical students in increasing students' medical communication self-efficacy, health professionals' communication skills, and perceived importance of medical communication.

MATERIALS AND METHODS

Participants

No a priori power analysis was conducted, as the pilot study relied on a small convenience sample of available students. As such, the findings should be interpreted with caution, as the limited sample size reduces statistical power and increases the likelihood of both Type I and Type II errors. Fifty-eight healthcare students completed the questionnaire. Five responses were excluded because the participants answered an attention check question incorrectly ("Do you think stones float on water?", Agree/Disagree). One case was excluded due to missing values. The final sample at the pre-test consisted of 42 women (80.8%) and 10 men (19.2%). Of these, 32 (61.5%) previously participated in other communication courses, and 45 (86.5%) did medical practice or volunteering in a hospital or clinic. The pilot study participants had an average age of 20.21 years with a standard deviation of .72. The posttest questionnaire was completed by 24 students. Among them, 16 (66.7%) were women, 8 (33.3%) were men, 21 (87.5%) had previously done medical practice or volunteering in a hospital or clinic, and 11 (45.8%) had participated in a communication course in the past. The students had an average age of 20.25 years with a standard deviation of .67.

To assess potential differences between the students who completed only the pretest and those who completed both the pretest and posttest, we conducted non-parametric and categorical comparisons. A Mann–Whitney U test showed no significant difference in age between the two groups (U = 334.00, Z = –.04, p = .97). However, Chi-square tests indicated significant differences in gender distribution ($\chi^2(1) = 5.71$, p = .02), with a higher proportion of male students among those who completed both assessments. There was also a significant association between attrition and prior participation in communication courses ($\chi^2(1) = 4.65$, p = .03), suggesting that students without previous communication training were more likely to remain in the study. No significant difference was found regarding prior clinical or volunteering experience ($\chi^2(1) = 1.01$, p = .32).

Procedure

The students participated in a 3-month course called Ethical Considerations in Communication with Patients. The first session, called Ethical Communication with Patients and Relatives, included topics such as active listening, assertive communication, conflict management, non-verbal communication, and patient-centered care. The second session, Ethical Communication in Oncology and Palliative Care, included the presentation of the WHO principles of patient care, case studies, and ethical dilemmas. The third session was about ethical considerations in organ transplantation. The fourth session addressed the ethical considerations in communication within the field of psychiatry and mental health. Finally, the last session was about social media, mass media, and public health issues.

The sessions were developed based on the European Psychiatric Association guide regarding ethical aspects of communication with patients and their families [19]. The basic principles of communication that were presented were: respecting the patients' autonomy, dignity, opinions, values, privacy, and confidentiality; sincere and clear communication; encouraging the expression of emotions; using a language adapted to the patient's age and culture; avoiding time constraints [19]. Regarding the communication about the diagnosis, four principles were proposed: informing patients about their diagnosis after analyzing what they know and what they want to know about the disease; the step-by-step and flexible approach to presenting the diagnosis, avoiding the communication of unreliable information; prior discussion with patients to clarify what can be communicated to relatives [19]. Finally, the recommendations regarding communication about treatments were followed: presentation of all relevant information about treatments (risks and benefits, duration, possible adverse events); presenting the advantages and disadvantages of refusing treatment; helping the patient to make an informed decision; informing the relatives about the treatment [19].

Participation in the ethical communication course was optional. Since the present pilot study included only students who voluntarily attended the course, there was no inter-group comparison performed, and variability between groups was not controlled for. The course sessions were delivered by faculty members from the medical university, specifically two psychologists and one psychiatrist with clinical and teaching experience. All lecturers were course coordinators with expertise in communication skills training and medical ethics. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the University (Approval No. 8371, dated March 29, 2024).

Measurements

Medical communication self-efficacy was evaluated with the scale developed by Parle, Maguire, and Heaven (1997) that measures self-efficacy regarding nine components of communication with the patient (e.g., helping a patient deal with the uncertainty of his/her situation, breaking bad news to a patient) [20]. Students reported on a scale from 0 (not at all confident) to 100 (totally confident) how confident they were that they could successfully manage each situation.

Health professionals' communication skills were measured with the Health Professionals Communication Skills Scale (HP-CSS) [21], which contains 18 items (e.g., When I give information to patients, I do so in understandable terms.) on a scale from 1 (seldom) to 6 (many times) that evaluates four communication skills dimensions (empathy, informative communication, respect, and social ability/assertiveness).

Perceived importance of medical communication was measured with a modified form of the medical communication self-efficacy scale [20], in which the students reported, on a scale from 1 (not important at all) to 5 (very important), how important they consider the behaviors in the respective scale (e.g., concluding a patient interview with an agreed problem list and a plan of action) in the physician-patient relationship.

The instruments were translated into Romanian by two psychologists with expertise in health psychology using the back-translation method. Internal consistency was evaluated using Cronbach's alpha coefficients for each scale at both pretest and posttest, indicating good reliability (values presented in Table 1).

RESULTS

Descriptive Statistics

We performed the descriptive analysis for the study variables at the pretest and posttest for the students who completed the questionnaires at both time points. Results are presented in Table 1.

The Differences between Pretest and Posttest

We analyzed differences between the pretest and posttest using the Wilcoxon signed-rank test. Students reported significantly higher levels of medical communication self-efficacy after the course, Z = –3.29, p = .001, with a large effect size (r = .67). Similarly, the perceived importance of medical communication significantly increased from the pretest to the posttest, Z = –2.26, p = .02, with a medium effect size (r = .46) (Table 2). No significant change was observed in health professionals’ communication skills, Z = –1.72, p = .09, although the effect size was in the medium range (r = .35).

Table 1: Descriptive Statistics for Pretest and Posttest (N = 24)

Variable	Condition	Mean	Standard Deviation	Cronbach's alpha
Medical communication self-efficacy	Pretest	55.99	13.76	.82
	Posttest	68.86	15.83	.89
Health professionals’ communication skills	Pretest	4.70	.61	.86
	Posttest	4.92	.58	.86
Perceived importance of medical communication	Pretest	4.23	.58	.87
	Posttest	4.43	.61	.91

Table 2: The Differences between Pretest and Posttest (N = 24)

Variable	Z	p	Effect size (r)	Interpretation
Medical communication self-efficacy	-3.29	.01	.67	Large effect
Health professionals’ communication skills	-1.72	.09	.35	Medium effect (non-significant)
Perceived importance of medical communication	-2.26	.02	.46	Medium effect

DISCUSSION

This pilot study aimed to assess the effectiveness of an ethical communication course for Romanian medical students in enhancing students' self-efficacy in medical communication, improving health professionals' communication skills, and increasing the perceived importance of medical communication. The course followed the European Psychiatric Association's guide on ethical communication [19]. Key principles included respecting patients' autonomy, dignity, privacy, and confidentiality; clear communication; encouraging emotional expression; using appropriate language; and avoiding time constraints. For diagnosis, principles included informing patients, using a step-by-step approach, avoiding unreliable information, and clarifying what can be shared with relatives. For treatment, recommendations included presenting all relevant information, discussing treatment refusal consequences, aiding informed decisions, and informing relatives.

Ethical communication is increasingly recognized as an essential competency in healthcare, yet many medical students receive inadequate formal training in this area [1,2,3]. Research shows that healthcare professionals frequently encounter ethical dilemmas that require effective communication skills, particularly in cases of informed consent or end-of-life decision-making [4]. The ICN Code of Ethics (2021) and the WMA International Code of Medical Ethics (2022) stress the importance of ensuring clear, transparent, and compassionate communication in patient care, yet studies indicate that many healthcare professionals struggle to implement these principles effectively [3,22]. Ethical stress and moral distress are also common among nurses and physicians who feel unprepared to navigate these complex interactions, leading to demoralization and job dissatisfaction [4]. Given these challenges, structured ethical communication training is essential for enhancing professionalism, patient trust, and ethical decision-making in clinical practice [6].

Students reported higher levels of medical communication self-efficacy and perceived importance of medical communication after completing the course. These results are in line with previous studies in which different types of communication courses had a positive effect on healthcare students’ communication self-efficacy and attitudes toward communication skills [11,12,13]. Our results are also in accordance with the studies highlighting that ethical interventions enhance ethical knowledge [17,18]. Research further suggests that integrating ethics training into medical curricula can reduce moral distress and improve ethical resilience, helping future healthcare professionals navigate value conflicts and ethical dilemmas with greater confidence [2,3].

No statistically significant differences were found in terms of health professionals’ communication skills. This result is in contradiction with other studies in the field [7,13,14,17]. There are two possible explanations for this result. Firstly, without ongoing reinforcement and opportunities to practice these skills in clinical settings, the impact of the course might diminish. It is possible that the students did not have enough opportunities to interact with patients and practice the new skills. Future courses could offer students more practical contexts in which to interact with patients. Secondly, the lack of effect may be because the course did not include didactic methods used in the training that had positive results in increasing communication skills (e.g., care scenarios with professional actors playing the role of patients, practicing patient interviews with peers, watching actors perform a theatrical piece depicting communication challenges between doctor and patient) [11,14]. Future courses could include a greater variety of teaching methods.

The present pilot study has several limitations. First of all, the sample is very small. Second, the study was based on a convenience sample. Thirdly, the sample lacked diversity, as most students shared similar educational backgrounds, age ranges, and academic experiences. This limited representativeness may affect the generalizability of the results to broader and more heterogeneous student populations. Fourth, some of the students did not complete the questionnaire at both time points. Additionally, the voluntary nature of participation may have introduced self-selection bias, as more motivated or engaged students were likely overrepresented in the post-test sample, potentially inflating the observed effects. These limitations regarding the participants make the conclusions of the study inconclusive. Future studies could be based on larger statistically representative samples. Fifthly, there was no control group of students to compare their scores with those of the students who attended the course. Therefore, it was not possible to test whether the increases in self-efficacy and the perception of the importance of communication were due to the participation in the communication course or due to other factors (e.g., the participation in other courses, and the experience with patients in hospitals). Future studies could also include a control group to test the effectiveness of the course. Another limitation of this study is the exclusive use of self-report questionnaires, which are subject to personal biases and may not accurately reflect actual improvements in communication skills. Without objective assessments, such as knowledge tests, role-playing evaluations, or clinical observations, it is difficult to determine whether students have effectively internalized and applied the skills learned. Future research should combine subjective and objective measures to better evaluate training outcomes. In addition, the statistical power of the study was limited due to the small sample size and lack of a priori power analysis. Future studies should conduct formal power calculations to ensure adequate sensitivity for detecting meaningful changes. Finally, although the students participated in the course, they did not have extensive practical experience with patients in hospitals. Therefore, the study participants could not evaluate whether they improved their communication skills in real contexts with patients. Future studies could test the effectiveness of such courses after students have more practical experience with patients. Future studies could also explore the integration of structured pedagogical frameworks (such as experiential or transformative learning models) into course design, to better support behavioral change and deepen students' engagement with ethical communication principles.

CONCLUSION

This pilot study explored the effectiveness of an ethical communication course for Romanian medical students, focusing on its impact on communication self-efficacy, perceived importance of communication, and communication skills. The results indicate that theoretical training can improve students' confidence in communicating with patients and raise their awareness of the ethical significance of medical communication. However, the course did not produce measurable changes in actual communication skills. These findings highlight the need to complement theoretical instruction with practical components such as role-playing, simulations, or supervised patient interactions to support skill development. Given the small, non-representative sample and lack of a control group, the results should be interpreted with caution. Future research should test similar interventions using larger, randomized samples and objective outcome measures. Additionally, future courses could consider incorporating structured pedagogical frameworks to enhance student engagement and better support the internalization and application of ethical communication principles.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, M.L., C.G.I., A.C.C. and A.C.; methodology, B.O.; software, D.M.P.; validation, M.L.., B.O., and A.C.; formal analysis, D.M.P.; investigation, S.G.; resources, M.L. and A.C.C.; data curation, C.G.I.; writing—original draft preparation, B.O..; writing—review and editing, M.L. and A.C.; visualization, B.O..; supervision, M.L.; project administration, L.S.; funding acquisition, M.L. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the University (Approval No. 8371, dated March 29, 2024).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Assessment of Suicide Risk in the Civilian Population, Active Military Personnel and Veterans Using Psychometric Instruments – A Literature Review

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Abstract: The assessment of suicide risk is a highly debated topic in the literature due to the complex interplay between social, psychological, cultural, and biological factors that contribute to the pathogenesis of self-harm. There is no unanimously accepted predictive model of suicide and no widely recognized evidence-based algorithm for psychiatric and psychological evaluation in clinical and non-clinical populations for early detection of self-harm. Therefore, this narrative review is focused on identifying the validated clinical instruments that may contribute to the construction of a possible screening and monitoring plan for suicide risk in military and civilian populations. Four electronic databases were searched (PubMed, CINAHL, Google Scholar, and Clarivate/Web of Science) for relevant reports on psychometric tools for detecting suicide risk published between the inception of each archive and January 2025. Seventeen instruments dedicated to the measurement of suicide and suicide-related aspects were reviewed, as well as three tools for the evaluation of depression severity that include specific items for assessing self-harm. The advantages and vulnerabilities of each instrument were assessed, and the particular features of using these tools in the military population were also explored. In conclusion, although a large number of validated instruments for the assessment of suicide risk exist, it is not possible to recommend the use of a single tool, either for clinical and nonclinical populations or for military and civilian personnel. The endeavor of finding an algorithm for the assessment of suicide risk is still far from reaching its end, as new psychometric instruments and possibly a new paradigm for the phenomenon of self-harm are sorely needed.

Keywords: suicide risk, self-harm, depression, military personnel, veterans, suicide prevention

INTRODUCTION

Suicide prevention is one of the most challenging phenomena in clinical psychiatry due to the social impact of self-harm, its high prevalence, and the lack of unanimously accepted predictive models. More than 720000 individuals die by suicide each year, and self-aggression is the third leading cause of death among individuals aged 15 to 29 years old [1]. More than one in every 100 deaths worldwide occurs due to suicide, and methodological improvements are needed in order to increase the accuracy of reports regarding complete suicide and suicide attempts [2]. In this context, structured assessment of suicidal ideation and behaviors is an important aspect of the psychiatric clinical practice, and the development of validated instruments (scales, inventories, and questionnaires) for assessment of suicidal risk is essential for both civilian and military populations.

Suicide rates are still high among military personnel, both active and retired [3]. The Army Study To Assess Risk and rEsilience in Servicemembers (Army STARRS) is a large investigation targeting the prediction of suicide in active duty soldiers who were hospitalized (N=53769 participants), in the next year after discharge [4]. The incidence of suicide in this population was 12% of all US Army suicides, equivalent to 263.9 suicides per 100,000 person-years vs. 18.5 suicides per 100,000 person-years in the entire US Army [4]. This data indicates the need for more careful post-discharge monitoring of active duty soldiers, but the specific means to be implemented for this purpose are far from being clear. A potential contributor to the higher risk of suicide among military personnel has been the existence of stigmatizing beliefs since these can act as deterrents for help-seeking [3]. In a cohort study (N=153,736 patients) of US veterans, the risk assessment suggested that only a few variables were associated with subsequent suicide, especially suicidal ideation, firearm access, and preparatory behaviors, which is completely insufficient to develop a risk prediction algorithm [5]. According to the US Department of Veterans Affairs, the risk of suicide in veterans was 1.57 to 1.66 higher than in non-veterans between 2017 and 2020 [6].

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Searching for psychological predictors of suicide is but only one side of the complex process of identifying relevant contributors to the pathogenesis of suicide. Biological factors, such as chronic diseases, and social vulnerability factors, like stigmatization, migration, or bullying, cultural and economic influences, e.g., poverty, acculturation stress, being part of a cultural minority, transgenerational conflicts, etc. [7-12]. However, all these potential pathogenetic factors have intricate relationships, and disentangling them is necessary as part of constructing an evidence-based approach to a predictive model for self-harm behavior.

Based on all these data, it becomes clear that finding adequate tools for detecting the risk of suicide is essential for creating preventative measures and thus decreasing the incidence of completed suicide. To attain this objective, the current review focuses on the detection of the most validated and widely used psychometric instruments for the detection of suicide risk in military personnel, veterans, and civilians. It is expected that such an endeavor could provide the basis of an algorithm for the evaluation of vulnerable populations, as a screening strategy, and for the monitoring of at-risk groups during and after exposure to severe stressors. This review does not target only clinical populations derived from civilian or military environments but also non-clinical populations, which may be exposed to significant risk of suicide ideation and behavior.

MATERIALS AND METHODS

A narrative literature review was conducted by searching four major databases – PubMed, CINAHL, Google Scholar, and Clarivate/Web of Science – for all primary and secondary reports published between the inception of each archive and January 2025, using as keywords “suicide” OR “self-harm” AND “scale” OR “inventory” OR “questionnaire” OR “profile” OR “psychometric instrument” OR “psychometric tool” AND “civilian” OR “military personnel” OR “veterans. Only papers in English were selected. The inclusion criteria were adolescents and adults, clinical and nonclinical groups, civilian and military populations, primary outcomes related to suicide, clinician-rated and self-rated instruments, both prospective and retrospective studies, but also systematic reviews, narrative reviews, and meta-analyses. Exclusion criteria were undefined measurement methods, exclusively non-structured methods of evaluation, poorly defined study design, and children or unspecified population characteristics.

RESULTS

A number of 17 instruments dedicated to the measurement of suicide and suicide-related aspects were found during the literature search, and three tools that include at least one item related to suicide, that were investigated in multiple studies were also included in the analysis.

The **Suicide Attempt Self-Injury Interview** (SASII) was developed by Linehan et al. (2006) to assess the multiple factors associated with suicide attempts and self-aggressive behaviors without a clear suicidal intent [13]. According to the authors of this instrument, its major strength is the use of a standardized definition of suicidal and other self-injurious activities, allowing the practitioners to assess the suicidality/suicide intent regardless of the particular aspects and consequences of the acts themselves [13]. This tool also offers individual ratings for the components of lethality, such as the method used, the consequences of the gesture, and the therapy needed [13]. The scales of SASII are „suicide intent”, „lethality”, „rescue likelihood”, „risk/rescue ratio”, „suicide communication”, „interpersonal influence”, and „emotional relief” [13]. SASII allows for each episode of non-suicidal self-injury behavior to be coded separately and described by its characteristic details. The major variables are assessed by the interview and the frequency of self-injury behaviors, the medical treatment required secondary to the behaviors, and a set of four factors for each episode of non-suicidal behavior - medical risk, suicidal intent, instrumental intent, and impulsivity [13].

A study investigating patients (N=60) with repetitive nonsuicidal self-injury (NSSI) tried to validate subtypes of these behaviors, based on multiple self-report scales, and two structured interviews, SCID-5-CV (Structured Clinical Interview for DSM-5 Clinical Version) and SASII [14]. The results supported a two-subtype model, i.e., (a) substance abuse and suicide attempt subtype- with a higher rate of lifetime suicide plans, number of lifetime suicide attempts, higher severity of self-harm, borderline personality features, anger, posttraumatic manifestations, and difficulties in regulation of own emotions; (b) cutting and scratching subtype [14]. The use of SASII in this study supported the validity of this model and, consequently, the existence of clinical heterogeneity of individuals with NSSI [14].

In another study, SASII was combined with the Beck Scale for Suicide Ideation (BSI), Beck Depression Inventory (BDI), and Beck Hopelessness Scale (BHS), to characterize the self-harm behaviors in a group of Pakistani patients (N=221) [15]. The results showed the possibility of common causal mechanisms and processes underlying self-harm between the investigated population and other groups, with factors such as financial, relationship, or interpersonal difficulties being considered [15]. SASII was administered to collect data on details regarding the time, methods, circumstances, motivations, and treatment for self-harming behaviors [15].

According to a systematic review that explored self-harm measuring tools, seven such validated instruments were identified, but there was a high variation detected in their overall quality [16]. Out of the identified tools, SASII appeared to be the most detailed and psychometrically solid instrument currently available for measuring self-harm behaviors [16]. The other instruments identified were Self-Harm Inventory (SHI), Deliberate Self-Harm Inventory (DSHI), Self-Harm Behavior Questionnaire (SHBQ), Self-Injury Questionnaire (SIQ), Self-Injurious Thoughts and Behaviors Interview (SITBI), and Inventory of Statements About Self-Injury (ISAS) [16]. The reported reliability of SASII was α=0.63-0.93, and the convergent validity with therapist notes (r=0.76-0.86) and the medical records (r=0.75-0.82) were also high [16].

A study investigating the influence of NSSI onset age in adolescents is associated with the frequency of subsequent episodes of self-harm (N=103 participants) showed that a lower age of onset and a longer duration of self-harm were both associated with an increased frequency of other NSSI episodes and a higher risk of a first suicide attempt [17]. Therefore, early identification of NSSI using validated instruments is an essential way to create adequate management strategies for these behaviors [17]. In this important study, SASII was used to collect data on most of the parameters targeted, including time, modality, and frequency of self-harm behavior [17].

A recent cross-sectional study evaluated the presence of factors associated with self-harm behavior during military service in the Israel Defense Forces and used SASII and the Columbia Suicide Severity Rating Scale (C-SSRS) to characterize this population [18]. A number of 1238 episodes of self-harm behavior were identified between 2017 and 2021 and included in the analysis [18]. NSSIs correlated with the presence of adjustment difficulties, and higher rates of previous psychiatric diagnoses were found in individuals with suicide attempts [18]. The risk of dying by suicide during military service was twice as high in soldiers with previous suicide attempts and psychiatric diagnoses [18]. Also, if these soldiers were serving in a combat unit, the risk of suicide was four times higher [18].

Another two-year prospective study explored the predictive value of NSSI for suicide attempts in a clinical sample of US active duty military personnel (N=152, mean age=27.53 years), using SASII and Beck Scale for Suicide Ideation (BSSI-C) [19]. All participants reported current suicidal ideation and/or had a suicide attempt during the last month [19]. The results showed 40% of soldiers with a history of NSSI and 25% of those with a history of suicide attempt made a suicide attempt during the two years of follow-up [19]. Soldiers with a history of NSSI were twice as likely to engage in another suicide attempt, but those with a previous suicide attempt had no increase in suicidal behavior vs. soldiers without a history of suicide attempts [19]. However, 30% of the soldiers with a suicide attempt in their history had engaged in NSSIs, and the total number of NSSIs was significantly associated with an increased risk for future suicide attempts [19].

The **Columbia Suicide Severity Rating Scale** (C-SSRS) is one of the most widely used instruments in Psychiatry to determine suicide risk, but it must be noted that it cannot replace a complete clinical assessment. The C-SSRS has very good convergent and divergent validity with other scales for assessing suicidal ideation and behavior, its subscales are sensitive to changes over time, and the suicidal ideation subscale has a moderately high internal consistency [20]. The C-SSRS is structured in such a way that it allows separate assessments of the severity and intensity of suicidal ideation, autolytic behavior, and lethality of the attempt [20]. The severity of ideation is rated on a five-point scale, the intensity of ideation subscale also on a 5-point ordinal scale (i.e., frequency, duration, controllability, deterrents, and reason for ideation), the behavior subscale is rated on a nominal scale (i.e., aborted, actual, and interrupted attempts, preparatory behavior, and NSSI), while the fourth scale is the lethality subscale for the assessment of actual attempts (there is a 6-point ordinal scale, and if the current lethality is zero, potential lethality of attempts is rated on a 3-point ordinal scale) [20].

The cut-off value of 27 on C-SSRS has very good sensitivity and specificity for classifying suicide attempts in the first three months, according to a prospective multicenter cohort study (N=804 patients with a recent episode of self-harm) [21]. The CSSRS and the Suicide Assessment Scale (SUAS) had a better chance to classify the suicide attempts in the first months but also during a one-year follow-up, although the cut-off value for long-term monitoring could not be established for either instrument [21]. The same study showed that the Suicide Intent Scale (SIS) was the only instrument able to classify suicides adequately at 3 and 12 months, with a cut-off score of at least 21 and at least 17, respectively [21].

C-SSRS had proven a sensitivity of 67% and a specificity of 76% for identifying suicidal behaviors at the follow-up (mean period of 64 days) in a large study (N=3776 patients) [22]. Negative life events were associated with a 2.4% subsequent risk of suicidal behavior, and patients reporting lifetime suicidal ideation with intent/prior suicide attempt had a 4 to 9 times higher risk to report prospectively suicidal behavior [22].

C-SSRS score includes information collected from the patient, but also from other significant sources, and has been validated in emergency settings, as well as in the outpatient population [23]. Also, C-SSRS has been validated in children and adolescents, military veterans, and patients with different psychiatric disorders [23]. The US Food and Drug Administration recommends the use of C-SSRS for clinical trials, and it has been approved by the Centers for Disease Control and Prevention to define and monitor suicidal ideation and behavior [23,24].

The administration of C-SSRS in a large sample of US veterans (N=15373) resulted in confirmation of concurrent validity by comparison with self-reports from the last three months and attempts documented in medical records [25]. Nevertheless, a significant number of discordant responses were reported [25]. The predictive value for future attempts was more than 80% [25]. Another study evaluated the psychometric assessment of C-SSRS and Suicidal Behaviors Questionnaire-Revised (SBQ-R) in 200 active-duty personnel and concluded acceptable reliability for both SBQ-R and C-SSRS ideation severity subscale, while the confirmatory factor analysis supported the SBQ-R, but not the self-reported version of C-SSRS [26]. Yet another study that included US military personnel with lifetime attempted suicide (N=121) used C-SSRS to test the hypothesis that NSSIs elevate the suicide risk; the results showed that a history of NSSI is relevant for increasing the service member's social alienation and acquired tendency for suicide, but did not correlate with the number of lifetime suicide attempts or the controllability of suicide ideation [27].

The **Scale for Suicide Ideation** (SSI) and its self-administered version, Beck Suicidal Ideation Scale (B-SSI), were developed by Beck et al. and are widely used in clinical studies, although their dimensional structure is debated in the literature [28-30]. For SSI, the internal consistency was high, and there were recorded moderately high correlations with clinical ratings of suicidal risk and self-administered measures of self-harm, while the sensitivity to changes in hopelessness and depression over time was also good [28]. For B-SSI, the

Cronbach alpha coefficient was 0.90, indicating high internal consistency for both paper-pencil and computer versions [29]. B-SSI has 21 items, and its items are scored on a 3-point Likert scale, with a total score varying from 0 to 38 [29,31]. The cut-off score of 6 for B-SSI was defined in a prospective study as presenting accuracy for future suicidal behavior, and a reduction to less than eight items with stochastic curtailment was sufficient for this purpose [32]. There is a version for the assessment of current ideation, i.e., the SSI-C, and another for the worst moment in the patient's life, i.e., the SSI-W, both with 19 items [33]. Both SSI-C and SSI-W were positively correlated with a diagnosis of mood disorder, a personality disorder, and measures of depression and hopelessness [33]. The internal consistencies of both scales were high, i.e., alpha 0.84 for SSI-C and 0.89 for SSI-W [33].

A study that involved US military service members showed that summing up five items of the B-SSI (i.e., 1, 2, 4, 6, and 15) is an acceptable strategy to shorten this tool while preserving the predictive value of 74% for a cut-off score ≥1 [34]. A study that included 39 Israeli soldiers (mean age 19 years) who were suicide attempters during their military service included evaluations using B-SSI, C-SSRS, and Suicide Intent Scale (SIS); the results supported seven items of the B-SSI, one of the C-SSRS and two of the SIS were associated with severe suicide attempts [35].

The **Beck Hopelessness Scale** (BHS) explains hopelessness through the concept of a cognitive schema focused on unfavorable expectations, both in terms of the short-term and long-term perspective. This instrument, developed by Beck et al. in 1974, includes 20 items with a dichotomous format, and its primary use is the evaluation of suicide risk in clinical and non-clinical adult populations [36,37]. The original version has three factors, i.e., affective, motivational, and cognitive, but other alternative factorial structures have been suggested [37]. The BHS can be self-administered, as well as administered by the clinician, to hospitalized and non-hospitalized subjects, in specialized outpatient clinics or in emergency hospitals [37]. The predictive validity of BHS has been confirmed by multiple studies in patients with psychiatric disorders [38]. The Cronbach alpha was 0.81 in the general population, according to a study (N=1500 participants) [39]. Shorter versions of BHS have been created, in order to increase its applicability for clinical populations, such as BHS-9 or BHS-4 [38,40,41].

A study focused on improving the detection and prediction of suicidal behavior among military personnel by measuring suicidal beliefs used a set of tools, BHS included, in order to evaluate a new instrument, i.e., the Suicide Cognitions Scale (SCS) [42]. Another study included BHS in the set of instruments dedicated to the assessment of psychological pain in suicidal veterans and concluded that psychological pain accounted for the most shared variance for suicidality, but most relevant correlations were determined for the C-SSRS [43].

The **SAD PERSONS scale** is a mnemonic with each letter representing a risk for suicide, and the total score varies from 0 to 10 [44]. The interpretation is low risk for scores from 0 to 4, medium risk from 5 to 6, and high risk from 7 to 10 [44]. Modified SPS (M-SPS) is characterized by a substitution of new factors and changes in scoring (low risk = 0-5, medium risk = 6-8, and high risk = 9-14) [45]. These are simple and easy-to-administer instruments developed for screening suicide risk in community mental health settings [46].

The SAD PERSONS Scale (SPS) was assessed in a systematic review (N=9 studies) and the data were considered insufficient to support its use in the assessment or prediction of suicidal behavior [47]. In a large study (N=5462 consecutive adults evaluated by psychiatrists in tertiary emergency departments), the suicide rates at six months, 12 months, and five years were determined [48]. Low-risk assessed by SPS at baseline died by suicide at 6 and 12 months, while high-risk scores were significantly associated with death by suicide over the five-year interval by both SPS and M-SPS [48]. Another study (N=4019 consecutive referrals to psychiatric services in emergency departments) used both SPS and M-SPS and showed that high-risk baseline values presented low sensitivity and low positive predictive power [49]. SPS did not predict suicide better than chance in this study, but when only the five original scale items that accounted for the higher proportion of suicide attempt variance were used, the sensitivity was 93.5%, and the predictability was 5-fold higher for suicide attempt presentation [49].

To evaluate the possibility of using SAD PERSONS in veterans, a study (N=271 participants from the Midwest Veterans Affairs Medical Center) concluded that the rate of false-positive and false-negative results is unacceptable [46].

The **Reasons for Living Inventory** (RFLI) was created by M. Linehan in 1983, and it consists of a checklist of motives for choosing to continue living, instead of self-harming with suicidal intent [50]. RFLI has 72 items (long version) or 48 items (short version). The six factors originally describing the structure were replicated by other authors, the internal consistency was proven acceptable (Cronbach alpha = 0.72-0.92), and the test-retest reliability was also demonstrated [51]. A high score on RFLI predicted fewer future suicide attempts in the next two years interval for initially hospitalized women with depression, but not for men, in a study (N=386 participants) [52].

RFLI was administered in a veteran male population (N=421) with various combat exposures and a history of suicide attempts, and the initial results were supportive of this tool being used in the specified population [53]. The reliability and validity of RFLI were confirmed by exploratory factor analysis and bi-factor analysis, correlates between total scores and subscale scores, and estimates of internal consistency reliability on the original instrument subscale scores [53].

The **Reasons for Living Scale-Military Version** (RFL-MV) was created later by Deutsch & Lande, who added 20 items to the items of RFLI-SV, items specifically designed to reflect the military environment characteristics [54]. RFLI-MV was evaluated on 200 patients from the military personnel, and a six-factor structure was defined, with the subscale of "Military Values" reflecting the coherence of the new dimension added to Linehan's version of the tool [54].

The **Reasons for Living versus Reasons for Dying Assessment** is another tool that combines the protective factors and triggers for suicide, to offer a more complete interpretation of the individual's risk [55]. In a study (N=49 suicidal university counseling center patients), 173 reasons for living and 145 reasons for dying were obtained and structured in eight and, respectively, nine categories, and different chi-square results for the two dimensions were obtained, suggesting a different salience of those dimensions for suicidal patients [55]. The evaluation of the reasons for dying vs. reasons for living (RFD-RFL) index in military psychiatric inpatients (N=167) following a suicide event showed that family was the most reported supporting factor (40% of the participants), while the most frequently invoked reasons for dying were general descriptors of self (27%), general statements about escape (19%), and others/relationships (19%) [56]. Higher values of this index were associated with a more significant wish to die vs. wish to live, higher levels of hopelessness, and a history of multiple suicide attempts [56]. When individuals can find more reasons for dying than for living, this could be indicative of a higher suicide risk [56].

Another study evaluated the risks of dying vs. the risks of living in patients admitted after a suicide attempt (N=60) and a monitoring period of 24 months, showing that the number of reasons for dying was the most relevant predictor for increased suicidal ideation at baseline, and reasons for dying, depressive symptoms and baseline suicide ideation predicted suicide reattempt up to 12 months later [57]. In the mediation analysis, reasons for dying mediated the effect of depressive symptoms at baseline and one-year follow-up on suicidal ideation [57]. However, reasons for living did not prove themselves a protective factor against suicide risk [57].

The **Suicide Opinion Questionnaire** (SOQ) was designed to assess the different approaches to the event of suicide in different cultures and demographic groups, being administered in multiple settings and populations, e.g., college students, medical students, and mental health professionals [58,59]. The test-retest reliability coefficient for SOQ was r=0.78 to 0.91, and the intercorrelations between the scales were in the range of r=0.20 [59]. An exploratory factor analysis detected a weak 2-factor structure in one study, and this model failed in the second study, thus raising doubts about the factorial structure of this instrument [60].

In a group of undergraduate volunteers (N=738), the responses to SOQ were compared between attempters, contemplators, and nonattempters, and a factor analysis indicated the existence of seven factors, with two significant functions discriminating between the respondents based on their gender and prior suicide history [61]. In another study (N=91 psychiatric inpatients admitted for suicidality), the SOQ was included in a battery of tests focused on suicidality, and the results showed that 20 items differed between patients with suicide attempts vs. those without at baseline and follow-up [58]. The linear discriminant analysis supported the sensitivity and specificity of the test for post-discharge suicide attempts for the 9-item scale [61]. The potential of SOQ to be useful in identifying prospectively the individuals with a risk of suicide is supported by the available data, but maybe including it in a module of tests would be more beneficial than using it alone, and using it in high-risk patients would increase its predictive power [58].

In a study (N=1758 Marine Non-Commissioned Officers) that evaluated the usefulness of SOQ in military personnel, a 4-factor structure was detected, which accounted for approximately 30% of the total variance, with sex, education, and prior exposure to suicide within the individual's military unit being significantly associated with suicide options [62].

The **Suicide Trigger Scale** (STS) is a self-report instrument to assess the trigger states for suicide, which showed a strong internal consistency, with a Cronbach alpha of 0.94, and good validity related to past suicidal behavior [63,64]. Its third version, STS-3, showed that scores may be predictive for post-discharge suicide attempts [63]. This scale has 42 items and three factors explaining 43% of the variance- Frantic Hopelessness, Ruminative Flooding, and Near-Psychotic Somatization [64]. In a validation study (N=183 adult psychiatric patients with suicidal ideation/attempt in the psychiatric emergency room) with a 1-year follow-up, suicidal subjects with suicide attempt history had a mean of 7 points more than those without such a history, and Frantic Hopelessness was a significant predictor for current suicide attempt, if only attempters requiring at least some medical attention were considered [64].

A subset of six items on the STS-3 improved the prediction of post-discharge suicide attempts, and patients with ultra-high scores differed significantly from those with ultra-low scores on mood intensity, depression, impulsiveness, abuse history, and attachment security, in a validation study (N=161 adult psychiatric patients admitted to hospital for suicidal ideation or attempt) [63].

The **Manchester Self-Harm Rule** (MSHR) was based on an exploration of multiple variables related to self-harm, i.e., those which include the nature of the act, details of self-harm episode, the main reason for current distress/precipitant to the episode of self-harm, social and demographic information, clinical history, and current medical state [65]. The four questions of MSHR refer to the history of self-harm, previous psychiatric treatment, current psychiatric treatment, and benzodiazepine taken as an overdose [65]. The tool has a sensitivity of 97%, a specificity of 26%, and a positive predictive value of 22% [65]. A related tool is the ReACT Self-Harm Rule, derived from a prospective cohort study, which analyzed 29571 self-harm presentations to hospital Emergency Departments [66]. Recent self-harm (in the last year), living alone/homelessness, cutting as a method of self-harm, and treatment for an ongoing psychiatric disorder were factors associated with higher risk [66]. This tool had 95% sensitivity and 21% specificity, with a positive predictive value of 30%, identifying 83 out of the subsequent 92 suicides [66].

The **Suicidal Behaviors Questionnaire** (SBQ) is a 34-item self-assessment instrument for suicidal thoughts and behaviors, developed by Linehan in 1981 [67]. Another, much shorter form of SBQ, containing only four items, and another 14-item tool [67-60]. Other versions of SBQ are available, but this multitude of variants precludes the finding of definite recommendations and psychometric properties for each population (adolescents vs. adults, clinical vs. nonclinical) [70,71]. SBQ 4-item version correlated with SSI scores significantly and it is recommended as a screening tool [71]. A revised version exists, SBQ-R, with four items, which benefits from empirical support as a risk measurement tool that differentiates between suicide-risk and non-suicidal risk individuals at a cut-off value of 7 (nonsuicidal sample) or 8 (clinical samples) [70]. SBQ-R has good internal consistency (Cronbach alpha = 0.97), convergent validity, a one-factor solution (89.5% of the total variance), good sensitivity (82%), and specificity (63%) when differentiating between serious planner/ideator and attempter groups [70]. SBQ-R has been translated and validated in numerous languages due to its brevity, ease of administration, and good psychometric properties [72,73].

The first item ("Have you ever thought about or attempted to kill yourself?") of the SBQ-R and the total scores have been recommended for use in both clinical and non-clinical populations [70]. A Bayesian approach to the SBQ-R supports the usefulness of item 1 for identifying other parameters of suicide, e.g., thoughts and behaviors [74]. The same analysis showed the relevance of each SBQ-R item when exploring the suicide elements and suggests that the individual item evaluation can offer clinically meaningful insights [74].

SBQ-R and the Risk-Taking Questionnaire 18 (RT-18) helped differentiate between individuals with suicide attempts and healthy controls by the variable "risk-taking" [75]. Individuals who reported a recent suicide attempt also had a higher tendency to sensation-seeking and impulsive behavior as correlates to risk-taking behaviors [75]. SBQ-R and other instruments were administered in a study (N=1310 medical graduate students) that suggested a significant risk of suicide (6.7%) among specific populations of students in China [76]. Poor relationships with the supervisor increased the risk of suicidality, suggesting more care is needed when educators are interacting with medical students, especially if the last ones are coming from families with low socio-economic status [76]. The administration of the original SBQ version in clinical populations was also explored in a study with patients diagnosed with bipolar disorder (N=98 participants); the association between anxiety dimensions and SBQ scores was relevant in a similar way to the association with depressive rumination [77]. Depressive rumination was a significant predictor of higher SBQ scores, and emotional processing was a significant predictor for lower SBQ scores for women and the entire sample [77].

In an exploration of the temperament dimensions most associated with the progression from ideation to suicide attempts, an adapted version of SBQ with 17 items was used together with the Affective and Emotional Composite Temperament Scale (AFECTS) [78]. The depressive, cyclothymic, and volatile were the most frequently associated temperaments with suicide attempters, while the cyclothymic, depressive, and euphoric temperaments were associated more with progression from ideation to suicide attempt [78].

The use of SBQ-R in a study that included post-9/11 veterans and service members seeking treatment for psychiatric symptoms (N=261 participants) reflected the high rates of suicidal ideation and behavior in this population [79]. A rate of 40% was obtained in this sample, based on the SBQ-R, indicating a high risk of suicide [79]. Negative posttraumatic thoughts about self, gender, military branch of service, higher levels of severity, anger and anger expression, less impulse control, and lower rank, all these factors were associated with a higher risk of suicidal ideation and behavior [79].

Another retrospective analysis included 3356 cases (age 55+) collected from the US National Health and Resilience in Veterans Study (2019-2020) and used SBQ-R in correlation with various demographical and personal history data [80]. The rate of suicidal ideation during the last year was 6.6%, for lifetime suicide plans was 4.1%, and for future suicide intent 0.9% [80]. A higher level of loneliness and a lower level of purpose in life was most strongly associated with last year's suicide ideation, while a lifetime history of major depressive disorder correlated with suicide plans and suicide attempts [80].

In another cohort study (N=3078 US military veterans), the rates of suicide ideation and suicide attempts did not present higher values during the COVID-19 pandemic vs. pre-pandemic level, but a small percentage of veterans (2.6%) developed new-onset suicidal ideation during the pandemic [81]. SBQ-R items 1 and 2 were used in this study, together with the UCLA Loneliness Scale, Alcohol Use Disorders Identification Test, Life Events Checklist for DSM-5, Adverse Childhood Experience Questionnaire, Medical Outcome Study Social Support Scale-5, and the Coronavirus Health and Impact Survey [81].

The **Military Suicide Attitudes Questionnaire** (MSAQ) is a 32-item instrument with a four-factor structure designed to be used by military service members [82]. The confirmatory analysis included 317 US military personnel, and good levels of test-retest reliability were found; the four factors identified were "individual-based rejection vs. acceptance", "psychache vs. pathological", "unit-based acceptance vs. rejection", and "moral vs. immoral" [82]. The validation study did not control for social desirability, which may be an important limitation [82].

Two different studies of MSAQ validation included 200 and 1116 individuals, respectively, from the military environments, and factorial analyses support the previously reported four-factor structure [3]. The reliabilities ranged from 0.77 to 0.83; men had more negative suicide-related beliefs vs. women, and discomfort and unacceptability beliefs were more positively associated with perceived barriers to care [3].

The **Suicide Probability Scale** was developed in 1982, it contains 36 items and is a self-report tool [83]. This scale was designed to assess suicide risk in adolescents and adults, and it only takes 5 to 10 minutes to complete [83]. The score is obtained by adding the answers using a 4-point scale; there are four factors: Hopelessness, Suicide Ideation, Negative Self-evaluation, and Hostility [83]. The Cronbach alpha for this scale was 0.92 in a sample of hospitalized patients with psychiatric disorders, and the results correlated with the scores on the Beck Depression Inventory-II and Adult Suicidal Ideation Questionnaire [83,84]. The utility of this scale did not confirm its utility in adolescents to assess suicide potential, and the factorial structure was not confirmed [85]. However, other studies support its utility for this population, with good convergent and discriminant validity [86].

The **Sheehan Suicidality Tracking Scale** (SSTS) has a standard version with 16 items that assess the seriousness of suicidality, the frequency of key phenomena, and the overall time spent in suicidality on a Likert scale (0-4) [87]. It is available in a patient and clinician-rated format, but a version that reconciles the eventual discrepancies between the previous two in specific items also exists [87]. There is a clinically meaningful change version of the SSTS and a pediatric version (P-SSTS) [87,88]. Another version of SSTS (v.10) has an 8-item instrument that measures variables related to self-injury, self-harm, suicide ideation and suicide attempt, with each item being scored on a Likert scale, from 0 (absent) to 4 (extremely) [89]. SSTS proved good convergent, divergent, internal consistency, and test-retest stability, according to a cross-sectional study (N=303 undergraduate students) [89]. All SSTS items were loaded on a single factor, suggesting it could be useful as a screening tool [89].

In a clinical study (N=82 subjects with generalized anxiety disorder) that assessed an experimental corticotropin-releasing factor antagonist vs. placebo and an active comparator (escitalopram), SSTS had a good sensitivity, as well as item 3 on the Hamilton Depression Rating Scale for Depression (HAMD) for identification suicidal thoughts or behaviors (100% and 63%, respectively) [90].

In another clinical trial, which investigated the evolution of depressive symptoms during citalopram + lithium vs. citalopram + placebo (N=80 patients), SSTS and Montgomery Asberg Depression Rating Scale (MADRS) were administered as primary measures, and the results showed a larger reduction on SSTS vs. MADRS (43% vs. 25%) [91]. A subgroup of patients who received lithium and citalopram had higher SSTS remission rates (45% vs. 19%) [91].

The **InterSePT scale for suicidal thinking** (ISST) has 12 items and assesses the current suicidal ideation in patients with chronic psychosis [92]. In two studies, with 22 inpatients with schizophrenia and schizoaffective disorders who had a recent suicide attempt or ideation, and 980 patients with the same disorders and a history of suicidal ideation in the past 36 months, respectively, ISST was used as a monitoring tool for two years, together with Clinical Global Impression for Severity of Suicide (CGI-SS), Calgary Depression Scale (CDS), Scale of Functioning (SOF) and Positive and Negative Symptom Scale (PANSS) [92]. The Cronbach alpha was high (0.86-0.89) for individual items, as well as the overall value (0.88) [92].

The ISST total score was highly correlated with the CGI-SS by the blind rater [92]. During the InterSePT trial that evaluated clozapine efficacy, ISST and CDS were administered to predict suicide attempts or hospitalizations [93,94]. The results of the InterSePT trial suggest both tools may be useful for assisting clinical decision-making regarding the suicide risk in patients with chronic psychoses [93].

The **Acquired Capability for Suicide Scale** (ACSS) is a 20-item tool administered based on the Interpersonal Psychology Theory of Suicide and assesses factors that facilitate individuals to acquire the ability to engage in suicidal acts, such as fearlessness about death and habituation to pain (which are considered needed to overcome the self-preservation reflexes) [95]. The items are scored on a Likert scale, from 0 to 4, and the reliability of the scale was calculated in a study to be alpha=0.80 [95]. In a study that included suicide ideators, depressed suicide attempters, and control participants (N=44 participants), based on the ACSS administration, suicide attempters showed the highest level of fearlessness and pain insensitivity and a greater history of painful and provocative life events [96].

A systematic review evaluated the use of ACSS among US military personnel and veteran samples (n=31 studies), and the results, based on a high risk of bias sources, indicated inconsistent findings across studies [97]. The authors of the respective analysis recommended caution in the interpretation of ACSS empirical data [97].

Regarding the instruments for the assessment of depression, which include items specific for suicidality, three tools were analyzed based on their wide use in clinical and epidemiological trials and their validated psychometric profile.

The **Beck Depression Inventory** (BDI) is among the most widely used self-rating scales for depression severity, with a long history of being administered in therapeutic and other various studies, starting from its publication in 1961 [98]. The internal reliability was confirmed in clinical and nonclinical samples and the average Cronbach alpha was 0.75; the factorial validity of this instrument showed a variable number of factors, from 1 to 9, and the convergent and discriminant validity was also proven [98]. Of interest for this review is the suicide item for its predictive value for deaths by suicide, which is scored from 0 (“I do not have any thoughts of killing myself”) to 3 (“I would kill myself if I had the chance”) [99].

A study that included 5319 patients showed that the single BDI item significantly predicted both deaths by suicide and repeated suicide attempts [100]. Each successive rating of the suicide item on BDI conferred greater risk, suggesting a direct proportionality between the two values, with an optimal cutoff score of ≥1 for suicide and ≥2 for suicide attempts providing the maximum equilibrium between sensitivity and specificity [100]. A value of ≥1 on item 9 on BDI-II was also used as a marker for suicide ideation in a study that concluded type D personality could be a risk factor for suicide ideation in individuals with major depression with (N=318) [101].

The **Hamilton Rating Scale for Depression** (HAMD) represents, in fact, a variety of scales that differ by the number of items and modalities of administration [102]. HAMD is a valid and sensitive clinimetric index and requires informed use, with the specific type of HAMD to be selected for each context [102]. The HAMD includes an item on the assessment of suicide risk (scored from 0 – “absent” to 4- “attempts at suicide”) [99]. It correlates significantly with the number of suicide attempts and the age at the first attempt in a study with 281 suicide attempters [103].

The **Montgomery Asberg Depression Rating Scale** (MADRS) is a 10-item tool dedicated to the measurement of depressive severity that includes an item dedicated to „suicidal thoughts” (scored from 0 – „enjoys life or takes it as it comes” to 6 – „explicit plans for suicide when there is an opportunity/active preparations for suicide”) [99]. This item of MADRS and the suicide item on HAMD correlated with the first five items on SSI at a significant level (r>0.80) in a study that assessed the efficacy of ketamine on the severity of depression [99].

DISCUSSION

Table 1 presents a synthesis of the instruments explored in this review. All of the reviewed instruments present benefits and shortcomings, making the unanimous recommendation of only one such instrument for evaluating suicide risk highly unlikely. However, based on the characteristics of each population or group that needs to be explored (screened or monitored) for suicide risk, a certain set of tools may be selected from the available resources, depending on their psychometric properties and previous studies' results available.

Table 1: Analysis of the psychometric tools for the assessment of suicide

Psychometric instrument	Studies on active military personnel and /or veterans	Studies on the civilian population	Advantages	Disadvantages	Observations
SASII	Yes [18,19]	Yes [14-17]	Standardized definition of suicidal and other self-injurious activities; individual ratings for the components of lethality, such as the method used, the consequences of the gesture, and the therapy needed.	More studies on military personnel are needed to confirm its psychometric properties in this population and its predictive power. The necessary time to administer is quite long.	Reliability and convergent validity were determined. Studies that compared this instrument with other validated instruments were performed. Evaluates both suicidal and non-suicidal self-injury behaviors.
C-SSRS	Yes [23,25-27]	Yes [21-23]	Separate assessments of the severity and intensity of suicidal ideation, autolytic behavior, and lethality of the attempt.	Data about the self-reported version of C-SSRS in military personnel is insufficient to recommend its use in this population.	Very good convergent and divergent validity with other scales used for the same purposes.
SSI, B-SSI, SSI-C, SSI-W	Yes [34,35]	Yes [28-20,33]	The set of SSI scales offers a more nuanced choice for different settings and populations.	The dimensional structure is debated.	Good internal consistency and sensitivity to changes for several important suicide factors.
BHS	Yes [42,43]	Yes [38]	Evaluation of suicide risk in clinical and non-clinical adult populations. Self-administered or clinician-administered.		Demonstrated predictive validity and reliability. Shorter versions of this tool have been created.
SPS, M-SPS	Yes [46]	Yes [47-49]	Ease of administration; the existence of a shorter version.	The predictive value of suicidal behavior was considered inconsistent.	A further refinement of the items included in this scale may be needed to increase its predictive value.
RFLI, RFL-MV	Yes [53,54]	Yes [51,52]	A self-administered version and a clinician-administered version exist. A military version of this scale also has been published.	The RFLI scales (both long and short versions) are quite long due to the large number of items.	A checklist of motives for living that can represent useful resources to work with in psychotherapy. Acceptable internal consistency and test-retest reliability.
RFD-RFL Index	Yes [56]	Yes [55,57]	It combines two distinct lists of reasons for living and reasons for dying.	It is a complex and long instrument to administer (17 domains).	Reasons for living are not a valid protective factor against suicide risk. However, reasons for dying may be useful as a predictor of suicide risk.
SOQ	Yes [62]	Yes [58,61]	It was constructed to be sensitive to cultural and demographic variability.	Different studies did not support the factorial structure.	Test-retest reliability was demonstrated. It was administered in different groups. It has the potential to help identify the risk of suicide.
STS	None were identified	Yes [63,64]	It has several versions, some of them presenting scores with good predictive values.	More studies are needed to support its utility, especially in military personnel.	Good internal consistency and validity related to past suicidal behavior. A subset of items could be further explored for higher predictive value than the overall scale.

MSHR	None were identified	Yes [65]	Based on multiple variables related to self-harm	Psychometric properties are poor.	Good sensitivity but poor specificity and low predictive value.
ReACT SHR	None were identified	Yes [66]	Based on a large populational study, and includes multiple variables related to suicide.	More research is needed to validate this instrument.	Good sensitivity but poor specificity and low predictive value.
SBQ, SBQ-R	Yes [79-81]	Yes [70,72,73,75-78]	Many versions of this instrument exist. It was explored in multiple studies that enrolled veterans.	Psychometric properties are poorly defined, except for SBQ-R.	It was extensively explored in both military and civilian populations.
MSAQ	Yes [3,82]	Not applicable	It was explored in two studies with US military personnel.	The currently available studies have methodological limitations.	More studies are needed to explore the validity of this instrument.
SUPS	None were identified	Yes [83-86]	It is a short instrument, easy to administer.	It is a self-report tool. The factorial structure is debated.	Good validity and reliability. The utility of this tool in adolescents to predict suicide was not confirmed.
SSTS	None were identified	Yes [89-91]	Patient and clinician-rated format, and a version that reconciles the differences between the two also exists. A shorter version exists.	No study was identified with military populations.	Good convergent, divergent, internal consistency, and test-retest stability. Only one factor was identified: good screening tools.
ISST	None were identified	Yes [92]	A valuable instrument for assessing suicidal ideation in patients with schizophrenia spectrum disorders.	It applies to a limited population, i.e., a clinical population with chronic psychoses.	High construct validity, good correlation with other scales.
ACSS	Yes [97]	Yes [95,96]	Short, easy-to-administer tool.	Caution is recommended in the interpretation of this scale's results in the military population.	Good validity and reliability.
Scales with single items evaluated for suicide predictive power					
BDI - item 9	None were identified	Yes [99-101]	The suicide item predicted deaths by suicide and suicide attempts.	Insufficient data to support scores on single items as independent predictors of suicide.	Good internal reliability in clinical and nonclinical samples for depressive disorders.
HAMD – item 3		Yes [103]	The suicide item correlated with the number of suicide attempts.		Valid and sensitive tool for depression.
MADRS – item 10		Yes [99]	Short and easy-to-administer tool. The suicide item correlated with items on SSI.		A useful tool for the quantification of depressive symptoms across age intervals.

SASII= Suicide Attempt Self-Injury Interview, C-SSRS= Columbia Suicide Severity Rating Scale, SSI= Scale for Suicide Ideation, B-SSI= Beck Suicidal Ideation Scale, BHS= Beck Hopelessness Scale, SPS= SAD PERSONS, M-SPS= Modified SAD PERSONS, RFLI= Reasons for Living Inventory, RFL-RFD= Reasons for Living versus Reasons for Dying Assessment, RFL-MV= Reasons for Living- Military Version, SOQ= Suicide Opinion Questionnaire, STS= Suicide Trigger Scale, MSHR= Manchester Self-Harm Rule, ReACT SHR= ReACT Self-Harm Rule, SBQ= Suicidal Behaviors Questionnaire, MSAQ= Military Suicide Attitudes Questionnaire, SSTS= Suicide Probability Scale Sheehan Suicidality Tracking Scale, ISST= InterSePT scale for suicidal thinking, ACSS= Acquired Capability for Suicide Scale, BDI= Beck Depression Inventory, HAMD= Hamilton Rating Scale for Depression, MADRS= Montgomery Asberg Depression Rating Scale

A prospective comparative analysis of multiple scales for the assessment of suicide risk included MSHR, ReACT Self-Harm Rule, SAD PERSONS, M-SPS, and Barratt Impulsivity Scale (BIS) in patients who would repeat self-harm within six months [104]. By evaluating 483 episodes of self-harm, the recurrence rate was 30%, and the sensitivity of these tools varied from 1% for SAD PERSONS, to 97% for MSHR [104]. The positive predictive value also varied, from 13% for M-SPS to 47% for the clinician assessment of risk [104]. The authors of the respective study concluded that risk scales for self-harm have limited clinical utility, as they are no better than clinician or patient ratings of risk [76]. A secondary analysis of data collected from multicenter prospective cohort studies showed that some individual items outperformed the scale in which they are included, but no items were superior to clinician or patient risk estimations

[105]. Based on the data from the same analysis, the use of suicide risk scales was less cost-effective than the clinician and patient ratings based on QALY (Quality-Adjusted Life Years) exploration [106].

A systematic review that evaluated the level of evidence for instruments assessing the suicide risk (n=21 studies) included 15 such tools and for the outcome suicide attempt, SPS had a sensitivity of 15% with a specificity of 97%, MSHR 97% and 20%, respectively, ReACT a similarly low specificity with MSHR; BHS presented a sensitivity of 89% and a specificity of 42% for the outcome suicide [107].

A direct comparison of the psychometric properties of CSSRS, STS, and SSTS in 199 psychiatric inpatients was conducted in a randomized manner by three trained judges [108]. All three assessments had very good accuracy for suicidal ideation and attempts (k=0.72 to 1.00, and 0.82 to 0.95, respectively), with an interrater agreement about CSSRS categories being more varied (k=0.48 to 1.00) [108]. Cronbach alpha value was less than 0.55 for CSSRS ideation and 0.78-0.92 for STS and SSTS [108]. The administration of any of these three instruments would improve reliability vs. unstructured assessment in the evaluation of suicidal ideation and behavior [108].

A study included 40 subjects with suicidal ideation and behavior who were interviewed using the InterSePT Scale for Suicidal Thinking-Plus, the SSTS, and the CSSRS, administered in a random sequence [109]. An agreement between these scales and the 2012 Food and Drug Administration (FDA) Classification Algorithm of Suicide Agreement categories was sought [109]. SSTS and InterSePT scale had an acceptable agreement with CSRSS in the detection of passive ideation, active ideation with method, intent, and plan, completed suicide, preparatory actions, and self-injurious behaviors [109]. However, these tools did not detect the presence or absence of other active suicidal ideation combination categories and aborted and interrupted attempts [109]. These results show the importance of validity exploration regarding the widely used scales for suicide risk assessment and the concordance with the FDA categories [109].

The use of single items from validated scales for depression as indicators for the assessment of suicidal ideation is a debated approach in clinical practice. A comparison of BDI and HAMD suicidal items and SSI scores was conducted in 281 suicide attempters and a positive correlation between the first two and the specific scale for suicide scores (SSI) [103].

The importance of searching for chronic diseases as predictors for suicide that can interfere with mental health due to negative impact on multiple levels (i.e., low functionality, poor quality of life, pain, low self-esteem) can not be overemphasized [110-113]. Therefore, any predictive model for suicide should take into account not only personality features, psychiatric symptoms, and actual psychological stressors but also organic diseases and their essential effects on mental health. This involves the necessity of a complex evaluation of individuals with a risk of suicide by a team of specialists, not only psychiatrists and clinical psychologists.

Treatment of the pathological background in patients with suicide risk can not be overemphasized [114-117]. Although significant advances in the field of psychopharmacology can be observed, as well as multiple psychotherapies focused on reducing the risk of self-harm, it is essential to note that no single intervention can be appropriate for all cases with self-harm tendencies, due to the unique configuration of vulnerability factors and triggers that exist in each case.

The current review's limitations are related to its narrative design, which explains why important sources may be missed from the analysis and the lack of assessing the quality of each report. The review's strengths are its focus on clinical tools used for military and civilian populations and the inclusion of psychometric properties together with relevant clinical and epidemiological studies for each instrument.

Directions for further research in this field are the need to further investigate the psychometric properties of some scales presented in this review, like MSAQ, which is dedicated to military personnel, and the comparative exploration of such instruments focused on clinical and non-clinical samples, to highlight the strengths and vulnerabilities of each. The final purpose of further research could be the construction of an algorithm able to detect individuals vulnerable to self-harm behavior.

CONCLUSION

Many good-quality, validated instruments allow for assessing the risk of suicide in civilian populations, and some of them have been explored in military personnel, like SBQ, C-SSRS, SASII, SOQ, etc., while others have been constructed especially for military populations, like MSAQ. Unfortunately, each of these tools has shortcomings that are expected to be corrected by further research.

Therefore, any algorithm for the detection of suicide risk based exclusively on the existing scales, interviews, and questionnaires would be vulnerable to a more rigorous analysis since the complex pathogenesis of the suicide phenomenon would impair its predictability.

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The author declares no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

As the only author, I assume the responsibility for data collection, writing, and revision of the manuscript.

Ethics approval and consent to participate

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SYSTEMATIC REVIEW

New Insights in Microcirculation Research in Sepsis

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Abstract: Sepsis remains a leading cause of morbidity and mortality worldwide, with certain high-risk populations—including elderly patients, neonates, immunocompromised individuals, those with chronic diseases, and pregnant women—experiencing worse outcomes. These groups exhibit distinct pathophysiological responses, which complicate diagnosis, treatment, and prognosis. Despite advancements in sepsis management, challenges persist in early risk stratification, individualized therapeutic strategies, and long-term recovery. Aim: This study aims to evaluate the impact of sepsis on high-risk populations, identify prognostic factors influencing clinical outcomes, and explore personalized treatment approaches to optimize patient management. Methodology: A systematic narrative review was conducted, following PRISMA guidelines, by analyzing peer-reviewed literature from 2015 to 2025. This study included the analysis of 80 scientific articles from eight international databases. Studies focusing on sepsis pathophysiology, microcirculatory dysfunction, diagnostic techniques, therapeutic interventions, and post-sepsis outcomes in high-risk populations were included. Results: Elderly patients demonstrated the highest sepsis-related mortality rates (>40%), while neonates and immunocompromised individuals exhibited delayed inflammatory responses, complicating diagnosis. Prognostic biomarkers such as IL-6 and IL-10 showed potential utility in these populations. Personalized fluid resuscitation, antimicrobial stewardship, and immune-modulating therapies were identified as critical to improving outcomes. Post-sepsis syndrome (PSS) was prevalent, particularly in survivors with chronic comorbidities and prolonged ICU stays. Conclusions: Optimizing sepsis management in high-risk populations requires a multidisciplinary, personalized approach, incorporating early biomarker-based diagnostics, precision-guided hemodynamic support, and tailored rehabilitation programs. Future research should focus on novel immunotherapies, antimicrobial resistance strategies, and long-term functional recovery in sepsis survivors.

Keywords: sepsis, microcirculatory dysfunction, prognostic biomarkers, post-sepsis syndrome, antimicrobial resistance

INTRODUCTION

Microcirculatory Alterations in Sepsis

Sepsis is a life-threatening condition characterized by a dysregulated host response to infection, leading to systemic inflammation, multi-organ dysfunction, and, in severe cases, death. One of the critical pathophysiological hallmarks of sepsis is microcirculatory dysfunction, which plays a pivotal role in tissue hypoxia and organ failure [1]. The microcirculation comprises small blood vessels, including arterioles, capillaries, and venules, responsible for oxygen delivery and metabolic exchange at the cellular level [2]. In sepsis, widespread endothelial activation, increased vascular permeability, and impaired autoregulation disrupt normal microvascular perfusion, exacerbating cellular distress and metabolic imbalance [3].

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Microcirculatory alterations in sepsis are driven by complex interactions between inflammatory mediators, coagulation disturbances, and endothelial dysfunction. Inflammatory cytokines such as TNF-α, IL-6, and IL-1β induce endothelial cell activation, promoting leukocyte adhesion and microvascular thrombosis [4,5]. Additionally, nitric oxide (NO) dysregulation contributes to an imbalance between vasodilation and vasoconstriction, further impairing tissue perfusion [6].

From a diagnostic perspective, bedside techniques such as sublingual videomicroscopy and sidestream dark-field imaging (SDF) have emerged as valuable tools for assessing microcirculatory abnormalities in critically ill patients with sepsis [7]. These imaging modalities allow real-time visualization of capillary flow, providing essential insights into the extent of microvascular dysfunction and guiding therapeutic interventions. Advanced imaging approaches, including contrast-enhanced ultrasound (CEUS) and near-infrared spectroscopy (NIRS), offer additional perspectives on micro-circulatory heterogeneity and oxygenation deficits in septic patients [8,9].

Understanding microcirculatory impairments in sepsis is crucial for developing targeted therapeutic strategies aimed at restoring tissue perfusion and mitigating organ dysfunction [10]. Recent research has explored the potential benefits of fluid resuscitation strategies, vasoactive agents, and adjunctive therapies such as corticosteroids and endothelial-targeted interventions in optimizing microvascular function.

Fundamental Pathophysiological Mechanisms – Microcirculatory Dysfunction and Endothelial Abnormalities in Sepsis

The pathophysiological mechanisms underlying microcirculatory dysfunction in sepsis are complex and involve a cascade of inflammatory, coagulative, and endothelial disturbances that ultimately lead to impaired oxygen delivery and tissue hypoxia [11].

Microcirculatory impairment is a defining feature of sepsis, characterized by altered capillary perfusion, increased heterogeneity in blood flow, and endothelial dysfunction [12]. Unlike systemic hemodynamic failure, which is typically reflected in macrovascular changes (e.g., hypotension), sepsis-related microcirculatory alterations occur independently of blood pressure changes and directly contribute to organ failure [13].

Inflammation plays a central role in disrupting microcirculatory homeostasis. The excessive release of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β leads to endothelial activation, increased vascular permeability, and leukocyte adhesion to the endothelium [14]. This inflammatory cascade results in endothelial swelling, capillary plugging by activated leukocytes, and microthrombi formation, all of which impede oxygen diffusion and exacerbate tissue hypoxia [15]. Additionally, dysregulated nitric oxide (NO) production contributes to vascular tone abnormalities, creating an imbalance between vasodilation and vasoconstriction that further disrupts blood flow distribution at the microvascular level [16].

From a structural perspective, capillary rarefaction and endothelial glycocalyx degradation are key pathological features of microcirculatory dysfunction in sepsis. The glycocalyx, a protective layer covering endothelial cells, plays a crucial role in vascular permeability and mechanotransduction [17]. In sepsis, glycocalyx breakdown due to enzymatic degradation (by heparanase and matrix metalloproteinases) results in increased vascular leakage, impaired shear stress sensing, and loss of anticoagulant properties, contributing to sepsis-induced coagulopathy (SIC) [18].

Endothelial dysfunction in sepsis is a result of direct cellular injury, oxidative stress, and immune activation. Endothelial cells, which normally maintain vascular homeostasis through the balanced release of vasoactive substances, become dysregulated, shifting towards a pro-inflammatory and pro-thrombotic phenotype [19].

One of the key mechanisms involved in endothelial dysfunction is increased expression of adhesion molecules such as ICAM-1, VCAM-1, and selectins, which facilitate leukocyte adhesion and transmigration into tissues, further amplifying inflammation [20]. This process leads to capillary plugging and increased diffusion distance for oxygen, creating a mismatch between oxygen delivery and cellular demand [21].

At the same time, mitochondrial dysfunction in endothelial cells exacerbates metabolic failure, reducing the ability of microvascular endothelial cells to regulate vascular tone and respond to local oxygen needs appropriately [22]. The resulting endothelial metabolic shutdown contributes to an irreversible cycle of hypoxia, organ dysfunction, and cellular apoptosis in severe sepsis cases [23].

Sepsis-induced coagulopathy (SIC) is a well-recognized phenomenon in which systemic inflammation disrupts the balance between coagulation and fibrinolysis, leading to widespread microthrombosis and perfusion defects [24]. The overactivation of the coagulation cascade is primarily driven by excessive tissue factor (TF) expression, leading to thrombin generation, platelet aggregation, and fibrin deposition within the microcirculation [25].

Additionally, antithrombotic mechanisms become suppressed due to reduced production of key anticoagulant proteins such as antithrombin, protein C, and thrombomodulin, further promoting a prothrombotic state [26]. The resulting microvascular thrombosis impairs capillary perfusion, leading to local ischemia, tissue necrosis, and multi-organ failure in severe septic shock cases [27].

Furthermore, fibrinolytic dysfunction exacerbates these complications. Sepsis induces the release of plasminogen activator inhibitor-1 (PAI-1), which suppresses fibrinolysis and prevents the clearance of microthrombi, further worsening microvascular obstruction

[28]. This shift towards a hypercoagulable state with impaired clot resolution represents a critical therapeutic target in sepsis management.

The long-term impact of microvascular dysfunction in sepsis extends beyond the acute phase of the disease. Persistent endothelial damage and capillary loss contribute to post-sepsis syndrome, characterized by prolonged inflammation, immune suppression, and residual organ dysfunction [29]. Chronic microvascular dysfunction has been linked to long-term cognitive impairment, cardiovascular complications, and persistent fatigue in sepsis survivors [30].

From a therapeutic standpoint, interventions targeting endothelial protection, micro-vascular flow optimization, and early coagulation modulation have shown promise in experimental and clinical studies [31]. Strategies such as glycocalyx preservation (e.g., fluid therapy with albumin, heparan sulfate analogs), endothelial stabilizing agents (e.g., steroids, angiopoietin modulators), and anticoagulant therapy in select patient populations are under investigation for their potential to mitigate sepsis-induced microvascular injury [32].

Prevalence of Microcirculatory Dysfunction in Sepsis

The prevalence of microcirculatory dysfunction in sepsis varies depending on disease severity, patient population, and the diagnostic techniques used. Studies using sublingual videomicroscopy and sidestream dark-field (SDF) imaging have shown that nearly 90% of patients with septic shock exhibit significant microvascular abnormalities, including reduced capillary density, increased flow heterogeneity, and impaired oxygen extraction [33,34]. Even in patients with less severe sepsis, microcirculatory alterations are observed in approximately 50–70% of cases, highlighting the early involvement of the microvasculature in disease progression [35].

The extent of microcirculatory dysfunction is closely linked to the severity of sepsis. In its early stages, microvascular perfusion changes may be subtle while overall hemodynamics remain stable. As sepsis advances to septic shock, severe capillary perfusion defects become more pronounced, leading to increased lactate levels, tissue hypoxia, and multi-organ failure [36].

Certain patient populations are particularly susceptible to profound microcirculatory disturbances. Elderly patients often experience more severe endothelial dysfunction due to age-related vascular changes and a diminished ability to compensate for inflammatory stress [37]. Patients with chronic diseases such as diabetes or chronic kidney disease face a heightened risk due to pre-existing endothelial dysfunction and oxidative stress, which exacerbate sepsis-induced microvascular damage [38]. Immunocompromised individuals, including cancer patients and transplant recipients, frequently exhibit atypical microcirculatory responses due to an altered inflammatory cascade, making early detection more challenging [39].

Microcirculatory dysfunction is significantly more prevalent in sepsis compared to other critical illnesses. In patients with non-septic shock, such as cardiogenic or hemorrhagic shock, global hemodynamic parameters may be compromised, but microvascular function remains relatively preserved in the early stages [40]. In contrast, sepsis induces early and widespread microvascular failure even when macrocirculatory parameters, such as blood pressure, appear normal [41].

In non-septic intensive care unit (ICU) patients, studies indicate that microcirculatory alterations affect only 10–30% of individuals, whereas in sepsis, this prevalence is three to five times higher [42]. This contrast highlights the unique impact of sepsis on the endothelium and microvascular network, making microcirculatory dysfunction a key prognostic indicator.

Special Categories of Patients with Sepsis

Sepsis represents a major challenge in medical practice, but certain categories of patients are at significantly higher risk of complications and mortality. These patients require tailored therapeutic approaches, as they have distinct physiological and immunological characteristics that influence their response to treatment and overall prognosis [43].

Older adults are particularly vulnerable to sepsis due to multiple physiological changes associated with aging. Their immune system undergoes a progressive decline, known as immunosenescence, which is characterised by a reduced ability to respond to infections and impaired production of pro-inflammatory cytokines [44]. Additionally, a state of chronic low-grade inflammation (inflamm-aging) contributes to dysfunctional immune responses, increasing the severity of sepsis.

From a cardiovascular perspective, elderly patients often exhibit increased vascular stiffness and reduced cardiac reserve, limiting their ability to compensate for septic shock [45]. Furthermore, frailty and sarcopenia negatively impact prognosis, increasing the risk of multiple organ failure.

The treatment of sepsis in elderly patients should be individualised, considering their increased risk of cardiovascular overload during fluid resuscitation and their predisposition to acute kidney injury. Close monitoring of haemodynamic status and careful adjustment of antimicrobial therapy based on renal function are essential to improving outcomes.

The presence of chronic diseases affects both the progression and management of sepsis, often complicating treatment strategies and patient outcomes.

Diabetic patients are more vulnerable to severe infections because persistent hyperglycaemia weakens neutrophil function and encourages the growth of resistant pathogens. Additionally, diabetes contributes to endothelial dysfunction, worsening the microcirculatory disturbances typical of sepsis [46].

Patients with chronic kidney disease experience a heightened risk of sepsis as uremia-induced immunosuppression weakens their immune response. The frequent use of vascular catheters further increases infection risks, while the accumulation of uraemic toxins disrupts immune function and slows antimicrobial clearance [47]. These factors make precise antibiotic dose adjustments essential, considering the patient's glomerular filtration rate.

Patients with chronic obstructive pulmonary disease are prone to respiratory sepsis because impaired mucociliary function and chronic airway inflammation promote recurrent infections. Prolonged corticosteroid use, a common treatment for COPD, can further suppress the immune system, making patients more susceptible to opportunistic infections [48].

Managing sepsis in patients with chronic conditions requires a careful approach that balances aggressive therapy with the risks posed by pre-existing diseases. Optimising antimicrobial therapy and fluid resuscitation plays a key role in preventing further deterioration of organ function [49].

Sepsis in children differs significantly from adult sepsis in terms of pathophysiology and clinical presentation. Neonates and infants have an immature immune system, making them more susceptible to severe bacterial and viral infections. Furthermore, infants have a limited ability to mount an effective inflammatory response, potentially delaying the diagnosis of sepsis [50].

Beyond immunological differences, children exhibit major variations in drug metabolism, necessitating careful adjustments in antibiotic and vasopressor dosing based on body weight and organ maturity [51].

Early recognition of sepsis is critical in paediatric patients, as hypotension—a hallmark of sepsis in adults—tends to appear late in children. Identifying early signs of impaired tissue perfusion is therefore essential for the timely initiation of treatment.

Immunosuppressed individuals face a significantly higher risk of severe sepsis and life-threatening complications, often with atypical clinical presentations that can delay diagnosis and treatment [52].

This group includes oncological patients, organ transplant recipients on immunosuppressive therapy, individuals with advanced HIV infection, and patients with autoimmune diseases undergoing biologic treatments. Due to a weakened immune response, sepsis in these patients is frequently caused by opportunistic pathogens, and the usual inflammatory signs may be absent. In cases of severe neutropenia, fever might not be present, making early detection more challenging [53].

Effective management relies on the prompt administration of broad-spectrum antibiotics, close monitoring of inflammatory markers, and immune support when necessary. Granulocyte-colony stimulating factor may be considered to enhance neutrophil recovery and improve outcomes in selected cases [54].

Pregnancy induces significant physiological changes that increase a woman's susceptibility to severe infections and septic complications. Increased placental blood flow and alterations in immune responses can influence both the progression of sepsis and the response to treatment [55].

Sepsis during pregnancy can have devastating consequences for both the mother and the fetus. Intrauterine infections, such as chorioamnionitis, may trigger preterm labour or result in fetal demise. Moreover, maternal hypotension and placental hypoperfusion can severely impact fetal development [56].

The management of sepsis in pregnancy must be prompt and aggressive, with rapid administration of antibiotics that are safe for the fetus and careful monitoring of maternal haemodynamic status. The timing and mode of delivery should be determined through a multidisciplinary approach, balancing maternal and fetal risks.

MATERIALS AND METHODS

This study employed a comprehensive approach to investigate the impact of chronic comorbidities on the progression and management of sepsis. A systematic narrative review methodology was chosen to integrate the structured rigor of a systematic review with a narrative synthesis approach, ensuring a nuanced analysis of a complex, multi-disciplinary topic. The review synthesizes existing literature on the interactions between sepsis and chronic conditions such as diabetes, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and immunosuppressive states, emphasizing clinical implications and future research directions.

The analysis focused on three key aspects:

Pathophysiological mechanisms underlying increased susceptibility to sepsis in patients with diabetes, CKD, COPD, and immunosuppression, highlighting immune dysfunction, endothelial damage, and microbiome alterations.

Impact of chronic diseases on sepsis outcomes, including prolonged hospitalization, increased mortality risk, and the need for individualized therapeutic strategies.

Optimization of sepsis management in these high-risk populations, exploring antimicrobial stewardship, fluid resuscitation strategies, immunomodulatory therapies, and personalized treatment approaches based on disease-specific factors.

A key question that shaped the direction of this review was: How do chronic comorbidities influence the pathophysiology, clinical presentation, and management of sepsis, and what strategies can optimize patient outcomes in these high-risk populations?

To ensure a systematic synthesis of findings, studies were selected based on predefined inclusion criteria, prioritizing clinical trials, observational research, and meta-analyses that explored sepsis in patients with chronic comorbidities. A comparative evaluation of treatment strategies was conducted, examining the effectiveness and safety of current therapeutic approaches while identifying gaps in knowledge and areas requiring further investigation.

Approach to Identifying Relevant Articles

A systematic literature search was performed across multiple academic databases, including PubMed, Scopus, Web of Science, Elsevier, Springer, Wiley Online Library, MDPI, and Frontiers, to investigate the role of microcirculatory dysfunction in sepsis. The search process adhered to PRISMA guidelines, employing Boolean operators (AND, OR) to enhance search precision and optimize the identification of relevant studies on microvascular changes associated with sepsis.

Key search terms included: "microcirculatory dysfunction in sepsis" (17,100 results), "sepsis-induced endothelial dysfunction" (17,800 results), "capillary perfusion in septic shock" (16,700 results), "microvascular alterations and organ failure" (18,300 results), and "sepsis-associated mitochondrial dysfunction" (10,800 results).

Structured search strings were applied to maximize relevance and retrieve high-quality studies, such as: "(microcirculatory dysfunction OR endothelial dysfunction) AND (sepsis OR septic shock) AND (capillary perfusion OR mitochondrial dysfunction)."

To ensure the inclusion of the most relevant and up-to-date studies, several selection filters were applied. The search was restricted to publications from 2015 to 2025, allowing for the incorporation of both recent developments and foundational research that contribute to the understanding of microvascular dysfunction in sepsis. Only full-text articles available in English were considered to ensure accessibility to studies with broad international relevance.

The review focused exclusively on peer-reviewed literature, including clinical trials, systematic reviews, and meta-analyses. Priority was given to review articles offering a comprehensive synthesis of current knowledge, while clinical studies were evaluated for their insights into the relationships between microvascular dysfunction, disease progression, and treatment outcomes.

Search queries were tailored for each database to account for variations in indexing and search functionalities. Beyond standard database searches, citation tracking and reference analysis of key studies were conducted to identify additional relevant research that may not have been captured in the initial search. This method helped enhance the dataset by incorporating essential studies that might have been overlooked through automated search strategies.

Eligibility Criteria for Study Selection

Inclusion Criteria

The selected studies needed to address the challenges and treatment approaches for sepsis in high-risk patient groups. Included publications were required to provide relevant information on these aspects:

Pathophysiological mechanisms, such as immune dysregulation, endothelial dysfunction, and metabolic alterations in neonates, elderly patients, immunocompromised individuals, and those with chronic diseases.

Diagnostic and monitoring strategies, including biomarkers, advanced hemodynamic assessment tools, and molecular diagnostics for early detection and risk stratification.

Therapeutic approaches, addressing personalized treatment strategies, the role of immune modulation, tailored fluid resuscitation, and organ-support therapies such as renal replacement therapy and extracorporeal membrane oxygenation (ECMO).

The review incorporated original research, systematic reviews, meta-analyses, and clinical studies published in peer-reviewed, indexed journals to ensure the selection of high-quality, reliable sources.

Exclusion Criteria

The following types of studies were not included in the review:

Research that did not adhere to PRISMA guidelines, as standardized reporting is crucial for ensuring methodological reliability.

Case reports that lacked generalizability or did not provide adequate data on sepsis treatment in high-risk patient populations.

Studies that focused only on general aspects of sepsis without addressing the specific challenges faced by neonates, elderly individuals, immunocompromised patients, or those with chronic illnesses.

Articles that were not based on original research or were published in non-peer-reviewed sources were excluded, ensuring that only high-quality, evidence-based studies were considered.

Selection Process

A structured approach was used to evaluate all identified articles based on predefined inclusion and exclusion criteria, ensuring the selection of high-quality, methodologically sound studies relevant to the research objectives. The PRISMA framework was implemented throughout the process to maintain transparency and consistency in study identification. The final selection offers a comprehensive analysis of sepsis patho-physiology, diagnostic methods, and treatment strategies in vulnerable patient populations.

The selection process was carried out in two main stages. In the initial stage, titles and abstracts were screened to determine relevance, prioritizing studies that focused on sepsis in neonates, elderly individuals, immunocompromised patients, and those with chronic conditions. Studies that did not specifically address these populations or lacked original research data were excluded at this point.

During the second stage, a full-text review was conducted on the remaining articles. Preference was given to studies that examined the relationship between sepsis severity, microvascular dysfunction, immune response alterations, and organ failure. Additionally, research discussing tailored treatment approaches—such as immune modulation, hemodynamic stabilization, and organ-support therapies—was prioritized.

To ensure the credibility and reliability of findings, all selected studies underwent a rigorous quality assessment to confirm adherence to PRISMA guidelines and alignment with the research scope. This approach helped minimize potential biases and strengthened the validity of the conclusions drawn.

Despite efforts to create a comprehensive selection, certain limitations must be acknowledged. The exclusion of grey literature may have led to the omission of valuable studies not indexed in major academic databases. Restricting the review to English-language publications may have excluded relevant research available in other languages. Additionally, limiting the search to specific databases may have overlooked studies published in other sources. Lastly, the decision to focus on research published between 2015 and 2025 may have resulted in the exclusion of older foundational studies that could provide historical insights into sepsis mechanisms and treatment advancements.

RESULTS

Overview of Selected Studies

To ensure transparency and traceability in the selection process, a PRISMA diagram has been included in the material, illustrating the number of studies identified and filtered at each stage of the analysis. This diagram initially presents the total number of articles retrieved through the search, followed by the number of those excluded after reviewing titles and abstracts, and finally, the number of studies selected for in-depth analysis. Additionally, the reasons for excluding certain articles are specified, providing clarity regarding the decisions made during the selection process.

The use of the PRISMA diagram is essential for maintaining transparency, as it allows readers to understand the decision-making process behind article selection and ensures compliance with the criteria established at the outset of the review [57,58].

The PRISMA flow diagram in Figure 1 outlines the selection process for studies examining microcirculatory dysfunction and sepsis management in vulnerable patient populations. A systematic search was performed across eight major databases, yielding a total of 80,700 results.

Following the removal of duplicate records (22,000) and studies excluded due to lack of relevance, incomplete data, or methodological limitations (502), a total of 700 articles remained for screening.

During the initial screening phase, 532 records were excluded based on predefined criteria, primarily for not specifically addressing sepsis in neonates, elderly individuals, immunocompromised patients, or those with chronic diseases. In the first review phase, 168 studies were assessed for eligibility, with articles evaluated for their relevance to sepsis pathophysiology, diagnostic tools, and treatment strategies.

In the second phase, a more detailed evaluation was conducted, applying stricter inclusion criteria related to immune dysregulation, microvascular dysfunction, and patient-specific therapeutic approaches. Studies that focused solely on general aspects of sepsis without addressing unique challenges faced by high-risk patient groups were excluded.

The final selection included 80 studies, covering a wide range of topics, including the pathophysiological mechanisms of sepsis, personalized treatment strategies, and emerging diagnostic technologies. These studies, published between 2015 and 2025, provide valuable insights into optimizing sepsis management in vulnerable populations.

Figure 1: PRISMA Flow Diagram of articles related to Microcirculatory Dysfunction and Sepsis Management in Vulnerable Patient Populations

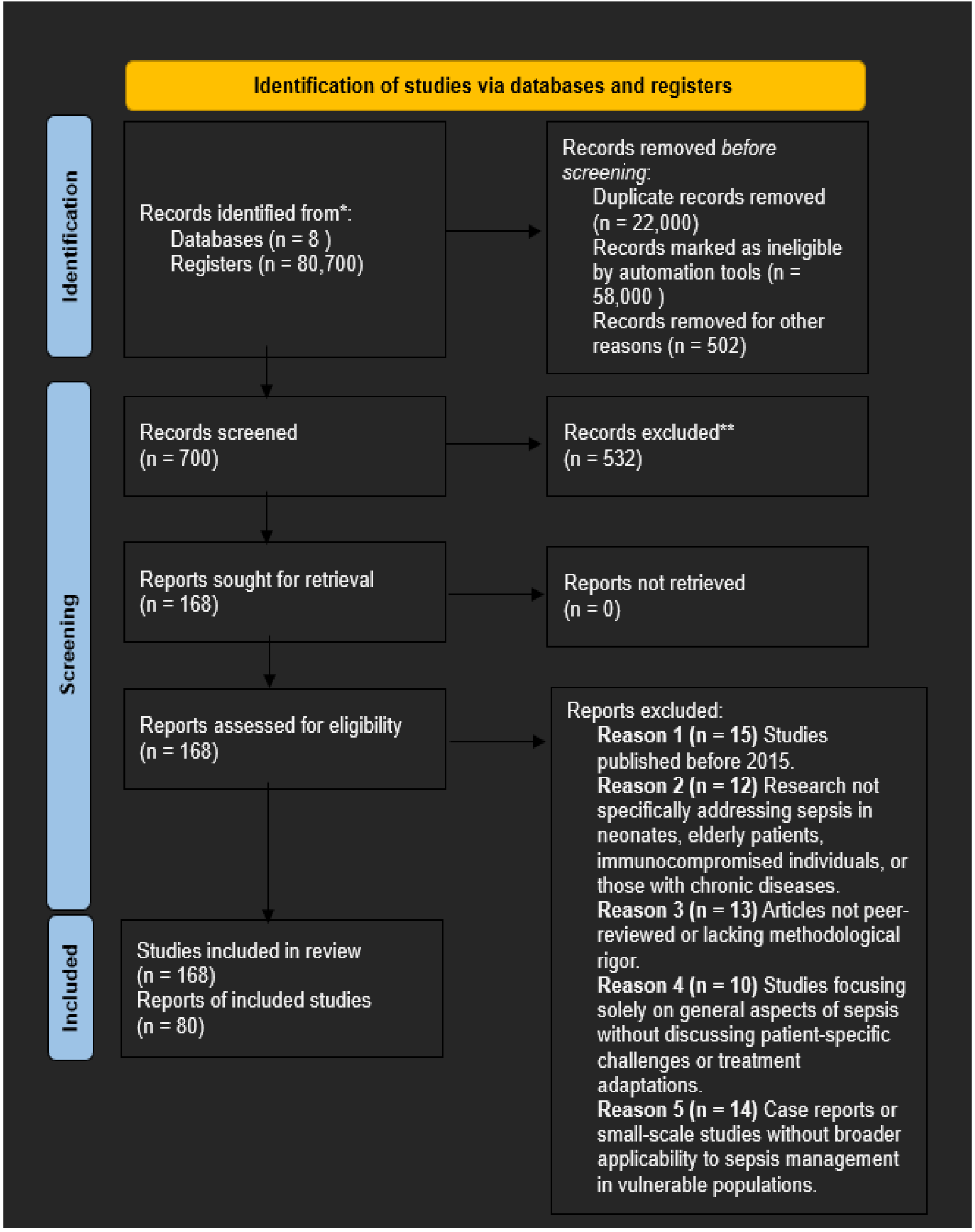
Identification of studies via databases and registers
Identification
Screening
Included
Records identified from*:
Databases (n = 8)
Registers (n = 80,700)
Records removed *before screening*:
Duplicate records removed (n = 22,000)
Records marked as ineligible by automation tools (n = 58,000)
Records removed for other reasons (n = 502)
Records screened (n = 700)
Records excluded** (n = 532)
Reports sought for retrieval (n = 168)
Reports not retrieved (n = 0)
Reports assessed for eligibility (n = 168)
Reports excluded:
Studies included in review (n = 168)
Reports of included studies (n = 80)

Reason 1 (n = 15) Studies published before 2015.
Reason 2 (n = 12) Research not specifically addressing sepsis in neonates, elderly patients, immunocompromised individuals, or those with chronic diseases.
Reason 3 (n = 13) Articles not peer-reviewed or lacking methodological rigor.
Reason 4 (n = 10) Studies focusing solely on general aspects of sepsis without discussing patient-specific challenges or treatment adaptations.
Reason 5 (n = 14) Case reports or small-scale studies without broader applicability to sepsis management in vulnerable populations.

This systematic review was conducted following the PRISMA 2020 guidelines. The PRISMA checklist and flow diagram are provided as supplementary materials.

This methodology enabled a comprehensive analysis of the specialized literature, synthesizing key findings on microcirculatory dysfunction and sepsis management in vulnerable patient populations. By incorporating data from multiple disciplines, this approach provided a well-rounded understanding of sepsis pathophysiology, diagnostic innovations, and personalized treatment strategies.

The studies included in the results section strictly followed the predefined inclusion criteria, ensuring the selection of high-quality and relevant research aligned with the core objectives of this review. These studies were systematically analyzed and synthesized to explore the relationship between microvascular alterations, immune dysregulation, and patient-specific therapeutic approaches, offering objective data and clinically significant insights to enhance sepsis management in high-risk groups.

Sepsis in High-Risk Patient Populations: Unique Challenges and Management Strategies

Patients at increased risk of sepsis belong to special categories, each with specific physiological, immunological, and clinical characteristics that significantly influence disease progression and response to treatment. In these groups, sepsis can progress rapidly, present with atypical symptoms, and have a more severe prognosis, requiring personalized management and close monitoring (Table 1).

Table 1: Categories of Patients at Increased Risk of Sepsis and Their Specific Characteristics

Patient Category	Specific Characteristics	References
Elderly Patients	- Immunosenescence: weakened immune response - Blunted inflammatory response (atypical symptoms) - Compromised cardiovascular and renal function - Increased risk of multi-organ dysfunction	[59], [60]
Patients with Chronic Comorbidities	- Impaired immunity due to preexisting conditions (diabetes, kidney disease, COPD, heart failure) - Increased oxidative stress and chronic inflammation - Risk of hemodynamic imbalances and fluid overload	[61]
Pediatric Patients	- Immature immune system - Increased risk of bacteremia and multi-organ dysfunction - Different drug metabolism (requires careful dose adjustment) - Nonspecific symptoms (lethargy, hypothermia, feeding difficulties)	[62], [63]
Immunocompromised Patients	- Weakened inflammatory response (atypical symptoms) - Increased susceptibility to opportunistic infections - Requires aggressive antibiotic therapy and intensive monitoring	[64], [65]
Pregnant Women	- Physiological changes increase infection susceptibility and excessive inflammation - Early signs may be masked by cardiovascular adaptations - Specific risks: chorioamnionitis, postpartum endometritis - Therapy must be carefully selected to ensure fetal safety	[66], [67]

As we can see in Table 1, elderly patients face a range of physiological and immunological changes that increase their vulnerability to sepsis. With aging, the immune system undergoes immunosenescence, characterized by a weakened innate and adaptive immune response. This leads to a reduced ability to combat infections and a delayed activation of defense mechanisms [59]. Additionally, the inflammatory response may be blunted, meaning classic symptoms of sepsis, such as fever and tachycardia, may be absent or less pronounced. Cardiovascular and renal functions are often compromised in elderly individuals, making hemodynamic balance and fluid management more challenging. Their overall frailty, combined with a higher risk of multi-organ dysfunction, necessitates a carefully tailored therapeutic approach to prevent rapid deterioration and severe complications [60].

Patients with chronic comorbidities represent another high-risk group, as preexisting conditions directly impact infection response and the body's ability to maintain homeostasis during sepsis. Diabetes mellitus, for example, impairs immune function by increasing oxidative stress, altering neutrophil function, and reducing phagocytic capacity, making severe infections and associated complications more likely [61]. Chronic kidney disease leads to the accumulation of uremic toxins that suppress immune function and increase susceptibility to opportunistic infections. Additionally, patients with chronic obstructive pulmonary disease or heart failure exhibit persistent inflammatory states and limited functional reserves, causing sepsis to exacerbate these conditions rapidly. For these patients, treatment strategies must balance infection control, organ protection, and the prevention of adverse effects, such as fluid overload from aggressive resuscitation.

Pediatric patients exhibit significant physiological and immunological differences compared to adults, affecting both the presentation and management of sepsis. The immune system of neonates and infants is underdeveloped, resulting in a reduced ability to mount an effective response against infections, which increases the risk of bacteremia and multi-organ dysfunction [62]. Additionally, drug metabolism differs in children, requiring careful dose adjustments for antibiotics and other therapies based on body weight and organ maturity. Clinical signs of sepsis in this group are often non-specific, including lethargy, respiratory distress, hypothermia, or feeding difficulties, making early diagnosis challenging. Close monitoring and rapid intervention are crucial to preventing severe complications and reducing mortality in pediatric sepsis [63].

Immunocompromised patients, including those with cancer, autoimmune diseases, or those undergoing immunosuppressive therapy, are highly vulnerable to sepsis due to an impaired immune system that cannot effectively control infections. The absence of a robust inflammatory response allows infections to spread quickly, and classic sepsis symptoms such as fever and leukocytosis may be absent, leading to delayed diagnosis [64]. These patients are also prone to opportunistic infections caused by pathogens that are typically non-threatening to immunocompetent individuals, complicating the choice of antimicrobial therapy. Sepsis management in immunocompromised patients requires an aggressive approach, including the rapid administration of broad-spectrum antibi-otics, intensive monitoring, and, in some cases, adjunctive therapies to support immune function [65].

Pregnant women represent a unique high-risk category for sepsis, as physiological changes during pregnancy can increase both infection susceptibility and exaggerated inflammatory responses. The immune system undergoes adaptations to tolerate the presence of the fetus, potentially reducing the ability to fight off infections effectively [66]. Simultaneously, increased plasma volume and cardiac output alter the body's response to infection and can mask early signs of sepsis. Obstetric infections, such as chorioamnionitis and postpartum endometritis, are common causes of sepsis during pregnancy and can have severe consequences for both the mother and the fetus. Treating sepsis in pregnant women requires careful consideration of fetal safety, necessitating the selection of appropriate antibiotics and close hemodynamic monitoring to prevent maternal and fetal complications [67].

Each of these special patient categories requires a personalized approach to sepsis management, tailored to their unique physiological and immunological characteristics. Early diagnosis, intensive monitoring, and prompt therapeutic interventions are essential to improving outcomes and reducing mortality in these vulnerable populations.

Tailored Therapeutic Approaches for Special Patient Populations in Sepsis

Managing sepsis in high-risk patient populations requires a personalized therapeutic approach that considers age, comorbidities, immune status, and physiological differences. Standard treatment protocols may not be fully applicable to these groups, necessitating dose adjustments, targeted antimicrobial strategies, and specialized supportive interventions to optimize outcomes while minimizing complications.

Table 2 presents the key therapeutic considerations for each of these high-risk patient groups, highlighting the necessary adjustments in sepsis management to optimize clinical outcomes and reduce complications.

Table 2: Tailored Therapeutic Approaches for Special Patient Populations in Sepsis

Patient Category	Key Therapeutic Considerations	References
Elderly Patients	- Adjust antibiotic dosages due to reduced renal and hepatic function - Lower doses or extended intervals for renally excreted antibiotics (e.g., aminoglycosides, vancomycin) to prevent nephrotoxicity - Monitor for fluid overload and hypotension with advanced hemodynamic tools (e.g., echocardiography)	[68], [69], [72]
Pediatric Patients	- Use weight-based dosing due to immature liver enzymes and different drug metabolism - Close monitoring of oxygenation, perfusion, and fluid balance - Early noninvasive ventilation (e.g., high-flow nasal cannula) to prevent respiratory failure - Adjust antibiotic regimens based on age-specific pharmacokinetics	[73], [74]
Patients with Chronic Comorbidities	- Modify medication regimens to prevent interactions (e.g., fluid overload in heart failure) - Adjust diabetes management to avoid sepsis-induced hyperglycemia - Consider early renal replacement therapy in patients with kidney disease - Balance infection control with organ protection strategies	[71], [75]
Immunocompromised Patients	- Use prolonged or higher-dose antibiotic courses if needed, balancing toxicity risks - Consider antifungal and antiviral coverage for broader protection - Utilize immunotherapies such as G-CSF for neutropenic patients - Monitor for opportunistic infections and tailor treatment accordingly	[70], [76], [79], [80]
Pregnant Women	- Select antibiotics carefully to avoid teratogenic risks (prefer beta-lactams, macrolides, cephalosporins) - Adjust dosages based on increased plasma volume and altered metabolism - Monitor fetal well-being and placental perfusion in maternal hypotension - Involve obstetric and neonatal specialists for optimal maternal-fetal outcomes	[83], [84], [85], [86], [87]

According to Table 2, antibiotic and medication dosages must be carefully adapted based on age, underlying conditions, and overall patient status. In elderly patients, reduced renal and hepatic function can significantly alter drug metabolism and clearance, increasing the risk of toxicity [68]. Lower doses or extended dosing intervals may be required for renally excreted antibiotics such as aminoglycosides and vancomycin to prevent accumulation and nephrotoxicity [69]. Pediatric patients have immature liver enzyme systems and differing body compositions, necessitating weight-based dosing and close monitoring to ensure therapeutic efficacy while avoiding adverse effects. In immunocompromised individuals, higher or prolonged antibiotic courses may be required due to impaired immune clearance of pathogens, but this must be balanced against the risk of toxicity and resistance [70]. Patients with chronic diseases, such as diabetes or heart failure, may require modifications in medication regimens to avoid interactions or worsening of underlying conditions, such as fluid overload with aggressive intravenous hydration [71].

Supportive interventions play a critical role in managing sepsis in these vulnerable populations. Elderly patients often have diminished cardiovascular reserve, making them prone to hypotension and fluid overload, requiring careful hemodynamic monitoring using advanced tools such as echocardiography or dynamic fluid responsiveness tests [72]. Pediatric patients, particularly neonates, have immature cardiovascular and respiratory systems, necessitating closer monitoring of oxygenation, perfusion, and fluid balance [73]. In critically ill patients with underlying respiratory diseases such as chronic obstructive pulmonary disease or in neonates with underdeveloped lung function, early implementation of noninvasive ventilation or high-flow nasal cannula therapy can help prevent respiratory deterioration [74]. Patients with renal impairment may require early initiation of renal replacement therapy to prevent worsening metabolic derangements and fluid overload [75].

Targeted antimicrobial therapy is essential to optimizing treatment outcomes while minimizing the risk of antibiotic resistance, particularly in patients with recurrent sepsis [76]. Broad-spectrum antibiotics should be initiated promptly but de-escalated based on culture results and antimicrobial susceptibility testing to avoid unnecessary prolonged exposure to broad-spectrum agents [77]. In patients with frequent hospitalizations or prior antibiotic exposure, local resistance patterns should guide empirical therapy to ensure adequate pathogen coverage [78]. Immunocompromised patients, such as those undergoing chemotherapy or organ transplant recipients, are at higher risk for fungal or viral infections in addition to bacterial sepsis, necessitating a broader diagnostic approach and potential inclusion of antifungal or antiviral agents in their treatment regimen [79].

Immunotherapy has emerged as a potential adjunct in managing sepsis, particularly in immunocompromised individuals. Strategies such as the administration of granulocyte colony-stimulating factor (G-CSF) can enhance neutrophil function in patients with chemotherapy-induced neutropenia, reducing the risk of severe infections [80]. Intravenous immunoglobulins (IVIG) may be beneficial in certain septic patients with antibody deficiencies, such as those with primary immunodeficiencies or hypogammaglobulinemia secondary to chronic diseases [81]. Additionally, emerging therapies targeting immune modulation, such as checkpoint inhibitors and cytokine blockade, are being explored to balance excessive inflammatory responses and prevent immune paralysis in sepsis [82]. However, these approaches require further clinical validation to determine their efficacy and safety across different patient populations.

Special considerations are necessary when treating sepsis in pregnant women to minimize risks to both the mother and fetus. Physiological changes in pregnancy, including increased plasma volume, altered renal clearance, and changes in drug metabolism, require careful selection and dosing of medications [83]. Certain antibiotics, such as tetracyclines and fluoroquinolones, should be avoided due to potential teratogenic effects or adverse fetal outcomes [84]. Beta-lactams, macrolides, and selected cephalosporins are generally considered safe and are preferred when treating infections in pregnancy [85]. Management of sepsis in pregnant patients also involves close fetal monitoring, particularly in cases of maternal hypotension, as reduced placental perfusion can lead to fetal distress [86]. Early involvement of obstetric and neonatal specialists is crucial to ensuring optimal maternal and fetal outcomes, particularly in cases requiring preterm delivery due to worsening maternal condition [87].

A tailored therapeutic approach is essential in managing sepsis in high-risk populations, as a one-size-fits-all strategy may not be effective or safe. Personalized dosing regimens, targeted antimicrobial selection, intensive supportive care, and emerging immunomodulatory therapies must be carefully integrated into treatment plans to improve survival and reduce complications in these vulnerable patient groups.

Outcomes and Prognostic Factors in Sepsis Among High-Risk Patient Populations

The prognosis of sepsis varies significantly among different patient populations, with special categories such as the elderly, pediatric patients, immunocompromised individuals, those with chronic diseases, and pregnant women facing distinct challenges that influence survival rates, recurrence risk, and long-term health outcomes. The interaction between individual patient characteristics, severity of illness, and therapeutic interventions plays a crucial role in determining overall outcomes.

Mortality and morbidity rates in sepsis differ across patient groups, with elderly individuals exhibiting the highest case fatality rates due to pre-existing frailty, immune senescence, and a diminished physiological reserve. Studies indicate that sepsis-related mortality in elderly patients can exceed 40%, particularly in those with multi-organ dysfunction or underlying cardiovascular disease [88]. Pediatric patients generally have lower mortality rates due to their greater physiological resilience and the ability to recover from severe infections when treated promptly [89]. However, neonates and premature infants remain highly vulnerable, with significantly higher sepsis-related mortality due to their immature immune systems and increased susceptibility to bloodstream infections [90]. Immunocompromised individuals, including cancer patients, transplant recipients, and those undergoing immunosuppressive therapy, also face elevated mortality risks, as their inability to mount an adequate immune response allows infections to progress more aggressively [91]. Patients with chronic diseases such as diabetes, chronic kidney disease, and chronic obstructive pulmonary disease experience worse outcomes due to baseline organ dysfunction, which limits their ability to compensate for sepsis-induced metabolic and inflammatory disturbances [92]. Pregnant women with sepsis generally have lower mortality rates compared to other high-risk groups, but severe maternal sepsis remains a leading cause of maternal morbidity and can result in adverse fetal outcomes, including preterm birth and stillbirth [93].

The success of treatment and the likelihood of sepsis recurrence depend on multiple factors, including early diagnosis, appropriate antimicrobial therapy, hemodynamic stabilization, and organ support. Patients in high-risk categories often require more aggressive and prolonged interventions, yet they may also experience higher rates of treatment failure. In elderly patients, delayed recognition of sepsis due to atypical presentations contributes to increased mortality, and even those who survive the acute phase frequently develop post-sepsis complications such as cognitive decline and functional impairment [94]. Pediatric patients, despite their potential for recovery, may experience long-term immunological and developmental consequences following severe sepsis. Immunocompromised individuals often require extended antimicrobial treatment due to persistent infection risks, but this increases their susceptibility to antimicrobial resistance and recurrent sepsis episodes [95]. Patients with chronic diseases frequently experience sepsis relapses, particularly when their underlying conditions are poorly controlled, leading to recurrent hospitalizations and a progressive decline in overall health status [96].

Prognostic factors vary based on patient category and the therapeutic approach used. In elderly patients, markers of frailty, pre-existing comorbidities, and the presence of multi-organ dysfunction strongly predict poor outcomes. In pediatric sepsis, factors such as gestational age, birth weight, and early initiation of antibiotics influence survival rates [97]. Immunocompromised patients with profound neutropenia or severe lymphopenia are at the highest risk of mortality, particularly when infections are caused by multidrug-resistant organisms or opportunistic pathogens [98]. The effectiveness of immunotherapy, such as granulocyte colony-stimulating factor (G-CSF) or intravenous immunoglobulin (IVIG), in improving survival in these patients remains an area of ongoing research. In patients with chronic diseases, glycemic control in diabetics, renal function stability in those with kidney disease, and optimal management of pulmonary disease play critical roles in determining outcomes [99]. Pregnant women with sepsis have better prognoses when infections are detected early and treated promptly, but complications such as septic shock, disseminated intravascular coagulation, and fetal distress significantly worsen both maternal and fetal outcomes [100,101].

The long-term impact of sepsis on quality of life has become an increasingly important topic of discussion, particularly with the recognition of post-sepsis syndrome (PSS), a condition characterized by persistent physical, cognitive, and psychological impairments following recovery from sepsis [102]. Many sepsis survivors experience chronic fatigue, muscle weakness, neuropathy, and reduced exercise tolerance, which significantly impact their ability to perform daily activities [103]. Cognitive dysfunction, including memory impairment and difficulty concentrating, is particularly common in elderly patients and those who require prolonged intensive care unit (ICU) stays [104]. Anxiety, depression, and post-traumatic stress disorder (PTSD) are frequently reported, further diminishing quality of life [105]. Pediatric patients who survive sepsis may experience developmental delays and long-term neurocognitive impairments, especially if they suffered from hypoxia or severe inflammation during the acute phase [106]. Immunocompromised patients often face ongoing infection risks and require long-term prophylactic antimicrobial therapy, increasing their burden of care. Individuals with chronic diseases who survive sepsis often experience accelerated disease progression, leading to increased dependence on healthcare resources [107]. Pregnant women who recover from sepsis may face complications such as placental insufficiency, preterm labor, or long-term cardiovascular effects, emphasizing the need for close postpartum monitoring [108].

The impact of long-term sepsis management on patient health and functionality underscores the importance of comprehensive post-discharge care. Early rehabilitation programs, including physical therapy and cognitive training, can help mitigate some of the adverse effects of post-sepsis syndrome [109]. Ongoing monitoring for secondary infections, organ dysfunction, and psychological distress is essential to improving the long-term well-being of sepsis survivors. Targeted interventions, such as personalized nutrition plans, immune-modulating therapies, and infection prevention strategies, may help reduce the risk of recurrent sepsis and promote a better quality of life for high-risk patients [110].

The prognosis and long-term outcomes of sepsis vary widely depending on patient characteristics, timely intervention, and post-sepsis care. While advances in early recognition and targeted therapies have improved survival rates, the high burden of post-sepsis complications highlights the need for continued research and multidisciplinary approaches to optimize long-term recovery in vulnerable populations.

Microcirculatory Dysfunction in Sepsis and Its Clinical Implications

Microcirculatory dysfunction plays a critical role in the progression and severity of sepsis, significantly influencing patient outcomes. Various diagnostic techniques are employed to assess alterations in microvascular perfusion, endothelial integrity, and oxygen delivery at the tissue level. Sublingual video microscopy, using techniques such as sidestream dark field (SDF) imaging, allows for direct visualization of capillary flow abnormalities [111]. Near-infrared spectroscopy (NIRS) is utilized to evaluate tissue oxygenation, while biomarkers such as lactate levels and endothelial activation markers provide additional insights into microvascular impairment [112].

The characteristics of microcirculatory dysfunction in sepsis differ significantly from those observed in other critical illnesses. Septic patients frequently exhibit extensive capillary heterogeneity, increased leukocyte-endothelial interactions, and impaired oxygen extraction, even when macrocirculatory parameters such as blood pressure appear normal [113]. Comparing microvascular alterations between septic and non-septic critically ill patients helps elucidate underlying pathophysiological mechanisms and guides the development of targeted interventions [114].

Table 3 summarizes key diagnostic techniques used to assess microcirculatory dysfunction in sepsis, highlighting their advantages, limitations, and clinical relevance in managing critically ill patients.

Table 3: Diagnostic Analysis of Microcirculatory Dysfunction in Sepsis

Category	Aspect Analyzed	Description	Advantages	Disadvantages	Examples	References
Diagnostic Techniques Used	Sublingual Video Microscopy (SDF/OPS Imaging)	Direct visualization of microvascular flow abnormalities and capillary perfusion deficits.	Real-time, bedside assessment of microcirculation.	Operator-dependent, limited standardization.	Identification of capillary heterogeneity and sluggish flow in sepsis.	[115], [116]
	Near-Infrared Spectroscopy (NIRS)	Measures tissue oxygenation to assess microvascular dysfunction in real-time.	Non-invasive, continuous monitoring.	Limited spatial resolution, indirect measurement.	Detects impaired tissue oxygen extraction in septic shock.	[117], [118]
	Plasma Biomarkers (Lactate, Endothelial Activation Markers)	Biochemical markers reflecting endothelial dysfunction and microvascular impairment.	Easily accessible, quantitative assessment.	Nonspecific, affected by multiple factors.	Elevated lactate and angiopoietin-2 levels indicating sepsis severity.	[119], [120]
Key Microcirculatory Features	Capillary Perfusion Deficits	Impaired blood flow distribution at the microvascular level, leading to tissue hypoxia.	Helps explain oxygen delivery failure in sepsis.	Difficult to correct solely with macrocirculatory support.	Decreased proportion of perfused capillaries in septic patients.	[121], [122]
	Endothelial Dysfunction and Glycocalyx Damage	Disruption of endothelial barrier function, increasing vascular permeability, and inflammation.	Critical factor in sepsis progression and organ failure.	No direct bedside measurement is currently available.	Degradation of the endothelial glycocalyx leading to capillary leakage.	[123], [124]
	Oxygen Extraction and Mitochondrial Dysfunction	Sepsis-induced alterations in cellular oxygen utilization and mitochondrial energy production.	Key insight into metabolic failure in septic shock.	Challenging to measure in clinical settings.	Reduced oxygen consumption despite adequate oxygen delivery.	[125], [126]

According to Table 3, the assessment of microcirculatory dysfunction in sepsis highlights the necessity of a multimodal approach for accurate diagnosis and patient management. Sublingual video microscopy remains a valuable bedside tool for directly visualizing microvascular perfusion abnormalities [115,116], while near-infrared spectroscopy provides non-invasive, real-time insights into tissue oxygenation deficits [117,118]. Biomarkers such as lactate and endothelial activation markers offer an accessible means of assessing microvascular impairment [119,120], though they lack specificity and require correlation with other clinical parameters.

The distinct microcirculatory alterations observed in sepsis, including capillary perfusion deficits [121,122], endothelial dysfunction [123,124], and mitochondrial impairment [125,126], underscore significant pathophysiological differences compared to other critical illnesses. These findings suggest that conventional macrocirculatory-focused management strategies may be insufficient, emphasizing the need for personalized interventions targeting microvascular restoration.

Integrating microcirculatory assessment with inflammatory and metabolic biomarkers can enhance early risk stratification, optimize therapeutic strategies, and improve patient outcomes. This underscores the importance of a multidisciplinary approach, incorporating intensivists, infectious disease specialists, and hemodynamic monitoring experts to refine sepsis management and reduce mortality in high-risk populations.

Challenges

The treatment of sepsis in high-risk patient populations presents significant clinical and scientific challenges. Despite advancements in early diagnosis, antimicrobial therapies, and supportive care, difficulties persist in tailoring treatment strategies to meet the unique needs of vulnerable groups. Two major challenges are the identification of reliable prognostic markers to predict disease progression and the absence of standardized protocols for the personalized management of sepsis in special populations [127].

A primary obstacle in managing sepsis in these high-risk groups is the lack of robust prognostic markers that enable early risk stratification and guide treatment decisions. In the general sepsis population, biomarkers such as procalcitonin, C-reactive protein, and lactate levels are commonly used to assess disease severity and response to treatment [128]. However, in elderly patients, these markers may be less reliable due to an altered inflammatory response and atypical sepsis presentation. Similarly, in immunocompromised patients, the absence of a strong inflammatory response can lead to falsely low biomarker levels, delaying diagnosis and appropriate intervention [129]. Pediatric patients, especially neonates, have distinct immune responses that differ from adults, making it difficult to apply standard sepsis biomarkers to this population [130]. Recent studies have explored cytokines such as IL-6 and IL-10 as potential markers for sepsis severity in immunocompromised and pediatric patients, but further research is needed to validate their clinical utility. The absence of well-defined biomarkers complicates early detection, treatment optimization, and outcome prediction in these vulnerable groups [131].

Another key challenge is the lack of standardized protocols for the individualized management of sepsis in special populations. Although general sepsis guidelines exist, they do not always account for the unique physiological and immunological characteristics of elderly patients, pediatric patients, those with chronic diseases, or immunocompromised individuals [132]. For instance, fluid resuscitation strategies must be carefully adjusted in elderly patients with reduced cardiac and renal function to prevent fluid overload. Pediatric patients require weight-based adjustments for antibiotics and vasoactive medications, but there is no universal protocol guiding optimal dosing in neonates with sepsis [133]. Immunocompromised patients often require broader anti-microbial coverage, yet prolonged exposure to broad-spectrum antibiotics increases the risk of resistance and secondary infections, making antimicrobial stewardship particularly complex [134]. Similarly, in pregnant women, balancing maternal treatment with fetal safety is a significant challenge, as some life-saving antimicrobial and vasopressor therapies pose potential risks to fetal development. The absence of tailored sepsis management guidelines for these groups leads to variability in clinical practice and inconsistencies in patient outcomes [135].

Standardizing diagnostic and treatment protocols is essential for improving sepsis management in high-risk populations. Without clear evaluation and treatment criteria, clinical decision-making often relies on physician experience rather than evidence-based guidelines, leading to disparities in care across healthcare settings [136].

DISCUSSION

The findings from this analysis highlight the complexity of managing sepsis in high-risk patient populations and underscore the need for a personalized, multidisciplinary approach. The strength of evidence supporting various therapeutic interventions, prognostic indicators, and post-sepsis outcomes varies across patient groups, influencing decision-making for healthcare providers, researchers, and policy-makers.

Early and targeted antimicrobial therapy remains the cornerstone of sepsis treatment, with strong evidence (Level A) supporting its role in improving survival rates. The literature consistently emphasizes the importance of initiating broad-spectrum antibiotics within the first hour of suspected sepsis to reduce mortality, particularly in immunocompromised patients and neonates who are highly susceptible to rapid disease progression [137]. However, antibiotic stewardship remains a critical concern, as prolonged or inappropriate antimicrobial use contributes to resistance, especially in patients with recurrent sepsis [138].

Supportive interventions such as hemodynamic monitoring and organ support therapies play a crucial role in managing sepsis in elderly patients, pediatric populations, and those with chronic comorbidities [139]. Evidence suggests that goal-directed fluid re-suscitation and vasopressor therapy improve outcomes in patients with sepsis-induced hypotension (Level A). However, the literature also indicates that fluid overload can worsen prognosis, particularly in elderly patients with cardiac or renal impairment, necessitating individualized resuscitation strategies [140]. Pediatric patients require weight-based hemodynamic adjustments, yet a lack of standardized protocols for fluid management in neonatal sepsis creates challenges in optimizing treatment [141].

The role of immunotherapy in sepsis treatment remains an evolving area of research, with emerging evidence suggesting potential benefits for immunocompromised patients. Studies exploring the use of intravenous immunoglobulins (IVIG) and cytokine-targeting therapies indicate possible improvements in immune function and infection control (Level B) [142]. However, data remain inconclusive, and the heterogeneity of immune responses in different patient groups complicates the identification of optimal candidates for such therapies [143].

Post-sepsis outcomes and long-term patient recovery have become increasingly recognized as critical aspects of sepsis care, particularly with the identification of post-sepsis syndrome (PSS) [144]. Studies show that sepsis survivors, particularly elderly patients and those with pre-existing comorbidities, frequently experience persistent physical, cognitive, and psychological impairments that reduce their quality of life (Level A) [145]. The literature highlights the need for early rehabilitation programs, including physical therapy and mental health support, to improve post-sepsis functional outcomes [146]. Pediatric patients, though generally more resilient, may experience long-term developmental delays, while immunocompromised individuals remain at high risk for recurrent infections [147].

The analysis of sepsis treatment and outcomes in high-risk populations underscores the need for a more personalized approach to management. While early antimicrobial therapy, hemodynamic support, and organ protection strategies are well established, optimizing treatment for specific patient groups remains a challenge [148,149]. Further research into immunomodulatory therapies, targeted resuscitation strategies, and post-sepsis rehabilitation programs could improve outcomes for vulnerable populations. For healthcare systems and policy-makers, prioritizing research funding and developing population-specific guidelines will be crucial in advancing sepsis care.

CONCLUSION

The findings of this analysis reinforce and expand upon the existing research on sepsis management in high-risk patient populations, emphasizing the distinct pathophysiological mechanisms and therapeutic challenges that differentiate these groups from the general sepsis population. The evidence strongly supports the fundamental role of age-related immune changes, comorbid conditions, and immune suppression in altering sepsis progression, response to treatment, and long-term recovery. These differences explain the variability in treatment outcomes and underscore the necessity for patient-specific, tailored therapeutic approaches.

From an antimicrobial therapy perspective, the literature confirms that early and targeted antibiotic administration remains the cornerstone of sepsis treatment, particularly in immunocompromised patients and neonates, where delayed intervention significantly worsens prognosis. However, the growing concern of antimicrobial resistance in patients with recurrent sepsis highlights the urgent need for enhanced antimicrobial stewardship programs and the integration of rapid molecular diagnostics to optimize therapy selection. The challenge of balancing broad-spectrum coverage with the risk of resistance remains a key consideration in long-term sepsis management.

The role of hemodynamic support and organ protection strategies in sepsis treatment is well established, with evidence favoring individualized fluid resuscitation and vaso-pressor therapy based on patient-specific factors such as age, renal function, and baseline cardiovascular status. While goal-directed resuscitation strategies improve survival, the risk of fluid overload in elderly and chronically ill patients necessitates a more precise, dynamic approach to fluid management. The increasing use of bedside ultrasonography and advanced monitoring techniques presents an opportunity to refine resuscitation protocols and improve patient outcomes.

The growing recognition of post-sepsis syndrome (PSS) highlights the need for a more comprehensive approach to sepsis care, extending beyond the acute phase to address long-term health complications. Studies confirm that sepsis survivors, particularly elderly individuals and those with preexisting conditions, frequently experience persistent physical, cognitive, and psychological impairments that significantly impact their quality of life. Post-sepsis rehabilitation programs, including structured physical therapy, cognitive assessments, and mental health support, are increasingly recognized as essential components of recovery. However, gaps remain in the availability and accessibility of these services, underscoring the need for healthcare systems to prioritize long-term sepsis care.

These findings highlight the importance of integrating personalized therapeutic strategies, advanced diagnostic technologies, and long-term rehabilitation programs into sepsis management.

Implications for Future Research

Given the limitations of standard sepsis management in high-risk populations, future research should focus on developing personalized treatment strategies that account for immune dysfunction, comorbid conditions, and age-related physiological changes. Studies investigating immunomodulatory therapies, such as IL-6 and IL-10 inhibitors, could provide new therapeutic avenues for immunocompromised patients who fail to mount an adequate immune response. Additionally, research into host-directed therapies, including immune checkpoint inhibitors and cell-based treatments, may help restore immune balance in patients with immune exhaustion or dysregulated inflammatory responses.

The growing threat of antimicrobial resistance, particularly in patients with recurrent sepsis, underscores the need for novel antibiotic stewardship strategies. Future studies should explore rapid molecular diagnostics and point-of-care pathogen identification tools to facilitate early, targeted antibiotic therapy while minimizing unnecessary broad-spectrum antibiotic use. Additionally, research into alternative antimicrobial approaches, such as bacteriophage therapy and antimicrobial peptides, could provide effective solutions for multidrug-resistant infections. The development of nanotechnology-based drug delivery systems to enhance antibiotic penetration into infected tissues and biofilms should also be a priority.

Hemodynamic monitoring and resuscitation strategies in sepsis require further refinement to improve outcomes in specific patient populations. Research into precision-guided fluid resuscitation, incorporating real-time hemodynamic assessment tools such as microcirculatory imaging and noninvasive cardiac output monitoring, could help tailor fluid therapy and prevent complications such as fluid overload in elderly and chronically ill patients. Additionally, studies comparing restrictive versus liberal fluid strategies in different patient populations could provide insights into optimizing volume resuscitation while minimizing adverse effects.

The long-term impact of sepsis, particularly post-sepsis syndrome (PSS), remains an area requiring further exploration. Future research should focus on identifying bi-omarkers that predict long-term cognitive, physical, and psychological impairments following sepsis. Large-scale longitudinal studies evaluating the effectiveness of rehabilitation programs, including physical therapy, neurocognitive interventions, and mental health support, are needed to establish standardized post-sepsis care protocols. Investigating the role of gut microbiome alterations in post-sepsis recovery may also provide opportunities for microbiome-targeted therapies to improve immune homeostasis and overall health outcomes.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, G.P.G. and D.R.T.; methodology, G.P.G and D.R.T; software, M.B.N and I.S.C.; validation, G.P.G and L.P; formal analysis, R.M.S and C.P.; investigation V.T.G and I.S.C.; resources G.P.G, L.P and V.T.G..; data curation, R.M.S., L.P. and M.B.N.; writing—original draft preparation G.P.G. and C.P.; writing—review and editing, G.P.G,. D.O.C. and V.T.G..; visualization, M.B.N and R.M.S.; supervision, G.P.G.; project administration, V.T.G and I.S.C. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLES

The risk of bioterrorist and biocrime attack in the contemporary world

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Abstract: *The biological attack is the artificial spread by various means of pathogens that can cause serious infectious and contagious diseases as well as the spread of germ toxins that can be used by an aggressor as a means of fighting in order to reduce the troops' fighting force, by causing serious disease outbreaks or by killing people, animals and/or plants.*

Biological agents are microorganisms and/or microbial, animal or plant toxins, used as specific ammunition for biological weapons or used by terrorists in "bio-chem" attacks.

The risks of bioterrorism and biocrime attack in the contemporary world are real, and the history of the 20th century and the beginning of the 21st century confirms this.

It is necessary for preventive measures to be implemented on the unlawful use of biological agents. Early medical and non-medical countermeasures must be prepared for the prophylaxis, treatment and cessation of the consequences of any biological attack.

Keywords: *biological attack, bioterrorism, biocrime, medical protection*

INTRODUCTION

The biological attack is the artificial spread by various means of pathogens that can cause serious infectious and contagious diseases as well as the spread of germ toxins that can be used by an aggressor as a means of fighting in order to reduce the troops' fighting force, by causing serious disease outbreaks or by killing people, animals and/or plants.

Biological agents are microorganisms and/or microbial, animal or plant toxins, used as specific ammunition for biological weapons or used by terrorists in "bio-chem" attacks.

Terrorism, according to the League of Nations (1937), can be defined as the totality of criminal acts directed against a state or made or planned in order to create a state of terror in the minds of certain individuals, a group of people or the general public.

The objectives of terrorism can be grouped as follows:
a) Achieving political goals.

b) Attracting the attention of domestic and international public opinion to the "noble goal" pursued.

c) Undermining the authority of political regimes in some countries by creating a state of inner strain, insecurity and uncertainty, economic and social chaos.

d) Forcing authorities to meet certain requests.

g) Revenge on some officials.

h) Achieving military goals: management disruption at a strategic or operational level, diminishing or partially destroying the opponent's military potential, the disruption of the logistics system, paralyzing communications and telecommunication systems.

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i) Religious fanaticism.

Bioterrorism consists in using or threatening to use the weapon of mass destruction for claims, with the declared intent of affecting the public health and/or the environment.

Currently, the following means are known for dispersing biological agents:
- Using saboteurs;
- Using vectors of microorganisms such as insects, mites, rodents or other infected animals;
- Incorporation of biological agents into explosive ammunition;
- Aerosolization – through aerosols released from the ground, air or sea – is considered the most effective means of contaminating large areas of territory within the shortest feasible time interval.

With regard to the production of biological weapons, both ordinary and resistant biological agents can be used, the latter being preferred, because they cause a form of the disease that can not be treated with the usual drugs, causing a disease outbreak of greater magnitude and severity.

In general, biological attacks are masked by natural epidemics, epizooties and epiphytosis, the latter two being generally considered to be economic attacks, with the aim of decreasing trade, implicitly, causing the decline of the standard of living in the affected area.

Biological attacks can also be manifested in the form of zooanthroponoses, affecting both animals and humans alike. [1, 2].

BRIEF HISTORY

"Read the history and, thus, discover what will be" Nicolae Iorga rightly said. The first "chemical weapons" - "toxic smoke" were used around 424 BC in the Peloponnesian War. The first users of the "biological weapon" (around 400 BC) can be considered the Scythians, who used arrows soaked in decomposing bodies or mixed with manure. The Spartans used to cast sieves of sulfur-moistened wood on sieged fortresses. Greek, Persian, and Roman literatures describe how dead animals were used for fountain-contaminate.

The Mongols, in the siege of the Kaffa fortress in Crimea, during the war waged between 1346 and 1347, used the corpses of those killed by the plague in order to infect or create a breach among the fortress defenders.

In the fifteenth century, the Spanish infested the French wine with blood from those infected with the pests. Also, at that time, Pizzaro distributed to the Americans in South America garments contaminated with smallpox. In the sixteenth century, the same virus was used by the English to impregnate the blankets distributed to the ameridian tribes that helped the French. In the war against Sweden in 1710, the Russian troops used the same method as the Tartar army did 400 years prior – namely, the use of the corpses of those killed by the plague.

Table 1. Cases of illicit activity with biological agents around the world, confirmed by the judicial system, 20th century (W.S. Carus)

No	Objectives	Category			TOTAL cases	Comments
		Terrorist	Criminal	Others/not specified		
1	Killing	4	17	0	21	
2	Terror	6	9	22	37	
3	Extortion	0	13	3	16	
4	Losses	0	5	0	5	
5	Anti-animal or cultures	1	2	0	3	
6	Mass murder	4	0	0	4	
7	Revenge	0	3	0	3	
8	Disability	2	0	0	2	
9	Political	1	0	0	1	The fewest
10	Unknown	9	7	72	88	The most
TOTAL		27	56	97	180	
Comments		The fewest		The most		

Table 2. The objectives of using biological agents in the 20th century (W. S. Carus) [α]

No	Activity	Category			TOTAL cases	Comments
		Terrorist	Criminal	Others/not specified		
1	Acquisition and use	5	16	0	21	
2	Acquisition (possession)	3	7	2	12	
3	Interest (attempt)	6	4	0	10	The fewest
4	Threat or false attack	13	29	95	137	The most
TOTAL		27	56	97	180	
Comments		The fewest		The most		

During the Vietnam War, Americans used defoliant, desiccant and sterilizing herbicides. These substances did not fall under the Geneva Protocol of 1925 nor in the Convention on the Prohibition of Chemical Weapons. In the 1970s, ricin was used to assassinate Bulgarian dissidents Gheorghe Markov and Vladimir Kostov.

In 1976, the mayors of several US cities were the recipients of letters that were delivered in envelopes on which had been used an adhesive impregnated with lethal germs.

In 1979, among people working in a military microbiology unit, was recorded an epidemic of lung anthrax, resulting in over 60 deaths.

In 1984, in the US, members of the Bhagwan Shree Rajneesh sect have contaminated the food in Oregon restaurants with Salmonella Tiphi, causing 750 cases of major disease outbreaks.

In 1988, Iraq was spreading toxic and biological substances in Halabja, causing victims among the civilian population, predominantly children and women. Also in 1995, Iraq admitted to having in its posession Bacillus anthracis, botulinum toxin and aflatoxins, declaring to be ready to use them. All this demonstrates that, although the Convention on the Prohibition of Chemical Weapons was signed in 1972, the biological weapons research continued.

Since the end of 1992, there have been many more complex epidemics in the US, Milwaukee area, with wide-ranging effects: 400,000 cases of crypto-sporidium outbreaks from water consumption; in the spring of 1993, a respiratory epidemic with unidentified causes; during the fall of 1994, 250,000 cases of salmonella food poisoning.

On October 5th, 2001, in the US, Florida area, after having inhaled the anthrax bacillus, a tabloid's editor passed away. On October 20th, 2001, three letters containing anthrax spores were delivered to Washington DC, USA, having been received by Tom Daschle, a member of the Senate, and by Tom Brokaw from NBC News, as well as by the newspaper "The New York Post ". On November 2nd, 2001, a letter containing anthrax spores was found in Karachi, Pakistan, which was addressed to the newspaper "Daily Jang". In Santiago, a similar postal mailing resulted in disease outbreaks of thirteen people.

Figure 1. Frequency of the illicit use of biological agents in the 20th century, on decades (W. S. Carus) [α]

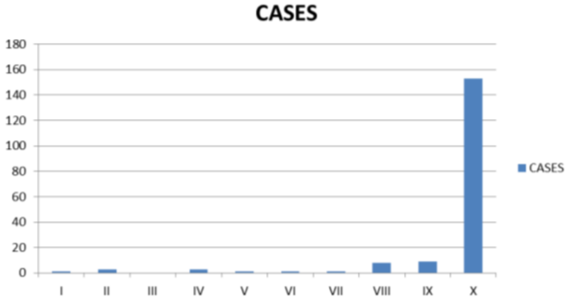

After September 11, 2001, the danger of massive terrorist attacks on the population of any part of the world is no longer just a working hypothesis. Chemical, biological or nuclear terrorism is, at present, one of the most serious threats to all states. Given the trend of interethnic and religious violence, as well as the number of cases of human rights violations in certain "high-risk" areas around the globe, analysts have warned against accentuating the risk of using these types of weapons in terrorist actions, underlining the imperative nature of urgently establishing non-proliferation measures.

Table 3. Type of biological agent used (W.S. Carus) [α]

1	Living agents	136 cases
2	Toxins	26 cases
3	Unknown	6 cases
TOTAL		168 cases

COUNTERMEASURES

Combating bioterrorist attacks involves, first of all, the intervention of the secret services, the police and the judicial system. Also, the intervention of the National Health System, the National Defense System, local communities and non-governmental organizations is crucial in the fight against these attacks. These non-medical contermeasures have an active, prophylactic role, preparing the population in the event of an attack. [3, 4, 5]

Table 4. Dissemination technique (W. S. Carus) [α]

No	Dissemination	Category			TOTAL cases	Comments
		Terrorist	Criminal	Others/not specified		
1	Aerosolization	2	0	0	2	The fewest
2	Injection/topic	6	10	0	16	
3	Food	1	20	1	22	
4	Water	4	0	2	6	
5	Natural vectors	0	1	1	2	The fewest
6	Not specified	10	13	79	102	The most
7	Unknown	5	10	2	17	
TOTAL		28	54	85	167	
Comments		The fewest		The most		

The responsibility for coordinating actions in these situations lies with the government of the country holding the EU Presidency, with the government of the country under attack and/or with the European Center for Infectious Disease Control.

The specific capabilities of the Medical Department of the Ministry of Defense are important for the medical protection against weapons of mass destruction and, in particular, for the fight against biological attacks. The Center for Military Medical Scientific Research has a Laboratory of Anti-infective Medical Protection and Epidemiological Emergencies, which functions as a specialized medical protection unit against biological weapons. It conducts military medical scientific research to protect troops and the civilian population against biological weapons and/or toxins, for biological warfare, bioterrorist attacks, or biological accidents.

Table 5. The mortality rate in the attack with biological agents (W.S. Carus) [α]

Purpose		Cases	Deaths
1	Bioterrorism	751	0
2	Biocrime	130	10
TOTAL		881	10

Source: Carus W. S. „Bioterrorism and Biocrimes. The illicit use of biological agents since 1990", Center for Counterproliferation Research, National Defense University, Washington DC, 2001. [6]

If we make a retrospective of the 20th century, the most warrior of all, it is noticed that the practical use of weapons/biological agents was a reality. [6]

It is noted that in the last decade of the 20th century there were more cases than in the rest of the century.

OBSERVATION

An important component in the fight against biological attacks is represented by medical espionage, a constantly updated data base of new agents and existing diseases, thus, becoming the key to finding the most effective treatment.

With regard to the measures required for the imminence of a biological attack, individual protection means are used such as the gas mask - which is not 100% effective, but it decreases the amount of inhaled substance, having a protective role, because for most substances in order for harmfull effects to hapen it is necessary for large quantities to be inhaled.

Chemotherapics present much wider benefits in regards to the protection against biological weapons, acting switftly, having a broad spectrum and being easily administered in large communities.

Also, a very impressive step after a biological attack is the decontamination by appropriate physical and chemical means for the equipment, rooms, clothes, water sources, soil, etc. [7, 8, 9, 10]

CONCLUSIONS

The risks of bioterrorism and biocrime attacks in the contemporary world are real, and the history of the 20th century and the beginning of the 21st century confirms this. It is necessary to implement preventive measures on the unlawful use of biological agents. Early medical and non-medical countermeasures must be prepared for the prophylaxis, treatment and cessation of the consequences of any biological attack.

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REVIEW

The Novelty of Contrast-enhanced Ultrasound in the Pathology of Liver Tumors. A Narrative Review of the Literature

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Abstract: Liver pathology represents, nowadays, a major cause of mortality and morbidity worldwide. Since MRI helps in limited cases in the differential diagnosis of various pathologies concerning liver tumors, other modern diagnostic strategies should be prioritized. Besides various tumoral biomarkers, contrast-enhanced ultrasound (CEUS) plays a substantial role in differentiating various liver pathologies due to its availability and specificity. Various contrast-enhanced substances used for CEUS assist mainly in detecting focal liver lesions and differentiating their malignant character. This review focuses on the main indications and limits of this novel investigation, considering that patients presenting with various liver pathologies are more challenging. We highlighted in our review the similarities with the CT/MRI imaging methods in reference to the specificity and sensitivity of the method, in its potential ability to detect various liver tumors. Moreover, the contrast agents used by CEUS are eliminated throughout the lungs, showing no evidence of hepatotoxic, nephrotoxic, or cardiotoxic events. In conclusion, the accessibility and safety of the procedure and the absence of the use of ionizing radiation point out that CEUS could be a stand-alone technique in the diagnosis and evaluation of complex liver pathologies.

Keywords: contrast-enhanced ultrasound; hepatocellular carcinoma; viral hepatitis; liver cirrhosis

INTRODUCTION

The diagnosis of various liver tumors remains, nowadays, challenging even if new diagnostic and prognostic tools are continuously developed [1–5]. In this context, contrast-enhanced ultrasound (CEUS) has been a widely used imaging method in Europe and Asia for over 10 years. CEUS consists of an ultrasound that employs contrast agents for increasing the accuracy of the investigation, typically injected intravenously. It can be compared with other contrast-enhanced imaging methods, such as CT or MRI, and it possesses the advantage of a real-time assessment of different focal liver lesions, improving their detection and characterization [1,2]. CEUS has become more popular in recent years than CT or MRI due to the fact that it has almost no adverse reactions, does not make use of any type of ionizing radiation, and can be employed successfully in patients with impaired renal function [1].

CEUS uses a special type of contrast agent that consists of a microsphere with an albumin, phospholipid, or surfactant-based shell and a gas filling, which subsequently forms a microbubble [2]. These microbubbles do not usually surpass 7 µm in size and have a mean diameter of 2.5 µm. These small sizes allow the microbubbles to freely circulate in the capillaries and impede them from passing through the vascular endothelium into the interstitial space, thus rendering them exclusively intravascular [2,6,7].

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The microbubbles are excreted in two different ways depending on their components – the shell is metabolized in the liver, while the gas filling is eliminated through the lungs in about 10 to 15 minutes, even in patients with chronic obstructive pulmonary disease. This characteristic allows the utilization of these contrast agents in patients with renal failure, thus posing no risk of nephrotoxicity, when other contrast-enhanced methods such as CT or MRI are not indicated [2,6,8,9].

There are many contrast agents available around the world, but the most conventional are the sulfur hexafluoride microbubbles (SonoVue, Bracco), the perflutren lipid microspheres (Definity, Luminity), and the perfluorobutane microbubbles (Sonazoid).

SonoVue is the contrast agent most often used in Western countries and comprises a phospholipid shell that surrounds the sulfur hexafluoride microbubbles, resulting in a diameter of 2.5 µm. The agent is usually injected as a bolus of 2.4 mL in a peripheral vein (the antecubital vein), together with a 5 mL to 10 mL saline solution, using a large enough needle (more than 20-gauge) to ensure that the bubbles do not rupture during the procedure [1,2,6].

Sonazoid is mainly employed in Japan, Norway, and South Korea, and uses perfluorobutane microbubbles. Although it is similar to the other contrast agents used in CEUS, unlike those, Sonazoid can be phagocytized by the Kupffer cells and allows an additional evaluation of the liver in the Kupffer phase or the post-vascular phase. The agent can persist up to several hours in the liver and spleen after its vascular phase [1,6].

INDICATIONS - GENERAL CONTEXT

Even though the most common clinical application of CEUS is the characterization of focal liver lesions and differentiating between malignant and benign masses, there are studies and guidelines that show there are numerous other uses for this technique. As an intravascular contrast-agent imaging method, CEUS can provide information about different vascular diseases that may affect the carotid arteries or the aorta and other abdominal blood vessels, or it can help assess the thrombosis of the portal vein [10,11].

CEUS plays an important role in the imaging of the extravascular structures, mainly cavities, either physiological, such as the peritoneal, pleural cavities, biliary and gastrointestinal tracts, or non-physiological, including cysts, abscesses, or diverticula [12,13]. Thus, CEUS is used in gynecology to evaluate the uterus and the fallopian tubes, in nephrology and urology to help diagnose ureteral reflux and to discern between bladder or renal tumors and cysts or testicular masses. Excluding the liver evaluation, CEUS also has other applications in the assessment of the gastrointestinal tract.

It is successfully used in patients with inflammatory bowel diseases to estimate disease activity or with fistulae and abscesses, and at the same time, it can be administered orally in order to examine the bowel lumen [12–14].

CEUS can be successfully used for assessing different focal liver lesions seen on standard ultrasound and is indicated for the following: hepatic nodules larger than 10 mm (for nodules smaller than 10 mm CEUS can be attempted by an expert examiner); LI-RADS-graded liver lesions as LR-3, LR-4 or LR-M on CT or MRI: monitoring enhancement pattern changes over time for LI_RADS-graded lesions as LR-3 or LR-4; detecting arterial phase hyperenhancement (APHE) when it is absent on CT or MRI, but still suspected; guiding liver biopsies or treatments for difficult to visualize lesions, aiding in choosing the right lesion to be biopsied or reevaluating prior biopsied lesions with inconclusive histopathology; assessing treatment response; making the differential diagnosis between a tumor inside a vein or a simple thrombus [15].

One of the most important indications for CEUS is liver mass characterization and not just detection, with some studies showing an equivalence or even a superiority compared to CT or MRI scans. This ultrasound can also help in differentiating benign from malignant nodules [16].

The most common benign lesions that can be diagnosed using CEUS are hemangiomata, focal nodular hyperplasias (FNH) and hepatic adenomata. Malignant masses which are evaluated by CEUS are hepatocellular carcinomata (HCC) and non-hepatocellular tumors, that include metastases, intrahepatic cholangiocarcinomata (ICC) and lymphomata [16–18].

Liver lesions in CEUS can be described using two features that appear in different phases of the ultrasound: arterial phase hyperenhancement (APHE) and washout [1,16].

If there is no washout present and the enhancement in the arterial phase is sustained, there is a high probability that the mass is benign. In comparison, malignant masses show a decline in the enhancement (washout) in the portal or late phase. Furthermore, the intensity and the timing of the washout can be employed to differentiate between types of liver malignancies.

HCC nodules are most likely to display a late (in over 1 minute) and weak washout, while non-hepatocellular malignancies exhibit early (in less than 1 minute) and intense washout. In order to detect liver metastasis, a sweep of the entire liver in the portal venous phase is essential [1,2,16,17,19]. The benign lesions are usually described by specific patterns of enhancement in the arterial phase. Some examples of these patterns are peripheral discontinuous nodular enhancement (hemangiomata), stellate vessels with centrifugal filling (FNH) and centripetal filling (some adenomata) [16,20].

TECHNICAL EXECUTION

To correctly perform an ultrasound using contrast agents, firstly, it is necessary to have a properly trained examiner who should be able to recognize focal liver lesions and vascular disorders, to assess the effect of a treatment over a lesion and to compare different findings with other imaging methods such as CT, MRI or PET-CT. Secondly, the ultrasound equipment needs to have appropriate hardware, for example, scanners that can cancel soft-tissue enhancement, and software that can display both the standard ultrasound image and the CEUS image simultaneously [15,16]. The patient should be in a supine position with the abduction of the right arm, and before injecting the agent, the examiner should be aware of the medical history of the patient, of the clinical aspects and also of any existing cysts or calcifications that may be wrongly interpreted as malignant [1,12,15,16,21].

For liver imaging, the recommended dose of SonoVue, the most frequently used contrast agent, is 2.4 mL. This value can be decreased by 20-25% if the patient has a low BMI in order to avoid the excessive enhancement of the liver or it can be increased if the lesion is very superficial [15]. The substance is then injected through a large IV line (20-gauge minimum) as a bolus in the span of 2-3 seconds, immediately followed by a saline flush of 5-10 mL [2,7,15].

Interpretation of CEUS is made by evaluating the three vascular phases that follow the injection of the contrast agent. These phases appear as a result of the double vascularization of the liver, namely the hepatic artery and the portal vein [21].

The arterial phase begins at 10 to 20 seconds after injection and ends at 30-45 seconds and appraises the arterial vascular supply of the liver lesion by degree and pattern [1,21]. The portal venous phase begins immediately after the arterial phase at 30-45 seconds and can last up to 120 seconds (2 minutes). This phase marks the entry of the contrast agent in the portal vein and is characterized by diffuse and maximal enhancement of the liver parenchyma [1,21]. The late phase is represented by the amount of time passed from the end of the previous phase until the clearance of the contrast agent from circulation and it can vary from 2 to 8 minutes (120–480 seconds), depending on the type and dose of the agent [1,21]. The post-vascular phase only appears if Sonazoid is injected and it is the result of the contrast loading of the Kupffer or other phagocytic cells. This particular phase usually starts after 8 minutes (480 seconds) following the initial injection and can be seen even after several minutes or hours [1,21] (Table 1).

Table 1: Vascular phases and their duration in contrast-enhanced ultrasound (CEUS)

	START	END
Arterial	10-20 s	30-45 s
Portal venous	30-45 s	120 s
Late	>120 s	Approximately 4-8 min (bubble disappearance)
Post-vascular	>480 s	Approximately 30 min

In the following images, an HCC nodule can be seen during a contrast-enhanced ultrasound. The hyperenhancement in the arterial phase and the late and mild washout in the later phases should be noted (Figure 1).

Figure 1: HCC nodule as seen during CEUS. (a) After 0 seconds (moment of contrast injection), (b) After 30 seconds (arterial phase); (c) After 90 seconds (portal venous phase); (d) After 140 seconds (late phase). Image credits: Teodora Isac.

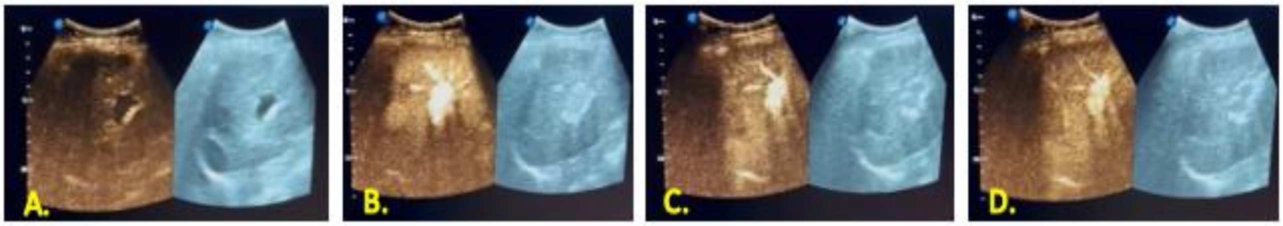

BENEFITS

The contrast agents used for CEUS have a few major advantages in comparison to those used for CT or MRI. The most important characteristic is the increased safety of administration, with almost no side effects reported in the literature. There is no evidence of either cardiotoxic, nephrotoxic or hepatotoxic events, nor any interference with the thyroid function as they do not contain iodine and no need for any specific laboratory testing or fasting before the procedure [2,15,21]. Therefore, these contrast agents can be harmlessly administered to patients with renal failure and CEUS can be considered the first-line imaging method for these particular cases [2,21]. Some studies show that when adverse reactions do appear, they are: headaches, chest pain or chest discomfort and nausea, all other symptoms being extremely rare [21,23,24].

Compared to CT contrast agents, CEUS agents have a significantly lower incidence of hypersensitivity events and the probability of an anaphylactoid-type reaction is extremely low, with a rate of 0.001% and a rate of 0.0006% of fatalities after SonoVue administration [2,21]. Some studies show that CEUS is also safe for use in pediatric patients or pregnant women [21]. Following these observations in the population, the 30-minute period of monitoring previously recommended after injection of contrast agents was eliminated [15].

CEUS has the advantage of being a real-time investigation with a higher sensitivity for detecting APHE in focal liver lesions than CT or MRI and is one of the most important imaging methods for identifying HCC nodules [6,21]. CEUS can categorize a rapidly enhancing hemangioma as LR-1, often wrongly diagnosed as LR-3 or LR-4 by CT or MRI, and can also differentiate an arterioportal shunt from a true HCC nodule, which can confuse the previously mentioned types of scanning [6].

CEUS was successfully employed for guiding treatment procedures or for evaluating treatment response. When performing a US-guided thermal ablation of a liver nodule, often an HCC one, CEUS showed a rate of 95.2% of patients with complete tumor ablation after a single procedure compared to 32% of patients after using a standard US. The rate of tumor recurrence was reduced to 16% as compared to 48% and the patients that benefited from CEUS guidance showed better progression-free survival rates [16,25,26]. The sensitivity of CEUS for post-treatment assessment is more than 80% and the specificity nears 100%, in some studies exceeding other imaging modalities, and, if used immediately after the ablation, is able to evaluate hemorrhages or hepatic infarctions [26].

Another treatment procedure after which CEUS can be used to determine residual tumor is transarterial chemoembolization (TACE). When employed after 4 weeks, it can successfully differentiate between a real active tumor and post-procedural inflammation. Some studies show that CEUS can visualize residual HCC nodules after only 1 day following the procedure, with higher rates of detection than CE-CT after 1 month [25]. It also has the advantage of detecting smaller areas of viable tumor that cannot be correctly appreciated by CT or MRI [26].

Though limited, there are studies that attest to the use of CEUS for evaluating patients one week after transarterial radioembolization (TARE) based on its ability to detect changes in tumoral perfusion [26].

Some studies evaluate CEUS as a monitoring tool for systemic therapies and their effects on tumor angiogenesis [25].

LIMITS

Even if CEUS is one of the most efficient imaging methods for liver nodules, there are some limits that need to be taken into consideration.

First of all, being a real-time evaluation, it is not possible to examine more than one lesion at the same time or to visualize the entire liver in the arterial phase; for this instance, a second bolus of contrast agents is needed for the same patient [2,15]. For patients with multiple liver nodules, contrast-enhanced CT or MRI is recommended [22].

Secondly, areas that are difficult to examine on a standard ultrasound are most likely the same when using CEUS and it can be troublesome when the lesion is smaller than 10 mm or is located subdiaphragmatic or very deep [15,22]. Small, simple cysts can be misinterpreted as metastatic lesions because both of them appear hypoechoic on CEUS [2].

For nodules that require complete imaging staging or monitoring of changes in size over time, CT or MRI are more likely to be of use, because CEUS alone is either not sufficient or it is very difficult to capture exactly the same image as the first time on serial US exams [6,22,27].

Even though CEUS is better than standard US, it is still examiner-dependent and the quality of the images may differ with movement artifacts or other interferences [2]. Some limits may also appear in the technique itself, for example, artifacts related to the dosing of the contrast agent, destruction of the microbubbles by the US beam, or inadequate tissue suppression as seen in a fatty liver [16].

CEUS LI-RADS and HCC

In order to create standardized reports for liver nodules, mainly HCC, seen on CEUS examinations, a scoring system has been designed called LI-RADS (Liver Reporting and Data System) [1,28,29]. It helps improve communication between different examiners and reduce confusion and diagnostic errors in patients with high risk for HCC [28]. The nodules observed with CEUS are then classified as: LR-1 (definitely benign), LR-2 (probably benign), LR-3 (intermediate malignancy probability), LR-4 (probably HCC) and LR-5 (definitely HCC) [1,28] (Table 2). Because CEUS is also able to evaluate other types of liver masses, additional categories have been added: LR-M (malignant, but not specific for HCC), LR-TIV (tumor present in the vein) and LR-NC (not categorized due to omission or image degradation) [1,15,30,31]. The sensitivity of CEUS for detecting HCC lesions is dependent on the mass size. For nodules of less than 2 cm, the sensitivity can vary in different studies from 53.6% to 83.3% and for nodules bigger than 2 cm, it ranges from 91.3% to 94.5%, with a mean of approximately 78.3% and a specificity of around 93.1% [32].

If a nodule shows rim-like APHE, which means the enhancement is more intense in the periphery, forming a halo around it, the category according to CEUS LI-RADS should be LR-M and they are usually liver metastasis or other malignant lesions, but not HCC. If the nodule has a peripheral discontinuous globular pattern, it should be included in the LR-1 category as a benign hemangioma [1,33].

Table 2: CEUS LI-RADS diagnosis (modified after [6])

APHE (arterial phase hyperenhancement)	**NO**		**YES** (not rim and not peripheral discontinuous globular)	
Rim APHE or early washout (<60 s) or marked washout – LR-M Peripheral discontinuous globular – LR-1 (hemangioma)				
Nodule size	Nodule size	≥20 mm	<10 mm	≥10 mm
No washout	LR-3	LR-3	LR-3	LR-4
Late and mild washout	LR-3	LR-4	LR-4	LR-5

In a study that took place between January 2010 and December 2018, 382 patients (with a total of 464 liver lesions) with chronic liver disease at high-risk for hepatocellular carcinoma were included [28]. The exclusion criteria were: transarterial chemoembolization, radiofrequency ablation, and systemic treatment for HCC [28]. The 464 liver lesions were divided into: 68 non-HCC non-malignant lesions (hemangiomata, simple cysts, fatty infiltration, regenerative nodules), 359 HCCs and 37 non-HCC malignant lesions (liver metastases, other types of malignancies). By using CEUS LI-RADS, all of the 464 nodules were classified and the percentage of HCC was established for each category. From the total number of lesions examined, 22 of them were part of the LR-1 category and the other 8 lesions were classified as LR-2. Among those, the HCC rate was 0%. 26 lesions were included in the LR-3 class, but 11 (42.3%) of them were HCC. From the 106 nodules that were included in the LR-4 category, 85 (80.2%) were also confirmed to be HCC. 264 nodules from the total of 464 were categorized as LR-5 by means of CEUS and the HCC rate in this category was 258 (97.7%). For the remaining lesions, 11 were classified as LR-TIV and all of them (100%) were confirmed as HCC, and 38 were classified as LR-M; only 5 (13.2%) of those actually were HCC [28].

The sensitivity of the LR-5 category for the diagnosis of hepatocellular carcinoma was 71.9%, with a specificity of 94.3%, a positive predictive value of 97.7%, a negative predictive value of 49.5% and an overall diagnostic accuracy of 76.9% [28].

By combining the LR-4 and LR-5 categories, they managed to improve the diagnostic accuracy to 90.7%. In this case, the sensitivity was 95.5%, the specificity 74.3%, the positive predictive value 92.7% and the negative predictive value 82.9% [28].

This study has once more shown that CEUS is comparable to CE-MRI and CE-CT in the diagnosis of hepatocellular carcinoma and can be used as a first step to describe liver lesions in high-risk patients during the HCC surveillance. Moreover, using CEUS instead of CT/MRI limits the use of unnecessary imaging techniques in the case of non-malignant tumors [28].

Differentiating an intrahepatic cholangiocarcinoma from a hepatocellular carcinoma in patients with liver cirrhosis can be difficult. The CEUS pattern of the intrahepatic cholangiocarcinoma in this study was a heterogeneous and rim-like enhancement in the arterial phase and a portal venous and late vascular phase washout. The key feature of the HCC is represented by the arterial hyperenhancement of the nodule, followed by a late (after one minute) or absent washout [28]. CEUS has shown a good performance in the diagnosis of HCC, with good sensitivity and specificity using the LI-RADS algorithm. However, an HCC nodule lower than 2 cm has shown a lower sensitivity (56.3%) as compared to 78.9%, representing the sensitivity of HCC nodules higher than 2 cm.

In spite of all the limitations of CEUS presented in this study, the CEUS LI-RADS algorithm for HCC diagnosis remains a useful non-invasive clinical practice [28].

In conclusion, for a nodule to be diagnosed as HCC, it needs to show an APHE, not rim-like and not peripheral discontinuous globular patterns and late and mild washout, as compared to other malignant lesions of the liver categorized as LR-M, that reveal a rim APHE and early, marked washout [30,31,33].

Specific HCC-related conditions and CEUS contribution

CEUS could help differentiate AFP-negative hepatocellular carcinoma from other liver malignancies. A total of 99 patients with liver disease and AFP-negative primary liver malignant tumors were included in a study that took place between January 2018 and February 2021. While the inclusion criteria were the following: serum AFP negative for all patients, the CEUS LI-RADS criteria applied to all patients and CEUS performed within 2 weeks before the liver surgery or biopsy, the exclusion criteria were the following: tumor lesion below 1 cm, thrombosis of the vein, multiple tumors, other treatment modalities in advance of the CEUS examination [28]. They performed CEUS using Siemens ACUSON Sequoia and Philips IU22/EPIQ7 and SonoVue as the contrast agent [33].

According to the results of the biopsy, the 99 intrahepatic lesions were divided into 38 cases of other malignancies (3 cases of HCC combined with ICC, 31 cases of ICC, 3 cases of lymphoma, 1 case of spindle cell sarcoma and 1 case of angiosarcoma) and 61 cases of HCC [33]. The study showed that there were no patients in the LR-1, LR-2 or LR-3 categories in both the HCC group and the OM group. In the HCC group, there were 13 patients in the LR-4 category, 38 patients in the LR-5 category and 10 cases in the LR-M category, while in the OM group, there were 3 patients in the LR-5 category and 35 cases in the LR-M category [33].

The table below shows the sensitivity, specificity and both positive and negative predictive values for the diagnosis of hepatocellular carcinoma or other liver malignancies using the CEUS features [33] (Table 3).

Table 3: CEUS characteristics and their efficacy in the diagnosis of both HCC and OM
(modified after [33])

HCC characteristics	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic compliance rate
APHE (but non-rim)	95.1%	63.2%	80.6%	88.9%	82.8%
Mild washout	88.5%	65.8%	80.6%	78.1%	79.8%
Late washout	75.4%	84.2%	88.5%	68.1%	78.8%
LR-4	21.3%	97.4%	100.0%	44.2%	51.5%
LR-5	62.3%	92.1%	92.7%	60.4%	73.7%

Other primary malignancies (OM)	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic compliance rate
Rim APHE	57.9%	96.7%	91.7%	78.7%	81.8%
Marked washout	65.8%	95.1%	89.3%	81.7%	83.8%
Early washout	84.2%	82.0%	74.4%	89.3%	82.8%
LR-M	92.1%	83.6%	77.8%	94.4%	86.9%

Although AFP is a serological marker of great importance in the early screening of HCC, there is a important number of patients who are AFP-negative and present intrahepatic nodules. Therefore, CEUS is a safe, convenient, cost-effective and radiation-free tool that can reveal microvascular vascularization of the tumor. However, it may be difficult to differentiate HCC nodules from ICC nodules in cirrhosis due to the overlap between the enhancement patterns [33].

This study shows that the LR-5 category has a specificity of 92.1% for HCC nodules that are AFP-negative and a specificity of 62.3%. The LR-M category represents an optimal way to differentiate other liver malignant lesions from HCC nodules. The LR-M category in this study revealed a sensitivity of 92.1% and a specificity of 83.6%. [33] Moreover, CEUS LI-RADS has a great application value for the diagnosis of HCC versus OM in patients at risk of HCC. CEUS LI-RADS can be used to determine the presence of focal intrahepatic lesions, even at an early stage when AFP is negative. Nevertheless, CEUS LI-RADS needs continuous improvement in order to diagnose and differentiate other liver malignancies efficiently [33].

Furthermore, CEUS proved to be helpful in the evaluation of the post-treatment response in patients who underwent transarterial chemoembolization (TACE) for HCC nodules. TACE is a therapeutic option widely used for unresectable hepatocellular carcinomas, given the fact that the majority of patients with HCC are usually diagnosed when the disease is at an advanced stage [34]. Patients who underwent TACE as treatment for HCC need to be accurately evaluated to establish the subsequent plan of therapy. CEUS can depict tumor necrosis after TACE, as early as 1-2 months, while the shrinkage of the tumor can take longer to occur [34]. This study wanted to describe the patterns of residual HCC on CEUS and compare the accuracy of CEUS with that of CT in the assessment of HCC response to TACE [34].

This study took place between February 2010 and June 2015 and included patients with HCC who had undergone TACE. They were divided into two groups: those who were assessed for the very first time after the procedure for tumor response and those who were suspected of tumor recurrence due to raised serum AFP.

The inclusion criteria in the study were the following: single mass or multinodular HCC less than 50% of the liver, entire nodule seen on standard US, nodule smaller than 15cm, while the exclusion criteria were represented by the presence of comorbidities (renal and heart failure), severe cirrhosis, HCC involving more than 50% of the liver and breastfeeding women. After applying the exclusion criteria, 50 patients with 70 liver lesions were evaluated by CEUS, but only 57 masses by CEUS, CT and MRI [34].

MRI detected residual disease in 36 masses out of 57; meanwhile, CEUS successfully identified 34 out of 36, resulting in a sensitivity of 94%. The specificity of CEUS compared to MRI was 100% regarding the 21 masses with no recurrent disease. The positive and negative predictive values of CEUS were 100% and 91% [34].

Compared to the multiphase CT, the sensitivity of CEUS was 94% (vs. 50%), while the specificity was 100% for both imaging tools. These results may be explained by the Lipiodol substance used for TACE, which may appear similar to a residual viable tumor. Therefore, the CT scan may be confusing, while CEUS, by not being affected by Lipiodol, can detect even tiny nodules as early as one week after TACE. After 4 weeks post-TACE, CEUS achieved superior sensitivity rates than CT and MRI (100% vs. 50%) [34].

CEUS could be considered an effective alternative technique for identifying early tumor response of HCC after TACE (using Lipiodol substance). However, it is recommended that, in the long term, CEUS be used together with MRI/CT for a better evaluation of the tumor response [34].

CONCLUSION

CEUS is a real-time imaging method used in many areas of interest, including the study of liver masses. It is shown in many studies that the sensitivity and the specificity of CEUS compared to CT/MRI are similar, or even superior, in the diagnosis and surveillance of hepatocellular carcinoma and other malignant liver nodules. The contrast agents used by CEUS are eliminated throughout the lungs, showing no evidence of hepatotoxic, nephrotoxic, or cardiotoxic events. In order to systematize the assessment of the HCC nodules, the CEUS LI-RADS classification was introduced.

Ultimately, although CEUS has its limitations, there are great perspectives in the appraisal of liver nodules using this imaging technique. The accessibility and safety of the procedure and the absence of the use of ionizing radiation reinforce the idea of using CEUS in the near future as a stand-alone technique in the diagnosis and evaluation of the response to TACE for the hepatocellular carcinoma nodules.

Conflicts of interest and sources of funding

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Authors' contribution

All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

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ORIGINAL ARTICLE

Correlational Analysis of Performance Outcomes during the Education of Combat Medics at the Tactical Medical Training Cell of the Belgian Defense

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Abstract: Combat medics must deploy multiple cognitive, manual, and social skills to perform efficient life-saving interventions and ensure the safe transportation of casualties. In 2021, the Tactical Medical Training Cell of the Belgian Army introduced an immersive and integrative educational program for Advanced Emergency Medicine Technicians (EMTa) certification. This study aims to provide a correlational analysis of performance outcomes during the certification period. While conducting their assessment, candidates were evaluated in three different but complementary roles: Medical Leader (ML), Medical Practitioner (MP), and Tactical Leader (TL). The individual outcomes in theoretical and practical examinations of one hundred thirty-seven candidates were collected during 2021 and 2022. The mean success scores for the theoretical and total practical examinations were 90.6% and 80.1%, respectively. Candidates scored significantly better as TL. Theoretical results were significantly correlated with the ML function, and performances in all three positions were correlated with each other. This study showed that within the combat medic team, individual performances, even if initially well-defined, were associated with the success rates of the other positions. This finding should be considered in the further consolidation of combat medic education.

Keywords: military medicine; education; first aid; combat medic; tactical leader; emergency medicine

INTRODUCTION

In today's operational theaters, combat medics must deploy multiple cognitive, manual, and social skills to perform efficient life-saving interventions, provide basic medical and nursing care, and ensure the safe transportation of casualties off the battlefield. In a contemporary warfare environment, first-line healthcare professionals face disruptive tactical challenges and novel technological threats [1]. Due to these particularly complex working conditions and this broad spectrum of responsibilities, educational and training programs must emphasize a strong knowledge base and intense practice of basic and advanced damage control resuscitation and surgical procedures. Furthermore, when combined, this knowledge and these skills should stimulate agile physiological thinking, appropriate clinical prioritization and time management, and the conservation of material and human resources, while considering cultural differences and ethical issues where necessary [2].

The integration of adult education principles can actively contribute to enhancing these learning performances. Adult education is defined as a teaching model that incorporates strategies of student self-direction toward goals of practical relevance for the learner [3]. According to Illeris, learning has individual cognitive, emotional, and external environmental dimensions [4]. An integrative and cross-fertilization process – the start of any learning process – takes advantage of five types of stimuli: (a) perception of the raw material; (b) transmission by another person (e.g., in lectures and instruction or through questions); (c) experience; (d) imitation; and (e) participation [5]. Consequently, accommodative and transformative learning that enlarges stimulation modes can contrast with restricted cumulative and assimilative learning. More specifically, theories of adult education aim to value the prior learning and experience of adults [6]. In a systematic review of faculty development initiatives designed to improve teaching effectiveness in medical education, the use of experiential learning, feedback, and effective peer relationships was found to be paramount to the success of these programs [7].

However, for a long time, the superiority of adult education principles in the specific domain of healthcare professionals' education was a matter of debate [8-10]. Interactive learning methods can be used in new contexts that do not resemble the original learning situation [8]. Scientific evidence supports the view that multimodal

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and interactive learning principles can lead to higher performance during medical education and training [11-13]. Additionally, problem- or case-based learning appears to promote greater critical thinking, better integration of facts, and improved self-evaluation compared to traditional teaching models with teacher-directed learning and non-interactive modes of teaching [14].

Although these principles of adult learning have been well-described for over 50 years, comparatively little is known regarding their integration into combat medic education or training programs.

In 2021, the Belgian Defense, Tactical Medical Training Cell (TacMed) introduced an integrative and immersive model of an educational program in the Advanced Emergency Medicine Technicians (EMT) certification. All Combat Medics candidates for the advanced course are systematically exposed to this learning model. The principles of the course shape theoretical and practical skills as support for individual professional development and, by extension, leadership maturing. The essential components for individual and collective medical training were identified following the NATO SOF doctrine [15]. Different training principles, such as Life Tissue training (LT) and simulation with role players (SIM), are combined. The last serves as a core education pivot because of the abundance of peer-reviewed evidence from military studies that clearly demonstrates that training methods using human patient simulators for teaching trauma care skills were superior and more cost-effective than many other learning techniques [16]. Finally, specific efforts are put into hyperrealistic and immersive simulation training scenarios as stimulators of team performance [17]. Like other authors [18,19], the course organizers conceived team performance as a multilevel process that includes the interrelation between individual-level and team-level taskwork and teamwork processes. The coordinated activities of a team of well-educated and trained individuals were considered the main objective [20,21].

The primary objective of the study was to establish, within an integrative hyperrealistic education model, the associative principles between theoretical and practical domains in order to explore global performance outcomes. As a secondary objective, success rates for specific achievements were inventoried, and a complementary analysis of their association profiles was performed.

MATERIALS AND METHODS

Study design

Advanced Combat Medic candidates presenting at the Medical Component Competence Centre, Belgian Defense, were included in this single-center retrospective cohort study.

Study setting and data collection

This study was carried out from January 2021 to December 2022. Instructors, evaluators, and Exercise Controllers systematically collected and processed all theoretical and practical examination results.

Population

A yearly refresher Tactical Combat Casualty Care (TCCC) educational program must be attended by all Belgian soldiers. The operational units of the Belgian Medical Component and combat units, including Special Operation Forces, detached their Combat Medic candidates. In their respective units, they applied voluntarily for a specific position as first-line responders. All candidates were consecutively included. To exclusively explore the course benefits, only students who failed the entrance examination were excluded from this study.

Intervention

A three-week theoretical module covering TCCC, Pre-Hospital Trauma Life Support (PHTLS), and Advanced Medical Life Support (AMLS) principles is preceded by an initial entrance exam on general anatomy and physiology. At the end of this sequence, and after succeeding in the theoretical exam in these domains, a three-week practical module is launched. This module includes dead tissue training, trauma patient simulation (using role players or manikins), clinical scenarios (individual or team-based), and a broad spectrum of clinical practicals. These are focused on amphibious medical incidents, mass casualty situations, complex casualty extrication, and CASEVAC (Casualty Evacuation). During a 72-hour final synthesizing on-field examination, all candidates were evaluated based on three independent secondary domains: their capability to act as Medical Practitioner, Medical Leader, and Tactical Leader.

Continuous monitoring by experienced healthcare professionals and psychologists during the on-field certification period ensured adherence to ethical guidelines and psychological safeguards.

Outcomes

Two main domains (total theoretical and practical scores at the final examination) and three practical secondary domains were considered as performance outcomes. The three practical secondary domains were designed to cover cognitive, procedural, and environmental dimensions. These three practical domains were linked to the following positions:

a. The Medical Leader (ML) was responsible for overall clinical assessment and management. The ML's responsibilities included: preliminary medical assessment, medical decision-making, guidance of medical activities, securing medical evacuation, and leadership of medical providers. Evaluation methods for this function assessed the quality of the medical approach (both diagnostic and therapeutic), the effectiveness of control over hard skills by team members, the quality of general clinical guidance, adequate and effective communication with the superior clinical echelon, control of safety rules, and final clinical responsibility.

b. The mission of the Medical Practitioner (MP) was to execute technical medical skills under the leadership and supervision of the ML. Particular attention was paid to the quality of material preparation, completeness, and safety in execution. The specific soft skills evaluated were: empathy towards the casualty, reactivity to ML demands, quality of reporting to the ML, and interaction and communication with the ML and other MPs.

c. Finally, the Tactical Leader (TL) was responsible for team security, controlling situational awareness, and coordinating the medical team's tactical actions. This function was evaluated through quality indicators of situational awareness, safety rules, planning of team actions, and consolidation of the medical evacuation plan proposed by the ML.

Evaluation tools and variables

During the theoretical evaluation, the candidates were evaluated on the percentage of correct responses at the end of the theoretical module. At least two independent evaluators from TacMed scored the respective ML, MP, and TL performances during the practical examination. Concerning the scenarios with role players, three main objectives were preliminarily defined and specifically evaluated through a scoring system reportable on a percentage scale. The first evaluation objective was always dealing with the hard skills, the second with the individual soft skills, and the third with the individual contribution to the collective dynamics.

The evaluators had received the same education and training for this purpose. The education of evaluators respects the NATO standards and includes additional skills from the civilian evaluation systems developed for health care. They regularly train for this function and are obliged to participate in all evaluation exercises organized by TacMed. At least one of the observers is educated and certified in an Observer/Trainer function following the NATO standards (International Military Medical Observer Trainer Course (IMMOT) course, NATO MED-MS-36887, NATO Centre of Excellence for Military Medicine). During the preparation of the scenario, the defined objectives and evaluation tools per objectives are revised together within the evaluator's team. All evaluators use the same evaluation scale.

Evaluators are blinded to theoretical scores and scores from the other scenarios run by the candidate.

The evaluation indicators were independently evaluated by each evaluator and compiled into a final score. The final score for each function was obtained by simple cumulation of the individual scores of all of the examinators. Each candidate went through the different positions at least two times.

The final global practical score corresponded to the sum of all scenarios' scores of the candidate.

In support of this scoring system materials, additional videos or pictures were captured.

At the end of each scenario, during the debriefing, individual and collective learning points were pointed out by each candidate as well as by the entire team.

Statistical analysis

An overall description of all populations/outcomes was statistically explored. Categorical variables were summarized using frequencies and percentages. Continuous variables were summarized using medians and standard deviations (SD). After checking for normality using the Kolmogorov-Smirnov test, the data were found to be not normally distributed (statistic = 0.127; df = 107, $p < 0.001$). The Wilcoxon Rank Sum test was used to compare two groups, paired or unpaired. The Mann-Whitney U and Kruskal-Wallis tests were used to compare a continuous variable with a categorical variable with two or more categories, respectively. Correlations between theoretical/practical results and individual results from the secondary domains were calculated using Spearman's rank correlation, two-tailed. The overall practical results (%) were defined as the dependent variable for multivariate linear regression, with multiple independent variables being demographic or individual results. A p-value < 0.05 was considered statistically significant. Biostatistical analyses were supported by IBM SPSS Statistics Version 29.0.1.0 (171) (License Ghent University, Belgium).

RESULTS

One hundred thirty-seven combat medic candidates for EMTa certification were recruited, and their individual scores were collected. Their participation was well-distributed during the two-year study period: from January 2021 to December 2022 (Table 1). With a mean seniority in the army of 8.99 years (SD = 5.13 years, range = 1-22 years), the candidates had a mean age of 30.30 years (SD = 5.89 years, range = 21-49 years). The vast majority of candidates were male (97.8%), with only 2.2% being female. In the total group, 71.5% of candidates succeeded in the final test, and 9.5% failed. Nineteen percent of the total group did not complete the entire course or final certification examination, mainly due to changed work schedules. Although not statistically significant, older candidates were found to be more resilient regarding non-completion of the course.

Theoretical and practical domains

A mean success score of 90.6% (SD = 6.5%) was found for the theoretical examination. On the other hand, the mean of the total practical examinations was 80.1% (SD = 4.8%) at the end of the 72-hour certification field exercise. All age and seniority groups presented this drop between theoretical and practical results, with no significant difference observed between younger and older candidates.

Secondary domains during practicals

The mean results displayed the following respective scores: for the ML function, the success rate was 79.1% (SD = 6.49%); for the MP, 80.8% (SD = 6.28%); and for the TL function, 81.1% (SD = 5.68%). Candidates scored significantly better as TL (p = 0.014) in comparison with their function as ML. When observing the secondary domain success scores, no significant difference was observed between the age or seniority categories.

Table 1: Population characteristics

Characteristic	N	Mean	StdDev	Range
Age (years)	137	30.20	5.89	21-49
Seniority (years)	137	8.99	5.13	1-22
		Count	**Percentage**	
Gender				
Male		134	97.8%	
Female		3	2.2%	
Year of completion				
2021		75	55.5%	
2022		61	44.5%	

Figure 1: Descriptive map of observed associations

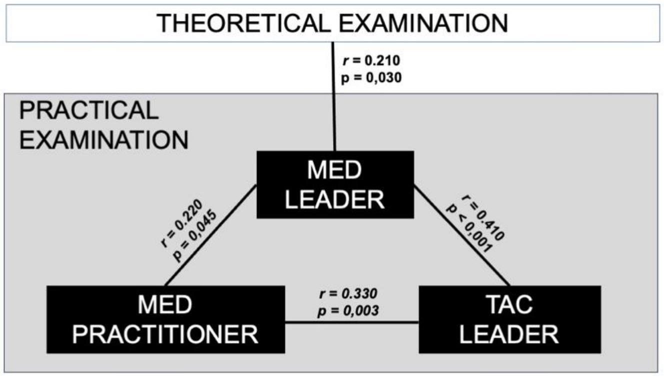

Correlations between main and secondary domains

Interestingly, the theoretical results were found to be weakly but significantly correlated not with the total practical score, but only with the ML function (r = 0.210, p = 0.030) (Figure 1). As expected, the total practical scores were significantly correlated with their secondary domains: ML (r = 0.210, p < 0.001), MP (r = 0.565, p < 0.001), and even more strongly with TL (r = 0.793, p < 0.001) functions. Furthermore, the secondary domains were found to be statistically correlated with each other: weakly or fairly but significantly correlated between ML and MP (r = 0.220, p = 0.045), and between MP and TL (r = 0.330, p = 0.003), while the correlation between ML and TL was found to be statistically stronger (r = 0.410, p < 0.001).

Multivariate linear regression

With the total results at the practicals as the dependent variable, the multivariate linear regression identified all practical secondary domains as independent predictors of the total practical results (p < 0.001), with the theoretical results as a weaker but significant independent predictor (p = 0.04) (Table 2). Candidate age and seniority were not found to be predictors of higher performance scores at the final practical examination.

DISCUSSION

In this study, we explored performance scores during military educational and training programs for combat medics. An integrative and immersive combat medic-specific training was proposed in a structured and stratified manner. During the practical examination, three different positions were tested, and this analysis shows how they were associated with each other. During the educational phase of the program, candidates were stimulated in their leadership (medical and tactical) positions through an integrative and cross-fertilization process, taking advantage of five types of stimuli identified by Jensen [5]. On the other hand, the role of the MP was not restricted to the adequate and safe execution of medical hard skills. Although well-structured assessment testing of medical hard skills is beneficial [22], additional soft skills such as communication, reporting, or mutual support were also added to the MP's educational program.

Table 2: Results of regression analyses performed to determine the effect of indicators in predicting performance scores at the practical examination

Coefficients*				
	Coefficient Std Error	**Standardized coefficients β**	**t**	**Sig**
Age	0.005	-0.005	-0.771	0.443
Seniority	0.006	0.002	0.254	0.800
Candidate category	0.041	0.003	0.876	0.384
Scores theoretical results	0.003	-0.008	-2.095	0.040
Medical leader	0.003	0.553	128.107	<0.001
Medical provider	0.003	0.353	83.922	<0.001
Tactical leader	0.003	0.412	103.293	<0.001

R^2 = 0.999
F Change = 10945.960
Sig F. Change <0.001
StdError of the estimate = 0.15587
N=137

** Dependent variable: Total scores at practicals*

Objectives of the Course

Our education and training principles were largely inspired by the eight learning fundamentals of Haraldseid-Driftland et al. (2023) – categorizing collaborative, practical, and content elements – to help develop learning principles and tools to translate resilience into healthcare practice [23]. We complemented these with some of the six guiding recommendations of Sousa et al., including simulation, milestones as course learning objectives, and a multi-dimensional, competency-based assessment system [24]. The ultimate goals of this approach were to optimize crew resource management on one hand, and the individual resilience of first-line responders on the other. Especially in healthcare, communication, teamwork, and leadership top the list of nontechnical skills pivotal for clinical success [25,26]. As an illustration, deficiencies in nontechnical skills are among the leading causes of adverse events in the operating room [27,28]. In the early 1990s, Crew Resource Management principles initially developed to reduce pilot errors were applied to the medical field by anesthesia instructors, who developed the concept of Anesthesia Crisis Resource Management [29,30], with later extension to other actors in the operating room [31]. Across levels and contexts, this approach was found to stimulate healthcare professionals to collaborate, adapt to change, maintain processes and functions, and improve quality and safety through interactions such as exchanging information, coordinating, negotiating, aligning needs, and developing buffers [32]. Increased resilience has been shown to positively impact nursing practice by Thomas and Asselin [33]. In this context, resilience appears to be an important asset [34], and resilience-enhancing interventions to prepare healthcare students for their future professional practice were found to be beneficial [35].

Conceiving a Balanced Educational Program

Observing performance results, this study identified statistical associations between different positions within a combat medic team. For instance, in our study, the TL position – even if primarily more oriented towards situational awareness – was associated not only with global performance scores but also with the quality of more specific medical functions. Over the past century, educational psychologists and researchers have posited many theories to explain how individuals acquire, organize, and deploy skills and knowledge [36,37]. The three different processes of learning identified by Illeris – cognitive, emotional, and social – can occur simultaneously, forming a holistic learning process [4]. Additional dimensions of learning, such as physical or spiritual, could probably be added to this scheme.

At a cognitive level, our study has shown that the execution of medical techniques is not simply linked to previous knowledge acquisition. MP performance was correlated with the presence of two leading functions. Based on our methodological limitation of using a retrospective observational protocol, we cannot establish any intrinsic causal strength. Cognitive learning theories support the view that learning is a targeted internal process focusing on thinking, understanding, organizing, and consciousness. Even if successful in many learning aspects, such as information processing, curiosity stimulation, and motivation, they were found not to be strong enough to transfer the required knowledge to all learners. Additionally, they require considerable time and a trained workforce [37]. This evidence justified our choice not to exclusively concentrate on hyperrealism or single structural fidelity. We hypothesized that it may be more productive from an educational standpoint to consider the fidelity of the simulation scenario relative to clinical task demands along with physical resemblance [38-41], referring to evidence from the literature in this field [39-44]. This advantage of using multimodal simulation was supported by recent literature [42,43,45,46].

The emotional dimension of learning was not specifically explored in our study. However, our educational approach took advantage of an additional social dimension by encouraging and persuading previously learned behaviors, drawing the attention of learners, and creating strong responses [39-44]. Thanks to selective attention and memory, humans learn more from their experiences and organize these experiences into internal representations that facilitate future behavior [40-45]. Passive formats, lack of relevance, and disconnection from students' needs are some arguments supporting the apparent lack of efficacy of formal lectures [38, 46]. It appeared that the candidate's age or seniority in the army was not correlated with performance scores. Seniority and its consequent experience in the army, initially considered as a possible confounding factor or indirect advantage in this integrated learning model, cannot exclusively support the entire learning process. This finding stressed that medical and tactical leadership functions within a Combat Medic team must be learned and trained in addition to acquiring the necessary medical hard skills.

Finally, to emphasize the social dimension of this learning process, we chose event-based assessment as an essential method to deal with the complexity of authentic clinical interactions [47]. It potentially overcomes the shortcomings of not being able to observe tacit behaviors and cognitions and allows team members to assess the unobservable elements of teamwork [48,49].

Holistic Approach for the Education of Combat Medics

Our results only describe performance patterns, and our study was not designed to measure inferences within intrinsic cognitive mechanisms. Further analyses should be conducted to describe causality between indicators, identify all influencers, and measure their respective effect sizes on performance. The reported interactions could be considered the basis for a virtuous circle between these three functional abilities: medical guidance, execution, and external situational vigilance.

First, this study confirmed existing scientific evidence stating that a strong integration of various learning methods or tools leads to solid theoretical and practical results, even when considering specific practical tasks.

Secondly, a strict tasking of ML, MP, and TL functions within a combat medic team facilitated the organization and the detection of medical or tactical issues. These multiple but complementary views on the same medical situation positively influenced the final performance of each individual in the group. This task was initially designed to avoid interference and improve the quality of healthcare. All these tasks appear not to act separately but can reinforce each other. However, this approach imposes complementary education and training in soft skills, such as communication and reporting.

Finally, these considerations are not restricted to the specific environment of the Belgian Army. The program developed by TacMed at Belgian Defense was based on existing national or international protocols and studies, whether developed by or for armed forces or not. It took into account the military culture in Belgium and existing programs in other professional communities. The combination of educational tools was tested and adapted within the military, but external civilian experts adjusted program content or assessment methods. For this reason, some of these findings can be generalized beyond the specific Belgian military context.

Limitations

This study has limitations. The education program benefited from knowledge accumulated from the trainers' expertise, general trends in adult education, and guiding principles in EMT practice. Although efforts were made to minimize their effects, bias and unmeasured factors due to this retrospective design are still an issue. Because of their retrospective and observational nature, correlations could not establish causation between parameters. The conclusions of this study are based on the 71.5% of candidates who successfully completed the certification process. This high attrition rate could introduce a bias towards overestimating competency and positive correlations. Furthermore, the study is subject to all the limitations of a monocentric study design. Finally, while this is a preliminary qualitative investigation of integrative practice and leadership views in a military prehospital context, the perspectives represented may not reflect the entire profession, given the small overall sample size. Despite these limitations, these results provide valuable insight into some performance indicators in a complex work environment.

CONCLUSION

Inspired by recent findings in adult education, our integrative and immersive education program for advanced combat medic certification has demonstrated high success rates at both theoretical and practical levels. Furthermore, the performance levels of the different functions within the Combat Medic team, even if initially well-delimited, were found to be correlated with each other. These reported interindividual interactions open new perspectives for the consolidation of learning outcomes.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, F.W., J.J. and J.B.W.; methodology, J.B.W.; validation, F.W., J.J. and N.D.; formal analysis, J.B.W.; investigation, F.W. and J.B.W.; resources, J.B.W.; data curation, J.B.W.; writing—original draft preparation, J.B.W.; writing—review and editing, F.W., J.J., N.D. and J.B.W.; visualization, J.B.W.; supervision, J.B.W.; project administration, F.W. All authors have read and agreed to the published version of the manuscript.

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The authors did not use a generative artificial intelligence (AI) tool or service to assist with preparation, data analysis, or interpretation of results. AI was utilized to refine the language of the main body text after initial editing and review. The authors take full responsibility for the content of this publication.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki, and approved by the institutional office for data protection and the Ethics Committee (EC) of Ghent University and Ghent University Hospital (UZ Gent) and registered under number ONZ-2023-0600.

Patient consent for publication

Not applicable. Our retrospective study used examination results which regularly collected data from the databases. The consent for the use of personal data was given by each candidate as a routine procedure of the Centre of Medical Expertise, Belgian Defense provision.

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ARTICLE

Expectations vs. Appropriate Testing in Preventive Care: A Discussion Based on Insights from Romanian Responders

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Abstract: Preventive medicine plays a crucial role in ensuring the efficiency of healthcare systems, and laboratory investigations represent one of its main pillars. However, achieving an optimal balance between the benefits and costs of testing remains a challenge. Caught between public expectations, appropriate test utilization, and the need for adequate physician training in test ordering, enhancing patients' understanding and knowledge becomes essential. To explore this aspect, a general online survey was conducted, completed by 321 respondents. The results show that, while respondents generally followed physicians' recommendations, many also sought laboratory testing on their own initiative. Direct access to laboratory services - facilitated by the widespread availability of private laboratories - has become a common practice. This raises concerns about the potential overuse of laboratory testing and underscores the importance of further investigating the factors driving patient self-empowerment. Enhancing shared decision-making between patients and physicians is essential to optimize the benefits of testing, minimize unnecessary costs, and reducing the risk of health-related anxiety.

Keywords: laboratory investigations; routine testing; public health; medical education; healthcare overuse

INTRODUCTION

Laboratory testing plays a critical role in the diagnosis, monitoring, prognosis, and treatment of diseases. In modern healthcare, best practice emphasizes the principle of shared decision-making. Although this approach is more commonly applied to therapeutic decisions, it is equally relevant to laboratory investigations and diagnostic testing, as highlighted by Watson et al. While patients rarely request blood tests directly, they often perceive laboratory investigations as a meaningful step in the management of their health condition and as an indication that their physician is taking their concerns seriously. For patients, laboratory results are expected to provide clear answers or solutions, whereas physicians typically use them as tools to rule out serious conditions and to guide clinical reasoning. Due to the discrepancy between patients' expectations and the lack of adequate explanations regarding the actual purpose of the investigations, some patients are disappointed when the tests do not provide a clear diagnosis or concrete solutions for their medical condition [1].

In the context of individuals' tendency to absolutize the role of laboratory testing - as either a means of validating their health status or resolving medical issues - direct-to-consumer laboratory testing emerges as a form of self-empowerment. This is a challenge for the safety and efficacy of healthcare because it allows patients to obtain test results without physician referral, clinical interpretation, or a clear understanding of the limitations related to the test's analytical and diagnostic performance or its overall quality [2].

In the context of an increased volume of test requests, there is a potential for negative consequences. When performed by physicians, ordering unnecessary tests may lead to overdiagnosis, which can result in unwarranted treatments, potential adverse effects, and increased costs for the healthcare system. Contributing factors

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include patient expectations and pressure, as well as physicians' fear of legal liability, all of which may influence the decision to order tests that are not clinically justified [3].

Furthermore, the education and training of physicians in ordering adequate laboratory investigations might be a challenge. According to a study conducted by Fung et al., the majority of residents in family medicine ordered appropriate tests from a list of 38 laboratory investigations for prevention and screening purposes; however, the majority of the participants ordered at least one inappropriate test per patient (average from 3.3 to 5.7 per patient), leading to excessive costs. Several factors have contributed to the tendency to order unnecessary laboratory tests, including patient requests, fear of overlooking important clinical details, working in solo practice, and concerns about potential litigation [4].

While the main reason why healthy individuals visit the primary care facilities is preventive check-ups, and in this context, the family physicians order a high number of screening tests, the overuse of laboratory investigations has decreased from 1978 [5].

Considering the major influence in balancing overuse and effective screening of individual perceptions, understanding, and emotional responses regarding the role of laboratory testing, this study aims to provide general insights from individuals living in Romania. It explores their opinions, habits, behaviors, and knowledge related to laboratory testing, intending to inform the design of future representative studies.

MATERIALS AND METHODS

A survey was conducted using an online data collection tool (Google Forms), which included questions with predefined answers. Respondents selected the answers they deemed most appropriate. The questions addressed various topics related to medical laboratory investigations, including knowledge, opinions, habits, and behaviors. Participation in the survey was voluntary; the link to the form was accessible to anyone, and respondents' completion of the survey was automatically considered as consent to participate in the study. An introductory section provided information about the study's purpose.

Data were collected using a general data collection instrument rather than a validated questionnaire, aiming to capture a broad perspective and highlight insights and new areas for research, rather than yielding quantitative and generalizable results.

The data were centralized and analyzed using Microsoft Excel and Google Sheets. Relative frequencies were calculated and presented in tables and charts. This study employed descriptive statistics only, reflecting its exploratory nature and the self-selected sample. Therefore, no inferential analyses were performed, and causal or generalizable conclusions cannot be drawn. Further research with representative samples and appropriate analytical methods is needed to investigate potential associations.

RESULTS

1. Characteristics of the study respondents and health status

A total of 321 respondents participated in the survey, with an average age of 29 years (ranging from a minimum of 18 to a maximum of 81 years). Among the participants, 85% identified as female and 15% as male. Geographically, 22% of respondents resided in rural areas, and 78% in urban settings. The educational and professional backgrounds of the respondents are detailed in Table 1. Notably, 27% reported having no relatives or close friends working in the healthcare field. Regarding self-reported health status, 77% of respondents considered themselves healthy, while 23% indicated they suffered from chronic conditions, as illustrated in Figure 1.

Table 1: Educational and professional profile of the respondents

Level of studies	Middle school	High school	Higher education
	1.5 %	27%	72%
Occupation	Student	Employee	Other
	45%	50%	5%
Study or work in one of the following fields: health, medicine, veterinary medicine, dentistry, pharmacy, biology		No	Yes
		55%	45%

The majority of respondents rated their overall health status as good or very good; however, most also reported being concerned or very concerned about their health, as shown in Figure 2.

Several behaviors were reported by respondents as being associated with their health-related concerns, as presented in Table 2.

Figure 1: The relative frequency of chronic diseases among respondents who reported having illnesses

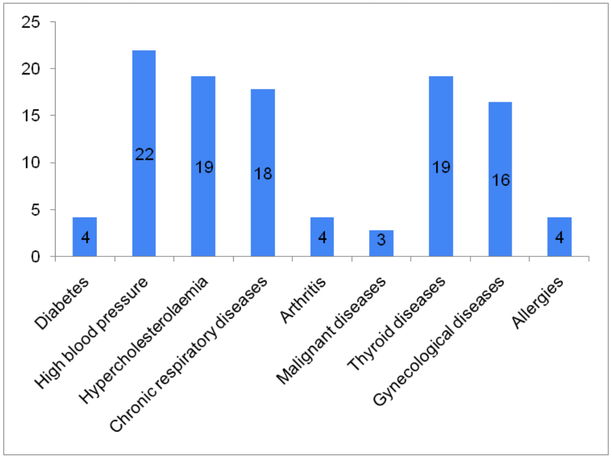

Figure 2. Self-perception of the overall health status (a) and level of concern related to the health status (b)

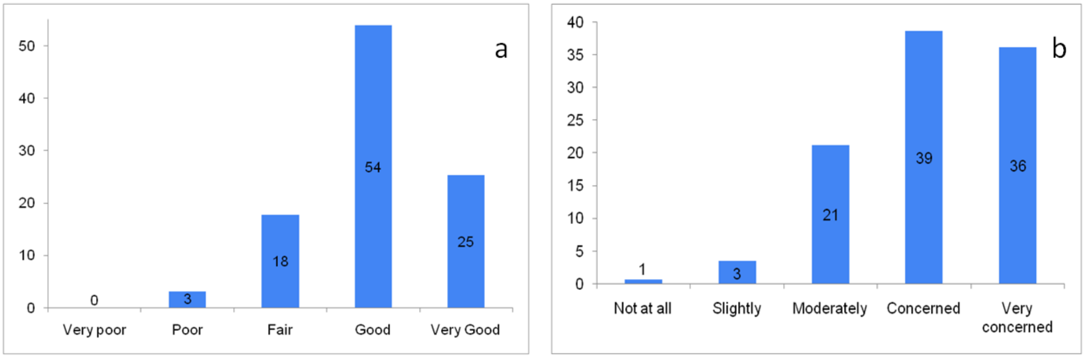

Table 2. Self-reported behaviors associated with health concerns – statements and percentages of respondents in agreement

Behavior linked to health concerns	Relative frequency
I don't consider myself concerned about my health.	4%
I frequently see a doctor for preventive purposes.	28%
I visit the doctor immediately when symptoms appear.	47%
I get medical tests done frequently.	50%
I get laboratory tests done as soon as I notice symptoms.	25%
I go directly to the pharmacy to request treatment if I notice something is wrong.	21%
Concern about my health makes me think about it all the time or frequently.	15%
Concern about my health makes me anxious.	20%
I adopt a healthy lifestyle to be in the best possible health.	36%
I'm afraid of catching infectious diseases in various everyday situations.	26%
I generally postpone going to the doctor for as long as possible.	11%
When I should go to the doctor, I prioritize it over other professional or personal matters.	38%
When I should go to the doctor, I first deal with other professional and personal matters and only then make an appointment.	13%

Opinions about laboratory investigations

Table 3 summarizes respondents' views and knowledge about medical laboratory investigations, their importance, and the recommendations they reported being familiar with regarding laboratory testing.

Table 3: Opinions about laboratory investigations – statements and percentages of respondents in agreement

How important do you consider routine laboratory tests to maintaining health?	
Very important, laboratory tests are essential for detecting and monitoring health problems.	87%
Moderately important, laboratory tests may be useful in certain situations, but are not always necessary.	12%
I do not consider laboratory tests essential for maintaining health.	1%
Do you think it is important to have regular laboratory tests, even if you are healthy?	
Yes, to detect potential health problems in their early stages.	90%
No, if I don't have symptoms, I don't need tests.	2%
It depends on each person's age and lifestyle.	7%
I am not sure.	1%
How well do you think laboratory test results indicate health status?	
The tests show exactly what health problems I have.	25%
Most of the time, the analyses are indicative.	41%
The tests cannot accurately indicate what health problems I have, regardless of their nature.	4%
Certain tests can indicate the exact disease, while others assess the general state of health.	29%
What do you consider to be the ideal approach?	
We should have all kinds of tests done frequently to make sure we are healthy.	51%
We should perform several more important types of tests frequently to ensure we are healthy.	48%
I do not believe that it is necessary to perform routine tests frequently to detect possible health problems.	1%
Do you think that your doctor recommends enough tests for you?	
I didn't need to see my family doctor/specialist.	8%
Yes, I believe I am receiving the necessary recommendations for my health.	63%
I am not sure.	8%
No, I think I am being recommended too many tests.	2%
No, I think that too few tests are recommended for me.	16%
I don't do laboratory tests.	2%
How frequently do you think laboratory tests are recommended for someone your age?	
3-4 times a year	6%
1-2 times a year	83%
Once every 2-3 years	2%
Less often	1%
No specific time frame is recommended	3%
What is your level of confidence in the results of laboratory tests?	
Very high	28%
High	61%
Moderate	11%
Low	0%
Absent	0%
How do you see the doctor's role in interpreting laboratory test results?	
As an essential guide in understanding the results.	90%
Like someone who should only be consulted in case of problems.	6%
I do not consider it necessary to have a doctor interpret it, as there is enough information available on the internet.	1%
No opinion.	2%

Do you think laboratory tests should be used more in prevention?	
Yes, it could help prevent some conditions.	62%
Yes, it could help detect diseases in their early stages.	31%
No, it could cause unnecessary worries.	1%
It depends on each individual case.	5%
How do you evaluate the experience during sample collection for laboratory analysis?	
Comfortable and without problems.	55%
Acceptable, there is potential for improvement.	40%
Uncomfortable and unpleasant.	4%
I have no personal experience in this regard.	1%
Which of the following aspects do you think could improve your experience while performing laboratory tests?	
Reducing waiting time for results.	12%
Lower costs or covered by health insurance.	60%
Better communication and explanation of results.	13%
Reducing waiting time for sample collection.	2%
Do you consider that laboratory results are managed with confidentiality in mind?	
Yes, I trust that they are treated confidentially	77%
I am not sure	15%
No, I have doubts about their confidentiality	2%
I have no personal experience with this	3%
No opinion	2%
Do you think there is enough public education about the importance and usefulness of laboratory tests?	
Yes, there is enough public education.	7%
No, more education would be beneficial.	87%
Not sure.	4%
I don't think public education would make a difference.	2%
No opinion.	1%
When you receive the results of laboratory tests, do you consider it useful to automatically receive explanations of the results in a formal setting?	
No, because I understand all the content and I don't need any other information.	7%
No, because I can find the necessary information by asking people I know or searching the internet.	1%
I do not know.	1%
Yes, I think it would be useful to receive explanations of the results every time I have my lab tests.	88%

Behavior of respondents

Regarding how the respondents deal with the results of medical laboratory investigations, most respondents reported either seeking medical advice or requesting feedback about their health, as shown in Figure 3.

Table 4 presents self-reported adherence to physicians' recommendations regarding laboratory tests, as well as respondents' motivation, testing frequency, and behaviors related to receiving laboratory results.

The majority of the responders reported that the fear of blood or the fear of blood collection does not prevent them from having their laboratory tests done, as presented in Figure 4.

With regard to the entity involved in laboratory investigations, the majority of the responders preferred private laboratories to have their tests done, as shown in Figure 5.

Access, availability, barriers and knowledge related to laboratory testing

In the group of responders who were employed, 48% mentioned that their employer does not provide access to routine laboratory testing, 47% that they have access to routine laboratory testing once a year or more often, and 4% that they have access to routine laboratory testing from their employer every few years.

The responders considered costs and lack of time as the main factors that prevent them from having their routine laboratory testing done, as presented in Figure 6.

Figure 3: Respondents' attitudes toward normal (a) and abnormal (b) laboratory results

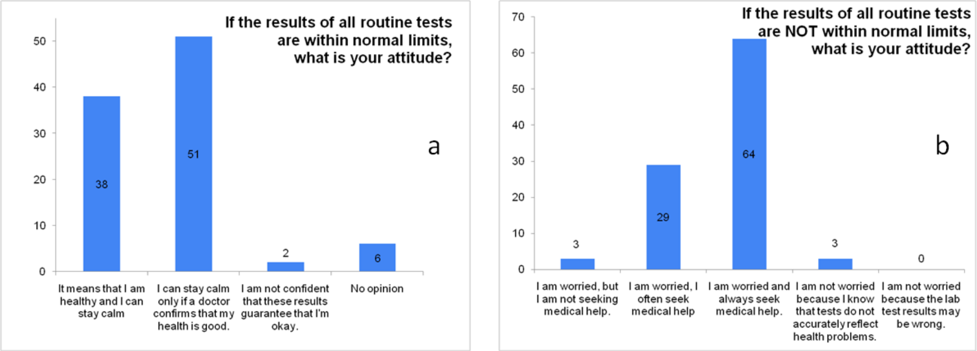

Table 4: Behaviors related to undergoing laboratory investigations, compliance with physicians` indications and dealing with lab test results – statements and percentages of respondents in agreement

Do you do laboratory tests on your own initiative or on the recommendation of a doctor?	
On the recommendation of a family doctor/specialist.	23%
On my own initiative.	24%
Both on the recommendation of a doctor and on my own initiative.	51%
I don't do laboratory tests.	2%
How often do you do your routine lab tests?	
Twice a year or more often	23%
Once a year	61%
Once every few years	6%
Only when I have a health problem	8%
Never	2%
When you do laboratory tests on your own initiative, what is the motivation? (multiple choice)	
I do not do my laboratory analyses on my own initiative.	14%
I want to make sure that my health remains good.	63%
I want to know if I have a health problem, even if I don't have symptoms.	52%
I have chronic illnesses or other conditions and for this reason, I want to have tests done.	13%
When you performed laboratory tests on the doctor's recommendation, for what purpose were you guided in this direction? (multiple choice)	
To establish a diagnosis.	46%
To follow up a disease.	24%
To perform routine tests without having a health problem.	69%
I did not do any tests recommended by a doctor.	6%
Do you always do your lab tests when your doctor recommends them?	
Always	74%
Often	21%

Rarely	3%
Never	2%
When you don't do lab tests following your doctor's recommendation, what is the motivation? (multiple choice)	
No medical tests were recommended to me.	26%
I was healthy and it was just routine tests.	30%
I did not have enough time.	19%
I did not have access to a collection point/medical analysis laboratory.	7%
I didn't see the usefulness of the analyses in that context.	6%
The costs were too high.	17%
What would you do if your tests are outside normal limits, and the doctor tells you that everything is fine with your health status?	
I turn to another doctor.	35%
I inquire into other sources and make my own decisions for diagnosis and treatment.	7%
I continue the investigations and repeat the tests.	40%
I trust the doctor and do nothing extra.	17%
What is your main source of information regarding laboratory tests? (multiple choice)	
My physician	89%
Acquaintances who work in the medical field	33%
Healthcare related websites	13%
Family, friends and acquaintances who do not work in the medical field	11%
I did not request/seek information about the analyses	3%
Books, magazines and specialized articles	17%
Websites of laboratories and clinics	28%
Social media groups	1%

Figure 4: The impact of the fear of blood or blood collection on having laboratory testing done

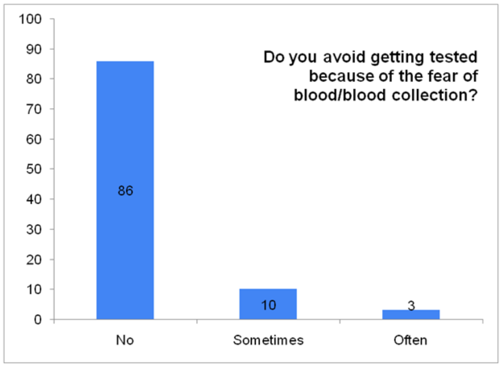

The knowledge and opinions about the laboratory investigations reimbursed by the public healthcare insurance system are presented in Table 5.

The majority of the responders have a sample collection facility or a medical laboratory within a reasonable walking distance from their home and reported that the distance from it does not influence their decision to have their routine tests done (Figure 7).

DISCUSSION

Overall, respondents were optimistic about their health status, with most rating it as good or very good. Nevertheless, the majority also reported being concerned or very concerned about their health. This particular perception may influence their attitudes and behaviors related to laboratory investigations. For example, health anxiety has a major impact on healthcare use [6].

Despite expressing concern about their health, fewer than one-third of respondents reported seeking preventive medical care. Approximately 40% reported adopting a healthy lifestyle, and less than half indicated that they consult a physician promptly upon noticing symptoms. More than 60% agreed that laboratory testing could be used more extensively for prevention, and over 30% believed that laboratory tests could be better utilized for early disease detection. However, half of the respondents considered that they already undergo laboratory tests frequently, while only a quarter reported getting tested when symptoms occur.

In general, respondents demonstrated awareness of the importance of laboratory testing for disease diagnosis (including early detection) and monitoring. They also acknowledged that the variety of available laboratory tests serves different purposes in healthcare. While less than one-third expressed very high confidence in laboratory test results, more than 60% reported having confidence in them overall. Additionally, nearly 80% of respondents stated that they trust laboratory investigations are handled confidentially.

Figure 5: Preferences for public and private laboratories

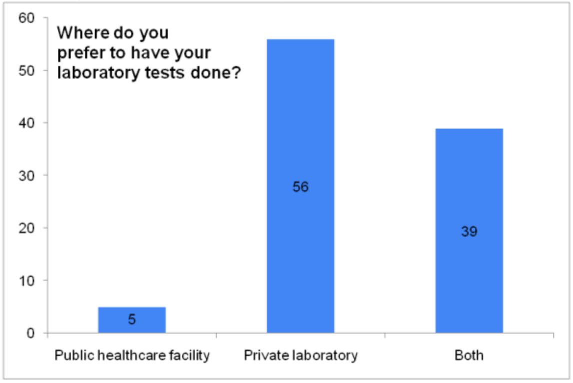

Figure 6. Main barriers to routine laboratory testing

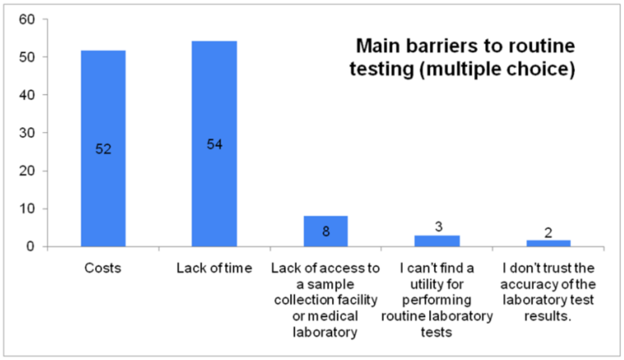

Table 5: Opinions and knowledge of the responders about the reimbursement of the costs for laboratory investigations – statements and percentages of respondents in agreement

Are laboratory tests reimbursed by the public healthcare insurance system?	
I don't know this information.	30%
Routine tests are reimbursed once a year.	26%
Laboratory tests are reimbursed, regardless of whether they are routine or not, once a year.	4%
Routine tests are reimbursed whenever a referral from the family doctor is used.	33%
Lab tests are reimbursed, regardless of whether they are routine or not, whenever needed.	3%
Lab tests are reimbursed only for those with an established diagnosis.	2%
Lab tests are reimbursed only for hospitalized persons.	1%
Have you ever benefited from laboratory tests reimbursed by the public healthcare insurance system?	
Rarely	15%
Every time/almost every time	21%
Often	36%
I do not know	10%
No	17%

Patients often perceive laboratory tests as inherently accurate and confidential, yet studies suggest gaps in their understanding of these concepts. Sankar et al. highlighted that misconceptions about confidentiality can undermine trust, with some patients overestimating privacy safeguards [7]. Educating patients about the accuracy limits of tests and existing privacy protections may strengthen their engagement and promote informed participation in healthcare decisions [8].

With regard to healthcare received from physicians and the medical system, most respondents considered that, in general, they are adequately recommended for laboratory testing. Overall, routine laboratory testing appears to be driven equally by the respondents' own initiative and by physicians' recommendations. Patients who undergo laboratory testing on their own initiative are motivated by various factors, including the desire to confirm their perceived good health status or personal experiences—such as witnessing family members or acquaintances being diagnosed or saved through timely laboratory testing [3]. The growing availability of direct-to-consumer testing has enabled patients to access laboratory analysis without a physician's mediation, but some studies highlight potential risks, including misinterpretation of results and unnecessary anxiety [9]. According to the results of Shaked et. al, in the case of patient-requested laboratory investigation, even after the doctor explained why the tests were unnecessary, the majority of patients expected to receive a referral for laboratory testing, likely due to the perception that blood tests are objective and reliable tools for validating one's health status [3].

Figure 7: The availability of sample collection facilities or medical laboratory close to the residence of respondents (a) and its impact on decisions regarding laboratory testing (b)

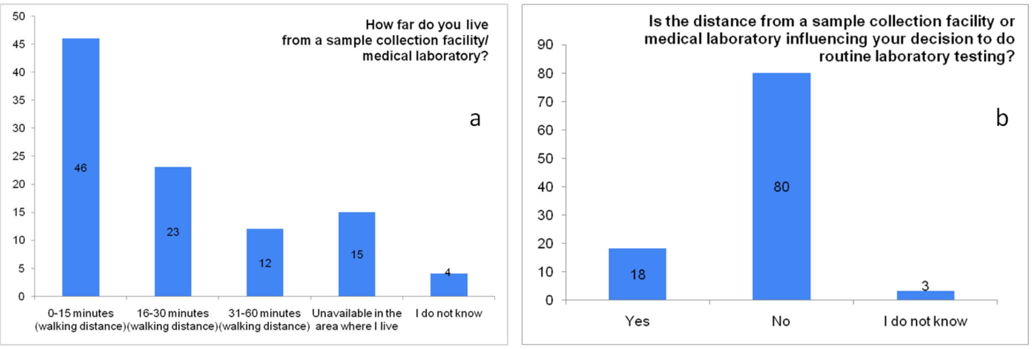

The main reasons for undergoing laboratory tests without a medical recommendation are to ensure good health and to detect early or subclinical diseases. Balancing preventive strategies with patient education about the limitations and risks of indiscriminate testing is essential to avoid fostering a culture of overmedicalization [10]. Approximately 90% of the respondents of this study acknowledged the importance of physicians in explaining laboratory test results. Similarly, nearly 90% believed it would be beneficial to receive explanations of their results in a formal setting each time they undergo laboratory investigations.

Around half of the participants reported that, when laboratory results fall within the biological reference interval, they feel reassured only after receiving confirmation from a physician that their health status is adequate. When routine test results fall outside the reference interval, more than 60% of respondents stated that they always seek medical help, while nearly 30% reported doing so often. According to a study performed by Marloes et al., patients overestimate the performance of blood testing, and results within normal limits were automatically seen as proof of an optimal health status. However, this study included only patients who voluntarily presented to the general practitioner for blood tests [11].

Furthermore, the vast majority of respondents (approximately 90%) identified physicians as their primary source of information regarding laboratory tests. Random online, non-professional sources were not commonly used. Instead, acquaintances working in healthcare and official websites of medical institutions were reported as the second and third most frequently cited sources of information, respectively. On the other hand, a survey by Cocco et al. revealed that an important part of respondents searched for explanations of lab results online before consulting a clinician, sometimes resulting in inappropriate self-diagnosis [12]. McMullan et al. found that patients using online resources for interpreting laboratory results often encounter conflicting or inaccurate information, leading to confusion and heightened anxiety [13].

When routine testing is recommended by a physician, the majority of respondents comply with the recommendation and undergo testing. Among those who do not comply, the main reasons reported are the perception of being in good health due to the routine nature of the check-up, lack of time, and associated costs. Adherence to recommended laboratory testing is essential for effective clinical management, but can be suboptimal. In patients with diabetes, a large registry study found that approximately 1 in 7 did not complete essential lab tests (such as HbA_1c or creatinine) within six months of ordering. Key predictors of better adherence included older age, female sex, and orders placed during face-to-face visits [14].

The majority of respondents considered that routine laboratory testing should be performed once or twice per year. Over 60% reported undergoing routine testing once annually, while more than 20% reported doing so twice a year or more frequently. The optimal frequency for routine blood testing depends on individual health status and clinical context. For healthy adults, many guidelines recommend annual basic panels during preventive visits, but more frequent testing is justified in chronic conditions or medication monitoring [15].

Although fear of blood or blood withdrawal is generally common [16], only 4% of respondents in this study described the sample collection procedure as uncomfortable or unpleasant. Additionally, nearly 90% reported that such fears do not influence their decision to undergo laboratory testing.

From the respondents' perspective, reducing waiting times for sample collection or results, as well as receiving more information and explanations about laboratory investigations and their outcomes, were not considered priorities for improvement. The primary issue identified was the cost of laboratory investigations and their coverage by health insurance.

Cost represents a significant concern, especially given that the majority of respondents reported preferring to have their laboratory tests performed in private laboratories rather than in public healthcare facilities. Additionally, lack of time and financial constraints were reported as the main barriers to undergoing laboratory testing.

Due to the widespread availability of private laboratories in Romania and access to their services without the need for a physician's referral, two specific aspects emerge: firstly, the healthcare system lacks control over potential overuse of testing, and secondly, the costs are often entirely supported by the patient. This situation may differ in other countries, where the overuse of laboratory investigations is potentially driven by the extensive coverage provided by public or private health insurance. When testing is widely accessible and patients and the healthcare provider are not directly burdened by the costs, the likelihood of overuse may increase [17].

Respondents appeared to have limited knowledge regarding the coverage and conditions for laboratory investigations under the public health insurance system, and few reported utilizing reimbursed laboratory tests.

Overall, respondents expressed the view that there should be increased public education regarding the importance and benefits of laboratory testing. Patients often struggle to understand the need for certain laboratory tests and their results, highlighting the importance of public education in laboratory medicine [18].

Overall, the responses to questions regarding laboratory medicine suggest that the general public in Romania may have high expectations for routine testing. Currently, there are no regulations in Romania that restrict access to direct-to-consumer testing. While direct-to-consumer access to routine laboratory investigations is generally permitted across countries, some nations have imposed restrictions on direct access to genetic testing [19]. Although most genetic conditions are rare, for example, familial hypercholesterolemia is a relatively common genetic disorder [20]. In the absence of regulatory limitations, such conditions may be perceived by the general public as suitable for direct-to-consumer testing - particularly given the high prevalence and awareness of hypercholesterolemia. However, this raises the risk of misinterpretation, since direct-to-consumer tests for this condition often lack optimal performance for accurate detection [21].

The current study has several limitations. First, the data collection instrument was not a validated questionnaire, which limits the generalizability of the findings. Additionally, respondents were self-selected, resulting in a sample that is not representative of the general population. Moreover, the sample did not uniformly or comprehensively cover all demographic backgrounds of Romanian citizens, with most respondents holding higher education degrees and residing in urban areas. Finally, all data were self-reported, relying on respondents' subjective perceptions and, in some cases, their memory and ability to estimate the frequency of events (e.g., how often they underwent routine laboratory testing).

However, it is essential to conduct this type of exploratory research, which is less quantitative in nature, to identify key areas for further investigation. This study provided a rapid assessment of various aspects related to patients and laboratory testing, offering a foundation for more detailed future research.

Future studies should build on the exploratory findings of this research by employing validated questionnaires and representative samples to enhance the generalizability of results. Further investigation is needed to understand the multifaceted factors influencing patients' adherence to physician recommendations for laboratory testing, including psychosocial, economic, and cultural determinants. Additionally, evaluating the impact of public education campaigns on patients' knowledge, trust, and behavior regarding laboratory investigations could provide valuable insights. Qualitative research methods, such as in-depth interviews and focus groups with both patients and healthcare providers, would offer a deeper understanding of the perceptions, fears, and informational needs surrounding laboratory testing. It is also important to explore disparities in access to laboratory services across urban and rural settings and among different socio-economic groups. Finally, examining the role of cost and health insurance coverage in shaping patients' decisions to undergo laboratory testing would contribute to the development of targeted interventions to reduce barriers and improve utilization.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

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Authors' contribution

Conceptualization, M.D., T.C., I.A.B and E.P.; methodology, M.D., T.C., I.A.B. and E.P.; investigation, M.D., T.C., I.A.B., I.I., R.M.U., G.T.L., R.R., A.S., M.M.C., E.P.; data curation, M.D., I.A.B., E.P.; writing—original draft preparation, M.D., T.C., I.A.B., I.I., R.M.U., G.T.L., R.R., A.S., D.O.C., E.P.; writing—review and editing M.D., T.C., I.A.B., I.I., R.M.U., G.T.L., R.R., A.S., M.M.C., E.P.; supervision, M.D., T.C., E.P. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

This study was conducted using an online questionnaire that was publicly available and open to the general population. Participation was voluntary, self-selected, and anonymous. No personal or identifiable data were collected, and no recruitment of patients was involved. Information about the purpose and nature of the study was provided at the beginning of the form. Completion and submission of the form were considered as an indication of informed consent to participate in the study. Considering all these aspects, the study was conducted in compliance with ethical guidelines.

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REVIEW

Oxidative Stress and Nutritional Antioxidants in Neurological Diseases

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Abstract: A significant number of neurological diseases are pathogenetically related to oxidative stress, including but not limited to cerebrovascular afflictions such as ischemic and hemorrhagic stroke, Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, and so on. Natural nutrients may help limit the impact of oxidative stress and, therefore, delay or prevent the impairment of these diseases. Although these natural components have not entered routine use, many have been studied in preclinical or even clinical settings with promising results. Therefore, the need to find the stage of the research in the field of validating the properties and clinical usefulness of such nutrients represents the main reason this narrative review was conducted. This analysis explored the PubMed database for papers related to the influence of natural nutrients on the onset and evolution of some of the most severe neurological disorders. The results of the review provide an overview of the pathways oxidative stress may undertake and how the use of nutrients may counteract these pathways. In conclusion, natural nutrients may have beneficial effects that can be impactful on clinical outcomes, but more good quality research in this field is needed before formulating any clear recommendation.

Keywords: oxidative stress, neurodegeneration, antioxidants, nutrients

INTRODUCTION

This narrative review aims to provide readers with a brief, yet pinpointed overview as to the therapeutic potential of nutritional antioxidants. Given that oxidative stress has a key involvement in the pathogenesis of many neurological diseases (neurodegenerative, cerebrovascular, or inflammatory), targeting oxidative stress through such components (known to medicine for hundreds, if not thousands of years) may prove efficient. Moreover, this paper should be viewed as an incentive to inspire future research, preclinical and particularly clinical, in this field, so that the usage of such components may one day become a validated treatment with a probable wide spectrum. A step-by-step approach to nutrient intervention in neurological diseases is provided in Figure 1.

MATERIALS AND METHODS

Comprehensive research has been conducted by consulting the PubMed database for relevant reports published between the inception of the archive and January 2025.

The following keywords have been used by themselves or in various combinations to achieve optimal results: "Parkinson's disease", "amyotrophic lateral sclerosis", "Alzheimer's disease", "multiple sclerosis", "small vessel disease", "ischemic stroke", "hemorrhagic stroke", "inflammation", "oxidative stress", "reactive oxygen species", "mitochondrial dysfunction", "apoptosis", "necrosis", "excitotoxicity", "lipid peroxidation", "vitamin C", "vitamin E", "coenzyme Q10", "black tea", "green tea", "taurodeoxycholic acid", "flavonoids", "curcumin", "polyphenol", "antioxidant", "Ginkgo biloba", "mecasin", "citicoline", "Artemisia", "Silybum", "curcumin", "melatonin", "saffron", "vitamin D3", "Gastrodia elata", "lavender oil".

Case reports, preprints, and standalone abstracts have been excluded. Only papers published in English were included in the selection.

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Figure 1: Addressing neurological diseases through nutrient intervention

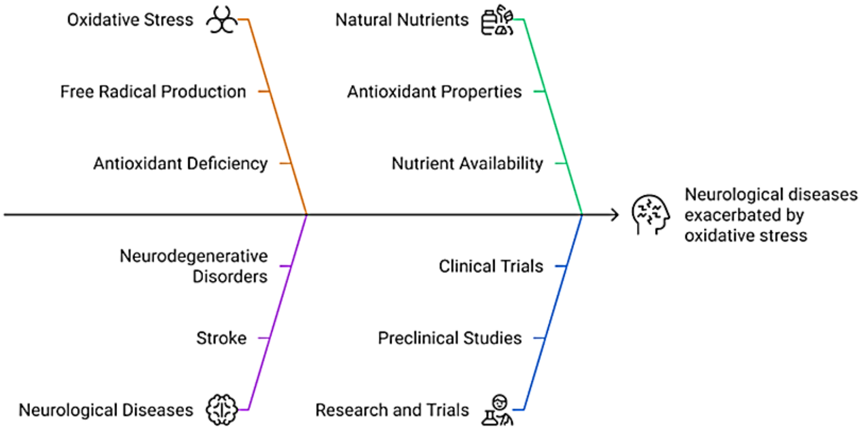

Oxidative stress is a central component in the pathophysiology of a plethora of neurological diseases. Nutrients may be an appealing method to target oxidative stress and, therefore, mitigate or perhaps even prevent these afflictions. However, the impact of nutrients has to be appropriately measured through validated, reproducible clinical trials before they can broadly enter therapeutic practice.
This figure has been generated using Napkin AI.

RESULTS AND DISCUSSIONS

Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative disease with a characteristic loss of the dopaminergic neurons in the pars compacta of the substantia nigra [1]. Literature has shown that environmental factors (herbicides, pesticides (such as paraquat), industrial pollutants, neurotoxins (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or MPTP) and its metabolite (1-methyl-4-phenylpyridinium), as well as genetic factors (mutations of DJ-1, Parkin, PINK1) [2] involved in PD pathogenesis induce oxidative stress (OxSt) in the mitochondria [3], reduce the production of adenosine triphosphate (ATP), facilitate the generation of reactive oxygen species (ROS), and finally lead to the apoptosis of dopaminergic cells [2].

Pathogenesis

1 Oxidative stress and mitochondrial dysfunction

OxSt contributes to the degeneration of dopaminergic cells in PD [4]. Also, OxSt is tightly linked to other pathogenic mechanisms involved in PD (mitochondrial dysfunction, excitotoxicity, and inflammation); therefore, it is difficult to ascertain whether it is a primary factor in PD or a consequence of the aforementioned mechanisms [3].

In PD, lipid peroxidation is upregulated, and antioxidant pathways (superoxide dismutase – SOD, catalase, glutathione peroxidase – GPx, ceruloplasmin, vitamin E, vitamin C, copper, zinc, selenium) may be insufficient to protect the cells from pathological oxidation [4]. Increased OxSt in association with diminished antioxidant protectors leads to the loss of neurons in the pars compacta, hence the development of PD [4]. Studies have proven lower levels of vitamin C and E in PD patients versus control groups, launching the hypothesis of increased consumption of these vitamins in PD due to OxSt [4].

Excessive ROS production, mitochondrial transport changes, and increased iron deposition in the pars compacta of the substantia nigra are key pathways in PD pathogenesis [1]. There is a close link between ROS formation and mitochondrial dysfunction [1]. Cellular death in PD is thought to be connected to excessive lipid peroxidation, occurring as a consequence of increased oxidation and expenditure of compensatory mechanisms [1]. ROS have a direct cytotoxic impact, as well as an indirect one by impacting mitochondrial complex I, with higher lipid peroxidation [1]. The reduction of this complex leads to the production of more ROS [1].

2 Neuroinflammation

In PD, α-synuclein deposition induces an activation of the microglia in the pars compacta, thus activating nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase) [1]. The latter's consequences are, on the one hand, ROS production in the extracellular compartment afflicting nearby neurons, and on the other hand, redox signaling in the microglia, amplifying the pro-inflammatory response with the induction of chronic inflammation [6].

The presence of excessive OxSt in PD is supported by studies that have proven an increase in carbonyl derivatives [7], in indicators of lipid peroxidation [8], and 8-hydroxy-2'-deoxyguanosine (a marker of nucleic acid damage) [9].

Nutrients

Given the involvement of OxSt in the occurrence of PD and the fact that PD treatment (especially L-dopa) may determine, after a long period of usage, the appearance of free radicals, the administration of multiple antioxidant agents in high dosage along with L-dopa may increase the latter's efficiency [10,11].

In experimental studies, vitamin E was proven to protect cells from OxSt-related damage in PD and the chronic administration of high doses of vitamin E may be a therapeutic strategy in PD treatment or prevention [3,12]. In other experimental studies, vitamin E diminished the OxSt induced by midbrain iron deposits in PD patients, suggesting it may have neuroprotective effects in these patients [13].

Other observational studies on human subjects suggested that a combined administration of high doses of vitamins E and C may determine a slowed PD progression through their antioxidant effect (14), although their efficacy was not proven in double-blind randomized clinical trials (RCTs) [15].

Taurodeoxycholic acid is an antioxidant agent, decreasing ROS reduction, an effect that has been proven when in vivo PD lab models were used [16].

Coenzyme Q10 has an important antioxidant role in the mitochondria and the lipid membrane, some of its antioxidant effects being mediated by its interaction with α-tocopherol. A phase 2 clinical trial on the efficiency of the antioxidant effect of the coenzyme on early PD patients (i.e., patients who did not require specific therapy) versus placebo showed a decrease in the treated patients' disability [17].

Black and green tea, due to high polyphenol concentration, exert an antioxidant effect and may have a neuroprotective and neuroregenerative effect in this disease, although clear evidence to support this assertion, derived from clinical research, is scarce [18].

Flavonoids enhance the endogenous activation of antioxidant enzymes, inhibit the peroxidation of lipids, and reduce the production of inflammatory mediators [19]. Due to all these actions, flavonoids exert a favorable effect on dopaminergic neurons and mitigate PD manifestations in laboratory studies [19].

An association between PD and a lack of adherence to the Mediterranean diet suggests a potential neuroprotective effect of this diet, through an undetermined mechanism that may attenuate the OxSt and inflammation, which are both recognized pathogenetic factors in PD [20].

Alzheimer's disease and small vessel disease

Alzheimer's disease (AD) is a neurodegenerative affliction characterized by the dysfunction of memory, attention, and other cognitive processes; it is the most common form of dementia [21]. Small vessel disease (SVD) may affect microvasculature systemically or mainly in the brain, the latter constituting a devastating cause of stroke and dementia worldwide [22,23].

Pathogenesis

AD mainly determines a synapse affliction, with the development of senile plaques mainly containing β-amyloid (Aβ). Aβ derives from the proteolysis of the amyloid precursor protein performed mainly by the enzymatic actions of β- and γ-secretase, with an imbalance between production and clearance causing the Aβ buildup [21]. The lesions produced by OxSt in AD are associated with an accumulation of Aβ [11]. Aβ buildup is also a central feature of the cerebral amyloid angiopathy form of SVD, to which AD is intimately connected; some might argue that the two constitute an inseparable spectrum [22]. The other form of SVD, deep perforator arteriolosclerosis, centrally features a blood-brain barrier (BBB) dysfunction, subsequent morphological and functional changes to the vascular wall, as well as myelin impairment [22,23].

Aβ determines the release of ROS by attacking mitochondrial enzymes (particularly cytochrome c oxidase), leading to mitochondrial dysfunction, increasing superoxide radicals, conversion to hydrogen peroxide (H2O2), the release of cytochrome, affecting the production of ATP and cellular apoptosis [21].

OxSt increases with aging and it is an important causal factor in the appearance of AD and other neurodegenerative age-related diseases, such as SVD. Moreover, OxSt is thought to bridge the connection between SVD and AD [24]. It is not yet fully understood how neurochemical factors influence the appearance of age-related cerebral lesions, but more and more data has shown the involvement of biometals (Cu, Fe, Zn) in Aβ accumulation. Copper is a potent mediator for the hydroxyl radical, and the high-affinity link between copper and Aβ (via histidine and tyrosine), as well as between zinc and the amyloid precursor protein and Aβ may contribute to the increased OxSt in AD [11].

Apolipoprotein E is a lipid transport molecule believed to be related to ROS and directly to lipid peroxidation in AD [25]. Carriers of apolipoprotein E4 more often have cerebral amyloid angiopathy [24].

To conclude, the involvement of OxSt in AD pathogenesis is supported by the high concentrations of Cu, Fe, Al, and Hg in the brain parenchyma, a high level of lipid peroxidation and DNA oxidation, as well as a decrease in polyunsaturated fatty acids, in cytochrome c oxidase activity, and energy metabolism [11]. OxSt could become a promising link between AD and SVD; therefore, research focusing on OxSt may decrease the neurological manifestations of both illnesses [24].

Nutrients

In preclinical research, caffeine has been associated with a tendency to lower the Aβ levels in early (familial) AD, because of its antioxidant properties [26]. Studies on cerebral amyloid angiopathy models are lacking.

Curcumin is an antioxidant and anti-inflammatory agent (inhibiting cyclooxygenase and lipoxygenase) that has been shown to reduce carbonyl derivatives, facilitating Aβ disaggregation, with possible beneficial effects in AD in animal studies [26]. Curcumin may also aid endothelial function, which is frequently impaired in SVD, as it has been shown to diminish the risk of stroke in animal models [27].

Vitamin E is a potent antioxidant that prevents oxidizing lesions induced by Aβ in cell cultures; it also delays memory impairment in animal studies on AD [28]. Some RCTs suggested vitamin E, through its antioxidant effects, may delay functional disability in patients with mild to moderate AD [29]. Vitamin E tocotrienols have demonstrated a positive impact on white matter lesions (associated with SVD) in an RCT [30].

Polyphenols (such as anthocyanins from berries, catechins and flavonoids from tea, curcumin from turmeric, and resveratrol from grapes) have an antioxidant effect with possibly associated neuroprotection, observed in preclinical models of AD [26]. Dizziness improved in SVD patients taking polyphenols [31].

The association between AD and a lack of adherence to the Mediterranean diet indicates that the latter may have a neuroprotective effect through an as-of-yet incompletely understood mechanism, potentially including an influence on OxSt and inflammation, both involved in AD pathogenesis [32]. Compliance with a Mediterranean diet lowered the burden of white matter lesions in SVD patients [33].

Due to their antioxidant properties, alkaloids such as groenlandicine, berberine, and palmatine from Coptis chinensis rhizomes may find their use in AD treatment [26]. Berberine promotes endothelial health and has been proven to support proper arterial elasticity in human subjects [34].

Milk thistle (Silybum marianum) diminishes OxSt and memory decline in laboratory studies of AD [35]. Milk thistle extracts also favored proper endothelial function in mice [36].

Ginkgo biloba, through its capacity to purge ROS, may reduce Aβ aggregation in AD, which is suggested by certain in vitro studies [26], but on the other hand, this theory has been contested in the literature [37]. Currently, there is little proof to support the routine use of Ginkgo biloba in AD patients [26]. Ginkgo extract had a beneficial yet limited effect on cognitively impaired mice models of SVD [38].

Melatonin overcomes apoptosis, prevents Aβ from interfering with mitochondrial DNA, diminishes lipid peroxidation, and, in high concentrations, protects from the OxSt induced by Aβ [39]. However, more studies are needed to prove its efficiency in delaying progression in early AD stages or in AD prevention [39]. Melatonin was shown to be a potential BBB integrity protector in cell cultures [40].

Coenzyme Q10 is an antioxidant factor that maintains mitochondrial membrane potential during OxSt, while also protecting neural cells from excessive Aβ deposition in laboratory studies of AD [26]. It also eliminates peroxyl radicals and may prove efficacious in AD [17].

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease defined by a loss of central and peripheral motor neurons [41].

Pathogenesis

The pathophysiology of ALS onset is quite complex, probably heterogeneous, and not fully understood [42]. OxSt is a common link between various pathogenetic mechanisms of ALS, contributing to: (a) mitochondrial dysfunction: high ROS levels may augment mutations in mitochondrial DNA, and mutations of the Cu-Zn-SOD1 mitochondrial gene are synchronous with increased OxSt;(b) protein aggregation: OxSt is a contributor to the mechanisms of abnormal aggregation of SOD1; (c) axonal transport: certain fragments of axonal neurofilaments are targeted by OxSt [43].

Environmental or modifiable risk factors may be the source of OxSt, incriminated in ALS pathogenesis: (a) smoking induces lipid peroxidation, DNA damage, and cellular death, smokers having a higher risk of ALS (sporadic form); (b) chemicals used in agriculture (pesticides, organophosphates) are linked to ALS and induce mitochondrial dysfunction and ROS production [44].

It is not clear whether OxSt is the main cause of ALS or if it is a consequence of all the other pathogenetic mechanisms involved, but the existence of SOD1 gene mutation among familial cases suggests OxSt is central to the pathophysiology of genetic forms of ALS, at the very least [43].

OxSt could also interfere with ALS pathophysiology by deteriorating the mitochondria that support presynaptic transportation and, hence, affecting presynaptic mechanisms involved in neurotransmission [45]. ROS and reactive nitrogen species (RNS) alter RNA processing, generating mitochondrial dysfunction that eventually leads to neuronal death [46].

In ALS, indicators of OxSt are increased, such as carbonyl derivatives (in sporadic forms), 3-nitrotyrosine (a marker of oxidative lesions, with a higher expression in sporadic forms as well as familial forms associated with SOD1 mutation), 8-hydroxy-2'-deoxyguanosine (a sign of damaged DNA), 4-hydroxynonenal (showing lipid peroxidation) [43].

Nutrients

Laboratory studies have proven that mecasin (also known as KCHO-1, a herbal extract) diminishes OxSt via the gp91phox and MAPK pathways in ALS patients. Mecasin improved motor function and delayed ALS onset in study models [47].

EGb761, a standardized Gingko biloba extract with antioxidant and neuroprotective effects, could prove useful in treating ALS patients [48].

To prevent OxSt from interfering in ALS pathogenesis, various antioxidant agents have been tested, such as vitamin E and vitamin B complex; however, there were no significant proven benefits [43].

Multiple sclerosis

Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system [49,50], defined by perivenous lymphocytic infiltration and macrophage presence in the cerebral parenchyma [51].

Pathogenesis

MS neuropathology is characterized by focal demyelinating lesions and axonal lesions. Neurodegeneration is induced by inflammation and mitochondrial dysfunction, with ROS production as a common element. ROS promotes transendothelial migration of leukocytes, contributing to axon and oligodendrocyte degeneration [52].

ROS occurs mainly by the activation of macrophages, microglia, and astrocytes and are produced through NADPH-oxidase, myeloperoxidase, and inducible NO-synthase. ROS leads to neuron, axon, myelin, and oligodendrocyte destruction and may contribute to mitochondrial dysfunction, which in turn exacerbates ROS production, augmenting MS lesion appearance [52].

Through lipid, protein, and DNA oxidation, OxSt leads to cellular dysfunction and, finally, to necrosis or apoptosis [53]. In the early stages of MS, OxSt is associated with microglial activation and the inflammatory process, whereas in advanced stages, it is linked to mitochondrial dysfunction, pathological iron accumulation in cerebral structures and its release in demyelinating lesions [54]. Myelin and oligodendrocytes are the most damaged by OxSt in this disease, a phenomenon that has been supported by the cytoplasmic accumulation of oxidated lipids and nuclear DNA alterations [54].

Nutrients

Melatonin presents neuroprotective effects and may decrease the OxSt in individuals with secondary progressive MS [55] by ROS neutralization and induction of antioxidant enzymes [49].

Coenzyme Q10 potentially diminishes OxSt and stimulates the activity of antioxidant enzymes in relapsing-remitting MS [56].

Saffron extract attenuated OxSt in experimental studies on MS [57].

Vitamin D3 has antioxidant properties and has been shown to halt lipid peroxidation in MS literature [58].

Ischemic stroke and intracerebral hemorrhage

Ischemic stroke (IS) is the most widespread type of cerebrovascular affliction and a relevant cause of death and disability worldwide. Intracerebral hemorrhage (ICH) is about 30% of all non-traumatic strokes worldwide, with a higher disability rate compared to IS [59]. The major cause of ICH is small vessel disease, a complex microvascular affliction of the brain [23].

Pathogenesis

Normally, the BBB protects against neurotoxins. After ischemia occurs, various mediators of inflammation, as well as other potentially neurotoxic molecules, cross the altered BBB to the brain parenchyma, contributing to neuronal lesions [60]. A breakdown of the BBB also occurs in ICH, as well as an enhanced proinflammatory response with a release of ROS [61,62].

OxSt is an important factor involved in the pathophysiology of IS [63]. Immediately after a cerebral injury is produced in IS as a result of vascular obstruction, the rapid increase of ROS determines the extension of the ischemic area [64]. OxSt also occurs in ICH and may surprisingly have a disseminated impact, such as the induction of glomerular dysfunction [65].

The brain is particularly sensitive to oxidative lesions. The main sources of ROS in the brain are the mitochondrial respiratory chain, NADPH-oxidase (especially isoforms 2,3,4 that are notably expressed in the central nervous system) and xanthine oxidase (XO). Superoxide (O2−), physiologically produced by the mitochondria, is converted via SOD to H2O2 and acts as a cellular messenger, with a role in neuronal signaling in the central and peripheral nervous system. Oxygen depletion in ischemic neurons favors anaerobic glycolysis, with higher lactate levels and acidosis. The latter contributes to OxSt, providing H+ and hence facilitating O2− conversion to H2O2 [60].

After cerebrovascular flow is reestablished, the mitochondrial respiratory chain is reestablished with the generation of a new peak of ROS [63], contributing to cerebral reperfusion lesions [60]. O2− is involved in reperfusion lesions and NADPH-oxidase contributes to its production [60]. Mitochondrial malfunction has also been detected in ICH around the hematoma [66].

During cerebral ischemia, ATP is broken down to hypoxanthine, which accumulates in the ischemic cerebral parenchyma, and during the reperfusion phase, XO facilitates hypoxanthine oxidation to xanthine and subsequently to uric acid, a process during which O2− and H2O2 are generated [60].

An increase in excitotoxic amino acids such as glutamate in cerebral lesions as well as in the perihematoma region in ICH also favors the production of ROS [63,67].

Excessive ROS leads to lipid peroxidation and a deterioration of proteins and nucleic acids, hence the lesion area is extended and finally, necrosis and apoptosis occur [63]. Lipid peroxidation is the main pathway through which ROS impacts neurons: phospholipase A2 is activated by ROS and subsequently releases arachidonic acid, further enhancing the production of ROS. By impacting lipids, ROS produces aldehydes, dienals, and alkanes, thus provoking neuronal apoptosis. The process of DNA oxidation has an early onset in ischemia, and, although it is initially reversible, then an irreversible deterioration of DNA and cellular death appear due to all the ROS production pathways and a blockage of antioxidant mechanisms [60].

Nutrients

Vitamin C, playing the role of an oxygen donor, can revert oxidative processes [60]. Vitamin E is a lipid antioxidant capable of inhibiting lipid peroxidation and acting as a ROS scavenger [68]. In an experimental study, vitamin C and E administration diminished lipid peroxidation and IS volume [69], but their therapeutic effects were not proven in human observational studies [68,70,71]. Vitamin C plasma levels are reduced in ICH patients [72] and administering vitamin C and E in combination with ICH rat models had positive effects [73].

Coenzyme Q10 is a component of the mitochondrial transport chain, the latter being involved in ROS production [74]. Coenzyme Q10 builds up in the mitochondria, having a powerful antioxidant effect, proving its efficacy in neuronal protection in IS experimental models [75]. Coenzyme Q10 therapeutic strategies in ICH are also under investigation [76].

Citicoline is a natural component that may reduce the release of fatty acids during lipid peroxidation [74]. Citicoline has demonstrated protective effects in experimental IS studies [77], but these results were not obtained in IS patients [78]. Small double-blind trials on citicoline in ICH have shown an increase in muscular strength, however, this needs to be proven in larger studies [79].

Wormwood (Artemisia absinthium), a plant of the Asteraceae family, increases ROS purging and diminishes lipid peroxidation, managing to reduce IS volume in experimental literature [80]. Eupatilin, an Artemisia-derived flavone with antioxidant properties, had neuroprotective effects by reducing inflammation in the microglia in ICH cellular models [81].

Ginkgo biloba extract reduces lipid peroxidation and protects neurons against OxSt in experimental studies [82]. There is no convincing data to support its routine use in IS patients [83]. A systematic review on ginkgo biloba extract in ICH patients has recently shown promise for this therapy, but further validation is needed [84].

Gastrodia elata is a plant belonging to the Orchidaceae family with antioxidant activity, capable of inhibiting glutamate neurotoxicity [85]. In animal studies, it confers neuroprotection against cerebral lesions caused by ischemia and reperfusion [86].

In animal models, lavender oil proved to have antioxidant properties, reducing ROS levels in ischemic or reperfusion lesions [87].

Cannabinoids, mainly cannabidiol, have been associated with neuroprotective effects and the studies with rat cortical neuron cultures exposed to toxic levels of glutamate support a further investigation of these compounds in different pathologies, including ischemic stroke [88,89]. Also, cannabidiol is increasingly investigated in other disorders, including psychiatric illnesses, due to its anxiolytic, antidepressant, and antipsychotic properties, and the benefit of being administered as an adjuvant to current treatment, thus potentially decreasing the rate of treatment resistance [90,91].

CONCLUSION

To summarize, whereas it may play a variety of roles in the pathogenesis of various neurological diseases, oxidative stress is a worthy target to consider in future therapeutic strategies. Natural nutrients may have beneficial effects that can be impactful, pending more validation in the literature. Vitamin E appears as a versatile option, with potential neuroprotective effects in PD, a possible functional role in AD, and an antioxidant capacity in both ischemic and hemorrhagic stroke, although its clinical role requires further, solid proof. Coenzyme Q10 could also be useful to PD, AD, MS, and stroke patients, with a vast potential awaiting exploration. Ginkgo biloba might also hold promise in AD and stroke, but there is limited evidence to support its routine use, despite clinicians nowadays often adding it to therapeutic regimens. Pending improved technologies to refine and standardize natural extracts, as well as solid and validated methodology usage in RCTs, novel therapies may include nutrients in a plethora of neurological diseases.

GLOSSARY

Aβ = β-amyloid; AD = Alzheimer's disease; ALS = amyotrophic lateral sclerosis; ATP = adenosine triphosphate; BBB = blood-brain barrier; GPx = glutathione peroxidase; ICH = intracerebral hemorrhage; IS = ischemic stroke; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NADPH = nicotinamide adenine dinucleotide phosphate; OxSt = oxidative stress; PD = Parkinson's disease; RNS = reactive nitrogen species; ROS = reactive oxygen species; SOD = superoxide dismutase; SVD = small vessel disease; XO = xanthine oxidase.

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Authors' contribution

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ORIGINAL ARTICLES

The concept of biological warfare and real biological attacks

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Abstract: *In the current military-political context, with the Cold War having ended, we find ourselves in full anti-terror war, in which Romania is a direct participant, as a member of N.A.T.O. and the E.U., and the issue of biological warfare and bioterrorism is again highly topical but bearing other valences.*

There is information that there are still laboratories and plants specializing in the research and manufacture of biological weapons. Most of the results of such research are not intended to be published; however, a number of research guidelines that testify to the trends in the improvement of biological weapons and their means of use can be deduced from the data published by researchers from several research institutes.

We believe that the threats posed by bioterrorism are real and that it is mandatory to be prepared at any time to prevent, combat and liquidate the consequences of "bio-chem" attacks, respectively the management of the consequences.

Keywords: *biological attack, biological warfare, biological agents, international legislation, medical protection*

INTRODUCTION

In the current military-political context, with the Cold War having ended (1947-1990), we find ourselves in full anti-terror war (since 2001), in which Romania is a direct participant, as a member of NATO and the EU, and the issue of biological warfare and bioterrorism is again highly topical but bearing other valences.

DEFINITIONS

War is a short or lasting conflict (whether military or not, declared or not), between two or more groups, social categories or countries, aiming to achieve financial, ethnic, territorial, economic and political interests.

The biological warfare is the artificial spread by various means of pathogens that can cause serious infectious and contagious diseases as well as the spread of germ toxins that can be used by an aggressor as a means of fighting in order to reduce the troops' fighting force, by causing serious disease outbreaks or by killing people, animals and/or plants.

Weapons of mass destruction (WMD) are those weapons, which, used by the aggressor, cause extensive material damage (destruction of buildings, constructions, machinery, installations, means of transport, etc.) as well as a large number of victims among employees and unprotected animals.

Chemical, biological, radiological and nuclear defence (CBRN defence) represents the protection measures taken during times of war or terrorist attacks, in the event of a chemical, biological or nuclear attack.

The biological weapon is a system of unconventional weapons of mass destruction, whose ammunition carries biological agents and contaminates the enemy in order to cause disease outbreaks for the latter.

Biological agents are microorganisms and/or microbial,

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animal or plant toxins, used as specific ammunition for biological weapons or used by terrorists in "bio-chem" attacks.

Total war is a large-scale conflict in which policy makers mobilize all available resources to ensure the destruction of the rivals' ability to defend itself. During times of total war, there are no actual non-combatants, meaning that all people in a particular country - civilians and military alike – are considered targets.

The hybrid war represents undeclared wars by those participating states, where the military component is not explicitly assumed and is by no means singular (they are executed in concert as part of a flexible strategy with long-term goals).

THE BIOLOGICAL ATTACK

Under the conditions of modern warfare, the effects of using the biological weapon can be amplified by successive or concurrent use with other WMDs. In a concrete battlefield, the biological attack can occur with:

a) The enemy on the offensive – can use the biological weapon to weaken the opponent's defence power. The most likely objectives to be attacked with biological agents would be:
1. Concentration of troops, resistance points
2. Reserve and support units
3. Air and sea troops found at bridge ends, traffic routes clogs, operational reserves, etc.

In the tactical and operative space, pathogens of diseases of short and virulent incubation, but non-transmissible from human to human, will be used. Infected animals or insects will not be used, as they would be dangerous for own troops.

In the strategic space, the aggressor will spread biological agents that cause many types of diseases to disrupt the battlefield, cause panic and aggravate material insurance.

b) The enemy on defence – could use the biological weapon against opposing troop concentrations to reduce their force of attack. Can be attacked with biological agents, the units in the offensive, those in the second line or the back-ups, the foreign military bases, etc.

In the operative space, infected insects and animals may also be used to target troop concentrations; the passes, roads, and crossing points may be contaminated to hinder the pursuit.

Living organisms are the sole targets of these attacks, thus buildings (for e.g.) being left intact, these being able to be decontaminated and further used. The biological agent is massively released into the area by the enemy and can contaminate a large number of humans and/or animals (anthroposoonosis) turning them into secondary sources of infection by releasing in their turn pathogens in the external environment. Except for those destined exclusively for humans and for those common to both humans and animals, there are also those biological agents destined exclusively for the destruction of domestic animals and cultivated plants.

THE PROVISIONS OF INTERNATIONAL LAW

From the point of view of International Law, biological weapons are expressely prohibited because they fall under the mass destruction weapons category, and their effect cannot be limited in time and space, with medium-term effects not always predictable. Moreover, the military use of biological weapons is currently considered to be tactically and operationally inefficient, being tactically difficult and risky from a medical point of view, because an outbreak once released can get out of hand.

Exceptions on using WMD

An important issue in controlling the Convention's implementation is the existence of certain exceptions because the Convention cannot completely ban the use of living agents and their toxins. So far, no convention banning the use of a certain type of weapon against the enemy has explicitly prohibited its use on its own population.

First of all, biological agents and some toxins can be used in vaccine manufacturing, for diagnostic and treatment purposes. For example, biological agents may be used in the treatment of inflammatory bowel diseases such as Crohn's disease inflammatory form with moderate or severe activity that have not responded to conventional immune-suppressive therapy or where immunosuppressant therapy is contraindicated. They may also be used in patients with moderate/severe RCUH who have not responded to corticosteroid and immunosuppressive therapy or severe/ fulminant colitis. These patients are required to undergo treatment with drugs such as Infliximab or Adalimumab containing a chimeric anti-TNFα agent consisting of IgG1 monoclonal antibodies, 25% murine and 75% human, respectively a fully humanized anti-TNFα agent, IgG1 type.

At the same time, the emergence of vaccines has greatly revolutionized medical science since they carry an important prophylactic role.

The vaccine is a biological product containing suspensions (antigens) of attenuated, inactivated (killed) viruses or living bacteria, or fractions thereof, which are administered in order to induce a specific immune response. Depending on the type of vaccine, fragments of pathogen agents were used differently:

1. Viral vaccines – corpuscular vaccines with living attenuated viruses (oral polio vaccine, rotavirus vaccine, measles, rubella, varicella, amaryllis) or inactivated viruses (inactivated polio vaccine, rabies, Hep A) and antigenic fragments or viral subunits (Hep B, influenza vaccine)

2. Bacterial-corpuscular vaccines with living attenua-ted germs (BCG) or killed (germs), (cholera vaccine, pertussis), polysaccharide subunits (meningococcal, pneumococcal, Hib, typhoid vaccine) or purified (pertussis acelular)

3. Anatoxin – diphtheria, tetanus

Thus, the BTWC allows all signatory countries to possess and use reasonably small (grams) quantities of BWA for defensive scientific research to obtain new means of diagnosis, prophylaxis and treatment. But a real problem is that these living biological agents can be multiplied on demand and in any quantity or can synthesize toxins as specific ammunition for biological weapons.

Secondly, one of the BTWC articles states that in case of force majeure, when a state itself is threatened, it may notify the UN three months before resuming the production of biological weapons to deter the enemy. This also explains why Israel, "an island in a sea of hostile Arabs", has not signed and ratified the convention.

And Romania, which joined since 1972, ratified it only in 1979, in order to have an extra advantage in the face of a possible assault of the USSR in the context after 1968. It should be underlined that in fact Romania has not researched, manufactured, stored, and has never used biological or toxin weapons, nor did it have such an intention, but it was indeed, the target of covert biological attacks in the 20th century and, we must continue to take preventive measures.

In this complex situation, effective legal measures for the application of the BTWC need to be implemented worldwide, but concrete actions are difficult to apply in practice. The UN is in a position, as an international forum, to impose and control compliance with the Convention, being helped by the Security Council, the World Health Organization as a specialized body.

STANAG are NATO standards for different areas that set technical or operational standards in certain situations, including in the field of biological warfare, and which need to be adapted and implemented by Allied countries for interoperability.

PREVENTION OF BIOTERRORISM ATTACKS

Preventing bioterrorist attacks is mainly a political and social problem, which is primarily achieved through the intervention of the secret services, police and the justice system. Combating bioterrorist attacks also involves the intervention of the National Health System, the National Defence System, etc. The specific capacities of the Medical Department of the Ministry of Defence are important for the medical protection against the weapons of mass destruction and especially for fighting against biological attacks, permanently having the forces and means necessary for the medical protection against the C.B.R.N agents.

The Center for Military Medicine Scientific Research (CCSMM) has a Laboratory of Anti-infective Medical Protection and Epidemiological Emergencies, which functions as a specialized medical protection unit against biological weapons. It conducts military medical scientific research to protect troops and the civilian population against biological weapons and/or toxins, for biological warfare events, bioterrorist attacks, or biological accidents targeting primarily: bacteria, viruses, fungi, etc. and their toxins, to establish medical protection procedures, through cooperation between different medical specialties: microbiology, epidemiology, etc.

A first stage subsequent to the occurrence of a biological attack is the identification of the causal agent, being performed solely in a microbiology laboratory, starting from correctly harvested samples (from the air, soil, water, pathological products from the sick, etc.). The identification operation must be quick and specific.

In the event of an immediate biological attack, the problem at hand is the immediate decontamination by appropriate physical and chemical means of the staff’s equipment, of the rooms, the water sources and the food, etc. The appropriate anti-epidemic measures to limit the spread of the infection are, along with the above, another important stage in the post-attack protocol, in which we will: isolate the sick and the suspects, take care of individual and collective hygiene, qarantine the D.D.D. actions and so on. If the agent has been identified, we can perform emergency immunoprophylaxis by using vaccines, by administering prophylactic or therapeutic antibiotics, etc.

The protection against biological attacks depends on the moment when the attack is detected, thus, when the media shall signal the imminence of an attack, the possibility to benefit from individual protection (ex-gas mask) and collective protective means (shelters) should be available. The biological means of protection and the emergency prophylaxis with appropriate substances (serums, vaccines, etc.) can be of good efficacy. Immunostimulators and chemo-therapeutic agents (wide-spectrum antimicrobials) can also be associated with these.

THE BIOLOGICAL WARFARE – A HISTORICAL REVIEW

There are very few data from the Prehistory and Antiquity that testify to the use of biological weapons during warfare. However, the latest research attests to the fact that the Hittites were the pioneers of bioterrorism. The Hittite people lived on a territory that today belongs mostly to Turkey. Three thousand years ago, they used infected sheep aiming at conquering enemy people. According to the study performed by the Italian microbiologist Siro Trevisanato, the bacterium considered the first weapon of mass destruction in history was Francisella Tularensis, responsible for tularemia, an infectious and contagious rodent disease, caused by a bacterium and transmitted to domestic animals and humans, manifesting itself by fever, chills, vomiting, etc. The disease still exists nowadays and, if not treated properly, can lead to death. Studying ancient papers, researchers have proven that tularemia first appeared in a Phoenician city (located on the border between Lebanon and Syria today) in the Middle East in the 14th century BC. The Hittites plundered this city in 1325, bringing with them infected animals, which may have spread the disease – also referred to as the "Hittite Plague" - throughout its territory, as explained by the researcher. Trevisanato points out that there are several papers attesting the epidemic. At the time of tularemia’s utter virulence, the Hittites came to the attention of a neighboring population of the city of Arzawa (Western Anatolia), who took advantage of their weakness to invade their territory.

During the Middle Age as a Mongol saw his comrades die of plague in the three years of the siege of Caffa's Black Sea city, founded by the Genovese, he had the brilliant idea of throwing corpses over the city walls. The Genoese left the city, but the plague spread across Europe.

In the Modern Age – the First World War brought a more sophisticated biological warfare, with the development of microbiology, many pathogens causing diseases could be identified that could then be cultivated in the laboratory. There are suspicions about the use of plague against Russian troops at St. Petersburg. Petersburg in 1915, about horses being infected in US ports for British and French armies.

In the Contemporary Age – extraordinary intelligence has been invested in the development and modernization of all categories of weapons. Each country classified biological programs at the highest level of secrecy; there is little evidence today about the details of the programs being carried out, with only little evidence on the existence of such programs in the current media. Specific anti-human, anti-animal and anti-plant programs have been developed, agents have been standardized for use as weapons, and insects have been studied for use as natural dispersion vectors.

PERSPECTIVES

The range of pathogens used as biological weapons is very wide, which gives rise to particular difficulties in the elaboration of the protection and treatment measures. Types of agents:

1. Bacteria: anthrax, plague, cholera etc;
2. Viruses: yellow fever, tick-borne encephalitis (TBE), smallpox, etc;
3. Rickettsia: Q fever, exanthema of typhus etc;
4. Pathogenic fungi: coccidioidoreo etiological agents, etc;
5. Toxins: bacterial (Botulinum, staphylococcal, etc.), fungal, of animals or plants (ricinotoxin).

These are only the pathogens found in nature. But new genetic techniques now allow us to obtain germs with new qualities, not naturally occurring (modified or hybridized) and which can be considered more dangerous by their pathogenic effect and increased resistance in the external environment.

Some agents have a lethal effect, while others have an incapacitating effect, leaving man out for a period of time ranging from a few hours to a few weeks.

They can be joined by immunosuppressive substances, which disrupt the mechanisms of the body's anti-infectious resistance, exposing it to current infections or to those deliberately disseminated.

In the case of a biological attack, it should be stressed that the possibility of using two or more biological agents simultaneously, whose effects combine or potentiate, or can be used with other weapons of mass destruction, is estimated.

We can also mention the existence of biological agents destined exclusively for destroying domestic animals and cultivated plants (e.g. avian influenza, barberry rust for plants) that can lead to large losses or can be used to destroy the economy of a competing country etc.

CONCLUSIONS

There is information that there are still laboratories and plants specializing in the research and manufacture of biological weapons. Most of the results of such research are not intended to be published; however, a number of research guidelines that testify to the trends in the improvement of biological weapons and their means of use can be deduced from the data published by researchers from several research institutes (e.g. the study of some of the most dangerous communicable diseases in humans and animals that currently have a very narrow spreading area). Another evidence of concern in the field of biological weapons is that the military personnel of some states are regularly exposed to prophylactic vaccines against some infectious diseases that are not circulating in the respective country.

We believe that the threats posed by bioterrorism are real and that it is mandatory to be prepared at any time to prevent, combat and liquidate the consequences of "bio-chem" attacks, respectively the management of the consequences.

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REVIEW

The Transformative Role of Artificial Intelligence in the Future of Radiology

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Abstract: Recent advances in technology and artificial intelligence have transformed radiology. Artificial intelligence, which uses machine learning, deep learning algorithms, and cellular neural networks, is now applicable to image analysis, enabling the analysis of large volumes of data and the early identification of lesion features, such as tumors. Integrating artificial intelligence into clinical workflows increases the efficiency of medical report generation and enables efficient prioritization of complex or urgent cases. Predictive analytics based on artificial intelligence uses extensive patient data, including demographics, medical history, tests, and imaging, to build more accurate predictive models. Among the areas where artificial intelligence is successfully implemented in radiology is the automated detection of lung nodules. In the field of neurology, artificial intelligence helps track progressive lesions through volumetric analysis of the brain and surveillance of demyelinating diseases. In oncologic radiology, artificial intelligence is used for automated image segmentation, lesion review, and standardized report generation. Therefore, in this ever-evolving landscape, radiologists must embrace emerging technological advances in order to occupy a unique position at the intersection of different diagnostic fields.

Keywords: artificial intelligence; radiology; magnetic resonance imaging; computed tomography

INTRODUCTION

The Current State of Radiology and Its Importance in Modern Medicine

Radiology has been closely linked to the degree of technological evolution and has experienced revolutionary developments in recent years. This review examines the implementation of Artificial Intelligence (AI) in radiology, examining its practical applications, associated challenges, and potential paths for progress.

Radiology is a medical field that, through several imaging technologies, including Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Positron Emission Tomography (PET), ultrasound, and conventional radiology, contributes to the formation of medical diagnoses [1].

Challenges facing radiology today include a high workload that is not adapted to the existing workforce, corporatization, streamlining of workflow, and burnout. All of these contribute to the “perfect storm,” creating pressure on radiologists and the institutions in which they work [2].

In this context, AI can contribute to the evolution of radiology, from improving image acquisition to image interpretation with the help of advanced image analysis [1].

Understanding Terminology: AI, ML, and DL

Artificial intelligence has a contribution in radiology through machine learning (ML) and deep learning (DL) algorithms that play a role in image analysis.

Artificial intelligence is the replication of human intelligence in machines programmed to simulate human thought and action, with the aim of creating intelligent systems that operate autonomously and have the ability to solve problems that would normally require human intelligence.

Machine learning algorithms have emerged in recent years and are based on the introduction of decision trees, support vector machines, and neural networks, with these algorithms forming the basis of image analysis.

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Deep learning is a subset of ML that uses multilayer artificial neural networks, which allow DL algorithms to understand complex data, which is successful in visual recognition. The layers are learned from the data, with architecture inspired by the human brain [1, 3].

HOW ARTIFICIAL INTELLIGENCE IS CHANGING THE FUTURE OF RADIOLOGY

AI is crucial to radiology's future in many aspects:

Intelligent image analysis algorithms

Through DL, AI can analyze large volumes of unstructured data, such as images, and learn patterns to automatically recognize features from the raw data. Through these technologies, and particularly through Convolutional Neural Networks (CNNs), AI has revolutionized the early identification of lesion patterns in CT scans [3].

Increased workflow speed

Integrating AI into clinical workflows gives radiologists quicker access to advanced imaging, speeding up results and helping prioritize complex or urgent cases [1]. There are areas where the use of artificial intelligence tools has reduced the workflow of radiologists by about 34%, such as in the case of breast cancer screening [4]. One of the main problems that arises when implementing AI in radiology is the lack of standardization between platforms and the fact that there are multiple AI tools with different user interfaces, which ultimately leads to an inefficient workflow, as radiologists must access different systems to reach the information provided by AI. The existence of a centralized AI platform, which integrates multiple diagnoses, with a single interface, can facilitate the workflow [5].

Predictions based on image analysis

The ability to make an accurate prediction of a patient's prognosis is essential for timely interventions. The emergence of predictive analytics through artificial intelligence allows the analysis of a large volume of patient data, including demographic data, medical history, and previous tests, which, together with imaging, can be used to establish predictive models with greater accuracy [6].

The role of AI in education

The current approach to education emphasizes a series of theoretical notions and a practical case-based experience. However, the method has its limitations, exposing learners to a limited number of cases compared to real-world work situations, as well as limited access to expert radiologists in certain fields. This is an area where AI can have a transformative impact through its ability to analyze large volumes of data and provide real-time feedback to complement the knowledge acquired through traditional education. Also, the existence of work platforms with simulations of real cases, connected to large databases, can increase the quality of training for students and residents [7].

Another transformative element is the inclusion of interdisciplinary education; radiologists must go beyond the traditional focus and have experience in fields such as genomics, pathology, laboratory medicine, oncology, molecular medicine, and immunology. Thus, the radiologist can understand the complex data that modern AI systems introduce into analysis to generate multimodal results. Radiologists occupy a unique position at the intersection of diverse diagnostic fields and play a central role in the diagnosis and care of patients, which is why their expertise in placing imaging data in an integrative clinical context is essential. Interdisciplinary collaboration is the way to ensure that radiologists are active users of artificial intelligence. Another area that can be improved is communication skills, an area in which the existence of structured reports also helps interdisciplinary communication and the transmission of clear conclusions [8].

Radiology training programs should prepare radiologists to understand artificial intelligence and to be able to make decisions under uncertainty without being influenced by cognitive biases, a situation that training in risk assessment and probabilistic reasoning helps to improve. This shift involves more than simply adapting the curriculum; it involves an integrative mindset focused on continuing education and an education that emphasizes interdisciplinary collaboration. These are the values that can help radiologists adapt to the complexity of the modern data they face [8].

AREAS WHERE AI IS SUCCESSFULLY INTEGRATED IN RADIOLOGY

Oncology

Correct interpretation of images, considering tumor heterogeneity and imaging characteristics, is a key factor in cancer diagnosis. By integrating AI into the oncology imaging workflow, multidimensional information about tumors can be extracted, which together can aid in tumor staging and stratification, molecular diagnosis, metastasis prediction and treatment response [9]. The main directions of focus for the inclusion of AI in oncology imaging include automatic image segmentation, lesion review, and the generation of standardized responses based on automated analysis. A significant aspect in oncology is the development of radiomics, but also the contribution of AI in radiotherapy.

Among the areas where artificial intelligence is successfully integrated into radiology is oncology, where techniques for automatic image segmentation, lesion review, generation of standardized responses based on automated analyses, and the development of radiomics have been successfully implemented.

Automated Image Segmentation

DL significantly contributes to image segmentation and classification and therefore increases the speed and accuracy of diagnosis. CNNs contribute significantly to the ability of artificial AI to learn complex patterns, which are found in fields with visual components, such as radiology. This is possible due to the complex, layered architecture of these networks, which have demonstrated increased ability in object detection and segmentation. CNNs have shown a key role in segmenting brain tumors in MRI examinations. Other applications where CNN segmentation has been successfully applied in radiology are the individualization of lung nodules, also distinguishing between benign and malignant tumors in mammography [1, 10, 11].

Review of lesions

Radiology exploits the most useful AI algorithms to automatically identify, locate, and recognize abnormalities found on medical images, whether they are tumors, fractures, or other types of injuries. Among these, DL and reinforcement learning help AI systems increase diagnostic accuracy, helping to halve the radiologist's workload and medical decision-making time. DL models can identify small lesions more easily through resolution enhancement techniques such as interpolation and super-resolution. To increase the performance of AI analysis algorithms in medical object detection, image pre-processing procedures are applied. Among the image preprocessing techniques used in artificial intelligence for object detection in radiology, there are some important ones, such as spatial resampling, intensity normalization, image registration, data augmentation, noise reduction, and segmentation. Data augmentation techniques include rotating, scaling, and flipping images to create larger data sets for use in AI models. Radiological applications of AI object detection apply to both tumors and other types of lesions in a wide range of examinations, including X-ray and CT scans, but one of the most innovative applications is CNN-based segmentation in MRI imaging [12].

Development of standardized reports based on automated analysis

The existence of structured reports in radiology improves workflow efficiency by faster generation of medical reports, especially when there are variable templates for various clinical aspects. The European Society of Radiology (ESR) emphasizes the need to use structured medical reports to streamline workflow and reduce the potential for errors. The use of structured reports that include a standardized list of issues reduces the risk of error by guiding the radiologist through a system of reviewing key points. At the same time, a structured approach ensures better communication between specialties and ultimately contributes to better patient care. With multiple AI tools, there is a risk that radiologists will be overwhelmed, but AI algorithms for rapid image analysis and standardized reporting can create consistency and make these disparate AI tools work together more effectively [5].

Radiomics

Among AI techniques, ML and radiomics involve extracting predefined features from radiological images through a data characterization algorithm. These features encompass various aspects of tumors, such as intensity-based measurements, shape characteristics, volume, heterogeneity, vascularity, and effect on surrounding structures. The entire process aims to increase diagnostic and predictive capabilities [1, 9].

Radiotherapy

Technological progress in diagnostic imaging has improved the efficiency of radiotherapy by introducing artificial intelligence software that optimizes the segmentation of tumors and organs at risk, prognostic assessment, treatment planning and optimization, thus saving considerable time for the radiation oncologist [13].

Lung nodule detection and classification

An advantage and a significant improvement of the workflow in radiology services appeared with the programs for automatic analysis and segmentation of pulmonary nodules [14]. These programs have become routine and are permanently attached to PACS systems, identifying, measuring dimensions, analyzing the structure, density and location of pulmonary nodules with dimensions between 3-30 mm [14].

There are also limitations in the automated analysis of pulmonary nodules, among which the most significant are the false identification of vascular trajectories as nodular hyperdensities, and other limits in identifying lesions in areas with ventilation disorders, with motion artifacts or with fibronodular sequelae. However, automated analysis programs are continuously improving and shortening the times for preparing medical reports, increasing work efficiency, and becoming working tools for the redistribution of highly qualified human resources in other directions where they are still irreplaceable [15].

Another useful contribution is the automated comparative analysis of the evolution of pulmonary nodule sizes, in accordance with the intervals specified by specialist guidelines, such as that of the Fleischner Society, an aspect that contributes to establishing the most appropriate therapeutic and follow-up conduct [16]. Automated analysis generates medical reports that enter patients' electronic records in parallel with the generation of a set of images with automatically marked lesions [15]. A concrete example in practice is that provided by CAD-type analyses in the identification of undetected tuberculous lesions.

Trauma with applications in military medicine

In imaging departments serving emergency rooms in civilian or military field hospitals, cerebral hemorrhagic lesions, or hemorrhages in any part of the body, represent the most common cause of post-traumatic death. Rapid recognition of hemorrhages and the most appropriate therapeutic interventions are of paramount importance. The role of AI systems using ML is to optimize diagnosis in these situations through algorithms that recognize hemorrhagic lesions and assess their risk potential through CT and FAST ultrasound methods [17]. These programs use semiological notions of lesion recognition, such as Language Models (LM) for automatic 3D labelling to recognize hemorrhage subtypes based on brain segmentation, midline deviation, mass effect, cerebral edema, and hyperdensity values. Diagnostic models in neurotraumatology utilize various emergency diagnoses, including hemorrhage, ischemia, cerebral infarction, space-occupying lesions, mass effect, hydrocephalus, and midline deviation. Neurotraumatology lesion recognition models based on imaging data obtained through radiography, computed tomography, and MRI can also be used for the rest of the body, through 3D anatomical segmentation, 2D CNNs software, and Recurrent Neural Network (RNN) [18].

Neurology

By using the Picture Archiving and Communication System (PACS) system, lesions with complex neurological substrates can be effectively detected and characterized through automatic algorithms that have entered routine analysis in some types of cerebral pathologies.

Automatic Cerebral Volumetry consists of an efficient automated analysis based on segmentation using the 3D T1 MPRAGE sequence. For reference, the images and measurement values are compared with those of a comparative database of patients from the same age groups for whom the brain volume is reported. Thus, this program provides cerebral volumetric assessment in case of suspected dementia, by evaluating the frontal, parietal and temporal lobes, and particularly the hippocampus. Brain volume assessment can also be extremely useful in multiple sclerosis and other degenerative diseases, such as Multiple Systemic Atrophy (MSA), Progressive Supranuclear Palsy (PSP), and Cortico-basal Degenerative Disease (CBD).

The program can also analyze the volume of specific brain areas such as the caudate nucleus, putamen, globus pallidus, cerebellar cortex, and midbrain [19].

The *Evaluation of Arterial Intracranial Vascular Lumen* uses a Time-of-flight (TOF) acquisition for analysis before being processed with the Maximum Intensity Projection (MIP) program. The analysis program is attached to the PACS system and generates a medical report that includes measurements and imaging of the identified lesions. In the case of intracranial aneurysms, it is estimated that 10-15% of them are underdiagnosed, and these automated analysis programs can improve the anomaly detection rate with an accuracy of almost 100% [20].

The *Characterization of a Demyelinating Lesion* is based on the analysis of 3D Fluid-Attenuated Inversion Recovery (FLAIR) sequences that highlight the location of the lesions. The analysis includes classification of the lesions using various reference systems, such as the McDonald and Fazekas criteria.

The program can generate a medical report that includes data on the location and size of the lesions, the presence of new lesions compared to previous examinations, and key illustrative images that are included in the report. Thus, this type of analysis can be extremely useful in all demyelinating diseases, including multiple sclerosis [20].

Brain tumor analysis allows for the automated assessment of tissue structures associated with tumor presence in entities with different anatomopathological substrates. Automated tumor lesion analysis is performed after intravenous administration of a contrast agent, which evaluates the behavior and uptake of the contrast. Automated lesion analysis is applied to highlight and differentiate various lesions and tissue components, which can be especially useful in the characterization of glioblastoma-type lesions, metastases and other tumor types, such as meningiomas. Also, complex data analysis allows for the possibility of integration into radiotherapy and neurosurgery programs [21].

Prostate

AI has become a commonly used tool for the diagnosis of prostate cancer, useful for both radiologists and urologists in multiparametric MRI examinations. The speed and efficiency of prostate cancer diagnosis and staging are among the greatest achievements for AI in prostate MRI evaluation [22].

Prostate cancer staging is performed using the PIRADS classification system and is integrated into the PACS system. The AI-generated medical report provides PIRADS classification, prostate volume, and detailed multiparametric analysis of prostate lesions, including nodule identification and categorization. In addition, when available, previous examinations can be evaluated comparatively. Medical reports also include appropriate graphical representations and dimensional assessments, as well as mapping of prostate lesions [22, 23].

When the patient's PSA analysis is also available, this aspect can be represented in tables and associated with the presentation of prostatic density in the workflow report. This medical report can also be effectively used for image-guided biopsy, depending on the representation and arrangement of the most representative lesions [22, 23].

CLINICAL IMPLEMENTATION OF ARTIFICIAL INTELLIGENCE, CASE STUDIES AND APPLICATIONS

Case 1. Detection and analysis of intracerebral aneurysms using artificial intelligence

By analyzing brain MRI, TOF sequences, AI software can analyze the intracerebral vascular lumen and thus identify the presence of intracerebral arterial aneurysms and estimate their location and size (Figure 1).

Figure 1: Brain MRI examination. (A) Axial TOF images. (B) Automated detection of an aneurysm in the anterior communicating artery.

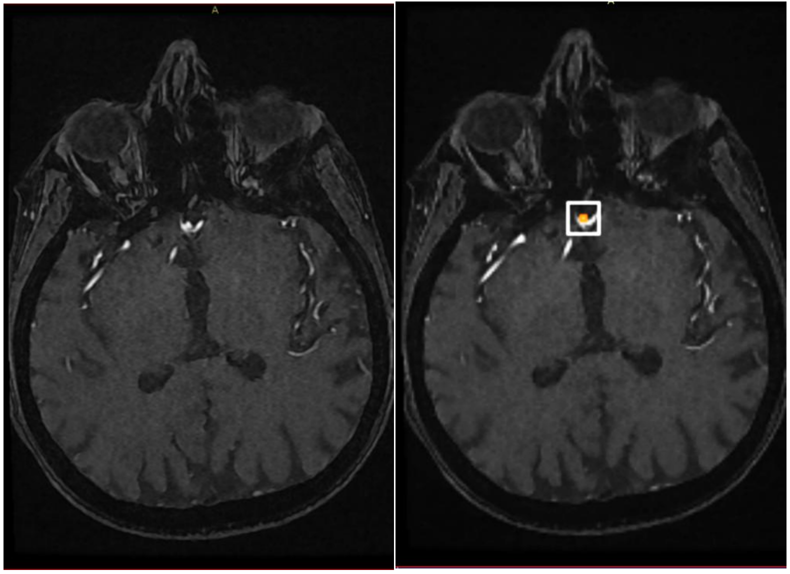

(A) **(B)**

Case 2. Automatic identification and dimensional monitoring of pulmonary nodular lesions

With the help of automated AI programs, in the case of thoracic CT examinations, the lung parenchyma can be analyzed for the presence of lesions, thus nodular lesions are identified, counted and measured automatically, and if there are previous examinations, the dimensional progression of the lesions can also be automatically assessed. Thus, with the help of AI tools, such as Rayscape, clinical impact improvement and shortening of diagnostic times are observed [24] (Figure 2).

Figure 2. CT examination of axial sections, lung parenchyma window. (A) Automatic identification of pulmonary nodular lesions and dimensional assessment. (B) Automatic comparative analysis of pulmonary lesions with dimensional progression assessment.

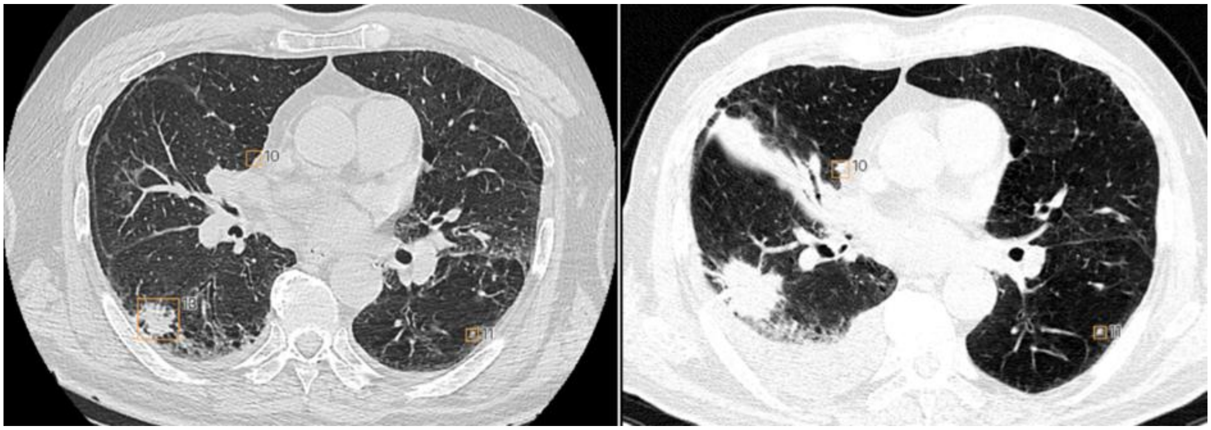

Case 3. Automated prostate volume analysis and lesion characterization

Automated prostate analysis on MRI examinations is an area where AI has demonstrated progress and efficiency, so that prostate volume is assessed, lesions are identified, measured and classified according to PI-RADS classification, and a mapping of the most significant lesions is made in the report (Figure 3).

Figure 3. MRI examination, coronal T2 images of the prostate with automated prostate volume analysis and characterization of nodular lesions.

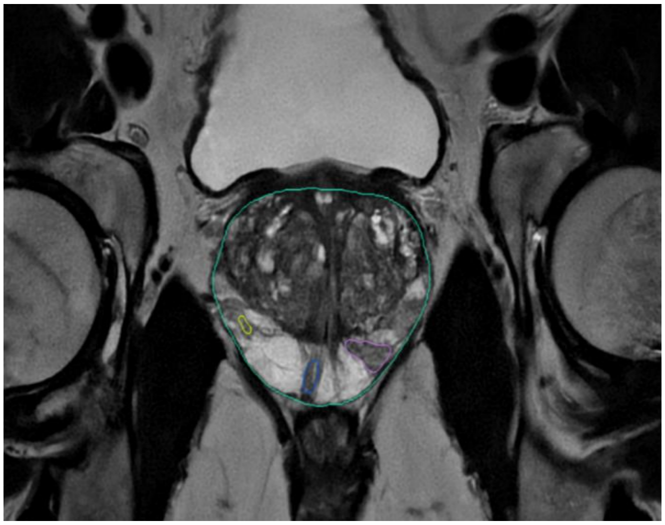

METHODS AND INDICATORS FOR EVALUATING THE EFFECTIVENESS OF AI PROGRAMS

The most useful evaluation method is the one that uses the Area Under the Curve (AUC) indicator in binary detection. The method is derived from the Receiver Operating Characteristic (ROC) curve, which uses sensitivity and specificity as parameters to state whether a lesion is present or not [17]. In the ideal mode, the optimal value of AUC is 1. Between 1 and 0.5 AUC has the appearance of a random model and below 0.5, it cannot be brought into discussion. At the current time, the interpretation in an efficient model has AUC values close to 0.9. The evaluation of the AUC indicator has, in turn, references and standards related to diagnosis established at the level of junior and senior radiologists with expertise. This level of over 0.9 is more easily achieved in lesions defined by few parameters and with clear values, such as hemorrhages, fractures, pneumothorax, lesions from mammographic examinations, while multiparametric defined lesions, such as prostatic or hepatic ones, require a senior radiologist-type standard of expertise. In the radiological evaluation of polytrauma in emergency services, high AUC values, above 0.9, and short processing time are required [25].

CONSIDERATIONS AND CHALLENGES

- Legal and ethical implications

The legal and social implications of artificial intelligence technologies in radiology need to be seriously assessed. One concern is the lack of transparency behind the decision-making process through artificial intelligence. Another key aspect in the implementation of artificial intelligence is the lack of a legislative system that regulates the level of involvement of artificial intelligence. Medical liability in case of malpractice is attributed to the radiologist. AI tools must meet rigorous validation and approval standards to establish their safety. Once approved, they must be continuously monitored to ensure that the results they provide are dependable. The implementation of AI systems requires adherence to established standards, appropriate regulatory approval, and awareness among radiologists and medical practitioners regarding their limitations, ensuring these factors are carefully considered [1, 26].

- Bias and generalization

Despite the development of artificial intelligence, when implemented in real life, its performance varies for certain subgroups. The question arises whether the goal should be fairness to the individual or fairness to the group. It has been observed that artificial intelligence takes shortcuts in the learning process, which can lead to biased results towards certain subgroups, for example, when multiple factors, including age, gender, and race, are taken into account in the diagnosis [27].

- Familiarization, incorporating AI into existing workflow systems

The integration of artificial intelligence into clinical radiology faces several challenges. One of these is the existence of robust hardware capable of managing the substantial amounts of data generated by medical imaging systems, which implies investment in such infrastructure. On the other hand, this new hardware and software must be able to integrate with existing radiology systems. There is also a need for training of radiologists and for them to collaborate with artificial intelligence developers [1].

A LOOK INTO THE FUTURE OF RADIOLOGY

The future transformation of radiology includes the integration of Augmented/Virtual Reality (AR/VR) and AI. AR/VR technologies are expected to play a significant role in radiological practice and radiological education by improving the visualization of radiological images, thereby contributing to diagnosis and treatment planning. Also, through machine learning, AI improves its image analysis capabilities, thereby contributing to reducing diagnostic errors. A tool based on AI is Computer-Aided-Diagnosis (CAD), which can contribute to the development of an integrative diagnosis by incorporating radiological, anatomopathological and genomic information into the AI-assisted workflow [1].

CONCLUSION

Artificial intelligence is developing rapidly, and understanding its use in medical practice is mandatory. Radiology is transforming, and specialists must keep up with it, adopting these innovative technologies and being integrated into multidisciplinary clinical teams, with collaboration between healthcare providers being paramount to fully capitalize on the benefits of this scientific knowledge.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research work received no external funding. No AI tools were used to draft or edit the manuscript.

Authors' contribution

Conceptualization, D.C., and A.S.C.; methodology, D.C., and A.S.C.; software, D.C., and A.S.C..; validation, D.C., and A.S.C.; formal analysis, D.C., and A.S.C.; investigation, D.C., and A.S.C.; resources, D.C., and A.S.C.; data curation, D.C; writing—original draft preparation, D.C., and A.S.C.; writing—review and editing, D.C., and A.S.C.; visualization, D.C.; supervision, D.C. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Not applicable.

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REVIEW

A Review of the Ethical and Legal Challenges in Clinical Trials

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Abstract: Clinical research is the cornerstone of the progress recorded in psychiatry in the last six decades, or of what is called the psychopharmacological revolution. Regardless of the stage of clinical research, there are sensitive ethical and legal aspects that may appear during this process, and such challenges have to be acknowledged in order to preserve its scientific value, integrity, and centered-on-the-patient-wellbeing core principles. Also, adequate strategies focused on preventing the risk of ethical misconduct and litigation have to be found to avoid a loss of trust in the results of clinical research, to protect vulnerable populations from abuse, and to ensure a legally stable environment for investigators. In order to explore these practical problems, a narrative review was performed through a search in PubMed and Google Scholar databases. The main ethical risks detected in clinical research were related to errors in the methodology of obtaining informed consent, monitoring the participant's safety during the clinical trial, and falsifying collected data. Several famous cases of ethical misconduct were found and analyzed, and methods to decrease the risk of the re-appearance of such problems have been listed. In conclusion, this review is an invitation to explore the complexity of the methodology of clinical research and its ethical and legal risks, as well as to find new ways to mitigate the possibility of such risks related to the research process.

Keywords: clinical trial; volunteers; informed consent; vulnerable population; pharmacological agents; psychopharmacology; ethical misconduct

INTRODUCTION

Clinical research is an essential component of the broader paradigm of evidence-based medicine that is focused on ensuring scientific progress in healthcare. Although the evolution of medicine is an important objective, it must always be kept in mind when discussing clinical trials that the ultimate goal is to increase the quality of services offered to patients and improve their prognosis and chances of regaining a normal life. To refer only to the field of psychiatry, the developments recorded in the armamentarium of psychotropics in the last six decades represent a veritable revolution that allowed a change in the nosological paradigm of psychiatric disorders. Due to this change, which showed there is a biological basis for mental illnesses and that pharmacological agents can mitigate their symptoms, the stigmatization and the tendency to institutionalize patients with such diagnoses decreased and are expected to diminish further in the near future.

The importance of clinical research is illustrated by the high number of trials identified by a search in the International Clinical Trials Registry Platform (ICTRP), a World Health Organization database, that reported the existence of 10606 depression studies in 2024 [1]. According to the US National Library of Medicine database for clinical trials (ClinicalTrials.gov), more than 530000 studies are registered, out of which over 200000 are exploring a drug or a biological product, regardless of the pathology targeted, at the beginning of 2025 [2].

The need to conduct clinical trials in psychiatry is supported by a large number of practical difficulties when confronting the reality of mental health problems. One of the most critical challenges for clinicians is to increase the rate of responsiveness in treatment-resistant psychiatric illnesses, being known that 30-35% of depressive disorders, 30% of schizophrenia spectrum disorders, and up to 25% of bipolar disorders do not respond well enough to the usually administered pharmacological agents [3-7]. Another significant motive to foster clinical research in the field of psychiatry is the continuous quest to find new formulae to enhance therapeutic adherence (e.g., other ways of administration for a particular substance, coupling

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the active drug with a vehicle that allows a slower release of the active substance so that the interval between administration could increase, or to find molecules with superior tolerability). Changes in the pharmacodynamic or pharmacokinetic properties of certain drugs that are already available for clinical use could lead to the discovery of new drugs with potentially superior properties. Covering emerging health needs, including mental health-related problems, such as the long COVID-19 syndrome, new psychoactive substances (NPS) use disorders, etc., is another important reason for conducting clinical trials.

Some of the above-mentioned directions of research have been more productive than others, while high expectations are related to the objectives insufficiently accomplished, like detecting pathogenic rather than symptomatic treatments or improving the tolerability of already existent drugs for treatment-resistant cases. For example, when discussing the improvement of treatment adherence, the discovery of long-acting antipsychotics that allow patients with schizophrenia to receive only two injections each year (i.e., paliperidone palmitate with 6-month release) could be considered significant progress [8-10]. The production of active metabolites as independent drugs, a process starting with tricyclic antidepressants, like nortriptyline from amitriptyline, or desipramine from imipramine, and continuing with new-generation antidepressants and atypical antipsychotics, like desvenlafaxine from venlafaxine, or paliperidone from risperidone, allowed for improving and diversification of pharmacokinetic and pharmacodynamic properties of the parent drugs [11-15]. Another direction of research is improving the pharmacodynamic properties of a racemic mixture by extracting only the most potent enantiomer, as was the case of escitalopram, levomilnacipran, or eszopiclone [16-20]. Even one of the most sensitive topics in psychopharmacology, i.e., the pathogenic vs. symptomatic treatments, acknowledged significant progress in the last years by the Food and Drug Administration (FDA) approval of monoclonal antibodies that target beta-amyloid peptides, like aducanumab, donanemab or lecanemab [20-23].

The responsibility for conducting clinical trials belongs to medical professionals, primarily doctors, but also to other categories of healthcare personnel – registered nurses, clinical psychologists, etc., who follow specific training programs before engaging in any research activity. Completing these training programs provides professionals with a common background of knowledge and skills necessary for participation in pharmacological studies. Specific certifications, such as Good Clinical Practice (GCP)– ICH Guidelines E6 R2, or qualifications for scoring scales and inventories used to measure the severity of clinical symptoms, have the aim to protect participants in clinical studies, reduce the risks of malpractice for investigators, increase the validity of data obtained from the research, and decrease the inter-rater variability of clinical scale results [24-27].

The Declaration of Helsinki regarding human experimentation originated in 1964 in the World Medical Association (WMA) statement and has been constantly revised ever since, with the last revision in 2024 [28-30]. This declaration is an essential step in the history of conducting research with human subjects, as it stipulates the fundamental principles of this domain, for example, the unconditioned respect for individuals and their right to self-determination and making informed decisions regarding their own health, including aspects pertaining to participation in clinical studies [28,30]. The primary duty of the investigators is to the patient or volunteer included in clinical studies, and the participant's well-being takes precedence over the interests of science and society [28-30]. This declaration also stipulates that ethical aspects are primordial and laws and regulations are only second in importance [28-30].

Ethical and legal errors in pharmacological research have the potential to harm not only study participants but also the integrity of the entire research process, thus indirectly affecting a very large number of patients who could benefit from a specific investigational product once it is approved. This narrative review is dedicated to highlighting the most relevant domains of clinical research that are vulnerable to malpractice risk and to investigate possible ways to decrease the probability of its onset.

MATERIALS AND METHODS

Two electronic databases (PubMed and Google Scholar) were searched for the terms „malpractice," OR „ethical issues," OR „methodological errors," OR „legal issues," AND „clinical trials," OR „pharmacological research," OR „drug research," OR „psychopharmacology." All primary and secondary reports published in English were selected if they corresponded to the inclusion criteria and did not meet the exclusion criteria. In order to ensure adequate coverage of this topic, grey literature was also searched for papers published in English. The period chosen for published papers was between the inception of each database and January 2025. Inclusion criteria: all types of research and commentaries, press releases and related sources of information; all stages of clinical trials, if related to the field of psychiatry; pharmacological interventions, either alone or in combination with other interventions. Exclusion criteria: non-pharmacological interventions used alone; reports that did not contain precise data on ethical or legal issues related to clinical trials in psychiatry; undefined outcomes or imprecise source of the referenced studies.

RESULTS

From the clinical research perspective, ethical and legal error problems appear when a doctor participating in clinical trials (hereinafter referred to as "investigator") does not comply with the correct way of obtaining adequate informed consent, mismanages patient safety measures, or falsifies research data. These actions can lead to serious consequences for participants, undermine the validity of clinical trial results, and negatively impact the medical community and society as a whole through the decrease of individuals' chances of getting the optimal cure.

Ethical and legal challenges related to obtaining informed consent

Signing informed consent is the first procedure a participant in a clinical trial has to perform prior to any study-related procedures [31]. This step is mandatory in the era of clinical trials conducted under rigorous ethical principles, according to the principles of the

Declaration of Helsinki [28-30]. The procedure of informed signed consent is mentioned in the GCP guidelines and reflects the principles of absolute respect for patients' autonomy and their right to be informed in every aspect of the trial that is relevant to their own health [24-27]. Also, the Declaration of Helsinki stipulates that whenever the participants are unable to give their own consent, surrogate consent may be obtained from an individual acting in the participant's best interest, but the consent of the participant still needs to be obtained if at all possible [28-30,32]. The cases when this procedure is applicable are also presented in the Declaration of Helsinki [28]. The investigator must ensure that this consent is given freely, without any form of coercion, and with a comprehensive understanding of the implications of the study [28].

Possible errors in this domain, which are compatible with a risk of malpractice, are (a) a failure to disclose potential risks of the trial, (b) providing false or intentionally incomplete information, and (c) coercing patients to participate in the study.

For example, if an investigator conducting a study fails to properly explain to participants the adverse events or health risks of the investigational product, patients could be exposed to a danger to their health that they did not voluntarily assume in the very beginning. A study exploring the proportion of participants in clinical trials (N=135 cohorts) who were aware of the different elements of informed consent showed that only 52-75.8% understood what they were signing [33]. According to this study, 74% of the participants were aware of potential benefits and less than 70% of the study's aim, while 66% understood the rule of confidentiality [33]. Also, the proportion of participants who understood the informed consent did not increase during the 30 years of the studies included in the review [33]. These data indicate the need for better communication between investigators and participants during the signing of the informed consent form.

According to the GCP-ICH, both the informed consent discussion and the written informed consent form and any other written information to be provided to subjects should include explanations of the testing hypothesis process involved by the clinical trial, which is the purpose of the trial, understanding the fact that the treatment is not validated and what is the probability of random assignment to each treatment/placebo [24-27]. Also, the participant must receive adequate information about the study procedures to be followed, including all invasive procedures, which are his/her responsibilities, those aspects of the trial that are experimental, and the reasonable risks that can be anticipated or inconveniences to the subject and, also, the reasonably expected benefits [24-27]. Other elements included in the informed consent, according to the same source, refer to the alternative procedure(s) or treatment(s) that may be available to the subject, and their potential significant benefits and risks, the compensation and/or treatment available to the subject in the event of study-related harm, and the anticipated payment, if any, to the subject for participation in the study [24-27]. The list of all elements that constitute an informed consent form is too extensive to be reproduced here, but this complexity explains the long time necessary for the investigator to explain the totality of the relevant aspects to each participant in order to obtain his/her approval to participate in the trial.

Patient safety and monitoring of adverse events

Investigators are responsible for carefully monitoring the psychobiological parameters of each participant in a trial, ensuring that subjects are not exposed to avoidable health risks [24-27]. This process presumes a regular verification for the existence of adverse reactions, offering adequate interventions if these appear and discontinuing participants' involvement if they develop a serious adverse event. Failure to comply with this principle is a severe form of malpractice and a clear violation of the core principles of the Declaration of Helsinki [28-30].

For example, if an investigator continues to administer an investigational product to a patient despite clear signs suggesting the onset of severe side effects, he or she is engaging in a form of negligence that can have severe legal consequences far beyond the violations of ethical conduct. In this area are placed the cases when participants who remain pregnant during the clinical trials are still receiving the investigational product or when adverse events to the experimental drugs are treated with medications not allowed by the study protocol, thus disregarding the potential of pharmacologically dangerous interactions. According to a study [34] focused on the understanding of potential trial participants regarding the monitoring and communication of serious adverse events during a clinical trial, it was observed that their awareness of safeguards or regulations in this domain was very scarce; also, many of them expressed the desire to be more informed about the potentially serious adverse events and short reporting deadlines. The same study reported on the concerns of participants about potential financial conflicts of interest in monitoring and further reporting serious adverse events during a clinical trial [34].

In the same direction, the 2024 revision of the Declaration of Helsinki notes that unproven interventions focused on alleviating pain or suffering must not bypass ethical safety measures or avoid evaluation by controlled clinical trials [35], highlighting the importance of conducting an accurate assessment of all health parameters even in the most severe patients.

New tools have been suggested for the most accurate monitoring of safety in clinical trials, including those using graphical visualizations that are congruent with the FDA/EMA (European Medicines Agency) guidelines [36]. Such tools are interactive and support regular medical monitoring and oversight [36]. The National Institutes of Health in the US require data and safety monitoring boards for all phase III clinical trials, which are conceived as a supplementary barrier to errors in safety monitoring [37].

Data falsification and breaking the research integrity

The accuracy of data collection in the clinical research process and the integrity of the analysis of information regarding the efficacy and tolerability of the investigational product are essential to provide valid results. Ethical breaks and legal issues can be detected in this field when an investigator manipulates or falsifies clinical data to obtain some desired results [38,39]. Data falsification exerts significant prejudice on the validity of research, endangers the patient’s safety, and violates the core ethical principles of medicine.

For example, such an unethical approach can lead to the approval by competent institutions of harmful or ineffective treatments for clinical use, which can significantly endanger future patients [38-41]. Furthermore, new research could be based on a false presumption, and this could exponentially amplify the negative economic and medical consequences of a single false study [38-41].

Several examples of data falsification and research integrity break are: (a) selective reporting, which is a form of reporting only data that supports the drug's efficacy while omitting negative or inconclusive results; (b) data harvesting, a phenomenon that refers to the selective analysis of certain variables that fit a desired outcome, ignoring others; (c) partial monitoring of tolerability data, which may be observed when there is a lack of monitoring or inadequate reporting of adverse effects that occur during the study; (d) lack of adequate monitoring of participants after the study ends to confirm that the investigator ensures that any long-term effects of the drug are documented and managed appropriately; (e) underestimation of risks, which refers to the situation when the investigators minimize or ignore potential risks that expose participants to short, medium, and long-term negative effects [38-43].

Another example of phenomena that negatively interact with research integrity is the participation in clinical trials of investigators with insufficient experience or without enough credentials, and this is a direct violation of the Declaration of Helsinki [28-30]. Thus, allowing individuals without the necessary certifications or knowledge to conduct or supervise critical aspects of a trial is an example of ethical misconduct [28-30]. Failure to provide adequate training to staff on ethical practices, safety protocols, or specific clinical trial procedures is also a breach of research integrity [28-30].

Ethical integrity can also be breached by improper recruitment and enrollment of participants [28-30,38]. For example, the inclusion of ineligible participants might refer to the enrollment of individuals who do not meet the inclusion criteria for the study, such as those with contraindicated health conditions or who are already taking other medications prohibited by the study protocol. On the other hand, but in the same category of ethical breaches, the exclusion of eligible participants refers to discrimination against specific populations, such as vulnerable groups, in order to distort results or save time. Such examples are the inclusion of participants diagnosed with major depression and active suicide ideation, although the study protocol prohibits their enrollment, and, on the other hand, exclusion of patients with severe depression due to the investigator’s fear they will be challenging to manage, although the study protocol allows their participation.

Ethical breaches may be derived from protocol deviations and protocol violations [44,45]. The first case refers to divergences between the study activities from the institutional review board (IRB)-approved protocol with minimal impact, while the second refers to divergences that reduce the quality or completeness of the data, inaccuracies in the informed consent form, or endanger the subject's safety, rights, or welfare [45]. For example, if the test procedures are modified or the treatment administration algorithm is changed during the trial without a clear rationale, these may affect the results and induce harmful reactions in the participants. Also, failure to adequately document protocol changes or deviations makes it impossible to assess their potential impact on the trial. Failure to report or address ethical concerns is a significant methodological and ethical or legal error that can be observed, for example, when an ethical review is neglected by failing to obtain approval from an IRB or ethics committee before the trial begins or failing to address ethical concerns raised during the review process [46].

Several ways to decrease the risk of protocol deviations are enhanced training for the investigators, regular monitoring visits, involvement of the Ethics committee in site monitoring, etc. [44]. In a study that explored 80 postgraduate dissertations, representing observational studies (74%) and interventional trials (10%), a significant proportion of protocol deviations (more than 42%) were due to nonreporting and incomplete documentation of the divergencies [47].

Consequences of malpractice in clinical trials

Clinical trial litigation has been rapidly escalating during the last decades, and defendants are not only principal investigators or sponsors but also IRBs, individual IRB members, study coordinators, etc. [48,49]. Beyond the legal repercussions, malpractice in clinical trials can have long-lasting effects on public opinion about medical research. Patients may become hesitant to participate in future clinical trials, fearing that their health may be at risk. As a consequence, the interest of the general public in research could decrease, and thus, the availability of new treatments for various disorders could be delayed, or potentially valuable drugs may even be abandoned.

Furthermore, the reputation of healthcare practitioners can be significantly damaged when cases of malpractice in clinical trials are discovered and exploited by mass media [50,51]. Such phenomena can fuel an environment of skepticism and mistrust, leading to the erosion of the relationship between the patient and the healthcare specialists. The impact is, consequently, negative not only at an individual level but also at a macro-social level, with consequences beyond the level of a research site or a certain clinical trial.

Examples of famous cases when ethical and legal violations of clinical research were detected

One of the first cases of ethical misconduct after the Nuremberg Trials, which brought to the general interest the dangers of experiments without ethical restraints, was related to the MKUltra studies (1955–1967) [52,53]. These involved „mind-control” techniques using LSD-25, and were sponsored by the CIA [52]. Many of the subjects were given LSD and other psychoactive substances without consent, and some were subjected to psychological manipulation; also, not all of the „investigators” were healthcare specialists, but they came from very diverse professional backgrounds, e.g., one of them was a magician, i.e., a student of Harry Houdini, while another was a CIA consultant with a shady history; tragically, this so-called experiment for mental illnesses became medical torture not very unlikely to the Nuremberg Trials [52,53]. Different reports describe the effects of these experiments on mental health, including the Veterans Health Initiative Report in 2003 [53].

The study 329 (1994–2001) investigated the use of paroxetine and imipramine in adolescents, and this research was later found to have serious ethical and scientific flaws, including selective reporting of results [54,55]. An independent reanalysis of the data from Study 329 (the RIAT initiative) showed that acute and long-term paroxetine and imipramine were harmful and ineffective in adolescents with major depression [54]. In the original study, paroxetine proved itself well-tolerated and effective in that population on multiple outcomes, i.e., the Hamilton Depression Rating Scale (HAMD), Schedule for Affective Disorders and Schizophrenia for Adolescents-Lifetime version (K-SADS-L), and Clinical Global Impression (CGI), while the withdrawal rates due to adverse events were 9.7% vs. 6.9% (paroxetine vs. placebo) [55]. The RIAT analysis presented several ways in which the presentation of safety data in clinical trials may influence the apparent tolerability of a drug, e.g., the use of an idiosyncratic coding system, failure to transcribe all adverse events from clinical records to an adverse event database, or filtering data on adverse events through statistical techniques [55]. This case is interesting because it represents an argument to make it available for independent analysis of all primary data available from a clinical trial [56].

The Ranbaxy Case (2006-2008) involved a major Indian pharmaceutical company in a significant controversy related to drug safety, more specifically to the quality of various generic drug products, including gabapentin and the antibiotic ciprofloxacin [57]. Ranbaxy was accused of falsifying data in clinical trials, including trials of generic drugs, related to the purity, strength and quality of these generics, and the case resulted in the company being fined $500 million by the US Department of Justice for selling counterfeit drugs and submitting false data to the FDA [57]. Incomplete testing records and inadequate programs focused on the assessment of the stability characteristics of drugs were detected by an FDA inspection in the Paonta Sahib facility in 2006 [57].

Other doubts about the regulation authorities failing to keep up with ethical issues in clinical research in India have been raised [58]. Although most of the alleged ethical violations were not confirmed, the controversy about the clinical trials in India has decreased dramatically the trust in clinical research originated in India [58]. Lack of informed consent from volunteers, conflict of interests surrounding the members of independent ethics committees, suspicions about the use of vulnerable populations, and lack of insurance or compensation in case of serious adverse events were subjects presented by activists [58]. In 2017, a guideline passed by the Indian Council of Medical Research was launched to improve the quality of clinical research in that country [59]. These guidelines approach sensitive topics, like the procedure to obtain informed consent, the respect for voluntariness, the definition of vulnerable population, the assessment of the benefits vs. risks balance, and the compensation for harm derived from participation in clinical trials [59]. Also, regularization of the Ethical Committee structure and functions are included in these revised guidelines [59].

Prevention of malpractice in clinical trials

The complexity of clinical research has attracted numerous debates over the last decades, and national institutions with responsibilities in the field of drug regulation have initiated their own rules and norms, besides international or regional ones. EMA, WHO (World Health Organisation), and WMA are the leading institutions with expertise in the regularization of clinical research [60-65]. National agencies, like the FDA in the US or the PMDA (Pharmaceuticals and Medical Devices Agency) in Japan, are also regulatory organizations with rich experience and ethical guidelines dedicated to clinical research [66,67].

In order to decrease the risk of ethical misconduct and litigations in this field, such institutions with responsibilities in the domain of monitoring the research activity must provide continuing education and training to physicians involved in clinical trials, ensuring that they understand the ethical, legal, and scientific responsibilities they bear. The activity of pharmacovigilance is also a topic that needs to be regularized because this stage is essential for ensuring post-marketing feedback from the patients. Phase IV studies are not at all less important than other clinical trials due to their importance in maintaining clinical use of only those drugs that prove themselves useful and tolerable, even after they pass the pivotal trials. The real safety profile of a certain drug may be defined only by continuing surveillance, and this requires an intense effort from patients, doctors, and regulatory institutions [68].

There is a need for effective oversight mechanisms, including local ethics committees, which evaluate the ethical, methodological, and safety aspects of clinical trials before and during their implementation. These committees act as a core protection for human research participants due to their independent review of the ethical acceptability or proposal for human research [69]. The importance of these organisms has been mentioned in the previous chapter, as avoidance of the conflict of interest within IRBs/Ethical Committees was a topic raised in the controversy surrounding clinical research in India [59].

Also, the transparency of research data implies compliance with rigorous protocols, with clear documentation of all procedures, decisions and results, which can be audited at any time by competent national and international institutions. This aspect's importance was highlighted by the Study 329 case, where an independent re-analysis of the original data provided different results [54].

DISCUSSION

The progress recorded in the treatment of psychiatric disorders in the last six decades could not be possible without clinical research based on the dedication of the investigators and volunteers who contributed in a significant way to this endeavor. Like any scientific domain, clinical research is not free of ethical and legal challenges, especially because, due to its sensitive nature, it mixes epistemological aspects (i.e., the experimental nature of the investigational process), with axiological (i.e., the liberty of the individual as a core value), and ontological ones (e.g., the biological vs. spiritual causation of our mental life). Thus, hypersensitive topics are related to each dimension; starting from the acknowledgment of the participants that they assume certain health risks in a clinical trial, continuing with the voluntariness vs. coercion dilemma when being recruited for a study, or with the liberty to decide when to withdraw from a trial, all these aspects may be negatively exploited and consequently may trigger ethical misconduct and litigation. Clinical research has received a lot of attention from both the scientific world and the public, not to mention the vehicle between the two, i.e., the media. Avoiding dogmatic thinking and understanding the complexity of clinical research is important for any investigator, as is the ability of scientists to maintain the transparency of the research to society, which is the final beneficiary of their work. The legislation in the field of clinical trials is an evolving organism, and the Declaration of Helsinki is the result of the conjugated effort of society and the scientists trying to prevent the repeating of tragic ethical misconduct as in the Nuremberg Trials, MK Ultra experiments, etc.

Because the number of clinical trials is continuously increasing as a result of the social pressure to find more performant and more tolerable pharmacological agents, not to mention the need to respond to newer threats to human health, there is an ever-higher need to control the potential risk factors for ethical deviations in clinical research. Therefore, the vulnerabilities related to the clinical research methodology explored in this review, i.e., those about the obtaining of informed consent, those connected to patients' safety, and those derived from data falsification, need to be continuously monitored and regulated by authorized institutions. The failure to do so would lead to a decrease of public trust in clinical research and virtually erase the line between scientific, validated-through-trials pharmacological agents and any other substance promoted by non-regularized commercial entities. This is a very important and actual topic because psychedelics (LSD included) or other substances with a risk of addiction are nowadays explored again for the use of various mental disorders, while various therapies with innovative mechanisms (e.g., focused on immune factors or gene modulation) are also emerging as potential solutions for multiple, psychiatric or somatic, illnesses [70-75]. Therefore, a continuous updating of ethical guidelines is needed to protect the participants in clinical trials from any type of abuse and to allow the investigators to work in a legally secure environment.

CONCLUSION

Pharmacological research activity is an essential component of the approach aimed at increasing the quality of medical practice by multiplying the existing therapeutic options for a specific medical condition. Evolution in the field of medicine is impossible to achieve, at least taking into consideration the current level of scientific knowledge, without clinical trials. Through this type of activity, progress in pharmacology is ensured, and the quality of patients' lives is increased while their chances of functional recovery and prognosis are improved.

Ethical and legal risks in clinical research derive mainly (but in no way exclusively) from (1) irregularities in obtaining informed consent of participants in the study, (2) failure of investigators to respect the safety of participants in the clinical study, and (3) incorrect reporting of data obtained from the research.

Controlling these risks involves respecting the stages of professional training of clinical trial investigators, as well as the efficient functioning of institutional control mechanisms in the field of pharmacological research.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, O.V, A.M.C.., and A.G.M.; methodology, B.M.P.; formal analysis, O.V, A.M.C.; investigation, O.V., A.M.C., B.P.M., A.G.M.; resources, O.V.; data curation, A.M.C..; writing—original draft preparation, O.V., A.M.C.; writing—review and editing, O.V., A.M.C., B.M.P.; visualization, A.G.M.; supervision, O.V., A.M.C.; project administration, O.V. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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REVIEW

Combatting HIV/AIDS in the Armed Forces: A Literature Review

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Abstract: The Human Immunodeficiency Virus (HIV) and the Acquired Immunodeficiency Syndrome (AIDS) remain significant public health concerns worldwide. This review seeks to present an updated perspective on the spread, consequences, and control measures of HIV/AIDS, focusing on military personnel as a particular at-risk group. It starts by outlining general information about the transmission of the virus and summarizing recent global epidemiological trends. This review further explores the difficulties that HIV/AIDS presents in military settings, including policies regarding the management of service members living with the virus. Additionally, it examines several prevention strategies implemented by militaries around the world, such as educational programs, provision of condoms, and efforts to reduce the stigma associated with the infection.

Keywords: HIV; AIDS; military population; prevention; treatment; management

INTRODUCTION

The Human Immunodeficiency Virus (HIV) was first presented in 1983 as a non-transforming retrovirus [1]. This virus targets CD4+ T lymphocytes and is responsible for Acquired Immunodeficiency Syndrome (AIDS) in individuals with the infection [2]. This condition is characterized by two human lentiviruses: HIV-1 and HIV-2, with transmission occurring exclusively from human to human. Typically, in the absence of a treatment, HIV-1 infection can lead to AIDS, a syndrome associated with significant mortality rates [3]. Both HIV-1 and HIV-2 are RNA viruses that carry the necessary information for the synthesis of the viral proteins essential for the replication and assembly of the virus [4].

HIV transmission occurs either horizontally or vertically [5]. Vertical transmission is the most common way for children to acquire the infection, either in utero, during childbirth, or through breastfeeding [5,6]. Horizontal transmission involves needle sharing or unprotected sexual contact [5,7,8].

One population that has been identified as vulnerable to acquiring HIV infection is military personnel, due to frequently reported high-risk sexual behaviour [9,10] or to their high mobility and exposure to biologically foreign environments [11,12].

Aim

This literature review explores the challenges faced by military populations in the context of HIV/AIDS and presents prevention strategies and proposed solutions for effective prevention and control in this high-risk group.

MATERIALS AND METHODS

This paper examines the medical literature concerning HIV/AIDS, as well as the public information disseminated by state agencies regarding programs and strategies aimed at preventing HIV transmission among military personnel.

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RESULTS

HIV/AIDS: General Overview and Key Statistics

At the end of 2022, the World Health Organization reported that 39 million people were living with HIV, including 1.5 million children. That year, 1.3 million people contracted the virus, reflecting a 38% decline compared to 2010, when 2.1 million new cases were reported [13].

Concerning HIV-related mortality, 2022 saw 630,000 deaths attributed to the virus, down from an estimated 1.3 million deaths in 2010. This represents a 51% decrease over a 13-year period.

In terms of treatment, around 29.8 million people received antiretroviral therapy (ART) in 2022. Among those infected that year, 86% were aware of their HIV status, 76% had begun treatment, and 71% had successfully achieved viral suppression [13].

The African continent remains the most affected globally by HIV, with an estimated 25.6 million individuals living with the virus. Within this group, 90% are aware of their status, 82% receive antiretroviral therapy (ART), and 76% have achieved suppressed viral loads. The region has also seen a significant drop in incidence per 1,000 uninfected individuals. Along with a 56% decrease in AIDS-related deaths since 2010, down to an estimated 380,000 annually [13].

The European Region has an estimated 3 million people living with HIV. Of these, 72% know of their status, 63% are undergoing treatment, and 60% have achieved suppressed viral loads. Roughly 1.9 million individuals are on ART. However, Europe has experienced a rise in HIV incidence over the past decade, from 0.16 to 0.20 per 1,000 uninfected people, alongside approximately 180,000 new infections reported in 2022. AIDS-related mortality has also increased by 37% since 2010, with an estimated 52,000 deaths recorded in 2022 [13].

In Romania, according to the latest data, 716 people were diagnosed with HIV/AIDS in 2023. Over the period from 1985 to 2023, a total of 18,389 individuals have been recorded as having the disease, with 8,556 losing their lives due to HIV-related causes [14].

Epidemiology of HIV in Military Populations

Military populations represent a unique demographic in the global HIV epidemic [11,12], being predominantly composed of young adults [15,16] and often deployed to environments where risk behaviors may increase - factors shown to contribute to new infections among U.S. Army Soldiers [17].

HIV incidence differs substantially across regions, with particularly high rates observed in certain areas of sub-Saharan Africa and Southeast Asia. In the United States, while overall HIV prevalence is lower, military personnel are not exempt. Despite strict screening protocols, they are not exempt [18]. As of March 2021, over 170,000 U.S. service members were deployed globally, marking the largest international military presence at that time[19]. By June 2024, approximately 166,000 active-duty U.S. troops- representing 12.8% of the total force- were still stationed overseas [20].

In 2021, the HIV incidence in active-duty U.S. military was 21 cases per 100,000 service members, slightly higher than that of the general U.S. population, which was approximately 13.9 per 100.000 [21].

Between 1990 and 2024, the Department of Defense conducted over 46 million HIV screening tests, reflecting an extensive and ongoing surveillance effort aimed at early detection and prevention. A total of 1,581 active-duty service members were diagnosed with HIV between January 2017 and June 2022, with 62% remaining on active duty following their diagnosis [22].

These trends are particularly relevant given the global mobility of U.S. forces and their deployment in diverse environments, often under operational stress or during humanitarian missions, which may complicate prevention efforts [23].

In sub-Saharan Africa, HIV/AIDS has disproportionately impacted military personnel compared to civilians. This increased vulnerability is linked to demographic factors- mostly young, sexually active men- and the specific conditions of military service, such as extended time away from home, deployment to unstable regions, and social dynamics that encourage risky sexual behavior [15,16,24].

In 2014, Lloyd et al. reported that the prevalence of HIV among military men from sub-Saharan Africa and other low-income settings was notably higher compared with civilian men of reproductive age, and even higher among longer-serving service members [25]. In 2024, Grillo et. al revealed that military members were frequently involved in dense HIV transmission networks, alongside the civilian population and female sex workers [26].

The Perspective on HIV/AIDS in the Romanian Army

Military personnel infected with HIV but without any functional deficiency (e.g., those who are asymptomatic, experiencing acute retroviral syndrome, or presenting with generalized persistent lymphadenopathy) or who have only low functional impairment (e.g., clinical manifestations due to HIV infection; constitutional symptoms; herpes zoster; idiopathic thrombocytopenic purpura; or peripheral neuropathy) are not classified as having a disability and are allowed to continue performing their military duties [27].

Risk Factors for HIV in the Military

Military populations are considered to be at an elevated risk for acquiring HIV infection [12] due to exposure to a multitude of risk factors. The composition of military forces- mostly young, sexually active males- places them in a higher-risk demographic compared to the general population [25]. Frequent deployments that often result in extended separation from stable partners may further increase the tendency to engage in unsafe sexual practices [28]. The mobility of military troops and exposure of personnel to high-prevalence settings also contribute to this risk [11, 26].

Sexual risk behavior is an essential driver of transmission. In 2015, Hakre et al. reported that among HIV seroconverters in the U.S. Army between 2012 and 2014, most reported multiple sexual partners, inconsistent condom usage, and casual sexual contact or anonymous partners in the year before diagnosis [10].

A culture of masculinity and peer pressure has been reported to contribute to the encouragement of sexual risk-taking behaviors among military personnel [29], influencing HIV-related practices and increasing vulnerability to infection [30].

Tran et al. reported in 2019 that in the Armed Forces of the Republic of the Congo, alcohol consumption, commonly reported among active-duty soldiers, has also been shown to encourage risky sexual habits such as a reduction in condom usage; an increase in transactional sex and multiple sexual partners compared to the lower risk peers [31]. Similar patterns were reported on by Harbertson et al. in 2019, in the U.S. Marine Corps, where a reduction of condom use was associated with alcohol abuse or drug use to enhance sexual experiences [32].

Institutional barriers that may delay diagnosis, prevention, and linkage to healthcare within the military population include concerns over confidentiality and fear of stigma or professional consequences [33], furthering a decrease to essential social and medical care, such as ART [34].

HIV Prevention Strategies and Program Implementation in Military Settings

HIV epidemiology and prognosis have improved substantially due to the universal use of ART, the availability of medications that are more effective and better tolerated by patients [35], and the introduction of preventive measures such as pre-exposure prophylaxis (PrEP) [36]. With a timely diagnosis followed by early initiation of ART, the life expectancy of individuals living with HIV has significantly increased [37, 38].

The Department of Defense (DoD) in the United States of America offers the TRICARE program as part of its efforts to reduce the spread of HIV. This program includes a range of services aimed at both HIV prevention and care. Key initiatives include coverage for pre-exposure prophylaxis (PrEP), as well as promoting access to treatment options such as ART. Additionally, TRICARE provides counseling and support services, and it covers HIV testing for beneficiaries identified as being at risk for contracting the infection [23, 39].

Nonetheless, public health initiatives still encounter obstacles. Misconceptions about HIV transmission are prevalent among people of reproductive age in developing countries and represent a barrier to effective prevention efforts [40,41]. Promoting medical education and raising awareness around HIV-related stigma may have a positive impact on lowering risky sexual behaviors, particularly among military populations [42, 43].

The Department of Defense HIV/AIDS (DHAPP) of the United States of America implements an extensive HIV/AIDS prevention program with various strategies and interventions. These include index case HIV testing, periodic updates to military HIV policies, promotion of condoms, and facilitating access to them. DHAPP also conducts training to prevent stigma and discrimination related to HIV/AIDS [44].

In 2011, Singh and Banerjee conducted a survey among regimental officers regarding hiv prevention in the armed forces and found that there were difficulties in program implementation, especially in the promotion of condom use [45]. In 2015, Tran et al. reported that distributing free condoms to military personnel with improved packaging and a more pleasant scent led to an increase in usage among servicemembers, thereby enhancing efforts to prevent sexually transmitted infections within the military community [46].

Anastario et al. conducted in 2012 a pilot study in a military unit in Barbados, where a modified version of the Popular Opinion Leaders (POL) intervention was used. This intervention involved selecting and training Popular Opinion Leaders within the military personnel to promote conversations on condom use. As a result, there was a noticeable rise in condom use among military personnel after the intervention [47].

CONCLUSION

HIV/AIDS continues to pose a serious health challenge on a global scale, even as progress has been made in recent years. The prevalence of the disease remains a significant social and public health concern, with millions of individuals affected worldwide.

In military settings, targeted prevention strategies are particularly important due to the specific occupational and behavioral risks associated with service. Various armed forces have adopted structured programs aimed at limiting transmission, supporting personnel living with HIV/AIDS, and addressing stigma within military environments.

By focusing on education, encouraging risk-reduction behaviors, and ensuring access to services such as PrEP and ART, military institutions play a key role in HIV control. Sustained implementation of these measures can help transition HIV/AIDS from a widespread public health threat to a manageable chronic condition.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

All authors have read and agreed to the published version of the manuscript

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ORIGINAL ARTICLES

Morphological characteristics of the celiac-mesenteric trunk

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Abstract: *The present paper describes the morphological characteristics of 12 celiac-mesenteric trunk cases highlighted by angiography-computed tomography (CT), characteristics met only in male cases (1.82% of the cases). In relation to the vertebral column, the origin of the trunk was found in the the upper half of the L1 vertebra – intervertebral disk between L1 and L2 vertebrae. At the level of its origin from the aorta, the celiac-mesenteric trunk had an external diameter with values ranging from 8.7-13.4 mm, the aortic ostium of the celiac-mesenteric had a vertical diameter ranging from 8.8 to 13.1mm, the horizontal diameter values ranging from 8.8 to 11.2mm. At the level of its origin, the celiac-mesenteric trunk and the aortic wall had an angle with values ranging from 30.0o to 90.2o. The length of the celiac-mesenteric trunk had values ranging from 21.8 to 42.5 mm, most frequently met values were ranging from 30.7 to 33.5mm. At the level of its bifurcation, the celiac-mesenteric trunk had an angle with values ranging from 82.2 to 120,7, most frequently met values were over 90o. The celiac trunk resulted from the celiac-mesenteric bifurcation had an exterior diameter of 6.2 – 10.2 mm, values that in relation to exterior diameter the celiac-mesenteric originated, it represented 65.57 – 92.47% of its external diameter. The celiac trunk up to the end of its ramification had a length with values ranging from 6.3 to 16.8 mm. In all cases being a hepatosplenic trunk, the left gastric aorta originated in the abdominal aorta in 10 cases (83,3% of the cases) and in the other 2 cases, the left gastric aorta originated in one case under the end bifurcation of the celiac trunk whereas the other case in the celiac-mesenteric trunk, before its end bifurcation. The superior mesenteric artery resulting from the ramification of the celiac-mesenteric trunk had an external diameter with values ranging from 4.4 to 8.5 mm that represented 44.44 – 85.06% of the external diameter of the celiac-mesenteric trunk.*

Keywords: *celiac-mesenteric trunk – morphological characteristics*

INTRODUCTION

The celiac trunk and the superior mesenteric aorta are collateral branches of the abdominal aorta that are originating independently from its trunk, assuring the vascularization of the biggest part of the digestive tube and its annexed glands as well as the vascularization of the spleen. There have been cases where both arteries originated from the aorta through a common trunk, the celiac-mesenteric trunk, with frequencies of [1] and 3% [2, 3] – most authors pointing a percentage between 1.0 and 2.5% of the cases. According to [4], during the embryonal development, out of the 4 roots of the omphalomesenteric artery, roots 2 and 3 will disappear and the persistent root will unite by longitudinal anastomosis resulting into left gastric artery. The persistent root will give birth to the celiac trunk and the other one will issue the superior mesenteric artery. In the case where the first and the fourth root disappear, then the celiac-mesenteric trunk will originate.

MATERIAL AND METHODS

The material for this study comprised of 16 angiography-computed tomography (CT), performed with GE LightSpeed VCT high image resolution 64-slice CT system and GE LightSpeed 16- slice CT system, both systems within the

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premises of the Clinical Emergency County Hospital "Sf. Andrei" in Constanta. The above-mentioned 12 cases were studied out of 2220 patients that presented for a specialist consult for various medical reasons, during the period between July 2012 and June 2016, in Dobrogea area of the country, south - eastern part of Romania. From the total number of patients, 830 were females (37.39%) and 1390 were males (62.61%). The study was conducted with respect to the following parameters: the level of origin of the celiac-mesenteric trunk from the aorta in relation to the vertebral column, the trajectory and the angle formed from the origin of the celiac-mesenteric trunk with the aortic wall, the exterior diameter as well as the endovascular diameter of the celiac-mesenteric trunk at the level of its aortic origin, its length up to its bifurcation, the angle formed between its two terminal branches as well as the diameter and the trajectory.

RESULTS

In the present study, we met 12 cases with celiac-mesenteric trunk (0.54% of the total cases), all male cases (0.86% of the male cases). In relation to the vertebral column, the origin of the trunk was located in the upper half of the L1 vertebra – intervertebral disk between L1 and L2 vertebrae. In 7 cases (58.33% of the cases) the origin was located at the level of the upper half of the L1 vertebra, most frequently near the upper margin of the vertebral body. In 3 cases (25% of the cases), the celiac-mesenteric trunk originated in the lower half of the L1 vertebra, whereas in 2 cases (16.67% of the cases) it originated at the level of the intervertebral disk L1 – L2.

The celiac- mesenteric trunks originating from the aorta was located higher than the origin of the renal arteries, at a distance of 8.9 – 20 mm from the right renal artery, respectively 9.2 – 22.8 mm from the left renal artery. This indicates that the right renal artery originates most frequently from the aorta than the left renal artery.

The celiac-mesenteric trunk originating from the aorta had an external diameter with values ranging between 8.7 to 13.4 mm, in 8 cases (66.67% of the cases) with a diameter ranging between 11.4 and 13.4 mm. By comparing the external diameter of the celiac-mesenteric trunk with the external diameter of the abdominal aorta at the level of its origin, we have discovered differences in values, ranging from 9.3 to 13.8 mm between the two diameters, representing 40.09 – 58.01% of the external aortic diameter.

The aortic ostium of the celiac-mesenteric trunk had a vertical diameter with values ranging from 8.8 to 13.1 mm – the horizontal diameter ranged between 8.4 to 11.2 mm. In all cases the aortic ostium had an oval shape. In 8 cases (66.67% of the cases) the long axis was horizontal and in 4 cases (33.33% of the cases), the axis was vertical. By comparing the vertical diameter of the aorta, the ostium trunk had a vertical diameter with values ranging between 34.92 to 55.26% of the aortic one, whereas in the case of the horizontal diameter it presented with values ranging between 41.79 to 54.37% of the horizontal aortic diameter.

At the level of its origin, the celiac-mesenteric trunk created and angle with the aortin wall, with values ranging between 30° to 90.2° – extreme values had been found in one for each value, most frequent value for the angle being of 60°. This explains the trajectory of the celiac-mesenteric trunk, most frequently position being transverse anterolateral to the right, an aspect met in 10 cases (83.33% of the cases) and only 2 cases having a horizontal trajectory (transversal) presenting with an angle of 90,2° respectively 90,3°.

The length of the celiac-mesenteric trunk had values ranging between 21.8 to 42.5 mm, most frequently met values ranging from 30.7 to 33.5 mm.

The bifurcation of the celiac-mesenteric trunk into the two arteries had an angle with values ranging from 82.2° to 120.7°, most frequently met values being over 90° (83.33% of the cases). In the case that, after the bifurcation, the superior mesenteric artery had a transverse anterolateral to the right trajectory, the celiac trunk would always be ascending to the right, sometimes close to a vertical position.

The celiac trunk resulting from the celiac-mesenteric bifurcation had an external diameter with values ranging from 6.2 to 10.2 mm, extreme values have been met in only one case for each value; the rest of the cases with values ranging between 8.0 to 9.2 mm. In relation to the external diameter of the celiac-mesenteric trunk from which it originated, the celiac trunk had an external diameter with smaller values by 0.7 to 4.2 mm, representing 65.57 – 92.47% of its external diameter.

The length of the celiac trunk up to its ending ramification had values ranging between 6.3 to 16.8 mm – extreme measures were found in only one for each case. All cases presented with a hepatosplenic trunk with the left gastric artery originating from the abdominal aorta in 10 cases (83.33% of the cases), at a distance of 15.1 – 18 mm above the origin of the celiac-mesenteric trunk. In one case, the left gastric aorta originated from the terminal bifurcation of the celiac trunk whereas in the other case it originated from the celiac-mesenteric trunk before its ending ramification.

The endovascular ostium of the celiac trunk had a vertical diameter with values ranging from 7.1 to 8.6 mm, with a horizontal diameter of 6.6 to 8.6 mm. In 11 cases (91.67% of the cases) the horizontal diameter was bigger than the vertical diameter by 0.5 – 1.3 mm, thus having an oval-shaped horizontal long axis. In only one case (8.33% of the cases) the diameter of the ostium was bigger vertically than horizontally, with a difference of 0.5 mm thus having an oval shape with a vertical long axis.

The superior mesenteric artery resulted by the ramification of the celiac-mesenteric trunk had an external diameter with values ranging between 4.4 to 8.5 mm, smaller by 1.3 – 5.9 mm, representing 44.44 – 85.06% of the external diameter of the celiac-mesenteric trunk.

Table: Percentage frequency of the celiac-mesenteric trunk

Author	CMT frequency	Differences
Adachi	2.4 %	- 1.86 %
Babu	2.79 %	- 2.25 %
Chen	0.7 %	- 0.16 %
Eaton	0.54 %	0
Ferrari	1.7 %	- 1.16 %
Jones	1.7 %	- 1.16 %
Kornafel	1.5 %	- 0.96 %
Lippert	3 %	- 2.46 %
Lipshutz	2.4 %	- 1.86 %
Matusz	0.68 %	- 0.14 %
Michels	2.5 %	- 1.96 %
Mu CG	0.98 %	- 0.44 %
Natsume	0.60 %	- 0.06 %
Nelson	2.0 %	- 1.46 %
Panagouli	0.76 %	- 0.22 %
Piquand	2.0 %	- 1.46 %
Rio Branco	3.0 %	- 2.46 %
Song	1.06 %	- 0.52 %
Yadov	2.5 %	- 1.96 %
Our study	0.54 % of the total cases	0.86 % male cases

The endovascular ostium of the superior mesenteric artery had a vertical diameter with values ranging from 6.8 to 8.5 mm, with a horizontal diameter of 6.2 to 8.5 mm. In 11 cases (91.67% of the cases) the horizontal diameter was bigger than the vertical diameter by 0.3 – 1.1 mm, having an oval shape with a horizontal long axis. In only one case (8.33% of the cases) the diameters of the ostium were equal, thus having a round shape.

By comparing the external diameters of the celiac trunk and the superior mesenteric aorta, we have discovered that in 11 cases, the superior mesenteric aorta had a smaller diameter than the celiac trunk, representing 47.83 – 83.33% of the diameter of the celiac trunk and only in one case the diameter of the celiac trunk was smaller than the one of the superior mesenteric artery, representing 83.73% of its diameter.

DISCUSSIONS

Analyzing the results, we had and comparing them to the data in the existing literature referring to the morphological characteristics of the celiac-mesenteric trunk, we have discovered there is no thorough description of these, most authors mentioning only the frequency as a vascular variation.

By comparing the results of the study with the data existing in the literature we had the possibility to consult, we discovered that our results were the same with the results of [1], whereas in the rest of the cases we found differences with values ranging from 0.06 to 2.46%. Compared with the statistics of [5, 6, 7, 8, 9, 10] our results were smaller with values ranging between 0.06% [5] and 0.52% [10]. Compared with [11] the results we had were smaller by 0.96% and values ranging from 1 to 2.25% we most frequently met [Adachi, cited by 8, 12, 13, 14, 15, 16, 17, 18, 19]. Babu [19] is the only author mentioning the frequency of the celiac-mesenteric trunk in both genders – our results on male cases being smaller by 0.46%. Unlike our study where the presence of the celiac-mesenteric trunk was met only in male cases, Babu [19] discovers the presence of celiac-mesenteric trunk having a wider spread within female cases (1.47% of the cases). In relation to the statistics of [2, 3], the results are lower, the difference being by 2.46%. Lippert [3] discovers the presence of the gastro-hepato-spleno-mesenteric trunk in 2% of the cases, a difference of 1.95% from our study, and the hepato-spleno-mesenteric trunk with the origin of the left gastric aorta from the aorta in 1% of the cases, a difference of 0.55% from our study. Babu [19] finds that in 3 cases (15.79% of the met celiac-mesenteric trunks), the origin of the left gastric aorta was in the celiac trunk resulted from the celiac-mesenteric bifurcation at the level of the abdominal aorta. In a case described by Anupama [20], from the celiac trunk resulted the hepatic artery, the splenic artery, the left gastric artery and a branch that participated in formation of the gastro-duodenal artery. In the specialist literature, we have not met celiac-mesenteric trunk cases where the left gastric artery originated anteriorly to the ending ramification of the celiac-mesenteric trunk, closer to its origin.

The length of the celiac-mesenteric trunk given by Anupama [20] is of 13.00 mm, Tugrul [21] specifics a length of 13.42 mm, both values being smaller than the ones we found in our study by 8.80mm, respectively 8.38 mm smaller than the minimum length and 29.5 mm, respectively 29.08 mm smaller than the length we have measured in our study.

With regards to the diameter of the celiac-mesenteric trunk, Tugrul [21] finds an external diameter of 13.98 mm, a diameter bigger by 0.58 mm than what we have measured for the maximum value and with 5.28mm bugger than the minimum diameter. Compared to our results Tugrul [21] finds the celiac trunk resulting from the celiac-mesenteric bifurcation with a diameter (7.09 mm) bigger by 0.89 mm from the minimum diameter and smaller by 3.11 mm from the maximum value of the diameter we have measured. At the level of the superior mesenteric artery Tugrul [21] finds a diameter of 5.25 mm, bigger by 0.86 mm from the minimum diameter and smaller by 3.25 mm than the values of the maximum diameter we have measured in the study. Hemanth [22] finds an external diameter of 8.7 mm for the superior mesenteric artery, bigger by 4.3 mm than the minimum diameter, respectively by 0.2mm than the maximum value within our study.

CONCLUSION

There is a relatively low frequency of celiac-mesenteric trunk cases described in the literature, our case having the lowest frequency. The differences between different authors is related to the number of cases they have studied and due to the fact that cases where the hepatic artery was the only one originating from the trunk weren't taken into consideration as well as the methods used for the research. There is a difference between the classical methods (dissections, intramuscular injections) in relation to the modern methods, especially MDCD that has better results than the conventional angiography [17, 23]. With modern exploration methods, it has been noted that a greater number of vascular variants and malformations have been observed than those reported in the specialist literature involving other methods. We could add as a cause to the differences and the probable existence of some peculiarities related to the geographical area, which exist between authors who carried out their research on different meridians of the world.

We consider the name of vascular malformation used by some authors when talking about the celiac trunk is not the correct name, whereas vascular variant seems a more appropriate name since all vascular branches of the two arteries are present, having the same morphological characteristics as in the case where they originated independently from the aorta, also because they don't affect the vascular territories they serve.

The present study is interesting not only for a morphologist but also, knowing the different types of anatomical variants is fundamental for the planning of abdominal surgical procedure and is important to the radiologist as well, as specified by [10, 23].

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REVIEW

The Effect of Biological Therapies on Psoriasis and Its Associated Comorbidities – New Facts and Future Options

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Abstract: Psoriasis is a chronic and complex disease that consists of characteristic cutaneous lesions and is frequently accompanied by systemic comorbidities caused by the chronic inflammatory state. The most common such comorbidities are hypertension, diabetes mellitus, dyslipidemia, inflammatory bowel diseases, depression, anxiety, neoplasia, and non-alcoholic fatty liver disease. Therefore, it is of utmost importance to simultaneously treat both psoriasis and the associated conditions with the best therapies available to improve patients' life quality. Therefore, this review aims to evaluate the effects of biological therapies on both psoriasis and its comorbidities and also to highlight other future options.

Keywords: psoriasis, chronic inflammation, biological therapies

INTRODUCTION

Psoriasis is a chronic, immune-mediated inflammatory disease that affects approximately 125 million people, or around 2% of the world population [1-3]. It has a bimodal incidence, with peaks between 16-20 years and between 57-60 years, with almost 70% of the cases before the age of 40 years [4]. On the other hand, due to an increase in life expectancy, the number of patients with psoriasis who are over 65 years is increasing, and that represents a therapeutic challenge, as the treatment has to be modulated in order to prevent drug interactions and not interfere with other comorbidities [4].

Psoriasis is mainly characterized by specific cutaneous lesions, but also by systemic involvement, the most frequent associated comorbidities being hypertension, diabetes mellitus, dyslipidemia, depression, anxiety, neoplasia, and non-alcoholic fatty liver disease [1,5]. The most common form of psoriasis is psoriasis vulgaris, which consists of well-defined erythematous plaques covered with silvery scales, most often found on knees, elbows, and scalp [6,7]. Other possible forms are guttate psoriasis, which affects most frequently children and young people, usually after streptococcal infections, and consists of small, teardrop lesions on trunk and extremities, pustular psoriasis, a rare form, which is characterized by sterile pustules, and inverse psoriasis, which affects flexion surfaces [7,8]. In severe cases, psoriasis can evolve toward an erythrodermic form affecting over 90% of the body surface, a condition that requires urgent medical intervention [9]. The pathophysiologic mechanisms are represented by an abnormal activation of the immune system, especially of T helper lymphocytes – Th1 and Th17, the next step being the production of proinflammatory cytokines like tumor necrosis factor alpha (TNF-α), interleukin 17 and 23 [10]. The result is the proliferation of keratinocytes and local inflammation, with the formation of the characteristic psoriasis plaques [10].

The diagnosis is mainly clinical, but there are also a few methods that can help in confirming the diagnosis, like reflectance confocal microscopy, which can show an increase in the size and number of dermal papillae, along with a dilation of capillary vessels, and dermoscopy [11,12].

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In this review, we aim to evaluate the psoriasis-associated comorbidities and to identify the best therapeutic approach in order to treat the patient as a whole.

PSORIASIS-ASSOCIATED COMORBIDITIES

The main link between psoriasis and all the comorbidities involved is the systemic inflammation, mediated by pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), and interleukin (IL) 17 and IL-23 [2]. This inflammation process involves mainly the vessels, aggravating atherosclerosis and making the cardiovascular diseases the most important comorbidities in patients with psoriasis, leading to a decrease in life expectancy of approximately 4-5 years [13-15]. Also, the risk of cardiovascular diseases is increased by over twofold in patients with psoriasis, independently of other risk factors, especially in severe forms and in patients under the age of 50 years, the mechanism involved being vascular inflammation that affects mainly coronary arteries and promotes early atherosclerosis [3,13,16]. Another very important aspect is that dyslipidemia, characterized by high levels of triglycerides and low-density lipoprotein cholesterol (LDL) and low levels of high-density lipoprotein cholesterol (HDL), has an essential role in the relationship between psoriasis and cardiovascular diseases [17]. Therefore, a correct management of the lipid levels is very important in order to lower the cardiovascular risk [17].

Obesity is another important comorbidity with a greater prevalence in patients with psoriasis, the severity of the last one being proportional to the body mass index [18]. The relationship between obesity and psoriasis is bidirectional. Psoriasis, due to chronic inflammation, leads to insulin resistance, promotes visceral fat deposition, and, in severe forms, limits mobilization, all of which increase the risk of becoming overweight. On the other hand, obesity is associated with a great number of pro-inflammatory adipokines, like leptin, and a low number of anti-inflammatory ones, an imbalance that aggravates local and systemic inflammation and also increases the risk of psoriasis by over one and a half times [18-21]. Another fact worth mentioning is that obesity can reduce the treatment efficiency due to a larger distribution volume and, therefore, indirectly aggravate the severity of psoriasis [19]. Moreover, almost 50% of the patients with psoriasis have metabolic syndrome, defined as the presence of at least three of the following: abdominal obesity (waist at least 102 cm in men and at least 88 cm in women), insulin resistance or increased level of blood glucose (at least 100 mg/dL), dyslipidemia (tryglicerides at least 150 mg/dL and/or HDL under 40 mg/dL in men and under 50 mg/dL in women), arterial hypertension (over 130/85 mmHg) [22]. The presence of metabolic syndrome is very important, as it is linked with a greater prevalence of cardiovascular diseases, fact that creates a vicious circle [20]. In addition, osteoporosis is more frequently encountered in patients with psoriasis due to the activation of the osteoclasts by the pro-inflammatory cytokines, and it also aggravates the prognosis [23].

Diabetes mellitus is one of the most frequent diseases worldwide and also a common comorbidity associated with psoriasis, both by chance and by similar pathophysiological mechanisms, the first link between these two diseases being noted as early as 1897 [14,24]. As it was already mentioned, psoriasis is characterized by systemic inflammation that can lead to insulin resistance and promote visceral fat deposition, mechanisms that can increase the risk for developing diabetes by up to two times compared with the general population [22]. Moreover, diabetes may be induced in certain patients with psoriasis by the use of corticosteroids in an attempt to control the severity of the disease, especially if other therapies are lacking [23].

Regarding mental ailments, patients with psoriasis have a greater risk than the general population, especially for depression, anxiety, and suicide [2]. The explanation is that the presence of cutaneous lesions leads to shame, low self-esteem, and isolation from other people, all of which result in a reduction of the quality of life [2]. In addition, a low level of melatonin is very frequent in this category of patients and may contribute to depression by inducing changes in the sleep pattern [25].

Table 1: Common hepatic adverse reactions of classical psoriasis treatment

Treatment	Effects on the liver	Sources
Methotrexate	• significant toxicity • can induce hepatic fibrosis and cirrhosis if used long time, especially in NAFLD	[32,33]
Cyclosporine A	• can increase lipid levels and favor hepatic steatosis • treatment should be carefully monitored in NAFLD	[34]
Acitretin	• can aggravate dyslipidemia and NAFLD in patients with metabolic syndrome	[35]
Dimethyl fumarate	• has a better safety profile and can reduce hepatic inflammation and oxidative stress • however, the liver function should be monitored carefully	[36]

NAFLD – non-alcoholic fatty liver disease

Last, but not least, one of the most common comorbidities recently associated with psoriasis is non-alcoholic fatty liver disease (NAFLD) [26]. It consists of excessive deposition of lipids in the liver that is not caused by alcohol consumption and that can progress to non-alcoholic steatohepatitis (NASH) and cirrhosis [27]. Epidemiological studies indicate that the prevalence of NAFLD is three times higher in this category of patients and also that the severity of psoriasis is proportional to that of NAFLD [28-30]. Moreover, an interesting study has revealed that almost 47% of the patients with psoriasis had NAFLD, and 22% even had biopsy-confirmed NASH

[29]. The diagnosis of NAFLD is established through imagistic methods like abdominal echography and elastography and laboratory tests for liver function, and fully confirmed by liver biopsy [31]. It is worth mentioning that NAFLD has a significant impact not only on the liver but also on other systems, including the heart, kidneys, and thyroid gland, which have to be carefully evaluated [29]. Thus, the combination of psoriasis and NAFLD leads to a greater cardiovascular risk and mortality, emphasizing once again the importance of treating the patient as a whole [28]. However, some of the therapies available for psoriasis can have a direct impact on the liver and be dangerous, especially if patients already have NAFLD [31]. Table I highlights the most common adverse hepatic reactions.

The dermatologist has an essential role in the early identification of psoriasis-associated comorbidities. The general management consists of carefully evaluation of the liver function and of cardiovascular risk using physical examination – body mass index calculation, arterial pressure, signs of dyslipidemia like the presence of xanthomas, imagistic methods – abdominal echography, liver elastography, coronarography in selected cases and laboratory tests, a wise choice of medication, especially in the presence of NAFLD, where biological therapies are the first recommended, and inclusion of non-pharmacological treatment options like a healthy diet with anti-inflammatory products and physical activity [13,17,18,27].

However, an integrated approach is mandatory for a correct and complete treatment, the most commonly involved medical fields being cardiology, gastroenterology, and psychology [37].

TREATMENT OF PSORIASIS AND ITS ASSOCIATED COMORBIDITIES

The treatment of psoriasis has significantly evolved in the last century, from empirical therapies to modern biological medications based on a more profound understanding of the pathophysiological mechanisms [19]. Initially, between 1920 and 1950, the treatment of psoriasis was focused on the clinical aspect, and the most common medications included arsenic, coal tar, salicylic acid, and dithranol [19]. In the 1950s, significant progress had been made by the development of topical corticosteroids, a very efficient form of medication, that rapidly became the gold standard of the treatment due to anti-inflammatory properties, but which lacked specific targets [38]. Moreover, if used extensively, adverse reactions occurred in the form of skin atrophy or tachyphylaxis [39]. A more important step toward better control of psoriasis was made when methotrexate and cyclosporine were discovered, both of them used in severe forms of psoriasis, but are also limited by the risk of significant hepatic and kidney adverse reactions or toxicity [32].

Table 2: Treatment of psoriasis: benefits on comorbidities, risks, and side effects

Medication	Benefits on comorbidities	Risks	Side effects	Sources
Methothrexate	↓systemic inflammation ↓cardiovascular risk	can aggravate NAFLD	hepatotoxicity	[32,33]
Cyclosporine	rapid control of severe psoriasis ↓ inflammation	arterial hypertension dyslipidemia	nephrotoxicity	[34]
TNF-α inhibitors (Infliximab, Adalimumab, Etanercept, Certolizumab)	↓ inflammation ↓cardiovascular risk ↓ risk of hepatic fibrosis ↓ insulin resistance first line in psoriatic arthritis	↓ efficiency in overweight patients modest weight gain	infections, especially TB autoimmune diseases: SLE or arthritis hepatotoxicity congestive HF rare: lymphoma and skin cancer	[49]
IL-17 inhibitors (Secukinumab, Ixekizumab, Brodalumab)	↓ inflammation improve dyslipidemia of choice in overweight patients	may ↑ IBD	fungal infections, especially local/systemic candidosis and respiratory infections pain, erythema, edema at the site of the injection rare: autoimmune diseases – SLE-like, anterior uveitis	[44,50]
IL-23 inhibitors (Guselkumab, Risankizumab, Tildrakizumab)	↓ inflammation ↓ insulin resistance improve hepatic function very good in the long term, with no negative impact on comorbidities	none	respiratory tract infections, especially viral pain, erythema, and edema at the site of the injection rare – autoimmune diseases – less frequent than with TNF-α or IL-17 inhibitors	[50]
Dual IL-12/23 inhibitors (Ustekinumab)	↓ vascular inflammation improve endothelial dysfunction	none		[51,52]
Small molecules (Apremilast)	↓ systemic inflammation improve glycemic metabolism modest weight loss, which can be of benefit	diarrhea		[53,54]

HF – heart failure; IBD – inflammatory bowel disease; IL – interleukin; NAFLD – non-alcoholic fatty liver disease; SLE – systemic lupus erythematosus; TB – tuberculosis; TNF-α – tumor necrosis factor alpha; ↓ - lower; ↑ - increase/aggravate

The true revolution of psoriasis treatment, especially of the severe forms, was provided by biological medication, which targeted specific inflammatory cytokines and modulated the pathophysiological mechanisms involved [40]. However, significant side effects are also common, therefore, a good collaboration with other subspecialities is mandatory [40]. The main representatives of this therapeutic class are: TNF-α inhibitors, IL-17 inhibitors, IL-23 inhibitors, and dual IL-12/23 inhibitors [41,42]. Of particular importance regarding comorbidities are the effects of these therapies on atherosclerosis, as a main cardiovascular risk factor. It is evaluated using the thickness of the intima and media of the vessels as measured by echography, the pulse-wave velocity, the aspect of the atherosclerotic plaques by CT angiography, and the level of C-reactive protein [43]. For example, it was revealed that IL-17 inhibitors like secukinumab significantly reduce the thickness of the intima and media of the vessels and also the arterial rigidity, much more than classical treatments like methotrexate and cyclosporine [44]. On the other hand, they seem to not affect the vascular inflammation evaluated by PET/CT or on metabolic markers like insulin or adiponectin [43]. Further studies are necessary to better explore these effects, especially in the long term, in order to simultaneously reduce both the severity of psoriasis and of cardiovascular risk, eventually by using therapies that target multiple cytokines at the same time [45].

As was mentioned before, another very frequent psoriasis-associated comorbidity is obesity, with almost 50% of the patients with psoriasis having metabolic syndrome [18,21,22]. Hence, a wise choice of medication is mandatory, especially because not all the therapeutic classes are effective in this context [13,37,46,47]. For example, TNF-α inhibitors have low efficiency in overweight patients and can even lead to an increase in weight [37,46,47]. IL-17 inhibitors, IL-23 inhibitors, and also small molecules (Apremilast) are of choice in this situation, due to their improvement in dyslipidemia, insulin resistance, and weight loss [47].

Last, but not least, NAFLD is a very important comorbidity that has to be taken into consideration when choosing a therapeutic approach, especially in patients with severe psoriasis, in whom this comorbidity also has a high prevalence [28,29]. Methotrexate or cyclosporine should be avoided if NAFLD is suspected, because they can aggravate it [28,47]. In this context, a much better solution nowadays is represented by the use of biological therapies, especially of TNF-α inhibitors, which can decrease the risk of hepatic fibrosis and decrease inflammation and dyslipidemia [1,48].

For an easier understanding, the most common therapies used in psoriasis are presented in Table 2, along with their benefits on psoriasis-associated comorbidities, risks involved, and the most important side effects.

NON-PHARMACOLOGICAL MANAGEMENT OF PSORIASIS AND ITS ASSOCIATED COMORBIDITIES

As was mentioned before, in this category of patients, it is of utmost importance to adopt an integrative approach, from modification of the lifestyle to a careful selection of the best medications in order to have as many benefits as possible with the minimum of side effects. Interventions on the lifestyle are mainly represented by aerobic exercises for at least 150 minutes per week, which reduce the risk of obesity and also improve the lean mass and the glucidic metabolism, management of the stress and of anxiety, and by adopting a healthy diet [46,55]. This last aspect is very important, as the Mediterranean diet is well documented to reduce inflammation and to prevent metabolic comorbidities [46]. It consists of healthy fats like olive oil, good quality proteins from fish, chicken, or eggs, fibers, vitamins, and minerals from fresh fruits and vegetables, and also of cereals and fermented dairy products [56]. In psoriasis, the Mediterranean diet has many important benefits: it reduces systemic inflammation, insulin resistance, and dyslipidemia and, consequently, reduces the risk of atherosclerosis, obesity, and NAFLD [46,55]. Regarding alcohol consumption, in modest quantities, the red wine has antioxidative properties due to resveratrol, but the frequent use has to be discouraged because it has a negative impact both on the severity of the disease, increasing the levels of pro-inflammatory cytokines like TNF-α and IL-17 that can aggravate the skin lesions and NAFLD, and on the treatment adherence and efficiency [46,57].

Table 3: Plants most commonly used in psoriasis

Plant	Effects	Sources
Aloe vera (Aloe barbadensis miller)	↓ local inflammation ↑ skin hydration prevents exfoliation	[58,64]
Turmeric (Curcuma longa)	– TNF-α – IL-6 ↓ local inflammation	[58,59]
Fish oil (Omega-3)	↓ systemic inflammation ↑ skin hydration ↑ PASI score	[56,58]
Chamomile (Matricaria chamomilla)	soothing skin effects ↓ local inflammation ↓ pruritus	[58,60]
Capsicum (Capsicum annuum)	↓ pain and pruritus ↓ local inflammation sometimes can irritate the skin	[58,65]
Fenugreek (Trigonella foenum-graecum)	↓ local inflammation ↑ skin barrier	[58,66]

IL – interleukin; TNF-α – tumor necrosis factor alpha; ↓ - decrease; ↑ - increase; – - inhibits

Medicinal plants are used in psoriasis as associated therapies due to their anti-inflammatory, antioxidative, and immune modulation properties. Even though the efficiency of these therapies is not well studied, some plants significantly reduced the symptoms of the disease when taken orally or applied topically [58]. Some of these plants are Aloe vera (Aloe barbadensis miller), Curcuma longa, omega-3 rich fish oil, Matricaria chamomilla, Capsicum annuum, and Trigonella foenum-graecum [58]. Aloe vera hydrates the skin and reduces local inflammation, while curcumin inhibits pro-inflammatory cytokines like TNF-α și IL-6 [58,59]. Omega-3 fatty acids from fish oil modulate the immune response and can decrease the severity of cutaneous lesions [56,58]. In addition, chamomile has soothing effects and is frequently topically utilized [60]. Last, but not least, capsaicine, which is a chili pepper extract, has analgesic and anti-inflammatory properties, decreasing pruritus and redness when used topically, while fenugreek can also be used orally, with similar anti-inflammatory and anti-oxidative characteristics [61–63]. On the other hand, future studies are needed to better explore their properties, and, because of this, patients must visit a specialist before taking any of these plants to prevent side effects or drug interactions. These plants and their properties in treating psoriasis are presented in Table 3.

CONCLUSION

Psoriasis is a complex disease, with a multitude of comorbidities that significantly affect the quality of patients` lives. Thus, the concomitant treatment is essential in reducing the risks, and a careful selection of the medication is mandatory, as not to aggravate the pre-existing or newly appeared conditions. The most common psoriasis-associated conditions are cardiovascular diseases, especially due to systemic inflammation that promotes early atherosclerosis, obesity, metabolic syndrome, dyslipidemia, NAFLD, diabetes, mental ailments like anxiety and depression, neoplasia, and inflammatory bowel disease. The greatest mortality is caused by cardiovascular diseases, the prevalence of them being over two times higher in patients with psoriasis. Consequently, pro-active management is essential, using both pharmacological and non-pharmacological measures, ideally in a personalized manner.

The novel, modern therapeutic options, which include biologic therapies, are very promising. They reduce systemic inflammation by targeting specific cytokines involved in the pathogenesis of psoriasis, like TNF-α, IL-17, IL-23, and, therefore, can improve both the cosmetic appearance and other conditions, especially when concurrently used with non-pharmacological interventions. Of them, IL-17 inhibitors seem to be of real benefit in reducing atherosclerosis and ameliorating cardiovascular diseases, the most dangerous risk in psoriasis patients. Also, IL-23 inhibitors and the small molecules (Apremilast) are of choice in overweight patients, decreasing dyslipidemia, insulin resistance, and obesity. Moreover, TNF-α inhibitors reduce the risk of NAFLD.

It can be concluded that nowadays there are many therapeutic options for treating psoriasis, and novel therapies targeting specific pathophysiological mechanisms that can modulate both psoriasis and its associated conditions. However, further studies are necessary to better identify the long-term effects of these modern, biological therapies.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization AFG, RSC, DOC and CC; methodology AFG, RSC, DOC and CC; validation MP, RSC, DOC, and CC; formal analysis AFG and CC; investigation AFG and DLC; resources AFG and DLC; data curation DOC; writing—original draft preparation AFG; writing—review and editing DOC, and CC; supervision MP, DOC and CC; project administration AFG, and DLC. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

Salivary Investigation of the Complex Relationship between NLRP3 Inflammasome, Leptin and Total Antioxidant Capacity in the Context of Periodontal Disease

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Abstract: Periodontal disease is a chronic pathology, resulting from the action of microbial communities that become dysbiotic, accompanied by immune response impairment in periodontal tissues, leading to an inflammatory process that promotes progressive tissue destruction. Abnormal activation of nucleotide-binding oligomerization domain (NOD-), leucine-rich repeat (LRR-), and pyrin domain (PYD)-containing protein 3 (NLRP3) promotes chronic inflammation. Research shows that leptin and oxidative stress (OS) are involved in the activation of the inflammatory pathways, including the activation of the NLRP3 inflammasome. In this context, the main objective of our study was to determine the levels of the NLRP3, leptin, and total antioxidant capacity (TAC) in the saliva of patients with periodontal disease. The present cross-sectional study included 44 patients with periodontitis, while the control group was represented by 19 clinically and biologically healthy adults. Saliva samples were collected from all participants in the study, and salivary levels of NLRP3, leptin, and TAC were determined using the Enzyme-linked immunosorbent assay (ELISA) and commercial kits. The results show that NLRP3 had statistically significantly increased levels in the study group versus the control group ($p< 0.00001$). Although TAC was also increased in the patients compared to the healthy subjects ($p=0.491$), no statistically significant differences were found. On the other hand, leptin levels were significantly lower in periodontitis patients compared to the control group ($p< 0.00001$). The results obtained in the present study are promising, but further studies are needed to obtain a more comprehensive understanding of the complex molecular mechanisms underlying periodontal disease.

Keywords: periodontal disease; NLRP3 inflammasome; leptin; TAC; saliva

INTRODUCTION

Around 700 species of oral bacteria form the oral microbiome [1], a term used by molecular biologist and Nobel Prize laureate Joshua Lederberg [2, 3] to define the mixture of symbiotic, commensal, and pathogenic microorganisms present in our organism [4]. Periodontal disease is a chronic inflammatory disease [5] with origins in the dental plaque [6], being a result of microbial communities that become dysbiotic, corroborated with immune response impairment in periodontal tissues, leading to an inflammatory process that promotes progressive tissue destruction [7, 8].

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In the beginning, the inflammation induced by anaerobic bacteria [6] leads to gingival bleeding, pain, and swelling, which are clinical traits of gingivitis, which affects almost 90% of the population and is considered to be the mildest form of periodontal disease [9, 10]. The progression of the disease, in the lack of appropriate treatment, leads to irreversible attachment loss of the periodontium and alveolar bone destruction, thus causing periodontitis [9], which, if left untreated, results in tooth loss [11, 12].

Periodontal disease affects not only the quality of life of the patients because of the associated symptoms and dysfunctions, but also has a considerable effect on society, even from an economic point of view [13], becoming a global health burden [14]. Moreover, by favoring the transfer of periodontal pathogens and pro-inflammatory agents to other tissues, periodontal disease has been linked to systemic diseases [15], including diabetes and heart disease [9], respiratory infections [16], Alzheimer's disease [17], neurodegenerative diseases[18], autoimmune diseases, and even malignancy [19].

Recent research has shown that the development of periodontal disease is not promoted only by bacterial, but also by viruses, environmental factors (e.g., smoking) and lifestyle of the host, and it involves genetic mechanisms and immune response [5, 7], new studies focusing on the development of alternative therapies such as vaccines or antioxidant agents to improve the prevention of the disease [20].

The activation of inflammasomes represents one of the core pathways linked to inflammatory diseases [21]. The nucleotide-binding oligomerization domain (NOD-), leucine-rich repeat (LRR-) and pyrin domain (PYD)-containing protein 3 (NLRP3) inflammasome [22], found in the cytosol, is a supramolecular complex that comprises receptor protein NLRP3, adapter protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and caspase-1 [23]. These three components of the inflammasome have different functions. While receptor protein NLRP3 can detect endogenous danger signals and enlist downstream targets [23, 24], ASC links NLRP3 to caspase-1, which is involved in cytokine release and pyroptosis (inflammatory cell death) [22, 24, 25] via gasdermin D [24]. NLRP3 can be activated by cellular alterations [23], microbial infections, or environmental stimuli [26]. The activation of NLRP3 leads to protease caspase-1 activation, which in turn promotes the production of cytokines IL-1β and IL-18, thus upholding inflammation [26]. Furthermore, abnormal activation of NLRP3 promotes chronic inflammation, hence being involved in the development of numerous diseases [25], such as cardiovascular diseases [27], metabolic diseases [25], autoimmune diseases [28], cancer [25], periodontal disease [29], and, as it was recently discovered, COVID-19 [30].

So, the NLRP3 inflammasome is responsible for the transformation of IL-1β, one of the most important pro-inflammatory cytokines linked to periodontal disease, into its biologically active form [31]. This mediator of inflammation can induce osteoclast differentiation, thus playing a crucial role in alveolar bone resorption, specific to periodontitis. Moreover, NLRP3 activation mediates osteoblasts' apoptosis via IL-1β and IL-18, affects the fibroblasts in the periodontal ligament via IL-1β and IL-6, and is involved in leukocytes' regulation in inflamed periodontal tissue [32].

Research shows that leptin and reactive oxygen species (ROS) can activate inflammatory pathways, including the NLRP3 inflammasome [33]. Leptin, a peptide hormone produced primarily by adipocytes [34], is involved in the regulation of food intake, additionally having an important role in proinflammatory immune responses [35]. It is also produced by the salivary glands, which are responsible for leptin storage and secretion as well [36]. In periodontal disease, leptin has been shown to be able to regulate the ratio between osteoprotegerin and receptor activator of nuclear factor kappa beta (RANKL) in gingival fibroblasts [37], hence influencing bone remodeling [38]. In a recent study conducted on mice, Han et al revealed that leptin promotes macrophage polarization in periodontitis via NLRP3 inflammasome [39]. Furthermore, leptin was found to promote oxidative stress (OS) [40].

OS has already been proven to be one of the key players in periodontal disease development and progression, contributing to tissue destruction and tooth loss [41]. OS is characterized by the loss of balance between oxidants and antioxidants, promoting the formation of ROS and free radicals, which can play an important role in inflammation, despite their reduced lifespan [42]. In periodontal disease, the activation of the innate immune system produces a storm of proinflammatory cytokines and overproduction of ROS [41]. On the other hand, in chronic inflammation, such as the one present in periodontitis, overproduction of ROS, mainly by neutrophils [43], can not only induce OS, and activate proteolytic enzymes but can also activate different signaling pathways [44].

In order to investigate the complex mechanisms involved in the pathogenesis of this condition, the main objective of our study was to determine the levels of the NLRP3, leptin, and TAC (total antioxidant capacity) in the saliva of periodontitis patients.

MATERIALS AND METHODS

Study groups

The present cross-sectional study included 44 patients with periodontitis, diagnosed according to the 2018 AAP/EFP Classification of Periodontal & Peri-implant Diseases [45, 46].

Inclusion criteria for the study group:

- Presence of at least 14 teeth on both dental arches combined;

- Interdental clinical attachment loss (CAL) is detectable in ≥2 non-adjacent teeth or buccal/ oral CAL ≥3 mm with periodontal pocket depth >3 mm is detectable in ≥2 teeth;

- CAL cannot be attributed to non-periodontal causes such as:

1) gingival recession of traumatic origin;

2) dental caries extending into the cervical area of the tooth;

3) presence of CAL on the distal aspect of a second molar and associated with malposition or extraction of a third molar;

4) a combined endo-periodontal lesion;

5) occurrence of a vertical root fracture [46].

Exclusion criteria for the study group:

- chronic alcohol consumption;
- administration of contraceptive medication;
- pregnancy or breastfeeding;
- periodontal treatment less than 6 months before sampling.

Among the 46 patients initially recruited from the Periodontology Department of the Faculty of Dentistry, "Carol Davila" University of Medicine and Pharmacy, and two private practices, 2 were excluded because they had active autoimmune diseases, which could have altered the results, given the fact that inflammation is also at the center of these pathologies.

The control group was represented by 19 clinically and biologically healthy adults with no periodontal disease, other oral disorders, and any known systemic pathology.

All the participants in this study were informed about the aim of the study and their voluntary participation. It was also explained to them that by agreeing to take part in the research, they would have no additional risks to their periodontal treatment, nor any costs or benefits of any kind. Moreover, the subjects included in the study were assured of their anonymity in the processing and publication of the data obtained. Furthermore, they were informed of the right to freely withdraw from this study at any time without any type of repercussions or penalties. After they completely understood the nature and purpose of this research, they signed the informed consent. This study was approved by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy (22976/17.08.2022).

Sample collection and biomarker determination

All subjects included in our study received written information regarding the collection procedure before sampling. Saliva was collected during their next appointment, in the morning, between 8 and 10. They were recommended to avoid food and drink intake, as well as any oral hygiene method, for 12 hours before the collection of the sample. If this was not possible, they had to refrain from eating and drinking for at least 60 minutes before sampling.

Additionally, smoking and chewing gum were also restricted in the last hour before the sample collection, while physical effort was forbidden 30 minutes before the collection. Another requirement was to avoid using lipstick or similar products that morning. In order to remove food scraps and exfoliated cells, each participant in the study rinsed their mouth with distilled water before the collection of the samples.

During the procedure, they were advised to relax, not swallow, and not communicate with others. Three mL of unstimulated total saliva was collected from each participant in the study. Once retrieved in sterile tubes, the saliva was transported to the laboratory on ice in a special transport box for biological samples. Samples were then centrifuged at 3000 rpm for 15 minutes and aliquoted. Until further analysis, they were kept in the freezer, at -80°C.

The three biomarkers investigated in this study were determined using the Enzyme-linked immunosorbent assay (ELISA) and commercial kits (DRG Instruments GmbH, Germany – TAC and leptin; Abbexa LTD, Cambridge, UK for NLRP3). The determination of all parameters was performed according to the instructions of the manufacturer.

Statistical analysis

All the data from the study were analyzed using IBM SPSS Statistics 26 (IBM, USA). Data were expressed as means ± std. Deviation, as well as medians, depend on the distribution's normality. The Shapiro-Wilk test was used to evaluate data distribution. The non-parametric Mann-Whitney U test was then applied to compare quantitative independent variables between the groups. Correlations were assessed using Spearman's rho correlation coefficient. A p-value lower than 0.05 was considered statistically significant.

RESULTS

The patient group included 44 subjects, among whom 21 were males (47%). The mean age was 51.8 ± 9.9 years, with a median of 51.5 years. The average number of remaining teeth in the patient group was 21.8 ± 4.6, with a median of 23. Eleven patients (25%) suffered from hypertension, diabetes mellitus, hyperthyroidism, or osteoporosis. The control group was represented by 19 healthy subjects, among whom 5 were males (26%). Their mean age was 28.5 ± 13.8 years, with a median of 19 years.

The results obtained show that when all patients with periodontal disease were compared with the control group, NLRP3 had statistically significantly increased levels in the study group (Table 1, Figure 1). Although TAC was also increased in the patients compared to the healthy subjects, no statistically significant differences were found (Figure 2). At the same time, leptin was significantly decreased in patients with periodontal disease compared to the control group (Figure 3).

Table 1: NLRP3, TAC and leptin salivary levels in periodontal patients and the control group

Biomarker	Group	Median	Mean ± Std. Deviation	p-value*
NLRP3	Patient	1.1372 ng/mL	1.117 ± 0.332 ng/mL	**< 0.00001**
	Control	0.7382 ng/mL	0.776 ± 0.208 ng/mL	
TAC	Patient	76.741 µmol/L	114.127 ± 119.398 µmol/L	0.491
	Control	70.838 µmol/L	94.553 ± 101.928 µmol/L	
Leptin	Patient	5.894 ng/mL	6.124 ± 2.064 ng/mL	**< 0.00001**
	Control	8.827 ng/mL	8.618 ± 1.478 ng/mL	

**Mann-Whitney U Test*

Figure 1: Salivary levels of NLRP3 in the patient group *versus* the control group

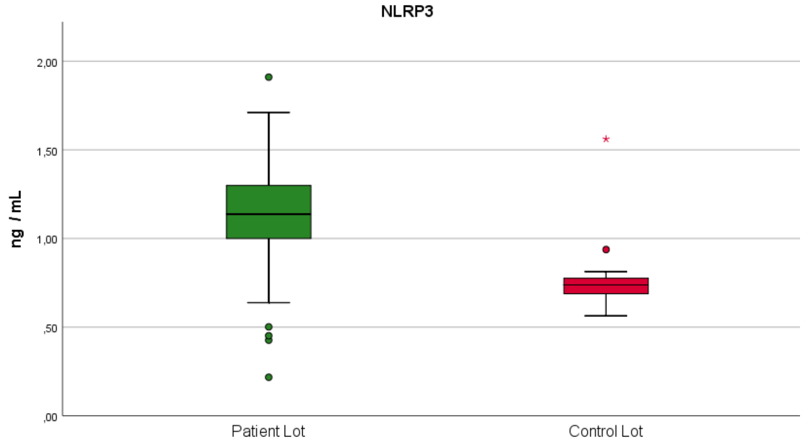

Figure 2: Salivary levels of TAC in the patient group *versus* the control group

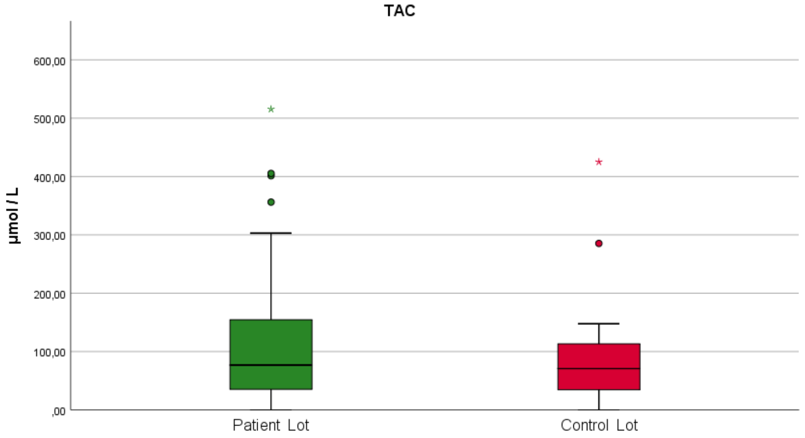

Figure 3: Salivary levels of leptin in the patient group *versus* the control group

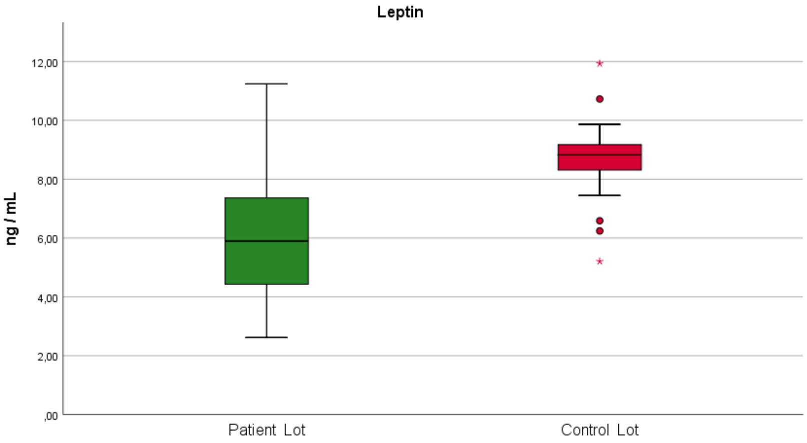

None of the three determined parameters displayed significant differences when comparing patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions (Table 2, Figures 4, 5, 6).

Spearman's rank correlation coefficient was used to test intragroup possible associations between salivary parameters. No significant correlation was found between salivary levels of leptin and NLRP3 when including all patients or when analyzing just patients with periodontal disease without associated systemic conditions. Also, no significant correlation was found between salivary levels of NLRP3 and TAC or TAC and leptin when including all patients or when analyzing just patients with periodontal disease without associated systemic diseases. No statistically significant correlations were found between the concentrations of the analyzed biomarkers and the number of remaining teeth when taking into consideration all periodontal disease patients, nor when including only the patients without associated systemic diseases (Table 3).

Table 2: Comparison of the biomarkers' salivary levels in periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

Biomarker	Group	Mean ± Std. Deviation	p-value*
NLRP3	No Associated Diseases	1.13 ± 0.369 ng/mL	0.356
	Associated Diseases	1.077 ± 0.189 ng/mL	
TAC	No Associated Diseases	112.041 ± 112.434 µmol/L	0.828
	Associated Diseases	120.387 ± 115.215 µmol/L	
Leptin	No Associated Diseases	6.346 ± 2.011 ng/mL	0.115
	Associated Diseases	5.457 ± 2.174 ng/mL	

**Mann-Whitney U Test*

Figure 4: Salivary levels of NLRP3 in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

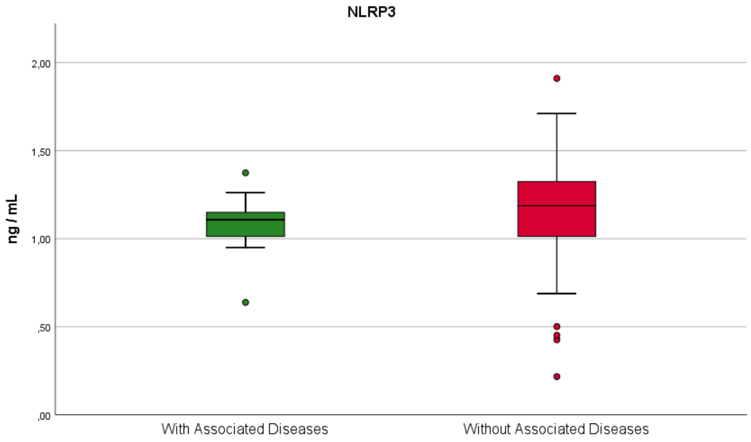

Figure 5: Salivary levels of TAC in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

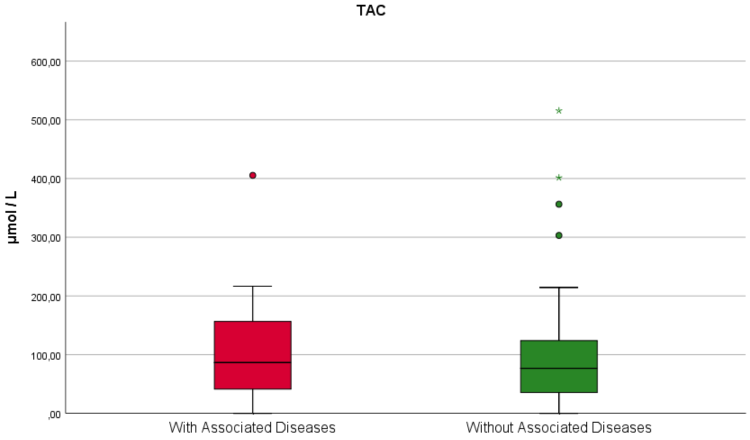

Figure 6: Salivary levels of leptin in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

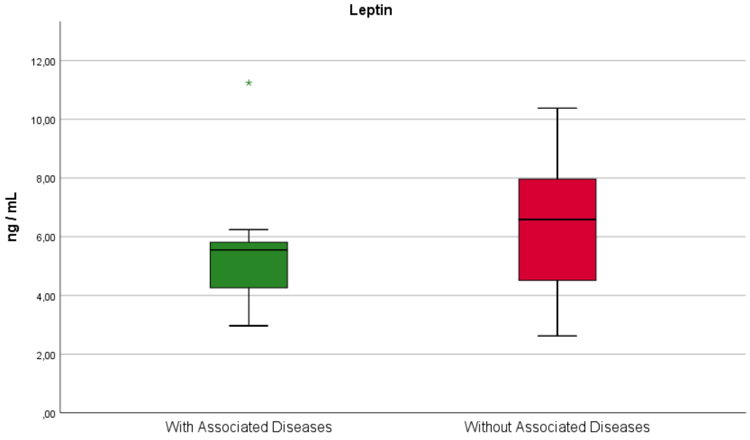

Table 3: Correlations between the determined biomarkers

Correlation	Spearman Rho	p – value
Between the number of remaining teeth and the NLRP3 levels for all patients	0.175	0.255
Between the number of remaining teeth and Leptin levels for all patients	0.095	0.541
Between the number of remaining teeth and the TAC levels for all patients	-0.158	0.305
Between the number of remaining teeth and the NLRP3 levels for patients without associated disease	0.223	0.213
Between the number of remaining teeth and leptin levels without associated disease	-0.063	0.729
Between the number of remaining teeth and leptin levels for patients with associated diseases	0.378	0.252
Between the number of remaining teeth and the TAC levels for patients without associated disease	-0.088	0.626
Between leptin and NLRP3 in all patients	0.147	0.340
Between leptin and NLRP3 in patients with no associated diseases	0.234	0.189
Between TAC and NLRP3 in all patients	-0.171	0.267
Between TAC and NLRP3 in patients with no associated diseases	-0.131	0.467
Between TAC and leptin in all patients	-0.168	0.276
Between TAC and leptin in patients with no associated diseases	-0.059	0.746

DISCUSSION

Saliva has already proven to be a reliable diagnostic, prognostic, and monitoring fluid for both oral and systemic diseases, being very rich in molecules and macromolecules that can be used as biomarkers [48]. Furthermore, the collection procedure is easy, it is more efficient in terms of costs, has a lower risk of cross-infection compared to the blood, and most importantly, the sampling is non-invasive [48, 49]. However, when determining the salivary levels of these biomarkers, we have to keep in mind that their concentrations are frequently decreased compared to the blood. Additionally, there are several factors that can influence their levels, including the moment of the day or the level of stress [49].

In recent years, inflammasomes have represented a major research interest due to their potential to determine an inflammatory response by integrating signals from different pathogens and altered cells [50, 51]. The most studied of the inflammasomes in systemic diseases, NLRP3, has also gained attention regarding its connection with periodontal disease [52]. The dysbiosis and inflammation associated with periodontal disease lead to the activation of the components of the NLRP3 inflammasome. Apart from promoting pyroptosis and neutrophil recruitment, once activated, NLRP3 promotes the overproduction of cytokines and antibodies, as well as the release of prostaglandin E2. In the end, all these events result in the destruction of the periodontium [53].

The results of previous studies performed on animal models were not conclusive. Yamaguchi et al induced periodontal disease in mice by orally injecting them with Porphyromonas gingivalis, showing that this microbial species, involved in the inflammatory process characteristic of chronic periodontitis, activated the NLRP3 inflammasome, leading to a response from the cells of the innate immune system and involvement of the IL-1 family of cytokines [54]. Nevertheless, a recent study by Rocha et al. performed on mice with induced periodontal disease revealed different results, showing that the inflammation associated with periodontitis and the consequent bone resorption was not determined by NLRP3, which played a minor role in the process, but rather by caspase-1 [55]. The results of our study showed statistically significant increased salivary levels of NLRP3 in patients with periodontitis compared to the control group ($p < 0.00001$, Table 1, Figure 1), suggesting a possible association of NLRP3 with the pathogenesis of this oral condition. Furthermore, the data we obtained did not show any correlation between NLRP3 salivary levels and the number of remaining teeth on the dental arches (Table 3).

When comparing the salivary levels of NLRP3 in patients with periodontitis and without systemic diseases to those who have associated systemic diseases, we were unable to find any statistically significant difference (Table 2). These results indicate that NLRP3 levels are mainly due to the presence of periodontal inflammation and not to the coexistence of other pathologies. Isola et al reported that patients with periodontitis and patients with periodontitis and type 2 diabetes had increased NLRP3 levels, both in the serum and saliva, not only compared to healthy controls, but also versus patients who had type 2 diabetes, but did not have periodontal disease [53]. Using samples from the gingival crevicular fluid and gingival biopsies, Garcia-Hernandez et al. showed that patients with periodontitis and uncontrolled type 2 diabetes displayed upregulated genes and proteins associated with NLRP3 activation compared to patients who had only periodontitis [56].

A systematic analysis from 2020 concluded that the levels of leptin both in plasma and saliva reported in the analyzed studies were too different, and although a decrease in salivary levels of leptin was observed in periodontal patients compared to healthy subjects, that data was not sufficient to establish the relationship between the disease and levels of leptin [57].

Similar to other studies, our results showed that leptin had significantly decreased levels in the saliva of patients with periodontal disease compared to those of the control group ($p < 0.00001$, Table 1, Figure 2) [58]. Leptin has already been shown to activate NLRP3 inflammasome in other inflammatory diseases, including systemic lupus erythematosus, and autoimmune disease characterized by the presence of the inflammatory process [59]. Despite both NLRP3 and leptin showing increased levels in periodontitis, we could not find a correlation between the two biomarkers.

TAC was defined by Miller as the "measure of the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound” [60]. Since ROS are naturally counteracted by antioxidants, determining the total antioxidant capacity has become one of the common and important ways to monitor the effect of oxidative stress in periodontal disease. However, the results regarding TAC levels in periodontal disease are controversial, with research showing both significant increases in periodontitis patients compared to healthy controls, as well as no significant differences [61].

On the other hand, Toczewska et al reported lower levels of TAC in both the saliva (which was a mixture of non-stimulated and stimulated saliva) and the gingival crevicular fluid of periodontal patients [62]. A decrease in the antioxidant capacity of patients with periodontal disease was also reported by Diab-Ladki et al [63]. Moreover, similar results were obtained by Brock et al, although their data did not show a statistically significant difference for salivary levels [64]. In our study, the determination of TAC displayed higher salivary levels in periodontitis patients compared to the control group ($p=0.491$, Table 1, Figure 3), but no statistical significance was found. This difference could be explained by the fact that the control group consisted of healthy subjects who had a mean age lower than that of the periodontitis patients. Limberaki et al previously reported that, contrary to expectations, younger people have lower TAC levels in the serum than older people [65].

Previous research revealed that while mitochondrial dysfunction and ROS overproduction can promote NLRP3 activation, their existence is not imperative for this to happen. Additionally, DNA damage caused by ROS can activate the inflammasome, but its release can happen downstream of the activation of the inflammasome [66]. This could explain why our results showed no statistically significant correlation between the salivary levels of NLRP3 and those of TAC.

Our study has several limitations, including the study groups being relatively small. Additionally, there was a significant mean age difference between the study group and the control group. Moreover, although saliva was collected following the same recommendations for all participants in the study, the salivary flow of each participant could have played a role in the level of the biomarkers [67]. Nevertheless, they reveal that the data obtained by our research group suggests the involvement of NLRP, leptin, and oxidative stress in periodontitis.

CONCLUSION

The results obtained in the present study are promising, but they incite further research that should be more focused on the relationship between these biomarkers and other factors that could influence the outcome, for example, the different types of bacteria involved in periodontal disease. Moreover, analyzing not just the saliva of the patients, but their gingival crevicular fluid could provide better insights. Future perspectives could be offered by determining these biomarkers in dynamic, before and after periodontal treatment, as well as correlating them to clinical parameters. All these represent objectives for our future research, which would offer a more complete picture of the mechanisms involved in periodontal disease development and progression and thus, show how NLRP3 activation can promote periodontal tissue destruction and bone loss.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, I.-I.S.-S., A.C.D; methodology, A.-S.D, D.M., B.-F.M; software, R.I., S.C.B; validation, T.-C.S., A.M.C..; formal analysis, D.M., S.C.B; investigation, T.-C.S., B.-F.M; resources, T.-C.S., A.M.C.; data curation, D.M., B.-F.M; writing-original draft preparation, I.-I.S.-S., T.-C.S., R.I.; writing-review and editing, I.-I.S.-S., D.M., A.-S.D, A.C.D; visualization, R.I., A.M.C.; supervision, A.-S.D, A.C.D; project administration, I.-I.S.-S., A.C.D.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy (22976/17.08.2022).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

The impact of combat stress on the coping strategies selected by mobilized military personnel with differing family statuses

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Abstract: Since February 24, 2022, military personnel of the Ukrainian Defense Forces have been resisting the military aggression of the Russian Federation. The fighting and the mobilization of men for military service complicated the well-being of Ukrainian families. The purpose of the article is to identify the relationship between the choice of coping strategies and post-traumatic stress disorder (PTSD) and maladaptation indicators in married and unmarried mobilized military personnel. Ukrainian Defense Forces military personnel (n=171 males, between 20 and 60 years of age) took part in this study. The "Strategic Approach to Coping Scale", "Mississippi Scale for Combat-Related Posttraumatic Stress Disorder", and "Disadaptation Express Questionnaire" were used. Correlation and exploratory factor analysis made it possible to identify the structure of coping strategies in mobilized servicemen with different family statuses and to determine the relationships between coping strategies, PTSD, and maladaptation indicators. The pre-war experience of using coping strategies by married and unmarried mobilized military personnel was not a sufficient barrier to the emergence of PTSD symptoms and maladaptation manifestations. Married and unmarried mobilized servicemen had a deficit structure of coping strategies characteristic of war.

Keywords: military personnel; coping strategies; PTSD; family; combat stress; mental health; mobilized soldiers; Russian-Ukrainian war

INTRODUCTION

The large-scale war in Ukraine, which began on 24.02.2022, has exacerbated the problem of mobilizing civilians and preparing military personnel to participate in hostilities to protect the sovereignty and lives of Ukrainians [1]. However, mobilization for military service has also complicated the well-being of families, who are forced to overcome problems arising from threats to the health and lives of family members, changes in family lifestyle, and everyday difficulties on their own, without men/women. This was also due to military actions on the territory of the country: experiencing threats of air raids, loss of housing, forced displacement, distance learning for children and their permanent stay at home, remote work or its absence, etc. [2,3]. These circumstances were factors that destroyed families, as well as additional stress for military personnel who, while defending their country, cannot protect and preserve their families.

Coping with potentially traumatic situations, including combat stress, involves the use of coping mechanisms and stress management strategies [4]. One of the models of stress management is the "Multiaxial Model of Coping", which comprises three axes: active–passive, prosocial–antisocial, and direct–indirect [5]. These axes represent dimensions of general coping strategies that allow for broader application across individualistic and collectivist cultures. The model assumes that active and avoidant strategies are not socially indifferent, and ignoring this fact may lead to a misinterpretation of their effects [6]. Prosocial coping involves strategies that positively utilize social resources. Antisocial coping includes behaviors that, while

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satisfying the individual’s needs, can be detrimental to others [7]. Aggressive coping is also effective as long as it does not become outright antisocial. Antisocial coping does not denote psychopathological behaviors, but rather coping methods that can be potentially harmful to others, involving the use of others or even exploiting them for personal gain [8].

Research has detailed the negative impact of combat deployments on service members and their families, the devastating effects of maladaptation and post-traumatic stress disorder (PTSD) symptoms on families, including violence against spouses and children [9,10]. PTSD couples reported clinically significant levels of relationship distress several times more frequently than comparison couples, both for general distress and across all specific problem areas (e.g., aggressive behavior, quality of leisure time together, sexual functioning, conflicts about finances and child rearing) [11]. Another study found that greater unit support, marital satisfaction, and psychological hardiness were associated with less anger, anxiety, depression, and PTSD symptomatology in soldiers [12]. The positive effects of intimate partner [13] and unit support reduced negative emotionality and traumatic stress only for veterans with less severe combat experiences [14].

However, the conducted studies did not examine the features of PTSD manifestations and maladaptation to the choice of coping strategies of mobilized servicemen with different family statuses. For such people, military service was not their own choice, and they were forced to adapt their civilian traumatic experience to overcome the effects of combat stressors and wartime stress factors.

The purpose of the article was to identify the relationship between the choice of coping strategies and indicators of post-traumatic stress disorder and maladaptation in married and unmarried mobilized military personnel.

MATERIALS AND METHODS

Study design and participants

This study was an exploratory descriptive study. All participants provided their informed consent for inclusion before engaging in the study. Ukrainian Defense Forces military personnel (n=171 males, aged 20 to 60 years, with a mean age of 41.84±6.49 years) participated in this study. The participants included 71% privates and 29% non-commissioned officers, all of whom participated in the Russian-Ukrainian war and had combat experience ranging from 6 to 10 months (8.75±2.58 months). Before the war, 62% of the participants had urban origins, while 38% had rural origins; 24% had completed secondary education, 57% had completed secondary specialized education, and 19% had attained higher education. The military personnel were sent to the rehabilitation center from combat positions to partake in a psychological recovery program lasting 14 days [15]. Two groups were formed to conduct the study: group 1 included 76 unmarried participants, while group 2 consisted of 95 married mobilized military personnel. The participants were identified based on various manifestations of acute stress reactions, significant negative experiences—including signs of depression and suicidal ideation—presence of PTSD symptoms, sleep problems (more than 50%), somatic complaints (more than 80%), and wounds and contusions (more than 75%); they also faced difficulties in returning to combat missions due to the consequences of illness, injury, and wounds. Female military personnel were excluded from this study because less than 0.5% of female combatants participated throughout the program period. Officers were also not included in the study due to their small number in the psychological recovery program (less than 1%). Participants were randomly selected for the study.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the National Academy of the National Guard of Ukraine, Kharkiv, Ukraine (protocol code 2024/12, dated September 24, 2024).

Instruments

To determine the relationship between the choice of coping strategies and indicators of PTSD and maladaptation in mobilized military personnel, the “Strategic Approach to Coping Scale” (SACS), “Mississippi Scale for Combat-Related Posttraumatic Stress Disorder” (MSCRPTSD), and “Disadaptation Express Questionnaire” (DEQ) were used.

The SACS was used to determine coping strategies and behavior patterns of military personnel to overcome stressful situations, adapted into Ukrainian [16,17]. The SACS consists of 54 statements, the answers to which were given on a 5-point Likert scale (Cronbach’s $\alpha = 0.846$). The scale allowed for the choice of coping strategies (behavior models) such as “Assertive Actions” (Cronbach’s $\alpha = 0.627$), “Social Joining” (Cronbach’s $\alpha = 0.736$), “Seeking Social Support” (Cronbach’s $\alpha = 0.746$), “Cautious Actions” (Cronbach’s $\alpha = 0.676$), “Instinctive Actions” (Cronbach’s $\alpha = 0.691$), “Avoidance” (Cronbach’s $\alpha = 0.669$), “Indirect Actions” (Cronbach’s $\alpha = 0.654$), “Antisocial Actions” (Cronbach’s $\alpha = 0.687$), and “Aggressive Actions” (Cronbach’s $\alpha = 0.724$). The results were analyzed by comparing the data on the degree of expression (low, medium, high) of a specific person’s coping behavior for each subscale with the average values of the coping stress models.

The MSCRPTSD [18] was used to diagnose PTSD in military personnel on missions in the war zone, adapted into Ukrainian [17, 19]. The scale consists of 35 statements (4 subsets), the answers to which were given on a 5-point Likert scale (Cronbach’s $\alpha = 0.88$). Subset 1 (11 statements) describes the symptoms of the “intrusion” group when the traumatic event is constantly repeated in the experience in one (or more) ways. Subset 2 (11 statements) relates to the symptoms of the “avoidance” group when there is a constant avoidance of stimuli associated with trauma, blocking of emotional reactions, and numbness, which was not observed before the trauma. Subset 3 (8 statements) describes the symptoms of “excitability” when persistent symptoms of arousal increase that were not observed before the injury. Subset 4 (5 statements) describes symptoms associated with guilt and suicidal tendencies. Despite the grouping of statements into 4 subsets, one general indicator was calculated taking into account the conversion of the answer into a score for direct and inverse statements, reflecting the severity of PTSD symptoms (range from 35 to 175 points, where 35-80 points is a variant of the norm; 81-114 points - separate symptoms of PTSD; 115-175 points - clinical manifestations of PTSD, a psychiatric examination and inpatient examination were recommended).

The DEQ was used to identify signs of a violation of the adaptability of the soldier’s personality: violation of the regulatory function of the emotional-volitional sphere and self-esteem; lack of prospects for continuing life and the ability to overcome life’s difficulties (probability of committing suicidal attempts); loss of moral convictions, the likelihood of committing addictive and delinquent acts; loss of communicative potential (comradely support, reduced ability to accept the help of one’s team) [19]. The DEQ consists of 45 statements included in 5 subscales (Cronbach’s $\alpha = 0.848$): “Sincerity of answers” (Cronbach’s $\alpha = 0.719$), “Violation of behavioral regulation” (Cronbach’s $\alpha = 0.736$), “Probability of committing suicide attempts” (Cronbach’s $\alpha = 0.743$), “Violation of moral normativity” (Cronbach’s $\alpha = 0.702$), and “Loss of communicative potential” (Cronbach’s $\alpha = 0.709$). Each positive response was worth 1 point, and each negative 0 points. The overall DEQ scale was calculated as the sum of scores on 4 scales (values on the “Sincerity of answers” scale were not included). The results of the overall DEQ scale were evaluated as follows: 1-10 points - high adaptation to combat operations, sufficient tolerance to adverse mental and physical stress, including under conditions of severe combat stress; 11-14 points - average adaptation, unstable level of performance, especially in combat conditions; 15 points or more - low adaptation (distress and adjustment disorders) that does not meet the requirements for soldiers in combat conditions.

Information on the implementation of a formal translation or back-translation protocol for SACS, MSCR-PTSD, and DEQ is unavailable. The authors of the article did not pilot test or validate the design of the psychodiagnostic instrument used in this study; we used data from the aforementioned Ukrainian sources. We are not aware of any published or internal references that describe the psychometric properties of the Ukrainian versions of these instruments in detail.

Statistical Analysis

The study was conducted in two stages. At the first stage, the use of correlation analysis allowed us to determine the relationships between the indicators of coping strategies, PTSD, and maladaptation in unmarried (group 1) and married (group 2) participants. At the second stage of the study, the indicators of coping strategies, PTSD, and maladaptation were compared in two participant groups. For the data presented, basic descriptive statistics were used (arithmetical mean M, standard deviation SD). The reliability of differences in the results of the mean values in two interrelated groups was determined using Student’s t-test. For the assessment of the statistical significance of differences, we used the level of significance from $p = 0.05$ to $p = 0.001$. No correction for multiple comparisons was applied.

Exploratory factor analysis was used to determine the structure of coping strategies in each participant’s group. Exploratory factor analysis of matrices was carried out using the extraction method (principal component analysis) and the rotation method (Varimax with Kaiser normalization). We did not perform the Kaiser-Meyer-Olkin (KMO) statistic and Bartlett’s test of sphericity for each group. Correlation analysis allowed us to determine the relationships between the identified factors in married and unmarried participants. The study results were statistically analyzed using SPSS 22.0 (IBM, Armonk, NY, USA).

RESULTS

Tables 1–2 present the results of the correlation relationship between the indicators SACS, MSCRPTSD, and DEQ scales by the group 1 and group 2 participants.

Table 3 presents the results of the coping strategies by the group 1 and group 2 participants.

Tables 4–5 present the exploratory factor analysis results of the coping strategies structure by the group 1 and group 2 participants.

Tables 6–7 present the correlation results between the factor structure of coping strategies and indicators of PTSD and maladaptation by the group 1 and group 2 participants.

Table 1: Correlation relationship indicators between the SACS, MSCRPTSD, and DEQ scales by the group 1 participants

Scale name	SACS								
	Assertive Actions	Social Joining	Seeking Social Support	Cautious Actions	Instinctive Action	Avoidance	Indirect Action	Antisocial Actions	Aggressive Actions
MSCRPTSD									
General indicator of PTSD	-0.10	-0.30**	-0.19	0.01	0.19	0.16	0.17	0.21	0.58**
DEQ									
Sincerity of answers	-0.02	0.06	0.03	0.21	0.06	-0.02	0.23	0.12	0.37**
Disruption of behavioral regulation	-0.13	-0.16	-0.14	0.12	0.27*	0.29**	0.25*	0.13	0.50**
The likelihood of committing suicide attempts	-0.15	-0.15	-0.18	0.09	0.30**	0.18	0.17	0.18	0.51**
Violation of moral norms	-0.08	-0.12	-0.19	0.12	0.18	0.18	0.26*	0.26*	0.36**
Loss of communicative potential	-0.07	-0.19	-0.12	0.15	0.17	0.20	0.30**	0.11	0.43**
General indicator of maladaptation	-0.13	-0.19	-0.18	0.14	0.28*	0.26*	0.28*	0.19	0.53**

*Note. *p = 0.05, **p = 0.01*

Table 2: Correlation relationship indicators between the SACS, MSCRPTSD, and DEQ scales by the group 2 participants

Scale name	SACS								
	Assertive actions	Social joining	Seeking social support	Cautious actions	Instinctive actions	Avoidance	Indirect actions	Antisocial actions	Aggressive actions
MSCRPTSD									
General indicator of PTSD	-0.42**	-0.31**	-0.24*	-0.10	-0.06	0.09	0.03	-0.02	0.42**
DEQ									
Sincerity of answers	-0.21*	-0.13	-0.16	-0.01	-0.20*	0.12	0.21*	0.09	0.14
Disruption of behavioral regulation	-0.40**	-0.27**	-0.29**	-0.10	-0.05	0.11	0.08	0.03	0.35**
The likelihood of committing suicide attempts	-0.34**	-0.30**	-0.34**	-0.06	-0.12	0.07	0.05	-0.02	0.31**
Violation of moral norms	-0.29**	-0.19	-0.28**	-0.05	-0.01	0.18	0.15	0.13	0.26*
Loss of communicative potential	-0.38**	-0.26**	-0.33**	0.02	-0.14	0.03	0.11	0.13	0.35**
General indicator of maladaptation	-0.43**	-0.31**	-0.38**	-0.06	-0.09	0.12	0.11	0.08	0.39**

*Note. *p = 0.05, **p = 0.01*

Table 3: Coping strategies indicators by the group 1 and group 2 participants (points, Student's t-test, Cohen's d)

Coping strategies name	Group 1	Group 1	t	p	d
Assertive actions	19.82±3.01	20.32±3.52	1.00	0.32	0.15
Social joining	21.96±4.86	23.56±4.66	2.17	0.03	0.34
Seeking social support	21.12±5.09	23.00±5.30	2.36	0.02	0.36
Cautious actions	21.34±4.08	21.80±4.19	0.72	0.46	0.12
Instinctive actions	18.47±3.66	18.61±3.47	0.25	0.74	0.05
Avoidance	17.14±3.99	16.89±4.36	0.39	0.73	0.05
Indirect actions	17.01±4.35	17.74±4.33	1.08	0.29	0.16
Antisocial actions	15.01±4.66	14.55±3.73	0.71	0.47	0.11
Aggressive actions	17.49±4.99	16.03±5.18	1.86	0.05	0.29

Table 4: Factor structure of coping strategies by the group 1 participants

Coping strategies name	**Factors**		
	1 (33.98%)	2 (24.92%)	3 (11.97%)
Assertive actions	0.393	0.047	0.776
Social joining	0.807	-0.058	0.210
Seeking social support	0.904	-0.040	-0.009
Cautious actions	0.755	0.226	0.032
Avoidance	0.481	0.359	-0.602
Indirect actions	0.167	0.862	0.107
Antisocial actions	-0.012	0.865	-0.018
Aggressive actions	-0.016	0.655	-0.281

Table 5: Factor structure of coping strategies by the group 2 participants

Coping strategies name	**Factors**		
	1 (31.61%)	2 (24.43%)	3 (13.40%)
Assertive actions	0.651	0.169	0.062
Social joining	0.812	-0.126	-0.172
Seeking social support	0.829	-0.238	0.095
Instinctive actions	0.167	0.154	0.815
Avoidance	-0.162	0.151	0.798
Indirect actions	0.013	0.874	0.178
Antisocial actions	-0.121	0.846	0.145

Table 6: The correlation relationship indicators between the factor structure of coping strategies and the scales MSCRPTSD and DEQ by the group 1 participants

Factors name	**PTSD and maladaptation indicators**					
	General indicator of PTSD	**Disruption of behavioral regulation**	**Likelihood of committing suicide attempts**	**Violation of moral norms**	**Loss of communicative potential**	**General indicator of maladaptation**
Factor 1 “Limited search for socially significant assistance”	-0.18	-0.06	-0.11	-0.09	-0.06	-0.09
Factor 2 “Destructive methods of gaining an advantage over others”	0.38**	0.36**	0.34**	0.37**	0.35**	0.41**
Factor 3 “Self-affirmation as a demonstration of decent behavior”	-0.21	-0.28*	-0.24*	-0.13	-0.17	-0.25*

*Note. *p = 0.05, **p = 0.01*

Table 7: The correlation relationship indicators between the factor structure of coping strategies and the scales MSCRPTSD and DEQ by the group 2 participants

Factors name	**PTSD and maladaptation indicators**					
	General indicator of PTSD	**Disruption of behavioral regulation**	**Likelihood of committing suicide attempts**	**Violation of moral norms**	**Loss of communicative potential**	**General indicator of maladaptation**
Factor 1 “Socially significant methods of attracting external resources”	-0.37**	-0.34**	-0.35**	-0.26*	-0.36**	-0.41**
Factor 2 “Socially inconsistent methods of attracting external resources”	0.01	0.09	0.07	0.19	0.19	0.16
Factor 3 “Uncertainty in one’s resources and careful attitude towards them”	0.10	0.12	0.06	0.14	-0.04	0.08

*Note. *p = 0.05, **p = 0.01*

DISCUSSION

At the first stage of the study, the use of correlation analysis allowed us to determine the relationships between coping strategies and indicators of PTSD, maladaptation in mobilized military personnel with different family statuses. Thus, productive coping “Assertive actions” (p = 0.01), “Social joining” (p = 0.01), and “Seeking social support” (p = 0.01) were associated with lower indicators of PTSD and maladaptation only in married participants (group 2). Such unproductive coping as “Instinctive actions” (p = 0.05), “Avoidance” (p = 0.05), “Indirect actions” (p = 0.05), “Antisocial actions” (p = 0.05) had relationships with the indicators of PTSD and maladaptation only in unmarried participants (group 1). The coping “Aggressive actions” (p = 0.01) was interrelated with the PTSD and maladaptation indicators in both groups of participants. The obtained results allow us to assume that such civilian experience as life in marriage influenced some features of the choice of coping strategies that determine the ability of mobilized servicemen to withstand combat stress factors. Similar studies showed that problem-solving coping reduced PTSD symptoms, while coping strategies aimed at avoiding stressful situations increased them [20]. Our results showed that this relationship is also be influenced by the family status of a serviceman.

As previously noted, social relationships such as intimate partners and military unit support reduced PTSD symptoms and negative emotionality and had a positive impact on military personnel’s well-being [14]. In our study, we assumed the possibility of other variants of these relationships: the maladaptation indicators could have been by receiving specific social assistance (“Seeking social support”), and the symptoms of PTSD were could reduced while maintaining or restoring the ability to engage in general activity (“Social joining”). We found that in group 2, the indicators of choosing such coping strategies as “Social joining” and “Seeking social support” were the highest among all coping strategies, and they differed from the indicators of group 1 (p = 0.05 for both values).

In the second stage of the study, exploratory factor analysis and correlation analysis were used to identify the tentative structure of coping strategies among mobilized military personnel with different family statuses, as well as to determine the relationship between coping strategies and indicators of PTSD and maladaptation.

In group 1, the identified tentative three-factor structure of coping strategies described 70.72% of the variance of features. The factor 1 content was determined by the coping strategies “Seeking social support,” “Social joining,” and “Cautious actions.” This factor was associated with the involvement of additional external resources. Perhaps, when asking for assistance, the group 1 participants were afraid of making a mistake, which could become an additional source of stress. This factor was designated as “Limited search for socially significant assistance.” The factor 2 content was determined by the coping strategies “Antisocial actions”, “Indirect actions”, and “Aggressive actions”. These coping strategies were associated with the abuse of actions to create one’s superiority over others, to create conditions for using the resources of colleagues for one’s purposes. This factor was designated as “Destructive methods of gaining an advantage over others”. The positive pole of factor 3 was determined by the coping strategies “Assertive actions”, and the negative pole “Avoidance”. These coping strategies were associated with one’s confidence and readiness to take responsibility. However, unlike assertive actions in group 2, where they may have been part of the interaction to overcome the consequences of stress factors. In group 1 assertive actions were possibly a component of self-affirmation. This factor was designated “Self-affirmation as a demonstration of decent behavior”.

It should be noted that the group 1 participants showed a tendency where destructive ways of gaining an advantage over others rather increased mental trauma than contributed to protection from the effects of stress factors. In this group, the factor “Self-affirmation as a demonstration of decent behavior” to a certain extent contributed to the reduction of “Disruption of behavioral regulation” (p = 0.05) and “Likelihood of committing suicide attempts” (p = 0.05). Indeed, the self-confidence and assertiveness that characterized the participants in this group may have been characteristics that allowed them to control themselves in a stressful situation.

In group 2, the identified tentative three-factor structure of coping strategies described 69.43% of the variance in features. The content of factor 1 included the coping strategy “Seeking social support,” “Social joining,” and “Assertive actions.” These strategies emphasized the importance of seeking external resources while considering the interests of fellow soldiers to mitigate the effects of combat stress factors. We referred to this factor as “Socially significant methods of attracting external resources.” The content of factor 2 was determined by the coping strategy “Indirect actions” and “Antisocial actions”. This factor was designated as “Socially inconsistent methods of attracting external resources” and had a statistically significant negative relationship with the consequences of the stress experienced: PTSD indicators (p = 0.01) and all maladaptation indicators (p = 0.01). Factor 3 was associated with the coping strategy “Instinctive actions” and “Avoidance”. This factor was designated as “Uncertainty in one’s resources and careful attitude towards them”. The main goal of the described factor structure of coping strategies in group 2 was associated with to attract additional resources and avoid unfavorable circumstances.

In studies that examined the factor structure of coping strategies, three main factors have often been identified: problem-focused coping, getting social support, and avoidance [21,22]. However, we found that all mobilized servicemen had a deficit structure of coping strategies. Moreover, married servicemen had a desire to obtain additional external resources by any means to overcome the effects of combat stress factors, as well as a careful attitude towards their resources. It can be assumed that married servicemen were more oriented towards interaction to overcome stressful situations, while unmarried servicemen were forced to rely more on their strength.

It is a common assertion that the effectiveness of stress attenuation is limited because it is often not possible to avoid stressors, and avoiding or minimizing stress can lead individuals to miss opportunities for performance and growth. Thus, during stressful situations, a more efficacious approach is to optimize stress responses (i.e., promote adaptive, approach-motivated responses) [23]. Another study concluded that an avoidant-focused coping style acts as a vulnerability factor for PTSD symptoms, whereas hardiness acts as a resilience factor against symptom development [24]. Thus, when interpreting coping strategies such as “Cautious actions” and “Instinctive actions”, it is necessary to take into account their role in the overall structure of coping and how personal resources for coping relate to the strength of stressful impacts. Our results partially confirm the conclusions that: (1) a robust latent variable named personal resources (indicated by social support, unit cohesion, and trait resilience) was negatively associated with PTSD severity; (2) personal resources were negatively associated with negative posttraumatic cognitions; (3) negative posttraumatic cognitions fully mediated the association between personal resources and PTSD severity [25].

Study limitations

This study certainly had limitations. 1) The study served as a generalization of experiences from the rehabilitation center focused on the psychological recovery of military personnel following participation in hostilities. This significantly influenced the amount of available empirical data (including sample size and psychodiagnostic survey methods), the study's structure, and the mathematical processing methods used. 2) The research was exploratory, aimed at generalizing the outcomes of surveys of military personnel involved in the psychological recovery program, as well as identifying potential hidden resources and methods for preventing PTSD and maladjustment. 3) Information on the implementation of a formal translation or back-translation protocol for SACS, MSCR-PTSD, and DEQ is unavailable. The authors of the article did not pilot test or validate the design of the psychodiagnostic instrument used in this study; we used data from the Ukrainian sources. We are not aware of any published or internal references that describe the psychometric properties of the Ukrainian versions of these instruments in detail. 4) The risk of false positive results was increased by the fact that no correction for multiple comparisons was applied. 5) Data on resources for building resilience against combat stress factors was limited among mobilized military personnel who did not choose military careers and received very brief training. 6) Existing data on family support for servicemen relates to scenarios where families are safe, a situation not observed in Ukraine, creating an ambivalent role for the family that can serve as both a source of anxiety and a resource. 7) The research was conducted on a sample of servicemen who participated in the psychological recovery program, specifically those exhibiting signs of mental and physical exhaustion, PTSD symptoms, and maladjustment. Consequently, the results related to the characteristics of coping structure deficits in both established variants—typical for married and single mobilized servicemen—should be specifically attributed to the corresponding samples. As a result, it is inappropriate to extend the findings of this study to servicemen who cope more effectively with the impact of combat stress. 8) The results obtained possess low practical value (Cohen’s d); however, they allow us to formulate valuable hypotheses for further research. For example, PTSD prevention may be linked not only to direct family support but also to shared life experiences such as joint problem solving, which remain relevant even in the absence of direct family support, highlighting its value. Additionally, an intriguing hypothesis is the assessment of individual coping: its position within the overall coping strategy framework (which may exhibit signs of collective or individual culture) and the general nature of this structure concerning the situation (for instance, a deficiency in the overall structure indicating a reliance on external assistance). 9) Female military personnel were excluded from this study because less than 0.5% of female combatants participated throughout the duration of the psychological recovery program. 10) The participant sample consisted solely of enlisted military personnel and sergeants; officers did not take part in this study. 11) This study faced limitations due to the brief duration of the psychological recovery program and the need to avoid overloading participants with additional activities that did not align with the program’s objectives, which limited the application of research methods and the repeatability of the survey, among other factors. Finally, the current study was constrained by the absence of an active comparison condition and a longitudinal follow-up. Thus, by summarizing some of the outcomes from the rehabilitation center's work on the psychological recovery of military personnel following military operations, we aimed to attract the scientific community's attention to addressing new urgent challenges in military medicine and psychology, particularly in the unique area of psychological support for mobilized military personnel.

CONCLUSION

The pre-war experience of using coping strategies by married and unmarried mobilized military personnel was a rather weak preventive measure for counteracting combat stressors, and not a sufficient barrier to the emergence of PTSD symptoms and maladaptation manifestations Assertive actions could be associated as a preventive factor for the negative consequences of combat stress only in married military personnel. Perhaps this was due to their structure of coping strategies, in which assertive actions were an integral part of interaction, while in unmarried servicemen it was self-affirmation. The creation of a wide range of social relationships had a positive impact on the preventive power of social interaction, which helped to overcome the consequences of PTSD and social maladaptation manifestations for both groups of mobilized military personnel. Aggressive actions, which became a source of additional stress and a method of responding to stress, were linked to higher PTSD scores.

Psychological support of the family among married mobilized servicemen was associated with the formation of resilience to the action of combat stress factors. In addition, compatible actions of married servicemen in stressful situations due to feedback formed an adequate stress response, reduced the likelihood of meta-experiences. Unmarried mobilized servicemen did not have family experience, so their coping strategies mainly came down to reacting and individual resilience to combat stress factors.

Married and unmarried mobilized servicemen had a deficit structure of coping strategies characteristic of war. They realized that their resilience to the effects of combat stress factors depended on the possibility of receiving help from others. And if married mobilized servicemen were more inclined to exercise caution when spending their resources, then unmarried servicemen tried to use self-assertive behavior as a method to raise their authority and thus attract additional external resources.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, Y.M., and N.Ku.; methodology, N.Kr.; software, V.F., and V.K.; validation, Y.M., O.H., and N.Ku.; formal analysis, Y.M.; investigation, N.Kr.; resources, V.F., and O.H.; data curation, Y.M., and N.Ku.; writing—original draft preparation, Y.M.; writing—review and editing, N.Ku., and Y.M.; visualization, V.K., and O.H.; supervision, N.Kr., and V.K.; project administration, N.Ku.; funding acquisition, V.F. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the National Academy of the National Guard of Ukraine, Kharkiv, Ukraine (protocol code 2024/12 and date of approval 24/09/2024).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Efficacy and Patient Satisfaction of Laser Labiaplasty for Labia Minora Hypertrophy: A Retrospective Analysis

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Abstract: Background: Labia minora hypertrophy can lead to aesthetic dissatisfaction and functional discomfort among women, affecting their quality of life and sexual self-esteem. Traditional surgical labiaplasty techniques are effective but are associated with higher complication rates and longer recovery times. This study aims to evaluate the efficacy, safety, and patient satisfaction of laser labiaplasty as a minimally invasive alternative for labia minora reduction. Methods: A retrospective analysis was conducted on 60 female patients who underwent laser labiaplasty. Preoperative assessments included medical history, gynecological examination, and patient-reported concerns. The procedure utilized a CO_2 laser for tissue excision without the need for sutures. Postoperative outcomes were evaluated through follow-up visits and telephone interviews, focusing on complications, recovery time, and patient satisfaction measured by the Subjective Global Aesthetic Improvement Scale (sGAIS). Results: The primary motivations for surgery were aesthetic concerns (85%) and functional discomfort (55%). The mean age was 34.7 years. Minor complications included transient postoperative bleeding (10%) and mild discomfort (25%), with no major complications reported. High satisfaction rates were observed, with 92% of patients reporting significant improvement (sGAIS scores of 4 or 5). Conclusions: Laser labiaplasty is an effective, minimally invasive procedure with high patient satisfaction and low complication rates. It offers a viable alternative to traditional surgical methods for women seeking labia minora reduction due to aesthetic or functional concerns.

Keywords: laser labiaplasty; labia minora hypertrophy; patient satisfaction; minimally invasive procedure; genital aesthetics

INTRODUCTION

Labia minora hypertrophy, characterized by the enlargement or elongation of the inner vaginal lips, has been a largely recognized condition [1,2]. Women experiencing this condition often report both aesthetic dissatisfaction and functional issues, such as discomfort during physical activities or sexual intercourse [3,4]. The increased visibility and awareness of genital aesthetics in contemporary society have led to a growing number of women seeking corrective procedures to address these concerns [5,6].

Genital aesthetics significantly impact a woman's sexual self-esteem and overall quality of life [7,8]. Studies have demonstrated that dissatisfaction with genital appearance can lead to psychological distress, reduced sexual function, and decreased confidence [9,10]. The rise of media portrayal of idealized genital images has further influenced women's perceptions, prompting many to pursue medical interventions to achieve their desired appearance [11].

Traditional surgical labiaplasty techniques, including edge resection, wedge resection, Z-plasty, and deepithelialization, have been employed to reduce labial tissue [12,13]. While effective, these methods are associated with risks such as scarring, altered sensation, asymmetry, and extended recovery periods. These potential complications have led both patients and clinicians to explore less invasive alternatives that offer effective results with fewer risks [14].

Laser labiaplasty has emerged as a minimally invasive option that utilizes laser technology to excise excess labial tissue precisely [15,16]. Introduced more than 15 years ago using a neodymium-doped yttrium aluminum garnet (Nd) laser, the technique has evolved with the adoption of carbon dioxide (CO_2) and erbium-doped yttrium aluminum garnet (Er) lasers [17]. These lasers offer advantages such as reduced intraoperative bleeding, minimal thermal damage, and enhanced healing, contributing to improved patient outcomes.

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Despite the growing popularity of laser labiaplasty, there is a paucity of comprehensive studies evaluating its efficacy, safety, and patient satisfaction. Most existing literature comprises small case series or focuses on surgical techniques without detailed analysis of patient-reported outcomes [18,19]. This gap underscores the need for robust clinical data to inform practitioners and patients about the benefits and limitations of this procedure. Therefore, the objective of this study is to retrospectively analyze our center's experience with laser labiaplasty in treating labia minora hypertrophy. By examining preoperative motivations, procedural details, postoperative outcomes, and patient satisfaction, we aim to provide valuable insights into the efficacy and safety of laser labiaplasty as a minimally invasive alternative to conventional surgical methods.

MATERIALS AND METHODS

This retrospective study analyzed data from 60 female patients who underwent laser labiaplasty between June 2020 and September 2024. Inclusion criteria encompassed women aged 18 years or older diagnosed with labia minora hypertrophy, who expressed aesthetic or functional concerns and completed a minimum of six months of follow-up. Exclusion criteria included active genital infections, uncontrolled systemic diseases, previous labiaplasty, or other genital surgeries.

All patients underwent a comprehensive preoperative evaluation. Medical and gynecological histories were obtained, focusing on reproductive history, menstrual cycle details, prior pregnancies, and any gynecological conditions or surgeries. Physical examination included measurement of labia minora dimensions using standardized calipers. Preoperative investigations included a Papanicolaou (PAP) smear, vaginal secretion analysis for infections, and a transvaginal ultrasound to assess pelvic anatomy.

The classification of labial hypertrophy identifies three types of labial hypertrophy, based on Stephane Smarrito's study [20]: Type I, known as the "flag," affects the anterior one-third and was found in 11% of the cases; Type II, or the "oblique," involves the middle third and accounted for 29% of cases; and Type III, termed "complete," includes the posterior third, observed in 60% of the patients. Symptomatically, Type I patients typically reported aesthetic concerns and discomfort from tight clothing without dyspareunia. Type II was noted for a fuller appearance, and Type III frequently presented with dyspareunia more so than the other types. Surgical approaches were tailored accordingly, with Type I cases treated using a superior pedicle flap technique for moderate labial resection, while Types II and III were approached with the lambda laser technique. This classification system allows for more targeted surgical strategies that align closely with patient symptoms and expectations, providing a significant improvement over the traditional method based solely on dimensional measurement.

The procedure was performed under local anesthesia. Initially, a topical anesthetic cream containing lidocaine 2.5% and prilocaine 2.5% was applied to the labia minora for 30 minutes. After the removal of the cream and disinfection with povidone-iodine solution, infiltration anesthesia was administered using 1% lidocaine with epinephrine 1:100,000 via a 30G needle. Preoperative markings were made to delineate the extent of tissue excision (Figure 1).

Figure 1: Preoperative markings

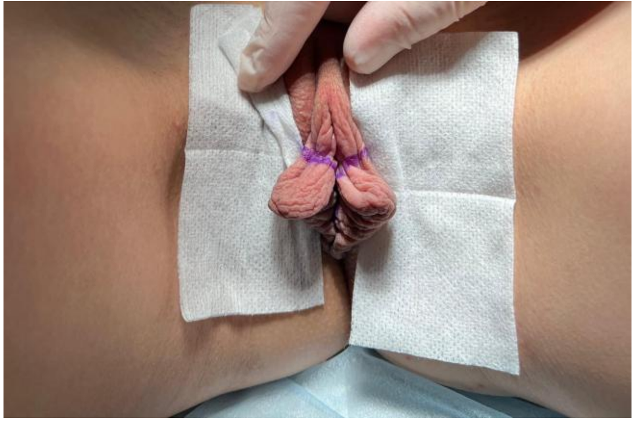

A CO_2 laser (CO_2RE, Syneron Candela) equipped with a surgical handpiece was set to a continuous wave mode at 10 Watts. The laser was used to excise excess labial tissue along the marked lines, ensuring symmetrical reduction and preservation of the natural contour (Figure 2). Hemostasis was achieved through the laser's coagulative properties, eliminating the need for sutures. After the procedure, the area was treated with topical fusidic acid ointment and covered with a sterile dressing.

Patients received detailed postoperative instructions, including applying fusidic acid ointment twice daily for one week; maintaining local hygiene with gentle cleansing using mild soap and water; avoiding sexual activity, strenuous exercise, swimming pools, and saunas for six weeks; and wearing loose-fitting clothing to minimize friction.

Follow-up visits were scheduled at one week, one month, three months, and six months postoperatively. Evaluations focused on wound healing, identification of any complications, and assessment of patient satisfaction using the Subjective Global Aesthetic Improvement Scale (sGAIS), a validated 5-point scale ranging from 1 (no improvement) to 5 (very significant improvement).

After the clamps were removed, the treated area was managed with targeted local applications of anti-inflammatory and antibiotic solutions. Specifically, Fucidin H cream, containing fusidic acid 20 mg/g and hydrocortisone acetate 10mg/g, was used in conjunction with Baneocin powder, which includes zinc bacitracin 250 UI and neomycin sulphate 500 UI/g. To control bleeding and further protect the site, a hemostatic sponge was placed over these topical agents, and the area was subsequently dressed. Pain management was addressed systemically through an intravenous administration of acetaminophen 10mg/ml, amounting to 100 ml, and an oral dose of Ketonal Forte, which contains ketoprofen at a concentration of 100 mg.

Figure 2: Excised excess labial tissue

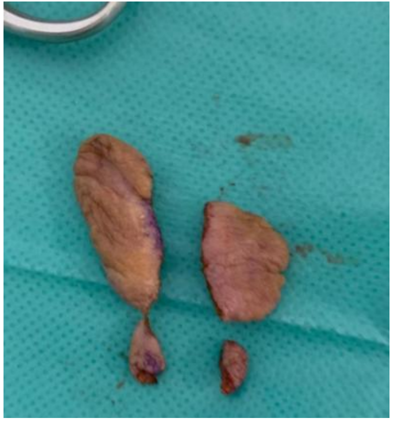

Discharge instructions provided to the patient emphasized crucial care steps to ensure proper healing. The patient was instructed to maintain local compression and restrict themselves to bed rest on the day of the procedure to minimize movement and stress at the site. For the initial three weeks post-procedure, the patient was advised to clean the area using Betadine and apply the previously mentioned Fucidin H cream and Baneocin powder twice daily. In the subsequent three weeks, the regimen shifted to cleaning with water and soap followed by the application of a protective repairing cream to foster skin restoration and barrier function.

Figure 3: Case 1. (a) Before and (b) six weeks after laser labiaplasty

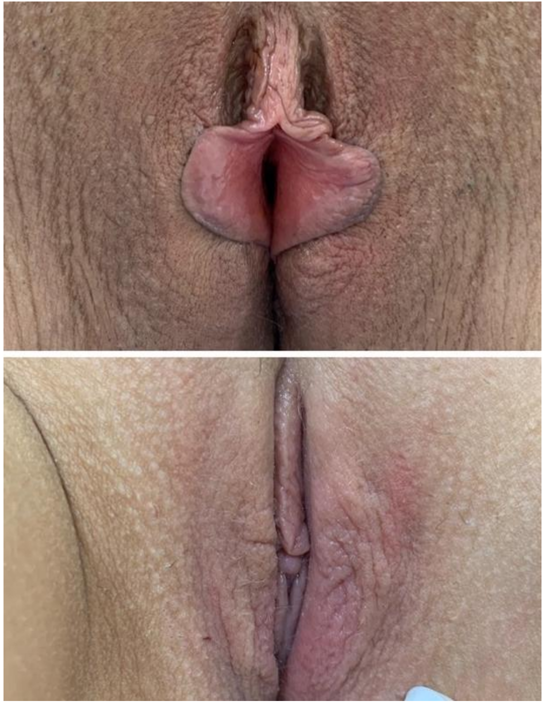

At the six-week post-procedure mark, a follow-up consultation was arranged to evaluate the healing progress and the cosmetic outcome of the laser labiaplasty. The provided figures (Figure 1 and Figure 2), display the condition of the area before the procedure and at the six-week follow-up. These images illustrate a notable reduction in labial size and improved symmetry, indicating a successful aesthetic enhancement. The area appeared to be well-healed without signs of infection or significant inflammation, underscoring the effectiveness of the postoperative care regimen.

Figure 4: Case 2. (a) Before and (b) six weeks after laser labiaplasty

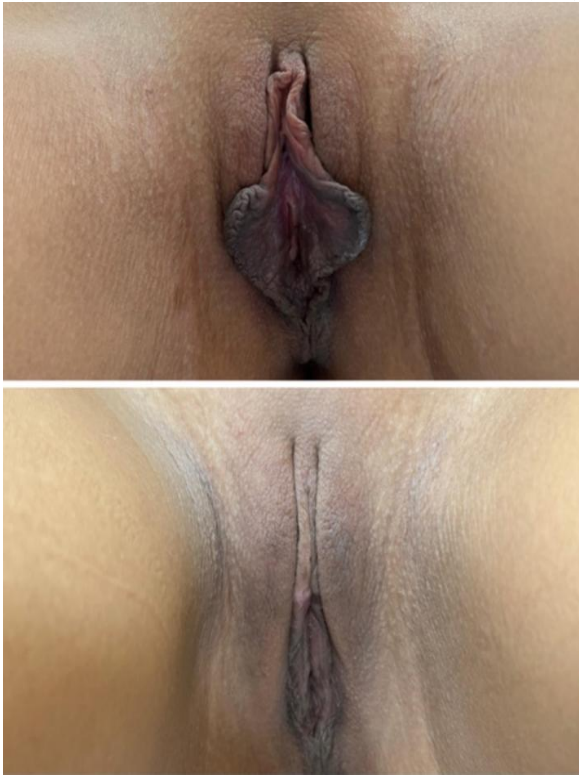

RESULTS

The study included 60 women aged between 19 and 52 years, with a mean age of 34.7 years. The average age at menarche was 13.1 years. Regarding reproductive history, 40% of patients were nulliparous, 35% had one pregnancy, and 25% had two or more pregnancies. Positive gynecological history, such as polycystic ovary syndrome, previous cesarean sections, or uterine fibroid removal, was present in 30% of patients. This demographic distribution indicates a diverse patient population seeking laser labiaplasty (Table 1).

Table 1: Demographic and Clinical Characteristics

Parameter	Mean (SD)	Range
Age (years)	34.7 (7.8)	19 - 52
Age at menarche (years)	13.1 (1.4)	11 - 16
Number of pregnancies	0.9 (1.1)	0 - 4
Positive gynecological history (%)	30%	N/A

SD – Standard Deviation

Aesthetic concerns were the primary motivation for 85% of patients, highlighting the significance of genital appearance in this cohort. Functional discomfort during daily activities, such as exercise or wearing tight clothing, was reported by 55% of patients. Discomfort during sexual intercourse affected 45%, indicating that labia minora hypertrophy can significantly impact sexual function. Recurrent infections or hygiene difficulties were less common but still noteworthy at 15% (Table 2).

Table 2: Preoperative Motivations

Motivation	Number of Patients (%)
Aesthetic concerns	51 (85%)
Functional discomfort during activities	33 (55%)
Discomfort during sexual intercourse	27 (45%)
Recurrent infections/hygiene issues	9 (15%)

Postoperative complications were minimal. Mild bleeding occurred in 10% of patients, resolving within an average of 2.3 days without intervention. Mild to moderate discomfort was experienced by 25% of patients, lasting approximately 4.2 days. Notably, statistical analysis revealed a significant association between previous pregnancies and postoperative discomfort ($p=0.03$), suggesting that multiparous women may experience more discomfort due to tissue changes from childbirth. No infections or hematomas were reported, indicating a favorable safety profile (Table 3).

Table 3: Postoperative Complications and Recovery

Complication	Number of Patients (%)	Duration (Days)	p-value
Mild bleeding - Total	6 (10%)	2.3 (1.1)	0.12
Mild to moderate discomfort – Nulliparous	6 (10%)	4.0 (1.5)	-
Mild to moderate discomfort – One pregnancy	5 (8.3%)	4.1 (1.6)	-
Mild to moderate discomfort – Multiparous	4 (6.7%)	4.5 (1.8)	0.03*
Infection	0 (0%)	N/A	N/A
Hematoma	0 (0%)	N/A	N/A

* *Student's t-test*

Table 4 provides a comprehensive overview of patient satisfaction following laser labiaplasty, as measured by the standardized Global Aesthetic Improvement Scale (sGAIS). The table categorized patient responses into five distinct levels, highlighting the overwhelmingly positive feedback received. Specifically, a majority of 33 patients, accounting for 55% of the total surveyed, reported a "Very significant improvement" in their condition, with an sGAIS score of 5. This indicated that these patients perceived the results of their surgery as greatly surpassing their expectations in terms of both aesthetic appeal and functional benefits. Additionally, 22 patients, representing 37% of the total, observed a "Significant improvement," scoring a 4 on the sGAIS. This suggests that for a large number of patients, the procedure substantially met their initial goals and resulted in marked enhancements.

On the lower end of the scale, 5 patients, making up 8% of the total, felt they had only "Moderate improvement," denoted by a score of 3 on the sGAIS. This group perceived the changes as noticeable but not as extensive as perhaps hoped for. Importantly, the table showed that no patients reported a lack of change or a worsening of their condition post-procedure, with zero reports in both the "No change" and "Worsened condition" categories (scores of 2 and 1, respectively). This absence of negative feedback underscored the effectiveness and safety of laser labiaplasty in achieving desired outcomes without adverse effects. The data clearly demonstrated a high level of patient satisfaction overall, with 92% of participants rating their improvement as significant or very significant, highlighting the procedure's ability to meet or exceed patient expectations in the vast majority of cases.

Table 4: Patient Satisfaction (sGAIS Scores)

Satisfaction Level	No/Mild Complications n (%)	Moderate Complication n (%)	Total	p-value*
Very Significant Improvement	32 (97%)	1 (3%)	33	
Significant Improvement	16 (73%)	6 (27%)	22	
Moderate Improvement	0 (0%)	8 (100%)	5	
p-value				<0.001

* *Chi-square test*

Among 60 patients, 34 who were satisfied and 17 who were less satisfied cited aesthetic concerns, against expected counts of 37.4 and 13.6, respectively. Functional discomfort during activities was reported by 28 satisfied and only 5 less satisfied patients, compared to expected figures of 24.2 and 8.8. For discomfort during sexual intercourse, 20 satisfied and 7 less satisfied patients were recorded, versus an expected count of 19.8 for satisfied and 7.2 for less satisfied. Lastly, recurrent infections or hygiene issues were less commonly reported, with 6 satisfied and 3 less satisfied patients, aligning closely with expected values of 6.6 and 2.4. The overall chi-square analysis resulted in a p-value of 0.306, indicating no significant statistical association across the groups (Table 5).

Table 5: Analysis of Complaints and Patient Satisfaction Post-Laser Labiaplasty

Complaint Type	Satisfied Patients (n = 40, %)	Less Satisfied Patients (n = 20, %)	Expected (Satisfied)	Expected (Less Satisfied)	p-value
Aesthetic concerns	34	17	37.4	13.6	
Functional discomfort during activities	28	5	24.2	8.8	
Discomfort during sexual intercourse	20	7	19.8	7.2	
Recurrent infections/hygiene issues	6	3	6.6	2.4	
p-value					0.306

Patients with Type III hypertrophy showed a substantially higher improvement in sexual discomfort ($p < 0.001$), aligning with their higher baseline rates of dyspareunia. Differences in aesthetic and functional improvements were not statistically significant across the three groups ($p = 0.43$ and $p = 0.07$, respectively), although Type II and Type III trended toward slightly greater gains than Type I. Reduction in hygiene-related complaints was similar among all types ($p = 0.73$). Overall satisfaction (sGAIS) remained high across all groups, with no statistically significant variation ($p = 0.56$), as presented in Table 6.

Table 6: Postoperative Quality of Life Differences by Labial Hypertrophy Type

Variable	Type I	Type II	Type III	p-value
Mean Improvement in Aesthetic Concerns (0-10 scale, higher = greater improvement)	4.7 ± 1.3	4.9 ± 1.1	5.1 ± 1.2	0.43
Mean Improvement in Functional Discomfort (0-10 scale)	5.4 ± 1.3	6.2 ± 1.4	6.3 ± 1.6	0.07
Mean Improvement in Sexual Discomfort (0-10 scale)	3.3 ± 1.0	4.6 ± 1.2	6.6 ± 1.5	<0.001
Patients Reporting Fewer Hygiene Issues (n, %)	2 (28.6%)	4 (23.5%)	10 (27.8%)	<0.001
Mean Postoperative sGAIS Score (1-5 scale*)	4.4 ± 0.5	4.3 ± 0.6	4.5 ± 0.4	0.56

** Standardized Global Aesthetic Improvement Scale (SGAIs): 1 – Worsened; 2 – No Change; 3 – Moderate Improvement; 4 – Significant Improvement; 5 – Very Significant Improvement.*

DISCUSSION

Literature findings

The study demonstrates that laser labiaplasty is a safe and effective minimally invasive procedure for treating labia minora hypertrophy. The high patient satisfaction rates align with existing literature, indicating that laser techniques offer significant advantages over traditional surgical methods. The predominance of aesthetic concerns as a motivation reflects societal trends emphasizing genital aesthetics and their impact on women's self-esteem and sexual health.

The low incidence of postoperative complications, such as bleeding and discomfort, highlights the safety profile of laser labiaplasty. The precise excision and coagulative properties of the CO_2 laser reduce intraoperative bleeding and eliminate the need for sutures, contributing to faster healing and reduced postoperative pain. The absence of infections and hematomas in our cohort further supports the procedure's safety.

The significant association between previous pregnancies and increased postoperative discomfort may be attributed to changes in tissue elasticity and sensitivity resulting from childbirth. This finding suggests that multiparous women may require additional preoperative counseling and postoperative support to manage expectations and enhance recovery.

In the realm of female genital cosmetic surgery, the studies by Pardo et al. [21] and Bizjak-Ogrinc et al. [22] provide valuable insights into the efficacy and patient satisfaction associated with different techniques of laser labiaplasty of the labia minora. Pardo et al. [21] reported on 55 cases conducted between October 2003 and November 2004, where the primary indications were moderate hypertrophy and labial asymmetry, with 67% of cases due to these reasons. They noted a high satisfaction rate, with 91% of patients reporting being very satisfied and 9% satisfied, despite a 7% incidence of minimal suture dehiscence during the early postoperative period. In a similar manner, the study by Bizjak-Ogrinc et al. [22], which analyzed 80 patients from February 2015 to April 2018, found an equally high level of patient satisfaction with the sutureless laser labiaplasty technique. Their approach, interestingly, reported minimal side effects—7 instances of wound infection, and one case each of bleeding and hematoma formation, yet no patients were partly satisfied or dissatisfied. sutures.

Similarly, Li et al.'s study on 17 Chinese women, with an average age of 36.4 years, demonstrated significant improvements in vulvar texture and pigmentation after three treatment sessions, with a scoring system showing substantial changes ($p < 0.05$) and no adverse reactions [23]. Patients self-reported enhanced satisfaction, indicating both perceived and observed effectiveness. Similarly, Lauterbach et al. [24] assessed vaginal CO_2 laser treatment in a cohort of 25 women with sexual dysfunction and pelvic floor issues. They reported significant increases in vaginal elasticity and tightening post-treatment (54.8 ± 5.2 vs. 41.5 ± 6.3, $P = 0.0027$; 1.97 ± 0.25 vs. 1.32 ± 0.31, $P = 0.0014$), as well as improvements in pelvic muscle contraction strength (25.9 ± 3.5 vs. 16.5 ± 4.2, $P = 0.0011$) and FSFI and VHI scores. This study utilized vaginal tactile imaging (VTI) for objective assessment, adding robust quantification to support the clinical effectiveness of CO2 laser treatment in enhancing vaginal biomechanical properties.

Moreover, Messas et al. [25] reviewed 40 studies involving 3466 participants to assess the efficacy of fractional CO_2 ($FrCO_2$) laser therapy in treating genitourinary syndrome of menopause (GSM) and vulvovaginal atrophy (VVA) [25]. Their findings highlight the therapy's good efficacy and safety, with high patient satisfaction noted across studies. However, they also pointed out the lack of level I evidence and called for more randomized, sham-controlled trials to validate these results further and address clinical questions such as cost-effectiveness and potential synergistic effects with other treatments. Similarly, Géczi et al. [26] conducted a systematic review and meta-analysis of various labiaplasty techniques, analyzing data from 86 studies. They reported a high overall satisfaction rate (94%, CI 93%-95%) with specific techniques like deepithelialization, reaching even higher satisfaction rates (97%, CI 85%-99%). Despite these high satisfaction rates, they noted variations in complication rates across different techniques, with some, like the wedge resection, showing higher incidences of complications such as dehiscence. Both studies underscore the importance of personalized treatment approaches and the need for high-quality research to optimize outcomes in cosmetic genital surgeries.

The study by Smarrito et al. [27] provided a focused examination of secondary labiaplasties, reporting on 44 cases split into two main categories: those involving wound dehiscence primarily from lambda laser procedures and those related to over-resections or amputations. The authors highlighted the use of local flaps and lipofilling techniques to address aesthetic and functional issues resulting from over-resection, emphasizing the importance of surgical precision and anatomical knowledge to prevent such complications. In a similar manner, the systematic review and meta-analysis by Escandón et al. [28] surveyed a much larger cohort of 3804 patients across 46 studies, reporting a high satisfaction rate of 99% for labia minoraplasty. However, they also noted significant complications, such as a 5% incidence of dehiscence from laser-assisted labiaplasty and up to 8% hematoma formation from W-shape resection. Both studies underscore the necessity of thorough training in surgical techniques and patient-specific approaches to optimize outcomes and minimize the need for subsequent corrective procedures. The findings from Escandón et al. [28] further support the observations of Smarrito et al. [27] by quantitatively demonstrating the impact of technique selection on complication rates and patient satisfaction, thereby reinforcing the call for meticulous surgical practice and adequate anatomical understanding in labiaplasty procedures.

Limitations

This study has several limitations. While the study provides valuable insights, limitations include its retrospective design and the reliance on patient-reported outcomes, which may introduce bias. The lack of a control group undergoing traditional surgical labiaplasty limits direct comparison of efficacy and safety between techniques. The retrospective design may introduce selection bias, as only patients who returned for follow-up were included. The sample size, while larger than some previous studies, is relatively small and may not represent the broader population. Reliance on subjective measures like the sGAIS may not capture objective functional improvements. Additionally, the absence of a control group limits the ability to compare laser labiaplasty directly with traditional surgical methods. Future prospective, randomized studies with larger sample sizes are warranted to validate these findings and optimize patient selection and procedural protocols.

CONCLUSION

Laser labiaplasty is an effective and safe, minimally invasive procedure for women seeking labia minora reduction due to aesthetic or functional concerns. The high patient satisfaction rates and low incidence of complications make it a valuable alternative to traditional surgical techniques. Personalized patient counseling and careful consideration of individual medical histories can further enhance outcomes. Future research should focus on long-term results and direct comparisons with other surgical modalities to establish standardized guidelines.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, F.D.; methodology, F.D.; software, F.D.; validation, M-M.C. .; formal analysis, M-M.C.; investigation, M-M.C..; resources, S.A.; data curation, S.A.; writing—original draft preparation, F.D., M.M.C. and S.A.; writing—review and editing, D.O.C. and F.D.; visualization, D.O.C. and FD.; supervision, D.O.C.; project administration, D.O.C. and F.D.; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Elos Clinic from Bucharest, Romania (protocol code NR 29 and date of approval 6 January 2020).

Patient consent for publication

Consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

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ORIGINAL ARTICLES

Local treatment options for management of loco-regional esophageal squamous cell carcinoma

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Abstract: *Aim: Surgical treatment is the main treatment method for esophageal cancer. The prognosis is poor due to high local recurrence and distant metastasis rates. Study aims to evaluate the most effective local treatment modality esophageal squamous cell carcinoma (SCC) according to real life data.*

Method: 136 patients were studied retrospectively. All patients were middle or lower esophageal cancer and had the SCC histology. Patients were divided into the surgical resection, definitive CRT (dCRT), and multimodal treatment groups according to curative local treatment they received.

Result: 32.4% were in the surgical, 36% were in the dCRT, and 31.6% were in the multimodal group. Median disease-free survival was 21 months (95% CI 14-27) in the surgical group, 8 months (95% CI 4-11) in the dCRT group, and 18 months (95% CI 0-39) in the multimodal group (p=0.059). The median overall survival was found to be 40 months (95% CI 0-92) in the surgical group, 19 months (95% CI 15-22) in the dCRT group, and 54 months (95% CI 11-96) in the multimodal group (p=0.012). In multimodal group, the number of patients receiving preoperative CRT was 25, and postoperative CRT was 18. Median OS was 47 months (95% CI 0-99) in the preoperative CRT group, and 64 months (NA) in the postoperative CRT group (p=0.302).

Conclusion: DFS and OS contributions of multimodal treatment in esophageal SCC have been shown in the present study. The addition of CRT to surgery in the preoperative or postoperative period has a contribution independently of the treatment sequence.

Keywords: *multimodal treatment, surgery, definitive chemoradiotherapy, squamous cell, esophagus cancer*

INTRODUCTION

Esophageal cancer is the seventh most common cause of cancer-related deaths worldwide. In 2012, 455,800 people were diagnosed with, and 400,200 people died from esophageal cancer [1].

Surgical resection has traditionally been the primary treatment for esophageal cancer in order to maintain local disease control and prolong life expectancy. The median survival is 15-18 months, and the 5-year survival rates are 20-25% after esophagectomy [2].

Failure in the treatment is generally due to recurrence and metastases. Developments in standard therapy are ongoing. Radiotherapy can provide locoregional control in esophageal cancer, while chemotherapy has both local and systemic antineoplastic effects. With the development of multidisciplinary treatment approaches, the use of

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chemoradiotherapy (CRT) in addition to surgery has become the most frequently used treatment method [3].

In esophageal cancer treatment, the results obtained from surgical treatment alone are not satisfactory in terms of locoregional recurrence or distant metastasis rates. This has paved the way for multimodal treatment approaches. Together with the multimodal treatment, chemoradiotherapy (CRT) added to surgery in the preoperative or postoperative period, aims to provide disease-free survival and an increase in life expectancy.

Preoperative chemoradiotherapy has begun to be used to increase the R0 resection rates by reducing the tumor stage before surgery, thereby contributing to survival [4-10]. The application of surgery after preoperative chemoradiotherapy is now a well understood and effective treatment [11, 12]. In the ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study (CROSS), a pilot study related to preoperative chemoradiotherapy, the preoperative chemoradiotherapy was reported to increase the median OS approximately 2-fold when compared to surgical treatment alone (49.4 vs 24.0 months) [4].

In the literature, there are data about the postoperative (adjuvant) activity of chemo-radiotherapy in addition to its preoperative activity. In the past, the major problem in applying chemoradiotherapy in the postoperative period was the inability of patients to complete the scheduled treatment due to poor performance of the patients after surgery, but currently developing surgical and radiotherapy techniques have partly removed this problem. Studies by Rice et al. [13] and Bedard et al. [14] have demonstrated the survival advantage of postoperative CRT. In the retrospective study by Rice, the median survival was stated to be 28 months with surgical postoperative CRT, and 15 months with surgery alone ($p<0.05$). In the study by Bedard, the median overall survival was reported to be 47.5 months with postoperative CRT and 14.1 months with surgery alone ($p=0.001$) [14].

Following the studies showing the benefits of preoperative and postoperative chemoradiotherapy, studies about treatment sequence emerged. Current data on the prognostic effect of local treatment sequence in esophageal cancer remain controversial [15, 16]. In a study based on the SEER data, it was determined that preoperative CRT ensured better survival compared to postoperative CRT [15], while a prospective study reported no difference in survival between taking the CRT in the preoperative or postoperative period [16].

In the light of this information, the prognostic implications of optimal multimodal treatment, surgery and chemoradiotherapy sequence in the treatment of esophageal cancer are still controversial. No standard guideline has been created in this particular. The main aim of this study was to evaluate the most effective local treatment modality in non-metastatic, middle and lower esophageal squamous cell carcinoma (SCC) according to real-life data.

METHODS

A total of 136 patients diagnosed between the years 2005-2016 in 3 different oncology clinics were included in the study. The patient data were reviewed retrospectively. Patients aged 18-70 years, without distant metastasis, treated for curative purposes, with ECOG performance status 0-2, with appropriate liver/renal reserve, middle and lower esophagus involvement, and with SCC histology were included in the study. Patients with adenocarcinoma histopathology, with cervical esophagus involvement, and those who only received RT as a local treatment were not included in the study.

All the patients were applied with thorax and entire abdomen computed tomography (CT) or positron emission tomography (PET-CT) or endoscopic ultrasonography (EUS) examinations for the purpose of staging prior to local treatment. The patients were divided into 2 groups as local and local advanced disease according to the radiological results at the time of diagnosis. Radiologically, patients with T4 tumors or lymph node positivity were considered to have locally advanced disease, while non-T4 patients with lymph node-negativity were considered to have local disease.

The patients were divided into 3 groups as those who underwent only surgical resection, only definitive CRT (dCRT), and multimodal treatment (preoperative CRT or postoperative CRT) according to the curative local treatments they received and were evaluated comparatively. Platinum-based combination therapies (fluorouracil or taxane) were administered concomitantly with the chemotherapy regimen in patients receiving chemoradiotherapy.

In CRT planning, a 3 mm interslice distance CT scan was performed on all patients in the supine position. In patients undergoing definitive and preoperative chemoradiotherapy, the gross tumor volume (GTV) was determined for primary mass and involved lymph nodes using thoraco-abdominal CT, PET-CT, endoscopy and EUS information, if available. The clinical target volume (CTV) was established by giving a margin of 4 cm in the superior-inferior direction and 1 cm in the radial direction to the primary mass. The planning volume was obtained by allowing 1 cm extra margin to this. The spinal cord, lungs, and organs at risk, such as the heart, remaining within the area were contoured. In postoperative therapy, bilateral supraclavicular or celiac lymph nodes were included in the area in the location of all mediastinum and primary masses.

The gastroesophageal intersection and proximal stomach were included in the area in distal localized tumors.

The radiotherapy (RT) plans were made by applying the 3-dimensional conformal technique. Patients undergoing definitive CRT (dCRT) received a total of 50.4 Gy radiotherapy, and those undergoing pre-operative or postoperative CRT received a total of 45 Gy radiotherapy, which was applied as 1.8 Gy daily for 5 days a week.

All patient records were reviewed clinically, pathologically and in terms of treatment characteristics. The long-term survival data of patients were obtained from the patient files and from the data of the Turkish civil registry office.

Approval for the study was granted by the Ethics committee of Sağlık Bilimleri University Dışkapı Yıldırım Beyazıt Training and Research Hospital.

The data were analyzed using SPSS 18.0 for Windows software. Descriptive statistics were expressed as mean ± standard deviation or median (minimum-maximum) for non-continuous numerical variables, and as number (n) and percentage (%) for categorical variables.

The progression and overall survival rates of all cases were investigated using Kaplan-Meier survival analysis. The 2-year and 3-year cumulative progression and overall survival rates together with mean lifespan and 95% confidence intervals for these terms were calculated. The demographic characteristics of the patients were assessed using descriptive statistical methods. Parameters that were significant in univariate analysis were assessed using Cox regression multivariate analysis. A value of p<0.05 was accepted as statistically significant.

RESULTS

A total of 136 patients were evaluated comprising 32.4% (n=44) in the surgical group, 36% (n=49) in the definitive CRT (dCRT) group, and 31.6% (n=43) in the multimodal treatment group. Of the total patients, 52.2% were female and 47.8% were male.

The demographic characteristics of the patients are shown in Table 1.

Table 1: Demographic characteristics of the patients

		Surgery (n=44)	**dCRT** (n=49)	**Multimodal Treatment** (n=43)	***p* Value**
Age (years)	Median (min-max)	52 (32-76)	58 (25-77)	50 (32-70)	0.001
Gender	Female	26 (59.1%)	21 (42.9%)	24 (55.8%)	0.249
	Male	18 (40.9%)	28 (57.1%)	19 (44.2%)	
Smoking	Smokers	20 (45.5%)	26 (53.1%)	12 (39.5%)	0.426
	Non-smokers	24 (54.5%)	23 (46.9%)	26 (60.5%)	
Alcohol Use	Users	15 (34.1%)	14 (28.6%)	19 (44.2%)	0.288
	Non-users	29 (65.9%)	35 (71.4%)	24 (55.8%)	
Comorbid disease	Present	15 (34.1%)	16 (32.7%)	8 (18.6%)	0.208
	Absent	29 (65.9%)	33 (67.3%)	35 (81.4%)	
Cancer history in family	Present	9 (20.5%)	14 (28.6%)	9 (20.9%)	0.581
	Absent	35 (79.5%)	35 (71.4%)	34 (79.1%)	
Tumor Grade	Well	2 (4.5%)	5 (10.2%)	4 (9.3%)	0.240
	Moderate	8 (18.2%)	6 (12.2%)	5 (11.6%)	
	Poor	3 (6.8%)	12 (24.5%)	4 (9.3%)	
Location of tumor	Mid-esophagus	18 (40.9%)	25 (51.0%)	25 (58.1%)	0.271
	Lower esophagus	26 (59.1%)	24 (49%)	18 (41.9%)	
Pre-clinical stage	Early	26 (59.1%)	11 (22.4%)	20 (46.5%)	0.001
	Locally advanced	18 (40.9%)	38 (77.6%)	23 (53.5%)	

When the postoperative stages of 44 patients treated with surgery alone were evaluated, it was seen that 15.9% (n=7) of these patients were stage 1, 40.9% (n=18) were stage 2, and 43.2% (n=19) were stage 3. Adjuvant chemotherapy with cisplatin/fluorouracil combination was administered to 27.3% (n=12) of the patients in this group.

One patient died in the intensive care unit due to surgical complications in the postoperative period.

In the group of patients receiving definitive CRT, 1 patient died due to complications during treatment.

The number of patients receiving preoperative CRT was 25 and the number of patients receiving postoperative CRT was 18 in the multimodal treatment group. In the multimodal treatment group, a pathological complete response was determined in 64% (n=16) preoperative CRT group. In this group, 4 patients died in the postoperative period due to complication. In postoperative CRT group, 11.1% (n=2) of the patients were stage 1, 16.7% (n=3) were stage 2, and 72.2% (n=13) were stage 3 after surgery.

The median follow-up time was 31 months (2-142) in the surgical group, 11 months (2-106) in the definitive CRT group, and 20 months (4-140) in the multimodal treatment group.

The median disease-free survival was 21 months (95% CI 14-27) in the surgical group, 8 months (95% CI 4-11) in the definitive CRT group and 18 months (95% CI 0-39) in the multimodal treatment group (p=0.059). DFS curve shown in Figure 1.

Figure 1: Disease Free Survival

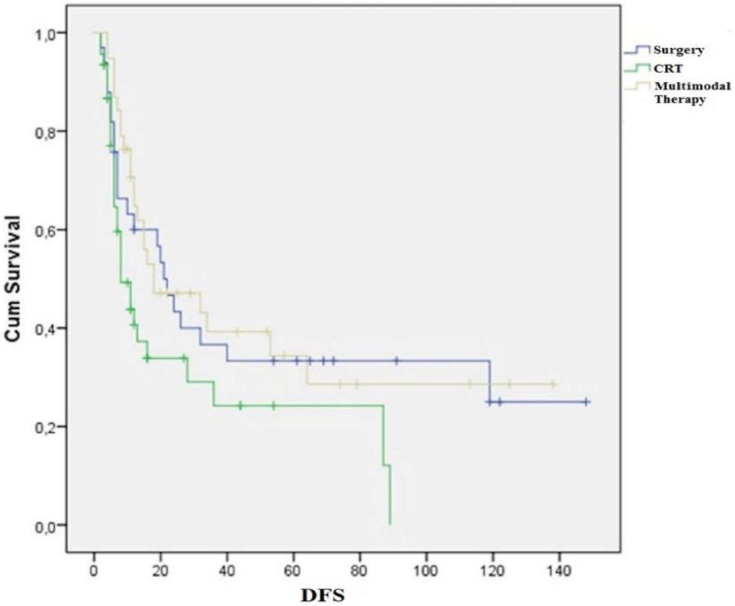

The 2-year disease-free survival (DFS) was 43% in the surgical group, 33% in the definitive CRT group, and 47% in the multimodal treatment group (p=0.05).

The median overall survival (OS) was 40 months (95% CI 0-92) in the surgical group, 19 months (95% CI 15-22) in the definitive CRT group and 54 months (95% CI 11-96) in the multimodal treatment group. The difference between the groups was statistically significant (p=0.012). Cumulative OS curve shown in Figure 2.

The 2-year OS was 68% in the surgical group, 40% in the definitive CRT group and 59% in the multimodal treatment group (p=0.01).

In the multimodal treatment group, the median OS was found to be 47 months (95% CI 0-99) in the preoperative CRT group, and 64 months (NA) in the postoperative CRT group. The difference between the groups was not statistically significant (p=0.302). OS curve when the multimodal group was divided into two as preoperative and postoperative CRT shown in Figure 3.

DISCUSSION

Surgical resection is the basic method that is conventionally effective in providing local disease control in non-metastatic esophageal cancer treatment. However, due to high locoregional recurrence and distant metastasis rates, surgical resection alone is insufficient. The median overall survival after esophagectomy is 15-18 months and the 5-year survival rate is 20-25% [2].

Figure 2: Overall Survival

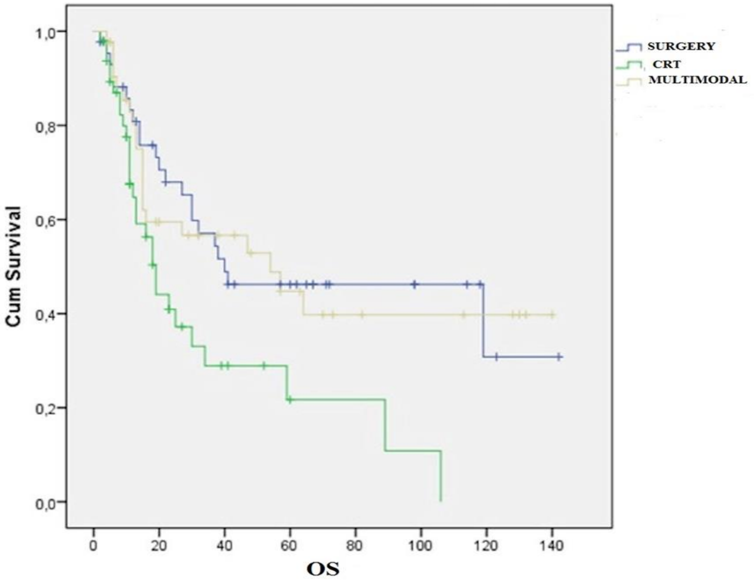

Figure 3: Overall survival in definitive chemoradiotherapy group

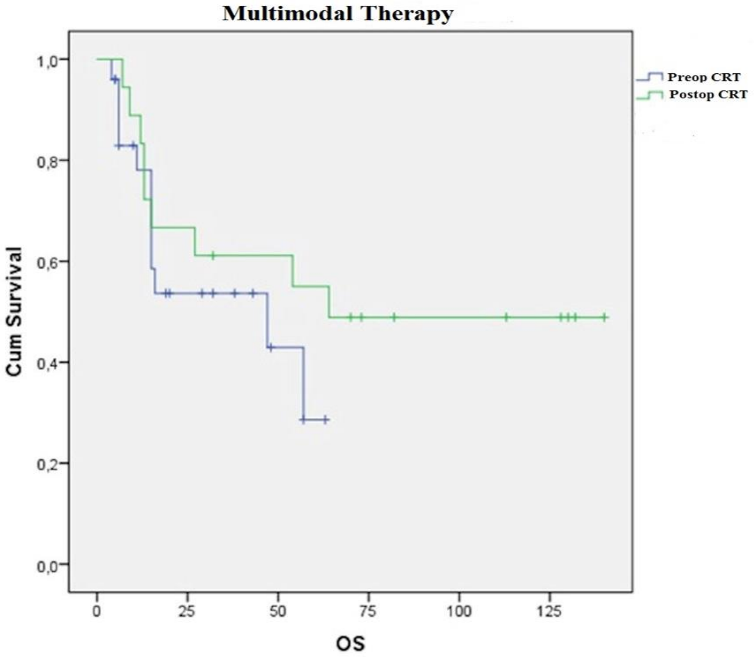

In a study by Orringer et al. conducted on 800 patients, the 5-year survival rates after esophagectomy were reported 23% [17]. In another study in which the surgery was compared with surgery after preoperative CRT, and patients were evaluated in the early stage, the median OS was reported as 18.6 months in the patients who underwent surgery [9].

In the present study, the disease-free survival was 21 months and the overall survival was 40 months in the group of patients who underwent surgery alone. In the surgical group, 27.3% of the patients (n=12) received adjuvant chemotherapy with Cisplatin/Fluorouracil combination. Compared to general literature data, the patients included in the current study had longer DFS and OS. Possible reasons for this may be the exclusion of upper esophageal tumors, which are known to have a worse prognosis, and the fact that approximately 25% of the patients who underwent surgery were administered adjuvant chemotherapy.

Although surgical resection is still the basic standard approach in non-metastatic esophageal tumors, definitive chemoradiotherapy is still an effective local treatment alternative for patients not suitable for curative surgery [18]. In one of the largest studies in which a direct comparison was made between definitive chemoradiotherapy and surgery, the two-year survival rates were 45.4% in the definitive CRT group and 56.2% in the surgical group [19]. This study supports the idea that all patients who cannot undergo surgery due to high risk may be recommended definitive chemoradiotherapy independent of the histology.

In the present study, the definitive CRT group showed a disease-free survival of 8 months, an overall survival of 19 months, and a 2-year survival rate of 43%. These results are consistent with the literature.

Although surgical resection is the curative treatment approach for esophageal cancer, the poor long-term survival outcomes have encouraged multimodal treatment approaches in this patient group. With preoperative chemoradiotherapy as one of the multimodal treatment methods, the treatment of micro metastases and regression of the tumor stage contribute positively to the overall survival rates of patients by increasing the rate of curative resection [4-10].

In the CALGB 9781 study, the median survival was 4.4 years vs. 1.7 years (p=0.002) and 5-year survival rates were reported to be 39% vs 16% in favor of multimodal treatment in esophagectomy after preoperative CRT, where the cisplatin/fluorouracil combination was used compared with esophagectomy only [10]. In the CROSS trial, patients who underwent surgery after preoperative chemoradiotherapy and patients who underwent surgery only were compared. The concomitant paclitaxel-carboplatin combination was used in this study. It was reported that the preoperative CRT improved the survival times compared to the surgery group (OS 49.4 months vs 24.0 months, HR for survival, 0.657; 95% CI, 0.495-0.871; p=0.003) [4]. The long-term results of the CROSS trial also showed that the contribution to overall survival continued [12].

Another multimodal treatment approach is postoperative adjuvant CRT. Many studies have shown the contribution to survival of postoperative chemoradiotherapy in patients who have undergone esophagectomy [13, 14, 20, 21, 22, 23]. In a study by Hwang et al., the 3-year OS rates in squamous cell esophageal cancer were 44.9% in patients who underwent postoperative CRT after esophagectomy, and 28.1% in patients who underwent surgery alone [20].

Following studies showing the contribution of preoperative and postoperative CRT in esophageal cancer, questions were asked about the prognostic contribution of surgery and the sequence of chemoradiotherapy and what should be the optimal treatment sequence. In a prospective study by Lv et al., where preoperative CRT, postoperative CRT, and surgery were compared, both 5-year OS (43.5% vs 42.3%) and 5-year DFS (37.5% vs 37.2%) were found to be similar between the preoperative CRT and postoperative CRT groups (p>0.05) [16]. Chen et al. emphasized that CRT together with surgery extended the overall survival independently of the treatment sequence [24].

In a retrospective study in which Hsu et al. evaluated the optimal treatment sequence for locally advanced esophageal cancer in 2017, the preoperative CRT and postoperative CRT groups were found to have similar median OS (26 vs 23 months, p=0.31) [25]. A statistically insignificant but clinically significant difference was found in disease-free survival (16.7 months vs 10.4 months, p=0.061). However, in that study, the rate of stage 3 patients was higher in the group receiving postoperative CRT.

Based on this information, in the present study, the multimodal treatment group was found to be superior to patients who underwent surgery only or definitive CRT in terms of both overall survival and disease-free survival. The treatment sequence in the multimodal group was not found to affect the overall survival and disease-free survival and these results are consistent with the current literature.

Since the present study was retrospective, it has some limitations. As patients from three different oncology clinics were included, it was not possible to obtain information on which criteria the patients were directed to different treatment groups when the initial treatment plan was made. No information could be obtained about why the patients with local disease could not be operated on. The fact that patients undergoing surgery were operated on by different surgical clinics was another limitation. Not all toxicity data could be obtained due to the retrospective nature of the study.

In conclusion, it was determined that the multimodal treatment of squamous cell medium and lower esophageal tumors increased the disease-free survival and overall survival, but the treatment sequence had no effect in this particular. However, there are questions that still need to be answered for operable esophageal tumors, such as what the ideal local treatment modalities and combinations are, whether it is preferable to apply the chemotherapy scheme synchronously with CRT, and the location of postoperative adjuvant chemotherapy. There is a need for further randomized phase studies on these subjects.

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ORIGINAL ARTICLE

Evaluating Arbitrary and Histological Margins in High-Risk Nonmelanoma Skin Cancers: A One-Center Experience

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Abstract: Background: Nonmelanoma skin cancers (NMSCs), including basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC), often occur in high-risk areas like the face, ears, and scalp. Surgical excision is standard, but achieving tumor-free margins while preserving function and appearance is challenging. This study evaluates the adequacy of arbitrary surgical margins versus histopathological clearance. Methods: A retrospective analysis was conducted on 147 patients with BCC or cSCC in high-risk areas treated at Elias Emergency University Hospital (2020–2024). Data included demographics, tumor type, size, location, excision margins, and histological clearance. Margins were grouped from 0 mm to >10 mm. Results: Most patients were male (59.2%) with a mean age of 70.6 years. BCC accounted for 70.7% of cases. Arbitrary margins averaged 5.4 mm; mean histological margins were ~2.5 mm. Complete excision was achieved in 91.2%; positive margins were most common on the nose and scalp. Discussion: Arbitrary margins in high-risk areas are often narrower than guidelines recommend, especially where tissue is limited, increasing the risk of incomplete excision. Conclusion: While aesthetic and functional considerations often necessitate smaller margins, improved intraoperative margin assessment could enhance oncological outcomes in high-risk NMSC.

Keywords: Nonmelanoma skin cancer (NMSC), Basal cell carcinoma (BCC), Cutaneous squamous cell carcinoma (cSCC), Surgical margins, Histopathological clearance, High-risk anatomical areas, Mohs micrographic surgery, Arbitrary excision margins, Incomplete excision, Oncologic safety

INTRODUCTION

Basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC) represent the two most common forms of nonmelanoma skin cancer (NMSC), with BCC being the most prevalent malignancy globally. While BCC is characterized by slow growth and minimal metastatic risk, its potential for local destruction and disfigurement, especially in anatomically sensitive areas, underscores the importance of timely and adequate treatment. cSCC, although less frequent, poses a relatively higher risk of local invasion and lymphatic metastasis, particularly in immunosuppressed patients [1].

The cornerstone of NMSC treatment remains surgical excision, favored for its efficacy, cost-effectiveness, and accessibility across healthcare systems. Surgical excision allows for histopathological evaluation of the margins and is generally sufficient for low-risk lesions. However, for high-risk tumors located in functionally and cosmetically sensitive areas (defined as Area H: central face, ears, eyelids, nose, genitals, and hands), standard excision can be technically challenging. In these zones, even small tumors may have subclinical extension, increasing the risk of incomplete excision and recurrence [2].

A surgical margin refers to the outer edge of the tissue removed during surgery. The tumoral involvement of the resected margins is a crucial prognostic element. Evaluating the tumoral invasion of the margins is essential for determining whether the excision is sufficient or incomplete. In instances of cancer, follow-up management choices depend on whether the margins are devoid of tumors. Consequently, precise and timely margin evaluation is essential. Attaining clear surgical margins guarantees the complete removal of cancerous tissues, minimizing the chances of local recurrence [3].

While standard excision remains widely used, it has notable limitations in high-risk areas. One of the primary drawbacks is the inability to assess 100% of the peripheral

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and deep margins during surgery. Mohs micrographic surgery (MMS) is considered the gold standard for treating high-risk NMSCs, particularly recurrent or infiltrative lesions. MMS provides complete circumferential and deep margin control (CCPDMA), resulting in superior cure rates—over 98% for primary BCCs and above 95% for recurrent cases. Moreover, MMS allows for maximal tissue conservation, which is crucial in cosmetically sensitive areas. However, its disadvantages include longer operative times, higher costs, and limited availability, which restrict its universal application [4].

This study aims to demonstrate that standard surgical excision can yield acceptable oncological outcomes in the management of high-risk nonmelanoma skin cancers, particularly in under-resourced hospitals where Mohs micrographic surgery is not available.

MATERIALS AND METHODS

This study included 147 patients who underwent surgical excision of non-melanoma skin cancer at the Plastic Surgery Department in „Elias Emergency University Hospital", Bucharest, Romania, between 2020-2024.

Inclusion criteria – all patients admitted to our plastic surgery department with skin lesions with high suspicion of non-melanocytic skin cancer/histopathological confirmation, in a high-risk area, who accepted the surgical treatment. The high-risk areas were defined using NCCN guidelines depending on anatomical localization and size: head, scalp, genital area, trunk, and extremities >2cm.

Exclusion criteria – patients with BCC/cSCC in low-risk areas, premalignancies, melanoma, psychological disorders, and refusal of future treatments.

Arbitrary surgical margins were defined as preoperative markings based on local tissue availability, skin elasticity, and the surgeon's experience. Data regarding sex, age, comorbidities, type of anesthesia used, type of reconstruction, surgical dissection plane, arbitrary surgical margins, peripheral and deep clean margins, tumor diameter, and ulceration were collected and analysed. Surgical standard excision was performed using a no.15 blade in a full-thickness fashion. The deep dissection plane was chosen depending on the case. Excisions were performed under local, regional, or general anesthesia. For wound closure, depending on defect size and location, we used primary closure, skin grafts, and/or flaps. Specimens were oriented using sutures as markings for histopathological evaluation. Excised specimens were sent for histopathological examination to assess tumor clearance.

The histological clearance margins of the lesions were measured peripherally and in depth, recorded in millimeters to one decimal place. They were categorized into five groups:

- zero clearance (indicating margin involvement)
- clearance under 1 mm (0.1–0.9 mm)
- clearance between 1 and 4.9 mm
- clearance between 5 and 9.9 mm
- clearance greater than 10 mm.

Those with zero clearance were advised to have a re-excision following their histopathological results. Patients with clearance margins under 1 mm underwent dermatoscopy surveillance every 3 months; in case of recurrence, the tumors were re-excised. Patients were monitored for wound healing and recurrence.

RESULTS

Demographic

In the 4 years, there were 147 patients, predominantly men with 87 cases (59.2%), with an average age of 70.6 years (see Table 1). BCCs were the predominant cancer, with 104 excisions (70.7%), while cSCCs had 43 excisions (29.3%).

Regarding comorbidities, cardiovascular diseases were predominant in 68 cases (46,2%), followed by diabetes mellitus in 11 cases (7.4%) and other malignancies in 10 cases (6.8%). The anesthesia of choice was local in 143 cases (97.3%), regional and general anesthesias were less prevalent at 2 and 3 cases, respectively. The preferred method of reconstruction was the local flap, with 54 cases (36.7%), followed by full-thickness skin grafts with 38 cases (25.9%), and direct sutures with 36 cases (24.5%). On the other hand, reconstruction techniques such as regional flaps and split-thickness skin grafts were rarely used.

The surgical plane of choice was subcutaneous with 62 cases (42.2%), suprafascial with 42 cases (28.6%), while deeper planes such as supracondylar (14.3%) and supraperiostal (8.2%) have been rarely used.

Incomplete excisions

There were 13 cases of incomplete excisions (see Table 2). The areas with incomplete excisions were located on the nose, with 5 out of 13 cases, followed by the scalp, 4 out of 13 cases. The cheek and peri-orbital regions had a lower rate of incomplete excisions, accounting for 2 cases. Notably, no incomplete excisions were recorded for lesions located on the lips, limbs, or trunk.

Table 1: Demographics and Clinical Characteristics

Sex - female - male	 60 (40.8%) 87 (59.2%)
Age	70.6 years (29 – 94 years)
Type - BCC - SCC	 104(70.7%) 43 (29.3%)
Comorbidities - CV - Diabetes Mellitus - other malignancies	 68(46.2%) 11(7.4%) 10 (6.8%)
Type of anesthesia - local - regional - general	 143 (97.3%) 2 (1.4%) 3 (2%)
Type of reconstruction - direct suture - skin graft -STSG -FTSG - local flap - regional flap	 36 (24.5%) 6 (4.1%) 38 (25.9%) 54 (36.7%) 3 (2%)
Surgical dissection plane - subcutaneous - suprafascial - supracondral - supraperiostal	 62 (42.2%) 42 (28.6%) 21 (14.3%) 12 (8.2%)
Arbitrary surgical margins (mean, mm)	5.4 mm (2-15mm)
Largest diameter (mean, mm)	21.6 mm (5 – 184mm)
Peripheral clearance margins (mean, mm)	2.52 mm (0-9.2mm)
Deep clearance margins (mean, mm)	2.65mm (0-20mm)

Table 2: Incomplete excisions expressed as % of all incomplete excisions depending on anatomic site

Anatomic site	Total Lesions	Incomplete Excisions	% of All Incomplete Excisions	Mean Diameter (mm)	Mean Arbitrary Margin (mm)	Presence of Ulceration (n)
Cheek	17	1	7.7%	17.11	4.3	16
Forehead	15	2	15.4%	18.6	4.4	9
Nose	33	5	38.5%	14.1	4.3	24
Peri-orbital	24	1	7.7%	15.45	5.2	15
Lips	8	0	0%	13.3	6.2	8
Scalp	26	4	30.8%	22.6	5.0	19
Limbs	14	0	0%	35.2	9.2	11
Trunk	10	0	0%	58.6	7.6	6
	147	**13**				

The majority of cancers (64.6%) had peripheral histological clearance margins between 1.0–4.9 mm, as we can see in Table 3. Margins with clearance under 1 mm were present in 15.6% of cases. Complete margin involvement (0 mm clearance) was identified in 4.8% of tumors, comparable to the 8.8% incomplete excision rate. No cases exceeded peripheral margins larger than 10 mm, balancing oncological safety with tissue preservation.

The most common deep margin range was 1.0–4.9 mm, present in 57.1% of cases. Deep margins between 0.1–0.9 mm were observed in 19% of cases. Complete deep margin involvement (0 mm clearance) was identified in 8.8% of cases, reinforcing the necessity of achieving adequate excision depth to minimize recurrence risk. Only 4.1% of lesions had deep clearance margins exceeding 10 mm.

Table 3: Margin Clearance Distribution Among Excised Tumors

Margins (mm)	Peripheral total	% total lesions	Deep total	% total lesions
0	7	4.8%	13	8.8%
0.1-0.9	23	15.6%	28	19.0%
1-4.9	95	64.6%	84	57.1%
5.0-9.9	13	8.8%	16	10.9%
>10	0	0%	6	4.1%

Peripheral Margins >1 mm

The highest histological clearance rates, with peripheral margins over 1 mm, were noted in lesions located on the limbs (100%) and scalp (73%), suggesting that these regions allow a wider excision. In contrast, the lowest rates of peripheral clearance beyond 1 mm were documented in lesions on the forehead (6.1%) and lips (4.8%), revealing the technical difficulties of obtaining wider margins in these areas.

Table 4: Distribution of Peripheral and Deep Histological Clearance Margins depending on anatomic site

Anatomic site	Total lesions	Peripheral histological clearance margins > 1mm	%	Deep histological clearance margins (0.5-4.9 mm)	%
Cheek	17	13	8.8%	17	11.6%
Forehead	15	9	6.1%	11	7.5%
Nose	33	21	14.3%	27	18.4%
Peri-orbital	24	16	10.9%	22	15.0%
Lips	8	7	4.8%	8	5.4%
Scalp	26	19	12.9%	21	14.3%
Limbs	14	14	9.5%	13	8.8%
Trunk	10	8	5.4%	10	6.8%
	147	**107**	**72.8%**	**129**	**87.8%**

Deep Margins (0.5–4.9 mm)

The nasal region had the greatest number of cases with deep margins in the 0.5-4.9 mm range, with 27 cases (18.4%), as seen in Table 4, explaining its elevated rate of incomplete excisions. Areas such as the peri-orbital region, presented 22 cases (15.0%), the scalp with 21 cases (14.3%), reveal the difficulties of achieving sufficient deep margins in said locations.

DISCUSSION

Basal cell carcinoma is the most prevalent cancer, presenting with a slow growth rate and a very low metastatic risk, located predominantly on the head and neck, but also on the limbs and trunk [5]. BCCs tend to be locally destructive and may cause deformity or recurrences if not fully excised [6]. The most important risk factor is long-term/chronic sun exposure, followed closely by a history of either BCC or SCC, with patients being more likely to develop another BCC compared to patients without a history of NMSCs. The rate of developing BCC increases with age, with an average age of 68 years; men have higher rates of BCC than women [7]. The treatment of choice is removing the tumor through different methods, such as Mohs micrographic surgery, peripheral and deep en face margin assessment, cryosurgery, electrodesiccation, and surgical excision. Surgical excision remains the staple method of removing the tumor [8,9].

Cutaneous squamous cell carcinoma, while not as prevalent as BCC, is still a frequent malignancy with similar risk factors as the BCC, such as sun exposure, Fitzpatrick skin types I-II, male gender, and other etiologies such as immunosuppression and chronic wounds (e.g., Marjolin Ulcer). Regarding the recurrence rate following surgical excision, cSCCs located in areas prone to prolonged sun exposure yielded a higher rate of recurrence compared to areas with less sun exposure. The primary risk of recurrence is invasion of the dermis. On the other hand, there was no noticeable difference between the 2 areas regarding the rate of metastases [10,11]. SCCs pose a low metastatic risk, the preferred metastatic site being the lymph node [12,13]. They can be more aggressive than BCCs, with a higher likelihood of local invasion and potential for metastasis, especially in high-risk cases. Understanding the local invasiveness of NMSCs is crucial for determining appropriate treatment strategies and improving patient outcomes [14].

Surgical excision of NMSCs aims to completely remove the tumoral tissue while sacrificing as little normal tissue as possible. This can be achieved through simple elliptical excision. However, Mohs micrographic surgery is the gold standard for treating high-risk tumors. Its primary benefit lies in conserving healthy tissue, preserving anatomical structures, and ensuring tumor-free margins. A recent randomized clinical trial found that Mohs surgery yielded superior outcomes for recurrent BCC compared to direct excision, although no significant differences were observed for primary BCC treatment [15].

Area H refers to high-risk regions, including the central face, ears, genitals, and hands, which require balancing oncologic control with cosmetic and functional outcomes [16]. Mohs surgery is an excellent option for preserving function and enhancing cosmetic outcomes. From a reconstructive perspective, imaging techniques help to identify optimal blood supply for flaps, minimizing complications and improving success rates. As a result, the focus of skin cancer surgery has evolved from simple excision and flap coverage to superior functional and aesthetic outcomes [17].

Peripheral and deep en face margin assessment (PDEMA), also known as complete circumferential peripheral and deep margin assessment (CCPDMA), is associated with the highest cure rates for NMSCs, due to complete peripheral and deep margin visualization, and re-excision of residual tumor [18]. Mohs is the most common method of PDEMA; however, there are also other techniques, such as the Tubingen method, which differs from Mohs by having a histopathological examination in a 1-5 day interval over Mohs's rapid frozen section and interpretation [19].

The mean age of patients with NMSCs in our study was 70.6, with a tendency to be more represented in males. Ciążyńska et al. observed no significant differences between genders. However, the mean age of men in their group was 70.9 +/- 11,4 years, and for women 71.4 +/- 11.9 years [20].

According to NCCN Guidelines Version 2.2025, high-risk criteria for both BCCs and cSCCs are: tumor diameters >= 2 cm in the trunk and extremities, any size pertaining to the head, neck, feet, pretibial and anogenital area, recurrence, immunosuppression, and poorly defined borders [21,22].

The issue with arbitrary surgical margins is that they can lead to either removing more normal tissue than necessary or less than required, and thus leading to recurrence. A study from the Hospital Universitari Vall d'Hebron in Barcelona reviewed histopathological reports from all BCCs treated by different specialties, aiming to determine the influence of a surgical specialty on assessing and excising a tumor with proper safety margins. It revealed that dermatologists had the lowest rate of positive margins out of other specialties, such as plastic surgery, general surgery, etc. attributed to their broader knowledge and expertise in evaluating tumor margins macroscopically. Therefore, proper identification of macroscopic tumoral margins for excision must be emphasized [23].

Australian Journal of Plastic Surgery conducted a study in which they monitored subjects with different levels of experience, as they were drawing a 3 mm, 5 mm, and 10 mm margin around different lesions without using a measurement device, with acceptable margins being considered to be approximately within 0.5 mm of the recommended surgical margin. Margins were underestimated, no matter the level of experience, being smaller in size for each bracket. Margins of 3 mm have been the most accurately estimated out of the 3, increasing the margin size leads to an increase in inaccuracy. This serves to enforce the importance of proper safety margins and the risk of re-excisions caused by improper measurements [24,25].

The majority of excised tumors (64.6%) displayed peripheral histological clearance margins ranging between 1.0–4.9 mm. Narrow peripheral margins (0.1–0.9 mm) have been observed in 15.6% of cases. Complete margin involvement (0 mm clearance) was identified in 4.8% of tumors. No cases exceeded peripheral margins larger than 10 mm, balancing oncological safety with tissue preservation. The most common deep margin range was 1.0–4.9 mm, present in 57.1% of cases. Deep margins between 0.1–0.9 mm were observed in 19% of cases. Complete deep margin involvement (0 mm clearance) was identified in 8.8% of cases, reinforcing the necessity of achieving adequate excision depth to minimize recurrence risk. Only 4.1% of lesions had deep clearance margins exceeding 10 mm. Khalid-Raja M. suggested identifying the peripheral margin of excision by using the wet blotting technique and applying a 4-mm margin of excision to all NMSCs. Their results showed a 95% successful excision rate and a peripheral clearance margin of 0.1-14 mm, a mean peripheral margin of 3.73 in the case of BCCs, and a 96% successful excision rate with peripheral margins varying from 0.5 to 19.0 mm, a mean peripheral margin of 4.86. Comparatively, our study showed a 92% successful excision rate and a mean peripheral margin of 2.52 mm, with the mean average surgical margin being 5.4 mm. The highest number of incomplete excisions was located at the nose and scalp with arbitrary surgical margins of 4.3 and 5.0 mm, respectively. Lesions over 2 cm lead to a 91.2% complete excision with a 4 mm margin while using the wet blotting technique. This coincides with our findings [26,27].

J Kiely et al. recommend the excision of poorly differentiated cSCCs with a minimum peripheral margin of 6 mm, well-differentiated cSCCs were excised with an average peripheral margin of 4.72 mm, and the poorly differentiated ones with an average margin of 6.42 mm. This led to an involvement of peripheral margins in 3% of well-differentiated lesions and 13.2% of poorly differentiated ones. Deep margins were also close or involved in 50% of poorly differentiated cSCCs, demonstrating that poorly differentiated cSCCs are not properly excised in both peripheral and deep planes, in spite of the guidelines [28].

Australian Journal of General Practice recommends for high-risk lesions over 5 mm margins for BCCs and a 6 mm margin for cSCCs, claiming selection of surgical margin should aim to balance between incomplete excision of the tumor and normal tissue sacrifice, affecting cosmetic and functional outcomes [29].

Eva Van Loo et al. found the recommended excision margins for BCC too defensive, evaluating the Dutch Guideline, which states that the margin for low-risk BCC should be 3-4 mm, and the high-risk BCC up to 20 mm, incorporating the H-zone as a high-risk BCC. Currently, the Dutch and European consensus is that high-risk BCC should have a minimum of 5 mm, or 5-15 mm (European guideline). The findings indicate that adherence to the guideline in high-risk BCC was not adequate, yet it had a limited effect on the incomplete excision rate. A margin of 3 mm was sufficient for 99.6% of low-risk BCCs, and a 5 mm margin for high-risk BCCs up to 94% [30].

High incomplete excision rates (8.8%) suggest that in certain areas, such as the nose and scalp, wider margins might be needed. The lowest incomplete excision rates were found in the peri-orbital region and the cheek, while no incomplete excisions were documented on the lips, trunk, and limbs. Ceder H found higher incomplete excision rates located on the face and scalp, the highest ones being on the nose, ear, and peri-orbital area; also noteworthy, circular excisions were also more often incomplete compared to elliptical excisions [31].

Despite its high cure rates and tissue-sparing advantages, Mohs micrographic surgery (MMS) presents several notable disadvantages that limit its universal application. One of the primary concerns is the limited availability of specialized centers and trained personnel, making access difficult in under-resourced or rural healthcare settings. MMS is also more time-consuming than standard excision, as it involves intraoperative histological analysis of multiple tissue layers, requiring patients to remain in the facility for extended periods. Additionally, the procedure is costlier due to the need for specialized equipment and trained histopathologists, potentially placing a financial burden on healthcare systems and patients alike. From a logistical standpoint, not all lesions are suitable for MMS—tumors with extensive subclinical spread, poorly defined borders, or aggressive histologic subtypes may require complementary or alternative approaches. Lastly, in settings where rapid frozen-section pathology is not feasible, delays in diagnosis and treatment can occur, reducing the practicality of MMS outside high-volume dermatologic surgery centers [32]. While Mohs surgery offers excellent cure rates, its use in elderly patients requires careful consideration. The prolonged procedure time, need for patient immobility, and potential for postoperative care challenges may be poorly tolerated by those with comorbidities or cognitive impairment. Therefore, treatment should be individualized based on the patient's overall health, functional status, and life expectancy [33].

CONCLUSION

This study highlights the challenges of achieving adequate surgical margins in high-risk anatomical areas affected by nonmelanoma skin cancers. Although arbitrary margins based on surgeon experience averaged 5.4 mm, histopathological evaluation revealed that a significant number of excisions had suboptimal peripheral or deep clearance, particularly in constrained areas like the nose and scalp. Despite a 91.2% overall rate of complete excision, the 8.8% rate of positive margins underscores the limitations of visual and tactile margin estimation alone. The findings support existing guidelines recommending wider margins ≥5 mm for BCC and ≥6 mm for cSCC, while also emphasizing the need to balance oncologic safety with aesthetic and functional preservation. In high-risk regions, individualized surgical planning, improved intraoperative margin assessment, and consideration of techniques like Mohs micrographic surgery in selected cases may optimize outcomes and minimize recurrence. Ultimately, refining margin estimation and tailoring surgical strategy to both tumor characteristics and anatomical context are essential for improving the quality of care in patients with high-risk NMSCs.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, S.M.R and K.A.J, methodology, A.L.P.; formal analysis, A.L.P.; data curation, P.A.C; writing—original draft preparation, K.A.J, A.L.P; writing—review and editing, S.M.R..; visualization, R.D.S..; supervision, R.D.S.; project administration, A.L.P. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Elias University Emergency Hospital, Bucharest, Romania (protocol code 3606 and 19.06.2025).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

New Study of the antimicrobial activity of natural extracts of Taraxacum officinale and Chelidonium majus

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Abstract: The rise of antimicrobial resistance poses a significant challenge to global health. This study investigates the antimicrobial properties of Taraxacum officinale and Chelidonium majus. Taraxacum officinale, commonly known as dandelion, has been utilized for centuries in traditional medicine for its various therapeutic benefits, including treating cholecystitis, enhancing hepatic function, regulating blood pressure and cholesterol levels, and exhibiting diuretic effects. Additionally, research has indicated that infusions derived from this plant offer favorable outcomes in managing renal diseases. This study aims to assess the antimicrobial activity of extracts from Taraxacum officinale and Chelidonium majus employing qualitative screening, quantitative determination, and in vitro evaluation of anti-biofilm properties. In vitro evaluation of the antibiofilm activity of plant products revealed their ability to inhibit the development of biofilms on inert substrate; plant extracts of C. majus were found to inhibit biofilm formation in all bacterial strains tested, while tincture of T. officinale showed very good activity on gram-negative and fungal strains. The results obtained are promising, supporting further research into the biochemical characteristics of these extracts in order to formulate strategies for the control of bacterial and fungal infections in humans.

Keywords: extracts, antimicrobial effect, antibiofilm, Taraxacum officinale, Chelidonium majus

INTRODUCTION

The modern era of antimicrobial chemotherapeutics began with the discovery of sulfonamides and penicillin, and in the following decades the spectrum of antimicrobial substances was broadened by the discovery of new active substances capable of preventing and/or treating infections caused by pathogenic microorganisms. Thus, the introduction of antibiotics was a crucial moment that underpinned the saving of millions of patients, while at the same time leading to significant advances in medicine [1].

However, less than a decade after the clinical use of antibiotics, as a result of inappropriate prescribing or overuse of antibiotics both in humans and in veterinary and zootechnical medicine, without certainty of the infectious agent determined by laboratory tests or to promote animal husbandry [2], the gains from the development of this treatment are threatened by increasing rates of pathogen resistance to antibiotics, now leading to the Antimicrobial Resistance Crisis (AMR) [3].

The global increase in the incidence of infectious diseases that can no longer be eradicated with the substances developed to date represents the evolving response of microorganisms to the widespread administration of antibiotics. It has been declared by multiple organizations, such as the World Health Organization (WHO),

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to be a public health problem of concern. Therefore, the AMR crisis has led to the need to investigate and discover new potential antimicrobial agents [4,5,6].

A new research direction is to exploit the antimicrobial properties of large varieties of medicinal plants, which represent a natural reservoir providing many compounds useful in the fight against multidrug-resistant microorganisms [7,8].

Taraxacum officinale (dandelion), is a medicinal plant that has been used for centuries for its benefits in the treatment of conditions such as cholecystitis, improving hepatic function, lowering blood pressure and blood cholesterol levels, or for its diuretic effect. In addition, studies in the literature have shown that infusions made from this plant have beneficial effects on renal diseases [8].

Chelidonium majus (celandine), one of the oldest and most widely used plants in traditional medicine, presents manyfold benefits, being often used in the treatment of eczema and condyloma caused by Human Papilloma Virus (HPV) infection, hepatic and gastric diseases, as well as having anti-inflammatory and analgesic effects. [9,10,11,12,13,14,15].

The aim of this work is to study the antimicrobial activity of extracts of Taraxacum officinale (tincture), Chelidonium majus (tincture). The following objectives were proposed to achieve this aim: qualitative screening of the antimicrobial activity of plant extracts, quantitative determination of the antimicrobial activity of plant extracts and in vitro investigation of the anti-biofilm properties of plant extracts. [16,17,18].

MATERIALS AND METHODS

Two plant extracts were analyzed in this study: Taraxacum officinale (dandelion) tincture and Chelidonium majus (tumbleweed) tincture.

Reference microorganisms were tested, two gram-negative bacterial strains: Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853, two gram-positive strains: Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212 and one fungal strain: Candida albicans ATCC 10231, according the American Type Culture Collection.

Qualitative evaluation of plant extracts was carried out by the drop method on solid agar medium by adapting the diffusimetric antibiogram method. Technique is: a 5µl volume of each plant extract was dropped onto the surface of a Plate Count Agar (PCA), non seleciv medium, inoculated with a standardized microbial suspension obtained from the strain to be tested by the cloth seeding technique. Sensitivity to a particular plant extract can be observed by inhibition of microbial growth around the deposited droplet. Interpretation of results: antimicrobial activity of the extracts was determined by assessing the formation of an inhibition zone around the deposited extract droplet and measuring its diameter using a millimeter ruler.

Quantitative determination of the antimicrobial activity of plant extracts was carried out by the microdilution method in liquid medium. Principle of the method: in 96-well ELISA microplates with liquid culture medium, 1/100 diluted microbial suspensions of the test strains are inoculated with an initial density of 0.5 McFarland in a gradient of plant extract concentrations. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values are determined by drop seeding on agar medium the first 8 wells corresponding to 50%- 0.195% concentrations of each plant extract.

Interpretation of results: antimicrobial activity of the extracts was determined by evaluating the growth of microbial cultures on agar medium. The MIC value represents the concentration at which the plant extract inhibits the growth and multiplication of microorganisms, having a bacteriostatic effect, and the MBC value represents the concentration at which the plant extract has a lethal action on microorganisms, having a bactericidal effect.

Evaluation of the antibiofilm activity of plant extracts was tested by the 1% crystal violet microtitration method: following incubation of sterile 96-well ELISA microplates with liquid culture medium in which microbial suspensions are inoculated in a gradient of concentrations of the tested plant extracts, the in vitro antibiofilm activity of different concentrations of tinctures and alcoholic extracts is analyzed on the basis of absorbance values of the 1% crystal violet dye at 492 nm. Interpretation of the results: the antibiofilm activity of the plant extracts could be evaluated by determining the absorbance value from the spectrophotometric reading at 492 nm. The concentration of the plant extracts required to inhibit biofilm growth corresponds to the last well at which the absorbance value is less than half the absorbance value of the positive control corresponding to the same plant extract.

RESULTS

In this study we aimed to investigate the antimicrobial properties of some plant extracts of Taraxacum officinale (tincture) and Chelidonium majus (tincture). Qualitative evaluation of the antimicrobial activity of T. officinale plant extract determined an inhibition of microbial growth against E. faecalis ATCC 29212, P. aeruginosa ATCC 27853 and C. albicans ATCC 10231 strains, showing inhibition halo values of 9 mm, 8 mm with weak microbial growth within the microbial inhibition zone and 8 mm with the presence of a large number of colonies within the inhibition halo, respectively. Resistance to the action of the plant extract was recorded for strains of S. aureus ATCC 25923 and E. coli ATCC 25922, with the presence of T. officinale tincture not inhibiting their microbial growth.

Microbial growth of the fungal strain was inhibited in the presence of the plant extract from C. majus, resulting in an inhibition zone value of 7 mm. The same growth inhibition zone diameter was also recorded for the E.coli strain ATCC 25922, and a value of 11 mm was observed for the gram-positive strain E. faecalis ATCC 29212 (Table 1). This extract showed no antimicrobial activity against S. aureus ATCC 25923 and P. aeruginosa ATCC 27853 strains.

The qualitative determination study of the antimicrobial activity of S. marianum tincture on reference strains reveals that on both gram-negative strains, E. coli ATCC 25922 and P. aeruginosa ATCC 27853, and fungal strain, C. albicans ATCC 10231, the plant extract showed no antimicrobial activity (Table I). Inhibition of microbial growth could however be observed for the gram-positive strains S. aureus ATCC 25923 and E. faecalis ATCC 29212, with growth inhibition zone values of 9 mm and 8 mm respectively.

Table 1. - Quality determination of antimicrobial activity.

Strain tested	**Growth inhibition zone diameter (mm) and CMI**					
	T. officinale **(tincture)**	**CMI**	**Interpretation**	***C. majus*** **(tincture)**	**CMI**	**Interpretation**
S. aureus ATCC 25923	0	N/A	resistant	0	N/A	resistant
E. faecalis ATCC 29212	9^1	52µg/ µL	sensitive	1^1	4298 µg/ µL	intermediate
E. coli ATCC 25922	0	N/A	resistant	7^1	87 µg/ µL	sensitive
P. aeruginosa ATCC 27853	8^1	66µg/ µL	sensitive	0	N/A	resistant
C. albicans ATCC 10231	8^2	66µg/ µL	sensitive	7^1	87 µg/ µL	sensitive

Legend: [1]weak/slight microbial growth within the inhibition zone. [2]presence of a large number of colonies within the inhibition zone.

Formula: $CMI=Q/V$ $Q=10000\mu L$ $V=\pi R^2 G$ (1)

The method of quantitative determination of the antimicrobial activity allowed to highlight the concentrations of the plant extracts at which the growth and development of the tested reference strains are inhibited, as well as the concentrations determining the bactericidal action. (Fig. 1)

Figure_1 - Results of antimicrobial activity testing: Agarized, drop-seeded medium from 96-well microplates with strains of *S. aureus, E. faecalis, E. coli, P. aeruginosa* and *C. albicans* after incubation for 18-24h at 37ºC

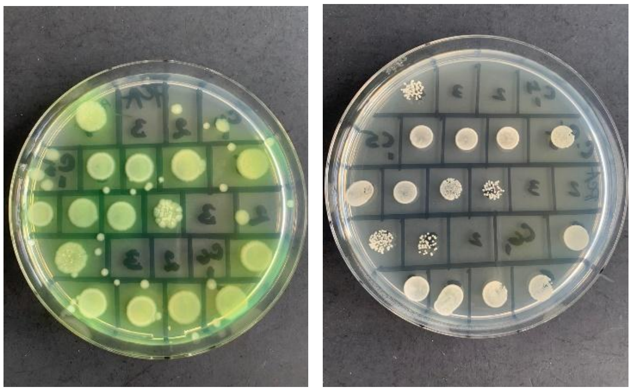

The plant extract of T. officinale shows a strong inhibitory effect on gram-negative P. aeruginosa ATCC 27853 and fungal strains, the bacteriostatic effect being achieved at a concentration of 3.13%, while on gram-positive strains and E. coli ATCC 25922, the concentration of the plant extract required to inhibit microbial growth is 12.5-25%. On gram-negative strains and E. faecalis strain ATCC 29212, the 12.5% concentration of C. majus extract has a bacteriostatic effect, thus a medium inhibitory effect was recorded, but a weak and a strong inhibitory effect of this extract was observed on C. albicans ATCC 10231 and S. aureus ATCC 25923 strains, with MIC values of 50% and 3.13% respectively.

The tinctures tested in the study showed lethal action on the reference microbial strains at an average concentration of 20.8%. For the plant extract of T. officinale, a concentration of 12.5% was required for bactericidal effect on S. aureus ATCC 25923, E. coli ATCC 25922 and C. albicans ATCC 10231 strains, and for E. faecalis ATCC 29212 and P. aeruginosa ATCC 27853, concentrations of 50% and 6.25%, respectively, were required. In the presence of C. majus tincture, the lowest CMB value recorded was 6.25% for S. aureus strain ATCC 25923. Bactericidal action on E. faecalis ATCC 29212 and P. aeruginosa ATCC 27853 strains was observed at a concentration of 12.5% of the plant extract, while concentrations of 25% and 50% were required for the gram-positive E. coli ATCC 25922 and fungal strains, respectively.

Analysis of spectrophotometric data revealed that T. officinale tincture shows little effect on Gram-positive strains, inhibiting biofilm growth at a concentration of 25%. The effect of this tincture was higher on gram-negative and fungal strains, with inhibition of biofilm growth observed at concentrations of 0.78%, 1.56% and 3.13% for C. albicans ATCC 10231, E. coli ATCC 25922 and P. aeruginosa ATCC 27853, respectively. The minimum concentration of C. majus tincture required to inhibit biofilm growth of C. albicans strain ATCC 10231 is 1.56%, while for E. faecalis ATCC 29212 and E. coli ATCC 25922 antibiofilm activity was achieved at a concentration of 12.5%, and for S. aureus ATCC 25923 and P. aeruginosa ATCC 27853 at 6.25%.

The chromatograms obtained in this study provide detailed information about the phytochemical composition of the extracts from Taraxacum officinale and Chelidonium majus. These analyses are essential for identifying the active compounds involved in the observed antimicrobial activity and for correlating them with the experimental results.

The attached figures present representative chromatograms for each extract, highlighting the main absorption peaks and specific compounds. This information contributes to a better understanding of the mechanisms through which the substances in the studied plants act against various microbial strains. The presence of active compounds is clearly demonstrated, supporting the qualitative and quantitative results obtained in the antimicrobial activity tests.

The chromatogram represents the analysis of hydroalcoholic extract of Taraxacum officinale, (Fig. 2) highlighting major phytochemical compounds such as luteolin glucoside (the main peak, indicating the dominant compound), caffeoyl derivatives, and luteolin. Additionally, chlorogenic acid and quercetin are present, known for their antioxidant and anti-inflammatory activities. This composition suggests a high therapeutic potential, supporting the antimicrobial activity observed in the study.

Figure_2 Chromatogram of hydroalcoholic extract of *Taraxacum officinalis*

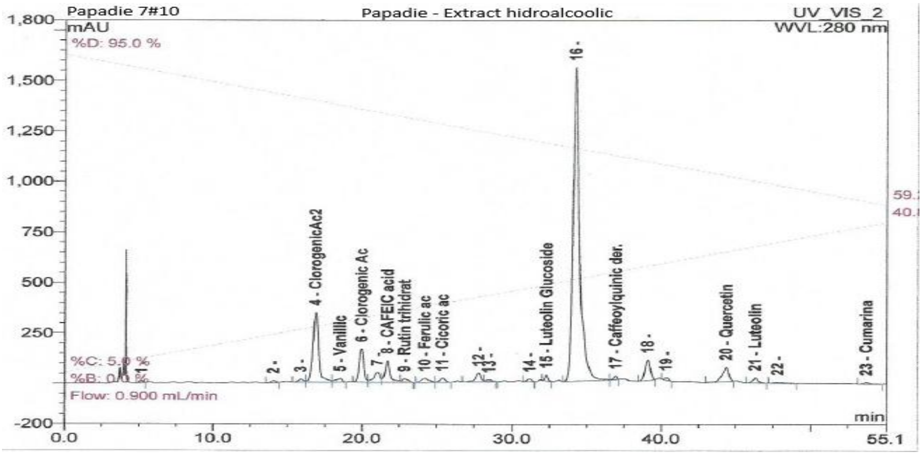

The chromatogram analyzes a hydroalcoholic extract of Chelidonium majus (Fig. 3), highlighting chelidonine as the main compound (retention time 6.667 minutes), suggesting a high concentration. Chelidonine is an alkaloid known for its antimicrobial and antitumor properties. The smaller peaks indicate the presence of other alkaloids, such as sanguinarine or berberine.

Figure 3_ Chromatogram of hydroalcoholic extract of *Chelidonium majus*

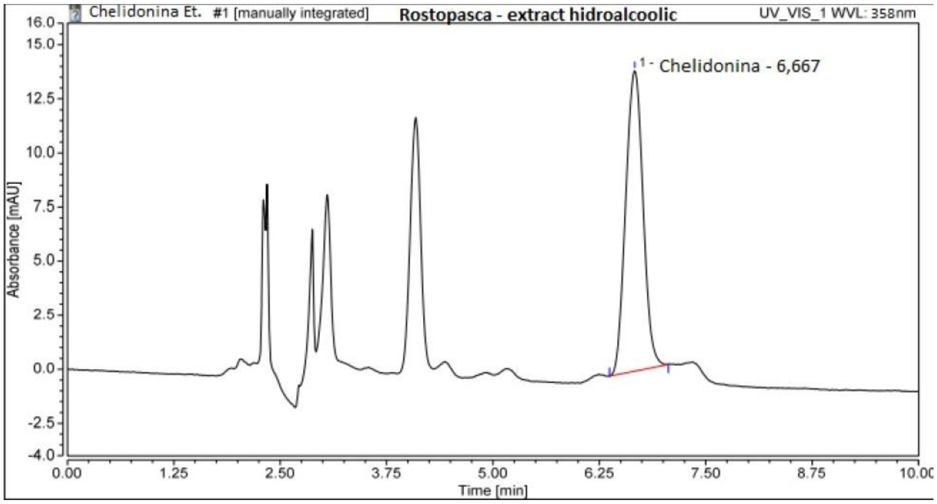

DISCUSSION

The present study investigated the antimicrobial properties of plant extracts tinctures from Taraxacum officinale and Chelidonium majus. The qualitative and quantitative evaluations of antimicrobial activity revealed differential effects of these plant extracts on different strains, shedding light on their potential therapeutic applications.

Taraxacum officinale L. is a flowering herbaceous perennial plant of the Asteraceae family. Interest in researching the biochemical characteristics as well as the pharmacological properties of this species increased considerably in the 1950s, and numerous active substances have now been identified, including flavonoid compounds, phenolic acids such as cyclonic, chlorogenic and caffeic acid, alkaloids, and terpenes. In modern medicine, this plant is marketed and used as an herbal treatment to relieve symptoms of conditions such as arthritis, hepatic disorders and to suppress oxidative stress [17,18, 19,20,21,22,23]. Dandelion exhibits a diverse array of pharmacological effects, making it a valuable medicinal plant with potential therapeutic applications. The study by Trinh et al. 2020, reported that ethanol-based plant extract from T. officinale exhibits bactericidal effect on S. aureus strain ATCC 25923 at a concentration of 20 mg/ml [24]. T. officinale extract tested in the study by Sangeetha and Ezhilarasan 2016, showed lethal action on E. faecalis strain ATCC 29212 at a concentration of 200mg/mL [25]. The study by Shah et al. (2011) [26] showed that S. marianum extracts did not exhibit antimicrobial activity against the tested gram-negative strains E. coli ATCC 25922 and P. aeruginosa ATCC 27853.

Chelidonium majus is a medicinal plant belonging to the Papaveraceae family that is found in the wild flora of Europe, Asia, North America, as well as in some regions of Africa. It is widely used in traditional herbal medicine to treat conditions including biliary and hepatic dysfunction, jaundice and to relieve pain caused by edema. It has antidiuretic, spasmolytic, sedative, and antimicrobial properties. The dried aerial part of C. majus is an official medicine in the pharmacology of European countries, but it is necessary to administer it under the supervision of authorized medical personnel due to the presence of a rich concentration of isoquinoline alkaloids. [27,28,29,30,31]

The antimicrobial activity of the studied plant extracts varied significantly depending on the microbial strain and the type of extract used. For instance, T. officinale exhibited inhibition against E. faecalis and P. aeruginosa strains but not against S. aureus and E. coli. Similarly, C. majus showed inhibition against E. faecalis and C. albicans but not against S. aureus and P. aeruginosa. These findings suggest that the efficacy of plant extracts may be strain-specific, highlighting the importance of considering multiple strains in antimicrobial studies. [32,33,34,35]

The results of this study are consistent with some previous findings but differ from others. For example, the antimicrobial activity of T. officinale against S. aureus strain ATCC 25923 reported by Trinh et al. (2020) aligns with our findings, whereas Tunç et al. (2019) reported no antimicrobial activity against E. faecalis strain ATCC 29212, contradicting our observations. Such discrepancies may arise from variations in extraction methods, microbial strains, or growth conditions, emphasizing the need for standardized protocols and comprehensive strain selection in antimicrobial research.

The determination of MIC and bactericidal concentration provided insight into the potency of the plant extracts against different microbial strains. Higher MIC values were often observed for gram-negative bacteria compared to gram-positive bacteria and fungi. This differential susceptibility underscores the complex interplay between microbial cell wall structures and the mechanisms of action of plant compounds.

The ability of plant extracts to inhibit biofilm formation is of particular interest due to the role of biofilms in antimicrobial resistance. Our study demonstrated varying degrees of biofilm inhibition by the tested extracts, with some showing promising activity against both gram-positive and gram-negative bacteria as well as fungi. These findings suggest the potential of plant extracts as adjuncts in combating biofilm-associated infections.

Despite the valuable insights provided by this study, several limitations should be acknowledged. The use of reference strains may not fully represent the diversity of clinical isolates, warranting further investigations with clinically relevant strains. Moreover, elucidating the underlying mechanisms of antimicrobial activity and conducting in vivo studies are crucial steps toward clinical translation.

The chromatographic analysis of the hydroalcoholic extracts of Taraxacum officinale and Chelidonium majus revealed the presence of major bioactive compounds, each contributing to the specific therapeutic potential of the plant. The chromatogram of Taraxacum officinale indicated Luteolin glucoside as the main compound, known for its antioxidant and anti-inflammatory activities, alongside caffeoyl derivatives. For Chelidonium majus, chelidonine (retention time 6.667 minutes) was identified as the major compound, recognized for its antimicrobial and antitumor properties.

These results emphasize that each extract possesses a distinct phytochemical composition that may synergistically contribute to the observed antimicrobial activity. The identification of specific compounds through chromatographic analysis supports the understanding of the mechanisms of action of the extracts and justifies further investigations into their use in antimicrobial, antioxidant, and hepatoprotective treatments. This study underscores the diverse antimicrobial potential of plant extracts against a range of microbial pathogens. Further research focusing on standardization, mechanism of action, and clinical efficacy is warranted to harness the therapeutic benefits of these natural products effectively.

CONCLUSION

In this experimental work, the antimicrobial properties of two plant extracts of Taraxacum officinale (tincture) and Chelidonium majus (tincture) were studied on 5 reference bacterial strains, two gram-positive (Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212), two gram-negative (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) and one fungal strain, Candida albicans ATCC 10231.

Qualitative testing of antimicrobial activity revealed that the plant extracts inhibited the growth and development of the tested microbial strains.

Quantitative determinations of antimicrobial activity allowed the determination of the concentrations of plant extracts responsible for the bacteriostatic and bactericidal effect, and a higher sensitivity of microbial strains could be observed in the presence of tinctures.

In vitro evaluation of the antibiofilm activity of plant products revealed their ability to inhibit the development of biofilms on inert substrate; plant extracts of C. majus were found to inhibit biofilm formation in all bacterial strains tested, while tincture of T. officinale showed very good activity on gram-negative and fungal strains.

The results obtained are promising, supporting further research into the biochemical characteristics of these extracts in order to formulate strategies for the control of bacterial and fungal infections in humans.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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Authors' contribution

Conceptualization, R.C.S., and V.O.; methodology, C.T and D.C.; software, M.C.R.; validation, M.M., V.O and D.C.; formal analysis, R.C.S. and M.C.R.; investigation, M.C.R..; resources, V.O.; data curation, D.C.; writing—original draft preparation, D.C and F.D.U.; writing—review and editing, M.C.R. and C.E.M; visualization, C.E.M.; supervision, V.O.; project administration M.M. .All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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REVIEW

Dermatoporosis. What We Know and What to Expect

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Abstract: With the increase of the medium lifespan in developing countries, skin aging rise attention more and more. In 2007, Kaya and Saurat rebranded the extreme expression of skin fragility under the name of ‘‘dermatoporosis’’ or chronic cutaneous insufficiency/fragility syndrome. Dermatoporosis is still underused in medical literature and nurses and wound specialists know also about this condition, but they call it "skin tears". There is obvious confusion cause the nursing literature talks about skin tears while dermatologists write about dermatoporosis. Clinical aspects comprise atrophy, purpura, and pseudo-stellate scars. Firstly, the British dermatologist Thomas Bateman described senile purpura in 1818 as purpuric patches arising in elderly people after minimal trauma, especially on the dorsum of the hands. Furthermore, between 1970 and the full documentation of Kaya and Saurat in 2007, we found medical papers about senile purpura, pseudoscars, skin tears, and so on. Starting from this historical data, they bring valuable information according to clinical aspects, histopathology, ultrasonographic aspects, pathophysiology, and therapeutics. This paper aims to analyze published data for improving the diagnosis and management of dermatoporosis.

Keywords: dermatoporosis

HISTORY

With the increase of the medium lifespan in developing countries, a new type of dermatosis was described in 2007 by Kaya and Saurat under the name of ‘dermatoporosis’ of chronic cutaneous insufficiency/fragility syndrome [1,2,3]. Clinical aspects comprise atrophy, purpura, and pseudo-stellate scars (Figure 1). Firstly, the British dermatologist Thomas Bateman described senile purpura in 1818 as purpuric patches arising in elderly people after minimal trauma, especially on the dorsum of the hands [4]. Later, in 1967 Colomb reported pseudo-stellate scars as fibrous scars with a stellate aspect arising on the forearms of the elderly [5]. One year after this description, Frederik and Zak made the connection between stellate pseudoscars and senile purpura, describing that both arise on the forearms of the elderly after minimal traumas. They also report that the shape of these scars can be round or linear, not only stellate [6]. In 1972, Colomb described the trade consisting of atrophy, Bateman's purpura, and spontaneous pseudo-scars The term pseudoscars seems to be related to the fact that there is no solution of continuity with the exterior, the lesions are produced by a compression trauma without true lesions in the extreme external part of the skin, the epidermis [5,7]. Furthermore, there is a lack of reports between 1970 and the full documentation of Kaya and Saurat in 2007. Starting from this historical data and personal clinical cases, they bring valuable information according to clinical aspects, histopathology, ultrasonographic aspects, pathophysiology, and therapeutics [3]. The prevalence of dermatoporosis is about 30% in patients older than 65 years, but this aspect can vary according to the recruitment group: non-hospitalized patients, inpatients, outdoor patients, and patients for rehabilitation centers. The aim of this paper is to analyze published existing data for improving the diagnosis and management of dermatoporosis.

RISK FACTORS AND COMORBIDITIES

Only the future will decide if dermatoporosis is the expression of the general process of senescence, chronic sun exposure or even both [3]. Firstly, some data indicate that patients could have a genetic predisposition according to the connection with atopic dermatitis [8]. Furthermore, analyzing some aspects of aquaporines and skin aging previous statement is increased [9]. Aging plays a major role, the aging process acts mainly through chronic sun exposure, especially the professional type [3].

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Figure 1: Clinical aspects of dermatoporosis: A) Dermatoporosis located on the forearms; B) Dermatoporosis located on the forearms and anterior thorax; C) Superficial hematoma after a minimal trauma; D) Deep hematoma in a patient with severe and extended dermatoporosis

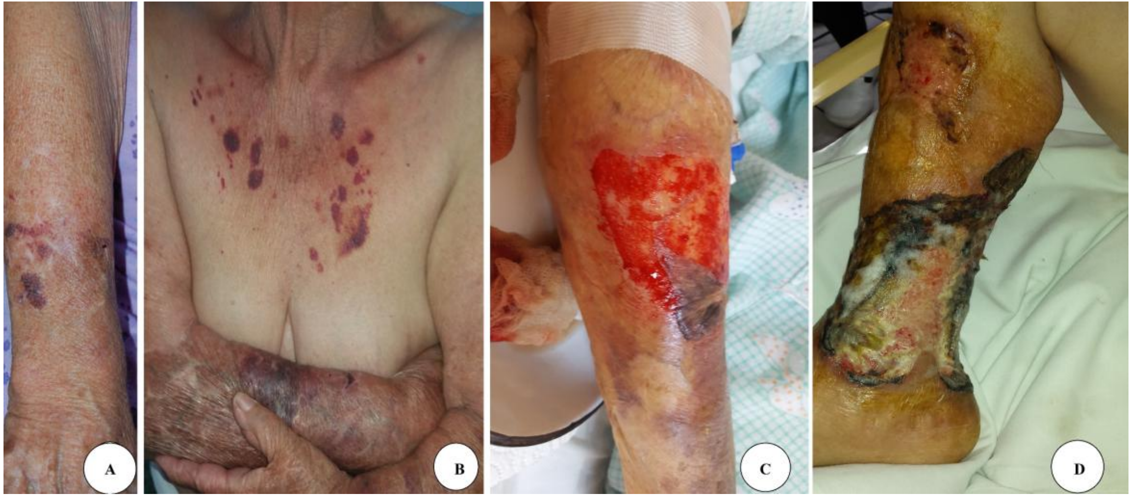

Figure 2: Bleeding after repeated procedures for peripheral vein catheterization in a female patient with primary dermatoporosis

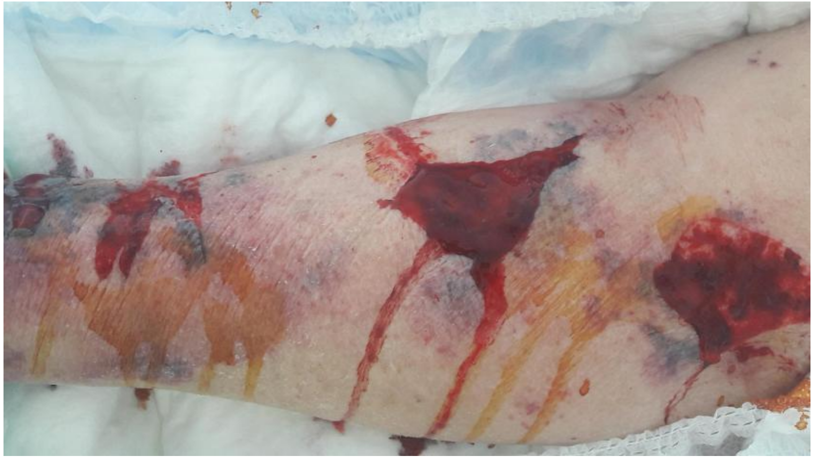

Intrinsic aging or endocrine aging plays a role, the disease is more frequent in postmenopausal women [10]. On the other way, seems that maternal age > 40 years at last pregnancy, breastfeeding for > 7 months per pregnancy and > 18 cumulative months, age < 20 years at first pregnancy, and menopause after 45 years play a protective role against dermatoporosis [11]. Iatrogenic factors like systemic steroid usage especially in patients with blistering diseases can induce dermatoporosis by decreasing the extracellular matrix leading to skin atrophy.

Regarding topical steroids seems that only clobetasol propionate 0.5% can induce dermatoporosis, can it be used for more than six months, this aspect can be visible on the forearms in patients with psoriasis (Figure 3) [12]. The author documented also an interesting case of a 42 female old patient suffering from a secreting cortisol tumor with a Cushing syndrome aspect and typical lesions of dermatoporosis on the forearms (Figure 4). Other medications could be anticoagulants, and epidermal growth factor inhibitors (EGFR) used in the treatment of different carcinomas (Figure 5). Associated comorbidities like osteoporosis, renal failure, and anemia were cited [13].

Moreover, there is a strong connection between the degree of osteoporosis and dermatoporosis [14,15]. It is not clear yet if osteoporosis has a direct influence on the onset of dermatoporosis or if both are the result of the general aging process having hormonal disturbances at the base. Additional information was brought by Kluger in 2016 describing the possible connection of dermatoporosis with the lack of vitamin C levels [16]. Laboratory data about skin expression or serum levels of vitamin C and dermatoporosis are lacking. This correlation is based only on the benefits of topical or systemic administration of vitamin C [16,17].

Recently, vitamin D deficiency was also proposed as a factor by Romano et al based on the connection between osteoporosis and dermatoporosis. Anyway, correlation data between decreased levels of vitamin D and dermatoporosis degree in the elderly are necessary for validation [18]. Finally, most of the patients present an association of these risk factors.

Figure 3: Secondary dermatoporosis: A) Secondary dermatoporosis in a female patient treated with 0.5 mg prednisone per kilo of body weight for 4 months for bullous pemphigoid; B, C) Secondary dermatoporosis in 2 patients treated with 1 mg per kilo of body weight prednisone for 5 months for pemphigus vulgaris.

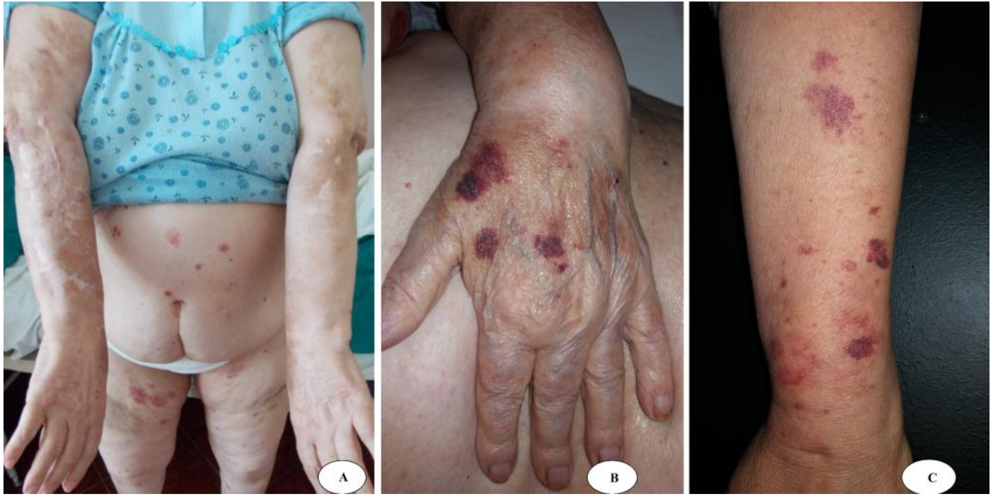

Figure 4: Secondary dermatoporosis in a 42-year-old-female patient diagnosticated with a secreting cortisol tumor: A) Cushing syndrome; B) Facial telangiectasia; C) Dermatoporosis on the forearms; D) Dermatoporosis on the shins.

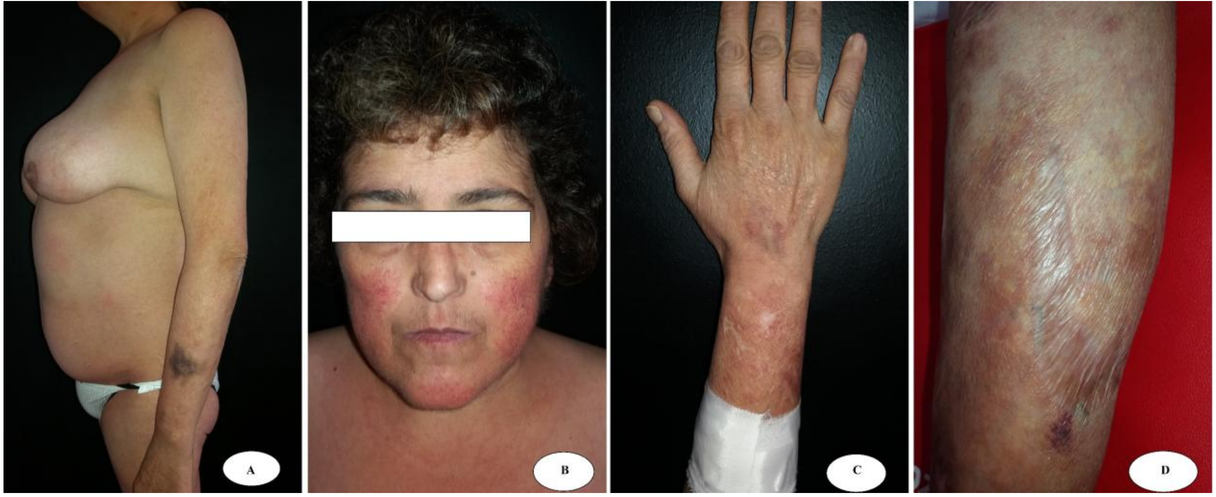

Figure 5: Dermatoporosis in patients undergoing erlotinib in a 67- year-old female patient undergoing erlotinib for lung cancer.

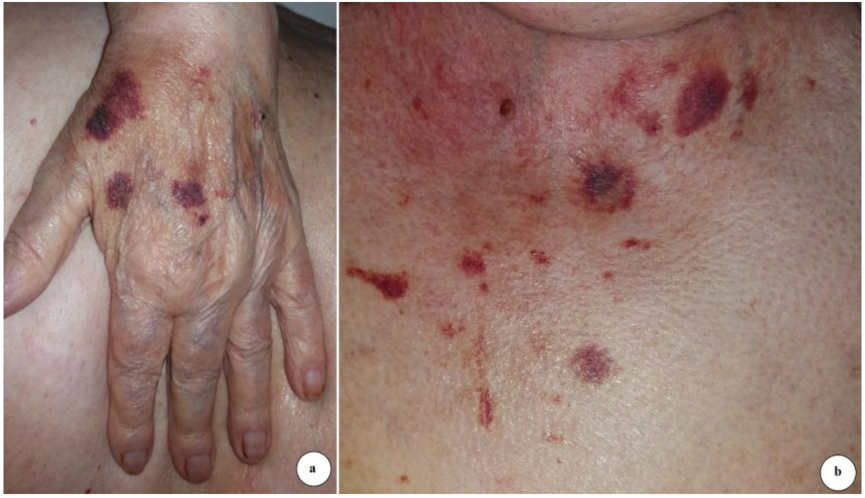

CLINICAL ASPECTS

The clinical changes are skin atrophy, purpura, pseudoscars, hyperpigmentation, and ulcerations. Due to atrophy, there is an increased risk of the appearance of superficial or dissecting hematomas of the skin that form by the accumulation of blood in between the hypodermis and the muscular fascia due to local traumas [3,19]. Most lesions are located at the level of the forearms and shins [3]. Other sites are the chest, arms, and dorsal parts of the hands. It is a tendency for females to develop lesions on the chest and face compared with men. The appearance of lesions at the level of the face and scalp is reported in rare and isolated cases [3,10]. Skin atrophy is the main sign of dermatoporosis. It is essential for the development of the other changes, so that, on the background of exposure to trauma of the underlying structures, purpura and skin ulcers develop. These heal with hyperpigmentation and scars [3]. Senile purpura (Thomas Bateman, 1818) occurs in the elderly, especially on the backs of the hands, and is often found in the context of dermatoporosis [3.4]. It has a complex mechanism, being produced by both exposure of blood vessels due to atrophic skin, and by a decrease in the elastic fibers of the vascular endothelium. An increase in capillary permeability with age is also taken into account [4,20]. Colomb stellate pseudoscars are found on the forearms of the elderly and are produced by defective remodeling of the extracellular matrix due to functional disturbances of enzymes such as metalloproteinases [21]. Appearance on the shins and other sites is also possible. They can be stellate, linear, or plaque-like. The stellate ones are the most common [3,10]. They are formed by the rupture of collagen fibers but without clinical evidence of a break in continuity at the level of the epidermis. Repair is done by excessive proliferation of fibrous tissue, which leads to a clinically hypertrophic scar appearance [5,7]. Pseudoscars are found in approximately 30% of people over the age of 70. Hyperpigmentations appear as residual lesions and as a sign of photodamaged skin [3,10,21]. Cutaneous ulcerations appear based on the cutaneous atrophy after some unnoticeable frequent minimal traumas. They appear at the level of the extremities [3].

Figure 6: Dermatoporosis in a 52-year-old-female patient undergoing erlotinib for breast cancer

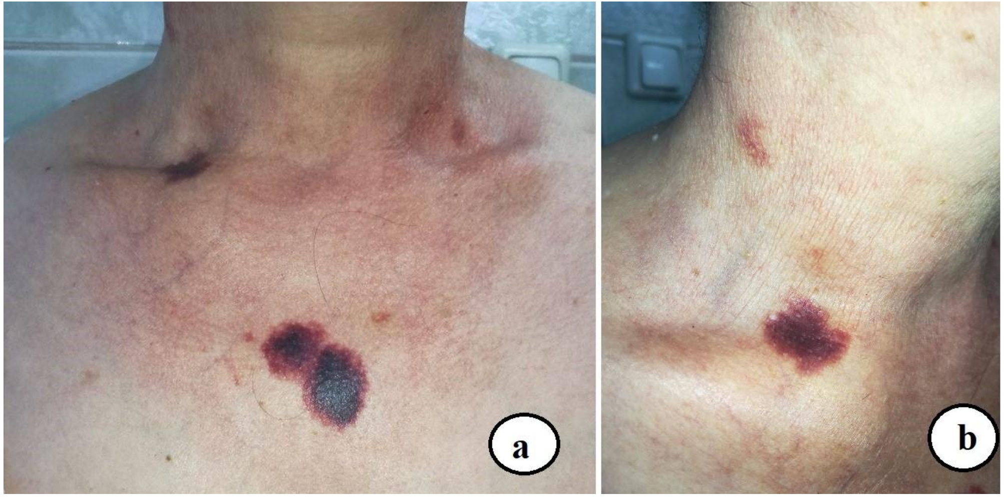

NATURAL COURSE AND COMPLICATIONS

When the cause is obvious like usage of corticosteroids, local or oral, disruption of the treatment can improve greatly and stop the development of further purpura. Superficial hematomas are due to blood accumulation between the dermis and the subcutaneous fat. Clinically appears as a detachment of a skin region from the subjacent adipose tissue. They appear in the advanced stages of dermatoporosis and are found at the level of the shins as well as at the dorsal surface of the forearms [3]. Deep Dissecting hematomas of the skin (DDH) represents the main complication of dermatoporosis. They form as the accumulation of blood between subcutaneous fat and muscular fascia. It represents a surgical emergency due to the risk of block detachment of the cutaneous structures with exposure to infections of major muscular tissue areas. Due to the profound localization of blood collection, the detachment of the skin does not occur immediately. Thus, the lesions mimic cellulitis, presenting as painful erythematous-edematous plaques. People over 80 years with are affected, especially the females and they are located strictly on the calves. The positive diagnosis is done through magnetic resonance imaging (MRI) that shows a hypoechogenic band in the virtual space located between the hypodermis and the muscular fascia. DHH represents a major complication of the disease, the emergency surgical drainage being necessary. The repair of the residual defects is done slowly by the application of topical hyaluronic acid or with skin grafting [3,19] The development of non-melanocytic skin cancer is possible also on dermatoporotic skin, the author saw a case of actinic keratosis and one of cutaneous horn arising in the area of dermatoporosis (Figure 6).

DIAGNOSIS

Clinical aspects are usually enough for the diagnosis. More than that, Saurat developed a self-diagnosis tool (IDA: Index Dermatoporosis Assessment) consisting of a two-module questionnaire that is addressed to individuals aged ≥65 years. The technique was validated in France and Mexico. Skin ultrasonography quantifies the grade of atrophy showing a thickness of skin approx. 0.7-0.8 mm at the level of the regions with dermatoporosis (normal value = 1.3-1.5 mm). The usefulness of reflectance confocal microscopy

(RCM) for the diagnosis of dermatoporosis and quantifying skin thickness was proven by Mezinger, but is expensive and not accessible for routine. Electronic microscopy highlights the disorganization of collagen with parallel exposure of the fibers on the surface of the epidermis (88). Anyway, skin biopsy is an invasive tool, so is recommended to be avoided in such patients and usually is unnecessary when the diagnosis is obvious but might be necessary to rule out other diagnoses in case of atypical location or isolated lesions. For these cases, a 3 mm punch biopsy can be performed on non-erosive/ulcerated lesions without any risk. A general blood count to exclude bleeding disorders, exploration of renal function, and osteoporosis assessment is strongly recommended [3,8]. The fragility of dermatoporotic areas with bleeding and delayed healing results in significantly limited skin biopsies and secondary histopathological and immunohistochemistry studies.

CLASSIFICATION AND STAGING

Depending on the main etiological factors, dermatoporosis can be classified as primary or secondary. Regardless of the form, the lesions are located on the same regions of election such as forearms, shins, anterior part of the thorax, and dorsum of hands [3,10]. Face and scalp location was also cited [10]. Primary dermatoporosis or actinic is due to chronic exposure to the sun, especially in patients over 60 years who are affected. Secondary dermatoporosis lies behind iatrogenic factors, especially the prolonged utilization of corticotherapy. It is characterized by the possibility of the lesions appearing under the age of 60 as well as by partial or complete resolution of the lesions after corticotherapy cessation [3]. The systemic usage as well as topic one can lead to the development of the lesions. Regardless of the way of administration or the cutaneous location in the case of local therapy, the lesions develop in s the same regions/ zones as well as in primary dermatoporosis [3,6]. The disease presents four evolutionary stages. The first stage is characterized by atrophies, purpura as well as pseudo scars, and adding the presence of cutaneous ulcerations leads to the second stage. In the third stage, the ulcerations are increased in size and number. Development of DDH is characteristic of the fourth stage [3,6,19].

PREVENTION AND TREATMENT

The prevention consists firstly in avoidance of the triggers. Regarding this, there is the necessity to use the factors for photoprotection and the avoidance of trauma in patients who are already suffering from primary dermatoporosis. Careful organization at home with the furniture (« coffee table syndrome »), prevention of falling, also proper information of nursing staff the way they handle the patients, no adhesive directly on the skin, avoid excessive application of potent or highly potent corticosteroids. The first topical was proposed by Kaya in 2007, at the same time as the disease was described. The application of hyaluronic acid 1% leads to the improvement of the lesions [3]. In a recent study, topical or oral hydrolyzed collagen does not improve precocious lesions of dermatoporosis – stage I [23]. Topical retinoids are cited to be efficient in the treatment of dermatoporosis since their role is the re-establishment of the differential cellular process. The use of the combination of hyaluronic acid and retinyl-aldehyde is more effective than the separate application of these two [24]. Alpha-hydroxy-acids (AHA) are efficient by diminishing the grade of atrophy [25]. Humbert and colleagues sustain that dermatopororis, especially the Bateman senile purpura, is due to a deficit of vitamin C skin level and it shows an abatement of the lesions after applying topically vitamin C 5% [17]. There is limited data regarding the efficacy of topical application of topical human epidermal growth factor or dehydroepiandrosterone [26,27]. Systemic administration of vitamins C and D could bring some benefits [16,18]. An isolated case of dermatoporosis in the upper limbs treated with polymethylmethacrylate (PMMA) using the BioSculpt®technique was reported.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, MAB; methodology, MAB, ACC; software, ACC; validation, DOC, ALT, NK; formal analysis, NK; investigation, MAB, ACC; resources, DOC, ALT, NK; data curation, ALT, NK; writing—original draft preparation, MAB, NK, ACC; writing—review and editing, DOC, ALT; visualization, ALT, NK; supervision, MAB, NK; project administration, DOC, ALT. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki.

Patient consent for publication

Consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

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ORIGINAL ARTICLES

Methods of assessing stable coronary artery disease by non-invasive imaging techniques

Carmen M. Voicu[1], **Tiberiu Nanea**[2]

Abstract: *Diagnosis of stable coronary artery disease is vital for prognosis, classification and early treatment. Current guidelines show that patients with stable angina who are suspected of CAD need to undergo a certain protocol for classification and further analysis. This review's aim is to present the most used non-invasive techniques for identification of CAD and to underline the current development of imaging technology and the possible reduction of invasive measures due to non-invasive techniques.*

Currently, non-invasive techniques used to diagnose stable coronary artery disease have a very high accuracy and newer methods seem to be comparable to the gold-standard. The majority of the methods discussed have an optimal performance for patients with PTP between 15-85%, and the future of diagnosis for these patients seem to involve less invasive measures and less radiation by improving the current devices and by usage of machine-learning algorithms.

Keywords: *coronary artery disease, PTP, stress echocardiography, SPECT, CMR, CCT*

INTRODUCTION

Diagnosis of stable coronary artery disease is vital for prognosis, classification and early treatment. Failure in doing so, might determine secondary events such as: disease progress, myocardial infarction or even death.

Current guidelines show that patients with stable angina who are suspected of CAD need to undergo a certain protocol for classification and further analysis.

This review's aim is to present the most used non-invasive techniques for identification of CAD and to underline the current development of imaging technology and the possible reduction of invasive measures due to non-invasive techniques.

METHODS

A PubMed review was performed, analyzing all publications from 1968 to 2019 concerning the topic "stable coronary artery disease" (keywords: coronary artery disease, PTP, stress echocardiography, SPECT, CMR, CCT). Human studies and meta-analyses, published in English, were cited in this review.

RESULTS AND DISCUSSION

Diagnosis and management

According to current guidelines, patients with suspected SCAD will follow a step-by-step approach for confirmation.

After the clinical examination and basic tests applied (ECG, bio-chemistry indicators, resting echocardiography), if the cause of chest pain is none other than CAD and the left ventricle ejection fraction is higher than 50%, the evaluation of suspected SCAD commences (patients with LVEF<50% have a high risk for cardiovascular events and they should have ICA without further testing). [1]

First step is to determine the pre-test probability of SCAD

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(PTP). This indicator is evaluated by the age, gender and the nature of symptoms for the suspected patient, and evaluates the probability of having a coronary artery disease before realizing any tests.

Non-invasive imaging techniques that assess coronary artery disease usually have sensitivities and specificities around the value of 85% (coronary computed tomography angiography – coronary CTA has the highest sensitivity (95%) [2] while stress echocardiography has the highest specificity (94.6%) [3]). This is why for 15% of the patients, most imaging techniques will be false positive and therefore, for these patients (with PTP < 15%) performing no non-invasive imaging tests will bring less incorrect results. Therefore:

- patients with PTP < 15% will not do any further investigations;
- patients with PTP between 15-65% are recommended to have an exercise ECG as the initial test and afterwards if necessary, perform imaging tests;
- patients with PTP between 66-85% must have a non-invasive imaging test for confirmation;
- patients with PTP over 85% are assumed to have SCAD, so they don't need further investigations. [1]

As such, patients with PTP between 15%-85% are required to be assessed with non-invasive techniques for risk stratification and selection for invasive measures (revascularization or ICA). [4]

Electrocardiogram exercise testing

As described above, for patients with PTP within 15-65% for suspected SCAD, an exercise electrocardiogram will be the first and best option.

According to the Bruce protocol, this test involves using a treadmill or an exercise bicycle in which patients are monitored using a continuous 12-lead ECG and their blood pressure measured, when they are exercising. The level of difficulty increases incrementally every 3-minutes until a certain target is reached (the predicted heart rate for the age of the patient) or the patient cannot continue the test. [5]

If ST-depression is present with a value of ≥ 0.1mV or 1 mm, in one or more ECG leads, which persist at least 0.06-0.08 seconds after the J-point, the test detects a possible CAD. [1]

Data shows that the usefulness of the test might come for its higher specificity, one study shows a sensitivity of 45%, and specificity of 85% [6] while another study shows higher values for men (sensitivity – 40%, specificity – 96%) than for women (sensitivity – 33%, specificity – 89%). [7]

Also, in a meta-analysis which compared the efficacy of exercise stress testing between 34 studies, results also showed that for men a positive exercise test results in a 89% probability of CAD, and for women results in a 69% probability of CAD. Whereas, a negative exercise test leads for men to a 37% probability of CAD and for women to an 18%. These values show that a large share of patients may be undetected after performing an exercise stress test. [8]

This method is useful only for patients that don't have ECG abnormalities at baseline. For example, patients with Wolff-Parkinson-White syndrome, paced ventricular rhythm, LBBB, use of digitalis, atrial fibrillation, left ventricular hypertrophy with repolarization changes or digoxin use, have a higher likelihood that the observed ST-segment depressions would happen because of the baseline pathology and not the CAD. Therefore, in these patients the ECG results aren't interpretable and exercise ECG testing should not be performed. [9]

Despite all the results, for low-risk patients, for example women, the standard ECG exercise treadmill test should be the first diagnostic strategy, because of its availability and lower costs of usage. Additional imaging techniques, like myocardial perfusion imaging (MPI) does not add any substantial benefit to the exercise treadmill test, but increases diagnostic costs significantly [10].

Also, it is safe to say that during exercise testing, the risk of having myocardial infarction because of the increase in the pro-coagulant activity, doesn't increase for patients with CAD (objectified angiographically) in comparison to those who don't have CAD, meaning that the method is in general safe. [11]

Although this method is accessible for diagnosis of CAD, in the near future it will probably be replaced with other more accessible, non-invasive imaging techniques. Cardio-goniometry (3D-ECG) was demonstrated to have a higher performance in diagnosis (sensitivity 75% vs sensitivity 68.1% - exercise ECG and specificity 74.4% vs specificity 38.1% - exercise ECG) of CAD in women, and being able to have this efficacy without having to undergo stress-testing and being practically free-of-risk, surely proves an improvement in easiness and performance in the diagnosis of CAD for the near future. [12]

Stress echocardiography

Another imaging technique for the assessment of stable CAD is stress echocardiography. This method usually involves a mechanical stressor (exercise performed on a treadmill or a bicycle) or a pharmacological agent.

The principle of stress echocardiography is the identification of functionality for all cardiac walls and further classification into normal, ischaemic, viable or necrotic myocardium:

- normal myocardium responds with normokinetic function at rest and during stress it may have a normokinetic or hyperkinetic function;
- ischaemic myocardium may have a normal function at rest, but during stress its response worsens, having a hypokinetic/akinetic/dyskinetic function;
- viable myocardium testing in dysfunctional myocardium at rest shows a improvement during stress response because it would indicate a region of hibernating myocardium that improves its function after revascularization;
- necrotic myocardium doesn't improve its function during stress, a region of dysfunctional myocardium at rest has its function fixed. [13]

Stress echocardiography using exercise stress is preferable because it provides additional data like exercise time, workload, heart rate and blood pressure variability. Pharmacological stress using dobutamine can be used for patients who are unable to exercise. [1]

Also using dobutamine bring the advantage of assessment of viability more accurately than exercise stress echocardiography. Ischaemic but viable myocardium at low doses of dobutamine (10-20 μg/kg/min) improves its function, but at high doses (40 μg/kg/min) its function worsens. This effect is due to the initial inotropic function of dobutamine at low doses, whereas in high doses its function changes to chronotropic and especially vasodilatory. [14]

Using additional contrast to the method (by using microbubbles of encapsulated gas) can bring enhancement of the images viewed, by improving image quality, accuracy of detection of CAD and reader confidence, especially when certain segments cannot be visualized at rest. [15]. Newer data show data coronary microvascular dysfunction (a potential cause for chest pain) can be observed using myocardial contrast echocardiography, a marker for possible CAD, patients with this issue having lower hyperemic perfusion and microvascular flux rate than patients with normal microvascular function. [16]

Diagnosis of CAD using stress echocardiography is quite efficient. Exercise stress echo, dobutamine and dipyridamole stress echo show sensitivities of 85%/80%/78% and specificities of 77%/86%/91% for detection of CAD, which are significantly better than electrocardiogram exercise testing, and similar to SPECT [17]

Also, using strain-rate imaging and tissue Dopple imaging can improve the quality of detection for CAD. Because the interpretation of images in most echocardiographies are subjective and prone to errors, measuring strain-rate can determine an objective view on the diagnosis. Voigt et al showed that ischemic segments had significantly higher peak systolic strain-rates and strains during ejection time during dobutamine stress than nonischemic segments [18] and Gupta et al., recently showed in a meta-analysis similar results, analysis of longitudinal strain imaging having higher AUC-ROC prediction of CAD (0.92) than assessment of wall motion (0.83). [19]

The newest advancement in echocardiography for detecting CAD markers is the 3D-speckle tracking echocardiography (3D-STE). One of the most used scoring systems to objectify CAD severity is the Gensini score. In one study, using 3D-STE to measure 4 parameters of CAD (global longitudinal strain – GLS, global circumferential strain – GCS, global radial strain – GRS and global area strain – GAS) it had been found that patients found with critical CAD after coronary angiography had significantly worse values of GLS,GCS,GRS,GAS than patients with noncritical CAD. Also the Gensini score had a significant positive linear correlation with GLS and GAD, meaning that 3D-STE was a very good method do identify the grade of severity of CAD. [20]

SPECT (Single photon emission computed tomography) – Myocardial perfusion scintigraphy

One of the most used imaging techniques, uses similar principles like the previously described methods. It uses either exercise stress or pharmacological stress (e.g. adenosine, dipyridamole, dobutamine, regadenoson) and measures the level of uptake of radioactive tracers at rest level and stress level from the myocardium. In ischemic tissue, due to the lower blood flow in stress response, the uptake of the tracer is reduced, meaning a positive test for diagnosis of CAD. [4]

Standard radiotracers are Technetium-99m (^{99m}Tc) or Thallium 201 (^{201}Tl), although the latter has a higher radiation dose. New developments in this technology (cadmium-zinc-telluride – CZT) show a significantly higher performance than conventional SPECT and a lower dose of radiation. [21] Also another radiotracer (for PET) that is frequently used is Rubidium 82 (^{82}Rb) that is associated with lower radiation exposure and high sensitivity (82%) and specificity (90%) for detecting ischaemia. [22]

The method also compares ischaemic areas of myocardium to normal areas. Some patients (with triple vessel disease) whom hearts have a widespread ischemia, will be detected false-negative for CAD because of the lack of inducing ischemia in the underperfused myocardium, proving a limitation of the method. [23]

Another limitation of the method would be diagnosis of CAD in patients with left bundle branch block (LBBB). In those patients, the intraventricular septum has a delay in contraction (being dyskinetic), and in the moment of image acquisition may have a lower thickness, indicating a LAD territory ischaemia, and thus a false-positive result. [24]

Also, for asthmatic patients that are undergoing SPECT, using adenosine is a contraindication because of the possible precipitation of bronchospasm due to activation of all adenosine receptors (especially A_1, A_{2B} and A_3). Regadenoson, a selective A_{2A} agonist (for producing hyperaemia) is currently used for these patients, because of its sufficient hyperaemic response, less side effects, and comparable efficacy to adenosine. [25]

The REASSESS study, in which in a prospective manner was assessed the diagnostic performance of coronary computed tomography angiography (CTA) vs. SPECT, showed that although many cases of stable CAD wouldn't have been diagnosed by SPECT if invasive measures (angiography) wouldn't have been taken, the overall performance of both of the methods (CTA and SPECT) was similar, in objectifying hemodynamically significant stenosis, having a similar accuracy (70% - CTA vs. 68% SPECT). [26]

The issue of false-negative results in SPECT, although present, is indicated to have a low prevalence and thus, a low impact. For example, in 133 patients with normal SPECT results, only 16% had anatomically and functionally significant stenoses, and from 180 vessels analyzed with normal SPECT results, only 8.33% had stenoses. As such, the prevalence of false-negative cases in normal SPECT remains low and a normal result stays important in diagnosis and follow-up. [27]

SPECT also predicts efficiently long-term outcomes. Patients with ischaemia burden, who had SPECT and also underwent early coronary revascularization had significantly lower all-cause mortality and cardiac mortality in comparison to patients who received only pharmacological therapy, indicating a significant diagnostic value for the severity of CAD by using SPECT. [28]

Usage of myocardial perfusion scintigraphy combined with positron emission tomography is more accurate in comparison to SPECT, having higher sensitivities (90% - PET, 85% - SPECT) and higher specificities (88% - PET, 85% - SPECT), also AUC-ROC prediction was higher for PET (0.95) in comparison to SPECT (0.90), according to Mc Ardle et al. [29]

A similar study showed better image quality and diagnostic accuracy of CAD for PET in comparison to SPECT (showing a percentage of 78%/79% excellent image quality of rest/stress PET scans vs. 62%/62% excellent image quality of rest/stress SPECT scans). [30]

Data also shows that using PET-MPI to determine myocardial blood flow is excellent and superior to the relative measurement of tracer uptake, being more efficient for the detection of CAD and eliminating false-negative patients with balanced ischaemia (triple vessel disease). [31]

New technology in PET-MPI for diagnosis of CAD show similar results as described above, for usage of flurpiridaz ^{18}F in comparison to SPECT, bringing more advantages for PET-MPI (higher quality images, diagnostic certainty of interpretation, and sensibility of detection of CAD). [32]

Cardiac magnetic resonance

One of the most advantageous methods of imaging techniques, because of the high-detail images, non-invasivity and lack of any ionizing radiation, cardiac magnetic resonance can identify CAD, even better than the previous methods discussed. Similar to all CAD detection methods, is uses a stress agent, exercise or pharmacological.

Stress cardiac magnetic resonance using both a bicycle [33] and a treadmill [34] seems feasible for detection of CAD. Recently, the EXACT study, which used stress CMR with a treadmill, showed a sensitivity of 79% and a specificity of 99% for detection of CAD after using a SPECT, also showed higher agreement of diagnosis with angiography (k=0.82) than SPECT (k=0.46), demonstrating as such excellent diagnostic value. [35]

Pharmacological stress uses a vasodilator (adenosine or regadenoson) or dobutamine. Added in the vasodilator method is the administration of i.v. gadolinium contrast, to enhance the perfusion defects that can be seen in ischaemic walls. Using dobutamine requires a similar method like in stress echocardiography, by viewing wall motion abnormalities in ischaemia response. [36]

In CMR the assessment of CAD can be either qualitative (by visual assessment of presence and degree of ischaemia) or quantitative by measuring absolute blood flow. A recent study showed that quantitative analysis had similar diagnostic accuracy (83%) as qualitative (80%) and observers considered a better approach to diagnose CAD using quantitative CMR. [37]

Recent data showed that CMR stress with perfusion in comparison with SPECT and PET showed good diagnostic accuracies, having a sensitivity of 89% (in comparison to SPECT – 88%, and PET – 84%) and a specificity of 76% (in comparison to SPECT – 61% and PET – 81%). Authors also considered that CMR could be a good alternative to PET, having a similar diagnostic accuracy. [38]

Nevertheless, current data shows that perfusion CMR has a better diagnostic performance value than SPECT, as shown in the MR-IMPACT trial [39]. Also, the CE-MARC study showed that CMR had better sensitivities than SPECT (88.7% for men vs. 50.9% for men – SPECT and 85.6% for women vs. 70.8% for women – SPECT) and similar specificities, and better diagnostic accuracies. [40]

The CE-MARC 2 study compared the efficacy of CMR, NICE guidelines and SPECT in reducing the percentage of unnecessary angiographies in patients with suspected angina. The results showed that the CMR strategy determined a lower probability of unnecessary angiographies within 12 months than the NICE guidelines strategy, CMR and SPECT reducing significantly the number of angiographies in comparison to NICE guidelines. [41]

The adverse effects for gadolinium-based contrast agents used in CMR have a low frequency (0.36%), the severe are even less (0.033%), and these adverse effects are more physiological than allergic (most frequently were dyspnea, hypersensitive reactions and emesis). [42].

Similar to SPECT, it had been shown that CMR can have long-term prediction values. Newer technology in CMR measures GLS (global longitudinal strain), this marker can be an independent predictor for long-term major adverse cardiac events (MACE): heart failure hospitalization, myocardial infarction, sustained ventricular tachycardia or death, in pacients with known or suspected coronary artery disease. That means CMR can identify patients with high risk of having dangerous adverse events in the near future because of CAD. [43]

Also, the newest data shows that CMR with quantitative myocardial perfusion mapping can detect coronary microvascular dysfunction separately from CAD, with high AUC-ROC prediction values (0.95 on vessels with MVD) with high sensitivity (90%) and specificity (89%), almost comparable with invasive procedures. [44]

More powerful magnets (3.0 Tesla), although not so widely available, used on CMR machines, have a net superiority over 1.5 Tesla CMR, having a far greater AUC-ROC prediction value (0.963 vs 0.645), sensitivity (90.5% vs. 61.9%) and specificity (100% vs. 61.9%). [45]

Computed tomography

Multidetector row CT systems used in cardiac CT are sufficient enough to visualize coronary anomalies, although this last method is preferable for measuring coronary anatomy and pathology occurring in this matter. There are two methods of using CT in the benefit of diagnosis for CAD: calcium scoring and CTCA (coronary computed tomography angiography).

Calcium scoring uses the principle of identifying coronary calcification, without use of contrast. Any pixel above 130 Hounsfield units is defined as coronary calcification and can be quantified using the Agatston score, which is dependent by the size of the plaque and the radiographic density. [46]

However, there can be identified obstructive coronary lesions which may be not calcified and vice-versa, critically calcified coronary arteries which aren't yet obstructed. Because of this matter, the preferable way to diagnose CAD is CTCA.

The North American Society for Cardiovascular Imaging (NASCI) agreed in their guidelines that patient selection is needed before usage of CTCA. As such:

- because of contrast usage, any patients with known history of severe anaphylactic reactions to contrast, have contraindication to this method;
- patients with history of acute myocardial infarction, severe hypotension, decompensated heart failure or renal impairment have also contraindication to this method;
- pregnant women or patients who are unable to cooperate to the breath-hold instructions are not able to have CTCA. [47]

The method involves usage of a bolus of contrast injected i.v., and after that realizing a coronary angiogram with complementary ECG gating. The ACCURACY trial showed that CTCA in comparison to ICA (invasive coronary angiography) had similar performances in measurements (CTCA sensitivity – 95% vs. 94%, CTCA specificity 83% vs. 83%, CTCA positive predictive value – 64% vs. 48% and CTCA negative predictive value 99% vs. 99%) for diagnosis of CAD with 50%/70% stenosis. [2]

Although the main issue in this non-invasive technique is usage of radiation and scan preparation, another problem is that presence of heavy calcification (objectively measured by an Agatston score > 400) in case of patients with severe CAD lowers the specificity, by creating beam hardening artefacts and excessive image noise. [48]

Analysis of outcome data, such as in the PROMISE trial, showed that patients with suspected CAD who did CTCA had in long-term observation, fewer catheterizations than patients who were functionally tested (using exercise electrocardiography, stress echocardiography or nuclear stress testing) although these patients hadn't any improvement in clinical outcome. [49], and also the SCOT-HEART trial showed that usage of CTCA reduced fatal and non-fatal myocardial infarction but not significantly in patients with suspected CAD. [50]

Improving the current performance in CTCA is possible. One method is measuring tthe fractional flow reserve (CTFFR). Although it is highly time-consuming and requires computer modeling, data shows that usage of CTFFR in CTCA is beneficial, increasing the diagnostic accuracy in comparison to standard CTCA. [51]

Additional usage of β-blockade and nitroglycerin before the exam seem to increase the diagnostic performance [52] and newer data show that CTFFR-CTCA is a safe alternative to ICA with a significantly lower rate of ICA in comparison to functional testing (61% cancelling rate), therefore lowering the clinical cost and having the same clinical outcomes. [53]

Machine-learning (based on a deep learning model) in CTFFR-CTCA seems to also make better the standard procedure, by reclassifying patients with nonsignificant stenosis and raising overall diagnostic accuracy (from 71% to 85%). [54]

CONCLUSION

Currently, non-invasive techniques used to diagnose stable coronary artery disease have a very high accuracy and newer methods seem to be comparable to the gold-standard. The majority of the methods discussed have an optimal performance for patients with PTP between 15-85%, and the future of diagnosis for these patients seem to involve less invasive measures and less radiation by improving the current devices and by usage of machine-learning algorithms.

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REVIEW

The Importance of Screening and Early Diagnosis for a Good Outcome in Patients with Colorectal Cancer

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Abstract: Colorectal cancer (CRC) ranks third worldwide in terms of morbidity and second in mortality. In Romania, CRC represents the second leading cause of malignancy, accounting for 13.3% of all diagnosed cancers and associated with a five-year survival rate close to 50%. Despite the availability of effective CRC screening programs proven to reduce incidence and mortality, low participation rates contribute to a high occurrence of advanced disease complications. As a result, bowel obstruction develops in approximately 25% of colorectal cancer cases, significantly worsening patient outcomes. This review aims to highlight the role of screening in reducing the incidence of such complications and to assess the outcomes associated with surgical management of malignant bowel obstruction. A comprehensive review of current literature was conducted, focusing on the incidence, clinical presentation, and management of intestinal obstructions in CRC patients. Emphasis was placed on studies evaluating screening programs, risk factors, and surgical interventions. Data indicate a continuous rise in emergency presentations due to obstructive CRC, correlating with low screening uptake. Early detection through organized screening significantly lowers the risk of obstruction and improves outcomes. Enhanced screening programs and early identification of high-risk individuals are crucial in preventing advanced CRC complications. Timely diagnosis not only reduces emergency surgical interventions but also improves prognosis and overall survival rates.

Keywords: colorectal cancer; screening programs; bowel obstruction; surgical management; early detection

INTRODUCTION

Colorectal adenocarcinoma is a significant global health concern, affecting approximately one million individuals annually and causing nearly 500,000 deaths each year [1-2]. Although rare before the age of 40, its incidence sharply increases after age 45, with most cases diagnosed beyond the age of 50. In Romania, colorectal cancer ranks second among all cancers, accounting for about 13% of cases and a five-year survival rate close to 50% [3-5]. Despite a declining population, Romania saw an increase in cancer incidence from 2011 to 2018, reflecting challenges in healthcare access and prevention [6].

Colorectal cancer is frequently diagnosed at advanced stages due to insufficient screening programs and common risk factors such as smoking, poor diet, and lack of physical activity [7-8]. Additionally, Romania's healthcare system faces significant challenges, including financial and logistical barriers, which hinder early detection and treatment [9-12]. Some studies indicate that approximately 40% of cases occur in the right colon, 31% in the left colon, and 29% in the rectum [13].

The rising incidence of early-onset colorectal cancer has led to changes in screening guidelines, with organizations such as the American Cancer Society lowering the recommended starting age from 50 to 45 years. This shift aims to enhance early detection and improve patient outcomes, though its overall impact remains under evaluation. The optimal screening approach, whether through non-invasive tests followed by confirmatory colonoscopy or direct colonoscopic screening, continues to be debated [14-15].

Effective screening methods such as colonoscopy, flexible sigmoidoscopy, CT colonography, capsule endoscopy, and stool-based detection tests play a crucial role in identifying and removing precancerous lesions, ultimately reducing cancer-related mortality. This paper asserts the importance of increasing awareness and implementing systematic screening programs to curb colorectal cancer incidence and mortality, potentially preventing severe complications such as bowel obstructions [16-19].

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MATERIALS AND METHODS

This narrative review systematically examines the existing literature concerning colorectal cancer screening, diagnosis, and the management of acute complications. A comprehensive search was conducted across four prominent electronic databases, namely PubMed, NCBI, Google Scholar, and MDPI. Relevant articles were identified using targeted keywords, such as "colorectal cancer", "screening", "early diagnosis", and "treatment." To ensure the inclusion of contemporary advancements, priority was given to recent publications, primarily from the past five years. Only studies specifically addressing methods for colorectal cancer screening, diagnostic processes, and therapeutic interventions for advanced stages were selected for detailed evaluation in this review.

RESULTS

Screening Methods

Colorectal cancer (CRC) screening is a critical tool in the early detection and prevention of the disease, significantly lowering both incidence and mortality rates. Several screening strategies are available, each with its own benefits and limitations (Figure 1). As technology advances, newer approaches are being incorporated to enhance detection accuracy, patient compliance, and accessibility. The current landscape of CRC screening includes invasive procedures such as colonoscopy, non-invasive stool-based tests, imaging modalities, molecular biomarker assessments, and emerging techniques such as artificial intelligence (AI) and liquid biopsies [20].

Figure 1: Screening guidelines

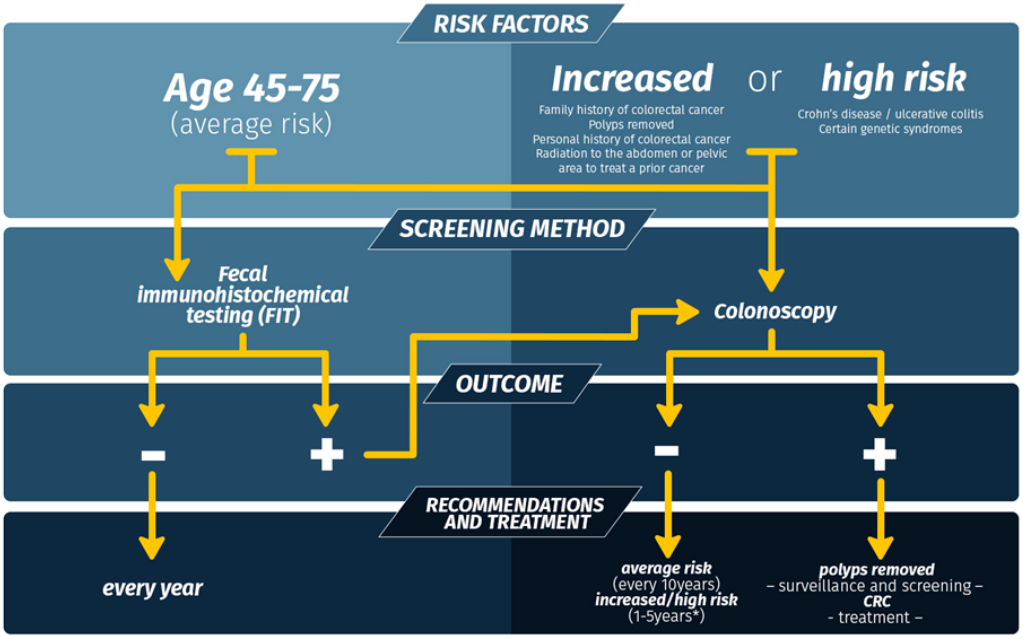
RISK FACTORS
Age 45-75
(average risk)
Increased
or
high risk
Family history of colorectal cancer
Polyps removed
Personal history of colorectal cancer
Radiation to the abdomen or pelvic area to treat a prior cancer
Crohn's disease / ulcerative colitis
Certain genetic syndromes
SCREENING METHOD
Fecal immunohistochemical testing (FIT)
Colonoscopy
OUTCOME
-
+
-
+
RECOMMENDATIONS AND TREATMENT
every year
average risk
(every 10years)
increased/high risk
(1-5years*)
polyps removed
– surveillance and screening –
CRC
- treatment –

**family history of colorectal cancer – screening starting before age 45*
polyps removed – 3 years
personal history of colorectal cancer - 1 year after surgery
radiation to the abdomen or pelvic area to treat a prior cancer – after age 35 or between 3 to 10 years
Crohn's disease or ulcerative colitis – 1 to 3 years
certain genetic syndromes - begin at a young age, more frequently depending on genetic syndrome

Colonoscopy remains the most effective and widely used screening method due to its ability to directly examine the entire colon and rectum. This procedure enables the detection and removal of precancerous polyps, thereby preventing progression to malignancy. With a sensitivity exceeding 95% for advanced neoplasia, colonoscopy is a highly reliable diagnostic and preventive tool. However, its drawbacks include the need for extensive bowel preparation, sedation, and the expertise of trained specialists. Additionally, although rare, complications such as perforation and post-procedural bleeding can occur. Given these factors, adherence to colonoscopy screening remains suboptimal, particularly in average-risk individuals. Nevertheless, it is strongly recommended for high-risk populations, such as those with a personal or family history of CRC or genetic syndromes like Lynch syndrome and familial adenomatous polyposis [21-22].

Flexible sigmoidoscopy is another visualization-based screening tool that examines only the rectum and the lower part of the colon. While it is less invasive and does not require sedation, it is limited in its ability to detect right-sided lesions in the proximal colon. Despite this limitation, studies have shown that sigmoidoscopy reduces CRC incidence and mortality, particularly for left-sided tumors. It is often used in conjunction with stool-based tests to improve overall detection rates [23].

Capsule endoscopy is another innovation being explored for CRC screening. This technique involves swallowing a small, camera-equipped capsule that captures images of the gastrointestinal tract as it moves through the digestive system. Unlike traditional colonoscopy, capsule endoscopy does not require sedation and is non-invasive. However, current challenges include incomplete visualization of the colon, difficulty detecting small lesions, and limitations in image interpretation. Research is focused on enhancing camera resolution, battery life, and image processing technology to improve its effectiveness as a screening tool [24-25].

Computed tomography (CT) colonography, also referred to as virtual colonoscopy, is a radiological screening technique that generates three-dimensional images of the colon and rectum using low-dose CT scans. This non-invasive method eliminates the need for sedation and is generally more tolerable for patients. CT colonography has demonstrated high sensitivity for detecting polyps larger than 10 mm but is less effective for smaller lesions. Unlike conventional colonoscopy, it does not permit biopsy or polyp removal, requiring follow-up colonoscopy for confirmation and intervention when abnormalities are detected. Radiation exposure is another factor that must be considered, particularly for younger individuals undergoing repeated screenings [26].

Blood-based testing has emerged as a promising direction in colorectal cancer (CRC) screening, providing a minimally invasive alternative to traditional methods. These assays aim to detect tumor-associated molecular alterations in circulation, offering improved patient compliance, especially among those hesitant to undergo invasive procedures such as colonoscopy. Among the most studied blood-based tools are circulating tumor DNA (ctDNA) tests, which identify somatic mutations, copy number alterations, and epigenetic changes shed by tumor cells into the bloodstream. ctDNA-based screening has demonstrated high specificity for CRC detection and holds potential for both early diagnosis and post-treatment surveillance. Despite these advances, the sensitivity of ctDNA for detecting early-stage CRC and precancerous adenomas remains limited [27]. This poses a challenge for its application as a standalone screening modality, particularly for detecting lesions before malignant transformation. Additional liquid biopsy strategies, including the analysis of circulating tumor cells (CTCs), exosomal RNA, and methylated DNA, are under active investigation. These approaches aim to enhance detection sensitivity by capturing a broader spectrum of tumor-derived signals, though most remain in the experimental phase and require extensive validation in large-scale prospective studies before being adopted into routine practice. Parallel to blood-based diagnostics, biomarker-driven innovations in stool and urine-based screening have gained traction. Advances in genomics and proteomics have led to the identification of novel molecular signatures associated with CRC, including aberrantly methylated DNA sequences, microRNAs, and tumor-specific proteins. These biomarkers have contributed to the development of next-generation assays that improve the performance of non-invasive tests. The multitarget stool DNA test (sDNA-FIT), which combines fecal immunochemical testing with molecular markers for DNA mutations and methylation, exemplifies this approach and has already been integrated into clinical practice. Nevertheless, ongoing research seeks to refine these assays further, expand biomarker panels, and improve sensitivity and specificity—particularly for early-stage lesions [27-28].

Stool-based screening tests offer an alternative for individuals who prefer non-invasive methods. The fecal immunochemical test (FIT) has largely replaced the traditional guaiac-based fecal occult blood test (gFOBT) due to its improved specificity and sensitivity. FIT detects human hemoglobin in stool samples, reducing the risk of false positives from dietary or upper gastrointestinal sources. This test is typically performed annually and has been associated with a reduction in CRC-related mortality. However, its lower sensitivity for detecting advanced adenomas necessitates frequent retesting. A more advanced stool-based approach, the multitarget stool DNA test (sDNA-FIT), combines FIT with molecular markers to detect genetic mutations and epigenetic changes linked to CRC. This method enhances sensitivity beyond FIT alone and improves the detection of precancerous lesions. However, it has a higher false-positive rate and requires a confirmatory colonoscopy for abnormal findings [29-31].

Artificial intelligence (AI) is increasingly being applied to CRC screening, particularly in colonoscopy and imaging-based methods. AI-enhanced colonoscopy utilizes deep learning algorithms to analyze real-time endoscopic images and improve the detection of adenomas. Studies have demonstrated that AI-assisted colonoscopy increases the identification of subtle mucosal abnormalities, reducing the risk of missed lesions. AI is also being integrated into stool and blood-based screening tests, where machine learning models optimize the interpretation of molecular signatures to improve accuracy. Additionally, computational models incorporating genetic, environmental, and clinical data are being developed to personalize CRC risk assessment and screening recommendations [32-33].

Despite the variety of available screening methods, adherence to CRC screening guidelines remains a significant concern, particularly in populations with limited healthcare access. Socioeconomic barriers, healthcare infrastructure, and patient awareness all play critical roles in screening participation. Efforts to improve accessibility include at-home testing kits, such as FIT and stool DNA tests, which allow individuals to collect and submit samples without visiting a healthcare facility (Figure 2). Increasing public awareness, healthcare provider engagement, and offering personalized screening options based on individual risk factors are essential strategies for enhancing participation in screening programs [34].

Diagnosis Methods

Colorectal cancer (CRC) diagnosis relies on a combination of clinical evaluation, laboratory testing, endoscopic procedures, and imaging techniques to confirm malignancy and determine disease stage. Despite advancements in screening and early detection, a significant proportion of patients are still diagnosed at advanced stages, often presenting with complications such as bowel obstruction. Colonoscopy remains the gold standard for CRC diagnosis, allowing for direct visualization, biopsy, and removal of suspicious lesions during a single procedure. Late-stage detection is associated with poorer outcomes, underscoring the need for timely and effective diagnostic strategies [35].

Imaging modalities, including contrast-enhanced CT scans, magnetic resonance imaging (MRI), and positron emission tomography (PET) scans, play a critical role in staging CRC and evaluating tumor spread. These techniques help determine the extent of local invasion, lymph node involvement, and distant metastases. Accurate staging is essential in guiding treatment decisions, including surgical resection, chemotherapy, radiation therapy, or a combination of modalities for advanced cases. Imaging is particularly important for assessing resectability in metastatic disease, as well as monitoring treatment response in patients undergoing systemic therapy [36].

Figure 2: Screening methods

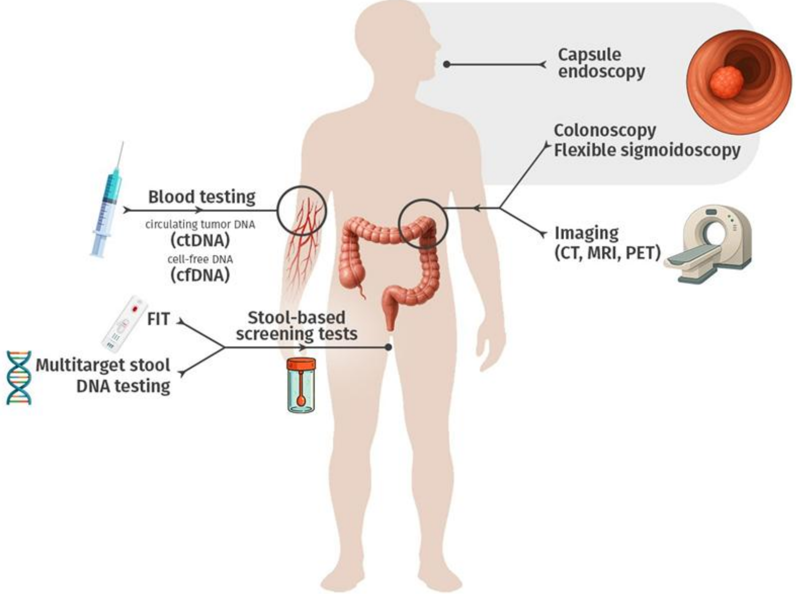

Despite these diagnostic advancements, many CRC cases are still identified at later stages when symptoms become severe. One of the most common emergency presentations of advanced CRC is bowel obstruction, occurring in approximately 20% of cases. This complication results from tumor-induced narrowing of the intestinal lumen, leading to symptoms such as severe abdominal pain, bloating, nausea, vomiting, and the inability to pass stool or gas. Bowel obstruction often necessitates urgent medical intervention, with many patients requiring emergency surgery to relieve the blockage and resect the affected segment of the bowel. Compared to elective surgery for early-stage CRC, emergency surgical interventions are associated with increased postoperative complications, prolonged hospital stays, and higher mortality rates. The occurrence of bowel obstruction as an initial manifestation of CRC highlights the ongoing challenges in early detection, particularly among individuals who do not undergo regular screening. Patients presenting with obstruction often have advanced tumors that limit treatment options, negatively impacting prognosis. Research has shown that individuals diagnosed with obstructive CRC have lower survival rates compared to those diagnosed through routine screening, emphasizing the need for widespread and consistent preventive measures [37-38].

Treatment

For patients diagnosed with early-stage CRC, particularly stage I and stage II, surgical resection is the primary treatment. In stage I, where the tumor is confined to the bowel wall without lymph node involvement, removal of the affected segment of the colon with regional lymphadenectomy is typically curative. The preferred surgical approach may be open or laparoscopic colectomy, with the latter offering benefits such as reduced postoperative recovery time and lower complication rates. In stage II disease, the cancer has penetrated the muscular layer of the colon but has not spread to regional lymph nodes. Surgery remains the standard of care, but adjuvant chemotherapy may be considered in cases where high-risk pathological features are present, including T4 tumors, lymphovascular invasion, perineural invasion, poor differentiation, or inadequate lymph node sampling. The decision to administer chemotherapy is individualized, considering both the potential survival benefit and the risk of treatment-related toxicity [39-41].

Management of stage III CRC, where the cancer has spread to regional lymph nodes, involves a multimodal approach integrating surgery and systemic chemotherapy. The primary surgical treatment is colectomy with complete mesocolic excision and lymphadenectomy to achieve optimal oncologic outcomes. Adjuvant chemotherapy is recommended in nearly all cases to eliminate micrometastatic disease and reduce recurrence risk. Standard chemotherapy regimens include FOLFOX (5-fluorouracil, leucovorin, and oxaliplatin) and CAPOX (capecitabine and oxaliplatin), with treatment duration typically spanning six months. In patients who cannot tolerate oxaliplatin, fluoropyrimidine monotherapy remains an option, though with slightly lower efficacy [39-40].

Stage IV CRC is characterized by distant metastases, most commonly involving the liver, lungs, or peritoneum. In these cases, systemic therapy is the cornerstone of treatment, intending to prolong survival and maintain quality of life. Chemotherapy regimens such as FOLFOX and FOLFIRI (5-fluorouracil, leucovorin, and irinotecan) are frequently utilized, often in combination with targeted agents. Bevacizumab, an anti-VEGF monoclonal antibody, is commonly added to chemotherapy to inhibit tumor angiogenesis, particularly in patients with RAS-mutant tumors. In contrast, EGFR inhibitors such as cetuximab and panitumumab are used in RAS-wild type tumors, demonstrating improved response rates when combined with chemotherapy. Molecular profiling, including assessment of KRAS, NRAS, and BRAF mutations, is crucial in guiding treatment selection, as tumors harboring certain mutations exhibit resistance to EGFR-targeted therapies. Additionally, immunotherapy has emerged as a novel treatment option for patients with microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR) tumors, with checkpoint inhibitors such as pembrolizumab and nivolumab demonstrating promising efficacy in this subset of patients [39-40, 42-43].

A significant proportion of CRC cases, particularly those diagnosed at advanced stages, present with bowel obstruction, a life-threatening complication resulting from tumor-induced luminal narrowing. This condition manifests as severe abdominal pain, nausea, vomiting, and bowel distension, often necessitating urgent medical intervention. The management of bowel obstruction is guided by the patient's overall prognosis, performance status, and treatment goals. In patients with potentially resectable disease and good functional status, surgical intervention, such as resection of the obstructing tumor or creation of a diverting ostomy, may be performed to restore bowel function and facilitate further oncologic treatment. However, in patients with widespread metastases or poor surgical candidacy, non-surgical approaches such as endoscopic stenting are preferred. Self-expanding metal stents (SEMS) can be placed to relieve obstruction and maintain luminal patency, serving as either a bridge to surgery in operable patients or a palliative measure in those with unresectable disease. While SEMS placement offers symptom relief with a lower risk of perioperative morbidity, potential complications include stent migration, re-obstruction, and perforation, particularly in patients receiving anti-angiogenic therapy [44-46].

Medical management plays an essential role in symptom control for patients with bowel obstruction. Corticosteroids may be administered to reduce tumor-associated edema and improve bowel patency. Antiemetics, including dopamine antagonists and serotonin receptor antagonists, are used to alleviate nausea and vomiting, while opioid and non-opioid analgesics help manage pain. In cases where bowel function cannot be restored, a venting gastrostomy tube may be placed to decompress the stomach and relieve symptoms of persistent vomiting. Total parenteral nutrition (TPN) may also be considered for select patients to prevent malnutrition when enteral feeding is not feasible. Given the complex nature of malignant bowel obstruction, a multidisciplinary team approach, including oncology, gastroenterology, surgery, and palliative care specialists, is necessary to ensure comprehensive management tailored to the patient's individual needs [47].

DISCUSSION

Colorectal cancer (CRC) remains a major health challenge despite advances in screening, diagnosis, and treatment. A key issue is the persistent late-stage diagnosis, which is often associated with complications such as malignant bowel obstruction. The need for improved screening adherence and the integration of novel technologies to enhance early detection is evident, as early intervention significantly improves survival outcomes. However, real-world implementation of screening recommendations remains suboptimal due to accessibility issues, patient reluctance, and limitations of existing tests.

The effectiveness of CRC screening depends on balancing sensitivity, specificity, patient compliance and healthcare system feasibility. While colonoscopy is the most definitive method, its invasiveness and preparation requirements often deter patients. Non-invasive alternatives like fecal immunochemical tests (FIT) and stool DNA testing provide practical options but require frequent testing and have limitations in detecting precancerous lesions. CT colonography offers another alternative but lacks the ability to remove polyps during the procedure. The future of CRC screening may lie in risk-adapted strategies that personalize screening intervals and methods based on genetic predisposition, lifestyle factors, and emerging biomarkers.

A significant portion of CRC cases are detected at advanced stages, often when symptoms such as bowel obstruction arise. These cases require urgent medical intervention, typically involving surgery or endoscopic stenting. While self-expanding metal stents (SEMS) are increasingly used for temporary relief, their risks, including perforation and migration, necessitate careful patient selection.

Addressing late-stage CRC diagnosis requires increased awareness of early warning signs, improved access to screening programs, and adherence to recommended screening guidelines. Healthcare providers play a crucial role in recognizing potential symptoms and encouraging high-risk individuals to undergo regular screening. Strategies to improve early detection include expanding non-invasive screening options, utilizing risk-based stratification models to identify individuals at greater risk, and integrating emerging technologies such as liquid biopsy and artificial intelligence-assisted diagnostics. By strengthening early detection efforts, the burden of late-stage CRC and its associated complications, including bowel obstruction, can be significantly reduced, leading to better patient outcomes and improved survival rates.

Disparities in CRC outcomes highlight the need for broader access to screening and timely treatment. Socioeconomic barriers, healthcare infrastructure disparities, and patient education gaps contribute to late-stage diagnoses. Efforts to integrate artificial intelligence (AI) in colonoscopy and risk prediction models could enhance detection accuracy and optimize screening protocols. AI-assisted imaging analysis has demonstrated the ability to improve adenoma detection rates, reducing missed lesions and potentially lowering CRC incidence.

The treatment of CRC is continually evolving, with research focusing on optimizing multimodal therapeutic strategies to improve survival and quality of life. While surgery remains the foundation for early-stage disease, systemic therapy plays a critical role in advanced CRC, particularly with the integration of targeted agents and immunotherapy. The management of complications such as malignant bowel obstruction requires a patient-centered approach, balancing aggressive interventions with supportive care to maintain comfort and dignity. Ongoing clinical trials continue to explore novel therapeutic approaches, including next-generation targeted therapies and personalized medicine strategies, which aim to refine treatment paradigms and improve outcomes for patients diagnosed with colorectal cancer.

CONCLUSION

While significant progress has been made in CRC screening, diagnosis and treatment, challenges remain in optimizing early detection, improving treatment outcomes for advanced-stage disease and addressing disparities in care. A multidisciplinary approach integrating surgery, systemic therapy and supportive care remains crucial in managing CRC across different disease stages. Ongoing research into AI, molecular profiling and liquid biopsies holds promise for further refining CRC management strategies and improving patient outcomes. Continued efforts to enhance screening adherence, personalize treatment approaches and expand access to novel therapies will be key in reducing the global burden of colorectal cancer.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization- M.T., J.C. and B.I.; methodology - M.T.; investigation - M.T., J.C. and B.I.; writing of the manuscript- M.T. and J.C.; review, editing - M.T., J.C. B.I. and H.S; data curation - J.C. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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REVIEW

Targeting Heart Disease in Cardiovascular–Kidney–Metabolic Syndrome: A Review of Novel Therapeutic Approaches with Prognostic and Quality-of-Life Impact

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Abstract: Cardiovascular-kidney-metabolic (CKM) syndrome describes the complex, bidirectional interplay among cardiovascular, renal, and metabolic dysfunctions, where impairment in one system accelerates decline in the others. This interconnected pathophysiology significantly elevates the risk and progression of heart disease, particularly heart failure, through mechanisms involving insulin resistance, systemic inflammation, neurohormonal activation, and endothelial dysfunction. In this context, therapeutic strategies targeting heart disease must address the multifaceted drivers of CKM. Recent advances highlight the need for an integrated, patient-centered approach that combines lifestyle interventions with pharmacological treatments tailored to individual cardiovascular risk. While foundational therapies such as RAAS inhibitors and statins remain essential, novel agents now offer additional prognostic and quality-of-life benefits. SGLT2 inhibitors have emerged as a cornerstone therapy, improving outcomes in heart failure with both preserved and reduced ejection fraction, independent of glycemic control. GLP-1 receptor agonists and dual GLP-1/GIP agonists like tirzepatide demonstrate cardiometabolic and renal protection, while finerenone shows promise in diabetic kidney disease and several heart failure phenotypes. These therapies not only target glycemic control but also reduce cardiovascular mortality, hospitalizations, and renal decline. Optimizing cardiovascular outcomes in CKM syndrome requires early, multifactorial therapeutic intervention, informed by evolving evidence and a deeper understanding of the heart–kidney–metabolism axis.

Keywords: heart failure, diabetes mellitus, obesity, chronic kidney disease, GLP-1 receptor agonists, SGLT2 inhibitors, mineralocorticoid-receptor antagonists, finerenone, cardiovascular-kidney-metabolic syndrome

INTRODUCTION

Heart disease is frequently observed in patients with CKM syndrome. There is a pathophysiological interplay between the cardiovascular, renal, and metabolic systems, where dysfunction in one organ can exacerbate impairment in others, which substantially increases the risk of developing heart failure. Patients with CKM syndrome usually present with overlapping conditions as type 2 diabetes, chronic kidney disease, and atherosclerotic cardiovascular disease, all of which contribute to functional and structural cardiac dysfunction.

Heart disease and cardiovascular mortality represent the main drivers of prognosis in all CKM syndrome comorbidities. Hence, special consideration should be directed toward understanding pathophysiological mechanisms through which all these metabolic risk factors converge toward cardiovascular impairment. Additionally, emphasis should be placed on the early and appropriate initiation of the right medication for the right patient, tailored to the estimated individual risk, particularly concerning the novel drug classes that have demonstrated beneficial effects across all components of the CKM syndrome: GLP1 receptor agonists, SGLT2 inhibitors, and the non-steroidal mineralocorticoid receptor antagonist finerenone.

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This review emphasises the role of the heart in the CKM spectrum, with particular interest in the therapeutic implications.

GLP-1 RECEPTOR AGONISTS

GLP-1 (glucagon-like peptide-1) is a natural hormone mainly released by intestinal L enteroendocrine cells residing in the distal jejunum, ileum, and colon, as a result to oral glucose intake. GLP-1 is classified as an incretin hormone because it stimulates insulin release from beta pancreatic cells, inhibits the release of glucagon and, along with that, hepatic glucose output, and reduces the rate of gastric emptying and promotes satiety by central appetite suppression. GLP-1 has a very short half-life of approximately 2 minutes, being quickly cleaved by dipeptidyl peptidase IV (DPP-4), a widely expressed enzyme, thereby limiting the strong insulin secretagogue effect of this hormone [1–3].

Owing to the very short half-life, the molecule of GLP-1 is not suitable for an adequate therapeutic agent. Nevertheless, researchers developed molecules capable of being adequate therapeutic agents: inhibitors of DPP-4, which are capable of prolonging the effects of natural GLP-1, and molecules that represent long-acting GLP-1 receptor agonists [1].

Apart from pancreas, GLP-1 receptors are expressed on various types of cells throughout the body and the finding of this widespread localization of receptors led to studies that demonstrated multiple effects beyond the well-known incretin actions, the so-called “pleiotropic effects”: in central nervous system, being involved in the regulation of appetite, in the cardiovascular system, being expressed both in cardiomyocytes and vascular cells, in the musculoskeletal system, gastrointestinal tract, regulating the motility or secretion, interfering in the gut-brain axis, in adipose tissue influencing fat metabolism through interaction with leptin, in kidneys at multiple levels (arterial and arteriolar smooth cells, glomerulus, juxtaglomerular cells, proximal tubule), modulating renal function both directly by altering glomerular hemodynamics and indirectly through less known direct interactions between gastrointestinal system and kidneys known as gut-renal axis [3–7]. Even in beta pancreatic cells, besides altering insulin or glucagon levels, the stimulation of GLP-1R could enhance beta-cell proliferation, inhibit apoptotic pathways, and induce expansion of islet mass [4].

Considering that diabetes mellitus and obesity serve as risk factors in the context of cardiovascular diseases, the effects of GLP-1 receptor agonists on the cardiovascular system are, to a considerable extent, mediated through modulation of these metabolic conditions.

Regarding their role as antidiabetic medications, all GLP-1RAs proved effective in glycemic control by reliably reducing fasting plasma glucose and HbA1c (up to a reduction of 56.2 mg/dl FPG levels and 2.1% for HbA1c levels, respectively) [8].

Obesity is strongly associated with the development of T2DM as excessive adipose tissue, particularly visceral fat, contributes to insulin resistance [9]. The central downregulation of appetite, accompanied by slowing the gastric evacuation, which induces a feeling of fullness, facilitates the reduction of overall calorie intake and the maintenance of a favorable long-term energy balance [3]. Large meta-analyses of the available RCTs proved that GLP-1 RA are effective in inducing weight loss and a reduction in the body mass index and in the waist circumference [8,10].

Conversely, an increasing body of evidence suggests that GLP-1RAs exert direct beneficial effects on various components of the cardiovascular system, independently of their impact on glycemic regulation and weight control.

As far as cardiomyocytes are concerned, the presence and the exact extent of GLP-1R expression are unclear, corroborated by limited evidence regarding the cellular localization of GLP-1R protein [11]. However, in cellular and animal models, besides metabolic effects, which could indirectly improve cardiac function, there is evidence that GLP-1 could restore crucial signaling pathways that could be altered by external stressors [7]. For example, GLP-1 could ameliorate the extent of ischemia-reperfusion injury by activating specific signaling pathways and inhibiting cardiomyocyte apoptosis [3]. Moreover, GLP-1 signaling is involved in reducing hyperglycemia-induced apoptosis of the cardiomyocytes by optimizing the signaling pathways from the sarcoplasmic reticulum and in lowering the extracellular matrix remodeling [12].

There is also important evidence that GLP-1 receptor stimulation influences various pathogenic mechanisms that are involved in atherogenesis. First of all, GLP-1 can mitigate endothelial dysfunction by inducing NOS-mediated vasodilation and by limiting the formation of advanced glycosylation end-products [3,13]. Additionally, GLP-1 is involved in improving lipid metabolism at several levels: with respect to gene expression, it downregulates genes of lipogenic enzymes and upregulates genes of lipolytic enzymes, reduces lipotoxicity by lowering lipid peroxidation, stimulates fatty acid oxygenation, inhibits HMG-CoA reductase and lowers cholesterol levels, ameliorates hepatic steatosis, lowers serum triglyceride levels and even reduces intestinal lipid absorption [3,14]. There are also described effects of enhanced cholesterol efflux from foam cells or suppression of foam cell formation [13]. Even an anti-inflammatory pathway is proposed with the downregulation of genes involved in inflammation and oxidative stress. Moreover, GLP-1 promotes an atherosclerotic plaque remodeling towards stabilization by reduction of matrix metalloproteinases (MMP), proteolytic enzymes involved in weakening of plaque fibrous caps and in increasing the risk of rupture [3,13].

GLP-1RA proved effects of blood pressure reduction both in preclinical and clinical studies, by as much as 6 mmHg (as it was found in DURATION-1 trial) [11,15]. There are several mechanisms possibly involved in this effect: the capacity of GLP-1RA to counteract the sympathetic nervous system hyperactivation, both at the central and peripheral nervous system level, the improvement in the endothelial function and therefore in vasodilation, and the possible effect on renal tubular mechanisms by increased natriuresis, which was observed in experimental models of hypertension [16,17]. The effect of lowering blood pressure was consistent throughout the entire spectrum of renal function, from normal to severely reduced [18].

Diabetes affected individuals are at high risk of chronic kidney disease (CKD) (as many as 40% diabetic patients develop chronic kidney disease), diabetic nephropathy representing the leading cause of chronic kidney disease worldwide. However, in the CKD population, cardiovascular events constitute the primary contributor to mortality, due to enhanced atherosclerosis, arterial and valvular calcification, and amplification of myocardial fibrosis [19]. There is a clear rationale that reducing both the risk and progression of chronic kidney disease could play a pivotal role in decreasing the burden of cardiovascular complications in diabetic patients.

Apart from indirect beneficial effects of glycemic control, weight loss and blood pressure reduction upon renal function, there are described direct effects: increased natriuresis and diuresis, at least partially explained by inhibiting sodium-hydrogen exchanger 3 in proximal tubular cells, anti-inflammatory effects by inhibition of cytokine production and chemotactic recruitment of inflammatory cells, free-radical scavenging effects and direct glomerular hemodynamic effects [5,17,19].

Another pathophysiological mechanism seemingly targeted by this therapeutic class is the modulation of the renin–angiotensin–aldosterone system, as there was been proven that GLP-1RA could induce a decrease in plasma levels of renin and angiotensin II. The effect of lowering the activity of this system could contribute to the effect of lowering blood pressure, the enhanced natriuresis, and could influence glomerular hemodynamics by vasodilation of the efferent arteriole and reduction of glomerular capillary pressure [18].

Beyond the pathophysiological effects demonstrated at the experimental level, the clinically relevant renoprotective effects include the reduction of albuminuria and, as evidenced by several studies, a reduction in eGFR decline [18,20].

Transitioning from experimental models and pathophysiological mechanisms to clinical evidence, GLP-1RA use in practice is supported by results from representative large-scale cardiovascular outcome trials. These studies have positioned GLP-1 receptor agonists as a distinct class among antidiabetic medications, with benefits that go beyond mere glycemic regulation.

There are 8 cardiovascular outcomes randomized controlled trials (CVOTs) for 7 different GLP-1RA (lixisenatide, liraglutide, semaglutide s.c., oral semaglutide, exenatide, albiglutide, dulaglutide, efpeglenatide) which included type 2 diabetic patients with high cardiovascular risk or previous cardiovascular disease. Four different GLP-1RAs, namely liraglutide, semaglutide s.c., dulaglutide, and efpeglenatide, proved efficient in reducing a composite end-point of major adverse cardiac events MACE (comprising cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke)[21]. However, analyzing the hazard ratio for individual components of MACE, there is a clear heterogeneity between these molecules. A meta-analysis which included all 8 RCTs, comprising a total of 60.080 patients (72.4% having established cardiovascular disease), concluded that GLP-1RA class reduce MACE by 14% (HR = 0.86, 95% CI 0.79-0.94, P = 0.006), with significant effects on the risk of cardiovascular death by 13% (P = 0.016) and nonfatal stroke by 16% (P = 0.007) [22]. Another meta-analysis, which included the same RCTs but ELIXA (the RCT examining lixisenatide), because lixisenatide is a short-acting agent that raised concerns regarding the lack of sustained GLP-1 inhibition in patients, GLP-1 RA proved effective in reducing MACE by 14% (HR 0.86 [95% CI 0.80–0.93]; p<0.0001), cardiovascular death by 13% (HR 0.87 [0.80–0.94]; p=0.0010), stroke by 17% (0.83 [0.76–0.92; p=0.0002), and, in addition to the other meta-analysis, myocardial infarction by 10% (0.90 [0.83–0.98]; p=0.020) [23]. Additionally, both meta-analyses proved a significant 12% reduction in all-cause mortality [22,23].

Table 1: Major CVOTs of GLP-1RA [21] (Although promising results, albiglutide was withdrawn from the market by the manufacturer due to commercial reasons)

TRIAL	**ELIXA**	**LEADER**	**SUSTAIN-6**	**EXSCEL**	**Harmony Outcomes**	**REWIND**	**PIONEER-6**	**AMPLITUDE-O**	**SELECT**
DRUG	***Lixisenatide***	***Liraglutide***	***Semaglutide s.c.***	***Exenatide***	***Albiglutide***	***Dulaglutide***	***Semaglutide oral***	***Efpeglenatide***	***Semaglutide s.c.***
POPULATION	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	BMI>27 kg/m^2
MACE HR (95% CI)	1.02** (0.89–1.17)	0.87 (0.78–0.97)	0.74 (0.58–0.95)	0.91 (0.83–1.00)	0.78 (0.68–0.90)	0.88 (0.79–0.99)	0.79 (0.57–1.11)	0.73 (0.58–0.92)	0.80 (0.72–0.90)
CV DEATH HR (95% CI)	0.98 (0.78–1.22)	0.78 (0.66–0.93)	0.98 (0.65–1.48)	0.88 (0.76–1.02)	0.93 (0.73–1.19)	0.91 (0.78–1.06)	0.49 (0.27–0.92)	0.72 (0.50–1.03)	0.85 (0.71–1.01)
MI HR (95% CI)	1.03 (0.87–1.22)	0.86 (0.73–1.00)	0.74 (0.51–1.08)	0.97 (0.85–1.10)	0.75 (0.61–0.90)	0.96 (0.79–1.15)	1.18 (0.73–1.90)	0.78 (0.55–1.10)	0.72 (0.61–0.85)
STROKE HR (95% CI)	1.12 (0.79–1.58)	0.86 (0.71–1.06)	0.61 (0.38–0.99)	0.85 (0.70–1.03)	0.86 (0.66–1.14)	0.76 (0.61–0.95)	0.74 (0.35–1.57)	0.80 (0.48–1.31)	0.93 (0.74–1.15)

Taking into account the various pleiotropic effects of GLP-1, there has emerged the hypothetical idea of using GLP-1RA in individuals without diabetes. In 2023, the results of the SELECT trial were published, a randomized controlled trial that included overweight patients (BMI>27 kg/m^2) with a history of cardiovascular disease but without diabetes. Subcutaneous semaglutide proved efficient, compared to placebo, in reducing MACE by 20% (HR 0.80 [0.72 to 0.90]; p<0.001), mainly driven by a statistically significant reduction of myocardial infarction risk by 28% [24].

These solid results, from large, reputable RCTs, have been translated into guideline recommendations. According to the 2024 ESC Guidelines for the management of chronic coronary syndromes and the 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes, GLP-1RAs have a class I (level of evidence A) recommendation in patients with diabetes mellitus type 2 and history of atherosclerotic cardiovascular disease to reduce incident MACE [25,26]. This recommendation stands regardless of the baseline or target HbA1c or any other antidiabetic medications the patient may be taking. Additionally, consistent with the SELECT trial, the 2024 ESC Guidelines for the management of chronic coronary syndromes (CCS) included a IIa (level of evidence B) recommendation for the utilization of subcutaneous semaglutide in overweight (BMI > 27 kg/m^2) CCS patients to mitigate the risk of cardiovascular mortality and myocardial infarction [24,25].

As far as kidney outcomes are concerned, there is an RCT specifically designed to assess renal endpoints. The FLOW trial included 3533 patients with type 2 diabetes mellitus and CKD (defined as eGFR 50-75 ml/min/1.73m^2 and macroalbuminuria UACR >300 mg/g or eGFR 25-50 ml/min/1.73m^2 and albuminuria UACR >100 mg/g) and compared subcutaneous semaglutide with placebo. The results showed that subcutaneous semaglutide reduced the risk of a primary outcome (defined as a composite of major renal adverse events comprising new onset of kidney failure, a reduction of eGFR of at least 50% from baseline, or death from kidney or cardiovascular-related causes) with 24% (HR 0.76; [0.66 to 0.88]; p=0.0003) [27]. There is also some evidence toward improving kidney outcomes emerging from the pivotal CVOTs of GLP-1RA and from the meta-analyses which assessed them, but with a lower degree of statistical power as the conclusions are derived from secondary outcomes or exploratory assessments, and the renal benefits were demonstrated through the reduction in the progression of albuminuria, with fewer effects observed on hard end-points [21].

With regard to heart failure, the aforementioned meta-analyses reported a 10% risk reduction of heart failure hospitalizations, but this result was driven only by the reduction reported in the AMPLITUDE-O trial, which investigated efpeglenatide [21–23]. As a result, this outcome could not be generalized as a class effect.

2 clinical trials specifically investigated GLP-1RA in HFrEF patients: FIGHT and LIVE. Neither of the two trials demonstrated the beneficial effects of GLP-1 receptor agonists in patients with HFrEF. Notably, the LIVE trial even raised concerns regarding a potential trend toward an increased risk of mortality, ventricular arrhythmias, and atrial fibrillation [21,28]. In terms of HFpEF, two complementary RCTs were published, which investigated subcutaneous semaglutide in obese patients (BMI>30 kg/m^2) without DM (STEP-HFpEF) and with DM (STEP-HFpEF DM). In both trials, there are reported similar results: significant reductions in primary end-points such as a reduction of 7.8 points in Kansas City Cardiomyopathy Questionnaire (KCCQ) (95% CI [4.8 to 10.9]; P<0.001) in STEP-HFpEF and a reduction of 7.3 points in KCCQ in STEP-HFpEF DM (95% [CI] [4.1 to 10.4]; P<0.001) and an average of 10.7% reduction in body weight (95% CI [−11.9 to −9.4]; P<0.001) in STEP-HFpEF and 6.4% reduction in body weight (95% CI [−7.6 to −5.2]; P<0.001) in STEP-HFpEF DM. There are also reported significant results concerning secondary end-points, such as an average improvement of 20.3 m at 6-minute walk distance (95% CI [8.6 to 32.1]; P<0.001) in STEP-HFpEF and 14.3 m (95% CI [3.7 to 24.9]; P=0.008] in STEP-HFpEF DM, respectively. Essentially, these two trials proved that semaglutide improves symptoms and exercise capacity in obese HFpEF patients with or without type 2 DM, but neither study was powered enough to evaluate the influence on hard clinical events, such as heart failure-related hospitalizations or urgent hospital visits [29,30]. It has been suggested that the clear weight reduction effect had a major contribution to the improvement of symptoms and exercise capacity in these patients [21].

Tirzepatide has recently emerged as a new star in the GLP-1 modulation field, being the latest discovered molecule in this class. More specifically, tirzepatide is a dual agonist that acts both on the receptors of GLP-1 and the receptors of GIP (glucose-dependent insulinotropic polypeptide). GIP represents another incretin hormone, naturally released by K-cells of the upper small intestine. Tirzepatide was validated as an effective antidiabetic medication (up to 2.4% reduction in HbA1c), as proved in the series of trials SURPASS 1-5, when tirzepatide was subsequently compared to placebo, or to active comparators, such as semaglutide, to which it was shown to be non-inferior or insulin degludec, to which it was shown to be superior in terms of glycemic control [31]. In terms of weight management, in SURMOUNT 1-4 clinical trials, tirzepatide proved a potent effect, with a mean weight loss of up to 26% [32]. In a recent network meta-analysis, compared to GLP-1 RA, tirzepatide proved higher efficacy in terms of glycemic control and weight control, probably owing to the dual incretin action [8]

Besides direct effects in the metabolic pathway, the SUMMIT trial yielded noteworthy findings in both the cardiovascular and, to some extent, the renal pathways of CKM syndrome. It included 731 obese patients (BMI over 30 kg/m^2) with HFpEF (LVEF of at least 50%), regardless of their diabetes status, who were randomized to receive either tirzepatide or placebo. The trial yielded positive results with regard to the both primary end-points: first one representing a composite end-point formed by death from cardiovascular causes or a worsening heart failure event (hazard ratio, 0.62; 95% confidence interval [CI], 0.41 to 0.95; P=0.026), a result mainly driven by a reduction in worsening heart-failure events (hazard ratio, 0.54; 95% CI, 0.34 to 0.85), and the second one a reduction in KCCQ score, with a between-group difference at the end of the study of 6.9 (95% CI [3.3 to 10.6]; P<0.001). There was no difference in cardiovascular death [33]. Therefore, for the first time, the SUMMIT trial offered evidence derived from an RCT that influencing metabolic pathways with tirzepatide, regardless of diabetes diagnosis, we could influence hard cardiovascular end-points.

An interesting, recently published subanalysis of the SUMMIT trial found that the beneficial effects of tirzepatide upon worsening heart failure events or various metrics pertaining to functional capacity or quality of life are not influenced by the presence or absence of CKD, namely, eGFR does not seem to influence the effects on major adverse heart failure outcomes. In a head-to-head comparison pertaining to the primary composite end-point of death from cardiovascular causes or a worsening heart failure event, tirzepatide showed a larger absolute risk reduction in the CKD group (preventing 3.6 primary endpoint events for 100 patients treated for 1 year) compared to the group without CKD (preventing 1.6 primary endpoint events for 100 patients treated for 1 year). This may probably be an effect at least partially explained by the larger global risk of heart failure events in CKD patients. Moreover, tirzepatide seems to significantly ameliorate glomerular function at 52 weeks (+2.9 [95% CI: 0.9-4.9]; P = 0.004), an effect which proved constant throughout the broad spectrum of baseline eGFR (between 15 ml/min/1.73 m^2 and 70 ml/min/1.73m^2), as well as UACR at 24 weeks (−25%; 95% CI: −35.5% to −12.7%; P < 0.001) [34].

Although we have a very probable class effect of GLP-1RA in reducing MACE and unique evidence for tirzepatide in influencing hard end-points in HFpEF, currently, there is no direct evidence from RCTs that the dual GLP-1R/GIPR agonist reduces MACE. There exists an ongoing trial (SURPASS-CVOT) which currently evaluates the effect of tirzepatide in comparison to dulaglutide on MACE in patients with type 2 diabetes mellitus.

In general, GLP-1RAs are considered relatively well-tolerated medications, and evidence derived from clinical trials supports their effectiveness. However, there are described side effects such as hypersensitivity reactions (including, in rare cases, anaphylactic shock), a hypothetical enhanced risk of C-cell thyroid neoplasm (based on animal studies and theoretical risk), gastrointestinal adverse effects (such as nausea or vomiting, especially during initiation or dose escalation, increased ileus risk), an increased risk of cholelithiasis, cholecystitis and pancreatitis, and concerns of increased pancreatic cancer risk emerging from observational studies. Generally, gastrointestinal adverse effects are the most common and the most frequent cause of drug discontinuation [35].

SGLT2 INHIBITORS

SGLT2 inhibitors were created to target the SGLT2 transporter, a protein mainly located in the first segment of the proximal convoluted tubule, which is responsible for the absorption of 90-97% of the filtered glucose. The reabsorption of one molecule of glucose is coupled with one sodium ion, the latter being transported along the transmembrane concentration gradient created by the Na/K ATP-ase pump [36]. The residual glucose that escapes reabsorption is transported via SGLT1, which is located in the more distal parts of the proximal convoluted tubule. In case of SGLT2 blockage, SGLT1 could partially compensate for its effects, with only 50-60% of the filtered glucose excreted in the urine [37]. Although SGLT2 is mainly located in the kidney, it is also found in the brain, liver, thyroid, and striated muscle [36,37].

The development of SGLT2 inhibitors was inspired by phlorizin, a naturally occurring compound known to be a SGLT2 inhibitor, with the therapeutic objective of promoting natriuresis and glycosuria in order to obtain glycemic control [37]. In the context of efforts to preserve the biologically active properties of phlorizin, while also improving its oral bioavailability and stability, a series of novel molecules were developed: canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin. Moreover, a dual inhibitor that blocks both SGLT1/SGLT2, namely sotagliflozin, was approved, which adds the effect of delaying intestinal glucose absorption by inhibiting SGLT1 at this level [37,38]. All molecules share very attractive pharmacologic properties: optimal oral absorption, once-daily administration as a result of the long half-life, metabolism occurring primarily in the liver, reduced renal clearance, and absence of clinically significant drug interactions [37].

Throughout studies, SGLT2 inhibitors have shown only modest effectiveness with regard to glycemic control, with reductions in HbA1c levels within the range of 0.5-1% [36,39]. Despite continuous glycosuria, inhibition of SGLT2 reduces insulin release and increases glucagon levels, which stimulates gluconeogenesis besides activation of lipolysis and lipid oxidation [40]. Similar to GLP-1 receptor agonists, and constituting an important observation for the largely overweight type 2 diabetes population, it was observed that SGLT2 inhibitors also exert a weight-reducing effect. This effect was observed to be approximately 2 to 4 kg, a consistent and durable reduction documented across all studies and for all molecules. To refer to specific figures, the inhibition of glucose reabsorption at the nephron level causes a loss of 75 g of glucose per day, which means nearly 300 kcal per day. It would be expected that a yearly weight loss of 10 kg, which is much more than was noted in clinical studies [40]. There are proposed mechanisms for this discrepancy between theory and practice, and the most prominent one is compensatory hyperphagia induced by a reduction in leptin, a hormone released by adipose tissue which inhibits appetite at a central level [40,41].

After the finding that rosiglitazone, a thiazolidinedione, is associated with an increased risk of adverse cardiovascular events, the scientific community requested that the new SGLT2i need to undergo cardiovascular outcome trials in order to prove their safety, similar to the aforementioned GLP-1RA.

The EMPA-REG OUTCOME trial tested empagliflozin in over 7000 patients with established cardiovascular disease and proved that this SLGT2i is associated with a significant 14% (HR 0.86; 95% confidence interval [CI], 0.74–0.99; p=0.04) reduction in the risk of major adverse cardiovascular events, a 32% reduction of all-cause mortality (HR, 0.68; 95% CI, 0.57–0.82]; p<0.001), and a 35% reduction of HF hospitalization risk (HR, 0.65; 95% CI, 0.50–0.85; p=0.002) [42,43]. Similarly, the DECLARE TIMI trial, for dapagliflozin, showed non-inferiority to placebo with respect to MACE (p<0.001) and a reduction with 17% of the risk for cardiovascular death or hospitalization for heart failure (HR, 0.83; 95% CI, 0.73-0.95; p=0.005) [42,44]. The CANVAS trial, for canagliflozin, showed a reduction with 14% of the combined risk of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke (HR, 0.86; 95% CI, 0.75 to 0.97; P<0.001 for noninferiority; P=0.02 for superiority) [42,45]. Ultimately, the VERTIS trial, which studied ertugliflozin, found a significant reduction in the risk of heart failure hospitalizations [42]. Based on these preliminary studies, the ESC guidelines for the management of heart failure mention a class I indication for the utilization of SGLT2i in diabetic patients at high risk for CV events to reduce the risk of hospitalization for heart failure events, MACE, end-stage renal dysfunction, or cardiovascular death [46].

These initial trials prompted the conduction of future dedicated trials in order to establish a potential role of SGLT2i in the management of heart failure patients.

The further DAPA-HF trial proved to be a pivotal study, which paved the way for the actual large indications of SGLT2i in heart failure with reduced ejection fraction (HFrEF). This trial included 4744 patients with II-IV HYHA class heart failure, with an ejection fraction of less than 40% and proved that dapagliflozin (10 mg single dose) is associated with the reduction of a primary composite outcome of cardiovascular death, HF hospitalizations, and worsening HF (HR, 0.74; 95% CI, 0.65 to 0.85; P<0.001), with all components of this composite outcome being positively and significantly influenced. This trial also found that the effect is not influenced by the diabetic status of the patient [42,47].

For empagliflozin, the EMPEROR-Reduced trial showed similar results: a reduced composite outcome of cardiovascular death and HF hospitalizations (HR, 0.75; 95% CI, 0.65–0.86; p<0.001), similarly independent of the diabetic status of the patient. In the case of empagliflozin, the effect on the primary outcome was mainly driven by the reduction of the rate of HF hospitalizations, as the reduction of cardiovascular death proved to be non-significant [42,48]. A meta-analysis of these 2 landmark trials found significant reductions in all-cause death (pooled HR 0.87, 95% CI 0.77-0.98; p=0.018) and in cardiovascular death (pooled HR 0.86, 0.76-0.98; p=0.027) [49].

These findings have been incorporated into guideline recommendations, as both the ESC guidelines and the ACC/AHA guidelines on heart failure mention dapagliflozin and empagliflozin as indicated in all HFrEF patients, irrespective of their diabetic status, to reduce the risk of heart failure-related death or hospitalization (class I level of recommendation A) [46,50].

Encouraged by the positive results in the HFrEF field and taking into account the lack of available medications with prognostic effects in HFpEF, the idea of investigating the efficacy of these medications in this prevalent HF phenotype has emerged. And initial evidence emerged from the SOLOIST-WHF trial, which evaluated the effects of sotagliflozin, the dual SGLT1/SGLT2 inhibitor, initiated soon after an episode of decompensation of heart failure; sotagliflozin proved a lower risk of a primary composite end-point formed by cardiovascular death or hospitalizations and urgent visits for heart failure (HR, 0.67; 95% confidence interval [CI], 0.52 to 0.85; P<0.001). The result, suggesting a potential effect of SGLT2i in HFpEF, was that the risk reduction of the primary end-point was consistent across the entire spectrum of ejection fraction [51].

Empagliflozin gained evidence for HFpEF and HFmrEF attributable to the results of the EMPEROR-Preserved trial (which included patients with an ejection fraction above 40%). Empagliflozin 10 mg once daily reduced a composite end-point of worsening heart failure or cardiovascular death (hazard ratio, 0.79; 95% confidence interval [CI], 0.69 to 0.90; P<0.001), a result driven by a significant reduction in the risk of worsening heart failure (with no difference in death from cardiovascular causes) [52]. Likewise, in the DELIVER trial (heart failure patients with an ejection fraction above 40%), dapagliflozin 10 mg od reduced the events of a similar primary composite end-point (hazard ratio, 0.82; 95% confidence interval [CI], 0.73 to 0.92; P<0.001). Once again, this effect is attributable to the reduction of the worsening heart failure events, without significant effects on cardiovascular mortality [53]. As a result, clinical guidelines have been harmonized and currently recommend empagliflozin and dapagliflozin (unique dose of 10 mg per day) in HFpEF and HFmrEF for the reduction of the risk of HF hospitalization or cardiovascular death (class I recommendation in European guidelines, class IIa recommendation in American guidelines) [50,54].

Important evidence for using SGLT2i in heart failure derives from the EMPULSE trial, in which empagliflozin was initiated in hospitalized acute heart failure after stabilization, regardless of ejection fraction or diabetes status. The primary analysis was a win ratio, being a composite of death, heart failure events, time to first heart failure event, and modifications in KCCQ. Empagliflozin proved a better win ratio, with a statistically significant result (win ratio 1.36, 95% CI 1.09-1.68, p=0.0054), with consistent results across the prespecified subgroups [55]. Besides this combination of prognostic and symptomatic benefits, empagliflozin also proved significant results on various indices of decongestion, suggesting that early initiation of empagliflozin in hospitalized acute heart failure patients also has the potential to contribute to an early and sustained decongestion [56].

Regarding the benefits of SGLT2 inhibitors in another acute setting—this time early after myocardial infarction—a recently published meta-analysis concluded that early administration of SGLT2i post-myocardial infarction may be beneficial by reducing the risk of recurrent major adverse cardiovascular events (MACE) or hospitalization for heart failure. However, this meta-analysis did not demonstrate a significant reduction in mortality among these patients [57].

Similar to GLP-1RA, SGLT2i is also an effective medication in chronic kidney disease. Based on findings from early studies on cardiovascular safety, specific trials have been designed to directly assess the potential beneficial effects of SGLT2i in CKD. 4 trials, CREDENCE (for canagliflozin), DAPA-CKD (for dapagliflozin), SCORED (for sotagliflozin), and EMPA-KIDNEY (for empagliflozin) proved that SGLT2i slow the progression of CKD. Whereas for canagliflozin and sotagliflozin, the effects were proven only in T2DM populations, in contrast, for empagliflozin and dapagliflozin, the effects were irrespective of the diabetic status, similar to HF trials. It is important to mention that the recommendation is to initiate dapagliflozin at an eGFR of at least 25 ml/min/1.73m^2 and empagliflozin at an eGFR of at least 20 ml/min/1.73m^2, but the administration may continue even if the renal function further deteriorates (this indication derives from the design of the studies and the characteristics of the included patients) [58].

Table 2: Major CVOTs of SGLT2i [42]

	Type 2 diabetes mellitus	**Heart failure**	**Chronic kidney disease**
Proved effects	In high-risk cardiovascular patients, it reduces the risk of major adverse cardiac events or cardiovascular death. (Besides improved glycemic control and weight loss)	Reduce the risk of heart failure-related death or hospitalization (across the entire ejection fraction spectrum) Improves decongestion in stabilized acute heart failure (empagliflozin)	Mitigate the progressive reduction of eGFR and lower the risk of renal or cardiovascular-related death
Drugs approved	Canagliflozin, Dapagliflozin, Empagliflozin, Ertugliflozin	Dapagliflozin, empagliflozin	Canagliflozin, Dapagliflozin, Empagliflozin
Guideline recommendations	Recommended as first-line add-on therapy to metformin, especially in cardiovascular high-risk, heart failure, or CKD patients	Class I recommendation in HFrEF Class I recommendation in ESC guidelines, class IIa recommendation in ACC/AHA guidelines for HFpEF	T2DM and CKD with eGFR>20 ml/min/1.73m^2 ACR>200 mg/g (and eGFR>20 ml/min/1.73m^2) ACR<200 mg/g with eGFR 20-45 ml/min/1.73m^2 CKD + heart failure

MINERALOCORTICOID RECEPTOR ANTAGONISTS (MRAs)

It is known that overactivation of the renin-angiotensin-aldosterone system and aldosterone receptors (mineralocorticoid receptors, MR) in particular, has effects beyond salt and water retention and hypertension. MR overactivation is capable of inducing inflammation and fibrosis in various organs, being involved in CKD and CV disease progression, situations in which RAAS is overly activated and aldosterone is in excess [73]. As far as metabolic derangement is involved, diabetes mellitus also induces a state of overactivation of MRs, and in obese patients there was observed a significant aldosterone production from adipose tissue was observed [74,75].

The deleterious effects of excess aldosterone are facilitated by the widespread expression of mineralocorticoid receptors throughout the body, as they are present not only in the kidneys but also in the heart, blood vessels, gastrointestinal tract, adipose tissue, and central nervous system [73].

The first therapeutic options to block MRs were the steroidal agents spironolactone and eplerenone, respectively.

The first results for these agents came from RCTs, which tested them in HFrEF, a common entity in advanced stages of cardio-renal-metabolic syndrome. In RALES trial, conducted in 1999, spironolactone reduced all-cause mortality by 30% (RR, 0.70; 95% CI, 0.60 to 0.82; P<0.001) and the risk of heart failure hospitalization by 35% (RR 0.65; 95% CI, 0.54 to 0.77; P<0.001), besides observed benefits on improvement regarding the symptoms of heart failure [76]. Eplerenone gained evidence from the EMPHASIS-HF trial, which proved a significant reduction of the primary composite endpoint of CV death or heart failure hospitalization (HR, 0.63; 95% CI, 0.54 to 0.74; P<0.001) [77]. Further trials provided evidence for steroidal MRAs, namely eplerenone, early after myocardial infarction. EPHESUS and REMINDER trials found that eplerenone, early initiated after MI, in patients with clinical signs and symptoms of heart failure and an ejection fraction of less than 40% could reduce all-cause mortality, length of hospitalization due to heart failure, ventricular arrhythmias, or the risk for deterioration of ejection fraction [78,79]. The beneficial effects of MR blockade in HFrEF and after myocardial infarction emphasize the important effects of aldosterone in cardiac adverse remodelling.

Regarding the HFmrEF and HFpEF, the available results are not as solid as in HFrEF. In the TOPCAT trial, 3,445 patients with symptomatic heart failure and a left ventricular ejection fraction of ≥45% were randomized to receive either spironolactone or placebo. Although the study narrowly failed to meet its primary composite endpoint (including cardiovascular death, aborted cardiac arrest, and hospitalization for heart failure), its results generated considerable debate [80]. The difference between a significant HR in American cohort (HR, 0.82; 95% CI, 0.69-0.98; p = 0.026) and a non-significant HR in Russian and Georgian cohort (HR, 1.10; 95% CI, 0.79-1.51; interaction p = 0.12) has led to speculation that the apparent neutral results of the TOPCAT trial may be due to issues with enrollment and drug adherence in the Eastern European cohort [81]. Based on the significant results on American cohort, there is a 2b recommendation in ACC/AHA guidelines on heart failure for steroidal MRA use in HFpEF and HFmrEF [50].

Hypertension in general and resistant hypertension in particular are common entities in chronic kidney patients or in obese, insulin-intolerant, or diabetic patients. Resistant hypertension is associated with a poor prognosis, causing target organ damage. The PATHWAY-2 RCT established spironolactone as a first-line agent in resistant hypertension, on top of maximum tolerated doses of ACEI/ARB, calcium-channel blocker, and thiazide diuretic [82].

The main limitation of steroidal MRAs' use is the risk of hyperkalemia, a risk observed since the early trials and considered relatively significant, particularly in a population with chronic kidney disease. A meta-analysis that included the landmark RCTs of steroidal MRAs proved that the risk of hyperkalemia and the benefits are inversely proportional to the eGFR (with unclear benefits compared to high risk of hyperkalemia in patients with an eGFR<30 ml/min/1.73m^2) [83].

Gynecomastia and breast pain are other well-known and relatively frequent adverse effects of spironolactone (with a 10% to 1% ratio observed in the RALES trial) [73].

There is a limitation in the steroidal MRAs' potential for broader cardiorenal protection, given the fact that their widespread use is limited by hormonal adverse effects or, in advanced CKD, by a significant risk of hyperkalemia. Moreover, data regarding the direct effects of steroidal MRAs on slowing CKD are unconvincing. There is a meta-analysis that proposes some benefits on intermediate endpoints such as albuminuria or eGFR slope, albeit at the cost of significant risk of hyperkalemia, worsening renal function, or gynecomastia [84].

The novel class of non-steroidal mineralocorticoid receptor antagonists (nsMRAs) has emerged in an effort to overcome these limitations while preserving the beneficial effects of the steroidal MRAs.

Finerenone is the most prominent agent of this pharmacological class, with several advantages over its steroidal counterparts: a comparable potency of MR inhibition but with a much higher selectivity, virtually no sexual side-effects, a more balanced tissue distribution between kidneys and the heart (in comparison with sMRA which are more concentrated at the renal level) and a shorter plasma half-time, which might contribute to a lower risk of hyperkalemia [85,86].

The first RCTs investigating finerenone were FIDELIO-DKD and FIGARO-DKD. FIDELIO-DKD primarily enrolled diabetic patients suffering from advanced CKD with prominent albuminuria, and there was a prespecified primary composite kidney endpoint. FIGARO-DKD enrolled diabetic patients with moderate albuminuria associated with a wider spectrum of CKD and stated a primary composite cardiovascular endpoint [85]. The results from these 2 RCTs and from their pooled analysis (FIDELITY) form the basis for the KDIGO guideline recommendation of finerenone in CKD: in patients with diabetic nephropathy, with an eGFR>25 ml/min/1.73 m^2 and with an albuminuria >30 mg/g, despite a maximum dose of ACEI/ARB [72]. Apart from reducing the kidney disease progression, finerenone reduced cardiovascular risk (HR 0.86, 95% CI: 0.78-0.95) and the risk of heart failure hospitalization (HR 0.78, 95% CI 0.66-0.92) in diabetic CKD patients [86].

The latter FINEARTS-HF trial provides the first quality results for MRAs (particularly nsMRA, finerenone) in HF with EF above 40%. In this RCT, finerenone reduced the risk of a primary composite endpoint formed by worsening heart failure events and cardiovascular death with 16% (rate ratio, 0.84; 95% confidence interval [CI], 0.74 to 0.95; P=0.007), driven by a significant reduction in worsening heart failure events, similar to SGLT2i results in the same patient population [87]. Compared to the previous ones, FINEHEARTS was a revolutionary trial also by the population included, with approximately 60% not having CKD or diabetes, suggesting that there may be important direct effects on the heart [88].

The recent FINE-HEART meta-analysis allows for the formulation of global conclusions regarding finerenone as far as cardiovascular and renal outcomes are concerned. This meta-analysis included the three RCTs, FIDELIO-DKD, FIGARO-DKD, and FINEARTS-HF, encompassing a total of 18991 patients. 12.1% of the included patients concurrently had HF, CKD, and diabetes, the main conditions encompassed in the CKM syndrome. The results indicate that finerenone reduces the risk of heart failure hospitalization (HR: 0.83; 95% CI: 0.75-0.92; $P < 0.001$) and of a composite kidney outcome, thus delaying CKD progression (HR: 0.80; 95% CI: 0.72-0.90; $P < 0.001$). Although not succeeding in proving risk reductions in cardiovascular death (HR 0.89; 95% CI: 0.78-1.01; $P = 0.076$), finerenone seems to reduce the risk of all-cause death (HR: 0.91; 95% CI: 0.84-0.99; $P = 0.027$) [88].

Currently, the sole recommendation for finerenone within cardiology guidelines is the class IA recommendation in the 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes, in patients with type 2 DM and CKD (UACR>300 mg/g or eGFR 25-60 ml/min/1.73m^2 and UACR>30 mg/g), in addition to ACE-I or ARB, to reduce the risk of cardiovascular events or kidney failure [26].

This recommendation, similar to those related to SGLT2i or GLP-1RA, empowers all medical doctors involved in the management of CKM syndrome to take proactive measures aimed at improving the prognosis of these patients. It remains to be seen what future heart failure guidelines will unveil regarding nsMRA indications, probably related to future trials that may investigate the effects of finerenone in this population.

CONCLUSION

Management of cardiovascular disease within the cardiorenal metabolic (CKM) syndrome requires a comprehensive, individualized approach, guided by cardiovascular risk stratification. While lifestyle interventions remain foundational, pharmacologic therapy plays a pivotal role in improving prognosis and quality of life.

Standard treatments such as RAAS blockade—with ACE inhibitors, ARBs, and steroidal MRAs—form the therapeutic backbone. However, recent advances support the early initiation of novel agents with proven benefits across all three CKM components.

GLP-1 receptor agonists and dual GLP-1/GIP agonists (e.g., tirzepatide) not only promote glycemic control and weight reduction but also lower the risk of major adverse cardiovascular events and may preserve renal function. SGLT2 inhibitors have demonstrated robust prognostic benefits in heart failure—regardless of ejection fraction—and chronic kidney disease, independent of glycemic status. Finerenone, a non-steroidal mineralocorticoid receptor antagonist, is recommended in diabetic kidney disease with albuminuria and shows emerging evidence of efficacy in HFpEF and HFmrEF, although formal guideline recommendations are still pending.

Optimizing therapeutic strategies with both established and novel agents is essential for altering the course of heart disease in CKM syndrome and addressing its complex, multisystem pathophysiology.

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Authors' contribution

Conceptualization, A.G.F., S.H. and S.M.S., methodology, M.J..; formal analysis, M.F.; data curation, E.R.; writing—original draft preparation, A.G.F., S.A.P.; writing—review and editing, E.C., R.D.G., M.F., E.R.; visualization, R.D.G.; supervision, S.M.S.; project administration, S.H. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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CLINICAL PRACTICE

Endoscopic eradication of nodular gastric vascular antral ectasia by using band ligation after argon plasma coagulation

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Abstract: *Gastric antral vascular ectasia (GAVE) is an important cause of gastro-intestinal bleeding. The most common clinical presentation of GAVE is chronic occult bleeding that leads to symptomatic iron deficiency anemia, but some cases could present with acute massive bleeding. Frequently, patients are dependent by iron suplimentation, or in severe cases even blood transfusions. Endoscopic therapy is frequently necessary in acute or chronic blood loss. Over the past several years, treatment for GAVE has continued to evolve as the number of available effective therapeutic interventions has increased. These included: YAG laser, argon plasma coagulation (APC), endoscopic band ligation, cryotherapy and surgical anterectomy. Argon plasma coagulation is the most commonly used technique, but has been associated with several complications like sepsis, post-APC bleeding, gastric outlet obstruction and increased incidence of hyperplastic polyps. Endoscopic band ligation (EBL), a mechanical procedure, has been reported in the past years as an effective salvage therapy for GAVE that is refractory to other approaches, or even as the first line treatment. We present a case of nodular GAVE treated succesfully with endoscopic band ligation after unsuccesufull sessions with argon plasma coagulation.*

Keywords: *gastric antral vascular ectasia, endoscopic band ligation, Argon plasma coagulation, gastro-intestinal bleeding*

INTRODUCTION

Gastric antral vascular ectasia (GAVE) or "watermelon stomach" is a relatively rare cause of gastrointestinal (GI) bleeding that mainly affects women aged 70 years and older. The incidence is estimated to be aproximately 4% of upper GI bleeding [1]. The term "watermelon stomach" is derived from the characteristic endoscopic appearance of longitudinal rows of flat, reddish stripes radiating from the pylorus into the antrum that resemble the stripes on a watermelon [2].

It usually presents as occult bleeding with chronic iron deficiency anemia. Even though GAVE was first diagnosed about six decades ago, its etiopathogenesis has not been fully established yet, with many hypotheses proposed such as mechanical stress, hormonal factors, and autoimmune factors. It is associated with liver cirrhosis, autoimmune disease, connective tissue disorders and collagen vascular disorders (eg, systemic sclerosis, Sjogren syndrome), chronic renal failure, bone marrow transplantation, acute myeloid leukemia and heart disease.

Frequently GAVE patients have chronic GI bleeding, but sometimes it can cause severe acute life-threatening bleeding especially in elderly with multiple chronic medical illnesses [2].

GAVE is typically located in the gastric antrum; however, it may be also found rarely in other areas of the GI tract, including cardia, duodenum, jejunum, and rectum. The

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involvement of the proximal part of the stomach is very rare and commonly located within a diaphragmatic hernia.

At endoscopy, GAVE may appear as 2 types. First type is the diffuse punctuate lesions in the antrum typically seen in male patients with cirrhosis and commonly accompanied by acute bleeding. Second one is the red lesions organized in stripes radially departing from the pylorus, known as “watermelon stomach”, mostly common in females with connective tissue diseases and usually present with occult bleeding [1].

Histopathologically, GAVE is characterized by vascular ectasia, spindle cell proliferation, and fibrohyalinosis. Immunohistochemical staining for CD61, a platelet marker, further confirms a diagnosis of GAVE [3].

Over the years, treatment for gastric antral vascular ectasia (GAVE) has continued to evolve and the number of available treatments has continued to increase, including surgical, medical and endoscopic therapies

Medical therapy has not clearly shown satisfactory results. Multiple drugs, such as hormonal (estrogen-progesterone) therapy, steroids, octreotide and tranexamic acid, have been tried to control GAVE-related bleeding. After all, no one has clearly shown satisfactory results in order to consider medical therapy as a valid alternative to an invasive approach.

Endoscopic therapies have rapidly become the first line therapy with argon plasma coagulation (APC) as the most common used method and more recently with radiofrequency ablation system (RFA) using Halo90 catheter and endoscopic band ligation (EBL). Both of them have been shown to be safe and effective for GAVE treatment. The latter two have been utilized in treatment of severe, diffuse, APC refractory GAVE. The clinical outcomes of ablative therapy such as APC treatment or HALO90 system were reported as 80%-100% success rate.

Other therapies include Nd:YAG (neodymium:yttrium-aluminum-garnet) laser coagulation, but with a higher risk of perforation given the deeper thermal effect. Endoscopic sclerotherapy, heater probe, cryotherapy have also been described in the literature. For unresponsive cases to endoscopic therapy, surgery with antrectomy can be considered but carries a high surgical risk, especially in the cirrhotic patients.

APC is most commonly used method, but has been associated with sepsis, post-APC bleeding, gastric outlet obstruction and increased incidence of hyperplastic polyps’ formation. It is needed multiple sessions of APC to reduce bleeding episodes and/or decrease transfusion dependence. APC has been found to be equally effective in the treatment of GAVE and is superior to ND:YAG laser in cost, convenience and complication rates(4).

The non-ablative treatment options is the endoscopic band ligation. EBL was firstly reported as the treatment for refractory GAVE in the patients who failed other treatment modalities such as APC or hormonal therapy by Sinha et al. [5]

Endoscopic band ligation has been reported as an alternative effective endoscopic therapy and the rate of complication is low in comparison with those reported for APC in retrospective studies [6].

A case series of 9 patients reported by Wells et al also showed superiority of EBL over endoscopic thermal therapy, which were APC and bipolar thermal probe therapy, for the rate of re-bleeding, duration of hospitalization and post procedure transfusion. The complications reported for this procedure were very small. Band ligation was prefered as the first line treatment regarding of the extensive involvement of the lesion in the patients. According to the result of the treatment mentioned above, endoscopic band ligation could be considered as first line treatment options for the GAVE patients especially for extensive area of involvement. [7]

A recent prospective study showed endoscopic improvement with the use of endoscopic band ligation in 91% of the patients, with a significant improvement in the hemoglobin and ferritin levels. Band ligation in GAVE has been associated with transient abdominal pain in a minority of the patients, but no major complications have been reported in the literature. [9]

Some studies suggest that the number of sessions required for GAVE eradication is inferior when using EBL compared to APC, resulting in inferior health care costs. However, randomized controlled trials are lacking to determine whether EBL is more cost effective than APC as the primary endoscopic therapy for GAVE. [9, 10]

Although initial reports of these endoscopic modalities are encouraging, well-performed, larger, prospective studies are needed before providing any definitive conclusion.

CASE PRESENTATION

Clinical data

We present a case of a 57-year-old female, diabetic with multiple microvascular complications – diabetic polineuropathy and chronic renal disease, hypertensive, with moderate hypochromic microcytic anaemia and a positive fecal occult blood test (FOBT), already in treatment with iron therapy who presented for abdominal pain, fatigability, phisical astenia and arthralgias. She denied no change in weight, bowel pattern, or stool color. The physical examination revealed paleness of the skin and mucosa and otherwise unremarkable.

Laboratory data

Labs indicates a moderate hypochromic microcytic anaemia (hemoglobine level – 9.4 g/dL, mean corpuscular volume – 70.2 fL, hematocrit – 31.1%, mean corpuscular hemoglobin – 21.2 picog and sideremia – 26 microg/L.

Endoscopy

Superior digestive endoscopy showed hypertrofic gastric antral vascular ectasia with mucosal eryhema, friability and visible small vessels extending radially in linear rows throughout the antrum and a duodeno-gastric reflux. She underwent serial argon plasma coagulation treatments and a second gastroscopy was performed next day which showed post argon plasma coagulation ulcers which were biopsied.

Histopathology data

Ulcer post argon plasma coagulation biopsy performed showed modifications of chronic inactive gastritis, but GAVE was not confirmed on biopsy.

Evolution

After two months and with iron therapy, the hemoglobin level was normalised (12.7 g/dL) with low mean corpuscular volume and mean corpuscular hemoglobin. At the endoscopic examination the macroscopic appearance persisted with multiple friable hypertrofic and nodular antral vascular ectasia and duodeno-gastric reflux.

At 3 months follow-up, hemoglobin level mantained in the normal interval (13.5 g/dL), but the endoscopic appearing was worst, with larger, friable, with edema and hypertrofic gastric atral vascular ectasia (Figure 1). We decided to apply 6 elastic bands on the biggest lesions with success (Figure 2). No complications of the procedure were reported, and the patient was discharged after 24h.

Figure 1: Endoscopic appearance from the index endoscopy, demonstrating multiple erythematous friable antral hypertrophic and nodular lesions, representing nodular gastric antral vascular ectasia (GAVE)

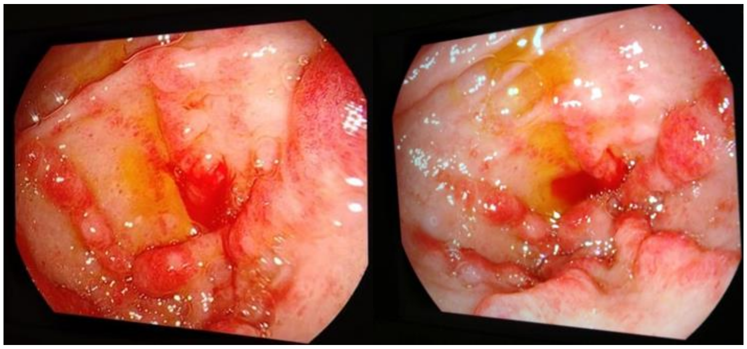

Figure 2: Endoscopic image from the band ligation of GAVE

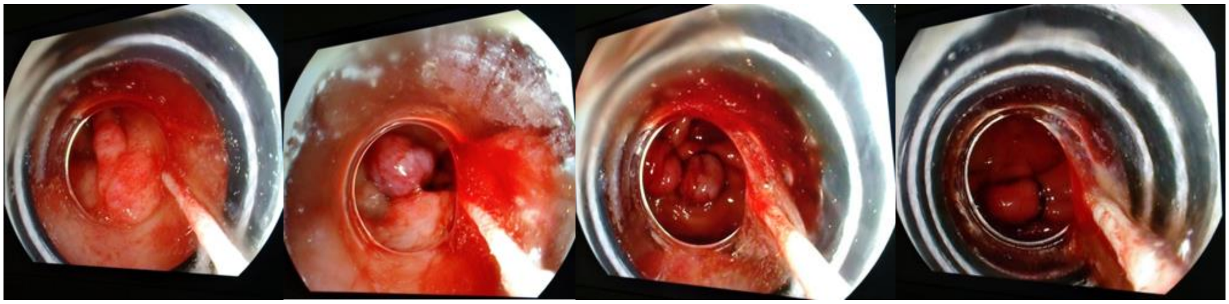

Two months later, hemoglobin level was stable (13.4g/dL). The upper endoscopy revelead small hyperemic antral circumferential disposed lesions with no signs of bleeding and no hypertrophy, with almost complete eradication of GAVE (Figure 3).

DISCUSSION

GAVE is a poorly understood entity, of unknown etiology, and an increasingly identifiable cause of chronic iron deficiency anemia. The pharmacological management of

GAVE had only poor results, so the mainstay of GAVE management remains endoscopic therapy.

Figure 3. Endoscopic appearance 2 months after endoscopic band ligation

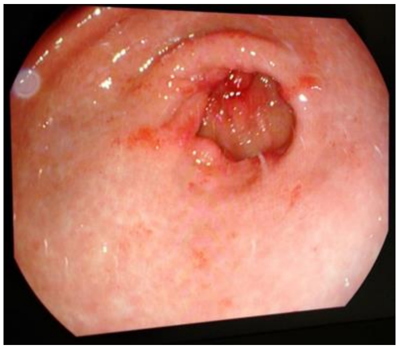

Figure 4. Suggested flow chart for treatment algorithm [14]. GAVE: Gastric antral vascular ectasia; APC: Argon plasma coagulation; RFA: Radiofrequency ablation; EBL: Endoscopic band ligation.

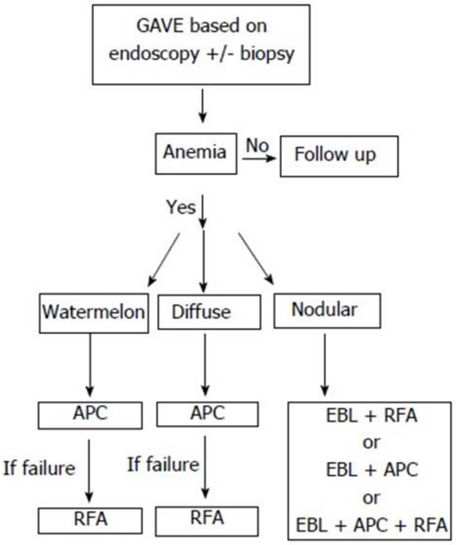

APC is a modality of non-contact electrocoagulation that applies high-frequency energy into tissue to cause thermal effects, which has been used successfully to treat GAVE with an effective transient response, but primary failure rates of therapy of up to 14% have been reported. Some authors suggest that this endoscopic method is insufficient in order to achieve medium and long-term treatment success, as it has been associated with a high recurrence rate (40–100%) [11].

In addition, APC might have complications, such as sepsis, pyloric stenosis and gastric outlet obstruction syndrome, in 20–33% of the patients [12].

EBL has been reported to be a relatively easy technique for GAVE therapy, has been shown to be safe and effective with lower complication rates in comparison with APC. Both Sato et al. [6] in 2012 and Prachayakul er al. [13] in 2013 conclude that EBL may be useful in the treatment of GAVE, to avoid the high recurrence rate after APC.

In this case, the patient was initially treated with APC after being diagnosed with GAVE as the cause of iron deficiency anemia, but this strategy was not successful. APC treatment might not be effective in some cases, especially in the case of hypertrofic and nodular type of GAVE, and this could be explained by the limited depth of thermal injury.

Since the histological changes are present in the mucosa and submucosal layer, EBL may be more effective for GAVE because of its ability to obliterate the submucosal vascular plexus like for esophageal varices. In this patient, the presence of extensive areas of the antrum affected with a high-density of both mucosal and submucosal vascular malformations is a likely explanation for the primary failure of the APC treatment.

It is becoming apparent that patients with severe, diffuse or refractory disease require multimodal therapy. Our case not only shows that, but also that patients specifically with nodular variant GAVE require and respond well to multimodal therapy.

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ARTICLE

Five-year Analysis of Head and Neck Non-Melanoma Skin Cancer Incidence, Demographics, and Site Distribution in a Tertiary Care Center

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Abstract: Introduction: Non-melanoma skin cancers (NMSC) represent a significant global public health burden, particularly in high-income countries. The increasing incidence of head and neck NMSC contributes to rising morbidity and necessitates multi-disciplinary management. Objectives: This study aims to evaluate the incidence, demographic distribution, histological subtypes, and seasonal variation of NMSC over a five-year period at a tertiary center in Romania. Materials and Methods: A retrospective observational study was conducted from January 2020 to December 2024, including 180 patients diagnosed and treated for malignant skin lesions. Data were extracted from clinical and histopathological records. Chi-square tests assessed seasonal distribution, and Mann-Whitney U tests compared lesion depth and surface area between demographic groups. Results: A total of 388 malignant lesions were recorded. Basal cell carcinoma (BCC) was the most common subtype (n = 168), followed by squamous cell carcinoma (SCC) (n = 25). Most patients resided in urban areas, particularly Bucharest. Males presented with significantly deeper lesions (mean: 1.5 mm) compared to females (mean: 1.25 mm; p = 0.009). Seasonal analysis revealed a significant variation in incidence, with the lowest number of cases in spring ($\chi^2 = 10.31$, $p = 0.016$). Conclusion: The incidence of malignant skin lesions increased from 2020 to 2023, followed by a decline in 2024. Seasonal and sex-related differences were observed, with the face being the most frequent site of involvement. While the sex distribution was relatively balanced, males exhibited significantly greater lesion depths compared to females.

Keywords: non-melanoma skin cancers; squamous cell carcinoma; basal cell carcinoma; seasonal variation; epidemiology; retrospective study; head and neck cancer; Romania

INTRODUCTION

Cutaneous malignancies accounts for most cancers across the world and they are primarily divided into melanoma (MSC) and non-melanoma (NMSC) skin cancers [1]. According to the Globocan (Global Cancer Observatory) 2022 data, non-melanoma skin cancer ranks as the fifth most commonly diagnosed cancer worldwide, with over 1.2 million new cases reported [2]. However, despite its high incidence, NMSC ranks only 22nd in terms of cancer-related mortality, highlighting the generally favorable prognosis of NMSC compared to other malignancies [2].

The incidence of head and neck MSC and NMSC is rising all over the world and can be the source of significant morbidity and mortality [3]. The necessity of a multidisciplinary team that includes dermatology, plastic and reconstructive surgery, medical and radiation oncology remains a financial problem in many countries, due to the comprehensive care that needs to be provided for patients with head and

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neck cutaneous malignancies [3,4].

The vast majority of non-melanoma skin cancers include basal cell carcinoma (BCC), followed by squamous cell carcinoma (SCC), both showing a rapidly increasing trend in their incidence in the last years and a significant health burden in many countries [5,6]. Although the exact global incidence of these two is unknown, an overview of the last years carried out by World Health Organization concluded that BCC comprises about 80% of all NMSC, while SCC comprise about 20%, with a BCC to SCC ratio ranging from 2 to 4:1 [5,6]. While this is a classic agreed ratio, the recent studies suggest a possible changing in the pattern due to aging of the population, with equal numbers of SCC and BCC being treated in the future [6,7].

Multiple etiological risk factors related to the individual or the environment were studied for understanding the cause of NMSC, but the main driving carcinogen remains ultraviolet radiation (UVR) [5,6]. This explains why over 80% of these malignancies occur on the sun exposed skin of the head and neck (followed by the hands and forearms), with higher prevalence of BCCs in the nasal area, while SCCs are preferentially located on the auricle [5,8,9]. UVR are responsible for the induced dinucleotide mutations at pyrimidine bases, exhibiting strong transcription strand bias and also showing a high mutational burden with the mutant genes varying between BCCs and SCCs [6].

Although its mortality is low and it rarely results in metastatic disease, BCCs can cause significant health burden through his great morbidity, generated by the destructive local spread [9]. These tumors are slow growing but can, however, develop large, causing locally tissular destruction and spreading into vital structures such as the central nervous system or important blood vessels [10]. Traditionally, the five most commonly accepted histological subtypes of BCC, described by Lang and Maize, include superficial, nodular, micronodular, infiltrative and morpheaform or sclerosing BCC [10,11]. Of note, a particular form of BCC, called the basosquamous cell carcinoma (BSCC) or the metatypical BCC, may arguably be a SCC subtype, as it shows both basal cell and squamous cell cancer differentiation in a continuous fashion [10,11]. The metatypical BCC, together with morpheiform and infiltrative BCC belong to the aggressive-growth subsets, while the others are considered indolent-growth variants [11]. Regarding their epidemiology, in one large retrospective study on 1039 consecutive cases of BCC, the histological analysis demonstrated 21% were nodular, 17% superficial, 15% micronodular, 7% infiltrative and just 1% morpheic [12].

In contrast with basal cell carcinoma, the squamous cell carcinoma has metastatic potential, and when present, the general prognosis is rather poor due to the mortality associated with regional and nodal metastases [9]. It is the most common skin cancer after BCC and generally has a good prognosis, the overall metastasis rate varying from 1% to 4% [13]. There are several risk factors attributed with the regional metastatic potential, like a poor differentiation, a perineural invasion, the presence of recurrency, a depth of invasion greater than 6 mm or a size bigger than 2 cm [14]. The SCC typically develop in a progressive manner, beginning with premalignant lesion -actinic keratosis (AK)-to in situ SCC (Bowen's disease) and eventually to invasive squamous cell carcinoma [13]. Usually, only a small proportion of the precursors develop into invasive cutaneous SCC [13,15]. Despite that, the high mortality of the metastatic variant and the high frequency of SCC in general remain of great concern and represent a financial burden for the health care services, as it needs a close clinical surveillance for many years [15].

Unlike the BCC variants, where the biological behaviors show little significant difference, there are a wide histopathological subtype of SCCs which demonstrates not just microscopic differences, but also a very diverse clinical behaviors [16]. There are several histologic subtypes of SCC and each of them carry an important prognostic significance [16,17]. The generic type (the simplex or conventional) has the greatest risk of metastasis, followed by lobular (acantholytic), Bowenoid, spindle (pleomorphic)-all three with a similar risk- and the last, verrucous carcinomas, has the most favorable prognosis [16,17]. Another predicting cancer behavior in cutaneous squamous cell carcinoma are the histopathologic parameters, which stage tumors into four categories primary based on their nuclear pleomorphism [17]. According to Broder, the tumors can be well-differentiated (less than 25% undifferentiated cells), moderately- well differentiated (less than 50% undifferentiated), poor differentiated (less than 75% undifferentiated) and anaplastic or pleomorphic (more than 75% undifferentiated) [17].

Nevertheless, the spectrum of head and neck cutaneous malignancies ranges from fairly well-behaved tumors (most of the BCCs) to potentially very aggressive cancers with high metastatic rate (usually melanoma, but also some histologic types of SCC) [3]. Their increasing incidence, the necessity of a multidisciplinary team and the need of a well-developed early screening evenly distributed among countries and regions, make the topic current and highly important in the present context.

The aim of this paper is to present the incidence of non-melanoma skin cancers of the head and neck in a single tertiary center in a retrospective study, from 2020 to 2024. Also, we'll describe the correlation between malignancies and gender, age, type of cancer, and its dimensions, together with trends in their frequency. Understanding temporal trends and potential fluctuations in incidence is essential for effective healthcare planning and resource allocation. Seasonal patterns in skin cancer diagnoses suggest that environmental factors, such as ultraviolet exposure, may influence disease occurrence and warrant further investigation. Additionally, variations in lesion characteristics between different demographic groups, including sex and geographic location, highlight the importance of exploring underlying biological, behavioral, and healthcare access factors. Investigating these aspects can contribute to improved prevention strategies, early detection, and tailored treatment approaches for skin cancer patients.

MATERIALS AND METHODS

A retrospective observational study was conducted on a database of patients diagnosed and treated for malignant skin tumors at a tertiary care center in Romania between January 2020 and December 2024. Due to the COVID-19 pandemic, there is a notable gap in data collection between April 2020 and June 2021, during which clinical activity was significantly reduced. The goal was to assess demographic distribution, histological types, anatomical localization, and treatment patterns of skin cancer patients in Romania in the last 5 years. Data were extracted from the institution's electronic medical records and physical registries, curated by the dermatology department.

The patients were selected using the following inclusion criteria: Patients with a clinical diagnosis of a skin lesion suggestive of malignancy; Hospital admission (i.e., full inpatient hospitalization, not outpatient consultation or day-case admissions); Histopathological confirmation of a malignant skin tumor.

Exclusion criteria consists of: Benign skin lesions and suspected malignancies for which no biopsy was performed (e.g., due to patient refusal or other contraindications).

Following the application of the inclusion criteria, a total of 388 malignant non-melanoma skin lesions were identified across 180 patients. While most patients presented with an average of approximately 2–3 lesions, a small subset exhibited over 10 malignant lesions each. Although not all lesions underwent histopathological confirmation, those that were not biopsied shared highly similar macroscopic features with confirmed cases, allowing for a reliable clinical diagnosis based on visual assessment.

The dataset includes both male and female patients residing in Bucharest as well as in 19 other counties across Romania. All patients sought medical evaluation and treatment at the tertiary facility. Of the 180 eligible patients, 125 (69.4%) originated from urban areas, while 55 (30.6%) were from rural settings. Sex distribution was relatively balanced, with 52% of the cohort being female and 48% male. Patient ages ranged from 35 to 93 years, with a median age of 69 years for females and 71 years for males.

The standard clinical pathway involved an initial dermatologic evaluation, during which a provisional diagnosis was made based on the morphological characteristics of the lesion. Patients then underwent a biopsy for histopathological confirmation. Upon receiving the diagnostic result, patients returned for definitive treatment, which typically consisted of complete surgical excision or electrocauterization, depending on the lesion's type, size, and location.

The following variables were systematically collected for each patient: demographic information (including age, sex, and place of residence); clinical characteristics (lesion location, provisional clinical diagnosis, year and month of diagnosis); histopathological data (tumor type and size); and treatment details, specifically the definitive procedure performed—either surgical excision or electrocauterization—as well as the occurrence of recurrence or the need for re-excision.

All tissue specimens were processed and analyzed by board-certified pathologists within the same department, using standard hematoxylin-eosin (H&E) staining tech-niques.

Data base was created using Microsoft Excel 2019. Statistical analysis was per-formed using JASP 0.19.3 program. Statistical analyses included Chi-square tests to as-sess seasonal distribution differences and Mann-Whitney U tests to compare lesion depth and surface area between demographic subgroups.

RESULTS

When analyzing the annual incidence of malignant skin lesions, a progressive in-crease was observed over the first four years of the study period: 18 cases in 2020, 25 in 2021, 40 in 2022, and a peak of 63 cases in 2023. This upward trend was followed by a decline in 2024, with 34 cases recorded (Fig. 1). A linear regression analysis revealed an average annual increase of approximately 7 cases (slope = 7), with a coefficient of determination (R^2) of 0.64, suggesting a moderate correlation between time and case number. However, the trend did not reach statistical significance ($p = 0.244$), likely due to the limited number of years analyzed and the variability introduced by external factors such as the COVID-19 pandemic in 2020–2021, the post-pandemic rebound in 2023, and the subsequent decrease observed in 2024.

The average hospitalization duration remained relatively stable over the past five years, starting at 4.2 days in 2020, slightly increasing to 4.5 days in 2021, then gradually decreasing to 4.3 days in 2022, and further declining to 4.1 days in both 2023 and 2024 (Fig. 2). The longest hospital stay was recorded in 2020, lasting 19 days, while the shortest stays of 2 days were observed in 2023 and 2024.

Figure 1.The trend line of malignant skin lesions

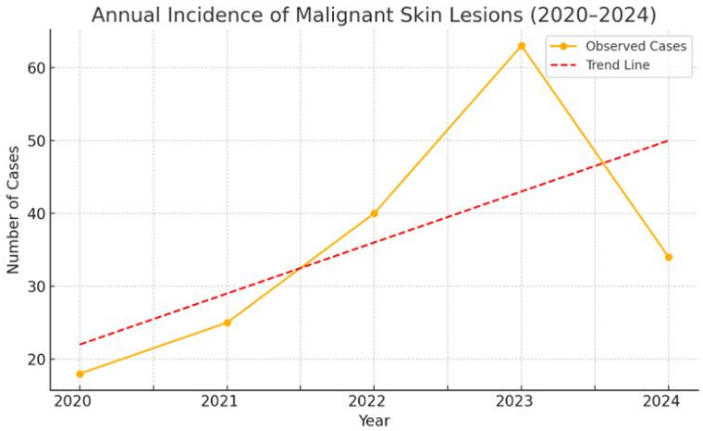

Figure 2. Evolution of the average hospitalization days (2020-2024)

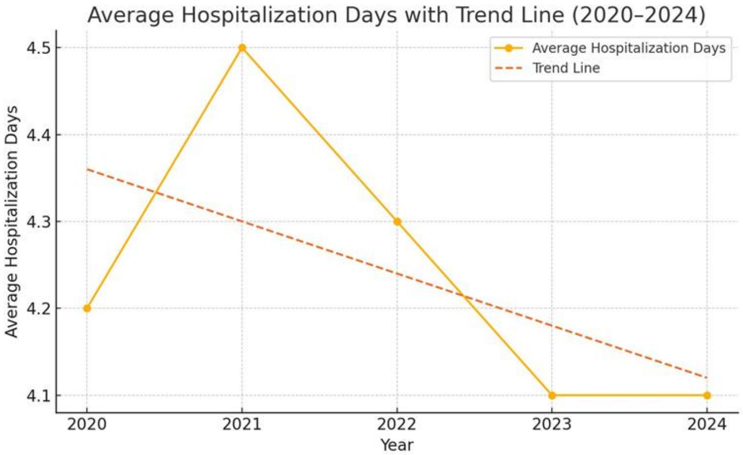

Of the 180 patients, 95 were residents of Bucharest, with the highest concentration coming from Sector 3 (n = 35) and Sector 4 (n = 32). Among patients from outside the capital, the neighboring counties of Ilfov (n = 16) and Giurgiu (n = 14) were the most represented.

When assessing seasonal distribution over the five-year period, the lowest incidence of diagnosed malignancies was observed in spring, with only 27 cases recorded. The other seasons showed relatively similar frequencies, with 47 cases in summer, 55 in autumn, and 51 in winter (Table 1, Fig. 3). In order to determine whether there is a significand difference between the observed frequencies and expected frequencies, we have applied the Chi-squeare test (χ^2). The test revealed a statistically significant difference in the seasonal distribution of skin cancer diagnoses ($\chi^2 = 10.31$, $p = 0.016$).

Table 1. Presentation period by month, year and season

Presentation period	**2020**	**2021**	**2022**	**2023**	**2024**	**TOTAL**	**No. of cases per season**
Jan.	6	Pandemic	1	9	5	**21**	**51 winter**
Feb.	9		1	5	5	**20**	
Mar.	3		2	3	6	**14**	**27 spring**
Apr.	Pandemic		2	1	3	**6**	
May			3	1	3	**7**	
Jun.			5	3	2	**10**	**47 summer**
Jul.		1	7	7	2	**17**	
Aug.		10	2	5	3	**20**	
Sept.		11	4	8	2	**25**	**54 autumn**
Oct.		1	6	6	3	**16**	
Nov.		1	4	9	0	**14**	
Dec.		1	3	6	0	**10**	
TOTAL	**18**	**25**	**40**	**63**	**34**	**180**	**180**

Figure 3. The percentage distribution of cases by season

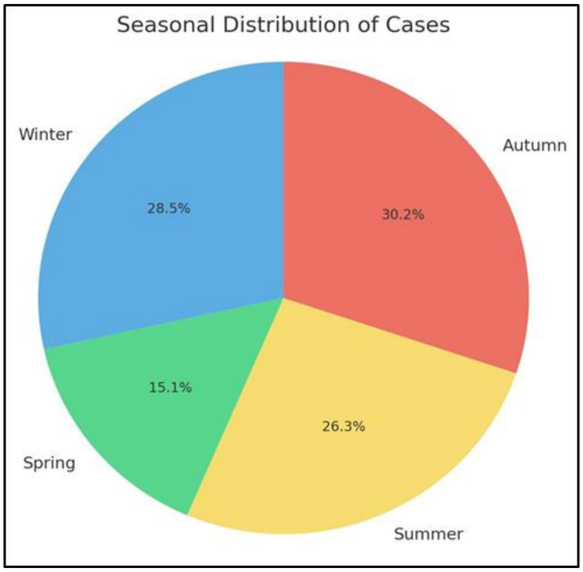

The malignant skin lesions identified in this study were categorized into basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. Out of the total 388 lesions, 168 were classified as BCC (Fig. 4). Histopathological evaluation allowed for further subtyping of BCC cases. The nodular subtype was the most prevalent (n = 68), followed by the adenoid type (n = 43), superficial BCC (n = 26), and the infiltrative variant (n = 23). Rare subtypes included basosquamous, pigmented BCC, Pinkus fibroepithelioma, and infundibulocystic BCC, each represented by one or two cases. Additionally, a single case associated with Gorlin syndrome was documented. Notably, no cases of micronodular BCC were observed (Fig. 5). Regarding squamous cell carcinoma, a total of 25 cases were identified. The majority were well-differentiated SCCs (n = 16), followed by moderately differentiated SCCs (n = 8) and poorly differentiated SCCs (n = 3). No anaplastic SCCs were detected during the study period.

Figure 4. Distribution of non-melanoma skin cancers cases

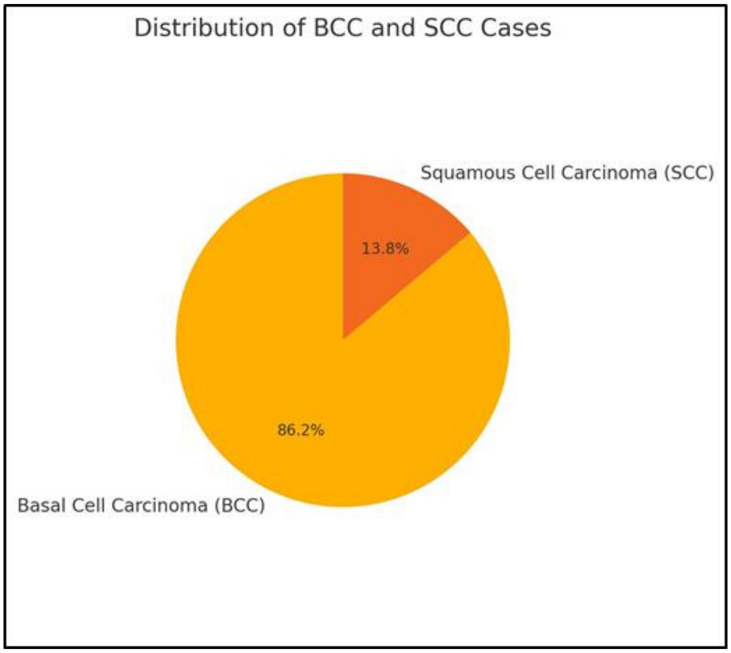

Figure 5. The incidence of histological subtypes of BCC

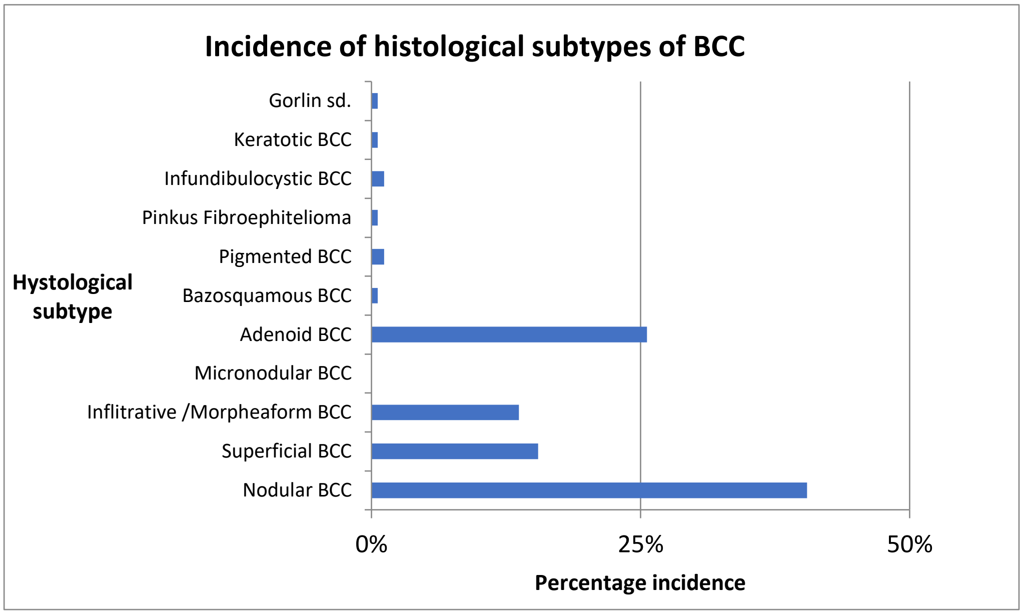

Based on the collected data, the majority of malignant lesions were located on the face (n = 152), a significantly higher number compared to those on the neck (n = 16) and scalp (n = 12). A more detailed anatomical breakdown of facial lesions revealed 20 cases on the forehead (10.3%), 23 on the nasal pyramid (11.8%), and 18 on the zygomatic region (9.2%). Additionally, 23 patients presented with multiple lesions on various facial subregions. Beyond the face, 24 other patients were identified with multiple malignant lesions distributed across the head and neck areas. Regarding the neck, the posterior cervical triangle showed a higher incidence (n = 11) compared to the anterior cervical triangle (n = 5). As for the scalp, lesion distribution was as follows: 2 cases on the vertex, 2 in the temporal region, 4 in the parietal region, 1 occipital lesion, and 3 patients with multiple scalp lesions.

When comparing the anatomical distribution of BCC and SCC, distinct patterns were observed. Several regions—including the anterior cervical triangle, vertex, temporal, , parietal, occipital, external canthus, upper and lower eyelids, chin (menton), perioral region, nasal area, auricle, and mastoid region—exhibited only BCC lesions, with no SCC cases recorded in these locations. On the forehead, 11% of all BCCs were located, compared to only 4% of SCCs, indicating a preferential distribution of BCC in this region. In contrast, the zygomatic region accounted for the highest proportion of SCC cases (20%), suggesting a predilection for SCC in this area. Within the posterior cervical triangle, SCC represented 8% of all cases found there, while BCC accounted for 5%, indicating a slightly higher occurrence of SCC in this particular region (Fig. 6).

The vertical dimension of malignant skin lesions showed notable variation across histological subtypes. Among basal cell carcinoma (BCC) types, the infiltrative/morphea form subtype had the highest mean depth (1.46 mm), followed closely by the superficial type (1.35 mm) and adenoid (solid adenoid) variant (1.30 mm). Less invasive subtypes such as nodular BCC had a mean depth of 1.21 mm, while rare variants like pigmented, Pinkus fibroepithelioma, and Gorlin syndrome-associated BCC displayed moderate vertical dimensions (ranging from 1.1 to 1.75 mm). Micronodular BCC was not reported in the dataset. In contrast, squamous cell carcinoma (SCC) subtypes tended to show greater vertical extension. Well-differentiated SCCs had the greatest average depth at 1.92 mm, while moderately differentiated SCCs averaged 0.97 mm, and poorly differentiated SCCs presented an intermediate value of 1.08 mm. These findings suggest a potential correlation between SCC grade and tumor depth.

Figure 6. Distribution of NMSC by anatomical location

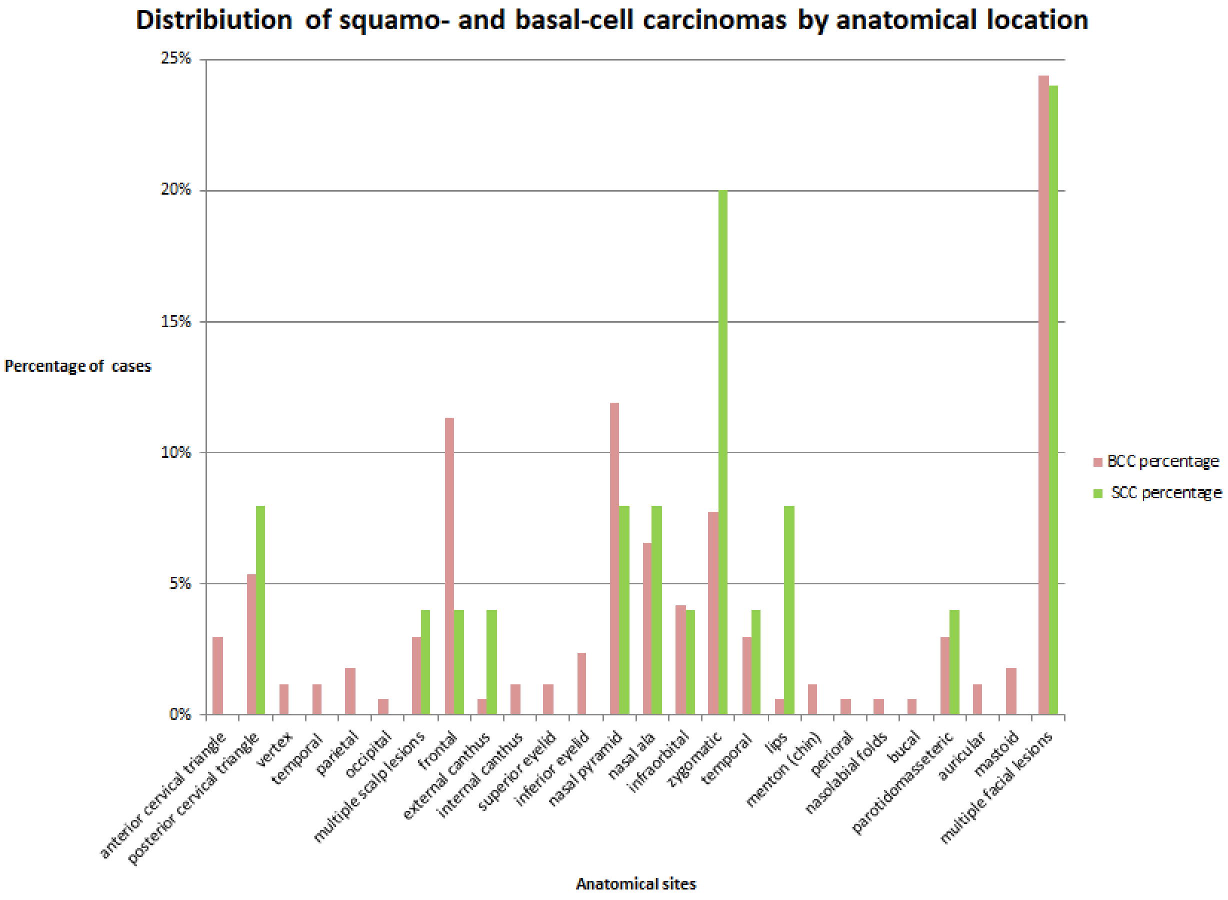

Temporal analysis revealed fluctuations in lesion depth over the five-year period. For nodular BCC, the vertical dimension ranged from 1.0 mm to 1.49 mm, indicating no clear increasing or decreasing trend. A more pronounced variation was observed in the superficial BCC subtype, which reached a peak of 1.85 mm in 2022 and dropped to 1.06 mm in 2023 before rising again to 1.61 mm in 2024. Infiltrative BCCs peaked at 2.52 mm in 2022—suggesting a possible diagnostic delay or more aggressive presentation in that year. Regarding SCC, well-differentiated lesions consistently displayed greater depth than other SCC subtypes, with a maximum of 2.5 mm recorded in 2022. However, sample sizes for some years and subtypes are limited, warranting cautious interpretation.

A Mann–Whitney U test was performed to compare the vertical depth of BCC and SCC. The median depth in BCC subtypes was 2.20 mm, while SCC subtypes showed a median of 1.32 mm. The test yielded a U statistic of 129.5 and a p-value of 0.797, indicating no statistically significant difference between the two groups.

In order to assess whether there is a correlation between age and the vertical di-mension of the lesion, we applied Spearman's rank correlation test. The results (Spear-man's ρ = 0.023, p = 0.744) suggest that there is no significant association between the two variables, indicating that lesion thickness does not appear to vary meaningfully with patient age in our dataset.

A Mann–Whitney U test (which is non-parametric) was conducted to determine whether there was a difference in vertical dimension between males and females. The results indicated a statistically significant difference between the groups (U = 6473.0, p = 0.009), suggesting that sex may influence vertical dimension. The vertical dimension mean in men was 1.5 mm, while in female was 1.25 mm.

When analyzing the lesion surface area across basal cell carcinoma (BCC) subtypes, we observed considerable variability. The infiltrative/morpheiform subtype demonstrated the largest mean area, with an average of 2.47 mm², followed by the adenoid type (1.58 mm²) and nodular type (1.43 mm²). The superficial BCC subtype had a slightly higher average area (1.85 mm²) than nodular, which may reflect the lateral spread characteristic of this variant. Rare subtypes such as basosquamous/metatypical carcinoma presented a notably high mean area of 74.46 mm², although this finding is based on a single case and should be interpreted cautiously. Other rare variants, including keratotic, fibroepithelioma of Pinkus, and infundibular-cystic subtypes, exhibited relatively small areas ranging from 1.2 to 1.43 mm². The Gorlin syndrome-associated lesion had an average area of 1.55 mm².

For squamous cell carcinoma (SCC), the mean lesion areas were generally larger than most BCC subtypes. Well-differentiated SCCs demonstrated the highest average area, reaching 2.41 mm², while poorly differentiated tumors averaged 1.07 mm². Moderately differentiated SCCs had the smallest mean area among SCC subtypes at 0.77 mm².

A Mann–Whitney U test was also conducted to compare the lesion surface areas between BCC and SCC tumors. The analysis yielded a U statistic of 133.5 with a p-value of 0.60, indicating no statistically significant difference in lesion surface area between the two groups. This suggests that, despite observed differences in average lesion sizes, such as larger surface areas in some well-differentiated SCCs. These differences may be attributed to sample variability rather than a true underlying disparity in tumor size between BCC and SCC in this cohort.

There was a significant difference in lesion area between males and females (U = 6290.0, p = 0.0095) (Fig. 7). While the median lesion area was higher in males (1.0 cm² vs. 0.7 cm²), a rank-based comparison showed a small-to-moderate effect (rank-biserial r = −0.21), likely influenced by high outliers in the female group. A lesion measuring 74.46 mm in a female case was excluded from the analysis to minimize the impact of outliers and ensure that the results more accurately reflect the central tendency and variability within the dataset.

Figure 7. Grouped bar chart showing lesion area distribution by sex

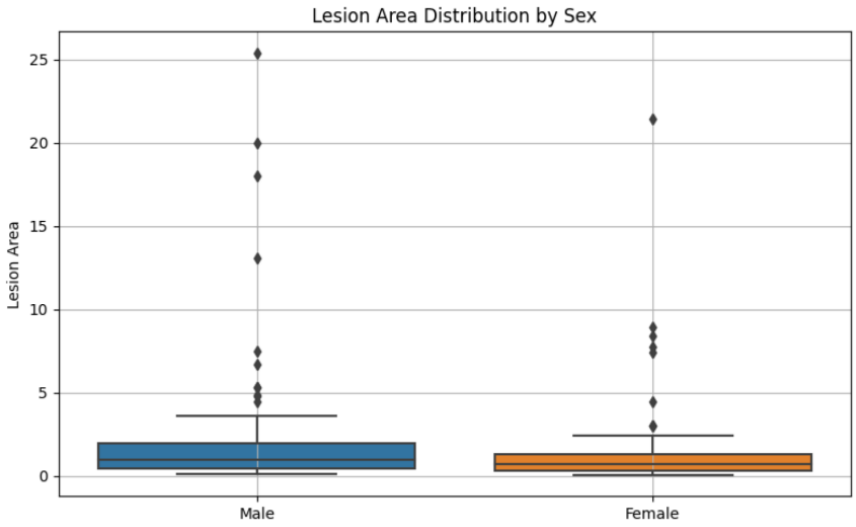

DISCUSSION

Comparing the data we collected with other clinical studies conducted in recent years in different regions of Romania, a consistent national trend in the incidence of non-melanoma skin cancers can be observed. Both a one-year 2022 study from South-Western part of the country (Craiova city) and a five-years 2014 study from the North-Eastern region (Iasi city) support and validates our findings, confirming their reliability and broader applicability. Thus, both studies indicate a prominence of basal cell carcinomas among the identified NMSC cases, with reported proportions of 55% in the 2014 study and 75,5% in the 2022 study [18,19]. Notably, the 75,5% figure closely aligns with our own findings, where BCC accounted for 86% of cases [18]. This may reflect either an increasing trend in the incidence of BCC over time, or differences in exposure to specific risk factors depending on region. Other similarities to our study include the predominance of the nodular subtype of BCC, which was the most frequently encountered variant in both comparative studies [18,19]. In our study, nodular BCC accounted for 40% of cases, closely matching the 40,4% reported in Iasi [19]. Additionally, all three studies identified the face as the most common anatomical site for tumor occurrence, which may support the correlation between UV exposure and lesion development [18,19]. Moreover, a slight female predominance was observed consistently: women represented 52% of cases in our study and 53% in the Craiova one [18]. These very similar findings highlight the potential role of common environmental and behavioral risk factors and support the need for continued epidemiological monitoring in Romania.

The observed seasonal variation, with higher incidence on non-melanoma skin cancers during autumn and lower numbers in spring, is consistent with the findings of previous studies. A 2017 study from North Carolina concluded that the diagnosis rates of BCC and SCC were highest in October and August, so at the end of the festival season, that confirmed the idea of delayed diagnosis after summer UV exposure [20]. According to Bianconi et al., the Umbria region of Italy had also a higher incidence of NMSC during autumn (mostly October), which was explained as a late cancer progression effect of UV exposure [21]. These similarities support the hypothesis that medical consultation is often delayed due to the clinical appearance of suspicious skin lesions following prolonged summer sun exposure. Consequently, many lesions are diagnosed in the following season- autumn- after cumulative sun damage becomes clinically. Given the seasonal pattern of delayed diagnosis, it is crucial to examine potential preventive measures, such as public health campaigns targeting periods of high UV exposure months. These interventions should aim to raise awareness about the risks of sun damage, promote early skin examinations, and encourage timely medical consultations.

The lack of statistical significance regarding the vertical dimension and area may be due to sample size limitations or uneven subtype representation within groups. For example, rare or extreme outliers like the metatypical BCC area (74 mm²) can skew averages and impact variability. Future studies with larger, more balanced samples may provide clearer insight into surface area differences between malignant skin lesion subtypes.

The recent Covid-19 pandemic impacted in a negative way the worldwide healthcare systems, leading to disruptions in routine medical services. As observed in Table I, there were no admissions of dermatology cases in our department between April 2020 and June 2021, which indicates that dermatological services were not operational through this period. There are several existing factors which contributed to the activity disruption. Firstly, the pandemic's health care focus likely caused resource redirection. Secondly, institutional policies mandating restriction consults of any case that is not an emergency severely capped support for patients. Another reason may be the fact that considerable sections of the medically unwell population opted not to attend medical facilities due to the high likelihood of contracting the virus, which adds even more weigh. This local experience aligns with global trends observed during the pandemic. A study analyzing data from the American Academy of Dermatology's DataDerm registry reported a significant decrease in in-person dermatology visits between April 2020 and June 2021, with a corresponding increase in tele-dermatology appointments [22]. However, expanding tele-dermatology services did not ascertain timely diagnosis and prompt treatment for a significant number of patients. Research shows lockdowns led to a diverse range of disruptions that caused cancer diagnoses to be missed on a global scale. The World Health Organization's International Agency for Research on Cancer estimated that about one million cases of cancer went undetected globally during the lockdown because of Covid-19 [23]. This statement emphasizes the need for strategies to maintain essential healthcare services even during public health crises. The increasing number of diagnoses of cutaneous cancer in the post-pandemic period, could stem from the backlog of cases that went undiagnosed when routine dermatological checkups were either postponed or unavailable.

In the present study, a statistically significant difference was observed in the vertical thickness of non-melanoma skin cancers (NMSC) between male and female patients, with mean measurements of 1.5 mm and 1.25 mm, respectively. This disparity, though numerically modest, holds substantial clinical relevance, as increased tumor thickness is associated with a higher risk of local invasion and recurrence.

Several factors may contribute to this gender-based difference in tumor presenta-tion. One of them could be considered the delayed medical consultation among men. Men are generally less likely to engage in preventive healthcare practices. According to a 2009 study, the white middle-class men were the most affected by melanoma because of the delay in seeking medical attention for skin lesions [24]. This tendency may result in the progression of tumors to greater thickness before diagnosis. Another contributing factor to the gender difference seems to be the sun protection behaviors. The American Association for Cancer Research reported in a 6 years study, that women are more likely to adopt behavioral changes as using sun protection measures like including sunscreen and protective clothing, to prevent future cutaneous cancers [25]. They would also limit more the outdoor activity and when outside, they would seek shade more than men [25]. This may contribute to earlier detection and thinner tumors at diagnosis.

Although our study shows a higher incidence of NMSC in urban areas, this finding is most likely explained by the location of the study center- a tertiary hospital in Bucharest. The majority of patients included in the study were residents of Bucharest, which leads to an overrepresentation of urban cases. This does not necessarily reflect a higher exposure to risk factors in urban settings, but rather the geographic bias introduced by easier access to specialized dermatological care in the capital. As shown in a retrospective U.S. study with patients recruited from two primary care practices in one rural Maine regional health system, rural patients often face significant barriers to dermatological evaluation and treatment [26]. That may include longer travel distances, limited availability of specialists and socioeconomic constraints.

Another point to discuss is the predominance of basal cell carcinoma in our studied population, with a higher incidence of the nodular subtype (40% of the BCC cases). These findings are aligning with the worldwide trend, as the BCC is well-known the most frequent skin cancer in adult patients with fair skin, the nodular lesions accounting for more than 60% of all BCCs [27]. Nodular subtype are usually slow growing, non-aggressive tumors, belonging to the indolent-growth subset [10]. Thus, its predominance in the studied population may indicate that most cutaneous neoplasms in fair-skin adults are having a favorable outlook. This may be explained by the commonly less aggressive nature of the nodular variant, or by the fact that they are being detected in an early stage, before becoming invasive. The last one is not a well-supported hypothesis, as we have to emphasize the frequent delay in making a definitive diagnostic due to several contributing factors like the still existing need for access to a good dermatological care for all socioeconomic groups.

Regarding squamous cell carcinoma, our study revealed a greater average vertical dimension of well-differentiated lesions (1,92mm), compared to all the mean depths of the different subtypes of BCCs. The bigger vertical growth of SCC can have potential implications in the tumor outcome, as may be an indicator for the cancer aggressiveness. According to a 2024 literature review, the cutaneous squamous cell carcinoma of the head and neck can present metastasis in approximative 2,5% of patients, most often localized in the level II lymph nodes and the parotid glands [28,29]. Unlike BCC, cSCC can spread both nearby and distant locations, one of the high-risk criteria for the metastasis being represented by a depth invasion greater than 6 mm [28,29]. Although none of the studied lesions were deeper than 6 mm, it is necessary to highlight the importance of correlating depth patterns of cSCCs with their metastatic rates and with the right choice of treatment. The primary method of treatment remains the surgical resection, but the tumor depth and size can dictate the type of therapy need to be used. The vertical dimension of cSCCs influences also the follow-up protocols, as patients with greater tumor depths (so high-risk groups) requires a tighter follow-up [29].

Lastly, although most NMSC cases are treated successfully with local excision, ad-vanced or recurrent head and neck lesions (particularly large or deep infiltrating squamous cell carcinomas) may require complex surgical resections. In such cases, reconstruction becomes critical not only for functional and aesthetic restoration but also for maintaining the patient’s quality of life. Free flaps and other advanced reconstructive techniques, such as the radial forearm or anterolateral thigh flap, offer valuable options. The success of these procedures underscores the importance of surgical expertise and a multidisciplinary approach, as emphasized in recent scoping reviews. [30]

Our study's possible limitations should be taken into account when interpreting the results as they may have introduced bias. Since only 180 patients were chosen from a single tertiary medical center, the study had a relatively small sample size and was carried out over a five-year period. Since it ignores possible variations in clinical, histo-pathological, or demographic characteristics among various populations or geographical areas, this may lower its statistical power and have an impact on the findings' generalizability. Furthermore, the COVID-19 pandemic coincided with a portion of the study, which disrupted data collection and resulted in fewer patients having access to medical services. Clinical activity was interrupted by these contextual factors, resulting in missing or insufficient data. Notwithstanding these drawbacks, our research still provides insightful information.

Nevertheless, we emphasize the very actual need of further prospective studies which should assess the targeted screening programs influence in early cancer detection and diagnosis, and the effectiveness of different treatment strategies in patients with head and neck non-melanoma skin cancers.

CONCLUSIONS

The study identified a progressive increase in the incidence of malignant skin lesions from 2020 to 2023, followed by a decrease in 2024. This trend could be attributed to factors such as the impact of the COVID-19 pandemic on healthcare access and a possible post-pandemic surge in cases. Continued monitoring is essential to validate whether this trend persists in subsequent years.

The analysis revealed a statistically significant variation in skin cancer diagnoses across seasons, with the lowest incidence observed in spring and relatively similar fre-quencies in summer, autumn, and winter.

The face was the most common location for malignant lesions, emphasizing the significant role of sun exposure in the etiology of NMSC. BCC was the most frequent histological subtype, with nodular BCC being particularly prevalent. In contrast, SCC, although less common, demonstrated greater metastatic potential and depth, especially the well-differentiated subtype.

Our analysis revealed statistically significant differences in lesion size between sexes, with both area and vertical dimensions being greater in males compared to fe-males.

The majority of NMSC cases were recorded in urban areas, particularly Bucharest, suggesting a potential link between urban living and higher skin cancer incidence or, conversely, a lack of adequate healthcare access in rural regions. Additionally, while the sex distribution was relatively balanced, males exhibited significantly greater lesion depths compared to females.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, M.M., M.D. and A.C.; methodology, I.A.T. and G.M.; software, A.Z.C.A. and D.V.; validation, M.M., S.R.G. and A.C.; formal analysis, M.D. and I.A.T.; investigation, M.M. and S.R.G.; resources, G.M. and A.Z.C.A.; data curation, M.D. and D.V.; writing—original draft preparation, M.M. and M.D.; writing—review and editing, M.M. and I.A.T.; visualization, G.M. and D.C.; supervision, S.R.G. and D.C.; project administration, A.C. and D.V.; funding acquisition, A.Z.C.A. and D.C. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Carol Davila University of Medicine and Pharmacy (protocol code 16341 from 1st July 2025).

Patient consent for publication

Not applicable, because it is a retrospective study.

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REVIEW

Multiple Intestinal Neuroendocrine Tumors: A Literature Review and Case Report

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Abstract: Neuroendocrine tumors (NETs) are a group of rare malignancies characterized by heterogeneous clinical and biological presentations. Their estimated annual incidence ranges from 3 to 6 cases per million inhabitants, with a notable rise in prevalence due to improved diagnostic capabilities and medical awareness. Gastrointestinal NETs predominantly affect the small intestine (~45%), followed by the rectum, appendix, colon, and stomach. Despite advancements in imaging modalities such as CT, MRI, and somatostatin receptor-based PET-CT scans, the diagnosis of small intestinal NETs remains challenging due to nonspecific symptoms, frequently leading to delayed recognition until advanced disease stages. Surgical resection with comprehensive lymphadenectomy remains the cornerstone of curative management for localized disease, offering improved survival outcomes when R0 resection is achieved. Nevertheless, recurrence rates remain high, underscoring the critical importance of postoperative surveillance. This review provides a comprehensive synthesis of recent literature regarding the epidemiology, clinical manifestations, diagnostic methods—including imaging and immunohistochemical markers—and therapeutic approaches, particularly emphasizing surgical management strategies. A clinical case example is integrated into the review to illustrate the real-world complexity and challenges in the diagnosis, treatment, and follow-up of patients with small intestinal NETs.

Keywords: multiple gastrointestinal neuroendocrine tumors, small intestine, nonspecific abdominal pain

INTRODUCTION

Gastrointestinal neuroendocrine tumors (NETs) are rare, slow-growing tumors characterized by specific histological, biological, and clinical features. These tumors have shown a significant increase in incidence and prevalence over recent decades, reaching an increase of over 100% in the past 40 years, due to advancements in diagnostic methods and increased medical awareness [1-6]. Gastrointestinal NETs represent an important subtype of neuroendocrine tumors, affecting various segments of the gastrointestinal tract, as follows: the small intestine (45%), rectum (20%), appendix (16%), colon (11%), and stomach (7%) [7].

One of the most notable characteristics of these tumors is their predominant setting in the small intestine, particularly in the terminal ileum, approximately 60 cm from the Bauhin valve. This makes them the most frequent malignancy of the small intestine [8]. Small intestinal neuroendocrine tumors are often diagnosed in advanced stages because clinical symptoms are frequently nonspecific and can include abdominal pain, intestinal obstruction, and symptoms caused by carcinoid syndrome [2]. Additionally, these tumors develop distant metastases more frequently than neuroendocrine tumors from other locations, further complicating their clinical management. Mesenteric lymph node metastases are found in over 80% of patients at the time of diagnosis, regardless of the primary tumor's size [9-14]. Imaging techniques such as contrast-enhanced CT, MRI, and somatostatin receptor-based PET scans have improved detection, while immunohistochemical markers such as chromogranin A and synaptophysin aid in confirming the diagnosis.

Histologically, gastrointestinal neuroendocrine tumors are characterized by the presence of neuroendocrine cells that secrete various peptides and biogenic amines. These substances can cause diverse clinical manifestations, including diarrhea, flushing, and wheezing, within the context of carcinoid syndrome [15].

Biologically, the tumors exhibit variable expression of neuroendocrine markers such as chromogranin A and synaptophysin, which are used in the diagnosis and monitoring of the disease [7, 16-18].

Recent studies have highlighted the genetic and molecular diversity of gastrointestinal neuroendocrine tumors. Mutations in the MEN1, DAXX, and ATRX genes are frequently associated with these tumors. The identification of these mutations has allowed for a better understanding of the tumor pathogenesis and opened new perspectives for targeted therapies [8, 19-21].

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The clinical management of patients with gastrointestinal neuroendocrine tumors involves a multidisciplinary approach, including advanced imaging, endoscopic techniques, surgery, and systemic therapies. Surgical intervention remains the treatment of choice for resectable tumors [22], while systemic therapies, including somatostatin analogs, mTOR inhibitors, and radionuclide therapies, are used for advanced or metastatic disease [23-29].

EPIDEMIOLOGY AND DEMOGRAPHICS

Gastrointestinal neuroendocrine tumors (NETs) represent a relatively rare group of malignancies, yet recent literature indicates a significant rise in their incidence worldwide. Small intestinal NETs account for approximately 45% of all gastrointestinal NETs, establishing these tumors as the most prevalent malignancy of the small bowel [1,7]. The estimated global annual incidence ranges between 3 to 6 cases per million inhabitants, with a clear upward trend observed over the last four decades, primarily attributed to improved diagnostic methods and increased physician awareness [2,4].

A comprehensive analysis of epidemiological data from the Surveillance, Epidemiology, and End Results (SEER) database (1977–2016) by Liu et al. (2023) demonstrated that gastrointestinal NETs have exhibited a dramatic increase of over 100% in diagnosed cases during recent decades [2]. This significant upward trend reflects advances in diagnostic imaging modalities and improved endoscopic techniques, facilitating earlier and more accurate diagnosis [2,7].

In line with findings reported by Taal and Visser (2004), small intestinal NETs show notable geographical variation, yet consistently demonstrate an annual incidence ranging from 3 to 6 cases per million inhabitants globally [4]. This consistency suggests a common biological behavior and potentially shared risk factors influencing the occurrence of these tumors [4-5].

Epidemiological patterns also reveal that the diagnosis of small bowel NETs most commonly occurs between the ages of 50 and 70 years, highlighting the importance of clinical suspicion within this age group when nonspecific gastrointestinal symptoms are present. The age distribution emphasizes the need for targeted diagnostic vigilance in middle-aged and older patients presenting with chronic abdominal complaints without an obvious etiology [6-7,12].

In addition, several retrospective studies have documented the high likelihood of delayed diagnosis due to nonspecific symptomatology, often resulting in advanced disease at initial presentation, with a high frequency of mesenteric lymph node involvement and distant metastasis at the time of diagnosis [6-7].

Therefore, awareness of these epidemiological trends, along with improved understanding of the disease's clinical behavior, is crucial for clinicians to achieve earlier diagnosis and intervention, ultimately enhancing patient outcomes.

CLINICAL PRESENTATION AND DIAGNOSIS

The clinical presentation of gastrointestinal neuroendocrine tumors (NETs), especially those of the small intestine, is highly variable and nonspecific, frequently contributing to significant delays in diagnosis. Commonly reported symptoms include chronic abdominal pain, often intermittent and vague, unexplained weight loss, changes in bowel habits such as chronic diarrhea or intermittent constipation, and episodes of intestinal obstruction due to either tumor mass or mesenteric fibrosis [7,25,27].

In certain instances, especially when tumors exhibit secretory activity (functional NETs), clinical presentation may include symptoms associated with the classic carcinoid syndrome. This syndrome occurs predominantly when liver metastases are present, allowing serotonin and other vasoactive substances to enter systemic circulation. Characteristic features include episodic flushing, chronic diarrhea, bronchospasm, and occasionally cardiac valvular lesions [15,29].

The nonspecific clinical picture typically leads to prolonged diagnostic latency, with literature reporting that many cases are incidentally identified during surgeries performed for unrelated reasons, such as intestinal obstructions or abdominal hernias, rather than direct suspicion of NETs [25,27].

Imaging plays a crucial role in diagnosing NETs and determining disease extent. Computed tomography (CT), Magnetic Resonance Imaging (MRI), and somatostatin receptor imaging (68Ga-DOTATATE PET/CT) are the mainstay diagnostic tools. Of these, somatostatin receptor PET-CT (68Ga-DOTATATE PET-CT) has demonstrated superior sensitivity and specificity, especially for detecting small primary tumors and distant metastases compared to conventional imaging methods [3,8].

Histopathological confirmation is indispensable for accurate diagnosis and characterization of NETs. Immunohistochemical markers such as chromogranin A, synaptophysin, and the transcription factor CDX2 are routinely employed and demonstrate high sensitivity and specificity for neuroendocrine differentiation [3,6].

Additionally, the proliferation index assessed by the Ki67 index is crucial in grading these tumors and assessing their malignant potential. The World Health Organization (WHO) classification categorizes NETs as G1 (Ki67 <3%), G2 (Ki67 3-20%), or G3 (Ki67 >20%), directly impacting the choice of therapeutic strategies and prognostic evaluation [6,12,22].

Given the nonspecific nature of the symptoms, the combination of clinical suspicion, advanced imaging modalities, and specific immunohistochemical analyses is paramount to timely diagnosis and improved outcomes for patients with small intestinal NETs.

Surgical management of small intestinal neuroendocrine tumors

Surgical intervention remains the cornerstone of management for localized small intestinal neuroendocrine tumors (SI-NETs). The primary goals of surgical treatment include complete tumor resection (R0 resection), adequate lymph node dissection, and alleviation of symptoms associated with tumor burden or obstruction.

Given that SI-NETs frequently present with multifocal lesions (25-44% of patients), a thorough exploration and palpation of the entire small intestine from the ligament of Treitz to the ileocecal valve is critical to achieve adequate surgical clearance. Consequently, open laparotomy remains widely accepted as the gold standard, as it allows complete assessment and palpation to detect subcentimeter tumors that are often missed by minimally invasive techniques [9,14,23-27,28].

Despite this, several studies have explored the efficacy of minimally invasive approaches such as laparoscopy. Figueiredo et al. (2014) described laparoscopic resection of small-bowel NETs in a cohort of 12 patients, demonstrating comparable outcomes in selected cases. Similarly, Reissman et al. (2013) successfully employed laparoscopic resections in 20 patients with primary midgut carcinoid tumors, emphasizing the feasibility and safety of this method in carefully selected cases. However, both studies highlighted critical limitations, notably the difficulty in detecting sub-centimetric lesions, which may necessitate additional techniques such as hand-assisted laparoscopy or open exploration to achieve complete resection [25-27].

Comprehensive lymphadenectomy is also a fundamental aspect of surgical treatment, as metastases in mesenteric lymph nodes are present in more than 80% of patients at initial diagnosis. Optimal lymph node dissection has demonstrated improved long-term outcomes and decreased recurrence rates. Motz et al. (2018), in a comprehensive retrospective analysis from the National Cancer Database (NCDB), emphasized that thorough lymphadenectomy significantly correlates with improved survival outcomes, particularly when combined with R0 resections [10-11,28].

Following curative surgery, long-term follow-up is essential due to the notably high recurrence rate, estimated around 50% even after achieving an R0 resection. Le Roux et al. (2011), in a large multicenter French retrospective analysis, highlighted that factors such as tumor size, presence of lymph node metastases, and microscopic residual disease significantly increase the risk of recurrence. Regular postoperative surveillance involving biochemical markers, such as chromogranin A, and imaging modalities, including CT and somatostatin receptor PET-CT, is therefore mandatory to ensure early detection of recurrence and prompt therapeutic intervention [29-33].

In summary, optimal surgical management of small bowel NETs requires careful evaluation and meticulous surgical techniques, aiming at complete resection (R0) and adequate lymph node clearance. Given the frequent recurrence even after successful surgery, ongoing surveillance and follow-up are critical components in the comprehensive management of patients with SI-NETs.

Case example: multifocal small intestinal net

A 68-year-old male, with history of right inguinal hernia surgery in 2005 and no significant family medical history, who has been exposed to toxic environment, presents to our department complaining of diffuse abdominal pain amplified in the left lower quadrant, particularly postprandial, anorexia, the presence of a left inguinal pseudotumoral mass, and 15 kg weight loss in the last 3 months.

The symptoms began approximately 2 months before admission, with severe abdominal pain, predominantly in the left lower quadrant, unresponsive to usual analgesics, anorexia, intestinal motility disorders – loose stools in the last 3 weeks, weight loss – 15 kg in 3 months, and 2 episodes of minor upper gastrointestinal bleeding.

The patient was evaluated in another medical center through upper gastrointestinal endoscopy, lower gastrointestinal endoscopy, and no significant findings. Abdominal ultrasound showed a left lower abdominal quadrant mass.

The patient presented to the Surgery Department of “Dr. Carol Davila” University Central Emergency Military Hospital, in an emergency setting, with a left lower quadrant mass, painful, enlarged (7/8 cm), irreducible, with normal overlying skin and a diffusely painful abdomen.

Subsequent investigations were performed. Thoracic-abdominal-pelvic CT scan revealed a left inguinal hernia with a 4.5 cm abdominal wall defect, narrow neck, and larger herniating sac containing omentum and a large bowel loop. There were proximal intestinal obstructive changes and colonic distension with stasis content and air-fluid levels. Additionally, a tumor mass was identified in the intramesenteric mesogastric region, antero-left para-aortic, cranial to the iliac bifurcation, measuring approximately 5/4/3 cm. The mass had a heterogeneous structure with hypodense areas included and no surgical cleavage plane to the loco-regional digestive loop.

The patient underwent emergency surgery, consisting of exploratory laparotomy. Intraoperatively, an intense adhesive process (Figure 1), multiple whitish jejunal tumor masses up to 3 cm in size, with two mesenteric tumoral masses measuring 3 cm and 5 cm in diameter with adherence to neighboring loops and incarcerated left inguinal hernia (Figure 2) were found, without ascites and no peritoneal or liver nodule. Extensive enterectomy with side-to-side entero-enteric anastomosis and non-mesh hernia repair were performed (Figures 3, 4). There were no signs of ischemia in the bowel loop contained within the herniated sac, hence, there was no need for resection. The non-mesh surgical technique was preferred in order to prevent the risk of infection associated with the mesh.

During hospitalization, the patient had a favorable evolution and was discharged on the 7th postoperative day.

Figure 1: Abdominal adhesions

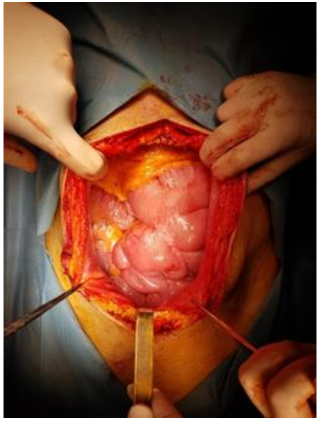

Figure 2: Mesenteric tumoral mass

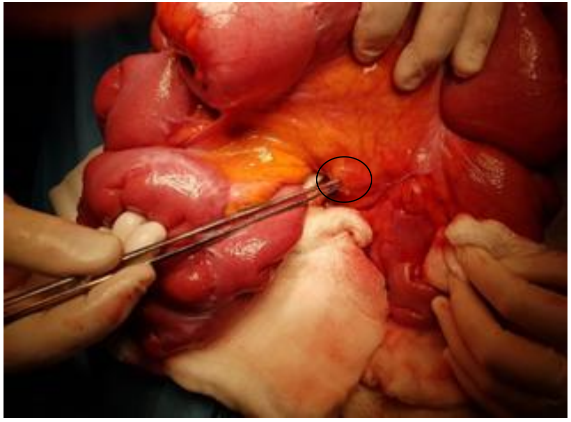

Figure 3: Side-to-side anastomosis

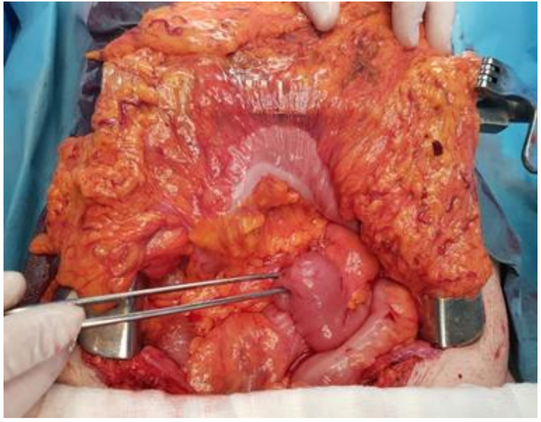

Figure 4: Resected specimen

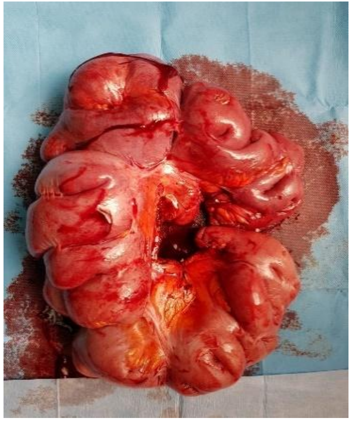

Histopathologic result confirmed the diagnosis of neuroendocrine tumor involving multiple levels of the small intestine with lymph node metastases (3/10) (TNM: pT4 N2 Mx).

The immunohistochemical examination revealed that CDX2 (EPR2764Y) exhibited a uniform positive reaction with both intensity and distribution in the tumor cells, indicating consistent expression throughout the sample. Similarly, CHROMO (LK2H10) showed a uniform positive reaction with intensity, while Synaptophysin (SP11) demonstrated a uniform positive reaction with diffuse intensity in the tumor cells, suggesting widespread expression. Furthermore, Ki67 (30-9) displayed an intense nuclear reaction in 1% of the tumor cells, indicating a low proliferative index within the examined sample. These findings collectively support the diagnosis and provide insights into the tumor's characteristics.

Table 1: Immunohistochemical results

CDX2 (EPR2764Y): Uniform positive reaction with intensity and distribution in tumor cells
CHROMO (LK2H10): Uniform positive reaction with intensity in tumor cells
Synaptophysin (SP11): Uniform positive reaction with intensity, diffuse in tumor cells
Ki67 (30-9): Intense nuclear reaction in 1% of tumor cells

Immunophenotypic data correlated with the morphological cells in the examined fragment are compatible with a neuroendocrine tumor of reduced histological grade (G1).

The patient did not receive chemotherapy, somatostatin analogues, or radiotherapy. He underwent routine follow-ups and regular imaging investigations. At the five-year evaluation, no tumor recurrences or new pathological findings were detected.

DISCUSSION

Primary tumors in the jejunum or ileum are frequently very small, requiring meticulous palpation of the small intestine from the ligament of Treitz to the ileocecal valve. In 25% to 44% of patients, there are multifocal primary tumors, many of which are subcentimeter in size and can only be detected through careful digital palpation [24]. The use of laparoscopy in these cases is controversial, as it may not allow the detection of such small tumors and does not enable the comprehensive palpation of the entire small intestine that is essential for identifying multifocal disease. Therefore, the limitations of laparoscopy must be taken into account when determining the surgical strategy for these patients. The literature contains limited studies on the laparoscopic resection of small bowel neuroendocrine tumors (SBNETs). Figueiredo et al. [25] reported successful laparoscopic resections in 12 patients, while Reissman et al. [26] documented similar success in 20 patients. Wang et al. [27] described successful laparoscopic or minimally invasive resections of ileal neuroendocrine tumors (NETs) in six patients who presented with NETs of unknown primary origin. In their study, the authors highlighted the critical role of palpation during minimally invasive surgery to identify the small, often multifocal primary tumors. To facilitate this, they employed a hand-assisted laparoscopic device or a soft tissue wound retractor to exteriorize the jejunum and ileum. This technique allows for comprehensive palpation, resection of the primary tumors, dissection of mesenteric lymph nodes and associated fibrosis, and subsequent intestinal anastomosis.

Current literature regarding the optimal management of neuroendocrine tumors recommends achieving an R0 resection to increase survival rates. However, an R0 resection is feasible in only 20% of cases due to the typically advanced stage at diagnosis [28]. It is important to highlight that the patient presented as an emergency case with symptoms caused by intestinal obstruction due to an incarcerated inguinal hernia, and not from the intestinal tumors, which were discovered incidentally. Therefore, it cannot be claimed that there was an intent for radical treatment, even though an R0 resection was achieved.

Adjuvant treatment options for neuroendocrine tumors may include chemotherapy, somatostatin analogues, and radiotherapy. However, following curative surgery for small intestinal neuroendocrine tumors, there is no established benefit for adjuvant systemic therapy. Somatostatin analogues are recommended for their antiproliferative properties as a first-line treatment for slowly progressive, well-differentiated metastatic small intestinal neuroendocrine tumors. Additionally, they are used for antisecretory purposes to manage persistent secreting syndromes or refractory diarrhea by inhibiting gastrointestinal secretions [29-31].

The overall survival rate appears favorable when compared to other malignancies. However, achieving reliable survival data is challenging due to variations in terminology, inconsistencies in coding practices, conflicting results from smaller cohort studies, and recent advancements in treatment modalities [32].

Several studies have indicated that the long-term recurrence rate for small intestinal neuroendocrine tumors (SI-NETs) following curative R0 resection, in the absence of distant metastases, is approximately 50%. This high recurrence rate underscores the need for vigilant postoperative surveillance and ongoing management strategies to monitor for disease recurrence. Factors contributing to this recurrence include residual microscopic disease and the inherent biological behavior of SI-NETs. Consequently, patients require comprehensive follow-up protocols, including regular imaging and biochemical assessments, to promptly identify and address any recurrence or progression of the disease [33].

CONCLUSION

The case highlights the importance of considering gastrointestinal neuroendocrine tumors in the differential diagnosis of nonspecific abdominal pain.

Advancements in diagnostic and treatment methods have significantly improved the outlook for many patients with NET, but challenges remain, especially for advanced or poorly differentiated cases.

Although neuroendocrine tumors are rare, their increasing incidence and clinical complexity underline the need for a multidisciplinary approach for effective diagnosis and treatment. Progress in the genetic and molecular understanding of these tumors has opened new avenues for targeted therapies, offering improved hopes for patients.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

MT, BI, JC, HS performed the surgical procedure. MT contributed to the manuscript's conceptualization, methodology, investigation, discussion, review, editing, and supervision. BI contributed to the conceptualization, investigation, data curation, review, and writing of the manuscript. JC contributed to the manuscript's conceptualization, investigation, review, and editing. HS contributed to the editing and review of the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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ORIGINAL ARTICLE

Physiological Demands of Real and Simulated Combat Flight

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Abstract: Combat aircraft pilots operate in highly demanding environments where their expertise and psychophysiological responses impact performance. This study evaluated the acute psychophysiological effects of simulated and real combat flight maneuvers. Twelve Spanish Air Force fighter pilots (mean age = 33.08 ± 5.21 years) participated, averaging 13.25 ± 5.15 years of military service, including deployments in international missions. Various physiological and metabolic variables—isometric hand strength, lower body strength, pulmonary capacity, blood oxygen saturation, urinary hydration levels, cortisol, and blood lactate concentrations— were measured before and after both simulated and real combat flights. Results showed no statistically significant differences between real and simulated flights for most variables, including blood oxygen saturation, lactate, glucose, cortisol, urine color, and physical fitness indicators. However, glucose levels significantly decreased after simulated flights ($p = 0.021$), and horizontal jump performance improved post-real flights ($p = 0.004$). The similarity in physiological responses suggests that simulators effectively replicate real combat conditions, reinforcing their value as a safe, effective training tool. Simulators enhance pilot preparedness while minimizing exposure to combat risks. These findings contribute to aviation psychophysiology, improving training protocols and operational readiness for both military and civilian applications, ultimately optimizing pilot performance and safety in high-risk environments.

Keywords: combat flight, fighter pilots, psychophysiological response, flight simulation, operational training

INTRODUCTION

The operational limits of military personnel are frequently challenged in a range of extreme scenarios, including parachute operations (both High Altitude High Opening [HAHO] and High Altitude Low Opening [HALO]), engagements in symmetrical and asymmetrical warfare, subterranean missions, and close-quarters combat situations. Among these, combat aviation is widely regarded as one of the most physiologically and psychologically taxing environments. Military pilots are exposed to both acute and chronic stressors, such as heightened autonomic nervous system activity, pronounced cardiovascular and hemodynamic responses, and sustained exposure to gravitational accelerations (G-forces), which can exceed 9G during high-intensity maneuvers. These conditions can substantially compromise functional capacity, manifesting in acute loss of consciousness, grey-out episodes, spatial disorientation, and blackouts, even when utilizing oxygen supplementation systems and G-suits. Furthermore, combat aviators must operate at altitudes exceeding 15 kilometers (~50,000 feet), with cabin pressurization and the administration of supplemental oxygen typically required above 7 kilometers (~20,000 feet) to mitigate the dangers associated with hypoxia and decompression illness during tactical missions [1,2,3,4,5].

Exposure to acute psychophysiological stress elicits pronounced sympathetic nervous system activation, increases in anaerobic metabolism—as evidenced by elevated blood lactate concentrations—and disturbances in several cognitive domains, including cortical arousal, memory retention, temporal perception, and perceived exertion. Repeated exposure to such stressors can induce habituation, which is associated with attenuated physiological responses and improved task performance. However, when stress exposure is prolonged and recovery is inadequate, this may result in deleterious long-term effects on both physiological and psychological health. As such, implementing training strategies that closely

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mimic the stressors encountered in combat within a controlled setting is essential to enhance both operational effectiveness and the overall well-being of military personnel and aviators [5,6,7,8].

In this context, simulation-based training has gained increasing recognition as a method to reproduce the multifaceted stressors characteristic of combat environments. In aviation, flight simulators are commonly employed to replicate conditions such as high-G exposure, complex tactical maneuvers, and the cognitive load inherent to aerial combat. These simulated environments enable pilots to adapt to task-specific challenges without facing the direct risks associated with live combat operations. Prior investigations have demonstrated the utility of such simulators in assessing variables like mental workload, spatial orientation, and perceptual illusions under stress-inducing conditions. Nonetheless, despite their widespread implementation, research remains limited regarding the extent to which simulators replicate the full spectrum of psychophysiological responses observed during actual combat aviation [6,7,8,9].

Existing literature examining the physiological demands of real combat aviation has largely focused on variables such as G-force exposure, hypoxia, and the cumulative stress of extended tactical engagement. These studies have documented substantial physiological strain on the respiratory and muscular systems, as well as on metabolic processes, with measurable alterations in biomarkers such as lactate and cortisol. In contrast, flight simulators are engineered to recreate these stressors predominantly at a cognitive level, often falling short of reproducing the mechanical and gravitational stresses encountered in real flight. Although some findings suggest simulators effectively induce mental stress, there is insufficient empirical evidence to support their capacity to fully emulate the psychophysiological impact of actual combat flying [10,11,12].

A comprehensive understanding of the differential psychophysiological responses elicited by real versus simulated combat flight is imperative for refining pilot training regimens and operational planning. Given the considerable implications for pilot health and mission effectiveness, accurate replication of combat-induced stress in training environments is of paramount importance. This study seeks to address a notable gap in the current literature by directly comparing the physiological and metabolic responses of fighter pilots engaged in both real and simulated combat maneuvers. The outcomes of this research have the potential to inform the development of optimized training protocols, thereby enhancing preparedness for the demands of aerial warfare.

Accordingly, the present study hypothesizes that real combat flight induces significantly greater acute psychophysiological stress in pilots compared to simulated flight conditions. This hypothesis is grounded in the premise that, although simulation technologies are capable of replicating many cognitive stressors, they are inherently limited in their ability to reproduce the physical elements of combat aviation, including the effects of G-forces and mechanical loading. Elucidating these distinctions is essential for the advancement of simulation-based training strategies that adequately reflect the realities of combat flight.

MATERIALS AND METHODS

Participants

To achieve the objective of this research we analyzed the physiological response of fighter pilots before and after a simulated and real attack flight maneuver.

A total of 12 fighter pilots (age=33.08±5.21) from the Spanish Air Force participated in this cross-sectional study. Participants had mean military service experience of 13.25 ± 5.15 years, with experience in international missions in the current conflict in Lebanon, Afghanistan, Bosnia, Kosovo, and Iraq. Before participation, the experimental procedures were explained to all the participants, who gave their voluntary written informed consent in accordance with the Declaration of Helsinki. In addition, All the procedures were approved by the University research ethics committee (approval number: 206/219), and all participants agreed and gave written consent to participate in the study. Participants were equipped with the standard flying suit and boots, combat gear composed of parachute harness, life support jacket, and G-suit with a total weight of 10kgs.

Procedures

Pilots were evaluated before and after a real fight mission and a simulated one. Conditions, protocols, and procedures were exactly the same either in simulated or real combat. F5 combat aircraft was used for the real manoeuvres (Figure 1). For the simulated maneuvers, an operational F-5 M (Indra Company, Madrid, Spain) flight simulator was used (Figure 2). All pilots conducted both protocols, and 48 hours of rest between them were assigned. The tactical combat maneuvers were performed in the Air Fighting School of the Spanish Air Force at Talavera de la Real Air Base (Badajoz, Spain), in the middle of April month, between 08:00 and 15:00, with temperatures between 20 and 24 ºC and clear sky. Order and type of protocol were also randomized, in both cases, the protocol consisted of:

In both cases, pilots conducted both maneuvers at altitudes between 8000 and 18,000 ft, with G force between 0.5 and 5.9, with oxygen mix supply over 8000 ft with a combat duration between 30 and 35 min. The offensive maneuver consisted of a fighter aircraft that started at a 1-mile separation and a speed of 400 knots (unit of speed equal to one nautical mile). Both aircraft maintained a sustained rate of 410 knots and 5.5 Gs for 30 to 40 seconds until the target aircraft (referred to as the 'bandit') was within effective gun range. By this point, the bandit had closed the distance to the offensive aircraft, causing the speed to drop to 350–300 knots and the G-load to decrease to 4–4.5 Gs. After the initial bullet stream, a repositioning maneuver could be performed to solve range, aspect, and closure. Speed will decrease to 300 knots and gravity forces to 3G's to return to a control position of effective gun employment. The exercise started at 16000 ft and it progressively decreased to 10,000 ft. In the defensive maneuver, the lead aircraft turned to one side to keep the pursuing offensive aircraft in sight. The exercise began at an altitude of 17,000 feet and a speed of 350 knots. Defensive aircraft executed a break turn at 4 G's slowing down at the same time, looking over the shoulder at the offensive aircraft to assess his pursuit curve. At 300 knots, the defensive aircraft executed turning maneuvers, gradually reducing G-forces from 3.5 to 2 Gs and speed to 200–250 knots. Depending on the progression of the air combat, the defensive aircraft could maintain an airspeed of 350 knots and a G-load of 3.5–4 Gs while descending. Exercise finished at 10000 ft.

Figure 1: F5 combat aircraft

Figure 2: F5 Indra Company (Madrid, Spain) flight simulator

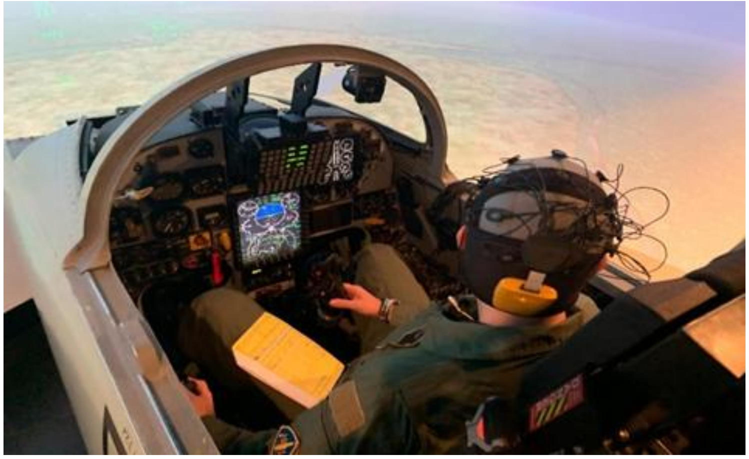

Materials

One hour before and thirty minutes after finishing the air combat maneuvers the following variables were measured:

Lower body muscular strength employing horizontal jump test. Subjects performed a standardized warm-up consisting of 2x10 vertical jumps and then, they performed two maximal horizontal jumps as in the previous report (-), and the best attempt was used for the statistical analysis.

The forced expiratory volume in the first second (FEV1) corresponds to the maximum volume of air exhaled in the first second of the FVC. And mean expiratory flow or FEF25-75, which is between 25% and 75% of the forced expiratory maneuver. Were analyzed using a QM-SP100 (Quirumed, Spain) spirometer in a maximum inhale-exhale cycle.

Urine samples were collected to analyze dehydration levels which were examined by the urine color (UC) chart (color range 1-8; where 1 = very pale-yellow urine, reflected a good level of hydration, and 8 = very dark yellowish-brown, reflected a significant level of dehydration); the number closest to the sample color was recorded.

Isometric hand strength (IHS) by a grip dynamometer (Takei Kiki Koyo, Japan).

Blood oxygen saturation ($SatO_2$) and heart rate (HR) by a pulse oximeter (Pulse Oximeter 30 Beurer Medical).

Lactate and glucose samples were collected by taking a sample of 5 µl of capillary blood from the fingers of the pilots and analyzed by Accutrend Plus (Roche Diagnostics, Switzerland).

Direct Salivary Melatonin ELISA (Bühlmann, Schönenbuch, Switzerland) was used to measure cortisol concentrations.

Statistical analysis

Wilcoxon signed-rank test was used to examine the difference between the pre and post-measures. The baseline was subtracted to post-values to normalize and compare the acute effects of real vs. simulated flight. Effect sizes [r] were calculated for the non-parametric tests which are classified as follows: 0.5 is a large.

RESULTS

The results of the study revealed notable differences between the acute psychophysiological effects of real and simulated combat flights on specific metabolic, respiratory, and physical fitness variables. A significant reduction in glucose levels was observed following simulated combat flights (p = 0.021, effect size = 0.668), while this effect was not present after real flights. This finding suggests that the metabolic demands in simulated conditions may differ from those experienced during real combat maneuvers. Additionally, a significant improvement in horizontal jump performance was found after real flights (p = 0.004, effect size = 0.839), indicating enhanced lower body strength or neuromuscular efficiency induced by real combat flight conditions.

No significant differences were detected in other variables, including blood oxygen saturation, blood lactate levels, cortisol concentrations, urine color, respiratory function (FEV1, FER25, FEF75), or handgrip strength, either between pre- and post-measurements or when comparing real and simulated flight conditions. These results highlight the comparable physiological demands of simulated and real combat flights across most variables, emphasizing the potential of simulation-based training to closely replicate real flight conditions. However, the observed differences in glucose levels and horizontal jump performance underline specific areas where real combat flight may impose unique demands.

These findings suggest that flight simulators are effective tools for replicating many of the psychophysiological demands of combat aviation, while also identifying areas where real combat conditions elicit distinct responses. This information is critical for optimizing training protocols and ensuring pilots are adequately prepared for operational demands.

Table 1: Acute effects of real and simulated combat flight on metabolic, respiratory and physical fitness variables

	Variables	Baseline Mean (SD)	Post Mean (SD)	Baseline vs post measure		Acute effects of a real vs a simulated mission	
				p-value	Effect Size	p-value	Effect Size
Real flight	Blood oxygen saturation (%)	98.58 (0.51)	98.00 (0.74)	.100	0.475	.476	0.205
Simulated flight		98.08 (0.67)	98.08 (0.67)	.527	0.182		
Real flight	Lactate (mmol/l)	1.92 (0.78)	2.86 (3.04)	.367	0.261	.790	0.077
Simulated flight		2.62 (1.78)	3.25 (2.97)	.533	0.180		
Real flight	Glucose (mg/dl)	96.42 (9.57)	96.25 (8.61)	.844	0.056	.084	0.498
Simulated flight		102.75 (14.04)	92.50 (4.74)	.021*	0.668		
Real flight	Cortisol (µg/dl)	9.70 (5.78)	7.56 (6.30)	.158	0.408	.445	0.220
Simulated flight		6.30 (4.83)	6.87 (7.01)	.386	0.250		
Real flight	Urine color (a.u.)	3.75 (1.71)	3.17 (0.94)	.176	0.391	.887	0.044
Simulated flight		3.08 (1.08)	2.58 (1.44)	.092	0.486		
Real flight	Fev1 (l)	5.11 (1.19)	4.81 (0.66)	.450	0.218	.142	0.424
Simulated flight		4.83 (0.68)	4.85 (0.65)	.123	0.445		
Real flight	FER25 (l)	3.92 (0.61)	4.08 (0.44)	.308	0.294	.722	0.103
Simulated flight		4.13 (0.64)	4.16 (0.75)	.260	0.324		
Real flight	FEF75 (l)	10.10 (1.89)	9.72 (2.07)	.477	0.205	1.000	<0.001
Simulated flight		9.98 (1.91)	9.27 (1.99)	.678	0.120		
Real flight	Handgrip (N)	52.62 (6.08)	51.92 (7.43)	.759	0.088	.386	0.250
Simulated flight		50.62 (7.23)	51.09 (5.47)	.858	0.051		
Real flight	Horizontal jump (cm)	141.33 (11.10)	147.42 (12.99)	.004*	0.839	.878	0.044
Simulated flight		137.42 (9.10)	144.09 (10.32)	.055	0.553		

**p-value <0.05*

DISCUSSION

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible.

Future research directions may also be highlighted.

This study aimed to analyze the acute psychophysiological effects of simulated and real combat flights in military pilots. The findings emphasize the utility of flight simulators for training purposes, as no significant differences were observed in most physiological and metabolic variables between simulated and real combat flights. These results align with previous research demonstrating that modern simulators effectively replicate many of the stressors encountered in real-world conditions, such as mental workload and physical demands while avoiding the risks associated with live operations [13,14,15]. The high fidelity of modern simulators in recreating operational environments supports their role as indispensable tools in pilot training.

Despite the overall similarity in physiological responses, certain differences were evident, providing nuanced insights into the unique demands of real combat flights. A significant improvement in horizontal jump performance following real flights was observed, suggesting that real combat maneuvers may elicit greater neuromuscular activation compared to simulated conditions. This response could be linked to the heightened cortical arousal and increased recruitment of fast-twitch muscle fibers required to cope with the physical and cognitive demands of real flight maneuvers. Similar findings have been reported in high-intensity military tasks, such as close-quarter combat and parachute jumps, where lower body strength improves due to acute neuromuscular adaptations to high-stress conditions [16,17,18]. These findings reinforce the hypothesis that real-world stressors induce unique physiological responses that are challenging to replicate entirely in simulated environments.

Interestingly, no significant changes were detected in handgrip strength, highlighting the distinct demands placed on upper body fine motor skills in piloting. Unlike lower body strength, which benefits from gross motor activation, piloting requires sustained precision and control over joystick or yoke movements, which rely on fine motor skills rather than maximal force. Previous research has similarly noted that tasks involving fine motor coordination are less affected by acute fatigue compared to those demanding gross motor output [19,20,21]. These results suggest that while simulators effectively mimic many neuromuscular demands, they may not fully capture the precise motor control required during real flight operations.

The absence of significant changes in blood lactate levels following real flights contrasts with findings in other high-stress combat scenarios, where anaerobic metabolism typically increases due to intense physical exertion [22,23]. This discrepancy may be attributed to the effects of G-forces during real flights, which redistribute blood flow, potentially reducing capillary perfusion at peripheral sampling sites. Simulated flights, lacking such mechanical stressors, exhibited lactate levels closer to the anaerobic threshold, further supporting the influence of G-forces on metabolic responses [24,25,26]. Research on the physiological effects of G-forces in combat aviation highlights their role in altering cardiovascular and metabolic regulation, with implications for pilot performance and endurance [27,28].

Respiratory function, as assessed by spirometry and blood oxygen saturation, showed no significant differences between simulated and real flights. The stability of these variables suggests that the participants' high level of operational experience and training enabled them to maintain respiratory efficiency across both conditions. Previous studies have identified respiratory fatigue as more prevalent among less experienced pilots or during extended operations [29,30]. The lack of observed respiratory impairments in this study underscores the resilience of experienced pilots. However, the reliance on spirometry alone may have limited the scope of respiratory assessments. Advanced techniques such as wearable strain gauge sensors or optoelectronic plethysmography could provide a more detailed understanding of respiratory patterns during flight [31,32].

Cortisol levels remained stable across both flight conditions, a finding that may reflect the effects of habituation associated with extensive operational experience. High-stress environments often elicit significant cortisol responses in less experienced individuals, but research has shown that habituation to stress reduces these responses over time in seasoned personnel [33]. This phenomenon is consistent with studies in other military contexts, where experienced individuals exhibit attenuated psychophysiological responses to acute stressors [34]. The stability of cortisol levels in this study further emphasizes the importance of experience in mitigating stress responses during high-pressure scenarios.

Hydration profiles, assessed through urine colorimetry, did not differ significantly between simulated and real flights. However, the hydration levels observed were close to dehydration thresholds, highlighting the need for improved pre-flight hydration strategies. Dehydration has been shown to impair both physical and cognitive performance, particularly in high-stress environments such as combat aviation [35,36,37]. The use of urine color charts for hydration monitoring has been validated in clinical and operational settings, offering a practical tool for maintaining optimal hydration status among pilots [38]. Addressing hydration deficits could enhance both safety and performance during flight operations.

The results of this study contribute to the growing body of literature emphasizing the effectiveness of flight simulators in replicating the majority of psychophysiological demands associated with real combat flights. However, the observed differences in neuromuscular and metabolic responses highlight specific areas where real flight conditions impose distinct challenges that simulators may not fully replicate. For instance, while simulators effectively mimic the mental workload and basic physiological demands of combat aviation, they may not accurately replicate the physical effects of G-forces or the fine motor requirements of real piloting.

These findings have important implications for the design and implementation of simulation-based training programs. By identifying the limitations of current simulators, training protocols can be refined to better prepare pilots for the unique demands of real combat scenarios. For example, incorporating supplementary training exercises that target neuromuscular activation and motor coordination could address the gaps identified in this study. Additionally, integrating advanced physiological monitoring tools, such as portable biosensors and electroencephalography, could enhance the precision and effectiveness of simulation-based training [39,40,41].

Limitations of the study

This study has several limitations that should be considered when interpreting the results. First, the relatively small sample size (n = 12) limits the generalizability of the findings, particularly given the elite status of the participants as experienced military pilots. Access to larger samples in similar operational contexts remains a challenge, but it would enhance the robustness of the conclusions. Second, the study did not control for gender differences, which may introduce variability in psychophysiological responses. Given the increasing presence of female pilots in military aviation, future studies should aim to include a gender-balanced sample.

Another limitation is the reliance on conventional physiological and metabolic measures, such as spirometry and blood lactate levels, which may not fully capture the complexity of pilot responses during flight. The lack of advanced monitoring techniques, such as electroencephalography (EEG) or respiratory inductive plethysmography, restricts the scope of insights into neuromuscular and respiratory dynamics. Additionally, the study did not evaluate psychological measures such as perceived exertion, stress, or workload, which are critical components of the psychophysiological response in high-stress environments.

Finally, while the study focused on acute effects, it did not assess the cumulative impact of repeated exposure to simulated and real flight conditions. Chronic stress and its potential long-term effects on pilot health and performance warrant further exploration.

Practical applications

Our research on the effects of experience on the findings of this study has direct implications for improving pilot training and operational readiness. The demonstrated effectiveness of flight simulators in replicating many psychophysiological demands of real combat flights supports their continued use as a safe, cost-effective, and practical training tool. Simulators allow pilots to acclimate to combat stressors without exposure to the risks associated with live operations, such as physical injury or equipment failure. These insights reinforce the value of simulators in enhancing pilot preparedness, particularly during the early phases of training.

The observed differences in neuromuscular activation and metabolic responses between real and simulated flights highlight areas where simulation-based training can be refined. Incorporating supplementary neuromuscular exercises, such as lower body strength training, may enhance physical preparedness for real combat scenarios. Additionally, addressing hydration deficits through pre-flight hydration protocols could mitigate the risk of dehydration-related performance decrements.

Moreover, the study underscores the importance of tailoring training protocols to individual pilot characteristics, including experience level and physiological baseline. Advanced monitoring technologies, such as wearable biosensors, could be integrated into training programs to provide real-time feedback on physiological and psychological responses, enabling personalized training adjustments.

Future research lines

Future research should build upon the findings and limitations of this study by addressing several key areas. First, future studies should include larger sample sizes and greater diversity, particularly through the inclusion of female pilots. This approach would improve the generalizability of findings and allow for a deeper exploration of potential gender differences in psychophysiological responses. Additionally, investigations into the cumulative effects of repeated exposure to both simulated and real combat flight conditions are essential. These studies could provide valuable insights into the long-term impact of operational stressors on pilot health, performance, and resilience, areas that remain underexplored.

Advanced physiological and psychological monitoring tools should also be integrated into future research. Techniques such as electroencephalography (EEG), respiratory inductive plethysmography, and portable biosensors could offer a more comprehensive understanding of pilot responses. These tools would enable the assessment of critical parameters, including brain activity, detailed breathing dynamics, and stress biomarkers, allowing for a more nuanced analysis of the interaction between physiological and psychological stressors.

Incorporating psychological measures, such as perceived exertion, workload, stress, and fatigue, will also enhance future studies by providing a holistic view of pilot performance under stress. These measures would complement physiological data and help elucidate the interplay between mental and physical demands in combat aviation. Furthermore, comparing psychophysiological responses between novice and experienced pilots would yield valuable insights into how training and habituation influence operational readiness, offering opportunities to tailor training programs to the specific needs of pilots at different stages of their careers.

Another important area for future research involves enhancing the fidelity of flight simulators. Efforts should focus on better replicating the physical effects of G-forces and other unique stressors encountered during real combat flights. Advanced simulation technologies that integrate neuromuscular and metabolic stressors could bridge the gap between simulated and real flight conditions, thereby improving training effectiveness.

Hydration and nutritional strategies represent another critical avenue for investigation. Tailored interventions to optimize hydration status and metabolic recovery could significantly enhance pilot performance during combat scenarios. Research in this area would address a practical operational need and contribute to the development of evidence-based protocols to support pilot health and performance.

Finally, cross-operational comparisons should be conducted to broaden the scope of research. Investigating psychophysiological responses across different aviation contexts, such as helicopter pilots or unmanned aerial vehicle (UAV) operators, would provide a more comprehensive understanding of aviation-specific stressors and their management. These comparisons would also facilitate the identification of universal stressors and strategies applicable across various aviation roles, contributing to the overall advancement of the field.

CONCLUSION

The present study underscores the essential function of flight simulators within pilot training frameworks, particularly in their capacity to replicate a substantial portion of the psychophysiological demands encountered in actual combat aviation scenarios. Nevertheless, the discrepancies identified in neuromuscular activation patterns and metabolic responses indicate that simulators may fall short in fully emulating the intricate physiological challenges of real-world flight operations. Bridging these limitations through the integration of targeted training interventions and sophisticated monitoring methodologies has the potential to enhance pilot preparedness and physiological resilience, thereby contributing to improved performance and mission safety. These results highlight the necessity of ongoing research aimed at refining training protocols to ensure that pilots are effectively equipped to meet the complex requirements of contemporary aerial warfare.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

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Authors' contribution

Conceptualization, V.J.C.-S., S.V. and J.P.F.-G.; methodology, V.J.C.-S., S.V. and J.P.F.-G.; formal analysis, J.F.T.-A., V.J.C.-S., S.V. and J.P.F.-G.; investigation, J.F.T.-A., V.J.C.-S., S.V., M.A.M.-G. and J.P.F.-G.; resources, J.P.F.-G. and V.J.C.-S.; data curation, J.F.T.-A., V.J.C.-S., S.V., M.A.M.-G. and J.P.F.-G.; writing—original draft, J.F.T.-A.; writing—review & editing, J.P.F.-G. and V.J.C.-S; visualization, M.A.M.-G.: supervision, V.J.C.-S. and J J.P.F.-G.; project administration, J.P.F.-G.; funding acquisition, J.P.F.-G. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the University of Extremadura (approval number: 206/219: July 24, 2019).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

Development and Implementation of Checklist Management in Manned Space Medical Rescue Transfer and Handover

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Abstract: Background Medical rescue transfer and handover in manned spaceflight is a critical component of space rescue operations, directly impacting the safety and well-being of astronauts. Objective This study aims to develop a comprehensive checklist for the medical rescue transfer and handover processes associated with manned spaceflight, thereby enhancing the efficiency of these procedures. Methods The checklist was designed and implemented during practical training sessions focused on manned space medical rescue. Participants were divided into two groups: one utilizing the checklist for transfer handover and another relying on verbal communication. Key metrics such as transfer time, quality of handover, and satisfaction scores were collected and analyzed for both groups. Results The group employing the checklist demonstrated significantly improved outcomes compared to the verbal group in terms of transfer time, quality of handover, and overall satisfaction scores ($P < 0.001$). Conclusion The implementation of checklist management has been shown to enhance both the efficiency and quality of transportation and handover processes, establishing a robust foundation for successfully executing manned space medical rescue missions.

Keywords: Manned Space Medical Rescue; Transfer; Handover; Checklist Management

INTRODUCTION

China's manned space program has advanced to the stage of conducting missions involving a space station, resulting in significantly extended durations for astronauts' stays. These missions are increasingly arduous and complex, which elevates the likelihood of accidental injuries and illnesses [1,2,3]. Space medical rescue is a critical component in ensuring astronaut safety and constitutes an essential aspect of manned space system engineering [4,5]. The efficiency and quality of transfer and handover during medical rescue directly impact the stability and prognosis of astronauts. Research indicates that standardized transfer handovers can reduce handover time, enhance accuracy and efficiency, and decrease the occurrence of adverse events. Checklist management has emerged as an effective strategy to mitigate errors arising from oversight; it offers advantages such as rapid comprehension, straightforward implementation, and precise handovers [6,7]. Currently, checklist management has been successfully employed in emergency evacuation and transfer protocols by foreign Marine Corps units, yielding positive outcomes in reducing adverse incidents while improving the quality of transfers and handovers [8].

This study aims to implement checklist management within nursing transfer processes during aerospace medical rescue training. A statistical analysis will be conducted on the time taken for these transfers, the quality achieved therein, as well as satisfaction levels among nursing staff compared with traditional oral handovers. This approach seeks to provide insights into methodologies for enhancing both the quality and efficiency of aerospace medical rescue operations alongside nursing transfers and handovers.

One population that has been identified as vulnerable to acquiring HIV infection is military personnel, due to frequently reported high-risk sexual behaviour [9,10] or to their high mobility and exposure to biologically foreign environments [11,12].

MATERIALS AND METHODS

1. Design of transfer handover list

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By convening a panel of eight experienced experts in the field of space medicine, we developed a comprehensive checklist for manned space rescue, transfer, and handover procedures. In designing the content, we prioritized the timeliness of first aid interventions prior to astronauts exiting their cabin. This checklist encompasses critical information including the arrival time of medical rescue personnel, the timing of astronaut cabin egress, on-site emergency treatment duration, astronaut evacuation timing, as well as vital signs monitoring and condition changes throughout the evacuation process [8]. As illustrated in Table 1, the handover list is categorized into four dimensions comprising fifteen items:

(1) Basic Information: This section includes data such as astronaut identification number, gender, age, exit time from the cabin, and entry time into the medical transport vehicle.

(2) Preparation Before Transport: This dimension covers essential vital signs of astronauts prior to transport along with safety measures for astronauts themselves; it also addresses preparations by medical staff and necessary equipment.

(3) Observation During Transport: Key observations during transportation include monitoring astronauts' vital signs—such as consciousness level, blood pressure readings, heart rate fluctuations, oxygen saturation levels—and noting any sudden changes in their condition.

(4) Transfer and Handover: The final category consists of data regarding consciousness levels and vital signs at transfer points; skin status assessments; past medical history including allergies; venous access details; artificial airway management; and medications administered during transit.

The signature section was duly signed off by both nursing staff involved in pre-cabin treatment and transport as well as receiving nursing personnel at the destination hospital. Additionally, precise records were maintained detailing each step of an astronaut's transfer to a ward down to minute-level accuracy. The transfer handover documentation exists in duplicate copies that are stored separately to serve as formal handover references.

Table 1: Astronaut transfer handover record sheet

<table>
<tr><td colspan="6">Astronaut transfer handover record sheet</td></tr>
<tr><td>Basic Information</td><td colspan="5">Number: Gender: Age: Astronaut out time: Into the medical carrier time:</td></tr>
<tr><td rowspan="4">Preparation</td><td colspan="5">Personnel Preparation: Doctor ☐ Nurse ☐ Departure time:</td></tr>
<tr><td colspan="5">Vital Signs: T: ℃; P: times/min; R: times/min; BP: mmHg; SPO_2: %</td></tr>
<tr><td colspan="5">Safety Protection:
Head and Neck Fixator ☐ Limb Protection ☐ Transport Equipment Good Performance Mouth ☐</td></tr>
<tr><td colspan="5">Equipment:
ECG Monitoring ☐ Oxygen Bottle ☐ Defibrillator ☐ Sputum Suction Equipment ☐ First-aid Case ☐</td></tr>
<tr><td rowspan="4">In transit (Observation)</td><td colspan="5">Vital Signs: T: ℃; P: times/min; R: times/min; BP: mmHg; SPO_2: %</td></tr>
<tr><td colspan="5">State of consciousness:
Awake ☐ Somnolence ☐ Lethargy ☐ Light Coma ☐ Deep Coma ☐ Delirium ☐ Agitation ☐</td></tr>
<tr><td colspan="5">Pupil Condition: Left ( ) Right ( )
Light reflex: Sensitive ☐ Slow ☐ Absent ☐</td></tr>
<tr><td>Changes in Condition</td><td colspan="4">No ☐ Yes ☐</td></tr>
<tr><td rowspan="3">Transfer & Handover</td><td colspan="5">Vital Signs: T: ℃; P: times/min; R: times/min; BP: mmHg; SPO_2: %</td></tr>
<tr><td>Past history</td><td colspan="2">No ☐ Yes ☐</td><td>History of Allergies</td><td>No ☐ Yes ☐</td></tr>
<tr><td>Pipe line</td><td>Artificial Airway</td><td colspan="3">No☐ Oxygen Tube ☐ Mask ☐ Oropharyngeal Airway ☐</td></tr>
</table>

			Tracheotomy ☐ Tracheal intubation ☐ Depth of intubation: Ventilator parameters:
		Venous Channel	No ☐ Yes ☐ Indwelling Needle ☐ Bone Marrow Puncture ☐ Site: Puncture Time:
		General	No ☐ Abdominal Cavity Drainage Tube ☐ Thoracic Cavity Drainage Tube ☐ Others:
			Catheterization Time: Leakage Length: Pipe Clamp End: Yes ☐ No ☐
	Medication	Special Drugs	Yes ☐ No ☐ Name/Dose：
		General Drugs	Yes ☐ No ☐ Name/Dose：
	Skin	No ☐ Yes ☐ Site:	

Signature of Pre-cabin Emergency Nurse: Signature of Post-cabin Hospital Nurse: Handover Completion Time:

2. Training of transfer handover list

After the completion of the design for the transfer and handover checklist, nursing staff training was conducted by the organizers in accordance with expert consensus on astronaut treatment before the re-entry module of the landing site of space station mission[4]. This training also adhered to the Standard Operating Procedure (SOP) for emergency medical care for astronauts[9,10,11], as well as operational specifications pertaining to medical care and support during space station missions[12,13]. Methods such as group discussions and scenario simulation demonstrations were employed to elucidate each item on the checklist systematically. The effectiveness of this training was assessed through on-site questioning, ensuring that participants comprehensively understood each item and minimizing potential errors in documentation.

3. Group study on the transfer handover list

To implement the transfer and handover checklist in manned space medical rescue training, a study was conducted with two groups: a control group utilizing traditional oral handover methods and an experimental group employing the checklist handover approach. Each group consisted of 15 participants. The metrics evaluated included transfer preparation time, transfer handover time, and total transfer time.

Transfer preparation time refers to the duration required for the simulated astronaut to enter the transport carrier, during which the medical team counts personnel, consumables, and equipment while confirming both the condition of the injured individual and the status of all equipment. Transfer and handover time encompasses the period needed for the simulated astronaut to exit from the transport carrier and be transported to the astronaut ward. This phase includes transferring essential information such as basic details, vital signs, level of consciousness, and any changes in condition between pre-cabin rescue nursing staff and posterior support hospital nursing staff.Total transit time is defined as the cumulative sum of both transit preparation time and transit handover time.

4.Evaluation

The handover process with the posterior branch hospital was systematically evaluated by the training team leader during each transfer. The quality of handover for both the experimental group and the control group was assessed based on three key components: preparation prior to transfer, the actual transfer and handover process, and comprehension of the patient's disease status. Each component was assigned a score ranging from 1 to 3 points, where 3 points indicated excellent performance, 2 points denoted good performance, and 1 point represented fair performance.

Concurrently, a satisfaction questionnaire was distributed to nursing staff at the backward branch hospital to gauge their satisfaction regarding the transfer and handover process across six dimensions: vital signs monitoring, level of consciousness, changes in condition, venous access management along with other pipelines, medication administration, and skin conditions. Each aspect received a score between 1 (minimum) and 10 (maximum).

5.Statistical analysis

All data were analyzed using IBM SPSS Statistics 24 . Measurement data that conformed to a normal distribution were presented as mean(M) ± standard(SD) deviation. Count data were expressed in terms of frequency (percentage). The comparison of normally distributed measurement data between the two groups was conducted using an independent samples t-test.

RESULTS

1. Transit time

The transfer time was significantly reduced in the experimental group compared to the control group. In the experimental group, the transfer preparation time averaged 13.27±1.10 minutes, while the transfer handover time was 9.73±1.67 minutes, resulting in a total transfer time of 23.00±1.89 minutes. Conversely, in the control group, the average transfer preparation time was 18.13±2.07 minutes and the transfer handover time was 14.80±1.97 minutes, leading to a total transport time of 32.93±2.84 minutes. The difference between these two groups was statistically significant (P <0.001). Detailed results are presented in Table 2.

Table 2: Comparison of transit times in different groups

Groups	Number	Transit Preparation Time (min)	Transfer Handover Time (min)	Total Transit Time (min)
Control group	15	18.13±2.07	14.80±1.97	32.93±2.84
Experimental group	15	13.27±1.10	9.73±1.67	23.00±1.89
T		10.21	13.65	15.1
P		<0.001	<0.001	<0.001

2. Quality of transfer handover

The scores of the experimental group surpassed those of the control group across three dimensions: preparation prior to transfer, the transfer and handover process, and mastery of disease status. As illustrated in Table 3, the mean scores for preparation before transfer, transfer handover process, and mastery of disease status in the experimental group were 3.00±0.00, 2.53±0.51, and 2.60±0.50, respectively. In contrast, the corresponding scores for the control group were 2.40±0.13, 1.67±0.49, and 1.60±0.63. The differences between these two groups were statistically significant (P < 0.001).

Table 3: The quality of transfer handover was compared in different groups

Groups	Number	Prepare Before Transfer	Transfer Handover Process	Mastery of Disease Status
Control group	15	2.40±0.13	1.67±0.49	1.60±0.63
Experimental group	15	3.00±0.00	2.53±0.51	2.60±0.50
T		-4.58	-6.5	-5.12
P		<0.001	<0.001	<0.001

3.Satisfaction

Table 4 presents the satisfaction survey scores of medical staff at the posterior branch hospital. The satisfaction scores for the experimental group regarding vital signs, state of consciousness, condition changes, venous access and various pipelines, medication, and skin conditions are as follows: 9.83 ± 0.27, 9.41 ± 0.37, 9.56 ± 0.38, 9.41 ± 0.37, 9.66 ± 0.38, and 9.67 ± 0.38 respectively. In contrast, the control group's satisfaction scores in these same categories were: vital signs (8.73 ± 0.45), state of consciousness (8.57 ± 0.39), disease changes (8.75), venous access and each pipeline (8.63 ± 0.46), medication (8.79 ± 0 .41), and skin conditions (8 .59± 0 .50). The results indicate that the experimental group exhibited significantly higher satisfaction levels compared to the control group (P < 0 .001).

Table4: Comparison of disability among soldiers with different reasons

Groups	Number	Vital Signs	State of Consciousness	Changes in Condition	Pipe Line	Medication	Skin
Control group	15	8.73±0.45	8.57±0.39	8.75±0.41	8.63±0.46	8.79±0.29	8.59±0.50
Experimental group	15	9.83±0.27	9.41±0.37	9.56±0.38	9.41±0.37	9.66±0.38	9.67±0.38
T		-9.39	-8.82	-8.84	-6.24	-8.56	-7.63
P		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

DISCUSSION

To ensure the life and health of astronauts is fundamental to manned space projects. A review of the history of manned space flight worldwide reveals a number of accidents that have resulted in significant losses. In the United States, there have been 19 incidents and failures during manned space flights, leading to 18 fatalities. The former Soviet Union conducted numerous manned space missions, which also resulted in injuries among astronauts, including six deaths [14,15]. To date, all of China's manned space missions have been successfully completed without any serious injuries. As our country embarks on a new phase with its space station program, the frequency of manned launch missions is increasing, while records for in-orbit flight durations continue to be extended. This rise introduces unforeseen factors and maintains an ongoing risk of accidental injury to astronauts. In the event of such an accident, medical rescue serves as the final safeguard for astronaut safety.

The transfer and handover process is a critical component of aerospace medical rescue, significantly influencing the prognosis of injured individuals and patients. The occurrence of adverse events during transfer and handover is closely associated with various factors, including equipment issues, patient conditions, communication barriers, and other challenges faced by medical personnel [16,17]. Implementing an appropriate transfer and handover checklist can enhance communication efficiency among medical staff, enabling them to quickly and effectively comprehend the patient's condition while minimizing the risk of overlooking essential information. This ultimately ensures the safety of patients during their transfer [18,19].

By utilizing a standardized checklist for transfer handovers that delineates key points and critical content in written form, a unified standard is established. This approach improves both the accuracy and efficiency of the transfer process while preventing omissions of vital items. Consequently, it facilitates evidence-based practices in transfer handovers and safeguards patient safety throughout this procedure [20]. Research indicates that employing a checklist for handovers can reduce the incidence of adverse events during transfers from 13.8% to 5.4%, thereby significantly enhancing both quality and efficiency in these processes [21].

Checklist management was implemented in the transfer and handover process of aerospace medical rescue training. Utilizing a transfer and handover checklist, each item was marked off systematically, resulting in a rapid and concise documentation process with accurate and clear recorded content. The critical links that are often overlooked during handovers were consolidated into a standardized format to mitigate the risks associated with oral communication, thereby reducing the likelihood of omissions or repetitions. This approach not only saves time during transfers but also ensures that medical personnel at receiving hospitals have comprehensive access to vital signs and overall injury assessments of simulated casualties throughout the entire evacuation process [22]. The findings from this study indicated that the checklist-based handover group (experimental group) outperformed the oral handover group (control group) significantly across two evaluation metrics: transfer handover time and quality. Furthermore, medical staff at the receiving hospital expressed markedly higher satisfaction regarding six key aspects of the transfer process compared to their counterparts in the oral handover group (control group).

The practice of aerospace medical rescue training has demonstrated that checklist management offers several advantages:

(1) Ensuring the accuracy of transfer and handover information. Traditional oral handovers often suffer from inaccuracies in information transmission, ambiguity regarding key content, omissions of critical disease data, and sometimes deficiencies in the objectivity, accuracy, and completeness of the conveyed information. These unclear handovers and existing shortcomings directly impact the quality of care following transfer. By utilizing a tabular format, a nursing transfer and handover checklist can effectively reflect the basic situation and condition of simulated astronauts in a simple, comprehensive, and intuitive manner. This checklist provides complete information—including vital signs, illness details, rescue efforts, and nursing considerations—for medical personnel at posterior support hospitals. It serves as both a reference point for handover tasks and contributes to making the process more standardized and evidence-based.

(2) Improving the quality and efficiency of transfers and handovers. The checklist facilitates an item-by-item review according to its contents during handover sessions; this approach significantly reduces omissions related to key content or data due to limitations in energy levels or memory capacity among medical staff—factors exacerbated by misunderstandings or time constraints. Consequently, it shortens overall handover duration while substantially enhancing its quality. Research findings indicate that groups utilizing checklists for handovers performed significantly better than those relying on oral communication concerning both transfer times and overall quality of transfers. Following implementation of checklist-based procedures for handing over responsibilities, there is clear evidence that both efficiency and quality are markedly improved while ensuring safety and timeliness throughout the process.

(3) To ensure the safety of astronaut transportation, space medical rescue is critical to both the life and safety of astronauts as well as the overall success or failure of the mission. In the event that an astronaut sustains an accidental injury, there are heightened risks associated with the transfer process. The implementation of a checklist handover serves as a safe nursing practice designed to facilitate effective communication before, during, and after the transfer based on a predetermined transfer handover list [23]. The self-designed transfer handover checklist plays a vital role in conducting risk assessments prior to transfer, thoroughly evaluating timing considerations, and emphasizing careful monitoring of conditions along with emergency interventions throughout the transfer process. Upon safely reaching their destination for transfer, this checklist is utilized once more to convey essential information

regarding vital signs, consciousness levels, venous access status, various drainage tubes in place, medication administered, and any changes in condition experienced by the astronauts. This systematic approach proves beneficial for medical personnel at receiving facilities by enabling them to swiftly assess conditions and execute necessary procedures effectively.

CONCLUSION

With the successful completion of the inaugural in-orbit rotation involving the astronaut teams of Shenzhou 14 and Shenzhou 15, China's space station has transitioned into a long-term operational mode. Consequently, space medical rescue and support missions will now be conducted under standard working conditions. This study demonstrates that effective checklist management can enhance both the efficiency and quality of the transfer handover process. The objective of this approach is to normalize, standardize, and systematize the procedures for space medical rescue transfers and handovers, thereby establishing a robust foundation for the successful execution of astronaut medical support and rescue operations.

Conflicts of interest and sources of funding

The authors declare no conflict of interest and this research received no external funding.

Authors' contribution

The initial draft was prepared and revised by JL, while GH, PS, XG, and LD contributed to the collection of information and the preparation of the dataset. YW further refined the analyses and provided essential support for the manuscript. LS and YT supervised all aspects of the study, including the design of the data analysis plan, oversight of the data analysis process, validation of results, and writing of the manuscript based on the first draft. All authors participated in reviewing and finalizing the manuscript; furthermore, all interested parties have read and approved it.

Ethics approval and consent to participate

This study does not consider pertinent ethical issues related to individuals.

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ORIGINAL ARTICLE

Investigating Digital Skills of Healthcare Professionals in Greek Military and Civil Hospitals: A Cross-Sectional Study

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Abstract: Background and Aim: Digital skills of healthcare professionals are recognized as critical for the successful digital transformation of healthcare. The present study aims to examine the relationship between the digital skills of healthcare professionals and the organizational structures of hospitals. Methods: In this cross-sectional study, using the convenience sampling method, 857 healthcare professionals, working in Greek military and civilian hospitals, voluntarily participated. Data were collected between July and November 2021 through a structured questionnaire, based on the Digital Competence framework. Digital skills were categorized into five subscales: information, communication, content creation, problem solving, and safety skills. Statistical analysis was performed with SPSS 26.0, with p-values <0.05 considered statistically significant. Results: Cronbach's alpha values for all subscales were (α)>0.8, indicating high reliability. Younger participants showed higher scores (p<0.001). The mean scores for information, communication, content creation, problem solving and safety skills in military hospitals were calculated as 52.1 (SD=32.3), 50.6 (SD=31.8), 48.4 (SD=33.3), 49.8 (SD =30.8) and 52.8 (SD=34.1) respectively, while in civilian hospitals they were calculated as 45.1 (SD=32.2), 44.5 (SD=29.5), 41.8 (SD=32.4), 41.5 (SD=30.8) and 47.1 (SD=34.5) respectively. Conclusions: Healthcare professionals in military hospitals exhibit higher digital competence compared to their counterparts in civilian hospitals. Identifying the organizational factors that promote greater digital competence among healthcare professionals provides essential information to policymakers and healthcare managers for implementing targeted organizational improvements.

Keywords: Digital skills, Healthcare professionals, Military hospitals, Digital Competence framework

INTRODUCTION

The management of competencies and skills in the healthcare sector has drawn significant attention from international organizations, recognizing that a well-trained workforce is essential for ensuring patient safety and delivering high-quality care [1,2]. In alignment with the continuous advancements in medical technology, the necessity for healthcare professionals to develop new skills has become increasingly urgent [3,4]. This need was starkly highlighted during the recent COVID-19 pandemic, where the heightened digital demands of healthcare services underscored the importance of sufficient digital capabilities [5,6].

In particular, digital skills are deemed critical not only for the effective management of healthcare organizations but also for medical diagnosis, treatment monitoring, patient care, and decision-making processes [7,8]. Within the healthcare domain, digital competence encompasses knowledge of digital literacy and technology as essential tools for the patient-centered provision of quality healthcare services. It reflects the dynamic interaction between healthcare professionals, patients, and colleagues, as well as the drive to acquire professional expertise in digital technology [9,10].

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It is widely recognized that digital competencies are essential for the effective integration of health technologies into routine clinical practice. Numerous processes are now carried out digitally, such as patient monitoring, where electronic health records and vital sign monitoring systems enable doctors and nurses to track patient status in real time. Material procurement is also facilitated through electronic systems, enhancing both accuracy and efficiency. Furthermore, telemedicine plays a pivotal role, particularly in military hospitals, by enabling the delivery of healthcare services to remote areas and military bases. Digital tools further support decision-making by allowing healthcare professionals to analyze data and make evidence-based decisions more efficiently [3,11,12].

In Greece, military hospitals offer more structured training programs for digital skills, supported by military frameworks that emphasize continuous professional development of their personnel. These programs encompass modules on the management of digital tools, telemedicine, and electronic patient record systems, reflecting the military's long-standing tradition of structured training and lifelong education. Additionally, military healthcare professionals are often encouraged to engage in e-learning programs to further enhance their expertise in healthcare technologies.

Conversely, in public hospitals, training programs for digital skills vary depending on the institution and the extent of technological integration. Significant advancements have been observed in large hospitals that have adopted electronic medical records; however, training opportunities remain limited due to resource constraints and time pressures. Healthcare professionals, including nurses and doctors, typically receive training through Ministry of Health initiatives or European-funded programs such as eHealth and Horizon 2020 [9,10].

Despite extensive research on digital skills, a literature review from the past decade indicates a scarcity of studies that examine the digital competencies of healthcare professionals within a specific and well-defined conceptual framework. One such framework is the European Digital Competence (DigComp) Framework, created by the European Union as a common umbrella of digital competences for its citizens, which defines digital competence domains, indicators for each domain, competence levels, examples of knowledge, skills, and attitudes, as well as examples of application [13]. Moreover, limited research has explored the associations between digital skills and the organizational work environment. To the best of our knowledge, no prior studies have investigated differences in this domain between military and civilian healthcare professionals.

Therefore, the objective of this paper is to address this research gap by examining the relationship between digital skills of healthcare professionals and the organizational structures of their work environments. We aim to contribute evidence on the organizational factors influencing healthcare professionals' digital competencies, which can inform policymakers and healthcare managers' efforts to implement targeted organizational improvements.

MATERIALS AND METHODS

Study Design

A cross-sectional survey utilizing a descriptive study design was conducted based on prior research, which demonstrated that the Digital Competence Indicator tool is a reliable and valid measurement scale applicable to Greek doctors and nurses [14]. The study addressed two primary research questions: (1) How are healthcare professionals' digital skills assessed? and (2) Are there differences in the digital skills of healthcare professionals between military and civilian hospitals?

The present study was conducted as part of the research activities of the Department of Nursing at the Hellenic Mediterranean University and received ethical approval from the Review Committee (Protocol No.: 4634/29-03-2021). Additionally, the necessary permissions were obtained from the Scientific Committees of all participating hospitals (Protocol No.: 5584/1973/26-06-2021). Data were collected using a self-assessment questionnaire, with the principal investigator maintaining sole access to the collected data. Participation was entirely voluntary, and all participants were informed both verbally and via an information/consent form that completing and returning the questionnaire constituted their consent to participate in the study. The information/consent form was approved by the above Scientific Committees. The research adhered strictly to the European Union's General Data Protection Regulation, ensuring full compliance with personal data protection standards.

Participants and settings

A convenience sample of doctors and registered nurses was selected from seven tertiary care hospitals in Greece. This particular sampling method was preferred because it offers time and cost savings, ease of access, and an abundance of qualitative data. However, it carries the risk of bias and systematic errors, and therefore, research findings cannot be accurately generalized beyond the study sample. The selection of hospitals was based on the diversity of organizational structures between military and civilian institutions, as well as the aim of achieving a large sample size. The study population size was calculated using a 95% confidence interval and a 5% margin of error, ensuring that the final sample accurately represented healthcare professionals in Greek hospitals. The target sample size was N>800. A total of 1207 questionnaires were distributed, and 876 were collected, of which 19 were invalid due to incomplete responses. Thus, the study population is N=857.

The inclusion criteria for participation were employment in either a military or civilian hospital, professional status as a doctor or nurse, and, for nurses, registration as per the European Qualifications Framework (EQF). The distribution of questionnaires was proportional to the size of each hospital, based on the number of medical and nursing staff within the institutions. Data collection occurred during staff meetings, where participants from various work positions within the hospitals were approached, provided with explanations of the study objectives, and given clarifications on completing the questionnaire. Data collection was conducted between July and November 2021.

Research tool

Data were collected using a validated 37-item paper-based questionnaire. It is built on the European DigComp Framework, which comprehensively defines the elements of digital competence as a combination of skills, abilities and attitudes essential for incorporating data processing into the professional careers of healthcare providers [13,15].

The questionnaire consisted of two parts. The first part captured demographic characteristics, including gender, age, educational level, profession, specialty, hospital type, professional experience, administrative responsibilities, and possession of digital skills certification. The second part assessed 26 digital skills, organized into five competency domains (subscales): information, communication, content creation, problem-solving, and safety skills (Table 1).

Table 1: Competence area and digital skills of the questionnaire. Adapted from the European Commission's DigComp framework [13]

Competence Area	Digital Skills
Information Skills	1. Finding information about goods and services
	2. Obtaining information from public authority websites
	3. Reading or downloading online news/newspapers/news magazines
	4. Copying or moving a file or folder
	5. Seeking health-related information
Communication Skills	6. Sending/receiving emails
	7. Telephoning over the internet/video calls (via webcam) over the internet
	8. Participating in social networks
	9. Posting messages to chat sites
	10. Uploading self-created content to any website to be shared
Content Creation Skills	11. Creating websites or blogs
	12. Writing a computer programme using a specialized programming language
	13. Using copy and paste tools to duplicate or move information within a document
	14. Creating electronic presentations with presentation software (e.g., slides), including e.g., images, sound, video, or charts
	15. Using basic arithmetic formulae to add, subtract, multiply, or divide figures in a spreadsheet
Problem-Solving Skills	16. Connecting and installing new devices
	17. Installing a new or replacing an old operating system
	18. Modifying or verifying the configuration parameters of software applications
	19. Doing an online course
	20. Buying or ordering goods or services for private use (last 12 months) over the internet
	21. Selling online
	22. Job search or sending an application
	23. Internet banking
	24. Making an appointment with a practitioner via a website
Safety Skills	25. Using any kind of IT security software or tool (anti-virus, anti-spam, firewall, etc.) in order to protect private computers and data
	26. Updating one or more security products at least occasionally

Subscale values were calculated on a scale ranging from 0 to 100, with scores approaching 0 reflecting a more negative evaluation of the variable and scores approaching 100 reflecting a more positive assessment.

The questionnaire provided a concise yet compelling assessment of the Information Technology (IT) skills of hospital doctors and nurses. Designed for ease of use and brevity, it was particularly suitable for hospital settings often characterized by high workloads and limited time for activities outside core medical and nursing responsibilities. Participants rated the extent of their computer usage for each activity on a five-point Likert scale, ranging from 1 ("not at all") to 5 ("very much").

Data Analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) version 26.0. Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables were presented as absolute numbers (n) and percentages (n%). The normality of variables was assessed using the Shapiro-Wilks test, alongside graphical methods such as the Normal Q-Q plot, Detrended Normal Q-Q plot, and Box Plot. For all statistical tests, differences were considered significant at $p < 0.05$. Effect sizes weren't reported. The independent t-test was employed to compare the means of two groups, while one-way analysis of variance (ANOVA) was used for comparisons involving more than two group means.

RESULTS

Participant's characteristics

The study population comprised 857 Greek healthcare professionals employed across seven tertiary care hospitals. The results indicate that 538 participants (62.8%) were female, while 319 (37.2%) were male, with a mean age of 39.7 years (SD = 10.0). Of the total, 657 participants worked in military hospitals, while 200 worked in civilian hospitals. Indicatively, it is reported that the number of military doctors and nurses in hospitals, at the present time, amounts to approximately 1,700 people.

Specifically, among the military hospitals, 384 participants were from the 251 Hellenic Air Force General Hospital, 110 from the 401 General Military Hospital of Athens, 66 from the Athens Naval Hospital, and 97 from the General Military Training Hospital. Among civilian hospitals, 82 participants were from the Sismanoglio General Hospital of Attika, 51 from the Asklepieio Voulas General Hospital, and 67 from the Henry Dunant Hospital Center.

The majority of participants were registered nurses (70.2%), with doctors comprising 29.8% of the sample. Considering the nature of the study population, where nurses outnumber doctors by more than twice, the preponderance of the female gender can be explained by the fact that the nursing profession in Greece has traditionally been considered female-dominated for many years. The average professional experience was estimated at 15.3 years. Regarding educational levels, 528 participants (61.6%) held a Bachelor of Science (BSc) degree, 275 (32.1%) had also completed a Master's degree, and 54 (6.3%) held a PhD degree. Examining the level of education by profession, doctors have a higher proportion of PhD holders (5.4%) than nurses (0.9%). Conversely, nurses have a higher percentage of master's degree holders (22.8%) than doctors (9.3%). The demographic characteristics of the study population are summarized in Table 2.

Table 2: Demographic characteristics of participants (N = 857).

		Hospital				**Total**		
		Civilian hospital		***Military hospital***				
		N	N %	N	N %	N	N %	p-value
Age (years)*		40.6 (9.9)		39.4 (10)		39.7 (10)		0.143
Gender	Female	134	67.0%	404	61.5%	538	62.8%	0.158
	Male	66	33.0%	253	38.5%	319	37.2%	
Educational level	BSc	136	68.0%	392	59.7%	528	61.6%	0.092
	MSc	52	26.0%	223	33.9%	275	32.1%	
	PhD	12	6.0%	42	6.4%	54	6.3%	
Profession	Medical Doctor	53	26.5%	202	30.7%	255	29.8%	0.250
	Registered Nurse	147	73.5%	455	69.3%	602	70.2%	

**Note. Age expressed as mean (standard deviation)*

Digital Skills

Regarding digital skills, the results are overall. The mean scores of all digital skills subscales indicate an average level and are as follows: information skills, 52.1 (SD = 32.3); communication skills, 50.6 (SD = 31.8); content creation skills, 48.4 (SD = 33.3); problem-solving skills, 49.8 (SD = 30.8); and safety skills, 52.8 (SD = 34.1). Median scores, interquartile ranges (IQR), and p-values of the above subscales are presented in Table 3.

The reliability of the subscales was confirmed, with Cronbach's alpha values indicating high internal consistency: information skills (5 items; $\alpha = 0.95$), communication skills (5 items; $\alpha = 0.95$), content creation skills (5 items; $\alpha = 0.95$), problem-solving skills (9 items; $\alpha = 0.97$) and safety skills (2 items; $\alpha = 0.92$). Cronbach's alpha was calculated by group. It is worth noting that the high Cronbach's alpha values (>0.9) observed in the study results are likely due to the narrowness of the aspect structure of the measurements' tool subscales. However, the measurement scale was taken as an existing structure from the European DigComp Framework and was not developed by the researchers. Future survey to explore the factor structure by removing some potentially redundant items or adding new ones is a challenge for the present investigators.

Table 3: Median scores, IQR, and p-values of subscales (information skills, communication skills, content creation skills, problem-solving skills, safety skills) per type of hospital

		Hospital		
		Civilian hospital	**Military hospital**	**Total**
Information skills	Median	45.00	60.00	55.00
	IQR	63.75	60.00	60.00
	p-value	<0.001		
Communication skills	Median	40.00	55.00	50.00
	IQR	50.00	65.00	60.00
	p-value	0.002		
Content creation skills	Median	40.00	50.00	50.00
	IQR	60.00	67.50	65.00
	p-value	0.001		
Problem-solving skills	Median	30.60	52.80	50.00
	IQR	58.30	52.80	55.60
	p-value	<0.001		
Safety skills	Median	37.50	62.50	62.50
	IQR	71.88	62.50	62.50
	p-value	0.007		

The mean scores across all five subscales of the measurement tool were found to differ significantly between military and civilian hospitals. Specifically, the mean score for the "Information skills" subscale in military hospitals was M = 54.2 (SD = 32.1), compared to M = 45.1 (SD = 32.2) in civilian hospitals, with t(855) = -3.522, $p < 0.001$. For the "Communication skills" subscale, the mean score in military hospitals was M = 52.4 (SD = 32.3), while in civilian hospitals it was M = 44.5 (SD = 29.5), with t(855) = -3.088, p = 0.002.

Similarly, the "Content creation skills" subscale showed a mean score of M = 50.4 (SD = 33.3) in military hospitals versus M = 41.8 (SD = 32.4) in civilian hospitals, with t(855) = -3.236, p = 0.001. For "Problem-solving skills," the mean score in military hospitals was M = 52.4 (SD = 30.4), compared to M = 41.5 (SD = 30.8) in civilian hospitals, with t(855) = -4.395, $p < 0.001$. Lastly, the "Safety skills" subscale yielded a mean score of M = 54.6 (SD = 33.8) in military hospitals and M = 47.1 (SD = 34.5) in civilian hospitals, with t(855) = -2.740, p = 0.006.

The mean values (SD) of digital skills (information, communication, content creation, problem-solving, and safety skills) by hospital type are illustrated in Figure 1.

DISCUSSION

Based on the theoretical framework outlined, the findings of this study align with existing research emphasizing the growing importance of digital skills for healthcare professionals. Digital competence is essential not only for the effective delivery of healthcare services but also for ensuring patient safety and enhancing the overall efficiency of healthcare systems [16,17]. These competencies enable healthcare professionals to manage patient data effectively, navigate electronic health records [18,19], process large volumes of information[20], and utilize telemedicine tools [21,22]—capabilities that are no longer optional, but essential and fundamental to healthcare professionals' expertise [10,23,24].

In the present study, digital skills of the Greek study population can be characterized as good, as they are proven to be above the median level, with safety and information skills gathering the highest averages, communication and problem-solving skills being at the median level, and content creation skills being the weakest, with a score just below the median level. It is noteworthy that content creation skills require particular IT knowledge and technical skills, such as developing or maintaining computer systems, hardware, or software, and writing a computer program using a specialized programming language. Thus, they appear as the digital skills category with the lowest proficiency rates in the majority of occupations in Europe [25].

The above levels of digital skills are in line with a similar Greek study, where the digital competence of healthcare professionals is moderate to good [26]. Research conducted on healthcare professionals in European countries shows that the majority have medium levels of digital competence. A study carried out in Moldova found advanced levels of digital skills in the areas of information and communication and moderate levels in the areas of content creation, safety, and problem solving[6]. In Finland, the levels of digital competence are relatively high[27], while in some regions in Finland and Sweden, digital skills are reported as adequate but with several shortcomings[16] and in Catalonia, they appear at a basic and intermediate level [28]. According to the European Centre for the Development of Vocational Training (Cedefop), in terms of digital intensity measurements in the EU for 2021, the level of digital skills for health professionals appears as basic to intermediate, and only in a percentage of 13.4% as advanced [29].

Figure 1: Clustered Bar Mean (95% CI) of Information skills, Mean of Communication skills, Mean of Content creation software skills, Mean of Problem-solving skills, Mean of Safety skills by Hospital (Error Bars: 95% CI).

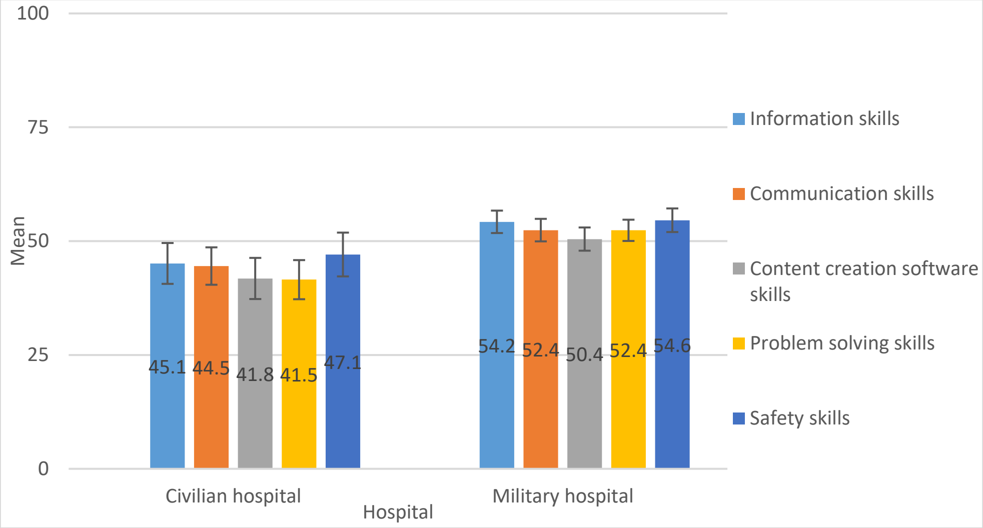

Consistent with prior research, this study also revealed that younger healthcare professionals demonstrated higher levels of digital competence, particularly in domains such as communication and content creation. Literature suggests that younger professionals tend to exhibit greater eHealth literacy and are more adept at using digital tools [30,31]. This generational disparity in digital skills underscores the need for targeted interventions that provide older healthcare professionals with the necessary training and support to enhance their digital proficiency. Hospital management can organize continuing education programs that are easily accessible and tailored explicitly to older professionals, such as online courses, interactive sessions, or specially designed workplace courses.

It is worth noting that, in the measurement tool, all possible demographic confounding factors were included to avoid biased conclusions. According to the literature review, the demographic factors found to be associated with digital skills levels were age, type of occupation, gender, professional experience, and educational level, which were studied in our research [30,32,33]. However, the results of this study found no significant differences in digital skills based on gender, professional experience, specialty, or educational level.

In addition, research underscores the critical role of the working environment in shaping digital literacy. For instance, De Veer and Francke (2010) observed that attitudes toward electronic health records varied significantly depending on the type of healthcare organization [11]. Similarly, Jarva et al. (2023) as well as Rippen et al. (2013), emphasized that strong organizational support—through timely interventions and the provision of adequate resources—is essential for the successful adoption of digital technologies by healthcare professionals [9,34]. Similarly, the findings of the present survey revealed significant differences in digital skills between military and civilian hospitals, with the predominance of health professionals in military hospitals.

This superiority can be attributed to several factors, including the structured nature of military institutions, where digital training and protocols are more systematically integrated into daily operations [35,36]. Military hospitals often place a stronger emphasis on technical proficiency and operational readiness [37,38], which likely fosters the development of higher digital literacy among their staff. Moreover, they prioritize digital training for their personnel as part of their operational strategies and compliance with applicable protocols. In contrast, civilian hospitals may face a broader array of challenges, including inconsistent administrative policies and slower technology adoption due to resource constraints [35,39].

In Greece, in recent years, the Ministry of Digital Governance, in coordination with the Ministry of Health and other bodies, has been working to strengthen the digital competence of doctors and nurses through the implementation of a national e-health strategy. Among the objectives are to improve the continuous education of health professionals and to ensure access to reliable digital information and tools [40]. However, training programs for civilian health professionals are usually their personal responsibility, as organizational procedures are inadequate. In contrast, in military environments, such programs are supported by the respective military education sectors, financed with their own resources and means, and organized on a regular and periodic basis for the training of all their personnel.

The operation of military hospitals, with adherence to hierarchical procedures, is a significant factor in their ability to implement and train staff in digital technologies efficiently. These institutions often adopt new systems at a faster pace, which may explain the consistently higher levels of digital proficiency observed among healthcare personnel in military settings. Such an organizational advantage aligns with the findings of Ludwick and Doucette (2009), who emphasized that organizational readiness and robust support systems are critical to the successful implementation of electronic health records [41].

Furthermore, the constant exposure to technology [36,42] and digital tools [43,44] observed in military settings provides healthcare professionals with more opportunities to enhance their digital skills. In contrast, civilian hospitals face a variety of challenges that may hinder the development of such competencies. In Greece, these challenges include diverse administrative structures, slower decision-making processes, and limited access to resources for digital training [45,46]. The inherent flexibility within civilian hospital operations may further contribute to inconsistencies in the application and development of digital skills among healthcare professionals. This discrepancy could reflect differences in organizational priorities and the availability of technological resources within these institutions.

A noteworthy area where military healthcare professionals excel is in safety skills, likely due to the stringent data protection and cybersecurity protocols mandated within military environments [47,48]. This focus on safeguarding sensitive patient and operational data, cultivates a more advanced understanding of cybersecurity measures among military hospital staff. Although civilian hospitals also prioritize data security, they often encounter greater challenges in implementing uniform protocols due to variations in organizational structure and resource constraints.

Another significant disparity lies in problem-solving skills. Military healthcare professionals appear to be better equipped to handle technical challenges, potentially due to the emphasis on problem-solving as an integral part of military training and operational effectiveness [49]. In contrast, the less regimented procedures commonly found in civilian hospital settings may provide healthcare professionals with fewer structured opportunities to develop and refine these skills.

We consider that the Ministry of Health or the EU frameworks could adopt military-style training modules emphasizing techniques such as extensive practical training, simulation exercises, the development of specialized centers of expertise, field training techniques, the use of existing training frameworks, needs- and context-based design, continuous harmonization with modern standards, and synergies between civilian and military professionals. One such model is the Common Security and Defence Policy (CSDP) Training Programme, an EU policy to provide senior civil and military officers with the necessary skills and knowledge in crisis management situations [50].

Future Implications

We believe that civil-military cooperation, by embracing strengths and avoiding weaknesses, could improve the overall digital capability of health professionals. As military hospitals provide a more conducive environment for developing digital skills, civilian hospitals could benefit from adopting some of the structured approaches used in military hospitals. With the continuous evolution of healthcare technology, it is essential that all healthcare professionals, irrespective of their work environment, are supported in acquiring and maintaining high levels of digital competence.

Digital skills among healthcare professionals represent a burgeoning field of research that has experienced rapid growth over the past decade. The findings of this study highlight the need to explore further the role of institutional support in fostering digital skills, as well as the factors contributing to digital competence gaps among healthcare professionals across different organizational settings. These results hold international relevance, given the global significance of digital transformation in the healthcare sector. With additional research conducted in similar settings across various countries, these findings could be generalized to further advance digital literacy and transformation in healthcare systems worldwide.

Limitations

This study is subject to three key limitations that may affect the generalizability of the findings. First, the participating nurses were all registered nurses (European Qualifications Framework - EQF 6) and not practicing nurses (EQF 5), and therefore, the measurement tool is validated and reliable only for registered nurses. Second, a self-assessment questionnaire was used as a measurement tool, which entails the risk of subjectivity due to overestimation or underestimation of the actual skills of the participants. Finally, the convenience sampling approach carries the risk of bias and systematic errors.

CONCLUSION

This study underscores the critical importance of digital skills among healthcare professionals, drawing attention to notable differences between staff in military and civilian hospitals. The findings indicate that military healthcare professionals generally demonstrate higher levels of digital competence, likely attributable to the structured and resource-rich environments characteristic of military hospitals. These organizational factors, which foster a more digitally skilled workforce, serve as a foundation for identifying and implementing necessary organizational improvements that healthcare managers can leverage.

However, it is important to note that both military and civilian healthcare professionals exhibited moderate levels of digital skills overall, signifying substantial room for improvement across both settings. Strengthening digital competence across the healthcare sector remains essential for meeting the demands of modern healthcare systems.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, A.K. and C.M.; methodology A.K. and C.M.; software, A.K. and C.C.; validation, A.K. and C.M.; formal analysis, C.C. and V.A.K.; investigation, A.K., D.K., V.A.K., M.M., V.T. and C.M.; resources, A.K. and C.M.; data curation, A.K., D.K., C.C. and C.M.; writing—original draft preparation, A.K.; writing—review and editing, A.K., D.K., C.C., V.A.K., M.M., V.T. and C.M.; visualization, A.K.; supervision, C.M., D.K. and C.C.; project administration, A.K., C.C. and C.M. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Hellenic Mediterranean University Ethics Committee (Nursing Department's Executive Board 4634/29-03-2021).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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CLINICAL PRACTICE

Death due to a rare posttraumatic complication: fat embolism

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Abstract: *Fat embolism is a rare complication of high or medium intensity trauma. It is caused by the formation of fat particles in the territories of small terminal circulation, especially at pulmonary, tegumentary and cerebral levels. The mechanism underlying the fat embolism is described by several different theories in literature. In legal medicine, fat embolism raises many controversies upon the diagnostic certainty of the thanatogenerator mechanism leading to death. The occurrence of fat embolism syndrome followed by death must be properly explained so that the fatality can be adequately framed from a legal point of view. In this paper the authors present three cases in which victims of trauma died due to clinically undiagnosed fat embolism, the diagnosis being established only by postmortem histopathological examination. The authors underline the fact that the diagnosis of fat embolism syndrome, although it is a clinical one, it is often established only during autopsy.*

Keywords: *fat embolism, trauma, death, autopsy, diagnosis*

INTRODUCTION

Embolism represents the motion through circulatory blood flow of a material which is not present in blood in normal conditions and its subsequent lodging inside the blood vessels. In the case of fat embolism, the material carried through the blood is represented by fat particles that reach the level of microcirculation. Fat embolism syndrome represents the systemic manifestation of the fat particles being present inside the microcirculation.

Fat embolism was first described by Zenker in 1862 in the case of a railroad worker who died of crushing injuries. In 1873 Bergmann diagnosed fat embolism in a living patient who had suffered a fracture of the femoral bone [1, 2].

The causes of fat emboli formation are multiple, both traumatic and non-traumatic. The most frequent traumatic causes are: long bone fractures, orthopedic procedures, soft tissue trauma (fat tissue laceration), liposuction and mastectomy, neurosurgical interventions. Non-traumatic causes include: acute pancreatitis, extensive burns, diabetes, fatty liver due to diets rich in extrinsic fats and oils [2, 3]. Exceptionally, fat embolism has been observed in cases of hepatic steatosis (in alcoholics and diabetic patients with hepatic damage) due to the destruction of hepatocytes and subsequent release of fat particles [4, 5].

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Fat embolism occurs in 90% of patients who have suffered a trauma, but only 2-5% of them develop the fat embolism syndrome; particularly at risk are those with fractures of the long bones. It is difficult to estimate the duration of this syndrome because the symptoms are often subacute or masked by other symptoms of trauma [2, 3].

Post-mortem studies have shown a different but a very high incidence of fat embolism. Thus, a study by Behn C. et al. on 527 autopsies demonstrated the presence of fat embolism in 92 of these [6]. Another study conducted by Hiss et al. in 1996, on 53 victims of aggression followed by death, showed a high incidence of fat embolism. All the subjects studied were young, male, victims of aggressions, suffering severe trauma 24 hours before death. In thirty two cases the presence of fat embolism was observed in the main organs, with no other obvious cause of death. The authors of the study hypothesized that the source of fat embolism was the mechanical disintegration of the subcutaneous adipose tissue [7].

In cases of fat embolism there is a poor correlation between post-mortem and clinical findings. This discrepancy ultimately gave rise to the concept of "iceberg effect of fat embolism" [8- 12]. This justifies, as well as cases with a insufficiently outlined clinical picture for a particular pathology, or a clinical picture that cannot explain the occurrence of death, the importance of forensic autopsy and postmortem laboratory investigations (histopathological and toxicological) in order to establish the thanatogenerator mechanism and the causal link between trauma / illness and death, and ultimately the legal nature of the case, i.e. suicide, accident or homicide [13- 19].

MATERIALS AND METHODS

We present three cases of trauma in which the cause of death was the fat embolism syndrome. All the cases are characterized by a rapid clinical deterioration leading to death, so that a clinical diagnosis of fat embolism syndrome could not be established antemortem.

Case no. 1

Male, 72 years old, chronic alcohol consumer, with past medical history of left femoral fracture and right sided craniotomy, is admitted to hospital for head trauma. At the time of admission physical examination revealed multiple ecchymoses, pain at the pelvic level, lower limbs and abdomen. Clinical and paraclinical investigations established the following diagnosis: minor craniocerebral trauma, facial trauma, posttraumatic subarachnoid hemorrhage, dura mater hygroma, abdominal contusion, pelvic contusion. Surgical intervention was performed to evacuate the dura mater hygroma 6 days after the admission. Evolution was initially favorable. On the 4th postoperative day, the patient developed a febrile syndrome. Laboratory analysis highlighted: elevated leucocyte count (13.390 of which 11.270 neutrofils); elevated serum amylase (225 U/L); lowered erythrocyte count (3.83 milion); hematocrit levels as low as 40.08 %; low thrombocytes count (137.700); increased level of the liver enzyme AST to 48 U/L; low creatinine serum levels (0.69 mg/dl) and reduced glomerular filtration rate to 85 mil/min/1.73 m2. The next day the patient suffered cardiorespiratory arrest that did not respond to resuscitation maneuvers, followed by death. The forensic autopsy was performed. The external examination of the body showed: multiple injuries denoting violence such as ecchymoses and abrasions; signs of medical treatment at the level of the skull, i.e. recent surgical incision and an old whitish scar. The internal examination showed: on the head, 2 craniotomy areas, one of them being recent, subarachnoid hemorrhage, areas of flaccid cerebral structure, small petechial hemorrhagic areas disseminated throughout the brain. The examination of the lungs showed: pulmonary stasis and edema, and areas of pulmonary condensation. Fragments of brain and lungs were harvested during autopsy and subsequently examined by optic microscopy using the Sudan III staining. The microscopic examination revealed fat emboli in the brain and lungs. Following the postmortem macroscopic and microscopic examination we established that the cause of death was the fat embolism syndrome.

Case no. 2

Male, 54 years old, was admitted to the hospital with the diagnosis of: head trauma (subarachnoid hemorrhage, fracture of the left temporal bone with extension to the mastoid bone), face trauma (fracture of right jaw, comminuted fracture of the nose) and chest trauma (left pneumothorax, multiple rib fractures).

The patient was hospitalized for 35 days, during which multiple pleurotomies and bronchoscopies were performed. Laboratory examinations showed: increased leucocyte count (25.170 of which 21.100 neutrofils); low red blood cell count (2.612 milions); low hematocrit (21.240 %); low hemoglobin level (7.793 g/dl); normal thrombocyte count; increased level of AST (103 U/L); increased serum creatinine (4.28 mg/dl); increased GGT levels (93 U/L) and reduced glomerular filtration rate (10.8 mil/min/1.73 m2). Clinical evolution was initially favorable. On day 34 after admission the patient developed a feverish syndrome. The next day he suddenly suffered cardiorespiratory arrest, followed by death. A forensic autopsy was performed. The external examination of the cadaver revealed bruises and signs of recent medical treatment. The internal examination showed: linear head fracture, subdural hematoma under partial resorption, small petechial hemorrhagic areas in the brain, pulmonary edema, and areas of pulmonary condensation. Fragments of organs were harvested (brain, lung) and examined by optic microscopy using Sudan III staining. Following the macroscopic and microscopic examinations we concluded that the cause of death was the fat embolism syndrome.

Case no. 3

Male, 20 years old, victim of a traffic accident, was admitted to the hospital with the diagnosis of: polytrauma due to road traffic accident, major craniocerebral trauma with diffused cerebral edema, multiple rib fractures, fracture of the left iliac wing and left ischial bones, fracture of the left femoral neck, and type III A fracture of the femoral diafisis. Patient was admitted in the hospital for 7 days and underwent a surgical intervention for the femoral fracture. During the first days after the surgery the patient was hemodynamically stable. On day 4 the patient showed tachypnea, tachycardia, and fever and his clinical condition suddenly worsen. The laboratory examinations showed: low leucocyte count (1.56 of which 1.37 neutrofils); reduced numbers of erythrocytes (2.31 milions); low hematocrit (23.3%); low hemoglobin (6.77 g/dl); reduced number of thrombocytes (58.600/ mmc); increase in liver enzyme AST (272 U/L) and ALT (250 U/L); increased serum creatinine (2.43 mg/dl); increased serum urea (94.16 mg/dl) and reduction of glomerular filtration rate to 27.21 mil/min/1.73 m2. Death occurred on the same day. At autopsy, the external examination of the corpse revealed: multiple ecchymoses, abrasions, hematomas, signs of medical treatment. The internal examination showed: brain contusion; multiple rib fractures; pulmonary edema, pulmonary condensation areas, pulmonary infarction; fractures of the pelvis and of the left femor. The microscopic examination of the fragments of brain and lungs collected during the autopsy and stained with Sudan III showed fat embolism. Therefore, based on macroscopic autopsy findings and histo-pathological examination of the brain and lung fragments, we established that the cause of death was the fat embolism syndrome.

RESULTS

In all the three cases the victims suffered various trauma: in the first case the victim suffered soft tissues and head injuries, in the second case the victim suffered a polytrauma with multiple fractures and soft tissue injuries and in the third case the victim suffered craniocerebral trauma, soft tissue injuries and long bone fractures. Therefore, in all the cases, the etiological conditions for the fat embolism occurrence were met. However, in none of the cases the diagnosis was clinically established antemortem. The examination by optic microscopy of the fragments of brain and lungs collected during autopsy and stained with Sudan III showed in all the cases the fat microemboli in the form of orange globes disseminated throughout the fields examined microscopically (figures 1-4) and allowed the postmortem diagnosis of fat embolism. Therefore, in all three cases presented, the results of the histopathological examination of the fragments harvested from the brain and the lungs during the autopsy were essential for determining the cause of death.

Figure 1: Microscopic aspect of brain tissue Sudan III stain x 40 (ice exam)

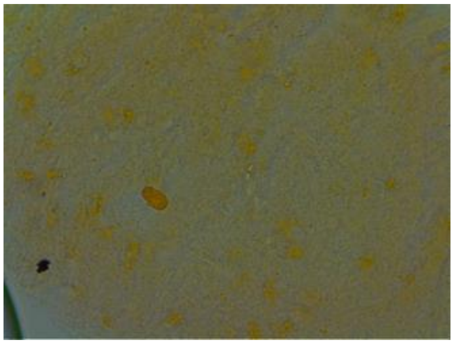

Figure 2: Microscopic aspect of lung tissue Sudan III stain x 20 (ice exam)

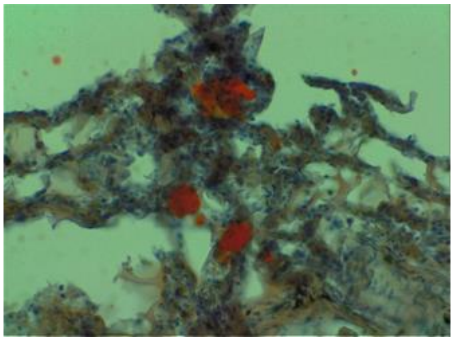

Figure 3: Microscopic aspect of lung tissue Sudan III stain x 40 (ice exam)

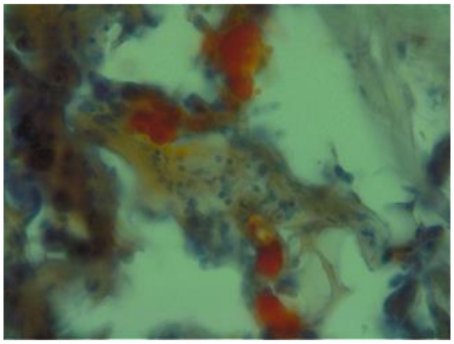

Figure 4: Microscopic aspect of lung tissue Sudan III stain x 10 (ice exam)

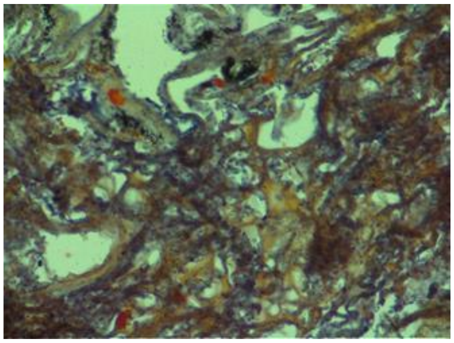

DISCUSSION

Fat embolism occurs due to the mobilization of fat cells or fat released by them, followed by their direct penetration into open blood vessels or lymphatic channels. Most emboli have a diameter of 15 to 40 µ; the fat emboli in the lungs are generally larger than those in the large arteries of the systemic circulation [11].

Several theories are mentioned in the literature to explain the pathogenesis of the fat embolism [20].

The mechanical theory shows that mobilization of fat particles occurs due to trauma to the bones or soft tissues. As a consequence, the fat particles released by the trauma enter the venous circulation from where they reach the lungs, where the majority remains, but some of them can also pass into the systemic circulation, reaching other organs.

The biochemical theory, also known as the emulsion instability theory, is mostly useful in explaining the non traumatic fat embolism.

The intravascular coagulation theory holds that in stressing conditions, substances that activate disseminated intra-vascular coagulation and aggregation of fat particles are released.

The toxic trauma theory claims that small blood vessels are affected by high concentrations of fatty acids in the plasma leading to increased vascular permeability.

The mechanical theory is based on the concept of the mobilization of fatty bone marrow from the long bones diaphysis. In the cases presented by us, only one patient suffered a recent fracture of the femur. In the other two cases we have to consider the trauma of soft tissues (hypodermic tissue) as the cause of the fat embolism, by noting that in both cases multiple ecchymoses were observed on the skin.

From a clinical point of view, Gurd and Wilson have developed the major and minor criteria which allow which allow for the diagnosis of fat embolism. The major criteria are: axillary or subconjunctival petechiae; hypoxaemia ($PaO2 <60$ mm Hg, $FIO2 = 0$); central nervous system depression disproportionate to hypoxaemia; pulmonary oedema; radiological signs and symptoms of respiratory insufficiency; cerebral changes unrelated to a cranial trauma or other illness; rash. The minor criteria are: tachycardia more than 110 bpm; pyrexia more than 38.5°C; fat globules present in urine; changes in renal function (reduced urine output); drop in haemoglobin level (more than 20% of the value upon admission); drop in haematocrit values; drop in platelet values (more than 50% of the value upon admission); increased erythrocyte sedimentation rate (greater than 71 mm per hour); fat globules present in the sputum; emboli present in the retina; tachicardia; fever; renal insufficiency; sudden thrombocytopenia. According to Gurd and Wilson the diagnosis of fat embolism can be established if either one major criterion and 4 minor criteria or 2 major criteria are met [21].

Lindeque suggested that the criteria of Gurd and Wilson may under-diagnose the syndrome, and proposed the following criteria based on respiratory parameters: Pao2 kPa of less than 8Fio2 0.21; Paco2 kPa or pH of less than 7.3 or more than 7.3; respiratory rate greater than 35 breaths/min−1 even after adequate sedation; increased breathing efforts showed by: dyspnea, use of accessory muscles, tachycardia and anxiety [22]. Any patient with a fractured femur and/or tibia, presenting one or more of these criteria, was diagnosed with fat embolism syndrome. These criteria led to the diagnosis of fat embolism syndrome in 29% of patients in a series of 55, which is higher than other series, especially since this study excludes patients with thoracic lesions, where some of Lindeque's clinical signs may appear in the absence of fat embolism [22].

Petechial eruption is considered a pathognomonic clinical sign for fat embolism syndrome. It may occur in about 60% of patients, usually on the conjunctiva, on the oral mucous membrane and on the skin of the neck and shoulders. This distribution can be explained by the drops of fat that accumulate in the aortic arch before the independent embolization at skin level through the subclavian and carotid vessels [20, 23-25]. The factors contributing to the petechial eruption are: stagnation of blood, loss of coagulation factors and platelets, and damage to the endothelial walls due to free fatty acids (FFA) leading to rupture of capillaries [25].

In the vast majority of cases, the fat embolism remains asymptomatic, and the fat emboli are dispersed into small cells and phagocyted by macrophages or embedded in hepatic cells [26, 27]. Thus, many cases remain undiagnosed before death.

In the three cases presented, the symptomatology before death was poor and unspecific (febrile syndrome, tachycardia, tachypnea), and remained undiagnosed. The diagnosis of fat embolism syndrome was established only after performing the autopsy, and the histopathological examination of the brain and lung fragments stained specifically with Sudan III. Therefore, for post mortem diagnosis of fat embolism syndrome, it is essential to perform a histopathological examination with special stains that highlight the presence of fat emboli in the tissues and organs.

In the cases presented in this paper, fat embolism at the pulmonary and cerebral level was observed. According to literature, brain and lung localizations are the most important, although in most cases pulmonary fat embolism remains clinically asymptomatic. In order to be able to certify fat embolism as the cause of death, it is necessary for histopathological examinations to find fat emboli on a large part of the examination fields. The gravity of the consequences of pulmonary embolism also depends on the size and amount of fat emboli, as well as whether or not they pass into the systemic circulation to the lung.

In the brain, emboli produce small petechial hemorrhages, areas of ischemic necrosis and hemorrhage or necrosis and demyelination. Cerebral embolism is considered in literature as a paradoxical event, because it requires the presence of cardiac atrial septal defects in order to be explained. In the presented cases, there was no autopsy finding that showed neither atrial or ventricular septal defects nor patent arterial canal that could explain the cerebral fat embolism. An explanation of the pathophysiological mechanism which determined the cerebral embolism could not be found in this study.

The cause of death in all the three cases presented in this paper was the fat embolism. The diagnosis was established with certainty only after autopsy, by a histopathological examination which revealed the presence of fat emboli in the lung and brain tissues. Of the cases presented only one suffered a recent fracture of the femur; another presented in addition to trauma paraclinical changes suggestive of acute pancreatitis as a contributing factor; none had cardiac malformations that could explain the paradoxical fat embolism in the brain; all of the cases showed multiple lesions of the subcutaneous tissue and death occurred shortly after the initial trauma or after surgical interventions.

Death due to fat embolism can fall into the category of violent or non-violent deaths. Regardless of the cause of fat embolism, from forensic point of view the fat embolism is considered a complication that leads to a secondary causal relationship; in other words between the trauma/ initial pathology and death an intermediate link is inserted represented by a complication (the fat embolism) which is connected to a greater or lesser extent to the initial trauma/ pathology [28-30].

CONCLUSION

From forensic point of view, the diagnostic certainty in posttraumatic lesions is a priority objective. From the diagnosis it is possible to reconstruct the thanatogenerator mechanism and finally it can be useful to respond to the legal aspects of the case, i.e. the causal relationship between trauma and death.

In the case of trauma with reduced thanatogenerator potential, which usually evolve without complications, the occurrence of fat embolism syndrome followed by death must be appropriately explained so that the case can be properly framed from a legal point of view. It is important to emphasize that the histopathological examination using specific stainings, such as Sudan III, has a fundamental and incontestable role in the diagnosis of fat embolism and in estimating its gravity, and therefore, its implication in the occurence of death.

Conflict of interest: *The authors declare that there is no conflict of interest.*

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REVIEW

From Genetic Links to Personalized Therapies: Understanding Melanoma and Urological Cancer Overlap

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Abstract: Malignant melanoma and urological cancers originate from different tissues and organs, yet several studies highlight connections between these malignancies, including common risk factors, genetic predispositions, and immunological pathways. Evidence from recent studies suggests that a prior diagnosis of melanoma may increase the likelihood of subsequently developing renal cell carcinoma (RCC), and, conversely, patients with RCC appear to face a heightened risk of being diagnosed with melanoma. Shared factors such as a personal or family history of cancer, UV radiation exposure, smoking, and obesity have all been linked to an increased incidence of various cancer types. A major link between malignant melanoma and urological cancers is the presence of shared genetic mutations and familial cancer syndromes. Key mutations, including germline mutations in BRCA1, MITF, CDKN2A, TP53, and alterations in the PI3K/AKT pathway, significantly contribute to the risk of both types of malignancies. Personalized medicine, which tailors prevention and treatment strategies to an individual’s genetic, environmental, and lifestyle factors, has significantly improved cancer care. The primary aim is to select the most effective treatment for each patient, maximizing therapeutic outcomes, reducing side effects, and minimizing the risk of drug resistance. Advances in genomics and immunology are driving the development of personalized therapies that target specific molecular pathways and immune responses common to both melanoma and urological cancers. Angiogenesis inhibitors and checkpoint inhibitors have demonstrated notable success in treating these cancers, with tumor mutational burden serving as a valuable biomarker for predicting the efficacy of immune checkpoint inhibitors.

Keywords: malignant melanoma; urological cancers; risk factors; shared genetic mutations; lifestyle changes; personalized treatment; tumor mutational burden; angiogenesis inhibitors; checkpoint inhibitors

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INTRODUCTION

Cancer currently ranks as the leading cause of mortality worldwide. The growing number of cases and related deaths is primarily driven by shifts in demographic patterns, such as population aging and growth, along with greater exposure to environmental and lifestyle-related carcinogenic factors [1].

Although malignant melanoma and urological cancers arise from different anatomical sites and tissues, multiple studies have identified links between them. These connections include overlapping risk factors, common genetic susceptibilities, and similarities in immune-related pathways involved in their development.

Gaining insight into the association between malignant melanoma and urological malignancies may contribute to the improvement of approaches for early diagnosis, risk reduction, and therapeutic management.

Although melanoma accounts for less than 2% of cancer cases globally, it remains one of the most frequently diagnosed malignancies across Europe (5th place). Survival outcomes have improved considerably in recent years, with the overall 5-year survival now approaching 93.3%. However, patients diagnosed with stage IV disease continue to face a poor prognosis, as the survival rate in advanced stages drops significantly, reaching only 29.8% [2].

Urological cancers (UC), which include kidney, bladder, prostate, testicular, penile, and urethral cancers, differ significantly in incidence, risk factors, and prognosis.

Renal cell carcinoma (RCC) represents roughly 3% of all malignant tumors diagnosed globally. The occurrence of this malignancy tends to be more frequent in industrialized nations, especially in Western societies, where lifestyle habits, environmental risk factors, and advancements in medical imaging likely play a role in higher diagnosis rates. Renal cell carcinoma represents a major public health issue due to its frequently silent evolution in early stages and the elevated risk of distant spread if not recognized in time [3]. Globally, it is estimated that more than 430,000 new cases are identified annually, underlining the increasing impact of this disease on healthcare systems. Of these cases, approximately 138,000 are reported in Europe alone, underscoring the significant prevalence of RCC across the continent. Such statistics emphasize the importance of ongoing efforts in early detection, prevention, and the development of effective treatment strategies. [4].

Urothelial carcinoma is the second most common urological malignancy in developed countries [5]. Bladder cancer (BC) accounts for 90–95% of the UC [6]. It ranks as the seventh most frequently diagnosed cancer among men globally [7].

Primary urethral carcinoma is considered a rare cancer, accounting for < 1% of all genitourinary malignancies [8].

Approximately 1.5 million new cases are reported worldwide each year. This high incidence places it as the second most commonly identified malignancy in the male population [9].

Testicular cancer (TC) accounts for 1% of adult neoplasms and 5% of urological tumors. Its incidence has risen in recent decades, particularly in industrialized countries, and continues to increase [10].

Penile cancer is a rare condition in industrialized countries, with an incidence of approximately 1 per 100,000 men in Europe and 0.5 per 100,000 in the USA [11].

The aim of this article is to evaluate the potential connections between malignant melanoma and the most common urological cancers—renal cancer, bladder cancer, and prostate cancer—from the perspectives of epidemiological factors, shared genetic mutations and molecular pathways, clinical implications, and personalized medicine.

EPIDEMIOLOGICAL LINKS

Environmental and Lifestyle Risk Factors for Malignant Melanoma and Urological Cancers

Advancing age, genetic predisposition, prior cancer diagnoses, radiation exposure (such as ultraviolet rays), contact with certain hazardous substances, tobacco use, alcohol intake, and excess body weight are all factors that have been linked to a higher likelihood of developing different forms of cancer.

Ultraviolet (UV) exposure and vitamin D

The role of ultraviolet (UV) sunlight radiation in the development of skin cancers, particularly malignant melanoma, is well-established. UVA (320–400 nm) penetrates deeper into the skin, leading to indirect DNA damage through reactive oxygen species (ROS), while UVB (280– 320 nm) directly damages DNA by inducing cyclobutane pyrimidine dimers [12]. UVB radiation can also induces mutations in genes such as TP53 and BRAF, contributing to melanomagenesis [13].

However, the association between UV exposure and RCC remains unclear, recent studies have attempted to clarify the relationship but have produced mixed results. Grasso et al. [14] found no statistically significant differences between sun exposure in patients affected by kidney cancer and controls, both during childhood and adult life. Contrary to this, Karami et al. [15] suggest that among males there is an inverse association between occupational UV exposure and renal cancer risk. Replication studies are warranted to confirm these results.

Similar to the RCC, the potential involvement of vitamin D in the development and progression of bladder cancer and upper urinary tract urothelial tumors remains underexplored. Recent epidemiological studies offer conflicting results regarding the potential link between blood levels of vitamin D and the risk of bladder cancer onset. However, several investigations suggest that individuals with a higher intake of vitamin D through diet, particularly when not accompanied by elevated calcium consumption, may have a reduced risk of developing bladder malignancies [16]. In a meta-analysis, Zhang et al. [17] concluded that Vitamin D deficiency has been linked to a heightened risk of bladder cancer. According to Gislefoss et al. [18] educed serum concentrations of 25-hydroxy vitamin D, along with obesity, may be linked to a higher risk of developing bladder cancer. The 2024 guidelines of the European Association of Urology (EAU) highlight that both insufficient and excessive levels of vitamin D have been linked to a higher likelihood of developing prostate cancer, with a particularly stronger connection observed in cases involving high-grade tumors [19,20]. Additionally, Murphy et al. demonstrated that Vitamin D deficiency was linked to higher Gleason scores and advanced tumor stages at diagnosis [21].

Smoking

The effect of smoking on melanoma outcome still remains an enigma [22]. In a recent study, Arafa et al. [23] found that individuals who currently smoke or have a heavy smoking history tend to exhibit a greater susceptibility to developing squamous cell carcinoma (SCC), while simultaneously displaying a lower probability of malignant melanoma. Interestingly, no significant relationship was observed between past smoking and the overall risk of skin cancer. In contrast to established risk patterns, a case-control study by Sondermeijer et al. [24] reported an inverse association between cigarette smoking and melanoma incidence in men. However, smoking remains a well-documented risk factor for various urologic malignancies.

Smokers face a significantly elevated likelihood—estimated at around 1.5 times higher—of being diagnosed with renal cell carcinoma (RCC) when compared to non-smokers [25]. Encouragingly, long-term smoking cessation has been shown to gradually diminish this elevated risk [26]. Multiple carcinogens (N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), polycyclic aromatic hydrocarbons (PAHs), Cadmium and lead (Pb), 2-naphthylamine) are absorbed through the lungs into the bloodstream and then lipophilic carcinogens may accumulate in renal tissue [27].

Cigarette smoking represents the leading modifiable contributor to bladder cancer development and is estimated to be responsible for nearly half of all diagnosed cases [28]. Low-tar cigarettes are not associated with a lower risk of developing BC. The risk associated with electronic cigarettes has not been adequately assessed. Research by Bjurlin et al. [29] revealed the presence of carcinogen-related biomarkers in the urine of individuals who use e-cigarettes, several of which have a well-established association with bladder cancer. Kispert et al. [30] recently presented a study showing that smoking is associated with increased accumulation of platelet-activating factor (PAF) and elevated expression of the PAF receptor (PAF-R), which may contribute to tumor progression and metastasis in smokers.

Current cigarette smoking has been linked to an increased risk of prostate cancer (PCa) mortality, as well as more aggressive tumor characteristics and a poorer prognosis, even after smoking cessation [31].

Obesity

Obesity promotes chronic inflammation, a recognized risk factor for cancer development. Inflammatory cytokines can stimulate the growth of both melanoma and renal cell carcinoma (RCC), potentially linking these two malignancies in obese patients [32].

Obesity appears to be associated with malignant melanoma, particularly in patients with a BMI >30 [2].

Several retrospective studies have shown that obesity appears to be a factor that positively influences the prognosis of patients with RCC, called the "obesity paradox" [33]. Recently, Graff and colleagues [34] provided a comprehensive review with the main purpose of disentangling the "obesity paradox" in renal cancer. The results support obesity with a BMI >35 as a risk factor for total and fatal RCC. They undermine the obesity paradox by suggesting that weight loss around diagnosis, and not low BMI itself, is associated with worse prognosis.

In the REDUCE study [35], obesity was associated with a lower risk of low-grade PCa and a higher risk of high-grade PCa.

Occupational Exposure

Occupational exposure to carcinogens can increase the risk of various cancers. One common risk factor for both malignant melanoma and urological cancers is exposure to polycyclic aromatic hydrocarbons.

In a 45-year follow-up study conducted across five Nordic countries, Pukkala et al. [36] found an elevated incidence of prostate cancer, malignant melanoma, non-melanoma skin cancer, and lung adenocarcinoma among firefighters, with skin absorption identified as a primary route of exposure. Similarly, individuals working in the petroleum industry are exposed to comparable risks [37].

According to the 2024 EAU Guidelines [38], occupational exposure to polycyclic aromatic hydrocarbons and other aromatic amines or hydrocarbons, mainly in the paint, dye, or petroleum industries, is the second most important risk factor for bladder cancer.

While certain studies have indicated a potential association between occupational exposure to polycyclic aromatic hydrocarbons and an increased risk of renal cancer [39,40], there is no high-quality evidence to definitively confirm this link [41].

SHARED GENETIC MUTATIONS AND MOLECULAR PATHWAYS

Integrating molecular profiling into clinical oncology practice, particularly through mutation-focused approaches, has become essential in the evolution of precision medicine. By identifying specific genetic alterations within tumors, clinicians can better tailor immunotherapeutic interventions that modulate immune checkpoints. Therapeutic approaches that focus on modulating immune checkpoints have identified key molecular pathways, such as those involving PD-1 and its ligand, as well as CTLA-4, as critical elements in regulating immune responses against tumors. Targeting these mechanisms has opened promising prospects for the treatment of a wide range of cancers [42]. These immunotherapeutic agents have significantly reshaped treatment approaches across several types of cancers with overlapping characteristics.. Such technology was entirely unavailable before the completion of the Human Genome Project in 2001 [43]. However, current advances now enable the routine identification of relevant therapeutic mutations in many cancers, particularly in malignant melanoma and also in UC.

Genetic Mutations in Malignant Melanoma

Melanoma exhibits one of the highest mutation frequencies among cancers [44]. The most frequent mutations are BRAF, NRAS, NF1, the TERT promoter, and CDKN2A, while KIT and TP53 mutations occur at a much lower frequency. [45].

- The BRAF mutation is the most common in malignant melanoma, occurring in 2/3 of these patients. [46]. This BRAF mutation (especially the BRAF V600 mutation) promotes cellular growth and inhibits apoptosis and is more commonly found in patients with intermittent sun exposure compared to those with chronically sun-exposed skin or unexposed skin [47].

- NRAS mutations. Among the three main proto-oncogenes of the RAS family (NRAS, KRAS, and HRAS), NRAS mutations play a key role in continuously activating pathways that lead to cell growth, proliferation, and cell cycle dysfunction. They are found in approximately 1/4 of patients with malignant melanoma, particularly in those with non-exposed skin, and are associated with aggressive clinical forms and poor prognosis [48,49].

- The neurofibromatosis type 1 (NF1) mutation leads to a loss of function in certain tumor suppressors. NF1 mutations appear to be correlated with high UV exposure, resulting in a high mutational burden [50,51].

- The TERT (telomerase reverse transcriptase) promoter mutation is a UV-induced mutation associated with poor clinical prognosis. This mutation typically occurs in the promoter region, resulting in increased expression of the gene [52].

- CDKN2A Mutation. The CDKN2A gene contributes to cellular homeostasis by limiting uncontrolled cell division through mechanisms that halt the progression of the cell cycle. Its function is essential in preventing the unchecked proliferation characteristic of tumor development. When mutations occur in this gene, normal growth regulation is disrupted, promoting tumor development. Individuals who carry CDKN2A mutations face a markedly increased risk of developing multiple primary melanomas, often at an earlier age. [53,54].

- KIT is a proto-oncogene receptor tyrosine kinase and binds to stem cell factor. By activating the KIT protein, cell growth, proliferation, survival, and migration can be affected. KIT mutations occur more commonly in areas of chronic sun damage [55].

- BAP1 Tumor Predisposition Syndrome (BAP1-TPDS) is an inherited cancer syndrome marked by germline mutations in the BRCA1-associated protein 1 (BAP1) gene [56]. The consequences include genomic instability, uncontrolled cell proliferation, and resistance to apoptosis. In the case of cutaneous melanoma, patients with germline mutations in BAP1 develop multiple atypical melanocytic lesions (dome-shaped, skin-colored to reddish papules), which are histologically distinct from conventional melanomas [57,58].

- TP53 Mutation. In human biology, the TP53 gene plays a major role in preserving genomic integrity and is often described as the "guardian of the genome." This designation stems from its crucial function in cellular defense mechanisms, particularly in detecting DNA damage and initiating appropriate responses such as cell cycle arrest, DNA repair, or apoptosis. By preventing the propagation of cells with genetic errors, TP53 serves as a key tumor suppressor and a vital component in maintaining cellular homeostasis and protecting against malignant transformation [10]. The TP53 mutation has been found in association with BRAF, NRAS, and NF1 mutations and seems to appear at a later stage in tumor development. Even though TP53 is rarely mutated in melanoma, reduced levels of p53 contribute to aggressiveness and resistance to therapy for patients with malignant melanoma [59,60].

Genetic Mutations in Urological Cancers

There is increasing evidence that genetic susceptibility factors and familial associations may influence the incidence of UC, although their role is not as well defined as in the case of malignant melanoma.

- BAP1 Tumor Predisposition Syndrome (BAP1-TPDS) can predispose individuals not only to cutaneous and uveal melanoma but also to renal cell carcinoma (RCC) [57].

In case of cutaneous melanoma, patients with Germline mutations in BAP1 develop multiple atypical melanocytic lesions (dome-shaped, skin-colored to reddish papules), histologically distinct from conventional melanomas [58].

Kapur et al [61] in a retrospective analysis on 145 patients with primary clear cell renal cell carcinoma (ccRCC) concluded that in RCC, the clear cell subtype (ccRCC) germline mutations occur in approximately 10–15% of ccRCC. This study concluded that these patients are associated with poorer overall survival compared to those without the mutation.

Studies on the expression and prognostic role of BAP1 in prostate cancer are currently lacking. However, Steurer et al. immunohistochemically analyzed more than 17,000 prostate cancer cases, assembled on a tissue microarray over 10 10-year period, and concluded that their study shows BAP1 upregulation is associated with prostate cancer progression and aggressiveness [62].

- CDKN2A Mutation. The role of CDKN2A mutations in renal cell carcinoma is less clear. Recently, in a study, Kiatprungvech and colleagues [63] demonstrated that CDKN2A mutations may be associated with higher tumor grade and poorer prognosis in RCC, development, and sarcomatoid changes.

Deletions of CDKN2A are a molecular risk factor for tumour progression in non-muscle-invasive bladder cancers, an indicator of increased aggressiveness and worse prognosis in muscle-invasive bladder cancers [64].

In a meta-analysis investigating the involvement of CDKN2A methylation in prostate cancer, Cao et al. [65] found no strong evidence supporting a significant association between this epigenetic alteration and the onset or advancement of the disease. Nevertheless, lower levels of CDKN2A expression were correlated with poorer disease-free survival outcomes, suggesting potential prognostic relevance.

- MITF Mutation. The Microphthalmia-associated Transcription Factor (MITF) regulates genes involved in melanogenesis, melanocyte survival and proliferation, and DNA repair. The MITF-E318K variant is a heterozygous missense mutation characterized by the replacement of glutamic acid with lysine at the 318th amino acid position in the protein sequence.. Carriers have a higher risk of developing early-onset melanoma [66].

This mutation was also identified in patients with RCC, suggesting a shared genetic risk factor with melanoma. In their study, Lang et al. [67] observed that the presence of the pathogenic MITF variant in a family with bilateral and multifocal type 1 papillary renal cell carcinoma reinforces its potential role as a genetic risk factor for RCC. These findings also highlight the need to consider MITF variant screening regardless of the tumor's histological subtype.

- TP53 mutation. The role of the p53 pathway in renal cancer is underestimated. In RCC, there is a low rate of p53 mutations, which leads to poor response to conventional therapies or even therapy resistance [68].

According to findings reported by Wu et al. [69], alterations in the TP53 gene were identified in half of the individuals diagnosed with bladder cancer. It is worth noting that these genetic alterations appeared with greater frequency in tumors that had penetrated the bladder's muscular layer, as opposed to those confined to the superficial, non-muscle-invasive stages. Patients with TP53 mutations were associated with lower TP53 mRNA expression levels, more advanced tumor stages, and higher histologic grades.

- Loss of function in the VHL gene, often caused by inherited mutations, is associated with the development of von Hippel-Lindau hereditary cancer syndrome. This rare genetic condition can predispose affected individuals to various tumor types, including, in some cases, clear-cell renal cell carcinoma [70].

- Alterations in the BRCA1 and BRCA2 gene pathways have been linked to more aggressive disease phenotypes and less favorable prognoses in individuals affected by metastatic castration-resistant prostate cancer [71]. Specifically, inherited BRCA2 mutations are frequently observed in early-onset prostate cancer and are recognized as independent predictors of metastatic progression and reduced prostate cancer-specific survival [72,73]. Nyberg and colleagues [74] in a prospective cohort study confirmed the BRCA2 association with aggressive PCa. This mutation does not occur in other urological cancers or malignant melanoma.

CLINICAL IMPLICATIONS AND PERSONALIZED MEDICINE

Prevention is the main principle in reducing morbidity and mortality, as well as in lowering healthcare system costs.

Personalized medicine, which customizes prevention and treatment strategies based on an individual's genetic, environmental, and lifestyle factors, has greatly advanced cancer care [75].

Understanding the link between malignant melanoma and urological cancers is a key advancement toward personalized medicine.

Identifying genetic mutations and syndromes that link melanoma to urological cancers highlights the importance of genomic profiling in cancer diagnosis and treatment. The development of next-generation sequencing (NGS) technologies has made it possible to identify particular genetic alterations that may increase a person's susceptibility to various types of cancer or impact how they respond to specific therapeutic interventions [76].

Tailoring Prevention Strategies (Table 1).

Reduce Ultraviolet Exposure and the Role of Vitamin D

Excessive UV exposure is widely recognized as the leading environmental risk factor for melanoma. In people with an increased hereditary predisposition to skin cancer [77], tailored prevention approaches may involve regularly applying sunscreens that offer protection against both UVA and UVB rays, opting for clothing that minimizes sun exposure, and refraining from the use of artificial tanning devices.

In a systematic review and meta-analysis, Song et al. [78] concluded that a moderate dietary vitamin D supplement to prevent serum 25(OH)D deficiency may be beneficial for the long-term survival of melanoma patients.

A prospective study conducted by Lin et al. [58] suggested that exposure to ultraviolet radiation may be associated with a reduced incidence of prostate cancer. Additionally, research has shown that both low and excessively high levels of vitamin D may contribute to a higher likelihood of developing prostate cancer, particularly in cases involving more aggressive tumor forms [19]. Therefore, maintaining normal vitamin D levels is essential.

Table 1: Recommendations for lifestyle changes

	Malignant melanoma	**RCC**	**Bladder cancer**	**Prostate cancer**
Smoking cessation	uncertain	yes	yes	yes
Reduce ultraviolet exposure	yes	-	-	no/increase
Vitamin D	moderate increase dietary	normal vitamin D levels		
Obesity	uncertain	yes	yes	yes
Dietary antioxidants	yes	yes	yes	yes

Smoking Cessation in Malign Melanoma and Urological Cancers Prevention

While the relationship between smoking and malignant melanoma remains inconclusive, tobacco use is widely recognized as a major contributor to the development of several urologic malignancies, especially bladder cancer and renal cell carcinoma. Smoking cessation programs, aided by pharmacological treatments such as nicotine replacement therapy (NRT), have proven effective in reducing the incidence or recurrence of progression of RCC [26] and bladder cancer [80]. In a network meta-analysis of 20 randomized controlled trials, Gou et al. [81] concluded that Varenicline and Bupropion increased the odds of smoking abstinence.

Obesity and Dietary Interventions in Malignant Melanoma and Urological Cancers Prevention

Studies that focus specifically on the link between obesity and melanoma prognosis are limited [61]. Obesity may be a risk factor for bladder cancer recurrence [83] and a higher risk of death from prostate cancer [84].

The use of dietary antioxidants has shown promising results in studies, supporting the potential role of antioxidants in the prevention of malignant melanoma related to UV exposure.

Obesity and dietary habits are well-established risk factors for prostate cancer and RCC.

Personalized weight management programs guided by genetic and metabolic profiles, and dietary recommendations, such as reducing red and processed meats and increasing intake of plant-based foods rich in antioxidants (e.g., lycopene), regular physical activity, are essential for individuals at high risk for prostate cancer and RCC [64,65].

Screening and Surveillance

Patients with a history of melanoma or urological cancer need careful monitoring for the potential development of additional malignancies. Routine screenings, such as skin examinations and urological assessments, can aid in early detection and improve patient outcomes. Screening for malignant melanoma is recommended for individuals with certain risk factors, including family or personal history, lifestyle or occupational predispositions, and being an older white male.

Patients with germline mutations in BAP1, CDKN2A, MITF, and other cancer-related genes may benefit from more extensive cancer screening protocols. These could include regular skin examinations for melanoma, imaging studies to detect urological cancers, and continuous monitoring for other associated malignancies.

Skin cancer screening remains a topic of debate.

There is an ongoing debate regarding the value of routine screening for skin cancer.

According to current assessments by major public health authorities, existing evidence is insufficient to clearly confirm or reject the benefits of regularly screening asymptomatic adults in the general population [87]. In contrast, screening is recommended for high-risk individuals by both the Australian Cancer Network [88] and the Canadian Cancer Society [89].

The group of patients at higher risk for developing skin cancer includes individuals with a personal history of skin cancer, those with light-colored skin, eyes, and hair, a high number of moles or freckles, and a history of multiple blistering sunburns during childhood [87]. In individuals at elevated risk, initial screening typically involves assessment of skin lesions using the "ABCDE" criteria, focusing on asymmetry, irregular borders, variations in color, and lesions exceeding 6 mm in diameter. Another widely used approach is the visual examination of the skin to detect pigmented lesions that appear noticeably different from the patient's other moles, a method commonly referred to as the "ugly duckling" sign [90].

The American Academy of Dermatology advises that patients diagnosed with cutaneous melanoma undergo cancer risk counseling if they present specific risk factors. These include having a family history involving three or more relatives on the same side affected by invasive melanoma or pancreatic cancer; the presence of three or more primary invasive melanomas, particularly if at least one was diagnosed before the age of 45; the occurrence of one or more melanocytic BAP1-mutated atypical intradermal tumors (MBAITs) accompanied by a family history of cancers such as mesothelioma, meningioma, or uveal melanoma; the presence of two or more MBAITs; or a broader family history that includes other forms of cancer. [91]. According to the National Comprehensive Cancer Network (NCCN), genetic testing for p16/CDKN2A mutations is advised in cases where an individual or family history includes three or more invasive cutaneous melanomas, or a combination of invasive melanoma, pancreatic cancer, and/or astrocytoma. Furthermore, individuals diagnosed with invasive cutaneous melanoma who also have a first-degree relative affected by pancreatic cancer are considered appropriate candidates for genetic evaluation. [92].

Renal cancer screening.

There is currently no evidence to support primary screening in the general population. The most pragmatic approach is to screen high-risk individuals or integrate this screening with other routine screening programs. This strategy improves the cost-effectiveness ratio, particularly for patients at risk of RCC [93]. Genetic assessment is advised in individuals under the age of 47, as well as in those presenting with tumors affecting both kidneys or multiple sites within the same kidney. It is also warranted for patients who have a direct or extended family history of renal cell carcinoma or relatives carrying a confirmed pathogenic genetic alteration. Additionally, testing is appropriate when histopathological characteristics raise suspicion of an inherited subtype of renal cancer [94].

Bladder cancer screening.

Current evaluations by major public health experts indicate a lack of sufficient high-quality evidence to clearly assess whether bladder cancer screening in asymptomatic adults provides more benefits than harms [95]. Similar recommendations are also found in the EAU Guidelines 2024 [96].

Prostate cancer screening.

Screening for PCa is still controversial. [97]. Prostate screening is associated with an increased diagnosis of prostate cancer (PCa), the detection of more localized disease, and fewer cases of advanced PCa. However, no PCa-specific survival benefit has been observed, and there has been no overall survival (OS) benefit [98]. Consequently, population-based screening has not been widely adopted.

According to the 2024 EAU Guidelines, PSA testing for prostate cancer screening is recommended for well-informed men at higher risk of PCa. It is generally recommended for men aged 50 and above. Earlier screening may be appropriate for those over 45 who have close relatives previously diagnosed with the disease or who belong to high-risk populations, such as individuals of African ancestry. Furthermore, men with confirmed BRCA2 mutations are advised to initiate screening starting at age 40 [99].

Hereditary genetic testing is recommended for men who have several relatives affected by prostate cancer at a relatively early age—specifically before turning 60, or in cases where a family member has died as a result of the disease. Genetic testing may also be appropriate for men who have a familial pattern of high-risk inherited mutations or a history of multiple cancer diagnoses occurring on the same side of the family [99].

Personalized Treatment

The goal of personalized medicine for cancer patients is to select the most effective treatment tailored to each individual, aiming for maximum efficacy, reduced side effects, a lower risk of drug resistance, and ultimately, improved overall survival. With advancements in technology, genetic testing has become increasingly accessible and is now part of routine clinical practice. [100]. Advancements in genomics and immunology are driving the development of personalized treatments that focus on specific molecular pathways and immune mechanisms common to both melanoma and urological cancers. The convergence of these genetic pathways offers promising potential for the creation of targeted therapeutic approaches.

In most cancer cases, treatment follows well-established standards that are outlined in specialized guidelines. It includes surgical therapy (nephrectomy, prostatectomy, radical cystectomy, melanoma surgery, etc.), radiotherapy, and/or hormone therapy.

In the final stages, palliative therapies are offered to patients with metastatic cancer, and these treatments can help enhance quality of life while potentially prolonging disease-free survival.

Angiogenesis inhibitors and immunotherapies have shown notable effectiveness in treating malignant melanoma and urological cancers, particularly kidney and bladder cancers. Most prostate cancers exhibit resistance to immunotherapies, particularly immune checkpoint inhibitors [101].

Angiogenesis inhibitors.

VEGF inhibitors function by blocking the interaction between VEGF and its receptors (VEGFRs) on endothelial cells, effectively stopping the signaling processes responsible for initiating new blood vessel formation [102]. VEGF inhibitors are primarily categorized into two groups: monoclonal antibodies, which bind directly to VEGF ligands to prevent receptor interaction, and tyrosine kinase inhibitors (TKIs), which target VEGFRs along with associated kinases to block angiogenesis signaling pathways [103,104]. These inhibitors have shown notable effectiveness in adjuvant treatment for renal cell carcinoma (RCC) and malignant melanoma, though their use is more restricted in other types of urological cancers.

Monoclonal VEGF antibodies, particularly Bevacizumab, have demonstrated effectiveness in the treatment of both renal cell carcinoma (RCC) and malignant melanoma [105,106].

Tyrosine kinase inhibitors are commonly prescribed as initial or subsequent targeted treatments for patients with kidney cancer that has progressed locally or spread to distant sites [107,108]. Initial treatment with sunitinib alone has demonstrated significant benefits by delaying disease progression and enhancing the overall tumor response. Some tyrosine kinase inhibitors, such as pazopanib, have shown similar effectiveness to sunitinib when used in the initial treatment of patients with metastatic kidney cancer [109]. Certain agents, including cabozantinib, have shown clinical benefit in patients with advanced clear-cell renal cell carcinoma that no longer responds to therapies targeting the VEGF receptor pathway. This option is particularly relevant for individuals who have not yet received immune checkpoint inhibitors and who have previously undergone one or two systemic treatment regimens [110].

For patients with malignant melanoma, sunitinib may show efficacy, particularly in those with KIT mutations [111].

Checkpoint inhibitors

Immunotherapeutic strategies—especially those directed at modulating checkpoint pathways—have shown significant efficacy in the treatment of various cancers. A prominent category within these therapies includes drugs designed to block the binding between PD-L1 and its associated receptor, PD-1, both of which are crucial in helping tumors evade immune surveillance. Interrupting this interaction enables the reactivation of T cells, thereby strengthening the immune system's capacity to detect and destroy malignant cells.

Tumor mutational burden (TMB) serves as an essential biomarker for predicting immunotherapy outcomes, representing the total count of somatic mutations within a tumor's DNA. Although tumor mutational burden (TMB) does not appear to impact the success of treatments aimed at inhibiting angiogenesis, it is considered an important biomarker in forecasting patient responsiveness to immunotherapeutic strategies. These treatments work by altering specific immune checkpoints involved in immune regulation, including pathways mediated by proteins like PD-1, PD-L1, and CTLA-4 [112]. Tumors with a high mutational burden typically generate a higher quantity of neoantigens (abnormal proteins created by genetic mutations). This abundance of neoantigens improves the chances of immune system recognition and response, thereby boosting the effectiveness of immunotherapy [113]. Cancers such as melanoma, lung cancer, and urothelial cancers often have a high tumor mutational burden (TMB), while prostate and breast cancers typically show lower TMB [69]. Tumors with high TMB tend to express a wider array of neoantigens, making them more prone to immune system recognition and response, particularly when treated with checkpoint inhibitors. As a result, immunotherapy is generally more effective in cancers with elevated TMB [114,115].

Both malignant melanoma and RCC are highly immunogenic tumors, meaning they can provoke an immune response. The strong immunogenic nature of both cancer types has contributed to their responsiveness to immunotherapeutic approaches, especially those involving checkpoint blockade with agents targeting PD-1 and CTLA-4. However, the application of PD-L1 inhibitors in the treatment of renal cell carcinoma remains relatively restricted [116].

After the introduction of ipilimumab in 2011 for the systemic treatment of melanoma, immunotherapy has made significant strides in treating both resectable and unresectable melanoma [117]. The CheckMate 037 [118,119]and CheckMate 066 trials established nivolumab's efficacy in patients with metastatic melanoma. Additionally, the KEYNOTE-006 trial, which compared pembrolizumab and ipilimumab for advanced melanoma, showed pembrolizumab's superiority in terms of the 2-year overall survival rate [120].

In the 2023 ASCO Guideline Update on Systemic Therapy for Melanoma, checkpoint inhibitors are positioned as the primary treatment choice for malignant melanoma. They are recommended for neoadjuvant therapy (in resected stage III B-IV melanoma), as adjuvant therapy (for resected stage II B or higher), and for unresectable melanoma. For patients with unresectable or metastatic cutaneous melanoma, or resected stage IV melanoma, the combination of ipilimumab and nivolumab serves as one of the primary treatment options [121].

In selected cases of metastatic kidney cancer, monotherapy with an immune checkpoint inhibitor, such as pembrolizumab, can represent a suitable therapeutic choice, particularly when combination regimens are not indicated or tolerated. However, in metastatic patients whose disease is categorized as intermediate or poor risk and who are suitable candidates for immunotherapy, current clinical guidelines tend to favor starting with a dual-agent immunotherapeutic regimen as the initial line of systemic treatment [122]. Currently, five immunotherapy-based combination treatments have received approval for clinical use. These include the pairing of ipilimumab with nivolumab, pembrolizumab with axitinib, as well as other therapeutic duos such as nivolumab combined with cabozantinib, avelumab administered alongside axitinib, and pembrolizumab used in conjunction with Lenvatinib [123]. Among these, the ipilimumab and nivolumab combination is specifically approved for intermediate- and poor-risk mRCC patients [124].

Despite their efficacy, these agents also cause immune-related adverse effects that may be life-threatening if not detected and controlled appropriately [125].

Targeting the PD-1/PD-L1 immune checkpoint pathway has demonstrated significant therapeutic benefits in patients with advanced or inoperable bladder cancer, including those with metastatic disease. Nivolumab has received FDA approval as adjuvant therapy in individuals with urothelial carcinoma who face an elevated risk of disease recurrence after undergoing surgery. Additionally, the PD-1 inhibitor pembrolizumab was authorized for use in patients whose condition has worsened despite prior treatment with platinum-based chemotherapy, a decision supported by findings from the phase III clinical study known as KEYNOTE-045 [126].

For prostate cancer, the use of angiogenesis and checkpoint inhibitors in therapeutic strategies is limited. Currently, pembrolizumab is the only FDA-approved treatment option, although it has not been approved by the EMA. [127].

Beyond the therapies previously mentioned, each of these pathologies benefits from specific treatments targeting different types of genetic mutations and molecular pathways.

For malignant melanoma with BRAF gene mutations (V600E/K), treatment combinations such as dabrafenib with trametinib (or encorafenib with binimetinib) are indicated [128,129].

In prostate cancer, besides androgen receptor pathway inhibitors, non-hormonal, non-cytotoxic treatments like poly-ADP ribose polymerase inhibitors (PARPi) have emerged, specifically designed to target cancer cells harboring BRCA mutations [130]. While no AKT inhibitors are currently approved, the investigational drug ipatasertib has demonstrated significant activity in clinical trials when combined with abiraterone acetate in patients with loss of the tumor suppressor protein PTEN, as confirmed by immunohistochemistry [131].

CONCLUSION

The link between malignant melanoma and urological cancers is underpinned by common epidemiological factors and genetic mutations, establishing a foundation for progress in personalized medicine. As understanding of the shared molecular pathways in these cancers expands, opportunities for tailored prevention strategies, screening practices, and individualized treatments become increasingly apparent. Identifying genetic predispositions—such as BAP1 and CDKN2A mutations—early on, along with employing targeted therapies and immunotherapies, is expected to meaningfully enhance patient care in the near future.

Personalized medicine holds great promise for patients with elevated risks of both melanoma and urological cancers. By leveraging genomic profiling alongside advancements in immunotherapy and targeted therapies, more tailored and effective treatment options become possible, enhancing patient outcomes. However, ongoing research and clinical trials are essential to fully understand the effects of shared genetic mutations and molecular pathways and to establish comprehensive, individualized treatment protocols. Further refinement in treatment sequencing approaches will also be necessary to optimize care for these patients.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

DPH contributed to the manuscript's conceptualization, methodology, discussion, supervision, and writing. MAB contributed to the manuscript's editing, data curation, and writing. NM contributed to the editing and data curation of the manuscript. OSC contributed to the conceptualization, discussion, and supervision of the manuscript. MDV contributed to the manuscript's conceptualization, editing, and data curation. CT contributed to the discussion, data curation, and writing of the manuscript. IA contributed to the methodology, review, and editing of the manuscript. BOF contributed to the conceptualization, discussion, review, and supervision of the manuscript. VMG contributed to the editing, data curation, and writing of the manuscript. OKIM contributed to the conceptualization, discussion, and supervision of the manuscript. CTM contributed to the conceptualization, methodology, investigation, discussion, review, editing, supervision, data curation, and writing of the manuscript.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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ORIGINAL ARTICLE

Therapeutic Particularities of Depression in Relapsing-Remitting Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) is an autoimmune disease in which the myelin sheath is damaged by the body itself. Because of the symptoms and progressive forms, in most cases, patients experience recurrent depressive disorder or anxious depression disorders. Depression treatment for MS patients stands in two categories, one is psychotherapy and the other is pharmacological. This article presents the research results on customized pharmacological treatment schemes applied to a group of relapsing-remitting multiple sclerosis (RRMS) patients with moderate, or severe, depression. All of them are included in the national treatment program for multiple sclerosis and their health state is continuously monitored. The therapeutic scheme based on four different antidepressants had encouraging results, meaning that any of the antidepressants used is efficient and with the patient's good tolerance. There were no side effects that could determine interruption of the treatment. Further research development would focus on improved therapeutic plans for patients with depression disorders in relapsing-remitting multiple sclerosis.

Keywords: depression disorder; therapeutic particularities; multiple sclerosis; HAM-D scale

INTRODUCTION

In 1868, the neurologist Jean-Martin Charcot mentioned for the first time multiple sclerosis (MS) as a neurological affection with unpredictable evolution varying from benign (mild) up to aggressive. This illness especially affects people, aged between 30-50 years, most of them being diagnosed in their mid-thirties. There are also teenagers affected, mostly the ones who smoke and the overweight young women. The official reports [1] mention the ratio of males and females as 1:3.

There is no identified specific factor to trigger MS, so the assumption is that of a series of factors that could cause the disease, like:

- genetic factors - usually, if one parent has a diagnosis of multiple sclerosis, the risk for their children to have MS is about (2 - 3)%, and, more importantly, if both parents have a diagnosis of multiple sclerosis, the risk for their children to have this disease is about (12 - 30)%;

- Environmental factors - are diverse and perhaps complementary, of which the following are considered relevant: prolonged sun exposure during childhood and adolescence - determines high serum vitamin D levels and thus lower risk of MS; smoke from smoking - affects systemic immunity and can damage the central nervous system (CNS); obesity - especially in teenage girls, when hormonal changes that occur secondary to obesity are related to inflammatory processes; sleep disturbance - wake cycle and poor quality of rest; brain trauma - when being a child or adolescent could cause the disease.

Multiple sclerosis is an autoimmune neurodegenerative disease that acts on and destroys the central nervous system [2,3]. It is an inflammatory disease that leads to demyelination and axonal loss, resulting in manifest cognitive, motor, or sensory symptoms such as depression, emotional distress, or anxiety. As mentioned in [4],

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the clinical course of MS is classified as follows: Clinically Isolated Syndrome (CIS); Relapsing-Remitting (RRMS); Primary Progressive (PPMS); Secondary Progressive (SSMS).

Once the evolution of MS is on, the predominant emotional disorder is stated to be [5,6] depression. Patients with depression also have a poor psychosocial quality of life, manifest diminished capacity to work, and manifest decreased therapeutic compliance.

Most of the studies [7,8,9,10,11] mention fatigue, depression, and pain as inherent disorders associated with structural and functional changes in the brain in MS. These changes have been noticed in the basal ganglia, prefrontal cortex, and limbic system [12,13,14].

Comorbid fatigue and/or depression symptoms reported in patients with MS are also linked to impairment of the hypothalamic-pituitary-adrenal (HPA) axis [15]. Because chronic fatigue and depression are low levels of cortisol and dehydroepiandrosterone (DHEA) it is possible to be endocrine contribution. Studies [16,17] have proved that MS patients state to have increased energy after taking corticosteroids as treatment for MS relapse.

As mentioned in [18,19] depressive disorder (MDD), or clinical depression, represents a debilitating disease exhibiting at least one major depressive episode for at least two weeks, with an evident bad mood, low interests and pleasure (anhedonia), poor cognition and neurovegetative symptoms (fatigue, loss of appetite, insomnia). There is a high focus on improving the quality of life for MS patients. Depression treatment usually is individualized and is split between associated pharmacological and non-pharmacological ones.

The pharmacological treatment with Desipramine (tricyclic antidepressant) 200 mg/day [20] and Paroxetine (selective serotonin reuptake inhibitor) 40 mg/day [21] is mentioned in comparison with placebo, for a period of five and, respectively, twelve weeks. As a result, there was noticed a slight improvement of depression symptoms but, not a statistically significant result.

In [22] it is analyzed how a specialized exercise training program with prescriptive guidelines [23, 24] having as background the social cognitive theory sustains the reduction severity of depressive symptoms in MS patients.

This article presents the research results on therapeutic particularities of depression, a survey carried out on a group of relapsing-remitting multiple sclerosis (RRMS) patients with moderate, or severe, depression forms. The therapy plan was developed based on the authors' expertise in the treatment of multiple sclerosis and mainly consists of the administration of one of the following antidepressants: venlafaxine; sertraline; mirtazapine; and trazodone. The diagnosis and the severity of depression were evaluated by the total Hamilton Depression Rating Scale (HAM-D).

Aspects of materials and methods used in research, as well as results, discussion, and conclusion of the obtained results, are evidenced in the next chapters. Further research development focus is also mentioned.

MATERIALS AND METHODS

There are different signs of multiple sclerosis [25,26] caused by the location and severity of the myelin sheath lesions. Troubles in moving arms or legs, disorders in equilibrium, visual disturbances, or, when severe, loss of ability for independent motion - these are the alarm signals for this illness.

For the moment, there is no specific and targeted treatment for curing MS, but there are therapeutic schemes usually focusing on fast and high recovery from attacks, reducing probabilities of relapse, slowing down the disease progression, and adequate managing of symptoms. There are also cases when the symptoms are mild and patients do not need specific treatment.

While MS attacks are on, for reducing inflammation of the nerves are used intravenous corticosteroids. If there is no positive response to corticosteroids and the symptoms are extremely severe, plasma exchange can be used for ease of symptoms (not for curing). There are also treatment schemes for lowering the rate of relapse and reducing the risk of brain atrophy, such as immunomodulatory medication, monoclonal antibodies, or antihistamines. Solutions for MS symptom relief are various and customized on patients, like: kynetotherapy to increase muscle strength; muscle relaxers for reducing pain in muscle rigidity; specific medication against pain, insomnia, and depression; and psychological counseling.

There is a common opinion regarding the risk of immobilization for patients with multiple sclerosis but, the highest risk for them is that of suicide. Psychological manifestations have been noticed since the 19th century [27], once the clinical signs of this illness have been described.

About 50% of patients with multiple sclerosis exhibit at least one depression symptom, and a percentage varying in-between (15 ÷ 30)% of these patients have severe depression disorder.

A comprehensive study [28] indicates that the majority of MS patients present with both anxiety and depression symptoms. However, due to the presence of multiple overlaps in diagnostic criteria, it is challenging to ascertain the exact frequency of each of these conditions. The predominant feature of their syndrome points towards depression, anxiety, or, mixed anxiety-depressive disorder. Even for now, it has not been identified yet a direct relationship between multiple sclerosis and depression, the main mechanisms are assumed to be the ones that follow:

- Depression is determined by the process of damaging specific brain zones, related to the control and manifestation of emotions. There are also immune and endocrine changes induced by MS that could determine bad mood feelings.

- Depression is triggered by the emotional impact of the patient when finding out the medical diagnosis of multiple sclerosis.

- Depression manifests as a side effect of pharmacological treatment, like interferon-based medications or corticosteroids.

The criteria for diagnosis of depression, as mentioned by the Diagnostic and Statistical Manual of Mental Disorders, DSM-5 [27] are evidenced in Table 1.

Table 1: Criteria of depression by DSM-5 [26]

No.	Criteria	Tick √ if YES within two weeks
1	Mood of depression - long time a day, almost every day	
2	Diminished interest/pleasure in most activities - long time a day, almost every day	
3	Significant weight loss/gain without specific dieting	
4	Slow down in decision-making and performing physical activities (relevant to the others)	
5	Fatigue and poor energy	
6	Bad feelings and guilt for most of the things done - almost every day	
7	Difficulty in concentration and thinking, indecisiveness - almost every day	
8.	Repeated thoughts of death, suicidal behavior	

An individual is diagnosed with depression if, at least, five of the above symptoms are exhibited within two weeks and one of them should be depressed mood and/or diminished interest or pleasure. Attention is to be given so that symptoms do not result from any substance abuse.

There are also two additional specifiers for the diagnosis, of depression:

- with mixed features – such as manic symptoms;

- with anxious distress – the presence of anxiety symptoms;

The depression causes are multiple and commonly classified in four main categories (A ÷ D), as follows:

A. Psychosocial stress - that represents the disability in MS because it is generated by: global physical impairment; illness evolution in pus, with no possible estimation in time; motor deficits; diminished social role / inadequate support from the family.

B. Neuro-immune-endocrine triad (see figure 1) - as in MS patients the severity of depression depends directly on the amount of cytokines, IFN⍰. In this research, there was noticed decrease in IFN⍰ amount after antidepressant treatment, once the depression symptoms diminished. The cytokines stimulate the axis: HHA (hypothalamic-hypophyseal-adrenal) ⍰ CRH (Corticotropin-releasing hormone) ⍰ ACTH (Adrenocorticotropic hormone) ⍰ CORTISOL. The cytokines disturb the metabolism of monoamines (serotonin, non-adrenaline, dopamine) by prostaglandins - in cerebral areas highly important for depression: the hippocampus and hypothalamus.

C. Relationship between brain injuries and depression - based on neuroimaging evidence (RMN). Multiple sclerosis illness does affect myelin and nerve conduction so depression could be a direct consequence of the disease. The severity of depression directly depends on the right temporal lesions of the frontal lobe and the area adjacent to the basal ganglia.

D. Pharmaceutical treatment - there is no relevant evidence of increased occurrence of depression symptoms caused by the MS pharmacological treatment. Mainly, if the depression gets worse it is caused by the worsening of disability. Depression treatment for MS patients stands in two categories, one is psychotherapy and the other is pharmacological. Psychotherapy commonly consists of: psychological counseling; support therapy and cognitive-behavioral techniques.

To diminish depression, there are some additional steps [29] that could be done by multiple sclerosis patients, complementary to psychotherapy and pharmacological treatment, such as:

- physical exercises - that determine higher endorphins rate and, as a consequence, reduce fatigue and better mood;

- stress management program - meditation;

- talk about feelings and emotions - to a friend, family member, or, priest;

- belong to a community - to take part in targeted activities (walks, talks, reunions);

- find something that makes you happy and enjoy the day;

- help other people in need - get a sense of worthiness and utility;

- pet adoption - if possible and allowed by doctors;

- excellent sense of humor.

Figure 1: Neuro-immune-endocrine triad scheme

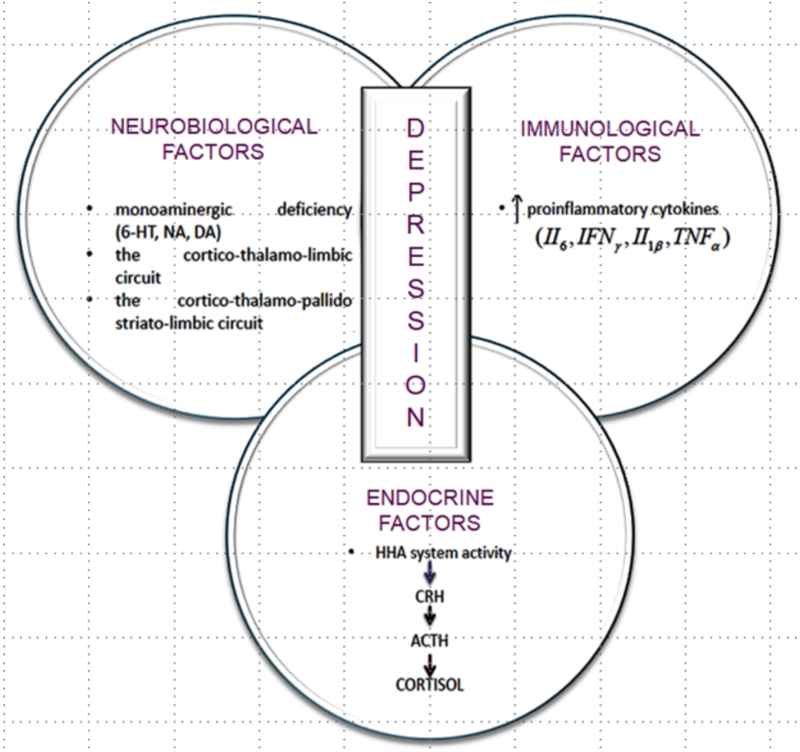
NEUROBIOLOGICAL FACTORS
• monoaminergic deficiency (6-HT, NA, DA)
• the cortico-thalamo-limbic circuit
• the cortico-thalamo-pallido striato-limbic circuit
DEPRESSION
IMMUNOLOGICAL FACTORS
• ↑ proinflammatory cytokines
$(Il_6, IFN_\gamma, Il_{1\beta}, TNF_\alpha)$
ENDOCRINE FACTORS
• HHA system activity
CRH
ACTH
CORTISOL

In this research the focus is not on either psychotherapy, or additional actions, even the patients in the sample group also practiced some of these therapies. This research is focused on the therapeutic particularities of depression, in relapsing-remitting multiple sclerosis (RRMS) patients.

Pharmacological treatment in depression for relapsing-remitting multiple sclerosis patients is evidenced in Table 2. These therapeutic schemes are particularly used in neurology clinics the authors work. All the patients of the survey are included in the national program for the treatment of multiple sclerosis and monitored by: neurological examination; brain imaging; psychological examination; and psychiatric examination.

Table 2: Pharmacological treatment in depression

Class	Representative	Dose [mg/day]	Comments
ADT (antidepressants)	Nortriptyline Desipramine	50 ÷ 200 100 ÷ 300	- limited use because of anticholinergic side effects (dry mouth, constipation, urinary and cardiovascular disorders)
SSRI (selective serotonin reuptake inhibitors)	Sertraline Fluoxetine S-Citalopram	50 ÷ 200 20 ÷ 80 10 ÷ 20	- good tolerance; - efficiency; - side effects (sexual dysfunctions, insomnia)
NaSSA (noradrenergic and specifically serotonergic antidepressants)	Mirtazapine	15 ÷ 45	- efficiency; -minimum effects on sexual function; - side effects (weight gain, drowsiness)
SARI (reuptake inhibitors and serotonin 2A antagonists)	Trazodone	150 ÷ 300	- efficiency; - minimum effects on sexual function; - side effects (drowsiness, orthostatic hypotension)
NDRI (noradrenaline and dopamine reuptake inhibitors)	Bupropion	150 ÷ 400	- efficiency; - minimum effects on sexual function; - side effects (convulsions, psychotic symptoms)
SNRI (serotonin and noradrenaline reuptake inhibitors)	Venalafaxine	75 ÷ 225	- efficiency; - side effects (intestinal disorders, psychotic symptoms, arterial hypertension)

The patients in the research group were diagnosed and subsequently admitted to the hospital when acute attacks occurred. Because of the relapsing attacks, patients develop anxious depressive disorder with panic attacks. Once the severe symptoms were relieved, they were discharged from the hospital and followed up the therapeutic scheme so that to get into the survey.

Initially, it was considered for the study a group of 102 patients, registered within a period of 4 years (2019 - 2023). Because of changes in their health conditions, mental and willingness to take part in the study, not the least family involvement, some of them had to retire. So, the size of the study (sample) group got down to 58, but its size remains fit for a research study (as the sample size is greater than 30).

The survey group is made of 58 relapsing-remitting multiple sclerosis patients with moderate, or severe, depression, out of which 18 are males with ages in between 18 and 30 years. The others are 40 females with ages between 30 and 40 years.

One can notice the higher percentage of females with MS diagnostic (40 out of 58, meaning around 69%) aspect revealed by most of the specific literature references. There has to be mentioned the fact that young people (above 25 years old) usually suffer from anxiety or anxious depression disorder, while mature people suffer from anxious depression disorders or recurrent depressive disorders. The survey period lasted for 16 weeks.

The customized therapeutic scheme applied for diminishing depression, based on the authors' expertise in the diagnosis and treatment of (relapsing-remitting) multiple sclerosis consists of the administration of one of the antidepressants (see Figure 2):

- venlafaxine - for 7 patients (12%), in flexible dosing of 75 ÷ 150 mg/day;
- sertraline - for 8 patients (14%), in flexible dosing of 50 ÷ 100 mg/day;
- mirtazapine - for 19 patients (33%), in a dose of 30 mg/day;
- trazodone - for 24 patients (41%), in a dose of 150 mg/day;

The selected percentages for medication considered the medical history of patients and estimated positive effects of the antidepressant. Also, previous experience of the authors (medical team) in the administration of these types of medicines is important. If there are multiple relapsing attacks, an increase in dosage has to be considered.

The antidepressant medication has to be administrated only in consideration of the following rules (see Figure 3):

- the patients who do not exhibit hypnic jerk were administered sertraline or venlafaxine which do not have sedative effects;
- the patients with hypnic jerk were administered mirtazapine or trazodone, as they also have sedative effects;
- the patients with appetite decrease/weight loss were administered mirtazapine.

Figure 2: Schematic representation of the depression medication for RRMS patients

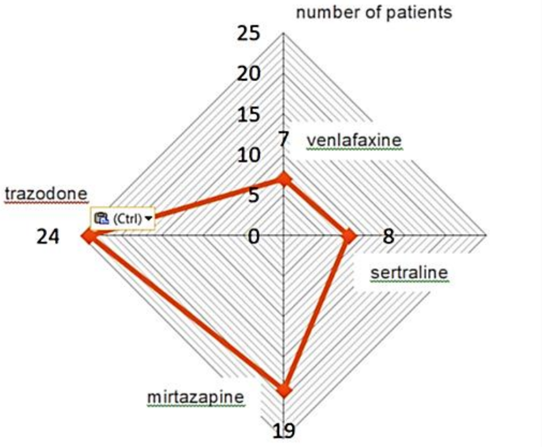

Figure 3: Customized therapeutic plan for RRMS patients

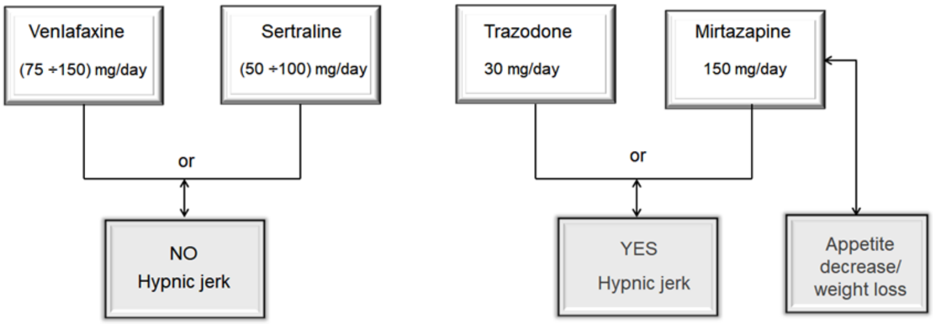

RESULTS

In diagnosing depression among patients with relapsing-remitting multiple sclerosis, it's essential to apply DSM-5 criteria tailored for MS. Recent guidelines from the National Multiple Sclerosis Society (2025) highlight the interplay between neurological symptoms and psychological distress in MS. These updated guidelines advocate for a nuanced diagnostic approach beyond traditional tools like the Hamilton Depression Rating Scale (HAM-D), ensuring diagnoses reflect current clinical insights.

This scale is a multiple-item questionnaire, providing a self-evaluation tool for depressive symptoms. The advantage of using HAM-D is that of high fidelity [30] in terms of clinical symptom assessment due to multiple questions for most of the items. It is compatible with DSM and provides an indicator for the severity of depression reported by an adult, based on a threshold score of clinical value.

Some relevant aspects of HAM-D and its scores are mentioned [31,32] next:

- the scale is scored between 0 and 4;

- scores of 0–7 suggest normal state;

- scores of 8–16 suggest mild depression;

- scores of 17–23 suggest moderate depression;

- scores starting from 24 suggest severe depression.

Before starting the customized depression disorder therapeutic schemes, based on the HAM-D test results, patients were divided into five groups, depending on test scores, as follows:

- 14 patients with HAM-D = 25 (group 1, G1);

- 5 patients with HAM-D = 21 (group 2, G2);

- 22 patients with HAM-D = 19 (group 3, G3);

- 14 patients with HAM-D = 18 (group 4, G4);

- 3 patients with HAM-D = 17 (group 5, G5).

Survey and evaluation after two weeks of treatment proved the improvement of depression symptoms, evidenced by psychiatric examination and HAM-D scores. The same trend was evidenced by further evaluation after six weeks of treatment, as evidenced in Figure 4). One can notice that patients with a HAM-D score of 25 at the beginning of the survey (severe depression, group G1) got to a score of 19 (moderate depression) after 2 weeks of treatment and, further, achieved a score of 11 (mild depression) by the end of the survey (within 6 weeks of therapeutic scheme applied). The same improvements in HAMD-D scores are observed for the patients classified in the other four groups (G2-G5).

Figure 4: HAM-D scores for RRMS patients after the therapeutic plan

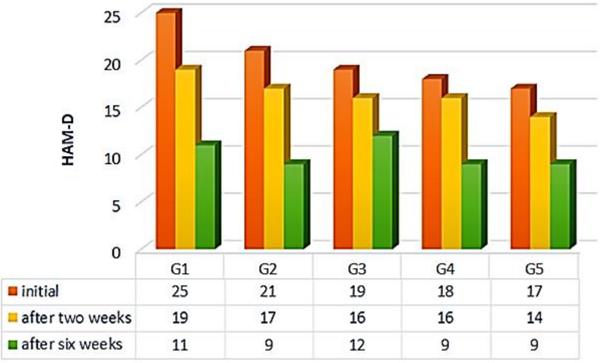

	G1	G2	G3	G4	G5
🟥 initial	25	21	19	18	17
🟨 after two weeks	19	17	16	16	14
🟩 after six weeks	11	9	12	9	9

DISCUSSION

There are different signs of multiple sclerosis caused by the location and severity of the myelin sheath lesions. Some alarm signals for this illness are: disorders in equilibrium, visual disturbances, and loss of ability for independent motion.

There is a common opinion regarding the risk of immobilization for patients with multiple sclerosis but, the highest risk for them is that of suicide. Depression represents a real problem in patients with multiple sclerosis, with about 50% of them exhibiting at least one depression symptom.

This research study is focused on therapeutic particularities of depression, in patients with relapsing-remitting multiple sclerosis (RRMS). Even though the patients in the sample group previously practiced either psychotherapy or additional actions for healing depression, the results did not prove to be fundamentally encouraging.

In the sample group, there are 18 males - from 18 up to 30 years old and 40 females aged between 30 and 40 years. This proves the higher percentage of females having the diagnosis of multiple sclerosis.

Depression disorder diagnostic was given to the 58 sample patients based on psychiatric and psychological examination by the total Hamilton Depression Rating Scale. There were considered five groups, depending on HAM-D test scores, all pointing toward severe to moderate depression disorders. The patients are included in the national program for the treatment of multiple sclerosis.

Analyzing the obtained results, important aspects of therapeutic schemes are evidenced. First, it is obvious that any of the four antidepressants are efficient and with patients good tolerance. Surveys and evaluations after two weeks of treatment proved the improvement of depression symptoms. The same trend was evidenced by further evaluation after six weeks of a customized therapeutic scheme.

Then, it is to be emphasized the lack of side effects that could determine interruption of the treatment. None of the sample group patients showed relevant side effects.

After two weeks of treatment, the HAM-D scores turn from severe depression up to moderate depression (for groups G1, and G2) and from moderate depression to mild depression (groups G3, G4, G5). After 6 weeks of the therapeutic scheme applied, in each of the groups, the result was that of mild depression.

The variation of HAM-D scores, through the survey, for each of the groups, G1-G5, is evidenced in Figure 5. Regression models (dash lines) that fit the survey data (curved lines) are marked along each of the regression lines, just to prove the tendency (by the negative slope) of decreasing the HAM-D results.

Statistical processing of data resulted in mean values of HAM-D scores as follows: 20.28 when starting the survey (initially); 16.69 after two weeks of customized therapeutic scheme and 10.67 at the end of the survey (after 6 weeks), as evidenced in Figure 6. It can be noticed that the trend was that of decreasing HAM-D scores so that the patients' symptoms of depression turned from moderate depression (scores of 17–23) to mild depression (scores of 8–16).

Figure 5: HAM-D models during the survey

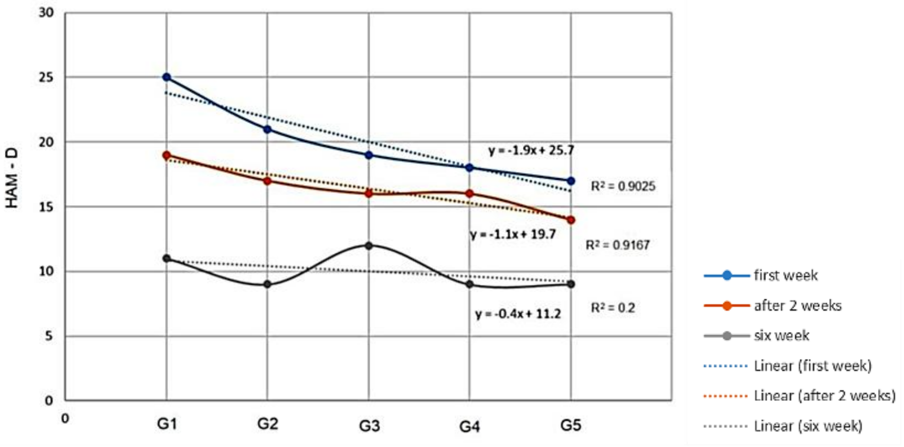

Figure 6: HAM-D mean scores

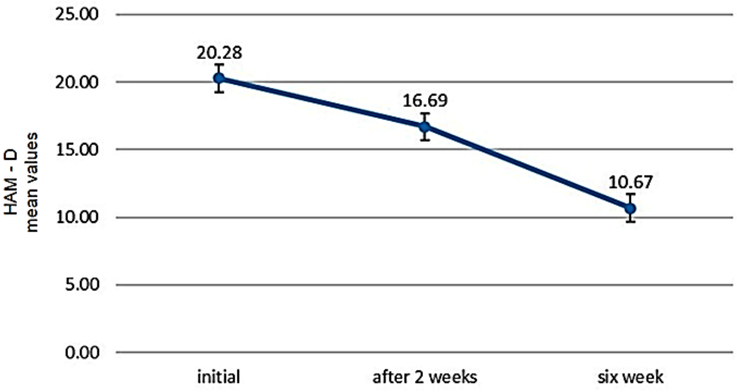

The items of the HAM-D scale that target somatic aspects (psychomotor retardation, agitation, depth of sleep, wake-up insomnia) are less specific when evaluating depression and anxious depression in patients with associated relapsing-remitting multiple sclerosis.

CONCLUSION

Depression is, relatively, common in MS patients, with approximately half of them being diagnosed with it. There is no exact knowledge of its relevance but it is assumed the influence of many factors like: psychosocial, immunological, and brain injuries. It is not sure if some MS patients have depression as a multiple sclerosis reaction or if depression is part of this illness's biology.

Many times, depression is underdiagnosed in MS patients because some of the depression symptoms also belong to the disease (fatigue, concentration difficulties, psychomotor slowness, sleep disorders, sexual disorders).

There is a negative relationship between multiple sclerosis and depression, as depression can be a factor for worsening MS and reverse. Most of the MS patients exhibit both anxiety and depression symptoms, the predominant feature of their syndrome being the one that points towards depression, anxiety, or, mixed anxiety-depressive disorder. The depressive symptoms are in remission with adequate treatment (psychotherapy and/or pharmacological) thus improving the quality of patient life.

Depression treatment for MS patients stands in two categories, one is psychotherapy and the other is pharmacological. Additionally, these, complementary actions could be performed by the patients as, for example: doing things that make them happy, activating a stress management program, belonging to a group of people that share similar problems, etc.

The research results on therapeutic particularities of depression are presented in this article. The sample group used in the study is made of 58 patients with relapsing-remitting multiple sclerosis, out of which 69% are females. They are between 18 to 40 years old.

The customized therapeutic scheme developed is based authors' expertise in the treatment of relapsing-remitting multiple sclerosis and mainly consists of the administration of one of the following antidepressants: venlafaxine; sertraline; mirtazapine; trazodone. The antidepressants are prescribed only considering particularities of the patient's health, like: exhibiting or not exhibiting hypnic jerk; evidence of appetite decrease, or weight loss.

The diagnosis and the severity of depression were evaluated by the total Hamilton Depression Rating Scale (HAM-D). Periodic evaluation of patients' depression symptoms evidenced improvement from severe to moderate to mild depression, after two weeks and, respectively, six weeks of therapeutic scheme.

Further research development would focus on different treatment schemes for depression disorders in MS patients, as well as on psychotherapy and sustained family involvement. It would also be pay attention to the complementary/additional steps in depression customized treatment as: stress management program - meditation; belonging to a community - so that to take part in targeted activities (walks, talks, reunions); find something that makes happy and enjoy the day; help other people in need - gets a sense of worthiness and utility.

Maybe, more adequate screening could be done using the Beck Depression Inventory (BDI) as it does exclude the items on somatic symptoms. It was not used in this research as the clinic where the survey was developed did not commonly apply the inventory for assessing depression severity.

Finally, it could be stated that a customized therapeutic scheme for depression disorders in patients with multiple sclerosis is considered to be the main tool for reducing symptoms and improving, even if possible, eliminating the depressed mood.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

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Authors' contribution

Conceptualization, E.F.L. and S.L.; methodology, D.G.C. and G.P.G.; software, A.B.; validation, G.P.G.; formal analysis, S.L.; investigation, H.M. and D.G.C.; resources, E.F.L. and S.L. data curation, G.P.G. and A.B.; writing—original draft preparation, D.G.C. and E.F.L.; writing—review and editing, E.F.L. and G.P.G.; visualization, S.L. and D.G.C.; supervision, G.P.G. and A.B.; project administration H.M. and S.L. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Clinical Hospital CF2, Bucharest, Romania; (protocol code 1121/12.10.2023).

Data Availability Statement

Data is available on reasonable request.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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CLINICAL PRACTICE

Papillary thyroid carcinoma arising on a hypertrofic pyramidal lobe

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INTRODUCTION

Development of thyroid gland starts by the pharyngeal epithelium thickening floor which later forms a diverticulum which, in its development is pushed caudally. The descent path is usually anterior to hyoid bone but it can also be posterior or through the hyoid bone and ends on the anterior surface of the first few tracheal rings.

This primitive steam that connects primordium with pharyngeal floor becomes thyroglossal duct. Until the second month of gestation thyroglossal duct devolve, leaving at its place a small lump – the foramen cecum, at the unification of third medium with posterior third of the tongue, but portions of the duct associated with thyroid tissue can persist at any site between tongue and thyroid [1, 2].

Cells in the lowest portion of the thyroglossal duct which comprises normal thyroid tissue differentiates in forming pyramidal lobe of the thyroid gland. Pyramidal lobe often comes out from the thyroid isthmus, but can also come from the medial side of one or both thyroid lobes [1]. The pyramidal lobe is thought to be present in 15-75% of the general population [1, 3]. Thyroglossal duct fails to involute in approximately 7% of the population [4].

Many remnants of thyroglossal duct are never detected clinically (2) and malignant transformation is uncommon [5].

CASE REPORT

We present the case of a 50 years old female patient with chronic renal failure of unknown etiology (probably secondary to hyper blood pressure), with Graves Disease (in treatment with antithyroid drugs for about 1 year) who was admitted in our department after she has initially presented in an ENT department (Otolaryngology) for the investigation of a tumoral mass located in the midline upper neck. At that moment physical exam revealed a 2/3 cm mass between the hyoid bone and the thyroid cartilage, mobile on swallowing, painless spontaneously and on palpation, covered by normal skin. IRM exam revealed a bilobate lesion 2.9/1.1 cm, very well encountered, in close relationship with the adjacent muscles and the hyoid bone, without any other pathological changes on the structures of the anterior neck: no enlarged lymph nodes. There were no reference about thyroid gland on the IRM report. The IRM conclusion was: expansive anterior cervical soft tissues tumoral mass. Surgical excision of the tumoral mass and simultaneous removal of the central portion of the hyoid bone (Sistrunk procedure) was performed and the pathology report showed papillary thyroid carcinoma arising from thyroglossal duct. In our department, three months later, thyroid ultrasound showed hypoechoic, inhomogeneous echotexture suggestive for chronic autoimmune process, hypoechoic nodule with discrete Doppler flow, without hypoechoic halo located at the connection of the right thyroid lobe with isthmus and two micro lymph nodes with intense Doppler flow, without hilum of 0.5/0.4 cm and 0.7/0.4 cm located anterior of the larynx. Tumoral mass previously removed in the ENT department was reviewed and the pathology report showed tumoral multilobulated pyramidal thyroid lobe with pattern

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of solid and sclerosant variant of papillary carcinoma, infiltrative into fibro conjunctive, adipose and muscular adjacent tissue (Figure 1 and Figure 2) and areas of micro angioinvasion of the capsule (Figure 3).

Figure 1: Papillary carcinoma infiltrative into fibroconjunctive, adipos and muscular adjacent tissue (x20 HE)

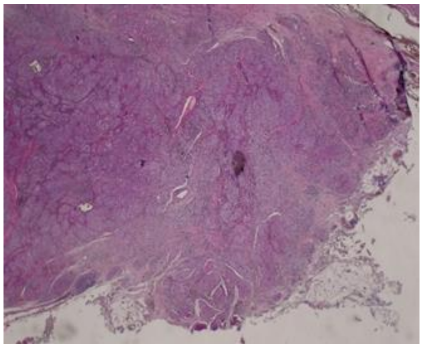

Figure 2: Papillary carcinoma infiltrative into fibroconjunctive adipose and muscular adjacent tissue (x40 HE)

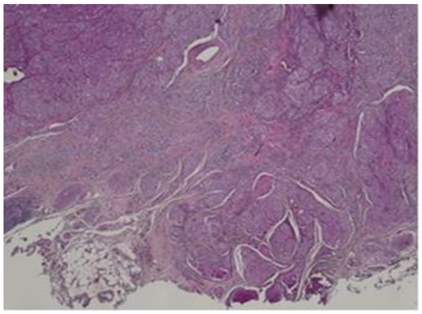

Figure 3: Micro invasion of the capsule (x200 HE)

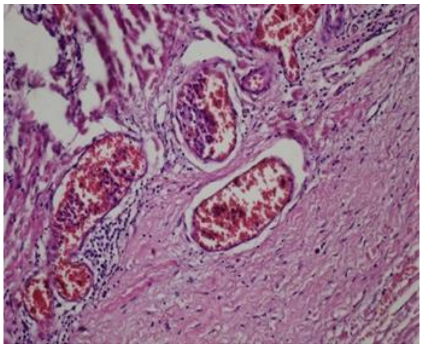

In this context, we decided total thyroidectomy (also as a treatment for Graves Disease) with dissection of central lymph nodes compartment. Histopathology report revealed embedded thyroid isthmus focus of papillary thyroid carcinoma 0.7/0.6 cm with tumoral pattern similar to that of tumoral pyramidal lobe previously removed (Figure 4), with areas of marginal invasion of the capsule (Figure 5) and micro angioinvasion of the capsule (Figure 6). The pathology report also showed reactive lymphadenopathy and post surgery staging was pT3N0.

Figure 4: Embedded thyroid isthmus focus of papillary thyroid carcinoma (x20 HE)

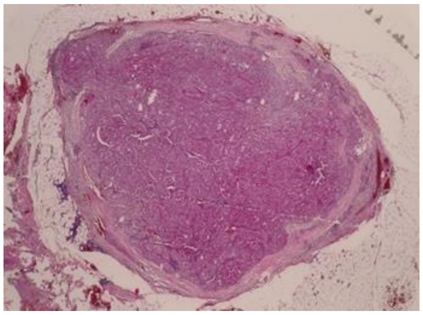

Figure 5: Marginal invasion of the capsule (x100 HE)

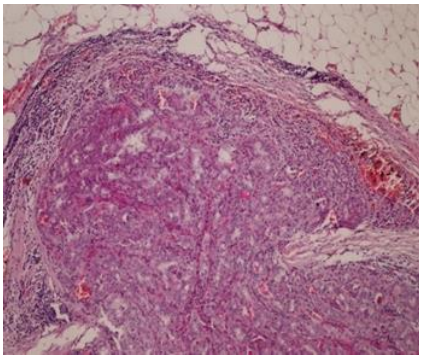

Figure 6: Microangioinvasion of the capsule (x100 HE)

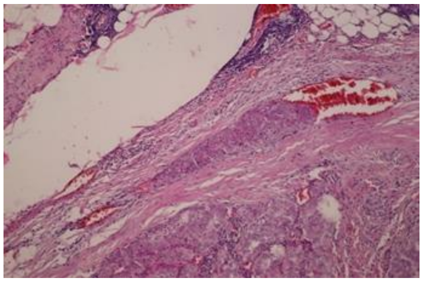

Radioactive iodine therapy was decided. Postsurgery, thyroglobulin level after Levothyroxine withdrawal is 0.2 ng/ml with unmeasurable thyroglobulin antibodies and 50 mCi 131I was administered. Post ablation thyroid scan showed no uptake of 131I in the thyroid bed or elsewhere in the body. Because of the association of BRAF mutation with papillary carcinoma of the pyramidal thyroid lobe, genetic testing of BRAF gene were performed and they were negative for somatic mutations in the 600 codon.

DISCUSSION

The detection of a pyramidal lobe or a thyroglossal duct in patients with hyperthyroidism is indicative of autoimmune hyperthyroidism. The vestiges of thyroglossal tract are more often seen in patients with Graves Disease compared with patients with autonomously functioning thyroid nodules and this is related with the presence of stimulating thyrotropin receptor antibodies [6, 7]. Pyramidal lobe can be the primary or secondary site of thyroid malignancy and during thyroidectomy, total excision of the pyramidal lobe is essential for patients with thyroid cancers who undergo radioactive iodine treatment (RAI) because the presence of pyramidal lobe prevents the increase in TSH and absorbs a large amount of the isotope and thus decrease the possibility to benefit from the treatment [8-10]. Residual pyramidal lobe can harbor cancer cells and from this point of view its total excision is indicated in thyroid cancers. In case of probable recurrence of malignant diseases due to pyramidal remnants, scintigraphy, ultrasound of the neck, computed tomography are indicated [11-13]

Thyroid pyramidal lobe can be the origin or recurrent site of papillary thyroid carcinoma. Malignant tumors arising from pyramidal lobe are rare and are associated with a high rate of concurrent thyroid cancer and it must be differentiated from thyroglossal papillary cancers because pyramidal cancer requires orthostatic thyroid surgery which is not always necessary in papillary thyroglossal cancers [14, 15]. Pyramidal lobe tumors are associated with poor prognostic factors such as: extra thyroidal extension, advanced T-stage, cervical lymph node metastasis, advanced AJCC stage (III, IV), BRAF mutation, multifocal thyroid cancer [16, 17]. In our patient there was one single embedded thyroid focus, no local lymph nodes metastasis and no distant metastasis. BRAF mutation was negative.

Malignant tumors arising from thyroglossal duct are also rare [18]. The majority are papillary carcinomas (about 94%) and less than 5% are squamous carcinoma [19]. The Sistrunk procedure that implies the simultaneous removal of the central portion of the hyoid bone to ensure the complete removal of the thyroglossal tract is enough for thyroglossal squamous carcinoma although they have a poor prognosis and a mortality rate of 30-40% [20]. On the other hand, papillary thyroglossal carcinomas are multicentric and multifocal and total thyroidectomy followed by 131I ablation and thyroid-stimulating hormone suppression is often required. There are still controversy regarding the need for total thyroidectomy, central or lateral compartment neck dissection and I131 ablation therapy in cases of papillary carcinoma of thyroglossal duct [21]. The decision depends on: tumor size (tumors larger than 1 cm require total thyroidectomy), abnormal findings of thyroid (multinodular goiter, cold nodule in a thyroid iodine uptake), histopathological findings, and the presence of enlarged lymph nodes or a history of neck irradiation [22].

In conclusion: in this report we have described a papillary thyroid carcinoma arising from a multilobulated pyramidal lobe including from a nodular peri-isthmic remnant of the pyramidal lobe (secondary to incompletely resected anterior tumor of the isthmus), in a patient with Graves Disease. The rest of the thyroid parenchyma is non tumoral. The complete resection of the thyroid is necessary because of tumor aggressiveness and the necessity of radioiodine ablation therapy.

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Atypical Cogan syndrome; case report

Gabriela C. Musat[1,2], Roxana E. Decusara[2], Ovidiu Musat[3]

Abstract: *Cogan syndrome is a rare disease characterized by the concomitance of non-syphilitic interstitial keratitis with Meniere-like vestibulo-auditory symptoms. There are criteria for the diagnosis of both typical and atypical Cogan syndrome. We present the case of a 40 years old woman with sudden onset of hearing loss, tinnitus, intense vertigo, instability associated with kerato-conjunctivitis. The concomitance of the symptoms, the onset, and the evolution under treatment are consistent with the diagnosis of atypical Cogan syndrome.*

Keywords: *Cogan syndrome, atypical Cogan syndrome; vertigo; hearing loss*

INTRODUCTION

Cogan syndrome is a disorder characterized by the association between the Meniere-like vestibulo-auditory symptoms and ocular symptoms (interstitial keratitis). Although the first to describe a disorder associating ocular and inner ear symptoms were Morgan RF, Baumgartner in 1934 [1], the name of the disease comes from Dr. David Cogan who published in 1945 a series of 4 cases of patients with non syphilitic interstitial keratitis and vestibulo-auditory symptoms [2]. In 1980 Haynes et al proposed the enlargement of the criteria for the diagnosis, defining typical and atypical Cogan syndrome. They proposed that other ophthalmologic inflammatory manifestations such as episcleritis, uveitis, conjunctivitis, can be considered as disease criteria for atypical syndrome. [3]

For a disease described such a long time ago there is very little knowledge about the etiology of the disorder. Until now, approximately 250 cases have been published but we still don't understand the etiopathogeny of the disease. It is considered an autoimmune disorder. This disease seems to affect young Caucasian adults with ages ranging between 25 to 35 years old, in most of the cases. [5]

CLINICAL FEATURES

Ocular manifestations: The main characteristic of the disease is the ocular involvement. Usually patients have red eye, eye pain and photophobia. The typical Cogan syndrome is defined by the presence of the non syphilitic interstitial keratitis. The examiner might notice granular and irregular infiltrate on the posterior part of the cornea. Neovascularization is also a possibility. Blindness and amaurisis can happen but usually the lesion regresses and the loss of visual acuity is moderate. In the majority of the cases both eyes are affected, the unilateral disease is infrequent [6]. In the atypical Cogan syndrome the vestibulo-cochlear manifestations can be associated with scleritis, episceritis, uveitis, optic neuritis, conjunctivitis or glaucoma. [7]

Vestibulo-cochlear symptoms: Cogan syndrome is classically characterized by sensory-neural hearing loss, vertigo and

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tinnitus in an association that resembles to Meniere's disease. The hearing loss might be profound, leading to cophosis in almost 52% of cases and usually it is bilateral. The auditory deficit is installed in days, months, too slow for a sudden nerosensorial hearing loss and too quick for a presbyacusys. The speech discrimination scores are poor. The hearing loss is associated with tinnitus. [4]

The vertigo can be important causing marked instability, ataxia, sometimes associated with nausea and vomiting. The nystagmus can be observed at the ocular examination. In most of the cases, complains are similar to Meniere's disease.

Other signs and symptoms

General manifestations of the disease are not rare, fever has been reported in many cases, weight loss and extreme asthenia can be found in patients with Cogan's.

Cardiac involvement, especially aortic insufficiency is present in as much as 15% of cases. Large vessels can also be affected causing heart murmur, abdominal pain, claudication of the members.

Musculo-scheletal involvement manifests as myalgia or arthritis (mono, oligo or polyarthritis).

Neurological signs appear in ¼ of cases [8], patients might have paresis, hemiplegia, aphasia, cerebellar syndrome, pyramidal syndrome, spinal cord disorders, epilepsy, and vigilance disorders. MRI can sometimes detect lesions of the white matter consistent with cerebral vasculitis.

Cutaneous lesions might appear during attacks taking the form of urticarial rash, vascular purpura, ulcerations or nodules.

Some patients might have gastro-intestinal or pulmonary symptoms.

Laboratory investigations

Biologic parameters can be modified in Cogan syndrome, especially during the attacks, but no laboratory test in pathognomonic for the disease. Leukocytosis, elevated ESR, anemia, hyperfibrinemia may appear. Several immunelogical modifications also can be noticed: rheumatoid factor, antinuclear antibodies, cryoglobulins, lupus anticoagulant but none of these are specific or relevant for the disease. [9]

In small series of patients, some authors determined the presence of specific antibodies for the inner ear or cornea but these studies were not relevant and could not be reproduced by other authors so cannot be used to support the diagnosis of Cogan.

Differential diagnosis

The first differential diagnosis one should bear in mind when facing a rapid onset hearing loss with vestibular symptoms and interstitial keratitis is syphilis. Another important differential diagnosis is Meniere's disease but in this case the ocular manifestations are absent.

Another diagnosis to be differentiated from Cogan is Susac syndrome, a retino-cochleo-cerebral vasculopathy involving the arterioles, manifested by central neurological disorders, visual acuity loss, and hearing loss.[10] Vogt- Koyanagi-Harada syndrome is characterized by uveitis, alopecia, vitiligo and audio-vestibular symptoms.[11] . Other systemic diseases such as Wegener granulomatosis, PAN, relapsing polychondritis, Behcet disease, and Sjogren syndrome can associate vestibulo-auditory symptoms with ocular involvement.

Evolution, prognosis

In some cases, the onset of the disease is preceded by upper respiratory tract viral infection.

The vestibular and auditory symptoms can be the first manifestations of the disease in 41% of cases. in 43 % of cases the Cogan syndrome debuts with the ocular symptoms. The involvement of the two organs is usually done in approximately 3 months [8]. In cases of atypical Cogan syndrome the complete symptomatology might be installed in a long period, even years.

Usually, after the first attack the disease enters a phase of remission without evident symptomatology. There is a possibility that there are recurrent episodes that repeat at variable intervals. Once installed the hearing loss is not remissible. The vestibular symptoms diminish as a result of the compensation mechanisms. The ocular symptoms have a variable evolution, but usually respond favorably to treatment.

Treatment

As the etiology and the pathogenic mechanisms are not known, there is not yet available a codified treatment for Cogan's syndrome.

Usually the first line of treatment is represented by corticosteroids. [12] In cases where the corticodependence is installed or in cases of corticoresistance there is the possibility of using other therapeutic agents such as immunosupressants (cyclophosphamide, azathioprine, and methotrexate). [13] The corticotherapy should be prescribed in high dosage (1-1.5 mg prednisone or equivalent) and interrupted in two weeks in cases where it is ineffective. Studies show that the vestibulo-cochlear symptoms respond to treatment only in one third of the cases (orphanet). Once the deafness is installed, it is usually non-reversible.

In the last years there were attempts to treat Cogan syndrome with TNF alfa blockers but there are not enough evidence based results [14, 15]

CASE REPORT

We present the case of a 40 years old woman with no remarkable medical history who presented at the emergency department of our hospital accusing sudden onset intense vertigo and dizziness, tinnitus and hearing loss in the right ear. The patient was admitted in the ENT department.

The physical examination of the patient yielded no relevant data, the ENT exam was within normal limits.

The vestibular examination pointed out to an important instability, the patient was unable to maintain orthostatic position or walk without support, no spontaneous nystagmus.

The cerebral IRM examination did not reveal any vascular or tumoral lesions. The neurologic examination did not discover any motor or sensorial deficit, no signs of localization.

The audiogram performed initially can be visualized in Figure 1. We diagnosed a profound sensoryneural hearing loss for the right ear and a medium sensory-neural hearing loss for the left ear.

Figure 1: Initial audiogram

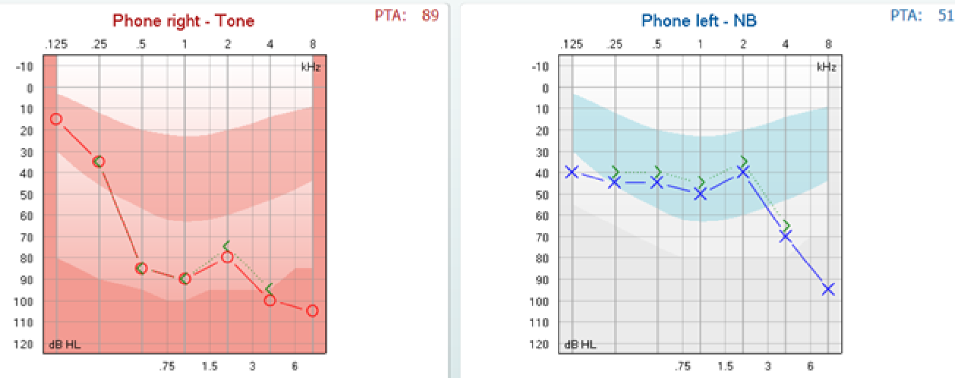

The rheumatologic examination revealed no remarkable findings: no arthritis, no cutaneous lesions, and no ocular symptoms.

The blood hematological and biochemical parameters were modified showing a slight leukocytosis with a white blood count of 11000/mm^3, the ESR also slightly elevated 25 mm/hour and the CRP had the value of 5. The modifications were interpreted as a reaction to an acute dental infection the patient had at that moment.

All the immunologic tests we performed were in normal limits, IgA, IgG, IgM, ANA, antibodies anti beta 2 glycoprotein, antibodies antiphospholipid were tested and the values were normal. Antibodies Ig M and Ig G for Epstein Bar virus, Toxoplasma, HIV, herpes virus, cytomegalovirus were all negative.

The ophthalmologic examination did not find any modifications of the anterior ocular pole at that initial moment.

The computerized posturography we performed at admission showed a severe vestibular deficiency pattern (Figure 2).

The videonystagmography with caloric testing evidenced a total right areflexia and an extremely important left hyporeflexia (Figure 3).

We started a treatment with high dose corticotherapy (solu-medrol), antiemetic (osetron), vestibular suppressant (diazepam), vasoactive agent (pentoxyphilin), vitamin (B1 and B6), plasma expander(dextran 40).

During the treatment, the patient presented a fluctuating evolution. The hearing level fluctuated especially on the left ear with PTA between 30 and 60 and on the right ear with PTA between 60 to 90.

In Figure 4 it can be noticed the aspect of two audiograms we performed during the treatment in which we could observe the fluctuant hypoaccusis in both ears.

The dizziness also fluctuated with episodes of severe vertigo. In these episodes, the direction of the nystagmus varied. We recorded horizontal rotatory nystagmus beating to the left but also to the right (Figure 5) alternating with periods of lack of nystagmus.

Figure 2: Computerized posturography

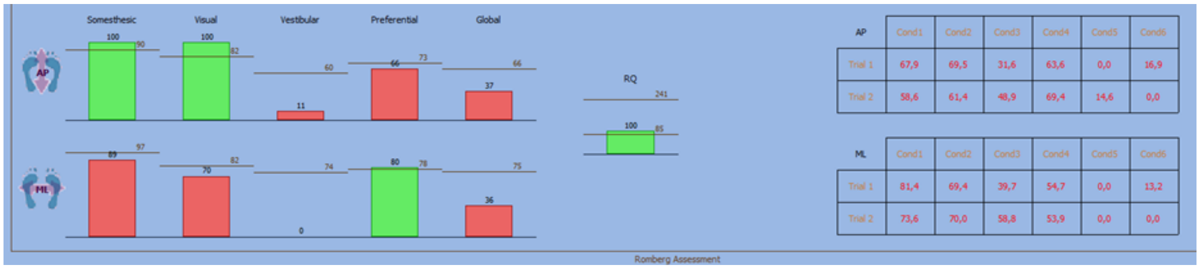
Somesthesic
Visual
Vestibular
Preferential
Global
AP
ML
RQ
100
90
100
82
60
11
66
73
66
37
241
100
85
89
97
70
82
74
0
80
78
75
36
Romberg Assessment

AP	Cond1	Cond2	Cond3	Cond4	Cond5	Cond6
Trial 1	67,9	69,5	31,6	63,6	0,0	16,9
Trial 2	58,6	61,4	48,9	69,4	14,6	0,0

ML	Cond1	Cond2	Cond3	Cond4	Cond5	Cond6
Trial 1	81,4	69,4	39,7	54,7	0,0	13,2
Trial 2	73,6	70,0	58,8	53,9	0,0	0,0

Figure 3: Videonystagmography

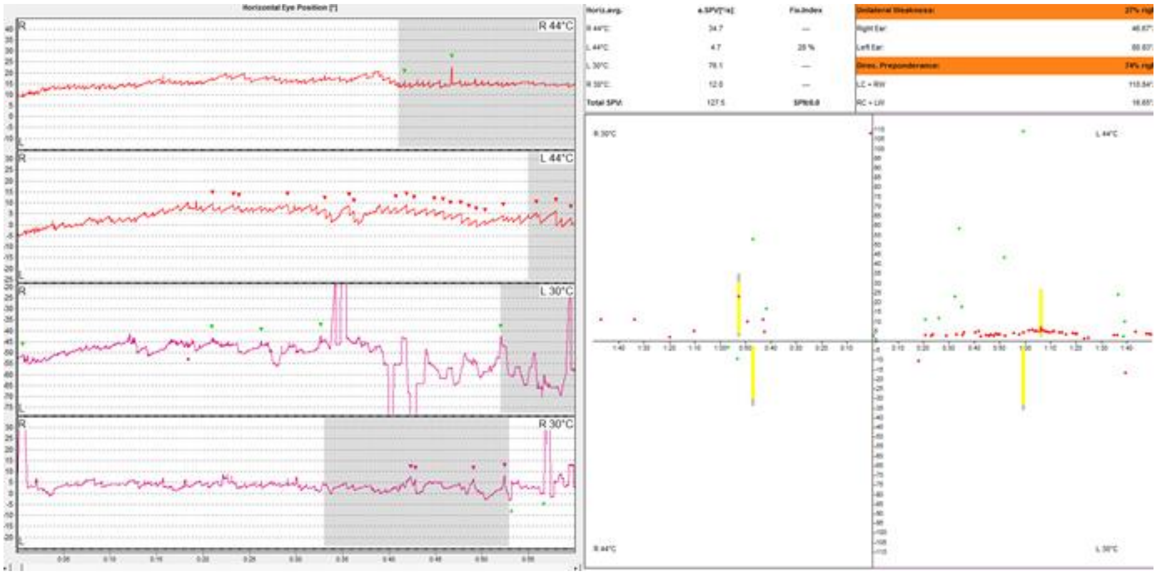

Figure 4: Audiograms during the treatment

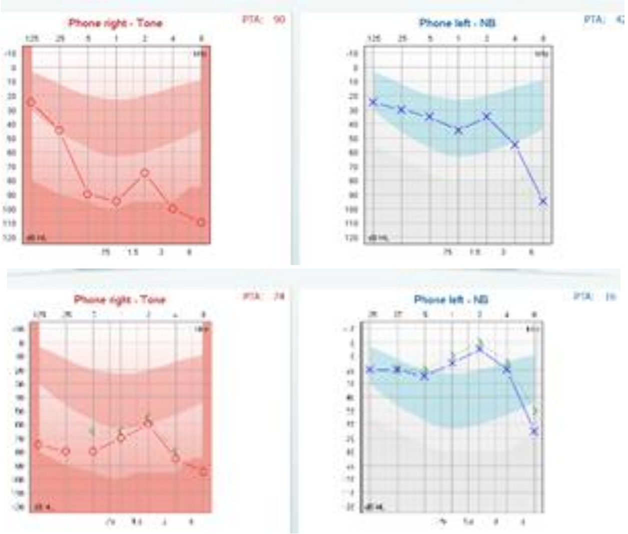

Figure 5: Horizontal rotatory nystagmus beating to the left but also to the right, alternating with periods of lack of nystagmus

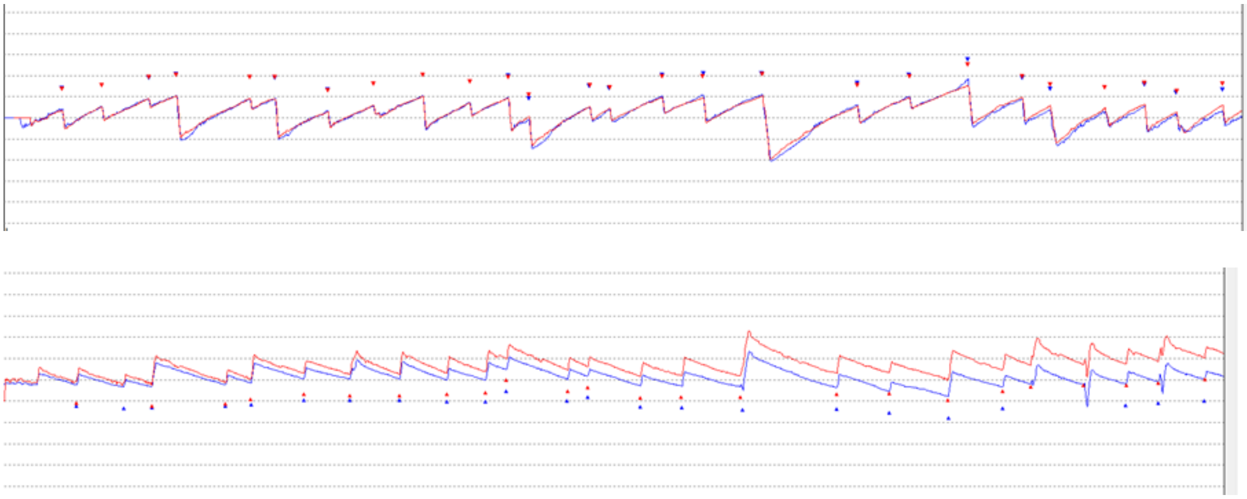

After 10 days of treatment in the hospital, the patient was discharged with an improvement of the hearing loss in the left ear, no vertigo and only a mild dizziness.

One month after this episode, the patient presented once again at the emergency department accusing intense vertigo, nausea, vomiting, tinnitus and aural fullness in the left ear. The audiogram evidenced a bilateral severe hearing loss (Figure 6).

Figure 6: Bilateral severe hearing loss

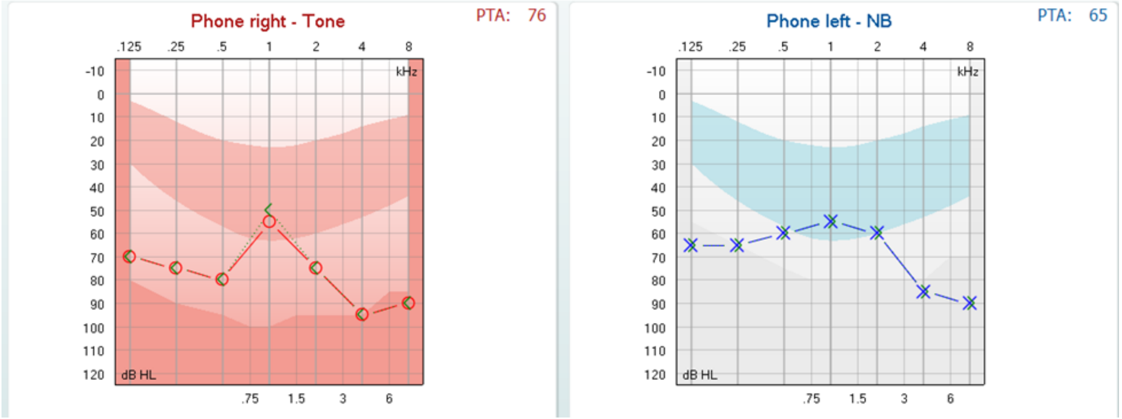

The rheumatologic examination did not reveal any modifications. The second day of hospitalization the patient suddenly presented eye pain with intense redness of the conjunctive. The ophthalmologic examination established the diagnosis of kerato-conjunctivitis (Figure 7).

In this moment, we were able to diagnose an atypical Cogan syndrome taking into account the association between the audio-vestibular symptoms with an inflammatory ocular disease.

We repeated the same treatment as in the first episode associating local eye topical corticosteroids and artificial tear solution with a major improvement of the hearing loss for the left ear PTA 10 (Figure 8).

The patient was discharged with a prescription of prednisone in low dose for a period of three months.

One year later, in the follow up, we noticed that the hearing in the right ear did not improve at all but the instability improved a lot so the patient was able to continue with everyday life. In this year she did not have any attack, no audiovestibular or ocular symptoms.

DISCUSSION

Haynes et al described atypical Cogan syndrome for the first time in 1980. The typical Cogan syndrome was described as an association between Meniere–like audio-vestibulary symptoms and non-syphilitic interstitial keratitis with an interval between the onsets of the symptoms of less than two years.

Figure 7: Kerato-conjunctivitis

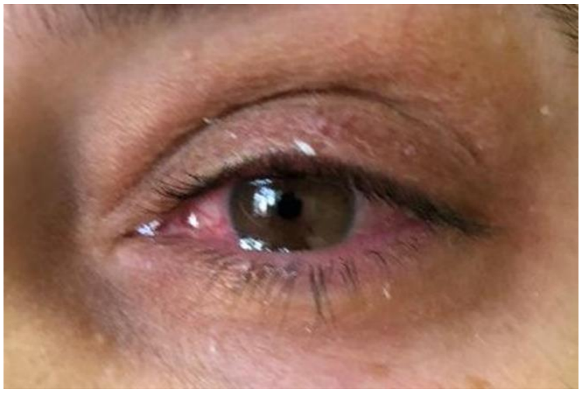

The atypical Cogan syndrome consists in an association of symptoms in which the disease criteria are grouped as follows:

- Inflammatory ocular manifestations with or without interstitial keratitis
- Typical ocular manifestations associated with audio-vestibular symptoms different from Meniere's
- A delay of more than 2 years between the onset of typical ocular and audio-vestibular manifestations.

The atypical Cogan syndrome with ocular manifestations other than interstitial keratitis tends to have a higher rate of systemic involvement with aortitis and has a worse prognosis. [3, 16]

It is the case of a 40-year-old woman with acute onset of intense audio-vestibulary symptoms with no remarkable medical history and no other general manifestations. At the first presentation the diagnosis of presumption was Meniere disease but there were characteristics of the evolution that did not entirely correspond. The disease was rapidly onset with bilateral and unequal involvement. The right ear had a profound hearing loss from the very beginning and practically did not respond to treatment. The left ear had a minor amelioration in the first episode and quite a good response in the second episode of the disease although the treatment was similar. During the admission we performed multiple audiograms evidencing the fact that the thresholds at both ears were in a continuous modification, not respecting the classical pattern of Meniere's. The videonystagmography with calorics showing bilateral vestibular lesion from the beginning of the disease, was also atypical for Meniere.

Figure 8: Major improvement of the hearing loss for the left ear

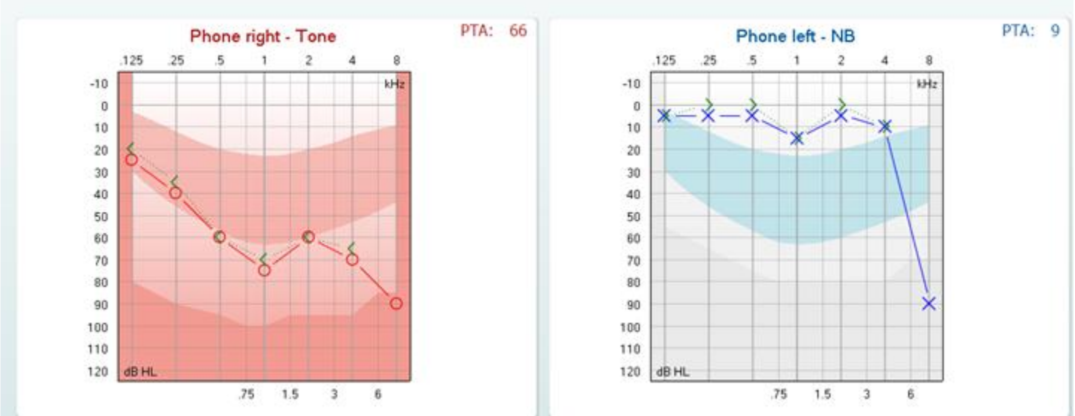

The onset and evolution of the disease made us believe that there is a autoimmune disorder of the inner ear but we did not have any disease criteria to classify. The laboratory tests were within normal limits, the minor leukocytosis and the slight elevated ESR were not noticeable. No other immunologic tests were modified or virus infections detected.

At the second episode of disease, the concomitance with the kerato-conjunctivitis was consistent with the diagnosis of atypical Cogan syndrome. We consider that this is the only disorder that can be taken into account for the diagnosis of this patient. Another remark is the fact that the patient is over the age of typical onset of the disease, but there are many reports of atypical Cogan with patients in the same age group. [17, 18]

The response to corticotherapy, was partial as the right ear did not recover, but the final result was considered satisfactory by the patient who could continue her daily activities.

CONCLUSION

Although Cogan syndrome is a rare disease, we must bear in mind that there is always a possibility of diagnosing it in the case of a patient with both vestibule-auditory symptoms and ocular manifestations.

Typical and atypical Cogan syndrome are diagnosed mainly based on clinical criteria as there are no laboratory tests able to evidentiate the disease.

The treatment consists mainly in corticotherapy. Immunosuppressants are an option in cases where corticotherapy is inefficient.

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VARIA

Patient-physician communication, an essential condition for an effective medical act

Carmen M. Voicu[1], Consuela M. Gheorghe[1]

An inconceivable reality for the medical world today is to consider the patient an active factor in his own healing process. Placement of the patient in the center of healthcare services aims at his loyalty, which means creating and maintaining correct and long-lasting relationships with him. In addition, in order to create such relationships with patients, both loyalty and stability, performance, and profitability strategies of the medical team must be thought on long-term.

Healthcare marketing situations differ, and medical units concerned with the essence of success must strive for a strategic alignment of relationships between all stakeholders (decision-makers, medical staff, patients, etc.) and the power of these relationships to improve their mutual value and even the relational context as a whole, turning patients into true brand promoters, thus enhancing marketing efforts [1].

The patient reaches to attitudes, judgements, and preferences about certain brands through a procedure of evaluating the features of these brands, developing a set of beliefs about the features that correspond to each brand [2].

Recognition of the strategic relationship that a patient desires is a medical marketing reality and involves greater commitment to accepting the challenge of creative thinking in often difficult situations, progressing through knowledge and understanding to mutual trust, converting emotions into balanced normality and the relationship thus created into an emotional-connected and loyal one for a certain period of time. This means concentrating efforts on patients, attention, and receptiveness to his wishes, but professionalism and rigor in such a way that his personal health expectations are not in vain. Moreover, it is very important to pay a close attention to communicating with him; it is even said that this type of communication is the broker of the relationship developed in a sensitive and dynamic environment like the healthcare field.

The complexity of providing healthcare services, on the one hand, and the wishes and expectations of patients, on the other hand, have as result special patient-physician communication valences and turn it into an essential condition. Permanent and effective communication with patients has become a condition of the existence of a qualitative medical act.

Given the continuous increase and diversification of health services, communication issues are becoming increasingly difficult and require much more laborious information efforts.

Due to the information explosion, patients tend to appreciate the quality of a product or service based on perceptions rather than on reality.

That is why efforts to optimize the technological and informational processes of communication between patients and medical staff, of exploring patients' behaviors regarding interpersonal communication in electronic environments in health services, of modeling negative emotions, of ways of transforming the healthcare infrastructure so that it offers an integrated insight of information through the optimized clinical and business processes, of welfare and health management, of patient-centered networks, of investigating the influence of virtual communities on the reputations of health organizations in Romania, has become a permanent necessity [3].

The relationship between the two sides, medical staff-patient, in the healthcare field, is much more complex, addressing social, psychological, and cultural aspects, which besides the base of the therapeutic level also implies a superior level of existential communication. The latter is found in medical communication because the medical act interferes with the destiny of the patient, linked in turn with elements of uncertainty and individual instability [4].

Interpersonal communication was the first spiritual instrument of the human being in the process of socializing, and is defined as the communication that occurs between two people in the context of their relationship and which, as it evolves, helps them negotiate and define the relationship [5].

Communication within the health organization takes place in a complex environment, where continuously modifying favorable and unfavorable factors coexist. Communication can take many forms and can be seen in different situations, but the most important of these is undoubtedly between patient and physician, which provide much of the data needed to establish the diagnosis and therapeutic attitude.

Interpersonal communication is formed by the combination of verbal forms (oral and written), nonverbal forms (gestures, mimics, posture, movement, aspect) and paraverbal forms (by voice attributes accompanying the word, such as intonation, rhythm, verbal flow), but, considering the importance of the information content of patient-physician communication (the diagnosis and treatment process), in the medical system, the emphasis is more on verbal communication. Non-verbal and paraverbal forms are important from the point of view of their emotional effect and the formation of sympathy.

In the health system, the relationship between the two sides, the medical staff-patient, is much more complex, involving a higher-level therapeutic type of existential communication. This type of communication [4] is involved in medical communication, because the medical act interferes with the destiny and evolution of the patient, being in turn linked to elements of uncertainty and individual instability.

On the other hand, the position of the two entities, namely the medical staff-patient, is different and unequal [6]. This relationship is established between members of two distinct social groups in terms of their prestige, power, and orientations. Thus, the physician has an extremely high status, given the level of information held and the specialized guidance through which he exercises his full authority.

The social role of the patient demonstrates his vulnerability, being forced to seek support whenever he needs. Thus, the patient is the most disadvantaged person, being influenced by physical and mental suffering, feeling the disease as a source of uncertainty and insecurity, while the physician is regarded as a person with multiple qualities, full of energy and sometimes with magical powers.

Gaining a high communicative competency needed by the specialist to create a real therapeutic alliance requires not only solid medical knowledge to diagnose and treat the disease but also the ability to obtain as much information as possible from the patient and interpersonal skills to respond to feelings and patient concerns and the ability to create and maintain the therapeutic relationship as a concrete offer of information and medical education [7].

Health communication is “the study and use of communication strategies to inform and influence the choices people make regarding their health”, and health information technology includes “digital instruments and services used to enhance self-care of patients, assisting patient-provider communication, informing about health behaviors, preventing health complications and promoting equity in health” [8].

A major concern of health services and primarily of hospitals has become communicating with patients, the quality of information provided to patients by physicians and the rest of the medical team. An essential component is the transmission of information, which has become increasingly important in medical deontology. This development is primarily driven by wishes of patients, more trained in health issues, and more cautious about the quality of the explanations they are given.

In the hospital environment, where the patient is subject to the attention of medical teams, the coherence of “what is said and what is not” becomes a permanent goal for patients and their relatives. Because, beyond anything “...there is an informational asymmetry between the physician and the patient: when they find themselves in the hospital (...), they feel ill, they do not feel what is going on with them, they do not know the possibilities of medical science, so they empower the physician with the freedom of choice” [9]. A good professional behavior is to state exactly how we are feeling and to write what we are saying. However, each physician is the supreme judge of how he or she does and can enrich his or her oral information by using information charts and, if considered necessary, by other documents or video support.

In healthcare services, access to online information has made patients more aware of their needs and desires so the result has materialized in a rigorous selection of service providers.

Therefore, healthcare managers and providers need to understand the stages a potential patient is going through in an online buying process or when expressing an intention to buy an online health product or service.

The winners will be those who build trust from the patient's perspective, with three progressive complex components in the relationship between the brand and him: credibility, care, and congruence. Certainly, what is very important is the amount of money spent by the patient for the recovery of health.

Nowadays, in health systems, the widened marketing mix enjoys considerable attention. It provides a simple organizational mechanism to understand the problems arising from the concerns of medical service providers. The widened mix has also developed as a partial solution to some of the most important problems faced by managers in terms of quality control of healthcare services provided and the relationship with patients in the process of providing these services. The widened mix explicitly recognizes the role of the staff and patients in the service delivery process, highlighting the role of marketing played by both sides.

The inclusion of Physical Evidence, Participants, and Process as distinct (contextual) elements underlines their importance and impact on marketing of health services. In addition, the key objectives are to increase patient satisfaction, quality of medical services offered and long-term patient gaining, which means that the widened marketing mix management is directly related to these objectives.

Due to the immaterial nature of healthcare service and the fact that, usually, they cannot be checked beforehand, patients are looking for tangible evidence of what they are about to experience in a particular immediate confrontation with the medical service. Even if a patient has a high experience with such a healthcare provider, variations, and contextual elements will affect patient's expectations related to the immediate confrontation with a medical service.

Elements of "Physical Evidence", such as noise level, smell, temperature, time, comfort, and even nutrition, will influence the perceptions of confronting with the healthcare service requested. Similarly, the attitudes and behavior of healthcare participants will influence the perceptions of current performance. Contextual elements can also influence satisfaction in the confrontation with the medical service, through their effects on attributions (of causes) for invalidation. Theory of attribution asserts that when results are not in line with expectations, people tend to look for reasons. The importance they give to "why", will influence the final evaluation of the outcome.

The communication process is very important within the medical unit; it is a process that affects the quality of the other processes. Through the positive impact on communication within the medical unit, the style oriented towards maintaining socio-emotional aspects and the leadership style oriented towards relationships is beneficial to the processes within the medical unit and to the emergence of positive socio-emotional states. This leadership style is associated with the existence of high quality communication relationships, which leads to the improvement of the quality of the other processes [10].

In the new context of health, development processes will need to focus not only on solving current problems, but also on anticipating future problems, not just on certain types of processes, but as many as possible, not just on technical resources, but more on human ones. Organizational-managerial excellence seeks not just the adaptation to circumstances, but also the becoming and the power to create the circumstances [11].

The depth of the process of change in health, its size, and its dynamics depend not only on the political will and on the subjective aspirations, but also on the existence and sufficiency of the necessary objective conditions, the managerial ones being the first.

In the context of today's healthcare problems, the special role of the manager is imposed by the need to create a general capacity for innovation, flexibility, stability, and ensuring success even in extreme situations. In this context, the theoretical but especially the methodological approach of the marketing of the medical system has special practical valences, requiring a change in the mental attitude of health managers towards an innovative strategic hospital marketing oriented towards and for the benefit of patients [12].

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VARIA

The tree we throw stones at

Mihail Mihailide

I stop to catch my breath on the crest of a hill in Predeluţ.

It's late autumn with a blue-diamond sky. Nevertheless, there is fresh snow fallen on the rugged tops of the mountains and on the hedges of dark fir trees at the foot of the Bucegi mountains – looking as if they were mounted lancers. The snow is glistening.

I feel the tall grass, dried by the season, under my feet. On the twisted and black branches of the old apple trees, red fruit swing in the scented wind. Around the roots of the some apple trees – there are spots of royal purple perennial irises. Although poisonous flowers, I can't resist their calling beauty and I lift a few threads.

Back on track, I turn my bicycle towards Bran, I descend by the Castel, pass by Vama Medievală, and ride along Turcu River to the place where it sweeps in Șimon stream. Then I turn left and head towards the Brandeberg guesthouse. Here I securely park my "Pegas". My destination is not the hotel but the building across the street, Parascheva Holy Church, on 340, Iancu Gonţea Street, whose frescoes were painted about three centuries ago by Nicolae Zugravu from Turcheş – Săcele. It is believed that he also put his talent at work at the Three Holy Hierarchs Monastery in Iași.

My objective as a traveller is not the church but the "cultural and medico-historical heritage" provided by the resting places around this holy edifice. Many important Romanian intellectuals – several of them physicians – were born in the village of Bran, today a tourist attraction, but also in the nearby villages; many sleep their eternal sleep in the cemetery of Şimon village, under crosses with their names engraved on them. I intend to leave the bunch of irises on Iancu Gonţea's grave… The surrounding mountains and the forests that reached the yard of my house when I was a child were like the lords of the land for me... It is at Şimon that I built a vault in order to make sure that I would not sleep my eternal sleep anywhere else. "Today, a commemorative plaque and the name of a street in Bran remind us of this son of the region", noted Emil Stoian, the brave chronicler of this beautiful mountain village, to whom the inhabitants owe a major contribution for preserving the image of those who really meant something for the Romanian people. Such people are numerous, in Bran: historians, philologists, lawyers, physicians, actors, officers, priests, professors and academicians; participants in the Great Union of 1918; heroes of the two World Wars. I have met some of the physicians...

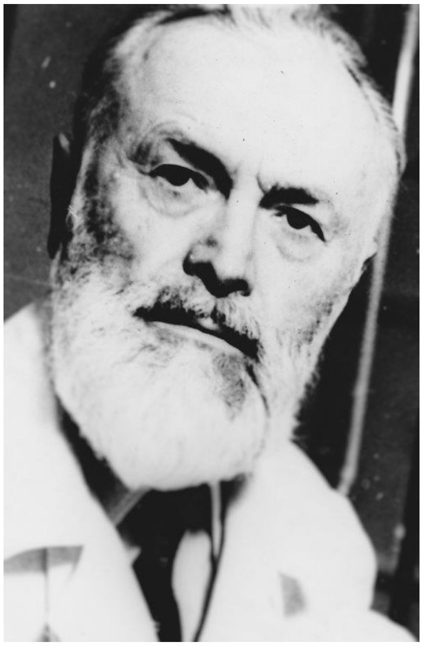

Found at the location, I feel disappointed: where is the tomb? I am told to go two kilometers uphill on the right side of the road. This time I decide to walk. Indeed, after passing the Primary School, on the yellowish wall of which a thin marble plaque reminds that the scholar attended his first classes "here", I pass by the house and the yard where he was born and spent his childhood, its gate always locked, and arrive at the church dedicated to St. Nicholas, standing a few meters retreated from the main street. Here, behind the building is a small cemetery. At its end, close to the hill, I finally find the vault: "Fam. Prof. Dr. Doc. Iancu Gonţea" with two rows of vertical crypts! It looks rather like a bunker. In the crypt by the side of the teacher lies his wife, Dr. Lucia L. Gonţea (1907-1998, born Popescu) buried 22 years after the professor.

From an oval photograph, embedded in the austere concrete wall, the professor gives me a harsh look. Where should I put the irises brought as a modest homage? It's difficult to find your way between the graves...

I have synthesized ergo-biographical data regarding Prof. Iancu Gonţea in a book [2], using sources coming mainly from the archives of the medical-historical documentation library of the National Public Health Institute of Bucharest, an institution whose enrichment with new information (manuscripts, photos, video tapes, books, magazines, etc.) and modern digitization was stopped for various reasons... Iancu Gonţea was one of my professors in my fourth year at the Bucharest Faculty of General Medicine. Decades have gone by. Before proceeding to "serious things", I ask the reader's permission to reproduce a few paragraphs, surely forgotten by the reader, perhaps funny, from the notes about the professor of the Food Hygiene Department, who was born in Şimon and returned there at the end of his life.

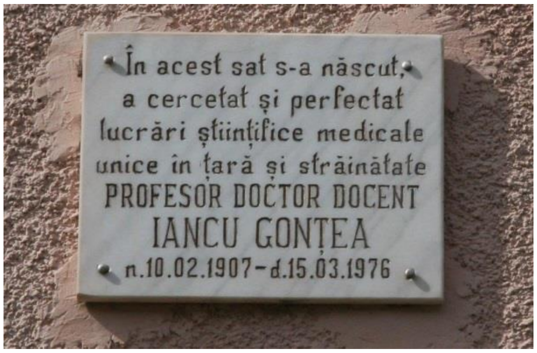

În acest sat s-a născut,
a cercetat și perfectat
lucrări științifice medicale
unice în țară și străinătate
PROFESOR DOCTOR DOCENT
IANCU GONȚEA
n.10.02.1907-d.15.03.1976

"We were two groups of fourth-year students of General Medicine, huddled in front of the laboratories of the Institute of Hygiene in Bucharest, where we were to take an oral exam in Food Hygiene. The professor – an unforgettable person... tall, slim, with penetrating eyes and a Hemingway-style beard, wearing an impeccable white gown – was sitting at the teacher's desk, on a podium, with the assistant group on his right, and us, the wretched students (oh, how we cried out for mercy knowing his exigency!), one step down, in front of the examiners, at a tiled table full of Erlenmeyer balloons, pipettes, tripods, test tubes and dropping bottles. Usually, this type of final exam was the responsibility of the associate professors. It was only exceptionally that the professor would waste his time with us. Well, there we were, living... the exception! Each of us picked from a jar the ticket with the subjects written on it, thought of them for a few minutes and answered the three questions, in the order in which they were typed.

After ending our monologues (rather scanty, more often than not), followed a dialogue with the professor, Iancu Gonţea himself. At the end, he wrote down the grade that each of us deserved in our grade-books and we tiptoed out of the lab. In the hall, we were approached by our classmates who, browsing through their notebooks, were trying to learn everything they hadn't learnt until then, before entering the lab room „to have their heads cut off".

– What subject did you pick? Does he also ask questions from «the practical experiments»? Is he harsh, is he kind? What grade did you get? Many of the questions were difficult to answer promptly... At a certain moment, one of the best students of our class came out of the lab, almost bursting into tears, obviously confused.

– What happened? What subject did you get?

– The third question, she explained us, as she started to feel better, was about the trophic content of the main foods. I explained how many proteins, lipids, fats and calories can be found in cow's milk, eggs, fish, chicken and I don't know what else I mentioned... I couldn't realize whether he was satisfied or whether I had gotten it all wrong. I finished what I had to say, but then he asked me additional questions, looking at me over his glasses:

– «What about ordinary vegetables, which of them have a higher vitamin content?» I gave the wrong answers, I stammered and then I said: carrots, spinach, tomatoes... Whatever!

– «What a-bout-par-sley?» he asked. I was as quiet as a fish. Total silence. He gave me the right answer and explained why his question was so important. Then he added:

– «Veronica, Veronica! You didn't really like Food Hygiene…» And then I saw him write 5 (five) in my grade-book and sign it. «You should come in the autumn to get a higher grade! he said, probably thinking that I would otherwise lose my scholarship…

The professor's question was not tricky, he had indeed told us in his lectures that parsley contains more vitamin C than lemon, orange or cabbage and, in addition to that, it also contains vitamins A, B1, B2, B6, K and E, as well as all the minerals useful to the body. Parsley is an antioxidant, antiallergic, antitumoral, which stimulates the gallbladder secretion, maintains elasticity of the vessels, and I can no longer remember now, more than half a century later, what other wonders we owe to this herb, commonly used for flavoring and adorning dishes!

Veronica had only remembered what the professor's assistant had told us at a seminar, smiling, about the qualities of this ancient vegetable: «It also ensures the good functioning of the genitals and even has a powerful aphrodisiac effect! »"[3]

Writing about himself in a curriculum vitae requested by the Academy of Medical Sciences (AMS), whose member he became, professor Iancu Gonţea believed that he had set a "new trend in the science he practiced, which was seen not only as food hygiene, studied separately from the man (in a narrow technical-sanitary concept), but as the science of biomedical, psycho-social, and economic relations between man and food"[4]. He has the merit of having contributed to determining the nutritional requirements in relation to one's occupational and environmental conditions, «for the first time demonstrating that physical activity increases protein and calcium needs, as well as vitamin C consumption, [and that] exposure to chemical noxae heightens the metabolism of thioamide acids and of ascorbic acid, increasing the needs of the body.»

In the same CV requested by the Academy of Medical Sciences, the professor listed the procedures he had devised for the biochemical control of vitamin nutrition status, as well as the methodology for studying the biological value of food, both highly appreciated by international scientific bodies.

The professor was to be awarded an important international prize (Maurel) as well as the silver medal in food science for his contribution regarding natural anti-nutritional substances in food and animal feed. I. Gonţea also demonstrated that no food is complete and set up an original classification of foods (by groups), embraced by experts of his time, in keeping with the benefits and the deficiencies of various food categories."[5]

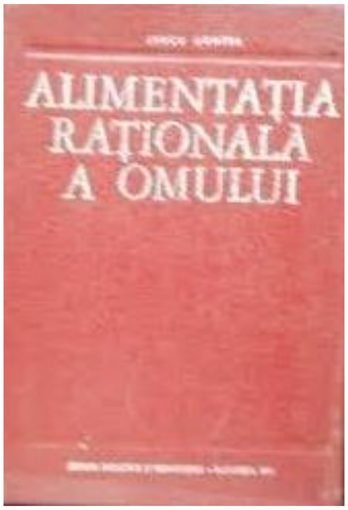

Given their originality and practical value, based on rigorous laboratory and field research, many of his writings have been translated into widely-circulated foreign languages. They cover the fields of effort physiology, pathophysiology, and medical clinic, but most of them include research on human nutritional needs under different physiological, occupational and environmental conditions: "Food Control" (1956), "Food Ration" (1956), "Rational Nutrition of Women during Maternity and Its Importance for Mother and Child Health" (1958) can be considered as major landmarks in medical literature, at least in the Romanian one. The last of the books mentioned were updated by the author, translated into German, and published by the prestigious publishing house

Gustav Fischer of Jena in 1965. Having received excellent reviews, this monograph was requested, from the German publishing house, by Dai-lichi Co. Ltd. Publishing House in Tokyo and became the first book by a Romanian scientist published in Japan, in 1974.

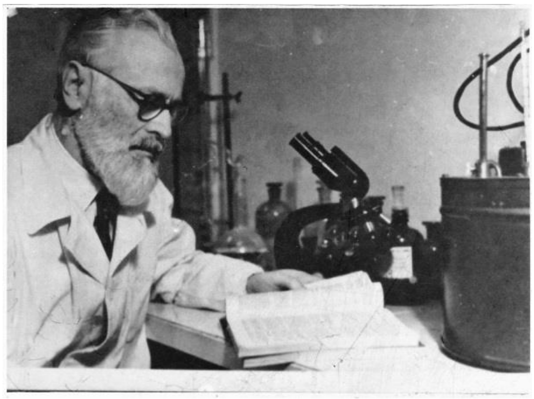

In 1963, the Medical Publishing House (Bucharest) released "The Bases of Nutrition". Three years later, another book was published: "Natural Anti-Nutritional Substances in Food and Animal Feed" (in association with Paraschiva Şuţescu, Ph.D.), subsequently translated into French and published by Vigot Frères Publishing House, in association with prof. Raymond Ferrando PhD (Paris, 1967), as well as into English, and published by Karger Publishers in Basel (Switzerland) [6] as well as in New York (1968). The Academy of the Socialist Republic of Romania awarded professor Gonţea the "Victor Babeş" prize for this book. Among the books that were published, almost every year, two titles still stand out, several decades later:" A Genetic Leap in the Nutritional Value of Corn – the Past and Present of this Cereal" (Ceres Publishing House) and "Alcoholism" (Medical Publishing House, 1976), an insight from the point of view of preventive medicine into the use and over consumption of alcoholic drinks – "considered to be a serious problem on a national level".

Professor Iancu Gonţea won high recognition in the scientific events he was invited to — international congresses and symposiums (Dresden, Moscow, where he accompanied the Romanian delegation as an expert in nutritional issues at C.A.E.R. meeting, 1969), at New Castle ("Women's Nutrition during the Reproductive Cycle", 1971), Mexico (where he presented a paper titled "Nutrition and Anti-Infectious Defense in Humans"), Austria ("The Effects of Sugar Abuse") etc. He received national and international awards and diplomas.

Under a Decree of the State Council of the Socialist Republic of Romania (153/1971), the professor was awarded the "Sanitary Merit", alongside other scientists rewarded "for the contribution to the Party's policy in the field of public health in our country". The chairman of the Council at the time was N. Ceauşescu, while the minister of health – Prof. Dr. Dan Enăchescu.

The fact that the romanian scholar did not also become a member of the Academy is rather amazing, since, forgive my insolence, many others were comfortably sitting in the "immortals"' seats of the Academy of the Socialist Republic for (scientific!) achievements, which, compared to his own, were a lot less significant...

*

Who would have thought that professor Iancu I. Gonţea – a former military physician (promoted major in 1948), hence a person whose past and family had been under the close scrutiny of the army's newly set-up personnel department and who had a "healthy" origin (being the son of a shepherd, born in the village of Şimon, on February 10, 1907), and therefore, seeming to be imbued with socialist "principles" and "ethics" – was to be placed under surveillance by the Securitate (being the subject of two files, "I. 498910")? [7]

He maintained correspondence with scientists from all over the world; often travelled to Western countries but also to the Soviet Union; he attended medical conferences; worked in the field of food hygiene, this having a great social impact. He represented the Socialist Republic of Romania in the World Health Organization. All this supposed that his loyalty to the "party and to the socialist homeland" were to be continuously tested by the "state bodies"...

The "vigilance" of the Securitate also translated into an internal decision issued on November 18, 1969, regarding the preservation of his "informative" file in the Operational Fund, under no. I. 715753, coming from the 3rd Directorate of the feared institution…

What was pursued Iancu Ion Gonţea accused of?

That "he was a member of the Iron Guard, working in the Sanitary Department of its Documentary Studies Center." That, "he was a major in the bourgeois army" (a member of the contingent of officers who took the military oath before the King). Later on that "he maintained correspondence with people from abroad, such as Prof. R. Ferrando (France) and foreign publishing houses." In an informative ("strictly secret") note obtained by major I. Ion, at agent "Gabriel" home and dated April 17, 1967, he declared that he met the person under surveillance during his studies at the Andrei Şaguna High School (1924-1927) and added that Iancu Gonţea (I.G.) "was a very good student, who graduated at the top of his class".

The note provided biographical data, generally consistent with that provided by Gonţea in his résumés. I.G. seems to been the son of poor peasants from Bran; he was not perform any legionary activities, although his uncle, a history teacher, dr. [Ioan] Moşoiu, "who was also the manager of the high school (the Andrei Șaguna Romanian Orthodox high school in Braşov) was known for his involvement in the legionary activity (he was the Commander of one of its local branches). After graduating from high school, I.G. joined the Medico-Military Institute (Bucharest) and "Gabriel" went to Prague. After 1948, they met again in Bucharest, on a daily basis, since I.G. taught at the Institute of Hygiene.

Despite being rather ill (suffering from diabetes), I.G. was very active, becoming a professor and the head of the Food Hygiene Department (Bucharest Institute of Hygiene). He was highly valued as a specialist, "Gabriel" convincingly using the communist jargon of the time: he "combines theory with practice and links laboratory research with practical activities, such as supervising in bakeries, canteens, etc." He had a vast journalistic activity, "Gabriel" citing in I.G.'s defense his contributions to Scânteia and Economic Life newspapers, ahead of the papers published in specialized magazines…

Next came a significant detail: although generally he did not have an active social life, I.G. celebrated his 60th birthday by organizing a "comradely lunch at the Faculty of Military Medicine". Among the guests featured professor [Nicolae] Nestorescu, a microbiologist and immunologist (who also was a former military physician) and lecturer Coman Petrescu, PhD (a pediatrician dealing with rational child nutrition). In "Gabriel"'s opinion, allegations that I.G. had been involved in the legionary movement activities as a student were simply "unfounded rumors."

However, in his Note, major I. Ion seemed keen to show his superiors how cautious he was: "Since I.G. is known by the Third Department (of the Securitate) as a former member of the Iron Guard, the agent will have to inform us of any data backing this assumption. In addition to that, the correspondence with foreigners will be kept under observation." Next came a list of names – French and German researchers and professors from (Paris) and (Heidelberg).

Here is what agent "Titi", who "kept him under observation during his teaching activities", wrote: "Always punctual, having a commanding attitude, he teaches the students notions of food hygiene during his two-hour lecture. During his lectures he seems to speak with passion, having a dignified attitude. From talking to other students, the source found out that professor Gonţea is very demanding during exams, asking the students to reproduce all the details, or better said the whole lecture, word by word. He does not hesitate to turn students out over the slightest mistake. His behavior gives the impression of a modest man, deeply involved in his profession."

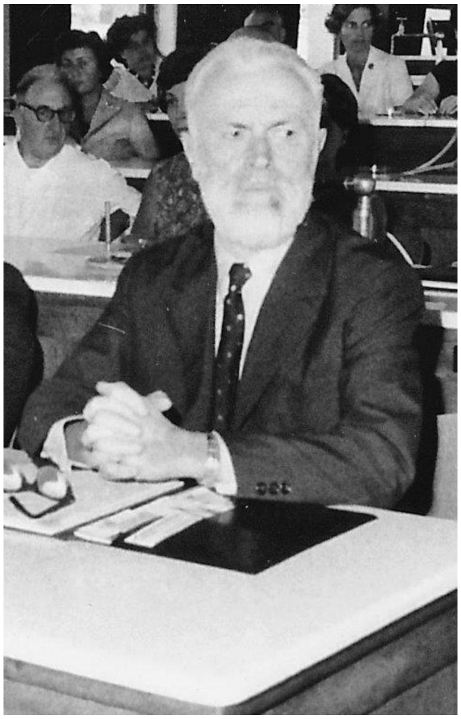

Elly – a professional informer

In Iancu Ion Gonţea's file, there is a note received by captainV. Ovidiu, on April 19, 1957, from agent "Elly", reeking of antipathy towards I.G., a text in which one can hardly tell the truth from "pure" lies. It is difficult, if not downright impossible, to check these allegations because the informer, hiding under the pseudonym given her by the Securitate, seems to be a professional of delation, citing people (giving their full names and the position held in the Institute) who could allegedly back her allegations, but who today, 60 years later, are most certainly dead...

"I inform you", wrote Elly, "that Dr. I.G., associate professor of nutrition at the Faculty of Medicine, living at 244, Dorobanti Street, phone 7.66.29, claims to be «untouchable» because he is backed by Acad. Ștefan Milcu and Acad. N. Lupu, who are «indebted» to him.

I.G. is the author of numerous fake public documents and characterizations (within the IMF – Institute of Medicine and Pharmacy), helped by former head of the personnel department At.). All these facts are known by the management. Dr. I. Per. from the Hygiene Institute, Laboratory of Food Chemistry, 1, Leonte Street, can provide references in this respect."

After this "introduction," "Elly" wrote that she knew I.G. since November 1954. "I know that he was a legionary, working in the cabinet of another Iron Guard physician, Dr. Ru, within the Legionary Ethics Committee – at the time, the steering committee of the Faculty of Medicine (?). At that time he worked wearing a green, Iron Guard shirt; at Iron Guard rallies he carried banners reading Nu merus nullus (sic!) and Nu merus claus (sic!). prof.dr. I.I.N., manager of the Institute of Physiology of the R.P.R. Academy, phone 4.20.59 (home) and Dr. Eug. D.), phone 7.31.16 (home) can give references about his activity at the time. Among the acts of hooliganism, I can mention the ones I heard about from Dr. Eug. D., namely that Assoc. Prof. Dr. I.G., under the legionary régime, tried to devastate the Cantacuzino Institute, kicking the doors when he entered the rooms, destroying laboratory equipment, etc. In the Military Sanitary Institute where he worked for some time, the whole institute knew him as a "passionate Iron Guard member" (Dr. Iac., Assistant at the physiology department, tel. 3.27. 90 ,home, can give references about this). In the bourgeois army, he was highly regarded because of the wealth he still holds, to a great extent:

- Mansion in Călimanesti
- Estate and mansion in Bran
- Houses in Bucharest
- Car etc.

Later on, Assoc. Prof. I.G. proved to be a hostile element, pleading in favor of Antonescu's criminal actions against the USSR. References: Dr. Eug. D. and Dr. P.

Thus, Assoc. Prof. Gonţea maintained ties with his former collaborators from the Legionary Nest, Assoc. Prof. Pr. Gh., from the Physiology Department, with Tudor G., also from the Physiology Department, with G. Maria from the same department, about whom I will tell you in detail further down. Assoc. Prof. I.G. has remained the same reactionary person, nurturing sympathy for the Iron Guard, and still indulging in hooliganism. Here are some facts:

1) He encourages the personnel not to apply for higher positions since exams are "based on the Russian model and have a temporary character". References can be given by C. Adrian, assistant at the Physics Faculty, phone 2. 68.09

2) Assoc .prof. I.G. sent two articles for publication to the Institute of Physiology of the Academy of the R.P.R. (People's Republic of Romania), registered with no. 36 / 6.III.1957 of the Nutrition Department:

- The bibliography of the first article includes 13 works, none of them Soviet.

- The bibliography of the second article includes ten works, all of them English and American.

- This amounts to hostile behavior towards Soviet science, and within the department, which contrary to the ideological orientation of the scientific research in the R.P.R.

3) He considers himself a "great patriot and a great Romanian". Under this mask, Assoc. Prof. I.G. hides his ferocious anti-semitism and hatred against elements dedicated to the working class. References: 1. Gabriela from Caritas, phone 2.90.40 who was an assistant at the Department of Nutrition, Dr. D. Eugen, phone 7.31.16; M. Maria. St. Constintin Street, No… Petre P. from the Cotroceni Hygiene Institute.

4) Assoc. Prof. Gonţea Iancu (hereafter names were blacked out by the CNSAS) – nurse G. Maria swore to me, in the autumn of 1955, on the health of her child, that, as head of the Nutrition Department, he had given her extra paid hours, the equivalent of a part-time job, [but] from the date of January 1, 1955 to September 1, 1955, he obliged her to give him 1/2 of her part-time wage. G. Maria told me this out of despair because (redacted by the CNSAS) his fabulous wealth helped him back the interests of the Iron Guard. [And] after she gave him this money, "which he literally grabbed from her and which were part of her due", as a reward, assoc.prof. Gonţea gave her the sack. The reason was another subordinate, dr. Şuţescu Paraschiva (anonymized words by the CNSAS) who [becoming a favorite] continued to work with I.G. G.Maria told me all this on the way between Ardealul Avenue and Plevnei Street, as she was going towards Progresului to the Rectorate of the Institute of Medicine and Pharmacy.

5) At the Nutrition Department, Assoc. Prof. Gonţea sells overpriced wool and veal meat from his estate in Bran to the staff, whom he obliges to buy, threatening to treat them badly if they don't. Assist. A. C. can give references, phone 3.11.10.

6) (Anonymised paragraph by the CNSAS) G. Maria was a notorious Iron Guard member, the daughter of the richest merchant in Constanța who lived in the countryside in Bulgaria until 1956, when she brought him back to Bucharest for good since "rich people are no longer oppressed as they used to be", according to her own words. G. Maria worked with the Legionnaires, being... (redacted). Thus she helped them set up camps in the V. Roaită resort and her father helped by providing food and transportation.

7) As far as his behavior towards students is concerned, I can mention that several of them have lodged complaints about his demagogic manifestations with the Dean’s Office of the

Faculty of Medicine, but to no avail. References can be given by student P. Aurel from the sixth year. His attitude towards the young employees of the Institute of Medicine and Pharmacy has remained fierily reactionary and he is doing his best to exclude those who had worked in ministries representing our régime. I am attaching the original statements by Prof. N., whom Assoc. Prof. Gonţea Iancu told that he would do his best so that Dr. Maria Mih. and her husband, C. Adrian, who had worked at the MAI [Ministry of Internal Affairs] would be sacked. C. Adrian was summoned to the C.C. of the P.M.R. for a mission, which Gonţea branded as a dangerous element for the reactionary clique he is a member of. (I should mention that the summons took place a few months after the appointment of assistant C. within the Medicine Faculty).

8) About Assoc. Prof. Gonţea Iancu's son, whose name is Gonţea Liviu, a fourth-year student at the Faculty of Medicine, I know that he was under investigation by the Ministry of Internal Affairs over hooliganism when he was a high-school student. I mention that Gonţea Iancu was connected to Lupaş Tudor, who provided him with articles translated from Hungarian, which had been broadcasted on the radio, which Gonţea Liviu handed to the students in his group. In a conversation with Tudor Geor., Lupas's uncle, who worked in the physiology laboratory, told me that «the guilty people are free while his nephew is in prison.»

When I asked him if Gonţea's son had been involved, he did not give a straight answer, but made me understand that he had."

(signed) Elly

We find ourselves in 1957, a few months after the Hungarian revolution (called "counter-revolution" by the Communists), when the vigilance and coercive measures of the Securitate got a new "boost", stimulating the activity of informants. Moreover, on October 27, 1956, in university cities, students were out in the streets demanding an improvement in living conditions, the removal of the Russian language from the educational curricula, and the liberalization of social life, demands which were followed by reprisals and arrests. Students and professors thus becoming a target of the newly established Ministry of State Security, an institution distinct from the Ministry of Internal Affairs, at the time led by Alexandru Draghici.

A year later, in June, a plenary session of the C.C. took place, announcing a return to the struggle against bourgeois ideology, criticism of intellectuals accused of snobbery, cosmopolitanism, apoliticism, negativity etc. Thus, the departure of the Soviet troops who had been stationed on Romanian territory for 14 years, did not seem to be auspicious to acts of dissent, especially from those who had had certain political views in the past, even if they had let themselves be carried away; on the contrary, it meant increased vigilance8.

Elly´s denunciations were obviously not based on "proletarian intransigence" or "ethical" reasons, but rather on personal, vindictive motives, which, after so many years, cannot be properly weighed. It is possible, however, that the "witnesses" mentioned by the informer may have considered themselves, for various reasons, harmed by the teacher (possibly by the exigencies that he imposed) or "stopped" in their desire for rapid ascension in the professional field or on the social ladder. In that period, the "weapon" at hand chosen by an informant in ensuring his success was invoking an alleged Iron Guard activity, or at least sympathy, on the part of the "target".

One of the graduates of the Faculty of Cluj (whose name was blacked out), was "promoted", as early as his student years, to a party activist, to the "Central Committee structure”! Starting in 1949, he held important positions in the state hierarchy: adviser to the minister of Health, then Sanitary General Inspector. He became pro-rector of the Institute of Medicine and Pharmacy in Bucharest (IMF) then, based on this rapid ascension and, of course, "being a valuable person", was named associate professor. (This rapid fast-lane promotion took barely six years).Since 1955 he engaged in research as head of department at the Hygiene and Public Health Institute in Bucharest. How could "Elly” not cite his name and indicate him as a credible witness in order to assess professor Gonţea's activity and behavior?

And what about another rival, born in 1884? The latter hoped that, thanks to the protection of his administrative superiors and by "revealing" I.G.'s alleged "Legionnary activity" and backing Elly's allegations, he could indefinitely extend his teaching term, in no matter what department, if Gonţea could be forced to give up the position he held «based on merit».

Nevertheless, we should mention that Elly´s detailed statements found in this file were not backed by any "notes" or testimonies given by the witnesses she cited.

After August 23, 1944, the issue of the legionnaires had become a major concern for the new power. In 1945, former legionnaire's members who were deemed as dangerous were arrested and sent to labour camps. Others were to be determined to become – and truly became – collaborators of the Communist Party, following Ana Pauker's intervention. On December 10, 1945, the Ministry of Internal Affairs issued an order "legalizing" some of the former Iron Guard members, who no longer posed a threat to the State and who had offered their services to the new régime. It was a bluff: the trio made up of Ana Pauker, Teohari Georgescu (minister of Internal Affairs) and KGB general Nicolski were later accused of having facilitated the infiltration of former Iron Guard members into the Communist Party. As a consequence, the three former Communist dignitaries were ousted.

At that time, however, the Legionnary Movement ghost had reappeared and informant "Elly" seized this opportunity to accuse Iancu Gonţea. It is true that many intellectuals, particularly younger ones, had sympathized with the Legionnary Movement in the 1930s, even though they later disavowed this affinity (see the notorious cases of Mircea Eliade, Eugen Ionesco, Emil Cioran.) Many intellectuals from the area of Braşov – Bran (eg, the great philologist Sextil Puşcariu), shared rightist, or even Iron Guard sympathies, an ideological confusion which they later regretted; among them there were also many physicians who had acquired some notoriety in their profession.

**

I once asked an older friend: why is there so much hatred towards a man who, through his knowledge and hard work, dedicated his life to medicine for the public welfare?

– "The he lazy man will throw stones only at the tree full of fruit, so that he can collect the fallen fruit effortlessly", my friend replied. "And then, we know from the Gospel that 'no good tree will bear bad fruit, nor will a bad tree bear good fruit.'"

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6. Ib. id. Reper 2
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8. Collective, coordinator Valentina Bilcea, „Istoria românilor. Date Fapte. Oarneni”; Foreword: Professor Adrian Cioroianu Ph.D., Meronia Publishing House, Bucharest, 2018

ADMINISTRATIVE ISSUES

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We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

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(5) Identifies trials with a unique number; and
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21 AUGUST - ZIUA MEDICINEI MILITARE

Şeful DIRECŢIEI MEDICALE
Col. medic conf. univ. dr. Marian MACRI

La 21 august 2007, se împlinesc 145 de ani de când, prin **Înaltul Decret Domnesc nr. 4629/21.08.1862**, semnat de Domnitorul Alexandru Ioan Cuza, a fost instituit *Corpul ofiţerilor sanitari ai armatei* şi *Direcţia Generală a Serviciului Sanitar Român*. S-a constituit astfel, cu începere din anul 1862, un organism specializat menit să asigure păstrarea sănătăţii efectivelor armatei române.

Încă de la începuturile ei, medicina militară a beneficiat de profesionalismul şi calităţile generalului de divizie medic dr. Carol Davila adus în ţară de Domnitorul Gheorghe Bibescu în anul 1852, care avea o împătrită responsabilitate: inspector general al serviciului sanitar al armatei, director al Şcolii de Medicină (întemeietorul învăţământului medico-militar şi medical superior din ţara noastră), inspector al adminstraţiei centrale sanitare şi efor al spitalelor.

Elaborarea *"Instrucţiunilor pentru funcţionarea ambulanţelor"* şi *"Deciziei relative la serviciul ambulanţelor în armată"* , precum şi promulgarea prin **Înaltul Decret nr. 1125/15.04.1882** a *„Legii pentru organizarea Serviciului Sanitar al Armatei"* au creat cadrul legislativ de funcţionare a serviciului sanitar militar, condus de o direcţie proprie în cadrul Ministerului de Război.

În decursul anilor, personalul medico-sanitar al armatei a devenit un corp de elită al medicinei româneşti, remarcându-se prin realizări importante în apărarea sănătăţii militarilor şi prin contribuţii însemnate la progresul ştiinţei şi practicii medicale.

Personalul medico-sanitar al Armatei României, dovedind profesionalism şi spirit de sacrificiu, s-a implicat în toate momentele cruciale ale istoriei poporului român începând cu Războiul de Independenţă, continuând cu Războiul de întregire naţională, Al doilea război mondial şi Revoluţia din Decembrie 1989.

Rezultatele obţinute pe tărâmul luptei cu moartea în campaniile menţionate, precum şi numeroasele jertfe din rândul personalului medico-sanitar motivează respectul oştirii şi al poporului nostru pentru serviciul medical al armatei, ai cărui eroi şi-au câştigat un binemeritat loc în rândul martirilor neamului.

Participările medicinei militare la misiunile de asistenţă umanitară sub egida O.N.U. din Somalia şi Angola, precum şi la suportul medical al misiunilor din cadrul Parteneriatului pentru Pace din Albania şi Bosnia, la misiunile sub egida NATO din Afganistan şi Irak, au evidenţiat profesionalismul şi devotamentul personalului medico-sanitar, capacitatea acestuia de cooperare cu structuri multinaţionale similare în soluţionarea problemelor de specialitate, făcând cunoscute valoarea medicinei naţionale şi încărcătura umană a activităţii medicale.

Cu toate că, potrivit concepţiei militare, medicina militară trebuie să se îndrepte spre specializări utile în timp de răboi şi situaţii de criză, foarte mulţi medici militari au abordat şi specialităţi deosebit de utile în timp de pace. Medicii militari de toate specialităţile au lucrat intens, susţinut şi cu eficienţă, devenind personalităţi recunoscute de lumea medicală internă şi internaţională. Mulţi medici militari au fost cooptaţi în învăţământul universitar medical devenind profesori, conferenţiari şi şefi de lucrări în Universitatea de Medicină şi Farmacie „Carol Davila" din Bucureşti, precum şi în alte facultăţi de profil din ţară, formând numeroşi specialişti şi chiar adevărate şcoli medicale naţionale, recunoscute şi pe plan internaţional. Totodată, o mare parte din aceşti specialişti ocupă funcţii în structurile de conducere în cadrul unor societăţi şi asociaţii profesionale interne şi internaţionale.

Medicina militară, dispunând de un corp medical de elită, s-a putut organiza şi păstra ca entitate remarcabilă de-a lungul anilor, rezistând tuturor greutăţilor şi vicisitudinilor vremurilor. Totodată, de-a lungul timpului, medicii militari au dovedit că reprezintă un corp de profesionişti solidar care şi-a cunoscut rolul şi menirea, manifestându-se ca autentice valori ale medicinei româneşti.

Începând cu anul 2001, reţeaua de medicină militară, asemenea întregii armate, cunoaşte un amplu proces de restructurare şi modernizare, început de la nivel central şi continuând la nivelul structurilor de execuţie – formaţiunile de asistenţă medicală -, proces continuu care urma să realizeze o intergrare cât mai concretă şi eficientă în sistemul naţional de asistenţă medicală bazat pe asigurările sociale de sănătate.

Astfel reorganizate, în concordanţă cu sistemul naţional de asigurări de sănătate şi cu reducerea efectivelor armatei, structurile noastre medico-militare au putut acoperi cerinţele tuturor solicitanţilor de servicii de sănătate – personalul armatei, pensionarii militari, veteranii de război -, au intervenit cu promptitudine, eficienţă şi eficacitate în situaţii deosebite (dezastre naturale, accidente tehnologice) şi au capacitatea ca în situaţii de criză şi/sau campanie să acorde asistenţă medicală de calitate trupelor, dând dovadă de manevrabilitate, maleabilitate şi fiabilitate.

Relaţiile de colaborare cu serviciile sanitare ale armatelor membre sau partenere NATO s-au dezvoltat şi diversificat, medicii militari şi formaţiunile medicale militare participând la exerciţii NATO/PfP, atât pentru organizarea şi conducerea acestora, cât şi pentru asigurarea asistenţei medicale reale.

Serviciile de sănătate puse la dispoziţia utilizatorilor de către formaţiunile medicale ale armatei se dezvoltă şi diversifică continuu pentru a putea face faţă concureţei altor prestatori de servicii din domeniul sanitar, proces obiectiv în beneficiul populaţiei, stabilit de sistemul asigurărilor sociale de sănătate în România, prevăzut de Legea nr. 95/2006 privind reforma în sănătate.

În strictă corelare cu aplicarea prevederilor Legii nr. 95/2006 trebuie depuse în continuare eforturi din partea tuturor factorilor de decizie direct implicaţi în transpunerea în practică a conţinutului Legii nr. 80/1995, privind statutul cadrelor militare, în special pentru asigurarea resurselor financiare necesare acordării medicamentelor gratuit în tratamentul ambulatoriu al beneficiarilor acestei legi, atât din fondul asigurărilor sociale de sănătate cât şi din bugetul M.Ap.

Anul 2007 aduce după sine noua Concepţie privind reorganizarea şi modernizarea sistemului medical militar, agreată de conducerea politică şi militară a ţării, conform căreia sarcinile specifice unităţilor şi formaţiunilor medicale în timp de pace se împletesc în mod firesc, necesar cu cele care privesc medicina operaţională, de campanie. În acest sens urmează ca pentru 6 spitale militare de urgenţă să se înfiinţeze servicii de medicină operaţională ce vor asigura resursele umane calificate pentru participarea cu eficienţă la misiunile ordonate în afara ţării.

Tradiţiile cu care medicina militară se mândreşte, ne obligă să ne angajăm cu toate forţele pentru îmbunătăţirea continuă a stării de sănătate a militarilor, pentru menţinerea capacităţii combative a armatei.

La aniversarea Zilei medicinei militare doresc să vă adresez felicitări şi urarea ca, prin abnegaţie, pricepere şi dăruire în muncă, să obţineţi rezultate cât mai bune în alinarea suferinţelor semenilor noştri, pentru formarea unor militari sănătoşi, permanent în măsură să facă faţă condiţiilor severe ale câmpului de instrucţie sau ale misiunilor pe care le au de îndeplinit.

MESAJUL
ŞEFULUI STATULUI MAJOR GENERAL

CU PRILEJUL ZILEI MEDICINEI MILITARE
- 21 AUGUST 2007 –

La 21 august 2007 sărbătorim 145 de ani de la înfiinţarea primelor structuri medicale în Armata României – Corpul ofiţerilor sanitari ai armatei şi Direcţia Generală a Serviciului Sanitar Român.

Beneficiind pe întreaga durată a existenţei de aportul unor corifei ai domeniului, medicina militară şi-a căpătat rapid un loc de cinste în conştiinţa militarilor români. Corpul medical al Armatei României şi-a dovedit calităţile profesionale, ştiinţifice şi umane de excepţie pe parcursul tuturor conflagraţiilor la care a participat, dând adevărate pilde de eroism în lupta cu rănile şi cu bolile necruţătoare ale acelor timpuri.

Spitalele militare organizate pe întreg teritoriul ţării şi-au probat pe parcursul a mai bine de un secol renumele de instituţii medicale de prim rang, apreciate ca atare de militarii activi, în rezervă sau în retragere, de familiile lor şi de toţi cetăţenii, care au găsit în acestea un loc de alinare a suferinţelor şi de redare a speranţei într-o viaţă liniştită şi fără dureri. Personalul medical din unităţi s-a distins printr-o conduită demnă, contribuind la dimensiunea profilactică a actului medical şi constituind întotdeauna interfaţa dintre militari şi instituţiile specializate.

Medicii militari au fost în permanenţă în avangarda specialiştilor în domeniu, de numele şi activitatea lor fiind legate nenumărate descoperiri ştiinţifice, lucrări şi tratate de specialitate, o activitate universitară laborioasă, pusă în slujba profesiei şi a oştirii.

Asemenea întregii armate, medicina militară trece în prezent printr-un proces de reorganizare şi restructurare, menit să o plaseze la nivelul cerinţelor unui sistem militar modern, urmând să fie în măsură să răspundă la standarde ridicate rigorilor profesionale şi ştiinţifice ale momentului, pentru a-şi îndeplini menirea.

Iată de ce, astăzi, în zi aniversară, mă înclin cu admiraţie în faţa memoriei celor care au pus bazele medicinei militare româneşti şi a urmaşilor lor, demni membri ai clubului select al celor pentru care viaţa umană este bunul suprem. Adresez sincerele mele urări de bine şi sănătate tuturor medicilor, asistenţilor şi personalului medical auxiliar din armată şi le urez să aibă parte numai de succese în bătălia nobilă pentru redarea speranţei într-o viaţă sănătoasă a tuturor celor care apelează la serviciile lor.

ŞEFUL STATULUI MAJOR GENERAL
Amiral dr.
Gheorghe MARIN

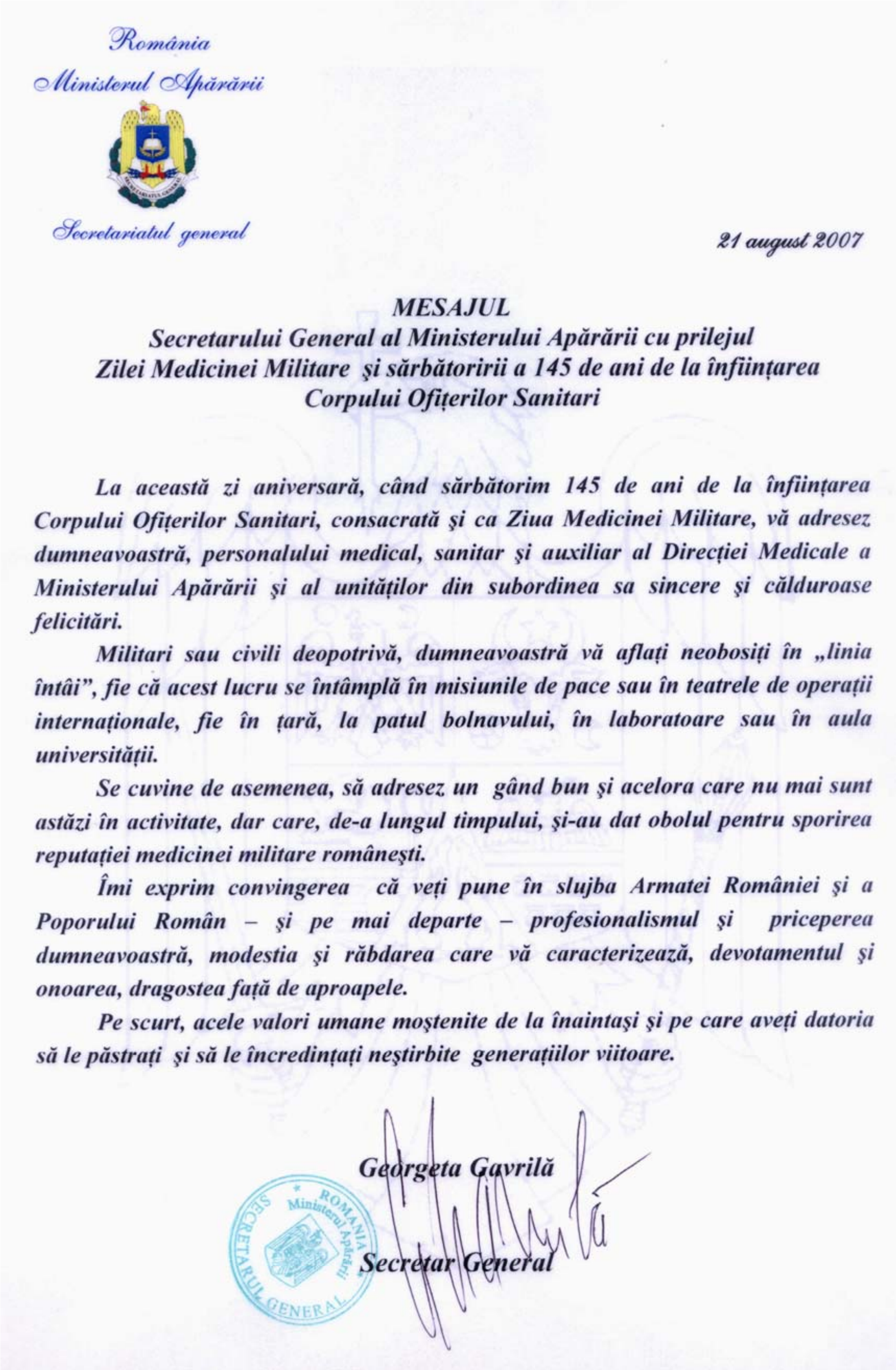

România
Ministerul Apărării
Secretariatul general

21 august 2007

MESAJUL
Secretarului General al Ministerului Apărării cu prilejul Zilei Medicinei Militare şi sărbătoririi a 145 de ani de la înfiinţarea Corpului Ofiţerilor Sanitari

La această zi aniversară, când sărbătorim 145 de ani de la înfiinţarea Corpului Ofiţerilor Sanitari, consacrată şi ca Ziua Medicinei Militare, vă adresez dumneavoastră, personalului medical, sanitar şi auxiliar al Direcţiei Medicale a Ministerului Apărării şi al unităţilor din subordinea sa sincere şi călduroase felicitări.

Militari sau civili deopotrivă, dumneavoastră vă aflaţi neobosiţi în „linia întâi”, fie că acest lucru se întâmplă în misiunile de pace sau în teatrele de operaţii internaţionale, fie în ţară, la patul bolnavului, în laboratoare sau în aula universităţii.

Se cuvine de asemenea, să adresez un gând bun şi acelora care nu mai sunt astăzi în activitate, dar care, de-a lungul timpului, şi-au dat obolul pentru sporirea reputaţiei medicinei militare româneşti.

Îmi exprim convingerea că veţi pune în slujba Armatei României şi a Poporului Român – şi pe mai departe – profesionalismul şi priceperea dumneavoastră, modestia şi răbdarea care vă caracterizează, devotamentul şi onoarea, dragostea faţă de aproapele.

Pe scurt, acele valori umane moştenite de la înaintaşi şi pe care aveţi datoria să le păstraţi şi să le încredinţaţi neştirbite generaţiilor viitoare.

Georgeta Gavrilă

Secretar General

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Chistul bronhogenetic cu localizare rară

Dr. SAVU C[*].,Dr. NICA Angelica, Dr. PIRVU A.

Chistul bronhogenetic este o leziune congenitală, întâlnită cel mai frecvent la nivelul mediastinului şi care se dezvoltă din regiunea şanţului laringotraheal. În majoritatea cazurilor este asimptomatic, simptomele care apar în evoluţie fiind determinate de complicaţiile pe care le determina asupra organelor din jur. Tratamentul de electie este cel chirurgical prin excizia completă a formaţiunii, pentru prevenirea recidivelor.

chist bronhogenetic chirurgie excizia

ABSTRACT

The bronchogenic cyst is a congenital disease, who is developed the most freqvently in the mediastinum from laringotracheal sulcus. In the most comun cases it has nosigns or simptoms. The clinical findings are caused by complication of this disease and the impact of surroundings organs. The treatment of choice is surgery with completely excision for prevents recurrences.

KEY WORDS: bronchogenic cyst, surgery

Chistele intratoracice sunt cele mai comune leziuni întâlnite la copii şi adulţi. Majoritatea sunt leziuni congenitale şi ocupa 20-32% din masele primare mediastinale. Jumătate sau chiar mai mult din acestea nu sunt identificate până în decada a 3-a sau a 4-a de viaţă (32% sunt descoperite înainte de 20 ani şi 68% după 20 ani). Clasificare lor se bazează pe etiologie, pe caracteristicile histologice şi embriologice.

CLASIFICARE

1. Congenitale – chiste mezoteliale, chiste dezvoltate din intestinul bronhogenic, chiste limfatice
2. Dobândite: inflamatorii, timice, teratogene, dermoide, dezvoltate din tiroida şi paratiroide, chistul hidatic.

EMBRIOGENEZA: Chistul bronhogenetic are ca punct de plecare sechestrarea celulelor din regiunea şanţului laringotraheal în timpul dezvoltării embriologice timpurii. În săptămâna a 3-a a gestaţiei, şanţul laringotraheal sau sistemul respirator primitiv se dezvolta în partea anterioară a intestinului primitiv, sub forma unui diverticul, situat caudal de punga faringiană.

Acest diverticul se transforma mai târziu într-un tub care devine arborele bronşic primitiv. După săptămâna a 4-a, apar distal 2 proeminente, care devin viitoarele bronşii şi mugurele pulmonar. În ziua a 35-a apare bronşia lobară. Înmugurirea anormală a arborelui bronşic poate conduce la mase chistice traheale şi bronşice numite chiste bronhogenetice.

Când aceasta înmugurire anormală apare devreme în timpul gestaţiei, chistele au tendinţa să se localizeze la nivelul mediastinului şi rareori comunică cu arborele bronşic. Chistele ce se dezvoltă mai târziu sunt mai periferice şi localizate în parenchimul pulmonar. Acestea comunică adesea cu bronşiile.

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Majoritatea chistelor bronhogenetice sunt în strânsă relație anatomică cu arborele bronşic, sau esofagul, în compartimentul visceral al mediastinului, ocazional se pot extinde sub diafragm.
Coselli şi colaboratorii au descris chiste bronhogenetice localizate în întregime în abdomen în regiunea cozii pancreasului.

HISTOLOGIE

Chisturile bronhogenetice sunt tapetate de un epiteliu ciliat columnar sau cuboid, unistratificat, având la baza fibre musculare netede. Mucoasa epiteliala poate suferi metaplazie scuamoasă. Când se produce infecția secundară, epiteliul poate fi distrus şi apare puroiul franc. Peretele chistului este subțire şi conține cartilaje hialine, glande bronşice, celule musculare netede si trunchiuri nervoase. Conținutul poate fi: lichidian clar, hemoragic, mucos, purulent.
În general, chisturile bronhogenetice sunt unice, dar pot fi şi multiloculare sau multiple.

LOCALIZARE

După Maier exista 5 localizari: 1-paratraheal, 2-carinal, 3-hilar, 4-paraesofagian si 5-variate. Raportat la bifurcația traheei ca linie de diviziune, s-a constatat ca 23% din chisturile bronhogenetice. sunt localizate deasupra carinei şi 77% distal de aceasta. După Roughly, o treime sunt situate în mediastinul mijlociu şi 2/3 in mediastinul posterior.
Localizări rare: mediastinul anterior, cavitatea pericardica, şanțul paravertebral, parenchimul pulmonar, ligamentul triunghiular, intratimic, subcutanat, presternal, supraclavicular, regiunea scapulară, transdiafragmatic şi chiar abdominal. Coselli sugerează ca aceste chiste rezultă din înmugurirea anormală a canalului primitiv care migrează în abdomen înainte de fuziunea cu membrana pleuroperitoneală.

Referitor la parenchimul pulmonar, mai frecvent, chistul bronhogenetic se întâlneşte la nivelul lobilor inferiori. Cu toate acestea, după Ribet şi colaboratorii, din 21 de cazuri de chist bronhogenetic la sugari şi copii, lobul superior drept şi lobul superior stâng au fost implicați fiecare în 7 cazuri, lobul inferior stâng în 6 cazuri şi lobul inferior drept într-un caz.

PATOLOGIE

20% din copii sunt asimptomatici. După Ribet şi colaboratorii, la 4 din pacienții lor, chistul bronhogenetic a fost identificat prin ultrasonografie prenatală. Chistele parenchimatoase comunică frecvent cu arborele traheobronsic şi de obicei prezintă semne de sepsis pulmonar.
La adulți, frecventa este mai mare la bărbați decât la femei. În general sunt asimptomatici. Peste jumătate devin simptomatici prin compresia căilor aeriene, a esofagului sau prin prezenta infecțiilor. Acestea din urma pot fi cauzate de perforația în fiecare din structurile învecinate, dar infecția apare cel mai des fără astfel de comunicații. După Sirivella 16 din 20 pacienți sunt simptomatici şi prezintă: tuse, dispnee, disfagie şi durere toracică. Simptome mai rar întâlnite sunt: febra, sputa purulenta, anorexia şi pierderea în greutate, hemoptizia.

În studiile efectuate de St-George şi colaboratorii, cel mai comun simptom a fost durerea substernală, care rezulta din iritația sau inflamația pleurei parietale sau mediastinale înconjurătoare.
Disfagia, dispneea şi tusea sunt date de compresia sau iritația bronşiilor sau esofagului de către chist.

Expectorația purulenta e dată de infectarea chistului, cu fistulizare sau mai probabil, de pneumonia din plămânul adiacent comprimat.

CAZ CLINIC

Pacienta C.S., în vârstă de 39 ani, nefumătoare, din mediu urban, având ca antecedente personale patologice: tiroidectomie totală pentru guşă polinodulara (efectuată în 2005 şi sub tratament cu Eutyrox 100µg), mixedem posttiroidectomie, hipoparatiroidism secundar iatrogen, histerectomie subtotală pentru fibrom uterin (2005), mastoza fibrochistica bilaterală, apendicectomie şi sechele fibronodulare pulmonare bilaterale, se internează în clinica noastră pentru: disfagie pentru solide şi lichide, dispnee de efort, astenie fizică marcată, scădere ponderală (6-7 kg în ultimele 2 luni), inapetența, fatigabilitate.

Examenul obiectiv pe aparate şi sisteme a fost in limite normale (pacienta subponderala).

Pe perioada internării s-au efectuat următoarele investigații: EKG- tahicardie sinusală; Ecografie cardiacă: Aorta inițiala-3,SIV-9, FE=60%; Consultul cardiologic a decelat prezenta unui prolaps de valva mitrală posterioară. Analizele hematologice au evidențiat: hemograma cu uşoară trombocitoza (441000/mmc), biochimie şi coagulograma normale, fibrinogen crescut (590,3mg/dl).

Pe radiografia pulmonară de față şi profil s-au remarcat leziuni fibronodulare bilaterale.

Endoscopia digestivă superioară nu a decelat modificări, cu excepția unei hernii gastrice transhiatale de dimensiuni mici.

Ecoendoscopia a pus in evidență o formațiune de aproximativ de 60,5/27mm, situată în mediastinul posterior, între aorta descendenta şi cord, care vine în contact cu pleura şi care nu pare sa o invadeze, cu conținut intens hipoecogen, relativ omogena, fără adenopatii peritumorale.(fig. 1)

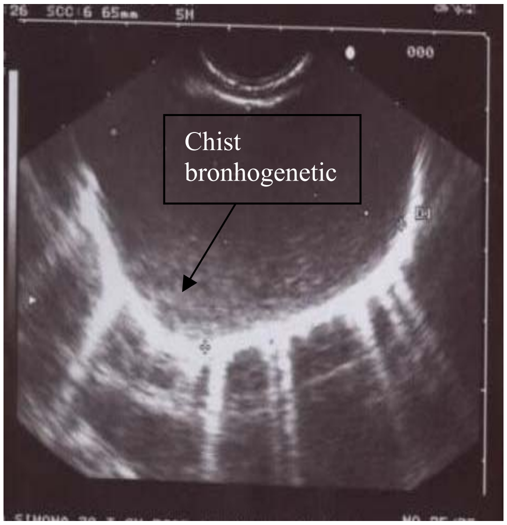

Fig.1.Ecoendoscopie.

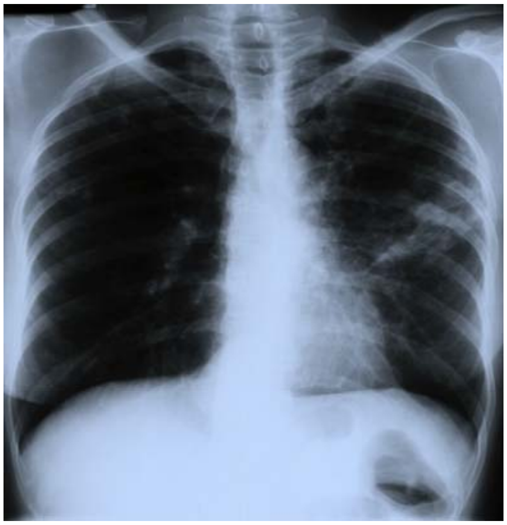

Fig.2.Radiografie toracica.

Concluzii: formațiune tumorală de mediastin posterior în observație etiologica (leiomiom, adenopatie giganta).

Tomografia computerizată cu substanță de contrast a evidențiat: formațiune hipodensă, omogena, neiodofila, ovalară, cu dimensiuni axiale de 5/3,2 cm, fără acumulare de contrast oral, situată în partea inferioară a toracelui drept, paraesofagian, compresivă moderat pe structura pleuropulmonară adiacenta; îndeosebi pe fereastra de parenchim, leziuni fibronodulare ce ocupa teritoriul apicodorsal al lobului superior stâng şi lingula; aceleaşi modificări, mai puțin extinse, dorsal în lobul superior drept şi în segmentul Fowler stâng; organele abdomenului superior în limite normale.

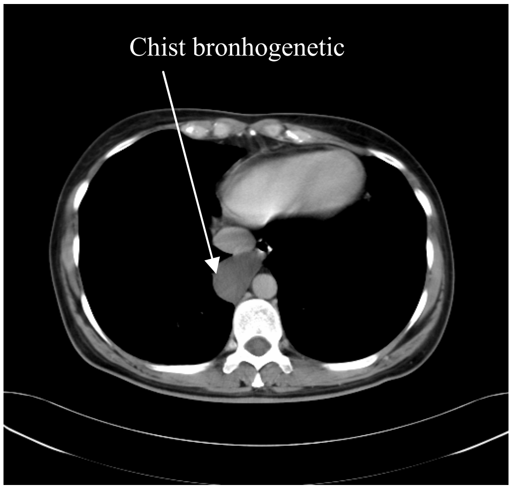

Fig.3 CT toracic

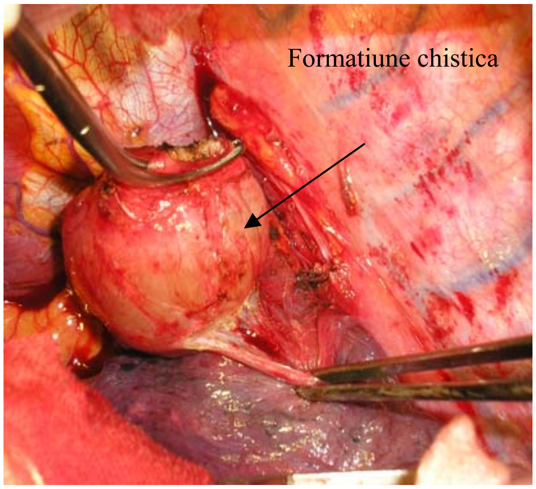

Fig. 4 Aspect intraoperator

Concluzii: formațiune chistică paraesofagiană dreapta, cu apartenența la structurile mediastinale posterioare (tumora neurala, chist de canal toracic) (fig.3).

Tranzitul baritat a descris: esofag normal, stomac alungit, hipoton, hipokinetic, cu polul inferior sub crestele iliace, pliuri ale mucoasei îngroşate, pilor permeabil, bulb duodenal normal, cadru duodenal normal.

În urma coroborării datelor clinice şi paraclinice s-a decis efectuarea intervenției chirurgicale şi s-a practicat chistectomie ideala (protocol operator nr.360/26.04.2007). S-a practicat toracotomie postero-laterala dreapta cu acces în cavitatea pleurală prin spațiul VII intercostal. La inspecție s-a constatat o formațiune chistica, cu diametre de 6/4 cm, localizată în mediastinul posterior şi dezvoltată paraesofagian, în ligamentul triunghiular, între aorta toracică şi vena cavă inferioară, înconjurată de parenchimul lobului pulmonar inferior drept. S-a disecat formațiunea de țesuturile învecinate şi s-a practicat chistectomie ideala(fig.4). Acest tip de localizare este rar întâlnită. Drenaj pleural cu 2 tuburi de dren 26CH şi 28CH cuplate la aspirație activă.

Evoluția postoperatorie a fost lent favorabilă prin persistenta pierderilor aeriene şi expansionare pulmonară lentă.

Examen histopatologic: perete chistic tapetat de un epiteliu de tip respirator şi cu glande de tip bronşic şi țesut muscular neted, aspect compatibil cu chistul bronhogenetic.

DISCUȚII

În studiul lui St-George 18 pacienți au avut chiste complicate, documentate atât la examinarea macroscopica cât şi la cea histopatologică, dar în nici una din aceste complicații nu au rezultat suferințe majore. În chistele bronhogenetice pot apare complicații mecanice date de compresia chistului asupra structurilor învecinate (vase mari, esofag, pericard, plămân, bronşii, etc).

Complicațiile cardiovasculare sunt rare. Watson şi Chaudhary citează 1 pacient cu tahicardie atrială şi ventriculară. Volpi şi colaboratorii raportează apariția fibrilației atriale paroxistice, data de compresiunea pe atrii. Watson şi Berkowits descriu compresia arterei pulmonare ce simulează stenoza arterei, Selke şi colaboratorii descriu compresia arterei pulmonare de lungă durată dată de artera pulmonară hipoplazica şi plămânul hiperlucent.

Sindromul de vena cavă superioară a fost întâlnit într-un singur caz.

Complicații infecțioase sunt reprezentate de inflamația peretelui chistic cu sau fără ulcerații ale mucoasei fiind întâlnită în 18% din cazuri.

Această descoperire a fost adesea asociată cu durerea toracica. Infecția bacteriană (pozitivată) a fost rară. Comunicarea franca cu arborele traheobronşic a fost întâlnită la 3 pacienți care prezentau dispnee, febră şi tuse productivă.

Comunicarea cu esofagul şi cavitatea pericardică nu a fost observată. Moor şi Jahnke au observat (ocazional) la chistele, a căror mucoasă prezenta epiteliu gastric, simptome legate de secreția acidă intrachistică.

Spock şi colaboratorii au descris apariția hemoragiei intrachistice exteriorizate prin hematemeza sau hemoptizie acestea fiind complicații hemoragice.

Riscul malignizarii este minim, s-a citat degenerarea în carcinom şi fibrosarcom. Greenfield şi Howe în 1965 şi Bemnheim şi colaboratorii în 1980 au descris transformarea în leiomiosarcom, dar se presupune că dezvoltarea s-a produs din peretele chistului. S-au citat şi asocieri cu tumori maligne: rabdomiosarcom, adenocarcinom, carcinom scuamos.

Cazul prezentat de noi a ridicat o serie de probleme de diagnostic diferențial cu: abcesul pulmonar (prezintă sindrom infecțios şi are perete gros), chistul hidatic (perete propriu), tumori chistice mediastinale (disgerminoame, chiste pericardice), tumori rare (chistadenocarcinom), leziuni traumatice (hematomul), tumori esofagiene extramucoase.

CONCLUZII

Atitudinea de expectativa nu se recomandă, chiar dacă chistul bronhogenetic este asiptomatic.

Procedura de electie este excizia completă, deoarece rezecția parțială sau aspirația chistului duc la recidivă. Se recomandă denudarea mucoasei chistului de pe structurile vitale, în condițiile în care nu se poate efectua rezecția completă.

Intervenția se poate efectua prin toracoscopie, chirurgie toracică videoasistată sau clasic. În funcție de localizarea chistului se recomandă toracotomie postero-laterala (pentru chistele localizate în mediastinul posterior) sau sternotomie (pentru chistele situate în compartimentul anterior al mediastinului).

Ginsberg a publicat un caz rezecat prin mediastinoscopie.

Avantajele chirurgiei toracice videoasistate: diminuarea durerii postoperatorii, durata scăzută a spitalizării, reintegrarea rapidă a pacientului în activitate.

Morbiditatea postoperatorie este scăzută prin complicații minime, mortalitatea zero.

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Factorii de risc în bolile cerebro-vasculare

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REZUMAT: Factorul de risc se defineşte în sens general ca orice condiţie sau caracteristică ce poate prevedea în cazul unui subiect, posibilitatea apariţiei unor boli manifeste clinic. Are prin urmare o semnificaţie predictivă. O altă definiţie este de factori ai ecosistemului uman care influenţează negativ starea de sănătate. Factorii de risc în bolile cerebro-vasculare pot fi majori (fundamentali, primari), adjuvanţi (favorizanţi) şi precipitanţi (declanşatori).

factori de risc majori adjuvanţi precipitanţi

ABSTRACT: The risk factor is generally defined as any condition or feature which can anticipate the possibility of the appearance of some clinical symptoms at patients. Therefore, it has a significance that can be predicted. Another definition is that of the elements of the human ecosystem which influence, in a negative way, the health condition. The risk factors in brain-vascular diseases can be very important (vital, primary), adjuvant and precipitant (activating).

Multiple date culese în urma studiilor epidemiologice au dus la elaborarea conceptului „factorului de risc” în bolile cerebro-vasculare, care prezintă în momentul actual importante implicaţii practice şi teoretice privind etiologia, patogenia şi prevenirea bolilor cerebro-vasculare. Am putea defini „factorul de risc” în bolile cerebro-vasculare, o deviere de la normal sau o afecţiune, care, evidenţiată la o persoană fără manifestări clinice, se asociază cu o probabilitate crescută de apariţie a bolilor cerebro-vasculare în viitorul mai apropiat sau mai îndepărtat.

Prin prezenţa unuia sau a mai multor factori de risc se poate evalua chiar cantitativ gradul de probabilitate pentru o persoană să facă o boală cerebro-vasculară. În acest fel suntem „susceptibili” de a face o boală cerebro-vasculară.

Prin analiza diverselor studii epidemiologice ca şi prelucrarea datelor statistice din studiul personal, se evidenţiază un număr mare de factori de risc care pot fi implicaţi direct sau indirect în producerea bolilor cerebro-vasculare sau pot reprezenta o relaţie statistică semnificativă cu incidenţa bolilor cerebro-vasculare: hipertensiunea arterială, ateroscleroza, dislipidemiile, diabetul zaharat, bolile cardiace (îndeosebi fibrilaţia atrială şi cardiopatia ischemică), alcoolismul, fumatul, obezitatea, ereditatea, bolile hematologice, malformaţiile vasculare intracraniene.

Rolul altor factori de risc este nesigur sau nedovedit concret: hiperuricemia, discriniile, sedentarismul, particularităţi constituţionale individuale, infecţiile, neoplaziile, radiaţiile, contraceptivele orale, substanţele psiho-active, cafeaua.

Ierarhizarea factorilor de risc privind importanţa lor în producerea bolilor cerebro-vasculare nu este uşoară şi cere încă studii suplimentare, dar este necesară pentru a putea constitui o bază de pornire în elaborarea unui program vast de prevenire şi combatere a bolilor cerebro-vasculare. Pe baza studiilor epidemiologice internaţionale, naţionale şi personale este posibil să conchidem că unii dintre factorii de risc au o relaţie primară cu bolile cerebro-vasculare şi putem să-i numim majori (fundamentali), în timp ce alţii ar juca un rol adjuvant (favorizant), iar alţii au un rol precipitant (declanşator).

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Factorii de risc majori (fundamentali, primari) în bolile cerebro-vasculare sunt: hipertensiunea arterială, ateroscleroza, dislipidemiile, bolile cardiace, diabetul zaharat, bolile hematologice, malformaţiile vasculare intracraniene, fumatul.

Factorii de risc adjuvanţi (favorizanţi, agravanţi) sunt: obezitatea, sedentarismul, istoricul familial (ereditatea), colagenozele, alcoolul, cafeaua, contraceptivele orale, discriniile, uricemia, solicitarea socio-profesională, neoplaziile, radiaţiile, substanţele psiho-active, vârsta, sexul.

Factorii de risc precipitanţi (declanşatori) sunt: salturile tensionale, infecţiile, excesele izolate sau combinate, suprasolicitarea socio-profesională, temperaturile extreme.

Factorii care au o relaţie primară sau favorizantă cu bolile cerebro-vasculare trebuie să fie prezenţi şi activi o perioadă lungă de timp, pentru a-şi produce efectul. Factorii precipitanţi (declanşatori) pot fi de scurtă durată de ordinul minutelor sau orelor. În absenţa factorilor majori (primari), factorii adjuvanţi (favorizanţi) singuri nu măresc semnificativ incidenţa şi prevalenţa bolilor cerebro-vasculare.

Datele studiilor Framingham au dus la conturarea unui profil de risc cerebro-vascular, care include 5 factori de risc întâlniţi la 10% din populaţia asimptomatică şi care determină ⅓ până la ½ din accidentele vasculare cerebrale: hipertensiunea arterială, hipercolesterolemia, toleranţa la glucoză anormală, dar nu diabetul, fumatul, hipertrofia ventriculară stângă.

Prezenţa tuturor factorilor creşte riscul de accident vascular cerebral de 8 ori. Trecerea în revistă a factorilor de risc în accidentele vasculare cerebrale evidenţiază ponderea hipertensiunii arteriale la toate vârstele şi la ambele sexe.

Temperaturile extreme (caniculă-ger) şi variaţiile mari şi rapide de temperatură constituie de asemenea un factor de risc, deşi nu există studii bine fundamentate. Din practica medicală s-a remarcat că acestea pot acţiona ca factori declanşatori ai unui accident vascular cerebral hemoragic sau ischemic.

Un studiu clinic şi paraclinic de Kory Calomfirescu şi colaboratorii referitor la factorii de risc în accidentele vasculare cerebrale (ischemice şi hemoragice) pe un lot de bolnavi până la 40 de ani a evidenţiat faptul că abuzul de nicotină şi alcool sunt cel mai frecvent întâlnite, apoi hipertensiunea arterială, agregabilitatea plachetară, dislipidemia (trigliceridele mărite), graviditatea, folosirea contraceptivelor orale, traumatismele cranio-cerebrale, encefalitele şi diabetul zaharat.

Identificarea factorilor de risc este un mijloc de scădere a riscului de boli cerebro-vasculare, prin reducerea factorilor de risc modificabili şi printr-o atitudine terapeutică mai adecvată datorită determinării cu acurateţe mai mare a stării de risc global.

În prezent în SUA există 4 milioane de oameni care au avut un accident vascular cerebral, iar în România există aproximativ 600.000 de bolnavi ce au suferit un accident vascular cerebral. Chiar şi după aceste cifre se impune pertinent identificarea factorilor de risc pentru accidentele vasculare cerebrale şi educarea comunitară privind accidentele vasculare cerebrale în vederea :

- reducerii impactului acestora;
- selectarea pacienţilor cu risc crescut la nivelul asistenţei primare;
- stabilirea unor scheme eficiente de profilaxie ce duc la scăderea incidenţei accidentelor vasculare cerebrale.

Psihologia omului contemporan în raport cu relaţia om sănătos - om bolnav este foarte complexă. Unii au o stare de linişte în legătură cu starea lor de sănătate, nesesizând nici o modificare notabilă, omiţând că în timp, firesc, organismul se uzează. Abuzul de alimente, stresul, sedentarismul, factorii ereditari predispozanţi, unele vicii, etc. predispun la tulburări care la început sunt imperceptibile clinic.

Încrederea în capacităţile ştiinţei medicale de a ne edifica asupra posibilităţilor reale ale organismului, dublată de o voinţă fermă de a acţiona conştient asupra vieţii noastre, ne poate face capabili să adoptăm o atitudine activă în menţinerea sănătăţii şi păstrarea cât mai îndelung posibil a acesteia. Aşa s-a născut şi s-a dezvoltat treptat acea concepţie nouă a medicinii denumită sanogeneză, care cuprinde totalitatea mijloacelor şi măsurilor pentru promovarea sănătăţii populaţiei.

Sănătatea şi boala reprezintă două concepte ce nu pot fi definite decât simultan, iar OMS a definit sănătatea ca fiind nu numai un concept negativ, adică absenţa bolii ci şi o stare completă de bine moral şi fizic. Există şi subiecte ce beneficiază de o „sănătate terapeutică” (ex. Sănătatea „insuliniană” a diabeticilor trataţi), pentru că se îngrijesc şi pentru atât timp cât se îngrijesc.

Depistările medicale prin acţiunile de control şi recontrol clinic şi paraclinic contribuie la depistarea bolnavilor necunoscuţi (ex. Controlul medical anual în organismele militare MAp, MI, SRI, CFR, Conel, etc.).

Trebuie subliniat faptul extrem de important, că în faţa bolii nu se poate face aproape nimic, dacă nu există o disciplină comportamentală şi terapeutică a bolnavului. În toate acţiunile întreprinse în scop profilactic (lupta împotriva fumatului, alcoolismului, sedentarismului, stress-ului, alimentaţiei neraţionale, obezităţii) aliatul de bază al medicului este omul, fiindcă el este responsabil şi beneficiar al stării proprii de sănătate şi al sănătăţii celorlalţi. Deci, acestui om trebuie să-i oferim surse de informare şi documentaţie pentru a înţelege care sunt adevăraţii săi duşmani care pot să-i decimeze sănătatea şi pentru a conştientiza aceşti factori pentru a lua o atitudine.

Prevenirea (profilaxia) înseamnă „ansamblul măsurilor care permit să se evite apariţia, apărarea şi extinderea unor boli” (definiţia OMS) şi ea se poate împărţi în mod schematic în prevenire: primară, secundară, terţiară.

Populaţia trebuie informată, sfătuită sau educată pentru a deveni cooperantă. Educaţia sanitară trebuie să înceapă de timpuriu, din perioada de şcoală şi să se desfăşoare fără discontinuităţi la locul său de muncă, în universitate, armată, spitale, cabinete medicale ambulatorii etc.

Medicii trebuie să înveţe şi să abordeze cu seriozitate problemele de depistare a factorilor de risc în diferitele boli, de evaluare a liniei evolutive, a traiectoriei patologice, să transforme relaţia medic-pacient, încât să înveţe să se exprime într-un limbaj comprehensibil pentru fiecare om, indiferent de gradul său de instruire, pentru stabilirea importanţei şi ierarhizarea factorilor de risc alcătuind chiar un „ profil de risc” pentru pacienţii săi.

Pentru clinician epidemiologia bolilor cerebro-vasculare oferă informaţii preţioase privind cunoaşterea frecvenţei diverselor tipuri de afecţiuni, în raport cu distribuţia lor geografică, naţională sau zonală şi a schimbărilor survenite în ultimul timp în patern-urile principalelor entităţi clinice.

Cercetările epidemiologice ale bolilor cerebro-vasculare au apărut în urma celor cardiace, cu toate că diagnosticul bolilor cerebro-vasculare este mult mai vechi din punct de vedere istoric. În acest sens de mare importanţă este evaluarea prognostică a factorilor potenţiali de risc, pe care îi prezintă orice bolnav în raport cu vârsta, sexul, tipul de afecţiune, încărcarea genetică, condiţiile de muncă şi viaţă, etc.

Pentru asigurarea unui sistem unitar de profilaxie, diagnosticare, tratament şi recuperare a bolnavilor cerebro-vasculari se impune cunoaşterea evoluţiei morbidităţii şi prevalenţei bolilor cerebro-vasculare, combaterea principalilor factori de risc din mediul de muncă şi viaţă, cât şi utilizarea cu randament corespunzător a bazei tehnico-materiale din dotarea unităţilor sanitare.

Profilaxia primară şi accidentele vasculare cerebrale se referă la depistarea precoce a factorilor de risc, la limitarea acţiunii unui factor de risc (ex. fumatul, obezitatea, sedentarismul etc.), care, deşi nu au un risc crescut de producere a accidentelor vasculare cerebrale decât numai în asociere cu alţi factori, sunt totuşi tratabili şi la dispensarizarea unor afecţiuni ce reprezintă factori de risc majori (hipertensiunea arterială, diabetul zaharat, bolile cardio-vasculare, bolile cu hematocrit crescut etc.). Tratamentul acestor afecţiuni poate contribui într-o măsură variabilă la reducerea incidenţei accidentelor vasculare cerebrale.

Există şi factori de risc recunoscuţi care nu pot să fie influenţaţi, cum ar fi spre exemplu: vârsta cronologică. Dintre factorii de risc majori cel mai important rămâne hipertensiunea arterială sistolică şi diastolică pentru toate decadele de vârstă care trebuie să fie corect susţinut şi permanent tratat.

Suntem în mileniul III şi se impune formarea unui nivel de cultură sanitară a populaţiei, indiferent de vârstă, statut socio-profesional şi cultural, pentru a stimula, prin cunoaştere gradul de cooperare în domeniul sanitar, fiind o garanţie a menţinerii şi promovării propriei sănătăţi la nivelul tuturor generaţiilor şi la cei ce ne vor urma (factorul de risc genetic-familial). O viaţă cumpătată în tinereţe, fără excese („a uza nu a abuza", „nici prea mult , nici prea puţin", „nici prea des, nici prea rar") cu o bătrâneţe activă şi frumoasă, poate să facă din fiecare om un longeviv care să fie util familiei, societăţii şi nu o povară sau factor perturbator în familie („ne naştem prin durere şi suferinţă, suntem investiţi cu speranţe şi perenitate de cei ce ne-au dat viaţă, ne dezvoltăm fizic şi psihic, ajungem la un apogeu-culme, apoi treptat coborâm panta spre ireversibilul firesc uman; depinde de noi, de medici şi societate ca această pantă să fie lină, suportabilă, lungă, fără obstacole şi prăbuşiri spectaculoase, cu o calitate a vieţii mulţumitoare").

CONCLUZII

1. Conţinutul noţiunii de „factori de risc" prezintă diferenţe faţă de cea de „factor etiologic" (factor cauzal). Numai o parte din factorii de risc se suprapun peste factorii etiologici;
2. Unii factori de risc nu presupun în mod necesar relaţii cauzale, ci favorizează alţi factori de risc în apariţia bolilor cerebro-vasculare;
3. Este important de amintit că prezenţa factorilor de risc nu implică în mod absolut o relaţie de cauzalitate directă, dar pot fi implicaţi în producerea bolii sau pot fi doar un marker nespecific al procesului patologic;
4. Identificarea factorilor de risc este un mijloc de scădere a riscului de boală cerebro-vasculară prin reducerea factorilor de risc modificabili şi printr-o atitudine terapeutică mai adecvată datorată determinării cu acurateţe mai mare a stării de risc global;
5. Reducerea factorilor de risc reprezintă abordarea clinică principală în prevenirea mortalităţii şi morbidităţii prin boli cerebro-vasculare;
6. Profilaxia multifactorială adresată simultan asupra unor combinaţii de factori de risc, întrerupând lanţurile de coincidenţe ce duc la boli cerebro-vasculare poate întârzia sau împiedica apariţia fenomenelor patologice;
7. Prevenirea primară a bolilor cerebro-vasculare este posibilă prin: controlul hipertensiunii arteriale (dispensarizare), scăderea concentraţiei colesterolului seric, combaterea fumatului, controlul diabetului zaharat, întrucât se însoţeşte de hipercolesterolemie;
8. A preveni este mai economicos şi mai eficient decât a trata.

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Proteina C Reactiva şi glaucomul

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Mr. Dr. A. NENCIU[*]

SCOP: Evidențierea posibilelor implicații ale proteinei C reactive (PCR) în mecanismele etiopatogenice ale glaucomului.
MATERIAL SI METODĂ: Studiu clinic prospectiv, observțional pe doua loturi de pacienți: lot A (14 pacienți) cu glaucom si lotul B (12 pacienți) fără istoric de boli oculare sau alte boli generale cunoscute ca fiind asociate cu nivele plasmatice crescute ale PCR – lot martor. Loturile au fost omogene privind distribuția pe grupe de vârsta şi sex la includerea in studiu. Nivelul PCR a fost determinat cu teste de înaltă specificitate.
REZULTATE: Din analiza nivelelor serice ale PRC pe cele doua loturi de pacienți s-au observat diferențe statistic semnificative (p<0,0001) in cazul pacienților cu glaucom comparativ cu lotul martor: 3,87±0,8mg/dl in lotul A, respectiv 0,68±0,13mg/dl in lotul martor.
CONCLUZII: Glaucomul se asociază cu nivele serice crescute ale PCR. În etiopatogenia multifactorială a acestei boli, in care factorul vascular are rolul său bine stabilit, modificările inflamatorii vasculare pot reprezenta o componentă importantă.

glaucomul proteina C reactivă etiopatogenie

ABSTRACT
Purpose: To observe the possible implications of the C reactive protein in pathogenesis of the glaucoma
Methods: A clinical observational prospective study on 2 groups: group A 14 glaucomatous patients and group B - control (12 normal subjects) without history of ocular pathology or general pathology known to be associated with high plasma levels of CRP. The groups were homogeneous about the age and sex distribution. CRP levels were evaluated by tests with high specificity.
Results: The plasma CRP level reveals important differences (p<0,0001) on the two groups: 3,87±0,8mg/dl in the group A and 0,68±0,13mg/dl in the control group.
Conclusions: Glaucoma is associated with high plasma levels of CRP. In the complex pathogenesis of glaucoma, in witch the vascular factor has his well known place, the inflammatory vascular modifications may represent an important component.
Key words: the glaucoma; CPR; pathogenesis.

Răspunsul inflamator acut apare în urma unei agresiuni exterioare, fiind produs de o serie de factori: infecţioşi, non-infecţioşi, chimici, fizici sau toxici. Acest răspuns inflamator se însoțeşte de prezenta serica a reactanților de fază acută dintre care cei mai importanți sunt proteina C reactiva (PCR), haptoglobina şi fibrinogenul. Reactanții de fază acută sunt modulați prin intermediul citokinelor. Interleukina-1 (IL1), interleukina-6 (IL6) şi factorul de necroză tumorală (TNF) sunt principalii stimulatori ai sintezei hepatice ai reactanților de fază acută.

Proteina C reactiva (PCR) este cel mai sensibil reactant de fază acută, fiind o proteină din clasa pentaxinelor; alcătuită din 5 subunități polipeptidice identice care se prezintă ca pentameri ciclici. Fiecare subunitate are un situs de legare pentru fosfocolină şi doua situsuri de legare pentru calciu. (vezi fig. 1)

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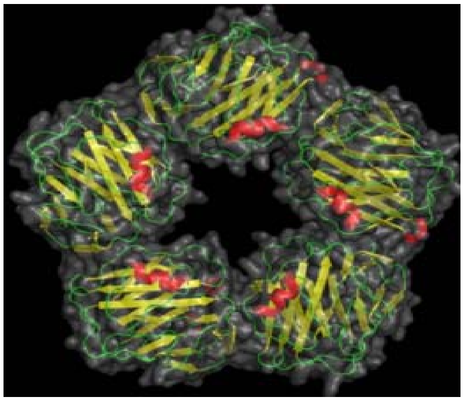

Fig.1. Proteina C reactiva

Patologia inflamatorie este incriminată într-o serie de afecţiuni generale precum ateroscleroza, boala Altzheimer sau boala Parkinson.

Multe studii recente arată asocierea între nivelele crescute ale markerilor inflamatori şi riscul de a dezvolta o patologie aterosclerotică chiar la pacienţii cu nivele bune ale colesterolului seric. În plus nivelele markerilor inflamatori, ai proteinei C reactive în particular , se asociază cu riscul de a dezvolta un episod recurent coronar la pacienţii cunoscuţi deja cu boala aterosclerotică cardiovasculară.

PCR reprezintă şi un marker important pentru sindromul metabolic, care la rândul lui influenţează prognosticul afecţiunilor cardiovasculare.

Actual exista 3 metode de a determina nivelul plasmatic al PCR: calitativ, semicantitativ şi cantitativ. Cel mai modern se folosesc testele cantitative, care sunt de înaltă sensitivitate: hs-CRP (''high sensitivity'' CRP test).

În ultima vreme se vorbeşte tot mai mult de rolul inflamaţiei într-o serie de afecţiuni, din care nici patologia ofatalmologică nu face excepţie. Patologia glaucomatoasă se caracterizează printr-o etiologie multifactorială, în care rolul inflamaţiei îşi pune amprenta.

În urma studiilor recente s-a demonstrat că diverse gene cu rol în codificarea proteinelor implicate în procese inflamatorii precum: chemochinele ligand 5/6, nucleotide imun asociate, receptorul pentru IL1, transtiretina sau haptoglobina se asociază cu glaucomul.

Întregul proces inflamator este întreţinut de expresia moleculelor inflamatorii la nivel tisular, care rezultă pe seama radicalilor liberi de oxigen, aceştia iniţiind şi contribuind la progresia leziunilor glaucomatoase.

Stressul oxidativ, mediat tot de radicalii liberi de oxigen produce leziuni pe moleculele ADN-ului celular din reţeaua trabeculară şi din celulele ganglionare retiniene, care în final conduc la progresia glaucomului.

Studii recente implică disfuncţiile endoteliale secundare scăderii biovalabilităţii oxidului de azot, prin creşterea stressului oxidativ, în acumularea de proteină C reactiva, cu acelaşi rezultat final – progresia leziunilor glaucomatoase. (vezi graficul nr.1)

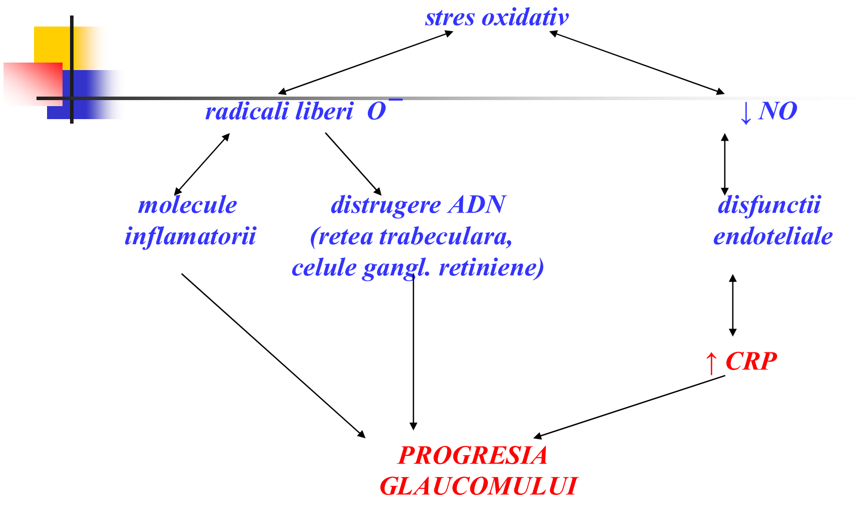
stres oxidativ
radicali liberi O¯
↓ NO
molecule inflamatorii
distrugere ADN (retea trabeculara, celule gangl. retiniene)
disfunctii endoteliale
↑ CRP
PROGRESIA GLAUCOMULUI

Grafic 1. Inflamația în glaucom

Pornind de la aceste premise scopul lucrării noastre este de a observa implicarea proteinei C reactive (PCR) în etiopatogenia si evoluția glaucomului.

Am realizat un studiu clinic prospectiv, observațional cu durata de un an, pe două loturi de pacienți:
- lotul A: alcătuit din 14 pacienți diagnosticați cu glaucom primitiv cu unghi deschis şi glaucom normotensiv, în acelaşi stadiu de evoluție;
- lotul B: lot martor, alcătuit din 12 subiecți clinic sănătoşi.

Criteriul de excludere, valabil pentru ambele loturi, a fost asocierea oricărei patologii oculare sau generale inflamatorii.

Evaluarea nivelelor plasmatice ale PCR a fost realizată cu teste de înaltă sensibilitate bazate pe principiul ELISA. Pentru fiecare pacient al fiecărui lot în parte au fost realizate câte trei determinări ale PCR: la includerea în studiu, la 6 luni şi la 12 luni.

Prelucrarea rezultatelor s-a realizat prin analiză statistică cu ajutorul testului Mann Whitney şi coeficientului de coreleție Spearmann.

Cele doua loturi au fost omogene privind distribuția pe vârstă si sex. Lotul A s-a alcătuit din două subgrupe: 9 pacienți cu glaucom primitiv cu unghi deschis şi 5 pacienți cu glaucom normotensiv. In tabelul 1 se observă ca raportul bărbați/femei şi media de vârsta a fost echivalent pentru ambele loturi.

Distributia dupa varsta si sex

	Group A				*Group B*	
	POAG		*NTG*		*normal*	
	M	*F*	*M*	*F*	*M*	*F*
	5	*4*	*2*	*3*	*7*	*5*
Age (years)	*69±4*	*67±6*	*72±5*	*70±3*	*68±7*	*73±5*

Tabel 1. Distribuţia pe vârste şi sex

Prima determinare a PCR de la începutul studiului a evidenţiat că in cadrul lotului martor peste 80% din subiecţi au prezentat valori plasmatice în limite normale: adică sub 1mg/dl. In lotul A 77% din pacienţii cu glaucom primitiv cu unghi deschis au valori ale PCR peste nivelul normal admis; iar pacienţii cu glaucom normotensiv în proporţie de 100% prezintă valori ridicate plasmatice. (vezi graficul 2)

CRP-prima evaluare

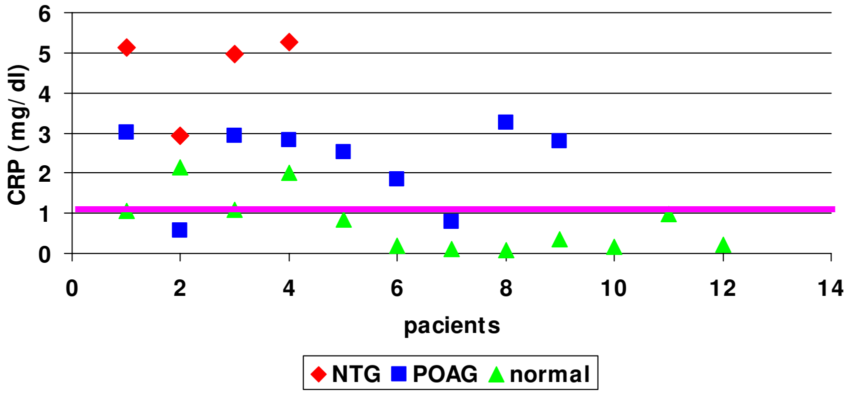

Grafic 2. PCR – prima determinare

Urmărind în dinamică evoluţia nivelelor plasmatice ale PCR se observă că atât la 6 luni, cât şi la 12 luni se menţin aproximativ aceleaşi valori ca la prima determinare, cu minime variaţii.(vezi graficul 3)

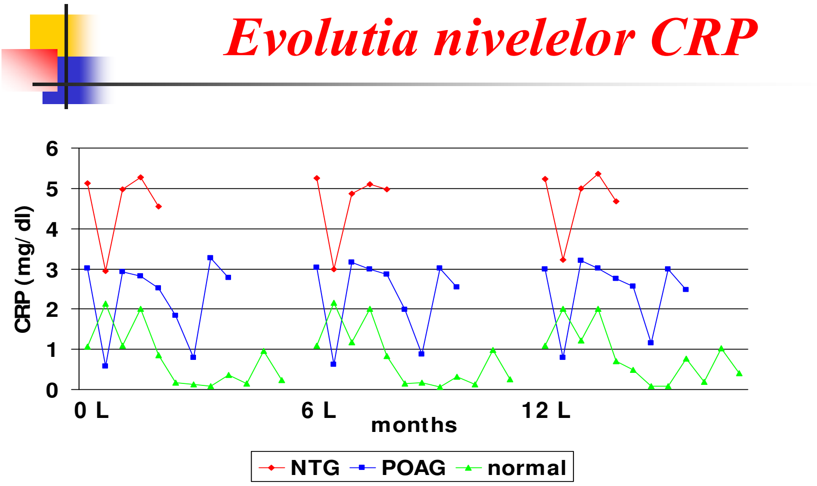

Grafic 3. Evoluţia PCR

În ceea ce priveşte distribuţia pe nivele a PCR pe loturi se observă că peste 80% din pacienţii clinic normali prezintă valori în limite normale (sub 1mg/dl), în timp ce doar 11% din pacienţii cu glaucom primitiv cu unghi deschis se găsesc în acest interval. Restul de aproximativ 77% au valori ale PCR moderat ridicate: între 1 şi 3mg/dl.

Cel mai spectaculos se prezintă lotul pacienţilor cu glaucom normotensiv la care peste 80% din pacienţi prezintă valori mult crescute ale PCR: peste 3mg/dl. (vezi graficul 4)

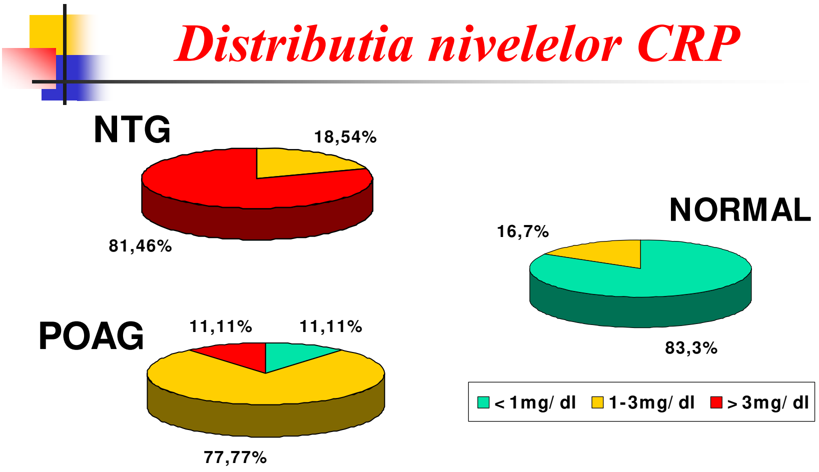

Grafic 4. Distribuţia pe nivele a PCR

Analizând media nivelelor proteinei C reactive se observă că subiecții lotului martor au o medie în limite normale (0,68mg/dl), pacienții cu glaucom primitiv cu unghi deschis au o medie moderat crescută (2,98mg/dl); în timp ce pacienții cu glaucom normotensiv prezintă nivelul cel mai ridicat de 4,93mg/dl. (vezi graficul 5)

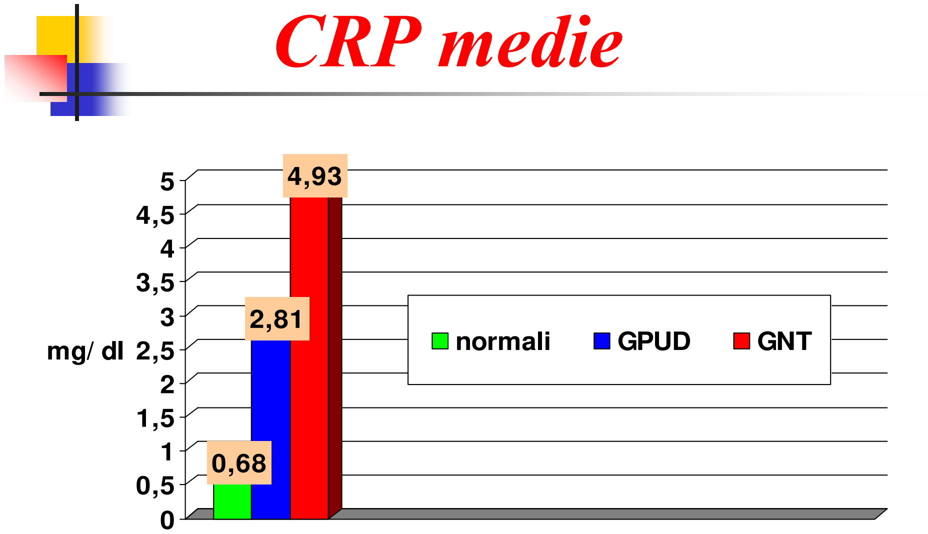

Grafic 5. Media nivelelor PCR

Studiul de față demonstrează faptul că nivele ridicate ale proteinei C reactive se asociază cu patologia glaucomatoasă. Se demonstrează o implicare mai activă a proteinei C reactive în patologia glaucomului normotensiv prin valori plasmatice mult mai mari fața de glaucomul primitiv cu unghi deschis.

Creşterea nivelelor serice ale proteinei C reactive este implicată direct în progresia leziunilor glaucomatoase.

Factorul vascular incriminat în glaucom, dar şi în alte patologii generale precum ateroscleroza, boala Altzheimer, boala Parkinson este amplificat de factorul inflamator.

Prin aceste descoperiri se doreşte deschiderea unor noi orizonturi de abordare terapeutică a bolii glaucomatoase.

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Iridociclita acută psoriazică

Lt.Col.Dr. Cornel STEFAN[*] Dr. Anna SAPUNDGIEVA*
Cpt. Dr. Adrian NENCIU[**] Dr. Diana MELINTE*

Rezumat: Prezentăm cazul unui ofițer de 33 ani, diagnosticat cu psoriasis in urmă cu 12 ani, care se prezintă în clinica de oftalmologie a S.C.U.M.C. cu iridociclita acută. Evoluția sub tratament a fost favorabilă, cu recuperarea funcțională completă. Considerăm apariția iridociclitei ca fiind un semn unic şi tardiv a bolii sistemice.

psoriazis iridociclita acută tratament recuperare

Abstract: We present the case of an officer, 33 years old, with anterior uveitis. The patient was diagnosed with psoriasis 12 years ago. With treatment the evolution was favorable and the recovery was complete. We consider that the apparition of anterior uveitis is an unique and late sign of the systemic disease.

Key words: Psoriasis, Anterior uveitis

Vă prezentăm cazul pacientului S.I., ofițer, 33 ani, sex masculin, cu domiciliu în Bucureşti, care se internează în clinica de oftalmologie a Spitalului Militar Central pe 26.02.2007, acuzând OS: dureri oculare, vedere încețoşată, fotofobie, lăcrimare, prim episod de ochi roşu. Din antecedentele personale patologice reținem - psoriazis vulgar, forma uşoară, diagnosticat in 1995, în tratament local cu Calcipotriol (Daivonex).

Istoricul bolii

Debutul suferinței oculare a fost în urma cu 3 zile, motiv pentru care pacientul s-a prezentat initial într-o altă clinica de specialitate. În momentul examinării în clinica noastră el a fost sub tratament local OS: cicloplegic (Mydriacyl 0.5%), timolol (Arutimol). Subconjunctival i s-a injectat Diprophos. Tratamentul general – Ederen, Voltaren retard.

În urma tratamentului menționat simptomatologia subiectivă nu se ameliorează si urmează prezentarea pacientului în clinica de oftalmologie a S.C.U.M.C.

Rezultatele examinărilor clinice obiective generale efectuate de noi constată prezența modificărilor active date de psoriazis doar la nivelul coatelor(eritem, scuame)(**Figura 1**)

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[*] Clinica de Oftalmologie- Spitalul Clinic de Urgență Militar Central

[**] Centrul medical de diagnostic si tratament "Academic Ştefan Milcu"

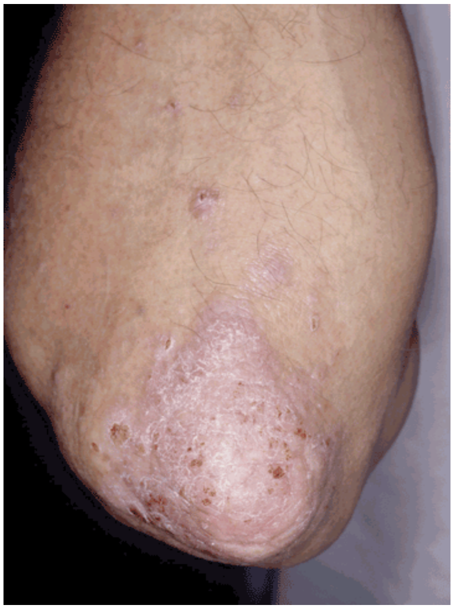

Figura 1: Modificările date de psoriazis la nivelul coatelor

Examenul oftalmolofic obligatoriu decelează:
VOD=1 cc – 0,75 D cyl ax 170°
VOS=1fc, cd (vedere încețoşată)
TOD=19 mmHg Goldmann
TOS=12 mmHg Goldmann
AO: percepție si proiecție de lumină în toate cadranele
Aspectul biomicroscopic al polului anterior*:*
OD: Aspect normal (**Figura 2**); OS(**Figura 3**)

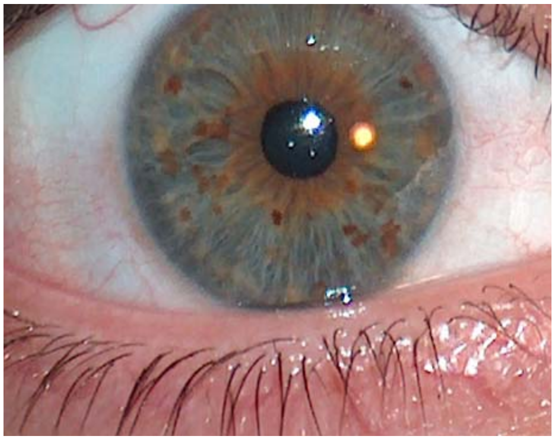

Figura 2: Examen pol anterior ochi drept

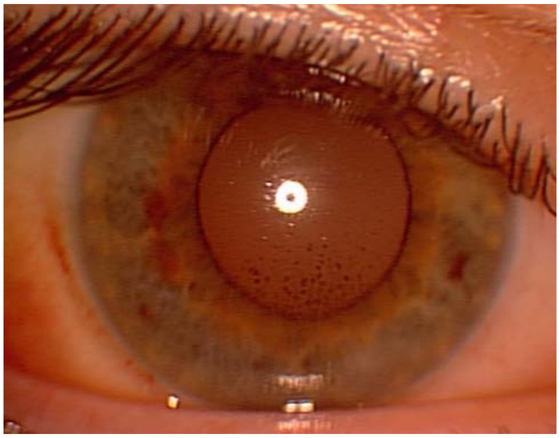

Figura 3: Examen pol anterior ochi stâng

- Conjunctiva – ochi roşu , fără secreții patologic
- Cornee – precipitate keratice endoteliale rotunde, nepigmentate, în ⅓ inferioară – medie a corneei.
- CA – de profunzime medie, Umor apos cu discret Tyndall
- Iris – desen stromal normal, guleras pigmentar integru
- Pupila – rotunda, centrala, semimidriaza fixă
- Cristalin – transparent

Examenul **fundului de ochi** la AO este fără modificări patologice (**Figura 4**)

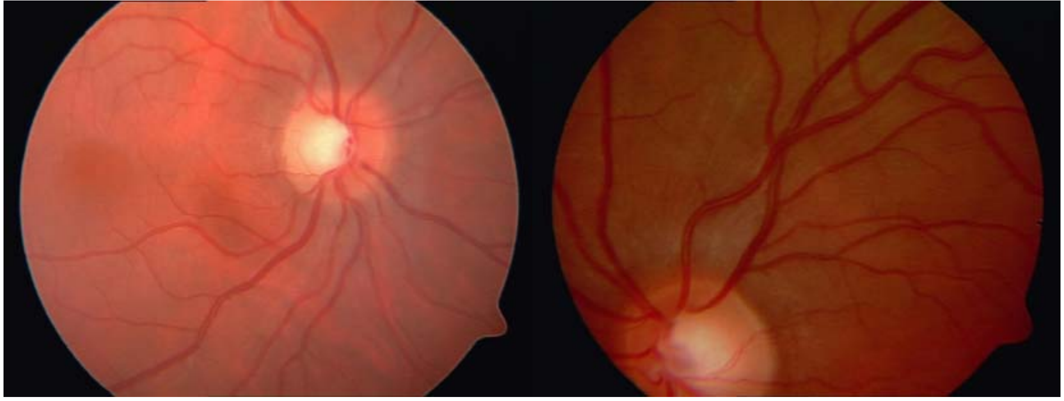

Figura 4 : Examen fund de ochi – fără modificări patologice

Ecografia oculo – orbitara nu a evidențiat modificări patologice (**Figura 5**)

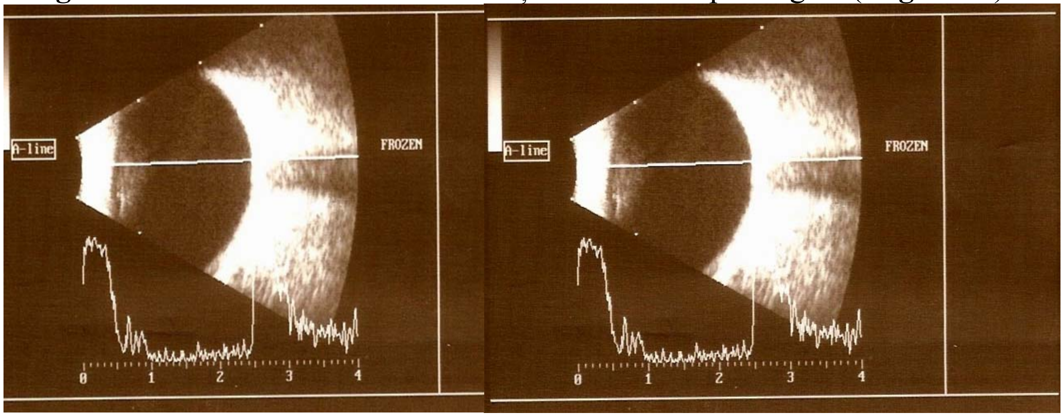

Figura 5: Ecografie oculoorbitară – fără modificări patologice

Pe baza anamnezei şi a examenului de specialitate ne-am orientat spre următorul **diagnostic de prezumție**:

- Psoriazis vulgar, forma uşoară
- OD: astigmatism miopic simplu
- OS: iridociclită acută

Pentru stabilirea diagnosticului pozitiv am solicitat: consult dermatologic(psoriazis vulgar, forma uşoară), ORL, reumatologic, urologic (in afara diagnosticului de psoriazis nu a fost diagnosticată nici o altă afecțiune) şi investigații de laborator cu următoarele rezultate:

VSH – normal
Proteina C reactivă, factor rheumatoid, VDRL, HIV – negative
HLG, glicemie - normal
Ac anti Chlamidia, Ac anti B.burgdorferi – absenți
HLA B27 – absent
Rx cardio – pulmonar, articulații sacro – iliace, craniu-fără modificări patologice
CT sinusuri anterioare si posterioare ale feței, articulații sacro – iliace – fără modificări
Sumar urina, urocultură, secreție uretrală – în limite normale
S – a stabilit următorul **diagnostic pozitiv**:
Psoriazis vulgar, forma uşoară
OD: astigmatism miopic simplu
OS: iridociclita acută psoriazică

S – a instituit tratament topic la OS si tratament general

Tratament topic - Midriatice (Mydriacyl)
- Corticosteroizi (Maxidex)
- Antibiotice (Tobradex)
- Antiinflamatorii nesteroidiene (Voltaren ophtha)

Tratament general - Antibiotic (Zinnat)
- Antihistaminic (Alerid)
- Vitamină C
- AINS (Voltaren 100 mg(retard), pansament gastric, 10 zile

În urmă eficienței deficitare a tratamentului (tradusă prin creşterea numărului de precipitate keratice), pacientului i s –a impus administrarea de Prednison per os 1 mg ⁄Kg corp o dată pe zi, cu scăderea treptată a dozei. Se externează cu ameliorarea simptomatologiei dar cu câteva precipitate corneene endoteliale restante. La primul control(10 zile după externare) nu se mai observă precipitate endoteliale (**Fig. 6, 7**).

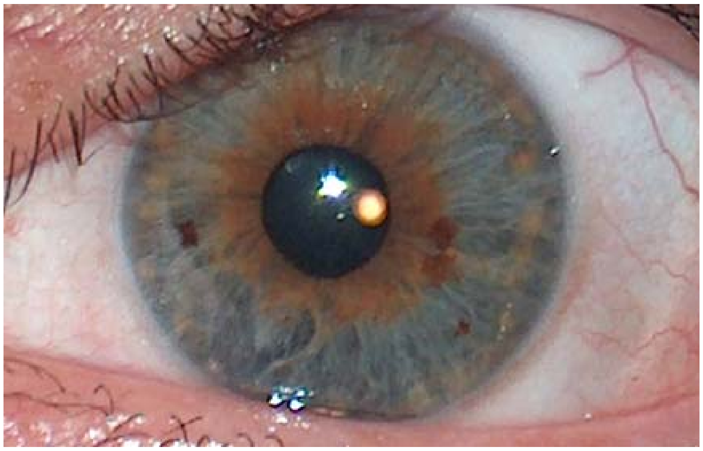

Figura 6: Evoluție ochi stâng sub tratament

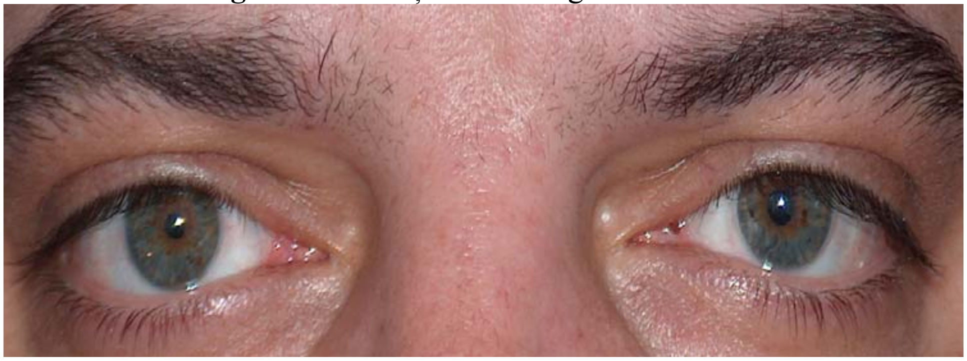

Figura 7: Evoluție după tratament

Particularitatea cazului şi concluzii

Iridociclita acută la un pacient cu psoriasis, dar fără artrită psoriazică, aflat la primul episode de ochi roşu şi care sub tratament are o evoluție net favorabilă cu recuperare funcțională de 100 %

Nu a avut repercusiuni medico–militare.

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Predicția riscului de moarte subită cardiacă prin alternanța undei T

Dr. Nicolae RĂDOI[*]

REZUMAT

Alternanța undei T (AT), de ordinul microvoltilor, este o nouă metodă clinică pentru evaluarea riscului de Moarte Subită Cardiacă. Alternanța undei T este rezultatul alternanței in durata potențialului de acțiune al celulei miocardice. Mecanismele celulare ale alternanței sunt legate de curba de restituire si perturbările cineticii intracelulare a ionilor de calciu cu afectarea schimbului Na –Ca sarcolemal. Alternanța discordantă, în care două regiuni miocardice învecinate prezintă alternanță în opoziție de fază, creează substratul electrofiziologic al reintrării prin dispersia marcată a refractarității miocardice. Testul AT este pozitiv când alternanța este susținută pentru cel puțin 1 minut, cu un voltaj mai mare de 1,9 µV. Studiile clinice publicate prezintă AT ca pe un marker important al riscului de aritmii ventriculare.

alternanața undei T metoda clinică Moarte Subită cardiacă

ABSTRACT

The alternation of T wave (AT) is a new clinical method to evaluate the sudden heart death risk. The alternation of T wave is the result of this alternation during the action potential of the myocardic cell. The cell mechanisms of the alternation are linked to curve restoring and perturbations of the intracell kinetics of calcium ions, altering the Na-Ca sarcolemmal exchange. The discordant alternation where two neighbouring myocardic zones have an alternation in phase opposition creates the electro-physiological re-entering substrate through the pronounced dispersion of myocardic refractory. The AT test is positive when the alternation is sustained for at least one minute, with a voltage higher than 1.9 µV. The clinical studies which are already published show the AT as an important marker of ventricular arrhithmias risk.

INTRODUCERE

Moartea Subită Cardiacă (MSC) rămâne o problemă majoră de sănătate publica, chiar şi în țările dezvoltate economic, unde reprezintă 11% din totalul deceselor şi cca 50% din decesele cardiace. 80% din cazurile de MSC survin la pacienții cu boala aterosclerotică coronariana. Singurul mijloc terapeutic în profilaxia primară sau secundară a MSC este defibrilatorul cardioverter implantabil (ICD). Predicția adecvata a riscului de MSC este foarte importanta pentru profilaxia primara, cost eficienta a MSC prin implant de ICD. Prețul mare al aparatului impune o selecție riguroasă a pacienților. Stimularea ventriculara programata (SVP), mult controversata in literatura de specialitate este invazivă şi costisitoare. Markerii noninvazivi clasici de risc aritmic, au specificitate şi valoare predictivă pozitivă mică.

Doar doi parametri noninvazivi se asociază semnificativ statistic cu riscul de MSC pentru luarea deciziei de a implanta profilactic un ICD. Aceştia sunt fracția de ejecție a ventriculului stâng (FEVS) şi alternanța undei T (AT) pe electrocardiograma. În SUA, metoda AT a primit aprobarea „Food And Drug Admnistration” (FDA), în anul 2000, pentru evaluarea riscului de MSC.

Detecția AT de ordinul microvolților pe ECG este asociată unui risc crescut de fibrilație ventriculară primară. În funcție de substratul anatomopatologic şi entitatea clinică, valoarea predictivă pozitivă pentru riscul de MSC se situează între 25 - 70%.

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ISTORIC

Alternanța undei T este alternanța electrică în care morfologia undei T se modifică de la o bătaie la alta într-o succesiune de tip ABABAB…..Primele observații despre AT aparțin lui Hering, în anul 1908[1]. În 1913, Windle[1] descrie pulsus alternans la pacienții cu cardiomiopatie severă şi conchide că "alternanța adaugă totdeauna la gravitatea prognosticului". În anul 1988, Smith, introduce în studiul AT metoda de analiză spectrală[2] care permite detectarea alternanței electrice de ordinul μV. În 1997 a fost publicat primul studiu în care determinarea AT s-a făcut prin test de efort ECG [3].

BAZELE ELECTROFIZIOLOGICE ALE AT

Studii de mapping optic au demonstrat că mecanismul care generează apariția AT este alternanța localizată în durata potențialului de acțiune (DPA). Conform ipotezei restituirii alternanța DPA apare atunci când înclinarea curbei de restituire (DPA exprimată în funcție de intervalul diastolic precedent) este mai mare decât 1 la valoarea curentă a intervalului diastolic[4]. Alternanța localizată a DPA se reflectă pe ECG în AT. Studiile de mapping optic ale lui Pastore şi Rosenbaum au evidențiat că AT pe ecg de suprafață este sincronă cu alternanța DPA[5].

MECANISMELE PRINCIPALE CELULARE ŞI SUBCELULARE

Se accepta trei mecanisme principale:

1. O panta > 1 a curbei de restituire, expresia relației intre DPA si intervalul diastolic anterior. Curba de restituire este dependenta de kinetica curenților repolarizării membranei celulare. Analiza stabilității DPA în contextul unor creşteri bruşte ale frecvenței cardiace impune folosirea curbei restituirii dinamice. Simulări computerizate ale efectelor schimbărilor de ritm cardiac asupra secvențialității DPA, prin folosirea curbelor de restituire dinamice obținute experimental au arătat că, reproducerea cu acuratețe a succesiunii DPA, necesită, de asemenea, includerea efectelor pe termen scurt ale memoriei cardiace, respectiv dependența DPA de durata PA şi intervalelor diastolice premergătoare. Pentru aceasta, Kalb[6] a recomandat folosirea „portretului restituirii" care cuprinde atât restituirea dinamică cât şi pe cea statică. Exista însă, forme de alternanță, ca de exemplu alternanța ischemică, în care înclinarea curbei restituirii este < 1.

2. Perturbarea reciclării intracelulare a ionilor de Ca, cu alternanță secundară a DPA prin afectarea princeps a schimbului Na-Ca. Studiile de mapping optic au arătat că alternanța spațială a calciului citosolic se corelează cu alternanța DPA. Perturbarea reciclării intracelulare a ionilor de Ca se poate realiza prin disfuncția receptorului pentru rianodina, Ca-ATP-azei reticulului sarcoplasmatic (SERCA) sau schimbului Na/Ca. Heterogenitatea spațială a alternanței calciului intracelular se corelează cu dispersia DPA în timpul AT. Diferențele de exprimare a SERCA 2a şi receptorului pentru rianodina pot constitui baza moleculara a diferențelor regionale în susceptibilitatea la alternanță[7].

Fluctuațiile Ca citosolic pot afecta secundar repolarizarea membranei prin intermediul curenților de membrana Ca – sensibili.

3. Alternanța în velocitatea conducerii miocardice prin modificările primare ale curbei de restituire ale vitezei de conducere miocardice. Acest mecanism poate fi legat de remodelarea primară a conexinelor miocardice.

ALTERNANȚA DISCORDANTA - mecanismul aritmogen

Alternanța în DPA nu este un proces cu distribuție uniformă în miocard. La fel cum DPA şi restituirea sunt distribuite ca şi gradienți de la apex la bază şi de la epicard la endocard, la fel sunt reprezentate magnitudinea şi faza alternanței la o anumită frecvența cardiacă (FC).

Potențialul de acțiune la baza ventriculului poate alterna în succesiunea lung – scurt - lung, iar la vârful ventriculului, simultan, alternanța să aibă succesiunea scurt – lung - scurt.

Pastore şi Rosenbaum[5] au folosit pentru descrierea acestui tip de alternanță, termenul de alternanță spațial discordantă (AD). În modelul experimental descris de Rosenbaum[5], prezența AD a fost condiția obligatorie pentru inițierea aritmiilor ventriculare.

Regiunile cu alternanță opusă sunt separate de zone de alternanță zero, cunoscute ca "noduri", care tind să fie orientate în direcția bază-apex. Odată cu depărtarea de zonele nodale magnitudinea alternanței creşte. Pot fi implicate in dezvoltarea alternantei discordante fluctuațiile calciului intracelular sau restituirea bifazica. Fox propune însumarea a trei factori pentru apariția AD şi instalarea blocului conductiv: prezența interacțiunilor electrotonice intercelulare; existenta unei funcții de recuperare a velocitatii conducerii; curba de restituire >1. In cadrul simulărilor sale computerizate, heterogenitatea restituirii este creată prin combinația dintre alternanta velocității conducerii şi curba abruptă a restituirii[6].

FACTORI MODULATORI AI ALTERNANȚEI

Ischemia, frecvența cardiacă şi nivelul tonusului simpatic sunt principalii factori care pot modula AT. Alternanța DPA poate fi privită ca o proprietate intrinsecă a miocardului ischemic[8]. Evaluarea riscului aritmic intrinsec prin AT se va face numai după excluderea ischemiei miocardice. Este însă foarte important de subliniat faptul că nu există dovezi că AT asociată unui risc crescut de aritmii ventriculare la om, ar fi în vreun fel dependentă de prezența ischemiei miocardice[8].

În general, o creştere de tonus simpatic determină o creştere a magnitudinii AT. Administrarea de beta-blocante scade nivelul de alternanță. Există opinii divergente în ceea ce priveşte necesitatea întreruperii beta-blocantelor înaintea efectuării testului. Recomandarea centrelor cu experiență este ca testul să se desfăşoare cu bolnavul sub medicația curentă.

Alternanța T este dependentă de FC. Chiar şi persoane fără afectare cardiacă pot prezenta AT semnificativă la FC mari, de obicei peste 120/min. S-a arătat că FC de debut a AT este mai redusă la pacienți cu afectare structurală cardiacă şi antecedente de aritmii ventriculare susținute, decât cei fără trecut cardiac sau antecedente aritmice. Rezultatele fals pozitive cresc la FC mai mari de 120/min. Condiția esențială pentru un test pozitiv este debutul AT susținute sub 110 bătăi/min.

INTERPRETARE, CLASIFICARE

În practica clinică, se foloseşte actualmente metoda analizei spectrale, dezvoltată de grupul de cercetători condus de Richard J Cohen de la M.I.T, Cambridge, USA. Pentru a calcula un spectru se utilizează 128 măsurători efectuate la nivelul punctelor corespunzătoare a 128 unde T consecutive. Frecvențele rezultate sunt exprimate în cicli/bătaie cardiacă (în loc de cicli/sec). Punctul din spectru, corespunzător la exact 0,5 cicli/bătaie indică nivelul de detecție al AT (Fig.1).

Acest colectiv a pus la punct tehnica necesară pentru evaluarea standardizată a AT prin proba de efort ecg obişnuită. Aparatura este comercializată sub denumirea "HEARTWAVE" şi este proprietatea firmei "CAMBRIDGE HEART". La noi în țară, de vânzarea aparatului, se ocupă firma "PHILIPS"(Fig.2)

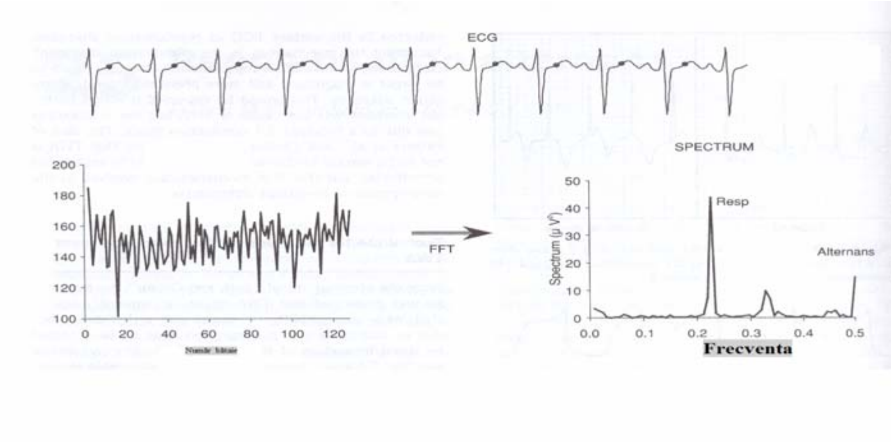

Figura 1 Transformarea Fourier a unei serii termporale pentru analiza spectrala a alternantei T

Rezultatele analizei sunt exprimate sub forma unor indici[9]:

- puterea alternanței(µV 2) : măsoară nivelul fiziologic real al AT;
- voltajul alternanței, exprimat în µV, reprezintă rădăcina pătrată a puterii alternanței;
- raportul alternanței, sau scorul K calculat ca raportul dintre puterea AT şi deviația standard a zgomotului în frecvența de referință. Alternanța T este considerată semnificativă dacă Indicele K este>3.

CLASIFICAREA TESTELOR de AT: Alternanța susținută: AT evident prezentă la frecvențe cardiace mai mari decât frecvența de debut; cu cel puțin 1 minut cu Valt >1,9 µV şi raport K >3 ; în oricare din derivațiile X, Y, Z sau VM (vector maxim) sau una din derivațiile precordiale, confirmată de o derivație precordială adiacentă (cu Valt>1,9µV). Alternanța poate fi considerată susținută chiar dacă magnitudinea scade la FC > 120/min[9].

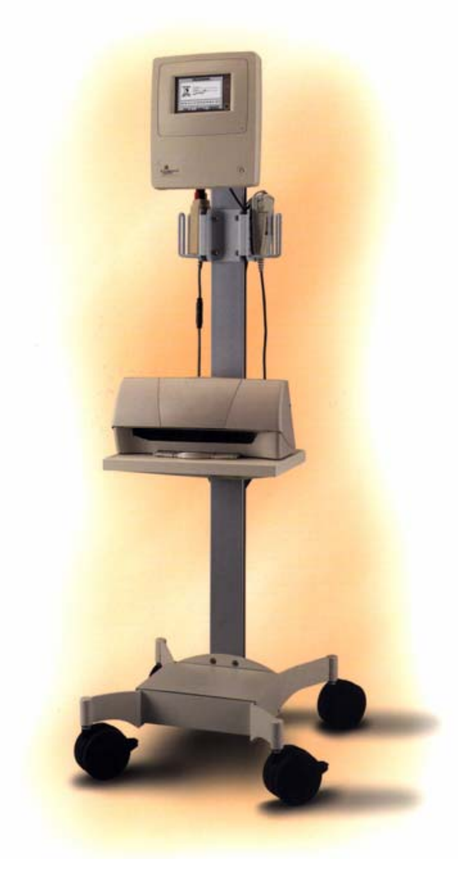

Figura 2 Aparatul "HEARTWAVE"

Criteriile de clasificare[9]: Test pozitiv (Fig. 3): AT susținută la o FC de debut (FCd) <110/min sau AT susținută la FC de repaus, chiar dacă aceasta este >110 b/min. Test negativ (Fig. 4): Regulile A: 1)Testul nu îndeplineşte criteriile pentru a fi pozitiv şi 2) frecvența cardiacă negativă maximă este >105b/min. Regulile B: testul este de asemenea clasificat ca negativ dacă pe timpul unui exercițiu maximal FC maximă este > 80 b/min şi dacă FC negativă maximă > (Fcmax - 5b/min). Test nedeterminat (echivoc): testul nu poate fi definitiv clasificat fie pozitiv, fie negativ.

Deoarece regulile B au fost testate clinic, prospectiv şi retrospectiv, Bloomfield[9] recomandă adoptarea lor în clinică, pe considerentul reducerii numărului de teste echivoce.

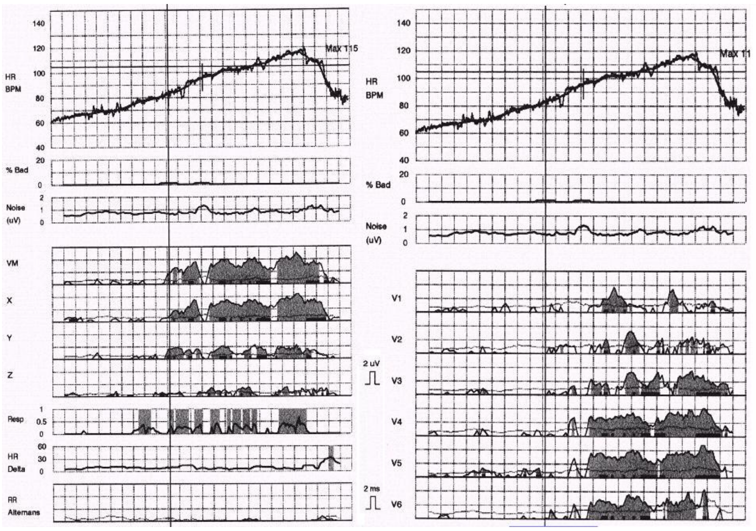

Figura 3. Test de AT pozitiv

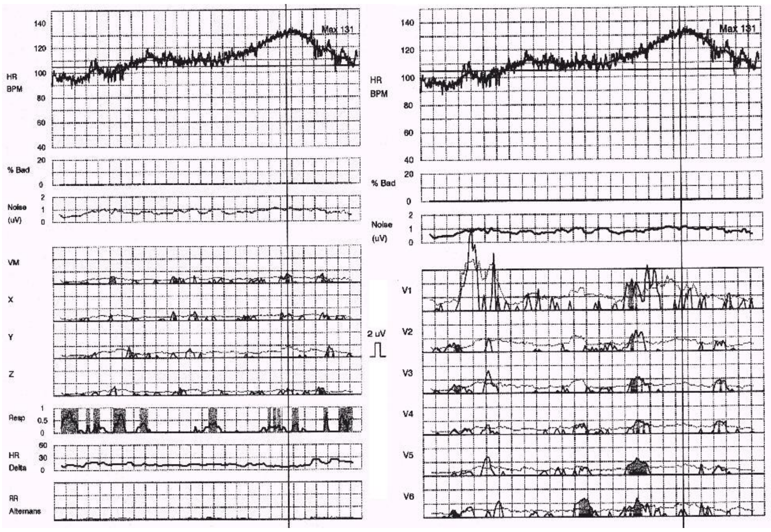

Figura 4. Test de AT negativ

Contraindicațiile pentru testarea AT sunt: fibrilația atrială, extrasistolia atrială frecventă, extrasistolia ventriculară, ritmurile electrostimulate ventriculare, reacțiile adverse la electrozii tegumentari. Reproductibilitatea pe termen scurt a metodei este satisfăcătoare. Turitto[10] compară înregistrările succesive de mAT efectuate la interval de 4 ore unul de altul. Din 42 de pacienți cu 2 înregistrări succesive, 39(93%) au prezentat rezultate concordante.

PRINCIPALELE REZULTATE CLINICE

Pacienți cu risc de aritmii ventriculare maligne (Tahicardie Ventriculară, TV, Fibrilație ventriculară, FV). Rosenbaum a publicat, în 1994, primul studiu clinic[11]: 83 de pacienți la care s-a determinat AT prin stimulare atrială concomitent cu SVP. La 32 pacienți cu SVP pozitivă, AT a fost un predictor al inductibilității TV cu o sensibilitate de 81% şi o specificitate de 84%.

Ca valoare predictivă, AT a fost echivalentă SVP. Ulterior, mai multe studii clinice[12-15] au dovedit valoarea predictivă a AT pentru riscul de aritmii ventriculare. Relevant este faptul că, după studiul lui Gold[13], FDA a aprobat oficial AT pentru evaluarea riscului de MSC în SUA.

Pacienți cu disfuncție de VS.

Studii clinice recente[16-21] au arătat că AT poate fi folosită pentru evaluarea riscului aritmic la pacienții cu Insuficiență cardiacă congestivă şi Cardiomiopatie dilatativă. În insuficiența cardiacă, pacienții cu AT (+) au avut o mortalitate de 10% la 2 ani, față de numai 1% la grupul cu AT negativă[17]. Valoarea predictivă negativă excelentă a AT este deosebit de utilă clinic la pacienții de tip MADIT II : date recente relevă că la bolnavii postinfarct miocardic cu FE <30% şi AT (-) nu este necesar implantul profilactic de ICD.

Pacienți cu Sincopă.

Bloomfield[22], analizând rezultatele AT la pacienți coronarieni cu sincopă, ajunge la concluzia a fost că AT poate identifica cel puțin la fel de bine ca SVP un subgrup de pacienți cu risc mare de MSC sau aritmii ventriculare. Pacienții cu AT (-) au un prognostic excelent şi de cele mai multe ori nu ar mai necesita investigații suplimentare.

Marangoni[23] a sugerat că utilizarea AT ca test de screening la pacienții coronarieni cu sincopă ar putea reduce foarte mult costurile de spitalizare. Argumentele au fost următoarele: metoda AT are sensibilitatea şi specificitatea similare SVP dar este de 10 ori mai ieftină şi fără nici un risc pentru pacient. Datorită faptului că în toate trial-urile clinice (2000 pacienți) valoarea predictivă negativă este aproape 99% (aproape nici un pacient cu AT negativă nu a prezentat MSC în următoarele 15 luni), este justificată economic doar internarea pacienților cu test AT(+).

Pacienți cu Infarct miocardic.

La 850 bolnavi, cu IM în antecedente, Ikeda[23] găseşte AT cel mai bun predictor de risc aritmic: un test de AT pozitiv ar indica un risc de aproximativ 20% pentru apariția în următorii 2 ani de MSC, FV sau TV.

Implicațiile AT in selecția pacienților cu indicație de implant de ICD.

Deoarece testul AT este util pentru relevarea riscului de aritmii ventriculare, poate fi folosit ca test de screening pentru identificarea bolnavilor care să fie adresați SVP şi să beneficieze de implantul de ICD. Testul poate delimita pacienții cu risc minim, care nu necesită implant profilactic de ICD.

Ezekowitz[25] analizează eficacitatea implantului de ICD la persoane cu risc crescut de MS, subliniind că deciziile referitoare la alocarea resurselor pentru ICD depind de stratificarea cu acuratețe a pacienților în funcție de risc.

Autorii rezumă rezultatele a 8 trial-uri care au inclus 4909 pacienți: AVID, CASH, CIDS, MADIT, CABG patch, MUSTT, MADIT II, CAT. ICD a redus riscul relativ pentru MSC cu aproximativ 50%, independent de riscul bazal. Analiza efectuată relevă o mare variabilitate a raporturilor de cost eficiență cu valori între 17,700 şi 138,800 dolari pe an de viață salvată. Marea variabilitate în raporturile de cost eficiență relevă importanța unei stratificări cu acuratețe a riscului aritmic, în vederea determinării pacienților care să beneficieze maximal de tratamentul prin ICD.

CONCLUZII

Alternanța undei T este un test clinic neinvaziv indicat în practica cardiologică pentru analiza riscului de aritmii ventriculare şi de MSC. Valoarea predictivă pozitivă, după diversele studii existente este variabilă, de la 25% la 80%, în funcție de afecțiunea de bază.

În anumite situații clinice, valoarea predictivă pozitivă poate fi crescută prin corelarea AT cu rezultatele SVP. Valoarea predictivă negativă este mai mare de 99%. Testul AT poate fi folosit in screeningul riscului de MSC la grupele de risc cardiovascular.

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Tulburarea de Stres Posttraumatic – o realitate a societății româneşti

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OBIECTIV: Importanța cercetării tulburării de stres posttraumatic (TSPT) pentru societatea românească ca urmare a creşterii numărului de militari şi angajați civili care au participat în diverse teatre de operațiuni şi manifestă simptome ale acestei tulburări psihice.
MATERIAL ŞI METODĂ: 3,2% din militarii care s-au întors din teatrele de război au fost diagnosticați şi au primit tratament pentru TPST; aproximativ 7% din cei care s-au reîntors şi nu au fost diagnosticați şi nu au solicitat consult psihiatric/psihologic de specialitate, având altă simptomatologie pentru care a fost solicitată evaluare psihologică, prezentau simptomele TSPT, motivând „ruşine" şi „frică" să recunoască starea lor în fața superiorilor. De asemenea am primit solicitări şi din partea familiilor pentru consiliere („nu reuşim să-l mai înțelegem", „s-au întors alți oameni decât cei care au plecat").
CONCLUZII: Cercetările indică faptul că TPST reprezintă o afecțiune psihiatrică importantă ce produce o suferință emoțională şi o inadaptare socială considerabilă, prezentând adesea un tablou clinic complex ce constituie o semnificativă provocare în ceea ce priveşte conduita terapeutică.

tulburare de stres posttraumatic **centru de recuperare** **militari şi angajați civili participanți la misiuni**

ABSTRACT
Purpose: The importance of study of Post-traumatic stress disorder (PTSD) in Romanian society, as a consequence of participation of Romanians troops in confliction areas.
Material and method: 3,2% of combatants were diagnosed with PTSD and were treated for this; 7% of combatants were diagnosed in time with PTSD, during another investigation; they justify "shame" and "fear" to admit disorder. Them family ask for support as well, as long as they can't communicate with them.
Conclusion: PTSD is an important psychiatric disease which can lead to endure and social inadaptability; it is a challenge in therapeutically assignment.
Key words: PTSD, recovery center, combatants

INTRODUCERE

„Şocul de bombardament" sau „sindromul de oboseală din timpul războaielor" cum a mai fost denumită tulburarea de stres posttraumatic (TSPT), se manifestă ca reacție la un eveniment catastrofal sever care se situează în afara limitelor experienței umane şi ar provoca oricui suferință. Tabloul clinic al TSPT este complex şi include simptome din sfera cognitivă, afectivă, fiziologică, comportamentală şi relațională. Terapia cognitiv-comportamentală, care pune un accent deosebit pe metode variate de expunere, s-a dovedit a fi cea mai eficace metodă de intervenție psihoterapeutică.

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Importanța cercetării în acest domeniu a devenit evidentă numai spre mijlocul anilor '70, când societatea americană s-a confruntat pentru prima dată cu problemele provocate de formele dezadaptative de comportament ale veteranilor războiului din Vietnam.

Datele obținute arătau că pentru aproximativ 25% din cei ce au luptat în Vietnam, experiența participării la acțiunile militare a fost cauza dezvoltării unor modificări negative de personalitate sub influența traumelor psihice.

Se estimează că peste 20% din totalul persoanelor care se întorc din taberele de război vor dezvolta TSPT şi că mult mai mulți vor dezvolta pe parcursul vieții tulburări psihice variate, de la depresie până la anxietate, vor trăi experiența divorțului, a consumului de droguri şi alcool, vor manifesta acte antisociale (Frank Ochberg, „Your Military Family Network”, 2007).

În 2005 cei aproape 216.000 de veterani ai SUA diagnosticați cu TSPT au beneficiat de 4,3 miliarde USD. Studiul din 2006 efectuat de revista Journal of the American Medical Association arată că 9,8% din combatanții din Irak s-au întors cu TSPT, iar 11,9% au dezvoltat TSPT în primul an de la întoarcerea acasă. În plus, peste 50.000 din veteranii din Irak şi Afganistan suferă de probleme mentale şi de sănătate, aproape jumătate dintre ei fiind diagnosticați cu TSPT; 22 dintre aceştia s-au sinucis, ridicând statistica la 1 din 5 necombatanți morți în 2005. În acest context Congresul SUA a cerut Departamentului Apărării să identifice şi să trateze membrii Serviciilor Armate suferinzi de TSPT.

În literatura de specialitate "stresul posttraumatic" sau "sindromul tulburărilor de stres posttraumatic" (TSPT) este descris ca fiind un stres întârziat şi care poate fi declanşat prin dezastre naturale, accidente grave, agresiuni, violuri şi alte evenimente critice cu impact traumatizant, atât fizic dar mai ales psihic.

Viața şi activitatea militară, în condiții de pace şi de criză şi cu atât mai mult în timp de război, constituie o sursă de trăiri emoționale pozitive şi negative complexe, niciodată univoce, uneori contradictorii sau ambivalente, dar întotdeauna aflate în situația de a genera şi de a menține stresul psihic.

Cercetările asupra stresului posttraumatic au debutat cu mai mult de jumătate de veac în urmă, încă în cel de-al doilea război mondial. Toate descrierile acestui fenomen includeau întotdeauna un şir de elemente comune: tulburări de somn, vise cu coşmaruri, depresii, excitabilitate crescută. Kardiner dă pentru prima dată o descriere complexă a simptomaticii acestui fenomen: 1) excitabilitate şi iritabilitate; 2) reacție nestăpânită la excitanți bruşti; 3) fixarea pe circumstanțele evenimentului traumatic; 4) fuga de realitate; 5) predispoziția pentru reacții agresive necontrolate.

În rezultatul cercetărilor a devenit clar că starea care se dezvoltă la cei ce au trecut prin situații critice generatoare de stres psihic (participarea la acțiuni militare, accidente, calamități naturale, diverse forme de violență) manifesta tendința nu numai să nu dispară cu timpul dar şi să devină tot mai pronunțată, debutând brusc pe fondul unei aparente bunăstări generale a persoanei.

Complexul de simptome ale tulburărilor cauzate de influența traumatizantă a diverşi factori stresanți asupra psihicului, de regulă ieşiți din comun în comparație cu experiența obişnuită a unui om, a fost descris în 1980 şi în DSM-III, ediția a 3-a, primind denumirea de sindromul tulburărilor de stres posttraumatic - TSPT. Caracteristic pentru sindromul descris este manifestarea sa tardivă, după o prelungită latență, putându-se vorbi de un stres psihic întârziat, şi faptul că sindromul poate fi intensificat prin alți factori stresanți adesea greu identificabili.

MATERIAL ŞI METODĂ

În DSM-IV[1] tulburarea posttraumatică de stres este definită prin şase criterii de bază:

(1) existența în antecedente a unui eveniment traumatic care implică atât iminența sau amenințarea cu moartea sau cu vătămarea importantă, cât şi un răspuns intens de frică, neajutorare sau groază;

(2) retrăirea persistentă a evenimentului traumatic prin intermediul amintirilor intruzive, a flashback-urilor disociative, a viselor chinuitoare recurente şi/sau a reactivității psihologice sau fiziologice la expunerea la unele activități sau situații care amintesc trauma;

(3) evitarea stimulilor asociați evenimentului sau o paralizare a responsivității generale, incluzând eforturile făcute de a evita gânduri sau sentimente legate de traumă, eforturi de a evita activități sau situații care trezesc amintiri ale traumei, pierderea interesului pentru activitățile importante, detaşare de alți oameni şi/sau tocire emoțională;

(4) existența unor simptome persistente ale unei stări de „hiperactivare" vegetativă ca, de exemplu, hipervigilență, tulburări de somn, iritabilitate sau izbucniri de furie, tulburări de concentrare şi/sau o reacție de surpriză exagerată;

(5) tulburarea are o durată de cel puțin o lună;

(6) efectele tulburării produc o suferință clinică semnificativă sau o afectare în funcționarea socială, ocupațională sau în alte domenii importante. Deşi simptomele TSPT sunt în mod obişnuit grupate în trei categorii primare (retrăire, evitare/paralizie, hiperactivare), simptomele tipice ce includ repetate retrăiri ale traumei împreună cu reactivitatea fiziologică asociată sunt cele care deosebesc cel mai bine TSPT de alte tulburări afective sau anxioase[2].

Haber şi colaboratorii[3] au constatat că tabloul clinic al TSPT include simptome din sfera cognitivă, afectivă, fiziologică, comportamentală şi relațională.

Dintre simptomele cognitive evidențiem următoarele: dificultăți de concentrare a atenției voluntare şi fixare a memoriei; retrăiri intense şi repetate ale experienței traumatice; prezența tulburărilor de senzorialitate (iluzii, halucinații); simptome disociative; amnezia psihogenă, incapacitatea de a-şi aminti aspecte importante ale traumei; evitarea gândurilor asociate cu evenimentul traumatic; vise cu coşmaruri repetate; percepție neobişnuită, adesea distorsionată a realității; afectarea viitorului în ceea ce priveşte profesia, orientarea profesională sau căsătoria; sentimente de autovinovăție; lipsa integrării cognitive a evenimentului traumatic perceput ca un factor de vulnerabilitate şi interpretare a unor situații neutre sau inofensive ca un pericol şi o amenințare; rezolvarea unor probleme se asociază cu crize de anxietate sau mânie.

În sfera afectivă putem regăsi următoarele simptome: stări de iritabilitate, explozii de mânie; disconfort intens apărut la confruntarea cu evenimente care simbolizează sau se aseamănă prin unele aspecte cu experiența traumatică; reducerea gamei reacțiilor emoționale; incapacitatea de a trăi sentimente de dragoste; anestezie emoțională sau tocire afectivă manifestată prin diminuarea capacității de răspuns afectiv; labilitate emoțională; sentimente de culpabilitate şi vinovăție; dispoziție depresivă, tristețe; stări de anxietate; incapacitatea de a trăi bucuria şi plăcerea unor activități relaxante.

Reacţiile fiziologice se pot manifesta astfel: dificultăţi la instalarea sau menţinerea somnului; răspuns exagerat în situaţii în care persoana este surprinsă sau speriată; reactivitate fiziolgică crescută în prezenţa unui stimul care reactivează amintirile, sentimentele sau senzaţiile asociate cu trauma; hipervigilenţă; modificarea parametrilor vitali prin creşterea frecvenţei cardiace şi a tensiunii arteriale.

În sfera comportamentală întâlnim o gamă de simptome variate, cum ar fi: evitarea fobică a situaţiilor care determină rememorarea evenimentului stresant; scăderea interesului şi reducerea participării în cadrul unor activităţi importante; fuga psihogenă manifestată prin calătorii neaşteptate departe de casă sau asumarea unor identităţi noi; nelinişte psihomotorie; dificultăţi în îndeplinirea sarcinilor cotidiene; schimbări ale stilului de viaţă sau a locuinţei; impulsivitate, episoade de agresivitate imprevizibilă; dependenţă medicamentoasă faţă de antialgice sau anxiolitice, utilizate pentru terapia disconfortului emoţional şi/sau a durerii fizice produse de evenimentele traumatice; abuz de substanţe psihoactive; tentative de autovătămare corporală.

În sfera relaţională se înregistrează o serie de simptome, de tipul: detaşare, înstrăinare şi izolare faţă de anturaj; afectarea capacităţii de trăire a sentimentelor de tandreţe şi intimitate; deteriorarea relaţiilor maritale şi parentale; distanţare excesivă exprimată în relaţionarea interpersonală cauzată de teama unei experienţe anterioare de trădare; evitarea unor dezvăluiri personale determinate de lipsa de încredere în alte persoane şi de teama de a fi respins.

Simptomele întâlnite în TSPT pot fi considerate, iniţial, o componentă a răspunsului normal al unei persoane la o experienţă copleşitoare. Dacă durata existenţei lor depăşeşte trei luni şi se prelungeşte o perioadă de luni sau ani de zile înseamnă că este vorba de o tulburare posttraumatică de stres. Indivizii care suferă de TSPT prezintă cel puţin un episod în care evenimentul traumatic revine în viaţa cotidiană. Uneori, acest fenomen se petrece brusc, evocarea revine clar în memorie şi se acompaniază de emoţii intense, dureroase. Alteori, se produce o retrăire a traumei în care amintirile sunt atât de puternice încât persoana revede scenariul traumei în faţa ochilor sau consideră că este vorba de o trăire autentică a traumei. Manifestările clinice descrise pot surveni în mod brusc, sub forma unui atac emoţional dureros, fără vreo cauză aparentă. Emoţiile pot exprima o stare de supărare care se însoţeşte de frică, mânie, crize de plâns. Experienţele emoţionale se produc în mod repetat sub forma reevocărilor evenimentelor traumatice sau a viselor cu coşmar.

În TSPT se manifestă pregnant fenomenul de evitare. Persoanele care au suferit traume se simt amortiţe, împietrite, evită relaţiile emoţionale cu familia, colegii de serviciu, prietenii şi îndeplinesc numai activităţile mecanice, de rutină, având o aparenţă rece, distantă, plictisită. Blake, Cook[4] descriu lipsa de afecţiune a acestor persoane faţă de propria familie, care se reflectă în conduita lor mecanică, inadaptată ambianţei. În acest context relatările membrilor familiei, ale prietenilor, ale colegilor de serviciu ajută la stabilirea diagnosticului şi a conduitei terapeutice.

Persoanele care prezintă TSPT evită situaţiile care le reamintesc de evenimentul traumatic, dezvoltând chiar o stare de teamă accentuată faţă de astfel de situaţii deoarece acestea pot agrava tabloul simptomatologic. Mulţi veterani de război refuză să accepte anumite responsabilităţi faţă de alte persoane, deoarece nu se consideră în stare să le asigure siguranţa şi protecţia lor. Alţii se simt vinovaţi pentru faptul că au supravieţuit dezastrului, în timp ce familiile lor sau prietenii au murit.

În studiile sale Symes[5] arată că depresia constituie o trăsătură comună a TSPT, ca o consecință a inabilității de rezolvare a emoțiilor dureroase, a stării de mânie şi supărare determinate de pierderile şi suferințele cauzate de evenimentul traumatic.

După încetarea acțiunii traumelor, comportamentul persoanelor cu TSPT este influențat în continuare de reacții emoționale dureroase, fără ca ele să conştientizeze acest lucru.

Starea de hiperexcitație cronică şi hipervigilență produce o modificare a comportamentului pacienților care suferă de TSPT, o scădere a capacității de concentrare a atenției voluntare şi de fixare a memoriei şi induce insomnii. Adesea, veteranii de război acționează într-un mod asemănător cu cel prezentat în perioada în care erau amenințați de traumă. Henry[6] arată că starea de hipervigilență întâlnită în TSPT determină o scădere a perfomanțelor profesionale şi o interrelaționare mai redusă cu familia şi prietenii.

Apariția unei reacții de alarmă biologică se exprimă prin declanşarea unor atacuri de panică, caracterizate printr-o teamă severă asemănătoare celei din timpul războaielor sau catastrofelor naturale, prin manifestări neurovegetative de tipul: epigastralgiilor, senzației de sufocare şi algiilor cefalice în cască[7].

Repartizarea celor mai semnificative simptome utilizate în evaluarea TSPT arată astfel:
1) prezența în anamneză a unei situații traumatizante (93% din experți i-au acordat nota cea mai mare);
2) întoarcerea permanentă la trăirile legate de circumstanțele traumei (78%);
3) visele şi coşmarurile pe tema traumei (77%);
4) amintirile obsesive (75%);
5) întoarcerile la situația traumatizantă prin asociații sau asemănarea circumstanțelor (73%);
6) "reactivitatea fiziologică" (mai mult de 66%).

METODĂ

Un model de tratament cognitiv-comportamental cu multiple componente este adecvat pentru majoritatea persoanelor cu TSPT. Acest model include un tratament multilateral destinat diverselor aspecte ale sindromului clinic asociat cu TSPT, cum ar fi reactivitatea emoțională şi fiziologică la anumite situații sau acțiuni care reamintesc trauma, simptomele intruzive şi comportamentul evitant, aptitudinile sociale deteriorate şi modularea emoțiilor (controlul furiei). Programul unifică abordarea terapeutică psihosocială a TSPT cu cea mai empirică susținere (terapia prin expunere) cu o componentă de exersare a aptitudinilor sociale şi a managementului anxietății[8].

Componentele principale ale acestui model sunt:

(1) Educația. Tuturor pacienților li se furnizează date generale despre TSPT cronică incluzând modalitățile frecvente de manifestare, problemele de diagnostic, comorbiditatea cu alte tulburări anxioase sau ale Axei I, etiologia şi o recapitulare a strategiilor actuale de tratament. Această componentă poate fi eficient realizată în 1-8 şedințe.

(2) Antrenamentul abilităților pentru managementul anxietății. Scopul acesteia este de a învață pacienții să-şi controleze anxietatea şi nivelurile stresului, inclusiv atacurile de panică. Exersarea aptitudinilor de management al anxietății poate servi unor funcții multiple şi probabil poate fi eficient realizată în 3-10 şedințe. Programele pentru suplimentarea terapiei prin expunere cu exersarea aptitudinilor de management al anxietății au fost cu succes realizate cu alte populații de victime ale traumelor[9] şi pot să includă elemente de exersare a relaxării, a respirației şi de control al panicii.

(3) Expunerea. Pacienţii trebuie expuşi la stimulii ce produc anxietate sau frică (legaţi de traumă) într-o maniera prelungită până există o reducere a fricii sau anxietăţii (până se obţine obişnuinţa sau stingerea) în cursul sedinţelor. Majoritatea persoanelor cu TSPT evită stimulii ce declanşează teama şi acest lucru ajută la creşterea intensităţii răspunsului de frică. Aşadar scopul expunerii este de a furniza un contact prelungit cu stimulii ce declanşează teama cu o durată suficientă ca obişnuinţa să se producă în cursul şedinţelor (o reducere cu 50% în reactivitatea din cursul şedinţei). Datele sugerează că această componentă poate fi eficient realizată în 4-15 şedinţe.

(4) „Temele pentru acasă". Componenta „temelor pentru acasă" (sau „practica programată") se poate aplica odată ce mai multe şedinţe de expunere au fost realizate cu succes şi reprezintă o formă de expunere care nu necesită prezenţa terapeutului. Trebuie imaginate experienţe care cer pacientului să se angajeze de la şedinţe de auto-expunere în imaginaţie realizate acasă care pot să servească drept pas iniţial către activităţile în vivo, ca de exemplu, vizionarea unor filme, discutarea cu alte victime ale traumelor sau cu persoanele dragi despre experienţele traumatice, etc. până la activităţi de care se teme şi care pot interfera cu calitatea vieţii: evenimente sociale (petrecerile, mesele împreună cu prietenii), cumpărături, mersul la filme, mese la restaurant, etc.

(5) Antrenamentul abilităţilor sociale. Scopul acestuia este de a forma abilităţi absolut necesare unor interacţiuni sociale eficace şi care pot antrena sentimentul de satisfacţie. Antrenamentul abilităţilor sociale poate fi deprins eficient în 6-15 şedinţe. Antrenamentul abilităţilor sociale vizează instruirea, modelarea, repetiţia comportamentală sau „jocurile de rol", feedback-ul şi întărirea. După fiecare şedinţă pacienţilor li se dau teme pentru acasă pentru a permite exersarea ulterioară şi consolidarea abilităţilor nou dobândite. Antrenarea abilităţilor sociale include următoarele elemente[10,11]:

Conştiinţa mediului social stabileşte nuanţele legate de când, cum şi de ce să iniţieze sau să întrerupă interacţiunile sociale. Aceasta include mecanismele verbale şi non-verbale ale întâlnirilor sociale de succes, incluzând identificarea unor subiecte de conversaţie adecvate, exerciţii specifice destinate să crească aptitudinile de atenţie şi ascultare, precum şi schimbările de subiect eficace[12].

Stimularea abilităţilor sociale urmăreşte formarea unor deprinderi de a iniţia şi de a menţine prieteniile, aptitudini adecvate pentru conversaţiile telefonice şi comunicarea asertivă. Această componentă este destinată să-i ajute pe pacienţi să înveţe acele abilităţi care le sunt necesare implicării în activităţi sociale diverse şi noi cu scopul de a-şi creşte repertoriul social şi posibilitatea ca interacţiunile sociale să devină intrinsec mai mulţumitoare[12].

Managementul furiei vizează controlul furiei şi al emoţiilor intense. Ar trebui să fie destinat reducerii izbucnirilor temperamentale şi manifestării problematice a furiei. În mod specific, această componentă trebuie să ajute prin a oferi pacienţilor o gamă de strategii pentru a-şi exprima furia, a-şi rezolva problemele, a-şi îmbunătăţi modularea emoţională şi a comunică asertiv cu ceilalţi, astfel încât violenţa fizică şi verbală să nu continue să le perturbe relaţiile sociale.

Managementul consecinţelor traumei îi învaţă cum să-şi îmbunătăţească comunicarea cu ceilalţi privind trauma, astfel încât să sporească înţelegerea acestora. Pacienţii trebuie învăţaţi cum să comunice asertiv când nu vor sau nu pot să discute cu alţii despre anumite subiecte sau evenimente. În plus, ei trebuie, de asemenea, învăţaţi să identifice şi să se opună modelelor de gândire negativă sau dihotomică (credinţa de a nu se încrede în toţi ceilalţi), ceea ce le limitează calitatea vieţii prin reducerea implicării în relaţii cu ceilalţi. În ultima instanţă, acest element trebuie să includă informaţii despre cum să evite să mai fie victime ale traumelor în viitor (sfaturi privind siguranţa personală).

Persoanele care dezvoltă simptomele TSPT nu trebuie să se aştepte ca 1-4 luni de terapie cognitiv-comportamentală intensă să „vindece" toate problemele. Mai curând tratamentul trebuie privit ca un mijloc de a ajuta pacienţii să-şi stăpânească şi să-şi controleze simptomele. Ţinând cont de acestea, este de aşteptat ca urmărirea continuă pe termen lung să fie necesară la majoritatea persoanelor cu TSPT.

CONCLUZII

Acest demers demonstrează că tulburarea de stres posttraumatic este o problemă a societăţii româneşti care, neglijată, poate avea consecinţe negative pe termen mediu şi lung asupra calităţii vieţii militarilor şi personalului civil angajat participant la misiunile din teatrele de război şi a familiilor acestora.

În acest scop propunem înfiinţarea unor centre pentru TSPT care să se adreseze acestei categorii sociale şi care să aibă drept scop:

- consilierea şi pregătirea psihologică a participanţilor în misiune, 3-4 zile;
- asigurarea îngrijirii medicale şi a reinserţiei sociale a participanţilor la misiuni;
- oferă educaţie şi pregătire în gestionarea stresului posttraumatic şi a altor tulburări legate de stres, stimularea resurselor şi a readaptării la mediul socio-profesional;
- informarea, educarea şi consilierea psihologică a familiilor;
- monitorizare pe o perioadă de 1 an de zile prin prezentări periodice (o dată la 3 luni sau la solicitare).

Considerăm că tratamentul şi recuperarea în TSPT poate fi realizată mult mai eficient imediat după revenirea din misiunile speciale, împreună cu familia, aproximativ o lună de zile într-o zonă cu caracteristici balneo-climaterice sedative, favorabil persoanelor sănătoase sau cu stări de surmenaj.

Aceste caracteristici bioclimatice, precum şi dotările moderne pentru recuperarea medicală (instalaţii pentru electroterapie, afuziuni, duşuri scoţiene, duş-masaj, duş subaqual, saună, aerosoli, sală de cultură fizică medicală şi recuperatorie, bazin de hidrokinetoterapie, băi cu apă clorosodică caldă, băi galvanice şi împachetări cu nămol de sendiment) sunt argumente care susţin înfiinţarea unui asemenea centru în staţiunea Bălţăteşti.

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Complianța la tratament a bolnavilor TB, cheia succesului terapeutic

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OBIECTIV: Tuberculoza reprezintă o problemă prioritară a sănătății publice în România. Controlul tuberculozei trebuie să devină astfel o prioritate a politicii de sănătate a Guvernului şi a programului de dezvoltare socio-economică pe termen mediu şi lung.
Creşterea complianței pacienților la tratamentul antituberculos în condiții de ambulator de specialitate a devenit o problemă în lumea medicală de specialitate, datorită importanței fenomenului „tuberculoză", în țară şi în lume. Dezideratul se poate materializa prin eforturi conjugate, strict medicale, coroborate cu acțiuni socio–economice, prin acordarea de stimulente sau facilități, în scopul creşterii gradului de aderență la tratament, ca urmare a eforturilor sporite de ridicare a nivelului de educație de sănătate şi a comunicării între pacient şi cadrele medicale.

tratament sub directa observare Strategie Națională de Control a tubercuzei complianță

ABSTRACT: Tuberculosis is a priority Public Health problem in Romania. Therefore, the control of tuberculosis must be a priority of the Government health policy and of the social and economical development programme on long-intermediate term.
The compliance development of the patients at the anti-phthisical treatment, in conditions of the out-patients department has been a problem in the medical specialty world because of the importance of „tuberculosis" phenomenum in the country and in the world. This wish can be achieved by uniting strictly medical efforts, corroborated with socio-economical activities, by according some stimuli or facilities, with the purpose of developing the adherence to the treatment, as a result of the increased efforts to increase the level of health education and communication among the patient and the medical staff.
Key words: tuberculosis, treatment under direct observation, National Strategy of Tuberculosis Control, compliance.

Strategia Națională de Control al Tuberculozei se încadrează în Strategia de țară a României privind atingerea până în 2015 a obiectivelor de dezvoltare ale mileniului (2015 MDGs) şi oferă cadrul necesar dezvoltării şi armonizării legislației şi reglementărilor naționale cu cele europene.

După adoptarea, începând cu anul 1998, a strategiei internaționale de tratament sub directă observație (DOTS) şi implementarea Programului Național de Control a Tuberculozei 2001-2005 (PNCT), România înregistrează in 2003, pentru prima dată în ultimii 20 de ani, o tendință de scădere a cazurilor noi de tuberculoză.

Scopul Strategiei Naționale de Control a Tubercuzei 2006-2010 este de a contribui la îmbunătățirea stării de sănătate a populației României, prin reducerea morbidității şi mortalității prin tuberculoză şi limitarea cât mai rapidă a răspândirii infecției şi bolii în populație, având la bază recomandările experților OMS, precum şi direcțiile strategice din Planul Global de Stopare a Tuberculozei 2006-2015.

Tuberculoza continuă să îsi etaleze istoria de peste 3000 de ani, infectând în momentul prezent o treime din populația globului şi ucigând aproximativ 2 milioane de oameni anual.

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Această boală rămâne în continuare să țină prima pagină a patologiei infecțioase de pe întreg mapamondul.

Se estimează că în ultimul deceniu al secolului trecut s-au înregistrat în lume peste 32 milioane de noi îmbolnăviri TB şi peste 10 milioane decese datorate TB; mai mult de 8 milioane de oameni fac TB/an şi aproximativ 2 milioane mor. Se apreciază că numărul cazurilor TB va crețte în ciuda eforturilor care se fac pentru controlul acestei boli şi va ajunge în 2020 la circa 10 milioane cazuri noi/an.

Dacă această boală nu va fi controlată în viitor, se estimează ca în perioada până în 2020, aproximativ 1000 milioane de oameni se vor infecta cu BK, peste 150 milioane vor face boala, iar 36 milioane vor deceda.

Distribuția geografică a tuberculozei este diferită de la un continent la altul şi chiar de la o țară la alta, incidența cea mai ridicată înregistrandu-se în Africa şi Asia.

În regiunea Europa, 16 din 51 țări se confruntă cu creşteri dinamice ale incidenței în ultimii 10 ani. În aceste țări se concentrează 77% din întreaga endemie europeană. România este pe locul 3 ca gravitate a endemiei. Cu toate că există o strategie numită DOTS, tuberculoza continuă să facă victime.

Tuberculoza reprezintă o importantă problemă de sănătate publică în România. Conform statisticilor WHO, în România sunt raportate zilnic 82 cazuri noi la adulți, 5 la copii şi 5 decese.

Tuberculoza afectează în principal populația activă a țării, înregistrându-se aproximativ 400 pacienți TB %oo, cu vârste între 45 - 49 ani.

Bolile contagioase care au fost controlate eficient în România, au reaparut în ultimii 10 ani, incluzând creşterea abruptă a frecvenței TB, ajungând de la 70%oo în 1990, la 138,7%oo în 2002, urmată de o scădere uşoară până în prezent.

Una din cauzele majore ale creşterii incidenței este complianța scăzută la tratamentul greoi şi de lungă durată. Deşi nimeni nu poate fi declarat vinovat că s-a îmbolnăvit de tuberculoză, de regulă, aceşti bolnavi nu sunt bine priviți în societate. Omul sănătos se teme să nu se îmbolnăvească şi, de aceea, când află de o rudă, coleg ori prieten că are tuberculoză, neştiind cum să se protejeze, se arată ostil față de pacient.

Există mentalitatea că TB e o boală ruşinoasă, încât pacientul sau familia se feresc să popularizeze situația. Sunt oameni cărora le vine greu să conceapă faptul că suferă de TB. Asta nu face decât să întârzie prezentarea la medic şi începerea tratamentului, timp în care pot infecta alte persoane.

Probleme apar şi la reintegrarea în muncă – este primit cu rezervă de colegi şi anturaj. Realitatea însă este alta: un pacient tratat corect se vindecă şi poate avea o viață absolut normală.ținând cont de plurifactorialitatea transmiterii acestei boli.

Este necesar să identificăm resursele necesare în vederea utilizării lor cu un raport cost-eficiență optim.

Tuberculoza este o boală care se poate preveni şi trata prin intervenții coordonate la nivel național şi local.

Lupta împotriva tuberculozei se abordează multidisciplinar şi intersectorial.

DATE EPIDEMIOLOGICE

Analiza datelor statistice arată, conform previziunilor, o scădere a incidenței tuberculozei în țara noastră, în ultimii ani.

Incidența în rândul populației generale a scăzut de la 138,8%00 în 1972 la 117,8 %000 locuitori în 2006, în timp ce media europeana este de 50 la 100.000.

Evolutia incidenţei TB 1972 – 2006

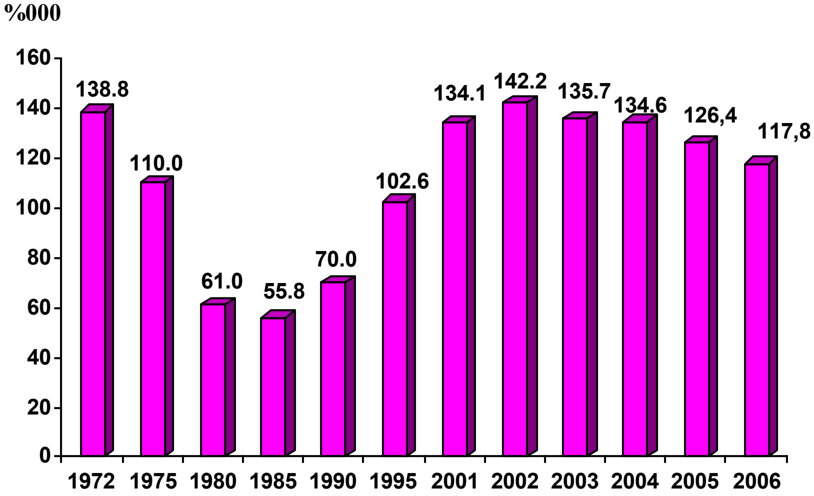

Iată în continuare situaţia incidenţei globale, pe judeţe, în anul 2006, Bucuresti-ul aflându-se uşor sub medie, pe primele locuri fiind judeţele Dolj, Ilfov şi Giurgiu.

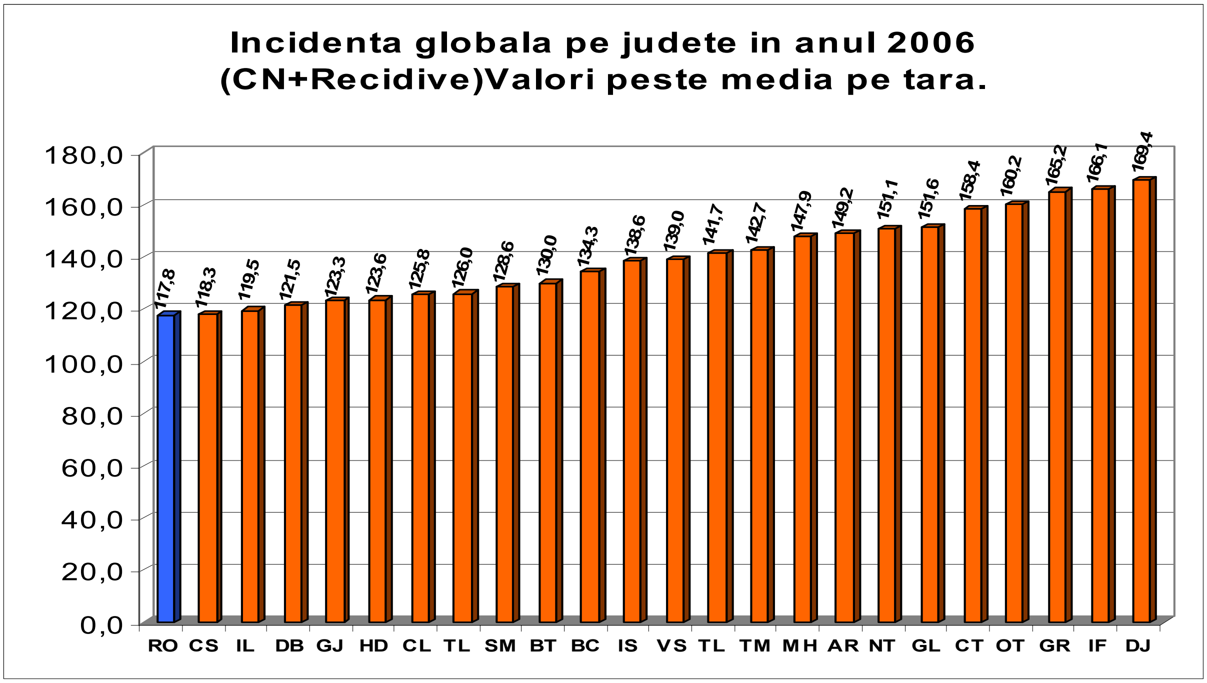

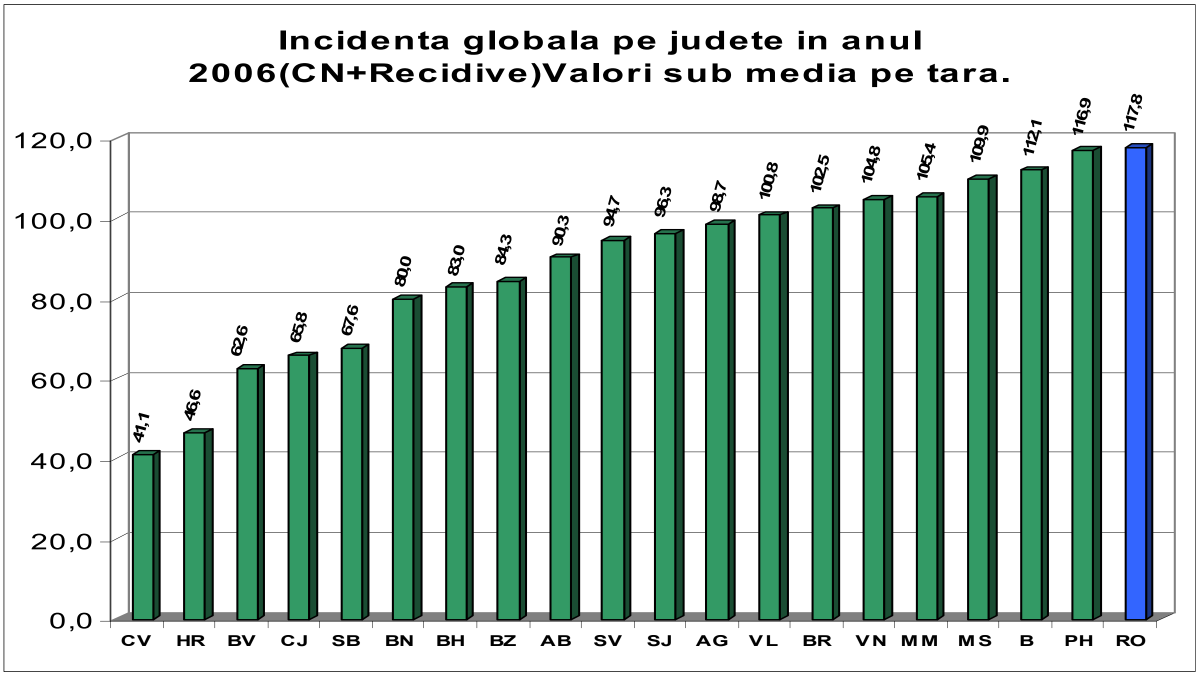

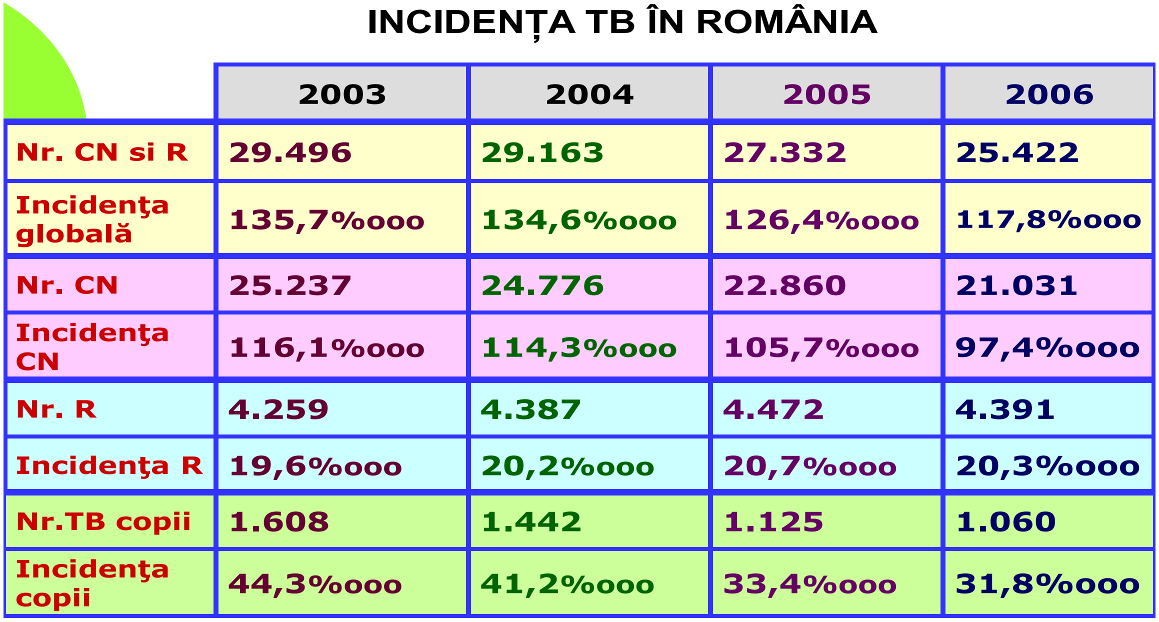

INCIDENȚA TB ÎN ROMÂNIA

	2003	2004	2005	2006
Nr. CN si R	29.496	29.163	27.332	25.422
Incidenţa globală	135,7%ooo	134,6%ooo	126,4%ooo	117,8%ooo
Nr. CN	25.237	24.776	22.860	21.031
Incidenţa CN	116,1%ooo	114,3%ooo	105,7%ooo	97,4%ooo
Nr. R	4.259	4.387	4.472	4.391
Incidenţa R	19,6%ooo	20,2%ooo	20,7%ooo	20,3%ooo
Nr.TB copii	1.608	1.442	1.125	1.060
Incidenţa copii	44,3%ooo	41,2%ooo	33,4%ooo	31,8%ooo

Această situație încurajatoare se datorează unui tratament eficace şi eficient, aplicat în condițiile sistemului DOT.

Caracteristicile acestui sistem, care îi conferă greutate şi credibilitate, sunt următoarele:

- Are tradiție încă din anii ’50 (sistem de înregistrare şi raportare a tuturor cazurilor de TB);
- Anii ’70: TSS (prefigurarea strategiei DOTS);
- 1998: instituirea strategiei DOTS în regim pilot;
- 2005: 100% acoperire cu DOTS;

- Urmăreşte recomandările OMS;
- Beneficiază de asistență tehnică din partea OMS;
- TB - prioritate de sănătate publică;
- Diagnosticul şi tratamentul TB sunt gratuite!
- Programul Național de Control al Tuberculozei este ordin al Ministrului Sănătății Publice.

Obiectivelele generale ale Strategiei Naționale a României de Control a Tubercuzei 2006-2010 sunt definite pentru atingerea Țintelor Globale propuse de către Planul Global de Stopare a Tuberculozei 2006-2015 (Obiectivele de Dezvoltare ale Mileniului):

1. Menținerea acoperirii 100% cu DOTS a populației afectate de tuberculoză;
2. Menținerea detectării a cel putin 70 % din totalul cazurilor de tuberculoză, până în 2010;
3. Extinderea şi menținerea ratei de succes terapeutic a cel putin 85% din totalul cazurilor noi de tuberculoză pulmonară, până în 2010.

Serviciile de sănătate de prevenire, diagnostic, tratament şi suport psiho-social în domeniul tuberculozei trebuie sa conlucreze si să fie accesibile, acceptabile şi eficace.

Strategia administrării tratamentului sub directă observare (DOT) este parte componentă a strategiei globale de control a tuberculozei (DOTS).

O atenție specială trebuie acordată grupurilor populaționale vulnerabile şi comunităților afectate (populația cu nivel socio-economic şi educațional scăzut, populația romă, deținuții, etc.), grupurile populaționale speciale, pentru intervenții specifice (persoanele infectate HIV care dezvoltă şi tuberculoză (HIV/TB), persoanele care efectuează tratamente imunosupresoare, pacienții cu tuberculoză rezistentă (MDR/TB).

Adminstrarea tratamentului poate fi plăcută atât pentru pacient cât şi pentru terapeut. Dacă pacientul simte că este tratat cu considerație, se poate dezvolta o relație ce va avea repercursiuni pozitive asupra comportamentului pacientului în administrarea DOT, dar şi pe termen lung, în viață.

Din păcate, non-complianța este un fapt dovedit cu care ne confruntăm. Un program coerent trebuie să se centreze pe pacient şi să țină cont de nevoile sale, diferențiat.

Asigurarea unui suport material al pacienților TB, în condițiile de afectare a unui individ prin această boală, determină îndepărtarea, sau marginalizarea din sfera preocuparilor de moment , cu dezinsertie sociala, profesionala şi familială, până la negativare şi reluarea activităților curente, poate creşte complianța la tratament.

Personalul care se ocupă de pacienții TB se confruntă cu probleme serioase pentru a obține complianța la tratament, cea mai gravă consecință fiind extinderea tuberculozei rezistente la tratament. Acest lucru reprezintă un argument major în sprijinirea ideeii ca pacienții TB, care nu-şi urmează corect şi complet tratamentul, trebuie să fie atent şi permenent monitorizați în privința complianței, iar măsurile trebuie rapid instituite.

ISTORIA NATURALA A FENOMENULUI

Dovezi istorice ale non-compliantei

Grupuri de risc la non-complianti

Personalul implicat în administrarea DOT apreciază non - complianța între 25-80%! Nu există un anumit grup sau categorie socială care să poată fi identificată ca având risc maxim de non-complianță. Chiar cei mai bine educați membrii ai societății pot deveni non-complianți, ținând cont de perioada lungă de tratament.

Nici sărăcia, nici locuința sub standard, nu înseamnă că aceşti pacienți sunt lipsiți de inițiativă şi nu vor copera. Nu există un element predictiv pozitiv al complianței.

În practica de zi cu zi rezultă că anumite modele comportametale sau informații anamnestice despre pacienți pot fi utile în prezicera complianței.

Din experiență ştim că urmatoarele grupuri de pacienți necesită o urmărire mai atentă, având un risc crescut de non-complianță:

- ✓ Eşecuri terapeutice anterioare. Persoanele care au eşuat anterior în completarea curelor terapeutice anti - TB sau a altor tratamente au risc înalt.
- ✓ Toxicomanii. Indivizii care abuzează de droguri de orice fel, suferă o alterare a stărilor cognitive şi prioritățile sunt inversate. Nu se renunță la starea de bine legată de drog în favoarea unui tratament coerent, care uneori provoacă chiar stare de rau! Mulți pacienți acuză greață şi vărsături cînd consumă alcool şi iau medicamente anti-TB.
- ✓ Afecțiuni mentale, emoționale, psihice, nedeclarate la persoane care nu înțeleg importanța tratamentului şi refuză medicamentele sau sunt susceptibile de a lua supradoze, ori au afecțiuni fizice care împiedică înghițirea medicamentelor (stricturi esofagiene).
- ✓ Persoanele care ar trebui să fie cele mai demne de încredere. Cel mai frustrant grup pentru personalul medical, deoarece riscul de non-complianță pare inițial mic, au intenții bune dar interesul scade o dată cu timpul. De regulă sunt oameni respectați în comunitate, instruiți, dornici de informații, politicoşi, punctuali, dar cu diferite slăbiciuni, cea mai neplăcută fiind faptul că nu îşi iau medicația conform prescripțiilor.
- ✓ Pacienții ostentativ cinstiti. Aceştia recunosc de la început dificultatea de a înghiți pastilele şi o mărturisesc. Deşi frustranți, atrag atenția personalului medical ceea ce, pe termen lung, economiseşte timp.
- ✓ Pacienți cu relații de slabă calitate cu personalul medical. Relația personal medical – pacient poate fi uneori stresantă. Studiile medicale nu dau şi certitudinea că se vor înțelege şi se vor agrea. Uneori pacientul preia „comanda” în detrimentul tratamentului.
- ✓ Alte situații sunt acelea în care pacienții ce primesc medicamentele dar trişează şi nu le înghit, dar vor să se asigure totuşi de eventualele beneficii obținute prin acordarea de stimulente.

MASURI IN ATITUDINEA FATA DE PACIENTII NON-COMPLIANTI

Există diferite căi prin care să poată fi realizată ameliorarea stării de complianță a pacientului la tratament, în scopul realizării DOT complet, cu consecințe favorabile pe termen lung asupra incidenței tuberculozei.

1.Educația pentru sănătate presupune:

- Educația individuală;
- Organizarea întrunirilor publice;
- Campanii media;
- Sprijinul autorităților publice locale şi poliție;
- Codul penal: Art. 383;
- Protocolul MSF - CNAS legat de tratamentul bolnavilor TB neasigurați/asistați social.

2.Ameliorarea relației pacient-terapeut

Tratamentul TB este lung şi plictisitor. Poate fi uneori agravant, prin reacțiile adverse la medicație. Pacientul trebuie să treaca peste aceste greutăți cu ajutorul cadrelor medicale, care trebuie să dezvolte şi să mențină o legătură solidă cu pacientul bazată pe comunicare clară şi eficientă, după un plan stabilit de terapeut de comun acord cu pacientul.

TB este o boală complexă. Personalul medical are nevoie de timp pentru a înțelege nevoile pacientului. Între pacient şi medic există o asimetrie informațională care pune pacientul în inferioritate: medicul trebuie să aibă capacitatea de a furniza pacientului informațiile de care are nevoie şi pe care le poate prelucra la nivelul său de cunostinte.

Din acest motiv, medicul trebuie să ştie şi să poată să se adapteze în permanență pentru ca, în cele din urmă, relația medic – pacient contează în completarea adecvată a terapiei.

Vor trebui făcute eforturi pentru a evita animozitățile între terapeut şi pacient; chiar dacă pacientul omite o doză, nu trebuie să se simtă pedepsit.

Atitudini de urmat:

- Îmbunătățirea canalelor de comunicare cu pacientul şi familia;
- Înțelegerea importanței tratamentului;
- TB se trateaza şi se vindecă;
- Folosirea unui vocabular tehnic adecvat;
- Eliminarea diferențelor culturale;
- La nevoie, constrângeri instituționale;
- Probleme de control.

3.DOT

Un pricipiu de bază este acela că dacă vrem să fim siguri că pacientul ia medicamentele, trebuie să privim la el când înghite. Deci strategia cea mai eficientă este DOT sau TSS. De aceea, pacienții trebuie convinşi să vină la cabinetul special destinat acestei activități. Un cadru medical face o evaluare profesională pacientului în vederea includerii în DOT. În funcție de complexitatea cazului şi de starea fizică a pacientului, asistenta medicală va administra DOT sau va delega altă persoană.

DOT-ul se administrează de 3 ori pe săptămână, la pacienții în faza de continuare a tratamentului standard sau zilnic, la pacienții cu TB-MDR, cu regimuri individualizate, de lungă durată.

Membrii personalului medical trebuie instruiți cu privire la asumarea responsabilității monitorizării tratamentului şi a complianței pacienților. Trebuie reținut că DOT-ul este o intervenție pozitivă şi nu punitivă, deci pacientului i se va explica necesitatea acestui tratament.

Este necesar crearea unui suport material pentru pacient, în condițiile în care afecarea unui individ prin TB determină îndepartarea din sfera preocupărilor obişnuite (sociale, profesioanele, familiale) urmat de dezinserție până la neglijare.

METODE DE REALIZARE ŞI IMPLEMENTARE A STRATEGIEI DOT

- **A crede în DOT**. Pentru a putea realiza DOT este nevoie în primul rând să credem în el şi în eficiența sa, deja dovedită. O atitudine pozitivă va da încredere pacientului că se află pe mâini bune.
- **Unde ?** Administrarea se face la cabinete special amenajate, cu personal special instruit în acest domeniu. Programul va fi flexibil pentru a putea asigura accesibilitate tuturor pacienților. Dacă pacientul nu poate veni la cabinet, trebuie să se găsească metode de a i se duce tratamentul la domiciliu. Consemnarea administrării prizelor de tratament se face pe foi speciale, unde sunt înscrise şi omisiunile, schimbările de tratament şi reacțiile adverse.
- **Confidentialitatea**. Se administrează tratamentul acolo unde se simte bine pacientul în condiții de confidențialitate absolută.

- **Delegarea**. În cazul în care pacientul nu poate ajunge la cabinet, se va găsi o persoană pentru a fi delegată să administreze tratamentul (asistent social, comunitar, rudă, vecin, etc.), după o prealabilă instruire şi transfer de responsabilitate.
- **Utilizarea de stimulente**. Unul din cele mai eficiente programe, utilizate astăzi în multe strategii de control al TBC este recurgerea la stimulente şi ajutoare pentru a încuraja completarea terapiei. Stimulentele sunt mici recompense date pacienţilor, pentru a-i stimula să-şi ia medicamentele sau să-şi respecte programările (la cabinetul medical sau acasă – pentru TSS). Ajutoarele sunt acele lucruri care "ajută" pacientul să-şi primească tratamentul (ca de ex.: bilete de autobuz, bani de taxi, baterii pentru maşină sau bani pentru babysitter astfel încât mama să se poată duce la cabimetul medical să-şi poata lua medicatia).

Stimulentele pot fi mici sau mari, pot fi alimente, bunuri sau servicii. Ceea ce reprezintă un stimulent pentru un pacient poate să nu reprezinte nimic pentru altul. De aceea, stimulentele trebuie adaptate fiecărui individ, altfel sunt ineficace. Un pacient căruia îi este foame va fi atras de unele alimente. Un pacient friguros va aprecia o pereche de ciorapi călduroşi sau un pulover. Unui copil i-ar plăcea o vizită la cofetărie cu sora sa sau sa-i fie citită o poveste. O mamă ar putea fi motivată de cadouri pentru copii ei, de exemplu caiete şi creioane pentru şcoală. Un buchet de flori este o pată de culoare în ziua unui pacient vârstnic.

Terapeutul, care este deosebit de sensibil la neovile fiecărui pacient, este cel care va avea cel mai mare succes în utilizarea stimulentelor şi ajutoarelor pentru completarea terapiei. După cum au constatat multe surori medicale, asistenţi sociali şi alţi terapeuti, că nu contează atât ce i se dă pacientului cât îi dai din tine insuti. Faptul că îti dai osteneala să alegi ceva semnificativ şi special pentru acea persoană este imediat înteles chiar de pacientul cel mai indiferent. Mulţi pacienţi, care luptă să-şi impună controlul asupra programului terapeutic şi care fac necazuri personalului, nu au avut niciodata o relaţie adevărată cu cineva. În consecinţă, utilizarea stimulentelor şi dăruirea de sine, devine o experienţă care schimbă viaţa, atât a pacientului cât şi a terapeutului.

Modalităţi de realizare practică:

- Educaţie de sănătate;
- Utilizarea aşa-ziselor carduri de memorie;
- Utilizarea stimulentelor constând în alimente, bonuri valorice, bani, îmbrăcăminte, articole de utilitate casnică, în scopul creşterii aderenţei la DOT;
- Administrarea medicamentelor la domiciliu, de către personalul medical, prin deplasare cu maşina din cadrul programului;
- Administrarea medicamentelor la domiciliu, de către o persoană din familie, instruită în acest sens;
- Administrarea medicamentelor la domiciliu prin intermediul unor asistenţi sociali sau comunitari, special instruiti;
- Acordarea de bilete de transport pentru facilitatea ajungerii la cabinetul medical;

Aplicarea unor metode de stimulare a complianţei la tratamentul antituberculos are ca rezultat negativarea pacientilor, vindecarea lor şi eliminarea surselor de îmbolnăvire. Consecinţa pe termen mediu şi lung este diminuarea focarelor epidemiologice TB şi scăderea endemiei tuberculoase.

Posibilatea generalizării la scară naţională ar putea deschide noi perspective asupra administrării strict supravegheate a tratamentului şi corectarea unor elemente ce se vor dovedi rentabile sub aspectul cost/beneficiu.

Studii făcute deja la noi în ţară, au fost derulate prin proiecte Global Fund, DOW si Crucea Rosie Romana (CRR). DOW şi CRR au colaborat cu succes la implementarea unui proiect în parteneriat, în anul 2005, intitulat *„Stabilirea unui sistem de distribuire de stimulente pentru îmbunătațirea tratamentului la pacienții TB în București, Ilfov şi Constanța”.* În cadrul proiectului au fost distribuite pacienților bonuri valorice pentru cumpărarea de alimente, în functie de consecvența prezentării la DOT. Acest proiect a fost reluat în august 2006 de CRR si extins în toată țara.

Un alt proiect realizat de organizația *Rromanicriss* a vizat formarea de mediatori comunitari care să activeze în raza sectoarelor 1 şi 6 din Bucureşti şi în județele Tulcea, Galați şi Giurgiu, demarat în 2005 şi derulat în continuare. Mediatorii sunt instruiți să poată furniza educație de sănătate precum şi informații pertinente despre tuberculoză în comunitățile de rromi şi nu numai, să administreze DOT la domiciliul pacienților nedeplasabili sau să recupereze pacienții TB.

Alt proiect în derulare aparține Samu-Social şi se referă la pacienții fara adăpost, suferinzi şi de tuberculoză.

Dezvoltarea unei strategii naționale pe termen mediu privind informarea- educarea-comunicarea în vederea schimbării comportamentelor, atragerea de resurse financiare şi colaborări cu instituții specializate în vederea implementarii IEC

Administrarea medicamentelor în tuberculoză, reprezintă cheia de boltă pentru succesul terapeutic. Tratamentul fiind de lungă sau foarte lungă durată, cu aasociere de mai multe medicamente, este greu de acceptat de catre pacient. De aceea s-au căutat mereu soluții pentru atragerea pacienților şi creşterea complianței la tratament.

În prezent se experimenteaz regimuri **noi** de tratament aflate încă în faza de studiu clinic, de scurtă durată (4 luni şi administrare zilnică) având o chinolonă administrată pe toata durata tratamentului.

Organizarea tratamentului trebuie să vizeze programarea administrării dozelor, convenabil pentru pacient, folosirea de stimulente, obținerea suportului familiei, îndrumarea pacienților către programe de asistenttă sau consiliere în caz de alcoolism, consum de droguri, probleme de sănătate mintală.

Principiile de tratament în tuberculoză au în vedere utilizarea combinațiilor de medicamente antituberculoase recomandate, administrarea lor sub directa observație, educarea pacientului şi familiei în sensul că numai un tratament CORECT şi COMPLET asigură vindecarea şi previne recidivele, atitudinea amabilă şi prietenoasă a personalului medical.

Posibilele cauze de întrerupere a tratamentului sunt: abuzul de alcool şi droguri, frica sau refuzul de a fi etichetat ca bolnav de tuberculoză, dispariția simptomelor bolii, neînțelegerea importanței tratamentului, motivele personale sau culturale (ex. tratamente alternative), efectele adverse, lipsa speranței de vindecare şi reintegrare, pacienți vârstnici sau cu boli mintale, probleme medicale asociate, teama de şomaj datorat bolii, relația defectuoasă cu personalul medical.

Atitudinea cadrelor medicale, în cazul întreruperii tratamentului, trebuie să cuprindă acțiuni care vizează convingerea pacientului să reia tratamentul.

Creşterea aderenței pacienților TB la trament implică, în primul rând, o foarte bună comunicare cu pacientul şi implicit o bună educație de sănătate a acestuia. Pacientul TB trebiue să înțeleagă boala pe care o are, să capete informații în acest sens, să cunoască tratamentul şi implicațiile acestuia, precum şi repercursiunule asupra semenilor săi, în cazul refuzului tratamentului. Pacientul poate renunța cu uşurință la tratament după 2-3 luni, mai ales în cazul în care începe să se simttă foarte bine, fapt ce ridică riscul recidivelor şi/sau al cronicizării bolii, până la transformarea în forme de tuberculoză MDR.sau chiar X-DR.

Implicațiile de ordin epidemiologic şi social sunt enorme, în cazul în care pacienților nu le sunt explicate aceste lucruri. Pe de altă parte, pentru ca pacienții ce au primit deja bagajul de informații de educație de sănătate, să accepte mai uşor tratamentul de lungă durată şi cu multe droguri, peste tot în lume se recurge la diferite modalități de stimulare a prezenței la tratamentul strict supravegheat.

În general se caută modalităţi şi metode în concordanţă cu nevoile pacienţilor sau se adaptează la specificul situaţiei din ţara respectivă.

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Istorie, prezent şi perspective pentru sănătate

Lt. Col. Ec. Panaite GRECU *

Nemţenii, locuitori ai Moldovei din cele mai vechi timpuri, au fost şi sunt creatori şi păstrători de autentică istorie.

Uniţi prin tradiţiile şi obiceiurile bine cunoscute din evocările lăsate de Ion Creangă, în ale sale „Amintiri”, locuitorii Depresiunii Neamţului au fost favorizaţi de un relief deluros, cu mici platouri şi pante domoale, pretabil habitatului sedentar, agriculturii; clima blândă, pânza freatică bogată în apă potabilă şi apă de sărătură, prezenţa unor întinse păduri au contribuit la dezvoltarea, în general a vieţii şi comunităţilor locale şi în particular a meşteşugurilor şi altor îndeletniciri ce au la bază prelucrarea lemnului, pietrei, cerealelor, fructelor, cărnii, lânii şi nu numai.

În microregiunea Târgu Neamţ, descoperirile arheologice confirmă pe deplin existenţa umană, încă înainte de Hristos; şantierele arheologice de la Ghindăoani, Tărpeşti, Davideni, au scos la lumină numeroase vestigii: monede, arme, unelte, locuinţe şi lăcaşuri de cult, morminte, toate de mare însemnătate istorică şi care conduc cu certitudine la concluzia că aceste teritorii sunt locul unei culturi şi civilizaţii străvechi.

Localitatea Bălţăteşti a fost atestată documentar din vremea Domnitorului Constantin Movilă care printr-un act de donaţie datat 01 septembrie 1608 donează vornicului său Pătraşcu „Satul domnesc Bălţăteşti.

În anul 1716, satul apare pentru prima oară pe o hartă, aceasta aparţinând Domnitorului Dimitrie Cantemir fiind intitulată „PRINCIPATUS MOLDAVIAE NOVA & ACCURATA DESCRIPTIO”, hartă unicat aflată în prezent în patrimoniul Bibliotecii Naţionale din Paris.

În perioada 1715-1849 Bălţăteştiul constituie sursa neînţelegerii privind dreptul de proprietate între familia Cantacuzino şi Mănăstirea Hangu.

În anul 1864 Bălţăteştiul trece în proprietatea domnitorului Dimitrie Barbu Ştirbei care o atribuie ca zestre fiicei sale Contesa Martha de Blöme.

În anul 1839 doctorul I. Chihac şi profesorul E. Humpel efectuează pentru prima oară analiza apelor minerale de Bălţăteşti extrase din trei fântâni de saramură; ulterior în 1850 cunoscutul savant român Petre Poni analizează aceste ape şi le stabileşte compoziţia chimică; toate aceste analize scot în evidenţă existenţa în conţinutul apelor de Bălţăteşti a unor cantităţi însemnate de sodiu, clor, magneziu, calciu, fier, potasiu, iod, acid clorhidric, bromură de magneziu, sulfaţi. Având la bază aceste analize, Principele Ştirbei, proprietar al Băilor, face considerabile investiţii pentru punerea în valoare a calităţilor curative şi de balneaţie ale celor trei izvoare de apă sărată, botezate: Olga, Elena şi Gheorghe.

Pe data de 19 octombrie 1878 doctor Dimitrie Cantemir, descendent al domnitorului moldovean, cumpără de la Principele Dimitrie Ştirbei băile , „Stabilimentul de slatină” compus dintr-o clădire principală în formă de potcoavă, şase bucătării şi şase cabine pentru băi.

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* Comandantul Sanatoriului de Balneofizioterapie şi Recuperare Medicală „Dr. Dimitrie Cantemir” Bălţăteşti

În anul 1883 doctor Konya, autor al analizelor apelor de la Baden-Baden şi de la Slănic Moldova, efectuează cea de-a patra analiză a apelor de Bălţăteşti, constatând că izvoarele de aici „sunt cele mai bogate în săruri din toate apele omologate de pe Continentul European”, comparabile ca nivel cu cele de la: KARLSBAD, MARIENBAD şi VICHY.

Timp de 15 ani doctorul Dimitrie Cantemir a derulat un program masiv de investiții care a avut ca obiective:

- captarea sistematică a izvoarelor,

- realizarea rezervoarelor şi batalurilor pentru depozitarea apei, nămolului,

- construirea clădirilor cu instalațiile necesare pentru funcționarea „Stabilimentului de băi”, a sălii de cură „cu inhalați de brad”, hoteluri şi vile pentru cazare, restaurantul, cazinoul, farmacia, poşta, parcul de brazi şi grădina.

În anul 1930 Stațiunea Bălţăteşti cuprindea:

- Baza de ape minerale cu patru izvoare:

- Izvorul nr. 1 - CAROL,

- Izvorul nr. 2 - INDEPENDENȚA,

- Izvorul nr. 3 - ELISABETA,

- Izvorul nr. 4 - CUZA-VODĂ

- „Hotelul mare” cu 48 camere;

- „Hotelul nou” cu 18 camere;

- „Vila Lili” cu 16 camere;

- „Vilele Cantemir şi Telegraf” cu câte 10 camere;

- „Vila Priveliştea”
-
- „Vila Coleandru”

După decesul doctorului Dimitrie Cantemir, văduva sa se căsătoreşte cu George Eduard Arapu, un moşier din județul Botoşani, care arendează stațiunea pentru zece ani lui Cristian Adolf administratorul moşiei Bălțăteşti.

În perioada 1916-1918 în incinta Băilor, din Ordinul Ministerului de Război, a funcționat Spitalul Militar 171 Buzău.

În anul 1920, George Eduard Arapu vinde Băile, doctorilor Lazăr şi David Goldhamer din Botoşani; aceştia au repus în stare de optimă funcționare baza de tratament şi baza de cazare.

În perioada celui de-al II lea Război Mondial în localul Băilor Bălţăteşti a funcţionat pentru o perioadă scurtă de timp Spitalul de zonă interioară nr. 622.

În baza ordinului nr. 3417 din 17 aprilie 1948, dat de prefectul judeţului Neamţ, comisia Prefecturii, primarul şi notarul comunei Bălţăteşti precum şi şeful Postului de Jandarmi, au preluat prin rechiziţie de la proprietarul David Golhamer „Băile Bălţăteşti".

În perioada 1948-1993 Staţiunea a fost administrată succesiv de către Staţiunea Balneoclimaterică Slănic Moldova şi întreprinderea de Turism Balnear-Baltur, societăţi comerciale care nu au putut susţine şi organiza eficient activitatea staţiunii, fapt ce a determinat Consiliul Judeţean Neamţ ca prin Decizia nr. 23 din 19 mai 1993 să transfere „fără plată" staţiunea în administrarea Ministerului Apărării, pentru o perioadă de cel puţin 15 ani.

Din momentul preluării, Ministerul Apărării prin Direcţia Medicală şi celelalte structuri de specialitate a derulat un program amplu de investiţii, dotare, modernizare, care să pună în valoare toate elementele de balneaţie:

- rezultatele binefăcătoare ale apelor sunt obţinute graţie acţiunii fiziologice a elementelor principale: clorura sodică, săruri sulfuroase, iod, fier cu efecte curative deosebite;
- aerul din regiune foarte bogat în ioni negativi are un efect reconfortant asupra organismului, de sedare şi de tonifiere totodată;
- datorită calităţii deosebite a factorilor naturali de cură, Sanatoriul Bălţăteşti oferă largi posibilităţi terapeutice, încadrându-se ca principala formă de tratament în: poliartrita reumatoidă, spondilita ankilopoetică, discopatii lombare, lombosciatică, spondiloza, coxartroza, gonartroza, artrozele mâinilor şi picioarelor, periartrita scapulo-humerală, osteoporoza, sechele posttraumatice, anexite, sterilitate, frigiditate, paralizii şi pareze, poliomelita, tromboflebita, dermatoze, rinite, sinuzite, astm bronşic, boli endocrine, nevroze, neuroastenii, tulburări de creştere la copii, rahitism.

Având în vedere:

- cadrul legislativ reprezentat de Legea nr. 95/2006 privind reforma în domeniul sănătăţii, Legea nr. 346/2006 privind organizarea şi funcţionarea Ministerului Apărării, Hotărârea Guvernului nr. 442/1992, completată cu Hotărârea Guvernului nr. 1580/2002 privind refacerea capacităţii de muncă a personalului armatei care lucrează în condiţii vătămătoare sau participă la misiuni în sprijinul păcii, în afara graniţelor ţării.
- unicitatea Sanatoriului, dată de starea complexă de mineralizare şi concentraţia apelor minerale, susţinute de gradul de dezvoltare atins de Sanatoriu prin: active, dotare, logistică, personal şi experienţa acumulată în mai bine de 150 de ani de funcţionare, cu performanţe interne şi externe recunoscute, deschid şi consolidează perspectiva dezvoltării viitoare a sanatoriului în cadrul administrativ, creat de Ministerul Apărării în colaborare cu structurile specifice din organica Consiliului Judeţean Neamţ şi în strânsă relaţie cu Agenţia pentru Dezvoltare Regională Nord-Est, existând premisa şi şansa formării unei nişe unice pe piaţa specializată pe recuperări şi reabilitări fizico-psihice, post operaţionale, a eşaloanelor combatante reîntoarse din teatrele de operaţii, precum şi pentru reinserţia profesională şi socială a personalului activ sau în rezervă, beneficiari ai serviciilor de sănătate oferite de Casa Asigurărilor de Sănătate a Apărării Ordinii Publice, Siguranţei Naţionale şi Autorităţii Judecătoreşti;
- resursele balneologice active, cu efecte deosebit de favorabile în tratarea afecţiunilor aparatului locomotor, sistemului nervos, sistemului circulator, respirator, afecţiunilor ginecologice şi a bolilor asociate;
- cadrul natural deosebit, având o atmosfera foarte bogată în ioni negativi, cu efecte reconfortante şi de tonifiere psihomotorie a organismului;
- obiectivele turistice unice în ţară:

■ ***Piatra Neamț*** oraș, reședință a județului cu importante izvoare ale istoriei şi culturii noastre: Muzeul de Ştiințe Naturale, Casa memorială Calistrat Hogaş, Fondul documentar Gh. T. Kirileanu, Bâtca Doamnei (cetate dacică), Curtea Domnească, Biserica şi Turnul cu ceas ctitorite de Ştefan cel Mare (1498-1499), Rezervația paleontologică „Cozla-Pietricica-Cernegura" (Faună fosilă), Căldările uriaşilor (monumente ale naturii).

■ ***Târgu Neamț*** oraş pe malurile râului Ozana, ia poalele Culmii Pleşu, are următoarele obiective: Cetatea Neamț, Muzeul de istorie, Casa Ion Creangă, Casa Veronica Micle, Şcoala Domnească, în imediata vecinătate a oraşului se află stabilimentul balnear al Stațiunii Oglinzi, colecția de artă populară Nicolae Popa din satul Tărpeşti la 10 km.

■ ***Mănăstirea Horaița*** ctitorită în jurul anului 1725 este refăcută la 1867 de arhimandritul Ermoghen Buhuş. În apropiere se află Schitul Horăicioara.

■ ***Mănăstirea Bistrița*** ridicată la 1400 este principală ctitorie şi necropolă a domnitorului Alexandru cel Bun. Mănăstirea Pângărați ridicată în timpul domniei lui Ştefan cel Mare, inițial a fost biserică din lemn. Devine mănăstire după 1560 sub domnitorul Alexandru Lăpuşneanu.

■ ***Mănăstirea Văratec*** fondată în 1785 de maica Olimpiada pe locul unei ctitorii din lemn a lui Ieremia Movilă. Dispune de: colecții de artă veche, mărturii despre oamenii de cultură ce au trecut pe aici - M.Eminescu, Al.Vlahuță, C.Hogaş, M. Sadoveanu, Ionel Teodoreanu; Statuia doamnei Safta Brâncoveanu - 1935 Ion Jalea, locul de veci al Veronicăi Micle, Rezervații naturale „Codrii de aramă" (Dealul Filioru), „ Pădurea de argint" (cu mesteceni de peste 200 ani).

■ ***Mănăstirea Agapia din Vale*** înălțată la 1644 de hatmanul Gavril Coci frate al domnitorului Vasile Lupu. În 1860 Nicolae Grigorescu execută pictura interioară cu tablouri în stil realist şi colorit de valoare deosebită. Aici au creat Bogdan Petriceicu Haşdeu şi fiica sa Iulia, I.L.Caragiale, Al. Vlahuță, G.Coşbuc. În zonă se află şi biserica Agapia din Deal, edificiu al sec. XIV, restaurată de către domnitorii Petru Rareş, Petru Şchiopu şi Duca Vodă.

■ ***Mănăstirea Neamț*** principal complex arhitectonic medieval de cult din Moldova, aparține sec.XIII, este succesiv ctitorită de Petru I Muşat, Alexandru cel Bun şi Ştefan cel Mare.

■ ***Mănăstirea Secu*** înălțată de vornicul Nicolae Ureche la 1602 este o construcție arhitectonică specifică Țării Româneşti, incintă fortificată cu turnuri de apărare.

■ ***Mănăstirea Sihăstria*** aşezământ din jurul anilor 1650 dispune de o biserică nouă, ridicată la 1734 de episcopul Ghedean de Huşi.

■ ***Rezervația Dragoş Vodă*** deține singurele exemplare de zimbri, faună de pădure şi de iaz. Este situată la 2 km sud de Mănăstirea Neamț.

■ ***Bicaz*** cu muzeul de istorie, Hidrocentrala Stejarul şi Portul Lacului Izvorul Muntelui ce asigură curse de agrement. În împrejurimi se pot vizita: Colecția de etnografie şi creație populară şi peştera Toşorog din comuna Bicazu Ardelean. Rezervațiile Cheile Bicazului - monument al naturii, Lacu Roşu (Stațiune Climaterică, lac natural de baraj).

- ■ ***Durău*** staţiune climaterică şi de practicare a sporturilor de iarnă, este situată la poalele versantului nordic al Masivului Ceahlău. Are ca obiective turistice: Schitul Durau pictat între anii 1935-1937, de pictorul Nicolae Tonița care a folosit tehnica encausticii (culori topite în ceară) procedeu antic. Se pot face drumeții până la Vf. Toaca.

Toate acestea reprezintă argumente puternice pentru păstrarea Sanatoriului de Balneofizioterapie şi Recuperare Medicală „Dr. Dimitrie Cantemir" Bălțăteşti în administrarea Ministerului Apărării şi subordinea Direcției Medicale, pentru înființarea Centrului de refacere fizică şi psihică a capacității de muncă a personalului din Sistemul Național de Apărare.

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Caracteristicile balneo-climaterice ale stațiunii Bălțătești în recuperarea şi tratamentul tulburării de stres posttraumatic

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OBIECTIV: Potențialul balnear al stațiunii Bălțătești prezintă o serie de particularități care o individualizează între celelalte stațiuni balneo-climaterice din țară, oferindu-i un loc special în recuperarea şi tratamentul unor afecțiuni ale aparatului locomotor, afecțiuni neurologice periferice, afecțiuni ale SNC, afecțiuni ORL, afecțiuni cronice ale aparatului respirator, dar şi în cele determinate de tulburările de stres posttraumatic.
MATERIAL ŞI METODĂ: Prezența unor grupe de substanțe terapeutice, ape minerale în conținutul cărora au fost determinate cantități importante de Na, Cl, Mg, Ca, Fe, K, I, acid carbonic şi clorhidric, şi factori climaterici cu acțiune sedativă asupra organismului uman cu o aeroionizare naturală predominant negativă au efecte benefice în diferite afecțiuni, anxietate, depresie, tulburări nevrotice, astm bronşic, hipertensiune arterială, etc.
CONCLUZII: Stațiunea Bălțătești este locul recomandat pentru recuperarea fizică şi psihică a participanților în teatrele de război, fiind un centru în care pot fi tratate atât problemele medicale, cât şi cele specifice.

factori climaterici recuperare tulburare de stres posttraumatic

ABSTRACT
Purpose: Bălțătești, as a balneotherapy place, is marked by several individualities, with recommendation in treatment of locomotion, peripheral and central neurological disorders, ENT disorders, chronically respiratory diseases, as well as for treatment for Post-traumatic stress disorder.
Material and method: The presence of therapeutically substances, mineral waters (dominated by Na, Cl, Mg, Ca, Fe, K, I, carbonic acid and hydrogen cloride), climaterical facts with sedative action, has a benefic action in diseases, anxiety, depresion, nevrotic deseas, bronchial asthma, hypertension, etc.
Conclusion: Bălțătești is recommended for physical and psychical recovery of veterans, where can be treated medical and specific problems.

Key words: climacteric facts, recovery, PTSD

INTRODUCERE

Potențialul balnear al stațiunii Bălțătești prezintă o serie de particularități care le individualizează între celelalte stațiuni balneo-climaterice din țară, şi anume:

- prezența unor grupe de *substanțe terapeutice*;
- *ape minerale* în conținutul cărora au fost determinate în cantități importante sodiu, clor, magneziu, calciu, fier, potasiu, iod, acid carbonic şi clorhidric; de asemenea există Izvorul Nr. 4 (părăsit) a cărui ape erau utilizate pentru cura internă deoarece aveau un conținut mare de sodiu, clor, brom, iod, calciu şi magneziu, apa având un gust sărat-amărui şi o greutate specifică de 1,0148 g;
    - ape iodurate şi clorurate-sodice
    - ape sulfatate cu un conținut de 1 g de sulfat la litru, combinat cu ioni de sodiu, potasiu, magneziu şi calciu;
    - ape minerale sulfatate, care conțin cel puțin 1 g la litrul de apă substanțe solide dizolvate, din care predomină anionul SO_4^{2-};
- *factorii climaterici:* cercetările de bioclimat au permis stabilirea unor corelații între caracteristicile climaterice şi reacțiile fiziologice ale organismului uman.

Cercetările efectuate au arătat că valorile elementelor climatice, moderate tot timpul anului, au acțiune de crutare (sedativ-indiferentă) asupra organismului uman, care nu primeşte stimuli prea intenşi. Numărul mediu de zile cu confort termic, calculat pe baza relației dintre temperatura aerului, umezeală si vânt, în condițiile în care un organism uman sănătos, îmbrăcat uşor, în repaus, nu pierde şi nu primeşte căldură, este în luna iulie, la amiaza, de 9.

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Numărul mediu de zile cu inconfort prin încălzire, tot în luna iulie, la amiaza, este de 7, iar numărul de zile cu inconfort prin răcire variază, în funcție de altitudine, între 5 şi 10. Numărul de zile cu confort prin adăpost de vânt, în luna iulie, la ora 13, este intre 10 si 15. La o viteza a vântului mai mare de 3,5 m/s, organismul uman resimte inconfort, chiar dacă temperatura este cuprinsă în limitele de confort.

Numărul mediu de zile cu inconfort datorat încălzirii prin adăpost de vânt este în luna iulie, la ora 13, între 10 si 13, iar a celor cu inconfort datorat răcirii prin adăpost de vânt este mai redus de 10. Indicele climato-turistic, calculat pe baza relației I=(S+T-5D)/5 dintre durata de strălucire a soarelui pe cer (S), temperatura medie lunară a aerului (T) şi durata precipitațiilor, excluzând precipitațiile din timpul nopții (D), evidențiază luna iulie ca fiind optimă (indice între 50 şi 80) şi pentru băi de aer.

Pe baza cunoaşterii valorilor elementelor climatice în limitele cărora organismul uman prezintă o stare medie de echilibru a sistemelor reglatoare, s-au calculat indicii de stres.

Depăşirea pozitivă sau negativă a valorilor optime determină stimularea sau stresarea organismului, antrenând mecanismele de autoapărare şi de adaptare pentru păstrarea homeostazei interne. Stresul bioclimatic cutanat este determinat de senzațiile de căldură şi de frig pe care le resimte organismul în procesul termoreglării. În cursul anului perioada noiembrie-martie este hiper-tonică, lunile iulie şi august sunt hipotonice, iar perioadele aprilie-iunie şi septembrie-octombrie sunt relaxante. Valoarea medie anuală a indicelui de stres bioclimatic cutanat este redusă (14). Stresul bioclimatic pulmonar se bazează pe schimburile respiratorii prin absorbția de oxigen şi eliminarea de bioxid de carbon. Poate fi determinat în condițiile în care tensiunea vaporilor de apă depăşeşte limitele optime şi acționează asupra mucoaselor căilor respiratorii. În condițiile în care tensiunea vaporilor de apă este cuprinsă între 7,5 şi 11,6 mb, climatul este echilibrat, relaxant, nestresant. Perioada noiembrie-martie, cu tensiunea vaporilor de apă sub 7,4 mb, are caracteristici deshidratante pentru mucoasele căilor respiratorii, iar perioada mai-septembrie este hidratantă, deoarece tensiunea vaporilor de apă depăşeşte 11,7 mb. Lunile aprilie şi octombrie sunt echilibrate.

Cercetările medicale au dovedit influența climatului aeroelectric asupra organismului uman, o aeroionizare naturală, bogată, predominant negativă are efecte benefice, de sedare în diferite afecțiuni: nevroze, astmul bronşic, hipertensiunea arterială, sporind astfel valoarea balneo-medicală a stațiunii.

Electricitatea atmosferei are influențe asupra organismului uman, mai ales prin ionizarea naturală a aerului. Predominarea ionilor pozitivi determină reacții nefavorabile ale organismului uman. Ionii negativi au o influență favorabilă în tratarea astmului bronşic, reumatismului, hipertensiunii, nevrozelor şi surmenajului.

La Bălțăteşti, măsurătorile privind nivelul aeroionizării au înregistrat un număr mediu total de 1055 ioni + şi - / cm^3 aer, din care 560 ioni -/cm^3 şi 495 ioni +/cm^3, coeficientul de unipolaritate fiind de 0,88[9]. Cercetările diferiților geofizicieni din ultimii ani indică o concentrație optimă de radon, care individualizează zona şi măresc efectele benefice ale curei balneare.

Plantele medicinale reprezintă factori naturali de cură foarte răspândiți şi din ce în ce mai solicitați în domeniul balneo-medical (fitoterapie), atât în prepararea unor medicamente, cât şi în cura externă, în băi cu plante. Medicamentele pe bază de plante sunt realizate şi în laboratorul PLANTAVOREL din Piatra Neamț, reprezentând un avantaj în tratament. În consecință, având în vedere tendințele manifestate pe plan internațional şi succesul de care se bucură aceste produse, ele ar putea fi valorificate cu succes la nivelul produselor balneare. Subliniem că existența unor produse secundare de tip extract obținute din ape minerale şi nămoluri, cum este sarea de Bălțăteşti, permite continuarea tratamentului la domiciliu.

Prezența unor substanțe minerale terapeutice, climato-terapia, marea diversitate a plantelor medicinale, amplasarea stațiunii balneare într-un cadru pitoresc, în apropierea unor obiective turistice naturale (Pădurea de fag şi molid de pe Înălțimea cu cota 706, cariera de Piatră), sau antropice, alte obiective turistice (mănăstirile Neamț, Văratec, Agapia, Secu). Fiind o zonă predominant agrară există legume şi fructe de sezon proaspete, localnicii preocupându-se intens să obțină produse ecologice care pot fi valorificate în realizarea unor produse 100% naturale şi servite în cadrul stațiunii.

În prezent Sanatoriul de balneo-fizioterapie şi recuperare medicală este dotat cu instalații moderne pentru electroterapie, afuziuni, duşuri scoțiene, duş-masaj, duş subaqual, saună, aerosoli, tratament ginecologic, sală de cultură fizică medicală şi recuperatorie, bazin de hidrokinetoterapie. Băile cu apă clorosodică caldă sunt completate cu băi galvanice şi împachetări cu nămol de sediment, masaj manual.

MATERIAL ŞI METODĂ

În prezent în stațiunea Bălțăteşti sunt tratate eficient următoarele afecțiuni:

- surmenaj fizic şi intelectual: stări de convalescență; anemii (tip Biermer); copii apatici, rahitici, convalescenți, debili, pulmonari, astenici, cu tulburări de somn, creştere, stări reumatismale, enuresis nocturn, reumatism cronic degenerativ;
- afecțiuni ale aparatului locomotor: afecțiuni reumatismale degenerative şi inflamatorii cronice (spondilita anchilozantă, artropatia psoriazică); afecțiuni discoradiculare (lombosciatică, discopatii lombare, recuperare post hernii de disc operate); artroze (coxatroză, gonartroză, artrozele mâinilor: artroze proximale tip Bouchard, artroze distale tip Heberden; toate stările preartrozice cu diverse localizări); sechele post-traumatice (sechele musculare: atrofii, hipotrofii, contracturi, retracturi; leziuni de nervi periferici; sechele vasculotrofice: tromboflebite, algoneuro-distrofii, limfedem; sechele articulare: redori, anchiloze);
- afecțiuni ginecologice în stări hiporeactive (procese metroanexiale; insuficiențe funcționale minore neuro-endocrine; sterilitate; deviații şi deplasări uterine; dischinezii hipotone; laxitate ligamentară; frigiditate) şi hiper-reactive (tulburări de pubertate şi menopauză; stări de convalescență; metroanexite cronice);
- afecțiuni neurologice periferice (paralizii, polinevrite, sindrom coadă de cal, poliradiculopatii sechelare);
- afecțiuni ale sistemului nervos central (sindrom hemiplegic, sindrom paraplegic);
- afecțiuni cronice ale aparatului respirator;
- afecțiuni asociate: boli vasculare, dermatologice, profesionale, endocrine, respiratorii cronice, ginecologice cronice, digestive cronice şi nevroze.

Apele sulfatate se utilizează în special în cura internă pentru tratarea afecțiunilor digestive şi hepatobiliare, datorită efectelor principale ale acestora: coleretic, colecistokinetic; conținutul de iod din aceste ape adaugă un plus de efecte care lărgesc indicațiile de cură. Astfel, în crenoterapie aportul de iod contribuie la stocarea sa în tiroidă, indicată în afecțiuni endocrine, tiroidiene şi ovariene, la care se adaugă şi efectele congestive şi stimulatoare asupra mucoasei digestive.

Stresul bioclimatic total, care prezintă influența globală a climatului asupra organismului uman, la nivelul pielii şi al plămânilor, este minim (40), caracterizând oraşul ca având un bioclimat sedativ, de cruțare, cu nuanțe de excitare. Acest tip de bioclimat este favorabil persoanelor sănătoase sau cu stări de surmenaj, persoanelor în vârstă sau pentru bolnavii care nu pot suporta căldura excesivă, amplitudinile termice mari sau umezeală mare a aerului.

Este cazul afecțiunilor cardiovasculare, reumatismului poliarticular acut sau al convalescenței de hepatită acută. Bioclimatul cu caracteristici sedative este indicat şi pentru copiii cu stări de nervozitate, fiind lipsit de contraindicații terapeutice, indiferent de sezon.

Datorită acestui bioclimat considerăm că staţiune Bălţăteşti poate fi o destinaţie pentru recuperarea fizică şi psihică a militarilor întorşi din teatrele de război, precum şi a celorlalţi militari.

Creşterea numărului de militari întorşi din teatrele de război care suferă de TSPT ne determină să susţinem necesitatea unei cure balneare şi de recuperare medicală fizică şi psihică.

CONCLUZII

În decursul anilor mulţi pacienţi şi-au găsit vindecarea în tratamentul oferit de această staţiune, ceea ce ne determină să susţinem că alături de recuperarea medicală poate fi făcută şi cea psihică, prin înfiinţarea unui Centru pentru tulburarea de stres posttraumatic adresat militarilor şi personalului civil participant la diverse misiuni. Activitatea centrului ar trebui să fie atât de prevenţie, cât şi de recuperare.

În acest sens propunem:

- pregătirea psihologică timp de 3-4 zile a militarilor selectaţi pentru misiuni, prin conceperea unui program virtual având drept scop transpunerea militarilor selectaţi pentru teatrele de război în faţa situaţiilor în care pot genera tulburarea de stres posttraumatic, petrecând experienţe similare celor stării de război. Prin monitorizarea bătăilor inimii, respiraţiei, transpiraţiei, pot fi urmărite efectele create de starea virtuală care genereazǎ răspunsurile emoţionale ce pot identifica potenţialii militari predispuşi tulburării de stres posttraumatic.

Ideea de bază constă în inducerea de stimuli asemănători stării reale pe o perioadă mare de timp şi încercarea de a păstra răspunsurile psihofiziologice la un nivel cât mai scăzut. Astfel, persoana poate învăţa înainte de misiune, într-un mediu terapeutic securizant, controlul reacţiilor emoţionale pentru ca apoi să exteriorizeze şi să generalizeze în viaţa de zi cu zi din timpul misiunii propriu-zise.

Noutatea acestei propuneri constă în educarea gestionării stresului înainte de misiune, comparativ cu specialiştii de la Centrul Medical Naval din San Diego, unde dr. Dennis Wood aplică acest procedeu după întoarcerea din misiune.

- la revenirea militarilor din teatrele de operaţiuni vor beneficia timp de aproximativ 14 zile, împreună cu familiile, de programe de consiliere şi psihoterapie, individuale şi de cuplu, care se vor centra pe următoarele aspecte:
- antrenarea în managementul anxietăţii generalizate (individual);
- terapie cognitiv-comportamentală de grup prin expunere şi antrenarea abilităţilor sociale;
- terapie de cuplu pentru reintegrarea în familie şi dezvoltarea relaţionării emoţionale şi a comunicării[*];

Simptomele de tulburare de stres posttraumatic pot să apară în primele luni de la revenirea din misiune, de aceea propunem ca toţi militarii să beneficieze şi de consiliere psihologică, concomitent cu recuperarea medicală.

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[*] un argument este tragicul accident al familiei comandantului batalionului 280 din 28 iulie 2006, în timp ce se îndreptau spre Focşani, la câteva ore de la revenirea batalionului din Irak.

ROLUL INFORMAȚIILOR MEDICALE ŞI PRELUCRAREA LOR ÎN VEDEREA PREGĂTIRII MISIUNII

Maior medic, instructor superior Bogdan SAVU[*]

Rezumat: Lucrarea îşi propune să discute pre-planificarea şi pregătirea asigurării medicale a forțelor aflate în deplasare şi în curs de desfăşurare. Există foarte multe informații/date, dar obiectivul esențial pentru această etapă constă în utilizarea lor cât mai eficientă. Informațiile trebuie să ajungă la momentul potrivit şi să contribuie la conceperea de măsuri care să protejeze populația expusă.

informații medicale asigurare medicală operații de susținere a păcii

ABSTRACT:
There is presented the force medical pre-deployment preparation in Peace Support Operations. There is a lot of information, but the main purpose is how to use it the most efficiently possible. So, the data must be processed in time and measures be established in order to prevent the excessive morbidity and mortality in both the civilians and response personnel.

Key words: Medical Intelligence, Medical Support, Peace Support Operations

Scopul asigurării medicale în Operațiile de Susținere(asigurare) a Păcii este de a îmbunătăți ori, cel puțin, de a menține starea de sănătate a populației. Planificarea adecvată a asigurării medicale a Operațiilor de Susținere a Păcii determină punerea în practică şi folosirea măsurilor de prevenire a morbidității şi mortalității excesive atât în rândurile civililor cât şi la nivelul efectivelor Forței implicate in operațiile de susținere a păcii. Lucrarea îşi propune să discute pre-planificarea şi pregătirea asigurării medicale. Există foarte multe informații/date, dar obiectivul esențial pentru această etapă constă în utilizarea lor cât mai eficientă. Informațiile trebuie să ajungă la momentul potrivit şi să contribuie la conceperea de măsuri care să protejeze populația expusă. Aşadar prezentarea se va axa pe Informațiile Medicale.

În timp ce restul personalului întreține tehnica militară pentru ca armele să fie folosite de mai multe contingente... serviciul medical trebuie să-şi concentreze efortul asupra efectivelor (luptătorilor) ... asupra celor care constituie “arma umană”.
Vor fi abordate:

- cerințele pentru serviciul medical, specifice activității din garnizoana de reşedință,
- evaluarea stării de sănătate şi nivelul individual de pregătire din punct de vedere medical (PIMR),
- cerințele pentru serviciul medical privind perioada anterioară desfăşurării efectivelor,
- controlul medical/activități specifice perioadei anterioare desfăşurării,

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- Informaţiile Medicale
- Şedinţele de lucru (informare, punere de acord, luare de decizii) pentru serviciul medical în perioada anterioară desfăşurării efectivelor.

De-a lungul istoriei sunt consemnate multe exemple în care pierderi de personal determinate de îmbolnăviri şi de o activitate neadecvată de protejare a sănătăţii forţelor au influenţat rezultatul operaţiilor militare. Multe dintre ele ar fi putut fi prevenite, iar istoria, cum este ştiută astăzi, ar fi putut arăta altfel.

Situaţia actuală din lume şi noul rol asumat de NATO, mai ales, cel al Forţei NATO de Răspuns (NRF) a complicat în mod evident utilizarea forţelor. Desfăşurarea rapidă este şi va continua să fie cuvântul de ordine. De aceea, cu mai puţine forţe disponibile ca număr, aparţinând tuturor naţiunilor, efectivele vor participa la deplasări mai frecvente şi de mai lungă durată. Pe durata operaţiilor externe există riscul îmbolnăvirii, al influenţelor mediului cu efecte necunoscute, există ameninţările activităţii teroriste şi mereu potenţiala expunere la acţiunea armelor de distrugere în masă.

Iar lista poate continua... Toate aceste posibile riscuri trebuie identificate şi trebuie stabilite măsuri de reducere şi chiar anihilare a impactului lor asupra sănătăţii personalului înainte ca prima unitate să fie desfăşurată. Acesta este obiectul de activitate al segmentului Informaţii Medicale.

În conflictele moderne, mai cu seamă în Operaţiile de Susţinere a Păcii (PSO) majoritatea pierderilor sunt datorate îmbolnăvirilor şi rănilor dobândite altfel decât în luptă (DNBI). De la primul rănit în luptă (WW) până la Desert Storm DNBI rămân cele mai importante. Aşa că este necesar ca acestea să fie studiate în primul rând. Responsabilitatea consilierului pe probleme medicale şi cea a celui care răspunde de planificarea medicală în raport cu Comandantul şi cu Forţa angrenată constă în a se asigura că este conceput şi pus în aplicare un program dinamic şi cuprinzător de protecţie a sănătăţii forţei. Unul dintre segmentele care participă la aceasta este cel al Informaţiilor Medicale.

Care sunt cerinţele segmentului medical, specifice activităţii din garnizoană înainte de deplasare? Efectivele răspund individual de menţinerea stării de sănătate. De exemplu, în cadrul Forţelor Aeriene ale Statelor Unite, fiecare formaţiune/unitate are un medic responsabil cu supravegherea stării de sănătate (Unit Health Monitor). Trupele îşi stabilesc vizitele medicale prin UHM. Acestea includ:

- Controlul preventiv (Preventive Health Assessment -PHA) – adică examinarea medicală şi actualizarea fişei, care trebuie făcute o dată la 12 luni
- Examenul stomatologic – adică examenul stomatologic profilactic şi detartrarea, care trebuie făcute o dată la 12 luni
- Examenul de laborator – testul HIV o dată la 24 de luni, G6PD, DNA, grupa sanguină, alte teste potrivit ordinelor in vigoare
- Vaccinări – fiecare trebuie să fie la curent cu ceea ce este de făcut
- Optometrie – purtătorii de ochelari trebuie să aibă mască de gaze adecvată.

Toate acestea conduc la Estimarea Preventivă a Sănătăţii / a Nivelului Individual de Pregătire din punct de vedere Medical, altfel zis PIMR.

Baza de date PHA(Control Preventiv)/ Nivel Individual de Pregătire din punct de vedere Medical (PIMR) poate fi folosită pentru a urmări nivelul de pregătire din punct de vedere medical al personalului, în totalitate, în vederea deplasării.

Procentul PIMR trebuie să fie foarte ridicat pentru a controla nivelul de pregătire din punct de vedere medical.

Aceste date pot fi postate pe web cu actualizări aproape în timp real; acest sistem este acum disponibil pentru comandanți.

Sistemul care se numeşte METRIC trebuie să fie inclus la nivelul Comandamentului şi al Forței Principale.

Activitățile medicale premergătoare deplasării sunt: revizuirea fişelor medicale individuale pentru ca solicitările de materiale medico-sanitare să fie in conformitate cu acestea; problematica de ordin medical nesoluționată să fie redirijată; evaluarea fiecărui participant la deplasare din punct de vedere medical, stomatologic, psihologic, cu vaccinările la zi; verificarea apartenenței fiecăruia la sistemul (de stat) de asigurare a sănătății; fişa medicală astfel actualizată însoțeşte fiecare participant la deplasare. Medicul care lucrează în cadrul segmentului Informației Medicale este cel care stabileşte imunizările specifice cele mai adecvate (febra galbenă, meningită, JE , variolă, antrax, gripă), prescrie medicația pentru prevenirea malariei dacă este necesar (clorochină, meflochină, doxiciclină).

La recoltarea de sânge un eşantion este dirijat către depozit – aşa încât dacă la testul HIV se constată că cineva este seropozitiv, atunci necesarul de sânge să fie asigurat. Trebuie să existe oportunitatea contactului cu un medic pe probleme de sănătate: sarcină, ochelari, medicație pentru boli cronice.

Definiția **Informațiilor Medicale** conform AFMIC: **IM analizează acea categorie de informații care are în vedere bolile infecțioase şi factorii de mediu care, influențând sănătatea, pot conduce la degradarea eficienței forțelor desfăşurate pe teritorii străine.**

Comitetul Medicilor Şefi ai Serviciilor Medicale ale țărilor membre NATO şi partenere (COMEDS) dă definiția următoare: **MEDINT este produsul prelucrării informațiilor medicale, bio-ştiințifice, ambientale raportate la sănătatea omului şi a animalelor. Această activitate fiind de natură tehnică specifică necesită o competență medicală binecunoscută pentru direcționarea şi prelucrarea datelor pe parcursul ciclului de informare**.

Pe de altă parte, în STANAG NATO 2481 **Informația Medicală constituie orice informație cu privire la amenințările de natură medicală sau de mediu sau la facilități ori capabilități medicale, primită pe alte canale decât cele specifice şi care nu a fost analizată prin prisma Serviciului de Informații.** Asemenea informație constituie o componentă esențială a planificării medicale operaționale şi trebuie făcută cunoscută membrilor Alianței. Acest tip de informație are o valoare potențială imensă pentru analiştii MEDINT, în măsura în care reprezintă o sursă importantă de „adevăruri de bază/inițiale” raportate la domeniul medical.

Elementul cheie este că Informația Medicală nu a fost analizată din punctul de vedere al serviciului de informații pentru a-i constata semnificația.

Cine este funcționarul superior responsabil cu Informația Medicală (MIO)?

Poate fi desemnat un medic ofițer de Sănătate Publică, sau un cadru mediu medical cu experiență în sănătate publică. Funcționarul superior responsabil cu Informațiile Medicale (Medical Intelligence Officer = MIO) trebuie să posede cunoştințe minuțioase de medicină şi fie instruit şi în domeniul medicinii preventive.

Importanţa MIO este mare.

Sursele Serviciului de Informaţii Medicale (un procent de 95% din Informaţia Medicală este disponibil ca SURSĂ DESCHISĂ) sunt, de exemplu, Centrele Statelor Unite pentru Prevenirea şi Controlul Îmbolnăvirilor, Centrul de Informaţii Medicale al Forţelor Armate ale Statelor Unite, Registrul Agenţiei Centrale de Informaţii pentru Evenimente din Lumea Întreagă, Organizaţia Mondială a Sănătăţii, Pro Med Digests, CDC Paginii Aurii, www.weather.com! Şi o sursă cu circuit închis provenind de la Serviciul de Informaţii şi SIPR AFMIC.

Este important de reţinut faptul că **NATO nu adună, analizează ori stochează informaţii de natură medicală pentru naţiunile aliate. Transferul de informaţie medicală în cadrul Alianţei este un rezultat al legăturii dintre parteneri.**

O abordare rapidă a domeniului Informaţiei Medicale presupune ca acesta să fie bine delimitat în ceea ce priveşte o posibilă ameninţare: pericol generat de mediu, de boli infecţioase, de boli contagioase, de igiena muncii, de floră sau faună; după cum pentru riscurile reale trebuie precizate: incidenţa/prevalenţa bolii, morbiditatea/mortalitatea, dacă este boală endemică sau epidemică, factorii de risc, condiţii de apariţie/scenarii şi măsuri de prevenire/combatere; esenţială este găsirea unei modalităţi de a atenua riscul: prin utilizarea echipamentului personal de protecţie, a sistemului de anihilare a vectorilor, prin profilaxie cum este pentru malarie, prin utilizarea repelenţilor, prin vaccinări adecvate, prin corectă hidratare, hrănire, mişcare, respectare a ciclului muncă/odihnă.

Determinantă este prevenirea prin cunoaşterea fenomenului şi a condiţiilor în care se declanşează, iar fără măsuri de combatere este imposibilă gestionarea sa!

Alte probleme de luat în discuţie sunt capabilităţile medicale ale ţării gazdă, informarea cu privire la reptilele veninoase pentru a căror muşcătură sunt necesare antidoturi specifice, precum şi căile aeriene de evacuare disponibile.

Ca exemplu se propune prezentarea activităţii de informare premergătoare desfăşurării trupelor americane în Irak. Toate subiectele abordate până acum sunt de interes permanent şi trebuie reluate pentru actualizare, lunar. Ceea ce trebuie să aibă loc cât mai frecvent posibil sunt instructajele/conferinţele în cadrul cărora se transmite şi se discută informaţia medicală.

Se identifică bolile generate de hrană, cele infecţioase şi cele transmisibile, sistemele de anihilare a vectorilor, stresul de luptă, antidoturile chimice şi biologice, existenţa/nonexistenţa bolilor profesionale, factorii de stres din mediu, animalele, plantele şi insectele periculoase. Se precizează problemele de mediu: temperaturi extreme, peste 130ºF, leziunile provocate de căldură (şocuri calorice, insolaţie, deshidratare) în timpul verii, furtuni frecvente de praf sau nisip, respectarea orarului de activitate şi odihnă şi a hidratării. Senzaţia de sete nu este un bun indicator al nivelului de hidratare. Nu trebuie aşteptat până apare senzaţia de sete pentru a consuma lichide. Este bine să se insiste pentru un consum de 250 ml/oră. Trebuie urmărită cantitatea de urină eliminată, trebuie purtate haine largi şi trebuie aplicat sistemul de îngrijire reciprocă (boddy–boddy).

Discutând despre bolile generate de hrană şi apă, trebuie spus că sistemul imunitar este destul de lipsit de apărare faţă de sursele de îmbolnăvire din hrana din ţările în curs de dezvoltare, apa şi gheaţa trebuind să fie considerate contaminate ca şi hrana şi apa vândute în sistemul economic local (deliberat sau accidental).

Trebuie optat între A LUPTA & A CÂŞTIGA şi A VOMA PÂNĂ LA ULTIMA PICĂTURĂ & A PIERDE.

Se foloseşte numai hrana, apa şi gheaţa provenite din surse aprobate.

Pericolul bolilor transmisibile cum ar fi chlamidia, gonorrhea, HIV poate fi controlat prin folosirea elementelor de protecţie individuale şi cel mai bine, prin abstinenţă. Nu se merge şi nu se înoată în apa rece pentru a evita pericolul generat de schistosomiază. Nu se admit în unităţi animale – mascotă pentru a evita pericolul turbării; se raportează orice muşcătură. Nu se consumă lapte sau produse din lapte nepasteurizat (lapte/brânză de capră).

Febra Q este rezultatul contactului cu oi infectate. Pentru a evita pericolul Antraxului nu trebuie consumată carne provenind din surse neaprobate. Apariţia sindromului Hantaviral este asociat cu excremente de rozătoare, după cum febra hemoragică de Crimeea este asociată expunerii la acarieni/căpuşe. Nici o medicaţie nu protejează împotriva leishmaniozei cauzată de înţepăturile vectorilor. Trebuie purtate uniforme tratate cu permetrin, cu mâneci lungi şi nesuflecate. Trebuie raportată orice leziune care nu se vindecă. Sistemul de anihilare a vectorilor presupune dotarea forţei cu uniforme şi aşternuturi tratate, ori/şi cu produse care se aplică direct pe piele (repelenţi)

.

Iată un exemplu de dotare cu vaccinuri şi medicamente împotriva malariei pentru profilaxia bolilor infecţioase într-un ipotetic teatru de operaţii unde sunt amplasate trupele.

• **Variolă**	**DA**
• **Antrax**	**DA**
• **Febra tifoidă**	**DA**
• **Febra galbenă**	**NU**
• **Meningococ**	**NU**
• **Influenţa**	**DA**

Pentru combaterea ameninţării NBC şi utilizarea corectă a antidoturilor este esenţial ca fiecare participant la misiune să fie corect informat. Igiena muncii urmăreşte modul în care fiecare participant la misiune îşi păstrează echipamentul individual de protecţie.

Activitatea în zona de desfăşurare (teatrul de operaţii) presupune existenţa unor potenţiale pericole pentru sănătate ca, de altfel, şi activitatea din garnizoană.

Condiţii aspre de mediu şi lipsa unei protejări a ochilor au condus la numeroase leziuni ale ochilor în rândul forţelor, mergând până la pierderea vederii. Deci, nu trebuie neglijat portul ochelarilor de protecţie şi al ochelarilor de soare, după caz.

Pericolul contactului cu plante periculoase poate fi evitat prin purtarea uniformei cu mâneci lungi şi nesuflecate şi a mănuşilor, dacă se poate. Se evită contactul mecanic cu cactuşi, cu plante cu tulpini subţiri şi ascuţite, cu buruieni şi bineînţeles nu se ingerează nici măcar de curiozitate.

Dacă în zonă este semnalată prezenţa şerpilor să se reţină că sunt mai activi în perioada mai rece din zi. Orice muşcătură de şarpe trebuie raportată imediat.

Contactul cu artropodele trebuie evitat. Prezenţa păianjenilor, a miriapodelor, a scorpionilor poate duce la înţepături dureroase şi potenţial periculoase. Orice înţepătură se aduce la cunoştinţa personalului medical. Dimineaţa se scutură hainele şi încălţămintea pentru a evita surprizele neplăcute.

Stresul de luptă, chiar fără a intra în detalii trebuie luat în consideraţie.

În concluzie

MENȚINEREA STĂRII DE SĂNĂTATE A FORȚEI ÎN GARNIZOANĂ +
+ EXAMINAREA MEDICALĂ PREMERGĂTOARE DEPLASĂRII FORȚELOR +
+ O BUNĂ INFORMAȚIE MEDICALĂ +
+ COMUNICARE =

= DNBI REDUSE

Pe la anul 400 î.c., Hypocrates din Cos, un medic şi profesor de medicină, grec de origine, afirma:

„ Trebuie prevăzute efectele anotimpurilor; dacă se studiază vântul, căldura, frigul, calitatea apei şi terenul unei zone ... se pot şti bolile care există, cum pot fi tratate acestea şi, ceea ce este cel mai important, cum să se prevină apariția lor.”

Cât am învățat cu adevărat în 2407 ani ?!!

BIBLIOGRAFIE

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2. PAM ADVON Team check lists
3. International Medical Operations Course EUCOM chapters 4-6, 11-15 September 2005, Georgia
4. Force Medical Protection – the Role of Medical Intelligence, B. SAVU, A. OLARU, Students and Young Doctors First European Congress of Military Medicine, ROMANIA, Bucureşti, octombrie 2005

Obiectivul informatizării unei biblioteci

Pcc Bibliotecar Cristina ŞCHIOPU[*]

Abstract
Informatica are avantajul de a reface rapid căile de comunicaţie atunci când beneficiază de programe specializate de abordare a datelor ierarhizate astfel încât să se acceseze prin mijloace la îndemâna cititorului informaţii cu caracter de ansamblu sau specializate.

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digitalizare organizarea informaţiei bază de date index program reţea pagină de prezentare

Noile sisteme de informare informatizate; instrumentele de informare tipărite: cataloage, bibliografii, lucrări de referinţă precum şi recomandările directe ale personalului specializat; împreună cu programe diverse de activităţi legate de carte şi documentul cu valoare ştiinţifică şi culturală aduce biblioteca în dialog cu cititorul.

Proiectarea unei baze de date începe cu decizia privind numărul şi tipurile de tabele pe care le va conţine baza de date.

Proiectarea şi crearea unei baze personalizate ţine cont de următoarele criterii:

- Utilizare şi dotare tehnică
- Ansamblul funcţiilor prelucrării de date
- Convivialitate
- Ergonomie
- Reţele
- Posibilităţi de întreţinere
- Asistare pentru diferite probleme
- Demonstraţii

Definiţii

Server: Calculatoare de mare putere, situate în general la distanţă, care satisfac cererile de prelucrare ale unor utilizatori.

Username:

Nume logic care desemnează un utilizator de sistem sau de bază de date, asociat în general la o parolă la momentul conectării.

Interesul unei biblioteci se manifesta pentru:

- suporturi noi, digitalizarea documentelor păstrate în bibliotecă
- interesul utilizării de documente electronice în bibliotecă: păstrare, consultare, asigurarea de documente înlocuitoare
- problemele juridice sau consultare pe loc.

Organizare şi planificare informaţională în slujba societăţii:

- Analiza situaţiei existente:
- Dezvoltarea structurii

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Posibila organizare a strategiei de informatizare:

1. Procedurile de prelucrare, circuitul documentelor;
2. Relațiile dintre cei care prelucrează documentele
3. Regulile de prelucrare
4. Organizarea pe fişiere, descrierea prelucrării intrare/ieşire
5. Relații – entități de lucru (indexuri)
6. Îmbunătățirea existentului pe baza obiectivă a probelor
7. Încadrarea în normele de funcționare (rapoarte de gestiune)
8. Metode de eliminare din stoc. Suport web
9. Baza de date şi achizițiile bibliotecii
10. Concluzii

Organizarea bazelor de date

Planul de aplicație şi metoda de lucru

- ordinea de introducere a datelor
- timpii de aşteptare
- cititorii

1. Proceduri conform circuitului datelor

Datele contabile (specifice evidenței de bibliotecă: data cererii de ofertă / prețurile; data facturării / plății; data introducere contabilitate; data BPTR; data introducerii în evidențele de bibliotecă)

Introducerea datelor la timp (decalajul de timp necesar circuitului documentelor)

2. Datele de gestiune. Schimbul de informații

Bazele de date au fost structurate după ce s-a stabilit dacă şi în ce măsură pe parcursul studiului intervine necesitatea schimbului de informații.

Colecțiile bibliotecii cuprind date din următoarele domenii:

- Medicina internă
- Hematologie
- Laborator şi Farmacie
- Oftalmologie
- Neurologie, Neurochirurgie
- Psihiatrie
- Dermatologie
- Histologie şi Atlase
- Homeopatie
- Pediatrie
- Stomatologie
- O R L
- Ginecologie
- Urologie
- Chirurgie
- Traumatologie
- Cardiologie
- Medicină sportivă
- Reumatologie
- Pneumologie
- Chirurgie toracică
- Radiologie şi imagistică
- Biografii

Gestiunea înregistrărilor programate (succesiunea etapelor pentru fiecare sector de evidență) .

3. Regulile de prelucrare

Introducerea datelor la timp

Datele de împrumut (nume, adresa, telefon)

Baza de date cu evidenta cititorilor

Datele privind cititorii, condiţiile şi regimul de împrumut

Gestiunea listelor cu cititori (datele de împrumut / restituire – ştergerea din evidenţă)

Baza de date a bibliotecii.

4. Organizarea pe fişiere

Prin număr şi indici (set de litere), bazele de date vor stabili tipul de date bibliografice pe care le va accesa personalul:

- Bibliotecă cu caracter ştiinţific, informaţional organizaţional şi economic
- Bibliotecă de documentare ştiinţifică cadre medii
- Bibliotecă cu caracter şcolar
- Bibliotecă cu caracter umanist (ştiinţe universale şi literatură)
- Bibliotecă cu caracter ştiinţific de cercetare.

5. Relaţii conform indexurilor

Analiza şi documentarea bazelor de date

6. Probe. Reactualizare

7. Raportări de gestiune

În multe cazuri, un raport răspunde la întrebări importante despre conţinutul bazei de date.

Pe lângă furnizarea de informaţii detaliative şi rezumative, care pot include calcule (câmpuri cu valoare calculată) ca în punctual 5, rapoartele au şi următoarele caracteristici:

- Raport uşor de citit şi de înţeles
- Anteturi şi subsoluri care tipăresc informaţii de identificare în partea de sus a fiecărei pagini
- Gruparea şi sortarea care organizează informaţiile

1.Date contabile (conturi contabile; solduri)

2.Date de gestiune (lucrul cu evidenţele)

3.Reguli de prelucrare

Conform etapelor de introducere stabilite

Sintaxa

Tools – Analize – Documentar

4. Proiectarea de tabele

Stocarea datelor într-o bază de date

Problemele de clasificare.

Problemele de "traseu" a unei cărţi de la înregistrare la împrumut şi la restituire.

Relaţionări

Verificări automate periodic prin condiţii de lansare în execuţie la Conectare automată (SQL analyzer)

Consultarea informaţiilor pe suport web (PHP)

Adaptate pentru structura noastră.

5. Filtrarea datelor

Prin combinarea clauzelor where. Where filtrează anterior grupării datelor, în timp ce Having filtrează după această operaţie, ceea ce reprezintă o diferenţă importantă.

Securitatea bazelor de date

Constrângerile reprezintă o componentă importantă a aplicării integrităţii referenţiale; indexurile putând contribui la îmbunătăţirea performenţelor operaţiei de prelucrări ante sau post-execuţie, iar opţiunile de securitate se pot folosi pentru gestiunea accesului la date.

Indexare. Sortare

Un index din Access este o structură de date invizibilă care stochează ordinea de sortare a unui tabel pe baza câmpului indexat, programul Access consultă indexul şi este capabil să sorteze tabelul foarte rapid.

După indexarea unui câmp, se pot vizualiza şi apoi modifica indexurile după cum este necesar.

Printre operațiile care sunt frecvent securizate se numără următoarele:

- Accesul la caracteristicile de administrare a bazelor de date (crearea tabelelor, modificarea sau ştergerea tabelelor existente etc.)
- Accesul la anumite baze de date sau tabele
- Tipul de acces (numai pentru citire, acces la anumite coloane etc.)
- Accesul la tabele numai prin intermediul descris al vederilor sau al procedurilor stocate
- Crearea unor niveluri multiple de securitate, permițând diferite niveluri de acces şi control, bazate pe conectarea la sistem
- Limitarea capacității de gestionare a conturilor de utilizator.

Salvarea datelor pe un suport extern contribuie la întreținerea datelor în format electronic.

Suporturile materiale de salvare sunt dischetele, CD şi micromemoriile (buffer) indicate pentru volum mare de date stocate.

9. Baza de date şi achizițiile bibliotecii

Se poate interoga baza de date pentru a stabili în ce măsură următoarea achiziție completează structura tehnică sau informația teoretică a domeniului pe care s-a prezentat raportul.

Astfel se stabilesc echivalențe şi efecte directe / indirecte asupra deciziilor referitoare la organizarea analitica a datelor.

Scopul strategiei de informatizare este să faciliteze definirea:

- Statutului administrativ al bibliotecii
- Autoritatea de decizie
- Publicul de deservire
- Locul său într-o rețea
- Mediul său informatic

10. Concluzii. Repartizări.

Trebuie să fii bibliotecar şi abia apoi să admiți cărțile şi internetul ca viață umanizată.

PAGINI DIN ISTORIA MEDICINII MILITARE ROMÂNEŞTI

Mărturii ale activității serviciului sanitar român în timpul Campaniei din Vest (1944-1945)

Mr. medic Elena Ramona POPA[*]

OBIECTIV: prezentarea unor documente de luptă ce dovedesc activitatea serviciului sanitar al Armatei 4-a române în timpul Campaniei din Vest (1944-1945) ;
DISCUȚII: Campania din Vest cuprinzând operațiunile militare duse de trupele româneşti pentru eliberarea Ardealului de Nord, a Ungariei şi a Cehoslovaciei de sub ocupația germană a însemnat pentru serviciul sanitar român eforturi susținute pentru menținerea efectivelor luptătoare în condițiile grele de război şi ale iernii anului 1944/1945 dar şi activități de ajutorare a populației civile din zonele respective amenințate şi de pericolul tifosului exantematic .
CONCLUZII: personalul medical şi sanitar inferior al Armatei 4- române, prin eforturi proprii, a contribuit la menținerea efectivelor combatante, mulți dintre medici şi sanitari pierzându-şi viața în timpul luptelor din cadrul Campaniei din Vest (1944-1945) ;

documente de luptă serviciul sanitar Campania din Vest

PURPOSE: presention of some war's documents that define the activity of the medical service of the 4-th's romanian Army during the West Campaign (1944-1945);
DISCUSSION: The West Campaign including the military operations done by romanians troups for gaining the freedom of North Ardeal, Hungary and Cehoslovacia from the german occupation, meant a difficult task for the romanian military medical service, due by the very hard winter of the 1944's but also because of the necessary activities for helping civilian people and because of the epidemy of typhos.
CONCLUSION: the medical staff of the 4-th's romanian Army, with their own meanings, sustained the health of the military forces, and many of them lost their life during the West Campaing.(1944-1945)

Răsfoind recenta lucrare „Jurnal de front - Armata a 4-a română în Campania din Vest (1944-1945)" a căror autori sunt general-maior dr. Ioan Ciupei şi colonel (r.) dr. Vasile Tutula, lucrare apărută la Editura Cluj Napoca în 2006 aflăm lucruri deosebite cu privire la activitatea serviciului sanitar român în perioada 5 septembrie 1944-12 mai 1945. Autorii şi-au concentrat eforturile asupra prezentării unor documente inedite aflate în arhivele militare referitoare la Campania din Vest, campanie ce a cuprins operațiile militare duse de către armata română pentru eliberarea Ardealului de Nord, a Ungariei şi Cehoslovaciei de sub ocupația germană.

Campania din Vest a fost „ o campanie care a durat 246 zile, atacând timp de 203 zile şi alte 137 de nopți de atac sau deplasări tactice pentru înlocuiri în dispozitiv". Nu a fost o campanie uşoară, au subliniat autorii lucrării, au fost condiții de luptă specifice; marşurile s-au desfăşurat în majoritatea cazurilor pe jos, pe timp de iarnă; în Cehoslovacia, zăpada fiind de 1-1,5 m cu troieni şi viscole mari, terenul a fost în majoritate muntos, la altitudini de 800-1500 metri; temperaturi ce s-au menținut continuu la minus 20 grade. Pierderile umane au fost mari, aproximativ 90.000 de militari români.

Prezentăm 2 documente ce ilustrează emoționant câteva aspecte ale activității sanitare desfăşurate de către medicii şi sanitarii români:

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[*] Unitatea Militară 01110 Str. Dr.Vicol nr. 2 Iaşi

Documentul nr.1
„Divizia 9 Infanterie
Comandantul

Ordin de Zi nr.71
Din 16 mai 1945

În grelele lupte pe care divizia le-a purtat în Ardeal, Ungaria şi Cehoslovacia, Ambulanța Militară nr.81 condusă de medicul căpitan dr. Menaide Ioan, funcționând în toate ocaziile cât mai aproape de linia de luptă şi desfăşurând o activitate neobosită şi-a adus reale şi neprețuite servicii frontului.

Aşa, în luptele de la Mureş (Ungheni, Cristeşti, Sântioara, etc) deşi încadrată numai cu doi medici a ridicat, operat, pansat şi evacuat în timp de 5 zile, printr-un efort supraomenesc, peste 1500 răniți făcându-şi un punct de onoare de a nu lăsa nici un rănit fără ajutor, în ciuda obstacolelor ce păreau de netrecut.

Mai târziu, organizându-se şi încadrându-se cu personal şi mijloacele necesare chiar pe câmpul de luptă, această formațiune aduce aportul unui adevărat spital în care răniții noştri găsesc dragostea camaraderească şi desăvârşită pricepere medicală îmbinate printr-o fericită împrejurare într-un deosebit elan al împlinirii datoriei.

Medicul căpitan dr. Menaide Ioan, el însuşi exemplu , în mijlocul subalternilor, înconjurat de o echipă de medici tineri, dar pricepuți şi pătrunşi de frumusețea misiunii pentru care au fost chemați aici, mereu prezenți la datorie, muncind ziua şi noaptea şi îmbărbătând răniții din grelele lupte de la Tisa, Megzaszo, Valea Hernadului, Perin, Jablonica, Brezno, Valea Hronului, Banska Bystrica, Valea Vahului etc, au reuşit prin curajoase şi pricepute intervenții chirurgicale să dea ajutorul necesar la peste 6500 răniți şi bolnavi dintre care 800 au fost tratați pe loc şi înapoiați frontului, iar restul puşi în situația de a suporta în cele mai bune condițiuni şi fără risc evacuarea către formațiunile sanitare dinapoi.

În toată această activitate, trupa sanitară a ambulanței, bine instruită şi bine condusă, a fost la înălțimea misiunii sale. Brancardierii acestei formațiuni, adânc pătrunşi de datoria lor şi disprețuind primejdia au dat ajutorul atât de necesar, chiar în linia de foc, sub ploaia de gloanțe şi obuze, plătind uneori cu viața.

Ajutorul medical a fost dublat şi de o deosebită atenție pentru curățenia, hrana şi cazarea răniților şi bolnavilor, fapt ce a determinat pe toți şefii ierarhici ca în desele inspecții ce au făcut să nu aibă decât cuvinte de laudă pentru comandantul, ofițerii şi trupa ambulanței, medicul şef al Armatei 4 calificând-o drept o formațiune ce face fală armatei.

Pentru conştiinciozitatea, curajul, spiritul de sacrificiu, priceperea şi munca neobosită puse în slujba celei mai nobile misiuni, redresarea fizică şi morală a răniților, pentru deosebit de frumoasele rezultate obținute,

ordon:

Citarea prin ordin de divizie a medicului căpitan dr. Menaide Ioan, medicul şef al Ambulanței Sanitare nr.81, precum şi a ofițerilor, subofițerilor şi trupei acestei formațiuni de serviciu, spre a-i servi ca exemplu în împlinirea datoriei în cel mai nobil înțeles al cuvântului.

Dat la Cartierul general al Diviziei 6 Infanterie, în Kadov (Moravia) la 18 mai 1945.

Comandantul Diviziei 6 Infanterie
General I. DIMULESCU

Arh.M.Ap.N., fond 4,dosar nr.82, f 118, **Copie** “

Documentul nr.2

„Ambulanţa 81-a Sanitară
Oficiul poştal Militar nr.54

Nr.5561
Din 18 mai 1945

Către

Divizia a 6 a Infanterie
Stat Major, Biroul 1

La ordinul Dvs.,nr.2410 din 16 mai 1945;

Am onoare a vă raporta următoarele :

Ambulanţa 81 Sanitară a căutat în toate împrejurările, indiferent de localitate şi naţionalitate, să vină în ajutorul populaţiei civile lipsită de asistenţă medicală şi de imposibilitate de a se trata din cauza condiţiilor şi distrugerilor grave ale războiului.

Ajutorul medical a fost dat neprecupeţit tuturor cetăţenilor care l-au cerut, deseori dându-se consultaţii gratuite şi asistenţă medicală necesară chiar la domiciliul cetăţenilor bolnavi netransportabili administrându-li-se în nenumărate cazuri unde era absolută nevoie şi erau în imposibilitate de a şi le procura, medicamentele necesare.

Se adaugă la aceste numeroase consultaţii şi distribuire de medicamente, facerea a numeroase pansamente civililor răniţi ca şi câtorva mari operaţii făcute pentru maladii care cereau imediata intervenţie chirurgicală, punând în caz contrar viaţa lor în pericol.

La această bogată asistenţă medicală care va fi ilustrată mai jos cu multiple cazuri, ambulanţa a contribuit deseori cu mijloacele sale proprii, să evacueze la Spitalele de Campanie româneşti, numeroşi răniţi civili, hrăniţi şi întreţinuţi în ambulanţa până la evacuare.

S-a contribuit de asemenea la prevenirea maladiilor contagioase din sate şi la stăvilirea epidemiilor, luându-se măsuri de izolare şi de parazitare a contagioşilor acolo unde au fost găsiţi.

Am ajutat de asemenea cu câteva căruţe la evacuarea civililor din comuna Kraşidobrocs, ajutând în acest fel la păstrarea bunurilor cetăţenilor:

Ca exemplu acum pentru asistenţa medicală acordată ostaşilor citām:

În Ungaria

În comuna Foro şi în comuna Garadna s-au dat consultaţii şi s-au făcut pansamente la 7 civili bolnavi şi răniţi din explozie de focos. În comuna Geci-Bator a fost operată o femeie dezarticulându-i-se degetul mare al piciorului stâng, cangrenat în urma unei plăgi de război.

A fost tratată de asemenea o apendicită acută cu plastron, care periclita viaţa bolnavei. Au fost vizitaţi şi patru civili cărora li s-au dat medicamentele necesare iar un grav rănit a fost pansat şi îngrijit în ambulanţă.

În comuna Percupa au fost consultaţi 3 civili, dându-li-se medicamentele necesare.

În Cehoslovacia

În comuna Dobrocs, unde ambulanţa a stat o lună de zile, s-a dat o bogată asistenţă medicală la civilii răniţi şi la bolnavii din întreaga regiune cetăţenii venind zilnic în număr mare la consultaţii. S-au dat peste 120 consultaţii medicale, făcându-se numeroase pansamente şi câteva mari operaţii.

Ilustrez cu cazurile mai importante:

1. Un copil din comuna Ianoşova cu un mare flegmon difuz al antebraţului drept, care punea întreg membrul superior în pericol, iar viaţa îi era ameninţată, a fost operat şi tratat până la vindecare în ambulanţă.

2. O fată de 20 ani rănită, cu fractura gambei drepte, fiind în pericol grav viaţa mamei şi a copilului, I s-a făcut o operaţie cezariană salvându-se copilul.
3. O familie întreagă din comună, compusă din 6 persoane rănită prin explozia unei grenade, a fost mult timp îngrijită în ambulanţă.
4. O femeie bolnavă grav de cord, a fost de asemenea tratată .
5. Bolnavi gravi de pleurezie, alţii cu tulburări gastro-intestinale, furunculoze, arsuri, etc. au fost trataţi.
6. S-au făcut numeroase extracţii dentare. În plus s-au dat numeroase consultaţii medicale, împărţindu-li-se şi medicamente la sugari, dându-li-se sfaturi pentru îngrijirea lor.
7. În comuna Poniki s-au dat peste 10 consultaţii şi s-au făcut pansamente la civili răniţi şi bolnavi.
8. A fost operată şi scăpată de la o moarte sigură o femeie din aceeaşi comună, cu o cangrenă umedă la gamba piciorului stâng după o plagă de război.
9. De asemenea un civil partizan cu o zdrobire a gambei drepte prin explozie de mină a fost operat făcându-i-se apoi multiple seruri şi transfuzii de plasmă salvându-l de la moarte.
10. Tot în această comună s-au luat măsuri pentru izolarea şi deparazitarea civililor la care se iviseră cazuri de boli contagioase.
11. În comuna Stranz au fost pansaţi şi îngrijiţi peste 10 răniţi veniţi din comuna Salkova, Sinita, etc. cu plăgi grave de război şi apoi evacuaţi cu maşina la spitalele româneşti din Nove Mesto.
12. Un băiat de 18 ani rănit grav prin explozie de mină cu o zdrobire a gambei stângi a fost salvat de la moarte prin operaţie şi transfuzii multiple de seruri şi plasmă.
13. În comuna Zaharoviţe, au fost operaţi şi îngrijiţi medical peste 15 răniţi civili şi apoi evacuaţi o parte la spitalele româneşti.

În concluzie: Ambulanţa 81 Sanitară, ori de câte ori a avut posibilitatea să dea un ajutor sub orice formă şi mai ales medical populaţiei civile a făcut-o, neţinând seamă de naţionalitate şi căutând cât a putut să îmbunătăţească suferinţa cetăţenilor.

Medic şef al Ambulanţei 81 Sanitară
Medic căpitan
/ss/Dr.Menaide Ioan”

Aceste documente arată doar câteva aspecte din activitatea serviciului Ambulanţei 81 Sanitare, parte a serviciului sanitar al trupelor române implicate în Campania din Vest (1944-1945)

BIBLIOGRAFIE

1. IOAN CIUPEI, VASILE TUTULA – *Armata a 4-a română în Campania din Vest (1944-1945)* - Ed. MEGA Cluj-Napoca, 2006 ;

ISTORICUL SPITALULUI MILITAR CRAIOVA

Comandantul Spitalului Clinic de Urgenţă Militar
„Dr. Ştefan Odobleja” – Craiova
Col. dr. Cornel TIŢA

Serviciul sanitar militar s-a născut odată cu primele formaţiuni militare organizate de către Generalul Kiseleff în principate, „Miliţia Românească” în Muntenia şi „Straja pământească” în Moldova.

Existenţa în oraşul Craiova, încă de la început, a unei garnizoane militare puternice, a impus organizarea unei asistenţe medicale autonome pentru militari, cadre şi familiile lor.

Spitalul Militar Craiova datează din anul 1882 prin transformarea fostei Infirmerii Colentina – înfiinţată în anul 1838, pe timpul domnitorului Bibescu Vodă. Ca urmare a obţinerii Independenţei naţionale, în anul 1883, armata română se reorganizează formându-se 4 (patru) corpuri de armată, iar numărul diviziilor se dublează. Spitalele din Craiova, Galaţi şi Iaşi s-au transformat în „spitale de corp de armată”, denumire care a datat până în anul 1927, când se schimbă denumirea în SPITALUL MILITAR CRAIOVA.

Corpul 1 Armată, cu reşedinţa în oraşul Craiova, cuprindea unităţi militare de pe raza fostelor judeţe Mehedinţi, Romanaţi, Dolj, Olt, Vâlcea, Argeş şi Gorj.

Serviciul sanitar al Corpului 1 Armată avea sub ordinele sale în afară de Spitalul de corp de armată din oraşul Craiova – încadrat cu toate secţiile – şi spitalele divizionare din Turnu Severin, Slatina şi Piteşti..

Terenul actual pe care se află Spitalul Militar Craiova, a devenit proprietatea Ministerului de Război în anul 1881, prin cumpărarea acestuia de la doamna Maria M. POENARU – actul nr. 677 – şi de la comuna Craiova – prin actul nr. 571.

În decursul anilor, pavilioanele din cadrul spitalului, au fost construite astfel :

- fostul pavilion administrativ – A – în anul 1893
- pavilionul B1 ORL şi Oftalmologie – pe atunci serviciul chirurgie – în anul 1885 şi restaurat după incendiu în anul 1892
- fostul pavilion B Boli interne în anul 1895 – demolat în anul 1991
- pavilionul B2 Boli contagioase şi actuala spălătorie a garnizoanei în anul 1885
- fostul pavilion farmacie, laborator, policlinică, triaj în anul 1895, sub denumirea de „Pavilionul D” şi ocupat de Depozitul sanitar al Corpului 1 Armată – demolat în 1991
- pavilionul G, în care este instalat punctul de control a fost construit în anul 1893, iniţial având destinaţia de capelă
- castelul de apă a fost construit în anul 1865
- în anul 1996 se dă în folosinţă pavilionul D1, administrativ şi policlinică, în care funcţionează şi secţia Radiologie şi farmacia spitalului.
- pavilionul ocupat în prezent de secţiile Dermato-venerice şi Neuropsihiatrie la etaj şi blocul alimentar la parter, a fost construit în anii 1952-1953.

În anul 1921, se înfiinţează serviciul de radiologie, dentistică şi laboratorul de bacteriologie. În acelaşi an se ocupă şi clădirea Companiei 1 sanitare şi funcţionează în acest pavilion serviciile ORL, Oftalmologie şi Dermato-venerice. Această clădire, după toate probabilitățile, era vechiul local al Infirmeriei Colentina – actualul pavilion B4 în care funcționează secția Chirurgie.

Serviciul Dermato-Venerice

Pavilionul Chirurgie

Împrejmuirea spitalului a fost realizată în anul 1886, instalația interioară de apă în anul 1889 odată cu a întregului cartier, numit atunci „Bariera Caracal”.

Între anii 1953 – 1962 s-au modernizat toate pavilioanele prin compartimentări în saloane mici şi rezerve, iar în anul 1965 se introduce încălzirea centrală, se construiesc alei asfaltate, se aduc o serie de îmbunătățiri la blocul alimentar, baie, spălătorie, se reamenajează serviciul de radiologie, stomatologie şi triaj, precum ți îmbunătățiri substanțiale în ceea ce priveşte dotarea materială cu mobilier, aparatură şi instrumentar sanitar, în toate serviciile medicale.

Perioada 1965 – 1970 este perioada în care spitalul a fost dotat cu aparatură medicală nouă, modernă, de mare tehnicitate.

Între anii 1930 – 1931 apar în cadrul spitalului primii subofițeri droghişti, iar în 1934 se angajează primele infirmiere. Până atunci atribuțiunile personalului mediu sanitar erau îndeplinite de subofițeri sanitari şi sanitari-brancardieri, recruți şi instruiți din rândul militarilor în termen.

În timpul celui de-al doilea război mondial, Spitalul Militar Craiova, a funcționat în actualul local până în primăvara anului 1944, când a fost bombardat şi mutat în cazarma „Alion” unde a funcționat până în anul 1945.

Până în anul 1949 Spitalul Clinic de Urgență Militar „Dr. Ştefan Odobleja” – Craiova a fost subordonat Companiei I-a sanitare din punct de vedere administrativ şi Corpului 1 Armată, din punct de vedere operativ.

Începând din anul 1950 Spitalul Clinic de Urgență Militar „Dr. Ştefan Odobleja” – Craiova devine organ de dotare şi mobilizare luând asupra sa o serie de responsabilități şi obligații privind unitățile teritoriale din regiunea Oltenia.

În decursul anilor au depus o activitate medico-militară susținută personalități de seamă în medicina românească, eminenți profesionişti şi organizatori de sănătate, printre care : general de brigadă doctor Potârcă Iacob, maior doctor Vercescu, primul autor al procedeului operator în hemoroizi, colonel doctor Gheorghe Ioan – medic primar ORL, căpitan doctor Mihăilescu Păunel – medic ORL, colonel doctor Vasilescu Mihai – medic chirurg, colonel doctor Panea Aurel – medic internist, colonel doctor Becherescu Nicolae – medic radiolog, maior farm. Mogoş Ştefan, locotenent colonel medic Ionescu Marcel, locotenent colonel medic Ionescu Mihai, locotenent colonel medic Vasilescu Ion şi mulți alții.

La conducerea Spitalul Clinic de Urgență Militar „Dr. Ştefan Odobleja” – Craiova s-au succedat următorii şefi (comandanți) de spital :

- 1875 – 1883 locotenent colonel doctor Vlasta
- 1883 – 1884 colonel doctor Vercescu Mihai
- 1885 – 1900 locotenent colonel doctor Caligari
- 1900 – 1903 colonel doctor Dumitrescu
- 1903 – 1905 colonel doctor Petrescu Grigore
- 1905 – 1906 colonel doctor Papilan Gheorghe
- 1906 – 1910 colonel doctor Condemin Nicolae
- 1910 – 1913 colonel doctor Antoniu Ilie
- 1913 – 1916 colonel doctor Potârcă Ion
- 1916 – 1917 colonel doctor Elian Ion
- 1917 – 1918 colonel doctor Grueascu M.

Personalul medical al Spitalului Militar Craiova 1920 - 1922

- 1918 – 1920 colonel doctor Ionescu Carp
- 1920 – 1921 colonel medic Baculescu Marin
- 1921 – 1922 colonel doctor Urdărescu M.
- 1922 – 1933 colonel medic Dobrinescu Ioan
- 1933 – 1937 locotenent colonel doctor Panea Aurel
- 1938 locotenent colonel doctor Sărdărescu Ioan
- 1939 – 1940 colonel medic Bălan Ion
- 1940 – 1941 colonel doctor Becherescu Nicolae
- 1942 locotenent colonel doctor Mitran Ilie
- 1943 – 1944 colonel doctor Oprean Gheorghe
- 1945 – 1946 colonel doctor Căpruciu Gheorghe
- 1946 colonel doctor Gheorghe Ioan
- 1946 – 1949 colonel doctor Hucan Constantin
- 1949 – 1953 colonel doctor Ştefănescu Ioan
- 1953 – 1954 colonel doctor Sava Vasile
- 1954 – 1955 colonel doctor Morărescu Aurel
- 1955 – 1958 colonel medic Popovici Radu
- 1958 – 1959 locotenent colonel medic Buzatov Petre

- 1959 – 1962 locotenent colonel medic Vlad Florian
- 1962 – 1968 locotenent colonel doctor Sporea A.
- 1968 – 1984 locotenent colonel medic Lupaşcu Victor
- 1984 – 1985 colonel doctor Predescu Constantin
- 1985 – 1991 colonel doctor Păun Nicolae
- 1991 – 1998 colonel doctor Dumitrescu Ion
- 1998 – 2005 colonel doctor Cheiță Nicolae

Începând cu anul 1968 prin efortul întregului personal, au fost obținute următoarele realizări :

- amenajarea drumului de acces din spital
- mutarea, amenajarea şi dotarea secției radiologie
- construirea şi mutarea secției triaj într-un local corespunzător
- construirea a două bordeie betonate
- amenajarea unui bordei carburanți
- amenajarea parcului auto
- extinderea serviciului stomatologic şi laborator
- construirea de trotuare la pavilionul policlinicii triaj
- reparații capitale şi reamenajarea blocului alimentar
- realizarea unui gard din prefabricate în latura estică a spitalului – 350 m.
- înfrumusețarea parcului spitalului prin plantări de pomi şi arbuşti ornamentali
- amenajarea unei baze sportive
- modernizarea băii spitalului.

Deşi împrejurările au fost dificile, spitalul şi-a asigurat ritmul activ şi a progresat sub diferite aspecte, că era bine încadrat cu personal medical, sanitar – în special corpul subofițerilor – şi administrativ.

Activitatea ştiințifică poate fi considerată sub aspect progresiv. Întreg personalul medical al spitalului şi al garnizoanei contribuie la susținerea activității cercului medico-militar local.

În timpul evenimentelor din decembrie 1989 obiectivul principal l-a constituit apărarea spitalului, evitarea evenimentelor grave , funcționarea spitalului în condițiile în care o parte din secții au fost scoase din funcțiune.

Pavilionul chirurgical avariat la Revolu\ia din Decembrie 1989

După 1990, în cadrul Spitalului Militar Craiova s-au realizat :

- redistribuirea unor spații de spitalizare şi tratament ambulatoriu pentru eficientizarea activității medicale
- s-au îmbunătățit condițiile de spitalizare a pacienților ținând cont de sarcinile spitalului privind asigurarea unui număr mare de unități militare teritoriale

- asigurarea funcţionării Dispensarului Policlinic Militar Caracal
- organizarea de manifestări ştiinţifice de prestigiu
- creşterea prestigiului profesional al spitalului prin impunerea instituţiei ca unitate spitalicească de prestigiu, prin profesionalismul cadrelor militare medicale şi sanitare şi a personalului civil.

În anul 1991 s-a demolat pavilionul B – Boli interne – şi s-a început construcţia actualului pavilion D, iar în perioada 1992 – 1996 s-a construit şi dat în funcţiune pavilionul D în care îşi desfăşoară activitatea Ambulatoriul de specialitate şi Serviciul Administrativ.

Serviciul Boili Interne

Sediul nou al Ambulatoriului de specialitate şi birourilor administrative

Din anul 1998 spitalul intră în relaţie contractuală cu CASAOPSNAJ asigurând servicii medicale şi investigaţii de specialitate de înaltă performanţă pentru personalul Ministerului Apărării Naţionale, Administraţiei şi Internelor, de Justiţie precum şi populaţiei civile din judeţele Dolj, Olt, Vâlcea, Gorj şi Mehedinţi .

După anul 2000 spitalul a fost dotat cu aparatură modernă - ecografe ,holter TA, fibrobronhoscop, laparoscop, aparatură pentru analize de laborator, iar în 2001 a fost s-a pus în funcţiune computerul tomograf .

TOMOGRAFIE COMPUTERIZATA
2002 10 2

Laboratorul de tomografie computerizata

Procesul de reorganizare al armatei a afectat şi Spitalul Militar Craiova reducându-se un număr mare de funcţii militare.

În continuare s-au reamenajat şi modernizat următoarele pavilioane :

- pavilionul B1 ocupat în prezent de secţia Boli Interne

- pavilionul B3 ocupat în prezent de secția Psihiatrie şi compartimentele Neurologie şi Dermato-venerice
- pavilionul C ocupat de Laboratorul de analize clinice prin crearea şi modernizarea spațiilor pentru realizarea circuitelor funcționale
- a fost refăcută împrejmuirea spitalului şi mutat parcul auto
- au fost amenajate spații de depozitare şi a fost renovat depozitul de alimente.

Începând cu anul 2005 spitalul a primit denumirea de Spitalul Clinic de Urgență Militar „Dr. Ştefan Odobleja” .

Dr. Ştefan Odobleja
1902 - 1978

Din anul 2006 a fost pus în funcțiune un nou pavilion în care îşi desfăşoară activitatea compartimentele ORL, Oftalmologie, Unitatea de primiri urgențe şi Serviciul internări-externări.

INSTRUCȚIUNI PENTRU AUTORI

Revista de Medicină Militară este o publicație de prestigiu, cu o vechime de peste 100 de ani. În scopul integrării revistei în circuitul internațional al publicațiilor științifice, autorii sunt rugați să respecte „*Cerințele uniforme pentru manuscrisele trimise jurnalelor biomedicale*” (stilul Vancouver de prezentare) (vezi: New England Journal of Medicine 1991, 324 : 424 – 428; British Medical Journal, 1991, 302; Romanian Jurnal of Gastroenterology 1992, 1/4, 1996, 5/1).

Revista de Medicină Militară este creditată de Colegiul Medicilor din România ca publicație științifică (2,5 credite pentru abonament), creditarea autorilor făcându-se la solicitarea acestora după prezentarea reprintului obținut ca dovadă a editării lucrării (solicitările vor fi adresate personal de autori Colegiului Medicilor din România – Bulevardul Timişoara nr. 15, sector 6, Bucureşti).

TRIMITEREA MANUSCRISELOR

Manuscrisele vor fi trimise redacției pe adresa: *Revista de Medicină Militară* – Direcția Mediclă, Str.Institutul Medico-Militar Nr. 3-5 sector 1, Bucureşti, tel. 021.319.60.06.

Manuscrisele vor fi însoțite de o scrisoare în care se menționează poziția şi calificarea tuturor autorilor, semnată de toți autorii şi conținând următoarele declarații:

1. Lucrarea este originală, nu a mai fost acceptată pentru publicare, nu este în prezent în considerarea vreunei alte reviste pentru publicare şi nu va fi publicată în altă parte fără permisiunea redacției.

2. În cazul articolelor cu mai mulți autori, trebuie menționat că toți au contribuit în mod direct la programarea, executarea sau analiza lucrării raportate, sau la redactarea acesteia. Autorii sunt rugați să facă aceste declarații cu multă grijă şi responsabilitate. Publicarea precedentă sub formă de rezumat trebuie, de asemenea, menționată.

Studiile pe pacienți umani sau studiile experimentale trebuie să respecte reglementările internaționale de etică. Sursele de finanțare sau suportul financiar trebuie menționat cu mulțumirile de rigoare.

În caz de litigiu este util ca autorii să menționeze numele şi adresa a doi experți pe care îi consideră recomandabili pentru a evalua manuscrisul respectiv.

Lucrările vor fi examinate de către doi revizori de specialitate din comitetul editorial. Dacă opiniile lor diferă, articolul este analizat de un alt revizor. Evaluarea articolelor se face în mod anonim, fără ca revizorii să cunoască numele autorilor lucrării.

Comitetul editorial poate solicita autorilor să modifice manuscrisul, în funcție de recomandările revizorilor. Comitetul editorial are dreptul să editeze (modifice) manuscrisele.

Autorii au întreaga responsabilitate pentru conținutul științific al lucrărilor lor.

Articolele care nu sunt conforme cu stilul revistei, sunt returnate autorilor fără a fi analizate de comitetul editorial.

Timpul de editare va fi cât de scurt posibil, în funcție de necesitățile științifice şi editoriale ale revistei.

Manuscrisele trimise spre publicare nu pot fi returnate. Când se primeşte un manuscris, autorii vor fi informați în scris, într-un interval de cinci-şase săptămâni, cu privire la acceptarea, nevoia de revizuire sau respingerea articolului.

Lucrările publicate vor deveni proprietatea revistei.

După publicare, primul autor va primi zece reprinturi ale articolului, în mod gratuit, pentru a fi distribuite celorlalți autori.

FORMATUL MANUSCRISULUI

Manuscrisul trebuie redactat în conformitate cu „*Cerințele uniforme pentru manuscrisele trimise jurnalelor biomedicale*” (stilul Vancouver de prezentare) (vezi New

England Journal of Medicine 1991, 324 : 424 – 428; British Medical Jurnal of Gastroenterology 1992,1/4, 1996, 5/1).

Articolele se redactează în Microsoft Word, Normal+Justf., Times New Roman, Font Size – 16 Bold pentru titlu, Font Size – 12 pentru text, iar rezumatul Font Size – 10 Bold. Pagina trebuie setată din Page setup la 2cm. marginile, format letter.

Autorii sunt rugați să trimită articolul pe dischetă şi două exemplare listate de manuscris (inclusiv pentru tabele şi figuri), rezumatul fiind scris atât în limba română, cât şi în engleză, pe adresa Redacției, tipărite pe o singură față a hârtiei A4, la un rând (inclusiv pentru tabele şi referințe bibliografice), cu margini largi. Pentru textul editat în limba română vă rugăm utilizați setarea de limbă („*Romanian”*) pentru tastatură (în „*Control Panel* ”din *Windows*) şi să nu utilizați alte tipuri de caractere româneşti. **Nu trimiteți manuscrisul în limba română fără caracterele specifice româneşti** (diacritice). Toate paginile trebuie numerotate consecutiv, începând cu pagina de titlu, jos pe centrul paginii.

MANUSCRISUL VA FI PREGĂTIT ASTFEL:

1. PAGINĂ DE TITLU

- Titlul lucrării (nu mai mult de 100 de caractere cu spații), fără abrevieri;
- Prenumele, inițiala celui de al doilea prenume, numele de familie al autorilor
- Numele complet al locului de muncă (instituție şi departament) pentru fiecare autor;
- Adresa de corespondență (cu detalii: grade ştiințifice didactice şi militare, persoana de legătură, telefon, fax, e-mail etc.)

2. PAGINĂ DE REZUMAT

- Rezumatul trebuie să aibă 150, până la 250 de cuvinte şi să cuprindă: obiectivul studiului, material şi metodă, rezultatele fără interpretarea datelor şi concluzii.
- Cuvintele-cheie: 3-4 cuvinte-cheie, după *Index Medicus.*

3. ARTICOLUL PROPRIU-ZIS

a. **Articol original** – cel mult 800-1000 de cuvinte (4 pagini).

b. **Prezentare caz** – cel mult 600-800 de cuvinte (2 pagini)..

c. **Referat general** – cel mult 2.000 de cuvinte (10 pagini).

- **INTRODUCERE**: prezentarea problemei, plasarea în context, scopul şi obiectivele studiului, ipoteza de lucru.
- **MATERIALE ŞI METODE**: pacienți, procedee, echipament (inclusiv indicații cu privire la producător), metoda de lucru, analiză statistică, tehnici operatorii, medicamente etc. (în funcție de specificul articolului), toate acestea fiind descrise în detalii suficient de amănunțite pentru a permite reproducerea studiului de către alți investigatori. În tot textul trebuie folosite aceleaşi nume pentru produse (cu specificarea numelui de marcă între paranteze la prima utilizare). Nu se recomandă numele pacienților.
- **REZULTATE**: fără interpretări.
- **DISCUȚII**: 1 – analiza rezultatelor prin prisma obiectivelor propuse ale studiului cu comentarii cu privire la aspectele lămurite (pozitive sau negative) sau nelămurite prin acest studiu;

2 – comparație între rezultatele obținute de autori şi alte studii din literatură, cu explicații, eventual ipoteze cu privire la diferențele observate, accentuând asupra progresului pe care cercetarea de față îl aduce;

3 – comentarii privind importanța studiului de față, precum şi o punere la punct a situației actuale a subiectului investigat atât în literatură, cât şi în practică; eventual se vor prezenta aici cele mai importante studii sau aspecte din domeniu.

4 – probleme nerezolvate, întrebări la care nu s-a răspuns şi care rămân deschise în viitor pentru studiile ce vor urma, eventuale direcții propuse pentru investigare în domeniul respectiv.

CONCLUZII

La nevoie, aceste capitole mari pot fi împărțite în subcapitole, după necesități.

Se recomandă utilizarea precaută a abrevierilor şi menţionarea numelui complet la prima apariţie în

text a abrevierii respective. Toate referinţele bibliografice vor fi citate în text cu cifre arabe, la exponent, fără paranteze, în ordinea apariţiei (de exemplu: Un alt studiu^{18} arată...). Toate figurile şi tabelele trebuie să fie citate în text în locul în care ele să apară (exemplu: Fig. 3, Tab. 2). Datele care apar în tabele, legendele tabelelor şi figurilor trebuie să fie rezumate, nu duplicate în text.

4. TABELELE

Tabelele trebuie să fie procesate la două rânduri, pe o pagină separată, numerotate consecutiv (cu referire în text în locul unde ele trebuie să apară) şi cu un titlu (exemplu: **Tabelul 1**. Distribuţia pe vârste şi sex a pacienţilor). Capetele de tabel se scriu cu caractere *italice.* Nu trimiteţi fotografii sau imagini ale tabelelor. Utilizaţi tasta „Tab” pentru a separa coloanele (nu bara de spaţiu) şi utilizaţi tasta „Enter” la terminarea liniei. Toate căsuţele goale din tabel trebuie să aibă o liniuţă. Dacă este nevoie, pentru cuvintele sau frazele lungi din căsuţe se vor utiliza abrevieri, însă cu explicarea acestora în subsolul tabelului. Asiguraţi-vă că toate tabelele sunt citate în text.

5. FIGURILE

Toate figurile (ilustraţiile), fie fotografii, diagrame sau grafice, trebuie să fie de calitate. Ele trebuie să fie numerotate în ordinea apariţiei în text (cu referire în text la numărul figurii în locul în care aceasta ar trebui să apară) şi, să poarte un titlu (exemplu: **Figura 1.** Distribuţia pe vârste şi sexe a pacientelor).

Pe această pagină referitoare la figuri, se va face o listă a tuturor numelor figurilor, în ordine numerică (la nevoie, cu legende explicative; identificaţi, în această legendă, orice literă sau săgeată utilizată pe figură).

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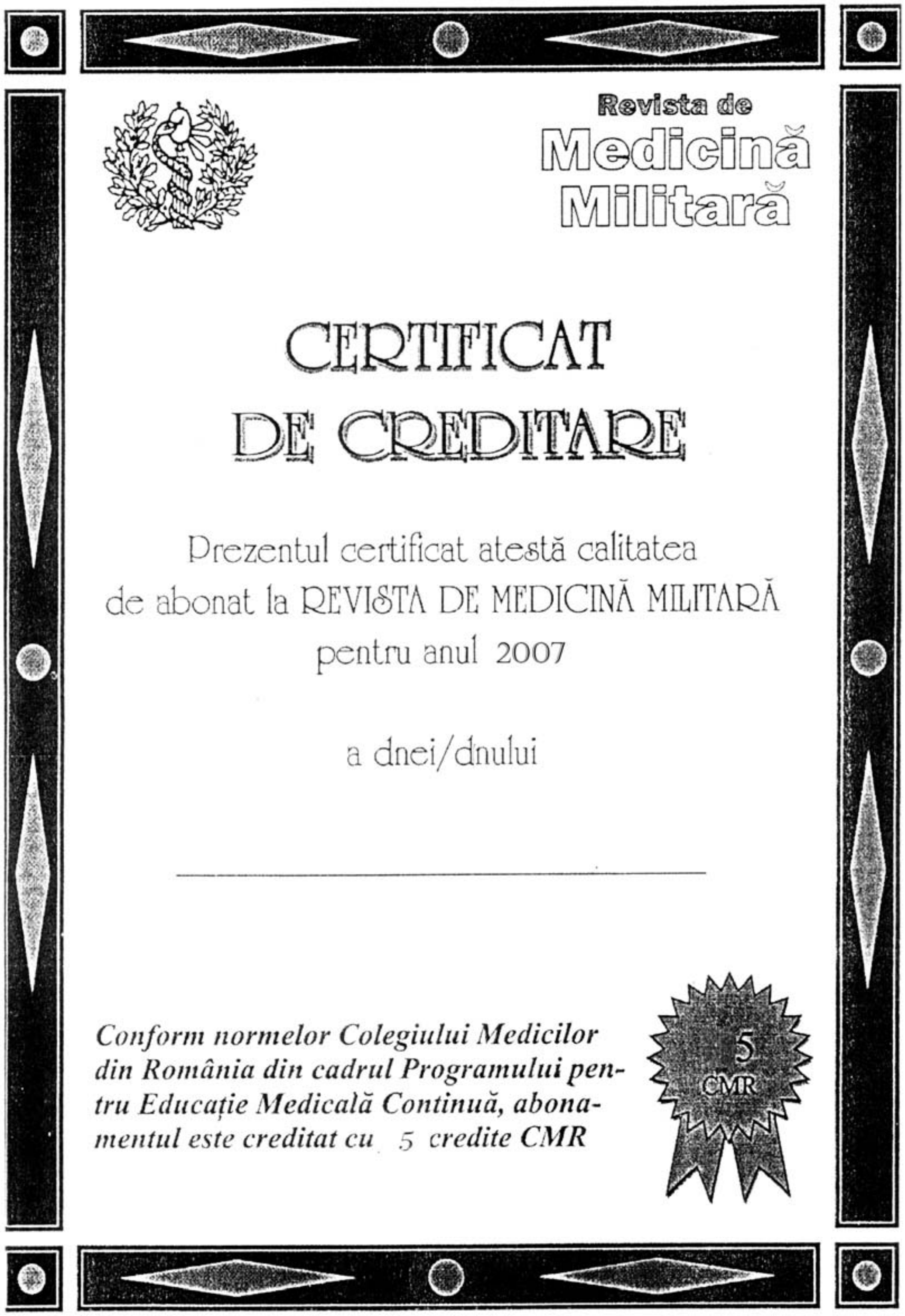

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Frecvența anodonțiilor parțiale la copiii din Oltenia

Dr. Anne-Marie RAUTEN*, dr. Petra ŞURLIN*,
mr (r) conf. univ. dr. Veronica MERCUŢ*,
dr. Catrinel GEORGESCU*, dr. Matei CUŢUI*

OBIECTIV: Determinarea frecvenței anodonțiilor la pacienții prezentând anomalii dentomaxilare, care au solicitat un tratament ortodontic.
MATERIAL ŞI METODĂ: Studiul a fost efectuat pe 1232 de pacienți, cu anomalii dentomaxilare, care s-au prezentat în Clinica de Ortodonție-Pedodonție a Facultății de Medicină Dentară a UMF din Craiova, între 2001 şi 2007. Au fost efectuate examene clinice şi radiologice.
REZULTATE: Din numărul total de pacienți studiați, 208 au prezentat unul sau mai mulți dinți lipsă. Din cele 208 cazuri de anodonții, în 152 de cazuri a fost afectată doar o grupă dentară, În 52 de cazuri au fost afectate cel puțin două grupe dentare diferite.
CONCLUZII: Incidența anodonției la lotul studiat a fost ridicată, iar forma cel mai frecvent întâlnită a fost anodonția bilaterală de premolari doi inferiori. Adresabilitatea către serviciul de ortodonție s-a făcut pentru anomaliile dentomaxilare asociate. În cazul anodonțiilor de premolari, diagnosticul a fost pus frecvent întâmplător cu ocazia examenului clinic şi radiologic efectuat la debutul tratamentului ortodontic.

anomalii dentomaxilare **anodonție parțială** **tratament ortodontic**

ABSTRACT
Objective: to determine the frequency of the anodonties in patients having dent maxillary anomalies, that solicited an orthodontic treatment.
Material and methods: the study was realized on 1232 patients that were present in Orthodontics-Paedodontics Clinic of Oral Medicine Faculty of Pharmaceutics and Medicine University in Craiova, between 2001 and 2007. The diagnostic was determined on clinical and radiological examinations.
Results: 208 of the cases presented one or more numerical deficiency of dents. In 152 cases was affected only a dental group, and in 52 cases – at least two different dental groups.
Conclusions: the incidence of anodontia was high, and the frequent was presented bilateral anodontia of inferior premolar two. The patients were solicited the orthodontics treatment for associate dent maxillary anomalies. In the anodonties of premolars, the diagnostic was determined accidentally on clinical and radiological examinations in start of the orthodontic treatment.
Key words: dent maxillary anomalies, partial anodontia, orthodontic treatment

INTRODUCERE

Absența unuia sau a mai multor dinți de pe arcada dentară, mergând până la lipsa totală a acestora, datorită neformării mugurilor dentari, este cunoscută în literatura de specialitate sub denumirea de anodonție.

De fapt terminologia utilizată pentru această anomalie dentară izolată este foarte variată, cei mai utilizați termeni fiind: agenezie, anodonție, aplazie, hipodonție, oligodonție, reducere numerică a dinților. După Stockton (1) termenii de hipodonție şi oligodonție desemnează de fapt două entități patologice diferite:

− hipodonția se referă la absența a mai puțin de 6 dinți de pe arcadă,
− oligodonția se referă la absența a mai mult de 6 dinți de pe arcadă.

Frecvența relativă a anodonțiilor, comparativ cu alte anomalii dentomaxilare diferă în literatura de specialitate fiind de: 3,5%-8% în funcție de rasă, dacă se exclud molarii trei, iar dacă se includ şi

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aceştia de 15%-25% după Dermaut (2) şi Gulzow (3). Arte şi colab. (4) găsesc o incidenţă de 5% a anodonţiei de incisivi laterali la populaţia europeană, iar Shapira (5) o proporţie de 77% la pacienţii cu despicături labio-maxilo-palatine. La noi în ţară Ionescu Ecaterina găseşte pentru anodonţii o incidenţă de 3,53% (6), iar Ţărmure Viorica de 8,03 % (7).

Prezenţa acestei anomalii are consecinţe negative asupra funcţiilor aparatului dento-maxilar, asupra fizionomiei, fonaţiei, masticaţiei şi determină tulburări de ocluzie (6), întârzieri în erupţia dinţilor învecinaţi (8), putând conduce astfel la complexe de inferioritate şi dificultăţi în încadrarea psihosocială a acestor pacienţi.

OBIECTIVE

Generale:

1. Determinarea frecvenţei anodonţiilor la pacienţii prezentând anomalii dentomaxilare, care au solicitat un tratament ortodontic.

Specifice:

1. Determinarea frecvenţei anodonţiei în funcţie de sex.
2. Determinarea frecvenţei anodonţiei în funcţie de maxilarul afectat.
3. Determinarea ordinului de frecvenţă în funcţie de tipul dintelui afectat.
4. Determinarea numărului total de dinţi absenţi/pacient.

MATERIAL ŞI METODĂ

Studiul a inclus toţi pacienţii prezentând anomalii dentomaxilare, care s-au prezentat în Clinica de Ortodonţie-Pedodonţie a Facultăţii de Medicină Dentară a UMF din Craiova în perioada 2001-2007, solicitând tratament de specialitate, adică un număr total de 1232 pacienţi. Fiecărui pacient i s-a întocmit o foaie de observaţie. Diagnosticul de anodonţie a fost pus pe baza datelor anamnestice culese de la pacient şi/sau aparţinători, pe baza examenului clinic, atrăgându-ne atenţia situaţiile în care lipseau de pe arcadă dinţi permanenţi după limita superioară de erupţie a acestora, concomitent cu existenţa unui proces alveolar pensat în regiunea respectivă sau cu existenţa unui dinte omonim nanic. Ulterior, diagnosticul a fost confirmat radiologic pe ortopantomograme.

REZULTATE ŞI DISCUŢII

Din numărul total de pacienţi studiaţi, 208 au prezentat unul sau mai mulţi dinţi lipsă, iar dintre aceştia 92 au avut doar anodonţie de molari trei, ceea ce conduce la o proporţie de 9,41% pacienţi cu anodonţie (excluzând molarii trei) şi respectiv 16,07% (incluzându-i şi pe aceştia). Incidenţa găsită de noi este asemănătoare cu cea raportată de Haavikko de 9,5% (9) şi de Bergstrom de 9,3% (10).

Distribuţia procentuală pe sexe este prezentată în tabelul de mai jos:

TABEL 1. Distribuţia pe sexe

SEX	FEMININ	MASCULIN
NR. DE PACIENŢI	116	92
FRECVENŢĂ	55,76%	44,24%

Mai afectate sunt fetele, ceea ce corespunde datelor din literatura de specialitate (10, 11, 12).

Distribuţia procentuală în funcţie de maxilarul afectat este prezentată în următor:

TABEL 2. Distribuţia în funcţie de maxilarul afectat

MAXILAR AFECTAT	MAXILAR SUPERIOR	MANDIBULĂ	AMBELE MAXILARE
NR DE PACIENŢI	69	77	62
FRECVENŢĂ	33,14%	37,01%	29,85%

Din cele 208 cazuri de anodonții, în 152 de cazuri a fost afectată doar o grupă dentară, procentele obținute fiind prezentate în următorul tabel:

TABEL 3. Frecvența în cazul afectării unei singure grupe dentare

DINTE ABSENT	NR.CAZURI	FRECVENȚĂ
IC inf.	4	2,63%
IL sup.	12	7,88%
PM 2 sup.	5	3,28%
PM 2 inf.	39	25,66%
M 3 sup.	52	34,21%
M3 inf.	40	26,34%

Dinții cei mai afectați au fost M3 superiori, urmați de M3 inferiori, PM2 inferiori, IL superiori, PM2 superiori şi IC superiori.

În 52 de cazuri au fost afectate cel puțin două grupe dentare diferite.

Din punct de vedere topografic anodonția de premolari doi inferiori a fost bilaterală în 69,24% dintre cazuri şi unilaterală în 30,76% dintre cazuri; cea de premolari doi superiori a fost bilaterală în 60% dintre cazuri şi unilaterală în 40%; anodonția de incisivi laterali a fost bilaterală în 58,33% dintre cazuri şi unilaterală în 41,66%.

Anodonțiile parțiale întinse găsite (puține numeric) au fost asimetrice.

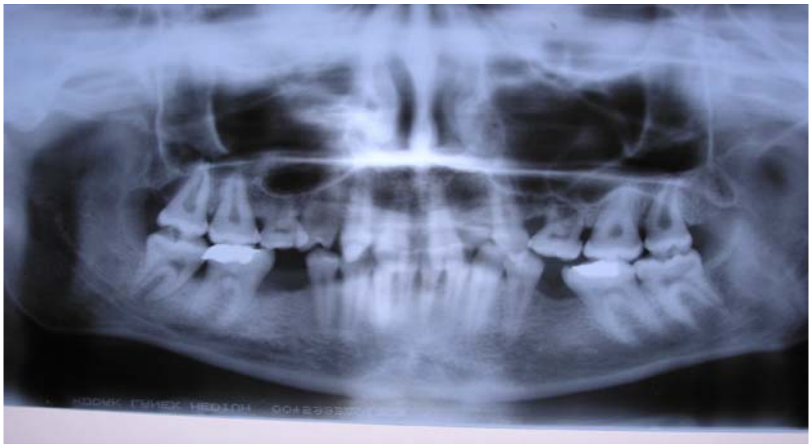

Fig. 1. Pacienta ML, 19ani, prezentând anodonție bilaterală de IL sup, PM1sup stg, bilaterală de PM2 sup, bilaterală de M1 sup, bilaterală de PM2 inf şi M1 inf

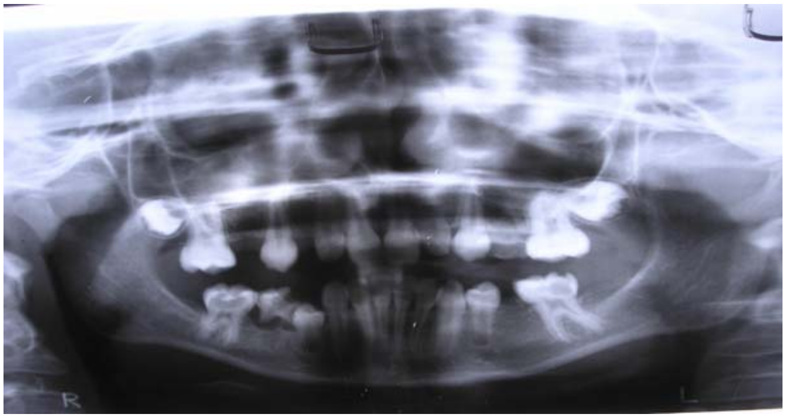

Fig. 2. Pacienta FA, 13 ani, prezentând anodonție bilaterală de M3 sup, a celor patru PMsup şi anodonții bilaterale inferioare de PM2, M2 şi M3.

Distribuţia procentuală în funcţie de numărul de dinţi afectaţi este prezentată în tabelul de mai jos:

TABEL 4. Distribuţia în funcţie de numarul de dinti afectaţi

NR. DINŢI ABSENŢI	NR.CAZURI	FRECVENŢĂ
1	61	29,32%
2	97	46,63%
3	16	7,69%
4	29	13,94%
5	2	0,88%
8	1	0,48%
11	1	0,48%
12	1	0,48%

Se constată că în majoritatea cazurilor sunt afectaţi unul sau doi dinţi.

CONCLUZII

1. Incidenţa anodonţiei la lotul studiat a fost ridicată, dacă nu luăm în calcul molarii trei, şi apropiată de limita inferioară a valorilor menţionate în literatura de specialitate dacă se includ şi aceştia.
2. Forma cel mai frecvent întâlnită, excluzând molarii trei, a fost anodonţia bilaterală de premolari doi inferiori.
3. Exceptând cazurile cu anodonţii parţiale întinse şi o parte dintre cele cu anodonţie de incisivi laterali superiori, adresabilitatea către serviciul de ortodonţie s-a făcut pentru anomaliile dentomaxilare asociate.
4. În cazul anodonţiilor de premolari, diagnosticul a fost pus frecvent întâmplător cu ocazia examenului clinic şi radiologic efectuat la debutul tratamentului ortodontic.

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HERNIE PARADUODENALĂ STÂNGĂ CU EVOLUȚIE PARTICULARĂ

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T.ROGIN , T.BÂTCĂ[**]

Hernia paraduodenală este o afecțiune rară, fiind citate în literatură circa 400 cazuri de la începutul secolului XX. Afecțiunea este dificil de diagnosticat , fiind descoperită întîmplător, cu ocazia unor complicații de natură ocluzivă. În etiopatogenia bolii este implicat procesul de malrotație intestinală, din perioada embrionară, cu apariția fosetelor paraduodenale patologice care generează herniile paraduodenale a căror evoluție poate fi grevată de apariția sindroamelor ocluzive. Este prezentat cazul unei paciente de 36 ani cu sindrom dureros trenant de etaj abdominal superior, etichetat inițial ca ulcer duodenal cronic acutizat, apoi ca pancreatită acută și în ultimă instanță ca un sindrom ocluziv înalt. Se intervine chirurgical confirmîndu-se diagnosticul de hernie paraduodenală stîngă strangulată, descoperindu-se anse intestinale subțiri cu modificări morfopatologice majore. S-a practicat degajarea anselor și desființarea spațiilor herniare prin burse etajate. Nu a fost necesară rezecția de intestin, ansa fiind la limita viabilității. Evoluția postoperatorie este dificilă, instalîndu-se un sindrom mixt de insuficiență hepatică cu coagulopatie și hemoragie digestivă difuză care au pus în pericol viața pacientei. Terapia intensivă complexă aplicată a dus în cele din urmă la o evoluție favorabilă. Raritatea afecțiunii, dificultățile de diagnostic precum și evoluția particulară, ne-au determinat să prezentăm acest caz.

hernie internă paraduodenală fosetă Landzert ocluzie

Paraduodenal hernia is a rare situation, less than 400 cases being published in literature. This condition is difficult to explore, diagnose appear within an occlusive syndrome. The ethiopathoplogy involves perturbation of intestinal rotation during the intrauterine life, producing paraduodenal fosses, which generates conditions for internal hernias and occlusive situations. We present 36 years women with a chronic epigastric pain, diagnosed as duodenal ulcer, later as acute pancreatitis and in the and as high occlusion syndrome. Open laparotomy is performed, discovering a strangulated left paraduodenal hernia, with severe morfopathological lesions of the intestinal loops. Kelotomy and purestring suture of parietal defect is performed. Postoperatory evolution has been difficult with a hepatic failure with coagulopathy and diffuse intestinal hemorrhage, threating the patient life. Intensive care leads eventually at a favorable condition. The rarity of this disease, difficulty to diagnose and the particular evolution are motives to present this case.

INTRODUCERE

Herniile interne au o incidență de 1-2% , ele fiind descoperite întîmplător cu ocazia unor laparotomii sau autopsii sau pot fi cauza unor complicații obstructive ale intestinului în 0,2-0,9% din cazurile de ocluzii intestinale.(1) Herniile paraduodenale reprezintă circa jumătate din herniile interne, entități clinice rare și dificil de diagnosticat.

Hernia paraduodenală are o etiologie congenitală, datorîndu-se unei erori în procesul de rotație al intestinului. Acesta are loc între săptămînile 5 și 10 ale vieții intrauterine datorită unei discordanțe între volumul anselor și capacitatea abdominală a embrionului, intestinele suferă o rotație antiorară de 270° precum și o fuzionare a peritoneului de fascia posterioară a trunchiului. În funcție de poziția duodenului și de orientarea deschiderii fosei paraduodenale se descriu henii paradudenale stîngi sau drepte.

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Cele stîngi sînt cele mai frecvente(75%) şi se presupune că se formează printr-un defect congenital al mezocolonului. Ansele intestinale se angajează într-un spaţiu denumit foseta Landzert, situat în stînga porţiunii a patra a duodenului, delimitată de ligamentul Treitz şi arcul vascular al lui Treitz, cu extensia sacului herniar retrogastric şi prepancreatic.(fig.1)

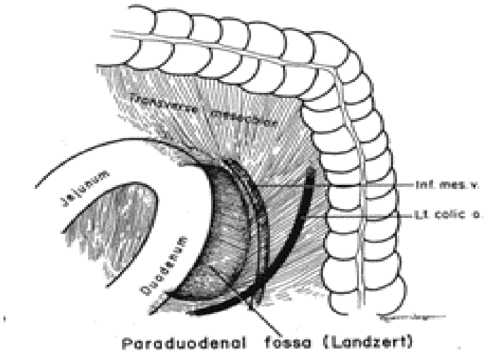

Fig.1 – localizarea orificiului herniar paraduodenal stîng

Herniile paraduodenale drepte se formează prin foseta Waldeyer, sacul herniar fiind delimitat anterior şi la stînga de vena colică dreaptă şi se complică în 50% din cazuri cu obstrucţia parţială sau totală a intestinului.(2)(3).

În literatura medicală s-au prezentat de la începutul sec.XX circa 400 de cazuri, din a căror analiză s-au evidenţiat următoarele concluzii : raportul M/F este de 3/1, vîrsta medie de diagnostic este de 38,5 ani, majoritatea cazurilor prezentîndu-se pentru suferinţă dispeptică cronică dar şi pentru complicaţii acute ce sugerau un sindrom ocluziv înalt.(4)(5)(6)(7)(8)(9)

Deoarece în cea mai mare parte din cazuri hernierea este intermitentă, diagnosticul este foarte dificil de stabilit, necesitând un grad înalt de susceptibilitate pentru coroborarea datelor clinice cu explorările imagistice. Examenul clinic nu evidenţiază niciun fel de modificări specifice, necesitând explorări radiologice(proba Pansdorf) şi tomografice pentru a surprinde ansa intestinală herniată.(10)(11)(12)

Situaţiile cu simptomatologie acută reprezintă de asemenea o încercare dignostică, uşurată de necesitatea efectuării laparotomiei care tranşează etiologia sindromului ocluziv, rezolvând totodată această patologie.(13)

PREZENTARE DE CAZ

Va prezentăm cazul unei paciente de 36 ani, fără antecedente personale patologice, care se internează pentru vărsături bilioalimentare, dureri abdominale colicative şi scădere ponderală de 15 kg. într-o lună. Simptomatologia a debutat în urmă cu 2 luni cu dureri predominant epigastrice, vărsături şi scaune diareice, iar CT-ul abdominal efectuat atunci a stabilit diagnosticul de pancreatită acută edematoasă. Sub tratament specific evoluţia a fost iniţial favorabilă, urmată de o perioadă de acalmie, pentru ca apoi, în urmă cu 7 zile, starea pacientei să se agraveze brusc, reapărând vărsăturile intense, bilioalimentare, precum şi febră 38°, scaune de aspect melenic.

La momentul internării în serviciul de boli interne cu diagnosticul de pancreatită acută recidivată şi sinuzită maxilară bilaterală, pacienta era intens deshidratată, oligurică, palidă, TA 95/50 mmHg. Examenul biologic a evidenţiat patologic Hb 10.2g/dl, leucocite 24100/µl (cu frecvente hematii în ţintă, anulocite, eritroitroblaşti 2%, mielocite 2%, metamielocite 5%, nesegmentate 7%, trombocitoză – 652000/µl), Na 126,4 mmol/L, K 2,5 mmol/L, timp de protrombină Quick 32,7 sec(martor 11,6sec), activitate protrombinică 31%, INR 2,82, fibrinogen 841 mg/dl. Celelalte valori biologice ,inclusiv amilazele erau în limite normale.

Examenul fizic evidențiază tegumente şi mucoase palide şi uscate, puls de 120b/min, slab bătut, abdomen suplu, fără distensie, uşor sensibil la palpare profundă în epigastru, fără semne de iritație peritoneale. Zgomote intestinale de tonalitate joasă, de frecvență şi intensitate redusă. Examenul genital nu evidențiază nimic patologic. Tuşeul rectal evidențiază hemoroizi externi în coroană, iar în ampula rectală materii fecale de aspect melenic coafate de sânge proaspăt. Examenul ORL confirmă diagnosticul de sinuzită maxilară bilaterală.

Se efectuează o EDS ce evidențiază mucoasa antrală intens infiltrată, edemațiată, congestivă, şi un reflux duodeno-gastro-esofagian foarte intens, ridicându-se suspiciunea unui proces infiltrativ antral. Se efectuează examenul radiografic toracopulmonar ce identifică prezența unui revărsat pleural bilateral în cantitate mică, iar abdominal numeroase nivele hidroaerice proiectate în hipogastru şi flancul drept.

Examinarea tomografică a abdomenului evidențiază anse de intestin subțire dilatate, pline de lichid, unele cu pereți groşi, stază la nivelul colonului ascendent cu nivel hidroaeric, fără mase tumorale, adenopatii, ascită sau pneumoperitoneu.(fig.2) (fig.3)

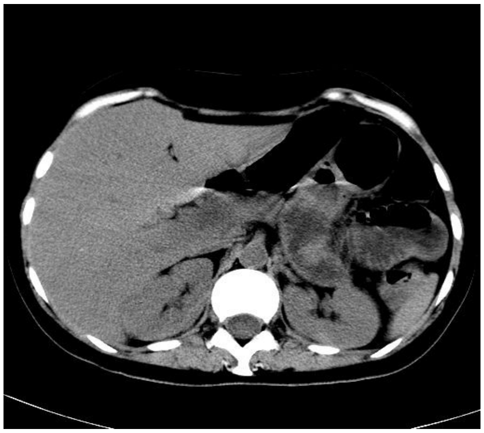

Fig.2 Evidențierea jejunului herniat retrogastric.

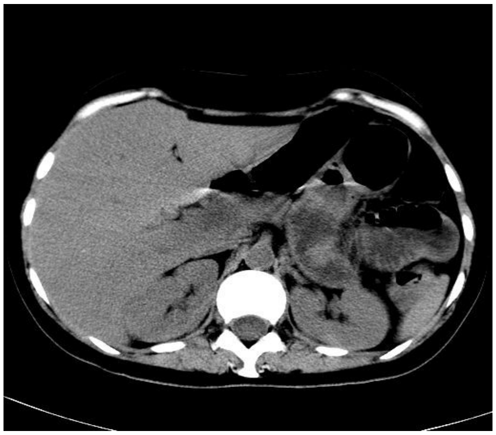

Fig.3 Aspect CT al anselor herniate retrogastric

Se instituie tratament intensiv de echilibrare hemodinamică şi electrolitică, se montează sondă urinară şi nazogastrică. După 24 ore starea pacientei se agravează, apar rectoragii cu sînge roşu, Hb ajunge la 5,6g/dl şi se solicită consultul chirurgical care, având în vedere toate datele, recomandă transferul în secția chirurgie şi intervenția chirurgicală în urgență.

Cu anestezie generală prin IOT, se efectuează o laparoscopie exploratorie care evidențiază anse enterale de aspect normal dar acoperite de false membrane, organe genitale interne şi apendice de aspect normal, etaj supramezocolic cu reacție inflamatorie acută intensă în regiunea pancreatică. Se decide conversia intervenției în laparotomie mediană xifo-ombilicală. Explorarea manuală a abdomenului evidențiază o breşă în mezocolonul transvers, în care se află încarcerată un segment jejunal de circa 40 cm., de la 10 cm. de unghiul dudenojejunal. După extragerea ansei herniate se evidențiază orificiul herniar situat la nivelul unei fosete paraduodenale Landzert, cu sac herniar ce ascensionează prepancreatic şi retrogastric până aproape de fornixul gastric, precum şi o altă fosetă, de mici dimensiuni, nelocuită, situată inferior de genunchiul duodenal.(fig.4)

Ansa enterală herniată este cu pereți groşi, edemațiați, congestivi , cu echimoze seromusculare, dar fără leziuni parcelare ischemice şi cu peristaltică prezentă.(fig.5)

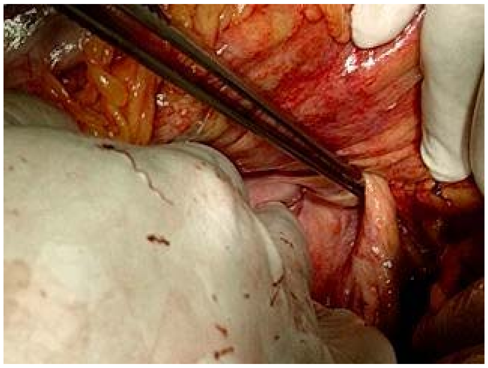

Fig.4 – Crificiul herniar paraduodenal stâng delimitat medial de duoden şi lateral de artera colică stângă.

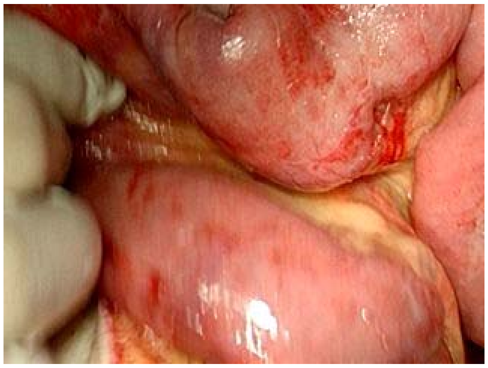

Fig.5 – Aspectul macroecopic al jejunului herniat.

Având în vedere starea biologică precară a pacientei, proximitatea unghiului duodenojejunal şi aspectul mai degrabă viabil al ansei herniate, se consideră că o rezecție ar avea un risc foarte mare de fistulizare, motiv pentru care intervenția se limitează la închiderea în burse etajate a celor două orificii herniare, lavajul cavității abdominale şi închiderea abdomenului.(fig.6) (fig.7)

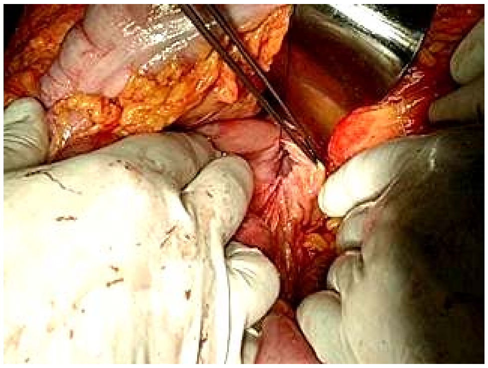

Fig.6 – Închiderea în bursă a celor două orificii herniare.

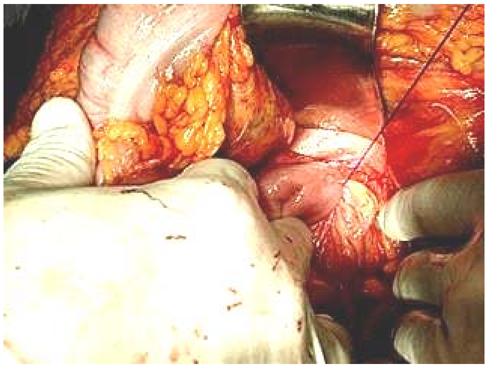

Fig.7 – Aspectul final al desființării orificiilor herniare.

Postoperator evoluția a fost lent favorabilă în primele 7 zile, cu îmbunătățirea semnificativă a stării generale, reluarea tranzitului pentru materii fecale normale, toleranță digestivă bună, corectarea valorilor biologice. Din ziua a 10a postoperator pacienta prezintă din nou câte 2-3 scaune melenice pe zi, survine o eviscerație blocată subcutanat de-a lungul întregii plăgi, probele hepatice se alterează treptat cu creşterea transaminazelor, a bilirubinei şi prăbuşirea coagulogramei. Hemogramele repetate indică o scădere a Hb până la 5,3g/dl, cu 20000leuc./µl şi trombocitopenie de 47000/µl. Se instituie tratament intensiv de reechilibrare, cu transfuzii repetate, plasmă proaspătă, antibioticoterapie cu spectru larg. Se efectuează o tomografie care evidențiază stomac cu conținut hidroaeric, anse intestinale cu pereți îngroşați, ficat cu consistență mult diminuată, aspect de eviscerație blocată, fără alte aspecte patologice intraabdominale.(fig.8).

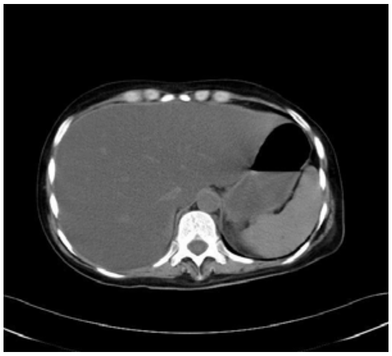

Fig.8 – Aspectul CT postoperaror cu hepatomegalie importantă.

Deoarece tratamentul intensiv aplicat pare ineficient, cu continuarea sîngerării digestive şi menținerea valorilor de Hb în jur de 5g/dl numai prin transfuzii zilnice de 1-2 unități de sânge, se considera această insuficiență hepatică cu punct de plecare un potențial focar septic intraperitoneal şi se hotărăşte o nouă intervenție chirurgicală, la 14 zile distanță de prima. Intraabdominal nu se găseşte nici un focar septic, ansa herniată inițial este de aspect aproape normal, cu pereți puțin îngroşați şi peristaltică bună, dar ficatul era mărit de volum şi cu consistență foarte scăzută, cu edem subcapsular. Se efectuează lavaj abundent şi cura eviscerației prin închiderea abdomenului intr-un strat cu fire de oțel.

Postoperator rezultatul hemoculturii sugerează o posibilă septicemie cu fungi pentru care se administrează tratament antifungic intravenos, concomitent cu terapia intensivă de susținere hepatică, alături de nutriția parenterală şi peros, considerându-se ca lanț etiopatogenic al insuficienței hepatice rezorbția masivă de compuşi toxici prin mucoasa segmentului intestinal afectat, prăbuşirea coagulării şi apariția sângerărilor difuze din întreg tractul digestiv.

Treptat prin continuarea tratamentului intensiv, starea pacientei se îmbunătățeşte, scaunele melenice dispar, coagulograma şi hemograma se normalizează, se reia toleranța digestivă, iar pacienta părăseşte spitalul la 30 zile de la internare, vindecată chirurgical.

DISCUȚII

Herniile paraduodenale sînt entități clinice rare, benigne, dificil de diagnosticat datorită unei simptomatologii nespecifice, prelungite, dar cu potențial evolutiv deosebit de grav.

Pe plan mondial s-au descris circa 400 de cazuri de hernii paraduodenale, majoritatea fiind identificate la bărbați, vîrsta medie fiind de 38,5 ani.

Clasificare

Ghahremani(14)a clasificat herniile interne în :

- hernii paraduodenale(50-55%)
- hernii prin hiatusul Winslow(6-10%)
- hernii transmezenterice(8-10%)
- hernii pericecale(10-15%)
- hernii intersigmoidiene(4-8%)
- hernii paravezicale(sub 4%)

La acestea se mai adaugă herniile perineale şi obturatorii.(fig.9)

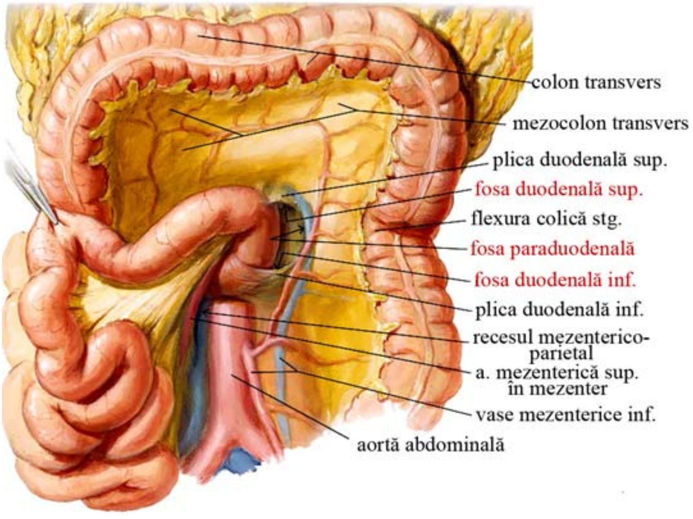
colon transvers
mezocolon transvers
plica duodenală sup.
fosa duodenală sup.
flexura colică stg.
fosa paraduodenală
fosa duodenală inf.
plica duodenală inf.
recesul mezenterico-parietal
a. mezenterică sup. în mezenter
vase mezenterice inf.
aortă abdominală

Fig.9 – Localizarea herniilor interne.

Orientarea asupra diagnosticului poate fi sugerată de istoricul simptomatologiei dureroase intermitent a etajului abdominal superior, în lipsa constatării unei patologii biliare sau gastroduodenale şi confirmată de proba Pansdorf sau o tomografie abdominală cu dublu contrast, care ar putea surprinde ansa herniată prin foseta paraduodenală, elementul fundamental reprezentându-l suspiciunea medicului asupra existenței acestei patologii.

În cazurile cu evoluție cronică, se poate lua în considerare tratamentul laparoscopic, atât timp cât intestinele nu sînt foarte destinse sau nu prezintă elemente de încarcerare şi se poate extrage ansa din sacul de hernie. Închiderea în bursă a orificiului herniar necesită o atenție sporită doar față de artera colică stângă.

Situațiile acute sau cele cu diagnostic incert, impun laparotomia de urgență care stabileşte diagnosticul şi tratează afecțiunea. Totodată este necesar explorarea amănunțită a întregului abdomen pentru a exclude prezența şi a altor orificii herniare.

Cazul prezentat a surprins evoluția foarte severă a acestei patologii, prin asocierea unei insuficiențe hepatice determinată de creşterea segmentară a permeabilității mucoasei jejunale şi rezorbția masivă de compuşi toxici, ceea ce a fost aproape de a produce decesul pacientei.

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Obezitatea factor de risc pentru litiaza biliară

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cpt. dr. Vasile MILOIU*

OBIECTIV: Prezentarea unui studiu care aduce date privind prevalența litiazei biliare şi rolul obezității ca factor de risc în apariția acestei boli, prin efectuarea unui triaj clinic şi ecografic asupra unui grup populațional semnificativ numeric (2348 de subiecți).
MATERIAL ŞI METODĂ: Lotul inclus in studiu a fost supus unui screening ecografic pentru depistarea pacienților litiazici. De asemenea, subiecților luați în studiu li s-au efectuat măsurători antropometrice, s-a determinat înălțimea, greutatea, indicele de masă corporală. Indicele de masă corporală a fost calculat după formula IMC= greutate (kg)/ înălțime(m)^2. S-a determinat frecvența obezității la subiecții cu litiază biliară comparativ cu cei fără litiază biliară considerându-se obeze persoanele cu IMC peste 30. Studiul a inclus persoane cu vârste cuprinse între 18 şi 90 de ani, pacienți internați, precum şi persoane care efectuau controale medicale periodice.
REZULTATE ŞI CONCLUZII: Datele obținute evidențiază prevalența crescută a obezității la subiecții litiazici comparativ cu cei nelitiazici (40,15%-19,65%). Detaliind pe sexe am obținut următoarele rezultate: la sexul feminin obezitatea este prezentă la 32,16% dintre pacientele cu litiază față de 19,48% la nelitiazice, în timp ce la sexul masculin 53,93% dintre litiazici sunt obezi față de 19,84% dintre nelitiazici. Rezultatele obținute arată că în zona studiată obezitatea este un factor litogen major la ambele sexe, cu un plus pentru sexul masculin la care peste 50% dintre litiazici au fost obezi. Obezitatea fiind un factor de risc influențabil, pentru prevenirea apariției calculilor biliari se impun o serie de măsuri de profilaxie primară dintre care un rol însemnat îl au igiena de viață şi cea alimentară cu combaterea obezității şi hiperlipidemiei.

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litiază biliară **obezitate** **factor de risc**

ABSTRACT
Objective: to present the relation between obesity and biliary lithiasis by clinical and echographic triage on 2348 patients.
Material and method: was achieved an echographic screening to discover the patients having lithiasis. Was determined the frequency of obesity in patients having biliary lithiasis versus group with no lithiasis. The age of the patients hospitalized and of the personnel which effected periodical medical control ranged from 18 to 90 years.
Results and conclusions: the results suggest the prevalence of obesity in subjects with lithiasis was higher than in patients without lithiasis (40,15%-19,65%). In women the obesity is present in 32,16% of the patients with lithiasis and in 19,48% of the subjects without lithiasis. In men the obesity is present in 53,93% of the patients with lithiasis and in 19,84% of the subjects without lithiasis. The obesity is an important lithogen factor both the men and the women, but in men over 50% of the patients having lithiasis were obese. The obesity is an important risk factor for development of biliary lithiasis. The primary prophylaxis – lifestyle and alimentary hygiene in correlation with the control of the obesity and the hyperlipidemy - prevents the development of biliary lithiasis
Key words: biliary lithiasis, obesity, risk factor

IPOTEZĂ DE LUCRU

Litiaza biliară a devenit o importantă problemă socioeconomică datorită prevalenței sale în continuă creştere. Interrelația dintre obezitate şi litiază a fost amplu dezbătută în literatura de specialitate. S-au efectuat numeroase studii epidemiologice, care au confirmat frecvența mai mare a calculilor biliari la obezi (1,2,3,4). Mecanismele sunt complexe. Masa adipocitară crescută duce la o creştere a sintezei de colesterol (5) şi la o suprasaturare a bilei în colesterol (6,7). Concentrația molară a colesterolului biliar *a jeun* la femeile obeze fără calculi este de 10,8% față de 6,8% la femeile normoponderale (8). Activitatea HMGCoA-reductazei creşte în obezitate în contextul unui aport alimentar şi a unei insulinemii crescute (9,10). Cu toate progresele efectuate, mecanismul complet al discoliei litogene în obezitate nu este elucidat.

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MATERIAL

În studiu a fost inclus un lot populațional de 2348 de subiecți cu vârste cuprinse între 18 şi 90 de ani, care s-au adresat laboratorului de ultrasonografie pentru examen ecografic abdominal. Studiul s-a derulat în perioada 2006-2007.

Din lotul global de 2348 de subiecți, 1236 au fost de sex feminin, iar 1112 de sex masculin. Lotul a cuprins persoane din toate mediile sociale, atât din mediul urban, cât şi rural. Au fost incluşi în studiu studenți, salariați din toate domeniile de activitate şi pensionari. Grupul studiat a inclus şi un număr de 75 de subiecți colecistectomizați pentru litiază biliară

METODĂ

Subiecților luați în studiu li s-au efectuat măsurători antropometrice, s-a determinat înălțimea, greutatea, indicele de masă corporală. Indicele de masă corporală a fost calculat după formula IMC = greutate (kg)/ înălțime (m)^2. S-a determinat frecvența obezității la subiecții cu litiază biliară comparativ cu cei fără litiază biliară considerându-se obeze persoanele cu IMC peste 30.

REZULTATE

Frecvența obezității la subiecții litiazici comparativ cu cei nelitiazici.

1. Date generale

Incidența obezității este prezentată mai jos comparativ la lititazici, respectiv nelitiazici.

TABEL 1. Incidența litiazei biliare în loturile studiate

Lot	Număr	Obezi	Non obezi
L.B.	232	94 (40,51%)	138 (59,49%)
Fără L.B.	2116	416 (19,65%)	1700 (80,35%)

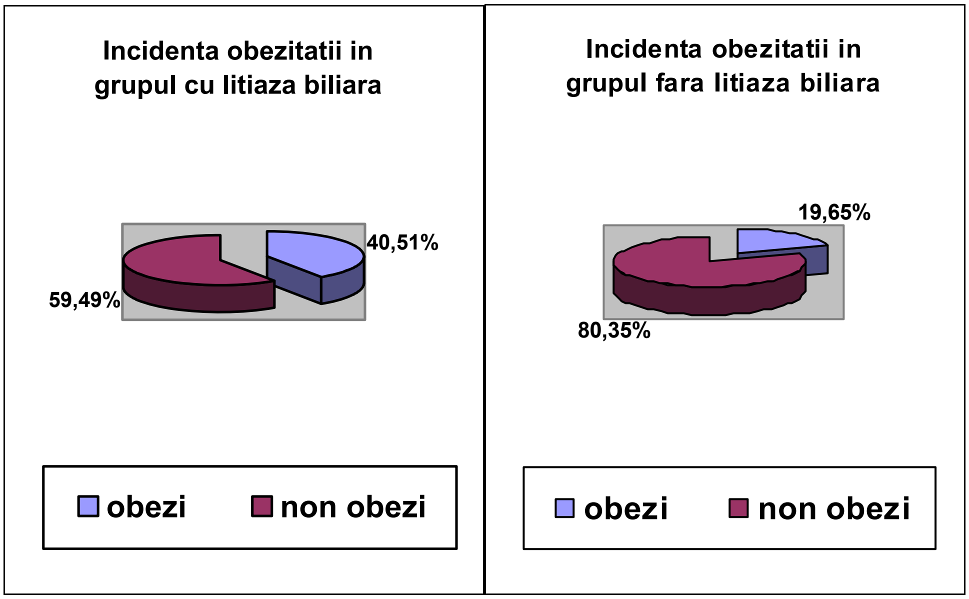

Incidenta obezitatii in grupul cu litiaza biliara

Incidenta obezitatii in grupul fara litiaza biliara

Figura 1

Datele obținute indică un procent aproximativ dublu de obezi în grupul litiazic comparativ cu cel nonlitiazic.

2. Date comparative pe sexe

Datele obținute la sexul feminin sunt redate în tabelul 2 şi figura 2.

TABEL 2. Prevalența obezității la femeile litiazice, respectiv nelitiazice

Lot	Număr	Obeze	Nonobeze
L.B.	143	46 (32,16%)	97 (67,84%)
Fără L.B.	1093	213 (19,48%)	880 (80,52%)

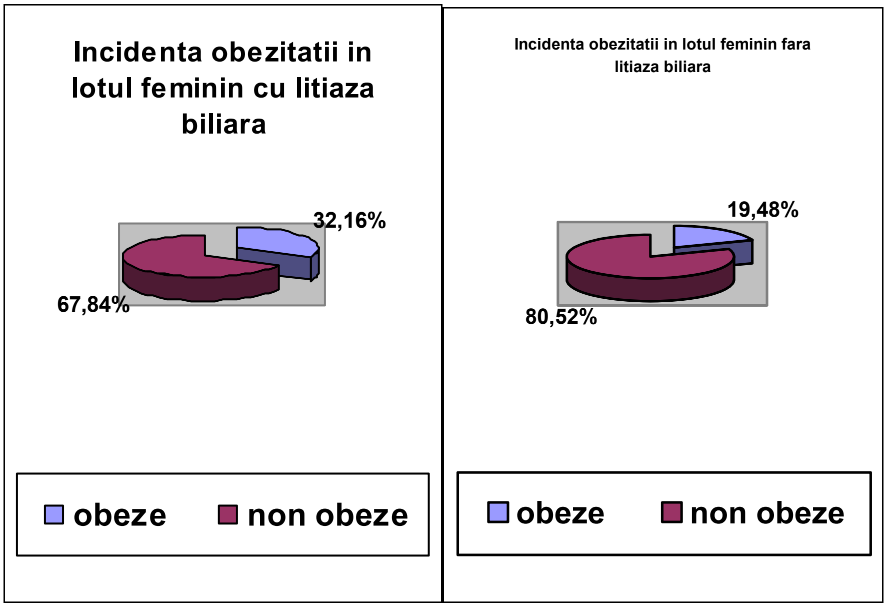

Figura 2

Putem observa la sexul feminin o diferență înalt semnificativă statistic în ceea ce priveşte prezența obezității între grupul litiazic şi cel nelitiazic.

Datele obținute la sexul masculin sunt redate în tabelul 3 şi figura 3.

TABEL 3. Prevalența obezității la bărbații litiazici, respectiv nelitiazici

Lot	Număr	Obezi	Non obezi
L.B.	89	48 (53,93%)	41 (46,07%)
Fără L.B.	1023	203 (19,84%)	820 (80,16%)

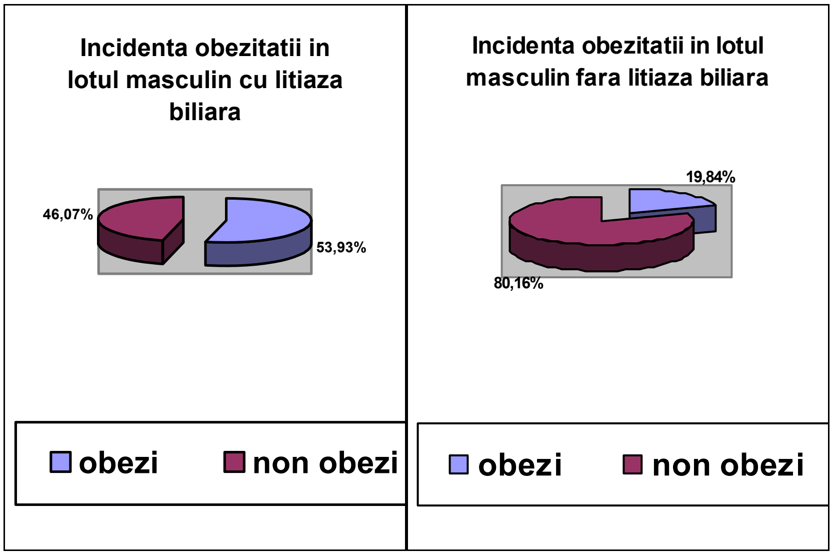

Figura 3

Se poate obseva la sexul masculin că mai mult de jumătate din litiazici prezentau obezitate (53,93%). Comparativ, la subiecții nelitiazici obezitatea era prezentă doar la 19,84%.

3. Date obținute pe grupele de vârstă

Datele obținute pe fiecare decadă de vârstă la lotul global sunt reprezentate în tabelul 4. Se poate observa pe fiecare grup de vârstă o frecvență mai mare a litiazei biliare la subiecții obezi comparativ cu cei neobezi.

TABEL 4. Frecvența obezității pe decade de vârstă la întreg lotul

Vârsta	Număr	L.B		Fără L.B	
		obezi	non obezi	obezi	non obezi
Sub 20 ani	67	1	0	5 (7,57%)	61
21-30 ani	215	2 (22,22%)	7	21(10,19%)	185
31-40 ani	392	6 (28,57%)	15	45(12,12%)	326
41-50 ani	416	21(41,17%)	30	71(19,45%)	294
51-60 ani	522	31(42,46%)	42	111(24,72%)	338
61-70 ani	495	22(43,13%)	29	116(26,12%)	328
71-80 ani	197	9 (45%)	11	39(22,03%)	138
81-90 ani	44	2 (33,33%)	4	8 (21,05%)	30

TABEL 5. Frecvența obezității pe decade de vârstă la femei

Vârsta	Număr	L.B.		Fără L.B.	
		obeze	non obeze	obeze	non obeze
Sub 20 ani	23	1	0	3(14,28%)	18
21-30 ani	68	1(16,66%)	5	12(19,35%)	50
31-40 ani	185	2(13,33%)	13	19 (11,17%)	151
41-50 ani	293	10(29,41%)	24	43(16,60%)	216
51-60 ani	310	16(37,20%)	27	63(23,59%)	204
61-70 ani	252	11(36,66%)	19	53(23,87%)	169
71-80 ani	82	4 (36,36%)	7	17(23,94%)	54
81-90 ani	23	1 (33,33%)	2	3 (15%)	17

La femeile litiazice observăm o frecvenţă crescută a obezităţii comparativ cu cele nelitiazice în decadele 5-9 de viaţă.

TABEL 6. Frecvenţa obezităţii pe decade de vârstă la bărbaţi

Vârsta	Număr	L.B.		Fără L.B.	
		obezi	non obezi	obezi	non obezi
Sub 20 ani	44	0	0	3(6,81%)	41
21-30 ani	147	2(66,66%)	1	26(18,05%)	118
31-40 ani	207	3(50%)	3	38 (18,90%)	163
41-50 ani	123	9(52,94%)	8	32(30,18%)	74
51-60 ani	212	16(53,33%)	14	47(25,82%)	135
61-70 ani	243	12(57,14%)	9	36(16,21%)	186
71-80 ani	115	5 (55,55%)	4	17(15,88%)	90
81-90 ani	21	1(33,33%)	2	4 (22,22%)	14

La bărbaţii litiazici, începând cu a treia decadă de viaţă frecvenţa obezităţii este semnificativ crescută comparativ cu lotul nelitiazic

DISCUŢII ŞI CONCLUZII

Numeroase studii situează obezitatea la loc de frunte ca factor de risc pentru apariţia litiazei biliare (11,12,13,14,15,16,17,18,19). Riscul litogen legat de obezitate variază în funcţie de vârstă, sex şi importanţa excesului ponderal. Un studiu efectuat la Cluj-Napoca pe 30 de pacienţi litiazici confirmă existenţa unei corelaţii pozitive între concentraţia colesterolului biliar şi greutatea corporală relativă (4).

Obezitatea duce la o secreţie şi excreţie crescută de colesterol, ce are drept consecinţă suprasaturarea bilei în colesterol (6,7). Aceasta, la rândul ei, crează condiţii favorabile pentru dezvoltarea calculilor de colesterol. Conform studiilor efectuate de Angel şi Herbst greutatea litiazicilor depăşeşte în medie cu 20% cifra ideală şi invers peste 30% dintre marii obezi au calculi. Într-un studiu efectuat la Clinica Medicală III a Spitalului de Gastroenterologie din Cluj-Napoca, 53,3% dintre pacienţii litiazici au fost obezi (11). Un alt studiu efectuat pe un lot feminin având în medie un surplus ponderal de 60 de kg a depistat prezenţa litiazei la 40% (20). Semnificativ s-a dovedit la femei excesul ponderal reprezentat la nivelul abdomenului (15).

Rezultatele obţinute în studiul nostru confirmă datele din literatură, care consideră obezitatea un important factor litogen. Am remarcat la lotul sudiat o incidenţă semnificativ mai mare a obezităţii la litiazici comparativ cu pacienţii nelitiazici (40,15%-19,65%). Detaliind pe sexe se observă aceeaşi corelaţie pozitivă între obezitate şi litiaza biliară. Astfel, la sexul feminin obezitatea este prezentă la 32,16% dintre pacientele litiazice faţă de 19,48% la nelitiazice, în timp ce la sexul masculin 53,93% dintre litiazici sunt obezi faţă de 19,84% dintre nelitiazici. Urmărind frecvenţa obezităţii la litiazici pe grupe de vârstă, observăm că aceasta creşte progresiv din decada a doua până în decada a opta. Acest fapt relevă ponderea dominantă a factorilor exogeni în litogeneză la vârstnici. Practic, cele două patologii se suprapun tot mai frecvent odată cu înaintarea în vârstă.

La lotul de femei litiazice frecvenţa maximă a obezităţii se remarcă în decadele 5-8 de viaţă. La sexul masculin frecvenţa obezităţii la litiazici este ridicată începând cu a doua decadă de viaţă şi se menţine până într-a opta.

Pe toate grupele de vârstă şi la ambele sexe, obezitatea este mai frecventă în grupul litiazic comparativ cu cel nelitiazic.

Rezultatele obținute ne duc la concluzia că în zona studiată obezitatea este un factor litogen major la ambele sexe, cu un plus pentru sexul masculin la care peste 50% dintre litiazici au fost obezi. Nu trebuie uitat faptul că obezitatea este un factor de risc influențabil, astfel pentru prevenirea apariției calculilor biliari se impun o serie de măsuri de profilaxie primară dintre care un rol însemnat îl au igiena de viață şi cea alimentară cu combaterea obezității şi hiperlipidemiei.

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Evaluare comparativă a tehnicilor de diagnostic în litiaza căii biliare principale

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Litiaza căii biliare principale (CBP) este o patologie cu care orice chirurg digestiv se confruntă. În literatura de specialitate s-au propus, de-a lungul timpului, mai multe soluţii terapeutice, dar şi instrumente matematice de tip scoruri, care ar permite selectarea pacienţilor cu risc de litiază hepatocoledociană, limitând necesitatea unor tehnici invazive. Studiul nostru are rolul de a determina, în mod retrospectiv, sensibilitatea şi specificitatea diferitelor tehnici diagnostice în scopul de a alege o baterie de teste care să prevadă, la un grad cât mai înalt, preoperator, riscul de litiază CBP.

Studiul a vizat un număr de 1074 pacienţi supuşi colecistectomiei la care s-a evaluat eficienţa prognostică a simptomatologiei, precum şi a unor explorări biologice şi imagistice pentru diagnosticarea litiazei CBP. Prelucrarea statistică a constatat că durerea în hipocondrul drept asociată icterului ($p<0,05$) ori descoperirea icterului la pacienţii internaţi în urgenţă ($p<0,01$) sunt singurele asocieri clinice cu valoare diagnostică. Bilirubina serică cu valori duble faţă de normal are valoare predictivă importantă ($p<0,005$) la pacienţii cu litiază a căii biliare principale, iar evidenţierea preoperatorie a litiazei coledociene (ultrasonografic ori computer tomografic) este un puternic factor predictiv pentru diagnosticul de litiază CBP ($p<0,001$). Constatarea dilatării hepato-coledocului, ecografic ori CT, nu a oferit rezultate semnificative în studiul nostru.

Concuzionând, afirmăm că litiaza căii biliare principale, fie că se dezvoltă *de novo* sau evoluează ca o complicaţie a litiazei veziculare, este o afecţiune a cărei simptomatologie a ieşit din standardele clasice, diagnosticul său necesitând obligatoriu o explorare imagistică preoperatorie sau intraoperatorie performantă.

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litiaza căii biliere principale (CBP) diagnostic

ABSTRACT

Common bile duct lithiasis is a frequent disease in a general surgical department. There were many diagnostic and therapeutic solutions proposed over the time in the literature. The aim of our study is to determine, by retrospective mode, sensibility and specificity of a few diagnostic features in order to predict the risk of common bile duct lithiasis.

We included in our study 1074 patients in which we achieved cholecystectomy and we evaluated the predicting efficiency of a symptomatology and a few biological and imagistical examens. Our results showed that abdominal right-upper quadran pain associated with jaundice ($p<0,05$) or jaundice at a patient in emmergency condition ($p<0,01$) has diagnosis role. Serum bilirubine with levels duble over the normal ($p<0,005$) and detecting (ultrasonographically or by CT scan) a lithiais inside of a CBD ($p<0,001$) represent other predictors of the disease.

Common bile duct lithiasis, even if is developing *de novo* or as a complication of vesicular lithiasis is a disease with a different symptoms compared with classical needing, for a correct diagnosys, a performant imagistic examination.

Key words: common bile duct stones, diagnosis

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INTRODUCERE

Litiaza căii biliare principale este o patologie cu care orice chirurg digestiv se confruntă. Chiar dacă metodele diagnostice, după cum şi soluţiile terapeutice, au evoluat spectaculos în acest domeniu, se constată, totuşi, o serie de dificultăţi în managementul hepatocoledocului litiazic.

Circa 10-20% dintre pacienţii prezentând litiază veziculară simptomatică au şi litiază de cale biliară principală (1). Simptomatologia acesteia din urmă este, adesea, greu de diferenţiat de cea a litiazei veziculare. Pe de altă parte implicaţiile clinice ale litiazei reziduale, deşi disputabile, pot fi serioase, detectarea litiazei căii biliare principale înainte de practicarea colecistectomiei laparoscopice fiind extrem de importantă (2,3).

Există mai multe strategii care propun managementul pacienţilor cu risc ridicat de litiază coledociană şi care urmează să fie supuşi colecistectomiei laparoscopice, prin abord primar endoscopic (colangiopancreatografie endoscopică retrogradă asociată ori nu sfincterotomiei endoscopice), pur laparoscopic, ori prin asocierea celor două căi fie în aceeaşi şedinţă operatorie, fie separat. Propunerea şi folosirea unor instrumente matematice de tip scoruri permite selectarea pacienţilor cu risc de litiază a căii biliare principale, limitând, totodată, efectuarea unor tehnici invazive (cum este ERCP) (4).

Studiul nostru are rolul de a determina, în mod retrospectiv, sensibilitatea şi specificitatea diferitelor tehnici diagnostice în scopul de a alege o baterie de teste care să prevadă, la un grad cât mai înalt, preoperator, riscul de litiază CBP.

MATERIAL ŞI METODĂ

Studiul s-a desfăşurat în cadrul Clinicii de Chirurgie a Spitalului Clinic de Urgenţă Militar „Dr. Ştefan Odobleja” din Craiova şi a parcurs două stadii. Primul stadiu a fost reprezentat de o cercetare retrospectivă, care a inclus pacienţi supuşi colecistectomiei laparoscopice în intervalul ianuarie 2002-decembrie 2006. În cadrul acestei etape s-a determinat sensibilitatea şi specificitatea mai multor date clinico-paraclinice în depistarea litiazei coledociene:

- vârsta;
- semne clinice şi simptome: durere tipică în hipocondrul drept, febră, frison, icter sclero-tegumentar, alterarea stării generale, greţuri, vărsături;
- date biologice: bilirubina serică, GOT, GPT, gamma-glutamiltranspeptidaza, fosfataza alcalină, albuminemia;
- date ecografice: diametrul căii biliare principale ori vizualizarea ecografică a calculilor;
- date computer-tomografice: evidenţierea calculilor, diametrul căii biliare principale, altă patologie asociată;
- colangiografia intraoperatorie.

Al doilea stadiu al studiului nostru s-a desfăşurat de-a lungul întregului an 2007 când, într-un mod prospectiv, au fost evaluate rezultatele obţinute în prima fază a cercetării, respectiv bateria de date cu eficienţă diagnostică predictivă pentru litiaza hepatocoledociană.

Au fost incluşi, în prima fază a studiului nostru, 1074 pacienţi, dintre care 812 au fost femei şi 262 bărbaţi, cu o vârstă medie de 57,1 ani (limite între 15 şi 92 de ani). Am exclus din studiu pacienţii care au prezentat sfincterotomie endoscopică anterioară, hepatită acută sau cronică, pancreatită cronică, boli inflamatorii intestinale ori antecedente de alcoolism. De asemenea, au fost excluşi din studiu pacienţi, care au prezentat neoplazii ce reduc speranţa de viaţă la mai puţin de 2 ani şi, de asemenea, sarcina în evoluţie.

Parametrii amintiţi mai sus au fost înregistraţi prin cercetarea foilor de observaţie la toţi pacienţii incluşi în studiu, fiind excluşi, de asemenea, acei pacienţi la care aceştia nu s-au putut obţine ori au fost neconcludenţi. Determinarea diametrului hepatocoledocului a fost efectuată de doi specialişti în ecografie, cu experienţă, considerându-se pozitivă o valoare mai mare sau egală cu 8 mm.

Raportarea rezultatelor s-a făcut faţă de constatările intraoperatorii şi relativ la colangiografia intraoperatorie ori la ERCP postoperatorie efectuate (Fig. 1 şi 2). Datele obţinute sunt prezentate în continuare.

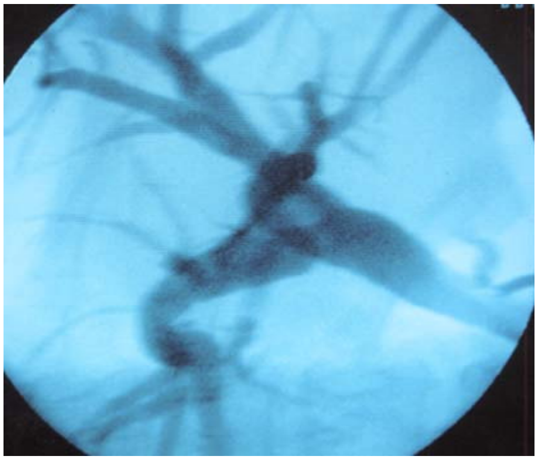

Fig. 1. Litiază coledociană – aspect colangiografie intraoperatorie (Colecția dr.Ianoşi)

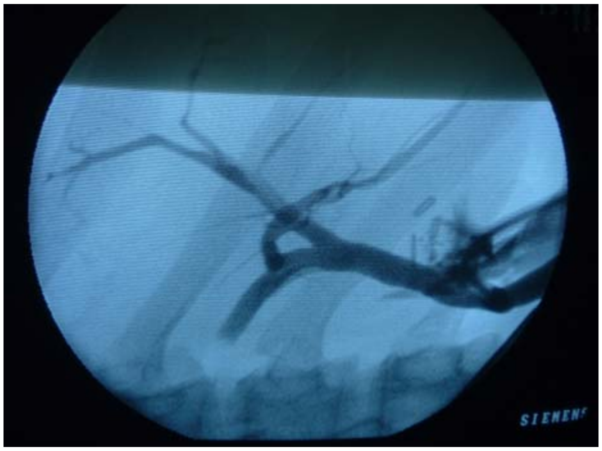

Fig. 2. Litiază coledociană – aspect colangiografie intraoperatorie (Colecția dr.Ianoşi)

Analiza statistică a loturilor de pacienți s-a făcut prin teste ale statisticii descriptive: diferențele dintre loturi au fost calculate folosind testul t, după folosirea inițială a testelor de normalitate Kolmogorov-Smirnov şi Shapiro-Wilk W, egalitatea variantelor fiind verificată prin testul F. În cazul testului t am folosit şi alternativa sa neparametrică, testul Mann-Whitney U, iar pentru a evalua diferențele dintre loturi multiple de pacienți am folosit testul ANOVA. Pentru stabilirea corelațiilor dintre variabile a fost utilizat calculul coeficientului de corelație.

REZULTATE

Din cei 1074 de pacienți supuşi colecistectomiei în perioada luată în studiu, aşa cum aminteam mai sus, au fost excluşi pacienții care au prezentat o sfincterotomie endoscopică anterioară, hepatită acută sau cronică, pancreatită cronică, boli inflamatorii intestinale ori antecedente de alcoolism, toate aceste situații putând altera concluziile, în special în ceea ce priveşte datele clinice culese. A rezultat un număr de 789 de pacienți, dintre care 612 femei şi 177 bărbați la care s-a efectuat evaluarea retrospectivă. Dintre aceştia 84 au prezentat litiază de cale biliară principală (50 de femei şi 34 de bărbați, cu un raport F:B = 1,5:1). Repartizarea acestora pe grupe de vârstă a arătat o incidență mai mare a litiazei hepatocoledociene la vârstele mai înaintate (peste 50 ani) după cum se constată în Fig.3a şi 3b.

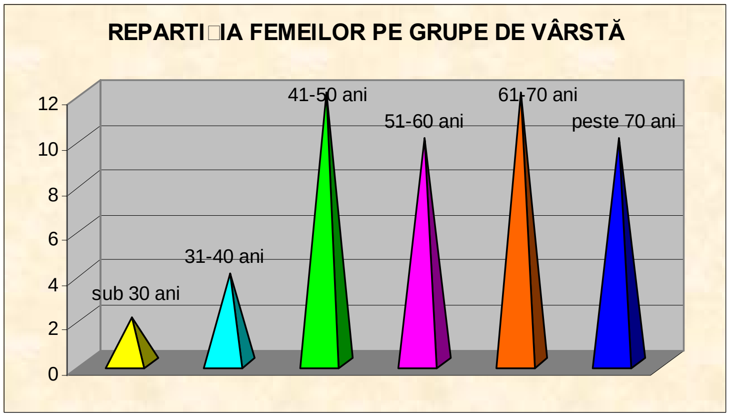

Fig. 3a. Repartiția femeilor cu litiază coledociană pe grupe de vârstă

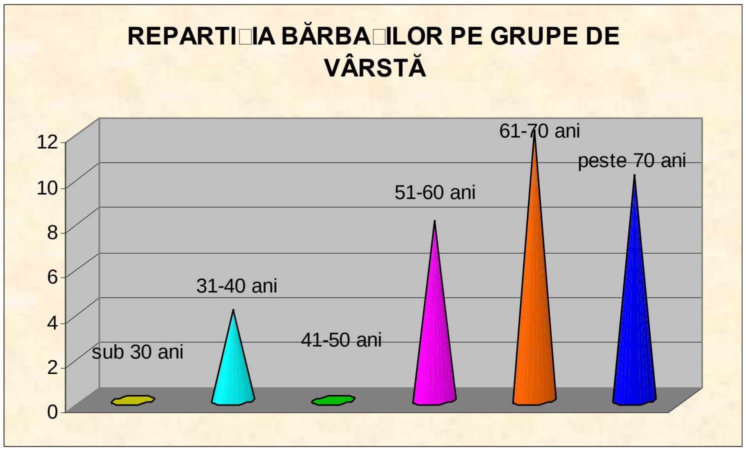

Fig. 3b. Repartiția bărbaților cu litiază coledociană pe grupe de vârstă

În ceea ce priveşte simptomatologia, chiar dacă, în termeni generali, datele vechi din literatură se păstrează, am constatat foarte rar triada clasică Charcot (durere – febră – icter) şi chiar mai rar pentada Reynolds. Durerea a constituit motiv de internare pentru doar 60% dintre pacienți, existând, însă diferențe în cazul asocierii colecistitei acute când durerea a fost prezentă la 81% dintre pacienți.

Febra a fost consemnată la 26 de pacienți (31%), iar frisonul la chiar mai puțini (19 pacienți – 24%). Icterul ar trebui să fie semnul clinic patognomonic pentru obstrucția căii biliare principale. Corelația cu nivelul bilirubinei serice este absolut, evidențiat şi în studiul de față, însă am constatat că o mare parte dintre pacienții incluşi în loturile noastre nu au prezentat acest simptom. Astfel, dintre cei 84 de pacienți cu litiază hepato-coledociană, numai 52 (62%) au prezentat icter, la 32 (32%) neconstatând acest simptom nici măcar în antecedente ori în cursul puseelor evolutive ale bolii. Situația s-a modificat radical, însă, în momentul în care am comparat cazurile internate prin urgență cu cele internate într-un mod programat. Dacă în cazul urgențelor simptomul principal care a condus pacientul la spital a fost icterul, la pacienții internați în mod programat, acest simptom a fost prezent doar în ceva mai mult de 50% dintre cazuri (16 pacienți din 30).

Dintre celelalte simptome constatate la internare, doar dispepsia de tip hepato-biliar, respectiv grețurile şi vărsăturile au fost consemnate într-un procent mai important (50, respectiv 26 dintre cei 84 de pacienți cu litiază a căii biliare principale). Restul simptomelor au fost prezente în procente mici, nesemnificative, adesea fiind incluse în cortegiul simptomatologic al afecțiunilor concomitente ori ale complicațiilor bolii litiazice hepatocoledociene.

Prelucrarea statistică a datelor clinice a constatat că doar durerea în hipocondrul drept ($p<0,05$) şi icterul la pacienții internați în condiții de urgență ($p<0,01$) sunt semnificative statistic pentru diagnosticul litiazei căii biliare principale, toate celelalte simptome oferind rezultate nesemnificative statistic.

Datele biologice efectuate la toți aceşti pacienți incluşi în studiu au fost: bilirubina serică, transaminazele serice (ALAT, ASAT şi gamma-glutamiltranspeptidaza), fosfataza alcalină şi albuminemia serică. Considerând drept valori de referință bilirubinemia peste valoarea normală am obținut un număr foarte mare de rezultate fals-pozitive ($p>0,5$), motiv pentru care am luat în calcul doar valorile acesteia peste dublul normalului. În aceste condiții bilirubina serică a devenit un indice cu valoare predictivă relativ mare pentru litiaza coledociană ($p<0,005$).

Fosfataza alcalină, enzimă de colestază, a avut valoare predictivă moderată ($p<0,1$) în studiul nostru, în timp ce valorile gamma-glutamiltranspeptidazei şi ale albuminei serice nu au avut semnificație statistică.

Determinarea diametrului căii biliare principale s-a efectuat ecografic în toate cazurile, iar în situații selective şi computer-tomografic; aceste metode imagistice au permis şi evidențierea calculilor la nivelul hepatocoledocului. Am înregistrat un singur rezultat fals-pozitiv în cazul constatării preoperatorii a unei litiaze coledociene, motiv pentru care considerăm (şi datele statistice o confirmă) că evidențierea litiazei coledociene este un factor predictiv puternic pentru diagnostic ($p<0,001$). Diametrul căii biliare principale determinat preoperator ecografic nu a fost dovedit ca factor predictiv. Aceeaşi determinare efectuată computer tomografic pare să aibă o eficiență diagnostică superioară, însă numărul de cazuri limitat nu ne permite să tragem o concluzie clară.

DISCUȚII

Formele asimptomatice de litiază a căii biliare principale sunt, probabil, frecvente, însă prevalența acestora este imposibil de precizat. Pentru ca un astfel de diagnostic să fie cât mai real este necesară efectuarea colangiografiei intraoperatorii în cursul oricărei colecistectomii. În realitate, colangiografia intraoperatorie se practică în manieră rutinieră în doar 10-15% dintre centrele cu experiență din lume, majoritatea clinicilor chirurgicale preferând efectuarea acestei manevre într-un mod selectiv, la pacienții care prezintă date preoperatorii sugestive pentru litiaza hepatocoledociană (5). În Clinica de Chirurgie a Spitalului Militar din Craiova practicăm colangiografia intraoperatorie, fie în cursul colecistectomiilor clasice, fie laparoscopice, în manieră selectivă.

În fața unui bolnav prezentând dureri în hipocondrul drept, însoțite sau nu de un sindrom dispeptic de tip hepatobiliar, cu modificări ale probelor hepatice este indicată, ca primă manevră diagnostică, practicarea unei ecografii abdominale (6). Este o tehnică sigură, ce nu presupune iradierea pacientului şi poate fi practicată indiferent de valorile pe care le constatăm în cadrul setului de analize biologice recoltate acestuia. Chiar dacă are o sensibilitate şi specificitate limitate comparativ cu alte explorări imagistice, tehnica are valoare prin disponibilitatea sa generală şi prin posibilitatea efectuării sale la orice pacient. Diagnosticul pozitiv ultrasonografic complet al patologiei căii biliare principale trebuie să răspundă la trei întrebări (7): dacă există un obstacol în calea fluxului biliar, să consemneze localizarea acestui obstacol şi care este natura sa (Fig.4).

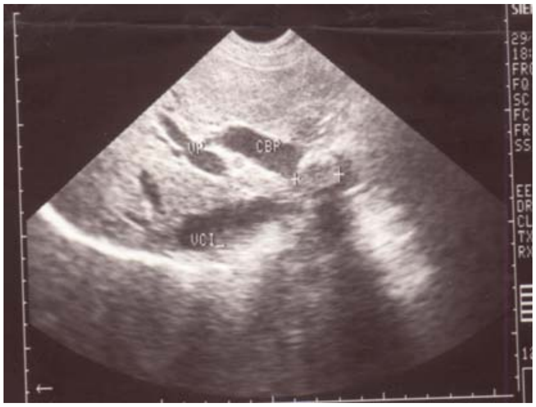

Fig. 4. Litiază coledociană – aspect ecografic (Colecția dr.G.Ianoşi)

Mai mult, investigația poate fi folosită ca screening înaintea efectuării altor explorări imagistice: dacă se constată dilatarea căilor biliare intrahepatice se continuă explorarea cu o colangiografie transparietohepatică, o cauză medicală de icter fiind în principal suspectată; dacă, din contră, se constată dilatarea căilor biliare extrahepatice, atunci se va prefera efectuarea colangiografiei retrograde endoscopice, la aceasta putând fi asociată şi o metodă endoscopică de drenaj biliar, sfincterotomia endoscopică cu extragerea calculilor (8). Ultrasonografia armonică este o tehnică ultrasonografică îmbunătățită, care permite ameliorarea calității imaginii, în special la pacienții la care explorarea este dificilă (9). Tot pe acelaşi principiu funcționează şi ecoendoscopia, care este o metodă neinvazivă introdusă relativ recent în arsenalul metodelor de investigare a căii biliare principale, reprezentând şi o importantă modalitate de evaluare a colestazei extrahepatice deoarece, prin plasarea transductorului în duoden, evită interferențele determinate de aerul intestinal ceea ce îi conferă o vizualizare foarte bună a căilor biliare extrahepatice. Rezoluția ecoendoscopiei biliare este sub 1 mm (chiar 0,1 mm) ceea ce o face utilă, la acest nivel, pentru detectarea microlitiazei biliare ori pentru evaluarea stricturilor benigne sau maligne ale ductului biliar.

Imagistica radiologică prin tomografie computerizată îmbunătăţeşte rezultatele obținute prin explorarea ultrasonografică, în special la nivel retroduodenopancreatic unde ecografia dă cele mai multe rezultate fals-negative. Explorarea căii biliare principale prin rezonanță magnetică nucleară se constituie într-o manevră diagnostică şi mai performantă ale cărei sensibilități şi specificități sunt cuprinse între 91-100%, respectiv 72-100% (10).

Colangiopancreatografia retrogradă endoscopică (CPRE) ar putea fi considerată drept cea mai veche investigație modernă a litiazei căii biliare principale (6). Este o explorare invazivă şi este cotată cu un risc de cca 1-2% de dezvoltare a unei pancreatite acute în special la tineri (11). Chiar dacă tinde să fie înlocuită în algoritmul diagnostic de către ecoendoscopie pe măsură ce aceasta devine accesibilă tot mai multor centre medicale, CPRE rămâne o explorare cu dublu rol, diagnostic şi terapeutic, fiind prima etapă, absolut necesară, în abordul terapeutic al litiazei CBP prin sfincterotomie endoscopică.

Studiul nostru s-a efectuat pe un număr relativ limitat de pacienți, fiind vorba despre experiența din ultimii 5 ani a unei singure clinici chirurgicale, însă trebuie remarcat faptul că datele obținute se apropie de ceea ce consemnează literatura de specialitate.

CONCLUZII

1. Litiaza căii biliare principale, fie că se dezvoltă *de novo*, fie că evoluează ca o complicație a litiazei veziculare, este o afecțiune a cărei simptomatologie a ieşit din standardele clasice, diagnosticul său necesitând obligatoriu o explorare imagistică preoperatorie sau intraoperatorie.
2. Durerea în hipocondrul drept, asociată icterului, ori descoperirea icterului la pacienții internați în urgență sunt singurele asocieri clinice cu valoare diagnostică.
3. Bilirubina serică cu valori duble față de normal are valoare predictivă importantă la pacienții cu litiază a căii biliare principale.
4. Evidențierea preoperatorie a litiazei coledociene (ultrasonografic ori computer tomografic) este un puternic factor predictiv pentru diagnosticul de litiază CBP; evidențierea dilatării hepatocoledocului, ecografic ori CT, nu a oferit rezultate semnificative în studiul nostru.

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GLIOBLASTOMUL MULTIFORM
- elemente de actualitate

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Glioblastomul multiform (GBL) poate fi considerat prin malignitatea sa înaltă „capul de serie" al astrocitoamelor difuze de tip fibrilar şi reprezintă o provocare neuro-oncologică. Autorii prezintă o trecere în revistă a principalelor caracteristici generale ale acestei entităţi nosologice şi evidentiază tratamentul complex multimodal aplicat pentru stoparea evoluţiei acestei tumori, prelungirea supravieţuirii şi asigurarea unei calităţi a vieţii acceptabile. Evoluţia GBL este gravă cu durată medie de supravieţuire sub 1 an.

glioblastom tratament multimodal calitatea vieţii

Abstract
The glioblastoma may be considered by its high malignity the "first of all" fibrillar-type diffuse astrocytoma and represents a neuro-oncological challenge. The authors present a main features review of this nosological entity and highlight the multimodal complex therapy to perform in order to stop the tumor evolution, life extension and the quality-of-life assurance. The GBL evolution is a severe one having an average of survival up to a year no more.

Key words: Glioblastoma, multimodal therapy, quality of life

Consideraţii generale

Definiţie. Glioblastomul este o tumoră cu origine neuroectodermală şi reprezintă cea mai malignă tumoră astrocitară cerebrală, fiind compusă, în cea mai mare parte, din astrocite neoplazice slab diferenţiate. Trăsăturile histopatologice includ polimorfism celular, atipii nucleare, activitate mitotică înaltă, proliferare microvasculară cu tromboze vasculare şi necroze. În general, glioblastomul afectează adulţii, cu predilecţie între 45 şi 70 ani şi de obicei, se localizează în emisferele cerebrale (10). Glioblastoamele se pot dezvoltă din astrocitoame difuze de gradul II, astrocitoame anaplazice (glioblastom secundar), dar în mod frecvent ele se manifestă după o evoluţie clinică scurtă, „de novo", fără prezenţa unei leziuni anterioare maligne (glioblastom primar).

Diferenţierea între GBL primar şi secundar a fost facută pentru prima oară de Scherer în 1940 care spunea: "din punct de vedere biologic şi clinic, GBL secundare se dezvolta din astrocitoamele difuze prin dediferenţiere şi trebuie deosebite de GBL primare (de novo)".

Acestea din urmă reprezintă majoritatea, apar în jurul vârstei de 55 de ani, sunt mai frecvente la bărbaţi şi au o istorie clinică scurtă, de obicei mai puţin de 3 luni, manifestându-se "de novo", fără evidenţă clinică sau histopatologică a unui astrocitom de grad mic preexistent(10).

Gradare. Glioblastomul multiform şi variantele sale (GBL cu celule gigante şi gliosarcomul) corespund gradului IV de malignitate (OMS).

Incidenta. Glioblastomul este cea mai frecventă tumoră cerebrală, reprezentând aproximativ 12-15% din toate neoplasmele intracraniene şi 50-60% din tumorile astrocitare (4). Vârsta medie de apariţie este 56 de ani, iar bărbaţii sunt mai frecvent afectaţi (sex ratio 1,5/1). În Europa, SUA şi Canada, incidenţa este de 2-3 cazuri noi la o sută de mii de oameni pe an (8).

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Localizare. Glioblastoamele se dezvoltă, de cele mai multe ori, din substanța albă subcorticală a emisferelor cerebrale (3).
În statistica noastră, localizările cele mai frecvente au fost: lob temporal 29%, lob parietal 25%, lob frontal 24% şi lobul occipital 16%. Localizările combinate fronto-temporale sau temporo-parietale sunt destul de frecvente(10). Tumora infiltrează structurile cerebrale adiacente: cortexul adiacent, ganglionii bazali, emisferul contralateral, pedunculii cerebrali, etc. Glioblastoamele intraventriculare sunt foarte rare, precum şi cele cerebeloase şi intraspinale (13).

Vârsta şi distribuția pe sexe. Glioblastomul poate apărea la orice vârstă, dar afectează în special adulții cu vârsta cuprinsă între 45 şi 70 ani, media fiind 56 ani (8). Bărbații par a fi mai frecvent afectați decât femeile (1,5/1).

Trăsături clinice. Istoricul clinic al bolii este de obicei scurt (mai puțin de 8 luni în mai mult de 50% din cazuri), dacă neoplasmul nu s-a dezvoltat din astrocitoame difuze de gradul II sau astrocitoame anaplazice (glioblastom secundar). Deseori, după o manifestare epileptică cu simptome neurologice nespecifice, pacienții prezintă dureri de cap şi tulburări de personalitate, iar semnele specifice ale hipertensiunii intracraniene devin rapid evidente (1).
Simptomatologia clinică a glioblastoamelor multiforme este similară cu a astrocitoamelor difuze anaplazice cu diferența ca primele se însoțesc de deficit neurologic focal cu debut brusc şi rapid evolutiv. Astrocitoamele difuze de grad II şi astrocitomul anaplazic infiltrează țesutul cerebral intact fără a-l distruge, comparativ cu GBL care pe lângă efectul de masă produce şi distrucția tisulară cerebrală, efectul clinic fiind deficitul neurologic focal. Hemoragia cerebrală acută poate sublinia debutul clinic al unui GBL dar aceasta se întâmplă în cazuri rare (3).

Neuroimagistica. Pe examinarea CT craniocerebrala, glioblastomul prezintă o formă neregulată, cu o zonă periferică circulară (ring like) care captează intens substanța de contrast şi o zonă centrală (hipodensă), ce reprezintă necroza – fig.1 (13).

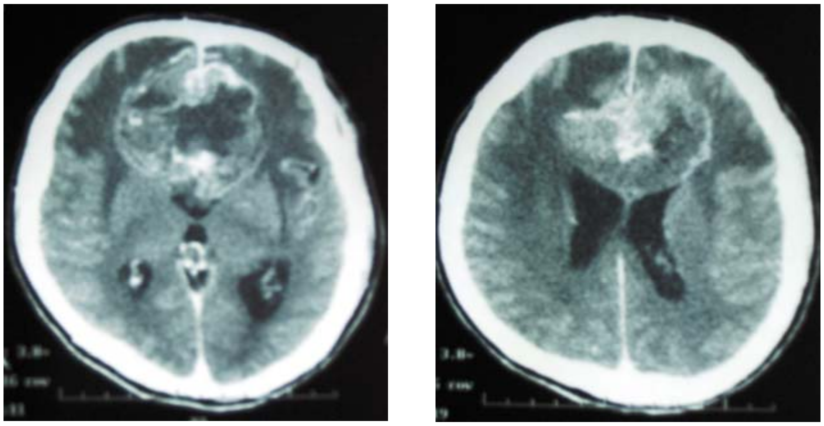

Fig. 1. Aspect CT-scan de glioblastom multiform bifrontal
(se observă tumora hipercaptantă a substanței de contrast marginal, cu necroză centrală şi intens edem peritumoral)

Imaginile obținute prin RM reflectă o marcată heterogenitate intratumorală, dată de hemoragie (de vârste diferite), necroze şi hipercelularitate în grade variabile. În secvența T1, imaginile RMN evidențiază o structură inelara periferică hipercaptantă de contrast, ce corespunde ariei tumorale cu celularitate crescută şi hipervascularizație (fig.2). Aceasta structură nu reprezintă marginea externă a tumorii, celule tumorale infiltrative putând fi uşor identificate chiar şi la 3 cm de marginea tumorală. În secvența T2 se observă focare de necroză chistică hiperintensă şi arii hipercelulare care au intensitate mai redusă, înconjurate de un edem vasogenic cu semnal crescut (5).

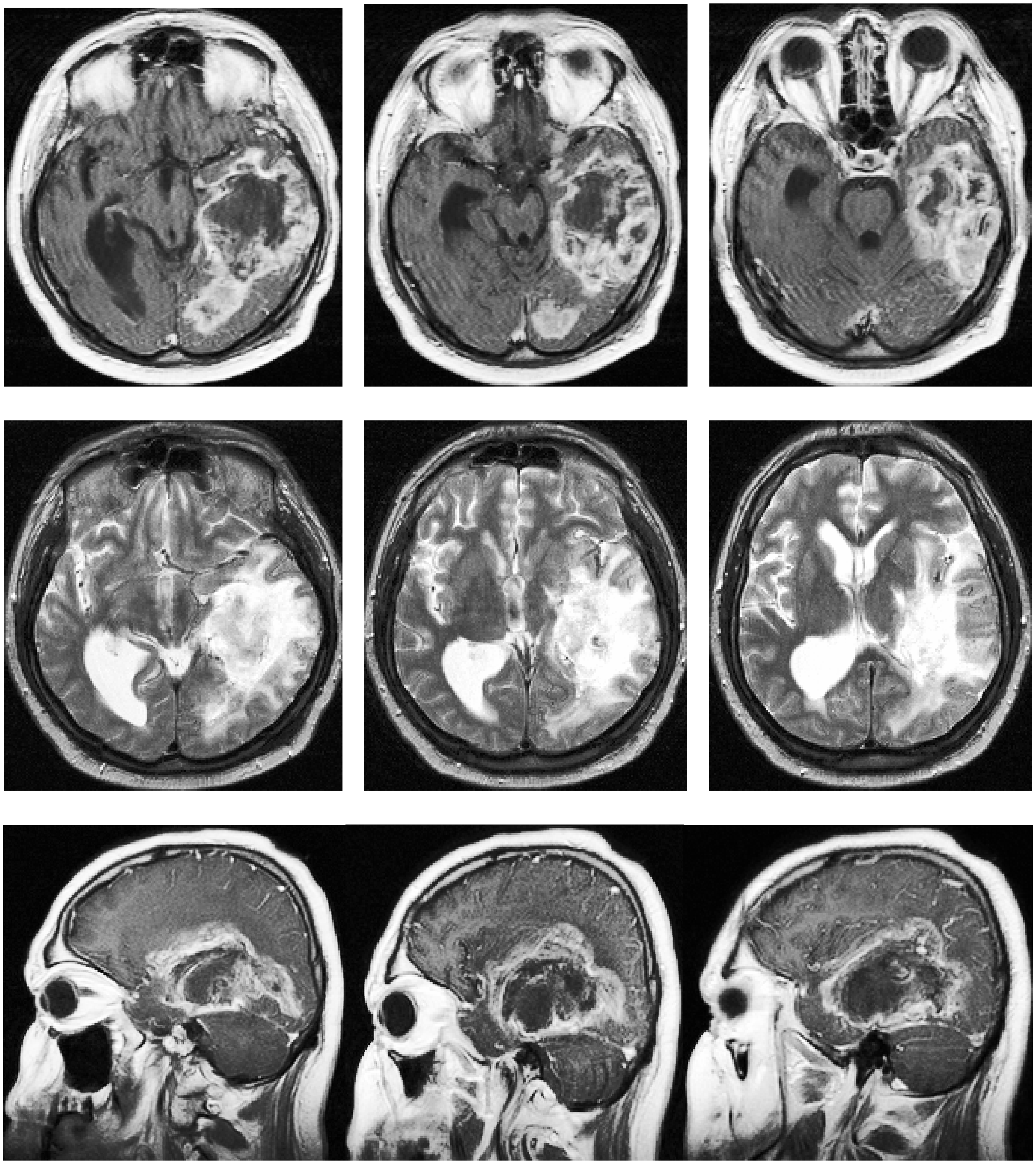

Fig.2. Aspect IRM de glioblastom multiform temporal stâng
(secvențe T1 axial şi sagital cu substanță de contrast şi T2 axial);

Imaginile obținute prin PET scan evidențiază consumul regional de glucoză, strâns corelat cu gradul de hipercelularitate. Trebuie făcută diferența dintre recidiva tumorii şi radionecroză, pe baza PET, care arată creşterea metabolismului glucozei în țesuturile recidivate față de hipometabolismul din radionecroză (7).

Uneori, GBL-ul este o leziune multicentrică, putând exista două tumori sincrone (simultan), detectate în focare independente în momentul primei evaluări sau metacrone, adică pot apărea la momente diferite în timp şi pretând la confuzii cu metastazele. O parte din aceste focare tumorale au aspecte histologice diferite, ceea ce ar pleda pentru originea lor policlonală (11) (fig. 3).

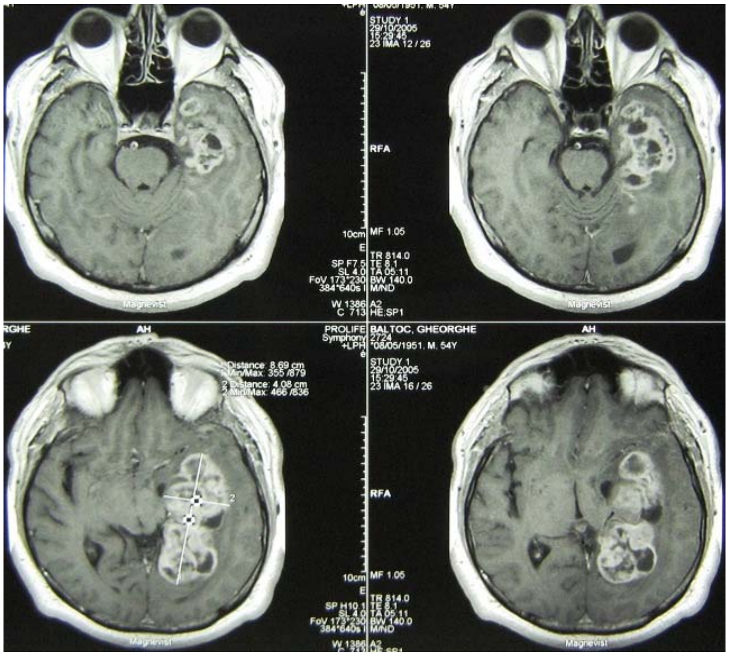

Fig. 3. Aspect IRM (secventă T1 – axial) de glioblastom multicentric temporal şi parietal, se observă extensia tumorală prin tracturile de substanță albă;

Neuropatologie. Macroscopic, GBL este o tumoră imprecis delimitată, suprafața de secțiune arătând zone de culoare gălbuie (necroză), ce poate ocupa 80-90% din masa tumorii, înconjurată de zone periferice gri-murdar, unde masa tumorală are o consistență moale şi zone brun-roşiatice care reprezintă hemoragii de vârste diferite (10). Uneori, hemoragiile mari pot produce confuzii cu accidentele vasculare hemoragice (fig. 4).

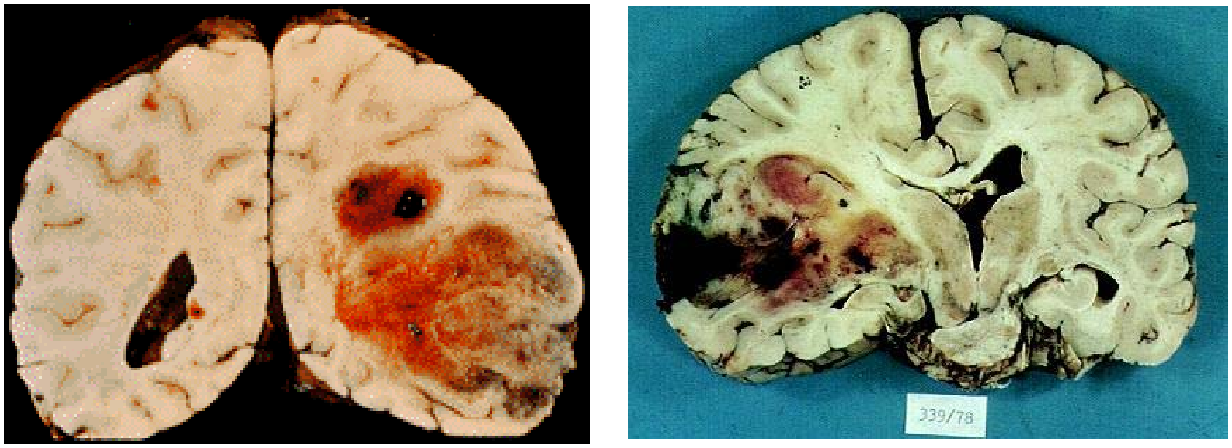

Fig. 4. Aspecte macroscopice de glioblastom multiform – secțiuni coronale;

Zona centrală pseudochistică vizibilă macroscopic conține un țesut tumoral necrotic lichefiat de culoare brun-roşiatică datorată hemoragiei recente şi produşilor de degradare ai hemoglobinei şi are pereți de obicei neregulați, spre deosebire de astrocitoamele de grad inferior care posedă un chist bine delimitat (14). Această zonă de necroză centrală este înconjurată de o zonă hipercelulară asemănătoare unui inel. Cele mai multe glioblastoame apar în emisferele cerebrale, intraparenchimatos, cu epicentru în substanța alba, dar pot ajunge în contact cu leptomeningele şi dura mater (4).

Aspectele microscopice sunt variabile; se prezintă ca o tumoră evident astroglială, datorită tramei fibrilare eozinofile, populată de o mare diversitate de celule astrocitare anaplazice având mari

diferențe de volum, de tinctorialitate, un aspect pleomorf, cu numeroase atipii celulare şi nucleare, precum şi celule multinucleate, gigante, aneuploide cu aspect monstruos (acestea din urmă fiind considerate ,,marca" de GBL). Dacă aceste celule gigante multinucleate domină aspectul histopatologic, denumirea ,,glioblastom cu celule gigante" este justificată (9).
Datorita anaplaziei crescute, uneori este greu de identificat originea astrocitară a tumorii (fig.5,6,7 şi 8).

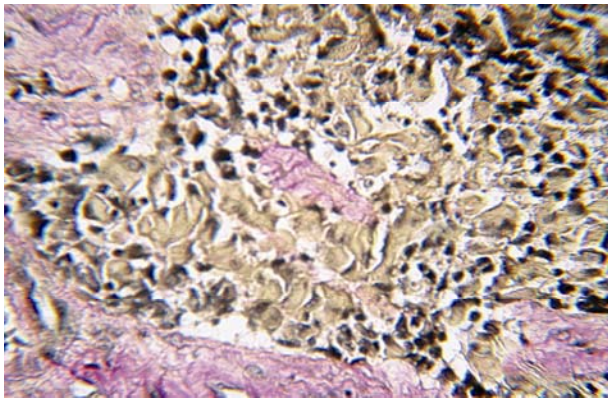

Fig. 5. Insulă celulară epitelioidă în GBL mitoze atipice) în GBL

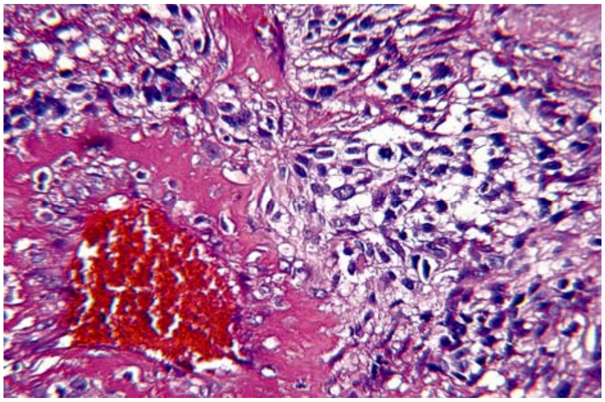

Fig. 6. Aspecte de anaplazie celulară (atipii, mitoze atipice) în GBL

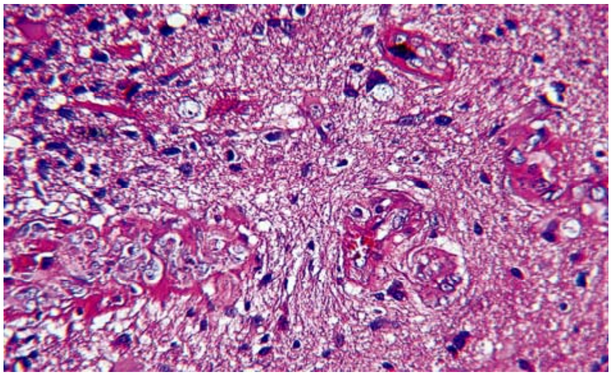

Fig. 7. Proliferari microvasculare

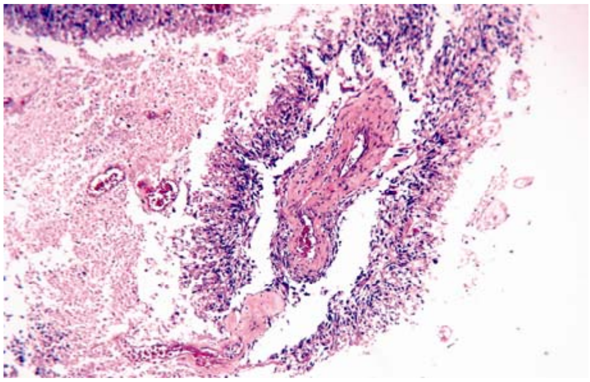

Fig.8. Zonă întinsă de necroză; rămân viabile celule tumorale dispuse perivascular

Pe lângă prezența celulelor gliale anaplazice, diagnosticul de GBL se precizează şi pe baza activității mitotice, a proliferării vasculare şi a necrozei. Proliferarea vasculară este mai exprimată în jurul focarelor necrotice şi la periferia zonei infiltrative (10) (fig. 5, 6, 7). Uneori pot fi recunoscute printre celulele anaplazice şi astrocite de tip fibrilar şi gemistocite, recunoscând tranziția de la grade inferioare la gradul IV. GBL-ul secudar prezintă centrul tumoral cu aspect de glioblastom, ce se pierde treptat într-un astrocitom de grad III şi ulterior într-o periferie de tip astrocitom fibrilar de grad II (13).

În ciuda evoluției clinice de scurtă durată, aceste tumori sunt de cele mai multe ori surprinzător de mari şi pot afecta mai mult decât un lob cerebral; unele pot infiltra pedunculul cerebral spre trunchiul cerebral sau corpul calos spre emisferul contralateral ("butterfly glioblastoma" fig .9).

Diseminare şi metastazare. Deşi tendința de a infiltra țesutul cerebral adiacent este o trăsătură comună tuturor tumorilor astrocitare difuze, GBL-ul are tendința naturală spre invazie, infiltrare şi distrucție a țesutului cerebral adiacent. O trăsătură caracteristică a GBL este extensia prin corpul calos spre emisferul cerebral contralateral, creând o leziune simetrică ("butterfly glioma").

Posibilitatea de extensie a fost observată şi prin capsula internă, fornix, pedunculi cerebrali, comisura albă anterioară sau radiaţiile optice etc.

Aceste structuri pot apărea lărgite, dilatate sau distorsionate şi pot servi drept cale de diseminare, permiţând extensia tumorii la distanţă şi sugerând de multe ori aspectul de GBL multifocal (11). "Guerilla cells", celule maligne călătoare, pot fi identificate şi la distanţă de 3-4 cm de tumora propriu-zisă, de obicei în zona edematoasă peritumorală. Aceste celule tumorale nediferenţiate pot migra şi la distanţe mult mai mari, uneori chiar la 6-7 cm de-a lungul tracturilor de substanţă albă sus menţionate (2) (fig.10).

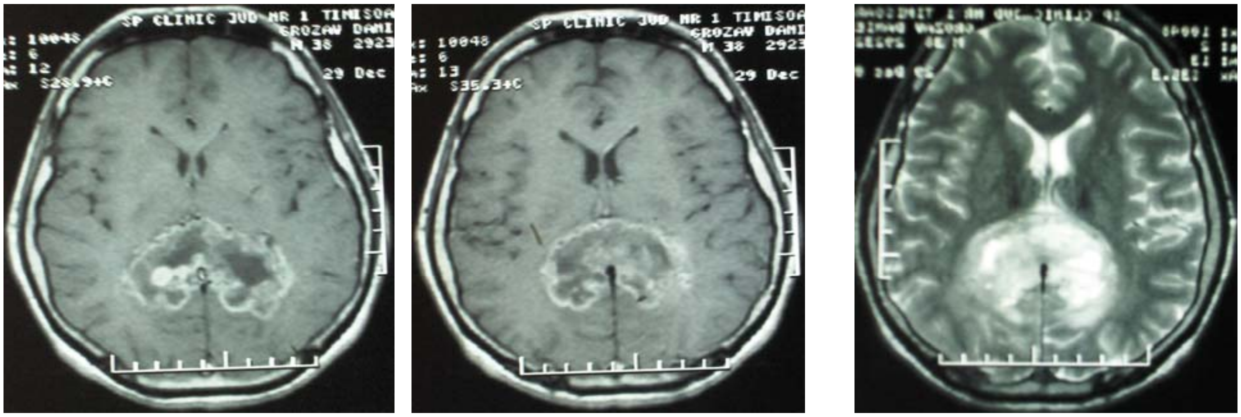

Fig.9. Aspect IRM de glioblastom multiform – „în fluture”(butterfly glioma), se observă extensia contalaterală prin spleniusul corpului calos;

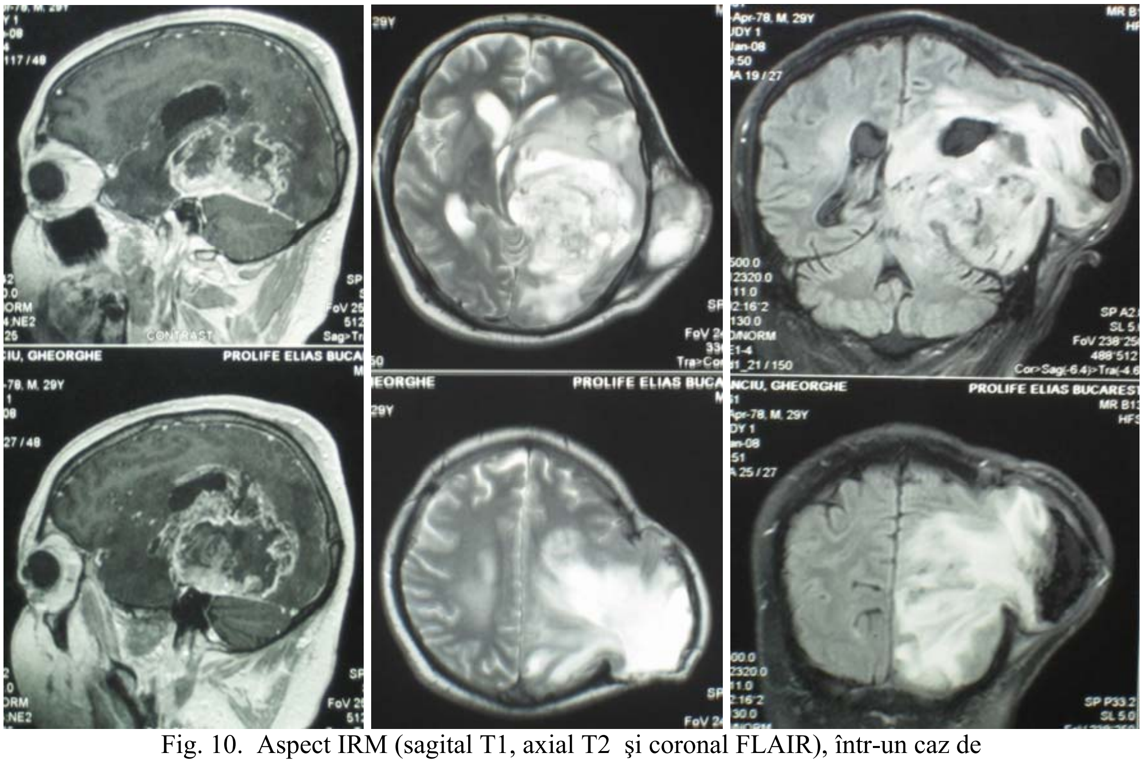

Fig. 10. Aspect IRM (sagital T1, axial T2 şi coronal FLAIR), într-un caz de glioblastom temporo-parietal stâng, operat iterativ şi recidivat;

Extensia de-a lungul spațiilor perivasculare este altă cale tipică de diseminare a GBL, dar invazia intraluminală vasculară este extrem de rară – celulele tumorale nu pot strabate elastina din structura peretelui vascular, nesecretând elastaza. Diseminarea pe cale hematogenă se produce doar în timpul manevrelor chirurgicale, spre țesuturi extraneurale (13). Metastazarea intraperitoneală via drenaj ventriculo-peritoneal a fost observată şi se poate produce (2).

În ciuda agresivității şi gradului crescut de infiltrare tumorală, tendința GBL de a invada spațiul subarahnoidian este destul de rară, metastazele via LCR la distanța fiind ocazionale (14). Penetrarea durei mater, sinusurilor venoase sau a osului se produce în mod excepțional (10).

Imunohistochimia. Imunoreactivitatea la GFAP este neomogenă şi inconstantă. Ea dovedeşte doar originea glială a celulelor tumorale, dar nu şi pe cea astrocitară. Celulele tumorale fixează mai omogen şi mai intens S100. Uneori GBL prezintă şi markeri epiteliali cum ar fi citokeratina. Vimentina este nespecifică fiind de folos doar ca indicator al imunoviabilității țesutului. Indicii de marcare ai proliferării celulare cu Ki67/MIB-1 evidențiază o fracție de creştere tumorală situată între 15-20% (3).

Genetica moleculară. Transformarea malignă a celulelor neuroepiteliale se produce diferit în cazul glioblastoamelor secundare comparativ cu glioblastoamele primare (11). În glioblastoamele secundare procesul dediferențierii celulare se realizează secvențial, prin achiziția seriată a modificărilor genice. Procesul începe prin afectarea genei ce codifică proteina supresoare tumorală p53 ("polițistul genomului"), însoțită sau nu de pierderea heterozigozității cromozomului 17p (care conține gena menționată). Aceasta este cea mai precoce modificare genică, detectabilă la majoritatea astrocitoamelor difuz infiltrative de grad II şi se asociază frecvent cu supraexprimarea PDGF (platelet-derived growth factor) si a receptorului său PDGFR (15). Transformarea în astrocitom anaplazic este consecința deletiilor alelice ale cromozomilor: 9p, 13q şi mai rar, a amplificării 12q. Toate aceste modificări fac parte dintr-un singur ciclu reglator complex, cu rol critic, al diviziunii celulare - ciclul reglator p16/p15/CDK4/CDK6/Rb. Afectarea ciclului menționat conduce la fosforilarea proteinei Rb, permițând celulei să avanseze din faza G1 spre faza S (inițiind astfel diviziunea mitotica).

În cele din urmă se produce amplificarea EGFR (epidermal growth factor receptor). Acesta este oncogena cel mai frecvent amplificată în astrocitoamele anaplazice şi în peste 50% din glioblastoamele de toate tipurile (11).

Glioblastoamele primare prezintă modificări genice de mare amploare care se produc brutal, fără secvențialitate. Ele asociază supraexprimarea EGFR cu deleția cromozomului 10. În afara acestor mutații genice principale se mai descriu şi afectări ale unor gene supresoare tumorale, cum sunt: DCC (deleted in colorectal cancer), deleția alelică a cromozomului 19q şi pierderea heterozigozității cromozomului 22q (11).

Tratament si prognostic.

Tratamentul este cel chirurgical, urmat de radioterapie convențională externă sau brahiterapie, chimioterapie. Tratamentul chirurgical trebuie să pună în balanță pe de o parte supraviețuirea mai lungă după rezecții tumorale mai largi, cu afectarea pe de altă parte a calității vieții. Excizia chirurgicală cât mai completă aduce o multitudine de avantaje printre care:

- scade efectul de masă;
- reduce frecvența hemoragiilor în patul tumoral (prin ablația vaselor de neoformatie);
- facilitează terapiile adjuvante (atât radioterapia cu variantele sale cât şi chimioterapia);
- îmbunătățeşte statusul neurologic al pacienților;
- îmbunătățeşte controlul medicamentos al crizelor epileptice etc.

Pacienții cu GBL multiform, tratați numai chirurgical, au un timp mediu de supraviețuire de cca.16 săptămâni (13).

Biopsia stereotactică poate fi luată în considerare în situațiile când:

- tumora este situată într-o zonă elocventă a creierului,
- tumora este de mici dimensiuni, cu localizare profundă,

- când pacientul prezintă deficit neurologic minim,
- pentru a face diferența între un GBL și o altă tumoră care poate fi tratată cu succes prin iradiere (ex. limfom), ambele fiind compatibile imagistic, pretând la confuzie (12)

Corticoterapia are eficiență în tratarea glioamelor cerebrale prin reducerea metabolismului cerebral al glucozei și reducerea edemului vasogenic (3).

Radioterapia produce modificări specifice, care trebuie recunoscute, atât pentru a putea urmări eficiența acesteia, cât și în scopul evitării unor confuzii diagnostice. Astfel, după un interval mai scurt pentru brahiterapie, mai lung pentru radioterapia convențională externă, tumora prezintă focare întinse, confluente de necroză, direct proporționale cu doza administrată (7). Necroza exprimă o perioada de repaus tumoral, caracterizată prin paucicelularitate, pleomorfism celular și mai ales prin apariția unei populații astrocitare gigantocelulare care reprezintă o formă de degenerescență tumorală. După câteva luni se produce selecția unei subpopulații celulare cu agresivitate ridicată, aproape lipsită de radiosensibilitate (2). Doza uzuală de raze X folosită este de 50-60 Gy. Radioterapia externă (whole brain) nu a dovedit o eficiență mai mare comparativ cu iradierea focală (7). Radioterapia convențională adițională chirurgiei poate extinde perioada de supraviețuire cu aproximativ 39 de săptămâni. Radiochirurgia prin "gamma-knife" are efect bun în tumorile profunde de volum mic (1).

Efectele chimioterapiei asupra aspectelor celulare și histologice în GBL sunt puțin cunoscute și încă discutabile. Se consideră că agenții alchilanți produc un beneficiu în 10% din cazuri, toți având o eficacitate similară. Carmustina (BCNU), procarbazina, cisplatinum, lomustina (CCNU), nimustina (ACNU), au fost primii agenți chemoterapeutici utilizați în glioamele maligne. În încercarea de a reduce efectele adverse a chimioterapiei sistemice s-au încercat mai multe metode de a administra cât mai local citostaticul. Administrarea intraarterială de Carmustina a provocat deteriorare vizuală, leucoencefalopatie, toxicitate retiniană etc.

S-a încercat administrarea locală peroperatorie, după excizia cât mai completă a tumorii, de carmustină - gel sau carmustina conținută în așa numiții "biscuiți" - wafers (7,7 mg în 200 mg polifeprosan). Unele studii randomizate au evidențiat că agenții alchilanți aplicați local pot crește supraviețuirea cu 9-13 săptămâni (7). Singurele complicații ale implanturilor cu citostatice constau în: edem cerebral, pot favoriza suprainfecția plăgii operatorii, creșterea incidenței crizelor epileptice, etc.

Temodal-ul (temozolomida) este un alt agent alchilant , derivat de nitrozuree, utilizat ca anti-angiogenetic. Eficacitatea lui a fost pusă la îndoială datorită depresiei leucocitare și trombocitare pe care o produce (8).

Terapia fotodinamică este o terapie bimodală ce implică injectarea preoperatorie a unui compus fotosensibilizant ce se localizează selectiv în celulele tumorale, urmată de excizia chirurgicală standard a tumorii, iar cavitatea restantă este iluminată (iradiată) cu raze laser cu o anumită lungime de undă și care sunt absorbite de celulele tumorale fotosensibilizate. Preparatul folosit este un derivat de hematoporfirină (Photofrin) și se administrează în doza de 5 mg/kgc cu 24 ore preoperator. Durata medie pentru fotoiradiere este de 30-60 minute modificând permanent direcția razei laser pe toată suprafața cavității de rezecție și irigând permanent cu ser pentru a preveni supraîncălzirea excesivă (1). Unele studii au evidențiat o prelungire a supraviețuirii cu cca. 18 săptămâni. Pacienții vor evita expunerea la soare pe o perioadă de 6-8 săptămâni după o astfel de procedură (1).

Excizia chirurgicală a recidivei tumorale poate crește supraviețuirea cu aproximativ 36 de săptămâni, cu mențiunea ca 10% din GBL pot recidiva la distanță de locația inițială (12).

Psihoterapia. Unele studii efectuate asupra profilului psihologic al unor pacienți tratați pentru GBL multiform au arătat că depresia și sentimentul lipsei de speranță de vindecare sunt asociate cu evolutie rapid infaustă, în timp ce comportamentul combativ și optimist este însoțit de rezultate mai bune (6). Datele semnalate sugerează că endorfinele (mediatori biochimici ai emoțiilor), sunt implicate în reglarea imună prin influențarea activităților celulelor "natural killer" (6).

Imunoterapia aplicată în cazul GBL multiforme, după injectarea intraveneoasă sau intracarotidiană de anticorpi monoclonali, produce o acumulare specifică la nivelul tumorii, dar ea este insuficientă pentru obținerea unui efect terapeutic (6). Bariera hematoencefalică poate constitui o piedică pentru pasajul anticorpilor monoclonali.

Încercările de depăşire a acestei piedici au constat în deschiderea barierei hemato-encefalice. Au existat încercări de injectare în restul tumoral, după excizia chirurgicală de limfocite "killer" sensibilizate, dar rezultatele favorabile nu s-au confirmat (2). Administrarea intravenoasă de imunomodulatori de tipul IL2, datorită creşterii edemului cerebral, nu poate constitui un tratament eficace împotriva GBL (6).

Durata medie de supraviețuire după tratament este aproximativ 8 luni (8). Pacienții mai tineri cu GBL secundar prezintă intervale ceva mai mari de supraviețuire, care pot ajunge până la 3-5 ani. Rezultatele tratamentului multimodal în GBL trebuie urmărite continuu clinic (statusul neurologic) şi paraclinic (CT-scan şi RMN repetate), asociate cu examen hematologic şi biologic general (1) (fig. 11).

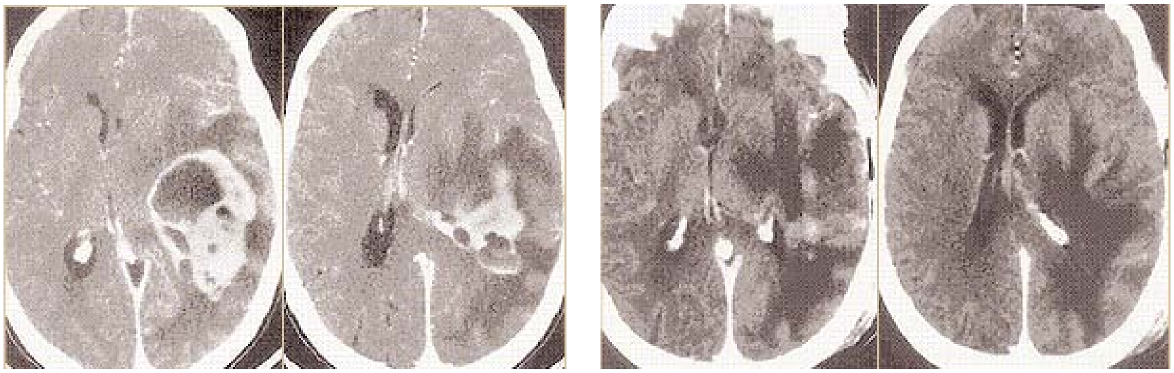

Fig. 11. Aspect pre- şi postopertaor, într-un caz de GBL multiform temporo-parietal stâng;

Cauza decesului provocat de acest tip de tumoră este neclară. Deşi o parte dintre pacienți decedează datorită efectului de masă şi hernierii cerebrale secundare, alții nu prezintă efect de masă semnificativ în momentul decesului (1). Deteriorarea neurologică progresivă şi decesul sunt cauzate de afectarea cerebrală de cauze necunoscute (12).

Concluzii

Diversitatea celulară determină o mare varietate de tipuri şi grade tumorale, care nu sunt uşor de identificat numai pe baza aspectelor histopatologice clasice (10). În plus, tumorile gliale sunt de obicei neomogene, atât în privința gradului tumoral, cât şi al tipului celular. Actul operator aplicat asupra unei tumori localizate intracerebral este autolimitat, fragmentele tumorale sunt, de obicei, mici şi adeseori nereprezentative pentru tipul sau gradul tumoral (7). Din acest motiv, diagnosticul se precizează prin coroborarea obligatorie a aspectelor clinice cu localizarea tumorii şi mai ales cu neuroimagistica (10). Este obligatorie separarea glioamelor de grade inferioare de cele superioare şi mai ales a celor neinfiltrative de cele difuz infiltrative, întrucât atitudinea chirurgicală este diferită de la caz la caz (12).

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Astmul bronşic indus de aspirină – o problemă de importanţă practică

Cpt. Dr. Mihăiţă PĂTRĂŞESCU[*]

Astmul bronşic indus de aspirină face parte din triada clasică Samter Vidal alături de polipoza nazală sau rinosinuzita. Este mai degrabă apanajul vârstelor adulte, fiind cel mai adesea o forma severă de astm. Tratamentul de desensibilizare cu aspirină este o alternativă terapeutică la îndemână pentru cazurile în care nu se poate apela la altă clasă de medicamente.

astmul bronşic indus de aspirină

Aspirin induced asthma is a part of the clasic Samter's triad along with nasal poliposis or rhinosinusitis. It is rather encountered in adult ages. It is a severe form of asthma. Desensitisation treatment with aspirin is a good therapeutic alternative to be used whenever another class of drugs aren't handy.

Astmul bronşic cunoaşte o prevalenţă în creştere în întreaga lume, cele mai mari rate fiind descrise în Noua Zeelandă şi Australia (aprox. 10%) . Este definit ca o suferinţă inflamatorie bronşică cronică cu predominenţa eozinofilelor care determină episoade recurente de wheezing, dispnee, senzaţie de constricţie toracică , tuse, în mod particular noaptea sau dimineaţa devreme şi la terminarea efortului fizic. Cel puţin în primele etape de evoluţie obstrucţia bronşică este total reversibilă, spontan, după administrarea unui beta2 agonist inhalator sau după o cură scurtă de corticosteroizi inhalator sau per os.

Caracteristica funcţională ventilatorie a astmului bronşic este hiperreactivitatea bronşică nespecifică la stimuli precum histamina, metacolina, manitolul. Deşi nu este nici suficient de specifică sau sensibilă pentru diagnosticarea astmului totuşi evoluează paralel cu severitatea acestuia şi cu inflamaţia bronşică . Monitorizarea fracţiei oxidului de azot în aerul expirator (FeNO) ca martor indirect al inflamaţiei eozinofilice poate să devină, într-un viitor foarte apropiat, o metodă neinvazivă la îndemână pentru managementul astmului bronşic:

- FeNO creşte înaintea deteriorării controlului astmului precedând modificările spirometrice care, adeseori nu evoluează paralel cu severitatea simptomelor
- FeNO este mult mai mare la pacienţii cu astm bronşic decât în alte suferinţe
- FeNO crescut anunţă responsivitatea la tratamentul corticosteroidian
- Modificări ale FeNO în cursul tratamentului permite ajustări ale acestuia.

În 75% din cazuri astmul bronşic este o problemă alergologică în sensul în care episoadele de bronhospasm pot fi declanşate de expunerea la alergeni specifici repiratori (polen, mucegaiuri, epitelii de pisică, câine, iepure, acarienii din praful de casă). În aceste situaţii poate fi demonstrată existenţa imunoglobulinelor E alergen specifice prin teste cutanate alergologice sau examene serologice. Chiar şi la aceşti pacienţi acţionează factorii declanşatori nespecifici: poluarea aeriană, efortul fizic, infecţiile virale respiratorii, fumul de tutun.

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O clasificare mai veche a astmului bronşic, încă funcțională din considerente didactice, identifică două forme etiologice:

- **astmul extrinsec** (cu referire la majoritatea cazurilor în care se identifică componenta alergică IgE mediată)
- **astmul intrinsec** (în care episoadele bronhospastice nu pot fi puse în asociere cu un alergen față de care să se demonstreze o reactivitate de tip IgE).

Astmul intrinsec este definit pentru întâia oară de Rackemann în 1947 care atrăgea atenția asupra existenței unui subgrup de pacienți adulți care prezentau:

- teste cutanate alergologice negative la majoritatea alergenelor comune
- fără istoric familial de boli atopice
- eozinofilie persistentă
- un curs evolutiv sever (cu spitalizări frecvente, episoade bronhospastice severe şi rezistente la tratament, responsivitate slabă la corticosteroizi)
- fără imbunătățire clinică imediată la internare.

În 1956 Samter adaugă observația că unii pacienți din acest subgrup prezintă:

- polipoză nazală
- sinuzită
- intoleranță la aspirină

Se defineşte astfel **triada Samter-Vidal**, aşa cum va fi cunoscută ulterior, care reuneşte ***polipoza nazală cu astmul bronşic şi intoleranța la aspirină*** . Cele trei elemente nu sunt prezente întotdeauna. Polipoza nazală poate lipsi fiind prezentă doar o rinosinuzită nonpolipoasă. În diagnosticul sinuzitei standardul imagistic de aur este reprezentat de examenul de tomografie computerizată iar nu de radiografia simplă a sinusurilor paranazale .

Din cele prezentate până acum se impun câteva precizări:

1. inflamația eozinofilică bronşică nu se întâlneşte exclusiv în astmul alergic (ea este prezentă şi astmul cu intoleranță la aspirină)
2. numărul eozinofilelor în sputa indusă se corelează cu FeNO şi cu şansa unui răspuns pozitiv la tratamentul antiinflamator corticosteroidian
3. predominența neutrofilelor în inflamația bronşică (forme de astm severe, astm cu crize bronhospastice predominent nocturne etc) se asociază cu un răspuns mai slab la corticosteroizi
4. polipoza nazală la un pacient astmatic impune explorarea toleranței la aspirină
5. indiferent de factorul cauzal şi de factorii declanşatori ai crizelor, inflamația bronşică în astm este persistentă cu evoluție şi în afara episoadelor de acutizare.

Acidul acetilsalicilic (aspirina) este utilizat în terapeutica umană de mai mult de 100 de ani. Împreună cu alte antiinflamatorii nesteroidiene (AINS) îşi exercită efectele antiinflamtorii, analgezice şi antipiretice (dar şi o parte din efectele adverse) prin inhibiția ciclooxigenazelor (COX1 şi COX2). AINS-urile induc o serie de efecte adverse printre care se numără şi cele din tabelul 1.

TABEL 1

Efecte adverse induse de Aspirină/AINS
Gastrită Ulcer gastroduodenal Hepatotoxicitate Anemie Trombocitopenie Anomalii de coagulare Nefrită interstiţială Sindrom Stevens-Johnson Eritem fix medicamentos Necroliză epidermică toxică Eritem nodos Erupţii maculopapulare Vasculită leucocitoclastică

În septembrie 2001 Stevenson publică în Annals of Allergy Asthma and Immunology o clasificare a reacţiilor allergice şi pseudoalergice la aspirină/AINS într-o încercare de sistematizare bazată pe posibile mecanisme. Primele patru tipuri de reacţii sunt legate de inhibiţia COX1, fiind astfel comune tuturor AINS. Ultimele patru tipuri cunosc o mediere imună şi, prin urmare, nu sunt reacţii adverse de clasă ci medicament specifice.

TABEL 2 *CLASIFICAREA STEVENSON A REACŢIILOR ALERGICE ŞI PSEUDOALERGICE LA ASPIRINĂ/AINS*

Tip de reacţie	**Caracteristici**
Tipul 1: astmul şi rinita la AINS induse la pacienţii astmatici	-Un subset de pacienţi astmatici prezintă la ingestia de AINS, crossreactiv, o combinaţie de rinoree, obstrucţie nazală, hiperemie conjunctivală, edem periorbital, bronhospasm -reacţia este doză dependentă
Tipul 2: urticaria şi angioedemul induse de AINS la pacienţii cu urticarie cronică	-apare mai frecvent la cei cu urticarie cronică activă -reacţia este doză dependentă
Tipul 3: urticaria şi angioedemul induse de AINS la indivizi normali	-apare, de asemenea, crossreactiv la AINS -unii pacienţi vor dezvolta urticarie cronică idiopatică ulterior
Tipul 4: reacţii mixte la indivizi normali	-cuprinde caracteristici din tipurile precedente
Tipul 5: urticarie şi angioedem induse de un singur AINS la indivizi normali	-crossreactivitatea AINS nu apare -atopia pare a fi un factor de risc
Tipul 6: anafilaxie indusă de un singur AINS	-de asemenea crossreactivitatea AINS nu se întâlneşte -demonstrarea IgE specifice pentru un singur AINS este dificil de demonstrat
Tipul 7: menigită aseptică la un singur AINS	-este un diagnostic de excludere -descris la naproxen, sulindac, tolmentin şi ibuprofen
Tipul 8: pneumonita de hipersensibilizare la un singur AINS	-nu a fost descrisă pentru aspirină.

Deci:

- pentru primele patru tipuri de reacţii mecanismul posibil incriminat este cel legat de inhibiţia COX1 ; din acest motiv reacţiile apar crossreactiv la toate AINS care inhibă această enzimă
- pentru primele patru tipuri de reacţii nu este necesară existenţa unei sensibilizări anterioare; reacţiile sunt doză dependente
- pentru ultimele patru tipuri de reacţii existenţa unui posibil mecanism imun face ca reacţia încrucişată între mai multe AINS bazată pe inhibiţia comună a COX1 să nu existe; aceste reacţii sunt medicament specifice şi nu sunt doză dependente.

Astmul bronşic indus de aspirină

Termenul de „indus" este impropriu câtă vreme intoleranța la aspirină apare la pacienții care suferă, deja, de astm bronşic. Actual se vorbeşte, astfel, de ***Boală respiratorie exacerbată de aspirină (AERD).*** Prevalența pare să fie mai mare la sexul feminin (2,3 / 1 feminin/masculin) iar debutul tinde să fie mai precoce iar severitatea bolii mai mare. Atopia nu este un factor de risc dar 1/3 din pacienți sunt atopici iar un istoric familial de AERD este prezent la 6% din bolnavi.

Prevalența AERD bazată doar pe istoricul pacienților este de aproximativ 10% printre pacienții astmatici peste vârsta de 10 ani. Dacă se ia în considerare un test de provocare orală, se ajunge la 20%. La subpopulația astmatică care asociază polipoza nazală sau rinosinuzita chiar fără un istoric personal de reactivitate la aspirină prevalența creşte la 40% dacă se efectuează teste de provocare orală. Dacă există, însă, un istoric personal de simptome respiratorii la expunerea la aspirină testul de provocare orală este pozitiv în peste 80% din cazuri.

Populația pediatrică sub vârsta de 10 ani are rar AERD.

De asemenea astmul bronşic şi urticaria declanşate de expunerea la aspirină se asociează rar la acelaşi individ.

Intoleranța la aspirină survine de obicei după o perioadă evolutivă de luni sau ani a patologiei respiratorii superioare (rinitosinuzită, polipoză nazală) şi inferioare (astm bronşic).

Administrarea de aspirină la un pacient astmatic nu este considerată un factor de risc pentru dezvoltarea de novo a intoleranței la aspirină.

Evitarea expunerii la aspirină/AINS nu modifică cursul bolii respiratorii, procesul inflamator cronic neputând fi în acest fel modificat.

Scurtă incursiune în patogeneza AERD

Semnele şi simptomele datorate expunerii acestor pacienți la aspirină şi alte AINS sunt în strânsă legătură cu interceptarea căilor de metabolizare ale acidului arahidonic. Inhibiția căii ciclooxigenazelor favorizează calea 5 lipooxigenazei (5 LO) cu producția în exces de leucotriene .

La pacienții cu AERD s-a observat:

- deficit constituțional al prostglandinei E2 (care inhibă 5 LO şi contrabalansează efectele negative ale leucotrienelor- LT)
- exces funcțional al LTC4 sintetazei
- nivele crescute bazal ale LTC4 în sânge şi lichidul de lavaj bronşic
- nivele crescute bazal şi după test de provocare orală cu aspirină ale LTE4 în urină
- nivele scăzute ale lipoxinei (mediator antiinflamator rezultat în urma metabolizării acidului arahidonic)
- producție crescută de acid 15 hidroxieicosatetraenoic (15 HETE) la stimularea in vitro a celulelor epiteliale ale polipilor nazali cu aspirină (această observație stă la baza unui test diagnostic in vitro numit ASPIT – aspirin sensitive patient identification test)
- diminuarea apoptozei eozinofilelor implicate în inflamația bronşică şi nazală.

LTC4 determină bronhospasm, creşterea permeabilității capilare cu edem, chemotaxie eozinofilică, creşterea producției de mucus.

Diagosticul AERD

Documentarea intoleranței la aspirină şi la alte AINS se face printr-un protocol de provocare orală cu doze progresiv crescătoare administrate la intervale de 2 ore. Protocolul se desfăşoară pe o perioadă de 2 zile. Testul este negativ dacă la 2 ore după doza de 650mg aspirină nu apar simptome rinobronşice. Testul este considerat pozitiv la o scădere a VEMS cu 20% sau dacă apare rinoree/obstrucție nazală.

TABEL 3 PROTOCOL DE PROVOCARE ORALĂ PENTRU AERD

Doza de aspirină (mg)			
Timp	**ziua 1**	**ziua 2**	**ziua 3**
7 a.m.	Placebo	30	100-150
10 a.m.	Placebo	45-60	150-325
1 p.m.	Placebo	60-100	650

Contraindicațiile testului de provocare orală sunt:
- istoric de reacții anafilactice severe precipitate de aspirină sau alt AINS
- boli severe cardiace, digestive, hepatice, renale
- infecții de tract respirator în intervalul a 4 săptămâni anterior testării
- sarcină
- tratament cu betablocante

Trebuie menționat faptul că adminstrarea anterioară testului de provocare oral cu aspirină a mai mult de 10 mg prednison sau a unui inhibitor de receptori de leucotriene poate bloca o reacție pozitivă.

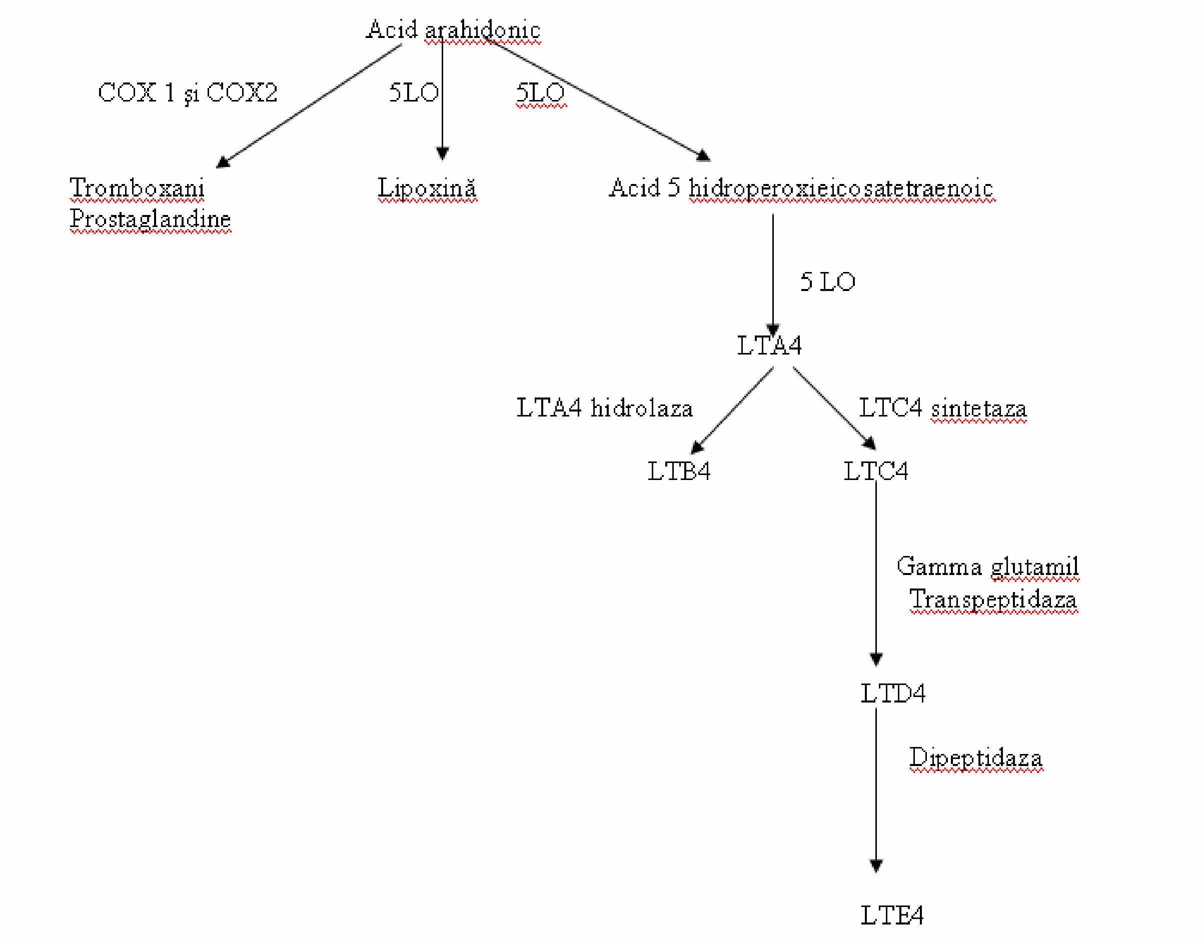
Acid arahidonic
COX 1 şi COX2
5LO
5LO
Tromboxani
Prostaglandine
Lipoxină
Acid 5 hidroperoxieicosatetraenoic
5 LO
LTA4
LTA4 hidrolaza
LTC4 sintetaza
LTB4
LTC4
Gamma glutamil
Transpeptidaza
LTD4
Dipeptidaza
LTE4

Tratament

Tratamentul AERD vizează în primul rând boala de bază : astmul bronşic si rinosinuzita.

Evitarea aspirinei dar şi a AINS neselective sau parţial selective este un gest imperativ.

Pacienţii cu AERD pot tolera inhibitori înalt selectivi de COX2 precum celecoxib sau etoricoxib.

Tratamentul de desensibilizare prin administrarea de doze progresiv crescătoare de aspirină până la doza terapeutică urmată de administrarea regulată a acesteia se poate face, fiind , însă, grevat de riscul efectelor adverse ale unei terapii cu AINS de lungă durată. ***Întreruperea aspirinei pentru o perioadă mai mare de 5 zile duce la reapariția intoleranței***. ***Desensibilizarea are caracter încrucişat putându-se efectua cu aspirină pentru toate celelalte AINS.***

În încheiere accentuez asupra necesităţii diagnosticării AERD, suspiciunea existenței intoleranței la aspirină/AINS fiind legată de existența unui astm sever, greu controlabil medicamentos asociat polipozei nazale sau rinosinuzitei.

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Rolul şi locul imagisticii medicale în diagnosticul pancreatitei acute

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OBIECTIV: Pancreatita acută rămâne o afecţiune cu prognostic incert cu toate cuceririle în domeniul diagnosticului şi tratamentului. Obiectivele care au stat la baza acestui studiu desfăşurat pe o perioadă de 13 ani au fost acelea de a constata şi de a evalua dificultăţile diagnostice în pancreatita acută.

MATERIAL ŞI METODĂ: Studiul a inclus 182 de pacienţi, strategia diagnostică implicând determinarea unui diagnostic pozitiv la internare urmată de determinarea diagnosticului de gravitate; acestea au fost completate cu datele imagistice, pentru ca în final să tentăm determinarea unui diagnostic etiologic.

CONCLUZIE: Determinarea cu acurateţe a unui diagnostic de pancreatită acută permite un abord terapeutic adecvat, centrat pe gravitatea bolii.

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pancreatita acută **diagnostic** **explorări imagistice**

ABSTRACT

Acute pancreatitis remains a diasease with bad prognosis, even diagnosis and treatment gains many advances. The aim of this study achieved on a period of 13 years was to evaluate the diagnostic difficulties in acute pancreatitis. We included in this study 182 patients, diagnostic strategy involving a good diagnosis at the admission followed by the severity diagnosis and completed by the imagery. Etiological diagnosis was tented for everyone. Results were shown on detail. We concluded that a good diagnosis assure a correct treatment for the severity of disease.

Key words: acute pancreatitis, diagnosis, imaging examens

INTRODUCERE

Pancreatita acută este o afecţiune severă în al cărei management sunt implicaţi, în diverse etape, chirurgi, internişti, gastroenterologi, medici care îşi desfăşoară activitatea în medicina de urgenţă, chiar medici de familie. De la prima descriere anatomoclinică de către Reginald Fitz (1889), pancreatita acută rămâne o afecţiune cu prognostic incert, distingându-se forme edematoase cu evoluţie benignă şi vindecare spontană şi forme necrotico-hemoragice, forme grave, a căror evoluţie rămâne totdeauna incertă (1). Debutează prin activarea prematură a zimogenelor intracelulare, inducţia factorilor de transcripţie, factorul nuclear kB (NF-kB) şi activatorul proteic 1 (AP-1), cu generare locală de cytokine şi chemokine.

Acestea conduc la inflamaţie şi leziuni celulare locale şi generale cu posibilă evoluţie spre insuficienţă multiorganică şi deces. Eliberarea citokinelor şi a altor mediatori proinflamatori reprezintă determinantul major al insuficienţei organice şi a mortalităţii precoce (2).

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Diagnosticul pancreatitei acute se bazează pe manifestările clinice şi pe rezultatele unor teste biochimice, dar acestea sunt numai câteva criterii din lungul şir de elemente de diagnostic ale acestei afecţiuni (3). Un studiu prospectiv multicentric realizat de AURC şi ARC, citat de Faginez (4) şi efectuat în Franţa arată că 4% dintre sindroamele dureroase acute ale abdomenului observate în cursul a 7 zile corespund unei pancreatite acute.

Tehnicile imagistice au ameliorat sensibil diagnosticul, scorurile clinice de diagnostic şi gravitate a bolii fiind îmbunătăţite prin implicarea ecografiei, computer tomografiei (CT), rezonanţei magnetice nucleare (RMN) etc. Introducerea CT ca metodă diagnostică standard pentru detectarea timpurie a necrozei a permis o înţelegere mai bună a relaţiei dintre modificările morfopatologice din pancreas şi evoluţia naturală a bolii (5).

Scopul studiului nostru a fost acela de a evalua dificultăţile diagnostice din pancreatita acută şi de a evidenţia rolul diagnostic major al computer tomografiei ca metodă imagistică de diagnostic, stadializare şi urmărire evolutivă.

MATERIAL ŞI METODĂ

Evaluarea s-a efectuat, retrospectiv, pe durata a 13 ani (ianuarie 1995-decembrie 2007) şi a inclus un număr de 211 pacienţi cu pancreatită acută internaţi în Clinica de Chirurgie a Spitalului Clinic de Urgenţă Militar din Craiova. Foile de observaţie ale tuturor pacienţilor incluşi în studiul nostru au fost evaluate, extrăgându-se elementele care au stat la baza diagnosticului de pancreatită acută. Strategia diagnostică, aşa cum a fost iniţiată încă de la internarea primului pacient, a parcurs stabilirea diagnosticului pozitiv pe baza datelor clinice coroborate cu câteva explorări paraclinice, urmată de stabilirea diagnosticului de gravitate pe baza unor criterii, în primul rând, bioclinice, urmărite în evoluţia primelor 24-48 de ore. Etapa diagnosticului imagistic a adus informaţii legate de distrugerea structurii pancreatice, devenind operativă după 4-7 zile de la debutul bolii. Supravegherea ulterioară, bioclinică şi imagistică a evoluţiei pancreatitei acute a impus strategia terapeutică. Diagnosticul etiologic s-a constituit într-un alt important factor de decizie a secvenţialităţii terapeutice medico-chirurgicale după stingerea puseului acut.

Trebuie remarcat faptul că tomografia computerizată a devenit un element extrem de important de diagnostic odată cu acumularea experienţei în domeniu, această tehnică imagistică fiind folosită şi cu rol de evaluare a evoluţiei bolii. Nicio altă explorare imagistică utilizată (cu referire la ecografia abdominală şi, atunci când a fost disponibilă, la rezonanţa magnetică nucleară) nu şi-a dovedit o eficienţă superioară acesteia. În cazul ecografiei am constatat adesea dificultăţi de descriere a pancreasului în contextul meteorismului abdominal din cadrul bolii, RMN fiind mai greu accesibilă, dar şi mai scumpă comparativ cu CT. În aceste condiţii am apreciat raportul calitate-preţ ca fiind adecvat pentru CT.

REZULTATE

Lotul de pacienţi studiaţi a fost alcătuit din 132 bărbaţi şi 79 femei, 101 provenind din mediul urban, iar 110 din mediul rural. Vârsta medie a fost de 59 de ani (cu limite între 18 şi 82 de ani, decadele cu incidenţă maximă fiind 41-50 de ani, urmată de aproape de decadele 51-60 şi 61-70 de ani.

În studiul nostru am constatat că profilul clinic al afecţiunii se evidenţiază conform cu cunoştinţele clasice, pancreatita acută fiind un sindrom dureros abdominal brutal, cu iradiere posterioară, decalaj termic, amestec de semne de ocluzie şi peritoneale şi o tendinţă de colaps cardiovascular.

În 2/3 din cazuri dozarea amilazemiei a constatat cifre superioare (de 4 ori normalul), aceste informaţii simple culese în urgenţă permiţând un diagnostic de certitudine.

În 1/3 din cazuri diagnosticul a fost de prezumţie, adesea prin eliminarea unor sindroame acute abdominale, în aceste cazuri fiind necesară lărgirea investigaţiilor biologice (amilazurie, lipazemie, leucocitoză, calcemie) completată cu alte mijloace diagnostice (scanner-ul abdominal, puncţia-lavaj a peritoneului şi laparotomia diagnostică).

Astfel, studiind datele clinice, am constatat prezenţa sindromului dureros acut abdominal tipic la 66,5% dintre pacienţi şi atipic la 33,5%. Alte date clinice semnificative statistic constatate sunt: alterarea stării generale (51,2%), sindromul ocluziv caracterizat de vărsături, meteorism abdominal şi oprirea tranzitului (42,5%), apărarea musculară localizată (48,2%) ori difuză (18,7%), diferite tulburări cardiovasculare (59,6%), acestea din urmă împreună cu oligoanuria, polipneea şi sindromul icteric constituindu-se într-o simptomatologie ce traduce insuficienţa de organ şi definind gravitatea bolii încă de la debut.

Elementele biologice caracteristice ce au susţinut diagnosticul au fost leucocitoza (71,5%), hiperamilazemia-amilazurie (39,4%), creşterea bilirubinei (22,3%), hiperglicemia (31,6%). Acestea au fost coroborate cu date clinice în cadrul indicilor bioclinici de gravitate a bolii.

La examenul radiologic simplu (abdominal şi toracic), utilizat de rutină, s-au constatat elemente semnificative în 6,7% din cazuri – ansa sentinelă în 5,6% din cazuri şi reacţie lichidiană pleurală stângă în 3,8% din cazuri – dar a permis excluderea altor sindroame acute abdominale.

Ecografia abdominală s-a efectuat de rutină, dar rezultatele au fost adesea parazitate de meteorismul abdominal. Utilitatea sa a fost efectivă în cazul etiologiei biliare şi pentru diagnosticul colecistitei acute ori a colecţiilor abdominale.

Pentru aprecierea gravităţii bolii am utilizat indici prognostici, bioclinici, peritoneali ori computer-tomografici (Imrie, Ranson, APACHE şi Balthazar). Conform acestora am încadrat cazurile ca pancreatite acute grave la 87 de pacienţi (41,2%) şi uşoare ori medii la 124 de pacienţi (58,8%).

Compararea datelor CT şi a datelor bioclinice a dat, uneori, rezultate divergente. CT nu este superioară testelor bioclinice; în schimb, rezultatul examinării iniţiale prezintă interesul furnizării unui document de referinţă susceptibil de a fi comparat cu examinările CT ulterioare pentru supravegherea evoluţiei şi stabilirea indicaţiei terapeutice.

S-a practicat tomografia computerizată în 88 de cazuri: la internare (19 pacienţi), după o zi la 21 de pacienţi, după 4 zile la 18 pacienţi, după 7 zile în 14 cazuri, postoperator (16 cazuri) şi repetat la 36 de pacienţi. Încadrarea conform clasificării Balthazar a fost următoarea: gradul A – 18 cazuri (20,4%), B la 11 cazuri (12,5%), C în 22 de cazuri (25%) (Fig.1), D la 26 de pacienţi (29,5%) şi E la 11 cazuri (12,5%) (Fig. 2, 3 şi 4).

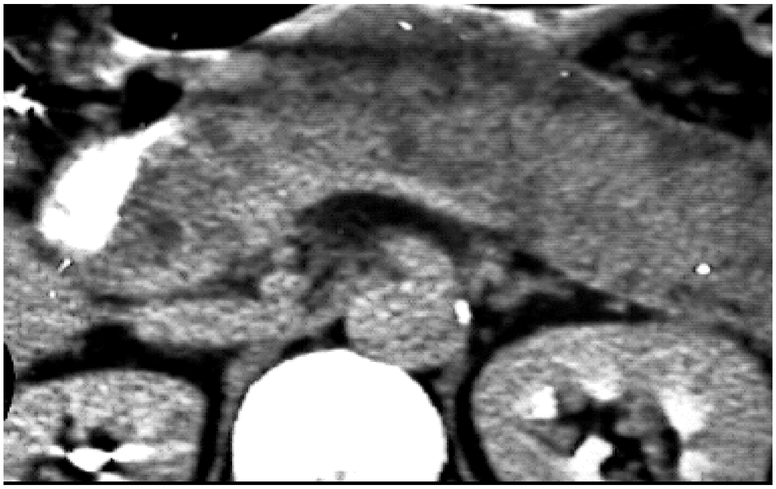

Fig.1. Pancres cu colecţie minimă peripancreatică şi zone minime de necroză. Scor Balthazar C

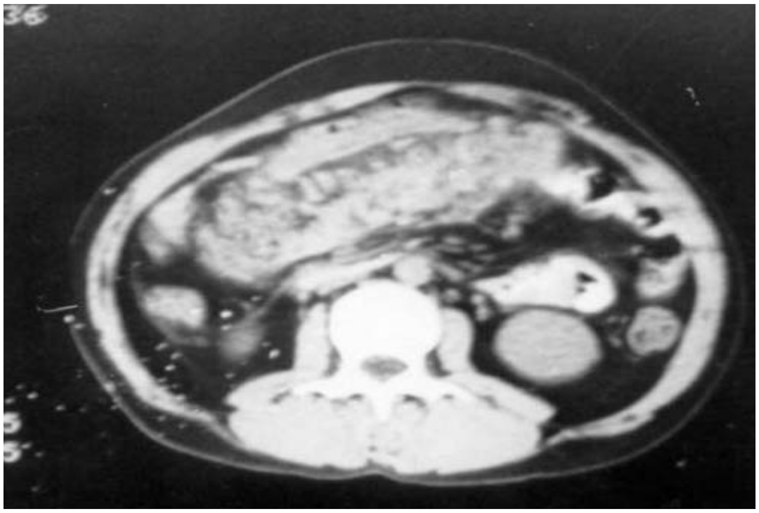

Fig.2. Pancreatită acută necrotico-hemoragică. Scor Balthazar E

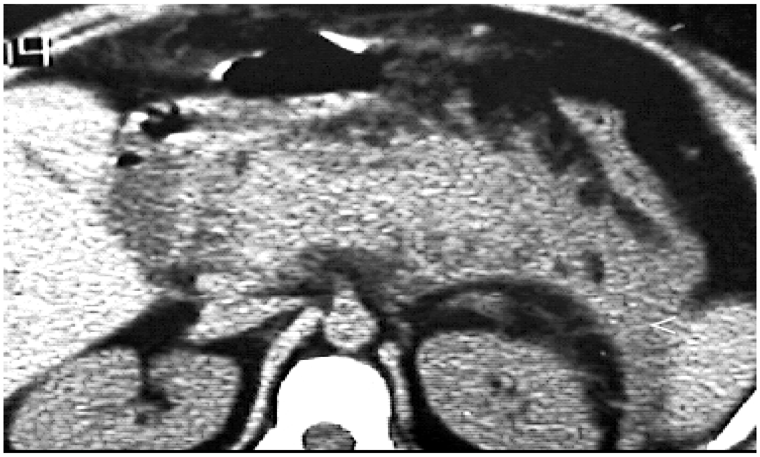

Fig.3. Pancreatită necrotică: pancreas cu zone de necroză cu fuzee peripancreatice, anterior şi pararenal stâng; semnul "haloului". Scor Balthazar E

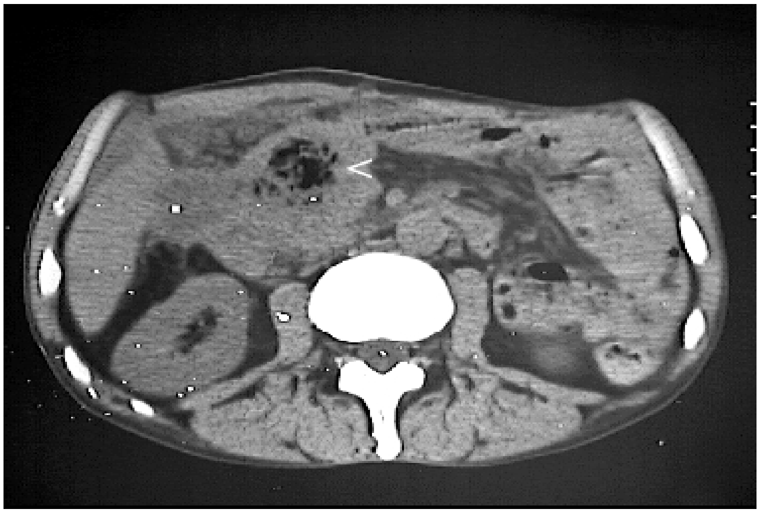

Fig.4. Necroză infectată – colecție fluidă cu pereți groşi contur difuz şi bule de gaz. Abces pancreatic infracefalic. Scor Balthazar E

Din punct de vedere etiologic am constatat etiologia biliară la 36,5% dintre pacienţi, alcoolică la 39,2%, pancreatita acută recurentă constatându-se la 54 de pacienţi (25,6%).

Diagnosticul de necroză infectată a avut la bază mai multe criterii: clinice (sindrom infecţios), biologice (leucocitoză), computer-tomografice (bule gazoase), puncţie eco-ghidată şi datele constatate intraoperator (aspecte macroscopice) (Fig. 5). Bacteriologia pozitivă s-a constatat la 41 de pacienţi (19,4%) prin prelevări intraoperatorii, *E.coli* fiind cel mai frecvent agent patogen (26%), urmat de *Enterococcus*, *Klebsiella*, *Enterobacter* şi stafilococ, fungii fiind descoperiţi izolat.

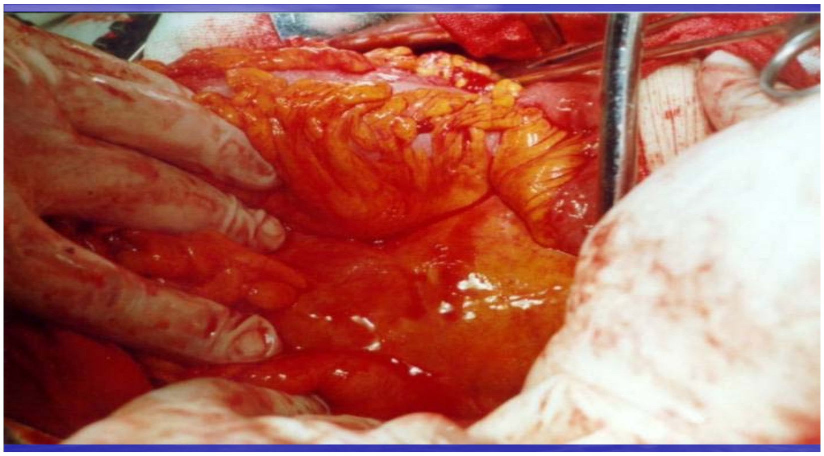

Fig.5. Pancreatită acută – fuzee de citosteatonecroză. Aspect intraoperator

DISCUŢII

Pancreatita acută rămâne una dintre marile probleme insuficient cunoscute datorită frecvenţei sale relative în chirurgia abdominală de urgenţă, dificultăţilor de diagnostic şi mai ales terapeutice ale căror principii comportă încă discuţii şi mortalitate crescută în formele severe. Mortalitatea generală a bolii variază între 2,1-7,8% (6). Evoluţia bolii este determinată de doi factori care reflectă severitatea acesteia: insuficienţa de organ şi necroza pancreatică. În general, decesul prin pancreatită acută severă se produce în primele două săptămâni. Mortalitatea tardivă se datorează infecţiilor, în particular infectării necrozelor pancreatice. Studii recente au demonstrat statistic reducerea mortalităţii în ultima perioadă ca rezultat al tratamentului intensiv modern (7).

Din punctul de vedere al strategiei diagnostice, cel mai important lucru la internarea bolnavului în spital este stabilirea diagnosticului pozitiv de pancreatită acută şi, apoi, în primele 24-48 de ore, a celui de gravitate (8). În experienţa noastră, diagnosticul pozitiv a fost posibil şi corect de la început la 69,78% dintre pacienţi, bazându-ne pe tabloul clinic caracteristic ori mai puţin specific, sprijinit de câteva explorări paraclinice: valori crescute ale amilazemiei, amilazuriei, leucocitozei, proteinei C reactive şi altele mai puţin semnificative (hiperglicemie, hiperbilirubinemie). Investigaţiile imagistice sunt nespecifice în primele 24-48 de ore (5).

Diagnosticul de gravitate se sprijină, în primul rând, pe criterii bioclinice (Ranson, Imrie ori APACHE), dar şi pe dozarea unor markeri ca lipazemia, proteina C reactivă, procalcitonina (8). Criteriile morfologice stabilite pe baza imaginilor computer-tomografice au permis o nouă clasificare a pancreatitelor acute şi încadrarea lor în forme uşoare-medii şi grave. CT permite, de asemenea, supravegherea evoluţiei colecţiilor, cercetarea gazelor (semne de infecţie a necrozei) în spaţiile retroperitoneale şi vizualizarea unui abces pelvin (9).

Există studii care arată superioritatea scorului Balthazar în faţa scorurilor bioclinice (10,11). Experienţa noastră confirmă faptul că nu există totdeauna o corelaţie absolută între clasa Balthazar D şi E cu un scor bioclinic Glasgow peste 3 (12).

Invers, un astfel de scor a fost totdeauna în concordanţă cu clasa D şi E Balthazar. Subliniem, în schimb, că întinderea necrozei este proporţională cu mortalitatea, dar trebuie făcută distincţie între necroza infectată şi abces. Intervenţiile chirurgicale asupra necrozelor sterile sunt discutabile (13,14).

CONCLUZII

1. Pancreatita acută este o boală relativ frecventă, diagnosticul său fiind dificil. Problema diagnostică impune, încă de la primirea pacientului, o strategie care să stabilească diagnosticul pozitiv de pancreatită acută, gravitatea acesteia, etiologia, iar în cazul formelor necrotice, dacă necroza este sterilă sau infectată.
2. Diagnosticul pozitiv iniţial depinde, în principal, de o anamneză precisă şi, în egală măsură, de un examen clinic îngrijit, sprijinit de câteva date de laborator simple (amilazemie, amilazurie, leucocitoză) şi completat cu o eventuală puncţie-lavaj peritoneală ori laparoscopie.
3. Diagnosticul formelor grave trebuie stabilit concomitent cu cel pozitiv ori în primele 24-48 de ore şi se sprijină pe existenţa scorurilor bioclinice, a insuficienţei de organ şi pe datele computer-tomografice, strategia terapeutică fiind subordonată gravităţii pancreatitei acute şi evoluţiei sale naturale.

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TUMORĂ RENALĂ STÂNGĂ CU INVAZIE ÎN MEZOCOLON ŞI COLONUL DESCENDENT

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col. conf. univ. dr. Dan L. MISCHIANU

Scop: evidențierea debutului atipic al unei tumori renale şi necesitatea cunoaşterii colectomiei în chirurgia rinichiului tumoral.
Material şi metodă: prezentare de caz clinic de tumoră renală cu debut atipic la care nefrectomia radicală a impus colectomie segmentară datorită invaziei colonului descendent.
Discuții şi concluzii:

- **Varicocelul apărut brusc poate fi primul semn în tumora renală stângă**
- **Chirurgia rinichiului tumoral impune uneori colectomia segmentară „de urgență”**
- **Computer tomograful orientează asupra posibilei extensii locale a suferinței tumorale dar aspectul intraoperator este definitoriu.**

tumoră renală, colectomie, tratament chirurgical

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tumora renală colectomie tratament chirurgical

Objective: describing acute onset of a renal tumor and the necessity of applying colectomy in renal tumor surgery
Material and method: case report of a renal tumor with uncommon onset in which radical nephrectomy have imposed segmentary colectomy due to descent colon tumoral invasion
Discusions and conclusions:

- varicocele with acute onset can be the first sign in left renal tumor
- renal tumor surgery may impose in some cases segmentary colectomy
- computer tomography shows the possibility of local extension of the tumor, but intraoperatory aspect is decisive.

Keywords: renal tumor, colectomy, surgical treatment

INTRODUCERE

Carcinomul renal tip Grawitz este tumora întâlnită cu frecvență maximă în decadele 5 şi 6 fiind de două ori mai frecventă la bărbați decât la femei. Cele mai frecvente manifestări clinice se grupează într-o triadă caracteristică reprezentată de durere, hematurie şi tumoră renală palpabilă. În afara simtomatologiei clasice este de reținut faptul că tumorile renale se pot manifesta prin semne, simptome şi sindroame extrem de variate, foarte multe fără legătură cu aparatul urinar. Dintre acestea varicocelul simptomatic secundar obstrucției venei spermatice poate apărea ca prim semn clinic. Tratamentul carcinomului renal parenchimatos este eminamente chirurgical şi constă în nefrectomia radicală perifascială.

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PREZENTARE CAZ CLINIC

Pacientul MN în vârstă de 53 ani, fumător, din mediu rural, fără antecedente personale patologice notabile se prezintă în Clinica de Urologie pentru mărire de volum şi jenă la nivelul hemiscrotului stâng apărute relativ brusc. Examenul obiectiv evidenţiază prezenţa unui varicocel stâng. Examenele de laborator în limite normale cu excepţia VSH-ului crescut. Este operat prin abord inghinal practicându-se ligatura venelor spermatice dilatate (procedeu Palomo). Evoluţia postoperatorie a fost simplă fiind externat vindecat chirurgical.

La aproximativ 30 zile de la externare pacientul prezintă brusc dureri colicative lombare stângi însoţite de hematurie macroscopică cu cheaguri motiv pentru care se prezintă în clinică pentru investigaţii şi tratament. Examenele de laborator evidenţiază patologic VSH crescut, hematurie microscopică. Ecografia arată prezenţa la nivelul rinichiului stâng, în treimea inferioară, a unei formaţiuni cu contur neregulat, neomogenă, cu diametrul 103/102 mm, vascularizată la examenul Doppler color, fără adenopatii subdiafragmatice. Radiografia pulmonară fără modificări patologice.
RRVS (fig.1) normală iar UIV (fig.2,3) evidenţiază absenţa secreţiei şi excreţiei rinichiului stâng (rinichi stâng mut urografic).

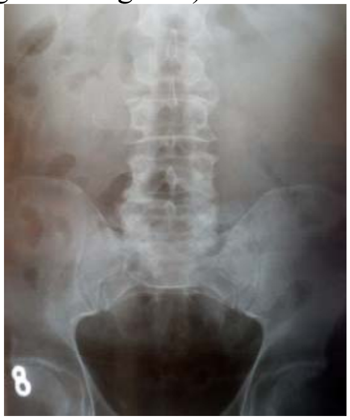
Fig. 1

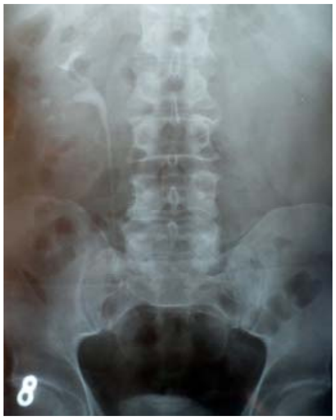
Fig.2

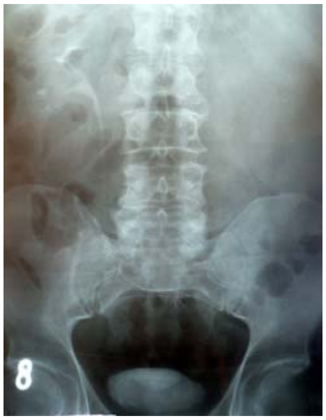
Fig.3

Fig.1 RRVS fără imagini de calculi radioopaci
Fig.2,3 absenţa secreţiei şi excreţei renale stângi – aspect urografic la 15 şi 60 de minute

Examenul CT (fig. 4,5,6) notează existenţa la nivelul rinichiului stâng a unei formaţi tumorale voluminoase de 11/10 cm ce deformează conturul renal în special în jumătatea inferioară, fascia renală îngroşată şi cu contur neregulat spre grăsimea din jur; nu se remarcă modificări de calibru ale lumenului venelor renale stângă şi cavă inferioară, nu se evidenţiază adenopatii locoregionale. Nu sunt prezente alte elemente de patologie a organelor din jurul rinichiului tumoral.

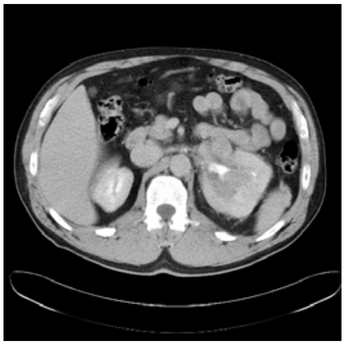
Fig. 4

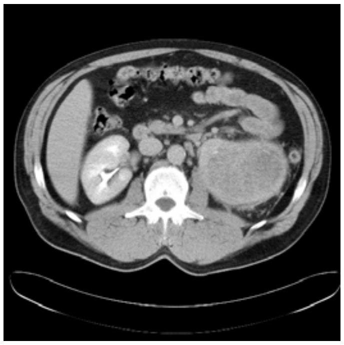
Fig. 5

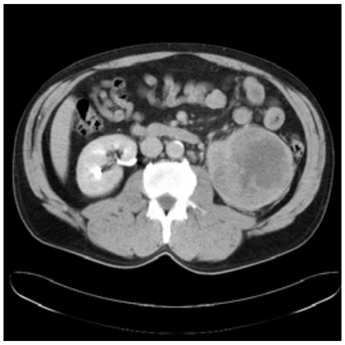
Fig. 6

Fig. 4,5,6 aspecte CT cu substanţă de contrast ale rinichiului tumoral

Se decide intervenția chirurgicală – nefrectomie stângă radicala perifascială prin abord anterior transperitoneal. Intraoperator se evidențiază o tumoră renală de aproximativ 10 cm în diametru ce infiltrează mezocolonul şi colonul descendent sub flexura splenică. S-a practicat extirparea în bloc a rinichiului tumoral împreună cu țesut celulogrăsos inclus în fascia lui Gerota, suprarenala stângă şi a mezocolonului şi colonului descendent interesat tumoral cu limită de siguranță, urmată de colorafie termino-terminală în dublu strat. S-au plasat tuburi de drenaj în loja renală, perianastomotic şi în fundul de sac Douglas. Evoluția postoperatorie a fost lent favorabilă cu reluarea tranzitului intestinal şi pentru gaze la aproximativ 5 zile postoperator.

În ziua a-12-a postoperator pe tubul de dren lombar se exteriorizează un lichid maroniu cu aspect fecaloid. Având în vedere starea generală bună a bolnavului şi prezența tranzitului intestinal pentru materii fecale şi gaze s-a decis tratamentul conservator al fistulei anastomotice cu suprimarea tubului de dren când acesta a devenit ineficient. Pacientul fiind externat vindecat chirurgical.

Examenul histopatologic – carcinom renal cu celule clare, multicentric grad I-II invaziv în grăsimea renală, seroasa şi musculara colonului şi în mezocolonul descendent.

Discuții şi concluzii:

- Varicocelul **stâng** apărut brusc poate fi prima manifestare a unei tumori renale iar examenul ecografic este absolut necesar în protocolul de investigații al varicocelului.
- Examenul CT orientează asupra extensiei locale tumorale dar aspectul intraoperator al leziunii este definitoriu şi poate modifica major intervenția chirurgicală propusă inițial.
- Decizia de a efectua colectomia a fost luată intraoperator fără să existe o pregătire preoperatorie a colonului şi de aceea colectomia segmentară „de urgență” trebuie bine cunoscută de orice urolog care abordează patologia chirurgicală tumorală renală.
- În cazul tumorilor renale mari pacientul trebuie informat (chiar în absența unor semne imagistice) despre posibilitatea existenței unei extensii locale care ar putea impune colectomia segmentară cu colorafie termino-terminală sau colostomie temporară (greu de acceptat de către bolnav).

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NEUROFIBROM INTRATORACIC GIGANT DE NERV VAG

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dr. AURORA FERA, dr. CORNECI

INTRODUCERE:

Deşi tumorile neurogene reprezintă cele mai frecvente tumori dezvoltate la nivelul mediastinului posterior, tumorile neurogene dezvoltate din nervul vag sunt foarte rare. Prezentăm cazul unui pacient în vârstă de 61 de ani internat în Clinica noastră cu diagnosticul de tumora pulmonara dreapta. Intraoperator s-a infirmat apartenența pulmonara a tumorii şi s-a constatat apartenența tumorii de nervul vag drept. S-a practicat excizia completa a tumorii, rezecția nervului vag şi esomiomectomie extramucoasa. Examen histopatologic: neurofibrom.

PREZENTARE DE CAZ

Pacientul in vârsta de 61 de ani se internează în clinica noastră cu diagnosticul de tumora pulmonara dreapta , diagnostic stabilit in urma efectuării unei radiografii toracice şi a examenului computer tomograf toracic. La internare prezintă dispnee la eforturi medii, tuse iritativă. Analizele hematologice au evidențiat: hemograma cu uşoara anemie (Hb=10,9g/dl), biochimie şi coagulograma în limite normale.

Examenul cardiologic stabileşte diagnosticul de cardiopatie ischemica silențioasă.

Probele funcționale ventilatorii externe au arătat disfuncție ventilatorie medie , cu reducerea VEMS cu 24%.

Radiografia toracică în incidență anteroposterioară şi de profil drept a evidențiat opacitate de intensitate subcostală, relativ bine delimitata, cu contur net ce ocupa jumătatea superioară a hemitoracelui drept (fig 1)

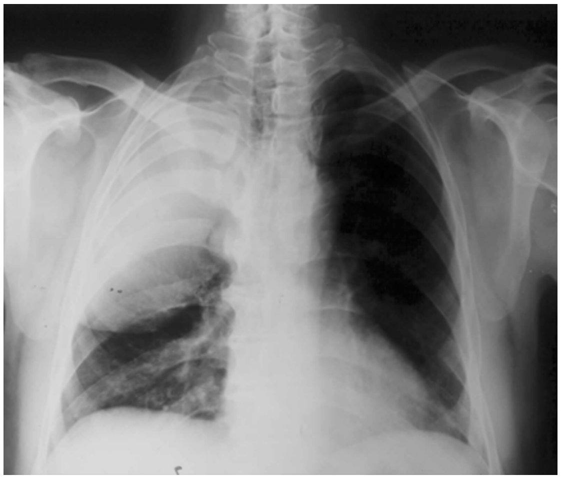

Fig.1: Radiografie toracică incidență anteroposterioară
(opacitate de intensitate subcostală, relativ bine delimitată
ce ocupă jumătatea superioara a hemitoracelui drept)

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Examenul computer tomograf toracic decelează masa cu structura tisulară, neomogenă, cu dimensiuni de 21/18/16 cm, localizată la nivelul lobului superior drept şi inferior drept; adenopatii de 2,7cm diametrul axial şi bloc adenopatic voluminos de aproximativ 6/3,8 cm cu zona de necroza centrală, localizate paratraheal drept.(fig.2, fig.3)

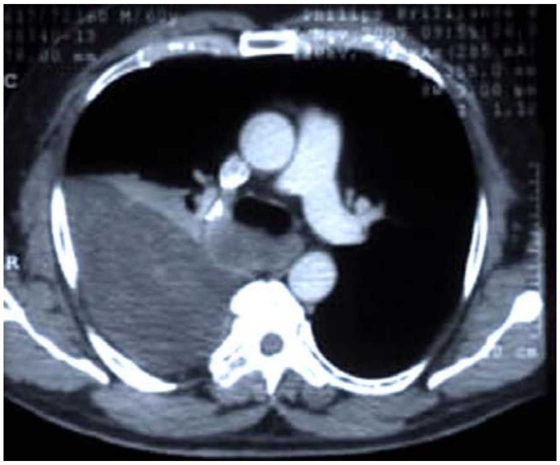

Fig. 2 Examen CT toracic
(tumora solidă localizată la nivelul lobului superior şi inferior drept; bloc adenopatic paratraheal şi retrotraheal drept)

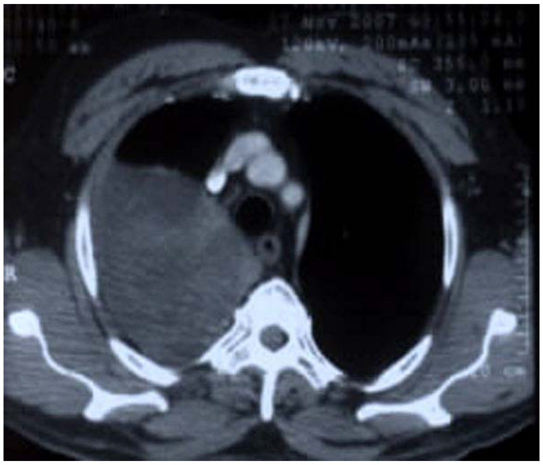

Fig. 3 Examen CT toracic
(tumora solidă localizată la nivelul lobului superior drept, în contact cu traheea)

Traheofibrobronhoscopia descrie: pintene la bronşia lobară superioară dreapta rotunjit cu obstrucţie prin compresiune extrinseca anterioară şi posterioară, cu edem zonal şi obstrucţie cvasicompletă la bronşia segmentară apicală a lobului superior drept.

S-a intervenit chirurgical prin toracotomie anterolaterală dreapta prin spaţiul 4 intercostal, intraoperator decelându-se(fig.4, fig.5) formaţiune tumorală solida, ovoida, albicioasa, voluminoasa, situată la nivelul mediastinului visceral şi posterior, ce comprimă parenchimul pulmonar al lobului superior drept şi inferior drept, comprimă traheea lateral drept şi posterior şi esofagul toracic superior faţă de care pare să nu aibă plan de clivaj.

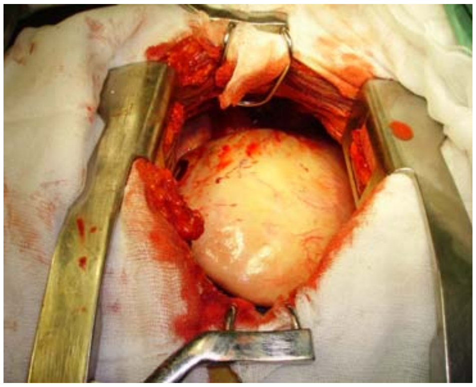

Fig.4 Aspect intraoperator
(tumora dezvoltată în cavitatea pleurala dreapta)

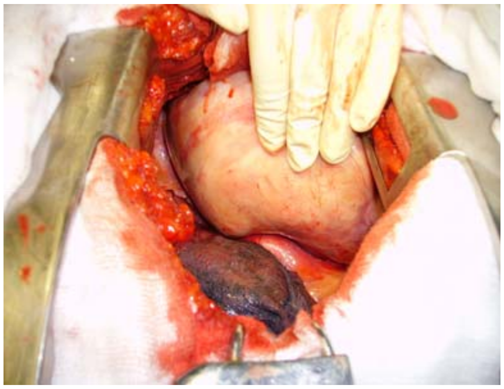

Fig.5 Aspect intraoperator
(tumora comprima parenchimul pulmonar pe care nu îl invadează)

Mobilizarea formațiunii tumorale decelează apartenența de nervul vag drept. Se constată că formațiunea tumorală prezintă două porțiuni(in iceberg): o porțiune de 6/5/3 cm. dezvoltată în mediastinul posterior în contact intim cu esofagul şi nervul vag drept din care formațiunea tumorală se dezvolta şi o a doua porțiune de dimensiuni mult mai mari, dezvoltată în mediastinul visceral şi cavitatea pleurală dreaptă pe care o ocupă în jumătatea ei superioară.

Cele două porțiuni sunt limitate de crosa venei azygos (fig.6). Se rezecă crosa venei azygos(fig.6), după care se procedează la extirparea formațiunii tumorale dezvoltată în cavitatea pleurală dreapta(fig.8), abandonându-se porțiunea dezvoltata în mediastinul posterior.

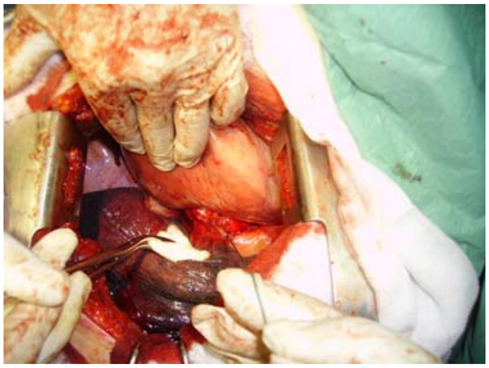

Fig.6 Aspect intraoperator
(rezecția crosei venei azygos)

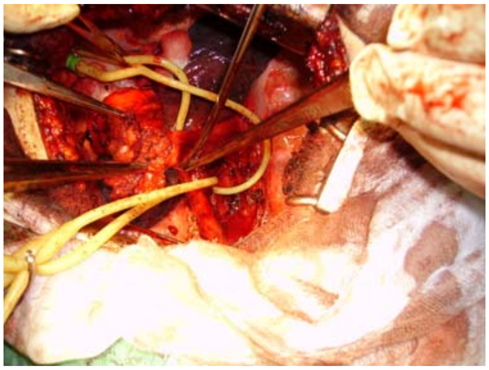

Fig.7 Aspect intraoperator
(rezecția nervului vag drept)

Se procedează la disecția formațiunii din mediastin fata de coloana vertebrala şi de trahee. Se confirmă originea din nervul vag drept(fig.7) care este rezecat. Disecția față de esofag se face în planul submucos esofagian, practicându-se esomiomectomie extramucoasă. Se practică astfel excizia în totalitate a formațiunii tumorale.(fig.9). Examen extemporaneu: tumora neurogena benigna.

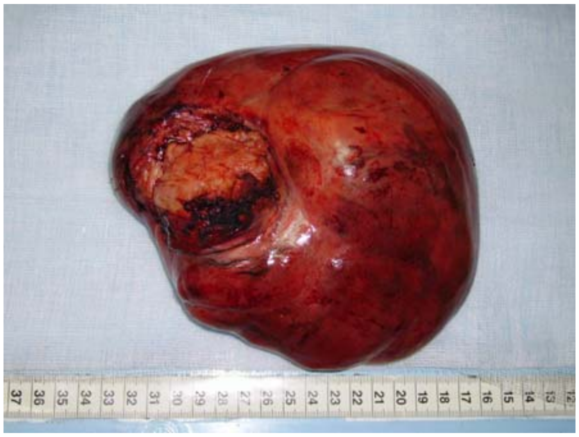

Fig.8 Piesa de rezecție 1
(porțiunea tumorală dezvoltată în
cavitatea pleurală dreapta)

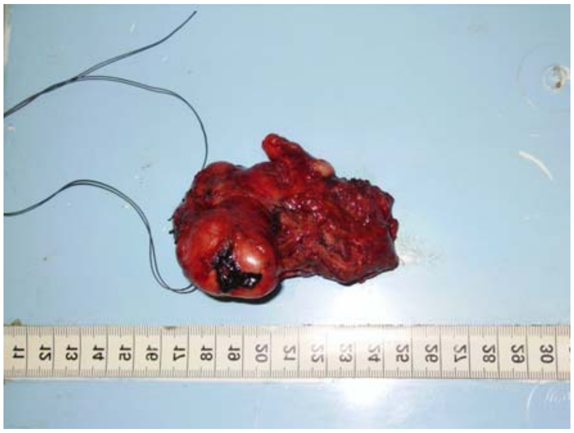

Fig.9 Piesa de rezecție 2
(porțiunea tumorală dezvoltată în
mediastinul posterior)

Evoluție postoperatorie favorabilă cu expansionare completă a parenchimului pulmonar(fig.10).

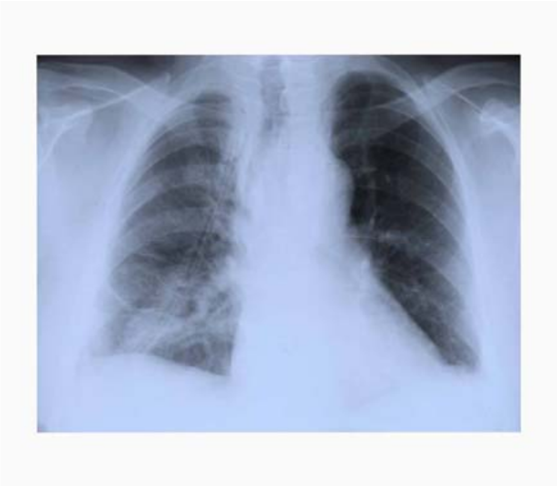

Fig. 10 Radiografie postoperatorie (expansionare completă a parenchimului pulmonar)

Examen histopatologic la parafina: neurofibrom

DISCUTII

Tumorile neurogene mediastinale îşi au originea în celulele ce derivă din creasta neurală, celulele din care se dezvoltă sistemul nervos periferic. Aceste celule cu origine ectodermala sunt: celule ganglionare din ganglionii spinali şi din sistemul nervos autonom, celule paraganglionare din sistemul simpatic şi parasimpatic, celule Schwann şi celule satelite. Țesutul conjunctiv de susținere, de origine mezodermala participă la formarea acestor tumori. Schwanoamele, neurofibroamele şi schwanomul malign se dezvoltă din nervii periferici, iar ganglioneuromul, ganglioneuroblastomul, neuroblastomul se dezvoltă din ganglionii nervoşi simpatici.

Copiii şi adulții tineri sunt mult mai susceptibili de a dezvolta tumori cu originea în ganglionii nervoşi autonomi, două treimi din aceste tumori având caracter malign. La adulți majoritatea tumorilor neurogene se dezvoltă din nervii periferici, 98% din acestea fiind benigne. Neurofibroamele reprezintă aproximativ 25% din tumorile neurogene mediastinale dezvoltate din nervii periferici. Neurofibroamele dezvoltate la nivelul nervului vag intratoracic sunt extrem de rare(cca. 1%).

Gayola şi colaboratorii au strâns din literatura de specialitate 20 de cazuri de neurofibroame dezvoltate din porțiunea intratoracică a nervului vag.

Neurofibroamele apar ca tumori boselate ce par a fi încapsulate, dar fără a avea o capsulă adevărată din țesut conjunctiv. Microscopic se descrie o proliferare de celule schwann şi de celule perineurale în proporție egală. Microscopia electronica evidențiază celule alungite, axoni mielinizați şi nemielinizați, stroma colagenică extensivă.

O proporție de 30-45% din pacienții cu neurofibrom mediastinal au boala Recklinghausen. Neurofibroamele multiple sau neurofibromul plexiform sunt patognomonice pentru neurofibromatoza. Riscul degenerării maligne a neurofibroamelor la această categorie de pacienți este crescut până la 10%.

Tumorile neurogene dezvoltate din porțiunea intratoracică a nervului vag sunt de cele mai multe ori asimptomatice şi sunt descoperite cu ocazia efectuării unei radiografii toracice. Simptomatologia este dată de compresiunea structurilor anatomice învecinate şi este reprezentată de tuse, disfonie, dispnee, dureri toracice. Simptomatologia ce ar putea fi atribuită interesării tumorale a fibrelor vagale destinate inervării cordului, musculaturii bronşice sau gastrointestinale nu a fost descrisă în practica şi în literatura de specialitate.

Examenul radiologic şi examenul computer tomograf pun în evidență formațiunea tumorală, apreciază caracterele tumorii şi relația cu structurile anatomice învecinate.

Examenul RMN poate aprecia etiologia tumorii neurogene: în neurofibroame fiind caracteristic un semnal de intensitate înalta în periferie şi de intensitate medie în centru în modul T2.

Excizia tumorală este recomandată nu doar pentru a confirma natura leziunii ci şi pentru a preveni continuarea evoluției şi compresia structurilor mediastinale învecinate. Pentru tumorile benigne, intervenția chirurgicală este curativă.

CONCLUZII

Tumorile mediastinale neurogene, considerate a fi cele mai frecvente tumori mediastinale, au fost devansate în ultimii ani de către timoame şi limfoame.

Dintre tumorile mediastinale neurogene, tumorile dezvoltate din nervul vag sunt extrem de rare, dar ele trebuie luate în considerație în diagnosticul diferențial al formațiunilor tumorale mediastinale.

Am considerat utila prezentarea acestui caz datorită rarității, volumului tumoral, cât şi al problemelor de tactică operatorie pentru excizia completă a formațiunii.

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Incompetența sfincterului uretral

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DEFINIȚII

Când o femeie tuşeşte şi pierde urină este vorba de incontinență la stres, termen inventat de Sir Eardley Holland în 1928. Cercetările au arătat că multe afecțiuni pot duce la incontinență la stres şi de aceea aste bine să folosim acest termen doar pentru simptome şi semne. În 1976, ICS a adoptat termenul „ incontinență reală la stres" (GSI) care a fost definit ca pierderea involuntară de urină prin uretră când presiunea intravezicală depăşeşte presiunea uretrală maximă în absența activității detrusorului (Abrams şi colaboratorii 1988). Se preferă termenul „incompetența sfincterului uretral" (USI) care indică mai exact fiziopatologia.

Folosim în continuare simptomul de „pierdere involuntară de urină la efort fizic" şi semnul de observare a pierderii de urină prin uretră în timpul activităților fizice.

Definitions:

When a woman coughs and loses urine, it means the stress insufficiency, a word invented by Sir Eardley Holland in 1928. The scientific research has indicated that many diseases can lead to stress insufficiency, and that is why it is good to use this word just for symptoms and signs. In 1976, ICS has adopted the notion "real stress insufficiency" (GSI) which was defined as the involuntary loss of urine by urethra when the intra-vesicle pressure passes beyond the highest urethral one in the lack of activity of the detrussor muscle (Abrams & co-workers, 1988). They would rather have the notion "urethral sphincter insufficiency" (USI) which indicates the physiopathology more accurately.

Further on we use the "involuntary loss of urine while physical effort" symptom and the sign of observation of the loss of urine by urethra during sports (physical activities).

ETIOLOGIE

Incompetența sfincterului uretral (ISD) are două cauze – coborârea gâtului vezicii şi a uretrei proximale, şi slăbirea rezistenței uretrale. În mod frecvent cele două cauze apar împreună. La cea dintâi avem de-a face cu o transmitere inegală a presiunii intraabdominale către uretra proximală, ceea ce duce la inversarea gradientului normal de presiune dintre vezică şi uretră, rezultând într-o închidere uretrală negativă.

Când este vorba de scăderea rezistenței uretrale, presiunea intrauretrală în repaus este mai mică decât presiunea intravezicală.

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Definiția uretrei de joasă presiune este: presiunea uretrală maximă este mai mică de 20 centimetri coloană de apă (Sand şi colaboratorii 2007au presiunea Valsalva în punctul de scurgere este sub 60 centimetri coloană de apă (McGuire şi colaboratorii 1993). Factorii care provoacă aceste stări sunt prezentați mai jos.

Slăbiciunea congenitală a gâtului vezicii e.g. epispadiadis

Ureta şi gâtul vezicii au formă imperfectă din cauza migrării defectuoase şi a fuzionării mezodermului pe linia mediană, ceea ce duce la un gât al vezicii mai lat, o uretră mai scurtă şi deficiențe ale muşchilor netezi şi striați. Simfiza şi clitorisul sunt despicate. Acest fel de incontinență nu poate fi eficient rezolvată prin procedurile convenționale de ridicare a gâtului vezicii, şi se corectează prin creşterea rezistenței uretrale, adică prin folosirea unui sfincter urinar artificial sau a unui agent de umflare a uretrei, e.g. colagenul.

Slăbirea congenitală datorată suportului slab al planşeului pelvian, poate duce la instalarea incontinenței de stres chiar din adolescență, şi aşa se explică procentul de 5-10% de fete cu incontinență (Nemir şi Middleton 1954; Thomas şi colaboratorii 1980). La femeile nulipare, care prezintă incompetența sfincterului uretral s-a observat o deficiență a formării de colagen care duce la reducerea colagenului total şi la scăderea colagenului de tip I (Keane şi colaboratorii 1992).

Provocarea mecanică

Presiunea intra-abdominală crescută, care se întâlneşte la femeile obeze şi la cele cu boli respiratorii cronice, poate duce la incontinență la stres. Obezitatea apare mai mult la femei nulipare aflate în premenopauză şi care prezintă incontinență la stres, decât la femeile pare şi care prezintă incontinență la stres (Creighton şi colaboratorii 1992). Obezitatea poate de asemenea să predispună la incontinență la stres femeile cu slăbiciune congenitală sau de dezvoltare a sfincterului uretral.

Naşterea

Mai multe naşteri pe cale vaginală cresc riscul de incontinență la stres (Foldspang şi colaboratorii 1992; Wilson şi colaboratorii 1996).

Chaliha şi colaboratorii (1999) au descoperit într-un studiu prospectiv efectuat pe 549 de femei nulipare că prevalența incontinenței la stres înainte, în timpul şi după naştere a fost de respectiv 3,6%, 43,7% şi 14,6%. Într-un studiu ulterior efectuat pe 286 de femei nulipare, care a urmărit investigarea urodinamică în al treilea trimestru, s-a observat o prevalență a incontinenței reale la stres de 9,1% şi 5% la cele 161 de femei care au revenit la 12 săptămâni după naştere (Charliha şi colaboratorii 1998). La acest grup de femei simptomele post-partum de incontinență la stres au fost asociate celui de-al doilea stadiu de travaliu activ de mai mare durată. Naşterea poate duce la incontinență la stres ca urmare a denervării componentelor netede şi striate ale mecanismului sfincterului, planşeului pelvian şi fasciilor pubocervicale (Snooks şi colaboratorii 1984; Sayer şi colaboratorii 1989) sau ca urmare a schimbărilor apărute în mecanismul de susținere a uretrei (Peschers şi colaboratorii 1996).

Menopauza

Deficitul de estrogen poate provoca slăbirea suportului gâtului vezicii şi pierderea închiderii ermetice a uroteliului. Cu toate acestea, studiile privind folosirea estrogenului nu au dovedit nici o îmbunătățire semnificativă a incontinenței la stres (Fantl şi colaboratorii 1994).

Traumatismul

Aceste implică fractura inelului pelvian şi diastaza simfizei cu desprinderea gâtului vezicii de ligamentele pubouretrale care îl leagă de spatele simfizei (Stanton şi colaboratorii 1981).

Operații în antecedente

Operațiile efectuate în apropierea gâtului vezicii pentru corectarea prolapsului sau a incompetenței sfincterului uretral pot duce la recidive ale incontinenței. Mecanismul nu este bine elucidat dar poate include afectarea sfincterului uretral, scăderea rezistenței uretrei sau a presiunii de închidere, fibroza post-operatorie ce afectează uretra şi țesutul parauretral, fixarea uretrei de spatele simfizei cu pierderea elasticității, şi eşecul de ridicare a joncțiunii uretrovezicale. În urma unui studiu efectuat pe 120 de femei cu incontinență reală la stres dovedită urodinamic, Hilton Stanton (1983) au descoperit că operațiile repetat nereuşite erau asociate cu presiunea uretrală scăzută.

Medicamente

Medicamentele hipotensive cum ar fi prazosin, reserpine, alpha-methyldopa şi phenoxybenzamine acționează prin inhibarea receptorilor $\alpha - 1 -$ adrenergici, şi duc la incontinență urinară (Kiruluta şi Andrews 1983; Dwyer şi Teele 1992).

Muşchi netezi ai uretrei proxime şi ai gâtului vezicii sunt inervați simpatic (Awad şi colaboratorii 1976) şi blocajul $\alpha -$ adrenergic are un efect relaxant prin scăderea presiunii uretrale (Anderson şi colaboratorii 1981).

Simptome

Simptomul clasic al incompetenței sfincterului uretral este incontinența la stres; totuşi, multe paciente acuză frecvența, incontinența de urgență şi incontinența la poziția stând în picioare (Cardozo şi Stanton 1980). În general incontinența la stres se agravează în săptămâna ce precede ciclul menstrual. Întreruperea jetului poate fi confirmată obiectiv la aproximativ 83% din pacientele cu incompetență a sfincterului şi la 87% din pacientele cu instabilitate a detrusorului, astfel încât acest simptom nu ne poate ajuta să diferențiem cele două tulburări. Pacientele sunt cel mai adesea multipare. Simptomele apar de obicei în timpul sarcinii şi se agravează în perioada postnatală şi în următoarele sarcini (Stanton şi colaboratorii 1980; Chalila 1999).

Semne

La examenul clinic nu apar trăsături speciale generale sau neurologice. Epispadis este uşor de recunoscut. Coborârea peretelui vaginal anterior (cistouretrocel) este prezentă la aproximativ 50% din femeile cu incompetența sfincterului. Trebuie evaluate capacitatea şi mobilitatea vaginului (care indică afectarea vaginului) deoarece acestea pot fi relevante în stabilirea variantei chirurgicale pentru redobândirea continenței. Trebuie notate şi alte probleme de prolaps genital sau de patologie uterină sau ovariană ca să se poată efectua chirurgia reparatorie şi pentru aceste elemente odată cu cea pentru continență.

Investigații

Vezica nu oferă dovezi sigure. Mulți clinicieni au demonstrat discrepanțe între rezultatele clinice şi cele urodinamice (Haylen şi Frazer 1987; Ng şi Murray 1989). Dificultatea constă în complexitatea acuzelor. O combinație a incontinenței la stres, incontinenței de urgență şi frecvenței sugerează foarte probabil instabilitatea detrusorului. În urma unui studiu urodinamic efectuat pe un număr de 800 de femei, aproximativ 50% au fost diagnosticate cu incompetența sfincterului uretral.

Din acestea doar 31% aveau simptomul şi semnul de incontinență la stres, iar 1,5% prezentau doar simptomul de incontinență la stres (Haylen şi Frazer 1987). Din tot grupul 85% prezentau iminență, incontinență la iminență şi la stres dar numai 25% au dovedit la testare instabilitatea detrusorului.

La fel s-a întâmplat şi cu tulburările de golire: 53% prezentau două simptome ale tulburărilor de golire care au fost confirmate doar în 10% din cazuri prin testare.

Scopurile testelor urodinamice sunt:

1. Să confirme simptomele (e.g. incontinenţa sau dificultatea la golire) şi gravitatea lor
2. Să ducă la stabilirea diagnosticului
3. Să depisteze alte anomalii, e.g. instabilitatea detrusorului
4. Să confirme modalitatea de rezolvare

Testele urodinamice trebuie precedate de efectuarea unei mostre negative de jet de urină pentru a evita procedurile invazive care ar agrava infecţia de la nivelul tractului urinar, deoarece altfel rezultatele ar fi neconcludente.

Cistometria şi videouretrografia

Diagnosticul cistometric de scurgere urinară indică prezenţa instabilităţii detrusorului sau a dificultăţilor de golire; doar dacă nu apare instabilitatea detrusorului se poate ajunge prin excludere la diagnosticul de incompetenţă a sfincterului uretral. Combinaţia cistometriei şi screeningului rediologic cu inregistrarea video, împreună cu comentariul ecografic, permite stabilirea altor diagnostice, inclusiv a cauzei incontinenţei la poziţia de stat în picioare, şi a prezenţei unui diverticul uretral, şi uneori prezenţa fistulelor urinare). Altfel contribuie prea puţin la stabilirea clară a incompetenţei sfincterului uretral (Stanton şi colaboratorii 1986).

Profilometria presiunii uretrale

Rolul profilometriei presiunii uretrale (UPP) în diagnosticarea incompetenţei sfincterului uretral rămâne controversat. Versi şi colaboratorii (1986) au declarat că „suprapunerea între normal şi GSI (USI) a fost atât de mare încât a făcut imposibilă diagnosticarea corectă". Sand şi colaboratorii (1987) au măsurat presiunea de închidere uretrală şi au stabilit că o scădere de 20 centimetri coloană de apă indică o uretră cu presiune scăzută, şi reprezintă un element important în eşecul chirurgiei reparatorii.

Hilton (1988) a cercetat conceptul de uretră instabilă ca şi cauză a incompetenţei sfincterului şi a descoperit că o schimbare relativă de 30% a presiunii maxime de închidere uretrală era elementul cel mai sigur în detectarea acestei afecţiuni. Principalul dezavantaj al presiunii uretrale este relativa instabilitate a cateterului, care duce la rezultate false sau suprapuse la pacientele continente şi incontinente.

Presiunile Valsalva în punctul de scurgere (VLPP)

Această tehnică de diagnosticare relativ recentă poate fi folosită pentru a măsura rezistenţa uretrală în diagnosticarea deficienţei intrinseci a sfincterului. O presiune de sub 60 de centimetri coloană de apă în poziţie stând în picioare la un volum de 150 ml dovedeşte deficienţa intrinsecă a sfincterului.

Testul este considerat foarte exact şi cu mare procent de repetare la aproximativ 80% din pacientele adulte cu USI (Bump şi colaboratorii 1995). În comparaţie cu UPP, VLPP dă rezultate similare dar testul este mai puţin costisitor şi durează mai puţin (Theofrastous şi colaboratorii 1995). Totuşi, Alcalay şi colaboratorii (1994) au găsit o specificitate crescută (100%) dar o sensibilitate redusă (69%) în diagnosticarea incontinenţei reale la stres, şi precizie redusă în diagnosticarea incontinenţei la stres în comparaţie cu variabilele UPP.

Ecografia

Ecografia depistează golirea incompletă a vezicii care poate duce la incontinenţă la stres. Poate permite evaluarea poziţiei şi deplasării gâtului vezicii, relevante în diagnosticarea incompetenţei sfincterului uretral şi poate fi utilă în analizarea eşecului chirurgiei convenţionale şi în alegerea finală a altei operaţii (Creighton şi colaboratorii 1994).

Concluzii

Multitudinea de proceduri de diagnosticare existente pentru evaluarea pacientelor cu simptome în zona tractului urinar inferior că unul sau două teste nu pot oferi informații decisive. În general, medicii trebuie să treacă de la testele mai simple la cele mai complexe numai dacă diagnosticarea corectă nu se poate realiza prin testele simple.

Testele cele mai eficiente din punctul de vedere al costului şi timpului şi care oferă cea mai concentrată informație, indicând direcția unei testări ulterioare sunt testul de tuse, cistometria pe un canal, Q tip test, şi reziduul după golire. După eliminarea posibilității de infecție şi după examinarea fizică şi anamnezică minuțioasă, aceste teste simple sunt obligatorii.

Toate testele trebuie reținute şi analizate cu grijă. Numai un clinician perspicace poate sintetiza aceste date şi poate decide un diagnostic corect şi un tratament rațional.

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SISTEMUL DE SERVICII DE ASISTENȚĂ SOCIO-MEDICALĂ ŞI ÎNGRIJIREA PERSOANELOR DE VÂRSTA A TREIA

Drd. ing**. Vasilica STRICHEA**[*]

Scopul protecției sociale este de a asigura, un trai decent categoriilor de persoane aflate în dificultate, permițându-lc în acelaşi timp să accepte şi să se adaple/e evyluțiiloF economice şi sociale.

Obiectivul sistemului de protecție socială constă în susținerea persoanelor aflate în dificultate care nu pot prin resurse proprii să ducă o viață normală.

Protecția socială este alcăluiul din diverse *beneficii sociale* (în natură sau în bani) care suplinesc un delicii de resurse ale persoanei, grupurilor şi colectivităților aflate în dificultate şi *servicii sociale* care se adresează unui *deficit de capacități* ale individului de a face față problemelor vieții.

Asistența socială asigură prin serviciile specializate punerea în aplicare a programelor şi măsurilor de protecție socială în vederea depăşirii perioadelor de criză şi refacerea forțelor proprii a personelor aflate în dificultate pentru a se integra în viața socială.

protecție socială **servicii sociale** **asistență socială**

În aplicarea măsurilor de protecție socială, statul român a urmărit respectarea prevederilor Constituției României referitoare la drepturile omului, precum şi o serie de principii, printre care se menționează: *protecția demnității umane, universalitatea măsurilor de protecție socială, promovarea principiilor solidarității şi justiției sociale, promovarea parteneriatului social ca mijloc central şi de eficientizare a tuturor măsurilor de politică şi protecție socială, trecerea treptată de la descentralizarea realizării protecției sociale şi, odată cu aceasta, angajarea în activitatea de protecție socială a agenților economici, a unităților adminstrației publice locale, a instituțiilor guvernamentale şi neguvernamentale, a societăților de caritate şi a persoanelor fizice prin contribuții materiale, băneşti şi sociale ale acestora într-un cadru legal adecvat.*

Scopul protecției sociale este de a asigura un trai decent categoriilor de persoane aflate în dificultate, permițându-le în acelaşi timp să accepte şi să se adapteze evoluțiilor economice şi sociale.

Obiectivul sistemului de protecție socială constă în susținerea persoanelor aflate în dificultate care nu pot prin resurse proprii să ducă o viață normală.

Protecția socială este alcătuită din diverse *beneficii sociale* (în natură sau în bani) care suplinesc un deficit de resurse ale persoanei, grupurilor şi colectivităților aflate în dificultate şi *servicii sociale* care se adresează unui *deficit de capacități* ale individului de a face față problemelor vieții.

Beneficiile sociale sunt şi ele la rândul lor de mai multe feluri:

- Beneficii sociale contributorii - se mai numesc şi *beneficiile de asigurări sociale* şi se acordă cu condiția plății contribuției la fondul de asigurări socialale – pensiile, ajutorul de şomaj, ajutorul de boală.

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[*] Direcția Medicală – Secția medicală şi relații internaționale

- Beneficile sociale non-contributorii – se mai numesc şi *beneficii de asistență socială* deoarece ele completează deficitul de resurse ale persoanelor aflate în dificultate, fără a plăti, în prealabil, o contribuție, iar acestea constau în alocațiile pentru copii, bursele de studii, gratuități sau reduceri la transport, subvenții pentru chirii, etc.

Serviciile sociale cuprind serviciile oferite de colectivitate, în mare măsură, în afara sistemului economic şi se împart în trei categorii: învățământ, sănătate şi asistență socială. Învătământul asigură dezvoltarea capacităților intelectuale şi tehnice al individului, sănătatea asigură prevenția şi integritatea capacităților fizice ale persoanei şi recuperarea acestora în caz de boală, serviciile de asistență socială sprijină persoanele aflate în dificultate şi pe cele care se confruntă cu probleme dificile de viață, susțin refacerea abilităților deteriorate şi încearcă reintegrarea socială a acestora.

Serviciile de asistență socială se acordă fie în *mediul natural de viață al individului sau colectivității*, de exemplu: acasă, la locul de muncă, fie în *instituții de asistență socială* – centre pentru copiii străzii, case pentru vârstnici, instituții pentru persoane cu dizabilități, etc.

Obiectivul central al asistenței sociale este de a sprijini prin diferite mijloace persoanele, grupurile şi comunitățile aflate în dificultate, care nu pot depăşi prin resurse proprii această situație, datorită unor cauze de natură economică, biologică sau psihologică, culturală, acordându-le ajutorul pentru a duce o viață decentă.

Categoriile de persoane beneficiare ale asistenței sociale sunt: copiii abandonați şi instituționalizați; persoanele vârstnice neajutorate, abandonate şi instituționalizate; persoanele dependente de droguri, de alcool; tinerii neintegrați social; familiile sărace; persoanele abuzate fizic, sexual; persoanele cu handicap fizic sau mintal; persoanele cu boli cronice fără susținători legali; persoanele care au suferit din cauza calamităților de orice fel.

Asistența socială asigură prin serviciile specializate punerea în aplicare a programelor şi măsurilor de protecție socială în vederea depăşirii perioadelor de criză şi refacerea forțelor proprii a personelor aflate în dificultate pentru a se integra în viața socială.

Serviciile sociale, în conformitate cu prevederile art.1 alin. 1 din Ordonanța Guvernului nr. 68/2003, *reprezintă ansamblul complex de măsuri şi acțiuni realizate pentru a răspunde nevoilor sociale individuale, familiale sau de grup,în vederea prevenirii şi depăşirii unor situații de dificultate, vulnerabilitate sau dependenta pentru prezervarea autonomiei şi protecției persoanei, pentru prevenirea marginalizării şi excluziunii sociale, pentru promovarea incluziunii sociale şi în scopul creşterii calității vițtii.*

Clasificarea serviciilor sociale

Serviciile sociale se organizează la nivel comunitar şi pot fi :

- servicii de asistență socială
- servicii de îngrijire social-medicale

Serviciile de asistență socială se clasifică în ***servicii sociale primare şi servicii sociale specializate*:**

➢ *Serviciile sociale primare* au un caracter general si urmaresc prevenirea sau limitarea unor situatii de dificultate sau vulnerabilitate. Principalele activitati desfasurate in cadrul serviciilor sociale primare sunt:

a) activități de identificare a categoriilor de persoane cu nevoi sociale;

b) activitati de depistare a situațiilor de risc şi de informare despre drepturile şi obligațiile persoanei beneficiare de servici sociale;

c) luarea de măsuri în vederea reducerii efectelor situațiilor de criză a persoanelor aflate în situații de risc;

d) măsuri şi acțiuni de sprijin pentru menținerea în comunitate a persoanelor în dificultate;

e) măsuri educative şi activităţi de consiliere a categoriilor de persoane beneficiare de servicii sociale;

f) alte măsuri şi acţiuni de protecţie socială care au drept scop prevenirea sau limitarea unor situaţii de dificultate ori vulnerabilitate, care pot duce la marginalizare sau excluziune sociala.

➢ *Serviciile sociale specializate* au ca scop menţinerea, refacerea şi dezvoltarea capacităţii de funcţionare socială a indivizilor şi familiilor. Activităţile specifice acestor servicii sunt cele de:

a) recuperare, reabilitare şi reinserţie socială a persoanelor aflate în situaţii de risc;
b) suport şi asistenţă pentru familiile şi copiii aflaţi în dificultate;
c) suport şi asistenţă pentru asigurarea unei vieţi autonome şi active persoanelor de vârsta a treia;
d) suport şi servicii de îngrijire acordate vârstnicilor aflaţi într-o situaţie de dependenţă;
e) sprijin pentru inserţia socială şi profesională a persoanelor sau familiilor în dificultate ori în situaţii de risc;
f) sprijin pentru integrarea, readaptarea şi reeducarea profesională, stabilite prin legislaţia în vigoare;
g) acţiuni de informare, susţinere şi consiliere, expertiză ori coordonare în vederea prevenirii oricărei forme de dependenţă;
h) acordarea de sprijin în primirea şi îngrijirea cazurilor de urgenţă, cu sau fără găzduire, a persoanelor sau familiilor în dificultate ori în situaţii de risc;
i) mediere socială;
j) alte măsuri şi acţiuni care au drept scop menţinerea, refacerea sau dezvoltarea capacităţilor individuale pentru depăşirea unei situaţii de nevoie socială.

Serviciile de îngrijire social-medicală se acordă în cadrul unui sistem social şi medical integrat şi au drept scop principal menţinerea autonomiei persoanei, precum şi prevenirea agravării situaţiei de dependenţă. Aceste servicii se acordă şi persoanelor care se găsesc în imposibilitatea de a realiza singure activităţile curente datorită unor afecţiuni fizice, psihice, mentale sau senzoriale sau care se află în faza terminală a unei boli incurabile.

Serviciile de îngrijire social-medicală pot fi de natură socială, medicală şi conexe, astfel:

I. serviciile de îngrijire social-medicală *de natura socială* pot fi următoarele:

a) servicii de bază: ajutor pentru igienă corporală, îmbrăcare şi dezbrăcare, igiena eliminărilor, hrănire şi hidratare, transfer şi mobilizare, deplasare în interior, comunicare;

b) servicii de suport: ajutor pentru prepararea hranei sau livrarea acesteia, efectuarea de cumpărături, activităţi de menaj, însoţirea în mijloacele de transport, facilitarea deplasării în exterior, companie, activităţi de administrare şi gestionare, activităţi de petrecere a timpului liber;

c) servicii de reabilitare şi adaptare a ambientului: mici amenajări, reparaţii.

II. serviciile de ingrijire social-medicală *de natură medicală* sunt reprezentate de activităţi complexe de diagnostic, tratament şi îngrijiri recomandate şi realizate în conformitate cu tipurile de afecţiuni pe care le prezintă beneficiarii acestor categorii de servicii sociale.

III. serviciile de îngrijire social-medicală *de natura serviciilor conexe*, sunt reprezentate de servicii de recuperare şi reabilitare, kinetoterapie, fizioterapie, terapie ocupaţională, psihoterapie, psihopedagogie, logopedie.

O clasificare pe domeniile de intervenţie şi sprijin ale serviciilor sociale poate fi facută din analiza structurii bugetare şi a transferului de competenţe între autorităţile publice. Astfel, în funcţie de adresabilitate, am putea clasifica serviciile sociale ca fiind destinate pentru:

- protecţia persoanelor vârstnice: servicii rezidenţiale, servicii de îngrijire temporară sau permanentă la domiciliu;
- protecţia copilului: centre de zi, centre de plasament, centre de primire în regim de urgenţă, centre maternale, servicii de tip familial;

- protecția familiei: centre de recuperare, centre de prevenire şi centre de informare pentru victimele violenței în familie;
- protecția persoanelor cu dizabilități: centre de îngrijire şi asistență, centre de recuperare şi reabilitare, centre de integrare prin terapie ocupațională;

Etapele procesului de acordare a serviciilor sociale:

- Evaluarea inițială - efectuată de către asistentul social din cadrul serviciului public de asistență socială din subordinea autorităților admininistrației publice locale, cu scop de identificare a nevoilor individuale sau de grup a persoanelor defavorizate social;

- Elaborarea planului de intervenție - cuprinde măsurile necesare soluționării situației de risc social, acordarea de prestații şi servicii sociale precum şi orice alte măsuri sociale;

- Evaluarea complexă – (în cazul în care există situații pentru a căror rezolvare se impune participarea mai multor profesionişti sau instituții specializate) se efectuează pentru identificarea şi stabilirea măsurilor de intervenție personalizate şi are ca scop elaborarea unei strategii de suport continând măsurile şi serviciile adecvate potrivit nevoilor sociale identificate;

- Elaborarea planului individualizat de asistență şi îngrijire cuprinde programarea serviciilor sociale, personalul responsabil precum şi procedurile de acordare; este elaborat de furnizorul de servicii sociale cu acordul şi participarea beneficiarului;

- Monitorizarea prin reevaluarea periodică a situației persoanei beneficiare şi cunoaşterea eficienței serviciilor sociale acordate pentru stabilirea continuării intervenției sau scoaterii din evidență a cazului.

SITUAȚIA ACTUALĂ A SISTEMULUI DE ASISTENȚĂ SOCIALĂ

Analiza profilului actual al sistemului de asistență socială pentru persoanele vârstnice aşa cum este prezentat în *Strategia națională de dezvoltare a sistemului de asistență socială pentru persoanele vârstnice în perioada 2005-2008*

În cazul persoanelor de vârsta a treia se întâlneşte o situație particulară, acestea având nevoie, în egala măsură, de două tipuri de suport :

- **servicii sociale**
- **servicii medicale**.

În aceste condiții, inițierea, dezvoltarea şi acordarea serviciilor sociale şi de sănătate, în special a celor de îngrijire personală, impun o politica unitară în domeniu pentru a realiza menținerea persoanelor vârstnice la domiciliu sau, dacă acest lucru nu este posibil, pentru a asigura suportul necesar continuării vieții într-o instituție de asistență socială.

În prezent, în România, funcționează:

a) 4 cămine pentru persoane vârstnice, cu o capacitate de 2.121 paturi, aflate în coordonarea metodologică a Ministerului Muncii Solidarității Sociale si Familiei;

b) 84 de centre de îngrijire şi asistență pentru adulții cu handicap, cu o capacitate de 10.230 paturi, reprezentând fostele cămine de bătrâni şi cămine spital aflate în coordonarea metodologică a Autorității Naționale pentru Persoanele cu Handicap (39 foste cămine de bătrâni si 45 foste cămine spital care nu şi-au schimbat profilul inițial);

c) 17 instituții de asistență socială (cămine pentru persoane vârstnice şi centre de zi) susținute financiar de organizații neguvernamentale, pentru care se acordă subvenții de la bugetul de stat, cu o capacitate totala de 776 locuri:

d) 39 de servicii la domiciliu subvenţionate de la bugetul de stat. (Sursa: Raportul anual de asistenta sociala al direcţiei de specialitate din cadrul Ministerului Muncii Solidarităţii Sociale si Familiei)

Principalele puncte slabe ale sistemului de asistenta sociala a persoanelor vârstnice:

În raport cu profilul global al sistemului:

- Numărul redus de studii şi cercetări privind tendinţele demografice, particularităţile vârstei a treia şi problematicile specifice vârstnicilor din ţara noastră ;
- Accesul persoanelor vârstnice la îngrijirea în sistem rezidenţial sau la domiciliu, este greoi, el realizându-se în baza mai multor metodologii, prevăzute de legislaţia specială pentru persoane vârstnice şi persoane cu handicap ;
- Dreptul la măsuri de asistenţă socială se acordă pe criterii diferite: evaluarea nevoilor persoanelor vârstnice este realizată de o echipa formată din medic şi asistent social care aplică grila naţională de evaluare a persoanelor vârstnice şi stabileşte gradul de dependenţă; persoana vârstnică cu dizabilităţi este evaluată de Comisia de expertiza a adultului cu handicap care încadrează persoana într-un grad de handicap; *este de subliniat faptul că legislaţia din ţările europene considera persoanele care au dobândit o dizabilitate generatoare de pierdere funcţională la vârsta a treia, drept persoane dependente şi nu persoane cu handicap;*
- Dezvoltare inegală şi inechitabilă a serviciilor sociale destinate persoanelor vârstnice la nivel naţional: exista judeţe cu concentrare masivă de ONG-uri furnizoare de servicii, iar altele în care nu este înregistrată nici o organizaţie activă;
- Absenţa activităţii de monitorizare şi control susţinută de criterii şi standarde clar definite, aplicată de personal calificat în domeniu;
- Servicii de prevenţie şi recuperare insuficiente ;
- Lipsa de colaborare între instituţiile administraţiei centrale şi locale din domeniul asistenţei sociale şi de sănătate, reprezentând principalul obstacol al dezvoltării serviciilor sociale şi medicale integrate, acordate la nivel comunitar;
- Prevederi legale depăşite sau dificil de implementat, cum ar fi: calcularea contribuţiei datorată de persoana vârstnică pentru acordarea serviciilor la domiciliu, facilităţile acordate îngrijitorilor proveniţi din familia beneficiarului, programul de formare mult prea complex faţă de atribuţiile reale ale unui îngrijitor la domiciliu, etc;
- Instituţii de asistenţă socială insuficiente faţă de solicitări, situaţie evidentă în protecţia persoanelor vârstnice, a persoanelor cu boli cronice în mod deosebit a celor cu afecţiuni neuropsihice.
- Incapacitatea sistemului de a rezolva probleme/situaţii personale extrem de grave care nu sunt deloc sau sunt marginal acoperite cu servicii sociale.

În raport cu organizarea:

- Regulamente de organizare şi funcţionale diferite ale instituţiilor care acordă acelaşi tip de servicii, pentru aceleaşi categorii de beneficiari;
- Disproporţie în alocarea de personal în raport cu complexitatea activităţii, precum şi lipsa unor normative de personal elaborate în baza unor evaluări prealabile, cu respectarea standardelor europene în domeniu;
- Existenţa mai multor sisteme de evaluare a deficitului funcţional;
- Sistem managerial informaţional slab dezvoltat, ceea ce conduce la decizii incorecte sau insuficient fundamentate;
- Incapacitatea sistemului de a oferi o imagine comprehensivă asupra numărului de beneficiari şi a serviciilor disponibile;

În raport cu finanțarea:

- Rigiditatea sistemului a generat imposibilitatea direcționării fondurilor în vederea soluționării unor situații de criză;
- Finanțarea instituțiilor de asistență socială din bugetele locale; deşi acestea asigură servicii pentru beneficiari care provin din toate localitățile județului, datorită bugetelor mici aceasta a condus la degradarea multor instituții sub aspect fizic şi funcțional;
- Neaplicarea legislației referitoare la convențiile ce se încheie pentru îngrijirea unei persoane vârstnice într-un cămin situat în alt judeţ, autoritatea locală unde îşi are domiciliul persoana fiind obligată să asigure cheltuielile necesare asistării acesteia;
- Nedefinirea clară a modalităților de finanțare a serviciilor sociale, astfel încât să fie asigurată sustenabililatea şi continuitatea acestora;

În raport cu resursele umane:

- Sub-dimensionarea necesarului de personal la nivel central şi local;
- Lipsa personalului calificat la nivelul serviciilor publice de asistență socială, dar şi la nivelul serviciilor şi instituțiilor de asistență socială destinate asistării persoanelor vârstnice;
- Reglementări insuficiente referitoare la nevoia de formare şi criteriile de acreditare a personalului care activează în domeniul serviciilor sociale pentru persoane vârstnice, respectiv lipsa standardelor profesionale care să permită atât includerea în Clasamentul Ocupațiilor din România, cât şi o salarizare corespunzătoare;
- Titulaturi diferite utilizate pentru personalul care are atribuții similare (asistent personal, îngrijitor la domiciliu);
- Insuficienta dezvoltare a sistemului de formare continuă în vederea dezvoltării de competențe;
- Lipsa unor profesii necesare managementului serviciilor sociale pentru persoane vârstnice: asistentul medical comunitar (echivalent al nursei comunitare), asistentul medical de geriatrie şi gerontopsihiatrie, managerul de caz, terapeutul ocupațional specializat în recuperarea persoanelor vârstnice, etc;
- Deşi cu tradiție în medicina românească, există blocaje în dezvoltarea specialității şi competențelor în geriatrie-gerontologie.

Instituții de asistență socială pentru persoane vârstnice

Definirea termenilor cămin-spital şi unitate medico-socială conform legislației

A. Cămine-spital

Organizarea şi funcționarea căminelor pentru persoane vârstnice:

Asistența socială pentru persoane vârstnice se acordă în conformitate cu prevederile Legii nr. 17/2000, unde se menționează că sunt considerate persoane vârstnice, persoanele care au împlinit vârsta de pensionare stabilită de lege.

- Căminul pentru persoane vârstnice este instituția de asistență socială unde se asigură condiții corespunzătoare de găzduire şi de hrană, îngrijiri medicale, recuperare şi readaptare, activități de ergoterapie şi de petrecere a timpului liber, asistență socială şi psihologică.
- Căminele pentru persoane vârstnice au secții pentru:
  a) persoane dependente;
  b) persoane semidependente;
  c) persoane care nu sunt dependente.
- Cheltuielile curente şi de capital ale căminelor pentru persoane vârstnice se asigură din venituri extrabugetare şi din subvenții sau transferuri acordate de la bugetul de stat.

Principalele obiective:

a) să asigure persoanelor vârstnice îngrijite maximum posibil de autonomie şi siguranţă;

b) să ofere condiţii de îngrijire care să respecte identitatea, integritatea şi demnitatea persoanei vârstnice;

c) să permită menţinerea sau ameliorarea capacităţilor fizice şi intelectuale ale persoanelor vârstnice;

d) să stimuleze participarea persoanelor vârstnice la viaţa socială;

e) să faciliteze şi încurajeze legaturile interumane, inclusiv cu familiile persoanelor vârstnice;

f) să asigure supravegherea şi îngrijirea medicală necesară, potrivit reglementarilor privind asigurările sociale de sănătate;

g) prevină şi să trateze consecinţele legate de procesul de îmbătrânire.

B. Unităţile medico-sociale

Beneficiarii serviciilor acordate în unităţile de asistenţă medico-sociale sunt persoane cu afecţiuni cronice care necesită permanent sau temporar supraveghere, asistare, îngrijire, tratament şi care, din cauza unor motive de natură economică, fizica, psihică sau socială, nu au posibilitatea să îşi asigure nevoile sociale, să îşi dezvolte propriile capacităţi şi competenţe pentru integrare socială.

- Unităţile medico-sociale sunt instituţii publice, specializate, cu personalitate juridică, în subordinea autorităţilor publice locale, care acordă servicii de îngrijire, servicii medicale precum şi servicii sociale persoanelor cu nevoi medico-sociale.
- Unităţile medico-sociale se înfiinţează ca instituţii rezidenţiale sau de zi, prin hotărâre a consiliului local sau judeţean, respectiv a Consiliului General al municipiului Bucureşti, prin reorganizarea unor unităţi sanitare, cu avizul Ministerului Sănătăţii Publice şi al Ministerului Internelor şi Reformei Administrative.
- Înfiinţarea de unităţi medico-sociale permite transferul unor servicii medicale – internări de lungă durată, îngrijiri la domiciliu – din activitatea spitalelor către aceste unităţi, astfel încât spitalele să se poată concentra pe furnizarea îngrijirilor medicale de durată scurtă/medie.
- Bunurile aflate în administrarea unităţilor sanitare care se reorganizează trec în administrarea unităţilor de asistenţă medico-sociale, conform protocolului de predare-primire încheiat între acestea.
- Personalul existent în unităţile sanitare care se reorganizează se preia de către unitatea de asistenţă medico-socială, cu încadrarea în statul de funcţii aprobat.
- Cheltuielile curente şi de capital se asigură din venituri proprii şi din subvenţii acordate din bugetul local, în funcţie de subordonare.
- Conducerea acestui tip de unităţi, este asigurată conform legislaţiei în vigoare, de către un director, sprijinit de un Consiliu Consultativ compus din 5 membri, desemnaţi din partea autorităţii publice locale prin a cărei hotărâre s-a înfiinţat unitatea medico-socială, a direcţiei de sănătate publică, a direcţiei generale de muncă şi solidaritate socială, a casei de asigurări de sănătate judeţene, respectiv a Municipiului Bucureşti şi din partea societăţii civile.
- Personalul unităţilor de asistenţă medico-socială este alcătuit din personalul medical preluat de la unitatea sanitară care s-a transformat. Pregătirea specifică acestui tip de activitate se va realiza prin cursuri de perfecţionare organizate de Ministerul Sănătăţii Publice, în colaborare cu Ministerul Muncii, Solidarităţii Sociale şi Familiei.
- Internarea persoanelor cu nevoi medico-sociale în unităţile cu acest profil se realizează la recomandarea unităţilor sanitare sau la solicitarea persoanelor fizice ori juridice, în baza unei grile de evaluare medico-socială, care constituie un instrument de lucru standardizat, prezentat sub forma unei fişe structurată pe componenta medicală şi componenta socială şi reprezintă instrumentul în baza căruia se realizează internarea în unităţile de asistenţă medico-sociale.

Finanțarea unităților medico-sociale

- Sume decontate de casele de asigurări de sănătate pentru servicii medicale furnizate pe bază de contract, conform Contractului-cadru anual;
- Contribuții personale ale beneficiarilor, după caz;
- Sponsorizări, donații, alte venituri potrivit legii.

Analiza posibilității înființării unor unități medico-sociale/cămine-spital în rețeaua sanitară militară

1. Înființarea unor astfel de unități medico-sociale permite transferul unor servicii medicale – internări de lungă durată, îngrijiri la domiciliu – din activitatea spitalelor militare către aceste unități, astfel încât spitalele militare să se poată concentra pe furnizarea îngrijirilor medicale de durată scurtă/medie şi pe activități specifice medico-militare.
2. Pentru intrarea în funcțiune a acestor unități medico-sociale în armată componenta medicală va fi susținută de Direcția Medicală şi CASAOPSNAJ, prin contractarea de servicii medicale cu aceste unități, iar componenta socială de structuri cu responsabilități în domeniu.
3. **Beneficiarii** serviciilor oferite în unitățile de asistenta medico-sociala sunt persoane cu afecțiuni cronice, care necesită, permanent sau temporar, supraveghere, asistare, îngrijire, tratament şi care au totodată şi *dependență socială*. Pentru unitățile medico-sociale ale M.Ap. beneficiari vor fi pensionarii militari şi membrii de familie ai acestora, veteranii şi văduvele de război, persoane care prezintă afecțiuni medicale cronice şi necesită perioade lungi de spitalizare, dar şi ca o formă de protecție socială pentru persoanele vârstnice şi singure.
4. **Internarea** persoanelor cu nevoi medico-sociale în unitățile cu acest profil se realizează la recomandarea unităților sanitare militare sau la solicitarea persoanelor fizice ori juridice, în baza unei grile de evaluare medico-socială, care constituie un instrument de lucru standardizat, prezentat sub forma unei fişe structurată pe componenta medicală şi componenta socială şi reprezintă instrumentul în baza căruia se realizează internarea în unitățile de asistență medico-sociale.
5. **Finanțarea unităților medico-sociale**: sunt cinci surse de finanțare, astfel:
    a) Fondul unic de asigurări sociale de sănătate sume decontate de casele de asigurări de sănătate, pe baza contractelor încheiate cu acestea, pentru finanțarea cheltuielilor de personal aferente medicilor şi asistenților medicali, precum şi a cheltuielilor cu medicamente şi materiale sanitare;
    b) Contribuții personale ale beneficiarilor sau ale susținătorilor legali ai acestora, aprobate în condițiile legii;
    c) Donații, sponsorizări, alte venituri;
    d) Subvenții şi transferuri de la bugetul de stat ;
    e) Fonduri puse la dispoziție prin bugetul M.Ap. pentru finanțarea cheltuielilor de reparare şi amenajare a clădirilor în care funcționează unitățile medico-sociale.

Pentru prevenirea, limitarea sau înlăturarea efectelor temporare sau permanente ale unor situații care pot genera marginalizarea sau excluderea socială a unor persoane, vrem să ne adresăm unei categorii defavorizate, persoanelor cu probleme sociale şi medicale care se internau în spitalele militare, aceste cazuri putând fi preluate de unitățile medico-sociale înființate în rețeaua sanitară militară.

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10. Legea nr. 17 din 6 martie 2000 privind asistența socială a persoanelor vârstnice
11. Ordonanța nr. 70 din 29.08.2002 privind administrarea unităților sanitare publice de interes județean şi local
12. Ordinul nr. 491 din 23 mai 2003 pentru aprobarea Grilei de evaluare medico-socială a persoanelor care se internează în unități de asistență medico-sociale
13. Ordonanța nr. 68 din 28 august 2003 privind serviciile sociale
14. Ordonanța de Urgență nr. 48 din 12.06.2003 privind unele măsuri în vederea întăririi disciplinei financiare şi a creşterii eficienței utilizării fondurilor în sistemul sanitar, precum şi modificarea unor acte normative
15. Legea 705/2001 privind sistemul national de asistenta sociala

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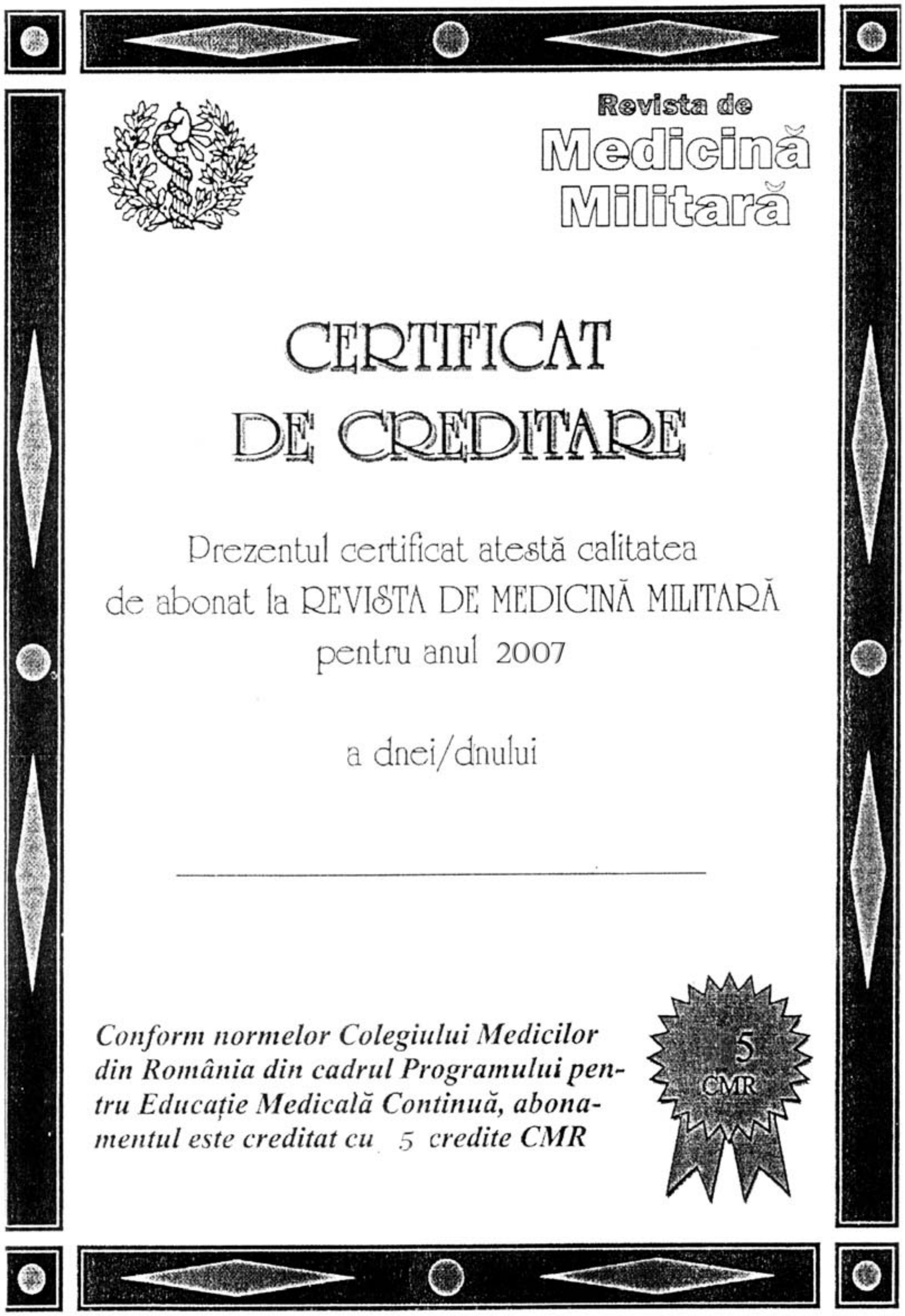

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DIN ROMÂNIA

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Diverticulul epifrenic

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Secţia Clínică Chirurgie Toracică - Spitalul Clinic de Urgenţă Militar Central „Dr. Carol Davila”

REZUMAT. Diverticulii epifrenici sunt cei mai frecvenţi diverticuli de corp esofagian (20% din totalul diverticulilor esofagieni). Prevalenţa reală nu este cunoscută datorită numărului mare de cazuri asimptomatice, care sunt descoperite întâmplător. Majoritatea bolnavilor au peste 50 de ani, în special decadele şapte şi opt, şi predomină sexul masculin. Odată descoperit şi în prezenţa unei simptomatologii corespunzătoare, indicaţia de elecţie este operatorie, diverticulectomie, esomiotomie extramucoasă extinsă. Efectuarea de rutină a unui procedeu antireflux (Belsey Mark IV) este încă controversată şi de aceea se practică doar când se pune în evidenţă reflux gastroesofagian sau hernie hiatală. Manometria preoperatorie devine obligatorie pentru a stabili cu exactitate tipul intervenţiei chirurgicale. Abordul minim invaziv este încă la început, şi în prezent sunt date relativ puţine datorită experienţei limitate. Rezultatele postoperatorii sunt foarte bune cu dispariţia simptomatologiei cu condiţia efectuării corecte a timpilor operatori.

Cuvinte cheie: diverticul epifrenic manometrie esofagiană diverticulectomie

ABSTRACT. Epiphrenic diverticula are the most common type of esophageal body diverticulum (20% of total esophageal diverticula). Real prevalence is not known, because there are many asymptomatic patients, which are discovered accidentally. Most of the patients are older than 50 years, and this condition present s especially during the seventh and eight decades of life, and is more frequent in men. Once it is diagnosed, in the presence of a adequate simptomatology, the treatment of choice is surgical: diverticulectomy, extended esophageal myotomy. Performing routine Belsey Mark IV antireflux procedure in this patients is controversial, and the patients undergo this operation, only when there is an evidence of gastroesophageal reflux or hiatal hernia. Preoperative manometry become compulsory for choosing the exact type of surgical intervention. The minimally invasive aproach is yet at beginning and in our days there are few results about this aproach because the lack of experience. Postoperative results are very satisfactory, with absence of the simptomatology, only on condition that the steps of the surgical intervention are performed correctly.

Diverticulii esofagieni se pot dezvolta la orice nivel al esofagului, dar etiologia, simptomatologia şi abordul lor chirurgical au condus la clasificarea diverticulilor esofagieni în trei grupe: faringoesofagian, parabronhial şi epifrenic. O a patra grupă – diverticuloza intramurală difuză – este descrisă, dar este destul de rară.

Din punct de vedere histopatologic, diverticulii esofagieni pot fi adevăraţi, care conţin toate straturile peretelui esofagian, şi pseudodiverticuli sau diverticuli „falşi”, formaţi doar din mucoasă şi submucoasă esofagiană.

Diverticulul epifrenic este un diverticul esofagian fals (un fund de sac căptuşit cu mucoasă esofagiană ce herniază prin punctele slabe ale muscularei), un pseudodiverticul al cărui perete este format doar din mucoasă şi submucoasă esofagiană.

Observaţia 1

Pacient în vârstă de 54 de ani se internează în clinica noastră acuzând disfagie pentru solide, dureri retrosternale, pirozis, vărsături postprandiale tardive, tuse iritativă nocturnă, scădere ponderală (7-8 kg în ultimul an).

Tranzitul baritat eso-gastro-duodenal a evidenţiat în treimea inferioară a esofagului toracic, pe partea stângă, o pungă diverticulară cu dimensiuni de circa 8/10 cm, cu colet larg, stomac cu pliuri îngroşate, bulb edematos, deformat cicatriceal.

Endoscopia digestivă superioară decelează diverticul gigant de 8-10 cm, cu mucoasă cu eroziuni şi conţinut alimentar; pe faţa posterioară duodenală postpiloric crater rotund de 8-10 mm şi adânc de 4-5 mm, înconjurat de mucoasa edemaţiată.

Examenul CT toracic cu substanță de contrast (fig.1, 2) pune în evidență formațiune pseudotumorală ovoidă cu margini regulate, cu dimensiuni de 8/9 cm, cu nivel lichidian, situată în mediastinul posterior, în contact cu esofagul toracic supradiafragmatic pe partea stângă.

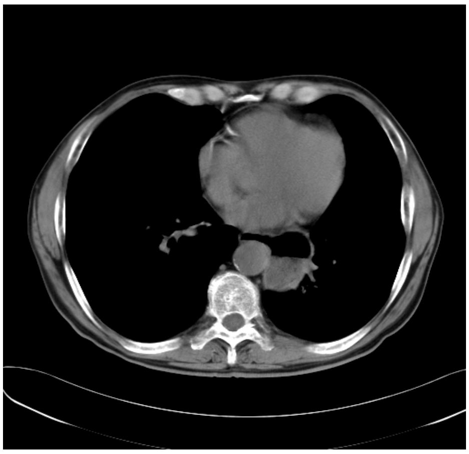

Fig.1. Examen CT:
diverticul esofagian cu nivel lichidian.

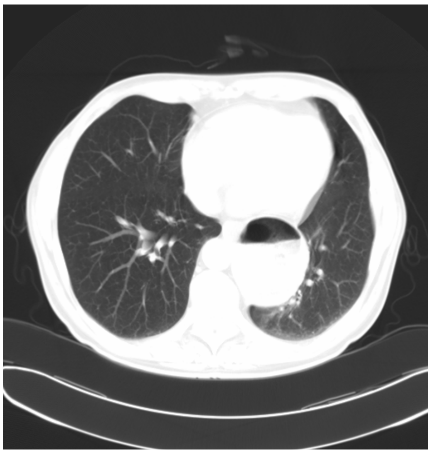

Fig.2. Examen CT:
diverticul esofagian cu nivel lichidian.

Se intervine chirurgical prin toracotomie posterolaterală stângă cu abordul cavității pleurale prin spațiul şapte intercostal. Secționarea ligamentului triunghiular pulmonar şi a pleurei mediastinale pune în evidență la nivelul esofagului inferior toracic o pungă diverticulară de mari dimensiuni, cu fenomene de periesofagită înconjurătoare. Se introduce o sondă Fauchet în esofag şi se disecă atent punga diverticulară, foarte aderentă pe toată lungimea de peretele esofagian (fig 3).

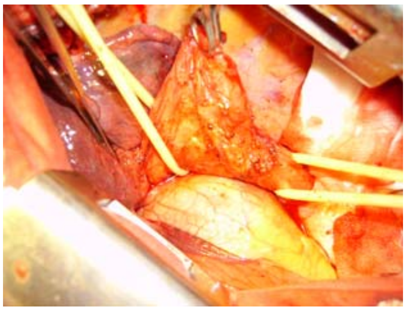

Fig 3. Aspect intraoperator.
Disecția esofagului cu
evidențierea diverticulului

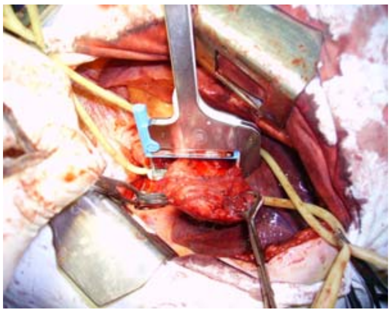

Fig. 4. Aspect intraoperator.
Staplarea la bază a diverticulului

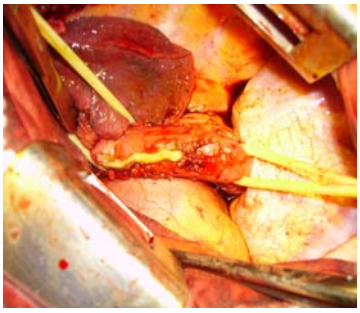

Fig 5. Aspect intraoperator.
Linia de sutură mecanică de la
nivelul peretelui esofagian

Se evidențiază coletul diverticular, cu dimensiuni de circa 5cm, şi se staplează razant la peretele esofagian. Secționarea pe pensă a diverticulului şi îndepărtarea din câmpul operator (fig.4, 5).

Întărirea tranşei de sutură mecanică cu surjet Biocryl 3.0. Esomiotomie extramucoasă pe versantul opus liniei de sutură pe o distanță de circa 6 cm, până la nivelul cardiei (fig.6).

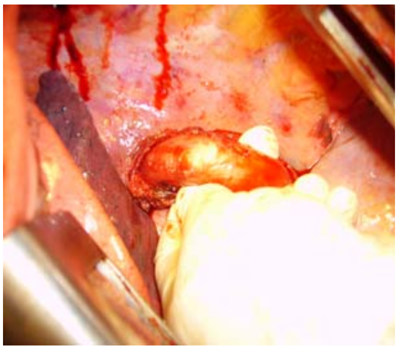

Fig.6. Aspect intraoperator.
Esomiotomie extramucoasă.

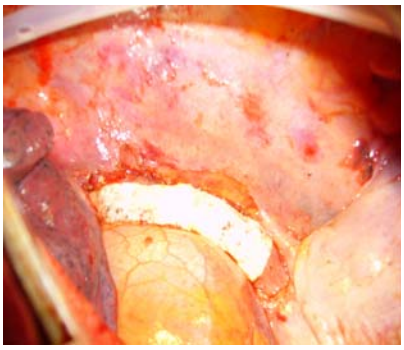

Fig.7. Aspect intraoperator.
Aplicare de TachoSil la nivelul esomiotomiei

Controlul hemostazei, verificarea integrității mucoasei esofagiene şi aplicarea de TachoSil la nivelul liniei de sutură mecanică şi esomiotomiei (fig.7). Se poziționează sonda nazogastrică în stomac, după verificarea integrității mucoasei esofagiene cu albastru de metilen. Drenajul cavității pleurale cu un tub de dren racordat la aspirație pasivă. Toracorafie anatomică.

Evoluția postoperatorie a fost favorabilă, cu reluarea alimentației pe sonda nazogastrică a doua zi postoperator. Suprimarea tubului de dren pleural în ziua a 7-a postoperator şi a sondei nazogastrice în ziua a 5-a postoperator, după control cu substanță radioopacă.

Observația 2

Pacient în vârstă de 62 de ani este transferat în Secția Clinică Chirurgie Toracică cu diagnosticul de diverticul esofagian epifrenic.

Tranzitul baritat esogastric pune în evidență la nivelul esofagului toracic distal o pungă diverticulară cu dimensiuni de circa 2/3 cm (fig.8).

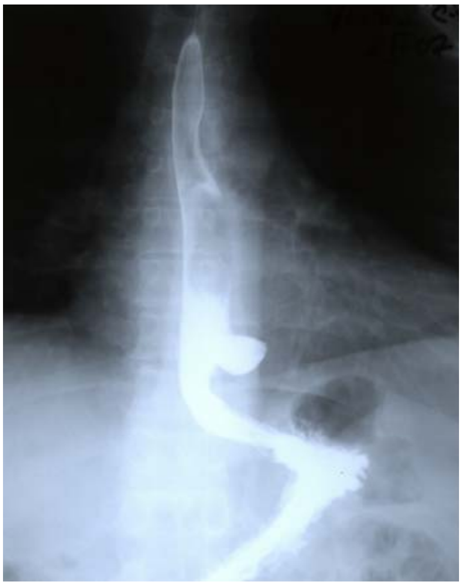

Fig.8 Tranzit baritat esogastric pungă diverticulară epifrenică.

Endoscopia digestivă superioară descrie diverticulul esofagian ca un fund de sac din care se aspiră resturi alimentare (fig.9).

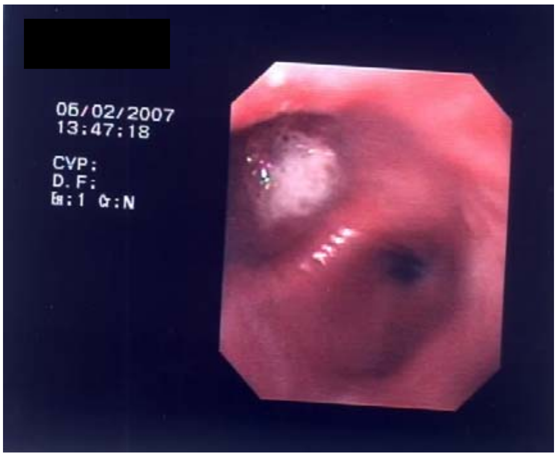

Fig.9 Endoscopie digestivă superioară: fund de sac esofagian inferior, cu resturi alimentare.

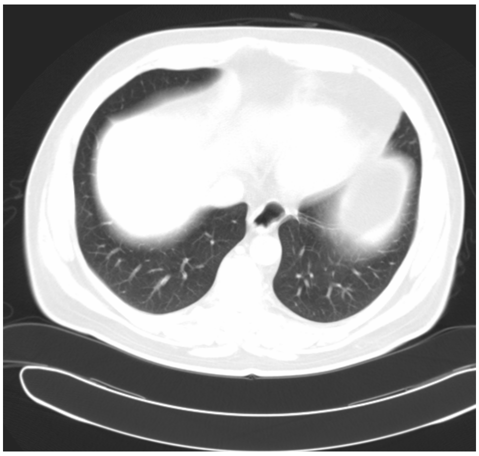

Fig.10. Examen CT: diverticul epifrenic de dimensiuni modeste.

Examenul CT evidențiază diverticulul epifrenic şi exclude eventualele patologii asociate (fig.10).

Atitudinea chirurgicală a fost aceeaşi, cu abordul cavităţii pleurale prin spaţiul şapte intercostal, secţionarea ligamentului triunghiular pulmonar şi a pleurei mediastinale, disecţia esofagului toracic terminal de la nivelul venei pulmonare inferioare până la nivelul hiatusului esofagian diafragmatic, punerea în evidenţa a diverticulului (fig.11).

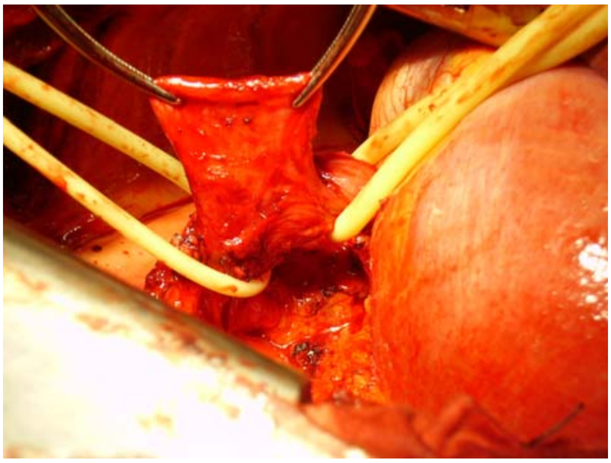

Fig 11. Aspect intraoperator:
disecţia şi evidenţierea diverticulului esofagian

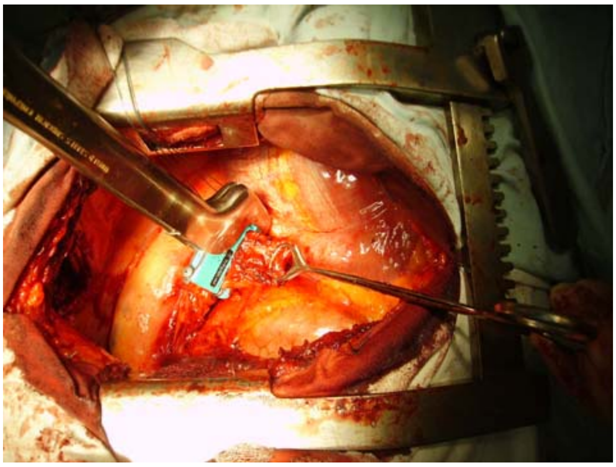

Fig 12. Aspect intraoperator:
Staplarea diverticulului la nivelul coletului.

Secţionarea diverticulului după staplare la nivelul coletului diverticular (fig.12).

Întărirea tranşei de sutură mecanică cu surjet Biocryl 3.0. Se practică esomiotomie extramucoasă pe versantul opus liniei de sutură pe o distanţă de circa 6 cm pînă la nivelul cardiei. Recalibrarea hiatusului esofagian cu 3 fire pe pilierii diafragmatici (fig.13).

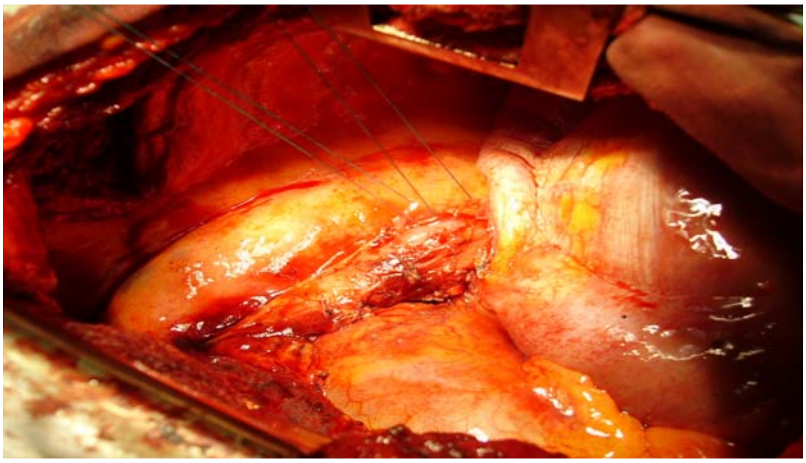

Fig 13. Aspect intraoperator.
Esomiotomie extramucoasă şi recalibrarea hiatusului esofagian

Observaţia 3

Pacientă, în vârstă de 58 de ani, este internată pentru regurgitaţii cu alimente nedigerate şi disfagie moderată. Tranzit baritat: esofag cu tranzit normal până în 1/3 inferioară, unde se evidenţiază un diverticul gigant cu diametrul de 8 cm. Tranzit prin cardia uşor încetinit. Stomac uşor alungit, cu pliuri îngroşate (fig.14, 15).

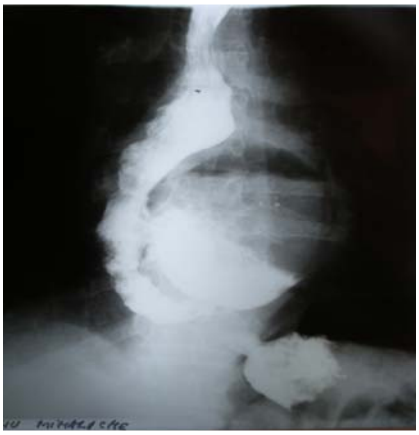

Fig 14. Tranzit baritat:
pungă diverticulară gigantă epifrenică.

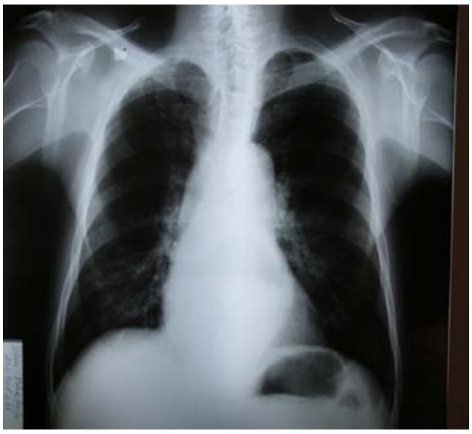

Fig 15. Radiografie toracică:
opacitate retrocardiacă cu nivel lichidian.

Tomografia computerizată cu substanță de contrast administrată oral evidențiază formațiune pseudotumorală de 8/10 cm, epifrenică şi cu comunicare cu esofagul toracic (fig.16, 17).

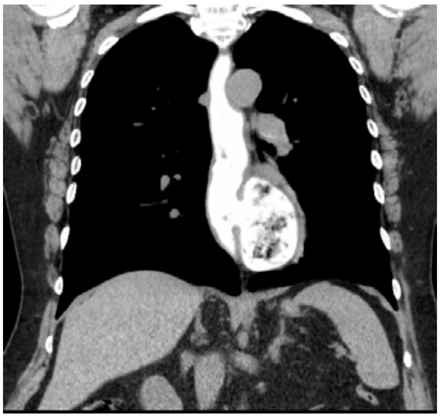

Fig 16. Examen CT cu subst. de contrast adm. oral – pseudotumoră cu nivel lichidian paraesofagian stâng supradiafragmatic.

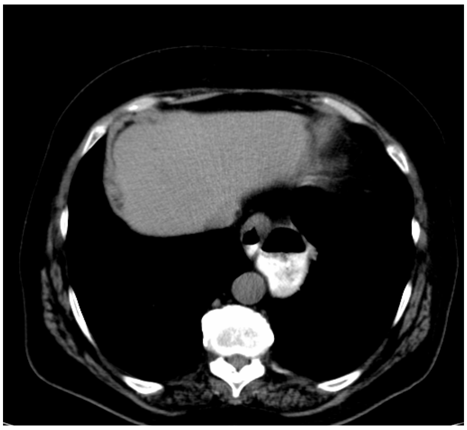

Fig 17. Examen CT cu subst. de contrast adm. oral – pseudotumoră cu nivel lichidian şi comunicare cu esofagul inferior toracic.

Se intervine chirurgical prin toracotomie posterolaterală stângă şi se evidențiază un diverticul esofagian cu diametrul de 10/7 cm, dezvoltat în 1/3 distală a esofagului (fig.18, 19). Se practică diverticulectomie, esomiotomie extramucoasă extinsă subaorto-cardial şi recalibrarea hiatusului esofagian (fig. 20, 21, 22).

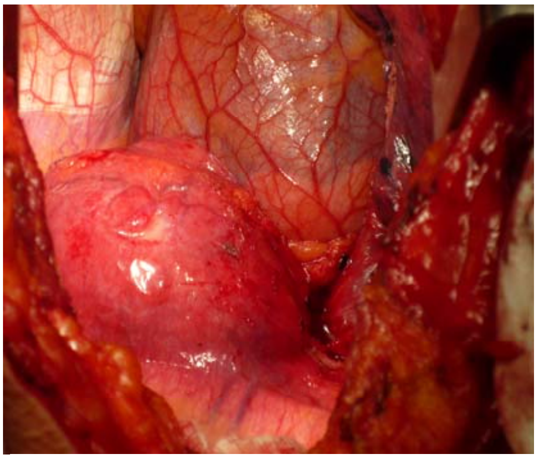

Fig 18. Aspect intraoperator – evidențierea diverticulului esofagian.

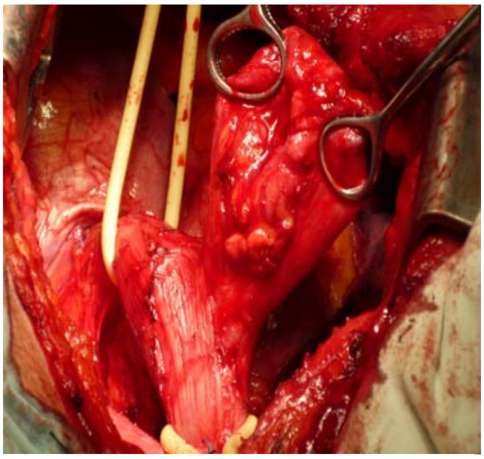

Fig 19. Aspect intraoperator – disecția diverticulului cu evidențierea coletului diverticular.

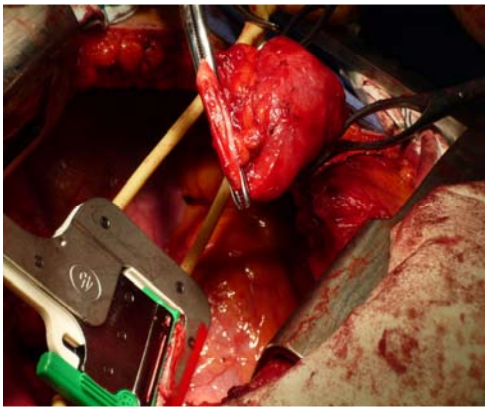

Fig.20. Aspect intraoperator – staplarea şi secționarea diverticulului la nivelul coletului.

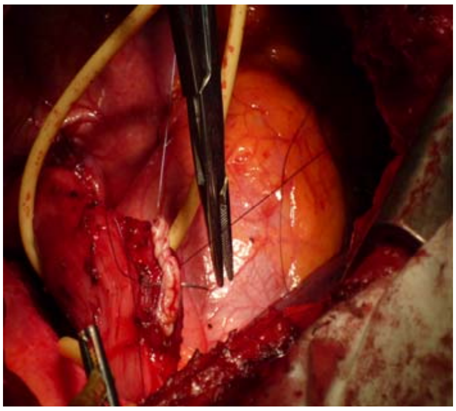

Fig.21. Aspect intraoperator – întărirea tranşei esofagiene cu surjet Biocril 3.0.

Piesa de rezecție se trimite la examen histopatologic (fig.23). Intervenția chirurgicală se termină în mod obişnuit cu drenaj pleural pasiv şi sondă nazogastrică.

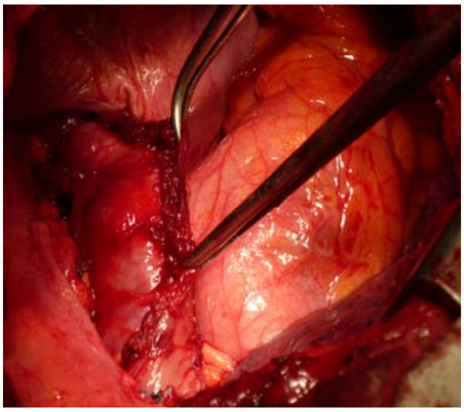

Fig.22. Aspect intraoperator. Esomiotomie extramucoasă subaorto-cardial

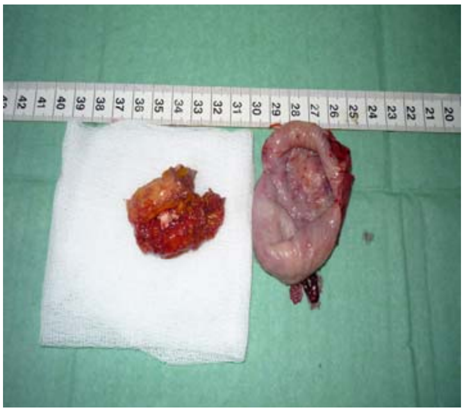

Fig.23. Piesa de rezecție. Diverticul esofagian şi conținutul său

Evoluția postoperatorie a fost favorabilă. Sonda nazogastrică, pe care s-a administrat alimentație corespunzătoare începând din a doua zi postoperator, se suprimă a cincea zi, după un control radiologic cu substanță de contrast.

Discuții

Diverticulul epifrenic este un diverticul esofagian fals (un fund de sac căptuşit cu mucoasă esofagiană ce herniază prin punctele slabe ale muscularei), un pseudodiverticul al cărui perete este format doar din mucoasă şi submucoasă esofagiană.

Diverticulii epifrenici sunt cei mai frecvenți diverticuli de corp esofagian (20% din totalul diverticulilor esofagieni). Prevalența reală nu este cunoscută datorită numărului mare de cazuri asimptomatice care nu sunt descoperite decât întâmplător. Majoritatea bolnavilor au peste 50 de ani, în special decadele şapte şi opt, cu o uşoară predominență a sexului masculin. Conform studiilor din ultimii 40 de ani ai Mayo Clinic, raportul diverticuli epifrenici/ diverticuli Zenker este de 1/5 (Trastek şi Payne, 1989).

Diverticulul epifrenic este un diverticul de pulsiune, luând naştere datorită unui gradient presional ce apare în lumenul esofagian şi care se răsfrânge transmural determinând hernierea mucoasei prin punctele slabe ale muscularei esofagiene. Deşi inițial simptomatologia a fost atribuită exclusiv diverticulului esofagian distal, Vinson (1934) raportează disfuncții ale motilității esofagiene subiacente. Acalazia, spasmul esofagian difuz, hipertonia sfincterului esofagian inferior şi alte anomalii motorii nespecifice au fost toate constatate în asociere cu diverticulul epifrenic.

Introducerea unei tehnici moderne de diagnostic, manometria ambulatorie timp de 24 de ore (Nehra şi colab., 2002), a permis studiul prospectiv al fiziopatologiei diverticulului epifrenic.

Orringer (1993) susţine că tulburările de motilitate esofagiene stau la baza apariţiei tuturor diverticulilor epifrenici. Acest concept, care afirmă că diverticulii epifrenici reprezintă de fapt o complicaţie în evoluţia tulburărilor de motilitate esofagiană mai degrabă decât o slăbiciune a peretelui muscular esofagian, a devenit general acceptat.

Majoritatea pacienţilor sunt asimptomatici, în timp ce restul au de la o disfagie uşoară până la regurgitaţie cu aspiraţie pulmonară. De obicei, diverticulul esofagian este descoperit întâmplător la un tranzit baritat efectuat pentru cu totul alte raţiuni.

Disfagia, durerea toracică, regurgitaţia, tusea nocturnă şi pneumopatia de aspiraţie se datorează mai degrabă disfuncţionalităţii motorii subiacente decât diverticulului epifrenic per se. Regurgitaţia şi aspiraţiile repetate determină pneumonii cu distrucţii însemnate de parenchim pulmonar.

Tranzitul baritat este metoda standard de evidenţiere a diverticulului, dar şi a eventualelor anomalii motorii esofagiene, putând decela şi o posibilă patologie asociată: cancer, hernie hiatală, stenoze esofagiene.

Endoscopia digestivă superioară trebuie efectuată de către endoscopişti cu experienţă şi poate diagnostica esofagitele şi cancerul esofagian, atunci când se suprapun peste patologia deja existentă (cazuri rare). Are un rol important în pregătirea preoperatorie a bolnavilor cu diverticuli voluminoşi, cu fenomene de retenţie şi regurgitaţie, prin îndepărtarea resturilor alimentare de la acest nivel.

Manometria esofagiană este importantă în descoperirea anomaliilor motorii, dar mai ales în indicarea obstrucţiei funcţionale şi a lungimii esomiotomiei ce urmează a fi efectuate.

PH-metria pe 24 de ore este indicată în momentul în care se suspicionează refluxul gastroesofagian şi impune efectuarea unui procedeu chirurgical antireflux.

Pacienţii asimptomatici şi cei cu o simptomatologie uşoară sunt de obicei trataţi conservativ, intervenţia chirurgicală impunându-se când apar retenţia alimentelor la nivelul pungii diverticulare cu regurgitaţie şi pneumoniile de aspiraţie consecutive. Totuşi părerile sunt împărţite: astfel, dacă Benacci şi colegii (1993) apelează la chirurgie în funcţie de mărimea, poziţionarea diverticulului dar şi de simptomatologie, Altorki şi colab. (1993) propun chirurgicalizarea tuturor pacienţilor cu diverticul epifrenic, pentru a preveni posibilele complicaţii.

Prima diverticulectomie a fost efectuată de Clairmont (1927), folosind calea de abord extrapleurală. Barett (1933) a efectuat prima diverticulectomie pe cale transpleurală. Tehnica chirurgicală curentă este cea prin toracotomie posterolaterală stângă, cu abordul cavităţii pleurale prin spaţiul şapte intercostal, disecţia sacului diverticular, diverticulectomie şi esomiotomie extramucoasă. Esomiotomia este necesară nu doar pentru a preveni apariţia fistulei esofagiene, ci şi pentru a ameliora simptomatologia datorată prezenţei tulburărilor de motilitate esofagiană asociate.

Esomiotomia se efectuează pe partea opusă diverticulectomiei şi trebuie extinsă câţiva milimetri pe stomac şi cranial până la nivelul venei pulmonare inferioare sau până la nivelul unde în timpul efectuării manometriei au fost semnalate tulburări de motilitate esofagiene. Unii chirurgi, precum Belsey (1966) sau Little (1988), au propus ca procedeu chirurgical adiţional esomiotomiei şi efecturea unei valve antireflux. Efectuarea de rutină a unui procedeu antireflux este încă controversată şi de aceea mulţi chirurgi îl practică doar atunci când preoperator se pune în evidenţă prezenţa refluxului gastroesofagian sau hernie hiatală. Procedeul antireflux de elecţie este Belsey Mark IV.

Recent au fost propuse tehnici chirurgicale miniinvazive în tratamentul diverticulului esofagian. Abordul toracoscopic – Rosati (1998), Van Der Peet (2001) şi laparoscopic – Rosati. Rolul toracoscopiei şi laparoscopiei în tratamentul diverticulului esofagian epifrenic rămâne să fie stabilit, în prezent existând date relativ puţine datorită experienţei limitate şi urmăririi pe termen lung a acestor rezultate.

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Imagistica prin rezonanță magnetică în evaluarea leziunilor traumatice ale gleznei

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REZUMAT. Glezna reprezintă segmentul membrului inferior care uneşte gamba cu piciorul, puțin acoperit de părțile moi, dar mult solicitat în statică şi locomoție. Datorită complexității structurilor anatomice ale gleznei, nevizualizabile prin tehnici radiologice şi scintigrafice, IRM rămâne metoda de elecție pentru vizualizarea multiplanară.

Cunoaşterea aspectului IRM normal al gleznei are o mare importanță în identificarea corectă a structurilor osteo-ligamento-tendinoase şi a patologiilor acestora. Având în vedere că în anumite activități sportive glezna şi în special tendoanele sunt foarte expuse, am efectuat un studiu retrospectiv IRM pe un lot de 170 de pacienți în scopul identificării şi evaluării leziunilor traumatice. Examinarea IRM a evidențiat leziuni pluritendinoase în majoritatea cazurilor. Dintre acestea cele mai multe au fost leziunile tendonului lui Ahile (predominant rupturi parțiale) şi leziunile flexorului lung al halucelui. S-au identificat şi alte leziuni tendinoase dar în procente mai mici.

În studiul nostru am folosit un aparat de rezonanță magnetică de 1T. Pacienții au fost poziționați în decubit dorsal cu piciorul de examinat în flexie plantară. Achizițiile s-au făcut în cele trei planuri: axial, sagital şi coronal, folosindu-se secvențele T1 SE, T2 SE, T1 TIRM, PD FS, T2 fl2D şi T2 de3D, cu secțiuni de 3 mm grosime şi un FOV de 16 cm.

Examenul IRM reprezintă o tehnică de elecție pentru vizualizarea multiplanară a tendoanelor şi a relațiilor acestora cu structurile din jur.

Cuvinte cheie: glezna, IRM, tendoane, tendonul lui Ahile, flexorul lung al halucelui

ABSTRACT. The ankle is a lower limb segment that joints the ankle with the foot, slightly covered by the soft tissues, but very much used while standing and in motion. Due to anatomical complexity of the ankle, not shown by radiography and scintigraphy, MRI remains the primary method for multiplanar imaging.

A good knowledge of the normal MRI aspect of the ankle has a great importance for a proper identification of the bony-meniscal-ligamentar lesions and their pathologies. Considering that in some sport activities the ankle and especially the tendons are very exposed to traumatic lesions, we have made a MRI retrospective study on a lot of 170 patients to identify and evaluate these lesions. The MRI exam showed associated tendinous lesions in most of the cases. Most of these lesions were injuries of the Achilles tendon (especially partial tears) and injuries of the flexor hallucis longus. We identified other tendinous lesions too but in a smaller percentage.

We used in our study a magnetic resonance machine of 1T. Patients were placed in supine position, with the examined foot in plantar flexion. The acquisitions were made in three planes (sagittal coronal and axial), using the T1 SE, T2 SE, T1 TIRM, PD FS, T2 fl2D and T2 de3D sequences, with 3 mm thick sections and a 16 cm FOV.

The MRI exam is the main technique for a multiplanar imaging of the tendons and their relations with the surrounding structures.

Glezna reprezintă segmentul membrului inferior care uneşte gamba cu piciorul, puțin acoperit de părțile moi, dar mult solicitat în statică şi locomoție. Aceasta este alcătuită din structuri anatomice complexe, nevizualizabile prin tehnici radiologice şi scintigrafice, astfel că IRM rămâne metoda de elecție pentru vizualizarea multiplanară.

În atletism, fotbal, basket, tenis, patinaj, hockey, precum şi în alte activități sportive şi de dans, glezna este suprasolicitată fie prin dorsiflexia forțată a piciorului contra muşchilor gambei contractați, fie prin hiperflexia plantară, prin schimbări rapide de picior, prin respingeri repetate de pe antepicior sau doar prin suprasolicitare.

Elemente de anatomie şi biomecanică ale gleznei

Din punct de vedere anatomic, glezna este un complex osteo-ligamento-tendinos compus din:

– structuri osoase;

– capsula şi sinoviala, ce cuprind articulația gleznei;

– structuri ligamentare şi tendinoase: împărțite în patru compartimente: anterior, posterior, lateral şi medial

Un rol foarte important îl are tendonul lui Ahile, din compartimentul posterior. Acesta este mărginit anterior de bursa retrocalcaneană şi de grăsimea Kager şi posterior de bursa calcaneană subcutanată. Este un tendon puternic, având o grosime de 4-6 mm, plat sau concav anterior în secțiune axială. La 2-6 cm deasupra inserției pe calcaneu prezintă o zonă slab vascularizată, fapt pentru care aici este locul de elecție al leziunilor.

Tendoanele sunt alcătuite din fibrile de colagen învelite în endotenon, formând mănunchiuri, acoperite la periferie de epitenon. Majoritatea tendoanelor sunt cuprinse la exterior într-o fascie, cu excepția tendonului lui Ahile, care e acoperit de peritenon, format din trei straturi (parietal, visceral şi mezotenonul între ele).

Din punct de vedere biomecanic, glezna are dublu rol: static şi dinamic. Prin rolul static păstrează echilibrul gambei pe picior iar prin cel dinamic realizează mişcările piciorului în mers de dorsiflexie, cu uşoară inversiune (ridică marginea medială a piciorului) şi o amplitudine de 15-20°, precum şi de flexie plantară cu uşoară eversiune (coboară marginea medială a piciorului) şi o amplitudine de 45°.

Anatomia IRM a gleznei

Cunoaşterea aspectului IRM normal al gleznei are o mare importanță în identificarea corectă a structurilor osteo-ligamento-tendinoase precum şi a patologiilor însoțitoare ale acestora. În acest studiu vom insista asupra tendoanelor gleznei, structuri deosebit de importante şi uşor de identificat cu ajutorul rezonanței magnetice. Ele sunt urmărite în toate cele trei planuri, axial, sagital şi coronal.

În plan axial putem identifica cele patru compartimente ale gleznei:

A. anterior:

a) tendonul **t**ibial anterior **(TA)**

b) tendonul **e**xtensor lung al halucelui **(ELH)**

c) tendonul extensor lung al **d**egetelor **(ELD)**

B. medial:

a) tendonul **t**ibial posterior **(TP)**

b) tendonul flexor lung al **d**egetelor **(FLD)**

c) tendonul flexor lung al **h**alucelui **(FLH)**

C. lateral:

a) tendonul peronier scurt **(PS)**

b) tendonul peronier lung **(PL)**

D. posterior

- tendonul lui Ahile

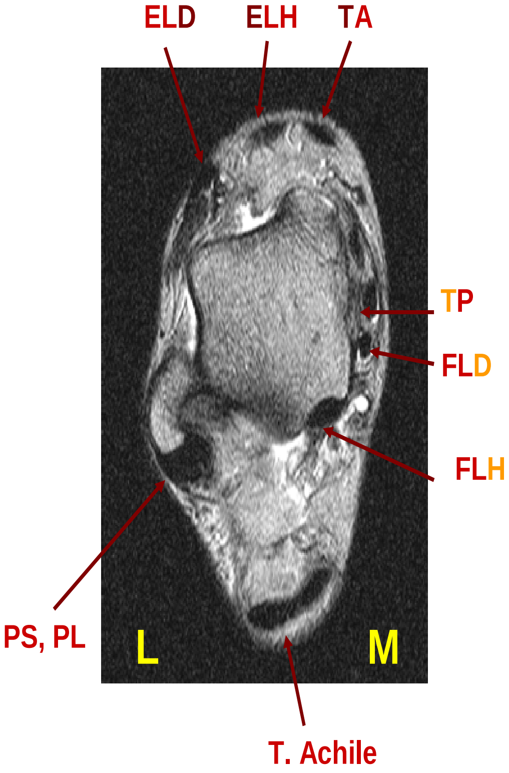

Deoarece planul axial este foarte important în identificarea compartimentelor şi a tendoanelor din acestea, modificările de contur dar şi cele de semnal intra- şi peritendinos, este necesară o bună cunoaştere a acestuia. Astfel, tendoanele compartimentului anterior se pot reţine uşor cu formula **TED**, iar ale celui medial folosind formula mnemotehnică **TDAH (Tom Dick and Harry)**

Compartimentul medial
Tom Dick and Harry
- **T**ibial posterior - **T**
- flexor lung al **D**egetelor - **D**
- **a**rtera, vena şi nervul tibial posterior - **a**
- flexor lung al **H**alucelui - **H**
- **T = 2 D** sau **2 H**

M = medial
L= lateral

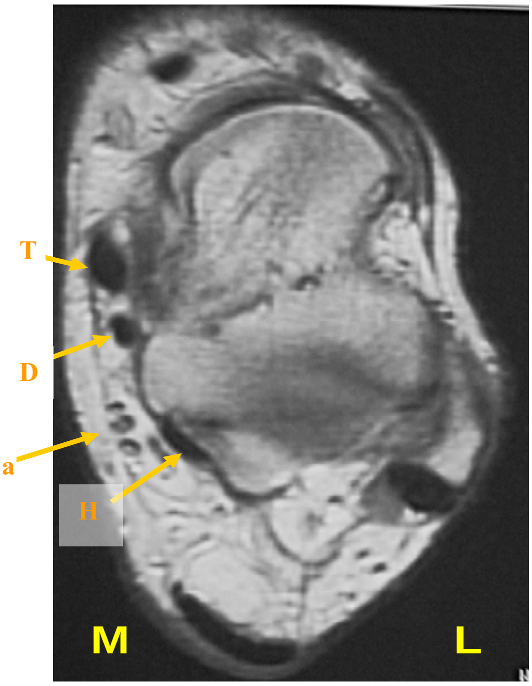

De asemenea, compartimentul anterior se reţine mai uşor cu ajutorul formulei **TED**:

Compartimentul anterior
TED
- **T**ibial anterior
- **E**xtensor lung al halucelui
- Extensor lung al **D**egetelor
- *Peroneus tertius* (parte din extensorul lung al degetelor) (Pt)

Compartimentul lateral
- Peroneul scurt (**Ps**)
- Peroneul lung (**Pl**)

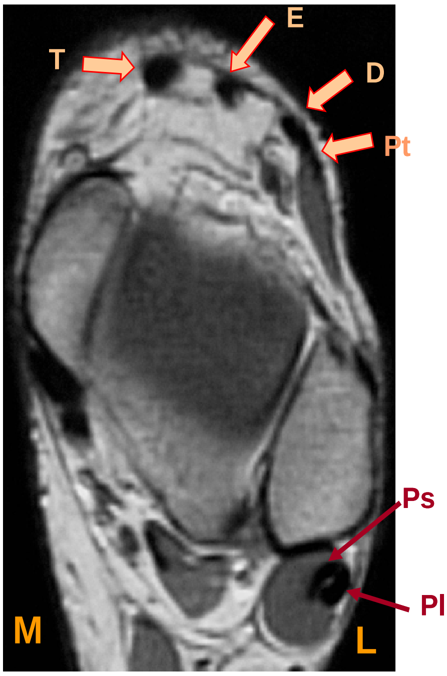

Compartimentul lateral cuprinde cei doi peronieri: peroneul scurt, de partea medială şi cel lung, de partea laterală.

Planul sagital este util în urmărirea traiectului tendinos în lungime, precum şi contururile anterior şi posterior, pe lângă modificările de semnal. Este important să coroborăm rezultatele obținute în plan axial cu cele din plan sagital pentru confirmarea diagnosticului imagistic.

Plecând dintr-un plan parasagital medial înspre lateral, putem identifica tendoanele după cum urmează: anterior se observă tendonul tibial anterior, iar posterior cele două tendoane din compartimentul medial, tibial posterior şi flexorul lung al degetelor (1).

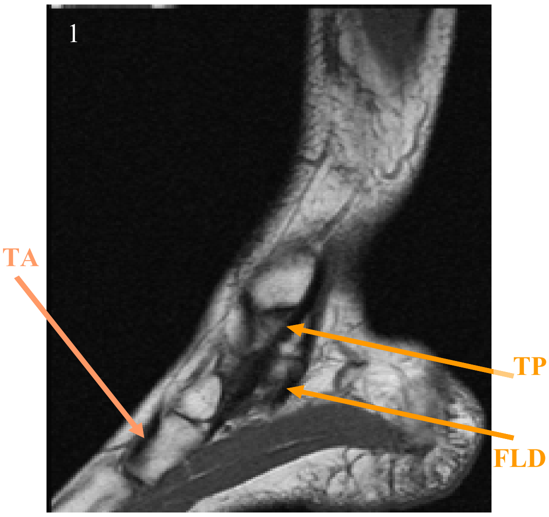

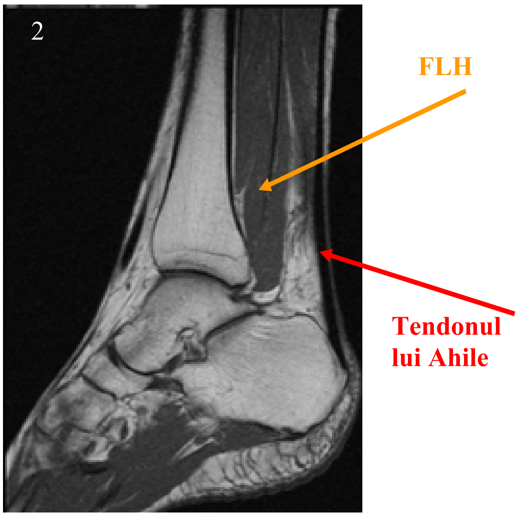

Posterior de flexorul lung al degetelor, tot în compartimentul medial se află tendonul flexor lung al halucelui. Acesta este mărginit posterior de grăsimea Kager şi de tendonul lui Ahile din compartimentul posterior (2).

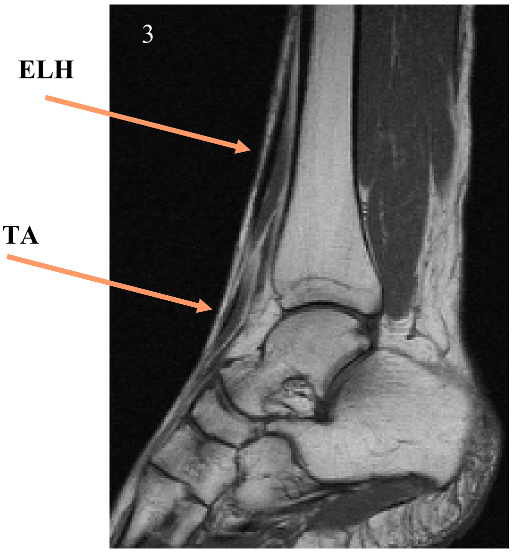

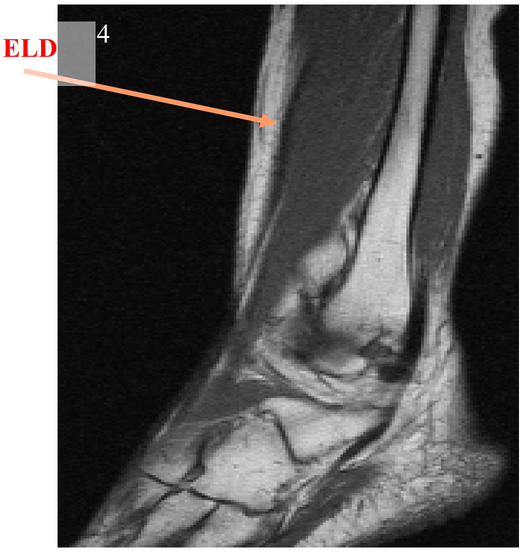

Dintre tendoanele compartimentului anterior mai putem observa anterior (3) o parte din tibialul anterior (TA) şi începem să vedem tendonul extensor lung al halucelui (ELH); pe următoarea imagine (4) se observă şi cel mai lateral tendon din acelaşi compartiment, extensorul lung al degetelor (ELD).

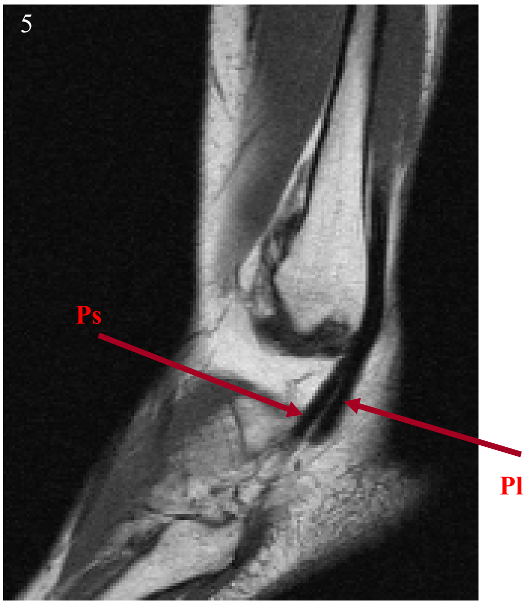

Compartimentul lateral cuprinde două tendoane: peronierul scurt (Ps), aflat medial şi peronierul lung (Pl), cel mai lateral.

Studiul nostru

Am realizat un studiu retrospectiv pe un lot de 237 de pacienți, în perioada septembrie 2004 – octombrie 2008, dintre care am selectat 170, 145 bărbați şi 25 femei, cu vârste cuprinse între 16 şi 67 ani, la care examenul radiologic a fost normal sau neconcludent.

Tehnica de lucru

Am utilizat un aparat Siemens, Magnetom, Harmony de 1T. Pacientul a fost poziționat în decubit dorsal cu piciorul de examinat în flexie plantară 20° sau hiperflexie pentru vizualizarea tendonului ELD ("efectul de unghi magic").

Antena folosită a fost una flexibilă, de suprafață, cu piciorul controlateral în afara FOV; se poate utiliza şi o antenă cadru pentru examinarea ambelor glezne.

Secvențele şi incidentele utilizate

Am folosit secvențele convenționale T1 SE şi T2 SE, secvența de inversie T1 TIRM, secvența PD FS, secvențele de gradient T2 fl2D şi T2 de3D, cu secțiuni de 3 mm grosime, în incidențele axială, coronală, sagitală şi un FOV de 16 cm.

Protocolul de examinare se poate modifica şi în funcție de patologia întâlnită.

Rezultate

La cei 170 de pacienți la care examenul radiologic a fost normal sau neconcludent, leziunile pluritendinoase au fost majoritare – 65,88%, cele monotendinoase fiind mai reduse – 26,47%. Leziunile de tendoane s-au asociat şi cu leziuni ligamentare (28,82%), leziuni de edem osos (18,23%) şi cu leziuni musculo-aponevrotice (14,7%). Alte leziuni importante întâlnite în studiul nostru sunt cele osteocondrale talare (15,88%).

Dintre afectările tendoanelor, cele ale tendonului lui Ahile au fost cele mai numeroase (61,76%), majoritatea fiind rupturi parțiale (37,64% dintre afectările ahilene), cele totale fiind într-un procent mult mai mic, 5,88%. Am întâlnit de asemenea într-un procent semnificativ leziuni de flexor lung al halucelui (22,94%). Restul leziunilor tendinoase au fost în procente mai mici.

Leziune		Nr.	Total pacienți
Tendonul lui Ahile 105 (61,76%)	**Ruptură parțială**	64(**37,64%**)	170
	Ruptură totală	10 (5,88%)	170
	Tendinite, para- şi peritendinite	23(13,52%)	170
	Tendinoză	8(4,70%)	170
FLH		39(**22,94%**)	170
TP		12 (7,05%)	170
FLD		11(6,47%)	170
PL		8(4,70%)	170
PS		6(3,52%)	170
TA		3(1,76%)	170

Rupturile tendonului lui Ahile (după *Magnetic Resonance Imaging in Orthopaedics and Sports Medicine* – David W. Stoller):

Tipul I – ruptură parțială ≤ 50%, pe fața anterioară sau pe cea posterioară.

Tipul II – ruptură totală, fie cu suprapunerea celor două capete tendinoase, fie cu retracția acestora şi apariția unui spațiu intertendinos ≤ 3 cm.

Tipul III – ruptură totală, cu un spațiu intertendinos 3 – 6 cm.

Tipul IV – ruptură totală, cu un spațiu intertendinos > 6 cm (rupturi neglijate).

Clasificarea rupturilor e foarte importantă deoarece orientează conduita terapeutică.

Rupturile se mai clasifică în:

► acute => 0-4 săptămâni;

► cronice => 1-3 luni.

În continuare vom exemplifica această clasificare prin cazuistica existentă în clinica noastră.

Ruptura parțială a tendonului lui Ahile

Aspectul IRM (incidențele sagitală şi axială)

1. arii intratendinoase lineare/ focale cu semnal crescut;
2. îngroşarea/ ondularea fibrelor fără spațiu intertendinos;
3. continuarea parțială a fibrelor tendonului pe imaginile sagitale;
4. ruptura parțială acută (hipersemnal T2 şi TIRM) suprapusă pe o tendinita ahileană cronică (semnal intermediar -scăzut).

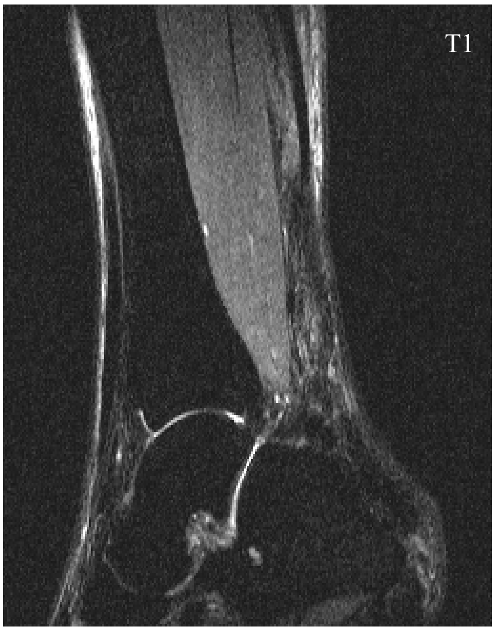

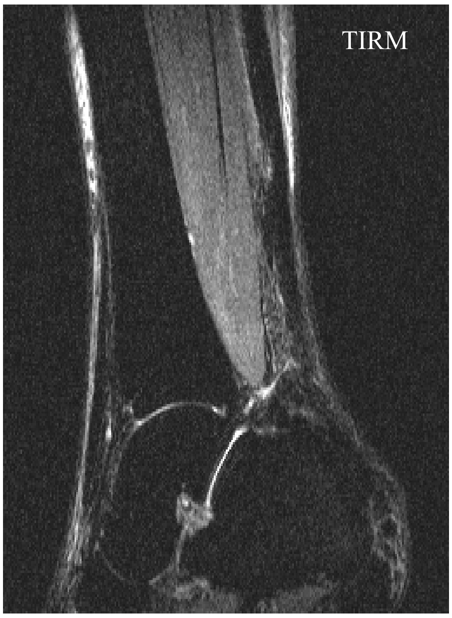

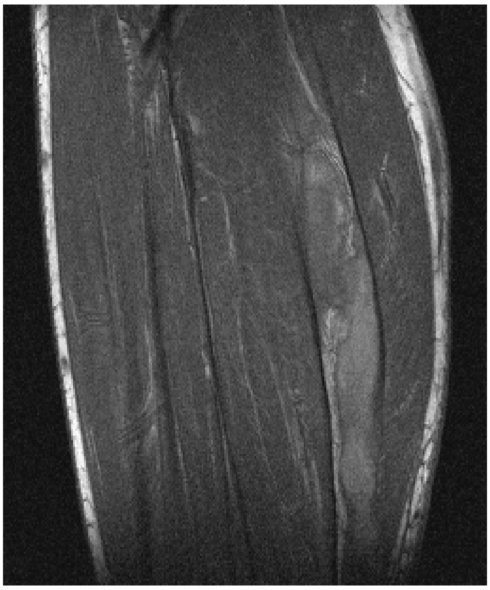
T1 SE

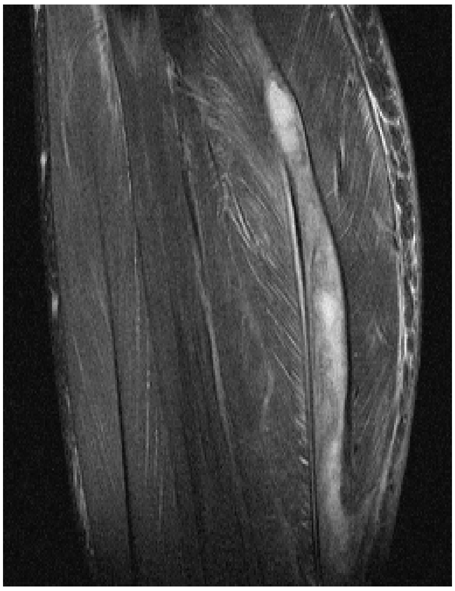
T2 SE FS

Cazul 1
N.R. 29 ani
Dg. trimitere: Traumatism gamba dr.
IRM: Pe secvențele: T1 TIRM sag. se evidențiază tendonul ahilean îngroşat, cu contur anterior neregulat şi cu modificări de semnal, predominant crescut pe un segment de 3 cm, sugestiv pentru infiltrație edematoasă. La nivelul gambei prezintă între mm. gastrocnemian şi solear o arie fusiformă cu semnal crescut T1SE şi hipersemnal T2 SE FS, dată de un hematom.

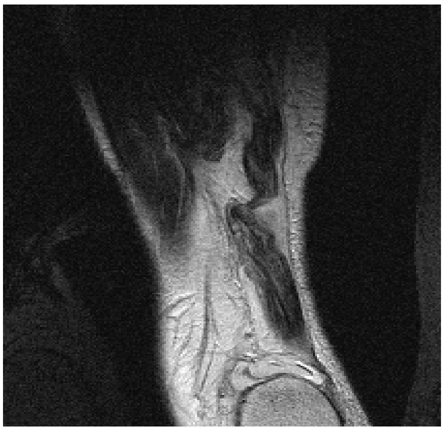

PD T2 TSE

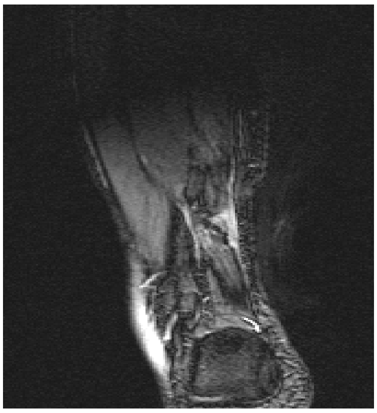

T2*

Cazul 2
F.A., 31 ani
Tendonul lui Ahile apare cu traiect angulat, îngustat, ondulat, cu semnal neomogen intratendinos, predominant crescut. În secvenţa T2* se evidenţiază o acumulare fluidă la nivelul focarului de ruptură. Sunt modificări subacute (3 săptămâni) de ruptură parţială de tendon ahilean.

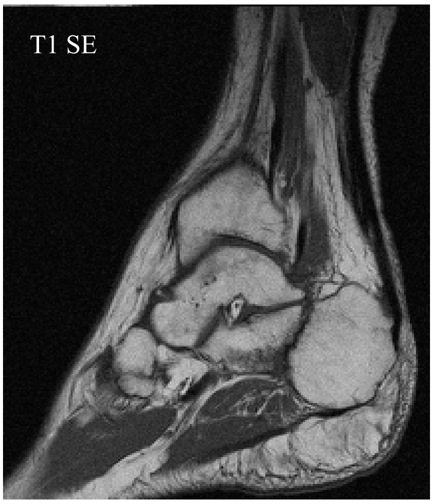

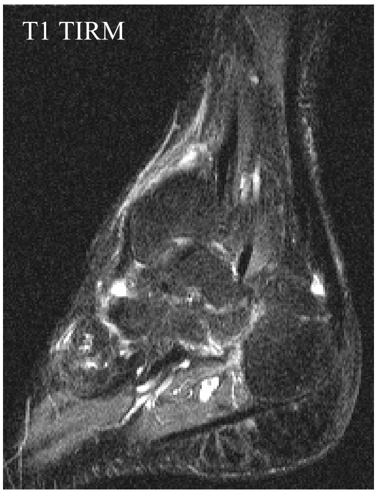

Cazul 3
T.M., 40 ani
Acest pacient a suferit un traumatism sever de gleznă şi picior. Examenul radiografic a arătat prezenţa fracturilor de calcaneu şi de maleolă peronieră. Datorită severităţii traumatismului s-a efectuat şi un examen IRM care a identificat în plus, pe T1SE, tendonul lui Ahile îngroşat, cu contur anterior neregulat, întrerupt şi o mică arie în hiposemnal anterior de acesta la nivelul inserţiei pe calcaneu. Secvenţa T1TIRM confirmă ruptura parţială anterioară a tendonului lui Ahile şi bursită retrocalcaneană (în hipersemnal).

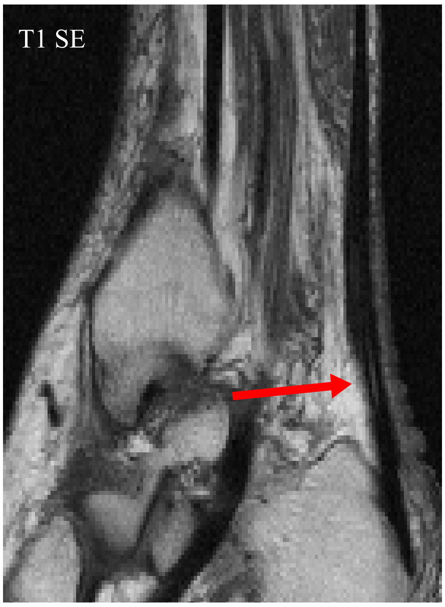

Cazul 4

M.D., 52 ani

Pacientul, care suferise un traumatism de gleznă dreaptă în urmă cu mai mult de o lună, a revenit la control. Examenul IRM a arătat tendonul lui Ahile îngroşat, cu contur neregulat anterior şi o imagine fusiformă cu semnal crescut intratendinoasă pe secvenţa T1 SE. Pe secvenţa de inversie observăm grăsimea Kager neomogenă, cu semnal predominant crescut, aspect sugestiv de infiltraţie fibro-adipoasă. Imaginea în plan axial confirmă modificările menţionate anterior de ruptură cronică parţială de tendon ahilean şi infiltraţie fibro-adipoasă a grăsimii Kager.

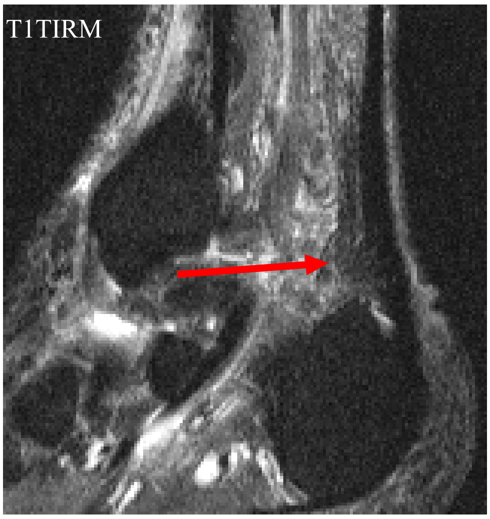

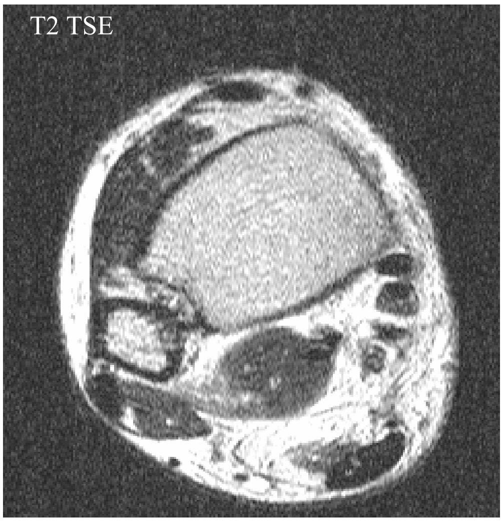

Ruptura totală a tendonului lui Ahile

Aspectul IRM:

- discontinuitate cu retracţia şi deşirarea capetelor tendinoase;
- T2*, T2 FS TSE, TIRM – arată prezenţa edemului şi a hemoragiei;
- faza acută: edem / hemoragie în spaţiul intertendinos şi peritendinos;
- faza cronică: ţesut cicatriceal hipointens sau grăsime hiperintensă în spaţiul intertendinos.

Aspectul RM postoperator:

- punte fibroasă de consistenţă mică între capetele rupte ale tendonului;
- aspect bulbos al capetelor rupte şi distanţă mare între acestea (vindecare suboptimă);
- artefacte de sutură în hipersemnal T2;
- spaţii fluide intratendinoase la 3-6 luni postoperator în hipersemnal;
- creşterea grosimii tendonului şi scăderea semnalului intratendinos la 12 luni postoperator.

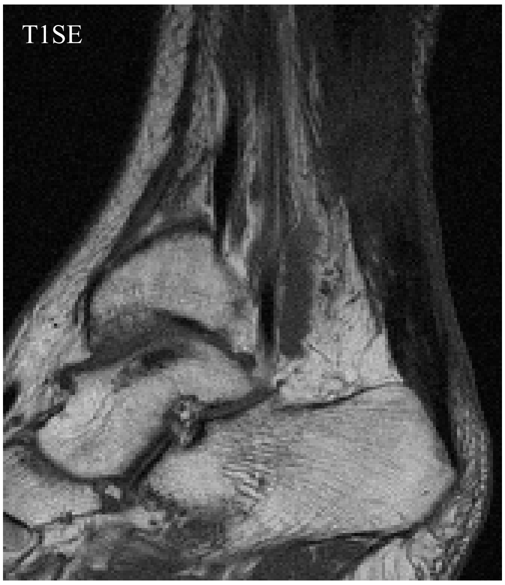

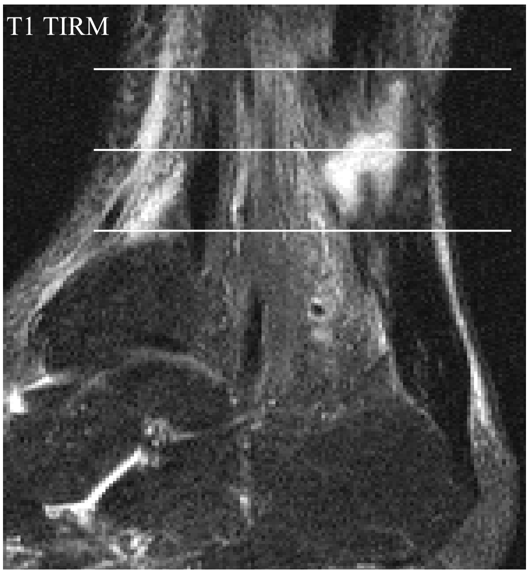

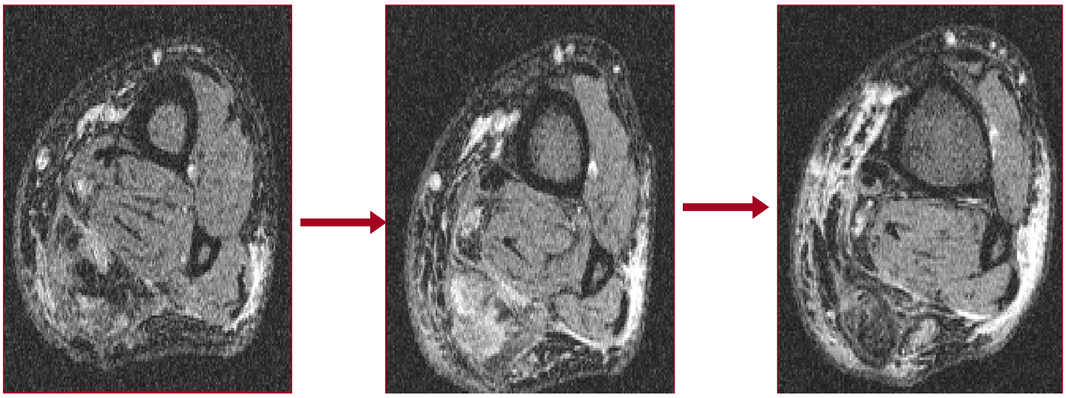

T2 fl 2D

Cazul 5

D.C.A., 39 ani

Secvenţa T1SE arată tendonul lui Ahile îngroşat, cu semnal neomogen, iar secvenţa de inversie demonstrează prezenţa rupturii totale a acestuia. Cele două capete tendinoase sunt cu margini neregulate, franjurate, separate de un spaţiu intertendinos de aproape 3 cm, unde se observă o arie în hipersemnal, sugestivă pentru infiltraţia edematoasă. Secvenţele în plan axial la nivelul capetelor tendinoase şi a spaţiului intertendinos demonstrează discontinuitatea tendonului, cu îngroşarea capetelor şi neomogenităţi de semnal la acest nivel.

Tendinita ahileană

Tendinita = inflamaţie intrinsecă a tendonului ahilean

Aspectul IRM

1. Îngroşare focală / fusiformă / difuză a tendonului lui Ahile pe toate secvenţele.
2. Modificări inflamatorii intratendinoase.
3. Tendonul apare îngroşat şi convex anterior pe incidenţa sagitală.
4. Fluid în bursa retrocalcaneană.

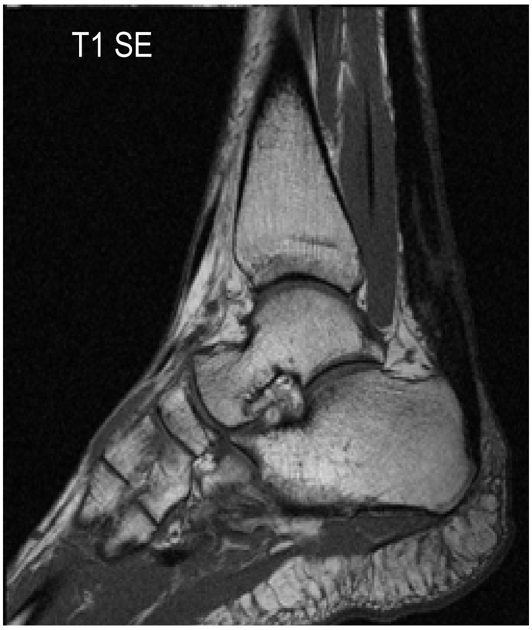

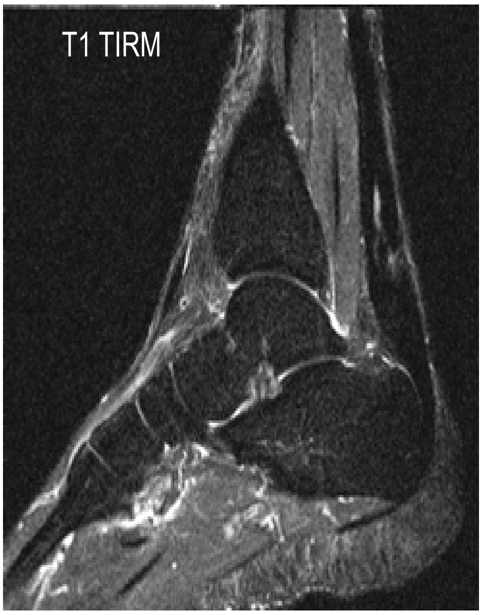

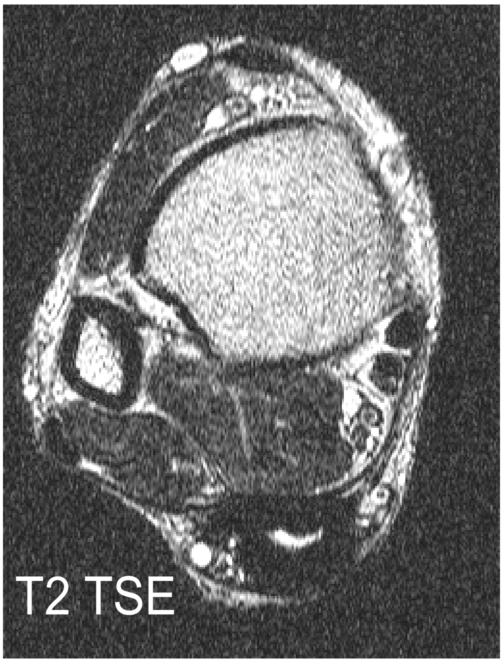

Cazul 6

I.I., 37 ani

Pacient cunoscut cu traumatism vechi de gleznă dreaptă revine pentru un nou traumatism. Examenul IRM arată tendonul ahilean mult îngroşat, convex anterior, cu zone fusiforme cu semnal crescut intratendinoase.

Peritendinita şi paratendinita ahileană

Peritendinita = inflamaţia peritenonului

Paratendinita = modificări inflamatorii în jurul tendonului

Ambele asociază modificări ale grăsimii preahilene Kager cu zone lineare sau difuze de semnal neomogen (determinate de edem sau de cicatrizarea peritenonului) cu sau fără morfologie anormală a tendonului.

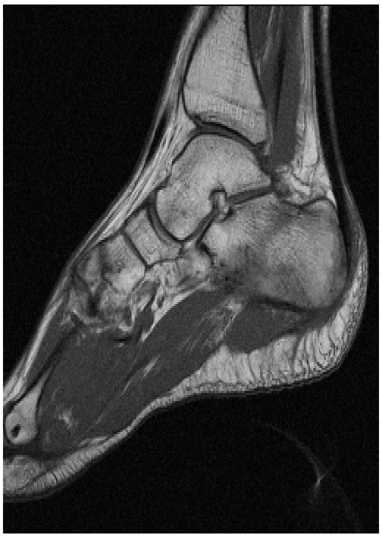

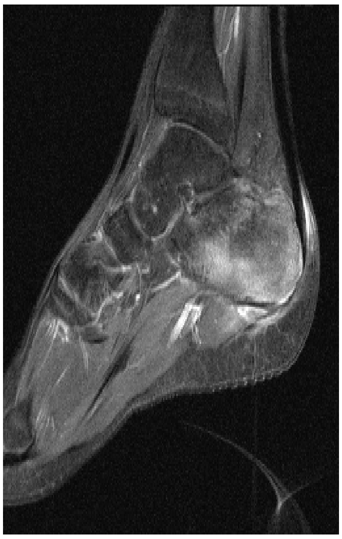

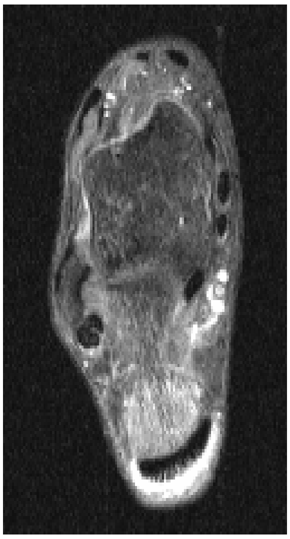

Cazul 7

C.E. 18 ani

Acest pacient tânăr s-a prezentat la camera de gardă pentru un traumatism forte de picior drept. Examenul IRM a evidenţiat edem osos la nivelul calcaneului (hiposemnal T1 SE şi hipersemnal T1 SE FS) şi infiltraţie edematoasă peri- şi paratendinoasă la nivelul tendonului ahilean.

Tendinoza ahileană

Modificări degenerative intratendinoase, fără substrat inflamator semnificativ. Clasificare:

1. degenerare fibromatoasă – îngroşarea tendonului, fără modificări de semnal;
2. degenerare mucoidă – îngroşarea tendonului şi semnal crescut IRM T1 şi T2 prin prezenţa depozitelor mucoide;
3. tendinopatie osoasă.

Cazul 8

P.I. 26 ani

La nivelul tendonului lui Ahile se observă modificări de degenerare mucoidă deasupra inserţiei pe calcaneu (semnal intermediar T1SE şi crescut T1 TIRM şi T2 fl 2D)

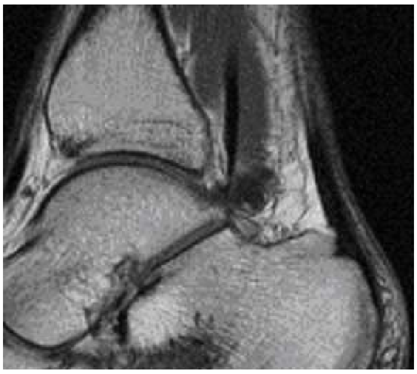

T1 SE

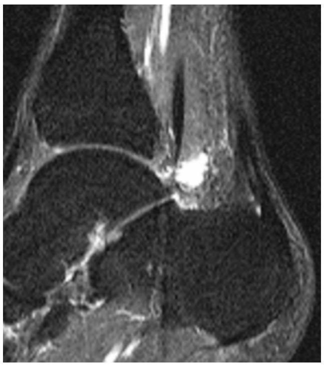

T1 TIRM

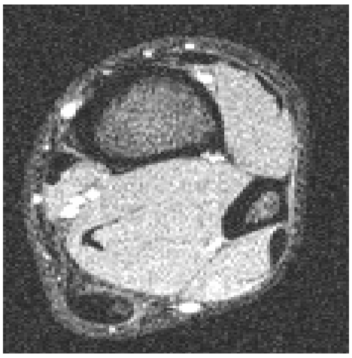

T2 fl 2D

Sindromul Haglund

Reprezintă o particularitate a tendinozei ahilene ce cuprinde: tendinoza ahileană de inserție = îngroşarea focală a tendonului lui Ahile la inserția calcaneana; bursită retrocalcaneană; bursită calcaneană superficială; proeminența tuberozității posterioare a calcaneului; fracturi avulsie ale tuberozității calcanene.

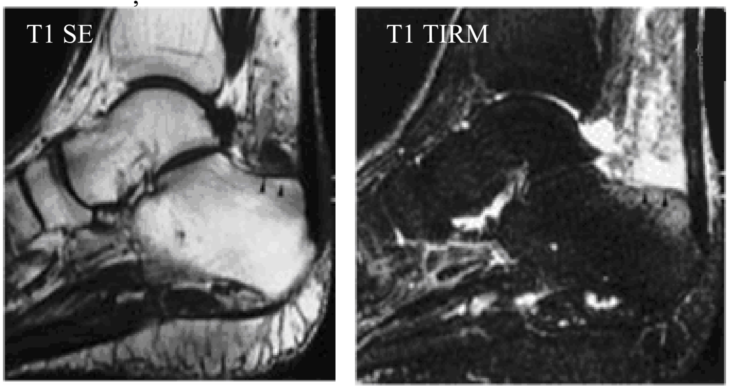

Cazul 9
I.C. 35 ani
Examenul IRM arată prezența tendinozei ahilene de inserție asociată cu bursita retrocalcaneeană, bursita calcaneeana superficială, proeminența tuberozității calcaneene şi edem osos la acest nivel.

Tenosinovitele. Afectează tendoanele şi fasciile lor. Se clasifică în: tenosinovite acute - fluid sinovial; tenosinovite cronice - fluid sinovial + îngroşarea tendonului cu semnal intermediar sau scăzut pe T2w; DD cu rupturile parțiale: pe secvențele cu TR şi TE lungi tendonul e în hiposemnal.

Cazul 10
C.B., 22 ani
Dg: Traumatism forte glezna dr.
IRM: Tenosinovita de TP, FLD, FLH, PL, PS, tendinită şi peritendinită cu ruptură parțială de tendon ahilean la inserția pe calcaneu.

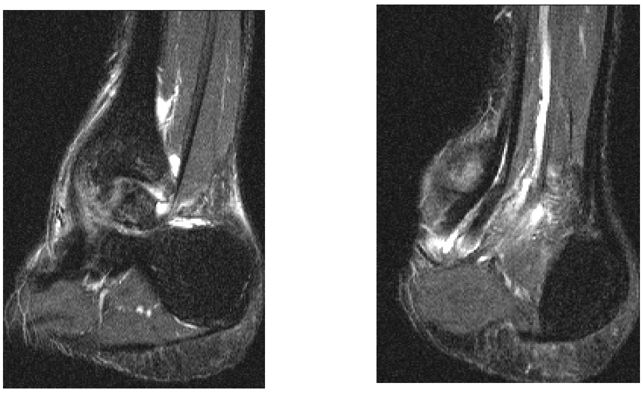

T1 TIRM

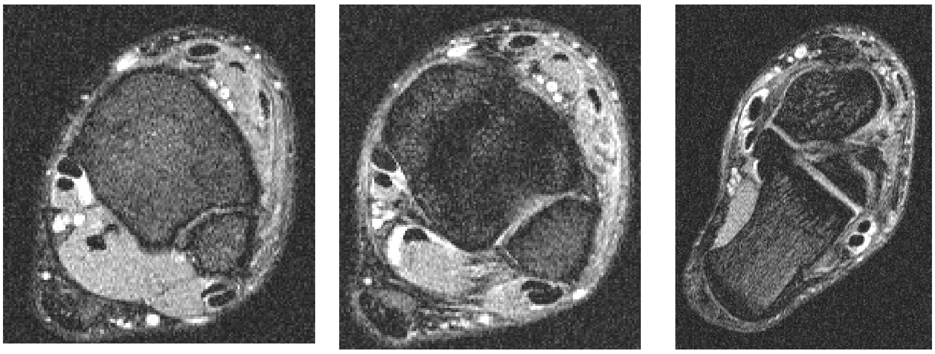

T2 fl 2D

Cazul 11
M.A.S., 16 ani

Tendonul tibial anterior apare îngroşat şi manşonat de o zonă în hipersemnal.
Aspect sugestiv de tendinită cu tenosinovită de tibial anterior.

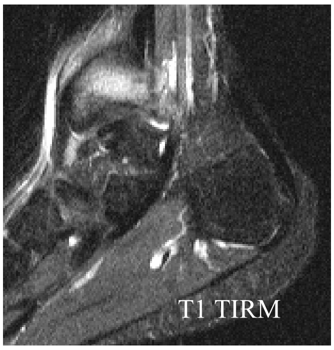

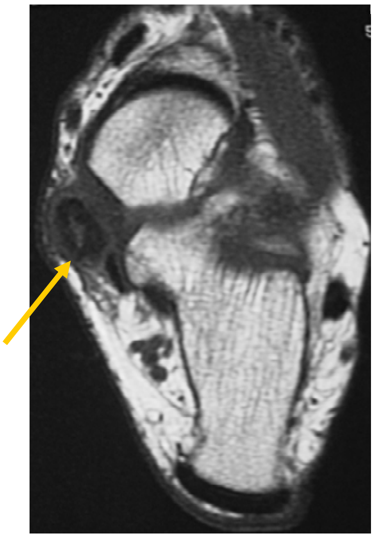

T1 SE

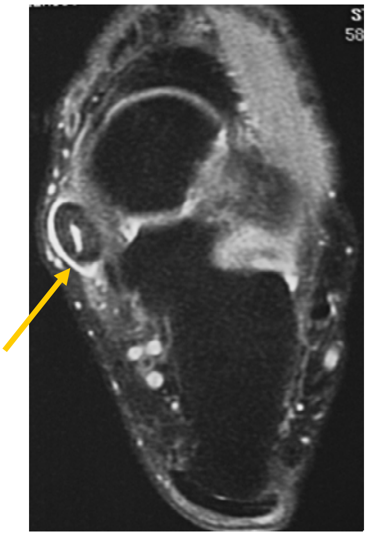

T1 TIRM

Cazul 12
G.T., 17 ani

Tibialul posterior prezintă modificări intra- şi peritendinoase în hiposemnal T1 SE şi hipersemnal T1 TIRM sugestive pentru tendinita asociată cu tenosinovita – tendinopatie de tibial posterior tipul I.

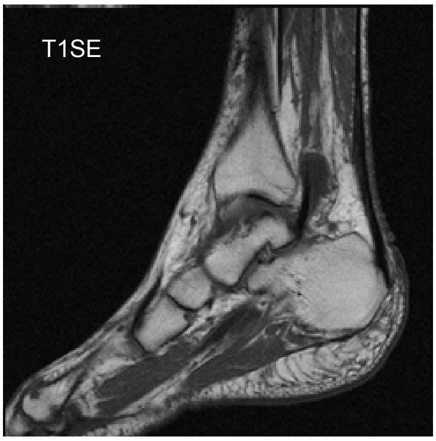

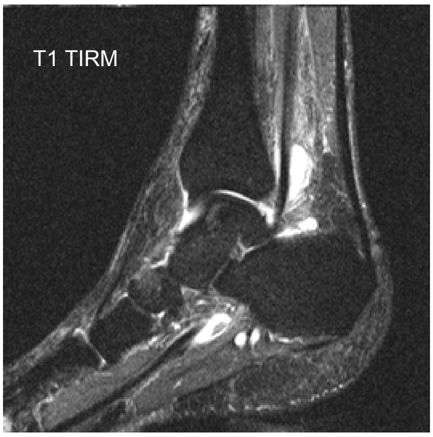

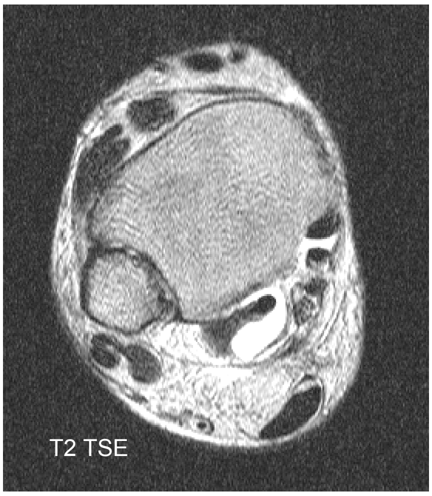

Cazul 13
M.D., 52 ani,

Tendonul flexor lung al halucelui este manşonat de o zonă în hiposemnal T1 SE şi hipersemnal T1 TIRM pe secvenţele în plan sagital. Această tenosinovită de flexor lung al halucelui este confirmată pe imaginile în plan axial, unde se observă de asemenea şi asocierea unei tenosinovite de tibial posterior.

Concluzii

– Pentru identificarea patologiei se impune o bună cunoaştere a anatomiei IRM a gleznei.

– Examenul IRM va fi adaptat patologiei suspectate. Astfel, pentru detecția edemului osos trebuie utilizate secvențele de inversie (STIR) şi cu supresia grăsimii (PD FS), aceleaşi secvențe putând fi utilizate şi pentru identificarea leziunilor de cartilaj.

– Cu ajutorul IRM putem identifica leziunile asociate tendinopatiilor, şi anume: edemul osos, leziunile osteocondrale, leziunile ligamentare, leziunile musculoaponevrotice, precum şi existența a două sau mai multe leziuni tendinoase simultane.

– Examenul IRM reprezintă o tehnică de elecție pentru vizualizarea multiplanară (axial, sagital şi coronal) a tendoanelor şi a relațiilor acestora cu structurile din jur, putând astfel aprecia gravitatea şi extensia leziunilor.

– În urma studiului efectuat în clinica noastră am identificat majoritar leziuni la nivelul tendonului lui Ahile (61,76%) şi la nivelul flexorului lung al halucelui (FLH - 22,94%) dar şi alte leziuni tendinoase (într-un procent mai mic), cum sunt: leziunile de tibial posterior (TP - 7,05%), leziunile de flexor lung al degetelor (FLD - 6,47%), leziuni de peronier lung (PL - 4,7%), leziuni de peronier scurt (PS - 3,52%) şi leziuni de tibial anterior (TA - 1,76%).

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Chirurgie orală şi maxilofacială - ediţia a III-a

Corneliu BURLIBAŞA
CHIRURGIE
ORALĂ ŞI
MAXILOFACIALĂ

Sub redacţia: Corneliu BURLIBAŞA

Tratatul de ***Chirurgie orală şi maxilofacială,*** aflat la cea de a treia ediţie, reprezintă o lucrare de referinţă în domeniu, care se adresează atât studenţilor în medicină dentară şi rezidenţilor în chirurgie orală sau maxilofacială, cât şi medicilor stomatologi sau chirurgi oromaxilofaciali.

Modul de redactare a tratatului permite ca fiecare cititor din categoriile enunţate să găsească foarte uşor informaţiile care îl interesează. Astfel, pe lângă noţiunile elementare, sunt prezentate şi unele aspecte de specialitate detaliate, precum şi o serie de metode şi tehnici de tratament intrate în arsenalul terapeutic în ultimii ani. Fără ca lucrarea să se transforme într-un manual de tehnici chirurgicale, sunt expuse şi numeroase tehnici operatorii, unele detaliate pe timpi operatori.

Meritul incontestabil al acestei prestigioase lucrări este că la elaborarea ei au participat colaboratori din toate clinicile de specialitate cu tradiţie din principalele centre universitare. În acest mod, a fost valorificată experienţa fiecărei şcoli de chirurgie orală şi maxilofacială din ţară, fără ca în acest fel să fie influenţată unitatea lucrării.

Ediţia actuală a volumului, tipărit în 2008, care are 1 312 pagini şi este ilustrat cu un număr de 981 figuri, abordează atât subiecte de chirurgie dento-alveolară, cât şi teme fundamentale de chirurgie maxilo-facială.

O trecere în revistă a cuprinsului cred că este edificatoare: Investigaţia clinică oro-maxilo-facială. Particularităţile intervenţiilor de chirurgie orală şi maxilofacială. Extracţia dentară. Chirurgia endodontică. Replantări şi transplantări dentare. Tratamentul chirurgical al parodontopatiilor marginale cronice. Chirurgia proprotetică. Patologia erupţiei dentare. Incluzia dentară. Infecţiile oro-maxilofaciale. Afecţiuni de origine dentară ale sinusului maxilar. Actualităţi microbiologice. Durerea în teritoriul oro-maxilofacial. Leziuni traumatice ale părţilor moi oro-faciale. Fracturile mandibulei. Fracturile de masiv facial. Traumatismele dento-parodontale. Chisturile maxilare. Chisturile părţilor moi cervico-faciale. Tumorile benigne şi hiperplaziile ţesuturilor moi oro-faciale. Tumorile benigne ale maxilarelor. Tumorile odontogene ale maxilarelor. Tumorile maligne oro-faciale. Tumorile maligne ale maxilarelor şi adenopatiile cervicale. Patologia glandelor salivare. Patologia articulaţiei temporo-mandibulare. Malformaţii congenitale ale feţei şi maxilarelor. Tratamentul chirurgical al anomaliilor dento-maxilare. Reconstrucţia osoasă în chirurgia oro-maxilo-facială. Lambourile de piele în chirurgia oro-maxilo-facială.

În aceste condiţii este evident că tratatul de ***Chirurgie orală şi maxilofacială*** elaborat sub redacţia Profesorului Corneliu Burlibaşa este cea mai valoroasă contribuţie în domeniu din ultimii ani, astfel încât nu ar trebui să lipsească din biblioteca oricărui specialist în medicină dentară sau chirurgie oro-maxilo-facială.

Conf.dr. Ion MAFTEI

Halitoza – aspecte clinice şi terapeutice

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REZUMAT. Halitoza (halena fetidă) defineşte mirosul neplăcut al gurii, perceput de anturaj odată cu eliminarea orală a aerului expirat. Aceasta reprezintă un fenomen care are implicaţii sociale, dar care poate fi corelat şi cu starea sănătăţii orale şi generale. Normele sociale curente subliniază importanţa imaginii personale, dar şi a relaţiilor interpersonale. În acest context, halitoza constituie un factor important în cadrul comunicării sociale şi de aceea poate fi un motiv de îngrijorare în sensul afectării stării de sănătate şi apariţiei unui handicap social pentru respectivul individ, cu repercusiuni asupra relaţiilor interpersonale.

Cuvinte cheie: halitoză, respiraţie neplăcută, pseudohalitoză, halitofobia, compuşi sulfurici volatili.

ABSTRACT. Halitosis, also known as breath malodour, is a condition that has health, but also social implications rendering it an area of oral science that opens medical and psychological issues. Breath malodour is a common concern for many patients and, therefore, dentists increasingly are being called on to help the patients with complaints of halitosis. The objective of this article is to review the etiology, classification, diagnosis and treatment strategies of halitosis.

1. INTRODUCERE

Halitoza (halena fetidă) defineşte mirosul neplăcut al gurii, perceput de anturaj odată cu eliminarea orală a aerului expirat. Aceasta reprezintă un fenomen care are implicaţii sociale, dar care poate fi corelat şi cu starea sănătăţii orale şi generale. Normele sociale curente subliniază importanţa imaginii personale, dar şi a relaţiilor interpersonale. În acest context, halitoza constituie un factor important în cadrul comunicării sociale şi de aceea poate fi un motiv de îngrijorare în sensul afectării stării de sănătate şi apariţiei unui handicap social pentru respectivul individ, cu repercusiuni asupra relaţiilor interpersonale.

Halitoza a fost un subiect neglijat mult timp, majoritatea specialiştilor punând mirosurile neplăcute de la nivelul cavităţii bucale pe seama problemelor digestive. În acest sens ar fi de amintit faptul că primul simpozion pe tema halitozei a avut loc abia în anul 1991.[1,3,5]

Problema halitozei este destul de frecvent întâlnită la pacienţii care se prezintă la medicul dentist. Astfel, conform informaţiilor provenite de la American Dental Association (ADA) din 1995, 92% dintre medicii dentişti chestionaţi au raportat faptul că au avut în tratament pacienţi cu halitoză anamnestică. Aproape jumătate dintre aceştia au declarat că au examinat săptămânal în medie 6 pacienţi cu respiraţie neplăcută.[13] Astfel, halitoza merită o atenţie sporită, cu atât mai mult cu cât, virtual, toţi indivizii prezintă episoade de respiraţie neplăcută.

2. ASPECTE EPIDEMIOLOGICE

Studiile au arătat că aproximativ 90% dintre cazurile cu halitoză au origine orală, în timp ce restul de 10% au cauze sistemice sau non-orale. În privinţa repartiţiei pe sexe, s-a constatat că halitoza apare cam în aceeaşi proporţie la femei şi la bărbaţi. Totuşi, se pare că femeile solicită tratamentul halitozei într-o proporţie mai mare comparativ cu bărbaţii, acestea fiind mai preocupate de sănătatea orală. [21,22]

În tabelul următor (1), am încercat să reunim atât aspectele legate de halitoză semnalate de către pacienţi, precum şi cauzele posibile ale acestora.

Tabelul nr.1

Prezența halitozei	**Cauze posibile sau surse ale halitozei**
Halitoza apărută după un regim alimentar îndelungat, după o cură de slăbire, la trezirea din somn, după administrarea unor medicamente, după efectuarea unor exerciții fizice sau după un discurs mai lung	Uscăciunea gurii, flux insuficient de salivă
Miros neplăcut în timpul vorbirii	Picături postnazale la nivelul zonei posterioare a feței dorsale a limbii
Depozite mici, albe, urât mirositoare pe suprafața limbii	Tonsiloliți proveniți din criptele tonsilare
Gingiile care sângerează şi/sau emană un miros neplăcut	Probleme parodontale, igienizare deficitară la nivelul zonelor interdentare
Miros neplăcut apărut la debutul menstruației	Inflamație parodontală
Miros neplăcut apărut la un moment dat în cavitatea bucală a copiilor mici	Debutul unei inflamații faringiene
Miros neplăcut apărut brusc în cavitatea natală a copiilor mici	Corpi străini în nas
Miros neplăcut emanat de corpul copiilor mici	Corpi străini în nas
Gust ori miros de peşte stricat	Trimetilaminurie (rar)
Halitoză la purtătorii de proteze mobile	Proteze menținute în cavitatea bucală în timpul nopții sau proteze care nu au fost igienizate corect
Miros neplăcut provenit din nas	Uscăciunea mucoasei nazale, corpi străini nazali, vegetații adenoide, sinuzite
Gust neplăcut pe tot parcursul zilei	Igienă orală precară, afecțiuni parodontale, activitate bacteriană excesivă la nivelul limbii

De asemenea, sursele sistemice ale halitozei includ afecțiuni generale şi ingestia unor medicamente. Cele mai cunoscute afecțiuni generale care pot duce la apariția halitozei orale sunt: diabetul zaharat, ciroza hepatică, uremia, neoplasmele digestive. Printre medicamentele care pot genera halitoză se numără antihipertensivele şi medicamentele folosite de obicei în tratarea virozelor respiratorii, dar şi cele utilizate în tratamentul unor afecțiuni sistemice (tabelul 2).[4,14,18]

*Tabelul nr. 2 (*Richard Miller)

	Cauze medicale posibile ale halitozei	**Cauze medicamentoase posibile ale halitozei**
Aparat respirator	Sinuzite	Amil nitrit
	Tuberculoze	Cloral hidrat
	Emfizem pulmonar	Paraldehidă
	Tonsilite	Metronidazol
	Tumori	Antidepresive
	Faringite	Antiparkinsoniene
	Pneumonie	Antipsihotice
	Bronşită	Narcotice
Ficat	Insuficiență hepatică	Decongestive nazale
	Ciroză hepatică	Antihistaminice
Rinichi	Uremie	Antihipertensive
Tract digestiv	Reflux esofagian	Anticolinergice
	Hernie hiatală	
	Neoplasm gastric	
	Sindrom de malabsorbție	
Sistemic	Diabet zaharat	

3. CLASIFICAREA HALITOZEI

Este foarte important ca atunci când ne confruntăm cu o respirație urât mirositoare, să putem face o diferențiere clară a formelor clinice de halitoză pe baza fenomenelor obiective care însoțesc aceste forme clinice. Astfel, Miyazaki[1,10] a realizat o clasificare simplă care permite diferențierea halitozei ca stare patologică de cea cu origine psihologică şi include şi necesarul de tratament.

Tabelul nr. 3. Clasificarea halitozei cu tratamentul necesar

Clasificare	Necesar de tratament	Descriere
I. Halitoza adevărată		Respirație urât mirositoare evidentă, cu o intensitate peste nivelul social acceptat
I. A. **Halitoza fiziologică**	TN - 1	1. Mirosul urât implică procese de putrefacție de la nivelul cavității bucale. Nu poate fi incriminată o boală anume sau un teren patologic. 2. Originea este în principal la nivelul regiunii posterioare a feței dorsale a limbii. 3. Trebuie exclusă halitoza temporară datorată factorilor alimentari (ex. consum de ceapă, usturoi etc.).
I. B. **Halitoza patologică**		
Orală	TN – 1 şi TN - 2	1. Halitoză datorată unei boli sau disfuncții la nivelul țesuturilor cavității bucale. 2. Halitoză derivată din cea fiziologică, modificată de condiții patologice (xerostomie, afecțiuni periodontale etc.).
Extraorală	TN – 1 şi TN - 3	1. Respirație urât mirositoare datorată afecțiunilor tractului pulmonar sau tubului digestiv superior. 2. Halitoză datorată afecțiunilor dismetabolice (diabet zaharat, ciroză hepatică, uremie etc.).
II. Pseudohalitoza	TN – 1 şi TN - 4	1. Respirație urât mirositoare care nu este percepută de anturaj cu toate că pacientul este convins de existența acesteia. 2. Se impun consiliere precum şi măsuri de igienă bucală elementară.
III. Halitofobia	TN – 1 şi TN - 5	O formă de halitoză orală care persistă în imaginația pacientului după ce a fost instituit tratamentul halitozei adevărate sau a pseudohalitozei.

4. ETIOPATOGENIA HALITOZEI

Halitoza se datorează prezenței gazelor urât mirositoare în aerul expirat prin cavitatea bucală şi, ca urmare, s-au făcut eforturi în sensul identificării acestor gaze. S-a demonstrat că gazele implicate în apariția halitozei sunt în principal compuşii sulfurici volatili (complexul VSC) precum hidrogenul sulfurat, metil-mercaptanul şi dimetil-sulfatul, însă au fost evidențiați şi compuşi aromatici volatili (indol, scatol), acizi organici (acid acetic, acid propionic), amine (cadaverină, putresceină).

Complexul VSC apare în urma unui proces de putrefacție a bacteriilor anaerobe Gram negative din salivă, şanțul gingival şi de pe suprafața dorsală a limbii. Prin efectuarea unor teste *in vitro* s-a demonstrat capacitatea unor bacterii de a produce complexul VSC. Dintre acestea se remarcă: *Prevotella intermedia, Fusobacterium nucleatum, Porphyromonas gingivalis şi endodontalis, Prevotella loescheii, Treponema denticola*, specii de *Veillonella, Klebsiella* pneumoniae, microorganisme incriminate în etiologia parodontopatiilor şi infecțiilor periapicale. Bacteriile anaerobe Gram negative pot fi izolate din şanțul gingival la pacienții cu gingivite şi alte afecțiuni parodontale, dar şi de pe suprafața dorsală a limbii la pacienții cu un parodonțiu indemn. [15,19,23]

Pe lângă prezența bacteriilor anaerobe Gram negative, în producerea gazelor urât mirositoare mai pot fi incriminate pH-ul salivar, fluxul salivar, dar şi nivelul oxigenului şi potențialul oxidoreducător al bacteriilor. În mod normal saliva este inodoră. Atunci când pH-ul salivar creşte, apare totuşi un miros neplăcut al cavităţii bucale.

Independent de statusul de sănătate al unui pacient, halitoza apare mai frecvent dimineaţa, după o perioadă de somn – „respiraţia matinală". Acest lucru se poate explica prin faptul că în timpul somnului, fluxul salivar provenit de la nivelul glandelor salivare mari este redus la minim, favorizând stagnarea şi iniţiind procesele de putrefacţie.[1]

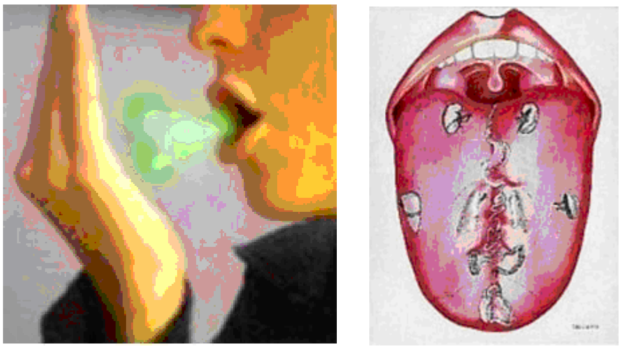

Fig. 1. Dorsul limbii implicat în apariţia VSC

De asemenea, în urma unor studii recente, se consideră că dorsul limbii (fig.1) reprezintă sursa primară implicată în apariţia complexului VSC, atât la pacienţii sănătoşi cât şi la pacienţii cu afecţiuni parodontale.[2,6,11,12] Astfel, morfologia feţei dorsale a limbii este foarte neregulată, prezentând multiple fisuri şi papile mucozale. Acestea pot crea un mediu în care microorganismele sunt bine protejate de acţiunea de curăţare a fluxului salivar, iar nivelul oxigenului este redus, având drept efect dezvoltarea bacteriilor anaerobe. Studiile demonstrează în primul rând că îndepărtarea depozitelor de pe limbă ar reduce producţia de VSC, iar în al doilea rând, din compararea mostrelor de aer rezultat după răzuirea limbii, periaj dentar şi clătire cu apă la pacienţii cu halitoză, reiese faptul că cea mai mare reducere a nivelului de VSC se înregistrează după răzuirea limbii.[22]

5. DIAGNOSTICUL HALITOZEI

Se cunosc trei metode principale de diagnosticare a halitozei:

1. metoda organoleptică;
2. gaz-cromatografia (GC);
3. monitorizarea sulfaţilor.

5.1. Metoda organoleptică

Este un test subiectiv care se bazează pe percepţia examinatorului referitoare la mirosul degajat de cavitatea bucală a subiectului. Se utilizează următoarele scoruri (Rosenberg, 1999):

0 = miros nedecelabil;
1 = miros greu decelabil;
2 = miros moderat;
4 = miros puternic;
5 = miros extrem de puternic.

Înaintea evaluării, atât subiectul cât şi examinatorul trebuie să urmeze anumite instrucţiuni pentru a se obţine un rezultat cât mai corect.

Instrucţiunile pentru pacient sunt următoarele:

- să nu consume alimente dure cu cel puţin 48 de ore înainte de examinare,
- să nu folosească produse cosmetice parfumate cu 24 de ore înainte de examinare,
- să nu mănânce, să nu bea, să nu fumeze sau să clătească gura cu ape de gură cu cel puţin 12 ore înainte de examinare.

Instrucţiunile pentru examinator sunt următoarele:

- să nu fumeze, să nu bea cafea, ceai sau sucuri înainte de examinare;
- să nu folosească produse cosmetice parfumate înaintea examinării.

5.2. Gaz-cromatografia

Este considerată cea mai bună metodă pentru evaluarea halitozei, deoarece măsoară specific nivelul complexului VSC, principala cauză a halitozei. Echipamentul necesar acestei evaluări este însă destul de costisitor, este nevoie de un operator experimentat, astfel încât această metodă se foloseşte doar în cercetare şi nu pentru uz clinic curent. [8,16,17]

5.3. Monitorizarea sulfaţilor

Această metodă utilizează aparate care analizează conţinutul total de sulfaţi ai aerului din cavitatea bucală a subiectului. Este o metodă ieftină, uşor de folosit, însă nu se pot decela toate componentele complexului VCS. Spre exemplu, halimetrul (Interscan Co. Chatsworth, California, fig.2) prezintă o senzitivitate pentru sulfaţii de hidrogen, însă are o senzitivitate redusă pentru metil-mercaptan, care contribuie semnificativ la apariţia halitozei datorată afecţiunilor parodontale.

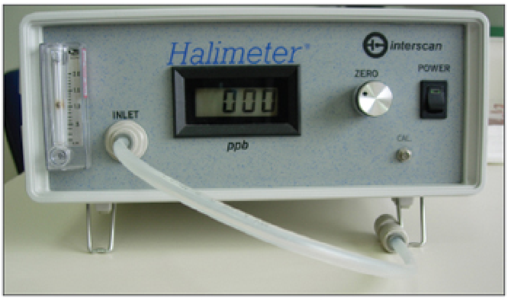

Fig.2 .Halimetrul.

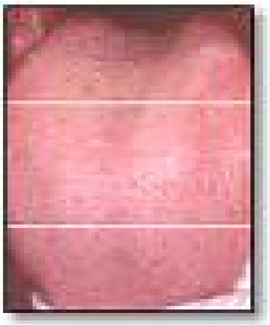

Fig.3 Încărcarea limbii

Cea mai sigură şi practică metodă de evaluare a halitozei orale este considerată metoda organoleptică efectuată de un clinician bine instruit. Cu toate acestea, nu este de neglijat utilizarea unui halimetru care poate cuantifica modificările gazelor din aerul expirat, astfel încât pacientului i se poate monitoriza evoluţia printr-o terapie adecvată.[10]

Desigur, pentru a realiza necesarul de tratament pentru fiecare pacient dar şi evaluările periodice, ar mai trebui studiate şi alte variabile clinice. Printre acestea amintim evaluarea statusului periodontal, inclusiv nivelul de igienă orală, precum şi gradul de încărcare a limbii.

Pentru evaluarea încărcării limbii este recomandat să se utilizeze un indice de cuantificare a nivelului de încărcare a limbii (fig.3).

Conform lui Yaegaki K. [1,10,20] scorurile acestui indice sunt următoarele:

- 0 = limbă neîncărcată;
- 1 = uşoară încărcare a 1/3 din dorsul limbii;
- 2 = uşoară încărcare pe 2/3 din dorsul limbii;
- 3 = încărcare peste 2/3 din dorsul limbii.

6. TRATAMENTUL HALITOZEI

În practica curentă, metodele de tratament al halitozei au fost împărţite în 5 clase (vezi tabelul III), astfel încât să constituie linii directoare pentru specialişti.

Liniile directoare ale tratamentului sunt direct corelate cu diagnosticul originii halitozei:

TN-1: explicaţii despre halitoză, educaţie pentru sănătatea orală individuală;

TN-2: profilaxie orală, periaj profesional şi tratamentul afecţiunilor orale, mai ales a celor parodontale;

TN-3: tratament în colaborare cu medicii specialişti generalişti;

TN-4: explicarea datelor rezultate în urma examinării, educaţie pentru sănătatea orală, asigurarea şi liniştirea pacientului că nu suferă de halitoză;

TN-5: tratament psihiatric.

Tratamentul halitozei fiziologice (TN-1), al halitozei orale (TN-1 şi TN-2) şi al pseudohalitozei (TN-1şi TN-4) sunt de competenţa medicului dentist. Tratamentul halitozei extraorale (TN-3) şi al halitofobiei (TN-5) trebuie condus de către medicul specialist ori de către psiholog sau medicul psihiatru.

În halitoza fiziologică (TN-1) trebuie pus accentul pe tehnicile de autoigienizare orală. Se instruieşte pacientul în privința unei tehnici corespunzătoare de curățare a dorsului limbii, dar şi de curățare interdentară. De asemenea, pacientul este încurajat să folosească ape de gură ori dentifrice cu activitate antibacteriană dovedită.

În halitoza de cauză orală (TN-2), pe lângă instrucțiunile de la TN-1, medicul dentist trebuie să acorde o atenție specială tratamentului afecțiunilor parodontale, dar şi tratamentului cariilor dentare şi restaurărilor dentare incorecte.

În halitoza de cauză extraorală, pacientului i se va explica că sursa nu este la nivelul cavității bucale şi va fi îndrumat către un medic specialist.

În pseudohalitoză (TN-4), pacienților trebuie să li se explice rezultatele examinării, diagnosticul şi să li se realizeze educația pentru sănătate orală cu mult tact, astfel încât să plece convinşi că au fost luate toate măsurile de prevenire a apariției halitozei. În acest context, pot fi utilizate cu succes halimetrele.

În halitofobie (TN-5), după ce au fost utilizate metodele mai sus amintite, pacientul va fi îndrumat către evaluare şi monitorizare psihologică sau psihiatrică[7,9].

7. CONCLUZII

1. Halitoza, modificare neplăcută a mirosului aerului expirat, poate avea cauze orale, digestive sau alte cauze generale.
2. Prin examen oral atent pot fi stabilite şi eliminate cauzele orale.
3. Persistența halitozei poate fi corelată cu cauze digestive sau alte afecțiuni generale sau medicația urmată, care impun consult şi tratament general de specialitate.
4. Depistarea şi tratamentul corect al halitozei trebuie să preocupe, în egală măsură, atât pe pacient, cât şi pe medic, având implicații sociale asupra pacientului şi relațiilor lui interpersonale.

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O carte profesională cu caracter de avertisment asupra nevoii de asistență interdisciplinară
Managementul psihiatric şi psihologic în cancerul de sân

O surpriză plăcută am avut de curând, când doamna psiholog Gabriela Grigorescu mi-a oferit cartea *Managementul psihiatric şi psihologic în cancerul de sân,* a cărei coautoare este împreună cu doamna doctor Alexandrina Baloescu. Le cunosc pe cele două distinse doamne de mai multă vreme, prin prisma valorii profesionale dovedite în cadrul Clinicii de Psihiatrie din S.C.U.M.C. ”Dr. Carol Davila” şi în calitate de colaboratoare apreciate ale regretatului profesor dr. M.D.Gheorghe. Am remarcat încă de atunci interesul constant al acestora pentru informația scrisă, dragostea pentru carte şi preocuparea permanentă de a fi în ton cu cele mai noi referințe din literatura de specialitate, aşa că abordarea unui subiect ce ține de latura profesională pare a fi un răspuns firesc la o atitudine permanent angajată ce vizează un interes comun medical- sănătatea celor aflați în suferință.

Boală complexă şi tramatizantă, cancerul de sân rămâne în continuare cea mai frecventă tumoră întâlnită la femei, constituindu-se în a doua cauză de deces prin tumori după cancerul pulmonar. Date recente ale Societății Americane pentru cancer relevă că una din opt femei dezvoltă cancer într-un moment al vieții ei, iar riscul de a muri din cauza acestei maladii este de 1 la 28.

Ceea ce aduce nou această carte pentru o temă destul de mediatizată, cel puțin din punctul de vedere al medicului oncolog, este caracterul de avertisment asupra nevoii de asistență interdisciplinară a pacientelor cu cancer de sân într-o echipă în cadrul căreia, uneori, psihiatrul şi psihologul capătă un rol decisiv. Este, în acelaşi timp, şi un răspuns la eforturile care s-au făcut în ultimii 30 de ani de a atrage atenția asupra aspectelor psihologice şi psihosociale asociate cancerului de sân.

Urmările psihosociale ale acestei boli care poate avea influențe covârşitoare pentru imaginea femeii, prin diminuarea atractivității, a feminității, a sexualității, a capacității de reproducere etc., sunt deosebit de traumatizante pentru paciente, psihologul devenind o adevărată placă de rezonanță, un suport eficient în luarea şi adoptarea unor decizii corecte.

În contextual înfățişat este lesne de înțeles de ce apariția unui astfel de titlu este binevenită atât pentru cititorul avizat, şi mă refer aici la personalul medical direct implicat în profilaxia şi tratamentul acestei boli, cât şi la cel mai puțin avizat, dar care, într-un fel sau altul, poate folosi informația în scop personal sau poate ajuta o persoană apropiată aflată în suferință.

Este demnă de menționat acuratețea cu care sunt expuse aspectele privind relația dintre depresie, anxietate, tulburările cognitive şi delirium, schizofrenie, durere, oboseală, tulburările de somn, alte suferințe psihologice şi cancerul de sân. Cu aceeaşi atenție sunt urmărite obiectivele şi nevoile de îngrijire ale pacientei cu cancer de sân, psihoterapiile ce i se pot aplica, datele despre alimentație. Bibliografia, extreme de bogată, aproximativ 400 de titluri, este încă o dovadă a manierei profesioniste în care autoarele au înțeles să trateze tema enunțată, adăugând în acest fel textului certitudine ştiințifică, credibilitate şi actualitate. Nu pot încheia scurtul îndemn la lectură a noii apariții fără a aminti gestul regretatului gl.bg.(rez)prof.dr. M.D.Gheorghe, gest cu profunde semnificații de generozitate şi calitate umană, de a însoți demersul colaboratoarelor sale cu un colegial preambul, din care reproduc un paragraf pe care îl consider definitoriu pentru oportunitatea editării acestei cărți: ”Elaborată într-un stil inconfundabil, prin care se recunoaşte contribuția profesională şi afectivă a celor două autoare, monografia este expresia pasiunii, experienței şi nu în ultimul rând a şcolii medicale pe care acestea o reprezintă. Pe de altă parte, este de apreciat curajul cu care este abordat un asemenea subiect, după ştiința mea în premieră națională”.

D.Nicolescu

Influența presiunilor ocluzale asupra menținerii și stabilității protezelor totale
(analiza cu MEF)

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REZUMAT. Cercetarea reprezintă o analiză numerică a stării de efort din substratul osos, ca rezultat al presiunilor transmise din zona ocluzală în procesul de masticație, urmărind gradul de influență al factorilor mecanici din zona de sprijin asupra menținerii și stabilității protezelor totale. S-a utilizat metoda de analiză MEF. În urma simulării pe modelul de calcul, a unor situații clinice funcționale și nefuncționale, s-a constatat că factorul modelator constant al câmpului de tensiuni este schema ocluzală, în funcție de care se produc influențe favorabile sau nu asupra menținerii și stabilității protezelor totale.

Cuvinte cheie: tensiuni, meținere, stabilitate.

ABSTRACT. The research represents a numerical analysis of stress, developed in bone support as a result of masticatory occlusal pressions, observing the influence of mechanical factors from bearing area, on retention and stability of complete denture.
Material and methode: It was used the FEM method. Was simulated on the calculus model, some clinical situations, functional and non functional and was found that the constant modulation factor on tension field is the occlusal design, which can produce positive or negativ influence in retention and stability of complete dentures.

Funcționalitatea protezelor totale se bazează pe binecunoscuta triadă de factori: sprijin, menținere și stabilitate, a căror acțiune asigură reușita tratamentului protetic.

Dacă în literatura există o vastă documentație asupra rapoartelor dintre proteză și mucoasa zonei de sprijin, unde o serie de mijloace fizice și chimice contribuie determinant la realizarea celor trei factori, în ce privește modificările biomecanice din suportul osos, studiile sunt în defășurare.

Scopul acestei lucrări este analiza numerică a stării de efort din substratul osos la edentatul total și stabilirea unor parametri de influență asupra menținerii și stabilității protezei totale maxilare, pentru care s-a utilizat o investigație digitală.

Material și metodă

Pentru evaluarea deformațiilor și tensiunilor din substratul osos maxilar s-a utilizat metoda elementelor finite MEF.

Cu ajutorul unui program FORTRAN s-a realizat un model geometric format din volume (fig.1)

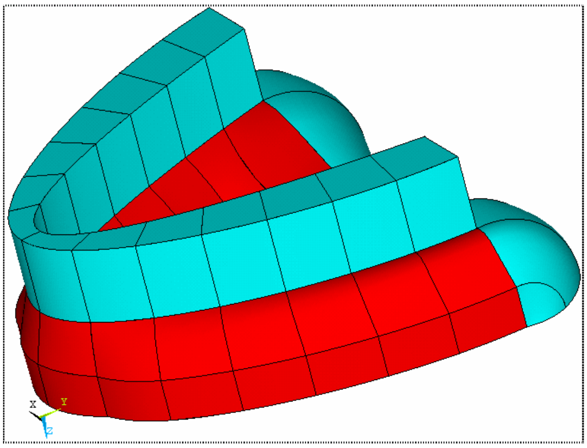

Fig.1. Model geometric global: arcada dentară artificială, baza protezei și substratul osos.

Volumele au fost discretizate în elemente finite; astfel, substratul osos, care constituie obiectul de studiu, a fost modelat ca volum şi discretizat cu elemente tridimensionale tetraedrice (fig.2).

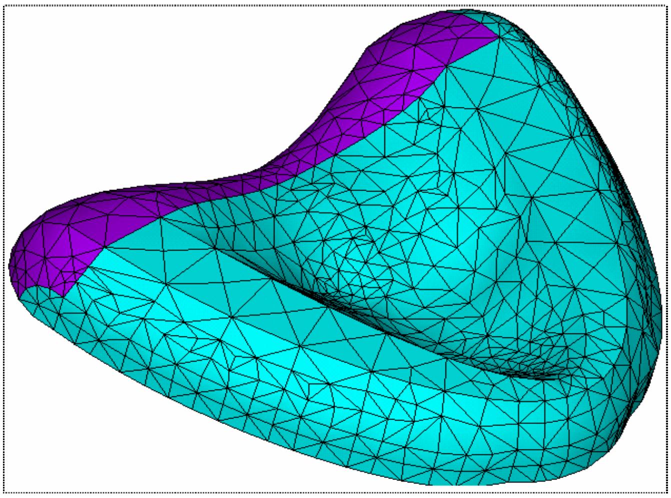

Fig.2. Discretizarea substratului osos cu elemente de volum.

Pentru încărcarea modelului s-a considerat o forţă de 100N, aplicată ca presiune masticatorie verticală pe o suprafaţă ocluzală localizată în funcţie de engrama masticatorie investigată. S-a operat în situaţii clinice diferite, din care exemplificăm trei situaţii funcţionale şi una nefuncţională.

Rezultatele sunt prezentate ca tensiuni echivalente Von Misses, afişate grafic şi numeric în MPa.

Rezultate

1. *Masticaţia unilaterală* - situaţie clinică funcţională, atribuită de Posselt la 12% din populaţie pentru dentat, la edentatul total procentul fiind mai redus.

Încărcarea s-a făcut pe hemiarcada superioară dreaptă a modelului de calcul, cu o presiune corespunzatoare forţei de 100N, care acţionează vertical pe suprafaţa ocluzală, în zona premolaro-molară, fără participare incizală (fig.3).

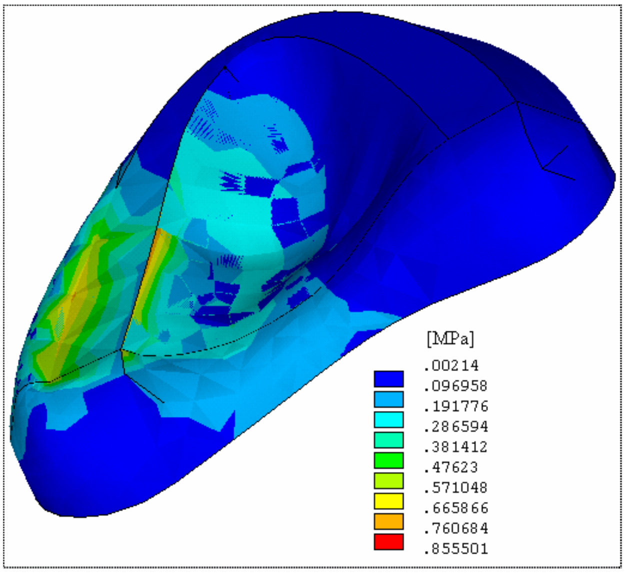

Fig.3. Tensiuni echivalente von Mises în substratul osos.

Rezultatele arată câmpul de deformaţii şi tensiuni ce se înscrie într-un perimetru cu o suprafaţă redusă, limitată între linia de mucoasă pasiv mobilă şi linia mediană a palatului dur, o treime din zona incisivă şi o treime a zonei distale a câmpului protetic maxilar.

În substratul osos, valorile tensionale scad de la centru spre periferie. Astfel, cifra maximă 0,85 MPa se înregistrează la nivelul crestei, pe muchia internă în dreptul molarilor şi celui de-al doilea premolar. Valorile descresc în zona vestibulară retrozigomatică - 0,47 MPa până la 0,19MPa.

Pe versantul palatinal descreşterea trece prin cifre medii spre linia mediană, unde se înregistrează 0,09MPa. În restul câmpului protetic osos, valorile tensiunilor sunt nesemnificative pe o plajă de 52%, fenomen care poate afecta stabilitatea protezei.

2. *Masticația bilaterală alternativă*. În acest stereotip dinamic de masticație bolul alimentar se transferă de la o hemiarcadă la alta, proces care continuă până la momentul deglutiției.

Încărcarea modelului s-a făcut pe întreaga hemiarcadă dreaptă.

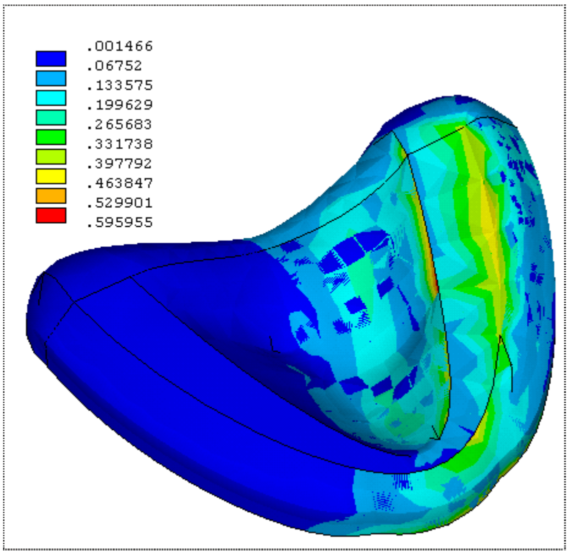

Fig.4. Configurația câmpului de tensiuni.

Imaginea distribuției tensiunilor (4) în substratul osos arată o extindere spre zona incisivă, iar la linia mediană se produce o uşoară depăşire a rafeului palatinal spre hemiarcada de balans. În zona premolaro-molară valorile tensionale sunt de 0,595MPa, iar în teritoriul incisiv de 0,33MPa şi în toată periferia de 0,006MPa, deci o scădere la toate reperele față de testul anterior. Acest tip de masticație se întâlneşte la pacienții cu o perioadă de adaptare mai mare cu noile proteze, timp în care se stabilizează anvelopa masticatorie pe baza noilor reflexe. Stabilitatea şi menținerea cresc odată cu scăderea zonelor cu presiuni nesemnificative, care aici este în proporție de 41%.

3. *Ocluzia în deglutiție*. La edentatul total, mecanica deglutiției se produce asemănător celei infantile. La începutul deglutiției, bolul insalivat este transportat cu ajutorul limbii şi aşezat pe fața ei dorsală. Urmează contracția muşchilor ridicători, care aduc arcadele în contact în R.C. pe toată suprafața ocluzală, după Ramfjiord. Durata contactului interdentar este de 1,5 sec. (Jankelson), ceea ce înseamnă o creştere de 10 ori față de durata contactului din timpul masticației. După acelaşi autor, forța de impact între arcade este mai mare ca în masticație.

Am considerat o solicitare medie de 150N, repartizată pe toată suprafața ocluzală a ambelor arcade maxilare.

Rezultatele arată distribuția echilibrată a tensiunilor în corpul protezei care are loc pe toata arcada artificială cuprinzând şi baza în întregime (fig.5).

Valorile maxime se situează în zona molară 1,182MPa şi scad uşor în bolta palatină spre 1,052MPa.

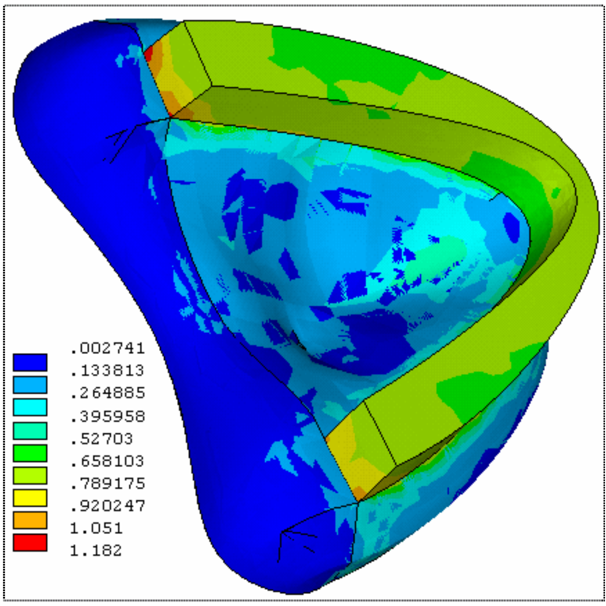
.002741
.133813
.264885
.395958
.52703
.658103
.789175
.920247
1.051
1.182

Fig.5. Tensiuni în corpul protezei.

În substratul osos valorile deformaţiilor şi tensiunilor sunt mai mici ca în placă, dar distribuţia lor este asemănătoare (fig.6).

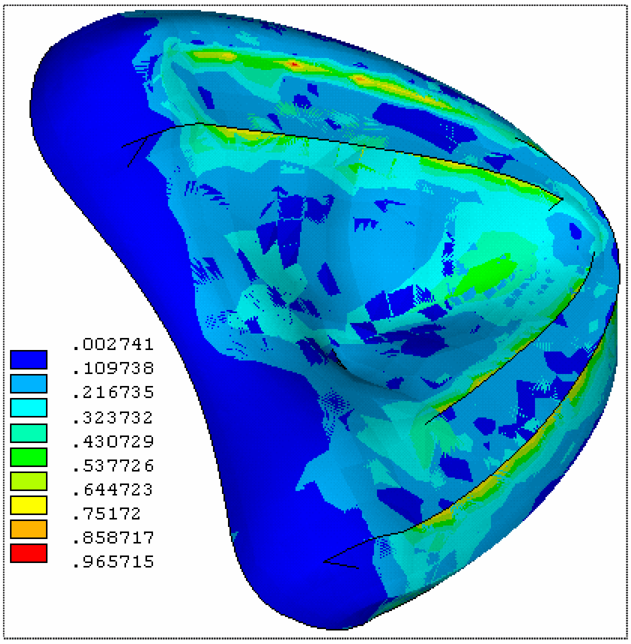
.002741
.109738
.216735
.323732
.430729
.537726
.644723
.75172
.858717
.965715

Fig.6. Tensiuni echivalente în substratul osos.

Astfel cota maximă este de 0,965MPa pe creasta alveolară la nivelul molarilor, diminuându-se spre 0,858MPa în bolta palatină.

În deglutiţie, distribuţia tensiunilor este cea mai echilibrată dintre toate situaţiile clinice simulate pe modelul de calcul, având o influenţă favorabilă asupra menţinerii şi stabilităţii.

4. *Ocluzia în propulsie cap la cap*. Este o situaţie clinică nefuncţională în care s-a modelat un tip de ocluzie cap la cap, care se produce în mişcarea de propulsie a mandibulei, fără a avea contacte în zonele laterale ale arcadelor. A fost considerată o forţă de 100N repartizată pe incisivi.

Prin rapoartele singulare, asimetrice şi mai ales inegale, se instalează o ocluzie neechilibrată cu localizare la creasta edentată frontală (fig.7).

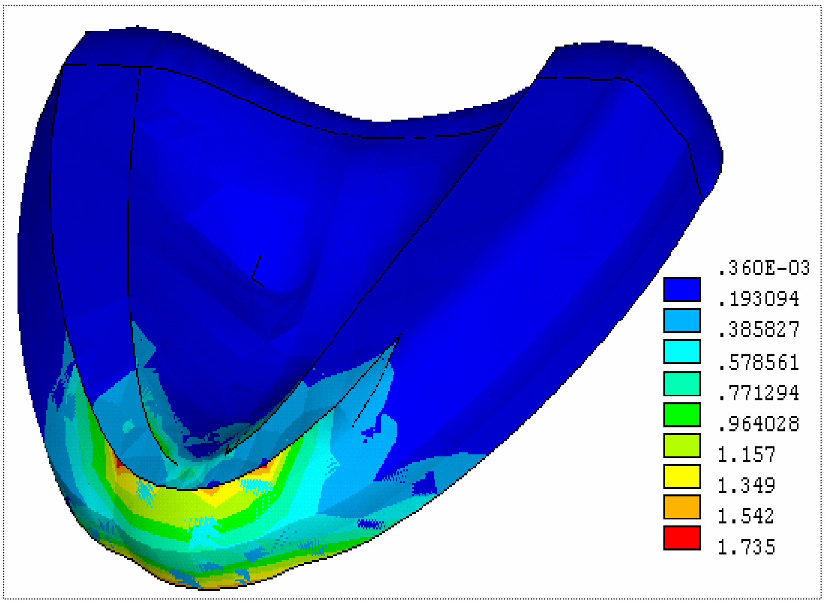

Fig.7. Tensiuni echivalente în substratul osos.

Asistăm la tensiuni foarte ridicate în zona incisivă centrală de 1,735Mpa, pentru ca în zona canină, înregistrările să arate valori de 0,36MPa, iar în restul substratului osos, pe o plajă întinsă de 71%, să nu apară modificări ale stării de efort. Presiunile exagerate pe creasta edentată în zona incisivă pe o porțiune restrânsă (muchia incizală) produc dislocarea protezei în porțiunea distală a câmpului protetic, unde menținerea şi stabilitatea sunt compromise.

Discuții

Un fapt de observație constant este că punctul de plecare în diferențierea rezultatelor îl constituie schema ocluzală modelată pe calculator.

În situațiile clinice funcționale, la presiuni ocluzale egale (100N), înregistrările valorice ale tensiunilor se înscriu într-un perimetru care închide o suprafață mai mică decât zona de sprijin osoasă.

Astfel rămân suprafețe libere de tensiuni înregistrabile, ca în masticația unilaterală (52%) sau în masticația bilaterală alternativă (41%).

În situația clinică nefuncțională, plaja cu valori nesemnificative este mai mare (71%).

Alt aspect al diferențierii este scăderea valorică a tensiunilor de la centrul de presiune masticatorie, din zona Pm-M 0,85MPa la 0,09MPa pe linia mediană a palatului dur. Deci, prin analiza numerică s-au obținut semnale de avertizare că în mecanismul masticației, chiar în situații clinice funcționale, nu se produce o dispersare totală a presiunilor pe suportul osos: transmiterea acestora nu este uniformă şi rămân zone în care tensiunile se micşorează până la un nivel în care înregistrările sunt nesemnificative.

Rezultanta distribuției diferențiate a tensiunilor în substratul osos are un efect negativ asupra stabilității şi în special a menținerii protezelor totale pe câmpul protetic, mai ales că tendințele dislocante pot fi influențate şi de structura neomogenă, anizotropă şi cu reziliență variabilă a mucoasei acoperitoare. În schimb în deglutiție asistăm la o distribuție echilibrată a stării de efort pe întreaga suprafață a zonei de sprijin osoase. Contactul ocluzal între arcade în RC şi contracția simultană şi simetrică a muşchilor ridicători determină transmiterea egală a presiunilor ocluzale şi inducerea în substratul osos a unui câmp de tensiuni uniform şi integral distribuit, care asigură menținerea şi stabilitatea optimă a pieselor protetice.

O situație particulară este analiza numerică în ocluzie propulsată cap la cap, la care valorile tensionale sunt mai mari numeric în zona crestei frontale maxilare, decât atunci când presiunea masticatorie se exercită în zona laterală. Efectul în timp al acestui tip de presiune nefuncțională este obiectivat în cazul sindromului combinației, la care coexistă:

- modificări resorbtive accelerate în zona frontală maxilară;
- proeminența tuberozităților în plan vertical;
- tendința la propulsie mandibulară;
- transformări fibroase ale suportului de sprijin al protezei în zona frontală, datorită desprinderii din distal şi rotației meziale.

Prin plasarea de presiuni repetate, cu valori semnificative şi durată mai mare, câmpul protetic de suport (substratul osos) se remodelează nefavorabil unui sprijin şi echilibru al piesei protetice supraiacente.

Situația clinică funcțională simulată prin aplicarea presiunilor masticatorii în zona laterală premolaro-molară produce tensiuni mai mici în substatul osos, care prin presare intermitentă nu modifică ritmul resorbției.

Concluzie

Investigația digitală poate fi considerată utilă prin operațiile de măsurare (cuantificare) a fenomenelor mecanice din substratul osos şi prin prezentarea unor parametri de referință într-un domeniu încă dificil, al rapoartelor dintre sprijinul, menținerea şi stabilitatea protezelor totale.

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Perturbările memoriei în tulburările psihice specifice

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REZUMAT. Tulburările memoriei sunt frecvent întâlnite în psihopatologie, iar evaluarea lor este necesar să fie multidimensională şi dinamică, pentru a putea diferenţia perturbările tranzitorii ale funcţiei mnezice, observabile la persoanele sănătoase, de disfuncţiile asociate tulburărilor psihice specifice. Memoria este unul dintre procesele cognitive intens studiate atât la nivel clinic, cât şi la nivel experimental, putând fi analizată din perspectiva neurobiologiei, a psihologiei cognitive, precum şi a psihologiei dezvoltării. Dimensiunea medico-legală este importantă atunci când discutăm despre memorie, deoarece existenţa perturbărilor mnezice este un motiv frecvent invocat în context criminologic. Distingerea dismneziilor din cadrul tulburărilor de invalidare cognitivă de modificările memoriei din depresie reprezintă un proces uneori dificil, care se bazează atât pe semne clinice, cât şi pe investigaţii psihologice specifice. Tulburările disociative cuprind, printre simptomele-cheie, disfuncţii ale memoriei, în special ale memoriei autobiografice. Schizofrenia asociază deficite mnezice şi prosexice importante, incluse în dimensiunea cognitivă a simptomatologiei, alături de celelalte patru dimensiuni, respectiv pozitivă, negativă, comportamentală şi afectivă. Tulburările mnezice pot fi induse şi de consumul de substanţe, după cum pot fi asociate bolilor organice.

Cuvinte cheie: tulburări mnezice, sindrom Ganser, demenţa, pseudodemenţa, simulare, schizofrenie

ABSTRACT. Memory disorders are frequently observed in psychopathology and their evaluation implies a multidimensional and dynamic evaluation, in order to differentiate transient memory dysfunctions in normal people from those associated with specific psychiatric disorders. The memory is one of the most extensive researched psychological processes, both at clinical and experimental level, from neurobiological, cognitive and developmental psychology perspectives. The forensic dimension is also important because of the high incidence of malingering in penitenciary settings. It's also important to differentiate dysmnesias associated to cognitive disorders from memory dysfunctions observed in depressive disorders, based on clinical signs and specific psychological investigations. Dissociative disorders involve, as key signs, memory impairments, especially at the autobiographic memory level. Schizophrenia has, beside the positive, negative, affective and behavioral dimensions, memory and attentional impairments, as components of the cognitive dimension. Memory disturbances could also be the consequences of drug abuse or they could be the results of various organic diseases.

1. Formele şi funcţiile memoriei

Perturbările obiectivabile ale memoriei şi acuzele subiective privind funcţionarea mnezică sunt frecvent întâlnite în clinica psihiatrică. Probleme tranzitorii legate de memorie apar şi în urma terapiei electroconvulsivante sau după administrarea unor medicamente psihotrope, iar alte puncte de interes pentru psihiatrie sunt reprezentate de implicarea acestui proces cognitiv în dezvoltarea personalităţii, menţinerea identităţii personale şi funcţionarea mecanismelor de coping.

Memoria este considerată capacitatea de achiziţionare, conservare şi evocare a informaţiilor. *Encodarea* este procesul de transformare a informaţiilor în semnale (reprezentări), care ulterior pot fi *stocate* de sistemul nervos pentru o perioadă variabilă de timp iar *evocarea* reprezintă etapa în care informaţiile păstrate sunt reactualizate sub forma reproducerii (realizată în absenţa elementelor ce necesită reactualizare) sau recunoaşterii (evocarea în prezenţa elementelor respective).

Memoria prezintă mai multe forme sau niveluri funcţionale, ea nefiind un proces singular, izolat, ci un mecanism complex, multidimensional, compus din mai multe tipuri de procese mnezice, fiecare dintre acestea având un substrat anatomic specific.

Există multiple criterii de clasificare a formelor de memorie, iar în continuare le vom prezenta pe cele importante din perspectivă clinică:

***(1) în funcţie de durată** distingem* (a) memoria imediată, cu durată de milisecunde (pentru informaţia vizuală, memorie iconică) sau câteva secunde (pentru stimulii auditivi, memorie ecoică), având o durată care coincide cu timpul de focalizare a atenţiei asupra stimulului respectiv; (b) memoria de scurtă durată (de aproximativ 15- 20 secunde), care implică şi o consolidare, însă incompletă, a informaţiei primite; (c) memoria de lungă durată (de la ore la ani), care se bazează pe mecanisme speciale de consolidare informaţională.

Înregistrarea informaţiilor senzoriale este asimilată conceptelor de „memorie primară", „memorie de lucru", „memorie imediată" sau „câmp prosexic" şi se referă la reţinerea iniţială a informaţiei, care poate să nu lase nicio urmă durabilă, odată ce focalizarea atenţiei voluntare este schimbată. De exemplu, o persoană care primeşte un număr de telefon pe care trebuie să-l reţină până ce-l va forma va folosi memoria imediată, ceea ce va determina uitarea acestei informaţii după ce motivaţia reţinerii ei a dispărut. Aceste informaţii sunt menţinute în modalitatea senzorială în care au fost recepţionate, de exemplu sunetele rămân în memoria ecoică, stimulii vizuali în memoria iconică etc. Dacă informaţia nu este procesată, transformată într-un mesaj cu semnificaţie semantică, de exemplu un cuvânt, o propoziţie, un simbol numeric, atunci ea nu va depăşi câmpul memoriei imediate.

Regiuni din cortexul parietal şi prefrontal sunt implicate în memoria de lucru. Durata memoriei vizuale de lucru, determinată experimental, este aproximată la 300 msec[1], iar cea a memoriei auditive este de circa 1.5-2 secunde[2].

Pentru verificarea, în situaţii clinice, a memoriei imediate, pacientului i se prezintă verbal trei itemi fără o legătură evidentă, de exemplu *„roşu, masă, haină"* şi i se cere acestuia să le repete imediat după ce le-a auzit. Dacă pacientul le poate repeta corect, atunci este evident că înregistrarea informaţiilor s-a produs, însă dacă pacientul nu reuşeşte să le redea exact, examinatorul le va repeta până când memorarea reuşeşte. Se notează numărul de încercări necesare memorării, considerându-se că existenţa a mai mult de două erori semnalează o înregistrare inadecvată.

O altă metodă de evaluare a înregistrării, care se foloseşte şi pentru determinarea funcţiei prosexice, este măsurarea câmpului atenţional. Pacientului i se prezintă cifre, incluse în seturi de dimensiuni crescânde. După fiecare serie, subiectul trebuie să le repete cu voce tare, fiindu-i interzis să şi le noteze pe hârtie. Se începe cu prezentarea unei perechi de cifre şi se continuă cu serii de cifre, până ce pacientul nu mai poate reproduce secvenţa corectă. Odată ce s-a atins pragul seriei de cifre, pacientul va fi invitat să reproducă în ordine inversă aceste serii. Limita pentru seriile de cifre reproduse în ordinea prezentării este de 6±1, rămânând stabilă la acelaşi individ, indiferent de vârstă, în condiţii fiziologice. Reproducerea în ordine inversă a seriilor de cifre este limitată, pentru un adult normal, în medie, la 5 elemente. Disocierile între performanţele la reproducerea cifrelor în ordinea prezentării, faţă de reproducerea acestora în ordine inversă, se întâlnesc în cazurile de leziuni ale lobului frontal, delirium şi demenţă incipientă.

Memoria de scurtă durată are o capacitate limitată (la 7±2 elemente), o durată limitată la 2-20 secunde, encodează informaţia verbal sau imagistic, iar actualizarea informaţiilor este serială.

Memoria de scurtă durată are sediul în structurile limbice şi este funcţională în condiţiile în care înregistrarea senzorială operează în parametrii normali. Memoria de scurtă durată conţine informaţii care pot fi folosite pentru decizii imediate sau care trec ulterior în memoria de lungă durată. Memoria de scurtă durată, având o capacitate limitată, este saturabilă informaţional.

Pentru a testa memoria de scurtă durată, pacientului îi sunt prezentate 3-4 cuvinte care vor trebui reproduse de acesta la 5 minute distanţă. După o prealabilă testare a memoriei imediate, pacientul este evaluat în funcţie de numărul de cuvinte pe care le poate reproduce după 5 minute. Se atribuie un punct pentru fiecare cuvânt reamintit corect de subiect.

Pentru a cuantifica severitatea deficitului memoriei de scurtă durată, pacientul este întrebat în legătură cu anumite cuvinte uitate din lista celor 3 itemi, folosind termeni categoriali. Astfel, de exemplu, pacientului care a uitat cuvântul „verde", i se spune de către investigator că este vorba de „o culoare". Dacă nici precizarea categoriei nu-l poate ajuta pe subiect în reamintirea cuvântului, atunci examinatorul îi poate prezenta o serie de cuvinte, în care să fie inclus şi cel din lista iniţială.

Memorarea unei liste scurte de cuvinte poate fi o altă metodă de testare a acestei forme de memorie. De exemplu, pacientul este invitat să memoreze două liste a câte 15 cuvinte, în prima fiind incluse cuvinte selectate după categorii, iar cealaltă cuprinzând cuvinte fără legătură între ele. Înainte de fiecare prezentare, pacientul este instruit să rețină cât mai multe cuvinte, fără ca ordinea reproducerii lor să fie importantă. Fiecare listă este supusă testării în mod separat, fiind citită de două ori de examinator, iar pacientul reproducând de două ori itemii fiecărei liste. Pacientul are la dispoziție un minut pentru a reda cât mai multe cuvinte, iar fiecare item reamintit se scorează cu câte un punct. Valorile normale sunt situate în jurul a 16 puncte per listă, fără diferențe semnificative între liste.

În sindromul amnestic, pacienții nu vor grupa cuvintele din prima listă în funcție de categorie, astfel încât performanțele vor fi similare la cele două liste. Un pacient cu depresie va avea performanțe relativ bune la prima listă, cea cu itemi categoriali, dar mai puțin bune la a doua, datorită scăderii motivației şi atenției, odată cu creşterea dificultății sarcinii. Un pacient cu demență va avea performanțe reduse la ambele liste. Cererea de reproducere a itemilor din liste după 20 minute permite evaluarea memoriei pe termen lung.

Memoria de scurtă durată auditivă non-verbală este testată prin prezentarea unui pattern de sunete înalte şi joase, urmând ca pacientul să reproducă pattern-ul respectiv. Se începe cu succesiuni mai simple de sunete, urmând modele mai complicate.

Memoria de lungă durată are capacitate şi durată practic nelimitată (respectiv pe durata întregii vieți), informația este encodată semantic, actualizarea se realizează în paralel, iar bazele sale neuroanatomice sunt ariile parieto-occipitale stângi.

În memoria de lungă durată informațiile sunt stocate permanent, ca urmare a procesului de consolidare. Informațiile stocate sunt distribuite la nivelul întregului cortex, cu suport din partea structurilor specifice fiecărui tip de informații (de exemplu, pentru conținutul emoțional se activează structurile amigdaliene, iar pentru conținuturile vizuale sunt activate ariile corticale vizuale asociative).

Memoria de lungă durată prezintă, conform modelelor cognitive, două subtipuri: *declarativă* şi *procedurală*, prima împărțindu-se în memorie semantică (responsabilă de stocarea informațiilor generale) şi memorie episodică (personalizată), iar a doua incluzând fenomenele de „priming” (amorsaj), învățare asociativă şi emoțională.

Fenomenul de *condiționare* implică învățarea asociativă prin experiențe conectate, aceste asocieri devenind reflexe şi neimplicând astfel rememorarea conştientă a situațiilor.

Fenomenul de *amorsaj* descrie fenomenul de facilitare a detectării sau identificării unui stimul senzorial pe baza experienței personale cu stimuli din aceeaşi categorie. Un stimul concordant cu expectațiile noastre este perceput mai rapid, deoarece acestea au amorsat reprezentarea lui în memorie, facilitând recunoaşterea. Pacienții cu amnezie pot recunoaşte fotografii cu obiecte prezentate anterior mult mai repede decât fotografiile care conțin obiecte noi, chiar şi după o săptămână de la prezentarea acestora. Amorsajul semnalează însă şi participarea unor mecanisme inconştiente, implicite. Există o relație între frecvența expunerii unui stimul şi aprecierea semnificației sau valorii sale. Pacienții amnezici şi subiecții normali se comportă similar în cadrul experiențelor de corelare a valorii unui stimul cu frecvența expunerii la acesta, ceea ce sugerează substratul inconştient al asocierii. La ambele grupuri, amorsajul se poate constata şi după mai multe săptămâni, ceea ce arată implicarea memoriei de lungă durată. Amorsajul are o funcție adaptativă prin creşterea vitezei de reacție la anumiți stimuli predictibili şi prin creşterea fluenței răspunsurilor la stimulii familiari.

Evaluarea memoriei de lungă durată se face atât la nivelul memoriei episodice, cât şi la cel al memoriei semantice.

În primul caz, pacientul este invitat să descrie detaliile unor experiențe personale semnificative, cum ar fi căsătoria, intrarea la şcoală, istoricul medical ş.a.m.d, urmărindu-se, pe baza comparării datelor provenite de la pacient cu cele provenite de la aparținători, corectitudinea secvențelor temporale, a circumstanțelor, poziționării spațiale etc. Evaluarea memoriei episodice poate fi realizată în timpul unui simplu interviu medical, în cursul anamnezei. Confabulațiile pot falsifica involuntar istoricul pacientului, fiind necesară specificarea naturii, a frecvenței de apariție şi a circumstanțelor apariției acestora.

Memoria semantică implică informații generale, pe care se presupune că orice persoană le cunoaşte. Întrebări de tipul „Care este capitala României?", „Care au fost ultimii 3 preşedinți ai țării?», « Care este Ziua Națională a României? » pot fi utilizate pentru a arăta existența disfuncțiilor memoriei semantice.

(2) în funcție de procesele de învățare şi recuperare implicate se disting: (a) memoria explicită, care stochează şi permite recuperarea informațiilor factuale şi evenimențiale; (b) memoria implicită, care se referă la învățarea inconştientă, de exemplu la cea implicată în dobândirea de noi abilități.

Memoria explicită corespunde cel mai bine conceptului comun de memorie, întâlnit în limbajul cotidian. Perturbările memoriei declarative sau explicite, amneziile, se prezintă sub forma deteriorării informațiilor privind drumurile, obiectele, fețele persoanelor cunoscute, melodiile, în general a conținuturilor verbale şi non-verbale, indiferent de modalitățile senzoriale în care s-au prezentat informațiile inițial.

Memoria non-declarativă sau implicită este păstrată în cazul leziunilor care conduc la pierderea memoriei declarative, astfel fiind menținute atât abilitățile dobândite anterior, cât şi capacitatea de învățare prin mecanisme simple, capabile să asigure supraviețuirea persoanei, prin adaptarea la medii noi, fără o recunoaştere explicită, conştientă, a strategiilor folosite în acest sens.

Pacienții amnezici pot avea aceste forme de memorie şi învățare complet operante, putând achiziționa abilități vizuale, vizuomotorii şi cognitive la fel de rapid ca persoanele normale. De exemplu, persoanele amnezice pot învăța să citească un text reflectat în oglindă, îşi îmbunătățesc performanțele de citire a unui text odată cu creşterea numărului de repetiții şi au o creştere a vitezei de citire a unor fragmente fără sens asemănătoare cu cea observată la subiecții normali. Sarcinile de clasificare şi categorizare pot fi îndeplinite normal, deşi pacienții amnezici nu-şi reamintesc itemii specifici care intră în componența categoriei respective.

În memoria procedurală sunt incluse cea mai mare parte a abilităților dobândite în cursul existenței personale, cum ar fi condusul maşinii, mersul pe bicicletă, schiatul, înotul. După o etapă inițială de practicare a acestor abilități, ele devin implicite, deci se situează sub pragul conştient.

O metodă de a evalua învățarea prin „priming", la pacienții cu amnezie profundă, este invitarea lor să încercuiască o anumită literă într-o listă de cuvinte. Pacientului i se spune că nu va trebui să-şi reamintească textul şi nici nu va trebui să-l recitească. Apoi subiectul completează restul cuvintelor dintr-o listă de fragmente semantice, în care sunt prezentate de exemplu primele 3 litere. Jumătate din cuvintele cuprinse în listă provin din lista originală, iar cealaltă jumătate conține doar cuvinte noi. Cuvintele care au fost văzute în lista originală sunt completate mai rapid decât cuvintele noi, deşi pacientul nu a recunoscut explicit contactul anterior cu acestea. Cu toate că subiectul nu-şi aminteşte cuvintele din prima listă, este evident că el realizează o recunoaştere implicită, reflectată în viteza cu care completează prima parte a sarcinii.

Memoria declarativă semantică se referă la informațiile stocate referitoare la fapte şi reguli, iar cea episodică este reamintirea conştientă a unor evenimente trecute. Pacienții cu amnezie nu pot reține evenimente recente sau mai vechi din viața lor, dar se pot descurca în viața de zi cu zi prin acțiuni reflexe. Memoria declarativă este consolidată de-a lungul timpului şi prezintă o deteriorare gradată în timp.

La pacienții korsakovieni se observă această disociere a compartimentelor mnezice, ei fiind incapabili să învețe informații noi (memorie explicită), dar reuşind să se orienteze în medii necunoscute anterior, după un anumit timp sau dezvoltând reflexe condiționate (memorie implicită). La aceşti pacienți există o disfuncție diencefalică, cu deficite profunde anterograde ale memoriei de lungă durată şi amnezie variabilă retrogradă, dar cu menținerea înregistrării şi a memoriei de scurtă durată. Pacienții pot reproduce stimulii prezentați după secunde sau minute, dar nu după trecerea mai multor ore sau zile.

Metamemoria se referă la cognițiile legate de memorie, respectiv la gândurile despre cum, de ce şi cât de adecvat funcționează procesele mnezice proprii. Perspectivele mai complexe asupra metamemoriei includ cunoaşterea mecanismelor memoriei, conştientizarea proceselor mnezice curente, convingerile privitoare la abilităţile mnezice şi afectele corelate cu memoria.

Din punct de vedere al psihologiei cognitive, metacogniţia reuneşte şi reflectă procesele de automonitorizare, luare a deciziilor, învăţare şi memorie, motivaţie, planificare şi formare de strategii, precum şi dezvoltarea cognitivă. De exemplu, Nelson şi Narens[3] au dezvoltat un cadru conceptual al metamemoriei, în care automonitorizarea şi procesele de autocontrol interacţionează în timpul achiziţiei, stocării şi recuperării informaţiilor.

Conceptul multidimensional[4] defineşte metamemoria ca fiind întreaga noastră cunoaştere, conştiinţă şi tot setul de convingeri legate de funcţionarea, dezvoltarea şi capacităţile memoriei personale şi general-umane.

Există 3 categorii principale de metamemorie:

(1) cunoaşterea declarativă a modului de funcţionare a memoriei, care include conştientizarea modului în care caracteristicile sarcinilor mnezice au impact asupra performanţei acestei funcţii cognitive, ce fel de strategii sunt necesare şi care pot fi aplicate în anumite situaţii particulare. Faptul că organizarea materialului de memorat conduce la o reţinere mai bună decât în cazul unui material nestructurat, constituie un exemplu de cunoaştere a proceselor mnezice .

(2) convingerile autoreferenţiale legate de capacitatea persoanei de a folosi memoria în mod eficient în situaţiile în care este nevoie de acest fapt constituie autoeficienţa mnezică. Această proprietate este legată de controlabilitatea memoriei de către individ. Credinţele personale asupra capacităţilor de reamintire pot determina (a) gradul în care persoana se plasează în situaţii care cer activitate menezică, (b) gradul de efort implicat în realizarea unei sarcini, (c) expectaţiile personale privind nivelul de performanţă mnezică şi (d) performanţele mnezice actuale. De exemplu, dacă o persoană consideră că nu este capabilă să-şi reamintească numele şi feţele persoanelor pe care le întâlneşte, ea poate evita situaţiile în care o astfel de abilitate ar putea fi necesară şi va face un efort mic de integrare mnezică, datorită expectaţiilor negative în aceste situaţii, astfel eşuând în continuare. Acest cerc vicios are la bază încrederea redusă în autoeficienţa mnezică.

(3) conştientizarea stării actuale a performanţelor mnezice este, de asemenea, integrată monitorizării mnezice. Pacienţii care îşi pot reaminti uşor informaţiile sunt capabili să-şi monitorizeze activ şi corect performanţele personale în relaţie cu proprietăţile sarcinilor cognitive. Un grad ridicat de corectitudine a predicţiilor performanţei şi raţionalizarea procesului de învăţare pot indica un pacient capabil să-şi reamintească rapid şi adecvat informaţiile stocate.

Patologia memoriei apare atunci când una sau mai multe dintre faţetele metacogniţiei sunt alterate: (a) cunoştinţele despre funcţionarea memoriei proprii sunt incomplete, eronate sau slab structurate; (b) autoeficienţa este redusă; (c) există incapacitatea de a monitoriza sau controla activităţile necesare reamintirii eficiente; (d) memoria este fluctuantă, necontrolată sau excesivă, legată de anxietate sau depresie.

Pacienţii depresivi vor avea tendinţa de a-şi aprecia funcţionarea mnezică mai slab decât este ea în realitate, răspunzând nediferenţiat la toţi itemii unui test autoadministrat, pe când amnezicii tind să răspundă mai nuanţat la itemi. Astfel, în cazul pacienţilor cu amnezie se poate vorbi de un pattern al deficitului mnezic: ei nu se plâng de dificultăţi în reamintirea unor evenimente petrecute demult sau în îndeplinirea unor instrucţiuni, dar au probleme în a-şi reaminti o informaţie după mai multe minute sau ore.

2. Tulburări psihice specifice

Sindromul Ganser este inclus, conform DSM IV TR, în categoria tulburărilor disociative. Termenul de „sindrom Ganser” este dat acestei patologii după descoperitorul ei, Sigbert Josef Maria Ganser care, în 1898, a descris această stare crepusculară la persoanele acuzate de comiterea unor infracţiuni şi care urmau să fie judecate.

Această patologie a mai fost numită şi „psihoză carcerală”, „sindromul răspunsului alături”, „pseudodemenţă”. Sindromul Ganser este rar şi pare a se întâlni mai ales la persoanele de sex masculin (în 75% din cazuri sau chiar mai mult), vârsta medie a pacienţilor fiind estimată la 30 ani, cu un interval de debut estimat între 15 şi 62 de ani.

(1) Termenul de sindrom Ganser este regăsit în literatura psihiatrică de la începutul secolului al XX-lea, majoritatea autorilor fiind de acord cu următorul tablou clinic al acestuia: (1) slăbirea capacităţii de percepţie a spaţiului şi timpului; (2) răspunsuri de tipul „nu ştiu” chiar la întrebări simple („suspendarea informaţiei” care se află la dispoziţia subiectului); (3) reducerea câmpului conştiinţei; (4) abilitatea de a înţelege întrebările este asociată cu incapacitatea de a răspunde corect; (5) răspunsurile oferite de subiect rămân în sfera semantică a întrebării; de exemplu, la întrebarea „Câte degete ai la o mână?”, pacientul va răspunde “Şase”; (7) pacientul prezintă halucinaţii şi poate reactiva sau retrăi într-un mod halucinator anumite experienţe traumatice; (8) analgezie parţială sau totală în unele zone ale corpului, care poate avea un caracter mobil, cu variaţii de la o zi la alta; (9) pacienţii nu sunt ofensaţi de natura simplă a întrebărilor care le sunt adresate, orice întrebare, chiar şi „cum te cheamă” sau „ce este acesta (ceas, pix etc)” determinând răspunsuri „pe lângă” subiect; (10) simptomele menţionate tind să apară în acelaşi timp, într-un cluster; (11) pacientul este surprins atunci când este informat în legătură cu simptomele prezentate în perioada „crepusculară” („vălul amneziei”, după Ganser); (12) nu există pierderi de memorie în legătură cu perioadele dinaintea fazei de instalare a simptomelor; (13) mulţi pacienţi au probleme legale; (14) simpla numărătoare devine dificilă; (15) pacienţii depun eforturi pentru a se concentra la întrebări şi încearcă să-şi depăşească starea de distractibilitate; (16) tranziţia de la sfera patologicului la normalitate este gradată, dar etapele se succed relativ rapid; (17) pacienţii revin la normal după faza acută a sindromului.

Mecanismele presupuse ale sindromului Ganser sunt disociative, deşi sunt autori care includ acest fenomen fie în categoria tulburărilor factice, fie în cea a psihozelor sau a simulării, dat fiind faptul că el apare mai ales în context medico-legal.

Amnezia psihogenă (disociativă) se caracterizează printr-un deficit sever şi extins al memoriei de tip retrograd. Amnezia disociativă implică prezenţa unor informaţii care nu sunt disponibile nivelului conştient pentru perioade variabile de timp. Pacienţii nu-şi reamintesc propriul nume, evenimente din copilărie sau perioade mai îndelungate din istoria lor.

Unii pacienţi cu amnezie psihogenă au o pierdere circumscrisă a memoriei de lungă durată, care poate acoperi doar zona autobiografică, pe o perioadă limitată. Există cazuri de pacienţi care pot relata evenimente publice trecute, dar nu şi evenimente private din istoricul lor.

Conform DSM IV TR, amnezia psihogenă (disociativă) reprezintă incapacitatea de a evoca informaţii personale importante, de regulă de natură traumatică sau stresantă, este prea întinsă pentru a putea fi explicată prin uitarea obişnuită[5]. Această tulburare mnezică este reversibilă, în condiţii speciale. Amintirea experienţei personale nu poate fi reprodusă în formă verbală sau, dacă este reprodusă, nu poate fi reţinută în totalitate în conştiinţă.

Cel mai frecvent, amnezia disociativă se prezintă ca o lacună sau serie de lacune raportate retrospectiv în evocarea unor aspecte din istoria personală. Aceste lacune se referă la evenimente de tipul automutilării, acceselor de furie sau tentativelor de suicid. Mai rar, amnezia psihogenă poate apărea ca un episod cu debut brusc, de exemplu în cursul războaielor sau ca reacţie la o catastrofă naturală. Există o relaţie între vârsta şi intensitatea traumei, pe de o parte, şi incidenţa amneziei, pe de alta. Astfel, amnezia disociativă este frecvent întâlnită în cazul abuzurilor cu durată lungă şi repetitive. Pacienţii cu istoric de abuz sexual au foarte frecvent amnezie totală înainte de recuperarea terapeutică a amintirilor. Într-un studiu s-a observat că până la 38% din supravieţuitorii unui abuz nu raportează amintiri legate de acele episoade, mulţi ani după ce acestea au survenit[6].

A existat o controversă în legătură cu substratul disocierii, respectiv dacă acesta este de natură defensivă sau o reacţie la stres. Freud a susţinut că disocierea este un fenomen defensiv care se activează în momentul în care Eul se consideră ameninţat şi care conduce la reprimarea sau separarea forţată a subsistemelor ideativ, pulsional şi mnezic. După Janet, disocierea este un deficit funcţional, o insuficienţă de legare a energiei, determinată de factori ereditari, factori stresori sau de interacţiunile acestora. Disocierea, conform teoriei lui Janet, corespunde unui deficit al integrării funcţiilor psihice ca reacţie la un factor de stres.

Din punctul de vedere al psihologiei cognitive, disocierea apare printr-o disfuncţie a memoriei autobiografice. Procesarea informaţiilor referitoare la experienţa proprie apare atât la nivel conştient, cât şi inconştient. Disocierea ar corespunde ştergerii informaţiilor referitoare la contextul spaţio-temporal, care sunt de obicei asociate cu memoria pentru evenimente, conducând la disfuncţii ale memoriei episodice şi autobiografice[7]. Traumele, experienţele hipnotice şi neconcordanţele între strategiile de procesare informaţională produc discontinuităţi la nivelul sentimentului de continuitate a propriei identităţi. Aceste experienţe deosebite nu sunt procesate într-o manieră integrativă, fiind encodate sub forma unei urme mnezice supraîncărcate emoţional.

Amnezia indusă de cauze medicale generale sau de consumul de substanţe este întâlnită în traumatismele cranio-cerebrale, accidentele vasculare cerebrale, intoxicaţiile cu CO, meningite, encefalite, infecţii cu HIV, sifilis, tumori cerebrale primare sau metastatice, intoxicaţiile cu alcool sau alte substanţe psihoactive, epilepsia de lob temporal, uremiile, insuficienţa hepatică, diselectrolitemiile, diabetul zaharat, boala Parkinson, hidrocefalia cu presiune normală, deficienţe vitaminice sau nutriţionale.

Amnezia organică se asociază cu probleme de învăţare, dar cu păstrarea memoriei pentru datele personale şi evenimentele din copilărie, exceptând cazurile de leziuni cerebrale temporale şi frontale. Pacienţii cu amnezie non-disociativă nu pot învăţa şi nici nu pot să-şi reamintească informaţii recente, având un deficit al memoriei anterograde. Acest deficit se poate asocia cu grade diferite de amnezie retrogradă, pe fondul unei funcţionări cognitive generale în limite normale.

În DSM IV TR, aceste forme de amnezie sunt clasificate alături de delirium şi demenţă, în categoria tulburărilor cognitive. Pentru a se stabili diagnosticul de „tulburare amnestică”, conform DSM IV TR, trebuie ca deficitul memoriei să fie suficient de sever pentru a induce perturbări ale funcţionării sociale sau profesionale şi trebuie să reprezinte un declin semnificativ de la nivelul anterior de funcţionare. Perturbările mnezice care survin exclusiv în cursul unui delirium sau care se suprapun demenţelor nu intră în această categorie.

Deficitul mnezic este mai evident în sarcinile care necesită o evocare spontană şi atunci când examinatorul oferă repere pentru ca persoana să-şi amintească o perioadă de timp mai recentă. În funcţie de ariile corticale afectate, deficitele mnezice pot fi mai importante în legătură cu stimulii auditivi sau vizuali.

Tulburarea amnestică apare frecvent în urma unui tablou clinic evolutiv care include confuzie şi dezorientare, care pot sugera un delirium. Pot exista elemente confabulatorii în prima etapă a acestui sindrom. Amnezia profundă poate conduce la dezorientare temporo-spaţială, mai rar autopsihică. Cei mai mulţi pacienţi nu au conştiinţa lacunelor mnezice şi pot nega existenţa acestora, chiar dacă le sunt prezentate dovezi. Alţi pacienţi pot fi conştienţi de aceste defecte de memorie dar asociază o stare de indiferenţă. Pacienţii cu tulburare amnestică tranzitorie pot fi perplecşi sau confuzi.

Amnezia tranzitorie datorată unei patologii carebrovasculare poate fi recurentă, cu episoade durând de la câteva ore la mai multe zile, iar cele apărute după un traumatism cranio-cerebral pot avea un pattern cu deficite mnezice foarte mari, imediat după eveniment şi ameliorare treptată în următorii 2 ani.

Abuzul prelungit de o substanţă, expunerea cronică la neurotoxice şi deficitele nutriţionale prelungite pot conduce la o amnezie cu debut insidios.

Tulburarea amnestică persistentă indusă de alcool este datorată deficienţei de tiamină (sindrom Korsakoff), asociată cu ingestia prelungită şi excesivă de etanol. Amnezia este însoţită de semne neurologice, cum ar fi neuropatia periferică, ataxia cerebeloasă, miopatia. Această amnezie urmează de obicei unui episod acut de encefalopatie Wernicke. Chiar dacă semnele encefalopatiei dispar, deteriorarea mnezică este persistentă. Debutul amneziei este de regulă brusc, dar unele persoane pot instala un debut mai insidios, pe parcursul mai multor ani, înaintea apariţiei unui episod deteriorativ final mai dramatic. Odată stabilizată, tulburarea amnestică persistentă indusă de alcool persistă indefinit, deşi poate exista o uşoară ameliorare în decursul timpului, iar într-un număr foarte redus de cazuri, simptomatologia se poate chiar remite (DSM IV TR).

Demenţele au fost intens studiate din punct de vedere al pattern-urilor deteriorării mnezice.

Memoria de scurtă durată sau memoria de lucru este deteriorată în demenţe datorită disfuncţiei sistemelor atenţionale frontale, dar şi a sistemelor subordonate. Alterarea sistemului executiv central este o observație constantă în cazul demențelor subcorticale de tipul demenței Huntington sau a paraliziei supranucleare progresive, fapt dovedit experimental prin reducerea performanțelor la sarcinile de tipul seriilor numerice crescătoare (în special atunci când acestea trebuie reproduse în ordine inversă), dar şi la sarcinile care necesită atenție distributivă[8]. Memoria episodică anterogradă este profund afectată în demența Alzheimer, mai puțin în demențele subcorticale, iar mecanismul de producere este diferit. Encodarea este mai puțin afectată în demențele subcorticale, rata de uitare este relativ normală în această patologie, iar recunoaşterea este relativ păstrată, ceea ce arată existența unui deficit în recuperarea informațiilor stocate[24]. Memoria retrogradă este afectată în special în demența Alzheimer, cu menținerea amintirilor din copilărie, în defavoarea celor mai recente. În demențele subcorticale nu există astfel de diferențe în privința conținutului mnezic afectat, corespunzător unui deficit de recuperare. Memoria semantică este afectată în demența Alzheimer, dar acest defect este depăşit ca importanță de dificultățile observate la nivelul memoriei episodice.

Memoria implicită este afectată diferit în cazul demențelor. Dacă pacienții cu demențe subcorticale au deficite ale performanțelor în sarcinile de învățare a abilităților, cei cu demență Alzheimer nu prezintă dificultăți la nivelul învățării motorii. Acest fenomen este rezultatul afectării neostriatului în demențele de tip subcortical (de exemplu, boala Huntington), dar nu în cele corticale (de exemplu, boala Alzheimer).

Fenomenul de priming a fost studiat la pacienții cu demențe, rezutând că priming-ul lexical este afectat în demențele corticale, dar păstrat în demențele subcorticale. Priming-ul semantic este afectat în mod similar, în cazurile de demență Alzheimer şi în cazul demențelor subcorticale.

Demența Alzheimer se instalează cel mai adesea insidios, primele semnale fiind cele legate de scăderea performanțelor mnezice. Este important de ştiut, din perspectivă clinică, faptul că nu toate aspectele memoriei sunt afectate egal în primele stadii ale bolii. Domeniul mnezic cel mai afectat este memoria episodică anterogradă, iar cea mai sensibilă determinare a acestei deficiențe este evaluarea capacității de reținere a informațiilor noi după un interval mai lung. Memoria pentru imagini este afectată într-o măsură relativ egală cu memoria cuvintelor.

Pacienții cu demență Alzheimer au performanțe marcat reduse la testele de memorie semantică, de tipul denumirii desenelor, denumirii itemilor după descrierea lor verbală, potrivirea imaginilor cu denumirile lor, vocabular şi cunoştințe generale, precum şi la potrivirea desenelor, care implică abilitatea de a deduce legătura asociativă între itemii reprezentați. În stadiile precoce ale bolii, există o afectare disproporționată a memoriei bazate pe categorii semantice, spre deosebire de performanțele obținute la testele bazate pe fluența literală. Ipotezele explicative ale acestor fenomene includ erori la nivelul accesului la memoria semantică sau distrugerea structurii memoriei semantice. Pe măsură ce boala progresează, spre stadiile moderate, distrugerea memoriei semantice devine importantă, ceea ce determină instalarea anomiei, golirea de sens a limbajului şi reducerea performanțelor la testele de cunoştințe generale.

Deliriumul este caracterizat printr-o instalare relativ rapidă a tulburărilor prosexice, însoțite de dezorientare, perturbări ale limbajului, halucinații sau iluzii. Deliriumul apare în contextul consumului sau sevrajului la o substanță cu risc de abuz, intoxicației cu alte substanțe, unei condiții medicale generale, unor cauze multiple sau unor cauze neprecizate.

Deteriorarea memoriei este evidentă cel mai frecvent în memoria recentă şi poate fi testată cerând persoanei să rețină denumirea unor obiecte fără legătură între ele sau o propoziție scurtă, pentru a le repeta după o perioadă scurtă, de câteva minute, fără distragerea atenției în acest interval. Capacitatea pacientului de a susține, deplasa ori focaliza atenția este perturbată, iar întrebările puse pacientului trebuie repetate, deoarece pacientul nu se poate concentra, iar răspunsurile sale tind să aibă un caracter perseverativ. Distractibilitatea pacientului este semnificativă, orice stimul irelevant de o anumită intensitate putând perturba focalizarea subiectului.

Simularea deficitelor mnezice este realizată în context medico-legal în două circumstanțe mai importante. Prima se referă la cazurile de omucidere, în care criminalul acuză pierderea amintirilor legate de evenimentul respectiv, unele evaluări arătând o incidență a acestui fenomen la 70% din persoanele care aşteptau să fie judecate şi la 25-45% din subiecţii care au fost deja condamnaţi[9]. A doua situaţie se referă la persoanele care afirmă că nu-şi pot aminti informaţii percepute după un anumit eveniment soldat cu un traumatism cranio-cerebral.

Dacă în DSM I (1952) simularea nu a fost inclusă, iar în DSM II (1968) acest fenomen era doar menţionat ca element de diagnostic diferenţial cu nevroza isterică, în DSM III (1980) apare ca diagnostic oficial. În DSM IV TR simularea este definită ca producerea intenţionată de simptome somatice sau psihice exagerate grosier, motivată de evitarea unor pedepse sau pentru obţinerea de avantaje.

Simularea amneziei este probabil una din cele mai frecvente forme de simulare a unui simptom psihiatric. Diagnosticul diferenţial trebuie să includă eliminarea cauzelor psihotice, conversiv-disociative, toxice, traumatice, epileptice. Argumentele în favoarea simulării sunt rezultatele normale la radiografia craniană, CT sau RMN cerebral, EEG, examenul neurologic, screening toxicologic, istoric personal de consum etanolic sau de tulburare disociativă.

Existenţa unor trăsături de personalitate histrionică sau istoric personal de episoade amnestice sugerează o cauză disociativă. O personalitate cu trăsături antisociale, pe de altă parte, creşte riscul de simulare.

Momentul debutului simptomelor poate corela cu apariţia motivaţiilor medico-legale, în cazul simulării. Pacientul poate însă anticipa anumite consecinţe cu mult timp înainte, începând să-şi construiască treptat tabloul de „amnezie”. Momentul dispariţiei simptomelor coincide, în cazul simulării, cu rezolvarea dificultăţilor legale.

Amnezia este fragmentară, mai curând decât globală, prezentând, de exemplu, focalizare mai ales pe domeniul autobiografic, dar fără modificarea altor domenii mnezice. Au fost raportate şi copieri ale unor simptome de tipul amneziei după cazuri celebre, intens mediatizate.

Schizofrenia se asociază cu o tendinţă generală de a obţine performanţe cognitive reduse, inclusiv în domeniul memoriei. O analiză controlată a 30 de studii care au evaluat funcţia mnezică la pacienţii cu schizofrenie efectuate în perioada anilor 1960-1980 a condus la concluzia că în episoadele acute de schizofrenie memoria este puţin afectată şi doar în unele arii, pe când în forma cronică performanţele slabe se întâlnesc relativ obişnuit şi pot avea severitate crescută[10]. Studiile ulterioare au confirmat prezenţa deficitelor mnezice semnificative în schizofrenie, care au corelat cu severitatea şi cronicitatea tulburării, precum şi cu deficitele intelectuale globale[11]. Aceste reduceri ale performanţelor au fost observate atât la pacienţii cronici, spitalizaţi, cât şi la unii pacienţi aflaţi în faza acută sau în remisiune. Deficitele mnezice au fost raportate atât la pacienţii care urmau tratament antipsihotic (± anticolinergic) dar şi la subiecţii fără istoric de tratament farmacologic. Gradul de afectare mnezică nu era diferit la pacienţii care primeau anticolinergice, comparativ cu cei care nu aveau aceste medicamente în schema terapeutică. Deficitele performanţei mnezice nu se datorează reducerii atenţiei sau a motivaţiei, existând variaţii pe aceste dimensiuni independent de rezultatele testării memoriei.

Referitor la tipurile de memorie afectate, majoritatea studiilor au constatat afectarea memoriei pe termen lung[11]. Investigarea memoriei de scurtă durată, deşi nu a fost realizată în studii foarte numeroase, a condus la observarea unor diferenţe între pacienţii cu schizofrenie şi subiecţii sănătoşi, în unele cercetări, în timp ce altele nu au reuşit să dovedească existenţa unor diferenţe între cele două loturi[12]. Unii autori susţin existenţa disocierii între memoria de scurtă şi cea de lungă durată la aceşti pacienţi, găsind diferenţe de 14% deteriorare în domeniul memoriei de scurtă durată versus 80% afectare a memoriei de lungă durată [13]. Există studii care arată disfuncţii la nivelul memoriei de lucru la pacienţii cu schizofrenie[14]. Memoria semantică este afectată în paralel cu memoria episodică[15]. În privinţa memoriei procedurale există un ritm mai redus de învăţare, după unii autori sau normal, după alţii. În favoarea ultimei constatări, experimentele conduse de Clare şi colab.[16] arată că învăţarea procedurală şi memoria aferentă acesteia rămân intacte chiar şi atunci când există un nivel global alterat al memoriei în schizofrenie şi chiar dacă nivelul de performanţă este diminuat de tendinţa generală de a avea performanţe slabe la testele cognitive, observabilă la aceşti subiecţi.

Pseudodemența depresivă este un fenomen frecvent întâlnit ca parte a tabloului clinic al tulburărilor afective. Un număr important de pacienți cu depresie relatează existența unor deficite mnezice, iar studiile actuale sugerează că a doua cauză de deteriorare cognitivă tratabilă la bătrâni, ca frecvență, este depresia. De cele mai multe ori, această pseudodemență are la bază o disfuncție a sistemelor atenționale, însă este neclar în ce proporție aceste disfuncții apar la nivel precoce (stadiul selectiv) sau tardiv (stadiul executiv). Studiile arată că, în depresie, există perturbări ale funcției vizuospațiale în contextul unor abilități verbale semantice neafectate, în timp ce perturbările funcțiilor executive survin în fazele mai avansate ale tulburării afective[17]. Distractibilitatea, definită neurocognitiv ca disipare a resurselor în cursul unei sarcini mnezice, are efecte catastrofale asupra generării de strategii de procesare informațională. Pe măsură ce depresia se ameliorează, deficitele mnezice se remit la majoritatea pacienților, cu excepția celor la care depresia a reprezentat calea de intrare într-o demență.

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Interrelații migrenă-epilepsie – aspecte actuale

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REZUMAT. Migrena și epilepsia se caracterizează prin simptome neurologice tranzitorii, examenul fizic general și neurologic fiind în limite normale intercritic, în ambele afecțiuni existând frecvent o componentă genetică sau o predispoziție familială.
Cercetări fundamentale recente au evidențiat existența unor mecanisme fiziopatologice comune între migrenă și epilepsie. De asemenea, studiile epidemiologice sugerează existența unei asocieri a celor două afecțiuni mai mult decât întâmplătoare.
Comorbiditatea poate crea oportunități terapeutice precum folosirea anticonvulsivantelor de tipul acid valproic sau topiramat în prevenția atât a crizelor epileptice, cât și a celor migrenoase.

Cuvinte cheie: migrenă, epilepsie, anticonvulsivante

ABSTRACT. In migraine and epilepsy transient neurological symptoms, normal neurological and physical findings between events and genetic/familiar disposition are common.
Recent basic research findings revealed pathophysiological links between migraine and epilepsy. Also, epidemiological studies suggested that the occurance of migraine and epilepsy in an individual could be beyond changes.
Comorbid conditions may create therapeutic opportunities, anti-epilptic agents like divalproex sodium and topiramate may prevent attacks of both migraine and epilepsy.

INTRODUCERE

Relația dintre migrenă și epilepsie a intrigat neurologii timp de peste 100 de ani, opiniile fiind divergente, de la asociere întâmplătoare la existența unei patogeneze comune.

Migrena și epilepsia se caracterizează prin simptome neurologice tranzitorii, examenul fizic general și neurologic fiind în limite normale intercritic, în ambele afecțiuni existând frecvent o componentă genetică sau o predispoziție familială.

Aproximativ 30% dintre migrenoși prezintă aură – disfuncție tranzitorie vizuală sau senzitivo-motorie care precede sau acompaniază cefaleea (migrenă cu aură). Aura migrenoasă reprezintă echivalentul la nivel uman al fenomenului de “cortical spreading depression” observat de Leão pe creierul de animal – depresia funcției neuronale, cu scăderea consecutivă a debitului sangvin cerebral (oligemia), deplasându-se dinspre lobul occipital spre cortexul frontal, fără a respecta un teritoriu vascular. Fenomenul de “spreading depression” este mecanismul prin care aura migrenoasă induce apariția epilepsiei (migralepsie).

Pe de altă parte, crizele epileptice pot constitui factorul precipitant în apariția migrenei la subiecții cu predispoziție genetică.

Substratul fiziopatologic este existența în ambele afecțiuni a unei stări de *hiperexcitabilitate cerebrală* cu modificări EEG între atacuri, subiectul prezentând un prag convulsivant – și respectiv migrenos scăzut. Acest prag este condiționat genetic și poate fi influențat de factorii de mediu și de terapia anticonvulsivantă.

În cazul migrenelor cu anomalii EEG s-a observat un *răspuns pozitiv al simptomatologiei la tratamentul anticonvulsivant,* ceea ce este o dovadă circumstanțială pentru o asociere între migrenă și epilepsie. De asemenea, în unele cazuri, *întreruperea tratamentului anticonvulsivant a determinat recrudescența atacurilor migrenoase*.

ASPECTE FIZIOPATOLOGICE

Există importante legături fiziopatologice între migrenă şi epilepsie. Este acum recunoscut că aceste afecţiuni sunt datorate unor mutaţii care duc la disfuncţia canalelor ionice, migrena şi epilepsia fiind în concepţia actuală considerate **canalopatii neuronale.**

Migrena hemiplegică familială (FHM) este o afecţiune rară, autosomal dominantă, caracterizată prin episoade de hemipareză tranzitorie urmată de cefalee. Există trei tipuri cunoscute de FHM:

– FHM tip1 – determinată de o mutaţie a genei CACNA1A (localizată pe braţul scurt al cromozomului 19p13) care determină o sinteză modificată a subunităţii α_1 a canalului de calciu voltaj-dependent de tip P/Q (Ophoff et all, Cell , 1996).

– FHM tip 2 – determinată de mutaţii ale genei ATP1A2 care codifică subunitatea α_2 a pompei Na^+/K^+ sunt asociate cu FHM (tip 2), mutaţia fiind localizată la nivelul cromozomului 1q23. (De Fusco M et all, Nat Genet ., 2003).

– FHM tip 3 – în care este implicat un nou locus p pe cromozomul 24 (2q24), ducând la o mutaţie heterozigotă missense (Gln1489Lys) a genei SCN1A care codifică canalul neuronal de sodiu voltaj-dependent. Această mutaţie a fost asociată atât cu FHM3 cât şi cu epilepsia, prin afectarea fluxurilor de sodiu de-a lungul membranelor neuronale şi ale celulelor gliale, confirmând încă o dată legăturile moleculare între migrenă şi eilepsie (Dichgans M et all, Lancet, 2005).

ASPECTE CLINICE

Multe studii au avut drept obiect relaţia dintre migrenă şi epilepsie, având în vedere **similarităţile** lor clinice: ambele sunt boli cronice în care sunt prezente atacurile paroxistice recurente caracterizate prin disfuncţie temporară a sistemului nervos central şi hiperexcitabilitate corticală interictală, putând fi sau nu precedată de aură.

Conform criteriilor IHS II diagnosticul de migrenă se pune pe baza repetării atacurilor, caz în care putem face distincţia între criză de tip migrenos şi boala migrenoasă.

În ambele afecţiuni este vorba despre existenţa unui *prag convulsivant/migrenos* condiţionat genetic şi influenţat de factorii de mediu şi de terapie anticonvulsivantă, iar starea de hiperexcitabilitate corticală intercritică poate fi obiectivată prin prezenţa anomaliilor electrice pe traseul EEG interictal.

Elemente clinice comune epilepsie - migrenă:

- Cronicitate
- Recurenţă sub formă de paroxisme
- Ictal – disfuncţie temporară a SNC
- Interictal – hiperexcitabilitate corticală (modificări EEG între atacuri)
- Existenţa unui prag convulsivant/migrenos condiţionat genetic şi influenţat de factorii de mediu şi de terapie (anticonvulsivantă)
- Aură +/-
- Alterarea tranzitorie a stării de conştienţă +/-
- Cefalee +/-

Diferenţierea migrenei şi epilepsiei poate fi dificilă având în vedere că ambele se caracterizează prin episoade de disfuncţie neurologică. Ambele afecţiuni pot determina alterare tranzitorie a stării de conştienţă, ca şi apariţia cefaleei. Cefaleea fără semne neurologice constituie o manifestare rară de epilepsie. Pe de altă parte, există şi varietatea de migrenă acefalalgică (aură migrenoasă fără cefalee).

Cel mai dificil este de diferenţiat migrena cu aură de crizele parţiale complexe. Dacă aura este scurtă (mai mică de 5 min) şi asociată cu alterare a conştienţei, automatisme şi alte semne motorii pozitive (mişcări tonico-clonice), diagnosticul este acela de epilepsie.

Dacă aura este de lungă durată (peste 5 min) şi prezintă asociate simptome pozitive (scintilaţii, parestezii) şi negative (scăderea acuităţii vizuale, pareze), diagnosticul mai probabil este acela de migrenă .

Cefaleea poate fi precipitată de o criză epileptică. Noua clasificare internațională a cefaleelor recunoaşte la secțiunea de cefalee secundare **cefaleea atribuită convulsiilor epileptice** cu două subtipuri:

- Hemicrania epileptică – care este cefaleea ipsilaterală cu trăsături migrenoase care apare sincron cu o criză epileptică sau descărcare electrică EEG.
- Cefaleea postictală – este asociată cu greață, vomă, survine în special după crizele epileptice convulsive.

ASPECTE EPIDEMIOLOGICE

Comorbiditatea se referă la o asociere mai mult decât incidentală a două condiții la acelaşi individ. Baza comorbidității poate fi genetică şi/sau bazată pe factori de mediu comuni. Uneori relația temporală nu este clară, astfel încât o afecțiune o poate cauza pe cealaltă.

În cazul migrenei există comorbiditate cu afecțiuni neuropsihice: epilepsia, depresia şi anxietatea; cu tremorul esențial şi cu accidentul vascular cerebral ischemic (în cazul femeilor tinere cu migrenă cu aură, în special în cazul celor fumătoare şi/sau care folosesc anticoncepționale orale). De asemenea, s-a descris asocierea migrenei cu tremorul esențial, cu vertijul şi cu tulburările de somn.

Pe de o parte poate exista o **asociere aleatoare** a celor două afecțiuni, dar pe de altă parte relația dintre migrenă şi epilepsie poate fi una de **tip cauzal.**

Coexistența migrenei cu epilepsia într-o relație de tip cauzal este sugerată de mai multe aspecte întâlnite în practica clinică: prezența epilepsiei în copilărie la pacienții migrenoşi, prezența patologiei subiacente ce determină migrenă şi epilepsie simptomatice, existența cefaleei migrenoase postictale, existența epilepsiei simptomatice induse de modificările determinate de migrena cronică şi a epilepsiei induse de aura migrenoasă.

Incidența epilepsiei la migrenoşi

Cifrele prevalenței epilepsiei la migrenoşi diferă între studiile populaționale prospective şi cele bazate pe cazuri selecționate din clinici specializate în tratamentul migrenei şi al epilepsiei.

Într-un studiu populațional, Bille nu a găsit asociere cu epilepsia la 73 de copii cu migrenă severă. Într-un studiu pe 500 indivizi, Lance şi Anthony au găsit o incidență mai mare a epilepsiei la pacienții cu cefalee tensională (2%), comparativ cu migrenoşii (1,6%). Mai multe studii bazate pe pacienți selecționați din clinici specializate în tratamentul migrenei au găsit o incidență scăzută a epilepsiei, cu o prevalență chiar mai mică decât în populația generală (<1%).

Pe un studiu populațional, Basser (1969) a obținut o incidență semnificativ mai mare a epilepsiei la 1830 de migrenoşi (5,9%) decât la cei 548 pacienți cu cefalee tensională (1,1%) şi a observat o incidență mai mare a alterării stării de conştiență la migrenoşi comparativ cu populația generală.

Date similare cu Basser a găsit Andermann (1987), care a obținut o *prevalență mediană a epilepsiei de 5,9% printre migrenoşi*, care depăşeşte cu mult prevalența din populația generală de 0,5%. Prevalența migrenei la epileptici variază între 8 şi 23%.

Incidența migrenei la epileptici

Ottman şi Lipton (1997) au studiat comorbiditatea migrenă–epilepsie folosind ”Columbia University's Epilepsy Family Study” şi au găsit printre subiecții cu epilepsie (probanți) o prevaleță de 24% a istoricului de migrenă. Rudele suferind de epilepsie ale probanților au avut o incidență de 26% a istoricului de migrenă, în timp ce la rudele fără epilepsie prevalența migrenei a fost de 15%. *Epilepsia creşte riscul relativ de migrenă de 2,4 ori , atât pentru probanți cât şi pentru rudele lor cu epilepsie*.

Riscul de migrenă nu a fost legat de vârsta de debut a epilepsiei, dar a fost mai mare la pacienții cu crize parțiale şi generalizate. Cel mai mare risc de migrenă îl au pacienții cu epilepsie post-traumatică (risc relativ = 4,1).

Există o incidenţă mai mare a migrenei în anumite forme de epilepsie, cum ar fi epilepsia benignă occipitală, epilepsia benignă rolandică şi epilepsia cortico-reticulată cu absenţe.

Riscul de migrenă este crescut atât înainte, cât şi după debutul crizelor epileptice, de aceea nu poate conta drept numai o cauză a epilepsiei şi nici drept numai o consecinţă a acesteia. Deoarece riscul de migrenă este crescut după epilepsia post-traumatică şi traumatismul cranio-cerebral este un factor de risc pentru ambele afecţiuni, se pare că factori de mediu comuni contribuie la comorbiditatea lor.

Totuşi, factorii de risc de mediu cunoscuţi nu pot conta în totalitate pentru această comorbiditate, câtă vreme riscul de migrenă este crescut şi la indivizii cu epilepsie idiopatică. Pe de altă parte, nici factorii de risc genetici nu pot conta complet pentru această comorbiditate, deoarece riscul de migrenă este ridicat atât la probanţii cu istoric familial pozitiv pentru epilepsie, cât şi la cei cu istoric negativ.

Probabil că *o stare alterată a activităţii cerebrale creşte atât riscul de migrenă cât şi cel de epilepsie, contribuind la comorbiditatea acestor afecţiuni*. Factorii de risc genetici sau de mediu pot creşte excitabilitatea neuronală sau pot scădea pragul de apariţie a celor două tipuri de atacuri. O scădere a magneziului cerebral sau alterări ale neurotransmiţătorilor, ale metabolismului mitocondrial (MELAS) pot reprezenta mecanismele plauzibile ale acestei alterări a excitabilităţii neuronale.

ASPECTE TERAPEUTICE

Asocierea între migrenă şi epilepsie are implicaţii clinice practice. Când tratăm pacienţii pentru una dintre aceste afecţiuni este important să menţinem un index mare de suspiciune pentru cealaltă afecţiune.

Comorbiditatea are un efect negativ pentru progresia bolilor, accentuând severitatea acestora.

Comorbiditatea migrenă–epilepsie determină un efect negativ asupra prognosticului epilepsiei, existând o probabilitate mai mică ca pacienţii cu epilepsie la care s-a asociat migrena să ajungă la remisiunea crizelor epileptice “seizure-free” la 10 ani de la diagnosticare, în acelaşi timp existând o mai mare incidenţă a cazurilor de epilepsie rezistentă la tratament în cazul acestui subgrup de pacienţi (Velioglu, 2005).

Afecţiunile comorbide implică limitări terapeutice, dar, de asemenea, pot crea şi oportunităţi terapeutice.

În tratamentul migrenei trebuie folosite cu prudenţă medicamentele care scad pragul convulsivant, cum ar fi antidepresivele triciclice, inhibitorii selectivi ai recaptării serotoninei şi neurolepticele.

Pe de altă parte, este avantajos să tratăm atât migrena, cât şi epilepsia cu un singur medicament. Astfel, divalproex sodium şi topiramatul previn atât atacurile de migrenă cât şi crizele epileptice.

Anticonvulsivantele (mai corect denumite agenţi stabilizatori neuronali sau neuromodulatori) şi-au demonstrat eficacitatea în tratamentul cefaleelor, acesta fiind în concordanţă cu teoria că migrena reflectă o afectare a creierului.

Dintre anticonvulsivante, *valproatul de sodiu şi topiramatul sunt oficial aprobate de FDA pentru tratamentul profilactic al cefaleei de tip migrenos*.

Acidul valproic este deosebit de util la pacienţii care prezintă comorbiditate migrenă-epilepsie, migrenă-tulburare anxioasă sau migrenă-boală bipolară.

Topiramatul a fost studiat în cele mai importante cercetări efectuate pentru profilaxia migrenei – MIGR-001, MIGR-002 (control placebo) care au inclus 970 pacienţi, şi MIGR-003, în care exista control activ cu propranolol.

Lamotrigina s-a dovedit a fi utilă în prevenirea aurei migrenoase într-un studiu open-label (Lampl. 2005).

Flunarizina – blocant al canalelor de calciu utilizat în tratamentul profilactic al migrenei – are o activitatea antiepileptică slabă. Carbamazepina şi oxcarbazepina nu s-au dovedit a fi utile în prevenţia migrenei.

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Caracterizarea fizico-chimică, testarea activității biologice şi farmacotoxicologice în vederea selecționării pentru terapia antiinfecțioasă a unor noi agenți antimicrobieni din clasa amidelor

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REZUMAT. La Facultatea de Farmacie au fost sintetizate trei serii de amide, cu câte şase structuri chimice diferite, care au fost testate comparativ în laboratorul de microbiologie pentru evidențierea efectului antimicrobian (conform normelor europene în vigoare), în vederea identificării celor mai activi compuşi. În acest scop au fost efectuate cercetări interdisciplinare privind obținerea unor noi agenți antimicrobieni, caracterizarea fizico-chimică şi testarea activității biologice în vederea utilizării în terapia antiinfecțioasă. Prin compararea rezultatelor şi prelucrarea statistică pentru selecționarea celor mai eficiente amide au fost identificate câteva amide care ar putea fi utilizate în terapia antiinfecțioasă, după parcurgerea etapelor de autorizare conform legislației în vigoare.
Testările au fost efectuate conform recomandărilor din „Metode şi tehnici uzuale în laboratorul clinic" editată de Ministerul Sănătății şi Academia de Ştiințe Medicale şi Standardele „Antiseptiques et desinfectants" AFNOR.

Cuvinte cheie: amide, farmacotoxicologie, substanțe antimicrobiene, terapie antiinfecțioasă

ABSTRACT. Three series of new amides were synthesised at the Faculty of Pharmacy, each one with six different chemical structures, that were comparatively tested in the microbiology laboratory to put in evidence the antimicrobian effect, according to EU norms, in order to identify the better quotient activity per toxicity for most active compounds. For this purpose, there were conducted interdisciplinary researches to obtain new antimicrobian agents, that were characterized in physical and chemical aspects and tested regarding their biological activity (antimicrobial in vitro and toxicological in vivo) and antitoxynological effect in vitro, in order to be utilized in the antiinfectious therapy. Using comparision of the results and statistical processing to select the most efficient amides, there were identified a few amides that might be utilized in the anti-infectious therapy after following the authorization steps according to existing law. The test were conducted according the recommendations found in "Usual methods and techniques for clinical laboratory", paperwork that was edited by the Health Minister, Medical Sciences Academy and the „Antiseptiques et desinfectants" AFNOR standards.

INTRODUCERE

Cea mai importantă strategie de prevenire şi combatere a fenomenului de rezistență microbiană față de medicamentele antimicrobiene este de a folosi substanțe active noi, la care încă nu s-a instalat rezistența. Aceasta implică un efort susținut de cercetare pentru a sintetiza noi substanțe potențial antimicrobiene, pentru a dispune permanent de un portofoliu de medicamente de rezervă pentru terapia antiinfecțioasă.

La Facultatea de Farmacie au fost sintetizate trei noi serii de amide, cu câte şase structuri chimice diferite, care au fost testate în laboratorul de microbiologie pentru evidențierea efectului antimicrobian conform normelor europene în vigoare, comparativ pe cele trei serii de compuşi, în vederea identificării celor mai activi. Au fost efectuate cercetări interdisciplinare privind obținerea unor noi agenți antimicrobieni, caracterizarea fizico-chimică şi testarea activității biologice în vederea utilizării în terapia antiinfecțioasă. Prin compararea rezultatelor şi prelucrarea statistică pentru selecționarea celor mai eficiente amide au fost identificate câteva amide care ar putea fi utilizate în terapia antiinfecțioasă, după parcurgerea etapelor de autorizare conform legislației în vigoare.

Testările au fost efectuate conform recomandărilor din lucrarea „Metode şi tehnici uzuale în laboratorul clinic” editată de Ministerul Sănătății şi Academia de Ştiințe Medicale şi Standardele „Antiseptiques et desinfectants” AFNOR.

Testarea efectuată a urmărit trei direcții: proprietățile fizico-chimice, toxicitatea acută pe şoareci şi efectul detoxifiant *in vitro*, ca posibil decontaminant în toxinologie.

1. TESTAREA PE ANIMALE DE LABORATOR

Au fost utilizați şoareci albi de laborator, fiind mamiferul cel mai comod de utilizat în experimente. Tehnica de lucru pentru screening-ul toxicologic constă în injectarea intra-peritoneală a 0,5 ml soluție de lucru (produs de testat) la şoareci adulți tineri, greutate 18-22 g, sănătoşi, de ambele sexe şi monitorizarea lor timp de minimum 3 zile: comportament, simptomatologie şi mortalitate. Preparatul testat poate fi considerat extrem de toxic dacă simptomele şi mortalitatea apar după câteva secunde sau minute, dacă este foarte toxic după câteva ore, moderat toxic după 1-2 zile, puțin toxic dacă simptomatologia se remite spontan şi animalul supraviețuieşte 3 zile, şi netoxic dacă în intervalul de monitorizare de minimum 3 zile nu apar modificări de comportament, simptome sau moarte. Acestea concordă în linii mari cu clasificarea toxicologică a substanțelor active şi constituie un test preliminar pentru orientarea testării farmacotoxicologice (tabel nr.1, adaptat după V.Voicu).

Tabel nr. 1. Clasificarea farmacotoxicologică a substanțelor active

Nr.crt.	DL50 mg/kg corp	Categoria de toxicitate	Observații
1	<5	Supertoxice	STL
2	5-49	Extrem de toxice	STL
3	50-499	Foarte toxice	Subst.active farmac.
4	500-4999	Moderat toxice	Subst.active farmac.
5	5000-14000	Cu toxicitate redusă	
6	>15000	Practic netoxice	

La diluția 1/10 şoarecii inoculați intraperitoneal cu 0,5 ml soluție C1.2 de concentrație 10% au murit în 2-3 minute prezentând semne nervoase. La examenul necropsic au fost remarcate în cavitatea peritoneală leziuni hemoragice, congestie şi hemoragii pe suprafața intestinelor.

La diluția 1/100 şoarecii inoculati intraperitoneal cu 0,5 ml soluție C1.2 de concentrație 10% au murit în intervale de timp relativ scurte (tabelul nr.2). La examenul necropsic au fost remarcate leziuni hemoragice în cavitatea peritoneală: congestie şi hemoragii pe suprafața intestinelor.

Material şi metodă

Testare de toxicologie acută pe animale (screening):

Au fost injectați intraperitoneal câte trei şoareci albi de laborator a 18-22g cu câte 0,5ml substanță dizolvată 10% în apa distilată şi un alt lot cu 10% substanță dizolvată în DMSO (dimetilsulfoxid), pentru o dizolvare completă. A fost urmărit comportamentul şi s-a notat mortalitatea timp de 3 zile, comparativ cu loturi martor, injectate cu apă distilată şi respectiv cu DMSO. La primul lot au murit doi şoareci la 5-7 minute şi al treilea după două zile, nu a existat nici un supraviețuitor. La lotul al doilea au murit doi şoareci la 5-7 minute şi a fost un supraviețuitor. La lotul martor cu apă nu a murit niciunul, la DMSO unul a murit la 6 minute, altul a intrat în comă cu convulsii şi a murit după o oră iar al treilea a murit după două zile. Putem considera că solventul DMSO (dimetilsulfoxid), deşi este foarte bun din punct de vedere fizico-chimic, nu poate fi utilizat în practică din cauza toxicității mari. În această situație va trebui să luăm în considerare numai testarea soluției apoase, deşi nu este o dizolvare completă. La examenul necropsic au fost remarcate în cavitatea peritoneală leziuni hemoragice, congestie şi hemoragii pe suprafața intestinelor. Substanța este toxică, dar acest fapt este de aşteptat la majoritatea substanțelor active farmacologic.

Tabel nr. 2. Test de toxicitate pe şoarece - amida C 1.2; inoculare ip. soluţie 1/100.

Nr. crt	Numărul matricol	Inoculare 0,5 ml ip
		Rezistă/minute
1	1	20
2	2	28
3	3	38
4	4	26
5	5	29
	suma	141
	media	28.2

Animalele inoculate cu doza 0,5 ml i.p., la diluţia 1/10 au murit la câteva secunde după inoculare. La diluţia 1/100, animalele au murit într-un interval de timp cuprins între 4 minute şi 38 de minute, prezentând o simptomatologie nervoasă (convulsii, pedalari în gol, ţipete stridente).

2. EFECTUL DETOXIFIANT

Determinarea efectului soluţiilor C1.1- C1.6 10% asupra aflatoxinelor

În acest scop s-a folosit o soluţie de aflatoxină brută extrasă dintr-o cultură fungică aflatoxigenă. Soluţia de aflatoxină cu concentraţia de 4,1mg% a fost pusă în contact direct cu soluţiile C1.1 - C1.6 (1ml aflatoxină + 1ml soluţie de testat).

Verificarea efectului detoxifiant s-a realizat după 24 de ore, folosind metoda cromatografiei în strat subţire. Aplicarea probelor pe cromatoplacă s-a realizat automat, cu aplicatorul Linomat IV, în volume fixe (10 µl/banda).

Condiţiile de cromatografie au fost: suport cromatografic – Silicagel 60 F 254 Merck HPTLC, 10/20 cm; developant – cloroform: acetonă: apă (140:20:0,3); migrare unidimensională ascendentă; vizualizare (estimare calitativă a benzilor fluorescente) – UV 254- 366 nm.

Trak-urile 1-6 prezintă probele de aflatoxină tratate cu soluţiile C1.1 – C1.6, iar tracul 7 reprezintă proba de aflatoxină netratată. Se poate evidenţia foarte bine că cele două benzi fluorescente reprezentate de AFB1 (aflatoxina blue 1) şi AFG1 (aflatoxina green 1) prezente în amestecul aflatoxinic de pe tracul 7 au rămas nealterate în cazul probelor tratate cu soluţiile de testat.

3. TESTAREA FARMACOTOXICOLOGICĂ

Obiectivul studiului: cercetările au avut ca obiectiv evaluarea toxicităţii acute a şapte compuşi farmacologic activi selectaţi din cele 3 serii de câte 6 compuşi cu acţiune antimcrobiană, în scopul introducerii în practica medicală.

Tabelul nr. 3. Soluţii de lucru: concentraţii, potenţial toxic, mod de preparare, doze volume şi căi de administrare

Sol. de lucru	Concentraţia soluţiilor de lucru (mg/ml)	Doze administrate (mg/kg/i.p.)	Volum administrat i.p. (ml/kg)	Potenţialul toxic al soluţiei	Mod de preparare
S1	7,94	317,73	40	3,20	503,14 mg ad 50 ml apă distilată
S2	6,30	252,26	40	3,30	7,94 ml S1 ad 25 ml apă distilată
S3	5,01	200,48	40	3,40	6,33 ml S1 ad 25 ml apă distilată
S4	4,46	178,55	40	3,45	5,62 ml S1 ad 25 ml apă distilată
S5	3,97	159,77	40	3,50	5,01 ml s1 ad 25 ml apă distilată
S6	3,54	141,70	40	3,55	4,46 ml S1 ad 25 ml apă distilată
S7	3,16	126,48	40	3,60	3,98 S1 ad 25 ml apă distilată

A fost testat experimental un compus chimic selectat ca fiind cel mai bun dintre compuşii cu acţiune antimicrobiană, având masa moleculară 503,14 g şi structura chimică prezentată mai jos, codificată cu indicativul C1.2.

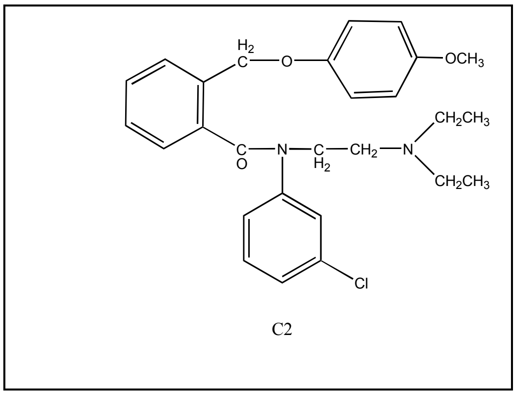

C2

Fig.1. Structura chimică a compusului testat

1. În vederea determinării DL_{50} a compusului selectat s-a utilizat un model experimental de toxicitate acută astfel: s-a preparat o soluție inițială de concentrație 7,94 mg/ml, corespunzătoare unui potențial toxic de 3,20, astfel: s-a cântărit la balanța analitică o cantitate de 397,15 mg din substanța testată. Într-un mojar, s-a adăugat peste substanța cântărită 2 ml alcool etilic şi 0,2 ml glicerină, picătură cu picătură, amestecând continuu. S-a transvazat amestecul într-un balon cotat de 50 ml şi s-a adăugat apă distilată până la semn; pH.-ul soluției astfel obținute măsurat cu un analizor electronic de pH a fost 5,20. Din această soluție mamă s-au preparat prin diluție soluții de lucru ale căror concentrații au fost redate în tabelul nr. 3.

2. Materialul biologic (100 şoareci albi, cu greutate cuprinsă între 18–25 g) a fost repartizat în loturi omogene de câte 10 animale (un număr egal de loturi de masculi şi femele), ținute în microclimat adecvat (vivariu) cu acces liber la hrană şi apă, în afara contactului cu insecticide.

3. Metodele de administrare a soluțiilor de lucru: Compusul testat a fost administrat intraperitoneal în volum de 40 ml/kgmc, imediat după cântărirea animalelor.

4. Monitorizarea animalelor a constat în urmărirea curbei ponderale şi a stării de sănătate pe o perioadă de 7 zile înaintea începerii experimentului. Cu 16 ore înainte de începerea experimentelor, animalelor li s-a oprit accesul la hrană, având acces liber la apă.

Monitorizarea animalelor în timpul experimentului a constat în urmărirea şi înregistrarea momentului apariției semnelor de intoxicatie, înregistrarea mortalității la 60 de minute şi la 24 ore.

Protocol experimental

În acest studiu farmaco-toxicologic a fost utilizat un model experimental de toxicitate acută pe o specie de rozătoare (şoareci albi) şi o cale de administrare (i.p.) pentru evaluarea DL_{50}:

Au fost introduse în experiment 10 loturi a câte 10 animale (5 femele şi 5 masculi): ca exemplu, Lotul 1, reprezentat de 10 animale (5 masculi şi 5 femele), la care s-a administrat i.p. un volum de 40 ml din soluția S1 de concentrație 7,94 mg/ml având un potențial toxic de 3,20. S-a urmărit şi înregistrat momentul apariției semnelor de intoxicație şi a mortalității la 60 de minute şi la 24 ore de la intoxicație.

Loturile la care au existat supraviețuitori au fost extinse pe 20 de animale, ca exemplu, Lotul 8, reprezentat de 10 animale (5 masculi şi 5 femele), la care s-a administrat i.p. un volum de 40 ml din soluția S4 (administrată lotului nr.4) de concentrație 4,46 mg/ml având un potențial toxic de 3,45. S-a urmărit şi înregistrat momentul apariției semnelor de intoxicație şi a mortalității la 60 de minute şi la 24 ore de la intoxicație.

Analiza statistică a rezultatelor experimentale s-a efectuat prin utilizarea programului de calcul statistic Origin 5.0, rezultând valoarea DL_{50}, ecuația dreptei de regresie şi coeficientul de corelație pentru $p< 0,05$.

Rezultate şi discuții

Rezultatele experimentale reprezentând mortalitatea loturilor introduse în experiment corespunzătoare dozelor administrate, înregistrată la 24 de ore, sunt redate în tabelul nr. 4.

Tabelul nr. 4. Rezultatele experimentale ale loturilor introduse în experiment la 24 ore după intoxicație

Nr. lot	Sol. de lucru	Concentrația soluțiilor de lucru (mg/ml)	Doze administrate (mg/kg/i.p.)	Volum administrat i.p. (ml/kg)	Potențialul toxic al soluției	Mortalitate procentuală
1	S1	7,94	317,73	40	3,20	100%
2	S2	6,30	252,26	40	3,30	100%
3	S3	5,01	200,48	40	3,40	100%
4	S4	4,46	178,55	40	3,45	80%
5	S5	3,97	159,77	40	3,50	40%
6	S6	3,54	141,70	40	3,55	20%
7	S7	3,16	126,48	40	3,60	0%
8	S4	4,46	178,55	40	3,45	70%
9	S5	3,97	159,77	40	3,50	40%
10	S6	3,54	141,70	40	3,55	10%

Valorile logaritmilor dozelor corespunzătoare loturilor la care au existat supraviețuitori şi probiturile mortalităților înregistrate la 24 ore la aceleaşi loturi au fost introduse în programul de calcul (Origin 5.0).

Rezultatele analizei statistice sunt reprezentate în tabelul nr. 5.

Tabelul nr. 5. Rezultatele analizei statistice a datelor primare

Nr.animale/lot	Doza administrata mg/kg i.p.	Log doza	Probitul mortalității înregistrate la 24 ore
20	178,55	2,25	5,67
20	159,77	2,20	4,75
20	141,70	2,15	3,96

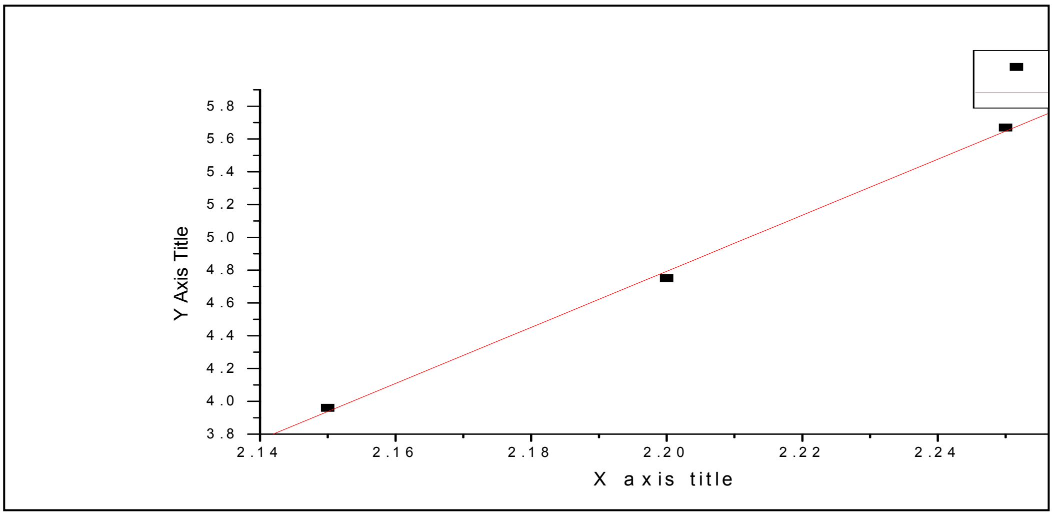

Fig.2. Reprezentarea grafică a dreptei de regresie.

Coeficientul de corelație este de 0,9935 pentru p=0,0279.

Ecuația dreptei de regresie şi coeficientul de corelație pentru $p \leq 0,05$ este de forma:

$$Y = 17,1*X - 32,82,$$

în care Y reprezintă probitul corespunzător efectului urmărit în experiment, respectiv mortalitatea, iar X, expresia matematică, respectiv logaritmul dozei de substanță care determină mortalitate. Pentru diferite valori ale mortalității (Y) rezultate experimental corespund diferite valori ale lui X, respectiv valori ale dozelor de substanță cu efecte letale. Corespondența matematică a corelației biologice doză (logaritm doza X) – efect (probit Y) este reprezentată de ecuația dreptei de regresie de forma prezentată mai sus. Coeficientul de corelație este o mărime rezultată din calcul care reprezintă corelația dintre doză şi efect. Limita inferioară acceptată este de 0,98.

Pentru Y=5, respectiv probitul corespunzător mortalității de 50%, valoarea lui X, respectiv valoarea log.DL_{50} este 2,21. Antilogaritmând, rezultă valoarea DL_{50} la şoarece de 162,18 mg/kg.

Limitele fiduciale ale DL-ului $_{50}$ calculate conform protocolului standardizat în Farmacopeea Română sunt reprezentate de următoarele valori: ± 1,01.

În acest studiu experimental a fost evaluată, prin determinarea DL_{50}, toxicitatea acută a unui compus farmacologic activ cu acțiune antimicrobiană.

Valoarea DL_{50} pe cale intraperitoneală la şoarece este de 162,18±3,23 mg/kg, corespunzatoare unui potențial toxic de 3,4759.

În acest context, din punctul de vedere al toxicității, substanța poate fi astfel încadrată în categoria substanțelor foarte toxice (vezi tabelul de clasificare).

A fost aplicată metoda de testare screening a efectului toxic al compuşilor, prin tehnica injectării intraperitoneale la şoareci; toți compuşii prezintă toxicitate acută.

Compuşii testați prezintă toxicitate mare, se încadrează în grupa "foarte toxici", ca majoritatea substanțelor active medicamentoase.

Animalele inoculate cu doza 0,5 ml i.p., la diluția 1/10 au murit la câteva minute după inoculare. La diluția 1/100 animalele au murit într-un interval de timp cuprins între 4 şi 38 de minute, prezentând o simptomatologie nervoasă (convulsii, pedalări în gol, țipete stridente).

Discuții

Animalele inoculate cu doza 0,5 ml ip., la diluția 1/10 au murit la câteva secunde după inoculare. La diluția 1/100 animalele au murit într-un interval de timp cuprins între 4 şi 38 de minute, prezentând o simptomatologie nervoasă (convulsii, pedalări în gol, țipete stridente).

A fost aplicată metoda de testare screening a efectului toxic al compuşilor, prin tehnica injectării intraperitoneale la şoareci; toți compuşii prezintă toxicitate acută.

Compuşii testați prezintă toxicitate mare, se încadrează în grupa "foarte toxici", ca majoritatea substanțelor active medicamentoase.

Considerăm că primele rezultate sunt pozitive şi că este utilă continuarea seriei de sinteze şi testări pentru selecționarea de noi substanțe antimicrobiene.

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Animalele inoculate cu doza 0,5 ml i.p., la diluția 1/10 au murit în multe cazuri la câteva minute după inoculare, prezentând o simptomatologie nervoasă (convulsii).

Testarea screening a efectului toxic al compuşilor, prin metoda injectării intraperitoneale la şoareci arată că toți compuşii prezintă toxicitate acută mare.

Compuşii testați prezintă toxicitate mare, se încadrează în grupa "foarte toxici", ca majoritatea substanțelor active medicamentoase.

Comparativ cu seriile anterioare, amidele din seria a treia prezintă activitate antibacteriană superioară şi pot fi considerate ca potențiale medicamente antibacteriene.

Concluzii

- La testarea screening a efectului antimicrobian a compuşilor prin metoda difuzimetrică pe mediu de cultură solid, toți compuşii prezintă activitate antibacteriană evidentă.
- La testarea în diluții succesive, substanțele au efect antibacterian slab, conform normelor pentru dezinfectante.

- Testarea screening a efectului toxic al compuşilor, prin metoda injectării intraperitoneale la şoareci, arată că toți compuşii prezintă toxicitate acută mare.
- A fost completată testarea cu metoda antibiogramei difuzimetrice, adaptată de noi pentru testarea substanțelor pulbere, cu rezultate bune.
- Compuşii testați C1.4, C1.5, C2.2, C2.3, C2.5, C3.5 şi C3.6 prezintă efect antibacterian maxim la concentrații de 1-10%.
- Nu apar mutante rezistente în zona de inhibiție, deci substanțele nu exercită efect presor pentru selectarea de tulpini rezistente.
- Compuşii testați nu prezintă efect evident antifungic, antiviral, sporicid sau detoxifiant.
- Compuşii testați prezintă toxicitate mare, se încadrează în grupa “foarte toxici”, ca majoritatea substanțelor active medicamentoase.
- Comparativ cu seriile anterioare, amidele din seria a treia prezintă activitate antibacteriană superioară şi pot fi considerate ca potențiale medicamente antibacteriene, în special C3.5 şi C3.6.

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Pe această pagină referitoare la figuri, se va face o *listă* a tuturor numelor figurilor, în ordine numerică (la nevoie, cu legende explicative; identificați, în această legendă, orice literă sau săgeată utilizată pe figură).

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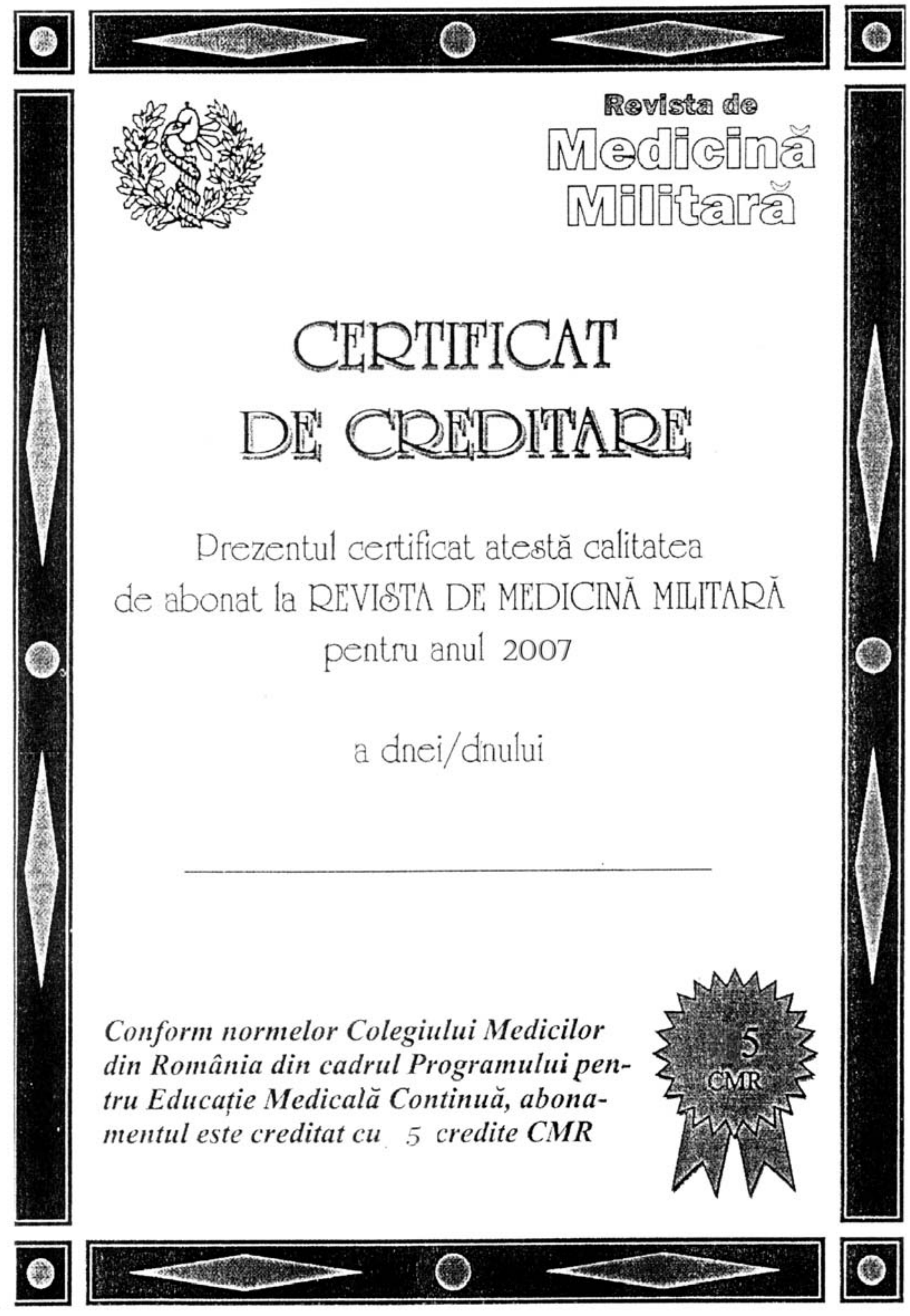

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DIN ROMÂNIA

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Aspecte particulare ale ischemiei miocardice aritmogene la pacienții cu angină pectorală stabilă

Lt.-col. şef lucrări dr. Liviu CHIRIAC, Col. şef lucrări Gabriel CRISTIAN, Lt.-col.dr. Romi BOLOHAN, Col.dr. Vasile MURGU, Dr. Mihai IACOB, Dr. Florina PINTE, Col. şef lucrări dr. Viorel GOLEANU, Col. şef lucrări Vasile GREERE, Dr. Dorin CÎRPACIU, Dr. Sorin DUMITRESCU, Col.dr. Ionel DROC, Gl.bg.(r) prof.dr. Ion ȚINTOIU, *Prof.univ.dr. Alexandru CÂMPEANU

Centrul Clinic de Boli Cardiovasculare al Armatei ,
***Spitalul Clinic Caritas Bucureşti**

Rezumat

***Obiectivul studiului.* Scopul studiului este de a stabili relația dintre datele clinice, electrocardiografice, ecocardiografice, angiografice la pacienții cu boală cardiacă ischemică (BCI) stabilă şi ischemie miocardică tranzitorie (IMT) asociată cu aritmii ventriculare potențial maligne (AVPM) – Complexe premature ventriculare – (CPV) cuplate, CPV > 10/oră, tahicardie ventriculară nesusținută (TV-NS).**

***Material şi metodă.* Lotul de studiu a fost alcătuit din 765 de bolnavi care aveau BCI asociată cu AVPM, investigați coronarografic şi ventriculografic. Toți pacienții prezentați au fost examinați clinic, electrocardiografic standard, test de efort ECG, monitorizare Holter, radiologic şi ecocardiografic.**

***Rezultate.* Tulburările de cinetică localizate pe peretele anterior al ventriculului stâng (scorul anterior) şi numărul segmentelor afectate sunt factori independenți de risc ai apariției AVPM. Profilul aritmogen al BCI stabile are corelate leziuni coronariene aterosclerotice severe extinse (tricoronariene), leziuni preferențial localizate la nivelul (trunchi principal stâng) TPS ≥ 50% şi ≥ 75% şi/sau în segmentul proximal al arterei descendente anterioare (ADA) ≥ 75% şi ≥ 95%, reprezentând factori independenți de risc ai apariției unor AVPM ($p < 0,02$ şi $p < 0,04$).**

***Concluzii.* Profilul aritmogen al BCI stabile este caracterizat de: leziuni tricoronariene, cu leziuni severe ≥ 75% la nivelul TCS şi ADA proximal, tulburări de cinetică parietală multiple interesând preferențial peretele anterior, infarct miocardic vechi, fracție de ejecție ventricul stâng (FEVS) scăzută, episoade ischemice repetate, prelungite şi importante care au alungit intervalul QTc > 450 ms.**

Cuvinte cheie: profilul aritmogen, ischemie miocardică tranzitorie, aritmii ventriculare potențial maligne, angină pectorală stabilă

Abstract

***Objectives.* The aim of study was to determine the relations between myocardic transitory ischemia, ventricular potential malign arrhythmia and angiocoronarographic, echographic, electrocardiographic data in patients (pts) with stabile coronary artery disease.**

***Methods.* 765 patients with stabile angina and ventricular potential malign arrhythmia were evaluated angiocoronarographic, echographic, standard ECG, Holter ECG, radiologic, and stress test.**

***Results.* Anomalies of wall motion preferential located on the anterior wall and number segments with contractility anomalies are independent risk factors for AVPM appearance. Arhytmogenic pattern of stabile CAD appeared at: 3 – vessel disease, LAD stenosis ≥ 75% and ≥ 95%, representing independent risk factors for AVPM appearance.**

***Conclusions.* Arhytmogenic pattern of stabile CAD is caracterisated by: tri-coronariane extensive lesions ≥ 75% preferential located LM and proximal LAD, multiple wall motion anomalies dominant localisated at anterior wall, prior myocardial infarction, low LVEF, important, prolonged ischemic episodes with increase of interval QTc > 450 ms.**

Introducere

Cercetările efectuate în ultimele decade au arătat că boala cardiacă ischemică (BCI) constituie o problemă majoră de sănătate în întreaga lume, fiind responsabilă de creşterea mortalităţii generale şi a morbidităţii extensive. Dacă în clasificarea BCI s-a individualizat şi o formă care se manifestă prin aritmii şi s-au efectuat numeroase cercetări privind fiziopatologia, diagnosticul şi tratamentul multiplelor tipuri clinice, aspecte ale relaţiei dintre aritmiile ventriculare şi BCI sunt neprecizate şi numeroase studii sunt în desfăşurare pentru clasificarea lor.

Obiectivul studiului

Studiul îşi propune să investigheze dacă există un "profil aritmogen" al bolii coronariane ischemice caracterizat prin asocierea – aritmii ventriculare potenţial maligne (AVPM), conform clasificării Morganroth (1) (CPV cuplate, CPV > 10/oră, TVNS), şi IMT depistate prin metoda Holter. Studiul va încerca să determine relaţia dintre datele clinice, electrocardiografice, ecocardiografice, angiografice la bolnavii cu IMT asociată cu AVPM. Studiul relaţiei dintre apariţia şi severitatea AVPM şi ischemia miocardică tranzitorie evaluată prin monitorizarea Holter, variabilitatea ritmului sinusal, durata, dispersia intervalului QT, potenţialele ventriculare tardive şi alţi markeri de risc ai apariţiei aritmiilor ventriculare.

Material şi metodă

În studiu au fost incluşi un număr de 765 de pacienţi care aveau APS asociată cu AVPM. Datele au fost obţinute pe baza unei "fişe de cercetare" special constituite şi care a inclus informaţii curente ale investigaţiilor efectuate în spital.

Criteriile de includere în lotul de studiu au fost: prezenţa ischemiei miocardice, diagnosticată clinic şi paraclinic (test de efort, monitorizare Holter, ecocardiografic), existenţa leziunilor aterosclerotice coronariene evidenţiate coronarografic, prezenţa AVPM, clasa funcţională NYHA I-II.

Criteriile de excludere din studiu au fost: sindrom coronarian acut, aritmii ventriculare maligne repetitive, prezenţa fibrilaţiei atriale, tratamentul concomitent cu substanţe aritmogene, valvulopatii, pecardite, cord pulmonar cronic, bolile congenitale, miocardite, cardiomiopatii dilatative documentate, noncomplianţa potenţială a pacientului.

Toţi bolnavii prezentaţi pentru AVPM (CPV cuplate, CPV > 10/oră, TV-NS) sau pentru angină pectorală au fost examinaţi clinic, electrocardiografic standard, test de efort ECG, Holter ECG, radiologic şi ecocardiografic la momentul iniţial în spital. Protocolul studiului a inclus 12 examinări clinice şi electrocardiografice, repetate periodic, urmărindu-se înregistrarea evenimentelor ischemice şi aritmice.

Analiza statistică

Datele obţinute au fost înregistrate într-un fişier (bază de date) în format *EPI INFO*.

Pentru compararea valorilor medii privind variabilele cantitative, s-a utilizat *testul "t" (testul "Student")*. Dacă valoarea calculată a testului a fost mai mică decât valoarea critică Tt (citită din tabele special întocmite pentru "distribuţia Student"), s-a considerat diferenţa dintre valorile medii ca statistic nesemnificativă, iar dacă valoarea calculată a testului a fost mai mare decât valoarea critică Tt citită din tabele (p-value < 0,05), s-a considerat diferenţa dintre valorile medii statistic semnificativă.

În cea de a doua situaţie, în funcţie de mărimile p-value au fost considerate cazurile: 0,01 < p-value < 0,05 – diferenţa este statistic semnificativă, 0,001 < p-value < 0,01 – diferenţa este statistic foarte semnificativă, p-value < 0,001 – diferenţa este înalt semnificativă.

Pentru măsurarea influenţei unor variabile considerate "variabile independente" sau "factori predictivi" asupra altor variabile considerate "variabile dependente" s-a utilizat *modelul regresiei logistice*.

Rezultate

Cei 765 de subiecți înrolați în studiu au fost monitorizați ECG continuu, ambulator, timp de 24 ore (metoda Holter), în medie la 6 luni, evidențiindu-se un grup de 333 bolnavi care au prezentat ischemie miocardică tranzitorie, dintre care 75 (29%) au avut AVPM. Caracteristicile bazale ale bolnavilor cu ischemie miocardică aritmogenă şi cu ischemie miocardică izolată sunt prezentate în tabelul nr.1. AVPM au apărut la 75 de bolnavi (29% din cazuri) cu episoade ischemice tranzitorii şi 258 de bolnavi (71% din cazuri) au prezentat episoade de ischemie miocardică fără să fie însoțite de aritmii ventriculare.

Tabelul nr.1. Caracteristicile bolnavilor din grupurile cu ischemie miocardică aritmogenă şi cu ischemie miocardică izolată

	Ischemie aritmogenă N = 75	Ischemie izolată N = 258
Vârsta (ani)	56,0 ± 9,3	58,0 ± 8,3
Sex (M/F)	52/23	172/86
Durata anginei pectorale stabile (luni)	31,4 ± 28,2	26,9 ± 31,7
Frecvența episoadelor anginoase (nr./săpt)	4,9 ± 6,3	3,6 ± 7,8
Consumul săptămânal de tablete de NTG	2,6 ± 4,9	2,3 ± 4,9
FC de repaus (bătăi/min)	79 ± 11,4	75 ± 9,5
TA sistolică (mm Hg)	134,5 ± 19,7	129,7 ± 23,5
TA diastolică (mm Hg)	85,2 ± 8,5	79,3 ± 8,5
Hipertensiune arterială (Nr.;%)	53 (70,2%)	184 (71,3%)
Diabet zaharat (Nr.;%)	29 (38,1%)	95 (36,9%)
Dislipidemie (Nr.;%)	32 (42,8%)	117 (45,2%)
Colesterolul total	219,3 ± 40,2	211,5 ± 42,5
LDL – colesterol	129,4 ± 31,4	127,6 ± 31,4
HDL – colesterol	41,9 ± 9,5	40,5 ± 9,8
Trigliceride	195,8 ± 62,4	197,9 ± 72,4

Numărul total de episoade ischemice înregistrate în 24 ore a fost de 529, *timpul total de ischemie* fiind de 2 144 minute. Cel mai scurt episod a durat 1 minut, iar cel mai lung, 48 de minute. Distribuția duratei şi a numărului episoadelor de ischemie/bolnav a fost variabilă: de la un episod ischemic aritmogen cu durata de 1 minut/24 ore, la un traseu cu 23 de episoade şi durata totală de ischemie de 48 minute.

Frecvența cardiacă medie la debutul episoadelor ischemice a fost de 106 ± 7 bătăi/minut la cei cu > 30 minute ischemie/24 ore şi de 90 ± 9 bătăi/minut la bolnavii cu < 30 minute ischemie/24 ore.

Ciclurile de ischemie miocardică aritmogenă au fost caracterizate de episoade scurte şi repetitive de subdenivelare a segmentului ST > 1 mm. În timpul crizelor anginoase tipice, 47% au asociat prelungirea duratei QRS şi subdenivelarea segmentului ST, în timp ce 65% dintre bolnavii cu angină pectorală stabilă au prezentat rareori prelungirea duratei QRS.

Episoade ischemice însoțite de aritmii ventriculare au un prim vârf de apariție la ora 6am, urmat de al doilea vârf la 1pm. Episoadele de ischemie fără aritmii ventriculare sunt predominante în prima şi în ultima parte a zilei (vârfuri la orele 6am, 2pm şi 10pm). Episoadele ischemice miocardice sunt de 2,6 ori mai frecvente la pacienții cu ischemie miocardică aritmogenă (8,5 ± 5,1 vs 3,2 ± 1,3)

Aritmiile ventriculare au apărut în timpul sau imediat după subdenivelarea de segment ST – grupul cu *aritmii ventriculare precoce* sau după normalizarea segmentului ST – grupul cu *aritmii ventriculare tardive.* Aritmii ventriculare au apărut tardiv la 46 bolnavi (61% din cazuri) şi precoce la 29 bolnavi (39% cazuri). *Aritmiile ventriculare precoce* au fost: CPV monomorfe ocazionale surprinse în timpul a 16 episoade ischemice, CPV monomorfe frecvente (>30/oră) apărute în 24 de episoade şi CPV polimorfe observate în 20 de episoade ischemice. Cupletele de CPV au apărut în 15 perioade ischemice, iar episoadele de tahicardie ventriculară susţinută au fost prezente la 21 de bolnavi. Fenomenul R/T a fost observat în 22 episoade ischemice.

Aritmiile ventriculare tardive care survin în timpul sau după perioada de normalizare a segmentului ST au fost: CPV < 30/oră în 32 episoade, CPV monomorfe >30/oră în 18 episoade şi polimorfe în 4 episoade ischemice. Cuplete de CPV au fost înregistrate în 35 de perioade ischemice şi TVNS în 34. Fenomenul R/T a apărut în 32 de episoade ischemice tardive.

Datele arată existenţa unei relaţii importante din punct de vedere statistic ($p<0,05$) între numărul arterelor coronariene cu stenoze semnificative şi incidenţa AVPM (de la 21% la unicoronarieni până la 45% la tricoronarieni), media CPV/oră (5,7 la unicoronarieni faţă de 48,1 la tricoronarieni).

Majoritatea bolnavilor cu ischemie miocardică aritmogenă au leziuni bi- şi tricoronariene, 53 bolnavi – 71% din cazuri. Aritmiile ventriculare complexe au fost mai frecvente la bolnavii cu stenoze tricoronariene (45% din cazuri) comparativ cu cei cu stenoze bicoronariene (24% din cazuri) şi unicoronariene (21% din cazuri) ($p < 0,01$). Procentul bolnavilor cu > 10 CPV/oră a crescut direct proporţional cu numărul arterelor coronare stenozate.

AVPM apar la 20% dintre bolnavii care au stenoze unicoronariene fără tulburări de contractilitate şi la 56% dintre cei care au tulburări de contractilitate, pentru ca la bolnavii cu leziuni tricoronariene AVPM să apară la 50% dintre cei care nu au tulburări de contractilitate şi la 85% dintre cei cu tulburări de contractilitate.

Rezultatele arată o relaţie strânsă între numărul segmentelor cu contractilitate miocardică anormală şi incidenţa CPV. CPV > 30/oră au fost prezente la 25% dintre bolnavii fără tulburări de contractilitate, la 44% care aveau afectate 1-4 segmente, la 77% la cei cu 5-10 segmente afectate şi la toţi bolnavii cu 11-17 segmente.

Indicele de scor (2) subdivizat într-un *scor anterior,* reprezentând mobilitatea pereţilor tributari distribuţiei arterei coronare descendente anterioare, şi un *scor posterior,* reprezentând mobilitatea segmentelor tributare teritoriului arterei circumflexe şi coronare drepte, a fost modificat diferit în cele două loturi studiate. Scorul anterior a fost crescut semnificativ ($p < 0,05$) în subgrupul cu ischemie miocardică aritmogenă (2,1 faţă de 1,5), pentru ca scorul posterior VS să fie crescut în subgrupul cu ischemie miocardică izolată (1,6 faţă de 1,8).

Durata episoadelor ischemice cardiace şi amplitudinea subdenivelării segmentului ST au fost mai mari în grupul bolnavilor cu ischemie miocardică aritmogenă (21,2 ± 15,1 minute vs 12,7 ± 5,7 minute) ($p < 0,05$) şi (2,5 ± 1,4 mm vs 1,8 ± 0,6 mm) ($p < 0,01$) ($p < 0,05$).

Antecedente de infarct miocardic au avut 40 de bolnavi (53% din cazuri) cu ischemie miocardică aritmogenă şi 85 de bolnavi (56,6% din cazuri) fără aritmii ventriculare. La bolnavii cu ischemie miocardică aritmogenă contractilitatea ventriculului stâng a fost anormală la 75% dintre bolnavii cu IM vechi faţă de 45% din cazurile fără antecedente de IM.

Derivaţia standard a tuturor intervalelor RR normale din 24 ore (SDNN) la bolnavii cu >30 CPV/oră a fost 44 ± 29 ms ($p < 0,05$) comparativ cu 72 ± 31 ms la cei cu 0 – 9 CPV/ora ($p < 0,05$), iar pNN50 la cei cu >30 CPV/oră a fost 9 ± 3 % versus 15 ± 6 % la 0 – 9 CPV/oră ($p < 0,05$).

În domeniul spectral VLF (*very low frequency*, 0,0033-0,04 Hz) a fost 935,3 ± 459,6 ms^2, la bolnavii cu > 30 ExV/oră faţă de 1 546,5 ± 1 377,6 ms^2 la bolnavii cu 0 – 9 ExV/oră, iar LF a urmărit tendinţa de scădere a acesteia la cei cu > 30 ExV/oră care a fost de 209,4 ± 291,8 ms^2 faţă de 339,6 ± 312,7 ms^2 la grupul cu 0 – 9 ExV/oră. Compararea valorilor medii ale HRV (*heart rate variability*) a arătat că aceste valori sunt semnificativ mai mari ($p < 0,01$) la bolnavii care au aritmii ventriculare precoce. Valorile medii ale LF (*low frequency*, 0,04-0,15 Hz) şi VLF sunt scăzute la bolnavii cu aritmii ventriculare tardive (918,3 ± 579,6 şi 209,4 ± 281,4 ms^2 vs 1 546,5 ± 1 377,6 şi

348,6 ± 323,3 ms^2) ($p < 0,05$), martore ale unei inbalanțe autonome datorate creşterii tonusului simpatic şi scăderii activității vagale.

Bolnavii cu stenoze semnificative coronariene în teritoriul TPS şi ADA care au aritmii ventriculare au prezentat reduceri importante ale tuturor componentelor HRV din domeniul timp şi prin analiza spectrală.

Parametri HRV în domeniul spectral la bolnavii cu ischemie miocardică aritmogenă VLF au fost de 1 546,5 ± 1 326,3 ms^2 şi de 1 846,5 ± 1 574,1 ms^2 la cei cu ischemie miocardică izolată, iar valorile LF au fost 348,3 ± 265,7 ms^2 versus 643,6 ± 342,5 ms^2, diferențele fiind semnificative din punct de vedere statistic ($p<0.01$).

SDNN şi SDANN (derivația standard a mediei duratei medii a intervalelor RR calculate pe segmente de înregistrare de câte 5 minute) au fost semnificativi mai scăzuți ($p < 0,01$) în grupul bolnavilor cu ischemie miocardică aritmogenă (61 ± 31 ms vs 81 ± 55 ms) şi (48 ± 28 ms vs 52 ± 34 ms).

Bolnavii cu stenoze semnificative coronariene în teritoriul trunchiului coronar stâng şi al arterei coronare descendente anterioare din lotul cu aritmii ventriculare ischemice au prezentat o reducere semnificativă a tuturor componentelor domeniului spectral .

Media intervalelor QTc a fost 441 ± 4 ms în timpul episoadelor ischemice tranzitorii, valoare similară cu intervalul QTc înregistrat în afara perioadelor ischemice tranzitorii ($p < 0,05$).

Media intervalului QTc a fost de 441,5 ± 5 ms la unicoronarieni, 442,2 ± 6 ms la bicoronarieni şi 444,9 ± 7 ms la tricoronarieni ($p<0.05$).

În timpul episoadelor ischemice tranzitorii se evidențiază o creştere semnificativă a mediei intervalelor QTc la pacienții cu ischemie miocardică aritmogenă (447,54 ± 5 ms versus 442,54 ± 6 ms), ($p<0.01$).

Rezultatele obținute evidențiază că atât intervalul QT, cât şi dispersia QT sunt crescute la pacienții ce prezintă ischemie miocardică aritmogenă, precum şi la cei cu funcție ventriculară stângă redusă. Dispersia QT a fost de 51,14 ms la bolnavii cu ischemie miocardică aritmogenă şi de 45,75 ms la cei cu ischemie miocardică izolată ($p < 0,01$).

Dispersia QT la bolnavii cu stenoze TCS ≥ 75%, ≥ 95% şi stenoze LAD în segmentul proximal ≥ 75%, ≥ 95% este semnificativ mai mare ($p<0.01$) față de dispersia în celelalte grupe, RCA şi LCX.

Evaluarea prin analiza de regresie logistică a relației dintre ischemia miocardică (aritmogenă sau izolată) şi durata episoadelor ischemice, scorul anterior de kinetică ventriculară, amplitudinea subdenivelării segmentului ST, infarct miocardic, VLF, LF, leziuni ADA, intervalul QTc şi dispersia QT au arătat că factorii analizați au o capacitate predictivă a bolii cardiace ischemice de 74,2%, mai puțin pentru cei cu forme asociate cu aritmii ventriculare (61,3% vs 80,6%) (tabelul nr.2).

Tabelul nr. 2. Analiza de regresie logistică

Observație			
	Ischemie miocardică		Procente corecte
	Aritmogenă	Izolată	
Step 1 Ischemie aritmogenă			
Izolată	46	29	61.3%
Procent total	29	121	80.6%
			74.2%

Analiza exponențială a coeficienților de regresie, de ischemie, contractilitate miocardică, VRS, QT şi PTV arată că o creştere a duratei episoadelor ischemice, a amplitudinii subdenivelării segmentului ST, stenoze LAD proximal ≥ 75% şi prezența infarctului miocardic vechi determină o creştere a şansei de a avea ischemie miocardică aritmogenă (tabelul nr.3). Durata episoadelor ischemice pare a avea cea mai mare şansă de ischemie miocardică aritmogenă crescând cu un factor de 3 465 în cazul adăugării unei unități la durata episoadelor ischemice.

Tabelul nr. 3. Regresia logistică a semnificației leziunilor coronariene, contractilității peretelui ventricular şi potențialelor tardive

	B	S.E.	Wald	Df	Sig.	Exp(B)
Step 1* Dur Ep	1.243	.137	.542	1	.012	3.465
Subdeniv ST	.032	.281	.032	1	.045	1.032
LAD proximal ≥ 75%	.583	.833	.798	1	.034	1.791
IM	-.831	.854	1.321	1	.012	3.435
VLF	.432	.035	3.456	1	.161	1.540
ScAnterior	1.961	.053	1.953	1	.051	7.106
VLF	.952	.091	.654	1	.342	2.590
QTc	-.453	.324	4.090	1	.402	0.635
QT	1.231	.134	3.675	1	.231	3.424
FEVS	.073	.421	1.623	1	.356	1.856
VLF	.776	.045	.654	1	.049	.887
SDNN	.412	.071	3.456	1	.171	2.572
Constant	.068	.288	.466	1	.032	.943

* Variabilele intrate în treapta 1: DurEI, SegST, LAD, IM, ScAnt, VLF, VF, QTc,

Rezultatele obținute în acest studiu au arătat că la monitorizarea Holter aritmiile ventriculare complexe apar semnificativ mai frecvente (8,5 vs 3,2, p<0,05) la bolnavii cu episoade de ischemie tranzitorie mai severă (subdenivelare segment ST 2,5 mm vs 1,8 mm; p < 0,01) şi cu durată mai lungă (21,2 min vs 12,7 min; p < 0,01).

Discuții

Leziunile coronariene aterosclerotice severe extinse (tricoronariene) sau localizate preferențial la nivelul TPS ≥ 50% şi ≥ 75% şi/sau în segmentul proximal al ADA ≥ 75% şi ≥ 95% reprezintă conform datelor obținute în studiu *factori independenți de risc* ai apariției unor aritmii ventriculare potențial maligne (p < 0,02 şi p < 0,04). *Califf şi colab.* au arătat că leziunea critică proximală , înainte de prima septală, a ADA a fost asociată cu o supraviețuire la 5 ani de 90% în comparație cu 98% la bolnavii cu leziuni distale. Supraviețuirea la 5 ani a bolnavilor cu leziune izolată de RCA a fost mai crescută (96%) decât la bolnavii cu leziune izolată de ADA (92%)[1].

Rezultatele studiului arată că AVPM apar semnificativ mai frecvent la bolnavii cu *tulburări de cinetică localizate pe peretele anterior al ventriculului stâng,* scorul anterior şi numărul segmentelor afectate reprezentând factori independenți de risc ai apariției aritmiilor ventriculare complexe. Între amploarea *tulburărilor de cinetică* şi incidența AVPM există o relație direct proporțională, conform datelor obținute.

Datele obținute sunt concordante cu recomandările actuale ale *ghidurilor ACC/AHA pentru criteriile de stratificare a riscului* la bolnavii cu BCI cronică, evidențierea de tulburări de contractilitate la mai mult de două segmente reprezentând un criteriu încadrat în categoria de risc înalt, cu rată a mortalității anuale mai mare de 3%.

Datele comunicate în literatură sunt contradictorii în ceea ce priveşte rolul predictiv aritmic al frecvenței CPV la bolnavii cu BCI cronică, dar cele mai multe studii evidențiază frecvența CPV > 10/oră ca un prag cu risc aritmic crescut. Mai mulți investigatori au subliniat că cel mai puternic factor predictiv aritmic la bolnavii cu BCI cronică este TVNS, relație contestată în prezent[1].

Semnificația TVNS ca factor predictiv de risc depinde de substratul lezional, fiind notabilă pe fond de necroză miocardică recentă şi prezența de cicatrici miocardice cu sau fără anevrism de VS. La pacienții coronarieni, importanța prognostică a TVNS depinde în primul rând de proximitatea față de perioada infarctului miocardic[2].

Conform datelor obținute în acest studiu, modificările tonusului autonom vegetativ sunt accentuate la bolnavii cu ischemie miocardică tranzitorie aritmogenă, evidențiindu-se o creştere semnificativă ($p < 0,01$) a duratei şi dispersiei intervalului QT. Scăderea semnificativă a parametrilor din domeniul spectral şi de timp a HRV, observată în prezentul studiu, nu a relevat însă o relație semnificativă statistic prin analiza de regresie logistică între modificările HRV analizate ca factori independenți şi şansa independentă de apariție a aritmiilor ventriculare complexe.

Conform studiului publicat de *Rotterdam Investigators,* s-a demonstrat că dispersia QT este un factor de risc independent pentru moartea cardiacă de cauză multicoronariană şi un predictor independent de aritmie postinfarct miocardic. *Trusz-Gluza şi colab.* au observat că la bolnavii coronarieni intervalul QT are un rol de predictor aritmic, fiind un marker cert al dispersiei repolarizării ventriculare[3]. Totuşi QTd nu este acceptată în nici un algoritm de evaluare a riscului aritmic sau are recomandări relativ controversate din cauza unor studii negative şi a numeroaselor sale probleme de natură metodologică şi tehnică .

Bigger şi Lombardi au arătat că valoarea predictivă a scăderii variabilității frecvenței cardiace rezultate din analiza spectrală este semnificativă la bolnavii postIMA (4,5), cei mai puternici predictori fiind valorile scăzute din regiunile ULF şi VLF, dar este controversată la bolnavii cu angină pectorală stabilă.

Concluzii

Corelarea datelor clinice, ecografice, electrocardiografice şi angiografice permite să se contureze o *formă aritmogenă a BCI stabile,* caracterizată prin: leziuni coronariene extensive *tricoronariene*, cu leziuni severe ≥ 75%, localizate preferențial *TPS şi LAD proximal*, *tulburări de cinetică parietală multiple, localizate dominant pe peretele anterior*, *antecedente de infarct miocardic* şi *disfuncție ventriculară şi FEVS scăzută.*

Rezultatele au arătat mecanismul complex al aritmiilor ventriculare ischemice, cu modificarea *refractarității miocardului ischemic* şi *scăderea activității vagale,* evidențiind rolul: *creşterii duratei şi dispersiei intervalului QT, scăderii parametrilor spectral şi timp dependenți HRV, duratei şi severității ischemiei miocardice.*

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O carte diferită de tiparele obişnuite:
Echinococoza pluriviscerală.
Chirurgia localizărilor toraco-abdominale

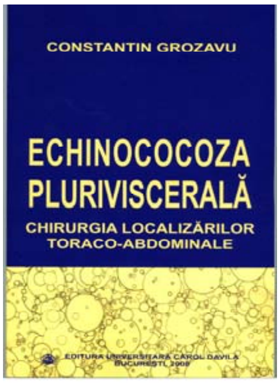
CONSTANTIN GROZAVU
ECHINOCOCOZA PLURIVISCERALĂ
CHIRURGIA LOCALIZĂRILOR TORACO-ABDOMINALE
EDITURA UNIVERSITARĂ CAROL DAVILA BUCUREŞTI 2008

Colonelul dr. Constantin Grozavu este şeful Secției clinice de Chirurgie Toracică din SCUMC, medic primar chirurgie toracică şi de chirurgie generală, doctor în ştiințe medicale, absolvent a peste 15 cursuri de pregătire postuniversitară, membru fondator al Societății Române de Chirurgie Toracică, membru al Asociației Medicilor şi Farmaciştilor Militari din România, membru al Societății Europene a Chirurgilor Toracici. O impresionantă carte de vizită, care relevă multă pasiune şi dăruire profesională. Doctorul Grozavu este însă şi un iubitor al cuvântului scris. Stau mărturie numeroasele sale colaborări la revistele de specialitate (peste 130 de lucrări publicate sau comunicate în țară sau străinătate) şi, mai nou, lucrarea *Echinococoza pluriviscerală. Chirurgia localizărilor toraco-abdominale*, apărută de curând (la finele anului 2008) în Editura universitară “Carol Davila”.

Studiul, desfăşurat pe o perioadă de şapte ani, este urmarea atenției speciale pe care autorul a acordat-o încă de la începuturile carierei sale chirurgicale bolii hidatice şi cuprinde un număr important de cazuri de echinococoză pluriviscerală diagnosticate şi operate în Clinica de Chirurgie Toracică din SCUMC. În absolut toate cazurile, autorul se regăseşte ca operator sau ca asistent operator, participând efectiv la îngrijirea şi urmărirea postoperatorie a pacienților.

Lucrarea este structurată în trei capitole. Inițial sunt abordate aspecte privind etiologia şi patogenia bolii prin prisma materialului clinic (84 de cazuri de echinococoză pluriviscerală), localizările echinococozei pluriviscerale şi clasificarea în funcție de compartimentul anatomic sau organul în care aceasta este dispusă. Este definită pentru prima dată în literatura de specialitate noțiunea de echinococoză pluriviscerală.

Capitolul al doilea este dedicat modalităților de diagnostic, în cadrul cărora examenul imagistic rămâne în continuare definitoriu, evidențiindu-se posibilitățile TC şi RMN, metode mai nou apărute, care ajută în mod real la diagnosticul pozitiv şi îndeosebi la diagnosticul diferențial al afecțiunii. În finalul capitolului autorul propune un algoritm de diagnostic plecând de la descoperirea unei echinococoze într-unul dintre cele două mari compartimente, toracic şi abdominal.

Capitolul trei se referă la tratamentul bimodal, medico-chirurgical, al echinococozei, considerat ca fiind atitudinea optimă, cu rezultatele cele mai bune. Sunt prezentate procedurile şi tehnicile operatorii, modalitățile de abord, evaluate prin filtrul experienței autorului, inclusiv o contribuție personală a acestuia de abord plecând de la un procedeu clasic. Studiul materialului clinic se încheie cu analiza mortalității şi morbidității postoperatorii.

Desigur, descrierea într-o manieră exhastivă, complexă, a echinococozei pluriviscerale ar presupune contribuția unor specialişti din mai multe domenii: chirurgie generală, chirurgie toracică, chirurgie cardiacă, neurochirurgie, ortopedie, parazitologie etc.

Doctorul Grozavu s-a oprit în lucrarea sa doar la localizările toraco-abdominale. Realizarea sa reprezintă doar un punct de reper pentru moment, o abordare ce poate fi continuată, aşa cum am spus, cu participarea şi a altor specialişti. Chirurgii care se confruntă cu această patologie vor găsi în prezenta carte un ghid valoros pentru activitatea lor practică, cu o iconografie sugestivă (69 de figuri), documente imagistice şi fotografii originale intraoperatorii sau necroptice care explicitează expunerea. O bibliografie pertinentă, ce cuprinde titluri (269) apărute într-un interval de timp ce se întinde din anii 60 până în prezent, ne oferă încă o dovadă a profesionalismului cu care autorul a tratat tema enunțată, oferind cititorului, aşa cum se specifică în prefața lucrării, “o carte cu un subiect ieşit din comun, total diferit de tiparele banale atât de obişnuite”.

Conf.univ.dr. Iulian BREZEAN

Ablația tumorală cu microunde a tumorilor pulmonare maligne – opțiune terapeutică nouă în tratamentul neoplasmelor pulmonare

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Rezumat. Deşi în prezent principalele metode de tratament al tumorilor maligne sunt de natură chirurgicală, chimioterapică şi radioterapică, o serie întreagă de tehnici miniinvazive ghidate imagistic, de tratament local tumoral, este acum accesibilă.

Ablația tumorală se defineşte prin inactivarea tumorală sau distrucția substanțială a tumorii prin aplicare intratumorală de substanțe chimice (etanol) sau energie termică (crioterapie, radiofrecvență, laserterapie).

Ablația tumorală cu microunde reprezintă cea mai recentă achiziție în acest domeniu. Prin această tehnică, abordul tumoral se poate face percutanat, laparoscopic sau chirurgical deschis.

Avantajele ablației tumorale folosind tehnici de ghidaj imagistic (ecografic, CT) constau în: morbiditate şi mortalitate scăzute, costuri scăzute ale procedurii, eliminarea posibilității de a interfera cu alte metode de tratament ale cancerului, posibilitatea de a fi repetate.

Cuvinte cheie: cancer bronhopulmonar, ablație tumorală in situ, microunde

Abstract. While surgery, radiation oncology, and systemic chemotherapy are still the three main pillars of tumor therapy, a broad range of minimally invasive, image-guided techniques for local tumor treatment is now available.

Thermal tumor ablation is a rapidly expanding treatment option for neoplasms of many organs, including the lung, liver, bone, and kidney. Tumor ablation has gained considerable attention due to its minimally invasive nature and the large number of patients who are not candidates for potentially curable surgical resection because of comorbidities or tumor number, size, or location. Microwave ablation (MWA) is a new thermal ablative technique that uses electromagnetic energy to produce coagulation necrosis. Microwave ablation shares many of the advantages of radiofrequency ablation but has additional theoretic advantages that may offer better and more consistent destruction of tumors. Microwaves have a much broader field of power density and a correspondingly larger zone of active heating. This may allow for more uniform tumor killing within a targeted zone and, more importantly, in perivascular regions.

Introducere

Ablația tumorală prin microunde – principii fizice

Microundele sunt radiații electromagnetice cu frecvențe cuprinse între 950-2450 MHz, cu spectru de acțiune ce se situează între cel al undelor infraroşii şi cel al undelor radio. Microundele acționează la nivelul moleculelor de apă intracelulare(din celulele tumorale) şi induc moartea celulară (necroza de coagulare) prin căldura degajată de mişcările de frecare produse la acest nivel.

Ablația tumorală prin microunde – avantaje

Ablația tumorală prin microunde are aceleaşi beneficii ca şi ablația tumorală prin radiofrecvență, dar oferă şi alte avantaje:

- temperaturi ridicate degajate intratumoral;

- timp de ablație scăzut;
- ablația tumorilor cu dimensiuni mai mari;
- posibilitatea de a folosi mai multe aplicatoare;
- repetitivitatea procedurii;
- durere postproceduralå de mică intensitate;
- procedura se poate aplica pe tumorile restante după chimio/radioterapie.

Ablația tumorală prin microunde – indicații

Ablația tumorală cu microunde a fost folosită cu succes, cercetată şi aplicată în tumorile hepatice. În acest sens există nenumărate studii clinice pe loturi de pacienți. În ultima perioadă ablația tumorală cu microunde îşi găseşte aplicabilitate şi în neoplasmele pulmonare. Pentru aceste localizări indicațiile de ablație sunt bine precizate şi efectuate în servicii de radiologie şi oncologie intervențională, în această echipă fiind cooptat şi chirurgul toracic. Indicațiile sunt următoarele:

- pacienți cu risc mare (comorbidități asociate, rezervă funcțională cardiorespiratorie scăzută);
- pacienți care refuză intervenția chirurgicală;
- metastaze pulmonare;
- recurența bolii după alte metode de tratament: radio/chimio-terapie.

Ablația tumorală prin microunde – tehnică

- acces percutanat, laparoscopic sau chirurgical;
- accesul percutanat – pacient sedat (midazolam, fentanil), rar anestezie generală;
- monitorizare puls, oximetrie, tensiune, ecg;
- anestezie locală cu xilină 1%;
- localizare precisă a tumorii prin examinare CT;
- inserția intratumorală a antenei sub ghidaj CT;
- expunerea tumorii la microunde (45 W, 915 MHz).

Prezentare de caz

Prezentăm cazul unui pacient de 83 de ani, internat în Clinica de Chirurgie Toracică SCUMC, fost fumător (50 PA), cu patologie cardiacă cunoscută: CMD ischemic, HTA, ateromatoză aortică şi carotidiană. Pacientul este asimptomatic din punct de vedere pulmonar. Radiografia toracică (solicitată de cardiolog) evidențiază opacitate imprecis delimitată, situată în câmpul pulmonar superior drept. Examen CT torace: tumoră pulmonară lob superior drept cu invazie limitată a pleurei parietale.

Probe funcționale ventilatorii normale.

Pacientul refuză intervenția chirurgicală

Având în vedere vârsta înaintată şi multiplele tare asociate, precum şi refuzul pacientului privind intervenția chirurgicală de rezecție pulmonară, de comun acord s-a hotărât efectuarea metodei de ablație tumorală cu microunde.

Intervenția s-a efectuat sub ghidaj CT, cu anestezie locală la nivelul spațiului 4 pe linia axilară anterioară dreaptă. Se inseră intratumoral antena, vârful acesteia poziționându-se la marginea internă a formațiunii tumorale (fig.1, fig.2). După cuplarea antenei la generator, se iradiază tumora cu microunde cu o putere de 45 W şi o frecvență de 915MHz, timp de 10 minute. În timpul procedurii, pacientul a fost monitorizat: alura ventriculară, tensiune arterială, oximetrie. După terminarea expunerii la microunde, pacientul se transferă pe secție, fără a prezenta vreo complicație imediată.

Evoluția postprocedură începând din a doua zi a fost marcată de: microhemoptizii ce au durat două zile şi care nu au necesitat tratament, subfebrilitate în primele 3 zile, pentru care s-au administrat antibiotice cu spectru larg, dureri de intensitate mică/medie în regiunea hemitoracelui drept pe linia axilară medie. S-au administrat analgetice uzuale. Reluarea activității zilnice din ziua a doua.

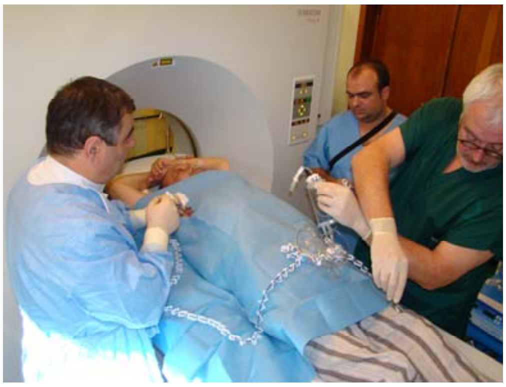

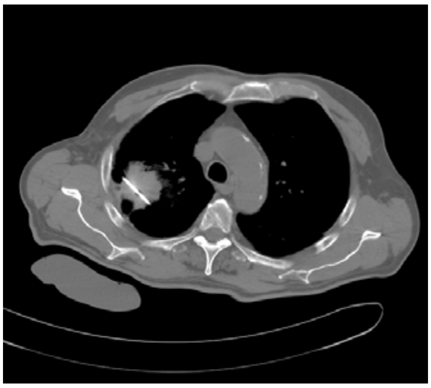

Fig. 1, fig. 2. Inserția antenei, sub ghidaj CT, în tumora pulmonară.

Fig. 3. Verificarea poziției sub ghidaj CT de către echipa operatorie.

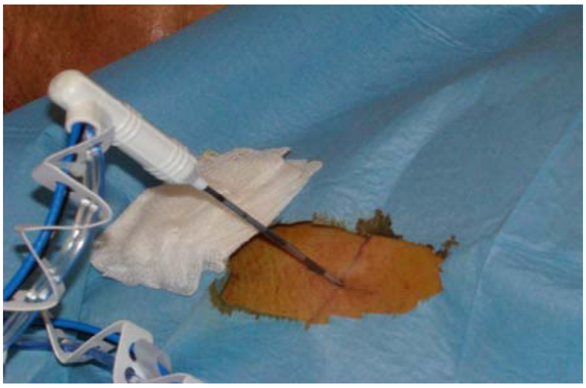

Fig. 4. Abordul percutanat al tumorii pulmonare

Evaluare imagistică postprocedurală:

- Examen CT torace prima zi (fig. 5): formațiunea tumorală din lobul pulmonar superior drept apare mai neomogenă şi discret mai mică, congestie difuză perilezională; mic pneumotorax local; discrete fenomene congestive şi în segmentul Fowler drept. Fără colecții hematice.
- Dimensiuni anterior ablației – 4,9/3,7 cm.
- Dimensiuni postablație – 4,6/3,7 cm.

Examen radiologic la externare: imagine nemodificată față de CT postablație.

Evaluarea tomografică la 30 de zile arată scăderea dimensiunilor tumorale şi prezența unui traiect transtumoral realizat de antena aparatului (fig. 6).

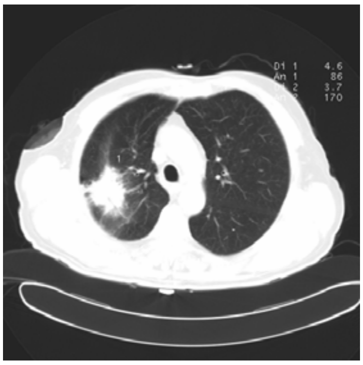

Fig 5. Control imagistic postoperator la 24 ore

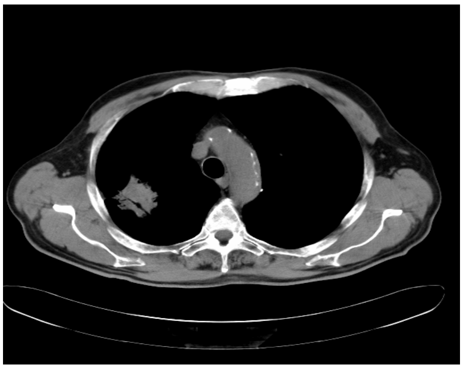

Fig 6. Control imagistic postoperator la 30 de zile

O mare parte dintre pacienţii cu tumori pulmonare (primare sau secundare) nu pot beneficia de intervenţie chirurgicală datorită tarelor asociate (cardiace, respiratorii, vârstă). Tratamentul convenţional la aceşti pacienţi constă în chimioterapie şi radioterapie. Datorită faptului că mulţi pacienţi se prezintă în stadii avansate ce nu pot beneficia de tratament chirurgical, tratamentul paleativ simptomatic la aceştia are un rol bine determinat. Ablaţia tumorală cu microunde a tumorilor pulmonare maligne este o procedură relativ sigură la aceşti pacienţi taraţi, ce creşte rata de supravieţuire şi controlul local al tumorii şi ameliorează simptomatologia.

Ablaţia tumorală cu microunde sub ghidaj CT s-a efectuat pentru prima dată în România în Spitalul Clinic de Urgenţă Militar Central, în Secţia Clinică Chirurgie Toracică şi Clinica de Radiologie, Imagistică Medicală şi Medicină Nucleară. Ea reprezintă o premieră medicală. Întrucât metoda este la început în România şi neputând trage concluzii pertinente, vă prezentăm un studiu realizat de departamentul de radiologie intervenţională din Rhode Island hospital (USA) pe 50 de pacienţi la care s-au efectuat 82 proceduri de ablaţie tumorală cu microunde.

Studiul a urmărit evoluţia tumorilor pulmonare după ablaţia cu microunde, prin evaluare CT la 1, 3, 6 luni. Aspectele CT au fost polimorfe, similar şi simptomatologia clinică. Cel mai frecvent aspect al tumorii a fost de "sticlă mată", diametrele iniţial crescute, ulterior în scădere. În 52% din cazuri a fost prezent aspectul de cavitaţie, îngroşare pleurală în 44%, retracţie pleurală în 8%, pleurezie minimă în 30%, pneumotorax în 39% din cazuri. Doar o treime dintre pacienţi au necesitat pleurotomie minimă postprocedură. Un număr mic din pacienţi au prezentat arsuri tegumentare (3%) şi dureri toracice (2%). A fost descris un sindrom postablaţie care constă din tuse productivă cu microhemoptizii, febră, mialgii, pentru care s-a administrat tratament antialgic şi antitusiv 3-4 zile. Un singur pacient (cu radioterapie şi vasculită secundară) a dezvoltat o suprainfecţie cu *stafilococus aureus,* care a dus în final la un sindrom ARDS, pentru care a necesitat terapie intensivă. Mortalitatea în primele 30 de zile postprocedură de ablaţie a fost zero.

Având în vedere tehnica relativ facilă a acestei proceduri, indicaţiile precise, considerăm că ea se va impune pe plan mondial ca o alternativă de tratament paleativ, alături de celelalte metode terapeutice, în terapia cancerului pulmonar.

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Particularități ale diseminărilor peritoneale în adenocarcinoamele pancreatice

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Rezumat. Carcinomatoza peritoneală reprezintă diseminarea locală/regională a neoplaziilor digestive, ginecologice sau a altor forme de cancer cu sau fără dovada existenței metastazelor sistemice. Pattern-ul carcinomatozelor este controlat de o serie de factori, dintre care proeminenți sunt cei fizici (locul tumorii primare, prezența sau absența ascitei, prezența peristalticii, absorbția lichidului peritoneal, gravitația, hidrodinamica lichidului intraperitoneal, vâscozitatea şi volumul fluidului) şi de o mai mică importanță sunt cei biologici (agresivitatea cancerului). Locurile cele mai frecvente de recurență după neopaziile tratate chirurgical sunt patul tumoral (50-100%) şi suprafețele peritoneale (20-70%) (15), efect ce poate fi explicat prin ipoteza "tumor cell entrapment". Am analizat 17 cazuri de neoplasm de pancreas. Dintre acestea 15 au avut localizare cefalică, 13 fiind neoperate iar două sunt cazuri prezentate la control după duodenopancreatectomie cefalică. S-a urmărit determinarea distribuției peritoneale a implantelor metastatice şi cuantificarea indexului neoplazic peritoneal (PCI), pe baza examinărilor imagistice şi explorării intraoperatorii prin laparoscopie sau laparotomie. Am stabilit distribuția şi redistribuirea carcinomatozelor în funcție de localizarea neoplaziei, sexul pacienților, intervenția chirurgicală şi condițiile patologice concomitente.

Cuvinte cheie: carcinomatoză peritoneală, adenocarcinom pancreatic, cancer peritoneal

Abstract. Peritoneal carcinosis is a local/regional dissemination of digestive malignancies, gynecological maloignancies or other forms of cancer with or without proof of the existence of systemic metastasis.

The pattern is controlled by several factors, prominent among which are the physical (instead of the primary tumor, presence or absence of ascites, peristaltics, peritoneal fluid absorption, gravity, intraabdominal liquid) and of more minor importance are the biological factors (aggressiveness of cancer).

We analyzed 17 cases of neoplasm of the pancreas. Sought to determine the distribution of peritoneal metastatic implants and quantification of peritoneal cancer index (PCI), based on imaging examinations and by surgical exploration (laparoscopic or laparotomy). We determined the distribution and redistribution in such conditions depending on tumor location, gender of patients, surgical intervention and concomitant pathological conditions.

Introducere

Carcinomatoza peritoneală reprezintă împrăştierea locală/regională a neoplaziilor digestive, ginecologice sau a altor forme de cancer cu sau fără dovada existenței metastazelor sistemice (1, 2).

Carcinomatoza peritoneală din tumorile gastrointestinale are o incidență între 13% şi 30% dintre pacienți (3). Dintre aceştia, aproximativ 60% prezintă metastaze sincrone şi 40% sunt metacrone. Cea mai comună cauză de prezentare este ascita sau ocluzia intestinală.

Suleiman (4) indică o apartenență a maselor peritoneale, în cazul pacienților solicitați pentru explorare imagistică, de carcinom ovarian în majoritatea cazurilor (46%), urmată de carcinomul colonic (31%), carcinom pancreatic (12%), carcinom de sân (4%) şi tumori de cauză primară necunoscută (8%). Locațiile principale de detectare a metastazelor peritoneale au fost marele oment, spațiul subfrenic drept, fundul de sac Douglas şi suprafețele diafragmului.

Pattern-ul de instalare al carcinomatozei este dependent şi de tipul neoplaziei.

Adenocarcinoamele nemucinoase ale tractului gastrointestinal au un pattern dependent de penetrarea tumorii până la nivelul seroasei. În acest moment se eliberează celule neoplazice care aderă imediat de peritoneu şi se dezvoltă în vecinătatea tumorii primare. Ar avea deci un pattern caracterizat ca din aproape în aproape (3). Ca atare, cancerele gastrice, pancreatice, colonice şi rectale neascitogene implică frecvent structurile aflate în imediata lor vecinătate, cum ar fi colonul, intestinul subțire şi marele oment. Metastazele peritoneale la distanță sunt rare în absența ascitei, în particular la nivelul etajului abdominal superior. Pattern-ul carcinomatozelor este controlat de o serie de factori, dintre care proeminenţi sunt cei fizici (locul tumorii primare, prezenţa sau absenţa ascitei, prezenţa peristalticii, absorbţia lichidului peritoneal, gravitaţia, hidrodinamica lichidului intraperitoneal, vâscozitatea şi volumul fluidului) şi de o mai mică importanţă sunt cei biologici (agresivitatea cancerului) (3).

Locurile cele mai frecvente de recurenţă după neoplaziile tratate chirurgical sunt patul tumoral (50-100%) şi suprafeţele peritoneale (20-70%)(15), efect ce poate fi explicat prin ipoteza "tumor cell entrapment".

Neoplasmele maligne ale stomacului, colonului, pancreasului şi ovarului care penetrează dincolo de marginile acestor organe pot disemina direct în lungul suprafeţelor viscerelor peritoneale adiacente şi pot implica alte structuri. Propagarea neoplazică în lungul suprafeţelor peritoneale poate cuprinde intestinul la distanţă de tumora principală. Mezocolonul transvers poate servi drept cale majoră de difuzare de la stomac, colon şi pancreas.

Meyers aduce în discuţie date interesante legate de compartimentarea peritoneală şi de comportamentul ligamentelor şi mezourilor, care rămân interconectate, drept căi de propagare a maladiilor. Reflecţiile peritoneale în abdomenul superior cuprind 11 elemente, ligamente şi mezentere: ligamentul coronar, gastrohepatic (omentul mic), hepatoduodenal, falciform şi gastrocolic, mezocolonul transvers, ligamentele duodenocolic, gastrosplenic, splenorenal, frenicocolic şi mezenterul intestinului subţire. Ţesuturile areolare din aceste structuri conţin vase de sânge şi limfatice. Aceste structuri realizează continuitatea unor planuri anatomice care permit diseminarea proceselor patologice între spaţiile peritoneale, cele extraperitoneale şi realizează conexiunile între structurile peritoneale şi cele extraperitoneale. De exemplu, micul oment justifică prezenţa unor determinări ale cancerului fundusului şi corpului gastric în lobul stâng hepatic. Prin ligamentul hepatoduodenal se pot extinde tumori retroperitoneale la ficat şi invers (5).

Inserţia mezocolonului transvers pe pancreas duce la extinderea leziunilor neoplazice pancreatice spre porţiunea inferioară a mezocolonului transvers. Continuitatea ligamentelor gastrosplenic şi splenorenal permite transmiterea maladiilor de la stomac spre splină şi coada pancreasului. Ligamentul frenicocolic stâng este în legătură cu splina, ligamentul splenorenal şi mezocolonul transvers şi poate explica interesarea unghiului splenic al colonului în cancerul cozii pancreasului.

După *Suk Kim* atât capsula Glisson, cât şi teaca Glisson se continuă cu spaţiul subperitoneal şi cu ligamentele gastrohepatic şi hepatoduodenal. Ca urmare, extinderea maladiilor, inclusiv a celei neoplazice, se poate face nu numai între structurile extraperitoneale, ci şi între cele extraperitoneale şi cele intraperitoneale. Ca urmare se poate stabili o cale de comunicare între retroperitoneu şi peretele abdominal anterior prin intermediul ficatului (6).

Material şi metodă

Am analizat 17 cazuri de neoplasm de pancreas. Dintre acestea 15 au avut localizare cefalică, 13 fiind neoperate, iar două sunt cazuri prezentate la control după duodenopancreatectomie cefalică. S-a urmărit determinarea distribuţiei peritoneale a implantelor metastatice şi cuantificarea indexului neoplazic peritoneal (PCI) (7), pe baza examinărilor imagistice şi explorării intraoperatorii prin laparoscopie sau laparotomie.

Am urmărit distribuţia şi redistribuirea carcinomatozelor în funcţie de localizarea neoplaziei, sexul pacienţilor, intervenţia chirurgicală şi condiţiile patologice concomitente.

PCI combină cantitativ distribuţia tumorală în 13 cadrane predefinite ale abdomenului şi pelvisului cu un scor lezional. Se stabilesc două planuri transverse şi două planuri sagitale care delimitează nouă regiuni abdominale. Planul transvers superior este localizat la marginea inferioară a rebordului costal, iar cel inferior este la nivelul spinei iliace anterosuperioare.

Planurile sagitale împart abdomenul în trei sectoare egale. Se evidenţiază astfel nouă compartimente, ce se numerotează în sensul acelor de ceasornic, 0 fiind la nivelul ombilicului şi 1 la nivelul spaţiului subdiafragmatic drept. Sunt numerotate astfel, 0-centrul abdomenului, 1-spaţiul drept superior (hipocondrul drept), 2-epigastrul, 3-spaţiul stâng superior (hipocondrul stâng), 4-flancul stâng, 5-spațiul stâng inferior (fosa iliacă stângă), 6-pelvis, 7-spațiul drept inferior (fosa iliacă dreaptă), 8-flancul drept. Regiunile de la 9 la12 separă intestinul subțire în jejun superior şi inferior şi respectiv, ileon superior şi inferior: 9-jejun superior, 10-jejun inferior, 11-ileon superior, 12-ileon inferior.

Scorul lezional este determinat după liza completă a aderențelor şi inspecția completă a suprafețelor peritoneale parietale şi viscerale din regiunile abdomino-pelvine. Se referă la cel mai mare diametru al implantului tumoral. Tumora primară sau recurențele locale care pot fi îndepărtate complet sunt excluse din evaluare. Dacă există confluențe de noduli tumorali, sunt caracterizate ca SL–3, chiar dacă este vorba de un strat fin (subțire) de implanturi tumorale:

–SL-0 înseamnă că nu sunt vizualizate depozite maligne;

–SL-1 implică prezența nodulilor tumorali sub 0,5 cm în cea mai mare dimensiune a lor; nu se cuantifică numărul nodulilor ci numai cel mai mare;

–SL-2 implică existența nodulilor între 0,5 şi 5 cm;

–SL-3 semnifică noduli tumorali peste 5 cm sau confluențe de noduli tumorali.

Se determină câte un scor lezional pentru fiecare dintre cele 13 regiuni abdomino-pelvine, suma acestora fiind PCI, care poate avea astfel valori de la 0 la 39.

Aprecierea intraoperatorie a PCI este un neajuns în cazul determinărilor unor valori mari, care contraindică gesturi chirurgicale complete. Ca urmare, determinarea preoperatorie a acestui index, prin computer-tomografie sau rezonanță magnetică poate evita o serie de gesturi chirurgicale inutile.

Explorarea CT centrată la nivel abdominal a fost realizată cu un aparat CT GE HiSPEED DX/i şi a presupus efectuarea de secțiuni în plan axial, cu respectarea următorilor parametri: colimare de 5mm pe secțiune scanată, viteza deplasării mesei - 5mm/secundă (pitch - 1,5), câmp minim posibil, 120 mA, 110 kV şi matrice 512X512. Pentru diferențierea formațiunilor şi identificarea arborilor vasculari a fost necesară administrarea intravenoasă a substanței de contrast. Pregătirea tractului gastro-intestinal urmăreşte posibilitatea identificării anselor intestinale şi diferențierea lor de eventualele mase tumorale ori ganglionare, deoarece o ansă neopacifiată poate fi o reală sursă de erori de interpretare, permițând însă şi evaluarea grosimii peretelui intestinal. Valorile de atenuare similare sau apropiate ale stomacului, colonului, ficatului, pancreasului, maselor musculare, adenopatiilor sau structurilor vasculare nu permit la explorarea nativă o identificare facilă a acestora.

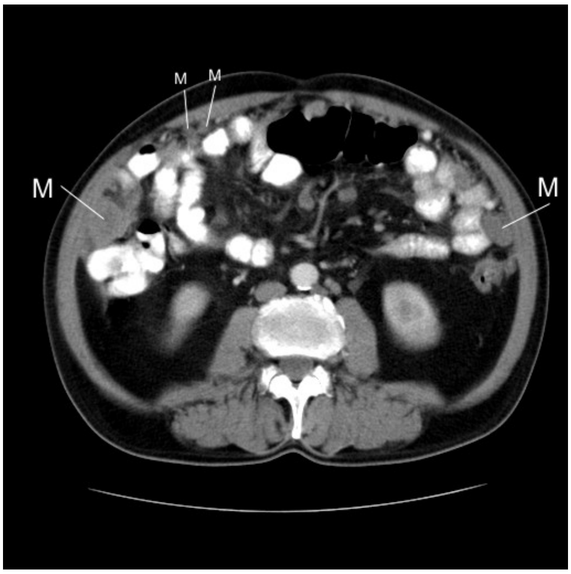

Fig.1. Metastaze peritoneale. Imagine CT axială, postcontrast iv ce evidențiază multiple formațiuni rotunde, de dimensiuni variabile situate la nivelul peritoneului anterior şi imagine lichidiană în spațiul perihepatic anterior. *M*-metastaze.

În acest scop se utilizează agenți de contrast care trebuie să asigure o creştere a densității cu cel puțin 40UH, comparativ cu structurile adiacente. Contrastul oral este folosit aproape fără excepție în identificarea abdomenului sub formă de soluții iodate hidrosolubile. Contrastul intravenos este de mare utilitate atât în caracterizarea țesuturilor anormale şi patologice, cât şi în aprecierea raporturilor acestora cu axele vasculare. Agenții cei mai folosiți sunt substanțe iodate nonionice. Imaginile obţinute prin computer-tomografie (CT) cu contrast maxim administrat intravenos şi per os conferă o rezoluţie spaţială deosebită. Astfel a fost posibilă aprecierea numărului, extensiei şi localizării acestora faţă de elementele anatomice (figura 1).

Imaginile obţinute prin IRM sunt adecvate pentru identificarea anatomică a spaţiilor şi ligamentelor peritoneale. Astfel am vizualizat pe secţiuni T1 axial, LT – ligamentul rotund, LV – *ligamentum venosum*, SSFDA – spaţiul subfrenic drept anterior, SSFDP – spaţiul subfrenic drept posterior, iar pe secţiuni coronale SFS – spaţiul subfrenic stâng, SHDA – spaţiul subhepatic drept anterior, LGC – ligament gastrocolic, LGS – ligament gastrosplenic, SHDP – spaţiul subhepatic drept posterior, BO – bursa omentală, SFS – spaţiul subfrenic stâng, demonstrând LF – ligament falciform, FW – Foramen Winslow (figurile 2,3).

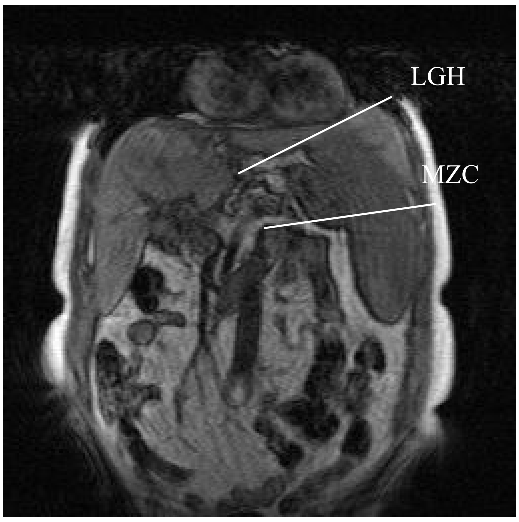

Fig.2. Imagine IRM. Secvenţă T1 coronală ce evidenţiază ligamentul gastrohepatic (LGH) şi mezocolonul (MZC).

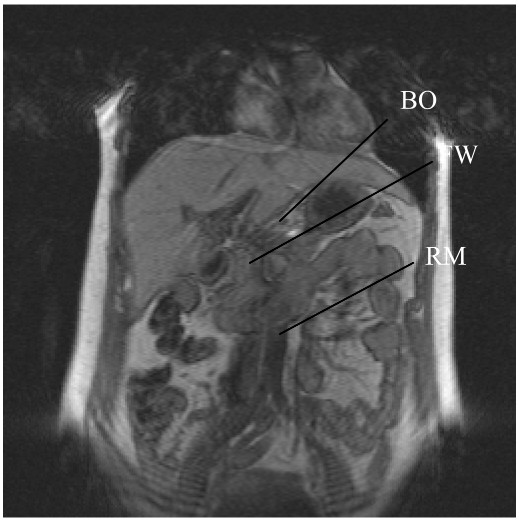

Fig.3. Imagine IRM. Secvenţă T1 coronală ce evidenţiază rădăcina mezenterului (RM), foramen Winslow (FW) şi bursa omentală (BO).

Rezultate

Pe lotul analizat am identificat 17 cazuri de neoplasm de pancreas. Dintre acestea, 15 au avut localizare cefalică, 13 fiind neoperate iar două sunt cazuri prezentate la control după duodenopancreatectomie cefalică.

Carcinom cefalopancreatic

Carcinom cefalopancreatic neoperat

Cazurile neoperate au fost în număr de 8 la bărbaţi şi 5 la femei.

Dintre cele 8 cazuri la pacienţii de sex masculin, 4 au prezentat forme ascitogene şi 4 nu au avut lichid intraperitoneal. Am identificat două cazuri cu ascită în cantitate mare şi câte un caz cu ascită în cantitate medie şi mică. Sunt evidenţiate două situaţii cu metastaze hepatice, pentru un caz cu ascită în cantitate mică şi un caz neascitogen şi două cazuri de prezentare cu icter, în situaţiile cu ascită în cantitate mare şi respectiv mică.

Cele 5 cazuri cu neoplasm pancreatic neoperat de sex feminin au fost diagnosticate cu ascită în 3 situaţii şi fără ascită în două situaţii. Am identificat două cazuri cu ascită în cantitate mare şi un caz cu ascită în cantitate mică. Pacientele cu forme ascitogene au prezentat două metastaze hepatice într-un caz cu ascită în cantitate mare şi unul cu ascită în cantitate mică şi o situaţie cu icter pentru pacienta cu lichid intraperitoneal în cantitate mică. Unul dintre cazurile cu lichid în cantitate mare a prezentat celularitate de tip reactiv la analiza frotiului. Pacientele cu forme neascitogene au fost icterice în ambele situaţii şi într-un caz am identificat metastaze hepatice la nivelul lobului drept.

Depozitele neoplazice apărute în cazurile de neoplasm cefalopancreatic neoperat *la **bărbaţi** cu **forme ascitogene*** prezintă diferenţe semnificative în funcţie de sectorul peritoneal interesat (p=0.0001).

Remarcăm în aceste situaţii o interesare statistic semnificativă a sectoarelor peritoneale în ordinea 0, 9, 10, 11, 12, 1, 3, 4, 5, 6, 7, 8, 2.

Se remarcă o corelaţie semnificativă a depozitelor neoplazice cu sectorul peritoneal implicat în cazurile cu neoplasm cefalopancreatic neoperat la bărbaţi (r=-0.49, p=0.000091, 95%CI). Acest aspect este explicat prin prezenţa depozitelor neoplazice importante în sectoarele peritoneale 9, 10, 11, 12.

Remarcăm în situaţiile de neoplasm cefalopancreatic neoperat la **bărbaţi** cu **forme neascitogene** o interesare statistic semnificativă a sectoarelor peritoneale în ordinea 0, 12, 1, 2, 7, 8, 9, 10, 11, 5, 6, 4.

Remarcăm în cazurile de *neoplasm cefalopancreatic neoperat la **femei** cu **forme ascitogene*** o interesare statistic semnificativă (p=0.029, 95%C) a sectoarelor peritoneale în ordinea 2, 0, 1, 3, 6, 4, 5, 7, 8, 9, 10, 11, 12. În cazul pacientelor de sex feminin cu neoplasm cefalopancreatic neoperat se remarcă o corelaţie inversă semnificativă a depozitelor neoplazice cu sectoarele peritoneale implicate (r=-0.54, p=0.000006). Putem spune că în acest caz depozitele neoplazice apar cu predilecţie pe sectoarele 0,1,2,3.

Remarcăm în situaţiile de *neoplasm cefalopancreatic **neoperat** la femei cu **forme neascitogene** în cantitate mare* o interesare statistic semnificativă a sectoarelor peritoneale în ordinea 2, 1, 3, 8, 0, 4, 5, 6, 7, 9, 10, 11, 12.

Factorii prognostici în apariţia depozitelor neoplazice peritoneale şi interesarea sectoarelor peritoneale în cazurile de neoplasm cefalopancreatic neoperat au fost analizaţi în funcţie de sexul pacienţilor şi de forma ascitogenă/neascitogenă.

Rezultatele corelaţiei multiple demonstrează corelaţia semnificativă (r=0.429, p=0.000, 95% interval de confidenţă) dintre prezenţa depozitelor neoplazice în cavitatea peritoneală în cazul neoplasmului cefalopancreatic neoperat în funcţie de forma de ascită prezentată şi sectorul peritoneal interesat.

Analiza coeficienţilor parţiali de corelaţie indică implicarea sectorului peritoneal, forma de ascită şi sexul pacienţilor în valoarea depozitelor neoplazice. Astfel, în cazul neoplasmului cefalopancreatic neoperat apariţia depozitelor neoplazice este influenţată în mod semnificativ de forma de ascită (r=0.42, p=0.000082, 95%CI) şi sectorul peritoneal interesat (r=-0.39, p=0.000237, 95%CI).

Un alt aspect evidenţiat este faptul că valoarea depozitelor neoplazice nu este influenţată de sexul pacienţilor (r=0.07, p=0.139, 95%CI).

Rezultatele testului neparametric de analiză predictivă indică faptul că factori importanţi de predicţie în apariţia depozitelor neoplazice sunt forma ascitogenă (ascitogenă/neascitogenă) (Wald stat.=12.48, p=0.0004) şi sectorul peritoneal interesat (Wald stat.= 8.16, p=0.0042). Pot exista însă şi alţi factori de predicţie ce nu au fost luaţi în studiu.

Carcinom cefalopancreatic operat

Cazurile prezentate la control după duodenopancreatectomie cefalică au fost în număr de două, ambele la femei şi ambele cu lichid de ascită prezent. Am notat o cantitate mare de revărsat într-un caz şi o cantitate medie în celălalt. Un caz a prezentat recidivă şi metastaze hepatice şi ambele au fost icterice la internare.

Carcinom corporeocaudal

Carcinom corporeocaudal neoperat

Pacienţii cu neoplasm corporeocaudal au fost ambii la prima prezentare, unul de sex feminin şi unul de sex masculin, ambii cu forme ascitogene şi cu metastaze hepatice. În cazul bărbatului cu ascită în cantitate mare am notat prezența unui lichid cu reacție celulară la examenul frotiului lichidului de ascită.

Distribuția depozitelor neoplazice pentru femeia cu neoplasm corporeocaudal neoperat la nivelul cavității peritoneale este ilustrată în tabelul nr.1. În cazul bărbatului nu a fost posibilă cartarea cavității peritoneale.

Tabelul nr.1. Repartiția valorilor depozitelor neoplazice în cavitatea peritoneală la pacienții de sex feminin cu neoplasm pancreatic corporeocaudal neoperat. S-sector, după Sugarbaker, 1,2,3 – LS1, LS2, LS3, IP- indice peritoneal al cancerului

S 12	**S 11**	**S 10**	**S 9**	**S 8**	**S 7**	**S 6**	**S 5**	**S 4**	**S 3**	**S 2**	**S 1**	**S 0**	**IP TOTAL**	
1	1	1	1	1	1	1	1	1	3	2	2	2	18	ASCITA CANTITATE MICĂ

Discuții

După Zervos, cancerul de pancreas este a cincea neoplazie gastrointestinală ca frecvență, dar se constituie în a treia cauză de deces prin cancer gastrointestinal (8).

Adenocarcinomul de pancreas este responsabil de aproximativ 5% din decesele prin cancer în SUA (9). Din cauza agresivității tumorii, stadiului avansat de boală la prezentare şi a lipsei terapiilor sistemice eficace, supraviețuirea la 5 ani este apreciată la 1-4% dintre bolnavi.

Pe o serie largă de piese de rezecție, Kayahara M. (10) propune stabilirea unui model de invadare ganglionară pentru cancerul de pancreas. Remarcă necorelarea mărimii tumorale cu prezența invaziei ganglionilor paraaortici ci numai cu ganglionii grupului pancreaticoduodenal posterior şi cei din jurul arterei mezenterice superioare, în cazul cancerelor capului de pancreas. Cel mai mare procent de invazie la nivelul ganglionilor paraaortici a fost în regiunea dintre trunchiul celiac şi artera mezenterică inferioară, atât pentru localizările la nivelul capului cât şi pentru cele la nivelul corpului pancreasului.

Metastazele peritoneale sincrone au incidență de 50-80%, cele metacrone de 30% şi durata medie de supraviețuire este apreciată de Windham la 6 luni (11).

Se apreciază că localizarea corporeocaudală este cea responsabilă de majoritatea determinărilor peritoneale (12).

Diseminarea peritoneală este indicată a fi calea majoră de recurență postoperatorie şi de mortalitate pentru adenocarcinomul de pancreas (13). Din acest punct de vedere localizarea tumorii la nivelul capului şi corpului, precum şi invazia perineurală extratumorală au valoare predictivă pentru dezvoltarea carcinomatozei.

De aceea, una din problemele care se ridică în cazul cancerului de pancreas este invazia perineurală. Caracteristica este încărcată, în aceste cazuri, cu o importantă semnificație clinico-evolutivă. Din acest motiv se înregistrează dificultăți însemnate în efectuarea unei rezecții curative. În plus, invazia perineurală este factorul care constituie elementul de rezistență la terapiile convenționale ale acestui tip de cancer, care de altfel se prezintă în forme histologice bine sau moderat diferențiate (14).

Raa raportează lavaje peritoneale cu citologie pozitivă în cazurile indicate pentru rezecții curative de până la 29% (15). Diseminarea peritoneală după intervenții cu intenție curativă este de 6-56%, iar recurența locoregională de 60%.

Makary arată într-un studiu prezența celulelor maligne în lichidele de lavaj ale cancerului de pancreas în 8-30% dintre cazuri (16). Cele mai multe dintre ele sunt notate în prezența altor metastaze macroscopice, dar într-o treime din cazuri nu se decelează metastaze macroscopice intraabdominale. Autorul presupune că un stadiu de boală mai avansat locoregional, detectabil computer-tomografic, este mai probabil să demonstreze şi implante peritoneale.

Ilka Vogel detectează imunohistochimic, în cancerul de pancreas stadiul II, prezența celulelor neoplazice în cavitatea peritoneală, situație în care a exclus clar orice comunicare a tumorii cu peritoneul (17). Aspectul este atribuit unei diseminări limfatice sau prin pori.

După Zervos, ascita se manifestă numai la 20% dintre bolnavii cu cancer de pancreas şi are o etiologie multifactorială (8). Poate apărea datorită obstrucției limfaticelor diafragmatice, creşterii producției de exsudat de către tumoră sau secreției de peptide active osmotic la nivel tumoral care modifică permeabilitatea vasculară. La mai mult din jumătate din pacienți celularitatea ascitei este negativă pentru celule neoplazice. Ca atare nu pot fi denumite în cel mai pur sens al cuvântului drept maligne, dar sunt indubitabil produse de neoplazie. Studii statistice regresive lineare nu au relevat caracteristici tipice pentru a prezice formarea ascitelor.

Slam citează un caz de adenocarcinom mucinos de coadă de pancreas, manifestat prin invazie a unghiului splenic al colonului, metastază ganglionară mezenterică şi o masă tumorală cecală de 6 centimetri, caz ce ridică posibilitatea realizării acestor determinări prin căi independente de exfolierea intraperitoneală a celulelor neoplazice (18).

Pancreasul este un organ retroperitoneal. Apariția metastazelor peritoneale în cazul acestei maladii readuce în atenție corelația dintre cavitatea peritoneală şi spațiul retroperitoneal, realizată prin intermediul ligamentelor şi mezourilor. *Meyers* aduce în discuție date interesante legate de compartimentarea peritoneală şi de comportamentul ligamentelor şi mezourilor, care rămân interconectate, drept căi de propagare a maladiilor.

Dată fiind gravitatea bolii, amploarea intervențiilor chirurgicale şi evoluția rapidă a acestor cazuri, numai o parte din rezultatele obținute au fost disponibile pentru prelucrare.

Astfel, din totalul de 17 cazuri analizate, 14 sunt de neoplasm cefalopancreatic, 8 la bărbați şi 5 la femei. Formele ascitogene sunt aproximativ egale (4 la bărbați şi 3 la femei). Numai două persoane de sex feminin s-au prezentat la control după duodenopancreatectomie cefalică, ambele ascitogene. Am înregistrat numai două cazuri de neoplasm pancreatic corporeocaudal (19).

Remarcăm în situațiile de neoplasm cefalic pancreatic la bărbați cu forme ascitogene o interesare statistic semnificativă a sectoarelor peritoneale în ordinea 0, 9, 10, 11, 12, 1, 3, 4, 5, 6, 7, 8, 2 (figura 4).

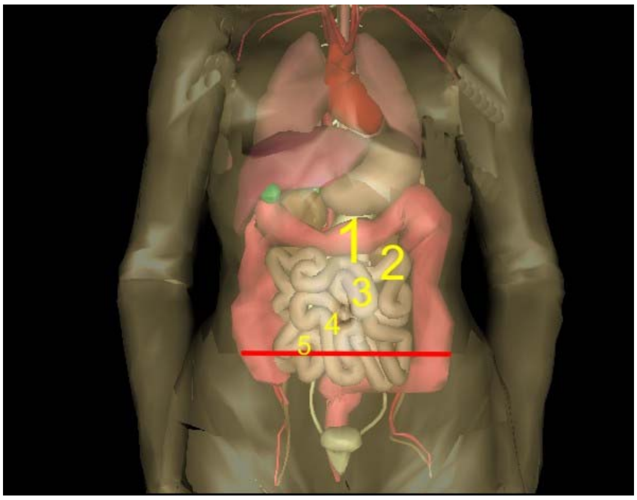

Fig.4. Distribuția metastazelor peritoneale sincrone în neoplasmul cefalopancreatic neoperat ascitogen la bărbați.

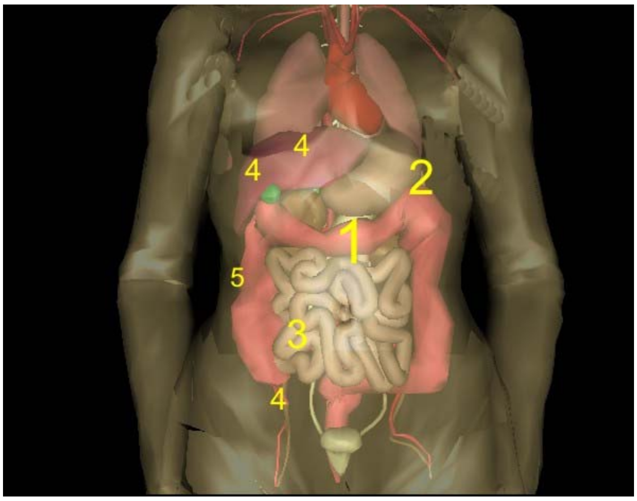

Fig.5. Distribuția metastazelor peritoneale metacrone în neoplasmul cefalopancreatic neoperat fără ascită la bărbați.

Depozitele neoplazice apărute în cazurile de neoplasm cefalopancreatic neoperat prezintă diferențe semnificative în funcție de sectorul peritoneal interesat.

Notăm în situațiile de neoplasm cefalic pancreatic la bărbați cu forme fără ascită o interesare statistic semnificativă a sectoarelor peritoneale în ordinea 0, 3, 12, 1, 2, 7, 8, 9, 10, 11, 5, 6, 4 (figura 5).

Se evidențiază în situațiile de neoplasm cefalic pancreatic la femei cu forme fără revărsat peritoneal o interesare statistic semnificativă a sectoarelor peritoneale în ordinea 2, 1, 3, 8, 0, 4, 5, 6, 7, 9, 10, 11, 12 (figura 6).

Am stabilit în situațiile de neoplasm cefalic pancreatic la femei cu forme cu revărsat peritoneal o interesare statistic semnificativă a sectoarelor peritoneale în ordinea 2, 0, 1, 3, 6, 4, 5, 7, 8, 9, 10, 11, 12 (figura 7).

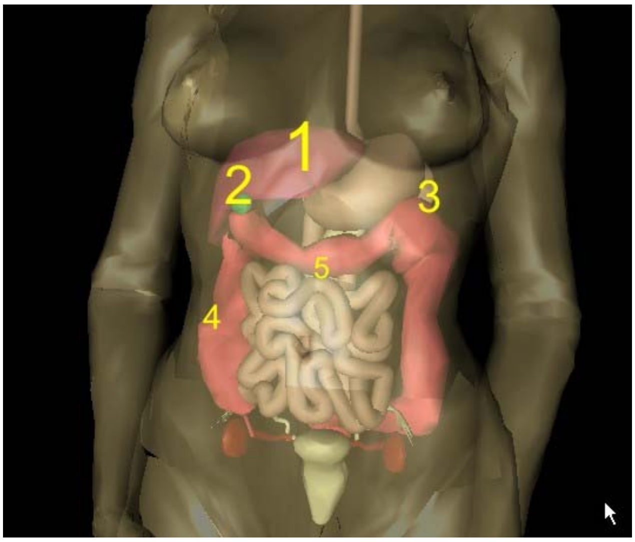

Fig.6. Distribuția metastazelor peritoneale sincrone în neoplasmul cefalopancreatic neoperat fără ascită la femei.

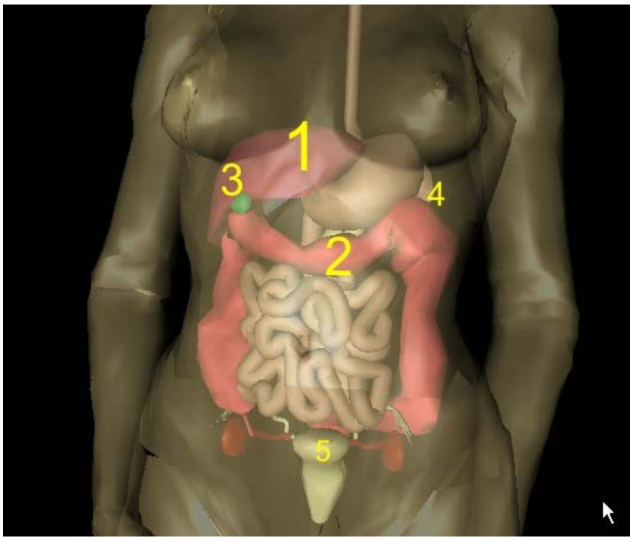

Fig.7. Distribuția metastazelor peritoneale sincrone în neoplasmul cefalopancreatic neoperat ascitogen la femei.

Rezultatele corelației multiple demonstrează corelația semnificativă (r=0.429, p=0.000, 95% interval de confidență) dintre prezența depozitelor neoplazice în cavitatea peritoneală în cazul neoplasmului cefalopancreatic neoperat în funcție de forma de ascită prezentată şi sectorul peritoneal interesat.

Analiza coeficienților parțiali de corelație indică implicarea sectorului peritoneal, forma de ascită şi sexul pacienților în valoarea depozitelor neoplazice. Astfel, în cazul neoplasmului cefalopancreatic neoperat apariția depozitelor neoplazice este influențată în mod semnificativ de forma de ascită (r=0.42, p=0.000082, 95%CI) şi sectorul peritoneal interesat (r=-0.39, p=0.000237, 95%CI).

Un alt aspect evidențiat este faptul că valoarea depozitelor neoplazice nu este influențată de sexul pacienților (r=0.07, p=0.139, 95%CI).

Analiza comparativă a acestor distribuții relevă tendința metastazelor peritoneale la sexul masculin de a se organiza centroabdominal şi de a implica ulterior ansele intestinului subțire. Nu sunt semnificative interesările de la nivelul cupolelor diafragmatice. Nu am înregistrat în aceste situații depozite vizibile la nivelul bursei omentale.

Cazurile diagnosticate cu ascită concomitentă sunt caracterizate şi prin demonstrarea unei interesări mai accentuate a anselor de intestin subțire, fapt concordant cu datele obținute şi în cazul altor patologii neoplazice asociate cu carcinomatoză analizate.

Tendința la sexul feminin este de a dezvolta depozite metastatice neoplazice mai importante la nivelul sectorului peritoneal 2, urmate de determinări subdiafragmatice şi centroabdominale. Acest fapt se corelează cu prezența mult mai însemnată pe acest lot de bolnavi a metastazelor hepatice (3 din 5 cazuri la femei față de numai 2 din 7 la bărbați).

Concluzii

Se remarcă tendința metastazelor peritoneale în cazul neoplasmului cefalopancreatic la sexul masculin de a se organiza centroabdominal şi de a implica ulterior ansele intestinului subțire. Nu sunt semnificative în aceste cazuri interesările de la nivelul cupolelor diafragmatice.

Pacientele diagnosticate cu această patologie au dezvoltat depozite metastatice neoplazice mai importante la nivelul sectorului peritoneal 2, urmate ulterior de determinări subdiafragmatice şi centroabdominale. Aspectele lezionale par a fi în concordanță cu prezența *metastazelor hepatice* la acest lot de paciente.

Ascita concomitentă a favorizat expunerea anselor de intestin subțire la implanturile neoplazice.

Valoarea depozitelor neoplazice nu este influențată de *sexul* pacienților

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Anestezia regională. Blocurile nervoase ale capului şi gâtului

Autori: Mihai Pricop, Ioan Sîrbu, Adrian Barbilian

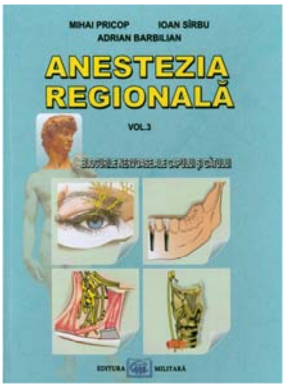

Lucrarea *Anestezia regională. Blocurile nervoase ale capului şi gâtului,* realizată de col. dr. Mihai Pricop, gl. bg. prof. univ. dr. Ioan Sîrbu şi col. conf. univ. dr. Adrian Barbilian, reprezintă o continuare a problematicii anesteziei loco-regionale iniţiată prin volumele *Blocurile nervoase ale membrelor* şi *Anestezia locală în patologia osteo-articulară a membrelor.* Acest nou tom se înscrie într-un efort deosebit de ambiţios, aflat în derulare, şi reprezintă un succes indiscutabil.

Cartea surprinde în mod plăcut prin maniera în care sunt prezentate informaţia şi iconografia, originală, cu referinţă strictă, deosebit de utilă şi care uşurează în mod direct efortul cititorului în desluşirea şi deprinderea tehnicilor prezentate.

Titlurile celor nouă capitole ne dau o imagine clară asupra tematicii abordate: Anestezia regională. Generalităţi, Blocurile nervului trigemen, Blocurile plexului cervical, Blocul nervului facial, Blocul nervului glosofaringian, Blocurile nervului vag, Blocul nervului spinal accesor, Blocurile nervilor occipitali, Blocurile retrobulbar şi peribulbar.

Ca şi în volumele anterioare remarcăm claritatea expunerii, ordinea logică a informaţiei, urmărindu-se într-o abordare aproape didactică redarea aspectelor anatomice, situaţiile în care se aplică anestezia generală, contraindicaţiile, echipamentul folosit, particularităţile care derivă din specificul intervenţiei propuse, terenul pacientului, metoda de anestezie aplicată, eventualele complicaţii.

Îndelungata practică a autorilor, faptul că aceştia îşi desfăşoară şi deţin informaţii în specialităţi medicale diferite (anestezie-terapie, chirurgie orală şi maxilo-facială, chirurgie), experienţa didactică a acestora, precum şi consultarea unei bibliografii moderne şi de dată recentă fac din această carte un ghid deosebit de util, cu largă adresabilitate, o sursă de referinţă pentru orice practician din domeniile enunţate.

Medicii din specialităţile anestezie-terapie, chirurgie orală şi maxilo-facială, oftalmologie, ORL, neurochirurgie, din unităţile de primire urgenţe, rezidenţii din aceste specialităţi, precum şi alte categorii de medici sau studenţi ai facultăţilor de profil vor găsi în noua apariţie editorială date extrem de folositoare în plan profesional.

Prezenţa în cadrul colectivului de autori a unor specialişti de renume din domenii conexe, eforturile acestora au dus la o remarcabilă reuşită în aria literaturii medicale, într-un teritoriu prea puţin abordat până în momentul de faţă, conturându-se într-un adevărat reper bibliografic.

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Modificări introduse de ghidul de resuscitare cardio-pulmonară ILCOR 2005

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Rezumat. De la fondare în 1992, ILCOR se întruneşte de două ori pe an. Primul ghid internaţional de resuscitare a fost realizat şi publicat în anul 2000 în colaborare cu American Heart Association (AHA), şi apoi un altul în 2005. Următoarea conferinţă de anvergură este aşteptată în februarie 2010, culminând cu elaborarea şi publicarea în octombrie 2010 a unui nou ghid de resuscitare. În prezentul articol se realizează o succintă analiză a modificărilor incluse în ghidul de resuscitare cardio-pulmonară din 2005 publicat de ILCOR.

Cuvinte cheie: resuscitare, şoc electric extern, tulburare de ritm, ventilaţie mecanică.

Abstract. Since 1992, ILCOR (International Liaison Committee on Resuscitation) meets twice each year. In collaboration with the American Heart Association (AHA), ILCOR produced the first International CPR Guidelines in 2000 and then again in 2005. The next Consensus Conference is scheduled in February 2010, followed by the publication of most recent evidence-based resuscitation findings in the form of a new resuscitation guideline in October 2010. This article presents a short review of major changes included in the 2005 cardiopulmonary resuscitation (CPR) guidelines published by ILCOR.

Introducere

Medicina de urgenţă este o specialitate relativ nouă, atât la noi în ţară (1991)[1,2] cât şi pe plan mondial. Aceasta s-a dezvoltat simultan cu progresul metodelor de intervenţie şi tratament şi al aparaturii medicale, precum şi cu accesul prompt şi optim al echipelor de intervenţie în prespital, prin dezvoltarea transportului medicalizat terestru şi aerian, dar şi a căilor de acces (autostrăzi, drumuri performante, extinderea urbanismului).

Manevrele de resuscitare s-au efectuat din cele mai vechi timpuri, începând din vremea profetului Elie, acestuia atribuindu-se prima resuscitare respiratorie prin respiraţie gură la gură la un copil. Ulterior au fost menţionaţi: Vesale, în 1543, cu ventilaţia artificială cu presiune intermitent pozitivă, Tossach, în 1771, prima ventilaţie gură la gură la un adult, iar în secolul al XIX-lea, Boehm (1878) şi Schiff (1882) cu masajul cardiac pe animale prin toracotomie, Maass (1892), masajul cardiac extern eficace, iar în 1900, Ogelsurd, Keen şi Zesas, care au efectuat primul masaj cardiac intern la om. Amintim că prima intubaţie translaringiană a fost făcută în 1880 de Macewn, iar prima defibrilare externă la om îi aparţine, în 1946, lui Gurvich[3]. Cu toate acestea, Peter Safar este unanim acceptat ca fiind părintele abecedarului resuscitării[3], cel care a integrat toate manevrele de resuscitare, creând astfel primul algoritm de resuscitare[4].

În ultimele decade s-au efectuat o multitudine de studii privind eficienţa metodelor de intervenţie, cât şi modul de producere a stopurilor cardio-respiratorii.

La mai mult de 300 000 de stopuri cardiace care se produc anual în SUA, rata de supravieţuire este estimată la mai puţin de 10% pentru stopurile survenite în prespital şi la mai puţin de 20% pentru stopurile din spital[5]. Studii suplimentare remarcă faptul că rata de supravieţuire coboară cu 10-15% pentru fiecare minut trecut de la producerea stopului cardiac, fără a se iniţia manevre de resuscitare[5].

Alte studii de gen arată că cele mai frecvente ritmuri de stop întâlnite par a fi fibrilaţia ventriculară (FV) şi tahicardia ventriculară (TV) fără puls (60-80% din cazuri), urmate de asistolă (20-40%) şi, respectiv, activitate electrică fără puls (PEA) (10%)[6]. În ciuda progreselor făcute în acest domeniu, unele studii arată rezultate chiar foarte slabe, raportând rate de supravieţuire de până la 4-9%[7]. Se pare că pacienţii cu fibrilaţie ventriculară au cel mai bun prognostic, cu o rată de supravieţuire de 4-33%, pe când cei cu activitate electrică fără puls au o rată de doar 1-4%, iar cei cu asistolă supravieţuiesc doar în cazuri excepţionale[6].

International Liaison Committee on Resuscitation (ILCOR)

ILCOR s-a format în 1992 în încercarea de a stabili o legătură între principalele organizaţii de gen de pe glob. Scopul declarat a fost acela de a întruni membri din mai multe specialităţi ale medicinei pentru a crea un ghid de resuscitare acceptat în cât mai multe ţări. În prezent principalele obiective ILCOR sunt [6,8]:

a) asigurarea unui forum de discuţii şi coordonare pentru toate aspectele legate de resuscitarea cardio-pulmonară şi cerebrală;

b) promovarea educaţiei şi antrenarea diverselor grupe de populaţie pe diverse căi pentru aplicarea corectă a protocolului de resuscitare;

c) încurajarea cercetării ştiinţifice în domenii ale resuscitării insuficient cunoscute;

d) asigurarea unor căi de colectare, reînnoire şi prezentare la nivel internaţional pentru informaţia ştiinţifică obţinută din studierea resuscitării;

e) comunicarea oficială a unor informaţii specifice despre resuscitare după ajungerea la un consens general.

De la fondare în 1992, ILCOR se întruneşte de două ori pe an. Primul ghid internaţional de resuscitare a fost realizat şi publicat în anul 2000 în colaborare cu American Heart Association (AHA), şi apoi un altul în 2005. Următoarea conferinţă de anvergură este aşteptată în februarie 2010, culminând cu elaborarea şi publicarea în octombrie 2010 a unui nou ghid de resuscitare.

Principalele modificări introduse în ghidul de resuscitare din 2005

Algoritmurile de resuscitare pentru suportul vital de bază („Basic life support" – BLS) şi pentru suportul vital avansat („Advanced life support" – ALS) sunt descrise pe larg în protocolul din 2005 şi sunt reprezentate schematic în fig. 1 şi, respectiv, fig. 2.

Algoritm BLS la adult

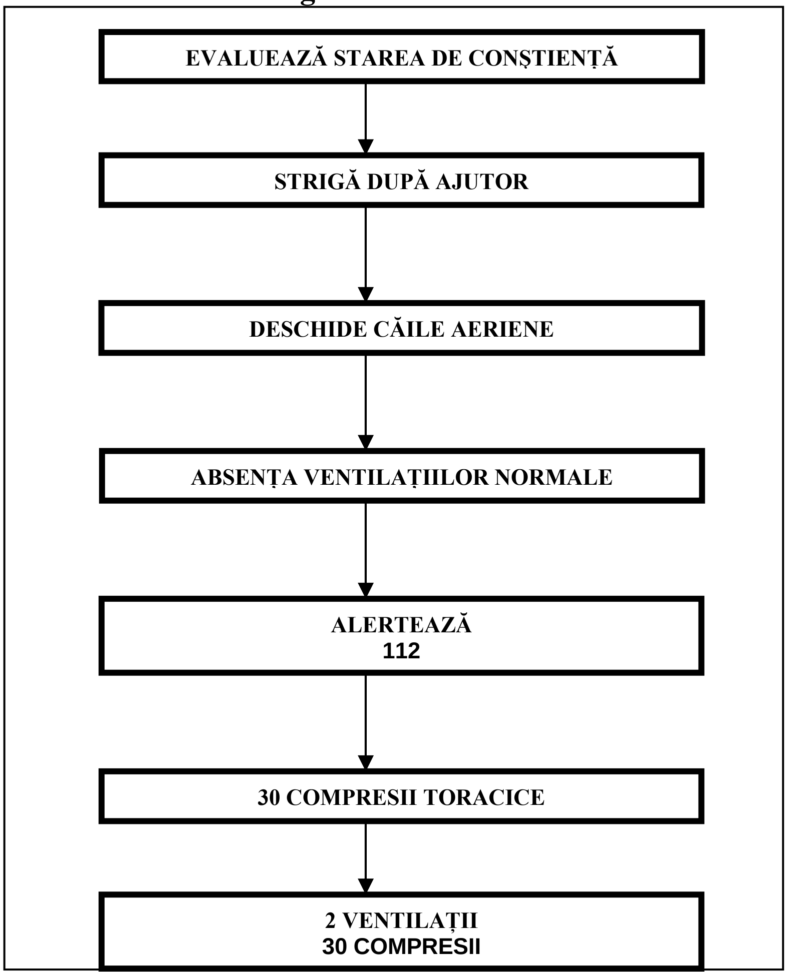

Fig. 1. Suportul vital de bază („Basic life support" – BLS)[8,13]

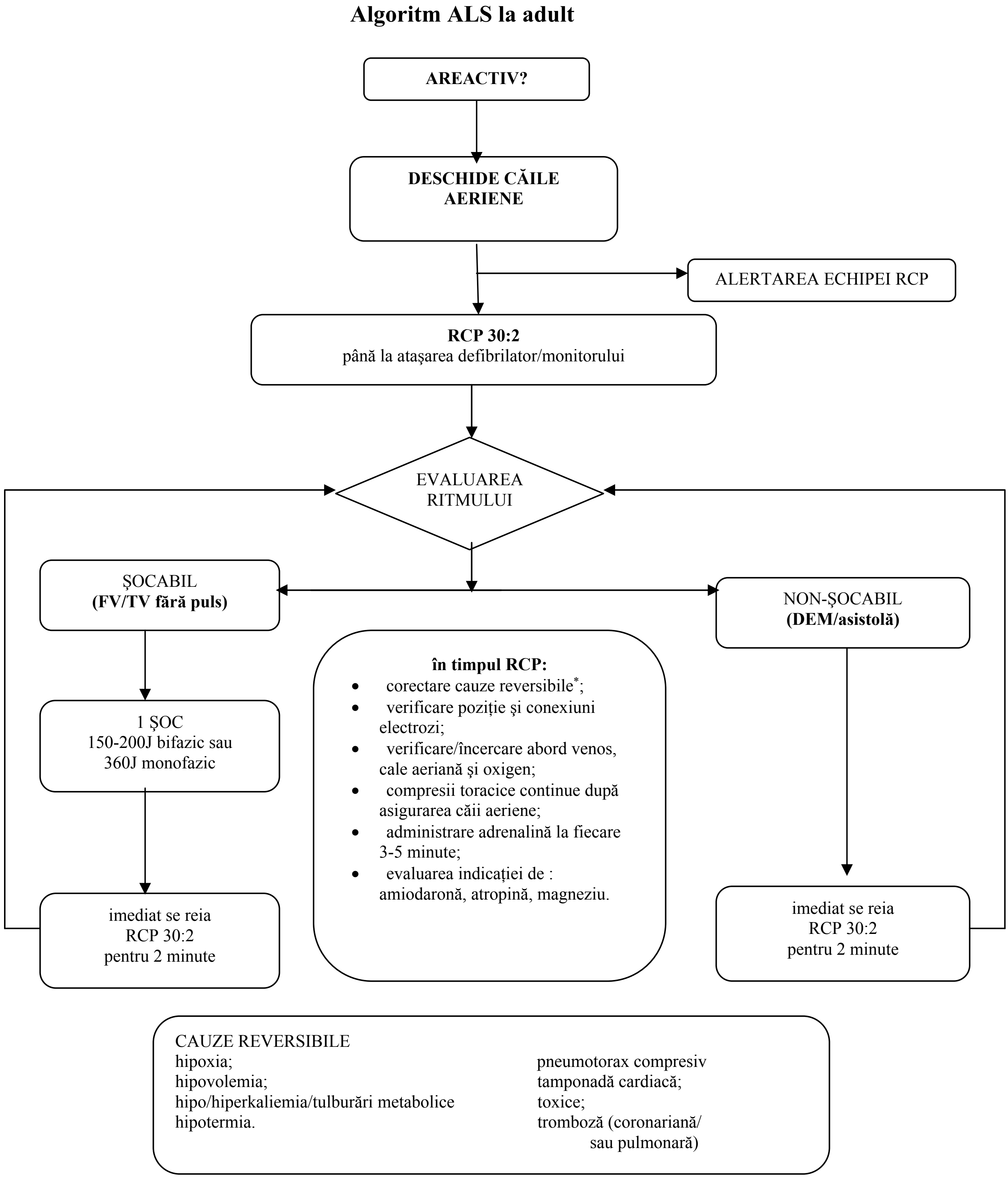
Algoritm ALS la adult
AREACTIV?
DESCHIDE CĂILE AERIENE
ALERTAREA ECHIPEI RCP
RCP 30:2
până la ataşarea defibrilator/monitorului
EVALUAREA RITMULUI
ŞOCABIL
(FV/TV fără puls)
NON-ŞOCABIL
(DEM/asistolă)
1 ŞOC
150-200J bifazic sau
360J monofazic
în timpul RCP:
• corectare cauze reversibile*;
• verificare poziție şi conexiuni electrozi;
• verificare/încercare abord venos, cale aeriană şi oxigen;
• compresii toracice continue după asigurarea căii aeriene;
• administrare adrenalină la fiecare 3-5 minute;
• evaluarea indicației de : amiodaronă, atropină, magneziu.
imediat se reia
RCP 30:2
pentru 2 minute
imediat se reia
RCP 30:2
pentru 2 minute
CAUZE REVERSIBILE
hipoxia;
hipovolemia;
hipo/hiperkaliemia/tulburări metabolice
hipotermia.
pneumotorax compresiv
tamponadă cardiacă;
toxice;
tromboză (coronariană/ sau pulmonară)

Fig. 2. Suportul vital avansat („Advanced life support" – ALS)[8,13]

1. Indicația de inițiere a resuscitării cardio-respiratorii (RCR)

Conform indicațiilor actuale, RCR se începe imediat ce se stabileşte că victima este inconştientă şi nu respiră normal. Se menționează că se indică începerea RCR şi pentru pacienții cu respirațiile de tip agonic (gaspuri, efort ventilator foarte mare, respirații superficiale) care apar în primele momente de la instalarea stopului[9].

2. Modificarea raportului compresii:ventilații pentru toate categoriile de vârstă, exceptând nou-născuții

Deşi nu există nici în prezent suficiente date care să stabilească un raport optim compresii: ventilații, s-a ajuns la concluzia că raportul compresii:ventilații 30:2 îndeplineşte cel mai bine aceste condiții. S-a luat în calcul necesitatea simplificării protocolului pentru a uşura instruirea şi pentru o memorare mai facilă a informațiilor[6].

3. Sublinierea importanței calității manevrelor de RCR

În plus față de recomandările din 2000, în ghidul din 2005 se fac referiri directe la importanța calității manevrelor de resuscitare cardio-respiratorie[9]. Se subliniază importanța limitării întreruperilor din timpul manevrelor de resuscitare şi de asemenea importanța corectitudinii compresiunilor toracice pentru reuşita resuscitării şi pentru limitarea suferinței pacientului pe termen lung, în special din punct de vedere neurologic.

4. Modificări în ventilația mecanică

În ghidul din 2005 se arată că managementul avansat al căilor aeriene nu reprezintă o prioritate. În caz că nu se reuşeşte intubația orotraheală se recomandă continuarea ventilației pe mască şi balon.

S-a arătat că hiperventilația cu sau fără intubație duce la creşterea presiunii intratoracice şi, prin aceasta, la scăderea întoarcerii venoase şi la scăderea debitului cardiac, cu implicații directe asupra perfuziei cerebrale şi miocardice. De aceea în cazul pacienților intubați frecvența ventilațiilor artificiale recomandată în cadrul ghidului din 2005 este de maxim 8-10/min [8], față de 10-12/min în 2000.

Avantajul major este acela că după intubație se pot efectua compresiile toracice în paralel cu ventilația mecanică, sporind astfel eficiența manevrelor de RCR.

5. Scăderea numărului de încercări de ventilație mecanică eficientă

În protocolul din 2000 se recomandau 5 încercări pentru a se obține două ventilații eficiente. În prezent se efectuează două ventilații, după care se trece imediat la compresiuni toracice. Nu se recomandă mai mult de două tentative de ventilație eficientă, subliniindu-se astfel importanța deosebită a efectuării compresiilor toracice cu întrerupere minimă pentru creşterea şanselor de supraviețuire a victimei[9,8].

6. Începerea RCR cu compresiuni toracice

S-a demonstrat că, inițial, în cazul unui SCR saturația arterială în oxigen este ridicată. În aceste condiții hipoxia tisulară este produsă în principal de scăderea debitului sanguin cardiac. De aceea se consideră că inițierea compresiunilor toracice este mai importantă pentru influențarea debitului cardiac şi reducerea hipoxiei cerebrale şi coronariene[8].

7. Renunțarea la verificarea pulsului central al pacientului

S-a observat că determinarea prezenței sau a absenței pulsului central se face cu dificultate, mai ales de către persoanele fără pregătire medicală. Ghidul din 2005 recomandă să se inițieze direct manevrele de RCR pentru orice victimă inconştientă cu respirație anormală[8]. Excepție de la regulă pot face persoanele cu pregătire medicală, care pot încerca să caute pulsul central, dar numai pentru 10 secunde şi, de preferat, în paralel cu evaluarea celorlalte semne vitale.

8. Modificări în schema de administrare a şocurilor electrice externe

În cazul ritmurilor şocabile o modificare importantă este aceea că se recomandă administrarea unui singur şoc electric extern, de preferat cu energie bifazică, renunțându-se astfel la suita de 3 şocuri electrice externe administrate succesiv cu energie crescătoare[6]. Se recomandă defibrilarea imediată dacă la locul intervenţiei există un defibrilator. Aici se reglementează şi folosirea defibrilatoarelor automate de către persoanele fără pregătire medicală. Se începe resuscitarea înaintea defibrilării dacă echipa de resuscitare întârzie mai mult de 5 minute. Fiecare şoc administrat este urmat imediat de manevrele de resuscitare, fără evaluarea ritmului. Se începe cu 30 compresii toracice, urmate de două ventilaţii în cicluri repetate timp de două minute, după care se evaluează ritmul cardiac. Energiile utilizate trebuie să fie de 360J pentru curent monofazic şi de 150-200J pentru defibrilatoarele bifazice, iniţial, apoi cu energie egală sau mai mare pentru şocurile următoare.

În cursul opririi cardiace au fost identificate 3 faze hemodinamice[3]: electrică, hemodinamică şi metabolică, ceea ce sugerează faptul că în primele 5 minute defibrilarea este prioritară. Între 4 şi 10 minute (faza hemodinamică) este prioritară asigurarea suportului circulator prin compresii toracice. În faza metabolică, administrarea medicaţiei şi inducerea hipotermiei ajung în prim plan.

Ca fiziopatologie, în evaluarea hemodinamicii opririi cardiace produse prin fibrilaţie ventriculară, apar o serie de secvenţe care susţin etapele ultimului algoritm de resuscitare.

Ulterior apariţiei fibrilaţiei ventriculare, ne confruntăm cu scăderea presiunii capilare pulmonare şi a fluxului retrograd, o mare cantitate de sânge fiind cantonată în sistemul venos, dilatându-se cordul drept. Ventriculul stâng se contractă, dar conţine puţin sânge, producând dischinezia septului interventricular. În acest moment, chiar dacă şocul electric extern restaurează ritmul sinusal, nu reapare însă şi fluxul, deoarece ventriculul stâng este gol, deteriorându-se metabolismul miocardic cu posibilitatea reapariţiei fibrilaţiei ventriculare. Contracţia toracică, produsă prin compresiile toracice, deplasează sângele din cordul drept în cel stâng, acest proces necesitând însă 1,3-3 minute de resuscitare cardio-pulmonară, timp exprimat în cele 2 minute de RCR, obligatorii, din algoritmul de resuscitare[3].

9. Modificări în schema de administrare a substanţelor în ALS (vasopresoare, antiaritmice, atropină)

În SCR cu FV sau TV fără puls este indicată administrarea de adrenalină, 1 mg, înainte de al treilea şoc electric, urmată de eliberarea şocului electric şi de cele 2 minute de RCR. În asistolă şi PEA administrarea de adrenalină se face cât mai curând posibil. Administrarea adrenalinei se face continuu la fiecare 3-5 minute[6,8].

Amiodarona se administrează în RCR dacă tulburarea de ritm (FV/TV) persistă după al 3-lea şoc electric[6].

Asistola este o condiţie care poate fi precipitată sau accentuată de un tonus vagal excesiv. De aceea administrarea de rutină a unei doze maximale de 3mg atropină în asistolă şi PEA este menţionată în ghidul din 2005 [6].

Dozele de medicamente utilizate în timpul resuscitării au rămas neschimbate faţă de cele din 2000. Nu se va întrerupe masajul pentru administrarea acestor substanţe.

10. Recomandări pentru fibrinoliză în timpul stopului cardio-respirator

Conform recomandărilor din 2005, se poate lua în considerare terapia fibrinolitică dacă se suspicionează un trombembolism pulmonar care să stea la baza stopului cardiac. Deşi din 2004 până în 2006 s-a desfăşurat în Europa un studiu[10] prospectiv, multicentric, randomizat, dublu-orb, placebo-controlat, pentru evaluarea eficacităţii şi siguranţei administrării de tenecteplază în SCR de origine presupusă cardiacă în comparaţie cu tratamentul standard, studiul a fost întrerupt prematur, deoarece analiza datelor obţinute până la momentul respectiv a arătat o foarte scăzută probabilitate ca studiul să demonstreze superioritatea terapiei cu tenecteplază în comparaţie cu placebo[11].

11. Modificări ale algoritmului pentru tratarea tahicardiilor cu puls

În cadrul protocolului din 2000 era prezentat un algoritm complex pentru abordarea acestor tulburări de ritm. În prezent în prespital terapia se restrânge[8] doar la tratarea cu manevre vagale sau adenozină a tahicardiilor cu puls şi cu complexe înguste. Tratamentul tahicardiilor cu puls, dar cu complexe largi, se recomandă a se efectua după aducerea pacientului la spital[8].

12. Recomandări pentru tratamentul pacientului după RCR

Managementul corect al pacientului după recăpătarea funcțiilor vitale („return of spontaneous circulation" – ROSC) este deosebit de important. Ghidul din 2005 subliniază acest lucru şi schimbă abordarea inițială axată pe tratarea principalelor tulburări de ritm (2000), focalizând eforturile terapeutice spre obținerea unor rezultate optime din punct de vedere neurologic. Se amintesc aici o serie de studii care arată avantajul inducerii hipotermiei controlate în primele 6 ore de la ROSC şi menținerea unei temperaturi centrale de 32-34°C timp de 12-24 ore, ulterior revenindu-se la temperatura normală. Se arată că această metodă reduce atât morbiditatea, cât şi mortalitatea la pacienții cu ROSC[12].

Ce a rămas nemodificat[9] din ghidul ILCOR 2000:

a) evaluarea siguranței salvatorului, a victimei şi a celor din jur;
b) evaluarea stării de conştiență a victimei;
c) solicitarea ajutorului/plecarea după ajutor;
d) numărul de compresii toracice/minut (100/minut);
e) forța de comprimare toracică (comprimarea planului toracic anterior cu 4-5 cm);
f) faza de relaxare a compresiei pentru a permite revenirea peretelui toracic anterior la poziția inițială.

Concluzii

În ghidul din 2005 s-au introdus, pe baza unor studii în domeniu, câteva modificări importante, cum ar fi: raportul compresii:ventilații de 30:2, limitarea la maximum a pauzelor din timpul masajului cardiac, defibrilarea precoce cu administrarea unui singur şoc electric extern, reglementarea folosirii defibrilatoarelor automate de către publicul larg şi, cel mai important, schimbarea abordării inițiale axate pe tratarea principalelor tulburări de ritm cardiac, focalizând eforturile terapeutice spre obținerea unor rezultate optime din punct de vedere neurologic, sporind astfel supraviețuirea pacientului pe termen lung.

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Ecografia obstetricală

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Rezumat. Pentru sarcină, ecografia este esenţială atât din punctul de vedere al faptului că este o metodă uşor de efectuat, neinvazivă, fără reacţii adverse, cât şi din punctul de vedere al performanţelor, întrucât permite o bună observare a evoluţiei sarcinii, pas cu pas, cu o acurateţe foarte mare. Ecografia folosită în timpul sarcinii s-a dovedit a fi foarte eficientă pentru a evalua dezvoltarea fătului, frecvenţa lui cardiacă, anexele fetale (placenta, cordonul ombilical), lichidul amniotic, pentru a investiga sarcina în momentele în care există riscul de pierdere.

Cuvinte cheie: ecografie ecografie fetală vârstă gestaţională

Abstract. During pregnancy, ultrasonography is essential because it is a easy to perform, non-invasive method, with no side-effects, and allows a good assessment of evolution, step-by-step, with a very high accuracy.
During pregnancy, ultrasonography is being used, because it has a prover efficiency in assessment of fetal developpement and amniotic fluid, a in order to exclude severe complications.

Este cunoscut faptul că pe parcursul sarcinii, medicul ginecolog recomandă o serie de investigaţii, pentru a evalua atât starea de sănătate a fătului, cât şi evoluţia sa. Una dintre aceste investigaţii este ecografia, tehnică care permite vizualizarea unor organe interne sau a fătului cu ajutorul ultrasunetelor şi care poate fi repetată de mai multe ori în timpul sarcinii, în funcţie de evoluţia acesteia.

În ultimii ani, ecografia a devenit o modalitate de diagnosticare aproape curentă şi asta în special datorită caracterului său neinvaziv pentru organismul uman, a preţului relativ scăzut şi a faptului că bolnavul nu necesită o pregătire specială sau spitalizare. În timp, această tehnică de diagnosticare a evoluat foarte mult sub raportul performanţelor, în prezent putându-se realiza în mod curent ecografii 4D, în care sunt observate, pe lângă caracteristicile morfologice ale organismului, şi acelea funcţionale. O astfel de ecografie oferă o imagine în mişcare a fătului şi poate furniza uneori mai multe detalii decât o ecografie bidimensională. Pentru sarcină, ecografia este esenţială atât din punctul de vedere al faptului că este o metodă uşor de efectuat, neinvazivă, fără reacţii adverse, cât şi din punctul de vedere al perfomanţelor, întrucât permite o bună observare a evoluţiei sarcinii, pas cu pas, cu o acurateţe foarte mare.

Efectuarea unei ecografii durează aproximativ 30 – 45 minute şi este lipsită de riscuri (chiar şi ecografia transvaginală) şi pentru mamă, dar şi pentru copil, fapt demonstrat de numeroase studii. Mai mult, oamenii de ştiinţă afirmă că această facilitate oferită părinţilor, de a-şi vedea copilul încă de când acesta se află în uter, este benefică pentru legătura emoţională care se creează între ei. Dacă mama are o legătură specială cu fătul, ea fiind cea care îi oferă „cadrul fizic“ de dezvoltare, tatăl poate să nu manifeste aceeaşi stare, însă aceasta se poate forma prin contactul vizual pe care îl are însoţindu-şi la ecografie partenera însărcinată.

Ecografia este folosită în timpul sarcinii şi s-a dovedit a fi foarte eficientă pentru a evalua dezvoltarea fătului, frecvenţa lui cardiacă, anexele fetale (placenta, cordonul ombilical), lichidul amniotic, pentru a investiga sarcina în momentele în care există riscul de pierdere a ei (durere, sângerare).

De regulă, medicii recomandă în timpul primului trimestru de sarcină (de preferat între săptămânile 11 şi 14) efectuarea unei ecografii transvaginale, o metodă mai invazivă, în sensul că este folosită o sondă vaginală pentru a vedea în interiorul uterului dezvoltarea fătului. În mod normal efectuarea ecografiei se face cu vezica plină. În acest scop este indicat consumul de apă sau al altor lichide chiar înainte de testare şi evitarea urinării înainte şi în timpul efectuării investigaţiei. În cazul unei ecografii transvaginale, transductorul este acoperit cu un manşon de latex şi cu un lubrifiant vaginal. Dacă persoana care urmează să fie examinată prezintă alergie la latex, ea va comunica acest lucru medicului înainte de investigaţie. Se recomandă consultul medical în legătură cu orice motiv de îngrijorare referitor la necesitatea efectuării ecografiei fetale, a riscurilor sale, a modului de realizare sau a rezultatelor care sunt indicate. În mod obişnuit o ecografie fetală este realizată de către un medic specialist cu atestat în ecografie obstetricală.

Ecografia abdominală

– necesită ingerarea unei cantităţi suficiente de apă pentru a umple vezica. O vezică plină ajută în transmiterea fasciculelor de ultrasunete şi împinge intestinele la distanţă de uter. Aceasta determină obţinerea unei imagini ultrasonografice mai clare;

– dacă ecografia este efectuată în ultima parte a sarcinii, o vezică plină poate să nu fie neapărat necesară, întrucât creşterea fătului va împinge intestinele de o parte;

– pacienta va fi poziţionată în clinostatism dorsal pe o masă de examinare. Dacă pacienta prezintă respiraţii scurte sau ameţeli în timp ce se întinde pe masă, partea superioară a corpului se va ridica sau se va întoarce gravida pe o parte;

– abdomenul este uns în prealabil cu gel, pentru a favoriza transmiterea ultrasunetelor;

– un instrument mic, manual, denumit transductor, va fi aplicat pe gel şi pe piele şi mişcat de-a lungul abdomenului de mai multe ori. Pacienta poate privi monitorul pentru a vedea imaginea fătului în timpul examinării. La finele investigaţiei se curăţă gelul de pe piele. Nu se recomandă micţiunea până ce investigaţia nu este realizată. Pentru o bună vizualizare a fătului, ecografia abdominală poate dura 30-60 de minute.

Ecografia transvaginală

– nu necesită ca vezica să fie plină;

– pacienta se întinde pe masă cu şoldurile uşor ridicate;

– un capişon (de forma prezervativului) va fi plasat peste transductorul vaginal. Transductorul este apoi inserat uşor în vagin şi apoi va fi mişcat şi rotat pentru a ajusta imaginea obţinută pe monitor. De obicei, ecografia transvaginală durează aproximativ 15-30 de minute.

Prima ecografie însă poate fi efectuată şi abdominal. În timpul primei ecografii, medicul va verifica dacă embrionul s-a implantat bine în uter şi nu se află în locuri improprii (sarcină extrauterină), stabilind totodată şi numărul de embrioni prezenţi.

Ecografia fetală este realizată pentru a evalua dezvoltarea fătului. Informaţii distincte ecografice necesare sunt obţinute în diferite trimestre pe parcursul sarcinii.

Astfel, în **primul trimestru de sarcină** ecografia fetală este realizată între 11 şi 13 săptămâni şi se urmăreşte:

– determinarea prezenţei şi evoluţiei sarcinii;

– viabilitatea embrionului;

– localizarea sarcinii;

– se determină dacă sarcina este multiplă sau nu;

– estimarea vârstei gestaţionale (vârsta fătului) prin măsurarea sacului gestaţional şi a lungimii craniocaudale a embrionului, sacul gestaţional poate fi observat şi măsurat la o vârstă a sarcinii de 4 - 5 săptămâni, embrionul putând fi vizualizat la 5-6 săptămâni; menţionăm că examinarea transvaginală permite vizualizarea prezenţei sarcinii sau a embrionului în sacul gestaţional cu aproximativ o săptămână mai devreme decât ecografia transabdominală;

– identificarea eventualelor malformaţii congenitale care pot afecta creierul sau măduva spinării. Această ecografie evaluează de asemenea şi vârsta sarcinii şi data concepţiei, făcând astfel posibilă determinarea datei probabile a naşterii. De asemenea, în timpul primei ecografii se va evalua în mod obligatoriu osul nazal şi, foarte important, se va măsura translucenţa nucală, un test care îi permite medicului să descopere dacă fătul prezintă riscul de a avea sindromul Down sau alte boli cauzate de anomaliile cromozomiale.

Medicul ginecolog va recomanda un nou examen ecografic între săptămânile 22 şi 24 de sarcină. Ecografia va fi abdominală, modalitate care permite evaluarea dezvoltării fătului şi a aspectului diverselor organe, motiv pentru care ea poartă denumirea de ecografie morfologică. Este considerată una dintre cele mai importante investigaţii din timpul sarcinii, întrucât pe parcursul ei se poate aprecia dacă organele fătului (inima, capul şi structurile intracerebrale, coloana vertebrală, bazinul, membrele, ficatul şi rinichii) s-au dezvoltat în mod corect.

În **trimestrul al doilea,** ecografia fetală urmăreşte:

– estimarea vârstei fătului (vârsta gestaţională);

– aprecierea dimensiunii şi poziţiei fătului, a placentei şi a fluidului amniotic; se vor măsura diametrul biparietal, lungimea femurală, circumferinţa craniană, circumferinţa abdominală, lungimea celorlalte oase lungi ale membrelor; în urma acestor măsurători se poate estima şi greutatea fătului;

– determinarea poziţiei fătului, a cordonului ombilical şi a placentei în timpul procedurilor gen amniocenteză sau a recoltării de sânge din cordonul ombilical;

– studierea, pe cât posibil, a mobilităţii fetale şi a cantităţii de lichid amniotic;

– realizarea unor măsurători ale circulaţiei sanguine la nivelul cordonului ombilical, al arterei cerebrale medii şi al arterelor uterine materne;

– detectarea malformaţiilor congenitale, ca de exemplu defectul de tub neural sau al afecţiunilor cardiace.

O altă ecografie va fi indicată în timpul ultimului trimestru de sarcină, între 33 şi 34 de săptămâni. Pe parcursul acesteia se evaluează dacă fătul s-a dezvoltat în mod corect şi dacă se încadrează în limite normale. Medicul va măsura, printre altele, circumferinţa capului şi lungimea femurului copilului şi va confrunta măsurătorile cu valori standard.

În cel de-al **treilea trimestru** ecografia fetală este realizată pentru:

– a se asigura că fătul este viabil şi pentru a determina poziţia acestuia;

– diagnosticarea unor anomalii morfologice fetale;

– evaluarea cantităţii de lichid amniotic;

– determinarea poziţiei placentei şi a gradului de maturizare;

– aprecierea dimensiunii şi a poziţiei fătului şi a greutăţii acestuia;

– aprecierea lungimii colului uterin;

– descoperirea unor posibile complicaţii (circulara de cordon, de exemplu).

În funcţie de evoluţia sarcinii, medicul poate recomanda în continuare efectuarea de ecografii periodice, care pot fi mai dese, dacă sunt particularităţi care trebuie urmărite la un interval de timp mai scurt, sau mai rare, dacă totul decurge normal. În cazul unei sarcini cu risc, dacă viitoarea mamă suferă de unele afecţiuni, cum ar fi diabetul sau hipertensiunea arterială, dacă a mai avut sarcini cu probleme, medicul va cere cu siguranță efectuarea mai multor ecografii (chiar şi una pe săptămână), pentru a monitoriza în mod constant sarcina şi a evita apariția de evenimente nedorite. O ecografie poate fi utilă şi în momentul naşterii, pentru a evalua poziția fătului şi a cordonului ombilical şi pentru a stabili necesitatea unei naşteri prin operație cezariană, pentru a evita complicațiile.

De asemenea, ea poate fi de un real folos în detectarea eventualelor probleme sau anormalități ale organelor copilului şi în localizarea fătului în cazul efectuării procedurilor pentru amniocenteză. Totodată, poate fi stabilită şi o eventuală patologie uterină, cum ar fi fibromul uterin sau malformațiile uterine.

Una dintre tainele pe care ecografia le poate dezvălui este şi sexul copilului. De regulă, în timpul ecografiei din trimestrul al doilea este posibilă şi stabilirea sexului copilului, care începe să fie vizibil începând cu luna a patra (dacă poziția lui în timpul ecografiei permite vizualizarea). Aparatura performantă folosită în majoritatea centrelor medicale permite, pe lângă efectuarea de fotografii ale micuțului, şi înregistrarea ecografiei.

De reținut!

– este demn de reținut faptul că rezultatele normale obținute la ecografia fetală nu garantează un copil normal, sănătos, numeroase studii desfăşurate în lume demonstrând că ecografia în cursul sarcinii nu detectează decât 60% dintre posibilele malformații şi doar 75% dintre fetii afectați de sindromul Down;

– medicul poate recomanda teste sau investigații suplimentare dacă rezultatele ecografiei fetale nu se încadrează în limite normale;

– bazându-ne pe efectuarea ecografiei precoce în timpul sarcinii, poate fi stabilită cu mai multă exactitate data naşterii, în funcție de dimensiunile şi dezvoltarea fătului;

– în al treilea trimestru, ecografia fetală nu determină cu exactitate vârsta gestațională sau greutatea fătului;

– efectele unei expuneri repetate a fătului la ultrasunete nu au fost determinate, totuşi nu se recomandă ecografii fetale repetate pentru motive nemedicale, cum ar fi determinarea sexului fătului sau pentru obținerea unor imagini „utile” doar pentru colecția personală;

– ecografia 4-D este testată pentru utilizarea în evaluarea anomaliilor fetale. Momentan nu este uşor disponibilă;

– ecografia Doppler (sau scanarea duplex) foloseşte fascicule de ultrasunete reflectate pentru a estima viteza şi direcția sângelui şi a fluxului sanguin de la la nivelul placentei la nivelul fătului.

Nu se cunosc riscuri asociate cu efectuarea ecografiei fetale, atât la mamă cât şi la făt. Totuşi, nu sunt excluse simptome de anxietate, dacă se depistează o afectare a sarcinii sau o malformație la nivelul fătului. Salvarea imaginii ecografice face posibilă folosirea ecografiei ca oportunitate a realizării primei fotografii a copilului.

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Rolul axei hipotalamo-hipofizo-corticosuprarenaliene în patogeneza tulburărilor de anxietate

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Rezumat. Analiza dovezilor experimentale, clinice şi preclinice, existente în prezent, susţine implicarea axei hipotalamo-hipofizo-corticosuprarenaliene (HHC) în fiziopatologia tulburărilor de anxietate, dar şi în a celor depresive. Glucocorticoizii sunt elemente esenţiale ale răspunsului de stres şi reprezintă agenţi de mobilizare a rezervelor energetice ale organismului în condiţii nefavorabile, contribuind la creşterea abilităţilor mnezico-prosexice, precum şi la reglarea funcţiei unor structuri nervoase cum ar fi hipocampul, amigdala, cortexul prefrontal. La nivel preclinic, hormonii axei HHC au fost implicaţi în medierea condiţionării prin frică, iar la nivel clinic aplicarea testului de supresie cu dexametazonă indică o secreţie modificată faţă de nivelul fiziologic al corticosteroizilor în tulburarea de stres posttraumatic. Din punct de vedere al aplicaţiilor practice pe care le implică analiza disfuncţiilor acestor mecanisme fiziopatologice, sunt importante de menţionat noile medicamente anxiolitice, bazate pe blocarea receptorilor hormonului de eliberare a corticotropinei (CRH) de tip CRH-1, al căror scop este oprirea cascadei cortizolice. Antalarmina este un astfel de agent care poate reduce eliberarea de ACTH ca răspuns la stresul cronic. Sistemul axei HHC interacţionează cu sistemul noradrenergic în procesele de consolidare a amintirilor traumatice, fenomen presupus a avea un rol important în crearea şi menţinerea simptomelor din tulburarea de panică şi tulburarea de stres posttraumatic. Astfel, o nouă posibilă ţintă farmacologică ar fi modularea interacţiunilor CRH- noradrenalină de la nivel hipocampic, pentru a putea preveni structurarea unor simptome cheie ale tulburărilor anxioase.

Cuvinte cheie: tulburări de anxietate, axa hipotalamo-hipofizo-corticosuprarenaliană, hormonul eliberator de corticotropină, anxiolitice

Abstract. Actual data analysis of both preclinical and clinical experiments supports the involvement of hypothalamic-pituitary-adrenal (HPA) axis in the pathophysiology of anxiety disorders, as well as in the pathology of depressive disorders. Glucocorticoids are essentials for the stress response and stand as energetic resources mobilizing agents in negative environment, where they act for increasing memory and attentional capacities, mediating regulatory functions of hippocampus, amygdala or prefrontal cortex. In the preclinical level, the hormones of HPA axis were involved in fear conditioning mechanisms, while the clinical level is characterised by modified secretion of corticosteroids, evoked by altered dexametasone suppression test, in posttraumatic stress disorder. From a psychopharmacologic perspective, the analysis of these pathophysiological mechanisms is important because of new anxiolytic drugs, designed to block the type I corticotropin releasing hormone receptors (CRH-1). Antalarmin is an experimental drug used for reduce the ACTH release in response to chronic stress. The HPA has a complex interaction with norepinephrine system in consolidation of traumatic memories, a phenomenon involved in the genesis and maintaining of panic disorder and posttraumatic stress disorder symptoms. A new possible pharmacologic target is modulating CRF- norepinephrine interactions at hippocampus, in order to lower the possibility of anxiety disorders key symptoms appearance.

1. Implicarea axei hipotalamo-hipofizo-corticosuprarenaliene în fiziopatologia anxietăţii

Cortizolul este un agent de mobilizare şi reumplere a rezervelor de energie, contribuind la creşterea vigilenţei, focalizarea atenţiei, formarea memoriei, inhibarea creşterii organismului şi a sistemului reproducător, controlul răspunsului imun. Cortizolul are efecte reglatorii importante asupra activităţii neuronilor de la nivel hipocampic, amigdalian şi din cortexul prefrontal. Glucocorticoizii pot creşte activitatea amigdalei, formarea de ARNm pentru CRF în amigdală, pot amplifica efectele CRH în apariţia fricii condiţionate şi pot facilita encodarea la nivel mnezic a stimulilor emoţionali. Aceste efecte pot explica reglarea mecanismelor adaptative induse de cortizol în timpul răspunsului acut la stres.

Expunerea acută la factorii stresori determină eliberarea de CRH, ACTH şi cortizol. Activarea axei hipotalamo-hipofizo-corticosuprarenale (HHC) în timpul stresului acut produce o creştere tranzitorie a cortizolului plasmatic şi o rezistenţă parţială la inhibiţia prin feedback a eliberării de cortizol, care persistă un timp scurt şi după dispariţia stresorului. Acest fenomen implică o down-reglare rapidă a receptorilor de glucocorticoizi, deoarece nivele înalte plasmatice ale acestor hormoni în timpul stresului scad numărul de receptori asociaţi în zona hipocampului, rezultând o creştere a secreţiei de corticosteroizi. După terminarea acţiunii factorului stresor, pe măsură ce nivelele de glucocorticoizi scad, densitatea receptorilor pentru aceşti hormoni creşte, iar sensibilitatea buclei de feedback se normalizează.

În timpul stresului cronic, apar modificări adaptative ale secreţiei de ACTH şi corticosteroizi, astfel încât nivelele plasmatice ale acestor hormoni sunt mai reduse decât cele atinse în timpul stresului acut. Anumite tipuri de stres cronic se asociază totuşi cu o creştere a secreţiei de corticosteroizi la şoarecii de laborator. Expunerea anterioară la stres produce răspunsuri mai ample ale descărcării de corticosteroizi la stimulări ulterioare, în cadrul unui fenomen de sensibilizare la stres.

Unii stresori care apar în perioade critice de dezvoltare exercită efecte pe termen lung asupra axei HHC. La şoarecii de laborator expuşi unor factori stresori puternici, induşi în perioada prenatală sau care au fost stimulaţi aversiv prin deprivare maternă timpurie, se observă creşteri ale concentraţiei plasmatice de corticosteroizi atunci când aceştia se confruntă ulterior cu alţi stresori, iar această tendinţă persistă şi la maturitate. Experienţele stresante care apar postnatal precoce sunt asociate cu alterări de durată ale concentraţiei bazale de ARNm pentru ACTH la nivel hipotalamic, deficite ale ARNm pentru receptorii hipocampici ai glucocorticoizilor, alterări ale răspunsului CRH, ACTH şi ale corticosteroizilor la stres. La primate, experienţele aversive precoce induc modificări ale comportamentului social atât în copilărie, cât şi la maturitate, caracterizate prin timiditate, reducerea interacţiunilor sociale şi creşterea submisivităţii. Aceşti subiecţi au de asemenea hiperresponsivitate la yohimbină, concentraţii crescute de CRH şi scăzute de cortizol la nivelul lichidului cefalorahidian, trăsături biologice asemănătoare cu cele ale adulţilor cu tulburare de stres posttraumatic.

Experienţe favorabile survenite în prima parte a dezvoltării pot avea consecinţe benefice pe termen lung asupra capacităţii organismului de a dezvolta răspunsuri adaptative la stres sau ameninţări. La nivel neurobiologic, consecinţele îngrijirii atente şi a stimulilor afectivi plăcuţi la şoarecii de laborator, în prima parte a vieţii acestora, au constat în creşteri ale densităţii receptorilor tip II pentru glucocorticoizi şi, consecutiv, ale sensibilităţii feedback-ului la corticosteroizi, precum şi leziuni mai reduse la nivel hipocampic mediate de hormonii de stres. Un nivel înalt al neuroplasticităţii există la sistemele nervoase implicate în reacţia la stres, în special în perioada prenatală şi imediat postnatală, sisteme care programează reacţiile biologice ulterioare la stres.

Diferenţele regionale în reglarea funcţiei CRF, produse de către stimularea receptorilor pentru glucocorticoizi şi stres, pot fi importante în medierea fricii şi a anxietăţii. Inhibiţia prin feedback a funcţiei CRH prin glucocorticoizi (pentru a suprima activitatea axei hipotalamo-hipofizo-suprarenaliene) apare la nivelul nucleului paraventricular al hipotalamusului (unde administrarea sistematică a glucocorticoizilor scade expresia CRH) şi la nivelul hipofizei anterioare (unde glucocorticoizii scad expresia receptorilor pentru CRH). Reglarea expresiei ARNm pentru receptorii CRH prezintă o specificitate regională, care se modifică atunci când stresul apare concomitent cu concentraţii crescute de glucocorticoizi. Atât după administrarea pe termen scurt, cât şi după administrarea pe termen lung a corticosteroizilor, expresia ARNm pentru receptorii CRH scade la nivelul nucleului paraventricular şi în hipofiza anterioară. După acţiuni repetate, pe modelele animale, ale stresorilor fizici suficient de puternici pentru a creşte mai mult nivelele plasmatice de cortizol, expresia ARNm pentru CRH scade în hipofiza anterioară, dar creşte în nucleul paraventricular. Nici stresorii fizici şi nici administrarea de cortizol nu alterează expresia receptorilor pentru CRH în nucleul central amigdalian sau *stria terminalis*. Secreţia de CRH nu este inhibată de glucocorticoizi în nucleul amigdalian central sau în *stria terminalis*, iar expresia de ARNm pentru CRH creşte în aceste arii în timpul administrării cronice de cortizol. Astfel, se consideră că feedback-ul pozitiv al glucocorticoizilor asupra funcţiei extrahipotalamice a CRH în amigdala şi *stria terminalis* poate contribui la producerea simptomelor anxioase.

Un alt mecanism prin care sistemul CRH-glucocorticoizi menţine homeostazia şi asigură mecanismele de modulare a reacţiilor adaptative la stres sau stimuli anxiogeni implică diferenţe funcţionale între variatele subtipuri receptorale pentru CRH. Receptorii CRH-1 şi 2 au roluri complementare în medierea responsivităţii la stresori şi a comportamentelor anxioase. CRH are afinitate diferită pentru cele două tipuri de receptori, mai mare pentru CRH1 (responsabili de apariţia anxietăţii), ceea ce susţine ideea că CRH determină efecte anxiogene atunci când este administrată exogen la animale sau când se eliberează endogen la şoareci modificaţi genetic pentru a supraexprima CRH.

Distribuţia diferită a receptorilor CRH-1 şi 2 are un rol în echilibrul efectelor facilitante şi a celor inhibitorii determinate de stimularea corticosuprarenalei în timpul stresului acut. La primate, densitatea de receptori CRH-1 este crescută în nucleii amigdalei, cortexul cingulat, cortexul prefrontal, cortexul insular şi parietal, girusul dinţat, cortexul entorinal şi este moderată în nucleul central amigdalian şi *locus coeruleus*. Receptorii CRH-2 sunt distribuiţi mai ales în cortexul cingulat, cortexul prefrontal medial, nucleul amigdalian central, aria CA-1 din hipocamp, nucleul paraventricular şi cel supraoptic hipotalamic.

2. Tulburarea de stres posttraumatic

Nivelele crescute de CRH în prima parte a vieţii corelează cu riscul de a dezvolta tulburare de stres posttraumatic după o nouă expunere la un eveniment nefavorabil de-a lungul vieţii. Acelaşi mecanism al secreţiei crescute de CRH răspunde şi de reducerea volumului hipocampic în tulburarea de stres posttraumatic. Capacitatea CRH de a creşte densitatea receptorilor de mineralocorticoizi hipocampici poate fi o explicaţie a nivelelor crescute de CRH şi normale sau reduse de cortizol, la unii pacienţi cu tulburare de stres posttraumatic.

Alterările funcţiei axei HHC au fost observate în tulburarea de stres posttraumatic, la pacienţii cu forma cronică a acestei tulburări fiind observată creşterea supresiei cortizolului la dexametazonă, opusul pattern-ului cortizolic din depresie. Rezultatele studiilor au fost contradictorii în privinţa concentraţiei de cortizol din urina de 24 ore şi plasmatică bazală, care a fost găsită scăzută, nemodificată faţă de lotul de control sau crescută la pacienţii cu tulburare de stres posttraumatic. Există diferenţe legate de sex, vârstă, tipul sau durata traumei, precum şi variaţii fiziologice legate de faza bolii. De exemplu, un studiu a arătat concentraţii crescute de cortizol în urina de 24 ore la bărbaţii, dar nu şi la femeile cu tulburare de stres posttraumatic, iar aceste anomalii au fost observate la o lună, nu şi la 6 luni după evenimentul traumatic.

Pacienţii de sex masculin cu tulburare de stres posttraumatic au o reacţie slabă a secreţiei de ACTH la stimularea cu factorul de creştere a corticotropinei (CRH), comparativ cu lotul de control. La femeile aflate în premenopauză, diagnosticate cu tulburare de stres posttraumatic au fost găsite nivele crescute de ACTH ca reacţie la stimularea cu CRH.

În timpul provocărilor comportamentale sau farmacologice ale axei hipotalamo-hipofizo-suprarenaliene, la pacienţii cu tulburare de stres posttraumatic nu au fost observate schimbări în concentraţia plasmatică de cortizol şi ACTH statistic semnificative între subiecţii sănătoşi, cei diagnosticaţi cu tulburare de stres posttraumatic şi cei expuşi traumelor dar care nu au dezvoltat tulburare de stres posttraumatic. Aceşti pacienţi cu tulburare de stres posttraumatic au un număr crescut de receptori pentru glucocorticoizi în limfocitele periferice şi o hiperfuncţie a acestor receptori în puncte cheie (hipotalamus) poate rezulta în sensibilitate crescută a feedback-ului la cortizol, apărând nivele scăzute de cortizol periferic. O reducere a secreţiei de cortizol după o traumă poate fi un marker de predicţie a dezvoltării unei tulburări de stres posttraumatic, ceea ce sugerează că o sensibilitate crescută a feedback-ului la cortizol poate apărea rapid după debutul sau chiar poate preceda instalarea tulburării.

Eliberarea la nivel central a CRH în lichidul cefalorahidian la pacienții cu tulburare de stres posttraumatic a fost evaluată în două studii care au găsit valori anormal de crescute la veteranii de război cu tulburare cronică. Răspunsul ACTH la stimularea cu CRH a pacienților cu tulburare de stres posttraumatic a fost redus comparativ cu subiecții sănătoşi, deşi concentrațiile de cortizol în periferie au fost găsite fie mai mici, fie normale sau crescute. Această neconcordanță se datorează controlului independent al secreției de CRH extrahipotalamice față de cea hipotalamică. Totuşi, studiile care au identificat reduceri ale concentrației sau nu au găsit creşteri ale cortizolemiei periferice la pacienții cu tulburare de stres posttraumatic reprezintă o provocare la adresa ipotezei că volumul hipocampic redus observat pe RMN la aceşti pacienți s-ar datora hipersecreției cortizolice. Dacă secreția de cortizol este crescută în imediata apropiere a momentului stresant, la pacienții cu tulburare de stres posttraumatic cronică, ipoteza reducerii volumului hipocampic în legătură cu glucocorticoizii se poate susține.

Studii longitudinale la pacienții cu tulburare de stres posttraumatic dezvoltată în urma accidentelor rutiere sugerează că secreția de cortizol este crescută la o lună după traumă, dar este normală la 6 luni după acțiunea factorului stresor. O ipoteză alternativă pentru reducerea volumului hipocampic în tulburările de stres este aceea că anomalia anatomică precede dezvoltarea tulburării şi poate fi considerată un factor vulnerabilizant.

3. Tulburarea de panică

La pacienții cu tulburare de panică, reacțiile slabe la stimularea cu CRH au fost raportate inconstant. Nivele normale şi crescute de nonsupresie a cortizolului la dexametazonă au fost de asemenea raportate. Responsivitatea axei HHC la stimularea simultană cu dexametazonă şi CRH a fost mai mare în panică decât la subiecții normali, dar mai redusă decât la depresivi. Nivelele urinare de cortizol liber au fost variabile. Pacienții cu tulburare de panică au avut secreție cortizolică crescută peste noapte şi cu o amplitudine mare a episoadelor secretorii ultraradiene, iar cei cu tulburare severă au asociat mai frecvent secreție amplă de cortizol. Răspunsul ACTH la stimularea cu CRH a fost redus în unele studii dar nu în altele, comparativ cu subiecții sănătoşi, deşi nivelul de CRH în lichidul cefalorahidian nu diferă semnificativ între pacienți şi subiecții sănătoşi.

4. Interacțiunile funcționale ale axei HHC cu sistemul noradrenergic

Achiziția unor comportamente adaptative la stres necesită conlucrarea sistemelor de neurotransmițători. Astfel, secreția de CRH creşte activitatea neuronilor din *locus coeruleus*, conducând la eliberare crescută de norepinefrină în mai multe zone cortico-subcorticale. Invers, eliberarea de noradrenalină stimulează secreția de CRH din nucleul paraventricular hipotalamic, sediul neuronilor care sintetizează CRH.

În timpul stresului cronic, *locus coeruleus* mediază eliberarea de noradrenalină în nucleul paraventricular, iar secreția de CRH de la acest nivel stimulează eliberarea ACTH din hipofiză. În continuare, stresul cronic creşte concentrația de cortizol care, printr-un feedback negativ, scade atât concentrația de CRH, cât şi de norepinefrină la nivelul nucleului paraventricular. Inhibiția mediată de glucocorticoizi a eliberării de CRH indusă de norepinefrină devine evidentă în perioadele de stres, ca un răspuns adaptativ ce diminuează amplitudinea efectelor cardiovasculare şi neuroendocrine mediate de nucleul paraventricular.

Interacțiunea dintre norepinefrină şi secreția de glucocorticoizi implică achiziția şi consolidarea amintirilor traumatice. O trăsătură caracteristică a tulburării de stres posttraumatic şi tulburării de panică este menținerea amintirii evenimentului traumatic, respectiv a primului atac de panică, precum şi reamintirea acestor informații ca reacție la multipli stimuli sau factori stresori.

Pe modelele animale, alterări ale nivelelor de catecolamine şi glucocorticoizi afectează consolidarea şi recuperarea informațiilor saturate emoțional stocate. Glucocorticoizii influențează stocarea mnezică prin activarea receptorilor specifici de la nivel hipocampic, iar efectele norepinefrinice sunt mediate prin stimularea receptorilor β amigdalieni. La oameni, supresia adrenocorticală blochează efectele de creştere a memoriei induse de amfetamine şi norepinefrină, iar propranololul afectează memoria unor evenimente cu rezonanță afectivă, nu şi a celor „neutre” din punct de vedere afectiv. Aceste date susțin că eliberarea acută de glucocorticoizi şi norepinefrină în reacțiile la traume pot modula encodarea amintirilor traumatice. Este posibil ca alterarea pe termen lung a acestor sisteme să determine distorsiunile mnezice observate în tulburarea de stres posttraumatic, cum ar fi memoria fragmentară, hipermnezia, deficite ale memoriei declarative.

5. Aplicații psihofarmacologice

Un număr important de companii farmaceutice au în programele de cercetare agenți terapeutici cu selectivitate ridicată pentru receptorii CRH-1, care urmăresc blocarea acestora şi reducerea cascadei cortizolice. Molecule cu greutate mică, non-peptidice, care modulează activitatea acestor receptori sunt studiate pentru tratamentul depresiei, anxietății şi tulburărilor de stres. Astfel de substanțe sunt, de exemplu, CP-154, 526 şi analogul său metilat, antalarmina, SC 241, R-121919, CR 1001.

Primul agent, CP-154, 526, a demonstrat efecte anxiolitice în unele studii preclinice, dar rezultatele sunt deocamdată contradictorii.

Antalarmina posedă proprietăți de blocare a receptorilor CRF-1, reducând consecutiv eliberarea de ACTH ca răspuns la stresul cronic. Antalarmina inhibă răspunsurile autonome simpatice, neurovegetative şi comportamentale la stresorii sociali, când a fost administrată la primate. Antalarmina poate fi un tratament de viitor pentru tulburările anxioase, hipertensiunea indusă de stres, reducerea simptomelor de sevraj după administrarea cronică de opioide.

Un alt agent farmacologic, R-121919, a demonstrat efecte anxiolitice în studii de fază II, în care pacienți cu depresie severă (scorul mediu pe Scala Hamilton pentru Depresie-HAMD a fost, la evaluarea inițială, 25) au primit doze de 5-40 mg/zi sau 40-80 mg/zi. La cei care au primit doze mai reduse, 50% au răspuns favorabil, adică au avut o scădere a scorului HAMD de minim 50%, iar 20% din subiecți au ajuns la remisiune (scor maxim pe HAMD 8). La cei care au primit doze medii, 80% au răspuns favorabil şi 60% au atins nivelul cerut pentru declararea remisiunii. Nu au fost raportate efecte adverse semnificative, iar nivelele bazale şi induse de stres nu au fost modificate, ceea ce sugerează că blocarea cronică a axei HHC nu produce neapărat efecte secundare. Totuşi, acest compus nu se mai află în cercetare deoarece studii ulterioare au demonstrat creşteri ale nivelului enzimelor hepatice.

În privința receptorilor CRH-2 sunt necesare încă studii de farmacologie fundamentală, care să stabilească mai precis proprietățile acestora. Astfel, agoniştii receptorilor CRH-2 se pare că ar poseda efecte anxiolitice, mai degrabă decât antagoniştii acestora.

6.Concluzii

Implicarea axei HHC în fiziopatologia tulburărilor anxioase a fost dovedită de studii efectuate la nivel preclinic, folosindu-se modelele animale ale anxietății şi condiționării prin frică, dar şi la nivel clinic, pe pacienții cu tulburare de panică şi tulburare de stres posttraumatic. Anomaliile testului de supresie a secreției cortizolice la dexametazonă, nivelele modificate de CRH şi corticosteroizi, precum şi a metaboliților urinari ai acestora, deşi inconstante, reprezintă elemente care susțin rolul axei HHC în patologia anxioasă. Deşi sunt necesare studii experimentale riguroase pentru evaluarea proprietăților receptorilor CRH în modularea anxietății, datele existente până acum au furnizat agenți farmacologici testabili pe modele preclinice şi chiar clinice. Totuşi, până în prezent, medicamentele care se adresează controlului disfuncțiilor axei HHC în patologia anxioasă nu au depăşit faza II de cercetare experimentală. În perspectivă, sunt necesare evaluări ale interacțiunilor axei HHC, CRH şi ACTH cu sistemul noradrenergic şi cu alte sisteme de neurotransmițători şi neuromodulatori, cum ar fi peptidul vasoactiv intestinal (VIP), implicați în modularea reacției de stres.

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Pandemia de gripă cu noul virus A(H1N1), riscul profesional şi prevenirea îmbolnăvirii

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La data de 11 iunie 2009, Organizaţia Mondială a Sănătăţii (OMS) a declarat pandemie de gripă cu noul virus A(H1N1), trecând la măsurile epidemiologice prevăzute pentru nivelul 6, cel mai înalt. Aceasta este prima pandemie a secolului XXI şi prima pandemie de gripă survenită după 41 de ani. După detectarea gripei "porcine" în Mexic şi identificarea virusului, în luna aprilie 2009, în mai puţin de două luni acest virus a infectat un mare număr de oameni, transmiterea virusului fiind considerată de OMS ca "nestopabilă". La data declarării pandemiei, dintre cazurile de gripă cunoscute, 29669 erau confirmate cu noul virus A(H1N1) în 74 de ţări, din care 145 au murit.

Din punct de vedere statistic, situaţia nu este gravă până acum, din populaţia totală doar 1/200000 a contractat virusul, iar dintre bolnavi au decedat sub 0,5%. Evoluţia bolii nu este gravă, până în prezent, fiind sub indicele de mortalitate mediu al gripei. Dar se pare că virusul a circulat timp de câţiva ani în populaţiile de porci, cu gripa porcină nediagnosticată, până când, probabil, în august 2008 s-a produs faimoasa hibridizare virală (şase componente de virus de gripă porcină, una de virus de gripă aviară şi una de virus de gripă umană), care a permis transmiterea interumană directă, noul virus provocând acum o epidemie şi nu o zoonoză. Există chiar şi ipoteze mai îndrăzneţe, că virusul ar fi, de fapt, un agent biologic creat special pentru bioterorism (pornind de la faptul că în natură hibridizarea se poate face numai între două specii înrudite şi niciodată între trei specii), sau că ar fi un experiment ratat pentru obţinerea de noi vaccinuri gripale etc., aceste ipoteze fiind în stadiul de "anchetă ştiinţifică" la nivelul OMS. În această situaţie se estimează că există un risc epidemic major ca extindere şi ca durată, iar măsurile trebuie să fie adecvate; categoriile socio-profesionale de risc vor fi reprezentate în primul rând de personalul medical, forţele de intervenţie în caz de urgenţă, şi de forţele de ordine, în cazul că se instituie măsuri de carantină. Încercăm să creionăm, prin acest articol, o serie de recomandări practice adresate în primul rând personalului militar, pe baza documentaţiei şi recomandărilor recente adresate specialiştilor şi publicului de către Ministerul Sănătăţii şi OMS.

Pandemia de gripă. Gripa este o infecţie comună în special în timpul lunilor de iarnă. Boala, cauzată de virusul gripal, poate fi uşoară sau severă şi, ocazional, poate conduce la deces. În general, unele grupuri de oameni pot fi mai susceptibile la complicaţiile gripei decât altele, în special persoanele în vârstă şi oamenii de orice vârstă cu anumite condiţii medicale. Acesta este motivul pentru care vaccinarea gripală este recomandată acestor grupuri de oameni anual. O pandemie este o epidemie răspândită în întreaga lume. Pandemia de gripă este diferită de gripa sezonieră deoarece ea survine atunci când apare un nou virus gripal la nivelul populaţiilor, faţă de care majoritatea oamenilor vor fi susceptibili şi care se poate răspândi uşor de la o persoană la alta în întreaga lume. Toate ţările vor fi afectate, motiv pentru care a fost declarată pandemia.

Semnele şi simptomele gripei. Este foarte posibil ca semnele şi simptomele gripei pandemice să fie similare cu cele ale gripei sezoniere, dar ele ar putea fi mai severe şi ar putea determina complicaţii mult mai serioase.

Cele mai semnificative simptome sunt debutul brusc, cu febră şi tuse şi/sau dificultăţi ale respiraţiei.

Alte simptome pot include: dureri de cap, oboseală, frisoane, dureri musculare, dureri în gât, secreţie nazală apoasă, strănut, pierderea apetitului (folosim termenii uzuali, din limbajul colocvial, şi nu termenii medicali, pentru ca acest ghid să poată fi bine înţeles şi uşor de urmat de oricare cititor).

Perioada de incubaţie (timpul între contactul cu virusul şi debutul simptomelor): intervalul este între o zi şi 4 zile, deşi la majoritatea oamenilor va fi de 2 sau 3 zile.

Perioada de infecţiozitate (cât timp eşti infecţios pentru alţii): oamenii bolnavi au infecţiozitatea maximă imediat după dezvoltarea simptomelor; ei pot continua să răspândească virusul, prin tuse şi strănut, până la 5 zile (7 zile la copii). Infecţiozitatea scade pe măsură ce simptomele încep să dispară, şi odată ce simptomele dispar, ei nu mai sunt infecţioşi pentru alţii.

Cum este gripa pandemică contractată şi răspândită. Este posibil ca o pandemie de gripă, ca şi gripa sezonieră, să se răspândească de la o persoană la alta prin contact direct. Câteva exemple de felul în care gripa se poate răspândi includ: picăturile mari expulzate prin tuse şi/sau strănut de o persoană infectată la o distanţă foarte mică (de obicei un metru sau mai mică) faţă de altcineva; atingerea sau strângerea mâinii unei persoane infectate şi apoi atingerea gurii, ochilor sau a nasului fără ca mâinile să fie spălate înainte; atingerea suprafeţelor sau a obiectelor (mânerul uşii, de exemplu) care s-au contaminat cu virusul pandemic şi apoi atingerea gurii, ochilor sau a nasului fără spălarea înainte a mâinilor.

Ce ar trebui să facă indivizii care au simptome sau care sunt bolnavi. Dacă un individ se simte bolnav sau are simptome compatibile cu gripa în timp ce se află la muncă, este important ca el sau ea să nu continue să lucreze. Simptomele lor trebuie să fie aduse la cunoştinţă imediat şefului/managerului lor sau departamentului de sănătate de la locul de muncă şi dacă ele sunt compatibile cu gripa, individul ar trebui să fie trimis acasă sau izolat. Se recomandă să contacteze "Linia Naţională pentru Pandemia de gripă" (care este operabilă în timpul pandemiei) şi să nu se întoarcă la muncă până la dispariţia simptomelor şi când se simt suficient de bine. Dacă indivizii dezvoltă simptomele când nu sunt la muncă, ei ar trebui să respecte următoarele sfaturi: să stea acasă (auto-izolare), să nu se reîntoarcă la muncă până când nu-şi revin total, să contacteze "Linia Naţională pentru Pandemia de gripă" pentru sfaturi şi pentru o evaluare iniţială a simptomelor, să informeze angajatorul sau departamentul de sănătate că sunt bolnavi.

Ce ar trebui să facem pentru a ne proteja pe noi şi pe alţii de gripa pandemică. Este important ca următoarele proceduri să fie respectate: să se folosească un şerveţel pentru a se acoperi nasul şi gura când se tuşeşte şi/sau se strănută, să se înlăture şerveţelul imediat după folosire şi apoi să ne spălăm pe mâini; şerveţelele ar trebui să fie înlăturate în containerele cu deşeuri menajere (ele nu necesită un tratament special); să nu se folosească batiste din materiale textile reutilizabile (batiste), sau refolosească şerveţelele de unică folosinţă, deoarece această manevră este însoţită de riscul de contaminare a buzunarelor şi a genţilor, care ar putea recontamina apoi mâinile de fiecare dată când se ating acele buzunare sau genţi; să ne spălăm pe mâini frecvent, în special după ce tuşim, strănutăm sau folosim şerveţele. Săpunul şi apa reprezintă o modalitate foarte eficientă de a curăţa mâinile; dar şi soluţiile de curăţat pe mâini (soluţiile antimicrobiene, în special cele pe bază de alcool) pot fi folosite ca o alternativă; este indicat să se evite atingerea gurii, a ochilor şi/sau a nasului, sau numai după ce ne-am spălat recent pe mâini; detergenţii şi apa obişnuită din gospodării ar trebui să fie folosite pentru a curăţa suprafeţele frecvent atinse cu mâna, este necesar să ne spălăm pe mâini imediat ce ajungem la muncă şi acasă.

RĂSPÂNDIREA BOLII. Pentru ca boala să fie răspândită în interiorul unei comunităţi trebuie să existe o sursă de infecţie, o cale prin care infecţia să se transmită, şi indivizi care sunt susceptibili la boală (lanţul epidemiologic).

Sursa: indivizii simptomatici. Este în general acceptat că o persoană poate fi considerată potenţial infecţioasă din momentul în care apar simptomele până în momentul în care simptomele dispar complet. În general, cu cât sunt mai severe simptomele, cu atât persoana poate să fie mai infecţioasă.

Transmiterea: prin intermediul picăturilor la o distanţă mai mică de un metru sau prin contact direct/indirect. Gripa este în general transmisă de la o persoană la alta prin contact direct şi la distanţe mici (cam la un metru). Modelul de transmitere este asociat cu răspândirea picăturilor respiratorii care provin din tuse şi strănut, prin contact direct cu o persoană bolnavă, sau indirect, prin intermediul obiectelor sau al suprafeţelor care sunt acoperite cu secreţii infectate cu virus. Virusurile gripale sunt uşor înlăturate şi distruse prin săpun şi apă, detergenţi obişnuiţi, sau soluţiile de spălat pe mâini (soluţii antimicrobiene, în special cele pe bază de alcool).

Cel care se infectează: individul susceptibil. Pentru a realiza transmisia virusului, indivizii susceptibili la boală trebuie să fie prezenţi. Până când un individ devine imun, prin infecţie naturală sau prin vaccinare, el prezintă risc de infecţie.

ACŢIUNI DE LIMITARE. Intervenţiile care blochează o parte sau întreaga cale de transmitere a virusului de la o persoană cu simptome compatibile cu gripa la o persoană susceptibilă au potenţial de a întrerupe lanţul transmiterii infecţiei. Aceste intervenţii au în general următoarele obiective: reducerea transmiterii infecţiei de la un individ (sau mai mulţi) cu simptome compatibile cu gripa la o persoana susceptibilă şi/sau reducerea riscului ca oamenii susceptibili să se infecteze.

Măsurile care ar trebui să fie luate în considerare în efortul de a reduce răspândirea virusului gripal în mediul de lucru se încadrează în trei categorii:

De mediu	Acţiuni desfăşurate în mediu pentru a reduce răspândirea virusului
Organizatorice	Acţiuni desfăşurate pentru a modifica comportamentul şi procedurile de la locul de muncă pentru a ajuta la reducerea răspândirii virusului
Comportament individual	Acţiuni desfăşurate la nivel de individ pentru a restricţiona răspândirea virusului gripal

Aceste acţiuni sunt clasificate în acest fel pentru a reflecta eficienţa lor potenţială.

Utilizarea echipamentului de protecţie personală, precum măştile de faţă, de către indivizi este considerată a fi cel mai puţin eficientă, deoarece se bazează pe respectarea şi pe interpretarea ghidului, şi este foarte uşor pentru indivizi să manipuleze greşit, să folosească incorect, sau să eşueze în folosirea lor. Ea tinde uneori să dea o impresie falsă de protecţie şi poate conduce la abandonul altor măsuri de control, mai eficiente. Dovada de bază cu privire la utilizarea măştilor de faţă de către publicul general este în momentul actual prea nesigură şi prea limitată pentru a sprijini puternic utilizarea măştilor de faţă de către public în timpul unei pandemii de gripă.

Măsuri la locul de muncă. Am încercat să identificăm măsurile pentru reducerea răspândirii virusului gripal la locul de muncă, acestea fiind grupate în două categorii mari, în funcţie de obiectivele intervenţiei subliniate anterior, iar procedurile sunt grupate în: comportament în mediu ambiant, organizatoric sau individual.

1. Reducerea transmiterii infecţiei de la un individ simptomatic. Principala acţiune va fi promovarea importanţei auto-izolării imediate şi eficiente de către indivizii cu simptome care sugerează o gripă; aceştia vor fi sfătuiţi să stea acasă până la dispariţia simptomelor. Acolo unde auto-izolarea nu este imediat posibilă, oamenii cu simptome de gripă vor adopta o igienă respiratorie şi a mâinilor adecvată şi, atunci când este posibil, vor evita, interacţiile apropiate şi contactul direct cu alţi oameni.

*Mediul ambiant***.** Semnele evidente ar trebui să descurajeze personalul şi vizitatorii care au simptomele gripei să intre la locul de muncă şi să amintească oamenilor de: semnele şi simptomele gripei, importanţa auto-izolării persoanelor cu simptome compatibile cu gripă, importanţa în orice moment a igienei respiratorii şi a mâinilor; suprafeţele ar trebui să fie curăţate frecvent cu materiale obişnuite de curăţat; acolo unde se poate, utilizarea eficientă a barierelor fizice (paravan sau tejghea) ar trebui să fie folosite pentru a ajuta la restricţionarea interacţiilor apropiate şi a contactelor directe cu vizitatorii sau clienţii potenţiali; este necesar să se acorde o atenţie sporită îmbunătăţirii accesului la facilităţi pentru igiena eficientă a mâinilor. Acolo unde se poate, se vor pune la dispoziţie soluţii de curăţare pe mâini (soluţii antimicrobiene, în special pe bază de alcool), la intrările în locurile care sunt folosite de vizitatori sau clienţi; unde este posibil, se va minimiza numărul corpurilor de mobilă şi al altor obiecte care ar putea să fie contaminate şi care sunt dificil de curăţat.

Organizatoric. Conştientizarea în rândul personalului expus a semnelor şi simptomelor gripei şi a necesităţii auto-izolării individuale (să stea acasă) dacă au simptome care sugerează o gripă; se ia în considerare procedura cea mai bună pentru oamenii care prezintă simptomele gripei la locul de muncă; se promovează regula prin care persoanele care nu se simt bine pot să plece acasă, unde vor sta până când se fac bine; se iau în considerare alternativele întâlnirilor directe şi ale vizitelor (conferinţe video sau prin telefon); atunci când contactele cu o persoană care are simptomele compatibile unei gripe (client/vizitator) nu pot fi evitate, se reduce riscul de transmitere a bolii la personal sau a altora printr-o igienă bună a mâinilor înainte de intrarea în locurile respective sau înainte de manevrarea bunurilor. Atunci când se lucrează cu documente sau bani, personalul va fi sfătuit să minimizeze contactul cu gura, ochii sau nasul până când se spală pe mâini; practicarea unor reguli de igienă respiratorie precum acoperirea gurii cu un şerveţel atunci când se tuşeşte sau se strănută; acolo unde se poate, contactul direct ar trebui să fie evitat şi, unde este posibil, va fi menţinută o distanţă de mai mult de un metru între personal şi client/vizitator; acolo unde interacţiile sociale nu pot fi evitate, indivizii cu simptome compatibile cu gripă vor fi sfătuiţi să minimizeze interacţiile apropiate şi/sau contactul direct cu alţi oameni. Alternativ, dacă contactele nu pot fi evitate, persoanele cu simptome de gripă sunt rugate să poarte o mască de faţă, dacă este disponibilă, în timp ce interacţionează cu personalul; strategiile sanitare ar trebui să asigure recunoaşterea timpurie a bolii şi nevoia ca lucrătorii bolnavi să rămână acasă. Strategiile sanitare este necesar să reflecte impactul unei pandemii asupra persoanelor aflate în întreţinere şi receptivitatea la necesităţile personalului care îngrijeşte un membru al familiei sau care are o pierdere ireparabilă.

Comportamentul individual. Dacă nu poate fi evitată o întrunire într-un loc public, atunci persoanele cu simptome compatibile cu gripă, acolo unde este posibil, vor fi sfătuite să se spele pe mâini sau să folosească o soluţie de curăţare a mâinilor înainte de a intra în locaţia respectivă; încurajarea persoanelor cu simptome similare unei gripe să menţină nivele ridicate de igienă respiratorie şi să arunce şerveţelele în mod corespunzător: “prinde-l, aruncă-l, distruge-l”; minimizaţi interacţiile cu oamenii: acolo unde interacţiile sociale nu pot fi evitate ar trebui să se acorde importanţă sfătuirii persoanelor care au simptome compatibile cu gripa să poarte o mască de faţă (dacă este disponibilă).

2. Reducerea riscului ca oamenii sănătoşi/susceptibili să fie infectaţi. Acţiunile principale vor urmări ca oamenii sănătoşi să reducă sau să evite contactele cu indivizii care au simptome similare unei gripe şi să adopte proceduri care reduc riscul de a contracta infecţia (de exemplu, măsuri de distanţiere socială şi o igienă eficientă a mâinilor).

Mediul ambiant. Evaluarea accesului la o igienă corespunzătoare a mâinilor, curăţarea suprafeţelor atinse frecvent cu mâna folosind agenţi de igienizare obişnuiţi.

Organizatoric. Conştientizarea importanţei igienei respiratorii şi a mâinilor; practicarea eficientă a distanţierii sociale în cadrul mediului de lucru dacă interacţiile sociale nu pot fi evitate. De exemplu, măsuri de reducere a frecvenţei interacţiilor, împărţirea pauzelor de prânz şi reducerea numărului de persoane din locurile închise, reducerea întâlnirilor faţă-în-faţă oricând acest lucru este posibil şi efectuarea de călătorii numai atunci când e absolut necesar, încurajarea folosirii conferinţelor şi comunicărilor video sau prin telefon, luarea în considerare a muncii la domiciliu pentru personalul pentru care acest lucru este o opţiune practicabilă, identificarea indivizilor care ar putea fi supuşi unui risc particular pentru efecte adverse ale gripei şi transferul lor în ariile în care contactele sunt minime.

Comportament individual. Este necesar să se adopte proceduri pentru o bună igienă a mâinilor şi să minimizeze atingerea mâinilor, a ochilor şi/sau a nasului; mărirea distanţierii sociale şi evitarea participării la aglomerări/întâlniri. Acolo unde nu se poate evita, adoptaţi o igienă bună respiratorie şi a mâinilor; minimizaţi orice contact cu o persoană care are simptome compatibile cu gripa: evitarea apropierii (la mai puţin de un metru) faţă de o persoană care are simptome compatibile cu gripa, dacă nu se poate evita, atunci luaţi în calcul folosirea unei măşti de faţă. Dacă poartă măşti, personalul trebuie să respecte procedurile pentru o singură utilizare, acordând o atenţie deosebită felului în care ele sunt scoase şi aruncate. Personalul s-ar putea expune la riscuri suplimentare de infecţie dacă nu foloseşte sau dacă nu înlătură corect măştile de faţă. Utilizarea măştilor nu trebuie să scadă aplicarea strictă a unor măsuri de control al infecţiei, mai relevante.

Modul de evaluare a riscului. Nu există doar o singură abordare sau măsură care poate fi adoptată pentru a reduce răspândirea gripei. Auto-izolarea promptă împreună cu igiena respiratorie adecvată şi igiena eficientă a mâinilor vor fi încurajate şi aplicate în mod activ. O abordare în care acţiunile care ţin de mediul ambiant, de organizare sau de individ sunt aplicate, va ajuta la reducerea răspândirii gripei la locul de muncă. Pentru a putea evalua dacă măsurile de limitare a răspândirii gripei sunt practice/practicabile, a fost dezvoltat un model. Acest model evidenţiază modalităţile de reducere a răspândirii virusului gripal prin combinarea diferitelor nivele de intervenţie, a felului în care ele ar putea fi folosite pentru a scădea numărul de persoane cu simptome compatibile cu gripa, dar şi minimizarea riscului de infecţie la persoanele susceptibile .

Un scenariu care arată cum poate fi aplicat modelul de evaluare este exemplificat în continuare. Acţiunile trebuie să ia în calcul aplicabilitatea oricărei măsuri la respectivul loc de muncă, să ajungă la soluţii şi să precizeze diferite intervenţii care depind de natura ocupaţiei şi a interacţiei cu publicul. Într-o şcoală, de exemplu, un profesor în clasa cu 30 de elevi aparent sănătoşi are responsabilitatea ca membru al personalului să aibă grijă de un copil bolnav în timp ce sunt aşteptaţi părinţii să-l ia; evident că o responsabilitate similară o au şi comandanţii sau şefii de unităţi, subunităţi şi formaţiuni militare faţă de subordonaţii lor.

Evaluarea scenariului. Un lucrător aflat într-un loc în care vine în contact direct cu publicul va avea un comportament adecvat pentru reducerea riscului de contaminare.

	Pentru a reduce transmiterea de la o persoană simptomatică la o persoană sănătoasă/susceptibilă	**Pentru a reduce riscul ca persoanele sănătoase/susceptibile să fie infectate**
De mediu (acţiuni desfăşurate în mediu pentru a reduce răspândirea virusului)	Încurajarea celor care sunt bolnavi să rămână acasă. Persoanele cu simptome similare unei gripe vor fi încurajate să stea departe de magazine. Dacă nu se poate evita acest lucru, persoanele simptomatice sunt sfătuite să se deplaseze în locurile menţionate în timpul perioadelor neaglomerate. Este indicată utilizarea tranzacţiilor electronice mai degrabă decât cu bani. Încurajarea utilizării sistemului poştal, telefonic şi internet pentru a efectua tranzacţii. Asiguraţi-vă, oricând este posibil, că există bariere fizice între client şi personal, cum ar fi o tejghea.	Folosirea indicatoarelor care să amintească de semnele şi simptomele gripei şi paşii care pot fi făcuţi pentru a minimiza riscul infecţiei. Acces uşor la facilităţile de igienă a mâinilor. Creşterea curăţării mediului ambiant cu agenţi normali de curăţare pentru acele suprafeţe care sunt frecvent atinse cu mâna. Oferiţi coşuri de deşeuri pentru şerveţelele contaminate!
Organizatoric (acţiuni desfăşurate pentru a modifica comportamentul şi procedurile de la locul de muncă în scop de reducere a răspândirii virusului)	Educarea personalului referitor la semnele şi simptomele gripei, pentru a realiza o recunoaştere rapidă a bolnavilor. Conştientizarea procedurilor organizatorice pentru tratarea persoanelor cu simptome similare unei gripe. Persoanele cu simptome similare unei gripe ar trebui să fie încurajate să stea acasă. Sunt indicate livrările la domiciliu pentru persoanele infectate, astfel încât acestea să se poată auto-izola şi să stea acasă. Promovarea regulilor prin care personalul care nu se simte bine să stea acasă până când se reface.	Educarea personalului referitor la semnele şi simptomele gripei pentru a promova o recunoaştere rapidă a bolnavilor Luarea în calcul a fezabilităţii limitării, restricţionării sau controlării numărului de clienţi din locaţia în care se vând produse la bucată, astfel încât să se diminueze interacţiile sociale şi să se mărească distanţierea socială.
Individual (acţiuni desfăşurate la nivel de individ pentru a restricţiona răspândirea virusului gripal)	Persoanele cu simptome corespunzătoare unei gripe vor fi încurajate să poarte măşti de faţă, dacă îngrijirea faţă în faţă nu poate fi evitată. Tuturor le va fi impusă o igienă corespunzătoare a mâinilor.	Încurajarea personalului să se spele pe mâini frecvent şi să nu-şi atingă gura, ochii şi/sau nasul numai dacă s-au spălat pe mâini. Dacă contactul apropiat cu o persoană cu simptome corespunzătoare unei gripe nu poate fi evitat, ar trebui să fie luată în calcul purtarea măştilor de către membrii personalului, ţinând cont de potenţiala cross-contaminare dacă ele nu sunt înlăturate adecvat.

Ghid pentru igiena mâinilor. Instruirea asupra igienei mâinilor este recomandată pentru a evita situațiile în care oamenii riscă să se contamineze singuri. Acest model de afiş a fost elaborat de Ministerul Sănătății:

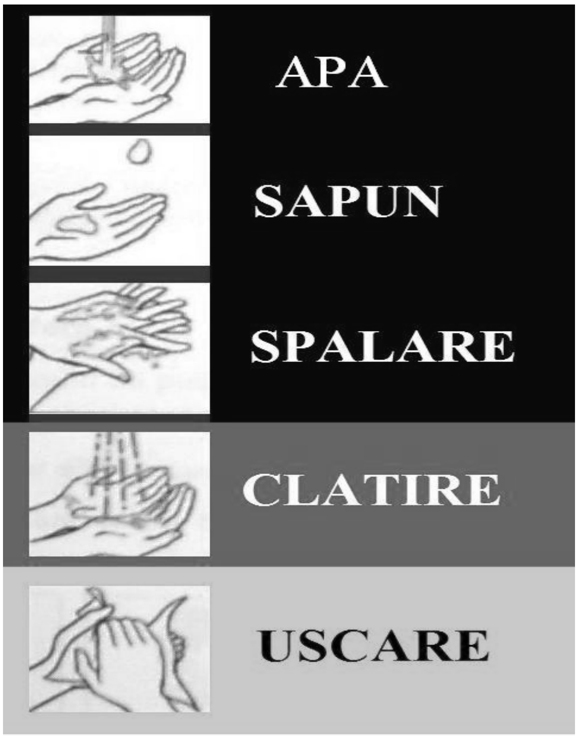

OPRIȚI RĂSPÂNDIREA GERMENILOR.
PUTEREA STĂ ÎN MÂINILE VOASTRE

Ghidul pentru utilizarea măştilor de față. Singura circumstanță în care măştile pot juca un rol în reducerea riscului de infecție ar fi situația în care o persoană sănătoasă a fost în contact inevitabil (la mai puțin de un metru) apropiat cu o persoană care are simptome compatibile gripei. Dacă apropierea (la mai puțin de un metru) de o persoană cu simptome compatibile gripei nu poate fi evitată, atunci ar trebui să fie luată în calcul utilizarea măştilor de față. Dacă poartă măşti de față, personalul trebuie să urmărească procedurile de utilizare sigură a lor, acordând o atenție specială felului în care ele sunt scoase şi înlăturate. Personalul s-ar putea expune unor riscuri suplimentare de infecție dacă nu foloseşte şi înlătură corect măştile. Utilizarea măştilor de față nu înlocuieşte aplicarea strictă a altor măsuri de control al infecției, care sunt mai relevante. Fără un sfat adecvat referitor la utilizarea adecvată a măştilor faciale, există riscul ca utilizatorii să se contamineze singuri de la exteriorul măştii contaminate, ca şi în cazul protecției NBC.

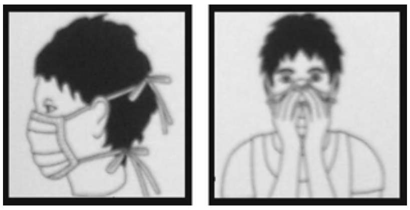

Aplicarea măştii de față. O mască de față ar trebui să fie pusă înainte de contactul cu o persoană cu simptome compatibile unei gripe. Mâinile vor fi spălate foarte bine înainte de manevrarea măştii. Benzile elastice se fixează la mijlocul capului şi al gâtului. Banda flexibilă se potriveşte la rădăcina nasului. Masca se potriveşte confortabil pe faţă şi sub bărbie.

Înlăturarea unei măşti de faţă. Masca de faţă va fi înlăturată odată ce nu mai există posibilitatea unui contact apropiat cu o persoană care are simptomele corespunzătoare unei gripe. Odată scoasă, masca va fi împăturită şi aruncată într-un container cu gunoi menajer. Mâinile vor fi spălate foarte bine după ce masca a fost scoasă şi aruncată. Presupunând că partea exterioară a măştii este contaminată: dezlegaţi sau tăiaţi legăturile de jos, urmate de cele de deasupra, sau de cele elastice, şi înlăturaţi masca prin folosirea numai a legăturilor, aruncaţi-o corespunzător, spălaţi mâinile foarte bine după ce atingeţi masca.

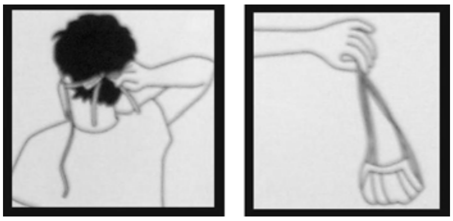

Alte puncte de reţinut. Masca de faţă este necesar să acopere nasul şi gura, să nu joace în jurul gâtului după sau la fiecare utilizare, este necesar să nu fie atinsă odată ce este pusă, să fie folosită numai o dată şi apoi aruncată.

Materiale de comunicare. Materialele de comunicare ale departamentului de sănătate, incluzând postere, trebuie să fie difuzate la toate instituţiile, unităţile şi organizaţiile.

Lista de control. Pentru a reduce transmiterea de la o persoană cu simptome corespunzătoare unei boli asemănătoare gripei la persoanele sănătoase/susceptibile.

Probleme de mediu care pot fi luate în consideraţie		**Bifaţi când este completat**
1	Utilizaţi postere în care se amintesc oamenilor semnele şi simptomele gripei şi măsurile care pot fi adoptate.	
2	Curăţaţi frecvent suprafeţele cu materiale obişnuite de curăţare.	
3	Acolo unde se pot aplica, faceţi uz de bariere fizice pentru a ajuta la restricţionarea interacţiilor apropiate şi a contactelor directe cu clienţii sau vizitatorii potenţial bolnavi.	
4	Luaţi în calcul îmbunătăţirea accesului la facilităţile de igienă a mâinilor şi disponibilitatea soluţiilor de curăţare a mâinilor.	
5	Minimizaţi numărul corpurilor de mobilă sau al altor obiecte care pot fi contaminate şi care sunt dificil de curăţat.	
Probleme organizatorii care pot fi luate în consideraţie		**Bifaţi când este completat**
1	Accentuaţi semnele şi simptomele gripei şi necesitatea de auto-izolare a unei persoane care are simptome corespunzătoare unei gripe.	
2	Gândiţi-vă la felul în care poate fi cel mai bine tratată o persoană cu simptome corespunzătoare unei gripe la locul de muncă.	
3	Promovaţi regula ca personalul care nu se simte bine să poată sta acasă până când se vindecă.	
4	Luaţi în calcul alternativele întâlnirilor şi vizitelor directe (conferinţe la telefon sau video)	

5	Când vizitele persoanelor cu simptome corespunzătoare unei gripe nu pot fi evitate, încurajaţi o igienă respiratorie şi a mâinilor adecvată.	
6	Acolo unde se poate, contactele directe vor fi evitate şi când nu se poate, ar trebui menţinută o distanţă mai mare de un metru între personal şi clienţi sau vizitatori.	
7	Acolo unde interacţiile sociale nu pot fi evitate, persoanele cu simptome corespunzătoare unei gripe vor minimiza interacţiile directe şi contactele directe.	

Probleme individuale care pot fi luate în consideraţie		**Bifaţi când este completat**
1	Încurajaţi o igienă a mâinilor corespunzătoare.	
2	Încurajaţi o igienă respiratorie corespunzătoare.	
3	Minimizaţi interacţiile cu oamenii.	
4	Luaţi în calcul recomandarea ca persoanele cu simptome corespunzătoare unei gripe să poarte măşti de faţă.	

1. Pentru a preveni riscul ca persoanele sănătoase/susceptibile să fie infectate

Probleme de mediu care pot fi luate în consideraţie		**Bifaţi când este completat**
1	Evaluaţi accesul la facilităţile de igienă a mâinilor.	
2	Curăţaţi frecvent suprafeţele care sunt foarte des atinse cu mâna.	

Probleme organizatorice care pot fi luate în consideraţie		**Bifaţi când este completat**
1	Conştientizaţi importanţa igienei respiratorii şi a mâinilor.	
2	Luaţi în calcul utilizarea eficientă a distanţierii sociale la locul de muncă.	
3	Reduceţi întâlnirile faţă-în-faţă oricând acest lucru este posibil şi faceţi numai călătorii absolut necesare.	
4	Încurajaţi utilizarea comunicărilor sau a conferinţelor prin telefon sau video.	
5	Luaţi în calcul utilizarea lucrului la domiciliu pentru cei care pot face acest lucru.	
6	Identificaţi persoanele care pot fi supuse unui risc particular pentru efecte adverse ale gripei şi deplasaţi-le în zonele în care contactele sunt minime.	

Probleme individuale care pot fi luate în consideraţie		**Bifaţi când este completat**
1	Încurajarea unor proceduri de igienă adecvată a mâinilor şi minimizarea atingerii gurii, ochilor şi/sau a nasului.	
2	Creşterea distanţierii sociale.	
3	Minimizarea contactului cu persoane care au simptome corespunzătoare unei gripe.	
4	Luaţi în calcul recomandarea ca persoanele cu simptome corespunzătoare unei gripe să poarte măşti de faţă.	

În loc de concluzii

Ne aflăm la începutul unei noi pandemii de gripă, după un interval de 41 de ani, şi prima pandemie a acestui secol.

Evoluția bolii nu este gravă, până în prezent, fiind sub indicele de mortalitate mediu al gripei.

Spre deosebire de epidemiile şi pandemiile precedente de gripă, acum dispunem de mijloace adecvate de diagnostic, tratament şi profilaxie.

Este necesar să se cunoască şi să se aplice măsurile de profilaxie, diagnostic şi tratament recomandate, pentru a reduce efectele medicale, sociale şi economice ale pandemiei de gripă.

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Scurte date istorice privind urologia în mediul medico-militar românesc

Col. conf. univ. dr. Dan MISCHIANU

Clinica de Urologie, SCUMC „Dr. Carol Davila”, Bucureşti

Primii paşi

Sunt convins că unii dintre cititorii rândurilor de faţă se vor întreba de ce tocmai acum această abordare, această preocupare. Un răspuns simplu, şi poate chiar sincer, ar fi că la un moment dat simţi nevoia să afli începuturile îndeletnicirii pe care o practici de ceva vreme, dacă ai un spirit scormonitor, şi că până la urmă vei afla ce şi cum ... Probabil de aici porneşte şi dorinţa mea, la care se mai adaugă un motiv, aş spune cum nu se poate mai nimerit – anul acesta se împlinesc 97 de ani de la înfiinţarea primului şi, până nu demult, a singurului serviciu de urologie dintr-un Spital militar românesc.

Doresc să mai fac nişte precizări. Odată cu trecerea timpului rememorarea faptelor şi a realizărilor înaintaşilor devine, aproape cu certitudine, o datorie pentru urmaşi. Colbul uitării se aşterne cu o viteză uimitoare chiar şi acum, într-un început de mileniu conectat la internet, şi în ceea ce mă priveşte, trebuie să recunosc că am rămas încă fidel “Galaxiei Gütenberg” şi mai puţin cuceririlor „Epocii IT”!...

Cititorul acestor rânduri nu va fi cred surprins când îi voi mărturisi că multe din datele cu “iz istoric” din acest material îşi trag seva din articole de revistă de mult arhivate, din ordine de ministru şi din capitolele unor cărţi. După cum la fel de bine cred că va fi de acord că simplul fapt de a pune o virgulă, chiar şi numai de a trage o linie („nulla dies sine linea!” – parcă aşa ar fi zis pictorul grec Apelles, citat fiind de Pliniu cel Bătrân!) şi de a preciza până la urmă lucrurile din punct de vedere istoric, poate fi chiar o întreprindere dificilă.

Dar intrând în subiect poate am să surprind cu o afirmaţie: prima operaţie urologică pe pământ românesc, atestată ca atare (conform Arh.St., Buc., Ştabul Oştirii, dos. 13/1836, f. 670), a fost efectuată de Col.Dr. Georg Johann Andreas von Grünau, primul comandant al Spitalului oştirii (1831-1838), instituţie care mai târziu va intra în conştiinţa publică sub numele de “*Spitalul Militar Central*”. Conform actului din arhivă, numitul doctor a efectuat în anul 1834 o operaţie de talie perineală pentru un calcul uretral. Să mergem însă pe firul datelor mai pe îndelete ...

Câte ceva despre înaintaşi şi despre Spitalul Militar Central

În Ţara Românească şi în Moldova la început au fost lazaretele. Data înfiinţării Lazaretului Regimentului 1 Bucureşti rămâne controversată: 26 iulie sau 13 septembrie 1831, sigur este că el reprezintă “embrionul” viitorului Spital Militar Central.

În ziua de 13 septembrie 2006 medicii militari români au sărbătorit 175 de ani de la înfiinţarea instituţiei numite Spitalul Clinic de Urgenţă Militar Central “Dr. Carol Davila”.

Consider că amănuntele referitoare la faptul că ostaşii au fost îngrijiţi timp de un an-doi la Mânăstirea Mărcuţa, mai apoi la Spitalul Sf. Pantelimon şi de abia din 1834 în clădirile Mânăstirii Mihai Vodă (singurul complex arhitectonic rămas din vremea Voievodului primei Uniri, parţial demolat, parţial “translat” de arhitecţii ceauşişti în 1986) sunt de acum date istorice.

Legat de istoria Spitalului Militar Central vom reaminti că doctorul Georg Johann Andreas von Grünau (1784-1857) era absolvent al Şcolii de Medicină din Göttingen, şi că se stabilise în Ţara Românească în 1813. La 1 septembrie 1831 a fost numit medic al Regimentului 1 Bucureşti şi Şef al Lazaretului .

Următoarea locaţie a Spitalului Militar Central este, începând cu 1858, Cazarma din strada Ştirbei Vodă (fost sediu al Comandamentului Infanteriei şi Tancurilor, în prezent sediul Direcţiei Naţionale Anticorupţie). Aici se va muta în 1859 Spitalul Militar de la Mănăstirea Mihai Vodă şi va funcţiona până către 1885-1889.

Solicitările fiind mari şi saloanele –tip lazaret– devenind neîncăpătoare, în 1883 Ministerul de Războiu va achiziţiona cu suma de 1 370 000 lei terenul de 82 802 m^2 din strada Francmasonă (fostă Ştefan Furtună, actuală Mircea Vulcănescu), având alături biserica cu hramul "Sf. Gheorghe") şi la scurt timp demarând construcţiile unui spital pavilionar "tip Bismark", aşa cum era în vogă pe atunci şi cum începuse "moda" pe la 1760 cu spitalul din Plymouth, Anglia. Menţionăm că la acea dată Spitalul Militar Central a fost primul spital european iluminat electric şi având în dotare o spălătorie cu aburi...

După ştiinţa noastră pavilionul în care sunt găzduite acum Secţia Clinică de Urologie şi cea de Ginecologie a fost terminat în 1903, iar în 1957 a avut loc compartimentarea saloanelor tip lazaret şi adăugarea sălilor de operaţie. În acest corp de clădire au funcţionat secţiile de chirurgie generală, care în 1979 au fost mutate în aşa-numitul Pavilion chirurgical, ridicat după cutremurul din 1977, în locul acestora venind "rudele mai sărace" – urologia şi ginecologia.

Nu am să reamintesc decât cu titlu de inventar – pentru cunoscători – că în acelaşi timp în care Profesorul Dr. Nicolae Turnescu (1819-1890) opera fimoze şi parafimoze, executa talii perineale şi cateteriza vezica urinară, iar Profesorul Dr. Constantin Dimitrescu Severeanu (1840-1930) – elev al "Şcolii de chirurgie" al strămoşului nostru comun Generalul Dr. Carol Davila şi mai apoi "părintele" de drept al Chirurgiei româneşti opera maladii urologice şi genitale, contemporanul lor – Generalul de brigadă Prof. Dr. Athanase Demosthen (1845-1925) – comandant al Spitalului Militar Central între 1899-1902 – era, din 1897, membru corespondent al Societăţii Franceze de Urologie, drept recunoaştere a faptului că terapeutica chirurgicală a bolilor urologice nu îi era străină. La baza bustului său, adăpostit în parcul din mijlocul Spitalului, există o placă pe care sunt inscripţionate următoarele cuvinte: "*Omagiu şi admiraţiune din partea camarazilor, colegilor şi elevilor săi*".

Începuturi întrepătrunse

Într-una din nuvelele sale, Marin Preda scria: "*…ceea ce pentru un civil are un sens, pentru un militar are cu totul altul!*". Plecând de la aceste cuvinte, amintesc, pe scurt, că părintele urologiei româneşti – Profesorul Dr. Petre Herescu (1867-1915), cel care devine la Spitalul Colţea primul Profesor român de Urologie, a activat ca extern al Spitalelor pariziene sub conducerea lui Jacoud şi Championière şi intern al aceloraşi Spitale în serviciul lui J.M.Albarran, Pierre Delbet şi Felix Guyon. Tot la Paris şi tot alături de celebrul şi "ciudatul" Joaquim Maria Albarran (1860-1912) – promotor şi creator al atâtor noutăţi în urologie (scăriţa lui Albarran, acul lui Albarran, legea şi semnul lui Albarran ş.a.) – învăţase timp de doi ani şi Căpitanul Dr. Eugen Christian (1882-1952). Doctorul Eugen Christian se instruise alături de Profesorul Jean Baptiste Camille Georges Marion (1869-1960), vestit urolog francez care, pe lângă boala ce îi poartă numele, a efectuat, în 1942, şi prima reconstrucţie vezicală pentru extrofie vezicală.

La revenirea în ţară, în 1912, Căpitanul Dr. Eugen Christian înfiinţează un serviciu de boli genito-urinare în Spitalul Militar Central. *Urologia medico-militară va împlini aşadar în acest an 97 de ani de la atestarea ei documentară*. Iniţial, serviciul va fi condus de Lt.Col.Dr. Gh.Mihăescu, Cpt.Dr. E.Christian este încă medic secundar, iar intern este Slt.Dr.Vasile Trifu.

La început vor avea la dispoziţie un salon al pavilionului chirurgical. După descrierea antecesorilor noştri un salon tip lazaret avea 15-20 de paturi frumos aliniate, cum astăzi mai vedem doar în filmele de început de veac XX. Vremurile tulburi şi schimbătoare rearanjează şi reorientează respectiva "structură" (vorba cronicarului: "*Nu sunt vremurile sub om!...*").

În campania din 1913 serviciul de Boli genito-urinare va fi condus de un anume doctor Moscu, apoi, când Spitalul Militar Central se va muta la Iaşi, în 1916, "Servicul genitourinar" îşi întrerupe activitatea şi şi-o reia în 1917 în componenţa Spitalului Militar al Corpului IV Armată Iaşi sub conducerea Mr.Dr. Eugen Christian, având alături pe secundarul Cpt.Dr. Ion Anghel.

Căutări

Frământările au repercusiuni şi asupra urologilor militari. Spitalul Militar Central revine în 1918 în "vechiul", de acum, sediu din strada Francmasonă, colţ cu Calea Plevnei şi serviciul "genitourinar" are la dispoziţie 64 de paturi, o sală de consultaţii şi alta "de spălături" pentru pacienţii din ambulatoriu dar trebuie să suporte totodată şi "tutela" serviciilor Chirurgie I şi/sau II din pavilioanele în care sunt găzduite împreună.

În 1920, după ce lucrurile se mai aşezaseră, Lt.Col.Dr. Eugen Christian, având alături pe Mr.Dr. Ion Anghel, Cpt.Dr. N. Constantinescu, Cpt.Dr. Marin-Popescu Buzeu ("sufletul" viitoarei Uniuni Medicale Balcanice!) şi Dr. Victor Vintici, începe să aibă drept obiective dezvoltarea şi înzestrarea secţiei.

În anul 1921 se înfiinţează un laborator propriu al Secţiei de Urologie a Spitalului Militar Central "Regina Elisabeta" cu trei sublaboratoare: bacteriologie (ce execută 2 500 - 3 000 de analize tip, având la dispoziţie microscop şi termostat), biochimie (circa 700 de dozări de uree/an, 1 300 - 1 500 examene de urină/an) şi anatomie patologică a pieselor recoltate operator. Mai apoi, Secţia de Urologie primeşte, prin "donaţie", trei cistoscoape, un uretroscop şi o trusă de litotriţie endovezicală. În 1932 este donată serviciului o instalaţie de radiologie prin grija altui "sponsor" – doamna Col. Simeon Marcovici.

În anul 1927 serviciul avea în componenţă 58 de paturi, un sector în care se acordau aproximativ 50 de consultaţii zilnic, o sală de operaţii şi o sală pentru cistoscopie. Din 1928 este "accesată" în serviciul uro-radiologic o ultimă achiziţie a epocii – cromocistoscopia.

Mai apoi, în 1931, serviciul uro-ginecologie ajunge doar la 55 de paturi. Sunt consultaţi, internaţi şi trataţi anual – în medie, aproximativ 1000 de pacienţi.

În 1937, Colonelul Dr. Eugen Christian este numit Inspector General al Serviciului Sanitar al Armatei (corespondent al Şefului Direcţiei Medicale de astăzi) şi predă studenţilor anului V ai Facultăţii de Medicină, ca agregat (profesor asociat), timp de 12 ani, noţiunile urologice de bază. După preluarea de către Dr. E. Christian a sarcinilor administrative ale Inspectoratului Medical, la conducerea Serviciului de Urologie este numit elevul său Col.Dr. Ion Anghel (1937-1940), acompaniat de doctorii secundari Marin Popescu Buzeu şi Eugen Mareş (i-am cunoscut, ca medici pensionari, aflaţi la finele carierei), iar mai apoi, după ce şi Col.Dr. Ion Anghel devine comandant al Spitalului Militar Central, la conducerea serviciului de urologie vine Col.Dr. Marin Popescu Buzeu (1940-1946), având drept secundari pe Cpt.Dr. Alexandru Augustin şi Dr. Daniel Ionescu.

În anul 1946 Col.Dr. M. Popescu Buzeu este trecut în rezervă iar Serviciul urologic este subordonat Secţiei Chirurgie III a Spitalului Militar Central. Vremurile sunt tulburi, structura este fragilă, Urologia românească ca atare nu se afirmase drept o specialitate de sine stătătoare (reamintim, ne aflăm în anii 1945-1950). Profesorul Dr. N. Hortolomei opera la Spitalul Colţea şi stenoze mitrale şi ulcer gastric şi calculi urinari, iar Profesorul Th. Burghele venea la direcţia Spitalului Panduri pe care îl va transforma într-o adevărată "Mecca urologiei româneşti" (*Enciclopedie medicală românească secolul XX*, pag. 1 103). Nici urologia din cadrul structurii medico-militare nu era prea bine lămurită asupra viitorului ei...

După Col.Dr. Marin Popescu Buzeu, şef al Serviciului Urologic devine Col.Dr. Traian Mihăilescu (1946-1947), apoi Col.Dr. H. Vascoboinic (1947-1948) şi Col.Dr. I. Atanasiu (1948-1957).

Între 4 noiembrie 1949 şi 19 iulie 1957, Serviciul genitourinar se găseşte în structura Secţiei III Chirurgie condusă de G-ral.Dr. Petre Costescu, chirurg polivalent, a cărui fotografie am văzut-o în biroul fostului şef al Clinicii Ortopedie-Traumatologie (G-ral.Prof.Dr. Gh. Niculescu) şi despre care ştiu că, după pensionare, a fost şef de echipă în Clinica Chirurgie la Spitalul de Urgenţă Floreasca – Clinica de Chirurgie. Era un şef permisiv, cu multe valenţe chirurgicale, un bun conducător.

La 19 iulie 1957 (*în curând se împlinesc 52 de ani!*) se organizează Secţia urologie, având în componenţă 100 de paturi şi şef pe Col. Dr. Alexandru Augustin (1957-1967), care din 1967 – devenit general maior dr. – conduce destinele Direcţiei Medicale a Ministerului Forţelor Armate

(M.F.A.). În data de 1 octombrie 1960, în cadrul unui proces simbiotico-saprofitic ce continuă şi azi Secţia de Urologie se uneşte cu Secţia Obstetrică-Ginecologie sub denumirea "Secţia ginecologie şi urologie", iar după cinci ani, pe 15 noiembrie 1965 secţiile amintite se separă şi redevin "Secţia de Urologie" (Şef Col.Dr. Alexandru Augustin) şi "Secţia de Ginecologie" (Şef Col.Dr. Mihail Vasiliade).

În 1966 Secţia de Urologie are în competenţa sa 65 de paturi iar prin plecarea G-ral.Dr. Alexandru Augustin la comanda Direcţiei Medicale la conducerea Secţiei urmează Col.Dr. Constantin Grigorescu (1967-1990). Colectivul Secţiei de Urologie a Spitalului Militar Central va număra, după 1970, sub conducerea Col.Dr. C. Grigorescu – doctor în ştiinţe medicale, pe Col.Dr. Ioan Marinescu – doctor în ştiinţe medicale, Col.Dr. Ioan Roman, Col.Dr. Anatolie Boldişor, Lt.Col.Dr. Mihai Nicolae Bînă –doctor în ştiinţe medicale– şi Lt.Col.Dr. Constantin Dumitrescu.

Secţia Urologie - Spitalului Militar Central, 1971

Poate că cele ce vom scrie în continuare sunt o încercare forţată de "paralelism". Ne aflăm însă în perioada în care Acad. Prof. Dr. Doc. Th. Burghele (1955-1972) este Rector al I.M.F. Bucureşti (actualmente U.M.F. "Carol Davila"), Ministru al Sănătăţii (1972-1976) şi în ultimul an al vieţii Preşedintele Academiei R.S.R. (1976-1977), iar mai apoi Acad.Prof.Dr.Doc. Eugeniu Gh. Proca este Rector al I.M.F. (1977-1979) şi Ministru al Sănătăţii (1979-1985). Şi fostul "şef de problemă" (termen particular medicinii militare) al urologiei – G-ral.Lt.Dr. Alexandru Augustin devine pentru câţiva ani (1967-1975) responsabil cu administraţia – adică medic şef al Serviciului Sanitar al Armatei. Oamenii îşi pun speranţe atunci când se schimbă şefii, dar...

Ce se poate afirma, cu certitudine, este faptul că în anii 1970-1989, ca şi după aceea, între Secţia de Urologie a Spitalului Militar Central şi Clinicile de Urologie Fundeni şi Panduri a existat o permanentă comunicare şi interdependenţă.

Conform relatărilor maeştrilor noştri, atunci când sălile de operaţie ale Spitalului Panduri erau în reparaţie Profesorul Dr. Doc. Valentin Neagu venea să opereze la Spitalul Militar Central, iar Doctorii Mihai Bînă şi Constantin Dumitrescu au efectuat – între anii 1980-1989 – cursuri la Clinica de Urologie Fundeni – sub "bagheta" Magistrului Prof. Dr. Doc. E. Proca.

În august 1990 G-ral.Dr. C. Grigorescu este pensionat şi conducerea Secției de Urologie este preluată, prin concurs, de către Col. Dr. Mihai Bînă (general maior de la 1 decembrie 1991).

Afirmări, priorități şi neîmpliniri

Anii 1990-1991 sunt ani ai "primenirii" colectivului Secției de Urologie care, sub conducerea G-ral. Bg. Conf. Dr. Mihai Bînă, devine Secție Clinică – pendinte de Facultatea de Specializare şi Perfecționare Postuniversitară a Institutului Medico Militar. Alături de şeful clinicii se află Col.Dr. C. Dumitrescu, Col. Dr. Dan Mischianu, Lt. Col. Dr. Mihai Tănăsescu, Lt. Col. Dr. A. Pantalon, Lt. Col. Dr. Marius Dinu şi Cpt.Dr. Pacu Ovidiu. Au mai activat, cu rezultate remarcabile, regretatul Lt.Col.Dr. Gavril Vlasin (decedat prematur la 07.06.2002) şi Lt.Col.Dr. Paul Vladu (în prezent la C.M.D.T.A – „Prof.Dr. Şt. Milcu"). După 1990, clinica de Urologie are în compunere 75 de paturi, dispuse în saloane pentru doi până la cinci pacienți.

În anul 2001 G-ral Prof. Dr. M. N. Bînă şi Col. Dr. C. Dumitrescu au trecut în rezervă, iar la 1 martie 2006, în cadrul procesului de restructurare al Ministerului Apărării părăsesc, din păcate, în plină putere creatoare şi structura medico-militară.

Din data de 22.11.2004 conducerea Secției este preluată de Col. Dr. Dan Mischianu, numit din 01.06.2005 şi Locțiitor al Comandantului / Director General Adjunct medical al Spitalului Clinic de Urgență Militar Central "Dr. Carol Davila". Începând cu anul universitar 2005/2006, Secția Urologie devine Clinică universitară în cadrul Facultății de Medicină a Universității de Medicină şi Farmacie „Carol Davila", iar colectivului condus de Col. Conf. Dr. Dan Mischianu – având în componență pe Col. Dr. Mihai Tănăsescu, Lt. Col. Dr. Adrian Pantalon, Lt. Col. Dr. Marius Dinu, Mr. Dr. Ovidiu Pacu, As. univ. Dr. Victor Mădan, As. Univ. Dr. Ilie Cristian, Lt. Dr. Ovidiu Bratu, Cpt. Dr. Florin Rusu – îi revin şi sarcini didactice privind învățământul universitar şi postuniversitar desfăşurat sub auspiciile Universității de Medicină şi Farmacie "Carol Davila" Bucureşti.

Colectivul Clinicii de Urologie a Spitalului Clinic de Urgență Militar Central "Dr. Carol Davila" – februarie 2007

Din 01.02.2009 Col. Dr. Mihai Tănăsescu este transferat în cadrul rețelei sanitare a Serviciului Român de Informații.

Una din prioritățile Secției Clinice de Urologie a Spitalului Clinic de Urgență Militar Central “Dr. Carol Davila” a fost utilizarea aparaturii laser (Alexandrit, Holmium) impact în tratamentul calculilor ureterali. Există speranța că şi terapia ESWL a litiazei urinare să fie reluată (ea a fost efectuată între 10.12.1992 – prima şedință – 2001) având la dispoziție alte aparate (între timp a fost achiziționată aparatura ESWL Storz, care urmează să fie pusă în funcțiune în scurt timp) şi, de asemenea, dorința să fie implementate şi alte tehnici noi de terapie a cancerului (brahiterapie prostatică, de exemplu).

În “arealul” medicinii militare române a existat şi există, la diferite spitale militare (Timişoara, Braşov, Galați, Piteşti, Iaşi şi Focşani), câte un medic specialist urolog, încadrat ca ofițer medic sau ca personal contractual civil (salariat civil). Din anul 2006 la Spitalul Militar de Urgență “Dr. C. Papilian” Cluj Napoca, în cadrul Secției Chirurgie Generală s-a creat şi un compartiment de urologie încadrat cu trei medici specialişti urologi, dintre care unul este şi specialist de chirurgie generală.

Cred că nu comit o impietate atunci când scriu aceste rânduri şi afirm că asertiunea sau sintagma din titlu privind “întrepătrunderea lucrurilor” este perfect reală. A dezvălui faptul că cel mai important urolog român al ultimului sfert de veac XX – Acad. Prof. Dr. Doc. Eugen Proca a fost student al Institutului Medico-Militar şi a fost trecut în rezervă în 1950 cu gradul de locotenent medic (Dora Petrilă, Martin S. Martin –*Spitalul Fundeni– istorie, destine*, Ed. Humanitas, 2004) ca şi faptul că elevul său, actualul Preşedinte al Asociației Române de Urologie – Prof. Dr. Ionel Sinescu, Prorector al UMF “Carol Davila” şi Şef al Centrului Clinic de Chirurgie Urologică, Dializă şi Transplant Renal Fundeni, a fost un strălucit absolvent şi şef de promoție al aceluiaşi Institut Medico-Militar, este un fapt de mândrie pentru medicii militari şi vine în sprijinul acestei aserțiuni.

De fapt, privind înapoi, contemplativ, *vis-à-vis* de eforturile, visele şi împlinirile înaintaşilor noştri, găsesc cât se poate de nimerit să trag următoarea concluzie: urologia aşa-zis “medico-militară” nu s-a deosebit şi nu diferă prin nimic de urologia “civilă” română. Rostul şi împlinirile ei au fost şi sunt totdeauna, chiar şi numai pentru a-l contrazice pe Marin Preda, alături de urologii civili.

Am scris aceste rânduri inspirându-mă, evident, din alte surse (cărți, articole, ordine interioare şi de ministru al apărării). Sunt convins că nimeni, chiar şi în materie istorică nu deține adevărul absolut! Sper că nu voi produce un disconfort major posibililor viitori cititori sau dacă am omis, prin neştiință, numele vreunuia din slujitorii breslei noastre ce au activat în mediul medico-militar.

Rămân convins însă că meseria noastră se bazează pe muncă cinstită, conştientă şi conştiincioasă pusă totdeauna în slujba bolnavilor şi de aceea închin aceste pagini efortului tuturor urologilor “aşa-zis militari” care, “fără de număr şi fără de istorie” au ținut “pe umerii lor” flacăra vie şi pasionantă a acestei specialități.

Cu o uşoară notă de pesimism, datorată probabil imprevizibilității efectelor posibile ale „crizei crizelor” ce va urma, închei cu următoarele cuvinte: acestea au fost odată misiunea, oamenii şi realizările Serviciului Urologic din Armata României... Acum, istorie este deja ceea ce se întâmplă sub ochii noştri...

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Urgențe epidemiologice posibile în cazul utilizării micotoxinelor ca arme biologice

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Centrul de Cercetări Ştiintifice Medico-Militare, Bucureşti

REZUMAT. Micotoxinele sunt metaboliți ai ciupercilor, în special ai mucegaiurilor, care apar la speciile toxinogene, în anumite condiții de mediu. Micotoxicozele sunt intoxicații acute sau cronice, care pot apărea la oameni sau animale, sub acțiunea micotoxinelor. Orice aliment de origine vegetală poate fi contaminat direct cu micotoxine, iar alimentele de origine animală pot fi contaminate direct sau indirect, prin consumarea de furaje contaminate.

Micotoxicologia reprezintă un domeniu nou şi important în medicina umană şi veterinară, cu implicații interdisciplinare: toxicologice, microbiologice, igiena alimentară, igiena mediului, oncologie etc. Datorită proprietăților pe care le au, micotoxinele ar putea fi folosite ca armă chimică în scopuri tactice, operative sau strategice ori în scop bioterorist. Sporii mucegaiurilor toxinogene ar putea fi diseminați ca armă biologică.

Finanțarea cercetării mijloacelor şi metodelor moderne de combatere a efectelor acestui pericol potențial nu este susținută cu fonduri corespunzătoare şi cu un interes proporțional cu importanța riscurilor asociate.

Având în vedere complexitatea şi gravitatea problemelor puse de micotoxicoze, este necesară aprofundarea micotoxicologiei din punct de vedere medico-militar, în special în privința protecției față de micotoxinele folosite ca posibilă armă de distrugere în masă.

Cuvinte cheie: arme biologice, costuri, micotoxine, protecție medicală.

ABSTRACT. Mycotoxines are metabolic products of the fungi, especially of the moulds, which appear to the toxinogenic species, in certain environmental conditions. Mycotoxicoses are chronic or acute intoxications appearing at humans or animals under the action of mycotoxines. Any aliment of vegetal origin can be directly contaminated with mycotoxines and the aliments of animal origin can be directly or indirectly contaminated by consuming contaminated feeds.

The mycotoxicology represents a new and important field in human and veterinary medicine, with interdisciplinary implications: toxicology, microbiology, food hygiene, environmental hygiene, oncology etc. Due to the property which mycotoxines posses, they can be used as chemical weapon with tactical, operative or strategic purposes or with bioterrorist purpose. The moulds spores can be disseminated on enemy territory, as biological weapon.

Financing the research of the modern methods and means to fight this potential threat is not sustained with corresponding founding and with an interest proportional to the importance of the associated risks.

Having in mind the complexity and the gravity of the problems posed by mycotoxicose, it is necessary to study thoroughly the mycotoxicology from the medico-military point of view, especially regarding the use of micotoxines as a possible weapon of mass destruction.

Micotoxicologia studiază intoxicațiile acute şi cronice (micotoxicoze) produse de toxinele secretate în anumite condiții de unele ciuperci patogene (biosinteza); cel mai adesea este vorba de micromicete din grupul fungilor filamentoşi (mucegaiuri): *Aspergillus, Penicillium, Fusarium* etc.

Micotoxinele cele mai importante în patologia umană şi veterinară sunt: aflatoxinele, ochratoxinele, zearalenonele şi trichotecenele.

Micotoxinogeneza este un caracter aleator, transmis genetic şi care poate fi stimulat prin tehnici speciale de cultivare. Din cultura de fungi toxigeni se poate preleva extractul brut „crude extract" sau poate fi purificat şi condiționat, mergând până la nivelul de standard de laborator.

Micotoxinele sunt printre cele mai puternice toxice cunoscute, cu excepția toxinei botulinice. Având toxicitate foarte mare, se realizează concentrații letale sau patogene la cantități foarte mici (μg), care nu pot fi sesizate organoleptic şi nici depistate prin metode curente de analiză toxicologică. În cazul folosirii ca STL, aceasta poate fi considerată ca o armă insidioasă, din cauza dificultăților de depistare cu metodele uzuale:

- în cazul depistării cu metode de înaltă tehnologie, faptul nu este relevant deoarece mucegaiurile toxinogene sunt ubicuitare;
- intoxicația letală se produce lent, în 1-3 zile, în acest interval de timp toxinele se metabolizează, apar metaboliți încă necunoscuți şi nedepistabili, ceea ce face ca diagnosticul de laborator al intoxicației cu micotoxine să fie greu de susținut;
- intoxicații cu doze subletale pot să dezvolte leziuni organice, de exemplu cancer hepatic, în interval de 5-10 ani, deci este greu de stabilit etiologia bolii;
- o formă particulară de întrebuințare militară poate fi diseminarea masivă în teritoriul inamic a sporilor de mucegaiuri toxinogene.

Micotoxinele pot fi produse în mod discret, cu forțe şi mijloace relativ reduse.

Pentru aceste motive, micotoxinele au fost utilizate ca armă chimică în războaie locale recente, de exemplu Irak-Iran şi U.R.S.S.- Afganistan.

1. Micotoxine. Ingerarea alimentelor conținând micotoxine, produşi toxici ai ciupercilor, în special ai mucegaiurilor, poate avea asupra sănătății umane efecte adverse grave. Ocazional, poate avea loc expunerea la micotoxinele aflate în suspensie în aer, îndeosebi în cazul folosirii lor ca substanțe toxice de luptă.

Prezența micotoxinelor în produsele alimentare depinde de excretarea lor ca metaboliți de către tulpini fungice specifice (toxinogene) şi de influența factorilor externi: substrat nutritiv, umiditate, temperatură, pH, compoziția atmosferei, vechimea culturii etc.

Contaminarea naturală a produselor alimentare de către aceste toxine variază în funcție de condițiile geografice, de metodele de producere şi stocare a alimentelor, ca şi de tipul de aliment implicat, anumite produse alimentare constituind substraturi favorabile proliferării fungice şi/sau toxinogenezei.

În practica medicală este importantă evaluarea riscurilor pe care le implică asupra sănătății umane expunerea la diferitele grupe de micotoxine, din care predomină patru:

1.Aflatoxinele, care fac obiectul unei analize mai detaliate deoarece sunt mai bine cunoscute comparativ cu celelalte micotoxine şi despre care s-a demonstrat că au, din punct de vedere epidemiologic, efecte mai grave asupra sănătății umane. Ele determină la om şi animale aflatoxicoze acute şi/sau cronice în câteva minute sau zile, respectiv cancer primitiv de ficat sau al altor organe.

2. Ochratoxinele – sunt incriminate în etiologia nefropatiei endemice balcanice.

3. Trichotecenele – sunt considerate ca agent etiologic al aleuciei toxice alimentare.

4. Zearalenonele – produc o patologie de tip hiperestrogenic.

Acestea creează o problemă importantă în medicina umană şi veterinară, sub aspect toxicologic, microbiologic, de igienă alimentară şi de igiena mediului, având şi implicații medico-militare, medico-legale şi economice.

2. Micotoxicoze. Toxicitatea anumitor ciuperci pentru om este cunoscută din preistorie, dar pericolul potențial pe care îl reprezintă micotoxinele unor ciuperci microscopice (mucegaiuri) a fost descoperită abia în anul 1850, odată cu asocierea semnelor clinice de ergotism cu ingestia de orez şi secară contaminate cu fungul „cornul secarei” – *Claviceps purpureea*.

Alte micotoxicoze umane au fost identificate, prin asocierea cu ingestia de pâine contaminată cu *Fusarium spp*, diagnosticarea stachibotoxicozei, relația cauzala dintre aleucia toxică alimentară şi ingestia de boabe de porumb avariate infestate cu *Fusarium graminearum* sau *Fusarium sporotrichoides*. Măsurile preventive luate ca urmare a acestor descoperiri au permis evitarea unor micotoxicoze chiar înainte de identificarea primelor micotoxine.

Descoperirea proprietăților hepatotoxice şi hepatocancerigene, şi posibil teratogene ale fungului *Aspergillus flavus*, la începutul deceniului 7, urmată de elucidarea structurii aflatoxinelor, a modificat strategia luptei contra tuturor micotoxicozelor. În prezent este posibil să se abordeze problemele mai exact, pe baza dozării chimice a toxinelor şi a studierii efectelor în funcție de doza primită. Majoritatea datelor actuale despre micotoxine şi micotoxicoze sunt furnizate de medicina veterinară.

Studiile de micotoxicologie, făcute pe teren sau în laborator, arată că problematica este complexă şi gravă, unele micotoxine având şi alte roluri în etiologia patologiei umane decât cele cunoscute până în prezent.

Aproape toate produsele vegetale sau animale pot să fie substrat favorabil pentru proliferarea fungică şi formarea ulterioară de metaboliți, inclusiv micotoxine. De aici decurge şi posibilitatea contaminării artificiale a alimentelor sau furajelor pentru oameni sau animale. Dar ingerarea lor de către animalele de fermă destinate producției alimentare, pe lângă efectul patogen direct asupra lor, cu implicații economice, va duce şi la contaminarea indirectă a omului prin consumul de lapte, organe şi carne de la aceste animale. Mai mult, există şi o aşa zisă expunere profesională la micotoxine, prin intermediul altor factori, de exemplu prin inhalarea aerosolilor din aer. Acest aspect are şi implicații medico-militare directe.

Dintre numeroasele grupe de micotoxine cunoscute până în prezent, numai aflatoxinele, ochratoxinele şi zearalenonele sunt bine definite sub aspect toxicologic la om şi la animal. Trichotecenele sunt controversate, iar alte micotoxine mai recent descoperite sunt în curs de cercetare: citreoviridina, citrinina, ciclocloproteina, luteoschirina, matorizina, patulina, toxina PR, rubratoxina, rugolozina, sterigmatocistina, tremorgenele etc.

Diagnosticul este dificil în medicina umană, boala fiind rară la om iar simptomele sunt necaracteristice. Laboratorul ar avea un rol esențial, atât pentru identificarea fungului micotoxigen cât şi pentru detecția, identificarea şi dozarea micotoxinei, dar nu există dotarea necesară în instituțiile sanitare. Din acest punct de vedere, medicina veterinară, care se confruntă adesea cu aceste situații, are forțele şi mijloacele adecvate pentru diagnostic, deci este utilă colaborarea. Medicina militară dispune, la Centrul de Cercetări Ştiințifice Medico-Militare, de un colectiv de Micotoxicologie, care este în măsura să pună diagnosticul de laborator în micotoxicoze.

Tratamentul a fost şi a rămas o mare problemă; intoxicația acută sau supraacută nu lasă timp suficient pentru diagnostic clinic şi etiologic, nu există mijloace specifice de tratament sau antidoturi. În intoxicația cronică simptomele apar foarte lent, chiar ani sau zeci de ani, sunt generale şi necaracteristice, iar când se instalează distrucții hepatice, renale, cancer hepatic etc. este tardiv. Cercetările noastre, legate de participarea armatei române la Operațiunea Furtuna deşertului (1991, Arabia Saudită), când exista pericolul folosirii de aflatoxine pulverizate din avion, de către armata irakiană, a condus la scheme de tratament de urgență cu doze masive de vitamina A administrată oral: înainte, în timpul sau imediat după expunere. Experimentele pe şoareci de laborator au dat rezultate foarte bune, aşa că am dotat farmacia de campanie cu un nou sortiment: „Lada cu vitamina A”. Din fericire nu a fost nevoie să o folosim, iar după terminarea războiului din Golf am reluat experimentele, am comunicat şi am publicat rezultatele la Conferința internațională de Toxicologie de la Londra, 1992, în Revista de Farmacie etc.

Profilaxia micotoxicozelor se face în primul rând prin respectarea igienei alimentare (alimente şi furaje), purtarea echipamentelor de protecție adecvate, administrarea de vitamina A în doze mari, iar în caz de forță majoră prin decontaminare specifică, prin diluția corespunzătoare a produselor contaminate cu necontaminate. Nu există vaccinuri sau seruri terapeutice, deoarece molecula foarte mică a micotoxinelor (haptene) nu îi conferă proprietăți imunogenice; pentru acest motiv sunt greu de obținut şi reactivii monoclonali, necesari pentru diagnosticul rapid; aceştia trebuie să fie cuplați în prealabil pe o macromoleculă pentru a fi suficient de mari ca să aibă capacitate imunogenică. Deoarece costurile medicale devin foarte mari, pentru o boală foarte rară, finanțatorii ar putea decide că este prea scump să investească în acest domeniu. Din păcate, uneori în medicină se aplică decizia politică incorectă, neformulată ca atare, dar aplicată în practică după principiul că „este mai ieftin să moară decât să fie tratat”.

3. Aflatoxina. Aflatoxinele sunt produse de către anumite tulpini de *Aspergillus flavus* şi de *Aspergillus parasiticus*. Aceste mucegaiuri sunt micromicete ubicuitare şi puterea lor de a contamina produsele alimentare destinate oamenilor şi animalelor este considerabilă.

Existenţa şi amploarea contaminării cu toxine variază cu diverşi factori geografici şi sezonieri, de asemenea cu condiţiile de cultivare, recoltare şi stocare a recoltelor.

În regiunile tropicale şi subtropicale acestea se contaminează mai uşor decât în regiunile temperate, deoarece predomină condiţii optime pentru dezvoltarea fungilor şi pentru formarea toxinelor: umiditate mare şi temperatură crescută. Ciupercile toxigene pot infecta o cultură care a fost atacată de insecte sau compromisă în alt fel, producând toxinele înainte sau după recoltarea şi stocarea produselor. Se pare că prima epidemie de aflatoxicoză s-ar fi produs cu aproape două decenii înainte de identificarea agentului etiologic: în cel de Al Doilea Război Mondial, în timpul asediului Leningradului de către armata germană, ruşii asediaţi şi înfometaţi au folosit pentru fabricarea pâinii făina din tot felul de vegetale, inclusiv mucegăite, deoarece nu aveau altă posibilitate; se estimează că o mare parte din cei consideraţi ca morţi de foame să fi fost de fapt micotoxicoze, agravate de malnutriţie şi condiţiile grele de trai.

Structura chimică a aflatoxinelor a fost elucidată, fiind puse la punct şi tehnici care permit decelarea şi dozarea unor cantităţi de ordinul a 1 μg/kg (1 ppb) şi chiar mai puţin, în produsele alimentare şi ţesuturile corporale.

Coexistă adesea aflatoxine de diferite tipuri (B_1, G_1, B_2, G_2), care au fost detectate în produse alimentare vegetale ca: porumb, arahide, nuci şi migdale, ca şi în alte produse destinate alimentaţiei omului sau animalelor. Denumirea lor provine de la culoarea fluorescenţei observată la examinarea în lumina ultravioletă: albastră (blue) sau verde (green).

La animale, aflatoxinele ingerate pot suferi o degradare metabolică. Aflatoxina B_1 poate fi convertită în aflatoxina M_1, care se poate regăsi în lapte (milk). Concentraţia sa în laptele de vacă este de aproximativ 300 de ori mai mică decât în furajul ingerat. La unele animale de experienţă, din aflatoxinele care le-au fost injectate au fost regăsite în ţesuturi, la 24 h după administrare, doar cantităţi mici de aflatoxine. În studii făcute pe porc, au fost decelate urme de aflatoxine în ficat, rinichi şi ţesuturile musculare ale animalelor care primiseră aflatoxine în regimul lor alimentar timp de mai multe luni. Se pare că până în prezent nu ar fi fost publicată nici o lucrare privitoare la resturile tisulare de aflatoxine la animale sacrificate.

Întrebuinţarea unor varietăţi rezistente de cereale faţă de mucegaiuri, ca şi aplicarea metodelor de uscare şi stocare adecvate pot reduce infestarea fungică şi implicit pot diminua contaminarea alimentară cu aflatoxine.

Dat fiind că nici prelucrarea termică obişnuită, nici procedeele de tratare uzuale nu elimină complet toxinele din produsele alimentare sau furajele destinate oamenilor sau animalelor, iar măsurile de precauţie luate înainte sau după recoltare nu asigură o protecţie absolută împotriva acestei contaminări, au fost puse la punct tehnici de decontaminare.

Toxina este, în general, concentrată într-o fracţiune mică a grăunţelor, care, adesea, se deosebesc de celelalte prin culoare. În aceste recolte (arahide, cafea etc.) trierea seminţelor decolorate reduce considerabil conţinutul de aflatoxine. Pe de altă parte, inspecţia vizuală în examinarea mucegaiurilor înaintea tratării permite o sortare iniţială, însă prezenţa ciupercilor toxinogene nu implică în mod necesar prezenţa de aflatoxine decelabile, şi nici invers. Deoarece distribuţia aflatoxinelor într-un produs contaminat netratat este inegală, eficacitatea supravegherii este subordonată calităţii eşantionării.

Se ştie că aflatoxinele sunt chimic degradabile *in vitro* sub acţiunea unor dezinfectante oxidante şi/sau alcaline. Decontaminarea chimică se poate face prin tratament cu apă oxigenată şi amoniac.

Dintre toate micotoxinele cunoscute numai grupul aflatoxinelor este recunoscut ca fiind asociat unor efecte bine definite asupra sănătăţii omului.

În numeroase regiuni au fost semnalate zoonoze de aflatoxicoză printre animalele de fermă. În cursul acestor epidemii, precum şi în cursul studiilor experimentale pe animale, inclusiv primate, s-a observat că organul cel mai afectat a fost ficatul. Reamintim că prima zoonoză cu această etiologie a fost înregistrată la o fermă de curcani, iar boala a fost numită iniţial „turk disease”.

Leziunile hepatice acute sunt caracterizate printr-o necroză progresiva a hepatocitelor şi o proliferare celulară la nivelul căilor biliare; cât despre leziunile cronice, ele se pot manifesta ca o fibroză hepatică, dar pot să evolueze spre cancer hepatic. Un nivel al dozei de aflatoxine de 300 µg/kg de aliment este capabil să inducă la porc, în 3-4 luni, o aflatoxinoză cronică. Aflatoxina B_1 este un cancerigen hepatic pentru om şi celelalte primate, ca şi pentru cel puțin alte opt specii de animale. Relațiile doză-răspuns au fost stabilite în cursul studiilor făcute pe şobolani şi pe păstrăv-curcubeu, incidența tumorală fiind de 10% pentru nivel al dozei de aflatoxină B_1 de 1 µg/kg aliment în primul caz şi de 0,1 µg/kg aliment în cel de-al doilea. În anumite studii au apărut la şobolanii tratați cu aflatoxine cancere de colon şi renale.

Aflatoxina B_1 provoacă aberații cromozomiale şi o ruptură a ADN-ului în celulele vegetale şi animale, precum şi, după activitatea microzomilor, mutații genetice în mai multe sisteme ale probei bacteriene. La doză mare, ea poate fi teratogenă.

Toxicitatea acută şi efectul cancerigen al aflatoxinelor sunt mai ridicate la şobolanul mascul decât la femelă; această diferență este legată de sex, putând fi de origine hormonală.

Starea nutrițională a animalelor, conținutul în lipotrope, proteine, vitamina A şi lipide (înțelegând acizi graşi ciclopropenoidici) pot modifica expresia toxicității acute şi/sau cancerogenicitatea.

Nu există date despre raportul dintre expunerea la aflatoxine şi hepatotoxicitatea acută la om. Au fost însă cazuri de leziuni hepatice acute care pot fi imputate unei aflatoxicoze acute.

O epidemie de hepatită acută care a atins mai multe sute de persoane din regiuni vecine a două state din NV-ul Indiei pare să fi fost cauzată de ingestia de porumb foarte contaminat, ale cărui eşantioane conțineau aflatoxine de ordinul a câteva mg/kg, cea mai mare concentrație relevantă fiind de 15 mg/kg.

Cancerul de ficat este mai frecvent în anumite regiuni ale Africii centrale şi Asiei de sud-est decât în alte părți ale lumii, fiind corelate cu consumul de orez şi alte alimente contaminate cu mucegaiuri toxinogene. Pe de altă parte, confruntând informațiile epidemiologice locale cu datele furnizate de experimentele făcute pe animale, s-ar părea că o creştere a expunerii la aflatoxine ar putea să crească riscul de cancer primitiv al ficatului. Ansamblul de date culese din Kenia, Mozambic şi Tailanda relevă o corelare pozitivă între aportul alimentar cotidian de aflatoxine (de 3,5 la 222,4 mg/kg greutate corporală şi incidența anuală brută a cancerului primitiv de ficat (de 1,2 la 13,0 cazuri /100 000 de locuitori). Unele indicii relevă de asemenea un rol viral în etiologia maladiei. S-a precizat, pe de o parte, că aflatoxinele au diverse efecte, în particular efecte cancerigene, pe mai multe specii de animale, iar pe de altă parte că există un raport între nivelurile expunerii la aflatoxine şi incidența cancerului primitiv de ficat, relevant în anumite părți ale lumii. De aceea este necesară menținerea, pe cât posibil, în afara expunerii la aflatoxine.

Concentrațiile maxime admise stabilite în mai multe țări pentru produsele alimentare trebuie să fie considerate ca un mijloc de a uşura aplicarea programelor de luptă contra aflatoxinelor şi nu ca limite de expunere a căror respectare ar garanta protejarea sănătății. În România, majoritatea laboratoarelor județene sanitar-veterinare au mijloacele pentru controlul micotoxicologic al alimentelor.

4. Ochratoxine. Ochratoxinele sunt produse de mai multe specii de ciuperci din genurile *Aspergillus* şi *Penicillum*. Aceste ciuperci sunt ubicuitare şi puterea lor de contaminare a produselor alimentare destinate omului şi animalelor este foarte mare; denumirea vine de la culoarea ocru pe care o prezintă mucegaiul.

Ochratoxina A, produsul principal al acestor mucegaiuri, a fost decelată în peste 10 țări europene şi în SUA. Producerea de ochratoxine de către speciile de *Aspergillus* nu este posibilă decât în condiții de umiditate mare şi temperatură crescută, în timp ce anumite specii de *Penicillum* sunt capabile să producă ochratoxine la temperaturi care nu depăşesc 5 C, deci inclusiv în frigider.

Tehnicile de analiză au fost puse la punct pentru identificarea şi dozarea ochratoxinelor. Rezidurile de ochratoxina A au fost identificate în țesuturi de porci sacrificați în abatoare şi s-a demonstrat că în condiții experimentale asemenea reziduri sunt încă decelabile în țesuturile porcului la o lună după întreruperea expunerii. Prezența ochratoxinelor B în aceste produse este rară.

În cazul ochratoxicozelor observate pe teren la animale de fermă (porci, păsări) semnalate în mai multe regiuni, prima manifestare de boală a fost nefropatia cronică. Leziunile constatate sunt următoarele: atrofia tubilor, fibroza interstițială şi, mai târziu, hialinizare glomerulară.

Ochratoxina A s-a dovedit a fi nefrotoxică la toate speciile de animale testate până acum, chiar la doză mică, adică 200 µg/kg aliment, la şobolan şi porc. Ea este de asemenea teratogenă la şoarece, şobolan şi hamster.

Nefropatia umană endemică era o maladie cu etiologie necunoscută, care până acum nu a fost observată decât în anumite regiuni ale Peninsulei Balcanice. În țara noastră este mai frecventă în Banat. În prezent se ştie că etiologia nefropatiei endemice balcanice este legată de intoxicația cronică cu ochratoxine; utilizarea în alimentație de nuci şi alune mucegăite prin depozitare improprie favorizează acest lucru.

Alterările renale pe care această maladie le induce sunt comparabile cu cele ale nefropatiei observate la porc în urma ingestiei de ochratoxina A, iar o expunere mare la această ochratoxină prin regimul alimentar a fost contestată în anumite regiuni ale Peninsulei Balcanice în care nefropatia endemică este întâlnită frecvent.

5. Trichotecene. Toxinele trichotecenice aparţin unui grup de compuşi chimici strâns înrudiţi, produşi de mai multe specii de *Fusarium, Cephalosporium, Myrothecium, Trichoderma* şi *Stachybotrys*. Patru trichotecene (toxina T-2, nivalenol, desoxinivalenol şi diacetoxicirpenol) au fost decelate în stare de contaminanți naturali într-un număr mic de eşantioane alimentare.

Aleucia toxică alimentară, maladie diagnosticată la om în deceniul 5, ar părea asociată ingestiei de grăunțe invadate de o specie de *Fusarium*. Prima epidemie a fost înregistrata în Ucraina: după ofensiva germană, porumbul a rămas necules pe câmp, iar în cursul iernii şi al primăverii, populația înfometată a cules porumbul mucegăit, l-a mâncat şi s-a îmbolnăvit. Nici un caz al acestei afecţiuni nu a mai fost semnalat de la sfârşitul celui de Al doilea Război Mondial; dispariția sa este după toate aparențele datorată ameliorării producției şi condițiilor de stocare a produselor alimentare. Este posibil să fi fost şi o reacție a organismelor slăbite de privațiunile de tot felul din vremea războiului. Nu există dovezi absolute pentru existența unui raport între trichotecenele recent identificate şi aleucia toxică alimentară observată altădată, sau orice altă boală umană.

6. Zeareleonone. Zeareleononele, metaboliţi produşi de diverse specii de *Fusarium*, s-a observat că sunt contaminantul natural al unor cereale, în particular porumbul, în numeroase țări ale Africii, Europei şi în S.U.A.

S-a demonstrat că zearalenona este un estrogen la diverse animale; în plus, pe teren, s-au observat cazuri de sindrom estrogenic specific la scroafă şi sterilitate printre vite, în raport cu un nivel al zearalenonei de 0,1 la 6,8 mg/kg în cazul vitelor mari. Aceasta induce de asemenea malformații congenitale ale scheletului la şobolan.

În anumite țări, eşantioanele de făină şi fulgi de porumb destinați consumului oamenilor au fost găsite contaminate cu zearalenona în proporție de până la 70 µg/kg, ceea ce corespunde la doze de 400 până la 600 ori inferioare celor care, în condiții experimentale, au efecte la maimuță şi şoarece. În anumite regiuni ale Africii, un nivel net superior al zearalenonei a fost ocazional decelat în berea şi terciul preparate din porumb sau sorg contaminate.

Până acum, nu a fost semnalat la om nici un efect advers imputabil prin aportul alimentar de zearalenona, dar merită reținută pe viitor în atenție posibilitatea unui risc pentru sănătate care rezultă dintr-un raport cotidian cu zearalenona, la nivelul celui care a fost observat în Africa în anumite preparate fermentate.

7. Micotoxinele ca armă de nimicire în masă. În ultimii ani s-au făcut cercetări şi chiar s-a folosit o nouă categorie de arme de nimicire în masă, la limita dintre agenții biologici şi cei toxici de luptă –micotoxinele– care sunt toxine extrase din ciuperci, în special din ciupercile microscopice (mucegaiuri).

Potrivit actualelor concepții ale specialiştilor militari, arma biologică poate fi folosită în orice operații militare pentru a provoca vătămări personalului, animalelor şi plantelor. Cu toate că agenții patogeni produc efecte foarte variate, scopul final al folosirii lor este scoaterea temporară sau totală a personalului din luptă.

Preocupările şi stocurile de muniții biologice sunt păstrate în secret. Se pare că de asemenea mijloace de luptă ar dispune marile puteri. S-a vorbit şi s-a scris mult în presa în urmă cu câţiva ani despre folosirea „ploii galbene” – de fapt a micotoxinelor – de către armata sovietică în Afganistan.

De asemenea, la Conferința de revizuire a Convenției privind interzicerea cercetării, producerii şi stocării armelor bacteriologice (biologice) şi cu toxine şi distrugerea acestora, desfăşurată în anul 1987 la Geneva, s-a menționat epidemia de la Sverdlovsk, considerată ca un accident la o instalație de producere a mijloacelor biologice de luptă.

Cu toate că din anul 1972 peste 150 de state au semnat Convenția privind interzicerea armelor biologice, în ultimii ani, în unele țări, au fost reluate preocupările pentru realizarea de noi mijloace biologice de luptă. Au fost dezvăluite preocupări irakiene şi iraniene, care cu sprijinul unor firme din Olanda, Canada şi Germania au pregătit micotoxine ca mijloace de luptă. Este vorba de un extract din mucegaiuri cu puternică acțiune toxică la nivel celular, ce provoacă scoaterea din luptă sau chiar moartea la doze mici prin micotoxicoze. Recent a fost judecat procesul de genocid prin care înalți demnitari irakieni erau învinuiți de atacarea populației minoritare kurde cu substanțe toxice. În fapt, este vorba de utilizarea de micotoxine pulverizate din avion în asociere cu levizita, care creează leziunea cutanată „poarta de intrare”.

Trebuie menționat şi faptul că în diferite țări se continuă cercetările în direcția realizării unor noi categorii de substanțe toxice, cum ar fi micotoxinele naturale şi de semisinteză. Sunt cunoscute preocupările unor state pentru utilizarea toxinelor de semisinteză, în principal micotoxinele, care se încadrează mai mult în domeniul armelor chimice decât al armelor biologice, de exemplu T-2 toxina. Spre exemplu, ar fi realizată o nouă categorie de substanțe toxice, folosind o tehnologie sofisticată de inginerie genetică, capabilă să neutralizeze pe timp limitat o armată de arme întrunite (fiind vorba, probabil, de micotoxine).

Pierderile sanitare probabile în urma utilizării toxinei botulinice asupra unor obiective, a căror populație este cuprinsă între 2 500 000 şi 50 000, sunt estimate în tabelele 1 şi 2.

Tabelul nr. 1. Contaminare pe cale aerogenă (aerosolizare)

Populația		Imbolnăviri		Decese	
Totală	Expusă	%	Cifre absolute	La cei tratați	La cei netratați
2.500.000	1.500.000	70	1.050.000	200.000	300.000
350.000	250.000	70	175.000	30.000	60.000
300.000	200.000	70	140.000	25.000	50.000
250.000	175.000	70	122.500	20.000	40.000
200.000	150.000	70	105.000	15.000	30.000
150.000	100.000	70	56.000	6.000	10.000
100.000	75.000	70	52.000	4.000	8.000
75.000	50.000	70	35.000	3.000	5.000
50.000	30.000	70	21.000	2.000	4.000

Tabelul nr. 2. Contaminare prin contaminarea surselor de apă

Populația		Imbolnăviri		Decese	
Totală	Expusă	%	Cifre absolute	La cei tratați	La cei netratați
2.500.000	1.600.000	50	800.000	80.000	200.000
350.000	230.000	50	115.000	11.500	30.000
300.000	300.000	50	100.000	10.000	25.000
250.000	150.000	50	75.000	7.500	21.000
200.000	130.000	50	65.000	6.500	18.000
150.000	100.000	50	50.000	5.000	15.000
100.000	70.000	50	35.000	3.500	10.000
75.000	50.000	50	25.000	2.500	6.000
50.000	35.000	50	17.000	1.700	3.500

O astfel de situație, teoretic posibilă – după unii autori este foarte greu de realizat în practică din cauza unei multitudini de factori care pot interveni. Dintre aceştia amintim:

–dificultatea realizării unei densități omogene a contaminării şi menținerii ei timp îndelungat;

–dispersia populației în zona contaminată;

–marea variație a sensibilității individuale a contaminărilor enterale;

–inactivarea ușoară după identificare cu dezinfectante fizice şi chimice (cloramină, permanganat de potasiu sau ozonizare).

Dacă o astfel de acțiune de luptă este puțin probabilă totuşi folosirea toxinelor botulinice pe anumite tipuri de obiective (zone) rămâne de mare actualitate, dată fiind uşurința cu care se poate realiza şi efectele pe care le poate da.

În legătură cu convenția privind interzicerea şi eliminarea armelor chimice, acestea se negociază de peste două decenii.

8.Considerente farmaco-economice privind utilizarea micotoxinelor ca arme biologice

După 11 septembrie 2001, pericolul unor atacuri teroriste de amploare asupra populației din oricare parte a lumii nu mai pare a fi un scenariu de neimaginat. Terorismul chimic, biologic sau nuclear nu mai este de domeniul filmelor *science-fiction*. Potrivit unor analişti, riscul utilizării acestor arme este în creştere, având în vedere sporirea violenței interetnice şi religioase şi a încălcării drepturilor omului. Potrivit altora, posibilitățile unui atac terorist cu arme biologice, de exemplu, sunt reduse, dar se trag semnale de alarmă asupra consecințelor dezastruoase, dacă nu se vor lua măsuri imediate.

Costurile în producerea armelor biologice sunt relativ mici, comparativ cu celelalte arme de distrugere în masă, astfel s-a consacrat expresia "armă atomică a săracului".

Studiile de farmaco-economie stabilesc că tratamentul bolilor induse de folosirea micotoxinelor se poate realiza cu cheltuieli mici, cu resurse din piața internă a medicamentului.

Aici intervine o situație care trebuie bine studiată în viitor: posibilitatea ca producția internă de medicamente să fie direcționată pentru ca un anume necesar să fie asigurat, în contextul în care principalii actori din piață – producători de medicamente - nu mai au un acționariat românesc.

Concluzii

Micotoxinele sunt metaboliți ai ciupercilor, în special ai mucegaiurilor, care apar la speciile toxinogene, în anumite condiții de mediu.

Micotoxicozele sunt intoxicații acute sau cronice, care pot apărea la oameni sau animale, sub acțiunea micotoxinelor.

Orice aliment de origine vegetală poate fi contaminat direct cu micotoxine, iar alimentele de origine animală pot fi contaminate direct sau indirect, prin consumarea de furaje contaminate.

Micotoxicologia reprezintă un domeniu nou şi important în medicina umană şi veterinară, cu implicații interdisciplinare: toxicologice, microbiologicie, igiena alimentară, igiena mediului, oncologie etc.

Datorită proprietăților pe care le au, micotoxinele ar putea fi folosite ca armă chimică în scopuri tactice, operative sau strategice ori în scop bioterorist.

Sporii mucegaiurilor toxinogene ar putea fi diseminați în teritoriul inamic, ca armă biologică.

Având în vedere complexitatea şi gravitatea problemelor puse de micotoxicoze, este necesară aprofundarea micotoxicologiei din punct de vedere medico-militar, în special în privința protecției față de micotoxinele folosite ca posibilă armă de distrugere în masă.

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2.Molly O'Meara Sheehan, *State of the World 2007*, Worldwatch Institute, New York, 2007.

3.Victor Lorin P., *Management în sistemul informațional spitalicesc*, Editura Universitara "Carol Davila", Bucureşti-2007.

4. Florian P., Theodor P.,Victor Lorin P., Monica R., *Marketingul serviciilor de îngrijire a sănătății*, Editura Universitară "Carol Davila", Bucureşti -2007.

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Articolele se redactează în Microsoft Word, Normal+Justf., Times New Roman, Font Size – 16 Bold pentru titlu, Font Size – 12 pentru text, iar rezumatul Font Size – 10 Bold. Pagina trebuie setată din Page setup la 2cm marginile, format letter.

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- **REZULTATE**: fără interpretări.
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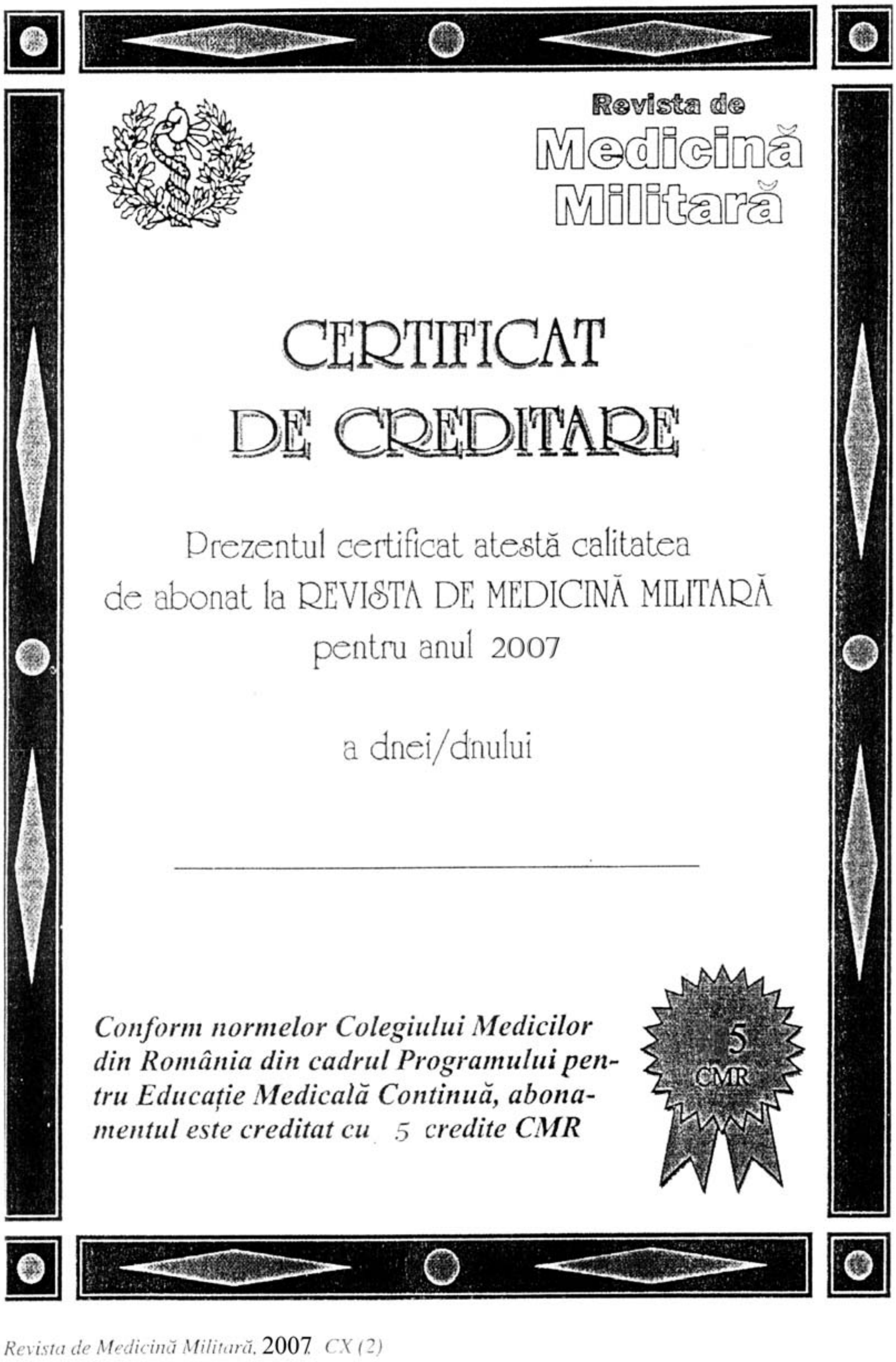

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(8 aprilie 1828 - 24 august 1884)

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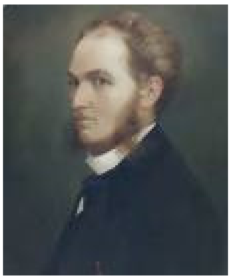

Anul acesta, în ziua de 24 august, s-au împlinit 125 de ani de la trecerea în neființă a Generalului Profesor Doctor Carol Davila, omul de numele căruia se leagă reorganizarea şi edificarea învățământului medical românesc, organizarea serviciului sanitar militar, întemeierea învățământului farmaceutic şi a celui veterinar, fondarea mai multor societăți şi reviste medicale, reforma sistemului balnear din țara noastră, întemeierea mai multor instituții cu un rol important în progresul societății româneşti.

Originile sale sunt învăluite în mister. S-a născut la 8 aprilie 1828 în Italia, lângă Parma, în localitatea Avila, de unde pare să-şi fi luat şi numele, Charles Antoine Francois sau, după alții, Carlos Antonio Francesco d'Avila. Cât priveşte părinții există surse care dau drept sigur faptul că ar fi fost fiul ilustrului muzician Franz Liszt şi al contesei Marie d'Agoult. Alte surse indică drept părinți pe acelaşi Franz Liszt, care în 1828 avea doar 17 ani!, şi pe domnişoara Crig (sau de Krick). Informațiile nu sunt prea clare şi nici Davila nu pare să-şi fi dorit prea mult să clarifice aceste lucruri. Primii zece ani ai vieții sale şi-i petrece la Frankfurt pe Main, în Germania, ulterior ajunge în Franța, la Limoges (unde începe Liceul şi învață şi limba franceză). Între 1843-1845 îl găsim la Nantes, ca elev stagiar în famacia lui Leon le Sant.

Se poate ca în acest moment al vieții sale să fi prins dragoste de tehnicile farmaceutice, care, mai târziu, aveau să-l conducă la prepararea faimoaselor picături Davila – tinctura compusă din ulei de mentă, revent, extract alcoolic de scorțişoară şi tinctură de opiu – atât de benefice în atenuarea vărsăturilor din holeră.

După anul 1845 îşi continuă studiile liceale la Angers, studii pe care le finalizează în 1847, şi lucrează ca stagiar în farmacia lui C.S.Olivier. Tot în localitatea Angers îşi desfăşoară şi desăvârşeşte instrucția medicală în cadrul Şcolii preparatoare de medicină şi farmacie (1 nov.1847 - 1850). Din 1850 îşi continuă studiile la Facultatea de Medicină din Paris pentru a ajunge doctor în medicină. În februarie 1853 absolvă facultatea şi-şi susține lucrarea de doctorat cu teza intitulată *Profilaxia sifilisului.*

Dar să vedem totuşi cum a ajuns Carol Davila pe pământ românesc. Se spune uneori că sfârşitul unei aventuri nu este decât începutul alteia şi că orice lucru are până la urmă o explicație!

În anul 1853, Barbu Ştirbei, domnitorul Țării Româneşti, face un anunț către Facultatea de Medicină din Paris prin care solicită un doctor care să organizeze serviciul medical al armatei şi să fie totodată şi medic personal al domnitorului. Consulul fracez Beclard (căsătorit cu una din fetele lui Barbu Catargiu şi fiu al decanului Facultății de Medicină din Paris) face cunoscut la Paris acest anunț, iar cel recomandat de către decan nu a fost altul decât Carol Davila. Ajunge în Țara Românească la 13 martie 1853, la nici 25 de ani, după ce-şi luase doctoratul şi ajutase la stingerea epidemiei de holeră din 1849 din Champigue şi Cherre. Intră în țară pe la Giurgiu şi deşi ar fi trebuit să stea numai trei ani va rămâne pentru totdeauna pe aceste pământuri (se spune că ar fi avut o solicitare să devină medic la curtea şahului Persiei, pe care a declinat-o). Uimeşte de la început pe domnitorul Ştirbei printr-o primă inspecție sanitară, când dispare fără urmă trei zile şi revine cu un bine fundamentat proiect de reformă sanitară. Ceea ce este mai interesant însă de abia acum urmează...

Pentru început locuieşte într-o locuință igrasioasă şi întunecoasă de pe cheiul Dâmboviței, unde contactează un reumatism articular acut, soldat cu o sechelă – anchiloza cotului drept. Aşa se explică şi atitudinea sa din cele mai multe ipostaze, aceea de a ține brațul drept la spate – poziție surprinsă şi de sculptorul Carol Storck în edificarea cunoscutei statui a lui Carol Davila din fața Facultății de Medicină (statuie inaugurată la 13 oct. 1903).

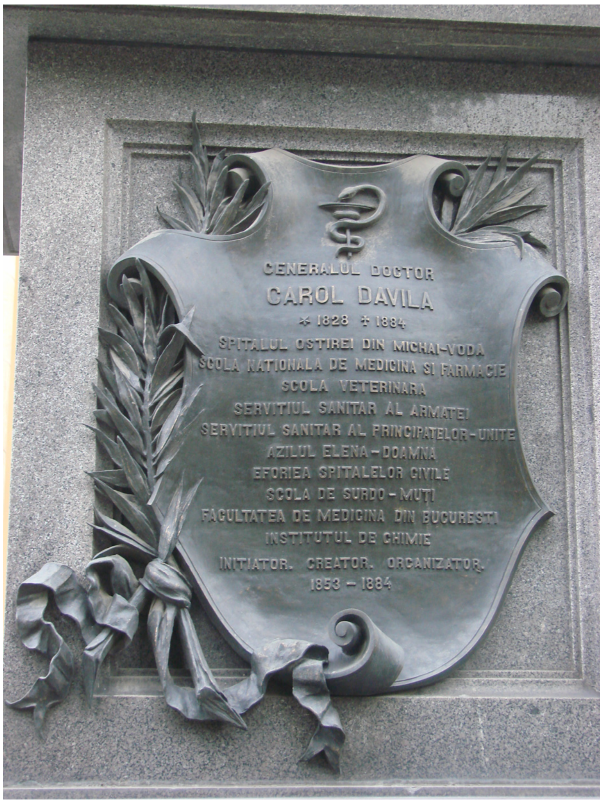
CENERALUL DOCTOR
CAROL DAVILA
✲ 1828 ✝ 1884
SPITALUL OSTIREI DIN MICHAI-VODA
SCOLA NATIONALA DE MEDICINA SI FARMACIE
SCOLA VETERINARA
SERVITIUL SANITAR AL ARMATEI
SERVITIUL SANITAR AL PRINCIPATELOR-UNITE
AZILUL ELENA-DOAMNA
EFORIEA SPITALELOR CIVILE
SCOLA DE SURDO-MUȚI
FACULTATEA DE MEDICINA DIN BUCURESTI
INSTITUTUL DE CHIMIE
INITIATOR. CREATOR. ORCANIZATOR.
1853 – 1884

A fost venirea lui Carol Davila în Țara Românească o aventură? Nicidecum. Totul a fost calculat şi gândit iar realizările sale rămân, peste veac, împliniri grandioase ale unei națiuni aflate încă la început de drum. El a edificat un sistem medical funcțional, organizând atât serviciul militar cât şi pe cel civil.

General Prof. Dr. CAROL DAVILA
ÎNTEMEIETORUL ÎNVĂȚĂMÎNTULUI
MEDICO-MILITAR ȘI MEDICAL
SUPERIOR DIN ȚARA NOASTRĂ

Imediat după sosirea sa în țară este numit şef al Spitalului oştirii de la Mihai Vodă, unde introduce spiritul de disciplină şi simțul răspunderii, noi metode de tratament (transfuzia de sânge, pentru prima oară la noi) şi vaccinarea antivariolică. În 1856 este avansat în funcția de supramedic al oştirii şi contractul de angajare îi este prelungit pentru încă şase ani.

În vara lui 1859, generalul dr. Carol Davila (înaintat la acest grad de către Al.I.Cuza) este numit, prin decret publicat în Monitorul Oficial, medic superior al tuturor trupelor din Principate, pentru ca în 1862 să fie numit Şeful serviciului sanitar al României unificate, funcție echivalentă în zilele noastre aceleia de ministru al Sănătății. Se dăruieşte cu întrega sa ființă activității pe care o desfăşoară. Următoarele cuvinte, care îi aparțin, sunt edificatoare în acest sens: ”Găsind pe malurile Dunării noua patrie, România, Franță a Orientului, în ideile tradiționale şi aspirațiunile de civilizațiune ale surorii celei mari, am devenit, cu inima şi cu fapta, cetățean român”.

Realizarea cea mai de seamă a lui Carol Davila rămâne reorganizarea şi edificarea învățământului medical românesc. Astfel el înființează în 1855 o şcoală secundară de chirurgie cu program şcolar teoretic liceal şi sanitar militar. Dar aşa cum se întâmplă de multe ori în viață, invidia îşi face apariția şi adversarii filoruşi încep să facă demersuri pentru închiderea şcolii. Davila însă este un luptător, el îşi apără crezul adresându-se guvernului francez, care se obligă să mențină şcoala pe cheltuiala sa. Este momentul în care caimacamul Alexandru Dimitrie Ghica dă un opis domnesc (decretul domnesc 1092/1857) pentru menținerea tinerei instituții, pe care Davila o transformă în scurt timp în Şcoala națională de medicină şi farmacie, formă de învățământ recunoscută destul de repede şi în Occident, printr-un decret dat de Napoleon al III-lea. După 10 ani, în 1869, va înființa Facultatea de Medicină. Între timp întemeiază învățământul farmaceutic şi cel veterinar, fondează numeroase societăți şi reviste de specialitate (Asociația medicilor români, „Monitorul medical”, „Gazeta spitalelor”), organizează concursuri medicale şi ţine prelegeri cu demonstraţii experimentale la Sfântu Sava, înfiinţează cu horticultorul austriac Ulrich Hoffman Grădina Botanică, cu un triplu scop: cunoaştere, recreere dar şi resursă pentru preparatele farmaceutice. Introduce concursurile medicale şi stagiile obligatorii în spitale.

Împreună cu farmacistul Hepites pune bazele farmaciei române. Contribuie major la redactarea *Farmacopeei Române*. Personalitate de mare ţinută morală, devotat învăţământului, preocupat de aprofundarea bazelor chimice, fiziologice şi farmacologice experimentale ale terapiei medicamentoase. A publicat o lucrare monumentală în patru volume *Tratatul de terapeutică şi materia medica.*

În calitate de şef al serviciului medical militar, va reforma sistemul balnear românesc, distrus şi ignorat de mai bine de 50 de ani. Dispune trecerea la sisteme mai moderne de exploatare a apelor minerale, precum şi la actualizarea unor analize. Propune şi planifică extinderea şi constuirea de noi instituţii balneare la Pucioasa (1864), realizează curăţirea izvorului 1 de la Căciulata, protejarea acestuia faţă de viiturile Oltului.

Dezvoltă cercetarea în domeniul balneologiei şi promovează rezultatele pe plan internaţional. „Revista ştiinţifică” nr. 6/1873 va publica un catalog al apelor minerale care va însoţi mostrele de ape de la Viena. Convinge un mare număr de medici pentru a participa la acţiunile de promovare a staţiunilor balneare şi stimulează curiozitatea studenţilor medicinişti pentru a cunoaşte, învăţa şi aplica mai târziu cunoştiinţele de balneologie şi balneoterapie. În urma demersurilor (participarea apelor româneşti la expoziţiile de la Viena (1873), Bruxelles (1893), Paris (1889), faima apelor româneşti depăşeşte graniţele ţării, apele de la Căciulata fiind recunoscute pentru efectele benefice asupra afecţiunilor renale, ele fiind recomandate în 1869 chiar şi împăratului Napoleon al III-lea.

Organizează cadrul legal şi ştiinţific de desfăşurare a activităţii de cercetare , tratament şi dezvoltare a balneoterapiei în ţara noastră. Are un aport important la înfiinţarea Societăţii de hidrologie (1868), având drept scop cercetarea şi punerea în valoare a staţiunilor din ţară şi contribuţia la apariţia legislaţiei balneare. A contribuit la formarea viitorilor specialişti în domeniu. Studenţii Şcolii de medicină din Bucureşti fac practică la Olăneşti şi studiază efectele curative ale acestor izvoare. Doctorul Carol Davila a fost un adevărat vizionar al reformelor sistemului sanitar şi învăţământului medical românesc.

A avut preocupări în domeniile medicinii interne, epidemiologiei, ortopediei, chirurgiei abdominale, a introdus în spitale tratamente moderne şi analizele de laborator. A creat în cadrul Spitalului Militar Central primul laborator de analize chimice. A contribuit la apariţia primei *Farmacopei Române* şi a fondat la Bucureşti (în 1876) filiala Română a Crucii Roşii Internaţionale. Tot lui Carol Davila îi aparţine ideea de a introduce consultaţiile gratuite din spitale pentru bolnavii săraci. Participă la stingerea unor epidemii din ţară, manifestă o grijă aparte pentru copiii orfani.

Modelează viaţa medicală sub patru domnitori, la trei dintre ei – Barbu Ştirbei, Alexandru Ioan Cuza şi Carol I – fiindu-le foarte apropiat. A crezut în inteligenţa poporului român. A dinamizat curentul de ascensiune socială a fiilor de ţărani prin şcoli şi universităţi. Un exemplu concludent este doctorul Constantin Severeanu, copil de ţăran din Mehedinţi, selectat de doctorul Carol Davila şi trimis cu bursă de stat la Facultatea de Medicină din Paris. Mulţi dintre elevii săi au devenit, peste ani, profesori ai Facultăţii de Medicină din Bucureşti.

În 1861 întemeiază un azil de orfane, în care adună 40 de fetiţe fără părinţi de prin mahalale. Constituit iniţial în casa soţiei sale, Ana Davila, orfelinatul de fete din Dealul Cotrocenilor , astăzi şoseaua Pandurilor 90-92, se mută într-un edificiu nou, construit din iniţiativa soţiei domnitorului Al. I. Cuza, luând numele de Elena Doamna. Peste câtiva ani înfiinţează un orfelinat şi pentru băieţi.

Venind din Occident şi cunoscând Occidentul, Carol Davila a consiliat şi convins patrioţii români să intre în Ordinul Francmasoneriei, organizaţie care avea în program eliberarea, emanciparea şi dezvoltarea micilor Naţiuni de sub ocupaţia marilor Imperii. După unele surse a fost iniţiat în1874 şi în scurt timp a ajuns Maestru Venerabil al Lojii Înţelepţii din Heliopolis. În 1875 era preşedinte al comitetului masonic pentru ajutorarea bucureştenilor afectaţi de inundaţii. Între 1879-1884 a fost ProMare Maestru al Marelui Orient al României.

Analizând datele avute la dispoziție și privind retrospectiv realizările Generalului Profesor Doctor Carol Davila, putem aprecia că a fost un om tenace, calm și răbdător în realizarea obiectivelor sale, modest, un om care a apreciat adevărul și a militat pentru cultivarea lui, că și-a respectat cu sfințenie crezul, pe care l-a caracterizat în următoarele cuvinte: „Operele noastre trebuie să supraviețuiască. A face bine este misiunea împlinirii umane".

CAROL DAVILA

Mormântul lui Carol Davila

Astăzi îi poartă numele, cu pioșenie și recunoștință, Universitatea de Medicină și Farmacie din București, Fundația și Editura Universitară Carol Davila, Spitalul Universitar de Urgență Militar Central și Spitalul Clinic de Nefrologie din București. Atunci când amintim de OPERA sa nu trebuie să uităm niciodată spiritul său organizatoric providențial, cultura sa inspirată din mai multe izvoare, prudența și supletea spiritului său perfect adaptate caracterului poporului român, în care s-a integrat pe deplin, hărnicia și curajul de a implementa și duce la bun sfârșit atâtea proiecte, așa cum magistral îl caracteriza George Călinescu: „În România el întemeie învățământul medical și fu în toate privințele un om excepțional".

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Colotorax spontan gigant drept

Col. dr. Constantin GROZAVU, Mr. dr. Adrian CIUCHE, Lt. dr. Dragoş MARIN, Cpt. dr. Augustin TUDOSE

Secția Clinică de Chirurgie Toracică
Spitalul Universitar de Urgență Militar Central ”Dr. Carol Davila”, Bucureşti

Rezumat. Hernia retrocostoxifoidiană (Morgagni-Larrey) este o afecțiune rară, dificil de diagnosticat (necesitând tehnologii speciale de explorare a integrității diafragmului), descoperită adesea întâmplător, cu ocazia unui control radiologic de rutină.

În etiopatogenia bolii un rol esențial îl joacă factorul congenital reprezentat de un defect embriologic de muscularizare a părții anterioare a diafragmului. De asemenea, în mecanismul de producere a hernierii viscerelor abdominale prin acest defect diafragmatic congenital un rol important revine şi factorilor favorizanți care acționează fie prin creşterea presiunii abdominale, fie prin scăderea tonusului muscular al cupolelor diafragmatice.

Este prezentat cazul unei femei de 72 de ani cu dispnee la eforturi medii, etichetată inițial ca o consecință a unei suferinței cardiace ischemice. Odată pus diagnosticul de hernie retrocostoxifoidiană cu colon prin examen CT-multislice cu reconstrucție în plan frontal şi sagital, este stabilită şi indicația chirurgicală cu caracter absolut pe care o implică această maladie. Intervenția chirurgicală practicată prin laparotomie xifo-ombilicală a dus la vindecarea bolii şi la ameliorarea simptomatologiei prin atingerea celor trei scopuri operatorii: reintegrarea colonului în abdomen, rezecția sacului herniar şi repararea defectului diafragmatic.

Raritatea afecțiunii, dimensiunile mari ale herniei (o mare parte din colonul transvers migrat în torace), dificultățile de diagnostic şi evoluția favorabilă prin tratament chirurgical (ameliorarea dispneei), ne-au determinat să prezentăm acest caz.

Cuvinte cheie: Colotorax, hernie diafragmatică

Abstract: **Morgagni-Larrey hernia represents a rare form of congenital diaphragmatic hernia, occurring in 1% to 6% of all cases of diaphragmatic defects. It is very difficult to diagnosticate this desease, because it need specials methods for diaphragmatic assessment.**

This type of hernia occurs in an area of weakness of the diaphragm, between the anterior sternal and costal fibers, a triangular region known as the Larrey space. The hernia is usually discovered incidentally in adults, whereas it can be accompanied by chest pain, nausea and vomiting in children.

The authors presented the case of a 72 year's women, with dyspnoea precipitated by exertion and relieved by rest. The first diagnosis was ischaemic cardiac desease. The final diagnosis was established on multislice CT examination, wich revealed the intrathoracic transdiaphragmatic and retrosternal colon herniation.

Surgery was performed immediately after diagnosis, consisting in a median laparotomy with closure of the diaphragmatic gap with separate stiches, with the hernia sac resection, with very good results consists in improvement of the respiratory function.

Introducere

Herniile diafragmatice pot fi congenitale sau dobândite. În funcție de natura orificiilor diafragmatice prin care se produc, întâlnim următoarele tipuri de hernii diafragmatice: anatomice, congenitale şi traumatice (1).

Herniile anatomice se produc prin orificiile naturale ale diafragmului (hiatul esofagian, orificiul venei cave inferioare, orificiul aortei, orificiile pentru venele lombare ascendente, orificiile pentru nervii splanhnici, orificiile paravertebrale pentru lanțul simpatic drept şi stâng); aceste orificii naturale se lărgesc progresiv permițând ascensionarea viscerelor abdominale în torace.

Dintre herniile diafragmatice dobândite cele mai frecvente sunt herniile hiatale, care se realizează prin ascensionarea în torace a stomacului şi mai rar a altor viscere abdominale, prin lărgirea progresivă a hiatului esofagian.

Herniile diafragmatice postraumatice sunt o altă variantă de hernii dobândite care se realizează însă printr-un orificiu diafragmatic anormal, postraumatic: ruptura diafragmatică (traumatism închis) sau plaga diafragmatică (traumatism diafragmatic deschis).

Herniile diafragmatice congenitale se realizează prin orificii anormale ale diafragmului care sunt rezultatul unei malformații congenitale. În funcție de localizarea acestor defecte la nivelul diafragmului, aceste hernii se pot subîmpărți în: hernii anterioare (hernii retrocostoxifoidiene tip Morgagni-Larrey), laterale (hernii ale cupolei diafragmului, cel mai frecvent postero-laterale tip Bochdaleck) şi mediale (hernii prin aplazie hiatală sau postero-mediale).

Herniile congenitale anterioare tip Morgagni-Larrey apar cel mai frecvent la adulți printr-un defect embriologic de muscularizare a părții anterioare a diafragmului (a septului transvers) ale cărui dimensiuni variază de la o simplă zonă de slăbiciune până la absența porțiunii sternale a diafragmului (orificiul Larrey foarte larg). Acest defect embriologic apare de obicei după fuzionarea membranelor pleuro-peritoneale cu septul transvers (hernii fetale, cu sac herniar)(8). În mecanismul de producere a hernierii viscerelor abdominale prin acest defect diafragmatic congenital un rol important revine factorilor favorizanți care acționează fie prin creşterea presiunii abdominale (obezitate, cifoscolioză, tumori abdominale, traumatisme abdominale, sarcină, eforturi fizice mari), fie prin scăderea tonusului muscular al cupolelor diafragmatice (scădere ponderală, importantă mai ales la vârstnici).

Apariția herniei Morgagni-Larrey la copii şi la nou- născuți este mult mai rară ca la adulți şi poate fi provocată de aplazia porțiunii musculare sternale a diafragmului (defect mare muscular retrosternal) însoțită de fuzionarea septului transvers cu membranele pleuro-peritoneale (hernii fetale, cu sac herniar) sau de absența acestei fuziuni cu persistența canalului peritoneo-pericardic (hernii intrapericardice, embrionare, în care sacul herniar lipseşte) (2), (14). Etiopatogenia de tip congenitală a acestor hernii este sprijinită de asocierea lor frecventă, atunci când apar la copii şi nou-născuți, cu alte malformații congenitale: sindrom Down, sindrom Turner, sindrom Noonan, pentalogia Cantrell, pectus excavatum/carinatum, malrotații digestive, malformații genito-urinare (6).

Studiile efectuate arată că hernia Morgagni-Larrey la adulți apare mai frecvent la femei, la nivelul hemitoracelui drept (defectul diafragmatic stâng este protejat de sacul pericardic), prezintă sac herniar (sunt hernii de tip fetal) iar dintre viscerele herniate cel mai des sunt prezente în sacul herniar marele epiploon şi colonul transvers, foarte rar stomacul, lobul stâng hepatic, intestinul subțire sau cecul.

Clinica acestor hernii este dominată de manifestările insuficienței respiratorii ce apar imediat după naştere (la nou-născuți) sau după efortul fizic (la copii şi adolescenți) sau de manifestări mixte (de tip respirator şi digestiv) la adulți. Între 30 şi 50% dintre pacienții adulți cu hernii Morgagni-Larrey sunt însă asimptomatici, boala fiind depistată în urma unui examen radiologic de rutină.

Prezentare de caz

Vă prezentăm cazul unei paciente în vârstă de 72 de ani, fără antecedente patologice semnificative, care se internează pentru dispnee la eforturi medii, astenie fizică, meteorism abdominal, constipație cronică şi pentru descoperirea radiologică, cu ocazia unui episod respirator intercurent, a unei opacități bine delimitate ce ocupă practic întreaga bază a hemitoracelui drept.

Examenul clinic arată o pacientă cu obezitate moderată, cifotică, echilibrată respirator în repaus, dispneică la eforturi medii, cu murmur vezicular diminuat la nivelul bazei hemitoracelui drept, submatitate percutorie la acest nivel, normotensivă, abdomen moderat destins de volum, meteorizat, nedureros, tranzit prezent pentru gaze dar absent pentru materii fecale.

Consultul cardiologic stabileşte diagnosticul de cardiopatie ischemică şi pune simptomatologia respiratorie pe seama unei insuficienţe cardiace clasa II NYHA, pentru care se recomandă tratament antianginos şi tonicardiac.

Examenul radiologic standard antero-posterior arată existenţa unei imagini radiologice semiopace, care ocupă întreaga bază a hemitoracelui drept, având marginea superioară convexă, bine delimitată. Imaginea descrisă este suprapusă pe opacitatea intensă, convexă, bine delimitată, dată de cupola hemidiafragmului drept şi peste umbra cardiacă (fig.1).

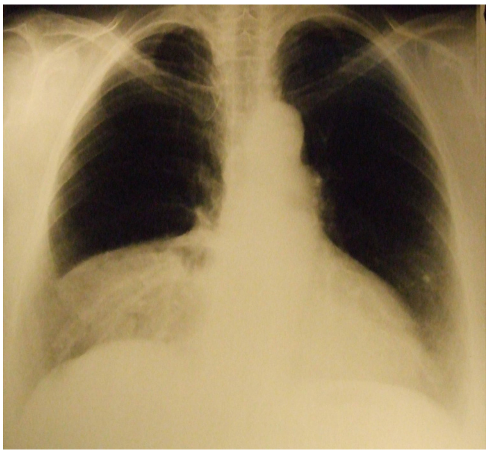

Fig. 1. Radiografie toracică faţă: opacitate ce ocupă baza hemitoracelui drept.

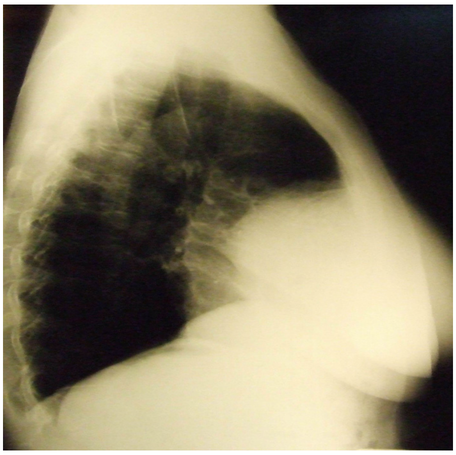

Fig. 2. Radiografie toracică de profil: opacitatea bazală proiectată anterior peste umbra cardiacă.

Radiografia de profil proiectează această imagine semiopacă de mari dimensiuni, anterior, în întregime peste opacitatea cardiacă (fig.2).

Examenul CT – multislice efectuat în Clinica de radiologie, imagistică medicală şi medicină nucleară din SCUMC arată pe secţiuni transversale prezenţa unei anse colice ascensionată paracardiac în dreapta, fără a putea aprecia pe acest tip de secţiuni, integritatea diafragmului (fig.3).

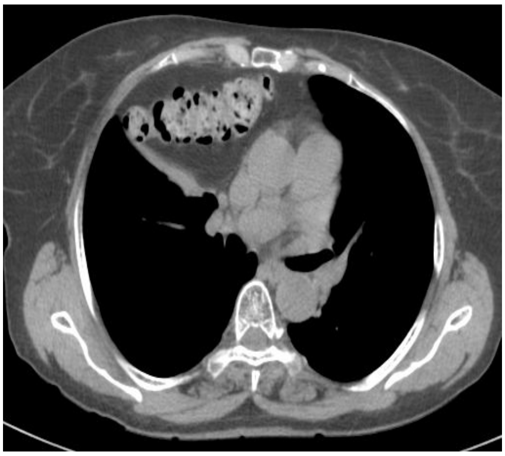

Fig. 3. CT multislice cu secţiuni transversale: ansă colică ascensionată paracardiac drept.

Pentru a putea diferenţia hernierea transdiafragmatică a unei anse colice de o posibilă relaxare a hemidiafragmului drept au fost deosebit de utile imaginile de reconstrucţie CT în plan frontal (fig.4) şi sagital (fig.5), realizate în cadrul aceleiaşi examinări. Acurateţea datelor legate de explorarea diafragmului, furnizate prin această performantă tehnologie imagistică, a permis stabilirea diagnosticului de hernie tip Morgagni-Larrey cu ansă de colon migrată transdiafragmatic drept. Se poate afirma că în acest caz examenul CT multislice, prin reconstrucţiile efectuate, a înlocuit cu succes examenul RM, considerat suveran în exploararea diafragmului (10).

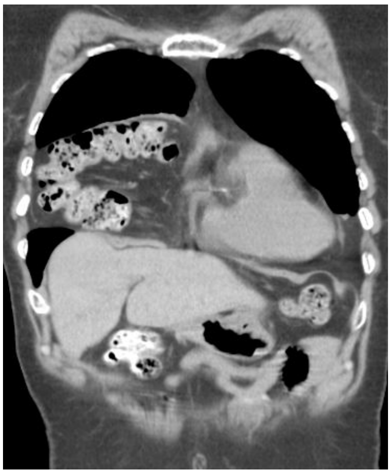

Fig. 4. Examen CT-multislice cu reconstrucţie în plan frontal: ansă colică intratoracică dreaptă cu întreruperea continuităţii hemidiafragmului drept la nivel paracardiac.

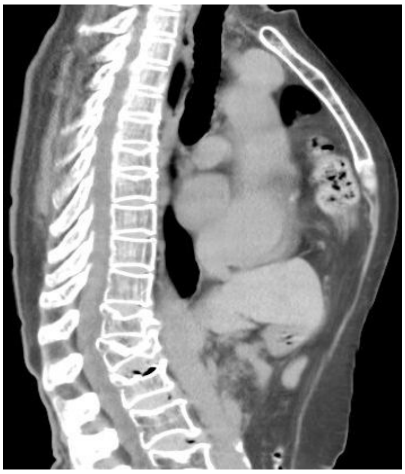

Fig. 5. Examen CT-multislice cu reconstrucţie în plan sagital: ansă colică intratoracică cu evidenţierea unei breşe de 4 cm la nivelul porţiunii retrosternale a diafragmului.

Odată pus diagnosticul de hernie diafragmatică tip Morgagni-Larrey s-a putut stabili şi indicaţia chirurgicală absolută a acestei boli.

Sub anestezie generală cu intubaţie selectivă se intervine chirurgical printr-o laparotomie xifo-ombilicală. Intraoperator se descoperă o porţiune importantă din colonul transvers herniată în hemitoracele drept, printr-o breşă diafragmatică de aproximativ 4 cm diametru, situată retrosternal drept, delimitată lateral de către ligamentul rotund hepatic (fig.7). Prin manevre uşoare de tracţiune se reintergează în abdomen ansa colică înconjurată de sac herniar peritoneal şi se observă absenţa semnelor de suferinţă ischemică ale acesteia (fig.6, 8, 9). Se rezecă sacul herniar (fig.10,11) şi se închide cu fire neresorbabile defectul diafragmatic, prin fixarea marginii diafragmului, anterior, la muşchiul drept abdominal, fără a se deschide cavitatea pleurală (fig.12,13). Operaţia se termină prin drenajul dublu al cavităţii peritoneale (subdiafragmatic şi sacului Douglas) şi prin laparorafie în planuri anatomice. Evoluţia postoperatorie a fost favorabilă, cu ameliorarea dispneei de efort şi externare în a 5-a zi postoperator.

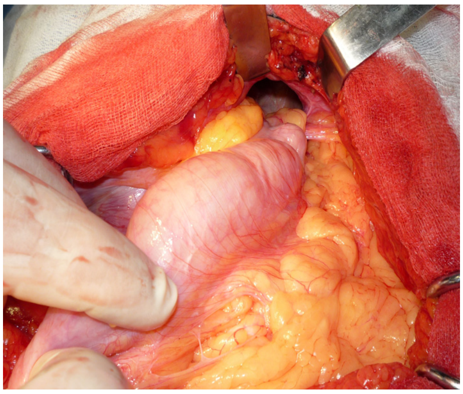

Fig.6. Prin manevre uşoare de tracţiune se reintergează în abdomen ansa colică herniată în hemitoracele drept

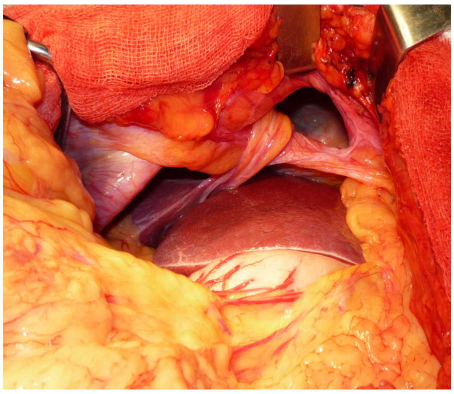

*Fig.7.*Breşă diafragmatică de aproximativ 4 cm diametru, situată retrosternal drept, delimitată lateral de către ligamentul rotund

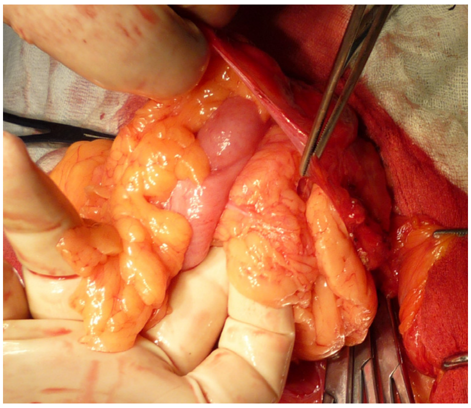

Fig. 8. Ansa colică înconjurată de sac herniar peritoneal.

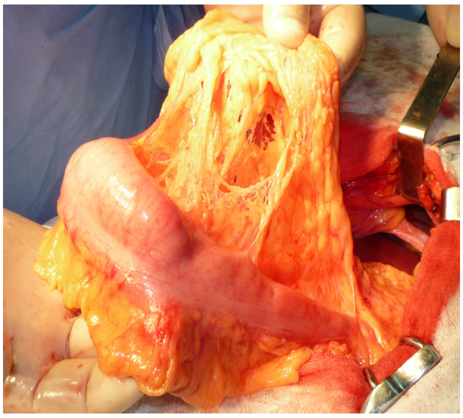

*Fig.9.*Porțiune importantă din colonul transvers şi din marele epiploon, reintegrate în abdomen; se observă absența semnelor de suferință ischemică.

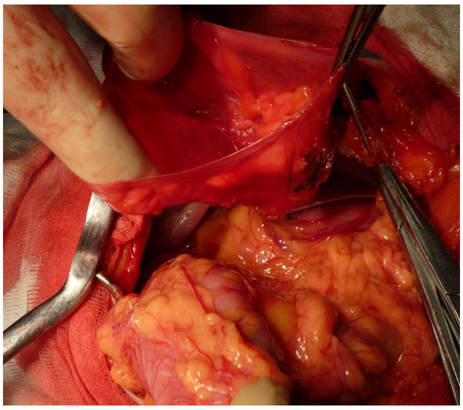

Fig. 10. Sac herniar peritoneal reintegrat în abdomen (formă fetală de hernie diafragmatică).

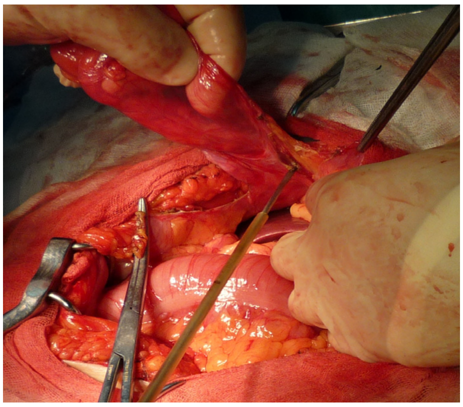

Fig. 11. Rezecția sacului herniar.

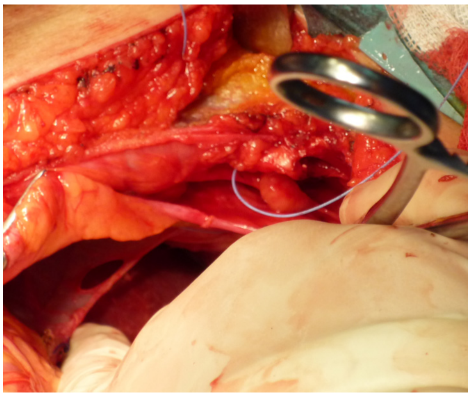

Fig. 12. Închiderea defectului diafragmatic prin fixarea marginii diafragmului, anterior, la muşchiul drept abdominal cu fire neresorbabile, fără a se deschide cavitatea pleurală.

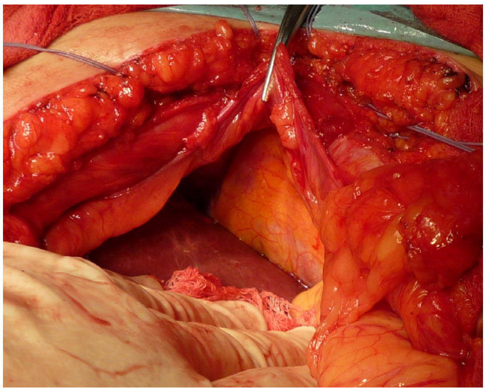

Fig. 13. Aspect final intraoperator: diafragm integru (breşă diafragmatică închisă).

Radiografia postoperatorie arată dispariția opacității de la baza hemitoracelui drept şi expansiunea pulmonară completă (fig.14).

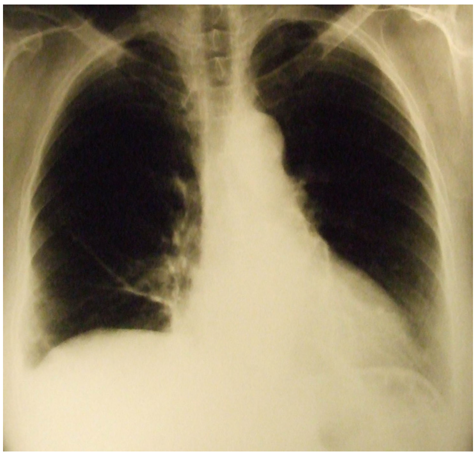

Fig. 14. Radiografie toracică antero-posterioară: dispariția opacității bazale drepte, diafragm drept cu morfologie normală.

Discuții

Cazul prezentat se individualizează prin dimensiunea importantă a herniei, o mare parte din colonul transvers fiind migrată intratoracic, printr-o breşă diafragmatică de dimensiuni relativ mici (4 cm), fără apariția suferinței intestinale de tip ischemic şi fără instalarea simptomatologiei ocluzive. Simptomatologia respiratorie accentuată la eforturi medii a fost practic singura suferință a pacientei.

Dispneea de efort, instalată progresiv de-a lungul anilor, a fost considerată şi tratată numai prin prisma decompensării suferinței cardiace de tip ischemic de care suferă într-adevăr pacienta. Ameliorarea postoperatorie a acestei simptomatologii este consecința eliberării plămânului drept de compresiunea pe care o exercita conținutul ansei colice.

În mecanismul de producere a herniei la această bolnavă, pe lângă factorul congenital, reprezentat de prezența defectului diafragmatic cu absența muscularizării părții anterioare a diafragmului, un rol important l-a avut şi hiperpresiunea abdominală generată de obezitate medie şi de cifoză.

Examenul CT- multislice cu reconstrucție în plan frontal şi sagital a fost esențial în stabilirea diagnosticului de hernie Morgagni-Larrey, înlocuind cu succes investigarea RM, considerată suverană în explorarea diafragmului.

Indicația chirurgicală a fost absolută, în condiții de programare, prevenind încarcerarea colonului cu apariția ischemiei şi a ocluziei intestinale.

Abordul prin laparotomie a fost de elecție, permițând realizarea în siguranță a principalelor obiective ale intervenției chirurgicale : reducerea ansei colice în abdomen, rezecția sacului herniar şi închiderea breşei diafragmatice.

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O sută de ani de urologie românească

ACADEMIA ROMÂNĂ
SECȚIA DE ȘTIINȚE MEDICALE
ACADEMIA DE ȘTIINȚE MEDICALE

ISTORIA UROLOGIEI ROMÂNEȘTI

Coordonator: Acad. Prof. Dr. L.M. POPESCU
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Prof. Dr. V. TODE

1909 - 2009
ARU
ASOCIAȚIA ROMÂNĂ DE UROLOGIE
100 DE ANI DE UROLOGIE ÎN ROMÂNIA

Editura Universitară "Carol Davila", 2009

Un gest de preţuire, o stavilă împotriva timpului care se scurge cu o viteză ameţitoare, o încercare de a îndepărta colbul uitării de pe munca, realizările, împlinirile şi neîmplinirile confraţilor înaintaşi. Aşa aş numi gestul urologilor români de astăzi prin care oferă colegilor şi tuturor celor pasionaţi o istorie cât se poate de sinceră a primului veac de Urologie de pe pământul românesc

Este vorba despre *Istoria urologiei româneşti*, Editura Universitară „Carol Davila”, 2009 – operă colectivă apărută sub egida a două mari instituţii academice române – Academia Română, Secţia de ştiinţe medicale şi Academia de Ştiinţe Medicale, coordonată de Domnul Acad. Prof. Dr. L.M. Popescu şi editată de Dl. Prof. Dr. Ioanel Sinescu, membru corespondent al Academiei Române şi Dl. Prof. Dr. Viorel Tode.

Sunt prezentate într-o manieră captivantă oameni, fapte şi întâmplări din primul secol urologic românesc. Iniţiativa editării acestei cărţi aparţine – şi este cuvenit să dăm Cezarului ce-i al Cezarului! – entuziastului Prof. Dr. Viorel Tode de la Universitatea „Ovidius” din Constanţa care, într-un cuprinzător capitol intitulat „Geneza urologiei româneşti”, prezintă începuturile întrepătrunse ale acestui fapt, ziua fastă de 14 octombrie 1909, când la Spitalul Colţea este oficial fondată Urologia Românească prin contribuţia Prof. Petre Herescu, discipol al marilor urologi francezi Felix Guyon şi Joaquin Maria Albarran. Sunt evidenţiate şi reliefate meritele ctitorilor – Profesorii Petre Herescu, Dimitrie Gerota, Nicolae Hortolomei, Emil Ţeposu şi a urmaşilor lor Theodor Burghele, Eugen Proca, Petru Drăgan şi a „urmaşilor urmaşilor” lor, fiindcă importanţi au fost şi sunt cu toţii – prin contribuţia tuturor sporind lucrarea urologică românească.

Deviza Asociaţiei Române de Urologie este dictonul lui Virgiliu: ”Labor improbus omnia vincit” iar apariţia acestei monografii, la un an de la apariţia monumentalului „Tratat de Urologie”, Editura Medicală 2008, completează şi reafirmă crezul profesional al urologilor români.

Dictonul latin „Fugit irreparabile tempus” poate fi adnotat: timpul urologic nu a fugit fără întoarcere pe pământ românesc – a existat şi există o continuitate a generaţiilor de urologi iar cei ce sunt astăzi în fruntea breslei au convingerea deja exprimată public că cei ce îi vor urma vor fi mai buni, mai competitivi, mai performanţi, mai europeni, conform devizei „O singură Europă, o singură urologie!”. Volumul constituie practic un rezumat al primilor o sută de ani de viaţă urologică românească, subliniindu-se deopotrivă atât faptele bune cât şi cele mai puţin bune, cu dorinţa subînţeleasă de a nu se mai repeta. Oricare ar fi tipul de inexactităţi strecurate în text cu siguranţă că acestea au fost comise fără voie sau din neştiinţă. Lipsesc relatările câtorva centre clinice care au realizări notabile în materie de îngrijire a bolnavului urologic, altele au făcut referire la activitatea recentă, postdecembristă, neajungând cu explorarea până la începuturi. Avem convingerea sinceră că nimic nu este perfect, totul este perfectibil şi ediţiile viitoare ne vor arăta aceasta!

Autorii şi coautorii *Istoriei urologiei româneşti* sunt convinşi că scriind-o şi-au făcut datoria faţă de breaslă şi faţă de „părinţii lor urologici”. „Fiindcă, dacă nu ar fi existat ei nu am fi existat noi!”. Păşind în secolul al doilea de viaţă urologică românească, nu vom spune decât: Vivat, Crescat, Floreat!

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Imagistica prin Rezonanță Magnetică în explorarea patologiei degenerative a coloanei vertebrale

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Rezumat. În clinica noastră am realizat, în perioada ianuarie 1999 - iulie 2009, aproximativ 35 183 explorări IRM (Imagistică prin Rezonanță Magnetică), iar circa 60% dintre examinări au fost dedicate coloanei vertebrale. În prezentul material am scos în evidență aspectele imagistice deosebite, elementele de prognostic, patologia asociată şi diagnosticul diferențial cu alte tipuri de afecțiuni cu semne clinice asemănătoare.

Cuvinte cheie: IRM, patologie degenerativă, hernie discală.

Abstract. Between january 1999 and july 2009 we have performed 35183 MRI examiantions and 60 % were dedicated for spine. Our paper shows special imaging aspects in MRI semiology, the associated elements and differential diagnosis with other lesions that mimic the same clinical aspect.

Cauza principală a durerii în spondiloza vertebrală se datorează modificărilor degenerative ale articulațiilor şi discurilor, cu afectarea structurilor anatomice inervate ale rahisului dorso-lombar (ligamente, periost, musculatură, vase sanguine, rădăcini nervoase, sinoviala articulațiilor interapofizare posterioare). Aceste modificări produc variații ale calibrului canalului vertebral şi neuroforamenelor cu repercusiuni asupra rădăcinilor nervoase şi măduvei.

După examenul clinic primul tip de examinare paraclinică este radiografia coloanei vertebrale. În cazul unor probleme de diagnostic cea mai utilă examinare este explorarea radiografică de profil comparativ în decubit şi în ortostatism.

În perioada ianuarie 1999 – iulie 2009 în clinica noastră au fost efectuate 35 183 examinări, dintre care 20 864 pentru coloana vertebrală. Dintre acestea 16 816, respectiv 80,16%, au fost efectuate pentru explorarea coloanei vertebrale lombare, 2 901, 13,6%, pentru coloana cervicală şi 1148 pentru coloana cervico-toracală sau toraco-lombară, adică 5,5%. Compartimentul extradural a fost afectat în 86,79%, în 18 107 cazuri, cel intradural extramedular în 1 844 cazuri, adică în 8,84% iar cel intramedular în 913 cazuri, adică în 4,37% din cazuri. Din punctul de vedere al clasificării tipului de afecțiune care afectează compartimentul extradural, spectrul patologiei a cuprins leziuni degenerative vertebrale, traumatisme vertebrale, leziuni infecțioase vertebrale, tumori vertebrale primitive sau secundare sau anomalii vertebrale. Asocierea de leziuni cu cele degenerative a fost prezentă în 70,3% dintre cazuri. 19 % dintre pacienți asimptomatici prezentau diverse modificări ale discurilor intervertebrale.

Examenul radiologic

Artrozele intervertebrale se evidențiază radiologic chiar şi la pacienții asimptomatici. Discartroza se traduce radiologic prin următoarele modificări: reducerea în înălțime a spațiului intervertebral, platouri vertebrale neregulate şi modificări de osteoscleroză. Se reduce înălțimea spațiului articular şi apar modificări de structură osoasă. Spondiloza dorso-lombară se caracterizează prin prezența difuză a osteofitelor, a căror semnificație clinică se corelează atunci când produc compresie radiculară sau chiar medulară, atunci când se dezvoltă exuberant.

Tabloul radiologic este dominat de diminuarea în înălţime a spaţiului intervertebral, de osteocondensare şi de prezenţa osteofitelor. Discartrozele mecanice sunt localizate în special în zonele de joncţiune între segmentele cervico-toracal şi toraco-lombar, iar cele degenerative sunt multiple, supraetajate.

Alte modificări radiologice asociate mai pot fi calcificările nucleului pulpos şi artroza interapofizară.

Tehnica de explorare în cazul explorării IRM

Protocolul standard de explorare cuprinde secvenţe de tip sagital de tip T1 şi T2, urmate de secvenţe axiale în planul discurilor de explorat, de obicei de tip T2. Nu trebuie neglijată valoarea secvenţelor de tip mielografic atât în plan sagital, coronal cât şi oblic, în planurile neuroforaminale. În funcţie de suspiciunea clinică mai pot fi folosite şi secvenţe care suprimă semnalul ţesutului adipos, precum şi injectarea substanţei de contrast în T1, cu şi fără supresie de grăsime şi eventual cu substracţie pentru evaluarea prizei de contrast. Secvenţele T2 ecou de gradient sunt utile pentru diferenţierea materialului discal de osteofite sau calcificări discale.

În vederea explorării eficiente a coloanei vertebrale şi pentru evitarea artefactelor produse de aortă sau de arterele carotide în explorarea cervicală se folosesc benzi de presaturaţie iar direcţia de codare în fază în sens cranio- caudal pentru explorarea sagitală şi dreapta- stânga pentru cea în explorarea axială.

În cazul unor neconcordanţe de diagnostic cu starea clinică a pacientului poate fi util examenul cu instalaţii IRM care investighează pacienţii cu coloana în alte poziţii şi care o pun în tensiune. În cazul în care nu se dispune de o astfel de instalaţie, examenul poate fi completat cu mielografie clasică şi migrarea substanţei de contrast în diverse poziţii.

Elemente de semiologie IRM

Aspectul IRM al discului intervertebral este de reducerere a semnalului în T2, reducerea înălţimii discului intervertebral, vacuum phenomenon, leziuni anulare, priză de contrast periferică la nivelul discului lezat datorită ţesutului de granulaţie

Terminologia folosită este: debord discal, protruzie, hernie. În funcţie de volumul materialului discal, de zona prin care acesta se deplasează faţă de structurile de fixare, explorarea IRM poate evidenţia dimensiunile şi raporturile cu structurile din vecinătate.

Platourile discale vertebrale şi măduva osoasă vertebrală suferă modificări ce pot fi clasificate conform Modic în următoarele grade :

- Modic I: edem şi inflamaţie cu semnal hipointens T1 şi hiperintens T2 şi cu priză de contrast locală;
- Modic II: degenerescenţă adipoasă cu semnal hiperintens T1 şi T2;
- Modic III: scleroză, semnal hipointens T1 şi T2.

Nodulii Schmorl reprezintă hernierea de material discal intraosos intervertebral.

Modificările degenerative ale faţetelor articulare se prezintă sub forma reducerii spaţiului articular, apariţiei osteofitelor, hipertrofiei proceselor articulare, şi a ligamentelor galbene, eroziunii subcondrale şi a chisturilor, precum şi a fluidului intra-articular şi, ocazional, a chisturilor sinoviale.

Elemente de apreciere a evoluţiei leziunilor discale, foarte importante în stabilirea conduitei terapeutice adecvate şi prognosticul tratamentului conservator, sunt reprezentate de priza de contrast din jurul materialului discal herniat în canalul vertebral, precum şi de semnalul discului în T2. Modificarea acestuia cu transformarea sa în hipersemnal predominant T2 are semnificaţia apariţiei fagocitozei locale.

În cazul suspiciunii clinice a compresiei medulare, IRM este examinarea de elecţie.

Protocolul de examinare se bazează pe realizarea de secvenţe sagitale ponderate în T1 şi T2, urmate de secvenţe de tip T1 post- injectare de gadolinium, precum şi axiale T2 sau T1 post-contrast la nivelul leziunii.

Rolul examenului IRM constă în recunoaşterea nivelului leziunii. Clinic, sindromul produs de leziune (de exemplu, anestezia în bandă sau abolirea unui reflex) nu trebuie confundat cu sindromul produs sublezional (sindromul piramidal şi tulburările senzitive ale membrelor inferioare).

- Compresia medulară poate fi produsă de cauze rahidiene, de exemplu o fractură tasare a unui corp vertebral poate fi produsă, post-traumatic sau spontan, de o hernie discală la diverse niveluri sau de o artroză în stadiu avansat.
- IRM poate pune în evidență o epidurită. Cauzele epiduritelor sunt de cele mai multe ori neoplazice şi rareori de origine infecțioasă - tuberculoză, spondilodiscită piogenă.
- Cauzele leziunilor intradurale extramedulare responsabile de o compresie medulară sunt meningioamele şi neurinoamele.
- Atunci când datele examenului clinic sunt insuficiente, putem realiza o secvență rapidă ce va explora întreaga coloană, iar pentru coloana toracală, pentru orientare, vom număra întotdeauna, în funcție de nivelul leziunii, fie primele vertebre cervicale, fie sacrul.
- Pentru orientare, în cazul variantelor anatomice ale vertebrelor de tranziție lombosacrate, confruntarea se va face cu radiografiile de față şi de profil sau ținând seama că, de obicei, vertebra L3 prezintă cele mai voluminoase procese transverse.

Uneori, descoperirea unei leziuni compresive medulare de origine inflamatorie poate sugera realizarea unei examinări IRM şi a creierului pacientului în căutarea de leziuni demielinizante.

Nevralgia cervico-brahială

În explorarea nevralgiei cervico-brahiale scopul examinării constă în evidențierea prin IRM a traiectului nervilor care formează plexul cervico-brahial, a măduvei cervicale şi a eventualelor compresii sau leziuni ce pot apărea pe traiectul acestora.

Protocolul se bazează pe realizarea de secțiuni sagitale T1 şi T2 ale coloanei vertebrale cervicale, urmate de secțiuni în plan axial în planul discurilor intervertebrale C5-C6, C6-C7, C7- T1 sau în planul oricărui disc în care este modificat calibrul canalului rahidian sau al găurilor de conjugare. În alegerea secțiunilor la nivel axial un rol foarte important îl are examenul clinic pentru determinarea nivelului leziunii, precum şi corelarea cu examenul radiografic cervical. Atunci când este posibil se recomandă realizarea examinării cu o componentă funcțională fie cu ajutorul unei instalații, fie cu comprese. Opțional se pot realiza secvențe mielografice, secțiuni în plan frontal sau în plan frontal oblic înclinate paralel cu traiectul emergenței rădăcinilor nervoase. Injectarea substanței de contrast paramagnetice este recomandată atunci când se suspicionează perezența unei leziuni tumorale sau infecțioase.

Obiectivele **examenului IRM sunt:**

- **Evidențierea herniei discale,** cu precizarea nivelului la care materialul discal se deplasează, a efectului asupra sacului dural, a găurilor de conjugare, a rădăcinilor nervoase, a măduvei spinale. Cazuri particulare prezintă debordurile discale circumferențiale, precum şi herniile discale apărute la mai multe niveluri sau cele apărute pe un canal rahidian cervical cu diametrul redus congenital. Diametrul sagital al canalului rahidian cervical normal este: la C1 ≥ 21 mm, C2 ≥ 20 mm, C3 ≥ 17 mm, C4-C7 = 14mm. Diametrul transversal al canalului rahidian la nivelul pediculilor vertebrali > 20 de mm.
- **Evidențierea cervicartrozei,** cu precizarea nivelurilor la care a apărut, a stenozei rahidiene consecutive, a găurilor de conjugare cu calibrul diminuat, a prezenței osteofitelor şi a efectului artrozei asupra măduvei. Atât modificările din cervicartroză, cât şi cele datorate herniilor discale pot produce leziuni medulare prin compresie mecanică şi perturbare a fluxului de LCR din spațiile subarhnoidiene. Aspectul este vizibil în special în secvențele de tip T2 şi se traduce prin zone de hipersemnal intramedular, imprecis delimitate.
- **Evidențierea unui proces expansiv intra-rahidian** – cel mai frecvent o formațiune tumorală de tip neurinom, meningiom sau un alt proces înlocuitor de spațiu de tip epidurită. IRM

trebuie să îi precizeze localizarea, apartenența la compartimentul extradural sau intradural, afectarea medulară sau radiculară.

- **Evidențierea de anomalii de semnal şi de morfologie vertebrală.** În majoritatea cazurilor, anomaliile de semnal se datorează prezenței insulelor intraosoase vertebrale adipoase (hipersemnal T1 şi T2) şi anulării semnalului la secvențele cu supresie de grăsime sau de osteocondensare (hiposemnal T1 şi T2), precum şi angioamelor vertebrale.

În suspiciunea de sindrom Pancoast Tobias examinarea regiunii cervicale are ca obiective participarea la diagnosticul de operabilitate şi măsurarea leziunilor înainte de tratament. Comparativ cu examenul CT, se apreciază mai eficient extensia tumorală la nivelul părților moi, vertebrelor adiacente şi, eventual, intrarahidian.

Protocolul obişnuit cuprinde secțiuni în plan transversal şi coronal între porțiunea superioară a câmpurilor pulmonare şi polii inferiori tiroidieni, cu şi fără injectarea substanței de contrast paramagnetice. În anumite situații sunt foarte utile şi secțiunile în plan sagital. Sincronizarea cardiacă şi tehnica de reducere a artefactelor respiratorii pot contribui la o mai bună calitate a imaginilor.

Rolul examenului IRM constă în aprecierea limitelor tumorale, a extensiei parietale şi a consecințelor formațiunii tumorale asupra structurilor vasculare de vecinătate.

- În cazul suspiciunii unei afectări mediastinale trebuie suspectate posibile leziuni la nivelul cavităților cardiace, la nivel traheal, esofagian, vertebral. Este de asemenea importantă măsurarea distanței dintre formațiunea tumorală şi mediastin, o distanță de minimum 3 cm asigurând o marjă de siguranță oncologică pentru chirurg.
- În vederea stadializării, IRM poate pune în evidență prezența adenopatiilor.

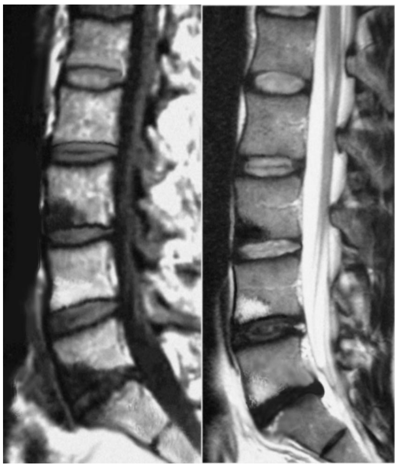

Fig.1. Toate tipurile de leziuni conform clasificării Modic, examinate comparativ în secțiuni sagitale T1 şi T2.

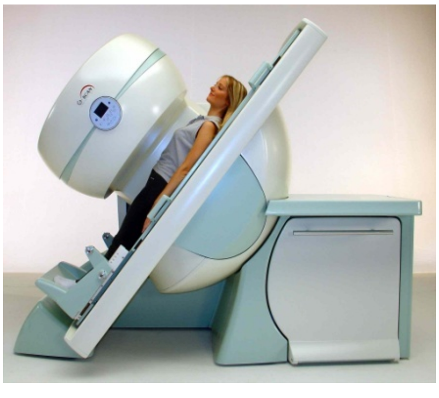

Fig. 3. Instalație care permite explorarea coloanei vertebrale în sarcină.

Fig. 2. Instalație IRM din SUUMC.

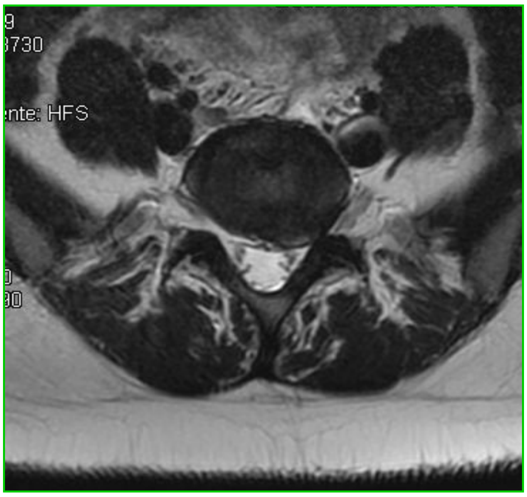

Fig. 4 a. Hernie discală posterolaterală stângă.

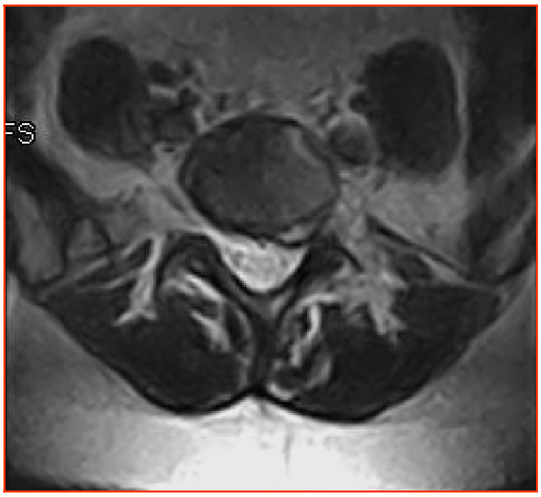

Fig. 4 b. Materialul discal îşi modifică semnalul–hipersemnal T2, element de prognostic favorabil

Concluzii: Explorarea IRM este metoda de elecție în explorarea patologiei coloanei vertebrale pentru că oferă cele mai multe şi mai utile informații despre această regiune. În evaluarea corectă a leziunilor, a stabilirii conduitei terapeutice adecvate sunt utile examinările comparative în dinamică, evaluarea semnalului discului intervertebral cu leziuni ale prizei de contrast din jurul fragmentului discal migrat în cazul herniei de disc cu fragmente rupte.

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Tratamentul prin trabeculoplastie LASER selectivă (SLT) al glaucomului cu unghi deschis

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Rezumat. Trabeculoplastia selectivă utilizează radiația emisă de cristalul de Yag-Nd, dublată în frecvență (532 nm lumină verde), care de asemenea este absorbită de pigmentul melanic situat în rețeaua trabeculară şi care se transformă în căldură. Dar prin durata foarte mică de acțiune a radiației emise de aparat căldura distruge numai fibrele încărcate cu pigment, fără să difuzeze masiv în jur. Consecutiv se produc două fenomene: fibrele distruse sunt îndepărtate de macrofage, dar în proporție mult mai mică, fără remanieri cicatriceale grave, iar căldura moderată propagată în jur determină şi remanieri nedistructive la nivelul colagenului trabecular, ducând la retracții şi subțieri ale fibrelor. Amândouă fenomenele duc la lărgirea ochiurilor rețelei trabeculare, efect durabil şi eficient. Astfel s-au constatat presiuni intraoculare durabil reduse la niveluri „de protecție” după 5 ani de la aplicarea terapiei la peste 85% dintre pacienți (5).
Consecințele sunt: pacienții nu mai au nevoie de terapie zilnică şi continuă cu picături antiglaucomatoase, calitatea vieții acestora creşte, se economisesc sumele de bani cheltuite cu terapia antiglaucomatoasă, susținută în prezent integral prin efortul societății, bani care s-ar putea redirecționa către alte programe de sănătate.

Cuvinte cheie: trabeculoplastie LASER selectivă glaucom cu unghi deschis

Abstract. Selective trabeculoplasty uses the radiation emited by the crystal Yag-Nd, dubled in the frequency (532 nm in green light), which is absorbed by the melanic pigment situated in the trabecular net transformed in the heat. The heat destroys only the fibers loaded with pigment using the short time of the action of the radiation emitted by the machine without massive diffusion around. As a consequence there are two phenomena; the destroyed fibers are removed by the macrophages but in a small percentage without significant scar remodeling and the moderate dissipated heat produces non-distructive remodeling at the level of the trabecular collagen that produce thinning and shortage of these fibers. Both phenomena produces enlarging of the trabecular network eyes an efficient and long-lasting effect.

În continuare vom prezenta un studiu realizat pe un număr de 34 de pacienți, 68 de ochi, la care s-a urmărit modificarea PIO după utilizarea SLT (6).

Date generale

Glaucomul este o afecțiune oculară gravă prin consecințele sale invalidante. Cunoscută în popor sub denumirea de „apa neagră”, se manifestă prin distrugerea iremediabilă a fibrelor nervului optic. Apar inițial modificări ale câmpului vizual prin constituirea de scotoame, mai întâi subclinice, apoi, prin lărgirea acestora, se ajunge la pierderea simțului luminos şi la orbire definitivă.

Boala este bilaterală, interesând ambii ochi, dar este mai frecvent asimetrică. Evoluează de cele mai multe ori insidios şi asimptomatic. Netratată, evoluția conduce inexorabil spre cecitate definitivă.

Date de anatomie şi fiziologie oculară

Retina este foița cea mai profundă a globului ocular. Aceasta conține: elemente sensibile la lumină (celulele cu conuri şi bastonaşe), elemente de transmitere a impulsului nervos (primul şi al doilea neuron din calea optică, respectiv - neuronul bipolar şi celulele ganglionare) şi axonii celulelor ganglionare înmănunchiați în structura care părăseşte globul ocular sub denumirea de nerv optic (NO). Suprafața discoidală observată la examenul fundului de ochi sub forma unei pete bine delimitate, de culoare roz pal, de pe care pleacă şi prin care intră vasele vizibile la nivelul retinei se numeşte papila NO.

În interiorul globului ocular se secretă de către procesele ciliare din camera posterioară (CP) situată înapoia irisului, umoarea apoasă (UA). Acest lichid clar are rol trofic şi plastic asupra structurii ochiului. Astfel, UA menţine un tonus intraocular convenabil schimburilor tisulare, menţine forma globului, hrăneşte structuri intraoculare, menţine transparenţa corneei la un nivel optim şi asigură un indice de refracţie necesar funcţiei optice a ochiului. Pe măsură ce aceasta se formează, UA părăseşte CP prin pupilă, pătrunde în camera anterioară (CA) înaintea irisului şi părăseşte globul pe la nivelul unghiului camerular, traversând trabeculul sclerocorneean (TS) prin canalul lui Schlem, ajungând, mai apoi, prin venele apoase, în circulaţia sistemică (1).

Jenarea acestui flux poate conduce la creşteri ale presiunii intraoculare (PIO). În general PIO nu trebuie să depăşească 20 mm Hg. Consecinţele creşterii îndelungate ale acesteia constau în modificări ireversibile ale metabolismul fibrelor ganglionare care formează NO, având drept urmare distrucţia acestora (2).

Clasificare. Conform Ghidului European de Glaucom, afecţiunea se împarte în glaucom primitiv şi glaucom secundar, funcţie de capacitatea noastră de a determina cauza care ar duce la modificarea secundară a stratului fibrelor nervului optic din retină şi, implicit, al aspectului şi funcţiei NO (3).

Fiziopatologie. În majoritatea cazurilor de glaucom are loc o modificare a evacuării UA din camera anterioară, în special prin obstrucţii la nivelul TS. Aceasta se poate produce prin diverse materiale care colmatează reţeaua (sânge, fibrină, material pseudoexfoliativ, corpi străini, tumori, neovase, membrane), situaţie specifică glaucomului secundar. Alteori obstacolul se realizează prin acoperirea unghiului cu rădăcina irisului (sinechii periferice prin particularităţi de morfologie a unghiului camerular) ori prin modificări primare ale ochiurilor reţelei trabeculare, care devin mai puţin permeabile la scurgerea UA. În acest caz suntem în prezenţa unor glaucoame primitive.

După lărgimea unghiului, glaucoamele pot fi cu unghi larg sau cu unghi îngust. Toate au în comun modificări caracteristice ale CV şi ale papilei NO însoţite de modificări ale presiunii intraoculare.

Există şi modificări caracteristice papilare şi de câmp vizual pentru glaucom, la care PIO nu depăşeşte valorile admise ca periculoase (aşa-numitele glaucoame cu presiune joasă), după cum există şi situaţii în care PIO se situează mult peste limita în general acceptată, fără însă a se înregistra niciun semn funcţional sau morfologic de glaucom (aşa- numita hipertonie intraoculară).

Indiferent de tipul de glaucom, atitudinea terapeutică vizează reducerea PIO la o valoare de la care să nu se mai observe modificări ale câmpului vizual.

Acest deziderat se bazează pe noţiunea de presiune ţintă (PT) şi ar fi acea nouă PIO la care modificările de CV să nu mai apară. În general se acceptă o scădere stabilă cu 25% faţă de presiunea iniţială şi, oricum, sub 20 mm Hg (3).

Modalităţile de micşorare a PIO sunt: reducerea formării de UA sau (şi) creşterea evacuării acesteia.

Aceste obiective se pot atinge prin trei modalităţi, care se pot aplica singure sau în combinaţie:

-terapie medicamentoasă, în special local, prin colire, dar şi prin medicamente administrate oral sau parenteral;

-terapie chirurgicală, având drept scop formarea de căi artificiale de drenaj extraocular al UA;

-terapie prin mijloace fizice care fie să reducă formarea de UA (distrugere parţială a corpului ciliar prin frig sau LASER), fie să crească rata de evacuare trabeculară prin trabeculoplastie LASER.

Dacă prima modalitate, cea prin medicamente, este cea mai veche, ea este grevată de mai mulţi factori, printre care se numără, în primul rând, costul medicaţiei, uneori ridicat, chiar dacă în prezent aceasta este gratuită. De asemenea, complianţa la tratament este deficitară. Întrucât terapia este necesar să fie urmată toată viaţa, ea generează perpetuarea senzaţiei de „bolnav” şi un complex caracteristic pacienților cu terapie îndelungată. Prezența reacțiilor adverse chiar şi la medicamentele topice poate impieta asupra complianței, a eficienței terapiei şi, în final, asupra calității vieții pacientului.

A doua soluție a fost îmbrățişată tocmai din cauza eşecurilor terapiei medicamentoase. Realizarea operatorie a unei căi de drenaj extraocular al UA este grevată de toate riscurile locale ale unei intervenții chirurgicale, precum şi de complicații tardive, cum ar fi cataractarea cristalinului sau închiderea căii de drenaj în timp. În ambele situații se impune efectuarea altor intervenții operatorii.

Al treilea grup de intervenții este cel mai recent: dacă intervențiile distructive asupra corpului ciliar se adresează cazurilor disperate, la care se vizează doar menținerea anatomică a unui glob nefuncțional, intervențiile care vizează remodelarea TC vor să elimine principala cauză a creşterii presiunii intraoculare, adică rezistența la scurgere a UA din ochi.

Inițial s-a utilizat efectul pe care radiația verde a laserului cu argon o are asupra pigmentului melanic observat la nivelul TS. Acesta absoarbe radiația de 514 nm (lumina verde) pe care o transformă în căldură (4). Consecința, dacă se iradiază cu o anumită durată şi putere, este distrugerea locală a rețelei trabeculare şi apariția de spații mari care permit filtrarea UA şi scăderea PIO. Dar s-a observat că acest efect este de scurtă durată (luni), reacțiile de cicatrizare locale anulând efectul hipotensor, ba chiar generând creşteri presionale prin dezvoltarea de sinechii în periferia irisului, capabile să închidă unghiul iridocorneean (2).

Trabeculoplastia selectivă utilizează radiația emisă de cristalul de Yag-Nd, dublată în frecvență (532 nm lumină verde), care de asemenea este absorbită de pigmentul melanic situat în rețeaua trabeculară şi care se transformă în căldură. Dar prin durata foarte mică de acțiune a radiației emise de aparat căldura distruge numai fibrele încărcate cu pigment, fără să difuzeze masiv în jur. Consecutiv se produc două fenomene: fibrele distruse sunt îndepărtate de macrofage, dar în proporție mult mai mică, fără remanieri cicatriceale grave, iar căldura moderată propagată în jur determină şi remanieri nedistructive la nivelul colagenului trabecular, ducând la retracții şi subțieri ale fibrelor. Amândouă fenomenele duc la lărgirea ochiurilor rețelei trabeculare, efect durabil şi eficient. Astfel s-au constatat presiuni intraoculare durabil reduse la niveluri „de protecție" după 5 ani de la aplicarea terapiei la peste 85% dintre pacienți (5).

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În continuare vom prezenta un studiu realizat pe un număr de 34 de pacienți, 68 de ochi, la care s-a urmărit modificarea PIO după utilizarea SLT (6).

Material şi metodă

S-a aplicat terapia LASER selectivă la 34 de pacienți (68 ochi) diagnosticați cu glaucom cu unghi deschis. S-a utilizat un laser Ellex Solo, lentila pentru trabeculoplastie Latina, anestezie topică cu Alcaine.

Criteriile de includere au fost:

-glaucom cu unghi deschis diagnosticat prin ex FO, CV şi gonioscopic;

-glaucom în tratament cu monoterapie, terapie dublă, combinație fixă de două antiglaucomatoase sau terapie triplă;

-dorința pacientului bine informat de a renunța la terapia prin picături.

Criteriile de excludere au fost:

-unghi camerular închis peste 180˚;
-ochi unic;
-glaucom acut;
-intervenții chirurgicale în antecedente pe ochi.

Tipurile de glaucom cu unghi deschis întâlnite au fost: glaucom primitiv -18 cazuri, glaucom secundar pseudoexfoliativ -5 cazuri şi glaucom secundar pigmentar -11 cazuri.

Presiunile intraoculare de la care s-a pornit terapia au fost: sub 21 mm Hg - 4 pacienţi, peste 21, dar sub 30 mm Hg - 29 pacienţi, peste 30 mm Hg -1 pacient.

Terapia medicamentoasă utilizată anterior aplicării SLT a fost: fără terapie - 8 pacienţi, cu terapie dublă sau combinaţie fixă - 17 pacienţi şi cu triplă terapie - 9 pacienţi.

Tehnica aplicării tratamentului: instilare conjunctivală: 1 picătură de Iopidin cu 20 de minute anterior terapiei, instilare de Alcaine (anestezic local) - 1 picătură cu 1 minut anterior terapiei, aplicarea de 50 +/-5 impacte de 400μ diametru contigue, pe 180°, la nivelul trabeculului sclerocorneean prin oglinda unei lentile Latina. Alegerea puterii impactelor s-a făcut ţinând cont de intensitatea pigmentării trabeculilor astfel: pentru trabeculii mai puţin pigmentaţi s-a pornit de la o energie iniţială de 0,8 mJ şi s-a crescut puterea treptat până la apariţia finelor bule de cavitaţie, apoi aceasta s-a redus cu 0,1 mJ. Pentru pacienţii cu trabeculi intens pigmentaţi puterea de plecare a fost de 0,4 mJ, urmându-se aceeaşi procedură.

Atitudinea postterapeutică: instilare de Maxidex - 1 picătură de două ori pe zi în următoarele 4 zile, păstrarea terapiei antiglaucomatoase avută anterior până la primul control al PIO, fixat după 7 zile. După această perioadă, s-a reevaluat PIO şi s-a renunţat la terapia antiglaucomatoasă. Un nou control al PIO s-a efectuat după alte 7 zile de la precedentul, cu pacientul fără medicaţie antiglaucomatoasă. La acest din urmă control s-a repetat şi examenul gonioscopic.

Rezultate: la primul control al PIO s-a constatat că 29 de pacienţi (88%) au avut PIO între 10 şi 20 mm Hg iar 5 pacienţi (12%) au avut PIO peste 21 mm Hg. După alte 7 zile în care pacienţii nu au mai avut terapie antiglaucomatoasă, s-au determinat presiuni sub 20 mm Hg la 27 de pacienţi şi presiuni peste 21 mm Hg la 7 pacienţi (cei 5 anterior la care s-au mai adăugat 2). La aceştia din urmă s-a completat terapia pe restul de 180° urmându-se protocolul de la prima şedinţă, cu acelaşi ritm şi aceeaşi procedură de urmărire, pentru alte 14 zile. Dintre aceştia, la sfârşitul celei de a doua perioade de urmărire s-a constatat că la 6 dintre ei presiunea intraoculară scăzuse sub 20 mm Hg, la un singur pacient situându-se peste 23 mm Hg.

Comentarii. S-a observat că după prima săptămână de terapie, presiunea intraoculară a pacienţilor supuşi SLT a scăzut în medie cu 8 +/- 3 mm Hg, adică cu circa 28% din presiunea iniţială. Aceste presiuni au crescut uşor în a doua săptămână, după retragerea medicaţiei antiglaucomatoase, dar s-au menţinut în limitele de 26-27% sub valoarea iniţială. Cazurile la care a fost necesară completarea terapiei (acele cazuri cu PIO peste 22 mm Hg) au fost cele la care PIO a fost mai aproape de 30 mm Hg (3 cazuri), au avut triplă terapie (două cazuri) sau au avut depuneri de material pseudoexfoliativ (un caz).

Pacientul la care presiunea a rămas peste 23 mm Hg şi după a doua şedinţă SLT a prezentat sinechii periferice de peste 90°, depuneri masive de material pseudoexfoliativ şi triplă terapie în antecedente. Acestuia i s-a recomandat tratament chirurgical al glaucomului - trabeculectomie sub volet scleral.

Pacienţii care au avut presiuni intraoculare sub 20 mm Hg cu sau fără terapie antiglaucomatoasă au avut cea mai marcată scădere a PIO, apropiindu-se de 10 mm Hg (45% din PIO iniţială).

Toti pacienţii cărora li s-a suprimat terapia antiglaucomatoasă au perceput acest lucru ca pe o evidentă îmbunătăţire a calităţii vieţii lor, înţelegând totuşi necesitatea de a se prezenta la controalele periodice conform planului de dispensarizare

Atitudinea în viitor. Pacienții supuşi SLT vor fi monitorizați din punctul de vedere al stabilității PIO la 3 luni, precum şi cu 2 CV în următoarele 6 luni, pentru a le compara cu cel de dinaintea aplicării terapiei.

Concluzii

1) SLT este o opțiune foarte eficientă în terapia antiglaucomatoasă care vizează scăderea presiunii intraoculare în cazuri selecționate .

2) Este sigură, repetitivă şi uşor de acceptat de către pacient.

3) Poate degreva casele de asigurări de sănătate de costurile necesare tratamentului cu picături a pacienților glaucomatoşi şi poate debloca fonduri utilizabile în alte sectoare ale îngrijirilor medicale.

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Dieta, stilul de viaţă, dislipidemiile şi bolile cronice noncomunicabile

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Rezumat: Rolul dietei şi al stilului de viaţă în determinismul bolilor cronice noncomunicabile (NCCD) a fost recunoscut în urma studiilor epidemiologice, a studiilor caz-control şi a studiilor efectuate de-a lungul anilor pe animale. Acestea au confirmat existenţa relaţiei dintre factorii alimentari, comportamentali şi cei de mediu şi de creştere a prevalenţei NCCD. S-au identificat, de asemenea, factorii de dietă şi de stil de viaţă care asigură o îmbătrânire sănătoasă. Consumul abuziv de alimente dense caloric, bogate în lipide şi glucide determină dismetabolismul postprandial, a cărui cronicizare, urmare a persistenţei defectului de dietă, determină modificări inflamatorii cronice care fac trecerea de la tulburarea de metabolism la NCCD. Speranţa de viaţă la naştere în România este cu 5,9 ani mai mică decât în ţările Europei de Vest, în mare parte datorită acestor factori de risc către care se îndreaptă eforturile politicilor de sănătate.

Cuvinte cheie: dietă, stil de viaţă, dislipidemii, boli cronice noncomunicabile

Abstract: The role played by diet and lifestyle in eliciting noncomunicable chronic diseases (NCCD) is now better recognized due to late epidemiologic, case-control and animal studies. Those studies conformed the relationship between dietary, behavioral and environmental factors, on one hand, and the steep raise of NCCD prevalence, on the other hand. There has also been shed light on the topic of dietary and lifestyle factors that could favor a healthier ageing process. The excessive eating of high caloric, fat and sugar rich foods results in postprandial dysmetabolism, which elicits chronic inflammatory disorders as a bridge to NCCD. Life expectancy at birth in Romania is 5.9 years shorter than that of Western European countries, mainly due to those risk factors presented above which represent the target for national health politics.

Dieta este un termen de origine latină, care înseamnă mâncarea şi băutura consumate de o persoană.

Termenul stil de viaţă a fost folosit pentru prima dată de Alvin Toffler în 1939, referitor la schimbările ce urmau să apară în sânul entităţilor etnice, culturale şi religioase, în societăţile post-industriale. Toffler preconiza că industrializarea va atrage şi o serie de schimbări: alimentare, culturale, comportamentale, în tradiţiile acestor entităţi. Aceste schimbări aveau să fie acceptate de un număr mai mic sau mai mare de persoane, dar aveau să fie recunoscute de entitatea respectivă ca fiind alte variante de viaţă, adică stiluri de viaţă, pe care autorul le numeşte subculturale, ca un derivat de la cultura de bază.

Actualmente stilul de viaţă este definit ca orice obicei, relaţie socială, de consum, stil vestimentar sau de recreere care este suficient de puternic pentru a influenţa semnificativ viaţa unui segment de populaţie. Referindu-se la acest aspect, profesorul Iulian Mincu aprecia că "în sfera civilizaţiei, obiceiurile alimentare, componentă importantă a stilului de viaţă, fac parte din categoria factorilor de natură utilitară ce asigură un anumit profil al stării de confort şi de securitate a individului, o serie de particularităţi legate de starea de sănătate şi longevitate".

Conceptul de “nutriţie optimă” (aport alimentar optim) se referă la recomandările dietetice care îşi propun să promoveze un aport adecvat de nutrienţi, după un plan de mese bine stabilit prin care să se asigure cele mai bune performanţe individuale şi cea mai lungă perioadă de viaţă într-o stare bună de sănătate.

În alimentaţia fiziologică, principiile nutritive fundamentale sunt asigurate atât de alimentele de origine vegetală (care ar trebui să aibă cel mai important rol), cât şi de cele de origine animală.

Aportul alimentar cuprinde două mari categorii de nutrienţi:

1.nutrienţi care furnizează energie:
  -glucide;
  -lipide;
  -proteine;
2.nutrienţi care nu asigură energie:
  -minerale (macro, micro şi oligoelemente);
  -vitamine (hidro şi lipo-solubile);
  -apa.

Ţinând seama de nutrienţii energogeni, optimul procentual din raţia calorică zilnică trebuie să fie astfel: 50-55% glucide, din care 90% glucide complexe (20% din fructe şi legume, 80% din cereale) şi 10% glucide simple, ≤ 30% lipide, din care: 1/3 de origine animală şi 2/3 de origine vegetală. Lipidele saturate trebuie să reprezinte < 10%, cele mononesaturate 12-14% iar cele polinesaturate 6-8%. Conţinutul în colesterol al dietei trebuie să fie < 300 mg/zi. 12-15% din raţia energetică va fi asigurată de proteine, cam 0,9 g/kgc/zi la adult, 1,3 g/kgc/zi la copii iar la gravide un plus de 50 grame/zi faţă de procentul admis pentru adulţi. Dacă ne referim la proteinele cu valoare biologică ridicată, atunci necesarul minim este de 0,53 g/kgc/zi la bărbaţi şi 0,50 g/kgc/zi la femei.

Fibrele din dietă trebuie să fie de 20 de grame/1 000 kcal.

Cea mai mare parte din aportul alimentar va fi constituită din cereale şi derivate, urmate de legume, grăsimi, lapte şi derivate, carne etc.:

-cereale şi derivate: 35%;
-grăsimi: 18%;
-legume şi fructe: 17%;
-lapte şi derivate: 12%;
-carne şi derivate: 8%;
-zahăr şi derivate: 8%;
-ouă: 2%.

Determinanţii biologici ai comportamentului alimentar pot fi clasificaţi în: fiziologici, fiziopatologici şi genetici. Între aceştia cei fiziologici sunt esenţiali.

Factorii fiziologici care influenţează consumul alimentar (dieta) sunt reprezentaţi de nevoile organismului legate de procesele de creştere, dezvoltare, perioadele speciale din evoluţia sănătoasă a individului - sarcină şi lactaţie (la femei) - adaptarea la diverse tipuri de activitate fizică şi intelectuală, adaptarea la diverse condiţii geo-climatice.

Studii genetice avansate au arătat că există variaţii genetice de răspuns al organismului la diverşi nutrienţi şi, de aici, şi nevoi optime proprii fiecărui individ, pentru diverşi nutrienţi. Aceste aspecte sunt studiate de o altă nouă ramură a ştiinţei numită nutrigenetica (genetica nutriţională).

Influenţele exercitate de alimente, efortul fizic şi mediul înconjurător asupra genomului uman sunt încă foarte puţin cunoscute, dar studiile de nutrigenomică constituie o reală provocare pentru mulţi cercetători, o speranţă de mai bine pentru umanitate.

Prezenţa bolii în organism poate fi răspunzătoare de apariţia unor dezechilibre nutritive, hidroelectrolitice şi acido-bazice, perceptuale (de gust, miros etc.), de tip imun. Faţă de aceste modificări, în organism se declanşează o serie de mecanisme adaptative complexe, inclusiv de schimbare a comportamentului alimentar, a senzaţiilor de foame şi sete.

Consumul abuziv de alimente dense caloric, uşor digerabile, bogate în lipide şi glucide conduce la dismetabolismul postprandial**.** Acesta se defineşte ca fiind un dezechilibru metabolic creat prin creşteri abrupte ale trigliceridemiei şi glicemiei postprandiale care are drept urmare un status inflamator proaterogen. Bolusul energetic peste necesităţile organismului depăşeşte capacităţile metabolice ale mitocondriei de la nivel muscular şi adipos. Glucoza şi acizii graşi liberi inundă ciclul Krebs stimulând producerea în exces de NADPH, fapt care amplifică formarea radicalilor liberi de oxigen, inclusiv creşterea oxidării LDL, creşterea tonusului simpatic, vasoconstricţie şi trombogenicitate.

Aportul sporadic crescut de lipide şi glucide după modelul prezentat mai sus determină modificări acute cu potenţial de reversibilitate, definind, astfel, dismetabolismul postprandial acut. Dacă excesul de aport este cronic sau intervin defecte genetice de metabolizare sau absorbţie ale lipidelor, se instalează modificări inflamatorii cronice ce se amplifică în cerc vicios şi fac trecerea de la dismetabolism la bolile cronice noncomunicabile.

Aportul excesiv cronic de lipide din alimentaţie determină creşterea producţiei intestinale de ApoB48, apolipoproteină inductibilă care este responsabilă de amplificarea absorbţiei la acest nivel a grăsimilor exogene. Excesul de trigliceride absorbite stă la baza depunerilor în ţesutul adipos şi declanşării insulinorezistenţei, responsabilă de cortegiul de fenomene fiziopatologice care duce la diabet zaharat, ateroscleroză, steatoză hepatică şi la accentuarea dislipidemiei.

Hiperlipemia postprandială (caracterizată prin concentraţii plasmatice crescute ale trigliceridelor, chilomicronilor şi remnanţilor lipoproteici) duce la alterarea raportului enzimatic dintre LCAT (care scade) şi CETP (care creşte), cu scăderea nivelului plasmatic al HDL colesterolului şi persistenţa remnanţilor bogaţi în colesterol.

O altă consecinţă a dismetabolismului postprandial cronic este mărirea producţiei de AGEs (*advanced glycation end products*) şi acumularea lor în ţesuturi, care duce la creşterea permeabilităţii vasculare, proliferarea matricei extracelulare şi a fibrelor musculare netede, constituindu-se în componente ale mecanismului fiziopatologic al aterosclerozei.

Statusul inflamator indus de dietă se reflectă în creşterea serică a nivelului PCR şi al endotelinei 1.

O stare inflamatorie sistemică este din ce în ce mai mult recunoscută ca mediator important al bolii coronariene. Într-un studiu din 2007 al lui Fito et al se arată cum o dietă inadecvată poate fi responsabilă de inducerea unei astfel de stări inflamatorii .

Dismetabolismul postprandial pare să fie un factor de risc independent pentru evenimentele cardiovasculare.

Este cunoscut faptul că 2/3 din pacienţii cu boală coronariană au o homeostazie anormală a glucozei. Există o relaţie de directă proporţionalitate între nivelul glicemiei după un test de provocare la glucoză şi riscul mortalităţii cardiovasculare.

Hiperlipemia postprandială se manifestă prin nivele crescute de trigliceride şi chilomicroni. Potenţează independent efectele adverse ale hiperglicemiei postprandiale.

Hipertrigliceridemia postprandială pare să fie asociată cu creşterea riscului evenimentelor cardiovasculare mai mult decât nivelul à jeun al trigliceridelor, aşa cum a arătat un studiu al lui Bansal et al publicat în 2007.

Un alt factor important al stilului de viaţă, care contribuie la o bună stare de sănătate, este activitatea fizică.

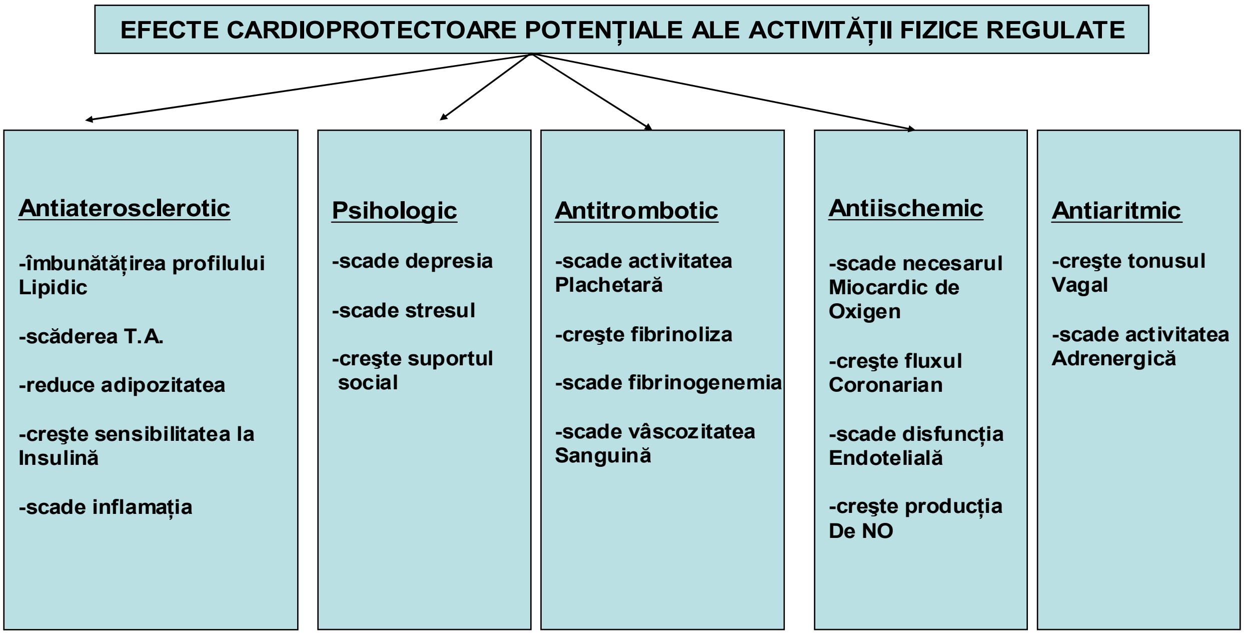
EFECTE CARDIOPROTECTOARE POTENȚIALE ALE ACTIVITĂȚII FIZICE REGULATE
Antiaterosclerotic
-îmbunătățirea profilului Lipidic
-scăderea T.A.
-reduce adipozitatea
-creşte sensibilitatea la Insulină
-scade inflamația
Psihologic
-scade depresia
-scade stresul
-creşte suportul social
Antitrombotic
-scade activitatea Plachetară
-creşte fibrinoliza
-scade fibrinogenemia
-scade vâscozitatea Sanguină
Antiischemic
-scade necesarul Miocardic de Oxigen
-creşte fluxul Coronarian
-scade disfuncția Endotelială
-creşte producția De NO
Antiaritmic
-creşte tonusul Vagal
-scade activitatea Adrenergică

Leisure time physical activity, competitive sports and ischemic heart disease by Borjesson M. Et al; European journal of Cardiovascular Prevention and Rehabilitation 2006, 13:133-136

În prezent nu există încă un consens asupra minimului de activitate fizică pentru menținerea stării de sănătate. Recomandările pentru adulți în scopul profilaxiei bolii ischemice coronariene şi pentru o bună capacitate respiratorie variază între: 150 minute/săptămână şi 30 de minute/zi de activitate fizică moderat-intensă. Dacă se urmăreşte păstrarea adecvată a greutății corporale, atunci activitatea fizică moderat-intensă trebuie să fie de 60 minute/zi. Pentru copii este necesar ca dincolo de activitatea lor zilnică să desfăşoare de trei ori/săptămână cel puțin 20 de minute de activitate sportivă intensă

Factorii socio-economici şi culturali joacă un rol foarte important în asigurarea unui aport alimentar şi a unui stil de viață adecvate. Stresul socio-profesional este un alt element ce influențează alimentația şi stilul de viață. Acesta, împreună sau separat de alți factori care caracterizează stilul de viață: activitatea fizică, fumatul, consumul de alcool sau droguri, sunt amplu studiate cu privire la rolul lor în determinismul bolilor cronice.

Prin boli cronice noncomunicabile (NCD= *Noncomunicable chronic diseases*) se înțelege patologia cronică nelegată de cauze infecțioase, determinată de factori genetici şi/sau de stil de viață. În lista acestor boli intră: ateroscleroza, hipertensiunea arterială, boala coronariană ischemică, neoplaziile, obezitatea, diabetul zaharat, unele afecțiuni psihice, astmul bronşic, boala Alzheimer.

OMS şi FAO subliniază faptul că în prezent la nivel mondial sunt două mari probleme de sănătate: malnutriția şi bolile cronice.

Aproximativ 30 % din populația Globului suferă de malnutriție. Principala cauză a malnutriției este sărăcia. Dar aspecte serioase ale patologiei legate de carențele vitaminice şi de oligoelemente întâlnim şi în țările dezvoltate şi în tranziție.

Rolul dietei şi cel al stilului de viață în determinismul îmbolnăvirilor au fost şi continuă să fie analizate în diverse moduri. Principalele studii efectuate pot fi grupate în: epidemiologice, clinice şi pe animale.

Câteva dintre marile studii epidemiologice efectuate de-a lungul anilor sunt:

Studiul NHANES (The National Health and Nutrition Epidemiologic Surveys), desfăşurat sub conducerea Centrului Național de Statistică Medicală, Centrului de Control al Îmbolnăvirilor, a început în anul 1956 şi se derulează şi astăzi . Inițial studiul a urmărit obținerea de informații cu privire la starea de sănătate a locuitorilor din SUA, vizând planificarea cheltuielilor pentru sănătate. Din 1959 şi până în prezent au fost desfăşurate opt studii în care au fost incluse peste 130 000 de persoane, de toate vârstele şi etniile. Cercetările efectuate cuprind date de anchetă privind starea de sănătate, modul de alimentație şi stilul de viață (efort fizic, comportament față de alcool, droguri, fumat), stare de bine, examen clinic – date antropometrice, TA, probe biologice de sânge (şi urină) care vizează glicemia, profilul lipidic, sideremia, concentrațiile unor vitamine şi alte microelemente, albuminemia, teste radiologice, densitometrie osoasă, teste pentru aprecierea capacității cognive, a depresiei, a comportamentului sexual. Studiul complex al acestor date a permis să se identifice prevalența şi incidența bolilor cronice non-comunicabile, să se poată defini existența unei reale interelații între factorii alimentari, comportamentali şi de mediu, astfel încât a fost posibilă întocmirea unor grile de risc pentru diverse afecțiuni, cum ar fi diabetul zaharat, obezitatea, unele boli cardiovasculare, osteoporoza, unele anemii, diverse hipovitaminoze şi carențe de minerale.

Framingham Heart Study, un studiu epidemiologic longitudinal care a început în anul 1948 sub conducerea Institutului Național al Inimii, Plămânilor şi Sângelui (National Heart, Lung and Bood Institute-NHLBI). Este în derulare şi astăzi, iar din 1971 se află şi sub patronajul Universității din Boston. Inițial au fost incluşi 5 029 de adulți din Framingham, Massachusetts, iar acum este la a treia generație de participanți (adică strănepoții primilor participanți). Datele acestui studiu au arătat legătura dintre nivelul colesterolemiei şi evoluția bolilor cardiace ischemice, au descoperit rolul particulelor de colesterol şi au permis stabilirea unui algoritm pentru stabilirea gradului de risc global cardiovascular, aşa-numitele Tabele Framingham, care stau la baza algoritmului de tratament al National Cholesterol Educational Treatment Panel – ATP III. De asemenea, studiul Framingham a permis crearea unor grile pentru aprecierea riscului coronarian, de fibrilație atrială, accidente vasculare cerebrale, claudicație intermitentă, grile care de asemenea stau la baza algoritmilor de tratament specifici)**.**

Studiul SENECA (Dietary Quality Lifestyle Factors and Healthy Ageing In Europe) a avut ca scop să identifice factorii de dietă şi stil de viață care asigură o îmbătrânire sănătoasă. A fost un studiu populațional longitudinal care s-a desfăşurat pe durata a 10 ani. Au fost urmăriți 1091 de bărbați şi 1109 femei, cu vârste cuprinse între 70 şi 75 de ani, şi cu o stare de sănătate bună, din 9 țări europene (Belgia, Franța, Danemarca, Italia, Olanda, Portugalia, Spania, Elveția şi Polonia). Studiul a urmărit starea de sănătate a acestor subiecți şi evoluția ei în timp în funcție de dietă şi de stilul de viață pe care le-au avut în trecut şi pe timpul derulării acestuia. S-a constat că persoanele care au avut şi şi-au păstrat o dietă de calitate (apreciată pe baza unei anchete alimentare care apoi a fost judecată folosind scorul dietei mediteraneene), nu au fumat şi au practicat activitate fizică constantă au prezentat o stare de sănătate fizică mai bună, un declin cognitiv mult mai scăzut şi au fost mai longevivi. O concluzie interesantă a fost aceea că fumatul îşi exprimă consecințele negative asupra stării de sănătate mai devreme în cursul vieții decât inactivitatea fizică.

Studiul EPIC (European Prospective Into Cancer), cu o durată mai lungă de desfăşurare , a început în 1992, cuprinde peste 500 000 de oameni din 10 țări europene şi vizează rolul dietei în apariția bolilor neoplazice. Din cei înrolați, 40 000 au făcut deja cancer.

În ceea ce priveşte obiceiurile alimentare, concluzia desprinsă până acum este că acestea diferă foarte mult de-a lungul Europei, ceea ce face din acest continent ” un adevărat laborator de studiu al obiceiurilor alimentare şi efectul lor asupra bolilor cronice, nu numai a cancerului", cum aprecia Ph. Dr. Elio Riboli, de la Imperial College din Londra.

În privința dezechilibrelor alimentare din Europa, studiile epidemiologice efectuate estimează că 1/3 din decesele premature se datorează dietei neadecvate.

Din datele statistice ale FAO(FAOSTAT) pentru Europa reiese că:

- aportul alimentar este mult peste necesarul caloric zilnic. În medie peste 2 700 kcal/zi, recordul fiind înregistrat în Portugalia, cu 3 768 kcal/zi, în 1999;
- în toate țările sursa energetică alimentară de bază este dată de produsele de origine vegetală, cerealele fiind sursa principală în toată Europa;
- cantitatea de energie adusă din produsele de origine animală este undeva în jur de 1 000 - 1 300 kcal/zi/persoană;
- consumul de proteine/zi variază între 127 grame (cel mai mare) şi 88 grame (cel mai mic). Sursele principale de proteine sunt în special cele animale, urmate de cele din cereale;
- sursa principală de lipide este asigurată predominent de produse de origine animală (în medie 83 grame/zi) față de produsele vegetale (în medie 66,7 grame/zi), cu excepția Greciei;
- consumul de zahăr ridicat de 18kg/an/persoană induce un risc foarte mare pentru carii dentare;
- creşterea consumului de calorii prin îndulcitori este o componentă în schimbările dietetice la nivel mondial. Aceasta este reprezentată de creşteri cu 74 kcal/zi progresiv între 1962 şi 2000. Urbanizarea şi creşterea veniturilor sunt considerate responsabile de 82% din schimbări .

În ceea ce priveşte alte obiceiuri alimentare şi de stil de viață, FAO concluzionează că în Europa rata sedentarismului a crescut semnificativ, fiind o caracteristică a grupelor tinere de populație. Astfel persoanele cu vârsta medie de 35 de ani sunt predominant sedentare, iar copii între 11 şi 13 ani sunt foarte sedentari. Este bine dovedit că sedentarismul şi privitul la televizor sunt asociate şi cu obiceiuri alimentare deficitare.

Într-un studiu din 2003 al European Opinion Research Group se emit o serie de concluzii referitoare la starea populației europene în privința activității fizice.

Zile de activitate fizică viguroasă în ultimele 7 zile pe grupe de vârstă

	15-25 ani %	**26-44 ani %**	**45-64% %**	**Peste 65 ani %**
Nimic	43,1	49,9	60,4	79,6
1 zi	9,8	8,4	7,9	5
2 zile	12,9	10,7	7,8	4,8
3 zile	11,8	8,2	5,8	3,3
4 zile	5,6	4	2,8	1,5
5 zile	6,3	7,7	5,1	1,4
6 zile	2,4	2,8	1,8	0,5
7 zile	6,5	6,8	6,9	3,1

Astfel, 80 % din populația de peste 65 ani nu a efectuat nicio formă de activitate fizică viguroasă în ultimele 7 zile , spre deosebire de cei între 15 şi 25 ani la care acest procent coboară la 43%. Doar 7 % din cei cu vârste cuprinse între 15 şi 64 ani raportează activitate fizică viguroasă în fiecare zi a ultimei săptămâni.

Timpul petrecut cu activități fizice viguroase în ultima săptămână scade cu vârsta. La grupa de vârstă 15-25 ani 14,5% raportează acest tip de activități desfăşurate pe 91-120 minute, la grupa 26-44 ani rata este de 10,7%, pentru 45-64 ani este de 8,9%, iar pentru grupa de vârstă de peste 65 ani această rată este de 4,5%.

Pentru activitățile fizice moderate se constată aceeaşi tendință descrescătoare. O treime (33,1%) dintre indivizii grupei de vârstă 15-25 ani nu au desfăşurat niciun fel de activitate fizică moderată în ultima săptămână, pentru ca pentru cei peste 65 ani această rată să fie de 55,9%.

500 000 de oameni mor anual în Europa din cauza fumatului, iar jumătate dintre aceştia au vârste cuprinse între 35 şi 69 de ani. Incidența fumătorilor în rândul adolescenților, în special a acelora a căror vârstă este sub 15 ani, este în creştere (între 25 şi 30%) .

În 2003, România a ocupat primul loc ca mortalitate prin accident vascular cerebral în lume, cu 177 decese la 100 000 locuitori, față de SUA, care înregistra 33 la 100 000 de locuitori. În 2005, statisticile au situat România între primele 10 țări ale lumii.

La nivelul continentului european în ultimele decenii ale sec. al XX- lea au apărut unele aspecte deosebite, observându-se o creştere marcantă a gradientului Vest - Est în ceea ce priveşte mortalitatea şi o adâncire a diferenței cu privire la durata medie de viață a populației din vestul Europei, față de cea din estul Europei.Toate țările Vest- Europene au înregistrat o creştere a duratei medii de viață, dar în țările Est- Europene s-a remarcat o scădere a acesteia cu creşterea semnificativă a numărului de decese în rândul adulților de vârstă medie.

Dacă în 1970 durata medie de viață la naştere era sensibil egală în toată Europa, în 1990 în țările foste comuniste aceasta era cu 5,5 ani mai mică, pentru ca în 1998 să fie cu 5,9 ani mai mică. Tendința de scădere a duratei medii de viață la naştere pentru țările foste comuniste a fost aceeaşi atât pentru bărbați cât şi pentru femei, cu păstrarea diferenței cunoscute între cele două sexe. Fenomenul a fost explicat prin transformările socio-economice substanțiale care au avut loc în aceste state, ceea ce a generat un stres foarte ridicat, care a dus la creşterea mortalității prin boli cardiovasculare (decesele prin BCV - infarct de miocard şi AVC - sunt de 10 ori mai mari în țările Est-Europene față de cele Vest-Europene). Acestor cauze li s-a mai adăugat una foarte importantă în rândul ambelor sexe: consumul exagerat de alcool. Între anii 1990-1994 țările fostei URSS au atins cea mai mare rată de mortalitate înregistrată vreodată în timp de pace. În Europa se înregistrează între 100 şi 300 accidente vasculare la 100 000 locuitori pe an, care în marea lor majoritate sunt ischemice, printre cauze aflându-se hipertensiunea arterială, dislipidemia, diabetul, fumatul, obezitatea. Potrivit statisticilor internaționale medicale, în 2002 pe primele locuri în lume la boli cardiovasculare, în general, se aflau Federația Rusă, urmată de Bulgaria şi România, iar în ceea ce priveşte accidentul vascular cerebral, potrivit OMS, România se afla pe primul loc, urmată de Bulgaria.

Conform celor mai noi raportări, în 27 de țări ale Europei s-a ajuns în prezent ca speranța medie de viață la naştere să fie de 75 de ani, în timp ce în alte 11 state aceasta este încă sub 70 de ani (în această categorie intră şi România).

60% dintre decesele datorate bolilor cardiovasculare aparţin țărilor din estul Europei.

Cancerul este responsabil de 30% din decese în țările din vestul Europei şi de 10% din cele din estul Europei.

Studiul Național cross-secțional “Cardio-zone”, efectuat de un grup de cardiologi de marcă din România , în anul 2006, pe un lot reprezentativ de adulți cu vârste între 18 şi 85 de ani, din opt regiuni ale țării (Bucureşti, Muntenia, Dobrogea, Banat, Crişana, Oltenia, Moldova, Transilvania) cu privire la prevalența bolilor cardiovasculare şi a factorilor majori de risc ai acestora, a arătat că în țara noastră factorii majori de risc pentru bolile cardiovasculare sunt: diabetul zaharat (39,1%), hipercolesterolemia (31, 4 %), obezitatea (26,3 %), fumatul (21,7 %). Din acest studiu a reieşit că obezitatea este mai frecventă în Banat, în timp ce diabetul zaharat este mai frecvent în Muntenia.

Pe baza rezultatelor studiilor clinice s-a ajuns în unanim consens la concluzia că în determinismul bolilor cronice non-comunicabile există două mari categorii de factori:

I.Nemodificabili:

1. vârsta;

2. sexul;
3. susceptibilitatea genetică.

II. Modificabili:
1. comportamentali: dieta, activitatea fizică, consumul de alcool şi tutun;
2. biologici: dislipidemia, HTA, obezitatea, hiperinsulinismul;
3. sociali: statusul socio-economic, cultural şi factorii de mediu înconjurător.

În prezent tot mai multe state, în politicile lor de sănătate, ţin seama de factorii de risc modificabili, adoptând diverse modalităţi de reducere a influenţei acestora asupra stării de sănătate a populaţiei. Astfel, s-a interzis fumatul în multe locuri publice aglomerate, etichetarea produselor alimentare este foarte riguroasă vizând informarea consumatorului cu privire la conţinutul în diverşi nutrienţi şi aditivi alimentari, s-a dezvoltat producţia de produse dietetice care au un conţinut scăzut de grăsimi şi zahăr, se face tot mai multă publicitate cu privire la efectul nociv al fumatului, al excesului de alcool, al poluării mediului, cultivarea plantelor şi creşterea animalelor se fac în tot mai multe state după anumite norme care vizează securitatea alimentară şi protecţia mediului.

Creşterea explozivă a bolilor cronice noncomunicabile şi în ţara noastră a făcut ca activitatea de depistare precoce a factorilor de risc pentru acestea să se materializeze într-un amplu program de investigaţii clinice şi biologice ale tuturor oamenilor (Programul Naţional de Sănătate - care se derulează prin medicii de familie începând din 2007 şi vizează crearea unei baze de date şi asigurarea măsurilor de profilaxie). De asemenea,în prezent se desfăşoară ample campanii de mediatizare a efectelor dăunătoare ale unui stil de viaţă neadecvat, precum şi o serie de activităţi pentru reducerea fumatului şi a unor forme de cancere.

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Sindroame psihotice induse de agenții farmacologici psihotropi

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Rezumat: Manifestările psihotice produse prin acțiunea la nivel farmacodinamic a substanțelor psihotrope, fie a celor folosite ca mijloace de tratament, fie a celor utilizate ilegal, cu potențial adictiv, reprezintă o problemă importantă de diagnostic diferențial şi atitudine terapeutică în practica medicală actuală. O gamă largă de agenți farmacologici utilizați în psihiatrie şi nu numai, cum ar fi antidepresivele, antipsihoticele, anxioliticele, antiparkinsonienele, timostabilizatoarele, pot fi responsabile, în anumite situații, de apariția unor episoade psihotice noi sau de producerea unor decompensări psihotice la pacienții cu tulburări psihice aflați în tratament de întreținere. O altă populație aflată la risc de dezvoltare a sindroamelor psihotice secundare este reprezentată de consumatorii de substanțe de tipul halucinogenelor, alcoolului etilic, cannabisului, preparatelor opioide sau cocainei, populație la care, în cadrul intoxicației sau sevrajului, se pot observa fenomene halucinatorii, delirante, tulburări la nivelul limbajului sau comportamentului. Anamneza centrată pe componenta farmacologică a tratamentului şi pe detectarea consumului altor substanțe cu potențial de abuz, examenul clinic şi toxicologic reprezintă elementele esențiale care pot îndruma medicul spre un diagnostic corect şi o atitudine terapeutică adecvată.

Cuvinte cheie: psihoză, antidepresive, antipsihotice, anticonvulsivante, antiparkinsoniene, intoxicație, sevraj

Abstract: Psychotic symptoms induced by actions at the pharmacodynamic level during treatment with psychotropics, or during illegal use of addictive agents, are an important phenomenon in the contemporary clinical practice due to the necessity of a correct differential diagnosis and the need to formulate an adequate therapeutic strategy. A large variety of pharmacological agents used in psychiatry and other domains of medicine, like antidepressants, antipsychotics, anxiolytics, antiparkinsonians and anticonvulsants could be responsible, in certain situations, for the onset of new psychotic episodes or worsening of previous psychotic symptoms in chronic psychiatric disorders. Another population at risk is formed by the users of hallucinogens, cannabis, alcohol, opioids or cocaine, which during intoxication or withdrawal phases could develop hallucinations, delusions, speech disorders or behavioral dysfunctions. A thoroughly conducted anamnesis, focused upon the psychopharmacologic treatment and detection of addictive behaviors, clinical examination and toxicology analysis help the physician to formulate an accurate diagnosis and to establish an efficient treatment strategy.

1. Introducere

Descrierea sindroamelor psihotice asociate ingestiei substanțelor cu acțiune psihotropă reprezintă o necesitate clinică, în contextul creşterii constante a numărului de agenți farmacologici de acest tip în practica terapeutică şi a prescrierii lor de către o categorie din ce în ce mai largă de specialişti. De asemenea, creşterea incidenței sindroamelor de dependență, a intoxicațiilor, sevrajelor şi consumului abuziv de substanțe psihotrope ridică necesitatea cunoaşterii tabloului clinic asociat patologiei psihotice secundare, cunoaştere fundamentală pentru efectuarea unui diagnostic diferențial corect şi a direcționării atitudinii terapeutice.

O gamă variată de substanțe psihotrope cu potențial de inducere a sindromului de dependență pot crea tabloul unei tulburări psihotice acute. Sevrajul şi intoxicația în cazul acestor agenți farmacologici pot fi însoțite de simptome psihotice cu intensități diferite, care se instalează în sfera percepției, a gândirii, comportamentului, limbajului etc. Alcoolul etilic, sedativele, hipnoticele şi anxioliticele pot induce o simptomatologie de tip psihotic atât în cadrul sindromului de sevraj, cât şi în cel de intoxicație, în timp ce amfetaminele, cannabisul, cocaina, halucinogenele, substanțele inhalante, opioidele şi fenciclidina sunt asociate cu fenomene psihotice în cursul intoxicației [1]. Halucinogenele, cum ar fi dietilamida acidului lisergic (LSD), 2,5-dimetoxi-4-metilamfetamina (mescalina) sau 3,4-metilenenedioxi-metamfetamina (MDMA, ecstasy) induc şi o tulburare perceptivă persistentă, caracterizată prin flashback-uri, sub forma retrăirii unor senzații şi percepții din timpul intoxicației, de tipul halucinațiilor şi iluziilor geometrice sau kinestezice, modificărilor intensității culorilor sau a strălucirii obiectelor din jur, postimaginilor pozitive, modificărilor dimensiunilor obiectelor şi persoanelor percepute (micro sau macropsii).

Pe lângă substanțele cu potențial adictiv, există şi medicamente folosite frecvent în practica psihiatrică, a căror utilizare poate fi responsabilă, în anumite contexte, de apariția unei simptomatologii de aspect psihotic. Astfel de substanțe sunt anticolinergicele, anticonvulsivantele, antidepresivele de tip triciclic, agoniştii dopaminergici, unele sedative şi hipnotice, psihostimulantele etc. (vezi tabelul 1). Principalele mecanisme implicate în geneza fenomenelor psihotice secundare sunt reprezentate de crearea unui dezechilibru dopaminergic sau colinergic la nivelul sistemului nervos central, interferența cu alte mecanisme neurofiziologice încă insuficient cunoscute (glutamatergice, GABA-ergice etc.) şi activarea unor tendințe latente, prodromale în populații cu vulnerabilitate crescută pentru o patologie de tip psihotic.

Desigur, inducția unor simptome sau sindroame psihotice nu este limitată la medicația psihotropă ci, dimpotrivă, dacă privim ansamblul cauzelor farmacologice ale acestor manifestări clinice vom putea observa că în majoritatea situațiilor sunt implicați unul sau mai mulți agenți chimici non-psihotropi. În literatura de specialitate sunt descrise asocieri cu inducția sindroamelor psihotice ale analgezicelor de tipul pentazocinei, chimioterapicelor, agenților antiinflamatori, antibioticelor, medicației cardiovasculare, preparatelor hormonale (tiroidine, suprarenaliene), imunosupresoarelor şi chiar a vitaminelor consumate în exces (vitamina A, D). Analiza tuturor acestor cauze depăşeşte însă cadrul teoretic propus aici şi reprezintă un domeniu de studiu interdisciplinar.

Tabelul 1. Asocierea manifestărilor psihotice cu utilizarea substanțelor psihotrope

Categoria de substanță psihotropă	**Substanțele specifice/ circumstanțele specifice**
Anticolinergice	trihexifenidilul, atropina, benztropina
Anticonvulsivante	fenitoina, etosuximida, primidona
Antidepresive	triciclice, IMAO
Agonişti dopaminergici	levodopa, bromocriptina, amantadina
Sedative şi hipnotice	benzodiazepine, barbiturice, în cadrul sevrajului, hipnotice non-benzodiazepinice
Antipsihotice	în cadrul sindromului de discontinuare
Psihostimulante	amfetamine, metilfenidat, pemolin
Alcoolul	în cursul fazelor de intoxicație sau sevraj
Cannabis, cocaina, halucinogene, droguri inhalante, opioide, fenciclidina	în cursul fazei de intoxicație

2. Principalele categorii de agenți psihotropi care induc sindroame psihotice

Psihostimulantele administrate în doză mare sau folosite abuziv pot determina apariția unor reacții psihotice. Amfetaminele sunt reprezentante ale acestui grup şi produc simptome asemănătoare celor din schizofrenia paranoidă, asociație descrisă încă din 1958 [2]. Psihoza indusă de amfetamine are un debut rapid, pacientul este lucid, iar experiențele pe care acesta le relatează au o calitate asemănătoare trăirilor din vis. În plus, există o stare de hipervigilență şi se pot instala halucinații vizuale. Tulburările de conținut ale gândirii sunt mai rare şi au un caracter persecutor. În cadrul tabloului clinic se poate observa coexistența unor comportamente compulsive stereotipe.

O anamneză atentă, cu evaluarea intervalului dintre consumul de substanță şi declanşarea manifestărilor psihotice, precum şi un screening toxicologic stabilesc, de regulă, diagnosticul diferențial cu psihozele non-farmacologice. Dacă simptomele persistă mai multe săptămâni, iar testele urinare rămân negative, atunci este posibil ca respectivul consum de amfetamine să fi declanşat sau decompensat o psihoză primară.

Metilfenidatul, pemolinul, metamfetamina şi fentermina pot declanşa şi ele reacții psihotice [3].

Antidepresivele au fost asociate cu amplificarea simptomatologiei psihotice preexistente sau precipitarea unor episoade psihotice de novo, în cadrul unor populații cu particularități neurobiologice şi clinice încă incomplet definite. Acest fenomen este valabil mai ales pentru antidepresivele care au proprietăți de stimulare a neurotransmisiei dopaminergice, cum ar fi bupropionul, triciclicele, inhibitorii monoaminoxidazei (IMAO), venlafaxina în doze mari, peste 225 mg/zi. Existența unei patologii cu o componentă psihotică, de tipul tulburării schizoafective, depresiei postpsihotice, depresiei cu manifestări psihotice, tulburării bipolare cu elemente psihotice sau a antecedentelor psihotice sunt elemente care favorizează instalarea sau agravarea unui tablou psihotic.

Un studiu care a inclus 150 de subiecți tratați cu nortriptilină sau imipramină a arătat că 13% dintre aceştia prezentau insomnie, hipomnezie şi perturbări în sfera gândirii [4]. Aceste efecte au crescut proporțional cu concentrația antidepresivelor respective, fiind mai frecvent întâlnite la vârstnici.

Antidepresivele pot exacerba simptomatologia schizofreniei [5]. Amoxapina, administrată în doze terapeutice, a fost asociată cu inducerea delirului, a halucinațiilor vizuale sau a simptomelor maniacale [6]. Există dovezi care susțin că antidepresivele pot întârzia remiterea simptomelor psihotice în schizofrenie, aratând şi că aceste substanțe nu sunt utile pentru terapia simptomelor depresive asociate decompensărilor psihotice din schizofrenie [7].

Reacții psihotice au fost observate şi în timpul terapiei cu IMAO [8].

Bupropionul a determinat, în cursul tratamentului, agitație psihomotorie, iritabilitate şi simptome psihotice la aproape 9% dintre pacienți [9]. Simptomele psihotice au constat mai ales în ideație paranoidă şi halucinații auditive, după cum s-a constatat în diferite studii de caz. Acest antidepresiv are o structură asemănătoare cu cea a amfetaminelor şi determină inhibiția recaptării noradrenalinei şi dopaminei, ceea ce ar explica suficient patogeneza sindromului psihotic secundar.

Agenții din clasa inhibitorilor selectivi ai recaptării serotoninei (ISRS) au fost asociați cu producerea unor halucinații vizuale complexe. Citalopramul, fluoxetina, fluvoxamina, paroxetina şi sertralina au indus halucinații şi ideație delirantă de persecuție. Combinația paroxetină- tramadol a condus la instalarea unui sindrom halucinator care nu a cedat decât la discontinuarea tratamentului.

Venlafaxina în combinație cu propafenona au produs o "psihoză organică" într-un studiu de caz, dar şi monoterapia cu venlafaxină a condus la instalarea unui sindrom paranoid la 3 zile după inițierea terapiei, în cazul unui pacient în vârstă de 85 de ani [10].

Benzodiazepinele au fost asociate cu simptome psihotice în faza de sevraj, chiar dacă intensitatea acestora a fost mai mică decât în cazul discontinuării administrării altor substanțe cu proprietăți sedative şi potențial de abuz (barbiturice, de exemplu). Un tablou clinic de depresie, însoțită de elemente psihotice, a fost descris la 4 pacienți aflați în perioada de sevraj benzodiazepinic [11]. Halucinații auditive şi vizuale au fost observate în perioada de oprire a consumului, după cum au mai fost descrise şi delirium sau manifestări catatonice. Mai rar au fost observate chiar şi simptome de schizofrenie de prim rang după Schneider [12,13]. Benzodiazepinele pot agrava psihozele prin creşterea concentraţiei GABA în anumite zone cerebrale.

Meprobamatul se poate asocia cu un sindrom de abstinenţă, în cadrul căruia pot fi întâlnite halucinaţii şi convulsii.

Hipnoinductoarele non-benzodiazepinice de tipul zolpidemului pot induce halucinaţii, în special la vârstnici sau la pacienţii cu insuficienţe hepatice severe, unde concentraţiile substanţelor sunt mai mari şi se menţin o perioadă mai îndelungată. Durata acestor episoade halucinatorii este de aproximativ o oră, dar atunci când se administrează concomitent zolpidem cu un ISRS, durata episodului halucinator se poate prelungi până la 7 ore, la persoanele cu această susceptibilitate.

Agenţii anticolinergici pot agrava simptomatologia pozitivă din schizofrenie [14]. Aceste substanţe pot induce efecte psihotice atât în caz de supradozare, cât şi la concentraţii terapeutice. Abuzul de anticolinergice a fost raportat în cazul benztropinei, trihexifenidilului sau prociclidinei. Pacienţii afirmă că, după consumul excesiv al acestor medicamente, resimt o stare de euforie, relaxare extremă, energie şi sociabilitate deosebite. Toleranţa la efectele euforizante se poate instala, în cazul trihexifenidilului, la doze ce variază între 15 şi 200 mg/zi.

Psihoza anticolinergică include semne neurologice, având un tablou clinic caracterizat prin dezorientare, confuzie, hipomnezie de fixare, agitaţie, dizartrie, vorbire incoerentă, presiunea vorbirii, halucinaţii, delir, ataxie, perioade de hiperactivitate care alternează cu perioade de somnolenţă, ideaţie paranoidă, anxietate şi comă. Halucinaţiile asociate cu delirul anticolinergic sunt, de obicei, vizuale, dar pot fi şi auditive sau tactile, după cum se pot regăsi şi combinaţii ale celor trei tipuri. Halucinaţiile vizuale constau în obiecte suspendate sau ataşate de îmbrăcămintea subiectului, pe care acesta are tendinţa să le strângă, înlăture sau să se agaţe de ele. Halucinaţiile tactile constau în senzaţia de mişcare a unor insecte pe sub tegumente.

Alţi agenţi antiparkinsonieni, de tipul amantadinei, levodopei sau bromocriptinei pot induce simptome psihotice. Aceste substanţe determină creşteri ale neurotransmisiei dopaminergice, agravând astfel simptomele pozitive ale psihozelor. De exemplu, amantadina stimulează eliberarea dopaminei la nivel sinaptic, levodopa este metabolizată şi se transformă în dopamină, iar bromocriptina este un agonist direct al receptorilor dopaminergici din sistemul nervos central.

Levodopa induce o creştere a incidenţei manifestărilor delirant-halucinatorii şi a depresiei [15]. Amantadina poate induce la pacienţi, în mod specific, halucinaţii vizuale şi agitaţie psihomotorie, după 2-3 săptămâni de tratament [16]. Bromocriptina induce simptome psihotice, precum şi exacerbări ale schizofreniei [17].

Antipsihoticele pot crea un tablou clinic de aspect psihotic, după oprirea rapidă a administrării lor, la un pacient care a urmat o perioadă îndelungată acest tip de tratament. Astfel de manifestări de recidivă însoţesc dischinezia tardivă [18]. Acest fenomen a fost atribuit suprasensibilizării receptorilor postsinaptici pentru dopamină, fenomen determinat de blocada cronică dopaminergică. Acest fenomen a fost denumit “psihoză de suprasensibilizare” [19].

Anticonvulsivantele au fost asociate cu efecte adverse comportamentale, inclusiv de aspect psihotic. Evaluarea relaţiei cauză-efect este însă dificilă, fiind cunoscută asocierea psihozei cu epilepsia. Simptome precum halucinaţiile, delirul, depresia şi confuzia pot fi observate în cursul tratamentului cu fenitoină [20].

Există şi raportări mai rare despre agravări ale psihozei sub terapia cu carbamazepină, acid valproic sau fenobarbital [21]. Anticonvulsivantele de nouă generaţie- gabapentin, felbamat, lamotrigina, tiagabina, topiramatul, vigabatrinul şi zonisamida- au fost şi ele asociate cu declanşarea unor fenomene de aspect psihotic [22].

Referitor la mecanismul fiziopatologic, a fost elaborată ipoteza “normalizării forţate” a traseului electroencefalografic sau a creşterii simptomelor psihotice odată cu reducerea crizelor convulsive. Conform acestei observaţii, diminuarea dozelor de anticonvulsivant ar putea agrava epilepsia dar ar reduce severitatea simptomelor psihotice.

Vigabatrinul a fost implicat în numeroase cazuri de psihoze farmacologice, explicaţia sugerată fiind aceea că acest agent inhibă GABA-transaminaza, crescând astfel concentraţia GABA şi deprimarea neurotransmisiei la nivelul substanţei negre.

Disulfiramul poate induce fenomene psihotice, raportate în absența interacțiunii cu alcoolul [23]. Au fost descrise halucinații, delir, stări confuzionale cu durate variabile. În studii de caz au fost descrise şi simptome caracteristice sindromului Capgras în asociere cu administrarea disulfiramului [24]. O analiză a evoluției clinice la un lot de 50 de pacienți tratați cu acest agent farmacologic a descoperit 9 cazuri de reacții psihotice, diferite de episoadele de delirium, care au fost mult mai frecvent observate [25]. Este posibil ca psihoza să se instaleze la doze mai mari decât cele folosite pentru tratamentul obişnuit, de prevenire a consumului de alcool.

Mecanismul fiziopatologic al acestor manifestări clinice este necunoscut, o ipoteză fiind acțiunea disulfiramului de inhibiție a dopamin-β-hidroxilazei, ceea ce ar conduce la un nivel crescut de dopamină intracerebrală. Un studiu care a evaluat activitatea dopamin-β-hidroxilazei a arătat că bărbații care aveau nivele mai reduse ale acestei enzime înaintea inițierii terapiei cu disulfiram au avut mai frecvent reacții psihotice [26].

Adăugarea la tratamentul cu disulfiram a terapiei antipsihotice poate agrava simptomatologia descrisă mai sus.

Simpatomimeticele pot induce halucinații tactile, de tipul senzației de « mişcare a unor viermi sub piele», apărute pe fondul neliniştii psihomotorii, anxietății şi a tulburărilor hipnice.

3.Concluzii

Pentru a realiza un diagnostic corect al acestor sindroame psihotice secundare este necesară obținerea unui istoric detaliat al expunerii persoanei (privind durata, cantitatea, contextul) la substanțele suspectate a fi responsabile de geneza respectivelor manifestări clinice. Dacă pacientul nu poate oferi informații valide, atunci realizarea unui interviu cu aparținătorii acestuia reprezintă o etapă absolut necesară a diagnosticului. Clinicianul trebuie să urmărească atât existența consumului anterior de psihotrope în scop terapeutic, cât şi utilizarea abuzivă a acestora sau autoadministrarea de substanțe cu risc de dependență de către pacient. Evaluarea comorbidităților somatice sau psihice şi a medicației concomitent administrate este, de asemenea, utilă pentru stabilirea unui diagnostic corect.

Examenul clinic oferă câteva indicii importante, care trebuie urmărite la toți pacienții: de exemplu, sindromul anticolinergic se asociază cu tegumente uscate, midriază, în timp ce amfetaminele induc tahicardie, hipertensiune şi agitație psihomotorie.

Examenul toxicologic urinar poate fi de ajutor, dar pacientul trebuie investigat în perioada propice acestei determinări, altfel existând riscul obținerii unor rezultate "fals negative". În cazurile dificile, o strategie eficientă ar fi oprirea administrării medicamentului suspectat şi observarea manifestărilor psihopatologice în timpul perioadei de eliminare a substanței respective.

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Cercetări pentru realizarea de noi produse decontaminante de combatere a efectelor atacului „bio-chem”

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Rezumat. Principalii compuşi chimici cu acțiune decontaminantă cuprind o gamă largă de substanțe: alcooli, aldehide (formaldehida, glutaraldehida), halogeni şi compuşi halogenați (clor, hipocloriți, cloramine, iod, iodofori), compuşi oxidanți (apă oxigenată, acid peracetic), fenoli, biguanide, argint şi compuşii săi, compuşi cuaternari de amoniu, substanțe tensioactive (cationice, anionice, neionice şi amfotere) etc. Aceştia au mecanisme de acțiune diferite, iar factorii care pot influența eficiența decontaminării sunt complecşi.

S-au efectuat cercetări teoretice şi experimentale în vederea realizării de noi decontaminanți bio-chem performanți care să îndeplinească următoarele condiții: să aibă un spectru larg de decontaminare bio-chem; să prezinte o toxicitate minimă față de subiecții umani, pentru a nu impune măsuri speciale de protecția muncii şi tehnica securității muncii pe timpul fabricației, condiționării şi utilizării; să nu necesite condiții speciale pentru depozitare; să aibă un caracter ecologic; să se poată fabrica în condiții tehnico-economice acceptabile etc.

În final au fost realizate opt formule de decontamianți bio-chem, cu efect microbiologic cert, cu toxicitate redusă şi care nu afectează suprafețele uzuale. Dintre acestea, cinci conțin substanțe active dezinfectante cunoscute, iar trei au substanțe nou sintetizate de colaboratori, respectiv cu alchilfosfat de la ICECHIM, compus cuaternar de amoniu şi sare de piridiniu de la ICTCM. La nevoie, prepararea şi utilizarea acestor decontamianante în teren este simplă, nepericuloasă şi nu necesită personal de specialitate. Dezinfectantele lichide pot fi utilizate şi ca dezinfectanți-detergenți, cu avizul Ministerului Sănătății.

Cuvinte cheie: atac bio-chem, bioterorism, decontaminare, protecție medicală CBRNE

Abstract. We present the results of the researches realized for producing efficient decontaminated products in treating the effects of the chemical and biological attack weapons. The decontaminations products must fulfill the following conditions: must have a large specter of bio-chemical decontamination with a minimum toxicity for the human subjects, no need for special storage, ecological features, acceptable technical-economical making conditions.

INTRODUCERE

Protecția medicală contra armelor de distrugere în masă (ADM) cu agenți chimici, biologici, radiologici, nucleari şi/sau explozivi (CBRNE) are în prezent o importanță deosebită nu numai pentru apărare în cadrul unui eventual război, ci şi pentru apărare antiteroristă, în acțiuni de luptă zise „asimetrice”. În ultimii ani, atacurile „bio-chem” au fost puse în practică de terorişti în diferite țări.

În cadrul unui proiect de cercetare de excelență pentru realizarea de produse decontaminante performante, tehnologii de obținere şi procedee de aplicare în vederea combaterii efectelor atacurilor chimice şi biologice, s-au efectuat cercetări teoretice şi experimentale în vederea realizării de noi decontaminanți bio-chem care să îndeplinească următoarele condiții: să aibă un spectru larg de decontaminare bio-chem; să prezinte o toxicitate minimă față de subiecții umani, pentru a nu impune măsuri speciale de protecția muncii şi tehnica securității muncii pe timpul fabricației, condiționării şi utilizării; să nu necesite condiții speciale pentru depozitare; să aibă un caracter ecologic; să se poată fabrica în condiții tehnico-economice acceptabile etc. Au fost prevăzute şi realizate mai multe etape: fundamentarea strategiei de obținere a produselor decontaminante performante, caracterizarea fizico-chimică şi domeniile de utilizare ale acestora în combaterea efectelor atacurilor teroriste chimice şi biologice; studii şi experimentări în vederea elaborării modelului experimental de laborator al tehnologiei de obținere a produselor decontaminante performante şi procedee de aplicare în combaterea efectelor atacurilor teroriste chimice şi biologice; elaborarea modelului experimental de laborator al tehnologiilor de obținere şi testare a compuşilor de decontaminare chimică şi biologică; prezentarea şi demonstrarea funcționalității şi aplicabilității modelului experimental de laborator al tehnologiei decontaminante performante în combaterea efectelor atacurilor teroriste chimice şi biologice; redactarea raportului final de activitate şi diseminarea informațiilor.

Principalii compuşi chimici cu acțiune decontaminantă biologică şi/sau chimică (bio-chem) cuprind o gamă largă de substanțe: alcooli, aldehide (formaldehida, glutaraldehida), halogeni şi compuşi halogenați (clor, hipocloriți, cloramine, iod, iodofori), compuşi oxidanți (apă oxigenată, acid peracetic), fenoli, biguanidine, argint şi compuşii argintului, compuşi cuaternari de amoniu, substanțe tensioactive (cationice, anionice, neionice şi amfotere) ş.a. Aceştia au mecanisme de acțiune diferite, iar factorii care pot influența eficiența decontaminării sunt complecşi.

MATERIAL ŞI METODĂ

Cercetări teoretice, experimentale şi tehnologice efectuate în scopul realizării de decontaminanți bio-chem performanți şi de simulatori de substanțe toxice de luptă (STL)

A. Cercetări pentru **sinteza chimică** efectuate în cadrul ICECHIM. Partenerul ICECHIM a sintetizat decontaminanți "bio-chem" pe bază de: săruri de amoniu ale O-mono şi O,O-dialchil [aril] ester fosfaților acizi; săruri de amoniu ale acizilor carboxilici halogenați (acid cloracetic) şi monoetanolamina sau cu 8-hidroxichinolina. Cel mai eficient a fost produsul cu cloracetat de 8-hidroxichinolina condiționat în etilenglicol, care a prezentat activitate antimicrobiană pe toate speciile de bacterii patogene testate.

1)**DECON – 01**, compoziție formată dintr-un amestec echimolar de O-monoalcoxi şi O,O-dialcoxiesteri-acizi fosfați sub formă de săruri de monohidroxialchil-monoamine, în care 25% dintre funcțiile acide (-OH) ale alcoxiester-fosfați-acizilor sunt libere (neneutralizate);

2)**DECON – 02**, compoziție formată dintr-un amestec echimolecular de O–monoalcoxi şi O,O-dialcoxiesterfosfați-acizi sub formă de săruri de polihidroxialchil-amine, în care 25% dintre funcțiile acide (-OH) ale alcoxiester-fosfați-acizilor sunt libere (neneutralizate);

3)**DECON – 03**, compoziție formată dintr-un amestec de săruri de aminice ale mixturii echimoleculare de O-monoalcoxi şi O,O-dialcoxi ester-fosfați în care 25% dintre funcțiile acide (-OH) au fost lăsate libere (fără a fi neutralizate);

4)**DECON – 04**, compoziție formată dintr-un amestec de O-mono şi O,O-dialchil ester-fosfați acizi în care grupările alcoxi sunt derivate de la polietilenglicol-300 (PEG-300): $HO-CH_2-(CH_2-O-CH_2)_3-CH_2-OH$. Grupările (funcțiile) acide (-OH) din structurile A, B, C sunt neutralizate în proporție de 75% cu monoetanolamină;

5)**DECON – 05,** un decontaminant care conține ca substanță activă cloracetatul de monoetanolamină: $Cl-CH_2-COO^-\ H_3N^+-CH_2CH_2-OH$;

6)**DECON – 06,** o compoziție similară din punct de vedere al structurilor moleculare pe care le conține cu DECON – 04 (structurile moleculare A, B, C); deosebirea constă în faptul că neutralizarea funcțiunilor acide din grupările fosfatice s-a făcut cu trietanolamină în proporție de 75%;

7)**DECON – 07,** substanța activă a decontaminantului este monocloracetatul de 8-hidroxichinolină:

8)DECON – 08, produsul activ al decontaminantului este boratul de 2-aminoetanol;

9)DECON – 09, decontaminantul este un amestec de mono şi dialchilesteri-fosfaţi acizi, conţinând structurile moleculare A, B şi C de la DECON -04, în care funcţiile acide (-OH) au fost neutralizate în proporţie de 70% (din care 42% cu 8-hidroxichinolina şi 28% cu 2-aminoetanol);

10)DECON – 30, decontaminant conceput pe baza rezultatelor foarte bune obţinute în cadrul testărilor în ceea ce priveşte capacitatea de decontaminare bio-chem a produsului DECON-09. În această variantă, funcţiunile acide (-OH) din structurile moleculare A, B şi C s-au neutralizat în proporţie de 70%, restul de 30% rămânând libere; neutralizarea s-a efectuat în proporţie de 35% cu 8-hidroxichinolină şi cu 35% monoetanolamină;

11)DECON – 31, un decontaminant bio-chem cu spectru larg de acţiune, care conţine ca produse active o mixtură pe bază de derivaţi anorganici de bor;

12)DECON – 32, o compoziţie în care componentele active sunt o mixtură formată din monocloracetat de 8-hidroxichinolină şi borat de monoetanolamină. Produsul se caracterizează printr-o mare capacitate de decontaminare, având, totodată, un spectru larg de acţiune bio-chem.

B. Cercetări pentru **sinteza chimică** efectuate în cadrul ICTCM. În cadrul ICTCM-DAS s-au sintetizat produse decontaminante din următoarele clase de substanţe: acizi şi peracizi organici cu număr mic de atomi de carbon; compuşi cu structura de amoniu cuaternar derivaţi de la alchilamine, chinolina, piperazina, imidazolina; săruri cuaternare de amoniu şi N-alchilpiridiniu; compuşi policuaternari de amoniu; copolimeri cu structura de amoniu cuaternar.

Elaborarea procesului tehnologic de laborator pentru obţinerea decontaminanţilor bio-chem de tip peracizi organici şi compuşi cuaternari de amoniu

În cadrul prezentei lucrări s-a avut în vedere elaborarea procesului tehnologic pentru două clase de produse: peracizi organici şi compuşi cuaternari de amoniu.

Peracizii organici (acidul peracetic) au acţiune rapidă asupra microorganismelor, inclusiv asupra sporilor. Prezintă avantajul că produşii de descompunere nu au efecte nocive sau toxice şi nu lasă reziduuri şi dezavantajul că au acţiune corozivă asupra metalelor şi suprafeţelor galvanizate. Acest neajuns poate fi diminuat prin adăugarea unor aditivi cu acţiune anticorozivă.

Acidul peracetic acţionează prin denaturarea proteinelor, afectarea permeabilităţii peretelui celular, oxidează legăturile sulfurice şi sulfhidril ale proteinelor, enzimelor şi ale altor metaboliţi. Unele studii consideră că activitatea antimicrobiană a acidului peracetic este comparabilă cu o glutaraldehidă, mai ales asupra sporilor şi a microbacteriilor.

Compuşii cuaternari de amoniu sunt cei mai numeroşi şi mai importanţi agenţi cationici cu acţiune bactericidă.

Absorbţia compuşilor cuaternari de amoniu la suprafaţa celulei este însoţită de modificări ale tensiunii superficiale, ceea ce determină creşterea permeabilităţii la nivelul membranelor externe şi alte modificări structurale.

Substanţele tensioactive cationice interferează metabolismul energetic al bacteriilor, acţiunea lor exercitându-se la nivelul structurilor membranelor externe ale acestora.

Substanţele tensioactive cationice, fiind încărcate cu sarcini electrice pozitive, sunt rapid atrase de elementele cu încărcătură negativă din peretele celular şi produc o reducere a tensiunii superficiale a membranelor celulare, mult sub valoarea de echilibru cu tensiunea din interiorul celulei, încât învelişul acesteia se rupe.

Tehnologia de obţinere a produselor de oxidare

Această tehnologie se bazează pe oxidarea alcoolului polivinilic în prezenţa acidului acetic şi a perhidrolului în mediu apos sau de perhidrol şi acid sulfuric în mediu apos. Astfel alcoolul polivinilic în prezența oxidanților suferă o scindare oxidativă a legăturilor C-C şi C-H cu formarea unor compuşi hidroxicarbonilici şi hidroxicarboxilici.

Tehnologia de obținere a compuşilor cuaternari de amoniu

Compuşii de tip "oniu", datorită particularităților structurale, pot avea şi proprietăți bactericide sau fungicide. Având în vedere datele din literatura de specialitate din acest domeniu şi rezultatele promițătoare obținute în faza a II-a a lucrării, s-au continuat lucrările de sinteză ale compuşilor de "oniu" de tipul sărurilor cuaternare de amoniu, în special cloruri.

Procedeul cel mai obişnuit de obținere a sărurilor cuaternare de amoniu sau piridiniu constă în tratarea aminelor terțiare cu halogenuri de alchil conform reacției:

$$(R)_3 N: \ + \ R'' Cl \qquad (R)_3 \longrightarrow R'' N^+ Cl^-$$

în care: R şi R' pot fi radicali alchil $C_1 - C_2$; R" poate fi radical alchil $C_1 - C_8$ până la $C_{18} - C_{20}$, benzil; X = halogen (de preferință clor).

Asemenea produse se pot obține într-o singură fază, pornind de la amina primară ($R\text{-}NH_2$) tratată cu un exces de agent de alchilare (R'Cl).

În locul clorurilor de alchil se poate folosi ca agent de alchilare pentru reacția de cuaternizare şi clorura de benzil.

Reacția este generală pentru toți compuşii organici care au în molecula lor o grupare de amoniu primară, secundară sau terțiară ca: etilendiamina, hexametilentetramina, precum şi pentru aminele care au atomul de azot în ciclu (piridina, chinoleina, morfolina etc.).

Decontaminanți bio-chem realizați la ICTCM

1) Produs Cod DC – 1, soluție apoasă de acizi organici obținuți prin reacții de oxidare (conc. 26 - 27%);
2) Produs Cod DC – 3, soluție apoasă pe bază de clorură de tetraalchilamoniu (conc. 15%);
3) Produs Cod DC – 4, soluție apoasă pe bază de compus policuaternar de amoniu (conc. 20%);
4) Produs Cod DC – 5, soluție apoasă pe bază de copolimer compus cuaternar de amoniu cu compus carboxilic nesaturat (conc. 20%);
5) Produs Cod DC – 6, soluție apoasă pe bază de copolimer cu structură de amoniu cuaternar (conc.10%);
6) Produs Cod DC – 1°, soluție apoasă de acizi organici şi peracizi obținuți prin reacții de oxidare (conc. 20%);
7) Produs Cod DC – 1b, soluție apoasă de acizi organici şi peracizi obținuți prin reacții de oxidare (conc. 10%);
8) Produs Cod DC – 6a, soluție apoasă pe bază de copolimer cu structură de amoniu cuaternar (conc. 20%); **9) Produs Cod DC – 7,** soluție apoasă pe bază de copolimer cu structură de amoniu cuaternar (conc. 20%);
10) Produs Cod DC – 10, soluție apoasă pe bază de polimer cu structură de amoniu cuaternar (conc. 20%);
11) Produs Cod DC – 15, soluție apoasă de acizi organici obținuți prin reacții de oxidare ale polialcoolilor (conc. 20%);
12) Produs Cod DC – 16, soluție apoasă pe bază de săruri de amoniu cuaternar şi acizi organici (conc. 25%);
13) Produs Cod DC – 17, soluție apoasă pe bază de sare de piridiniu şi substanță tensioactivă cationică (conc. 25%);
14) Produs Cod DC – 18, soluție pe bază de sare de chinoliniu şi substanță tensioactivă cationică (conc. 20%);
15) Produs Cod DC – 19, soluție pe bază de derivat heterocarbonic cu azot (conc. 20%);
16) Produs Cod DC – 20, soluție apoasă pe bază de compus cuaternar de amoniu (conc. 20%);
17) Produs Cod DC – 21, soluție pe bază de substanță tensioactivă cationică (conc. 10%).

S-a realizat şi prezentat modelul experimental al sistemului polivalent de decontaminare chimică şi biologică pentru sinteza decontaminanților de tip DECON şi de tip peracizi organici şi compuşi cuaternari de amoniu. S-au elaborat tehnologiile de laborator pentru obținerea decontaminanților care au dat cele mai bune rezultate în cadrul testărilor efectuate în ceea ce priveşte capacitatea de distrugere a substanțelor toxice de luptă şi a agenților biologici ce pot fi folosiți de terorismul internațional. Verificarea structurilor chimice pentru compuşii nou-sintetizați a fost efectuată de către Laboratorul de analize fizico-chimice din cadrul UMF.

REZULTATE ŞI DISCUȚII

Testarea microbiologică şi toxicologică a compuşilor realizați. Testările au fost efectuate conform recomandărilor din „Metode şi tehnici uzuale în laboratorul clinic” editată de Ministerul Sănătății şi Academia de Ştiințe Medicale şi Normele „Antiseptiques et desinfectants” AFNOR.

După testarea microbiologică şi toxicologică *screening* a substanțelor sintetizate de ICECHIM şi ICTCM şi selecționarea celor mai eficiente din punctul de vedere al activității antimicrobiene, au fost realizate mai multe formule de condiționare (CCSMM şi UMF) care, la rândul lor, au fost testate ca decontaminante, rezultatele fiind prezentate în rapoartele de cercetare.

A fost selecționat un set de produse noi, potențial utilizabile ca decontaminante polivalente, cu efect antibacterian, antiviral, antifungic, sporicid, antitoxinic şi cu toxicitate redusă pentru mamifere, pe baza testărilor de laborator *in vitro* efectuate în **laboratorul de microbiologie**. În principal, produsele selecționate au fost: clorigeni sub formă de pulberi adsorbante şi de soluții apoase de alchilfosfați, chinoline, săruri de amoniu cuaternar şi peracizi.

Aceste decontaminante potențiale au fost testate în continuare *in vitro* în faza de **micropoligon**, respectiv, în laboratorul de microbiologie au fost efectuate contaminări şi decontaminări cu agenți biologici şi toxine pe diferite suprafețe: metal, lemn, plastic, piele naturală, textile şi apă potabilă. Am urmărit reconfirmarea efectului antimicrobian în condiții care să simuleze realitatea din teren şi eventualul efect distructiv asupra unor materiale uzuale.

Aceste substanțe au fost testate şi *in vivo* pe **animale de experiență** în biobaza laboratorului: injectare intraperitoneală la şoareci albi de laborator a câte 0,5ml soluție de lucru pentru toxicitate acută; aplicarea pe pielea epilată a 1g substanță pentru toleranța cutanată la iepuri şi prin instilare oculară a substanței pentru iritare conjunctivală la iepuri. Nu au fost observate aspecte deosebite.

Experimentarea în poligon a modelelor experimentale ale noilor decontaminanți biologici

Având în vedere comportarea bună, conform aşteptărilor a decontaminantelor selecționate, am organizat un **minipoligon** pentru continuarea testărilor în condiții reale, apropiate de acțiunile de lupta tactice. Astfel, la Fortul 31 Cernica a fost pus în funcțiune un minipoligon biologic temporar, format din: o autospecială militară casată (ca țintă), platformă betonată, culturi microbiene cu simulatori de agenți biologici (pentru ca în eventualitatea că rezultatele nu sunt corespunzătoare, să nu rămână zona contaminată), decontaminante pentru testare, condiționate şi încărcate în sisteme de aplicare adecvate, truse pentru recoltare microbiologică în teren, echipament de protecție specific, materiale de stat major şi personal calificat şi specializat, antrenat corespunzător.

În funcție de rezultatele bune ale testărilor în minipoligon, după recepția fazei s-a propus factorilor de decizie militară experimentarea în condiții reale, în **poligonul NBC** specializat, pe maşini de luptă şi cu mijloace de decontaminare existente în dotarea armatei.

Tulpinile microbiene utilizate reprezintă principalele grupe de bacterii patogene:

- coci gram-pozitivi: *Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus fecalis;*

- bacili gram-pozitivi sporogeni: *Bacillus anthracis (tulpina vaccinala)*, *Bacillus cereus, Bacillus subtilis*;
- bacili gram-negativi: *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa;*
- vibrioni*: Vibrio cholerae.*

Exteriorul autovehiculului țintă a fost marcat cu suprafețe numerotate, de circa 0,1 mp, pentru contaminare şi decontaminare. Suprafețele au fost alese astfel:

- tablă vopsită orizontală, pentru decontaminanții pulbere;
- tablă vopsită verticală, pentru decontaminanții soluție apoasă;
- ferestre de sticlă, pentru decontaminanții soluție apoasă;
- anvelope de cauciuc, pentru decontaminanții soluție apoasă.

Contaminarea microbiană s-a făcut prin stropire pe suprafața alocată, separat cu fiecare tulpină şi cu mixtura rezultată din amestecul lor. Am utilizat suspensia microbiană cu circa 1 milion de germeni vii pe mililitru, câte 1 ml pe dmp.

Decontaminarea s-a făcut cu pulberi adsorbant-dezinfectante, prin acoperire completă a zonei contaminate şi ştergere cu tampon (burete) pentru îndepărtarea agentului biologic, circa 10 g pe dmp. Pentru decontaminante lichide s-a efectuat pulverizarea soluției apoase 10% până la acoperirea completă a zonei contaminate şi începerea curgerii excesului de lichid, în medie circa 10 ml pe dmp la suprafețe netede (geam, tablă vopsită) şi circa 20 ml pe dmp la suprafețe rugoase (tablă scorojită, pneuri).

Au fost recoltate probe microbiologice prin metoda tamponului igienico-sanitar (TIS) folosind trusa de recoltare microbiologică în teren model CCSMM. Momentele de recoltare au fost: înainte de contaminare (pentru a stabili nivelul inițial de contaminare naturală), imediat după contaminare (pentru a verifica nivelul de contaminare experimentală), după decontaminare la fiecare decontaminant cu fiecare agent biologic, la interval de 10 minute (conform cerinței pentru decontaminantele de uz militar) şi la interval de 45 minute (ca pentru dezinfectante de uz general). În plus, la terminarea experimentului au fost recoltate probe de pe diferitele piese ale echipamentului de protecție al operatorilor şi din mediul ambiant pentru a detecta eventuala contaminare reziduală.

Toate probele recoltate au fost imediat transportate, în condiții de biosiguranță, în **laboratorul de microbiologie securizat cu nivel P2+** şi au fost însămânțate pe mediu lichid (bulion nutritiv IC) şi medii solide (agar Mueller-Hinton IC şi geloză-sânge CCSMM). Mediile însămânțate au fost incubate la 37°C şi au fost examinate la 24 şi 48 ore, după care au fost lăsate pe masa de lucru la temperatura camerei alte 24 şi 48 ore, pentru interpretarea definitivă a rezultatelor.

La terminarea experimentului, toate materialele infecțioase au fost autoclavate şi apoi incinerate, pentru a evita riscul de contaminare, în special pentru speciile sporogene.

Se observă că, în general, decontaminarea nu este completă, în majoritatea cazurilor se reduce nivelul de contaminare microbiană cu 3-4 ordine de mărime, ceea ce ar putea fi considerat ca suficient pentru decontaminare. S-au obținut rezultate bune la substanțele DECON 7, 9, 30 şi 32 de la ICECHIM.

La soluția DC 18 ICTCM, 10% (cu substanța activă - săruri de chinoliniu) efectul antimicrobian la 10 minute este atât de bun, încât se produce „sterilizare la rece”. Ca urmare, aceste produse pot fi propuse ca decontaminanți biologici şi, eventual, ca decontaminanți polivalenți şi pot fi testate în poligonul specializat NBC, fără pericol de contaminare reziduală pentru utilizatori sau pentru mediu. Efectul antimicrobian se menține şi este mai evident după 45 de minute. S-au mai calificat şi soluțiile DC 7 şi 19 10% de la ICTCM (cu substanța activă săruri de amoniu), fapt care sugerează că toate aceste produse pot fi propuse ca eventuale dezinfectante tehnice de uz medical sau igienico-sanitar, conform Legii medicamentului.

Dintre produsele testate cea mai bună activitate microbiologică o manifestă produsul DC-7, care are şi un efect sinergic în amestec cu alte produse, DC-18 si DC-19. De asemenea, produsele

DC-4 şi DC-10 au activitate de decontaminare bună. O altă categorie de decontaminante cu acţiune promiţătoare o reprezintă produsele DC-1, DC-1a şi DC-1b, dar cu un spectru mai restrâns de acţiune.

Substanţele active confirmate prin testările anterioare ca fiind dezinfectante cu spectru larg pe microorganisme patogene, inclusiv pe agenţi biologici de luptă, au fost comparate şi evaluate ţinând cont de activitatea microbiologică şi de toxicitatea acută. Scopul a fost de a alege, în final, pe cele mai performante din fiecare grup farmacologic, în vederea formulării de decontaminante bio-chem, în colaborare cu Catedra de Industria medicamentului şi biotehnologii farmaceutice din Facultatea de Farmacie a Universităţii de Medicină şi Farmacie „Carol Davila” din Bucureşti.

Dintre substanţele active noi, sintetizate de parteneri, am ales cele mai eficiente de la fiecare, respectiv Decon 07 de la ICECHIM (alchilfosfat cu 8-hidroxichinolina), DC-7 (compus cuaternar de amoniu) şi DC-18 (sare de chinoliniu) de la ICTCM.

În final, au fost testate în teren opt formule de decontaminante, realizate pe baza celor mai eficiente substanţe nou-sintetizate, în paralel cu altele condiţionate cu substanţe cunoscute, pentru comparare, dar şi pentru a dispune de o gamă cât mai largă de decontaminante, ca substanţe active, mod de condiţionare şi destinaţie: Decontaminant lichid organofosforic semispumant (*Alchifos*), Decontaminant lichid compus cuaternar de amoniu semispumant (*Amodec*), Decontaminant lichid cu sare de chinoliniu (*Chinodec*), Decontaminant lichid formolat semispumant (*Fordec*), Decontaminant lichid cu glutaraldehida semispumant (*Glutadec*), Decontaminant pulbere cu clorigeni (*Clordec*), Detergent-dezinfectant pentru filtroventilaţie (*A/C Cleaner*), Dezinfectant-detergent pentru filtroventilaţie (*A/C Disinfecter*); pentru decontaminarea incintelor (laboratoare de microbiologie, cabinete de biosecuritate, arii de producţie aseptice, aeronave, nave, autospeciale, adăposturi etc.). Am realizat un *amestec superoxidant* care se aerosolizează cu un dispozitiv special, sub formă de ceaţă uscată, cu efect sterilizant; în final rezultă apă şi oxigen, deci este ecologic şi nu atacă instalaţiile; acest produs urmează să facă obiectul unor cercetări ulterioare.

Formulele menţionate sunt în portofoliul CCSMM şi au fost preparate prin microproducţie de decontaminante, pentru uzul propriu sau, la ordin, al altor formaţiuni militare.

Concluzii

Lucrările s-au desfăşurat conform planului de realizare a proiectului, cu participarea tuturor partenerilor, care a cuprins activităţile tehnico-ştiinţifice prevăzute şi în final s-au obţinut rezultatele prezentate.

S-au efectuat cercetările ştiinţifice teoretice şi experimentale la nivel de laborator în vederea conceperii şi realizării de decontaminanţi bio-chem polivalenţi, cu ajutorul cărora să se distrugă cvasitotalitatea agenţilor chimici şi biologici care pot fi folosiţi de terorismul internaţional.

S-au elaborat tehnologiile de laborator pentru obţinerea decontaminanţilor care au dat cele mai bune rezultate în cadrul testărilor efectuate în ceea ce priveşte capacitatea de distrugere a substanţelor toxice de luptă şi a agenţilor biologici ce pot fi folosiţi de terorismul internaţional.

Produsele au fost condiţionate sub formă de soluţii apoase, realizându-se amestecuri sinergice cu substanţe tensioactive cationice, testate în minipoligon în concentraţii de 10%.

În final au fost realizate opt formule de decontaminanţi bio-chem, cu efect microbiologic cert, cu toxicitate redusă şi care nu afectează suprafeţele uzuale. Dintre acestea, cinci conţin substanţe active dezinfectante cunoscute, iar trei au substanţe nou sintetizate de colaboratori: alchilfosfat la ICECHIM, respectiv compus cuaternar de amoniu şi sare de chinoliniu la ICTCM.

La nevoie, prepararea şi utilizarea decontaminantelor în teren poate fi simplă, nepericuloasă şi nu necesită personal de specialitate.

Dezinfectantele lichide testate pot fi utilizate şi ca dezinfectanţi-detergenţi, cu avizul Ministerului Sănătăţii.

În concluzie, apreciem că obiectivele prevăzute în planul de realizare a proiectului au fost îndeplinite la un nivel tehnico-ştiinţific şi tactico-operativ adecvat, iar realizările obţinute vor contribui semnificativ la amplificarea capacităţilor de protecţie medicală contra eventualelor atacuri bio-chem.

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Considerente istorice privind ocrotirea sănătății în România

Lt.-col (r). ec. Dumitru COSMESCU

Centrul de Cercetări Ştiinţifice Medico-Militare

Tradiția organizării şi finanțării îngrijirilor medicale în România este generată de apariția relativ timpurie a unor reglementări privitoare la organizarea asistenței medicale şi de ocrotire socială, precum şi a asigurărilor sociale de sănătate.

Prima lege sanitară românească, promulgată în 1874, are un puternic caracter de administrație centralizată pe teritoriul României. Legea a fost modificată de mai multe ori, cea mai importantă modificare datând din 1893, vizînd regimul consumurilor de produse medicale şi servicii.

Apar şi primele personalități medicale, care se poate considera că au inițiat sistemul de igienă socială şi sănătate publică:

- dr. Constantin Caracaş (1773-1828), medic al oraşului Bucureşti (1804), autor al lucrării *Topografia Țării Româneşti*. A contribuit la organizarea ştiințifică a activității medico-sanitare, fiind şi unul dintre fondatorii spitalului Filantropia din Bucureşti. A fost de asemenea tipograf şi editor, astfel a înființat la Bucureşti prima tipografie laică din Țara Românească (1817);
- dr. Ştefan Stâncă (1865-1897) este autorul lucrării *Mediul social ca factor patologic*;
- dr. Carol Davila (1828-1884) este cunoscut ca fiind medic român de origine franceză. În 1853 se implică în reorganizarea Spitalului Oştirii de la Mihai Vodă împreună cu farmacia, iar în 1855 înființează în Bucureşti o şcoală de mică chirurgie. Organizator al Serviciului sanitar militar - inspector general în timpul războiului de independență - şi al ocrotirii sănătății publice. A pus bazele învățământului medical din țara noastră, alături de Nicolae Kretzulescu, înființând, în 1857, Şcoala Națională de Medicină şi Farmacie. Şi tot acum, Carol Davila fundamentează primele instrucțiuni oficiale pentru apărarea sănătății muncitorilor din fabrici şi ateliere, şi organizează primele arondismente sanitare din țară.

Tot din inițiativa doctorului Carol Davila,a fost înființată, în1870, pe lângă Facultatea de Medicină, Grădina Botanică din Bucureşti. Intențiile creării acestei grădini aveau scop atât didactic - instruirea elevilor Şcolii Naționale de Medicină şi Farmacie - cât şi economic - aprovizionarea cu plante proaspete a farmaciilor. Experimentată în epidemia de holeră ce apăruse în tabăra turcească de la Dunăre, *tinctura Dr. Davila* ("antiholerică") s-a bucurat, timp de un secol, de o mare popularitate. Interesante sunt ingredientele din care este compusă tinctura: ulei de mentă, tinctură de opiu, extract de scorțişoară şi revent - toate cu rol calmant. Având în vedere complexitatea activității întreprinse de dr. Carol Davila, au luat ființă în țara noastră o serie de societăți ştiințifice: "Societatea medicală" (1857), "Societatea de Cruce Roşie" (1876), "Societatea ştiințelor naturale" (1876), precum şi două reviste medicale: "Monitorul medical" (1862) şi "Gazeta medicală" (1865);

- dr. Iacob Felix (1832-1905) , medic de origine cehă, şi-a făcut studiile la Viena. El este cel care a realizat primele înfăptuiri în domeniul igienei ştiințifice. Medic al oraşului Oltenița (1858-1859), este chemat la Bucureşti în 1860, la inițiativa doctorului Carol Davila. Devine profesor de igienă la Şcoala Națională de Medicină, aceasta transformându-se ulterior în facultate, iar dr.Iacob Felix urmând să fie titular la catedra de igienă. A studiat, alături de Al. Marcovici, epidemia de tifos exantematic şi de febră recurentă la Petersburg (1865). Pentru meritele ştiințifice, Academia

Română îl numeşte în 1879 membru al secţiei de ştiinţe[1]. Ocupă funcţia de director al Serviciului Sanitar, reuşind astfel să reglementeze problema alienaţilor, a combaterii conjuctivitei granuloase, să organizeze eficient ierarhia sanitară, să introducă cele dintâi măsuri de igienă a muncii; de asemenea, are grijă de reglementarea controlului alimentar şi al farmaciilor şi îşi aţinteşte privirile şi asupra serviciului veterinar. Una dintre cele mai mari izbânzi a fost combaterea cu succes a epidemiei de holeră din anul 1893.

În calitatea sa de profesor, dr. Felix Iacob, pune bazele ştiinţei igienei. Cercetător original, prin lucrările sale scrise în limbi străine, prin participarea la numeroasele congrese şi conferinţe internaţionale, a făcut cunoscută mişcarea ştiinţifică românească şi a contribuit în mod competent la soluţionarea marilor probleme de sănătate publică. A fost un savant progresist al epocii de tranziţie în medicina românească punând bazele ştiinţei igienei în ţara noastră. El a elaborat primele lucrări vaste de documentare asupra începuturilor şi dezvoltării medicinii româneşti.

Lucrarea *Geografia medicală a României,* din 1897, este rodul unor studii urmărite perseverent de-a lungul deceniilor. *Igiena publică şi poliţie sanitară* (în două volume) vol. 1, 1870, este primul tratat românesc oferit ca material didactic studenţilor, fiind valoros mai ales prin referinţele la realităţile din ţara noastră; al doilea volum apare în 1889. Studiile despre *Istoria igienei în România* reprezintă repere bibliografice deosebit de importante pentru cei ce vor să aprofundeze această latură a medicinii. În lucrarea *Medicul poporului* se ocupă intens de problemele sanitare ale muncitorilor şi ţăranilor, de bolile meseriaşilor şi de condiţiile în care ei muncesc;

- dr. Victor Babeş (1854-1926), născut la Viena, a fost fiul lui Vicenţiu Babeş, originar din Banat. Cu o carieră ştiinţifică remarcabilă, lucrând alături de Louis Pasteur la Paris, apoi în laboratoarele lui Rudolf Virchow şi Robert Koch la Berlin, profesor asociat la Universitatea din Budapesta, din 1897 este chemat ca profesor la Bucureşti. Este fondatorul şcolii româneşti de microbiologie (şi autor al primului tratat de bacteriologie din lume), devine membru al Academiei Române din 1893. Pe lângă activitatea ştiinţifică a fost preocupat de îmbunătăţirea stării sanitare a populaţiei, în special de combaterea şi prevenirea pelagrei;

- dr. Nicolae Kretzulescu (1812-1900), iniţiator al învăţământului medical din ţara noastră. Este fondator al unei şcoli de mică chirurgie la Spitalul Colţea din Bucureşti. Fruntaş în Revoluţia din 1848, ministru în mai multe rânduri şi prim-ministru (1859, 1862-1863, 1865-1866);

- dr.Ioan Cantacuzino, savant român, medic şi bacteriolog, prin calităţile sale de cercetător, organizator şi animator, a realizat în ţară o operă foarte importantă în domeniul bacteriologiei, imunologiei, epidemiologiei şi igienei sociale, contribuind, în acelaşi timp, la afirmarea ştiinţei româneşti pe plan internaţional. Aplecat asupra studiului filosofiei, fiziologiei şi ştiinţelor naturale, mai târziu, se va dedica studiului medicinii. Face parte din cea de-a treia generaţie de biologi , alături de E. Racoviţă, D. Voinov şi P. Bujor, patrioţi convinşi, animaţi de marile idealuri ale epocii. A fost profesor la Facultatea de Medicină din Bucureşti, membru al Academiei Române, întemeietor al Institutului care-i poarta numele şi fondator al Şcolii române moderne de microbiologie şi medicină experimentală. Profesorul Ioan Cantacuzino este întemeietorul medicinii experimentale la noi în ţară. Susţinând colaborarea clinicii cu laboratorul, a înfiinţat, în afara de Institutul de medicină experimentală, un serviciu spitalicesc. Cantacuzino continuă cercetările fostului său profesor, I. I. Mecinikov, în problema fagocitozei, a imunităţii la nevertebrate şi, în general, a mecanismului de apărare a organismului împotriva agenţilor patogeni. Contribuie în mod remarcabil la studiul holerei, tifosului exantematic, şi al tuberculozei. A creat noţiunea de imunitate prin contact. În campania din 1913 a iniţiat măsurile de combatere a epidemiei de holeră, iar în Primul Război Mondial, în calitate de conducător al serviciilor sanitare militare şi civile, a luat măsurile de combatere a marii epidemii de tifos exantematic. Ca director general al Serviciului Sanitar (1908-1910) luptă împotriva tuberculozei, organizează servicii sanitare model în opt judeţe şi laboratoare de bacteriologie în principalele oraşe ale ţării. Sintetizează ideile moderne de medicina curativă şi preventivă pe care le raportează la nevoile şi posibilităţile ţării din acea perioada în legea sanitară din 1910 (legea "Cantacuzino"). Realizează o serie de lucrări care au ca temă: descoperirea imunității celulare şi umorale, sensibilitatea şi lipsa de imunitate a organismului față de scarlatină, precum şi studii recunoscute la nivel mondial asupra holerei şi vaccinoterapiei. Introduce vaccinul lui Calmette, iar în 1912 creează vaccinul antitific. A întemeiat "Revista ştiințelor medicale" (1905). Împreuna cu prof. Ioan Athanasiu înființează o revistă ştiințifică, în limba franceză, "Archives de Biologie", fiind a doua mare revistă medicală românească în mişcarea ştiințifică internațională. În 1906 fondează, alături de C. Stere şi G. Ibrăileanu, revista "Viața românească".

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[1] Dr. Dorina N.Rusu, *Membrii Academiei Române 1866-1996. Mic dicţionar*, Fundaţia Academică "Petre Andrei", Ed. A 92, Iaşi, 1996, pg.126.

Sub directoratul sanitar al lui Ioan Cantacuzino apare în anul 1910 „Legea sanitară“, care instituie Consiliul sanitar superior – organ tehnic consultativ central şi consilii de igienă municipale şi județene. Pentru o populație medie de 15 000 locuitori se constituie o circumscripție sanitară, iar instituțiile sanitare de bază pentru mediul rural devin dispensarele medicale. La nivel central, în cadrul Direcției generale a serviciului sanitar se vor organiza servicii de epidemii, statistică sanitară, igienă şi sănătate publică.

Organizarea asistenței medico-sanitare în perioada dintre cele două războaie mondiale era reglementată, în principal, prin Legea sanitară şi de ocrotire socială nr. 236/1930, potrivit căreia:

- serviciile medico-sanitare erau conduse după norme stabilite de Ministerul Muncii, Sănătății şi Ocrotirii Sociale, indiferent de autoritatea de care depindeau din punct de vedere financiar;
- profesia de medic se exercita pe baza autorizației acordate de minister, publicată în Monitorul Oficial, sub condiția înscrierii persoanei ca membru în Colegiul Medicilor din România;
- resursele financiare se colectau în Fondul Sanitar de Ocrotire şi se gestionau sub administrarea unei regii autonome. Ele erau formate din:
  - venituri proprii (venituri din loterii şi jocuri de noroc, venituri din regie, 70% din taxele pentru autoritatea vânzării produselor medicamentoase străine şi indigene, 90% din veniturile nete ale monopolului stupefiantelor, taxele din îngrijire ale spitalelor, veniturile întreprinderilor autorizate de lege, donații etc.);
  - contribuții din partea statului.

Fondul sanitar era utilizat pentru:

a) asistența medico-sanitară a cetățenilor români, dovediți a fi săraci, cheltuieli de îngrijire şi costul medicamentelor pentru bolnavii de boli contagioase şi venerice, pentru îngrijirea unor categorii defavorizate din personalul medico-sanitar şi membrii lor de familie;

b) lucrări de investiții medico-sanitare şi de ocrotiri sociale prin subvenționarea județelor, comunelor, instituțiilor publice sau particulare medico-sanitare sau de ocrotire socială ale căror mijloace materiale nu erau suficiente;

c) credite, acordate instituțiilor publice medico-sanitare sau de asistență şi ocrotire socială pentru executarea de lucrări edilitare sau de salubritate;

Administrarea fondurilor se făcea de către Regia Autonomă a Fondului Sanitar şi prin fondurile județene, comunale, orăşeneşti şi municipale;

Eforii medico-sanitare[1] funcționau în oraşele reşedință de județ şi municipii, sub autorizarea şi controlul ministerului, pentru sprijinirea, dezvoltarea şi administrarea aşezămintelor şi operelor medico-sanitare şi de ocrotire locale, având dreptul de a primi donații pentru sănătatea publică şi de a administra fondurile puse la dispoziția lor.

Lipsa fondurilor necesare funcționării sistemului a obligat Guvernul să elaboreze, în anul 1933, „Legea asigurărilor sociale”. Ea a modificat vechea lege din anul 1912 şi a dus la reorganizarea serviciului medico-sanitar.

În România a funcționat un sistem de asigurări sociale care includea şi asigurarea de boală (modelul „Bismark” – Germania), reglementat de Codul Asigurărilor Sociale şi de Legea Asigurărilor Sociale (Monitorul Oficial nr. 298/22.10.1938).

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[1] Instituție administrativă de utilizare publică în domeniul medical.

Câmpul asigurării: persoanele asigurate obligatoriu erau acelea care aveau salariul de 8 000 lei/lună (curs 1938) şi persoanele asigurate pe baza unor legi speciale (salariaţii instituţiilor publice, precum şi pensionarii şi salariaţii agricoli).

Dreptul de asigurare consta în: îngrijire medico-sanitară, medicamente, pansamente şi accesorii medico-sanitare, tratament balneo-climateric, ajutoare băneşti de boală, accidente, pensii de bătrâneţe, pensii de urmaş.

Finanţarea se făcea prin Fondul General şi Fondurile judeţene ale asigurărilor sociale.

Sursele formării fondurilor de asigurare erau cotizaţiile salariaţilor, contribuţia patronală, contribuţia statului şi alte venituri.

Cotizaţiile se stabileau pe clase de salarizare şi reprezentau 8% din salariul mediu al clasei respective, salariaţii plăteau aceste cotizaţii împreună cu patronii lor în părţi egale. Patronii erau obligaţi ca, la întocmirea listelor de plată, să verse cotizaţiile şi contribuţiile aferente şi să aplice timbrele în carnetele de asigurare. Statul contribuia cu o sumă globală de 80 de milioane lei anual. În plus, patronii plăteau 1,2%, calculat la totalul salariilor, pentru asigurarea de accidente şi completarea fondului de rezervă. Alte venituri rezultau din costul carnetelor de asigurare, taxa pentru viza anuală, donaţii, amenzi, dobânzi, suprataxe etc. Gestionarea fondului general al asigurărilor sociale se făcea astfel: 60% pentru fondul caselor de asigurări sociale, 5% pentru fondul administraţiei casei centrale, 35% pentru pensii. Fondul caselor de asigurări sociale se administra în proporţie de 75% direct de casele de asigurări sociale, iar 25% se administra de către Casa Centrală de Asigurări Sociale, pentru ajutorarea acelora care nu puteau să-şi achite obligaţiile legale.

Organizarea administrativă cuprindea circumscripţii cu cel puţin 15 000 asiguraţi şi oficii pentru aproximativ 500 de asiguraţi.

Se poate concluziona că, în România, între cele două războaie mondiale, a existat un sistem legislativ elaborat foarte meticulos, ce permitea funcţionarea unui sistem de sănătate pentru populaţie. Acest sistem legislativ era bine armonizat cu celelalte sisteme juridice, permiţând o bună gestiune a banului; la vremea respectivă el era un sistem modern, recunoscut pe plan european.

Sănătatea publică, ca disciplină de învăţământ, a apărut în România în urmă cu aproximativ 50 de ani, la Bucureşti şi Cluj, respectiv la sfârşitul anului 1953.

Şcoala bucureşteană a dezvoltat aspecte teoretice, conceptuale, în timp ce şcoala clujeană a dezvoltat partea metodologică. O personalitate a şcolii bucureştene de medicină socială a fost Prof. Th. Ilea.

Sistemul serviciilor de sănătate din România de până în 1989, care nu a suferit nici după 1990 modificări substanţiale, a fost inspirat la apariţia sa (la sfârşitul anilor '40 şi în anii '50) din modelul sovietic (Semaşko), având însă similitudini şi cu sistemele existente în unele ţări din Europa de Vest - Marea Britanie, ţările nordice.

Principiile sistemului, multe dintre ele concordante cu recomandările făcute de Organizaţia Mondială a Sănătăţii, în anii '70 aveau la bază caracterul de stat, profilaxia, unitatea de conducere a întregului sistem, planificarea, gratuitatea şi accesibilitatea largă la asistenţă medicală, caracterul ştiinţific al politicii sanitare şi participarea conştientă a populaţiei la apărarea propriei sănătăţi.

Dacă multe din aceste principii sunt greu de criticat, unele din ele au fost folosite ca lozinci, aplicarea lor în practică devenind din ce în ce mai dificilă şi limitată la nivel declarativ.

În primii ani după instituirea puterii comuniste, accentul cade pe medicina primară, politicile vizând creşterea accesibilităţii populaţiei rurale la serviciile sanitare. Medicii sunt obligaţi să efectueze un stagiu de câtiva ani „la ţară”, astfel încât tot teritoriul este acoperit în ceea ce priveşte serviciile primare. Pe lângă acordarea asistenţei sanitare, medicii sunt obligaţi să îndeplinească şi servicii de medicină preventivă (vaccinări, supraveghere epidemiologică, supravegherea naşterilor, depistarea bolilor transmisibile, educaţia sanitară).

Eficacitatea sistemului în perioada iniţială a fost dată de faptul că România ieşise dintr-o criză profundă, generată de război, secetă, ocupaţia sovietică şi schimbările de după 1948, astfel încât putea mobiliza mai multe resurse în domeniu.

Eficienţa a fost strâns legată de capacitatea autorităţilor de a impune medicilor şi întregului personal sanitar, dar şi autorităţilor locale sarcini şi obiective precise.

Investițiile în fabrici care furnizează medicamente ieftine, în construcția unor instituții sanitare şi formarea personalului se fac simțite: speranța de viață creşte permanent, ajungând în jur de 70 de ani în 1975.

Este "*perioada de aur a sistemului sanitar de tip Semaşko*" în opinia specialiştilor sociologi care au cercetat domeniul[1].

Serviciile de sănătate constituie doar un subsistem al sistemului de sănătate şi se referă la unitățile şi prestațiile preponderent medicale pe care le produc aceste unități.

În esență, sistemul serviciilor de sănătate a fost şi este un sistem organizat şi finanțat de către stat, fiind alcătuit dintr-o rețea de unități sanitare proprietate a statului, administrate într-o structură centralizată, având la vârf Ministerul Sănătății. Finanțarea se face din impozite şi taxe generale prin bugetul de stat, iar medicii sunt salariați ai statului.

Examinarea critică a finanțării, organizării şi funcționării sistemului serviciilor de sănătate scoate în evidență, pe lângă neajunsurile sale, şi o serie de avantaje. O asemenea perspectivă este extrem de importantă pentru ceea ce se doreşte a fi în viitor asistența medicală şi ea a apărut în ultima vreme tot mai evidentă în lucrările de specialitate elaborate mai ales în mediile academice, ale experților şi consultanților occidentali, chemați să propună soluții de schimbare.

Dintr-un asemenea unghi de vedere, sistemul care a funcționat în România a avut şi o serie de elemente pozitive care au fost mai evidente cu deosebire în perioadele de creştere economică rapidă.

Sistemul a fost dezvoltat pe principii de echitate, asigurând accesul la servicii de sănătate cuprinzătoare pentru cvasitotalitatea populației. Accesul la servicii nu a fost influențat de capacitatea de a plăti a pacientului. Avantajul absenței plății în momentul utilizării s-a erodat însă pe măsura ce veniturile profesiunii medicale au devenit (şi mai ales au fost percepute) tot mai necorespunzătoare şi s-a ajuns ca sumele pretinse sau primite suplimentar de la pacienți de către personalul sanitar să fie considerate socialmente acceptabile sau chiar îndreptățite.

Finanțarea şi organizarea sistemului au permis un control eficace al costurilor. Proporția cheltuielilor pentru sănătate din PIB a fost relativ scăzută. "Prețul" unei asemenea limitări a fost şi lipsa sau limitarea accesului la unele servicii, în special cele necesitând cheltuieli mari pentru echipamente sau materiale (de exemplu, dializa renală, chirurgie cardiovasculară, transplante, proteze articulare, tomografie computerizată). Sistemul a funcționat cu structuri şi costuri administrative reduse.

Infrastructura serviciilor a fost dezvoltată extensiv, existând o rețea integrată de dispensare (teritoriale şi de întreprindere), policlinici şi spitale. Spre deosebire de alte foste țări socialiste, rețeaua de asistență medicală primară (dispensarele de medicină generală) a fost dezvoltată din punct de vedere organizatoric, dar neglijată ca dotare. Aici au fost cele mai evidente şi inegalitățile în privința asigurării personalului medical, legate de disparități de dezvoltare economică între județe, mediu urban/rural etc., în condițiile includerii dispensarelor în structura organizatorica a spitalelor, competiția pentru resurse a dat în general câştig de cauză serviciilor de specialitate, în detrimentul medicinii generale. Aceasta a avut un efect defavorabil asupra eficienței sistemului, cazurile care ar fi putut fi rezolvate la nivel primar încărcând spitalele şi consumând resurse scumpe.

Asigurarea asistenței medicale a populației cu paturi a fost bună, chiar supradimensionată față de nevoile reale, de posibilitățile acoperirii cheltuielilor curente şi față de densitatea paturilor existentă în țările occidentale. Spitalele consumă peste 70% din bugetul acordat ocrotirii sănătății şi rezolvă cca. 20% din cererile sanitare ale populației.

A existat suficient personal medical calificat şi un învățământ medical de calitate. Şi în acest domeniu, dacă se are în vedere densitatea medicilor raportată la PIB, România are o poziție convenabilă. Posibilitatea ingerințelor politicului a dus însă la disfuncții legate de formarea personalului în special în anii '80 (sistarea pregătirii medicilor specialişti şi a accesului acestora în oraşe, desființarea pregătirii postliceale pentru cadre medii).

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[1] Mihai Păunescu şi colectiv, *Management public în România*, Editura Polirom, Iaşi, 2008, pg.186.

Până la jumătatea anilor '70 s-au înregistrat ameliorări evidente ale nivelului stării de sănătate, ilustrate prin creşterea duratei medii de viaţă, eradicarea sau reducerea multor boli transmisibile, reducerea mortalității infantile, inițierea unor programe naționale în domeniile bolilor cardiovasculare, cancerului, tuberculozei, sănătății mentale şi sănătății dentare. La aceste îmbunătățiri ale sănătății au contribuit în mare măsură şi serviciile medicale.

În ciuda acestor avantaje, sistemul serviciilor de sănătate a avut şi o seamă de ***aspecte negative***, care începând cu anii '80 au devenit din ce în ce mai vizibile, deteriorându-se atât starea de sănătate a populației, precum şi asistența medicală.

Unele din caracteristicile sistemului sanitar au devenit după revoluție discordante față de cerințele dezvoltării unei societăți democratice, ale unui alt tip de stat şi concomitent cu trecerea la economia de piață, în perioada de tranziție, datorită specificului acesteia, o serie de aspecte negative au devenit acute.

Sistemul de sănătate care a funcţionat şi care este în continuare dominant în România are, la o examinare atentă, o serie de slăbiciuni si dezavantaje. Probabil că principala limită conceptuală a fost neînțelegerea faptului că sănătatea este o "afacere a întregii societăți", care are drept consecință încorporarea ei ca o țintă sau ca o variabilă în planurile de dezvoltare economică şi socială. De aici a decurs a doua consecință nefastă şi anume creditarea serviciilor sanitare cu responsabilitatea şi putința ameliorării stării de sănătate a populației, când partea imputabilă acestui sector nu reprezintă nicăieri mai mult de 10-15%. Urmarea firească a constituit-o culpabilizarea medicilor şi a sectorului sanitar pentru toate insatisfacţiile generate de deteriorarea stării de sănătate care era preponderent rezultanta acţiunii conjugate a altor factori (social-economici, comportamentali, biologici, ambientali, ş.a.m.d.).

- Medicalizarea excesivă a sănătății, influențată şi de modul de organizare şi conducere a serviciilor, marea majoritate a posturilor de conducere fiind ocupate de persoane fără nici o pregătire managerială, a redus mult şansele unor abordări intersectoriale, care să țină seama de toți factorii cu influență asupra sănătății.
- Planificarea rigidă, la care s-a adăugat sistemul centralizat şi de comandă au sufocat inițiativele şi responsabilitatea în gestiunea serviciilor de sănătate şi mai ales în adaptarea lor la nevoile sanitare în schimbare. Modelul centralizat, impunând pe cale administrativă norme şi standarde rigide, de multe ori arbitrare, a împiedicat adaptarea serviciilor la nevoile locale ale populației, diferite de la o zonă la alta. Aceste norme au dus la un amestec ineficient de abundență şi risipă în unele regiuni şi de lipsuri în altele.
- Planificarea de tip normativ a fost ineficace şi ineficientă. Deşi s-a încercat cu obstinație să se reducă prin metode administrative dezechilibrele prin uniformizarea distribuirii personalului şi a infrastructurii sanitare, rezultatul a fost un eşec. Se poate constata şi astăzi că sistemul nu a reuşit să reducă inechitatea teritorială, nici în nivelul stării de sănătate, nici în asigurarea populației cu medici şi nici în cea cu paturi. Finanțarea după criterii istorice, netinând seama de nevoile sanitare diferite ale populației, a accentuat disparitățile dintre județe.
- Subfinanțarea serviciilor a determinat întârzieri considerabile în introducerea tehnologiilor noi necesare diagnosticului sau tratamentului patologiei actuale, generând ulterior carenţe grave în asigurarea cu medicamente şi materiale sanitare de uz curent. Toate acestea au descurajat preocupările serviciilor de sănătate în domeniul asigurării şi evaluării calității îngrijirilor medicale, element fundamental al performanței sistemului.

INSTRUCȚIUNI PENTRU AUTORI

Revista de Medicină Militară este o publicație de prestigiu, cu o vechime de peste 100 de ani. În scopul integrării revistei în circuitul internațional al publicațiilor științifice, autorii sunt rugați să respecte *Cerințele uniforme pentru manuscrisele trimise jurnalelor biomedicale* (stilul Vancouver de prezentare) (vezi: *New England Journal of Medicine* 1991, 324 : 424 – 428; *British Medical Journal*, 1991, 302; *Romanian Journal of Gastroenterology* 1992, 1/4, 1996, 5/1).

Revista de Medicină Militară este creditată de Colegiul Medicilor din România ca publicație științifică (2,5 credite pentru abonament), creditarea autorilor făcându-se la solicitarea acestora după prezentarea reprintului obținut ca dovadă a editării lucrării (solicitările vor fi adresate personal de autori Colegiului Medicilor din România – Bulevardul Timişoara nr. 15, sector 6, Bucureşti)

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Studiile pe pacienți umani sau studiile experimentale trebuie să respecte reglementările internaționale de etică. Sursele de finanțare sau suportul financiar se vor menționa cu mulțumirile de rigoare.

În caz de litigiu este util ca autorii să menționeze numele şi adresa a *doi experți* pe care îi recomandă pentru a evalua manuscrisul respectiv.

Lucrările vor fi examinate de către doi revizori de specialitate din comitetul editorial. Dacă opiniile lor diferă, articolul este analizat de un alt revizor. Evaluarea articolelor se face fără ca revizorii să cunoască numele autorilor lucrării.

Comitetul editorial poate solicita autorilor să modifice manuscrisul, în funcție de recomandările revizorilor. Comitetul editorial are dreptul să editeze (modifice) manuscrisele.

Autorii poartă întreaga responsabilitate pentru conținutul ştiințific al lucrărilor lor.

Articolele neconforme cu stilul revistei sunt returnate autorilor fără a fi analizate de comitetul editorial.

Timpul de editare va fi cât mai scurt posibil, în funcție de necesitățile ştiințifice şi editoriale ale revistei.

Manuscrisele trimise spre publicare nu pot fi returnate. Când se primeşte un manuscris, autorii vor fi informați în scris, într-un interval de cinci-şase săptămâni, cu privire la acceptarea, nevoia de revizuire sau respingerea articolului.

După publicare materialele devin proprietatea revistei.

După publicare, primul autor va primi zece reprinturi ale articolului, în mod gratuit, pentru a fi distribuite celorlalți autori.

FORMATUL MANUSCRISULUI

Manuscrisul trebuie redactat în conformitate cu *Cerințele uniforme pentru manuscrisele trimise jurnalelor biomedicale* (stilul Vancouver de prezentare) (vezi *New England Journal of Medicine* 1991, 324 : 424 – 428; *British Medical Journal,* 1991, 302; *Romanian Journal of Gastroenterology* 1992,1/4, 1996, 5/1).

Articolele se redactează în Microsoft Word, Normal+Justf., Times New Roman, Font Size – 16 Bold pentru titlu, Font Size – 12 pentru text, iar rezumatul Font Size – 10 Bold.

Pagina trebuie setată din Page setup la 2cm marginile, format letter.

Autorii sunt rugaţi să trimită articolul pe dischetă şi două exemplare listate de manuscris (inclusiv pentru tabele şi figuri), *rezumatul fiind scris atât în limba română, cât şi în engleză*, pe adresa Redacţiei, tipărite pe o singură faţă a hârtiei A4, la un rând (inclusiv pentru tabele şi referinţe bibliografice), cu margini largi. Pentru textul editat în limba română vă rugăm utilizaţi setarea de limbă („*Romanian”*) pentru tastatură (în „*Control Panel* ”din *Windows*) şi să nu utilizaţi alte tipuri de caractere româneşti. **Nu trimiteţi manuscrisul în limba română fără caracterele specifice româneşti** (diacritice). Toate paginile trebuie numerotate consecutiv, începând cu pagina de titlu, jos pe centrul paginii.

MANUSCRISUL VA FI PREGĂTIT ASTFEL:

1. PAGINĂ DE TITLU

- Titlul lucrării (nu mai mult de 100 de caractere cu spaţii), fără abrevieri;
- Prenumele, iniţiala celui de al doilea prenume, numele de familie al autorilor
- Numele complet al locului de muncă (instituţie şi departament) pentru fiecare autor;
- Adresa de corespondenţă (cu detalii: grade ştiinţifice didactice şi militare, persoana de legătură, telefon, fax, e-mail etc.)

2. PAGINĂ DE REZUMAT

- Rezumatul trebuie să aibă 150, până la 250 de cuvinte şi să cuprindă: obiectivul studiului, material şi metodă, rezultatele fără interpretarea datelor şi concluzii.
- Cuvintele-cheie: 3-4 cuvinte-cheie, după *Index Medicus.*

3. ARTICOLUL PROPRIU-ZIS

a. **Articol original** – cel mult 800-1 000 de cuvinte (4 pagini).

b. **Prezentare caz** – cel mult 600-800 de cuvinte (2 pagini).

c. **Referat general** – cel mult 2 000 de cuvinte (10 pagini).

- **INTRODUCERE**: prezentarea problemei, plasarea în context, scopul şi obiectivele studiului, ipoteza de lucru.
- **MATERIALE ŞI METODE**: pacienţi, procedee, echipament (inclusiv indicaţii cu privire la producător), metoda de lucru, analiză statistică, tehnici operatorii, medicamente etc. (în funcţie de specificul articolului), toate acestea fiind descrise în detalii suficient de amănunţite pentru a permite reproducerea studiului de către alţi investigatori. În tot textul trebuie folosite aceleaşi nume pentru produse (cu specificarea numelui de marcă între paranteze la prima utilizare). Nu se recomandă numele pacienţilor.
- **REZULTATE**: fără interpretări.
- **DISCUŢII**:

1 – analiza rezultatelor prin prisma obiectivelor propuse ale studiului cu comentarii cu privire la aspectele lămurite (pozitive sau negative) sau nelămurite prin acest studiu;

2 – comparaţie între rezultatele obţinute de autori şi alte studii din literatură, cu explicaţii, eventual ipoteze cu privire la diferenţele observate, accentuând asupra progresului pe care cercetarea de faţă îl aduce;

3 – comentarii privind importanţa studiului de faţă, precum şi o punere la punct a situaţiei actuale a subiectului investigat atât în literatură, cât şi în practică; eventual se vor prezenta aici cele mai importante studii sau aspecte din domeniu;

4 – probleme nerezolvate, întrebări la care nu s-a răspuns şi care rămân deschise în viitor pentru studiile ce vor urma, eventuale direcţii propuse pentru investigare în domeniul respectiv.

CONCLUZII

La nevoie, capitolele mari pot fi împărţite în subcapitole, după necesităţi.

Se recomandă utilizarea precaută a *abrevierilor* şi menţionarea numelui complet la prima apariţie în text a abrevierii respective. Toate referinţele bibliografice vor fi citate în text cu cifre arabe, la exponent, fără paranteze, în ordinea apariţiei (de exemplu: Un alt studiu[18] arată...). Toate figurile şi tabelele vor fi citate în text în locul în care ele urmează să apară (de exemplu: *Fig. 3*, *Tab. 2*). Datele care apar în tabele, legendele tabelelor şi figurilor trebuie să fie rezumate, nu duplicate în text.

4. TABELELE

Tabelele trebuie să fie procesate la două rânduri, pe o pagină separată, *numerotate* consecutiv (cu referire în text în locul unde ele trebuie să apară) şi *cu un titlu* (exemplu: *Tabelul 1*. Distribuţia pe vârste şi sex a pacienţilor). Capetele de tabel se scriu cu caractere *italice.* Nu trimiteţi fotografii sau imagini ale tabelelor. Utilizaţi tasta „Tab” pentru a separa coloanele (nu bara de spaţiu) şi utilizaţi tasta „Enter” la terminarea liniei. Toate căsuţele goale din tabel trebuie să aibă o liniuţă. Dacă este nevoie, pentru cuvintele sau frazele lungi din căsuţe se vor utiliza abrevieri, însă cu explicarea acestora în subsolul tabelului. Asiguraţi-vă că toate tabelele sunt citate în text.

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Toate figurile (ilustraţiile), fie fotografii, diagrame sau grafice, trebuie să fie de calitate. Ele se *numerotează* în ordinea apariţiei în text (cu referire în text la numărul figurii în locul în care aceasta ar trebui să apară) şi *vor purta un titlu* (de exemplu: *Figura 1***.** Distribuţia pe vârste şi sexe a pacienţilor).

Pe această pagină referitoare la figuri, se va face o *listă* a tuturor numelor figurilor, în ordine numerică (la nevoie, cu legende explicative; identificaţi, în această legendă, orice literă sau săgeată utilizată pe figură).

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1. Blann D.W., Prien S. D.: *Estemation of fetal....*, Am J of Obst and Gynec, 2000, 182 (5): 1117-1120.

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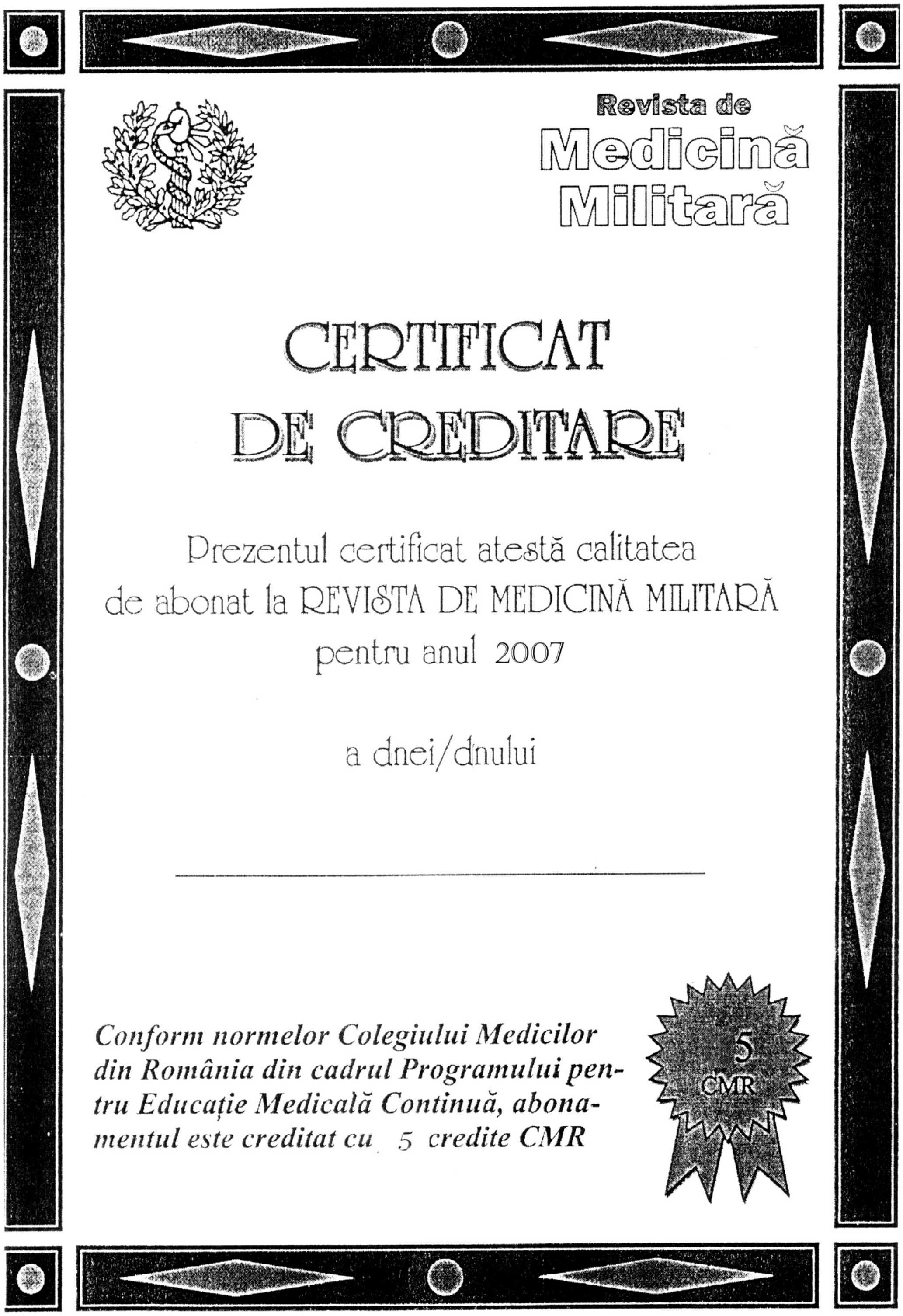

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DIN ROMÂNIA

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Dreptatea, bunul simț, înțelepciunea, maturitatea şi înțelegerea dezideratelor etapei în care ne aflăm...

Acestea ar fi, în linii mari, principiile care i-au direcționat activitatea noului şef al Direcției Medicale a Ministerului Apărării Naționale, Generalul maior medic profesor universitar doctor Ioan Sîrbu. Un profesionist de excepție dublat de un minuțios artizan al actului de conducere, fapt dovedit de-a lungul celor cinci ani cât s-a aflat la comanda Spitalului Universitar de Urgență Militar Central „Dr. Carol Davila”.

Şi-a dorit cu ardoare să practice actul medical. A absolvit Institutul Medico-Militar, Facultatea de stomatologie, din cadrul Universității de Medicină şi Farmacie „Carol Davila” Bucureşti, promoția 1995, fiind declarat şef de promoție pe țară cu media generală de absolvire 10. Din clipa în care a optat pentru o carieră medicală, doctorul Sîrbu şi-a dedicat întreaga existență performanței profesionale. A obținut rând pe rând titlurile de medic specialist, medic primar, doctor în ştiințe medicale, membru al unor societăți internaționale de profil; concomitent a desfăşurat o amplă activitate ştiințifică şi publicistică - comunicări, lucrări şi articole ştiințifice în reviste de specialitate-, parcurgând treptele didactice de la simplu stomatolog la şef de lucrări, la conferențiar şi la profesor universitar în cadrul Catedrei de Implantologie Orală a Facultății de Stomatologie a U.M.F. din Bucureşti.

Încă din anul 1997 a fost cooptat în cadrul Academiei Americane de Implantologie Orală, iar din 1998 devine membru al Comitetului Internațional de Implantologie Orală. Din anul 2000 încoace, în fiecare an, la lucrările Academiei Americane de Implantologie Orală din SUA participă şi medicul doctor Ioan Sîrbu, specializat la New York în clinica profesorului doctor Leonard Linkow, unul dintre părinții implantologiei mondiale. Este incontestabil faptul că în acest uriaş efort de înlocuire a protezelor clasice dentare cu implanturi, Şcoala de Implantologie Orală de la Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila” s-a înscris prin activitatea medicului doctor Ioan Sîrbu ca un adevărat pionier al introducerii în sistemul de sănătate din țara noastră a unei ramuri ştiințifice moderne, implantologia orală, născută şi dezvoltată peste ocean, în Statele Unite ale Americii. Această nouă disciplină a înlăturat toate inconvenientele generate de tehnici care schimbau fizionomia pacientului şi produceau un disconfort extrem de mare, redând oamenilor satisfacția pe care au avut-o cândva cu dinții lor naturali.

Aşa cum aprecia profesorul doctor Leonard Linkow de la Clinica de Implantologie Orală – Universitatea New York – „doctorul Sîrbu a depăşit numărul de 10 000 de implanturi şi poate fi considerat numărul unu în implantologia din România, motiv pentru care este solicitat anual de înaltul for academic din SUA să se alăture colegilor de breaslă americani în eforturile lor de cercetare într-un domeniu de strictă specialitate”.

Timp de peste cinci ani, Generalul de brigadă medic profesor universitar doctor

Ioan Sîrbu s-a aflat la comanda Spitalului de Urgență Militar Central „Dr. Carol Davila". Rezultatele spectaculoase obținute în activitatea de comandă a acestei instituții de elită a medicinii militare româneşti îl atestă ca pe un lider cu calități manageriale de excepție. Preluarea comenzii a avut loc într-o perioadă când la orizont se prefigurau obstacole dintre cele mai mari legate de accelerarea transformării structurale a armatei: reducerea şi reîmprospătarea personalului, aplicarea legislației sanitare şi promovarea cadrelor tinere, îmbunătățirea managementului resurselor umane, profesionalizarea şi supraspecializarea tinerilor medici, reconfigurarea priorităților impuse de unele standarde euroatlantice.

A fost nevoie de multă energie, inițiativă, răbdare şi căutări pentru a învinge obiceiurile păguboase în utilizarea fondurilor sau căderile în capcanele delăsării în perfecționarea pregătirii profesionale. Preceptele care l-au condus în această perioadă de încercări dure pe comandantul spitalului, Generalul de brigadă medic prof. univ. dr. Ioan Sîrbu, au stat şi la baza inițiativei de a transforma Spitalul de Urgență Militar Central din spital clinic în spital UNIVERSITAR, în accepțiunea sa aceasta fiind singura denumire care i se potriveşte prestigioasei unități sanitare de învățământ medico-militar.

Astăzi Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila" se află înscris definitiv şi ireversibil pe drumul transformării sale într-o unitate medicală modernă a mileniului trei, în care se tratează, prin internare sau ambulatoriu, peste 200 de mii de pacienți şi în care se pregătesc, anual, în medie, circa 600-700 studenți medicinişti şi îşi desăvârşesc pregătirea peste 400 de medici rezidenți militari şi civili. Cadrele tinere au preluat conducerea secțiilor şi clinicilor de specialitate, dovedind că sunt apte să le asigure managementul de performanță, trimiterile la cursurile de specializare şi supraspecializare s-au triplat, pavilioanele s-au reînnoit, s-au înlocuit tehnica şi aparatura veche, spațiile de cazare a bolnavilor seamănă tot mai mult cu cele europene moderne, ambulatoriul de specialitate a devenit multifuncțional, logistica unității se apropie tot mai mult de standardele euroatlantice. De asemenea, Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila" este una dintre bazele clinice cele mai importante ale Universității de Medicină şi Farmacie „Carol Davila" din Bucureşti, având şapte clinici universitare iar impactul mediatic reflectă creşterea continuă a calității asistenței medicale acordate.

Referindu-ne la atitudinea novatoare a Generalului maior medic prof. dr. Ioan Sîrbu, nu putem să nu amintim, în cadrul proiectelor de viitor, preocuparea sa permanentă de a pune la dispoziția colegilor săi o bază modernă de tratament în care aceştia să-şi poată exercita actul medical cu rezultate performante. Repetatele sale propuneri către forurile diriguitoare din Ministerul Apărării Naționale, Ministerul Finanțelor şi cel al Sănătății au găsit un răspuns pozitiv: unul dintre cele 8 spitale de urgență, care vor fi finanțate din programul special al Guvernului, va deveni noul Spital Universitar de Urgență Militar Central „Dr. Carol Davila". În mod cert noul sediu al spitalului, dotat corespunzător, va duce la creşterea performanței şi la diversificarea serviciilor medicale furnizate pacienților.

Acestea sunt doar câteva dintre realizările importante înregistrate de Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila" sub conducerea Generalului maior medic prof. univ. dr. Ioan Sîrbu. Obiectivele pe care şi le-a propus şi îndeplinit în perioada când a fost numit în funcția de comandant al acestei unități de elită a medicinii militare îl definesc ca pe un lider care posedă acele calități indispensabile care îi permit să atragă şi să-i mobilizeze şi pe alții pentru a îndeplini sarcini pe care nici nu au visat că le pot înfăptui. Suntem convinşi că numirea la conducerea Direcției Medicale a Ministerului Apărării Naționale va constitui pentru Generalul maior medic profesor universitar doctor Ioan Sîrbu o nouă provocare, un imbold de a contribui la ridicarea standardelor medicinii militare româneşti la nivel european şi mondial. Aşa cum îl cunoaştem, nu ne îndoim nicio clipă că o va face! Îi urăm şi pe această cale mult succes în nobila sa misiune!

Redacția

Tumorile articulației temporomandibulare. Considerații clinice şi imagistice

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Spitalul Militar Clinic de Urgență Iași

Rezumat. Articulația temporomandibulară (ATM) este cea mai complexă articulație a organismului uman. Patologia sa este vastă şi cuprinde aspecte etiopatogenice, traumatologice, inflamatorii acute sau cronice şi tumorale, exprimate sub un larg ansamblu de simptome şi semne clinice. Patologia tumorală a ATM este extrem de rară. Cazurile din studiu sunt reprezentate de osteom şi hiperplazie de condil. În patologia tumorală, CT helical unislice are capacitatea de a furniza detaliile necesare tratamentului chirurgical, folosind şi avantajele reconstrucției 3D. În acest context explorarea imagistică a articulației este de o importanță deosebită pentru precizarea diagnosticului şi a conduitei terapeutice.

Cuvinte cheie: articulație temporomandibulară, tumori, CT helical unislice

Abstract. Temporomandibular joint is the most comprehensive articulation of the human body. Pathology is extensive and includes trauma, acute or chronic inflammation and tumor, expressed as a broad set of clinical signs and symptoms. Tumors of the ATM are extremely rare. Cases in this study are osteoma and hyperplasia of condil. In tumor pathology, unislice helical CT has the ability to provide the details necessary surgical treatment, and benefits of using 3D reconstruction. In this context the joint exploration is of particular importance to specify the diagnosis and therapeutic conduct.

Introducere

Articulația temporomandibulară este una dintre componentele de bază ale sistemului stomatognat, contribuind la funcțiile de bază ale acestuia: masticație, deglutiție, fonație. Patologia sa este vastă şi cuprinde aspecte etiopatogenice, traumatologice, inflamatorii acute sau cronice şi tumorale exprimate sub un larg ansamblu de simptome şi semne clinice. În acest context explorarea imagistică a articulației este de o importanță deosebită pentru precizarea diagnosticului şi a conduitei terapeutice.

Material şi metodă

Examinarea radiografică a articulației temporomandibulare a fost efectuată cu un aparat de tip Telediagnost PHILIPS (2000).

În cadrul studiului de față examinarea pacienților a fost realizată cu un computertomograf GE-HiSpeed Dx/I helical.

Poziția pacientului este în decubit dorsal, cu mâinile pe lângă corp, capul în hiperextensie, fixat în tetiera special destinată studiului, bine imobilizat. Topograma stabileşte limitele anatomice ale studiului: de la cavitatea glenoidă a osului temporal până la incizura sigmoidiană a mandibulei. S-au practicat două studii axiale, fără contrast, în pozițiile „gură închisă" şi „gură deschisă" (secțiuni fine, 1-2 mm, alăturate, achiziționate cu tehnică „rezoluție înaltă"). Examinarea nativă convențională a articulației temporomandibulare se face [1] prin secțiuni axiale (transversale) de 3 mm grosime cu interval 0, de obicei 8-10 secțiuni paralele cu planul orbitomeatal (ce uneşte conductul auditiv extern cu unghiul extern al orbitei - punctul orbital inferior), în cele două poziții cu gura deschisă şi gura închisă. Studiul imaginilor CT l-am efectuat pe „fereastra de os" şi „ferestre de părți moi".

Imaginile pe care le-am obținut cu un aparat helical unislice permit identificarea reperelor anatomice osoase (figura 1).

Pentru examenul coronal direct poziția pacientului este în decubit dorsal sau ventral cu capul în hiperextensie, bine imobilizat pe tot parcursul examinării. Topograma este laterală. Prin înclinarea stativului aparatului se realizează secțiunile coronale şi se evită cuprinderea în planul de secțiune a lucrărilor dentare metalice, generatoare de artefacte. Secțiunile coronale (frontale) de 3 mm cu pas 0 se obțin când pacientul are capul în hiperextensie, ca pentru incidența Hirtz, ceea ce necesită o bună mobilitate a coloanei cervicale, cupele fiind realizate într-un plan perpendicular pe planul orbitomeatal.

Am practicat două studii coronale, fără contrast, în pozițiile „gură închisă" şi „gură deschisă" (figurile 2, 3).

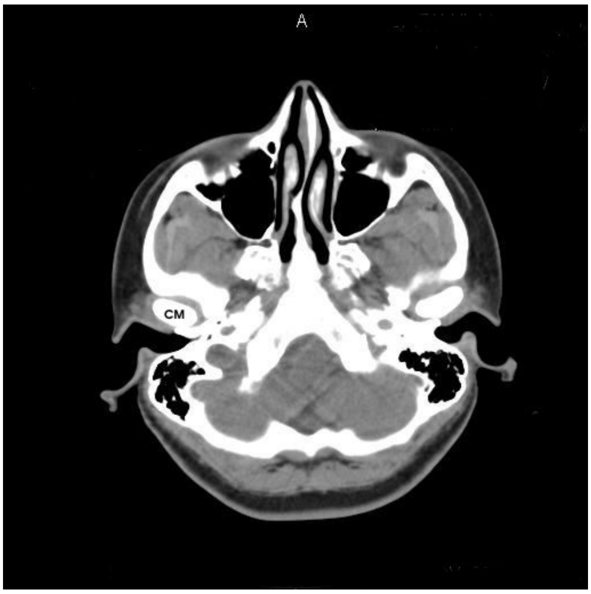

Fig. 1. Imagine CT axială nativă normală la nivelul condililor mandibulari (CM), în fereastră de os, poziția „gură închisă*"*.

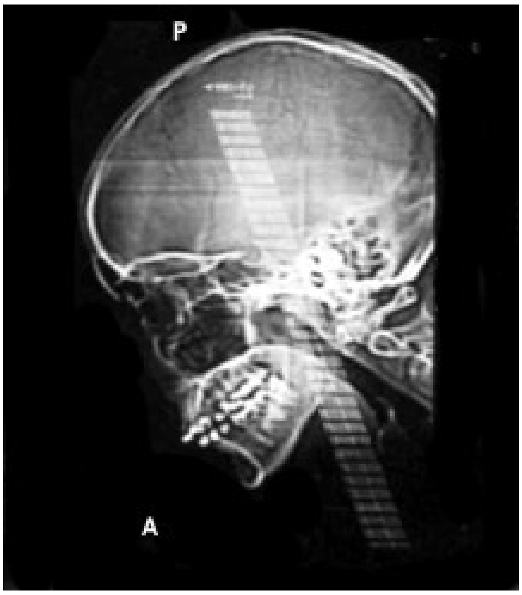

Fig. 2. Topograma laterală cu alegerea planului de referință.

A fost posibilă şi efectuarea unui studiu sagital direct al articulației. Pentru aceasta poziția pacientului se stabileşte în decubit lateral pe partea bolnavă cu capul ridicat la 20 de grade şi stativul aparatului înclinat în sens cranial cu 20 de grade. Examinările prin secțiuni sagitale sunt cele mai importante dar şi cel mai dificil de obținut, grosimea fiind de 3 mm cu pas 0, pacientul în decubit lateral cu capul înclinat sub un unghi de 20-25° timp de 10-15 minute fără să se mişte. Am realizat 10-20 de secțiuni cu gura închisă apoi cu gura deschisă, pentru fiecare articulație temporomandibulară în parte [1]. Am efectuat două studii sagitale, fără contrast, în pozițiile „gură închisă" şi „gură deschisă" (figurile 4, 5).

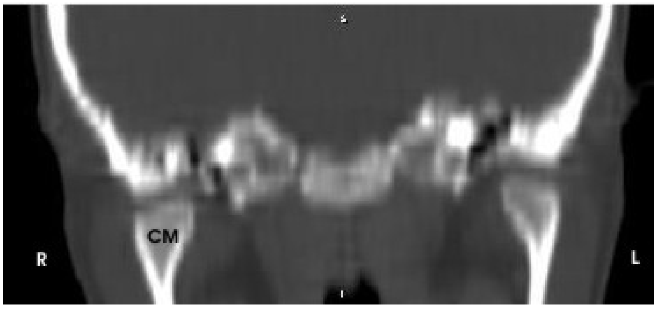

Fig. 3. Imagine CT coronală nativă normală la nivelul condililor mandibulari (CM).

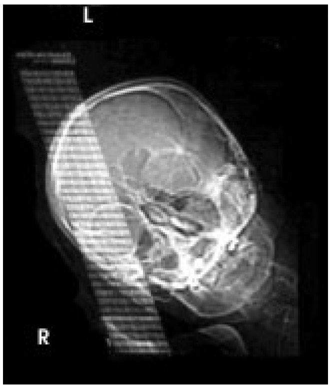

Fig. 4. Topograma anteroposterioară cu alegerea planului de referință.

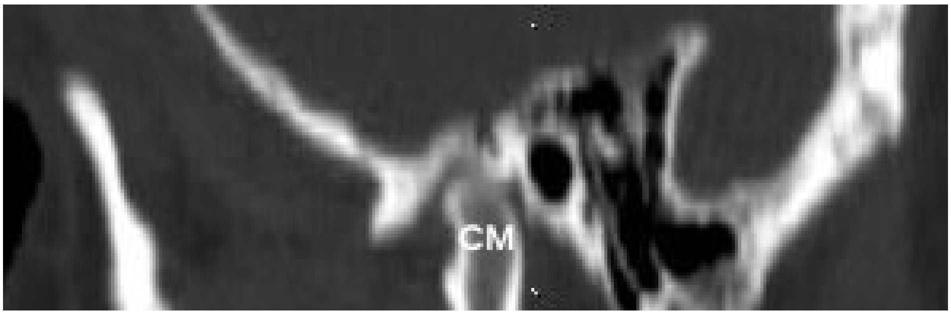

Fig. 5. Imagine CT sagitală nativă normală cu evidențierea condilului mandibular (CM) la nivelul ATM drepte în poziție „gură închisă”.

Pentru a se evita solicitarea pacientului într-o poziție incomodă, în vederea obținerii imaginilor coronale şi sagitale, este posibilă reconstituirea acestor incidențe, atât bidimensional cât şi tridimensional, folosind secțiunile axiale efectuate în decubit dorsal. Pentru reformatare am folosit secțiuni axiale adiacente cu increment de 1,5 mm care au permis obținerea unor imagini asupra modificărilor structurale osoase.

Reconstrucția tridimensională (3D) volumetrică spațială este de mare interes datorită relațiilor pe care le oferă în conduita preoperatorie, mai ales în traumatologie, malformații şi procese tumorale.

Pentru reconstituire am efectuat o achiziție a secțiunilor special adaptate, apropiate şi subțiri "în bloc" de aproximativ 40 de secțiuni apropiate de 1,5 mm grosime, cu interval de 0,5 mm.

Cantitatea de produs de contrast intravenos pe care am administrat-o a fost în doza obişnuită de 2 ml/kg cu 37% iod, folosind un injectomat. Această doză este diminuată în funcție de vârstă, starea de hidratare, prezența afecțiunilor renale.

Rezultate

Cazurile de tumori ale articulației temporomandibulare sunt rare, dar suspiciunea lor trebuie luată în seamă, deoarece simptomele şi semnele lor pot fi identice cu cele ale altor suferințe ale ATM [2, 3].

Debutul tumorilor poate fi şi din interiorul articulației cu invadarea țesuturilor înconjurătoare. Dacă sunt maligne, pot fi metastaze de la tumori de la distanță.

Tumorile benigne articulare cele mai întâlnite [4, 5] sunt osteoamele, condroamele, osteocondroamele [6].

Tumorile maligne articulare sunt rare [7]. Simptomele obişnuite pot fi durere, deformare locală dureroasă, anestezii, parestezii, pierderea auzului.

Diagnosticul diferenţial al tumorilor benigne de cele maligne şi precizarea naturii lor este dificil de făcut din punct de vedere imagistic.

Patologia tumorală a articulaţiei temporomandibulare este extrem de rară. Pe lotul studiat (633 cazuri) am identificat numai două cazuri .

Explorarea unei astfel de patologii mobilizează, în context clinic, un arsenal complex de investigaţii (tabel 1, figura 6).

Tabelul 1. Metode de explorare a tumorilor articulaţiei temporomandibulare pe lotul de studiu.

TUMORI Explorare	Osteom		Hipertrofie	
	Nr cazuri	%	Nr cazuri	%
RX	1	100%	1	100%
CT	1	100%	1	100%
Total	**1**		**1**	

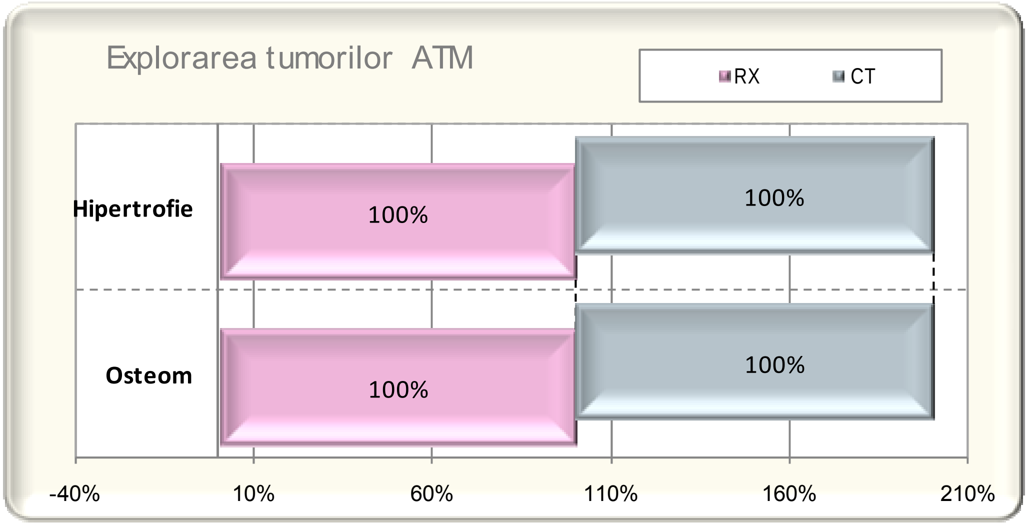

Fig. 6. Metode de explorare a tumorilor articulaţiei temporomandibulare pe lotul de studiu.

Cea mai frecventă tumoră a articulaţiei temporo-mandibulare (ATM) este osteomul.

Osteomul condilului

Originea lui este încă discutată, fiind incriminaţi *factori traumatici* (traumatisme directe sau indirecte, soldate cu fracturi şi calus exuberant), *infecţioşi* (artrite cronice) şi *endocrini*.

Este întâlnit mai frecvent la nivelul condilului şi la oasele feţei. În teritoriul maxilo-facial majoritatea osteoamelor sunt localizate la mandibulă.

Primul caz de osteom cu localizare în condilul mandibular a fost descris de Ivy în 1927; de atunci alte 12 observaţii au fost raportate în literatura de specialitate.

Experienţa clinicii se referă la un caz de "osteom gigant" al condilului mandibular.

Caz clinic: M.C, de sex masculin şi în vârstă de 55 ani, s-a internat cu asimetrie facială importantă, tulburări de ocluzie dentară şi grave tulburări de masticaţie.

De aproximativ 10 ani persoanele din anturajul său au sesizat o deviere a mentonului spre stânga, deviere ce s-a accentuat treptat. În plus pacientul în ultimii 2 ani acuză instalarea unor importante tulburări de masticație şi imposibilitatea obținerii unei ocluzii dentare normale.

Examenul clinic la internare evidenția asimetrie facială cu laterodeviație, mentonul deplasat spre stânga cu 1,5 cm (figura 7).

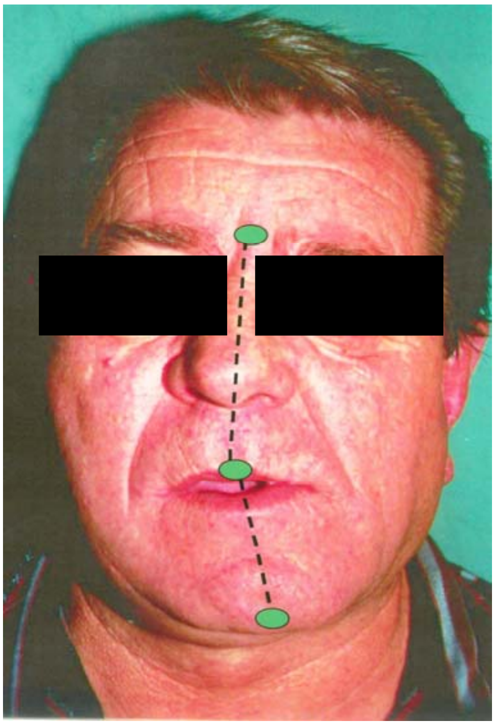
[redacted] [redacted]

Fig. 7. Caz clinic cu asimetrie facială cu laterodeviație, mentonul deplasat spre stânga.

Uşoară cădere a comisurii labiale drepte; în poziție de repaus ocluzia fantei labiale incompletă; la efortul de închidere a gurii se realizează treaptă labială inversă cu proieminarea jumătății stângi a buzei inferioare. Ramul vertical drept al mandibulei apare alungit, cu ştergerea reliefului unghiului extern. Distanța de la pupilă la marginea bazilară este cu 5-6 mm mai mare în dreapta decât în stânga, iar înălțimea verticală a etajului inferior al feței este mărită cu aproximativ 1 cm. În regiunea pretragiană dreapta, sub arcada zigomatică se palpează condilul mandibular mărit de volum, nedureros. Deschiderea gurii se realizează cu o marcată latero-deviație spre stânga, cu cracmente şi crepitații în ATM dreaptă.

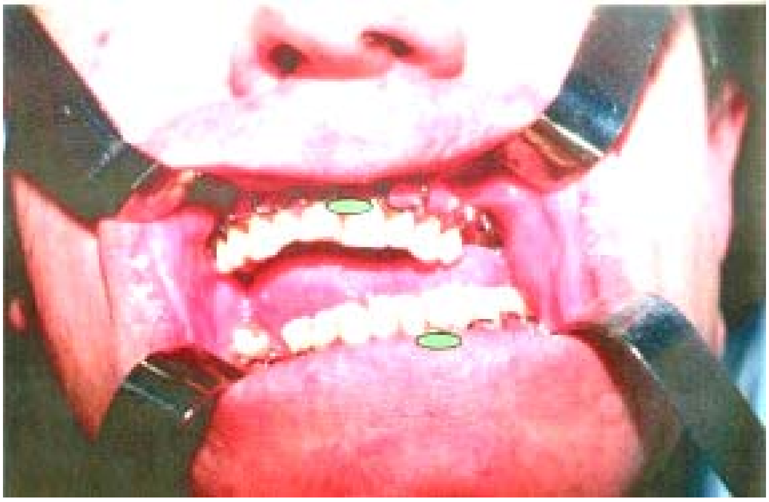

Fig. 8. Acelaşi caz, examenul intraoral remarcă devierea liniei mediane spre stânga.

La examenul intraoral se remarcă devierea liniei mediane spre stânga cu 1,5 cm (figura 8); vestibularizarea dinților de pe hemiarcada stângă; ocluzia dentară grav tulburată: muşcătură inversă pe stânga cu inocluzie verticală de 0,5 cm, ocluzie deschisă frontală de 1 cm şi inocluzie verticală dreaptă de 1,5 cm.

Pacientul prezintă importante tulburări de masticație, prin imposibilitatea punerii în angrenaj normal a suprafețelor ocluzale - masticație incomodă, ineficientă, tulburări de fonație şi fizionomice.

Radiografiile de față şi de profil dreapta ale mandibulei (figura 9), ortopantomografia (figura 10) şi CT (figura 11, 12) au pus în evidență o mărire exagerată de volum a capului condilului mandibular drept cu deformarea sa, având în ansamblu aspectul unei piramide cu baza în sus şi vârful în jos, depăşind spre anterior cavitatea glenoidă.

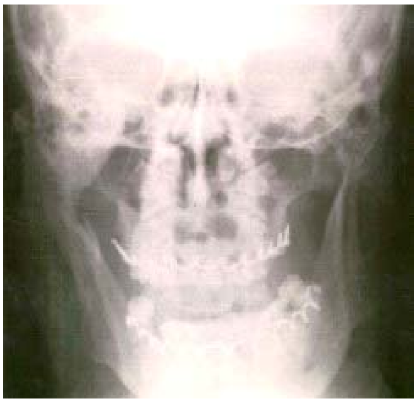

Fig. 9. Radiografie a articulației temporomandibulare drepte, imagine de față ce evidențiază la acest nivel o imagine radioopacă de 58/50 mm, rotundă, neomogenă, care deformează condilul mandibular şi depăşeşte cavitatea glenoidă.

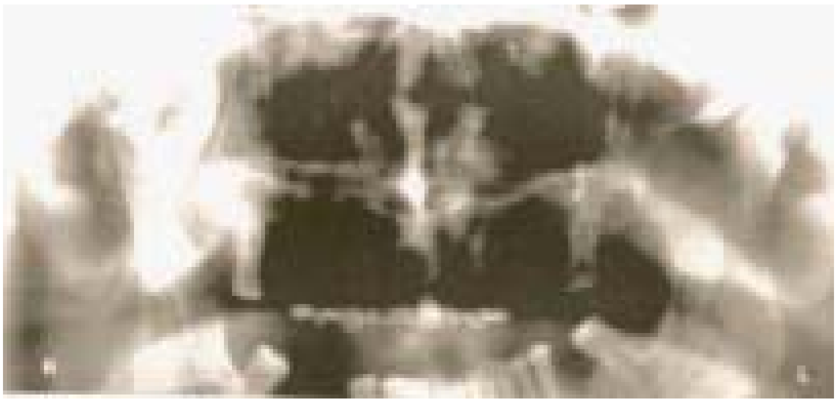

Fig.10. Ortopantomografie a articulației temporomandibulare care relevă o mărire exagerată de volum a condilului mandibular şi depăşeşte cranial şi anterior cavitatea glenoidă cu dispariția interliniului articular. Gâtul condilului este alungit.

Gâtul condilului apare mai alungit; incizura sigmoidă liberă; ramul vertical drept alungit şi îngustat, laterodeviație accentuată spre stânga mandibulei.

Examinarea CT nativă a pus în evidență un proces expansiv voluminos, de 50/60 mm, hiperdens, neomogen, la nivelul condilului mandibular drept cu contur boselat. La nivelul formațiunii se descriu calcificări centrale şi în periferie. Leziunea determină deformarea colului mandibular şi reacție de osteoliză, fiind imprecis delimitată la nivelul fosei pterigomaxilare şi muşchiului pterigoidian lateral. Procesul expansiv depăşeşte cranial cavitatea glenoidă, iar lateral nu se evidențiază limită de demarcație la nivelul capsulei articulare şi al părților moi periarticulare. Examinarea computertomografică postcontrast intravenos nu modifică aspectul densitometric al formațiunii, dar pune în evidență extensia la nivelul fosei pterigomaxilare şi al arterei maxilare interne, având interfață cu aceasta.

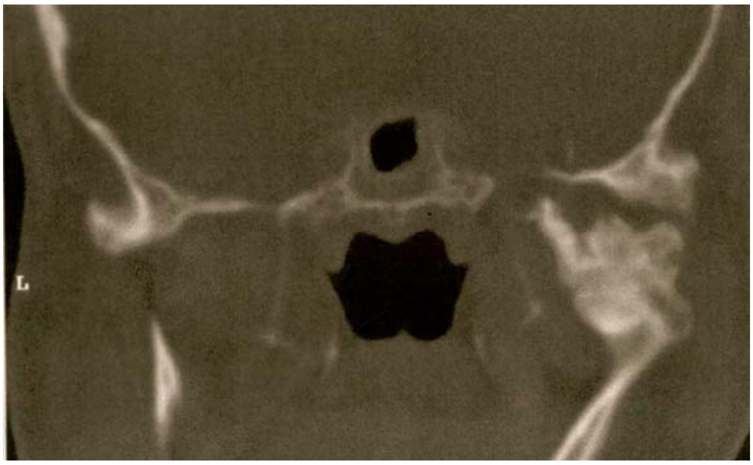

Fig. 11. Examinare CT coronală nativă a articulației temporomandibulare ce evidențiază o mărire a diametrelor condilului mandibular drept, hiperdensă nativ, cu zone hipodense, imprecis delimitată la nivelul părților moi periarticulare.

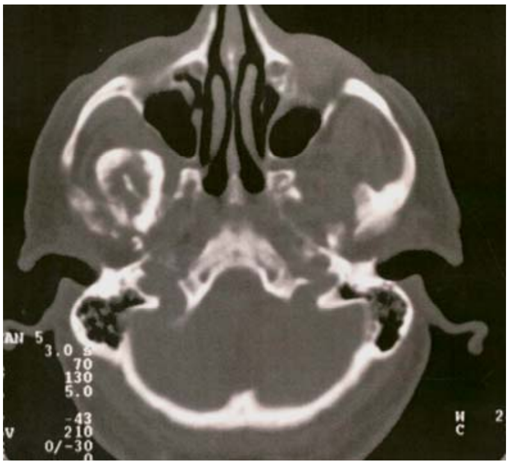

Fig. 12. Examinare computertomografică axială nativă a articulației temporomandibulare ce evidențiază un proces expansiv hiperdens, neomogen, voluminos, de aproximativ 50/60 mm al condilului mandibular drept, dezvoltat spre fosa pterigomaxilară dreaptă şi muşchiul pterigoidian lateral. Se notează producerea unei reacții de osteoliză la nivelul ramului mandibular vertical drept.

Sub anestezie generală prin intubație nazotraheală s-a abordat ATM dreaptă prin incizie preauriculară Ginestet [8, 9, 10, 11, 12]. Se evidențiază fața externă şi cea anterioară ale condilului mandibular mărit de volum. Cu fierăstrăul electric s-a realizat secțiunea osoasă la nivelul gâtului condilului, sub foseta pterigoidianului intern, apoi, cu foarte mare greutate, se îndepărtează condilul modificat de tumora ce se extindea spre baza craniului şi spre linia mediană a feței (figura 13).

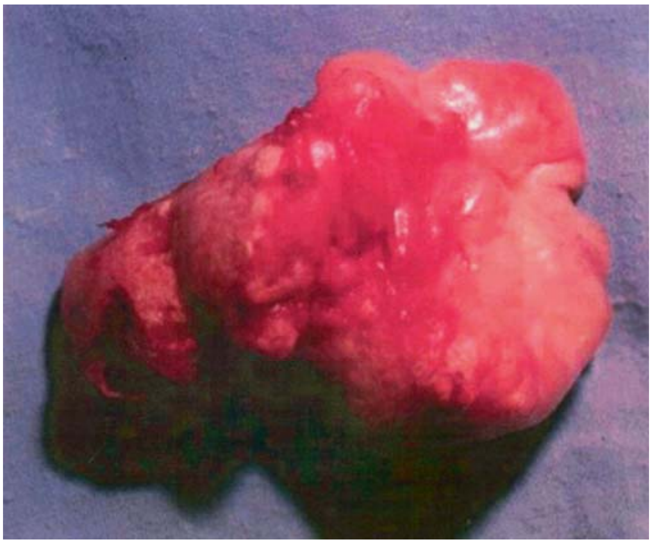

Fig. 13. Condilul modificat de tumoră.

Examenul macroscopic al piesei operatorii a evidențiat o tumoră voluminoasă de 50/60 mm care înlocuia complet capul condilului mandibular, cu suprafața boselată, alb-sidefie ca "scoica de mare" şi zone de aspect cartilaginos.

Din punct de vedere histologic formațiunea era constituită din țesut osos trabecular cu elemente fibroadipoase (figura 14). Aspectul corespunde unui "osteom spongios".

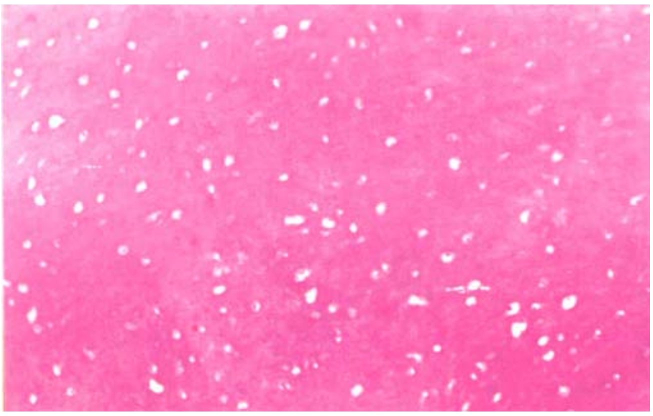

Fig. 14. Acelaşi caz, histologic formațiunea era constituită din țesut osos trabecular cu elemente fibroadipoase. Aspectul corespunde unui "osteom spongios".

La 2 ani postoperator vindecarea se menține, funcțiile ATM fiind reluate complet.

Hiperplazia de condil mandibular

Cunoscută în literatură şi sub denumirea de hipertrofie de condil, hipercondilie, laterognație sau prognatism unilateral inferior, hiperplazia de condil mandibular este o afecțiune benignă rară, caracterizată clinic printr-o asimetrie facială cu latero-deviație şi tulburări de ocluzie dentară. Primul caz a fost descris de Adams în 1836, de atunci şi până în 1968 fiind publicate în literatură în jur de 150 observații.

Se întâlneşte la orice vârstă, în proporții aproximativ egale la ambele sexe, se instalează insidios, de regulă la persoane tinere, cu vârsta cuprinsă între 15 şi19 ani.

Boala se manifestă printr-un complex de simptome ce rezultă din creşterea în volum, fără modificări histologice, a capului condilului şi alungirea colului, cu sau fără schimbarea de formă, cu sau fără transformări dimensionale ale ramului sau capului mandibular.

Etiologia afecțiunii este necunoscută, cel mai adesea sunt incriminate leziunile inflamatorii cronice otice sau articulare, leziunile traumatice ale capului condilian şi ale țesuturilor de vecinătate.

Tratamentul are ca scop ameliorarea leziunilor ce au dus la asimetria facială, corectarea ocluziei şi stoparea procesului de creştere dizarmonică a apofizei condiliene cu întregul ansamblu osos al feței. Procedeele utilizate sunt multiple, dependente de gradul de evoluție al bolii, de vârstă şi intensitatea tulburărilor produse de forma histopatologică a leziunii.

Observație clinică: J.A., de sex masculin şi vârstă de 16 ani, sesizează, înainte cu 1 an de spitalizare, laterodeviația dreaptă a mentonului, coborârea unghiului mandibular, alungirea ramului ascendent stâng, ştergerea uşoară a şanțului labiogenian şi rotunjirea marginii bazilare a mandibulei în dreptul comisurii labiale, lungirea cu 6-8 mm a distanței între pupilă şi marginea bazilară a mandibulei spre stânga. La palpare, condilul stâng era mărit de volum, nedureros, mobil în cavitatea glenoidă. Deschiderea gurii uşor limitată şi cu o marcată deviație spre dreapta.

S-a înregistrat o inocluzie marcată în sens vertical în 1/2 stânga, ocluzie laterală dreapta şi o muşcătură deschisă frontal cu deviație de 0,8 mm spre dreapta a liniei interincisive inferioare (figura 15).

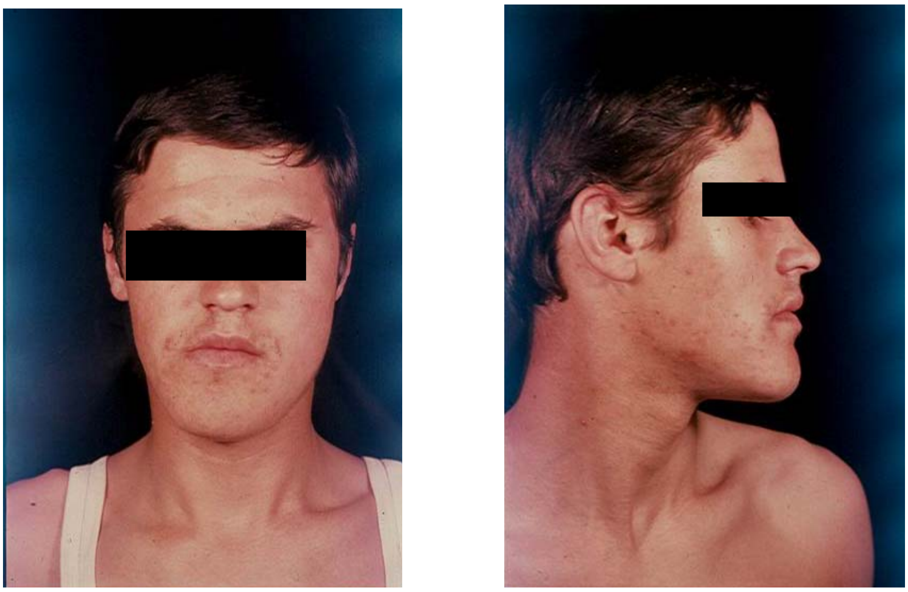

Fig. 15. Aspect clinic cu laterodeviația dreaptă a mentonului, coborârea unghiului mandibular, alungirea ramului ascendent stâng, ştergerea uşoară a şanțului labiogenian şi rotunjirea marginii bazilare a mandibulei în dreptul comisurii labiale, lungirea cu 6-8 mm a distanței între pupilă şi marginea bazilară a mandibulei spre stânga

Radiografic s-a evidențiat o mărire de volum a condilului şi o îngroşare a colului condilian, o deformare a incizurii sigmoide, alungirea şi îngustarea ramului vertical şi rotunjirea unghiului de partea stângă.

Tratamentul a constat în rezecția condilului stâng şi modelarea unui nou cap articular din bontul restant, aplicarea şi fixarea materialului de interpoziție Dacron peste capul articular, repunerea în ocluzie normală a arcadelor dentare şi imobilizarea intermaxilară timp de 6 săptămâni.

Examinarea nativă computertomografică coronală şi sagitală a evidențiat o creştere în diametre a condilului mandibular stâng cu scăderea neomogenă a densității osoase şi cu mici zone bine delimitate hipodense nativ (figurile 16, 17, 18). După administrarea de substanță de contrast intravenos, la examinarea CT axială nu s-au evidențiat modificări densitometrice la nivelul condilului mandibular stâng şi al cavității glenoide, leziunea fiind bine delimitată.

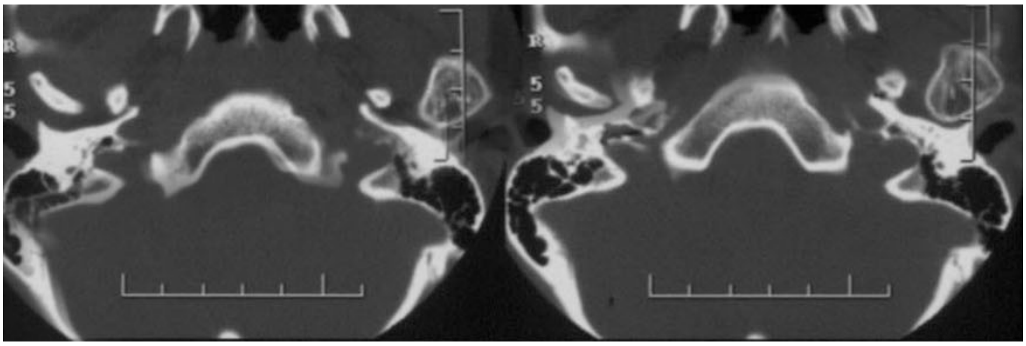

Fig. 16. Examinare CT axială a ATM pre şi postcontrast intravenos, în poziția „gură închisă”, care evidențiază o creştere în diametre a condilului mandibular stâng de aproximativ 20/22mm, cu contur neregulat şi cu zone centrale hipodense nativ şi postcontrast.

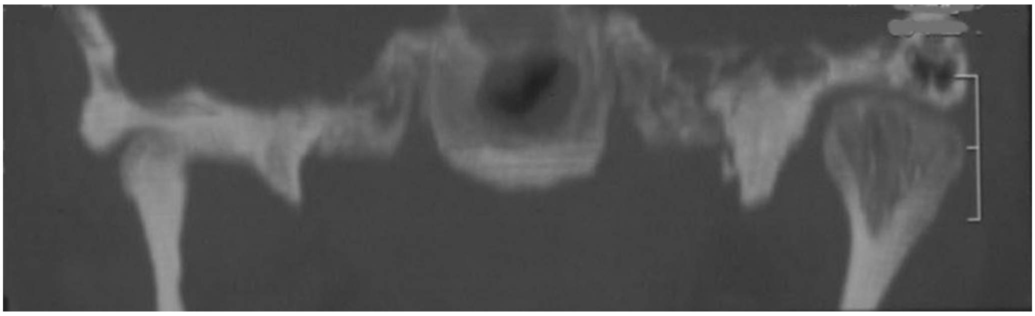

Fig. 17.Examinare CT coronală nativă a ATM, în poziția „gură închisă”, ce evidențiază o mărire de volum a condilului mandibular stâng, cu pierderea sfericității şi a conturului. Condilul mandibular stâng este neomogen, cu zone centrale hipodense, spațiul interarticular intern este lărgit, iar colul condilian este îngroşat.

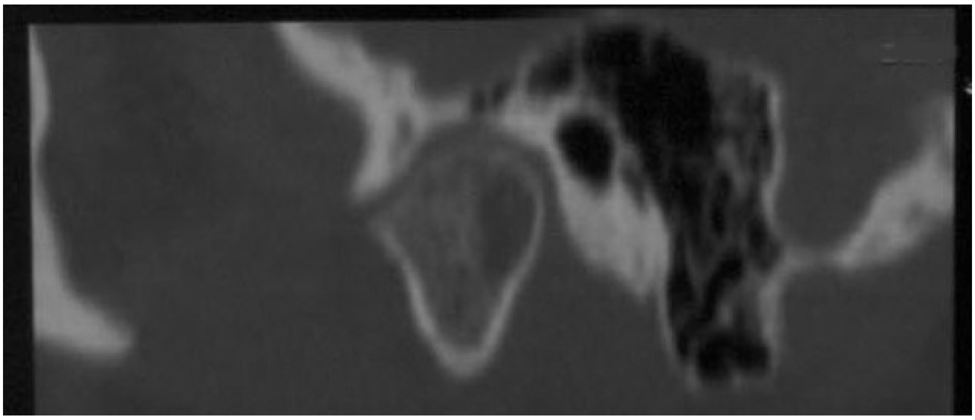

Fig. 18. Examinare CT sagitală nativă a ATM stângi, în poziția „gură închisă”, ce evidențiază o mărire de volum a condilului mandibular stâng cu structură neomogenă, având zone centrale hipodense. Colul mandibular este lărgit.

Examenul anatomopatologic: *macroscopic* se evidențiază un condil mărit de volum în toate sensurile fără schimbări evidente de formă; *microscopic* - o structură normală specifică apofizei condiliene.

Rezultatele se mențin la 2 ani postoperator.

Discuții

Tumorile articulației temporomandibulare sunt extrem de rare. Mostafapour [13] identifică 6 cazuri în 7 ani. Simptomatologia lor de debut se suprapune peste cea a sindromului de disfuncție a articulației temporomandibulare [13]. Ca urmare sunt dificil de diagnosticat, iar timpul de la debut la prezentare a variat de la 3 luni la 8 ani.

Tumorile benigne cele mai frecvent întâlnite la nivelul ATM sunt osteocondromul, condromul, osteomul, sinovita pigmentară vilonodulară şi condromatoza sinovială [14]. Din puținele date existente în literatura de profil se pare că osteocondromul este cel mai frecvent (40-45% din cazuri).

Tumorile maligne primitive ale articulației temporomandibulare sunt extrem de rare. Mai frecvent sunt citate invazii de vecinătate la acest nivel.

Simptomatologia inițială este nespecifică, similară pentru toate. Bolnavul se prezintă în general pentru tumefacție şi dureri la nivelul regiunii temporale. Deşi acest tip de patologie este rar (tabel

32), ea trebuie luatã în seamã, deoarece simptomele şi semnele acesteia pot fi identice cu cele ale altor suferințe ale ATM [2, 3].

Tabelul 2. Patologia tumorală a ATM

PATOLOGIA TUMORALĂ A ATM
Tumori primitive Tumori secundare
Tumori ale sistemului osos Tumori ale sistemului dentar Tumori ale părților moi învecinate (meningioame, condroame, sinovialoame)
Tumori benigne
Chisturi ale condilului mandibular
Osteom al condilului mandibular
Condrom
Osteocondrom
Leiomiom
Fibromixom
Tumori neurogene
Tumori maligne
Sarcom (condrosarcom, fibrosarcom)
Schwanom
Limfom
Carcinom cu celule gigante
Mielom multiplu

Imagistic se traduc prin leziuni osteoblastice şi osteolitice, cele maligne fiind frecvent osteolitice.

Tumorile de vecinătate evoluează cu resorbție osoasă târzie, determinând simptome la nivelul ATM.

Debutul tumorilor poate fi din interiorul articulației cu invadarea țesuturilor înconjurătoare. Dacă sunt maligne, pot fi metastaze de la tumori de la distanță.

Tumorile benigne articulare cele mai întâlnite [15] sunt osteoame, condroame, osteocondroame. Simptomele lor sunt: durerea, limitarea deschiderii arcadelor dentare si ocluzie deschisă de partea bolnavă. Condilul mandibular este mărit în volum. În condromatoza sinovială, focare cartilaginoase se dezvoltă în membrana sinovială şi uneori apar mase radioopace în interiorul articulației.

Tumorile maligne articulare sunt rare [7]. Simptomele obişnuite pot fi durere, deformare locală dureroasă, anestezii, parestezii, pierderea auzului.

Imaginile radiologice pot obiectiva osteoliză sau modificări de tip osteoblastic, în funcție de tipul tumorii [12].Examinarea CT a oferit informații topografice de mare interes pentru chirurgie.Se notează mărire de volum a condilului mandibular (mai mult sau mai puțin omogenă), depăşind fosa glenoidă, ceea ce determină o limitare a mişcărilor mandibulare. Se face diagnosticul diferențial cu hiperplazia de condil mandibular în care apare mărit dimensional dar păstrându-şi forma normală.

Investigațiile menționate ajută la delimitarea lezională şi evaluarea unei recidive.Diagnosticul diferențial al tumorilor benigne de cele maligne şi precizarea naturii lor este dificil de făcut din punct de vedere imagistic, totuşi osteocondroamele tind să se dezvolte într-o direcție supero-inferioară rezultând o laterognație şi arcuirea mandibulei spre partea afectată, iar osteoblastoamele tind să se dezvolte latero-median rezultând o extindere în țesuturile moi şi durere preauriculară.

Protocolul de investigație paraclinic cuprinde ortopantomografia, computertomografia şi rezonanța magnetică nucleară. În momentul diagnosticului majoritatea pacienților au expresie avansată la nivel radiologic [13]. Dos SANTOS [16] constată, pe 2 cazuri cu finalitate chirurgicală, că un examen computertomografic 2D detectează excelent calcificările tumorale, interesarea corticală şi, de cele mai multe ori extensia în țesuturile moi şi intramedulare. Examinarea CT 3D cu protocol vascular evidențiază cu acuratețe crescută extensia leziunii şi rapoartele cu structurile anatomice de vecinătate. Examinarea prin rezonanță magnetică este extrem de eficace în documentarea componentei tumorale intramedulare şi extraosoase mai ales pe secvențe T2.

În cazul de osteom descris în lotul de studiu remarcăm un debut clinic prin asimetrie facială de aproximativ 10 ani, dar cu instalarea unei simptomatologii mai exacerbate de aproximativ 2 ani.

La prezentare examenul radiografic a precizat o mărire exagerată de volum a capului mandibular drept cu deformarea sa depăşind spre anterior cavitatea glenoidă. Gâtul condilului apare mai alungit, la fel ca şi ramul vertical drept. Se notează distrucţii osoase de tip osteolitic la nivelul colului şi condilului mandibular, toate acestea exprimând o leziune avansată radiologic.

Examinarea CT nativă a pus în evidenţă un proces expansiv voluminos de aproximativ 50/60 mm, hiperdens, neomogen cu contur boselat al condilului mandibular drept. Se disting calcificări centrale şi în periferie. Tumora este imprecis delimitată la nivelul fosei pterigomaxilare şi muşchiului pterigoidian lateral. Se notează deformarea colului mandibular şi reacţii de osteoliză la acest nivel. Procesul expansiv depăşeşte anterior cavitatea glenoidă şi nu se evidenţiază limită de demarcaţie la nivelul capsulei articulare şi părţilor moi extraarticulare. Examinarea CT postcontrast intravenos nu modifică aspectul formaţiunii şi descrie extensia la nivelul fosei pterigomaxilare şi spre artera maxilară internă. Cazul a beneficiat de sancţiune chirurgicală cu rezultate stabile apreciate la 2 ani postoperator.

Concluzii

Patologia tumorală a ATM este extrem de rară. Cazurile din studiu sunt reprezentate de osteom şi hiperplazie de condil. Ortopantomografia a constituit baza pentru indicarea examenului computertomografic. Investigaţia a fost efectuată pe un aparat helical unislice cu folosirea unui artificiu tehnic – CT axial, coronal, sagital. A fost apreciată astfel, în cadrul studiului cu contrast, vascularizaţia şi infiltraţia tumorală în structurile adiacente.

Aportul ortopantomografiei în investigarea tumorilor articulaţiei temporomandibulare este ca de orientare pentru stabilirea indicaţiei unor examene mai complexe.

În patologia tumorală, CT helical unislice este dependentă de posibilitatea examinării prin artificiul tehnic CT axial, coronal, sagital. Are capacitatea de a furniza detaliile necesare tratamentului chirurgical, folosind şi avantajele reconstrucţiei 3D.

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Supraprotezarea mandibulară pe implante restante la un pacient cu boala Parkinson (prezentare de caz)

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Rezumat. Articolul prezintă cazul unei paciente în vârstă de 54 ani care s-a prezentat în cabinet cu două implante poziţionate bilateral pe mandibula edentată total; la maxilar prezintă o restaurare protetică compozită formată dintr-o punte metalo-compozită şi o proteză scheletată menţinută şi stabilizată unilateral. Ca antecedente patologice importante semnalăm prezenţa maladiei Parkinson de la vârsta de 35 ani. Pacienta a solicitat realizarea unei restaurări protetice cu utilizarea implantelor prezente la arcada mandibulară. Luând în considerare numărul implantelor, precum şi mişcările incontrolabile ale limbii şi musculaturii orale asociate, cu absenţa protezării, i s-a prezentat pacientei şi a fost acceptată soluţia unei supraproteze pe implante. În concluzie, soluţia de tratament s-a dovedit a răspunde cerinţelor protetice (echilibru, funcţionalitate, confort) şi a adus astfel satisfacţie pacientei.

Cuvinte cheie: supraproteză mandibulară, implante restante, maladia Parkinson.

Abstract. This article presents the case of a 54 years old female patient coming in the dental office with 2 implants already positioned on the mandibular arch. The maxillary arch is also already restored with a combined fixed-removable partial denture. The main problem in the patient's general state of health was the Parkinson disease (after age 35). The patient wants to restore the mandibular arch using the 2 implants. Considering the number of the implants, the movements of the tongue and oral muscles and the lack of temporary prosthetic treatment, the prosthetic solution that was explained to the patient was an overdenture on implants. In conclusion, this treatment fulfils the prosthetic goals (stability, function, comfort) and brought satisfaction to patients.

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1. INTRODUCERE

În practica curentă a proteticianului apar frecvent situaţii clinice care nu sunt neapărat tipice din punct de vedere conceptual. Aceste situaţii, mai puţin „ortodoxe”, solicită în mod deosebit atât medicul cât şi tehnicianul, în încercarea de a oferi pacientului cea mai bună soluţie posibilă. Mai mult decât oricând, în aceste situaţii-problemă coeziunea echipei medic-tehnician este la fel de importantă ca şi profesionalismul fiecăruia dintre aceştia.

2. PREZENTARE DE CAZ

Spre exemplificare vom prezenta rezolvarea unui astfel de caz, pentru a ilustra principiile care au stat la baza conceperii şi realizării tratamentului.

Evaluare iniţială

Pacienta, în vârstă de 54 ani, s-a prezentat la cabinet cu dorinţa de a realiza o restaurare protetică a arcadei mandibulare edentate total, cu prezenţa a două implante, plasate de o parte şi de alta a liniei mediene.

La examenul clinic s-a constatat prezenţa, pe arcada maxilară antagonistă, a unei restaurări protetice compozite (o punte metalo-compozită - fig. 1) şi o proteză scheletată menţinută şi stabilizată unilateral (fig. 2.). De asemenea, s-a constatat o reducere a spaţiului vertical disponibil atât prin examinare, cât şi prin evaluarea cu ajutorul modelelor de studiu şi diagnostic montate în ocluzor, cu ajutorul unui prim şablon de ocluzie confecționat în acest scop pe modelul mandibular. La maxilar angulația şi dimensiunea dinților artificiali accentuează dificultățile legate de lipsa de spațiu.

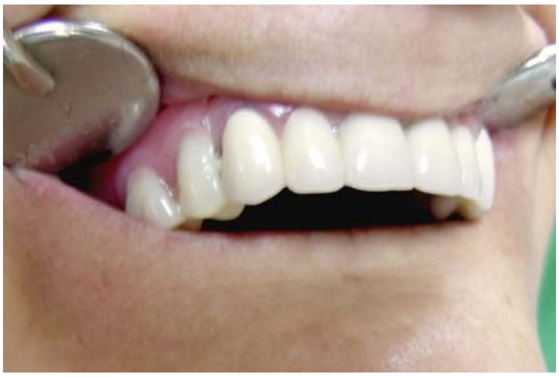

Fig. 1. Aspectul arcadei antagoniste

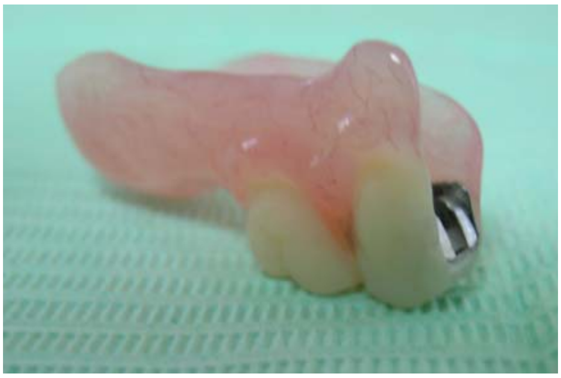

Fig. 2. Proteza scheletată menținută unilateral

La mandibulă, în prezența a două implante tip şurub, poziționate bilateral, deşi insuficiente ca număr, iar poziția implantului din cadranul 3, ca fiind nefavorabilă, s-a hotărât realizarea unei supraproteze menținute şi stabilizate cu ajutorul unei structuri de bară cu călăreți şi capse distale (1).

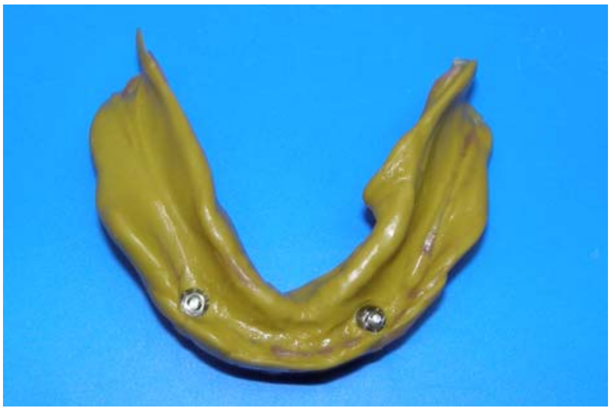

Fig. 3. Amprenta funcțională

Etape de tratament

Pacienta a refuzat inserarea mai multor implante la arcada mandibulară, dar a fost de acord cu soluția de supraproteză mobilizabilă menținută şi stabilizată pe implante cu ajutorul unei structuri cu sisteme speciale.

Amprenta preliminară mandibulară a fost luată utilizând o lingură universală pentru edentat total. Pe modelul preliminar s-a confecționat lingura individuală din compozit fotopolimerizabil. În dreptul implantelor aceasta a fost perforată, astfel încât să nu aibă contact cu capele de vindecare. În cabinet lingura individuală a fost probată şi adaptată conform regulilor de amprentare muco-dinamică a edentatului total. Menționăm că lingura individuală trebuie să fie intim adaptată pe creasta edentată şi corect adaptată marginal. Au fost înşurubate bonturile de transfer şi s-a verificat încă o dată ca lingura individuală să nu interfere cu acestea. Amprenta funcțională a fost luată cu un silicon de adiție de consistență medie (fig. 3), iar după spălare şi dezinfectare a fost îndiguită şi cofrată în laborator pentru conservarea integrală a funcționalizării marginilor cu ajutorul unui ring şi al unui alginat. S-a constatat că implantul utilizat în cadranul 3 prezenta un profil intern octogonal, iar cel din cadranul 4 unul hexagonal. Pentru implantul din cadranul 4 a fost folosită o capă calcinabilă cu profil hexagonal. Pentru implantul din cadranul 3 nu a fost posibilă găsirea pe piață şi utilizarea unei cape calcinabile cu profil octogonal; această indisponibilitate a dus la adoptarea unei alte soluții de confecționare a structurii prin frezarea unui bont de transfer.

Bontul de transfer a fost frezat şi scurtat maximum posibil având în vedere poziția nefavorabilă vestibularizată a implantului. Modelul funcțional a fost turnat din ghips IV şi a fost montat la paralelograf pentru a analiza şi decide axa de inserție-dezinserție a viitoarei supraproteze, axă în care se va confecționa bara.(3,4)

Pentru confecționarea structurii metalice s-au utilizat machetele prefabricate ale firmei producătoare de sisteme speciale. Macheta barei a fost confecționată din două bucăți pentru a respecta forma crestei edentate dintre cele două implante. Ambele au fost poziționate la paralelograf în axa de inserție stabilită utilizând pensa specială pusă la dispoziție de firmă. Macheta a fost definitivată, ambalată şi turnată, apoi prelucrată şi aşezată pe model (fig.4) pentru a fi trimisă în cabinet. Mezostructura a fost probată, adaptată şi verificată în cavitatea bucală, precum şi ca pasivitate prin strângerea şurubului în cadranul 4.

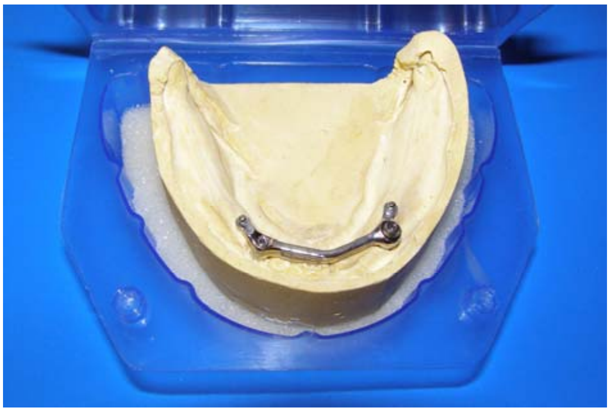

Fig. 4. Mezostructura pe model

S-a trecut la confecționarea suprastructurii. Pe bară au fost inserate machetele prefabricate ale lăcaşelor pentru călăreți şi pentru matricile capselor. A fost completată macheta suprastructurii utilizând elemente prefabricate secționate cât mai corect şi lipite cu ceară fotopolimerizabilă (utilizată pentru duritate şi pentru o contracție scăzută) în cantitate cât mai redusă. Şablonul de ocluzie realizat pe modelul funcțional a fost secționat în dreptul implantului din cadranul 3 din cauza poziției extrem de nefavorabile a acestuia (mult în afara crestei, spre vestibular). S-a realizat o montare inițială pentru a vizualiza posibilitățile de poziționare a dinților artificiali ținând cont de spațiul vertical deosebit de redus şi de poziția extrem de nefavorabilă a implantului din cadranul 3. S-a observat că dinții artificiali vor trebui reduşi şi vor fi evident mai mici decât antagoniştii. A fost realizată macheta finală a supraprotezei peste supra şi mezostructură.

Poziționarea dinților artificiali a fost deosebit de dificilă din cauza spațiului vertical redus, a poziției vestibularizate a implantului din cadranul 3, a angulației şi dimensiunii mari a antagoniştilor. Această machetă finală a fost amprentată înainte de a scurge ceara. S-a turnat un model peste care a fost confecționată o gutieră transparentă care să fie utilizată ca un ghid pentru a vizualiza atât spațiul disponibil pentru metalul suprastructurii, cât şi zonele unde este necesară aplicarea agenților de mascare. În interiorul dinților artificiali, pe fața mucozală, s-au realizat orificii în scopul de a obține o retenție suplimentară.

O atenție deosebită trebuie acordată feței mucozale a suprastructurii (fig.5) în sensul că lăcaşele pentru matricile capselor şi pentru călăreți trebuie să nu prezinte plusuri şi să fie doar sablate, în niciun caz lustruite, deoarece îşi pierd fricțiunea cu călărețul şi cu matricile. Porțiunile intermediare se lustruiesc normal.(5,6)

Supraproteza se prelucrează, se lustruieşte şi se finisează având grijă să nu se intervină asupra structurii metalice (fig. 6). Supraproteza totală a fost concepută şi realizată respectând etapele şi principiile de protezare a edentatului total (fig.7), pentru a putea asigura un grad de menținere şi stabilitate precum şi un sprijin muco-osos eficient care să completeze şi să susțină din punct de vedere biodinamic acțiunea implantelor. La final supraproteza se trimite în cabinet împreună cu toate componentele pentru a fi aplicată în cavitatea bucală (fig.8).

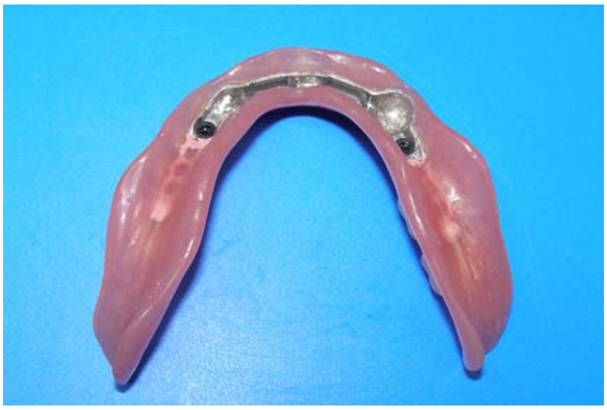

Fig.5. Fața mucozală a suprastructurii

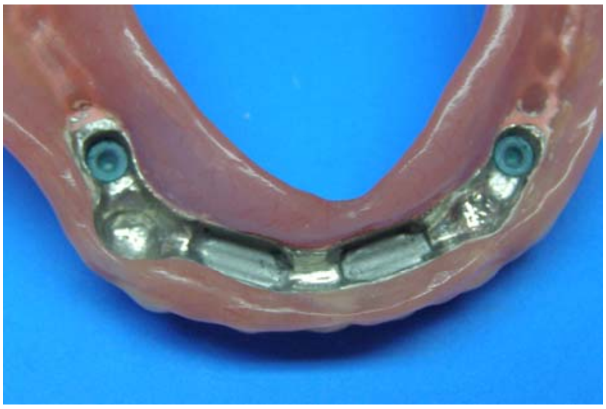

Fig. 6. Detalii ale suprafeței interne a protezei

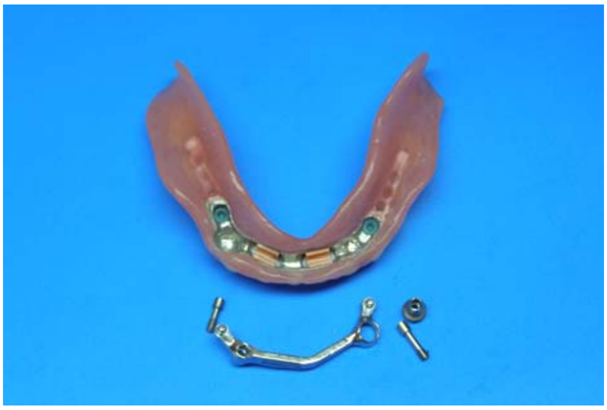

*Fig.7.*Sistemul de ancorare

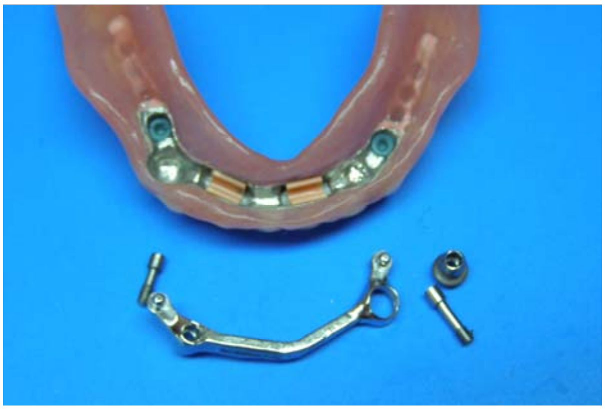

*Fig.8.*Detalii privind sistemul de ancorare

Supraproteza va conține astfel suprastructura, iar mezostructura va fi cimentată (fig.9) pe implantul din cadranul 3, în cavitatea bucală, utilizând un ciment special (metal-metal) după ce va fi înşurubată în implantul din cadranul 4. Pacienta a fost instruită în ceea ce priveşte manipularea şi igienizarea supraprotezei în şedința de aplicare (fig.10). (7)

Discuții

Cazul prezentat se distinge prin dificultatea deosebită apărută în elaborarea şi executarea planului de tratament din cauza spațiului vertical redus, a poziției şi dimensiunii dinților antagonişti şi a poziției şi profilului implantului inserat în cadranul 3, precum şi statusul general (boala Parkinson, cu toate inconvenintele protetice şi în manevrele de tratament) şi refuzul pacientei la intervenții suplimentare. De asemenea, gradul de atrofie al crestei reziduale mandibulare era destul de avansat.

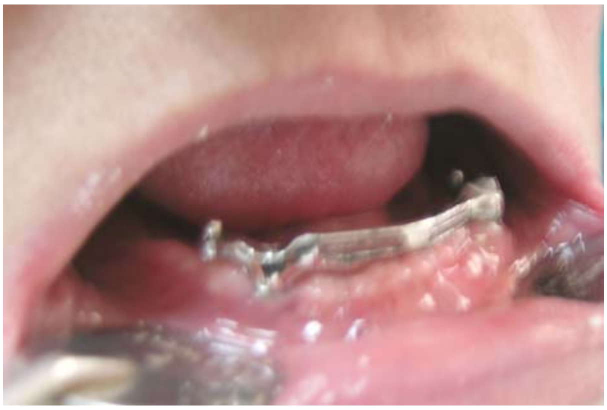

Fig.9. Mezostructura în cavitatea bucală

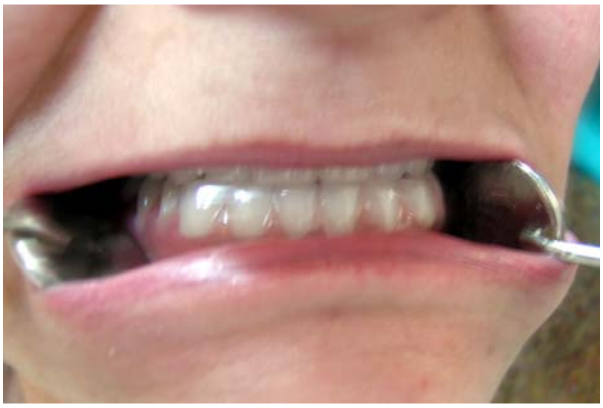

Fig.10. Supraproteza în cavitatea bucală

Supraproteza a fost concepută şi realizată astfel încât să aibă sprijin mixt, muco-osos şi implantar. Matricile şi călăreții prezintă un spațiu în sens ocluzal care permite înfundarea supraprotezei sub presiune ocluzală.

Designul barei nu este uniform ci prezintă un anumit profil (drept sau rotund) în funcție de care se optează pentru a realiza o legătură rezilientă sau rigidă cu călărețul. Bara a fost montată cu marginea dreaptă spre călăreț, astfel încât să realizeze o legătură rezilientă cu suprastructura incorporată în supraproteză.(8)

Concluzii

Terapia prin implante este din ce în ce mai frecvent utilizată, devenind o opțiune solicitată atât în rândurile pacienților, care nutresc visul „celei de-a treia dentiții", cât şi în rândurile medicilor care încearcă să răspundă solicitărilor pacienților. Deşi nu a devenit încă o terapie de masă, protezarea pe implante pare să-şi părăsească treptat statutul de excepţie şi în medicina dentară românească.

Fără a elimina logica clinică şi principiile proteticii clasice, implantologia completează valoros paleta de opţiuni oferite pacientului de către medic.

În terapia edentatului total este important să subliniem avantajele protezării mobile pe implante, care pe lângă un cost mai redus raportat la protezarea fixă pe implante oferă pacientului un confort deosebit şi o posibilitate de igienizare optimă.

Toate acestea, împreună cu faptul că pacientul poate efectua o masticaţie mult mai eficientă, recomandă supraproteza pe implante ca pe o soluţie ce poate fi frecvent folosită.

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Principii de triaj şi evacuare la spitalele de campanie (ROLE 1 şi ROLE 2)

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Rezumat. Dezastrul din punctul de vedere medical este caracterizat de disproporţia dintre capacitatea de răspuns medical, resursele medicale disponibile şi cererea de ajutor medical. Obiectivul managementului medical al dezastrelor este de a asigura cât mai rapid beneficiul optim pentru cel mai mare număr de victime şi de a reduce mortalitatea, morbiditatea şi efectele indirecte. Nu toate victimele pot primi imediat ajutorul de care au nevoie, o selecţie a lor este inevitabilă. Triajul este o metodă de management medical care permite identificarea cazurilor, introducerea lor în categoriile de triaj mărind, astfel şansele de supravieţuire. Cartela de triaj propusă are rolul de a îmbunătăţi identificarea, marcarea victimelor, de a stabili o legătură între diferitele etape ale lanţului de ajutor medical, de a uşura urmărirea victimelor şi nu în ultimul rând are importanţă medico-legală.

Cuvinte cheie: triaj, categorii de triaj, proceduri de triaj, cartele de triaj.

Summary. From a medical point of view a disaster is characterized by the disproportion between the medical response capacity and the actual medical resources available in order to manage the casualties. The objective of medical disaster management is to provide as rapid as possible the greatest benefit for the largest number of casualties in order to achieve a critical reduction in mortality, morbidity and indirect effects within the affected population. Not each individual victim can therefore immediately receive the necessary care, a certain selection of casualties is inevitable. Triage is a medical management tool which has the property to identify, to tag the disaster victims and in this order to facilitate the salvageable victims. The new proposed tagging system has the purpose to improve the victims identification, categorization, and to make a link between the different stages of the medical chain of care, to lighten the victims pursuing and it has also a forensic importance.

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I. INTRODUCERE

Dezastrele de orice tip sunt evenimente care determină un număr mare de morţi şi răniţi, afectarea sau distrugerea facilităţilor care susţin şi asigură necesităţile umane. Aceste evenimente determină o cerere foarte mare de necesităţi medicale care se răsfrâng asupra sistemului de urgenţă medical local/naţional, dar în acelaşi timp afectează pentru o lungă perioadă de timp activitatea economică generală, precum şi capacitatea locală/naţională de a începe/susţine activităţile de răspuns. În aceste condiţii este necesară existenţa unui instrument medical care să rezolve această discrepanţă între cererea foarte mare de servicii medicale şi capacitatea de răspuns a sistemului medical. Astfel a apărut în secolul al XIX-lea termenul de TRIAJ.

Din punct de vedere medical un dezastru se caracterizează printr-o disproporţie între capacitatea de răspuns medical şi resursele medicale disponibile pentru a conduce cazurile. Disproporţia poate fi cantitativă şi/sau calitativă atât în ceea ce priveşte personalul şi echipamentul, cât şi în ceea ce priveşte problemele medicale şi cele de organizare.

Obiectivele managementului medical al dezastrelor

Obiectivele managementului medical al dezastrelor sunt următoarele:

- să asigure cât mai rapid cu putinţă cel mai mare beneficiu pentru un număr cât mai mare de răniţi pentru a obţine o reducere maximă a mortalităţii, morbidităţii şi a efectelor indirecte în populaţia afectată;
- să identifice minoritatea de răniţi cu stare critică, dar care pot fi salvaţi, necesitând însă îngrijire imediată în cel mai scurt timp;
- să asigure folosirea eficientă a resurselor de personal, de echipament, de aprovizionare, de mijloace de transport, dar şi a facilităţilor medicale de tratament.

Managementul medical al victimelor unui dezastru vizează o serie de aspecte care pot fi împărţite în patru mari grupe:

- tipul şi severitatea leziunilor;
- numărul victimelor;
- distribuţia spaţială şi temporală a răniţilor;
- managementul cazurilor într-un sistem medical de urgenţă:
  - salvarea şi retragerea cazurilor din scena dezastrului;
  - punerea la punct a unui sistem de triaj;
  - măsurile de stabilizare;
  - distribuţia şi evacuarea cazurilor;
  - internarea şi îngrijirea definitivă a victimelor în facilităţi medicale de ROLE 2, 3 sau 4.

II. TRIAJUL MEDICAL

1. Istoric. Cuvântul triaj provine din limba franceză - „trier," care înseamnă a sorta, a selecta. Cel mai mare credit este acordat medicului francez Dominique Jean Larrey, chirurg în armata lui Napoleon, care a pus bazele unei metode rapide de evaluare şi introducere într-o categorie de prioritate a răniţilor de pe un câmp de luptă, pentru a-i evacua pe cei care aveau nevoie de îngrijire medicală urgentă. El a instituit aceste metode în timp ce bătălia era în desfăşurare şi a triat pacienţii indiferent de gradul lor militar.

2. Definiţie. Triajul (la scena dezastrului/pe câmpul de luptă din teatrul de operaţii) poate fi definit ca un proces medical care determină ordinea priorităţilor de evacuare şi tratament cu scopul de a folosi cât mai bine resursele disponibile pentru resuscitarea, stabilizarea, distribuţia şi transportul cazurilor şi toate acestea în beneficiul unui număr cât mai mare de cazuri (răniţi, pacienţi) şi pentru a reveni cât mai curând la situaţia normală.

În consecinţă, conceptul de triaj trebuie văzut într-un context mai larg şi are la bază următoarele elemente:

- evaluarea rapidă a tuturor victimelor dezastrului/conflictului armat;
- evaluarea naturii şi severităţii leziunilor;
- categorizarea/clasificarea (introducerea într-o categorie de triaj) cazurilor;
- resuscitarea, stabilizarea şi pregătirea pentru transport;
- distribuţia şi evacuarea cazurilor.

3. Procesul de triaj trebuie să fie:

-*Progresiv* – trierea iniţială va fi refăcută la nivelul ariilor de triaj desemnate pe parcursul lanţului evacuării (PMA - punctul medical avansat, spitalul ROLE 1 sau ROLE 2 etc.).

-*Continuu* – trebuie aplicat atâta timp cât există disproporţie între cererea de ajutor medical şi resursele medicale disponibile.

-*Dinamic* – pentru a asigura un triaj adecvat se vor lua lua în considerare o serie de factori:

- ○ numărul pacienţilor;
- ○ tipul, extensia şi severitatea leziunilor;
- ○ existenţa leziunilor asociate sau combinate (a poli-traumei);
- ○ mecanismul de producere şi prognosticul leziunilor;
- ○ modificările stării pacientului pe parcursul lanţului de ajutor medical;
- ○ efectul tratamentului;
- ○ vârsta pacientului;
- ○ cultura şi religia locală.

Decizia de triaj medical în dezastre devine mai complexă atunci când medicul trebuie să ia în considerare şi alţi factori, cum ar fi: efectele dezastrului, locaţia şi accesul la cazuri, condiţiile ostile de mediu (folosirea de arme chimice, condiţiile meteo nefavorabile etc.), întârzierea tratamentului, resursele limitate (personal, materiale, mijloace de transport), distanţa până la spital şi respectiv capacităţile de tratament al facilităţilor medicale primitoare.

De fiecare dată când va face triajul, personalul medical va avea de înfruntat două probleme majore şi de luat două decizii:

- ▪ Este resuscitarea şi/sau intervenţia chirurgicală necesară sau fezabilă?
- ▪ Care este prioritatea de tratament şi/sau de evacuare?

Pentru aceste motive principiul triajului este frecvent considerat o procedură medicală impersonală, inumană şi tipic militară, care este greu de asumat de medici şi mai ales de cei tineri care vor să-i salveze pe toţi pacienţii pe care îi au în îngrijire.

4. Consideraţii etice. Principiul triajului cere ca personalul medical să acţioneze în afara codului etic normal care are la bază buna stare medicală a individului, să ia în considerare prognosticul tuturor cazurilor şi să îi selecteze pe aceia care au cele mai bune şanse de recuperare, necesitând însă timpul minim de îngrijire şi cu folosirea unei cantităţi minime de resursele disponibile.

De aceea personalul medical implicat în procesul de triaj va trece *de la un sistem bazat pe etica individuală,* care are la bază o relaţie confidenţială aleasă liber de către pacient, o libertate de diagnostic şi terapeutică a medicului, precum şi folosirea a maximului de resurse medicale pentru fiecare pacient, *la un sistem bazat pe etica colectivă,* în care un „colectiv” de victime ale dezastrului va fi condus de un personal medical variat la diferite eşaloane ale lanţului de ajutor medical (LAM), tratamentul se va baza pe protocoale simplificate şi standardizate, iar resursele limitate vor fi alocate pentru un maximum de cazuri. Selecţia criteriilor care stau la baza triajului *nu trebuie lăsată pe seama personalului medical de la locul dezastrului*, deoarece în acele condiţii de la locul dezastrului pot apărea erori care pot duce la creşterea mortalităţii şi morbidităţii.

Principiile de Triaj şi Protocoalele pentru Situaţiile cu Cazuri Multiple (de masă) trebuie dezvoltate şi promulgate de autorităţile medicale competente în coroborare cu legislaţia în vigoare (Ministerul Sănătăţii, Direcţia Medicală a M.Ap.N.).

Implementarea deciziilor de triaj este o atitudine învăţată şi exersată care presupune cunoştinţe de:

- ❖ patologie (de evaluare a leziunilor);
- ❖ fiziopatologie (determinanţi anatomici şi fiziologici);
- ❖ cu privire la prognosticul leziunilor;
- ❖ şi cunoştinţe clare despre sistemul de urgenţă, resursele de personal şi materiale disponibile, dar şi despre capacitatea de tratament a facilităţilor medicale receptoare (primitoare) – spitalele de ROLE 1 şi 2 din teatrele de operaţii (TO).

Personalul medical desemnat pentru triaj trebuie să fie antrenat frecvent în procedurile de triaj, fiecare în funcția pe care urmează să o îndeplinească în echipa de triaj. Metodele de antrenament pot varia de la exerciții în sala de clasă, la exerciții pe câmp, cu simularea de cazuri reale (pacienți cu mulaje).

5. Modul de realizare a triajului medical. Triajul medical are la bază evaluarea numărului cazurilor, a naturii şi a severității leziunilor. Este esențială evaluarea rapidă a numărului leziunilor şi a leziunilor predominante. Această informație va fi comunicată de către comandantul detaşamentului medical eşaloanelor superioare responsabile de comandă şi coordonare pentru a uşura mobilizarea resurselor adecvate.

Examinarea medicală va fi întotdeauna simplă şi rapidă, fără a folosi mijloace de diagnostic avansate (doar măsurarea PA, a SpO_2 etc.), de aceea este foarte important:

- să fie realizată rapid pentru a nu întârzia evacuarea şi tratamentul definitiv al cazurilor;
- să aibă o acuratețe deosebită, să fie de încredere, deoarece fiecare eroare inițială de clasificare poate introduce un pacient critic într-o categorie de triaj uşoară, greşeală ce poate fi fatală pacientului, având în vedere afluxul mare de victime;
- să fie completă prin îndepărtarea hainelor şi a pansamentelor, lucru foarte important la pacienții cu poli-trauma, având în vedere că fiecare dintre leziuni contribuie la tabloul clinic general al acestora.

Ofițerul de triaj. Procedura de triaj este realizată de *ofițerul de triaj*, personaj învestit cu această funcție de comandantul unității/facilității medicale sau de şeful UPU.

Ofițerul de triaj trebuie să evalueze criteriile anatomice şi fiziologice, plus mecanismul de producere a traumei şi bolile asociate, să le interpreteze semnificația şi să-şi folosească experiența şi judecata clinică pentru a adopta cea mai bună decizie, luând în considerare şi capacitatea de ajutor medical pe care o are la dispoziție. Sarcina se va atribui unei persoane cu o experiență adecvată, prin aceasta înțelegând cea mai experimentată persoană şi nu cea mai în vârstă sau cea mai calificată din punct de vedere profesional.

Ofițerul de triaj poate fi:

- *la locul dezastrului* - un asistent medical experimentat sau un paramedic ;
- *în zona de triaj (zona de evacuare, zona de tratament)* – un medic de medicină de urgență şi un asistent medical;
- *în spital* – de obicei este un chirurg experimentat.

În orice caz, pentru ca o *echipă de triaj* să fie eficientă, membrii ei trebuie să fie antrenați în rolurile lor şi să aibă posibilitatea de a-şi menține aptitudinile profesionale prin practicarea profesiei lor într-un spital.

Luarea deciziei de triaj. Pentru a lua această decizie personalul medical implicat va ține seama de o serie de factori, care pot fi:

- *externi*: situația tactică şi misiunea de îndeplinit, posibilitatea de aprovizionare, vremea etc.;
- *interni*: aprovizionarea cu dispozitive şi materiale medicale, spațiul avut la dispoziție, capacitatea de îngrijire, personalul disponibil, existența stresului etc.

5.1. Clasificarea (categorizarea) victimelor dezastrelor şi principiile care stau la baza acestora

Proceduri de clasificare militară

a) Proceduri de triaj convenționale

Obiectivul procedurilor de triaj convenționale este de a utiliza efectiv şi eficient potențialul de tratament chirurgical şi medical, precum şi al mijloacelor de transport, pentru a asigura îngrijirea medicală optimă. Triajul se aplică la fiecare punct/facilitate medicală din lanţul de ajutor medical (LAM). Există 5 nivele de prioritate: urgent, imediat, întârziat, minim şi expectant (în aşteptare – muribund sau decedat).

Nivelul priorităţii determină momentul în timp la care se începe tratamentul şi/sau evacuarea, cu presupunerea că **TOATE CAZURILE** vor primi îngrijirea adecvată într-un interval de timp fixat anterior (de obicei primele 24 de ore).

URGENT (cod de culoare - roşu). Pacienţii din acest grup au nevoie de intervenţie medicală urgentă pentru a supravieţui. În această categorie intră pacienţii cu: asfixie, obstrucţie de CAS (căi aeriene superioare) de cauze mecanice, plăgi toracice aspirative, pneumotorax compresiv (“în tensiune”), plăgi maxilo-faciale cu asfixie sau cu risc de dezvoltare a asfixiei şi cu hemoragie internă ex-sangvinantă care nu răspunde la repleţia volemică agresivă. În această categorie mai intră majoritatea leziunilor cardiace, precum şi leziunile SNC cu alterarea statusului neurologic.

IMEDIAT (cod de culoare - roşu). Cazurile din această categorie au plăgi grave, care ameninţă viaţa, dar care necesită, pentru a supravieţui, proceduri chirurgicale de durată medie-scurtă. Aceste cazuri au o probabilitate mare de supravieţuire. Cazurile tind să rămână temporar stabile în timpul terapiei de repleţie volemică şi a evaluării (faza de supraveghere primară). Cuvântul cheie este ***temporar***. Din această categorie fac parte pacienţii cu următoarele patologii: plăgi toracice şi abdominale instabile, plăgi vasculare inaccesibile cu ischemie de membru, amputaţiile incomplete, fracturile deschise ale oaselor lungi, arsurile cu fosfor alb şi arsurile de gradul II şi III (clasificarea americană) pe ≥ 15-40% din suprafaţa corporală.

ÎNTÂRZIAT (cod de culoare - galben). Cazurile din această categorie pot tolera o întârziere înainte de intervenţia chirurgicală, fără a fi afectat însă prognosticul acestor pacienţi. Atunci când resursele medicale sunt depăşite, pacienţii din această categorie sunt ţinuţi pe loc/întârziaţi până când pacienţii din primele două categorii sunt îngrijiţi. Din această categorie fac parte:

- plăgile abdominale stabile cu leziuni viscerale probabile, dar fără hemoragie semnificativă; aceste cazuri pot fi întârziate 8-10 ore, după care există o corelaţie directă între timpul scurs până la intervenţie şi apariţia complicaţiilor;
- leziunile de ţesuturi moi care necesită debridare, plăgi maxilofaciale fără afectarea CAS, leziuni vasculare cu circulaţie colaterală adecvată, rupturi de tract urinar, fracturi care necesită intervenţie chirurgicală, debridare şi fixare externă şi majoritatea leziunilor oculare şi ale SNC.

MINIMAL (cod de culoare - verde). Această categorie cuprinde cazuri care sunt atât de uşoare, încât necesită toaletă, debridare minimă sub anestezie locală, administrarea de toxoid tetanic şi respectiv pansament de tip primul ajutor. Ei trebuie îndrumaţi rapid din aria de triaj spre zonele special amenajate, unde li se va acorda primul ajutor şi unde există personal medical nespecializat. În această categorie intră:

- arsurile pe mai puţin de 15% din suprafaţa totală a corpului, cu excepţia celor care afectează faţa, mâinile şi organele genitale;
- fracturile de membre superioare, luxaţiile, abraziunile, fazele iniţiale asimptomatice ale expunerii la radiaţii necuantificate, suspiciunea de leziuni prin explozie (perforaţie de timpan), modificări de comportament şi alte afecţiuni psihiatrice (PTSD).

EXPECTANT (cod de culoare - negru). Cazurile din această categorie au plăgi/leziuni atât de extinse încât chiar dacă ar fi singurul caz şi ar beneficia de îngrijirea medicală optimă, supravieţuirea ar fi puţin probabilă. Aceste cazuri vor fi separate de celelalte categorii de pacienţi, fără a fi însă abandonate. De aceea se vor lua măsurile paleative necesare pentru a le asigura confortul de către un personal competent. În această categorie intră următoarea patologie:

- TCC penetrante cu pacienţi în comă;
- leziunile înalte de măduva spinării;
- plăgile provocate de explozii care implică organe şi regiuni anatomice diferite;
- arsurile de gradul II-III pe suprafeţe de peste 60% BSA;
- convulsiile şi vărsăturile sub 24 de ore de la expunerea la radiaţii;
- şocul profund asociat cu leziuni multiple, respiraţia agonică.

b) Proceduri de triaj în situaţii cu cazuri multiple (de masă)

Existenţa unui aflux masiv şi brusc de cazuri, destul de mare pentru a depăşi resursele medicale disponibile, necesită o apropiere diferită pentru a tria cazurile. În aceste circumstanţe ***obiectivul serviciilor medicale*** este de a asigura îngrijirea medicală a unui număr cât mai mare de pacienţi – „de a face cât mai mult pentru cei mai mulţi”.

Termenul de „situaţii cu cazuri multiple” poate include „situaţii compensate”, atunci când resursele sunt adecvate şi „situaţii decompensate”, care apar mai ales în domeniul militar. Aceste situaţii pot fi temporare sau pentru o perioadă mai lungă de timp. Este la latitudinea comandantului local de a introduce în practică triajul în aceste situaţii.

Clasificarea în situaţiile cu cazuri multiple recunoaşte 4 categorii de triaj: imediat (roşu-T1), întârziat (galben-T2), minim (verde-T3) şi în aşteptare (negru-T4).

IMEDIAT – T1. Acest grup include acele victime care au nevoie de intervenţii chirurgicale pentru a le salva viaţa. Intervenţiile chirurgicale trebuie să nu fie consumatoare de timp şi se vor adresa pacienţilor cu cele mai bune şanse de supravieţuire (de exemplu, obstrucţia respiratorie, cazurile instabile cu leziuni toracice sau abdominale, amputaţiile de urgenţă etc.). Ordinul Ministrului Sănătăţii nr.487/2000 prevede categoriile de triaj pentru aceste situaţii cu cazuri multiple şi dezastre. Categoria de triaj T1- roşu include următoarele afecţiuni şi situaţii clinice:

- insuficienţa respiratorie acută, stopul cardiac resuscitat;
- hemoragia severă (> 1 litru);
- pacientul inconştient sau obnubilat, TCC sever;
- plăgile penetrante toracice/plăgile abdominale profunde;
- fracturile de pelvis, de torace, de coloană cervicală, de membre sau luxaţiile cu leziuni arteriale;
- arsurile complicate cu leziuni de căi aeriene, leziunile termice de inhalaţie;
- intoxicaţia cu CO.

ÎNTÂRZIAT – T2. Acest grup include acei răniţi care au nevoie de intervenţii chirurgicale consumatoare de timp, dar a căror stare generală permite o întârziere a intervenţiei chirurgicale fără a le pune în pericol viaţa.

Pacienţilor din această categorie li se va administra un tratament susţinut, intensiv care are la bază: abordul venos periferic, repleţia volemică agresivă, imobilizarea fracturilor, administrarea de antibiotice, cateterizarea vezicii urinare şi a stomacului, tratamentul analgezic etc. Afecţiunile care fac parte din această categorie sunt:

- arsurile de gradul II > 30% BSA (suprafaţa totală a corpului);
- arsurile de gradul III pe 10% BSA;
- arsurile de gradul III pe mâini, picioare, perineu şi faţă fără complicaţii respiratorii;
- arsurile complicate cu leziuni severe de ţesuturi moi sau fracturi minore;
- hemoragia moderată (între 500 - 1000 ml);
- leziuni ale spatelui cu / fără leziuni mielice;
- victime cu TCC (cu hematom subdural, cu confuzie) cu următoarele caracteristici:
  - i. licvoree (pierderi de LCR prin nas sau urechi);
  - ii. vărsături; modificarea frecvenţei respiratorii;
  - iii. hematoame periorbitale;
  - iv. anisocorie; răspuns motor absent (reflexele profunde absente);
  - v. bradicardie < 60/minut; creşterea rapidă a PA sistolice; colaps;
  - vi. pacient obnubilat (stupor).

MINIM - T3. Aceste cazuri au leziuni relativ minore, sunt pacienţi care pot avea singuri grijă de ei sau pot fi ajutaţi de personal non-medical. În această categorie intră:

- fracturile digitale şi dentare, contuziile, plăgile minore;
- arsurile minore, arsurile de gradul II pe ≈ 15% BSA;
- arsurile de gradul III pe ≈ 2% BSA;

- ○ arsurile superficiale (gradul I) > 20% BSA, cu excepţia mâinilor, picioarelor, perineului şi feţei;
- ○ PTSD (sindromul de stres post-traumatic).

Ultima categorie de afecţiuni nu exista în O.M.S. nr. 487/2000, a fost introdusă de subsemnatul pentru a clasifica şi acest tip de afecţiune, care se întâlneşte frecvent în toate tipurile de situaţii cu cazuri multiple/dezastre (de exemplu, atacurile teroriste, cutremurele etc.).

EXPECTANT – T4. Din această categorie fac parte două tipuri de victime: pacienţii care au răni aşa de grave încât chiar dacă ar fi singurul caz şi ar beneficia de toate resursele medicale, supravieţuirea lor nu ar fi posibilă şi, respectiv, pacienţii decedaţi. Aceste cazuri nu trebuie abandonate, ci vor fi separate de celelalte cazuri sub supraveghere medicală. Afecţiunile medicale care duc la încadrarea în această categorie sunt următoarele:

- ➢ arsurile de gradul II şi III > 40% BSA;
- ➢ arsurile de gradul II şi III > 40% BSA asociate cu:
    - fracturi importante;
    - TCC sever;
    - TCC deschis cu expunerea ţesutului cerebral şi în comă;
    - TCC sever asociat cu fracturi severe;
    - leziuni toracice;
    - leziuni de măduva spinării cu lipsa sensibilităţii şi motricităţii;
- ➢ leziunile severe la persoane cu vârsta > 60-65 de ani.

Categorii speciale de triaj. Sunt considerate categorii speciale de triaj următoarele tipuri de pacienţi, care apar în urma unor condiţii deosebite de luptă sau au un statut special:

- ➢ răniţii contaminaţi într-un mediu de luptă în care s-au folosit arme biologice şi/sau chimice;
- ➢ răniţii într-un război nuclear;
- ➢ prizonierii de război.

Pacienţii încadraţi în aceste categorii speciale necesită un management deosebit, deoarece personalul care intervine are nevoie de costume de protecţie personală speciale pentru a se putea face extragerea din mediu şi decontaminarea, după care urmează carantinarea (pentru pacienţii care au suportat un atac cu arme biologice). Prizonierii de război au un statut special deoarece în plus ei trebuie îngrijiţi sub pază armată.

Categorizarea cazurilor în situaţiile de dezastre civile

Majoritatea sistemelor de clasificare împart cazurile apărute în urma unui dezastru în patru mari grupe:

- ○ **"Prioritatea întâi - P1, categoria I, "imediat", "urgenţă" sau culoarea roşie – T1** indică nevoia imediată de intervenţie medicală;
- ○ **„Prioritatea a doua – P2", categoria a II-a, "întârziat" sau culoarea galbenă – T2** defineşte pacienţii care pot fi întârziaţi o perioadă limitată de timp (6-18 ore) fără a le pune viaţa în pericol imediat;
- ○ **„Prioritatea a treia – P3", categoria a III-a, "minim" sau culoare verde – T3** indică pacienţii care nu au răni serioase şi care vor supravieţui chiar dacă îngrijirea lor este întârziată ore sau zile (maximum 36 de ore);
- ○ **"Expectant" culoarea neagră /albastră, categoria a IV-a sau T4**, a fost adăugată pentru a defini pacienţii care sunt muribunzi, decedaţi, nu au şanse de a supravieţui sau sunt într-o stare atât de proastă încât pot fi salvaţi doar dacă se consumă toate resursele disponibile la acel moment, lăsând astfel fără îngrijire ceilalţi pacienţi. O parte din personalul medical are dificultăţi în a clasifica un pacient ca "expectant", deoarece într-o situaţie normală ar fi încercat să le salveze viaţa printr-un efort medical extraordinar. De

altfel este o decizie foarte grea, care este necesară atunci când există multe cazuri care necesită mai multe resurse (de orice tip) decât sunt disponibile.

Categorizarea cazurilor în situațiile de dezastre civile/militare în funcție de principiile ATLS

Principiile ATLS (Advanced Trauma Life Support) pot fi de asemenea folosite pentru a stabili prioritățile de tratament atât la facilitățile de ROLE 1 şi 2, cât şi la cele de ROLE 3. Categoriile de triaj sunt următoarele:

- **URGENT**: această categorie de triaj include pacienții cu risc de a deceda rapid după o traumă care afectează CAS şi care produce insuficiență respiratorie sau şoc care nu răspunde la tratamentul de stabilizare prevăzut de principiile ATLS. Intervenția chirurgicală de urgență va fi realizată în minute, pentru a putea acorda câteva şanse de supraviețuire acestui tip de pacient;
- **IMEDIAT**: această categorie de triaj include majoritatea cazurilor cu plăgi toracice sau abdominale care răspund la manevrele de salvare a vieții tip ATLS, dar şi pe cei cu leziuni extensive de țesuturi moi şi osoase, mai ales atunci când există şi o leziune majoră vasculară. Intervenția chirurgicală este necesară sub 6 ore de la traumatism;
- **ÎNTÂRZIAT**: această categorie de triaj include majoritatea cazurilor cu fracturi sau leziuni de țesuturi moi. Nu impune intervenții de tip ATLS, dar necesită intervenție chirurgicală între 12-24 de ore de la traumă;
- **MIMIM sau AMBULATOR**: această categorie de triaj include cazurile care sunt doar înregistrate. Ele se pot rezolva în ambulatoriu şi nu necesită evacuarea la eşaloanele superioare;
- **ÎN AŞTEPTARE - EXPECTANT**: această categorie de triaj include cazurile ale căror leziuni sunt atât de severe încât nu se aşteaptă supraviețuirea chiar în condițiile în care li se acordă îngrijirea medicală disponibilă. În această categorie intră cei cu moartea creierului sau cei cu arsuri profunde pe o suprafață mare a corpului. Aceste cazuri nu se evacuează de la eşaloanele care semnalează că au astfel de pacienți.

Principiile care stau la baza triajului - scoruri de care evaluează severitatea leziunilor

Pentru a putea evalua severitatea leziunilor, se impune ca personalul medical care realizează triajul să posede cunoştințe temeinice de anatomie, fiziologie, fizio-patologie şi cu privire la efectele bolilor asociate asupra prognosticului pacienților.

Severitatea leziunilor poate fi evaluată prin folosirea următoarelor criterii:

- criterii anatomice: regiunea anatomică afectată, gradul leziunii etc.;
- criteriile fiziologice (variabilele fiziologice) – presiunea arterială, pulsoximetria, pulsul periferic, statusul neurologic etc.;
- combinația de indicatori fiziologici, criterii anatomice şi mecanismul de producere al leziunilor (leziuni nepenetrante /penetrante);
- factorii morbizi asociați (vârsta, greutatea, prezența diferitelor afecțiuni – diabet zaharat, HTA, tratament anticoagulant etc.).

Personalul implicat în activitatea de triaj trebuie să cunoască o serie de sisteme de scor de evaluare a gravității traumei, cum ar fi: Trauma Score (TS), Revised Trauma Score (RTS), Injury Severity Score (ISS), scorul Teuşdea etc.

Champion a folosit scorurile de severitate pentru a evalua severitatea leziunilor pacienților în situațiile de dezastru obținând următoarele date (vezi tabelul nr.1)

.

În cursul procesului de triaj, dacă criteriile enumerate nu sunt aplicate corect pot apărea două tipuri de greşeli de triaj, şi anume “subtrierea” şi respectiv “supratrierea”. *Subtrierea* apare atunci când un pacient cu leziuni severe este triat şi trimis la facilităţi medicale unde resursele şi tratamentul sunt inadecvate (de exemplu, pacientul are TCC care necesită intervenţie chirurgicală şi ajunge la un spital de ROLE 2). Apare dacă criteriile de triaj nu sunt destul de sensibile.

Supratrierea apare atunci când un pacient cu leziuni minore este direcţionat spre un centru regional de trauma. Apare dacă criteriile de triaj nu sunt specifice.

Tabelul nr.1 Corelaţia între valoarea punctajului şi categoria de triaj

Prioritatea /categoria de triaj	**Scorurile de gravitate folosite**	
	TS (puncte)	**RTS (puncte)**
Imediat - T1	4-10	4-9
Intârziat - T2	11 – 12	10 – 11
Minim - T3	15 – 16	12
Expectant - T4	< 3	≤ 2

Oricare dintre cele două tipuri de greşeală de triaj poate creşte mortalitatea, morbiditatea sau poate determina folosirea inadecvată a resurselor limitate avute la dispoziţie.

Sisteme de marcare/etichetare (cartele de triaj) - TAGGING SYSTEMS

Sunt mai multe sisteme de marcare – cartele de triaj disponibile, una dintre cele mai cunoscute fiind METTAG – SUA (Medical Emergency Triage Tag – Cartela de Triaj pentru Urgenţe Medicale), care conţine o secţiune cu benzi colorate (roşu, galben, verde, negru) care se pot detaşa/rupe pentru a clasifica starea pacientului în respectivul moment al examinării.

În acest articol propun o ***cartelă de triaj*** (vezi figura nr.1) adaptată legii nr.95/2006 şi noilor condiţii de dotare ale sistemului medical militar de urgenţă din România. La alcătuirea cartelei au contribuit pe lângă studierea celorlalte tipuri de cartele de triaj şi cunoştinţele acumulate cu ocazia celor două mastere europene în medicina dezastrelor, precum şi cei 6 ani de studiu pentru finalizarea tezei de doctorat care a avut drept subiect poli-traumatismele şi scorurile de gravitate a leziunilor.

Cartela de triaj va fi completată şi agăţată de gâtului pacientului, ultima bandă colorată prezentă reprezentând categoria de triaj care dă prioritatea respectivului pacient.

Colţurile cartelei sunt numerotate pe o parte, iar pe cealaltă parte există simbolul unei ambulanţe (colţul va rămâne la şoferul ambulanţei) sau o cruce roşie (colţul va rămâne la medicul care a triat pacientul). Astfel va exista o evidenţă a pacienţilor cu privire la cine anume i-a îngrijit şi respectiv transportat, deoarece colţurile se pot detaşa. De asemenea cartela de triaj are şi importanţă medico-legală prin toate informaţiile pe care le conţine.

Noua cartelă de triaj propusă poate fi folosită şi în cercetarea ştiinţifică, deoarece conţine suficiente informaţii pentru a studia diferitele tipuri de dezastre şi conflicte militare, modul de producere a traumei, tipurile de leziuni care apar, evoluţia pacientului etc.

Acest tip de cartelă de triaj propusă de subsemnatul are două feţe şi mai multe secţiuni (vezi figura nr.1).

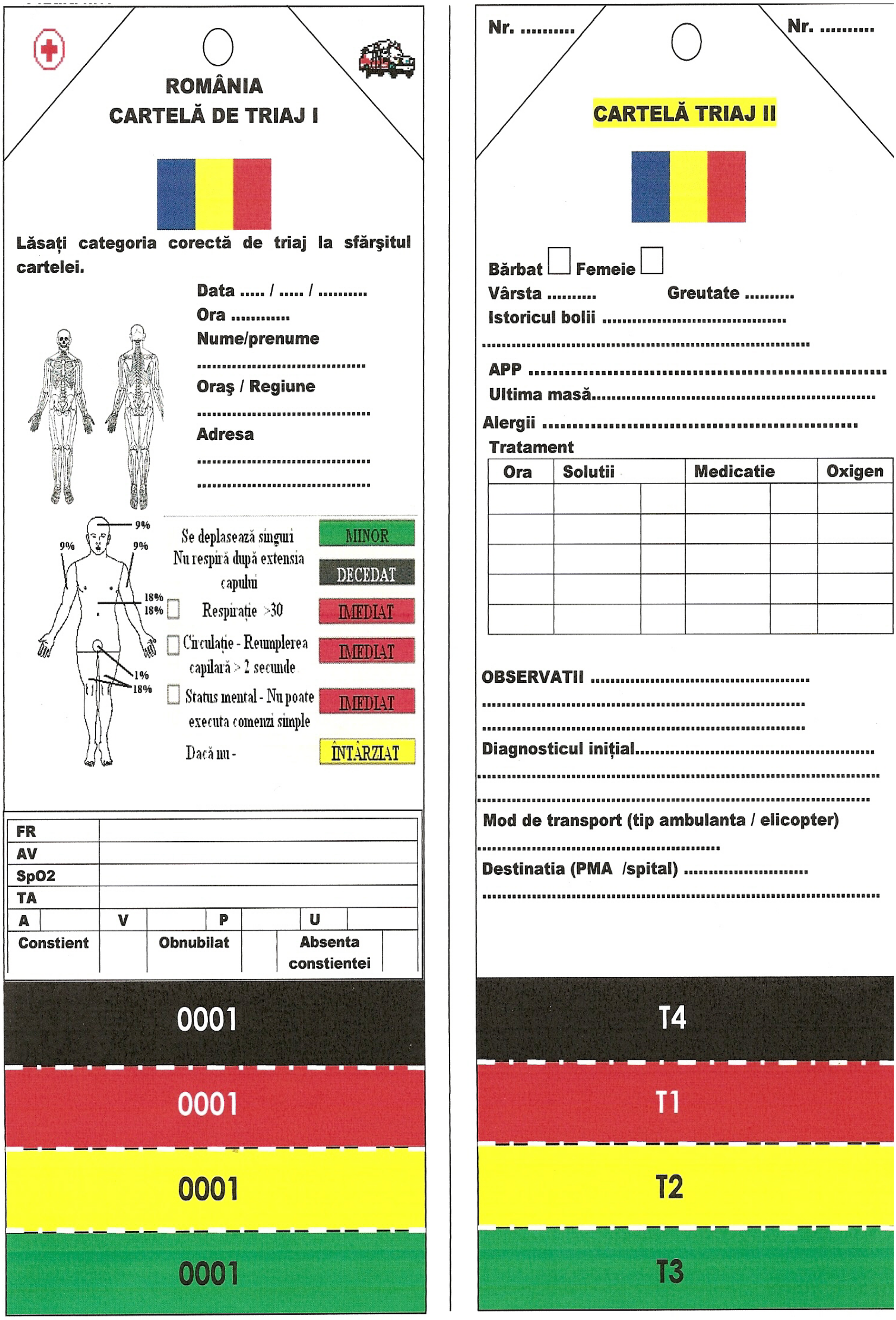
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ROMÂNIA
CARTELĂ DE TRIAJ I

Lăsați categoria corectă de triaj la sfârşitul cartelei.

Data ..... / ..... / ....
Ora ....
Nume/prenume
....
Oraş / Regiune
....
Adresa
....
....

Se deplasează singuri — MINOR
Nu respiră după extensia capului — DECEDAT
☐ Respirație >30 — IMEDIAT
☐ Circulație - Reumplerea capilară > 2 secunde — IMEDIAT
☐ Status mental - Nu poate executa comenzi simple — IMEDIAT
Dacă nu - ÎNTÂRZIAT

FR							
AV							
SpO2							
TA							
A		V		P		U	
Constient		Obnubilat		Absenta constientei			

0001
0001
0001
0001

Nr. ....
Nr. ....

CARTELĂ TRIAJ II

Bărbat ☐ Femeie ☐
Vârsta .... Greutate ....
Istoricul bolii ....
....
APP ....
Ultima masă...
Alergii ....
Tratament

Ora	Solutii		Medicatie		Oxigen

OBSERVATII ....
....
....
Diagnosticul inițial..
....
....
Mod de transport (tip ambulanta / elicopter)
....
Destinatia (PMA /spital) ....
....

T4
T1
T2
T3

Fig. 1

Prima faţă – CARTELĂ DE TRIAJ I are următoarele componente:

- ○ data şi ora examinării pacientului;
- ○ date paşaportale: nume, prenume, adresa, oraşul, numărul de telefon (atunci când este posibilă obţinerea acestora);
- ○ un tabel legat de aplicarea algorimului S.T.A.R.T., pentru a uşura trierea pacientului: dacă pacientul se deplasează singur – se va clasifica verde (T1), dacă nu respiră după extensia capului – se va clasifica negru (T4), dacă Frecvenţa Respiratorie (FR) este > 30/minut, va fi clasificat ca roşu (T1), dacă reumplerea capilară este > 2 secunde, va fi clasificat ca roşu (T1), dacă nu poate executa comenzi simple - va fi clasificat ca roşu (T1), iar dacă poate executa comenzi simple, dar are timpul de reumplere > 2 secunde, va fi clasificat ca galben (T2);
- ○ schema corporală care conţine şi scheletul osos, pentru a uşura evidenţierea leziunilor;
- ○ schema corporală cu „regula lui 9", pentru a uşura desenarea arsurilor atunci când ele există;
- ○ un tabel cu constantele vitale ale pacientului: frecvenţa respiratorie (FR), alura ventriculară/pulsul (AV), saturaţia periferică în oxigen (SpO_2), presiunea arterială sistolică (PAs), formula mnemotehnică AVPU şi referiri clare cu privire la statusul mental exact al pacientului – conştient, obnubilat, absenţa conştienţei (în comă);
- ○ la sfârşitul cartelei cu corespondenţă pe ambele părţi se află patru benzi (din care 3 se pot detaşa) care indică categoria de triaj. Cartela care rămâne ultima indică categoria de triaj. Pe benzi sunt înscrise numerele de identificare ale cartelei, care sunt înscrise şi pe unul dintre colţurile cartelei.
- ○ **Cartela conţine în partea superioară şi o indicaţie – „Lăsaţi categoria corectă de triaj la sfârşitul cartelei",** pentru a nu apărea confuzii cu privire la modul de semnalare a categoriei de triaj.

A doua faţă (revers) – CARTELĂ DE TRIAJ II - are următoarele secţiuni:

- ○ sexul, vârsta şi greutatea pacientului;
- ○ istoricul bolii – se vor nota modul de producere a leziunilor (plagă împuşcată, explozie, accident rutier, de tren, accident chimic etc.), semnele şi simptomele pe care le are pacientul;
- ○ antecedentele personale patologice (APP) – se vor nota antecedentele patologice ale pacientului, tratamentul cronic (anticoagulant, antiHTA etc.);
- ○ ultima masă – se va nota ora (cu aproximaţie) la care pacientul a luat ultima masă, informaţia este importantă pentru managementul CAS (căilor aeriene superioare), al intervenţiilor salvatoare de viaţă şi al celor chirurgicale de urgenţă;
- ○ alergii – se vor nota alergiile pacientului;
- ○ tratamentul aplicat pacientului (sub formă de tabel). Se vor nota: ora administrării, soluţiile perfuzabile, medicamentul, dacă s-a administrat oxigen;
- ○ observaţii – se vor nota elemente legate de starea pacientului, particularităţi ale cazului, leziunile majore, evoluţia acestuia;
- ○ diagnosticul iniţial;
- ○ modul de transport – se va nota mijlocul de transport: ambulanţă (tipul ambulanţei), elicopter, avion etc.;
- ○ destinaţia pacientului – se va nota destinaţia pacientului: spital, centru trauma etc.;
- ○ la sfârşitul cartelei cu corespondenţă pe ambele părţi se află patru benzi (din care trei care se pot detaşa) care indică categoria de triaj. Cartela care rămâne ultima indică

categoria de triaj. Pe benzi sunt înscrise categoriile de triaj: T4 pe banda neagră, T1 pe banda roşie, T2 pe banda galbenă şi respectiv T3 pe banda verde.

Cartela de triaj propusă poate fi folosită atât la nivelul facilității de ROLE 1, cât şi la nivelul facilităților de ROLE 2 şi respectiv 3. De asemenea, cartela (care însoţeşte obligatoriu pacientul) are şi calitatea de a face legătura dintre diferite structuri ale LAM, deoarece conține şi informații cu privire la starea pacientului la momentul primului triaj, la evoluția pacientului şi la tratamentul administrat.

5.2.Modul de realizare a triajului victimelor dezastrelor de orice tip. Triajul poate fi folosit în două scopuri principale: pentru tratament şi, respectiv, pentru evacuare.

Triajul pentru tratament

Sisteme fiziologice. Pentru a realiza triajul este necesar un sistem simplu, sigur, rapid şi reproductibil care să poată fi aplicat atât de medici, cât şi de asistenții medicali/paramedici. Se pot folosi sisteme fiziologice care caută evidențierea consecințelor leziunilor şi anume modificările semnelor vitale: frecvența respiratorie, frecvența pulsului şi timpul de reumplere capilară. Aceste sisteme sunt mult mai de încredere decât sistemele anatomice, care necesită cunoştințe clinice multiple, dezbrăcarea completă a pacientului şi investigarea paraclinică a acestuia.

Triajul tip "sită" – Triage Sieve

O metodă fiziologică de triaj larg acceptată (pentru tratament şi nu numai) este *triajul tip „sită”.* Acesta evaluează mobilitatea pacientului, libertatea CAS, respirația şi circulația.

Acest tip de triaj, şi nu numai, realizează doar o imagine de moment a cazului la ora evaluării. Pentru a identifica modificările în starea cazului, acesta va fi repetat la fiecare etapă a LAM. Este important ca inițial să ***nu*** încerci să prezici evoluția nefavorabilă a cazului, deoarece acest lucru va duce la „supra-triere”, care va încărca sistemul cu cazuri T1 şi T2.

Triajul Simplu şi Tratamentul Rapid - S.T.A.R.T. (Simple Triage and Rapid Treatment)

Pentru a putea realiza triajul este nevoie de o unealtă medicală care să faciliteze luarea deciziei de triaj, în acest scop fiind dezvoltate mai multe tipuri de astfel de unelte, dintre care cea mai folosită este S.T.A.R.T.

Sistemul S.T.A.R.T. (vezi tabelul nr.2) a fost dezvoltat mai ales pentru asistenții medicali şi pentru paramedici şi este un algoritm de triaj care aplicat corect permite introducerea victimelor dezastrelor în grupe de tratament adecvate. Aceştia trebuie să aibă cunoştințe temeinice de BLS – SVB (Basic Life Support –Suport de Bază al Vieții). Algoritmul permite evaluarea respirației, circulației şi statusului mental al pacientului în 60 de secunde sau mai puțin.

Pacientul trebuie triat înainte de a începe tratamentul, se stabileşte prioritatea, se poate scrie prioritatea pe fruntea pacientului, după care se trece la următorul pacient.

Evaluarea statusului mental se poate face de către medici cu scala Glasgow pentru come, dar restul personalului va folosi formula mnemotehnică AVPU, astfel:

- A – ALERT, pacientul este conştient, orientat temporo-spațial;
- V – VOCAL, pacientul este somnolent, răspunde doar la stimuli vocali;
- P – PAIN (durere), pacientul este obnubilat, răspunde doar la durere;
- U – UNRESPONSIVE (fără răspuns), pacientul este în comă, nu răspunde la durere sau alți stimuli.

Tabelul nr.2. Sistemul S.T.A.R.T. de triaj

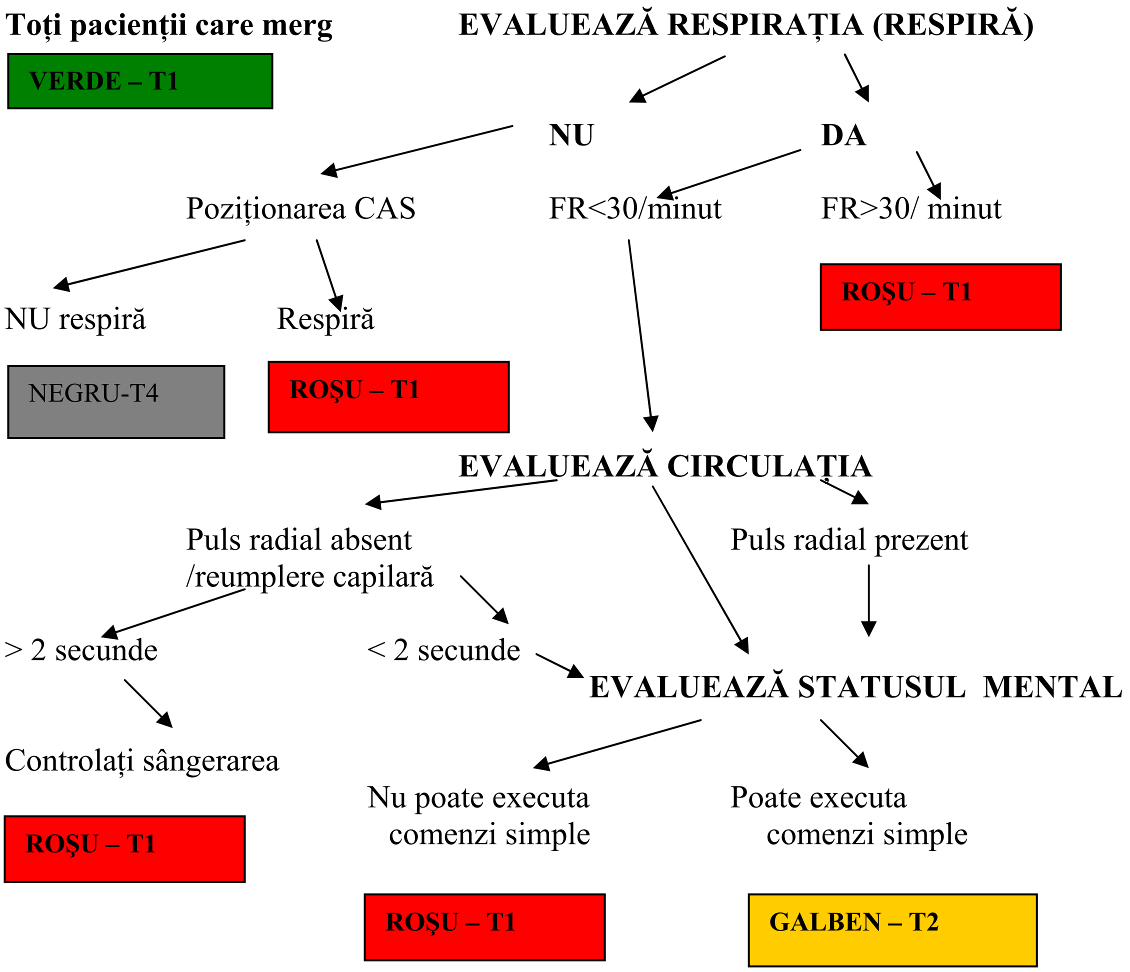
Toți pacienții care merg
VERDE – T1
EVALUEAZĂ RESPIRAȚIA (RESPIRĂ)
NU
DA
Poziționarea CAS
FR<30/minut
FR>30/ minut
ROŞU – T1
NU respiră
Respiră
NEGRU-T4
ROŞU – T1
EVALUEAZĂ CIRCULAȚIA
Puls radial absent /reumplere capilară
Puls radial prezent
> 2 secunde
< 2 secunde
EVALUEAZĂ STATUSUL MENTAL
Controlați sângerarea
Nu poate executa comenzi simple
Poate executa comenzi simple
ROŞU – T1
ROŞU – T1
GALBEN – T2

Tratamentul care poate fi administrat răniților la nivelul facilităților medicale în urma aplicării triajului fiziologic este următorul:

- **Facilități de ROLE 1** – se aplică tratament pentru a restaura şi stabiliza funcțiile vitale;
- **Facilități de ROLE 2** - se realizează resuscitarea, stabilizarea cazurilor şi intervenții chirurgicale de tip „damage control – control al leziunilor”, iar pacienții pot fi reținuți câteva zile;
- **Facilități de ROLE 3 -** se pot realiza spitalizarea pacienților şi intervenții chirurgicale care salvează viața, dar şi intervenții chirurgicale definitive;
- **Facilități de ROLE 4** - se realizează intervenții chirurgicale de înaltă specializare consumatoare de timp şi de îngrijiri medicale pe termen lung.

Triajul pentru evacuare – Triage Sort

Atunci când personalul şi resursele sunt limitate şi nu se poate face Triage Sieve se va folosi Triage Sort. Acest sistem de triaj foloseşte scorul Revizuit pentru Trauma (RTS), şi anume: frecvența respiratorie, presiunea arterială sistolică şi scorul Glasgow pentru come, pentru a da puncte (de la 0 la 12) cazurilor. În acest fel răniții pot fi introduşi într-una dintre categoriile de triaj, stabilind astfel prioritatea pentru evacuare şi/sau nevoia pentru intervenții suplimentare (vezi tabelul nr.1). Este o metodă mai complexă, deoarece necesită cunoştințe solide de evaluare a statusului neurologic (GCS).

Modul de realizare a procesului de triaj este asemănător, în timpul procesului de triaj urmărindu-se constantele fiziologice (mobilitatea pacientului, respirația, circulația şi statusul neurologic).

Metodele de evaluare sunt însă diferite, personalul medical urmărind: mobilitatea pacientului, frecvenţa respiratorie, presiunea arterială sistolică/prezenţa pulsului la artera radială/reumplerea capilară, statusul neurologic (AVPU sau GCS). În text este descris separat triajul pentru tratament şi respectiv pentru evacuare doar din motive didactice. Utilizarea lor separată pentru tratament şi respectiv evacuare este indicată atunci când se folosesc la nivelul facilităţilor de ROLE 2 şi respectiv 3, care au metode de evaluare mai avansate şi personal cu pregătire superioară.

Sistemele anatomice. Sunt greu de întrebuinţat în condiţiile de pe câmpul de luptă din teatrele de operaţii. Criteriile anatomice folosite definesc leziuni specifice ale organelor şi sistemelor de organe, lucru ce presupune un diagnostic definitiv, ceea ce înseamnă folosirea acestui tip de scor în faza spitalicească a ajutorului medical, la nivelul UPU (Unitate de Primiri Urgenţe).

Criteriile anatomice sunt în legătură directă cu regiunea lezată: cap şi gât/torace/ abdomen/regiunea dorsală/pelvis/piele şi extremităţi, gravitatea fiind corelată cu aceasta. Scorurile anatomice utilizează criterii anatomice de evaluare, unelte care apreciază severitatea leziunii în corelaţie cu organele lezate sau locul leziunii.

6. Resuscitarea, stabilizarea şi pregătirea pentru evacuare şi transport a victimelor dezastrului

Resuscitarea şi stabilizarea la locul dezastrului sunt necesare datorită impactului deosebit pe care îl au asupra prognosticului cazurilor: prevenirea numeroaselor decese timpurii, prelungirea timpului limită pentru tratamentul definitiv şi, nu în ultimul rând, schimbarea categoriei de triaj, ceea ce înseamnă schimbarea priorităţilor de tratament şi evacuare.

Procedurile folosite sunt similare celor utilizate în sistemul de urgenţă obişnuit de zi cu zi (la nivelul UPU) pentru a obţine un răspuns imediat şi eficient.

6.1. Organizarea, personalul şi intervenţia propriu-zisă. Toate cazurile trebuie să treacă printr-o *arie de triaj unică* şi este necesar să fie supuse unei evaluări rapide de către *un ofiţer de triaj iniţial* care face parte dintr-o echipă de triaj.

În zona de triaj **NU SE FAC TRATAMENTE MEDICALE DE NICIUN FEL**, aici se face doar triajul medical al pacienţilor rezultaţi în urma dezastrelor. Cazurile vor fi trimise/transportate rapid în aria de tratament adecvată pentru îngrijire.

O zonă de triaj (Z.T.) ideală îndeplineşte următoarele condiţii:

- este învecinată cu zona de primire a cazurilor - punctul de adunare răniţi, cu zona de evacuare şi, respectiv, cu zona de decontaminare;
- are un sens unic spre şi în interiorul zonei de triaj, pe rute separate care să fie uşor identificate, marcate (semne, culori, lumini chimice etc.) şi de aici spre zona de tratament;
- spaţiul pentru triaj va fi bine luminat, acoperit, suficient de mare, pentru a permite un acces uşor, o evaluare adecvată şi un transport rapid şi uşor al cazurilor în şi în afara acestui perimetru;
- are desemnat un spaţiu dedicat pentru identificarea, marcarea şi înregistrarea dispoziţiei iniţiale de triaj (de la locul dezastrului);
- are asigurat un număr suficient de tărgi, de toate tipurile (controlate de un subofiţer sanitar), astfel încât să permită un flux continuu al cazurilor.

Ideal, un chirurg sau un medic de medicină de urgenţă /de medicină generală cu atestat în urgenţe cu experienţă în plăgi de război trebuie folosit în Z.T. din facilitatea de ROLE 2. ***Din nefericire***, folosirea unui chirurg în afara sălii de operaţie este un lux pe care puţine unităţi medicale şi-l pot permite (doar facilităţile de ROLE 3 şi respectiv ROLE 4). De aceea, este esenţial ca o altă persoană cu experienţă clinică să fie antrenată pentru a prelua această sarcină, şi anume un (o) asistent(ă) medical(ă), mai ales la facilităţile de ROLE 1 şi 2.

Aceste antrenamente se pot realiza prin organizarea de exerciţii civile/militare cu simularea de diferite situaţii cu cazuri multiple (de masă), în cursul lor putându-se identifica persoana/persoanele capabile să îndeplinească acest rol în situaţii reale în absenţa unui chirurg.

6.2. Misiunea de dispecer şi de evacuare a cazurilor. Reglementarea medicală a cazurilor (dispeceratul) este un proces care coordonează evacuarea cazurilor de la locul dezastrului la facilitățile medicale adecvate, permiţând folosirea eficientă a resurselor din spitale. Victimele dezastrului trebuie evacuate în mijloace de transport adecvate, sub supraveghere medicală, folosind aceleaşi categorii de triaj de mai sus, astfel: cei care au categoria **T1 - roşu** vor fi transportaţi primii, urmaţi de categoria **T2 – galben** şi, respectiv, celelalte categorii de triaj, **T3** şi, ultimii, **T4**.

Procesul de dispecerat poate fi influenţat de numeroşi factori, cum ar fi:

- ➢ capacitatea de tratament a facilităților medicale primitoare;
- ➢ capacitățile de tratament specializat;
- ➢ capacitățile de transport din zona afectată;
- ➢ limita de timp pentru tratament definitiv;
- ➢ distanța până la facilitățile medicale primitoare;
- ➢ numărul şi starea clinică a cazurilor.

Comunicațiile eficiente sunt critice în acest proces de reglementare. De aceea trebuie să existe o *rețea de comunicație dedicată*, care să fie disponibilă pentru coordonarea medicală la locul dezastrului şi pentru procesul de dispecerat şi, respectiv, de transport al cazurilor la facilitățile medicale primitoare.

III. CONCLUZII

1. ***Triajul este un proces*** şi nu o procedură singulară realizată o singură dată de-a lungul LAM. Procedura de triaj trebuie realizată de către cei mai experimentaţi medici/asistenți medicali la diferitele nivele ale acestui lanţ.

2. ***Triajul în situații de dezastre nu este identic cu triajul într-o UPU în situații obişnuite***. Prioritatea se va acorda acelor pacienți care au cel mai mare beneficiu în urma tratamentului. Pacienții care au probabilitate mare de a deceda chiar cu un tratament adecvat şi cei care vor trăi fără tratament au prioritate scăzută (mică).

3. ***Triajul este un proces dinamic***. Decizia de triaj este influenţată atât de criteriile anatomice şi fiziologice, cât şi de factori care țin de organizare şi respectiv de mediu.

4. ***Triajul este un proces continuu***, el va fi repetat la fiecare nivel al LAM.

5. ***Triajul este un proces progresiv,*** începând cu un triaj preliminar la locul dezastrului şi un triaj mai amănunţit al cazurilor pe măsură ce pacienții avansează pe lanțul de ajutor medical (LAM).

6. În timp ce principiile de triaj sunt aceleaşi la diferite nivele ale LAM, ***aplicarea lor este flexibilă,*** în funcție de natura dezastrului şi de o serie de alți factori. Judecata clinică şi organizarea sistemului, asociate cu antrenamentul şi experiența personalului implicat au o valoare inestimabilă.

7. ***Metodele de triaj militare,*** care au o eficacitate dovedită în timp, par să aibă cea mai mare valoare în caz de dezastre. Aceste proceduri de clasificare militare este necesar să fie adaptate la tipul de patologie care apare în dezastrele civile, precum şi la logistica civilă. Pentru a obține cel mai mare beneficiu pentru cel mai mare număr de victime (răniți), cazurile trebuie introduse într-una dintre următoarele categorii de triaj:

- ○ **T1 – roşu - grupul de tratament imediat**. Cazurile din această categorie au nevoie de manevre medicale şi/sau intervenții chirurgicale care salvează viața cu şanse foarte bune de a supraviețui. Procedurile de tratament nu trebuie să fie consumatoare de timp.
- ○ **T2 – galben - grupul de tratament întârziat**. Cazurile necesită tratament în spital, dar starea lor permite o întârziere a tratamentului fără a le pune în pericol viața. Măsurile de suport medical administrate au rolul de a reduce efectele întârzierii tratamentului medical.
- ○ **T3 – verde - grupul de tratament minimal**. Sunt pacienți răniți uşor şi /sau care se pot deplasa singuri, care au nevoie de un tratament în ambulatoriu sau de îngrijiri minore care pot fi acordate şi de personalul nespecializat.
- ○ **T4 – negru - grupul de tratament în aşteptare**. Sunt pacienți răniți grav, frecvent cu multiple leziuni care au nevoie de tratament extensiv şi intensiv cu şanse mici sau zero de supraviețuire. Aceştia însă nu vor fi abandonați, ci li se va crea o zonă specială, unde li se

va administra un tratament paleativ, sub supraveghere. Dacă situaţia de dezastru intră sub control, tratamentul definitiv se va administra cât mai repede posibil celor care mai au şanse de supravieţuire.

8. *Sistemele de triaj trebuie standardizate* atât la nivel naţional, cât şi la nivel internaţional. Astfel politica de triaj standardizată va putea fi aplicată atât la nivelul situaţiilor de dezastre/cu cazuri multiple (de masă), cât şi al conflictelor militare.

9. *Cartela de triaj propusă* este uşor de completat, este clară şi conţine toate informaţiile necesare pentru a evalua pacientul şi a-l introduce într-o categorie de triaj. Ea mai conţine şi toate informaţiile necesare cu privire la modul de producere a traumei, tratamentul aplicat, modul de transport al pacientului etc., precum şi informaţii cu caracter medico-legal. De asemenea cartela de triaj face legătura între diferitele etape ale LAM, având toate informaţiile necesare cu privire la starea pacientului, evoluţia acestuia, tratamentul aplicat etc., uşurând urmărirea acestuia. În plus, ea mai poate fi folosită şi în cercetarea ştiinţifică a traumei, a patologiei dezastrelor etc.

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Esofagita eozinofilică
(aspecte etiopatogenice, clinice, diagnostice şi terapeutice)

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Rezumat. Esofagita eozinofilică este o suferință inflamatorie cronică a esofagului ce se prezintă cu simptome nespecifice diferite în funcție de vârstă: disfagie, vărsături, scădere ponderală, durere retrosternală, impactarea esofagiană a alimentelor solide. Apare în egală măsură atât în copilărie, cât şi la vârsta adultă. Caracteristica histopatogenică este infiltratul inflamator bogat în eozinofile la nivelul mucoasei esofagiene. Pacienții prezintă, cu o frecvență mai mare decât în populația generală, boli atopice precum: astmul bronşic alergic, rinita alergică, dermatita atopică. Alergia alimentară de tip imediat sau de tip întârziat este recunoscută ca fiind implicată în patogenia bolii, ceea ce face ca o mare parte din managementul esofagitei eozinofilice să fie reprezentată de dietele restrictive alergen specifice. Consultul alergologic permite abordarea terapeutică optimă a bolii.

Cuvinte cheie: eozinofile, esofagită, alergie alimentară, disfagie

Abstract. Eosinophilic esophagitis is a chronic inflammatory disorder of the esophagus which presents with unspecific and age related symptoms: dysphagia, vomiting, weight loss, chest pain, esophageal solid food impaction. Equaly targets children and adults. The pathogenic feature is the inflammatory eosinophilic infiltrate located in esophagian mucosal layer. The prevalance of atopic diseases (asthma, allergic rhinitis, atopic dermatitis) is increased. Food allergy (inmediate type and delayed type) is involved in disease pathophisiology, which renders dietary manipulation important position in the management of eosinophilic esophagitis. Allergy exam permits optimal therapeutical decisions.

Esofagita eozinofilică (E.E.) este un sindrom clinicopatologic a cărui recunoaştere este din ce în ce mai evidentă în mediul medical. În ultimii ani a crescut în mod simțitor numărul publicațiilor care relatează despre cazurile de E.E., despre aparițiile intrafamiliale, despre patogeneza şi opțiunile terapeutice. Histopatologic, suferința se caracterizează printr-un infiltrat inflamator eozinofilic dens limitat la nivelul mucoasei esofagiene[12]. Implicarea eozinofilului la nivelul altor segmente ale tractului digestiv a dus la definirea şi a altor entități nosologice: gastrita eozinofilică, enterita eozinofilică, gastroenterita eozinofilică, colita eozinofilică. Majoritatea studiilor estimează că prevalența esofagitei eozinofilice în populația generală variază între 1 şi 4 din 10 000 indivizi de toate vârstele. Un studiu demografic recent, din 2007, apreciază că 1 din 2 000 copii au E.E, prin incidență apropiindu-se de boli deja consacrate precum boala Crohn sau colita ulcerohemoragică, care, de asemenea, implică, într-o manieră de mai discretă amploare, inflamația eozinofilică. Datorită lipsei de sensibilizare a comunității medicale față de această problemă, diagnosticul E.E. este, adesea, întârziat, fapt care implică şi o aplicare tardivă a măsurilor terapeutice specifice acestei boli (dietetice şi medicamentoase).

Celelalte boli eozinofilice mucosale digestive enunțate sunt mult mai rare.

Eozinofilul este o celulă produsă în măduva hematoformatoare, transportată pe cale sanguină în țesuturile periferice sub influența unor factori chemotactici (interleukina 5, eotaxina), a cărei maturare se desăvârşeşte la acest nivel , îndeosebi în teritoriul mucoaselor (această poziționare este în legătură cu rolul major al eozinofilului la interfața dintre mediul extern şi cel intern, cu precădere fiind cunoscută implicarea lui în apărarea antiparazitară şi în derularea inflamației alergice). Principalele boli în patogeneza cărora intervine eozinofilul, grupate după organul specific interesat, sunt prezentate în tabelul următor.

Se poate observa caracterul extrem de heterogen al suferințelor în patogeneza cărora este implicat, în măsuri diferite, eozinofilul. Infiltrarea mucoasei esofagiene cu eozinofile poate fi primară (ca în esofagita eozinofilică primară, ce constituie subiectul lucrării de față) sau poate fi secundară (în cadrul sindromului hipereozinofilic sau în cadrul refluxului gastroesofagian; în această din urmă variantă numărul de eozinofile este doar discret crescut).

Principalele boli în patogenia cărora intervine eozinofilul

Specificitatea de organ	**Boli**
Tegumentul şi țesutul subcutanat	Dermatita atopică, pemfigoidul bulos, pemfigusul vulgar, dermatita herpetiformă, urticaria, foliculita eozinofilică pustulară, angioedemul cu eozinofile, reacții alergice medicamentoase
Plămânul şi tractul respirator	Rinita alergică, astmul bronşic alergic, sindromul Loeffler, aspergiloza bronhopulmonară alergică, pneumonia eozinofilică, sindromul Churg Strauss, granulomul eozinofilic
Tractul gastrointestinal	Esofagita eozinofilică, gastrita eozinofilică, enterita eozinofilică, colita eozinofilică, infecții parazitare, infecții fungice, refluxul gastroesofagian, alergia alimentară, enteropatia indusă de proteine, boala inflamatorie intestinală
Afecțiuni neurologice	Infecții ale SNC, şuntul ventriculoperitoneal, variate reacții adverse induse de medicamente
Afecțiuni reumatologice	Sinovita eozinofilică, sindromul mialgie-eozinofilie, vasculite eozinofilice (ex. Sindromul Churg-Strauss)
Cordul	Afectarea cordului este citată secundar unor suferințe sistemice precum sindromul hipereozinofilic sau sindromul Churg-Strauss (miocardită, pericardită, endocardită, cardiomiofibroză, vasculită coronariană)
Rinichiul	Cistita eozinofilică, nefrite interstițiale

Esofagita eozinofilică a fost descrisă pentru prima dată, la un pacient adult, în 1975. În următorii 20 de ani, au fost raportate în literatură cazuri sporadice şi studii pe loturi mici de pacienți. Abia în 1995, Kelly[3] prezintă 10 pacienți cu eozinofilie esofagiană izolată, nonresponsivă la antiacide dar care s-a ameliorat la formula dietetică elementală (formată numai din aminoacizi). Acest raport a fost primul care a sugerat că E.E. este o entitate patologică distinctă. După 1995 s-a înregistrat o creştere dramatică a numărului de cazuri diagnosticate şi a numărului de articole publicate în legătură cu E.E. În Japonia, abia în 2006 a fost raportat primul caz de E.E. şi nu există în prezent vreun continent, cu excepția Africii, în care să nu fi fost semnalată. Rapoartele apărute în literatură sugerează prevalența în creştere a E.E. Astfel, Noel[2] a descris o incidență a E.E. de 1 la 10 000 copii cu o creştere a prevalenței de 4 ori din 2000 până în 2003. Straumann[5] a prezentat date similare la adult, cu o incidență de 6 la 100 000 şi cu o creştere de la două cazuri în 1994 la 72 cazuri în 2003.

6,8% din pacienții cu esofagită prezintă caracteristici histologice de E.E., aşa cum arată Fox[1] într-un studiu din 2002.

Deoarece pediatrii, interniştii şi medicii de familie sunt în prima linie de abordare a pacienților cu afecțiuni cronice, este bine ca aceştia să cunoască modul de prezentare şi de evaluare a pacienților susceptibili să aibă E.E. Deşi evoluția naturală a E.E. este necunoscută, ea afectează persoane de orice vârstă. Astfel, copiii prezintă mai ales simptome de reflux gastro-esofagian (vomă, regurgitaţie, epigastralgii), nou-născuţii - iritabilitate, refuzul alimentaţiei şi întârziere în creştere iar adolescenţii şi adulţii - disfagie, durere toracică sau impactarea intra-esofagiană a alimentelor. Diagnosticul de E.E. poate fi precizat doar cu ajutorul endoscopiei digestive şi al biopsiilor. Deşi eozinofilele se pot identifica la nivelul laminei propria a tractului gastrointestinal căptuşit cu epiteliu unistratificat columnar, ele nu se găsesc în mod normal la nivelul epiteliului scuamos esofagian. Prezenţa lor la acest nivel este patologică. În trecut E.E. era frecvent confundată cu boala de reflux gastro-esofagian (BRGE). E.E. este o afecţiune distinctă şi nu răspunde la medicaţia antireflux indiferent de dozele de inhibitori de pompă de protoni utilizate. În majoritatea cazurilor, gradul eozinofiliei esofagiene la pacienţii cu E.E. este semnificativ mai mare (peste 15 eozinofile pe câmp microscopic) comparativ cu pacienţii care au BRGE (frecvent sub 5 eozinofile pe câmp microscopic, şi numai în esofagul distal). Gastroenterita eozinofilică (G.E.) determină la rândul ei creşterea eozinofilelor din esofag, dar acestea sunt crescute şi în alte segmente ale tractului digestiv.

Rolul eozinofilului în inflamaţia de tip alergic, coroborat cu observaţii referitoare la efectele benefice ale dietelor hipoalergenice (elementale şi restrictive) la pacienţii cu E.E., dar şi studiile care arată o prevalenţă crescută a hipersensibilizărilor de tip imediat (IgE mediate) la alergene alimentare şi respiratorii, precum şi frecvenţa mai mare faţă de populaţia generală a bolilor alergice cunoscute (astmul bronşic, rinita alergică, dermatita atopică), reprezintă tot atâtea motive pentru care E.E. a fost considerată o boală alergică. Poziţia actuală a comunităţii ştiinţifice medicale nuanţează acest punct de vedere. 80% din populaţia pediatrică şi 60% din populaţia adultă cu E.E. prezintă concomitent una sau mai multe din bolile alergice cunoscute.

E.E. este considerată o afecţiune cronică tratabilă care necesită restricţionare alimentară persistentă sau terapie medicală constantă.

Caracteristici clinice

Deşi majoritatea pacienţilor diagnosticaţi cu E.E. sunt tineri, caucazieni (>90%) şi bărbaţi (>65%), E.E. poate apărea la orice vârstă. Pacienţii prezintă unul sau mai multe dintre următoarele simptome: vomă, regurgitaţii, greţuri, epigastralgii sau dureri retrosternale, disfagie, hipersalivaţie, senzaţie de nod în gât sau apetit alimentar redus. Mai puţin frecvente sunt deficitul de creştere, hematemeza şi dismotilitatea esofagiană. Simptomele pot fi frecvente şi severe la unii pacienţi sau cu totul uşoare şi rare la alţii. Peste 50% dintre pacienţi, aşa cum am arătat, au concomitent afecţiuni atopice, incluzând astmul, dermatita atopică sau rinita alergică. Aproximativ jumătate dintre pacienţi au unul sau ambii părinţi cu istoric de boli alergice[12].

E.E. va trebui luată în consideraţie întotdeauna la pacienţii cu simptome severe sau refractare de reflux gastroesofagian în pofida utilizării maximale a inhibitorilor de pompă de protoni.

În mod curent, mulţi adulţi cu E.E. sunt diagnosticaţi la prezentarea în urgenţă datorită impactării esofagului cu bolul alimentar. Cum însă tot mai mulţi gastroenterologi şi imuno-alergologi sunt sensibilizaţi faţă de posibilitatea prezenţei acestei boli, simptome necorelate altădată cu E.E. (pirozisul, hipersalivaţia, epigastralgia) pot fi depistate la aceşti pacienţi. Stenoza subglotică şi edemul laringian refractar la tratamentul anti-reflux pot să mascheze E.E. concomitentă. Expunerea profesională a fost implicată în apariţia E.E. la doi adulţi, un brutar cu alergie la grâu şi un antrenor de călărie cu alergie la fân (Dauer, 2006)[6].

Istoricul familial este de asemenea important şi va fi luat în consideraţie. Un studiu recent prezintă 17 pacienţi cu disfagie, eozinofilie sau inel Schatzki din 7 familii în care E.E. a fost depistată pe parcursul a trei generaţii (Zink, 2007)[7].

Fiziopatologie

Iniţial etiologia E.E. a fost considerată multifactorială. Chiar din start, a fost incriminat refluxul gastroesofagian dar, după ce s-a observat că medicaţia antiacidă şi chirurgia antireflux nu au dat rezultate, au fost propuse alte cauze. Modelele de laborator au sugerat că aeroalergenii pot fi responsabili. Deşi alergenele inhalate (aeroalergene) pot avea un rol, nu au fost încă direct implicate drept cauză primară a E.E., deşi sunt câteva studii care sprijină această ipoteză. Astfel, pe modele murine Mishra[8] a indus eozinofilia la nivel esofagian la şoarecii cu sensibilizare alergică la un aeroalergen fungic (Aspergilus) după ce aceştia au fost expuşi intranazal la aeroalergenul incriminat. Fogg [9] raportează cazul unui pacient ce prezintă exacerbare sezonieră a E.E. (simptome şi modificări histologice) în strânsă relație cu evoluția sezonieră a nivelului polenic în atmosferă. Alergenele alimentare, în schimb, au fost legate direct de această afecțiune. Mai multe studii au demonstrat rezoluția simptomatologiei şi normalizarea histologiei țesutului esofagian la pacienții plasați pe dietă bazată strict pe aminoacizi.

Un studiu recent desfăşurat la Madrid (Lucendo, 2007)[10] pe 35 pacienți adulți cu E.E. a folosit imunohistochimia şi stereologia (observarea tridimesională a celulelor) şi i-a comparat cu pacienții cu BRGE şi cu lotul normal. Densitatea eozinofilelor din esofag a fost de 300 de ori mai mare la pacienții cu E.E. comparativ cu loturile de control. Mai mult, eozinofilele erau activate. Deşi limfocitele T şi mastocitele erau în număr crescut la pacienții cu BRGE, în cazurile de E.E. numărul lor era şi mai mult crescut.

Numărul mastocitelor şi gradul lor de activare sunt în strânsă legătură cu gradul de activitate al E.E. Este cunoscut faptul că proteina bazică majoră (MBP) produsă de eozinofil induce activarea şi degranularea mastocitului (cu eliberarea mediatorilor preformați - histamina, triptaza, chimaza, carboxipeptidaza A -, dar şi a celor formați de novo- interleukinele 4, 5, leucotrienele). Legătura dintre mastocit şi eozinofil este complexă, se realizează în ambele sensuri (Interleukina 5 produsă de mastocit asigură chemotaxia şi maturarea eozinofilului) şi se desfăşoară într-un mediu umoral şi celular inflamator în care predomină limfocitele T helper 2 şi citokinele acestui profil limfocitar (IL4, IL5, IL13, eotaxina), toate acestea fiind caracteristice inflamației alergice de tip imediat (IgE mediate). Mastocitele, unele prezentând pe suprafața lor IgE alergen specifice orchestrează acest proces inflamator, rolul lor detaliat în patogeneza E.E. nefiind însă pe deplin caracterizat, deoarce s-a observat că o mare parte dintre ele se activează prin mecanisme non imunologice, IgE nespecific; acest fapt este pus în legătură cu o ipoteza patogenică predominantă ce consideră componenta alergică de tip imediat (IgE alergen specifică) importantă, însă fără a-i acorda credit plenar. Prezența testelor cutanate alergologice de tip patch pozitive la diverse alimente la pacienții cu E.E. sugerează implicarea în fiziopatogeneză şi a unui mecanism alergic de tip întârziat (nonIgE mediat). Plasmocitele intraepiteliale au fost observate numai la pacienții cu E.E.

Un studiu al lui Yamazaki[11] din 2006 a analizat răspunsul specific la alergen *in vitro* (la alimente şi aeroalergeni) al celulelor mononucleare din sângele periferic la 15 pacienți cu E.E. comparativ cu 9 subiecți de control, sănătoşi. Producția de IL-5 a crescut la lapte, soia, arahide, praf, polen de Ambrozia, ceea ce sugerează existența unei sensibilizări la aceste alergene.

În 2007 Aceves[2] a observat remodelare semnificativă esofagiană la copiii cu E.E. S-au efectuat analize imunohistochimice cantitative şi au fost studiate mai multe chemokine la pacienții cu E.E., cu BRGE şi la subiecți normali. Biopsiile esofagiene au revelat, în cazul E.E., nivele crescute de fibroză subepitelială, de TGF-beta, precum şi creşterea densității vasculare şi a moleculelor de adeziune vasculară. Rezultatele obținute sugerează că modificările esofagiene pot conduce la formarea de stenoze, aceasta fiind complicația principală a E.E.

Se acumulează tot mai multe date cu privire la rolul geneticii în etiologia E.E. Astfel, s-a observat (Ganapathy, 2008)[12] că gena care codifică eotaxina 3 (una din chemokinele importante pentru traficarea eozinofilelor) are nivelul de inducție crescut şi că un singur polimorfism nucleotidic este asociat cu susceptibilitatea crescută de apariție a E.E.

O serie de mediatori mastocitari (histamina, leucotrienele, factorul de activare a plachetelor) au capacitatea de a acționa pe componentele neuromusculare ale tractului digestiv; în acest fel pot juca un rol important în anomaliile de peristaltism observate la nivelul esofagului în E.E. Mann şi Leung implică direct mastocitele în geneza inelelor esofagiene concentrice observate adesea la examenul endoscopic al pacienților cu E.E., construind, astfel, ipoteza potrivit căreia activarea acetilcolinei de către histamina mastocitară declanşează contracția fibrelor musculare netede din *muscularis mucosae* esofagiană cu apariția aspectului inelar specific endoluminal („trahealizarea esofagului”).

Toate aceste date referitoare la fenomenele complexe imunoinflamatorii de la nivelul mucoasei esofagiene obligă reconsiderarea rolului esofagului de la tradiționalul tub muscular ce asigură doar pasajul bolului alimentar către stomac la acela de organ imunoactiv capabil să inițieze reacții imunoalergice în urma expunerii la aero sau trofalergenele la care există demonstrată, prin teste alergologice, o hipersensibilizare.

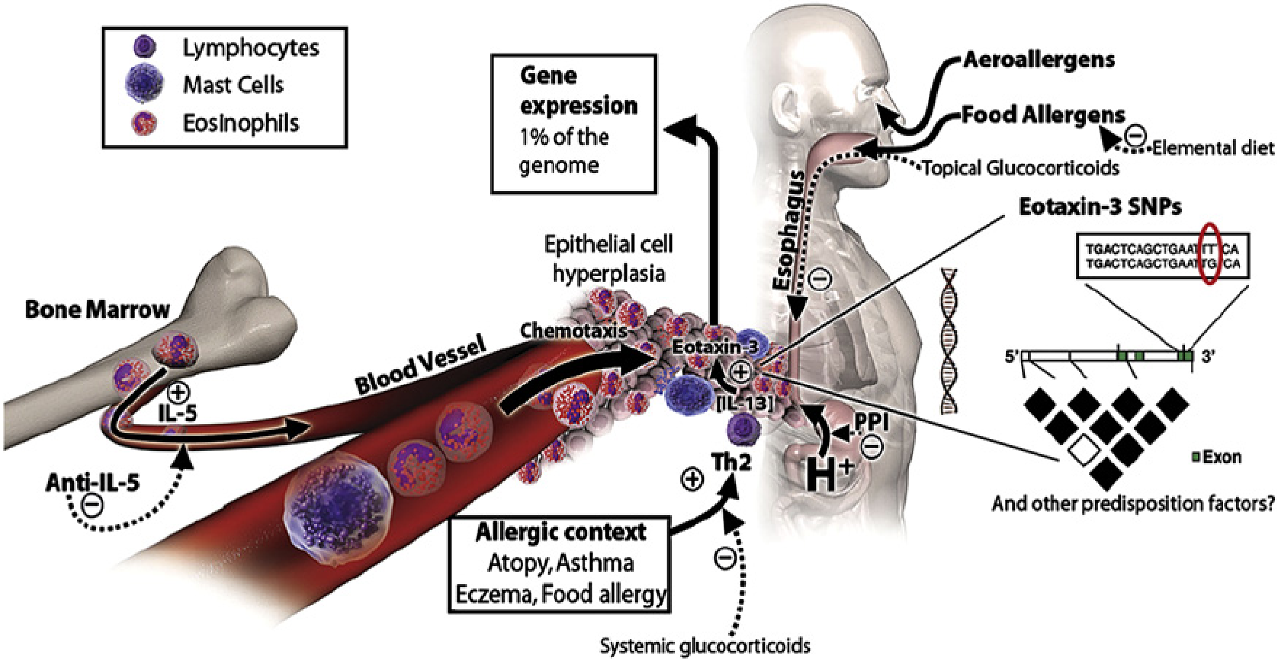
Lymphocytes
Mast Cells
Eosinophils
Gene expression
1% of the genome
Aeroallergens
Food Allergens
Elemental diet
Topical Glucocorticoids
Esophagus
Eotaxin-3 SNPs
TGACTCAGCTGAATTTCA
TGACTCAGCTGAATTGCA
5'
3'
Epithelial cell hyperplasia
Bone Marrow
Blood Vessel
Chemotaxis
Eotaxin-3
IL-13
IL-5
Anti-IL-5
PPI
H+
Th2
Exon
And other predisposition factors?
Allergic context
Atopy, Asthma
Eczema, Food allergy
Systemic glucocorticoids

Fig. 1. Mecanisme moleculare şi celulare implicate în patogenia esofagitei eozinofilice, după Ganapathy A. Prasad, MD, MSa, Nicholas J. Talley, MD, PhD, *Eosinophilic Esophagitis in Adults*, Gastroenterology Clin N Am 37 (2008) 349-368[12].

Diagnosticul E.E.

Acesta începe întotdeauna cu recunoaşterea simptomelor. Adesea însă, severitatea şi cronicitatea simptomelor E.E. trec neobservate. Intervalul dintre debutul simptomelor şi prima endoscopie este, în medie, de 3 ani.

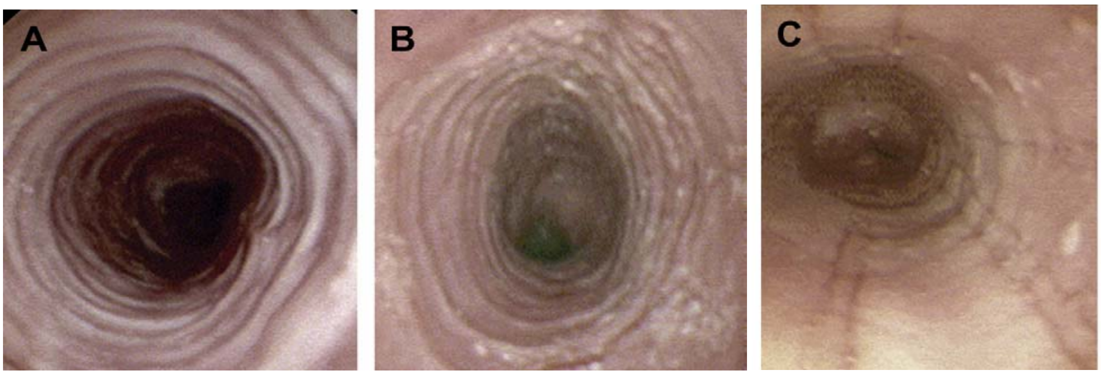

Fig. 2. Aspecte endoscopice ale mucoasei în esofagita eozinofilică:
A) Inelele mucozale suprapuse („trahealizare”); B) plăcile albe; C) fisurile longitudinale.

Impactarea esofagului cu bolus alimentar este prima manifestare la mulți adulți[12], cu o frecvență estimată de 13 cazuri la 100 000. Întotdeauna când impactarea alimentară sau disfagia severă apar la adulți, se va lua în considerare E.E. şi în decursul aceleiaşi endoscopii digestive superioare, după îndepărtarea bolului alimentar, se vor preleva biopsii endoscopice. Simpla inspecție vizuală nu este suficientă deoarece chiar şi esofagul aparent normal poate avea eozinofilie semnificativă. O treime dintre pacienții cu E.E. pot avea aspect esofagian normal[12].

Istoricul familial de boli atopice (astm bronşic, rinită alergică, dermatită atopică) este întâlnit la 30-50% dintre pacienți.

Dată fiind prezența acestor suferințe şi în istoricul personal al pacienților cu E.E. dar şi a implicării mecanismelor imunoalergice în patogeneza bolii este necesar consultul medicului alergolog şi efectuarea de teste cutanate alergologice prick (la aeroalergene şi trofalergene) sau patch (la trofalergene).

Eozinofilia serică crescută este observată la aproximativ jumătate din cazurile de E.E.

Alte aspecte endoscopice sugestive sunt: fisurile longitudinale, stricturile, inelele mucozale (trahealizare) şi plăcile albe (fig.2). Examenul histologic esofagian descrie în cazul E.E., pe lângă numărul mare de eozinofile (peste 15 elemente celulare pe câmp microscopic), mucoasă îngroşată, hiperplazia stratului bazal şi papile alungite. Sunt necesare prelevări de biopsii multiple deoarece aspectele histologice sunt variabile la acelaşi pacient iar sensibilitatea şi specificitatea diagnosticului cresc cu numărul biopsiilor prelevate. S-a observat că numărul eozinofilelor din mucoasa esofagiană se corelează doar cu severitatea disfagiei şi nu şi cu celelalte simptome, vârstă sau sex (fig.3).

Toate aceste modificări histologice sunt refractare la maximum de medicație inhibitoare a secreției gastrice.

Nu sunt interesate mucoasele gastrice şi duodenale, ceea ce diferențiază E.E. de gastroenterita eozinofilică (G.E.). Această diferențiere este importantă din punct de vedere terapeutic: corticosteroizii topici sunt eficienți în E.E. dar nu şi în G.E.

Ph-metria esofagiană permite diferențierea de boala de reflux gastroesofagian.

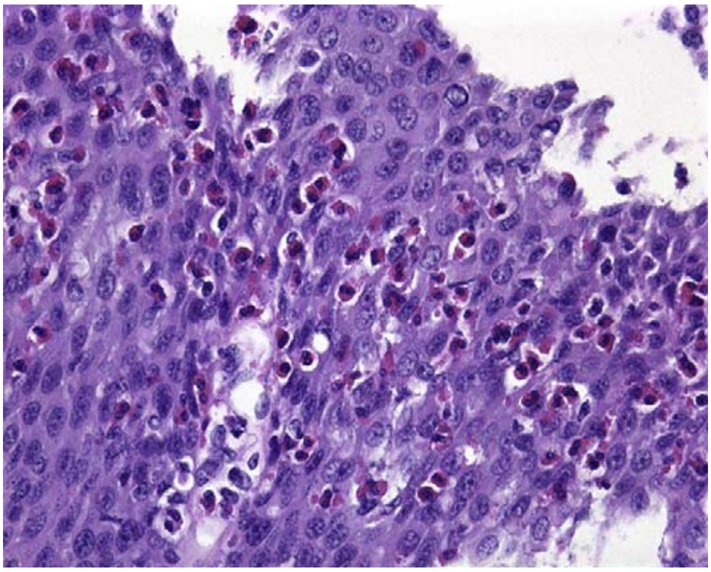

Fig. 3. Infiltrarea cu eozinofile a mucoasei scuamoase esofagiene.

Tratamentul E.E.

Deşi E.E. are o istorie naturală necunoscută şi este o afecțiune cronică, prognosticul pacienților este bun pe termen lung. În practică apar dificultăți în identificarea acelora care vor evolua cu timpul spre complicații față de pacienții care vor avea doar simple simptome cronice. În fiecare caz este importantă recunoaşterea simptomelor care influențează negativ calitatea vieții. În acest moment, opțiunile terapeutice constau în dilatare esofagiană, regim igieno-dietetic şi tratament farmacologic.

Dilatația esofagiană este utilă pentru ameliorarea imediată a simptomatologiei dată de impactarea esofagului cu bolus alimentar la pacienții care prezintă anomalii anatomice esofagiene semnificative. Dilatația nu este însă recomandată ca tratament de primă linie datorită efectelor adverse semnificative (durere, hemoragie, perforație) şi pentru că nu are valențe fiziopatologice. Ca regulă, de fiecare dată, când este posibil, se vor utiliza alte mijloace terapeutice înaintea efectuării dilatației esofagiene.

Dieta. Studii recente confirmă rolul alergiei alimentare în vasta majoritate a pacienţilor cu E.E. Dacă aeroalergenii sunt doar sugeraţi ca fiind factori etiologici ai E.E., rolul alergenilor alimentari este, neîndoielnic, demonstrat. Pacienţii răspund nu numai la dieta orientată de testele cutanate, ci şi la dieta empirică rezultată prin îndepărtarea alergenilor alimentari cunoscuţi. Alimentele cele mai frecvent implicate sunt: laptele, oul, grâul, secara şi carnea de vită. Se consideră că E.E. este datorată unei combinaţii de reacţii la alimente mediate de IgE şi non-IgE. Ca urmare, testarea combinată, prick testul pentru reacţiile mediate IgE şi patch testul pentru reacţiile non-mediate IgE, este eficientă pentru ghidarea tratamentului dietetic. Combinarea celor două tipuri de teste are o valoare predictivă negativă de 88-100% pentru toate alimentele, cu excepţia laptelui, unde este scăzută, iar valoarea predictivă pozitivă este mai mare de 74% pentru majoritatea alimentelor care dau E.E[12]. Prin urmare, când pacienţii sunt trimişi pentru evaluare alergologică, vor fi testaţi pentru alimente cu metoda "prick" şi " patch" şi pentru alergenii din mediul înconjurător, după o anamneză detaliată. Recomandările terapeutice subliniază evitarea tuturor alimentelor testate pozitiv şi a altora cunoscute ca având potenţial alergen chiar dacă testul nu s-a pozitivat şi la ele, prin recurgerea la dieta restrictivă sau de eliminare ori instituirea de la început a dietei elementale. S-a remarcat că îndepărtarea antigenelor din alimentaţie prin dieta elementală a ameliorat simptomele clinice şi histologia esofagiană în 98% cazuri (Liacouras, 2005). Alţi autori au avut succes cu dietele de eliminare bazate pe excluderea celor mai cunoscute alimente implicate în apariţia E.E. Normalizarea histologică apare la o lună după iniţierea dietei elementale. În multe cazuri, modificările dietei sunt dificile, complianţa fiind o problemă atât la copii cât şi la adulţi. Formulele alimentare pot fi foarte costisitoare. Deşi modificările dietetice trebuie considerate ca terapie de primă linie pentru copiii şi adulţii cu E.E., mulţi pacienţi au nevoie şi de alte modalităţi terapeutice.

Deoarece atât complianţa cât şi calitatea vieţii sunt importante, pentru adulţii şi adolescenţii cu E.E. se administrează corticosteroizi cu acţiune topică.

Se recomandă de asemenea şi managementul bolilor alergice induse de aeroalergeni datorită rolului aeroalergenilor în inducerea E.E. (Fogg, 2003, Mishra, 2001)[9,8].

Un interesant studiu (Simon, 2006)[14] a căutat să răspundă la întrebarea dacă aeroalergenii care reacţionează încrucişat cu alimentele pot cauza sau agrava E.E. Polenul de graminee reacţionează încrucişat cu alergenii alimentari derivaţi din grâu şi secară. Pacienţii cu E.E. studiaţi aveau atacuri lunare de disfagie, prezentau teste cutanate prick pozitive pentru polen de graminee dar şi pentru secară şi grâu. Au fost supuşi unui test de provocare orală la secară şi grâu (considerat pozitiv dacă disfagia apărea în intervalul a 30 minute) urmat de o dietă de eliminare a acestor alimente pentru o lună. Toate testele de provocare cu aceste cereale au fost negative şi nici nu au fost observate modificări histologice sau reducerea simptomelor la excluderea grâului pentru 6 săptămâni. Concluzia studiului a fost că sensibilizarea încrucişată între polenul de graminee (ce determină simptome de rinită alergică) şi alergenele de grâu şi secară nu are relevanţă terapeutică pentru E.E., nefiind astfel necesară recomandarea automată a unei diete alimentare de restricţie cu această specificitate la pacienţii care asociază o boală alergică respiratorie cu sensibilizare la polenul de graminee .

Terapia medicală. Corticoizii se dovedesc eficienţi în tratamentul E.E[12]. Corticoizii sistemici au fost primii care au demonstrat atât ameliorare clinică, cât şi histologică. Datorită reacţiilor adverse pe care le implică, utilizarea lor pe termen lung este prohibită. În cure scurte însă, sunt utili la pacienţii cu simptomatologie severă. Corticoizii ingeraţi (de exemplu, fluticazona, 88o mg x 2/zi, 6 săptămâni) sau inhalaţi sunt utili atât la adulţi, cât şi la copii. Încercările terapeutice cu cromoglicat de sodiu nu au înregistrat ameliorări clinice sau histologice. Terapia combinată este promiţătoare deoarece dieta restrictivă şi corticoizii topici la care se adaugă un inibitor de pompă de protoni induce ameliorare endoscopică în toate cazurile şi rezoluţie clinică în 87-93% din cazurile tratate 6 săptămâni. Recăderile sunt însă posibile în 25-40% din cazurile cu răspuns iniţial pozitiv la tratament. Dacă reapare simptomatologia, terapia se poate extinde la 12 săptămâni sau se poate iniţia corticoterapia orală.

Terapia biologică utilizează anticorpi anti-receptori de interleukină-5 şi este în curs de evaluare. Anti-IL-5 s-au dovedit eficienţi în sindromul hipereozinofilic care asociază esofagita eozinofilică. Se administrează trei doze i.v. de anti-IL-5. Efectul terapeutic benefic este consemnat prin reducerea simptomelor clinice, de scădere a eozinofilelor circulante şi a celor esofagiene, fără efecte adverse notabile.

Antagoniştii de leucotriene (montelukastum) reduc severitatea disfagiei fără a reduce eozinofilia mucosală esofagiană[12]. Sunt necesare însă doze mari, de 100 mg pe zi.

Istoricul natural al E.E.

Informaţiile ce privesc prognosticul pe termen lung sunt limitate. Strauman[15] a urmărit 30 de pacienţi adulţi pe o perioadă de 11 ani şi a observat că, deşi inflamaţia eozinofilică persistă la toţi cei simptomatici, numărul elementelor celulare se reduce în timp fără a se normaliza. Procesul inflamator nu a progresat către stomac şi duoden. Nici un pacient nu a dezvoltat sindrom hipereozinofilic, neoplazii sau esofag Barett. La aproximativ două treimi dintre pacienţi nu se constată nicio modificare a severităţii disfagiei.

În concluzie, E.E. afectează toate grupele de vârstă. Datorită interesului crescut pentru această entitate clinică patologică, există un număr impresionant de articole publicate în literatura medicală de specialitate. S-a înfiinţat şi un grup internaţional de lucru, denumit ”TIGER” (The International Gastrointestinal Researchers), pentru creşterea interesului, nivelului de educaţie şi de cercetare ştiinţifică cu privire la E.E. şi la alte afecţiuni gastrointestinale eozinofilice în rândul medicilor pediatri şi ai celor care activează în medicina adultului. Continuarea cercetărilor şi creşterea interesului pentru E.E. ca şi sporirea gradului de cunoaştere a fiziopatologiei acestei afecţiuni vor fi benefice pentru tratamentul optim al acestor pacienţi.

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Implicarea conducerii spitalelor în monitorizarea şi combaterea fumatului pasiv

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Rezumat. Studii şi cercetări ştiinţifice din toată lumea atestă rolul nefast al tabagismului asupra organismului uman. Aceleaşi investigaţii relevă şi costurile ridicate pe care este obligată societatea să le suporte pentru a-i trata pe cei care se îmbolnăvesc din cauza acestui obicei nesănătos. Prezentul articol prezintă principalele efecte ale fumatului asupra stării de sănătate susţinute prin statistici şi studii efectuate de importante organisme de sănătate din lume.

Cuvinte cheie: tabagism fumat pasiv fumat activ

Abstract. Worldwide studies and scientific researches certifie the bad influence of tabacosis upon human body. The same studies point out, also, communities the expensive treatment costs in tabacosis addicted people. This article depicts the main effects of smoking habit upon human health in statistics and studies made by significant health organisations from all over the world. There are also mentioned the healthy units and organisational required measures at society level.

Problema tabagismului capătă pe zi ce trece o importanţă din ce în ce mai mare din punctul de vedere al sănătăţii publice.

Majoritatea fumătorilor dezvoltă în timpul vieţii una sau mai multe boli datorate acestui viciu dăunător. Aproximativ 90% dintre cancerele pulmonare, 56-80% dintre bolile respiratorii cronice şi 22% dintre bolile cardiovasculare sunt atribuite fumatului.

Fumatul este o cauză majoră a morţii premature, afectând 54% dintre bărbaţii şi 32% dintre femeile de vârstă medie. Rata mortalităţii din cauza fumatului este estimată a fi în România de 522 decese/100 mii locuitori, faţă de 273/100 mii locuitori la nivel european.

Importantă ca o primă măsură de îmbunătăţire a stării de sănătate a populaţiei este reducerea prevalenţei fumatului la adolescenţi, obiectiv pe care şi l-au propus multe guverne (de exemplu, în Marea Britanie obiectivul s-a tradus printr-o diminuare a prevalenţei la grupa de vârstă 11-15 ani de la 13% în 1998 la 11% în 2003 şi 9% în 2005).

Stadiului actual al cunoştinţelor şi atitudinilor legate de consumul de tutun în România obiectivează din plin necesitatea de a interveni cu toate pârghiile pe care le deţine statul român pentru controlul acestui fenomen cu caracter epidemic, fenomen care implică foarte multe riscuri şi se reflectă în costuri foarte ridicate la nivelul societăţii româneşti.

Ca o consecinţă a consumului de tutun în România a fost şi lansarea unei campanii împotriva fumatului pasiv care a inclus şi reclama antifumat, pentru prima dată afişată în locuri publice din Bucureşti, campanie realizată în cadrul programului implementat de Centrul pentru Politici şi Servicii de Sănătate şi finanţat de Uniunea Europeană prin programul Phare – Dezvoltarea Societăţii Civile 2000. Astfel, se realizează un deziderat mai vechi, de abordare şi pe această cale a tineretului prin intermediul reclamei antifumat, care, aşa cum arată şi alte investigaţii în domeniu, determină un răspuns similar din partea acestora, indiferent în ce ţară se aplică.

Fumatul activ, dar şi cel pasiv, reprezintă în continuare o problemă gravă cu multiple consecinţe economice. O anchetă atentă, care ar încerca să descifreze aspecte multifactoriale, cu implicaţii legislative, sociale, medicale ar putea contribui şi la relansarea programelor de luptă antifumat în unităţile sanitare ale Ministerului Apărării. Informaţiile disponibile privind fumatul în unităţile medicale din M.Ap.N. sunt insuficiente, datele legate de prevalenţă, consecinţele fumatului şi chiar despre mortalitate lipsind cu desăvârşire.

Unităţilor spitaliceşti le revin o serie de sarcini majore în lupta pentru reducerea consumului de tutun şi a efectelor sale nocive asupra sănătăţii. Aceste obligaţii nu se reduc doar la crearea unui mediu fără fumat, cu scopul de a-i proteja pe nefumători, ci şi la acordarea unui sprijin activ fumătorilor în procesul de renunţare la fumat. Este important ca aceste aspecte să se afle atât în vederea pacientului, cât şi a tuturor categoriilor de personal sanitar.

În acest context spitalele au o serie de responsabilităţi care includ pe lângă activităţile de îngrijire, prevenire şi cercetare şi politici de control al tabagismului. Implementarea cu succes a unei politici de control al tabagismului este dependentă de conţinutul acestei politici şi de buget, de informaţiile comprehensive şi de procesele de pregătire, de participarea întregului personal, nu doar a fumătorilor, precum şi de stabilirea unui program de evaluare pe termen lung. Cu cât este mai mare participarea, cu atât sunt necesare mai puţine măsuri restrictive.

Este important ca politica spitalului şi acţiunile sale să fie în concordanţă cu alte activităţi de prevenire a fumatului în alte servicii de sănătate şi în comunitate în general.

Transformarea spitalului într-un loc de luptă împotriva fumatului, un spital fără fumat, este o încercare grea şi necesită un efort continuu şi susţinut pe o perioadă lungă de timp. Politica de luptă împotriva fumatului a spitalului (ca orice altă politică) necesită implicarea promptă a conducerii medicale şi administrative a instituţiei. Implicarea înseamnă rezolvarea tuturor problemelor iar pentru a fi eficientă aceasta trebuie susţinută de un management medical coerent. Aplicarea unei politici fără fumat nu necesită un buget specific, deşi acesta ar fi de dorit pentru a oferi posibilitatea conducerii de a organiza evenimente specifice şi de a difuza documente cu caracter antifumat.

Câteva argumente privind implicarea conducerii spitalelor în interzicerea fumatului în unităţile sanitare:

- personalul angajat, pacienţi sau vizitatori fumători reprezintă principala cauză de producere a incendiilor în spitale, fapt de care conducerea spitalului este responsabilă;
- dreptul la un spaţiu liber, pe care îl solicită fumătorii, poate fi cerut de către nefumători;
- când apar manifestări pe seama simptomelor de sevraj şi nu primesc tratament cu substituenţi de nicotină, fumătorii pot deveni agresivi sau pot fuma în zone unde acest lucru nu este permis. Astfel pot lua naştere conflicte între fumători şi nefumători;
- scrumul de ţigară distruge pardoselile încăperilor şi le conferă un aspect murdar, neîngrijit;
- pereţii se murdăresc mai repede în spaţiile unde se fumează;
- dispozitivele electronice medicale şi nemedicale se defectează mult mai frecvent într-un mediu cu fum de ţigară. Interiorul computerelor sau al altor dispozitive electronice existente într-un mediu cu fum de ţigară este mult mai murdar decât cel al dispozitivelor plasate într-un mediu în care nu se fumează;
- un spital în care este implementată o politică de interzicere a fumatului atestă faptul că interesul pentru sănătatea pacienţilor este maxim;
- acolo unde persnalul din conducerea spitalului fumează, unitatea sanitară respectivă este percepută ca o instituţie care nu-şi poate rezolva propriile probleme;
- existenţa unei politici de netolerare a fumatului într-un spital este un atu important privind acreditarea acestuia.

Un spital în care este interzis fumatul nu este împotriva fumătorilor care doresc să renunţe la acest obicei dăunător, dar nu o pot face, deoarece sunt dependenţi şi au nevoie de ajutor în acest sens.

Un spital curat este un spital fără fumat.

Epidemiologia fumatului în spitalele în care nu se fumează

Numeroasele date existente arată că, în marea majoritate a ţărilor europene, lucrătorii din domeniul sanitar fumează mai puţin decât populaţia generală, dar în cele mai multe cazuri sunt “fumători înrăiţi”.

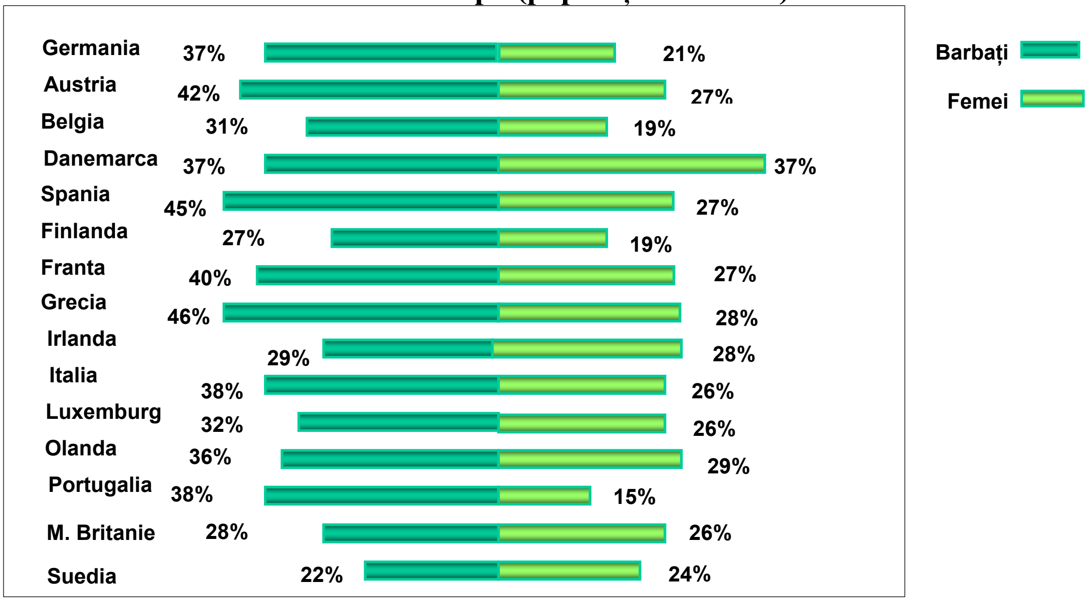

Consumul de tutun în Europa (populaţie ≥ 15 ani)

sursa: U.E. OMS March 1996

Prezentăm spre exemplificare câteva date statistice cu privire la fumatul activ şi pasiv la personalul medical din unele spitale europene:

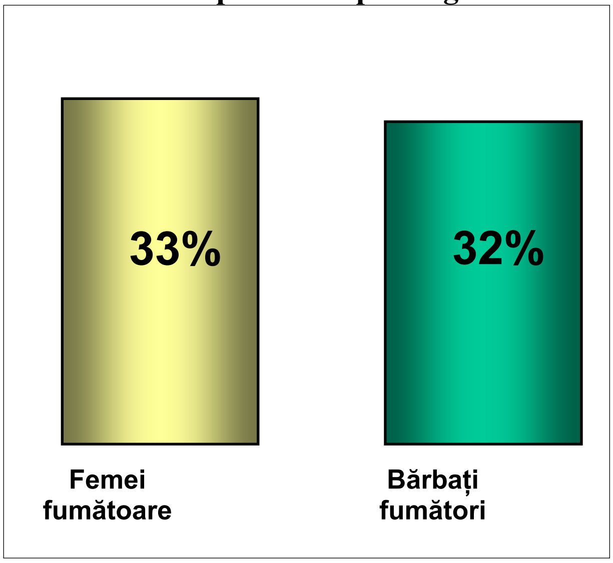

Obiceiul fumatului la spitalul Bispebierg din Danemarca*

*După Olsen AD et al. Fumatul activ si pasiv la personalul sanitar din spitalul Bispebjerg 1992-1999.

Obiceiul fumatului la personalul sanitar mediu din spitalele Italiei*

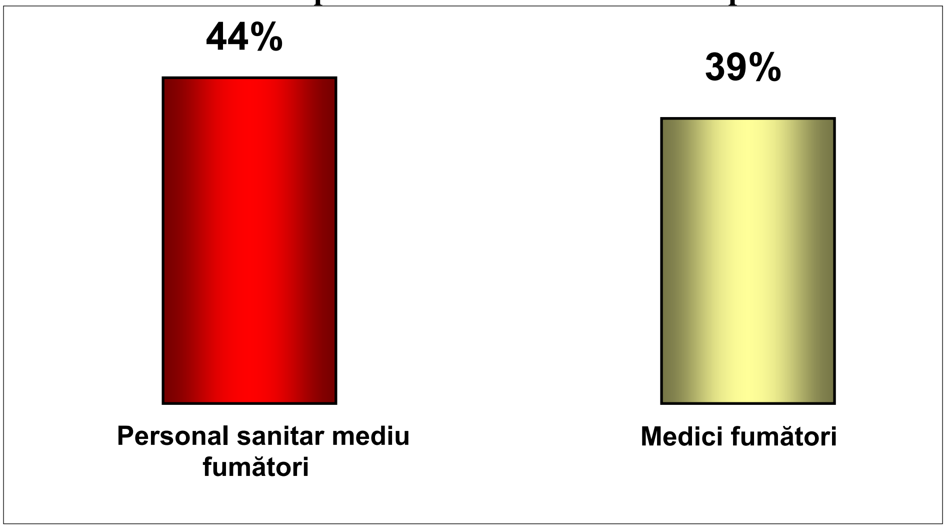

*După Nardini S. et al. Obiceiul fumatului si atitudinea vizavi de fumat a personalului sanitar mediu a unui spital general.

Obiceiul fumatului la asistentele medicale din Spania*

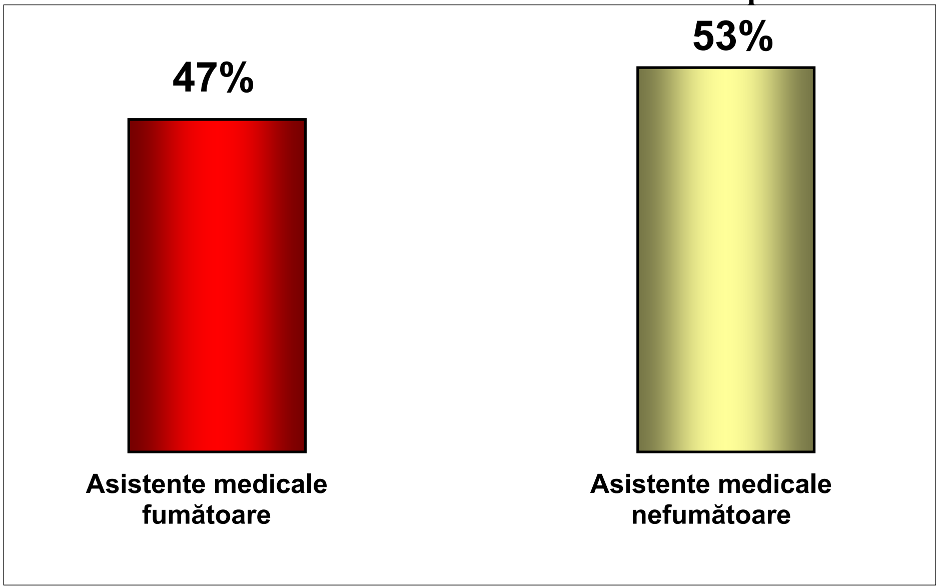

*După Fernandez Ruiz ML, Sanchez Bayle M. Prevalenta fumatului la femeile doctor si asistentele medicale din Madrid.

Obiceiul fumatului la personalul sanitar mediu din spitalele din Franța

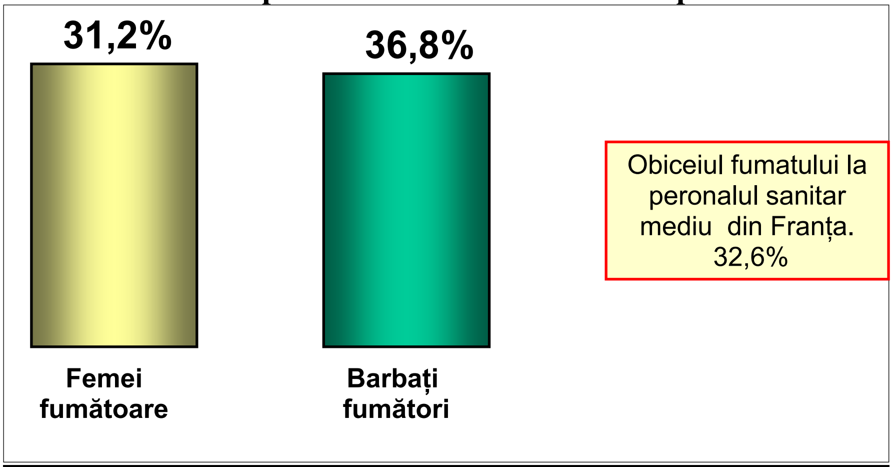

După Grizeau D et al. Atitudinea si comportamentul personalului sanitar mediu vizavi de tabagism

Fumatul pasiv - o amenințare pentru sănătate

Lucrătorii din domeniul sanitar trebuie să cunoască atât efectele fumatului activ cât şi pe cele ale fumatului pasiv.

Disconfortul provocat de fumatul pasiv este o realitate. Un studiu recent (*Baromètre santé*, 2000) a evidențiat că dintre nefumători 72 % sunt deranjați de fumul de țigară (*second hand tobacco).* Aceste rezultate demonstrează nevoia nefumătorilor de protecție față de fumul de țigară.

Un studiu recent a arătat faptul că 53% dintre fumători au început să fumeze pentru că erau deranjați de fumul de țigară. Necesitatea unui mediu liber de fumat este unanim acceptată atât de nefumători, cât şi de fumători.

Disconfortul provocat de fumatul pasiv în Europa*

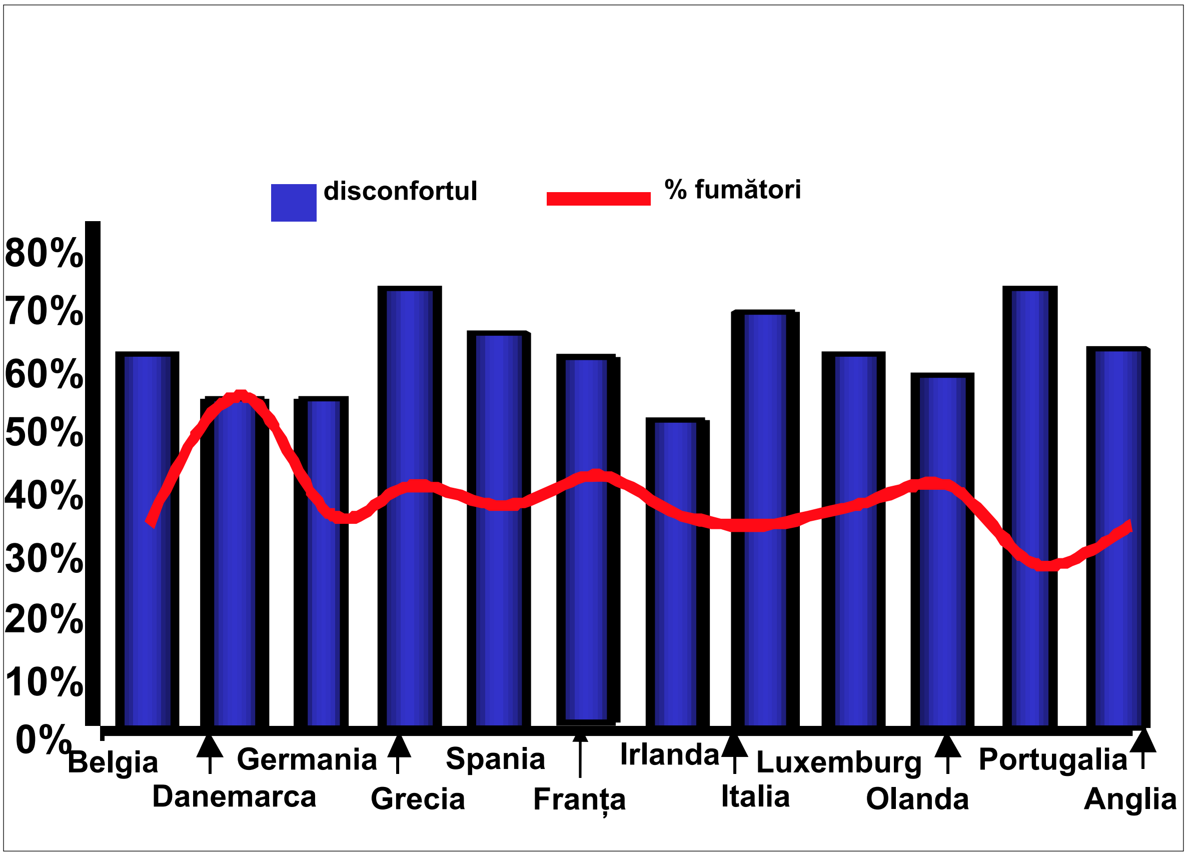

*Sursa: Europe against cancer, 1993 and WHO Europe, 1992.

În virtutea principiului toleranței reciproce între oamenii unei societăți civilizate, am putea accepta ca nefumătorii şi fumătorii să trăiască împreună în bună înțelegere. O atitudine de toleranță mutuală şi-ar avea locul dacă fumatul pasiv nu ar afecta sănătatea celor care sunt nevoiți să-l suporte.

Din ce în ce mai multe studii indică faptul că fumatul pasiv dăunează grav sănătății, inhalarea fumului de către nefumători având consecințe serioase. Mai mult, aceste consecințe sunt cu atât mai grave cu cât petrecem mai mult timp în preajma fumătorilor. Ştiind aceste lucruri, nu mai este pur şi simplu posibil să admitem în casele noastre fumatul partenerului sau al unuia dintre părinți. Este necesar să limităm acest punct al toleranței mai ales în mediul profesional, unde foarte rare sunt cazurile când ne putem alege noi mediul de lucru. Fumatul pasiv ne priveşte pe noi toți, întrucît el îi expune şi pe membrii familiei (copiii sunt cei mai afectați) la o întreagă serie de urmări extrem de periculoase.

Disconfortul provocat de fumatul pasiv raportat de fumător prin răspunsul la întrebarea: sunteți deranjat de fumul celorlalți?

Sursa: Baromètre santé 2000, CFES

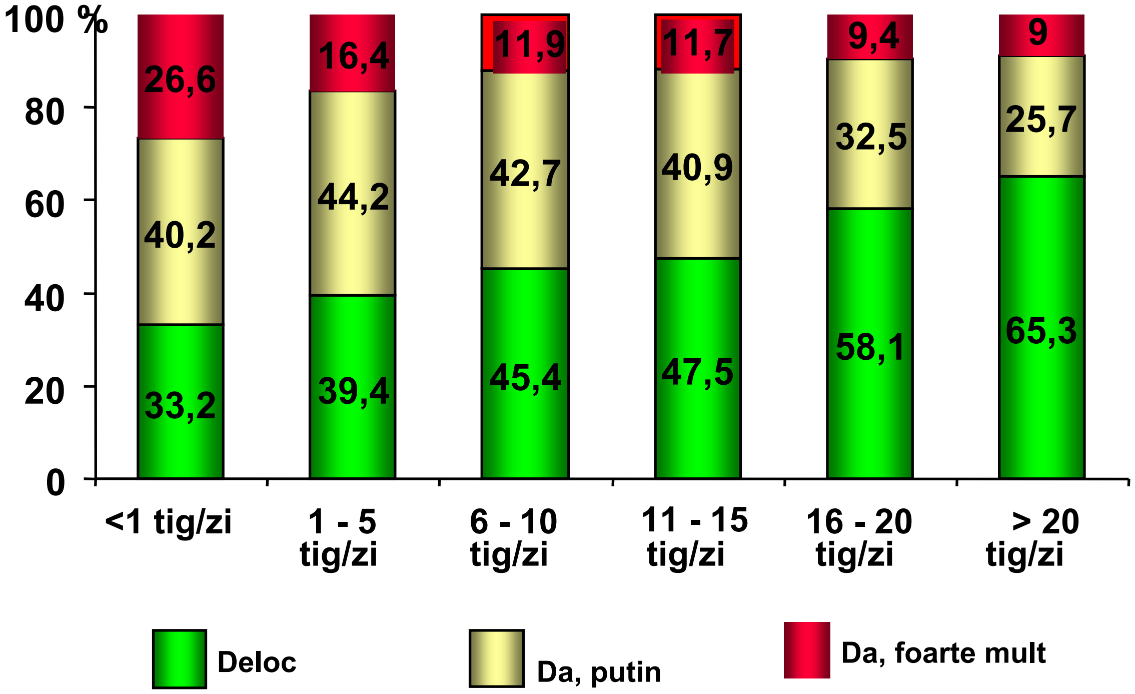

Studii recente demonstrează riscurile expunerii pasive la fumul de țigară pentru nefumător. Fumătorii sunt victimele principale ale fumului de țigară (500 000 decese anual în Europa). Date ştiințifice recente, majoritarea publicate în ultimii 15 ani, atestă faptul că expunerea la fumul de țigara determină îmbonăviri atât la copii, cât şi adulți.

Aceste dovezi necesită acțiuni restrictive în locurile publice, pentru a se evita expunerea la fumul de țigară.

Expunerea pasivă la fumul de țigară este asociată cu creşterea riscului de:

- *infecții de tract respirator inferior la copil (+ 72 % dacă mama fumează);*
- *otite medii recurente la copil (+ 48 % dacă fumează ambii părinți);*
- *crize de astm şi emoționale la copil;*
- *deficiențe de creştere intra-uterină şi greutate mică la naştere (pot apărea şi la mamele nefumătoare expuse pasiv fumului de țigară);*
- *moarte subită infantilă (riscul creşte de două ori);*
- *spasme coronariene (+ 25 % şi este principala cauză de deces datorată fumatului pasiv);*
- *cancer pulmonar (+ 26 %).*

Diferit de aceste efecte bine cunoscute, sunt presupuse mult mai multe urmări cu implicare în apariția unor boli non-maligne şi agravarea unora preexistente (maligne şi non-maligne), dar date ştiințifice noi sunt necesare pentru a confirma aceste aspecte.

Desigur, fumatul poate fi considerat „o plăcere periculoasă”, în funcție de hotărârea fiecăruia, iar societatea trebuie să tolereze diferențele existente între membrii ei. Fumatul este, deja de mult timp, cunoscut drept principala cauză evitabilă care dăunează grav sănătății.

Sănătatea nefumătorilor, constrânşi să inhaleze fumul tutunului (fumatul pasiv) este, de asemenea, amenințată de aceleaşi pericole. Date recente înregistrate în Elveția scot în evidență efectele pe care le are fumatul pasiv asupra sănătății din punct de vedere statistic:

- fumătorii pasivi suferă mai frecvent de tulburări respiratorii, cum ar fi respiraţia şuierătoare, tusea cronică şi simptome ale bronşitei cronice;
- riscul de a se îmbolnăvi de astm bronşic sau de bronşită cronică este net mai ridicat;
- cu cât nefumătorii sunt mai expuşi fumului de tutun, cu atât probabilitatea apariţiei unor boli respiratorii şi diminuarea capacităţilor fizice cresc.

Nimeni sau aproape nimeni nu mai contestă astăzi că fumatul este nefast pentru sănătate. Dacă numărul fumătorilor este atât de mare, acest lucru este o urmare a faptului că ei sunt dependenţi, fizic şi psihic, de ţigară.

Fumatul este cunoscut de mult timp ca principal factor care stă la baza apariţiei a numeroase boli, în mod deosebit a cancerului şi că în mod evident este responsabil pentru numeroase decese.

Aproape 90% dintre persoanele care suferă de cancer pulmonar au fumat de-a lungul multor ani.

Cercetările au demonstrat că persoanele nefumătoare sunt supuse aceloraşi riscuri ca şi persoanele fumătoare atunci când ele sunt expuse, împotriva voinţei lor, fumului unuia sau mai multor fumători din anturaj. În acest caz este vorba de aşa-numitul *tabagism pasiv* sau, cum mai este numit, *fumatul pasiv*.

Fumul de tutun este o formă comună de poluare a aerului din spaţiile închise. Milioane de adulţi dar şi de copii suferă zilnic din cauza fumului, acasă, la locul de muncă sau în locuri publice.

1. Cancerul şi fumatul pasiv

Fumul conţine substanţe care favorizează apariţia tumorilor maligne. Aceste substanţe nu sunt prezente numai la fumători, ci au fost detectate şi în organismul fumătorilor pasivi. În plus, cu cât expunerea la fum este mai prelungită, cu atât concentraţia substanţelor creşte în anumite organe, în sânge şi urină.

Studiul efectelor pe care le are fumatul pasiv asupra apariţiei cancerului este dificil. De fapt, substanţele toxice produse prin arderea tutunului trebuie să acţioneze o perioadă lungă de timp înainte ca o eventuală tumoră canceroasă să fie detectată, însă cercetările ştiinţifice au demonstrat că acest risc există.

2. Afecţiunile respiratorii şi fumatul pasiv

Pe lângă rolul pe care îl are fumul în apariţia tumorilor, cercetătorii au vrut să afle dacă fumătorii pasivi prezintă mai frecvent boli ale căilor respiratorii.

S-au studiat efectele fumatului pasiv în primul rând la copii. A fost dovedită existenţa unor diverse consecinţe negative, mai ales o susceptibilitate mai mare ca aceştia să sufere de infecţii ale tractului respirator, de inflamaţii ale canalelor auditive şi de astm, precum şi de o încetinire a dezvoltării capacităţii pulmonare.

Copiii expuşi în mod constant la fumul de ţigară sunt spitalizaţi mai des decât ceilalţi copii şi lipsesc frecvent de la şcoală din cauza dificultăţilor respiratorii.

În timp ce numeroase studii ştiinţifice au demonstrat efectele nocive ale fumatului pasiv asupra sistemului respirator la copii, literatura medicală, cu excepţia studiilor asupra bronşitei cronice, emfizemului pulmonar şi a cancerului pulmonar, nu furnizează decât puţine informaţii tratând efectele fumatului pasiv la adulţi. În plus, aceste studii se bazează adesea pe eşantioane restrânse de subiecţi şi rezultatele lor nu sunt întotdeauna corespunzătoare.

Deşi studiul ştiinţific al efectelor fumatului pasiv nu este simplu, diverse studii au arătat că fumatul pasiv creşte riscul apariţiei cancerului pulmonar, aşa după cum nu mai există nicio îndoială în ceea ce priveşte relaţia strânsă dintre fumatul pasiv şi numeroasele boli respiratorii.

3. Efectele fumatului asupra sugarilor şi copiilor mici

Există numeroase studii care tratează efectele fumatului pasiv la nou-născuţi şi la copii până la vârsta de 5 ani. S-a constatat că nou-născuţii fumătoarelor au adesea o greutate mai mică la naştere, adică în jur de 2 500 de grame, iar greutatea va fi cu atât mai mică faţă de cea normală cu cât femeia însărcinată fumează mai mult.

Nu mai puţin important este şi faptul că rata mortalităţii copiilor în ultimele zile ale sarcinii sau în primele zile după naştere este mai ridicată în cazul mamelor fumătoare decât în cazul mamelor nefumătoare.

S-a ajuns, de asemenea, la concluzia că sugarii cu greutate inferioară celei medii sunt predispuşi să se îmbolnăvească în primul lor an de viaţă.

Totodată la aceşti nou-născuţi există şi riscul de „moarte subită”, ale cărei cauze însă nu sunt încă în mod clar edificate. Se presupune că ar fi vorba despre pauzele lungi între respiraţii care duc la deces.

Din motive necunoscute, probabilitatea morţii subite a sugarului este strâns legată de consumul de tutun al mamei. Pe scurt, copiii care au părinţi fumători suferă mai frecvent de bronşite acute decât cei ai căror parinţi sunt nefumători. În plus, riscurile apariţiei bolilor sunt cu atât mai mari cu cât părinţii, şi în mod deosebit mama, sunt fumători.

4. Efectele fumatului asupra copiilor şi adolescenţilor

La copii şi adolescenţii a căror vârsta este cuprinsă între 5 şi 16 ani şi care trăiesc într-un mediu în care sunt obligaţi să inspire în mod constant fum de ţigară, se pot observa diverse forme de iritare a căilor respiratorii: tuse, expectoraţii, insuficienţă respiratorie, wheezing. Aceşti tineri suferă, de asemenea, în mod frecvent de repetate inflamaţii la nivelul canalului auditiv şi al amigdalelor. În plus, tot la aceşti copii a fost posibilă evidenţierea unei capacităţi pulmonare ceva mai reduse, dar totuşi semnificativă din punct de vedere statistic.

Atât Organizaţia Mondială a Sănătăţii, cât şi autorităţile sanitare din Statele Unite şi Marea Britanie au constatat că numărul cazurilor de astm şi gravitatea acestora la copii depinde de consumul mai mic sau mai mare de tutun din cadrul anturajului lor. Fumatul pasiv la copii şi la adolescenţi intensifică riscul acestora de a fi predispuşi la astm.

5. Efecte fumatului asupra adulţilor

Efectele fumatului pasiv asupra adulţilor sunt mai dificil de stabilit decât la copii, şi asta pentru că aceştia sunt, în genere, mai expuşi la influenţe nocive asupra căilor respiratorii, aşa cum se întâmplă, spre exemplu, la locul de muncă. Nu rămâne decât, pe baza studiilor ştiinţifice, să stabilim o legătură între fumatul pasiv şi diverse afecţiuni respiratorii, cum ar fi bronşita.

Primele studii privind apariţia cancerului pulmonar la adulţii expuşi în mod constant la fum au fost publicate la începutul anilor 80. În anul 1986 autorităţile sanitare din Statele Unite şi Marea Britanie, precum şi OMS au declarat că fumatul pasiv creşte în mod semnificativ probabilitatea apariţiei cancerului pulmonar.

Fumatul pasiv creează o serie de dificultăţi sau afecţiuni respiratorii:

- respiraţie şuierătoare;
- insuficienţă respiratorie chiar şi pe parcursul unui efort puţin pronunţat;
- bronşita acută cu tuse sau cu expectoraţii;
- bronşita cronică cu tuse sau cu expectoraţii purulente care durează de cel puţin doi ani.

Persoanele nefumătoare care pot trăi într-o atmosferă neviciată de fumul de ţigară respiră mai liber şi au mai puţine probleme de ordin respirator decât persoanele nefumătoare închise în spaţii pline de fum.

Doar 4% dintre persoanele nefumătoare care au posibilitatea să respire un aer curat au respiraţie şuierătoare, o parte suferă de tuse cronică şi 5% prezintă simptome de bronşită cronică (tuse sau expectoraţie); după o expunere îndelungată într-o atmosferă îmbibată cu fum de ţigară, aceleaşi persoane prezintă: respiraţie şuierătoare - 7%; tuse cronică - 11%; bronşită cronică - 7%.

Beneficiile angajaților din restaurantele în care fumatul este interzis

Simptomele a 67 barmani înainte şi după interzicerea fumatului

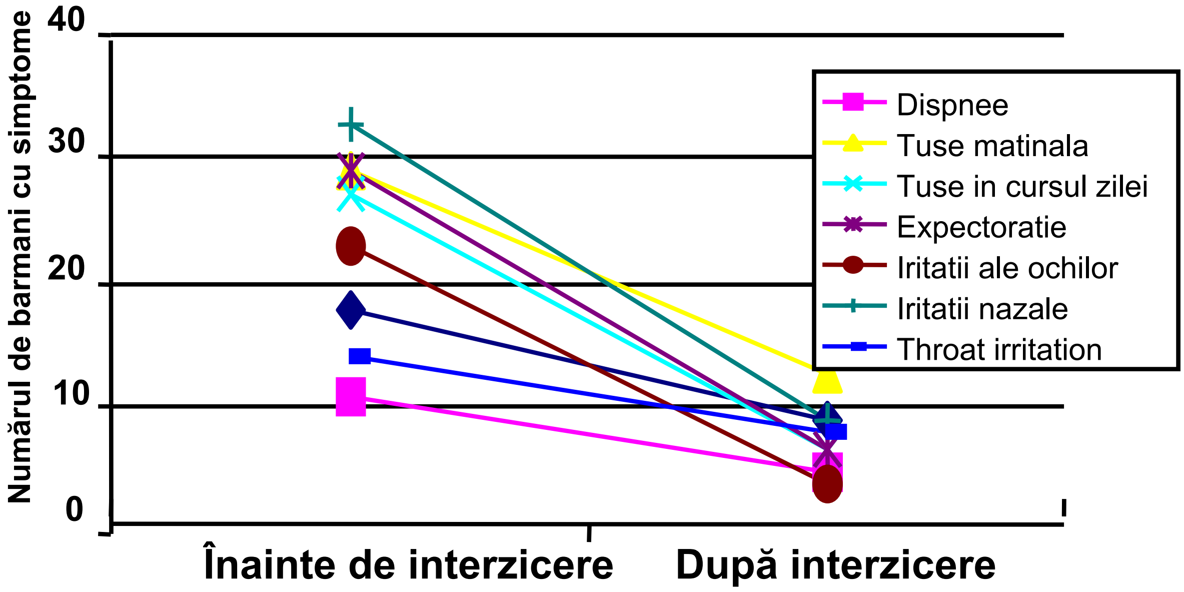

Sursa: **Eisner M et coll., JAMA 1998, 280, 1909-1914**

Dificultăţile respiratorii (tuse, bronşită acută, bronşită cronică, astm, insuficienţă respiratorie în timpul efortului fizic) sunt cu atât mai frecvente la nefumători cu cât aceştia sunt constrânşi să respire în încăperi pline de fum.

Monitorizarea biologică a fumatului pasiv

Componente	Neexpus		Expus		Fumător	
C0	3 ppm ± 1,5		7–11 ppm		>10–20 ppm	
HbCO	> 1,7 % CG	RIA	2-3 % CG	RIA	> 5 % CG	RIA
Cotinina						
Salivara (ng/ml)	0,4	3,6 – 31,3	0 – 8			
Plasmatica (ng/ml	nedetectabila	Nedetectabila	5 –7,33	1		
Urinara (ng/ml)		0,3 – 392	0–250			3 – 6810
Urin. (ng/mg creat.)			30			
Nicotina Salivara (ng/ml)	5,8 ± 4,35		10–43.6		150,6 ± 155	
Plasmatica (ng/ml)			2,49			
Urinara (ng/ml	7,47 ± 8.25		21.5 ± 28.7		396	

CG = cromatografie gazoasă ; RIA = radio-immuno-assay

a) Astmul şi fumatul pasiv zilnic. Cercetările arată, pentru persoanele nefumătoare, creşterea probabilităţii îmbolnăvirii de astm bronşic, diagnosticat din punct de vedere medical în funcţie de durata expunerii la fumul de ţigară. Această probabilitate este de două ori mai mare dacă durata expunerii zilnice la fum depăşeşte trei ore.

b) Bronşita cronică şi fumatul pasiv. Pentru fumătorii pasivi, riscul de a se îmbolnăvi de tuse cronică creşte de-a lungul anilor. Riscul prezintă o importanţă de două ori şi jumătate mai mare când fumatul pasiv este practicat de mai mult de douăzeci de ani. În plus, riscul este cu atât mai ridicat cu cât sunt mai mulţi fumători care poluează mediul ambiant, tusea cronică reprezentând primul simptom al bronşitei cronice sau al emfizemului pulmonar.

Ca regulă generală, simptomele apar după mai mulţi ani de expunere pasivă la fumul de ţigară, dar după apariţia primelor simptome, boala are o evoluţie gravă.

Dacă se află fumători în apropierea unui nefumător, atunci probabilitatea pentru acesta din urmă să fie victima unei bronşite cronice creşte, cu cât durata de expunere la fumul de ţigară este mai lungă.

Probabilitatea apariţiei bronşitei cronice este cu atât mai ridicată cu cât persoana nefumătoare este obligată să stea în preajma fumătorilor la locul de muncă.

Pe scurt, cu cât persoanele nefumătoare sunt mai mult timp expuse la fumul de ţigară, cu atât creşte riscul ca ele să sufere de afecţiuni ale căilor respiratorii şi o diminuare a capacităţilor fizice.

Statusul general şi tratamentele dentare. Clasificare ASA

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Rezumat. Sănătatea orală este parte integrantă a stării de sănătate generală, între cele două existând multiple interacţiuni. Una dintre cele mai folosite metode pentru aprecierea importanţei aspectelor patologice generale identificate, din perspectiva riscului medical prezent, utilizează Sistemul de clasificare a stării generale enunţat de ASA (American Society of Anesthesiology). Acesta este constituit din VI grade, pacienţii care solicită tratament dentar aparţinând în general primelor IV. Fiecare clasă prezintă o caracterizare specifică şi e corelată cu modificări ale tratamentului de o amploare variabilă. Clasificarea ASA este un instrument util, uşor de folosit de către medicul dentist, care orientează asupra gravităţii afecţiunii prezente şi implicaţiilor ei.

Cuvinte cheie: tratament dentar, status general, clasificarea ASA

Abstract. Oral health is integral part of general health. Between the two of them there are multiple interactions. One of the most currently used methods for assessing the importance of general pathological aspects identified (in terms of health risk factors) is the classification system set out by ASA (The American Society of Anaesthesiology). It consists of VI grades. Patients seeking dental treatment usually belong to the first IV. Each class has a specific characterization and is correlated with changes in treatment on a scale variable. ASA classification is a useful, easy to use by your dentist, which focus on disease severity and the implications they present.

Introducere

Sănătatea reprezintă o dimensiune cu impact deosebit asupra societăţii, atât prin implicaţiile ei asupra calităţii vieţii, cât şi prin contribuţia la buna desfăşurare a activităţilor populaţiei. Este privită astăzi mai mult ca o resursă pentru viaţă decât ca un scop al vieţii.

Ea presupune existenţa unui echilibru între toate „părţile componente” ale organismului uman. În acest context, sănătatea orală devine parte integrantă a stării de sănătate generală, între cele două existând multiple interacţiuni. Modificarea statusului general poate contribui uneori la apariţia unor aspecte patologice la nivelul cavităţii bucale. Acestea pot constitui semnale de alarmă ale unei afectări sistemice. Prezenţa eroziunilor dentare poate fi un indiciu al refluxului gastroesofagian sau bulimiei. Sindromul Sjögren, deshidratarea şi bulimia pot contribui la apariţia hiposialiei. În sens reciproc existenţa unor modificări la nivelul statusului oral poate fi corelată cu aspecte patologice sistemice. Se presupune că există o legătură între parodontita marginală cronică şi bolile cardiovasculare.

Astfel, medicina dentară este parte integrantă a medicinei generale. Medicul dentist trebuie să aibă permanent în vedere că aspectele patologice tratate reprezintă doar un aspect limitat al unui ansamblu mult mai complex. În momentul instituirii oricărui tratament este necesar să se aibă în vedere posibilele interacţiuni etiologice reciproce dintre aspectul local şi o afectare sistemică, riscul apariţiei unor complicaţii sistemice în timpul efectuării diferitelor manopere stomatologice, influenţele pe care le poate avea statusul general şi medicaţia asociată asupra evoluţiei şi prognosticului.

Identificarea modificărilor statusului general şi semnificaţia lor în practica medicală dentară

Orice tratament dentar începe printr-o analiză amănunţită a situaţiei prezente. Datele privind starea de sănătate generală sunt identificate în principal prin anamneză şi printr-un examen general sumar. Indicii suplimentare pot fi observate din comportamentul pacientului şi din examenul intraoral. Dacă medicul dentist consideră că unele aspecte identificate sunt neclare, acest lucru poate fi cel mai uşor şi sigur lămurit prin colaborarea cu medicul de familie al pacientului.

După culegerea şi analiza tuturor datelor despre statusul medical general şi local, medicul dentist este în măsură să ia o serie de decizii privind tratamentul specific. Aici un rol important îl are şi medicul generalist, care poate da indicaţii preţioase privind stabilirea modificărilor necesare în cursul tratamentului.

În primul rând existenţa unei patologii generale trebuie apreciată din perspectiva riscului tratamentului dentar asociat. În unele cazuri se consideră că în momentul respectiv pacientul nu este apt să suporte tratamentul dentar. Afectările sistemice pot face ca tratamentul stomatologic să prezinte un risc vital considerabil şi din acest motiv să fie necesară amânarea lui. În alte cazuri existenţa unei patologii generale se corelează cu modificarea planului de tratament, ale condiţiilor de desfăşurare (cabinet dentar sau unitate spitalicească), ale manoperelor de rutină (momentul instituirii, selectare individualizată a substanţelor şi materialelor folosite), utilizarea sedativelor, premedicaţii cu antibiotice etc.

Aceste atitudini sunt impuse de desfăşurarea în condiţii de siguranţă a actului medical dentar (pentru reducerea riscului ca pacientul să prezinte un episod acut în timpul tratamentului) şi obţinerea unui rezultat care să răspundă cerinţelor funcţionale şi estetice, cu un prognostic bun şi implicaţii negative asupra stării de sănătate minime (să nu agravăm afecţiunea sistemică).[i]

Sistemul de clasificare a stării generale enunţat de ASA (American Society of Anesthesiology)

Una dintre cele mai folosite metode pentru aprecierea importanţei aspectelor patologice generale identificate, în contextul tratamentului medical, utilizează Sistemul de clasificare a stării generale enunţat de ASA (American Society of Anesthesiology) (tabel I.). Criteriul principal este statusul general al pacientului, care este privit ca grad de risc medical în cazul tratamentului chirurgical, în care este integrată şi o subetapă în care se realizează anestezia (generală, loco-regională, locală).

Tabel I. Clasificarea ASA[ii]

CLASIFICAREA ASA (American Society of Anesthesiology)	
GRAD	**SEMNIFICAŢIE**
ASA I	Pacient sănătos
ASA II	Pacient cu boală sistemică moderată
ASA III	Pacient cu boală sistemică severă
ASA IV	Pacient cu boală sistemică severă, cu risc vital permanent
ASA V	Pacient muribund, care nu are şanse să supravieţuiască fără operaţie
ASA VI	Un pacient în moarte cerebrală, ale cărui organe sunt recoltate pentru a fi donate
Dacă tratamentul chirurgical este o urgenţă, indicativul corespunzător stării fizice va fi urmat de litera “E” (exemplu “5E”)	

Tratamentul dentar, pentru cei mai mulţi pacienţi, este o situaţie însoţită de anxietate, care se manifestă prin apariţia stresului, şi poate fi asociat cu tulburări ale homeostaziei. Totodată este de cele mai multe ori invaziv, fiind considerat în unele ţări ca aparţinând specialităţilor chirurgicale. În acest context, riscul operator şi anestezic este apreciabil şi în cursul tratamentului medical dentar, fiind deosebit de important ca pacientul să fie corect evaluat şi apoi să se practice individualizările necesare.

În practica medicală dentară de rutină, în cabinetele cu acest specific, pacienții aparțin în general primelor patru clase. În mod excepțional pacienții care aparțin ultimelor două clase pot necesita tratament specific dentar, dar aceste situații se realizează într-o unitate spitalicească.

Pentru o diferențiere mai clară a clasificării în scopul utilizării ei, sunt folosite caracterizări descriptive pentru fiecare grad. Exemple ale unor afecțiuni sistemice cu gradul ASA corespondent sunt prezentate în tabelul II.

Tabel II. Exemple de afecțiuni sistemice cu gradele ASA corespunzătoare

GRAD	AFECȚIUNI SISTEMICE
ASA II	Primele 2 trimestre de sarcină Pacient foarte anxios cu antecedente de sincopă în cabinet dentar Hipertensiune arterială (HTA) controlată: TA sistolică<140 mmHg/TA diastolică<90 mmHg Angină pectorală stabilă Infarct miocardic în antecedente mai vechi de ½ an fără simptomatologie şi reluarea activităților normale de către pacient Diabetul zaharat non-insulino dependent (tip 2) bine controlat (medicamentos sau prin dietă) Epilepsie controlată medicamentos, fără crize în ultimul an Astm bronşic controlat Afecțiuni tiroidiene Alergii manifeste Obezitate moderată
ASA III	Ultimul trimestru de sarcină (anumite poziții împiedică întoarcerea venoasă, stres) HTA gradul II ESC/ESH: TA sistolică: 160-179 mmHg/ TA diastolică: 100-109 mm Hg Infarct miocardic la mai mult de ½ an, cu simptomatologie (fatigabilitate, dispnee) Episoade de angină pectorală în antecedente (dar nu în ultimele 2 luni) Terapie anticoagulantă Terapie steroidă prelungită Diabet zaharat insulino-dependent (tip 1) controlat prin tratament cu insulină Pacienți compromişi, cu afectări pulmonare obstructive (inclusiv bronşite cronice, emfizem pulmonar) Obezitate cu grad ridicat
ASA IV	Hipertensiune arterială gradul III ESH/ESC: TA sistolică >180 mmHg/ TA diastolică >110 mmHg Angină pectorală instabilă Infarct miocardic sau accident vascular cerebral în ultimele 6 luni Diabet zaharat necontrolat Boală renală cronică în faza finală (pacienți cu dializă renală) Hemofilie Epilepsie necontrolată

Gradul **ASA I** înseamnă un pacient sănătos. În urma realizării anamnezei şi examenului clinic general realizat în cabinet nu reiese afectarea stării generale. Tensiunea arterială sistolică este mai mică de 140 mm Hg, iar cea diastolică mai mică de 90 mm Hg. Gradul de anxietate este mic, este nefumător, iar consumul de băuturi alcoolice are un nivel mic. Din această categorie nu fac parte pacienţii aparţinând vârstelor extreme. Poate alerga cel puţin 2 minute sau urca două etaje fără să apară dispnee sau fatigabilitate. În concluzie, nu există afectări organice, biochimice sau psihiatrice. Procesul patologic e localizat şi pacientul nu e compromis sistemic.

La indivizii consideraţi ca prezentând un grad **ASA II** a fost identificată o afecţiune sistemică, dar aceasta este bine controlată şi nu are risc vital. În această categorie sunt introduşi şi cei care consumă băuturi alcoolice în cantitate mare sau cei care prezintă un grad de anxietate ridicat legat de tratamentul dentar. Aceste persoane pot urca două etaje, dar apoi simt nevoia să facă o pauză datorită disconfortului apărut.

Gradul **ASA III** este corelat cu prezenţa unei afecţiuni severe, care limitează activităţile, dar nu reprezintă o incapacitate. Poate prezenta risc vital. Pacientul poate urca două etaje, dar pe parcurs se va opri datorită apariţiei dificultăţilor în respiraţie, disconfortului sau anginei pectorale.

Pacienţii prezentând gradul **ASA IV** manifestă o boală sistemică severă, puţin controlată sau într-un stadiu avansat. Limitează activităţile zilnice (nu pot desfăşura activitate fizică uşoară nici măcar pentru o perioadă scurtă de timp) şi au risc vital permanent. Disconfortul, dificultăţi în respiraţie, durerile în piept apar chiar şi în repaus, în lipsa efortului fizic, uneori chiar în timpul somnului.

Categoriile de risc sistemic pentru diferite manopere medicale dentare

După ce pacientul a fost încadrat în unul dintre gradele clasificării ASA avem formată o primă impresie generală privind implicaţiile stării generale asupra tratamentului dentar ce urmează a fi instituit. Riscul apariţiei complicaţiilor sistemice în cursul tratamentului dentar este variabil în funcţie de manopera realizată (tabel III).

Tabel III. Exemple de categorii de risc sistemic pentru diferite manopere medicale dentare

MANOPERA	Riscul apariţiei complicaţiilor sistemice
Examinare intraorală, examen radiologic, realizarea modelelor de studiu	Minim sau absent
Anestezie locală, proceduri de odontoterapie restaurativă, profilaxie, endodonţie fără prezenţa simptomatologiei, extracţii simple, tratament ortodontic	Mic
Endodonţie cu simptomatologie, extracţii multiple, inserarea unui implant, chiuretaj parodontal şi detartraj subgingival	Mediu
Proceduri chirurgicale de mare amploare, plasarea mai multor implanturi, anestezie generală	Mare

Cu cât ordinea clasei este mai mare, implicaţiile asupra tratamentului sunt mai complexe şi riscul medical este mai mare (tabel IV). În general, dacă pacientul prezintă un grad III sau IV ASA, este bine să se realizeze şi un control medical general înainte de tratamentul dentar. De exemplu, dacă un pacient neagă existenţa oricărei afecţiuni sistemice, dar nu poate urca două etaje fără pauză şi uneori apar dureri în piept, este indicat să fie examinat pentru a vedea dacă nu prezintă probleme cardiace.

Discuţii şi exemple

Pentru fiecare situaţie există anumite protocoale de lucru pentru desfăşurarea în condiţii de maximă siguranţă a actului medical dentar. De exemplu, în cazul bolnavilor epileptici, înainte de anestezie sau extracţie li se vor administra barbiturice, extracţia la diabetici se face sub protecţie de antibiotice şi planificarea tratamentului se va face ţinându-se cont de ora mesei. În cazul prezenţei problemelor cardiace se va utiliza anestezic cu nivel minim sau fără vasoconstrictor etc.

În prezenţa unui pacient cu o stare generală alterată medicul dentist va decide dacă nu este în interesul pacientului ca terapia stomatologică să fie amânată. Există, de exemplu, câteva situaţii clare în care se contraindică efectuarea extracţiilor dentare, preferându-se amânarea lor. Acestea sunt infarctul miocardic sau accidentul vascular cerebral în ultima jumătate de an, leucemiile acute, by-pass-ul aorto-coronarian. Motivaţia este legată de faptul că s-a constatat că riscul de a suferi infarct miocardic este mai mare la cei care l-au făcut o dată şi acesta se pare că scade după prima jumătate de an. Totodată, există o perioadă post infarct de 21 zile în care pot apărea mai frecvent complicaţii cum sunt tulburările de ritm, ruptura de pereţi ventriculari sau valve. Pacienţii cu by-pass aorto-coronarian prezintă aritmii aproximativ jumătate de an după intervenţia chirurgicală.

În situaţiile mai puţin clare se recurge la colaborarea cu medicul generalist care are pacientul respectiv în îngrijire.

Pentru cei mai mulţi pacienţi o vizită la medicul dentist este o situaţie însoţită de anxietate, care se manifestă prin apariţia stresului, ce duce la tulburarea homeostaziei. În prezenţa stresului apar modificări fiziologice la nivelul organismului, datorită eliberării unei clase de substanţe denumite cathecolamine. Efectul este de creştere a ritmului cardiac, şi astfel, prin scurtarea diastolei, la creşterea nevoii de oxigen la nivelul miocardului. Stresul apare frecvent cu câteva zile înaintea consultaţiei, însoţindu-se de tulburări de somn. În momentul aşezării pe fotoliul dentar semnele şi simptomele se înmulţesc. Frecvent apare o creştere a tensiunii arteriale, a ritmului cardiac (tahicardie) şi a celui respirator (hiperventilaţie). În astfel de cazuri, percepţia durerii este mult crescută, în ciuda cantităţii mai mari de substanţă anestezică. În aceste condiţii tratamentul dentar devine o experienţă neplăcută atât pentru pacient cât şi pentru medic.

Tabel IV. Corelaţii între clasa ASA şi implicaţiile asupra tratamentului medical dentar

GRAD	Implicaţii asupra tratamentului medical dentar
ASA I	Se poate efectua tratamentul dentar de rutină, fără a fi necesară vreo modificare
ASA II	Tratamentul dentar de rutină poate necesita unele modificări, dar afecţiunea, fiind sub control, frecvent acestea nu sunt de mare amploare. Ele vizează: realizarea tratamentului în cursul dimineţii, înainte ca pacientul să obosească, scurtarea şedinţelor de tratament, solicitarea unui consult medical general al pacientului, utilizarea sedativelor sau premedicaţiei cu antibiotice, reglarea fotoliului într-o poziţie favorabilă.
ASA III	În tratamentul dentar de rutină modificările sunt indicate aproape sigur şi pot fi mult mai complexe. Frecvent pacientul necesită consult general în scopul reevaluării, modificări ale planului de tratament. Datorită bolii severe face mai greu faţă situaţiilor stresante din cabinetul medical dentar.
ASA IV	Pacientul fiind compromis sever din punct de vedere medical, este indicat să se realizeze doar tratamentul dentar de urgenţă, acesta trebuind să fie efectuat într-o unitate spitalicească. În orice moment poate apărea un episod acut şi trebuie să existe în acel moment posibilitatea acordării tratamentului adecvat. Când este posibil, tratamentul dentar va fi amânat până când starea medicală a pacientului s-a îmbunătăţit până cel puţin la gradul ASA III.

În cazul suprapunerii anxietăţii peste un organism compromis sistemic apar reacţii mai severe. Printre acestea se pot enumera crize de angină pectorală, edem pulmonar acut, tulburări respiratorii mai grave la pacienţii cu astm bronşic, crize de hipoglicemie la pacienţii cu diabet insulino-dependent, criza adrenocorticală la pacienţii cu medicaţie cortizonică. În aceste situaţii se impun măsuri speciale pentru managementul anxietăţii. Acestea includ: explicarea pacientului a tot ce urmează să i se facă, medicaţie pentru reducerea anxietăţii înainte de începerea tratamentului, şedinte scurte sau şedinţe mai lungi cu pauze, reducerea timpului de aşteptare a pacientului, sedarea, analgezie suplimentară, plasarea la îndemână a medicamentelor şi dispozitivelor folosite de pacient în momentul crizelor (inhalator astm, nitroglicerină) etc.

Concluzii

Pentru desfăşurarea în condiţii de siguranţă a actului medical dentar şi obţinerea celor mai bune rezultate, tratamentul medical dentar trebuie să fie personalizat, realizat în concordanţă cu statusul general prezent.

Prezenţa unei patologii la nivelul stării generale de sănătate creşte în general gradul de dificultate a tratamentului deoarece: poate avea un rol etiologic în apariţia unor modificări locale, poate modifica planul de tratament, poate necesita modificări ale unor manopere (realizarea într-o unitate spitalicească, contraindicarea extracţiilor sau folosirea vasoconstrictorului etc.).

O metodă utilă pentru aprecierea riscului medical dentar în contextul tratamentului specific foloseşte Sistemul de clasificare a stării generale enunţat de ASA (American Society of Anesthesiology). Acesta este indicat mai ales pentru manoperele care prezintă un risc de apariţie a complicaţiilor sistemice apreciat ca fiind mediu şi mare.

Clasificarea ASA este un instrument util, uşor de folosit de către medicul dentist, care orientează asupra gravităţii afecţiunii generale prezente şi implicaţiilor ei.

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Tulburarea posttraumatică. Abordări

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Rezumat. Trauma reprezintă un eveniment care presupune o ameninţare percepută sau reală la adresa individului şi care atrage după sine o reacţie emoţională de intensitate extremă. Pentru a trăi sentimentul de eveniment, este necesar ca un aspect al realităţii să dea naştere unei surprize şi unei semnificaţii care să facă întâmplarea notabilă. Fără aspectul notabil conştiinţa nu va reacţiona şi nu va rămâne nimic, nici măcar sub forma unei amintiri. Nu putem vorbi despre traumatism decât dacă există o intruziune, dacă surpriza copleşeşte subiectul şi îi depăşeşte capacitatea sa de adaptare, îl zdruncină sau îl înscrie într-o direcţie în care el nu a vrut să meargă.

Stresul posttraumatic este descris in literatura de specialitate ca reacţie la un eveniment traumatizant ce depăşeşte capacitatea persoanei de a se adapta. Manifestările tulburării includ: vise recurente şi intruzive, reamintiri ale traumei, retragere socială, dificultăţi în a iniţia şi menţine relaţii intime, tulburări de somn. Aceste simptome persistă luni sau ani după traumă, adesea apărând după o lungă întârziere; ele pot conduce la dificultăţi severe în readaptarea la viaţa civilă: alcoolism, divorţ, şomaj. Doi factori apar ca fiind critici pentru magnitudinea răspunsului: primul şi cel mai evident este intensitatea traumei iniţiale. Al doilea şi mai puţin evident dar absolut vital este natura suportului social disponibilă pentru militarul traumatizat. Severitatea şi incidenţa tulburarii de stres posttraumatic cresc sau scad în funcţie de contextul social. Intensitatea traumei şi gradul de suport social merg împreună şi se amplifică una pe cealaltă. Când societatea pregăteşte un soldat să-şi înfrângă rezistenţa de a ucide un semen şi îl plaseaza într-un mediu în care el va face acest lucru, atunci societatea are obligaţia să se confrunte deschis, inteligent şi moral cu traumele şi cu repercusiunile acestora. Deşi tulburarea poate fi un efect al câmpului de luptă, societatea care îi primeşte înapoi pe militari îi poate ameliora condiţiile. Numindu-ne soldaţii "eroi" sau "mercenari care luptă pentru bani" ar putea face o mare diferenţă în reîntoarcerea lor acasă.

Cuvinte cheie: stres posttraumatic, vulnerabilitate genetică, intervenţie psihoterapeutică.

Abstract. Post-traumatic stress disorder (PTSD) is an emotional illness that develops when a person is exposed to a highly dangerous, very terrifying, possibly life-threatening event. Trauma can be relative depending on the reaction of the person experiencing it. Seemingly minor or "normal human events" can cause extreme trauma responses in some people, for example. Likewise, not all people exposed to war, for example, will develop PTSD.

The early intervention is crucial in the treatment' success. Treatment with medications alone does not and cannot transform PTSD, it can only control or alleviate some symptoms, and symptom control remains dependent upon the medication regimen. At their best, these drugs only serve to delay the inevitable confrontation with the pain, suffering, grief and guilt, buried deep inside.

Genetics explains a substantial part of the vulnerability in developing this disorder.

ISTORICUL TULBURĂRII DE STRES POSTTRAUMATIC (TSPT)

TSPT reprezintă o condiţie care a acompaniat nu doar războaiele moderne, ci toate războaiele. Clusterul său de simptome a fost prima oară diagnosticat ca "nostalgie" printre soldaţii suedezi în 1678. Medicii germani numeau această stare "heimweh" iar francezii "maladie du pays", ambele însemnând "boala de ţară", adică angoasa militarului care îşi doreşte să scape de zona de luptă şi să se întoarcă acasă. Spaniolii au denumit-o "estar rote", expresie care descrie condiţia sufletului ca urmare a războiului-"a fi sfărâmat". În Primul Război Mondial s-a vorbit despre"şocul obuzului", considerându-se că explozia unui obuz schimbă presiunea atmosferică din preajma soldaţilor, afectându-le sistemul nervos.

Soldații care manifestau simptome ale TSPT erau judecați ca fiind laşi sau ca având o slăbiciune personală, tulburarea fiind recunoscută ca "nevroză de luptă" sau "nevroză traumatică", denumiri care erau o reflectare a stigmei sociale. 306 militari britanici au fost executați în timpul războiului pentru laşitate, mulți dintre ei fiind victime ale "şocului de obuz". În al Doilea Război Mondial se vorbea despre "oboseala de luptă". Deşi TSPT a fost neluată în considerare decenii la rând, războiul din Vietnam a adus în atenția publicului această tulburare când medicii au început să o diagnosticheze ca "sindrom post-vietnam", veteranii din Vietnam obligând astfel comunitatea medicală să o recunoască în mod legitim.

Deşi TSPT a existat de secole, abia în 1980 Asociația Psihiatrică Americană a adăugat această tulburare oficial în DSM.

DSM I a apărut în jurul perioadei războiului din Coreea şi includea atunci o condiție numită "reacție la un stressor major". DSM II descria o "tulburare situațională tranzitorie". În acest timp, medicii şi specialiştii în sănătate mentală au învățat mai multe despre condițiile tulburării iar stigma aplicată militarilor începea să dispară. Atunci a fost pentru prima oară când s-a înțeles că simptomele erau încercări ale corpului şi minții de a procesa trauma: cu alte cuvinte, tulburarea era o funcție a unui proces normal de vindecare. DSM III apărea după războiul din Vietnam.

Criticii diagnosticului spuneau că politicienii şi atitudinile anti-război influențează formarea şi apariția diagnosticului. Această atitudine încă persistă printre unii veterani ai războiului din Irak care văd TSPT ca pe o problemă a perioadei Vietnam şi au dificultăți în a-şi conştientiza propriile simptome. În acelaşi timp în care DSM III începea să se dezvolte, mişcarea feministă a vorbit pentru prima oară despre violențele familiale, violuri şi traumele suferite de femei şi copii. Atunci s-a înțeles faptul că nu doar veteranii de război ci şi alte victime ale traumelor dezvoltă aceleaşi simptome ori similare.

Cauza TSPT, aşa cum este descrisă în DSM III, include o traumă dincolo de capacitatea persoanei de a- i face față. În DSM IV, o traumă era văzută ca eveniment care poate cauza moartea, rănirea dar nu este în mod necesar dincolo de capacitatea persoanei de a o depăşi, aşa cum este în DSM III. DSM IV a fost revizuit în anul 2000 şi una dintre schimbări a fost redefinirea conceptului de traumă în TSPT. Se stipulează în DSM IV-TR că evenimentul este calificat drept traumă dacă inspiră frică intensă, neajutorare sau oroare în victimă.

Acest lucru include evenimente care altădată nu au fost gândite că ar cauza TSPT. S-a adăugat de asemenea că individul nu trebuie să experimenteze evenimentul direct, ci că poate fi şi martor la el sau să fie expus într-un alt mod, spre exemplu, să audă despre el, lucru care de asemenea poate duce la dezvoltarea tulburării. Aceste schimbări reflectă noi înțelegeri despre traumă, cum ar fi faptul că răspunsul la traumă poate fi transmis din generație în generație, aşa cum apare la copiii şi nepoții supraviețuitorilor Holocaustului. De asemenea, copiii care au fost martori la violența parentală pot avea TSPT chiar dacă nu au fost ținte ale violenței. DSM-V, care va apărea în mai 2012, va conține ultimele studii legate de TSPT (Lisa DeLuca, 2009).

EXPLICAȚII PSIHOBIOLOGICE ŞI GENETICE ALE EXPERIENȚEI TRAUMATICE

Stimulii care amenință viața, potențiali traumatici, activează, printre altele, centrii de baraj din hipotalamus ai sistemului nervos autonom şi duc la sinteza hormonilor de stres. Regiunea hipocampică şi cortexul cingular sunt mai degrabă inhibate în stări extreme de excitație, astfel că ele nu îşi mai pot îndeplini funcția lor de filtru iar impresiile nu mai sunt percepute şi ordonate categorial. Fragmente senzoriale ieşite din context, în care domină impresii olfactive, vizuale, acustice şi kinestezice iau locul imaginilor percepute ordonate. Aceste impresii senzoriale, părți neurocognitive ale "schemei traumei" rămân timp îndelungat vii şi dacă sunt stimulate din nou, fie prin stimuli situativi, fie la retrăirea dispoziției traumatice, ele se întorc sub formă de imagini mnezice intruzive, care repetă adesea peste ani acelaşi scenariu. Caracterul de atemporalitate al acestor fragmente de memorie traumatice sunt puse, ipotetic, pe seama faptului că eşuează categorizarea şi contextualizarea impresiilor senzoriale, astfel că pot fi reproduse fidel fragmente de amintiri acauzale, atemporale şi aspaţiale.

Şi alte structuri ale sistemului nervos central care se ocupă cu integrarea informaţiei emoţionale şi cognitive sunt alterate ca urmare a experienţei traumatice. De exemplu, în corpul calos sunt integrate informaţii din emisferele dreaptă şi stângă şi astfel sunt coroborate caracteristicile gândirii simbolice, orientate spre rezolvarea problemelor, analitice (de la emisfera stângă), cu indicaţiile de integritate ale comunicării şi percepţiei nonverbale (prelucrate în emisfera dreaptă).

O indicaţie despre tulburarea acestei functii se conturează în cercetările care stabilesc o lateralizare emisferică pronunţată pentru informaţiile traumatice, în sensul unei activităţi sporite în partea dreaptă şi unei activităţi slăbite în emisfera stângă. Studii au demonstrat că pacienţii traumatizaţi prezentau o activitate crescută în emisfera dreaptă, mai ales în amigdala şi lobii temporali mediali. În acelaşi timp a fost stabilită o activitate sporită în cortexul vizual drept iar emisfera stângă, mai ales zona stângă inferioară, în care se află centrul vorbirii expresive, a fost comparativ mai puţin activată. Această descoperire a demonstrat că persoanele care trec printr-o situaţie traumatică o retrăiesc imagistic, nefiind capabile să o exprime în limbaj.

Comparaţiile între starea de dinainte şi starea de după o psihoterapie a traumei prin *Eye Movement Desensitation and Reprocessing* (EDMR) au demonstrat că după o terapie reuşită creşte activitatea în cortexul cingular dar se diminuează activitatea amigdalei, ceea ce reprezintă faptul că impresiile traumatice rămân neschimbate dar se poate face diferenţa între trecut şi prezent, între amintire şi ameninţare actuală. Astfel, se pare că activitatea cortexului cingular este hotărâtoare pentru diferenţierea între informaţia internă şi cea externă. EDMR este un proces care stimulează integrarea informaţiei pe cele două niveluri, psihic şi fiziologic şi care conduce la o elaborare imagistică şi verbală a traumei. Mişcările oculare ritmice laterale se opun lateralizării produse de traumă, susţinând astfel confluenţa dintre informaţia care vine din emisfera dreaptă şi cea care vine din emisfera stângă (Fischer, Riedesser, 2001).

FACTORI DE VULNERABILITATE GENETICĂ

În societatea modernă, mai mult de 80% din populaţie experimentează evenimente de viaţă cu severitate extremă, însă numai 10% dintre femei şi 5% dintre bărbaţi vor dezvolta TSPT în cursul vieţii. Pe de altă parte, persoane cu adevărat predispuse la TSPT nu vor dezvolta această tulburare pentru că din fericire nu se vor întâlni cu niciun eveniment stresant sever. Se consideră că simptomele TSPT reflectă modificări induse de stres în sistemul neurobiologic, reprezentând o adaptare neadecvată a acestuia la stresori severi. Nu este foarte clar dacă anumite modificări neurobiologice din TSPT reflectă o vulnerabilitate preexistentă sau consecinţele expunerii la traumă. Este cunoscut faptul că evenimente de viaţă din copilăria timpurie au efecte persistente asupra structurii şi funcţionării SNC, fenomen denumit "programarea dezvoltării". De asemenea mai este cunoscut că mediatorii hormonului glucocorticoid pot fi implicaţi în acest proces. Studii recente sugerează că mecanismele epigenetice pot juca un rol important în influenţarea reciprocă dintre expunerea la stres şi vulnerabilitatea genetică. Au fost descoperite diferenţe între receptorii glucocorticoizi (NR3C1) prelevaţi din hipocampusul sinucigaşilor cu istoric de abuz fizic în copilărie şi prelevaţi de la sinucigaşi neabuzaţi în copilărie, indicând astfel că aceste mecanisme sunt implicate în adaptarea umană la stres (Alja Videtic, 2009).

Conceptul de "co-variaţie gene-mediu", aparţinând geneticii comportamentale clasice, explică modul în care predispoziţiile genetice pot fi conectate la factori de mediu într-o manieră bilaterală: pasivă (când copilul moşteneşte gene specifice de la părinţi), reactivă (când părinţii reacţionează pozitiv sau negativ la trăsăturile copilului) şi proactiv (când persoana pare să selecteze un mediu care să se potrivească specificului genetic personal).

Rezultate recent publicate despre interacţiunea gene-mediu şi TSPT au demonstrat că proteina FKBP5, implicată în reactivitatea receptorului de cortizol, poate fi un predictor important pentru TSPT, explicând legătura dintre severitatea abuzurilor din copilărie şi această tulburare. Un eveniment traumatic e mult mai probabil să ducă la apariţia TSPT la adulţii care au experimentat traume în copilărie şi au FKBP 5 iar trauma a fost fizică sau sexuală (Vsevolod Rozanov, 2009). Un alt predictor pentru dezvoltarea TSPT este numărul de evenimente traumatice trăite de subiect.

Natura rezilienţei[1] rămâne însă mult mai obscură decât natura vulnerabilităţii în termeni de mecanisme genetice, pentru că se bazează mai mult pe cele psihologice, sociale şi spirituale.

ABORDAREA PSIHOTERAPEUTICĂ A TRAUMEI. INTERVENŢIA ÎN CRIZĂ

Presupune în primul rând asigurarea securităţii celor afectaţi, ceea ce înseamnă adesea îndepărtarea de la locul traumei, lucru care însă nu se poate realiza întotdeauna în practică. Sentimentul de siguranţă poate fi susţinut prin contact corporal nemijlocit, de exemplu prin ţinerea de mână sau cuprinderea spatelui. Victimelor care se află într-o poziţie neajutorată trebuie să li se spună că sunt în siguranţă şi că vine un ajutor medical de exemplu. Sentimentul de siguranţă este favorizat şi de informaţii despre evoluţia rănilor dacă cei afectaţi o doresc. Cei care ajută trebuie să întreprindă mereu o încercare de explicaţie, apoi să observe cu grijă reacţia celor afectaţi, care poate fi foarte diferită, după cum ei se află în stadiul de evitare sau de invadare de excitaţii. În cel din urmă caz, relaxarea şi liniştirea trebuie să primeze în faţa informării. În faza de negare, cel care ajută va acţiona cu precauţie şi nu va corecta decât pozitiv, dacă cei afectaţi relatează evenimentul fragmentat. Mecanismele de apărare vor fi respectate în faza acută, dat fiind faptul că ele reprezintă o încercare de autoapărare la care nu se poate renunţa.

Când este restabilit un sentiment bazal de siguranţă şi există posibilitatea de a vorbi, victimei i se poate spune că prin reacţia sa la traumă răspunde absolut normal la o situaţie neobişnuită. Cel care ajută trebuie să perceapă pozitiv izbucnirile emoţionale ca manifestare a comportamentului de coping expresiv. Dacă victimele îşi fac reproşuri pentru comportamentul lor necontrolat, poziţia de susţinere emoţională poate acţiona despovărător. În cazul paraliziei emoţionale, retragerii şi apatiei victimei în faza de evitare, cel care ajută trebuie să stea la dispoziţie ca simplu partener de discuţie, fără a i se impune.

Relatarea verbală a evenimentului traumatic va fi realizată cât mai curând posibil după producerea acestuia. Cu cât subiectul va pune în acţiune comportamente de evitare, cu atât anxietatea va fi mai puternică. În timpul crizei subiectul se află într-o stare de puternică activare emoţională, fapt ce îl determină să fie mai dispus la dezvăluiri faţă de un ascultător atent şi suportiv. El manifestă tendinţa de a repeta relatarea faptelor, fenomen care trebuie încurajat pentru că are un efect cathartic. Sub aspect terapeutic, descrierea evenimentului şi relatarea cogniţiilor şi a stărilor afective trăite reprezintă prima forma de expunere. Expunerile incomplete sau spontane (amintiri intruzive, coşmaruri) deşi sunt încercări spontane ale psihicului de a dobândi catharsis-ul şi de a se vindeca, au un efect terapeutic mai slab, deoarece nu se includ toţi stimulii condiţionali. Cu cât sunt abordate mai multe detalii în timpul expunerii pe plan imaginativ, cu atât aceasta va fi mai completă şi va facilita procesul de stingere a simptomelor de stres.

Obiectivul terapeutului constă în a-l ajuta pe subiect să-şi reamintească evenimentul traumatic, să accepte faptul că acesta a avut loc şi să proceseze gândurile şi stările afective negative.

Scopul unei convorbiri de susţinere este acela de a favoriza elaborarea traumei care corespunde procesului natural de vindecare a rănilor psihice şi, pe de altă parte, de a-i împiedica să cadă într-una dintre extreme, fie în copleşirea emoţională neajutorată, fie în negare. În faza de intervenţie post-traumatica trebuie să fie implicaţi specialişti în psihologie clinică, psihiatri şi psihoterapeuţi specializaţi pe traumă (Fischer, Riedesser, 2001).

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[1] Rezilienţa reprezintă un proces care permite reluarea unui tip de dezvoltare, în ciuda existenţei unui traumatism şi în condiţii potrivnice; o sfâşiere refăcută. Nu putem vorbi de rezilienţă decât dacă a existat un traumatism. Trebuie să evaluăm un număr cât mai mare posibil de factori din istoria personală a subiectului şi din contextul acesteia, pentru a descoperi care situaţie i-ar declanşa rezilienţa şi pentru a o evita pe aceea care i-ar degrada şi mai tare condiţia.

Critical Incident Stress Debriefing (CISD) este o intervenţie în criză care include primul ajutor psihologic după un eveniment traumatizant. Metoda este bazată pe o filosofie de rezistenţă, rezilienţă şi recuperare rapidă ce are ca efect scăderea simptomelor şi a riscului dezvoltării unei tulburări de stres posttraumatic. CISM nu este o terapie şi nici un substitut al acesteia, însă efectele sale au fost observate şi la mai mult de trei luni de la eveniment.

PSIHOTERAPIA POSTTRAUMATICĂ

Deoarece PTSD are simptome atât de variate, o combinaţie de psihoterapie şi medicaţie[2] este adesea necesară. Programele psihoterapeutice cu cea mai mare eficienţă demonstrată includ psihoterapia cognitiv comportamentală, variante ale terapiei prin expunere, inocularea stresului, EMDR şi multe combinaţii ale acestor proceduri. Bazată pe evidenţa unui studiu controlat, armata ucraineană a plasat EMDR în categoria celor mai eficiente proceduri folosite în tratamentul PTSD, alături de terapia cognitiv-comportamentală (Oleg Udovyk, 2009).

Psihoterapiile de orientare psihodinamică şi cele eclectice, deşi folosite la scară largă, nu au fost suficient testate ca tratament pentru PTSD. Totuşi tehnicile experienţiale sunt considerate de unii autori (Edward Tick, 2005) mult mai potrivite în abordarea traumelor cauzate de război întrucât favorizează un nivel ridicat al catharsis-ului, purificării emoţionale şi însănătoşirii. În terapia tradiţională, punctul de vedere prevalent este că vindecarea apare dacă terapeutul rămâne detaşat emoţional de viaţa şi povestea clientului.

Terapiile traumei şi implicit terapiile cu veteranii de război sunt opuse: dacă terapeutul menţine detaşarea şi manifestă poziţia tradiţională de neutralitate terapeutică, elaborarea traumei este îngreunată, povestea rămâne doar povara pacientului şi acest lucru poate duce din nou la retraumatizare. Dacă terapeutul manifestă o poziţie angajată, ne-neutră, atunci tocmai în aceste cazuri se poate ajunge la o modificare spectaculoasă.

Deşi terapia prin expunere şi-a dovedit eficienţa[3], există pacienţi care nu doresc sau nu pot efectiv să vizualizeze evenimentul traumatic pentru a da un răspuns emoţional. În acest punct se poate interveni cu ajutorul realităţii virtuale (RV), metodă care, începând cu anul 2003, a fost aplicată în armata americană pentru pregătirea psihologică a personalului militar înainte de misiune şi pentru tratarea tulburărilor emoţionale şi cognitive cu care militarii se întorceau din teatrul de operaţii.

Se consideră că limitele RV ar fi legate de faptul că unii pacienţi nu se simt prezenţi în RV şi că acest procedeu poate fi eficient mai mult în desensibilizare şi nu în tratarea PTSD, care este o tulburare mult mai profundă. O altă critică adusă RV este faptul că nu se stabileşte o relaţie terapeutică ori alianţa terapeutică reprezintă polul opus experienţei traumatice, ea oferă un cadru sigur, conferind protecţie şi încredere fără de care succesul unei terapii este compromis.

De curând, armata americană a trecut la promovarea unui model comprehensiv care optimizează capacităţile fizice şi cognitive. Conceptul de "total fitness" oferă cadrul necesar pentru obţinerea rezilienţei forţei militare capabile să îndeplinească cerinţele unui război prelungit şi asimetric în care militarii se află sub presiune crescândă. "Total fitness" reprezintă un concept care integrează şi optimizează componentele fizice, psihologice, sociale şi spirituale ale dimensiunii umane pentru ca militarii să fie pregătiţi să reuşească în orice situaţie operaţională (Todd Yosick, 2009).

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[2] Tratamentul doar cu medicamente nu transformă TSPT, poate doar să controleze sau să uşureze unele simptome iar controlul simptomelor rămâne dependent de regimul medicamentos. În cel mai bun caz, medicaţia poate doar să întârzie inevitabila confruntare cu durerea, suferinţa şi vina, îngropate adânc în suflet.

[3] Cele mai recente studii (Spira, Wiederhold, Wood, 2009) au demonstrat o rată a scăderii simptomelor PTSD de 72%.

ABORDAREA PSIHOLOGIEI MILITARE ROMÂNEŞTI A STRESULUI DE LUPTĂ ŞI TRAUMATIC

Secţia de psihologie militară a S.M.G. a dezvoltat un program complex de asistenţă psihologică a militarilor români participanţi la misiunile din Irak şi Afganistan. Programul cuprinde patru etape: I. Selecţia psihologică; II. Pregătirea psihologică pentru misiune; III. Asistenţa psihologică permanentă pe întreaga perioadă a misiunii şi IV. Evaluarea psihologică post-misiune.

I. Selecţia psihologică: se evaluează nivelul intelectual, personalitatea şi motivaţia militarului de a pleca în teatrul de operaţii. Dacă unul din aceste criterii este negativ, subiectul este respins din programul de pregătire.

II. Pregătirea psihologică pentru misiune. Pregătirea psihologică a detaşamentelor româneşti pentru participarea la misiuni în T.O. constă într-un ansamblu de activităţi teoretico-aplicative pentru însuşirea cunoştinţelor, formarea deprinderilor şi atitudinilor care să asigure stabilitatea şi echilibrul psihic al militarilor pe timpul executării misiunii.

Prin activităţile planificate se urmăreşte informarea detaliată a militarilor asupra specificului operaţiilor şi misiunilor ce urmează a fi desfăşurate şi creşterea nivelului de cunoaştere a acestora cu privire la factorii de risc din teatrul de operaţii, în scopul dezvoltării capacităţilor adaptative şi optimizării conduitelor de răspuns la solicitările misiunii. Pregătirea psihologică a efectivelor se desfăşoară:

1. Lunar, prin teme şi informări care descriu cât mai fidel misiunea, teatrul de operaţii şi riscurile psihologice specifice luptei. Astfel, şedinţele de informare se referă la:

- forme ale comportamentului dezadaptativ în situaţii de luptă şi modalităţi de contracarare;
- modificările psihosomatice cauzate de acţiunea factorilor de mediu (socio-culturali, geo-climatici, floră, faună), caracteristici teatrului de operaţii;
- metode şi tehnici pentru controlul şi managementul stresului, astfel încât, la sfârşitul pregătirii, fiecare militar să poată utiliza cel puţin un procedeu de autoreglare a stărilor psihice;
- tensiuni emoţionale şi reacţii de răspuns ale militarilor din contingentele care au participat anterior în teatrele de operaţii.

2. Lunar, prin teme de informare şi discuţii cu militarii şi familiile acestora privind implicaţiile psihologice ale separării temporare de familie;

3. În etape, ce cuprind activităţi de dificultate crescândă:

a) instruirea militarilor în condiţii normale, cu o intensitate medie a solicitărilor fizice şi psihice, într-un interval de timp determinat şi într-un mediu relativ cunoscut;

b) antrenarea în condiţiile acţiunii factorilor stresanţi specifici câmpului de luptă modern, până la realizarea performanţelor obţinute în condiţii normale. Instrucţia implică modificări esenţiale în ceea ce priveşte ritmul de execuţie, intensitatea solicitărilor fizice şi psihice, durata şi dificultatea misiunilor, mediul de desfăşurare şi crearea de situaţii problematice care să inducă stări psihice negative. Obiectivul acestor antrenamente este de a pregăti militarii pentru confruntarea cu situaţii stresante, diminuând astfel potenţialul pentru reacţii psihologice negative ca TSPT.

III. Asistenţa psihologică permanentă pe întreaga perioadă a misiunii. Cea mai importantă latură a programului este prezenţa psihologului în campul românesc, unde acesta poate imediat evalua şi asista un soldat care prezintă semne şi simptome ale stresului de luptă; pe de altă parte, psihologul poate avea un mai mare control asupra evoluţiei stării acestuia.

În urma incidentelor soldate cu militari răniţi, decedaţi, capturaţi, psihologul aplică următoarele măsuri de intervenţie:

1. La nivel individual:

a) Intervenţia în criză, fiind fundamentală pentru detensionarea subiectului, se va desfăşura în momentul sosirii militarului în câmp; când este restabilit un sentiment bazal de siguranţă şi există posibilitatea de a vorbi, cel afectat trebuie să pună în cuvinte trauma pe care a trăit-o. Militarul poate manifesta tendinţa de a repeta relatarea faptelor, lucru care va fi încurajat, pentru că are un efect detensionant. Odată verbalizată trauma, intensitatea emoţională scade şi cu cât se evită relatarea, anxietatea creşte ajungându-se la cronicizarea simptomelor. *Şedinta va dura până la stabilizarea emoţională a militarului, indiferent de cât timp presupune acest lucru;*

b) psihologul stabileşte un plan terapeutic pentru subiect până când acesta se va recupera şi va putea să-şi reia misiunile;

c) dacă militarul nu se poate recupera în scurt timp, el va fi repatriat, pentru a evita fenomenul de contagiune.

Militarii nu vor fi obligaţi să participe la misiunile imediat următoare incidentului, dacă nu sunt complet stabilizaţi psihoemoţional.

2. La nivel de colectiv:

- identificarea unui grup de sprijin emoţional printre camarazi, care să transmită militarilor afectaţi mesaje suportive de încurajare şi înţelegere, de toleranţă faţă de reacţiile acestora, ca urmare a situaţiei excepţionale prin care au trecut;
- împiedicarea victimizării militarilor afectaţi, pentru a le facilita readaptarea la programul misiunii şi a le activa mecanismele naturale de procesare emoţională a traumei.

IV. Programul de recuperare postmisiune cuprinde patru etape:

Etapa I, presupune **pregătirea psihologică a militarilor pentru readaptarea la viaţa de familie, socială şi profesională** şi se execută de către psihologul din teatrul de operaţii, cu 10 zile înainte de repatriere. În paralel, preşedintele grupului de sprijin familial şi psihologul arondat organizează în ţară întâlniri cu familiile militarilor, în vederea transmiterii de informaţii cu privire la readaptarea şi reinserţia celor care urmează să fie repatriaţi. În acelaşi timp, psihologul din teatrul de operaţii pune la dispoziţia comisiei de evaluare postmisiune, în vederea pregătirii etapei a II-a, informaţii atât cu privire la problematica psihologică ce a caracterizat misiunea, cât şi la existenţa militarilor afectaţi psihic de condiţiile misiunii.

Etapa a II-a, **evaluarea psihologică propriu-zisă**, cu o durată de 10-14 zile, este realizată prin desfăşurarea unui interviu semistructurat, în scopul identificării prezenţei tulburărilor de stres post-traumatic, de adaptare, de anxietate şi depresiilor. Etapa debutează în primele două zile de la repatrierea militarului.

Etapa a III-a, **readaptarea psihologică** treptată la condiţiile familiale şi sociale prezente, la grupul profesional şi activităţile de bază. Etapa se desfăşoară la unităţile de provenienţă ale militarilor şi este realizată de către psihologul unităţii, cu sprijinul medicului şi comandanţilor/şefilor de structuri. După 1-3 săptămâni de la repatriere, militarii care în urma evaluării psihologice postmisiune au fost diagnosticaţi cu diferite tulburări şi au fost îndrumaţi către psihologul de unitate, sunt incluşi într-un program de asistenţă psihologică. După 12 zile de la reluarea activităţii, psihologul unităţii, în colaborare cu medicul unităţii şi comandanţii/şefii de structuri, monitorizează modul în care militarii întorşi din teatrul de operatii îşi reiau activităţile şi se readaptează la sarcini.

Etapa a IV-a, **evaluarea post-inserţie în grupul profesional, familial şi social**. După 2 luni de la reluarea activităţii, psihologul unităţii evaluează nivelul de adaptare şi reintegrare, prin desfăşurarea unui interviu semistructurat atât cu militarii, cât şi cu membrii familiilor acestora. În funcţie de severitatea problemelor de adaptare şi de simptomatologia dezvoltată, militarii sunt incluşi într-un program de asistenţă psihologică. Tot în această etapă se realizează monitorizarea militarilor care au fost spitalizaţi (Prisăcaru, Macarenco, 2009).

În prezent armata română nu înregistrează niciun caz oficial de TSPT şi are un procent scăzut de tulburări emoţionale cauzate de condiţiile teatrelor de operaţii, lucru care se datorează printre altele:

- selecţiei psihologice înainte de misiune;
- intervenţiei imediate în urma unui eveniment traumatizant;
- asistenţei psihologice permanente a militarilor în teatrul de operaţii, indiferent dacă problematica este cauzată de misiune sau este mai veche;
- evaluării şi intervenţiei imediate după repatriere.

CONCLUZII

Reacția individului joacă un rol mai important decât evenimentul în sine: evenimente aparent “normale” pot cauza un răspuns traumatic unor persoane de exemplu, la fel cum nu toți cei expuşi războiului vor dezvolta TSPT.

Genetica explică o parte substanțială a vulnerabilității în apariția tulburării de stres posttraumatic.

Intervenția timpurie este crucială în succesul tratamenului, pentru că reduce tensiunea acumulată, împiedică instalarea sindromului posttraumatic, evită fixarea reacțiilor patologice.

Soldații doresc să vorbească despre coşmarurile lor, despre amintiri şi prietenii morți decât să fie întrebați cum le merge medicația. Ei au nevoie să fie ascultați de un terapeut atent şi suportiv care să-i ajute să-şi amintească trauma, să o accepte şi să proceseze gândurile şi emoțiile negative.

Există cazuri când terapeutul (cu siguranță un novice) a sfătuit militarul să nu-şi amintească trauma pentru a evita stresul. Acest lucru poate doar să blocheze amintirile în suflet, însă fără a dezvălui războiul îngropat, persoana nu este pregătită să meargă mai departe. Să consiliezi militarii să evite stresorii sau să folosească medicamente pentru a masca simptomele are un efect contrar, acela de a lăsa trauma îngropată în suflet şi nu de a o îndepărta ori a o reduce.

Reprimarea şi negarea emoțiilor par să fie cauzele majore ale TSPT. Trauma este responsabilă pentru intensitatea reacției la evenimentul stresor şi nu pentru debutul tulburării. Reprimarea şi negarea emoțiilor duc la alt mecanism al apărării de urgență: simptomul, fenomen observabil care exprimă o parte din universul intim invizibil. De îndată ce subiectul poate identifica imaginea propriei nefericiri, o poate numi şi poate da o formă confuziei pentru a face comunicabilă suferința.

Fundamental este ca alegerea strategiei terapeutice să se potrivească nevoilor victimei pentru ca aceasta să dobândească reechilibrarea psihică, morală şi spirituală.

Cultura noastră nu are mijloacele de a ajuta veteranii să se purifice din experiențele de luptă înainte de a intra în viața civilă, astfel încât aceştia iau energiile şi emoțiile războiului direct înapoi în cotidian, fără a se curăța emoțional şi fără o perioadă de tranziție. Întoarcerea acasă reprezintă astăzi un proces destul de brusc în care soldații care şi-au finalizat misiunea se întorc “înapoi în lumea reală”, deseori la o distanță de câteva zile de ultima lor luptă. Acum au nevoie să se readapteze la un mediu total schimbat, la o nouă lume, în care nimic nu este aşa cum ei au trăit pentru atâta timp. Această stare de fapt este confuză şi periculoasă atât pentru veterani cât şi pentru cei care îi aşteaptă iar alcoolul anesteziază temporar sentimentele şi energiile pe care soldații le cară înapoi acasă.

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Articolele se redactează în Microsoft Word, Normal+Justf., Times New Roman, Font Size – 16 Bold pentru titlu, Font Size – 12 pentru text, iar rezumatul Font Size – 10 Bold.

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3. ARTICOLUL PROPRIU-ZIS

a. **Articol original** – cel mult 800-1 000 de cuvinte (4 pagini).

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- **INTRODUCERE**: prezentarea problemei, plasarea în context, scopul şi obiectivele studiului, ipoteza de lucru.

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- **REZULTATE**: fără interpretări.

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1 – analiza rezultatelor prin prisma obiectivelor propuse ale studiului cu comentarii cu privire la aspectele lămurite (pozitive sau negative) sau nelămurite prin acest studiu;

2 – comparaţie între rezultatele obţinute de autori şi alte studii din literatură, cu explicaţii, eventual ipoteze cu privire la diferenţele observate, accentuând asupra progresului pe care cercetarea de faţă îl aduce;

3 – comentarii privind importanţa studiului de faţă, precum şi o punere la punct a situaţiei actuale a subiectului investigat atât în literatură, cât şi în practică; eventual se vor prezenta aici cele mai importante studii sau aspecte din domeniu;

4 – probleme nerezolvate, întrebări la care nu s-a răspuns şi care rămân deschise în viitor pentru studiile ce vor urma, eventuale direcţii propuse pentru investigare în domeniul respectiv.

CONCLUZII

La nevoie, capitolele mari pot fi împărţite în subcapitole, după necesităţi.

Se recomandă utilizarea precaută a *abrevierilor* şi menţionarea numelui complet la prima apariţie în text a abrevierii respective. Toate referinţele bibliografice vor fi citate în text cu cifre arabe, la exponent, fără paranteze, în ordinea apariţiei (de exemplu: Un alt studiu^{18} arată...). Toate figurile şi tabelele vor fi citate în text în locul în care ele urmează să apară (de

exemplu: *Fig. 3*, *Tab. 2*). Datele care apar în tabele, legendele tabelelor şi figurilor trebuie să fie rezumate, nu duplicate în text.

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Toate figurile (ilustraţiile), fie fotografii, diagrame sau grafice, trebuie să fie de calitate. Ele se *numerotează* în ordinea apariţiei în text (cu referire în text la numărul figurii în locul în care aceasta ar trebui să apară) şi *vor purta un titlu* (de exemplu: *Figura 1***.** Distribuţia pe vârste şi sexe a pacienţilor).

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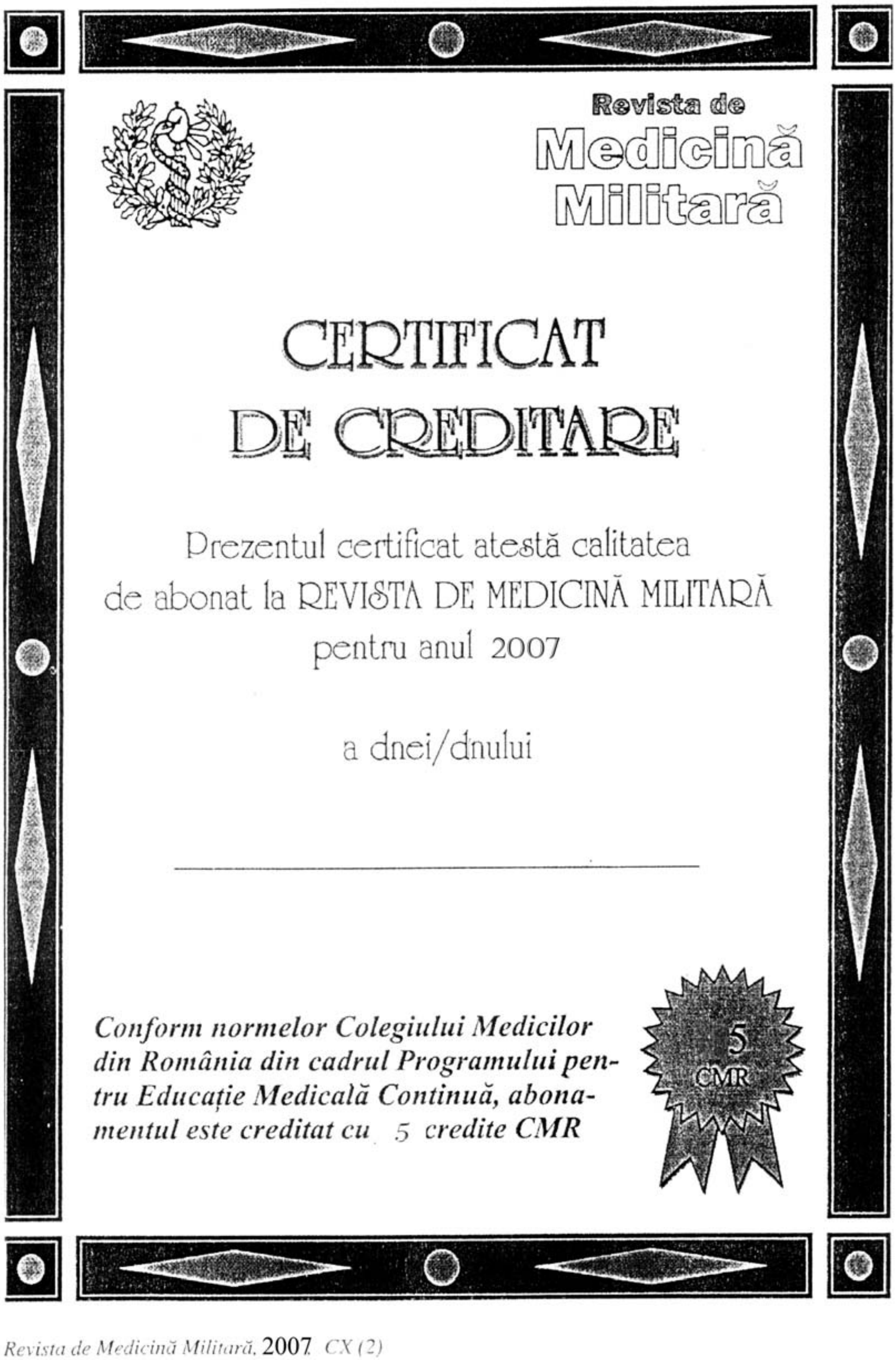

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O lume în continuă schimbare

Într-o lume în continuă schimbare, adaptarea şi evoluţia sistemului medical fie civil, fie militar sunt fenomene constante. Şi, în mod firesc, fiecare dintre noi, cititori ai acestei reviste, reprezentăm o parte componentă a unei organizaţii în care putem să fim obstacole sau motoare ale schimbării.

Zilnic primim mesaje prin intermediul mass media, pentru moment majoritar pesimiste, ceea ce în mod evident ne îndeamnă şi la găsirea unor soluţii pentru depăşirea unei astfel de situaţii. Cu momente de criză am fost mai mereu confruntaţi atât în calitate de pacienţi, cât şi de furnizori ai serviciilor de sănătate. Există o cale pentru a ieşi din impas şi de această dată? Din păcate o modalitate universal valabilă stă sub semnul întrebării, dar în toate vremurile continuarea investiţiei în pregătirea profesională şi predicţia personală asupra oportunităţilor lumii de mâine sunt soluţii general recunoscute. Desigur în acest proces un rol foarte important revine persoanelor care conduc sistemul, modului în care acestea sunt capabile de a proiecta o viziune şi de a orienta sistemul în direcţia dorită, de a avea scopuri eficiente şi bine definite, obiective clare şi cu o largă recunoaştere.

Indiferent de conjunctura de moment, *medicina militară* are de îndeplinit în continuare aceleaşi misiuni clare: să ofere asistenţă medicală şi să efectueze controale medicale periodice şi preventive pentru întreg personalul din armată, să acorde asistenţă medicală de urgenţă pentru toţi cetăţenii, să desfăşoare activităţi de învăţământ, de cercetare medicală şi sanitar veterinară, precum şi de pregătire operativă pentru luptă şi de specialitate, să asigure servicii medicale pentru toţi asiguraţii de la medicina primară la cea de ultraspecialitate. Tot acestui compartiment îi revin atribuţii pe linia misiunilor cu specific medical militar intern şi internaţional, expertizei medicale militare, asistenţei medicale pentru demnitari, asigurării logistice şi cu materiale sanitar farmaceutice, activităţilor de recuperare medicală, identificării factorilor de mediu, inclusiv a celor cu risc profesional şi cu impact direct asupra stării de sănătate, precum şi în elaborarea şi aplicarea măsurilor de prevenţie. Îndeplinirea acestor obiective se face cu un suport logistic şi informatic adecvat. Evident, medicinii militare îi revin o serie de atribuţii şi în situaţiile excepţionale: conflicte militare, cataclisme etc.

Aşa cum aminteam în primele rânduri ale materialului, sistemul economic al ţării noastre, ca şi al altor ţări de pe mapamond, se confruntă în prezent cu o criză cât se poate de gravă, cu repercusiuni evidente şi la nivelul sistemului medical. Limitarea drastică a fondurilor financiare impune căutarea unor soluţii alternative, investigaţii pentru găsirea acelor căi care să permită o utilizare raţională şi nu în exces, uneori chiar în abuz a resurselor medicale. Şi, în acest sens apelul la informaţie se dovedeşte cât se poate de bine venit. Pentru a demonstra rolul benefic al informaţiei este suficient ca fiecare dintre noi să facă recurs la memorie şi să-şi amintească de primii paşi făcuţi în specialitatea pe care o desfăşoară, la cât de utile i-au fost cele câteva sfaturi primite de la mentorul sau de la colegul cu mai mulţi ani de practică.

Experienţa colegilor din alte unităţi spitaliceşti, din ţară sau din străinătate, materializată în cărţi, articole publicate în reviste de specialitate, comunicările ştiinţifice, informaţiile de pe internet etc. sunt în egală măsură tot atâtea surse importante de îmbogăţire şi de reîmprospătare a cunoştinţelor care, aplicate în practică, pot conduce la rezultate benefice. În acelaşi context, viabilitatea sistemului medical militar impune şi o analiză pertinentă a mediului social, o evaluare atentă a resurselor umane şi financiare necesare şi, totodată, a posibilităţilor de a duce la bun sfârşit proiectele pe care şi le-a asumat. Pentru a răspunde pozitiv unor asemenea deziderate este nevoie de diversificarea serviciilor medicale şi sociale oferite, de redundanţă, adică de asigurare permanentă cu rezerve, cu delegare de competenţe şi responsabilizare cu sisteme de comunicare şi informare comună, cu transparenţă, flexibilizare şi elaborare de prognoze pe termene scurte şi cât de lungi posibile.

Cooperarea şi asumarea unor sarcini complementare cu sistemul medical civil, cu învăţământul universitar, cercetarea, manifestările ştiinţifice la nivelul tuturor structurilor din Armată sunt tot atâtea soluţii spre care îşi îndreaptă atenţia sistemul medical militar. Faţă de provocările actuale ale sistemului medical, medicina militară nu poate rămâne insensibilă. Ea este confruntată cu tendinţele extrem de actuale impuse de modernizare, schimbări la care însă trebuie să răspundă folosind eficient resursele de care dispune.

Informatizarea, precum şi sistemele complementare de valorificare a resurselor materiale şi umane şi accesarea de fonduri prin proiecte de cercetare sunt câteva dintre obiectivele ample vizibile care urmează să funcţioneze în viitorul apropiat iar noua denumire a Spitalului Clinic de Urgenţă Militar Central, de Spital Universitar de Urgenţă Militar Central este în acest sens doar un pas. Extinderea informatizării şi conexiunea structurilor componente prin infrastructura informatică compatibilă cu alte sisteme la nivel naţional reprezintă elemente cheie în vederea interoperabilităţii soluţiilor. Dosarul Electronic al Pacientului, asociat cu proceduri generalizate, precum şi arhivarea cu registre informatice la nivelul întregului sistem medical sunt tot atâtea modalităţi de a veni în sprijinul modernizării şi creşterii performanţei sistemului medical militar.

Prin paginile sale, *Revista de Medicină Militară* a încercat şi va încerca şi de acum înainte să pună la dispoziţia cititorilor săi materiale ce prezintă o cazuistică personală mai deosebită sau referate generale îmbogăţite cu date rezultate din experienţa proprie a autorilor, toate în ideea de a se constitui într-un real sprijin în activitatea pe care o desfăşoară fiecare dintre noi. În acest fel paginile revistei devin un spaţiu prietenos şi colegial de comunicare pentru întreg personalul medical din armată şi chiar pentru colegii din viaţa civilă, un cadru propice pentru un schimb fructuos de idei, de confruntare directă şi verificare a rezultatelor obţinute.

Acestea sunt câteva din gândurile pe care am ţinut să le transmit cititorilor în primul număr din anul 2010 al revistei noastre şi să le urez totodată mult succes în activitatea pe care o desfăşoară, precum şi invitaţia sinceră de a fi alături de noi atât în calitate de colaboratori, cât şi de beneficiari ai materialelor publicate.

Redactor şef
Col. dr. Dragoş CUZINO

Contribuții la studiul biopatogenezei ischemiilor. Cercetări experimentale şi pe biopsii miocardice interoperatorii

Dr. Dezideriu LAKY*, **Col. dr. Iancu MOCANU****

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****Centrul Clinic de Urgență de Boli Cardio - Vasculare al Armatei**
„Constantin Zamfir"- Bucureşti

OBIECTIV: Studiul biopatologiei miocardului ischemic în vederea aplicării metodelor adecvate de protecție a miocardului în chirurgia cardiacă.
MATERIAL ŞI METODE: Pe material experimental şi pe biopsii miocardice intraoperatorii s-au efectuat studii histologice şi ultrastructurale. Experimental s-au reprodus ischemii miocardice acute, tranzitorii cronice similare sindroamelor ischemice umane, urmărindu-se cronologic evoluția leziunilor.

Cuvinte cheie: studiu experimental, biopatologia miocardului ischemic

Abstract Purpose: The biopathology of ischemic myocardium was studied for apply the adeguate method of myocardial protections in cardiac surgery.
Matherial and methodes: On experimental matherial and on myocardial intraoperatories biopsies on a effected histological, histoenzimological and ultrastructural studies. Experimental on a reproduced acute, tranzitory and chronic syndroms similas to human pathology, to follow chronological the evolution of lesions. On intraoperatory succesive effeted on accnired and congenital patients, on are follow the consrquences of non physiological extracorporeal circulation and the influence of cardioplegie influence on cardiomyocytes. Intraoperatory biopsies from diskinetic myocardial zones from chronic atherosclerotique coronaropathies, followed the amplitude of evolutives lesions, apoptosis and the adaptative myocardial ischemic new formes as stunning and hibernation for the maintenance of the viability of these zones and theire prognosis and protective treatement. On non produced postoperatory complications after intraoperatory biopsies.

INTRODUCERE

În cadrul cercetărilor multidisciplinare asupra ischemiilor miocardice nu trebuie omise investigațiile morfopatologice, îndeosebi electronomicroscopice, pentru analiza structurilor mecanismelor subcelulare, în vederea elucidării disfuncționalităților miocardice.

Fundamentarea acestor procese patologice a fost efectuată la Institutul „Victor Babeş", în anii pionieratului acestor studii, în perioada 1971-1979, pe 100 de cazuri necroptice şi 347 de experimente, simulând sindroame ischemice umane, urmărite la intervale scurte, mai ales ultrastructural şi biochimic, în vederea reconstituirii imagistice a evoluției tabloului lezional. În baza acestor cunoştințe, în perioada 1982-2001, am studiat electronomicroscopic şi histoenzimologic, pe biopsii miocardice succesive, efectuate intraoperator de colectivul condus de V. Cândea, pe un număr de 267 de bolnavi cu valvulopatii şi malformații cardiace, evoluția leziunilor cardiomiocitare sub influența CEC şi efectele perfuziilor cardioplegice.

În perioada 1997-2000 s-au efectuat intraoperator de către M. Pătruț, V. Goleanu, I. Mocanu un număr de 30 de biopsii din zonele diskinetice, consecutive coronaropatiilor aterosclerotice, în scopul evidențierii gradului de viabilitate şi aplicării adecvate a protecției miocardului.

MATERIAL ŞI METODĂ

Pe material experimental şi pe biopsii miocardice intraoperatorii s-au efectuat studii histologice, histochimice şi ultrastructurale. Experimental, s-au reprodus ischemii miocardice acute, tranzitorii, cronice similare sindroamelor ischemice umane, urmărindu-se cronologic evoluţia leziunilor. Pe biopsii miocardice intraoperatorii la bolnavi cu cardiopatii dobândite şi congenitale, înainte şi succesiv, în timpul by-pass-ului cardiopulmonar s-a urmărit, cronologic, influenţa CEC şi a cardioplegiei asupra cardiomiocitelor. Biopsiile intraoperatorii din zonele diskinetice cu ischemii cronice ateromatoase au urmărit ponderea leziunilor şi modificările adaptative siderotice şi hibernarea în menţinerea viabilităţii acestor zone şi utilităţii lor prin by-pass-uri aorto-coronariene şi prognosticului lor.

Atât pe material experimental cât şi uman s-au aplicat, la Institutul ”Victor Babeş”, numeroase tehnici histologice: HE, VG, Lie, PAS – Alcian, tehnici biochimice pe suspensii mitocondriale, privind enzimele-marker mitocondriale precum: SDH, citocrom-oxidaza, şi lizozomale: fosfataza acidă şi catepsina D, şi mai ales electronomicroscopice, de transmisie.

1. Materialul experimental este reprezentat de un număr de 347 de animale, mai ales câini, supuse unor operaţii (ligaturi coronariene) de către N.M.Constantinescu, simulându-se sindroame ischemice umane.

1.1. Ischemii miocardice acute complete 5-60 de minute;

1.2. Ischemii miocardice acute urmate de reperfuzii;

1.3. Ischemii tranzitorii acute (metoda adaptării la ischemie) prin succesiuni scurte de obstrucţii şi revascularizări coronariene;

1.4. Precondiţionări ischemice precoce (ischemii tranzitorii, urmate de ischemii de 10-35 de minute);

1.5. Precondiţionări tardive, ischemii tranzitorii, urmate de ischemii de 20 şi sacrificări după 24 de ore de revascularizare;

1.6. Ischemii cronice prin obstrucţii coronariene incomplete şi sacrificări după 60 de zile.

2. Materialul uman

2.1. Biopsii miocardice succesive intraoperator efectuate la clamparea aortei şi periodic, în timpul CEC şi a perfuziilor cardioplegice (între 5-54 de minute) la un număr de 267 de pacienţi valvulopaţi şi cu malformaţii cardiace. La un număr de 90 de bolnavi cu valvulopatii s-au efectuat concomitent biopsii pulmonare, înainte şi după CEC, iar pe un număr de 62 de bolnavi biopsii hepatice înainte şi după by-pass-ul aorto-pulmonar. Aceste studii publicate sunt priorităţi în literatură.

2.2. Biopsii intraoperatorii din zonele diskinetice viabile la un număr de 30 de bolnavi din lotul de 186 de pacienţi studiat de I. Mocanu, cu cardiopatii ischemice aterosclerotice, supuşi revascularizării prin by-pass-uri aorto-coronariene.

REZULTATE

1. Pe material experimental:

Ischemiile acute

Obstrucţiile coronariene complete determină progresiv leziuni reversibile în primele 20-30 de minute, vizibile ultrastructural, interesând mitocondriile şi enzimele lor care scad şi sistemul tubular (dilatări), parcelar sarcolema, determinând edem sarcoplasmatic, disociind miofilamentele, mitocondriile, depleţii glicogenice, activări lizozomale cu eliberări de enzime hidrolitice.

După 20-30 de minute apar leziuni ireversibile mitocondriale, se accentuează leziunile mitocondriale, tubulare, depleţie glicogenică, numeroşi fagolizozomi, migrări ale granulelor natriuretice, edem marcat şi omogenizări ale miofilamentelor şi structurii sarcomerelor a cărei periodicitate se şterge. Se constată apariţia de corpi mielinici, leziuni nucleare cu depuneri de granule de calciu şi în mitocondrii, finalizându-se după 60 de minute, când cuprinde şi zona subepicardică. Procesul lezional se poate extinde în vecinătatea unde cardiomiocitele prezintă siderări şi leziuni evolutive până la apoptoze şi necroze care sunt substituite fibros.

Revascularizarea zonelor ischemice agravează tabloul lezional prin disfuncţii metabolice, îndeosebi prin radicali liberi de oxigen.

Ischemiile tranzitorii acute determină iniţial doar leziuni reversibile (figura 1).

Precondiţionările ischemice precoce (figura 10) se soldează cu leziuni reversibile, prin efectul citoprotector determinat de caracterul adaptativ al procesului.

Precondiţionările ischemice tardive induc leziuni heterogene cu limitate necroze în etapele finale, neapărând infarcte.

Ischemiile cronice

Obstrucţiile coronariene incomplete timp de 60 de zile, prin ligaturi parţiale determină imagini lezionale reversibile, la limita reversibilităţii, ireversibile şi modificări adaptative cardiomiocitare de siderare şi hibernare miocardică.

2. Pe material uman:

În condiţii de CEC, biopsiile miocardice succesive indică comparaţia cu zona martor (recoltat în momentul clampării aortei), accentuarea progresivă a leziunilor (figura 8), îndeosebi mitocondriale (figura 1), sarcotubulare (figura 2), depleţia glicogenică, formarea fagolizozomilor (figura 3), agravarea edemului interstiţial, disocierea sarcomerelor prin edem şi lize ale miofilamentelor, apoptoze (figura 14).

Acţiunea benefică a perfuziilor cardioplegice nu a putut jugula aceste leziuni evolutiv, însă a împiedicat în multe cazuri apariţia necrozei. Acest proces s-a realizat doar în cazuri de ICC cl. III, IV NYHA care au depăşit pragul reversibilităţii, instalându-se treptat leziuni ireversibile (figurile 5, 6, 7, 8). Menţionăm faptul că tabloul morfopatologic ischemic miocardic uman este similar cu cel experimental, motiv pentru care nu le mai descriem nici pe cele histoenzimologice.

Biopsiile recoltate din zone diskinetice au prezentat, în funcţie de durata şi gradul obstrucţiei ateromatoase ale coronarelor, leziuni heterogene (figurile 11, 14), intricate şi cu modificări de siderare şi hibernare (figurile 9, 12.a, 12.b), asigurând viabilitatea teritoriului supus grefării. Menţionăm faptul că enzimele lizozomale au fost concordante cu datele ultrastructurale.

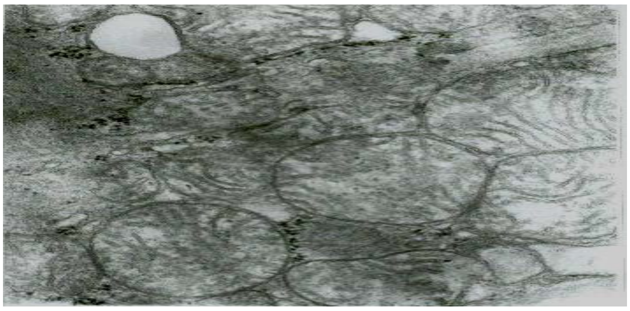

Fig.1. Biopsie intraoperatorie de miocard la 5 minute de la instalarea CEC: uşoare leziuni mitocondriale. Imagine electronomicroscopică, 25 000 X.

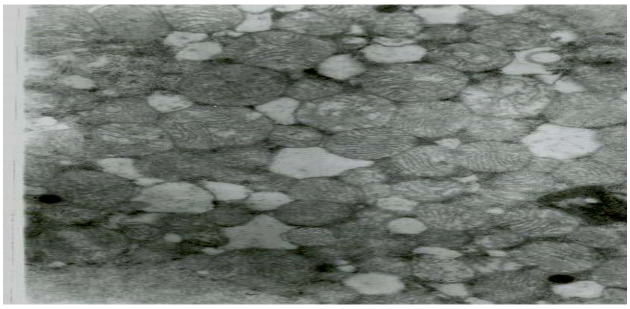

Fig.2. După 7 minute de CEC: apar şi dilatări ale reticulului sarcoplasmatic şi un lizozom activat.

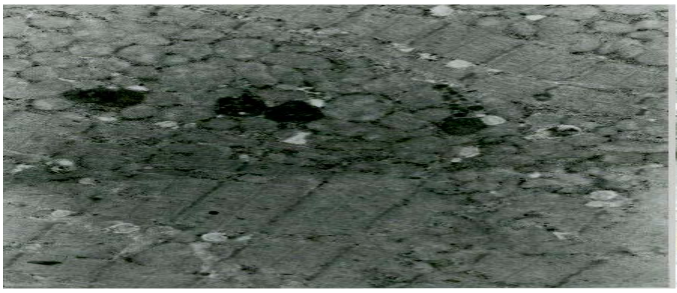

Fig.3. După 15 minute de CEC: mitocondrioză, hiperplazie de lizozomi activați. Imagine electronomicroscopică, 11 000 X.

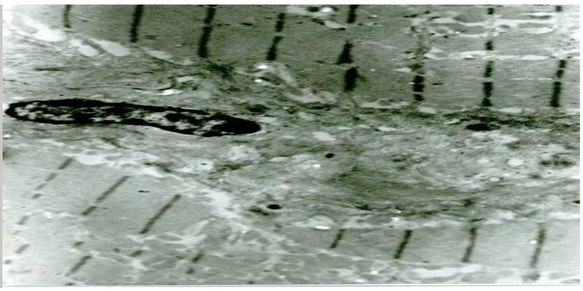

Fig.4. După 20 de minute de CEC: omogenizări ale sarcomerelor perinuclear.

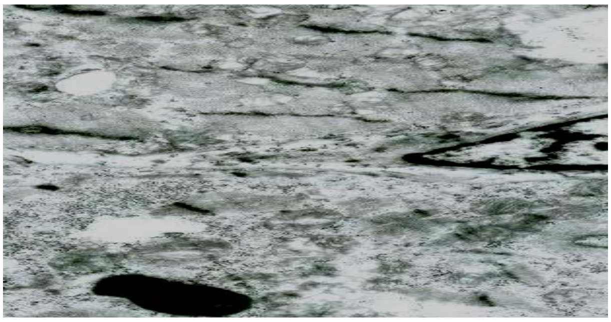

Fig.5. După 30 de minute de CEC: dezintegrări de sarcomere perilizozomal, marginații cromatiniene nucleare, vacuolizări mitocondriale, întreruperi ale liniilor Z, 6 000 X.

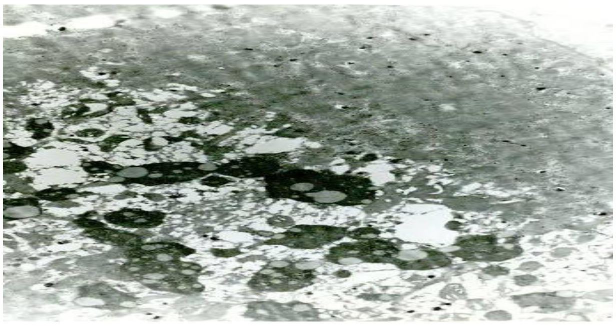

Fig.6. După 35 de minute de CEC: numeroşi fagolizozomi într-o zonă dezintegrată, 2 000 X.

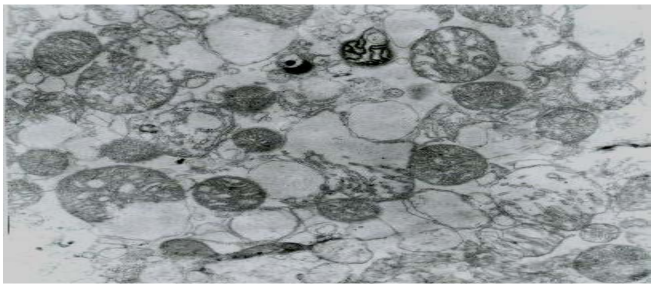

Fig.7. Numeroase mitocondrii cu dezintegrări, balonizări (swelling), după 30 de minute de CEC. Suspensie mitocondrială, 11 000 X.

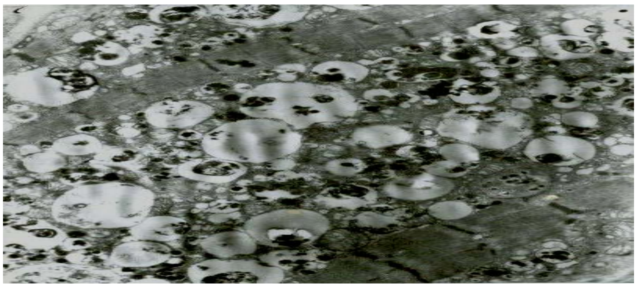

Fig.8. După 40 de minute de CEC: leziuni ireversibile dezintegrând total sarcomerele, cu vacuolizări, depuneri de calciu, fagolizozomi, 11 000 X.

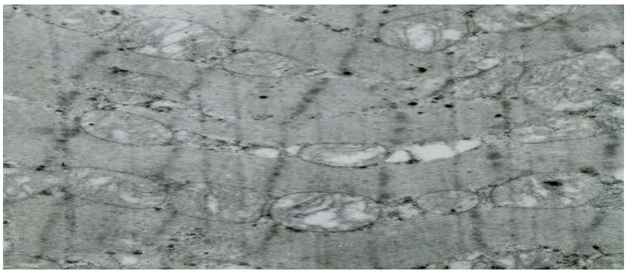

Fig.9. Miocard câine, ischemie tranzitorie acută: leziuni mitocondriale vacuolizante reversibile, 9 500 X.

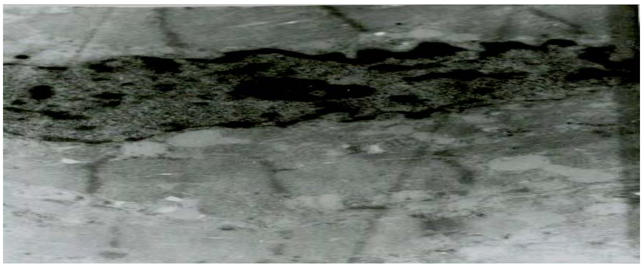

Fig.10. Miocard câine, precondiționare ischemică precoce: leziuni reversibile cu dilatări tubulare, dezintegrări parcelare ale unor sarcomere, 11 000 X.

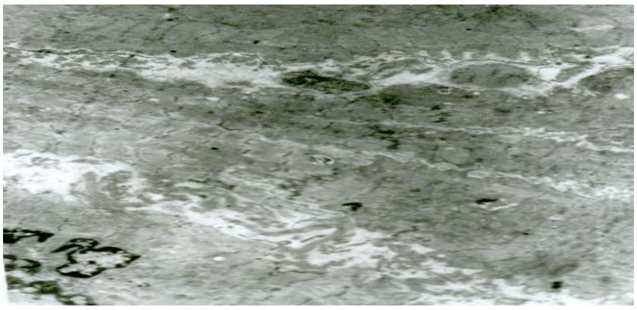

Fig.11. Biopsie miocardică la bolnav cu cardiopatie ischemică dureroasă (CICD) în timpul by-pass-ului aorto-coronarian: leziuni heterogene, 3 400 X.

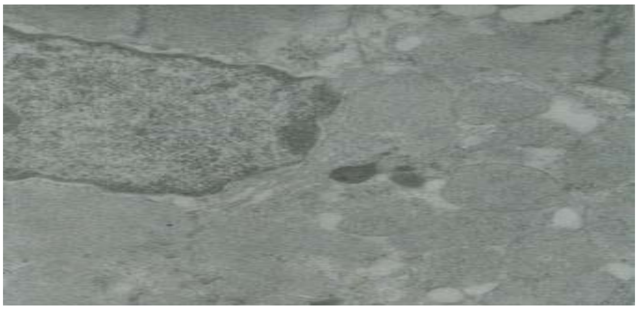

Fig.12a. Zonă din miocard cu modificări adaptative de hibernare miocardică: aspect granular fin al cromatinei nucleare, 11 000 X.

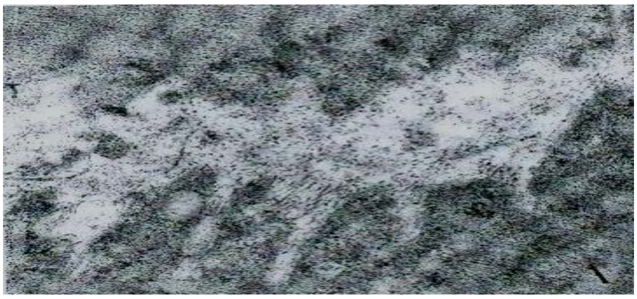

Fig.12b. Altă zonă din miocardul hibernant cu acumulări de granule de glicogen, 500X.

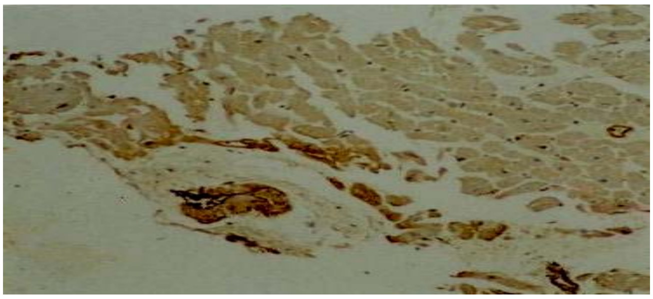

*Fig.13.*Zonă de miocard hibernant investigat imunohistochimic: prezența actinei muşchiului neted (colorat brun închis), 400 X.

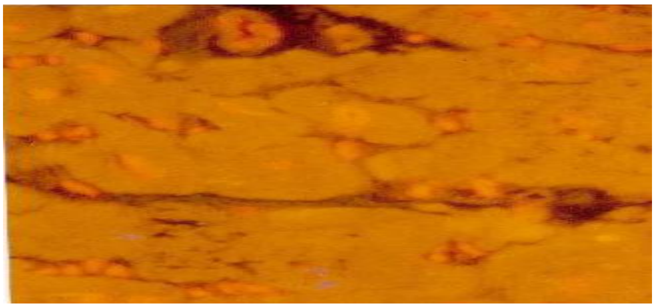

Fig.14. Zonă de miocard cu CICD cu leziuni avansate: prezența de apoptoze, corpi apoptotici (colorați intens portocaliu). Colorația cu iodură de propidiu, 400 X.

DISCUȚII

Cunoaşterea structurilor subcelulare şi a mecanismelor lor funcționale, în condiții de ischemie miocardică, prezintă importanță nu numai din punctul de vedere al datelor ştiințifice, ci, mai ales, din aplicarea lor în practica medico-chirurgicală.

Durata scurtă a manifestărilor sindroamelor ischemice, reversibilitatea acestora (1, 2), totală sau parțială, nu permite vizionarea substratului lor patologic decât experimental (7).

De aceea am recurs la efectuarea unei game de studii experimentale similare patologiei umane ischemice pentru descrierea cronologică (prin multiple sacrificări) a tabloului morfopatologic, îndeosebi electronomicroscopic şi biochimic enzimatic (8, 9, 10).

În ocluzii coronariene complete, trecerea la glicoliza anaerobă determină, în primele 20 de minute, leziuni reversibile urmate de alterări ireversibile predominente, între 30-35 de minute, necrozele extinzându-se subepicardic după 60 de minute (8, 10).

În vecinătatea ariei de infarct miocardic acut (IMA) există cardiomiocite cu modificări siderotice şi lezionale evolutive, putându-se, astfel, extinde teritoriul infarctizat, impunând clinicianului monitorizarea bolnavului.

Agravarea leziunilor cardiomiocitare, după revascularizări postischemice, se datorează multiplelor disfuncţii metabolice, îndeosebi formării de radicali liberi de oxigen (1, 2, 7).

Ischemiile tranzitorii acute experimentale simulează sindroamele anginoase, determină leziuni reversibile (7, 11), repetarea ischemiilor după revascularizare inducând precondiţionări ischemice precoce şi tardive, benefice.

Precondiţionările ischemice (15) sunt fenomene adaptative, de citoprotecţie care permit tolerarea mai bună a ischemiilor ulterioare, protejând cordul de aritmii.

Se observă scăderea mai lentă a ATP-ului în raport cu glicoliza anaerobă, acidifierea mai lentă a sarcoplasmei prin scăderea producerii de lactat. La bolnavii cu precondiţionări întârziate, instalarea unui infarct miocardic are, deseori, o evoluţie mai bună, întinderea mai redusă, fapt la care contribuie, probabil, dezvoltarea circulaţiei colaterale (2).

Obstrucţiile experimentale, incomplete ale coronarelor sunt similare celor aterosclerotice umane (12), din cardiopatiile ischemice cronice dureroase. Ele determină leziuni heterogene, intricându-se leziuni reversibile, ireversibile, fibroze, dar şi modificări adaptative embriofetale, de hibernare, menţinând viabilitatea acestor zone, care, în absenţa revascularizării, devin leziuni evolutive (6, 16).

Pe material uman, biopsiile miocardice succesive intraoperatorii efectuate în cursul CEC (3) au trebuit să fie permanent comparate cu miocardul martor recoltat imediat după clamparea aortei. Aspectul acestuia a fost variabil la diverşi bolnavi, în raport cu vechimea cardiopatiei, a clasei de I.C.C. NYHA, a gradului de HTP şi a altor afecţiuni. Repetatele biopsii miocardice, între 5-54 de minute, nu au prezentat complicaţii intra şi postoperatorii. În condiţiile unei circulaţii nefiziologice, respectiv CEC, cardiomiocitele au prezentat leziuni progresive, ajungând, după 20 de minute la limita reversibilităţii lezionale. Au apărut apoi numeroase granule natriuretice în ventricul, leziuni ireversibile diseminate la bolnavi cu I.C.C. cl. III-IV NYHA, şi mai ales IV NYHA, cât şi în valvulopatii septice, malformaţii severe. Perfuziile cardioplegice benefice (3) nu au putut împiedica apariţia şi evoluţia acestor leziuni, cordul oprit fiind în stare de siderare (4) globală şi preoperator, deja, variat lezat (13).

Biopsiile intraoperatorii efectuate în zonele diskinetice (14) supuse grefării aorto-coronariene nu au prezentat complicaţii. Viabilitatea lor, stabilită preoperator, a fost atestată electronomicroscopic prin ponderea leziunilor ischemice reversibile şi ireversibile intricate cu modificări de siderare, de hibernare (6, 16), cu cardiomiocite cu depleţii de miofilamente, aglomerări de granule de glicogen, diminuarea, până la dispariţie, a sistemului tubular, dediferenţierea embriofetală fiind atestată imunohistochimic prin prezenţa actinei muşchiului neted (6, 14, 16).

CONCLUZII

1. Cunoaşterea structurilor şi mecanismelor subcelulare în ischemii miocardice impune studii morfopatologice, cu tehnici moderne, pe material experimental şi uman.
2. Metodele experimentale simulând sindroame ischemice umane evidenţiază cronologic, mai ales, electronomicroscopic, evoluţia leziunilor acute, tranzitorii, de precondiţionare ischemică precoce şi tardivă.
3. Biopsiile intraoperatorii succesive efectuate, fără producere de complicaţii, evidenţiază agravarea progresivă a leziunilor în condiţii de CEC şi importanţa perfuziilor cardioplegice.
4. Biopsiile intraoperatorii din zone diskinetice viabile, de cauză ateromatoasă, permit aprecierea ponderii leziunilor evolutive şi de hibernare în menţinerea viabilităţii acestor teritorii şi rolul salvator al by-pass-urilor aorto-coronariene.
5. Lucrarea este primul studiu complex şi multidisciplinar, furnizând noi date ştiinţifice şi perspective de îmbunătăţire a protecţiei miocardului în chirurgia cardiacă.

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O LUCRARE ORIGINALĂ PRIVIND STUDIUL ANATOMOPATOLOGIC ŞI CARDIOCHIRURGICAL AL ISCHEMIEI MIOCARDICE:

„BIOPATOLOGIA MIOCARDULUI ISCHEMIC ŞI PROTECȚIA MIOCARDULUI ÎN CHIRURGIA CARDIACĂ”

Dr. Dezideriu Laky şi Dr. Iancu Mocanu, Editura medicală, Bucureşti 2008

Dezideriu Laky
Iancu Mocanu
BIOPATOLOGIA MIOCARDULUI ISCHEMIC ŞI PROTECŢIA MIOCARDICĂ ÎN CHIRURGIA CARDIACĂ
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Bucureşti - 2008

Literatura medicală, inclusiv cea medico-militară, s-a îmbogățit cu o monografie originală, scrisă, după experiențe de decenii, respectiv ani, de dr. Dezideriu Laky, cercetător principal gr. I la Institutul „Victor Babeş” Bucureşti şi de col. dr. Iancu Mocanu, medic primar la Centrul Clinic de Urgență de Boli Cardio-Vasculare al Armatei „Constantin Zamfir”. Cuvântul introductiv aparține unuia dintre fondatorii Centrului Clinic de Urgență de Boli Cardio-Vasculare al Armatei „Constantin Zamfir”: gl.bg. (r) prof. univ. dr. FESC Ion Țintoiu. Tratând o problemă de actualitate, prioritară în asistența medicală internațională, autorii selecționează din vasta cazuistică a clinicii, începând cu anul 1982 până în prezent, sute de bolnavi prezentând, pe fondul altor afecțiuni cardiace, valvulopatii, malformații, coronaropatii aterosclerotice, bolnavi care au fost supuşi, în cursul intervențiilor pe cord deschis sub circulație extracorporală, unor biopsii cardiace, repetate, intraoperator.

Lucrarea nu se doreşte o monografie didactică dedicată ischemiei miocardice şi protecției lui intraoperatorii, ci prezintă aspecte originale, personale ale acestor problematici.

În primele capitole sunt redate rezultatele obținute, prin îndelungate şi originale studii de către dr. Dezideriu Laky, privind morfopatogeneza acestei afecțiuni. Pornind de la o cercetare efectuată pe material necroptic, studiat histopatologic, prin microscopie optică, la Institutul „Victor Babeş”, încă din anul 1971, s-a continuat din acelaşi an cu diverse cercetări experimentale. Acestea vizau simularea unor sindroame ischemice din patologia umană, pentru a decela cronologic, din primele minute, biopatologia acestor leziuni, care, pe material necroptic, din cauza autolizei, nu puteau fi studiate electronomicroscopic şi histoenzimologic. Este de subliniat faptul că în cursul acestor experimente, de tipul ocluziei-reperfuziei coronariene şi în urmărirea efectelor circulației colaterale, s-a descris doar morfologic ischemia tranzitorie prin siderare, hibernare miocardică, precondiționare ischemică. Aceste stări ischemice au fost descrise pe larg, ca entități nosologice, în secolul trecut, la sfârşitul anilor 70 şi începutul deceniului 80, de importante personalități ca Rahimtoola, Brauner, Kloner ş.a., beneficiind de tehnici multidisciplinare moderne.

Autenticitatea acestor cercetări experimentale electronomicroscopice şi biochimice, pe animale, a fost atestată pe material uman, prin studii inițiate de gl.bg. (r) prof.univ.dr. Vasile Cândea, prin biopsii repetate, intraoperatorii, înainte şi după C.E.C., precum şi la bolnavi valvulari şi cu malformații cardiace. Descrierea evoluției acestor leziuni ischemice, precizate prin investigații electronomicroscopice, histoenzimologice, biochimice reprezintă o prioritate pe plan mondial, privind suferința miocardului în cursul circulației extracorporale. Pot fi menționate şi alte aspecte ultrastructurale în cadrul C.E.C., precum modificările matricei extracelulare, prezența granulelor natriuretice atriale dar şi la nivelul ventriculilor, influența unor medicamente cardioprotectoare precum fosfocreatina, a imaginilor de siderare, de hibernare miocardică. Corelându-se datele clinice cu cele morfologice şi cu alte investigații paraclinice, s-a certificat gradul insuficienței cardiace congestive, prognosticul şi terapia postoperatorie.

Acest studiu s-a lărgit, prin inițiativa aceluiaşi vizionar, gl.bg. (r) prof. univ. dr.Vasile Cândea, prin efectuarea de biopsii repetate pulmonare şi hepatice, în cursul operațiilor cardiace, reprezentând, de asemenea, o prioritate ştiințifică pe plan mondial.

S-a demonstrat faptul că, în cursul circulației extracorporale, are loc o intensă activitate fagocitară la nivel pulmonar, în schimb, la nivelul ficatului se produc accentuări progresive ale leziunilor anterioare, generate de staza cronică, impunând, postoperator, o atenționare specială.Studiul, efectuat pe bolnavii cu cardiopatii ischemice cronice, prin biopsii intraoperatorii recoltate din zonele ischemice dischinetice, a decelat modificări viabile precum: siderarea, hibernarea miocardică şi leziuni evolutive intricate ca apoptoze, necroze şi insule de fibroză, cu importanță prognostică şi terapeutică.

Ultimul capitol al monografiei este consacrat, pe baza documentării morfofiziologice, celui mai important factor al studiului, respectiv, protecția miocardului, în timpul intervenției cardiace. Aceasta este prezentată de către col. dr. Iancu Mocanu, care, timp de aproape un deceniu, pe un număr semnificativ de cazuri, pe analiza coronarografiei preoperatorii, prin introducerea algoritmului de calcul al scorului SYNTAX, al indicelui de revascularizare oferă soluții optime intervenționale care, împreună cu circulația colaterală, determină o nouă anatomie a circulației coronariene. Sunt analizate datele privind C.E.C., cardioplegia şi utilitatea metodelor de administrare a substanțelor cardioplegice folosite, precum şi reducerea necesarului energetic, prin hipotermie. Este subliniat rolul monitorizării electrocardiografice, tehnica şi tactica chirurgicală abordată. Această lucrare anatomo-clinică cuprinde 276 pagini grupate în nouă capitole, este bogat ilustrată, având imagini histologice color şi alb/negru şi numeroase imagini electronomicroscopice.

Concluzionând, această inedită monografie se adresează nu numai cardiochirurgilor, cardiologilor, ci şi interniştilor, având obiective diagnostice şi terapeutice redate într-un complex amplu anatomo-clinic mai rar întâlnit în literatura de specialitate.

Prof. univ. dr. Ion ȚINTOIU

Instalațiile de Rezonanță Magnetică de 3 Tesla

Colonel dr. Dragoş CUZINO, General bg.(r) prof. univ. dr. Ioan CODOREAN

Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila", Bucureşti

Rezumat. Tesla (T) este unitatea de măsură a câmpului magnetic. Înaintea instalațiilor de 3 T, instalațiile de 1,5 T erau standardul de câmp magnetic. Astfel, dacă o imagine este mai sugestivă decât 1 000 de cuvinte, IRM de 3 Tesla poate fi comparată cu o enciclopedie. Claritatea imaginii oferite de 3 Tesla este foarte utilă în creşterea performanței în examinarea creierului, măduvei spinale, sistemului musculo-scheletal, şi a screening-ului de tip whole-body. Există şi alte avantaje ale IRM de 3 T, cum este, spre exemplu, angiografia RM, care poate înlocui studiile de cateterism invaziv intervențional. Timpul mai scurt de scanare permite creşterea confortului pacientului iar calitatea imaginii oferă posibilitatea unei mai bune diferențieri a tipurilor de leziuni tumorale.

Cuvinte cheie: IRM, 3 Tesla, spectroscopie RM, tractografie RM, IRM a întregului organism

Abstract.Tesla (T) is the unit of measurement quantifying the strength of a magnetic field. Prior to the 3 Tesla Machine, the high-field standard was 1.5 Tesla. Thus, if a picture is worth a thousand words, the 3 Tesla MRI is an encyclopedia. The increased image clarity revealed by 3T is particularly beneficial for pathological conditions involving the brain, spine, musculoskeletal system and for whole body screening. The benefits of the 3T scanner are not confined to Magnetic Resonance Imaging. The increased spatial resolution of the 3T scanner allows for high-quality vascular imaging. Thus, 3 Tesla MR Angiogram studies may often supplant the need for invasive interventional catheter studies. Utilizing shorter scan times, the 3T machine maximizes patient comfort without compromising quality. The superb reliability of high-field MRI allows the radiologists to differentiate between benign and potentially hazardous medical conditions with confidence.

Impactul IRM asupra conduitei clinice şi protocoalelor de diagnostic a început odată cu progresele tehnologice importante din anii „70". La început valoarea cea mai des întâlnită a câmpului magnetic era de 0,6 T şi se credea că va fi utilizată ca metodă doar pentru țesuturile şi organele care nu produc artefacte de mişcare. A urmat o altă etapă în care instalațiile de 1T au fost cele mai utilizate datorită prețului convenabil şi calității suficiente a imaginilor şi studiilor de rutină. Scăderea prețului instalațiilor de 1,5 T a continuat, în prezent fiind cele mai vândute, dar viitorul apropiat poate să ne ofere ocazia de le a utiliza pe scară largă pe cele de 3T, pentru început în centrele de cercetare şi în spitalelele universitare.

Neurologii şi neurochirurgii au fost primii care au recomandat beneficiile câmpului magnetic ridicat, urmați de ortopezi, chirurgii vasculari şi de oncologi.

Aspectele care la început păreau dificile, legate de producerea antenelor de suprafață, limitele de siguranță ale undelor de radiofrecvență, de zgomotul din incinta aparatului au fost depăşite. Se mai mențin însă unele probleme legate de omogenitatea câmpului magnetic, greutatea mai mare a sistemului, artefactele de tip *chemical shift* şi diferența de contrast între unele țesuturi, dar şi acestea sunt în curs de rezolvare.

Rata de absorbție specifică - *Specific absorption rate (SAR)* - reprezintă cantitatea de energie eliberată într-o anumită masă sau un țesut. Comisia Tehnică Electrotehnică a impus ca valoare maximă 8 watt per kg W/kg pe țesut pentru fiecare 5 minute de scanare, sau 4W/kg pentru întreg corpul după 15 minute. Energia rezultată din undele de radiofrecvență disipată produce încălzirea tisulară. Dublarea intensității câmpului magnetic va produce o valoare a SAR de 4 ori mai mare. Din acest motiv poate fi uneori alungită perioada de scanare.

Imagistica paralelă este o altă metodă robustă de reducere a expunerii la undele de radiofrecvență prin reducerea paşilor de codare în fază realizați pentru o scanare completă. Aplicații practice pentru acest tip de imagistică sunt: Angiografia prin Rezonanță Magnetică, explorarea cardiacă şi abdominală sau toracică cu respirație blocată.

În **neuroimagistică** cel mai mare impact al instalației de 3 Tesla constă în creşterea calității şi consistenței IRM de tip BOLD şi funcțional. Față de 1,5 Tesla, datorită creşterii susceptilității şi senzitivității şi a valorii raportului semnal/zgomot, poate spori cu până la 40 % în activarea centrilor cerebrali. Aceste proceduri sunt folosite pentru producerea unei hărți - *brain mapping* - pentru identificarea cortexului sensorimotor sau a ariilor de limbaj. Aceste arii identificate şi cuplate cu un scanner şi cu un sistem de transmitere către pacient a paradigmelor pot fi folosite pentru evaluarea unor afecțiuni de tipul demenței şi al maladiilor psihiatrice.

Creierul normal conține diferite concentrații de metale, dintre care cel mai abundent este fierul. În funcție de concentrație sunt clasificate de la I la IV, gradația I având semnificația unei concentrații crescute în fier şi o întâlnim în *globus pallidus* şi în substanța neagră, în timp ce stadiul IV corespunde măduvei spinării şi substanței albe cerebrale. Spre deosebire de imaginea obținută la 1,5 Tesla, la 3 Tesla efectul paramagnetic şi cel diamagnetic sunt mult mai puternice, astfel încât studiile comparative au arătat diferențe ale aspectului cortexului motor între imaginile obținute cu cele două tipuri de instalații.

Tractografia este metoda care evidențiază traiectele nervoase prin utilizarea unor secvențe speciale IRM şi a unor soft-uri dedicate de vizualizare 2D şi 3 D pentru a fi puse în evidență atât fibrele lungi cât şi cele scurte de legătură cortico-subcorticală. Metoda de lucru se bazează pe anizotropie. Aceasta se defineşte ca proprietatea unui corp de a prezenta caracteristici variate iar în acest caz se referă la proprietatea de a difuza asimetric.

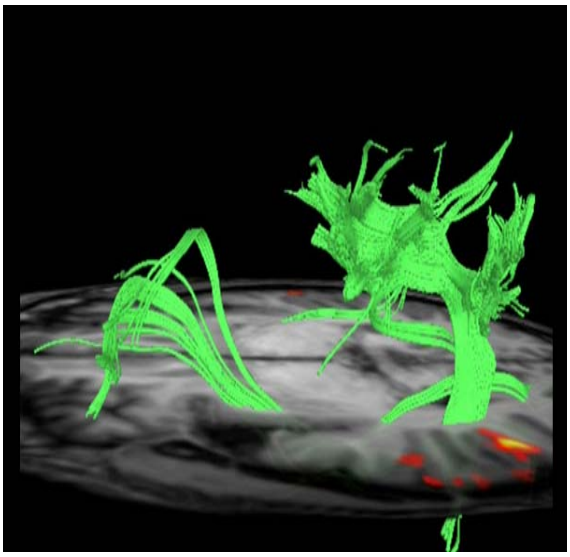

Tractografie RM - conexiune între ariile Wernicke şi Broca.

În privința **Angiografiei prin Rezonanță Magnetică** prin tehnica TOF- *Time of Flight-* la 3 Tesla T1 țesuturilor staționare este mai lung şi se foloseşte un unghi de basculă mai scăzut pentru optimizarea semnalului dat de mişcarea sanguină. De asemenea matricele mai mari cu 512 sau 1 024 pot fi mai eficient utilizate datorită SNR mai mare. Prin cuplarea tehnicii de imagistică paralelă şi a bobinelor îmbunătățite se obține un timp de scanare similar cu cel de la 1,5 Tesla cu o rezoluție mult mai bună.

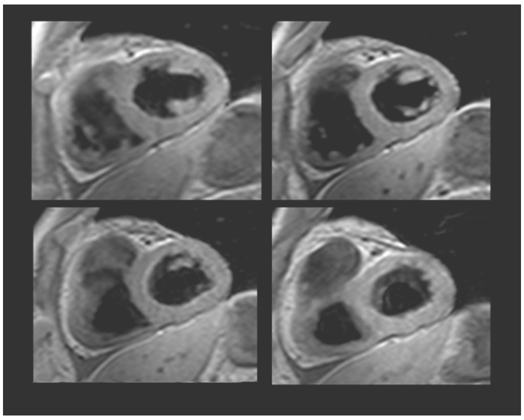

Imagini cu gating cardiac obținute în axul scurt al inimii.

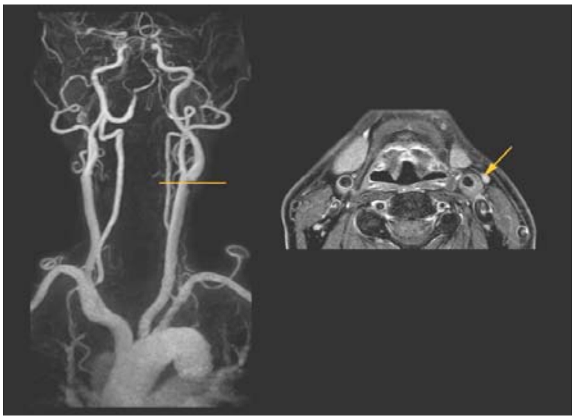

Angiografie RM după injectarea substanței de contrast Stenoza arterei carotide interne.

Explorarea cardiacă, încă o examinare dificil de realizat datorită contactului dintre țesutul pulmonar plin cu aer cu cel cardiac în mişcare, alături de vase sanguine şi fenomenul de flow-void prin utilizarea tehnicii black-blood şi bright-blood şi de imagistică funcțională oferă, asociate cu valoarea de 3 T a câmpului magnetic, perspective valoroase în evaluarea miocardului, a cineticii cardiace şi a arterelor coronare.

Spectroscopia IRM. Rezoluția spectrală se îmbunătăţeşte atunci când se trece de la 1,5 la 3 Tesla, datorită dublării intensității fenomenului de *chemical shift*. În acest fel sporeşte evaluarea de rutină a metaboliților care nu sunt evidențiați la 1,5 Tesla iar spectroscopia multinucleară poate oferi mult mai multe informații utile în ghidarea diagnosticului histopatologic.

IRM abdomen şi pelvis. Energia eliberată de sistemele 3T prin undele de radiofrecvență este foarte mare, ceea ce limitează numărul de secțiuni care pot fi realizate la o scanare în timpul unei secvențe. Au fost astfel dezvoltate achizițiile multiple cu respirația blocată- *breath hold-,* în special 2D EG. Pot fi obținute şi secțiuni subțiri de tip 3 D EG volumetrice funcționale izotropice ale întregului abdomen cu bobine de tip „high-density" şi la o singură achiziție cu respirația blocată. Imagistica de tip paralel ameliorează restricțiile date de valori crescute ale SAR.

IRM în ortopedie. Sunt responsabile de aproximativ 20% dintre examinările de ansamblu în centrele IRM în care se efectuează investigații de toate tipurile. IRM 3T prezintă şi în aceste cazuri calitate superioară prin raport semnal zgomot ridicat, iar bobinele de suprafață devin tot mai eficiente în a oferi rezoluție spațială crescută cu timp de examinare scăzut şi furnizează detalii anatomice de finețe.

"Whole"- body IRM. Într-un moment în care CT a înlocuit examenul radiografic obişnuit, cei care recomandă această investigație trebuie să pună în balanță riscul potențial al iradierii şi al injectării unei cantități sporite de substanță de contrast iodată. *Whole body CT* este pentru moment metoda de elecție dedicată explorării pacientului politraumatizat şi a celor din secțiile de anestezie şi terapie intensivă. Cerințele tehnologice necesare pentru o astfel de instalație sunt: magnet principal cu intensitate crescută, sistem de gradienţi rapid şi puteric, sistem de radiofrecvenţă omogen. Mai este necesar un sistem de mişcare a mesei de examinare de tip multistaţii şi "workstation" care să recompună imaginea. Rolul acestei metode este valoros şi indicaţiile în afara oncologiei pentru depistarea metastazelor pentru screening şi chiar pentru autopsie virtuală urmează să fie evaluate.

Concluzii

Faţă de aspectul IRM, considerat până de curând gold- standardul de 1,5 Tesla, instalaţiile de 3 Tesla permit obţinerea unei rezoluţii spaţiale, temporale şi de contrast mult mai ridicate şi astfel studiile morfologice şi funcţionale de difuzie, perfuzie, spectroscopie şi activare corticală oferă informaţii mult mai utile pentru activitatea de rutină şi cea de cercetare. Aplicaţiile sunt complexe şi valoarea imaginilor este foarte bună în explorarea creierului şi a patologiei traumatice, a accidentelor vasculare cerebrale, a bolilor substanţei albe, a bolilor Parkinson şi Alzheimer, în spectroscopia tumorală cerebrală dar şi în toate celelalte tipuri de afecţiuni, inclusiv whole-body.

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Interrelația afecțiunilor generale cu stomatita protetică. Studiu de literatură

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Rezumat. Stomatita protetică reprezintă o patologie a mucoasei bucale, frecvent asociată pacientului purtător de proteze mobile, existând o multitudine de factori locali şi generali incriminați în apariția ei. O serie de afecțiuni generale, prezente la purtătorii de proteze, sunt asociate stomatitei protetice. Raportat la vârstă, numeroase studii au arătat că o dată cu aceasta creşte numărul afecțiunilor generale, dar şi frecvența stomatitei protetice. Stomatita protetică, pe lângă efectul de traumă determinat de proteză, este frecvent corelată cu xerostomia, indusă de medicația specifică afecțiunilor generale. Pornind de la aceste observații, sunt analizate afecțiunile generale (cu modificările induse), dintre care unele specifice pacienților geriatrici, considerate ca favorizante pentru apariția stomatitei protetice. Cunoscând aceste afecțiuni, medicul generalist sau dentist poate adopta atitudini terapeutice corecte în cadrul tratamentului general la purtătorii de proteze mobile.

Cuvinte cheie: stomatită protetică, proteze mobile, afecțiuni generale

Abstract. Denture stomatitis is a disease of the oral mucosa, associated with the denture wearer, with a multitude of local and general factors that lead to its development. A number of general disorders of the denture wearers is associated with denture stomatitis. Many studies have shown that with age, the number of general affections increases, but also the frequency of denture stomatitis. Adding to the effect of trauma caused by the prosthesis, denture stomatitis is often correlated with dry mouth (xerostomia), induced by drugs specific in general diseases. General medical conditions are analyzed (with the induced changes), some of which are specific to geriatric patients, considered as favorable for the development of denture stomatitis. Therefore, the general practitioner or the dentist can adopt the correct therapeutic attitude in the general treatment of denture wearers.

Introducere

În pofida numeroaselor preocupări şi abordări sub diverse aspecte ale progreselor terapeutice obținute în ultimii ani, stomatita protetică rămâne o realitate clinică prezentă la un număr mare de pacienți protezați mobil, marea majoritate vârstnici, indiferent de tipul protezării mobile: totală, parțială sau supraprotezare pe dinți sau pe implante. Raportările legate de frecvența cu care este întâlnită în practica medicală prezintă o gamă largă de valori semnificative, de multe ori în relație directă cu o serie de afecțiuni generale sau cu medicația acestora, prin implicații directe asupra calității mucoasei sau indirect prin reducerea fluxului salivar (1, 2, 3, 4, 5, 6).

Etiologia stomatitelor asociate protezelor mobile s-a demonstrat că este extrem de variabilă, de tip plurifactorial, vizând o serie de factori locali, dar şi generali (8). Literatura de specialitate de actualitate priveşte ca factor determinant, în cea mai mare parte, agentul microbian de origine fungică, respectiv specii din genul Candida (6, 9), alături de factori locali, care enumeră xerostomia şi condiții legate de protezarea mobilă cu pondere dominantă (8). Nu există unanimitate în privința rolului protezei ca factor traumatic (10), pe de-o parte sunt studii care incriminează microtraumatismele date de instabilitatea protezelor (8), cu alterarea membranei bazale a epiteliului mucoasei orale (9), cărora li se opun autori ce consemnează contrariul, adaptarea perfectă pe câmpul protetic maxilar şi implicit stabilitatea protezei, privând mucoasa subiacentă de rolul protector al salivei (11). Desigur, există o tendință de a studia parametrii la modul individual.

Nivelul ridicat de contradicții stârnit de etiologia multiplă a stomatitei protetice poate fi explicat prin interpretarea diferită a multiplelor interacțiuni care se stabilesc între numeroşii factori etiologici (12) .

Clinic, există diferite grade de inflamație a mucoasei orale, situată sub baza protezei. Tabloului clinic de stomatită protetică i s-au propus numeroase clasificări, dar clasificarea de referință pentru stomatita protetică rămâne cea sugerată în 1962 de către Newton, bazată exclusiv pe criterii clinice, astfel:

- Tipul I - corespunde inflamației uşoare, localizate. Este prezentă o hiperemie punctiformă, petele roşii, lenticulare, de mărimea acului de gămălie corespunzând orificiilor glandelor salivare inflamate.
- Tipul II - formele generalizate la întreaga zonă de sprijin protetică descriu un eritem difuz (inflamație simplă generalizată).
- Tipul III - formele granulare, papilare includ leziuni de tip hiperplazic, concentrate mai frecvent în 1/3 anterioară a palatului dur (zona rugilor palatine sau papilare).

Stomatita protetică este prezentă numai la purtătorii de proteze mobile. Zonele congestive de la nivelul mucoasei bucale sunt în relație directă cu fața internă a protezei, sau asociate cu modificări la nivelul comisurilor bucale (stomatita angulară), mai ales în prezența Candidei (fig. 1).

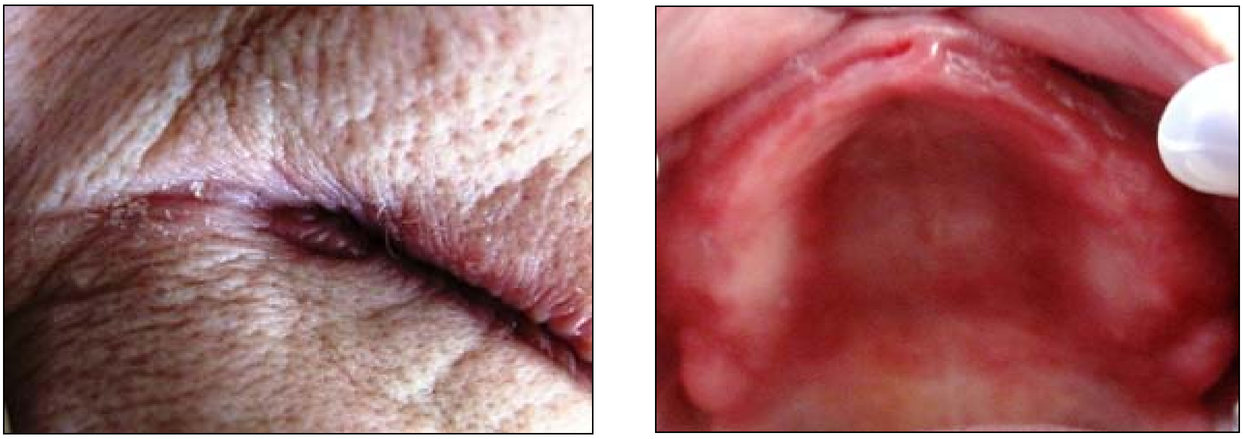

Fig. 1. Pacientă în vârstă de 74 ani, protezată total, cu stomatită protetică tip II, asociată cu cheilită angulară.

Afecțiuni generale prezente:

- sindrom depresiv sub tratament cronic antidepresiv cu compuşi din gama benzodiazepinelor (denumire comerciala d.c-bromazepam);

- cardiopatie ischemică sub tratament medicamentos cu vasodilatatoare tip trimetazidin (d.c.preductal);

- TA oscilantă sub tratament cu inhibitori ai enzimei de conversie a angiotensinei - Perindopril (d.c.prestarium);

- sindrom de hipercoagulabilitate cu două AVC - uri în antecedente, sub medicație anticoagulantă (d.c.tromboas).

Stomatita protetică poate fi prezentă şi în cazurile supraprotezării pe implante (fig.2), pe dinți (fig. 3) sau la purtătorul de proteze parțiale (fig. 4).

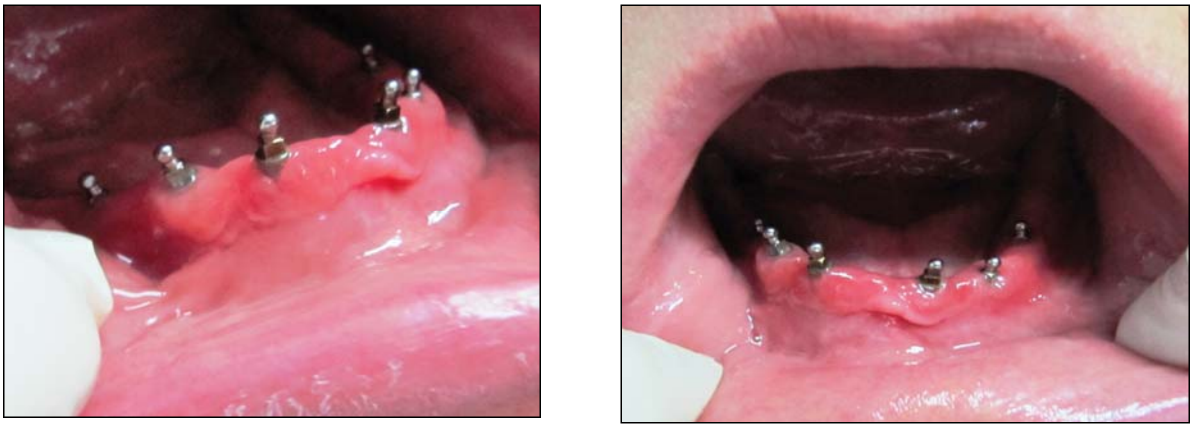

Fig. 2. Pacientă în vârstă de 58 de ani cu stomatită protetică tip I, cu supraprotezare pe implante

Afecțiuni generale prezente:

- tulburări de coagulare, fără tratament medicamentos;
- hemoleucograma indică: neutropenie, monocitoză, VSH crescut;
- hipercolesterolemie pentru care nu urmează medicație.

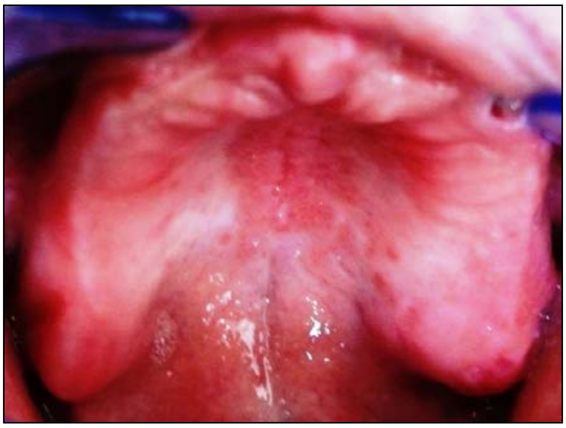

Fig. 3. Pacient în vârstă de 77 de ani, cu stomatită protetică tip I, cu supraprotezare pe dinți.

Afecțiuni generale prezente:

- hipertensiune arterială gr. III sub tratament cu medicație din grupa blocantelor canalelor de calciu- lercanidipin (d.c. leridip), betablocantelor selective tip nebivolol (d.c.nebilet), antagoniştilor angiotensinici tip telmisartan (d.c.pritor);
- angină pectorală sub tratament vasodilatator cu medicație din grupa nitraților organici, tip Isosorbid mononitrat (d.c.olicard retard);
- tulburări de coagulare sub tratament anticoagulant cu clopidogrel (d.c.plavix);
- insuficiență circulatorie periferică sub tratament cu alcaloizi ergotaminici, tip nicergolina (d.c.sermion);
- gastrită sub tratament cu medicație din grupa inhibitorilor pompei protonice, pantoprazol (d.c.controloc).

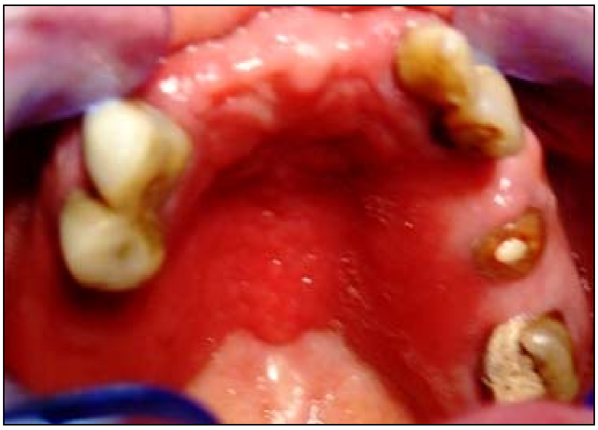

Fig. 4. Pacientă în vârstă de 63 de ani, protezată parţial, cu stomatită protetică tip III.

Afecţiuni generale prezente:

- hipertensiune arterială gradul III grup de risc înalt, sub tratament cronic cu diuretice din grupa sulfonamidelor, respectiv indapamid (d.c. tertensif)/ antagonişti ai angiotensinei, respectiv irbesartan (d.c.aprovel);
- boală artrozică vertebro-periferică sub tratament cronic cu alcaloizi din ergotamine, nicergolina (d.c. sermion);
- ateromatoză difuză sub tratament cronic hipolipidemiant cu rosuvastatina (d.c.crestor);
- sindrom depresiv, osteopenie, pentru care nu urmează tratament medicamentos.

Pacienţii cu stomatită protetică prezintă o prevalenţă crescută a co-morbidităţilor şi implicit un consum crescut de medicamente, cazurile prezentate anterior spre exemplificare fiind selectate aleatoriu, dintr-un lot mai mare de pacienţi cu stomatită protetică. Medicaţia urmată pentru diferite afecţiuni generale intervine direct, prin inducerea unor dezechilibre microbiene sau indirect, prin modificări cantitative şi calitative ale fluxului salivar. În acest context, cunoaşterea de către clinician a afecţiunilor sistemice, metabolice şi a deficienţelor sistemului imun, ca stări predispozante în producerea afecţiunilor mucoasei orale, reprezintă o necesitate. Omiterea rolului factorilor sistemici, lipsa colaborării interdisciplinare antrenează un risc de recidivă a acestei afecţiuni (13).

Elemente de patologie generală corelate cu creşterea frecvenţei stomatitei protetice

Există o varietate de entităţi patologice raportate în diverse studii de specialitate, care contribuie direct la sensibilizarea mucoasei orale aflate sub baza protezei şi la pregătirea terenului pe care se grefează stomatita protetică, sau indirect, ca efect advers al tratamentului medicamentos, urmat pentru afecţiunea de bază. Acestea includ:

- **Afecţiuni metabolice**

Diabetul zaharat

Diabetul zaharat este o tulburare frecventă, complexă, caracterizată prin diminuarea sau absenţa secreţiei de insulină şi cu rezistenţă la acţiunea metabolică a insulinei asupra ţesuturilor ţintă (A.J.Garber, 1998). Caracteristic pacientului geriatric este diabetul de tip II, non-insulino-dependent sub tratament cronic cu sulfamide sau biguanide antidiabetice. Alături de complicaţii precum neuropatiile, nefropatiile, retinopatia diabetică şi afectarea micro - respectiv macrovasculară, favorizarea unor afecţiuni orale prin diabet este raportată de către majoritatea studiilor. Tot acestea consemnează că vârsta pacientului, durata afecţiunii, precum şi gradul de control metabolic sunt implicate într-o mai mare măsură în apariţia manifestărilor diabetice orale decât tipul propriu-zis al diabetului (14). Acestea includ: gingivite, parodontite, carioactivitate crescută, xerostomie, infecţii fungice candidozice, sindromul de "gură arsă" (stomato- şi glosodiniile), lichenul plan oral, precum şi vindecarea întârziată, defectuoasă a plăgilor de orice natură (15). În privinţa stomatitei protetice determinate de specii de Candida, diabetul acţionează ca stare favorizantă prin mai multe mecanisme. Acesta induce disfuncţii salivare cu instalarea xerostomiei, deficienţe ale sistemului imun oral prin afectarea IgA-ului dimeric salivar, scăderi ale pH-ului salivar (16, 17) şi nu în ultimul rând hiperglicemia salivară, creându-se astfel un mediu favorabil pentru infecţiile fungice (18, 19). De asemenea, s-a constatat o capacitate crescută de adeziune a Candidei faţă de celulele mucoasei bucale la pacienţii cu diabet spre deosebire de cei care nu sufereau de această boală (20).

- **Afecţiuni endocrine**

Hipotiroidismul

Este o condiţie patologică des întâlnită, afectând în special sexul feminine, la care sinteza de hormoni tiroidieni este scăzută. Complicaţiile în sfera orală includ: macroglosie, disgeuzie, erupţie dentară întârziată, status parodontal deficitar, respectiv vindecare întârziată a plăgilor. Literatura de specialitate menţionează o frecvenţă semnificativă a stomatitei protetice la pacienţii geriatrici cu hipotiroidism, comparativ cu cei ce nu suferă de această afecţiune, cel mai probabil datorită medicaţiei hormonale substitutive, care are ca efect secundar neutropenie, respectiv xerostomie, ambele corelate cu scăderea imunităţii mucoasei orale (21). Creşterea capacităţii de adeziune a *Candidei albicans* la suprafeţele acrilice ale protezelor mobile permite transformarea aceluiaşi agent comensal într-unul patogen, prin perturbări profunde în ecosistemul oral (22).

Hipoparatiroidismul

Reducerea secreţiei de parathormon (PTH) survine pe cale iatrogenă, în urma ablaţiei chirurgicale a glandelor paratiroidiene sau ca proces de distrucţie autoimună a acestor glande. Deşi unele studii afirmă o corelaţie cu stomatita protetică, există rare sindroame, precum sindromul DiGeorge sau sindromul endocrino - candidozic, care pot fi asociate acestei condiţii (14).

Boala Addison

Afecţiune rară, cunoscută şi sub denumirea de insuficienţă cronică a glandelor suprarenale sau hipoadrenalism, reprezintă afectarea cortexului suprarenalian şi implicit a secreţiei de glucocorticoizi, mineralocorticoizi, respectiv a hormonilor sexuali. Printre factorii etiologici multipli, incriminaţi în distrucţia cortexului suprarenalian, alături de alte tipuri de infecţii, demnă de menţionat este invazia fungică a glandelor. Simptomatologia este multiplă, incluzând hipoglicemie, hipotensiune arterială, astenie, hipotonie, dezechilibre hidroelectrolitice, iar la nivel oral, caracterizată prin apariţia de leziuni pigmentare la nivelul mucoasei jugale, sau pe faţa dorsală a limbii. O serie de autori citează existenţa unei corelaţii între stomatita protetică de origine candidozică şi boala Addison (6, 23, 24), cel mai probabil interrelaţia datorându-se tratamentului cortizonic de substituţie. Prin deprimarea generală a reacţiilor mezenchimale, glucocorticoizii scad apărarea organismului la infecţie. Sunt inhibate răspunsul prin inflamaţie şi răspunsul imunologic la agenţii infecţioşi iar vindecarea ţesuturilor lezate, cicatrizarea, au loc cu întârziere. Astfel, în condiţii clinice, sub tratament corticoid creşte frecvenţa şi gravitatea multor infecţii (25).

Sindromul Cushing

O endocrinopatie de asemenea puţin frecventă, ce rezultă în urma expunerii organismului la hipercorticism endogen (producţia exagerată a corticoizilor la nivelul corticosuprarenalelor) sau exogen, în urma tratamentelor cronice pe bază de corticoizi. Şi în cazul acestei afecţiuni, prin acţiunea cortizonului în exces s-ar putea explica corelaţia cu stomatita protetică (26, 18), pe fondul unei imunități precare, a diminuării mecanismelor de fagocitoză (18), respectiv potențării gravității multor infecții şi vindecării deficitare.

- **Afecțiuni neoplazice**

Pacienții geriatrici cu afecțiuni maligne sunt frecvent edentați purtători de proteze mobile. Pe fondul unei imunități generale scăzute, infecțiile fungice orale se dezvoltă în special la pacienții cu forme avansate de cancer. Colonizarea fungică a mucoasei orale se caracterizează printr-o prezență crescută a coloniilor non - Albicans (27), în general rezistente la tratament. Turner, în 1996, afirma că în urma chimioterapiei apar modificări ale mucoasei orale, în sensul subțierii şi ulcerării acesteia, între a 4-a şi a 7-a zi de la inițierea tratamentului. Aceste aspecte se complică prin prezența plăcii microbiene. Mucoasa este invadată microbian, favorizând riscul apariției stomatitei protetice (28). Radioterapia regiunii cervico-faciale afectează direct țesuturile orale, inclusiv structurile mucoase şi glandele salivare, xerostomia fiind o complicație frecventă. Prin scăderea cantității salivei, pacienții cu xerostomie protezați mobil dezvoltă mai frecvent stomatită protetică, pe fondul infecțiilor fungice (29). Pacienții cu neoplasme sunt de cele mai multe ori sub tratament medicamentos analgezic. Analgezicele opioide, dar şi non-opioide, au la rândul lor ca efect secundar reducerea secreției salivare, cu instalarea xerostomiei (30).

- **Afecțiuni imunosupresive**

Modificările imunitare legate de vârstă sunt caracterizate prin pierderea unor funcții ale sistemului imunitar şi hiperactivarea altora. Răspunsul imunitar ineficient induce modificări ale mucoasei orale, în sensul favorizării suprapunerii factorului microbian (31). Imunosupresiile care apar în urma tratamentelor pentru neoplasme, post transplante hepatice, renale sau de măduvă osoasă, favorizează de asemenea dezvoltarea infecțiilor fungice şi apariția stomatitei protetice (6, 23, 32, 33). În cazul H.I.V., imunitatea mediată celular este deficitară: infecții polifungice invadează multiple situsuri la pacienții seropozitivi iar în particular, *Candida albicans* interacționează şi cu alți agenți microbieni, în cadrul stomatitei protetice (34). Majoritatea pacienților purtători de proteze mobile infectați cu H.I.V. prezintă tabloul clasic de stomatită protetică, printre alte manifestări la nivel oral, precum cheilita angulară, candidoza cronică hiperplazică, candidoza pseudomembranoasă sau candidoza eritematoasă (35). Tot la pacienții seropozitivi s-au constatat o frecvență crescută a xerostomiei, modificări în compoziția chimică a salivei, respectiv valori scăzute ale pH-ului salivar (36).

- **Afecțiuni sanguine**

Studii de specialitate enumeră în această grupă de afecțiuni leucemiile acute şi agranulocitoza, ca factori predispozanți pentru stomatita protetică (6). Neutropeniile sunt de asemenea stări favorizante, fagocitoza elementelor microbiene fiind afectată prin scăderea activității candidacide, microbicide a neutrofilelor (37). La vârstnici, scăderea numărului neutrofilelor salivare şi sanguine, alterarea funcționalității acestora predispun către apariția unor afecțiuni orale. În acest context, modificările calitative sau cantitative ale neutrofilelor au ca rezultat o susceptibilitate mai mare în privința stomatitei protetice (38).

- **Afecțiuni cardiovasculare**

Până în prezent nu apar date concrete care să vină în sprijinul unei corelații directe între acest tip de patologie, frecventă la pacientul geriatric, şi stomatita protetică. O relație de cauzalitate se poate stabili însă prin reacțiile adverse induse de medicația cronică de bază. Din această categorie fac parte: antiangiotensinele generatoare de xerostomie, uneori leucopenie sau chiar agranulocitoză, alfa şi beta blocantele, blocantele canalelor de calciu, diureticele, toate acţionând indirect asupra mucoasei orale, provocând xerostomie (30).

- **Afecţiuni psihice**

Pacienţii cu patologie de acest tip prezintă o frecvenţă marcată a stomatitei protetice, pe de-o parte datorită ritmului purtării protezelor, a modului precar de igienizare a acestora şi prin adresabilitatea scăzută la tratamentul stomatologic, iar pe de altă parte datorită medicaţiei psihotrope, generatoare de xerostomie. Din acest grup de medicamente fac parte antipsihoticele, antidepresivele, anxioliticele, miorelaxantele, respectiv sedativele (30).

- **Afecţiuni ale glandelor salivare**

Acestei categorii îi aparţin câteva boli autoimune, rar întâlnite în clinică şi anume sindromul Sjögren şi sindromul Mikulicz. Având o simptomatologie relativ similară, vom prezenta câteva caracteristici ale sindromului Sjögren, datorită frecvenţei sale mai crescute. Sindromul Sjögren primar este o afecţiune autoimună caracterizată prin pierderea cronică, progresivă a funcţionalităţii glandelor salivare, respectiv lacrimale, având drept rezultat instalarea xerostomiei şi a xeroftalmiei. Afectarea glandelor exocrine este rezultatul invaziei cu celule inflamatoare mononucleare a regiunilor focale, periductale glandulare şi dispariţiei celulelor secretoare epiteliale. În sindromul Sjögren secundar este implicată o altă patologie autoimună, respectiv artrita reumatoidă (39). Ambele tipuri afectează predominant sexul feminin de vârstă medie, iar ca frecvenţă a afecţiunilor reumatoide la nivel mondial se clasează pe al doilea loc (40). Studii recente raportează o frecvenţă crescută a colonizării cu *Candida albicans* a mucoasei orale la pacienţii cu sindrom Sjögren (41), explicată pe fondul scăderii pH-ului şi a capacităţii de tampon a salivei parotidiene, al alterării compoziţiei proteice salivare, cu creşterea alfa 2-microglobulinei, a lactoferinei, a lizozimului C, a cistatinei C, respectiv scăderea amilazei salivare şi a anhidrazei carbonice. Mucoasa orală de sprijin se subţiază, devine susceptibilă la infecţii, traumatisme, pregătind terenul pentru apariţia stomatitei protetice (42, 43).

Discuţii

Prin consultarea a 43 de publicaţii de specialitate, am găsit 15 grupe de afecţiuni generale corelate cu stomatita protetică, dintre care un număr de 7 mai frecvente la persoanele geriatrice. Ca mecanisme incriminate în apariţia stomatitei protetice sunt citate xerostomia (7 grupe de afecţiuni: diabetul zaharat, hipotiroidismul, neoplasmele, imunosupresiile, cardiopatiile, bolile psihice, respectiv cele autoimune ale glandelor salivare), modificări ale mucoasei orale (4 grupe de afecţiuni: diabetul zaharat, boala Addison, neoplasme, imunosupresii), ale imunităţii locale şi generale (6 afecţiuni: diabetul zaharat, hipotiroidismul, neoplasmele, imunosupresiile, afecţiuni sanguine şi ale glandelor salivare) sau scăderi ale pH-ului salivar (3 grupe de afecţiuni: diabetul zaharat, imunosupresii, afecţiuni autoimune ale glandelor salivare). Cea mai frecventă asociere a stomatitei protetice a fost cu xerostomia, în relaţie directă cu medicaţia. Din categoria substanţelor care induc scăderea fluxului salivar enumerăm ca relevante în acest context antidiabeticele, diureticele, antihipertensivele, sedativele, antidepresivele, anxioliticele, antipsihoticele, miorelaxantele, respectiv analgezicele (30).

Numeroase afecţiuni de ordin general au manifestări la nivel oral. Cavitatea orală poate fi privită ca o „oglindă“ în care se reflectă o serie de boli sistemice, de care uneori pacientul nu este în cunoştinţă de cauză. Din acest punct de vedere, între stomatita protetică şi unele afecţiuni de ordin general aduse în prim plan în cadrul acestui articol, medicaţia specifică, respectiv efectul acesteia asupra ţesuturilor orale, se poate stabili o legătură relevantă.

Concluzii

Stomatita protetică este o afecţiune orală caracterizată prin inflamaţia cronică a mucoasei orale, de diferite grade, strict legată de contactul acesteia cu faţa internă a protezei mobile. Frecvenţa acesteia este variabilă, ea putând fi influenţată de prezenţa unor afecţiuni generale, în primul rând prin intermediul medicaţiei aferente.

Hiposialia, indusă de unele afecţiuni generale şi de medicaţie, poate fi privită ca un important factor favorizant în apariţia stomatitei protetice.

Medicului dentist îi revine rolul de a recunoaşte manifestările orale din cadrul unor dezechilibre sistemice, imunologice, nutriţionale, farmacologice. Identificarea grupelor de risc trebuie să ghideze medicul dentist în adoptarea unor atitudini ce pot preveni apariţia stomatitei protetice în prezenţa unor afecţiuni generale sau medicaţiei aferente, ca factori favorizanţi.

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Ecoul somatic al tulburărilor emoționale din perspectiva psihoterapeutică

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Rezumat. Numesc tulburare emoțională inhibarea emoției, indiferent de natura ei, pozitivă sau negativă, de către forța rațiunii. Educația noastră este cea responsabilă pentru a nu lăsa emoțiile să se exprime. Am fost obişnuiți să nu dăm curs emoțiilor, în special celor negative (tristețe, iritabilitate, furie, teamă etc.). Nici în ceea ce priveşte emoțiile pozitive nu putem spune că ne simțim în măsură să le exprimăm deschis. Aşa încât ne hrănim cu ideea că, dacă învestim destul în inteligență, vom deveni suficient de puternici pentru a face față provocărilor vieții. Uităm deci de hrana pe care ar trebui să o dăm sufletului. Răspundem provocărilor vieții prelucrând mental esența acestora, simțindu-le însă foarte puțin cu inima. Nimeni nu ne-a învățat că vocea inimii şi cea a rațiunii ar trebui să se împletească pentru a ne putea bucura de o viață sănătoasă din punct de vedere psihic şi fizic.

Inima pare să aibă propria ei rațiune. Deschizând poarta către ea, putem păşi într-o lume nouă, cea a cunoaşterii şi dezvoltării interioare, care aduce cu ea întregirea ființei. Numai primind emoțiile care curg inevitabil în corpul nostru devenim mai puternici, reuşind să trăim în armonie cu noi şi cu ceilalți. Adevăratul echilibru interior este asigurat de acest dans permanent între rațiune şi simțire.

Cuvinte cheie: tulburare emoțională, emoție negativă, emoție pozitivă

Abstract. I see the emotional disorder like being an emotion inhibition, though it is positive or negative one, due to the power of mind. Our education is responsible for that. We have been told not to let emotions to go free. So, we strongly belive in the ideea that if our mind is powerfull enough, we'll be prepare to face life. We lose contact with our soul. We face life challenges with mind not with the heart. No one have thought us that a healthy life means hearing both voices: mind and heart.

The heart seems to have its own law. Learning it, we will take part of a new world, a world of self growthing.

Only receiving the emotions that are flowing in our body we become much stronger, succeeding to live life in harmony with us and others. The heathy way is to learn some steps of this heart and mind dance.

Numesc tulburare emoțională inhibarea emoției, indiferent de natura ei, pozitivă sau negativă, de către forța rațiunii. Educația noastră este cea responsabilă pentru a nu lăsa emoțiile să se exprime. Am fost obişnuiți să nu dăm curs emoțiilor, în special celor negative (tristețe, iritabilitate, furie, teamă etc.). Nici în ceea ce priveşte emoțiile pozitive nu putem spune că ne simțim în măsura să le exprimăm deschis. Mă refer aici, în special, la împărtăşirea cu ceilalți a sentimentelor pe care le simțim vis a vis de ei sau de anumite situații pe care le trăim. Cunoaştem cu toții deja celebra credință "sărută copilul când doarme", pentru a nu-i arăta prea multă iubire, ca şi când iubirea ar putea fi nocivă. Să nu mai vorbim despre credința că bărbații nu trebuie să plângă, această atitudine nefiind altceva decât un semn de slăbiciune din perspectiva sus-menționată. Purtăm în spate, aşadar, povara slabei disponibilități de exprimare emoțională care ne este transmisă din generație în generație datorită etichetei pe care a primit-o, cu foarte mare uşurință, emoția. În general, suntem tentați să interpretăm registrul afectiv în termeni de vulnerabilitate, care ar fi bine să rămână mai puțin cunoscută în exterior, el dând naştere la neplăceri. Uităm de înțelepciunea pe care o ascunde emoția. Aşa încât ne hrănim cu ideea că, dacă învestim destul în inteligență, vom deveni suficient de puternici pentru a face față provocărilor vieții. Uităm deci de hrana pe care ar trebui să o dăm sufletului. Răspundem provocărilor vieții prelucrând mental esența acestora, simțindu-le însă foarte puțin cu inima. Nimeni nu ne-a învățat că vocea inimii şi cea a rațiunii ar trebui să se împletească, pentru a ne putea bucura de o viață sănătoasă din punct de vedere psihic şi fizic.

Cu toate acestea, nu puţine sunt momentele din viaţă care ne reamintesc de originea noastră, ce nu se află în mental, ci în simţire. Aşa putem să ne explicăm generozitatea, blândeţea, iertarea, iubirea, compasiunea, în primul rând vis a vis de propria persoană şi apoi faţă de ceilalţi, ce îşi găsesc sursa nu în minte, ci în inimă. Tot astfel înţelegem de ce dezvoltăm nenumărate tulburări emoţionale, deşi mintea noastră este de-a dreptul sclipitoare.

În virtutea profesiei de psiholog, clinician şi psihoterapeut, sunt martora unor probleme emoţionale mai mult sau mai puţin grave ale celor cu care relaţionez zilnic în cabinet. Pe parcursul activităţii pe care o desfăşor în clinică întâlnesc, din nefericire, oameni ale căror manifestări emoţionale ajung să se cronicizeze, devenind adevărate tulburări. Şi când fac această afirmaţie am în vedere în special tulburările depresive şi anxioase. Cum tulburarea depresiv-anxioasă prezintă echivalente somatice, cei care o dezvoltă interpretează aceste simptome ca fiind semnele unor boli serioase de natură organică. Frecvent întâmpin rezistenţa lor de a înţelege că un simptom (cefalee, ameţelă, dificultăţi de vorbire, tulburări de echilibru etc.) resimţit în plan somatic poate avea cauza pur psihogenă. De multe ori, am constat preferinţa oamenilor de a se confrunta cu o suferinţă fizică mai degrabă decât cu una psihică, pentru că cea din urmă se află pe un tărâm necunoscut. Cum examinarea psihologică este secundară celei medicale, care elimină organicitatea suferinţei cu care pacientul se adresează cadrelor medicale, nu mai rămân prea multe de făcut decât acceptarea unei suferinţe emoţionale. Desigur nu toţi recepteză această idee cu uşurinţă, alegând să continue şirul investigaţiilor medicale pe care l-au început deja, de multe ori în incinta altor instituţii medicale. Cei care însă integrează posibilitatea unei suferinţe emoţionale aleg să o interpreteze în termenii unei boli psihice ce necesită un tratament adecvat chiar şi atunci când afecţiunea lor nu impune neapărat o intervenţie medicamentoasă. Asta pentru că le este foarte dificil să gestioneze lumea lor interioară. Foarte puţini se angajează într-un demers psihoterapeutic. Preferă să fie amorţiţi de tratamentul care le oferă confort, prin anestezierea trăirilor emoţionale. Deci, putem vorbi despre un fenomen de amorţeală care contribuie atât la cronicizarea suferinţei prin reprimarea trăirilor emoţionale, cât şi la anestezierea lor prin alegerea medicaţiei atunci când aceste trăiri reprimate dau pe dinafară, manifestându-se din plin. Din propria experienţă pot spune că persoanele care solicită ajutorul psihoterapeutic sunt fie cele care au un istoric de tratament medicamentos psihiatric, care le permite să observe că fără nicio schimbare nu pot spera la vindecarea lor, ci numai la o ameliorare a simptomelor, fie aparţin categoriei care tolerează foarte greu ideea unei medicaţii.

Din perspectiva psihoterapeutică, simptomul care însoţeşte tulburarea nu este decât o expresie a unui conflict interior. Orice încercare a inconştientului nostru de exprimare prin intermediul corpului care nu reuşeşte să pătrundă în câmpul conştiinţei poate fi interpretată în termeni de conflict interior. Poate fi vorba de o dificultate de comunicare, de relaţionare, de acceptare a unei situaţii, de exprimare a unor emoţii sau orice altă dificultate. În activitatea psihoterapeutică am constatat că toate simptomele care apar în tulburările emoţionale au echivalent în comportamentul celui care le resimte vis a vis de situaţiile de viaţă pe care le traversează. Cu alte cuvinte, corpul are propriul său limbaj şi dacă este descifrat se poate păşi pe treptele procesului de vindecare. Aşa încât, senzaţia de ameţeală care apare într-o tulburare emoţională poate fi simbolul unei tendinţe de supraresponsabilizare a individului care nu reuşeşte să-şi delimiteze spaţiul personal sau, dimpotrivă, expresia unei dificultăţi de a răspunde solicitărilor vieţii ce se naşte din tendinţa de a-i responsabiliza pe ceilalţi cu problemele personale. Teama de moarte, care apare foarte des într-un atac de panică, poate fi simbolul unei vieţi ce nu e trăită într-un mod satisfăcător. Dificultăţile de vorbire de natură psihogenă pot fi indicatorul unei comunicări disfuncţionale, fie în sensul unui blocaj în exprimare, fie în sensul unei exagerări în comunicare care este frânată de Inconştient. Aritmia cardiacă, de natură psihogenă, poate fi semnalul tras pentru închiderea individului în sfera raţionalului. Şi exemplele pot continua în această ordine de idei. Altfel spus, Inconştientul nostru, sursa noastră creativă, e întotdeauna prezent, călăuzindu-ne paşii întru vindecare. Aşadar, simptomul dezbracă vechea haină patologică şi o îmbracă pe cea nouă, cea a deschiderii spre Sine, care oferă climatul pentru cunoaşterea şi dezvoltarea personală a omului. Psihoterapia, indiferent de forma ei, oferă cadrul în care individul se angajează cu toată fiinţa în acest drum, al deschiderii către Sine.

Inima pare să aibă propria ei raţiune. Deschizând poarta către ea, putem păşi într-o lume nouă, cea a cunoaşterii şi dezvoltării interioare, care aduce prin sine întregirea fiinţei. Numai primind emoţiile care curg inevitabil în corpul nostru devenim mai puternici, reuşim să trăim în armonie cu noi şi cu ceilalţi. Adevăratul echilibru interior este asigurat de acest dans permanent între raţiune şi simţire.

De cele mai multe ori, în demersul terapeutic în care mă angajez împreună cu pacienţii , cel mai dificil moment este cel al deschiderii către emoţie. Nu suntem obişnuiţi să identificăm emoţiile şi dacă acest lucru se întâmplă, ne este foarte dificil să le acceptăm. Trăim cu impresia falsă că emoţiile negative (frica, furia, tristeţea etc.) fie nu îşi au rostul, fie devin o a doua natură a noastră. Aşa încât, ori ne simţim vinovaţi pentru prezenţa lor, încercând să scăpăm de ele, ori rămânem tributari acestor emoţii pe care le găsim fireşti, şi în felul acesta nu păşim o nouă treaptă pe scara dezvoltării interioare. Aşa înţelegem diferenţele care există între tristeţe şi depresie, între furie şi agresivitate, între teamă şi frica patologică.

Tristeţea este o emoţie absolut firească, care poate fi vindecătoare, dacă e primită şi lăsată să se manifeste cu încredere. În schimb, tristeţea reprimată din motive diverse, cel mai adesea din teamă, poate deveni stare depresivă. La fel stau lucrurile şi cu furia, teama etc. Aşadar, la origini, aceste emoţii sunt absolut naturale şi deloc periculoase, ba dimpotrivă, chiar vindecătoare. Ele ar putea rămâne aşa dacă nu ar interveni forţa minţii, care începe să emită judecăţi: “nu e firesc să plâng aşa”, “nu e firesc să simt asta”, “dacă mai plâng aşa, poate o să mă îmbolnăvesc” şi exemplele ar putea continua în această ordine de idei. În acest fel, deşi mintea noastră este uneori extrem de preţioasă, alteori poate fi o piedică în calea deschiderii către emoţie.

Calea terapeutică îşi propune să antreneze clientul să primească toate aceste emoţii, să le identifice, să le accepte şi apoi să le transforme nici mai devreme, nici mai târziu decât e momentul dat de procesul interior în care se găseşte el. Este vorba de aşa-numitul proces terapeutic de integrare. Numai reuşind să ne conectăm cu emoţiile noastre, primindu-le, fără a pune nici o judecată asupra lor, neidentificându-ne cu ele, intrăm în contact cu adevărata noastră natură. Teama, furia, tristeţea etc. pot fi adevărate surse de creativitate dacă sunt acceptate, decodate ca mesaj şi integrate. Ele ne pot călăuzi paşii pe calea unei adevărate naşteri simbolice. Această naştere simbolică îşi găseşte originea în principiile de bază ale procesului terapeutic care se referă la dezbrăcarea vechilor modele parentale şi îmbrăcarea unora noi, care să ne permită să vedem viaţa prin ochii noştri şi nu prin cei ai părinţilor noştri. Cu siguranţă, prin aceasta înţelegem sensul evoluţiei spirituale.

Vorbeam la începutul acestui eseu despre slaba disponibilitate de a împărtăşi chiar şi emoţiile pozitive cu cei din jurul nostru. Sunt de părere că, aşa cum Întunericul nu poate exista fără Lumină, nici emoţia pozitivă nu poate respira fără cea negativă. Dacă emoţia negativă se loveşte de teamă şi respingere, tot astfel emoţia pozitivă nu poate fi simţită şi împărtăşită la un nivel profund. Cum poate exista bucurie fără tristeţe? Cum îţi dai seama că simţi fericire dacă niciodată nu ai fost conştient de suferinţă? Cum poţi să oferi dacă nu ştii să primeşti? Cum poţi face pe cineva fericit dacă tu nu ai simţit ce înseamnă fericirea? Cum poţi rezona cu tristeţea celorlalţi dacă tu nu ai simţit ce înseamnă tristeţea?

Deci numai dacă reuşim să primim emoţiile negative vom fi capabili să ne bucurăm şi de cele pozitive. Ele reprezintă un întreg: două feţe ale aceleiaşi monede. Aşadar să avem încredere în ceea ce simţim, să ne bucurăm de emoţiile noastre, să le luăm cu noi şi să le preţuim ca pe nişte adevărate resurse. Să ne obişnuim să ne antrenăm mintea în a primi emoţiile şi în a le prelucra în profunzimea şi autenticitatea lor. Cu alte cuvinte, să începem să exersăm câţiva paşi în acest dans între raţiune şi simţire. Odată ce am învăţat câţiva paşi din acest dans este foarte greu să nu îi simţim ritmul, ori de câte ori vom simţi că am luat ceva distanţă de el.

Dar cum să facem să începem să-i simţim ritmul? Cum ne dăm seama dacă avem suficiente resurse? Cum ne dăm seama că suntem pe drumul cel bun, când deja suntem angajaţi într-un proces terapeutic. Iată câteva întrebări care încearcă a-şi găsi răspuns în rândul clienţilor care se situează într-un cadru terapeutic.

Poate că ar fi bine să începem să privim Inconştientul prin alţi ochi decât am fost obişnuiţi până în prezent. De multe ori ne lovim de sintagma "cine ştie ce ascunde Inconştientul nostru?", "eu nu sunt conştient de asta… dar poate, cine ştie?... în Inconştientul meu lucrurile stau altfel" etc. Ca şi când distanţa dintre noi şi Inconştientul nostru ar fi de neparcurs. Parcă am vorbi de ceva străin de noi. Mai mult, parcă se simte o teamă cu privire la această forţă văzută cam obscură.

Dacă am vedea în Inconştient forţa noastră creativă, care se afla la îndemâna noastră poate că nu am mai fi aşa de străini de noi, de propriile noastre emoţii. În această accepţiune, Inconştientul se transformă într-un adevărat izvor de resurse, altfel spus, devine puterea noastră vindecătoare. Accesul la el este permis de îndată ce devenim deschişi la prezenţa acestuia. Aminteam mai sus de faptul că emoţia pozitivă nu poate respira fără cea negativă. Tot aşa se întâmplă şi cu starea de conştienţă. Nu putem deveni pe deplin conştienţi de ce se întâmplă în jurul nostru dacă nu devenim conştienţi de ceea ce se petrece în interiorul nostru, mai precis în Inconştient. Universul nostru interior dă naştere celui exterior. Vedem lumea înconjurătoare prin lumea noastră interioară iar în lumea noastră interioară se află condensat Întregul Univers. Aşadar cele două Universuri se interconectează reciproc. Privind din acest unghi, devenim conştienţi de faptul că suntem înzestraţi cu toate resursele de care avem nevoie. Tot ce avem de făcut este să ne deschidem către această lume interioară şi să avem încredere în ceea ce vom primi de la ea. Psihoterapia este o cale către această deschidere. Prin intermediul ei omul învaţă să ia contact cu propriile resurse. Aşadar, problema nu se mai pune în termeni de suficiente resurse, ci de deschidere către resursele pe care le avem incontestabil. Aşa putem simţi înţelepciunea vechilor vorbe populare: "Nu ştiu cum am reuşit să trec prin atâtea suferinţe", "Să nu îţi dea Dumnezeu cât poţi să duci", "Nu ştiu de unde mai sunt, după atâtea necazuri" etc. Dacă suntem suficient de atenţi la aceste expresii, vom observa că există şi aci o fină delimitare între raţiune şi simţire: verbul "nu ştiu" conţine în el aspectul cognitiv, specific aşadar minţii, ce nu poate desluşi tainele puterii care a permis ca existenţa să mai fie posibilă.

Probabil că vechile generaţii erau mai bine conectate la Natură. Credinţa lor în Divinitate era foarte puternică. Cine ştie? Să fie asta o explicaţie pentru o incidenţă mai scăzută a afecţiunilor psihiatrice în trecut? Ar putea fi o temă demnă de a fi luată în considerare pentru studiile viitoare. În această viziune, Inconştientul pierde din obscuritatea fabricată de mintea noastră şi prinde un contur ceva mai luminos. Oricum, fie că-i permitem sau nu, Inconştientul nostru îşi face simţită prezenţa în noi. Din nefericire, de cele mai multe ori sub forma unor simptome somatice, care ascund acele conflicte interioare amintite mai sus. Şi asta, pentru că nu suntem conştienţi de forţa lui. Aşa încât, dacă am fi mai deschişi la prezenţa lui, am putea să ne bucurăm de energia lui creatoare, de puterea lui vindecătoare. Am putea vorbi de asemenea de prezenţa forţei Inconştientului în noi, nu numai în sensul unui simptom al cărui mesaj se vrea a fi decodat, ci şi în sensul unor situaţii pe care ni le putem explica foarte puţin, uneori deloc. Mă refer aici la anumite alegeri pe care le facem în viaţă, cum ar fi acceptarea unor persoane şi respingerea altora la o primă întâlnire cu ele, fără a avea mental vreo justificare pentru aceste preferinţe. Singura motivaţie care o avem pentru aceste preferinţe este simţirea. Cui nu i s-a întâmplat măcar o dată în viaţă să perceapă o persoană necunoscută până la acel moment ca fiind agreabilă sau deloc agreabilă fără a înţelege prea bine de ce? Până când relaţia cu acea persoană se derulează, asfel încât să permită integrarea mentală a atracţiei sau a respingerii, nu există, practic, o explicaţie palpabilă din punctul de vedere al prelucrării mentale. Cel mai elocvent exemplu este relaţia de atracţie dintre doi oameni care se îndrăgostesc. În acel moment, suntem mai puţini prezenţi în mental şi mai conectaţi la ceea ce simţim.

Există o întreagă dinamică a simţurilor. Parcă întregul Univers lucrează pentru noi, participă la acea magie a momentului. Poate că de aceea începutul fiecărei relaţii de iubire este fermecător. El se naşte din puritatea simţurilor. Pe parcursul relaţiei, uneori ne deconectăm de la ceea ce a făcut ca relaţia să ia fiinţă. Mintea începe să fabrice, să pună etichete, nouă, partenerului, relaţiei, problemelor cu care ne confruntăm zilnic în relaţie şi, încetul cu încetul, ne îndepărtăm de la ceea ce e consistent şi autentic. Este momentul în care devenim conştienţi de anumite probleme care apar în cuplu. Nu chiar întâmplător, terapia de cuplu îşi propune drept obiectiv conectarea partenerilor de relație cu resursele fiecăruia în parte şi apoi cu resursele relației. Deci, putem observa cum lumea noastră interioară este prezentă clipă de clipă la realitatea pe care o trăim, fie că suntem sau nu conştienţi de acest aspect. Încercând să devenim conştienţi de noi, participăm, de fapt, la întregirea ființei. Poate fi un cadou, pe care ni-l facem nouă înşine, pentru că… de ce nu? Chiar merităm!

Astăzi, există o serie de metode terapeutice menite să deschidă poarta către acest Univers Interior: tehnica respirației controlate, terapia prin dans, terapia prin sunet, prin desen, tehnicile de meditație, transele hipnotice etc. Totul e să nu rămânem indiferenți, la noi şi la ceilalți, cu alte cuvinte să ne dorim să deschidem această poartă, să fim curioşi să învăţăm câţiva paşi din acest celebru dans al rațiunii şi simțirii. Pentru că am ajuns să cred, din toată ființa, că nimic nu e întâmplător, că şi Universul lucrează pentru noi. Am certitudinea că există un moment pentru fiecare dintre noi pentru a ne deschide inima şi pentru a putea primi ceea ce vine.

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Terapia migrenei – aspecte actuale

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Rezumat: Migrena este o cefalee cronică caracterizată de episoade de durere severă (cu caracter pulsatil, unilateral localizată), asociată cu fenomene de disfuncţie a sistemului vegetativ (greaţă, vomă) şi hipersensibilitate la aferenţe de tipul luminii (fotofobie), a sunetului (fonofobie) sau la mişcarea capului (migrena fără aură).
Aproximativ 30% dintre migrenoşi prezintă aură – semne de disfuncţie vizuală sau senzitivo-motorie tranzitorii care preced sau acompaniază cefaleea.
Există acum disponibile noi opţiuni terapeutice atât în tratamentul atacului migrenos, cât şi în profilaxia migrenei.

Cuvinte cheie: migrenă, atac migrenos, profilaxie.

Abstract: Migraine is a common, chronic disorder characterised by episodes of severe headache (often throbbing and frequently unilateral), associated with autonomic nervous system dysfunction (nausea, vomiting) and with sensitivity to afferents, such as light, sound and head movement (migraine without aura).
Approximately 30% of migraine sufferers experience aura - transitory visual or sensorio-motor dysfunction that precedes or accompanies the headache (migraine with aura).
New therapeutical options are available for drug treatment of migraine attacks andmigraine prophylaxis.

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Există două forme majore de migrenă după criteriile de definiţie ale Societăţii Internaţionale de Cefalee (1):

- migrena fără aură – sindrom clinic caracterizat prin cefalee cu trăsături specifice şi simptome asociate;

- migrena cu aură – care apare la aproximativ 30% dintre pacienţi – este caracterizată prin simptome neurologice focale tranzitorii reversibile (vizuale, senzitivo-motorii), care preced şi uneori acompaniază cefaleea .

În terapia migrenei se folosesc atât mijloace farmacologice, cât şi nefarmacologice.

►**Tratamentul nefarmacologic al migrenei** se bazează pe măsuri igieno-dietetice şi de modificare a regimului de viaţă, în vederea evitării factorilor precipitanţi sau agravanţi ai cefaleei.

►**Tratamentul farmacologic** al migrenei poate fi **:**

I. acut (simptomatic) - abortiv al atacului migrenos, care utilizează medicaţie:

- **a)** nespecifică (antiinflamatoare nesteroidiene, antiemetice);
- **b)** specifică antimigrenoasă **(**derivaţii de ergot "secară cornută" şi triptanii).

II. preventiv (profilactic) – administrat zilnic, chiar dacă este prezentă sau nu cefaleea (2).

I. TRATAMENTUL ATACULUI MIGRENOS

Conform Ghidurilor Europene de Management Neurologic (3), definiţia succesului terapeutic în tratamentul atacului migrenos – aşa cum reiese din studiile clinice randomizate – se bazează pe:

- lipsa durerii la 2 ore de la administrare;
- ameliorarea severităţii durerii – trecerea de la forma moderată la cea uşoară sau lipsa durerii la 2 ore (Pilgrim, 1993);
- consistenţa eficacităţii la două din trei atacuri;
- lipsa recurenţei sau lipsa administrării ulterioare de medicaţie în decurs de 24 ore după tratament.

a) Medicaţia nespecifică (antiinflamatoare nesteroidiene, antiemetice)

► Analgezicele

Analgezicele reprezintă medicaţia de primă alegere în atacurile uşoare sau moderate. *Atacurile uşoare* pot fi tratate cu analgezice sau antiinflamatoare nesteroidiene, în timp ce atacurile severe răspund mai bine la medicaţie antimigrenoasă specifică (triptani – nivel de evidenţă de clasă A).

Dovezi ale eficacităţii analgezicelor sunt prezentate în tabelul de mai jos:

Tabel nr.1. Analgezice folosite în atacul migrenos

Substanţa	Doza (mg)	Nivel	Comentariu
Acid acetil salicilic (ASA)	1 000 (oral) 1 000 (i.v.)	A A	Reacţii adverse gastrointestinale Risc de sângerare
Ibuprofen	200-800	A	Reacţii adverse ca la ASA
Naproxen	500-800	A	Reacţii adverse ca la ASA
Diclofenac	50-100	A	Inclusiv Diclofenac -K
Paracetamol	1 000 (oral) 1 000 (supozitoare)	A A	Atenţie în insuficienţa hepatică şi renală
ASA plus	250 (oral) 200-250	A	Ca la ASA şi paracetamol
Metamizol	1 000 (oral) 1 000 (i.v.)	B B	Risc de agranulocitoză Risc de hipotensiune
Phenazon	100 (oral)	B	Ca la paracetamol
Acid tolfenamic	200 (oral)	B	Reacţii adverse ca la ASA

(R. Hughes, M.Brainin, N.E. Gilhus – *European Handbook of Neurological Management.* First Edition, 2006 , Blackwell Publishing Ltd)

Aspirina trebuie evitată la copiii sub 12 ani. Aspirina intravenous este mai eficientă decât ergotamine subcutanată (Limmroth, 1999).

Combinaţia ASA, paracetamol şi cafeină este mai eficientă în tratamentul migrenei decât substanţele singure sau în asociere fără cafeină (Lipton, 1998; Diener, 2005). Pentru a preveni cefaleea indusă medicamentos, analgezicele trebuie utilizate nu mai mult de 15 zile pe lună, iar combinaţiile de analgezice nu mai mult de 10 zile pe lună.

Coxibii nu sunt recomandaţi pentru tratamentul acut al migrenei, având în vedere posibilele evenimente adverse cardiovasculare.

Antiinflamatoarele nesteroidiene, rapid absorbabile, sunt deosebit de utile când apare *sensibilitatea scalpului şi a musculaturii cervicale.* Vor fi evitate la pacienţii cu ulceraţii gastrointestinale şi la cei cu diateză hemoragică

Opioizii au o eficacitate minoră şi nu există studii moderne controlate pentru aceste substanţe. Analgezicele opioide (codeina, petidina) sunt rareori folosite. Având în vedere riscul de apariţie a dependenţei, se pot utiliza la pacienţii cu atacuri severe neresponsivi dar care nu pot utiliza triptanii (gravidele).

► Antiemeticele

Folosirea antiemeticelor este utilă în tratamentul simptomelor vegetative asociate atacului migrenos, precum şi datorită efectului acestora de a îmbunătăţii absorbţia gastrică a analgezicelor. Agenţii antiemetici şi prokinetici *favorizează absorbţia antiinflamatoarelor nesteroidiene* la un pacient migrenos cu stază gastrointestinală şi cu o rată scăzută de evacuare gastrică.

Metoclopramid 20 mg este recomandat la adulţi şi adolescenţi; la copii se poate folosi domperidone 10 mg, datorită posibilelor reacţii adverse extrapiramidale ale metoclopramidului. Domperidone nu traversează cu uşurinţă bariera hematoencefalică, astfel încât apar rareori reacţii la nivelul SNC.

Tabelul nr. 2. Antiemetice utilizate în atacul migrenos

Substanţa	Doza (mg)	Nivel	Comentariu
Metoclopramid	10 – 20 (oral) 20 (supozitoare) 10 (i.m.; i.v.; s.c.)	B	Reacţii adverse: diskinezie, contraindicată la copii şi în sarcină.
Domperidone	20-30 (oral)	B	Reacţii adverse mai puţin severe ca la metoclopramid; poate fi folosit la copii.

(R. Hughes, M.Brainin, N.E. Gilhus – *European Handbook of Neurological Management.* First Edition, 2006, Blackwell Publishing Ltd)

b) Medicaţia specifică antimigrenoasă (derivaţii de ergot şi triptanii)

►Derivaţii de ergot

Derivaţii de ergot sunt *agonişti serotoninici neselectivi care au şi efect alfa adrenergic* şi sunt folosiţi în tratamentul atacurilor *moderat-severe* de migrenă în cazul în care analgezicele nu au un efect satisfăcător.

Deşi au fost folosiţi de mult timp în tratamentul migrenei, există puţine studii care să ateste eficacitatea derivaţilor de ergot în tratamentul migrenei (Tfelt-Hansen, 2000). Singurul derivat de ergot la care există dovezi ale eficienţei este ergotamine tartrat 2 mg (oral sau supozitoare).

Întrucât derivaţii de ergot au o absorbţie intestinală foarte scăzută, este preferabilă administrarea rectală sub formă de supozitoare.

Avantajele folosirii derivaţilor de ergot se bazeză pe faptul că prezintă un *timp de înjumătăţire îndelungat (>10 ore) şi o legare ireversibilă de receptori,* fiind indicaţi în cazul **atacurilor migrenoase de lungă durată** sau al **existenţei recurenţelor după administrarea de triptani.**

Reacţiile adverse ale derivaţilor de ergot sunt frecvente: greaţă, vomă, diaree, crampe musculare, parestezii şi senzaţie de răcire a extremităţilor. Comparativ cu sumatriptanul determină *un grad mai mare de constricţie coronariană,* fiind contraindicaţi în cardiopatii şi boli vasculare cerebrale. Se recomandă efectuarea unei electrocardiograme înainte de prima doză de ergotamină, în special la subiecţii a căror vârstă este peste 40 de ani.

Contraindicaţiile folosirii derivaţilor de ergot sunt: bolile cerebro-vasculare şi cardiovasculare, boala Raynaud, hipertensiunea intraarterială, insuficienţa renală, sarcina şi lactaţia.

Supradozarea acută sau cronică determină o intoxicaţie ("ergotism") manifestată prin vasospasm sever generalizat cu gangrenă.

Folosirea cronică, recurentă a ergoţilor determină apariţia "cefaleei de abuz medicamentos" – indusă foarte rapid şi la doze foarte mici, astfel că folosirea lor trebuie limitată la 10 zile pe lună.

► Triptanii (agoniştii serotoninei 5-HT$_{1B/1D}$)

Triptanii - sumatriptan, zolmitriptan, naratriptan, rizatriptan, almotriptan, eletrip-tan, frovatriptan - sunt medicamente specific antimigrenoase, care nu trebuie folosite în alte tipuri de cefalee (cu excepţia cluster-ului).

Lansat în Europa în 1991 ca prim medicament special conceput pentru tratamentul atacului migrenos, **sumatriptanul** a adus un important beneficiu, comparativ cu clasicii derivaţi de ergot, alte 6 molecule din aceeaşi clasă fiind puse ulterior pe piaţă.

Triptanii sunt derivaţi serotoninici având o acţiune selectivă, de agonişti ai receptorilor 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1F}$ şi într-o oarecare măsură 5-HT$_{1A}$.. Există trei **mecanisme farmacologice** distincte care explică efectul lor antimigrenos:

1) *Inhibarea inflamaţiei neurogenice durale* - stimulează receptorii presinaptici 5-HT$_{1D}$ ai fibrelor trigeminale A-delta şi inhibă eliberarea de CGRP şi stimulează receptorii presinaptici 5-HT $_{1D}$ ai fibrelor C, inhibând eliberarea de substanţă P şi neurokinină A.

2) *Vasoconstricţia meningeală, durală, cerebrală sau a vaselor piale, mediată prin stimularea receptorilor 5-HT $_{1B}$* – cu riscul apariţiei *coronaroconstricţiei.*

3) *Atenuarea directă a excitabilităţii celulelor nucleului trigeminal prin stimularea receptorilor 5-HT $_{1B/1D}$ de la nivelul trunchiului cerebral.*

Pentru toţi triptanii există forme de prezentare orale, pentru rizatriptan şi zolmitriptan există şi *comprimate dispersabile*, care se dezintegrează la nivelul cavităţii bucale dar se absorb la nivel intestinal. Sumatriptan şi zolmitriptan se prezintă şi sub formă de *spray nazal.* Sumatriptanul poate fi administrat şi *subcutanat* (autoinjector).

Sumatriptanul ***parenteral*** are cel mai bun profil farmacologic, cu un timp de apariţie a concentraţiei maxime de 10 minute şi o biodisponibilitate de 96%, având cea mai bună eficienţă clinică, cu o rată de răspuns de 76% şi o rată de dispariţie a durerii de 48% la 60 minute de la injectare şi o consistenţă intrapacient a răspunsului la atacuri multiple de 90%. În plus, este **util şi în atacul acut de cluster.** Limitările folosirii lui subcutanate provin de la faptul că pacientul trebuie să se autoinjecteze şi că reacţiile adverse sunt mai intense şi mai frecvente decât la sumatriptanul oral.

Eficacitatea şi tolerabilitatea sumatriptanului administrat rectal sau intranazal sunt similare sumatriptanului oral. **Sumatriptanul administrat sub forma de *spray intranazal* (20 mg) este singurul "triptan" care şi-a demonstrat eficienţa la *adolescenţi,*** la care atacurile migrenoase sunt, de obicei, de scurtă durată, fiind asociate cu simptomatologie gastrointestinală marcată şi o rată înaltă de răspuns la placebo.

Când un anumit triptan nu poate fi tolerat, trebuie încercat un altul!

Triptanii sunt eficienţi la aproximativ 60 % dintre non-responsivii la antiinflamatoare nesteroidiene (Diamond, 2004).

Triptanii pot fi eficienţi în orice moment în timpul atacului migrenos. Există dovezi că triptanii sunt cu atât mai eficienţi cu cât sunt administraţi mai precoce (Burstein, 2004; Dowson, 2004). Datorită aspectelor de siguranţă triptanii nu trebuie administraţi în timpul aurei, deşi nu s-au raportat evenimente adverse specifice.

Folosirea triptanilor este limitată la maximum 10 zile/lună, existând riscul de apariţie a cefaleei induse medicamentos.

Tabelul nr. 3. Triptanii folosiţi în atacul migrenos

Substanţa	Doza (mg)	Nivel	Comentariu
Sumatriptan	25, 50, 100 (oral – inclusiv forme rapid dispersibile) 25 (supozitor) 10, 20 (spray nazal) 6 mg (subcutanat)	A A	100 mg sumatriptan este referinţa pentru toţi ceilalţi triptani
Zolmitriptan	2,5; 5 (oral, inclusiv forme cu dezintegrare orală) 2,5 ; 5 (spray nazal)	A A	
Naratriptan	2,5 (oral)	A	Eficacitate mai redusă dar mai îndelungată decât la sumatriptan
Rizatriptan	10 (oral, inclusiv forma de vafe)	A	Doza este de 5 mg atunci când se administrează cu propranolol
Almotriptan	12, 5 (oral)	A	Probabil cu mai puţine reacţii adverse decât sumatriptanul
Eletriptan	20, 40 (oral)	A	80 mg permise în cazul în care 40 mg nu sunt eficiente
Frovatriptan	2,5 (oral)	A	Eficacitate mai redusă dar mai îndelungată decât la sumatriptan

(R. Hughes, M.Brainin, N.E. Gilhus – *European Handbook of Neurological Management.* First Edition, 2006 , Blackwell Publishing Ltd)

Reacţiile adverse generale ale triptanilor sunt: simptomele toracice, greaţa, paresteziile distale, oboseala.

Contraindicaţiile generale ale triptanilor sunt: hipertensiune arterială netratată, boala coronariană cardiacă, bolile cerebrovasculare, boala Raynuad, sarcina şi lactaţia, vârsta sub 18 ani (cu excepţia sumatriptan spray nazal) şi peste 65 de ani, insuficienţa hepatică sau renală severă.

Într-o ***meta-analiză care a cuprins 24 089 pacienţi incluşi în 53 studii clinice*** s-a folosit ca unitate de referinţă doza de 100 mg sumatriptan oral şi s-au urmărit următorii **parametri:**

- **ameliorarea la 2 ore a cefaleei** – comparativ cu 100 mg sumatriptan oral au fost *mai eficienţi 100 mg rizatriptan şi 80 mg eletriptan*, în timp ce au fost mai puţin eficienţi 2,5 mg naratriptan, 20 mg eletriptan şi 2,5 mg frovatriptan;
- **dispariţia completă a cefaleei la 2 ore** - au prezentat rate mai înalte la *80 mg eletriptan, 12,5 mg almotriptan, 10 mg rizatriptan.* Pentru 2,5 mg naratriptan şi 20 mg eletriptan ratele au fost mai reduse comparativ cu 100 mg sumatriptan;
- **consistenţa răspunsului** – eficacitatea la cel puţin două din trei atacuri tratate a fost de 67 % pentru 100 mg sumatriptan oral şi de 65% la doza de 50 mg sumatriptan oral.*Cele mai ridicate rate de consistenţă la 3 atacuri s-au observat la rizatriptan;*
- **tolerabilitatea** – extinderea efectelor secundare supărătoare, dar fără o semnificaţie clinică importantă (parestezii, senzaţie de presiune, ameţeală) prezintă mici diferenţe între diverşii "triptani". Cele mai joase valori ale reacţiilor adverse de acest tip s-au găsit *pentru 2,5 mg naratriptan şi 12,5 mg almotriptan, ratele de apariţie a acestor reacţii adverse nefiind diferite de placebo*;
- **siguranţa** – se referă la apariţia unor efecte secundare importante din punct de vedere medical. "*Triptanii" diferă între ei în ceea ce priveşte tolerabilitatea nu şi siguranţa.*

II. TRATAMENTUL PREVENTIV (PROFILACTIC)

Migrena produce o importantă *dizabilitate* (conform OMS, ocupă poziția 19 în ierarhia afecțiunilor cauzatoare de dizabilitate), motiv pentru care tratamentul profilactic îşi are justificarea. Migrena este o boală cronică care poate fi progresivă la unii pacienți, ceea ce susține de asemenea necesitatea unui tratament preventiv.

Tratamentul profilactic se administrează în absența cefaleei, pentru a reduce frecvența şi severitatea atacurilor migrenoase anticipate. Decizia de introducere a terapiei preventive se ia în funcție de frecvența, severitatea, durata atacurilor migrenoase, precum şi în funcție de răspunsul acestora la tratament.Tratamentul profilactic al migrenei se recomandă în următoarele situații:

-la pacienții la care calitatea vieții, activitatea profesională sau şcolară sunt sever afectate de către migrenă;

-frecvența atacurilor este de două sau mai multe ori pe lună;

-atacurile migrenoase nu răspund la tratamentul acut medicamentos;

-apariția de aure frecvente, foarte îndelungate care creează disconfort.

Se consideră ca succes terapeutic situația în care frecvența atacurilor migrenoase într-un interval de o lună scade cu cel puțin 50 % în 3 luni. Pentru evaluarea terapiei este necesară completarea unui jurnal. Aproximativ 2/3 dintre pacienții aflați sub tratament preventiv prezintă o reducere de 50% a frecvenței atacurilor migrenoase.

Fiecare medicament va fi început cu o doză minimă care, ulterior, va fi treptat crescută. În introducerea terapiei preventive este util de evaluat comorbiditățile migrenei la un anumit pacient. Pacienților le vor fi explicate reacțiile adverse ale preparatelor folosite. Metisergida va fi evitată datorită complicațiilor fibrotice, iar în cazul valproatului se va atrage atenția asupra riscului de teratogenitate.

Studiile randomizate, dublu-orb, placebo-controlate au arătat ca fiind eficiente în prevenția migrenei următoarele clase de substanțe:

a) beta – blocantele;
b) blocantele canalelor de calciu;
c) antidepresivele;
d) medicația antiepileptică (neuromodulatoare).

Tabelul nr. 4. Medicamente de primă alegere în tratamentul profilactic al migrenei

Substanța	Doza (mg)	Nivel
Beta-blocante		
Metoprolol	50-200 (oral)	A
Propranolol	40-240 (oral)	A
Blocante ale canalelor de calciu		
Flunarizină	5-10 (oral)	A
Antiepileptice		
Acid valproic	500-1 800 (oral)	A
Topiramate	25-100 (oral)	A

(R. Hughes, M.Brainin, N.E. Gilhus – *European Handbook of Neurological Management.* First Edition, 2006, Blackwell Publishing Ltd)

Tabelul nr. 5. Medicamente de a doua alegere în tratamentul profilactic al migrenei

Substanța	Doza (mg)	Nivel
Amitriptilina	50-150 (oral)	B
Naproxen	2x250-500 (oral)	B
Bisoprolol	5-10 (oral)	B
Petasites	2x 75 (oral)	B

(R. Hughes, M.Brainin, N.E. Gilhus – *European Handbook of Neurological Management.* First Edition, 2006, Blackwell Publishing Ltd)

a) Beta-blocantele

S-a demonstrat că sunt eficiente în profilaxia migrenei în studii randomizate, placebo-controlate, cele mai multe dovezi având beta-blocantul selectiv metoprolol.

De asemenea, bisoprolol, timolol şi atenolol pot fi eficiente, dar cu dovezi mai puțin convingătoare comparativ cu propranolol şi metoprolol.

Beta-blocantele acționează prin creşterea tonusului vascular cu creşterea rezistenței vasculare periferice, reducând vasodilatația din procesul migrenos. Sunt de elecție în cazul *atacurilor migrenoase cu frecvență relativ scăzută dar* ***intensitate mare.*** Reprezintă medicația de primă alegere în cazul *asocierii migrenei cu HTA sau angină pectorală şi al comorbidității migrenă – anxietate şi atacuri de panică, sau în cazul asocierii tremorului esențial.*

Sunt contraindicate în bradicardia sinusală, blocul atrio-ventricular, insuficiența cardiacă congestivă, sindromul Raynaud, astmul bronşic. Sunt de evitat la pacienții cu diabet zaharat, depresie. Beta-blocantele prezintă *interacțiuni medicamentoase cu rizatriptanul şi blocantele canalelor de calciu.*

Când unul dintre beta-blocante nu se dovedeşte a fi eficient poate fi înlocuit cu un altul. Doza de la început trebuie să fie mică şi crescută treptat, la interval de o lună. Propranololul tinde să aibă cele mai multe reacții adverse – fatigabilitate, bradicardie, hipotensiune arterială ortostatică, disfuncții sexuale. Fatigabilitatea poate creşte frecvența cefaleei, situație în care doza de beta-blocant va fi redusă.

b) Blocantele canalelor de calciu

Blocantele canalelor de calciu cresc pragul dureros prin afectarea transmisiei sinaptice şi e posibil să interfere cu mecanismele inflamației neurogene. Acestea sunt utilizate în cazul pacienților cu *contraindicații la beta-blocante*, în cazul migrenei cu *aură prelungită*, al *infarctului migrenos* sau al *migrenei vestibulare.*

În câteva studii, s-a demonstrat că blocantul de calciu non-specific flunarizina poate fi eficient, în doză de 5-10 mg, în profilaxia migrenei. Pacientele beneficiază de doze mai mici decât pacienții de sex masculin. Folosirea ei este limitată de apariția efectelor adverse din sfera ganglionilor bazali, durere abdominală şi creştere în greutate. Se poate folosi atât în tratamentul atacului de migrenă cu aură, cât şi în tratamentul profilactic al migrenei hemiplegice familiale sau al migrenei asociate cu hipertensiune.

Cyclandelate – un alt blocant al canalelor de calciu non-specific studiat în profilaxia migrenei - nu este recomandat, rezultatele studiilor fiind contradictorii.

c) Antidepresivele

Antidepresivele triciclice – potențează efectele serotoninei la nivelul SNC, prin urmare inhibă transmiterea durerii, crescând pragul migrenos. Antidepresivele sunt utile în tratamentul profilactic al pacienților cu atacuri migrenoase cu **frecvență mare**, dar nu foarte intense. Antidepresivele sunt de primă alegere în cazul comorbidității migrenei cu depresia, anxietatea, tulburările de somn sau al asocierii cu cefalee de tip tensional.

Singurul antidepresiv cu eficacitate consistentă în profilaxia migrenei este amitriptilina în doze 10-150 mg. Efectele adverse sunt de tip anticolinergic: sedare, uscăciunea gurii şi tulburare cognitivă. Este de evitat la pacienţii supraponderali, întrucât determină creşterea apetitului cu creştere în greutate. Amitriptilina este contraindicată în infarctul de miocard, aritmii, hipotensiunea ortostatică, glaucomul cu unghi închis, adenomul de prostată şi în epilepsie.

Fluoxetina în doze între 10-40 mg a fost găsită ca fiind eficientă în trei studii şi ineficientă într-un studiu placebo-controlat.

d) Medicaţia antiepileptică (neuromodulatoare)

Datele actuale privind patogenia migrenei susţin faptul că aceasta este o afecţiune primară a SNC în care există anomalii atât ale activităţii corticale, cât şi ale trunchiului cerebral, iar din punct de vedere genetic poate fi o canalopatie (migrenă hemiplegică familială).

Folosirea medicaţiei antiepileptice pentru profilaxia migrenei se justifică pornind de la ideea că ambele sunt afecţiuni cronice, caracterizate prin atacuri recurente de disfuncţie a SNC.

Substratul fiziopatologic este existenţa în ambele afecţiuni a unei stări de *hiperexcitabilitate cerebrală* cu modificări EEG între atacuri, subiectul prezentând un prag convulsivant – şi respectiv migrenos scăzut. Acest prag este condiţionat genetic şi poate fi influenţat de factorii de mediu şi de terapia anticonvulsivantă.

În plus, în unele forme de migrenă (sindrom Bickerstaff) poate apărea alterarea tranzitorie a stării de conştienţă.

În cazul migrenelor cu anomalii EEG s-a observat un *răspuns pozitiv al simptomatologiei la tratamentul anticonvulsivant,* ceea ce este o dovadă circumstanţială pentru o asociere între migrenă şi epilepsie. De asemenea, în unele cazuri, *întreruperea tratamentului anticonvulsivant a determinat recrudescenţa atacurilor migrenoase*.

S-au folosit iniţial în profilaxia migrenei fenitoinul şi carbamazepina, abandonate datorită reacţiilor adverse. Cel mai utilizat medicament anticonvlusivant în profilaxia migrenei este **acidul valproic** sub forma de divalproex, dar şi anticonvulsivante de nouă generaţie: topiramat, lamotrigină, gabapentină.

Efectul acidului valproic şi al topiramatului a fost demonstrat prin studii mari de profilaxie a migrenei (nivel de evidenţă clasa I). Recent, topiramatul şi-a demonstrat utilitatea şi în cazul altor cefalee cronice refractare la tratament, precum şi în profilaxia migrenei populaţiei pediatrice. Mecanismul de acţiune al valproatului şi topiramatului în prevenţia migrenei este legat de inhibarea transmisiei centrale a durerii prin potenţarea efectului inhibitor GABA-ergic.

Acidul valproic în doze de cel puţin 600 mg şi topiramatul în doze de 25-200 mg şi-au demonstrat eficienţa în studii placebo-controlate.

Lamotrigina nu reduce frecvenţa atacurilor migrenoase, dar este eficientă în reducerea frecvenţei aurelor migrenoase (Steiner, 1997; Lampl, 1999).

Gabapentin şi-a demonstrat eficienţa într-un studiu palcebo-controlat la doze între 1 200- 1 600 mg (Mathew, 2001).

► **Acidul valproic (***divalproex sodium***)** - Divalproex - este deosebit de util la pacienţii care prezintă *comorbiditate migrenă-epilepsie, migrenă-tulburare anxioasă sau migrenă-boală bipolară*. Mecanismul său de acţiune se explică prin prezenţa receptorilor GABA în trunchiul cerebral şi în ganglionul trigeminal. Administrarea de valproat accentuează efectele GABA-ergice având drept consecinţă blocarea inflamaţiei neurogene şi a celei induse de substanţa P.

Poate fi administrat la pacienţii la care sunt contraindicate beta-blocantele. Acidul valproic în doze de 500-1 000 mg/zi (sub dozele utilizate în epilepsie) reduce frecvenţa atacurilor migrenoase. *Hepatotoxicitatea* este cel mai sever efect advers, fiind necesară dozarea enzimelor hepatice la începutul tratamentului. Este contraindicat în caz de afectare hepatică, pancreatică, de trombocitopenie, precum şi în caz de sarcină, datorită riscului teratogen. Principalele efecte secundare sunt: sedarea, căderea părului, creştere în greutate, dezvoltarea ovarelor polichistice.

►**Topiramatul (Topamax)** - este un medicament antiepileptic de ultimă generație, cu multiple potențiale mecanisme de acțiune: este blocant al canalelor de sodiu, stimulator al transmisiei mediate de GABA şi inhibitor al receptorilor AMPA ai glutamatului. În două studii dublu-orb, placebo-controlate s-a dovedit a fi util în profilaxia migrenei, în doze (75-150 mg) inferioare celor din epilepsie. Reacțiile sale adverse sunt: parestezii, anorexie, scădere în greutate, alterarea memoriei. Este util în special la persoanele obeze şi este contraindicat în sarcină.

Topiramatul a fost utilizat cu succes în profilaxia crizelor la pacienții cu *migrenă refractară la adulți* (Krusz and Scott, 1999 – doza medie de topiramat 325 mg/zi; Storey et al, 1999 - doza medie de topiramat 104 mg/zi; Shuab at al., 1999 - doza de topiramat cuprinsă între 25-100 mg/zi). Deoarece frecvența reacțiilor adverse creşte odată cu mărirea dozei de topiramat se recomandă o *titrare lentă* a acestuia (25 mg/săptămână).

Hershey at al. a folosit o doză medie de 84 mg +/-38 mg/zi la copii, obținând o reducere cu 46% a frecvenței cefaleei. În cefaleea copilului, topiramatul este mai bine tolerat şi este eficient în doze mai mici decât cele necesare în tratamentul epilepsiei (Phillips TM, 2003).

La o doză de Topamax între 100 şi 200 mg/zi s-a observat scăderea frecvenței crizelor migrenoase, scăderea numărului de zile cu migrenă /lună şi scăderea folosirii medicației pentru terapia acută a migrenei. În plus, s-a remarcat faptul că efectele benefice apar din prima lună de tratament.

Topiramatul a fost studiat în cele mai importante cercetări efectuate pentru profilaxia migrenei (MIGR-001, MIGR-002) – nivel de evidență de clasa I. Topiramatul, alături de acidul valproic sunt singurele neuromodulatoare aprobate în SUA de FDA (Food and Drug Administration) în profilaxia migrenei. În plus**, topiramatul poate modula semnalizarea trigemino-vasculară** implicată în patogenia migrenei (Storer RJ, 2003).

Tabelul nr.6. Mecanisme de acțiune ale topiramatului

LOCUL DE ACȚIUNE	MECANISM DE ACȚIUNE
Canale de Na^+ voltaj dependente	Limitarea descărcărilor repetitive
Receptorii GABA $_A$	Potențează inhibiția mediată GABA la nivelul receptorilor GABA $_A$, nivel care nu este influențat de benzodiazepine sau barbiturice
Receptorii glutamat AMPA/kainate	Blochează neuroexcitotoxicitatea indusă prin glutamat influențând activitatea receptorilor non-NMDA
Canalele de Ca^{++} voltaj dependente	Uşoară reducere a curentului de calciu la nivelul canalelor voltaj dependente
Anhidraza carbonică	Inhibă izoenzimele II şi IV ale anhidrazei carbonice

Bazil S. Neurolog. 2002 Mar;8(2);71-81; Shank RP et al. *Epilepsia.* 2000;(suppl 1) S3-S9

► **Gabapentinul (Neurontin)** – în doză de 600-1 800 mg, într-un studiu deschis s-a dovedit a fi eficient în reducerea frecvenței şi a duratei episoadelor migrenoase. Este cunoscut pentru efectele sale analgetice în durerile neuropatice şi în profilaxia migrenei. Din punct de vedere chimic este un analog structural al GABA, mecanismul său de acțiune nu este încă pe deplin elucidat, el creşte rata de sinteză a GABA şi concentrația acestuia la nivel cerebral, având un loc de legare diferit de GABA la nivelul SNC. Posibil ca în migrenă să acționeze la nivelul unui component polipeptidic al canalului de calciu voltaj dependent de tip P/Q implicat în apariția migrenei. Este recomandat în migrena hemiplegică familială (care este sigur o canalopatie), alături de acidul valproic.

După o perioada de 12 săptămâni de tratament scade durata, intensitatea şi severitatea atacurilor migrenoase, în paralel cu diminuarea cantităţii totale de medicamente antimigrenoase, cu prevenirea apariţiei cefaleei induse medicamentos.

► **Lamotrigina (lamictal)** este un nou medicament antiepileptic care inhibă eliberarea patologică a aminoacizilor excitatori de tipul glutamatului şi aspartatului care s-a demonstrat a fi eficient în reducerea aurei migrenoase şi în scăderea frecvenţei atacurilor de migrenă cu aură (D'Andrea, 1999; Lampl,1999, Lampl C., 2005).

► **Levetiracetamul (keppra)** este un medicament antiepileptic de ultimă generaţie cu un profil de siguranţă deosebit de bun, cu un mecanism de acţiune încă incomplet elucidat (se pare că exercită un efect inhibitor asupra canalelor de calciu de tip neuronal) care, recent, s-a dovedit a fi util în profilaxia migrenei cu aură, alături de lamictal (Brighina F., 2006).

Alte terapii utilizate în profilaxia migrenei:

- *metisergida* (6-8 mg/zi) – agonist serotoninic administrat cu pauză de o lună la fiecare 6 luni de tratament datorită riscului de fibroză retroperitoneală şi retrouretrală;
- *antiinflamatoarele nesteroidiene* – utile în asocierea migrenă-artrită; magneziul dicitrat - indicat în profilaxia migrenei în cantitate de 300mg x 2/zi;
- *magneziul* este un stabilizator al pragului migrenos, indicat în profilaxia migrenei în cantitate de 300mg x 2/zi ;
- *riboflavina (vitamina B2) – 200 mg x 2/zi* şi *acidul lipoic* sunt benefice în migrenă prin corectarea disfuncţiei mitocondriale ;
- *cyclandelate* - inhibă acumularea de calciu intraneuronal, contracţia fibrelor musculare netede din peretele arterial, precum şi agregarea plachetară; de asemenea, inhibă eliberarea serotoninei din plachete. Se pare că acesta antagonizează specific chiar canalele de calciu voltaj dependente P/Q implicate în patogenia migrenei;
- *cyproheptadina* este un antihistaminic care are şi proprietăţi antiserotoninice; este folosit ocazional în migrenă. Efectele sale adverse includ sedarea, creşterea ponderală, senzaţia de gură uscată, ameţeală ;
- *clonidina* este un agonist al receptorilor adrenergici α centrali cu valoare limitată în profilaxia migrenei la femeile aflate la menopauză care nu doresc să urmeze tratament hormonal de substituţie;
- *toxina botulinică* – injectarea (25 unităţi) în regiunea glabelei, frontal şi temporal bilateral, reduce frecvenţa şi severitatea atacurilor migrenoase.

Toxina botulinică îşi exercită efectul prin inhibarea exocitozei acetilcolinei (Ach) de la nivelul terminaţiilor nervoase colinergice presinaptice, producând bloc presinaptic.

După injectare, toxina botulinică este preluată prin endocitoză în neuronul presinaptic al joncţiunii neuromusculare şi clivează proteinele esenţiale în exocitoza Ach. Locul de acţiune al toxinei botulinice de tip A este SNAP-25 *(synaptosome-associated protein with a molecular weigt of 25 kD),* care este o proteină a membranei presinaptice, iar pentru toxina botulinică de tip B, locul de acţiune îl reprezintă VAMP *(vesicle-associated membrane protein, synaptobrevin),* care este o proteină localizată în peretele veziculelor presinaptice care conţin acetilcolină.

Toxina botulinică este folosită în tratamentul spasticităţii musculare şi al distoniilor focale. Conform ipotezei de formare a punctelor trigger miofasciale, lezarea muşchiului la nivelul plăcii terminale determină eliberarea excesivă de Ach. Acetilcolina în exces determină contractură locală cu compresia micilor vase sanguine şi ischemie tisulară cu eliberare de bradikinină (BKN) care produce sensibilizarea nociceptorilor. În acest caz toxina botulinică este tratamentul cauzei, stopând eliberarea în exces a Ach.

În spasm şi distonie, toxina botulinică ameliorează durerea prin relaxare musculară. În plus, se pare că toxina botulinică are şi o acţiune analgetică directă, inhibând probabil şi exocitoza altor neuromediatori în afară de Ach, prevenind eliberarea de neuropeptide din terminaţiile nervoase nociceptive. În acest sens toxina botulinică este indicată în cefaleele în care inflamaţia neurogenă joacă un rol central. Astfel, toxina botulincă inhibă eliberarea de substanță P şi CGRP din terminațiile nervoase trigeminale în migrenă, ameliorând cefaleea.

Toxina botulinică este utilă în profilaxia migrenei în cazul migrenei cronice – cu mai mult de 15 zile de migrenă/lună pentru mai mult de 3 luni şi la care celelalte terapii au fost ineficiente. Mecanismul de acțiune al toxinei botulinice în profilaxia migrenei nu este complet cunoscut, folosirea acestei opțiuni trebuie să se alăture măsurilor preventive unanim accceptate.

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Abordări psihofarmacologice ale sistemului melatoninergic în tulburările hipnice

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Rezumat. Sistemul melatoninergic reprezintă o componentă esențială în cadrul substratului neurofiziologic al ritmului circadian, importanța sa în reglarea ciclului somn-veghe fiind dovedită de numeroase studii clinice. Agoniștii receptorilor melatoninergici prezintă avantaje farmacokinetice și farmacodinamice superioare melatoninei, putând acționa atât în insomnie, cât și în tulburările ritmului circadian de somn (asociate schimbărilor de fus orar, lucrului în ture etc). Pe lângă un număr de molecule existente astăzi în practica psihiatrică (ramelteon, agomelatina), al căror efect activator asupra sistemului melatoninergic a fost confirmat, există mai mulți agenți promelatoninergici aflați în diferite faze de cercetare, capabili să indice noi dimensiuni ale implicării acestui sistem în patologia somnului.

Cuvinte cheie: sistem melatoninergic, insomnia, tulburările ritmului circadian de somn, ramelteon, agomelatina

Abstract. The melatoninergic system stands as an essential element in the sleep circadian rhythm neurophysiology and melatonin's importance in regulating the sleep-wake cycle has been proven by many clinical trials. Melatonin receptors agonists have certain pharmacokinetic and pharmacodynamic advantages over melatonin itself because they could act therapeutically in insomnia, as well as in the circadian sleep rhythm disorders (jet lag, shift work and other similar phenomena). Beside active psychopharmacologic agents used nowadays in psychiatry (ramelteon, agomelatine), which have activating effects over the melatoninergic system validated through clinical research data, there are a number of promelatoninergic drugs, in different stages of controlled investigations, that are able to uncover new dimensions of this system involvement in sleep pathology.

1. Neurobiologia sistemului melatoninergic

Până în prima jumătate a secolului XX, glanda pineală a fost considerată un apendice epitalamic al creierului vertebratelor, cu funcții necunoscute. Descoperirea unei substanțe organice noi în extractul de epifiză de bovină, precum și identificarea structurii sale chimice (N-acetil-5-methoxi-triptamină), de către Aaron B. Lerner, au reprezentat un pas major în decelarea funcțiilor glandei pineale. Evidențiată pentru prima dată în extractul de epifiză, melatonina s-a dovedit a fi o moleculă cvasi-ubicuitară în ceea ce privește distribuția tisulară (fapt ce dovedește complexitatea rolurilor ei în organism) și prezența la diverse specii de nevertebrate și vertebrate.

O caracteristică importantă a acestui hormon cu structură indolică constă în faptul că sinteza sa are loc în timpul nopții, într-o strânsă legătură cu absența stimulului luminos și cu ritmul circadian, ceea ce i-a făcut pe unii autori să o descrie ca fiind un "hormon al întunericului" sau o „moleculă cronobiotică" [1].

Biosinteza melatoninei are loc în principal în pinealocite, în timpul nopții, pe parcursul a patru etape. Într-o primă etapă, precursorul L-triptofan este preluat din sânge în pinealocit și convertit în 5-hidroxitriptofan de triptofan-5-monooxigenaz-hidroxilaza și, ulterior, decarboxilat de L-aromatic-amino-acid-decarboxilaza, pentru a forma serotonină (5-HT). Serotonina este apoi acetilată în penultima etapă de către serotonin-N-acetiltransferaza (S-NAT)- enzimă cheie în reglarea ratei biosintezei melatoninei. N-acetil-serotonina este metilată de hidroxiindol-O-metiltransferază pentru a forma melatonina.

Magnitudinea şi durata creşterii nocturne a sintezei melatoninei este dependentă de lungimea fazei de întuneric a ciclului zi-noapte (ciclul geo-fizic extern). Ritmul sintezei hormonului epifizar este dependent de interacţiunea dintre sistemele genelor ritmului circadian localizate în nucleul suprachiasmatic (SCN), pacemaker-ul/generatorul de ritm circadian aflat în hipotalamus. Durata ritmului intern al SCN depăşeşte cu puţin 24 de ore, dar este resetat la 24 de ore pe zi datorită ciclului natural lumină-întuneric, prin stimularea retinei de către stimulul luminos. Acest stimul trimite semnale circadiene de-a lungul unei căi neurale polisinaptice, care include terminaţii simpatice, proiectându-se de la ganglionul cervical superior la glanda pineală şi determinând secreţia pulsatilă a melatoninei. Pigmentul fotoreceptor principal, cu rol în sincronizarea circadiană, se pare că este reprezentat de melanopsina din celulele ganglionare retiniene [2].

Nucleul suprachiasmatic este centrul reglator major al activităţii enzimei S-NAT. Lumina provoacă un declin rapid în activitatea acesteia printr-un mecanism complex, care este asociat cu controlul catabolismului prin intermediul fosforilării enzimei de către proteinkinaza A (PKA), Rho-kinaza, CHK1 şi apoi asocierea enzimei fosforilate cu "chaperon-like 143.3.3 protein", dimerizare care conduce fie la proteoliză mediată proteozomal, fie protejează complexul de disociere. Catabolismul S-NAT este limitat la glanda pineală, în timp ce la nivelul retinei enzima pare să fie protejată împotriva proteolizei în timpul zilei [3].

Fibrele nervoase simpatice post-ganglionare care trimit terminaţii în jurul glandei pineale eliberează noradrenalină, aceasta având, de asemenea, un rol esenţial în controlul sintezei melatoninei. Creşterea stimulării noradrenergice nocturne prin receptorii β şi α1 prezenţi la nivelul pinealocitelor creşte concentraţia AMPc intracelular şi, prin aceasta, activează S-NAT, rezultând niveluri crescute de melatonină [4]. Reglarea secreţiei de melatonină este de asemenea influenţată de serotonină, monoamin-oxidază, cortizol, aldosteron, testosteron şi estradiol - mecanismele moleculare nefiind cunoscute.

Melatonina este produsă în timpul nopţii, în lipsa stimulului luminos şi eliberată de la nivelul pinelocitelor în ventriculul III şi de acolo în lichidul cerebrospinal şi în circulaţie. Nivelurile hormonului la nivelul ventriculului III depăşesc de câteva ori, aşa cum arată date recente, nivelurile aflate în circulaţie [5]. Ulterior, are loc primul pasaj hepatic (timpul de înjumătăţire al melatoninei în serul uman este de mai puţin de o oră) şi peste 80% este excretată în urină ca 6-sulfatoximelatonină (6-SMT). Astfel, hormonul dispare din circulaţie la scurt timp după încetarea secreţiei, fapt care face ca excreţia urinară de 6-SMT şi nivelurile plasmatice ale melatoninei să fie strâns corelate la oameni [6].

În prezent există multe date care arată că melatonina are un rol important în favorizarea somnului la oameni. Nivelurile endogene ale hormonului sunt corelate cu perioadele scurte de somn diurn la nevăzători [7]. Înregistrările electrofiziologice au demonstrat că vârful somnolenţei nocturne este în strânsă legătură cu creşterea excreţiei urinare de 6-SMT [8]. Îmbătrânirea, anumite afecţiuni (neuropatia diabetică, boala Alzheimer etc.) şi anumite medicamente (beta-blocantele, clonidina, naloxona, AINS) diminuează secreţia nocturnă a hormonului epifizar şi sunt asociate cu tulburări de somn [9]. Melatonina exogenă induce toate efectele fiziologice care apar seara, în timpul secreţiei endogene a hormonului: creşte somnolenţa, scade temperatura corporală şi modulează ritmul ceasului biologic (SCN) [10].

Hormonul epifizar acţionează prin intermediul unor receptori specifici, membranari– MT1, MT2, care fac parte din familia receptorilor cu şapte domenii transmembranare asociaţi cu proteinele G şi sunt implicaţi în reglarea ritmurilor circadiene şi funcţiei hipnice. Un locus de legare al melatoninei, de afinitate joasă, denumit MT3, a fost recent identificat ca fiind la nivelul quinon-reductazei 2, dar rolul său fiziologic nu a fost pe deplin elucidat; există teorii conform cărora acesta ar fi implicat în acţiunea antioxidantă a melatoninei [11].

Un alt argument în favoarea rolului central al SCN în reglarea funcţiilor circadiene şi hipnice ale melatoninei este expresia la nivelul său a unei densităţi crescute de receptori MT1 şi MT2. Legarea melatoninei de receptorii MT1 modulează semnalele intracelulare prin inhibiţia adenilatciclazei şi stimularea hidrolizei fosfoinozitolului cauzând inhibiţia activităţii electrice neuronale la nivelul SCN, mediind vasoconstricţia, în timp ce activarea MT2 inhibă formarea a doi mesageri secundari -AMPc şi GMPc- la nivel celular, mediind vasodilataţia şi prin aceasta având rol esenţial în acţiunea reglatoare de ritm a hormonului epifizar [12].

Melatonina este capabilă să defazeze ceasul biologic circadian şi, în absenţa luminii, să întreţină ritmurile somn-veghe şi neuroendocrine. Administrată exogen, ea poate să întreţină sau să defazeze ritmul indus de melatonina endogenă, precum şi să modifice capacitatea fluxului luminos de a defaza ritmul biologic. Aceste efecte se presupune că îşi au sediul tot la nivelul SCN [1].

Circuitele cerebrale care sunt afectate de hormonul epifizar au început să fie mai bine cunoscute în prezent datorită studiilor ce folosesc investigaţii imagistice funcţionale şi moleculare. Efectele melatoniei administrate după-amiaza asupra funcţiilor cerebrale au fost evidenţiate printr-un studiu randomizat, dublu-orb, controlat placebo cu ajutorul rezonanţei magnetice funcţionale [13]. Respectivul studiu a arătat că melatonina atenuează activarea în zona rostromedială din cortexul occipital în timpul unei sarcini de căutare vizuală şi în cortexul implicat în auz în cursul unei sarcini muzicale; aceste efecte s-au corelat cu aprecierea subiectivă a oboselii. Rezultatele obţinute sugerează că melatonina modulează paternurile activităţii cerebrale la subiecţii vigili într-o manieră asemănătoare cu somnul natural, prin inducerea unei stări ce favorizează adormirea. Aceste efecte ale melatoninei sunt în mod esenţial diferite de cele decelate după deprivarea de somn, deşi ambele au ca efect inducerea unei stări de oboseală [19].

Hormonul epifizar nu creşte cantitativ perioada de somn cu unde lente, care este considerată un marker homeostatic al nevoii subiective de somn, astfel încât efectele de favorizare a somnului par să fie atribuibile componentei circadiene a reglării funcţiei hipnice [14].

2. Tulburările hipnice în care a fost implicat sistemul melatoninergic

Tulburările ritmului circadian de somn

Tulburările de ritm circadian reprezintă afecţiuni cronice determinate de relaţia inadecvată între orarul somn-veghe cerut de ambianţa în care se află persoana respectivă şi tiparul individual al ritmului somn - veghe. Aceste afecţiuni produc un declin important în funcţionarea persoanei respective în diverse arii de activitate (social, ocupaţional, familial), nu survin exclusiv în cadrul evoluţiei altei tulburări de somn sau tulburări psihice şi nu sunt rezultatul efectelor fiziologice ale unei substanţe (drog, medicament) ori al unei condiţii medicale generale. Aceste tulburări se clasifică în mai multe tipuri (conform DSM IV TR): tipul de fază de somn întârziat, tipul asociat cu lucrul în ture, tipul de decalaj de fus orar şi tipul nespecificat, care include sindromul de fază de somn avansat, sindromul de pattern de vigilitate neregulat, sindromul pattern-ului de somn-vigilitate de non-24 de ore (la nevăzători) etc. [14].

Insomnia

Noţiunea de insomnie este utilizată ca termen atât pentru un simptom, cât şi pentru o tulburare. Simptomul “insomnie” este definit ca acuza subiectivă de dificultate în adormire, menţinere a stării de somn sau calitate diminuată a somnului. În DSM-IV-TR insomnia ca simptom este inclusă drept criteriu de diagnostic pentru unele tulburări psihice, dintre care amintim tulburarea depresivă majoră şi tulburarea de anxietate generalizată. Insomnia, ca tulburare primară, este definită în DSM-IV-TR ca simptom de insomnie însoţit de tulburări majore în funcţionarea personală, cu durata de cel puţin o lună. Pentru a susţine diagnosticul de insomnie primară, conform sursei citate, simptomele nu trebuie să coexiste cu o altă tulburare a somnului sau cu alte tulburări medicale sau mentale. DSM-IV-TR include, de asemenea, diagnosticul de “insomnie secundară”, categorie în care este cuprinsă insomnia ce produce suferinţă semnificativă sau necesită abordare clinică independentă, dar este în strânsă legătură cu o tulbure mentală, medicală sau este datorată efectelor substanţelor sau medicaţiei [14].

3. Utilizarea în clinică a melatoninei şi a agoniştilor melatoninergici în tulburările de ritm circadian şi insomnie

În prezent, melatonina/agoniştii de melatonină par să fie singurul tratament medicamentos eficient în tratamentul tulburărilor de ritm circadian. În afară de efectul de favorizare a somnului, melatonina exogenă ameliorează funcția hipnică prin defazarea ceasului biologic [1]. Această acțiune a fost demonstrată în studii clinice la pacienții cu tulburare de ritm circadian tipul de fază de somn întârziat, caz în care ritmurile endogene ale melatoninei sunt decalate.

În aceste studii melatonina a fost administrată oral în doză unică de 5 mg pe zi timp de 28-30 de zile. Într-unul din studii [15] melatonina a fost administrată la toți pacienții seara, la ora 22, în timp ce în celelalte studii pentru fiecare pacient s-a determinat momentul începerii secreției de melatonină endogene [16,17]. În toate studiile administrarea melatoninei a avansat debutul somnului şi momentul trezirii, comparativ cu placebo [15-17] şi a îmbunătățit vigilența şi funcțiile cognitive [17].

Melatonina şi-a dovedit eficacitatea, în doze de 3-5 mg pe zi, la pacienții nevăzători cu tulburări de ritm circadian cu sindromul pattern-ului de non-24 de ore, dovedindu-se utilă în sincronizarea ciclului somn-veghe şi a ritmurilor neuroendocrine la ciclul zi- noapte [18]. Aceeaşi doză a fost propusă pentru îmbunătățirea somnului în sindromul de fus orar. Există evidențe sporadice ale eficienței acestui tratament [1], dar mecanismele implicate, momentul optim de administrare şi doza ramân să fie stabilite prin rezultatele studiilor viitoare.

Mai multe studii au încercat să determine utilitatea administrării de melatonină la bătrâni sau la bolnavii cu insomnie [19,20]. În cele mai multe dintre aceste studii, terapia a scăzut latența somnului comparativ cu placebo. Pacienții de vârstă medie si vârstnicii care au avut niveluri nocturne de excreție scăzute de 6-SMT sunt mai predispuşi să răspundă la terapia substitutivă cu melatonină [19]. În populația adultă cu tulburări pervazive ale dezvoltării, subgrupul la care s-a determinat o secreție nocturnă scăzută de melatonina a beneficiat mai mult în urma tratamentului substitutiv [21]. Alte dovezi indică faptul că melatonina este benefică pentru îmbunătățirea calității vieții la vârstnici [22].

Melatonina exogenă este rapid absorbită după administrare orală, cu vârful plasmatic al concentrației situat între 20 de minute şi 2 ore. Pentru a menține concentrații plasmatice eficiente pe parcursul nopții a devenit necesară dezvoltarea unei formule cu eliberare prelungită.

În studii clinice în care pacienții suferind de insomnie primară au primit melatonină cu eliberare prelungită timp de 3 săptămâni au fost demonstrate beneficii comparativ cu placebo, care constau în scăderea latenței apariției somnului şi evaluarea subiectivă superioară a calității somnului şi funcționării diurne, fără efecte la nivelul vigilenței şi chiar cu unele îmbunătățiri ale performanței matinale. Este notabil faptul că nu au fost remarcate modificări la nivelul arhitecturii sau duratei REM a somnului [23].

Efectele melatoninei retard de facilitare a somnului sunt similare în magnitudine cu cele ale altor hipnotice (de exemplu, zopiclona), fără efecte adverse în ceea ce priveşte performanța psihomotorie, condusul autovehiculelor sau memoria şi fără simptome de sevraj [24]. Melatonina potențează acțiunile modulatorilor receptorilor GABA-A (hipnotice benzodiazepinice şi non-benzodiazepinice), iar coadministrarea ei pare să faciliteze discontinuarea acestor medicamente. Efectele adverse cele mai citate ale melatoninei retard sunt faringita, durerea de spate şi astenia, fără însă ca amplitudinea acestora să pună probleme majore de siguranță a administrării [25].

Unele efecte ale melatoninei retard, nedemonstrate la agoniştii melatoninergici, sunt îmbunătățirea comportamentului după trezire, a atenției matinale şi a calității somnului. Aceste efecte sunt parțial explicate de acțiunea melatoninei cu eliberare prelungită asupra ritmului secreției de cortizol, la pacienții cu insomnie mai vârstnici de 55 de ani. La acest subgrup, secreția de cortizol debutează mai devreme în timpul somnului (comparativ cu tinerii) şi îl poate deteriora, melatonina retard având efectele mai sus menționate prin întârzierea momentului secreției de cortizol [26].

În concluzie, melatonina este un hormon produs noaptea, producția sa fiind continuă pe perioada lipsei fluxului luminos. Melatonina are rol în sincronizarea ceasului biologic la ciclul fizic extern (zi-noapte), produce o stare de anticipare a somnului modulând pattern-urile de activare ale creierului şi constituindu-se prin aceasta într-un factor reglator endogen al funcţiei hipnice. Melatonina exogenă este utilă în unele tulburări de ritm circadian, în timp ce forma sa cu eliberare prelungită capătă semnificaţie în tratamentul insomniei la vârstnici (peste 55 de ani), prin efectele benefice asupra calităţii somnului, îmbunătăţirea capacităţilor cognitive matinale, lipsa efectelor adverse şi a sindromului de discontinuare, relativă siguranţă în administrare.

Ramelteon

Ramelteonul este un agonist selectiv pentru receptorii MT1/MT2 ai melatoninei, indicat pentru tratamentul insomniei. Ramelteonul are afinitate neglijabilă pentru receptorii MT3 şi nu interacţionează cu receptorii GABA sau cu alţi receptori (adrenergici, dopaminergici, histaminergici, exceptând 5-HT1A, muscarinici sau opioizi) [27].

Doza orală recomandată este de 8 mg/zi, iar compusul se asoarbe rapid, concentraţia serică maximă atingându-se după 0.5-1.5 ore şi este echivalentă cu de 20-40 de ori nivelurile nocturne plasmatice ale melatoninei. Deşi se absoarbe în proporţie de 84%, biodisponibilitatea sa orală este cu mult mai mică datorită metabolizării extensive la care este supus după primul pasaj hepatic. În plasmă, medicamentul se leagă de proteine în proporţie de 82% (70% de albumină) şi este eliminat complet urinar la 96 de ore de la administrarea orală [28]. Unul din metaboliţii săi hepatici, denumit M-II, prezintă activitate biologică, legându-se de receptorii MT1, MT2 şi HT-2B; afinitatea sa faţă de receptorii melatoninei este mult mai slabă decât cea a ramelteonului (1/5-1/10), dar se crede că, datorită concentraţiilor plasmatice ridicate, ar putea contribui la instalarea efectelor biologice ale precursorului său [29].

Subiecţii mai în vârstă metabolizează medicamentul semnificativ mai încet decât subiecţii tineri, de aceea expunerea lor la orice doză este de două ori mai mare. Expunerea este de 4-10 ori mai mare la cei cu disfuncţie hepatică moderată, dar nemodificată la cei cu disfuncţie renală [28]. Metabolismul hepatic este legat de complexul enzimatic CYP 1A2 şi de aceea medicamentele care inhibă sau induc CYP 1A2 influenţează nivelurile plasmatice ale ramelteonului [29].

Ramelteonul se leagă de receptorii MT1 şi MT2, pe care îi activează, inhibând producţia de AMPc în culturi de celule clonate să exprime receptori melatoninici, afinitatea sa pentru receptori fiind mai mare decât cea a melatoninei [27].

Prin administrarea medicamentului în doze terapeutice concentraţiile plasmatice iniţiale sunt de sute de ori mai mari decât cele generate de secreţia nocturnă de melatonină. Având în vedere potenţialul concentraţiilor suprafiziologice ale melatoninei de a desensibiliza receptorul MT1 şi de a cauza o down-reglare a receptorilor MT2 în nucleul suprchiasmatic, ar putea exista posibilitatea apariţiei desensibilizării receptorilor la cei care iau ramelteon mai mult de câteva zile, având ca o consecinţă agravarea insomniei [30]. Desigur, aceste consideraţii teoretice, deşi impun prudenţă, trebuie documentate riguros din punct de vedere clinic.

Din păcate există foarte puţine date din literatură care să susţină sau să dezavueze ideea utilizării ramelteonului pentru tratamentul insomniilor. Într-unul din studii, subiecţii sănătoşi au primit o singură doză de medicament [31]; într-un alt studiu, subiecţii suferind de insomnie au primit ramelteon doar pentru două zile consecutive [32], în timp ce într-un al treilea studiu au fost descrise doar efectele subiective ale drogului [33]. De asemenea, nu există studii care să compare eficienţa ramelteonului cu melatonina sau studii care să documenteze consecinţele administrării îndelungate (>35 de zile) a medicamentului.

Având în vedere sărăcia informaţiilor referitoare la acest nou medicament şi faptul că acesta interacţionează cu receptorii MT1, MT2 ai melatoninei, pare rezonabil să presupunem că mecanismele de acţiune şi efectele clinice ale ramelteonului sunt asemănătoare cu cele ale melatoninei. Ramelteonul are o afinitate de legare de receptori de 3-16 ori mai mare decât hormonul epifizar. Există autori care consideră că această proprietate se traduce într-o potenţă mai mare faţă de melatonină [27].

În ceea ce priveşte efectele adverse, ramelteonul pare să aibă un profil favorabil: efectele adverse par să fie minore şi comparabile cu cele ale lotului placebo: somnolenţă, greaţă, fatigabilitate, cefalee, insomnie [30]. Nu s-au decelat modificări ale investigaţiilor de laborator sau electrocardiogramei. Totuşi, au fost semnalate efecte asupra sistemului de reproducere la adulţi, de aceea amenoreea, galactoreea, scăderea libidoului sau problemele de fertilitate pot fi puse pe seama modificării nivelurilor serice ale testosteronului sau creşterii prolactinei [34].

Un studiu ce a cuprins 14 subiecţi a comparat ramelteonul cu triazolamul şi placebo în ceea ce priveşte potenţialul de abuz. Nu a existat nici o evidenţă de abuz, nici chiar la doze de până la 20 de ori mai mari decât doza uzuală de tratament [35].

Ramelteonul este un agonist potent al receptorilor MT1 şi MT2 ai melatoninei, cu care împărtăşeşte acţiunea de scădere a latenţei somnului. Profilul clinic favorabil al efectelor adverse şi lipsa potenţialui de abuz îl impun ca o posibilă nouă terapie în tratamentul insomniei, dar nu există suficiente date care să susţină faptul că în administrarea cronică acest medicament îşi va menţine eficacitatea şi spectrul benign al efectelor adverse.

Agomelatina

Agomelatina este un agent antidepresiv recent apărut, cu un mecanism nou de acţiune, fiind agonist potent al receptorilor melatoninergici MT1 şi MT2 şi antagonist slab al receptorilor 5-HT2c. Acţiunea antidepresivă a agomelatinei a fost testată la om prin studii care au inclus mai mult de 2 400 de pacienţi, în cadrul cărora a fost urmărită eficienţa pe termen lung şi scurt a compusului. Toţi pacienţii trataţi cu acest agent farmacologic sufereau de tulburare depresivă majoră.Studiile au arătat că agomelatina administrată o dată pe zi, seara, este cel puţin la fel de eficace în tratamentul tulburării depresive majore ca inhibitorii selectivi ai recaptării serotoninei, cum ar fi paroxetina şi inhibitorii recaptării serotoninei şi noradrenalinei, cum ar fi venlafaxina, dar cu efecte adverse mai puţine decât acestea [36].

Folosirea agomelatinei ca tratament al tulburării depresive majore pare cu atât mai oportună cu cât majoritatea pacienţilor depresivi prezintă tulburări de somn cum ar fi insomnia, hipersomnia şi diminuarea calităţii somnului. Această substanţă, în afară de eficacitatea antidepresivă, a îmbunătăţit calitatea şi continuitatea somnului la pacienţii depresivi şi a crescut capacitatea de concentrare diurnă [37]. Agomelatina este bine tolerată şi pare să aibă efecte adverse sexuale mai puţine decât restul antidepresivelor.

Alţi liganzi ai receptorilor de melatonină

TIK-301, β –metil-6-clor- melatonina, este un agonist de înaltă afinitate a receptorilor MT1, MT2, care a fost elaborat pentru a avea o potenţă mai înaltă şi un profil farmacokinetic mai bun ca al melatoninei. Acesta reduce latenţa somnului într-un mod dependent de doză, fără a afecta negativ alţi parametri ai somnului. Spre deosebire de melatonină nu este asociat cu schimbări ale temperaturii corpului, pulsului sau tensiunii arteriale [38]. **VEC-162** este un agonist de melatonină MT1, MT2, aflat în studii de faza III pentru tratamentul insomniei [39]. **BMS-214778** (Tasimelteon) este un agonist de MT1, MT2 care ar putea fi folositor în tratamentul tulburărilor de ritm circadian [40]. **GR 196429** este un agonist MT1, MT2 asemănător ca structură cu ramelteonul, cu afinitate înaltă pentru receptorii de melatonină, având efecte pe activitatea electrică a neuronilor din nucleul suprachiasmatic asemănătoare cu cele ale melatoninei. Efectele asupra ritmului circadian ale **S-22153**, un antagonist nonselectiv al receptorilor de melatonină, au fost evaluate, deocamdată, doar la nivel preclinic.

4. Concluzii

Sistemul melatoninergic deţine un rol central în cadrul mecanismelor de reglare a ritmului nictemeral, astfel încât medicaţia care modulează activitatea acestui sistem prezintă o largă aplicabilitate clinică, atât în cazul insomniilor, cât şi în cel al tulburărilor ritmului circadian. Deşi în prezent există un număr limitat de agenţi farmacologici cu acţiune agonistă la nivelul receptorilor melatoninergici (ramelteon, agomelatină), care să-şi fi dovedit utilitatea în clinica tulburărilor hipnice, un număr important de molecule se află în diferite faze de cercetare, sugerând posibilitatea apariţiei în viitorul apropiat a unui arsenal important pentru combaterea acestei patologii frecvent întâlnite.

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Organizarea asistenței medicale în România. Activitatea unităților sanitare în anul 2008

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Sănătatea publică are un conținut diferit de alte discipline, prezentând ca o particularitate obiectul de preocupare: grupul sau grupurile de persoane şi nu persoana (individul). De asemenea, vizează rolul factorilor sociali care influențează sănătatea şi are o tentă preventivă. Sistemul de asistență medico-sanitară a fost pus recent pe noi baze, prin adoptarea legii asigurărilor pentru sănătate, a Legii nr. 95/2006 privind reforma în domeniul sănătății, cu modificările şi completările ulterioare. Implementarea noului sistem, pe lângă dezvoltările instituționale necesare, implică însă prezența unui corp nou de specialişti, administratori, manageri ai sistemului de sănătate.

Piața serviciilor de sănătate necesită îmbunătățiri şi modificări permanente, determinate de incertitudinea consumului, fluctuațiile cererii şi ofertei, limitele existente atât sub aspectul resurselor, cât şi în apariția mai multor furnizori datorită costurilor ridicate ale echipamentelor medico-sanitare.

În opinia specialiştilor în domeniu[1], restructurarea sectorului sanitar – prin regândirea funcționării acestuia pe principiile eficienței în condițiile accesului tuturor persoanelor la servicii medicale de calitate şi prin extinderea componentei private în domeniul sanitar – presupune nu numai măsuri de ordin organizatoric, ci şi pregătirea personalului pentru a asigura gestiunea resurselor existente în funcție de cerințele eficienței economice şi cele impuse de specificul sectorului sanitar.

În concordanță cu aceste aprecieri, analiza unor indicatori specifici ai serviciilor de sănătate cum sunt: suma alocată de la Bugetul asigurărilor sociale pentru sănătate sau proporția în PIB, numărul unităților sanitare, distribuția principalelor unități sanitare pe categorii de proprietate, evoluția şi structura personalului medico- sanitar pe profesiuni şi nivele de pregătire, baza materială permite direct şi indirect caracterizarea stării de sănătate.

Organizarea asistenței medicale în România este marcată profund de reforma serviciilor de sănătate care a continuat şi în anii 2008-2009 producând schimbări în infrastructura sistemului sanitar şi în structura personalului implicat în asigurarea serviciilor de sănătate, atât în sectorul public, cât şi în cel privat.

Rețeaua unităților sanitare a înregistrat în anul 2008 modificări importante. Astfel, a crescut cu 11 numărul spitalelor, cu 6 numărul policlinicilor, cu 70 al ambulatoriilor integrate spitalului, cu 5 numărul dispensarelor medicale, cu 668 numărul cabinetelor medicale de specialitate, cu 493 cel al cabinetelor stomatologice, cu 231 numărul cabinetelor medicale de familie şi cu 309 numărul farmaciilor şi cel al punctelor farmaceutice. De asemenea, s-au înființat 40 de noi societăți medicale civile, 68 de societăți civile medicale de specialitate şi 82 de societăți stomatologice civile medicale.

Datele statistice prezentate[1] în continuare s-au obținut pe baza informațiilor colectate, printr-o cercetare statistică exhaustivă, de la Autoritățile Județene de Sănătate Publică şi de la unitățile ministerelor care au rețea sanitară proprie. Cercetarea statistică se realizează cu periodicitate anuală[1] şi asigură colectarea informațiilor referitoare la rețeaua sanitară publică şi privată, existentă în anul precedent celui în care se face colectarea.

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[1] Florian Popa şi colectiv, *Marketingul serviciilor de îngrijire a sănătății*, Editura Universitară "Carol Davila", Bucureşti, 2007, pg.64-65.

[1] Institutul Național de Statistică, *Activitatea unităților sanitare în anul 2007*, Bucureşti, 2008, pag.3.

Comparativ cu anii precedenți, în anul 2008 distribuția principalelor unități sanitare se prezenta astfel:

Tabelul nr. 1. Numărul unităților sanitare pe principalele categorii de unități, anii 2006-2008

Unități sanitare				**Anul 2008 față de 2006**
	2006	**2007**	**2008**	**2007** **diferențe (+/-)**
Spitale	436		458	+11
Policlinici	260	263	269	+6
Dispensare medicale	211	208	213	+5
Sanatorii TBC	7	5	5	-
Preventorii	5	4	4	-
Sanatorii balneare	10	7	6	-1
Unități medico-sociale	60	66	66	-
Ambulatorii de specialitate	100	98	69	-29
Ambulatorii de spital	293	305	375	+70
Cabinete medicale de familie	10 969	11048	11 279	+231
Cabinete de medicină generală	1007	1036	1 033	-3
Cabinete stomatologice	9 948	10532	11 025	+493
Cabinete medicale de specialitate	6 878	8370	9 038	+668
Societăți medicale civile	54	66	106	+40
Societăți stomatologice civile medicale	95	74	156	+82
Societăți civile medicale de specialitate	102	156	224	+68
Farmacii şi puncte farmaceutice	6 209	6906	7 215	+309

Sursa: Institutul Național de Statistică, *Activitatea unităților sanitare în anul 2007*, Bucureşti, 2008, pag.3-39 .
Institutul Național de Statistică, *Activitatea unităților sanitare în anul 2008,* Bucureşti, 2009, pag.3-37

Unitățile înființate în cursul anului de referință au fost incluse în cercetare, numai dacă au acordat asistență medicală populației până la sfârşitul anului. Pentru stabilirea gradului de utilizare a paturilor din spitale, a numărului de internați şi a numărului zilelor de spitalizare s-au luat în considerare numai bolnavii internați, fără însoțitorii acestora. Personalul sanitar a fost înregistrat o singură dată, la unitatea sanitară unde desfăşoară norma de bază. Pentru calculul indicatorilor privind activitatea sanitară s-a utilizat populația la 1 iulie 2008.

În *sectorul public,* modificările intervenite în structura rețelei sanitare s-au datorat măsurilor luate în cadrul programului de restructurare, prin transformări ale tipologiei unităților sanitare, prin desființarea unora devenite ineficiente şi prin înființarea altora noi. Ca urmare a acestor modificări au apărut 3 spitale noi: unul în Municipiul Bucureşti, unul în județul Bihor, format prin transformarea centrului de sănătate Ştei în spital şi cel de-al treilea în județul Mureş, prin reorganizarea Spitalului Clinic Județean de Urgență Târgu Mureş în două spitale cu personalitate juridică, cu un număr mai mic de paturi (un spital județean de urgență şi un spital; clinic). Ordinul Ministrului Sănătății nr. 39/2008 privind reorganizarea ambulatoriului de specialitate al spitalului a dus la transformarea a 28 de ambulatorii de specialitate în ambulatorii integrate spitalelor şi la înființarea altor 40 ambulatorii integrate spitalelor. În anul 2008 au funcționat 274 creşe publice (236 cu regim zilnic şi 38 cu regim săptămânal) cu 13 569 paturi şi 16 664 copii înscrişi; în medie, un copil a fost înscris în creşă pe o perioadă de 139 zile din an (cu 7 zile mai mult faţă de anul anterior).

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[1] Institutul Național de Statistică, *Anuarul statistic al României*, Bucureşti, 2008, pag.366-372.

Reţeaua sanitară din *sectorul privat* a continuat să se dezvolte. Astfel, numărul spitalelor a crescut cu 8 unităţi, ajungând la 30. Spitale nou înfiinţate sunt în judeţele: Braşov (trei unităţi), Cluj (două unităţi), Constanţa (două unităţi) şi Buzău (o unitate). De asemenea, a crescut cu 189 numărul cabinetelor medicale de familie, cu 514 numărul cabinetelor stomatologice şi cu 520 cel al cabinetelor medicale de specialitate. În anul 2008 au funcţionat 246 policlinici (cu 5 mai multe decât în anul anterior), 812 laboratoare medicale (cu 63 mai mult decât în 2007) şi 1759 laboratoare de tehnică dentară (cu 96 mai multe decât în anul precedent). Faţă de anul 2007, numărul farmaciilor şi cel al punctelor farmaceutice a crescut cu 299 unităţi, iar numărul depozitelor farmaceutice a scăzut cu 11 unităţi.

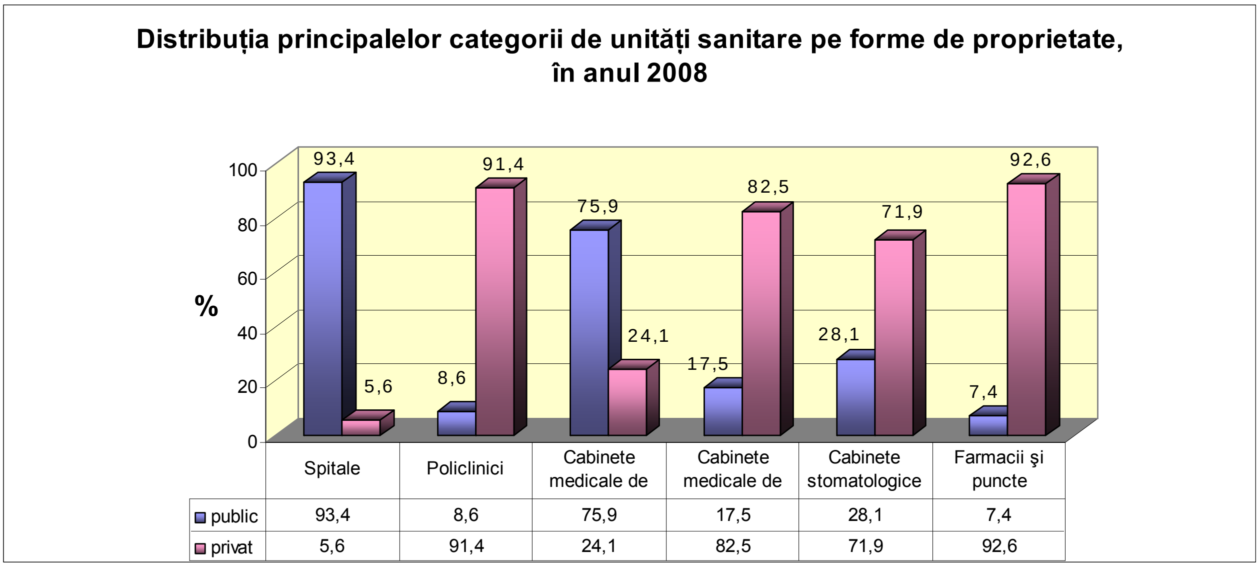

Figura nr.1

Sursa: Institutul Naţional de Statistică, *Activitatea unităţilor sanitare în anul 2008*, Bucureşti, 2009, pag.3-14 şi documentarea autorului la Ministerul Sănătăţii Publice - Centrul Naţional pentru Organizarea şi Asigurarea Sistemului Informaţional şi Informatic în Domeniul Sănătăţii

Comparativ cu reţeaua unităţilor din sectorul public, care include 93,4% din totalul spitalelor şi 75,9% din totalul cabinetelor medicale de familie, sectorul privat deţine majoritatea policlinicilor (91,4%), cabinetelor medicale de specialitate (82,5%), cabinetelor stomatologice (71,9%), precum şi a farmaciilor şi punctelor farmaceutice (92,6%).

Distribuţia *reţelei unităţilor sanitare pe medii de rezidenţă* evidenţiază faptul că în *mediul urban* se află majoritatea unităţilor din reţeaua sanitară: 88,6% din numărul total al spitalelor, 83,3% din totalul sanatoriilor balneare, 89,2% din cel al dispensarelor medicale, 90,6% din totalul cabinetelor de medicină generală, 59,3% din cabinetele medicale de familie, 78,6% din cel al farmaciilor, 93,3% din centrele de diagnostic şi tratament, 86,7% din cabinetele stomatologice, 98,3% din cabinetele medicale de specialitate, 94,6% din laboratoarele medicale, 95,8% din laboratoarele de tehnică dentară, toate centrele medicale de specialitate şi policlinicile.

În *mediul rural* funcţionează 60,6% din numărul unităţilor medico-sociale, 75,0% din preventorii, 51,1% din totalul centrelor de sănătate şi două treimi din punctele farmaceutice. Schimbările intervenite în structura şi numărul unităţilor sanitare au condus la modificări ale *numărului de paturi* din diversele categorii de unităţi. Astfel, înfiinţarea unor spitale noi a condus la creşterea cu 180 a numărului paturilor de spital. Scăderi ale numărului de paturi s-au înregistrat în: centrele de sănătate (cu 21 paturi de spital), prin transformarea unui centru de sănătate în spital (judeţul Bihor), în staţionarele de dispensar medical (cu 42 paturi), în sanatoriile de nevroze sau de neuropsihiatrie (cu 20 paturi) şi în unităţile medico-sociale (cu 97 paturi).

Ca urmare a apariţiei creşelor private şi a măririi capacităţii creşelor publice a crescut cu 594 numărul paturilor din aceste unităţi.În *sectorul public* cele mai importante modificări ale numărului de paturi s-au înregistrat la sanatoriile balneare, unde a crescut cu 290 numărul total de paturi, prin înfiinţarea sanatoriului balnear de la Bălţăteşti (jud Neamţ) cu 500 paturi, prin desfiinţarea unui sanatoriu balnear cu 185 paturi în judeţul Maramureş şi prin reducerea cu 25 a numărului de paturi din sanatoriul balnear din judeţul Constanţa.

Înfiinţarea a 8 spitale noi în *sectorul privat* a condus la o creştere cu 163 a numărului de paturi de spital. Numărul paturilor de spital destinate pacienţilor internaţi a fost completat de un număr de 4 421 paturi de spital, destinate cazurilor de zi. Dintre acestea, numai 46 paturi pentru cazurile de zi au fost înregistrate în spitalele din sectorul privat.

Tabelul nr. 2. Numărul paturilor din reţeaua sanitară

Paturi în unităţi sanitare	**2006**	**2007**	**2008**	**Anul 2008 faţă de 2007**
				diferenţe (+/-)
Paturi de spital (inclusiv paturile de spital, din centrele de sănătate)	142 034	138 025	138 184	+159
Sanatorii TBC *	1 437	909	918	+9
Sanatorii balneare	1 563	1 275	1 565	+290
Sanatorii de nevroze	-	260	240	-20
Preventorii	495	420	420	-
Unităţi medico-sociale	2 365	2 823	2 726	-97
Creşe	13 220	13 135	13 729	+594

* numai din unităţile din sectorul public

Sursa: Institutul Naţional de Statistică, *Activitatea unităţilor sanitare în anul 2008*, Bucureşti, 2009, pag.14-25 şi documentarea autorului la Ministerul Sănătăţii Publice - Centrul Naţional pentru Organizarea şi Asigurarea Sistemului Informaţional şi Informatic în Domeniul Sănătăţii.

Distribuţia paturilor pe medii de rezidenţă se prezintă astfel: mediul urban dispune de 92,5% din totalul paturilor de spital (inclusiv paturile de spital din centrele de sănătate), 68,1% din totalul paturilor din sanatoriile balneare, de 66,7% din paturile din sanatoriile de neuropsihiatrie sau de nevroze şi de 61,3% din totalul paturilor din sanatoriile TBC. În mediul rural, reţeaua unităţilor sanitare dispune de 85,7% din paturile preventoriilor şi de 57,0% din paturile unităţilor medico-sociale.

Ca observaţie generală numărul de paturi de spital este într-o sensibilă creştere, fenomen care ar trebui să se manifeste invers, în sensul descreşterii proporţionale cu tendinţele de evoluţie a populaţiei active. Reducerea numărului zilelor de spitalizare ca urmare a dezvoltării unor tehnologii moderne, respectiv a tendinţelor europene de scădere a numărului de paturi, ar trebui să fie o preocupare a managementului oricărui spital.

Particularitatea sistemului sanitar românesc prin care numărul de paturi asigură şi crearea/menţinerea unor structuri şi implicit a consecinţelor ce generează drepturi aferente (drepturi salariale sau titluri ştiinţifice).

În anul 2008, repartizarea paturilor de spital, pe specialităţi medicale, se prezenta astfel:

*Tabelul nr. 3.*Numărul paturilor din spitale, pe specialități medicale, în anul 2008

Paturi de spital pe specialități Medicale	**Total**		**Public**		**Privat**	
	număr	**%**	**număr**	**%**	**număr**	**%**
Total, din care:	**138 184**	**100,0**	**137 061**	**100,0**	**1123**	**100,0**
Interne	16 389	11,9	16 216	11,8	173	15,4
Psihiatrie	16 572	12,0	16 554	12,1	18	1,6
Chirurgie	15 074	10,9	14 875	10,8	199	17,7
Obstetrică-ginecologie	10 149	7,3	9 982	7,3	167	14,9
Pediatrie	9 330	6,8	9 270	6,8	60	5,3
Pneumoftiziologie	9 572	6,9	9 572	7,0	-	-
Boli infecțioase	6 190	4,5	6 190	4,5	-	-
Cardiologie	6 456	4,7	6 433	4,7	23	2,1
Neurologie	5 971	4,3	5 971	4,4	-	-
Recuperare medicală fizică şi balneologie	5 663	4,1	5 496	4,0	167	14,9
Neonatologie	4 872	3,5	4 792	3,5	80	7,1
ATI (anestezie, terapie intensivă)	4 768	3,4	4 706	3,4	62	5,5
Alte specialități	27 178	19,7	27 004	19,7	174	15,5

Sursa: Ministerul Sănătății Publice - Centrul Național pentru Organizarea şi Asigurarea Sistemului Informațional şi Informatic în Domeniul Sănătății, Unități-Paturi 2008, Bucureşti, 2009, pag.3-8

După specialitatea pentru care au fost alocate, din numărul total de 138 184 paturi de spital, 12,0% au fost folosite la specialitatea psihiatrie, 11,9% pentru interne, 10,9% pentru chirurgie, 7,3% pentru obstetrică-ginecologie, 6,9% pentru pneumologie, 6,8% pentru pediatrie, 4,7% pentru cardiologie, 4,5% pentru boli infecțioase, 4,3% pentru neurologie, 4,1% pentru recuperare, medicină fizică şi balneologie, 3,5% pentru neonatologie, 3,4% pentru ATI, iar 19,7% sunt paturi destinate altor specialități: ortopedie-traumatologie, ORL oftalmologie, boli cronice etc.

Distribuția paturilor de spital pe specialități este diferită în sectorul public şi în cel privat. Astfel, în *sectorul public,* cel mai mare număr de paturi de spital a fost alocat următoarelor specialități: psihiatrie (12,1%), interne (11,8%) şi chirurgie (10,8%).

În *sectorul privat* numărul cel mai mare de paturi de spital a fost alocat pentru chirurgie (17,7%), interne (15,4%), obstetrică-ginecologie (14,9%) şi recuperare, medicină fizică şi balneologie (14,9%).

În *mediul rural,* din numărul total de 10 413 paturi de spital (sector public şi privat), 49,1% (5116) au fost paturi pentru secțiile de psihiatrie, 21,8% (2272) pentru cele de pneumologie, iar 29,1% (3025) au fost paturi destinate celorlalte specialități. Din totalul paturilor pentru cazuri de zi 98,6% au funcționat în spitale din *mediul urban.*

În anul 2008, la 1 000 locuitori au revenit 6,4 paturi de spital (inclusiv cele din centrele de sănătate), la fel ca în anul precedent. Gradul de utilizare a paturilor de spital (şi centre de sănătate) a fost de 294 zile/an, a paturilor din sanatoriile TBC de 284 zile/an, a paturilor din sanatoriile balneare de 200 zile/an, iar a celor din sanatoriile de neuropsihiatrie sau nevroze de 280 zile/an. În preventorii, gradul de utilizare a paturilor a fost de 231 zile/an, iar a celor din unitățile medico-sociale de 296 zile/an.

Numărul pacienților internați în spitale a fost de 5 374 026 persoane, iar durata medie de spitalizare a fost de 7,6 zile. Au beneficiat de îngrijire medicală, fiind spitalizați cu internare de zi, un număr de 1 841 281 pacienți.

Rețeaua sanitară publică a acordat asistență medicală persoanelor internate în: spitale – 5 333 438 pacienți (cu 154 393 mai mulți decât în 2007), cu o durată medie de spitalizare de 7,6 zile, la fel ca în anul precedent; sanatorii TBC – 7 155 pacienți (cu 547 mai mulți decât în anul 2007), cu o durată medie de internare de 36,5 zile față de 39,4 zile în anul precedent. De asistență medicală acordată în sistemul sanitar public au beneficiat și persoanele internate în preventorii -1 165 persoane (cu 171 mai puține față de anul precedent), durata medie de internare fiind de 83,4 zile față de 74,0 zile în 2007 și în unitățile medico-sociale – 10 645 pacienți (cu 378 mai mulți decât anul precedent) și o durată medie de spitalizare de 75,7 zile față de 86,0 zile în 2007. În sanatoriile de neuropsihiatrie sau de nevroze au fost îngrijiți un număr de 2 251 pacienți, durata medie de spitalizare fiind de 29,8 zile, iar în sanatoriile balneare un număr de 18 383 persoane, cu o durată medie de internare de 17 zile.

În cele 30 spitale din *sectorul privat* au fost internați, în cursul anului 2008, un număr de 40 588 pacienți (cu 8 531 mai mulți decât anul precedent) cu o durată medie de spitalizare de 5,9 zile față de 6,2 zile în anul precedent.

În anul 2008, sistemul sanitar a fost deservit de 280 863 *cadre sanitare.* Dintre acestea 29,2% (82 060 persoane) au fost cadre sanitare cu pregătire superioară (medici, stomatologi, farmaciști, fiziokinetoterapeuți, asistente medicale și alt personal sanitar: biologi, chimiști etc.), 47,2% (132 464 persoane) au fost cadre medicale cu pregătire sanitară medie și 23,6% (66 339 persoane) a fost personal sanitar auxiliar.

Ponderea personalului sanitar de sex feminin în totalul medicilor a fost de 69,4%, în cel al stomatologilor de 63,5%, iar în al farmaciștilor de 91,5%. Un număr de 593 fiziokinetoterapeuți și 2 791 asistente medicale cu studii superioare au asigurat îngrijirea medicală în unitățile din sistemul sanitar public și privat. Dintre aceștia, 68,1% din fiziokinetoterapeuți și 91,7% din asistentele medicale cu studii superioare sunt de sex feminin. Din totalul personalului sanitar mediu 91,5% este de sex feminin, iar din al personalului sanitar auxiliar de 84,6%.

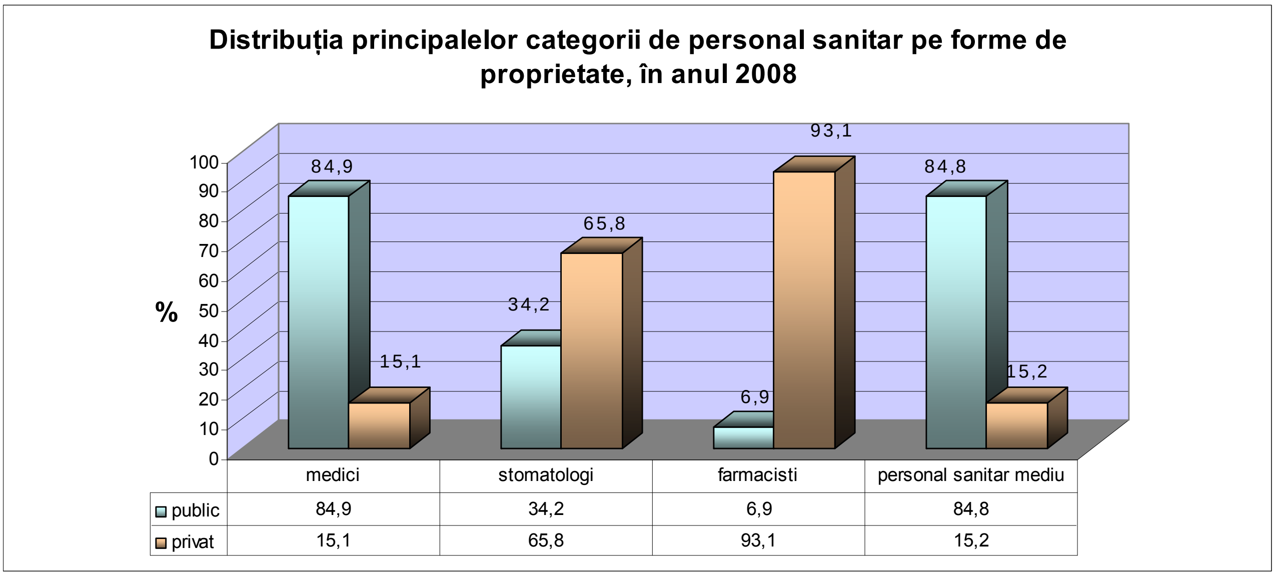

Distribuția principalelor categorii de personal sanitar pe forme de proprietate, în anul 2008

	medici	stomatologi	farmacisti	personal sanitar mediu
■ public	84,9	34,2	6,9	84,8
■ privat	15,1	65,8	93,1	15,2

Figura nr.2

Sursa: Ministerul Sănătății Publice - Centrul Național pentru Organizarea și Asigurarea Sistemului Informațional și Informatic în Domeniul Sănătății, Unități-paturi 2007, București, 2008, pag.3-8

Distribuția personalului sanitar după forma de proprietate a unității sanitare arată că în sectorul public își desfăşoară activitatea 86,6% din totalul medicilor, respectiv 77,8% din totalul medicilor de familie.

De asemenea, în sectorul public lucrează şi majoritatea asistentelor medicale cu studii superioare - 88,9%, fiziokinetoterapeuților - 88,9% şi majoritatea personalului sanitar mediu şi auxiliar: 86,9% din personalul sanitar mediu şi 97,6% din personalul sanitar auxiliar.

În sectorul privat își desfăşoară activitatea 65,1% din totalul medicilor stomatologi şi 92,4% din totalul farmaciştilor.

Distribuția personalului sanitar pe medii de rezidență este determinată de repartizarea teritorială a unităților sanitare.

Tabelul nr. 4. Distribuția principalelor categorii de personal sanitar pe medii de rezidență şi după forma de proprietate a unității, în anul 2008

Personalul sanitar	Total	Public	Privat
Medici	50 267	42 699	7 568
- urban	44 697	38 037	6 660
- rural	5 570	4 662	908
din care: medici de familie	11 567	8 825	2 742
- urban	6 993	4 822	2 171
- rural	4 574	4 003	571
Stomatologi	11 901	4 067	7 834
- urban	10 427	3152	7 275
- rural	1 474	915	559
Farmaciști	11 704	808	
- urban	10 099	780	9 319
- rural	1 605	28	1 577
Personal sanitar mediu	132 464	112 321	20 143
- urban	118 948	101 623	17 325
- rural	13 516	10 698	2 818
Personal sanitar auxiliar	66 339	64 405	1 934
- urban	58 109	56 459	1 650
- rural	8 230	7 946	284

Sursa: Institutul Național de Statistică, *Activitatea unităților sanitare în anul 2008,* Bucureşti, 2009, pag.3-10 şi documentarea autorului la Ministerul Sănătății Publice - Centrul Național pentru Organizarea şi Asigurarea Sistemului Informațional şi Informatic în Domeniul Sănătății

Unitățile sanitare din *mediul urban* dispun de 88,9% din totalul medicilor, 87,6% din totalul stomatologilor, 86,3% din farmacişti, 89,8% din personalul sanitar mediu şi 87,6% din personalul sanitar auxiliar.

Din totalul medicilor 23,0% sunt medici de familie, dintre aceştia 60,5% îşi desfăşoară activitatea în mediul urban şi 39,5% în mediul rural.

În ultimii cinci ani numărul populației ce revine la un cadru medico-sanitar (sector public şi privat) s-a redus semnificativ. Astfel, în anul 2004 reveneau 450 locuitori la un medic; 1 882 locuitori la un medic de familie, 2 188 locuitori la un stomatolog; 2 473 locuitori la un farmacist; 178 locuitori la un cadru sanitar mediu.

În anul 2008, numărul populației ce revine la un cadru medico-sanitar (sector public şi privat) se prezintă astfel: 428 locuitori la un medic; 1 859 locuitori la un medic de familie; 1 807 locuitori la un stomatolog; 1 837 locuitori la un farmacist; 162 locuitori la un cadru sanitar mediu.

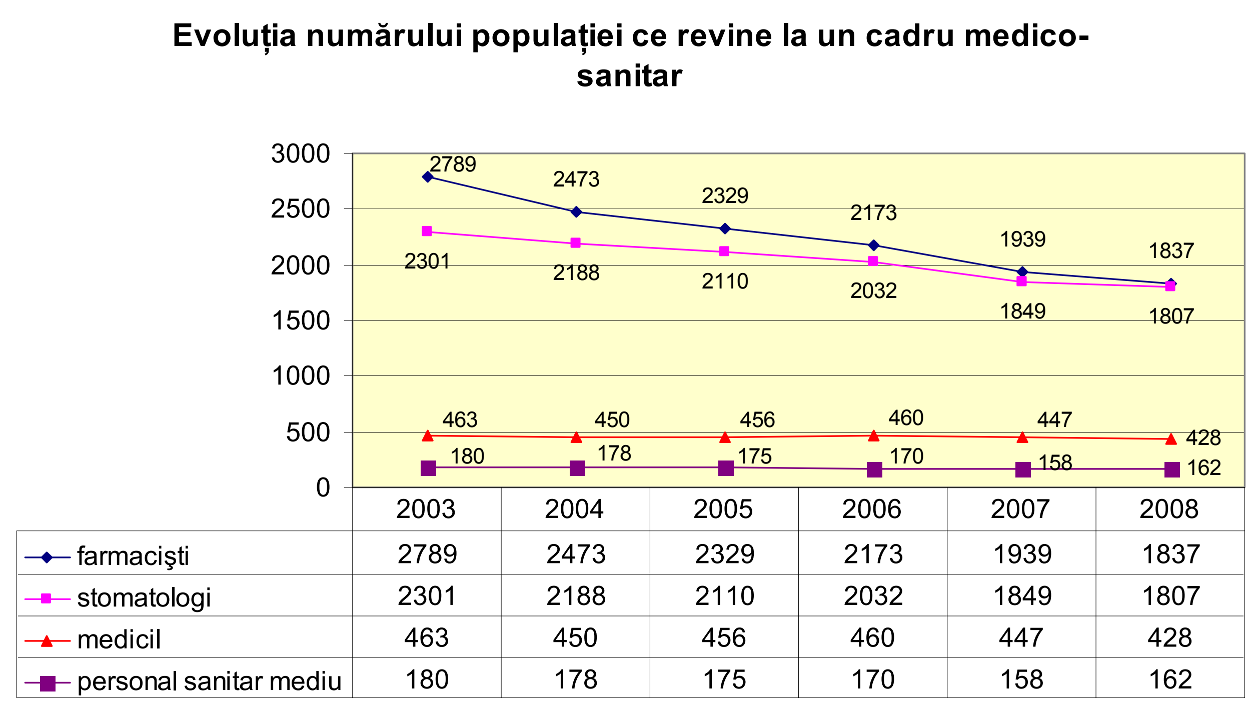

	2003	2004	2005	2006	2007	2008
◆ farmacişti	2789	2473	2329	2173	1939	1837
■ stomatologi	2301	2188	2110	2032	1849	1807
▲ medicil	463	450	456	460	447	428
■ personal sanitar mediu	180	178	175	170	158	162

Figura nr.3

Sursa: Institutul Național de Statistică, *Activitatea unităților sanitare în anul 2007*, Bucureşti, 2008, pag.3-18 şi documentarea autorului la Ministerul Sănătății Publice - Centrul Național pentru Organizarea şi Asigurarea Sistemului Informațional şi Informatic în Domeniul Sănătății.

În medie la 1 000 de locuitori revin: 5 farmacişti, 6 stomatologi, 23 medici şi 62 persoane cu pregătire sanitară medie.

La sfârşitul anului 2008, rețeaua instituțiilor de ocrotire medico-socială pentru persoanele adulte cu handicap înregistra un număr de 331 unități specializate care ofereau îngrijire pe timpul zilei sau cu spitalizare. Aceste unități au funcționat în principal în sectorul public, în sectorul privat înregistrându-se numai două unități. În aceste unități pacienții au dispus de un număr de 18 386 paturi. Pe parcursul anului 2008 au beneficiat de asistență medicală specială cu internare un număr de

18 030 persoane, fiecare dintre aceste persoane beneficiind în medie de asistență de specialitate 339 zile/an.

Personalul sanitar din unitățile de asistență medico-socială pentru persoane adulte cu handicap a fost format din: 242 medici, 8 stomatologi, 4 farmacişti; numărul personalului sanitar mediu a fost de 2 802, iar cel al personalului sanitar auxiliar de 5 874 persoane.

INSTRUCȚIUNI PENTRU AUTORI

Revista de Medicină Militară este o publicație de prestigiu, cu o vechime de peste 100 de ani. În scopul integrării revistei în circuitul internațional al publicațiilor științifice, autorii sunt rugați să respecte *Cerințele uniforme pentru manuscrisele trimise jurnalelor biomedicale* (stilul Vancouver de prezentare) (vezi: *New England Journal of Medicine* 1991, 324 : 424 – 428; *British Medical Journal*, 1991, 302; *Romanian Journal of Gastroenterology* 1992, 1/4, 1996, 5/1).

Revista de Medicină Militară este creditată de Colegiul Medicilor din România ca publicație științifică (2,5 credite pentru abonament), creditarea autorilor făcându-se la solicitarea acestora după prezentarea reprintului obținut ca dovadă a editării lucrării (solicitările vor fi adresate personal de autori Colegiului Medicilor din România – Bulevardul Timişoara nr. 15, sector 6, Bucureşti)

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Autorii poartă întreaga responsabilitate pentru conținutul ştiințific al lucrărilor lor.

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Articolele se redactează în Microsoft Word, Normal+Justf., Times New Roman, Font Size – 16 Bold pentru titlu, Font Size – 12 pentru text, iar rezumatul Font Size – 10 Bold.

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- **REZULTATE**: fără interpretări.

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1 – analiza rezultatelor prin prisma obiectivelor propuse ale studiului cu comentarii cu privire la aspectele lămurite (pozitive sau negative) sau nelămurite prin acest studiu;

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3 – comentarii privind importanţa studiului de faţă, precum şi o punere la punct a situaţiei actuale a subiectului investigat atât în literatură, cât şi în practică; eventual se vor prezenta aici cele mai importante studii sau aspecte din domeniu;

4 – probleme nerezolvate, întrebări la care nu s-a răspuns şi care rămân deschise în viitor pentru studiile ce vor urma, eventuale direcţii propuse pentru investigare în domeniul respectiv.

CONCLUZII

La nevoie, capitolele mari pot fi împărţite în subcapitole, după necesităţi.

Se recomandă utilizarea precaută a *abrevierilor* şi menţionarea numelui complet la prima apariţie în text a abrevierii respective. Toate referinţele bibliografice vor fi citate în text cu cifre arabe, la exponent, fără paranteze, în ordinea apariţiei (de exemplu: Un alt studiu[18] arată...). Toate figurile şi tabelele vor fi citate în text în locul în care ele urmează să apară (de

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Tabelele trebuie să fie procesate la două rânduri, pe o pagină separată, *numerotate* consecutiv (cu referire în text în locul unde ele trebuie să apară) şi *cu un titlu* (exemplu: *Tabelul 1*. Distribuţia pe vârste şi sex a pacienţilor). Capetele de tabel se scriu cu caractere *italice.* Nu trimiteţi fotografii sau imagini ale tabelelor. Utilizaţi tasta „Tab" pentru a separa coloanele (nu bara de spaţiu) şi utilizaţi tasta „Enter" la terminarea liniei. Toate căsuţele goale din tabel trebuie să aibă o liniuţă. Dacă este nevoie, pentru cuvintele sau frazele lungi din căsuţe se vor utiliza abrevieri, însă cu explicarea acestora în subsolul tabelului. Asiguraţi-vă că toate tabelele sunt citate în text.

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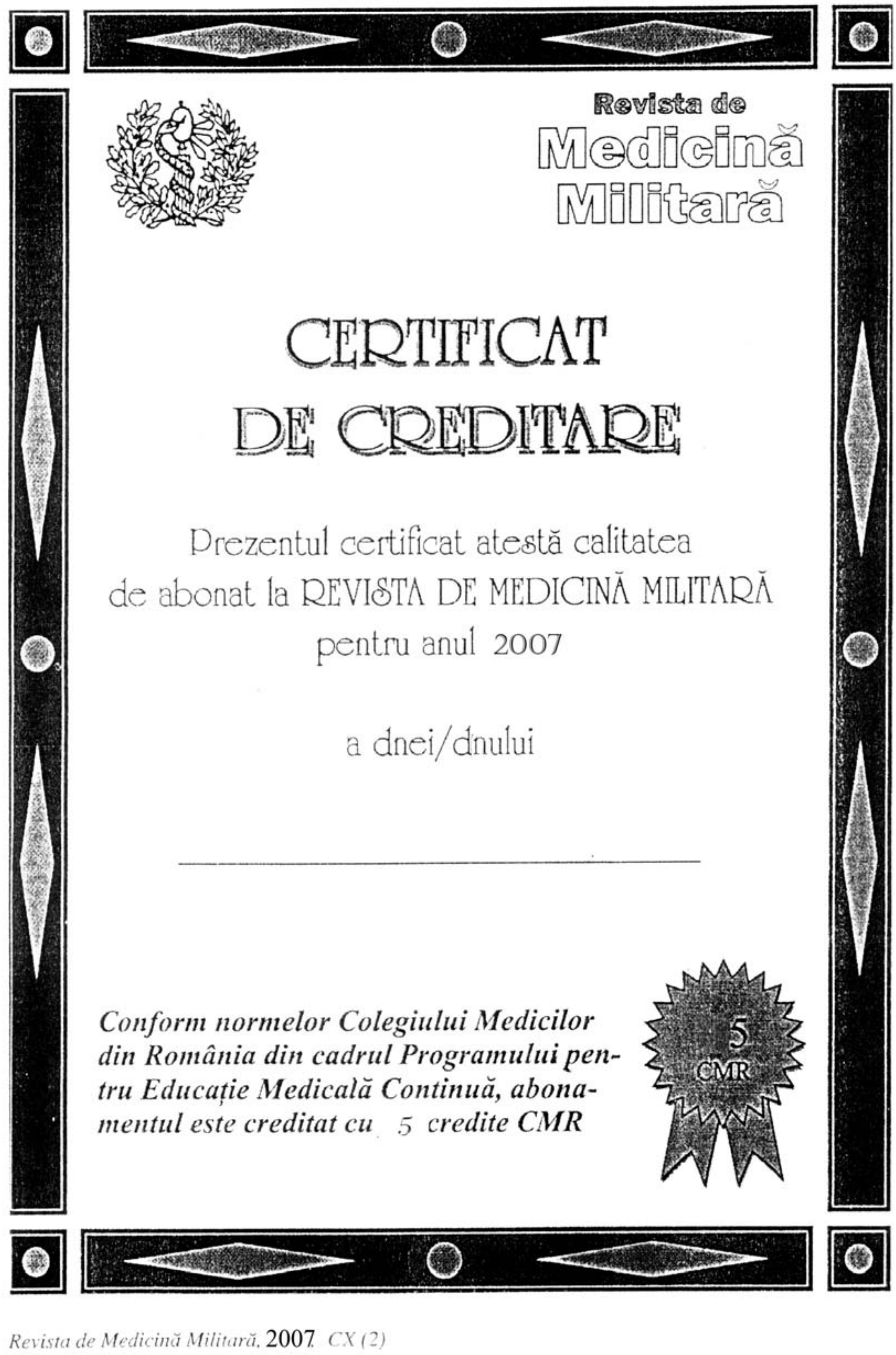

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(REVISTA SANITARĂ MILITARĂ, FONDATĂ ÎN ANUL 1897)

Anul CXIII
Nr. 2
Aprilie - Iunie 2010

EDITATĂ DE DIRECȚIA MEDICALĂ A
MINISTERULUI APĂRĂRII NAȚIONALE
ŞI ASOCIAȚIA MEDICILOR ŞI FARMACIŞTILOR MILITARI
DIN ROMÂNIA

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Valoarea imagisticii de fuziune (PET-CT) în diagnosticul tumorilor vertebro-medulare

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Rezumat. Imagistica de fuziune (PET/CT), utilizată pe scară largă în ţările dezvoltate, permite identificarea diferitelor leziuni la nivel molecular, făcând posibilă apariţia unui nou domeniu al medicinei de vârf – "imagistica moleculară". Marcarea cu un radiotrasor a unui component din lanţul acizilor nucleici, de exemplu, permite identificarea cancerului la nivel molecular şi tratamentul acestuia prin tehnicile radioimunoscintigrafice. Având o mare sensibilitate în detecţia precoce a unor leziuni într-un spectru larg al patologiei, procedurile de Medicină Nucleară sunt mult solicitate de către clinicieni.

Cuvinte cheie: imagistică de fuziune (PET/CT), tehnici radioimunoscintigrafice

Abstract. Fusion imaging (PET/CT), widely used in the developed countries, allows the identification of different lesions at the molecular level, enabling the emergence of a new field of advanced medicine - "molecular imaging". For example, marking with a radiotrasor of a nucleic acid chain component allows the identification of the cancer at the molecular level and its treatment by radioimuno scintigraphic techniques. Having a great sensitivity in the early detection of some lesions in a wide spectrum of pathology, Nuclear Medicine procedures are much sought by the clinicians.

Introducere

Cancerul debutează la nivel molecular, subcelular, prin modificarea unei gene care controlează creşterea şi comportamentul celular, în particular existând o creştere marcată a sintezei de ADN şi un consum local exagerat de glucoză ca suport energetic al multiplicării celulare anarhice. Aceste modificări care se "propagă" din aproape în aproape, astfel încât după un anumit interval de timp (ani) - în care boala evoluează de obicei asimptomatic - ajung să fie implicate ţesuturi sau organe. Atunci când numărul de celule canceroase este suficient de mare pentru a fi detectate vizual, prin tehnicile convenţionale (Rx, ecografia, CT, imagistica prin RM), boala a depăşit deja stadiul în care poate fi tratată cu succes.

Dezideratul activităţii medicale este de a depista cancerul înaintea apariţiei simptomelor clinice şi a diseminării în alte organe, în vederea instituirii unui tratament precoce, eficient.

Epidemiologie

Cancerul este una dintre principalele cauze de mortalitate în lume. Studii statistice recente, efectuate sub auspiciile Organizaţiei Mondiale a Sănătăţii, arată că una din 9 femei va dezvolta o formă de cancer de sân, că 1 din 7 bărbaţi va face o formă de cancer de prostată, iar 1 din 10 bărbaţi un cancer pulmonar. Tratamentul eficient depinde în principal de detecţia precoce a procesului tumoral (8).

Prognostic

În momentul diagnosticării prin mijloacele actuale existente la noi în țară, inclusiv prin explorările imagistice de înaltă performanță, cancerul nu poate fi vindecat. Tratamentul instituit, chirurgical, chimioterapic sau prin iradiere, poate asigura în raport cu stadiul evolutiv o supraviețuire ce nu depăşeşte 4-5 ani (2).

Imagistica medicală este un domeniu de vârf al medicinii moderne care cuprinde un grup de tehnici medicale cu principii diferite, dar care au în comun faptul că oferă date diagnostice prin intermediul imaginii unor sisteme anatomice, organe interne sau leziuni. Aceste tehnici imagistice pot fi grupate în:

- tehnici anatomice (structurale): ecografia, tomografia computerizată (CT), imagistica prin Rezonanță Magnetică (IRM), angiografia, radiografia digitală;
- tehnici funcționale (metabolice): scintigrafia planară, tomoscintigrafia (SPECT), imunoscintigrafia cu anticorpi monoclonali, tomografia cu emisie de pozitroni (PET).

Imagistica de fuziune anatomo-metabolică: sisteme integrate PET-CT, SPECT-CT, PET-RM sunt tehnici de înaltă performanță care permit printr-o singură examinare atât vizualizarea procesului metabolic patologic, cât şi localizarea cu precizie a acestuia, prin co-înregistrarea celor două categorii de informații (13, 14).

Datorită exapansiunii din ce în ce mai largi, chiar a introducerii în protocoalele de diagnostic sau de decontare pe scară largă în lume, ne vom referi în cele ce urmează la tehnica PET-CT.

Tehnica PET-CT este tehnica imagistică ce realizează dezideratul detecției precoce a cancerului prin vizualizarea consumului exagerat de glucoză a celulelor canceroase (PET) şi localizarea anatomică precisă a acestui proces prin scanarea simultană PET şi CT. Începând cu anul 1991, au fost instalate peste 2 – 3 000 de echipamente în întreaga lume. OMS estimează un necesar de aproximativ 1 echipament PET la 1 milion de locuitori. Tehnica PET se bazează pe marcarea unui constituent normal al organismului (de exemplu, deoxiglucoza, analog al glucozei) cu un radiotrasor cu emisie pozitronică (^{18}F, ^{14}C, ^{16}O, ^{13}N, dar în mod uzual ^{18}F), care, după injectarea i.v., se distribuie în tot corpul uman, în funcție de proprietățile de distribuție ale constituentului radiomarcat. Odată injectat în organism, pozitronii emişi prin dezintegrarea radionuclidului migrează la distanță mică în țesuturi, pierd o parte din energie, îşi încetinesc mişcarea şi se anihilează în urma coliziunii cu electronii prezenți. Prin procesul de anihilare, vor rezulta doi fotoni gamma emişi în direcții opuse (180^0), fiecare cu energie de 511 keV. Ambele radiații pot fi ulterior detectate prin dispunerea unui inel de detectori în jurul pacientului, astfel încât devine posibilă cartografierea distribuției izotopului emițător de pozitroni în organism. În acest fel se poate studia distribuția unui număr extrem de mare de substanțe al căror metabolism este afectat în diverse situații patologice (2, 12, 13).

De departe cel mai utilizat radionuclid emițător de pozitroni este ^{18}F, cuplat cu deoxiglucoza – analog al glucozei. Celulele canceroase active metabolic vor capta glucoza marcată de circa 30-40 de ori mai mult decât celulele sănătoase adiacente. Sistemul computerizat al instalației de detecție a fotonilor emişi în urma anihilării pozitronului cu un electron permite identificarea spațială a locului de fixare a radiotrasorului pozitronic şi vizualizarea procesului patologic.

Radiotrasorii emițători de pozitroni şi utilizabili pentru examinarea PET sunt cuprinşi în tabelul de mai jos:

^{18}F CU T1/2 FIZIC	**110 MIN**
^{11}C	**20 MIN**
^{13}N	**10 MIN**
^{15}O	**2 MIN**

Datorită timpului fizic de înjumătățire scurt, radionuclizii cu emisie pozitronică necesită a fi produşi “on site”, într-un ciclotron de uz medical *(baby cyclotron)*. Există şi radionuclizi emițători de pozitroni produşi în generator – de exemplu, ^{68}Ga, care, prin cuplarea cu diferite peptide, permite evaluarea şi stabilirea conduitei terapeutice în cazul tumorilor neuroendocrine, alte aplicații clinice fiind însă în stadiul de cercetare pentru moment (10).

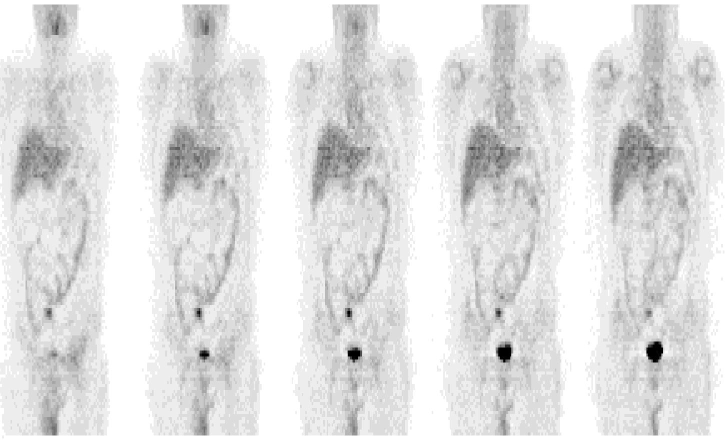

Fig. 1 . FDG-PET, aspect normal.

Metoda, dezvoltată în anii '70 (Phelps şi Hoffman), a fost utilizată timp de două decenii, predominant în cercetarea clinică a diferitelor procese celulare la nivel molecular, prin încorporarea izotopului pozitronic în diferite molecule cu mesaj (enzime, hormoni, anticorpi, peptide şi oligonucleotide). Imagistica apoptozei, aceea a neurotransmițătorilor şi a neoangiogenezei au fost transpuse de la probe experimentale în practica clinică (fig. 1). Progresele deosebite obținute în imagistica moleculară l-au făcut pe W.Wagner jr. (clinica J.Hoppkins - Baltimore) să afirme că "medicina moleculară a revoluționat medicina modernă, medicina nucleară vine să revoluționeze medicina moleculară”. În 1998 Townsend a avut ideea de a combina (suprapune) imaginile metabolice PET cu cele anatomice CT, realizând localizarea anatomică precisă a leziunilor prin ceea ce se numeşte "imagine de fuziune". Primele echipamente integrate PET-CT produse de marile companii au fost introduse în practica clinică în jurul anului 2000, fiind larg acceptate de comunitatea medicală.

Echipamentul PET-CT permite într-o singură examinare obținerea unei imagini de fuziune ce conține atât informații metabolice (PET), cât şi detalii anatomice precise (CT).

Indicațiile principale ale PET-CT sunt:

- detecția şi precizarea substratului malign sau benign al diferitelor tipuri de tumori;
- determinarea extensiei reale a tumorilor maligne, stadializarea preoperatorie şi restadializarea posterapeutică;
- diferențierea leziunii de fibroza postoperatorie sau necroza postiradiere de tumora reziduală sau recidivă;
- stabilirea prognosticului tumorii în raport cu nivelul captării glucozei şi cu gradul histopatologic;
- dirijarea abordurilor terapeutice: intervenția chirurgicală minimă, ablația transcutanată ghidată CT, chemoembolizarea;
- dirijarea biopsiei selective în identificarea componentei viabile a tumorilor parțial necrozate.

Aparatura necesară cuprinde: ciclotron pentru obținerea radioizotopilor cu emisie pozitronică; laborator de radiofarmacie pentru radiomarcarea moleculelor active (fluordeoxiglucoza, ^{11}C-metionina, ^{11}C-timidina, ^{18}F-deoxitimidina, ^{18}F-cholina etc.). Cu siguranță există posibilitatea achiziționării radiotrasorului de la o radiofarmacie, care, însă, având în vedere timpul de înjumătățire deosebit de scurt al radionuclizilor, trebuie să se găsească la o distanță rezonabilă de unitatea PET-CT şi este posibilă doar pentru radiotrasorii marcaţi cu 18F (T1/2 = 110 min, faţă de 11C cu T/2 = 20 min sau 15°, al cărui T/2 = 2 min!) (fig. 2).

Fig. 2. Echipament integrat PET-CT.

Scanarea PET/CT combină detaliile metabolice ale unei scanări PET (activitatea celulară a tumorii, viabilitatea tumorală etc.) cu cele anatomice oferite de o scanare CT (mărimea şi localizarea tumorii, masa, raporturi anatomice etc.), oferind o imagine mult mai precisă decât cea PET, respectiv CT, folosite separat. Studiul PET începe cu injectarea intravenoasă de FDG (18-fluor dezoxiglucoză), care este o substanță similară glucozei ce se ataşează de izotopul F^{18}. Țesuturile active din punct de vedere metabolic sau cele cu rată de multiplicare anormal crescută (tumorile) consumă zahărul radioactiv în cantitate mult crescută. Acumularea radiotrasorului duce la emisia de radiații gamma care sunt transformate de computer în imagini. Aceste imagini indică zonele "fierbinți" din punct de vedere metabolic, semnalând țesuturile cu activitate metabolică crescută (rata de multiplicare anormal crescută), deoarece celulele canceroase consumă mai mult zahăr/energie decât alte țesuturi sau organe. Scanarea de tip CT se bazează pe absorbția în grade diferite a razelor X de către structurile examinate şi pe procesarea computerizată a datelor cu obținerea de secțiuni fine ce pot fi reconstruite tridimensional, reproducând imagini detaliate ale structurilor organismului. Întreaga examinare durează, de obicei, 30 de minute, punând la dispoziție informații detaliate şi exacte ce permit medicilor care tratează pacientul să ofere un diagnostic rapid, corect şi complet. În oncologie, investigația PET/CT oferă posibilitatea de a diagnostica şi localiza din timp, de a monitoriza şi de a ține sub control evoluția cancerului. Cu ajutorul PET-CT, chirurgii, oncologii şi alți medici specialişti vor fi capabili să detecteze cu mai multă precizie cancerul, să evalueze recidivele, să realizeze o stadializare corectă şi completă şi să evalueze răspunsul probabil al organismului pacientului la terapie, inclusiv să urmărească mai eficient rezultatele chimioterapiei şi radioterapiei. În oncologie, PET-CT oferă informații vitale pentru diagnosticare, care permit monitorizarea rezultatelor tratamentului şi ajută adesea la evitarea tratamentului chirurgical. Scanarea PET- CT prezintă o multitudine de beneficii comparativ cu alte mijloace imagistice utilizate până în prezent :

- determină cât de răspândită este boala;
- determină localizarea tumorii pentru efectuarea biopsiei şi a tratamentului chirurgical sau pentru planificarea schemei de tratament;
- analizează răspunsul la tratament şi evaluează eficiența acestuia;
- detectează urme reziduale ale bolii sau recăderile;
- ajută la evitarea procedurilor de diagnostic invazive.

Fiind perfect compatibil cu sistemele de radioterapie oncologică (*Micromultileaf, Cyberknife, Tomotherapy*), PET/CT permite elaborarea urgentă de planuri de tratament optimizate pentru iradierea selectivă a țesuturilor tumorale active din punct de vedere metabolic, prezervând la maximum țesuturile sănătoase.

În neurologie şi neurochirurgie, PET - CT este folosită pentru a identifica zonele din creier care provoacă crizele epileptice şi pentru a stabili dacă tratamentul chirurgical reprezintă o variantă viabilă. De asemenea, această tehnologie permite diagnosticarea maladiei Alzheimer în stadii în care boala mai poate fi controlată medicamentos. Înainte de efectuarea investigației, pacienții sunt rugați: să nu mănânce şi să nu bea nimic (sau doar apă plată) cu cel puțin 6 ore înainte de examinare, deoarece aceasta ar putea afecta rezultatele (interdicția se referă şi la guma de mestecat fără zahăr, bomboanele mentolate, bomboanele şi băuturile de orice natură); în cazul în care iau medicamente, să bea doar atâta apă cât este necesară pentru a le înghiți; să reducă la maximum activitățile fizice în ziua în care sunt examinați (de exemplu: gimnastică, jogging etc.), deoarece ar putea afecta captarea radiotrasorului; să aducă, în ziua în care sunt investigați, examinările anterioare de tip CT, radiografii, scintigrafii, RMN (rezonanță magnetică nucleară), markeri tumorali, biletele de externare din spital şi rezultatele examenelor anatomo-patologice pe care le au (sub formă de rapoarte scrise, imagini sau CD-uri); să anunțe din timp dacă sunt diabetici, pentru a permite consultarea medicului curant cu privire la pregătirea şi desfăşurarea examinării; înainte ca examinarea propriu-zisă să înceapă li se va măsura oricum nivelul glicemiei; în cazul în care se înregistrează valori excesive ale glicemiei, valori care persistă şi după ce sunt luate măsuri pentru a le menține la un nivel acceptabil, investigația nu va putea fi efectuată; să anunțe dacă au temperatură, să anunțe existența sau suspiciunea stării de graviditate sau dacă alăptează; să telefoneze medicului specialist în cazul în care au întrebări referitoare la procedura de examinare pentru care au fost programați (fig. 3).

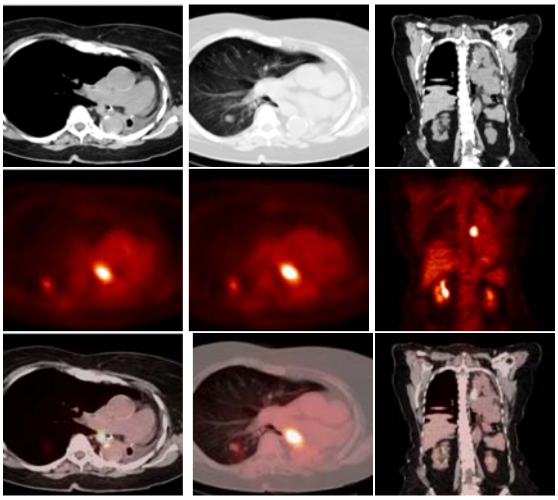

Fig. 3. Femeie de 78 ani cu adenocarcinom pulmonar scuamos operat; status post-pneumectomie. Indicația PET-CT: restadializare; examen PET-CT: Recidiva tumorală; metastază pulmonară dreaptă, anterior şi lateral stânga de corpul vertebral T5.

Examinarea va începe cu un test pentru a determina nivelul glicemiei. Pacienților li se va face apoi o injecție ce conține o cantitate foarte mică de glucoză radioactivă (zahar radioactiv), care se va distribui în întregul organism. Radiotrasorii folosiți în scanarea PET/CT îşi pierd foarte rapid radioactivitatea (în două până la trei ore), motiv pentru care sunt injectate doar doze foarte mici. La şase-opt ore după injecție, radioactivitatea restantă este neglijabilă. În majoritatea cazurilor, pacienții vor trebui să aştepte circa o oră pentru ca medicamentul radioactiv să se distribuie în organism. La aproximativ 60 de minute de la injecție, pacienții vor merge la toaletă pentru a urina. Pacienții vor fi rugați să stea nemişcați în timpul scanării, deoarece mişcările pot afecta rezultatul investigației. Pe parcursul scanării pacienții vor respira normal. În timpul achiziției imaginilor nu vor simți nimic neobişnuit, exceptând mişcările mesei, pe măsură ce anumite zone din organism sunt scanate. Pe tot parcursul examinării pacienții vor fi monitorizați de un operator şi vor putea comunica cu acesta prin microfon şi prin intermediul telecamerelor. Scanarea PET-CT durează între 20 şi 30 de minute. Procedura de examinare poate fi diferită, în funcție de zona care este examinată (fig. 4).

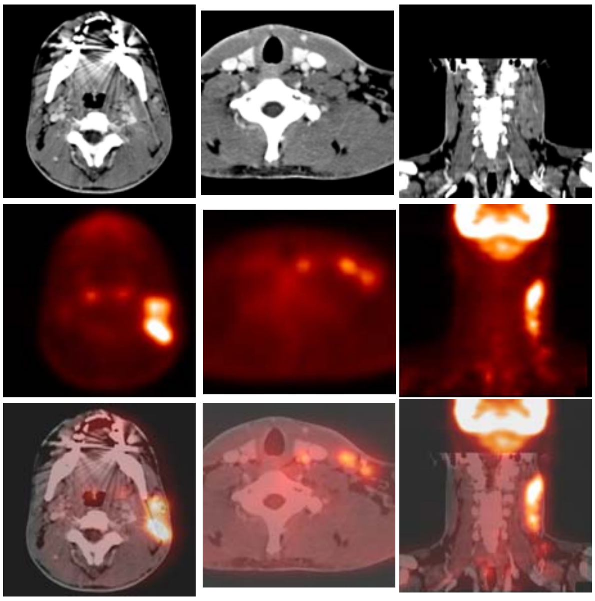

Fig. 4. Pacientă, 37 ani, prezintă astenie fizică, subfebrilitate, tumefacţie laterocervicală stângă.
Indicaţia PET/CT: diagnostic-stadializare.
Ex. anatomopatologic - *Limfom Hodgkin.*

Riscurile şi precauţiile în timpul examinării nu sunt neglijabile şi trebuiesc cunoscute: cu excepţia imobilizării din timpul desfăşurării examinării, nu este înregistrată nicio senzaţie specială, precum dureri, zgomote supărătoare, diferenţe de temperatură etc.; nu au fost niciodată semnalate reacţii nedorite în urma administrării pe cale venoasă de FDG, necesară pentru efectuarea examenului PET/CT; nu există contraindicaţii pentru această administrare şi numai în cazurile de insuficienţă renală severă sau de hiperglicemie ce nu se încadrează în valori mai mici de 180-200 mgr/dl examinarea nu se efectuează, deoarece aceste condiţii fac imposibilă utilizarea corectă a radiotrasorului. Uneori, injectarea intravenoasă a substanţei se poate dovedi dificilă. În acest caz se va introduce în prealabil o branulă; eventuala stare de graviditate, presupusă sau constatată, reprezintă o contraindicaţie în efectuarea examinării. Într-o astfel de situaţie, necesitatea efectuării investigaţiei va fi stabilită de comun acord cu medicii curanţi; alăptarea trebuie să fie întreruptă timp de cel puţin 6 ore după terminarea investigaţiei; insuficienţa renală determină o reducere a calităţii imaginilor examinării PET/CT, chiar dacă nu reprezintă o contraindicaţie a efectuării acesteia; pacienţii purtători de proteze ortopedice, stimulatoare cardiace, proteze arteriale, proteze venoase implantate etc. nu se expun niciunui risc, dar este oportun să informeze medicul care înregistrează datele de anamneză (istoricul medical) despre existenţa acestora (fig. 5).

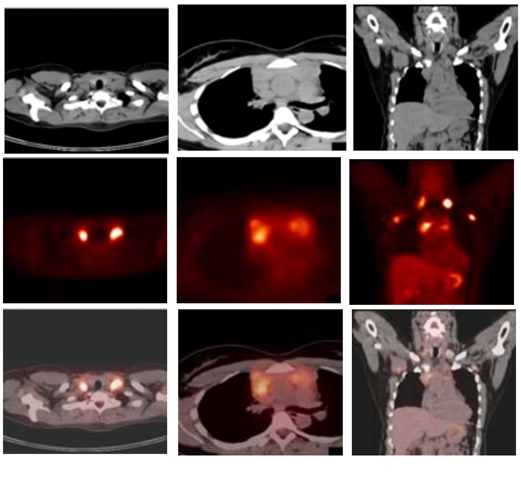

Fig. 5. Femeie de 39 ani, subfebrilitate, astenie fizică, prurit, ganglioni supraclaviculari palpabili, rahialgii toracale; se observă captarea FDG la nivelul mai multor focare costale, vertebrale, axilare, ganglionare; biopsie ggl.supraclaviculară - *limfom Hodgkin;*
Indicaţia PET-CT: stadializare.

După administrarea FDG urmează o aşteptare medie de 60 de minute pentru a permite distribuirea optimă a substanţei în organism. Durata de achiziţie a imaginilor cu aparatul PET - CT este de circa 30 de minute, dar în unele cazuri poate să se prelungească în funcţie de patologie (de exemplu, melanoame) sau de urmărirea unor anumite aspecte. Ultima parte a procedurii durează aproximativ 15 minute (fig. 6).

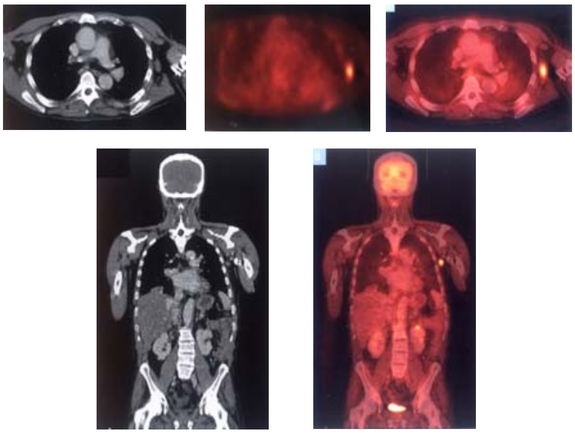

Fig. 6. Bărbat, 51 ani, cu limfom non-Hodgkin cu diseminare mediastinală, pulmonară, vertebrală şi cerebrală, a urmat multiple cure chimioterapice.
Indicaţia PET-CT: evaluare post-chimioterapie la 6 luni de la ultima cură de citostatice.
Diseminare limfatică axilară post limfom non-Hodgkin chimiotratat.

Prezenţa întregului echipament integrat PET-CT în România permite detecţia cancerului (şi a altor boli) la debut, înainte de diseminarea în întregul corp cu eficienţă terapeutică maximă.

Organizaţia Mondială a Sănătăţii şi Agenţia internaţională de luptă împotriva cancerului recomandă o instalaţie PET-CT la circa 1- 2 milioane de locuitori. Prezentăm câteva cazuri clinice de pacienţi trataţi în Clinica de Neurochirurgie a Spitalului Universitar de Urgenţă Militar Central "Carol Davila" din Bucureşti şi investigaţi PET-CT la Clinica Euromedic – Fundeni (fig. 7, 8, 9, 10, 11, 12).

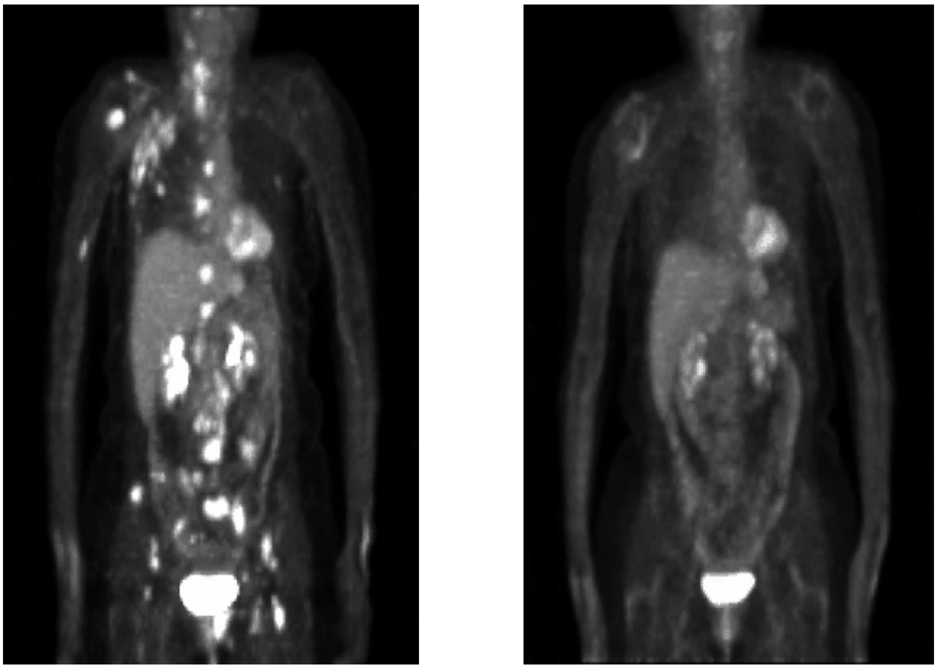

Înainte de chimioterapie

După chimioterapie

Fig. 7. Pacientă, 67 ani, metastaze cancer mamar, evaluarea răspunsului la chimioterapie.

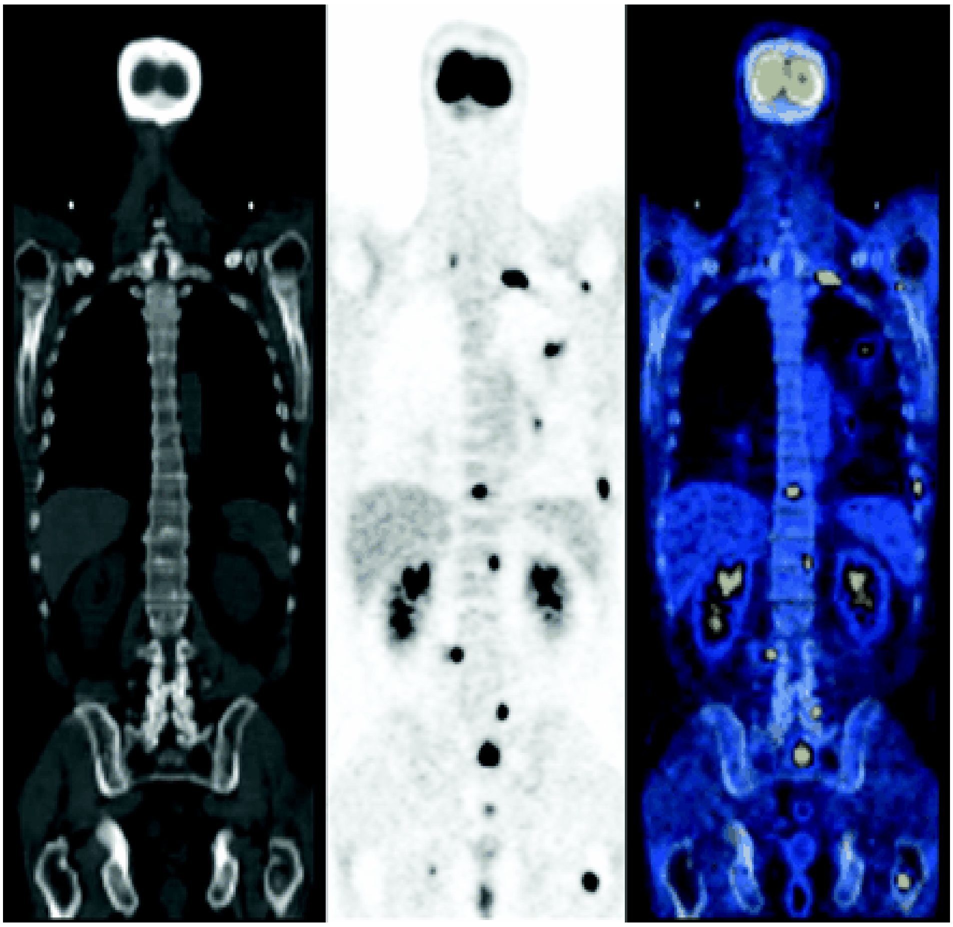

Fig. 8. Multiple metastaze osoase la PET şi PET/CT (CT-scan toraco-abdominal cu substanţă de contrast în limite normale), bărbat, 52 ani, suferind de cancer bronho-pulmonar.

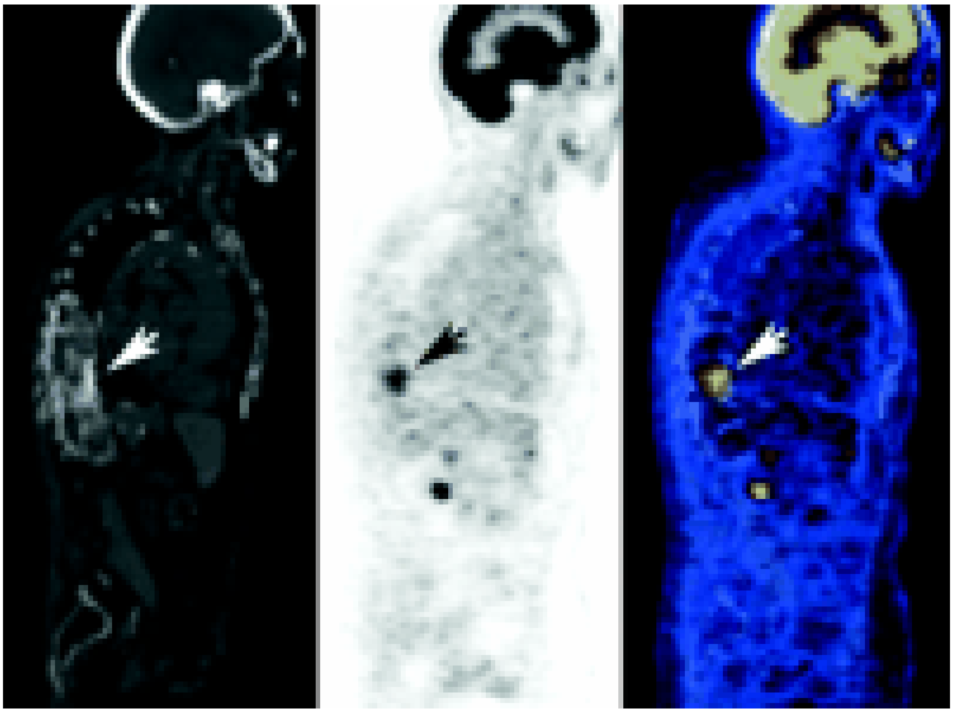

Fig. 9. Compresiune T11 la o femeie, 63 ani, cu multiple leziuni osteoblastice.

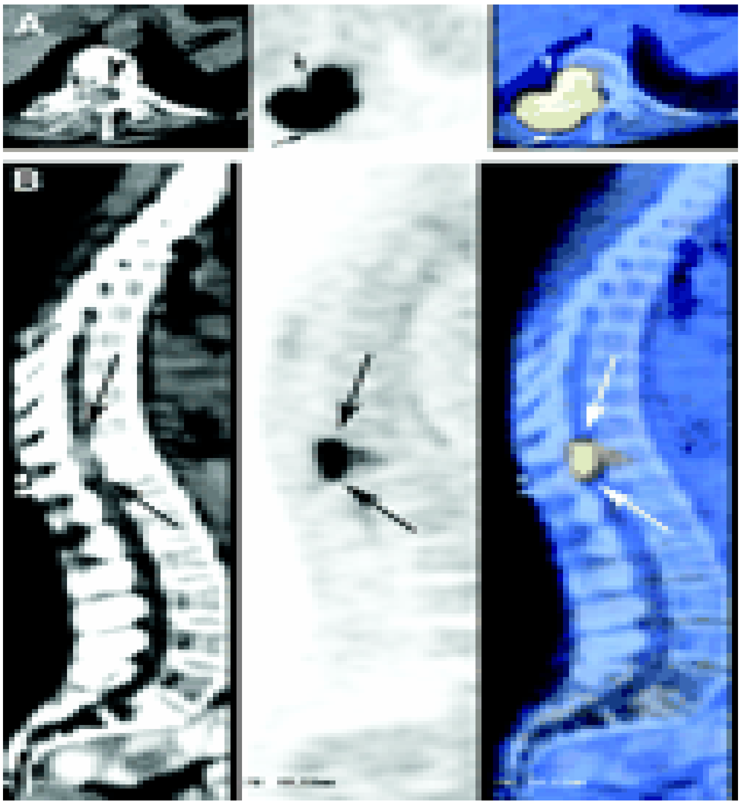

Fig. 10. Derminare secundară epidurală şi la nivelul corpului şi al pediculului vertebral T10, într-un caz de neoplasm mamar, femeie, 63 ani.

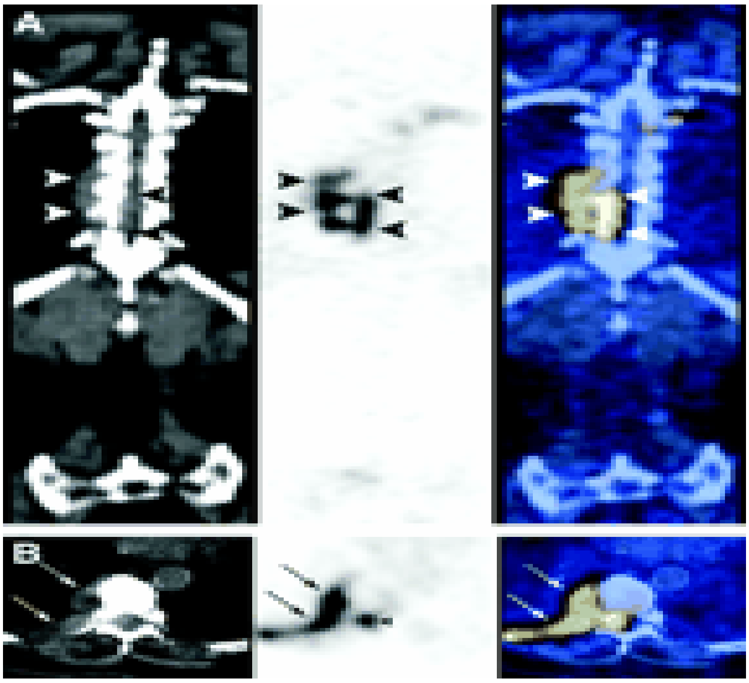

Fig. 11. Femeie, 52 ani, cancer colon, cu proces implicând porțiunea posterioară corp T11 şi elementele posterioare, cu extensie epidurală.

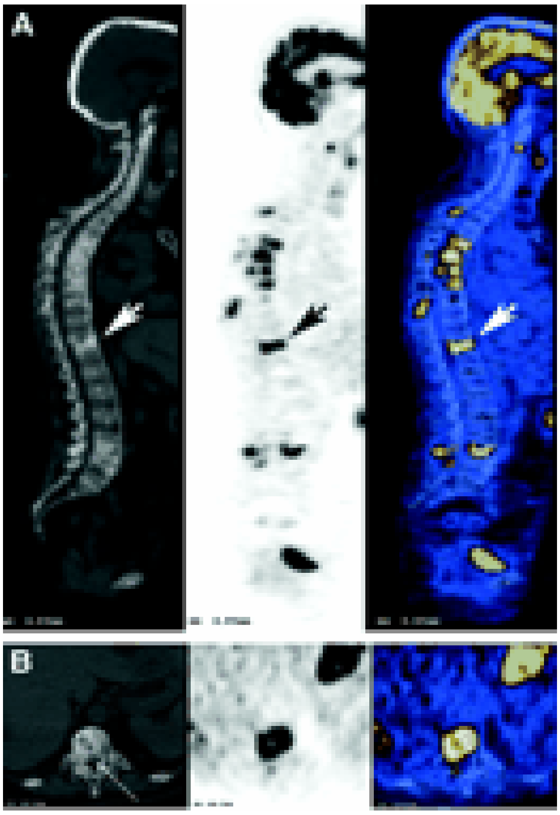

Fig. 12. Masă tumorală cu extensie epidurală prin gaura de conjugare T11 stânga intervertebrală, la o pacientă de 51 ani cu limfom.

Concluzii

În prezent patologia vertebro-medulară beneficiază de aportul diagnostic al unor noi tehnici, dezvoltate şi aplicate în ultimele două decenii. În ansamblu aceste proceduri sunt cunoscute sub numele de tehnici imagistice, care au în comun faptul că oferă informaţii diagnostice asupra substratului lezional cu ajutorul imaginilor prelucrate în sistem computerizat. În ordinea aplicabilităţii clinice s-au impus: imageria prin rezonanţă magnetică (IRM), tomografia computerizată (CT), tomoscintigrafia (SPECT) şi tomografia prin emisie de pozitroni (PET-CT). Aceste tehnici computerizate au revoluţionat neuroradiologia, înlocuind sau limitând mult aplicabilitatea metodelor de radiodiagnostic convenţional: radiografia simplă sau cu contrast (mielografia), tomografia convenţională şi arteriografia (12).

Scopul tratamentului chirurgical este reprezentat de o îmbunătăţire a calităţii vieţii, nefiind întotdeauna posibilă vindecarea bolii de fond. Se urmăreşte: decompresiunea nervoasă, încercând să se îmbunătăţească statusul neurologic al pacientului; stabilizarea, ameliorarea durerii, pacientul să fie capabil să menţină ortostatismul şi să meargă; să obţină un diagnostic histopatologic, pentru a urma un tratament oncologic ţintit. În investigarea şi tratamentul leziunilor vertebro-medulare tumorale este necesară respectarea unor cerinţe optime de conduită neurochirurgicală. Aceste cerinţe sunt impuse de cunoaşterea actuală a achiziţiilor în domeniul chirurgiei spinale, reflectate în literatura de specialitate. Scopul lor principal este evitarea numeroaselor erori şi complicaţii ce pot interveni în timpul tratamentului. Îndeplinirea acestor cerinţe va aduce un plus de claritate în relaţia medic-bolnav, în general şi în special în cazurile în care după efectuarea tratamentului neurochirurgical intervin situaţii litigioase legate de calitatea actului medical (7).

În patologia tumorală spinală investigaţiile trebuie să precizeze obligatoriu localizarea şi, pe cât posibil, etiologia leziunii. Localizarea precisă a zonei afectate dictează calea de abord, iar suspectarea etiologiei permite alegerea unei tehnici chirurgicale adecvate. De asemenea, pentru decizia operatorie este obligatoriu ca localizarea leziunii să fie în concordanță cu simptomatologia neurologică, adică leziunea, prin localizarea sa, să explice sindromul neurologic (11).

Pacientul trebuie investigat exhaustiv, înțelegând prin aceasta nu efectuarea unei suite de investigații abuzive, fără țintă, ci un protocol de investigații complete, adecvate cazului şi în măsură să stabilească un diagnostic precis. În acord cu standardele actuale internaționale, metodele de diagnostic în patologia spinală este necesar să fie focalizate pe investigații minim invazive, capabile să furnizeaze maximum de informații.

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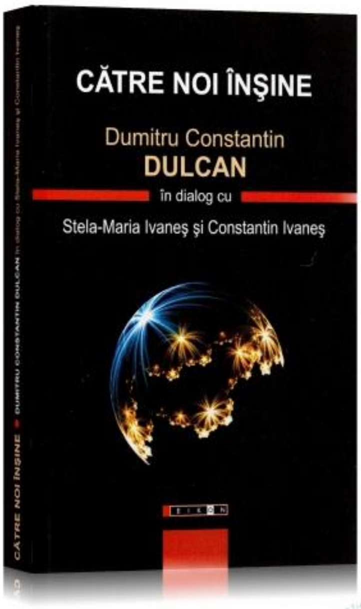

Un dialog fermecător despre valorile reale ale omenirii

Autori: **Prof. univ. dr. Dumitru Constantin Dulcan**, reputat neurolog şi psihiatru român, filosof al ştiinţei, autor şi al apreciatelor volume *Inteligenţa materiei* şi *În căutarea sensului pierdut* , apărute în cadrul editurii Eikon în anii 2009, respectiv, 2008, **Stela-Maria Ivaneş**, doctor în Filosofia ştiinţei, scriitor, psiholog, realizator radioTV, şi **Constantin Ivaneş** (alias Sergiu Alex, pentru melomani şi nu numai), istoric, scriitor, traducător, realizator radioTV. Ultimii doi sunt şi autorii binecunoscuţi ai spectacolelor de suflet pe care le-am savurat cu toţii în sălile Radiodifuziunii Cluj, ale Universităţii de Medicină şi Farmacie "Iuliu Haţieganu" din Cluj-Napoca, sau în cadrul Galelor Medicină-Artă-Cultură.

Cea mai recentă apariţie în plan editorial a Prof. dr. Gen. (r) Dumitru Constantin Dulcan, intitulată *Către noi înşine,* Editura EIKON, Cluj-Napoca, 2009, se înscrie în suita cărţilor de reală valoare pe care binecunoscutul autor le continuă şi în ultimii ani. Astfel, după ediţiile revizuite ale best-seller-ului *Inteligenţa materiei* şi volumului *În căutarea sensului pierdut*, vine cu această realizare, o chintesenţă a căutărilor şi înţelesurilor de o viaţă.

Dintr-un dialog incitant purtat cu alţi doi maeştri ai spiritului, Stela-Maria Ivaneş şi Constantin Ivaneş, rezultă o carte care încearcă să răspundă, îndemnând în acelaşi timp cititorul la meditaţie, la întrebările simple dar chinuitoare ale omenirii. Un veşnic neliniştit, căutător al valorilor spirituale, al cunoaşterii, al adevărului, împărtăşeşte cu generozitatea-i caracteristică concluziile şi părerile despre valorile reale ale omenirii, trecute prin filtrul unui om de ştiinţă, reputat neurolog şi psihiatru, fost şef al Clinicii de neurologie din SCUMC Bucureşti, până în anul 2005.

Einstein spunea: „pentru a afla înţelesul vieţii omeneşti, trebuie să ai credinţă. Omul care nu găseşte răspuns la întrebarea asta şi nu-şi găseşte scopul vieţii sale va fi nu numai nefericit, ci şi neputincios de viaţă!”. Iată că Domnul Profesor, împreună cu ceilalţi doi colegi de spirit, erudiţi, temerari, rafinaţi, se întrec într-un dialog fermecător, un *brainstorming* cu efect anxiolitic, din care se desprinde sensul miraculos al vieţii şi care are ca final un adevărat salt în conştiinţa cititorului. Este o pledoarie pentru adevărata viaţă, într-o lume aculturală şi despiritualizată, „o lume a trăirismului care nu vrea să ştie că mai există şi moarte, acum când totul îi este permis de către morala vremii şi totul îi este oferit într-o societate consumistă’’. *Ce este viaţa?, Miracolul numit Om, De unde venim?, Încotro ne ducem?, De ce nevoia de iubire, de frumos şi de Adevăr?, A fi sau a avea?, De ce boala?, De ce moartea?, Cum este posibilă vindecarea?, Dar prelungirea tinereţii? Şi ... O schimbare ce vine - anul 2012.* Sunt doar câteva dintre subiectele celor treisprezece capitole, care incită la lectură, oferind un adevărat regal pentru un segment elevat de cititori. După parcurgerea cărţii cu o nestăpânită curiozitate, veţi fi devenit cu siguranţă mai frumoşi, mai buni, mai toleranţi, mai împliniţi, mai înţelepţi, mai fericiţi!

Dr. Carmen Adella SÎRBU, MD, PhD
Secţia Neurologie, SCUUMC “Dr. Carol Davila”, Bucureşti

Atitudinea chirurgicală în icterul mecanic – studiu clinic retrospectiv

G. STĂTESCU, V. BĂDESCU, C. IVAŞCU, R. TIUTIUCA, Carmen VAIDA, F. ATANASIU

Spitalul Clinic Militar de Urgenţă "Dr. Iacob Czihac", Iaşi

Rezumat. Pentru chirurg cea mai importantă problemă este dacă pacientul are un icter "medical" sau unul "chirurgical", acesta din urmă fiind determinat de o obstrucţie care trebuie eliminată printr-o procedură chirurgicală.

Material şi metodă. Studiul efectuat este unul retrospectiv şi cuprinde 34 de cazuri internate cu diagnosticul de icter mecanic şi operate în serviciul de chirurgie al Spitalului Clinic Militar de Urgenţă "Dr. Iacob Czihac" din Iaşi între anii 2003-2008. În principal, cauzele de icter mecanic au fost reprezentate de patologia tumorală malignă, 23 de cazuri fiind datorate neoplasmului de cap de pancreas, 3 de tumori ale CBP tip Klatskin, 3 cazuri de icter mecanic postoperator, 2 cazuri de pancreatită pseudotumorală şi 3 cazuri de litiază restantă postcolecistectomie a CBP. Am analizat pe fiecare grupă de cazuri tehnicile chirurgicale pe care le-am aplicat.

Rezultate: etiologia după frecvenţa cazurilor (în medie, 1 caz la 1,5 luni) a fost reprezentată în principal de tumori maligne, dar şi de litiază a căii biliare principale. Tratamentul medical a avut în vedere pregătirea pentru intervenţia chirurgicală. Tratamentul chirurgical a fost adaptat pentru fiecare caz în parte. Rezultatele au fost în funcţie de gravitatea leziunii, vârstă, starea generală, întârzierea intervenţiei chirurgicale şi de natura leziunii.

Concluzii: unul dintre cele mai importante lucruri în aceste cazuri este adoptarea tacticii şi tehnicii chirurgicale individualizat, în funcţie de etiologia şi de nivelul obstrucţiei mecanice.

Cuvinte cheie: icter mecanic, derivaţie bilio-digestivă, duodenopancreatectomie cefalică

Abstract. The most important issue a surgeon is faced with is to establish whether the patient displays a "medical" or a "surgical" jaundice, the latter being caused by an obstruction, which must be removed through a surgical procedure.

Material and method: This is a retrospective trial which includes 34 patients admitted to hospital with a mechanical jaundice diagnosis, who had been operated on in the Surgery Ward of the Military Emergency Clinical Hospital "Dr.Iacob Czihac" in Iaşi (Romania) during 2003-2008. The mechanical jaundice causes had mainly to deal with a malignant tumoral pathology as follows: 23 were caused by pancreatic head neoplasms, 3 were common bile duct Klatskin tumors, 3 were post-surgery mechanical jaundice cases, 2 pseudo-tumoral pancreatitises and 3 cases of common bile duct post-cholecystectomy reformed lithiases. In each group of cases, a different surgical technique was applied, which the author analyzed in greater detail in the paper.

Outcomes: Based on the frequency of cases (an average of 1 case to each 1.5 months), the etiology was mainly represented by malignant tumors, but also by common bile duct lithiases. The medical treatment geared up to prepare the surgical intervention. The surgical treatment adjusted to each individual case. The outcomes had to deal with the severity of the lesion, patient's age, general condition, how early or late the surgical intervention occurred and the nature of the lesion.

Conclusions: One of the most important measures taken in these cases was to adopt a customized surgical strategy and technique in each individual case, based on its etiology and the level of mechanical obstruction.

Introducere

Icterul mecanic este un termen care se referă la excesul de pigmenţi biliari în ser şi în ţesuturi şi este evident clinic atunci când nivelul de bilirubină este de 2-3mg/dl.

Pentru chirurg cea mai importantă problemă este dacă pacientul are un icter "medical" sau un icter "chirurgical", acesta din urmă fiind determinat de o obstrucţie care trebuie eliminată printr-o procedură chirurgicală. Diagnosticul de icter se pune relativ uşor datorită coloraţiei specifice a tegumentelor. Concentraţia în ser a bilirubinei depăşeşte 2-3mg%. Ceea ce este cu adevărat important însă este eliminarea cauzelor medicale ale icterului. Diagnosticul diferenţial începe odată cu examenul clinic, când din anamneză putem deduce succesiunea apariţiei simptomatologiei caracteristice icterului mecanic prin durere, febră, icter (triada lui Charcot), iar la examenul local putem evidenția semnul Courvoisier-Terrier (vezicula biliară dilatată se palpează).

Important din punct de vedere chirurgical este momentul când trebuie să facem diferența între un icter litiazic şi un icter tumoral. Pe lângă anamneză un rol important îl au explorările paraclinice (ecografia, CT cu substanță de contrast, ERCP), care pot preciza natura obstacolului.

Material şi metodă

Am efectuat un studiu clinic retrospectiv care cuprinde 34 de cazuri internate cu diagnosticul de icter mecanic şi care au fost operate în secția de chirurgie generală a Spitalului Clinic Militar de Urgență "Dr. Iacob Czihac" din Iaşi între anii 2003-2008.

Obiectivele şi scopul studiului au constat în evaluarea problemelor de diagnostic, evaluarea tratamentului chirurgical cu studierea posibilităților de îmbunătățire a rezultatelor terapeutice, posibilitatea abordului laparoscopic, eficiența antibioterapiei, precum şi analiza complicațiilor postoperatorii şi a fatalității acestor cazuri.

În principal, cele mai multe cazuri au fost reprezentate de patologia malignă, dintre acestea 23 fiind reprezentate de neoplasmul de cap de pancreas. Diagnosticul a fost uşor de pus, pe lângă simptomatologia clinică fiind confirmat preoperator de examenul CT.

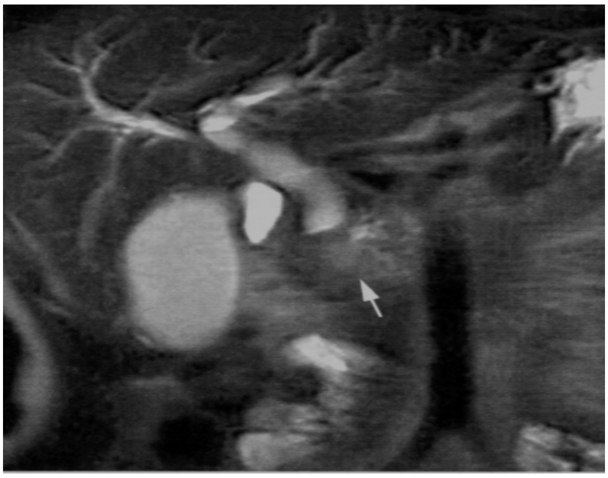

Fig.1. Tumoră de cap de pancreas la examenul CT efectuat cu substanță de contrast (cazuistică Spital Militar Iaşi).

Soluția terapeutică chirurgicală aplicată acestor cazuri a fost anastomoza bilio-digestivă hepatico-jejunală în 21 de cazuri şi coledoco-duodenală în restul cazurilor.

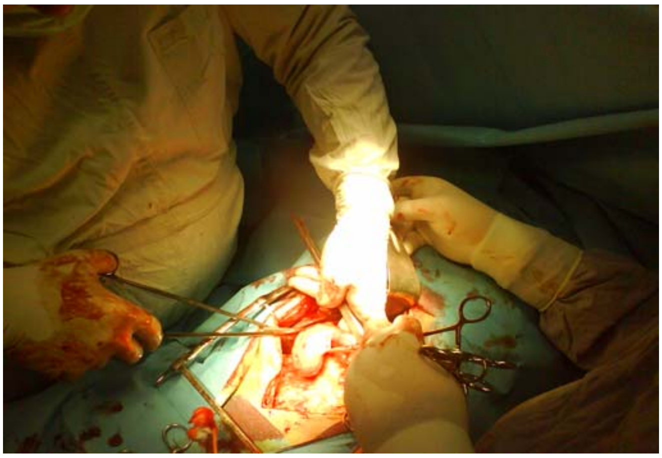

Fig.2. Colecistectomie tactică

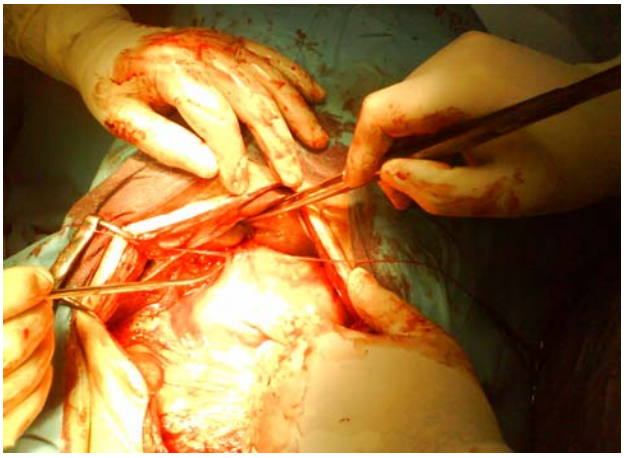

Fig.3. Coledocotomie longitudinală

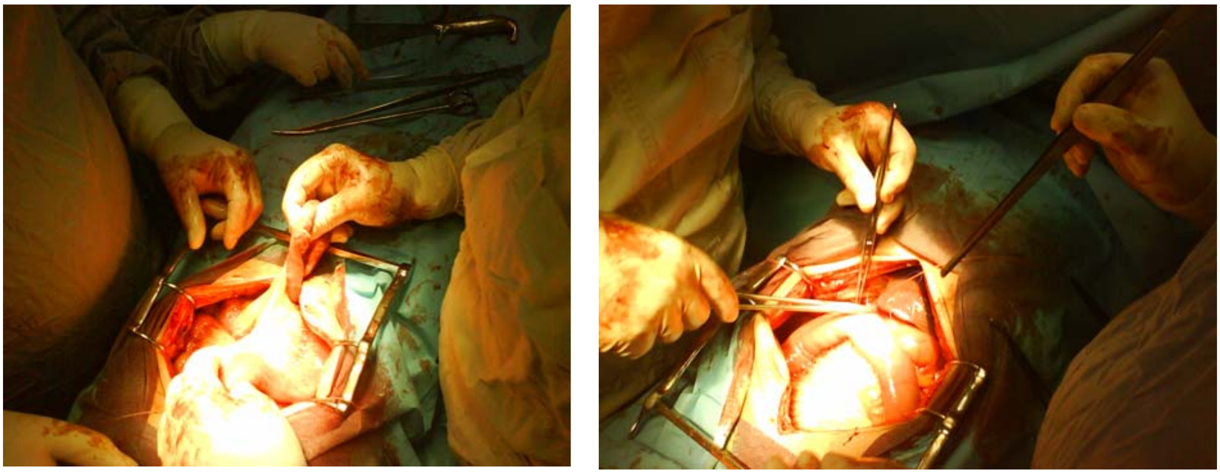

Fig.4. Pregătirea ansei jejunale în « Ω » şi efectuarea anastomozei hepatico-jejunale LL

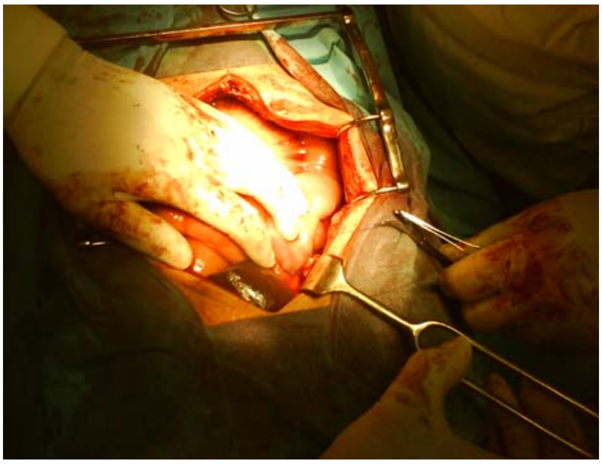

Fig.5. Anastomoza terminată

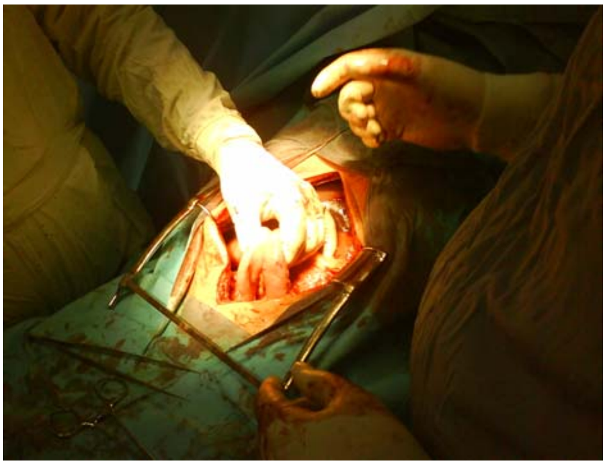

Fig.6. Anastomoza tip Braun

În două cazuri s-a putut efectua duodenopancreatectomie cefalică tip Wipple, tumora fiind mobilă pe planul posterior.

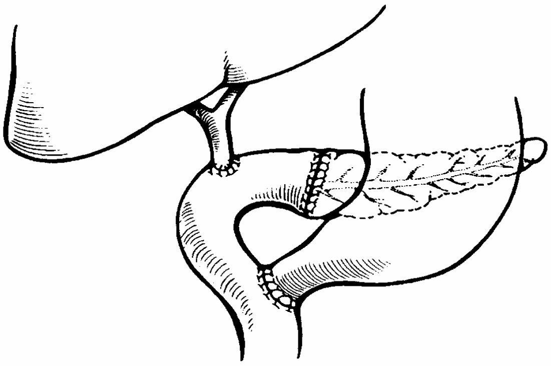

Fig. 7. Schema operației Wipple

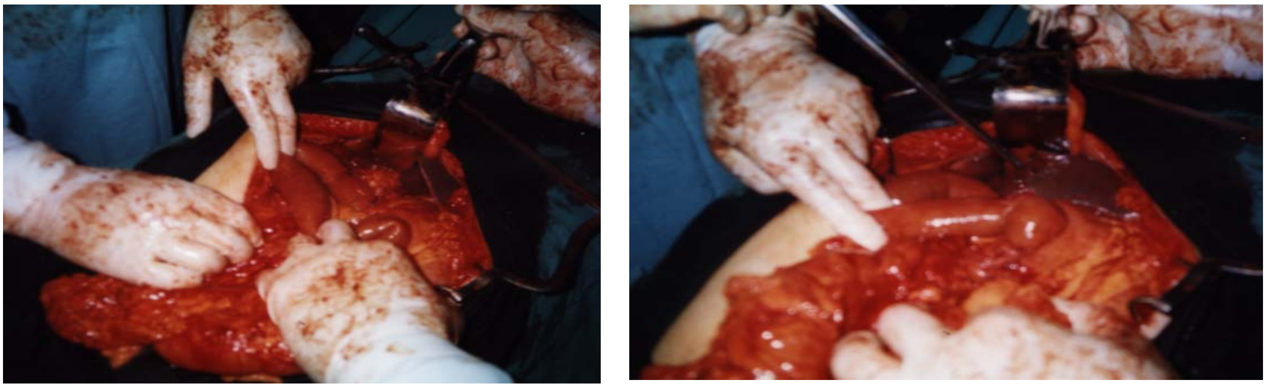

Fig. 8. Aspect final al anastomozelor efectuate pe ansa jejunală.

Am preferat să intubăm bontul pancreatic în ansa jejunală, după care la o distanță de 14-15 cm s-a efectuat anastomoza hepatico-jejunală termino-laterală iar gastroenteroanastomoza s-a practicat la aproximativ 40 cm. În imaginile din figura 8 se observă cum se aşează ansa jejunală după efectuarea celor 3 anastomoze într-un montaj tip Child. Biopsiile efectuate au confirmat diagnosticul de adenocarcinom de cap de pancreas.

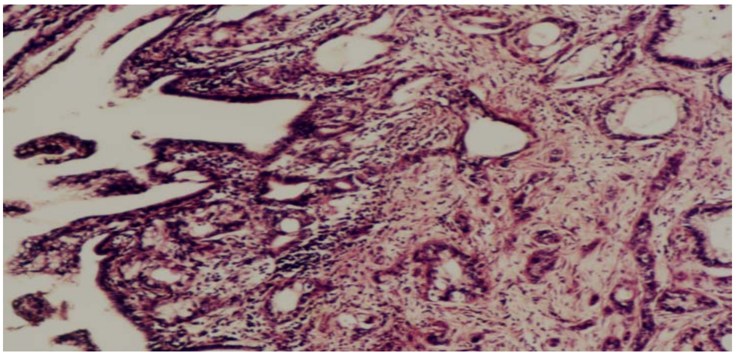

Fig. 9. Adenocarcinom de pancreas.

Alte tipuri de intervenții au constat în foraj tumoral, cu tub pierdut într-un caz, dublă derivație bilio-digestivă coledoco-duodenală şi gastroenteroanastomoză într-un caz şi două laparotomii exploratorii.

În ceea ce priveşte cele două cazuri de stenoze ale căilor biliare survenite după colecistectomii am practicat de asemenea derivații bilio-digestive pe ansă în "**Ω**".

Locul stenozei fiind mai jos de 2 cm de carefurul căilor biliare.

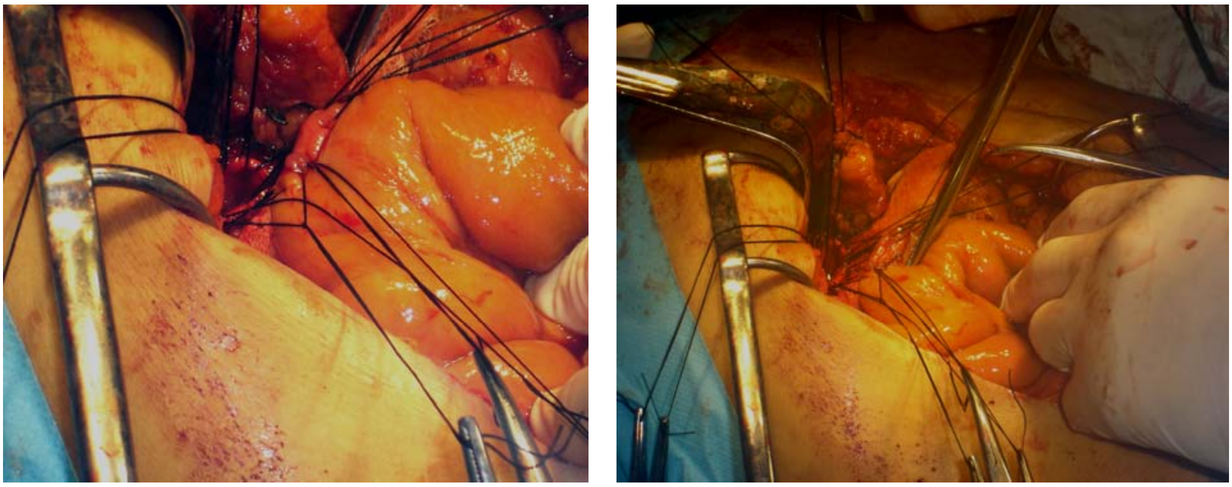

Fig.10. Calea biliară principală dilatată şi efectuarea anastomozei bilio-digestive pe ansă în "**Ω**

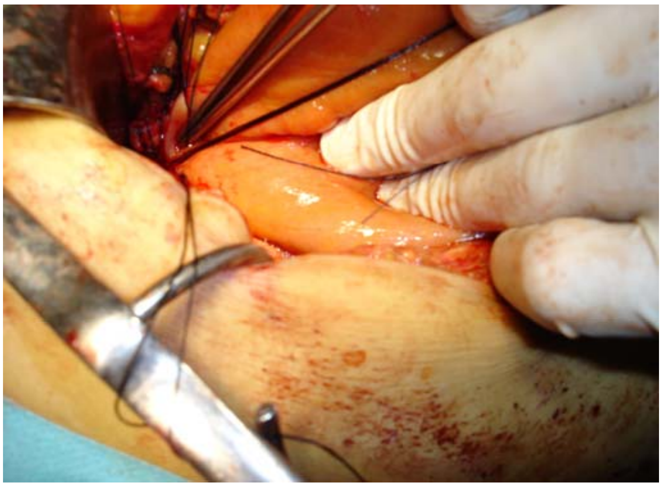

Fig.11. Anastomoza bilio-digestivă terminată.

Complicațiile postoperatorii au fost destul de numeroase.

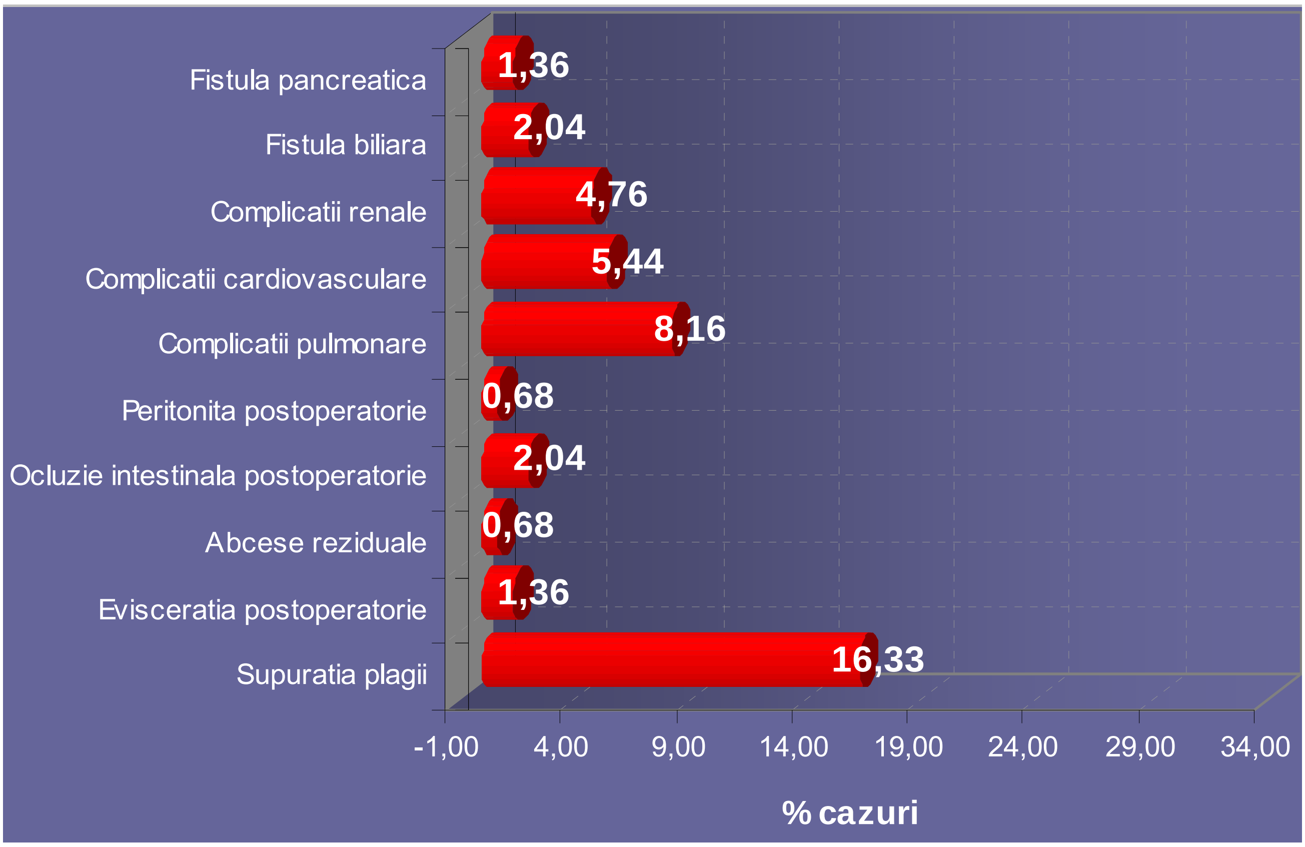

Discuții

Se poate spune că mortalitatea operatorie este ridicată la aceste tipuri de intervenții chirurgicale. Conform statisticilor, ea reprezintă aproximativ 20%.

Operațiile radicale pot fi efectuate numai dacă avem şi criterii intraoperatorii de rezecabilitate (în special mobilitatea tumorii față de planul posterior vascular şi absența metastazelor local şi la distanță).

Riscul de apariție a fistulelor este de asemenea ridicat față de alte intervenții chirurgicale.

Vârsta de peste 70 de ani nu constituie o contraindicație şi nu reprezintă un factor agravant, dar trebuie considerată ca un factor de risc suplimentar în cadrul riscului general.

Concluzii

Tratamentul medicamentos, reechilibrarea hidro-electrolitică şi antibioterapia au fost începute şi au reprezentat pregătirea în vederea intervenției chirurgicale.

Derivația bilio-digestivă a fost principala intervenție chirurgicală efectuată.

Operațiile radicale efectuate au fost reprezentate de duodenopancreatectomia cefalică (tip Wipple). Alte intervenții chirurgicale paliative efectuate au fost reprezentate de forajul transtumoral cu tub pierdut, gastroenteroanastomoză.

Abordul laparoscopic a prezentat avantajul unui diagnostic corect şi complet.

Rezultatele au fost în funcție de vârsta pacientului, de starea generală, de întârzierea diagnosticului şi după natura leziunii. Prognosticul a fost bun în afecțiunile benigne şi grav în cele maligne.

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Incidența unor semne obiective în sforăitul cronic cu risc major de apariție a sindromului de apnee în somn

Col. conf . univ. dr. Vasile Gh. MOSCALIUC*, Dr. Otilia EVA*, Dr. Ionuț Gabriel HĂNȚĂSCU**

***Spitalul Clinic Militar de Urgență „Dr. I. Czihac”, Iaşi**
****Spitalul Clinic „Sf. Spiridon”, Iași**

Rezumat. Lucrarea îşi propune să analizeze incidența unor semne obiective în diagnosticul sforăitului cronic cu potențial de evoluție către sindromul de apnee în somn. Este analizat un lot de 113 bolnavi internați în Spitalul Militar de Urgență ”Dr. I. Czihac” din Iaşi. Sunt descrise o serie de semne patognomonice descoperite în urma examenului O.R.L. şi modalități concrete de sancționare terapeutică.

Cuvinte cheie: sforăit (snoring), sindrom de apnee în somn, semne patognomonice O.R.L.

Abstract. This paper tries to analyse the incidence of some objective signs in chronical snoring diagnosis with a potential evolution to the sleep apneea. We have assesed a group of 113 pacients admitted in the Emergency Military Hospital „Dr. I Czihac” from Iasi. We described some pathognomonical signs discovered after performing E.N.T. examination and practical therapeutical aspects.

Introducere

Sindromul de Apnee în Somn (S.A.S.), aşa cum este cunoscut în literatura de specialitate, apare după 10–15 ani de sforăit aproape permanent. Odată instalat şi depistat prin procedurile polisomnografice, acesta devine invalidant pentru pacient, singura posibilitate de tratament fiind folosirea diverselor tipuri de aparate de oxigenare cu presiune pozitivă sau alternantă.

Mecanismele fiziopatologice prin care se ajunge la insuficiențe funcționale multiple ale organelor vitale, începând cu creşterea presiunii şi hipercapnia din mica circulație, sunt foarte bine descrise şi analizate de colegii pneumologi.

Etiopatogenia şi posibilitățile de prevenire a apariției S.A.S. se află în mâinile specialistului O.R.L., care poate colabora foarte bine cu medici din alte specialități, cum sunt: epidemiologia, endocrinologia, boli de nutriție şi diabet, neurologia etc.

Sforăitul apare, conform legilor lui Bernoulli, atunci când fluxul de aer inspirat trece printr-o serie de cavități tubulare unde poate întâlni şi diverse obstacole, producând curenți turbionari care pun în vibrație vălul palatin şi alte structuri moi de la nivelul faringelui, rezultând zgomotul caracteristic. Acesta are un spectru foarte larg, întinzându-se dincolo de limitele percepției auditive, având armonice sub 16 Hz, adică în spectrul infrasunetelor şi armonice peste 16 000 Hz, adică al ultrasunetelor. Acestea din urmă produc la nivelul citoplasmei miocitului din structurile faringelui aşa-numitul fenomen de cavitație. Fenomenul de cavitație nu apare spontan, ci printr-un efect aditiv în timp al ultrasunetelor emise de sforăitorul cronic.

Diverse studii epidemiologice ale S.A.S. arată că boala se declanşează după aproximativ 15 ani de sforăit cronic. În acest interval de timp, citoplasma şi corpusculii PALADE (ribozomii) sunt distruşi prin fenomenul de „cavitație”. Miocitul este distrus, iar țesutul muscular din vălul palatin, peretele posterior al faringelui, pilierii lojei amigdaliene şi baza limbii devine flasc, vibrând şi mai mult la trecerea coloanei de aer inspirat, în plus, putând fi aspirat cu uşurință, producându-se în acest fel obstrucția glotei şi apariția apneei. Infrasunetele emise ca armonice în timpul sforăitului se pare că au şi ele o acțiune distructivă la nivelul țesutului muscular al faringelui, acționând în special asupra plăcii neuro-motorii şi a desmozomilor.

Recunoscând din timp sforăitul cronic cu potențial de evoluție spre S.A.S., putem interveni activ terapeutic.

Material şi metodă

Examenul O.R.L. şi examenul general al unui pacient ne pot releva o serie de semne demne de luat în consideraţie.

Am studiat o serie de semne clinice generale şi locale la un număr de 113 pacienţi cu snoring cronic sau cu forme uşoare de S.A.S. internaţi în secţia O.R.L. a Spitalului Clinic Militar de Urgenţă „Dr. Iacob Czihac" din Iaşi, în perioada 01.01.2008 – 20.12.2009.

Aceste semne ne pot reda tabloul sforăitorului cronic cu potenţial evolutiv spre S.A.S.

Semne generale:

OBEZITATEA. Din totalul pacienţilor studiaţi, 78% erau obezi, prezentând cel puţin 15 kg peste indicele ponderal. Etiologia endocrinologică sau bolile de nutriţie nu au prezentat mai mult de 10% drept cauză a hiperponderabilităţii lor. 90% dintre aceşti obezi reprezintă rezultatul unui aport alimentar inadecvat faţă de consumul caloric zilnic.

SEMNELE DE IMPREGNAŢIE ALCOOLICĂ: facies vultuos, obosit, edeme palpebrale, conjunctive hiperemice, nasul şi pometii obrajilor cu teleangectazii etc. le-am întâlnit la 32% dintre pacienţii studiaţi.

OBOSEALA CRONICĂ: mai ales matinală, după somn neodihnitor, mergând de la mici picoteli până la adormirea spontană de tip narcoleptic, a fost regăsită la 92% dintre bolnavii noştri.

SCĂDEREA PERFORMANŢELOR INTELECTUALE şi a celor legate de munca cotidiană a fost remarcată la 90% din cazuri.

Semnele locale, depistate în urma examenului O.R.L. au fost:

- deformaţii ale piramidei nazale şi asimetria facială - 20%;
- aspectul de profil „bird face"- 28%;
- deviaţia septului nazal - 54%;
- rinopatia hipertrofică - 40%;
- polipoză rinosinuzală - 5%.

O serie de aspecte ale examenului de specialitate le-am considerat patognomonice în diagnosticul de degradare rapidă a musculaturii faringiene şi instalarea iminentă a S.A.S.

EDEMUL LUETEI: aceasta, indiferent de mărime, are un aspect translucid, marginile ei par dedublate, iar uneori dă impresia că este învelită într-o cămaşă gelatinoasă (fig. 1, 2).

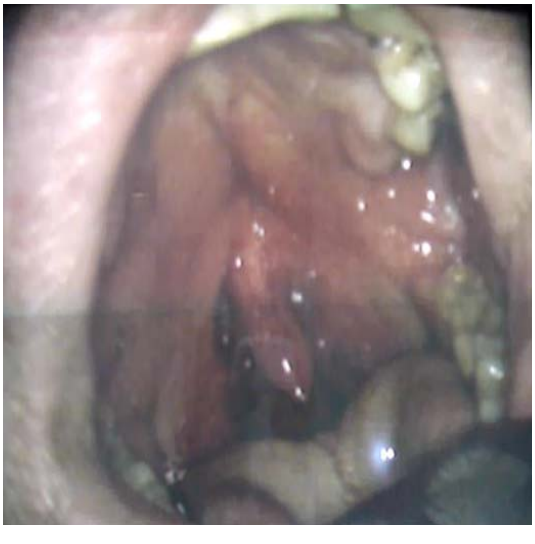

Fig.1. Edem al luetei

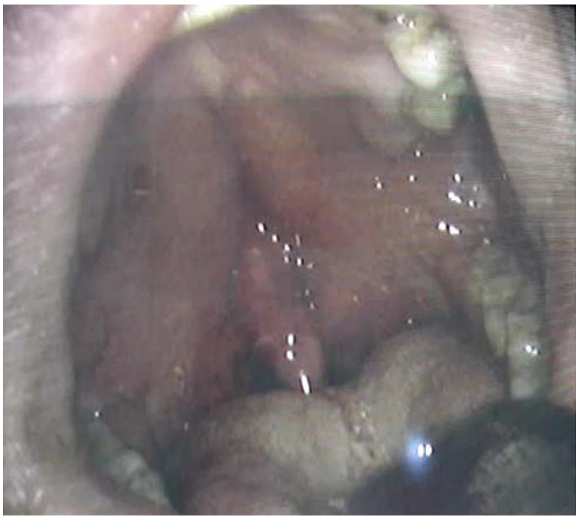

Fig. 2. Edem al luetei

MACROGLOSIA: limba acoperă întregul planşeu bucal, până la nivelul apical al danturii de pe arcada inferioară. Pare a se revărsa peste arcada dentară, fiind însoţită de o bogată sialoree. La baza acesteia se remarcă o proeminentă vascularizaţie venoasă. Depresia pe linia mediană, cu ajutorul spatulei, provoacă o răsfrângere a marginilor limbii, astfel încât apăsătorul poate fi complet acoperit (figurile nr. 3 şi 4).

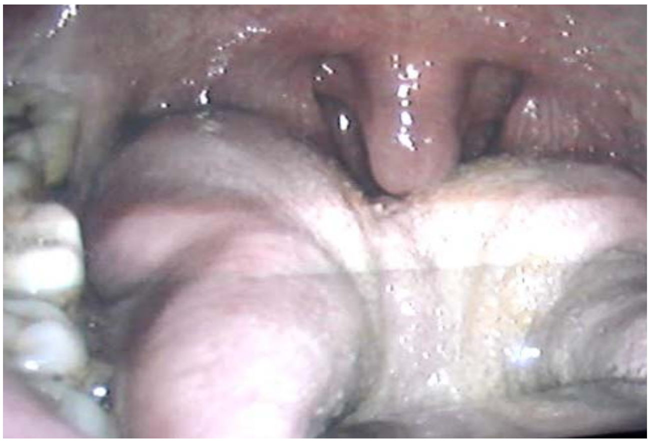

Fig. 3. Macroglosie cu depresia centrală a limbii.

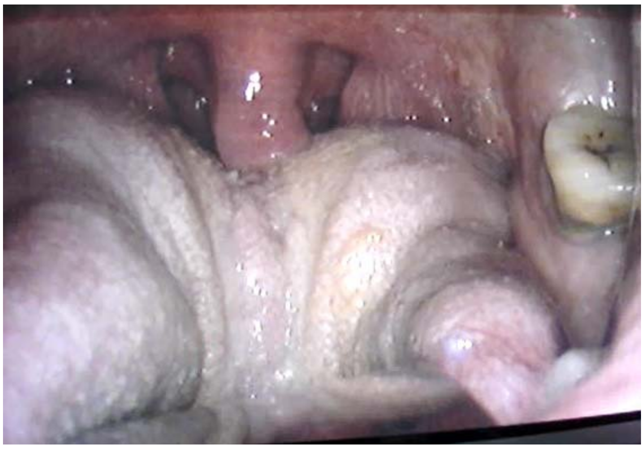

Fig. 4. Macroglosie cu depresia centrală a limbii.

DEPOZITELE MICELIENE: la nivelul limbii şi al peretului posterior faringian sunt de asemenea prezente, ca o consecință a metabolismului uşor acid. Acestea sunt rebele la orice formă de tratament.

PERETELE POSTERIOR AL FARINGELUI: are un aspect roşu granulat, strălucitor în lumină şi aderent de spatulă.

VĂLUL PALATIN ATONIC: sau cu tonicitate scăzută are tendința de a se acola de baza limbii şi peretele posterior al faringelui (fig. 5, 6).

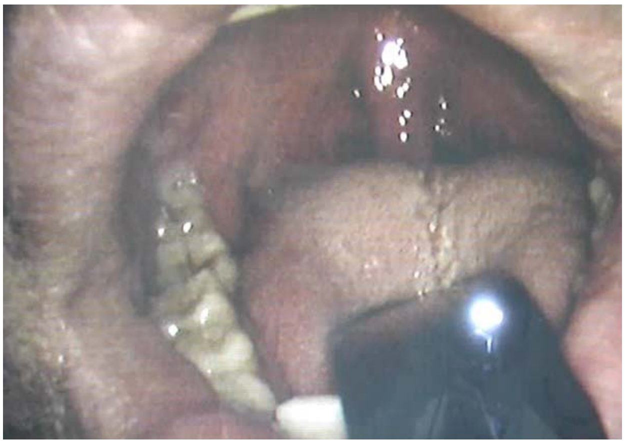

Fig. 5. Floră miceliană în sforăit cronic.

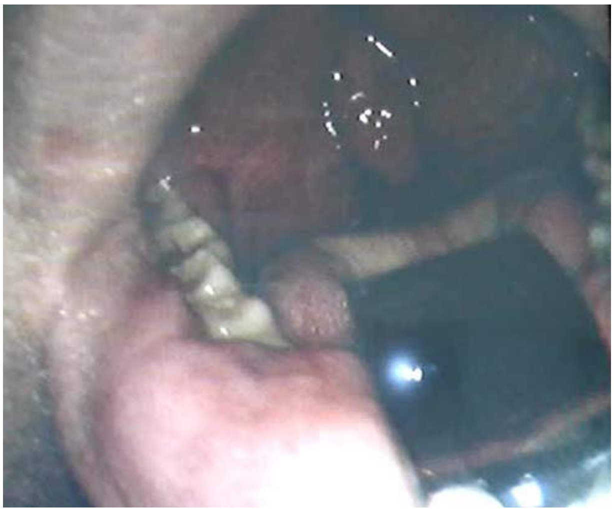

Fig. 6. Floră miceliană în sforăit cronic.

Aceste semne le-am considerat patognomonice deoarece au fost descoperite la toți pacienții studiați. În final, acestea oferă imaginea de îngustare a filierei aerodigestive prin creşterea în dimensiuni a bazei limbii, scăderea distanței dintre pilierii posteriori ai lojelor amigdaliene şi a coborârii hipotonice a vălului palatin.

Discuții

În funcție de existența semnelor menționate, medicul practician poate lua o serie de măsuri:

A. Dacă ne aflăm în fața unui bolnav care prezintă toate aceste semne patognomonice şi examenul polisomnografic arată că sindromul de apnee în somn este manifest, singura posibilitate terapeutică este administrarea de oxigen în timpul somnului, cu ajutorul aparatelor cu presiune pozitivă continuă sau alternantă. De obicei, nu se recomandă acestor pacienți procedurile chirurgicale de tipul uvulo-faringo-palato-plastia.

B. În caz că unele semne sunt prezente iar investigația polisomnografică arată rare accidente apneice, putem lua o serie de măsuri de o reală eficiență în tratarea sforăitului cronic şi prevenirea S.A.S. Aceste măsuri pot fi grupate astfel:

FIZIOKINETOTERAPIA
- mişcarea în aer liber;
- gimnastica şi efortul fizic progresiv şi controlat;
- masajul general şi segmentar;
- poziția ventrală în timpul somnului;
- folosirea dispozitivelor de menținere a mandibulei în contact cu maxilarul pe durata odihnei;
- dotarea dormitorului cu aparate de purificare a aerului (tip SALIN).

CONDUITA MEDICALĂ
- tratamentul unor afecțiuni conexe precum alergia, bronşita cronică etc.;
- evaluarea şi tratamentul unor afecțiuni endocrine şi neurologice;
- regimuri de slăbire prin scăderea aportului alimentar hipercaloric şi reaşezarea orarului de masă astfel încât cina să fie servită cel târziu la ora 18.00;
- interzicerea consumului de alcool şi tutun;
- evaluarea şi corecția parametrilor biologici;
- folosirea unor proceduri pentru menținerea funcțională a căilor respiratorii superioare, precum lavajul foselor nazale cu soluții saline şi gargară cu soluții de bicarbonat de sodiu;
- utilizarea melatoninei ca supleere a deficitului acesteia prin abolirea fazei de somn profund.

CONDUITA CHIRURGICALĂ

- tehnici de uvulo-faringo-palato-plastie, numai în condițiile în care pacientul a ajuns la o greutate normală;
- amigdalectomii, amigdalotomii şi rezecții parțiale ale bazei limbii folosind laserul sau radiofrecvența;
- proceduri chirurgicale de avansare şi pexie a mandibulei;
- infiltrațiile cu toxină botulinică în vălul palatin şi peretele posterior al faringelui;
- ramforsarea cu silicon a peretelui posterior al faringelui şi a pilierilor posteriori ai lojelor amigdaliene etc.

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Supraexpresia P53 în carcinoamele gastrice şi corelarea acesteia cu clasificarea Lauren şi Goseki

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Rezumat. Scop: Cu toată importanța lor, factorii de prognostic în cancerul gastric nu sunt bine cunoscuți încă. Material şi metodă: Studiul cuprinde piese anatomice de la 40 de pacienți cu carcinoame gastrice. Toate cele 40 de piese anatomice au fost fixate în formaldehidă tamponată cu pH neutru 10% şi în parafină. Secțiunile au fost preparate folosind tehnicile curente: hematoxilină-eozină, Van Gieson tricromic, şi albastru de Alsacia. Am folosit clasificarea Lauren cu două tipuri de carcinom gastric: intestinal şi difuz şi de tip Goseki cu patru tipuri principale de clasificare. Am studiat imunoreactivitatea p53 (DO 7 Biogenex).
Rezultate: Folosind clasificarea Lauren, imunoreactivitatea a fost pozitivă în 62,5% din cazuri: 7 cazuri bine diferențiate de carcinom gastric forma intestinală – indice scăzut un caz de carcinom de tip intestinal slab diferențiat, indice slab spre moderat, 15 cazuri de carcinom gastric forma difuză –indice moderat crescut. Folosind clasificarea Goleski, imunoreactivitatea a fost pozitivă pentru p53 în 62,5%: 3 cazuri de carcinom gastric intestinal –indice slab, 1 caz cu indice moderat de tip I, 3 cazuri cu indice scăzut de tip II, 9 cazuri cu indice de la scăzut la ridicat de tip III şi 6 cazuri de carcinom gastric cu indice de la scăzut la ridicat de tip IV.
Concluzii: Testul pozitiv pentru p 53 a fost exprimat în 66% dintre carcinoamele gastrice cu un pattern de intensitate al colorației de la tipul bine diferențiat până la formele slab diferențiate. Procentul de pozitivitate al tumorilor la p 53 nu se corelează cu gradul de diferențiere.

Cuvinte cheie: Cancer gastric, P 53

Abstract. Aims: Despite their importance, prognostic factors in gastric cancer are not well known, yet. Material and methods: The study included surgical specimens from 40 patients with gastric carcinoma. All specimens were fixed in 10% buffered neutral pH formaldehyde and paraffin embedded. Histological sections were stained using current techniques: haematoxylin-eosin, tricromic van Gieson, and Alcian blue. We used Laurén histological classification with two main types of gastric carcinoma: intestinal and diffuse and Goseki classification with four main types of gastric carcinoma.We assessed the p53 immunoreactivity. (DO 7 Biogenex).
Results: Using Lauren classification, immunoreactivity was positive for p53 in 62,5% cases: 7 cases of well differentiated gastric carcinoma intestinal type – low index, 1 case of poorly differentiated gastric carcinoma intestinal type – low to moderate index, 15 cases of gastric carcinoma diffuse type – moderate to high index. Using Goseki classification, immunoreactivity was positive for p53 in 62,5% cases: 3 cases with low index and 1 case with moderate index in type I, 3 cases with low index in type II, 9 cases with low to high index in type III and 6 cases with low to high index in type IV.
Conclusions: The positivity for p53 was expressed in 66% of the gastric carcinomas with a coloring intensivity growth pattern from the well differentiated types to the poorly differentiated types. The percentage of tumors positive for p53 did not correlate with the degree of differentiation of them.

Adenocarcinoamele gastrice reprezintă 90-95% din totalul tumorilor maligne ale stomacului (1).

Corelațiile endoscopice cu aspectele histopatologice au dus la apariția formelor incipiente de adenocarcinom cu prognostic favorabil, complet diferit de carcinomul gastric avansat. De asemenea, datele epidemiologice, imunohistochimice (IHC) şi evolutive confirmă cele două variante histologice frecvente, carcinomul gastric de tip intestinal şi carcinomul gastric de tip difuz (2).

La fel ca şi clasificarea OMS, clasificarea Goseki (1992) se bazează pe aspecte histologice. Aprecierea gradului de diferenţiere tubulară şi a cantităţii de mucus intracitoplasmatic permite distincţia a 4 categorii (3):

-tipul I - tubi bine diferenţiaţi şi mucus redus;
-tipul II - tubi bine diferenţiaţi, mucus abundent;
-tipul III - tubi slab diferenţiaţi, mucus redus;
-tipul IV - tubi slab diferenţiaţi, mucus abundent.

Numeroase studii au prezentat IHC în vederea aprecierii prognosticului corelat cu produsele proteice ale genelor.

Proteina P53 normală prezintă o viaţă extrem de scurtă şi este găsită în cantităţi extrem de mici în interiorul celulelor. Ea nu poate fi detectată în celulele normale folosind tehnicile IHC. Proteina P53 produsă de gena anormală p53 prezintă o viaţă mult mai lungă decât P53 normală, acumulându-se în interiorul celulelor maligne şi poate fi detectată IHC cu ajutorul Ac anti P53. În teorie, supraexpresia P53 ca un marker surogat al mutaţiilor genei p53 reprezintă un „test” IHC pentru stabilirea neoplaziei – displaziei. În practică totuşi, nu putem spune acelaşi lucru pentru că o corelaţie între colorare şi anormalităţile genei p53 nu este precisă.

Având în vedere cele relatate, acest studio îşi propune evaluarea imunoreactivităţii pentru p53 în carcinoamele gastrice şi corelarea acestuia cu clasificarea Lauren şi Goseki.

Material şi metodă

Materialul de studiu este reprezentat de 40 de piese de rezecţie chirurgicală provenite de la pacienţi diagnosticaţi cu neoplasm gastric. Întregul material a fost fixat în formol 10% şi inclus la parafină. Investigaţiile morfologice de rutină s-au efectuat pe secţiuni colorate, prin metode uzuale: hematoxilin-eozină, van Gieson, Albastru Alcian, PAS, care au permis încadrarea leziunilor şi stabilirea gradului de diferenţiere. Am folosit clasificarea Lauren şi Goseki pentru aprecierea tipurilor histologice. (4)

Coloraţiile imunohistochimice prin metoda Avidin-Biotin Complex s-au efectuat cu ajutorul autostainerului Optimax produs de Biogenex. Pretratamentul s-a efectuat prin fierbere, timp de 12-15min, în soluţie citrate, după care a urmat incubarea, 2 ore, cu antigen p53 (DO7 Biogenex – Ready to use). Vizualizarea cu DAB, urmată de colorarea uşoară (1min) cu hematoxilină, a permis evidenţierea p53 sub forma unui produs de reacţie de culoare brună cu localizare nucleară (5,6,7).

Prelucrarea statistică s-a efectuat utilizând testul de corelaţie Spearman Rank R, Chi-pătrat şi Anova.

Rezultate

Expresia p53 la cele 40 de cazuri studiate a fost gradată după o scală ce ţine cont de procentul celulelor tumorale cu nucleu pozitiv (8):

- 0 coloraţie negativă;
- + reacţie pozitivă în mai puţin de 10% din nucleii celulelor tumorale;
- ++ reacţie pozitivă în mai mult de 10% din nucleii celulelor tumorale dar mai puţin de 33%;
- +++ reacţie pozitivă în mai mult de 33% din nucleii celulelor tumorale.

S-a interpretat o reacţie p53 pozitivă colorarea nucleară distinctă, omogenă sau granulară. Produsul de reacţie a prezentat o culoare brună. (9)

Am considerat de asemenea că tumorile supraexprimă proteina p53 numai când am obţinut o colorare nucleară intensă prezentă în unul din cele 3 paternuri. Cazurile cu o coloraţie nucleară echivocă sau slabă le-am considerat negative. Tot negative au fost apreciate şi cazurile cu nuclei pozitivi extrem de rari sau izolaţi.

Nu am semnalat celule p53 pozitive pe epiteliul gastric normal adiacent tumorii şi pe lamele de control negative. Am detectat supraexprimarea proteinei P53 în 25 (63 %) din cele 40 de tumori gastrice studiate, remarcând o heterogenitate a intensităţii colorării şi modelelor de colorare (tabel nr. 1).

Tabelul nr. 1. Supraexprimarea p53 în cele 40 de cazuri studiate

P53	*Nr cazuri*	*%*
0	15	37.5%
+	9	22.5%
++	6	15%
+++	10	25%
Total	40	

Corelând supraexprimarea p53 cu clasificarea histologică Lauren, am identificat următoarele:

- 8 din cele 12 ADK de tip intestinal (66,6%) au prezentat supraexpresia p53. Această supraexpresie a fost cuantificată cu + pentru 7 cazuri şi cu ++ pentru 1 caz (acest caz reprezentând varianta moderat diferențiată, figurile 1, 2);

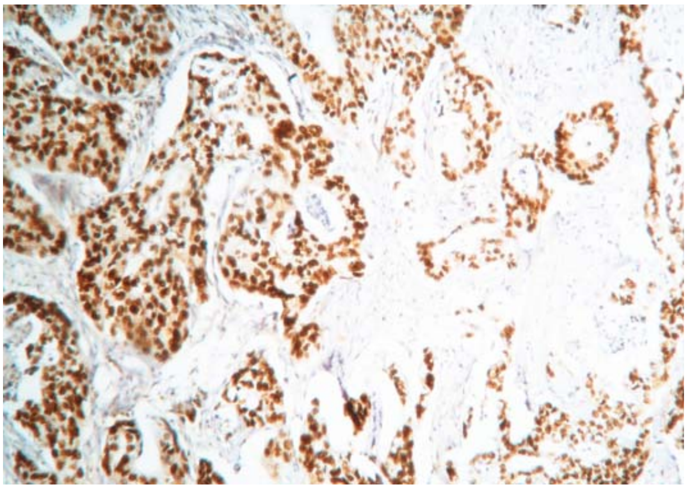

Fig. 1. Colorare nucleară distinctă omogenă sau granulară pentru p53 (++) într-un ADK moderat diferențiat, IHC x40.

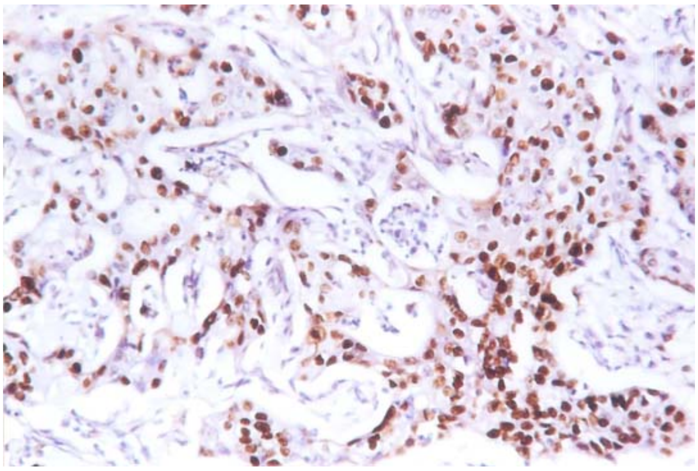

Fig. 2. Colorare nucleară distinctă omogenă sau granulară pentru p53 (++) într-un ADK moderat diferențiat, IHC x40.

- 15 din cele 26 de carcinoame gastrice difuze (57,6%) prezentau supraexpresia p53. Această supraexpresie a fost cuantificată cu ++ pentru 5 cazuri şi cu +++ în 10 cazuri (figura 3);

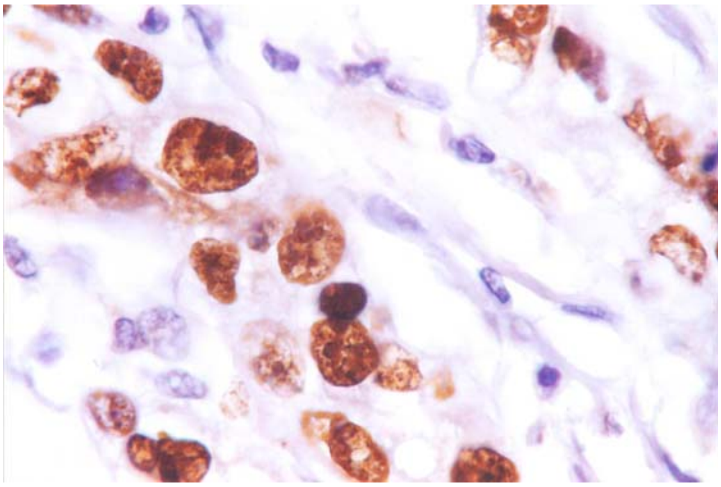

Fig. 3. Colorare nucleară distinctă omogenă sau granulară pentru p53 (+++) într-un carcinom gastric difuz, IHC x100.

- cele două forme mixte au prezentat amândouă supraexpresia proteinei P53, una fiind cuantificată cu + şi cealaltă cu ++.

*Tabelul nr. 2***.** Rezultatul testului de corelație p53 vs. clasificarea Lauren

	r-coeficient de corelație (95% interval de confidență)	*p*
Testul Spearman Rank R	0.5450	0.025434

Tabelul nr. 3. Supraexprimarea p53 vs. clasificarea Lauren

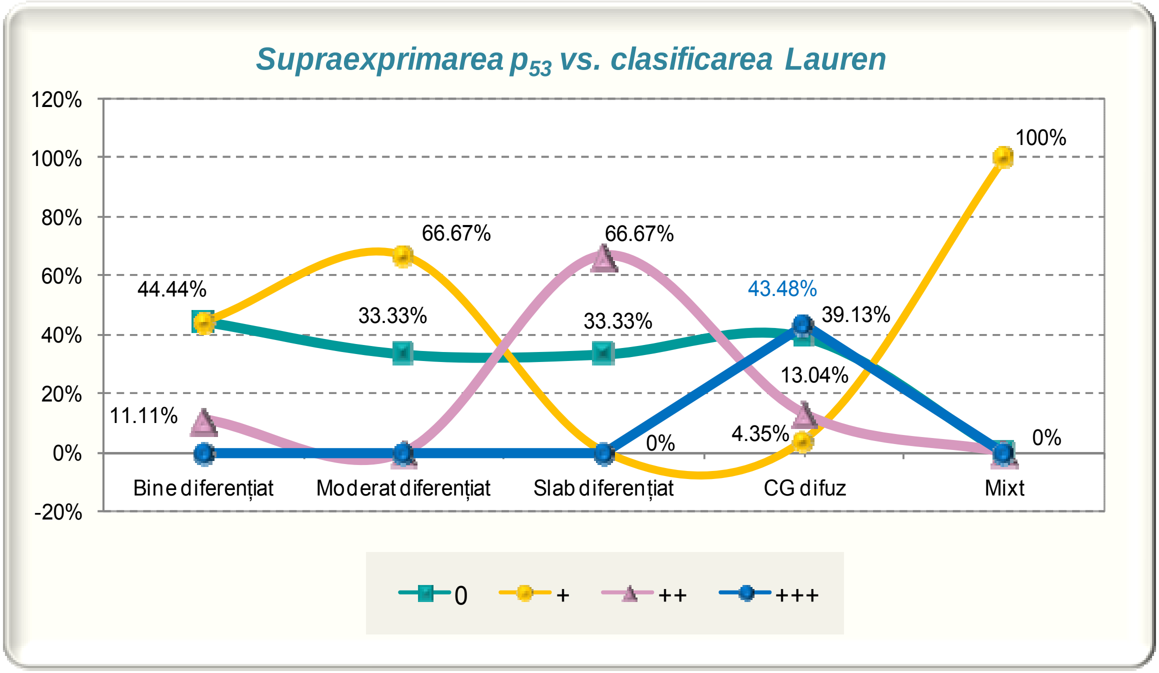

*Tabelul nr. 4***.** Parametrii estimați în testarea asocierii p53 vs. clasificarea Lauren

	Chi-pătrat $\chi 2$	*df*	*p* *95% interval de încredere*
Chi-pătrat - $\chi 2$	28.44874	df=12	p=0.00476
Yates Chi-square	29.71917	df=12	p=0.00308
Coeficient de corelație (Spearman Rank R)	0.630572		p=0.01088

Atât testul parametric Chi-pătrat, cât şi coeficientul de corelație neparametrică demonstrează prezența unei corelații semnificative între supraexprimarea p53 şi clasificarea Lauren ($\chi 2$=28.44, p=0.00476, r=0.6319, 95%CI) (tabelele 2, 3, 4).

Corelând supraexpresia p53 cu clasificarea Goseki, am identificat următoarele:

-tipul I – din 8 cazuri, 4 (50%) au prezentat supraexpresia p53. Această supraexpresie a fost cuantificată cu + pentru 3 cazuri şi ++ pentru 1 caz (care a avut corespondent forma moderat diferențiată din clasificarea Lauren) (figura 4);

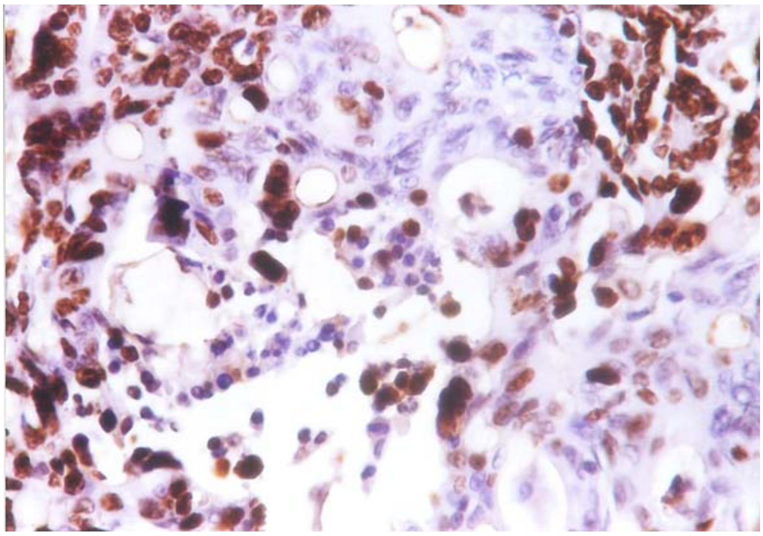

Fig. 4. Colorare nucleară distinctă omogenă sau granulară pentru p53 (++) într-un ADK moderat diferențiat, IHC x40

- tipul II – din 4 cazuri existente, 3 (75%) au prezentat supraexpresia p53, aceasta fiind cuantificată cu +;
- tipul III – din 16 cazuri existente, 11 (68.8%) au prezentat supraexpresia p53, aceasta fiind cuantificată cu + în 2 cazuri, ++ în 2 cazuri şi cu +++ în 7 cazuri;
- tipul IV -din cele 12 cazuri existente, 7 (58,3%) au prezentat supraexpresia p53, aceasta fiind cuantificată cu + un caz, ++ în 3 cazuri şi cu +++ în 3 cazuri (figura 5).

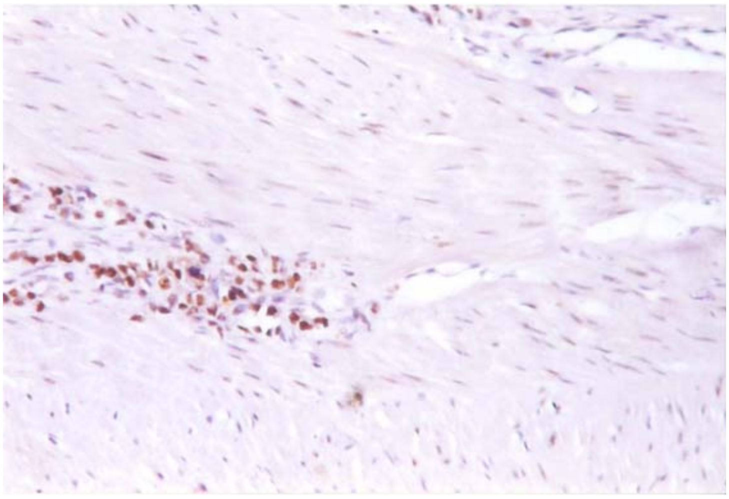

Fig. 5. Colorare nucleară distinctă omogenă sau granulară pentru p53 (+++) într-un carcinom gastric difuz. Invazie în pătura musculară, col. IHC x20.

Cele două cazuri mixte din clasificarea Lauren în clasificarea Goseki au reprezentat un amestec de formele I şi III, ambele prezentând supraexpresia p53, +.

Tabelul nr. 5. Parametrii estimaţi în testarea asocierii p53 vs. clasificarea Goseki

	Chi-pătrat $\chi 2$	*df*	*p* *95% interval de încredere*
Chi-pătrat - $\chi 2$	44.95370	df=9	0.009223
Yates Chi-square	46.47770	df=9	0.005757
Coeficient de corelaţie (Spearman Rank R)	0.5450		0.025434

Studiul asocierii p53 vs. clasificarea Goseki demonstrează existenţa unei corelaţii semnificative între supraestinarea p53 şi clasificarea Goseki ($\chi 2$=46.47, p=0.009, 95%CI), explicată printr-un procent ridicat de cazuri ce nu prezintă p53 şi aparţin clasei I şi a unui procent ridicat de cazuri ce prezintă p53 (++, +++) şi aparţin clasei IV (tabelele 5,6).

Tabelul nr. 6. Rezultatul testului de corelaţie p53 vs. clasificarea Goseki

	r-coeficient de corelaţie (95% interval de confidenţă)	p
Testul Spearman Rank R	0.5450	0.025434

Discuţii

Gena supresoare p53 (tipul „wild") induce apoptoza, în timp ce proteina mutantă blochează funcţia formei „wild" inhibând inducţia apoptozei. Acumularea proteinei p53 mutante se poate comporta ca un imunogen, care poate produce anticorpi anti-p53. Imunoreacţia pozitivă la p53 este corelată cu cantitatea genei p53 mutante putând evidenţia proliferarea celulară şi inhibiţia apoptozei; ea este observată predominant în nucleu ca un precipitat brun.

În condiții normale, p53 este considerată „gardianul genomului” (proteina p53 este un factor de transcripție care monitorizează stabilitatea genomului) sau „genă duală”, deoarece în stare normală funcționează ca antioncogenă, stopând ciclul celular şi permițând repararea ADN modificat, iar modificată devine oncogenă.

Alterarea ADN determină acumularea proteinei p53, care stimulează gena cip1, genă care codifică o proteină numită p21 (21 KDa) şi care inhibă kinazele ciclic-dependente (p21 se mai numeşte cip-cdk inhibitor protein). Astfel este blocată proliferarea celulară. Dacă repararea mutației este insuficientă prin oprirea ciclului celular în G1, atunci p53 induce moartea celulară prin apoptoză, pentru a se evita perpetuarea erorii la celulele fiice. (10)

Mutațiile genei p53 (gena supresoare a tumorilor) sunt întâlnite în 50% dintre cancerele mamare. Pierderea şi/sau alterarea proteinei p53, ca urmare a rearanjamentelor genei, poate determina dereglarea creşterii celulare, prin erori de replicare şi acumulări genetice.

În celulele în care p53 este inactivată, nu este oprită replicarea, cu o consecutivă instabilitate genetică. Această instabilitate se exprimă în timpul diviziunii celulare, favorizând o selecție rapidă de clone aneuploide sau de celule rearanjate.

Mutația punctiformă a genei p53 reprezintă evenimentul genetic cel mai frecvent întâlnit în cancerele umane, aceasta conducând la inactivarea funcției de legare specifice proteinei p53 la ADN.

Pierderea alelică a TP53 apare în peste 60% dintre cazurile cu carcinoame gastrice, iar mutațiile sunt identificate în aproximativ 30-50% dintre cazuri, procentele depinzând de metoda de screening mutațional şi de mărimea, dimensiunile, probei. Mutațiile TP53 sunt identificate în unele metaplazii intestinale, majoritatea alterațiilor afectând tumorile avansate. (11)

Mutațiile TP53 din cazul leziunilor gastrice sunt asemănătoare cu cele observate în alte cancere cu predominantă de tranziții de baze, mai ales la nivelul nucleotidelor CpG.

Mutațiile punctiforme determină prin modificarea secvenței aminoacizilor schimbarea configurației spațiale a proteinei p53. Modificările conformaționale induse de aceste mutații (considerate etape critice în transformarea malignă a celulelor) se asociază cu stabilizarea produsului genei p53 (12) şi cu acumularea proteinei mutante în nucleii celulelor tumorale în cantități suficiente pentru a fi detectate IHC. (13)

Pentru a studia semnificația prognostică a supraexprimării proteinei P53 şi a implicării sale prognostice, am analizat expresia acesteia utilizând anticorpul monoclonal anti-p53 (Biogenex DO7-AM).

Imunoreacția p53 pozitivă, semnalată în 62,5% dintre cazuri, a permis nu numai identificarea tumorilor cu acumulare nucleară a proteinei, ci şi subclasificarea tumorilor p53 pozitive în raport cu paternul imunocolorării şi procentul de celule pozitive din fiecare leziune.

Folosind clasificările Lauren şi Goseki corelate cu supraexpresia p53, s-a observat că în formele bine diferențiate (tip I Lauren, tip I şi II Goseki) a predominat o intensitate a colorării cuantificată cu + sau ++. Procentul tumorilor pozitive pentru p53 din această categorie a variat, fiind reprezentat de 66% pentru tip I din clasificarea Lauren şi 50, respectiv 75% pentru tipul I şi II din clasificarea Goseki.

În formele difuze (tipul II, clasificarea Lauren şi III, respectiv IV, clasificarea Goseki) s-a observat o intensitate a colorării cuantificată cu ++, respectiv +++. Procentul tumorilor pozitive a variat, fiind reprezentat de 57,6% (tip II, clasificarea Lauren) şi 64,2, respectiv 50%, în tipul III şi IV Goseki.

Dacă din punctul de vedere al intensității paternului de colorare putem spune că acesta a crescut de la formele bine diferențiate, cuantificate cu +, spre formele slab diferențiate, cuantificate cu +++, anticipând un comportament agresiv din punct de vedere al procentului tumorilor pozitive, observăm că avem un număr mai mare de tumori pozitive p53 în formele bine diferențiate (tip I Lauren, 62,5%, tip I – II Goseki, 50-75%) comparativ cu formele slab diferențiate (tip II Lauren, 57,6%, tip III, IV Goseki, 64,2%, respectiv 50%). Acest fapt ne face să ne gândim că p53, ca unic indicator de prognostic, nu are o valoare consistentă, fapt explicat de acel polimorfism la nivelul codonului 72, şi anume că acesta este codificat mai frecvent de o prolină decât de o arginină. Ori se ştie că aceasta din urmă este prezentă frecvent în cancerele gastrice antrale.

Altă concluzie la care ajungem este că aceste aspecte ar putea explica de ce o tumoră cu aspecte histologice bine diferenţiate de la care ne-am aştepta la un prognostic bun poate avea un comportament agresiv în totală neconcordanţă cu descrierea histopatologică.

Concluzii

Pozitivitatea pentru p53 în lotul studiat de 40 de cazuri a fost exprimată în 66% dintre carcinoamele gastrice, cu o creştere a intensităţii paternului de colorare de la formele bine diferenţiate cuantificate cu + spre formele slab diferenţiate cuantificate în +++. Această evoluţie anticipează comportamentul agresiv al tumorilor.

Din punctul de vedere al procentului de tumori pozitive, pentru p53 nu există o corelaţie între procentul de tumori pozitive şi gradul de diferenţiere al acestora, ceea ce ne face să ne gândim că p53, ca unic indicator de prognostic, nu are o valoare consistentă.

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Reacția morfogenetică in vitro la specia *Hypericum perforatum* L.

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Rezumat. Ca urmare a inițierii culturii in vitro la sunătoare, au fost inoculate explante pe medii cu diverse formule hormonale. Testarea reacției morfogenetice a vârfurilor de lăstari și a nodurilor a evidențiat cea mai bună reacție caulogenetică pe mediile B (BAP: 0,2-2mg/l), BK (1,0mg/l BAP+0,5mg/l kinetină) și MS, iar cea mai bună reacție rizogenetică pe A_2 (2mg/l IAA). Dintre explantele testate, vârfurile de lăstari și nodurile au generat neoplantule.

Cuvinte cheie: sunătoare, micropropagare, reacție morfogenetică, regulatori de creștere.

Abstract. To initiate the St. John's wort in vitro culture and to decipher the morphogenetic reaction of this species, explants of various origins were inoculated on culture media with different hormone formulae. The best caulogenetic response was induced by the apexes of young shoots and the stem nodes placed on B medium (BAP 0.2 – 2.0 mg/l), BK medium (1.0 mg/l BAP + 0.5 mg/l kinetin), and MS medium, while a significant rhizogenesis response was evidenced on A2 medium (IAA 2.0 mg/l). From the tested explants, the apexes of young shoots and the stem nodes generated neoplantlets.

Introducere

Hypericum perforatum este o cunoscută plantă medicinală, având acțiune coleretică, colagogă, antimicrobiană, antimicotică, antidepresivă. În medicina modernă specia este recomandată în special pentru efectul ei antidepresiv și studiată intens pentru efectul antiviral (1, 2, 3, 4). Având în vedere valoarea farmaceutică a sunătoarei, am considerat util să îi cunoaștem reacția morfogenetică in vitro, în ideea elaborării unei tehnologii de micropropagare a unor forme și linii valoroase obținute prin metode clasice de ameliorare.

Material și metodă

Primul pas în inițierea culturilor in vitro l-a constituit obținerea de plantule din semințe germinate septic, în luna decembrie, 2004. Am utilizat ca explante cotiledoane și plantule, care, după sterilizare și spălare în 2 - 3 băi de apă distilată sterilă, au fost inoculate pe mediile de inițiere Murashige-Skoog (1962), B_{02} (0,2 mg/l BAP) și B_2 (2 mg/l BAP), generând neoplantule sterile ce au servit ca sursă de explante. Reacția acestor explante (vârfuri de lăstari, noduri) a fost testată pe diverse formule de medii, în funcție de scopul urmărit. Sterilizarea mediilor de cultură s-a efectuat prin autoclavare la 121°C, timp de 15 minute. Toate operațiunile de transfer, inoculare și subcultivare s-au făcut cu instrumentar steril, în boxa cu flux laminar.

Culturile s-au realizat în flacoane Erlenmeyer de 100 ml (tip B). Ca sursă de carbon am folosit zaharoza (25 g/l mediu), iar pentru a solidifica mediile de cultură am utilizat agarul (8 g/l). Vasele inoculate au fost incubate într-o cameră cu condiții semiclimatizate (temperatură de 23 - 25°C, intensitatea luminii de neon de 2 500 lucşi, iluminare continuă). Neoplantulele obținute pe unele formule de mediu au fost acomodate la condiții septice în sistem hidroponic. Subculturile au fost efectuate la 3 - 4 săptămâni, interval după care se consideră că rezervele nutritive ale mediului s-au epuizat.

Menționăm că în stabilirea protocolului pentru inițierea şi realizarea culturilor in vitro am urmat unele recomandări făcute de o serie de autori (5, 6, 7).

Rezultate şi discuții

Trebuie menționat faptul că introducerea sunătoarei (*Hypericum perforatum* L.) în sistemul de cultivare „in vitro” nu a întâmpinat dificultăți. Explantele inițiale (plantule şi cotiledoane provenite din semințe germinate) au fost sterilizate cu $HgCl_2$, timp de 5 minute şi cu cloramină-T, timp de 10 minute. Aceşti agenți de aseptizare s-au dovedit eficienți, numărul de infecții fiind mic în cultura in vitro.

Mediul nutritiv de inițiere a facilitat continuarea creşterii, dezvoltării lăstarilor şi generarea de neoplantule ce au servit ca sursă de explante pentru testarea reacției morfogenetice a sunătoarei pe diferite formule hormonale ale mediului MS (*Murasige Skoog).* Deşi nodurile şi vârfurile de lăstari au fost cele mai folosite explante, am testat de asemenea reacția internodurilor şi calusurilor de internod pe o serie de formule de mediu (tabelul nr. 1).

Mediul de bază MS, deşi fără adaos de fitoregulatori de creştere, a produs o reacție caulogenă foarte bună, stimulând lăstărirea multiplă (figura 3). Lăstarii sunt foarte bine dezvoltați, cu frunze mari, de culoare verde-închis. Şi rizogeneza a fost bine reprezentată pe acest mediu, rădăcinile (fără ramificații secundare) înregistrând o viteză de creştere medie.

Am observat că inoculând vârfuri de lăstari şi noduri pe medii de cultură care conțineau între 0,2 şi 2,0 mg/l BAP, reacția a fost bună, şi anume formarea de lăstari. Aceştia au crescut şi au generat rădăcini nu foarte puternice, neramificate (figura 1). De asemenea, BAP favorizează fenomenul de lăstărire multiplă (figura 5). Lăstarii au o culoare verde mai închis decât pe alte formule de mediu. La concentrații crescute de BAP (până la 2 mg/l) este favorizată calusogeneza, formându-se un calus granular verde-închis. Din acest calus este generată lăstărirea multiplă abundentă, lăstarii având 2-3 ramificații bazale, însă o înălțime redusă.

În cazul combinării BAP(benzilaminopurina) cu 2,4-D (acid diclorfenoxiacetic), explantele reprezentate de noduri şi vârfuri de lăstari au produs o cantitate mai mare de calus friabil verde cu aspect granular. Reacția caulogenă a fost una slabă, lăstarii generați fiind puțini la număr şi firavi. Spre deosebire de mediul suplimentat doar cu 2,4-D, calusul produs pe BD a fost regenerativ (caulogen), pe când cel de pe mediul D, deşi în cantitate mai mare, s-a dovedit a fi neorganogen şi cu tendințe de degenerare. Asociind BAP cu NAA, explantele au avut o reacție modestă în ceea ce priveşte lăstărirea multiplă. Am remarcat prezența calusului granular verde, din care se formează lăstari cu 2-3 ramificații bazale.

Mediul MS suplimentat cu două citochinine, şi anume BAP şi kinetină, a fost caracterizat de o reacție caulogenă foarte bună, favorizând lăstărirea multiplă. Lăstarii au aspect de tufă şi sunt viguroşi pe acest mediu, fiind apăruți atât direct, cât şi indirect (din calus friabil verde-închis cu proliferare moderată).

Deşi mediul cu BAP şi acid giberelic (BG) determină tot lăstărirea multiplă, reacția fiind mai slabă decât pe cel descris anterior, în sensul că lăstarii nu sunt viguroşi, prezintă frunze mai mici, adesea au aspect vitrozat.

Prin asocierea kinetinei cu o auxină (2,4-D), caulogeneza nu a fost total inhibată, însă lăstarii apăruți au avut o dezvoltare redusă, la baza lor putând fi observat un manşon de calus friabil verde-deschis, cu tendințe de degenerare. Utilizând kinetina combinată cu o altă auxină, NAA, reacția a fost mai bună, remarcând caulogeneza (atât directă, cât şi indirectă). Lăstarii apăruţi nu erau ramificaţi şi au înregistrat o creştere lentă.

Nodurile şi vârfurile de lăstari au fost cultivate şi pe medii în care s-au înglobat numai auxine. Cea mai bună reacţie s-a obţinut pe mediul suplimentat cu IAA (0,5-2,0 mg/l), caz în care lăstarii apăruţi au fost viguroşi, neramificaţi bazal, cu viteză de creştere bună. Pe acest mediu s-au obţinut rădăcini bine dezvoltate (3-4/lăstar) şi lungi (figura 2). Am constatat o uşoară calusare la baza lăstarilor (foto 4).

Formula înglobând doar NAA (0,5-2 mg/l) a favorizat în special calusogeneza, apărând un calus friabil verde cu proliferare medie, uneori rizogen (producând rădăcini scurte, groase, cu peri absorbanţi). Varianta cu 2 mg/l NAA a generat lăstari prin caulogeneză indirectă, spre deosebire de varianta conţinând cantităţi mai mici de NAA, caz în care creşterea şi dezvoltarea lăstarilor sunt inhibate. Mediul suplimentat doar cu 2,4-D a blocat complet formarea lăstarilor, doar calusul fiind singura formaţiune care apare pe acest mediu.

Ca explante am utilizat şi internodurile. Când fragmentele de internod au fost inoculate pe medii cu auxine (A_1, A_2, D_2, N_2), explantele au calusat (fie doar la capete – conferindu-le aspect de haltere, fie pe toată suprafaţa), calusul fiind friabil, cu proliferare medie sau slabă, uneori cu tendinţe degenerative. De multe ori acest calus este rizogen. Pe mediul în care am înglobat BAP internodurile au generat calus friabil verde la capete, din care sporadic au fost generaţi lăstari.

Reacţia unor caluşi de internod obţinuţi pe unele formule hormonale a fost testată prin pasarea pe alte formule de medii, în felul acesta încercând inducerea diferenţierii, adică testarea capacităţii organogene a acestor caluşi.

Pe medii cu auxine, reacţia a fost variată. Formulele cu IAA au dus la proliferarea acestui calus friabil verde ce a produs lăstari cu dezvoltare medie, dar mai ales rădăcini (în special pe mediile cu cantităţi mai mari de IAA, cum este A_2, unde rădăcinile au fost viguroase, cu peri absorbanţi). Pe mediul suplimentat doar cu NAA, calusul a arătat tendinţe de degenerare.

De asemenea, o reacţie slabă au avut-o caluşii pe mediul înglobând BAP şi NAA, nefiind regenerativi. Reacţie caulogenă medie s-a obţinut pe mediul cu BAP şi kinetină, cele două citochinine combinate favorizând apariţia de lăstari, însă cu dezvoltare modestă.

Cea mai bună reacţie a calusului de internod obţinut pe medii cu BAP am înregistrat-o la transferul acestuia pe mediul cu kinetină şi 2,4-D, combinaţia aceasta ducând la formarea de lăstari bine dezvoltaţi, cu viteză de creştere bună (foto 6). Am verificat şi reacţia unor caluşi spumoşi de noduri, cu proliferare intensă, obţinuţi pe mediul BN şi transferaţi pe BAD, o combinaţie între o citochinină (BAP) şi două auxine (IAA şi 2,4-D) în cantităţi mici. Acest calus a fost organogen, facilitând apariţia fenomenului de lăstărire multiplă de intensitate medie (figura 7).

Luând în considerare cele menţionate anterior, putem afirma că numeroasele formule hormonale testate, incluzând aici şi mediul „martor” MS, generează formarea de neoplantule din explantele reprezentate de vârfurile de lăstari şi noduri, mai dificil din alte tipuri de explante. Specia reacţionează bine la condiţiile de cultivare „in vitro”, doar pe mediul cu GA3 (acid giberelic) am constatat apariţia hiperhidriei, lăstarii având aspect vitrozat.

Neoplantulele obţinute au fost acomodate în sistem hidroponic la temperatura camerei (circa 20°C), însă acest proces întâmpină dificultăţi (figura 8). În concluzie, cea mai bună reacţie caulogenă am obţinut-o pe mediile cu BAP şi pe cele cu BAP în combinaţie cu kinetina (Kin.), urmată de reacţia pe MS lipsit de fitoregulatori, iar din punct de vedere al rizogenezei, pe mediul A_2 (cu o concentraţie mai mare de IAA, mai precis 2 mg/l).

Deoarece interesul pentru utilizarea hipericinei ca agent terapeutic a crescut, în ultimul timp s-a investigat potenţialul culturii de ţesuturi a speciei *Hypericum perforatum* L. în scopul stabilirii unui sistem de propagare, pentru selecţia liniilor celulare cu înaltă productivitate.

În studiile efectuate asupra *Hypericum perforatum* L., Broderick et al. (8) au dezvoltat un sistem de producere a hipericinei în cultura in vitro, o tehnică rapidă de propagare a țesuturilor calusate şi de multiplicare a lăstarilor.

Utilizând ca explante pentru inițierea culturii in vitro muguri axilari şi apicali (8), autorii citați au obținut, prin organogeneză directă, muguri care au regenerat în urma subcultivării pe medii de regenerare. Lăstarii transferați pe mediul de înrădăcinare au evidențiat capacitate rizogenetică bună. Autorii şi-au axat studiile în direcția optimizării proceselor de caulogeneză şi rizogeneză la această specie.

Rezultatele experimentale obținute constituie premisa utilizării micropropagării pentru obținerea unor indivizi cu caracteristici bioproductive superioare.

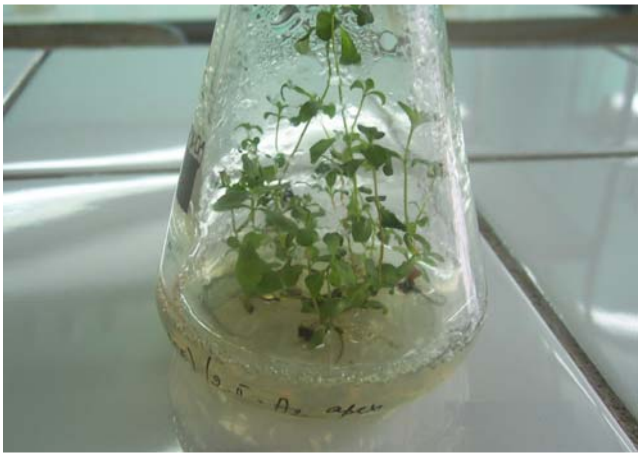

Fig. 1. Aspectul lăstarilor şi rădăcinilor secundare (A_2).

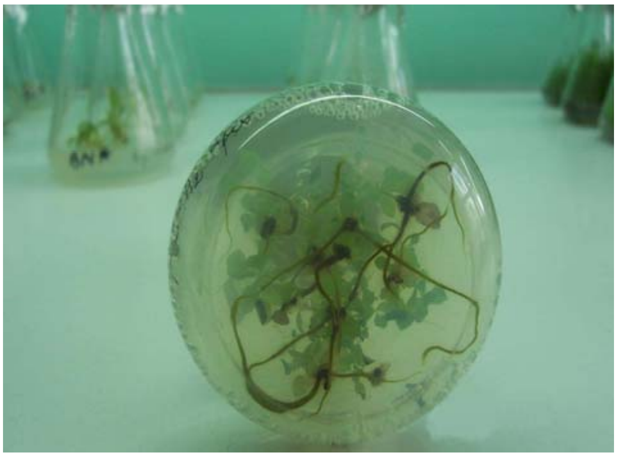

Fig.2. Rădăcini puternice, fără ramificații după circa o lună de la inițierea culturii (B_{02}).

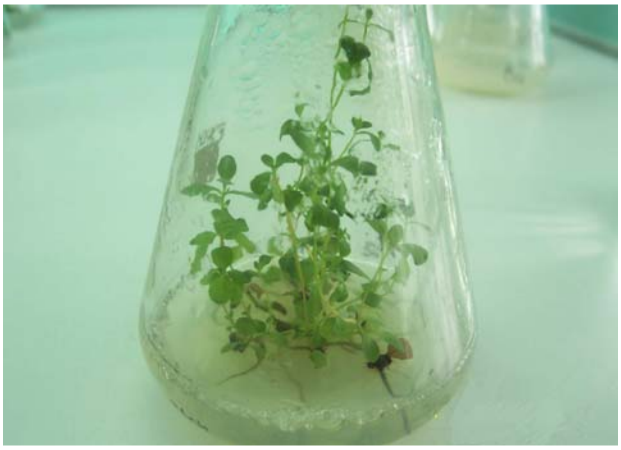

Fig. 3. Lăstărire multiplă intensă pe mediul MS fără hormone.

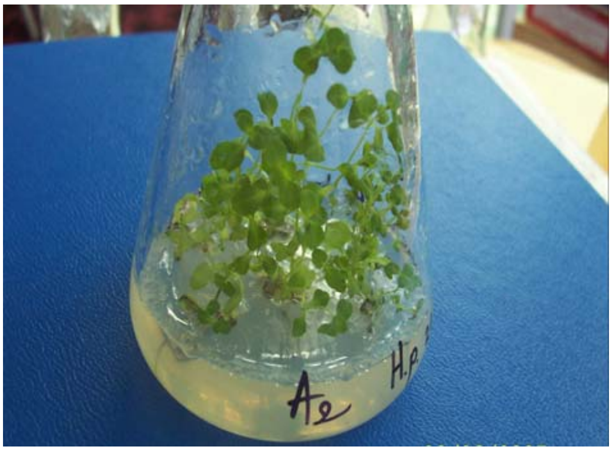

Fig. 4. Neoplantule obținute din apex caulinar pe A_2.

Tabelul 1. Reacția morfogenetică a unor explante de *Hypericum perforatum* L. în cultura in vitro

Var	Explantul	Formula hormonală	Regulatori de creştere (mg / l)							Reacția morfogenetică a explantelor şi viteza de proliferare
			BAP	2,4-D	GA3	IAA	IBA	Kin	NAA	
1	Vârfuri de lăstari Noduri	MS	-	-	-	-	-	-	-	Lăstărire multiplă (+++); lăstarii sunt bine dezvoltați, cu frunze mari, de culoare verde-închis; rizogeneză (++), cu rădăcini de circa 2 cm lungime, neramificate secundar, filamentoase, de culoare verde – deschis.
2	—″—	A	-	-	-	0,5-2,0	-	-	-	Lăstari viguroşi, neramificați bazal, cu înălțime de până la 8 cm (++) ; rizogeneză tot mai bine reprezentată o dată cu creşterea concentrației de IAA (+++), cu rădăcini bine dezvoltate (3-4/lăstar), lungi (3-4 cm) ; uşoară calusare la baza lăstarilor.
3	—″—	B	0,2-2,0	-	-	-	-	-	-	Lăstărire multiplă (++), lăstarii au culoare verde mai intens față de alte formule de mediu; rizogeneză (+) cu rădăcini slab dezvoltate, neramificate secundar; pe B2: calus granular (++), de culoare verde - închis, generat de noduri, ce generează lăstărire multiplă abundentă (+++), lăstarii au 2-3 ramificații bazale şi înălțime redusă (circa 5 cm).
4	—″—	BD1	1,0	0,5	-	-	-	-	-	Caulogeneză indirectă (+), reacție slabă; toate explantele nodale au calusat, producând calus friabil granular verde (++), caulogen; sporadic rizogeneză adventivă (+), cu rădăcini scurte, firave.

5	—″—	BN	1,0	-	-	-	-	-	0,01-0,5	Lăstărire multiplă (+), frunze curbate spre interior ; calus (++) granular verde-închis din care cresc lăstari cu 2-3 ramificații bazale.
6	—″—	BK	1,0	-	-	-	-	0,5	-	Lăstărire multiplă (++), lăstarii au aspect de tufe, sunt viguroşi, cu 2-5 ramificații bazale, apăruți fie prin caulogeneză directă, dar şi indirectă, din calus (++) granular verde-închis generat de noduri
7	—″—	BG1	1	-	0,5	-	-	-	-	Lăstărire multiplă (++), aspect de tufe, însă lăstarii nu sunt viguroşi, prezintă frunze mici, uneori având aspect vitrozat
8	—″—	KD1	-	0,5	-	-	-	1,0	-	Reacție slabă (+), lăstari cu dezvoltare redusă, cu un manşon de calus friabil vernil la bază; calus (+) verde friabil cu aspect granular ce tinde să degenereze
9	—″—	KN1	-	-	-	-	-	1,0	0,5	Caulogeneză (+), lăstari cu dezvoltare medie apăruți prin caulogeneză directă şi indirectă (din calus (++) granular verde); lăstarii (cca 4 lăstari/calus) nu sunt ramificați, prezintă creştere lentă
10	—″—	D2	-	2,0	-	-	-	-	-	Calusogeneză (++), calus friabil verde neorganogen cu tendințe degenerative
11	—″—	N	-	-	-	-	-	-	0,5-2,0	Caulogeneză (+); formare de calus (++) friabil verde ca un manşon la baza explantelor; rizogeneză cu rădăcini scurte, groase, cu perişori absorbanți albi; sporadic rizogeneză adventivă (+)
12	Internoduri	A1	-	-	-	1,0	-	-	-	Calusogeneză la capetele explantelor, calus friabil verde (+) ; sporadic internodurile au generat lăstari slab dezvoltați, cu rădăcini (+) şi rădăcini adventive (+)

13	—″—	A2	-	-	-	2,0	-	-	-	Calus pe toată suprafaţa explantelor, mai mult la capetele internodurilor ; calusul e rizogen, generând rădăcini viguroase (++)
14	—″—	B2	2,0	-	-	-	-	-	-	Calus (+) friabil verde la capetele internodurilor ; sporadic din calusul de internod apar lăstari
15	—″—	D2	-	2,0	-	-	-	-	-	Calus (+) friabil verde la extremităţile explantului, conferindu-i aspect de halteră ; calusul prezintă tendinţe degenerative
16	—″—	N2	-	-	-	-	-	-	2,0	Calus friabil vernil pe toată suprafaţa internodurilor (++)
17	—″—	BD1	1,0	0,5	-	-	-	-	-	Calus granular verde (+) pe toată suprafaţa explantelor, cu tendinţă de necrozare
18	—″—	KN	-	-	-	-	-	1,0	0,5	Calus (++) compact verde, cu aspect granular şi viteză de proliferare redusă, neorganogen în majoritatea cazurilor ; sporadic caulogen (+),
19	Calus de internoduri	A1	-	-	-	1,0	-	-	-	Calus (++) granular verde de internoduri, din care au diferenţiat lăstari (++) cu dezvoltare bună, dar şi rădăcini (+)
20	—″—	A2	-	-	-	2,0	-	-	-	Calus (+) granular verde mai ales la capetele explantului ; calusul generează rădăcini viguroase, lungi (4-5 cm) ; calusul este acoperit cu o pâslă fină de peri absorbanţi ; este şi caulogen, dând naştere la lăstari (++)
21	—″—	B02	0,2	-	-	-	-	-	-	Calus de internoduri obţinut pe BK, verde, friabil, care transferat pe B02 generează lăstărire multiplă (++)
22	—″—	N2	-	-	-	-	-	-	2,0	Calus (++) de internoduri cu mici lăstari – nu proliferează, degenerează; dezvoltare slabă a lăstarilor

23	—"—	BN	1,0	-	-	-	-	-	0,01-0,5	Calus (++) de internoduri obținut pe D2, spumos, verde, transferat pe BN nu se mai multiplică, nu diferențiază, se brunifică
24	—"—	BK	1,0	-	-	-	-	0,5	-	Calus (++) de internod spumos verde, obținut pe BD1, pasat pe BK duce la formarea de embrioizi şi lăstari firavi
25	—"—	KD1	-	0,5	-	-	-	1,0	-	Calus de internod obținut pe B02, verde, cu aspect granular, la transferul pe KD generează lăstari (++) bine dezvoltați, cu viteză de creştere bună (++)
26	Calus de noduri	BAD	1,0	0,5		0,1				Calus spumos verde de noduri, cu proliferare intensă, obținut pe BN. Transferat pe BAD, creşte mult în dimensiuni (++), e caulogen, generând lăstărire multiplă (++)

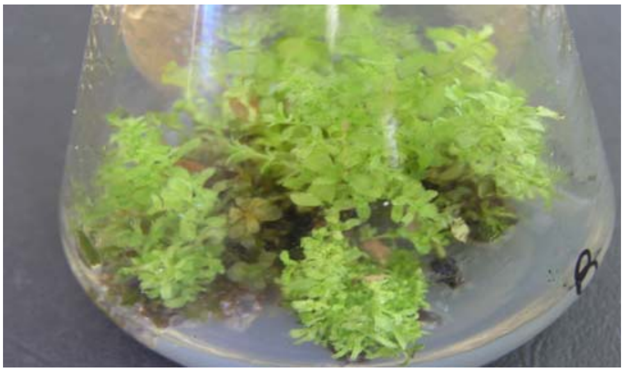

Fig. 5. Lăstărire multiplă pe mediu cu BAP (B2).

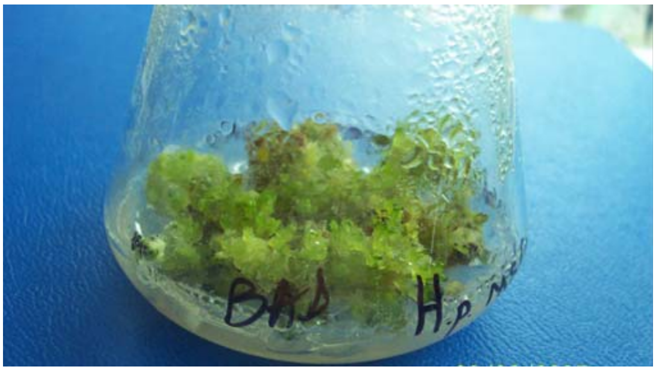

Fig. 6. Lăstari din calus de internod obținut pe B02 şi pasat pe mediul KD.

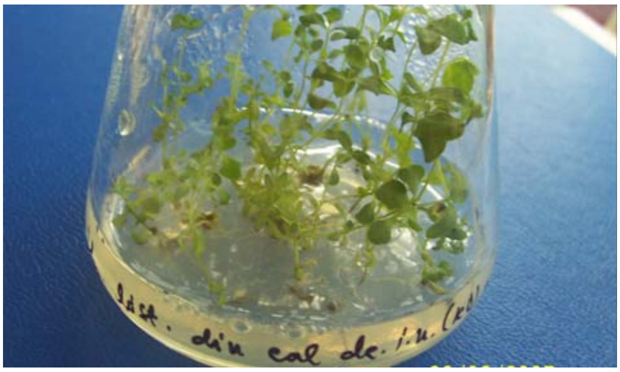

Fig. 7. Calus spumos verde de noduri cu proliferare intensă pe mediul BAD.

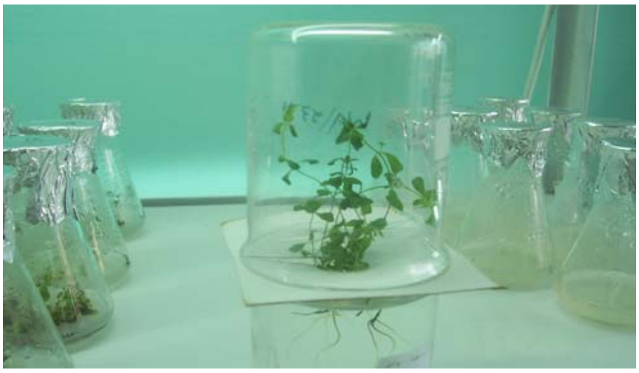

Fig. 8. Acomodarea neoplantulelor în sistem Hydroponic.

Concluzii

- Inițierea culturii in vitro de *Hypericum perforatum* nu ridică probleme, specia reacționând bine la condițiile de cultivare.
- Testând reacția morfogenetică a explantelor (vârfuri de lăstari şi noduri), am observat că cea mai bună reacție caulogenetică şi lăstărire multiplă (directă şi indirectă) se înregistrează pe mediul MS cu adaos de citochinine (BAP şi kinetină).
- Cea mai intensă rizogeneză a fost indusă pe mediul MS lipsit de fitoregulatori de creştere sau suplimentat cu 2 mg/l IAA.
- Principala reacție a explantelor internoduri a fost calusogeneza pe medii cu auxine sau cu citochinine, precum şi combinații ale acestora. Calusul de internod transferat pe mediile B_{02} (cu BAP) şi KD (kinetină +2,4-D) a fost caulogen.
- Neoplantulele sunt sensibile la trecerea în mediul ex vitro, acomodarea în sistem hidroponic realizându-se cu pierderi de material biologic.

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Evaluarea mecanismelor defensive la pacienții cu dublu diagnostic- fibromialgie şi tulburare mixtă depresiv- anxioasă

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Rezumat. ***Fundamentare teoretică:*** **În cazul tulburărilor din spectrul depresiv evaluarea rolurilor şi a organizării mecanismelor de apărare a fost extensiv realizată, însă în cazul patologiei psihosomatice, de exemplu al fibromialgiei, datele furnizate de literatura de specialitate privind importanța defenselor sunt încă insuficiente.**

Obiective: **Evaluarea organizării mecanismelor defensive la pacienții cu dublu diagnostic, fibromialgie şi tulburare mixtă anxios- depresivă.**

Metode: **Un lot format din 12 pacienți, diagnosticați cu fibromialgie, care prezentau şi diagnostic de tulburare mixtă anxios-depresivă, au fost evaluați folosindu-se un interviu orientat psihodinamic şi Chestionarul Mecanismelor Defensive - versiunea cu 40 de itemi (DSQ-40). Pacienții au fost evaluați din perspectiva severității simptomelor depresive şi anxioase cu ajutorul scalei Hamilton pentru anxietate (HAMA) şi a scalei Hamilton pentru depresie - versiunea cu 17 itemi (HAMD); severitatea simptomelor fibromialgiei a fost evaluată pe o scală vizuală analogică (VAS), scorată de la 1 la 10.**

Rezultate: **Evaluarea principalei variabile a arătat existența unui procent ridicat de mecanisme de apărare imature şi nevrotice, în special a somatizării, formațiunii reacționale, des-facerii, disocierii, deplasării şi proiecției. A fost observată o corelație între severitatea simptomelor şi numărul defenselor imature şi nevrotice folosite de pacienți, întrucât pacienții cu simptome severe de fibromialgie (VAS>7) au avut cel puțin 4 astfel de mecanisme de apărare.**

Concluzie: **Pacienții diagnosticați cu fibromialgie şi tulburare mixtă depresiv anxioasă prezintă un procent ridicat de mecanisme defensive disfuncționale; intensitatea simptomelor afective corelează direct proporțional cu factorul "defense imature", iar severitatea fibromialgiei corelează cu factorul "defense imature" şi "defense nevrotice".**

Cuvinte cheie: mecanisme defensive, fibromialgie, tulburare mixtă depresiv anxioasă, evaluare psihodinamică

Abstract. ***Background:*** **While in the depressive spectrum disorder the roles and structure of the defense mechanisms have been intensively studied, in fibromyalgia there are insufficient data regarding the importance of defensive mechanisms.**

Objective: **To assess the organization of defense mechanisms in patients with dual diagnosis, fibromyalgia and mixed anxiety-depressive disorder.**

Methods: **A group of 12 patients, diagnosed with fibromyalgia, who also presented mixed anxiety-depressive disorder, were evaluated using a psychodynamic oriented interview and the Defense Style Questionnaire -40 items version (DSQ-40). Our patients were also evaluated for the depressive and anxiety symptoms severity, using Hamilton Anxiety Rating Scale (HAMA) and Hamilton Depression Rating Scale 17-items version (HAMD), as well as for the fibromyalgia symptoms severity, using a 1 to 10 Visual Analogic Scale (VAS).**

Results: **The evaluation of the main variable showed a high rate of immature and neurotic defenses, especially somatization, reaction formation, undoing, dissociation, displacement and projection. There was a consistent correlation between the severity of symptoms and the number of immature and neurotic defenses used by individuals, as patients with severe symptoms of fibromyalgia (VAS>7) have at least 4 such defenses.**

Conclusion: **Patients diagnosed with fibromyalgia and mixed anxiety-depressive disorder have a high rate of dysfunctional defenses; the severity of affective symptoms correlates proportionally with the immature factor, while the severity of immature and neurotic factors correlates proportionally with fibromyalgia severity.**

Rolul mecanismelor de apărare în patologia depresivă şi psihosomatică

Mecanismele defensive reprezintă strategii psihice inconştiente care au funcţia de a proteja Eul de anxietatea derivată din conştientizarea unor pulsiuni inacceptabile, dar şi de disconfortul antrenat de perceperea sancţiunilor Supraeului sau de frustrările realităţii. Astfel, mecanismele de apărare operează ca răspuns la presiunile interne şi externe exercitate asupra Eului, pentru protecţia stimei de sine, a structurii şi a identităţii sinelui. Un mecanism defensiv devine disfuncţional atunci când este utilizat persistent şi antrenează comportamente dezadaptative, perturbând starea de echilibru psihic a individului.

Ierarhizarea mecanismelor defensive a fost pentru prima dată propusă de Anna Freud şi ulterior operaţionalizată de mai mulţi cercetători, dintre care amintim contribuţiile lui George Vaillant şi ale colaboratorilor săi de la Universitatea Harvard. Mecanismele defensive evoluează odată cu vârsta, trecând în spectrul maturităţii, iar stilurile de apărare mature corelează cu nivelul de sănătate psihică şi chiar fizică [1,2]. Bond şi colaboratorii au creat un instrument de măsurare a mecanismelor de apărare (« Defense Style Questionnaire », DSQ) [3], care a fost ulterior revizuit de Vaillant şi alţi practicieni [4]. Evoluţia defenselor la pacienţii cu depresie majoră a fost monitorizată cu ajutorul DSQ şi s-a dovedit că, sub tratament, folosirea mecanismelor mature a crescut de la un nivel redus la un nivel normal, iar pe parcursul a 2 ani de terapie utilizarea defenselor imature a scăzut treptat [5,6].

Percepţia durerii, modalităţile de exprimare a acesteia şi toleranţa la stimulii algici sunt fenomene supuse modelării sociale şi psihologice. Mecanismele de apărare, alături de constructe la care aceste mecanisme contribuie, cum ar fi imaginea de sine, sentimentul de autooeficienţă şi nivelul de socializare pot fi elemente importante ale strategiilor de a face faţă durerii fizice [7]. De aceea, considerăm că explorarea strategiilor inconştiente de apărare este un demers util în cazul fibromialgiei.

Obiectivul şi metodologia cercetării

Obiectivul actualei cercetări a fost evaluarea structurării mecanismelor defensive la pacienţii care prezintă dublu diagnostic - fibromialgie şi tulburare mixtă depresiv-anxioasă.

În această cercetare au fost incluse 12 persoane, 8 de sex feminin şi 4 de sex masculin, cu vârsta medie 45.6 ani (interval 18-65 de ani), care au fost diagnosticate de cel puţin un an cu fibromialgie şi de minim 6 luni cu tulburare mixtă depresiv-anxioasă. Diagnosticul de fibromialgie a fost stabilit pe baza criteriilor Colegiului American de Reumatologie (1990), iar diagnosticul de tulburare mixtă depresiv-anxioasă pe baza criteriilor Manualului de Diagnostic şi Statistică a Tulburărilor Mentale (DSM IV TR, 2000).

Pacienţii au fost evaluaţi în regim ambulatoriu (n=7) sau pe durata spitalizării pentru una dintre cele două afecţiuni (n=5). Criteriile de excludere au fost reprezentate de vârsta sub 18 sau peste 65 de ani, existenţa unor comorbidităţi psihiatrice de axă I sau II, existenţa bolilor organice şi a tratamentelor farmacologice concomitente. Au fost incluşi în acest studiu doar acei subiecţi care au oprit administrarea medicamentelor pentru una sau ambele afecţiuni cu minim o lună anterior evaluării.

Deoarece mecanismele de apărare reprezintă principala variabilă urmărită, instrumentul de evaluare agreat a fost forma revizuită a chestionarului pentru defense, « Defense Style Questionnaire-40 », care permite cuantificarea a 20 de mecanisme, structurate în 3 factori : factorul « defense mature », compus din suprimare, umor, anticipare şi sublimare ; factorul « defense nevrotice », compus din formaţiunea reacţională, idealizare, pseudoaltruism, des-facere; factorul « defense imature », compus din raţionalizare, fantezie autistă, deplasare, izolare, disociere, devalorizare, negare, agresivitate pasivă, somatizare, acting out, proiecţie şi scindare. Scorurile pentru fiecare defensă sunt distribuite pe o scală Likert şi sunt cuprinse între 1 şi 9, iar scorurile mari arată o utilizare crescută a mecanismelor individuale sau a factorului respectiv. Scorul este direct proporţional cu gradul de acceptare a afirmaţiilor care descriu mecanismele defensive.

Scalele Hamilton pentru evaluarea depresiei (HAMD), versiunea cu 17 itemi şi pentru evaluarea anxietăţii (HAMA) sunt instrumente validate şi utilizate în mod curent în clinica psihiatrică, dar şi în domeniul psihoterapiei, pentru monitorizarea evoluţiei simptomatologiei afective.

Am utilizat şi o scală vizuală analogică, scorată de la 1 la 10, pentru determinarea severităţii simptomatologiei fibromialgiei. Fiecare pacient a fost instruit să-şi scoreze severitatea acuzelor somatice folosind puncte de reper comune (durată, întindere, intensitate, rezistenţă la tratament, frecvenţă). Perioada pentru care pacientul a făcut evaluarea a fost ultima săptămână înaintea prezentării la medic. Scorurile mari au fost atribuite acuzelor somatice mai severe.

Rezultate

În urma evaluării psihodinamice a pacienţilor se observă că mecanismele defensive cele mai folosite de aceştia (fig.1) sunt somatizarea (n=9), formaţiunea reacţională (n=9), des-facerea (n=8), disocierea şi deplasarea (n=6 pentru fiecare mecanism), precum şi proiecţia (n=5). Existenţa somatizării poate sta la baza exprimării conflictelor intrapsihice prin cenestopatii, astfel justificându-se prezenţa acuzelor fără un substrat organic definit, iar formaţiunea reacţională sugerează un stil de coping rigid şi predominant pasiv.

Fig.1. Reprezentarea mecanismelor defensive disfuncţionale cele mai frecvent întâlnite la pacienţii cu fibromialgie şi tulburare mixtă depresiv-anxioasă

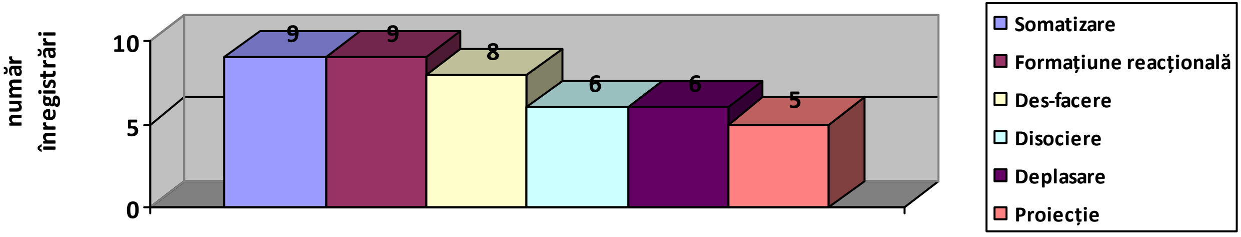

Factorii DSQ-40 intitulaţi "defense imature" şi "defense nevrotice" au valori semnificativ mai mari decât factorul "defense mature" (fig.2), ceea ce sugerează că pacienţii cu fibromialgie şi tulburare mixtă depresiv-anxioasă tind să folosească, într-o proporţie importantă, strategii de apărare disfuncţionale. Aceste mecanisme întreţin simptomatologia algică şi afectivă, creând dificultăţi în adaptarea socială, profesională şi familială a subiecţilor, influenţând insight-ul şi răspunsul terapeutic.

Fig.2. Reprezentarea factorilor DSQ-40 la pacienţii cu fibromialgie şi tulburare mixtă depresiv-anxioasă

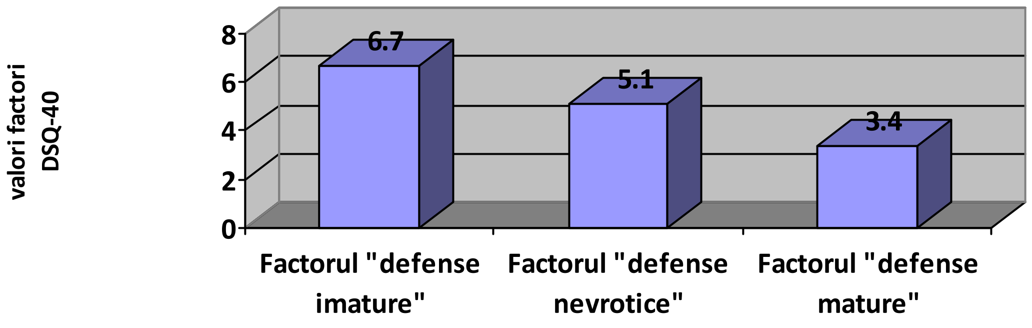

Severitatea fibromialgiei corelează cu numărul defenselor imature şi nevrotice folosite de pacienţi (fig.3). Astfel, dacă o fibromialgie autoevaluată de subiecţi ca având o severitate moderată (VAS 4-7) se asociază cu mai puţin de 4 mecanisme defensive imature sau nevrotice, persoanele care au un scor VAS>7 au prezentat minim 4 astfel de mecanisme.

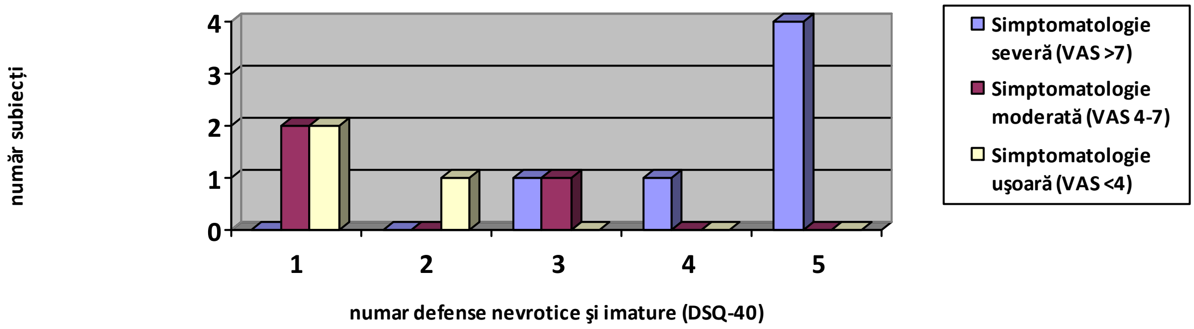

Fig.3. Asocierea mecanismelor defensive imature şi nevrotice cu nivelul de severitate a fibromialgiei

De asemenea, factorii “defense imature” şi “defense nevrotice” corelează cu nivelul de severitate a fibromialgiei (fig.4). Defensele mature sunt cel mai frecvent observate în grupa subiecţilor cu fibromialgie autoevaluată ca având o intensitate uşoară, în timp ce defensele imature şi nevrotice sunt des întâlnite în cazul persoanelor cu fibromialgie moderată şi severă.

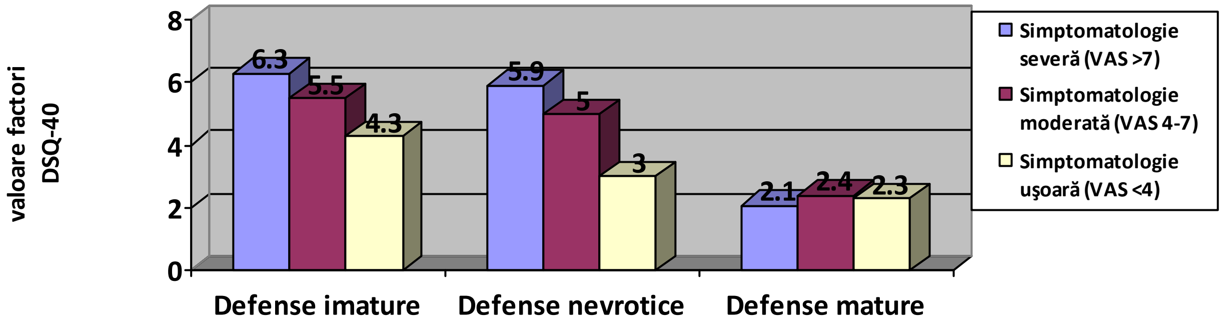

Fig.4. Asocierea factorilor "defense imature" şi "defense nevrotice" cu nivelul de severitate a fibromialgiei

Simptomatologia afectivă corelează cu stilurile defensive utilizate de pacienţi, astfel încât severitatea ridicată a anxietăţii şi a depresiei este mai frecvent asociată cu defensele imature, urmate de cele nevrotice (fig.5).

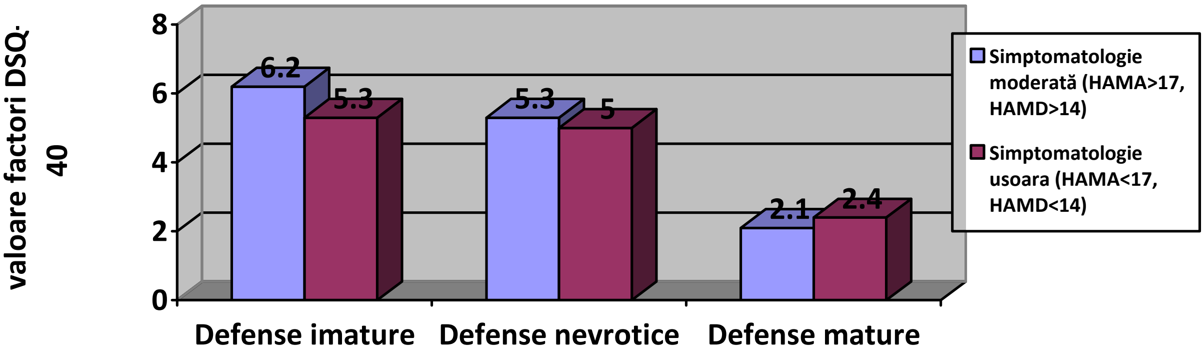

Fig.5. Asocierea factorilor DSQ-40 cu nivelul de severitate a patologiei afective

Concluzii

Evaluarea structurii sistemelor defensive la pacienții cu fibromialgie şi tulburare mixtă depresiv-anxioasă evidențiază prezența, într-o proporție ridicată, a mecanismelor de apărare imature şi nevrotice, în special a somatizării, deplasării, disocierii, des-facerii, formațiunii reacționale şi a proiecției. Există o relație de proporționalitate între severitatea simptomatologiei fibromialgiei şi numărul şi severitatea defenselor imature şi nevrotice. De asemenea, simptomatologia afectivă mai severă se asociază cu defense predominant imature.

Un demers psihoterapeutic va trebui să țină seama de mecanismele de apărare folosite de pacienți, astfel încât o evaluare inițială a acestora va putea ajuta terapeutul în construirea unei strategii eficiente. O astfel de evaluare este utilă şi din punctul de vedere al monitorizării unui tratament farmacologic, întrucât fenomene precum complianța terapeutică, transferul – inerent oricărei relații medic-pacient, reinserția socială, satisfacția pacientului privind tratamentul, percepția unor efecte adverse etc. depind, într-o măsură importantă, de dinamica defenselor.

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Evaluarea riscului chirurgical la pacienții hepatici

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Rezumat: Pacienții cu afecțiuni hepatice care necesită chirurgie au un risc mai mare de complicații chirurgicale şi anestezice comparativ cu cei fără patologie hepatică. Magnitudinea riscului depinde de tipul şi severitatea hepatopatiei, de procedura chirurgicală şi de tipul anesteziei. Pacienții hepatici pot avea afecțiuni concomitente (cardiovasculare, pulmonare etc.) care influențează evoluția chirurgicală. Toți pacienții care urmează să fie operați necesită efectuarea minuțioasă a anamnezei şi a unui examen fizic, pentru excluderea elementelor sau factorilor de risc pentru hepatopatie. Procedura chirurgicală este determinantul cel mai important al disfuncției hepatice postoperatorii la pacientul cu hepatopatie.

Cuvinte cheie: Hepatopatie, evaluare risc chirurgical, management.

Abstract: The patients suffering from liver diseases who may require surgery have an increased risk of anesthetic and surgical complications then those free of liver diseases. The amplitude of the risk depends upon the type and severity of liver disease, the surgical procedure and the type of anesthesia provided. Liver suffering patients may have comorbidities that influence the outcome of surgery. All the patients who are going to suffer a surgical procedure need to have their pathological history taken and to get through a thorough physical examination in order to exclude the elements and risk factors for liver diseases.The surgical procedure is the most important determinant of postoperation liver disfunction in liver suffering patient.

Pacienții cu afecțiuni hepatice care necesită chirurgie au un risc mai mare de complicații chirurgicale şi anestezice comparativ cu cei fără patologie hepatică. Magnitudinea riscului depinde de tipul şi severitatea hepatopatiei, de procedura chirurgicală şi de tipul anesteziei. Pacienții hepatici pot avea afecțiuni concomitente (cardiovasculare, pulmonare etc.) care influențează evoluția chirurgicală. Toți pacienții care urmează să fie operați necesită efectuarea minuțioasă a anamnezei şi a unui examen fizic, pentru excluderea elementelor sau factorilor de risc pentru hepatopatie. Întrebările sunt specifice : despre transfuzii anterioare, tatuaje, utilizarea de droguri ilicite, relații sexuale riscante, istoric familial de icter sau hepatopatie acută, istoric personal de icter sau febră postanestezie, consumul de alcool (curent, anterior, cantitate) şi despre medicația curentă. Vor fi evaluate aspectele clinice obiective sugestive de hepatopatie (fatigabilitate, prurit, mărire de volum a abdomenului, icter, eritem palmar, steluțe vasculare, splenomegalie, ginecomastie şi atrofie testiculară la bărbați).

Evaluarea prechirurgicală a hepatopatiei. Este discutabil dacă anterior chirurgiei, candidații, altfel sănătoşi, trebuie supuşi screeningului biochimic pentru hepatopatie. Majoritatea pacienților depistați cu teste biochimice hepatice anormale nu prezintă hepatopatie avansată. Astfel, este improbabil ca efectuarea de rutină a profilului biochimic hepatic la pacienți aparent sănătoşi, fără factori de risc pentru hepatopatie, să amelioreze evoluția clinică perioperatorie. De aceea, aceste testări nu sunt recomandate.

Efectele anesteziei şi chirurgiei asupra ficatului depind de tipul anesteziei, de procedurile chirurgicale specifice şi de severitatea hepatopatiei. În plus, evenimente perioperatorii ca hipotensiunea, sepsisul sau administrarea de medicamente hepatotoxice pot leza ficatul în timpul procedurii chirurgicale. Deşi creşteri uşoare ale aminotransferazelor serice, fosfatazei alcaline sau bilirubinemiei sunt frecvente după intervențiile chirurgicale, indiferent de tipul de anestezie folosit, ele se rezolvă de obicei fără urmări. Disfuncția hepatică, clinic semnificativă, este infrecventă şi mai probabilă la pacientul cu hepatopatie preexistentă. Un studiu efectuat pe 733 pacienți cu ciroză hepatică operați la Clinica Mayo a consemnat o mortalitate perioperatorie de 11,6% şi o morbiditate (complicații) de 30%, care au fost superioare procentelor consemnate la cei fără hepatopatie. **(1)** Factorii de risc identificați pentru mortalitate sau complicații au fost: scorul Child-Pugh, prezența ascitei, creatininemia crescută, hemoragia digestivă superioară preoperatorie şi încadrarea în scala Societății Americane a Anesteziştilor (tabel nr. 1).

Tabel nr. 1. Scala statusului fizic al Societății Americane a Anesteziştilor (ASA)

Clasa 1	Individ sănătos
Clasa 2	Pacient cu afecțiune sistemică uşoară
Clasa 3	Pacient cu afecțiune sistemică severă fără invaliditate
Clasa 4	Pacient cu afecțiune sistemică invalidantă cu risc vital constant
Clasa 5	Muribund cu speranță de supraviețuire de≤ 24 ore, cu sau fără operație

Tipul de chirurgie este probabil determinantul cel mai important al disfuncției hepatice postoperatorii la pacientul cu hepatopatie. Argumentele generale sunt următoarele:

- laparotomia se asociază cu reducerea mai mare a fluxului arterial hepatic față de chirurgia extraabdominală, mai ales datorită tracțiunii pe viscerele abdominale care determină hipotensiune sistemică reflexă prin dilatarea vaselor de capacitanță;
- procedurile asociate cu pierderi mari de sânge cresc riscul ischemic hepatic;
- ciroticii cu antecedente chirurgicale abdominale pot avea aderențe vasculare perihepatice care pot sângera excesiv intra-operator;
- chirurgia de urgență se asociază cu mortalitate şi morbiditate mai mari comparativ cu chirurgia electivă;
- colecistectomia, chirurgia gastrică şi colectomia se asociază cu mortalitate particular crescută la pacienții cu ciroză decompensată. Date mai recente sugerează o evoluție favorabilă postcolecistectomie. Într-un studiu relativ recent **(2)** scorul MELD ≥ 8 s-a asociat cu morbiditate crescută la 30 şi 90 de zile. Abordarea laparoscopică poate fi fezabilă în cazuri selectate de ciroză compensată. Însă, vor fi luate în considerare opțiuni mai puțin invazive, cum ar fi colecistostomia;
- chirurgia cardiacă la pacienții cirotici este de asemenea asociată cu o mortalitate crescută.

Efectele hemodinamice ale anesteziei asupra ficatului. În studii pe pacienți fără hepatopatie s-a demonstrat că efectele anesteziei generale asupra circulației hepatice sunt variabile. Ca regulă generală, multe anestezice inhalatorii sunt asociate cu reducerea fluxului sanguin portal şi a debitului cardiac. Efectul pe fluxul arterial hepatic depinde de anestezicul specific folosit şi de tipul de operație. Spre exemplu, anestezicele volatile halotan şi enfluran reduc fluxul arterial hepatic datorită vasodilatației sistemice şi a efectului inotrop negativ uşor, pe când isofluranul creşte sau are efect neutru pe fluxul arterial hepatic, motiv pentru care este de preferat la hepatici**. (3)** Deoarece aportul sanguin majoritar hepatic este asigurat de vena portă, efectul net al acestor modificări hemodinamice constă de obicei în reducerea fluxului sanguin hepatic şi a oxigenării hepatice. Magnitudinea acestor modificări la pacienții cu hepatopatie avansată nu a fost suficient studiată. Aceşti pacienți au anomalii circulatorii de fond incluzând indexul cardiac crescut şi rezistența vasculară sistemică redusă, care pot influența fluxul sanguin hepatic pe durata anesteziei. Cirotícii pot fi mai sensibili la hipoperfuzia hepatică față de condițiile bazale. Ca urmare, pot fi mai susceptibili la injuria hepatică, mai ales în fața unor agresiuni hepatice suplimentare (de exemplu, medicamente hepatotoxice sau suprapunerea infecției).

Metabolismul hepatic al anestezicelor inhalate. Agenții anestezici pot fi hepatotoxici prin mecanisme variate. Ca regulă generală, gradul metabolizării hepatice se corelează cu probabilitatea reacției toxice. Isofluranul şi mai noii haloalkani (desfluranul şi sevofluranul) sunt mai puțin metabolizați hepatic față de halotan sau enfluran şi prin urmare se asociază cu un risc mai redus de hepatită. Dintre haloalkanii utilizați frecvent, halotanul se asociază cu cel mai mare risc de hepatotoxicitate. Incidența hepatitei fulminante după expunerea la halotan este de la 1 la 6 000 la 1 la 35 000 după o singură expunere şi creşte la 1 la 3 000 după expuneri multiple. **(4)** Alți factori de risc de hepatotoxicitate severă sunt: sexul feminin, vârsta de peste 50 ani şi obezitatea. Hepatotoxicitatea halotanului a impus eliminarea sa la adulții din SUA. Această toxicitate nu se regăseşte la copii. Datorită potenței sale şi mirosului tolerabil, halotanul continuă să fie larg folosit la copii în SUA, pentru inducția anesteziei. Mai mult, probabil este cel mai utilizat anestezic inhalator la pacienții din întreaga lume.

Ventilația mecanică. Hepaticii au respirația compromisă datorită unor cauze legate direct de hepatopatie (de exemplu, sindrom hepato-pulmonar, colecții pleurale, hipertensiune pulmonară) sau datorită unor afecțiuni asociate, de tipul bronhopneumopatiei cronice obstructive. Hipoxemia severă (pO2 < 60mmHg) este considerată obişnuit o contraindicație relativă pentru chirurgie (exceptând transplantul hepatic în caz de sindrom hepato-pulmonar). Hipercapnia trebuie evitată la hepatici în timpul ventilației mecanice deoarece inițiază stimularea simpatică a vascularizației splanhnice, reducând astfel fluxul sanguin portal. Pacienții cu hepatopatie avansată au de obicei alcaloză respiratorie uşoară, compensată. Astfel, pCO2 trebuie menținut intraoperator între 35-40 mmHg**.** **(5)** De asemenea, în timpul anesteziei la cirotici se va asigura o concentrație de oxigen mai mare ca de obicei.

Tabel nr. 2. Factorii de risc pentru morbiditatea şi mortalitatea cirotícilor care urmează să fie operați

Tipul procedurii chirurgicale
Abdominală (în special colecistectomie, rezecție gastrică sau colectomie) Cardiacă De urgență Rezecție hepatică
Caracteristicile pacientului
Anemia Ascita Clasa Child (C>B) Encefalopatia Hipoalbuminemia Hipoxemia Infecția Icterul Malnutriția Hipertensiunea portală Timpul de protrombină prelungit (>2.5 secunde peste control, necorectat cu vit. K)

Agenții blocanți neuromusculari pot contribui la modificările fluxului sanguin hepatic din timpul anesteziei. Mai mult, efectele lor pot fi prelungite la hepatici datorită excreției biliare reduse, activității reduse a pseudocolinesterazei şi a volumului de distribuție crescut. Antracurium şi Cisatracurium sunt preferați la hepatici sau la cei cu obstrucție biliară deoarece metabolismul lor nu depinde de ficat sau de rinichi. Doxacurium (relaxant nondepolarizant cu acțiune prelungită) este recomandat pentru intervenții prelungite cum este transplantul hepatic**. (6)**

Analgezice şi sedative. La hepatici sunt preferate cele cu durată de acțiune mai scurtă, deoarece agenții cu durată mai lungă cresc riscul postoperator de delirium şi de encefalopatie hepatică. Efectele hepatopatiei pe metabolismul acestora este variabil. Astfel, deşi metabolismul tiopentalului este scăzut la cirotici, legarea de proteinele plasmatice este de asemenea redusă, aşa încât clearence-ul total corporeal este nemodificat. Pacienții hepatici au un risc crescut de reacții hepatotoxice rare la benzodiazepine sau barbiturice. Paternul injuriei este de obicei colestatic şi uneori include un sindrom febril, hepatită, limfadenopatie, eozinofilie şi dermatită (sindrom de hipersensibilitate la anticonvulsivante).

Estimarea riscului chirurgical la pacienții cu hepatopatie include: aprecierea severității hepatopatiei, a urgenței actului chirurgical (şi alternativelor la chirurgie) şi a comorbidităților medicale. Dacă pentru prevenirea decesului este necesară chirurgia de urgență, evaluarea riscului chirurgical este mai puțin relevantă. Dar, de obicei, majoritatea deciziilor sunt luate pentru proceduri elective sau semi-urgente pentru care există timp de evaluare a riscului, de optimizare a statusului medical al pacientului şi de considerare a abordărilor alternative. Majoritatea studiilor de risc chirurgical au fost pe pacienți cirotici (tabel nr. 2). **(7)** Există mai puțină informație despre riscul chirurgical al formelor mai puțin severe de hepatopatie sau despre pacienții cu tipuri specifice de hepatopatie.

Tabel nr. 3. Contraindicațiile chirurgiei elective la pacienții cu hepatopatie

Hepatită acută etanolică
Hepatită acută virală
Ciroză din clasa Child C
Insuficiența hepatică fulminantă
Hepatita cronică severă
Coagulopatia severă (timp de protromobină > 3 secunde, necorectat de vit. K; Număr trombocite < 50 000/ml)
Complicații extrahepatice severe: Insuficiență renală acută Cardiomiopatie, insuficiență cardiacă Hipoxemia

Pacienții la care chirurgia este contraindicată. Sunt cazurile asociate cu mortalitate chirurgicală inacceptabilă. Aceste situații sunt obişnuit considerate contraindicații pentru chirurgia electivă (tabel nr. 3).

Hepatita acută sau fulminantă este contraindicată pentru chirurgia electivă. Recomandarea se bazează pe studii mai vechi, în care mortalitatea operatorie era de 10-13% printre pacienții icterici supuşi laparotomiei ca parte a evaluării diagnostice şi care au fost apoi diagnosticați cu hepatită acută virală**. (8)** Similar, pacienții cu hepatită fulminantă sunt cazuri grave, improbabil să suporte efortul operator cu excepția transplantului hepatic.

Hepatita alcoolică este contraindicată pentru chirurgia electivă la pacienții documentați histologic. Mortalitatea de 55 la 100% a fost semnalată la astfel de pacienții supuşi biopsiei hepatice deschise, şuntului chirurgical porto-sistemic sau laparotomiei exploratorii. Este însă posibil ca evoluția tehnicii chirurgicale şi a îngrijirii postoperatorii să poată ameliora prognosticul acestor pacienți comparativ cu studiile anterioare, unele publicate acum 20-30 de ani. **(9)** Chirurgia electivă va fi amânată cel puțin 12 săptămâni sau va fi repetată biopsia hepatică, pentru confirmarea rezoluției histologice. Severitatea hepatopatiei de fond va fi reevaluată înaintea formulării recomadării finale.

Hepatita cronică severă. Riscul chirurgical se corelează cu clinica, biochimia şi severitatea histologică a bolii hepatice. Riscul chirurgical este crescut în cazul hepatitei cronice severe, histologic şi simptomatic, mai ales la cei cu afectarea funcției de sinteză şi excreție, cu hipertensiune portală sau cu necroză în punți ori multilobulară la puncția hepatică.

Pacienți cu risc crescut variabil. Riscul chirurgical la cirotici depinde de severitatea bolii, starea clinică şi tipul procedurii chirurgicale. De peste 30 de ani, principalul predictor al riscului operator la cirotici este clasificarea Child, dar studii mai recente sugerează că scorul MELD (Model for End Stage Liver Disease) poate fi superior.

Tabel nr. 4. Clasificarea Child – Turcotte

Parametru	**Clasa A**	**Clasa B**	**Clasa C**
Ascita	Absentă	Uşor controlată	Slab controlată
Bilirubinemie	<2 mg/dL (<34,2 micromol/litru)	2-3 mg/dL (34,2 -51,3 micromol/litru)	>3 mg/dL (>51.3 micromol/litru)
Albuminemie	>3.5 g/dL (>35 g/litru)	3.0-3.5 g/dL (30 - 35 g/litru)	<3.0 g/dL (<30 g/litru)
Encefalopatia hepatică	Nu	Uşoară	Avansată
Status nutrițional	Excelent	Bun	Precar

Tabel nr. 5. Clasificarea Child-Pugh a severității hepatopatiei (modificată)

Parametru	**Puncte desemnate**		
	1	2	3
Ascita	Absentă	Uşoară	Moderată
Bilirubinemie	<2 mg/dL (<34.2 micromol/litru)	2-3 mg/dL (34,2 -51,3 micromol/litru)	>3 mg/dL (>51.3 micromol/litru)
Albuminemie	>3.5 g/dL (>35 g/litru)	3.0-3.5 g/dL (30 -35 g/litru)	<3.0 g/dL (<30 g/litru)
Encefalopatie	Nu	Grad 1-2	Grad 3-4
Timp de protrombină(secunde peste limita maximă)	<4	4-6	>6
INR	<1.7	1.7-2.3	>2.3

Scorul total 5-6 este considerat de grad A (hepatopatie bine compensată); 7-8 este în gradul B (compromis funcțional semnificativ); 10-15 este grad C (hepatopatie decompensată). Aceste grade se corelează cu supraviețuirea la 1-2 ani astfel: gradul A – 100-85%, gradul B – 80-60%; gradul C – 45-35%.

Clasificarea Child. Studii retrospective au demonstrat că mortalitatea şi morbiditatea în ciroză se corelează cu clasificarea Child-Turcotte (tabel nr. 4) sau Child-Pugh (tabel nr. 5). Într-unul din studii, mortalitatea perioperatorie a fost de 10, 31 şi 76% la 100 pacienți cu ciroză alcoolică predominantă supuşi chirurgiei abdominale, aflați în clasa Child-Pugh A, B şi respectiv C. În analiză multivariată, clasificarea Child-Pugh a fost cel mai bun predictor al mortalității şi morbidității chirurgicale. Pacienții cu ciroză clasa Child A şi hipertensiune portală au risc postoperator crescut de ascită, icter şi encefalopatie. **(10)** Observații limitate sugerează că morbiditatea postoperatorie poate fi redusă prin plasarea preoperatorie a unui şunt porto-sistemic intrahepatic transjugular.

Măsurarea funcției hepatice şi scorul APACHE. Deşi au fost propuse mai multe teste funcționale hepatice ca predictori ai mortalității şi morbidității perioperatorii la cirotici (de exemplu, teste dinamice funcționale, cum ar fi capacitatea de eliminare a galactozei, testul respirator la aminopirină, clearence-ul cu verde de indocianină), acestea nu au adus informații utile suplimentare pe lângă clasificarea Child-Pugh, neintrând astfel în practica clinică curentă. APACHE III (Acute Physiology, Age and Chronic Health Evaluation System) poate prezice supraviețuirea ciroticilor din unitățile de terapie intensivă, dar nu a fost încă folosit în studii adresate specific ciroticilor operați.

Scorul MELD este un model statistic de predicție a supraviețuirii ciroticilor, folosit mai ales pentru selecția pacienților în vederea transplantului hepatic. Deşi predicția riscului chirurgical pentru intervenții nontransplant este promițătoare şi ar putea în final suplini clasificarea Child, ca metodă principală de determinare a riscului chirurgical, MELD necesită studii suplimentare pentru înțelegerea perfomanțelor sale ca model predictiv în chirurgie. **(11)** Adresa de pe internet: *www.mayoclinic.org/meld/mayomodel9.html* poate fi utilizată pentru calcularea estimativă a mortalității postchirurgicale la 7, 30, 90 zile, la 1 şi 5 ani, pe baza vârstei, clasei ASA, INR, bilirubinei şi creatininei serice.

Tabel nr. 6. Factorii de risc pentru morbiditatea şi mortalitatea pacienților cu icter obstructiv care urmează să fie operați

Azotemia
Colangita
Hematocrit < 30 %
Hipoalbuminemia
Obstrucția biliară malignă
Bilirubinemia > 11 mg/dL (188 μmol/litru)

Icterul obstructiv creşte riscul unor complicații perioperatorii ca: infecțiile (urmare a colonizării bacteriene a arborelui biliar, a disfuncției celulelor Kupffer şi a neutrofilelor şi a frecvenței crescute a endotoxinemiei), ulcerele de stres, coagularea intravasculară diseminată, dehiscența plăgilor şi insuficiența renală. În diverse studii, mortalitatea perioperatorie ajunge de la 8 la 28%. Analiza multivariată a identificat trei predictori ai mortalității postoperatorii:

- hematocritul inițial < 30%;
- bilirubinemia inițială > 11mg/dl (200 μmol/L);
- obstrucția malignă (carcinom pancreatic, colangicarcinom).

Când toți cei trei factori sunt prezenți, mortalitatea se apropie de 60% iar dacă lipsesc toți, mortalitatea este de doar 5%. Alți factori de predicție a evoluției chirugicale nefavorabile semnalați sunt: azotemia, hipoalbuminemia şi colangita (tabel nr. 6). Prezenţa hipertensiunii portale creşte de asemenea riscul chirurgical.

Pentru reducerea morbidităţii şi mortalităţii la aceşti pacienţi, s-a recurs la metode variate:

- administrarea i.v. perioperatorie de antibiotice cu spectru larg micşorează frecvenţa infecţiilor postoperatorii dar nu influenţează mortalitatea;
- drenajul biliar extern prin abord transhepatic nu ameliorează morbiditatea sau mortalitatea;
- drenajul biliar endoscopic are avantajul restaurării circulaţiei enterohepatice a acizilor biliari, evitând totodată complicaţiile puncţiei percutane. Dar ca şi drenajul extern, nu ameliorează mortalitatea chirurgicală în obstrucţia biliară malignă, deşi drenajul biliar preoperator este recomandat în rezecţiile hepatice extinse. În caz de colangită şi coledocolitiază, antibioterapia i.v. de spectru larg şi drenajul endoscopic se asociază cu mortalitate şi morbiditate mai reduse comparativ cu decompresia chirurgicală. Deşi sfincterotomia endoscopică se asociază cu creşterea complicaţiilor la cirotici, morbiditatea şi mortalitatea sunt reduse chiar şi la ciroticii din clasa C, dacă se reuşeşte decompresia biliară **(12);**
- o cauză majoră de morbiditate în icterul obstructiv este insuficienţa renală postoperatorie datorată de obicei necrozei acute tubulare. Frecvenţa generală este de 8% şi se explică mai ales prin absorbţia endotoxinelor din intestin. La subiecţii normali, absorbţia endotoxinelor este limitată de efectul detergent al sărurilor biliare asupra moleculei lipopolizaharidice a endotoxinei. Acest efect protector este absent în cazul icterului obstructiv cu secreţie biliară minimă. Ca urmare, pacientul dezvoltă vasoconstricţie renală exagerată. S-a sugerat că administrarea de săruri biliare sau lactuloză în icterul obstructiv poate preveni atât endotoxinemia, cât şi vasoconstricţia renală exagerată. Într-un studiu, 102 pacienţi cu icter obstructiv şi cu bilirubinemie > 5,8 mg/dl (100 µmol/L) au primit fie lactuloză, fie deoxicolat de sodiu sau nimic specific, preoperator. Deteriorarea postoperatorie a funcţiei renale la pacienţii cu funcţie renală nemodificată preoperator a fost semnificativ mai frecventă la cei care nu au primit niciun tratament specific preventiv. **(13)** Altă tentativă de reducere a incidenţei insuficienţei renale constă în administrarea postoperatorie de manitol. Deşi prezintă beneficiu, teoretic cel puţin, se pare că menţinerea volumului intravascular şi evitarea medicaţiei nefrotoxice de tipul aminoglicozidelor constituie probabil elementele cele mai importante de management;
- antibioterapia orală profilactică cu rifaximin reduce efectele adverse ale endotoxinemiei, dar un beneficiu clar nu a fost încă demonstrat. Mai mult, endotoxinemia ar putea spori prin eliberarea crescută de endotoxină datorită distrucţiei organismelor gram-negative. Pe de altă parte, antibioterapia i.v. de spectru larg trebuie, în general, administrată perioperator pentru că reduce incidenţa infecţiilor postoperatorii, deşi nu influenţează mortalitatea **(14);**
- nu este bine documentat dacă pacienţii cu hepatopatie colestatică (ciroză biliară primară, colangită sclerozantă primitivă) au risc crescut de necroză tubulară acută postperatorie. Interesant este că ciroza biliară primitivă pare să aibă risc redus de instalare a sindromului hepato-renal postoperator comparativ cu alte hepatopatii. O posibilă explicaţie ar putea fi acţiunile natriuretică şi vasodilatator renală ale sărurilor biliare retenţionate.

Chirurgia cardiacă se asociază cu creşterea mortalităţii la cirotici comparativ cu alte proceduri chirurgicale. Printre factorii de risc ai decompensării hepatice în chirurgia cardiacă se includ: timpul total de bypass cardiopulmonar, utilizarea bypass-ului cardiopulmonar nonpulsatil comparativ cu cel pulsatil şi necesitatea suportului presor perioperator. Bypass-ul cardiopulmonar poate exacerba coagulopatia subiacentă prin inducerea disfuncţiei plachetare, fibrinoliză şi hipocalcemie. Prin urmare, la ciroticii avansaţi care necesită intervenţie chirurgicală cardiacă se vor lua în considerare opţiuni mai puţin invazive, cum ar fi angioplastia, valvuloplastia sau tehnicile miniinvazive de revascularizare. **(15)**

Rezecţia hepatică la pacienţii cu ciroză, pentru carcinom hepatocelular sau alte tumori hepatice, prezintă risc crescut de decompensare hepatică comparativ cu alte tipuri de intervenţii chirurgicale. Acestor pacienți li se îndepărtează o parte din masa funcțională hepatocelulară în situația în care au deja rezerva funcțională hepatică compromisă. În trecut, ciroza era o contraindicație pentru rezecția tumorală hepatică datorită mortalității perioperatorii de peste 50%. În ultimul timp, mortalitatea perioperatorie pentru rezecția hepatică s-a redus la 3-16%, deşi morbiditatea postoperatorie este încă de aproximativ 60%. **(16)** Ameliorarea rezultatelor este atribuită unei mai bune selecții a pacienților (inclusiv detecția tumorală precoce), pregătirii preoperatorii meticuloase, monitorizării intensive intra- şi postoperatorii şi ameliorării tehnicilor chirurgicale.

Traumatismul la ciroticii laparotomizați prezintă risc crescut de morbiditate şi mortalitate, chiar şi pentru pacienții cu traumatisme minore (mortalitate generală 45% față de 24% la grupul de control). **(17)** Din acest motiv se recomandă ca pacienții depistați cu ciroză în timpul laparotomiei să fie admişi la terapia intensivă indiferent de severitatea traumatismului.

Pacienții cu risc crescut minimal. Pacienții cu hepatopatie cronică uşoară-moderată, fără ciroză, tolerează de obicei bine intervenția chirurgicală. Terapia medicală va trebui însă optimizată preoperator.

Hepatita cronică uşoară. Pacienții asimptomatici cu hepatită cronică uşoară au un risc redus de complicații. Spre exemplu, un studiu **(18)** arată că nu au existat complicații majore în cazul a 34 intervenții chirurgicale, efectuate la 24 pacienți cu hepatită cronică uşoară spre moderată. La doi dintre aceşti pacienți s-a înregistrat hiperbilirubinemie susținută, dar ambii aveau preoperator bilirubinemia ≥ 2,5mg/dl (35,91 μmol/L).

Steatoza hepatică şi steatohepatita nonalcoolică. Deşi aspectul histologic al steatohepatitei nonalcoolice (NASH) este asemănător cu al hepatitei alcoolice, pacienții cu NASH nu par să aibă mortalitate excesivă după chirurgia electivă.Totuşi, steatoza hepatică moderată spre severă tinde spre creşterea mortalității în urma rezecției hepatice. NASH este relativ frecventă în obezitatea morbidă la care se efectuează bypass gastric. Ciroza datorată NASH a fost depistată oarecum surprinzător la 6% dintre cazuri, cu o mortalitate perioperatorie de 4%. Deoarece nu toți pacienții recunosc consumul de alcool, este recomandată o perioadă de abstinență preoperatorie la toți pacienții cu aspect histologic de steatohepatită sau la cei suspectați de consum semnificativ de alcool deoarece alcoolicii au risc crescut de complicații perioperatorii, cum ar fi sindromul de abstinență şi hepatotoxicitatea la dozele terapeutice de acetaminofen (larg utilizat ca analgetic postoperator) chiar dacă nu prezintă hepatopatie. Mai mult, alcoolul potențează toxicitatea halotanului.

Hepatita autoimună. Chirurgia electivă este bine tolerată de obicei în cazul hepatitei autoimune fără decompensare funcțională hepatică. Pacienții aflați în tratament cu prednison vor primi hidrocortizon perioperator (doze de ”stres”).

Hemocromatoza. Pacienții cu hemocromatoză trebuie evaluați înaintea intervențiilor chirurgicale pentru depistarea complicațiilor evolutive, cum ar fi diabetul sau cardiomiopatia, care au impact asupra managementului perioperator. Dacă, în trecut, prognosticul mai puțin favorabil al transplantului hepatic la aceşti pacienți era atribuit cardiomiopatiei subiacente, acesta a fost ulterior ameliorat printr-o selecție mai atentă a pacienților pentru transplant.

Maladia Wilson. Pacienții cu afectare neuropsihică pot fi incapabili să-şi asume consimțământul informat necesar pentru chirurgie. De asemenea, intervenția chirurgicală poate precipita sau agrava simptomele neurologice. D-penicilamina, utilizată la aceşti pacienți pentru chelarea cuprului, interferă cu colagenizarea plăgii chirurgicale. De aceea, posologia acestui medicament va fi redusă preoperator şi încă două săptămâni după intervenția chirurgicală.

Optimizarea terapiei medicale. Pe lângă evaluarea riscului chirurgical, toți pacienții cunoscuți cu afecțiuni hepatice vor fi investigați pentru documentarea icterului, coagulopatiei, ascitei, diselectrolitemiei, disfuncției renale și encefalopatiei, deoarece toate vor necesita tratament specific preoperator.

Pacienții cu timpul de protrombină prelungit vor fi tratați preoperator cu vitamina K şi plasmă proaspătă, pentru normalizarea acestuia cu 3 secunde sub limita maximă normală. Este acumulată experiență tereapeutică şi cu factorul recombinant VII A, care poate corecta temporar timpul de protrombină. Timpul prelungit de sângerare poate fi corectat temporar cu diamino-8-D-arginin vasopresină (DDAVP). Tehnica chirurgicală optimă şi menținerea redusă a presiunii venoase centrale (PVC) pot diminua pierderile de sânge. Ascita va fi tratată intensiv pentru a reduce riscul dehiscenței plăgii şi a hernierii peretelui abdominal. În acest scop pacienții vor primi diuretice mai ales dacă au şi edeme periferice. Dacă nu prezintă edeme periferice sau dacă nu este timp suficient pentru tratamentul diuretic, ascita poate fi drenată total în cursul laparotomiei.

Diselectrolitemiile, mai ales hipopotasemia şi alcaloza metabolică, vor fi corectate preoperator şi monitorizate ulterior pentru a reduce riscul de aritmii cardiace şi de encefalopatie hepatică. Factorii care exacerbează encefalopatia hepatică vor fi corectați. Tratamentul profilactic nu previne însă encefalopatia postchirurgicală. Funcția renală va fi evaluată de asemenea, determinarea ureei şi creatininei serice fiind suficientă în majoritatea cazurilor. Valorile acestora pot însă supraestima funcția renală la pacienții cu hepatopatie avansată unde are loc reducerea sintezei ureei şi creatininei. Pacienții care prezintă varice esogastrice vor primi tratament profilactic adecvat. Deşi actul chirurgical nu creşte de obicei riscul de hemoragie variceală, se recomandă evitarea supraîncărcării cu fluide postoperator. Montarea preoperatorie a unui şunt tranjugular porto-sistemic intrahepatic (TIPS) la pacienții cu hipertensiune hepatică semnificativă clinic poate fi luată în considerare, dar este insuficient studiată.

Pacienții cirotici sunt adesea malnutriți. Suportul nutrițional perioperator poate reduce frecvența complicațiilor postoperatorii şi mortalitatea pe termen scurt. Beneficiul pe termen lung este însă incert. **(19)** O atitudine rezonabilă constă în asigurarea unui aport caloric total de 1,2 peste consumul energetic de repaus şi 1g/kg/zi de proteine. Din totalul aportului energetic, 30-35% va fi asigurat de lipide iar restul (50-55%) de către glucide. Se va suplimenta de asemenea şi aportul de vitamine liposolubile (A, D, E, K). Gastrostoma percutană (PEG) este contraindicată în prezența ascitei şi va fi evitată la pacienții cu hipertensiune portală, datorită riscului de dilacerare a varicelor parietale abdominale în timpul plasării PEG-ului.

Postoperator, pacienții hepatici vor fi monorizați atent pentru depistarea decompensării hepatice care se prezintă de obicei cu agravarea icterului, encefalopatie şi ascită. Testele biochimice uzuale pentru funcția hepatică sunt timpul de protrombină şi bilirubinemia. Dar bilirubinemia creşte de obicei şi datorită complicațiilor chirurgicale, transfuziilor multiple, pierderilor excesive de sânge, instabilității hemodinamice sau infecțiilor sistemice. Vor fi monitorizate de asemenea cu atenție: funcția renală, ionograma serică şi glicemia.

Sumarul recomandărilor. Pe baza datelor prezentate şi a experienței clinice acumulate, pentru evaluarea riscului chirurgical în intervențiile elective sau semiurgente la pacienții hepatici, credem că merită reținute următoarele recomandări:

- terapia medicală ar trebui optimizată la toți pacienții hepatici cunoscuți sau depistați preoperator;
- mortalitatea operatorie poate fi estimată pe baza clasificării Child şi a scorului MELD, luând în considerare şi alți factori, cum sunt : vârsta pacientului, scorul ASA şi comorbiditățile asociate;
- intervențiile chirurgicale elective şi semiurgente nu vor fi efectuate în caz de hepatită acută sau fulminantă, hepatită alcoolică, hepatită cronică severă, ciroza de clasă Child C sau scor MELD > 15, coagulopatie severă sau manifestări extrahepatice severe de hepatopatie (de exemplu, hipoxie, cardiomiopatie sau insuficiență renală acută) (grad de recomandare 1B);

- pentru ceilalți pacienți, cu hepatopatie mai puțin severă, riscul chirurgical este evaluat individual, în funcție de statusul clinic şi de tipul de procedură chirurgicală:

- intervenția operatorie este bine tolerată la pacienții cu ciroză din clasa Child A sau scorul MELD < 10, precum şi la pacienții cu hepatopatie cronică uşoară, noncirotică;

- actul chirurgical poate fi permis la pacienții cu ciroză din clasa Child B sau cu scor MELD 10-15 (cu excepția rezecției hepatice extensive sau a chirurgiei cardiace) dacă au beneficiat de o pregătire preoperatorie adecvată şi consistentă.

Pentru candidații aflați pe lista de transplant (ortotopic sau de la donator viu) se va lua în considerare posibilitatea amânării intervenției chirurgicale (elective sau semi-urgente) până după efectuarea transplantului hepatic**. (20)**

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Factori cu influență asupra metastazelor pelvine în carcinomatozele peritoneale din adenomul gastric

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Rezumat. Carcinomatoza peritoneală reprezintă diseminarea locală/regională a neoplaziilor digestive, ginecologice sau a altor forme de cancer. Similar tumorilor primitive, tumorile secundare peritoneale păstrează acelaşi caracter definitoriu de dispersie a caracterelor clinice, patogenice şi morfologice. Am luat în studiu 56 de cazuri de neoplasm gastric, antral şi corporeocaudal care au avut ca manifestare carcinomatoza peritoneală cu implicarea pelvisului. Rezultatele analizei multivariate indică factorii predictivi ai indexului neoplazic peritoneal al sectorului pelvin în funcție de localizarea neoplaziei şi sexul pacienților, feminin/masculin.

Cuvinte cheie: Carcinomatoză peritoneală, neoplasm gastric, neoplasm antral, neoplasm corporeocaudal.

Abstract. Peritoneal carcinosis consists of local / regional spreading of digestive and gynecological neoplasia and of other cancers. As initial primitive tumors, secondary tumors retain the same character defining peritoneal dispersion, clinical characteristics, pathogenicity and morphology. We studied 56 cases of gastric cancer who had show the carcinomatosis involvement of peritoneal pelvis. Results of multivariate analysis indicate factors predictive of peritoneal cancer index of pelvic structures per neoplasia location and sex of patients (female / male).

Introducere

Carcinomatoza peritoneală reprezintă împrăştierea locală/regională a neoplaziilor digestive, ginecologice sau a altor forme de cancer cu sau fără dovada existenței metastazelor sistemice (1,2). Majoritatea cancerelor prezente la nivelul abdomenului şi al pelvisului vor disemina pe trei căi diferite. Apar astfel metastaze hematogene, limfatice şi prin spațiile peritoneale (diseminare peritoneală), spre diferite suprafețe din abdomen şi pelvis (3,4).

Similar tumorilor primitive şi tumorile secundare peritoneale păstrează acelaşi caracter definitoriu de dispersie a aspectelor clinice, patogenice si morfologice, ceea ce îngreunează diagnosticul diferențial, întârzie decizia terapeutică (5) şi grevează prognosticul tumorii primitive (6).

Material şi metodă

În studiul nostru au fost incluse 56 de cazuri diagnosticate cu neoplasm gastric, antral şi corporeocaudal (4) şi care au avut ca manifestare carcinomatoza peritoneală cu implicarea pelvisului. Dintre acestea, 20 de cazuri au avut localizare antrală şi 36 de cazuri au fost diagnosticate corporeocaudal.

Toate cazurile au fost evaluate prin prisma indicelui neoplazic peritoneal (PCI), noțiune care caracterizează cantitativ şi calitativ interesarea unui sector peritoneal, cu accent pe sectorul 6, corespondent al pelvisului şi structurilor pelvine. PCI combină cantitativ distribuția tumorală în 13 cadrane predefinite ale abdomenului şi pelvisului cu un scor lezional (7,8).

Scorul lezional este determinat după liza completă a aderenţelor şi după inspecţia completă a suprafeţelor peritoneale parietale şi viscerale din regiunile abdomino-pelvine. Se referă la cel mai mare diametru al implantului tumoral. Tumora primară sau recurenţele locale care pot fi îndepărtate complet sunt excluse din evaluare. Dacă există confluenţe de noduli tumorali, sunt caracterizate ca SL-3, chiar dacă este vorba de un strat fin (subţire) de implanturi tumorale:

- SL-0 înseamnă că nu sunt vizualizate depozite maligne;
- SL-1 implică prezenţa nodulilor tumorali sub 0,5 cm în cea mai mare dimensiune a lor; nu se cuantifică numărul nodulilor, ci numai cel mai mare;
- SL-2 implică existenţa nodulilor între 0,5 şi 5 cm;
- SL-3 semnifică noduli tumorali peste 5 cm sau confluenţe de noduli tumorali.

Pentru lotul de studiu ales a fost realizată o cartare completă a cavităţii abdominale prin explorare chirurgicală, completată cu investigaţii de ecografie, computertomografie şi imagistică prin rezonanţă magnetică pentru zonele inabordabile chirurgical.

Studiul de faţă a urmărit în special cartarea sectorului 6 al cavităţii peritoneale al bolnavilor cu carcinomatoză din neoplasm gastric, corespunzător structurilor pelvine.

Dintre cazurile de neoplasm gastric antral neoperat, 5 sunt de sex masculin (IP sector 6 cu valorile 2,3,1,0) şi 4 de sex feminin (IP sector 6 cu valorile 0,2,0,2).

Dintre cazurile de neoplasm gastric antral operat, 4 sunt de sex masculin (IP sector 6 cu valorile 3,1,1,0) şi 7 sunt de sex feminin (IP sector 6 cu valorile 2,2,0,1,0,0,0).

Dintre cazurile de neoplasm corporeocaudal neoperat, 15 sunt de sex masculin (IP sector 6 cu valorile 1,1,3,1,1,1,0,0,0,0,0,0,0,0,0) şi 11 sunt de sex feminin (IP sector 6 cu valorile 1,3,2,1,1,1,1,0,0, 1,3).

Dintre cazurile de neoplasm corporeocaudal operat, 6 sunt de sex masculin (IP sector 6 cu valorile 0,1,2,0,0,0), iar 4 sunt de sex feminin (IP sector 6 cu valorile 3,1,0,0).

Rezultate

Rezultatele analizei multivariate indică factorii predictivi ai IP în funcţie de localizarea neoplaziei şi de sexul pacienţilor, feminin/masculin. În acest studiu s-au luat în discuţie rezultatele corelaţiei multiple, având-se în vedere modificările indexului IP.

Rezultatele corelaţiilor parţiale ce privesc localizarea – antral/corporeocaudal, feminin/masculin indică o asociere nesemnificativă a acestor parametri cu indexul IP. Astfel, localizarea prezintă o corelaţie inversă nesemnificativă (r=-0.265, p=0.358, 95%CI, RR=1.04), cu o uşoară scăderea a indexului IP pentru localizarea corporeocaudală.

La pacienţii de sex feminin s-a constatată o valoare a indexului IP uşor mai ridicată, dar nesemnificativă din punct de vedere statistic (r=0.38, p=0.161, 95%CI) comparativ cu pacienţii de sex masculin (RR=1.32).

Discuţii

Majoritatea cancerelor care apar în abdomen şi pelvis vor disemina pentru a constitui metastaze peritoneale pe trei căi diferite.

În dezvoltarea unei carcinomatoze peritoneale se consideră că metastazarea hematogenă se dezvoltă în etape secvenţiale care presupun penetrarea în vasele de sânge, eliberarea celulelor tumorale din conglomeratul tumoral şi circulaţia celulelor maligne (citemia), oprirea la nivelul patului capilar parenchimatos, pătrunderea în ţesut prin străbaterea barierei endoteliale, multiplicarea şi creşterea în stroma vasculară şi expansiunea tumorală. O formă particulară a metastazării hematogene o constituie mecanismul de producere al metastazelor embolice. În acest context extensia limfatică joacă un rol minor în diseminarea intraperitoneală a carcinomului metastatic. Metastazarea prin diseminare peritoneală se presupune a fi în general produsă prin eliberarea de celule din tumora primară în cavitatea peritoneală, reimplantarea şi creşterea lor în petele lăptoase ale marelui oment sau peritoneului mezenteric. Diseminarea intraperitoneală a neoplasmelor depinde şi de fluxul natural al fluidului în cavitatea peritoneală, care este guvernată de compartimentalizarea spaţiilor peritoneale asociată cu efectele gravitaţiei şi cu schimbările în presiunea intraabdominală produse de respiraţie.

Cele mai frecvente locuri de plasare a ascitelor (pooling) (4) şi, în consecinţă, de fixare şi creştere a maselor tumorale peritoneale metastatice sunt reprezentate de fundul de sac Douglas, mezenterul intestinului subţire de lângă valva ileocecală (partea superioară a ileonului terminal şi porţiunea medială a cecului), mezocolonul sigmoid (sau porţiunea superioară a sigmoidului), şanţul paracolic drept, spaţiul subhepatic şi subfrenic drept (5). Ca atare, studiul de faţă propune analiza retrospectivă a metastazelor pelvine la un lot de pacienţi diagnosticaţi cu carcinomatoză peritoneală din neoplazii gastrice.

Analiza statistică a datelor pe cazurile selectate arată că valoarea IP corespunzătoare sectorului 6 peritoneal, adică pelvisului, nu este dependentă de localizarea neoplaziei primare (neoplasm gastric antral sau corporeocaudal), fapt exprimat printr-o corelaţie inversă nesemnificativă (r=-0.265, p=0.358, 95%CI, RR=1.04).

Asemănător, sexul pacientului, masculin sau feminin, nu are un răsunet asupra gradului de implicare a structurilor pelvine în carcinomatozele cancerului gastric. La pacientele de sex feminin s-a constatat o valoare a indexului IP uşor mai ridicată, dar nesemnificativă din punct de vedere statistic (r=0.38, p=0.161, 95% CI) comparativ cu pacienţii de sex masculin (RR=1.32). Ambele sexe, indiferent de localizarea neoplaziei, au aceeaşi şansă de a dezvolta implante tumorale în structurile pelvisului.

Concluzii

Implicarea în procese de carcinomatoză peritoneală a pelvisului în neoplaziile gastrice nu este dependentă de localizarea neoplaziei primare (neoplasm gastric antral sau corporeocaudal).

Sexul pacientului nu are un răsunet asupra gradului de implicare a structurilor pelvine în carcinomatozele cancerului gastric.

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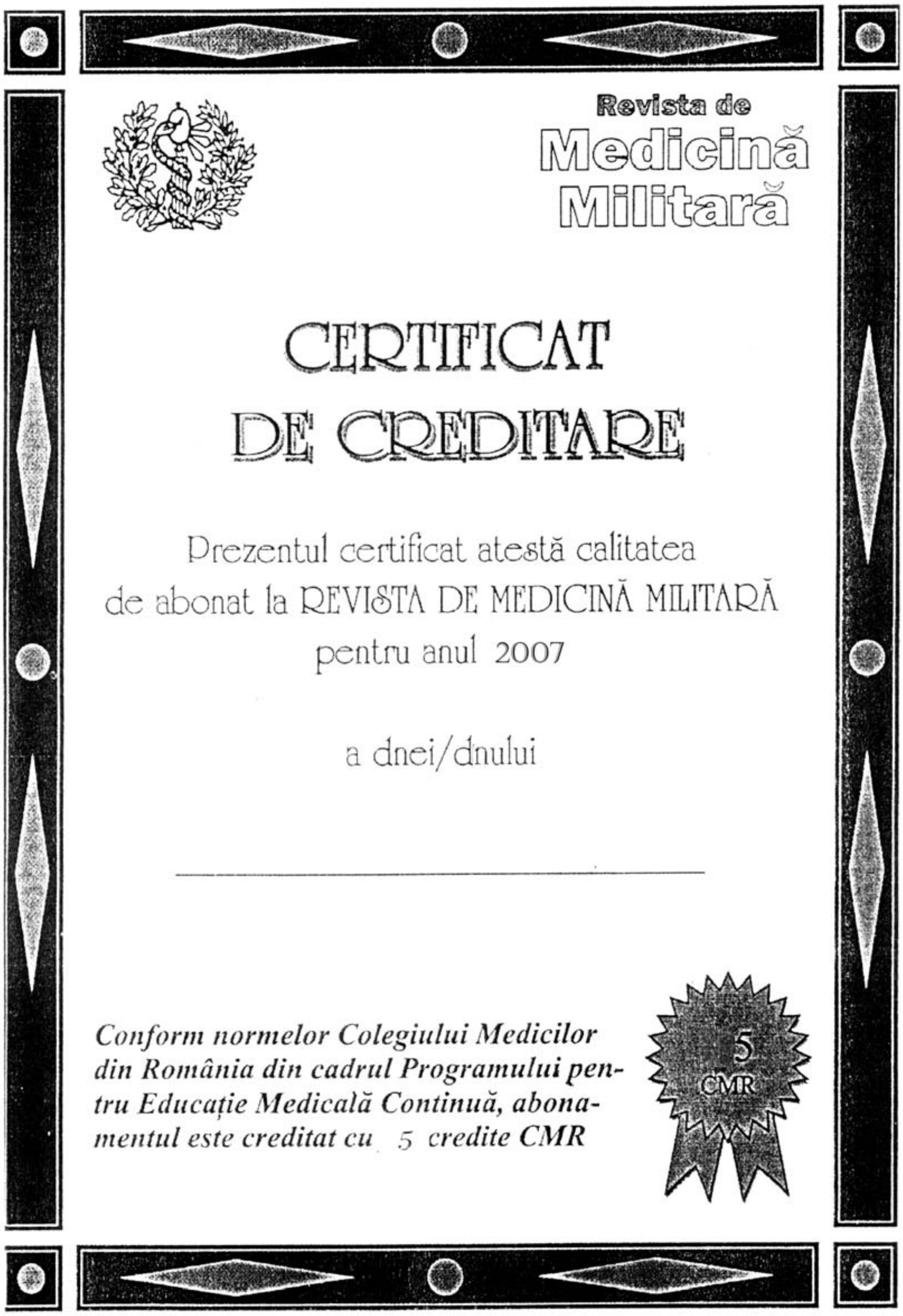

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Sternul înfundat – rezolvare chirurgicală minim invazivă

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Introducere

Sternul înfundat este cea mai frecventă malformaţie congenitală a peretelui toracic anterior. Malformaţia se accentuează odată cu creşterea în vârstă şi devine maximă în adolescenţă, când simptomatologia clinică se acutizează şi afectarea psihică devine importantă. De-a lungul timpului s-au imaginat nenumărate tratamente, fie conservatoare, fie chirurgicale, de corecţie. Procedeul Nuss, procedeu minim invaziv de corecţie a sternului înfundat, dezvoltat după 1986, este şi în momentul de faţă cel mai folosit pe plan mondial.

Material şi metodă

Sunt prezentate repere istorice legate de rezolvarea chirurgicală a sternului înfundat, avantaje şi dezavantaje ale chirurgiei deschise. Evaluarea clinică şi cea paraclinică, inclusiv măsurătorile şi indicii antropometrici, sunt redate în amănunt. Sunt prezentate indicaţiile şi contraindicaţiile procedeului Nuss, precum şi posibile complicaţii intraoperatorii şi postoperatorii recente şi tardive. Din cazuistica Secţiei Clinice de Chirurgie Toracică sunt înfăţişate 40 de cazuri de pacienţi diagnosticaţi şi investigaţi cu stern înfundat şi trataţi chirurgical după procedeul Nuss.

Rezultate şi concluzii

Sunt redate beneficiile clare ale procedeului Nuss, precum şi îmbunătăţirea parametrilor funcţionali şi estetici. Postoperator, pacienţii urmează un protocol specific recomandat de medicul curant.

Cuvinte cheie: stern înfundat, procedeu minim invaziv, procedeul Nuss

Introduction

Pectus excavatum is most frequent anterior thoracic wall congenital malformation. This malformation is increasing its effects with aging process and has the pick during the tees age, when the clinical symptoms become more acute and psychological effects are real important. During the time many treatment technique have been proposed, conservatory technique or surgical correction technique. The minimally invasive repair technique of pectus excavatum, "Nuss technique", developed after 1986, is the most used technique world wide.

Method

Here will be presented historical bench-mark related to surgical repair of pectus excavatum, advantages and disadvantages of open surgery. Also we will present in detail clinical and Para clinical evaluations including anthopometric indexes. We will present as well the indications and contraindications of Nuss technique and possible intra operational and post op recent and tardy. We review 40 cases from the Thoracic Surgery Clinic, patients being diagnosed and investigated for pectus excavatum, and, after confirming the diagnosis, surgical treated using Nuss procedure.

Results and Conclusions

Here are presented the real benefits of Nuss technique and improving of the functional and aesthetical indexes. The patients who have been treated with Nuss technique are following a strict and specific post op protocol recommended by the physician.

Definiţie

Sternul înfundat sau toracele în pâlnie (*pectus excavatum*) reprezintă o malformaţie congenitală a peretelui toracic anterior. Se caracterizează printr-o înfundare a corpului sternal şi mai puţin a cartilajelor costale adiacente, rezultând aspectul de pâlnie (fig. 1, 2).

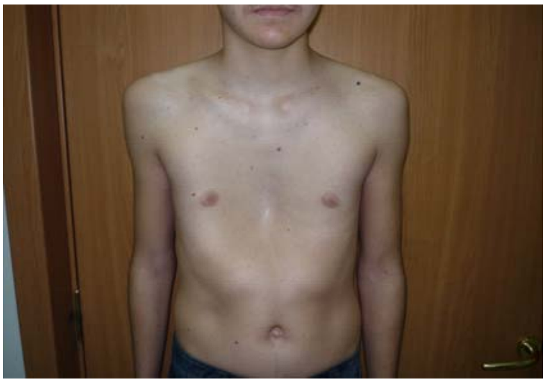

Fig. 1. Torace înfundat - aspect anterior

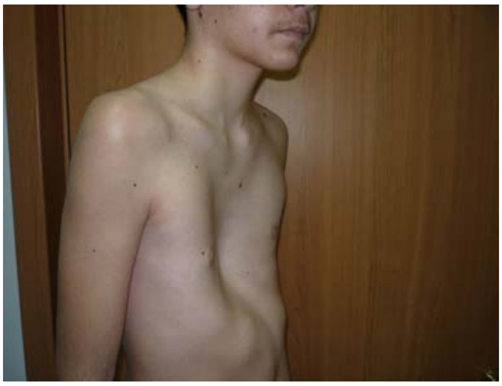

Fig. 2. Torace înfundat - aspect profil

Malformaţia este mai puţin observată la naştere şi se accentuează odată cu creşterea în vârstă, devenind foarte evidentă în perioada adolescenţei.

Incidenţă. Etiologie

Rata de apariţie a acestei malformaţii, după unii autori, este de un caz la 1 000 de nou-născuţi. Alţi autori, apreciază că este mai frecventă (Ravitch – 1 la 400 de nou-născuţi vii).

Sternul înfundat poate fi prezent ca malformaţie singulară, de sine stătătoare, sau în asociere cu alte malformaţii congenitale cardiace, cu astmul bronşic, cu sindromul Marfan, cu sindromul Ehlers-Danlos sau cu sindromul Poland.

Dacă pâlnia sternală este pe linia mediană, vorbim de *pectus excavatum* simetric (fig. 1, 2) iar dacă aceasta se găseşte la stânga sau la dreapta liniei mediane, vorbim de un *pectus excavatum* asimetric (fig. 3, 4). Acest fapt trebuie bine stabilit de la început, pentru că în funcţie de acest aspect se va stabili procedeul operator de corecţie.

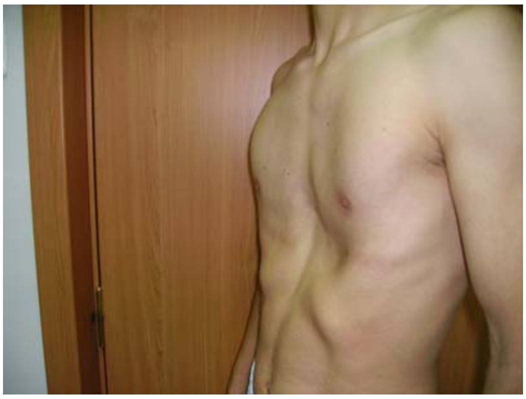

Fig. 3. Stern înfundat asimetric - preoperator

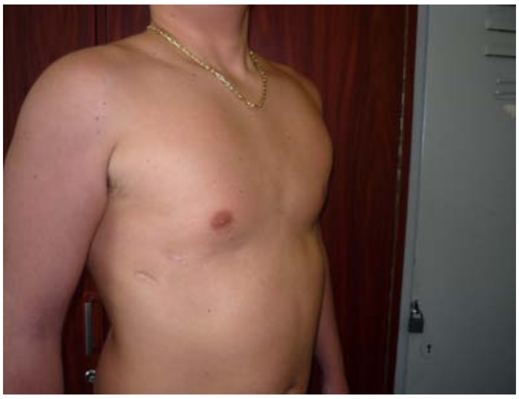

Fig.4. Stern înfundat asimetric - postoperator

Sunt mai multe teorii asupra mecanismelor etiopatogenice. Majoritatea autorilor sunt de acord cu teoria discondroplazică (creşterea anarhică a cartilajelor costale), dar mai sunt luate în discuţie şi alte teorii, precum traumatismul intrauterin, rahitismul, malformaţii ale diafragmului etc. Asociază frecvent şi afectări musculoscheletale, precum scolioza, cifoza, amastie, mameloane supranumerare ş.a.m.d.

Fiziopatologie

Toate tulburările fiziopatologice pe care aceşti pacienţi le prezintă se datoresc pe de-o parte compresiunii pe care o realizează sternul înfundat (groapa sternală) asupra organelor intratoracice sau mediastinale (în mod deosebit asupra inimii şi a plămânilor) sau deplasării acestora faţă de situaţia lor anatomic normală, iar pe de altă parte aspectului estetic inadecvat pe care respectivii pacienţi îl percep în special în perioada adolescenţei.

În ceea ce priveşte disfuncţiile ventilatorii şi modificările cardiovasculare produse de sternul înfundat, rezultatele studiilor şi cercetărilor sunt contradictorii. Unii autori afirmă că malformația nu duce la alterări importante ale funcţiilor cardiace şi pulmonare. Alții însă au demonstrat acest lucru prin teste funcţionale preoperatorii care s-au îmbunătățit semnificativ după intervenția chirurgicală.

Compresia cordului poate duce la modificări de EKG prin deplasarea acestuia în special către stânga, iar prin modificarea poziției inelului mitral se realizează prolapsul de valvă mitrală, care se asociază frecvent sternului înfundat.

Simptomatologie

Există două categorii de pacienți purtători ai acestei afecţiuni, unii simptomatici şi alții asimptomatici. Acest lucru este stabilit de către medicul de familie la prima examinare. Cei simptomatici vor fi îndrumaţi către un serviciu de chirurgie toracică, pentru evaluare completă clinică şi paraclinică. Pacienților asimptomatici, care rămân în observația medicului de familie, li se recomandă un tratament fizic pentru corectarea posturii, urmând a fi reevaluați la fiecare 6 luni. Aceştia vor fi încadrați în categoria pacienților simptomatici când vor fi prezente următoarele manifestări: adâncirea excavației sternale, creşterea gradului de intoleranță la efort, durere toracică, dispnee, palpitații, iar evaluarea paraclinică arată deplasare cardiacă şi deplasare pulmonară.

Vârsta ideală pentru intervenția chirurgicală este între 6 şi18 ani, dar ea poate fi extinsă chiar şi la pacienți care depăşesc 30 de ani.

Evaluarea clinică şi paraclinică

Presupune inspecția toracelui, analiza deformației din punctul de vedere al simetriei sau asimetriei față de linia mediană, asocierea cu alte malformații congenitale ale peretelui toracic. Evaluarea clinică stabileşte şi mărimea lamei care va fi utilizată, prin măsurarea distanței la locul de maximă adâncitură a deformație între linia axilară medie dreaptă şi linia axilară medie stângă. Măsurarea circumferinței toracelui (cirtometria) la nivelul adânciturii maxime este un indice antropometric utilizat de către chirurgii care practică acest procedeu, pentru o evaluare calitativă a performanțelor fiziologice postoperatorii ale pacienților operați.

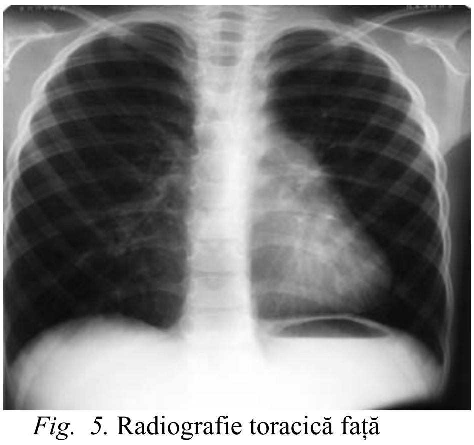

Fig. 5. Radiografie toracică față

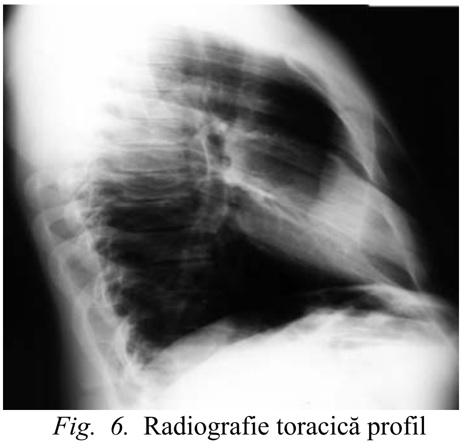

Fig. 6. Radiografie toracică profil

Radiografia toracică este prima investigație imagistică ce se efectuează. Pe radiografia de față putem semnala poziția cordului față de linia mediană, mărimea acestuia, dacă este sau nu asociat şi cu malformații cardiace. Radiografia de profil ne permite să apreciem gradul adânciturii sternale, precum şi distanța până la coloana vertebrală (fig. 5, 6).

Tomografia computerizată este obligatorie, aducând toate informațiile necesare stabilirii indicației operatorii. De asemenea, ea poate preciza cu exactitate dacă suntem în fața unei malformații simetrice sau asimetrice față de linia mediană (fig. 7, 8).

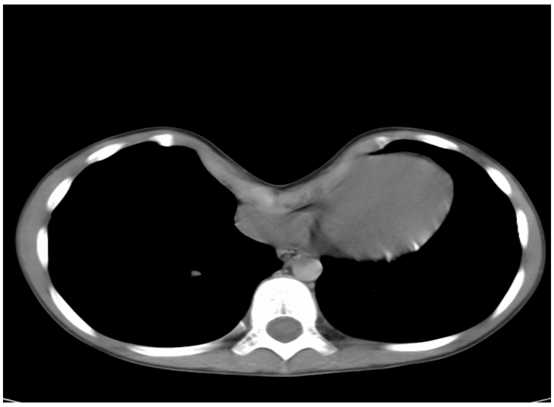

Fig. 7. Stern înfundat simetric

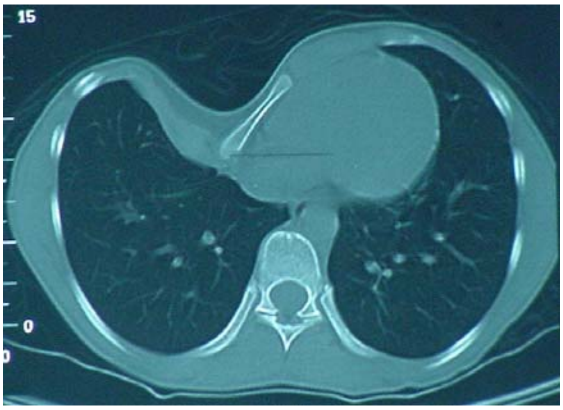

Fig. 8. Stern înfundat asimetric

Opțiuni pentru tratament

În prezent, opțiunile terapeutice chirurgicale cele mai utilizate s-au redus la trei metode: corecția minim invazivă a sternului înfundat (procedeul Nuss), utilizată în malformațiile simetrice sau uşor asimetrice; pentru formele complexe sau asimetrice se utilizează procedeul de sternocondroplastie Ravitch; chirurgii plasticieni intervin prin umplerea adânciturii sternale cu implanturi de silicon, urmărind astfel doar corectarea aspectului estetic al acestor tineri.

Procedeul minim invaziv de corecție a sternului înfundat (procedeul Nuss) – MIRPE

Este un procedeu minim invaziv de corecție, în care se utilizează trusa de toracoscopie şi trusa specială pentru stern înfundat. A fost folosit pentru prima dată de Dr. Donald Nuss, în 1980, în colaborare cu Walter Lorenz Surgical Inc. şi a fost practicat inițial la copii şi adolescenți. Procedeul constă în plasarea retrosternală, sub control toracoscopic, a unei lame speciale din oțel, titan şi nichel precurbate. Inițial, lama se introduce cu concavitatea în sus, ulterior, prin rotația la 180º, concavitatea lamei vine în jos, corectând deformația prin ridicarea sternului (fig. 9, 10).

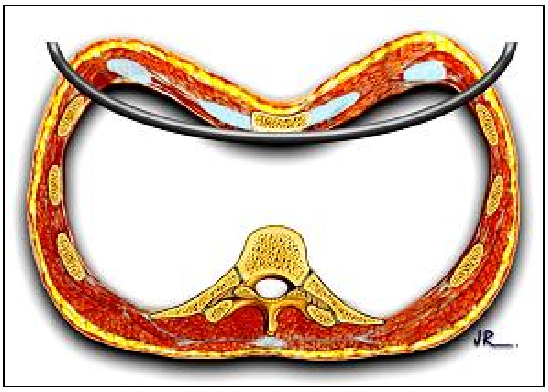

Fig. 9. Lama metalică precurbată introdusă toracoscopic cu concavitatea în sus

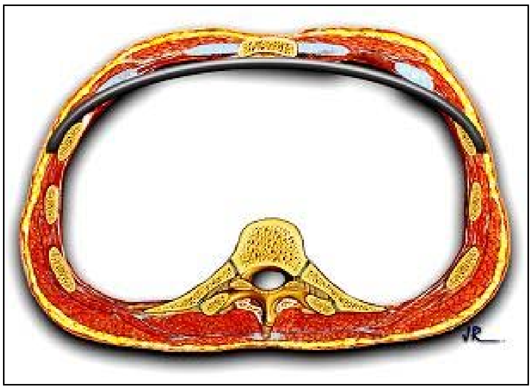

Fig. 10. Rotirea lamei la 180º, sub control toracoscopic corectează deformația

Trusa specială pentru stern înfundat

În prezent există mai mulți producători pentru astfel de truse, care diferă prin câteva componente de la un producător la altul. Trusa specială conține următoarele elemente:

- lamele de pectus numerotate de la 7 inch (17.8 cm) la 17 inch (43.2 cm), adecvate tuturor tipurilor de *pectus excavatum*, acoperind astfel toate vârstele de pacienți cu această malformație;
- trusa conține similar lamelor un număr identic de şabloane cu ajutorul cărora se modelează lama din metal, conform cu deformația;
- stabilizatoare din acelaşi material, cu ajutorul cărora lamele se fixează de peretele toracic;
- în funcție de vârsta pacientului, toracele acestuia, mărimea lamei, poate fi folosit unul sau două stabilizatoare la ambele capete ale lamei. În prezent, sunt firme care au scos pe piață stabilizatoare resorbabile;
- dispozitiv de modelat lama (bender). Există două tipuri de dispozitive, unul care se foloseşte manual, iar altul mecanic, care se poziționează pe o masă de instrumentar;
- dispozitiv pentru rotit lama (flipper) din poziția 0º, cu concavitatea anterior, în poziția 180º, cu concavitatea posterior, corectând astfel deformația;
- un număr de trei disectoare *(introducer*), unul mic (*small*), pentru pacienți cu vârsta cuprinsă între între 4 şi 8 ani, unul mediu *(medium*), pentru pacienți între 9 şi12 ani, şi unul mare (*large*), pentru pacienți de peste 13 ani.

Tehnica operatorie. În preziua operației se fac **măsurătorile** pentru determinarea lungimii lamei. Acestea se fac la nivelul adânciturii maxime, între linia axilară medie dreapta şi linia axilară medie stânga. Lama aleasă va fi cu 2 cm mai mică decât măsurătorile inițiale, deoarece şablonul măsoară diametrul extern al toracelui, în timp ce lama traversează diametrul intern al toracelui.

Anestezia este generală, cu intubație orotraheală selectivă a plămânului drept. Pentru controlul durerii, perioperator şi postoperator se foloseşte un cateter epidural. Preoperator se administrează o doză de antibiotic.

Poziția pacientului este în decubit dorsal, cu brațele în abducție la nivelul umerilor (fig. 11). Aplicarea "sterildrape"-ului (fig. 12) şi marcarea pacientului: se vor marca punctul cel mai decliv al excavației, precum şi punctele cele mai înalte parasternale corespunzător acestuia.

Planul orizontal care uneşte aceste trei puncte se va extinde spre peretele toracic bilateral şi se marchează între linia axilară anterioară şi linia axilară medie, locul viitoarelor incizii laterale.

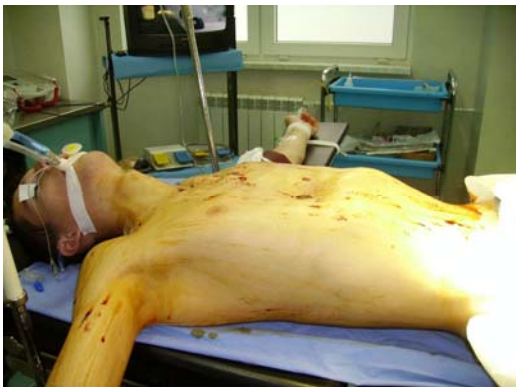

Fig. 11. Poziția pe masă, anestezia

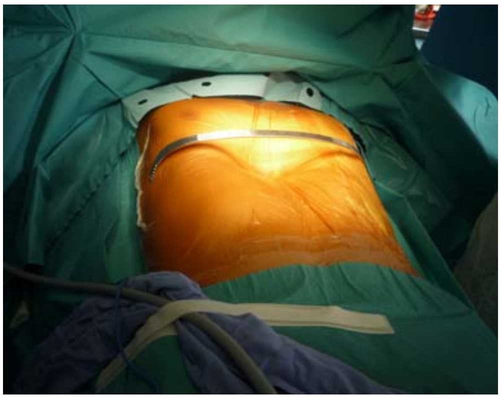

Fig. 12. Aplicare "sterildrape" şi lama modelată

Alegerea şablonului corespunzător, urmată de modelarea lamei metalice, utilizând dispozitivul special de îndoire: lama se modelează dinspre centru spre periferie, prin îndoiri mici, folosindu-se inițial poziția zero a dispozitivului de încurbare (raza minimă), apoi poziția unu (raza medie), iar în final, spre capetele barei, poziția 2 (raza maximă). Se evită curbarea capetelor laterale ale lamei pentru a se putea asigura fixarea stabilizatoarelor.

Inciziile laterale de 2.5 cm se fac pe linia axilară medie, la nivelul spațiului intercostal, pe orizontala marcată anterior. Inciziile pot fi orizontale, oblice sau verticale, în funcție de preferința şi experiența chirurgului operator. Urmează **tunelizarea tegumentului** la nivelul spațiului intercostal până la punctele cele mai înalte ale excavației, la nivelul spațiului intercostal marcat în prealabil.

Toracoscopia poate folosi un toracoscop de 5 sau 10 mm, 0 sau 30°. Se introduce prin hemitoracele drept la unul sau două spații intercostale sub incizia laterală. Toracoscopia poate fi folosită şi în hemitoaracele stâng prin incizia laterală făcută sau printr-o nouă incizie, la unul sau două spații inferior. Unii autori (Prof. Wilm) pătrund cu toracoscopul în hemitoracele drept prin incizia laterală unică. Se inspectează cavitatea toracică, se evidențiază eventualele leziuni asociate, după care se confirmă malformația sternală şi zona cu adâncitura cea mai mare, relația cu pericardul şi pachetul vascular mamar intern drept (fig. 13, 14).

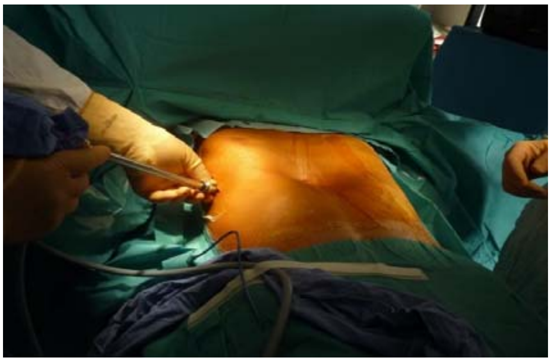

Fig. 13. Introducerea toracoscopului de 1o mm şi 30 de grade în hemitoracele drept

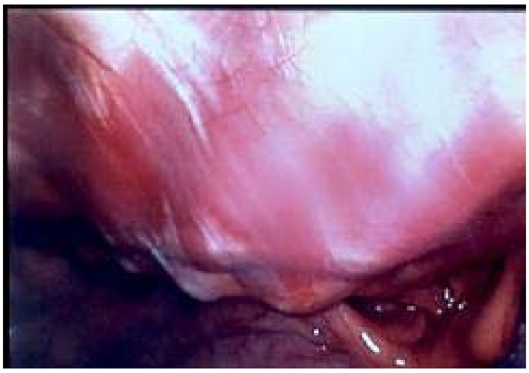

Fig. 14. Aspectul toracoscopic al deformației toracice - deformație în “U”

Toracoscopia poate să ne furnizeze o informație importantă, şi anume aspectul deformației. Aceasta poate fi în “U”, când suprafața sternală deformată este mai lungă, sau în “V”, când suprafața deformată este în unghi ascuțit. În funcție de această caracteristică a deformației vom avea în vedere modul de stabilizare a lamei de osteosinteză, care este mult mai fixă pe o deformație în “U” şi mai puțin fixă pe o deformație în “V”, ceea ce implică fixarea ei exterioară cu două stabilizatoare şi în mai multe puncte.

Trecerea retrosternală a disectorului la nivelul adânciturii maxime din dreapta în stânga trebuie făcută fără a leza pachetul vascular mamar intern, pericardul sau cordul. Până la marginea dreaptă a deformației, trecerea disectorului se poate vizualiza toracoscopic, mai departe prin mediastin, grăsimea retrosternală precardiac până în stânga, în spațiul similar celui din dreapta, timpul este orb şi de aceea trebuie făcut cu mişcări blânde, pentru a se evita orice tip de accident intraoperator. Unii autori, după cum am mai precizat, vizualizează toracoscopic şi pleura stângă sau chiar mediastinul retrosternal printr-o a treia (toracic stâng) şi a patra (subxifoidian) incizie. Se pot folosi cele trei disectoare (*large, medium, small*), în funcție de vârstă şi de dezvoltarea fizică a pacientului (fig. 15).

După trecerea disectorului din dreapta în stânga se procedează la **modelarea sternală** prin presiuni manuale făcute deasupra şi dedesubtul deformației şi a rebordului costal, pentru a obține forma dorită. Se repetă manevra de câteva ori, cu scopul de a întinde țesutul conjunctiv şi de a corecta excavația. Pentru deformațiile complexe se pot trece şi două disectoare pentru a corecta deformația, deci montarea a două bare.

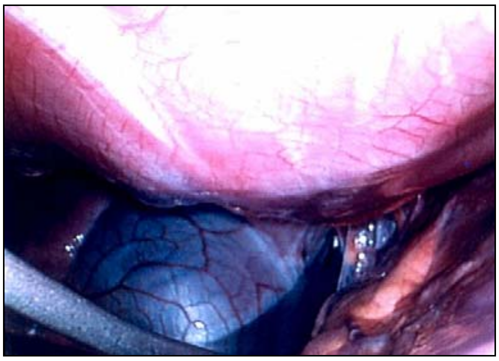

Fig. 15. Progresia disectorului retrosternal dreapta în stânga

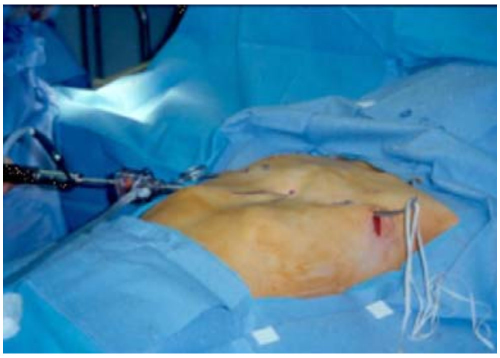

Fig. 16. Ataşarea unui şnur de vârful disectorului şi retragere acestuia la acelaşi nivel

Retragerea disectorului şi a unui şnur ataşat de vârful acestuia (fig. 16)**:** unii autori folosesc în locul şnurului un tub de dren ancorat de capătul disectorului, pentru a se evita leziunile vasculare de şnur sau marginile neregulate ale lamei de osteosinteză. Din aceste motive alţi producători au renunţat la lamele cu margini dinţate, schimbând în acest sens şi sistemul de fixare (MedExpert).

Lama de osteosinteză precurbată este ataşată de şnur sau de tubul de dren şi este împinsă şi uşor trasă de şnur din dreapta către stânga, cu concavitatea în sus (fig. 17).

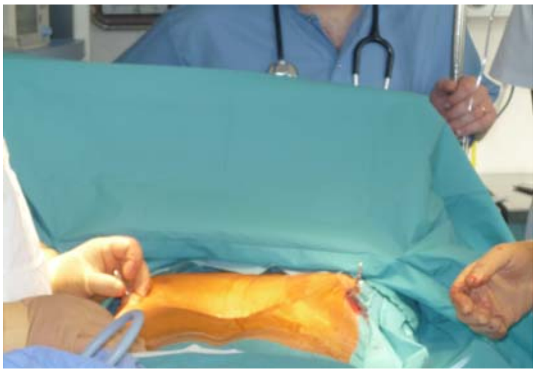

Fig. 17. Lama de corecţie trecută retrosternal, cu concavitatea în sus

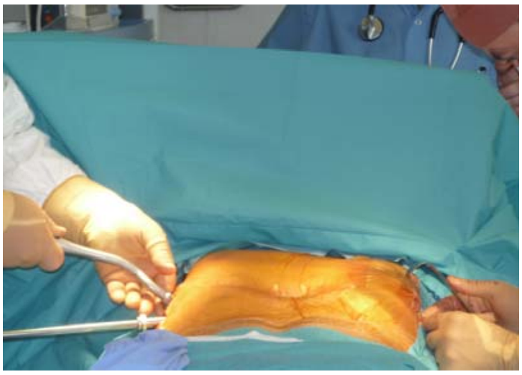

Fig. 18. Rotirea lamei la 180º, corectând astfel deformaţia

Rotirea lamei la 180º se face cu rotitorul din trusa specială (flipper), astfel încât lama din poziţia iniţială cu concavitatea în sus devine cu concavitatea în jos, spre coloana vertebrală, corectând în felul acesta deformaţia sternală. În cazul în care deformaţia nu s-a corectat convenabil, se poate proceda la montarea unei a doua lame, mai sus sau mai jos de prima lamă (fig. 18).

Stabilizarea lamei la peretele toracic se face prin utilizarea unor stabilizatoare din trusa specială, care se fixează pe de-o parte de capetele barei, iar pe de altă parte de musculatura peretelui toracic. În funcţie de dezvoltarea fizică, vârsta pacientului, aspectul în "U" sau "V" al deformaţiei se pot folosi unul sau două stabilizatoare. Unii producători au dezvoltat şi stabilizatoare resorbabile. Pentru montarea stabilizatorului se creează în timpul efectuării inciziei laterale un buzunar subcutanat. Forma stabilizatoarelor poate fi diferită, în funcţie de producător (rectangulare, triunghiulare etc.). Şi sistemul de prindere dintre lamă şi stabilizator diferă de la un producător la altul, unii renunţând la marginile dinţate ale lamei, necesare pentru fixarea stabilizatorului.

După plasarea lamei, evacuarea pneumotoraxului din hemitoracele drept sau stâng se poate face conservator, prin poziţionarea pacientului în Trendelenburg, ventilarea plămânilor cu presiuni pozitive, sau se pot lăsa 24-48 de ore tuburi de dren în cavitatea pleurală dreaptă şi/sau stângă, dacă s-a creat un pneumotorax şi pe stânga.

Radiografia postoperatorie imediată este obligatorie pentru vizualizarea poziției finale a lamei şi eventual a pneumotoraxului. Pacientul imediat postoperator poate fi transferat într-un salon de urmărire sau ATI pentru 24 de ore.

Accidente şi incidente intraoperatorii

Fiind un procedeu minim invaziv, este de dorit să nu existe accidente şi incidente intraoperatorii. Cel mai redutabil incident intraoperator este hemotoraxul, care poate fi cauzat în timpul tehnicii operatorii de mai multe surse de sângerare, la trecerea disectorului sau a lamei de corecție: pachetul vascular intercostal, parenchimul pulmonar (plaga pulmonară), pachetul vascular mamar intern drept, vase pericardice, cord (plagă cardiacă), pachetul vascular mamar intern stâng şi pachetul intercostal simetric de partea stângă (care nu pot fi vizualizate decât printr-o altă toracoscopie stânga). Un alt incident, destul de frecvent, este pneumotoraxul stâng produs prin efracția pleurei mediastinale stângi de către disector sau lama de corecție. Rezolvarea acestuia se poate face conservator, evacuându-se aerul pe lângă lama de corecție în hemitoracele drept sau, în situația unui pneumotorax important, prin efectuarea unei pleurotomii minime stângi pentru 24-48 de ore.

Complicații precoce (în primele zile postoperator)

Mai frecvent am întâlnit, în primele zile postoperator, apariția unei pleurezii drepte reziduale, serocitrină sau hemoragică, datorită neplasării unui tub de dren în cavitatea pleurală dreapta sau neevacuării complete a pneumotoraxului şi revărsatelor pleurale la finele intervenției chirurgicale. Pericardita serocitrină sau hemoragică este o altă complicație care apare în primele zile postoperator, ca urmare a leziunilor de decubit create de lamă asupra vaselor pericardice.

Complicații tardive

Cea mai frecventă complicație tardivă este migrarea lamei (*bar displacement*), care apare imediat ce pacientul începe să se mobilizeze şi să nu respecte întocmai indicațiile prescrise în acest sens. În asemenea situații pacientul trebuie să se prezinte de urgență la spital pentru repoziționarea lamei. Hemotoraxul tardiv şi pericardita tardivă sunt cauzate de leziuni de decubit ale lamei de corecție care pot pune în pericol viața bolnavului (insuficiență respiratorie, tamponadă). În literatură se descriu alergii la metale, eroziuni tegumentare sau infecții ale pielii cauzate de lama de corecție. Pentru fiecare dintre acestea se aplică, în momentul apariției, tratamentul corespunzător. De asemenea, se descrie ca şi complicație tardivă supracorecția, care presupune modelarea în exces a lamei, o curbură prea mare şi care transformă sternul înfundat în stern carenat. Recurența bolii este o altă complicație tardivă, care poate apărea în cazul indivizilor voluminoşi, cu structură osoasă solidă sau datorită calității îndoielnice a lamei de corecție (refolosire). Cel mai frecvent, recurența apare după extragerea lamei, în special dacă aceasta se face într-o perioadă mai mică de 2 ani de la operație.

Evoluție şi îngrijiri postoperatorii

În perioada imediat postoperatorie pacienții trebuie să respecte indicațiile prescrise de medicul curant, pentru a obține un rezultat favorabil funcțional şi estetic cât mai bun. Astfel, în primele 3 zile postoperator, când de regulă pacientul este internat, repausul se face cu fața în sus, fără să execute mişcări de îndoiri din talie sau piept, fără răsuciri. Se mobilizează progresiv, chiar din prima zi şi execută mişcări de gimnastică respiratorie. De regulă, a 4-a zi postoperator pacientul poate fi externat.

Recomandările la externare prevăd pentru primele 6 săptămâni mobilizare progresivă prin plimbări dese şi lungi, exerciții respiratorii profunde, evitarea aplecărilor, rostogolirilor, răsucirilor. Nu va face sport, nu va ridica greutăți şi nu va dormi pe o parte sau cu fața în jos. După 6 săptămâni, pacientul se prezintă la un prim control, iar dacă lama este în poziția corectă, pacientul va putea să îşi înceapă activitățile obişnuite, chiar şi sportul. Excepție vor face sporturile de contact, până la extragerea lamei.

Pacientul va trebui să cunoască şi să transmită personalului medical, în situaţia în care se impune efectuarea unei defibrilări sau a unui IRM, despre prezenţa lamei de osteosinteză.

Extragerea lamei se face între 2 şi 4 ani de la implantare. Pacientul este internat pentru 24-48 de ore. Procedeul de extragere se face cu anestezie generală şi intubaţie oro-traheală normală. După extragerea lamei, pacientul poate părăsi spitalul în câteva ore.

Experienţa noastră în MIRPE

În luna octombrie 2007, Secţia Clinică de Chirurgie Toracică din SUUMC a organizat un curs postuniversitar cu tema "Chirurgia miniinvazivă a malformaţiilor sternale – Pectus Excavatum", cu scopul de a introduce şi dezvolta acest procedeu şi în secţia noastră. La acest curs a fost invitat să conferenţieze profesorul Kamil Kaynak de la Facultatea de Medicină din cadrul Universităţii "Cerrahpasa" – Istanbul. La manifestare au participat un număr important de chirurgi toracici, care au asistat la prezentările orale, precum şi la demonstraţiile practice din sala de operaţie.

Între octombrie 2007 şi iunie 2010, în Secţia Clinică de Chirurgie Toracică a SUUMC au fost operaţi un număr de 40 de pacienţi (36 bărbaţi şi 4 femei) utilizându-se procedeul Nuss pentru *pectus excavatum*. Vârsta medie a pacienţiilor operaţi a fost 17,9 ani (variind între 8 şi 38 ani).

Media zilelor de spitalizare a fost de 4,8 zile.

La 38 de pacienţi s-a folosit o singură lamă, utilizându-se toate numerele, de la numărul 8 la numărul 16, fără extreme (numerele 7 şi 17). La 2 pacienţi am fost nevoiţi să introducem o a doua lamă de osteosinteză, pentru o corecţie adecvată.

Durata medie a operaţiei a fost de 58 de minute. La începutul dezvoltării tehnicii, intervenţiile durau mai mult (până la 2-3 ore), pe măsura efectuării unui număr mai mare de cazuri, timpul operator s-a redus substanţial, ajungând chiar şi la 40 de minute.

Pe cazuistica personală, singura complicaţie precoce întâlnită a fost pneumotoraxul drept, la primele cazuri, când evacuarea pneumotoraxului se făcea pe masa de operaţie, fără ca bolnavii să fie drenaţi aspirativ. Ulterior, am adoptat atitudinea de a drena pacienţii pentru 24 de ore, nemaiîntâlnind această complicaţie.

Cea mai frecventă complicaţie tardivă pe care am întâlnit-o a fost mobilizarea lamei (3 cazuri). Într-un caz s-a reintervenit şi repoziţionat aceeaşi lamă, cu corecţia deformării; într-un alt caz, după repoziţionarea primei lame şi corecţia parţială a deformaţiei s-a implantat o a doua lamă, corecţia fiind completă. Un al treilea caz, cu mobilizare de lamă, nu s-a mai prezentat la repoziţionarea lamei.

În două cazuri, la nivelul stabilizatorului au apărut cicatrici cheloide cu eroziune parcelară tegumentară, care s-au rezolvat chirurgical, cu anestezie locală.

Extragerea lamei înainte de 2 ani s-a efectuat în două cazuri, în principiu din cauza durerilor intense, care nu au cedat la analgeticele obişnuite, la un pacient cu o lamă, în vârstă de 38 de ani, la care capetele lamei erau inclavate în spaţiul intercostal împreună cu pachetul vasculo-nervos, şi la un pacient cu două lame, la care la unul dintre stabilizatoare a apărut o supuraţie tegumentară urmată de exteriorizarea acestuia. La cererea insistentă a familiei au fost extrase ambele lame. Aspectul post extragere la ambii pacienţi a fost mulţumitor. La alţi 3 pacienţi s-au extras lamele la peste 2 ani de la implantare, rezultatele fiind foarte bune.

După efectuarea celor 40 de cazuri, putem să afirmăm că procedeul Nuss de corecţie a sternului înfundat simetric şi uşor asimetric este unul bun şi că metoda prezintă reale beneficii, cum ar fi: operaţie minim invazivă, timp operator redus, pierderi sanguine minime, reintegrare socio-profesională rapidă, reabilitarea funcţiilor respiratorie şi cardiacă, rezultate excelente din punct de vedere estetic pe termen lung (fig. 19, 20).

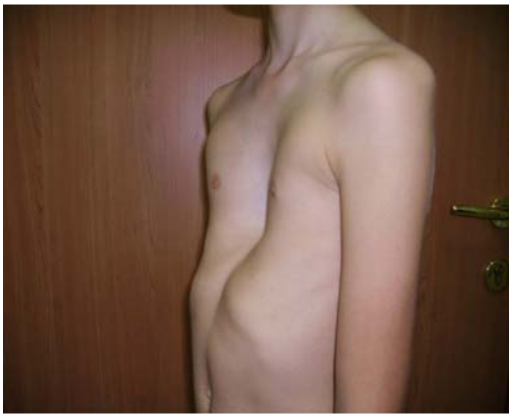
Fig. 19. Stern înfundat asimetric - preoperator

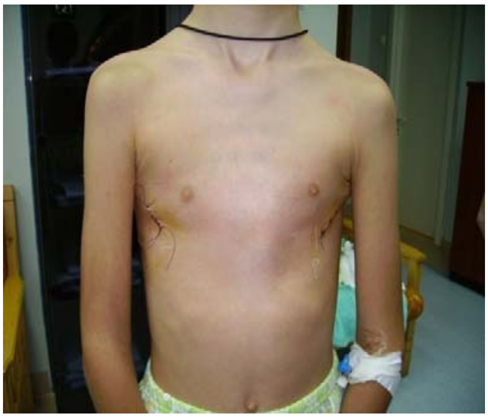
Fig. 20. Stern înfundat asimetric - postoperator

În **concluzie**, metoda de rezolvare minim invazivă a sternului înfundat – "procedeul Nuss" – este o soluţie eficientă, cu rezultate estetice şi funcţionale foarte bune, care permite reintegrarea socială şi profesională rapidă a pacientului, cu rezultate bune şi foarte bune în ceea ce priveşte propria imagine a corpului, precum şi în îmbunătăţirea funcţiei pulmonare şi a toleranţei la efort.

În studiul nostru, în urma analizei tuturor cazurilor, am constat că gradul de satisfacţie al pacienţilor şi al familiilor acestora a fost foarte bun la peste 80% din cazuri, ceea ce ne face să considerăm că indicaţia chirurgicală a acestei afecţiuni este pe deplin justificată.

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Masă tumorală mediastinală mare - hiperplazie foliculară timică. Prezentare de caz

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Abstract. Hiperplazia timică este o afecţiune foarte rară; poate fi întâlnită atât la copii, cât şi la adulţi şi pune probleme de diagnostic diferenţial cu neoplaziile timice. Corelarea aspectului imagistic al leziunii timice cu aspectele clinico-biologice permite stabilirea unui diagnostic preoperator cât mai exact şi adoptarea unei atitudini terapeutice adecvate. Hiperplazia timică prezintă două forme histologice distincte, care se manifestă independent: hiperplazia timică adevărată şi hiperplazia timică foliculară (limfoidă) a timusului.

Prezentăm cazul unei paciente în vârstă de 74 de ani, cu simptomatologie nespecifică: scădere ponderală - 7 kg în 2 luni, anorexie progresivă, astenie fizică marcată, suspectată de miastenia gravis. Examenul fizic iniţial, endoscopia digestivă superioară, colonoscopia, examenul neurologic şi examenul electromiografic nu au decelat modificări patologice.

Examenul computer-tomografic toraco-abdominal a relevat existenţa unei formaţiuni timice de aproximativ 2 cm, ce a captat substanţa de contrast. S-a intervenit chirurgical efectuându-se timectomie totală. În urma examinării microscopice s-a formulat diagnosticul histopatologic de hiperplazie foliculară timică. Evoluţia postoperatorie a fost favorabilă, cu ameliorarea asteniei fizice şi a stării generale şi chiar îmbunătăţirea apetitului alimentar.

Deoarece o tumoră de mediastin anterior nu poate fi diagnosticată cu certitudine prin metode neinvazive, este esenţială efectuarea unei biopsii incizionale prin mediastinoscopie anterioară sau toracotomie limitată, sau a unei biopsii excizionale prin toracotomie postero-laterală pentru a se putea formula diagnosticul histopatologic şi a se putea exclude alte afecţiuni ale timusului cu prognostic nefavorabil.

Am prezentat cazul întrucât această entitate histopatologică este foarte rară, în special la vârste înaintate, precum şi pentru importanţa efectuării diagnosticului diferenţial cu o proliferare neoplazică cu origine timică.

Cuvinte cheie: timus, hiperplazie timică, mediastin anterior

Abstract. Thymic hyperplasia is a very rare disease, which may appear both in children and adults, and could generate differential diagnosis problems with thymic neoplasias. The correlation of the imaging aspect of the thymic lesion with the clinico-biological aspects allows an accurate preoperative diagnosis and an adequate therapeutic attitude. Thymic hyperplasia occurs in two distinct histological forms, which manifest separately: true thymic hyperplasia and follicular (lymphoid) thymic hyperplasia.

We present the case of a 74-years-old patient, with non-specific symptoms: weight loss – 7 kg in 2 month, progressive anorexia, marked physical asthenia, suspected of myasthenia gravis. Physical examination, superior digestive endoscopy, colonoscopy, neurological examination, and electromyography did not identify pathological changes.

Thoracic-abdominal computer tomography identified the existence of a thymic tumoral mass, approximately 2 cm in diameter, capturing the contrast substance. A total thymectomy was performed. Postoperatively, the evolution was favorable, with diminishing of the physical asthenia, general status and appetite improvement.

Since an anterior mediastinal tumor cannot be diagnosed precisely through non-invasive methods, it is essential to perform an incision biopsy by anterior mediastinoscopy or limited thoracoscopy, or an excizional biopsy by postero-lateral thoracotomy, allowing histopathological diagnosis and a differential diagnosis with neoplastic proliferations of thymic origin.

We presented this case due to the rarity of this histopathological entity, especially in old patients, and for the importance of the differential diagnosis with neoplastic proliferations of thymic origin

Introducere

Dintre structurile mediastinale, timusul prezintă o probabilitate redusă de transformare neoplazică. Hiperplazia timică (HT) este o afecţiune foarte rară, putând afecta atât copiii, cât şi adulţii. În general, evoluţia este asimptomatică, deşi, în puţine cazuri, s-au raportat simptome de compresie mediastinală: dispnee, disfagie, obstrucţie de căi aerifere sau infecţii pulmonare acute sau recurente[1].

Hiperplazia timică pune problema diagnosticului diferenţial cu neoplaziile timice, deoarece unele dintre acestea au un prognostic rezervat şi o evoluţie biologică agresivă. Corelarea aspectului imagistic al leziunii timice cu aspectele clinico-biologice permite stabilirea unui diagnostic preoperator cât mai exact şi o atitudine terapeutică adecvată.

Hiperplazia timică prezintă două forme histologice distincte, care se manifestă independent: hiperplazia timică adevărată şi hiperplazia timică foliculară (limfoidă) a timusului[2,3,4]. De cele mai multe ori, aceste două tipuri histologice nu pot fi diferenţiate strict pe baza datelor clinice, evolutive sau imagistice. De regulă, HT adevărată poate apărea: (1) în anumite situaţii patologice – de exemplu, după arsuri, post-chimioterapie (tumori cu celule germinale, boală Hodgkin), post-radioterapie; (2) asociată unor afecţiuni endocrine (boală Graves); şi (3) în absenţa oricărui stres şi a altor asocieri comorbide, de regulă la copii şi tineri. În schimb, hiperplazia limfo-foliculară a timusului apare întotdeauna în contextul unei dezordini imunitare majore (miastenia gravis, LES, sclerodermia, tiroidita Hashimoto, artrita reumatoidă, scleroza multiplă şi pacienţii cu SIDA).

În *hiperplazia timică adevărată*, examenul macroscopic decelează creşterea în volum şi greutate a timusului. Deoarece greutatea glandei creşte continuu de la naştere (10-35g) până la pubertate (10-50g), suferind ulterior o atrofie progresivă (5-15g la vârstnici), evaluarea diagnosticului de hiperplazie timică adevărată se realizează pe baza tabelelor de greutate standard raportată la vârstă pentru timusul normal[1,5, 6,7]. Unii autori consideră că o greutate mai mare de două deviaţii standard faţă de valoarea medie defineşte limita stastistică a hiperplaziei timice[8]. Când greutatea timusului depăşeşte 100 de grame, hiperplazia timică este denumită masivă[8]. În mod caracteristic, examinarea microscopică decelează o proliferare a parenchimului timic cu aspect şi arhitectură lobulară normale, cu păstrarea structurilor corticale şi medulare şi minim ţesut adipos.

În *hiperplazia timică foliculară*, timusul are dimensiuni normale, dar greutatea poate fi mărită. Histopatologic, se constată un număr crescut de foliculi limfoizi de dimensiuni mărite, de tip secundar, cu prezenţa de centri germinativi, situaţi în spaţiile perivasculare şi paraseptale. Foliculii limfoizi sunt alcătuiţi în cea mai mare parte din limfocite de tip B, majoritatea acestora conţinând imunoglobuline din clasele IgM şi IgD[9]. Numărul acestor foliculi variază foarte mult atât de la pacient la pacient, cât şi în cadrul aceleiaşi glande; astfel se explică de ce diagnosticul poate fi eronat pe o singură secţiune sau pe fragmente biopsice de dimensiuni mici.

Hiperplazia foliculară a timusului are loc sub acţiunea hormonilor tiroidieni şi a modificărilor imunologice caracteristice afecţiunilor asociate. În cazul miasteniei gravis, centrii germinativi din foliculii timici produc anticorpi împotriva receptorului pentru acetilcolină; evoluţia cazurilor după timectomie este net favorabilă.

Prezentare de caz

Redăm cazul unei paciente în vârstă de 74 de ani, cu simptomatologie nespecifică: scădere ponderală - 7 kg în 2 luni, anorexie progresivă, astenie fizică marcată, suspectată de miastenia gravis. Examenul fizic iniţial, endoscopia digestivă superioară şi colonoscopia nu au decelat modificări patologice. Examenul neurologic, coroborat cu examenul electromiografic au infirmat asocierea unei posibile miastenii.

Examenul computer-tomografic toraco-abdominal a relevat existenţa unei formaţiuni timice de aproximativ 2 cm, care a captat substanţa de contrast. În urma stabilirii diagnosticului imagistic de tumoră timică, s-a intervenit chirurgical sub anestezie generală şi s-a efectuat timectomie totală prin sternotomie parţială înaltă, cu trimiterea piesei de rezecţie pentru investigare histopatologică. Evoluţia postoperatorie a fost favorabilă, pacienta externându-se în a 4-a zi de la operaţie. S-a constat ameliorarea asteniei fizice şi a stării generale, şi chiar îmbunătăţirea apetitului alimentar.

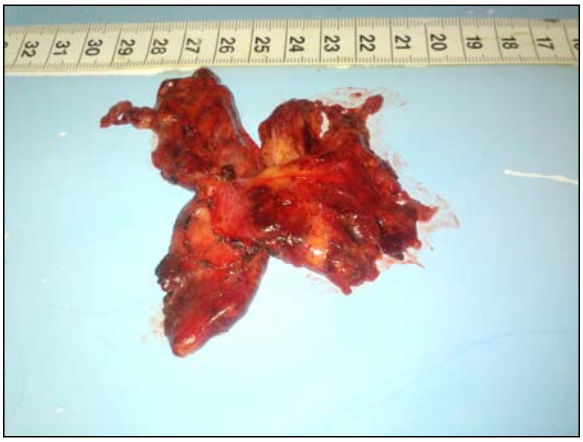

Fig. 1. Aspectul macrosopic al piesei de timectomie totală: suprafață externă netedă, focal lobulată.

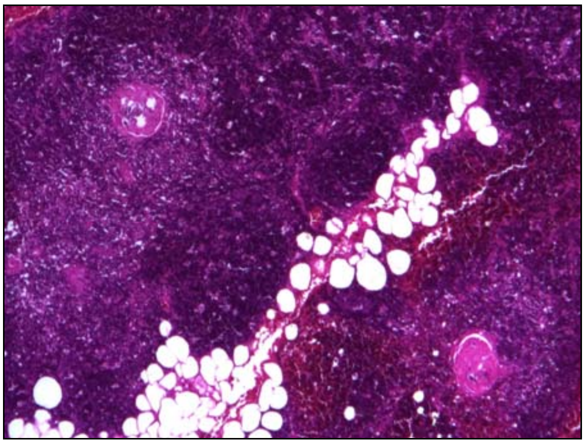

Fig. 4. Foliculi limfoizi cu centri germinativi, corpusculi Hassal, țesut adipos interfolicular. Colorație H&E, ob. 4x.

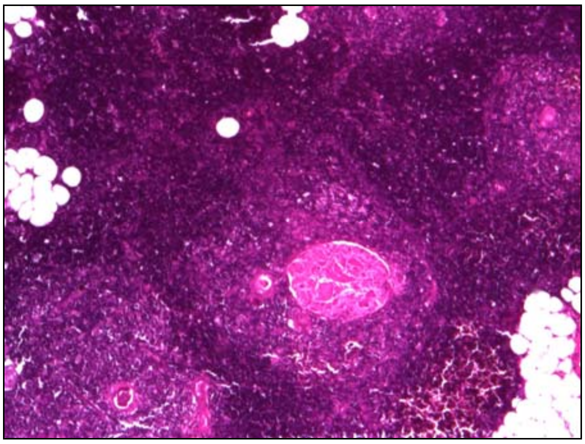

Fig. 2. Timus cu foliculi limfoizi secundari, cu centri germinativi şi corpusculi Hassal. Colorație H&E, ob. 4x.

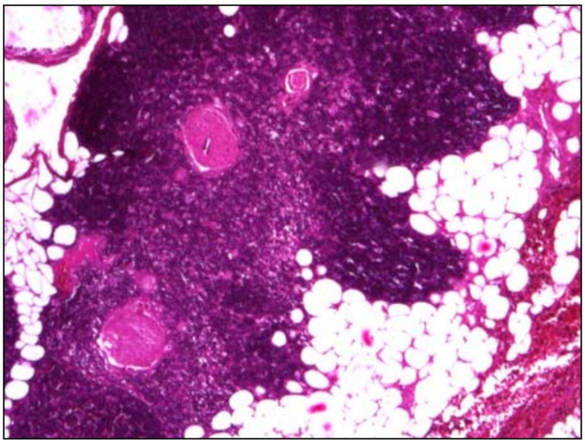

Fig. 5. Foliculi limfoizi cu centri germinativi, corpusculi Hassal şi zone întinse de țesut adipos interfolicular. Colorație H&E, ob. 4x.

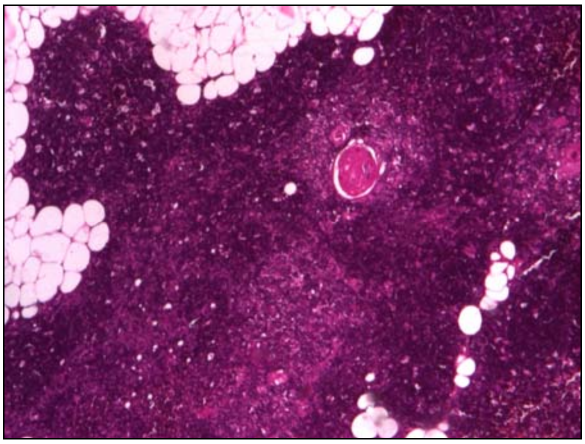

Fig. 3. Foliculi limfoizi cu formare de centri germinativi, corpusculi Hassal şi interpunere de țesut adipos interfolicular. Colorație H&E, ob.

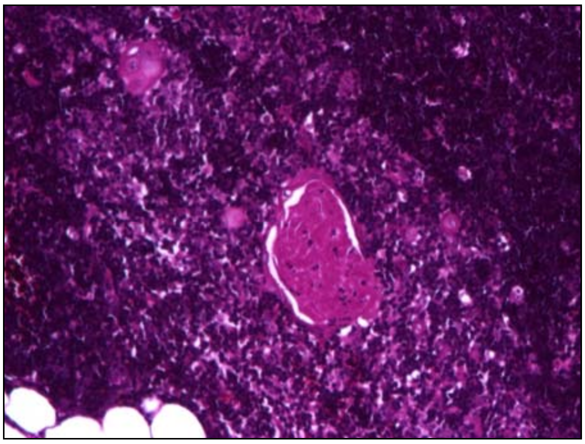

Fig. 6. Folicul limfoid secundar, corpusculi Hassal. Colorație H&E, ob. 10x.

Aspecte histopatologice

Examenul macroscopic. Piesa de excizie (timusul) a avut greutatea de 55g şi dimensiuni de 7,5/5,5/2,3 cm, suprafaţă externă netedă, focal lobulată, părţile superioare şi inferioare ale timusului prezentând aspect bombat (fig. 1). Pe secţiune, s-au observat numeroşi noduli albicios-gălbui, compacţi, diseminaţi în masa glandulară timică.

Examenul microscopic. Examinarea histopatologică a evidenţiat prezenţa a numeroşi foliculi limfoizi reactivi, cu formare de centri germinativi, situaţi la nivel medular timic, precum şi un număr crescut de corpusculi Hassal (fig. 2- 6). În urma examinării microscopice s-a formulat *diagnosticul histopatologic* de hiperplazie foliculară timică.

Diagnosticul diferenţial este necesar din cauza impactului prognostic pe care îl au unele dintre neoplaziile ce pot afecta timusul (timoame, limfoame, timolipoame, tumori neuroendocrine, teratoame, sarcoame, limfangioame, hemangioame, tumori tiroidiene substernale etc.)[10,11,12].

Concluzii

Deoarece o tumoră de mediastin anterior nu poate fi diagnosticată cu certitudine prin metode neinvazive, este esenţială efectuarea unei biopsii incizionale prin mediastinoscopie anterioară sau toracotomie limitată, sau a unei biopsii excizionale prin toracotomie postero-laterală pentru a se putea formula diagnosticul histopatologic şi a se putea exclude alte afecţiuni ale timusului cu prognostic nefavorabil. Am prezentat cazul datorită rarităţii acestei entităţi histopatologice, în special la vârste înaintate, precum şi pentru importanţa efectuării diagnosticului diferenţial cu o proliferare neoplazică cu origine timică.

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Observații asupra modificărilor semnalului IRM la nivelul spațiului presacrat şi retrorectal în neoplaziile rectale

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Rezumat. Localizarea anatomică, fixarea la nivelul grăsimii pelvine şi lipsa peristalticii fac din rect un organ ideal pentru explorarea prin IRM. La nivelul pelvisului şi organelor din acest teritoriu se descriu o serie de structuri cu importanță chirurgicală deosebită, posibil a fi explorate prin IRM. Spațiul retrorectal, fascia rectosacrată, spațiul presacrat, plexul presacrat şi fascia mezorectului sunt structuri ce pot fi evidențiate şi explorate prin IRM pelvin.

Evidențierea preoperatorie a unora dintre aceste structuri, particularitățile lor de semnal sau identificarea celor care nu se decelează în mod obişnuit, observații efectuate şi analizate corelativ pentru cazurile expuse, sunt elemente ce furnizează informații valoroase despre extinderea proceselor patologice, a evenimentelor ce pot influența atitudinea terapeutică şi tehnica chirurgicală.

Cuvinte cheie: neoplazii rectale, semnal IRM, atitudine terapeutică, tehnică chirurgicală

Abstract. Anatomical localization, fixity at the pelvic fat and lack of rectal body mobility are ideal for MRI exploration. In the pelvis and around pelvic organs are described a series of structures with particular surgical significance, that may be explored by MRI. Retrorectal space, rectosacralis fascia, presacral space, presacral plexus and mezorectal fascia are structures that can be identified and explored by pelvic MRI.

Preoperative highlight some of these structures, their signal features or identifying those who do not normally reveal, facts analyzed correspondingly in exposed cases, are elements that provide valuable information about the extension of pathological processes, events that may influence the therapeutic approach and surgical technique

Introducere

Localizarea anatomică, fixarea la nivelul grăsimii pelvine şi lipsa peristalticii fac din rect un organ ideal pentru explorarea prin IRM. La nivelul pelvisului şi organelor de la acest nivel se descriu o serie de structuri cu importanță chirurgicală deosebită, posibil a fi explorate prin IRM.

Spațiul retrorectal este definit ca fiind delimitat posterior de fascia parietală presacrată şi anterior de fascia mezorectală (1). Disecția la nivelul spațiului retrorectal observă la nivelul celei de-a patra vertebre sacrate apariția din fascia parietală presacrată a unei alte fascii, invariabil prezente, denumită fascia rectosacrată, care se va uni cu fascia viscerală ce înveleşte mezorectul posterior. Conexiunea este situată la 3-4 cm superior de joncțiunea anorectală.

Spațiul presacrat este definit ca un spațiu retroperitoneal localizat între peritoneul parietal al peretelui abdominal posterior sau fascia presacrată şi sacrum. Începe la bifurcarea aortei şi se întinde periferic până la planşeul pelvin. Lateral este delimitat de vasele iliace interne. Conținutul său este reprezentat de țesut lax areolar şi țesut conjunctiv, vase arteriale, plexul venos presacrat (2,3), plexul nervos superior hipogastric şi nervii hipogastrici, precum şi de porțiuni din plexul hipogastric inferior. Hemoragiile din plexul presacrat sunt potențial amenințătoare de viață în timpul intervenţiilor pe pelvis (4,5) şi sunt greu de controlat atunci când venele se retrag în găurile sacrate (6).

Din punct de vedere al imagisticii prin IRM, dintre structurile ligamentare pelvine fascia presacrată şi ligamentul sacrogenital sunt singurele observabile ca structuri normale. Restul structurilor sunt evidenţiate numai când sunt îngroşate de procese patologice (7). În acest spaţiu nodulii limfatici nu sunt vizibili în mod normal. În cancerele rectale diseminarea limfatică are loc iniţial prin ganglionii limfatici mezorectali şi presacraţi adiacenţi tumorii şi abia apoi se notează determinări de-a lungul ganglionilor de la nivelul vaselor rectale superioare.

Fascia presacrată se vede pe secţiunile sagitale IRM ca o structură liniară cu semnal scăzut ce acoperă vasele presacrate (8). Pe secţiuni T2 fast spin echo zona de semnal înalt anterior de fascia presacrată este ţesut adipos, iar lipsa de semnal din spaţiul retrorectal este dată de vasele sacrate mijlocii.

Fascia mezorectului este evidenţiată ca o structură de hiposemnal ce înconjoară mezorectul (1,9). Mezorectul este o structură vizibilă în IRM axial în hipersemnal (similar cu grăsimea) ce este dispusă în jurul rectului şi conţine vase (unele fără semnal) şi limfatice. Este bine delimitată de hiposemnalul muscularei propria a peretelui rectal (9). Ganglionii limfatici din mezorect apar ca structuri ovoidale în hipersemnal.

Fascia rectosacrală este o structură de forma unei benzi, de grosime variabilă, ce se întinde între sacrum şi fascia mezorectului la nivelul celei de a patra vertebre sacrate, structură ce are şi ea reprezentare IRM. Este denumită şi fascia lui Waldayer. Grosimea acesteia variază de la o membrană transparentă subţire până la o structură groasă, opacă. În aceste ultime situaţii poate conduce eronat disecţia fie în mezorect, cu relizarea unei excizii incomplete, fie în posterior de fascia presacrată, cu hemoragii dificile.

Prin IRM se pot furniza informaţii valoroase despre extinderea proceselor patologice, evenimente ce pot influenţa atitudinea terapeutică şi tehnica chirurgicală.

Material şi metodă

Am examinat o serie de pacienţi cu neoplasm de rect. Evaluarea a implicat un examen clinic, colonoscopie cu biopsie şi examen anatomopatologic, precum şi examinare prin IRM pelvin nativ şi postcontrast.

Evaluarea endoscopică a fost efectuată cu o trusă de videoendoscopie Olympus Exera 165.

Examinarea cazurilor studiate s-a efectuat pe un aparat PICKER OUTLOOK PROVIEW cu magnet deschis de 0,23T (low field), accesibil mai ales la pacienţii claustrofobi, anxioşi şi obezi.

Secvenţele utilizate au fost T2FSE (TR/TE de 4100/108 ms), T1FSE (TR/TE de 1500/24 ms) nativ şi postcontrast, STIR (*spectral presaturation with inversion recovery*), (TR/TE de 900/23 ms), pentru suprimarea semnalului dat de grăsime, în cele trei planuri axial, sagital şi coronar. Protocolul de examinare prevede secţiuni în secvenţe multiple, respectiv T2 FSE cu secţiuni cu grosime de 7 mm şi interval de 5 mm. Următoarea secvenţă de examinare a fost T1 FSE cu secţiuni cu grosime de 7 mm şi interval de 5 mm. Examinarea STIR a presupus secţiuni cu grosime de 7 mm şi interval de 7 mm.

Rezultatele au fost comparate cu aspectul intraoperator şi aspectele tehnice impuse de particularităţile cazului.

Rezultate

Cazul 1. Prezentăm cazul unui bărbat de 45 de ani, diagnosticat clinic cu neoplasm rectal inferior. Colonoscopia evidenţiază formaţiune vegetant ulcerată ce ocluzionează lumenul rectal, extinsă de la 3 cm la 9 cm de marginea anală. Formaţiunea tumorală este localizată rectal inferior până la nivelul rectului mijlociu. Investigaţiile prin IRM pelvin au diagnosticat în secvenţele T2 sagitale modificări de semnal de tip edematos, bine sistematizate, localizate în spațiul presacrat şi exprimate difuz în spațiul retrorectal. Fascia Waldayer nu este bine exprimată în investigația IRM a pelvisului (fig. 1).

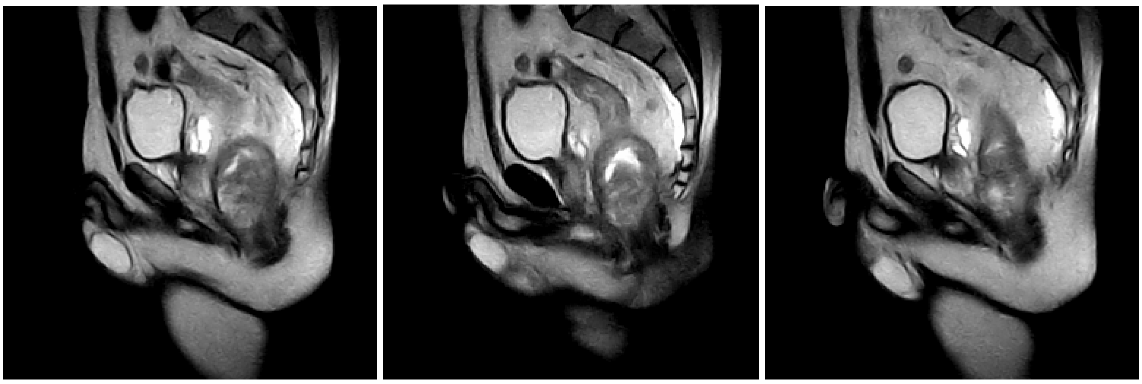

Fig. 1. Examinare IRM în T2 sagital cu modificări de semnal de tip edematos, bine sistematizate, localizate în spațiul presacrat şi cu caracter difuz în spațiul retrorectal.

Explorarea intraoperatorie a afirmat posibilitatea rezecției, care a decurs fără incidente tehnice deosebite. Examinarea intraoperatorie a feței anterioare a sacrumului a evidențiat edemațierea fasciei presacrate şi evidențierea prin transparență a vaselor presacrate. Disecția mezorectului nu a ridicat probleme deosebite la nivelul fasciei Waldayer (fig. 2).

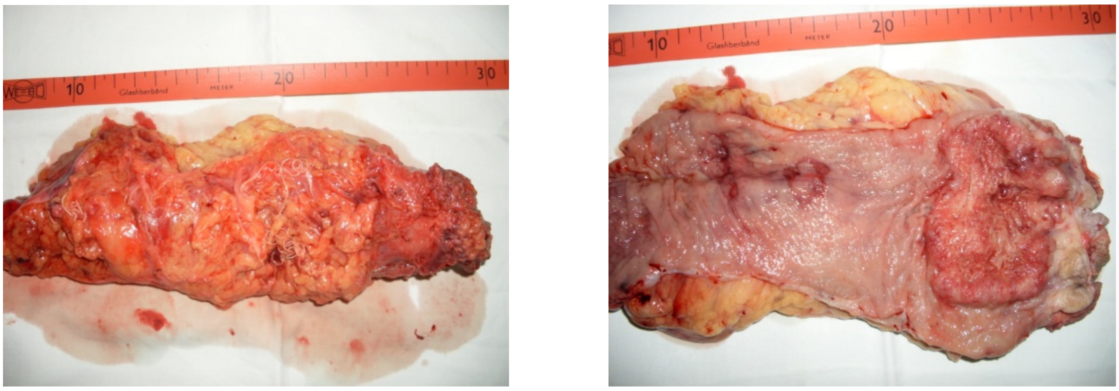

Fig. 2. Apectul piesei operatorii după amputație abdominoperineală de rect. Mezorectul este intact şi complet, iar tumora este cu extindere de la nivelul rectului inferior spre mijlociu.

Cazul 2. Ilustrăm cazul unei paciente de 57 de ani ce prezenta o formațiune tumorală la nivelul rectului mijlociu. Stenoza realizată de procesul patologic nu a fost depăşită de examinarea endoscopică. La examenul prin rezonanță magnetică se obține o imagine IRM T2 sagital care evidențiază o formațiune tumorală voluminoasă ce ocupă în totalitate lumenul rectului mijlociu şi superior, infiltrează posterior spațiul retrorectal, fascia mezorectului, fascia presacrată şi spațiul presacrat, iar anterior peretele posterior al vaginului şi uterului. Fascia Waldayer nu este bine exprimată în investigația IRM a pelvisului (fig. 3).

Explorarea intraoperatorie apreciază nerezecabilitatea formaţiunii, urmată de decizia de a practica un anus iliac stâng definitiv în continuitate. Nu am avut posibilitatea tehnică de a explora structurile pelvine.

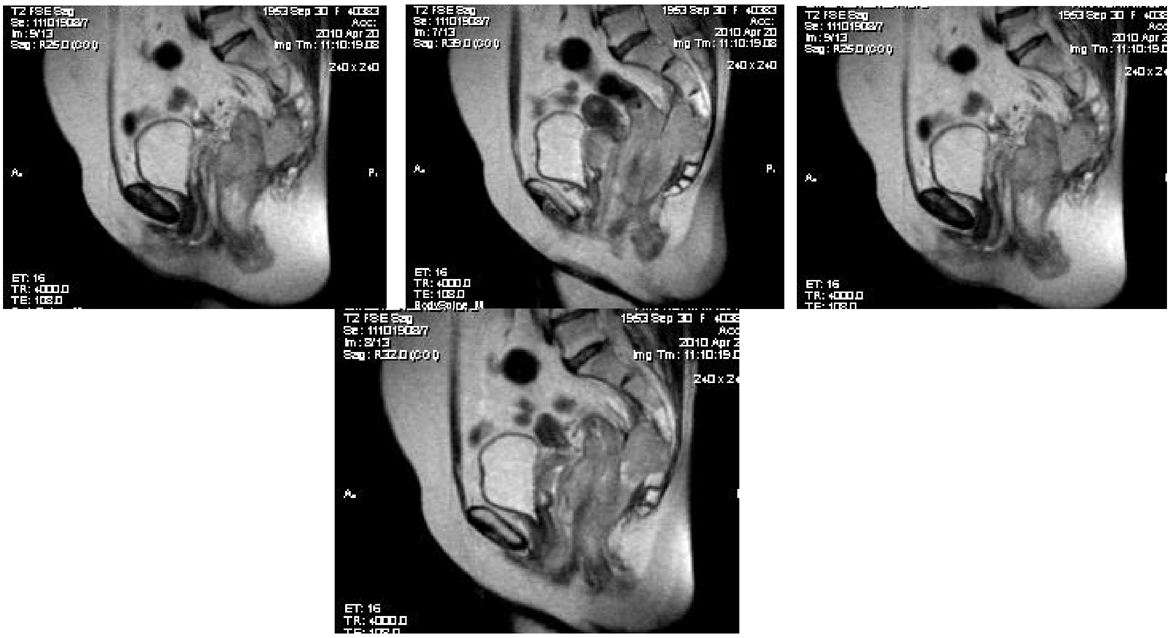

Fig. 3. Imagine IRM T2 sagital care evidenţiază o formaţiune tumorală voluminoasă ce ocupă în totalitate lumenul rectului mijlociu şi superior, infiltrează posterior spaţiul retrorectal, fascia mezorectului, fascia presacrată şi spaţiul presacrat.

Cazul 3. Obiectivăm situaţia unui pacient de 56 de ani, cu neoplasm la nivelul joncţiunii rectosigmoidiene. Colonoscopia descrie formaţiune ulcerant vegetantă la 13 cm de marginea anală, din care se prelevează biopsii. Imaginea în T2 sagital relevă formaţiune tumorală la nivelul joncţiunii rectosigmoidiene, bine delimitată la nivelul pereţilor rectului, la care se asociază edem la nivelul spaţiului presacrat şi arii nesistematizate difuze în hipersemnal T2 în spaţiul retrorectal între cele două fascii (fascia mezorectului şi fascia presacrată). Fascia Waldayer nu este bine exprimată în investigaţia IRM a pelvisului (fig. 4).

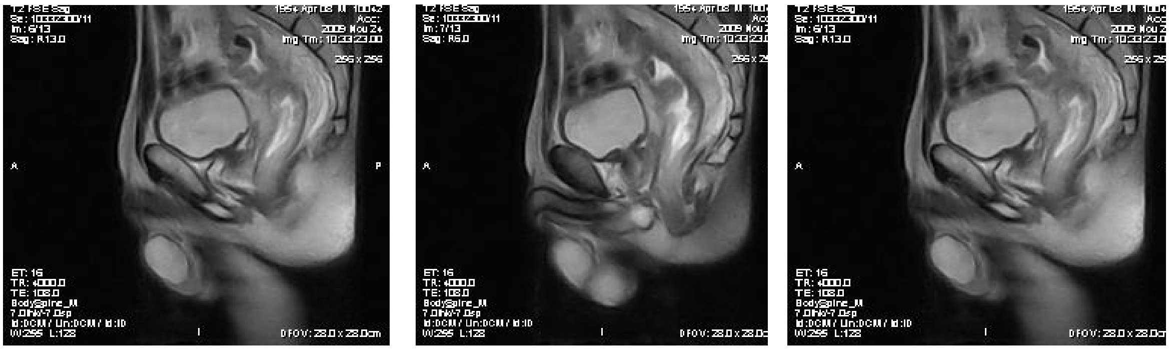

Fig. 4. Formaţiune tumorală la nivelul joncţiunii rectosigmoidiene, bine delimitată la nivelul pereţilor rectului, la care se asociază edem la nivelul spaţiului presacrat şi arii nesistematizate difuze în hipersemnal T2 în spaţiul retrorectal între fascia mezorectului şi fascia presacrată.

Explorarea intraoperatorie apreciază fezabilitatea unei rezecții Dixon joase cu anastomoză terminoterminală. Decolarea mezorectului a fost realizată fără dificultăți tehnice iar anastomoza a fost efectuată în condiții tehnice normale. Examinarea vizuală a spațiului presacrat a remarcat nevizualizarea prin transparența fasciei presacrate a plexului sacrat. Fascia Waldayer demonstrează consistență redusă intraoperator (fig. 5).

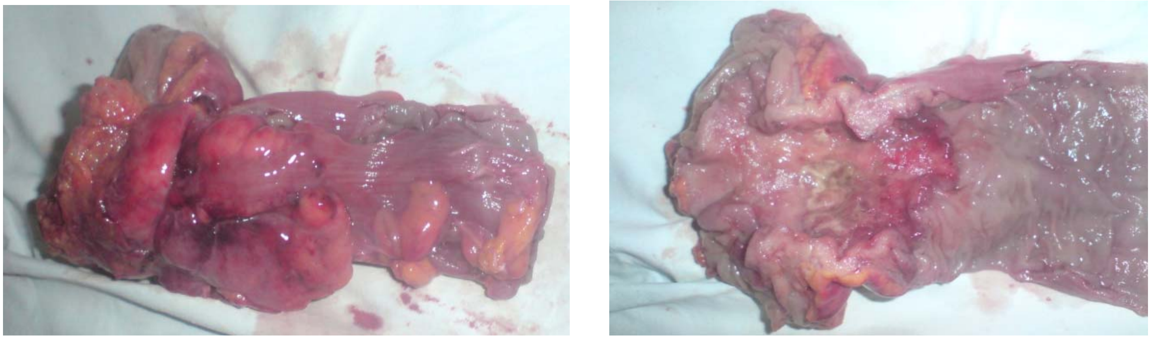

Fig. 5. Aspectul piesei de rezecție.

Cazul 4. Pacient în vârstă de 49 de ani, diagnosticat clinic cu neoplasm rectal inferior. Colonoscopia apreciază dezvoltarea unui proces vegetant şi ulcerat la 3 cm de marginea anală ce se extinde până la 7cm. Investigația IRM în T2 sagital evidențiază edem la nivelul spațiului retrorectal. Imaginile T2 axial demonstrează edem al spațiului presacrat, iar T1 sagital efectuată cu contrast intravenos detectează îngroşarea fasciei presacrate şi hipersemnal la nivelul fasciei. Imagine T2 sagitală care evidențiază edem la nivelul spațiului retrorectal la un pacient cu tumoră rectală inferioară. Pe o imagine T2 axială se evidențiază edem la nivelul spațiului presacrat, iar imaginile în T1 sagital cu contrast intravenos arată o îngroşare a fasciei presacrate. Explorarea IRM T1 sagital cu contrast intravenos identifică fascia prescrată şi fascia mezorectului, precum şi hipersemnal în spațiul retrorectal, în contextul unei formațiuni tumorale rectale inferioare, vascularizate şi infiltrative în fascia mezorectului. Fascia Waldayer nu este bine exprimată în investigația IRM a pelvisului (fig. 6,7,8).

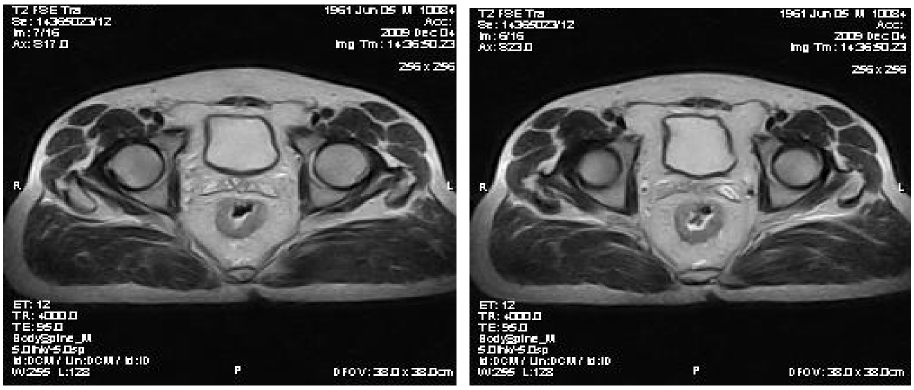

Fig. 6. Imaginile T2 axial ce demonstrează edem al spațiului presacrat.

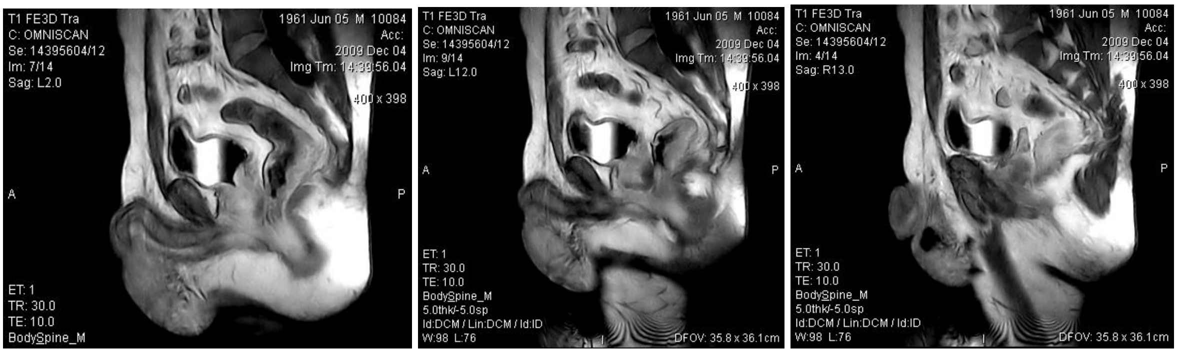

Fig. 7. Explorarea IRM T1 sagital cu contrast intravenos identifică fascia prescrată şi fascia mezorectului, precum şi hipersemnal în spaţiul retrorectal.

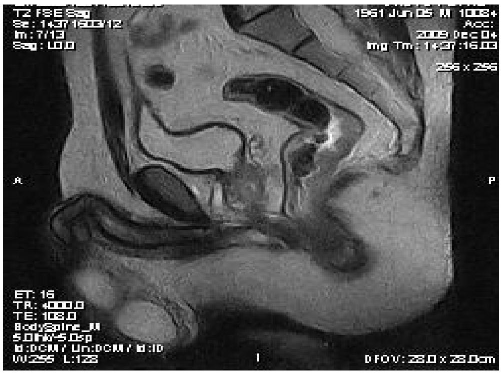

Fig. 8. Imagine T2 sagitală care evidenţiază edem la nivelul spaţiului retrorectal.

Explorarea intraoperatorie afirmă posibilitatea practicării unei amputaţii abdominoperineale de rect, care decurge din punct de vedere tehnic fără incidente. În timpul intervenţiei nu se remarcă exprimarea plexului presacrat şi nici dificultăţi de disecţie produse de fascia Waldayer (fig. 9).

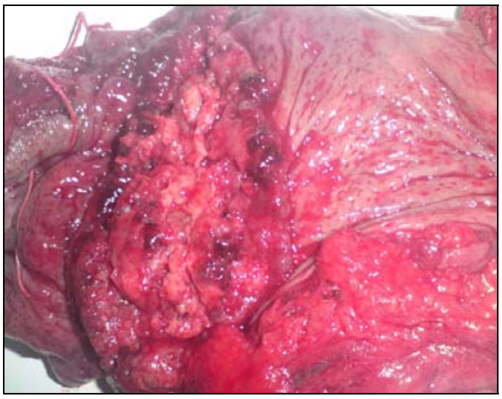

Fig. 9. Piesa de rezecţie după amputaţie abdominoperineală de rect la acelaşi pacient, cu evidenţierea tumorii rectale inferioare.

Cazul 5. Ilustrăm patologia neoplazică rectală printr-o imagine STIR sagitală care evidenţiază edem în spaţiul presacrat şi retrorectal la un pacient cu tumoră rectală inferioară. Pe o imagine T2 sagitală se evidenţiază edem al spaţiului presacrat, iar investigaţia mai relevă linii de hipersemnal, imprecis delimitate, sugestive pentru edem al spaţiului retrorectal (fig. 10, 11).

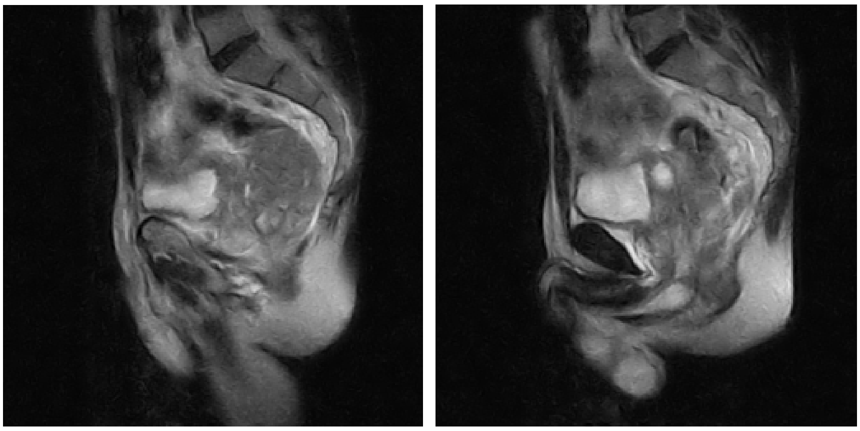

Fig. 10. Imagine T2 sagitală ce evidențiază edem al spațiului presacrat şi linii de hipersemnal, imprecis delimitate, sugestive pentru edem al spațiului retrorectal.

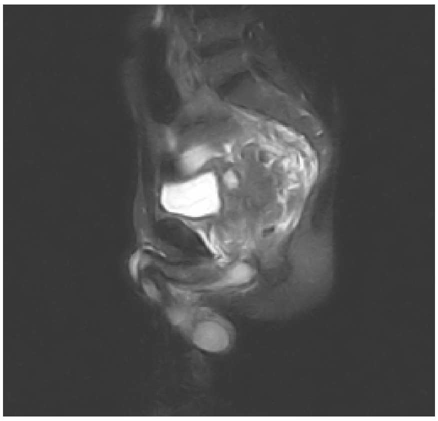

Fig. 11. Imagine STIR sagitală care evidențiază edem în spațiul presacrat şi retrorectal. Intraoperator se apreciază nerezecabilitatea leziunii.

Cazul 6. Pacientă, 72 de ani, cu diagnosticul clinic de neoplasm inferior. Colonoscopia relevă formațiune tumorală ulcero-vegetantă circumferențială, ocluzivă şi hemoragică situată la 4 până la 8 cm de marginea anală. Explorarea IRM demonstrează o imagine T1 sagitală cu contrast intravenos, care evidențiază fascia Waldayer. Imaginea în T2 sagital la acelaşi caz evidențiază cele două fascii (fascia mezorectului şi fascia presacrată) şi discret edem în spațiul presacrat. Se evidențiază un aspect T2 sagital ce relevă fascia Waldayer (fig. 12, 13).

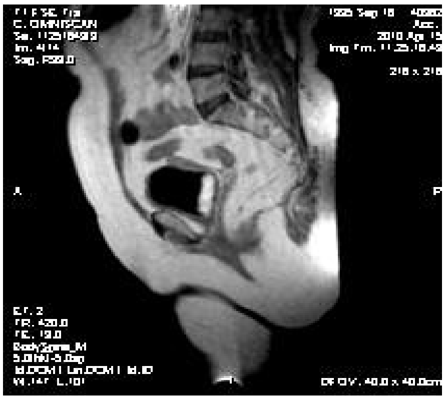

Fig. 12. Imagine T1 sagitală cu contrast intravenos care evidențiază fascia Waldayer la o pacientă cu tumoră rectală inferioară.

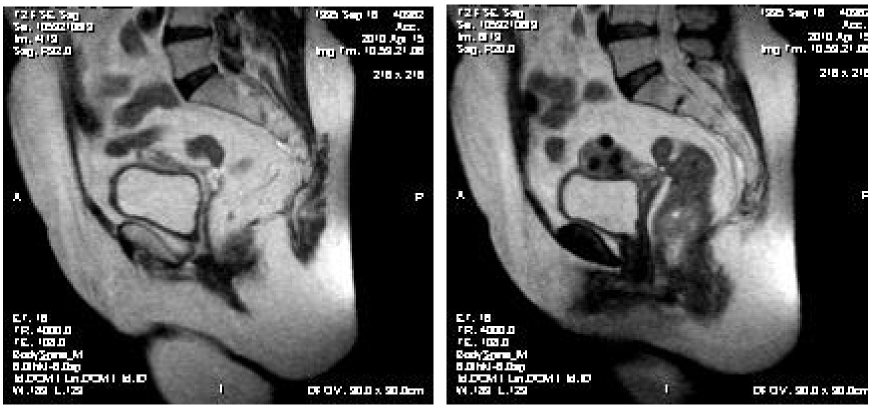

Fig. 13. Imaginea în T2 sagital la acelaşi pacient la care se evidenţiază cele două fascii, discret edem în spaţiul presacrat şi fascia Waldayer.

Explorarea intraoperatorie decide posibilitatea rezecţiei şi se practică amputaţie abdominoperineală de rect cu anus iliac stâng definitiv. În timpul intervenţiei este vizibil prin transparenţă plexul presacrat. Din cauza existenţei unei fascii Wadayer deosebit de bine exprimate disecţia mezorectului avansează în spaţiul presacrat cu deschiderea plexului presacrat şi instalarea unei hemoragii ce necesită meşarea spaţiului operator (fig. 14).

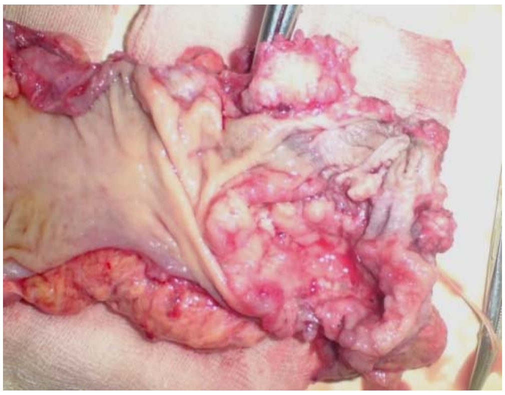

Fig. 14. Piesa de amputaţie abdominoperineală de rect la pacienta de mai sus.

Discuţii

Rectul este un organ ideal pentru explorarea prin IRM, fapt favorizat de localizarea anatomică, fixarea la nivelul grăsimii pelvine şi lipsa peristalticii. La nivelul pelvisului se descriu o serie de spaţii şi structuri cu importanţă chirurgicală. Astfel, spaţiul retrorectal este delimitat posterior de fascia parietală presacrată şi anterior de fascia mezorectală (1).

Disecţia la nivelul spaţiului retrorectal observă la nivelul celei de-a patra vertebre sacrate apariţia din fascia parietală presacrată a unei alte fascii, invariabil prezente, care se va uni cu fascia viscerală ce înveleşte mezorectul posterior, joncţiune situată la 3-4 cm superior de joncţiunea anorectală (4,5), denumită fascia rectosacrată.

Spaţiul presacrat este definit ca un spaţiu retroperitoneal localizat între peritoneul parietal al peretelui abdominal posterior sau fascia presacrată şi sacrum. Începe la bifurcarea aortei şi se întinde periferic până la planşeul pelvin. Lateral este delimitat de vasele iliace interne. Conţinutul său este reprezentat de ţesut lax areolar şi ţesut conjunctiv, vase arteriale, plexul venos presacrat, plexul nervos superior hipogastric şi nervii hipogastrici, şi porţiuni din plexul hipogastric inferior. Artera mediosacrală este prezentă constant.

Plexul venos presacrat rezultă din anastomozele dintre venele sacrate laterale şi mediane şi îşi are traiectul în fascia pelvină ce acoperă faţa anterioară a corpului sacrumului. Ca structură anatomică nu este vizibilă direct în timpul intervenţiilor de chirurgie rectală (2). Plexul venos sacrat începe caudal de la joncţiunea lombosacrată, în mod obişnuit prin formarea unui plex venos hexagonal ce drenează în ramul lateral al venei hipogastrice. Simetria bilaterală este prezentă în această zonă indiferent de prezenţa sau absenţa hexagonului venos (3). Porţiunea caudală a plexului venos sacrat constă într-o structură scalariformă formată din vene paralele longitudinale întretăiate de vene dispuse transversal la nivelul fiecărui segment notocordal. Ocazional sunt prezente vene pereche mediane longitudinale, venele sacrale mediale, care se anastomozează şi ele cu venele transverse. Cantitatea de vene vizualizate este variabilă de la pacient la pacient. Patern-ul descris este în general constant, iar gradul de vascularizaţie este bilateral simetric. În 47% din cazuri putem vorbi de un aspect de hipervascularizaţie (vase multiple suprapuse peste pattern-ul de bază), 23% din cazuri pot fi descrise ca moderat vasculare şi 30% ca hipovasculare (cu puţine vene sau fără vene deloc). Venele principale sunt constante ca apariţie şi poziţie.

Hemoragiile din plexul presacrat sunt potenţial ameninţătoare de viaţă în timpul intervenţiilor pe pelvis (5) şi sunt greu de controlat atunci când venele se retrag în găurile sacrate (6). Vena sacrată mijlocie drenează în vena iliacă comună stângă, în timp ce vena sacrată laterală se deschide în iliaca internă. În final, aceste vene drenează în sistemul cav. Plexul venos sacrat primeşte tributare de la venele lombare ale peretelui abdominal posterior şi din venele basivertebrale care trec prin foramenele osului sacrat. Acestea din urmă sunt vene cu perete fin ce descriu traiecte tortuoase în corpul vertebral (6). Ele comunică anterior şi lateral pe corpul vertebral cu plexul vertebral anterior. Venele sacrate laterale, mediale şi basivertebrale de o parte formează anastomoze extensive cu cele din partea opusă şi formează astfel plexul sacrat de pe faţa anterioară a sacrului.

Sunt autori care descriu şi o arie fără vase la nivelul spaţiului presacrat.

Ca repere de identificare reţinem o serie de caracteristici. Astfel, cel mai apropiat vas la nivel cefalic de promontoriu (MSP) este vena iliacă comună stângă (LCIV) (în medie 27 mm cu variaţii de la 9 la 52 mm). Cel mai apropiat vas lateral de promontoriul mediosacrat este de asemenea LCIV, la o distanţă medie de 22 mm (9-35 mm). Distanţa medie de la artera şi vena mediosacrată şi MSP este de 4 mm (0-15mm) şi respectiv 7 mm (0-17 mm). Distanţa medie de la plexul venos presacrat la MSP este de 34 mm (4-86 mm) (6).

Din punct de vedere al imagisticii prin IRM, dintre structurile ligamentare pelvine fascia presacrată şi ligamentul sacrogenital sunt singurele observabile ca structuri normale, în timp ce restul structurilor sunt evidenţiate numai când sunt îngroşate de procese patologice (7). În acest spaţiu nodulii limfatici nu sunt vizibili în mod normal. În cancerele rectale diseminarea limfatică are loc iniţial prin ganglionii limfatici mezorectali şi presacraţi adiacenţi tumorii şi abia apoi se notează determinări de-a lungul ganglionilor de la nivelul vaselor rectale superioare.

Fascia presacrală se vede pe secţiunile sagitale IRM ca o structură liniară de semnal scăzut ce acoperă vasele presacrate. Pe secţiuni T2 fast spin echo zona de semnal înalt anterior de fascia presacrată este ţesut adipos, iar lipsa de semnal din spaţiul retrorectal este dată de vasele sacrate mijlocii. Această structură a fost demonstrată indemnă sau invadată de procesele patologice în toate cazurile prezentate. În majoritatea situaţiilor analizate, investigaţia prin IRM a evidenţiat edem la nivelul spaţiului presacrat. Aspectul tehnic intraoperator indică valabilitatea aspectului pentru neoplaziile rectului mijlociu şi mai puţin pentru cele ale rectului inferior sau joncţiunii rectosigmoidiene.

Fascia mezorectului este evidenţiată imediat anterior de această structură, iar spaţiul potenţial dintre acestea două este spaţiul retrorectal. Fascia este evidenţiată ca o structură de hiposemnal ce înconjură mezorectul (1,9,10). Mezorectul este o structură IRM axial în hipersemnal (similar cu grăsimea) ce este dispusă în jurul rectului şi conţine vase (unele fără semnal) şi limfatice (9,11). Este bine delimitată de hiposemnalul muscularei propria a peretelui rectal (9). Ganglionii limfatici din mezorect apar ca structuri ovoidale în hipersemnal. Fascia mezorectului este bine evidenţiată în aceste patologii.

Fascia rectosacrală este o structură de forma unei benzi, de grosime variabilă, ce se întinde între sacrum şi fascia mezorectului la nivelul celei de a patra vertebre sacrate, structură ce are şi ea reprezentare IRM. Este denumită şi fascia lui Waldayer. Grosimea acesteia variază de la o membrană transparentă subţire până la o structură groasă, opacă. În aceste ultime situaţii poate conduce eronat disecţia fie în mezorect, cu realizarea unei excizii incomplete, fie în posterior de fascia presacrată, cu hemoragii dificile. Este cazul ilustrat de situaţia descrisă la poziţia 6 în care, din cauza unei structuri fasciale Waldayer bine reprezentate, disecţia a decurs în spaţiul presacrat cu instalarea unei hemoragii ce a necesitat hemostază prin meşare. Din punct de vedere al imagisticii prin IRM această structură este evidenţiabilă în cazuri patologice. Semnalarea ei ar putea dirija tehnica pentru evitarea conducerii unei disecţii eronate.

Concluzii

Rectul este un organ ideal pentru explorarea prin IRM, fapt ce poate influenţa tehnica chirurgicală. În majoritatea cazurilor analizate, investigaţia prin IRM a evidenţiat edem la nivelul spaţiului presacrat. Aspectul tehnic intraoperator indică valabilitatea aspectului pentru neoplaziile rectului mijlociu şi mai puţin pentru cele ale rectului inferior sau joncţiunii rectosigmoidiene. Explorarea prin IRM a pelvisului a evidenţiat structurile normal prezente (fascia presacrată şi ligamentul sacrogenital) dar şi pe cele care devin vizibile numai în momentul dezvoltării unor procese patologice (fascia mezorectului, fascia rectosacrată).

Fascia rectosacrală (fascia lui Waldayer) este o structură de forma unei benzi, de grosime variabilă. Prin particularităţile ei structurale poate conduce eronat disecţia fie în mezorect, cu realizarea unei excizii incomplete, fie în posterior de fascia presacrată, cu hemoragii dificile din plexul presacrat ce sunt potenţial ameninţătoare de viaţă şi sunt greu de controlat atunci când venele se retrag în găurile sacrate. Poate fi evidenţiată corespunzător la examinarea IRM, aprecierea ei ghidând intervenţia chirurgicală.

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Cea mai frumoasă dintre „profesiuni" ?!....

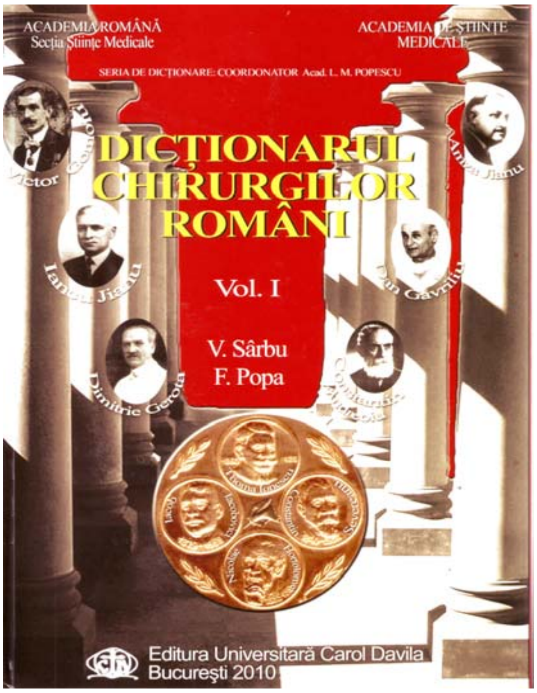
ACADEMIA ROMÂNĂ
Secţia Ştiinţe Medicale
ACADEMIA DE ŞTIINŢE MEDICALE
SERIA DE DICŢIONARE: COORDONATOR Acad. L. M. POPESCU
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Vol. I
V. Sârbu
F. Popa
Iancu Jianu
Dimitrie Gerota
Amza Jianu
Editura Universitară Carol Davila
Bucureşti 2010

Am o îndoială, pe care o afirm de la bun început. Încercând să dau acestei cronici un titlu uşor dubitativ, îmi permit să formulez o întrebare aparent simplă: este posibil ca meseria (oare aşa se cheamă?) sau profesia (conform nominalizării Ministerului Muncii) de chirurg să fie una dintre cele mai pasionante, mai consumptive şi mai pline de satisfacţii dintre profesiuni? Să fie oare, chiar cea mai frumoasă dintre ele? Sper ca răspunsul la această întrebare să-l aflaţi la sfârşitul acestor rânduri.

Cartea pe care vă sfătuiesc să o căutaţi în biblioteci şi să o răsfoiţi măcar, unică în arealul publicistic medical de la noi, aş zice chiar „tulburătoare" prin anvergură! , este un veritabil „Who's who... chirurgical", se intitulează „Dicţionarul chirurgilor români" şi a apărut, sub redacţia Domnului Profesor Dr. Vasile Sârbu şi a Domnului Profesor Dr. Florian Popa – Rectorul Universităţii de Medicină şi Farmacie „Carol Davila", Bucureşti şi sub auspiciile Academiei Române – Secţia de Ştiinţe Medicale şi a Academiei de Ştiinţe Medicale conduse de Domnul Academician Profesor Dr. Laurenţiu M.Popescu, la Editura Universitară „Carol Davila", în anul 2010.

Dicţionarele, cu siguranţă că pun în dificultate un recenzent, dispus să sublinieze performanţele sau neîmplinirile unei astfel de întreprinderi livreşti.

Travaliul autorilor a fost, în mod indubitabil imens; cartea este monumentală, în fond! Chiar şi numai a încerca, a descoperi şi a aduna biografiile tuturor slujitorilor de seamă ai specialităţilor chirurgicale, a chirurgilor „din tot spaţiul românesc, de la Baia Mare până la Chişinău, de la Suceava până la Severin, de la Oradea până la Constanţa şi Cernăuţi" a însemnat, pentru cine are percepţia fenomenului, un efort cu adevărat sisific!

Ni se perindă, prin faţa ochilor, destinele şi realizările multor profesionişti de succes din chirurgia de ieri şi de azi !

Rămân totuşi la final, ca umil cititor, cu o senzaţie de neîmplinire, cu siguranţă neimputabilă autorilor: cunosc eforturile, orele de nesomn, bucuriile aferente aşa zis-ului succes vremelnic în lupta cu boala şi frustrările autoimpuse unei vieţi de chirurg! Le înţeleg şi mi le-am subsumat disciplinei muncii mele de zi cu zi, la fel ca toţi chirurgii, indiferent de specialitatea lor, prezenţi în Dicţionar. Din păcate întâlnim multe articole la care „curriculum vitae" lipseşte, chiar şi pentru cei actuali. Pentru cei deveniţi (glumă sinistră, *mea culpa*!) „intratereştri" este un lucru de înţeles.

Să fie acesta un semn, în fond, că după extincţie nu mai rămâne nimic? Probabil.....

Marele critic literar Eugen Lovinescu (31 oct. 1881 – 16 iulie 1943) prezenta într-un articol referitor la moartea altui mare critic – Garabet Ibrăileanu (23.oct.1871-11.martie.1936), o chestiune absolut tulburătoare, adevărată până la urmă, pe care o reproduc din memorie (din păcate motoarele Google nu te pot ajuta întotdeauna!): „Atunci când ai dispărut şi tu şi cei care ţi-au dat viaţă, cu siguranţă că dispari definitiv din Univers!”, Garabet Ibrăileanu, fost celibatar, a decedat înaintea mamei sale!

După un travaliu de peste un deceniu antamat de alte cărţi anterior publicate, travaliu care a însemnat trierea, scanarea, citirea şi filtrarea – chiar şi cu ochii minţii – a numelor şi a activităţii a mii de oameni, de ieri şi de azi, care au avut şansa/sau neşansa? de a practica meseria de chirurg (generalist, urolog, toracic, plastician, neurochirurg, ORL-ist, maxilo-facial, pediatru ş.a.) autorii au gândit şi transpus în operă o idee superbă: fiecare dintre cele trei volume ale „Dicţionarului chirurgilor români” îşi deschide coperta şi oferă „ochilor aflaţi în spatele lentilelor” o alegorie – „arborele chirurgiei româneşti”. Trunchiul îşi trage seva din „rădăcini” şi o pulsează în „ramuri”, „crengi” şi „frunze”, lămuritor nominalizate.

Chirurgii sunt fiinţe perisabile, ca toţi oamenii. Adeseori au puterea de a oferi suferinzilor ce li se încredinţează spre tratament, în mod direct şi necondiţionat, o parte din sufletul lor, din generozitatea şi din „puterea magică” deprinsă de la maeştrii lor. Chiar cu preţul neglijării propriilor suferinţe! Acest Dicţionar, mult trudit în fond, are menirea să ne prezinte şi să ne responsabilizeze şi cu alte înţelesuri: chirurgii de azi, ca şi cei de ieri, nu reprezintă o „generaţie spontanee”; din nimic, cu siguranţă nu se va naşte nimic! Exemplul politicienilor români de azi este cel mai ilustrativ. Ei vin parcă de niciunde! Nimic nu este de înţeles, pentru cei mulţi, din aşa-zisa lor „întreprindere”! Sper, cu sinceritate şi încredere în viitor, ca breasla noastră, a chirurgilor români de toate specialităţile, să nu cunoască astfel de destine şi lucrări debusolate!

Chirurgia română de performanţă a început cu Constantin Dumitrescu Severeanu, a continuat cu marele Thoma Ionescu, cu Iacob Iacobovici, cu Nicolae Hortolomei, cu profesorii Jianu (Amza şi Iancu), cu cei doi mari Gerota (Dimitrie şi Dan), cu Theodor Burghele, Eugen Proca, Pavel Simici şi continuă din fericire pentru acest popor, cu truditorii de azi ce au învăţat perfect lecţia predată de maeştrii lor de mai ieri.

Răspunsul la îndoiala mea exprimată în debutul acestei cronici îmi este oferit, cum nu se poate mai bine, de cele inspirat scrise de „ctitorii” acestui **Dicţionar**: „Chirurgia este cea mai frumoasă profesiune lăsată de Dumnezeu pământenilor, o profesiune eternă, primul protocol operator având ca primă mână pe însuşi Dumnezeu, unicul chirurg şi anestezist desăvârşit”.

Prof. Dr. Dan MISCHIANU
U.M.F. „Carol Davila”, Bucureşti

În căutarea iubirii de sine

Psiholog Diana BĂLAN

Secția Neurologie
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Rezumat. Este iubirea suficientă pentru o relație de cuplu armonioasă? Dacă între doi parteneri există iubire, de ce apar probleme? Cum ajunge să moară o relație care se naște din iubire?

Iată câteva întrebări care încearcă să găsească un răspuns în demersul terapeutic pe care îl traversează persoanele ce se confruntă cu probleme de cuplu. Pentru a ne implica împreună într-o soluționare corectă, este necesar, mai întâi, să conturăm cât mai exact definiția iubirii. Considerăm această clarificare necesară pentru simplul motiv că, de cele mai multe ori, există o confuzie între dependență și iubire.

Iubirea pură conține libertatea. A deveni conştient de faptul că fiecare dintre noi este liber, evident sub raport opțional, este déjà un pas mare către regăsirea iubirii. Acceptarea libertății celuilalt înseamnă asumarea propriei libertăți interioare şi, totodată, momentul de întâlnire cu iubirea.

Cum se poate ajunge la iubirea de sine? Eu cred că prin acceptare a tot ceea ce suntem ca ființă. Asta înseamnă să primeşti cu încredere şi iubire tot ceea ce există acolo, în interiorul nostru: neîncredere, furie, tristețe, dependență, vulnerabilitate, critică, tot ceea ce putem recepta dacă suntem suficient de deschişi. Numai acceptând cu blândețe tot ceea ce descoperim ca fiind prezent în noi, vom reuşi să şi transformăm. Aceasta este vindecarea. Să nu uităm că Universul înseamnă mişcare şi că noi suntem parte din acest Univers. Inconştientul nostru, ca forță vindecătoare, funcționează după aceleaşi principii ca şi Marele Univers. Aşadar să lăsăm emoțiile să curgă, să ne deschidem către ele, să le creăm spațiu, hrănindu-ne din forța lor.

Cuvinte cheie: iubire, dependență, libertate, iubire de sine

Abstract. Is love all that we need for a healthy relationship? If it's really love why do we have problems in relations? What is the explanation for a relation to die if it was born from love?

There are some questions which needs an answer through all people, especially those who are engaged in psychoterapy.

For reaching the answer we need to define love because it happens to make confusion between love and dependence. I think love is freedom. To be aware that everybody is free it's a big step in finding the love. Accepting the other's freedom means to accept your own. This is the moment when we meet love.

How can we rich our love? I think that we can rich our love if we accept ourselfs. That means to be open with love and trust to our fear, sadness, vulnerability, judge, dependence and so on. Only if we are open to us, accepting our feelings kindly we can transform them. This is the cure. We know that the Universe is movement. We are a part of it. So, let's be open to our emotions, let them flow and feed ourselfs with their clever.

Este iubirea suficientă pentru o relație de cuplu armonioasă? Dacă între doi parteneri există iubire, de ce apar probleme? Cum ajunge să moară o relație care se naşte din iubire? Iată câteva întrebări care încearcă să găsească un răspuns în demersul terapeutic pe care îl traversează persoanele care se confruntă cu probleme de cuplu.

Din perspectiva psihoterapeutică se impune o redefinire a conceptelor care intră în această discuție: cum e văzută şi simțită iubirea de către cel care îi trăieşte absența, în prezent, şi cum ar arăta pentru el o relație de cuplu vie? Asta pentru că nu de puține ori întâlnim o atitudine confuză vis a vis de tema iubirii, de altfel un subiect mult discutat în literatură, şi nu numai în cea de specialitate. Din ce motive sunt atât de greu de disociat, uneori, stări cu un conținut absolut diferit, cum ar fi sentimentul de iubire şi starea de dependență, iubirea condiționată față de cea necondiționată.

Nu de puține ori întâlnim sintagma: ”dacă mă iubeşte, renunță, pentru mine, la ce-şi doreşte el”, “nu cred că este cazul să îi comunic eu despre ceea ce simt…ar fi trebuit să mă cunoască până acum.”

Iubirea pură înseamnă şi libertate. A deveni conştient de faptul că fiecare dintre noi este liber este déjà un pas mare către regăsirea iubirii. Acceptarea libertății celuilalt înseamnă confirmarea propriei libertăți interioare şi, totodată, momentul de întâlnire cu iubirea.

Avem credința că o dată ce simțim iubirea celuilalt nu o vom putea pierde vreodată. Şi lucrurile ar decurge aşa, dacă am fi conştienți de faptul că adevărata iubire se naşte din iubirea de Sine. Oare putem iubi pe cineva dacă nu ne iubim propria ființă? Putem fi prezenți într-o relație cu altcineva dacă nu suntem prezenți în noi înşine? Cum am putea dărui cuiva dragoste dacă nu suntem capabili, mai întâi, să ne-o oferim nouă înşine? Cum poți răspunde nevoilor celuilalt dacă nu eşti în stare să înțelegi pe deplin propriile-ți cerințe? Poți oare respecta spațiul personal al celuilalt dacă nu eşti conştient de spațiul propriu? Poți oare avea grijă de o altă persoană dacă nu ai capacitatea să te îngrijeşti de propria ființă?

Numai respectând calea cunoaşterii şi a iubirii de Sine reuşim să ne angajăm cu toată ființa într-o relație de iubire. Numai urmând această cale, un partener devine într-un cuplu oglinda celuilalt. Ceea ce vom oferi o să şi primim la un moment dat. Suntem responsabili pentru ceea ce suntem şi avem relația pe care noi o ființăm, nimeni altcineva în afara noastră. Suntem autorii propriei noastre realități. Aşa încât, dacă dăruim iubire şi încredere vom primi iubire şi încredere.

Cum se poate ajunge la iubirea de sine? Eu cred că prin acceptarea a tot ceea ce suntem ca ființă. Asta înseamnă să primeşti cu încredere şi iubire tot ceea ce există acolo, în interiorul nostru: neîncredere, furie, tristețe, dependență, vulnerabilitate, critică, şi tot ceea ce simțim printr-o atitudine de deschidere adecvată. Numai acceptând cu blândețe tot ceea ce descoperim ca fiind prezent în noi, vom putea şi transforma. Aceasta este calea spre vindecare. Să nu uităm că Universul înseamnă mişcare şi că noi suntem parte din acest Univers. Inconştientul nostru, ca forță vindecătoare, funcționează după aceleaşi principii ca şi Marele Univers. Aşadar să lăsăm emoțiile să curgă, să ne deschidem către ele, să le creăm spațiu hrănindu-ne din forța lor.

Se poate afirma că într-un cuplu apar tensiuni şi pentru că partenerii nu sunt obişnuiți să accepte, în egală măsură, atât propria ființă cât şi ființa celuilalt. Numai printr-un demers deliberat putem să întelegem şi să armonizăm în mod constructiv diferențele care există între noi. O relație de cuplu reprezintă o legătură între doi oameni ce poartă în spate “bagaje” care în mod firesc sunt diferite. Şi am în vedere aici educația, sistemul de valori, modelele comportamentale preluate de la părinți etc. Sunt exact tocmai “calitățile” care ne atrag la o anumită persoană, motivându-ne să construim o relație, şi pe care ajungem să le respingem la un moment dat. Şi asta pentru că suntem dependenți de aceste bagaje cu care venim din urmă, pe care încercăm să le aşezăm, cu mare grijă, într-un loc pe care tindem să-l extindem, ajungând, pe parcursul relației, să ocupe tot mai mult din spațiul cuplului. Mai mult chiar, încercăm să ținem închise căile de acces către aceste resorturi intime chiar şi pentru noi. Şi nu lăsăm pe nimeni să le deschidă. Ca şi când ar fi în contradicție cu bagajele celuilalt… Evident, printr-o astfel de atitudine ne va fi destul de greu, dacă nu chiar imposibil, să primim mirificul cadou al punerii lor în comun, în sensul împărtăşirii realității interioare cu celălalt. Dacă am privi lucrurile din acest unghi, am putea acorda mai multă atenție momentului în care întâlnim o anumită persoană. Ar trebui, poate, să căutăm răspunsul şi la aceste întrebări: De ce suntem atraşi de ea? De ce o respingem pe alta? Apare ea întâmplător în viața noastră? Dacă am încerca să fim deschişi sufletului nostru, am simți că nimic nu este întâmplător. Persoana acceptată reprezintă, de fapt, umplerea unui gol existent în noi. Mai exact, trăsăturile de personalitate ale persoanei alese vin să le completeze pe ale noastre (firea emotivă poate întâlni pe cea îndrăzneață, firea dominatoare, pe cea submisivă, firea blândă, pe cea furioasă etc.).

Dificultățile de relaționare apar din tendința de a-l schimba pe celălalt, ca şi când bagajul nostru ar fi cel mai cuprinzător. Nu suntem obişnuiți cu ideea de a da şi a primi, de a-l accepta pe celălalt cu tot Universul lui şi de a ne simți binecuvântați că suntem invitați în el. Dacă am fi mai prezenți în noi şi în celălalt ne-am putea hrăni din acest schimb permanent de energie. Acest schimb reprezintă adevărata dezvoltare a ființei şi a cuplului. Astfel, ceea ce numim diferențe devin adevărate resurse pentru fiecare în parte şi pentru cuplu. Relația de cuplu va deveni vie, va respira şi va fi respirată de partenerii care o ființează.

Dacă am deveni conştienți că nu suntem separați de celălalt ci că suntem părți dintr-un Întreg, am simți binecuvântarea pe care o primim cu fiecare întâlnire pe care o avem cu celălalt. Universul celuilalt nu e diferit de al meu. Ele fac parte din acelaşi Univers Interior şi în acelaşi timp din Marele Univers. Cu alte cuvinte ceea ce resping în celălalt, resping, de fapt, în mine. Ceea ce mă atrage la celălalt ar putea fi ceea ce încă nu a devenit viu în mine, cu toate că există acolo şi am nevoie de el. Suntem conținuți şi conținem toate ființele din jurul nostru. Suntem schițe ale aceluiaşi portret.

Calea nu e deloc dificilă. Este nevoie doar de deschidere, prezentă în Sine, încredere şi acceptare. Vindecarea vine de la sine… şi iubirea odată cu ea…

Psihoterapia, indiferent de forma pe care o îmbracă, facilitează reflectarea imaginii proprii în oglinzile celorlalți. Pot spune deschis că în demersul terapeutic cu clienții mei nu numai ei sunt cei care se simt îmbogățiți în urma acestei experiențe, ci şi eu. Cu fiecare client în parte, persoana cu care lucrez e una singură de fiecare dată, şi acea persoană sunt eu!

Actualități în terapia sclerozei multiple

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Rezumat. În prezent sunt aprobate 6 medicamente care modifică cursul natural al bolii la pacienții cu scleroză multiplă forma recurent-remisivă. Acestea includ 3 beta interferoni, glatiramer acetat, natalizumab şi mitoxantrone. Însă tratamentul injectabil cronic cu aceste medicamente modificatoare ale evoluției bolii scade aderența la tratament. Ca atare intrăm într-o nouă decadă terapeutică, cea a medicației cu administrare orală. Odată ce această nouă clasă va fi valabilă pe piață, rolul ei va fi determinat de balanța risc/beneficiu.

Cuvinte cheie: scleroza multiplă forma recurent-remisivă, tratament imunomodulator, administrare orală

Abstract. For patients with relapsing-remitting multiple sclerosis (RRMS), there are currently six approved medications that have been shown to alter the natural course of the disease. The approved medications include three beta interferon formulation, glatiramer acetate, natalizumab and mitoxantrone. But a lifetime of therapy with an injectable disease-modifying therapy alter adhesion to treatment. So a new therapeutic decade became available: oral medication. Determining a clinical role for the various oral disease-modifying therapies-once they reach market-will depend on balancing documented efficacy with safety.

Introducere

Scleroza multiplă (SM) este cea mai frecventă afecțiune neurologică care determină invalidități majore la adultul tânăr, cel puțin în țările Europei şi Americii de Nord. În țara noastră se estimează o prevalență de 35-40 la 100 000 locuitori. Debutează la adultul tânăr (20- 40 ani), fiind de 2-3 ori mai frecventă la femei decât la bărbați, cu consecințe dramatice în plan funcțional, mental, psihologic, social, familial.

Scleroza multiplă, afecțiune cronică a sistemului nervos central (SNC), se caracterizată prin episoade de inflamație şi demielinizare focală cu localizări multiple diseminate în timp şi printr-un proces de degenerescență axonală (care se desfăşoară în paralel, având însă o evoluție progresivă) la o persoană cu susceptibilitate genetică pentru boală.

Episoadele de inflamație/demielinizare focală se manifestă prin puseele clinice (recăderi urmate cel mai adesea de remisiuni, cel puțin în prima parte a evoluției bolii la majoritatea pacienților), în timp ce degenerescența axonală este cauza majoră a invalidității progresive şi ireversibile, fiind procesul dominant în formele progresive de boală (primară şi secundară), dar prezent încă din stadiile cele mai precoce în toate formele cunoscute ale afecțiunii.

În prezent, pe plan internațional sunt acceptate ca medicamente ce modifică evoluția bolii 4 *imunomodulatoare* - interferonul beta 1b (REBIF), cu administrare s.c. 3 doze pe săptămână, interferonul beta 1a (AVONEX), cu administrare i.m. 1 doză pe săptămână, interferonul beta 1b (BETAFERON), cu administrare s.c. 1 doză la 2 zile, glatiramerul acetat (COPAXONE) cu administrare s.c. câte 1 doză în fiecare zi – şi *imunosupresore* – mitoxantrona (Novantrone) şi *anticorpi monoclonali* – natalizumab **(**TYSABRI) cu administrare în PEV , 1 cură la 4 săptămâni.

Există şi alte forme de tratament imunomodulator sau imunosupresor care pot fi folosite în tratamentul sclerozei multiple în situații particulare, dar acestea nu sunt încadrate şi înregistrate de către autoritățile naționale şi internaționale, în concordanță cu rezultatele studiilor clinice desfăşurate până în prezent, ca agenți care modifică evoluția bolii.

Medicaţia modificatoare a evoluţiei bolii scade frecvenţa puseelor (măsurate clinic sau prin IRM), scade severitatea bolii (apreciată ca „încărcare“ de leziuni în secvenţa T2 a examenului IRM) şi încetineşte rata de progresie a invalidităţii.

Ca urmare a unei experienţe de aproximativ 10 ani în managementul pacienţilor cu scleroză multiplă (primii pacienţi trataţi în cadrul secţiei – septembrie-octombrie 2000), putem constata că ne îndreptăm către o nouă decadă în privinţa tratamentului ce vizează această afecţiune. Medicaţia modificatoare a evoluţiei bolii, care se administreaza toată viaţa, cu o frecvenţă variind de la zilnic la săptămânal, în funcţie de preparat, aşa cum am arătat mai sus, şi-a dovedit eficacitatea scontată în majoritatea cazurilor. Limitele ei au fost date, printre altele, de gradul redus de adeziune a pacienţilor la tratament sau de reacţiile adverse generale (manifestări pseudogripale, leucopenie, tulburări psihice, citoliză) ori locale (eritem, durere, necroze, liposidtrofii). Decada care se conturează vine cu noi medicamente de uz oral care pe de o parte rezolvă unele probleme (complianţa) dar determină alte reacţii adverse specifice, făcând decizia terapeutică dificilă atât din partea medicului, cât şi din partea pacientului, care are dreptul la opinie după informarea corectă şi completă. Vom enumera câteva dintre noile medicamente pentru tratamentul sclerozei multiple, care încep să-şi facă intrarea timid pe piaţa farmeceutică.

Cladribine (Merck Serono) este un medicament antineoplazic folosit în oncologie de peste 15 ani. În prezent, FDA îl evaluează în vederea obţinerii aprobării de a fi utilizat şi în tratamentul sclerozei multiple recurent remisive (SMRR). Administrat i.v., el a demonstrat efecte similare interfereonului, însă cu riscul imunosupresiei (herpes zoster) sau al efectelor teratogene. Studiul CLARITY (*cladibrine tablets treating MS orally*) pe 1 326 pacienţi ce au primit doze de 3,5 sau 5,25 mg/kg, cu o durată de urmărire de aproximativ 4 ani, a demonstrat o scădere a ratei puseelor la cei trataţi activ faţă de grupul placebo, un interval mai mare între pusee, reducerea progresiei invalidării ca şi micşorarea numărului de leziuni cerebrale. Reacţiile adverse au fost însă notabile. Limfocitopenia a fost prezentă la 21,6% din grupul tratat cu 3,5 mg şi la 31,5% la grupul tratat cu 5,25 mg, faţă de 1,8% la grupul placebo. Herpesul zoster a apărut la 8 şi respectiv 12 pacienţi dintre cei trataţi. În prezent se studiază durata optimă a tratamentului pentru a obţine rezultate bune, fără riscurile majore ale imunosupresiei.

Fingolimod (Novartis) alt medicament imunosupresor ce activează un mediator extracelular lipidic S1P1, având rol cheie în sistemul imun. Previne migrarea limfocitelor din ţesutul limfoid şi scade autoagresiunea limfocitelor asupra SNC. De asemenea, el traversează bariera hematoencefalică, având efect direct în SNC. Studiul TRANSFORMS, efectuat pe 1 292 de pacienţi cu SMRR, a urmărit efectele administrării orale a 1,25 mg/zi, 0,5mg/zi şi 30 mµg interferon beta 1a o dată pe săptămână, timp de 12 luni. Rata puseelor a scăzut semnificativ la pacienţii trataţi cu Fingolimod. Rezultate semnificative au fost obţinute şi din punct de vedere imagistic. S-au înregistrat însă două reacţii adverse fatale: varicelă şi encefalită cu herpes simplex. Alte reacţii adverse înregistrate au fost: infecţii nonfatale cu virus herpetic, bradicardie, bloc AV, HTA, edem macular, cancer cutanat, citoliză, cancer de sân. Un alt studiu, FREE-DOMS, derulat pe 1 272 pacienţi, urmăriţi timp de 24 luni, a demonstrat reducerea progresiei dizabilităţii.

Laquinimod (Teva) este un imunomodulator care modulează shift-ul Th1/Th2 fără riscul imunosupresiei. După 3 luni de tratament al pacienţilor cu SMRR cu o doză de 0,6 mg Laquinimod s-a observat o creştere de 11 ori a factorului neurotrofic din ser, sugerându-se şi un posibil rol neuroprotector. Reduce de asemenea inflamaţia, demielinizarea şi leziunile axonale pe modelele de encefalomielită autoimună experimentală.

Dalfampridine SR (Ampyra, 4-aminopiridina) are un mecanism de acţiune încă incomplet cunoscut, fiind un blocant al canalelor de potasiu ce determină mărirea duratei şi a amplitudinii potenţialelor de acţiune. Creşte doar conductibilitatea impulsului nervos în fibrele nervoase demielinizante, având ca rezultat sporirea vitezei de deplasare a pacienților. Acest medicament este unul simptomatic, neaparținând categoriei de medicamente ce modifică evoluția bolii, așa cum greşit interpretează unii dintre pacienți. A fost aprobat de FDA în ianuarie 2010 pentru tratarea pacienților cu SM. Medicamentul, cunoscut inițial sub denumirea de Fampridine, nu creşte durata complexului QRS sau a intervalului QT. Se foloseşte fie singur, fie împreună cu terapia modificatoare a evoluției bolii. Un studiu de fază III pe 540 pacienți cu SM arată efecte pozitive ale Dalfampridinei SR, administrată în doză de 10 mg, de două ori pe zi, crescând viteza de deplasare a pacienților tratați. Reacția adversă cea mai raportată a fost infecția urinară, altele mai rare fiind : amețeli, cefalee, insomnie, obstrucție nazală, durere toracică. Se recomandă evitarea medicamentului la cei cu IRC moderat-severă şi la cei cu crize de epilepsie. Reacțiile adverse sunt dependente de doză. Rezultatele benefice apar la aproximativ un sfert dintre pacienții tratați.

Concluzii. Apariția medicamentelor cu administrare orală pentru tratarea SM reprezintă un salt calitativ mult aşteptat atât pentru pacienți, cât şi pentru medici. Acestea vor fi utilizate pe măsură ce vor fi strânse suficiente dovezi ale tolerabilității şi siguranței în administrarea pe termen lung. Singura certitudine în prezent este creşterea adeziunii la tratamentul oral (față de cel injectabil, folosit în prezent) şi eliminarea reacțiilor adverse de la locul injectării.

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Managementul situațiilor de risc nuclear
(prima parte)

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Rezumat. Arma nucleară are o capacitate de distrugere în masă, prin efectele sale determinând afectarea gravă a vieții sociale şi a sănătății populației dintr-o anumită zonă, atât pe termen scurt, cât şi pe termen lung. Rezultatele acțiunii constau în contaminarea radioactivă a zonei respective şi într-un număr foarte mare de victime într-un interval de timp foarte scurt, ceea ce impune intervenția rapidă a structurilor abilitate ale sistemului național de urgență. Managementul victimelor va începe cu aplicarea algoritmului ABC, urmat de triajul medical, decontaminarea şi respectiv tratarea acestora în unitățile medicale adecvate.

Cuvinte cheie: armă nucleară, decontaminare, triaj medical, leziuni de iradiere

Summary. The nuclear weapon is a mass destruction weapon which by its physical effects affects seriously the social life from a specific area; also it has short and long term health effects. The result of the physical effects is the radioactive contamination of that specific area and a great number of victims in a very short time which are in need of rapid intervention of the national emergency system. Victim's management shall start with the ABCs algorithm applying, followed by medical triage, decontamination and their treatment in the medical facilities.

Generalități

Arma nucleară (A.N.) se bazează pe puterea distructivă a imensei cantități de energie care se degajă în timpul exploziei nucleare. Efectul ei depăşeşte de mii de ori puterea distructivă a celei mai puternice muniții încărcate cu substanțe explozive obişnuite.

Explozia nucleară se deosebeşte de cea convențională atât prin cantitatea de energie degajată, cât şi prin natura transformărilor care au loc în substanța încărcăturii. Pentru explozia nucleară, sursa de energie o constituie procesele care au loc în nucleele atomilor elementelor chimice.

Cauzele expunerii la radiații şi contaminare. Există la ora actuală mai multe scenarii de apariție a accidentelor şi urgențelor nucleare. Astfel, sunt posibile supraexpuneri accidentale la reactoare industriale, accidente medicale în urma terapiei cu radiații, sursele radioactive industriale şi medicale (pierdute, furate, folosite necorespunzător), accidentele în timpul transportului materialelor radioactive şi accidentele reactoarelor nucleare (Cernobîl, 1986). Impactul acestor accidente depinde de mărimea expunerii la radiații, de contaminare şi de numărul persoanelor afectate. În prezent se pot folosi ca arme de către terorişti aşa numitele „dirty bombs” – RDD (*radiological dispersal device* – dispozitive de dispersare radiologice), care sunt o combinație între un exploziv convențional (plastic sau dinamită) cu un material radioactiv, dar şi arme nucleare de mici dimensiuni. RDD determină victime prin explozia inițială a bombei, la care se adaugă şi contaminarea radioactivă a supraviețuitorilor şi a salvatorilor. Nuclizii care pot fi folosiți sunt ^{137}Cs, ^{60}Co, ^{239}Pu, ^{192}Ir, ^{90}Sr şi ^{241}Am.

Detonarea de către terorişti a unei arme cu putere mică într-o zonă populată determină pierderi masive de vieți omeneşti şi contaminare extensivă. Încărcăturile nucleare, după caracterul reacțiilor care au loc în interiorul armelor atomice la detonare, se pot împărți în trei tipuri:

- încărcături nucleare la care energia de explozie se obține numai pe seama reacției de fisiune, substanța fisionabilă fiind uraniul (U) 235 sau plutoniul (Pu) 239;
- încărcături nucleare în care pe lângă reacția de fisiune are loc o reacție de sinteză termonucleară a elementelor uşoare. La acest tip de încărcătură se mai foloseşte, pe lângă

^{235}U sau ^{239}Pu, un amestec de deuteriu şi tritiu sau o combinaţie de deuteriu cu litiu (deuterura de litiu);
- încărcături nucleare la care energia de explozie se obţine ca urmare a trei reacţii nucleare: reacţia de fisiune a nucleelor ^{235}U sau ^{239}Pu din compunerea focosului nuclear, reacţia de sinteză termonucleară a izotopilor de hidrogen (deuteriu şi tritiu) şi reacţia de fisiune a nucleelor de ^{238}U sub acţiunea neutronilor rapizi formaţi în timpul reacţiei de sinteză.

Fenomenele fizice care însoţesc explozia nucleară pe măsură ce aceasta evoluează sunt următoarele: unda de şoc, emisia de lumină, radiaţia penetrantă şi respectiv impulsul electromagnetic. Rezultatul acţiunii fenomenelor fizice mai sus amintite este contaminarea radioactivă a zonei respective (a terenului), a tehnicii, a personalului şi a obiectivelor.

Consecinţele medicale care apar în urma folosirii armei atomice depind de 4 factori majori: puterea armei (kilotone/megatone), înălţimea la care are loc explozia, condiţiile de mediu în care se produce explozia (apă, aer etc.) şi respectiv distribuţia şi adăpostirea populaţiei/trupelor în zona ţintă. La factorii enunţaţi anterior se mai adaugă şi tipul de dispozitiv utilizat, tipul materialului radioactiv folosit, cantitatea (activitatea) de material radioactiv, numărul de persoane afectate şi răspunsul sistemului de urgenţă.

Leziunile traumatice care apar în urma unei explozii nucleare sunt următoarele: leziuni provocate de explozie - 50%, leziuni termice (arsuri) - 35%, leziuni produse de radiaţiile ionizante iniţiale - 5% şi cele reziduale - 10%.

Tabelul nr.1. Leziunile provocate în funcţie de puterea armei nucleare şi distanţa de la epicentrul exploziei

Efectele armei nucleare	**Puterea armei (kilotone)/ Distanţă (metri)**			
	1 kt	**10 kt**	**100 kt**	**1,000 kt**
Leziuni provocate de explozie (50% din cazuri)	140 m	360 m	860 m	3.100 m
Arsuri (50% arsuri profunde)	370 m	1.100 m	3.190 m	8.020 m
Radiaţii ionizante (50% ineficienţa imediată tranzitorie)	600 m	950 m	1.400 m	2.900 m
Radiaţie ionizantă (50% mortalitate)	800 m	1.100 m	1.600 m	3.200 m

Tipuri de radiaţii ionizante. Există 5 tipuri cunoscute de radiaţii ionizante, astfel:
- alfa – energie înaltă, dar cu putere de penetrare scăzută (câtiva centimetrim în aer şi câţiva microni în ţesuturi);
- beta - energie înaltă cu potenţial de penetrare mai mare (câţiva metri în aer şi milimetri în ţesuturi);
- gamma – energie electromagnetică cu potenţial înalt de penetrare (mai mulţi metri în aer şi câţiva metri în ţesuturi);
- razele X - energie electromagnetică cu potenţial înalt de penetrare (mai mulţi metri în aer şi câţiva metri în ţesuturi);
- neutronii – particule ionizante, fără sarcină electrică, cu potenţial înalt de penetrare (mai mulţi metri în aer şi câţiva metri în ţesuturi).

Măsurarea cantităţii de radiaţii. În tabelele nr. 2, 3 şi respectiv 4 se regăsesc unităţile de măsură tradiţionale ale radiaţiilor, alte unităţi de măsură, precum şi dozele tipice de radiaţii eliberate de investigaţiile paraclinice uzuale.

Măsurarea iradierii se va face cu o serie de instrumente de monitorizare foarte sensibile şi care pot detecta prezenţa radiaţiilor chiar şi la nivele foarte scăzute.

Cele mai folosite instrumente pentru detectarea radiaţiilor sunt contorul Geiger-Mueller şi camera de ionizare. Contorul Geiger (CG) poate detecta nivele scăzute de radiaţii gamma şi majoritatea radiaţiilor beta, dar mai important este faptul că poate diferenţia cele două tipuri de radiaţii. Aparatul de măsurare numit camera de ionizare determină cu acurateţe nivelul expunerii la radiaţii începând cu nivele foarte joase (mR/h) şi terminând cu nivele foarte înalte de expunere (R/h). Pentru a determina cu acurateţe doza de radiaţii pe care a primit-o un pacient, un rol important îl are timpul de expunere la radiaţii.

Dozimetrele personale sunt dispozitive care măsoară doza cumulată de radiaţii primită de persoana care poartă acel dispozitiv. Există două tipuri: ecusoane (care conţin un film, material termoluminiscent etc.) sau dozimetre automate.

Tabelul nr. 2. Unităţi de măsură a radiaţiilor

Măsurători	Cantitate	Unităţi
Cantitatea de material radioactiv	Activitate radioactivă	Curie (Ci)
Ionizarea în aer	Expunere	Roentgen (R)
Energia absorbită pe unitate de masă	Doza absorbită	Rad
Doza absorbită în funcţie de tipul de radiaţie	Doza echivalentă	Rem

Tabelul nr. 3. Unităţi tradiţionale de măsură a radiaţiilor

1 rad	= 1 centigray	$=1\times10^{-2}$ Gray
1 Curie	$= 3.7\times10^{10}$ Becquerel	$=3.7\times10^{10}$ dezintegrări / secundă
1 Gray (Gy)	= 100 rad	
1 Becquerel	= 1 dezintegrare / secundă	
1 rad	=1 roentgen	=1 rem (pentru marea majoritate a radiaţiilor)

Alte unităţi de măsură. $LD_{50/60}$ este doza letală de radiaţie care va ucide 50% dintr-o populaţie în 60 de zile de la expunere. **$LD_{50/60}$** se poate clasifica în funcţie de mărime, astfel:

- $LD_{50/60}$ este **3–4 Gy** pentru cei care au doar leziuni de iradiere şi nu au nevoie de îngrijire medicală semnificativă;
- $LD_{50/60}$ este **≥6 Gy** pentru o populaţie care are doar leziuni de iradiere şi beneficiază de cea mai bună îngrijire medicală (inclusiv antiemetice, antivirale, antibiotice, citokine hematopoetice şi transfuzie sanguină);
- $LD_{50/60}$ este mult mai scăzută atunci când leziunile de iradiere sunt asociate cu traumă şi/sau arsuri.

Tabelul nr. 4. Doze de radiaţii tipice

Tipul de iradiere	Cantitatea de radiaţii
Zbor cu avionul	2,5 mrem
Radiografie toracică	5 mrem
Osteodensitometrie	400 mrem
CT abdominal	760 mrem
Clismă baritată	870 mrem
Cateterism cardiac	45 000 mrem

EFECTE MEDICALE SPECIFICE - LEZIUNI POTENŢIALE DETERMINATE DE ARMA NUCLEARĂ

Managementul eficient al victimelor rezultate în urma unei explozii nucleare presupune înţelegerea problemelor medicale ce pot apărea, deoarece tipurile de leziuni sunt diferite de cele produse de armele convenţionale. Efectele medicale specifice sunt datorate energiei termice (arsuri), exploziei, respectiv radiaţiei ionizante.

1. Leziunile termice sunt urmarea temperaturilor extrem de înalte provocate de explozia nucleară. Acest tip de energie este capabilă să producă arsuri şi la distanţe apreciabile de epicentrul exploziei.

Arsurile provocate de lumină sunt cauzate de radiaţia infraroşie, dar mortalitatea este mult mai mare datorită suprimării activităţii măduvei osoase (datorită iradierii) şi infecţiei. Iniţial apar probleme majore datorită faptului că există un număr mare de pacienţi care trebuie evacuaţi. Datorită afluxului mare de cazuri, triajul medical, având la bază gravitatea cazurilor (procentul de suprafaţă arsă - BSA şi profunzimea arsurilor), va urmări încă de la început conservarea resurselor, precum şi salvarea unui număr cât mai mare de pacienţi.

În funcţie de procentul de suprafaţă arsă (BSA), există următoarele posibilităţi de conducere a pacienţilor, după cum urmează:

- arsuri ≤ 20% BSA – se vor trata în ambulatoriu;
- arsuri care afectează zone critice (capul, gâtul, mâinile sau picioarele) – se vor interna în spital, chiar dacă suprafaţa arsă este mai mică de 20%;
- arsuri >20% BSA asociate sau nu cu leziuni de explozie - se vor interna pentru tratament intensiv şi chirurgical;
- arsurile > 50% BSA au şanse scăzute de supravieţuire şi vor avea o prioritate scăzută în condiţiile în care există foarte multe cazuri. Se vor interna în spital şi vor fi trataţi în limita posibilităţilor existente.

În funcţie de puterea armei, pacienţii cu arsuri pot avea asociate şi leziuni de iradiere. Aceşti pacienţi vor dezvolta insuficienţa măduvei osoase hematogene la 2-6 săptămâni de la internare, cu apariţia leucopeniei (limfopeniei) şi vor avea o mortalitate şi morbitate mult mai mari datorită riscului de infecţie. În astfel de situaţii, pacienţii vor fi temporizaţi şi se vor executa doar intervenţii care salvează viaţa în perioada de supresie medulară.

2. Leziuni provocate de explozie: trauma este provocată în mod direct de către valul de suprapresiune (unda de şoc) şi/sau în mod indirect de către obiectele zburătoare şi suflul exploziei.

Există 4 tipuri de leziuni provocate de explozie:

- ***leziunea primară prin suflu*** - apare în urma modificărilor rapide de presiune datorate mişcărilor undei de explozie şi afectează mai ales organe cavitare (plămânii);
- ***leziunea secundară prin suflu -*** apare datorită proiectilelor secundare: fragmente de arbori sau de metal care sunt antrenate de suflul exploziei. Majoritatea sunt leziuni penetrante, dar sunt posibile şi leziuni nepenetrante;
- ***leziunea terţiară prin suflul exploziei -*** rezultă din deplasarea întregului corp al victimei şi din impactul traumatic cu obiectele din jur;
- ***leziunile asociate exploziei;*** sunt injurii care rezultă în urma modificărilor mediului înconjurător, determinate de explozie, şi care includ incendiile, combustia de compuşi toxici, scurgerile de substanţe toxice, la care se adaugă şi modificările produse de energia radiantă. Leziunile care apar sunt arsuri, intoxicaţii, asfixii şi leziuni de iradiere.

Leziunile de explozie directe sunt rare, deoarece pacienţii care sunt îndeajuns de aproape de epicentru vor suferi leziuni termice şi indirecte letale. Cei care supravieţuiesc vor avea leziuni asociate (leziuni traumatice ale organelor interne), inclusiv leziuni de iradiere care vor creşte mortalitatea şi morbiditatea datorită supresiei medulare. Suflul exploziei nucleare nu seamănă cu suflul produs de exploziile convenţionale, deoarece puterea exploziei nucleare este mult mai mare, iar deplasarea maselor de aer se poate face cu viteze de sute de km/oră, capabile să antreneze atât fragmente de construcţii, care se transformă în adevărate proiectile, cât şi victime. Tipul leziunilor variază în ceea ce priveşte gravitatea, dar experienţa arată (explozia de la Hiroshima şi Nagasaki) că leziunile penetrante profunde sunt prezente în număr mic. De reţinut că în condiţiile afluxului mare de victime aceste cazuri pot trece neobservate până la apariţia semnelor clinice evidente de şoc.

3. Leziuni de iradiere. Detonarea unei arme nucleare produce cantităţi foarte mari de radiaţii ionizante sub două forme: radiaţie electromagnetică (gamma) foarte penetrantă şi radiaţii sub formă de particule (alfa, beta şi neutroni). Leziunile de iradiere se datorează radiaţiilor ionizante eliberate în momentul exploziei nucleare şi un timp considerabil după detonare.

Radiaţia emisă în momentul detonării se numeşte ***„radiaţie promptă”*** şi este produsă de reacţia nucleară de fisiune şi fuziune. Cea mai mare parte a acestei radiaţii este formată de radiaţia gamma şi neutroni, şi este emisă la câteva secunde de la detonare, timp de aproximativ un minut. Radiaţia emisă la un minut de la detonare se numeşte ***„radiaţie reziduală”*** şi este formată din produşi de fisiune (radioizotopi ai U şi Pu). Produşii nefisionaţi sunt radioactivi datorită bombardării cu neutroni.

Produşii de fisiune sunt hazardul principal deoarece emit radiaţia gamma şi au T de ½ de la câteva secunde la câţiva ani (majoritatea au T de ½ de zile la săptămâni). Mici părţi de uraniu sau plutoniu folosit la fabricarea armei nu vor fisiona şi vor emite radiaţii alfa, devenind periculoase în momentul ingestiei/inhalării acestora. Datorită temperaturilor foarte înalte generate de explozia nucleară, toţi produşii de fisiune şi reziduurile armei se vor vaporiza iniţial, după care se vor solidifica în particule foarte mici.

Dacă armele nucleare sunt testate în atmosferă, aceste particule vor ajunge în stratosferă şi vor coborî în ani de zile, dar dacă arma se detonează la o distanţă critică de suprafaţa terestră, explozia va antrena şi cantităţi mari din teren. În această ultimă situaţie, particulele radioactive se vor amesteca cu particulele din terenul respectiv şi vor cădea rapid înapoi pe pământ, la locul de detonare a bombei, determinând o creştere importantă a radioactivităţii locale.

Principalul risc într-o astfel de explozie este contaminarea externă a întregului corp de către izotopii care emit *radiaţii gamma*. Acest tip de radiaţie este o radiaţie penetrantă şi poate afecta inclusiv personalul din autovehicule (într-un tanc doza este de 4-10% din doza exterioară). Leziunile provocate de radiaţiile gamma depind de timpul petrecut în zona contaminată, precum şi de doza prezentă. Izotopii care emit *radiaţii beta* nu reprezintă un risc semnificativ, decât în cazul unui contact direct sau al ingestiei acestora. Contaminarea externă determină leziuni cutanate asemănătoare arsurilor termice, iar contaminarea internă poate fi urmată în ani de afectarea organelor, carcinogeneză şi îmbătrânire accelerată.

Având în vedere cele menţionate, protecţia împotriva radiaţiilor ionizante se va face purtând îmbrăcăminte de protecţie pentru particulele alfa sau adăpostindu-ne în adăposturi din materialele solide (pereţi de beton sau alt tip), pentru particulele beta; pentru a ne feri de radiaţiile gamma şi de neutroni vom folosi adăposturile cu pereţi de plumb sau construite din materiale echivalente.

4. Leziuni oculare - orbirea prin explozia de lumină/ arsurile retiniene - apar ca rezultat al observării în privirea periferică a energiei luminoase care rezultă după detonarea armei nucleare.

SINDROAME DE IRADIERE

Boala de iradiere, provocată de efectul radiaţiilor asupra întregului organism, poate fi letală, intervalul de timp variind de la zile la săptămâni, în funcţie de dozele de expunere. Clinic apar trei sindroame, determinate de organul sau sistemul de organe afectat şi de doza de expunere :

- *neurovascular* – provocat de doze foarte mari, foarte rar şi întotdeauna fatal în 2-4 zile ;
- *gastrointestinal* - provocat de doze ceva mai mici, este rar şi aproape întotdeauna fatal;
- *hematopoetic* - provocat de doze mici, este cel mai frecvent întâlnit şi asociat cu şanse de recuperare şi supravieţuire foarte mari.

Cele trei sindroame au unele caracteristici comune şi anume: un răspuns iniţial nespecific, o perioadă de latenţă şi o fază clinică.

1. Faza de răspuns iniţial nespecific. După o expunere la radiaţii, indiferent de sindromul care se va dezvolta ulterior, iniţial apare o perioadă de stare de rău, slăbiciune, anorexie, vărsături şi diaree. Toate aceste manifestări se datorează, probabil, leziunilor celulare provocate de iradiere, durează până la câteva ore şi sunt urmate de o perioadă de latenţă. Severitatea şi durata manifestărilor nu au nicio legătură cu gradul expunerii la radiaţii şi uneori chiar pot lipsi cu desăvârşire.

2. Faza de latenţă. Se caracterizează printr-o stare de bine relativ şi este prezentă în toate cele trei sindroame. Durata variază între câteva zeci de minute şi zile (3 zile) pentru sindromul neurovascular, câteva zile pentru sindromul gastrointestinal şi 2-6 săptămâni pentru sindromul hematopoetic.

3. Faza clinică. De obicei, pacienţii care ajung în această fază sunt internaţi doar dacă au şi alte leziuni traumatice asociate (arsuri, fracturi etc.).

3.1. Sindromul neurovascular. Apare la o expunere foarte mare (≥3 000 de cGy) şi este foarte rar, deoarece de obicei personalul militar este adăpostit. Poate fi prezent la personalul neprotejat, situat foarte aproape de punctul de detonare al unor arme puternice. Iniţial se înregistrează o uşoară deprimare a stării de conştienţă, urmată de ataxie, convulsii, stare de comă profundă şi deces. Nu sunt depistate modificări hematologice, deoarece evoluţia este foarte rapidă, cu mortalitate 100%.

3.2. Sindromul gastrointestinal. Însoţeşte expunerile la radiaţii ce depăşesc 1 000 de cGy, în general la 4-5 zile după detonarea armei. Diagnosticul diferenţial cu diareea infecţioasă este iniţial greu de realizat, dar pe măsură ce apare deprimarea măduvei osoase cu limfopenie, leucopenie şi anemie diagnosticul este evident. Pacienţii vor evolua spre deprimarea ireversibilă a măduvei osoase, iar tratamentul intensiv cu antibiotice, soluţii de repleţie volemică, soluţii electrolitice etc. nu va face altceva decât să le prelungească la câteva săptămâni durata de viaţă.

3.3. Sindromul hematopoetic. Pacienţii expuşi la doze mai mici de radiaţii vor dezvolta acest sindrom în 2-6 săptămâni (medie de 2-3 săptămâni), tabloul clinic depinzând şi de existenţa leziunilor traumatice asociate. Gradul de afectare a măduvei osoase depinde de doza de radiaţii la care a fost expusă victima. Pacienţii prezintă hemoragii de diferite gravităţi, începând cu apariţia de peteşii şi echimoze şi terminând cu hemoragii digestive. La aceste manifestări hemoragice se adaugă scăderea rezistenţei la infecţii, care este prima cauză de deces. Tratamentul constă în măsuri de suport al funcţiilor vitale (antibiotice, soluţii electrolitice etc.), transfuzii de sânge şi derivate. Evoluţia pacienţilor depinde de capacitatea de regenerare a măduvei osoase.

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Maladia celiacă şi autoimunitatea

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Rezumat. Maladia celiacă (M.C.) este o enteropatie mediată imun cu o prevalență de aproximativ 1% din populația SUA şi a Europei. Prezența sa este larg răspândită şi în restul lumii. Deşi consecințele clasice ale acestei enteropatii sunt malabsorbția cu diaree, scădere ponderală şi deficiențe nutriționale, diagnosticul este dificil, mai ales datorită formei latente care afectează majoritatea pacienților. Simptomele clinice generale sunt relativ moderate şi nespecifice (fatigabilitate, cefalee, artralgii), astfel că pot întârzia momentul diagnosticului.

Studii recente americane multicentrice semnalează prevalența acestei afecțiuni în grupurile cu risc crescut ca, de exemplu, diabetul zaharat autoimun insulino-necesitant, sindromul Sjogren, osteoporoza şi rudele de gradul I ale pacienților cu M.C. Pe lângă diaree, manifestările cel mai frecvent raportate sunt durerea abdominală şi constipația. Ca urmare a dificultăților diagnostice datorate formelor clinice latente sau uşoare, identificarea grupurilor de risc înalt în vederea testării serologice poate reduce semnificativ momentul diagnosticului şi gravitatea complicațiilor evolutive ale bolii netratate. Numeroase studii au demonstrat cost-eficiența testării pacienților cu intestin iritabil pentru depistarea M.C. Rezultate recente subliniază posibilitatea ca mecanisme imunologice, declanşate de expunerea la gluten, să contribuie la declanşarea şi întreținerea simptomelor intestinului iritabil care ar reprezenta astfel o afecțiune "celiac-like".

Afecțiuni autoimune variate au istoricul asociat cu M.C. Aceste date pun în discuție fie că răspunsul imun anormal, la nivelul mucoasei digestive expuse antigenelor din mediu, are rol în boala sistemică autoimună, fie că asocierile semnalate reflectă de fapt o predispoziție genetică. Mecanismele propuse ale acestei asocieri includ reglarea anormală a permeabilității intestinale şi producția crescută de autoanticorpi pe fondul inflamației cronice intestinale. Articolul de față analizează afecțiunile autoimune ale țesutului conjunctiv, endocrine şi dermatologice asociate maladiei celiace şi de asemenea modificările inflamatorii digestive asociate maladiei celiace refractare, enteropatiei autoimune, enteritei şi colitei colagenice.

Cuvinte cheie: boala celiacă, autoimunitate, gluten

Abstract: Coeliac disease (C.D.) is an immune mediated enteropathy with a prevalance of 1% within the U.S.A. and Europe populations. There is a worldwide disease distribution. Although the classic consequences of this enteropathy are diarrhea with malabsorbtion, weight loss and nutritional deficiencies, the difficulty in diagnosis is largely due to silent form which affects the majority of patients. Overall mild clinical symptoms with nonspecific complaints such as fatigue, headaches, and arthralgias are common and can delay diagnosis.

A large multicenter study in the United States showed an increased disease prevalence in high-risk groups, including patients with autoimmune insulin-dependent diabetes mellitus (AIDDM), Sjogren's syndrome, osteoporosis, and first-degree relatives of patients with celiac disease. In addition to diarrhea, abdominal pain and constipation were among the most highly reported symptoms. Because of the diagnostic difficulties inherent in this often mild or clinically silent presentation, the identification of high-risk groups for serologic screening may decrease the time to diagnosis and lessen the complications of untreated disease. Several studies have demonstrated the cost-effectiveness of screening the population with irritable bowel syndrome for celiac disease. The results from one recent study addressed the possibility of immunologically based mechanisms following gluten exposure contributing to irritable bowel syndrome symptoms that may represent a celiac-like disorder.

Various autoimmune diseases have been historically associated with celiac disease. These findings raise interesting questions as to whether abnormal immune responses at the level of the gut mucosa, when exposed to environmental antigens, have a role in systemic autoimmune disease, or whether these associations reflect more an underlying genetic predisposition. Proposed mechanisms of association include abnormal regulation of intestinal permeability and increased autoantibody production in the setting of chronic gut inflammation. This review focuses on the autoimmune connective tissue diseases, endocrine, and dermatologic conditions associated with celiac disease, as well asthe related gut inflammatory disorders of refractory celiac disease, autoimmune enteropathy, collagenous enteritis, and collagenous colitis.

IMUNOGENEZA INTESTINALĂ A MALADIEI CELIACE

Maladia celiacă este rezultatul reacției imune necontrolate la gluten. Acest răspuns determină inflamația intestinului subțire proximal, unde are loc contactul dintre proteinele glutenului, parțial digerate, şi sistemul imun digestiv. Acest răspuns imun depăşeşte limita unei reacții normale față de substanța exogenă, declanşând, la rându-i, un răspuns imun multifațetat față de autoantigene ce are drept rezultat afectarea substanțială a structurii şi funcțiilor tubului digestiv şi a altor organe. Manifestările clinice sunt heterogene, de la absența lor completă până la o multitudine de manifestări extraintestinale, semne de malabsorbție şi, în cazuri rare, apariția jejunitei ulcerative şi a limfomului cu celule T asociat enteropatiei.

În mucoasa intestinului subțire, stimularea celulelor CD4+T de către peptidele gliadinei are loc numai dacă sunt expuse de către moleculele DQ2 sau DQ8 MHC aflate pe suprafața celulelor cu rol de prezentator de antigene. Acest proces declanşează proliferarea celulelor T gluten-specifice, un răspuns inflamator puternic cu atrofie vilozitară subsecventă. Asociat, se produce un răspuns umoral aproape universal față de transglutaminaza intestinală (tTg). Rolul acestui proces nu este pe deplin înțeles. Curioasă este şi abilitatea tTg de pe suprafața celulelor de a lega şi dezamida peptidele gliadinei. Peptidele dezamidate se vor lega ulterior strâns de moleculele DQ2/DQ8, potențând astfel activarea şi proliferarea celulelor T CD4+. Atât răspunsul celulelor T, cât şi al celulelor B se diminuează după îndepărtarea glutenului, cu restaurarea arhitecturii viloase normale. Deşi, de mai mulți ani, sunt cunoscuți anticorpi direcționați împotriva proteinelor din grâu, descoperirea unor anticorpi, specific direcționați împotriva antigenelor extracelulare, a revoluționat diagnosticul maladiei celiace. Datorită înaltei specificități a anticorpilor anti-endomisium (Ac.AEM) şi a sensibilității mari a IgA-tTg, utilizarea conjugată a ambelor teste conferă o valoare pozitivă predictivă mare. Pentru screening se recomandă totuşi numai testul IgA-tTg, deoarece testarea Ac-AEM creşte semnificativ costurile. Urmărirea în dinamică a titrului IgA-tTg oferă informații despre expunerea ocultă la gluten sau despre lipsa de complianță la dieta fără gluten. Timpul de normalizare a titrului IgA-tTg în cadrul unei diete fără gluten este variabil, de la câteva săptămâni la mai multe luni.

Maladia celiacă este unică printre alte afecțiuni autoimune atât pentru că factorii precipitanți majori şi necesari sunt cunoscuți, cât şi pentru că factorii de mediu declanşatori trebuie să fie mereu prezenți, altfel atât maladia cât şi autoanticorpii regresează. S-a observat că unii pacienți cu predispoziție genetică (DQ2 sau DQ8) care consumă gluten nu dezvoltă totuşi maladie celiacă. S-a concluzionat că trebuie să fie implicați şi alți factori în afara celor genetici. Aceşti factori adiționali contributivi sunt mai puțin cunoscuți dar, probabil, includ un element de stimulare a unui răspuns imun înnăscut față de un factor din mediu, derivat din gluten sau față de un alt element prezent în lumenul intestinal.

FACTORII DE MEDIU

Momentul expunerii şi cantitatea de gluten implicat pot fi factori importanți ai instalării bolii. Afirmația are la bază studii observaționale. În anii 1980, incidența maladiei celiace printre copiii suedezi a sporit semnificativ şi a rămas crescută aproximativ 10 ani. Ulterior, prin modificarea, la nivel național, a practicilor de alimentație prin reducerea expunerii la gluten, incidența M.C. a scăzut, revenind la nivelele pre-epidemice. Această ”epidemie suedeză” a generat ipoteza că, introducerea unor mari cantități de gluten după încetarea alimentației la sân a fost responsabilă de insuficienta dezvoltare a toleranței imune la gluten a acestor copii. Alimentația la sân, ca factor singular, nu a părut să fie factorul de risc major. Expunerea la glutenul alimentar, mai devreme de 3 luni sau după a 6-a lună de viață, a fost găsită ca factor de risc major în cadrul unui studiu longitudinal efectuat pe un lot de copii în Denver, Colorado.**(1)** Aceste cercetări sugerează că există o fereastră crucială în care apare toleranța la gluten şi că suprapunerea introducerii glutenului cu alimentația la sân poate oferi cea mai bună protecție împotriva maladiei celiace la copil. Nu se cunoaşte însă dacă această practică va putea preveni şi apariția M.C. sau a altor boli autoimune de-a lungul vieţii. S-a arătat **(2)** că riscul apariţiei M.C. creşte odată cu numărul infecţiilor gastrointestinale din primele 6 luni de viaţă şi printre copii născuţi între lunile martie-iulie. Acest risc sezonier a fost demonstrat consistent, an de an, pe durata celor 10 ani ”epidemici”. Infecţiile frecvente cu rotavirusuri au crescut riscul de M.C. la 54 de copii cu vârste de până la 4 ani.

RISCUL BOLII AUTOIMUNE

Un studiu din 1999 **(3)** a demonstrat prevalenţa afectării autoimune printre 909 pacienţi care au avut M.C., comparativ cu lotul de control, deşi nu s-au găsit diferenţe semnificative în comparaţie cu 163 pacienţi cu boala Crohn. Analiza prin regresie logistică a arătat că, vârsta la care s-a pus diagnosticul de M.C. a fost un predictor semnificativ al apariţiei afecţiunii autoimune ulterior, în decursul vieţii. Alţi autori **(4)** au identificat prevalenţa afecţiunii autoimune (4,8%) printre rudele de gradul I al pacienţilor cu M.C. comparativ cu rudele de gradul I al subiecţilor de control (0,86%). Un subset dintre rudele de gradul I ale pacienţilor cu M.C. a fost diagnosticat cu boală latentă. Printre aceştia prevalenţa bolii autoimune era mai mare comparativ cu rudele sănătoase (20% faţă de 3,8%). Autorii sugerează că acest risc crescut se corelează cu prevalenţa mai mare a bolii celiace latente. Nu se ştie dacă pentru incidenţa în creştere a bolii autoimune este responsabilă M.C. netratată dar clinicianul este bine să fie avertizat despre posibilitatea acestor asocieri, cel puţin atunci când apar în practica clinică.

ASOCIERILE CU BOALA AUTOIMUNĂ

Sindromul Sjogren. Mai multe studii indică prevalenţa M.C. la pacienţii cu S. Sjogren. Într-unul dintre acestea, la 14,7% din 34 pacienţi iar mai recent la 4,5% din 111 pacienţi, maladia celiacă a fost confirmată prin biopsie de intestin subţire. **(5)** Atunci când s-au determinat autoanticorpii anti-transglutaminază tisulară (AuAc-tTg) **(6)** în serul pacienţilor cu lupus eritematos sistemic (LES), cu sindrom Sjogren şi artrită reumatoidă (AR), 12% dintre pacienţii cu S.Sjogren au fost pozitivi pentru IgA-tTg faţă de 4% în lotul de control. Cinci din şase pacienţi cu IgA-tTg aveau atrofie viloasă pe biopsia intestinului subţire, confirmând diagnosticul de maladie celiacă. Nivele semnificativ mai reduse de Au.Ac-tTg au fost depistate la pacienţii cu LES, AR şi scleroză sistemică.

Artrita inflamatorie. Dacă într-un studiu **(7)** prevalenţa M.C. printre 151 copii cu artrită juvenilă idiopatică a fost de 6,7% faţă de 0,6%, într-un alt studiu prevalenţa confirmată bioptic a fost de 2,5% printre 119 copii cu artrită cronică juvenilă. Această asociere nu s-a extins însă şi la adulţii cu artrită reumatoidă. Dieta fără gluten poate fi utilă la unii pacienţi cu simptome musculo-scheletale. Într-un lot de 200 de pacienţi cu M.C. **(8),** 26% aveau fenomene artritice. Prevalenţa artritei a fost semnificativ mai mare şi mai severă în grupul cu dietă normală (nemodificată) comparativ cu pacienţii aflaţi de 58 de luni pe dietă fără gluten. Aceste date confirmă observaţii mai vechi care arătau ameliorarea simptomelor articulare la pacienţii nou diagnosticaţi cu M.C. care urmau ulterior dietă fără gluten. Mecanismul subiacent manifestărilor artritice în M.C. este necunoscut, deşi sunt incriminabile modificările permeabilităţii intestinale în cazurile netratate de maladie celiacă. Literatura consemnează puţine cazuri de M.C. manifestă clinic la pacienţi cu LES. Dacă la copii, diagnosticul de maladie celiacă precede cu 15 ani pe cel de LES, la adulţi dimpotrivă, diagnosticul de M.C. urmează după 3,8 ani pe cel de LES. Asocierea LES cu maladia celiacă este mai degrabă rară. Deşi manifestările gastrointestinale ale LES sunt comune atât la adulţi cât şi la copii, M.C. este rară la această populaţie, dar trebuie luată în considerare în prezenţa simptomelor de malabsorbţie. În maladia celiacă netratată pot coexista Ac-antinucleari fals pozitivi.

AFECŢIUNI ENDOCRINE AUTOIMUNE ASOCIATE

Boala Addison. În studii europene, prevalenţa M.C. printre pacienţii cu boală Addison autoimună este între 1,2 şi 8%. Mai recent, italienii consemnează prevalenţa de 5,4% M.C. printre

109 cazuri de maladie autoimună Addison față de 0,06% la lotul de control. **(9)** Asocierea B.Addison cu M.C. a fost confirmată şi de un studiu pe 14 000 pacienți cu M.C. din registrul național suedez, care arată riscul crescut de B. Addison printre aceştia.

Diabetul zaharat autoimun insulino-necesitant (DZAIN) are prevalență crescută la pacienții cu M.C. În 40 de studii (efectuate mai ales în Europa şi SUA) screeningul serologic pentru M.C. a arătat o prevalență de 4,1%. **(10)** Manifestările gastrointestinale atribuite M.C. la pacienții cu DZAIN sunt în general uşoare. Malabsorbția activă poate determina răspunsuri glicemice impredictibile după mese, creând dificultăți în controlul diabetului. Deficiența de fier şi folat este frecventă. Diagnosticul de M.C. urmează după un an de la diagnosticul de DZAIN. Screeningul M.C. în DZAIN nu este acceptat universal dar este sugerat la copii, datorită prevalenței şi consecințelor potențiale ale întârzierii diagnosticului. Odată cu corecția malabsorbției, prin ameliorarea subsecventă a absorbției glucidelor, doza totală de insulină poate fi mai mare. În orice caz, medicul care tratează pacienți cu DZAIN trebuie să fie sensibilizați cu privire la oportunitatea testării pentru M.C.

Afectarea autoimună tiroidiană. Un studiu recent **(11)** a revelat o incidență de 2-7,8% (media=4,1%) la pacienții cu boală tiroidiană autoimună, maladie Hashimoto, boală Graves şi tireopatie autoimună pediatrică. În boala celiacă la adult poate apărea atât hipertiroidismul (0,1-5,2%; media=2,3%), cât şi hipotiroidismul (0-5,8%; media=2,6%). Deşi datele din prezent nu susțin screeningul pentru maladia celiacă la pacienții tiroidieni, conştientizarea posibilei asocieri obligă la reducerea pragului deciziei pentru testarea diagnostică.

Hepatopatia autoimună asociată. În cazul maladiei celiace netratate, transaminazele pot creşte uşor în absența unei boli hepatice primare. Pacienții sunt adesea asimptomatici şi prezintă inflamație periportală uşoară care se remite cu dietă fără gluten. Studii multiple au demonstrat asocieri variabile între ciroza biliară primară, hepatita autoimună, colangita sclerozantă şi maladia celiacă. Astfel, prevalența M.C. confirmată bioptic a fost de 1,3-7% la populația cu ciroză biliară primară şi de 4-6,4% la bolnavii cu hepatită autoimună. **(12)** În registrul național suedez, la 13 800 pacienți cu M.C., riscul de colangită sclerozantă primară este crescut de 4-8 ori. Testarea pentru maladia celiacă ar trebui inclusă în strategia de investigare a transaminazelor crescute. Au-Ac-tTg fals pozitivi pot apărea la hepatici dar titrul Ac-AEM este înalt specific chiar şi într-o astfel de situație şi este de ales pentru detecția maladiei celiace posibil asociate.

MANIFESTĂRILE DERMATOLOGICE

Maladia celiacă se poate asocia cu dermatita herpetiformă, psoriazis, vitiligo, alopecia aerata, maladia Behcet, lichen plan, dermatomiozita, *pyoderma gangrenosum* şi altele. Dermatita herpetiformă este strâns asociată cu M.C., este prevalentă la sexul masculin şi prezintă leziuni veziculare intens pruriginoase pe suprafețele de extensie ale extremităților, pe spate şi în regiunea fesieră. Într-un studiu finlandez, unul din patru pacienți cu M.C. are dermatită herpetiformă iar într-altul **(13)** s-a găsit o prevalență de 12,6% pentru M.C. şi de 22% pentru patologie autoimună sistemică, printre 226 pacienți cu dermatită herpetiformă. Diagnosticul este obiectivat prin vizualizarea în imunofluorescență a depozitelor granulare de IgA la joncțiunea dermo/epidermală pe material bioptic prelevat din apropierea (dar nu exact din) ariei afectate. Transglutaminaza tisulară epidermală (eTg) este considerată principalul autoantigen. Inflamația intestinului din M.C. se poate asocia cu producție crescută de anticorpi direcționați împotriva eTg. Leziunile regresează după terapia cu dapsonă şi dietă fără gluten.

Prevalența M.C. printre 92 pacienți cu psoriazis a fost de 4,34%. **(14)** Leziunile psoriazice s-au ameliorat cu dieta fără gluten, în special la cei cu titruri mari de Ac-anti gliadină şi creşteri uşoare ale limfocitelor epiteliului duodenal sau fără enteropatie. Studiile au sporit interesul cu privire la mecanismele imunologice care conectează afecțiunile gastrointestinale şi cutanate, precum şi oportunitatea unor tratamente eficiente. Au fost propuse mecanisme multiple, printre care permeabilitatea intestinală crescută, deficiența de vitamină D şi activarea celulelor T indusă de gluten.

MANIFESTĂRILE NEUROLOGICE

În 10-12% dintre cazuri, pacienţii cu boală celiacă au manifestări neurologice, incluzând ataxie cerebeloasă, neuropatie periferică, convulsii şi mielopatie.**(15)** Alte manifestări descrise sunt declinul cognitiv, demenţa, miopatia, epilepsia şi calcificările cerebrale. Ataxia glutenică poate apărea cu sau fără enteropatie şi se asociază cu un titru crescut de Ac-antigliadină. Simptomele gastrointestinale au fost prezente la doar 13% dintre aceştia. Vârsta medie de apariţie a manifestărilor neurologice este 48 de ani. Ac-antigliadină apare frecvent şi în alte afecţiuni cerebrale degenerative, cum este chorea Huntington şi ataxia spino-cerebeloasă condiţionată genetic. Termenul de neuropatie glutenică a fost propus pentru desemnarea pacienţilor cu neuropatie idiopatică şi Ac-antigliadină pozitivi, cu sau fără evidenţa enteropatiei. Terapia cu excluderea glutenului poate ameliora simptomele neuropatice, deşi nu s-a dovedit definitiv că aceasta modifică istoria naturală neurologică în absenţa adevăratei maladii celiace.**(16)** Etiologia sindroamelor neurologice legată de expunerea la gluten, nefiind direct asociată cu maladia celiacă, rămâne controversată.

DEFICITUL DE IgA

Prevalenţa maladiei celiace la pacienţii cu deficit de IgA este de peste 10 ori mai mare comparativ cu persoanele de control. Cum deficitul de IgA nu permite detecţia maladiei celiace prin măsurarea titrului tTg-IgA, se recomandă determinarea nivelului IgA total pentru a exclude existenţa acestei deficienţe. În prezenţa deficitului de IgA, tTg-IgG este adesea, deşi nu întotdeauna, crescut, indicând necesitatea efectuării biopsiilor de intestin subţire iar ulterior poate fi orientativă pentru expunerea ocultă la gluten sau pentru indiscreţii de dietă. Pacienţii cu deficit de IgA au mai frecvent forme latente de M.C., precum şi asocieri alergice ca astmul bronşic, dermatita atopică şi alergiile alimentare.

ANEMIA PERNICIOASĂ

Prevalenţa deficienţei de vit. B12 printre 159 pacienţi cu M.C., în cel mai amplu studiu publicat **(17),** a fost de 12%. Prevalenţa anemiei pernicioase nu a fost crescută. Evaluarea s-a efectuat pe datele de histologie gastrică, testarea Ac-anti factor intrinsec şi nivelul gastrinemiei. Deşi deficitul de vit. B12 este frecvent, se datorează foarte probabil altor cauze din afara M.C.

AFECŢIUNI AUTOIMUNE GASTROINTESTINALE ASOCIATE MALADIEI CELIACE

*Maladia celiacă refractară.*Unele cazuri de M.C. nu răspund la dieta fără gluten. Ca urmare, atrofia viloasă persistă, simptomele clinice evoluează iar riscul malignităţii este crescut prin activarea imună continuă. Maladia celiacă refractară este o complicaţie relativ rară care apare la 2-5% dintre pacienţi şi este de două tipuri, I şi II. Distincţia este importantă în termeni de predicţie ai morbidităţii şi mortalităţii, de strategii de terapie imunosupresivă şi de implicaţii pentru screeningul limfomului cu celule T asociat enteropatiei (EATL). Prezentarea clinică şi histologică a celor două tipuri prezintă similitudini în stadiile iniţiale, mai ales persistenţa simptomelor gastrointestinale, a atrofiei vilozităţilor, în pofida complianţei cu dietă strictă fără gluten de peste 12 luni şi titruri normale ale Ac-anti tTg. Distincţia între tipul I şi II de maladie celiacă refractară şi implicaţiile asupra supravieţuirii pacienţilor se bazează pe transformarea monoclonală a populaţiilor celulelor T intraepiteliale din fenotipul policlonal caracteristic tipului I de maladie celiacă refractară. Diagnosticul se precizează prin analiza imunohistochimică a markerilor specifici de suprafaţă ai celulelor T şi a analizei PCR a rearanjamentului genei receptorului celulei T (TCR- T cell receptor). Această testare se poate efectua numai în centre specializate. Maladia celiacă refractară tip II creşte riscul apariţiei jejunitei ulcerative şi a EATL. Dacă utilizarea capsulei endoscopice este limitată prin inabilitatea obţinerii de material bioptic, enteroscopia cu dublu-balon poate fi eficientă în diagnosticarea sau excluderea EATL cu sau fără jejunită ulcerativă în M.C. refractară. Scanarea PET (tomografie computerizată cu emisie de pozitroni) aduce un beneficiu adițional în diagnosticul precoce al EATL şi în identificarea manifestărilor extraluminale ale limfomului. **(18)** Combinația PET-CT oferă o sensibilitate chiar mai mare pentru detecția precoce. Factori de risc adiționali asociați EATL includ diagnosticarea tardivă, statusul HLA DQ2 homozigot şi sexul masculin. Datorită mortalității ridicate a EATL, diagnosticul precoce şi intervenția terapeutică subsecventă capătă un interes aparte.

Tipul I de maladie celiacă refractară răspunde în general la corticoterapie, care constituie astfel prima linie terapeutică. Azathioprina poate fi utilizată după inducția remisiunii clinice. Corticoterapia poate fi încercată şi în tipul II, unde însă cazurile corticorezistente implică introducerea regimurilor imunosupresive mai agresive, inclusiv administrarea de infliximab. **(19)** În tipul II de M.C. refractară, riscul apariției clonei celulare maligne T prin imunosupresia cronică este însă accelerat. Budesonidul este eficient în ameliorarea simptomelor clinice în M.C. refractară de tip I şi II, deşi nu s-a evidențiat normalizarea arhitecturii enterale. **(20)** Budesonidul este metabolizat semnificativ la primul pasaj hepatic, ceea ce minimizează efectele sistemice asociate corticoterapiei cronice. Înaintea diagnosticării maladiei celiace refractare, vor trebui excluse alte etiologii, cum sunt colita limfocitară, enteropatia autoimună, insuficiența pancreatică, enterocolita colagenică, suprapopularea bacteriană şi deficitul de lactază. Se recomandă de asemenea screeningul şi tratamentul deficitelor nutriționale, incluzând zincul, cuprul, vit. B12, folatul, fierul şi albumina. Uneori, în prezența hipoalbuminemiei severe asociate cu sindrom edematos şi infecții oportuniste, este necesară instituirea nutriției parenterale.

Enteropatia autoimună este o afecțiune rară, care predomină la copii deşi au fost semnalate şi cazuri cu debut la vârsta adultă. Au fost comunicate şi variante de enteropatie autoimună asociată cu gastrită atrofică, enterocolită autoimună, enteropatie autoimună asociată cu boli sistemice autoimune (sindromul sicca, tiroidita, miastenia gravis) şi enteropatia autoimună asociată cu artrita reumatoidă sau artrita psoriazică. Fiziopatologia enteropatiei autoimune este insuficient clarificată. Importante în patogeneză par să fie producția de TNF-alfa de către celulele T activate în mucoasa intestinală şi un subset unic de celule CD-T din lamina propria. Un studiu pe 15 adulți **(21)** cu vârstă medie de 55 ani, predominant caucazieni (87%), a consemnat atrofia vilozitară subtotală enterală proximală, limfocitoză crescută în lamina propria, anticorpi anti-enterocitari sau anti-celulă caliciformă (goblet cell) în serologie şi reducerea numărului de limfocite intraepiteliale. Clinic pacienții prezentau diaree cronică, scădere ponderală, lipsa răspunsului la excluderea glutenului din dietă şi absența cauzelor secundare adiționale de atrofie viloasă.

Criteriile pentru diagnosticul enteropatiei autoimune la adult propuse de acest studiu efectuat de Mayo Clinic sunt:

1) diaree cronică de peste 6 săptămâni;
2) semne şi simptome clinice de malabsorbție;
3) histologie specifică pe biopsia enterală;
4) excluderea cauzelor secundare de atrofie viloasă şi
5) serologie pozitivă pentru anticorpi anti-enterocitari sau anti-celule caliciforme.

Pentru diagnostic sunt necesare primele 4 criterii. Serologia negativă nu exclude diagnosticul de enteropatie autoimună. În maladia inflamatorie intestinală nespecifică sau maladia celiacă nu au fost depistați anticorpi anti-enterocitari. 60% dintre pacienți au răspuns pozitiv la doze mari de steroizi, dar 66% dintre aceştia au devenit corticodependenți sau au necesitat terapie imunosupresivă adjuvantă.

Enterita colagenică (Sprue colagenic) este strâns legată de maladia celiacă, atât histologic cât şi clinic. Pacienții prezintă simptome clasice de malabsorbție şi atrofie vilozitară la biopsia enterală. Se discută, încă, dacă enterita colagenică este o variantă a maladiei celiace. Elementele distinctive sunt prezența benzilor de colagen subepitelial şi lipsa de răspuns la îndepărtarea glutenului din dietă. Elemente comune sunt serologia pozitivă anti-endomisium şi riscul crescut de limfoame T şi B. **(22)** Cauzele enteritei colagenice nu sunt cunoscute iar tratamentul necesită de obicei corticoterapie prelungită. Enterita colagenică poate apărea în asociere cu colita colagenică.

Colita microscopică. Există o asociere clară între maladia celiacă şi colita microscopică, incluzând variantele de colită de colagen şi colită limfocitară. Aproximativ 30% dintre pacienţii cu maladie celiacă prezintă elemente de colită microscopică pe biopsia colonică iar 2-10% dintre pacienţii cu colită microscopică au atrofie viloasă sugestivă de maladie celiacă. De aceea se recomandă un prag redus de screening pentru maladia celiacă în rândul acestei populaţii.

Concluzii

Cele mai strânse asocieri între maladia celiacă şi bolile sistemice autoimune includ maladia Addison, tiroidita autoimună, diabetul zaharat autoimun insulino-necesitant, sindromul Sjogren, ciroza biliară primară şi hepatita autoimună (fig.1). Până la finalizarea unor analize de cost-beneficiu pentru screeningul bolii celiace şi promovarea unor algoritmuri diagnostice, se recomandă un prag redus pentru screening şi un grad înalt de suspiciune clinică. Există o asociere clară între dermatita herpetiformă şi maladia celiacă, astfel că diagnosticul definitiv de dermatită herpetiformă prin imunofluorescenţă directă este suficient pentru justificarea iniţierii dietei fără gluten, iar prezenţa autoanticorpilor tTg implică atrofie viloasă substanţială.

Testarea pacienţilor artritici este mai puţin clară şi aceasta în special datorită loturilor mici studiate, a diferenţelor de prevalenţă dintre cazurile prezente la adulţi şi la copii şi a tipurilor variate de artrită luate în studiu. De aceea se recomandă menţinerea unui prag redus de screening pentru copii comparativ cu populaţia adultă. Nu este indicat însă screen ingul pentru maladia celiac la toţi pacienţii cu lupus eritematos sistemic, chiar dacă el poate fi luat în considerare la pacienţii cu lupus care asociază simptomatologie gastrointestinală. Pacienţii neurologici, cu spectrul larg de simptome clinice descrise, vor fi evaluaţi pentru maladia celiacă dar şi pentru cazurile dependente de gluten care apar în absenţa enteropatiei. În absenţa unui răspuns clinic la restricţia glutenului din dietă şi a unor criterii diagnostice recomandate pentru maladia celiacă, vor fi luate în consideraţie şi alte boli

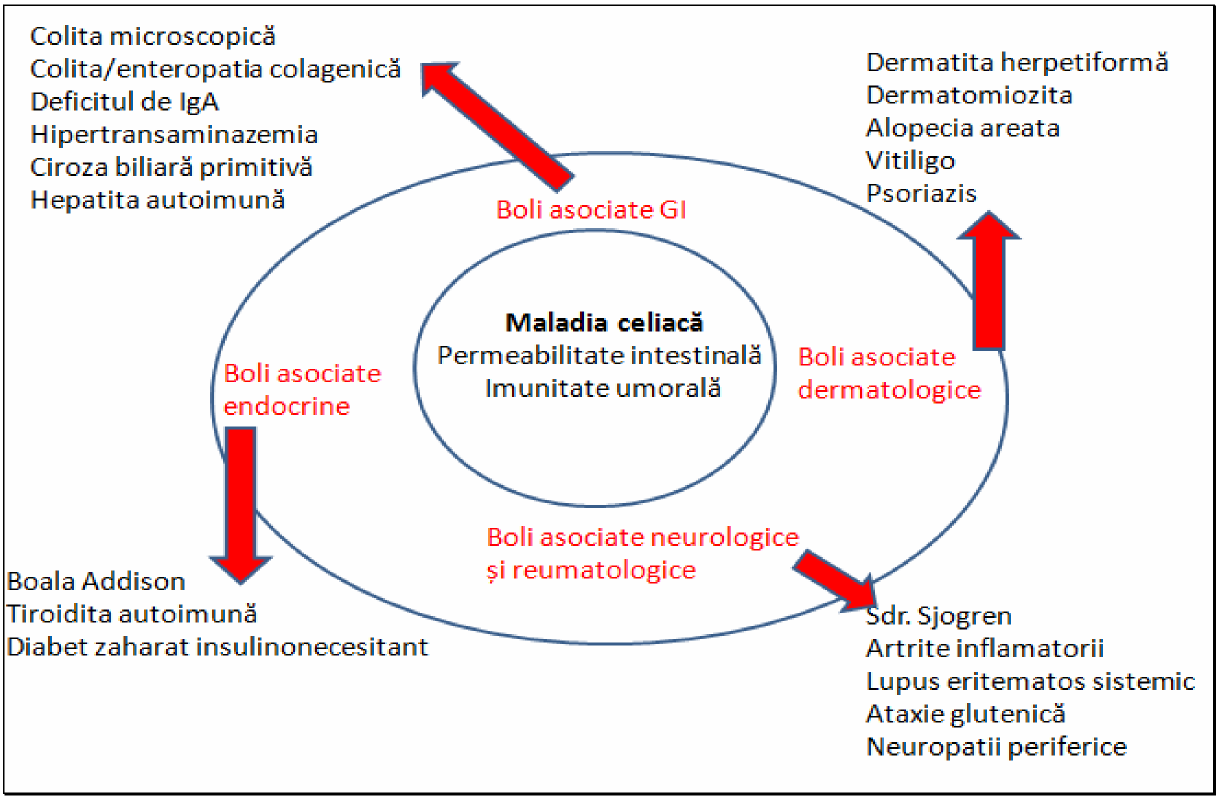
Colita microscopică
Colita/enteropatia colagenică
Deficitul de IgA
Hipertransaminazemia
Ciroza biliară primitivă
Hepatita autoimună
Dermatita herpetiformă
Dermatomiozita
Alopecia areata
Vitiligo
Psoriazis
Boli asociate GI
Maladia celiacă
Permeabilitate intestinală
Imunitate umorală
Boli asociate endocrine
Boli asociate dermatologice
Boli asociate neurologice şi reumatologice
Boala Addison
Tiroidita autoimună
Diabet zaharat insulinonecesitant
Sdr. Sjogren
Artrite inflamatorii
Lupus eritematos sistemic
Ataxie glutenică
Neuropatii periferice

Figura 1

autoimune ale intestinului subțire. Conexiunea dintre intestin, bolile sistemice autoimune şi factorii de mediu care le influențează constituie o zonă de cercetare în continuă expansiune. Înțelegerea aprofundată a mecanismelor imunologice subiacente va permite apariția unor terapii care să aibă drept țintă permeabilitatea intestinală, celulele T reglatoare din mucoasa intestinală şi neutralizarea citokinelor inflamatorii. Imunologia maladiei celiace oferă un mare avantaj pentru înțelegerea relațiilor dintre factorii genetici şi factorii de mediu în patologia autoimună în general.

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Riscul transmiterii virusului West Nile în România, în condițiile invaziei de țânțari din vara anului 2010

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Rezumat. Virusul West Nile (WNV) este un virus din grupul IV ((+)ss ARN), familia *Flaviviridae*, genul *Flavivirus*, specia *West Nile virus.* Este asemănător cu virusul encefalitei japoneze (complexe antigenice) şi se poate întâlni în regiuni tropicale şi temperate. Microscopia crioelectronică vizualizează virioni de 45-50 nm, sferici, acoperiți cu un înveliş proteic relativ neted. Structura microscopică este similară cu *Dengue Fever Virus,* cu care se înrudeşte.

De obicei infectează păsările, dar poate să infecteze şi mamifere, chiar oameni, calea de transmitere la oameni fiind înțepătura țânțarului infectat. Circa 90% dintre infecțiile umane cu WNV sunt asimptomatice. Virusul a fost identificat în anul 1937, la o femeie din Uganda, provincia West Nile; în prezent este răspândit pe toate continentele, cu mai multe tulpini. Infecția cu WNV la om poate să prezinte trei forme clinice: infecție asimptomatică (purtător aparent sănătos), sindrom febril (febra West Nile) şi, uneori, forma neuroinvazivă (meningita, encefalita sau meningoencefalita West Nile).

Nu se poate stabili cu precizie numărul de cazuri în întreaga lume; în SUA a pătruns în 1999 (s-a presupus un atac bioterorist mascat) şi până în 2003 a cuprins 45 de state, în Europa a fost identificat în sud: în Peninsula Balcanică, în Italia şi în Franța. În România a fost identificat prima dată în epidemie în 1996. Sursa: păsări şi mamifere infectate, vectorul: țânțari, în primul rând *Culex pipiens*, receptorul: animale neinfectate, inclusiv oameni, înțepați de țânțari infectați.

Nu există încă vaccin de uz uman, numai un vaccin sintetizat pentru uz veterinar, dar nu este disponibil pe piață. Combaterea vectorilor: dezinsecție, asanarea zonelor umede, plase la ferestre, haine cu mâneci lungi, substanțe insectifuge etc.

Cuvinte cheie: arbovirusuri, țânțari, West Nile Virus

Abstract. West Nile virus (WNV) is a virus group IV ((+)ssRNA) of the family *Flaviviridae*, genus *Flavivirus*. Part of the Japanese encephalitis antigenic complex of viruses, it is found in both tropical and temperate regions. Cryoelectron microscopy reveal a 45–50 nm virion covered with a relatively smooth protein surface. This structure is similar to the dengue fever virus; both belong to the genus *Flavivirus* within the family *Flaviviridae.*It mainly infects birds, but is known to infect humans, horses, dogs, cats and domestic rabbits.

The main route of human infection is through the bite of an infected mosquito. Approximately 90% of West Nile Virus infections in humans are without any symptoms. WNV was first isolated from a feverish woman in the West Nile District of Uganda in 1937; virus is now widespread on all continents, with more strains.There is no way for the government to accurately measure the number of worldwide cases at this time; entered the U.S. in 1999 (we assumed a bioterrorist attack masked) until 2003 included 45 countries. In Europe has been identified in the south: in the Balkan peninsula, in Italy and France. The virus is transmitted through mosquito vectors (*Culex pipiens*), which bite and infect birds and mammals. The birds are amplifying hosts, developing sufficient viral levels to transmit the infection to other biting mosquitoes which go on to infect other birds and also humans.

There is no vaccine for humans. A West Nile virus vaccine for horses was recently licensed, but its effectiveness is unknown.West Nile control is achieved through mosquito control, by elimination of mosquito breeding sites, larviciding active breeding areas and encouraging personal use of mosquito repellents. The public is also encouraged to spend less time outdoors, wear long covering clothing, apply bug repellant and ensure that mosquitoes cannot enter buildings.

Introducere

Biologie. Virusul West Nile (WNV) este un virus din grupul IV ((+)ss ARN), familia *Flaviviridae*, genul *Flavivirus*, specia *West Nile virus.* Este asemănător cu virusul encefalitei japoneze (complexe antigenice) şi se poate întâlni în regiuni tropicale şi temperate. Microscopia crioelectronică vizualizează virioni de 45-50 nm, acoperiţi cu un înveliş proteic relativ neted. Structura microscopică este similară cu *Dengue Fever Virus,* cu care se înrudeşte.

Epidemiologie. De obicei infectează păsările, dar poate să infecteze şi mamifere, chiar oameni, calea de transmitere la oameni este înţepătura ţânţarului infectat. Circa 90% dintre infecţiile umane cu WNV sunt asimptomatice.

Istoric. Virusul a fost identificat în anul 1937, la o femeie din Uganda, provincia West Nile; în prezent este răspândit pe toate continentele, cu mai multe tulpini.

Clinica. Infectarea cu WNV la om poate să prezinte trei forme clinice:

1. infecţie asimptomatică (purtător aparent sănătos);
2. sindrom febril (febra West Nile); perioada de incubaţie 2-8 zile, urmată de febră, cefalee, frisoane, sudoraţie, slăbiciune, limfadenopatie, somnolenţă, dureri articulare şi simptome asemănătoare gripei iar uneori pot să apară rash truncal de scurtă durată, simptome digestive (greaţă, vomismente, scăderea apetitului sau diaree); aceste simptome dispar după 7-10 zile, dar persistă o stare de oboseală câteva saptămâni iar limfadenopatia peste două luni;
3. boala neuroinvazivă (meningita, encefalita sau meningoencefalita West Nile) are aceleaşi simptome, dar scade nivelul de conştienţă până la precomă, reflexele tendinoase sunt hiperactive în prima fază şi apoi diminuate, apar tulburări extrapiramidale, convalescenţa este lungă şi persistă fatigabilitatea; rareori poate fi infectată şi măduva spinării, cu mielită, cu sau fără encefalită, corioretinită, hepatită, miocardită, nefrită, pancreatită, splenomegalie.

Morbiditate şi mortalitate. Nu se poate stabili cu precizie numărul de cazuri în întreaga lume. În SUA a pătruns în 1999 (s-a presupus un atac bioterorist mascat) şi până în 2003 a cuprins 45 de state, cu 9 306 cazuri confirmate şi 210 decese; până în 2005 erau raportate 16 298 cazuri confirmate; în 2009 au fost spitalizate 663 cazuri de febră West Nile, din care 335 cu encefalită sau meningită, dintre care 30 au murit, însemnând o rată de mortalitate de 4,5% din cazurile grave; bolnavii simptomatici sunt doar "vârful aisbergului", proporţia faţă de bolnavii asimptomatici fiind de 1 la 150, circa 100 000, adică o mortalitate de 3 la 10 000 de oameni infectaţi.

În Europa, virusul a fost identificat în sud: Peninsula Balcanică, Italia şi Franţa. În România a fost identificat prima dată în 1996 (A. Ungureanu/Alexse, CCSMM Bucureşti); au fost 393 cazuri spitalizate, din care 17 au murit, faţă de un total estimat de peste 10 000 de persoane bolnave, majoritatea din sud-estul ţării. În perioada 1997-2000 au mai fost confirmate 39 cazuri, din care 5 au murit. În 2009 au fost confirmate două cazuri iar în 2010 au fost doi suspecţi din Tulcea, care nu au fost confirmaţi pozitiv pentru anticorpi WNV la INCDMI Cantacuzino.

Transmitere

- sursa: păsări şi mamifere infectate (sălbatice sau domestice), cum sunt raţe, gâşte, găini, curci, ciori, porumbei, cai, câini, pisici, veveriţe, iepuri, oameni etc., unii incriminează şi reptile;
- vectorul: ţânţari, în primul rând *Culex pipiens,* unii presupun şi transmiterea prin căpuşe; riscul creşte în sezonul cald, dacă există umiditate (ploi, furtuni) şi căldură excesive, care favorizează înmulţirea ţânţarilor (aşa cum sunt condiţiile în momentul de faţă);
- receptorul: animale neinfectate, inclusiv oameni, înţepaţi de ţânţari infectaţi.

Deşi s-a considerat că nu există transmitere directă interumană, se pare că aceasta este posibilă în expunerea ocupaţională, expunerea conjunctivală cu sânge infectat, transfuzii de sânge, transplant de organe, transmitere transplacentară sau alăptare; ca măsură de siguranţă, băncile de sânge din SUA fac şi screening de rutină pentru WNV. Termenul de **virus transmis de artropode** a fost utilizat pentru prima dată în 1942, iar din 1963 termenul de **arbovirus** a fost adoptat oficial la recomandarea Subcomitetului Internaţional de Nomenclatură Virală, denumirea exprimând un aspect epidemiologic (arthropod-borne-viruses). Arbovirusurile se menţin în natură datorită transmiterii biologice între gazdele vertebrate sensibile prin intermediul artropodelor hematofage sau prin transmitere transovariană la artropode. Specificitatea vectorială netă se întâlneşte rar. Un virus poate fi transmis de mai multe specii de țânțari sau căpuşe. Capacitatea vectorială depinde de: gradul de sensibilitate al artropodului, capacitatea de transmitere a virusului, longevitatea, abundența, posibilitatea contactului cu gazdele vertebrate.

Vectorul se infectează atunci când gazda vertebrată pe care se hrăneşte este în plină viremie. Artropodele rămân infectate toată viața şi pot transmite virusul transovarian şi transfazial, fiind în acelaşi timp atât vector, cât şi rezervor de virus. Afecțiunile produse de arbovirusuri sunt infecții cu focalitate naturală transmise prin intermediul artropodelor hematofage, sunt răspândite pe toate continentele, dar distribuția lor este strict determinată de biologia artropodului vector.

Răspândirea actuală. În Grecia au fost 32 de cazuri confirmate în iulie, din care trei decese, ajungând la 80 cu opt decese în august; în Bulgaria sunt zeci de suspecți internați, dar neconfirmați încă. În România, pe data de 30 aug a. c., INCDMI Cantacuzino a confirmat două decese cu meningoencefalită virală având ca etiologie WNV şi încă şapte cazuri (în diferite județe) , care se pare că sunt doar debutul unui episod epidemic. Ca urmare, la 31 august autoritățile locale au demarat campania de dezinsecție pentru țânțari.

Diagnostic. În cazul pacienților internați în secții de neurologie sau boli infecțioase, cu **diagnostic clinic** de encefalită sau meningită, care prezintă la puncția lombară LCR clar (menigo-encefalită nebacteriană) se trimit probe la INCDMI Cantacuzino, Laboratoarele Naționale de Referință (LNR) pentru Laboratorul Infecții Transmise prin Vectori şi Entomologie Medicală (biolog Cornelia Ceianu). **Analiza de laborator** se face cu tehnica ELISA şi/sau PCR. Laboratorul de virusologie din CCSMM Secția 1 (biolog Aurora Ungureanu şi biolog Diana Popescu) a efectuat în anii anteriori aceste analize, pe care le-au raportat periodic, pe bază de convenție, la MS, dar în prezent analizează numai ser de cai şi păsări suspectate de infecție WNV, pentru monitorizarea surselor de contaminare. În caz de epidemie, laboratorul se poate implica imediat.

Tratament. Tratamentul etiologic (chimioterapice antivirale) nu există în uz curent, dar se practică tratamentul simptomatic şi de susținere a funcțiilor vitale şi tratament igieno-dietetic.

Profilaxie. Nu există (încă) vaccin de uz uman. Există un vaccin obținut prin biosinteză, pentru uz veterinar, dar nu este disponibil pe piață. Se practică monitorizarea portajului şi circulației virusului la păsări şi mamifere, domestice şi sălbatice, la care participă şi echipa noastră de cercetare (raportări la MApN/DM şi INCDMI Cantacuzino, comunicări ştiințifice şi articole în reviste de specialitate). Combaterea vectorilor: dezinsecție, asanarea zonelor umede, plase la ferestre, haine cu mâneci lungi, substanțe insectifuge (de exemplu, Autan pentru uz extern şi Vitamina B2 pentru uz intern) etc.

CCSMM se poate implica la ordin, prin Secția de Protecție medicală antiinfecțioasă şi Urgențe epidemiologice, cu forțe şi mijloace specifice:

- echipa de virusologie pentru confirmare de diagnostic la oameni şi la animale (există forțele şi mijloacele, trebuie achiziționați reactivi specifici ELISA şi PCR pentru WNV);
- echipa mobilă de intervenție biologică EMI-BIO pentru expertize în teren (există forțele şi mijloacele, trebuie alocat un mijloc de transport);
- echipa de microproducție pentru preparate specifice antițânțari (există forțele şi mijloacele pentru acțiuni punctuale, în folosul unor unități militare, împreună cu CMP al DM).

Influența schimbărilor climatice asupra epidemiilor. În epidemiologie ne atrag atenția mai ales zoonozele, zooantroponozele şi antropozoonozele, cunoaşterea **vectorilor** şi a rezervoarelor care determină dinamica bolii. Modificările factorilor climatici pot influența distribuția bolilor, în corelație cu latitudinea, longitudinea, multe maladii având o distribuție geografică supusă liniei izotermelor şi izochetelor. Prezicerea modificărilor şi descrierea zonelor de risc țin cont tot de valorile izochetelor şi izotermelor. Prezicerea utilizează curent modelul biologic, prin care se încearcă modificarea diferitelor variabile implicate în transmitere, ca şi modelul statistic, ce corelează distribuția bolilor cu factorii de mediu şi cu cei climatici. Modelul statistic este mai performant în practică, mecanismele ecologice necesitând investigații la scară mare, care să țină seama de fiziologie şi etiologie, în cazul fiecărui element al ciclului vieții, de circulația agenților infecțioşi în complexul patogen (sinecologia structurală şi dinamică). Modificările climatice sunt frecvent incriminate în extensia unor boli sau a vectorilor lor; invazia este de regulă corelată cu mişcarea bunurilor, animalelor, persoanelor, care străbat distanţe enorme prin traficul aerian. Un factor major al climatului, vizibil, mai ales, în zonele temperate, este reprezentat de **sezonalitate**. Previziunile actuale atrag atenţia asupra încălzirii (în ipoteza că încălzirea globală ar fi un fenomen real), a creşterii temperaturilor nocturne, a reducerii zilelor cu îngheţ, precocitatea toamnelor şi deci asupra dinamicii populaţiei şi a numărului de generaţii ale vectorilor.

Cercetările pe plan mondial au drept scop elucidarea relaţiilor dintre arbovirusuri şi artropodele vectoare. Astfel, s-a stabilit că dintre cele aproximativ 534 arbovirusuri izolate 220 provin din 233 specii de culicide (ţânţari), cuprinse în 20 de genuri. Restul de până la 534 sunt transmise de ixodide (căpuşe), flebotomide (flebotomi), ceratopogonide şi simuliide (tăuni). Speciile de culicide vectoare pentru arbovirusuri în România sunt încadrate în 6 genuri şi sunt larg răspândite pe tot teritoriul ţării noastre. Arbovirusurile transmise de culicidele vectoare sunt: West Nile, Tahyna şi Batai. Circulaţia acestor virusuri a fost semnalată în România prin izolarea virusului din culicide capturate în natură (Drăgănescu 1979, Arcan et al. 1974, Ungureanu et al. 1988, Nicolescu et al. 1991) şi prin depistarea anticorpilor la om şi animale (mamifere, păsări sălbatice şi domestice), stabilindu-se o schemă generală de distribuţie a acestora pe teritoriul ţării noastre.

Dacă virusul Batai nu este prezent la om, Virusul West Nile are o distribuţie destul de uniformă pe teritoriul ţării, cu o seropozitivitate de 4,4-7,1%, dar ajungând la 10,2% în populaţia umană; în medie seropozitivitatea este de 5,5%, dar atinge şi 17,3% la animalele domestice. În perioada iulie-octombrie 1996, în partea de sud-est a României s-a înregistrat cea mai mare epidemie de meningoencefalită cu virusul West Nile de până în prezent (Tsai et al. 1998, Nicolescu 1998). Aspectele relevate de epidemia de meningoencefalită provocată de virusul West Nile în România arată că **febra West Nile** (gripa de vară) este o boală care îşi face apariţia şi în Europa, unde se poate manifesta sub forme grave. De aceea, în prezent este necesară extinderea şi aprofundarea cunoaşterii populaţiilor de culicide potenţial vectoare pentru arbovirusuri, inclusiv pentru WNV, ca şi a sistemelor vectoriale existente la noi în ţară şi a implicării lor în patologia umană, în vederea prevenirii şi combaterii unor îmbolnăviri grave la om.

Din cele peste 500 arbovirusuri cuprinse în Catalogul Internaţional al Arbovirusurilor, peste 90 pot provoca infecţii clinice şi subclinice la om, iar dintre acestea aproximativ 31 pot fi asociate cu sindroame clinice ca: boli febrile generalizate, febre hemoragice, meningite, encefalite, meningoencefalite. Din rapoartele OMS reiese că în ultimii 15 ani s-a constatat o tendinţă de extindere a infecţiilor produse de arbovirusuri. S-au semnalat de asemenea cazuri de infecţie cu virusuri de import ca: febra West Nile, febra hemoragică Crimea-Congo, Denga hemoragică, febra Văii de Rift, febra Lassa, boala Marburg. Unele dintre aceste virusuri sunt importante pentru sănătatea publică la scară mondială sau pe mari zone geografice prin morbiditatea şi mortalitatea induse, cât şi prin măsurile care se impun pentru prevenirea epidemiilor, iar toate aceste virusuri sunt considerate şi potenţiali agenţi biologici.

Distribuţia geografică a infecţiilor arbovirale, atât ca zoonoze cât şi ca infecţii umane, este determinată strict de biologia artropodelor vectoare. Practic sunt răspândite pe toate continentele cu particularităţi legate de condiţiile geoclimatice, vectori şi gazdele vertebrate. Factorii care favorizează propagarea interregională a acestor virusuri sunt: modificările ecologice care influenţează creşterea populaţiilor de artropode şi alte gazde; pătrunderea omului în habitatele artropodelor şi rozătoarelor, despăduririle, irigaţiile, urbanizarea extensivă necontrolată, mişcările de populaţie, intensificarea mijloacelor de transport, comerţul cu animale şi păsări.

S-a semnalat extinderea geografică a unor arboviroze: encefalita japoneză s-a extins din Japonia în sud-estul Siberiei, până în India şi Malaezia; virusul *encefalitei ecvine de Est* a fost izolat şi în Filipine, Tailanda, Europa; virusul *Chikungunya* , izolat iniţial în Tanganyka, are astăzi un rol important în etiologia unei febre hemoragice din Asia de sud-est; virusul *febrei Văii de Rift* s-a extins în Egipt până la bazinul Mării Mediterane; febra galbenă s-a semnalat în 2001 în Peru, regiunea Amazonului şi în Siberia; forme de febră hemoragică Crimea-Congo s-au raportat în Albania şi Kosovo (zona musulmană, în care populaţia refuză vaccinarea). Interesant pentru febra galbenă este faptul că deşi în Asia de sud-est există vectorul *Aedes aegypti* şi populaţie receptivă, această boală nu este întâlnită. Se presupune că prezența altor virusuri din grupul B ar putea produce o excluziune. Interesantă este şi absența parțială sau totală a encefalitelor în anumite regiuni ale globului, absență care ține doar în parte de distribuția vectorilor specifici, dar cel puțin pentru Orientul Apropiat se consideră că este vorba despre o rezistență încrucişată a virusurilor înrudite antigenic (JE), ca urmare a infecției inaparente produse de virusul *West Nile.*

Arbovirusuri care produc meningite, encefalite, meningoencefalite. După inocularea virusului prin înțepătura vectorului, acesta se multiplică la nivelul țesutului subcutan, de unde, prin vasele limfatice, ajunge şi se fixează în ganglionii limfatici regionali. În această fază plasma conține un număr mare de virioni. Simptomatologia caracteristică în faza viremică este asemănătoare tuturor infecțiilor arbovirale: cefalee, febră, mialgii, artralgii. Probabilitatea invaziei sistemului nervos central (SNC) depinde de nivelul viremiei şi se manifestă prin fotofobie, cefalee puternică, redoarea coloanei vertebrale, fenomene digestive. În SNC leziunile constau în invazia de mononucleare şi formarea de manşoane perivasculare, inflamație meningeală, degenerare neuronală, necroză. Letalitatea este cuprinsă între 0,5-60%, în funcție de agentul patogen cauzal. Meningoencefalitele arbovirale pot fi transmise atât prin intermediul țânțarilor, cât şi prin căpuşe. De cele mai multe ori, aceste virusuri provoacă îmbolnăviri benigne, febrile sau cel mult un sindrom meningeal cu lichid cefalorahidian (LCR) clar.

Se cunosc şi arbovirusuri care în mod obişnuit determină îmbolnăviri de tipul febrei generalizate sau al celor hemoragice, dar care pot produce ocazional encefalite în funcție de condițiile particulare (cantitatea de virus, receptivitatea şi rezistența organismului uman, vârsta). În această categorie de virusuri se încadrează virusul West Nile, Denga, Febra Galbenă, Bhanja, Febra de căpuşe de Colorado etc. În zonele de mare endemicitate a unor encefalite, aproape toată populația poate fi infectată, dar majoritatea infecțiilor sunt inaparente (se citează în cazul encefalitei de St. Louis că, pentru un caz clinic se înregistrează 500-1 000 infecții subclinice). Sezonalitatea encefalitelor ca şi repartiția geografică reflectă în mare măsură sistemul ecologic care asigură ciclul evolutiv al infecției şi în mod deosebit depinde de biologia artropodului vector.

Sursa de infecție sau rezervorul natural de virus îl constituie gazdele vertebrate, nevertebrate şi artropodele hematofage. Rezervorul vertebrat de virus: mamifere mici, rozătoare mici, reptile, lilieci, amfibieni şi păsări. Persistența virusului în focarul natural este asigurată de ciclul gazdă vertebrată → artropod → gazdă vertebrată sau om. Cârtițele, liliecii, reptilele, rozătoarele mici asigură persistența virusurilor peste iarnă, fiind sursa de infecție în anul următor. Păsările au un rol deosebit în special în epidemiologia arbovirusurilor, pentru că ele reprezintă atât rezervorul de virus (au o fază viremică lungă de 100 zile), cât şi mijlocul de răspândire a acestora. Artropodele rămân infectate toată viața şi transmit virusul transovarian la generațiile următoare, fiind în acelaşi timp rezervoare şi vectori de arbovirusuri. Este cunoscută categoria de **gazde de amplificare,** care sunt gazde vertebrate ce favorizează creşterea numărului de îmbolnăviri la om şi care de regulă preced apariția unei epidemii. Păsările şi porcii sunt gazde de amplificare pentru virusul encefalitei japoneze, caii şi păsările pentru virusul *West Nile,* maimuțele pentru virusul febrei galbene, ovinele şi bovinele pentru virusul Crimea-Congo şi virusul febrei Văii de Rift etc.

Căile de transmitere în infecțiile arbovirale

Transmiterea biologică. Vectorul se infectează când gazda vertebrată pe care se hrăneşte se află în plină viremie. Virusul se multiplică inițial în tubul intestinal al artropodului, după care traversează bariera intestinală şi pătrunde în glandele salivare, unde se multiplică masiv. După 10-12 zile, vectorul infectat poate transmite virusul la alte gazde. Acest ciclu este esențial pentru transmiterea arbovirusului de către vector. Ciclul de viață al unui artropod este dependent de temperatură. Activitatea maximă a țânțarilor se desfăşoară la 25-35^{0}C, la 6^{0}C femelele mor în 24 h, iar la 15-16^{0}C nu mai înțeapă pentru a se hrăni. Dacă s-au infectat sugând sânge de la bolnavi, multiplicarea virusului în cadrul perioadei de incubație extrinsecă încetează dacă țânțarii sunt menținuți la 18^{0}C.

Arealul infecțiilor cu arbovirusuri este influențat de anumite obiceiuri ale vectorilor, şi anume: intervenția țânțarilor în transmiterea febrei galbene este legată de nivelul la care trăiesc şi activează aceştia. Pentru ca un vector să fie eficace trebuie să se infecteze uşor şi să transmită virusul, contactele cu gazda vertebrată să-i asigure infectarea, iar acesta să transmită virusul la noi gazde sensibile şi să aibă toate condițiile climatice favorabile de multiplicare. Specificitatea vectorială se întâlneşte foarte rar; în mod obişnuit țânțarii nu se infectează cu virusuri transmise de căpuşe şi viceversa, dar sunt posibile excepții, care au fost confirmate experimental.

Transmiterea mecanică. S-a demonstrat în laborator posibilitatea transmiterii mecanice a unor virusuri (virusul encefalitei ecvine poate fi transmis de un țânțar din genul *Aedes* în primele 7 ore de la prânzul infectant cu acelaşi randament ca în cazul folosirii unui ac contaminat), modalitate care ar putea fi realizată şi în natură, explicând arbovirozele explozive în cursul cărora mii de gazde vertebrate sunt infectate în câteva săptămâni.

Transmiterea digestivă. Infecția cu virusul encefalitei de căpuşă poate fi transmisă prin consumul de lapte crud de oaie, capră, vacă sau preparate proaspete de brânză. S-a demonstrat că virusul poate persista în produsele lactate aproximativ 60 de zile.

Transmiterea aerogenă. S-au semnalat infecții prin inhalare de praf contaminat cu dejecte de rozătoare sau căpuşe, ca şi la personalul care manipulează tulpini de arbovirusuri în cursul anumitor procedee de laborator. Transmiterea aerogenă ar putea face posibilă utilizarea unor arbovirusuri ca armă biologică deosebit de periculoasă.

Receptivitatea omului. La infecțiile cu arbovirusuri receptivitatea omului este generală, dar gravitatea bolii este determinată de agentul patogen cauzal pe de o parte şi de particularitățile ce țin de individ pe de altă parte. Cu excepția febrei galbene urbane, denga şi infecția cu virusul Chikungunya, infecția umană constituie un accident biologic care închide de regulă ciclul celor mai multe arboviroze. Arbovirozele sunt afecțiuni cu focalitate naturală, omul infectându-se atunci când pătrunde în focarele naturale, în zone epidemice sau endemice pentru anumite arbovirusuri. Receptivitatea este generală, dar particularitățile epidemiologice se răsfrâng asupra grupelor de risc din care fac parte pădurarii, muncitorii forestieri, vânătorii, agricultorii, turiştii, militarii aflați în campanie sau în diverse activități specifice armatei în timp de pace sau campanie. Există posibilitatea migrării infecției pe zone largi prin circulația grupurilor de maimuțe şi deplasarea țânțarilor duşi de vânt, astfel răspândindu-se infecția cu febră galbenă în America Centrală şi de Sud. S-a demonstrat şi în Africa migrarea infecției la maimuțe prin țânțarii transportați de vânt (aşa se explică apariția infecției pe o insulă din Lacul Victoria situată la 70 de km de țărm).

În regiunile de endemicitate se instalează o rezistență naturală a populației pentru virusurile care circulă în zonă, pericolul apare pentru cei care pătrund în aceste zone cu diferite ocazii. Imunitatea în infecțiile arbovirale este de lungă durată. Imunitatea dobândită prin boală pare să fie protectoare şi de lungă durată, însă nu există studii care să confirme această ipoteză.

Emergența bolilor transmise prin vectori. Pe plan global se desfăşoară un intens proces de emergență şi reemergență a unor boli infecțioase. De importanță maximă între acestea sunt bolile transmise prin vectori, deoarece ele se instalează pe teritorii foarte întinse, în urma unor procese îndelungate de adaptare prin care agentul patogen devine parte integrantă a unor cicluri de transmitere complexe în care sunt implicate gazde vertebrate şi nevertebrate din ecosistemele locale, iar odată instalate în teritoriile respective ele sunt extrem de greu de eliminat. Recrudescența bolilor transmise prin vectori este în principal determinată de schimbările climatice şi de mediu actuale, ca rezultat al unor cauze antropogene.

În România se înregistrează reemergența unor arboviroze şi o creştere a riscului de reintroducere a malariei, boli cu implicații deosebite pentru sănătatea publică. După epidemia din anul 1996, peste 800 de cazuri de infecții neurologice grave cu WNV şi peste 5% decese, şi peste 100 000 infecții de intensitate medie (gripa de vară) şi inaparente, pe un teritoriu depăşind 80 000 km în sudul țării, infecțiile cu virusul *West Nile* au continuat să se înregistreze până în prezent în fiecare an. În perioada 1997-2001, s-au confirmat 62 de infecții neurologice cu WNV, cu 7% decese şi adesea cu sechele la foştii bolnavi. În ultimii doi ani numărul lor a crescut: în 2001 s-au înregistrat 22 de cazuri grave. Numărul infecțiilor de intensitate medie sau inaparente a rămas necunoscut, dar se poate presupune că a fost de câteva mii sau mai mult. Circulația permanentă în ultimii ani a virusului *West Nile* în România a avut loc în contextul intensificării răspândirii acestui virus pe plan global, concretizată în epidemii şi epizootii, unele persistând mai mulți ani, în Italia, Franța, Rusia, Israel, Tunisia, Maroc, care s-au extins începând cu 1999 şi în SUA. În acest an virusul a fost pus în evidență în Canada şi în insulele Cayman. Pe lângă infecțiile cu virusul *West Nile*, în România au fost înregistrate în ultimii ani şi infecții cu virusul *Tahyna*, mai ales în zone situate de-a lungul Dunării. De asemenea, în apropiere de Mediaş persistă un focar de encefalită de căpuşă, iar pe zone întinse din sud-estul țării apar numeroase cazuri de febră butonoasă.

Concomitent, circulația altor arbovirusuri se intensifică pe plan global, exemplul cel mai apropiat geografic fiind apariția bolii *blue tongue*, produsă de un orbivirus transmis de Culicoizi, în Macedonia şi Kosovo, şi focare de febră hemoragică Crimea-Congo în Albania şi Kosovo. Circulația arbovirusurilor transmise de țânțari (fam. *Culicidae*) şi căpuşe (fam. *Ixodidae*) este favorizată de încălzirea globală actuală şi de modificările din mediu. Aceste fenomene determină atât extinderea arealelor de distribuție a artropodelor vectoare şi apariția unor populații ale acestora cu efective foarte ridicate, cât şi modificări în ecologia şi biologia unor specii de gazde vertebrate (păsări care transportă virusurile respective la mari distanțe, mamifere, populații umane tot mai numeroase care trăiesc în condiții favorizante pentru amplificarea şi transmiterea acestor virusuri, inclusiv în mediul urban).

Reemergența puternică a unor arboviroze şi creşterea riscului de reemergență a bolilor transmise de țânțari în Europa sunt favorizate de încălzirea globală actuală şi de modificările din mediu, care determină atât extinderea arealelor de distribuție a artropodelor vectoare şi apariția unor populații ale acestora cu efective foarte ridicate şi asigură posibilitățile de completare a ciclului biologic, de amplificare sau de transmitere a agenților patogeni în condițiile de mediu respective, cât şi modificări în ecologia şi biologia unor specii de gazde vertebrate: păsări care diseminează virusuri la mari distanțe; mamifere şi populații umane tot mai numeroase care trăiesc în condiții favorizante pentru menținerea agenților patogeni, inclusiv în mediul urban, existența unor populații umane lipsite de imunitate față de agenți patogeni importați etc. Un alt factor ce contribuie la reemergența acestor boli este circulația actuală intensă a unor grupuri mari de oameni din motive economice, turistice, militare, refugiați etc. care favorizează transportul, introducerea şi transmiterea unor agenți patogeni în noi teritorii, anterior indemne.

În România, pe lângă aceste elemente globale se adaugă factori locali, împreună ducând în prezent la reemergența şi/sau la creşterea riscului de reemergență a unor boli. Elementul local cel mai important este reprezentat de fauna de insecte **şi/sau alte artropode vectoare, reprezentate prin populații ale căror caracteristici ecologice (efective numerice, distribuție spațială, fenologie etc.) variază în timp şi spațiu în funcție de modificările factorilor climatici şi de mediu locali. În ultimii ani variația acestor factori, determinată de cauze naturale şi/sau economico-sociale, a avut caracter favorizant pentru menținerea şi dezvoltarea populațiilor de vectori, determinând o evoluție permanent îngrijorătoare a acestei faune. Efectivele populațiilor de vectori au crescut din cauza proliferării habitatelor de dezvoltare a larvelor atât în ecosistemele natural, cât şi în cele antropizate (prin fragmentarea ecosistemelor agrare în urma revenirii la agricultura de tip tradițional pe loturi mici de pământ; pe rețeaua de canale de irigație nefuncționale; în urma degradării unor amenajări hidrotehnice sau a celor de pe lacurile de acumulare şi agrement, în gospodăriile individuale rurale unde se cresc vite, se face grădinărit, în mediul urban unde există o mare varietate de habitate larvare). În plus, acțiunile de dezinsecție sunt adesea sporadice şi ineficiente.**

Favorizarea dezvoltării speciilor de țânțari vectori de arbovirusuri şi a **anofelismului urban** din cauza existenței a numeroase şi variate tipuri de habitate de dezvoltare a larvelor: subsoluri inundate, gospodării de tip rural în interiorul oraşelor, inclusiv în Bucureşti, unde uneori se cresc păsări, porci şi chiar vite şi unde apar numeroase focare larvare în habitate legate de rețelele de termoficare şi cele de apă şi canalizare, de degradarea amenajărilor în zonele de agrement, existența unor zone de tip "maidan" cu depozite de gunoaie etc. Populațiile de vectori cu efective ridicate s-au extins în zone degradate de inundații, alunecări de teren, defrişări etc.

Condițiile climatice şi de mediu actuale favorizează şi dezvoltarea populațiilor de căpuşe vectoare nu numai în ecosistemele naturale, ci şi în cele antropice; în ultimii ani se înregistrează extinderea arealelor de distribuţie a ixodidelor în ecosistemele antropice. Condiţiile actuale de mediu favorizează în mare măsură dezvoltarea şi menţinerea unor populaţii abundente de culicide vectoare şi adesea de căpuşe în imediata vecinătate a omului, atât în mediul rural, cât şi în cel urban.

Toate aspectele actuale privind bolile transmise prin vectori, prezentate şi concretizate prin intensificarea circulaţiei globale a unor arbovirusuri şi creşterea riscului de reapariţie a malariei mai ales în Europa, capătă noi dimensiuni în contextul internaţional actual. Prezenţa unor populaţii abundente de insecte şi a altor artropode vectoare reprezintă unul dintre factorii de risc importanţi pentru sănătatea publică prin diseminarea şi transmiterea unor agenţi patogeni care ar putea avea rolul de armă biologică.

Profilaxie. *Măsurile de protecţie individuală* vizează în primul rând evitarea înţepăturilor de ţânţari sau de căpuşe. În cazul apariţiei primelor semne de boală, individul trebuie să se prezinte imediat la serviciul de specialitate, unde medicul va lua măsurile ce se impun în această situaţie. Măsurile de protecţie împotriva agenţilor vectori presupun, în primul rând, măsuri individuale de protecţie. Protecţia faţă de ţânţari: plasă de sârmă, naylon sau tifon la ferestre, uşi, guri de aerisire, intrarea în corturi; pologuri de tifon deasupra paturilor, eventual impregnat cu insecticide persistente de tipul piretroizilor de sinteză; protecţia feţei şi a gâtului cu plasă şi a mâinilor cu mănuşi. *Măsurile de protecţie generală* ar fi ideal dacă s-ar aplica înainte de a se pătrunde în zonele respective şi în funcţie de caracteristicile epidemiologice ale acestora.

Măsurile generale prevăd şi utilizarea produselor insecticide conform instrucţiunilor

WHO/CTD/WHO PES/97,2 şi care presupun:

❖ insecticide pentru tratamente reziduale în spaţii închise

Insecticidul	**Structura chimică**	**Cantitate (g s.a.*/m^2)**	**Remanenţă (luni)**	**Mod de acţiune**
Malathion	OP	2	2 – 3	Contact
Deltamethrin	PY	0,02 – 0,05	2 – 3	Contact
Lambda–cyhalothrin	PY	0,02 – 0,03	3 – 6	Contact

s.a. = substanţă activă, OP = compus organofosforic, PY = piretroid de sinteză

❖ insecticide pentru tratamente în exterior

Insecticid	**Structura chimică**	**Doza de s.a. g/ha**	
		Aerosoli reci	**Ceţuri**
Doclorvos	OP	150	200 – 300
Malathion	OP	112 – 693	500 – 600
Pirimiphos–metyl	OP	230 – 330	180 – 200
Deltamethin	PY	0,5 – 1,0	–
Permetrin	PY	5	10

Spaţiile exterioare sunt dezinfectate şi dezinsectizate cu aerosoli sau ceaţă caldă, folosind, în funcţie de întinderea ariei de tratat, aparate portabile, echipament montat pe maşini, elicoptere.

❖ insecticide utilizate în combaterea larvelor de ţânţari

Insecticid	**Structura chimică**	**Doza s.a. 1g/ha**	**Formulare**	**Durata efectului (săptămâni)**
Malathion	OP	220 – 1000	CE,GR	1 – 2 (2100)
Piriproxyfen	Regulator de creştere	5 – 10	CE,GR	4 – 12 >5000
Temephos	OP	56 – 112	CE,GR	2 – 4
Diflubenzuran	GR regulator de creştere	25 – 100	GR	2 – 6 >4640
Bacilul thuringensis	Insecticid bacterian	în funcţie de formulare	Suspensii, granule	1 – 2 >30.000 1 – 2 >5000

Pentru distrugerea cuiburilor larvare, în special a celor din apele stătătoare, se pot folosi tehnici de aspersare cu produsele insecticide menționate.

BOLI INFECȚIOASE TRANSMISE DE ȚÂNȚARI

Febra West Nile. **Virusul West Nile face parte din familia *Flaviviridae*, genul *Flavivirus*, serogrupul virusului encefalitei japoneze (JE). Este transmis în natură într-un ciclu enzootic pasăre (rezervor) la țânțari (vector). Puii din cuiburi şi păsările tinere dezvoltă viremii cu titru ridicat şi de lungă durată şi constituie principalul rezervor de virus. Competența vectorială a țânțarilor este determinată de caracteristicile de specie: capacitatea de a se infecta şi a deveni infectant şi preferințele de hrănire care influențează nivelul contactului cu gazda vertebrată viremică. Speciile ornitofile sau segmente de populație cu preferințe ornitofile sunt implicate ca vectori atât în ciclurile enzootice şi epizootice, cât şi în transmiterea virusului la om. S-a observat la anumite specii de țânțari considerate ornitofile că odată cu creşterea densității populațiilor preferința de hrănire nu mai este aşa de strictă şi femelele se hrănesc, cu rată ridicată, şi pe mamifere, inclusiv pe om.**

Creşterea densității populațiilor de țânțari şi reducerea perioadei de incubație extrinsecă odată cu creşterea temperaturii ambiente determină desfăşurarea cu rapiditate a ciclurilor de transmitere a virusului şi amplificarea cantității de virus circulant în natură (transmitere epizootică) care inițiază ciclul de transmitere epidemică. Omul şi mamiferele în general, devenind gazde accidentale pentru WNV, nu se constituie în verigi ale ciclului de transmitere, deoarece dezvoltă o viremie de scurtă durată şi titru scăzut, sub nivelul pragului de infectare pentru țânțari. Vectorul reprezintă veriga slabă a ciclului de transmisie, verigă ce poate fi atacată prin acțiuni țintite de control al populațiilor de țânțari, cu efect direct de întrerupere a transmiterii virusului.

Păsările reprezintă rezervorul de virus din cadrul ciclului de transmitere a virusului WN. Datele de filogenie moleculară obținute pentru tulpinile de virus WN izolate în Europa demonstrează originea africană a virusului. Se presupune că virusul este introdus în Europa de păsări migratoare. Mecanismele acestui proces nu sunt încă elucidate: fie păsările care au o viremie îndelungată se constituie ca rezervor de virus în zonele în care staționează vara, fie poartă virusul în stare latentă şi acesta este reactivat în urma scăderii rezistenței păsărilor după efortul şi consumul de energie uriaş făcut în timpul migrației. De asemenea, păsările pot transporta pe corpul lor acarieni, căpuşe care odată infectați cu virus WN devin purtători tot restul vieții, îl pot transmite transovarian la descendenți şi pot infecta alte păsări în special în cuiburi.

Studiul serologic la păsări oferă informații valoroase cu privire la nivelul de activitate al virusului în natură, informații ce au implicații practice imediate în prevenția transmiterii la om. Astfel, în cazul unui arbovirus înrudit care circulă de asemenea într-un ciclul păsări țânțari (virusul encefalitei de St. Louis) experiența a demonstrat că există un interval de 2-3 săptămâni între înregistrarea seroconversiei la păsările sentinelă şi momentul apariției cazurilor de îmbolnăvire la om. Acest timp poate fi folosit cu eficiență pentru operațiunile de combatere a vectorilor, având ca rezultat final întreruperea transmiteri virusului. Țânțarii reprezintă **veriga slabă** a ciclului de transmitere, singura care poate fi controlată de către om.

Virusul WN a fost izolat prima dată din sângele unei femei febrile din districtul West Nile, Uganda în anul 1937, apoi a fost izolat de la oameni bolnavi, păsări şi țânțari începând cu anul 1956. Virusul a fost încadrat în serogrupul encefalitei japoneze, iar distribuția lui geografică include, pe lângă Africa, Europa, Rusia europeană şi asiatică, Asia de Sud, Orientul Apropiat şi din 1999 şi pe continentul american în SUA şi Canada. Epidemii produse de virusul WN: Israel 1950-1954, 1957, 2000; Africa de Sud 1974,1983,1984; Franța 1962-1964,1966; România 1996.

Izbucnirile epidemice provocate de virusul WN în ultimi cinci ani subliniază o creştere a patogenității acestui virus ca şi o extindere a ariei de răspândire. După 1996 s-au semnalat epidemii sau epizootii în: Tunisia 1997; Cehia 1997; Italia 1998; Israel 1998; Rusia 1999; SUA 1999; Franța 2000; Israel 2000; Canada (păsări) 2001; SUA 2001. Specialiştii sunt de acord că păsările sălbatice sunt instrumentul de introducere a virusului în arealele temperate din Europa în timpului zborului migrator. Virusul este inoculat la populație prin femelele de țânțari care se hrănesc cu sângele păsărilor infectate; specialişti din Israel au afirmat că izbucnirea epidemică cu WNV din anul 2000 a oglindit aproape exact ruta păsărilor migratoare. Deşi focarele naturale pentru infecţia cu virusul WN sunt în principal situate în ecosisteme umede (delta râurilor, zone inundate) şi sunt caracterizate prin ciclul păsări-ţânţari, căpuşele pot servi la substituirea vectorilor şi forma ciclul păsări-căpuşe în habitate încălzite. În anumite circumstanţe uneori poate funcţiona şi ciclul broaşte-ţânţari.

În Europa circulaţia virusului WN este confirmată în două tipuri de bază ale ciclurilor şi ecosistemelor: ciclul rural (silvatic) – păsări sălbatice obişnuite de apă şi ţânţari ornitofili; ciclul urban – păsări sinantrope sau domestice şi ţânţarii, în principal *Culex pipiens* sau *Culex modestus*, care se hrănesc pe păsări sau oameni. Principalul ciclu este cel rural, dar în Bucureşti în 1996, în cursul epidemiei cu virus WN a predominat ciclul urban de transmitere. Persistenţa virusului WN în focarele endemice de boală din zona temperată a Europei s-ar putea face prin două mecanisme: virusul poate persista în femele de Culex care hibernează, larvele infectate transovarian sau infecţiile cronice în gazdele vertebrate (păsări sau broaşte); virusul mai poate fi reintrodus continuu prin păsările migratoare infectate cronic de la focarele naturale tropicale sau subtropicale. În consecinţă, creşterea activităţii virusului WN în Africa poate fi urmată, cel puţin în următorii ani, de epidemii reale de febră WN în Europa.

Infecţia produsă de virusul WN la om este de regulă o boală febrilă, asemănătoare cu gripa, având o perioadă de incubaţie de 3-6 zile, cu debut brusc, febră moderată 3-5 zile, uneori bifazică, cefalee frontală, mialgii, artralgii,conjunctivită, dureri retroorbitale, uneori erupţie (maculopapulară sau rozeolară), limfadenopatie, anorexie, dureri abdominale, diaree şi simptome respiratorii. Sub 15% din cazuri pot evolua cu meningite sau encefalite acute cu lichid clar (asociate cu rigiditate cervicală, vomă, confuzii, somnolenţă, tremurul extremităţilor, reflexe anormale, convulsii, pareze şi comă), hepato-splenomegalie, hepatită, pancreatită şi miocardite. Vindecarea este completă, mai rapidă la copii şi persoanele tinere – adulte, uneori acompaniată timp îndelungat de mialgii şi slăbiciune. Nu s-au semnalat sechele permanente, iar cazurile mortale s-au întâlnit la persoane de peste 50 de ani.

Circulaţia virusului şi implicarea lui în neuroinfecţiile umane au fost semnalate în România încă din anii ‘60. S-au identificat anticorpi în populaţia umană, la păsări şi la animale domestice localizate în vestul, estul şi sudul ţării. În urma **studiului seroepidemiologic** efectuat în colectivul de virusologie din Centrul de Cercetări Ştiinţifice Medico-Militare faţă de unele arbovirusuri s-a stabilit că circulaţia actuală a virusului West Nile în România este semnalată practic în toate zonele ţării ca şi în Bucureşti, producând infecţii la om dar şi la animale domestice.

În România sunt condiţii propice de climă, temperatură, umiditate şi o populaţie bogată de culicide care determină biotopi favorabili constituirii de focare naturale pentru virusul WN. Delta Dunării, lacurile din nordul Dobrogei, care constituie zone de cuibărit sau de tranzit pentru numeroase păsări migratoare şi ubicvitare, ca şi traseele de migraţie ce străbat ţara venind din Africa, creează condiţii de introducere în fiecare an a virusului WN. Pe de altă parte, multitudinea de gazde şi vectori care menţin şi transmit virusul descendenţilor constituie mecanismul de menţinere a virusului WN în focarele naturale.

În aceste condiţii a apărut epidemia de meningoencefalită din Bucureşti şi judeţele din sudul ţării în vara-toamna anului 1996. În perioada iulie-octombrie au fost spitalizaţi 835 de bolnavi cu neuroinfecţie acută virală. Diagnosticul etiologic a fost stabilit în Laboratorul de Arbovirusuri din Centrul de Cercetări Ştiinţifice Medico-Militare, prin examinarea serurilor de la bolnavi faţă de antigenul virusului WN prin testul clasic al hemaglutinoinhibării (HAI). Rezultatele serologice au evidenţiat anticorpi specifici cu creştere în dinamică faţă de virusul WN, stabilind că infecţia a fost transmisă prin înţepătura de ţânţari care conţineau virusul.

Diagnosticul de infecţie arbovirală acută produsă de virusul WN a fost apoi confirmat şi de Laboratorul de Febre Hemoragice şi Arbovirusuri Pasteur (Paris, Franţa). Ulterior, după stabilirea virusului implicat în epidemie, echipa de la CDC (SUA), venită în România să studieze epidemia, a testat prin ELISA IgM WN probe de ser de la 509 pacienţi; la 393 s-a confirmat o infecţie acută cu virusul WN. S-au înregistrat 42 de morţi, dintre care 17 au fost diagnosticaţi cu infecţie WN.

Studiile efectuate în acea perioadă în Bucureşti prin testarea anticorpilor anti virus WN în sânge au stabilit că în perioada epidemiei cel puțin 100 000 de persoane au fost infectate cu virus, dar au dezvoltat o formă uşoară de boală. În aceeaşi perioadă s-a capturat un număr mare de țânțari (specialişti din Institutul Cantacuzino şi CDC-Fort Collins, SUA). S-au recoltat seruri de la păsări domestice şi sălbatice, inclusiv vrăbii, stârci etc. care au fost testate. Cercetătorii americani au izolat o tulpină de virus WN dintr-un lot de 50 de țânțari capturați din scările a trei blocuri din sectorul 3 al Capitalei la laboratoarele CDC în 1997 – tulpină RO 97-50 şi care este, pe baza analizei moleculare, asemănătoare cu tulpina Senegal 1993. Izolările de tulpini din anii 1994 şi 1996 din nordul Africii sugerează creşterea transmisiei enzootice a virusului WN.

Programul de supraveghere pentru infecția cu virus WN inițiat de Ministerul Sănătății pentru Bucureşti şi 15 județe din sud-estul României, începând cu 1997, a permis monitorizarea anuală a infecțiilor acute virale ale SNC şi examinarea probelor de ser şi LCR pentru virusul WN. Astfel, în perioada 1997-2001 s-au înregistrat 1 906 cazuri clinice spitalizate cu infecții virale acute ale SNC din Bucureşti şi 15 județe din sud-estul României. S-au identificat 61 de pacienți cu boală acută produsă de virusul WN (meningite 67,7%, meningoencefalite 29%, encefalite 3,4% şi un procentaj de mortalitate de 10,3%). Distribuția pe ani a cazurilor pozitive WN a fost următoarea: 15 cazuri în 1997; 5 cazuri în 1998; 7 cazuri în 1999, cu 2 decese; 13 cazuri în 2000, cu 2 decese; 22 cazuri în 2001. Debutul îmbolnăvirilor s-a situat între lunile aprilie şi octombrie, factorii determinanți fiind creşterea temperaturii, umidității şi densitatea populației de vectori. Rezultatele investigațiilor demonstrează că arbovirusul WN este activ pe teritoriul României şi este implicat în etiologia unor meningite şi meningoencefalite acute virale.

În condiții favorabile desfăşurării ciclurilor de amplificare virală în natură, virusul WN îşi menține un potențial de declanşare a focarelor epidemice. În consecință este necesară: continuarea supravegherii cazurilor de îmbolnăviri acute virale ale SNC nu numai în zona de sud, ci şi în alte zone, în special în estul şi vestul țării; supravegherea şi controlul populației de țânțari vectori de arbovirusuri; informarea populației din zonele de risc în care s-au identificat cazuri pozitive cu privire la acțiunile de prevenire a îmbolnăvirilor prin măsuri personale de limitare a contactului cu țânțari sau căpuşe în locuințe sau în mediul înconjurător; forurile de decizie trebuie să cunoască în permanență distribuția focarelor de îmbolnăviri cu arbovirusuri pentru a recomanda măsurile de protecție pentru cei care pătrund pentru diverse activități în aceste zone.

Până în urmă cu 10 ani, bolile transmise prin vectori erau o problemă a câtorva zone pe glob. În ultimii ani aceste boli au devenit o problemă de sănătate pe plan mondial atât prin epidemiile provocate de unele virusuri cunoscute a fi benigne cum este virusul WN, cât şi prin apariția unor afecțiuni noi şi deosebit de periculoase datorită transmiterii interumane, ca şi extinderea în alte teritorii. Apariția infecției cu virusul WN pe continentul american, din anul 1999, a dus la studii intense, măsuri de urmărire şi prevenire şi o strictă monitorizare a cazurilor de boală la om, păsări (în special ciori), populații de țânțari; acum 49 state americane au WNV prezent.

Concluzii

În România există premise pentru izbucnirea unei epizootii dublate de epidemie de febră West Nile, în luna septembrie 2010. Dintre aceste cazuri ar putea să apară simultan câteva zeci sau sute de complicații meningo-encefalitice, care vor necesită internare în secții de boli infecțioase, recoltare de LCR şi diagnostic virusologic. Centrul de Cercetări Ştiințifice Medico-Militare dispune de forțe şi mijloace specifice pentru a participa activ, în cadrul Sistemului Național de Sănătate, la managementul unei eventuale crize biologice cauzate de virusul West Nile.

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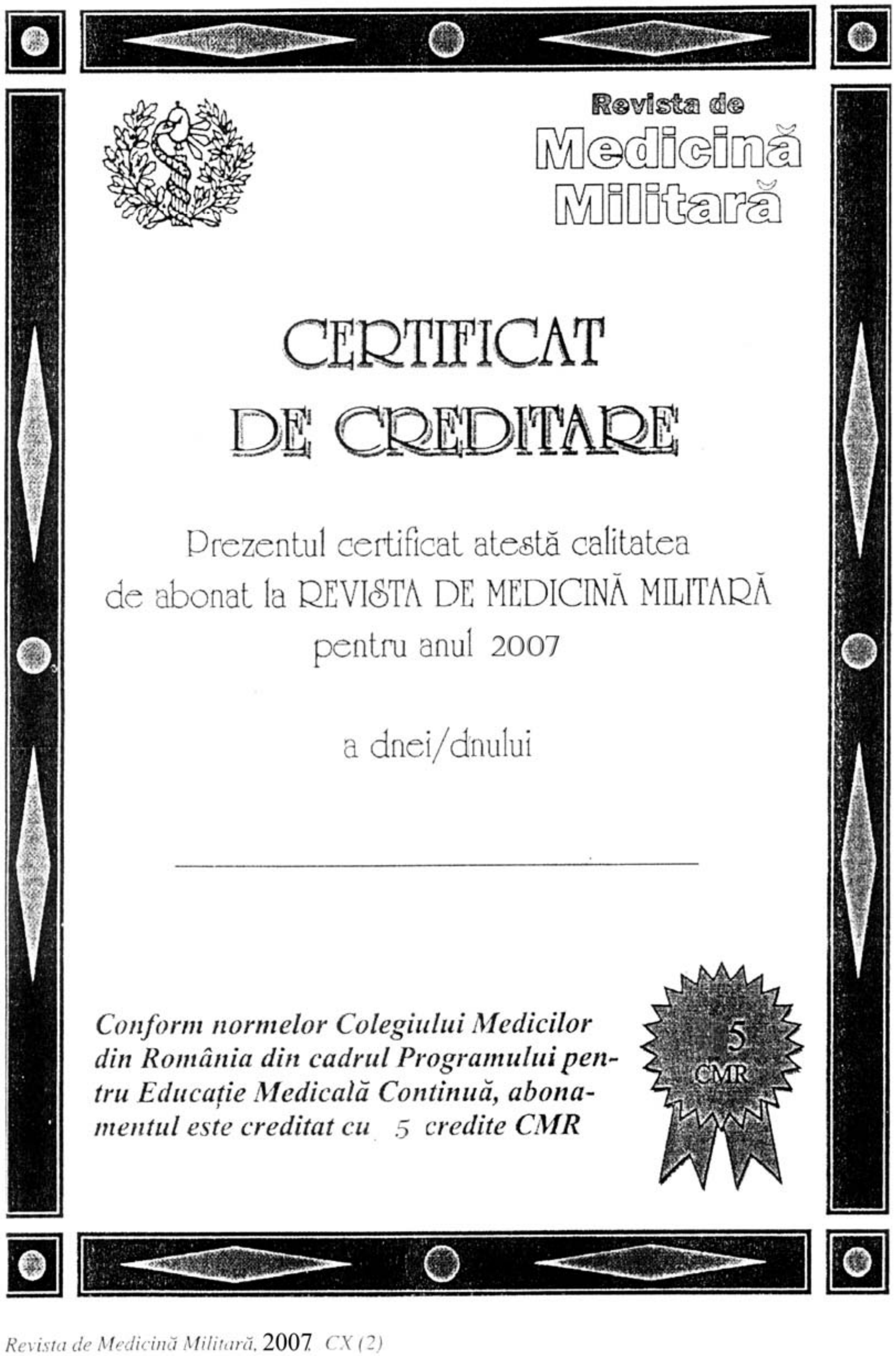

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DIN ROMÂNIA

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Manifestări digestive în lupusul eritematos sistemic

Col. dr. Florentina IONIȚĂ RADU*, Cpt. dr. Mihai PĂTRĂȘESCU**

***Clinica Medicală II, **Ambulatoriul de specialitate**
Spitalul Universitar de Urgență Militar Central "Dr. Carol Davila"

Rezumat. **Lupusul eritematos sistemic (LES) poate interesa multiple organe şi sisteme. Poliartrita, ulcerele orale şi rash-ul cutanat reprezintă manifestări clinice întâlnite frecvent. Manifestările gastrointestinale sunt comun întâlnite fie ca urmare a evoluției naturale a LES, fie ca urmare a efectelor adverse ale medicației. Aproximativ jumătate din pacienții cu LES prezintă anorexie, greață, vărsături. În literatură, incidența manifestărilor gastrointestinale variază în limite largi: între 1 şi 27 %, şi se referă la: pancreatita lupică, enterita lupică, pneumatosis cystoides intestinalis, ulcere orale, pseudoobstrucția intestinală, gastropatia cu pierdere de proteine, ischemia mezenterică. Recunoaşterea posibilității interesării acestor organe în cursul evolutiv al LES este importantă din management diagnostic şi terapeutic optim.**

Key words: lupus, digestive, autoimmunity

Abstract. Systemic lupus eritematosus (SLE) may involve different viscera and systems. The most frequent clinical manifestation is represented by poliarthritis, oral ulcers, rash. Digestive manifestations are related to natural development of SLE or to side effects of medication. Almost half of SLE suffering patients complain of anorexia, nausea, vomiting. The incidence of digestive manifestations vary largely between 1 and 27% and consists of: lupus pancreatitis, lupus enteritis, pneumatosis cystoides intestinalis, oral ulcers, intestinal pseudoobstruction, protein loosing gastropathy, mesenteric ischemia. The aknowledgement of digestive involvement in SLE carries an important significance in relation to an optimal diagnostic and therapeutic management of the disease.

Lupusul eritematos sistemic (LES) este o boală autoimună sistemică care cuprinde o gamă foarte largă de manifestări clinice. Se caracterizează printr-o dereglare funcțională imună care determină producerea de autoanticorpi îndreptați împotriva mai multor țesuturi. Morbiditatea şi mortalitatea acestei boli sunt semnificative(1).

Cursul clinic al bolii se caracterizează prin perioade de exacerbare, alternând cu perioade de remisie. Incidența LES variază de la 2 la 7,6 cazuri la 100 000 de indivizi pe an, prevalent identificându-se la populația afro-caraibiană, urmată de cea asiatică. Distribuția pe sexe este de 9:1 în favoarea sexului feminin. Debutul bolii este plasat între 15 şi 55 ani. Agregarea familială a LES este cunoscută de multă vreme, studii pe gemeni au demonstrate o rată mare de concordanță la gemenii monozigoți. În dezvoltarea bolii au fost implicate mai multe gene, în mod particular genele complexului major de histocompatibilitate HLA clasa II, şi anume: DR2 şi DR3. În plus față de această susceptibilitate genetică, la pacienții caucazieni şi africani s-a descris cu o frecvență semnificativă statistic şi cu implicații patogenice defectul de expresie a componentelor complementului (C1q, C4, C3)(2;3;4).

LES poate interesa multiple organe şi sisteme. Poliartrita, ulcerele orale şi rash-ul cutanat reprezintă cele mai frecvent întâlnite manifestări clinice. Afectarea organică cu prognosticul cel mai nefavorabil este la nivel renal şi cerebral. Manifestările hematologice (anemie hemolitică, trombocitopenie, limfopenie) sunt frecvent întâlnite. Adeseori debutul este marcat de manifestări clinice nespecifice, precum fatigabilitatea, febra, anorexia(5;6;7).

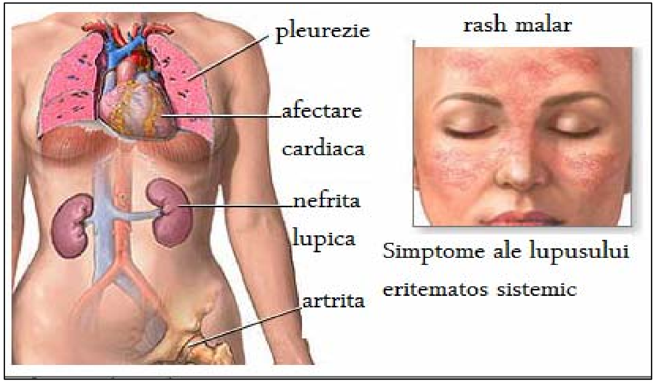

Colegiul American de Reumatologie a publicat în 1982 un set de criterii de diagnostic ale LES cu largă utilizare în practica medicală curentă. Aceste criterii au fost ulterior revizuite pentru a include şi anticorpii antifosfolipidici (aPL), porninduse de la observația prezenței frecvente a acestora la pacienții cu LES, cu implicații în patogenie.

Prognosticul pe termen lung al pacienților cu LES s-a îmbunătățit de-a lungul anilor, ca urmare a unei combinații între diagnosticul precoce şi terapia eficientă.

Investigațiile imunologice au contribuit semnificativ la ameliorarea înțelegerii LES în sensul unui diagnostic corect şi al descoperirii unor tratamente mai bine direcționate către mecanismele patogenice ale bolii. Se cunoaşte astăzi că autoanticorpii anti ADN pot fi prezenți, în medie, cu 2,7 ani anterior diagnosticului. Anticorpii antinucleari (ANA) pot apărea mai devreme decât anticorpii anti AND(6;7).

Manifestările gastrointestinale sunt comun întâlnite fie ca urmare a evoluției naturale a LES, fie ca urmare a efectelor adverse ale medicației. Adeseori însă sunt trecute cu vederea, de cele mai multe ori datorită mascării simptomatologiei de semnele şi simptomele afectării renale sau cerebrale. Incidența manifestărilor gastrointestinale variază, în literatură, în limite largi: între 1 şi 27%. Par a fi mai frecvente la populația de origine asiatică decât la caucazieni. În continuare voi prezenta doar aceste manifestări(1;8;9).

Aproximativ jumătate din pacienții cu LES prezintă anorexie, greață, vărsături. Aceste simptome pot fi determinate de boală per se, de uremia asociată insuficienței renale, de tratamentul citostatic cu azatioprină, micofenolat mofetil, ciclofosfamidă.

Serozita este un criteriu important de diagnostic şi cuprinde pleurita, pericardita, ascita. Este prezentă la 10-12% dintre pacienți şi, etiologic, poate fi diferențiată într-o formă inflamatorie (serozita propriu-zisă) şi o formă non inflamatorie (determinată de hipoalbuminemie- sindrom nefrotic, ciroză hepatică, enteropatia cu pierdere de proteine).

Criterii de diagnostic ale Colegiului American de Reumatologie pentru lupusul eritematos sistemic*

Criterii
1. Rash malar 2. Rash discoid 3. Fotosensibilitate 4. Ulcere orale nedureroase 5. Artrită 6. Serozită - pleurezie -pericardită 7. Afectare renală -proteinurie persistentă -cilindri celulari 8. Afectare neurologică -psihoze -confuzii 9. Afectare hematologică -anemie hemolitică -leucopenie -limfopenie -trombocitopenie 10. Afectare imunologică -anticorpi anti ADN -anticorpi antiSm -anticorpi antifosfolipidici 11. Anticorpi antinucleari

*Această clasificare cuprinde 11 criterii de diagnostic. Pentru stabilirea diagnosticului sunt necesare cel puțin 4 criterii, identificate simultan sau discontinuu în perioada de observație.

Ulcerele orale sunt frecvent întâlnite la pacienții cu LES şi afectează între 6 şi 52% dintre aceştia. De obicei sunt nedureroase şi apar predominant la nivelul palatului dur, a cavității nazale şi a peretelui faringian. Faptul că majoritatea pacienților cu LES primeşte terapie imunosupresoare implică riscul unei cauze infecțioase pentru aceste ulcere. Candidoza, infecția cu virusuri herpetice, leucoplakia orală pot fi recunoscute după ulcerațiile orale sau leziunile albicioase sub formă de placarde(8;9;10;11).

Nu este rar întâlnit în LES nici sindromul sicca, care evoluează cu xerostomie şi xeroftalmie şi poate afecta până la 20% din toți pacienții la un moment dat, pe parcursul evoluției bolii. Xerostomia favorizează boala periodontală şi ulcerele aftoase, eritemul, hemoragia şi hipertrofia gingivală. De obicei, tratamentul cu ciclosporină A exacerbează hipertrofia gingivală. Biopsia tisulară poate pune în evidență modificări histopatologice specifice lupusului, similare cu cele cutanate. Acestea includ: infiltrat inflamator perivascular limfohistiocitar, spongioză, hipercheratoză, depozite de IgG, IgM, complement şi fibrinogen subepitelial. Aceste leziuni sunt, uneori, dificil de diferențiat de lichenul plan(12;13).

Tratamentul de elecție pentru leziunile orale este cu antimalarice de tipul clorochinei şi hidroxiclorochinei, dar şi corticosteroizii topici. Corticosteroizii sistemici şi/sau azatioprina sunt rezervați cazurilor foarte severe. Ciclosporina A este medicație de linia a doua în cazurile refractare la tratamentul de mai sus. Nu există, în momentul de față, studii care să permită recomandări bazate pe dovezi clare în ceea ce priveşte utilizarea tratamentului sistemic în ulcerele orale asociate LES. Guma de mestecat fără zahăr, saliva artificială, pilocarpina administrată sistemic sunt tot atâtea posibilități de tratament care suplinesc deficitul de salivă la pacienții cu sindrom Sjogren secundar(14;15).

Simptomele esofagiene la pacienții cu LES includ disfagia şi pirozisul. Este dificil însă să fie apreciată riguros contribuția relativă a bolii sau a medicației la tabloul clinic. La 2/3 dintre pacienți au fost raportate anomalii manometrice constând în hipoperistaltică la nivelul 1/3 superioare a esofagului, nu întotdeauna însă asociate cu simptome relevante clinic. Interesarea sfincterului esofagian inferior este rar întâlnită. Nu există o corelație foarte bună între rezultatele investigațiilor manometrice şi tabloul clinic. Tratamentul convențional simptomatic (inhibitori de pompă de protoni asociați meselor mici şi de volum redus) reprezintă o soluție terapeutică mai bună decât medicația antiinflamatorie. Dacă însă lupusul se află într-o perioadă evolutivă, documentată serologic şi bioptic, atunci tratamentul specific al acestuia trebuie început sau amplificat(14).

Afectarea gastrică este, cel mai adesea, rezultatul efectelor adverse ale medicației antiinflamatorii. Prin urmare, pacienții care urmează pentru perioade lungi de timp tratament cu antiinflamatorii nesteroidiene (AINS) trebuie să asocieze un inhibitor de pompă de protoni. Gastrita cu citomegalovirus poate fi urmarea tratamentului de lungă durată cu imunosupresoare precum micofenolatul mofetil.

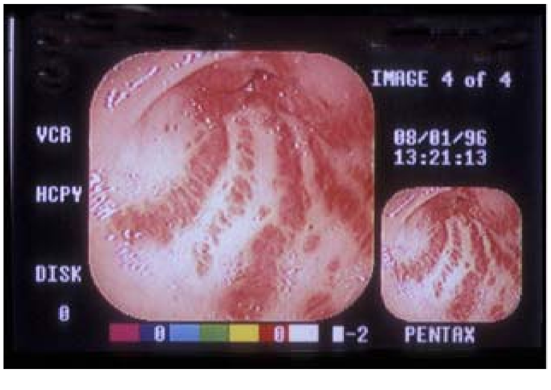

GAVE- aspect endoscopic

O malformație vasculară rară precum ectazia vasculară antrală gastrică (GAVE) se întâlneşte, uneori, la pacienții cu LES şi poate fi responsabilă de hemoragie digestivă superioară acută sau cronică şi, implicit, de anemie cronică sideropenică. Tratamentul recomandat este cu doze moderate de prednison sau, în formele mai severe, metode de endoscopie intervențională sau antrectomie.

Principalele sindroame gastrointestinale în LES

Durerea abdominală

Durerea abdominală este un simptom nespecific relativ frecvent întâlnit la pacienții cu lupus. Abdomenul acut reprezintă o provocare importantă diagnostică şi terapeutică şi poate avea mai multe cauze, aşa cum rezultă din tabelul de mai jos.

Tratamentul imunosupresiv şi cotricosteroizii pot masca semnele şi simptomele ischemiei sau perforației intestinale (principalele cauze de abdomen acut la pacienții cu LES).

Cauze de durere abdominală în LES

În relație cu LES	**În relație cu tratamentul**	**Cauze non LES**
Tromboză de venă renală Tromboză mezenterică Colecistită acalculoasă Perforație intestinală Vasculită Sarcină ectopică Pancreatită Serozită Infarct splenic Boală ischemică intestinală Angioedem	Gastrită, duodenită Pancreatită Sepsis Ulcer peptic Perforație Enterocolită Infecție cu Salmonella	Apendicită Hepatită virală Pancreatită biliară Diverticulită Aderențe chirurgicale

Durerea abdominală, cu grade variate de severitate, poate apărea pe parcursul tratamentului cu: AINS, azatioprină, ciclosporină, tacrolimus, micofenolat mofetil. Evaluarea activității bolii pe baza scorului SLEDAI (*Systemic Lupus Erythematosus Disease Activity Index*) permite medicului curant o abordare terapeutică corectă. Este bine cunoscută asocierea dintre un scor SLEDAI mare şi vasculita intraabdominală şi tromboza mezenterică (două complicații abdominale severe ale LES). Pentru un diagnostic corect se impune examenul CT şi ecografia abdominală iar în cazurile cu tablou clinic foarte zgomotos, laparotomia exploratorie efectuată precoce poate fi preferată altor mijloace de investigare, datorită mortalității mari la acest subgrup de pacienți(18;19).

Suprapopularea bacteriană a intestinului subțire este frecvent cauză de durere abdominală nonchirurgicală la pacienții cu LES nespitalizați.

Insuficiența mezenterică sau aşa-numita angină intestinală reprezintă o problemă, uneori trecută cu vederea, la pacienții cu LES. Este ştiut faptul că aceşti pacienți au un risc mai mare decât populația generală pentru ateroscleroză cu instalare prematură, care afectează diverse teritorii: cerebral, coronarian dar şi splanhnic.

Afectarea arterială splanhnică determină un sindrom dureros abdominal cronic intermitent debutat, de obicei, post prandial şi care evoluează în episoade persistente de 1-3 ore. Datorită durerii, pacienții prezintă scădere ponderală şi teama de a se alimenta. Standardul de aur este angiografia convențională, deşi informații utile se pot obține şi din investigații imagistice mai puțin invazive, precum examenele CT sau RM cu angiografie.

Factorii identificați care favorizează dezvoltarea şi progresia aterosclerozei la pacienții cu LES sunt: evoluția de lungă durată a bolii, insuficiența renală, tratamentul de lungă durată cu corticosteroizi, prezența factorilor de risc cardiovascular (hipertensiune, fumat, hiperlipidemie, diabet zaharat). Managementul factorilor de risc cardiovascular la aceşti pacienți plus tratamentul cu statine pot reduce probabilitatea apariției şi evoluției aterosclerozei.

Opțiunile terapeutice pentru insuficiența mezenterică includ: revascularizare chirurgicală, angioplastie mezenterică transluminală percutană cu sau fără montare de stent. De asemenea, în schema de tratament trebuie inclusă şi medicația antiplachetară şi anticoagulantă, funcție de raționamentul clinic(20).

Enterita lupică

Enterita lupică, numită şi vasculita gastrointestinală, este una dintre cele mai severe complicaţii ale LES. Deşi unii autori cred că prezenţa anticorpilor antifosfolipidici (*aPL-antiphospholipid antibodies*) este asociată cu această complicaţie, cei mai mulţi sunt de părere că aceşti autoanticorpi sunt în relaţie cu evenimentele trombotice arteriale sau venoase şi nu cu fenomenele vasculitice. Astfel, există o legătură între tromboza mezenterică şi aPL dar nu şi între vasculita mezenterică şi aceşti anticorpi.

Enterita lupică este acompaniată de ischemia intestinală iar la examenul CT se pot observa anse intestinale dilatate, îngroşare difuză sau focală a peretelui intestinal, edem mezenteric şi ascită. Toate aceste semne sunt însă nespecifice.

Ariile anatomice cel mai frecvente implicate sunt jejunul şi ileonul. Rectul este relativ rar implicat, deşi este un teritoriu al tractului digestiv bine vascularizat.

Diagnosticul de vasculită intestinală care determină ischemie intestinală reversibilă poate fi susţinut şi de o probă terapeutică cu prednisolon în puls terapie (1 g pe zi intravenos timp de 3 zile). Uneori este necesar diagnosticul diferenţial cu bolile inflamatorii intestinale.

În tabloul clinic al pacientului cu enterită lupică, pe lângă durerea abdominală, apar şi elemente ce indică boala activă în alte organe, febra, hemoragii oculte în scaun sau chiar melenă. Tratamentul de elecţie este cel cu glucocorticosteroizi, singur sau în combinaţie cu agenţi imunosupresori de tipul ciclofosfamidei. Tratamentul chirurgical este indicat în cazurile foarte severe(21;22).

Pseudoobstrucţia intestinală

Această entitate nosologică este o complicaţie gastrointestinală rară şi puţin înţeleasă a LES. Poate fi definită de prezenţa tabloului clinic sugestiv de obstrucţie intestinală, dar fără a se reuşi punerea în evidenţă a unei leziuni organice obstructive. Pseudoobstrucţia intestinală reflectă o disfuncţie a musculaturii netede a viscerelor sau a sistemului nervos autonom visceral. Nu numai intestinul este astfel afectat, ci şi alte organe cavitare: pacienţii pot prezenta ureterohidronefroză, pseudoobstrucţie de tract biliar. Această constatare susţine ideea unei implicări a tulburării de motilitate a musculaturii netede.

Aproape jumătate dintre pacienţii cu această complicaţie a LES sunt asiatici, ceea ce poate sugera existenţa unei componente genetice, deşi aceasta nu a fost încă identificată.

Tabloul clinic include durere abdominală, meteorism abdominal, încetinirea tranzitului intestinal. Abdomenul acut cu semne de iritaţie peritoneală este rareori întâlnit.

Pseudoobstrucţia intestinală (PI) poate fi prima manifestare a LES, dar de cele mai multe ori apare ca o complicaţie evolutivă a bolii. Mortalitatea în PI nu este în legătură directă cu aceasta, ci se datorează, mai degrabă, sepsisului indus de medicaţia imunosupresoare agresivă.

Majoritatea cazurilor evoluează cu atacuri recurente frecvente, cu interesarea cu predilecţie a intestinului subţire şi, mai rar, a colonului.

În cursul atacurilor, ecografia abdominală şi examenul CT cu substanţă de contrast evidenţiază adesea dilatare ureterală bilaterală cu reducerea volumului vezicii urinare.

În unele cazuri se poate identifica şi o cistită interstiţială cronică, boală asociată cu depunerea de complexe imune circulante, complicaţie evolutivă recunoscută a LES. Suspiciunea clinică de PI este, clasic, confirmată prin manometria antroduodenală şi esofagiană. Se poate, astfel, demonstra prezenţa aperistalticii esofagiene şi a hipomotilităţii intestinale (de natură neurogenică sau miogenică).

Un posibil mecanism patogenic, sugerat de fiziopatogeneza LES, poate fi cel prin vasculita mediată prin complexele imune circulante sau prin autoanticorpii îndreptaţi împotriva sistemului nervos autonom enteric sau musculaturii netede intestinale. Unele rapoarte histopatologice în PI identifică fibroză de perete intestinal, serozită şi vasculită mezenterică. Nu au fost identificaţi autoanticorpi specifici pentru PI dar majoritatea pacienţilor au anticorpi antinucleari şi anticorpi anti Ro.

Tratamentul de elecție al PI este cu doze mari de corticosteroizi administrați intravenos. Ca tratament inițial sau la nonresponderi se poate apela la: ciclosporina A, ciclofosfamidă sau azatioprină. În combinație cu tratamentul imunosupresor se mai utilizează antibioticele cu spectru larg (pentru a diminua suprapopularea bacteriană) şi medicamente prokinetice la nivelul intestinului subțire (de tipul eritromicinei, care are activitate prokinetică prin acțiune pe receptorii de motilină). În formele refractare sau deosebit de grave sunt citate ca opțiuni terapeutice: octreotidul (analog de somatostatină) şi rituximabul (anticorpi monoclonali anti CD20 - marker fenotipic de suprafață pentru limfocitele B). Se apreciează că este foarte importantă recunoaşterea precoce a acestei complicații gastrointestinale a LES datorită reversibilității bune a PI la medicația imunosupresivă. Tratamentul chirurgical trebuie evitat ori de câte ori este posibil(22;23;24).

Gastroenteropatia cu pierdere de proteine

Gastroenteropatia cu pierdere de proteine (GEPP) este un sindrom clinic caracterizat prin hipoalbuminemie datorată pierderilor proteice la nivelul tubului digestiv în absența unor astfel de pierderi la nivel renal sau malnutriției sau reducerii aportului proteic alimentar sau unei cauze hepatice. Prin urmare, diagnosticul de GEPP este, prin excelență, de excludere. Chiar şi alte suferințe gastrointestinale pot să evolueze cu pierdere de proteine (limfomul intestinal, limfangiectazia intestinală).

Tabloul clinic al GEPP include: edeme generalizate hipoproteice, durere abdominală (prin edem de perete intestinal, urmare a scăderii presiunii oncotice intravasculare), diaree severă.

GEPP poate fi prima manifestare a LES dar de cele mai multe ori apare tardiv şi rar în cursul evoluției bolii.GEPP (la fel ca şi PI) este mai frecvent întâlnită la pacienții de origine asiatică, deşi baza genetică a acestei predispoziții nu este încă identificată. Patogeneza acestei complicații a LES nu este bine cunoscută. Vasculita mezenterică poate, totuşi, juca un rol fiziopatogenic important. Nu au fost identificați autoanticorpi specific asociați cu această suferință dar s-a sugerat rolul complexelor imune circulante în creşterea permeabilității capilare la nivelul mucoasei digestive.

Diagnosticul de GEPP este suspectat clinic, dar trebuie confirmat prin investigații suplimentare. Standardul de aur este scintigrafia cu albumină serică umană marcată cu Tc 99m. Alte investigații utile diagnosticului sunt: endoscopia digestivă, examenul CT abdominal, testul de malabsorbție la D xyloză.

Majoritatea pacienților cu GEPP au un răspuns bun la tratamentul cu glucocorticosteroizi dar, în formele refractare, se poate apela la puls terapia cu ciclofosfamidă. Combinația prednisolon-azatioprină s-a dovedit eficientă în îmbunătățirea prognosticului(25;26;27).

Pneumatosis cystoides intestinalis

Pneumatosis cystoides intestinalis (PCI) este o complicație foarte rar întâlnită la pacienții cu LES, dar care prezintă importanță practică prin gravitatea ei. Se caracterizează prin prezența de gaz cu dispoziție lineară sau chistică în teritoriul submucos sau subseros al intestinului. Segmentele intestinale cel mai frecvent afectate sunt colonul stâng şi ileonul. Această entitate nosologică a fost citată şi în alte boli autoimune sistemice, în plus față de LES, şi anume : în sclerodermia sistemică, în dermatomiozită.

Au fost propuse mai multe mecanisme patogenice; cele mai frecvent citate sunt : microdilacerări ale mucoasei intestinale, infecții mucosale, ischemie prin vasculit. PCI evoluează, cel mai adesea, asimptomatic dar se poate prezenta şi ca pneumoperitoneu, aseptic, ce trebuie diferențiat de perforația intestinală. Relevanța descoperirii sale trebuie interpretată doar în context clinic. De cele mai multe ori, nu are nicio semnificație în managementul general al LES şi, prin urmare, nu impune nici un fel de intervenție terapeutică particulară. Examenul CT cu substanță de contrast a abdomenului contribuie la precizarea diagnosticului. Investigația bioptică nu este necesară după punerea în evidență radiologică a aspectului caracteristic al PCI.

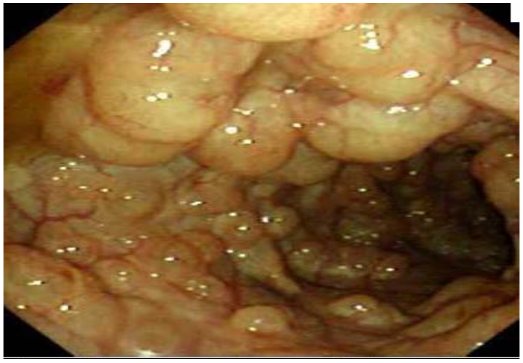

Aspect endoscopic al PCI

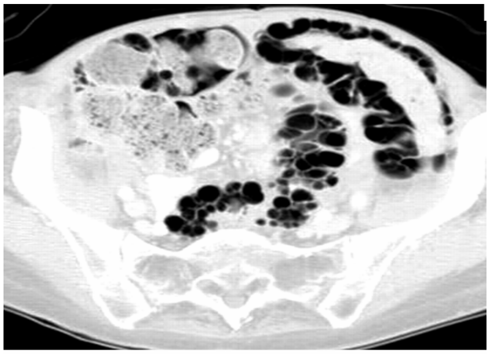

Aspect CT al PCI

Tratamentul, în situația în care s-au exclus cauze potențial amenințătoare de viață, precum necroza intestinală sau perforația intestinală, include oxigenoterapia şi antibioticoterapia. Prognosticul este excelent(27;17).

Pancreatita lupică

Este o complicație rară a LES. În populația generală cele mai frecvent întâlnite cauze de pancreatită includ: coledocolitiaza, abuzul de alcool etilic, cauze medicamentoase. Pacienții cu lupus pot dezvolta pancreatită de origine neprecizată, posibil legată fiziopatogenic de boala autoimună per se. Cauzele enunțate pentru populația generală sunt valabile şi aici şi trebuie să facă obiectul unui diagnostic diferențial riguros.

Dintre medicamentele frecvent utilizate în tratamentul LES este cunoscut faptul că azatioprina şi glucocorticosteroizii pot determina pancreatită medicamentoasă, deşi relația de cauzalitate este dificil de demonstrat în fiecare caz în parte. De cele mai multe ori, pancreatita este o complicație a LES, de regulă în legătură cu gradul de activitate al bolii.

Mecanismele patogenice posibile propuse sunt: autoimunitatea per se, vasculita, tromboza asociată anticorpilor anti fosfolipide, sindromul sicca.

Tabloul clinic este cel clasic, cu durere abdominală severă, greață şi vărsături, dar mortalitatea pare să fie mai mare în cazul pacreatitei asociate LES.

Tratamentul include controlul fazei evolutive a LES şi mijloacele terapeutice standard suportive pentru pancreatită(28;3).

În concluzie, manifestările digestive din LES sunt variate şi pot reprezenta o adevărată provocare diagnostică şi terapeutică. Recunoaşterea lor permite optimizarea eforturilor medicului clinician în managementul corect al acestei suferințe.

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Influența statusului general asupra stării de sănătate orală la edentatul total

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Rezumat. Edentația totală, în ciuda progreselor înregistrate în domeniile medical, tehnologic şi al biomaterialelor, continuă să rămână o stare clinică cu o frecvență mare la nivelul populației în vârstă. Se constată o prevalență variabilă a afecțiunii în funcție de regiunea geografică, vârsta şi statusul socio-economic. Prezența edentației totale este asociată reducerii calității vieții individului prin afectarea funcțiilor aparatului dento-maxilar, cu consecințe negative asupra stărilor de sănătate orală şi generală, cu influențarea negativă a percepției asupra propriei persoane şi diminuarea interacțiunilor sociale. Stările de sănătate generală şi orală sunt conectate prin posibilele interacțiuni reciproce şi prezența factorilor de risc comuni. La nivel oral, cele mai frecvente modificări prezente concomitent edentației totale sunt: xerostomia, candidoza bucală, hiperplaziile mucoasei bucale, stomatodiniile şi cancerul oral. În condițiile creşterii vârstei medii de viață, a numărului de pacienți purtători de proteze mobile şi a utilizării vreme îndelungată a protezelor, se impune o nouă abordare a edentatului total la pacientul geriatric, în vederea folosirii optime a protezei şi, în consecință, a îmbunătățirii calității vieții.

Cuvinte cheie: edentat total, status general, xerostomie, candidoză

Abstract. Despite the advances present in medical care and biomaterials, edentulism still has a high frequency in elderly individuals. Prevalence is variable and depends on a number of factors including geographic region, age and socio-economic status. Edentulism is associated with reduced quality of life, due to oral functional involvement, with negative consequences on oral and general health, with negative influence on self-perception and social interactions. Oral and general health is connected by mutual interactions and common risk factors. The most frequent oral conditions present in edentulous patient are: xerostomia, candidosis, hyperplasia and oral cancer. With increasing life expectancy and numbers of complete edentulous patients, a new approach for treating the complete edentulous geriatric patient is necessary, in order to increase patient satisfaction with dentures and quality of life.

Introducere

Edentația totală este o situație clinică prezentă la pacienți în societatea actuală, cu efecte negative asupra „stării de bine” a individului, element central al percepției actuale privind starea de sănătate. Ea este definită prin absența tuturor dinților naturali şi este caracteristică în special persoanelor în vârstă. Cauza edentației este datorată în mod deosebit extracțiilor dentare, realizate cel mai frecvent consecutiv afectării odontale prin carie şi boala parodontală, afecțiuni cu o prevalență crescută în populație. Dintre persoanele a căror vârstă depăşeşte 65 de ani, 34% prezintă procese carioase şi 40% afecțiuni parodontale. Pierderea dinților are atât influențe negative asupra sănătății orale şi generale, cât şi efecte sociale şi psihologice. Edentația totală este asociată cu reducerea calității vieții individului prin afectarea funcțiilor orale (în primul rând scăderea capacității masticatorii), a esteticii faciale, cu influențarea negativă a percepției asupra propriei persoane şi diminuarea interacțiunilor sociale.

DATE EPIDEMIOLOGICE

Prevalenţa edentaţiei totale

Deşi edentaţia totală este întâlnită la aproape orice vârstă, prevalenţa este maximă la adultul în vârstă. Raportările Organizaţiei Mondiale a Sănătăţii din 2004 indică valori crescute (maxim 78%) la populaţia a cărei vârstă depăşeşte 65 de ani, existând o mare variabilitate în funcţie de regiunea unde s-a realizat ancheta epidemiologică (tabelul nr. 1). În România aproximativ 1 din 4 indivizi cu vârsta cuprinsă între 65 şi 74 ani prezintă edentaţie totală.

Tabelul 1. Raportări ale Organizaţiei Mondiale a Sănătăţii privind edentaţia totală, 2004

ŢARA	persoane edentate (%)	grupa de vârstă (ani)
Canada	58	65+
Statele Unite ale Americii	26	65-69
Albania	69	65+
Austria	15	65-74
Bosnia şi Herzegovina	78	65+
Bulgaria	53	65+
Finlanda	41	65+
Ungaria	27	65-74
Italia	19	65-74
Polonia	25	65-74
România	26	65-74
Slovenia	16	65+
Anglia	46	65+

În ţările dezvoltate, prin eforturile serviciilor de sănătate publică, s-a înregistrat o scădere a frecvenţei edentaţiei totale. De exemplu, în Statele Unite ale Americii prevalenţa edentaţiei totale la persoanele cu vârsta peste 65 ani a scăzut de la 50% în 1950, la 42% în 1990 şi la 26% în 2004. Aceste constatări sugerează că edentaţia totală nu este o situaţie inevitabilă, o consecinţă obligatorie a procesului de îmbătrânire. O explicaţie pentru scăderea prevalenţei acestei afecţiuni s-ar putea regăsi în măsurile preventive instituite şi susţinute de serviciile de sănătate publică (cum sunt sigilările dentare, fluorizarea apei, folosirea apelor de gură şi pastelor de dinţi cu fluor).

Previziunile actuale indică o frecvenţă crescută a edentaţiei totale şi în viitor. Acest lucru este corelat cu o speranţă de viaţă mai mare, element asociat creşterii segmentului populaţional în vârstă, alături de scăderea natalităţii. Se preconizează că numărul persoanelor în vârstă va spori cu 10% la fiecare zece ani, edentaţia totală rămânând, cel mai probabil, o afectare destul de des întâlnită în rândul populaţiei.

Prevalenţa edentaţiei totale în funcţie de vârstă

Conform raportărilor „National Center for Chronic Disease Prevention and Health Promotion”, SUA, 2002, există o corelaţie pozitivă între vârstă şi prezenţa edentaţiei totale. În grupa de vârstă 25-44 ani prevalenţa edentaţiei totale a fost de 2%, pentru adulţii de 45-64 ani de 10% şi 25% în rândul celor cu vârsta mai mare de 65 ani. În general, se consideră că persoanele în etate sunt cele care prezintă riscul cel mai mare pentru această afecţiune, procentul crescând o dată cu vârsta. Există diferenţe legate şi de sex, pentru perioada pre-postmenopauză numărul femeilor edentate total fiind de aproape trei ori mai mare decât al bărbaţilor. Totuşi, este important să subliniem că edentaţia totală, deşi frecventă în rândul populaţiei, nu reprezint o consecinţă inevitabilă a procesului de îmbătrânire.

Prevalența edentației totale în funcție de statusul socio-economic

Studiile epidemiologice au arătat că există o asociere clară între edentația totală şi statusul socio-economic. Persoanele cu un nivel de educație mai ridicat şi cu un venit mai mare prezintă un procent mai mic de edentați totali. Conform raportărilor „National Center for Chronic Disease Prevention and Health Promotion” din 2002, în toate grupele de vârstă există o corelație negativă între prevalența edentației totale şi gradul de educație. La nivelul pacienților cu vârstă mai mare de 65 de ani, cei cu mai puțin de 12 clase prezintă o prevalență a edentației de 39%, cei cu 12 clase de 25%, iar cei cu mai mult de 12 clase de 13%. O explicație probabilă ar putea fi legată de interesul crescut față de sănătatea şi igiena orală, precum şi accesul mai mare la serviciile de sănătate al celor cu un grad de educație sporit şi un status socio-economic ridicat.

STATUSUL GENERAL AL PACIENTULUI EDENTAT TOTAL

Edentația totală, ca afecțiune caracteristică în primul rând adulților în vârstă, este necesar să fie integrată stării generale de sănătate, cu care stabileşte multiple interacțiuni. Odată cu înaintarea în vârstă creşte şi posibilitatea ca o persoană să sufere de una sau mai multe boli cronice. Afectările sistemice cu cea mai mare prevalență în rândul populației a cărei vârstă depăşeşte 65 ani sunt redate în tabelul nr.2. Existența lor este asociată cu prezența dizabilităților şi cu scăderea calității vieții.

Tabelul 2. Afecțiuni sistemice cu prevalență crescută în grupa de vârstă de peste 65 de ani

Cele mai frecvente afectări sistemice la adulții în vârstă	
Artrită	Alergii
Boli cardiovasculare	Diabet
Hipertensiune arterială	Afectări musculo-scheletale
Deficiențe senzoriale	Cancere
Afectarea auzului si vazului	Incontinență urinară
Boli psihice	Boli infecțioase

Un studiu privind statusul general - realizat în Clinica de Protetică Dentară din Bucuresti, pe un număr de 24 de edentați total, femei şi bărbați, cu vârsta cuprinsă între 50 şi 85 ani - a arătat că probleme legate de statusul general au fost reprezentate în special de hipertensiunea arterială, cu o frecvență egală la bărbați şi femei (33,33%), şi de osteoporoză, boală ce se manifestă exclusiv la femei - 41,67% (fig. 1). Pacienții au mai prezentat afecțiuni cardio-vasculare (16,67% la femei, 33,33% la bărbați), diabet (în procent egal, 16,67%, la femei şi bărbați), epilepsie (16,67% dintre bărbați) şi boli psihice (16,67% dintre femei).

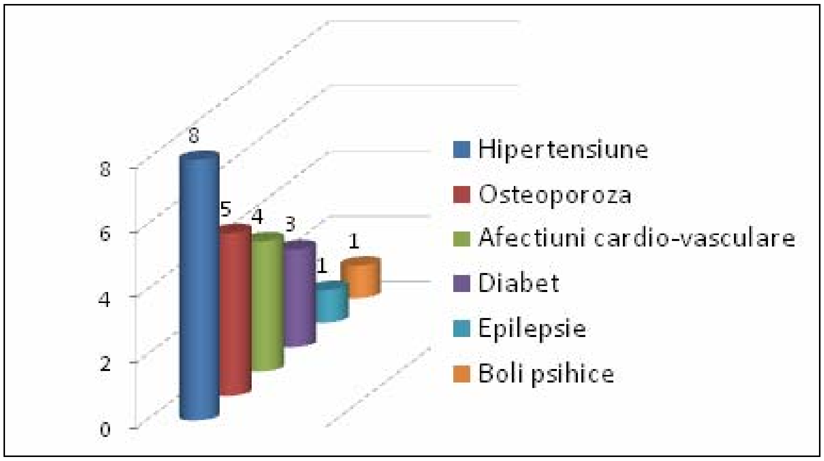

Fig. 1. Grafic privind statusul general la edentatul total

Stările de sănătate generală şi orală sunt interconectate prin posibilele interacţiuni reciproce. Altfel spus, între starea de sănătate a structurilor orale şi starea de sănătate generală există un proces de intercondiţionare reciprocă, fiind bine ca ele să fie percepute ca un întreg. Acest lucru a fost dovedit ştiinţific prin cercetările în domeniu. De exemplu, boala parodontală este asociată cu diabetul, insuficienţa cardiacă şi afecţiunile cronice ale aparatului respirator. Pierderea dinţilor a fost de asemenea asociată cu un risc crescut de accident vascular cerebral şi afecţiuni psihice. Prezenţa afecţiunilor reumatismale, afectarea văzului şi auzului la un edentat total pot fi asociate cu dificultăţi în înţelegerea planului de tratament, cu un acces mai dificil la serviciul medical şi cu scăderea capacităţii de a-şi igieniza protezele. Totodată, datorită scăderii sistemului imunitar, apare un risc crescut pentru infecţiile acute. La persoanele de gen feminin edentate total, se va avea în vedere că 30% dintre acestea suferă de osteoporoză, boală care predispune la resorbţia crestei după pierderea dinţilor şi creşte dificultăţile în tratamentul protetic pe implante.

Afectările stării de sănătate orală şi generală sunt interconectate şi prin prezenţa factorilor de risc comuni. Fumatul este un factor de risc în apariţia cancerului, afecţiunilor cardio-vasculare, accidentelor vasculare cerebrale, emfizemului, bronşitelor. La nivelul cavităţii bucale fumatul este asociat cancerului oral şi bolii parodontale. Totodată, anumite boli se pot caracteriza concomitent atât prin manifestări generale, cât şi orale (lichenul plan).

Un aspect care nu trebuie neglijat este legat de medicaţia pe care cei mai mulţi dintre pacienţii edentaţi total o urmează. Este cunoscut faptul că pot apărea modificări/efecte secundare manifestate la nivelul cavităţii orale. De exemplu, Aspirina poate creşte riscul de sângerare, iar Nifedipinul, Ciclosporinele, Fenitoina pot avea un rol în apariţia hiperplaziilor gingivale. Totodată, mai mult de jumătate dintre respectivii pacienţi iau cel puţin 4 tipuri de medicamente, existând posibilitatea ca între acestea să apară interacţiuni. Astfel de manifestări sunt posibile şi odată cu administrarea medicamentelor prescrise de medicul dentist (cel mai frecvent antibiotice, analgetice, sedative). Chiar dacă aceste interacţiuni sunt de cele mai multe ori nesemnificative, se recomandă ca ele să fie avute permanent în vedere.

În concluzie, realizarea unui plan de tratament corect, care să răspundă cerinţelor funcţionale, necesită înţelegerea stării de sănătate generală a pacientului şi a interrelaţiei afectare sistemică - afectare orală. Evaluarea stării generale a pacientului trebuie să preceadă atât examenul local, cât şi planul de tratament. Se impune totodată consemnarea afecţiunilor generale cu care pacientul este sau nu în evidenţa medicului de familie sau specialist, medicaţia administrată, eventualele alergii la medicamente sau anestezice.

MODIFICARI ALE STATUSULUI ORAL LA EDENTATUL TOTAL

Funcţionarea optimă a aparatului dento-maxilar este strâns legată de integritatea dentiţiei şi a structurilor orale. Variaţiile stării de sănătate orală la adulţii în vârstă rezultă din diferenţele culturale, particularităţile sociale, dietă, acces la serviciile de sănătate, gradul de educaţie şi altele. Dinţii sunt un simbol al statutului, autorităţii, puterii, percepţia variind în cadrul diferitelor culturi. Edentaţia totală este starea clinică cu cea mai profundă afectare a statusului oral, pe lângă modificările morfo-funcţionale loco-regionale ireversibile însoţindu-se de implicaţii generale, sociale şi comportamentale. De exemplu, un studiu realizat în Anglia privind impactul pierderii dinţilor asupra masticaţiei şi statusului nutriţional arată că edentatul total prezintă un consum redus de fibre, proteine, fructe, legume şi respectiv calciu. „Starea de bine” la edentatul total este legată atât de caracteristicile acestei stări particulare, cât şi de unele afecţiuni asociate (fenomene involutive, afecţiuni generale, medicaţia aferentă) şi de calitatea tratamentului instituit. Multe dintre schimbările fiziologice şi patologice sunt reflectate la nivelul mucoasei orale.

Protezarea convenţională a edentaţiei totale, cel mai utilizat remediu până la ora actuală, este un tratament cu o dificultate crescută, care nu întodeauna reuşeste să răspundă pe deplin cerinţelor pacienţilor. Într-un studiu privind satisfacţia în protezarea totală, realizat pe 907 bătrâni edentaţi total din Ontario (Canada), sunt raportate ca principale motive de nemulţumire: probleme legate de aspectul fizic şi socializare (22%), deficienţe masticatorii (14%) şi fonatorii (6%). Observaţiile noastre au arătat că modificările estetice legate de edentația totală sunt de cele mai multe ori îmbunătățite prin protezare, dând pacientului un confort psihic (figurile 2,3).

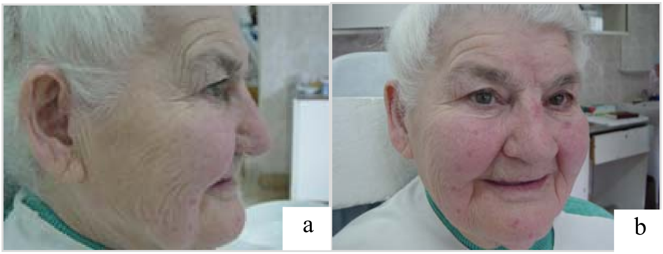

Fig. 2. Aspectul facial al edentatului total neprotezat a. normă laterală b. normă frontală

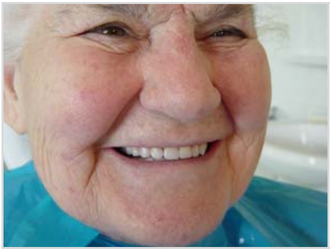

Figura 3. Aspectul facial al edentatului total protezat

Cele mai frecvente modificări orale, asociate edentației totale, prezente la persoanele în vârstă, purtătoare de proteze mobile, sunt: resorbțiile severe ale crestei alveolare ce creează mari dificultăți în protezare, stomatita protetică, candidozele, hiperplazii ale mucoasei, xerostomia, cancere etc. (figurile 4,5,6). Resorbția severă a crestei sau apariția crestei balante (fibroase) sunt legate de presiunile excesive asupra structurilor de suport. Astfel, cauza cea mai frecventă pentru producerea crestei balante în zona frontală la maxilar este reprezentată de „sindromul combinației" descris de KELLY (1972). Mecanismul etiologic este explicat prin presiunile mari exercitate de dinții antagoniști şi deplasarea câmpului masticator în zona frontală, unde se exercită presiuni excesive ce conduc treptat la pierderea suportului osos.

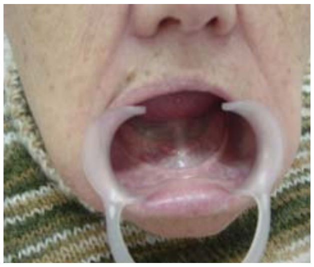

Fig. 4. Resorbția severă a crestei

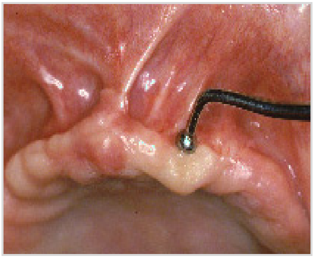

Fig. 5. Creasta fibroasă

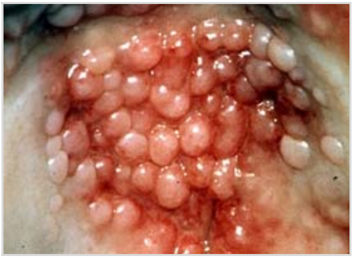

Fig. 6. Hiperplazie papilară

Xerostomia

Xerostomia este definită ca senzația de gură uscată, diagnosticul pozitiv rezultând prin raportarea simptomatologiei de către pacient. Inițial, existența acestei manifestări a fost asociată cu scăderea cantitativă a fluxului salivar. Totuşi cercetările în domeniu au arătat că nu există o corelație pozitivă între acestea. Într-un studiu realizat în Australia, pe o populație în vârstă, 20,5% prezentau xerostomie, 22,1% hiposalivație şi doar 5% ambele afecțiuni. Se apreciază că pacienții acuză uscăciunea gurii atunci când fluxul salivar scade cu peste 50%.

Xerostomia are o frecvență crescută în rândul persoanelor în vârstă, raportările diverselor studii indicând o prevalență între 10 şi 40%. Este mai des întâlnită la femei, în toate grupele de vârstă. Această afecțiune este mai frecventă la persoanele care sunt sub medicație (mai mult de 400 de agenți medicamentoşi se presupune că au efecte secundare asupra funcției salivare). Prevalența xerostomiei creşte odată cu sporirea numărului de medicamente luate concomitent.

Cauza xerostomiei nu este pe deplin elucidată. Se presupune că un rol important îl au afectările sistemice, efectele secundare şi interacțiunile medicamentoase. Printre condițiile sistemice frecvent asociate xerostomiei sunt: alcoolismul cronic, boli autoimune, boala Parkinson, boli psihice, deshidratarea, iradieri ce interesează glandele salivare, diabetul, boala Sjogren, respirația orală. Printre medicamentele frecvent asociate xerostomiei se numără: sedativele, antipsihoticele, antidepresivele, antihistaminicele, diureticele şi unele antihipertensive. Frecvenţa mai mare a xerostomiei la adulţii în vârstă nu este asociată hiposalivaţiei, ca parte a tabloului clinic al pacientului geriatric. Deşi la nivelul glandelor salivare apar modificări odată cu înaintarea în vârstă, la adulţii sănătoşi fluxul salivar nu este afectat de procesul de îmbătrânire.

În cazurile cu scăderea fluxului salivar, asociată sau nu xerostomiei, apar modificări importante locale şi generale. Funcţia salivară are un rol important în percepţia gustativă (corelată cu plăcerea alimentară), protecţia şi repararea părţilor moi, având şi proprietăţi antibacteriene. Hiposialia este asociată unor tulburări funcţionale, creşterii susceptibilităţii la infecţii (candidoza) şi deficienţelor nutritive (digestie, masticaţie, deglutiţie). Xerostomia e corelată cu apariţia unui grad de disconfort, tulburări de somn şi scăderii calităţii vieţii. La pacienţii purtători de proteze totale, modificările cantitative ale fluxului salivar pot avea consecinţe în direcţia adaptării cu protezele (disconfort, manifestări datorită absenţei efectului lubrifiant al salivei), menţinerea protezelor (prin scăderea adeziunii, prin reducerea tensiunii de suprafaţă) şi risc mai mare de apariţie a stomatitelor (frecvent candidoza).

Candidoza bucală

Candidoza bucală, cauzată în majoritatea cazurilor de Candida Albicans, afectează preferenţial bătrânii şi purtătorii de proteze dentare. Este cunoscut rolul pe care îl au în etiopatogenia bolii dezechilibrele florei bacteriene orale şi deficienţele imunitare. Diabetul zaharat, antibioterapia incorect condusă, corticoterapia sau chimioterapia neoplasmelor pot iniţia infecţia. Protezele oferă un mediu prielnic de multiplicare a ciupercilor, iar xerostomia (medicamentoasă, din sindromul Goujerot-Sjogren sau după iradiere) este legată de formele atrofice ale afecţiunii, cu variantele ei de stomatită de proteză şi cheilită angulară (perleşul). În stomatita de proteză este esenţială curăţirea mecanică urmată de dezinfectarea protezei cu soluţii antiseptice (clorhexidină 0,12%, hipoclorit de sodiu 1:50). Proteza poate fi un suport pentru unguentele antifungice, asigurând un contact intim şi de mai lungă durată cu mucoasa afectată.

Hiperplaziile mucoasei orale

Leziunile mucoasei bucale asociate cu purtarea protezelor pot fi reacţii acute sau cronice la acţiunea plăcii microbiene, la constituienţii materialului bazei protezei sau pot fi leziuni prin traumă mecanică. Interesarea mucoasei de către hiperplazie este o afectare cronică, se poate limita la ţesuturile din jurul marginilor protezei sau poate cuprinde mucoasa de sub proteză (în special de la nivelul bolţii palatine, în treimea anterioară).

Hiperplazia prezentă la nivelul bolţii palatine se caracterizează prin apariţia unor papile proeminente ce acoperă suprafeţe variabile ale mucoasei bolţii palatine, mai ales la nivelul porţiunii centrale, pe torus. Leziunile sunt în general nedureroase sau cauzează un uşor disconfort. Pe lângă acţiunea traumatică a protezei, este incriminat şi factorul microbian (Candida Albicans), întreţinut prin purtarea continuă a protezelor şi igienizarea lor deficitară.

Stomatodiniile (sindromul de gură arsă)

Stomatodiniile se caracterizează printr-o senzaţie de arsură la nivelul cavităţii bucale. Este relevantă o diferenţiere între senzaţia de gură uscată şi stomatodinii. În marea majoritate a cazurilor, stomatodinia apare la pacienţii care poartă proteza totală şi mai ales la femeile a căror vârstă depăşeşte 50 de ani, la care senzaţiile de arsură reprezintă simptome frecvente în perioada de după menopauză. De obicei, semnele clinice nu sunt evidente, dar simptomele apar des, de multe ori în asociere cu plasarea unei noi proteze. Ca o caracteristică, manifestările sunt prezente dimineaţa şi tind să se agraveze pe parcursul zilei. Calitatea durerii este dată de senzaţia de arsură asociată cu senzaţia de gură uscată şi senzaţii de modificare a gustului. Alte simptome posibile sunt durerile de cap, insomniile, iritabilitatea sau depresiile.

Cancerul oral la purtătorii de proteze

Cancerul este una dintre principalele cauze de mortalitate la ora actuală, prevalența cea mai crescută fiind în segmentul de vârstă de peste 60 ani. Principalii factori de risc în apariția cancerului oral sunt fumatul şi consumul de alcool. Alți factori asociați cu o frecvență mai mare a cancerului sunt sexul (bărbații prezintă o prevalență mai crescută a acestei afecțiuni, posibil în corelație cu consumul mai mare de tutun şi alcool) şi statutul socio-economic.

Cancerul oral este diagnosticat anual la mai mult de 30.000 de indivizi în SUA, luând 10.000 de vieți în fiecare an. Pentru 96% dintre cancerele orale, carcinomul scuamos este precedat de leziuni ale mucoasei bucale. Transformarea malignă, care este impredictibilă, are loc la 1-90% din leziuni în timp de 5 ani.

Legat de situația particulară de edentat total, acțiunea traumatică cronică a protezei asupra mucoasei este des prezentată ca un factor de risc în apariția leziunilor maligne la nivel oral. Această situație se constată mai ales atunci când protezele sunt realizate incorect şi uneori apare suprapunerea infecției cu Candida, teorie nesusținută încă prin studii clinice. Totuşi, existența unor leziuni ale mucoasei fără tendință la vindecare după corectarea protezei ridică suspiciunea de cancer şi necesită examene complementare pentru diagnosticul de certitudine.

CONCLUZII

- Îmbătrânirea populației, conform numeroaselor raportări, are consecințe socio-economice, dar şi asupra sănătății generale şi orale a acestor pacienți.
- Edentația totală, în ciuda progreselor înregistrate în domeniile medical (ştiințific şi al serviciilor de sănătate), tehnologic şi al biomaterialelor, continuă să rămână o stare clinică cu o frecvență mare la nivelul populației în vârstă.
- Evoluția îndelungată a edentației totale, cu modificări profunde loco-regionale, în prezența unor afecțiuni generale şi medicația specifică, face să crească dificultățile în protezarea totală a pacientului geriatric.
- În edentația totală şi protezarea ei trebuie să se ia în calcul aspectele de ordin general (fiziologice, de patologie generală, medicația) în interrelație cu aspectele locale şi implicațiile lor asupra statusului nutrițional şi general, precum şi asupra calității vieții.
- În condițiile creşterii vârstei medii de viață, a numărului de pacienți purtători de proteze mobile şi a utilizării vreme îndelungată a protezelor, se impune o nouă abordare a edentației totale la pacientul tânăr edentat, dar şi la cel geriatric.
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“Lecțiile de anatomie” ale lui Rembrandt

Rembrandt (Harmenszoon van Rijn Rembrandt) este unul dintre cei mai valoroşi artişti olandezi ai secolului al 17-lea. S-a născut în anul 1606, la Leiden, localitate situată în apropierea Rinului (de unde şi porecla „van Rijn”), şi era cel de-al nouălea copil al unui morar.

Prin opera sa, Rembrandt revoluționează canoanele tradiționale ale picturii, transpunând pe pânză într-un mod inconfundabil calitățile şi defectele, frumosul şi urâtul. A fost în egală măsură un iscusit gravor, un magician al culorilor, un colecționar pasionat de picturi, sculpturi, gravuri şi antichități ce aparțineau marilor maeştri din trecut sau erau contemporani cu el , dar şi negustor de artă.

Veşnic atent la ceea ce se petrecea în jurul său, Rembrandt, în vârstă de 26 de ani, nu putea să rămână indiferent la un fenomen inedit care-i pasiona pe contemporanii săi: disecțiile publice, care se bucurau de o importanță aparte în Olanda acelor vremuri. Aşa că în momentul când breasla medicilor chirurgi i-a cerut să imortalizeze pe pânză o astfel de şedință, pictorul a răspuns cu destul entuziasm. Tabloul avea să fie cunoscut peste timp sub numele de *Lecția de anatomie a doctorului Tulp* şi va fi prima operă în care artistul va aborda domeniul portretului de grup din secolul al 17-lea olandez. Doctorul Nicolaes Pietersz(1593-1657), cunoscut sub numele Tulp, datorită însemnului familial reprezentat printr-o lalea, numit în 1628 profesor de anatomie (*prelector anatomiae*) de către Consiliul chirurgilor din Amsterdam, este înfățişat în timpul prelegerii sale despre tedoanele care permit flexarea degetelor, el însuşi îndoindu-şi degetele într-un gest demonstrativ. Celebra pictură redă un grup de şapte persoane cu gulere albe care asistă extrem de atente la o lecție de anatomie ținută pe cadavrul unui criminal renumit şi spânzurat la Amsterdam în 1632 (este vorba de lecția publică de anatomie ținută în anul 1632 de către doctorul Nicolaes Tulp). Este o disecție publică, la care numărul asistenților era limitat şi pentru care se plătea un bilet (la o astfel de lecție a participat, în 1697, şi țarul Petru I al Rusiei, în periplul său prin țările Europei occidentale. Se spune că întrucât ceilalți ruşi care îl însoțeau au refuzat să participe la manifestare, țarul i-ar fi pus să guste câte o bucățică din cadavru, drept pedeapsă!). Disecția se efectua în lunile răcoroase, pentru a evita răcirea artificială a cadavrului.

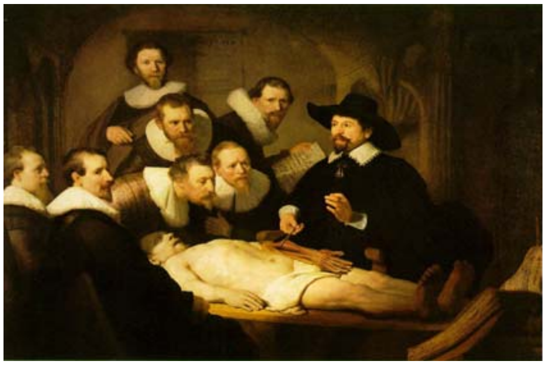

Dacă acest tablou este îndeobşte cunoscut, el figurând destul de des pe coperțile diverselor tratate de anatomie, mai puțin intrată în conştiința publică este o altă pictură a lui Rembrandt inspirată din acelaşi univers tematic - *Lecția de anatomie a doctorului Joan Deyman*, realizată de artist în 1656 pentru acelaşi consiliu al medicilor. În anul 1653 doctorul Deyman îl înlocuieşte pe doctorul Tulp în funcția de inspector al Colegiului Medicilor din Amsterdam. Din această funcție, Deyman apelează la Rembrandt să picteze o disecție rară, cerebrală, disecție realizată pe cadavrul unui delincvent spânzurat la 28 ianuarie 1656 şi disecat în zilele de 29, 30 şi 31 ale aceleiaşi luni. Acest tablou fascinant este în prezent în Muzeul Regal din Amsterdam, însă dimensiunile lui sunt mult mai mici decât ale originalului, datorită deteriorărilor suferite într-un incendiu din 1723. În fragmentul salvat, doctorul Deyman apare în centrul tabloului, în spatele cadavrului, iar celelalte personaje sunt dispuse pe cele două laturi, stând jos sau în picioare.

Dincolo de valoarea artistică indubitabilă, cele două capodopere ale lui Rembrandt reprezintă o transpunere a unor scene dintr-un domeniu vital al vieții ştiințifice din Europa secolului al 17-lea, şi anume practica medicală.

Dumitru NICOLESCU

Imagistica prin Rezonanță Magnetică în tromboza venoasă cerebrală

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Rezumat. Comparativ cu infarctul de cauză arterială, infarctul venos apare mult mai rar dar recunoaşterea lui este foarte importantă deoarece permite instituirea unui tratament adecvat şi cât mai precoce. Simptomatologia în tromboza venoasă este frecvent nespecifică şi deseori pacientul prezintă migrenă şi leziuni ale nervilor cranieni. IRM permite o recunoaştere rapidă şi uşoară a trombului venos şi a leziunilor cerebrale. Tromboza venoasă se asociază de asemenea cu alte complicații ce necesită intervenție terapeutică.

Key words: MRI, thrombus signal, MR venography

Abstract. Thrombosis of the venous system of the brain is an uncommon cause of cerebral infarction relative to arterial disease but is an important consideration because of its potential morbidity. The symptomatology in venous thrombosis is frecquently unspecific and may occur with headache and cranial nerve palsies. MRI permit easier and early recognition of venous sinus thrombosis, offering the opportunity for early therapeutic measures. Venous thrombosis also may be associated with other medical complications that require therapeutic intervention.

Scopul investigației: diagnosticarea unei tromboze venoase cerebrale şi analiza consecințelor sale asupra parenchimului cerebral în perioada inițial următoare, precum şi în repermeabilizarea sinusului trombozat la distanță (după circa o lună). Când este posibil, va fi realizată ca examen de primă intenție. IRM aduce informații atât asupra sinusurilor venoase, cât şi asupra parenchimului cerebral. Majoritatea trombozelor venoase se produc la nivelul sinusului sagital superior şi al sinusurilor laterale. Trombozele izolate corticale sau profunde sunt dificil de evidențiat. Boala veno-ocluzivă cerebrală este o afecțiune cu potențial letal ridicat. Aspectul clinic este de multe ori nespecific, astfel încât IRM are un rol important în stabilirea cât mai rapidă a diagnosticului.

Fiziopatologie. Cunoaşterea anatomiei sistemului venos este esențială pentru evaluarea pacienților cu tromboză venoasă. Infarctul cerebral apare atunci când este lezată o venă sau un sinus venos datorită congestiei sau obstrucției. Tromboza unui sinus lateral asociază clinic migrenă, iar extensia către bulbul venei jugulare produce sindromul de foramen jugular. În tromboza sinusului cavernos pot apărea şi leziuni ale nervilor cranieni. Leziunea se poate complica şi cu hemoragie cerebrală. Metaanaliza ne arată că raportul între numărul de infarcte venoase şi cele arteriale este de circa 1: 62,5, că mortalitatea este între 13,8 şi 48 % în cazurile netratate şi că între 25 şi 30 % dintre pacienți prezintă restitutio ad integrum.

Clinic pacienții pot prezenta crize de tip epileptic, alterarea nivelului de conştiență până la comă, deficite neurologice focale în funcție de zona afectată. Hemipareza şi deficite ale ambelor membre inferioare pot apărea în tromboza sinusului sagital superior. Afazia, ataxia, amețeala şi hemianopsia pot apărea împreună cu sindroamele afectării nervilor cranieni de tipul neuropatiei vestibulare, tinitusului pulsatil, tulburări de auz, vederea dublă. În cazul trombozei sinusului cavernos asociate cu cea a venei oftalmice poate apărea proptoza şi edemul periorbital ipsilateral. Pot fi prezente hemoragiile retieniene şi edemul papilar asociate cu leziuni ale mişcărilor extraoculare şi ptoză.

Protocolul. Secvenţele folosite de obicei sunt de tip sagital şi coronal T1 şi T2, orientate paralel sau perpendicular pe planul sinusurilor venoase şi cu secvenţe de tip SE sau GE, în funcţie de protocolul ales, realizând un raport optim între timpul de examinare şi numărul de secvenţe realizate. Injectarea produsului de contrast paramagnetic precum şi secvenţele de tip angiografic venos, cu flux lent sunt obligatorii pentru realizarea diagnosticului. Secvenţele cu protocol de rutină trebuie să completeze examenul pentru evaluarea corectă a leziunilor parenchimatoase cerebrale. Secvenţa FLAIR este sensibilă la prezenţa trombusului.

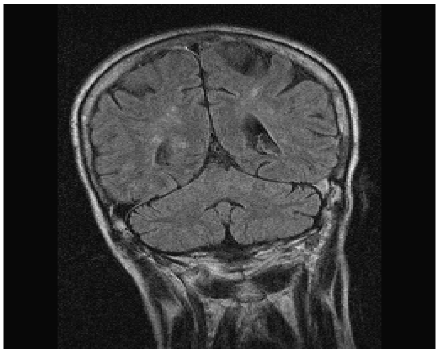

Fig.1. FLAIR coronal. Tulburare de flux în sinusul transvers de partea stângă

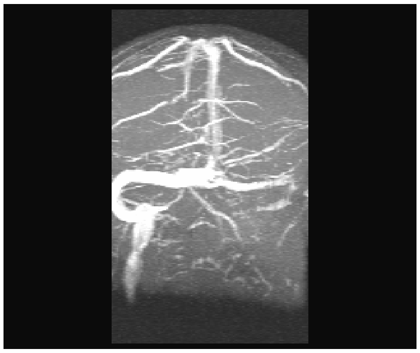

Fig.2. Flebografie RM. Reconstructia MIP evidenţiază diminuarea fluxului în sinusul transvers stâng

Rolul IRM constă în vizualizarea de semne directe, respectiv vizualizarea trombusului venos, şi indirecte, cum sunt leziunile parenchimatoase nesistematizate.

Venele cerebrale sunt avalvulare şi nu prezintă ţesut muscular parietal. Sistemul venos cerebral prezintă două părţi componente, una centrală iar cealaltă periferică. Sistemul venos periferic drenează în sinusul sagital superior, inferior şi în sinusurile transverse prin venele anastomotice sau cele de drenaj. Cele mai importante vene anastomotice sunt **vena cerebrală superficială mijlocie,** care drenează în sinusul cavernos, **vena anastomotică mare,** care drenează în sinusul superior sagital, şi **vena anastomotică posterioară,** care drenează în sinusul transvers.

Sistemul venos profund este format din marea venă Galen, venele cerebrale interne, vena bazală Rosenthal, afluenţii acestora, inclusiv cele transcerebrale şi subependimare.

Tromboza unei vene corticale este evocată atunci când se pune în evidenţă o imagine cu semnal hiperintens T1 şi FLAIR.

Injectarea substanţei de contrast regăseşte semnul delta descris şi CT, şi care constă în evidenţierea trombusului asociat cu priză de contrast periferică parietală.

- Semnalul trombusului venos se comportă la fel ca orice hematom intracerebral. Putem sistematiza evoluţia trombusului venos în două etape:

- înainte de primele 5 zile cu stadiul de oxi- şi dezoxihemoglobină cu aspect de izosemnal T1 şi izo- hipersemnal T2, între 5 şi 30 de zile stadiul de methemoglobină cu semnal hiperintens T1 şi T2;

-după 30 de zile, spre deosebire de hematomul intracerebral, fie apare o repermeabilizare a sinusului venos, evidenţiat prin lipsa de semnal (*flow void*), fie acesta se fibrozează, aspect vizibil prin apariţia unei prize de contrast la nivelul unui sinus venos la nivelul unei structuri cu semnal de fibroză - semnal intermediar T1 şi T2.

- Angiografia prin rezonanță magnetică evidențiază fie o lipsă de semnal la nivelul unui sinus venos în primele 5 zile în TOF (*time of flight*) sau PC(*phase contrast*) , fie după primele 5 zile un semnal slab ce atestă o diminuare a fluxului sanguin în TOF sau o lipsă de semnal în PC. Trebuie avut în vedere faptul că după primele 5 zile semnalul methemoglobinei hiperintens T1 şi T2 poate fi integrat în reconstrucțiile MIP (*maximum intensity projection*) ale sinusurilor venoase, ceea ce poate crea confuzii cu aspectul normal al fluxul venos.

- Semnele indirecte ale leziunilor parenchimatoase cerebrale sunt edemul vasogenic şi transformarea hemoragică mult mai frecventă în cazul infarctului venos față de cel arterial.

- La nivel meningeal putem evidenția priza de contrast anormală la nivel de dura-mater şi o stagnare sanguină intrameningee sau apariția unei circulații de colateralitate.

- IRM poate evidenția uneori şi cauza local declanşatoare sau favorizantă a trombozei.

- Modificările de flux evidențiate IRM sunt în principal datorate tromboflebitei cerebrale infecțioase asociată cu determinări septice cerebrale secundare sau trombozei aseptice.

-Procesele septice asociză simptomatologie clinică sugestivă şi provin în general din vecinătatea feței, meningitei septice sau unor procese septice generalizate.

- Tromboza aseptică apare secundar în cadrul sindromului paraneoplazic, unei deshidratări intense asociate sau nu cu alți factori de risc, cum sunt sarcina, afecțiunile cardiace congenitale sau dobândite, coagulopatiile, vasculita, chimioterapia, prezența tumorilor cerebrale.

-Venograma IRM are limite date de fenomenele de flux din anumite zone ce produc absența semnalului cu rezultat fals pozitiv de tromboză şi de variante constituționale cu diferențiere dificilă între hipoplazia sinusului transvers şi hipoplazia.

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Dicționar de psihiatrie – ALOGIA

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Originea termenului se află în limba greacă, α- însemnând “fără”, iar λόγος “vorbire”, semnificația psihopatologică fiind aceea de reducere semnificativă a fluenței discursului sau a productivității verbale. Alături de aplatizarea afectivă, reducerea gamei de emoții trăite, avoliția şi asocialitatea, alogia reprezintă unul dintre simptomele negative observate în schizofrenie [1]. Aceste simptome pot fi primare (intrinseci procesului schizofren) sau secundare (depresiei, anxietății, fenomenologiei pozitive, deprivării senzoriale, fenomenelor extrapiramidale etc). Manifestările negative se asociază cu spitalizări prelungite şi cu un impact negativ asupra funcționării generale a individului. Alogia face parte şi din sindromul de deficit al schizofreniei, alături de restricționarea afectului, scăderea gamei de emoții şi incapacitatea de a trăi emoții plăcute, reducerea sferei intereselor, diminuarea sentimentului că viața are un sens, reducerea dorinței de interacțiune socială [2]. Poate fi întâlnită şi în episoadele depresive severe, delirium, demențe (în cadrul afaziei) sau tulburările conversive. Mutismul electiv observat la copii este temporar şi selectiv, având o cauză psihologică.

La un pacient cu alogie poate fi remarcată reducerea vorbirii spontane, răspunsurile fiind monosilabice şi lipsite de orice detalii din partea pacientului. In extremis, se poate ajunge la mutism, situație în care contactul verbal cu subiectul nu poate fi stabilit. În cazul unui pacient cu alogie interviul directiv este deseori singura soluție de obținere a unor răspunsuri, în cazul unor întrebări deschise pacientul putând refuza să colaboreze.

O altă variantă a alogiei este cea în care subiectul, deşi stabileşte un contact psihoverbal şi are un debit verbal în limite normale, nu oferă informații relevante, având un discurs vag, circumstanțial, repetitiv, stereotip, excesiv de abstract sau concret. Pacienții cu alogie pot avea o latență crescută a răspunsurilor, par detaşati şi preocupați de altceva decât de circumstanțele interviului.

Hipodopaminergia mezolimbică/mezocorticală a fost implicată în geneza simptomatologiei negative. De asemenea, dereglări ale sensibilității receptorilor glutamatergici NMDA, precum şi disfuncții în mediația colinergică şi hipofuncția sistemului noradrenergic pot contribui la apariția şi menținerea simptomelor negative. Rezultatele terapeutice în cazul simptomelor negative (deci inclusiv al alogiei) sunt modeste, chiar după introducerea antipsihoticelor atipice în arsenalul psihofarmacologiei. Modulatorii glutamatergici, de tipul agoniştilor NMDA glicină şi D-cicloserină au redus, în unele studii, simptomatolgia negativă, atunci când au fost adăugați la antipsihoticele atipice, cu excepția clozapinei [3]. Augmentarea terapiei antipsihotice cu inhibitori de acetilcolinesterază a fost încercată, dar datele nu sunt, până în prezent, concludente [4].

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Abordări psihofarmacologice în prevenția secundară a stresului posttraumatic[1]

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Rezumat. Pacienții expuși la evenimente traumatice au un risc crescut de a dezvolta o tulburare de stres posttraumatic (TSPT). Această analiză a literaturii de specialitate a avut ca obiectiv evaluarea gradului de susținere a unor recomandări privind profilaxia secundară a TSPT prin metode psihofarmacologice. Populația țintă a prezentei analize este constituită din pacienți recent expuși la un eveniment psihostresor sever, care au primit profilaxie secundară de tip farmacologic a TSPT. Acești pacienți au fost ulterior reevaluați, pentru a se detecta procentul de apariție a TSPT. Cele mai importante clase de substanțe, clasificate în funcție de principalele sisteme de neurotransmițători pe care acestea acționează, au fost morfina, anticonvulsivantele, benzodiazepinele, antidepresivele serotoninergice, antipsihoticele, glucocorticoizii, beta-blocantele, alfa 2-agoniștii și alfa 1-antagoniștii. Există diferențe semnificative în privința numărului și a calității studiilor care au evaluat aceste substanțe, iar majoritatea cercetărilor sunt realizate pe loturi restrânse sau reprezintă serii de cazuri. Până în prezent, cele mai bune rezultate au fost obținute prin administrarea beta-blocantelor, urmate de glucocorticoizi și morfină, acestea din urmă doar pe subpopulații bine definite.

Cuvinte cheie: tulburarea de stres posttraumatic, profilaxia secundară a TSPT, intervenții precoce, profilaxie psiho-farmacologică

Abstract. Patients confronted with a traumatic event have a high risk for developing posttraumatic stress disorder (PTSD). This literature review was conducted in order to find the level of evidence for possible psychopharmacologic oriented recommendations in the secondary prophylaxis of PTSD. The target population of this review is represented by patients in the aftermath of a trauma, that received PTSD psychopharmacological prophylaxis and who were reevaluated at a follow-up visit. The main classes of drugs, matched to their neurobiological mediators, were morphine, anticonvulsants, benzodiazepines, serotoninergic antidepressants, antipsychotics, glucocorticoids, beta-blockers, alpha 2-agonists and alpha 1-antagonists. There are significant differences in the number and quality of clinical trials involving these drugs and the majority of data still comes from small scale studies and series of cases. It appears that beta-blockers have the most support from the literature, followed by glucocorticoids and morphine, but only in special subpopulations.

1.Introducere

Există raportări în literatura de specialitate cu privire la o perioadă definită de timp ("golden hours"), în care o intervenție specifică ar putea preveni apariția de sechele somatice sau cel puțin ar putea reduce amploarea consecințelor negative ale unui factor traumatic. Primele luni după expunerea la o traumă sunt importante pentru prevenirea tulburării cronice de stres posttraumatic (TSPT cronic) [1].

Potrivit DSM IV – TR ("Diagnostic and Statistical Manual of Mental Disorder, fourth edition- revised") diagnosticul de TSPT este stabilit dacă există simptome de hipervigilență, rememorări dureroase ale evenimentului traumatizant și evitare a stimulilor legați de traumă, care persistă mai mult de o lună [2]. Toate aceste simptome cauzează suferinţă clinic semnificativă sau deteriorare în funcţionarea socială, ocupaţională sau alte arii importante ale funcţionării individului.

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TSPT este clasificată ca fiind acută (durată mai mică de 3 luni) sau cronică (durată mai mare de 3 luni), existând şi o formă de TSPT cu debut tardiv, în care simptomele apar după 6 luni de la evenimentul traumatizant. Pacienţii care au trăit un eveniment traumatizant şi care au simptome de hipervigilenţă, evitare şi reamintiri dureroase ale evenimentului care au dispărut în mai puţin de o lună după expunere sunt diagnosticaţi ca având reacţie acută de stres (RAS), conform DSM IV TR.

Cele mai multe studii care au evaluat eficacitatea profilaxiei secundare a TSPT sunt de mică amploare, nerandomizate, necontrolate placebo. Datele decelate din cazuri seriate, studii de caz şi studii deschise se constituie în majoritatea informaţiilor pe care le putem folosi pentru o analiză sistematică. Studiile clinice având această temă, sunt de obicei non-comparative, utilizează un singur medicament şi sunt realizate pe o perioadă scurtă de timp.

Un alt aspect important pentru a aprecia utilitatea abordărilor terapeutice este heterogenitatea populaţiei selectate. Cele mai multe studii clinice realizate pe populaţii de veterani, utilizând ca abordări terapeutice atât psihoterapia cât şi terapia medicamentoasă au arătat că tratamentul este mai puţin eficient decât la grupurile selectate din restul populaţiei. Există multe variabile care ar putea explica aceste diferenţe, începând cu natura traumei, expunerea repetitivă la aceasta, contextul profesional, atitudini diferite ale partenerilor de viaţă în populaţiile de militari faţă de cele de civili etc.

Din analiza literaturii rezultă că au fost investite mai multe resurse, cel puţin până acum, în cercetarea prevenţiei prin metode pshoterapeutice a TSPT decât prin intervenţie psihofarmacologică. Numărul studiilor care compară psihoterapia cu terapia medicamentoasă în populaţia cu expunere recentă la o traumă este de asemenea foarte redus. Pentru a fi eficientă, o intervenţie precoce după expunerea la un eveniment traumatizant ar trebui să aibă ca obiective: controlul rapid al manifestărilor neurovegetative comune reacţiei la stres, diminuarea suferinţei emoţionale, încurajarea întoarcerii la funcţionalitatea completă [1]. Utilizarea medicamentelor în această populaţie este dificilă datorită efectelor secundare care ar putea încetini reintegrarea în viaţa socială şi profesională, ar putea scădea calitatea vieţii şi interfera cu activităţile zilnice. De aceea, evaluarea balanţei între eficacitate şi tolerabilitate este importantă şi recomandarea bazată pe dovezi ar trebui să cântărească ambele aspecte ale urmărilor administrării de agenţi psihofarmacologici.

Bazele neurofiziologice ale genezei TSPT sunt încă incomplet elucidate, dar neurotransmiţătorii şi hormonii cel mai probabil implicaţi în această patologie par a fi norepinefrina, serotonina, glutamatul, acidul gama-amino-butiric (GABA), dopamina, neuropeptidul Y, opioidele endogene, glucocorticoizii şi factorul eliberator de corticotropină (CRF). O mare parte a cercetărilor axate pe fiziopatologie, neuroendocrinologie, farmacodinamică, neuroimagistică şi farmacologie clinică au detectat dovezi convingătoare pentru prezenţa hiperactivităţii sistemului noradrenergic la pacienţii cu TSPT [3]. Această hiperactivitate este observată ca răspuns la o multitudine de factori stresori, dar nu apare în condiţii bazale sau de odihnă. Alterarea activităţii neuronilor noradrenergici este asociată cu simptomele de hiperactivare şi retrăire caracteristice TSPT [4].

Serotonina jocă un rol important în reglarea activităţii cortexului prefrontal, amigdalei şi hipocampului, arii cerebrale implicate în fiziopatologia TSPT şi a altor tulburări anxioase [5]. De asemenea, serotonina interacţionează cu neurotransmisia noradrenergică şi modulează nivelul sinaptic al catecolaminelor prin heteroreceptorii presinaptici 5HT1A.

Glutamatul şi GABA sunt cei mai importanţi neurotransmiţători cu proprietăţi excitante şi, respectiv, inhibitorii. Expunerea la factori stresori şi la substanţe corticoide creşte eliberarea de glutamat la nivel cerebral, neurotransmiţător care este implicat în mecanismele de condiţionare şi în consolidarea memoriei. Plasticitatea pe termen lung glutamat-mediată este implicată în consolidarea amintirilor traumatice în TSPT [6]. GABA are efecte anxiolitice, iar stresul sever ar putea altera complexul receptoral GABA-A/BZD [7]. Tratamentul cu benzodiazepine, agonişti GABA şi inhibitori ai recaptării GABA scade simptomele anxioase în TSPT.

În TSPT s-a găsit o creştere în excreţia urinară a dopaminei şi a metabolitului ei principal, dopamina fiind cunoscută ca modulatoare a reacţiilor de condiţionare a fricii. Eliberarea excesivă de dopamină este importantă în hiperactivare, hipervigilenţă şi stările paranoide scurte din cadrul TSPT.

Neuropeptidul Y are proprietăţi anxiolitice şi de diminuare a stresului. O scădere a transmisiei neuropeptidului Y reduce adaptarea la stres şi se corelează cu hiperactivitatea noradrenergică în TSPT [8].

Endorfinele şi enkefalinele sunt neuropeptide endogene implicate în geneza unor simptome cum sunt paresteziile, analgezia şi disocierea din TSPT. Pacienţii suferind de această tulburare au niveluri crescute de beta-endorfine în lichidul cefalorahidian, de aceea o creştere a activării sistemului opioid endogen se crede a fi implicată în genezaTSPT [9].

Pacienţii cu TSPT au niveluri plasmatice şi urinare de cortizol scăzute comparativ cu grupurile de pacienţi care au alte diagnostice psihiatrice şi cu grupurile de control [10]. La datele amintite anterior se asociază creşterea concentraţiei CRF în LCR.

1. Formularea obiectivului cercetării

Scopul principal al acestei analize este de a găsi dovezi în sprijinul abordării psihofarmacologice în prevenţia secundară a TSPT. De asemenea, au fost evaluate datele care asociază medicamentele incluse în studiile clinice privind intervenţia precoce în TSPT cu neurotransmiţătorii sau sistemele hormonale prin care aceste medicamente acţionează. Datorită faptului că TSPT este o tulburare severă, incapacitantă, deseori rezistentă la tratament şi cu tendinţă la cronicizare, considerăm că este important să subliniem importanţa cercetărilor privind prevenţia bolii.

Deoarece abordările psihoterapeutice sunt mai dificil de standardizat şi datorită faptului că mai multe cercetări au fost efectuate în legătură cu abordările psihoterapeutice în profilaxia secundară a TSPT, incluzând două analize sistematice Cochrane (din 2009 şi 2010) [12,13], am analizat doar date referitoare la abordările psihofarmacologice.

2. Metodologie

Această cercetare este o analiză a literaturii care a inclus studii clinice, cazuri seriate şi studii de caz din bazele de date Pubmed, Medscape, Embase şi Cochrane, publicate între 1995 şi 2010. S-au căutat cercetări clinice după cuvintele cheie “TSPT”, “tulburare de stres posttraumatic”, “reacţie acută la stres”, combinate cu “profilaxia secundară”, “prevenţie”, “intervenţie precoce” şi “farmacoterapie”. De asemenea, am analizat Ghidurile de tratament al TPST, detectate prin căutarea în principalele baze de date psihiatrice (American Psychiatric Association, National Institute of Clinical Excellence, Australian Center for Posttraumatic Mental Health).

Toate studiile selectate au inclus populaţii adulte, civile sau militare (veterani ori personal activ) sau populaţii heterogene expuse la traume psihologice. Pacienţii au fost trataţi cu substanţe medicamentoase, dar am inclus şi studii care au implicat o formă de psihoterapie pentru comparaţia eficienţei. Rezultatele analizei literaturii au fost aliniate mecanismelor neurobiologice ale TSPT: fiecare factor neurobiologic implicat în geneza TSPT a fost alăturat medicamentului corespunzător (bazat pe mecanismele farmacodinamice de acţiune a medicamentelor).

3. Rezultate

Toţi cei 9 factori neurobiologici menţionaţi în introducere (norepinefrina, serotonina, glutamatul, GABA, dopamina, neuropeptidul Y, CRF, opioidele endogene şi glucocorticoizii) ca având rol în geneza TSPT au fost identificaţi prin cercetarea literaturii de specialitate. Toţi factorii sunt asociați cu intervenții farmacologice, deşi nu toți agenții terapeutici respectivi sunt întâlniți în mod curent în practica clinică.

Tabelul 1. Criterii de includere şi excludere ale studiilor în/din analiza sistematică

Criterii	**Includere**	**Excludere**
Populația	Populație adultă, militari, civili sau populații amestecate expuse la traume psihologice. Studii, serii clinice sau raporturi de cazuri axate pe prevenția secundară a TPST. Ghiduri Naționale de tratament al TPST.	Studii care au inclus persoane cu comorbidități severe somatice sau psihiatrice. Studii neavând specificată vârsta populației țintă şi rezultate generale care nu ar fi permis formularea de concluzii pentru persoanele peste 18 ani.
Intervenția	Studii care au evaluat farmacoterapia sau farmacoterapia vs. psihoterapie în profilaxia secundară a TSPT; studii deschise, randomizate sau nerandomizate, cu sau fără control placebo.	Studii fără o formulare a intervenției folosite sau având insuficiente criterii pentru a recunoaşte metoda de tratament.
Locul intervenției	Spital, ambulatoriu, unități de îngrijire de zi	Lipsa precizării locului de desfăşurare.
Rezultate	Reducerea incidenței sau diminuarea simptomelor TSPT la vizitele ulterioare (numărul simptomelor, severitatea, nivelul de disfuncții asociate) evaluate de clinician sau autoevaluate.	Studii neavând ca rezultat primar reducerea incidenței/simptomatologiei TSPT.

Unul dintre cele mai recente studii (ianuarie 2010) cu privire la prevenția secundară a TSPT a fost publicat în *New England Journal of Medicine*, de Lisa Troy Holbrook şi colaboratorii [14] şi a implicat o populație de militari. Personalul rănit, aparţinând Marinei şi Infanteriei marine a Statelor Unite, dislocat în Irak, a fost tratat precoce (de obicei sub o oră) post-resuscitare şi post-traumatism, cu morfină pentru durere. Urmarea acestei abordări a fost un risc semnificativ mai mic de a dezvolta TSPT, observat la pacienții care au primit morfină. Importanța acestui studiu nu este dată numai de numărul mare de participanți (n=696), ci şi de acuratețea metodologiei şi de omogenitatea populației
studiate (extrase din *The Navy-Marine Corps Combat Trauma Registry Expeditionary Medical Encounter Database*). Dintre pacienții care au dezvoltat TSPT, 61% au primit morfină; dintre pacienții care nu au prezentat TSPT, 76% au primit morfină (O.R. 0.47; $p<0.001$). Asocierea a rămas semnificativă după ajustarea în funcție de severitatea traumei, vârstă, prezența/absența amputației, precum şi a altor factori clinici selectați legați de traumatism.

Datele de mai sus sunt susținute de un alt studiu [15], care a evaluat pacienți admişi în spital după apariția unui traumatism fizic. Pentru aceşti subiecți (n=155) au fost evaluate: existența tulburărilor psihice, durerea şi doza de morfină în săptămâna inițială după apariția traumei şi apoi la 3 luni post-eveniment (n=120). Subiecții au fost reevaluați pentru a decela apariția TSPT şi a altor tulburări psihice. Pacienții care au îndeplinit criteriile pentru TSPT au primit semnificativ mai puțină morfină decât cei care nu au dezvoltat TSPT. Analiza statistică regresivă a indicat că severitatea TSPT la 3 luni a fost prezisă în mod semnificativ de durerea acută, leziunile cerebrale traumatice uşoare şi doza de morfină în primele 48 de ore după traumă, în urma echilibrării variabilelor după severitatea traumei, sex, vârstă şi tipul traumatismului.

Deoarece rănirea fizică după o traumă (în special leziunile asociate cu dezvoltarea ulterioară a durerii severe) este un factor de risc pentru TSPT, asemenea rezultate sugerează ca potențială metodă de profilaxie reducerea rapidă şi eficientă, în zonele de acordare inițială a îngrijirilor, a durerii persoanelor traumatizate, atât în cazul personalului militar cât şi al populației civile.

Din punct de vedere neurobiologic, activarea adrenergică post-traumă ar putea fi blocată de morfină, prin acțiunea la nivelul receptorilor µ opioizi, localizați atât în amigdală cât şi în locus coeruleus (care găzduieşte cei mai mulți neuroni adrenergici ai sistemului nervos central). Durata tratamentului ar trebui să fie atent monitorizată deoarece riscul de instalare a dependenței de substanțe la pacienții cu TSPT este important.

Glutamatul joacă un rol crucial în răspunsul la stres la om şi în formarea amintirilor traumatice şi, de aceea, investigarea sa în profilaxia secundară a TSPT este justificată. Din nefericire, cele mai multe dintre cercetările referitoare la transmisia glutamatergică în TSPT sunt în stadiul preclinic. Importanța glutamatului în activarea amigdalei este susținută de cercetările de laborator. Agonistul parțial al receptorului NMDA D-cicloserina s-a dovedit a îmbunătăți extincția fricii la rozătoare, dar şi la pacienții cu fobii, cărora le-a fost aplicată terapia prin expunere [16,17]. D-cicloserina oferă oportunități de sporire a efectelor terapiei cognitiv-comportamentale în tulburările anxioase [18].

Anticonvulsivantele care acționează asupra glutamatului sunt posibile instrumente în prevenția secundară a TSPT. Valproatul, carbamazepina, lamotrigina, topiramatul, zonisamida, gabapentinul şi pregabalina acționează asupra neurotransmisiei glutamatergice, fie specific (stimulând eliberarea neurotransmițătorului sau activând receptorii N-metil-D-aspartat), fie nespecific. Există studii care sugerează eficacitatea topiramatului, lamotriginei şi acidului valproic în tratamentul TSPT [19], dar nu există niciun studiu privind profilaxia acestei patologii.

GABA este cunoscut a inhiba activarea indusă de stres a amigdalei, iar receptorii GABA aflați în aria bazolaterală a amigdalei diminuează excitația glutamatergică. Aceasta sugerează că benzodiazepinele sau anticonvulsiantele care potențează transmisia GABA-ergică ar putea fi utile pentru tratamentul acut posttraumatic.

Un studiu mai vechi , realizat de Gelpin et al. (1996) [20], a comparat două benzodiazepine cu potență înaltă în intervenția precoce pentru prevenția TSPT. Deşi la o lună şi la 6 luni nu a existat nicio diferență în scorurile pentru TSPT şi anxietate între cele două benzodiazepine sau între ele şi grupul de control, s-a evidențiat o mică reducere în expresia clinică a hiperactivării neurovegetative.

În al doilea studiu de mică amploare, realizat de Mellman et al. (1998) [21], pacienții tratați cu tetrazepam au avut doar o îmbunătățire a somnului din prima noapte, fără ca benzodiazepina să aibă un impact semnificativ asupra scăderii ratelor TSPT la 6 săptămâni.

"Ghidul Practic pentru Tratamentul Pacienților cu Tulburarea Acută de Stres Posttraumatic" (Asociația Psihiatrică Americană, 2004)[22] recomandă ca "benzodiazepinele să fie evitate la pacienții cu TSPT, pe cât posibil". Un alt ghid, "Managementul TSPT la Adulți şi Copii în Îngrjirea Primară şi Secundară" (Institutul Național pentru Excelență Clinică, Marea Britanie, 2005) [23], nu menționează benzodiazepinele ca o terapie validată pentru prevenția TSPT.

Sistemul serotoninergic are o rețea complexă de interacțiuni atât cu sistemele GABA-ergic, adrenergic, glutamatergic, dopaminergic, cât şi cu axa hipotalamo-hipofizară. De asemenea, s-a observat că administrarea pe termen lung a unui inhibitor selectiv de recaptare a serotoninei (ISRS) ca paroxetina creşte volumul hipocampal la pacienții cu TSPT, având proprietăți de stimulare a neurogenezei [24].

Un studiu comparativ, de mare amploare, randomizat, care a inclus supraviețuitori adulți ai evenimentelor traumatice care au dezvoltat TSPT acută şi au beneficiat fie de terapie cognitiv-comportamentală, terapie prin expunere prelungită sau de terapie cu escitalopram, a arătat că ambele forme de psihoterapie au scăzut prevalența TSPT la 5 luni. Indivizii care au primit tratament în faza acută cu escitalopram nu au diferit ca beneficiu de pacienții tratați cu placebo, sau de cei care s-au recuperat spontan (incluşi în listele de aşteptare) [25].

Tabelul 2. Studii clinice care au folosit benzodiazepine pentru profilaxia secundară a TSPT

Autori	**Număr de pacienți. Design-ul studiului**	**Momentul intervenției post-traumă**	**Medicamente**	**Rezultate**
Gelpin E, Bonne O, Peri T, Brandes D, Shalev AY. [20]	Studiu prospectiv 13 supraviețui-tori ai unei traume	6.7 +/- 5.8 zile dupa traumă (interval, 2-18)	**clonazepam** (N = 10, 2.7 +/- 0.8 mg/zi) sau **alprazolam** (N = 3, 2.5 mg/dzi).	Subiecții din grupul ce a primit benzodiazepine nu a diferit față de grupul de control în ceea ce priveşte scorurile la o lună şi 6 luni pentru TSPT şi anxietate.
Mellman, T. A., Bustamante, V., David, D., et al. [21]	Studiu mic descriptiv (n=4)	Nespecificat	30 mg **temazepam** zilnic pentru 5 zile, zilnic pentru 2 zile.	Fära diferențe statistice în ratele TSPT la punctul de urmărire 6 săptămâni, deşi a fost găsită o tendință în grupul placebo(k = 1; n = 22; RR = 3.2; 95% CI 0.54–18.98). Grupul care a primit tetrazepam a avut o îmbunătățire semnificativă a somnului din prima noapte după eveniment , dar nu au existat diferențe semnificative între grupuri la următoarele vizite.

Având în vedere numeroasele tipuri de receptori de serotonină, o analiză a literaturii ştiințifice sugerează că intervenția precoce psihofarmacologică ar putea fi realizată mai ales cu agenți care activează receptorii 5HT1A, cum ar fi ISRS-urile, venlafaxina, antidepresivele triciclice sau inhibitorii mnoamin-oxidazei. În plus, medicamentele care blochează receptorii 5HT2, cum ar fi nefazodona sau trazodona, ar putea fi de asemenea utile. Din păcate, nu există date la ora actuală care să susțină eficacitatea agenților serotoninergici în prevenția secundară a TSPT. Există şi dezavantaje ale antidepresivelor serotoninergice: au o latență crescută a acțiunii teraputice, majoritatea studiilor clinice sugerând o perioadă de cel puțin două săptămâni până la apariția efectului clinic; unele medicamente, cum ar fi ISRS-urile, ar putea exacerba anxietatea în prima săptămână de administrare, la pacienții cu TSPT.

Dopamina este implicată în răspunsul la stres al amigdalei şi administrarea de antagonişti de dopamină cum ar fi antipsihotice atipice sau cele convenționale ar putea să amelioreze reacția acută la stres. Nu există, până în prezent, niciun studiu despre eficacitatea antipsihoticelor în prevenția secundară a TSPT.

Neuropeptidul Y inhibă eliberarea atât de norepinefrină, cît şi de CRF, de aceea este implicat în fiziopatologia reacției de stres. Datele din cercetarea clinică şi preclinică sugerează că medicația care stimulează secreția acestui neuropeptid ar putea ameliora RAS, TPST şi alte probleme induse de stres. Concentrațiile plasmatice ale neuropeptidului Y par să fie direct asociate cu recuperarea din TSPT, pentru că erau mult mai mari la veteranii de război care avuseseră TSPT decât la veteranii care aveau TSPT în momentul determinării sau la veteranii care nu au dezvoltat niciodată TSPT [26]. În prezent, în practica medicală nu există niciun drog care să acționeze prin intermediul neuropeptidului Y.

Glucocorticoizii influențează simptomele TSPT, cum ar fi coşmarurile cu tematică legată de traumă, flashback-urile, reamintirile din timpul zilei. Pacienții care au fost tratați într-o unitate de terapie intensivă (ATI), acuză adesea amintiri traumatice asociate cu dezvoltarea TSPT. Pacienții grav bonavi din ATI prezintă de multe ori cantități scăzute de glucocorticoizi endogeni. Mai multe studii controlate efectuate pe pacienți din ATI cu insuficiență severă multiorgan, boli cronice complicate, şoc septic sau status post-chirurgie cardiacă au arătat că administrarea de hidrocortizon determină o reducere semnificativă a simptomelor TSPT pe termen lung la supravieţuitori şi îmbunătăţiri în calitatea vieții acestor pacienți [27]. Există motive întemeiate să presupunem că antagoniştii CRF ar putea avea efecte benefice asupra simptomelor TSPT. Aceşti agenți sunt în prezent utilizați în cercetări pe animale şi sunt în curs de dezvoltare de către companiile farmaceutice, fără a fi însă disponibili pentru uzul clinic.

Norepinefrina este implicată în consolidarea reminiscențelor memoriei traumatice după acțiunea unui stimul negativ puternic. Această reminiscență supraconsolidată este motivul pentru generarea TSPT. Scopul terapeutic urmărit de acțiunea asupra sistemului adrenergic este de a inhiba activarea excesivă a receptorilor alfa-1 şi beta adrenergici, precum şi de a augmenta influența inhibitorie a receptorilor alfa-2 adrenergici. Rezultatul unui asemenea tratament ar fi acela de a reduce activarea amigdalei, de a potența funcția PFC şi de a inhiba stimularea locus coeruleus sau activarea secundară a altor structuri corticale şi subcorticale.

Tabelul 3. Beta-blocantele în prevenția secundară a TSPT

Autori	**Nr. de pacienți. Design studiu**	**Momentul intervenției după traumă**	**Medicamente**	**Rezultate**
Vaiva G, Ducocq F, Jezequel K şi al. [28]	N=19	2 până la 20 de ore (media =9.5)	7 zile **propranolol** 40 mg de 3ori pe zi ;doza scade progresiv în 8-12 zile, voluntarii tratați (n=11) versus efectele lipsei tratamentului la cei care au refuzat medicația, dar au fost de acord să participe la studiu (n=8).	La 2 luni scorurile Scalei Rezulatului Tratamentului TSPT au fost mai mici la cei care au luat propranolol comparativ cu cei care nu au urmat tratamentul (p=0.037), la fel ratele TSPT (unul din 11 versus 3 din 8, p=0.012). 37,5 % din cei ce au refuzat propranololul au avut simptome de TSPT, în contrast cu 9% din cei care au acceptat medicația.
MB Stein, CKerridge, J.Dimsdele , DB Hoyt. [29]	n=48 dublu-orb	În primele 48 de ore de la traumă	14 zile cu betablocant **propranolol** (n=17), anticonvulsivantul anxiolitic **gabapentin** (n=14) sau **placebo**(n=17).	Niciunul dintre droguri nu a arătat beneficii semnificative față de placebo, pe simptomele depresive sau de stres posttraumatic la 1,4 şi 8 luni.
Pitman RK, SandersK B, Zusman RM [30]	N=41,pro-spectiv, controlat placebo	În primele 6 ore după eveniment	**propranolo**l 40 mg de 4 ori pe zi (n=18) versus placebo (n=23), 10 zile.	La 4 săptămâni simptomele tulburării au fost detectate la 30 % din subiectii care au primit placebo şi la 18 % din cei care au primit propranolol prin aplicarea Clinician-Administered TSPT Scale (CAPS) (p=0.15).
Krausenec T,Padberg F.Roozen-daal B[31]	N=108, observati-onal study	Nespecificat	**metoprolol**	La 6 luni femeile tratate cu metoprolol aveau un nivel semnificativ mai mic al memoriilor traumatice şi al scorurilor simptomelor TSPT decât femeile ce nu au primit beta-blocant (p=0.02).

Într-un studiu realizat de Vaiva et. al., administrarea precoce de propranolol a fost mai eficientă, semnificativ statistic, decât lipsa tratamentului, după 8 săptămâni, deoarece a redus ratele TSPT (9% vs. 37.5 %, p=0.012). Un studiu de mai mare amploare (n=108) a arătat rolul favorabil al metoprololului în prevenția TSPT , în cazul femeilor expuse la traumă [31].

Există, de asemenea, un studiu dublu orb care nu a arătat nicio diferență de evoluție la subiecții tratați cu propranolol sau gabapentin față de placebo [29]. Într-un alt studiu, deşi la 4 săptămâni nu erau diferențe semnificative între propranolol şi placebo, la 12 săptămâni pacienții tratați cu substanță activă au arătat scăderea responsivității fiziologice la probele de imagerie legată de evenimentul traumatic [30].

Kinzie şi Leung au descris unele beneficii ale utilizării clonidinei (agonist alfa-2) în managementul TSPT la refugiații sever răniți din Cambodgia, deşi profilul tolerabilității scăzute a acestui drog a contribuit la o lipsă a investigărilor suplimentare ale acestei abordări [32].

Prazosinul (alfa1- blocant) a scăzut incidența coşmarurilor la personalul militar diagnosticat cu TSPT [33], dar nu există studii în domeniul prevenției TSPT.

O prezentare sintetică a datelor din analiza noastră şi a nivelului de evidență este redată în tabelul următor.

Tabelul 4. Prezentare sintetică şi gradul de susținere a recomandărilor terapeutice

Sistemele de neurotransmițători	**Studii clinice (SC)**	**Nivelul de susținere (GRADE)**	**Recomandări**
Endorfine	2 SC (+), mai multe raportări de caz	C	Pacienți cu leziuni fizice, durere, discomfort somatic sever
Glutamatul, NPY, CRF, dopamina	Niciunul	-	Niciuna
GABA	2 SC (1+, 1-)	D	Temazepam, ca hipnotic
Serotonina	1 SC(-, escitalopram vs. psihoterapie)	D	Niciuna
Glucocorticoizi	1SC (+)	C	Hidrocortizon pentru populații selectate (ATI, şoc septic)
Norepinefrina	4 SC (2+, 1-, 1?) pentru beta-blocante	B	Beta-blocantele ar putea fi utile (propranolol, metoprolol), posibile diferențe legate de sex ale efectului

Gradul:

A: calitate înaltă - cercetări ulterioare este foarte puțin probabil să schimbe încrederea în estimările efectului;

B: calitate moderată - cercetările ulterioare este probabil să aibă un impact important în încrederea în estimarea efectului şi ar putea schimba estimarea;

C: calitate joasă - cercetările ulterioare este foarte probabil să aibă un efect important asupra încrederii în estimările efectului şi este probabil să schimbe estimarea;

D: calitate foarte joasă - orice estimare a efectului este foarte incertă.

5. Comentarii

Există anumite limitări ale acestei cercetări deoarece am inclus doar pacienți adulți, fără a include abordarea combinată psihoterapie şi psihofarmacologie. Numărul mic de studii (n=10) şi heterogenitatea metodologiei utilizate indică necesitatea explorării suplimentare în câmpul farmacoterapiei aplicate pentru prevenția secundară a TSPT.

Interesul pentru acest subiect este reflectat de numărul mare de studii clinice care sunt în desfăşurare. Nu mai puțin de 15 studii sunt listate pe cunoscutul site oficial de profil http://clinicaltrials.gov, implicând medicamente ca sertralina, escitalopramul, mifepristona, hidrocortizonul. Toate aceste studii au fost găsite după cuvintele cheie "TSPT" şi "prevenție", în urma excluderii cercetărilor axate pe abordări psihoterapeutice.

În timp ce neurobiologia TSPT a dezvoltat în continuu noi modele de înțelegere a acestei afecțiuni, cercetarea farmacologică pentru intervenția precoce în TSPT este încă la începuturile sale.

6. Concluzii

Căutarea unui medicament miraculos, care ar putea opri dezvoltarea TSPT în urma expunerii la un traumatism este, în mod evident, de mare importanță, deoarece această patologie este ridicată în rândul persoanelor expuse la incidente critice. Până acum, un număr important de medicamente au fost studiate în cercetări clinice de amploare mică şi medie, serii de cazuri şi studii de caz, dar aceste date nu sunt foarte convingătoare atunci când se pune problema formulării unor indicații terapeutice. Expectațiile din partea cercetării vizează descoperirea de noi medicamente, cu efecte secundare mai puține şi eficacitate mai mare, dar şi evaluarea în studii mari, randomizate, a medicamentelor existente.

Recomandarea bazată pe cele mai multe dovezi, privind profilaxia secundară a TSPT, este administrarea unui beta-blocant, deşi clinicianul ar trebui să țină cont de efectele adverse posibile ale acestei clase de medicamente.

Morfina este recomandată la pacienții cu traume fizice, pentru ameliorarea durerii şi evitarea consolidării amintirilor traumatice.

Medicamentele GABA-ergice, de tipul benzodiazepinelor, ar putea fi utilizate cu prudență pentru simptome ca insomnia, dar proprietățile profilactice privind TSPT ale acestor medicamente nu sunt susținute de dovezi. Hidrocortizonul este util pentru pacienții din ATI, cu şoc septic şi poate şi în alte condiții în care se decelează niveluri scăzute ale cortizolului endogen.

Agenții serotoninergici vor primi în continuare probabil mai multă atenție pentru prevenția secundară a TSPT, dar deocamdată nu există suficiente date pentru a formula o astfel de recomandare.

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Tratamentul dependenței de tutun și metode de stopare a fumatului

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REZUMAT. Există o gamă variată de opțiuni de tratament care s-au dovedit eficiente, inclusiv terapiile comportamentale şi farmacologice. Niciuna dintre terapii nu poate fi utilizată în mod individual, excluzându-le pe celelalte, pentru că variază semnificativ eficacitatea acestora, accesibilitatea, eficiența şi costul individual. De exemplu, farmacoterapiile tind să solicite mai puțin timp de la indivizi şi sunt mai eficiente decât terapiile comportamentale, dar pot avea un cost mai crescut. Oricum, pentru anumiți indivizi (gravide, persoane cu boli cardiace) costurile se justifică datorită faptului că beneficiile renunțării sunt excelente. Intervențiile minimale ale personalului medical oferă nu doar o importantă abordare din punct de vedere al raportului cost-eficiență, ci ajută la schimbarea culturii într-una în care aceştia devin modele de nefumători. La baza varietății de metode demonstrate şi a nevoilor existente pe plan intern şi extern poate lua naştere principiul conform căruia țelul ideal ar fi maximizarea opțiunilor şi oportunităților intervențiilor de stopare, atât asupra întregii populații, cât şi asupra fiecărui individ.

Cuvinte cheie: nicotină dependență abstinență sevraj receptori nicotinici

ABSTRACT. There is a wide range ot treatment options that have been proved effective, including behavioural and pharmacological therapies. There is no single approach that should be emphasized to the exclusion of others because the therapies vary widely in their efficacy, their acceptability, their cost-effectiveness and their cost on an individual and population basis. For example, pharmacotherapies tend to require less human resource time to deliver and are more effective than many behavioural therapies, but can cost more in material resources. However, for some populations (pregnant women, and persons with heart disease) for whom there is an especially great benefit of cessation, the costs may be more readily justified. Minimal interventions by health professionals offer not only an important and cost-effective approach, such interventions can help to change the culture from one in witch the health professionals are models for non-smoking. On the basis of the variety of provern methods and variety of needs within and across coutries, it would appear that the ideal goal would be to work towards maximizing the options and occasions for smoking-cessation interventions, both population and individual based.

PREAMBUL

Terapia comportamentală şi farmaceutică împotriva dependenței de tutun şi în scopul renunțării la fumat poate contribui substanțial la îmbunătățirea stării de sănătate prin acceptarea ideii de stopare a fumatului. Chiar şi pentru persoanele care au încercat recent abținerea de la fumat, fără terapie, beneficiile pot fi însemnate dacă tratamentele le ajută să dobândescă abstinența mai devreme, pentru că riscul îmbolnăvirii este legat de perioada fumatului.

Sunt discutate mai multe concepte care să contribuie la importanța plasării terapiei în cadrul "triunghiului" de control: TUTUN-BOALĂ-EFORT. Primul concept pentru a înțelege dispoziția în tratarea unei boli este cauza bolii, iar anumiți factori pot împiedica eficacitatea şi utilizarea tratamentului. În cazul dependenței de tutun, sunt cel puțin 3 elemente importante de luat în considerație:

-primul element este considerat faptul că dependența de tutun este un puternic proces biologic şi social care impietează mult dobândirea şi menținerea renunțării la tutun;

-al doilea este acela că dependența de tutun este adesea însoțită de o teamă nerealistă în ceea ce priveşte tratamentul, în aceeaşi măsură în care se presupune că tratamentul nu este necesar. Acest lucru este tipic pentru procesele de dependență, uneori fiind asociat ca factor *"de negare"* sau *"rațional"* datorită tendinței persoanei dependente de a nega faptul că este cu adevărat dependentă, că are nevoie de ajutor, precum şi pentru considerentul că îşi imaginează că tratamentul este mai riscant decât boala în sine;

-al treilea element este acela că boala este promovată de către industria de tutun într-o manieră fără precedent, ceea ce angajează toate metodele de presiune economică, socială, politică şi de reglementare care să-i permită menținerea şi susținerea dependenței de tutun.

Tratamentul poate fi posibil, dar rareori uşor de realizat în implementarea renunţării la fumat. Întrucât produsele de tutun sunt comercializate pentru a obţine o maximă atractivitate din partea consumatorilor, medicamentele pentru tratarea dependenţei de tutun sunt produse în scopul siguranţei sănătăţii şi a eficacităţii, acordându-se atenţie deosebită şi asupra diminuării folosirii lor în mod repetat sau a apariţiei dependenţei. Acest lucru înseamnă că tratamentul este, prin concepţia sa, *"apelat"* în mai puţine rânduri decât tutunul şi, întrucât problema principală în ceea ce priveşte tutunul este permiterea şi susţinerea stopării consumului acestuia, un aspect important al majorităţii tratamentelor este acela de a favoriza respectarea şi folosirea adecvată în a dobândi şi a susţine abstinenţa de la tutun. Mai mult, industria de tutun oferă posibilitatea ca produsele sale să fie uşor de găsit, atractive şi foarte permisibile, în timp ce farmacoterapia este mai greu de accesat, adesea fiind disponibilă numai prin prescripţie medicală sau la puncte cu vânzare limitată şi, în plus, este mai scumpă, deoarece, în general, se găseşte în pachete care includ schema de tratament şi suficiente elemente care să descurajeze uzul ocazional, ca un susbstituent temporar pentru tutun.

Prin similitudine, terapia comportamentală nu este adesea uşor disponibilă, este consumatoare de resursă umană şi a fost apelată de sub-populaţii şi fumători.

Statistica, în ceea ce priveşte farmacoterapia, oferă multiple opţiuni indivizilor, importante pentru a se integra cu variatele preferinţe individuale. Acestea includ mai multe forme de terapie a înlocuirii nicotinei (gumă de mestecat, tabletă, plasture, spray nazal sau inhalant oral) şi bupropion.

Eficacitatea generală, dovedită, a medicamentelor este similară cu furnizarea unei dublări aproximative a probabilităţii pe termen lung a stopării fumatului. Tratamentul comportamental poate avea efect la propriu şi poate contribui la creşterea substanţială a succesului farmacoterapiei.

Tratamente eficiente şi extrem de scumpe au fost revizuite în multe ţări şi instituţii care pledează asupra necesităţii ca întreg personalul medical şi clinicienii să intervină în mod constant în scopul stopării fumatului la pacienţii lor.

Aria tratamentului comportamental, începând de la intervenţii scurte ale personalului medical specializat în a încuraja şi a menţine stoparea fumatului, până la programe cu o structură complexă privind încetarea fumatului, are ca domeniu eliminarea apetitului pentru tutun, precum şi recidiva folosirii acestuia.

Preocupări majore ale secolului XXI vizează tratamente relevante din punct de vedere cultural şi specializate pentru populaţie, adaptate fiecărui individ, cu un mare grad de accesibilitate, similar modalităţilor de obţinere a produselor de tutun. Tratamentul ar trebui oferit ca o componentă a conştientizării eforturilor societăţii de a controla consumul de tutun, ceea ce presupune: taxe crescute la ţigări, restricţii asupra fumatului în spaţiile publice, activităţi educative mult mai convingătoare asupra pericolului pe care-l reprezintă fumatul şi beneficiile renunţării la acest viciu, restricţii asupra comercializării produselor de tutun. Aceste elemente sunt hotărâtoare în cadrul eforturilor de stopare, a tratamentul utilizat şi în menţinerea abstinenţei faţă de tutun.

Terapia comportamentală

Eficacitatea terapiilor comportamentale în tratamentul dependenţei de tutun a fost revizuită, pe înţelesul tuturor, într-un ghid clinic practic, publicat în SUA, intitulat *"Tratarea fumatului şi a dependenţei"*, în rapoarte ale medicilor generalişti americani, din cadrul Departamentului de Sănătate şi Servicii Medicale din SUA (2000) şi englezi, de la Academia Medicală Regală a Marii Britanii, precum şi prin tratamente online.

O varietate de terapii comportamentale şi-a dovedit eficacitatea pentru mulţi fumători. Aria terapiilor comportamentale este complexă, de la simplul sfat oferit de un specialist, până la modalităţile extinse oferite de clinici specializate în consilierea stopării fumatului. În mod evident, odată cu nivelul de complexitate al terapiei creşte şi costul pe care fumătorul îl suportă, precum şi poliţa de asigurare, iar accesibilitatea terapiilor descreşte, în special în ţările în curs de dezvoltare.

1. Sfatul medicului specialist

Simplul sfat al unui specialist s-a dovedit util în creşterea ratei de abstinență cu până la 30%, în raport cu absența consilierii. Chiar dacă nu au existat studii sistematice de consiliere efectuate de către clinicieni, se constată o sporire similară a ratei de abstinență.

Ghidurile din SUA recomandă 5 abordări:

1. întreabă despre utilizarea tutunului;
2. sfătuieşte-i pe toți fumătorii să renunțe;
3. probează-i bunăvoința de a renunța rapid;
4. urmăreşte pacientul să renunțe;
5. pregăteşte contactul următor.

Întrucât efectul absolut al unui scurt sfat este relativ mic, această intervenție poate avea un impact considerabil atunci când numărul celor care apelează la medicii specialişti este mai mare. Medicii specialişti sunt de neînlocuit datorită abilității lor de a-i consilia în egală măsură pe pacienții care vor să renunțe, precum şi pe cei care nu au această intenție. Fumătorii care nu intenționează să renunțe la acest obicei pot primi informații mai documentate de la medici, fiind sensibilizați atât asupra efectelor nocive, cât şi a modalităților de stopare a fumatului, în mod special prin relevarea beneficiilor rezultate.

Pentru fumătorii care sunt nesiguri de abilitatea lor de a renunța, specialiştii pot utiliza strategii motivaționale, cum ar fi discutarea despre barierele asupra stopării fumatului şi soluțiile pentru depăşirea acestora. Beneficiile unor astfel de intervenții pot fi întărite cu elemente particulare, cum ar fi încurajarea pacientului de a selecta o anumită zi pentru a renunța la fumat şi personalizarea câştigului potențial.

Pentru fumătorii pregătiți să renunțe, specialistul poate oferi un suport motivațional puternic care să-i ajute să stabilească o dată calendaristică de renunțat, să le prescrie terapii farmaceutice pentru dependența de nicotină şi să le sugereze strategii comportamentale pentru a preveni recidiva.

2. Materiale de ajutor individual

Materialele universale de ajutor individual nu sunt mai utile decât consilierea oferită de specialist, dar sunt mai eficiente decât lipsa oricărei intervenții şi prezintă avantajul de a fi accesibile unui număr mare de persoane cu venituri modeste, datorită costului relativ scăzut. Prin urmare, ele pot avea o anumită valoare, chiar dacă nu prezintă aceeaşi eficacitate ca medicația. Materiale cu mesaj de încurajare a fumătorilor de a renunța la fumat şi de a-i ajuta în acest demers sunt în continuă creştere, fiind larg răspândite în țările dezvoltate.

În alegerea unor anumite materiale, spre furnizare sau recomandare către pacient, este important să se țină cont de accesibilitatea acestora. Este util ca ele să conțină un limbaj specific, adecvat unui anumit nivel de instruire şi acces cultural. De exemplu, în unele culturi trebuie să existe o corelație între beneficii, respectiv partea rațională asupra stopării fumatului, şi familie. Majoritatea materialelor sunt în mod obişnuit tipărite, iar aria lor de cuprindere poate fi de la o pagină, cu un scurt ghid şi trucuri, până la un manual structurat cu exerciții îndreptate spre o renunțare rapidă. Dintre resurse pot fi incluse casete audio, video, sau programe pe calculator, precum cele larg răspândite pe site-urile web disponibile. Accesibilitatea crescută a internetului poate crea oportunități pentru terapii de ajutor, structurate individual.

Modalități recente de abordare sunt concentrate pe executarea unor materiale de ajutor individual adecvate nevoilor proprii ale individului. Materialele individualizate se bazează pe răspunsul individual al fumătorului, în acest caz putând furniza persoanelor o reacție (autocontrol) interactivă, informații despre stadiul lor de schimbare, considerații privind balanța decizională pro sau contra renunțării la fumat, tentații care pot dezvolta, la majoritatea fumătorilor, situații şi tehnici specifice cu care aceştia se confruntă. Contactul telefonic este un mijloc economic care trebuie adăugat la lista materialelor de ajutor individual. Şase experiențe analizate de Cochrane cuprind beneficiile obținute în urma consilierii primite prin telefon.

3. Intervenţii la nivel comportamental şi psihologic

Suportul comportamental, incluzând numeroase şedinţe de consiliere individuală sau de grup, ajută la stoparea fumatului. Ambele terapii (individuală şi de grup) au contribuit la îmbunătăţirea ratei de renunţare, în comparaţie cu utilizarea materialelor de ajutor individual. Rata de renunţare este similară în cazul ambelor terapii folosite. În consecinţă, oricare terapie poate aduce beneficii.

De regulă, terapiile de grup sunt mai ieftine, dar utilitatea lor poate fi limitată de dificultăţile întâmpinate în recrutarea şi păstrarea participanţilor. Trei modalităţi de consiliere şi terapie comportamentală au demonstrat rate crescute de abstinenţă:

1) furnizând fumătorilor rezolvarea problemei/instruirea dobândirii acestor deprinderi (de exemplu: evitarea situaţiilor unde există fumători, identificând piedici pentru fumat);

2) acordând suport social, ca parte a tratamentului;

3) ajutând fumătorii să obţină suport social în afara tratamentului.

O altă componentă a terapiei comportamentale este fumatul care vindecă prin sublinierea efectelor nocive. Acest tip de terapie îi vizează pe pacienţii care fumează intensiv, adesea până la punctul de disconfort, greaţă sau vomă. Terapia de aversiune împleteşte stimulul plăcerii, obţinute prin fumat, cu stimulii dezagreabili. Obiectivul este eliminarea impulsului de a fuma. O astfel de terapie poate fi eficientă, dar necesită suport mai complex decât alte terapii comportamentale şi pare a nu fi pe deplin acceptată.

Un principiu de bază al terapiei de contact persoană-persoană îl constituie principiul *”mai mult este mai bine”*. Întrucât contactul minim (mai puţin de 3 minute), sau contactul cu un medic specialist, poate mări rata de abstinenţă până la 30%, comparativ cu lipsa oricărui contact, consilierea pe o durată mai lungă (peste 10 minute) poate dubla rata de abstinenţă.

4. Campanile de comunicare prin mass-media

Impactul mass-mediei a fost studiat intens în Australia, Canada, Finlanda şi SUA. Campaniile mass-media pot creşte conştientizarea în ceea ce priveşte efectele nocive ale fumatului asupra sănătăţii şi beneficiile care rezultă din stoparea acestuia. Acestea pot schimba şi întări atitudinea asupra stopării fumatului, furnizează indicaţii pentru o acţiune simplă şi influenţează comportamentul fumătorului.

5. Linii telefonice speciale sau servicii de internet pentru campania de renunţare la fumat

Liniile telefonice speciale au costuri reduse şi sunt uşor accesibile serviciului de stopare a fumatului. Acestea furnizează suport confidenţial şi anonim fumătorilor care doresc să renunţe. Pot fi disponibile toată ziua, inclusiv seara şi în weekend.

Liniile telefonice speciale folosesc două moduri de abordare: ***reactiv***, în care fumătorul poate apela direct, şi ***proactiv***, în care consilierii sună înapoi solicitanţii acordându-le suportul de care au nevoie.

Eficienţa şi preţul de cost ale consilierii telefonice proactive pentru stoparea fumatului sunt larg recunoscute.

Eficacitatea consilierii telefonice reactive este mai greu de demonstrat, datorită preluării şi controlului aleator al probelor ce conţin un serviciu reactiv. Oricum, este din ce în ce mai evident că alte metode de evaluare pot fi mai adecvate pentru serviciile reactive, deşi consilierea telefonică reactivă poate fi eficientă şi necostisitoare.

Liniile telefonice speciale destinate renunţării la fumat sunt populare şi uşor de accesat de către fumători. Spre exemplu, un studiu din Marea Britanie concluzionează că printr-o singură intervenţie s-a identificat 4,2% din totalul populaţiei adulte fumătoare din Anglia, ceea ce a reprezentat un succes important.

Liniile speciale pot fi apelate de către grupuri care au dificultăţi în accesarea serviciilor destinate stopării fumatului. Linia telefonică asiatică a Marii Britanii primeşte 20.000 de apeluri/an şi identifică 10% dintre fumătorii din Asia de Sud. Pentru marea majoritate a apelanților, linia telefonică reprezintă primul lor contact cu orice formă de serviciu pentru stoparea fumatului.

Liniile telefonice speciale destinate renunțării la fumat intensifică dezvoltarea unei game de servicii de consiliere, de calitate, folosind internetul, care sunt integrate cu liniile telefonice de ajutor. Un număr de astfel de linii în țări europene ca Danemarca, Germania, Olanda şi Suedia dezvoltă servicii inovatoare construite şi adaptate la tendința generală a acestora.

În prezent există numeroase site-uri disponibile pe internet, capabile să ofere fumătorilor ajutor şi suport. Două dintre cele mai cunoscute sunt www.uitnet.org (care rulează în asociație cu Departamentul de Sănătate Publică a Universității din Boston) şi www.stop-tabac.ch (înființat de Universitatea din Geneva). Alt site este www.netdoctor.com.

În Marea Britanie fumatul este răspândit în special în rândul muncitorilor necalificați, aceştia având acces redus la internet (27%, față de 70% în rândul celor calificați). Oricum, odată cu apariția noii tehnologii, mai rapide, este evident că accesul la internet va fi în curând mult extins. Ca un mijloc de livrare a informării şi suportului de îngrijire a sănătății, internetul are potențialul de a identifica o arie răspândită de fumători şi consumatori de tutun. Internetul oferă potențial în găsirea unui număr mare de fumători, având costuri reduse, iar competiția constă în dezvoltarea unei arii de servicii web inovatoare şi eficiente pe care fumătorii să dorească să le folosească.

6. Competiții de renunțat cu succes la fumat de tipul "*renunță şi câştigă*"

Reprezintă o metodă, dovedită şi necostisitoare, de stopare a fumatului în rândul unei mari părți din populație, aceasta susținând tangențial şi controlul național al fumatului la locul de muncă. Campaniile *"renunță şi câştigă"* utilizează metode inovatoare de comunicare, parteneriate, organizații comunitare şi implicări în serviciul de sănătate din care să rezulte o rată de renunțare la fumat în jur de 20%.

Metodele conservatoare de evaluare a participanților, după 1 an, arată că între 10 şi 30% dintre participanți au rezistat fără nicio țigare în acest interval, folosind campaniile "*renunță şi câştigă"*.

"Renunță şi câştigă" este o campanie de stopare a fumatului în continuă creştere şi conține mesaje pozitive pentru fumători. Participanții se opresc din fumat pentru cel puțin o perioadă competitivă de 4 săptămâni şi, dacă reuşesc, câştigă premii. Organizatorii campaniilor *"renunță şi câştigă"* din diverse țări furnizează resursele financiare şi premiile pentru campanile locale, urmând un protocol internațional standard.

Au prioritate în concurs toți participanții care au folosit zilnic tutunul timp de cel puțin 1 an şi care au vârsta peste 18 ani.

Centrul internațional de coordonare *"Renunță şi câştigă"* de la Institutul Național de Sănătate Publică din Finlanda şi campaniile organizate sub acest titlu sunt în strânsă colaborare cu Organizația Mondială a Sănătății.

7. Locurile speciale pentru fumat

Spațiile speciale destinate fumatului la locul de muncă reprezintă, în domeniul sănătății publice, o modalitate eficientă ce încurajează pe termen lung motivația privind renunțarea la utilizarea tutunului. Mai mult, această măsură poate include un număr mare de indivizi la un cost minim. Lucrul individual cu fumătorii este un factor important, dar are un impact limitat dacă nu se iau în calcul şi factorii înconjurători care promovează şi susțin fumatul.

Amenajarea locurilor speciale pentru fumat, în special a amplasamentelor care conțin acest mesaj, reprezintă o componentă esențială a oricărei strategii care promovează stoparea fumatului.

SUA au o experiență de peste 20 de ani în încercarea de schimbare a politicii publice de promovare a locurilor speciale pentru fumat. Crearea politicilor publice şi private de restricționare a fumatului s-a dovedit a fi un mod de abordare extrem de eficace în promovarea stopării fumatului. Numeroase studii relevă efectele acestor restricții asupra comportamentului angajatului fumător, demonstrând o comprimare a consumului mediu zilnic de țigări şi o scădere a ratei globale de prevalență a fumatului.

Procentul fumătorilor care intenţionează să renunţe la fumat este mai mare acolo unde există astfel de amplasamente care, la rândul lor, par să promoveze renunţarea pe termen îndelungat. Documentele deţinute de industria de tutun dezvăluie faptul că restricţiile impuse celor care fumează au fost considerate o ameninţare majoră la profitul acesteia, din momentul în care s-a aflat că *"fumătorii care se confruntă cu restricţii la locul de muncă au o rată de renunţare mai mare cu 84% faţă de medie"* şi s-a aşteptat *"un declin al consumului de tutun cu 10%"* în cazul în care fumatul ar fi fost interzis peste tot la locul de muncă. Legislaţia care restricţionează fumatul în locuri publice este, de asemenea, o strategie eficace în susţinerea stopării fumatului, de când s-a constatat un beneficiu adiţional al acestei reglementări.

Legile privind purificarea aerului în încăperi sunt asociate cu o prevalenţă a fumatului mai redusă, proporţional cu creşteri ale numărului intenţiilor de renunţare la fumat.

Statele care impun numeroase restricţii privind monitorizarea fumatului alături de taxe crescute înregistrează cele mai scăzute rate de consum. Aceste corecţii nu se pot realiza doar printr-o lege sau prin stabilirea unor politici restrictive. Politica trebuie implementată cu grijă, alături de furnizarea serviciilor de susţinere a stopării fumatului şi întărirea procedurilor stabilite şi aplicate.

Terapia farmacologică

Numeroşi indivizi sunt capabili să renunţe cu succes la fumat cu forţe proprii, apelând la un ghid comportamental de ajutor individual. Oricum, cei mai mulţi fumători sunt dependenţi de nicotină şi ar putea beneficia în urma intervenţiilor privind aspectele psihologice, în special dereglările cauzate de dependenţa de tutun şi sevrajul, recunoscute de ICD 10 - Clasificaţia Internaţională a Bolilor.

Dezvoltarea farmacoterapiei în tratarea dependenţei de nicotină se concentrează pe alinarea simptomelor sevrajului. De exemplu, medicamentele cu nicotină reduc aceste simptome prin înlocuirea parţială a nicotinei acumulate prin fumat.

Antidepresivele, cum sunt bupropionul şi nortriptilina, pot fi eficace în stoparea fumatului datorită acţiunii lor asupra depresiei cauzate de renunţarea indusă, ceea ce este similar cu renunţarea la nicotină. Au fost experimentate încercări clinice de promovare a renunţării prin produse farmacologice, în scopul reducerii altor simptome de sevraj, ca anxietatea.

Eficacitatea produselor

În prezent, sunt două categorii de medicaţie disponibile în renunţarea la fumat: medicamente ce conţin nicotină şi medicaţia non-nicotină.

Printre medicamentele fără nicotină, bupropionul a fost acceptat (acreditat) în stoparea fumatului, în timp ce altele sunt considerate eficiente chiar dacă nu au fost acreditate de autorităţile în drept ca fiind produse pentru tratamentul renunţării la fumat. Bupropionul şi diversele produse folosite în terapia de înlocuire a nicotinei au fost vast studiate, iar concluzia acestor cercetări este că toate sunt eficiente, păstrându-se anumite limite şi condiţii, toate putând dubla rata abstinenţei, prin placebo.

Compararea eficienţei produselor este problematică, deoarece există foarte puţine studii interne privind compararea produselor în mod direct şi pentru că rata de succes a produselor variază destul de mult. Oricum, putem concluziona că produsele sunt eficiente în funcţie de compoziţia acestora, dar acest efect poate fi îmbunătăţit cu un suport comportamental intensificat.

Nu există un sistem validat de îmbinare perfectă între forma de tratament aplicată fumătorilor şi cel mai de succes efect. Indivizii variază în alegerea celei mai acceptabile forme de tratament, iar cei care au încercat să renunţe şi au eşuat cu un anumit produs potapela la alte produse mai eficiente. De aceea, este important să existe cât mai multe produse disponibile.

MEDICAMENTE DE LINIA I

1. Terapia de substituție nicotinică (TSN)

1.1. Plasturele transdermic

Diversitatea din sistemele cu plasturi este descrisă în tratate iar diferențele de farmacocinetică au fost ilustrate într-un experiment clinic *”față-n față”*. Pentru că este imposibil de previzionat care fumători răspund la acțiunea plasturilor, este util să se asigure accesul acestora la toate tipurile de plasturi. De exemplu, unii fumători pot apela la un plasture care eliberează niveluri crescute şi rapide ale nicotinei, în timp ce alții preferă plasturi care eliberează gradual cantități mai mici de nicotină.

Introduşi în uz din anul 1991, în prezent, pe piața farmaceutică mondială se găsesc trei tipuri de plasturi, care diferă între ei prin: concentrația de nicotină conținută şi eliberată (5,10,15 mg sau 7,14,21 mg), durata de aplicare (16 sau 24 de ore) şi nivelele de nicotină obținute în sânge.

Plasturii se adresează subiecților adulți de peste 18 ani, fumători a 10-15 țigări/zi, cu dependență moderată de nicotină.

Terapia cu plasturi nicotinici se recomandă pentru aproximativ 3 luni, se începe cu terapia maximă timp de o lună, apoi, în următoarea lună se continuă cu doza medie şi, în ultima lună, se administrează plasturi cu concentrația minimă.

Principalul avantaj al formulelor de plasturi acreditate este că au eficiență, chiar dacă pacientul îl utilizează dimineața sau în cursul zilei. Din acest motiv, complianța la terapia cu plasturi tinde să fie mai crescută decât pentru alte produse folosite în această terapie.

Plasturele eliberează nicotină mai lent decât schemele obişnuite de substituție ale nicotinei, deşi concentrațiile plasmatice ale nicotinei cresc în cursul zilei prin folosirea plasturelui, mai ales dacă pacientul nu apelează la produsele obişnuite de substituție a nicotinei de atâtea ori cât îi sunt recomandate în cursul unei zile.

Cele mai frecvente *reacții adverse* produse de folosirea plasturelui cu nicotină sunt de natură dermică, locale, întâlnite la peste 50% dintre utilizatori. Prin schimbarea zilnică a locului de aplicare a plasturelui, în conformitate cu instrucțiunile de utilizare, precum şi prin folosirea unguentelor de tip Triamcinolon, se reduce incidența acestor reacții dermice. Tulburări de somn au fost de asemenea întâlnite la folosirea plasturelui timp de 24 de ore, observându-se o rată mai mare la plasturii de 21 mg, față de cei de 7-14 mg. Medicii pot recomanda plasturi cu concentrații mai mici persoanelor care manifestă tulburări de somn, precum şi îndepărtarea acestora după 16 ore şi pe timpul cât dorm.

Alte reacții adverse: cefalee, vertij, vise anormale, grețuri, palpitații, stări care pot fi induse şi de sindromul de abstinență însuşi.

1.2. Guma cu nicotină

Prima terapie de substituție a nicotinei disponibilă consumatorilor a fost polacrilexul cu eliberare transmucosală de nicotină (guma cu nicotină). Inițial, acest produs a fost aprobat pentru a fi prescris în câteva țări europene, în Canada şi în SUA, la începutul anilor 1980, dar şi în multe alte țări la sfârşitul aceluiaşi deceniu.

În anii ‘90, multe țări au aprobat comercializarea gumei, fără prescripție medicală, chiar şi în farmacii, în alte țări fiind catalogată ca un produs nemedicamentos.

În compoziția gumei cu nicotină au fost introduse arome de mentă sau portocală, în efortul de a creşte complianța pacienților la utilizarea acesteia, mai ales că aroma inițială era neplăcută.

Au fost efectuate sute de cercetări, care au la bază studii clinice şi primare, în ceea ce priveşte siguranța şi eficacitatea produselor pentru abstinență şi în tratarea simptomelor de sevraj, inclusiv dorința puternică de a fuma.

Guma cu nicotină nu se mestecă ca o gumă obişnuită, ci intermitent, ținându-se în cavitatea bucală aproximativ 30 de minute, pentru a elibera nicotina.

Acest preparat se indică persoaneleor cu vârste de peste 18 ani, care fumează cel puțin 10 țigarete/zi, totalizând un scor Fagerstrom ≥ 4 şi care sunt motivate să oprească fumatul.

Este disponibilă în concentraţii de 2 şi 4 mg, fiecare dintre acestea eliberând aproximativ 50% din nicotină după o perioadă de 15-30 minute de la utilizare.

Folosirea gumei determină nivele medii sanguine ale nicotinei de 7-10 ng/ml, considerate suficiente pentru a stimula receptorii nicotinici din creier şi a reduce astfel intensitatea sindromului de sevraj. Timpul optim de consumare nu trebuie să fie mai mic de 30 de minute. Dacă guma este administrată corect, respectând toate aceste mici amănunte şi durata standard a curei terapeutice, ea este extrem de eficientă. Se mai impun precauţii în cazul consumului de băuturi acide, dovedite a interfera absorbţia nicotinei, motiv pentru care se indică evitarea ingestiei de sifon, apă minerală, sucuri acidulate, cola, cafea, bere, timp de aproximativ 15 minute înainte şi în timpul cât se mestecă guma cu nicotină. Astfel, atunci când cantitatea de gumă de mestecat administrată este stabilită la 10 bucăţi pe zi, fumătorul recepţionează aproximativ 10 sau 20 mg de nicotină zilnic, folosind gumă de concentraţie 2-4 mg. Absorbţia medie de nicotină din ţigări este de aproximativ 30 mg/zi. În acest fel, cei care utilizează guma nu ating nivelul zilnic de nicotină pe care l-ar obţine din ţigară. Mai mult, datorită absorbţiei lente a nicotinei din gumă, comparativ cu cea din fumat, dozele individuale nu produc niveluri arteriale crescute ale nicotinei, aşa cum se întâmplă în cazul fumatului. Fumătorii care folosesc mai puţine gume dobândesc concentraţii mai mici de nicotină, ceea ce poate reduce eficacitatea tratamentului.

Datorită importanţei substituirii nicotinei în cantităţile adecvate, succesul tratamentului pe bază de gumă cu nicotină depinde parţial de numărul de bucăţi pe care fumătorul le utilizează zilnic.

Fumătorii dependenţi şi-au crescut şansele de dobândire a abstinenţei la o doză de 4 mg, în loc de 2 mg. După câteva săptămâni sau luni, numărul dozelor zilnice se reduce gradual, până la eliminare. Această fază terapeutică durează câteva săptămâni, în cazul celor mai mulţi utilizatori, dar tratamentul va fi continuat cât va fi necesar, pentru a permite atât adaptarea graduală a organismului la nicotina redusă, cât şi pentru a împiedica reluarea fumatului.

Persoanele cărora li se recomandă folosirea gumei cu nicotină trebuie să fie capabile să-şi administreze corect medicamentul, pe principiul *“mestecă şi parchează”,* şi este necesar să nu posede condiţii medicale coexistente din categoria: diabet insulino-dependent, afecţiuni coronariene, ulcer peptic, HTA, boli ale articulaţiei temporo-maxilare, sarcină, alăptare, afecţiuni dentare, care se pot agrava datorită mestecării gumei.

Per total, durata recomandată a tratamentului este în medie de 14 săptămâni, cu o limită inferioară de 8-10 săptămâni, pentru indivizii cu dependenţă redusă, şi cu o limită superioară de 3-6-12 luni, pentru cei sever şi foarte sever dependenţi.

1.3. Tableta orală cu nicotină

Tableta se prezintă în concentraţii de 1, 2 şi 4 mg, nu se mestecă şi nici nu se înghite. Instrucţiunile de folosire şi dozajul sunt similare cu cele ale gumei cu nicotină; se dizolvă în aproximativ 30 de minute, cu diverse variaţii, în funcţie de indivizi.

Ca şi la guma cu nicotină, nicotina din tabletă este absorbită lent prin mucoasa bucală şi eliberată în sistemul circulator. Tableta este o alternativă a gumei cu nicotină la indivizii care necesită o administrare intermitentă şi controlabilă a nicotinei, dar care nu agreează guma de mestecat.

Cantitatea de nicotină absorbită dintr-o tabletă pare a fi mai mare decât cea eliberată de gumă. Studiile privind administrarea în doză unică arată un nivel al concentraţiei plasmatice maxime cu 8-10% mai mare şi un nivel al concentraţiei medii a agentului terapeutic din organism cu 25-27% mai mare faţă de gumă, în concentraţiile de 2 şi 4 mg, acest lucru datorându-se retenţiei reziduale de nicotină în gumă.

Principala *contraindicaţie* o reprezintă infecţiile orofaringiene. Tableta orală cu nicotină este, de asemenea, contraindicată copiilor şi gravidelor şi impune aceleaşi precauţii cardiovasculare ca şi inhalerul nicotinic.

Cura durează 12 săptămâni şi nu se recomandă mai mult de 20 de tablete zilnic.

1.4. Tableta sublinguală

Un comprimat conține 2 mg de nicotină, fiind produs şi comercializat în numeroase țări europene. Produsul este conceput să fie administrat sublingual, unde se dizolvă în aproximativ 15-20 minute.

Ca şi tableta cu nicotină, tableta sublinguală are avantajul că nu se mestecă. Nivelurile de nicotină obținute prin folosirea unei tablete de concentrație 2 mg sunt similare cu cele rezultate de la o gumă care are aceeaşi concentrație.

Durata totală a tratamentului trebuie să fie ≥ cu 12 săptămâni, cu reducerea progresivă ulterioară, după caz.

Într-un experiment aleator, placebo dublu orb, cu tablete sublinguale de 2 mg, ratele de succes privind obținerea unei abstinențe totale în cazul activ, versus placebo, au fost 50% vs. 29% în 6 săptămâni, 42% vs. 23% în 3 luni, 33% vs. 18% în 6 luni. Rata de renunțare este dublă față de alte forme ale terapiei de substituție a nicotinei. În acest experiment, efectele adverse au fost minore şi tolerabile, cele mai comune fiind sub formă de iritații şi de inflamații în gât şi în gură.

Efecte adverse: dureri bucofaringine. Contraindicația principală fiind infecțiile cu aceeaşi localizare.

1.5. Inhalatorul cu nicotină

Inhalatorul cu nicotină constă într-un dispozitiv pentru cavitatea bucală şi un cartuş de plastic cu nicotină. A fost prima dată comercializat în SUA, în 1998, ca medicație prescrisă pentru stoparea fumatului, şi conceput astfel încât să satisfacă aspectele comportamentale ale fumatului numite *"ritual de ducere la gură a mâinii"*, cu eliberarea nicotinei în scopul reducerii simptomelor psihologice de sevraj.

Medicamentul este contraindicat femeilor gravide şi tinerilor sub 18 ani şi trebuie utilizat cu precauții la pacienții cardiaci, mai ales la cei care au avut IMA în ultimele 2-3 săptămâni, ca şi la cei care au aritmii severe sau angină pectorală instabilă.

Dozajul este de 6-16 cartuşe/zi, în condițiile în care un cartuş poate elibera 4 mg de nicotină.

Durata recomandată a terapiei este de 6 luni, cu mențiunea ca în ultimele 3 luni doza să fie ajustată treptat.

Este important de specificat că cea mai mare parte din nicotină este eliberată în cavitatea bucală (36%), în esofag şi în stomac (36%), foarte puțină ajungând până în plămâni (4%).

Deoarece absorbția se realizează mai întâi prin mucoasa bucală, rata de absorbție este similară cu aceea a gumei cu nicotină.

Fiecare cartuş din inhalant conține 10 mg de nicotină, din care peste 4 mg pot fi eliberate şi 2 mg pot fi absorbite prin *"ritual de pufăit"*.

Pacienții pot seta inhalantul la nivelul de nicotină de care au nevoie.

În experimentele clinice, majoritatea fumătorilor care au reuşit să devină abstinenți au folosit între 6-16 cartuşe pe zi.

Reacții adverse: iritație la locul administrării (gât şi cavitate orală), tuse iritativă, rinită.

Deocamndată produsul nu este comercializat în România.

1.6. Sprayul nazal cu nicotină

Sprayul nazal cu nicotină a fost conceput să elibereze fumătorilor cantități de nicotină mai rapid decât se realizează prin folosirea gumei sau a plasturelui.

Dispozitivul disponibil consumatorilor este o sticlă multi-doză cu un mecanism de pompare adaptat, care eliberează 0,5 mg de nicotină la un jet de 50 ml. Un recipient conține 100 de doze.

Doza uzuală este de 0,5 mg/narină, inițial 1-2 doze/oră, cu posibilitatea creşterii dozajului atât cât este necesar pentru îndepărtarea simptomelor de sevraj.

Cu o doză pe oră (1mg nicotină) timp de 10 ore se produc concentrații medii în plasmă de 8 ng/ml.

Limitele între care se situează dozarea zilnică sunt de 8-40 doze/zi, în total durata terapiei este de 3-6 luni.

Nicotina din spray este absorbită în sânge relativ rapid, comparativ cu cea din gumă sau plasture. Chiar dacă rata absorbţiei nicotinei în plasmă, cu ajutorul spray-ului, este apropiată celei din ţigări şi inhalantul oral, magnitudinea creşterii concentraţiei de nicotină în plasmă este mai redusă.

Conform prospectului, dozele din sprayul nazal ar trebui individualizate pentru fiecare pacient în concordanţă cu nivelul de nicotină de care acesta depinde, precum şi cu incidenţa simptomelor cauzate de excedentul de nicotină.

Nici acest substitut nicotinic nu este permis gravidelor şi tinerilor sub 18 ani, impunând aceleaşi precauţii cardiovasculare ca şi tableta/inhalerul cu nicotină.

Reacţii adverse: iritaţie nazală, congestie nazală, modificări tranzitorii de gust şi miros.

Nu se recomandă pacienţilor cu hiperreactivitate bronşică.

2. Bupropion SR

Una din simptomele renunţării la fumat este starea de dispoziţie negativă, care poate fi luată în calcul la eşecurile apărute în procesul de renunţare sau de reluare a fumatului, după o perioadă de abstinenţă. Din acest motiv, numeroase antidepresive au fost testate în scopul sprijinirii stopării. În anul 1989 Ferry a deschis calea tratamentelor non-nicotinice ale dependenţei nicotinice. El a observat că dintre fumătorii care urmau un tratament antidepresiv cu bupropion, un procent important îşi schimbă „opinia" despre fumat, găsindu-l neplăcut ori, pur şi simplu, nu mai simţeau nevoia de a fuma şi renunţau spontan la consumul de tutun.

Compania producătoare a **bupropionului a refuzat, iniţial, să cerceteze fenomenul, nefiind convinsă că un astfel de abord ar fi util, astfel că Ferry şi-a finanţat un mic studiu pilot: 44 de fumători au primit bupropion ori placebo. Niciunul dintre cei care au primit placebo nu a re**nunţat să fumeze, în schimb, 50% dintre cei care au primit bupropion nu mai fumau la 3 luni. Cu acest studiu compania farmaceutică a fost convinsă să înceapă cercetările, astfel că, în anul 1997, este înregistrat primul tratament non-nicotinic cu bupropion.

Bupropionul SR (**Zyban**, GlaxoSmithKline) este un medicament antidepresiv omologat. Acesta este primul produs non-nicotină care a fost acreditat de FDA din SUA şi este menţionat ca primă terapie în Ghidul practic clinic din SUA *"Tratarea fumatului şi a dependenţei"*. Este cunoscut în lume din anul 1997, în România fiind disponibil din anul 2002.

Nivelul plasmatic sigur al bupropionului se instalează în aproximativ 5 zile, sau mai mult, motiv pentru care este indicat ca un fumător să înceapă utilizarea acestuia cu aproximativ o săptămână înainte de data de renunţare.

Primele trei zile se administrează câte un comprimat de 150 mg per os, apoi 150 mg x 2/zi, la intervale de cel puţin 8 ore pentru restul curei, în total o cură de 7- 9 - 12 săptămâni.

Cum poate combate un astfel de medicament un „obicei" precum fumatul? Răspunsul nu este cu certitudine desluşit, însă există destule studii care arată că acţionează la nivelul receptorilor din sistemul nervos de care se leagă nicotina. Individul îşi administrează nicotină dar aceasta nu îşi mai face efectul la nivelul creierului. Rezultatul: persoana nu mai simte nici plăcerea şi nici nevoia de a fuma, rămânând doar nevoia de a face gestul, ritualul, tabietul cu care s-a obişnuit.

Rezultatul este spectaculos, mai ales la persoanele care fumează în exces, cu atât mai mult cu cât există şi un oarecare efect de scădere a apetitului alimentar. Chiar dacă este sigur (medicamentul trecând şi proba timpului, el fiind comercializat de mai bine de 25 de ani), utilizarea lui trebuie avizată de un medic specialist, deoarece face parte din clasa antidepresivelor şi, în plus, modul de administrare este special. Astfel, o singură tabletă nu are niciun efect, fiind necesare 5-7 zile până când medicamentul atinge concentraţia sanguină eficientă. Din acest motiv, el nu se recomandă pentru tratarea imediată a simptomelor de sevraj.

Contraindicaţii: vârsta sub 18 ani, sarcina, alăptarea, hipersensibilizare la bupropion sau excipienţi, tulburări convulsive sau antecedente, tumori cranio-cerebrale etc.

Reacţii adverse: insomnia, cefaleea, gura uscată (cele mai frecvente).

Există statistici conform cărora prin folosirea bupropionului în combinaţie cu plasturele cu nicotină poate creşte eficacitatea faţă de utilizarea individuală a produselor. Oricum, în prezent nu este menționată nicio recomandare pentru aplicarea lor combinată, iar metodele complexe de acreditare, alături de dificultățile în comercializare, o fac indezirabilă pentru producători.

3. Vareniclina

Este cel mai nou medicament de renunțare la fumat, disponibil în țara noastră, intrând în uz, în lume, cu aprobarea FDA, în anul 2006. Medicamentul este produs şi comercializat sub denumirea de **Champix** (în Europa), **Chantix** (în SUA).

Aprobarea intoducerii tratamentului cu Champix s-a bazat pe un vast program de studii clinice, ce a cuprins peste 4 000 de pacienți. Subiecții incluşi în studii fumaseră în medie 21 de țigări/zi, pe o perioadă medie de 25 ani. Rezultatele acestor studii au arătat că peste 44% dintre pacienți au renunțat la fumat la terminarea tratamentului.

Studiile clinice de fază 3 au inclus peste 2 000 de pacienți şi au evaluat eficiența în renunțarea la fumat a vareniclinei în doză de 1 mg de 2 ori pe zi, bupropion, 150 mg de 2 ori pe zi şi placebo. După 3 luni de tratament, ratele de abstinență continuă, sub tratamentul cu vareniclină, au fost semnificativ mai mari comparativ cu bupropion sau placebo: 44% vareniclina versus 30% bupropion, versus 18% placebo.

Stanley Mendon, Country Manager - Pfizer Romania spunea: *"Champix reprezintă un pas înainte în lupta împotriva dependenței de tutun, fiind rezultatul multor ani de cercetare şi dezvoltare. Pfizer aduce soluții inovative în multe dintre ariile terapeutice pentru care nu există încă tratamente. Champix este un medicament unic pentru toți cei care sunt hotărâți să renunțe la fumat. Din punct de vedere al procentului de fumători, România este astăzi în topul țărilor din Uniunea Europeană. Este pentru prima dată când un tratament inovator se lansează în România, practic în acelaşi timp cu celelalte state europene. Ne dorim să contribuim activ la sprijinirea tuturor celor implicați în lupa anti-fumat din România, prin sprijinirea programelor derulate de autorități, dar şi a proiectelor dezvoltate în sectorul privat"*.

Mecanismul de acțiune al vareniclinei presupune o activitate de agonist parțial, precum şi de antagonist al receptorilor nicotinici din creier, cu precădere la nivelul receptorului α 4 β 2 – considerat ca principalul mediator care determină addicția de nicotină. El se leagă specific de receptorul de nicotină, blocând acțiunea nicotinei inhalată din țigară şi interferând astfel cu efectul de recompensă al fumatului: persoana fumează şi nu mai simte plăcerea, relaxarea, calmarea, „recompensa" adusă de tutun. În acelaşi timp, este un agonist parțial al receptorilor de nicotină, adică induce o eliberare de dopamină, la fel ca şi nicotina, dar în cantități mai mici (previne simptomele de sevraj).

Vareniclina se administrează pe cale orală, indiferent de ingestia de alimente, în două etape:

- ❖ **doza inițială:** 1 cpr. de 0,5 mg/zi, primele 3 zile de tratament, apoi 1 câte 1 cpr. de 0,5 mg x 2/zi, în zilele 4-7 de tratament, iar în zilele 8-14 câte 1 cpr. de 1 mg x 2/zi;
- ❖ **doza de continuare:** 1 cpr. de 1 mg x 2/zi zilnic, între săptămânile 3-12.

Vareniclina este, în general, foarte bine tolerată.

Contraindicații: hipersensibilitate la substanța activă su excipienți, vârsta sub 18 ani, sarcină şi alăptare.

Cea mai frecventă *reacție adversă* sub tratamentul cu vareniclină este greața, dar rata întreruperii tratamentului din această cauză a fost sub 3%. Este urmată de dureri abdominale, constipație, balonări, precum şi de tulburări neuropsihice (insomnii, cefalee, vise anormale).

Creşterea în greutate, după 12 săptămâni, raportată sub tratamentul cu vareniclină, a fost de 2,4 -2,9 kg, similar cu placebo.

Fumătorii care nu pot tolera reacțiile adverse pot beneficia de reducerea temporară sau definitivă a dozei la 0,5 mg de 2 x pe zi.

MEDICAMENTE DE LINIA A II-a

1. Clonidina

Clonidina diminuează simptomele sevrajului la sedative şi alcool, putând fi utilă şi pentru micşorarea simptomelor de sevraj cauzat de tutun. Deşi neacreditată de FDA, studiile efectuate au acordat clonidinei un nivel “A”, ca dovadă a elementelor pozitive aduse de folosirea acestei substanțe în cadrul unor multiple experimente clinice. Se poate administra zilnic în doze de 0,15-0,75 mg în forma orală şi 0,1- 0,2 mg în cea transdermică.

Este indicată începerea terapiei cu aproximativ 3 zile înaintea datei fixate pentru oprirea fumatului. Ea nu a fost aprobată de FDA în tratarea dependenței de tutun.

2. Nortriptilina

Este un antidepresiv triciclic, eficace în renunțarea la fumat, ca medicație de linia a 2-a cu nivel de dovadă “A”. Cunoscută în primul rând ca antidepresiv, nortriptilina nu a fost aprobată de FDA în terapia dependenței nicotinice. Patru studii randomizate recomandă nortriptilina ca medicație eficace în sevrajul tabagic, de administrat sub supraveghere medicală, la un dozaj inițial de 25 mg/zi, crescut progresiv până la doza țintă de 75-100 mg/zi, cu o durată medie a curei de 12 săptămâni.

Tratamentul se va începe cu 10-28 zile înainte de data preconizată pentru oprirea fumatului.

Alte produse utilizate în tratamentul dependenței nicotinice

1. Vaccinul antinicotinic este studiat şi el în ultimii 10 ani şi se pare că nu mai este mult până la obținerea produsului final. Principiul este următorul: nicotina este o moleculă mică ce poate depăşi bariera hemato-encefalică, motiv pentru care apar atât de uşor efectele centrale (psihotrope şi dependență). Dacă se introduce o cantitate mică de nicotină modificată (vaccinul), se generează răspuns imun specific (producerea de anticorpi față de o anume porțiune a nicotinei). La introducerea ulterioară a nicotinei, se produce rapid o cantitate mare de anticorpi care se leagă specific de nicotină, astfel încât dimensiunile ei cresc şi nu mai poate traversa bariera hemato-encefalică. Consecința: persoana nu mai resimte efectele centrale, nervoase ale nicotinei (nici pe cele psihice, de recompensă, nici dependență) iar consumul de tutun îşi pierde sensul.

Implicațiile sunt foarte complexe: vaccinul nu numai că este util pentru fumători în demersul lor de a renunța mai uşor şi mai repede la acest obicei dăunător, dar îi ajută şi pe foştii fumători să nu mai reia fumatul şi pe nefumători să nu înceapă consumul de tutun. Mai mult, vaccinarea poate avea implicații şi pentru femeile însărcinate, prin blocarea efectelor nicotinei asupra fătului (chiar dacă mama nu fumează, în sângele ei există nicotină provenită de la fumătorii din jur).

Cercetările sunt foarte avansate iar specialiştii estimează că în 3-4 ani va fi disponibilă şi această metodă de tratament. Oricum, 3 companii îşi dispută întâietatea pe piață.

2. Rimonobant, molecula cu efect “anticanabis”, antagonist al receptorilor din sistemul nervos central pentru acest drog, anulează apetitul alimentar crescut din perioada sevrajului tabagic şi produce o creştere de dopamină (cu efect în inducerea plăcerii sau dependenței), având ca rezultat scăderea dorinței de a fuma. Medicamentul a fost comercializat mai mult în Franța, fiind apreciat pentru efectele sale benefice în privința obezității şi a reîngrăşării după curele de slăbire. Administrarea lui în renunțarea la fumat nu a fost însoțită de o creştere în greutate. Medicamentul nu se comercializează în România.

3. În ultimii ani s-au încercat şi *alte produse*, deja cunoscute (amitriptilina, bromocriptina, naltrexona, glucoza) sau noi (selegilina, reboxetina etc.), însă eficiența lor nu a fost dovedită de studii. De asemenea, *terapia genică* este promițătoare, dar, pentru a fi aplicată, trebuie să identificăm corect genele implicate. Progrese remarcabile s-au făcut în ultimii ani, însă până la dezvoltarea unui tratament eficient şi sigur mai este mult timp.

4. Metodele naturiste tip acupunctura, homeopatia, reflexoterapia, fitoterapia sunt foarte populare, mai ales în România, însă eficiența lor nu a fost dovedită ştiințific.

Cochrane a concluzionat, în anul 2004, după cercetarea studiilor publicate de-a lungul timpului, că sunt dovezi foarte puține şi neconcludente ale eficienței reale a acestor terapii. Cu siguranță există oameni care au beneficiat de pe urma uneia sau alteia dintre metode, însă nu se poate afirma, cu certitudine, că rezultatul se datorează metodei în sine sau faptului că fumătorul îşi vede terapeutul săptămânal, discută cu el, îi sunt îndreptate greşelile şi este încurajat. Atâta timp cât metoda nu îi afectează sănătatea ori bugetul, iar fumătorul crede în ea, singura reținere în a urma o astfel de terapie este ca fumătorul să nu se descurajeze, să nu-şi piardă încrederea în sine şi în reuşită.

Îmbunătățirea procesului de eliberare a nicotinei

1. Plasturi cu concentrații crescute

Folosirea plasturilor cu concentrații crescute ar fi indicată pentru a simula mai bine dozele de nicotină acumulate în timpul perioadei de fumat, în special printre fumătorii înverşunați.

În anul 1995 a fost realizat un studiu aleator, dublu-orb, printre 504 fumători care au primit plasturi de 22 sau de 44 mg, în condiții de prescripție diferită. Printre aceia care au primit un contact minim, doza de 44 mg a produs o abstinență mai mare în 4 săptămâni decât cea de 22 mg (68% față de 45%). De asemenea, s-a realizat un studiu aleator dublu-orb în care 1 039 de fumători au primit 0, 21, 35, 42 m/zi timp de 6 săptămâni, urmând ca, peste 10 săptămâni, procedeul să fie reluat. Ratele de abstinență la concentrații de 0, 21, 35, 42 mg au fost de 16%, 24%, 30%, 39% la sfârşitul tratamentului (12 săptămâni). Nu s-au înregistrat diferențe statistice semnificative între tratamentele active.

Un alt studiu deschis a fost efectuat printre 40 de fumători pentru a determina siguranța şi tolerabilitatea a 44mg/zi pentru stoparea fumatului. Subiecții au primit această cantitate timp de 4 săptămâni, urmate de alte 4 săptămâni în care s-au administrat 22 mg/zi. Rata de prevalență a renunțării la fumat, confirmată biochimic, a fost de 65 % şi 55% în săptămâna 4 şi, respectiv, în săptămâna 8 de terapie cu plasturi. Asamblate, rezultatele confirmă faptul că plasturii cu concentrații ridicate de nicotină pot îmbunătăți reuşita tratamentului pentru indivizii foarte dependenți.

2. Forme de combinare a plasturelui cu formele de administrare obişnuită

O strategie pentru îmbunătățirea pe viitor a eficacității terapiei de substituție a nicotinei constă în combinarea unei medicații care permite eliberarea pasivă a nicotinei (de exemplu, plasturele transdermic) cu o medicație care permite eliberarea nicotinei după dorința individului (de exemplu, guma, sprayul nazal, inhalantul). Rațiunea combinării este aceea că fumătorii ar putea avea nevoie atât de o eliberare lentă, pentru a acumula o concentrație constantă de nicotină şi a uşura simptomele de sevraj, cât şi de o reacție rapidă, care se poate administra la cerere. Această viteză de reacție a efectelor poate fi importantă în special dimineața, când simptomele de sevraj şi dorința de a fuma sunt în punctul culminant pentru cei mai mulți fumători.

Plasturele furnizează nicotină în mod regulat şi pasiv, în timp ce guma poate fi utilizată conform nevoilor utilizatorilor. Astfel, combinând plasturele cu nicotină, care poate preveni apariția sevrajului sever, cu guma, care poate conferi o stare de acalmie în contextul *„trigger-to-smoke”*, se obține un tratament excelent peste terapia în sine. Studii clinice demonstrează o creştere graduală a eficacității plasturelui combinat cu guma, comparativ cu orice alt produs utilizat în mod individual. Într-un studiu care compară eficacitatea inhalantului alături de plasturele cu nicotină, versus inhalant combinat cu plasturele placebo, în lupta împotriva fumatului, s-a descoperit o rată de abstinență mai crescută a celor care au folosit, pe parcursul unui an, pachetul combinat

Niciun studiu nu a analizat utilizarea combinată a plasturelui cu tableta cu nicotină, cu sprayul nazal sau cu inhalantul, deşi putea fi preconizată o creştere graduală a eficacității.

Neluând-se în calcul posibilitatea obţinerii unei eficacităţi crescute, actualele prospecte ce privesc terapia de substituţie dau un semnal de avertisment împotriva utilizării combinate. Fără a ignora aceste avertismente, strategiile de stopare vor fi mult limitate de către specialişti şi clinicieni. Gradul de complexitate privind obţinerea aprobării combinării medicaţiilor, alături de dificultatea comercializării combinate a produselor au încetinit încercările fabricanţilor de a câştiga acreditarea combinării terapiilor.

Gradul de protecţie şi toxicitate la nicotină

Deşi nicotina poate fi toxică, administrată în doze mari, în corelaţie cu dozele eliberate prin folosirea tutunului sau a medicaţiilor de substituţie, efectele toxice ale acesteia, rezultate prin fumat, sunt considerate modeste comparativ cu agenţii cancerigeni şi alte toxine prezente în tutun. De exemplu, nicotina nu este cancerigenă, iar substituţia acesteia nu este un factor riscant în apariţia cancerului. Cu toate acestea, nicotina eliberată de produsele de tutun nu este benignă. Ea poate produce o diversitate de efecte adverse potenţiale, în funcţie de concentraţia şi tipul de administrare. Astfel, fumatul în timpul sarcinii produce un risc mai crescut al efectelor datorate consumului mărit de nicotină şi a substanţelor adiţionale din fumat, ca monoxidul de carbon. La nicotina obţinută prin medicaţia de substituţie, efectele adverse ale acesteia în timpul sarcinii sunt mai puţine decât prin fumat. Deoarece apariţia riscurilor nu poate fi exclusă, este recomandată consultarea unui medic privitor la utilizarea medicaţiei în timpul sarcinii. În mod similar, nicotina se constituie într-un factor de risc pentru artrita coronariană, ceea ce duce la inscripţionarea pe prospectele medicaţiilor cu nicotină a avertizării indivizilor în privinţa consultării unui medic înainte de utilizarea produselor.

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Managementul situaţiilor de risc nuclear
(partea a doua)

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Rezumat. Arma nucleară are o capacitate de distrugere în masă, prin efectele sale determinând afectarea gravă a vieţii sociale şi a sănătăţii populaţiei dintr-o anumită zonă, atât pe termen scurt, cât şi pe termen lung. Rezultatele acţiunii fenomenelor amintite constau în contaminarea radioactivă a zonei respective şi într-un număr foarte mare de victime într-un interval de timp foarte scurt, ceea ce impune intervenţia rapidă a structurilor abilitate ale sistemului naţional de urgenţă. Managementul victimelor va începe cu aplicarea algoritmului ABC, urmat de triajul medical, decontaminarea şi respectiv tratarea acestora în unităţile medicale adecvate.

Cuvinte cheie: armă nucleară, decontaminare, triaj medical, leziuni de iradiere

Summary. The nuclear weapon is a mass destruction weapon which by its physical effects affects seriously the social life from a specific area; also it has short and long term health effects. The result of the physical effects is the radioactive contamination of that specific area and a great number of victims in a very short time which are in need of rapid intervention of the national emergency system. Victim's management shall start with the ABCs algorithm applying, followed by medical triage, decontamination and their treatment in the medical facilities.

MODUL DE RĂSPUNS LA UN DEZASTRU NUCLEAR

Factorii de identificat în cazul unui dezastru nuclear sunt: tipul radiaţiei ionizante, doza de radiaţii în zona respectivă, cantitatea contaminării radioactive şi acumularea dozelor la victime şi salvatori.

1. Zona dezastrului (zona fierbinte). În această zonă trebuie identificate toate riscurile radioactive sau de altă natură (incendii, prăbuşirea clădirii, explozie secundară etc.). Se va identifica sursa radioactivă şi modul cum a fost eliberată – explozie, incendiu sau emisie gazoasă. Se va determina timpul scurs de la producerea accidentului/atentatului.

1.1. Siguranţa personalului salvator. Personalul salvator va folosi echipamentul de protecţie, va mânui orice material cu atenţie, nu va mânca, nu va bea şi nu se va dezechipa în zona fierbinte sau înaintea decontaminării pentru niciun motiv. Întotdeauna se va considera oricine şi orice contaminat până la proba contrarie.

1.2. Zona cu radiaţii se va carantina obligatoriu, ţinând seama de direcţia vântului, de tipul materialului radioactiv (solid, lichid sau gaz), dar şi de prezenţa altor riscuri. Se vor stabili două linii în jurul sursei de radiaţii, una *internă* (în apropierea sursei) şi alta *externă* (zonă tampon între zona dezastrului şi zona neprotejată).

Linia internă de carantină. Se consideră că tot ce există între această linie şi sursă este contaminat. În interiorul acestei zone vor intra doar personalul şi echipamentul esenţial pentru îngrijirea şi transportul pacienţilor. Personalul care va pătrunde în zonă va fi dotat cu echipament protector şi cu dispozitive de monitorizare a radiaţiilor. De reţinut că tot materialul contaminat va rămâne în interiorul acestei linii.

Linia de carantină externă. Această zonă va funcţiona ca o zonă tampon pentru personalul de salvare, de siguranţă şi pentru cel de ordine publică între locul dezastrului şi public. Aici vor staţiona echipamentul şi restul personalului de salvare. În această zonă se va organiza punctul de control pentru monitorizarea radiologică, se vor realiza decontaminarea şi transferul pacienţilor.

1.3. Pregătirea facilităţilor medicale pentru primirea pacienţilor victime ale unui dezastru nuclear. După anunţarea dezastrului nuclear, fiecare unitate medicală implicată în răspunsul la dezastru trebuie să-şi activeze: planul intraspitalicesc, planul de control al radiaţiilor şi planul de protejare a personalului împotriva radiaţiilor.

Planul intraspitalicesc va conţine: modul de obţinere a dispozitivelor de control al radiaţiilor, modul de alertare a personalului, personalul adiţional de suport (personalul din departamentul de medicină nucleară, de oncologie, de la siguranţa radiaţiilor), locul zonei de triaj, locul zonei decontaminare pentru persoanele fără leziuni, precum şi instrucţiuni generale de protecţie a personalului.

Planul de control al radiaţiilor va conţine: locul de amplasare a containerelor pentru materiale contaminate, protejarea podelelor şi precauţiile universale.

Planul de protejare a personalului împotriva radiaţiilor se referă la supravegherea mâinilor şi hainelor personalului expus cu dispozitive de măsurare, înlocuirea mănuşilor şi a halatelor (dacă se contaminează şi de la un pacient la altul), menţinerea contaminării radioactive în interiorul zonelor de decontaminare şi de tratament. În comparaţie cu situaţia folosirii armelor chimice şi biologice, în cazul utilizării armelor nucleare salvatorii au posibilitatea de a detecta foarte uşor prezenţa radiaţiilor apelând la un contor Geiger-Mueller, care este foarte sensibil şi, totodată, permite alertarea personalului în cazul în care echipamentul pe care îl poartă este contaminat peste limita admisă, el putând fi schimbat sau decontaminat.

2. Evaluarea zonei dezastrului nuclear. Triajul medical al pacienţilor iradiaţi

2.1. Evaluarea zonei. În primul rând se va determina dacă există posibilitatea contaminării radioactive, iar dacă există vreo îndoială, se consideră zona ca fiind contaminată. Toate autovehiculele vor staţiona într-o zonă sigură, cu vântul în spate, la cel puţin 50 de metri de linia de carantină internă. Se va cere ajutorul iar spitalele din zonă vor fi anunţate de existenţa unui posibil dezastru radioactiv/nuclear.

Triajul iniţial se va face pentru a determina existenţa victimelor, numărul lor şi gravitatea leziunilor. Se vor evalua leziunile care ameninţă viaţa şi se va asigura suportul de bază al vieţii (SVB) dacă este posibil şi necesar. Cazurile care nu reprezintă urgenţă imediată trebuie întârziate până după evacuarea din zona fierbinte. Se vor muta, cât mai rapid posibil, toate victimele într-o zonă necontaminată, pentru a evita apariţia de noi leziuni. Pacienţii cu leziuni urgente (care ameninţă viaţa) vor fi transportaţi la spital, anunţând conducerea spitalului care îi primeşte despre posibilitatea contaminării radioactive, despre severitatea şi tipul leziunilor acestora. Pacienţii cu leziuni mai uşoare pot rămâne în zona tampon pentru decontaminare şi tratament medical obişnuit.

2.2. Triajul medical constituie prioritate cea mai înaltă, iar în condiţiile folosirii armei nucleare este diferit, deoarece leziunile de iradiere la pacienţii care supravieţuiesc *nu se manifestă înainte de câteva săptămâni de la expunere.* **Triajul medical iniţial** se bazează pe prezenţa: leziunilor convenţionale, a semnelor şi simptomelor (greaţă, vărsături, diaree, oboseală), istoric ale punerii la radiaţii (când şi cum?) şi supravegherea contaminării. De aceea aceste leziuni vor fi tratate primele, fără a fi omise modificările provocate de nivelul radiaţiilor. Pacienţii cu politrauma vor fi resuscitaţi şi stabilizaţi, iar pregătirea pre-operatorie va conţine şi o fază de decontaminare radioactivă. Se va pune şi un diagnostic preliminar de leziune prin iradiere doar pentru cei care au simptome de expunere (greaţă, vărsături, diaree, hipertermie, eritem, hipotensiune arterială, disfuncţie nervos centrală). În post-operator se va evalua expunerea radioactivă cu mai multă atenţie.

Categorii de triaj la pacienții iradiați:

- imediat (roşu) – T1: cei care necesită intervenție imediată pentru salvarea vieții. Se vor evacua primii;

- întârziat (galben) – T2: cazurile care au doar leziuni prin iradiere, fără simptome neurologice majore. Dacă coexistă şi trauma, intervențiile chirurgicale trebuie realizate în primele 36-48 ore de la expunere sau la 2 luni după traumatism;

- minim (verde) – T3: cazurile se decontaminează, plăgile se curăță şi se închid cu atenție;

- expectant (negru) – T4: leziunea pură prin iradiere nu amenință acut viața, doar dacă iradierea este masivă şi atunci pacientul este clasificat **negru - expectant**. Pacienții primesc tratament de susținere (paleativ) compatibil cu resursele existente. Se pot folosi doze mari de analgetice la nevoie.

Supraviețuirea individuală a pacienților iradiați este pusă sub semnul întrebării dacă**:**

- expunerea întregului corp depăşeşte 200 rad;
- expunerea unui segment major al corpului (capul, regiunea toracică etc.) este de ordinul a sutelor sau miilor de rad;
- există leziuni combinate – leziuni fizice (traumatice) grave şi respectiv de iradiere.

După ce pacienții au fost triați şi tratați în funcție de leziunile traumatice convenționale, triajul va fi făcut în funcție de gravitatea leziunilor de iradiere, pentru a putea realiza managementul cazurilor în condițiile unui dezastru de asemenea anvergură. Pentru aprecierea expunerii la radiații se pot utiliza *dozimetrele tactice*, care însă nu pot oferi personalului medical prea multe informații datorită expunerii diferite a trupelor la radiații. De aceea se poate folosi determinarea numărului de limfocite (în primele 24 de ore de la eveniment) ca *dozimetru biologic* la pacienții clasificați ca „leziune de iradiere posibilă” sau „leziune de iradiere probabilă”, astfel:

- limfocite >1 500/mm^3 – nu necesită tratament;
- limfocite între 500-1 000/ mm^3 – iradiere severă care necesită spitalizare;
- limfocite >500/ mm^3 – iradiere care poate fi fatală, se spitalizează pentru complicațiile care pot apărea datorită pancitopeniei;
- limfocite aproape de zero – pacienții au fost iradiați supraletal, nu supraviețuiesc.

În aceste condiții este greu de pus un diagnostic precoce, de aceea pacienții care au doar leziuni de iradiere pot fi clasificați apelând la un sistem bazat pe trei categorii posibile determinate de prezența sau absența unor simptome asociate leziunilor de iradiere apărute în prima zi după expunere. Simptomele caracteristice produse de iradiere sunt: greața şi vărsăturile (apar la 6-12 ore după expunere, doză >100-200 cGy), hipertermia (este prezentă în primele ore, doză ≥200 cGy), eritemul (apare în prima zi, doză de 1 000-2 000 cGy), hipotensiunea arterială (asociată cu un prognostic prost, doză de sute de rad supraletală) şi disfuncția neurologică (dacă apare în prima oră de expunere doza este supraletală, deces în 48 de ore).

Categoriile de pacienți care au doar leziuni de iradiere şi care pot fi folosite pentru triaj sunt:

- leziune de iradiere improbabilă – nu are simptome de iradiere, se triază folosind leziunile convenționale;

- leziune de iradiere probabilă – are simptome prodromale (anorexie, greață, vărsături), se realizează algoritmul ABCDE, nu necesită tratament în primele zile, dacă nu are leziuni traumatice, se determină limfocitele repetat în primele 48 de ore;

- leziune de iradiere severă – pacientul este iradiat fatal, prezintă toate simptomele de iradiere, va primi tratament simptomatic şi se vor determina limfocitele repetat.

În situațiile cu aflux mare de cazuri, pentru a scădea presiunea asupra spitalelor specializate şi a centrelor trauma, unele dintre spitale se pot transforma în spitale de triaj şi respectiv spitale în care victimele vor fi reținute (spitale de ROL 2), acordându-se un tratament minimal. Victimele fac parte din următoarele categorii: arsuri minime, poli-trauma medie, leziuni chimice uşoare şi leziuni severe cu şanse scăzute de supraviețuire sau care au nevoie de măsuri suportive în aşteptarea posibilității de tratament adecvat.

3. Decontaminarea. Decontaminarea constă în utilizarea celor mai judicioase procedee pentru neutralizarea, eliminarea sau diminuarea efectelor armelor de distrugere în masă (N.B.C.) care au contaminat oamenii, animalele, echipamentele, terenul şi/sau apa.

3.1. Decontaminarea imediată individuală (D.I.I.). Decontaminarea imediată individuală constă în: îndepărtarea produşilor radioactivi de pe porţiunile descoperite ale corpului, de pe mucoase (ochi, nas şi gură), neutralizarea/îndepărtarea agenţilor toxici de luptă de pe porţiunile descoperite ale corpului şi respectiv distrugerea/îndepărtarea agenţilor biologici de pe porţiunile descoperite ale corpului. Operaţiunea se va executa cu ajutorul pachetelor de decontaminare individuală, fără a afecta îndeplinirea misiunii de luptă, astfel: în cazul contaminării radioactive şi biologice, de regulă, după ieşirea din raionul contaminat, imediat ce permite situaţia, iar în cazul contaminării chimice, imediat după producerea contaminării.

3.2. Decontaminarea operaţională (D.O.). Acest tip de decontaminare este activitatea executată de către indivizi şi/sau echipe, restrânsă la părţile operaţionale ale echipamentelor, materialelor şi/sau zonei de interes (de acţiune, de lucru), în vederea reducerii riscului de contaminare prin contact sau prin transfer, pentru a putea permite desfăşurarea acţiunilor militare. D.O. poate cuprinde decontaminarea individului după D.I.I., decontaminarea unor părţi ale subansamblurilor esenţiale ale tehnicii de luptă şi a porţiunii de teren necesare îndeplinirii misiunii. Această activitate se execută cu echipamentele de decontaminare din înzestrarea subunităţilor şi/sau unităţilor până la eşalonul batalion (similare) inclusiv.

3.3. Decontaminarea completă (totală) (D.C.) este activitatea executată de unităţi, cu sau fără sprijin extern, cu scopul de a reduce contaminarea personalului, echipamentelor, materialelor şi/sau raioanelor (zonelor) de interes la nivelul cel mai scăzut posibil.

Decontaminarea completă (totală) cuprinde: decontaminarea completă a personalului, a tehnicii de luptă, a echipamentului şi a altor materiale, iar în anumite situaţii, decontaminarea totală poate cuprinde şi decontaminarea terenului.

Decontaminarea completă a personalului se va executa în cel mai scurt timp, aceasta constând în: executarea D.I.I., spălarea întregului corp cu apă caldă şi săpun, schimbarea obligatorie a lenjeriei, iar la nevoie a întregului echipament.

Decontaminarea totală a personalului se execută în raioanele de decontaminare, dar şi prin folosirea echipamentelor, instalaţiilor şi a altor posibilităţi existente în zonă (băi publice special amenajate, ape curgătoare necontaminate etc.).

Zona (raionul) de decontaminare. În principiu se va crea o arie de decontaminare cu perimetru de siguranţă şi cu acces doar pentru persoanele autorizate; podelele şi pereţii se acoperă cu material antiderapant, se instalează filtre pentru aparatele de ventilaţie, iar echipamentul se acoperă. Zona va fi departe de trafic, cu intrare şi ieşire uşor de identificat, prevăzută cu materiale necesare pentru o decontaminare adecvată şi destul de mare pentru a permite o decontaminare de masă.

Personalul care deserveşte zona de decontaminare va avea echipament special cu dublu înveliş (mănuşi lungi, cizme etc.) care poate fi înlăturat la părăsirea acestei zone. Echipamentul se va schimba frecvent. În zonă se va afla un *ofiţer responsabil cu siguranţa radiaţiilor* (numit de şeful zonei) care va avea un dozimetru şi/sau un contor Geiger.

În afara zonei de decontaminare va exista şi un *ofiţer pentru supraveghere radiologică* care va controla atât personalul, cât şi echipamentul care vine din zona de decontaminare.

Decontaminarea pacienţilor cu expunere radioactivă. Personalul medical care acordă primul ajutor nu are de suferit dacă aplică algoritmul ABCDE unui pacient care provine dintr-un mediu contaminat nuclear, dacă acesta este decontaminat extern corect.

Decontaminarea va începe după stabilizarea pacientului, folosind o procedură pas cu pas. Nu întârziaţi intervenţii chirurgicale sau alte proceduri medicale necesare până la terminarea decontaminării! Îndepărtarea hainelor pacientului (dezbrăcarea acestuia) înlătură până la 90% din contaminarea radioactivă. În cadrul decontaminării de suprafaţă au prioritate plăgile deschise. Plăgile vor fi irigate abundent cu ser fiziologic, timp de câteva minute. Personalul medical va purta echipament chirurgical (măşti/scuturi faciale, mănuşi, şorţuri, protecţie pentru încălţăminte etc.), pentru a reduce posibilitatea de contaminare inclusiv prin ingestia sau inhalarea particulelor radioactive. Se vor schimba mănuşile de la un pacient la altul, precum şi protecția pentru încălțăminte la ieşirea din zona contaminată a UPU, se vor spăla frecvent mâinile şi brațele, iar la ieşirea din tură, echipamentul folosit va fi pus în saci speciali pentru a fi monitorizat din punct de vedere al radiațiilor. La 24 de ore de la contactul cu pacienții contaminați personalului medical i se vor recolta probe de urină şi hormoni tiroidieni în funcție de radionuclizii prezenți.

Proceduri de decontaminare externă. Se va realiza supravegherea primară notând zonele cu contaminare crescută, zonele descoperite şi plăgile. Se vor colecta probe din gură, nas şi plăgi, urina şi fecalele, inițial în zona de decontaminare, după care se va continua procedura şi în afara ei în primele 24 de ore. Se vor trata toate leziunile care amenință viața sau alte afecțiuni anterioare decontaminării. Se vor examina plăgile pacientului pentru a identifica prezența de materiale radioactive în sau pe plagă. Se vor îndepărta aceste materiale şi se vor depozita în containere speciale. Se vor decontamina întâi plăgile cu agenți obişnuiți şi se vor usca. Se va repeta procedura până când plăgile nu vor mai avea radiații detectabile, după care se vor acoperi cu pansamente impermeabile. Se va spăla întregul corp cu apă, bureți şi săpun (alți agenți de curățare). Se va freca uşor pielea pacientului şi se va clăti cu multă apă călduță până când nu mai rămâne nicio radiație detectabilă. Se va transfera pacientul din zona de decontaminare în zona de îngrijire după ce i se va schimba halatul folosit până în acel moment. De asemenea i se vor schimba şi pansamentele (compresele, feşile) deoarece în cazul plăgilor contaminate acestea absorb secrețiile contaminate radioactiv.

FORME DE AFECTARE A ORGANISMULUI UMAN

1. Iradierea externă. Iradierea externă reprezintă expunerea la radiații cu origine externă şi care de obicei nu este în contact cu suprafața corpului.

Tabelul nr.5. Iradierea penetrantă – doze, semne şi simptome, modul de conducere a cazurilor

Doză (rad)	**Semne şi simptome**	**Măsuri**
0 – 5	**Fără**	Se înregistrează pacienții.
5 – 25	**Fără. Examene speciale de laborator**	Se anunță organele ierarhic superioare în 24 de ore. Se înregistrează pacienții.
25 – 100	**Simptome tranzitorii > 2 ore (limfocitopenie după 1-4 zile)**	Se anunță organele ierarhic superioare în 24 de ore. Se înregistrează pacienții. Documentați expunerea, semnele, simptomele şi recuperarea.
100 – 200	**Simptome evidente (greață, vărsături, sindrom hematopoetic în ore/zile) < 2 h**	La fel ca mai sus. Scade performanța trupelor. Tratament simptomatic.
400	**Simptome persistente, severe (greață, vărsături etc.) < 1 oră**	La fel ca mai sus. Mortalitate 50% dacă nu se tratează. Tratament simptomatic.
600	**Simptome evidente persistente în 10-30 minute**	La fel ca mai sus. Mortalitate 100% dacă nu se tratează. Tratament simptomatic.
1 000	**Vărsături < 10 minute, diaree, hematemeză, melenă**	La fel ca mai sus. Mortalitate 100% chiar dacă se inițiază tratament.
5 000	**Semne şi simptome cardiovasculare şi SNC, vărsături < 10 minute**	La fel ca mai sus. Mortalitate 100% chiar dacă se inițiază tratament în mai puțin de 48 de ore.

2. Contaminarea externă. Contaminarea radioactivă înseamnă prezența unui material radioactiv într-un loc nepermis, de obicei amestecat cu praf sau reziduuri. Se consideră contaminată extern o suprafață a corpului dacă la măsurarea radiațiilor se descoperă la controlul direct cu contor Geiger > 450 picocurie de radiații beta – gamma sau 50 picocurie de radiații alfa.

Priorități în activitatea de decontaminare externă: 1) decontaminarea plăgilor; 2) începeți cu zonele cu contaminarea cea mai crescută; 3) protejarea plăgilor necontaminate; 4) spălarea (irigarea) şi curățarea uşoară cu un burete steril; 5) realizați debridarea plăgilor numai în cazuri excepționale; 6) schimbați frecvent pansamentul.

Obiectivul principal al decontaminării pielii este de a preveni contaminarea internă prin ingestie sau inhalare. De aceea, pentru a evita acest lucru zona contaminată trebuie acoperită cu o compresă etc. până când măsurătorile directe scad < 450 picocurie pentru emiţătoriii de radiaţii beta – gamma sau < 50 picocurie pentru emiţătorii de radiaţii alfa.

Un obiectiv secundar este de a minimaliza doza la nivelul pielii prin decontaminare urgentă.

Astfel, în cazurile de contaminare externă se vor rezolva întâi aspectele medicale acute, conform algoritmului ABCDE, după care se va evalua extinderea contaminării pielii. Se vor îndepărta hainele, bijuteriile, se vor lua probe din nas, gură urechi şi se vor depozita în saci de plastic sau cutii de sticlă pentru identificarea radionuclizilor şi analiză ulterioară. După aceşti primi paşi se va trece la decontaminarea zonei afectate, astfel:

- se extinde procesul de decontaminare, asigurând o balanţă între riscul de a leza pielea pe parcursul activităţii şi posibilitatea de a fi lezată de pulberile radioactive. *Nu se va leza pielea!;*
- decontaminarea ochilor, urechilor, nasului şi a leziunilor va fi supravegheată de personalul medical;
- părul care este foarte contaminat şi este rezistent la acţiunea de decontaminare se îndepărtează cu foarfeca sau cu un aparat electric.

În continuare se va aplica bandă adezivă pe zona contaminată, pentru a îndepărta materialul radioactiv de pe piele. Este cea mai bună metodă de decontaminare în cazul contaminării cu pulberi radioactive. Nu se va aplica banda adezivă pe zonele acoperite cu păr mult sau pe pleoape sau pe alte ţesuturi fragile. Se va evita banda adezivă foarte aderentă.

După îndepărtarea benzii adezive se va spăla zona cu săpun şi apă 1- 3 minute sau cu soluţie de curăţat mâinile care nu conţine apă tot 1-3 minute, iar în cazurile în care contaminarea este excesivă, porţiunea respectivă se spală 1-3 minute folosind un abraziv uşor, săpun şi apă.

Tabelul nr. 6. Contaminarea externă (iradierea nepenetrantă) – doze, semne şi simptome, modul de conducere a cazurilor

Doză (rad)	Semne şi simptome	Măsuri
0 – 50	Fără	Se înregistrează pacienţii expuşi.
50 – 250	Fără	Se anunţă organele ierarhic superioare în 24 de ore. Se înregistrează pacienţii expuşi.
250 – 400	Fără	Se procedează la fel ca mai sus.
400 – 800	Eritem tranzitor (aspect de arsură solară) la 1 – 3 zile de la expunere	Se procedează la fel ca mai sus.
800 – 1 500	Eritem rapid (8-24 de ore) cu / fără flictene, asemănătoare arsurilor solare severe.	Se procedează la fel ca mai sus. Tratament simptomatic.
≥1 500	Flictene severe, cu afectare tisulară care necesită timp îndelungat pentru vindecare	Se procedează la fel ca mai sus. Management dificil cu apariţia de complicaţii pe termen lung.

***Dacă se suspectează contaminarea cu radioizotopi chimic activi se vor folosi metode şi tehnici de decontaminare mai agresive la nivelul zonei contaminate, astfel*:**

- se va utiliza o soluţie de înălbitor comercial în diluţie de 1/10-1/20 în apă (soluţie 5-10 % de hipoclorit de Na) sau detergent combinat cu făină de porumb (în raport de 1:1, sub formă de pastă) în jurul feţei, a plăgilor etc.;
- se va spăla zona 1-3 minute cu o soluţie 1% de acid citric;
- se va spăla zona 1-3 minute cu o soluţie 1% de EDTA / DTPA;
- se mai poate folosi rumeguş amestecat cu apă sau 65% Na_3PO_4/4,5 carboxilmetilceluloză combinat cu detergent, în apă (raportul amestec: apă 1/20)
- se mai poate folosi detergent 1,5% amestecat cu apă şi 30 de grame de diatomit (se livrează comercial sub denumirea de kisselgur (silice pulverulente) / de făină fosilă / tripoli.

Notă: Apa folosită în scopul decontaminării poate fi aruncată direct în sistemul de canalizare.

3. Contaminarea plăgilor. Decontaminarea plăgilor se consideră o urgenţă deoarece la acest nivel absorbţia radionuclizilor se poate face foarte rapid. Se consideră o plagă contaminată dacă materialul radioactiv prezent în interiorul sau la limita unei soluții de continuitate la nivelul pielii are un potențial mijloc de acces sau de reținere în organism.

Mod de acțiune:

-Se vor trata leziunile care amenință viața conform algoritmului ABCDE.

-Se vor monitoriza plăgile pentru prezența contaminării, astfel:

-monitorizare directă pentru radiații beta şi/sau gamma;

-comprese mici uscate sau bureți chirurgicali pentru particulele alfa;

-se va iriga plaga cu ser fiziologic sau apă sterilă până când contaminarea plăgii va fi îndepărtată sau nu se va mai nota nicio modificare în nivelul de contaminare. Se foloseşte pentru decontaminare soluția de Ca-DTPA/ Ca-EDTA asociată cu lidocaină 2% (10 ml) în 100 ml ser fiziologic.

De obicei, se absoarbe doar o mică parte din materialul radioactiv de la nivelul plăgilor. O escoriație sau o arsură contaminată poate fi curățată cu săpun şi apă şi cu anestezie topică dacă este necesară o curățare mai viguroasă.

Notați pe fişa de urgență care se întocmeşte la nivelul UPU regiunea afectată, tipul radionuclidului, forma chimică a radionuclidului, metoda de monitorizare, nivelele de contaminare şi metodele folosite pentru a decontamina plaga.

4. Contaminarea internă. Se consideră că o persoană este contaminată intern atunci când materialul radioactiv a intrat în organism prin inhalare, ingestie sau absorbție. Pentru a realiza o decontaminare eficientă, se vor avea în vedere următorii factori: absorbția, excreția, concentrația, T de ½ biologic şi T de ½ efectiv. Se pot folosi agenți chelatori care scad cantitatea încorporată şi cresc eliminarea, cum ar fi DTPA (acid dietilentriaminopentaacetic) sau KI (iodura de potasiu în cazul contaminării cu ^{131}I). Timpul de la expunere este critic şi tratamentul urgent, dacă un examen inițial al secrețiilor nazale şi al întregului corp arată o posibilă absorbție de radionuclizi.

Măsurile care se vor lua în aceste situații sunt asemănătoare cu cele adoptate în cazul contaminării externe. Astfel, leziunile care amenință viața se vor trata conform algoritmului ABCDE, se va determina şi se va controla contaminarea externă. În plus, se va estima nivelul contaminării în funcție de tipul contaminantului şi de poarta de intrare. Dacă se suspectează o contaminare internă, se vor recolta imediat probe nazale şi pacientul va fi programat pentru a i se recolta urina/24 de ore şi materiile fecale, pentru determinarea radionuclizilor.

Iodura de potasiu (KI) poate fi folosită pentru prevenirea încărcării tiroidei cu izotopi radioactivi după accidentele reactoarelor nucleare.

Agenții chelatori pot fi utilizați pentru a elimina metalele din circulația sanguină înainte ca acestea să se fixeze în organele țintă. Se pot folosi şi diuretice, pentru a creşte excreția contaminanților interni. Albastrul de Prusia este util pentru a îndepărta radionuclizii de tipul ^{137}Cs sau taliul (Tl) din patul capilar intestinal şi pentru a împiedica reabsorbția lor.

Tabelul nr.7. Substanțe folosite pentru decontaminarea internă

Medicament	**Radionuclid**	**Dozaj**	**Principiul de acțiune**
DTPA (Ca sau Zn)	Am, Cf, Co, Cm, Pu, Y	i.v. 1 g/zi sau infuzie 1g/250ml glucoză 5%	Chelare
KI	I	130 mg /zi oral	Blocare
Albastru de Prusia	Cs, Rb, Tl	1-3 g/zi oral	Adsorbție
Penicilamina	Metale grele	300-900 mg/8 h oral	Chelare
Fosfați de Al	Sr	100 ml gel oral	Adsorbție/demineralizare
Alginați	Sr	10 g oral apoi 4 g/zi	
NH_4Cl	Sr	1-2g la 6 h oral	

Dacă se suspectează un contaminant de tip emițător alfa (praf, ceață, fum), se vor obține probe nazale şi orale anterior decontaminării (duş sau spălare). Dacă aceste probe nu se pot recolta, contaminarea se va determina din materiile fecale.

Atunci când se suspectează o contaminare pulmonară şi/ sau gastro-intestinală se va face o contorizare Geiger parţială sau totală a întregului organism. Contaminarea externă trebuie îndepărtată anterior determinării pentru a evita o determinare fals pozitivă.

În situaţia în care se suspectează şi se detectează o contaminare gastro-intestinală şi doza aşteptată este > 15 rad, pentru a creşte eficienţa decontaminării se va minimaliza absorbţia prin administrarea de antiacide şi se va accelera evacuarea prin administrarea unui catartic.

În cazul în care se suspectează şi se detectează o contaminare pulmonară cu un agent cu tropism osos (Ra, Sr, Ac, Th, Pu etc.) se vor lua următoarele măsuri: se vor iriga nările uşor cu soluţie salină sau apă şi se va monitoriza sputa pacienţilor pentru a determina contaminarea.

În concluzie, primele ore de la expunerea la radionuclizi sunt esenţiale pentru a preveni absobţia acestora fie prin decontaminare locală, chelare, tratament fiziologic, fie prin limitarea absorbţiei.

5. Expunerea la arme cu neutroni. Expunerea la neutroni determină efecte diferite de expunerea la radiaţii gamma, deoarece neutronii au efecte biologice mai puternice. Ei activează mici cantităţi de material organic în organism determinând unele elemente să devină radioactive, uşurând punerea diagnosticului. Personalul medical va lua următoarele măsuri:

- va trata pacientul simptomatic, se va decontamina pielea;
- dacă expunerea a fost însoţită de o reacţie de fisiune nucleară, cu producerea de iod radioactiv ce dă o expunere de > 10 rad, se va administra KI (iodură de potasiu) pentru a bloca captarea iodului radioactiv în tiroidă;
- se vor recolta ~10 ml de sânge şi 100 ml de urină pentru o analiză spectrală pentru ^{24}Na (radioactiv);
- se va recolta ~ ½ g de păr pentru determinarea ^{32}P (radioactiv);
- se vor lua de la pacienţi obiectele de metal pentru a determina activarea radioactivă a respectivului obiect şi se va nota locul de unde a fost luat.

TRATAMENTUL LEZIUNILOR PROVOCATE DE ARMA NUCLEARĂ

1.Tratamentul de urgenţă. În cazurile de expunere la radiaţii victimele vor fi tratate aplicând algoritmul ATLS (ABCDE), cu realizarea manevrelor salvatoare de viaţă, inclusiv resuscitarea cardio-respiratorie şi cerebrală, chiar şi respiraţie gură la gură, posibilitatea de contaminare a salvatorului de la victimă fiind foarte mică.

Se vor evalua şi reevalua semnele vitale şi starea de conştienţă pe tot parcursul procesului de decontaminare. Este necesar ca personalul medical de urgenţă să fie pregătit pentru a interveni rapid în cazul în care apare o schimbare în starea pacientului. După realizarea supravegherii primare, a fazei de resuscitare (acolo unde este cazul) se va trece la supravegherea secundară, pentru evaluarea completă a tututor leziunilor. Pentru a realiza documentarea acestor leziuni se vor face toate investigaţiile radiologice care sunt necesare. La acest tip de pacient nu trebuie scăpat din vedere nivelul anxietăţii, existenţa sindromului de stres post-traumatic (PTSD), precum şi nevoia de intervenţie precoce a psihologului sau chiar a medicului psihiatru.

2. Tratamentul leziunilor de iradiere. Îngrijirea medicală semnificativă poate fi necesară la 3-5 săptămâni de la expunere pentru 10-50% din personal. Problemele pot include infecţii, hemoragii, febră, vărsături şi diaree.Tratamentul va conţine:

- fluide şi electroliţi pentru pierderile gastro-intestinale (GI);
- citokine pentru pacienţii cu imunosupresie, care au < 300-500 leucocite/ml sau cei cărora le scade numărul de leucocite. Anterior administrării tratamentului se va repeta hemograma;
- reducerea orelor de program. Se va interzice expunerea la radiaţii, intervenţiile chirurgicale elective şi traumele.

De reţinut că dacă pacientul are > 1 700 leucocite/ml la 48 de ore de la expunere, este puţin probabilă expunerea la radiaţii fatale. Pacienţiii asimptomatici care au primit doze letale de radiaţii vor fi în serviciu de zi până când devin simptomatici.

Amputaţia trebuie luată în considerare atunci când contaminarea radioactivă este foarte mare şi există posibilitatea de a apărea radionecroza.

3. Tratamentul leziunilor combinate. Clasic, plăgile de luptă sunt lăsate deschise pentru a se vindeca per secundam, dar în cazul pacienţilor iradiaţi, aceastea vor servi ca focare de infecţie. Aceste plăgi vor fi debridate şi închise la 36-48 de ore, după ce vor fi explorate chirurgical din nou. Leziunile radiologice cresc morbiditatea şi mortalitatea datorită compromiterii răspunsului normal hematopoetic şi imun. Intervenţiile chirurgicale elective trebuie întârziate pe timpul suprimării răspunsului imun, dacă acest lucru este posibil.

Evacuarea pacientului la eşaloanele medicale superioare va fi întârziată până când acesta va fi stabil din toate punctele de vedere.

Tratamentul leziunilor care ameninţă viaţa se va face anterior procedurilor de decontaminare, de estimare a dozelor de iradiere şi respectiv anterior tratamentului simptomelor de iradiere.

Nu uitaţi! Trataţi pacienţii conform algoritmului trauma ATLS şi anume ABCDE anterior măsurilor de decontaminare!

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Cercetări privind obținerea unor noi agenți antimicrobieni din clasa tioureidelor acidului 2(2`-feniletil)-benzoic: sinteza, caracterizarea fizico-chimică şi screening-ul biologic

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Centrul de Cercetări Ştiinţifice Medico-Militare, Bucureşti

Rezumat. Lucrarea este o sinteză selectivă a unui Proiect de cercetare pentru studiul unor noi compuşi din clasa tioureidelor acidului 2-(2'-feniletil)-benzoic. A fost efectuat screening, conform normelor europene în vigoare, pe bacterii aerobe, bacterii anaerobe, virusuri, fungi, levuri, spori şi toxine. A fost efectuata si testarea toxicologică acută pe animale de laborator.

Rezultatele obținute au fost prelucrate statistic, în vederea evaluarii comparative a efectelor precum şi pentru încadrarea în grupe farmacologice. La testarea efectului antimicrobian al compuşilor, prin diluții succesive în mediu de cultură lichid, conform normelor pentru dezinfectante, compuşii nu evidențiază activitate antibacteriană. Majoritatea tioureidelor testate cu această tehnică au prezentat efect antibacterian, inegal, de la foarte slab la slab. Efectul obținut a fost mai bun pe bacteriile gram-pozitive decât pe cele gram-negative. Prin metoda de testare screening a efectului antibacterian al compuşilor, prin metoda difuzimetrică pe mediu de cultură solid, compuşii nu prezintă activitate antibacteriană. Compuşii testați nu relevă efect antifungic, antiviral, sporicid sau antitoxinic. La testarea prin metoda antibiogramei difuzimetrice, adaptată de noi în acest scop, unii compuşi prezintă efect antibacterian, ceea ce ne conduce la concluzia că aceste cercetări trebuie să fie continuate şi aprofundate. Metoda de testare screening a efectului toxic al compuşilor, prin tehnica injectării intraperitoneale la şoareci, demonstrează toxicitate acută normală pentru substanțele farmacologic active, fapt confirmat prin testarea farmacotoxicologică. Inexistența unui efect antimicrobian evident *in vitro* nu înseamnă că substanțele nu pot avea alte efecte farmacologice.

Cuvinte cheie: agenți antimicrobieni, sensibilitate microbiană, terapie antiinfecțioasă, tioureide

Abstract. The study presents a concise selection of applicative research of pharmaceutical microbiology for the purpose of testing the biological activity of certain new thioureides having potentially anti-infectious effects.

Objectives: the synthesis, physical-chemical analysis and biological testing of the thioureides (3 series), screening on aerobic bacteria, anaerobic bacteria, viruses, fungi, yeasts, spores and toxins, testing the antimicrobial effect according to the European norms in force, acute toxicological testing on laboratory animals, comparing the results and statistical processing for the classification in pharmacological groups.

The majority of the thioureides tested presented antibacterial effect; however, this was unequal, varying from very weak to very good; we deemed very weak the average effect occurring by the inhibition area having a different diameter. We registered substances having a very weak effect, weak, good and with very good effect. The effect is better on the Gram-positive bacteria, rather than on the Gram-negative ones, almost double, which could lead us to conclude that the effect would take place via the alteration of the bacterial wall structure, probably through the inhibition of the peptidoglycan synthesis. On average, the value of the diameter of the inhibition area of some thioureides as weighed against the standard bacteria tested, as compared with the mean for the phenol standard. The compounds tested do no indicate antifungi, antiviral, sporicidal or antitoxin effect.

Lucrarea este o sinteză selectivă a Proiectului de cercetare intitulat "Cercetări privind obținerea unor noi agenți antimicrobieni din clasa tioureidelor: sinteză, caracterizarea fizico-chimică şi screening biologic" ANTIMIC, Contract nr. P3 61-046 /2007 (2007 – 2010) cu Centrul Național de Management Programe (CNMP), Unitatea de Management Programe (UMP): domeniul 6 Biotehnologii, coordonator de proiect Universitatea de Medicină şi Farmacie "Carol Davila" Bucureşti, care a efectuat sinteza şi analiza fizico-chimică a noilor compuşi, la Catedra de Chimie Farmaceutică din Facultatea de Farmacie. Centrul de Cercetări Ştiințifice Medico-Militare, în calitate de partener al proiectului, a efectuat testarea biologică a tioureidelor acidului 2-(2'-feniletil)-benzoic (3 serii). Obiective**:** Screening pe bacterii aerobe, bacterii anaerobe, virusuri, fungi, levuri, spori şi toxine; testarea efectului antimicrobian conform normelor europene în vigoare;. testarea toxicologică acută pe animale de laborator; compararea rezultatelor şi prelucrarea statistică pentru încadrarea în grupe farmacologice.

BAZA TEORETICĂ ŞI ORGANIZAREA TESTĂRILOR. Dezinfecția este acțiunea de distrugere şi îndepărtare a marii majorități a microorganismelor de pe suprafețe şi obiecte, din aer, apă, alimente etc., cu mențiunea expresă că în urma acestui demers pot rămâne anumite microorganisme, mai ales spori, care însă, în condițiile date, nu pun în pericol starea de sănătate a pacienților. Metoda se adresează celei de a doua verigi a procesului epidemiologic, căilor şi mecanismelor de transmitere ale infecției. Dezinfecția foloseşte un complex de metode şi mijloace (mecanice, fizice, chimice) menite să îndepărteze sau să distrugă agenții patogeni sau condiționat patogeni existenți în mediu şi capabili să genereze infecții.

Dezinfecția poate fi *profilactică,* atunci când se urmăreşte îndepărtarea unor eventuali germeni (potabilizarea apei, pasteurizarea laptelui, prelucrarea alimentelor, dezinfecția în unitățile sanitare, colectivități etc.), sau în *"în focar"*, când acțiunea este îndreptată împotriva unor germeni cunoscuți, care sunt eliminați în mediu de către bolnavi sau de către purtătorii de germeni.

Eficacitatea dezinfecției este influențată de numeroşi factori: felul şi nivelul contaminării microbiene, timpul de expunere la dezinfectant şi concentrația utilizată, temperatura ambiantă, interreacția dintre dezinfectant şi suportul material pe care se aplică etc.

Centers for Diseases Control (CDC) din Atlanta, SUA, a definit mai multe nivele de dezinfecție: *dezinfecția de nivel înalt*: distrugerea tuturor microorganismelor, cu excepția sporilor unor microorganisme; *dezinfecția de nivel mediu (intermediar):* se inactivează majoritatea germenilor, inclusiv *Mycobacterium tuberculosis,* unele virusuri şi unii fungi, fără afectarea sporilor; *dezinfecția de nivel scăzut,* care poate distruge marea majoritate a bacteriilor, virusuri şi fungi dar nu are acțiune pe unii germeni rezistenți în mediu, cum ar fi bacilul Koch; *curățarea* reprezintă îndepărtarea diferitelor substanțe (praf, pământ, substanțe organice) de pe suprafețe; se obține, de regulă, utilizând apă, detergenți, factori mecanici. Are rolul de a favoriza acțiunea substanțelor dezinfectante. **Antisepticele** sunt acele substanțe cu acțiune antimicrobiană astfel condiționate încât să poată fi folosite prin aplicare externă pe piele sau țesuturi. Din punct de vedere structural, dezinfectantele sunt extrem de variate. Pentru dezinfecție sunt utilizați: alcooli, halogeni şi compuşi halogenați, aldehide, fenoli, compuşi cuaternari de amoniu, peroxidul de hidrogen etc.

ASPECTE DETERMINANTE ÎN STABILIREA STRATEGIEI DE CERCETARE

Rezistența microbiană la antibiotice poate fi definită ca abilitatea germenilor patogeni de a supraviețui şi de a se multiplica în prezența uneia sau mai multor substanțe antimicrobiene. Germenii rezistenți sunt sau devin "toleranți" la antibiotice, sustrăgându-se prin variate modalități efectului antibacterian scontat, după administrarea de doze terapeutice, netoxice pentru organism. Se descriu: *rezistența naturală, de specie*, la antibiotice, care este caracteristică unor specii bacteriene şi *rezistența dobândită la antibiotice*, definită ca fenomen adaptativ al speciilor microbiene față de mediul extern nefavorabil şi care se realizează prin mecanisme diverse, determinate în final de specia microbiană implicată şi de tipul de substanță antimicrobiană utilizată.

Antibiograma, atât prin procedeul clasic, difuzimetric, cât şi prin mijloace moderne, automatizate, prin metode calitative sau cantitative, reprezintă modalitatea cea mai simplă de a evalua acțiunea substanțelor antimicrobiene asupra germenilor. În acelaşi timp, antibiograma, cu toate varietățile şi modernizările ei, cum ar, de exemplu, E-testul, oferă indicații valoroase asupra tratamentului antimicrobian ce trebuie aplicat în cazul unei infecții. În acelaşi timp, antibiograma va fi interpretată doar ca un „ghid în alegerea terapiei antimicrobiene" şi nu ca un „ordin de prescriere" (M. Voiculescu), ea fiind un instrument util atât timp cât „nu i se cere mai mult decât poate oferi" (M. Balş). Ca urmare, este necesar să se efectueze permanent cercetări pentru sinteza şi testarea unor noi substanțe potențial antimicrobiene, pentru a contracara această competiție dintre microbi şi chimioterapicele antimicrobiene.

MATERIAL ŞI METODĂ

Experimentarea unor tehnici de lucru pentru stabilirea procedurilor de operare standard. Testările au fost efectuate conform recomandărilor din „Metode şi tehnici uzuale în laboratorul clinic", lucrare editată de Ministerul Sănătății şi Academia de Ştiințe Medicale, şi Standardele „Antiseptiques et desinfectants" AFNOR.

PROTOCOL DE TESTARE. Tulpini bacteriene utilizate: *Escherichia coli* CIP54 127 (1) (ATCC 10 536); *Pseudomonas aeruginosa* CIP A 22 (1); *Staphylococcus aureus Oxford* CIP 53 154 (1) (ATCC 9 144); *Bacillus anthracis 34F2 Sterne,* forma vegetativă şi spori. Tulpinile bacteriene listate au fost cultivate în bulion peptonat, cultură statică în tuburi de 16/160 mm, respectând următori parametri: temperatură de incubare 37°C; timp de cultivare 24 h; aerare lipsă; agitare lipsă. La sfârşitul cultivării, din fiecare tulpină bacteriană au fost efectuate frotiuri (colorație Gram) care au fost examinate la microscop în vederea evaluării purității bacteriene şi a sterilității fungice. Culturile pure au fost utilizate la testarea activității bactericide a produsului la diluțiile 1/1, 1/2; 1/4 şi 1/8 după 5, 10 şi 15 minute de contact (cultură + dezinfectant). Din fiecare diluție, după 5 - 15 minute de contact, au fost efectuate însămânțări cu ansa în plăci Petri cu agar nutritiv. Plăcile au fost incubate 48 h, fiind examinate la 24 şi 48 h pentru evaluarea creşterii bacteriene. Evaluarea dezvoltării culturilor bacteriene a fost făcută după următoarea schemă: **+** Creştere (cultură) abundentă pe linia de însămânțare; **±** Creştere (cultură) slabă sub formă de colonii rare; **-** Lipsa creştere culturii; *** -** Cultura de 24 h. A fost selecționat un set de tulpini microbiene standard pentru testarea efectului bactericid, virulicid, fungicid şi sporicid; a fost adaptată metoda de testare a efectului antimicrobian a compuşilor, prin diluții succesive şi, comparativ, prin tehnica antibiogramei difuzimetrice; a fost aplicată procedura de operare standard pe o grupă de clorigeni şi pe una de oxidanți; a fost pregătită baza materială specifică pentru testarea activității antimicrobiene; a fost antrenat personalul implicat în testare; raportul preliminar de experimentare constituie o bază pentru etapele următoare.

TESTAREA TIOUREIDELOR METIL DIN CELE TREI SERII

Testare ca substanțe dezinfectante. Au fost testate trei loturi a câte cinci substanțe, cu codurile de sinteză: M1 la M78 (aleator). Testarea efectuată a urmărit trei direcții, şi anume: proprietăți fizico-chimice; pulberi grosiere de culoare albă, greu solubile în apă, uşor solubile în DMSO; efectul bactericid *in vitro* pe medii de cultură al substanțelor față de un set de bacterii aerobe gram-pozitive şi gram-negative, anaerobe şi spori bacterieni; toxicitatea acută pe şoareci, prin injectare intraperitoneală.

Tehnica de lucru. Medii de cultură utilizate: bulion nutritiv, geloză nutritivă, Agar Mueller-Hinton. Tulpini bacteriene utilizate: *Escherichia coli* CIP54 127 (1) (ATCC 10 536); *Pseudomonas aeruginosa* CIP A 22 (1); *Staphylococcus aureus* Oxford CIP 53 154 (1) (ATCC 9 144); *Bacillus subtilis; Bacillus cereus; Bacillus anthracis 34F2 Sterne; Clostridium botulinum* (anaerob strict). Tulpinile bacteriene aerobe au fost cultivate în bulion nutritiv, cultură statică în tuburi de 16/160, respectând următorii parametri: temperatura de incubare 37°C, timp de cultivare 24 şi 48 h. Tulpina anaerobă de *Cl. botulinum* a fost cultivată în bulionVF 72 h. La sfârşitul cultivării, din fiecare tulpină bacteriană au fost efectuate frotiuri (colorație Gram), care au fost examinate la microscop în vederea evaluării purității bacteriene şi sterilității fungice. Culturile de 24 h pure au fost utilizate la testarea activității bactericide a tioureidelor cercetate la diluțiile 1/10; 1/50; 1/100; 1/200; 1/400; şi 1/800, după 5 şi 10 minute de contact (cultură + dezinfectant). De notat că din cele 6 substanțe, două sunt solubile în apă la 1/10, două sunt greu solubile în apă la 1/10, iar alte două se solubilizează doar parțial, dând soluții suprasaturate cu care nu s-a putut lucra la această concentrație. Din fiecare diluție, după 5 şi 10 minute de contact, au fost efectuate însămânțări cu ansa în plăci Petri cu agar nutritiv. Plăcile au fost incubate 48 de ore, fiind examinate la 24 şi 48 h pentru evaluarea creşterii bacteriene. Evaluarea dezvoltării culturilor bacteriene a fost făcută după următoarea schemă: + Creştere (cultură) pe linia de însămânțare = 0 puncte, ± Creştere (cultură) sub formă de colonii rare = 1 punct, L - Lipsă creştere cultură = activitate bactericidă eficientă = 2 puncte. Pentru comparare am aplicat un indice global, respectiv suma şi media aritmetică a efectului antimicrobian observat.

REZULTATE ŞI DISCUȚII

Datorită faptului că testările în aceste condiții au arătat rezultate slabe şi coroborând cu faptul că nu testăm substanțe dezinfectante certificate, ci facem numai un screening al unor noi substanțe potențial antimicrobiene, am aplicat şi alte tehnici de testare, pe care le-am considerat adecvate.

Proprietăți fizico-chimice. Pulberi grosiere de culoare albă, greu solubile în apă la diluțiile 1/10 – 1/100, chiar sub încălzire (la temperatură de 40-45C° şi agitare). Solubile în dimetilsulfoxid (DMSO).

Testare efect bactericid - prezentăm un exemplu în tabelele nr. 1 şi nr. 2.

Tabel nr. 1. Testare efect bactericid M71 – timp de contact 5 min

Nr. crt	Tulpina	Diluții				
		1/50	1/100	1/200	1/400	1/800
1	*E. coli*	+	+	+	+	+
2	*P. aeruginosa*	+	+	+	+	+
3	*S. aureus*	+	+	+	+	+
4	*B. anthracis*	+	+	+	+	+
5	*B.ant.(spori)*	+	+	+	+	+
6	*Cl. bot.*	+	+	+	+	+
	SUMA	0	0	0	0	0
	MEDIA	0	0	0	0	0
	Observații					

Tabel nr. 2. Testare efect bactericid M71 - timp de contact 10 min

Nr. crt	Tulpina	Diluții				
		1/50	1/100	1/200	1/400	1/800
1	*E. coli*	+	+	+	+	+
2	*P. aeruginosa*	+	+	+	+	+
3	*S. aureus*	+	+	+	+	+
4	*B. anthracis*	+/-	+/-	+	+	+
5	*B.ant.(spori)*	+	+	+	+	+
6	*Cl. bot.*	+	+	+	+	+
	SUMA	0	0	0	0	0
	MEDIA	0	0	0	0	0
	Observații					

Testarea pe animale. La diluția 1/10, şoarecii inoculați cu 0,5 ml intraperitoneal soluție de concentrație 10% au murit în câteva minute, prezentând semne de neurotoxicitate; oferim câteva exemple în tabelul nr. 3. La examenul necropsic au fost remarcate în cavitatea peritoneală leziuni hemoragice discrete: congestie şi hemoragii pe suprafața intestinelor. Aceleaşi rezultate se obțin la toate substanțele din cele trei serii.

Tabel nr. 3. Efectul toxic al tioureidelor din seria 3, aplicate ca soluție 10% în DMSO câte 0,5ml (echivalent 50 mg s.a.), injectat i.p. la şoareci

Nr.crt.	Cod subst.	Mortalitate imediată	1 zi	2 zile	3 zile	Total	Media	Observații
1	FBT 71	+						f.toxic
2	FBT 72	+						f.toxic
3	FBT 73	+						f.toxic
4	FBT 77	+						f.toxic
5	FBT 78	+						f.toxic
M1 pozitiv	Fenol 5%	+						f.toxic
M2 negativ	AD							netoxic
M3 solvent	DMSO							netoxic

Deoarece efectul antibacterian este slab, am adaptat metoda antibiogramei difuzimetrice pentru testare. Astfel, în plăci Petri de 15 cm cu mediu pentru antibiograme Mueller-Hinton însămânțate cu următoarele tulpini de referință INCDMI Cantacuzino: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* şi *Bacillus anthracis* (forma vegetativă şi sporulată), am format 4 serii de testare. Am poziționat cu şablonul câte 19 substanțe în fiecare placă (3 plăci pe serie), cu o microspatulă a circa 10 mg substanță pură. După incubare aerobă la 37°C, plăcile au fost examinate macroscopic la 24 şi 48 h şi nu au fost observate zone de inhibiție a creşterii bacteriene. Deoarece este posibil ca efectul să nu se manifeste, întrucât substanțele sunt greu solubile, deci difuzează doar parțial în mediul de cultură (care conține 96% apă), am realizat soluții apoase, dar substanțele nu s-au solubilizat. Având în vedere faptul că substanțele testate sunt greu solubile, am considerat că ar fi posibil să avem concentrații reale mai mici decât cele estimate şi am efectuat o nouă serie de teste, în care solventul folosit a fost dimetilsulfoxidul (DMSO). În acest solvent solubilizarea a fost rapidă şi completă şi am repetat testarea în aceleaşi condiții. Am poziționat cu pipeta automată de 50µl soluție din fiecare substanță de testat circa 5%, echivalentul a 2,5 mg substanță activă, pentru compararea directă cu etalonul clasic, fenol 5% în apă distilată, ca martor pozitiv. Am utilizat ca martori apa distilată şi DMSO, folosind aceeaşi tehnica (placă, mediu, cultură, şablon, incubare, citire) şi nu s-au observat zone de inhibiție. Deoarece toate testele de screening efectuate de noi, cu diferite metode şi tehnici de lucru, nu au evidențiat un efect antibacterian cert, nu am continuat testările pentru CMI, CMB etc.

Tabel nr. 4. Rezultatele testării cu pulberi 10 mg exprimate în diametrele zonei de inhibiție în milimetri

Nr.crt.	Cod subst.	*Staf.a.**	*B.cereus***	*E.coli**	*Ps.aer.**	Suma	Media	Observații
1.	FBT 71	0	0	0	0	0	0	f.slab
2.	FBT 72	0	0	0	0	0	0	f.slab
3.	FBT 73	0	0	0	0	0	0	f.slab
4.	FBT 77	7,5	0	0	7,5	15	3,75	slab
5.	FBT 78	8	0	0	0	8	2	slab
	Total mm	15,5	0	0	7,5	23	5,75	
	Media mm	3,1	0	0	1,5	4,6	1,15	slab
M1	Fenol 5%	21	13	18	18	70	17,5	bun
M2	AD	0	0	0	0	0	0	
M3	DMSO	0	0	0	0	0	0	
Blank		0	0	0	0	0	0	

- *tulpină standard, colecția națională INCDMI Cantacuzino
- **tulpină colecția laboratorului

Tabel nr.5. Rezultatele testării cu soluție 5% DMSO 50µl exprimate în diametrele zonei de inhibiție în milimetri

Nr.crt.	Cod subst.	*Staf.a.**	*B.cereus***	*E.coli**	*Ps.aer.**	Suma	Media	Observații
1	FBT 71	0	0	0	0	0	0	f.slab
2	FBT 72	0	0	0	9	0	0	f.slab
3	FBT 73	0	0	0	0	0	0	f.slab
4	FBT 77	10	0	0	12	22	5,5	slab
5	FBT 78	0	0	0	0	8	2	slab
	Total mm	10	0	0	21	31	7,75	
	Media mm	2	0	0	4,2	6,2	1,55	slab
M1	Fenol 5%	21	13	18	18	70	17,5	bun
M2	AD	0	0	0	0	0	0	
M3	DMSO	0	0	0	0	0	0	
Blank		0	0	0	0	0	0	

- *tulpină standard, colecția națională INCDMI Cantacuzino
- **tulpină din colecția laboratorului

COMENTARIU. Tioureidele testate au prezentat caracteristici fizico-chimice similare, fiind pulberi, de diferite dimensiuni, de culoare albă; toate sunt greu solubile în apa distilată, dar solubile în dimetilsulfoxid; nici una nu prezintă activitate antibacteriană certă; animalele inoculate cu doza 0,5 ml i.p., la diluția 1/10 au murit la câteva minute dupa inoculare, deci prezintă toxicitate mare, de altfel caracteristică majorității substanțelor farmacologic active; este necesară testarea efectului antibacterian şi în alte condiții decât cele standardizate pentru dezinfectante; majoritatea tioureidelor testate cu această tehnică au prezentat efect antibacterian, inegal, de la foarte slab la slab; am considerat foarte slab efectul mediu manifestat prin zona de inhibiție cu diametrul mai mic sau egal cu 6 mm (cât zona de aplicare a substanței), slab între 6 şi 10 mm, bun între 10 şi 17,5 mm (echivalentul etalonului) şi foarte bun peste această valoare; efectul este mai bun pe bacteriile gram-pozitive decât pe cele gram-negative, de unde s-ar putea concluziona că mecanismul de acțiune ar fi prin alterarea structurii peretelui bacterian, probabil prin inhibarea sintezei de mureină; având în vedere efectul în general slab, nu am continuat testările.

EFECTUL ANTIFUNGIC. Testarea efectului inhibitor al soluțiilor asupra dezvoltării fungice la *Aspergillus parasiticus*. Experimentul s-a realizat pe plăci Petri cu ϕ = 10 cm, cu mediul solid Czapek-Dox. Soluțiile de testat, cu concentrația 10%, au fost adăugate în condiții de sterilitate în volume de 0,5 şi 1 ml/placă. După absorbția soluțiilor s-a adăugat suspensia fungică de conidii = 1 ml/placă, acelaşi volum şi densitate sporală ca şi în placa Martor, urmărindu-se dezvoltarea culturilor fungice, după o incubare la 26^0 C, pe parcursul a 7 zile. Aceste observații sunt valabile pentru toate soluțiile testate. Putem concluziona că soluțiile nu afectează dezvoltatea fungilor din genul *Aspergillus* (tabel nr. 6).

Tabel nr. 6. Testare micologica pe *Aspergillus parasiticus* aflatoxigen (exemplu)

Soluția de testat (ml/placă)	Caracteristici micologice macroscopice la 7 zile de la incubare
Cultura Martor *A. parasiticus*	Pânza miceliană compactă, densă, sporulată, pigmentat verde închis.
M71	Se constată o dezvoltare miceliană normală, tipică genului *Aspergillus*, fără modificări sau alterări ale procesului de sporulare sau pigmentare a miceliului.

EFECT SPORICID. S-au folosit culturi sporulate de *Aspergillus parasiticus,* pentru a se testa efectul soluțiilor asupra viabilității sporilor. Soluțiile testate, cu concentrația 10%, au fost aplicate pe o cultură fungică sporulată de *Aspergillus parasiticus*, permițându-se contactul direct între fung şi soluția de testat 5 şi respectiv 20 de minute. Suspensia de spori fungici a fost folosită ca inocul pentru cultivare pe mediul solid Czapek Dox, urmărindu-se dezvoltarea tulpinilor pe o perioada de 7 zile. Cultivările s-au realizat pe plăci Petri cu ϕ = 8 cm, aplicându-se câte 3 ml din soluția de testat pe fiecare placă. Incubarea culturilor s-a realizat la 26^0 C, timp de 7 zile.

S-au constatat următoarele aspecte: culturile fungice provenite din 12 suspensii sporale tratate, 5, respectiv 20 de minute cu soluțiile 10%, prezintă aceleaşi caracteristici morfologice macroscopice ca şi cultura Martor provenită din suspensie sporală netratată.

Putem afirma că soluțiile 10% nu au efect sporicid. Aceleaşi rezultate se obțin şi la celelalte substanțe, fără excepție.

EFECT DETOXIFIANT. Determinarea efectului soluțiilor 10% asupra aflatoxinelor. În acest scop s-a folosit o soluție de aflatoxină brută extrasă dintr-o cultură fungică aflatoxigenă. Soluția de aflatoxină cu concentrația de 4,1 mg%

a fost pusă în contact direct cu soluțiile 1 ml aflatoxină + 1ml soluție de testat. Verificarea efectului detoxifiant s-a realizat după 24 de ore, folosind metoda cromatografiei în strat subțire. Aplicarea probelor pe cromatoplacă s-a realizat automat cu aplicatorul Linomat IV în volume fixe (10 µl/ bandă). Condițiile de cromatografie au fost: Suport cromatografic -Silicagel 60 F 254 Merck HPTLC, 10/20 cm; Developant Cloroform: acetonă: apă(140:20:0,3); Migrare unidimensională ascendentă; Vizualizare (estimare calitativă a benzilor fluorescente) UV 254-366 nm; Tracurile 1-6 prezintă probele de aflatoxină tratate cu soluțiile iar tracul 7 reprezintă proba de aflatoxină netratată. Se evidențiază foarte bine cele două benzi fluorescente reprezentate de AFB1 şi AFG1 prezente în amestecul aflatoxinic de pe trac, rămase nealterate în cazul probelor tratate cu soluțiile de testat. Aceleaşi rezultate se obțin şi la celelalte substanțe.

EFECT ANTIVIRAL. Efectul antiviral a fost pus în evidență prin acțiunea pe care o manifestă aceste substanțe asupra virionilor: virusul West Nile (WNV), virusul encefalitei de căpuşă (TBE) şi virusul hepatitei B (HBV), cultivate pe culturi celulare.

Efectul citopatic este prezent îndeosebi în culturile care provin de la păsări şi mamifere. Se manifestă în 24 h - 7 zile, prin rotunjirea celulelor, monostratul prezentând arii largi de desprinderi celulare. Leziunile celulare constau în alterații mitocondriale, vacuolizarea citoplasmei şi, caracteristic, hipertrofia membranelor reticulului endoplasmatic, în care se acumulează particule virale. În celulele de țânțar, efectul citopatic poate fi minim sau absent (deşi virusul este sintetizat în concentrații mari) ori de tip sincițial. La examenul ultramicroscopic se constată hipertrofia membranelor interne celulare, ca şi la celulele de mamifere. Până în prezent nu s-a reuşit cultivarea virusului HB în culturi celulare. S-au făcut numeroase încercări, utilizându-se: culturi primare de hepatocite umane adulte sau fetale şi linii stabilizate de hepatom celular. Celulele infectate nu prezintă alterări tipice sau efect citopatic, iar nivelurile de virus sunt minime.

TESTAREA PE ARBOVIRUSURI. Pentru cultivarea flavivirusurilor am folosit linia celulară Vero. Din linia celulară Vero 186 (1:3) am efectuat subculturi în kolle de 25 ml. Pentru desprinderea monostratului am utilizat tripsină Versen, iar pentru cultivare, mediul Eagle îmbogățit cu ser fetal de vițel 5%. Kollele au fost incubate la 37^{0}C, timp de 24 h, iar a doua zi au fost examinate la microscopul inversat, pentru a observa formarea monostratului. Culturile au fost urmărite din punct de vedere morfologic la 24, 48 şi 72 h. Am obținut următoarele rezultate (tabel nr. 7).

Tabel nr. 7. Testarea virusologică pe compuşi (exemple)

Proba	Efectele apărute după intervalul de timp:		
	24 h	48 h	72 h
M	Aspect normal, monostrat celular complet.	Aspect normal, monostrat celular complet.	Aspect normal, monostrat celular complet.
P	Desprindere celule 95%, celule rotunde translucide plutesc libere în mediu, decolorarea mediului de cultură.	Desprindere celule 100%, celule rotunde translucide plutesc libere în mediu, decolorarea mediului de cultură.	Desprindere celule 100%, celule rotunde translucide plutesc libere în mediu, decolorarea mediului de cultură.

Rezultatele obținute au fost concludente. Atât virusurile, cât şi cele trei substanțe dezinfectante au distrus monostratul celular, iar prin punerea lor în comun se presupune că efectele s-au însumat. De aceea am efectuat un alt test, imunoenzimatic, în care am folosit suspensii virale (WN şi TBE) şi suspensie din creier normal. Rezultatele obținute demonstrează că substanțele dezinfectante nu au acțiune certă asupra suspensiilor virale WN şi TBE, godeurile care conțineau amestecurile de suspensii şi dezinfectanți având reacție de culoare.

TESTAREA PE HBV. Pentru virusul HB am folosit kit-ul BioELISA HBsAg, care pune în evidență prezența antigenului HBs în diferite probe. Controlul pozitiv din trusă a fost pus în contact cu cele trei substanțe dezinfectante. Am folosit o probă pozitivă de hepatită B (diluată în alcool), care a fost şi ea amestecată cu cele trei substanțe dezinfectante. În urma acestei testări am ajuns la concluzia că substanțele testate ca dezinfectante nu acționează asupra AgHBs.

TESTAREA FARMACOTOXICOLOGICĂ. **Obiectivul studiului:** evaluarea toxicității acute (prin injectare i.v. la şobolani) a unui compus farmacologic activ selectat din cele trei serii de compuşi tioureide metil cu posibilă acțiune antimicrobiană, în scopul introducerii în practica medicală. Având în vedere că niciunul dintre compuşi nu s-a calificat ca potențial dezinfectant, nu am mai efectuat acest test laborios decât la unul dintre compuşi, respectiv la M78. Conform cu Raportul farmacotoxicologic, substanța are toxicitate mare (foarte toxic), dar acest fapt este comun majorității substanțelor farmacologic active. Rezultatele concordă cu testarea screening pe şoareci.

DISCUȚII. A fost aplicată mmetoda de testare screening a efectului antibacterian al compuşilor, prin metoda difuzimetrică pe mediu de cultură solid; toți compuşii nu prezintă activitate antibacteriană. A fost aplicată metoda de testare screening a efectului toxic al compuşilor, prin metoda injectării intraperitoneale la şoareci; toți compuşii prezintă toxicitate acută, ceea ce este normal pentru substanțele farmacologic active. A fost aplicată metoda de testare a efectului antimicrobian al compuşilor, prin diluții succesive în mediu de cultură lichid, conform normelor pentru dezinfectante, toți compuşii nu prezintă activitate antibacteriană. Majoritatea tioureidelor testate cu această tehnică au prezentat efect antibacterian, inegal, de la foarte slab la slab. Efectul este mai bun pe bacteriile gram-pozitive decât pe cele gram-negative. Compuşii testați nu prezintă efect antifungic, antiviral, sporicid sau antitoxinic. La testarea prin metoda antibiogramei difuzimetrice, adaptată de noi în acest scop, unii compuşi prezintă efect antibacterian, ceea ce ne conduce la concluzia că aceste cercetări trebuie să fie continuate şi aprofundate. Oricum, faptul că nu există un efect antimicrobian marcat *in vitro* nu înseamnă că substanțele nu pot avea şi alte efecte farmacologice.

Concluzii

La testarea efectului antimicrobian a compuşilor, prin diluții succesive în mediu de cultură lichid, conform normelor pentru dezinfectante, compuşii nu prezintă activitate antibacteriană. Majoritatea tioureidelor testate cu această tehnică au prezentat efect antibacterian, inegal, de la foarte slab la slab. Efectul este mai bun pe bacteriile gram-pozitive decât pe cele gram-negative.

Prin metoda de testare screening a efectului antibacterian a compuşilor, prin metoda difuzimetrică pe mediu de cultură solid, compuşii nu prezintă activitate antibacteriană certă.

Compuşii testați nu prezintă efect antifungic, antiviral, sporicid sau antitoxinic.

La testarea prin metoda antibiogramei difuzimetrice, adaptată de noi în acest scop, unii compuşi prezintă efect antibacterian, ceea ce ne conduce la concluzia că aceste cercetări trebuie să fie continuate şi aprofundate.

Metoda de testare screening a efectului toxic a compuşilor, prin injectare intraperitoneală la şoareci, demonstrează că prezintă toxicitate acută, ceea ce este normal pentru substanțele farmacologic active, fapt confirmat de testarea farmacotoxicologică.

Inexistența unui efect antimicrobian evident şi superior etalonului fenolic *in vitro* nu exclude posibilitatea ca substanțele să poată avea efect antiinfecțios ori alte efecte farmacologice, ceea ce se poate verifica prin cercetări ulterioare.

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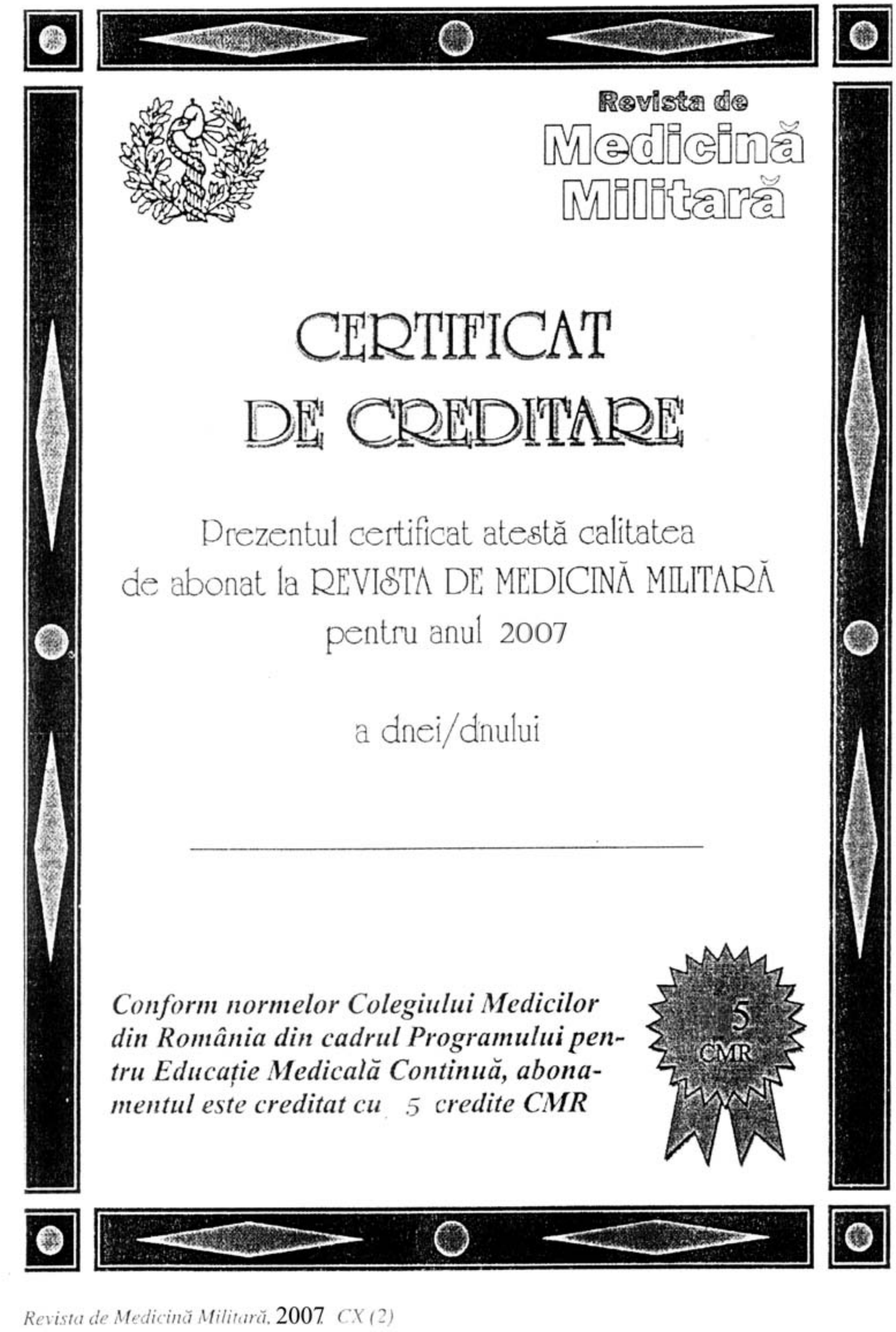

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Tumorile de sân. Mamografia prin Rezonanță Magnetică (MRM)

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Rezumat. Cancerul mamar este cauza cea mai frecventă a cancerului la femei, des implicată în decesul femeilor sub 50 de ani. Riscul unei femei de a dezvolta cancer de sân este de circa 12 % iar mortalitatea prin cancer de sân este crescută. Riscul de transmitere ereditară a fost evaluat mai exact odată cu descoperirea genelor BRCA1 şi BRCA2. Mamografia prin Rezonanță Magnetică este cea mai sensibilă metodă pentru detecția şi stadializarea carcinomului invaziv de sân. Această metodă deschide căi noi în diagnosticul cancerului de sân.

Cuvinte cheie: cancer de sân, IRM, MRM, priză de contrast.

Abstract. Breast cancer is the most common cancer in women and one of the leading cause of death in women under age 50. The chance of a woman developing invasive breast cancer is about 12%, and breast cancer is one of the leading causes of cancer mortality. Hereditary breast cancer has been increasingly recognized with the discovery of the *BRCA1* and *BRCA2* genes. MR Mammography is the most sensitive method for detecting and staging invasive breast carcinomas. It has gained widespread acceptance for clinical applications from high-risk screening evaluation to cancer evaluation and therapy response. This method is opening a new way in the diagnosis of breast cancer.

Scopul examinării constă în completarea examenului clinic, ecografic şi mamografic atunci când acestea nu sunt concludente în vederea localizării şi caracterizării cât mai exacte a tumorii. Examenul IRM nu înlocuieşte mamografia şi vine cu date complementare pentru stabilirea conduitei terapeutice adecvate. Alte indicații ale examinării IRM de sân sunt investigarea integrității implantului de sân, evaluarea efectelor chimioterapiei, distingerea țesutului cicatriceal de tumorile recidivate. IRM evaluează în profunzime anomaliile detectate ecografic sau mamografic şi poate detecta în stadii incipiente tumorile de sân în cazul țesutului mamar dens precum şi la pacientele cu risc crescut de apariție a acestei afecțiuni.

Protocolul de examinare foloseşte secvențe cu supresie de grăsime de tip T2 şi pre- şi post contrast T1. Utilă este şi tehnica de substracție şi reconstrucțiile MIP. Agentul de contrast poate fi injectat la mână sau cu injector automat. Pentru a evidenția kinetica la substanța de contrast, este necesar ca examinarea să înceapă rapid după injectarea substanței de contrast. Rezoluția trebuie să pună în evidență leziunea de minim 3 mm. Atunci când se suspicionează şi componentă axilară tumorală, protocolul va fi adaptat nevoilor de evaluare. Examinarea se efectuează cu pacienta în pronație, cu brațele pe lângă corp şi cu o linie venoasă antecubitală. Antenele folosite trebuie să ofere raport semnal zgomot optim. Antenele de tip „phased array" pot oferi semnal uniform iar pentru a obține o rezoluție eficientă în timp scurt este nevoie de FOV reduse şi secțiuni cât mai subțiri. Secvențele cu supresie de grăsime necesită shimming adițional. Artefactele respiratorii pot produce probleme de examinare dar sunt mult reduse de examinarea în pozița de pronație. Mişcările cardiace şi cele datorate fluxului vascular din vasele mamare pot produce imagini cu artefacte iar schimbarea direcției de codare în fază poate îmbunătăți calitatea imaginii. Mai pot fi folosite metodele de creştere a numărului de achiziţii (*oversampling*), precum şi tehnicile de presaturare. Bobina dedicată de examinare este cea de tipul phased-array iar aceste dispozitive prezintă design adaptat şi pentru acces de ambele părţi la biopsie şi localizare. Pacienta este introdusă cu membrele inferioare înainte.

În prezent sunt folosite următoarele tipuri de substanțe paramagnetice pe cale intravenoasă : Gadolinium Dimeglumine (Magnevist Bayer-Schering), Acid Gadoteric (Gd DOTA, Dotarem - Guerbet), Gadodiamida (Gd-DTPA–BMA , Omniscan Nycomed), Gadobenat de dimeglumina(GD- BOPTA Multihance Bracco). Ideală este adminstrarea cu un injector automat compatibil cu instalația IRM a substanței de contrast paramagnetice şi în bolus rapid cu un flux de 2ml /sec , urmat de 20ml de ser fiziologic. Chiar dacă nu oferă un standard comparabil, injectarea manuală a substanței de contrast poate fi de asemenea utilizată.

Pentru secvențele de tip Ecou de gradient 2 D, doza de 0,16 mmol/kg oferă o sensitivitate mai bună față de standardul de 0,1mmol/kg, în cazul câmpurilor magnetice mai mici de 1 Tesla, iar pentru câmpuri magnetice mai mari de 1 Tesla, doza standard utilă este de 0,1 mmol/kg, iar secvențele utilizate sunt de tipul 3D.

Este preferabil ca declanşarea achiziției de imagini să fie făcută cu o primă fază în intervalul de timp de 30-60 de secunde de la debutul injectării, iar obținerea de imagini să se realizeze în 1-2 minute. Se mai recomandă începerea injectării cu 15 secunde înainte de achiziția primelor imagini, astfel încât umplerea aproximativ a jumătate din spațiul K să se producă în fază arterială

Rolul examenului IRM constă în analiza tumorală a răspunsului la terapie sau în evaluarea reziduului tumoral. Postoperator, absența sau prezența prizei de contrast poate fi echivocă, deoarece fibroza post tratament poate avea priză de contrast. IRM poate caracteriza leziunile chistice, hematoamele, ductele mamare lărgite, hematoamele, precum şi ganglionii limfatici. Foarte importantă este analiza invaziei peretelui toracic în cazul leziunilor maligne. Analiza dinamică a kineticii contrastului face distincția dintre caracteristicile benigne şi cele maligne din punct de vedere morfologic, al configurației tumorale, marginilor, arhitecturii interne tumorale, caracteristicilor prizei de contrast, formei, intensității acesteia, a progresiei spațiale a contrastului. La pacientele tinere leziunile false pot apărea relativ frecvent datorită reactivității hormonale crescute. Se recomandă ca administrarea substanței de contrast să fie făcută între zilele 10-15 ale ciclului menstrual, pentru a reduce la minimum priza de contrast a țesutului normal. Cancerul invaziv de sân poate fi evidențiat datorită activității neo-angiogenetice intense. Aspectul tipic de cancer invaziv constă în creşterea marcată a intensității substanței de contrast în prima fază, urmată de stagnare în platou şi de fază wash-out. Priza de contrast periferică este aproape patognomonică pentru cancerul de sân. Aspectul neregulat de tip stelat cu priză cel puțin intermediară este sugestiv pentru leziune malignă. O leziune regulată şi bine delimitată cu enhancement rapid şi hiposemnal T2 creşte suspiciunea de tumoră malignă. IRM evaluează eficient implanturile mamare pentru excluderea unor eventuale rupturi. IRM evaluează eficient sânul dens mamografic, precum şi răspunsul la chimioterapie. IRM poate diferenția țesutul cicatricial de tumora recidivată. Totuşi doar 50% dintre carcinoamele ductale in situ prezintă priză de contrast. Masele care prezintă hipersemnal T2 pot fi : chisturi, ganglioni limfatici, fibroadenoame, carcinoame mucinoase, tumori necrotice. Hipersemnalul T2 sugerează probabilistic benignitatea.

S-au făcut progrese în identificarea femeilor cu risc deosebit de apariție a cancerului de sân. Acesta poate apărea la purtătoarele de gene BRCA1 şi BRCA2 iar testele care le identifică le pot selecționa pentru explorarea acestor femei prin IRM. Detecția carcinomului in situ este una dintre indicațiile de bază, alături de biopsia ghidată IRM, analiza eficienței chimioterapiei adjuvantei, existența leziunilor maligne contralaterale şi evaluarea adenopatiilor axilare.

Mai puțin de 0,5 % dintre cancerele de sân pot apărea la bărbați. O masă în această regiune apărută la un bărbat se poate încadra în unul dintre următoarele diagnostice: ginecomastie, abcesul de sân, metastază la nivelul sânului, sarcom, cancer primar. IRM poate evidenția extensia tumorală la peretele toracic.

Localizarea tumorală poate fi periareolară, în unul dintre cadranele glandei mamare, sau poate avea o prelungire axilară. Evaluarea locală trebuie să țină seama de piele, mamelon şi peretele toracic.

Ganglionii limfatici regionali sunt ipsilaterali şi îi includ pe cei axilari, mamari interni infra- şi supraclaviculari. Alți ganglioni limfatici care îi includ pe cei cervicali sau contralaterali sunt considerați a fi metastaze.

Metastazele hematogene sunt frecvent localizate în plămâni, oase, ficat, creier, glande suprarenale şi pleură. Whole body IRM poate fi foarte utilă în depistarea şi evaluarea metastazelor în cazul tumorilor maligne de sân cunoscute.

Criteriile de efectuare anuală a IRM de sân a American Cancer Society:

- prezența mutației genetice BRCA;
- rude de gradul I purtătoare de gene BRCA;
- riscul de 20 -25 % pe toată durata vieții;
- iradierea în regiunea toracelui la vârste cuprinse între 10 şi 30 de ani;
- sindromul Li-Fraumeni (afecțiune ereditară autozomal dominantă ce predispune la boli maligne) şi rude de gradul I cu acest sindrom;
- sindromul Cowden (al hamartoamelor multiple- afecțiune autozoal dominantă);
- sindromul Bannayan-Riley-Ruvalcaba (multiple hamartoame şi lipoame subcutanate asociate cu macrocefalie şi hemangioame-afecțiune autozomal dominantă).

Argumente insuficiente pentru sau împotriva IRM de sân (recomandare echivocă):

- riscul estimat al 15-20 % pe durata vieții – definit de criteriile BRCAPRO;
- carcinomul lobular in situ sau hiperplazie lobulară atipică;
- hiperplazie ductală atipică;
- sânul dens sau heterogen caracterizat mamografic;
- femei cu istoric personal de cancer de sân, care include şi carcinomul in situ.

Avantajele examinării IRM față de alte tipuri de examinări imagistice sunt legate de lipsa utilizării radiațiilor ionizante, achiziția directă a imaginilor în toate planurile disponibile, posibilitatea scanării întregului perete toracic, reconstrucțiile de tip 3D de tip MIP cu evaluarea prizei de contrast a leziunilor şi a ductelor galactofore. Senzitivitatea metodei este crescută (peste 90 %, conform meta-analizei) la carcinoame invazive, poate depista zone de malignitate ocultă multifocală reziduală, estimează cu acuratețe dimensiunile invaziei, rezoluția spațială este foarte bună, are abilitatea de a evidenția nodulii limfatici.

Contraindicațiile examinării IRM sunt legate de administrarea substanței de contrast IRM, incapacitatea de a sta în poziție de procubit, cifoză accentuată sau cifoscolioză, obezitate, sâni cu dimensiuni foarte mari, claustrofobie. O atenție deosebită se va acorda pacienților care prezintă risc deosebit de dezvoltare a dermopatiei nefrogenice fibrozante după administrarea substanței de contrast paramagnetice.

Secvența ideală ar trebui să aibă ca parametrii o rezoluție temporală sub 30 de secunde, achiziție volumetrică şi fără gap-uri de intersecție, rezoluție spațială de sub 1mm hipersensitivitate la agentul de contrast, anularea omogenă a grăsimii şi posibilitatea de a obține imagini ale ambilor sâni la o singură scanare.

După administrarea substanței de contrast, întreaga examinare trebuie să se încheie într-un interval de 5-7 minute, deoarece materialul de contrast paramagnetic difuzează normal în țesuturi. Planul de achiziție al imaginilor va ține seama de direcția de codare în fază, care este antero-posterioară pentru planul axial şi cel sagital, în vederea reducerii artefactelor de mişcare şi a celor datorate mişcărilor respiratorii. În planul coronal, schimbarea direcției de codare în fază în sens cranio-caudal permite utilizarea unui Field of View-FOV dreptunghiular.

Evaluarea intensității semnalului tumoral prin folosirea ROI (Region of Interest) şi aprecierea curbei temporale de variație este metoda cea mai folosită în cuantificarea leziunilor mamare. Pot fi identificate 3 tipuri de curbe:

- tipul I lent ascendent, în care priza de contrast se pune în evidență după 5 minute. La rândul său este împărțit în Ia, constant şi lent progresiv ascendent, şi Ib, cu o curbă lentă şi un platou tardiv;
- tipul II în platou - cu priză de contrast intensă în primele 1-2 minute, urmată de un platou tardiv subsecvent. Acest tip de priză de contrast sugerează caracterul malign al leziunilor;
- tipul III prezintă priză de contrast intensă după 1-2 minute, urmată de o scădere bruscă (washout) care produce un vârf caracteristic malignității.

Analiza pixel cu pixel a curbei de încărcare se face cu ajutorul stației de lucru-workstation, care utilizează soft special şi o hartă specială de culoare pentru analiza curbelor prizei de contrast a leziunilor foarte util în cazul focarelor multiple.

Aspect postoperator şi post-biopsie

Un aspect deosebit îl prezintă glanda mamară după biopsie. Țesutul normal nu se modifică după biopsia de rutină. Pot fi evidente focare hemoragice sau fragmente metalice cu hiposemnal T1 şi T2 mai uşor vizibile în secvențele de tip ecou de gradient. **Hematoamele şi seroamele** sunt vizibile la scurt timp după intervențiile chirurgicale, au aspect fluid şi nu prezintă priză de contrast. În funcție de concentrația de produşi hemoragici şi de vârsta produşilor de degradare a hemoglobinei, aspectul poate fi diferit. Prezența prizei de contrast, precum şi o banda de hiposemnal T1 şi T2 sugerează prezența țesutului de granulație ce poate masca leziuni maligne reziduale. Priza de contrast intensă postoperatorie este întâlnită în primele 28 de zile iar după această dată aspectul prizei de contrast capătă specificitate crescută. Necroza adipoasă are ca aspect IRM de zone de hipersemnal T1. Apare sub forma unor focare de țesut de granulație de tip stelat şi neregulat care prezintă priză de contrast intensă şi care se comportă specific în cazul secvențelor cu supresie de grăsime.

Aspectul post-radioterapie în primele 9-12 luni evidențiază o creştere globală a permeabilității capilare, având drept rezultat intensificarea prizei de contrast cu aspect difuz, care după circa 18 luni se transformă, gradual, în fibroză de tip peteşial. Orice priză de contrast nodulară sugerează recidivă tumorală.

Aspectul post-chimioterapie este foarte important prin analiza prizei de contrast care arată evoluția şi răspunsul leziunilor sub influența tratamentului. Dispariția prizei de contrast arată răspunsul favorabil la chimioterapie.

Leziunile benigne - fibroza perichistică, în care leziunile apar în T2 cu hipersemnal uniform, sunt bine delimitate, bine circumscrise, fără priză de contrast şi cu conținut fluid. Leziunile mai vechi pot apărea cu aspect vâscos şi cu semnal hiperintens în T1 şi discret hipointens în T2.

- **ectazia de duct mamar** poate fi vizibilă în secvențele precontrast datorită concentrației crescute de secreții cu hipersemnal T1 dacă sunt desicate-deshidratate şi ductele retroalveolare dilatate. Administrarea substanțelor de contrast pune în evidență aspectul acestui tip de leziuni.
- **adenozita sclerozantă** trebuie investigată corect în dinamică, pentru că leziunea prezintă tardiv priză de contrast intensă. Fără contrast poate fi dificil de diferențiat de parenchimul glandular.
- **fibroadenoamele** pot prezenta priză de contrast intensă şi puternică în cazul femeilor tinere cu adenoame mixoide. Distincția față de țesutul malign se face prin analiza curbei de tip washout, care nu apare în cazul adenoamelor, prin forma regulată sau polilobată şi delimitată net față de țesutul adipos din jur. Caracteristicile sunt echivalente criteriului ecografic, în care raportul diametrelor trebuie să fie de aproximativ 0,5 în cazul leziunilor benigne şi în jur de 1 în cazul leziunilor maligne. Fibroadenoamele pot avea septuri în interior care nu prezintă priză de contrast. Atunci când asociază şi prezența de țesut hialin prezintă priză de contrast cu accentuare mai lentă şi mai puțin intensă.

- papiloamele sunt dificil de distins de fibroadenoame. Comportamentul lor la injectarea substanţei de contrast este diferit şi pot prezenta priză de contrast intensă, moderată sau slabă. În general au margini bine definite şi pot fi diferenţiate de carcinoamele medulare sau intra-ductale.

- lipoame sau fibroadenolipoame (hamartoame) - sunt leziuni benigne de tip mezenchimal, diagnosticate de obicei prin imagistică de tip convenţional.

Leziunile de tip infecţios de tipul mastitei pot fi investigate prin metode convenţionale iar **MRM** arată priza de contrast intensă cu aspect difuz şi slab delimitată din punct de vedere regional. Diagnosticul diferenţial se face cu mastita granulomatosă sau cu afectarea în alte boli de tipul celor produse de diferite mycobacterii sau actinomicete sau în sarcoidoză şi granulomatoză Wegener.

Anomaliile de graniţă(borderline lesions) de tipul displaziei proliferative, hiperplaziei ductale atipice leziunilor sclerozante radiale, papilomatozei juvenile şi carcinomului lobular in situ sunt dificil de investigat prin toate metodele imagistice şi trebuie urmărite periodic şi biopsiate. Aspectul este dificil de evaluat datorită neregularităţii leziunilor şi prizei de contrast intense.

Leziunile maligne

Carcinomul ductal in situ – prezintă aspecte variate ale prizei de contrast care includ priza minimă de contrast sau chiar lipsa ei totală. Aspectele diverse ce pot apărea mai pot fi de tip focal, nodular ductal regional. Mamografia RM nu poate evalua eficient microcalcificările. Înşelător poate fi aspectul pseudo-benign, în care priza de contrast este minimă şi nu poate fi diferenţiată de aspectul normal al parenchimului.

Carcinomul ductal invaziv apare neregulat, spiculiform sau multilobulat. Priza de contrast este intensă şi creşte rapid. Există şi cazuri rare în care poate să prezinte priză de contrast lentă şi cu intensitate redusă. În jurul leziunii poate exista displazie floridă iar în leziunile multifocale se evidenţiază extensia la peretele toracic sau mamelon.

Carcinomul lobular invaziv prezintă în majoritatea cazurilor priză de contrast rapidă şi intensă. MRM este metoda de elecţie pentru stadializare preoperatorie.

Alte tumori maligne sunt **carcinomul coloid sau mucinos**, care se poate prezenta cu un aspect asemănător fibroadeneomului, dar cu septuri cu priză de contrast în interior, **carcinoamele papilare sau tubulare,** care au priză de contrast intensă şi rapidă în corelaţie cu starea angiogenezei tumorale, **limfoamele non-Hodgkin,** rare şi, de obicei, secundare extensiei a bolii şi care pot fi sincrone sau metacrone bilateral, **sarcoamele** primare sau rezultat al transformării sarcomatoase a carcinoamelor metaplazice, tumora filoidă cu aspect lobulat şi zone chistice intratumorale.

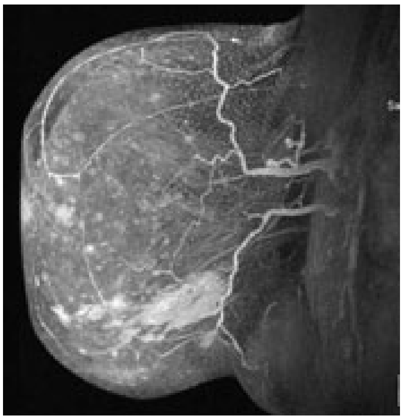

Fig.1

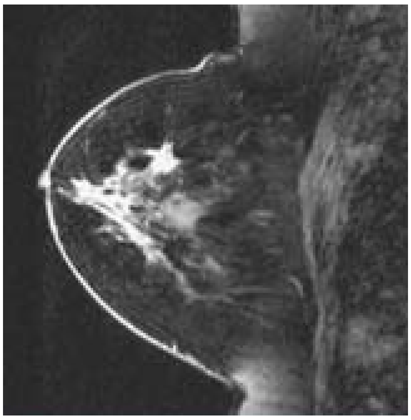

Fig.2

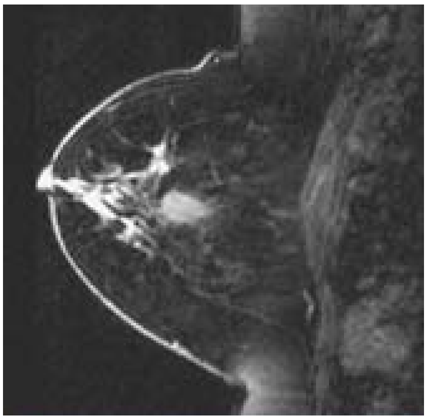

Fig.3

Fig. 1. Aspecte ale IRM în cazul Carcinomului Ductal in situ. Se observă priza de contrast intensă de la nivel tumoral.
Fig. 2. Aspect comparativ al prizei de contrast la nivelul coexistenţei unui fibroadenom situat caudal şi a unui carcinom mamar situat caudal de primul.
Fig. 3. Aspect comparativ între fibroadenom şi tumora de tip carcinomatos situată caudal cu priză intensă de contrast.

În concluzie, dacă priza de contrast intensă şi rapidă ar fi tipică țesutului malign, MRM ar fi metoda standard de explorare în practica clinică. Deoarece atât leziunile benigne, cât şi cele maligne se pot comporta surprinzător de diferit, specificitatea metodei este destul de scăzută (se apreciază prin metaanaliză la circa 37%). În consecință, diagnosticul paraclinic se bazează pe valoarea complementară a metodelor imagistice. IRM oferă rezoluție spațială crescută şi analiză foarte bună a arhitecturii leziunii. Metoda este de asemenea foarte importantă pentru că are valoare foarte mare prin repetabilitate şi comparație în timp a evoluției leziunilor, şi pentru că poate conduce foarte eficient calea biopsiei .

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Şuntul transjugular intrahepatic portosistemic, metodă de tratament minim invazivă care prelungeşte supravieţuirea pacienţilor cu ciroză hepatică

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Rezumat. Şuntul portosistemic intrahepatic transjugular reprezintă o tehnică de radiologie intervenţională minim invazivă prin care se realizează o comunicare directă, la nivel intrahepatic, între o ramificaţie a venei porte şi una dintre venele hepatice, aparţinând sistemului cav inferior. Este o soluţie terapeutică salvatoare pentru stadiile avansate ale cirozei hepatice, care scade frecvenţa complicaţiilor hemoragice, a decompensărilor prin ascite refractare la tratamentul clasic şi prelungeşte viaţa pacienţilor, oferindu-le astfel o şansă de supravieţuire până când transplantul de ficat necesar devine accesibil.

Cuvinte cheie: şunt portosistemic intrahepatic transjugular, flux hepatofug, ciroză hepatică

Abstract. Transjugular intrahepatic porto-systemic shunt represents an interventional radiologic miniinvasive technik, which make an intrahepatic direct comunication between a branch of the portal vein and one of the hepatic veins, belonging to the systemic venos circulation. It is a liferiscue therapeutic solution for the end stage liver desease, which dramatically drop the frequency of the haemorragic events, refractary ascites and delay the moment of death, giving the pacients a real chance to survive untill the moment when the liver transplant is available.

Şuntul portosistemic intrahepatic transjugular reprezintă o anastomoză intrahepatică latero-laterală ("side-to-side") între o vena suprahepatică şi un ram al venei porte, determinând scăderea semnificativă a presiunii portale. Scopul acestei metode este de a crea un flux hepatofug în vena portă. Astfel, se şuntează anastomoza dintre reţeaua sinusoidală portală şi cea capilară sistemică, care este "jugulată" de fibroza hepatică. Acest şunt preia cea mai mare parte din sângele adus de vena portă la ficat, care astfel nu mai reflueazǎ în circulaţia venoasă perigastrică şi esofagiană, determinând formarea varicelor gastrice şi esofagiene, sursa celor mai redutabile complicaţii, adesea letale, ale cirozei hepatice: hemoragiile variceale. Este o tehnică imaginată în urmă cu 40 de ani, în scopul ameliorării rezultatelor terapeutice din complicaţiile hipertensiunii portale - în particular hemoragia variceală, care este grevată şi în prezent de o mortalitate extrem de mare.

În 1969, Rosch a efectuat pentru prima dată o conexiune percutană între vena portă şi vena hepatică, cu aport jugular. În 1977, Reich a realizat un traiect intrahepatic între aceste două repere vasculare, iar în 1983 Colapinta a montat pentru prima oară în lume un stent metalic care a creat o cale artificială de comunicare între vena portă şi vena hepatică dreaptă. Această protezare s-a constituit în ceea ce numim astăzi tehnica TIPS (**T**ransjugular **I**ntrahepatic **P**ortosistemic **S**hunt). Timp de 10 ani această modalitate de scădere a hipertensiunii portale a fost experimentată, perfecţionată, lăudată şi criticată până la dezavuare din cauza numeroaselor riscuri postintervenţionale pe care le presupunea şi care nu au putut fi evitate sau anulate în primii ani de experimentare a metodei.

Începând din anul 1993, această metodă minim invazivă, care substituia intervenţiile chirurgicale laborioase de şunt spleno-renal (cu riscurile de mortalitate postoperatorii deloc de neglijat), a început să câştige teren în tot mai multe centre medicale din Europa şi America, devenind o manevră radiologică intervenţională de top, la fel de spectaculoasă şi eficientă precum montarea stenturilor coronariene. TIPS-ul a determinat prelungirea supravieţuirii pacienţilor care se aflau pe lista de aşteptare pentru un transplant hepatic, crescându-le sensibil şansa de a primi un ficat nou şi odată cu el o nouă viaţă. De obicei, durata de funcţionare a unui stent porto-venos este de câteva luni până la un an, din cauza obstrucţiei acestuia cu material biologic. S-au raportat însă cazuri când pacienţii cu TIPS au supravieţuit cu această proteză mai mulţi ani, fără a fi nevoie să fie înlocuit stentul montat iniţial. Aşa se face că între anii 1993-2008 TIPS-ul a fost acceptat în toată lumea ca metodă de elecţie, prin gradul minim de invazivitate (comparativ cu tehnicile clasice chirurgicale) care poate preveni complicaţiile cele mai redutabile din ciroza hepatică - hemoragiile variceale şi sindromul hepato-renal.

În 1994, U.S. National Digestive Deseases Advisory Board a stabilit următoarele indicaţii ale TIPS, care sunt valabile şi astăzi:

- sângerare variceală esofagiană acutanecontrolată prin metode clasice;
- sângerare variceală recurentă şi refractară la pacienţi ce nu pot tolera tratament endoscopic sau farmacologic;
- stand-by în vederea transplantului hepatic în sindromul hepato-renal;
- hidrotorax hepatic;
- HTP din sindromul Budd-Chiari;
- ascită refractară.

Contraindicaţii absolute TIPS:

- insuficienţă cardiacă dreaptă cu creşterea PVC;
- boală polichistică hepatică;
- insuficienţă hepatică severă.

Contraindicaţii relative:

- infecţie acută sistemică sau intrahepatică (colonizarea stentului);
- encefalopatie hepatică severă;
- tumori hepatice hipervasculare;
- tromboza de venă portă.

Complicaţii:

- pneumotorax
- fistulă arterio-venoasă;
- aritmii cardiace severe (produse de trecerea cateterului prin atriul drept);
- lezarea arterei hepatice sau a căilor biliare intrahepatice;
- puncţionarea venei hepatice în teritoriul extrahepatic (hemoragii);
- agravarea encefalopatiei (25% din cazuri);
- stenoza şuntului (ocluzarea);
- edem pulmonar acut/insuficienţă cardiacă congestivă (creşte D.C. şi PVC).

Pregătirea pacientului pentru intervenţie:

- antibioterapie cu spectru larg pre şi post intervenţie (2-3 zile şi respectiv 7 zile);
- evaluarea preoperatorie cu ecografie Doppler (vena portă, măsurarea velocităţii);
- portografie arterială, venografie RMN;
- transfuzie cu masă trombocitară (dacă PLT< 50 000/mmc);
- plasmă proaspătă congelată la INR>2;
- efectuarea unui CT hepatic sau RMN, eventual şi ecografie abdominală pentru evidenţierea ramurilor sistemului port intrahepatic cu decelarea eventualelor anomalii anatomice şi situaţii particulare cu importanţă în efectuarea TIPS-ului;
- pacientului i se explică principiul metodei, avantajele, precum şi incidentele ce pot apărea intraoperator, reacţiile secundare şi riscurile potenţiale care decurg din această intervenţie. Apoi se obţine consimţământul scris al acestuia.

Tehnica realizării TIPS-ului implică 4 timpi (la pacient aflat sub anestezie generală), care sunt efectuaţi de către o echipă formată din doi medici operatori, o asistentă şi un medic anestezist. Se respectă aceleaşi reguli ale asepsiei ca şi la o intervenţie chirurgicală obişnuită. Etapele operatorii sunt redate în figurile de mai jos:

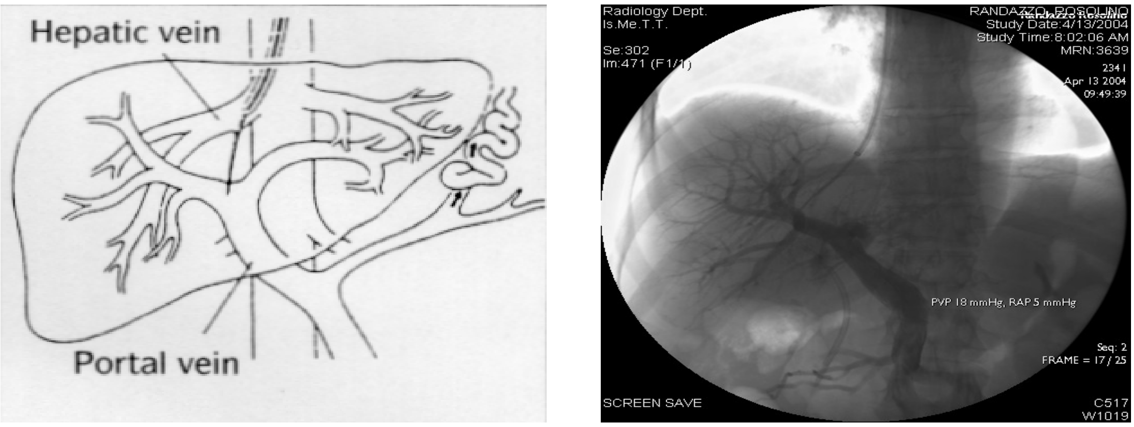

Fig. 1. Prin abord transjugular drept, un ac-cateter este dirijat sub control fluoroscopic în vena cavă inferioară şi se cateterizează optim o venă hepatică (obişnuit, dreaptă sau medie).

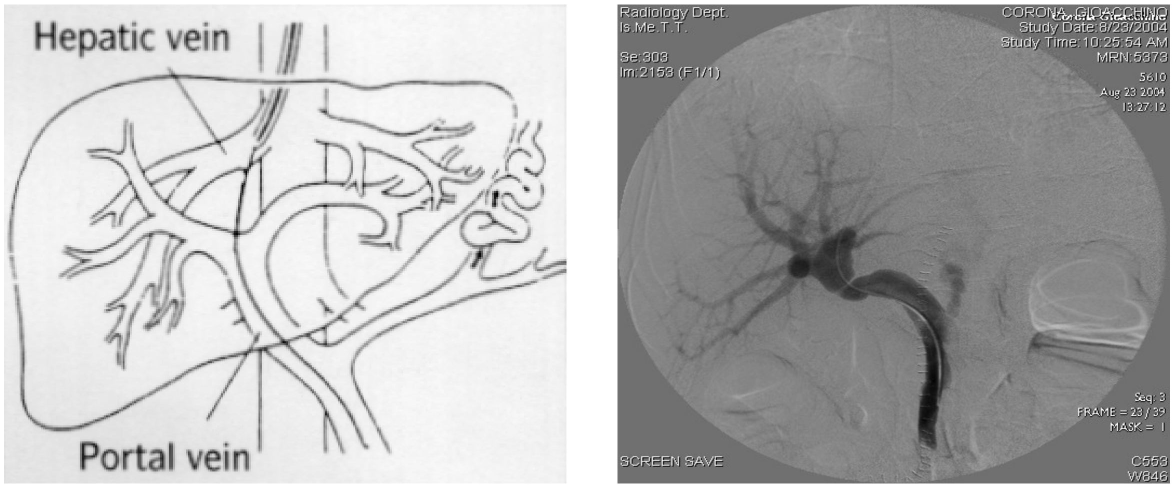
Hepatic vein
Portal vein
Radiology Dept.
Is.Me.T.T.
Se:303
Im:2153 (F1/1)
CORONA, GIOACCHINO
Study Date:8/23/2004
Study Time:10:25:54 AM
MRN:5373
5610
Aug 23 2004
13:27:12
Seq: 3
FRAME = 23 / 39
MASK = 1
SCREEN SAVE
C553
W846

Fig. 2. Din vena hepatică acul cateter este avansat transparenchimatos spre un ram corespondent al portei, accesul acestuia probându-se prin injectare de substanță de contrast (portografie). Odată identificat sistemul port, prin acul-cateter este plasat în portă un fir-ghid.

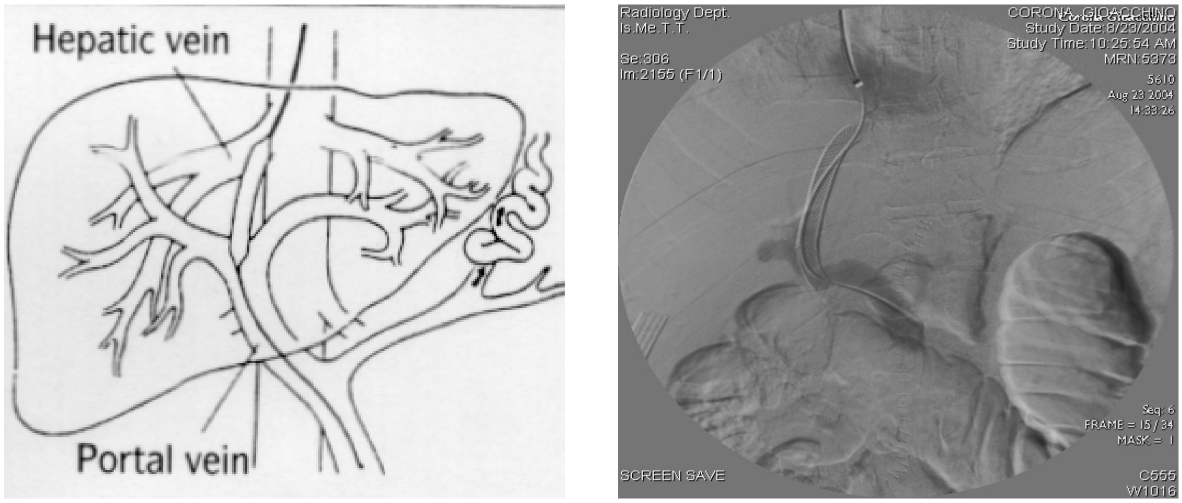
Hepatic vein
Portal vein
Radiology Dept.
Is.Me.T.T.
Se:306
Im:2155 (F1/1)
CORONA, GIOACCHINO
Study Date:8/23/2004
Study Time:10:25:54 AM
MRN:5373
5610
Aug 23 2004
14:33:26
Seq: 6
FRAME = 15 / 34
MASK = 1
SCREEN SAVE
C555
W1016

Fig. 3. Pe firul ghid este translatat un cateter-balon de angioplastie care se poziționează echilibrat (cu reperele la limita radioopace situate în vena hepatică, respectiv ramul port) în segmentul intraparenchimatos anterior tunelizat.

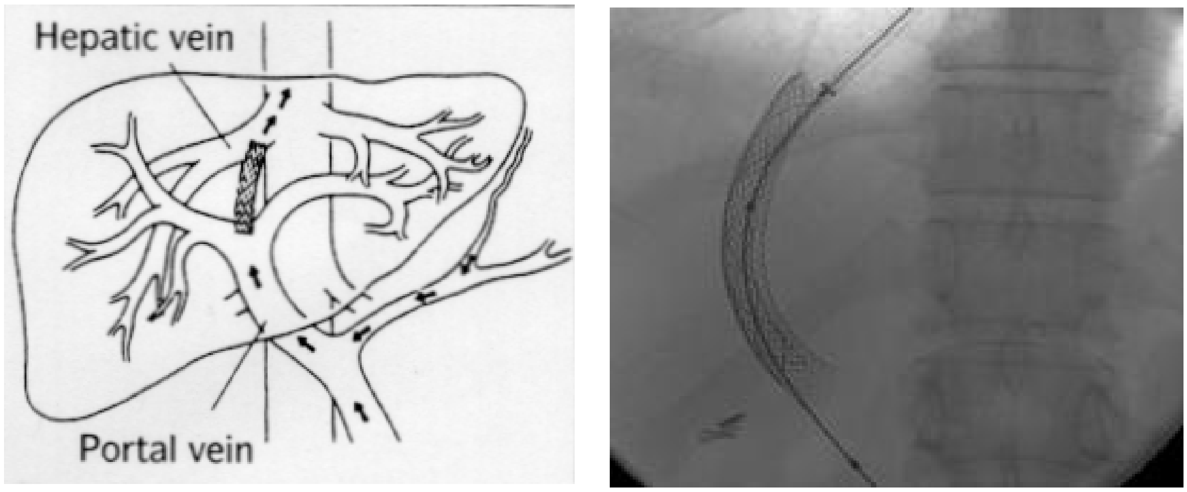
Hepatic vein
Portal vein

Fig. 4. Pe traiectul format se plasează un stent metalic expandabil, selectat dimensional corespunzător asigurării unui șunt permeabil, pentru a menține funcționalitatea șuntului.

În România nu există în prezent un centru de radiologie intervenţională în care să se efectueze de rutină o astfel de intervenţie, cu toate că morbiditatea şi mortalitatea prin ciroze hepatice virale şi toxic-etanolice este foarte mare, iar complicaţiile redutabile ale acestei boli (hemoragiile variceale, ascitele refractare şi sindromul hepato-renal) sunt responsabile de majoritatea deceselor prin această afecţiune gravă.

Pe de altă parte, activitatea de transplant hepatic, care s-a dezvoltat continuu în ultimul timp în România, prin proiectul care se derulează la Institutul de hepatologie şi transplant hepatic de la Spitalul Fundeni, reclamă administrarea cât mai eficientă a aspectelor legate de lista de aşteptare cu pacienţii eligibili pentru transplant, iar TIPS-ul permite, prin prelungirea supravieţuirii acestor pacienţi, candidaţi pentru intervenţie, accesul unui număr cât mai mare de bolnavi la transplantarea unui ficat nou, salvator. Iată de ce secţia de Boli interne-gastroenterologie din Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila" şi-a propus să demareze un proiect medical ambiţios care să asigure efectuarea de şunturi porto-sistemice intrahepatice transjugulare la pacienţi cirotici selectaţi, cu indicaţie medicală şi socială pentru această intervenţie, nu în ultimul rând a acelora care se află pe lista de aşteptare pentru un transplant hepatic.

Primul lot de 4 pacienţi a fost deja supus acestei intervenţii în cadrul laboratorului de endoscopie al secţiei noastre, iar trei dintre aceştia au primit un nou ficat, prezentând o evoluţie bună postoperatorie. Aceste prime 4 TIPS-uri au fost efectuate în colaborare cu Departamentul de Radiologie Intervenţionala al Spitalului Beilison din Petach Tikva, Israel. Un al cincilea pacient cu ciroza hepatică a fost supus de curând intervenţiei prin TIPS la spitalul Hradec Kralove din Praga, Cehia, de către prof. dr. Antonin Krajina, cu care am stabilit continuarea acestui proiect medical.

Profesorul Krajina este unul dintre cei mai apreciaţi experţi din Europa în acest domeniu şi urmează să vină în spitalul nostru începând din luna martie a anului acesta, pentru a efectua intervenţii TIPS la pacienţii selectaţi şi pregătiţi pentru protezare hepatică în cadrul unui protocol de investigaţii bine stabilit. Din echipa mixtă ce se va constitui vor face parte profesorul Krajina, un medic radiolog cooptat din Secţia de Radiologie a spitalului şi un medic endoscopist din secţia noastră. Dorinţa noastră este ca după un număr de TIPS-uri efectuate în comun să ajungem să facem această intervenţie pe cont propriu, astfel încât tot mai mulţi pacienţi din SUUMC să poată beneficia de această indicaţie.

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Identificarea prin analiză moleculară a virusului Encefalitei de Căpuşă

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Rezumat. Encefalita de căpuşă este o infecţie determinată de un arbovirus transmis prin muşcătura căpuşelor infectate sau prin consumul de lapte sau produse din lapte insuficient prelucrate termic de la animale aflate în fază viremică. Flavivirusurile care determină această infecţie sunt în general transmise de căpuşele *Ixodes ricinus*. Apariţia în România, în anul 1999, a unei epidemii determinate de acest virus demonstrează că poate crea serioase probleme de sănătate publică. Virusul encefalitei de căpuşă (TBEV) este implicat în dezvoltarea unor infecţii neurologice cu diferite grade de severitate, iar analiza de laborator este bazată pe detecţia anticorpilor specifici sau a acidului nucleic viral în probe de ser şi lichid cefalorahidian recoltate de la persoane infectate. Virusul TBE prezintă un genom ARN de polaritate pozitivă (infecţios) şi poate fi izolat de la om doar în condiţii speciale, din acestă cauză numărul de izolate virale umane fiind redus în toată lumea. De aceea, izolarea virusului TBE se poate realiza prin detecţia acestuia în vectorii de transmitere (căpuşele din genul *Ixodes*), care reprezintă rezervorul natural de virus. Prezenţa virusului TBE în căpuşele *Ixodes ricinus* colectate dintr-o zonă epidemică din Transilvania a fost demonstrată prin metode de biologie moleculară. Pentru a detecta ARN-ul viral în izolatele virale obţinute de la căpuşe, am folosit o metodă de revers transcriere PCR asociată cu nested PCR. Primerii utilizaţi au fost specifici pentru regiunea 5′ non-codantă (5′ NCR) a genomului viral, iar ampliconii obţinuţi au fost similari cu tulpina de referinţă TBE Hypr.

Cuvinte cheie: virusul encefalitei de căpuşă, flavivirus, RT-PCR, *Ixodes ricinus*

Abstract. Tick Borne Encephalitis (TBE) is a disease produced by an arbovirus transmitted by the bite of infected ticks or by the consumption of raw milk or dairy products from viremic animals. Flaviviruses that cause tick-borne encephalitis (TBE) are generally transmitted by the tick *Ixodes ricinus*. In Romania, an outbreak of TBE virus has been detected in 1999 in Sibiu country, proving that the virus can be a public health problem. TBE virus is involved in neurological infections, with different degrees of severity and actually laboratory analysis is based on detection of specific antibodies or virus/nucleic acid detection in patients' sera and cerebrospinal fluid. The TBE virus present ARN genome with positive polarity (infectious viruses) and can be isolated from the infected humans only in special situations. That is the reason why the number of strains isolated is reduced in the hall wade world. In this case the only way that can be done for the isolation of the TBE virus strains is the detections in terrestrial vectors (Ixodidae), because those have a very important part as natural reservoir. The presence of tick-borne encephalitis (TBE) virus in the *Ixodes ricinus* tick collected from epidemic area by Transylvania was set out using molecular methods. To detect viral RNA in the viral isolates obtained from tick, we are use an RT–PCR method associated with nested PCR. The primers used were specific for the 5′ non-coding region (5′ NCR) of the viral genome and the obtained amplicons was similar with the TBE Hypr strain.

Introducere

Virusul encefalitei de căpuşă este un arbovirus care aparţine subgrupului TBE (*tick-borne-encephalitis* – encefalita transmisă de căpuşă), complex antigenic distinct al familiei Flaviviridae. El este agentul etiologic al encefalitei transmise de căpuşă la om ca urmare a înţepăturii căpuşelor contaminate (vectori) sau prin consumul de lapte şi produse lactate insuficient prelucrate termic provenite de la animale domestice parazitate de artropodele vector infectate. A fost clasificat de

Center for Diseases Control (CDC) şi National Institute of Allergy and Infectious Diseases (NIAID) în Categoria C de agenţi biologici utilizabili în bioterorism, pe baza gradului de transmisibilitate la om şi animale, dozei infectante, impactului economic şi vaccinurile disponibile.

Este responsabil de apariţia unor infecţii neurologice (4) la om, mai mult sau mai puţin severe, în funcţie de subtipul antigenic implicat. Asistăm astăzi la creşterea incidenţei encefalitei de căpuşă în vestul şi centrul Europei, Scandinavia şi ţările Baltice (5), în strânsă corelaţie cu modificările arealului artropodelor vector (căpuşele din familia *Ixodidae*) şi schimbările climatice globale. Persoanele care trăiesc, muncesc sau călătoresc în zone endemice prezintă risc crescut de infecţie cu acest virus (3).

În România există condiţii geoclimatice şi ecologice care permit menţinerea şi circulaţia virusului TBE în focare naturale, fapt care a condus la apariţia unei epidemii în anul 1999 (9). De aceea trebuie să fim pregătiţi pentru supravegherea şi detecţia arbovirusurilor, implicit a virusului TBE, ca potenţiali agenţi biologici utilizaţi în bioterorism, situaţie în care profilul epidemiologic al izbucnirii este diferit de cel al epidemiilor spontane.

Sistemele vectoriale căpuşă-agent patogen sunt foarte sensibile la schimbările condiţiilor de mediu, incluzând factorii abiotici şi biotici, considerându-se că încălzirea globală a fost principalul element ce a declanşat schimbările epidemiologice înregistrate în ultimii ani (6). Ciclurile enzootice ale virusului encefalitei de căpuşă sunt extrem de fragile şi depind de particularităţile sezoniere ale dinamicii populaţiilor de ixodide, care la rândul ei este strict dirijată prin combinarea nivelurilor critice ale temperaturii şi umidităţii, niveluri ce sunt foarte uşor afectate de către schimbările climatice generale.

Distribuţia şi sezonalitatea infecţiilor transmise de vectori vor fi şi ele afectate de schimbările climatice. Creşterea temperaturilor în Europa ar putea permite stabilirea aici a unor specii tropicale sau semitropicale de vectori, dar şi apariţia infecţiilor transmise de aceştia. Schimbarea distribuţiei unor importante specii vectoriale ar putea fi considerată primul semn al efectului modificărilor climatice globale cu repercusiuni asupra sănătăţii populaţiei.

Metodele de investigare a virusului TBE includ determinarea prezenţei acestuia la artropodele vector (7, 8), prin realizarea unor teste specifice cu ajutorul gazdelor animale sensibile sau culturilor celulare (2). Caracterizarea unor izolate virale de la ixodide, prin metode moleculare (1), poate furniza informaţii utile cu privire la prezenţa acestui arbovirus în România.

Material şi metodă

Testările au fost realizate pe izolate virale de căpuşă din colecţia laboratorului, obţinute în urma epidemiei de meningoencefalită din anul 1999 (Brateiu, Sibiu) de către echipa de cercetare a colectivului de Virusologie, prin inocularea mojaratelor de artropode pe familii de şoareci sugari.

Începând cu anul 2006, am verificat viabilitatea acestora, în vederea stabilirii unei metode de investigare a prezenţei virusului TBE la artropodele vector, folosind metodele serologice. Pentru aceasta au fost selecţionate 6 loturi de izolate virale din colecţia laboratorului: 1/4; 1/3; 2/5; 2/4; 5/3; 5/5. Am efectuat suspensii virale în PBS cu albumină serică bovină 0,5% (suspensie stoc 1/1) pentru fiecare lot de studiu. Acestea au fost inoculate pe familii de şoareci sugari în diluţii de 1/10 şi 1/50.

Am selecţionat loturile 2/4, 2/5, 5/3, 5/5 deoarece acestea au produs îmbolnăvire şi mortalitate ridicate la animalele de experienţă şi am realizat 5 serii de pasaje care au avut ca scop obţinerea unei cantităţi crescute de particule virale. Din creierul acestora a fost extras antigen (posibil viral) cu ajutorul tehnicii de extracţie cu sucroză-acetonă (Clarke&Casals).

Pentru a putea avea un martor pozitiv, am realizat multiplicarea tulpinii Central Europene (TBE Hypr), obţinută de la Centrul de Referinţă din Bratislava, prin realizarea de inoculări intracerebrale cu diluţia 1/100 pe creier de şoareci sugari. În urma acestor inoculări, şoriceii care au prezentat fenomene caracteristice de boală sau au murit au fost recoltaţi şi menţinuţi la $-80^{o}C$ pentru conservare. Am obţinut antigen TBE pozitiv, prin intermediul aceleiaşi tehnici de extracţie, care a fost utilizat în reacţiile imunoenzimatice.

În urma testărilor antigenelor obținute din izolatele de căpuşă (loturile 5/3, 5/5) s-a constatat că acestea au avut un comportament asemănător cu antigenul extras din tulpina de referință TBE Hypr, spre deosebire de cel obținut din loturile 2/4, 2/5 care a prezentat reacție negativă, asemănătoare martorului negativ al reacției.

Deoarece sensibilitatea reacției ELISA este mai scăzută în comparație cu reacțiile de analiză moleculară, am continuat testarea în paralel a tulpinii de referință TBE Hypr şi a izolatelor de artropode vector.

Scopul acestui studiu a fost detecția prin tehnici de biologie moleculară a acestor izolate. Am utilizat metode de revers transcriere şi nested PCR. Testările inițiale au presupus generarea unui ADN complementar din ARN-ul genomic şi o reacție de amplificare cu ajutorul unor primeri specifici (Pp1: 5'- GCGTTTGCTTCGGACAGCATTAGC - 3, Pm1: 5'- GCGTCTTCGTTGCGGTCTCTTTCG - 3) regiunii 5'NC (5'noncodandă). Reacțiile s-au desfăşurat într-o singură etapă şi au fost urmate de un nested PCR (pentru creşterea specificității), în care au fost utilizați alți primeri (Pp2n: 5'- TCGGACAGCATTAGCAGCGGTTGG - 3, Pm2n: 5'- GTCTCTTTCGACACTCGTCG - 3), iar ampliconii obținuți au fost vizualizați prin electroforeză în gel de agaroză în sistem submers.

Reacția de reverstranscriere PCR a fost realizată cu ajutorul One Step RT-PCR kit (Qiagen) cu un program de amplificare dedicat, iar cea de nested PCR a presupus utilizarea Ampli Taq Gold kit (Applied Biosystems). Fragmentele ADN obținute au fost vizualizate pe un gel de agaroză în lumină ultravioletă şi supuse unor reacții de purificare în vederea eliminării contaminanților, dimerilor şi primerilor rămaşi neîncorporați în urma reacției de amplificare. Procedura de purificare s-a realizat cu ajutorul kitului Wizard PCR Preps DNA Purification System (Promega), în conformitate cu recomandările producătorului

Rezultate şi discuții

Regiunea țintă a fost reprezentată de zona 5' NCR (regiunea 5' noncodandă) a genomului viral.

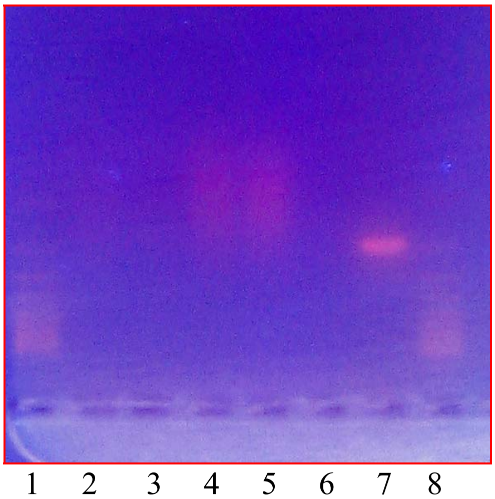

Fig. 1. One Step RT-PCR pentru izolatele virale C2/4, C2/ 5, C5/3, C5/5 şi Hypr TBE (original). Electroforeză în gel de agaroză LMP (low melting point) 2%.
(Linia 1-marker greutate 100pb; Linia 2,3 - C2/4, C2/ 5; Linia, 4, 5- Amplicon 220 pb C5/3, C5/5 – banda difuză; Linia 6-Martor negativ; Linia 7- Hypr TBE; Linia 8-marker greutate 100pb)

În urma reacțiilor PCR s-au obținut ampliconi de 220 perechi de baze pentru izolatele C5/3, C5/5 şi pentru tulpina de referință TBE Hypr, care a fost folosită ca martor pozitiv în reacție.

Pentru creşterea sensibilității reacției, fragmentele ADN obținute în urma realizării primei reacții PCR pentru izolatele C5/3, C5/5 şi pentru tulpina de referință au fost amplificate cu ajutorul altei perechi de primeri în vederea eliminării amplificărilor nespecifice. Prin nested PCR s-au evidențiat benzi ADN cu lungimea de aproximativ 178 pb care corespund regiunii țintă analizate şi care confirmă rezultatul pozitiv al acestei reacții. Reacția nested PCR şi-a atins ținta, deoarece la izolatele C5/3, C5/5 obținute în urma primului PCR, benzile ADN vizualizate au fost difuze, iar prin reamplificarea fragmentelor am obținut benzi ADN clare. Comparativ cu tulpina de referință sunt mult mai slabe, dar acest lucru se poate explica prin cantitatea de încărcătură virală, care la izolatele de căpuşă este mult mai mică.

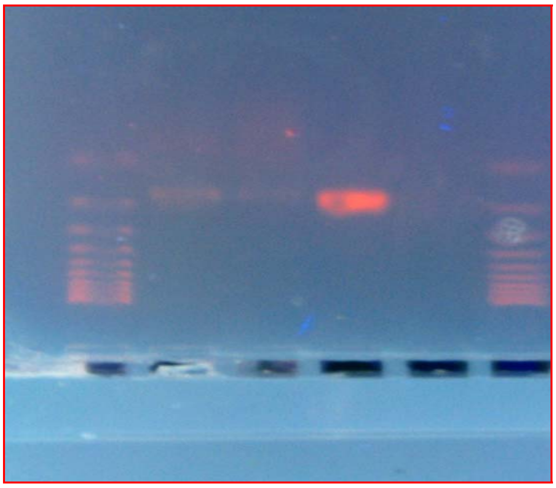

Fig. 2. Nested RT-PCR pentru izolate virale C5/3, C5/5 şi Hypr TBE (original). Electroforeză în gel de agaroză LMP (low melting point) 2%. (Linia 1-marker greutate 100pb; Linia 2, 3 - Amplicon 178 pb C5/3, C5/5; Linia 4-Amplicon 178 pb Hypr TBE; Linia 5 - Martor negativ; Linia 6 - marker greutate 100pb)

Purificarea ampliconilor direct din produsul PCR a condus la obținerea unei benzi ADN clare doar pentru tulpina de referință. Pierderea ADN-ului extras din izolatele virale C5/3, C5/5 se datorează cantității scăzute de material genetic obținut ca urmare a multiplicării pe şoarecii sugari.

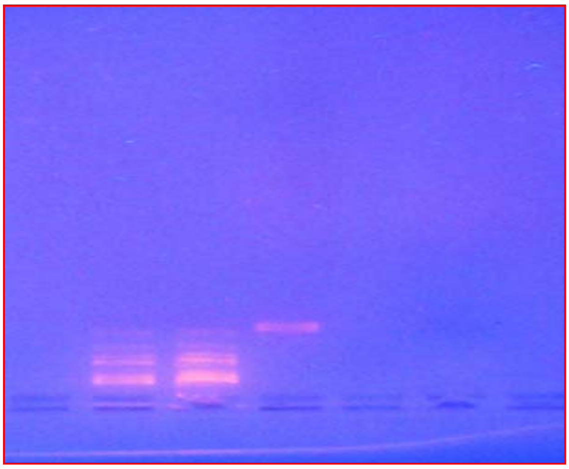

Fig. 3. Purificare ADN izolate virale C5/3, C5/5 şi Hypr TBE (original). Electroforeză în gel de agaroză LMP (low melting point) 2%. (Linia 1-marker greutate 100 pb; Linia 2, Amplicon purificat 178 pb Hypr TBE)

Diagnosticul de laborator al etiologiei encefalitei de căpuşă are şi importanță medico-militară, având în vedere faptul că acest virus este inclus pe lista de agenți biologici (armă biologică virală incapacitantă), iar datorită endemicității în centrul şi estul Europei, ar putea să apară şi atacuri bioteroriste mascate. Specificitatea înaltă a acestui test de diagnostic viral constituie în acelaşi timp şi analiza de confirmare a diagnosticului microbiologic, cerință a regulilor de bună practică de laborator şi a instrucțiunilor NATO pentru identificarea agenților biologici.

Concluzii

Testarea izolatelor de artropode vector prin **analize serologice a demonstrat prezența anticorpilor neutralizanți pentru virusul TBE** în serurile pozitive, atât pe **antigenul pozitiv** (extras din tulpina de referință), cât şi pe **antigenul obținut din izolatul viral C5.**

- **Izolatele virale**, obținute de la căpuşele recoltate de pe capre din gospodăriile bolnavilor cu encefalită de căpuşă la epidemia din 1999 din Brateiu/Sibiu şi **tulpina de referință TBE Hypr**, au fost testate prin metode de biologie moleculară şi **s-a demonstrat prezența virusului TBE**, prin obținerea unui fragment ADN de aproximativ 178 pb care corespunde regiunii 5' noncodantă a genomului virusului encefalitei de căpuşă.
- Prezența virusului TBE în izolatele virale de căpuşă confirmă diagnosticul infecțiilor umane în epidemia din 1999.
- **Concordanța între testările serologice şi moleculare** demonstrează corectitudinea rezultatelor cercetărilor inițiale.
- Evidențierea prezenței virusului TBE la căpuşele vector şi semnalarea cazurilor de infecție acută la om confirmă faptul că **în România există condiții favorizante circulației virusului encefalitei de căpuşă.**

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Abordări terapeutice în dependența de substanțe
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Factori de vulnerabilitate în toxicomanii
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Cele două lucrări, semnate de col.conf.dr. Daniel Vasile şi mr.dr. Octavian Vasiliu, abordează o tematică de mare interes pentru psihiatria actuală, în contextul propagării alarmante a fenomenului toxicomaniei, fenomen cu implicații profunde sociale, psihologice şi medicale. Factorii de vulnerabilitate în toxicomanii sunt evaluați din perspectivă clinică, fiind abordate conexiunile personologice, neurobiologice, genetice şi social-interacționale ale acestora.

Autorii prezintă studii de caz şi propun un model structurat de evaluare a pacientului diagnosticat cu dependență de substanță. De asemenea, sunt prezentate sugestii de abordare terapeutică şi implicațiile dublului diagnostic toxicomanie-tulburare de personalitate pentru evoluția şi prognosticul terapiei.

Lt.col. dr. Bogdan VASILE
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Abordarea neuropatiilor optice

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Rezumat. Neuropatiile optice sunt provocate de afectarea nervului optic din cauze diferite. Fibrele nervului optic părăsesc retina la nivelul discului optic, mergând la nivelul cortexului vizual, unde se formează imaginea. Principalul simptom este scăderea/pierderea vederii la ochiul afectat. Cauzele neuropatiilor optice pot fi: ischemice, demielinizante, compresive, infiltrative, traumatice, toxice, nutriționale, mitocondriale şi ereditare. Diagnosticul impune examinarea discului optic prin oftalmoscopie, teste sanguine, rezonanță magnetică nucleară şi tomografie computerizată cerebrală, tomografia în coerență optică, potențiale evocate vizuale, fluoroangiografie etc. Investigațiile clinico-paraclinice adecvate permit un tratament prompt şi măsuri preventive atunci când acestea se impun.

Cuvinte cheie: neuropatie optică etiologie diagnostic diferențial

Abstract. Optic neuropathy refers to damage to the optic nerve due to any cause. The optic nerve contains axons of nerve cells that emerge from the retina, leave the eye at the optic disc, and go to the visual cortex where input from the eye is processed into vision. The main symptom is loss of vision, in the affected eye. The optic neuropathy may be caused by ischemic, demielinating, compressive, infiltrative, traumatic, nutritional, toxic, mitochondrial and hereditary conditions. Diagnosis involves examination of the back of the eyes by ophthalmoscope, blood tests, magnetic resonance imaging (MRI) or computed tomography (CT) of the brain, optical coherence tomography (OCT), *v*isual evoked potentials, fluoroangiography, etc. Appropriate clinical and paraclinical investigation, lead to prompt treatment and preventive measures.

Abordarea neuropatiilor optice

Etiologia neuropatiilor optice (NO) se poate stabili pe baza anamnezei, a examenului clinic, imagistic, electrofiziologic, serologic şi genetic. Scăderea vederii poate fi cauzată de afecțiuni ale nervului optic, retinei sau globului ocular. În general, în neuropatia optică întâlnim scăderea acuității vizuale, afectarea vederii pentru culori, defect pupilar aferent relativ (DPAR, pupila Marcus-Gunn), afectarea câmpului vizual, cu sau fără modificarea discului optic.

Anamneza

Pacientul recunoaşte rapid scăderea vederii când este afectată vederea centrală. În cazul afectării vederii periferice deficitul este mai greu perceptibil de către pacient, ducând la prezentarea tardivă la medic. La fel şi scăderea vederii la un singur ochi este recunoscută mai greu, deoarece este compensată de vederea binoculară. Scăderea vederii poate avea o progresie lentă, ca în cazul leziunilor compresive, sau rapidă, cum este cazul în nevrita optică sau în neuropatia optică ischemică, cu deosebirea că în primul caz ameliorarea se produce spectaculos de cele mai multe ori.

Examinarea clinică şi paraclinică

Pacientul cu NO prezintă modificări ale vederii colorate, ale fundului de ochi şi defect pupilar aferent relativ (*relative afferent pupillary defect*-RAPD, *Marcus-Gunn pupil*). În cazul în care aceşti parametri sunt conservați, diagnosticul de NO este pus sub semnul întrebării. Trebuie excluse viciile de refracție.

Vederea colorată necesită o transmisie foarte fidelă, cu un raport de 1:1 între celulele cu con (responsabile de vederea colorată) şi cele ganglionare. Axonii celulelor ganglionare intră în componenţa NO într-un procent semnificativ şi de aceea perturbarea vederii colorate este un indicator fidel al afectării nervului optic. Pacientul acuză deficit pentru vederea colorată la ochiul afectat, mai ales pentru albastru, verde, violet. Se folosesc testele necesare pentru diagnosticarea discromatopsiei: Hardy-Rand-Rittler şi Ishihara. Deficitele campimetrice pot sugera diferite etiologii ale neuropatiei optice. Nevrita optică şi leziunile compresive determină un deficit campimetric central, în timp ce NO ischemică produce un deficit segmentar ce cuprinde pata oarbă cu respectare meridianului orizontal, în acord cu dispunerea fibrelor retiniene ce merg spre discul optic.

Defectului pupilar aferent relativ (DPAR) ajută la diagnosticarea NO asimetrice : deoarece un nerv optic afectat contribuie mai puţin la reflexul consensual pupilar, o dilataţie paradoxală a ambelor pupile apare când se stimulează de acea parte. Detaliem acest test, având în vedere importanţa lui practică în neurooftalmologie.Un stimul luminos aplicat unui ochi determină o constricţie pupilară promptă la ambii ochi (reflex direct). Dacă stimulul luminos se mută rapid la celălalt ochi, ambele pupile trebuie să menţină constricţia (răspuns pupilar normal). În cazul afectării unui nerv optic, balansul luminii la ochiul afectat determină dilatarea pupilei datorită transmiterii deficitare a luminii prin nervul compromis. Revenirea stimulului la ochiul normal duce la contracţia pupilei prin creşterea transmisiei luminii. Testul este sensibil şi poate detecta disfuncţii minore ale NO unilateral. DPAR se obţine la ochiul cu acuitate compromisă, dar nu şi în cazul afectării periferice severe a câmpului vizual. Prezenţa DPAR la ochiul care are un defect campimetric sugerează mai degrabă o leziune a tractului optic decât a nervului optic.

Aspectul discului optic

Poate fi edemaţiat, palid sau normal. Edemul papilar trebuie diferenţiat de pseudoedemul papilar ce apare în anomalii drusen. Aici vasele se disting bine printre fibrele nervoase, au pulsaţii vizibile, deşi acestea pot lipsi în 10% din cazuri. Uneori, pseudoedemul papilar poate fi însoţit de hemoragii subretiniene.

1. Nevrita optică (papilita, nevrita retrobulbară): se caracterizează prin scăderea bruscă a vederii la unul sau la ambii ochi (10% din cazuri), simultan sau succesiv, cu evoluţie, în ore sau zile, spre amauroză. Apare de regulă la adulţii tineri. Deficitul vizual se însoţeşte de dureri la mobilizarea globilor oculari, sensibilitate la palparea lor şi perceperea diferită a luminii. Se accentuează la efort sau după o baie caldă (fenomenul Uthoff). Este afectată şi vederea colorată. Discul optic şi retina sunt de regulă normale. Numai dacă este afectat capul nervului optic poate apărea un grad de edem papilar (1/3 din cazuri). Cu timpul însă, discul optic capătă o paloare mai ales temporală, cu extensie ulterioară peripapilar, sugerând că demielinizarea a fost acompaniată şi de pierdere axonală. Examinarea prin RMN confirmă edemaţierea nervului optic şi captarea de godolinium, în secvenţele T1 demonstrând inflamaţia retrobulbară. Tomografia în coerenţă optică (OCT) poate cuantifica gradul atrofiei nervului optic. Este un marker structural obţinut printr-o metodă noninvazivă. Examenul lichidului cefalorahidian poate decela limfocitoza (10-100), proteinorahie, gamaglobuline şi benzi oligoclonale.

În majoritatea cazurilor nu se evidenţiază nicio cauză de neuropatie optică. În 2/3 din cazuri, pacientul îşi revine spontan sau, după administrarea i.v. a unor doze mari de corticosteroizi, în câteva săptămâni. Această terapie determină revenirea mai rapidă a vederii, corectarea câmpului vizual şi a sensibilităţii la contrast, fără a influenţa prognosticul vizual pe termen lung. S-a demonstrat că administrarea orală a corticoizilor, practică încă des întâlnită, favorizeaza recăderile. Pot rămâne sechele de genul discromatopsiilor, scotoamelor, diminuarea luminozităţii sau, rar, amauroză definitivă. Nevrita optică este asociată cel mai frecvent cu scleroza multiplă (SM), boală demielinizantă ce apare între 20 şi 50 de ani, predominant la femei. De aceea, examinarea RMN trebuie să exploreze şi creierul, pentru a evidenţia lezunile demielinizante centrale, în cazul când acestea există, deoarece riscul dezvoltării SM după 15 ani de la un episod de nevrită optică este de 72% la cei cu una sau mai multe leziuni demielinizante centrale şi doar de 25% la cei cu RMN normal. La 15% dintre pacienţii cu SM, debutul a constat într-un episod de nevrită optică retrobulbară.

Un caz particular îl reprezintă neuromielita optică (NMO) sau Boala Devic, caracterizată prin leziuni demielinizante necrozante ale nervului optic şi măduvei spinării. Un marker al NMO, a cărui specificitate rămâne a fi demonstrată, este autoantigenul aquaporina 4.

Deci cea mai frecventă cauză de nevrită optică este scleroza multiplă, alte cauze mai rare fiind demielinizările postinfecţioase sau cele idiopatice. Anumite caracteristici atipice fac diagnosticul de nevrită optică improbabil. Profilul temporal al pierderii AV (progresie mai mare de 2 săptămâni sau absenţa remisiunii în decurs de 1 lună), absenţa durerii, un scotom atipic (defect altitudinal), un fund de ochi nespecific (edem marcat, atrofia nervului optic, hemoragii, inflamaţie sau exudat retinian). Toate acestea necesită continuarea investigaţiilor pentru stabilirea diagnosticului.

Diagnosticul diferenţial al NO unilaterale se face cu :

- afecţiuni ale retinei în care pacienţii acuză metamorfopsii (distorsiuni) sau fosfene;
- sindromul idiopatic acut de mărire al petei oarbe în care apar modificări peripapilare pigmentare;
- retinopatia seroasă centrală se prezintă cu pierderea bruscă a vederii, în absenţa durerii prin dezlipirea retinei;
- ocluzia de arteră centrală retiniană se caracterizează prin albirea retinei cu un spot roşu macular determinat de conservarea circulaţiei coroidale;
- ocluzia venei centrale a retinei se caracterizează prin hemoragii retiniene şi turgescenţa venelor retinei.

Diagnosticul diferenţial al NO *bilaterale* se face cu afecţiunile caracterizate de *edemul papilar* bilateral, ca urmare a *creşterii presiunii intracraniene*. Se produce blocarea fluxului axoplasmic în fibrele nervoase cu creşterea volumului axoplasmei în discul optic. Deşi edemul papilar e bilateral de obicei, uneori poate fi asimetric prin diferenţele anatomice din meningele ce acoperă NO intracranian. În faza acută există o discordanţă între edemul papilar marcat şi conservarea vederii, mai ales cea centrală. Când acuitatea vizuală scade, înseamnă că lichidul s-a extins între fibrele NO, ajungând la maculă. În timp, axonii nervului optic sunt tot mai afectaţi şi vederea se compromite. Edemul se estompează şi se instalează paloarea discului, rezultând edem papilar atrofic. Apar modificări campimetrice de tip constrictiv şi modificarea discului optic.

Creşterea presiunii intracraniene se poate produce în:

- tumori cerebrale;
- accidente vasculare cerebrale, mai ales la tineri fără atrofie cerebrală;
- compresiunea sistemului ventricular în fosa posterioară;
- tromboza de sinus venos;
- meningita criptococică este asociată şi cu edem papilar;
- pseudotumori cerebri sau hipertensiunea intracraniană idiopatică pot duce la edem papilar şi la pierderea lentă a vederii (tabelul nr. 1).

*Tabel nr. 1.*Cauzele neuropatiei optice (după Adams R.D., Victor M.)

1. **Demielinizantă**
    - Scleroza multiplă
    - Neuroretinită postvirală sau infecţioasă
2. **Ischemic**
    - aterosclerotic
    - boala Horton

  - arterita sifilitică
3. **Parainfecțioasă**
   - tromboză de sinus cavernos
   - sinuzită
4. **Droguri + toxine**
   - Methanol
   - Ethambutol
   - Clorochina
   - Streptomicină
   - Clorpropamid
   - Cloramfenicol
   - Derivați Ergot
5. **Carențe**
   - Vitamina B_{12} (ciancobalamina)
   - Vitamina B1 (tiamină)- ambliopia tabaco-alcoolică
6. **Ereditare şi de dezvoltare:**
   - Atrofia optică juvenilă dominantă
   - Atrofia optică Leber
7. **Compresive şi infiltrative:**
   - Meningiom de aripă de sfenoid
   - Metastaze de nerv optic ± chiasmă
   - Gliom de nerv optic
   - Atrofie optică după edem papilar îndelungat
   - Oftalmopatie tiroidiană
   - Sarcoidoză
   - Granulomatoză Wegener
   - Limfoame, leucemii

2. Neuropatia optică ischemică (NOI) este cauzată de întreruperea vascularizației în vasa-nervorum, care irigă nervul optic, secundar unei boli inflamatorii sau aterosclerozei arterei oftalmice sau a rr sale ciliare posterioare. Clinic apare scăderea brutală a acuității vizuale şi un deficit de câmp vizual, de obicei altitudinal.

Neuropatia Optică Ischemică Anterioară (NOIA) nonarteritică este cauza cea mai frecventă a NO unilaterale la adulții peste 45 de ani. Poate fi prima manifestare a ocluziei ACI. În astfel de împrejurări pot apărea simultan NOI ipsilateral şi infarct cerebral. Sunt situații când infarctul nervului optic rezultă din reducerea fluxului sanguin datorat ocluziei carotidiene, anastomozelor ineficiente şi modificărilor locale la nivelul circulației piale a nervului optic. Alteori cauza este embolismul în aa. ciliare posterioare, vasele piale sau ambelor. Totuşi, în majoritatea cazurilor NOI non-arteritică este o boală a vaselor mici legată de factori de risc precum HTA, DZ, fumat şi mai puțin de patologia carotidiană. Zona afectată este porțiunea prelaminară, laminară şi imediat retrolaminară a nervului optic care primeşte sânge din rr. terminale ale AO, aa. ciliare posterioare şi din circulația pială. Discul optic poate fi palid sau hiperemic, edemațiat, cu hemoragii peripapilare şi exudate. Tardiv, edemul e înlocuit cu atrofia capului nervului optic, papila devenind alb sidefie. Unele cazuri de NOIA pot fi asociate cu alte semne de ischemie oculară: infarct coroidal, emboli retinieni sau neovascularizația camerei anterioare sau a irisului. Aceste leziuni găsite în absența unei retinopatii diabetice sau a arteritei granulomatoase sugerează ocluzia carotidiană ipsilaterală. NOIA nonarteritică este posibil să apară după chirurgia oculară, creşterea presiunii intraoculare putând compromite perfuzia capului nervului optic. Poate apărea şi după pierderi masive de sânge sau alte cauze de ischemie şi hipotensiune. Debutul este brusc şi fără durere, uneori însă scăderea vederii poate progresa peste o lună. Ameliorări spontane sunt posibile în primele 6 luni. Unele studii relevă efectul benefic al corticoterapiei. Riscul de apariție la celălalt ochi este de 30-40 %. Uneori un edem papilar poate fi semnul premonitor pentru instalarea deficitului vizual. Recurenţa la acelaşi ochi este rară, posibil prin faptul că atrofia optică ce urmează evenimetului iniţial decomprimă nervul. Nu se corelează cu risc de stroke, având mecanisme patofiziologice diferite (tabelul nr. 2).

Neuropatia Optică Ischemică Anterioară (NOIA) arteritică se asociază cel mai frecvent cu arterita cu celule gigante (arterita temporală sau boala Horton), ce apare mai frecvent la femei. Arterita temporală afectează arterele mari şi medii care au lamina elastică ce reprezintă sediul iniţial al inflamaţiei. Trebuie suspectată la persoanele cu vârste de ≥ 60 ani care prezintă semne de arterită temporală (dureri temporomandibulare, claudicaţia mandibulei, cefalee, anorexie, dureri articulare, scădere în greutate, V.S.H. crescut, polimialgie reumatică, depresie, afectare mnezică). Uneori semnele arteritei cu celule gigante pot fi absente, pierderea acută a vederii fiind unicul semn de boală. Se asociază aproape întotdeauna cu edem papilar. Coexistenţa ischemiei retiniene cu spoturi albicioase ce seamănă cu lâna sau bumbacul şi edemul papilar sunt patognomonice. Ischemia este cauzată de ocluzia inflamatorie a aa. ciliare scurte posterioare, ce hrănesc porţiunea retrolaminară şi laminară a discului optic. Pierderea vederii cortizonică e de obicei unilaterală dar poate fi şi bilaterală simultană, sau apare la celălalt ochi după zile, săptămâni sau luni, mai ales în absenţa corticoterapiei. Creşterea VSH-ului, prezenţa proteinei C reactive şi prezenţa celulelor gigante şi a inflamaţiei cu ocazia biopsiei de arteră temporală stabilesc diagnosticul. Este una din puţinele boli pentru care putem iniţia terapia în cazul suspiciunii diagnosticului, salvând vederea pacientului. Odată vederea afectată, recuperarea ei este incertă, în ciuda tratamentului.

Tabel nr.2. Neuropatii optice

	Nevrita optică	**Neuropatie optică ischemică anterioară nonarteritică (NOIA)**	**Compresiune de nerv optic sau chiasma optică**
Debut	Acut, aproximativ 1 zi	Hiperacut, aproximativ 1 oră	Cronic
Grupul de vârstă	<45 ani	>45 ani	Fară diferenţe legate de vârstă
Durere	La mişcarea globilor oculari	Preponderent difuză	De obicei cefalee
Aspectul discului optic la debut	Normal sau edem papilar	Intotdeauna edem papilar (exceptie: NOIP*)	Normal, edem papilar sau atrofie
Aspectul discului optic în evoluţie	Normal sau atrofie	Atrofie sectorială sau globală	Normal sau atrofie, rareori cu persistenţa edemului
Câmp vizual	Defecte ale nervului optic, Scotom central, Îngustare generalizată	Defecte ale fibrelor arcuate, De obicei amputare inferioară altitudinala	Defecte ale nervului optic, Scotom central, Îngustare generalizata
Simptome generale	Deficite neurologice variabile	Boli vasculare ischemice	Posibil endocrinopatii, procese expansive
RMN	Demielinizare	Nimic deosebit	Evidenţiază formaţiuni înlocuitoare de spaţiu
Tratament	Corticoterapie in doze mari sau fara tratament	Reducerea factorilor de risc	Decompresie chirurgicală, radioterapie sau fara intervenţie
Prognostic, evoluţie naturală	Recuperare importantă	Fără modificări sau recuperare paţtiala	În cazul tumorilor benigne recuperare posibilă

Neuoropatia Optică Ischemică Posterioară (NOIP) este mult mai rară şi în acest caz discul optic este normal, fiind afectată porţiunea retroorbitară a nervului optic. După 4-6 săptămâni se poate instala atrofia NO cu paloarea discului. Poate fi rezultatul unor pierderi sanguine severe, anemiei, hipervâscozității sanguine, procedurilor chirurgicale prelungite (chirurgia spinală). Se prezintă cu scotom central, prin întreruperea vascularizației nervului optic posterior (prin plexurile piale circumferențiale), producând ischemie în centrul nervului, unde își au traiectul fibrele maculare. Poate apărea după procedeele chirurgicale, ca rezultat al creşterii presiunii venoase orbitare cauzate de edemul orbitar şi de hipotensiunea arterială simultană.

3. Alte condiții

- Bolile inflamatorii: neurosarcoidoză, lupus eritematos sistemic, Sindromul Sjogren pot cauza NO subacută.
- Boli infecțioase: *Bartonella henselae, Treponema pallidum, Borrelia Burgdorferi*.
- Alte infecții: HIV, toxoplasmoză, Citomegalovirus, Criptococcus, sinuzita sau mucocel.
- Alte cauze de NO: compresiuni intraorbitare sau extraorbitare (meningiom de bază de craniu, adenom pituitar, craniofaringiom), anevrisme, boala Graves, limfoame, leucemii, meningite carcinomatoase, metastaze ale NO (cancerul mamar şi pulmonar).
- Radiațiile pot cauza NO mai ales tardiv, la 6-24 luni după iradiere cu 50 Gy. Progresia deficitului este de săptămâni sau luni iar remisiunile spotane sunt rare. Corticosteroizii pot reduce edemul NO.
- Neuropatia optică paraneoplazică trebuie suspectată la pacienții cunoscuți cu neoplazie. În acest caz se însoțeşte de alte disfuncții neurologice.
- Neuropatia ereditară Leber este rară, cu debut subacut în a 2-a şi a 3-a decadă. Este cauzată de mutația ADN-ului mitocondrial. Este cu transmitere maternă şi penetrare incompletă intrafamilială. Bărbații sunt afectați în 80-90% din cazuri, nefiind o explicație pentru aceasta. 55% din cazuri debutează cu scăderea vederii bilateral, în celelalte cazuri celălalt ochi va fi afectat în aproximativ 9 luni. Este un pseudoedem papilar şi fluoroangiografia confirmă absența circulației capilare. De obicei, deficitul vizual persistă, remisiuni spontane fiind mai rare şi depinzând de tipul mutației (T 14484C). Nu există tratament eficient, dar trebuie îndepărtat rolul potențial toxic al alcoolului şi al fumatului.
- NO cu transmitere dominantă se prezintă în copilărie cu scăderea acuității vizuale insidios, asimetric. Boala se datorează mutației genei OPA1 cu 90 variante descoperite până acum.
- Altă NO ereditară este sindromul Wolfram (diabet insipid, diabet juvenil, atrofie optică, surditate, boli mitocondriale, precum MELAS, MERRF).
- NO glaucomatoasă apare în cazul creşterii presiunii intraoculare. Glaucomul cu unghi închis se poate prezenta cu pierdere acută a vederii însoțită de dureri, mimând nevrita optică. Aici însă durerea este foarte intensă, însoțită de ochi roşu, pupilă nereactivă. Glaucomul cu tensiune oculară normală este dificil de recunoscut. Apar defecte campimetrice de tip constrictiv şi modificarea discului optic.
- NO posttraumatică cu avulsia sau secțiunea nervului este o altă cauză. ½ dintre pacienți se pot ameliora spontan. Corticoterapia în primele 8 ore poate fi benefică. Examenul FO relevă hemoragii extensive intraoculare. În contrast, NO traumatică posterioară indirectă se prezintă cu pierderea vederii, dar cu fundul de ochi normal. Poate rezulta prin forțe de forfecare şi edem al NO în canalul optic.
- Alte cauze: saltul hipertensiv, diabetul zaharat tip I, toxic nutriționale (scotom central)-ambliopie alcoolo-tabagică (NO nutrițională sau ambliopie carențială), deficit de vitamina B12, substanțe toxice: etambutol, metanol, cloramfenicol, isoniazida, streptomicină, clorpropamidă, deviați ergotamină.

În concluzie neuropatiile optice trebuie atent investigate. În funcție de orientarea diagnostică pe care o avem, putem solicita următoarele teste: MRI cerebral + orbite cu contrast, AC antiquaporin - la cei cu NO recurentă bilaterală, tomografia în coerență optică (OCT), electroretinogramă, angiografie cu fluorescență, potențiale evocate vizuale, teste serologice pentru sifilis, boala Lyme, toxoplasmoză, HIV, herpes, anticorpi antinucleari, AC Sjogren, puncție lombară, teste genetice pentru NO ereditare.

În final propunem un algoritm de diagnostic adaptat după Andrew G. Lee şi Paul W. Brazis.

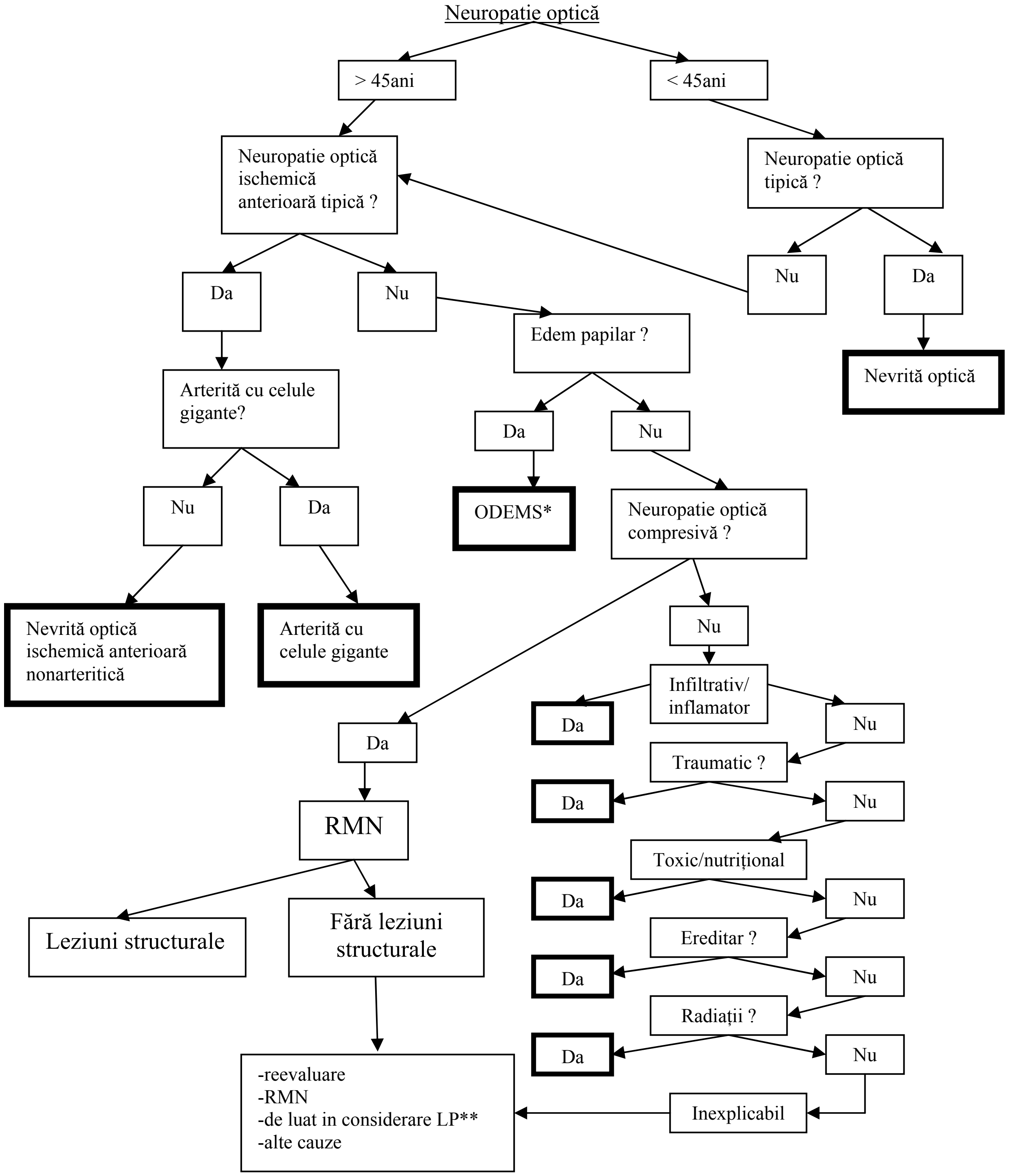
Neuropatie optică
> 45ani
< 45ani
Neuropatie optică ischemică anterioară tipică ?
Neuropatie optică tipică ?
Da
Nu
Nu
Da
Edem papilar ?
Nevrită optică
Arterită cu celule gigante?
Da
Nu
Nu
Da
ODEMS*
Neuropatie optică compresivă ?
Nevrită optică ischemică anterioară nonarteritică
Arterită cu celule gigante
Nu
Infiltrativ/ inflamator
Da
Nu
Da
Traumatic ?
Da
Nu
RMN
Toxic/nutrițional
Da
Nu
Leziuni structurale
Fără leziuni structurale
Ereditar ?
Da
Nu
Radiații ?
Da
Nu
-reevaluare
-RMN
-de luat in considerare LP**
-alte cauze
Inexplicabil

** Edem papilar recurent cu stea maculară (Recurrent optic disc edema with a macular star)*
*** Puncție lombară*
Schema nr.1 : Algoritmul de diagnostic al neuropatiei optice, adaptat dupa Andrew G.Lee şi Paul W. Brazis

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O temă actuală - hepatita cronică cu virus C

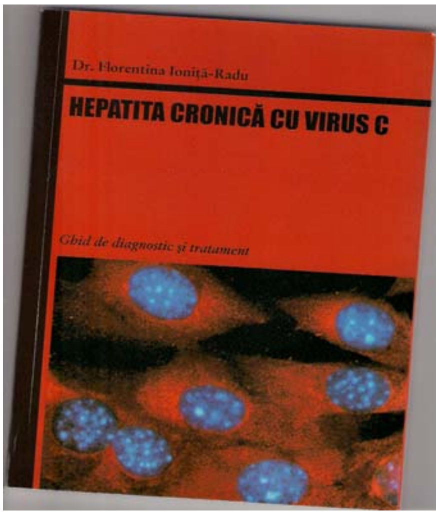

Dr. Florentina Ioniță-Radu

HEPATITA CRONICĂ CU VIRUS C

Ghid de diagnostic şi tratament

Abordarea unui astfel de subiect nu putea fi asumată decât de un specialist în domeniu, având în vedere importanța acestei teme care preocupă în mod constant lumea medicală, din pricini şi pe căi diferite, încă din deceniul al optulea al secolului 20. Colonelul dr. Florentina Ioniță-Radu, autoarea lucrării, dedică această carte „uneia dintre cele mai importante probleme de sănătate publică, cu implicații personale, sociale şi economice”, încercând în fapt o incursiune în spațiul destul de vast al cunoştiințelor circumstanțiate până în prezent hepatitei C, pentru a pune la dispoziția cititorilor un material cât se poate de sintetic legat de diagnosticul, managementul şi tratamentul uneia dintre cele mai frecvente infecții prezente în rândul populației, 170 000 000 persoane la nivel global (22 de milioane în Europa), şi cu o extindere extrem de rapidă - 3-4 milioane de persoane nou infectate pe an.

În contextul menționat, lucrarea *Hepatita cronică cu virus C* este o carte cu incontestabile merite dacă ținem seama de metoda riguroasă pe care o foloseşte autoarea în organizarea materialului, de modul rațional în care dezvoltă ideile, de spiritul didactic în care îşi desfăşoară demonstrația, de noutatea informației şi de caracterul pregnant practic al concluziilor sale. Şefă de secție în SUUMC „Carol Davila”, medic primar boli interne, medic primar gastroenterolog, cu competențe în nefrologie şi endoscopie digestivă, membră a mai multor societăți medicale - Societatea Română de Gastroenterologie şi Hepatologie, Societatea Română de Endoscopie Digestivă, Asociația Română pentru studiul ficatului, Societatea Română de Nefrologie, membră fondatoare a Fundației Române a Rinichiului, membră fondatoare a Fundației Române de Endoscopie Digestivă, preşedintă a Fundației Române de endoscopie Digestivă, membră a Uniunii Medicale Balcanice, membră a Asociației Europene pentru studiul ficatului, membră a Societății Europene de Oncologie Digestivă – col. dr. Florentina Ioniță-Radu abordează subiectul după ce consultă o bogată şi recentă bibliografie, pe care însă o filtrează prin propria-i experiență. Pe parcursul întregii lucrări, autoarea procedează cu onestitate profesională, înlăturând digresiunile şi concentrându-se cu exactitate ştiințifică asupra temei propuse, plecând de la modalitățile de diagnostic, urmărind manifestările extrahepatice, formele particulare de boală la pacienții imunocopromişi, acordând o atenție deosebită tratamentului afecțiunii, efectelor sale adverse , terapiei formelor speciale de boală şi a nonrespoderilor.

Deşi într-un scurt preambul autoarea este tentată să nu acorde prea multe şanse abordării subiectului propus într-un moment în care informațiile legate de evoluția hepatitei cronice cu virus C şi mai ales despre tratamentul antiviral apar în cascadă, când de la zi la zi se ivesc schimbări în modul de tratament, când rolul factorilor genetici se dovedeşte tot mai important, faptul că diagnosticul, tratamentul bolii şi al complicațiilor sunt împărțite între mai multe categorii de medici (gastroenterologi, infecționişti, internişti, medici de familie, epidemiologi), existența unui număr mare de pacienți care întrerup tratamentul sau nu doresc să-l înceapă datorită reacțiilor adverse reale sau presupuse au convins-o de necesitatea de a oferi o şansă în plus atât suferinzilor, cât şi medicilor printr-un material care sintetizează cunoştințele validate până în prezent legate de hepatita cronică cu virus C. A rezultat o carte extrem de utilă, cu un pregnant caracter practic, care se adresează nu numai medicilor din disciplinele amintite, ci şi studenților medicinişti, personalului mediu sanitar, precum şi publicului larg.

Prof. dr. Carol STANCIU

Circulaţia şi implicarea virusului West Nile în patologia infecţioasă din România

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Rezumat. Virusul West Nile (WNV) face parte din familia *Flaviviridae,* genul Flavivirus. Face parte din complexul antigenic al encefalitei japoneze şi a fost găsit atât în regiunea temperată, cât şi în cea tropicală. Este transmis la om prin vectori (ţânţari, în special din genul Culex). Principalul rezervor natural de virus îl constituie păsările migratoare. Virusul WN produce în general o boală febrilă benignă cu simptomatologie caracteristică, dar poate avea şi o evoluţie gravă, ducând la meningită, encefalită, meningoencefalită, uneori cu forme mortale, în special la bătrâni. Susceptibilitatea la infecţie este generală, boala fiind urmată de imunitate îndelungată.

Diagnosticul epidemiologic are caracter orientativ şi se referă la cazurile clinice cu simptomatologie caracteristică asociate cu activitatea desfăşurată într-o zonă cu focalitate naturală pentru infecţia cu arbovirusuri, creşterea populaţiei de ţânţari, sau indicii referitoare la un atac biologic cu aerosoli care să conţină arbovirusuri. Diagnosticul de certitudine este stabilit de laborator prin examinarea probelor de la bolnavi: sânge (ser şi cheag), LCR, exudat faringin, fragmente necropsice din creier, ficat, splină, miocard.

Nu există niciun tratament specific pentru infecţia cu WNV. În cazurile mai severe se administrează tratament de susţinere, inclusiv lichide intravenos.

Cuvinte cheie: agenţi biologici, ELISA, ţânţari, virus West Nile

Abstract. West Nile virus (WNV) is a virus of the family *Flaviviridae.* Part of the Japanese encephalitis (JE) antigenic complex of viruses, it is found in both tropical and temperate regions. The main route of human infection is through the bite of an infected mosquito (Culex). The main natural reservoir of the virus is the migratory birds. Generally, WN virus causes a benign febrile disease with characteristic symptoms, but also have a serious development leading to meningitis, encephalitis, meningoencephalitis sometimes deadly forms, especially the elderly. Susceptibility to infection is general, the disease followed by prolonged immunity.

Epidemiological diagnosis is indicative and refer to events characteristic clinical symptoms associated with the activity in a natural focal area for infection with arboviruses, mosquito population growth, or evidence for a biological attack with aerosol containing arboviruses. Certainty diagnosis is established by laboratory examination of samples from patients, blood (serum and clot), CSF exudate faringin, necroptice fragments in the brain, liver, spleen, myocardium.

There is no specific treatment for WNV infection. In more severe cases are given supportive treatment including intravenous fluids.

Introducere

Virusul West Nile (WN) a fost izolat pentru prima dată în anul 1937 de Smithbourne, din sângele unei bolnave febrile din districtul West Nile, Uganda. Virusul are o dimensiune de ~ 70 nm, genomul viral fiind o moleculă de ARN monospiralat infecţios inclus într-un strat proteic alcătuind nucleocapsida, învelită într-un dublu strat lipidic (înveliş sau peplos) în care sunt inclavate două proteine capsidale cu rol în ataşare, antigenice şi imunogene (1, 7).

În 1956 virusul a fost încadrat în serogrupul encefalitei japoneze şi a fost inclus pe lista de agenţi biologici virali incapacitanţi. Prima epidemie înregistrată a fost în Israel în 1950-1954, apoi în 1957, după care au urmat epidemii sau epizootii în Franța 1962-1964, 1966 în delta Ronului, Camargue, în Africa de Sud 1974, 1983, 1984, România 1996, Tunisia şi Cehia 1997, Italia şi Israel 1998, Rusia 1999, Israel 2000, SUA 1999-2003 (când a fost suspectat şi un atac bioterorist mascat), Canada 2001-2003 (5,6).

În România, în epidemia din 1996, din Bucureşti şi 21 județe din sud-estul țării, s-au înregistrat 835 cazuri de meningo-encefalite virale, din care s-au examinat serologic 509, rezultând un număr de 393 infecții acute produse de virusul WN cu 17 morți; transmiterea virusului s-a făcut prin ciclul urban (2,9). În vara anului 2010 a existat o alertă epidemiologică de febră West Nile în România, dar din fericire nu a fost urmată de epidemie.

Persistența infecției cu virusul WN în focarele naturale din zona temperată a Europei este bine stabilită. Arbovirusurile supraviețuiesc peste iarnă în femelele de țânțari care hibernează (Culex), în larvele infectate transovarian, în gazdele vertebrate infectate cronic (păsări şi broaşte). Ca o alternativă, virusul poate fi reintrodus din focarele tropicale şi subtropicale prin păsările migratoare infectate cronic (10).

Izbucnirile epidemice din ultimii 10 ani în țări din Europa şi din Africa, ca şi extinderea infecției pe continentul Nord American produc serioase probleme de sănătate publică şi impun măsuri deosebite de protecție.

Aplicațiile şi alte activități specifice personalului militar, care se desfăşoară în țară sau în alte zone ale lumii, încadrează acest segment de populație în grupa de risc pentru infecția cu arbovirusuri.

Manifestări clinice. Infecția produsă de virusul WN la om este de regulă o boală febrilă. După înțepătura țânțarului infectat virusul pătrunde în circulația microvasculară şi se multiplică în țesutul dermic local, după care este transportat prin vasele limfatice la ganglionii limfatici regionali. Viremia este urmarea replicării virale în țesuturile limfoide.

După o perioadă de incubație de 3-5 zile boala debutează brusc cu febră mare bifazică, cefalee frontală, mialgii, artralgii, conjunctivită, durere retroorbitală, limfadenopatie, uneori erupție cutanată, diaree şi simptome respiratorii. Sub 15 % din cazuri pot evolua cu meningite sau meningoencefalite acute cu LCR clar asociate cu rigiditate cervicală, fotofobie, vomă, confuzii, somnolență, tremurul extremităților, reflexe anormale, convulsii, pareze, hepatosplenomegalie, hepatită, pancreatită, miocardită (7).

Vindecarea bolii febrile este completă, dar uneori poate fi însoțită timp îndelungat de mialgii şi slăbiciune.

Diagnostic. Diagnosticul clinic se referă la sindromul major de febră generalizată întâlnit în majoritatea virozelor şi nu este în măsură să precizeze o infecție arbovirală.

Diagnosticul epidemiologic are caracter orientativ şi se face în cazurile clinice cu simptomatologie caracteristică asociate cu activitatea desfăşurată într-o zonă cu focalitate naturală pentru infecția cu arbovirusuri, creşterea populației de țânțari sau indicii referitoare la un atac biologic cu aerosoli care să conțină arbovirusuri.

Diagnosticul serologic se bazează pe evidențierea anticorpilor anti virus WN din ser şi LCR, prin testul ELISA. Prezența anticorpilor de tip IgM într-o probă de ser şi în LCR indică o infecție acută produsă de virusul WN. Pentru evidențierea anticorpilor de tip IgG este necesară examinarea unei a doua probe de ser prin tehnicile clasice de hemaglutinoinhibare (HAI) şi seroneutralizare (SN) pe culturi celulare sau prin testul ELISA IgG/WN.

Nu există terapie specifică antivirală, tratamentul este unul de susținere. Bolnavii cu infecție febrilă WN necomplicată sunt tratați cu analgezice, pentru a reduce cefaleea şi mialgia, antipiretice, anticonvulsive; este necesară menținerea echilibrului electrolitic şi evitarea complicațiilor bacteriene. În faza acută bolnavii pot fi sursă de infecție pentru țânțari, de aceea camerele în care sunt spitalizați trebuie să aibă plasă la geamuri sau pereții să fie tratați cu substanțe insecticide remanente (6).

Nu este necesară izolarea şi carantinarea bolnavilor pentru că boala nu se transmite interuman, sunt suficiente măsurile standard de control şi evitarea contactului cu vectorii. Virusul poate fi distrus prin căldură, 30 de minute la 80^0C, prin autoclavare (materialele care se pretează sau cele de unică folosință), dezinfectantele obişnuite pe bază de hipoclorit, cloraminele, produse dezinfectante mai noi ca Gigasept FF, Primasept, Mikrozid s.a.

Receptivitatea este generală, dar mai sensibile sunt persoanele peste 60 ani. Imunitatea este de lungă durată. Având în vedere aceste considerații, normele de biosiguranță în laboratoarele medicale recomandă ca diagnosticul de VWN să fie efectuat în laboratoare de microbiologie securizate BSL3.

Material şi metodă

Probele examinate au fost: seruri umane din zonele în care s-au identificat infecții acute cu arbovirusuri în anii precedenți şi seruri de păsări şi animale domestice din jurul locuințelor sau din zonele în care s-au identificat infecții acute cu arbovirusuri.

Antigenele utilizate în tehnica ELISA indirectă de detecție a anticorpilor IgG au fost preparate în laborator, prin tehnica Clarke şi Casals, şi au fost inactivate cu beta-propiolactonă. Antigenele obținute s-au caracterizat pentru antigenitate şi specificitate prin reacțiile de hemaglutinare (HA) şi hemaglutinoinhibare (HAI) cu hematii proaspete de gâscă şi serurile omoloage în condițiile de temperatură şi pH specifice tulpinii virale utilizate.

Probele umane de ser şi LCR au fost examinate pentru evidențierea anticorpilor de tip IgM prin testul ELISA de captură a anticorpilor anti virus West Nile de către laboratorul nostru până în anul 2005, iar apoi de către Laboratorul infecții cu transmitere prin vectori şi entomologie medicală din cadrul INCDMI Cantacuzino, cu care suntem într-o strânsă colaborare.

Diagnosticul de laborator în infecția umană acută cu virusul West Nile se bazează pe evidențierea anticorpilor specifici de tip IgM prezenți în probele de ser şi LCR de la cazuri clinice de neuroinfecții virale, prin testul ELISA IgM (CDC – Fort Collins – USA) (13,14).

Metoda se bazează pe reacția antigen – anticorp, culoarea este generată prin interacția dintre un substrat enzimatic cromogen şi o enzimă cuplată cu un anticorp de detecție.

Testul detectează anticorpii specifici pentru virusul WN, produşi timpuriu în cursul unei infecții primare cu virus WN. Testul imunoenzimatic de captură IgM este testul de elecție pentru detecția IgM specific, deoarece este simplu, sensibil şi aplicabil atât serului, cât şi lichidului cefalorahidian.

Serurile de animale domestice (cai) s-au examinat pentru evidențierea anticorpilor de tip IgG anti virus WN, utilizându-se testul ELISA IgG WN. Conjugatul îl constituie anticorpii anti IgG de specie cuplați cu peroxidază (anti IgG cal) (11). Pentru evidențierea anticorpilor anti virus WN în *serurile de păsări* s-a utilizat tehnica de blocare ELISA. Testul utilizează un anticorp monoclonal anti virus WN a cărui activitate poate fi blocată de anticorpii prezenți în serurile de examinat.

Rezultate şi discuții

S-au evidențiat anticorpi de tip IgM anti virus WN la 41 bolnavi, în anul 2010, stabilind o infecție acută produsă de virusul WN. Ceea ce se observă este apariția târzie a primului caz de infecție acută West Nile, şi anume luna august (tabel 1).

Au fost înregistrate 4 decese, toate acestea survenind la persoane vârstnice care sufereau de mai multe boli cronice asociate: diabet zaharat - insulinodependent, hipertensiune arterială şi boală cardiacă. Pentru prezentarea unei statistici a cazurilor umane de infecție cu virusul West Nile în perioada 2000 – 2010 am sintetizat datele din cercetările proprii şi date furnizate din comunicările Ministerului Sănătății (tabel 2).

După epidemia de infecție cu virusul West Nile din anul 1996, Ministerul Sănătății a implementat în teritoriul considerat de risc (județele riverane Dunării), în perioada de activitate a vectorului (mai - octombrie), sistemul de supraveghere şi control al infecției cu virus West Nile.

Tabel 1. Cazuri de infecţie cu virusul West Nile în România, anul 2010

Judeţul	Nr. cazuri	Data confirmării	Decese
Dolj	1	30.08.2010	
Alba	1 1	30.08.2010 15.09.2010	
Buzău	1	09.09.2010	
Cluj	1 1	30.08.2010 20.09.2010	
Ialomiţa	1 1 1 1	31.08.2010 07.09.2010 09.09.2010 13.09.2010	
Galaţi	1	30.08.2010	
Sibiu	1 1 1	30.08.2010 13.09.2010 15.09.2010	
Bucureşti	1 3 2 3	30.08.2010 07.09.2010 15.09.2010 20.09.2010	Bărbat, 75 de ani, boli cronice asociate
Teleorman	1	30.08.2010	
Mureş	1 1 1	30.08.2010 07.09.2010 15.09.2010	
Constanţa	1 3 2 2	30.08.2010 09.09.2010 15.09.2010 20.09.2010	Bărbat, 79 de ani, boli cronice asociate femeie, 60 de ani, boli cronice asociate
Călăraşi	1	07.09.2010	
Iaşi	1 1 1	07.09.2010 09.09.2010 13.09.2010	
Vrancea	1	13.09.2010	
Mehedinţi	1	13.09.2010	Bărbat, 64 de ani, boli cronice asociate
Total	**41**		**4**

Prezenţa aproape constantă a cazurilor de infecţie West Nile la om în unele judeţe (Galaţi, Tulcea, Ilfov) şi în municipiul Bucureşti, seroprevalenţa ridicată a anticorpilor faţă de acest virus la păsări şi cai ridică ipoteza unei endemicităţi a infecţiei.

În perioada 2002 – 2008 au fost efectuate de către ANSVSA investigaţii serologice la cabaline şi păsări pentru detecţia anticorpilor specifici (IgG) faţă de acest virus. Probele de seruri de la cabaline au fost prelevate din judeţele: Bucureşti, Dâmboviţa, Dolj, Galaţi, Giurgiu, Ialomiţa, Ilfov, Olt, Prahova, Teleorman, Tulcea, iar procentul mediu de animale seropozitive a fost de 20,7% (tabel 3). S-a constatat că procentele de seropozitivitate mai mari de 8% la păsări domestice au fost corelate cu apariţia unor cazuri umane de infecţie cu virusul WN, grupate în microfocare, aşa cum s-a întâlnit în ultimii ani în judeţele Tulcea, Dolj, Olt şi Bacău (tabel 4). Procentajele de seroprevalenţă de 4% au fost corelate cu cazuri izolate de infecţie la om. Interesul mare acordat studiului păsărilor nu este determinat doar de necesitatea de a cunoaşte mai bine ecologia virusului, ci şi de faptul că studiul serologic la păsări oferă informaţii valoroase cu privire la: circulaţia virusului West Nile în natură, nivelul de activitate al virusului în fiecare sezon, posibilitatea transmiterii tangențiale la om.

Tabel 2. Situația infecțiilor cu virus West Nile în perioada 2000-2010

Județ	Cazuri pozitive										
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Alba	-	-	-	-	-	-	-	-	-	-	2
Bucureşti	-	7	-	1	2	1	-	-	-	-	9
Buzău	-	-	-	-	2	1	-	1	1	-	1
Brăila	-	2	-	-	-	-	-	-	1	-	-
Călăraşi	-	1	-	-	-	-	1	-	-	-	1
Cluj	-	-	-	-	-	-	-	-	-	-	2
Constanța	-	3	-	1	3	-	-	-	-	-	8
Dolj	-	-	1	-	1	-	1	-	-	1	1
Galați	7	1	-	4	1	-	-	1	-	-	1
Giurgiu	-	-	-	-	-	1	-	-	-	-	-
Ialomița	3	-	-	-	1	1	-	-	-	-	4
Iaşi	-	-	-	-	-	-	-	-	-	-	3
Mehedinți	-	-	-	-	-	-	-	-	-	-	1
Mureş	-	-	1	-	-	-	-	-	-	-	3
Olt	-	-	1	-	-	-	-	-	-	-	-
Prahova	-	2	-	-	1	-	-	-	-	1	-
Sibiu	-	-	-	-	-	-	-	-	-	-	3
Teleorman	-	3	-	-	-	-	-	-	-	-	1
Tulcea	3	2	-	-	-	-	-	1	-	-	-
Vaslui	-	-	-	-	-	-	-	-	-	-	-
Vrancea	-	1	-	-	-	-	-	-	-	-	1
Total	**13**	**22**	**3**	**6**	**11**	**4**	**2**	**3**	**2**	**2**	**41**

Elementul local cel mai important este reprezentat de fauna de artropode vectoare ale căror caracteristici ecologice se modifică în funcție de variația factorilor climatici şi de mediu locali.

În ultimii ani variația acestor factori a avut un caracter favorizant pentru menținerea şi dezvoltarea populațiilor de vectori, determinând şi evoluția permanentă îngrijorătoare a acestei faune.

Populațiile de vectori s-au extins în spațiu cuprinzând şi zone în care au apărut degradări ale mediului produse de inundații, alunecări de teren, defrişări, amenajări hidrotehnice, lacuri de acumulare şi altele.

Prezența unor populații abundente de artropode vectoare reprezintă unul din factorii de risc importanți pentru sănătatea publică prin diseminarea şi transmiterea unor agenți patogeni, care ar putea avea şi rolul de armă biologică.

Vectorii transmit biologic sau mecanic diferite virusuri. Transmiterea biologică în cazul arbovirusurilor este un proces extrem de complex, cuprinzând modalități de transmitere orizontală şi verticală.

Transmiterea orizontală se referă la circulația virusului între vectori prin intermediul unei gazde vertebrate, şi anume inocularea virusului în gazda vertebrată de către vectorul artropod hematofag şi apoi contaminarea altui vector femelă prin hrănirea sa pe gazda vertebrată infectată.

În cazul transmiterii verticale vectorii femele pot transmite virusul la progeniturile lor pe cale transovariană. Artropodele pot transmite mecanic virusul de la o gazdă vertebrată la alta, fără ca acesta să se fi multiplicat în vector. Transmiterea mecanică se realizează prin intermediul pieselor bucale ale vectorului contaminate în timpul hrănirilor succesive cu sânge şi are loc într-un interval de câteva ore de la hrănirea cu sânge, după care virusul trece în faza de eclipsă în intestinul țânțarului (2 – 3 zile).

Tabel 3. Seroprevalența anticorpilor față de virusul West Nile la cabaline (2002-2008)

Județ	Nr.probe pozitive/ Nr.probe testate (seroprevalența %)							
	2002	2003	2004	2005	2006	2007	2008	2002-2008
Bucureşti	-	-	-	7/51 (13,7%)	40/296 (13,5%)	22/65 (33,8%)	1/36 (2,8%)	70/448 (15,6%)
Dolj	-	-	-	-	-	2/100 (2%)	-	2/100 (2%)
Dâmbovița	-	-	-	-	-	5/100 (5%)	-	5/100 (5%)
Galați	-	-	-	-	-	45/150 (30%)	-	45/150 (30%)
Giurgiu	-	-	43/157 (27,4%)	-	-	-	-	43/157 (27,4%)
Ialomița	-	-	-	-	-	16/100 (16%)	-	16/100 (16%)
Ilfov	-	-	-	175/1216 (14,4%)	186/1073 (17,3%)	71/484 (14,7%)	46/294 (15,6%)	478/3067 (15,6%)
Olt	-	-	-	-	-	0/86 (0%)	-	0/86 (0%)
Prahova	-	-	1/138 (0,7%)	-	-	1/48 (2,1%)	-	2/186 (1,1%)
Teleorman	78/707 (11%)	6/231 (2,6%)	73/803 (9,1%)	-	-	25/157 (15,9%)	-	183/1898 (9,6%)
Tulcea	-	-	-	264/823 (32%)	214/666 (32,1%)	443/1031 (43%)	193/624 (30,9%)	1114/3144 (35,4%)
Total	**78/707** **(11%)**	**6/231** **(2,6%)**	**117/1098** **(10,6%)**	**446/2090** **(21,3%)**	**440/2035** **(21,6%)**	**630/2321** **(27,1%)**	**240/954** **(25,2%)**	**1957/9436** **(20,7%)**

Transmiterea mecanică are mare importanță în condițiile actuale de climă şi mediu din România, condiții care favorizează dezvoltarea unor populații de artropode vectoare cu efective foarte ridicate, crescând probabilitatea transmiterii. Se pare că transmiterea mecanică are un rol important în declanşarea epidemiilor explozive.

Concluzii

Decelarea anticorpilor specifici West Nile la om şi la animale domestice şi sălbatice confirmă că acest virus este prezent în România încă din anul 1955. Circulația virusului West Nile s-a intensificat în ultimii 10 ani ca urmare a presupusei încălziri globale şi a altor schimbări de natură antropogenă.

Numărul total de infecții neurologice la om în România în intervalul 1997-2009 a fost de 68, iar în anul 2010 au fost raportate 41 de infecții umane cu virusul West Nile. Cele mai multe cazuri, 9 la număr, au fost întâlnite în municipiul Bucureşti. Au fost înregistrate 4 decese, la persoane vârstnice care prezentau diverse boli cronice. Se observă modificarea profilului epidemiologic al infecției cu virusul West Nile față de anii anteriori prin apariția unor cazuri în arii neafectate până acum, Transilvania şi Moldova.

Tabel 4. Seroprevalența anticorpilor față de virusul West Nile la păsări domestice (2002-2008)

Județ	Nr.probe pozitive/ Nr.probe testate (seroprevalența %)							
	2002	2003	2004	2005	2006	2007	2008	Total 2002-2008
Bacău	-	-	-	-	-	-	15/96 (15,6%)	15/96 (15,6%)
Bucureşti	1/14 (7,1%)	-	-	2/85 (2,4%)	25/322 (7,7%)	79/533 (14,8%)	-	107/940 (11,4%)
Buzău	0/2 (0%)	-	-	0/2 (0%)	-	-	12/100 (12%)	12/104 (11,5%)
Călăraşi	0/19 (0%)	-	-	-	0/5 (0%)	-	0/51 (0%)	0/75 (0%)
Dolj	5/93 (5,4%)	5/70 (7,1%)	17/229 (7,8%)		43/176 (24,4%)	4/239 (1,6%)	26/111 (23,4%)	100/918 (10,9%)
Dâmbovița	1/66 (1,5%)	-	-	-	-	3/75 (4%)	-	4/141 (29,1%)
Galați	-	-	-	-	-	0/19 (0%)	-	0/19 (0%)
Giurgiu	9/243 (3,7%)	55/575 (9,6%)	26/375 (6,9%)	6/218 (2,7%)	15/190 (7,9%)	106/214 (49,5%)	20/126 (15,8%)	237/1941 (12,2%)
Ialomița	-	-	-	-	-	0/105 (0%)	-	0/105 (0%)
Ilfov	1/33 (3%)	-	-	73/517 (14,1%)	129/1262 (10,2%)	232/2306 (10%)	60/573 (10,4%)	495/4691 (10,6%)
Olt	-	-	0/96 (0%)	-	-	-	5/34 (14,7%)	5/130 (3,8%)
Prahova	2/234 (0,8%)	-	1/112 (0,9%)	-	22/197 11,2%)	55/56 (98,2%)	8/109 (7,3%)	88/708 (12,4%)
Sibiu	-	-	-	-	6/65 (9,2%)	-	-	6/65 (9,2%)
Teleorman	36/409 (8,8%)	5/75 (6,7%)	-	-	-	49/97 (50,5%)	-	90/581 (15,5%)
Tulcea	1/15 (6,7%)	-	-	9/139 (6,4%)	43/184 (23,4%)	19/240 (7,9%)	46/186 (24,7%)	118/764 (15,4%)
Vaslui	1/96 (1%)	-	-	-	-	-	-	1/96 (1%)
Vrancea	2/130 (1,5%)	-	-	-	-	-	-	2/130 (1,5%)
Total	**59/1354 (4,3%)**	**65/720 (9%)**	**44/812 (5,4%)**	**90/961 (9,3%)**	**283/2401 (11,8%)**	**547/3884 (14%)**	**195/1416 (14,8%)**	**1283/11548 (11,11%)**

Decelarea anticorpilor anti West Nile la mamiferele domestice (cai) pe teritoriul țării noastre demonstrează prezența şi implicarea virusului West Nile în neuroinfecții la animele. Extinderea în ultimii ani a epizootiilor şi pe plan mondial, deşi mamiferele sunt considerate gazde accidentale, ridică un semn de întrebare. Se pare că ecvidele ar fi "gazde de amplificare" pentru virusul West Nile.

Păsările sălbatice şi domestice sunt implicate în circulația virusului West Nile pe teritoriul țării noastre. Rolul lor este în diseminarea la distanță (verigi de legătură) şi amplificare a virusului (rezervor natural).

Pentru eliminarea riscului de transmitere a virusului West Nile şi a altor arbovirusuri la om se impun măsuri de supraveghere şi combatere a populațiilor de culicide vectoare care reprezintă "veriga slabă" a ciclurilor de menținere, transmisie şi amplificare arbovirală.

- Supravegherea şi controlul populațiilor de vectori ai arbovirusurilor (țânțari) printr-un program continuu care vizează reducerea habitatelor de dezvoltare a larvelor, combaterea stadiilor larvare, combaterea adulților în cazul în care au fost scăpate de sub control populațiile larvare sau în cazul speciilor de țânțari care migrează pe distanțe mari. Vectorul este "veriga slabă" a lanțului epidemiologic prin controlul căruia se poate întrerupe ciclul de transmitere a virusului.
- Informarea populației din zonele de risc, în care s-au identificat cazuri pozitive WN, cu privire la acțiunile de prevenire a îmbolnăvirilor prin măsuri personale de limitare a contactului cu vectori, apelarea la serviciile de specialitate.
- Informarea şi avizarea permanentă a populației asupra pericolului îmbolnăvirilor transmise prin vectori.
- Supravegherea şi controlul persoanelor care şi-au desfăşurat activitatea în alte țări cu zone endemice pentru infecția cu arbovirusuri.

Rezultatele investigațiilor de supraveghere a circulației virusului West Nile, pe o perioadă de mai mulți ani, duc la concluzia că intensitatea circulației depinde în cea mai mare măsură de nivelul cantitativ al populațiilor de culicide vectoare, dependent la rândul său de factorii climatici. Transmiterea la om poate avea un nivel scăzut în anumite perioade, dar chiar şi în condiții mai puțin favorabile, ea s-a înregistrat în microfocare diseminate pe teritorii foarte largi. Apariția acestor microfocare confirmă permanența circulației enzootice a virusului, demonstrată de nivelele mai mult sau mai puțin ridicate ale seroprevalenței anticorpilor față de virusul West Nile la păsări şi la cai în aceste teritorii.

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Epidemiologia consumului de tutun pe glob şi în România

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Rezumat. Aproximativ o treime din populaţia Uniunii Europene fumează în mod curent, fie ţigări, fie trabucuri sau pipă. De asemenea, aproape jumătate din populaţia Europei pretinde că nu a fumat niciodată, în timp ce aproximativ 22% declară că a renunţat la fumat. În ţările din sud-estul Europei, în special în Grecia, proporţia fumătorilor depăşeşte 40%. Pe următoarele locuri se află Bulgaria (39%), Ungaria (38%), Turcia şi Macedonia (37%). Dimpotrivă, în ţările din nordul Europei, precum Suedia şi Finlanda, procentul fumătorilor este cel mai scăzut, 16% şi, respectiv, 21%. Din punct de vedere socio-demografic, bărbaţii fumează mai mult ca femeile, au vârsta sub 54 ani, nivel de trai scăzut, fără ocupaţie sau cu pregătire profesională scăzută.

Cuvinte cheie: consum de tutun prevalenţă producţie industrială fumători pasivi

Abstract. Almost one third of citizens in the European Union currently smoke either cigarettes, cigars or a pipe. Almost half claim that they have never smoked, while the remainder have given up smoking (22%). Southern Europe countries see the greatest proportion of smoker especially Greece, where the proportion of smokers exceeds 40%. Smoking is the next highest in Bulgaria (39%), Hungary (38%), Turkey (37%) and the Republic of Macedonia (37%). Conversely, in the Northern Member States of Sweden and Finland the proportion of smokers is the lowest, between 16% and 21% respectively. Socio-demographically, smokers are more likely to be male than female, under 54 years of age and from lower social groups. In terms of occupation, smokers are more likely to be unemployed, manual workers or self-employed.

1. CONSECINŢELE FUMATULUI ÎN LUME

Tabagismul defineşte consumul prelungit de tutun, în special sub formă de ţigarete, precum şi consecinţele care derivă din acesta.

Ce este *fumătorul?* În anul 2004, Centrul pentru Politici şi Servicii de Sănătate preia definiţia fumătorului (Departament of Health and Human Services - The health benefits of smoching cesatation. A report of the Surgeon General, 1990), ca fiind *„persoana care în momentul studiului fumează, utilizând orice produs de tutun fie zilnic, fie ocazional”.*

În prezent, fumatul a devenit un obicei extrem de frecvent astfel că, pe glob, s-a ajuns la un consum de 6 000 de miliarde de ţigarete anual. Se estimează că în lume sunt peste 1,3 miliarde de fumători, dintre care peste 800 de milioane se află în ţările în curs de dezvoltare. Aproximativ 3 milioane de persoane mor anual din cauza fumatului, de unde rezultă o frecvenţă medie a deceselor îngrijorătoare: un deces la fiecare 10 secunde. În anul 1995, numărul deceselor datorate fumatului în lume a fost de 1 915 000 în ţările dezvoltate şi de 1 210 000 în ţările în curs de dezvoltare, dintre care 440 000 numai în China. Datorită unei asemenea răspândiri a fumatului, mulţi au denumit vremurile pe care le trăim *“epoca bolilor şi deceselor produse de tutun”*, îmbrăcând caracterele unei *“epidemii generale”,* precum şi un *“veritabil flagel al acestui sfârşit de veac şi crescândă ameninţare la adresa omenirii”.*

Fumatul s-a răspândit mai întâi la sexul masculin şi apoi la cel feminin. Pretutindeni, fumatul s-a dezvoltat în raport invers cu nivelul socio-economic şi gradul de instruire a populaţiei. În lume, zilnic, câteva sute de tineri încearcă prima ţigară, devenind apoi dependenţi de nicotină. Milioane de copii, aflaţi în viaţă acum, vor muri de boli datorate consumului de tutun. Există mai multe victime ale nicotinei decât cele provocate de cel de-al doilea război mondial, astfel că la nivel mondial tutunul produce zilnic decesul a circa 10 000 de persoane.

În anul 1993, proporţia fumătorilor în ţările dezvoltate era de 20-35%, iar în cele în curs de dezvoltare de 2-10%. Paradoxal, în multe ţări numărul fumătoarelor creşte continuu comparativ cu fumătorii de sex masculin. În ţările dezvoltate, femeile fumătoare sunt mai ales tinere, cu instruire elementară şi cu nivel socio-economic scăzut.

Datele epidemiologice prezentate la a doua Conferinţă europeană şi prima Conferinţă iberico-americană privind *“Tutunul sau Sănătatea”* sunt alarmante: în Cuba, 30% din cauzele de deces sunt datorate bolilor determinate de fumat, în SUA, reprezintă 25%, iar în Marea Britanie 15-20%. Anual se înregistrează un număr de decese datorate tutunului: 400 000 în SUA, 10 000 în Marea Britanie, 70 000 în Italia, 45 000 în Spania etc. În lume fumează în prezent aproximativ 200 000 000 de femei, cu vârfuri de prevalenţă ce ating 28% în unele ţări foste socialiste din estul Europei sau 21% în multe ţări latino-americane şi insulele Caraibe. Conform unor studii epidemiologice, cazurile de mortalitate la toate vârstele sunt de 2-3 ori mai numeroase la fumători decât la nefumători, ceea ce înseamnă că jumătate din numărul fumătorilor vor deceda din cauza fumatului. În condiţiile menţinerii consumului actual de tutun, circa 50 000 000 de persoane fumătoare, adică aproximativ 10% din populaţia globului, vor deceda din cauza tutunului. Jumătate dintre fumătorii ucişi de tutun sunt în deplină maturitate, la aceştia speranţa de viaţă diminuându-se cu aproximativ 20-25 de ani.

La sfârşitul secolului al XX-lea, 62 000 000 de persoane din ţările dezvoltate au decedat din cauza consumului de tutun, dintre care 52 000 000 bărbaţi şi 10 000 000 femei. Tutunul ocupă cel de-al şaselea loc între factorii de deces din ţările dezvoltate. Această proporţie tinde să ia amploare, mai cu seamă prin dezvoltarea epidemiei de tabagism la femei, ceea ce duce la concluzia că dintre cei 1,2 miliarde locuitori din ţările dezvoltate cam 200 de milioane vor muri din cauza tutunului.

În ţările dezvoltate, 30% din decesele datorate cancerului pulmonar, adică 40-45% din bărbaţi şi 10-15% din femei, au la origine fumatul, iar decesele prin cancer pulmonar provocate de fumat sunt de 90-95% la bărbaţi şi de 70-75% la femei.

Începând cu anul 1990, tabagismul produce 3 000 000 de victime anual. În anul 1995, fumatul a cauzat decesul a 514 000 de persoane prin cancer pulmonar şi a 625 000 prin boli cardiovasculare, în ţările dezvoltate. Tot în acelaşi an, conform estimărilor OMS, fumatul a produs aproximativ 1 440 000 de decese în rândul bărbaţilor şi 475 000 de decese la femei, ceea ce reprezintă 25% din decesele la bărbaţi şi 10% din decese la femei în ţările dezvoltate.

În prezent, din cei 1,3 miliarde de fumători de pe glob, aproape 1/3 din populaţia lumii cu vârste mai mari de 15 ani, 47% sunt bărbaţi şi 12% femei, din care, în ţările în curs de dezvoltare, fumatul a cuprins 48% bărbaţi şi 7% femei, faţă de 42% bărbaţi şi 24% femei în statele dezvoltate.

Statisticile OMS arată că un mare fumător pierde în medie 8,3 ani din viaţă, iar unul care fumează moderat pierde 4,6 ani din durata medie de viaţă. În schimb, la foştii fumători, după circa 10 ani de la abandonarea fumatului, decesul apare în aceeaşi proporţie ca la nefumători.

Piaţa tutunului este dominată de 7 companii multinaţionale, iar din suprafaţa globului, 4-5 milioane de hectare de pământ arabil sunt alocate cultivării tutunului. În perioada 1962-1964 producţia mondială de tutun a fost de circa 4 400 mii de tone, în anul 1976 aproape 5 700 mii tone (cu aproximativ 20% mai mult decât în anul 1970), ajungând, după anul 1985, la peste 6 000-6 500 mii tone, cu variaţii între ţările dezvoltate şi cele în curs de dezvoltare. Cele mai importante ţări producătoare de tutun sunt China (fiind un mare consumator) şi SUA, urmându-le îndeaproape India, fostele ţări ale Uniunii Sovietice, Turcia şi Brazilia, tutunul fiind cultivat în peste 120 de state de pe glob.

În ţările în curs de dezvoltare producţia de tutun a crescut mai rapid datorită cererilor crescute de consum şi a încurajării producţiei în vederea reducerii importului. Aşadar, ţinând cont de amploarea consumului de tutun şi de consecinţele sale asupra sănătăţii omului, tabagismul a devenit astăzi *“cea mai răspândită şi cea mai ucigătoare dintre toxicomaniile socialmente acceptate”*. Consumul cronic de tutun este, practic, o pandemie ce ameninţă sănătatea tuturor locuitorilor.

2. CONSUMUL DE TUTUN ÎN CHINA

China, ţara cea mai populată din lume, este, în prezent, cel mai mare producător şi consumator de tutun. Pe parcursul anilor '80, consumul total de ţigări a crescut într-un ritm anual de 7%. Din cauza fumatului, în China se înregistrează anual moartea a aproximativ jumătate de milion de oameni, pentru perioada 2020-2029 estimările ajungând la două milioane de decese, datorate aceleiaşi cauze.

Prima anchetă naţională, efectuată în anul 1984, pe un eşantion de 519 600 persoane arăta că 33,9% din populaţia de peste 15 ani fumează curent (61% bărbaţi şi 7% femei). Altfel spus, în acel an, în China erau 300 de milioane de fumători, mai mult decât întreaga populaţie a SUA. De asemenea, se estima că din cauza fumatului vor muri prematur un număr de aproximativ 50 de milioane de copii. În rândul personalului medical din China, dintre medici fumează 60% bărbaţi şi 6,3% femei (probabil datorită religiei şi obiceiurilor), iar dintre asistente fumează între 7,1% şi 33,3 %, cel mai numeros fiind personalul medical care îşi desfăşoară activitatea în serviciile de pediatrie.

Relevant este faptul că în China o singură societate de producere, prelucrare şi desfacere a tutunului poate înregistra încasări de două ori mai mari decât întreaga sumă alocată serviciilor de sănătate. Între anii 1985 şi 1992, în timp ce consumul de ţigări la adulţi scădea în ţările puternic dezvoltate, în China acesta creştea cu 20%, astfel că pentru fiecare ţigară produsă mai puţin în ţările industrializate, în China se vindeau cu trei mai mult. Analize recente indică scăderea consumului de tutun printre adulţii de vârstă mijlocie, la ambele sexe, în schimb creşte numărul fumătorilor printre tinerii de ambele sexe.

În noiembrie 1998 au fost publicate rezultatele celei mai mari statistici naţionale din lume, pe decese cauzate de fumat, care a inclus 1,25 milioane de chinezi. Studiul a descoperit că nu doar rata deceselor prin fumat este mai mare decât se aştepta, dar şi tipurile de boli diferă faţă de cele din vest. În această ţară, 2/3 din bărbaţi devin fumători înainte de vârsta de 25 de ani, deci tutunul va determina decesul a 100-300 milioane bărbaţi aflaţi sub 30 de ani. La femei însă fumatul este în scădere, de la 10%, prevalenţa dinainte de anul 1950, la 1%, cât reprezintă în prezent.

În China sunt circa 9 milioane bolnavi cu hipertensiune arterială, 6 milioane de supravieţuitori ai unui accident vascular cerebral, la care se adaugă, în fiecare an, 1,5 milioane de cazuri noi de accidente vasculare cerebrale. În anul 1996 procentul de fumători pasivi era de 53,3%, dintre care 68,4% erau expuşi zilnic fumului de ţigară, majoritatea fiind copii şi femei. Se estimează că în circa 30 de ani vor deceda din cauza fumatului aproximativ 2 milioane de chinezi, adică 900 mii pe an.

Wang Kean, cercetător şi preşedinte al Academiei Chineze de Medicină Preventivă din Beijing, afirma că *"dacă nu vom acţiona imediat, vom suferi după 20-30 de ani, atunci fiind prea târziu..."*. Acelaşi cercetător estimează că, în prezent, numărul deceselor pe an va ajunge la 750 000, crescând la 3 milioane pe an pe la mijlocul secolului următor, când tinerii fumători de azi vor depăşi vârsta mijlocie.

Richard Peto, şeful echipei de cercetători de la Oxford, spunea: *"în timp ce China are 20% din populaţia lumii, chinezii fumează 30% din ţigaretele produse în lume"*.

Îngrijorător este faptul că, la întrebările din chestionare adresate chinezilor, două treimi au răspuns că fumatul este puţin sau deloc dăunător. Realitatea este că jumătate dintre fumători sunt ucişi de tutun, iar cum 2 din 3 tineri chinezi fumează, fumatul va ucide aproape o treime din tinerii chinezi.

3. CONSUMUL DE TUTUN ÎN SUA

În această ţară fumatul este principala cauză a bolilor prevenibile şi a morţii premature. Anual, mai mult de 1 000 000 de copii şi adolescenţi americani încep să fumeze iar majoritatea fumătorilor abandonează cu greu acest obicei. În SUA fumatul este responsabil pentru aproximativ 390 000-400 000 de decese premature în fiecare an, adică mai mult decât 1 din 6 morţi.

Riscul de decese datorate tabagismului s-a estimat anual la circa 7 000 la 1 milion de fumători, de 10 ori mai mare decât riscul deceselor cauzate de alcool (541%ooo), de 60 de ori mai mare decât riscul deceselor prin accidentare la locul de muncă, de 1 000 de ori mai mare decât riscul generat de poluarea atmosferică şi de 3 500 de ori mai mare decât riscul de electrocutare.

În anul 1984, OMS afirma că: *"tabagismul face în 3 ani mai multe victime decât toate războaiele la care a participat SUA"*.

Studiile efectuate pe o perioadă de 12-20 de ani, cuprinzând peste 1 500 000 de persoane, arată că frecvenţa cancerului pulmonar la fumători este, în medie, de 10 ori mai mare decât la nefumători. Alarmant este faptul că, în SUA, ţigările se vând şi în farmacii, 57% dintre farmacişti permiţând fumatul în aceste spaţii.

Şi în SUA fumatul este influenţat de nivelul socio-cultural al populaţiei: 23,6% în vestul SUA, faţă de 26% în statele din est, fiind mai frecvent la lesbiene şi homosexuali, precum şi la grupele de vârstă cuprinse între 25 şi 30 de ani. Cu excepţia persoanelor care au un nivel de educaţie de 0-8 ani, procentul fumătorilor este invers proporţional cu nivelul de educaţie, cel mai mare procent fiind în rândul populaţiei cu 9-11 ani de educaţie (38,2%). De asemenea, procentul de fumători este mai mare în rândul populaţiei sărace (34,7%), faţă de cei care trăiesc la nivelul sau peste pragul de sărăcie (24,1%).

În perioada 1955-1988, prevalenţa fumatului în SUA este prezentată în tabel, conform datelor OMS.

Evoluţia prevalenţei tabagismului în SUA

Sex (%)	1955	1965	1970	1976	1980	1983	1987	1988
Bărbaţi	53	50	44	42	38	35	32	31
Femei	25	32	31	31	29	29	27	26

Sursa: date OMS

În perioada 1964-1974, Centrul Naţional de Statistică din SUA a constatat un plus de îmbolnăviri de 14% la bărbaţii fumători şi de 21% la femeile fumătoare, studiile referindu-se la boli respiratorii banale: gripa şi alte boli infecţioase, boli digestive etc.

Epidemiologii au estimat recent că numărul bolnavilor cu BPOC este de 16 milioane, această boală fiind a 6-a cauză principală de deces.

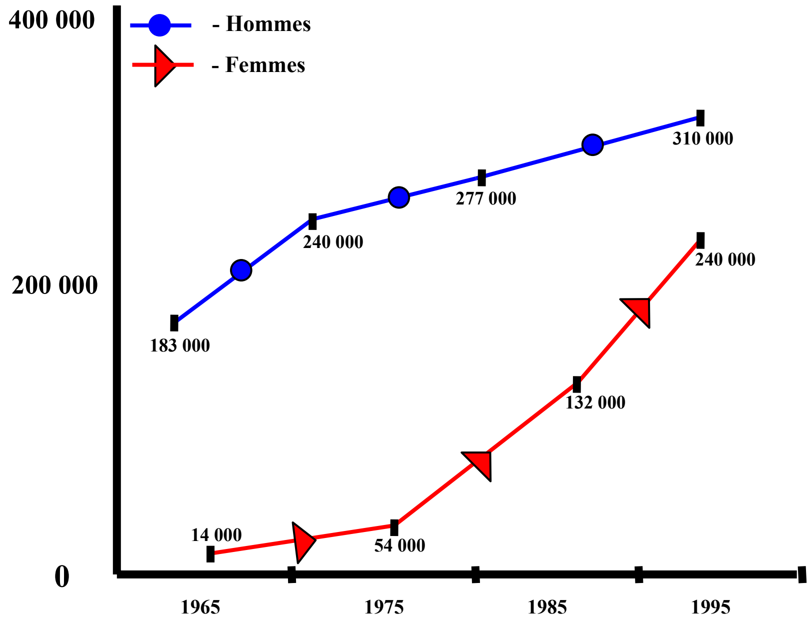

Numărul deceselor datorate fumatului în SUA, în perioada 1965-1995 (date OMS)

În privinţa tinerilor, numai în anul 1989, peste 3 000 de adolescenţi s-au apucat să fumeze, estimându-se că dintre aceştia 750 vor muri prin boli determinate de fumat. Rolul reclamelor în acest sens este nefast şi foarte important: 85% dintre fumătorii tineri alegând primele trei mărci de ţigări care fac obiectul unei publicităţi masive.

În anul 1994, 48 milioane adulţi (25,5%) fumau în mod curent, dintre care 25,3 milioane bărbaţi şi 22,7 milioane femei, adică 21% fumau permanent şi 4,6% fumau ocazional.

Din punctul de vedere al grupărilor rasiale/etnice procentul mai mare, adică 42,2%, îl reprezintă amero-indienii şi nativii din Alaska, iar cel mai mic procent, respectiv 13,9%, este reprezentat de populaţia asiatică şi a insulelor Pacificului. Totuşi, cu vârsta, numărul fumătorilor scade de la 45,5%, între 21 şi 30 de ani, la 26,3%, la vârstele de peste 65 ani iar, din anul 1988, rata fumătorilor studenţi, având vârste cuprinse între 17-18 ani, a scăzut la 18%, de la un maxim de 30%.

Starea de sănătate este influenţată şi prin expunerea involuntară la fumul de tutun, adică fumatul pasiv, astfel că, în SUA, dintr-un milion de copii astmatici, 200 000 îşi datorează boala expunerii pasive la fumul de tutun. De asemenea, în primii doi ani de viaţă, riscul infecţiilor căilor respiratorii inferioare este cu 50-60% mai mare, iar până la 18 luni, riscul este de 10-15%. La copii fumatul pasiv se asociază cu supuraţiile urechii mijlocii, iritarea căilor respiratorii superioare, diminuarea semnificativă a funcţiei pulmonare.

Dintr-o analiză economică a urmărilor tabagismului în SUA se estimează că anual se cheltuiesc 501 miliarde de dolari (6 000 de dolari pe fumător sau fost fumător) pentru tratarea bolilor rezultate în urma fumatului. De exemplu, costul tratamentului cancerului pulmonar este de 200% din produsul naţional brut pe cap de locuitor pentru fiecare an de viaţă câştigat. În anul 1960, ca urmare a afectării sănătăţii prin consumul de ţigări, în SUA s-au pierdut 77 milioane zile de muncă, iar în anul 1974, 81 milioane, fiind înregistrate 150 milioane zile de spitalizare în plus, iar durata medie a spitalizării, la fumători, este mai mare cu 2 zile. S-a estimat că la o tonă de tutun corespund 990 de cazuri noi de îmbolnăviri şi 650 de decese, iar pierderile prin mortalitate şi morbiditate, instalate prematur, sunt de 27 200 000 dolari, la un beneficiu de 2 600 000 dolari. Pierderile sunt dispersate, fiind înregistrate în rândul populaţiei, iar câştigul aparţine unor magnaţi ai tutunului.

4. CONSUMUL DE TUTUN ÎN INDIA

În anul 2000, New Delhi a găzduit 150 reprezentanţi din ţări diferite, tema adunării fiind fumatul. În India sunt aproximativ 250 milioane de consumatori de tutun: 69% fumători, 30,7% cei care mestecă tutun şi 0,03% cei care prizează tutun. Aceştia *"ingurgitează"* 94 700 milioane ţigarete şi 700 miliarde de produse de mestecat şi prizat. La începutul secolului India britanică a fost a doua mare producătoare şi consumatoare de tutun, astăzi fiind a treia de acest fel din lume. Dacă orăşenii şi bogaţii fumează ţigarete, săracii şi tinerii din mediul rural fumează *"bidi"*, ţigări speciale, fabricate din tutun neprelucrat aflat într-o cantitate mai mare şi cu un conţinut crescut de nicotină. Se fumează de 8 ori mai multe *"bidi"* decât ţigări obişnuite.

În India, circa 43% din bărbaţi fumează, consumând peste 90 miliarde ţigări. Ţigările indiene conţin cantităţi mai mari de monoxid de carbon şi nicotină decât cele obişnuite, putând avea diverse arome: de căpşuni, vanilie, ciocolată etc. Ţigările sunt, de obicei, fără filtru, iar pachetele nu au tipărit avertismentul obişnuit, de unde şi riscul crescut în rândul tinerilor şi al copiilor.

Femeile de la oraş nu fumează, spre deosebire de cele din mediul rural, depinzând însă şi de zonă. În Bihar se fumează *"hookah"* sau pipă, pe coasta de sud femeile fumează *"khutta",* însă în cea mai mare parte a Indiei se foloseşte tutunul de mestecat (un amestec ce conţine flori sau frunze de tei, lămâie şi tutun). Femeile din Maharashtra folosesc *"mishri"*, o pudră neagră obţinută din prăjirea şi triturarea tutunului. Datorită acestor obiceiuri, se constată o creştere a incidenţei cazurilor de cancer oral şi de leucoplazii bucale în rândul adulţilor de la 0,2% la 4,9% iar datorită fibrozării submucoasei orale, 1 din 10 indieni acuză senzaţie de arsură şi nu poate deschide gura.

Anual mor, datorită consumului de tutun, aproximativ 635 000 de adulţi. Ca măsură, în anul 1990, guvernul Indiei a elaborat un ordin administrativ prin care se interzice fumatul în locuri publice : spitale, instituţii educaţionale, săli de conferinţe, trenuri, metrouri, autobuze etc.

5. CONSUMUL DE TUTUN ÎN AUSTRALIA

În Australia, cele mai multe persoane încep să fumeze în adolescenţă, cu toate că unele abordează acest obicei atunci când sunt angajate sau când îşi continuă studiile. Ultimele statistici arată că mai puţin de 1/3 din populaţia adultă a Australiei fumează. În această ţară, industria de ţigări a cheltuit 4 bilioane de dolari pentru reclame însă, tot aici, au fost câştigate procese pentru daune aduse fumătorilor pasivi, în procese fiind convocaţi şi experţi. Prevalenţa consumului de ţigarete în Australia, în perioada 1945-1989, este prezentată în tabelul de mai jos.

Prevalenţa consumului de ţigarete în Australia pe perioada 1945-1989

	1945	1964	1969	1972	1980	1986	1989
Bărbaţi (%)	72	58	45	40	40	32	30
Femei (%)	26	28	28	31	31	29	27

Sursa: Viaţa medicală 1993

Din datele statistice ale consumului de tutun în Australia rezultă următoarele:

- cei mai mulţi australieni sunt nefumători;
- din procentajele populaţiei care a fumat mai mult de un an rezultă că fumatul devine din ce în ce mai nepopular, tot mai multe persoane aleg să nu fumeze, iar fumătorii abandonează fumatul (aproximativ 1 500 000 de australieni se lasă de fumat în fiecare an);
- marea majoritate a tinerilor aleg să nu fumeze;
- fumatul este mai popular printre fete decât printre băieţi, cu toate că după vârsta de 20 de ani numărul bărbaţilor fumători îl depăşeşte pe cel al femeilor nefumătoare;
- la sfârşitul anului 1980 rata tinerilor fumători a scăzut la toate categoriile de vârstă, la începutul anilor '90 constatându-se totuşi o creştere a numărului acestora;
- datorită fumatului experimental, la băieţi creşterea a fost mai mare la grupa de vârstă cuprinsă între 12-15 ani.

6. CONSUMUL DE TUTUN ÎN EUROPA

Scurt istoric

Primii europeni care au văzut oameni fumând au fost marinarii lui Cristofor Columb, în anul 1492, după debarcarea în Lumea Nouă. Amerindienii fumau cu ajutorul unor pipe din lemn, obiceiul având semnificaţii magice, fiind folosit şi în scopuri terapeutice şi sociale.

În anul 1558, în Europa, respectiv în Portugalia, se cultivă pentru prima dată tutunul, fiind folosit iniţial numai pentru prizat, precum şi ca medicament.

Primele seminţe de tutun au fost introduse în Franţa, prin anul 1556, de către călugărul Thévet, care le-a adus din Brazilia, dar folosirea tutunului s-a generalizat abia prin anul 1560, când Jean Nicot (1530-1600) trimite Catherinei de Medicis pudră de tabac pentru a-i vindeca migrenele.

Olandezii au cultivat tutunul prima dată în anul 1615, iar în Saxonia, s-a cultivat în anul 1531.

În Anglia, tutunul a fost introdus în anul 1565 de către Sir John Hawkins, deşi se vehiculează şi numele lui Sir Walter Raleigh şi chiar al Reginei Elisabeta.

Folosirea tutunului devine obişnuinţă în Europa pe la 1600.

Producţia de tutun în Europa

Fumatul afectează peste 40% din populaţia Europei, consumul de ţigarete în ţările europene fiind de peste 600 000 milioane pe an, aici înregistrându-se mai mult de jumătate din decesele cauzate de fumat în lume. Una din constatările principale, care s-a desprins din lucrările Conferinţei consacrate unei Europe fără tutun *"Tutun sau Sănătate"*, din 2-4 octombrie 1996, Helsinki, Finlanda, este aceea că *"tutunul este drogul utilizat cu cea mai mare plăcere în Europa, 36% din adulţi fumând zilnic"*.

În perioada 1989-1992, într-o serie de state ale Comunităţii Europene (CE) s-au constatat menţineri, la valori relativ apropiate de la an la an, ale suprafeţelor cultivate cu tutun (în cazul Franţei, Germaniei, Belgiei şi Portugaliei), scăderi ale acestora în Italia şi Spania, precum şi creşteri, de 12,8%, în Grecia (102 317 ha în 1992). În aceleaşi ţări, producţia de tutun brut a înregistrat evoluţii similare, cu excepţia Spaniei, unde, la o suprafaţă mai mică cu circa 25%, s-a înregistrat o producţie mai mare cu câteva sute de tone. Supremaţia o deţin Italia şi Grecia, care asigură 80% din producţia de tutun a CE.

Începând cu anul 1990, în Europa de Est, fumatul se extinde îngrijorător, odată cu tranziţia spre o economie de piaţă şi apariţia companiilor internaţionale de tutun care, în absenţa unei legislaţii în domeniu, îşi oferă promoţional produsele, concomitent cu o reclamă agresivă la adresa acestora. Dat fiind faptul că în ţările din Europa Centrală şi de Est tehnologia de fabricare a ţigărilor este perimată, calitatea ingredientelor din compoziţia ţigării (mentol, glicerină, cacao, esenţe etc.) fiind inferioară, s-a dezvoltat un import masiv de ţigări, legal şi prin contrabandă, de unde atracţia marilor companii ale industriei tutunului spre această zonă, cu investiţii care se ridică la sute de milioane de dolari, cu mult peste cele alocate serviciilor de sănătate, conform datelor OMS. Presiunile făcute de investitorii străini asupra guvernelor acestor ţări sunt enorme. De exemplu, în Rusia s-au investit câteva zeci de milioane de dolari americani pentru construirea unei fabrici de ţigări. În Cehia şi Slovacia s-au investit, de aceleaşi mari companii, 396 milioane de dolari americani, fapt ce a dus la obţinerea de către acestea a 30% din acţiunile monopolului asupra tutunului. De asemenea, în Ungaria s-au investit 34 milioane de dolari americani în acelaşi scopuri.

În CE producţia de tutun se subvenţionează, în anul 1990 acesteia revenindu-i 62% din bugetul CE destinat politicii agricole comunitare, aproximativ 1 232 milioane ECU, adică 2,8% din totalul bugetului, cheltuielile de subvenţie a culturilor de tutun crescând în 11 ani (1980-1991) de 4,3 ori.

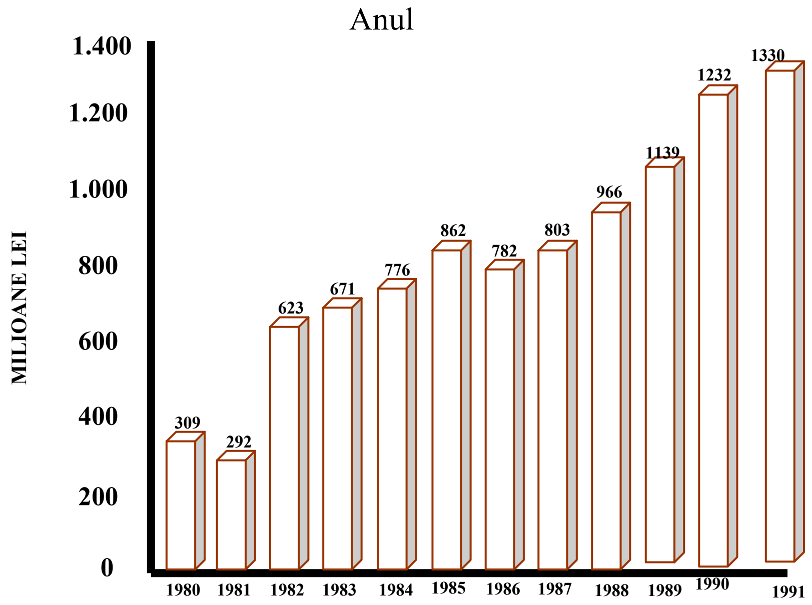

Subvenţii ale tutunului în CE în perioada 1980-1991 (date OMS)

De remarcat că în anul 1990 valoarea subvenţiei/hectar la cereale a fost de 129 ECU, în timp ce subvenţia/hectar pentru tutun a fost de 4 527 ECU. În anul 1991, în CE se importau 528 000 tone tutun brut, adică 75% din tutunul necesar şi utilizat. În anul 1990, în CE se importau din SUA 102 000 tone de tutun, la un preţ mediu de 5,7 ECU/kg, iar preţul tutunului exportat de CE a fost de 1,2 ECU/kg. În anul 1990, Italia a exportat 103 000 tone tutun, după cu urmează: în CSI -3 000 tone la

0,02 ECU/kg, în România - 9 000 de tone, la acelaşi preţ, Benin - 3 000 tone, la 0,10 ECU/kg, apoi Albaniei, Ungariei, Poloniei, Bulgariei, precum şi altor ţări.

Aspecte epidemiologice

În cadrul celui de-al V-lea seminar OMS, consacrat unei *"Europe fără tutun"*, desfăşurat la Varşovia în perioada 26-28 octombrie 1995, s-a făcut o trecere în revistă a situaţiei pandemiei tabagice în Europa, trăgându-se şi concluzia îngrijorătoare că în centrul şi estul continentului se înregistrează 700 000 de decese datorate fumatului:

Prevalenţa fumatului în câteva ţări din Europa

Nr. crt.	Ţara		Vârsta	Ambele sexe	% Anchetaţi	
					Bărbaţi	Femei
1	**România**	1994	≥ 15 ani	28,0	42,7	15,2
2	**Republica Cehă**	1994	≥ 15ani	37,0	43,1	31,2
3	**Danemarca**	1994	≥ 15 ani	42,0	44,7	39,1
4	**Finlanda**	1994	≥ 14-64 ani	24,0	30,0	19,0
5	**Franţa**	1994	≥ 18ani	33,0	40,3	27,3
6	**Grecia**	1994	≥ 15ani	37,0	46,0	28,0
7	**Italia**	1994	≥ 15ani	32,0	38,0	26,0
8	**Luxemburg**	1994	≥ 15 ani	27,0	32,0	26,0
9	**Olanda**	1994	≥ 15 ani	33,0	36,0	29,1
10	**Polonia**	1994	≥ 16 ani	39,3	50,9	29,1
11	**Portugalia**	1994	≥ 15 ani	36,0	38,0	15,0
12	**Regatul Unit**	1994	≥ 16 ani	28,0	29,0	28,0
13	**Suedia**	1994	≥ 15 ani	23,0	23,0	23,0

Sursa: date OMS

Constatând că în ţările fostului bloc comunist se face tot mai simţită penetraţia companiilor multinaţionale de industrie a tutunului, comparativ cu ţări din vestul şi nordul Europei (Marea Britanie, Franţa, Belgia, Finlanda), companiile de publicitate sunt foarte active, agresive şi rafinate în mesaje. Astfel, în anul 1990, chestionându-se mai mult de 9 000 de tineri cu vârste cuprinse între 11 şi 15 ani, din 12 ţări ale CE, la întrebarea *"Cunoaşteţi vreun produs de tutun?"* au răspuns pozitiv 44% dintre ei, în Italia, 41% în Belgia, 36% în Germania, 13% în Anglia şi 8% în Irlanda, în condiţiile în care în aceste ţări este interzisă prin lege reclama la produsele de tutun. În 1995, la o prevalenţă a fumatului de 46% la bărbaţi şi 26% la femei, în Europa, numărul deceselor ce incriminează fumatul era de 1,5 milioane, numărul femeilor decedate fiind în continuă creştere:

Numărul deceselor atribuite fumatului în rândul bărbaţilor şi femeilor de 35-39 ani în statele Regiunii Europene a OMS

Anul	Bărbaţi	Femei	Ambele sexe
	35-39 ani	35-69 ani	35-69
1985	696.228	65.721	671.947
1990	665.069	75.387	740.456
1995	663.647	78.309	741.956

Sursa: Didilescu, Marica

În anul 2004, prevalenţa fumatului în Europa Centrală şi de Est varia între 29,1% în Cehia şi 41,75% în Ungaria, România, cu 44%, apropiindu-se de Bosnia-Herzegovina, cu 48%, şi de Serbia, cu 47%, situându-se departe de ţările Uniunii Europene.

Pe sexe, prevalenţa fumatului în unele ţări din Europa Centrală şi de Est, de la începutul şi până la mijlocul anilor '90, este prezentată în tabelul de mai jos, conform datelor OMS:

Prevalenţa fumatului la adulţi în câteva ţări din Europa Centrală şi de Est
(la începutul şi până la mijlocul anilor 1990)

Ţara	Anul	Masc.	Fem.	Total	Observaţii
Bulgaria	1989	49	17	33	Zilnic şi ocazional
Cehia	1994	43	31	36	Regulat şi ocazional ≥ 15 ani
Estonia	1994	52	24	36	Zilnic ≥ 16 ani
Polonia	1993	51	29	39	≥ 16 ani
România	1994	43	15	28	Zilnic şi ocazional
Rusia	1995	67	27	47	Estimat de Ministerul Sănătaţii şi industria medicală
Slovacia	'91-'92	43	26	34	Regulat + 10% ocazional
Slovenia	1994	34,7	22,7	28,2	-
Ungaria		40	37	39	Estimat

Sursa: date OMS

De remarcat valorile considerabile consemnate pentru bărbaţi în Rusia - 67%, Estonia - 52% şi Polonia - 51%, iar pentru femei, cele din Ungaria - 37%, Cehia - 31% şi Polonia 29%.

6.1. Consumul de tutun în Marea Britanie

În Marea Britanie, *"markerii pentru fumători sunt: rasa albă, părinţi singuri, cei cu puţină educaţie, aflaţi în evidenţa serviciilor de securitate socială, cei ce depun o muncă manuală şi meserii cu stres"*. Fumatul produce de 4 ori mai multe decese decât drogurile, alcoolul, sinuciderile şi accidentele rutiere cumulate.Tinerii, cu vârste între 11 şi 15 ani, consumă aproximativ 17 000 000 de ţigări săptămânal. La 1 000 de tineri cu vârsta de 20 ani, care fumează în mod regulat, 250‰ vor muri înainte de vârsta de 70 ani şi alţi 25‰ după această vârstă (durata medie de viaţă este mai mare de 70 ani).

Ultimul studiu al lui Richard Doll şi Richard Peto & colab. arată că riscul fumatului este mult mai mare decât se anticipa, adică între 1/2 şi 2/3 dintre fumători mor constant prin cancer pulmonar, BPOC şi emfizem, rata mortalităţii crescând odată cu deprinderea viciului la vârste tinere. Posibilitatea unui nefumător de 70 ani de a ajunge la 90 ani a crescut din 1961 în 2001 de la 12% la 33%, pe când pentru fumători a scăzut, în aceeaşi perioadă, la 10% la 7%.

În anul 1974 mureau, din cauza fumatului, prin cancer pulmonar, 37.000 de persoane iar, în prezent, mor aproximativ 100.000 de persoane anual, adică 300/zi.

Evoluţia prevalenţei tabagismului în Regatul Unit, în perioada 1956-1988, este prezentată în tabel:

Evoluţia prevalenţei tabagismului în Marea Britanie (%)

Fumători \ Anul	1956	1961	1965	1972	1976	1980	1984	1988
Bărbaţi	75	72	68	52	46	42	36	33
Femei	42	44	43	41	38	36	32	30

Sursa: date OMS

În privinţa femeilor fumătoare, Marea Britanie poate oferi datele cele mai sugestive: după Epoca Victoriană (1920), eliberate de rigorile legii, numărul acestora a început să crească, iar emanciparea a crescut în plus după al doilea război mondial.

În anul 1952, A. Bradford şi Richard Doll sunt primii care, în studiile lor, asociază fumatul cu cancerul bronho-pulmonar la femei.

6.2. Consumul de tutun în Franţa

La început, tutunul a fost folosit sub formă de decoct, apoi prizat iar în secolul XVII au apărut pipa şi obiceiul de a se fuma, pentru ca în anul 1811 să fie creat monopolul tutunului de către Napoleon, care, astăzi, cunoaşte o dezvoltare fără precedent.

În anul 1975, Chicon arăta faptul că, în Franţa, 48% din fumători consumă 10-20 ţigarete zilnic, iar 7,5% fumează peste 30 de ţigarete pe zi.

În anul 1985, 18 milioane de fumători, din care 82% bărbaţi şi 32% femei, au consumat 94,7 miliarde de ţigări. Tot în acel an, s-a constatat că, printre cauzele de deces, locul 5 era ocupat de BPOC, procentul decedaţilor din această cauză fiind, în 1970, de 1,6% la bărbaţi şi 0,9% la femei. Anual, numărul deceselor cauzate de fumat, este de 3 ori mai are decât cele produse prin accidente rutiere. Tot anual se înregistrează 20 000 cazuri de cancer bronhopulmonar, precum şi peste 30.000 de bronşitici cronici care decedează datorită fumatului iar din totalul fumătorilor 25% mor prin infarct miocardic acut.

În anul 1990, o treime din populaţia Franţei fuma, 40% din bărbaţi şi 27% din femei, fumatul fiind responsabil de 60 000 de decese pe an, dintre care 3 000 de femei. Chiar şi din rândul salariaţilor Institutului Francez de Cancer au fost înregistraţi 21% bărbaţi şi 30% femei fumători obişnuiţi, 18% medici fumători şi 23% ex-fumători, iar printre studenţii din anul I la medicină au fost identificaţi 11% fumători ocazionali şi 25,5% fumători obişnuiţi, vârsta debutului fiind de 14,2 ani. Dintre aceştia din urmă, după 6 luni de la primirea unei scurte informări asupra pericolelor pe care le implică consumul de tutun, 32,8% au încercat să întrerupă fumatul.

În anul 2010, primele rezultate ale Barometrului de sănătate publicate de către *Institut national de prévention et d'éducation pour la santé (INPES)* sunt consacrate evoluţiei prevalenţei tabagismului în Franţa. Cifrele arată prima creştere semnificativă a tabagismului de la adoptarea Legii Evin. Astfel, scăderea tabagismului observată la bărbaţi, începând cu anii '70, rămâne constantă. În schimb, în perioada 2005-2010 se constată o creştere a tabagismului la femei, mai ales la cele născute între anii 1945 şi 1965. Prevalenţa consumului de tutun la fumătoarele zilnice, cu vârste între 45-64 ani, a cunoscut o creştere de 7%, de la 16%, în anul 2005, la 22,5%, în anul 2010.

24% dintre femeile însărcinate se declară fumătoare zilnice în cursul sarcinii. Această cifră rămâne stabilă din 2005, reprezentând un semn mai degrabă pozitiv, în contextul general al creşterii consumului de tutun. Populaţia tânără a Franţei deţine recordul european la fumat, aceasta şi datorită faptului că nu există nicio politică educaţională care să descurajeze fumatul, consecinţă directă a sumelor mici de bani alocate pentru combaterea acestui viciu.

6.3. Consumul de tutun în alte ţări din Europa

Cu toate că statele Comunităţii Europene recunosc pericolul consumului de tutun, tot ele sunt cele mai mari importatoare de ţigări, ţările dezvoltate închizând pieţele autohtone şi creând desfacere masivă în ţările în curs de dezvoltare.

În ţările Europei de Est, vestul pătrunde prin reclame, importul de tutun aducând bani, producând modificări legislative şi *"lobby"* în parlamente, alături de creşteri ale incidenţei bolilor provocate de fumat şi moarte prematură. Este evocatoare în acest sens deviza marilor concerne R.J.Reynolds şi Ph. Morris: *"urăsc fumatul, dar iubesc banii"*.

Să urmărim câteva aspecte particulare din diferite ţări ale Europei:

-*în Italia*, din rândul personalului sanitar sunt fumători 20-43% bărbaţi şi 22-44% femei, medicii fumând mai mult decât populaţia;

-*în Finlanda*, unde legislaţia antifumat a dat rezultate, din populaţia care fumează, 8-10% sunt bărbaţi şi 6% sunt femei. Prevalenţa la medici este de 60%, la asistentele medicale de 79,6% iar la personalul care lucrează în sectorul administrativ de 68%, medicii tineri fumând mai puţin decât cei vârstnici. În Finlanda, în perioada 1965-1989, prevalenţa fumatului este prezentată în următorul tabel:

Prevalenţa tabagismului în Finlanda

Sex (%)	**1965**	**1970**	**1975**	**1980**	**1985**	**1989**
Bărbaţi	57	44	40	37	35	34
Femei	14	16	17	16	17	20

Sursa: date OMS

-*în fosta Cehoslovacie,* unde există 39% fumători în rândul populaţiei, 33% sunt fumători medici şi 49% asistente medicale, iar grupa cu riscul cel mai mare de debut al fumatului este vârsta de 15-18 ani ;

-*în Polonia,* la sfârşitul deceniului opt al secolului, şapte întreprinderi de stat producătoare de tutun controlau 95% din piaţa internă şi doar 5% era cota firmelor străine (Tomasz Sienivtycz). Ulterior, producţia autohtonă a scăzut cu 10% în favoarea importului, devenind, după anul 1990, una dintre cele mai căutate pieţe de desfacere a firmelor străine producătoare de tutun. În anul 1970 Polonia a înregistrat cel mai mare consum de ţigări din lume (peste 3 500 ţigări/persoană/an), pentru ca la sfârşitul aceluiaşi an să scadă datorită crizei economice. În aceeaşi perioadă, fumătorii polonezi cu vârste cuprinse între 20 şi 60 ani reprezentau 60-75%. În intervalul 1972-1982 rata femeilor fumătoare a crescut de la 20 la 30%, iar în anii 1981-1983, când muncitorii primeau ţigări, indiferent dacă erau sau nu fumători, la femeile tinere rata fumatului a crescut la 50%. În 1990 apare *Declaraţia Kazimierz*, care stipulează reguli stricte cu privire la comercializarea produselor de tutun: preţuri minime, reguli pentru apariţia reclamelor, educarea publicului, în special a tinerilor, cu privire la efectele nocive ale consumului de tutun. Urmare a acestei politici, în anii '90 consumul de tutun scade pentru prima dată după cel de-al doilea război mondial, apoi, pâna în anul 1998, cu încă 10%, în ciuda alocării de către companiile de tutun a unei sume de 100 milioane dolari anual pentru reclame la produsele de tutun. Polonia continuă politica antitabac, astfel că în anul 2000 Parlamentul a votat noua lege antifumat, alocându-se 0,5% din produsul brut pentru aceste activităţi. Dacă în anul 1970, în Polonia erau 14 milioane de fumători (62% bărbaţi şi 32% femei), în anul 1990 numărul lor a scăzut la 10 milioane (40% bărbaţi şi 20% femei) ;

- *Suedia* este singura ţară din Europa în care datorită campaniilor riguroase antifumat prevalenţa fumătorilor este în scădere, ajungându-se astăzi la un procent de sub 20% fumători ;

- *Irlanda* este prima ţară din Europa care interzice reclamele pro-tutun, mergând până la “prohibirea totală a fumatului în orice loc public, de muncă sau de distracţii” ;

- *în Ungaria,* un studiu al misiunii WHO din 1997, denumit “Tutunul sau sănătatea”, trage un semnal de alarmă: rata mortalităţii din cauza tutunului este cea mai ridicată din Europa, aproape cea mai mare din lume, în fiecare an decedând, din cauza bolilor asociate fumatului, peste 30 000 de unguri, situaţie comparabilă, din acest punct de vedere, cu cea din Africa subsahariană (1/3 dintre bărbaţi şi 45% dintre decedaţii de vârstă mijlocie). La începutul anului 1990 consumul de ţigări/locuitor era de 52,9% la bărbaţi şi de 31,2% la femei. În anul 1999 numărul fumătorilor în rândul femeilor crescuse la 31,2%, de la 22%, în anul 1986. Un sondaj efectuat de CDC în 1996 demonstrează că jumătate dintre elevii cu vârsta de 18 ani erau fumători. De asemenea, în anul 1998, s-au cheltuit pentru reclame şi publicitate pro-tutun aproape 10 milioane USD, sumă de 60-70 ori mai mare decât fondurile acordate anual, pentru controlul asupra tutunului, Ministerului Sănătăţii. După anul 1999 se trece la adoptarea unor legi de control al tutunului şi de combatere a fumatului, Ungaria reuşind, sub îndrumarea OMS, să creeze, pe scheletul Societăţii Respiratorii Maghiare, un program de sevraj şi un număr mare de centre de asistenţă a fumatului.

6.4. Evoluţia pandemiei tabagice pe glob

În situaţia în care nu se iau măsuri eficiente de combatere a fumatului şi se păstrează ritmul actual al consumului de tutun, urmările tabagismului vor constitui o catastrofă mondială. Există o rapidă escaladare a numărului de fumători şi a dependenţei tabagice pe întreg mapamondul. În anul 1994, la deschiderea celei de-a 2-a Conferinţe Mondiale asupra Tutunului şi Sănătăţii, dr. Hiroshi Nakajima, directorul general OMS, declara că: “*tabagismul nu este o epidemie ca oricare alta, în fiecare an, în lume, se produce un deces la fiecare 10 secunde. Dacă ritmul actual al epidemiei se menţine, în 30-40 de ani tutunul va ucide 10 milioane de persoane pe an”*.

Anastasatu făcea observaţia că punctul critic al extinderii fumatului este de 50%, sub acest procent există şanse de limitare a flagelului printr-o luptă susţinută.

Între anii 2020-2030, se estimează că vor fi circa 10 milioane decese din cauza fumatului, 70% dintre acestea înregistrându-se în ţările dezvoltate. Altfel spus, 1 din 6 fumători va muri, iar 7 din 10 decese vor fi din rândul persoanelor cu venituri mici şi mijlocii. În anul 2025, la nivelul planetei, numărul deceselor prin boli cauzate de fumat va fi de circa 8,4 milioane.

Dacă în ţările dezvoltate tabagismul a înregistrat scăderi în rândul adulţilor, industria tutunului s-a orientat rapid către alte pieţe, prevalenţa tutunului crescând în ţările în curs de dezvoltare, în special pe seama sporirii numărului de femei fumătoare. David Byrne, comisar european pentru problemele sănătăţii, afirma că: *“în ţările Uniunii Europene, companiile de tutun internaţionale sunt nevoite să recruteze anual 500 000 noi fumători care să-i înlocuiască pe cei ce mor prematur din cauza bolilor provocate de fumat”*.

În anul 2025, numărul fumătorilor va fi, probabil, de 1,6 miliarde.

Spre exemplu, dacă nu se ia nicio măsură, în Franţa, până în anul 2025 numărul celor morţi din cauza fumatului va creşte de la 60 000/an la 165 000/an, dintre care 55 000 femei. De asemenea, în Uniunea Europeană se vor înregistra 1 100 de morţi/zi.

O treime dintre adulţii tineri tind să devină fumători permanenţi, în ţările industrializate, provocând decesul prematur a circa jumătate din numărul de persoane cu vârste cuprinse între 35 şi 69 de ani; jumătate din ei vor muri după vârsta de 70 ani, scurtându-şi speranţa de viaţă cu aproximativ 8 ani. Dacă această tendinţă continuă, peste 10% din cei 120 milioane de tineri care au atins vârsta adultă vor muri anual prematur.

Se estimează că până în anul 2020, numărul deceselor anuale prin boli cauzate de fumat va ajunge la 10 milioane, iar 500 milioane de persoane aflate astăzi în viaţă vor deceda, număr echivalent cu 9% din populaţia globului. Studiul *Global Burden of Disease* apreciază că, tot până în anul 2020, BPOC va fi responsabilă pentru mai mult de 6 milioane de decese pe an, această boală devenind a 3-a cauză de deces din lume şi a 5-a cauză de invaliditate.

Karl Oloff Fagerström, directorul Centrului de Informaţii asupra fumătorilor din Helsinborg (Suedia) şi autorul celebrului chestionar care estimează dependenţa de nicotină, aprecia că: *"în anul 2000, o singură ţară europeană va ajunge la un nivel de 20% al prevalenţei fumătorilor, printr-o activitate susţinută şi sistematică de reducere a fumatului şi aceasta va fi Suedia",* ceea ce s-a şi întâmplat. Dar, în Suedia, mortalitatea imputabilă fumatului era mică oricum.

Este cunoscut faptul că fumatul constituie o gravă ameninţare la adresa sănătăţii omului. Statistic, un mare fumător îşi plăteşte această *"plăcere"* cu 8,3 ani din viaţă iar un fumător moderat pierde 4,6 ani din durata medie a vieţii. În schimb, la cei ce renunţă la fumat, după aproximativ 10 ani de la abandonarea acestui viciu, decesul survine în aceleaşi proporţii ca la nefumători.

Prejudiciul asupra îmbolnăvirilor produse de tutun este mult mai profund decât arată statisticile, fiind la fel de nefast atât asupra sănătăţii, cât şi asupra economiei mondiale. Un economist al Băncii Mondiale a estimat pierderile anuale nete datorate tabagismului la 200 miliarde de dolari, din care jumătate în ţările în curs de dezvoltare. Fiecare creştere a consumului de tutun cu 1 000 de tone se reflectă prin pierderi nete de 27,2 milioane de dolari pentru economia mondială. Cu un consum mondial anual de tutun care depăşeşte 7 milioane de tone, consum aflat în continuă creştere, amploarea pierderilor economice este uriaşă, fiind egalată şi chiar depăşită de suferinţa şi bolile produse de fumat.

6.5. Consumul de tutun în România

Romania are o populaţie estimată la 22,4 milioane locuitori, cu o creştere în 1980, apoi încă una, de 2,2 % în perioada 1985-1990, urmând o diminuare prin emigrare şi scăderea natalităţii cu menţinerea mortalităţii (în anul 2000 sporul natural la 1 000 locuitori era de 0,95 ‰, natalitate - 10,45‰, mortalitate - 11,4‰).

Date generale

Datorită producţiei industriale masive a ţigaretelor şi a generalizării obiceiului de a fuma ca urmare a emancipării populaţiei, mai ales după cel de-al doilea război mondial, consumul de tutun din România a crescut rapid:

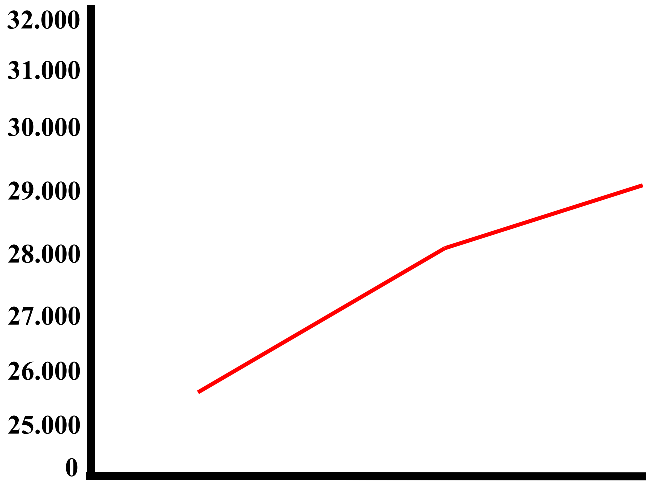

Consumul de tutun în România (date de la Centrala de industrializare a tutunului)

Barnea şi colab. arată că în anul 1978, conform datelor furnizate de Centrala de industrializare a tutunului, consumul mediu de tutun pe o persoană mai mare de 15 ani este estimat la 1,82 kg, cu variații pe județe între 0,98 kg şi 2,58 kg:

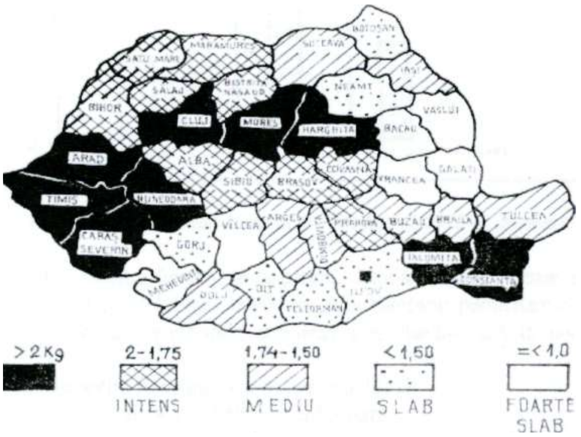
BOTOȘANI
MARAMUREȘ
SATU MARE
SUCEAVA
SĂLAJ
BISTRIȚA NĂSĂUD
NEAMȚ
BIHOR
CLUJ
MUREȘ
HARGHITA
VASLUI
BACĂU
ARAD
ALBA
COVASNA
GALAȚI
TIMIȘ
HUNEDOARA
SIBIU
BRAȘOV
VRANCEA
TULCEA
CARAȘ SEVERIN
VÎLCEA
ARGEȘ
PRAHOVA
BUZĂU
BRĂILA
GORJ
IALOMIȚA
CONSTANȚA
MEHEDINȚI
DOLJ
OLT
TELEORMAN
> 2 Kg
2-1,75
INTENS
1,74-1,50
MEDIU
< 1,50
SLAB
=< 1,0
FOARTE SLAB

Consumul de tutun în România pe județe în 1978
(date de la Centrala de industrializare a tutunului)

În anul 1957 România producea cca. 20 000 tone de tutun iar, la începutul lui 1980, producția de tutun în România, la o suprafață cultivată cu tutun de aproximativ 40 000 ha, era de aproape 40 000 tone, din care jumătate era exportată, importând la rândul nostru tutun din Bulgaria, China, Albania etc.

În anul 1970, tot Barnea şi colab., într-o anchetă pe 37 050 de persoane din 12 localități rurale şi semirurale, au constatat un procent de 27,4% la bărbații fumători şi de numai 0,32% la femeile fumătoare iar, la bărbați, 40,7% reprezentau vârste peste 20 de ani şi numai 7,1% sub 20 de ani:

Frecvența fumatului în mediul rural şi semirural

Autorul	Nr. persoane	Vârsta	Mediul	Fumători %	
				Bărbați	Femei
M. Barnea 1974	37.050	peste 15 ani	12 localități rurale şi	27,4	0,32
			semirurale	>20 = 40,7	
V. Golli 1971	2.942	peste 15 ani	Craiova	< 20 = 7,1	
P. Manu 1970	1.000	30-59 ani	Bucureşti	33,3	4,3
St. Duțu 1972	1.804	peste 40 ani	Bucureşti	36,5	11,9
E. Barnea 1980	11.328	adulți	Slatina	33,5	9,2
			Piteşti	34,5	3,9
			Petroşani	49,1	12,9
			Hunedoara	54,7	18,1
				56,1	10,3

Sursa: *Pneumoftiziologia, 1994, 3-4*

Golli, în anul 1971, a obținut date asemănătoare pe un lot de 2 942 persoane de ambele sexe, în vârstă de peste 15 ani: 33,3% bărbați fumători şi 4,3% femei fumătoare. În Bucureşti, P. Manu constata o frecvență a fumatului de 36,5% la bărbați, cu vârste cuprinse între 30 şi 59 de ani, şi de 11,9% la femeile de aceeaşi vârstă.

În anul 1972, Ştefan Duțu, pe un lot de 1 804 persoane, obține date asemănătoare, studiind vârstele de peste 40 ani: 33,5% bărbați fumători şi 9,2% femei fumătoare.

După anul 1975 consumul de tutun în România cunoaşte o creştere rapidă. Astfel, în anul 1980, pe un lot de 11 328 de adulți din 4 oraşe, Elena Barnea constată proporții cu mult mai mari, mai ales în rândul bărbaților şi mai ales în centrele puternic industrializate: Bucureşti, Hunedoara, Petroşani. La Petroşani, din 828 de salariați ai unei întreprinderi, proporția fumatului la bărbați era de 53,2% şi la femei de 21,9%. În anul 1980, în Valea Jiului, aceiaşi autori constatau o frecvență ridicată comparativ cu restul localităților din țară. Paralel cu creşterea frecvenței fumătorilor creşte şi intensitatea fumatului. E.

Barnea şi colab. ajunge la această concluzie comparând, în perioada 1979-1980, numărul de ţigări consumate/zi de fumători, faţă de perioada 1969-1971:

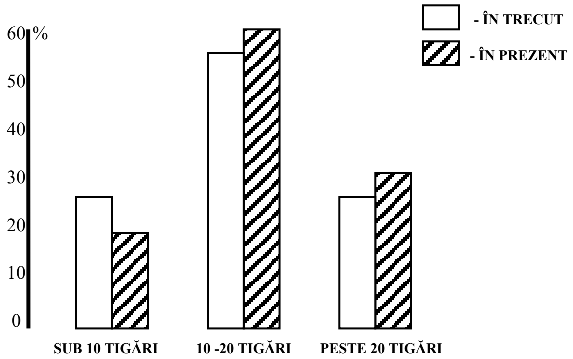

Intensitatea fumatului în 1980 faţă de 1972 (E. Barnea)

După 1989, în ţara noastră fumatul ia amploare datorită acţiunii publicitare efervescente a marilor concerne de ţigarete, cointeresate material, precum şi ca urmare a contraofensivei timide duse de organizaţiile antifumat care funcţionează la noi: de ex. AMETOS şi Liga antitabagică - Cluj.

În anul 2004, statisticile pentru România sunt dezamăgitoare:

- 46,4% dintre bărbaţi şi 24,1% dintre femei fumează;
- 64% dintre persoanele sub 16 ani fumează, cu o creştere de 11% faţă de anul 1999, simultan cu scăderea vârstei de debut;
- 32% dintre elevi au fumat prima ţigară înainte de 14 ani.

Consumul de ţigarete în perioada 1990-2001 a crescut îngrijorător, conform datelor din următoarele tabelele:

Consumul de ţigarete din perioada 1990-2001 în România

	Milioane de bucăți	% schimbare anual	Index 1990-100.0
1990	18190	-18,1	100.0
1991	25780	+41,7	141.7
1992	27220	+5,6	149.6
1993	29760	+15.4	163.6
1994	29530	+0.8	162.3
1995	30500	+3,2	167.7
1996	31860	-4,5	175.2
1997	30509	-4,2	167.7
1998	33740	+10,6	185.5
1999	22070	-19,8	148.8
2000	30710	+13,4	168.8
2001	32250	+5,0	177.3

Sursa: *Estimări ERC bazate pe vânzări înregistrate*

Consumul per capita de ţigarete în România în perioada 1990-2001

	Populație (milioane)	**Bucăți /zi**	**Bucăți/săpt.**	**Bucăți /an**	**Schimbare (%)**	**Index 1990-100.0**
1990	23,21	2,1	15,0	784		100,0
1991	23,19	3,0	21,3	1112	+14,3	141,8
1992	22.79	3,3	22,9	1194	+7,3	159,3
1993	22,76	3,6	25,1	1308	+9,5	166,8
1994	22,73	3,6	24,9	1299	-0,7	165,7
1995	22,68	3,7	25,8	1345	+3,5	171,6
1996	22,61	3,9	27,0	1409	+4,8	179,7
1997	22,55	3J	25,9	1353	-4,0	172,6
1998	22,50	4,1	28,8	1500	+10,9	191,3
1999	22,46	3,3	23,1	1205	-19,7	153,7
2000	22,41	3,8	26,3	1370	+13,7	174,7
2001	22,36	4,0	27,7	1442	+5,3	183,9

Sursa: *Estimări ERC*

În România (din 1989 până în 2001), prevalența fumatului a crescut de la 25,9% la 36%, numărul femeilor fumătoare fiind în ascensiune cu aproximativ 10%, consumându-se anual 2 000 țigarete per capita.

În anul 1995, Biroul Regional al OMS pentru Europa consemnează că numărul fumătorilor în România era de aproximativ 43% (16% la barbați şi 25% la femei), iar în anul 2004 se ajunsese la 44%.

În anul 2001, România ocupa un loc fruntaş în privința mortalității: 63,08%$_{000}$ (87,48%$_{000}$ la sexul masculin şi 44,02%$_{000}$ la sexul feminin). În utimele statistici, ocupă locul II în Europa, ca număr de fabrici de țigarete, ce produc o multitudine de mărci pe piață. Conform unor studii, la noi în țară fumează aproape 50% dintre bărbați şi peste 15 % dintre femei, procent în continuă creştere:

Populația fumătoare în perioada 1995-2000 din România

	1995	**1996**	**1997**	**1998**	**1999**	**2000**
Populația totală	22,68	22,61	22,55	22,50	22,46	22,41
(milioane) -	8,75	8,78	8,80	8,81	8,82	8,84
Bărbați adulți	9,29	9,33	9,37	9,39	9,42	9,45
(+15)	**18,04**	**18,12**	**18,17**	**18,20**	**18,23**	**18,28**
Din care fumători						
(%) –	42,7					48,0
Bărbați adulți (+15)	15,2					25,0
Femei adulți (15+)	**28,5**	**31,2**	**34,0**	**34,7**	**35,4**	**36,1**
Număr de fumători						
(milioane) –	3,74					
Bărbați adulți (+15)	1,41					
Femei adulți (15+)	**5,15**	**5,66**	**6,18**	**6,32**	**6,46**	**6,60**

Sursa: Estimări ERC bazate pe date demografice UN/USCB

Conform studiilor OMS, tot la 4 ani, în țara noastră se înregistrează câte 100 000 de decese provocate de fumat sau, aşa cum am amintit, în anul 1995 *"în România pierdem, tot la 5 ani, câte 150 000 de oameni în plină vigoare, victime ale unei deprinderi de care s-au legat din pură naivitate...".*

În anul 1990, în România, din totalul de 62 000 de decese survenite la bărbații cu vârste cuprinse între 35 şi 69 de ani, 32% (aproximativ 20 000 de decese) au fost atribuite fumatului iar, dacă generalizăm, 11% din decesele de toate cauzele (aproximativ 247 000) survenite în acest an, în țara noastră, sunt generate de fumat. La un număr de 46 000 de decese datorate cancerului pulmonar, 4 200 sunt generate de fumat, iar din cele aproximativ 27 000 de decese de cauză vasculară, în 8 100 a fost incriminat fumatul.

Tot în anul 1990, 24% din totalul cancerelor şi 11% din totalul deceselor se datorau fumatului:

România: decese în anul 1990, toate vârstele.
Număr de decese atribuite fumatului (total decese - mii)

	Barbați	**Femei**	**Barbați + femei**
Toate cancerele	7,3/19 (39%)	0,6/14 (4%)	7,8/33 (24%)
Toate cauzele	24/132 (18%)	2,9/115 (3%)	27/247 (11%)

Sursa: Didilescu, Marica

În anul 1994, în România, conform unei anchete a Centrului Național de Statistică Medicală a Ministerului Sănătății, prevalența fumatului era de 28% la populația cu vârsta de peste 15 ani inclusiv (în creştere față de 25,9%, cât se înregistra în anul 1989) iar pe sexe, prevalența era de 42,7% la bărbați şi 15,2% la femei.

Studiul populațional realizat în anul 2003 de către Centrul pentru Politici şi Servicii de Sănătate, pe un eşantion reprezentativ la nivel național, format din 1 209 persoane, cu vârste cuprinse între 15 şi 59 ani, a arătat că mai mult de 1/3 din populație (35,1%) este reprezentat de persoane fumătoare. Acestea sunt persoane care au fumat cel puțin 100 de țigări în viața lor şi care în ultima lună au fumat zilnic sau ocazional.

Referitor la statutul de fumător, există o diferență foarte mare pe sexe - aproape ½ (46,4%) dintre bărbații cu vârste cuprinse între 14 şi 60 ani este reprezentată de fumători, față de 24,1% dintre femeile din aceeaşi categorie de vârstă. 12,7% din procentul total (7,95% dintre femei şi 17,7% dintre bărbați) sunt persoane care au fost încadrate la categoria ex-fumători. Persoanele din această categorie au fumat în viața lor mai mult de 100 de țigări, dar la momentul realizării studiului (în ultima lună) nu au fumat deloc, deoarece s-au lăsat de fumat. Nefumătorii reprezintă 52,1% dintre persoanele incluse în lot (68% dintre femei şi 35,8% dintre bărbați). Această categorie este formată din persoane care nu au încercat niciodată să fumeze sau, dacă au încercat acest lucru, nu au fumat mai mult de 100 de țigări pe parcursul vieții.

În anul 2007 a fost repetat studiul din anul 2003, realizat de către Centrul pentru Politici şi Servicii de Sănătate, pe un eşantion de 2 434 persoane cu vârste cuprinse între 15 şi 59 ani, din mediul urban şi rural, reprezentativ la nivel național. Studiul a arătat că aproape 1/3 din populația vizată de studiu (30%) este reprezentată de persoane fumătoare (persoane care în ultima lună au fumat zilnic sau ocazional). Cea mai mare prevalență a fumatului se întâlneşte la grupele de vârstă 15-24 ani (34,8%) şi 25-34 ani (32,9%). Cea mai mare parte a fumătorilor (61,6%) fumează între 10 şi 20 țigări/zi. O persoană din 10 fumează mai mult de 20 de țigări/zi, 3 din 5 persoane au încercat măcar o dată să fumeze în viață. Din totalul fumătorilor 58% au declarat că doresc să se lase de fumat, iar aproape ½ dintre fumători a încercat să se lase de fumat cel puțin o dată în viață.

Fumatul la tineri în România

Un studiu al profesorilor R. Vlaicu şi Şt. Hăgăuş, efectuat în anul 1972, pe un lot de 1 900 de elevi şi studenți din Cluj, arată o medie a fumătorilor de 29% la băieți şi 17% la fete, cu următoarea distribuție pe grupe de vârstă: 18% între 14 şi 16 ani, 21% între 16 şi 18 ani şi 34% între 18 şi 25 de ani.

În anul 1979, pe un lot de 1 170 de studenți din Petroşani şi 1 860 de studenți din Braşov, E. Barnea şi colab. evaluează frecvența fumatului la băieți, la 48,2%, respectiv 50%, iar la fete, la 27,7% şi, respectiv, la 32,7%. Tot ei semnalează apariția fumatului la copiii de vârsta pubertății (10-14 ani). Un studiu din anul 1981, efectuat în trei şcoli din capitală, arată că 43,2% din băieți şi 27,4% din fete au încercat să fumeze, iar 11,1% şi respectiv 4,4% fumează aproape sistematic, față de un deceniu în urmă când V. Golli şi E. Barnea constatau că băieții sub vârsta de 15 ani fumau în proporție de 5,8%, respectiv 7,3%, iar fetele fumau 1,5% şi respectiv 1,2%.

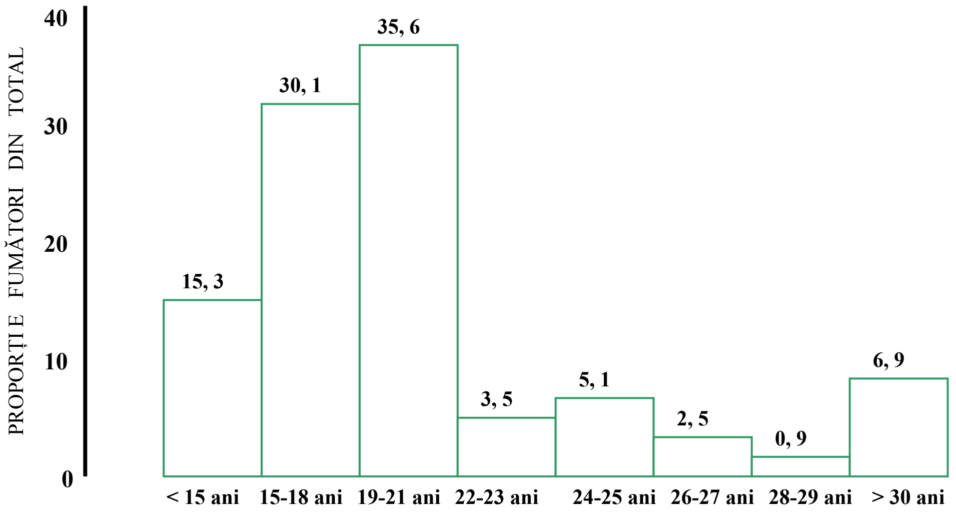

Vârsta de debut a fumatului (E. Barnea)

În anul 1987, P. Mihăilescu arăta, într-o anchetă personală pe 247 de elevi din clasa a X-a şi 284 de elevi din clasa a XII-a, de la trei licee din Bucureşti, frecvența fumătorilor în rândul acestora iar, în acelaşi an, o altă anchetă s-a adresat unui lot de 701 studenți din Cluj şi Timişoara:

Fumatul în rândul elevilor şi studenților

An de studii (clasa)	Nr. persoane	Fumători %		
		băieți	fete	total
Cls. a X-a, Bucureşti	*247*	*14,0*	*6,4*	*7,7*
Cls. a XII-a, Bucureşti	284	34,2	12,3	21,4
Anul I, Cluj, Timişoara Anul V,	395	25,9	11,7	5,4
Cluj, Timişoara	306	43,0	26,8	31,8

Sursa: Pneumoftiziologia, 1994, 3-4

În anul 1995, o anchetă a Institutului de Pneumoftiziologie asupra unui lot de 8 117 elevi din clasele a Vlll-a, a X-a şi a Xll-a, cu vârste cuprinse între 14-18 ani, din nouă reşedinţe de judeţ (Vaslui, Constanţa, Hunedoara, Maramureş, Caraş-Severin, Dolj, Vâlcea, Dâmboviţa) şi Bucureşti, arată o prevalenţă a fumatului de 2,8% la clasa a VIII-a (3 137 elevi), 12,3% la clasa a X-a (3 299 de elevi) şi de 28.5% la clasa a Xll-a (2 381 de elevi).

După un alt studiu, la copiii între 8-14 ani se înregistrează 10,1% fumători, proporţia băieţi/fete fiind de 4/1, aceştia alegându-şi exemplele din rândul părinţilor (98% fumători) şi prietenilor (50% fumători).

În anul 1997 Ciufecu, în urma unui sondaj de tip statistic aleator nerepetat, făcut cu ajutorul unui chestionar de tip cognitiv-comportamental, pe un grup de 123 de elevi din clasele IX-XII ale Liceului *"Jean Monet"* Bucureşti, cu rezultatele prelucrate pe calculator, a concluzionat următoarele:

- 39,8% din totalul tinerilor chestionaţi (49 de elevi) sunt fumători;
- procentual, fetele fumătoare îi domină pe băieţii fumători: 40,5% faţă de 38,6%;
- 42% din băieţi au *"gustat"* prima ţigară sub 14 ani, iar 80,4% dintre fete au început *"aventura"* între 14 şi 17 ani;
- jumătate dintre fete au renunţat apoi la fumat, rămânând 40,5% fumătoare consecvente;
- motivele pentru care aceştia au început să fumeze sunt: 59,2% din curiozitate, 14,3% pentru integrare, 12,2% din cauza modei;
- peste 57,1% din tinerii fumători provin din familii în care ambii părinţi fumează, iar 28,6% au şi fraţi sau surori care fumează;
- 59,2% din aceştia au ambii părinţi intelectuali şi 91,8% din părinţii fumătorilor au funcţii de conducere;
- 26% din elevi fumează pe ascuns, 90% din ei aşteptându-se la mustrări din partea părinţilor, dacă i-ar depista;
- cantitativ, fetele fumează mai puţin decât băieţii: 43,7% din ele fumează sub 5 ţigări/zi, 35% din băieţii fumători fumează 10-20 de ţigări/zi.

Un studiu similar efectuat pe un lot de 125 de elevi (57 fete şi 68 de băieţi), cu vârste cuprinse între 13-14 ani, din două şcoli elementare din Constanţa, completează şi aduce noi elemente:

- 27,2% sunt atraşi sau tentaţi de fumat, 62% au fumat din curiozitate, 18% au aflat de la confraţii fumători că ţigara îi dă individului o notă de personalitate şi teribilism, iar 20% şi-au găsit consolarea în ţigară în urma unor satisfacţii sau decepţii personale;
- 11,2% fumează ocazional: când au bani, când li se oferă ţigări la diverse evenimente;
- privind informaţia despre nocivitatea tutunului, 55% din elevi au fost informaţi în familie, 40% în şcoală şi 5% din mass-media;
- 35% din elevii din lotul studiat cumosc faptul că tutunul este cancerigen, numai 10% ştiu că fumatul generează şi alte boli şi numai 6% pot atribui simptome personale tutunului.

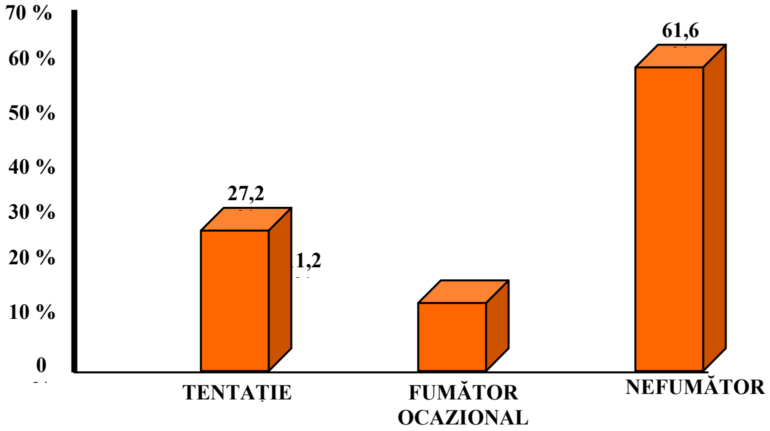

Atitudinea faţă de consumul de tutun

Din acest studiu rezultă următoarele concluzii:

-la 13-14 ani se fumează masiv în rândurile tinerilor;

-nici familia, nici şcoala şi nici mass-media, nu oferă suficiente noţiuni privind nocivitatea imediată şi consecinţele îndepărtate ale fumatului;

-toate localurile publice în care tinerii îşi petrec timpul liber sunt poluate cu tutun.

După anul 1990, adolescenţii ocupă ţinta numărul doi a marilor concerne de ţigări, ca o consecinţă a reclamelor pro-fumat, 11% dintre elevii claselor mici şi până în clasa a XII-a încercaseră deja o ţigară în momentul efectuării studiului (1994), iar 4% din cadrele didactice fumau în prezenţa acestora.

În anul 1995, C. Didilescu arată că prevalenţa fumatului în rândul elevilor de 14 ani este de 2,8%, ea crescând la 12.3% la vârsta de 16 ani şi la 28,5% la vârsta de 18 ani.

În anul 1999, un studiu ESPAD, desfăşurat în rândul elevilor de 16 ani, arată că 18% erau fumători, 19% fumători ocazionali, 57% au fumat cel puţin o dată şi 42,7% nu fumaseră niciodată.

Interesante sunt şi concluziile studiului efectuat în anul 2000 de Institutul Naţional de Statistică:

-populaţia cu vârstă peste 15 ani fuma zilnic în procent de 21%, 4% renunţaseră la fumat;

-în intervalul de vârstă 15-24 ani fumau 18,8% dintre băieţi şi 8,8% dintre fete, 10,4% devenind fumători înainte de 15 ani, iar 71,4% dintre aceştia între 15 şi 19 ani;

-5,5% din totalul fumătorilor au început să fumeze înainte de 15 ani şi 47,5% între 15 şi 19 ani;

-în mediul urban, înainte de 15 ani, au intrat în rândul fumătorilor 5,2%, iar 45,6% au început să fumeze între 15 şi 19 ani. Realitatea este că în colectivităţile şcolare şi studenţeşti are loc o contaminare masivă, o adevărată epidemie tabagică, contribuind la întreţinerea şi amplificarea pandemiei tabagice.

Fumatul în rândul personalului medical din România

Din această categorie fac parte medicii, asistenţii medicali, farmaciştii, profesorii şi studenţii facultăţilor de medicină.

În anul 1972, C. Anastasatu şi colab., într-un studiu efectuat pe un lot de 634 de studenţi în medicină din Bucureşti, constatau că 59,8% dintre băieţi şi 17,4% dintre fete erau fumători obişnuiţi, iar 13,9% dintre băieţi şi 28% dintre fete erau fumători ocazionali.

Studiul lui V. Golli de la Craiova, efectuat pe un număr de 3 290 de studenţi în medicină, arată că frecvenţa globală a fumătorilor era de 28,5%, iar raportul băieţi/fete fumători este de 4/1.

P. Mihăilescu, în perioada 1988-1991, efectuând o anchetă pe un lot de 114 medici generalişti, rezidenţi în pneumologie, constată următoarele:

- dintre aceştia, 54 (47,4%) erau fumători, jumătate începând să fumeze înainte de debutul studiilor medicale;
- 1/3 au început să fumeze în cursul facultăţii, iar 16,4% după ce au devenit medici;
- există o progresie a fumatului, de la 15,4%, în primul an de studii medicale, la 31,8%, în anul V, ajungând la 47,4%, la tinerii medici, după 5-10 ani de la terminarea studiilor;
- deşi la absolvirea cursurilor de pneumologie medicii ştiau riscurile la care predispune fumatul, doar 5,6% din fumători au abandonat fumatul şi 9,3% au redus cantitatea de ţigări fumate zilnic.

În anul 1995, o echipă de medici (G. Petrescu, I. Baican, T. Drugan) a efectuat o anchetă în rândul medicilor din 11 secţii din Cluj-Napoca. Cei 219 medici au fost repartizaţi pe sexe (103 bărbaţi şi 116 femei), apoi pe categorii de vârstă: sub 30 de ani (91 persoane), între 30 şi 50 de ani (62 persoane), între 51 şi 70 de ani (59 persoane), apoi pe specialităţi, incluzând şi medicii stagiari generalişti (103 persoane). Concluziile acestei anchete au fost următoarele:

- majoritatea absolută a medicilor (99,8%) consideră fumatul ca fiind dăunător sănătăţii;
- toţi consideră că pacienţii trebuie avertizaţi întotdeauna cu privire la pericolul fumatului asupra sănătăţii (92,12%);
- există relaţii tutun-sănătate insuficient cunoscute de medici, de exemplu decesul neonatal în tabagismul parturientei;

- există diferenţe pe clinici în ceea ce priveşte numărul de ţigări fumate zilnic de către medici, primul loc ocupându-l chirurgii (92,86%), oftalmologii (90%), dermatologii (88,89%), ultimul loc fiind atribuit secţiilor de recuperare (23%), media fiind de 6,05 ţigări/zi, per ansamblu, prevalenţa fumatului ridicându-se la 58,9% (55,24% la bărbaţi şi 61,4% la femei):

Repartiţia medicilor pe sexe şi specialităţi

Specialitatea	Total	Total fumători	%	Bărbaţi	Femei	Fumători	%	Fumătoar	%
Boli lnfectioase	12	6	50	3	7	3	60	3	42,86
Boli	10	8	80	5	7	2	66,67	6	85,71
Chirurgie	14	13	92,86	8	6	7	87,5	6	100
Dermatologie	9	8	88,89	5	4	4	80	4	100
Medicală	15	10	66,67	8	7	5	62,5	5	71,43
Oftalmologie	10	9	90	5	5	4	80	5	100
ORL	19	12	63,16	8	11	3	37,5	9	81,82
Recuperare	13	3	23,08	7	3	3	42,86	0	0
Radiologie	13	8	61,54	6	7	3	50	5	71,43
Stagiari	103	49	47,57	46	57	22	47,83	27	47,37
Urologie	4	2	50	4	0	2	50	0	?
TOTAL	219	129	58,90	105	114	58	55,24	70	61,40

Sursa: Pneumoftiziologia, 1991, 3-4

-funcţie de mediul de provenienţă s-a constatat că cei născuţi în oraşe mari fumează mai mult (5,1 ţigări/zi) decât cei proveniţi din mediul rural (3,1 ţigări/zi) sau din oraşe mai mici (3,2 ţigări/zi):

Repartiţia medicilor funcţie de provenienţă (mediu) şi consumul de ţigări/zi

Specialitatea	Ţigări/zi	Număr ţigări/zi funcţie de provenienţă		
		Oraş mare	**Oraş mic**	**Sat**
Boli infecţioase	7,92	8.33	10	0
Chirurgie	3,55	6,57	1,42	0
Dermatologie	0,30	0,40	6	0
Medicală	8,25	7,8	0	0
Oftalmologie	3,63	6,57	3,33	0
ORL	5,68	4,44	10	0
Recuperare	5,14	7,12	7,5	0
Stagiari	5,8	6,08	0	0
Spit. studenţesc	6,62	5,6	1,66	0
Urologie	6,38	3,33	0	15
TOTAL	6,05	5,81	3,27	3,14

Sursa: Pneumoftiziologia, 1991, 3-4

- majoritatea medicilor sunt de acord cu avertizarea fumătorului despre riscurile fumatului prin: texte imprimate pe pachetele de ţigări, ridicarea taxelor pentru fumat, interzicerea fumatului în unităţile sanitare, limitarea fumatului în locuri speciale şi spaţii închise, interzicerea vânzării tutunului la copii.

C. Ionescu şi colab., studiind prevalenţa fumatului la medicii care predau în facultăţile de medicină, au găsit o incidenţă de 31,9% la fumătorii obişnuiţi şi 12,7% la cei ocazionali, cel mai mare număr de fumători fiind întâlnit la grupa de vârstă 30-49 de ani şi în secţiile de pediatrie (41,2%), chirurgie (38,7%) şi laborator (26,8%).

În anul 1998, Mihălţan identifică, în rândul medicilor de familie, un procent al fumătorilor de 40% la bărbaţi şi 19% la femei, iar în anul 2000, în rândul studenţilor medicinişti din anul IV la UMF Bucureşti erau 32,7% fumători: 36,5% bărbaţi, 30% femei şi 7,8% ex-fumători.

În anul 1999, o anchetă organizată de C. Didilescu în rândul medicilor demonstrează că 43,2% erau fumători şi 16,6% ex-fumători, rata cea mai mare (44,3%) întâlnindu-se la grupa de vârstă cuprinsă între 30-49 ani. Totodată, aceeaşi anchetă a demonstrat că prevalenţa consumului de tutun este cea mai mare (62,6%) în rândul specialiştilor.

O anchetă efectuată în anul 1997 în rândul pneumologilor din România a evidenţiat un procent foarte mare de fumători: 60,5% dintre bărbaţi şi 4% dintre femei.

În anul 2005, cu prilejul *"Zilei Mondiale Fără Tutun"*, se arăta că, în rândul medicilor din unităţile sanitare, 51,4% sunt bărbaţi fumători şi 38% femei fumătoare.

O altă categorie importantă de fumători, în rândul personalului medical, o constituie asistentele medicale. Având în vedere faptul că majoritatea sunt femei, concluzia este uluitoare: incidenţa ajunge la 22,5% (22,2% pentru băieţi şi 22,4% pentru fete) cu fluctuaţii, în funcţie de vârstă, între 17,6% şi 28,6%, majoritatea debutând la 17-19 ani.

În anul 1996, o anchetă, condusă de Mihălţan, în rândul personalului care lucrează în farmacii, arată că 21,9% sunt fumători activi, 10,7% sunt foşti fumători şi 8,9% sunt fumători pasivi.

În concluzie, se observă faptul că, tocmai cei care trebuie să dea exemplu şi sfaturi pacienţilor, fiind documentaţi în privinţa patogenităţii tutunului, abuzează de consumul de tutun, fiind mari fumători.

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ANUNŢ

Anul acesta va avea loc, la Bucureşti, ediţia a XVI-a a Congresului Comitetului Balcanic de Medicină Militară (BMMC).

Evenimentul se va desfăşura în perioada **1 - 4 IUNIE 2011**, la Cercul Militar Naţional şi va reuni specialişti din domeniul medicinei militare aparţinând serviciilor medicale militare din Albania, Bulgaria, Grecia, România, Turcia, Serbia.

La ediţia de anul acesta se vor organiza şi următoarele mese rotunde:

- Regenerarea celulară - aplicaţii în managementul pacienţilor cu traume complexe;
- Îngrijirea traumelor multiple şi medicina de urgenţă - o abordare comprehensivă;
- Diagnosticul multiaxial în tulburările depresive - dimensiuni clinice şi terapeutice;
- Medicină aeronautică.

Personalul medical militar şi civil din reţeaua sanitară a Ministerului Apărării Naţionale, interesat să participe la această activitate, va trimite, până la data de **15 MARTIE 2011**, rezumatele lucrărilor propuse, pe adresa de e-mail aurelianmoraru@yahoo.com.

Rezumatele vor fi redactate în Microsoft Word XP (minim 200 de cuvinte, maxim 250 de cuvinte), folosind caracterele Times New Roman, corp 12 , respectând următoarele indicaţii:

- titlu: sugestiv, redactat în limba engleză, maximum 10 cuvinte;

- autori: maxim 5 autori pentru fiecare lucrare, în care se va specifica, după caz: gradul militar, titluri academice (asistent, conferenţiar, profesor etc.), prenume (iniţială la bărbaţi şi nume întreg la femei);

- instituţia;

- cuvinte cheie (maxim 5, relevante pentru conţinutul lucrării);

- rezumatul propriu-zis care va urmări scopul, materialul şi metoda, rezultatele şi concluziile lucrării;

- punct de contact (telefon fix şi mobil, adresa de e-mail).

Se va menţiona modalitatea de prezentare a lucrării (oral sau poster).

La lucrările si dezbaterile congresului pot asista cu statut de participanţi şi membrii cotizanţi ai Asociaţiei Medicilor şi Farmaciştilor Militari din România (AMFMR). Taxa de participare este de 50 de EUR, echivalentul sumei în lei la cursul BNR din ziua plăţii.

Comitetul Balcanic de Medicină Militară Balcanică (BMMC) este o structură medico-militară înfiinţată la Sofia în mai 1993 la iniţiativa şefilor serviciilor medicale ai ministerelor apărării din Bulgaria, Grecia, România şi Turcia.

Cu ocazia celei de a XI-a ediţii a Congresului Balcanic de Medicină Militară, care a avut loc la Atena, în luna iunie 2006, s-a semnat şi acordul de acceptare a Serbiei în cadrul BMMC. Anul aceasta, odată cu organizarea la Bucureşti a ediţiei a XVI-a, va fi semnată şi aderarea Albaniei la acest forum regional medical.

Scopul acestei organizaţii este de a dezvolta cadrul adecvat cooperării în domeniul medicinii militare, de a împărtăşi experienţa practicării medicinei între profesioniştii din serviciile medicale ale ţărilor membre, schimbul de idei, inovaţia. Dialogul ştiinţific, promovat sub egida BMMC, contribuie la creşterea vizibilităţii activităţilor medico-militare în această parte a Europei. Se are în vedere şi dezvoltarea procesului de interoperabilitate între serviciile medicale ale armatelor ţărilor din sud-estul Europei, cu scopul de a constitui o unitate mobilă multinaţională de ROL 2 pentru asistenţă umanitară la dezastre şi calamităţi naturale.

BALKAN MILITARY MEDICAL COMMITTEE BMMC 1995

ILCOR 2010 – Principalele modificări introduse de ghidul de resuscitare cardio-pulmonară

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Rezumat. Ghidurile de resuscitare 2010, ca şi ghidurile precedente, au la bază cele mai recente Consensuri Internaționale asupra Resuscitării Cardiopulmonare (RCP) împreună cu Recomandările de abord Terapeutic (CoSTR) privind noile terapii care pot influența semnificativ evoluția pacienților. Această ediție a Ghidurilor în RCP a Consiliului European de Resuscitare (*ERC*) actualizează ediția publicată în 2005 şi menține ciclul de 5 ani stabilit pentru reevaluarea şi modificarea ghidurilor. În prezentul articol se realizează o succintă analiză a modificărilor incluse în ghidul de resuscitare cardio-pulmonară din 2010 publicat de Comitetul Internațional de Legătură pentru Resuscitare (*ILCOR*).

Cuvinte cheie: resuscitare, şoc electric extern, tulburare de ritm, ventilație mecanică.

Abstract. Resuscitation Guidelines 2010, as the previous guidelines, were based on the most recent International Consensus on Cardiopulmonary resuscitation (CPR) with recommendations of Therapeutic approaches (CoSTR) on new therapies that can significantly influence the evolution of the patients. This edition of the Guidelines in CPR of European Resuscitation Council (ERC) updated edition published in 2005 and maintains the cycle of five years set for reevaluation and modification of guidelines. In this article provides a brief analysis of the changes included in the guidelines for cardio-pulmonary resuscitation in 2010 published by International Liaison Committee on Resuscitation (ILCOR).

I. INTRODUCERE

Medicina de urgență este o specialitate clinică medicală care începând cu anul 1991 şi până în prezent a reprezentat un punct de reper pentru medicina din România prin echipele de medici tineri experimentați care realizează cu succes intervenții de mare complexitate, comparabile cu standardele de performanță internațională. În Unitatea de Primiri Urgențe activează, alături de medicii de medicină de urgență, şi medici specialişti anestezie terapie intensivă promovând împreună standarde şi ghiduri de resuscitare[1,2], pentru a realiza astfel alinierea cu noile tendințe europene privind modul de abordare a RCP.

Ca metodă, RCP are drept obiective principale înlocuirea respirației şi circulației normale la un pacient în stop cardiorespirator (SCR). Respirația normală este înlocuită de ventilația mecanică a pacientului, iar circulația prin compresiunile toracice externe (CTE). Mecanismele care stau la baza CTE sunt pompa cardiacă şi respectiv toracică.[3,4] Examinarea căilor aeriene trebuie realizată rapid, eliberarea acestora reprezentând o parte importantă a RCP. Eliberarea CAS se poate realiza prin metode simple, folosind sau nu adjuvanți ai căilor aeriene (pipe orofaringiene etc.), sau complexe (combitubul, intubația orotraheală)[3,4].

Primele date privind respirația artificială şi masajul cardiac toracic au fost publicate de către Safar şi Kouwenhoven în 1958 şi, respectiv, 1960[6,7]. Prima încercare privind evaluarea tehnicilor de resuscitare a avut loc la conferința națională a Institutului de Medicină American în 1966[7]. Asociația Cardiologilor din America a publicat în 1974, 1980 şi 1986 standardele şi ghidurile privind RCP şi îngrijirea cardiacă de urgență[8-10]. Ulterior, în 1992, prin reunirea mai multor asociații a fost format Comitetul Internațional de Legătură pentru Resuscitare (*ILCOR*) care avea ca scop crearea unui ghid de resuscitare acceptat de cât mai multe țări[11]. Primul ghid de resuscitare a fost realizat şi publicat în 2000[12] şi revizuit la fiecare 5 ani. De la publicarea recomandărilor în 2005[15], rata de supraviețuire s-a dublat şi chiar triplat în întreaga lume ca rezultat al implementării noilor protocoale.

În prezentul articol se realizează o succintă analiză a modificărilor incluse în ghidul de RCP din 2010, publicat de Comitetul Internațional de Legătură pentru Resuscitare (*ILCOR*), referitor la suportul vital de bază şi cel avansat.

II. PRINCIPALELE MODIFICĂRI INTRODUSE ÎN GHIDUL DE RESUSCITARE DIN 2010[2]

A. ADULŢI

Resuscitarea cardiopulmonară reprezintă o serie de manevre salvatoare care îmbunătăţesc ratele de supravieţuire după un stop cardiac [3,4,5,13]. Deşi metoda optimă de resuscitare poate varia în funcţie de resuscitator, victimă şi de resursele disponibile, întrebarea fundamentală privind resuscitarea rămâne: „cum se poate obţine o resuscitare cardio-pulmonară precoce şi eficace?”.

O resuscitare eficientă după un stop cardiac necesită un set integrat de acţiuni coordonate reprezentate de Lanţul supravieţuirii (*Chain of Survival*): recunoaşterea persoanelor în situaţia de a face stop cardiac sau în stop cardiac, solicitarea de ajutor, integrarea RCP şi a defibrilării ca şi componente fundamentale ale resuscitării precoce, precum şi terapia adecvată post-resuscitare.

Ca şi celelalte ghiduri de resuscitare, cel din 2010 se bazează pe ghidurile elaborate anterior, fiind punctate modificările efectuate în cadrul fiecărui capitol al ghidului[4,14]. Algoritmurile de resuscitare pentru **S*uportul Vital de Bază*** (*„**Basic life support**” – **BLS***) şi pentru ***Suportul Vital Avansat*** (*„**Advanced life support**” – **ALS***) sunt descrise pe larg în protocolul din 2010 şi sunt reprezentate schematic în *fig.nr.1* şi, respectiv, *fig.nr. 2*.

1. Suportul Vital de Bază (SVB)

Modificările principale ale liniilor directoare faţă de ghidul din 2005 se referă la:

- modul de adresare a întrebărilor: dispecerii (de la nivelul Dispeceratelor Integrate de Urgenţă şi nu numai) trebuie instruiţi cum să adreseze întrebări apelanţilor folosind protocoale stricte pentru a obţine informaţiile necesare: recunoaşterea lipsei de conştienţă, absenţa respiraţiilor spontane, sau orice altă anormalitate în respiraţia victimei. Prezenţa acestor semne, la care se asociază recunoaşterea gaspingului (ca semn de SCR), trebuie să ducă la iniţierea algoritmului de SCR ;
- toţi salvatorii, cu pregătire anterioară sau fără, trebuie să facă compresii toracice externe (CTE) victimelor aflate în SCR;
- ghidarea prin telefon a RCP se va face pentru salvatorii neinstruiţi şi doar pentru a realiza compresiile toracice;
- se încurajează utilizarea aparaturii care oferă instrucţiuni şi feedback imediat salvatorului în timpul manevrelor de resuscitare. Datele stocate se vor folosi pentru a îmbunătăţi calitatea RCP şi pentru a asigura feedback-ul profesional al salvatorilor în timpul şedinţelor de analiză a activităţii.

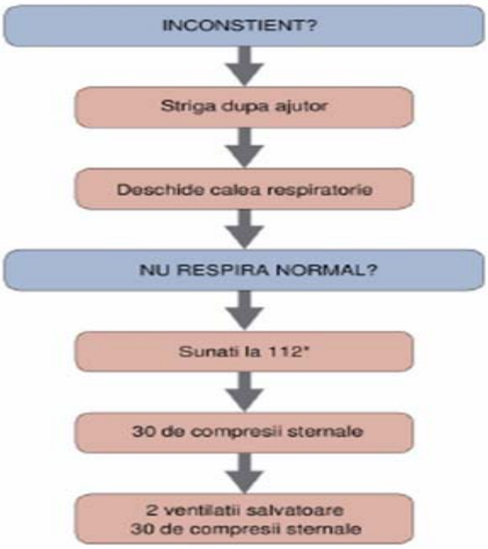

*Sau numarul national de urgenta

Fig.1. Algoritmul pentru suportul vital de bază la adult [2]

- ➢ Se subliniază importanţa deosebită a compresiilor toracice corect efectuate.
- ➢ **Obiectivele CTE** sunt: coborârea sternului cu cel puţin 5 cm la o frecvenţă de cel puţin 100 compresii/min, care permite revenirea toracelui la forma iniţială şi respectiv de a reduce perioada de întrerupere a compresiilor toracice.
- ➢ Salvatorii instruiţi în resuscitare ar trebui să administreze şi ventilaţie mecanică victimei, la o rată compresie-ventilaţie de 30:2.

2. Terapia electrică: defibrilatoarele automate, defibrilarea, cardioversia şi pacing-ul extern

Cele mai importante schimbări în Ghidurile ERC 2010 în privința terapiilor bazate pe electricitate cuprind:

- *administrarea precoce a CTE* (cât mai repede) şi fără întreruperi;
- în cazul terapiei SCR în pre-spital, personalul ambulanței (publice, SMURD, SMMU) trebuie să realizeze corect manevrele de RCP în tot timpul necesar pentru conectarea şi încărcarea defibrilatorului şi pregătirea administrării şocului electric (SE);

> ➢ *Scăderea la minimum cu putință a pauzelor pre şi post SE* prin efectuarea neîntreruptă a CTE;
> ➢ Se recomandă continuarea CTE în timp ce defibrilatorul se încarcă;
> ➢ Se vor relua imediat CTE după defibrilare;
> ➢ Administrarea şocului electric trebuie să determine o întrerupere de maximum 5 secunde în efectuarea CTE.

- *siguranța salvatorului* rămâne un element fundamental. Se admite că riscul pentru salvator în cazul utilizării defibrilatorului este minim, mai ales dacă salvatorul poartă mănuşi. Se pune accent pe scăderea duratei pre-şoc electric;

> ➢ Compresiile toracice se opresc pentru foarte puțin timp şi doar pentru a facilita intervenții specifice.
> ➢ Continuarea compresiilor toracice pe timpul necesar încărcării defibrilatorului, fapt ce va determina scăderea la minimum a pauzei pre-SE.

- A*dministrarea şocurilor electrice (SE)* - se pot administra până la 3 SE, unul după altul, dacă apare FV/TV în timpul cateterismului cardiac sau în perioada post-operatorie precoce în chirurgia cardiacă;
- strategia administrării a 3 SE poate fi folosită şi în cazul unui SCR cu martori, când defibrilatorul manual este deja conectat iar ritmul cardiac este FV/TV;
- se recomandă dezvoltarea programelor pentru defibrilatoarele semiautomate, precum şi amplasarea acestora în mai multe zone, atât publice, cât şi rezidențiale.

> ➢ *Nu se mai recomandă* efectuarea manevrelor de RCP pentru o perioadă de timp prestabilită (două-trei minute) înainte de analiza ritmului cardiac şi administrarea şocului electric;
> ➢ Având în vedere lipsa datelor concrete care să sprijine sau să infirme această strategie, acele echipaje SMU care au implementat deja algoritmi de intervenție ce cuprind efectuarea manevrelor RCP pentru o perioadă pre-stabilită pot continua să utilizeze algoritmul respectiv.

3. Suportul avansat al vieții (SVA)

Cele mai importante modificări ale principiilor privind SVA în ghidul ERC 2010 sunt:

- *CTE de calitate neîntrerupte* - întreruperea la minimum a compresiilor toracice corect administrate în cursul procedurilor specifice SVA;
- folosirea sistemelor "*track and trigger*" pentru a identifica orice alterare în starea pacientului şi a putea administra terapia necesară prevenirii SCR intraspitalicesc;
- descoperirea semnelor care anunță instalarea SCR în afara spitalului,
- *defibrilare precoce în prespital* - se renunță la recomandarea pentru manevre RCP cu timp prestabilit înainte de defibrilare la pacienții aflați în stop cardiac, în afara spitalului, şi fără martori din cadrul SMU la instalarea stopului;

- *Administrarea drogurilor prin sonda de intubație oro-trahealǎ* nu se mai recomandă;
- *Administrarea intraosoasă a drogurilor* - dacă nu se poate asigura acces intravenos, drogurile se vor administra intraosos;
- *Adrenalina* – se administrează în terapia SCR cu FV/TV, 1mg iv repetat, după cel de-al treilea şoc electric, odată cu reluarea CTE. Se continuă administrarea ei la fiecare 3-5 minute (în timpul ciclurilor alternante de RCP).
- *Amiodarona* (300 mg) se administrează, de asemenea, după cel ce-al treilea şoc electric.
- *Atropina* nu se mai recomandă în terapia de rutină pentru asistolă sau activitate electric fără puls.

- *lovitura de pumn precordială* nu mai este recomandată ;
- *intubația oro-trahealǎ precoce* - se reduce importanța intubației oro-traheale (IOT) precoce, cu excepția cazului în care este efectuată de o persoană foarte experimentată, cu o minimă întrerupere a compresiilor toracice ;
- *medicamentele folosite în resuscitare* - apar modificări în calea de administrare şi în schema de administrare a substanțelor în ALS (adrenalină, vasopresoare, antiaritmice, atropină);
- *capnografia* – se poate folosi pentru confirmarea şi monitorizarea plasării corecte a sondei traheale, a calității manevrelor RCP şi pentru a detecta semnele precoce de restabilire a circulației spontane (ROCS);

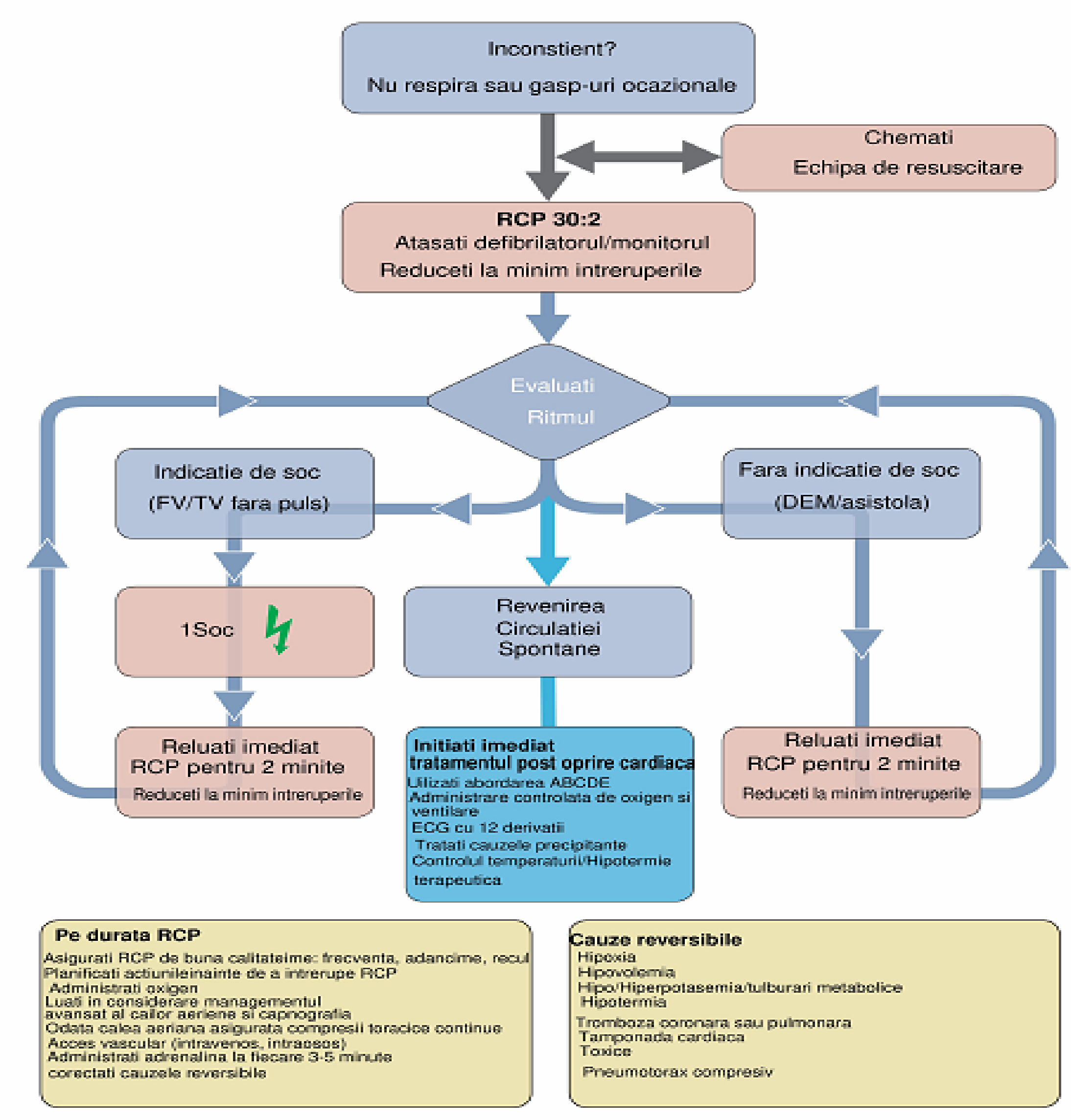
Inconstient?
Nu respira sau gasp-uri ocazionale
Chemati
Echipa de resuscitare
RCP 30:2
Atasati defibrilatorul/monitorul
Reduceti la minim intreruperile
Evaluati
Ritmul
Indicatie de soc
(FV/TV fara puls)
Fara indicatie de soc
(DEM/asistola)
1Soc
Revenirea
Circulatiei
Spontane
Reluati imediat
RCP pentru 2 minite
Reduceti la minim intreruperile
Initiati imediat
tratamentul post oprire cardiaca
Utilizati abordarea ABCDE
Administrare controlata de oxigen si ventilare
ECG cu 12 derivatii
Tratati cauzele precipitante
Controlul temperaturii/Hipotermie terapeutica
Reluati imediat
RCP pentru 2 minite
Reduceti la minim intreruperile
Pe durata RCP
Asigurati RCP de buna calitateime: frecventa, adancime, recul
Planificati actiunileinainte de a intrerupe RCP
Administrati oxigen
Luati in considerare managementul
avansat al cailor aeriene si capnografia
Odata calea aeriana asigurata compresii toracice continue
Acces vascular (intravenos, intraosos)
Administrati adrenalina la fiecare 3-5 minute
corectati cauzele reversibile
Cauze reversibile
Hipoxia
Hipovolemia
Hipo/Hiperpotasemia/tulburari metabolice
Hipotermia
Tromboza coronara sau pulmonara
Tamponada cardiaca
Toxice
Pneumotorax compresiv

Fig. 2. Algoritmul pentru suportul vital avansat la adult [2]

- *ultrasonografia cardiacă* - se recomandă folosirea ultrasonografiei în timpul SVA, datorită rolului său potențial;
- *hiperoxia* - se recunosc posibilele probleme pe care le poate pune hiperoxemia după momentul ROCS. După restabilirea circulației spontane, iar nivelul saturației oxigenului poate fi măsurat cu acuratețe (prin pulsoximetrie sau analiza gazelor din sângele arterial), se va menține SaO_2 la 94-98 % prin titrarea procentului de oxigen inspirat;

> ➢ *Ecografia cardiac*. Deşi nu s-a demonstrat că această metodă îmbunătăţeşte supraviețuirea, ecografia poate detecta cauzele reversibile ale SCR (tamponada cardiacă, embolia pulmonară, disecția de aorta, hipovolemia, pneumotoraxul). Când este disponibilă pentru folosirea de către clinicieni instruiți, ecografia poate ajuta la diagnosticarea şi tratarea cauzelor reversibile de SCR. Este recomandat abordul subxifoidian.
>
> ➢ Absența mişcărilor cordului în timpul RCP obținute ecografic este înalt predictivă pentru deces; cu toate acestea nu există date despre sensibilitatea şi specificitatea acesteia.

- *protocolul post-resuscitare* - succesul restabilirii circulației spontane (ROSC) este doar primul pas spre obiectivul principal, recuperarea completă în urma stopului cardiac. Sindromul postresuscitare, care cuprinde leziunea cerebrală postresuscitare, disfuncția miocardică postresuscitare, răspunsul sistemic ischemie/reperfuzie şi persistența patologiilor preexistente, adesea complică faza postresuscitare.
- *Intervenția coronariană primară percutanată* – se va pune accentul pe această metodă la pacienții cu ROSC susținută după un SCR, inclusiv la pacienții comatoşi, dacă condiția clinică a pacientului o impune;

> ➢ Prin implementarea unui protocol bine structurat şi explicat de terapie postresuscitare se poate creşte rata supraviețuirii pacienților aflați în ROCS după un stop cardiac.
>
> ➢ Se revizuiesc recomandările referitoare la nivelul glucozei în sânge: la adulții cu ROCS susținută post SCR, glicemia >10 mmol/l (>180 mg/dl) ar trebui tratată, dar hipoglicemia trebuie să fie evitată.

- *Hipotermia indusă terapeutic* se poate utiliza pentru pacienții aflați în comă ce au supraviețuit unui SCR cu un ritm cardiac cu, sau fără, indicație de şoc electric.

B. COPII [2,16].

La copii modificările majore ale principiilor Suportului pediatric al vieții sunt următoarele:

- *recunoaşterea SCR* – salvatorii nu pot determina absența sau prezența pulsului la un copil în mai puțin de 10 secunde, de aceea ei trebuie să caute alte semne de viață. Dacă stăpânesc foarte bine tehnica palpării pulsului, ei o pot adăuga la celelalte semne pentru a pune diagnosticul şi pentru a decide începerea manevrelor de resuscitare. Se va palpa în funcție de vârsta copilului, astfel: a. carotidă (copil), a. brahială (copii mici) sau a. femurală (copii sau copii mici);
- *raportul CTE/ventilație* – raportul folosit la copii depinde de prezența a 1 sau 2 salvatori. Cei care acționează singuri vor folosi raportul 30/2 care este la fel în SVB (BLS) la adult sau copii. Paramedicii trebuie să învețe să folosească şi raportul 15/2, dar pot recurge şi la raportul 30/2, dacă sunt singuri şi dacă nu pot să atingă numărul cerut de CTE. Dacă salvatorii nu pot să realizeze ventilația gura la gură, trebuie încurajați să realizeze măcar RCP tip compresie toracică;
- *tehnica de compresiune toracică* – coborârea toracelui trebuie să reprezinte cel puțin 1/3 din diametrul anteroposterior, la copii mici CTE se va realiza cu 2 degete pentru cazurile cu un salvator şi respectiv tehnica cu cele 2 police (mâinile înconjoară toracele) pentru cazurile cu 2 sau mai mulți salvatori. Pentru copii mai mari se poate folosi atât tehnica cu o mână, cât şi cu două mâini;
- *defibrilatoarele semiautomate (DSA)*– se pot folosi cu succes şi sunt sigure la copii cu vârsta > 1 an. Padelele speciale sau software-ul DSA atenuează energia eliberată la 50-75 J, care este recomandată pentru copii de la 1-8 ani; la aceşti copii se poate folosi la nevoie şi un DSA cu software pentru adulți. La copii < 1 an se va folosi însă un DSA cu atenuator de doză;
- *defibrilatoarele manuale* - dacă se utilizează un defibrilator manual în timpul dintre încărcări, nu se vor întrerupe CTE decât pentru aplicarea SE. Acesta va avea o valoare a energiei de 4 J/kg (de preferat bifazic) pentru defibrilarea copiilor;
- *intubația traheală* – se pot întrebuința şi sonde cu balonaş de etanşeizare la copii, diametrul fiind calculat după formula Penligton. Se va monitoriza E_TCO_2 pentru a confirma poziția corectă a sondei de IOT şi pentru a optimiza RCP;

- *Ventilația* - rămâne o componentă foarte importantă a RCP în cazul SCR asfixic.
- *CTE* - Se va pune accent pe CTE de calitate foarte bună, cu întreruperi minime, iar profunzimea CTE să fie de 4cm (copii mici) şi respectiv 5cm (copii>3 ani).
- Se pune accent pe relaxarea toracică post CTE.
- *Compresiile* trebuie să fie ≥100/minut, dar nu > 120/minut.
- *DSA* - se pot folosi DSA pentru adulți la copii cu vârsta > 1 an, chiar dacă nu există atenuator de energie eliberată.

- *ROSC* – după restabilirea ROSC se va titra administrarea de oxigen pentru a reduce riscul hiperoxiei.

C. NOU-NĂSCUȚI[2]

La nou-născuți, la naştere, modificările protocolului de resuscitare sunt următoarele:

- *pentru nou-născuții necompromişi* - se recomandă o întârziere de aproximativ 1 minut de la naşterea copilului şi până la clamparea cordonului ombilical;
- *pentru nou-născuții la termen* - se va folosi pentru resuscitare la naştere doar aer, oxigenul folosindu-se doar dacă oximetria nu este acceptabilă;
- *pentru prematuri <32 săptămâni de gestație* - se va folosi pentru resuscitare la naştere un amestec de aer-oxigen, sub un control judicios al oximetriei;
- *prematurii <28 săptămâni de gestație* – se vor înveli complet într-o folie de plastic sau într-un sac imediat după naştere, fără a fi măcar uscați. Temperatura în sala de naşteri va fi de cel puțin 26^0C. Vor fi îngrijiți la incubator cu o sursă de căldură şi stabilizați. Vor rămâne înfăşați până când temperatura lor se va stabiliza;
- *nu se recomandă aspirarea meconiului din gura nou-născutulu*i atunci când extremitatea cefalică a acestuia se află încă la nivelul perineului mamei;
- *nou-născut apneic şi cianotic* prin meconiu după naştere se va aspira cu rapiditate şi va fi intubat traheal la nevoie. Dacă se prelungesc manevrele de IT sau nu au succes, se va începe ventilația pe mască, mai ales dacă apare bradicardia;
- *adrenalina* se poate administra intravenos în doză de 10-30 µg/kg sau pe sonda de IT 50-100µg/kg pentru un efect similar al administrării iv a unei doze de 10 µg/kg.
- *detectarea CO2 în aerul expirat* în asociere cu examenul clinic este recomandată pentru detectarea IT la nou-născutul cu ROSC ;
- *hipotermia terapeutică* - se foloseşte la nou-născuții cu encefalopatie moderată-severă de cauză hipoxică-ischemică. Această metodă nu afectează resuscitarea imediată, dar este importantă pentru îngrijirea postresuscitare.

- *Raportul CTE/ventilație* rămâne 3/1 pentru RCP a nou-născutului.
- *Adrenalina* se administrează iv în doză de 10-30 µg/kg şi respectiv 50-100µg/kg pe sonda de IOT pentru un efect similar al administrării iv a unei doze de 10 µg/kg.
- *Ventilația* se va realiza pe mască dacă nou-născutul nu poate fi intubat sau se bradicardizează.
- *E_TCO_2* este cea mai bună metodă de confirmare a IOT la nou-născutul cu ROSC.
- *Hipotermia terapeutică* - se foloseşte la nou-născuții cu encefalopatie moderată-severă de cauză hipoxică-ischemică

III. CE A RĂMAS NEMODIFICAT DIN GHIDUL ILCOR 2005

O serie de principii au rămas nemodificate după ultima revizuire din octombrie 2010, astfel:

- evaluarea siguranței salvatorului, a victimei şi a celor din jur;
- evaluarea stării de conştiență a victimei şi solicitarea ajutorului;
- numărul de compresii toracice/minut (100/minut);
- forța de comprimare toracică (comprimarea planului toracic anterior cu 4-5 cm);
- faza de relaxare a compresiei pentru a permite revenirea peretelui toracic anterior la poziția inițială;
- SVA împarte ritmurile cardiace în ritmuri şocabile şi non şocabile. Fiecare ramură a protocolului este similară, cu 2 minute de RCP, efectuate înainte de analiza ritmului şi, când este cazul, verificarea pulsului.

- terapia fibrinolitică nu ar trebui folosită de rutină în resuscitare, fiind luată în considerare doar dacă SCR s-a produs ca urmare a emboliei pulmonare confirmate sau suspectate.

IV. RESUSCITAREA ÎN SPITAL

După instalarea SCR în spital, diferenţa dintre suportul vital de bază şi cel avansat este arbitrară, resuscitarea fiind un proces continuu, bazat pe algoritmi şi protocoale care trebuie cunoscute de întreg personalul medical din secţii.

Pentru ca pacientul să aibă o evoluția favorabilă, de o importanţă vitală sunt următoarele:

- SCR să fie recunoscut imediat;
- să se solicite ajutor folosind numărul de telefon standard;
- RCP să fie începută imediat folosind adjuvanți de cale aeriană dacă este indicat, iar defibrilarea va fi realizată precoce în primele 3 minute.

Un algoritm recomandat de noile ghiduri pentru managementul inițial al stopului cardiac intalat în spital este prezentat în *Fig.nr.3.*

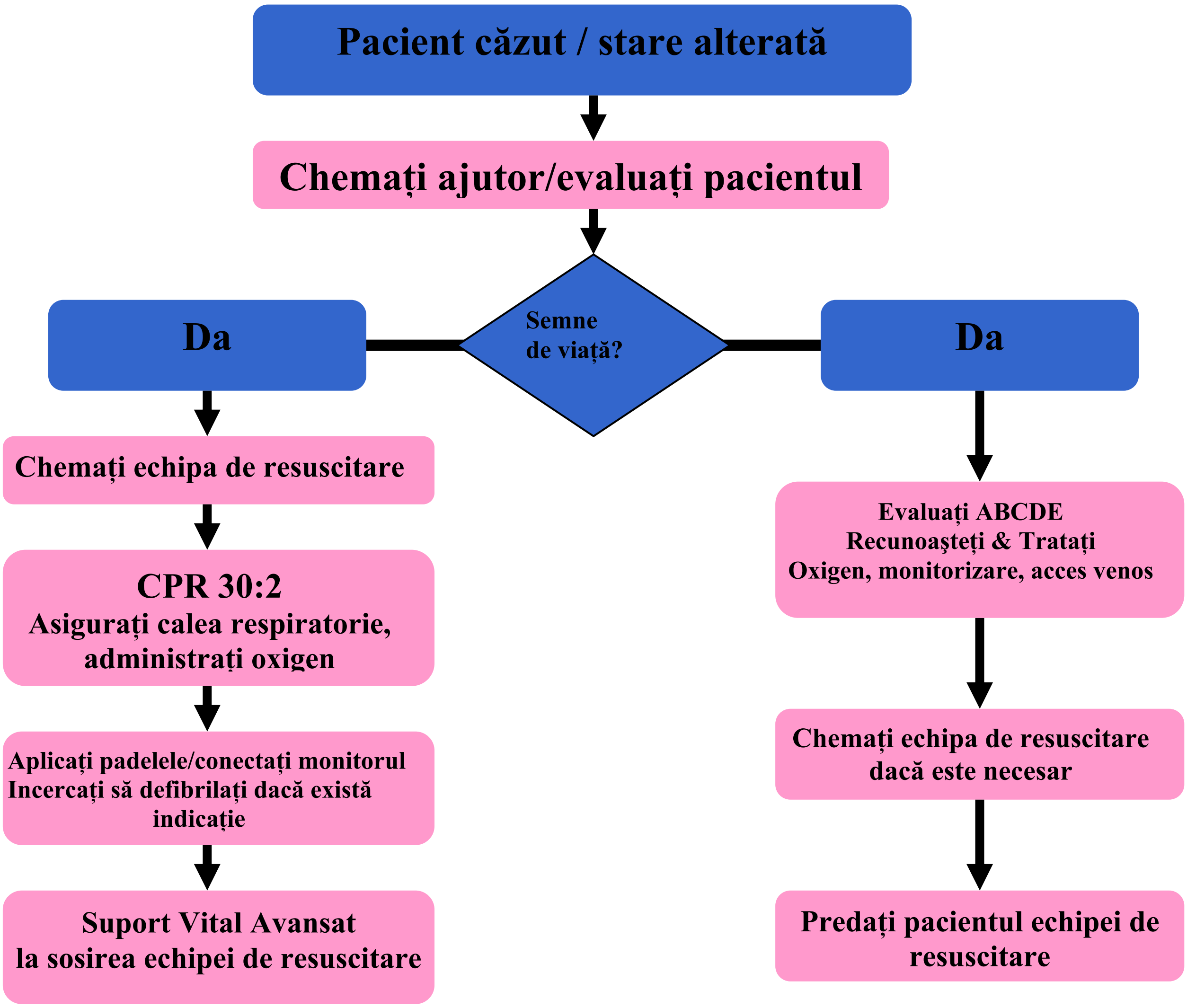
Pacient căzut / stare alterată
Chemaţi ajutor/evaluaţi pacientul
Semne de viaţă?
Da
Da
Chemaţi echipa de resuscitare
CPR 30:2
Asiguraţi calea respiratorie, administraţi oxigen
Aplicaţi padelele/conectaţi monitorul
Incercaţi să defibrilaţi dacă există indicaţie
Suport Vital Avansat
la sosirea echipei de resuscitare
Evaluaţi ABCDE
Recunoaşteţi & Trataţi
Oxigen, monitorizare, acces venos
Chemaţi echipa de resuscitare dacă este necesar
Predaţi pacientul echipei de resuscitare

Fig. 3. Resuscitarea în spital la adult [2]

Toate secţiile spitalului trebuie să aibă acces la echipament de resuscitare şi la medicaţie corespunzătoare pentru a facilita resuscitarea rapidă a pacientului în SCR. Echipa de resuscitare poate fi tradiționala echipă de resuscitare, alertată doar atunci când s-a produs stopul cardiac (din secția UPU sau ATI). Alternativ, spitalele pot avea strategii în recunoaşterea pacientului cu risc de a dezvolta stop cardiac şi alertarea unei echipe specializate (EMU sau EIR) înaintea producerii acestuia.

V. CONCLUZII

-*Compresiile toracice* ar trebui să fie primul pas în abordarea stopului cardiac. Prin urmare, acum se recomandă ca A-B-C-ul resuscitării cardiopulmonare (Airway-Breathing-Compressions) să fie modificat în C-A-B (Compressions-Airway-Breathing);

-*Compresiile toracice* se vor realiza cu o viteză crescută, la o rată de cel puțin 100 de ori pe minut. În plus, compresiile toracice ar trebui să coboare sternul cel puțin 5 cm la adulți şi copii, şi respectiv 4 cm la sugari;

-*Tehnicile de lucru în echipă* trebuie învățate şi practicate în mod regulat;

-*Capnografia cantitativă*, utilizată pentru a măsura de dioxid de carbon la ieşire, ar trebui să fie folosită pentru a confirma intubarea şi monitorizarea calității resuscitării;

-*Hipotermia terapeutică* ar trebui să fie parte a unui sistem global interdisciplinar de îngrijire, după resuscitarea unui stop cardiac;

-*Atropina* nu mai este recomandată pentru utilizarea de rutină în gestionarea şi tratarea activității electrice fără puls sau asistoliei;

-*Intubația trahealӑ* se va realiza doar atunci când se poate executa de o persoană înalt calificată, pentru a întrerupe cât mai puțin compresiile toracice.

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Articolele se redactează în Microsoft Word, Normal+Justf., Times New Roman, Font Size – 16 Bold pentru titlu, Font Size – 12 pentru text, iar rezumatul Font Size – 10 Bold. Pagina trebuie setată din Page setup la 2cm marginile, format letter.

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- Titlul lucrării (nu mai mult de 100 de caractere cu spații), fără abrevieri;
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- Rezumatul trebuie să aibă 150, până la 250 de cuvinte şi să cuprindă: obiectivul studiului, material şi metodă, rezultatele fără interpretarea datelor şi concluzii.
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3. ARTICOLUL PROPRIU-ZIS

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b. **Prezentare caz –** cel mult 600-800 de cuvinte (2 pagini).

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- **INTRODUCERE**: prezentarea problemei, plasarea în context, scopul şi obiectivele studiului, ipoteza de lucru.
- **MATERIALE ŞI METODE**: pacienţi, procedee, echipament (inclusiv indicaţii cu privire la producător), metoda de lucru, analiză statistică, tehnici operatorii, medicamente etc. (în funcţie de specificul articolului), toate acestea fiind descrise în detalii suficient de amănunţite pentru a permite reproducerea studiului de către alţi investigatori. În tot textul trebuie folosite aceleaşi nume pentru produse (cu specificarea numelui de marcă între paranteze la prima utilizare). Nu se recomandă numele pacienţilor.
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4 – probleme nerezolvate, întrebări la care nu s-a răspuns şi care rămân deschise în viitor pentru studiile ce vor urma, eventuale direcţii propuse pentru investigare în domeniul respectiv.

CONCLUZII

La nevoie, capitolele mari pot fi împărţite în subcapitole, după necesităţi.

Se recomandă utilizarea precaută a *abrevierilor* şi menţionarea numelui complet la prima apariţie în text a abrevierii respective. Toate referinţele bibliografice vor fi citate în text cu cifre arabe, la exponent, fără paranteze, în ordinea apariţiei (de exemplu: Un alt studiu^{18} arată...). Toate figurile şi tabelele vor fi citate în text în locul în care ele urmează să apară (de exemplu: *Fig. 3*, *Tab. 2*). Datele care apar în tabele, legendele tabelelor şi figurilor trebuie să fie rezumate, nu duplicate în text.

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Tabelele trebuie să fie procesate la două rânduri, pe o pagină separată, *numerotate* consecutiv (cu referire în text în locul unde ele trebuie să apară) şi *cu un titlu* (exemplu: *Tabelul 1.* Distribuţia pe vârste şi sex a pacienţilor). Capetele de tabel se scriu cu caractere *italice.* Nu trimiteţi fotografii sau imagini ale tabelelor. Utilizaţi tasta „Tab" pentru a separa coloanele (nu bara de spaţiu) şi utilizaţi tasta „Enter" la terminarea liniei. Toate căsuţele goale din tabel trebuie să aibă o liniuţă. Dacă este nevoie, pentru cuvintele sau frazele lungi din căsuţe se vor utiliza abrevieri, însă cu explicarea acestora în subsolul tabelului. Asiguraţi-vă că toate tabelele sunt citate în text.

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Pe această pagină referitoare la figuri, se va face o *listă* a tuturor numelor figurilor, în ordine numerică (la nevoie, cu legende explicative; identificaţi, în această legendă, orice literă sau săgeată utilizată pe figură).

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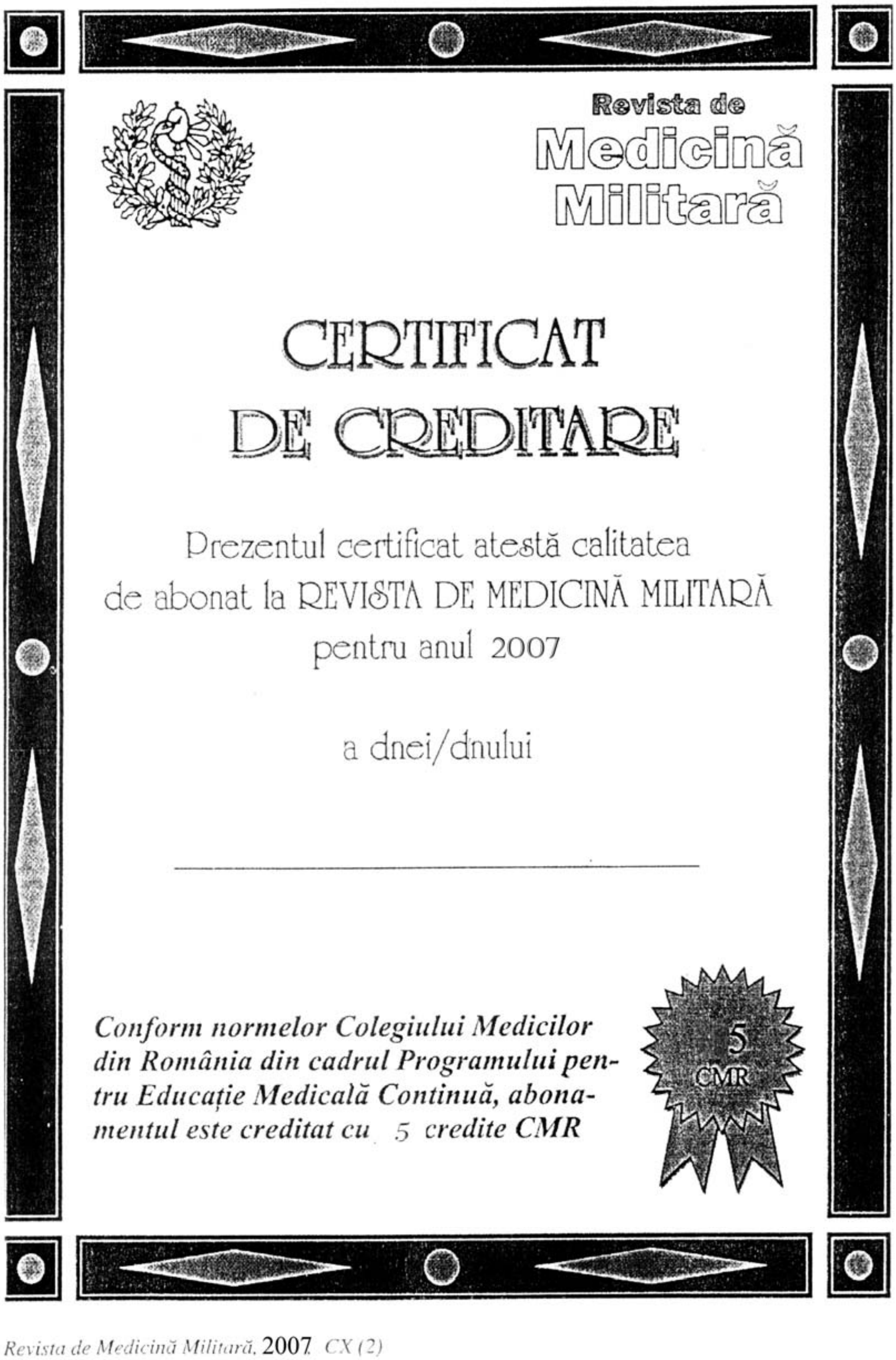

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Chistul hidatic cardiac

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Rezumat. Chistul hidatic cardiac este o afecțiune gravă, cu potențial letal, această localizare fiind neobişnuită. În principal, boala este asimptomatică, apariția simptomatologiei poate fi cauza unei complicații survenite în evoluția acestuia şi poate duce până la moarte. Orice suspiciune ridicată la un examen cardiac sau eco-cord impune stabilirea unui diagnostic de certitudine prin toate metodele imagistice existente în prezent. Tratamentul este obligatoriu pentru a preîntâmpina apariția complicațiilor şi este medicamentos (paraziticid) şi chirurgical. Tratamentul medicamentos poate fi pre- şi postoperator. Cel chirurgical este singurul care îndepărtează în totalitate parazitul, abordul fiind prin sternotomie mediană şi by-pass cardio-pulmonar în majoritatea cazurilor; pe cazuri selecționate fiind posibil şi un abord prin toracotomie. Diagnosticarea şi aplicarea tratamentului cât mai timpurii sunt foarte importante pentru a se evita complicațiile, care pot avea chiar şi o evoluție fatală.

Cuvinte cheie: chist hidatic cardiac, etiologie, tratament

Abstract. Cardiac hydatid cyst is a serious, potentially fatal disease, this location being unusual. The disease is mainly asymptomatic, symptoms usually due to complications during its evolution; some complications can even lead to patient's death. Any suspicion of a hydatid cyst raised during a periodical exam (clinical examination, cardiac ultrasound) requires certainty, through all the current imaging methods. Treatment is required in order to prevent medical complications (drug therapy and surgery). Drug therapy may be initiated pre- and postoperatively. The surgery is the only way to completely remove the parasite. The surgery addresses the cyst through median sternotomy and cardio-pulmonary by-pass in most cases; in selected cases is possible through a thoracotomy approach. Diagnose and implement of early treatment are very important as to avoid complications that may even have a fatal outcome.

Scurt istoric al hidatidozei

Boala hidatică este o zoonoză larg răspândită, capabilă să infecteze un număr mare de animale domestice sau sălbatice şi chiar oameni. Agentul etiologic este un parazit al câinelui şi al altor canide, stadiul larvar al acestui parazit realizând chistul hidatic, formă ce afectează doar accidental omul. Boala este cunoscută ca entitate clinică încă din timpuri străvechi. Lichidul hidatic a fost descris în antichitate de Hippocrates şi Galen, dar sursa şi natura acestuia nu erau cunoscute. „Talmudul" menționează câteva leziuni chistice în viscerele unor animale sacrificate; Goeze (1872) descrie scolecşii din lichidul hidatic. Viermele adult a fost descoperit şi descris în intestinul câinilor de către Batsch, în 1786, care i-a dat numele de *granulosus*, iar în 1805 Rudolphi l-a plasat în genul *„echinococcus"*. Legătura dintre acest vierme adult şi chistul hidatic a fost stabilită mai târziu, de Leuckart. În 1972 o nouă specie, *Echinococcus vageli,* a fost descrisă de Rausch, în America de Sud, la canidele sălbatice. Ulterior, în Columbia a fost diagnosticată hidatidoza umană datorată acestor specii.

Etiologie

Agentul etiologic (parazitul) al echinococozei este tenia *Echinococcus granulosus*, ce aparține genului *Echinococcus*, familia *Taenide*, clasa *Cestode*. În forma sa adultă parazitul trăieşte în intestinul subțire al câinelui, lupului, şacalului şi vulpii, care dețin rolul de gazdă definitivă. În stare larvară se găseşte în organele gazdei intermediare: ierbivore, porc, maimuţe şi numai accidental la om, unde este cunoscut drept chist hidatic (hidatida). Forma adultă din intestinul subțire al gazdelor definitive măsoară aproximativ 3-5 mm. Morfologic este constituit dintr-un cap (scolex) şi un gât, care se continuă cu corpul (strobila). Scolexul este organul de fixare; acesta are un aspect globulos şi măsoară aproximativ 0.4-0.6 mm. El prezintă pe proeminența sa patru ventuze rotunde înconjurate la bază de o coroană de până la 50 de cârlige dispuse concentric. Corpul continuă gâtul şi este format din trei sau patru segmente (proglote). Prima proglotă mică este proglota tânără, a doua proglotă este cea matură, iar a treia proglotă (bătrână) are dimensiunile cele mai mari, prezentând un aspect de uter în care se pot dezvolta până la 2 000 de ouă. Ouăle sunt sferice şi au dimensiuni între 35 şi 38 de µm, au un înveliş subțire, neted şi o membrană centrală brună striată. Ele conțin embrionul hexacant, cu şase croşete, care reprezintă elementul infectant al parazitului, care ajunge în mediul extern odată cu proglotele bătrâne şi cu dejecțiile animalului parazitat.

Gazda

Parazitul adult de *Echinococcus granulosus* trăieşte în partea superioară a intestinului subțire al gazdei canide, cu scolecşii fixați pe vilozități. În infecțiile severe, intestinul subțire al câinelui conține câteva sute de asfel de paraziți adulți. Alături de câinele domestic, *Echinococcus granulosus* mai infestează şi câini sălbatici: dingo (Australia), şacali (Africa), coioți (America), precum şi alte animale: lupul, hiena, vulpea. Nu există o gazdă specifică pentru stadiul larvar (gazda intermediară) a teniei *Echinococcus granulosus*. Acesta este găsit la mai multe mamifere, accidental şi la om.

Morfologia chistului hidatic

Chistul hidatic are o structură concentrică, fiind alcătuit dintr-o membrană cuticulară la exterior, laminată, elastică, alb-lăptoasă şi o membrană germinală la interior, granuloasă, subțire, puțin rezistentă. Peste cuticulă chistul este înconjurat de o adventice de natură fibroasă – rezultat al reacției organului gazdă. Adventicea (perichistul) este separată de membrana cuticulară printr-un plan de clivaj virtual, ce devine real după evacuarea chistului. Lichidul hidatic care umple interiorul chistului este limpede, incolor, inodor (ca ”apa de stâncă”), are densitatea de 1008-1015, iar pH 6.7-7.2. Lichidul este steril atât timp cât cuticula este intactă. Are calități antigenice puternice, putând produce atunci când este evacuat un şoc anafilactic. În interiorul chistului se mai găsesc capsule proligere rezultate în urma unui proces de înmugurire a membranei germinative. La început, capsulele sunt prinse de membrana germinală printr-un mic pedicul care ulterior se poate rupe, veziculele fiind eliberate în lichidul hidatic, unde, mai târziu, pot sedimenta, intrând în compoziția nisipului hidatic. În interiorul capsulelor proligere se formează prin înmugurire protoscolecşii, care sunt de obicei invaginați, cu cârligele spre interior. Eliberați în lichidul hidatic, ei devin evaginați, cu cârligele dispuse la exterior; ulterior acestea se vor detaşa şi vor forma împreună cu protoscolecşii şi veziculele proligere nisipul hidatic.

În interiorul sau exteriorul chistului hidatic se mai pot găsi şi veziculele fiice (libere), care apar din veziculele proligere sau prin vezicularea protoscolecşilor. Ele pot fi endogene, când se formează în interiorul chistului, sau exogene, când se formează în exteriorul acestuia. Veziculele fiice exogene sunt cauza hidatidozei multiloculare.

Localizarea echinococozei

Boala hidatică poate afecta întreg organismul uman, practic chisturile hidatice pot fi cantonate în orice organ sau viscer, țesuturi sau cavități. Cel mai des afectat organ este **ficatul**. În majoritatea studiilor, aşa cum sunt şi cele ale lui Tode şi asociații (1960), incidența dezvoltării hepatice este între 50 şi 60%; Saidi (1976) a raportat o incidență între 70 şi 80%. **Plămânii** reprezintă a doua localizare ca frecvență, cum au precizat Barett (1947) şi Dew (1928), cu o incidență ce variază între 10 şi 30 de procente. Rămâne o proporție de 10-15% din cazuri în care parazitul poate fi localizat în orice alt organ, viscer, țesut sau cavitate (cord, pancreas, creier, splină, rinichi, peritoneu, țesut muscular, oase, tiroidă, țesut subcutanat etc.).

Interesarea **cardiacă** este neobişnuită (0.05-2% - după Omer Tetik) [1, 2]. Primul caz de echinococoză cardiacă a fost descris în 1836 de Williams [3]. Larvele ajung în cordul drept pe calea ductului toracic sau a venei cave inferioare. De aici sunt aruncate în circulația pulmonară sau pot migra prin foramen ovale în cordul stâng. Pe calea circulației coronariene larvele ajung în pereții cordului. Cel mai frecvent este interesat ventriculul stâng (55-60%), care primeşte cea mai mare cantitate de sânge, urmat ca frecvență a localizărilor cardiace de ventriculul drept (15%), atriul stâng (8%), pericard (8%), septul interventricular (5-9%) şi atriul drept (3-4%). Simptomatologia pacienților cu hidatidoză cardiacă este nespecifică, de multe ori pacienții sunt asimpotmatici, dar se asociază frecvent cu complicații fatale, de aceea tehnicile imagistice joacă un rol foarte important în diagnosticarea interesărilor cardiace. În cazurile asimptomatice, rata de mortalitate este mai mare, datorită reacțiilor anafilactice acute şi a şocului cardiogenic, ca urmare a perforației chistului. Cazurile asimptomatice pot exprima de asemenea ca prim simptom embolizare periferică.

În cazul pacienților simptomatici, simptomatologia este dată de numărul şi localizarea chisturilor hidatice, dar şi de integritatea acestora şi, în plus, de afecțiunile cardiace preexistente. Chistul hidatic cardiac poate produce efect de masă, efuziune pericardică sau fenomene de embolizare; compresiunea pe elementele sistemului excitoconductor se poate asocia cu apariția blocurilor sau a aritmiilor; compresiunea pe coronare poate da angină. Alte modificări ale contracției cardiace sunt disfuncțiile valvulare şi reducerea volumelor vehiculate. Ruperea chistului în cavitățile cardiace conduce la embolizare în circulația pulmonară sau sistemică şi se poate asocia cu reacții alergice majore, hipertensiune pulmonară şi infestări secundare. Ruperea chistului în pericard produce pericardita acută, însoțită chiar de fenomene de tamponadă cardiacă sau, în timp, de pericardită cronică constrictivă. Examenul clinic toracic şi abdominal poate fi normal.

Până la apariția metodelor moderne de investigație (CT, IRM), examenul radiologic era singurul care sugera un diagnostic de echinococoză cu localizare toracică. Se descrie o opacitate rotundă sau ovalară ”ca trasă cu compasul” de intensitate subcostală. Introducerea computer-tomografiei în arsenalul de diagnostic al echinococozei a adus avantaje uriaşe. Computer-tomografia evidențiază zone de hipodensitate omogenă cu valori densitometrice de tip lichidian (0-15 UH), de formă rotundă sau ovalară, cu contur precis delimitat. În plus, față de radiografia clasică, investigația CT ne poate informa despre stadiul evolutiv al chistului hidatic (Khannous, 1993). Tomografia computerizată este practic obligatorie pentru stabilirea unui diagnostic corect şi a unui diagnostic diferențial al chisturilor hidatice față de alte formațiuni înlocuitoare de spațiu.

Examenul prin rezonanță magnetică (IRM) este valoros în diagnosticul chistului hidatic complicat, cu evoluție îndelungată, întrucât pune în evidență mai bine imaginile polimorfe; nu aduce însă întotdeauna date semnificative în plus față de computer-tomografie. Kotoulas afirmă, în 1996, că IRM-ul este o metodă foarte eficientă de diagnostic în echinococoza pluriviscerală cu localizări cardiace, vase mari, pulmon, permițând o evaluare preoperatorie cât se poate de fidelă.

Angiografia cu măsurarea presiunilor intracavitare poate fi de folos, dar reprezintă o manevră invazivă cu risc crescut.

Examenele de laborator pot sugera afectarea miocardică: LDH, CK, troponina, sau proces alergic: eozinofilie. Testul ELISA la antigen echinococotic poate fi pozitiv în 80% din cazuri. Diagnosticul diferențial se face cu tumori cardiace, tumori de mediastin, chisturi pericardice dar şi cu anevrismul de ventricul.

Tratamentul chirurgical

Îndepărtarea chisturilor hidatice cardiace este necesară dacă se are în vedere recuperarea completă şi prevenția recurențelor. Intervenția chirurgicală are ca scop excizia completă a chistului urmată, eventual, de repararea chirurgicală a structurilor lezate. Se impune luarea tuturor măsurilor de siguranță: analiza completă a statusului biologic al pacientului, monitorizarea completă a pacientului intraoperator, administrarea de medicație antialergică, rezerve de sânge. Calea de abord recomandată este sternotomia totală. By-pass-ul cardiopulmonar scade riscul diseminării generale a parazitului şi permite tehnici reparatorii. Riscul de embolizare pulmonară este foarte mare în timpul şi după operaţie, în special pentru chisturile din cavităţile drepte. Pentru acest motiv este preferabil by-pass-ul complet cardio-pulmonar prin clamparea aortei şi a arterelor pulmonare. În plus, se recomandă plasarea în cavitatea ventriculară a unei comprese îmbibate în soluţie paraziticidă, pentru a preveni contaminarea cordului cu conţinutul chistului. Tehnica chirurgicală impune succesiunea riguroasă a 4 timpi: **P**: puncţionarea; **A**: aspirarea conţinutului; **I**: instilarea agentului paraziticid; **R**: reaspirarea conţinutului chistului (în engleză **PAIR**: **P**=puncture, **A**=aspiration, **I**=infusion, **R**=reaspiration) .

Puncţionarea se face cu un ac fin, după izolarea cu comprese îmbibate. Inactivarea parazitului se poate face cu alcool, soluţie salină hipertonă sau iodină. În cazul chistului hidatic cardiac se preferă folosirea soluţiei saline hipertone, deoarece nu are efect asupra sistemului excitoconductor. Tot cu soluţie salină hipertonă se face şi lavajul pericardului. Se aşteaptă 5 minute, apoi se reaspiră conţinutul. Cu un bisturiu foarte subţire se incizează perichistul, evitând coronarele. Se extrage membrana proligeră şi se face toaleta locală a cavităţii perichistice.

Se evaluează leziunile generate de chist la nivelul septului interventricular: defecte parietale, edem, calcificări ale miocardului şi se practică corectarea chirurgicală. Cavitatea restantă poate fi abandonată sau se poate desfiinţa. Cavitatea poate fi lăsată deschisă spre pericard sau spre cavităţile cardiace. Tehnicile de desfiinţare a cavităţii pot produce distrucţia suplimentară a ţesuturilor, hemoragie şi diverticul al septului interventricular.Tehnicile de plastie cu petec au un risc crescut de apariţie a abcesului şi pot scădea performanţa cardiacă prin deteriorarea motilităţii septului. Chiar dacă în alte localizări s-a luat în discuţie şi aspirarea percutanată a chistului, acest lucru este contraindicat în CHM. Drenajul cavităţii pericardice şi parietorafia sunt ultimii timpi operatori. Pe cazuri atent selecţionate abordul chistului se poate face şi prin toracotomie dreapta sau stânga, în funcţie de dezvoltarea chistului, timpii operatori ulteriori fiind aceiaşi.

Tratamentul medical

Tratamentul medical constă în administrarea albendazolului 10mg/kg/zi. Cura începe imediat după testele serologice, precede şi succede intervenţia chirurgicală. În felul acesta se scurtează perioada de tratament medicamentos postoperator şi se previne interesarea şi a altor organe. Se repetă 4-5 cure de câte 30 zile, cu pauză de 2-3 săptămâni. Complicaţiile administrării de albendazol sunt: subţierea sau ruptura membranei hidatice, tulburări gastro-intestinale, tulburări neurologice, neutropenie, alopecie. Reevaluarea ecocardiografică şi serologică se face la 6 luni, în primul an şi apoi anual.

Prezentare caz clinic

Prezentăm în continuare cazul unui pacient în vârsta de 35 ani, asimptomatic, din mediul urban, subofiţer. Pacientul a fost internat iniţial în secţia cardiologie, în urma unei evaluări medicale de rutină care a evidenţiat radiologic o formaţiune tumorală mediastinală.

Anamnestic pacientul infirmă contactul cu câinii, tuberculoza sau alte afecţiuni organice.

Examinările radiologice anterioare obligatorii anuale nu au arătat modificări.

Examenul clinic la prezentare: afebril, stabil hemodinamic şi respirator, TA=120/80mmHg, AV=80b/min, fără raluri pulmonare, fără congestie venoasă sistemică, fără sufluri cardiace sau arteriale.

Ecocardiografia cardiacă (figurile 1 şi 2): cavităţi cardiace şi grosimea pereţilor ventriculului stâng în limite normale, FEVS>60%, valve şi aparate subvalvulare normale morfologic şi funcţional. La nivelul SIV anterior, în 1/3 medie se obiectivează o formaţiune chistică bine delimitată ce creşte progresiv în diametru către baza SIV, comprimând septul către VS cu prelungiri către pericard, adiacentă trunchiului arterei pulmonare.

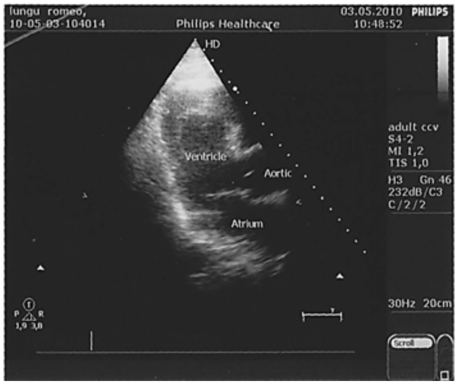
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Fig. 1. Ecografie cardiacă.
Imagine chistică în septul interventricular

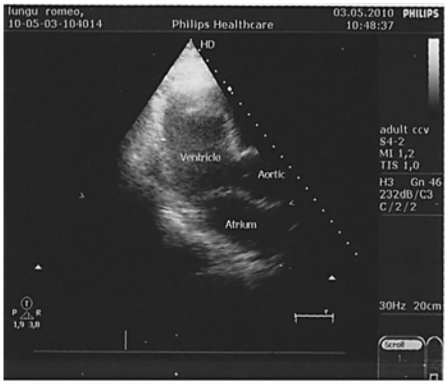
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TIS 1,0
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232dB/C3
C/2/2
30Hz 20cm
Scroll

Fig. 2. Ecografie cardiacă.
Valve şi aparate subvalvulare normale

Examenul CT torace cu contrast iv (figurile 3 şi 4) relevă o formaţiune chistică de 61,3/54,7/60 mm, restul structurilor anatomice examinate de aspect normal CT.

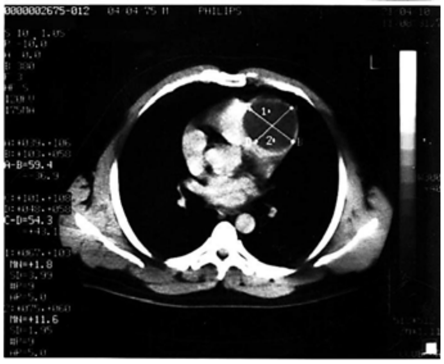
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1
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A-B=59.4
C-D=54.3

Fig. 3. CT torace cu contrast iv.
Formaţiune chistică de 61,3/54,7/60 mm

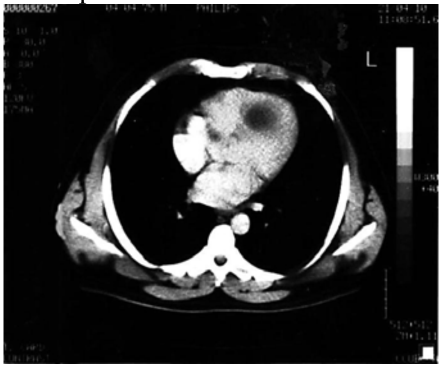

Fig. 4. CT torace cu contrast iv.
Localizare la nivelul septului interventricular

Intervenţia chirurgicală s-a desfăşurat cu intubaţie selectivă şi s-a ales ca modalitate de abord toracotomia antero-laterală stângă, cu abord prin spaţiul III intercostal.

La inspecţie s-a constatat pleură liberă, bombarea pericardului antero-superior de hilul pulmonar. S-a practicat pericardotomia la acest nivel (figura 5).

La nivelul peretelui interventricular, se observă o formaţiune chistică cu dimensiuni de aproximativ 6/5 cm cu maximă corticalizare. S-a izolat zona cu comprese îmbibate în betadină, s-a puncţionat chistul, s-a aspirat lichid clar şi s-a optat pentru abţinerea de la inactivarea parazitului (figura 6).

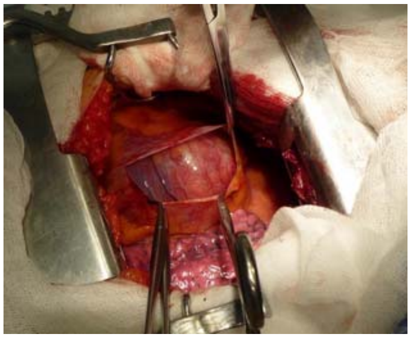

Fig. 5. Aspect intraoperator.
Pericardotomia

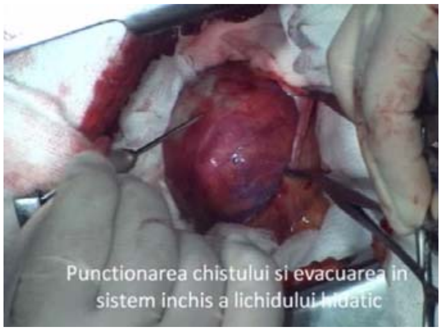
Punctionarea chistului si evacuarea in sistem inchis a lichidului hidatic

Fig. 6. Aspect intraoperator.
Puncţionarea chistului şi aspirarea lichidului hidatic

Secţionarea perichistului s-a efectuat într-o zonă avasculară, pe o lungime de circa 1,5 cm, prin care s-a extras membrana proligeră (figurile 7, 8). S-a efectuat un lavaj abundent cu betadină al cavităţii restante, al cavităţii pericardice şi al cavităţii pleurale (figura 9).

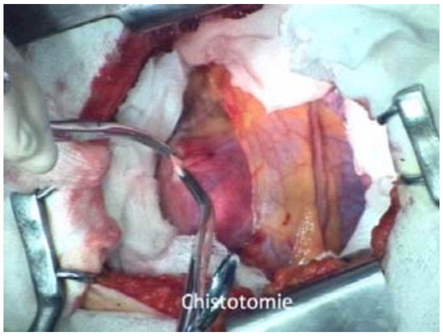

Fig. 7. Aspect intraoperator .
Secționarea perichistului între pense

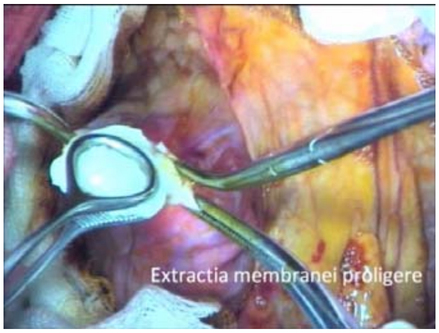

Fig. 8. Aspect intraoperator.
Extragerea membranei proligere

Cavitatea perichistică a fost abandonată (figura 9), iar cavitatea pleurală a fost drenată cu două tuburi, urmând închiderea toracelui.

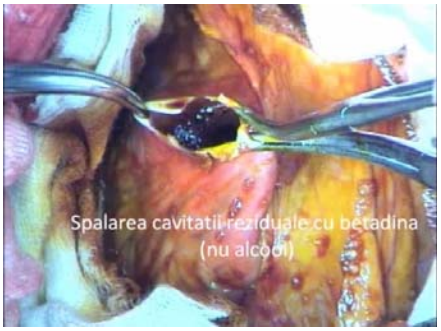

Fig. 9. Aspect intraoperator.
Lavaj cu betadină al cavității restante

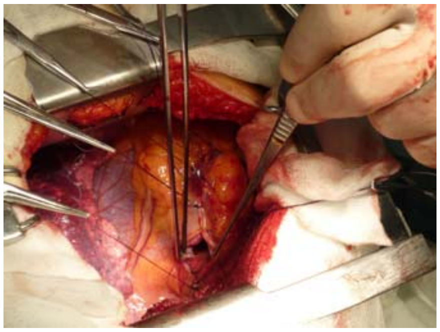

Fig. 10. Aspect intraoperator.
Pericardorafie incompletă (fereastră)

Evoluția post-operatorie a fost favorabilă sub tratament antibiotic, aintiinflamator şi antisecretor gastric. Tuburile de dren pleurale s-au suprimat la 3, respectiv 5 zile.

La externare pacientul a fost preluat de serviciul de parazitologie, urmând 3 cure de albendazol.

Controalele radiologic şi ecocardiografic la 1 lună arată un aspect în limite normale post-operator.

În concluzie, diagnosticarea precoce a chistului hidatic cardiac este foarte importantă. Îndepărtarea chirurgicală a chisturilor hidatice cardiace este necesară dacă se are în vedere recuperarea completă şi prevenția recurențelor.

Riscul de embolizare pulmonară este foarte mare în timpul şi după operație, în special pentru chisturile din cavitățile drepte.

După diagnosticul pozitiv, chistul trebuie îndepărtat fără a se contamina zona adiacentă, țintind un maxim de eficiență cu un minim de incidente şi efecte adverse.

În ceea ce priveşte cazul prezentat, putem specifica câteva particularități ale acestuia: chist hidatic cardiac unic (fără alte localizări); dezvoltare probabil rapidă (la examinările radiologice anuale nu s-a observat); dezvoltarea la nivelul septului interventricular, către pericard, pe marginea stângă a cordului, fără afectarea contracției cardiace a permis abordul prin toracotomie stângă, fără by-pass-ul cardiopulmonar; cavitatea perichistică a fost abandonată iar drenajul s-a făcut indirect prin breşa de pericardotomie în pleura stângă; evoluția clinică şi paraclinică a demonstrat că şi toracotomia poate fi o cale de acces în chistul hidatic cardiac.

În Secția Clinică de Chirurgie Toracică a Spitalului Universitar de Urgență Militar Central “Dr. Carol Davila” există o preocupare reală asupra acestei patologii – echinococoza. Practic, în acest serviciu s-au abordat toate localizările echinococozei pluviscerale, mai puțin localizarea cerebrală. În ceea ce priveşte localizarea cardiacă, acest caz nu este singular. Au mai fost operate în serviciu încă 4 cazuri în context plurivisceral.

Un caz a prezentat o dublă localizare cardiacă şi multiple localizări secundare pleurale – chisturile din cord fiind situate în atriul şi în ventriculul drept. Unul dintre chisturile cardiace s-a complicat prin fisurare, ceea ce a dus la realizarea unei pleurezii hidatice drepte. S-a intervenit chirurgical prin toracotomie axilară dreaptă, s-a confirmat pleurezia hidatică dreaptă. Chistul hidatic din peretele atriului drept era rupt în cavitatea pleurală dreaptă, din el evacuându-se resturi de membrană hidatică. Pe întreaga arie a plămânului drept se constată multiple diseminări secundare chistice infracentimetrice. În mediastinul antero-superior se mai constată prezența unui alt chist de dimensiuni mari ce se dezvoltă mult spre hemitoracele stâng. Acesta se abordează clasic prin evacuare, inactivare, extragere membrană proligeră, cavitatea reziduală lăsându-se deschisă în pleura dreaptă. Celălalt chist cardiac, situat în peretele ventricului drept, de dimensiuni 4/4 cm, intact, fără o corticalizare prea evidentă nu se abordează, bolnavul fiind trimis într-un serviciu de chirurgie cardio-vasculară pentru rezolvarea sub circulație extracorporeală.

Un alt caz se referă la o pacientă în vârstă de 16 ani care prezintă un chist hidatic cardiac la nivelul peretelui antero-lateral al ventriculului stâng şi multiple diseminări secundare pulmonare bilaterale. Chistul hidatic din cord se abordează într-un serviciu de chirurgie cardiacă, sub circulație extracorporeală, abordul chisturilor multiple toracice se efectuează în serviciul nostru prin toracotomii succesive. S-au abordat majoritatea chisturilor după procedee clasice, evoluția fiind favorabilă.

Un al treilea caz este al unui pacient cu o localizare pulmonară dreaptă şi una miocardică. Pacientul este operat pentru chistul din miocard, ventriculul stâng, cu dezvoltare mult intrapericardică, prin toracotomie în Secția de Chirurgie Toracică, similar cazului prezentat mai sus. Ulterior se abordează cele două chisturi de la nivelul plămânului drept.

Toți aceşti bolnavi au fost îndrumați către tratament paraziticid postoperator sau între şedințele operatorii.

Eficacitatea tratamentului medicamentos asociat celui chirurgical este dovedită.

Într-un al patrulea caz de localizare cardiacă stângă pacientul a refuzat intervenția chirurgicală, leziunea intracardiacă de aproximativ 2/3 cm dispărând ecografic la administrarea terapiei medicamentoase. Cealaltă localizare, pulmonară stângă, a fost abordată în mod clasic.

Discuții

Echinococoza cardiacă este în principal simptomatică. Când este vorba de cazuri simptomatice, simptomele includ angina, datorită presiunii pe arterele coronare, dispnee şi palpitații, în literatură fiind descrise chiar şi cazuri de chisturi hidatice cardiace ce au mimat un infarct miocardic acut [4]. În cazurile asimptomatice rata de mortalitate este mai mare datorită reacțiilor anafilactice acute şi a şocului cardiogenic, ca urmare a perforației chistului. Cazurile asimptomatice pot exprima de asemenea ca prim simptom embolizare periferică. Dintre metodele diagnostice, scanarea completă CT şi IRM cardiacă sunt foarte importante în vederea planificării intervenției chirurgicale [5]. Începerea tratamentului cu albendazol imediat după rezultatul pozitiv al testelor serologice scurtează perioada de tratament medicamentos postoperator şi previne interesarea şi a altor organe [6]. Îndepărtarea chisturilor hidatice cardiace este necesară dacă se are în vedere recuperarea completă şi prevenția recurențelor.

Riscul de embolizare pulmonară este foarte mare în timpul şi după operație, în special pentru chisturile din cavitățile drepte. Pentru acest motiv este preferabil by-pass-ul complet cardio-pulmonar prin clamparea aortei şi a arterelor pulmonare. O compresă cu soluție NaCl 20% poate fi plasată în ventriculul drept pentru a preveni contaminarea cordului cu conținutul chistului. În multe centre, interiorul chistului este spălat cu o soluție iodină. Alții preferă soluția de NaCl, deoarece este ieftină, uşor de procurat, mai puțin vâscoasă şi netoxică [7]. Se evită închiderea cavității restante. Chisturile localizate în septul interventricular pot cauza slăbiri excesive ale peretelui, edem şi calcificări ale miocardului, şi de aceea prin sutura cavității chistului acul poate cauza distrugeri musculare şi hemoragie. Plasarea unui patch este de asemenea o tehnică des folosită în multe centre, dar aceasta nu este o părere unanim acceptată, alți autori considerând că o cavitate reziduală duce la creşterea riscului de abcedare. Plasarea unui patch sau suturarea cavității va scădea performanța cardiacă, cauzând retracția septului interventricular şi afectând mobilitatea acestuia.

Ca o **concluzie** putem afirma că diagnosticarea timpurie a chistului hidatic cardiac este foarte importantă. După diagnosticul pozitiv, chistul trebuie îndepărtat fără a se contamina zona adiacentă, țintind un maxim de eficiență cu un minim de incidente şi efecte adverse.

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Evaluarea IRM a tulburărilor de flux ale lichidului cefalorahidian

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Rezumat. Hidrocefalia rezultă în urma unui dezechilibru între formarea, circulaţia şi reabsorbţia lichidului cefalorahidian (LCR) care are drept consecinţă un exces în volumul şi presiunea acestuia cu urmări asupra parenchimului cerebral şi a funcţiilor acestuia. LCR este dispus în jurul creierului şi al măduvei spinării. Este conţinut şi în ventriculii cerebrali şi în spaţiul subarahnoidian. Serveşte ca un strat protector al creierului faţă de agresiunile mecanice, dar este şi un vehicul de transport pentru neurotransmiţători şi pentru alţi metaboliţi. Imagistica prin rezonanţă magnetică (IRM) este modalitatea cea mai bună şi eficientă de investigare a hidrocefaliei în ceea ce priveşte cauzele şi consecinţele acesteia. Protocolul imagistic trebuie să fie adecvat fiecărui pacient pentru eficienţa maximă.

Cuvinte cheie : hidrocefalie, LCR , IRM.

Abstract. Hydrocephalus is a condition in which imbalance of formation, flow, or reabsorption of cerebrospinal fluid (CSF) leads to excess CSF volume/pressure, which then compresses the brain substance and impairs its development and function. CSF surrounds the brain and spinal cord. It is contained within the ventricles and the arachnoid. It serves as a cushion for the brain and protects the neural structures from mechanical damage.CSF also serves as a transport vehicle for different neurotransmitters and other metabolites to and from the brain (sink effect). MR imaging is by far the best tool to investigate hydrocephalus, its causes, and its consequences. The *imaging protocol* must be adapted to the patient for maximum efficiency.

Scopul investigaţiei constă în evidenţierea unei dilataţii a sistemului ventricular precum şi în precizarea, atunci când este posibil, a cauzelor acesteia. Tipul hidrocefaliei, obstructivă sau comunicantă, poate fi precizat analizând căile de drenaj ale ventriculilor. Spaţiile cu lichid cefalo-rahidian (LCR) sunt sistemul ventricular şi spaţiile arahnoide care înconjoară creierul şi măduva spinării. Volumul de LCR este în mod normal de circa 150 de cc. IRM poate evalua eficient aspectul morfologic al creierului iar prin protocoalele de evaluare a cineticii LCR prin secvenţele în contrast de fază poate evalua fluxul LCR. În fiecare minut se produce o cantitate de 0,4 ml de LCR şi de aproximativ 500 ml pe zi.

Elemente de anatomie. Creierul şi măduva sunt învelite în trei membrane meningeale : dura mater sau pahimeningele, arahnoida şi pia-mater, ultimele două constituind leptomeningele. *Dura mater* este formată din două straturi dense de ţesut conjunctiv. Stratul extern funcţionează ca şi un periost şi este ferm ataşat de os iar cel intern este cel al învelişului meningeal şi constituie una dintre suprafeţele spaţiului subdural. Arterele meningeale sunt cuprinse între aceste suprafeţe. Cea mai voluminoasă este artera meningeală mijlocie şi este ram din artera maxilară, care este ramură din artera carotidă externă. *Arahnoida* este o structură delicată şi dură. Este ataşată de sinusurile durale prin granulaţiile Pacchioni şi prin vili. Oricare nerv părăseşte craniul sau canalul spinal dura şi arahnoida îl însoţeşte pe un scurt traiect. În cazul nervilor optici dura însoţeşte canalul optic şi când ajunge în orbită stratul extern devine periost. Aceste aspecte sunt importante deoarece hemoragia subarahnoidiană se poate extinde anterior de-a lungul segmentului orbital al nervului optic iar în cazul meningeoamelor intracraniene acestea se pot extinde din vecinătatea neuroforamenului optic până în spaţiul subarahnoidian în jurul porţiunii orbitale a nervului optic. Tumorile maligne de tipul retinoblastoamelor sau melanoamelor pot metastaza prin continuitate pe această cale. *Pia mater* constă în trei straturi de celule de origine mezodermală. Spre deosebire de arahnoidă această membrană căptuşeşte toate suprafeţele vizibile ale creierului şi canalului spinal, cu excepţia suprafeţei ventriculilor laterali.

Spaţiul subarahnoidian este plin cu lichid cefalorahidian. Toate vasele de sânge precum şi nervii cranieni şi spinali străbat aceste zone cu fluid. Ca urmare, în caz de infecţie, extinsă şi la spaţiile leptomeningeale, vasele şi nervii cranieni pot fi implicaţi în acest proces inflamator. Spaţiul subarahnoidian trebuie privit în continuitate din zonele parietale ale creierului caudal către *cauda equina* în regiunea coccigiană. Spaţiul subarahnoidian nu comunică cu spaţiul subdural. Acolo unde spaţiul este larg, zonele respective poartă denumirea de cisterne. *Sistemul ventricular* este format din ventriculii laterali, precum şi din ventriculii III şi IV. Fiecare dintre ventriculii laterali are un corn anterior, o regiune denumită cella media, un corn posterior şi un alt corn inferior sau temporal. Ambii ventriculi laterali se conectează cu ventriculul III prin foramen Monro iar prin apeductul Sylvius cu ventriculii III şi IV.

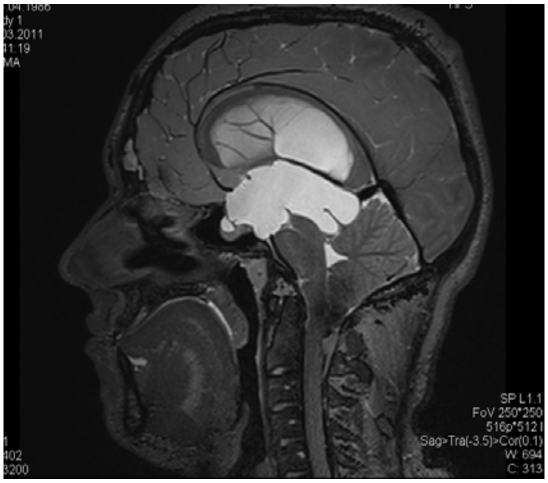

Fig. 1. Secvenţă de tip T2 în plan sagital. Se vizualizează dilataţia ventriculilor laterali şi a ventriculului III prin obstrucţia fluxului LCR la nivelul apeductului Sylvius. Ventriculul IV are volum normal.

Ventriculul IV comunică cu spaţiul subarahnoidian prin trei deschideri – două foramen Luschka şi foramen Magendie. Cele două foramene Luschka sunt situate la nivelul unghiurilor pontocerebeloase şi pot fi identificate cu ajutorul plexurilor coroide din vecinătate. Foramen Magendie este situată posterior de bulb şi în faţa cisternei magna. Fiecare dintre cei patru ventriculi are un plex coroid. Plexurile ventriculilor laterali sunt cele mai voluminoase.

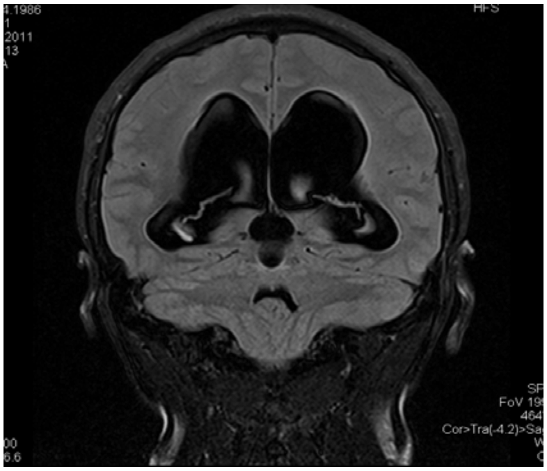

Fig. 2. Secvenţa FLAIR în plan coronal. Pot fi evaluaţi eficient ventriculii laterali şi aspectul parenchimului periventricular din lobii temporali.

Protocolul de realizare a examenului constă în secvenţe axiale T2, sagitale T1, coronale sau sagitale FLAIR, completate în funcţie de necesităţi de secvenţe coronale şi axiale postinjectare T1 de substanţă de contrast. Pot fi utile şi secvenţele de tipul celor care evaluează fluxul LCR. **Rolul** constă în precizarea tipului, obstructivă sau comunicantă. Chiar şi în cazul hidrocefaliei necomunicante există un grad de absorbţie al LCR. LCR este un filtrat produs în principal de plexurile coroide.

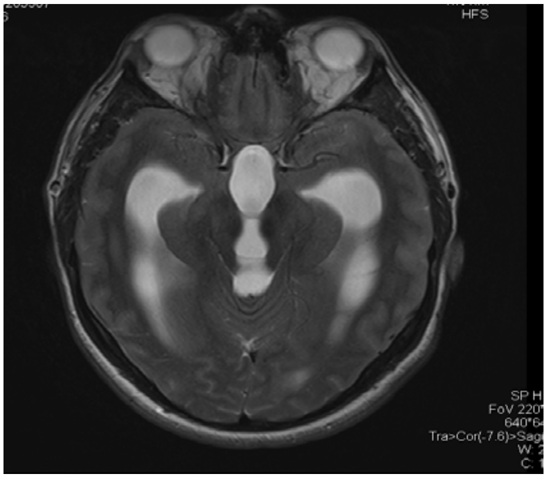

Fig. 3. Secvenţă T2 în plan axial. Se vizualizează eficient parenchimul cerebral şi volumul ventriculului III .

Fluxul de LCR se face dinspre ventriculi spre spațiile subarahnoidiene cu ajutorul contracției şi expansiunii parenchimului cerebral în diastolă şi sistolă. După ce o parte din LCR părăseşte ventriculul IV şi pătrunde în canalul spinal, o altă parte se deplasează cranial către convexitate. Acest tip de flux LCR-bulk flow nu este singurul mecanism de mişcare al acestuia. Secvențele FLAIR evaluează aspectul pereților ventriculari şi al parenchimului cerebral din vecinătate ținând cont de artefactele care pot apărea, astfel încât este foarte utilă utilizarea de secvențe FLAIR în mai multe planuri. Distensia ventriculară este denumită hidrocefalie internă hipertensivă. Atunci când distensia ventriculară se datorează lipsei parenchimului cerebral din malformații sau atrofie, ea este denumită hidrocefalie internă ex vacuo; când se datorează atrofiei circumvoluțiilor cerebrale, cum este cazul bolii Pick, poartă denumirea de hidrocefalie externă ex vacuo.

- Hidrocefalia obstructivă apare atunci când există un obstacol între ventriculi şi căile de drenaj (orificiul Monro, apeductul mezencefalic, orificiul Magendie şi Lushka). Poate fi mono-, bi, tri sau tetra-ventriculară, în funcție de localizarea obstacolului. Puncția lombară poate antrena acest gradient de presiune şi poate produce angajament tonsilar. LCR produs de plexurile coroide este resorbit de granulațiile Pachioni şi este drenat în sinusul sagital superior.
- Hidrocefalia comunicantă poate apărea atunci când există un efect de resorbție a LCR prin intermediul granulațiilor Pacchioni sau atunci când există hipersecreție de LCR la nivelul plexurilor coroidiene (papilom coroidian). Cei patru ventriculi apar dilatați, iar orificiul Magendie apare beant. În acest caz puncția lombară nu comportă niciun risc.
- Cauzele hidrocefaliei obstructive sunt tumorile sau alte procese ocupatoare de spațiu ce comprimă structurile ventriculare sau căile de scurgere ale LCR, precum şi hemoragia intraventriculară urmată de formarea cheagului sanguin.
- Cauzele hidrocefaliei comunicante sunt inflamația lepto- sau pahimeningee şi papilomul coroidian dezvoltat la nivelul plexurilor coroide.
- Semnele unei toleranțe dificile a hidrocefaliei sunt resorbția transependimară şi angajarea temporală sau amigdaliană cerebeloasă.
- Hidrocefalia acută este mult mai puțin tolerată față de cea cronică.
- În diagnosticul diferențial cu atrofia cerebrală poate fi utilă analiza comparativă a coarnelor frontale şi temporale ale ventriculilor laterali, care sunt mai puțin dilatate în atrofie față de hidrocefalie.
- Există o entitate numită hidrocefalie cu presiune normală a LCR care se prezintă sub forma dilatației tuturor ventriculilor fără apariția tulburărilor de resorbție a LCR periventriculare şi care beneficiză eficient de tratament prin şunt. Tratamentul prin şunt trebuie monitorizat pentru că un drenaj prea accentuat poate provoca apariția hematoamelor subdurale şi colabarea ventriculilor.
- Atunci când evaluarea se extinde şi asupra măduvei este necesar să fie evaluat şi canalul ependimar, care este de fapt o prelungire a ventriculului 4. În mod normal canalul central medular nu este vizibil; el devine vizibil când este dilatat. Această modificare este numită hidromielie, iar atunci când afectează şi măduva - siringomielie. Evaluarea poate fi făcută suplimentar față de secvențele clasice cu secvențe de tip phase contrast flow imaging.
- Presiunea intracraniană este în mod normal de circa 100 de ml de apă. Valoarea ei este determinată de volumul țesutului cerebral, al LCR, al sângelui şi al altor țesuturi situate în cutia craniană. Aceste structuri sunt în permanentă interacțiune, orice creştere de volum într-unul dintre compartimente duce într-o primă etapă la scăderea compensatorie a celorlalte, dar în final la creşterea presiunii intracraniene.
- Colapsul ventricular este o complicație hidrostatică a tratamentului prin şunt ventricular. Această complicație apare în special în poziție ortostatică şi este rareori diagnosticată în decubit, adică în timpul examinării IRM.

Concluzii

IRM este cea mai eficientă modalitate de investigare a hidrocefaliei prin utilizarea secvenţelor subţiri cu grosimea de 1mm. T1 şi T2 sagitale pot evalua structurile line mediane , T2 în plan coronal evaluează conţinutul ventriculilor laterali şi în special al coarnelor temporale, FLAIR pune în evidenţă edemul cerebral, secvenţele de tip CISS şi FIESTA evaluează apeductul şi aspectul chistic ale leziunilor. Aspectul de flow-void este evaluat de secvenţele de tip FSE sau TSE T2 şi completat de secvenţele Cine-Phase Contrast Imaging, care, în plus, pot şi cuantifica acest flux. Secvenţele de difuzie pot evalua leziunile ischemice recente asociate şi pot diferenţia chisturile epidermoide. Secvenţele angiografice rapide sau pentru fluxul venos pot fi utile, dar nu intră în protocolul curent. Administrarea de contrast paramagnetic intravenos poate fi utilă în identificarea tumorilor sau leziunilor infecţioase iar administrarea intracisternală nu este foarte utilizată datorită tendinţelor minim invazive. Explorarea hidrocefaliei fetale se poate face doar după 18 săptămâni, datorită volumului mic al creierului. Fătul este în continuă mişcare, astfel încât secvenţele eficiente trebuie să fie scurte, de ordinul a 30 de secunde. Se utilizează atât secvenţe T2, pentru anatomie, cât şi T1 ultrafast, pentru evaluarea leziunilor hemoragice sau a calcificărilor.

IRM este util atât pentru evaluarea primară diagnostică, cât şi pentru urmărirea evoluţiei leziunilor. În unele cazuri, IRM spinală poate depista cauze ale hidrocefaliei atât datorită unor tumori, cât şi unor malformaţii. Hidrosiringomielia poate fi asociată iar protocolul cuprinde atât secvenţe sagitale T1 şi T2, cu şi fără supresie de grăsime, şi uneori cu utilizarea substanţei de contrast paramagnetic.

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Hemoragia digestivă superioară
(abordarea diagnostică şi terapeutică actuală)

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Rezumat. Pacienţii cu hemoragie digestivă superioară acută se pot prezenta cu hematemeză, melenă şi/sau hematochezie. Evaluarea riscului pe criterii clinice şi endoscopice sprijină predicţia prognosticului evolutiv. Stigmatele endoscopice de hemoragie recentă sunt predictive pentru evoluţia clinică. Ghidurile clinice sunt utile pentru prognosticul evolutiv, inclusiv pentru durata spitalizării, pentru recidiva hemoragiei şi pentru mortalitate. În hemoragia digestivă superioară acută, endoscopia prezintă valenţe diagnostice, prognostice şi terapeutice. Ulcerul peptic este cea mai frecventă cauză de hemoragie digestivă superioară. Majoritatea ulcerelor peptice sunt datorate infecţiei cu *Helicobacter pylori* (H.p.) sau medicaţiei antiinflamatoare nesteroidiene (AINS). Hemoragia digestivă superioară acută poate fi elementul clinic inaugural al ulcerului peptic. Endoscopia digestivă superioară constituie cea mai eficientă metodă diagnostică. Sindromul Mallory-Weiss se asociază cu eructaţii şi vomă repetitive. Hemoragia variceală se asociază cu mortalitate mare şi risc de 80-90% de resângerare în lipsa intervenţiei terapeutice prompte.

Cuvinte cheie: hemoragie digestivă superioară ulcer peptic diagnostic tratament

Abstract. Patients suffering from acute upper GI bleeding can present with hematemesis, melena, hematochezia. Risk assessment based on clinical and endoscopic criteria can help predict risk for good and bad outcomes. Stigmata of recent hemorrhage are endoscopic findings that predict outcome. Clinical guidelines are used to predict outcomes, including length of hospital stay, rebleeding, and mortality. Endoscopy provides diagnosis, prognosis, and therapy. Peptic ulcers are the most common cause of upper GI bleeding. Most peptic ulcers are due to H. pylori infection or NSAID use. Upper GI bleeding may be the first sign of peptic ulcer. Digestive endoscopy may be the most important diagnostic test. Mallory-Weiss tears are associated with repeated vomiting and retching. Bleeding from esophageal varices is associated with high level of mortality and a 80-90% risk of re-bleeding without proper intervention .

CONSIDERAŢII CLINICE GENERALE

Hemoragia digestivă superioară (HDS-NV) nonvariceală constituie un motiv obişnuit de prezentare la camera de gardă pentru urgenţe dar şi pentru spitalizare. Numai în SUA, este responsabilă pentru 300 000 de internări anual, la care se adaugă alte 100 000-150 000 cazuri de HDS survenite în cursul spitalizării pentru alte patologii. Prin definiţie, sursa sângerării digestive superioare se localizează proximal de ligamentul Treitz. Evoluţia naturală a HDS-NV consemnează că aproximativ 80% se vor opri spontan, fără a fi necesară intervenţia de urgenţă. Dar dacă un pacient resângerează atunci mortalitatea creşte de zece ori!

În cazul HDS-NV, mortalitatea generală este de 5-10%, rămasă neschimbată din 1945, cu toată noua apariţie a medicaţiei moderne, a endoscopiei diagnostice şi terapeutice, a unităţilor de terapie intensivă şi a progreselor chirurgicale! Mortalitatea se datorează în mod curent unor factori independenţi de sângerarea digestivă şi apare în primul rând la pacienţi mai vârstnici şi care urmează tratamente de tipul antiinflamatoarelor nonsteroidiene sau antiagregantelor plachetare (gen clopidogrel). Administrarea aspirinei în doze mici pe termen lung creşte de două ori riscul HDS comparativ cu placebo, cu o incidenţă anuală a

HDS majore de 0,13%! Comparativ cu aspirina administrată singular, combinaţia aspirină-clopidogrel creşte de 2-3 ori numărul cazurilor de HDS majoră! Pentru pacienţii care se tratează cu aspirină şi clopidogrel, factorii de risc direct implicaţi pentru HDS sunt istoricul de ulcer peptic şi de hemoragie digestivă. Factorii de risc adjuvanţi sunt: sexul masculin, vârsta de peste 70 ani şi infecţia cu *Helicobacter pylori*. Mortalitatea la pacienţii cu HDS se datorează frecvent complicaţiilor cardiovasculare şi comorbidităţilor mai degrabă decât hemoragiei digestive incontrolabile.

MANIFESTĂRILE CLINICE ALE HDS

Semne şi simptome. Caracterizarea, pentru început, a tipului de hemoragie este utilă. Astfel, hematemeza se defineşte ca sângerare deschisă, cu vomă conţinând sânge proaspăt, eventual asociat cu cheaguri. Melena se prezintă cu scaune negre şi lucioase „ca smoala” şi având un miros distinct. Termenul de „zaţ de cafea” descrie aspectul aspiratului gastric sau vomei cu resturi de sânge vechi, coagulat. Hematochezia constă în pasajul transrectal de sânge proaspăt cu/fără cheaguri. Deşi sângele roşu deschis evacuat transrectal este de obicei indicativ pentru hemoragia digestivă inferioară, poate fi întâlnit şi în hemoragia digestivă superioară masivă!

Concomitent cu evaluarea clinică iniţială, în cazul suspiciunii de HDS, se efectuează resuscitarea în scopul realizării stabilităţii hemodinamice. Sunt evaluate semnele vitale, prezenţa sau absenţa şocului şi hipovolemiei, precum şi comorbidităţile medicale asociate (neoplaziile, maladia obstructivă pulmonară cronică, cardiopatia ischemică etc.). Pacienţii cu hipotensiune posturală au o pierdere de sânge de minim 10% iar cei în stare de şoc au pierdut minim 20% din volumul total de sânge, ceea ce semnifică un prognostic rezervat! Este important şi bilanţul medicaţiei de fond utilizate de către pacient, cu atenţie specială pentru anticoagulante (heparină, acenocumarol, warfarină), agenţii antiplachetari (clopidogrel, aspirină) şi antiinflamatoarele nesteroidiene (AINS).

Evaluarea riscului.

Predictorii clinici. Factorii de predicţie ai prognosticului în HDS-NV pot fi utili în triajul şi managementul pacienţilor. Rolul aspiraţiei pe tub nasogastric este controversat. Aspiratul poate oferi informaţii în funcţie de conţinut şi poate fi corelat cu culoarea scaunului. Mortalitatea este de 6% dacă aspiratul este clar (fără sânge), 18% dacă hematemeza sau aspiratul conţine sânge proaspăt şi de 30% dacă se asociază şi hematochezia. Dar aspiratul nasogastric poate fi fals negativ în ulcerul duodenal hemoragic asociat cu spasm piloric sau chiar în ulcerul gastric dacă tubul nu este poziţionat decliv.

Există o serie de factori de predicţie ai evoluţiei clinice severe în HDS (tabelul nr. 1). Astfel, HDS survenită în afara spitalului prezintă un risc de mortalitate de 7,1% comparativ cu 32,7% dacă hemoragia apare la persoanele spitalizate din alte motive. De asemenea, mortalitatea este de 8,7% la pacienţii de până la vârsta de 60 ani, pentru a ajunge la 13,4% la cei care au depăşit această vârstă. Mortalitatea creşte cu numărul comorbidităţilor. Astfel, dacă la pacienţii cu două comorbidităţi mortalitatea este de 2,6%, la cei cu 4 comorbidităţi ajunge la 9,9%, la cei cu 6 comorbidităţi la 27% şi la 66,7% în cazul a 8 comorbidităţi asociate.

Tabelul 1. Factorii de risc pentru evoluţia nefavorabilă a hemoragiei digestive superioare

- Vârsta de peste 60 ani;
- Şocul hipovolemic (TAs < 100mmHg; pulsul > 100 b/min);
- Hemoragie din sursă neoplazică sau variceală;
- Declanşarea HDS în spital;
- Comorbidităţi asociate;
- Sângerare activă (hematemeză, aspirat nasogastric de sânge proaspăt sau hematochezie);
- Sângerare recurentă;
- Coagulopatie severă.

Ghidurile clinice optimizează managementul pacienţilor cu HDS-NV. Ele identifică pacienţii cu risc redus, care pot fi externaţi fie direct din departamentul de urgenţă, fie după o scurtă perioadă de spitalizare, precum şi pacienţii cu risc crescut care vor beneficia de resursele terapeutice suplimentare. Majoritatea ghidurilor clinice reunesc date clinice şi informaţiile obţinute la endoscopie. Aceste ghiduri pot fi parte a managementului clinic de rutină, în sprijinul direct al deciziilor de triaj, având impact semnificativ în ameliorarea eficienţei actului medical, fără reducerea calităţii sale.

Scorul Rockall este predictiv pentru riscul de resângerare şi mortalitate în cazul pacienţilor cu HDS-NV, pe care-i clasifică în trei grade de severitate, în funcţie de următoarele variabile: vârsta, prezenţa şocului, comorbidităţi, diagnostic şi stigmate endoscopice (tabelul nr. 2). Pacienţii cu risc redus au un scor ≤ 2, reprezentând 29,4% dintre toţi pacienţii. Grupul cu risc redus are un risc de resângerare de 4,3% şi de mortalitate de 0,1%. Pacienţii cu scoruri Rockall între 3 şi 5 prezintă risc intermediar de resângerare şi de mortalitate (2,0-7,9%) iar cei cu scoruri de ≥ 6 au risc şi mai mare de resângerare şi de mortalitate (15,1, respectiv 39,1%).

Tabelul 2. Scorul de risc Rockall

Scorul/Variabila:	0	1	2	3
Vârsta (ani)	< 60	70 -79	≥ 80	-
Şoc	Nu	Tahicardie (P>100 b/min; TAs > 100 mmHg)	Hipotensiune (P>100 b/min);(TAs<100mmHg)	-
Comorbidităţi	Fără comorbidităţi majore	-	Insuficienţă cardiacă congestivă, arteriopatie coronariană,orice comorbiditate majoră	Insuficienţă renală sau hepatică, cancer metastatic
Diagnostic	Sindrom Mallory-Weiss, fără stigmate de hemoragie recentă	Orice alte diagnostice	Cancer digestiv superior	-
Stigmate majore de hemoragie recentă	Cu/fără spot hemoragic	-	Cheag, vas vizibil, hemoragie difuză	-

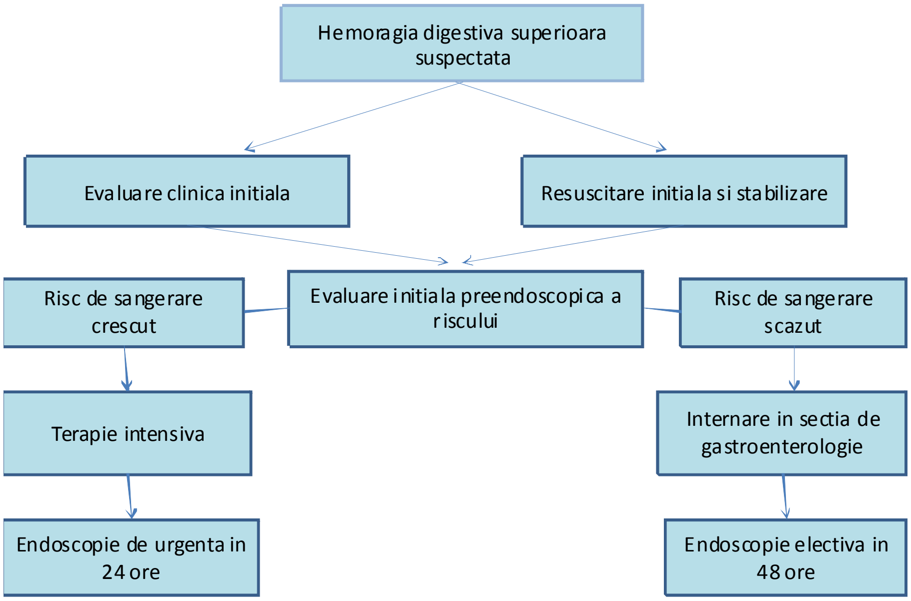
Hemoragia digestiva superioara suspectata
Evaluare clinica initiala
Resuscitare initiala si stabilizare
Risc de sangerare crescut
Evaluare initiala preendoscopica a riscului
Risc de sangerare scazut
Terapie intensiva
Internare in sectia de gastroenterologie
Endoscopie de urgenta in 24 ore
Endoscopie electiva in 48 ore

Fig. 1. Algoritm pentru managementul hemoragiei digestive superioare

TRATAMENTUL HEMORAGIEI DIGESTIVE SUPERIOARE

TERAPIA MEDICALĂ

Terapia iniţială. Pacienţilor li se montează două catetere venoase periferice sau pentru acces venos central. Pentru menţinerea unei presiuni sanguine adecvate vor fi administrate de la început soluţii cristaloide (ser fiziologic sau Ringer lactat). Administrarea de oxigen va fi aplicată de rutină. Pacienţii care nu pot respira spontan adecvat sau cei cu hematemeză severă şi cu risc de aspiraţie secundară vor fi intubaţi endotraheal. Pacienţii cu risc crescut, ca de exemplu vârstnicii sau cardiacii, vor fi transfuzaţi pentru menţinerea hematocritului peste 30%. Tinerii şi pacienţii fără comorbidităţi vor fi transfuzaţi pentru menţinerea hematocritului peste 20%. Pacienţii cu coagulopatie vor fi perfuzaţi cu plasmă proaspăt congelată sau li se vor administra vitamina K. Transfuzia cu masă plachetară se impune dacă trombocitele sunt < 50 000/ml.

Medicaţia utilizată pentru terapia HDS-NV este variată şi include: antiacide, antagonişti de receptori H-2, inhibitori de pompă de protoni şi octreotid. S-a dovedit că antiacidele şi antagoniştii receptorilor H-2 nu modifică evoluţia clinică a pacienţilor cu hemoragie digestivă superioară.

INHIBITORII POMPEI PROTONICE (IPP) au demonstrat, în studii recente pe pacienţi selectaţi cu HDS-NV, un beneficiu terapeutic cert, motiv pentru care sunt larg acceptaţi. IPP sunt singurele care menţin pH-ul gastric peste 6.0, prevenind astfel fibrinoliza cheagului din craterul ulcerului hemoragic. Există numeroase studii care demonstrează beneficiul IPP la pacienţii cărora li s-a efectuat hemostază endoscopică. Lau şi colaboratorii au studiat pacienţi cu hemostază eficientă prin combinarea injectării de epinefrină şi electrocauterizarea bipolară. Pacienţii au fost randomizaţi pentru infuzie in bolus de 80 mg omeprazol, urmată de infuzie continuă de 8 mg/oră, versus placebo. La pacienţii care au primit omeprazol, resângerarea în general şi aceea din primele trei zile au fost mai reduse. Mai mici au fost şi tendinţa recurgerii la chirurgie şi rata deceselor. Prin urmare, resângerarea este redusă la pacienţii cu terapie combinată (endoscopică şi IPP în doză mare) comparativ cu pacienţii trataţi exclusiv endoscopic.

Alte studii arată că, la pacienţii trataţi iniţial cu infuzie în bolus de omeprazol urmată de infuzie continuă, recurgerea la endoscopie este mai redusă, numărul pacienţilor cu sângerare activă la endoscopie este mai mic iar numărul cazurilor de ulcer cu baza clară (curată) în momentul endoscopiei este crescut. Datele actuale sugerează că beneficiul terapeutic al IPP este de clasă şi că posologia trebuie să fie similară pentru IPP-urile disponibile i.v. Recomandarea curentă este pentru 80 mg in bolus iniţial, urmată de infuzie cu 8 mg/oră pentru încă 72 ore după terapia endoscopică. Pacienţii stabili în acest interval vor fi trecuţi pe doza orală standard.

OCTREOTIDUL poate fi utilizat în terapia HDS-NV. Deşi larg folosit în HDS variceală, există date care confirmă utilitatea sa şi în HDS-NV. O metaanaliză efectuată de Imperiale şi Birgisson pe 1 829 pacienţi, reuniţi din 14 trialuri care au folosit octreotid sau somatostatină în HDS-NV, a relevat că riscul relativ de resângerare a fost de 0,53 (0,43-0,63), sugerând că octreotidul reduce riscul de sângerare continuă în hemoragia digestivă superioară nonvariceală. Deşi util ca tratament adjuvant, octreotidul nu trebuie, în general, utilizat de primă intenţie în HDS-NV. Introducerea sa va fi luată în considerare în cazul sângerărilor persistente în pofida terapiei medicale optimale care a inclus IPP, la pacienţi cu risc chirurgical crescut (de exemplu, cei spitalizaţi cu comorbidităţi multiple).

TERAPIA ENDOSCOPICĂ

Scopul terapiei endoscopice este să stopeze sângerarea continuă şi să prevină resângerarea. Resângerarea este cel mai important factor determinant al morbidităţii şi mortalităţii. Obţinerea unei hemostaze susţinute depinde de accesul la vasul hemoragic, de calibrul relativ mic al acestuia şi de absenţa coagulopatiei semnificative. Endoscopia digestivă superioară (EDS) are valenţe diagnostice, prognostice şi terapeutice în hemoragia digestivă superioară acută iar efectuarea sa este rezonabilă în acest context (50% dintre complicaţii sunt cardio-pulmonare). EDS localizează sângerarea în 90-95% cazuri. Sensibilitatea diagnostică creşte dacă se efectuează cât mai aproape de debutul sângerării şi scade cu îndepărtarea de acest moment. Printre opţiunile terapeutice oferite de EDS sunt incluse injectarea, cauterizarea, montarea de endoclips-uri sau terapia combinată care le include. EDS este de asemenea predictibilă pentru probabilitatea sângerării persistente sau recurente, pe baza recunoaşterii stigmatelor endoscopice de hemoragie recurentă.

Stigmatele hemoragiei recente sunt aspecte endoscopice depistabile la pacienţii cu ulcer peptic hemoragic iar recunoaşterea lor prezintă semnificaţie prognostică majoră (tabel nr. 3). Pacienţii care sângerează activ în momentul endoscopiei au un risc foarte mare de continuare a sângerării (de până la 90%) dacă nu se intervine terapeutic. Pacienţii care prezintă vase vizibile dar fără sângerare actuală vor resângera în aproximativ 45% din cazuri. Pacienţii cu cheag aderent au o probabilitate intermediară de resângerare. Astfel, 25% din cazurile cu cheag aderent vor resângera în funcţie de ceea ce se află subiacent. Dacă sub cheag se află un vas vizibil, riscul resângerării este mai mare decât dacă se află pe o bază curată. Pacienţii cu crater ulceros curat sau cu sindrom Mallory-Weiss şi care nu au coagulopatii concomitente sau medicaţie care modifică coagularea au un risc foarte redus de resângerare (< 5%). Vasele vizibile non-hemoragice pun cele mai multe probleme. Ele sunt protuberanţe într-un crater ulceros, cu culoare variabilă. La rândul lor, acestea pot reprezenta vase, pseudoanevrisme sau cheaguri.

Tabelul 3. Stigmatele hemoragiei recente: risc de resângerare, mortalitate şi prevalenţă.

Stigmatul	**Risc de resângerare (%) (numai cu terapie medicală)**	**Mortalitate (%)**	**Prevalenţă (%)**
Sângerare arterială activă	55 – 90	11	10
Vas vizibil, fără	40-50	11	25

sângerare			
Cheag aderent	20-35	7	10
Hemoragie difuză (fără vas vizibil)	10-25	-	10
Spot plat	< 10	3	10
Ulcer cu bază curată	< 5	2	35

UTILITATEA TERAPIEI ENDOSCOPICE în ulcerele peptice hemoragice a fost controlată placebo folosind electrocoagularea multipolară comparativ cu terapia endoscopică simulată. Terapia endoscopică a determinat o ameliorare semnificativă a hemostazei, reducerea numărului de unităţi de sânge transfuzat, a numărului de intervenţii de urgenţă, a zilelor de spitalizare şi a costului spitalizării. De asemenea, s-a semnalat tendinţa spre reducerea mortalităţii în grupul tratat endoscopic. Astfel, terapia endoscopică a devenit standardul de îngrijire pentru pacienţii cu stigmate de hemoragie recentă cu risc crescut. Pacienţii cu sângerare activă sau cu vas vizibil non-hemoragic vor fi trataţi prin hemostază endoscopică, pentru ameliorarea evoluţiei clinice şi a prognosticului. Cheagurile aderente se regăsesc în 10% din ulcere şi se asociază cu un risc de resângerare de 20-35% (mai mic la tinerii fără comorbiditaţi şi mai mare la vârstnicii cu comorbidităţi). Abordarea terapeutică a acestor cheaguri aderente este încă controversată. Dacă iniţial, ghidurile terapeutice recomandau abţinerea de la tratarea lor, ulterior s-a demonstrat eficacitatea terapiei endoscopice. Tehnica abordării lor presupune injectarea de adrenalină în patru cadrane, la baza cheagului. Urmează ghilotinarea cheagului cu ansă la rece, la 3-4 mm de baza acestuia. Atenţia este sporită pentru a nu fi forfecat cheagul, pentru a nu fie iritată şi mai mult zona de intervenţie şi pentru a nu fi declanşată sângerarea. Baza ulcerului este apoi irigată suficient pentru evidenţierea oricărui stigmat subiacent. Această tehnică pare sigură şi facilitează terapia endoscopică. Orice vas vizibil sau orice sângerare activă vor fi tratate prin electrocauterizare.

METODE ENDOSCOPICE PENTRU CONTROLUL SÂNGERĂRII. Modalităţile curente terapeutice pentru HDS-NV includ injectarea de substanţe farmacologice (mai ales adrenalină diluată), terapia de contact termic prin termocoagulare sau electrocoagulare, metodele termale non-contact (mai ales coagularea în plasmă de argon sau cu laser Nd:YAG), terapia mecanică prin tehnica ligaturii sau plasării de endoclipsuri şi modalităţi de terapie combinată (mai ales injectarea asociată uneia din celelalte modalităţi enumerate).

Terapia prin injectare reduce fluxul sanguin mai ales prin tamponadă locală. Agenţii vasoconstrictori însă (adrenalina diluată 1/10 000-1/20 000) pot reduce suplimentar fluxul sanguin. Pot fi utilizaţi şi alţi agenţi sclerozanţi (etanolamina) sau trombogeni, deşi sunt mai puţin eficienţi. Aceste terapii pot fi ineficiente în sângerarea activă a ulcerelor fibrotice sau penetrante. Ca monoterapie, injectarea nu pare la fel de eficientă ca alte monoterapii şi nu trebuie utilizată singular. Date recente indică că o doză mai mare de soluţie injectată (20-30 ml în total) ar fi mai eficientă decât dozele mai mici (< 10ml) folosite anterior. Dar în acelaşi timp, doze mai mari de adrenalină cresc riscul efectelor adverse cardiovasculare, mai ales dacă injectarea este efectuată în regiunea esofagiană.

Utilizarea metodelor termice pentru terapia HDS-NV este foarte răspândită. În prezent, electrocauterizarea bipolară este folosită extensiv, având avantajul eficienţei atât din incidenţă perpendiculară, cât şi tangenţială. Procedura se consideră eficientă atunci când vasul sanguin s-a aplatizat complet iar sângerarea s-a oprit.

Terapia combinată este utilizată frecvent şi implică obişnuit tehnica de injectare de adrenalină diluată 1/10 000 asociată unei proceduri de termocoagulare. Există catetere care permit atât injectarea cât şi coagularea. Terapia combinată este sigură şi eficientă. Lin şi colaboratorii au comparat injectarea de adrenalină singură faţă de cauterizarea bipolară singulară şi faţă de terapia combinată la 96 pacienţi cu sângerare activă sau vase vizibile fără sângerare. Deşi rata hemostazei iniţiale a fost similară în cele trei grupe, rata resângerării a fost semnificativ mai redusă în grupul în care s-a aplicat terapia combinată. Prin urmare, terapia combinată asigură un control mai durabil al hemoragiei comparativ cu monoterapia.

Larg utilizate în alte ţări, endocliopsurile s-au răspândit mai recent şi în România. Studii comparative ale utilizării lor arată o eficienţă primară similară în ceea ce priveşte hemostaza iniţială. Mai mult, rata de sângerare poate fi redusă prin utilizarea endoclipsurilor comparativ cu alte modalităţi terapeutice. În prezent, pacienţii care necesită terapie endoscopică pentru hemoragia nonvariceală vor fi trataţi fie cu terapia combinată care asociază injectarea de adrenalină cu termocoagularea, fie cu endoclipsuri, cu sau fără injectare de adrenalină.

Endoscopia de control (second-look) presupune repetarea endoscopiei digestive la un interval bine precizat, după prima endoscopie terapeutică. De obicei, este efectuată la 24 ore de la endoscopia iniţială. La a doua endoscopie sunt abordate terapeutic orice stigmate de hemoragie, cum ar fi vasul sanguin vizibil. Endoscopia second-look este benefică cel puţin în anumite circumstanţe.

TRATAMENTUL HEMORAGIEI DIGESTIVE SUPERIOARE RECURENTE

Evoluţia naturală a pacienţilor trataţi endoscopic arată că dacă la 80-90% dintre ei hemostaza efectuată este definitivă, 10-20% vor resângera în pofida succesului terapeutic endoscopic iniţial (fig.2). În acest grup, doar 50% vor beneficia de controlul permanent al hemoragiei prin a doua terapie endoscopică.

Pentru restul, va fi necesară recurgerea la alte modalități terapeutice hemostatice, cum ar fi angiografia sau chirurgia.

În cazul pacienților care resângerează, după succesul terapeutic endoscopic inițial, este important să se răspundă la întrebarea dacă vor beneficia de al doilea tratament endoscopic sau dacă ar fi mai bine să li se acorde tratamentul chirurgical. Dacă studiile efectuate pe acest subiect arată că mortalitatea, durata spitalizării şi numărul transfuziilor efectuate au fost similare la grupul cu terapie endoscopică repetată şi la cel tratat chirurgical, rata complicațiilor este mai mare în acest ultim lot (16 față de 6, P=0.03). Predictorii eşecului repetării terapiei endoscopice sunt: ulcerul de peste 2 cm şi prezența hipotensiunii arteriale. Tratamentul endoscopic reduce necesarul chirurgical fără să crească rata complicațiilor. De aceea, în majoritatea cazurilor repetarea terapiei endoscopice este recomandată înaintea altor opțiuni terapeutice.

Importantă este şi identificarea corectă a pacienților care vor beneficia în ultimă instanță de chirurgie pentru ca adresarea acestora spre acest tip de intervenție să se facă în timp optim. De aceea este importantă implicarea precoce a chirurgului în managementul pacienților care ar putea fi candidați la intervenția chirurgicală, în special în cazul pacienților etichetați cu risc crescut.

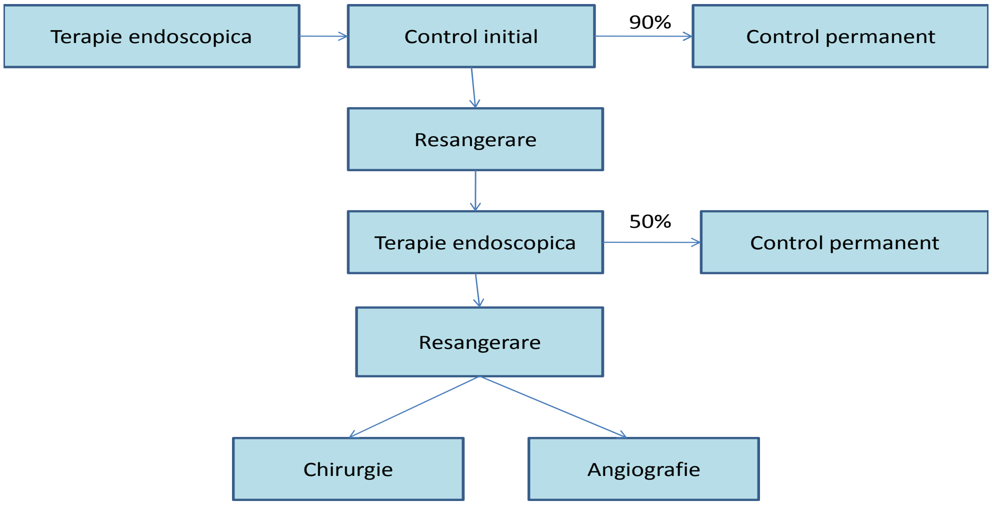

Fig. 2. Evoluția naturală a pacienților cu hemostază endoscopică

IMPACTUL ANTICOAGULĂRII ASUPRA RESÂNGERĂRII

Utilizarea tot mai largă a medicației anticoagulante în ultimii ani conferă o dificultate crescută în managementul hemoragiilor digestive. Sunt incluse atât anticoagulantele mai vechi, ca de exemplu warfarina şi heparina, cât şi agenții antiplachetari mai noi, larg utilizați în bolile cardiovasculare, mai ales după montarea stenturilor coronariene. Deşi datele cu privire la ghidarea managementului acestor pacienți sunt relativ puține, în general se recomandă menținerea pe cât posibil a anticoagulantului cu reducerea sau chiar normalizarea INR-ului. Cele mai frecvente cauze de HDS sunt descrise în tabelul de mai jos.

Tabelul 4: Cauzele frecvente şi prevalența hemoragiei digestive superioare (studiu al Societății Americane de Endoscopie Gastrointestinală pe 2 225 cazuri cu HDS)

Sursa de sângerare	**Prevalența (%)**
Ulcerul duodenal	24,3
Eroziunile gastrice	23,4
Ulcerul gastric	21,3
Varicele esogastrice	10,3
Sindromul Mallory-Weiss	7,2
Esofagita	6,3
Duodenita erozivă	5,8

Se poate observa că, dintre cauzele de HDS non variceală, pe primele locuri sunt ulcerele peptice şi gastritele eroziv-hemoragice. Sindromul Mallory-Weiss, caracterizat prin fisuri la nivelul joncțiunii esogastrice asociate vărsăturilor intempestive, de obicei la tineri şi la consumatorii de alcool, este o cauză relativ frecventă, dar care rămâne adeseori nediagnosticată dacă endoscopia digestivă superioară se efectuează la mai mult de 48 ore (în acest interval, leziunile se vindecă spontan, fără sechele).

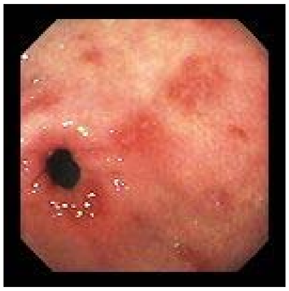

Fig. 3. Gastrită antrală erozivă indusă de AINS

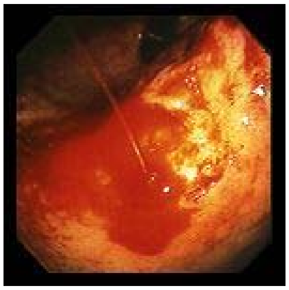

Fig. 4. Ulcer gastric cu hemoragie arterială

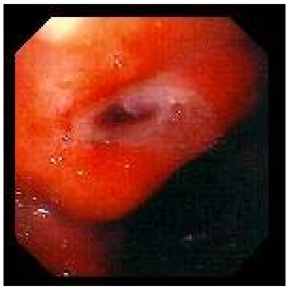

Fig. 5. Ulcer bulbar cu cheag vizibil

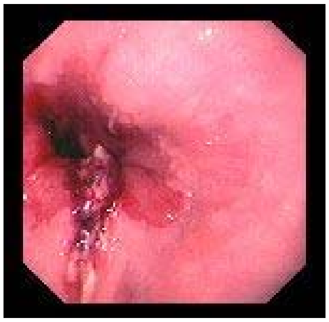

Fig. 6. Sindrom Mallory-Weiss (joncțiunea esogastrică)

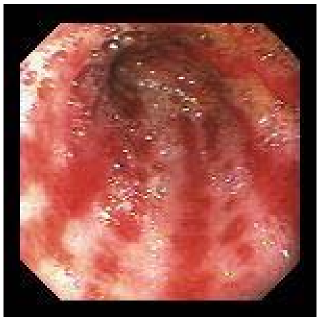

Fig.7. Ectazie vasculară gastrică

HEMORAGIA VARICEALĂ

Considerații generale

Varicele esofagiene şi gastrice pot apărea în afara oricăror manifestări clinice. La pacienții cirotici, mai ales în stadiile avansate, se instituie măsuri de screening pentru detecția varicelor în vederea reducerii riscului hemoragiei inițiale. Hemoragia acută variceală diferă de sângerarea nonvariceală prin mai multe aspecte. Astfel, doar 50% dintre hemoragiile variceale se opresc spontan, comparativ cu 80-90% dintre hemoragiile digestive superioare non-variceale. După oprirea hemoragiei active variceale, riscul recurenței sângerării este de până la 70% în primele 6 săptămâni. Riscul maxim de recurență hemoragică este în primele 48-72 ore. Peste 50% dintre toate episoadele hemoragice recurente apar în primele 10 zile. Supraviețuirea în cele 6 săptămâni după hemoragia inițială este direct corelată cu resângerarea. Factorii de risc ai recidivei hemoragiei variceale includ vârsta de peste 60 de ani, varicele mari, sângerarea inițială severă (cu Hb < 8g/dl la internare) şi insuficiența renală. Mortalitatea este între 15-50% pentru fiecare episod hemoragic şi de 70-80% în cazul persistenței sângerării. Hemoragia variceală este răspunzătoare pentru 1/3 din toate cazurile de deces din ciroza hepatică.

Patogeneză

Varicele esofagiene şi gastrice apar ca urmare a hipertensiunii portale. Aceasta poate avea cauze prehepatice (tromboza venei porte, schistosomiaza etc.), cauze intrahepatice (ciroza, mai ales) şi cauze posthepatice (tromboza venei hepatice, insuficiența cardiacă şi pericardita constrictivă). Varicele sunt colaterale venoase dilatate cu perete subțiat şi slăbit care are tendința să se rupă şi să sângereze masiv. Varicele pot fi izolate la nivelul stomacului în tromboza venei splenice, în pancreatita acută sau în tumorile pancreatice.

Manifestări clinice

Semne şi simptome. Simptomele varicelor hemoragice sunt nespecifice şi includ hematemeza, melena şi hematochezia. Pacienţii pot prezenta ameţeli, cefalee iar cei cu hepatopatie severă, encefalopatie hepatică. Alte manifestări clinice sunt cele asociate cirozei hepatice, şi anume icterul, steluţele vasculare, eritemul palmar, ascita şi contractura Dupuytrene.

Modificările de laborator. Enzimele hepatice (alanin-aminotransferaza - ALAT şi aspartat-aminotransferaza - ASAT) sunt modificate în prezenţa hepatopatiei active. Pacienţii pot avea hiperbilirubinemie şi afectarea funcţiei de sinteză, cu hipoalbuminemie şi INR crescut. În cazul supresiei medulare de cauză etanolică, pacienţii pot prezenta pancitopenie. Cei cu hipersplenism pot avea trombocitopenie. În cazul coexistenţei insuficienţei hepatice poate apărea hipoglicemia iar sindromul hepato-renal se însoţeşte cu uree şi creatinină serice crescute.

Manifestări imagistice. Endoscopia digestivă superioară este modalitatea primară de diagnostic, permiţând şi terapia endoscopică. Criteriile Societăţii Japoneze pentru Studiul Hipertensiunii Portale clasifică varicele în funcţie de mărimea şi forma lor. Astfel, varicele mici şi drepte sunt clasificate ca F1, cele largi şi tortuoase care ocupă < 1/3 din lumen sunt F2, iar cele mai mari, care ocupă peste 1/3 din lumenul esofagian, sunt F3. Examenul baritat esofagian poate detecta varicele medii şi mai ales mari, dar nu prezintă sensibilitate pentru varicele mici iar utilizarea lui împiedică alte manevre diagnostice şi terapeutice ulterioare (endoscopia, angiografia etc.). CT-ul abdominal sau ecografia abdominală pot detecta prezenţa vaselor colaterale. Angiografia venei porte poate demonstra prezenţa colateralelor şi recanalizarea venei ombilicale. Gradientul de presiune hepatic venos de peste 12 mm Hg este predictiv pentru riscul hemoragic.

Diagnosticul diferenţial include toate cazurile de hemoragie digestivă superioară nonvariceală. Pacienţii cu ciroză şi HDS sângerează la nivelul varicelor în 50-90% cazuri. Ei pot prezenta însă şi alte surse de sângerare, cum ar fi esofagita, ulcerul peptic, sindromul Mallory-Weiss etc.

Complicaţiile varicelor esogastrice hemoragice pot fi severe, până la sângerare masivă şi deces. Hemoragia variceală acută pe teren cirotic poate agrava hepatopatia de fond, cu apariţia sau agravarea encefalopatiei hepatice şi a sindromului hepato-renal. Infecţiile bacteriene sunt prezente la 20% din ciroticii care sângerează variceal şi ajung la 50% din cazurile internate pentru hemoragie variceală. S-a dovedit că antibioterapia în hemoragia variceală acută scade rata infecţiei ulterioare, a peritonitei bacteriene spontane, a bacteriemiei şi a decesului.

Tratamentul hemoragiei variceale acute necesită utilizarea unor modalităţi multiple, simultane, care includ terapia medicală şi endoscopică. Va fi evitat excesul de resuscitare deoarece supraîncărcarea de volum se asociază cu persistenţa sau recurenţa sângerării. Complementar, se poate recurge la intervenţii radiologice şi chirurgicale.

Tratamentul medical este iniţiat înaintea evaluării endoscopice. Antibioprofilaxia predecesoare endoscopiei, la cirotici, reduce complicaţiile infecţioase şi scade mortalitatea. Medicaţia reduce sângerarea activă variceală. Terlipresina este un analog sintetic al vasopresinei cu abilităţi similare de control al sângerării şi de reducere a mortalităţii ca ale acesteia, fără multe din efectele sale sistemice. Somatostatina şi analogul său, octreotidul, au efect vasoconstrictor indirect şi de reducere a fluxului sanguin portal. Octreotidul se administrează in bolus i.v. de 25-50mcg, urmat de infuzie continuă i.v. de 25-50mcg/h. S-a dovedit că octreotidul şi somatostatina sunt utile pentru obţinerea hemostazei şi prevenirea resângerării precoce. După stabilizarea pacienţilor cu episod hemoragic variceal acut se introduce terapia beta-blocantă, pentru profilaxia resângerării.

Tratamentul endoscopic hemostatic este de elecţie în hemoragia digestivă variceală. Obiectivele endoscopiei constau în depistarea şi tratarea sursei hemoragice. Varicele sunt evaluate pentru existenţa semnelor de hemoragie activă sau a marcherilor de hemoragie recentă. Aceşti marcheri includ varice mari tortuoase, varice pe varice, spoturi roşu-vişinii plate şi spoturi hemocistice uşor supradenivelate pe varice. Trombii fibrino-plachetari sunt protruzii mamelonate pe varice, indicative pentru sediul unei hemoragii recente şi pentru riscul foarte crescut de resângerare. Terapia endoscopică este aplicată iniţial în esofagul distal la joncţiunea esogastrică şi apoi extinsă proximal. Majoritatea hemoragiilor variceale se situează în esofagul distal pe ultimii 5-10 cm. Varicele gastrice răspund mult mai slab la terapia endoscopică. Mai recent s-au obţinut rezultate satisfăcătoare prin injectare de cianoacrilat, de fibrină sau aplicarea de endoclipsuri. Scleroterapia endoscopică este metoda consacrată de injectare intra şi/sau perivariceală a 1-3 ml agent sclerozant (moruat de sodiu, etanolamină etc.) la nivelul fiecărui sediu hemoragic. Deşi eficientă, nu este lipsită de unele complicaţii, precoce sau tardive, cum ar fi: ulceraţiile, resângerarea, disfagia, stenozele, mediastinita sau emboliile pulmonare sau cerebrale (pentru cianoacrilat). Ligatura prin bandare variceală este mult mai eficientă, asociată cu mai puțină resângerare, mai puține complicații, cu mortalitate mai redusă și necesită un număr mai redus de ședințe pentru obliterarea variceală totală. Bandarea variceală controlează hemoragia acută în 90% dintre cazuri, cu o rată de resângerare de 30%, fiind astfel metoda de elecție în hemoragia variceală acută. Prezintă dificultăți de execuție și de depistare a sediului hemoragiei în sângerarea activă mare. De asemenea, aplicarea metodei în retroflexie endogastrică este prohibitivă.

Tamponada cu balon este eficientă pentru hemostaza pe termen scurt și este rezervată în cazul hemoragiilor necontrolate endoscopic sau în cazul hemoragiilor masive care contraindică endoscopia. Cel mai utilizat este tubul Sengstaken-Blakemore, prevăzut cu un balon gastric mare, de 250 ml și un altul, esofagian, precum și un sistem de aspirație gastrică. Balonul gastric este insuflat, după verificarea radiologică a poziționării corecte intragastrice. Și balonul esofagian poate fi insuflat la nevoie, dar cu riscul de perforație esofagiană, care crește semnificativ mortalitatea. În lipsa hemostazei definitive, resângerarea, după deflația balonului, este aproape regula. De aceea, tamponada cu balon este o metodă temporară, de stabilizare a pacientului înaintea aplicării unei metode hemostatice definitive.

Terapia radiologică realizează un șunt porto-sistemic transjugular intrahepatic conectând, cu ajutorul unui stent, sistemul venos port cu una dintre venele hepatice. Complicațiile includ encefalopatia datorată șuntului, ocluzia șuntului cu resângerare și migrarea șuntului. Metoda este rezervată în general pacienților cu hemoragie variceală, persistentă în pofida intervențiilor medicale și endoscopice anterioare.

Terapia chirurgicală este variată și poate fi utilizată pentru controlul hemoragiei variceale, atât esofagiene cât și gastrice. Se poate realiza un șunt porto-sistemic fie neselectiv, porto-cav, fie selectiv – spleno-renal distal. Alternativ, se poate efectua transsecția esofagiană, cu ligatura variceală și devascularizarea joncțiunii gastroesofagiene (procedura Sugiura). Intervenția ultimă pentru controlul hemoragiei variceale este transplantul hepatic. De această procedură beneficiază mai ales pacienții trecuți pe lista de așteptare pentru transplant datorită insuficienței hepatice semnificative. În general, pacienții cu evoluție favorabilă după efectuarea șuntului chirurgical au funcția hepatică bine prezervată (Clasa A Child-Turcotte-Pugh), dar nu au răspuns eficient la terapia endoscopică.

Evoluție și prognostic. Hemoragia variceală este o cauză semnificativă de resângerare și de deces. Sângerarea variceală se oprește în aproximativ 50% din cazuri, dar la cei cu sângerare persistentă, mortalitatea se apropie de 80%. Riscul de resângerare este mare (până la 70%) până la efectuarea obliterării varicelor eso-gastrice. În cazul în care pacientul supraviețuiește episodului inițial hemoragic, bandarea endoscopică se repetă la fiecare două săptămâni, până la eradicarea totală variceală. Tehnica se combină cu terapia medicală cu blocant beta-adrenergic. Prognosticul pacienților cu hemoragie variceală gastro-esofagiană este rezervat, chiar dacă se reușește controlul hemoragiei, deoarece ea însăși este indicativă pentru stadiul avansat al hepatopatiei. Pacienții pot deceda prin decompensare hepatică, resângerare, infecții, insuficiență renală sau alte complicații.

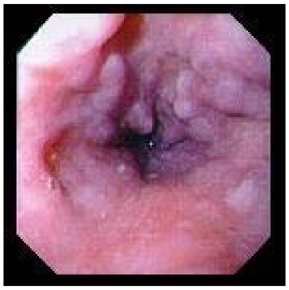

Fig. 8. Varice esofagiene medii

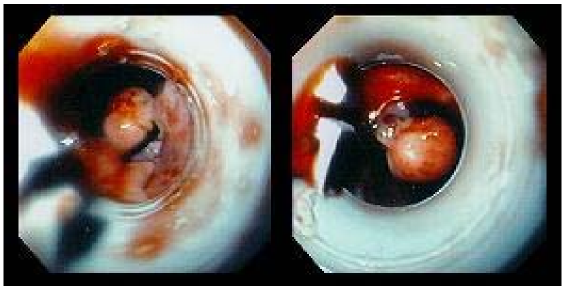

Fig.9. Bandarea endoscopică a varicelor esofagiene

În concluzie, hemoragiile digestive superioare acute reprezintă un subiect important de patologie în practica gastroenterologică, care pune în valoare valențele diagnostice și terapeutice ale endocopiei esofagogastroduodenale.

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Caracterizarea tulpinii *Bacillus anthracis* 1190 R prin tehnici proteomice

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Rezumat. Tulpina vaccinală *Bacillus anthracis* 1190 R a fost utilizată ca model experimental pentru caracterizare proteomică, prin spectrometrie de masă MALDI-TOF (Matrix Assisted Laser Desorbtion Ionization – Time of Flight), cu sistemul de identificare pentru agenţi biologici Microflex LT 20. Sistemul Microflex LT 20 este un spectrometru de masă, echipat cu baze de date pentru identificarea agenţilor biologici, procesele de achiziţie, salvare şi stocare a informaţiilor fiind asistate de calculator. Pentru a fi analizate cu spectrometrul de masă Microflex LT 20, agenţii biologici din genul *Bacillus spp.,* proveniţi din colecţia INCDMI Cantacuzino, au fost în primul rând cultivaţi şi caracterizaţi morfo-tinctorial şi cultural, utilizând metode bacteriologice clasice. Culturile de *Bacillus anthracis* 1190 R, identificate prin metode bacteriologice clasice, au fost testate cu aparatul Microflex LT 20 (programul Bio Profiler Expert) pentru obţinerea spectrelor specifice tulpinii analizate (semnătura proteică). Spectrele obţinute au fost analizate cu programul Flex Analysis pentru caracterizarea proteomică a tulpinii *Bacillus anthracis* 1190R. Rezultatele au evidenţiat faptul că tulpina analizată a prezentat un domeniu de masă caracteristic genului Bacillus, cuprins între 1272-11534 Da, precum şi picuri caracteristice tulpinii *Bacillus anthracis*, 9643,48 Da şi 9960,72 Da. Spectrele de masă examinate au fost identificate ca aparţinând speciei bacteriene *Bacillus anthracis,* practic fiind confirmate rezultatele obţinute prin tehnicile bacteriologice clasice.

Cuvinte cheie: agenţi biologici, *Bacillus anthracis*, spectrometrie de masă

Abstract. Vaccinal strain of *Bacillus anthracis* 1190 R it was used as an experimental model for proteomics characterization trough mass spectrometry, MALDI-TOF (Matrix Assisted Laser Desorption Ionization – Time of Flight), with identification system for biological agents Microflex LT 20. Microflex LT 20 system, is a mass spectrometer complete with data base for biological agents identification, the information's acquisition, saving and storing processes are computer controlled. In order to be analyzed using mass spectrometer Microflex LT 20, genus Bacillus spp. Biological agents, resulted from INCDMI Cantacuzino collection, was, first of all, cultivated and morfotinctorial and cultural characterized, using classic bacteriologic methods. *Bacillus anthracis* cultures1190 R, identified trough classic microbiological methods, was tested with Microflex LT 20 machine, (Bio Profiler Expert program) for having spectrums specific for analyzed strain (proteic signature). The resulted spectrums were analyzed with Flex Analysis program for proteomic characterization of *Bacillus anthracis* 1190R strain. The results, have revealed that the analyzed strain have presented a mass domain characteristic to Bacillus genus, between 1272 Da and 11534 Da, and also peaks specific to Bacillus athracis strain, 9643,48 Da and 9960,72 Da. Examined mass spectrums was identified as belonging to bacterial species *Bacillus anthracis*, in fact, the results obtained trough classic bacteriological methods are now confirmed.

Introducere

Antraxul este o maladie cunoscută de oameni din timpuri străvechi, fiind menţionată în însemnările din papirusurile egiptene, în scrierile arabe, ca şi în operele clasicilor antici (Homer, Virgiliu, Ovidiu). Posibilitatea de transmitere a bolii nu a fost luată în calcul de primii cercetători care au studiat mai amănunţit boala. În monografia lui Fournier "Observation et experiences sur le charbon malign", apărută în anul 1869 la Dijon, sau tratatul lui Chabert "Traite du charbon ou anthrax" au fost enumeraţi mai mulţi factori de mediu (uscăciunea, umiditatea, natura păşunilor) ca principali agenţi cauzali ai bolii. La sfârşitul secolului al XVIIIl-lea şi mai ales în prima jumătate a secolului al XIX-lea se deschide o nouă etapă în cercetarea bolilor transmisibile prin demonstrarea modului de propagare a acestora. Lista personalităţilor care au abordat acest subiect este foarte lungă, însă nu putem să-i omitem pe Robert Koch, cercetător genial care a utilizat *Bacillus anthracis* pentru elaborarea primului model complet al infecţiei experimentale, reproductibilă în laborator. În aceeaşi perioadă Luis Pasteur, împreuna cu tânărul discipol Emile Roux, a realizat primul vaccin eficace contra antraxului la animale, fapt demonstrat în celebra experienţă din anul 1881 în localitatea Poully le Fort. În România în secolul al XIX-lea boala a avut o distribuţie foarte largă, fiind întâlnită pe tot teritoriul ţării, producând pagube importante dar şi numeroase cazuri de îmbolnăviri la oameni. Aceste lucruri au determinat autorităţile ca în anul 1808 să impună crescătorilor de animale măsuri sanitar-veterinare restrictive fără precedent (restricţii de circulaţie, izolarea animalelor, interzicerea tăierilor). După anul 1935, prin munca asiduă a profesorului Stamatin s-a reuşit obţinerea unei tulpini de *Bacillus anthracis* asporogenă, edematogenă şi bună imunogenă, punându-se astfel bazele primului vaccin românesc contra antraxului, vaccin finalizat în anul 1946 cu sprijinul doctorului Isopescu, cercetător la Institutul Pasteur din Bucureşti. Începând cu anul 1948, măsurile generale precum şi vaccinările aplicate planificat au condus la diminuarea cazurilor de antrax la om şi la animale. În ţara noastră se utilizează de peste 60 de ani vaccinul cărbunos de uz veterinar, care conţine spori viabili ai tulpinii *Bacillus anthracis* 1190 R (Stamatin).

Bacillus anthracis este agentul biologic cel mai cunoscut şi folosit pentru fabricaţia de arme biologice, utilizat şi de către terorişti. Statele lumii au luat în serios riscurile bioterorismului şi au elaborat planuri de măsuri pentru lupta împotriva acestui flagel. În cadrul acestor măsuri o preocupare majoră o constituie şi găsirea unor metode rapide şi precise pentru detecţia şi identificarea agenţilor biologici potenţial utilizabili în bioterorism (*Bacillus anthracis, Yersinia pestis, Francisella tularensis, Variola major* etc.). Pe de altă parte, ONU a adoptat mai multe rezoluţii împotriva terorismului, dintre care amintim: "Tratatul cu privire la neproliferarea armelor nucleare", din 01.07.1968, şi „Convenţia cu privire la interzicerea perfecţionării, producţiei şi stocării armelor bacteriologice (biologice) şi cu toxine şi la distrugerea lor" (BTWC 1972).

Tulpina vaccinală *Bacillus anthracis* 1190 R a fost utilizată ca model experimental pentru caracterizarea proteomică prin spectrometrie de masă, MALDI-TOF (Matrix Assisted Laser Desorbtion Ionization – Time of Flight), a unui agent biologic din genul *Bacillus spp*., cu sistemul de identificare a agenţilor biologici Microflex LT 20. Sistemul este compus din: elementul de bază, aparatul Microflex LT 20, software aferent (Bio Typer, Bio Profiler, Flex Control, Flex Analisys şi software control-analiza aparat), calculator pentru preluare şi prelucrare date, imprimantă. Elementul de bază este un MALDI TOF, având domeniul de masă 1 000-20 000 Daltoni. Controlul instrumentului se efectuează cu ajutorul unui computer care utilizează sistemul de operare Windows, fapt ce permite efectuarea proceselor de achiziţie şi evaluare a datelor. Computerul controlează sistemul, precum şi procesele de achiziţie, salvare şi stocare a informaţiilor spectrale şi permite procesarea ulterioară a informaţiilor şi accesul la bazele de date.

Material şi metodă

Genul *Bacillus spp.* cuprinde bacili gram-pozitivi, în culturile tinere, unii gram-variabili, în culturile bătrâne, sporulaţi; cei mai mulţi mobili prin flageli peritrichi, aerobi sau facultativ anaerobi, oxidazo-pozitivi şi o mare parte catalazo-pozitivi. Au o diversitate a caracterelor fiziologice: specii psihrofile, mezofile sau termofile, alcalofile sau acidofile, halotolerante ori halofile. Experimentul a fost efectuat folosind tulpina vaccinală *Bacillus anthracis 1190 R* Stamatin, provenită din colecţia Institutului Naţional de Cercetare-Dezvoltare Cantacuzino (INCDMI Cantacuzino). Tulpina de bacil cărbunos a fost cultivată pe medii de cultură uzuale, folosind tehnici bacteriologice clasice pentru obţinerea de colonii izolate. Coloniile microbiene din genul *Bacillus spp.* obţinute au fost identificate prin metode bacteriologice clasice, operaţiune validată ulterior cu sistemul de identificare a agenţilor biologici Microflex LT 20.

Caracterizarea tulpinilorde *Bacillus anthracis* prin metode bacteriologice clasice (caractere culturale şi morfotinctoriale)

Din tulpina de colecţie au fost efectuate însămânţări pe medii de cultură uzuale, bulion nutritiv, agar nutritiv şi geloză-sânge. Mediile de cultură au fost incubate timp de 72 de ore la temperatura de 37ºC. La intervalele de 24, 48 şi 72 de ore culturile au fost examinate macroscopic şi microscopic. *Bacillus anthracis* prezintă pe substraturile de cultivare aspecte caracteristice. În bulion simplu cultura apare sub forma unui depozit floconos, greu omogenizabil, mediul de cultură rămânând limpede. La examenul microscopic (coloraţia Gram) bacilii sunt coloraţi în albastru-violet şi sunt grupaţi în lanţuri lungi cu aspect de bastonaş caracteristic. În culturile pe agar apar colonii mari, rugoase, cu contur neregulat, bacilii fiind grupaţi în lanţuri mai scurte sau izolaţi. Marginile coloniilor, examinate cu lupa, apar înconjurate de prelungiri laterale, ondulate, asemănătoare cu şuviţele de păr (aspect de „cap de meduză"). Pe mediul geloză-sânge, *Bacillus anthracis* formează, după incubare peste noapte, la temperatura de 37°C, colonii mari, netede, cu diametrul de 3-5 mm, alb-cenuşii, turtite, nehemolitice ori slab hemolitice.

Culturile obţinute au prezentat toate caracterele specifice genului *Bacillus spp.,* fapt care a permis să tragem concluzia că tulpina analizată aparţine speciei *Bacillus anthracis*.

Caracterizarea tulpinilor de *Bacillus anthracis* prin spectrometrie de masă MALDI-TOF

Condiţiile de cultivare a microorganismelor pentru generarea de spectre proteice sunt foarte importante în economia tehnicii de identificare Maldi-Tof. În acest sens pot fi utilizate medii minimale (geloză simplă, geloză sânge), dar şi medii speciale (mediul Plet), respectând temperaturile optime de cultivare a speciilor bacteriene luate în studiu, pentru a obține o reproductibilitate fidelă şi termeni egali de comparare între tulpini şi baza de date. Pentru a obține rezultate finale corecte se recomandă utilizarea de materiale proaspăt crescute peste noapte (culturi de 24 de ore), dacă este posibil, sau bacterii crescute lent, cultivate timp de mai multe zile. Dacă plăcile cultivate sunt stocate la temperatura de 4°C, calitatea spectrelor obținute scade relativ repede (în termen de câteva zile). Trebuie menționat în mod deosebit aspectul privind puritatea culturilor, care poate fi controlată prin executarea de frotiuri colorate Gram dublu şi examinate la microscopul optic, cu obiectivul cu imersie.

Extracția proteinelor 16S din ribozomi

Culturile de *Bacillus anthracis* au fost însămânțate prin scarificare cu ansa (tehnica epuizării pe placă) pe geloză simplă şi geloză sânge, în plăci Petri, în vederea obținerii de colonii izolate. Incubarea s-a realizat la temperatura de 37°C, timp de 24 de ore. Din fiecare placă Petri au fost prelevate câte 3 colonii, fiecare colonie constituind în final o probă dublă. Extracția proteinelor ribozomale 16S a fost efectuată prin tehnica “Microorganisme Profiling “, extracție TFA 80 %, după cum urmează: se prelevează de pe placa de cultură materialul biologic, se descarcă ansa în tubul Eppendorf şi se adaugă 50 µl TFA 80%. Suspensia obținută se pipetează până la dizolvarea completă. Se aşteaptă 10-30 de minute. În continuare, se adaugă 3 Vol H_2O (150 µl) şi Vol 100% AN (200 µl). Se amestecă prin centrifugare la viteza maximă aproximativ 2 minute şi se transferă supernatantul într-un tub Eppendorf nou. La pasul următor se prelevează 1-2 µl din supernatantul anterior, se etalează pe plăcuța țintă (în godeu) şi se usucă cu aer. După uscare, fiecare godeu se acoperă cu 2 µl soluție de matrice, se usucă din nou cu aer şi se examinează la spectrometrul de masă Microflex LT 20

Prepararea soluției Matrix

Soluția matrice utilizată pentru obținerea spectrelor are aceeaşi compoziție indiferent de tehnica de extracția folosită. Matrixul este o soluție saturată de α-HCCA, 50% acetonitril (AN), 2,5% acid tri-fluoracetic (TFA). Compoziția de 50% AN / 2,5% TFA plus este denumită solvent organic de bază (OS). Pentru preparare se prelevează câteva cristale de α-HCCA, care se pun într-un tub de plastic Eppendorf. Peste cristale se adaugă 200-500 µl solvent organic de bază. Se agită energic câteva minute, apoi se ține la temperatura camerei până ce soluția se saturează. Pentru a elimina cauzele nereuşitelor este indicat a se utiliza produse chimice de cea mai mare puritate chimică (potrivite pentru MALDI sau HPLC). Matrixul odată preparat poate fi stocat la frigider până la o săptămână.

Tehnica de lucru

Analizarea probelor începe prin aplicarea unei cantități mici de material biologic direct pe placa țintă, sub forma unui film subțire. Materialul de start poate fi o colonie unică sau o parte din materialul biologic obținut în urma extracției proteinelor ribozomale conform tehnicii descrise anterior. Filmul microbian subțire se acoperă cu 2 µl de soluție matrice şi se usucă cu aer. Ca matrice este folosită o soluție saturată de acid alfa-ciano-4-hidroxicinamic (AACHC) într-un solvent organic (SO). Solventul organic se compune din 50% acetonitril (ACN), 2.5% acid trifluoroacetic (ATF) în apă distilată. Plăcuța țintă cu materialul biologic etalat şi uscat se introduce în docul aparatului Microflex LT 20 şi se începe examinarea. Din fiecare placă Petri cu cultură de *Bacillus anthracis* 1190 R au fost prelevate câte două colonii care, după extracția proteinelor ribozomale 16S, au fost etalate pe 4 godeuri ale plăcuței țintă. Pe fiecare probă (godeu) au fost executate cel puțin trei până la 5 trageri, rezultând aproximativ câte 10 spectre pentru fiecare colonie prelevată. Aceste spectre au fost analizate şi comparate cu spectrele din baza de date a aparatului, selectând scorurile ≥80.

Rezultate şi discuții

Analiza spectrelor agenților biologici din genul Bacillus, reprezentând fragmentele polipeptidice 16S din ribozomii, a relevat un domeniu de masă cuprins între 1 000 şi 12 000 daltoni, cu vârfuri (picuri) cuprinse în domeniul 1 200-11 500 Da. Media picurilor caracteristice genului a fost cuprinsă în următoarele domenii de masă: 3 550-3 650; 4 600-4 950; 5 400-5 900; 6 300-7 300; 9 300-10 300; 11 500-12 000; 10 500-11 500 Da. Scorul mediu luat în calcul pentru introducerea spectrelor în baza de date a fost de ≥80; este reprezentativ şi dă certitudinea unei identificări corecte.

Compoziția diferită a mediilor de cultură (geloză-sânge sau geloză nutritivă) nu are efect semnificativ asupra modelului de distribuție a picurilor, remarcându-se totuşi anumite diferențe ale spectrelor, în special în domeniile de masă cuprinse între 3 000-10 000 Da, diferențe care pot fi atribuite componentelor variate ale mediilor de cultură testate şi implicațiilor acestora în metabolismul bacterian. Trebuie remarcat şi faptul că prezența mediului de cultură aderent la colonii (prelevat odată cu colonia de bacterii) nu se traduce în vreun semnal care să demonstreze influența sa asupra rezultatului testării.

Faza de dezvoltare (creştere) a celulelor influențează într-un grad mic rezultatele obținute. Celulele aflate în faza de creştere lag prezintă un model foarte asemănător ca în cazul celulelor aflate în faza staționară, sau în faza care succede imediat moartea celulelor. Mai mult, datorită faptului că pregătirea probelor şi măsurarea acestora se desfăşoară în condiții standardizate, profilurile spectrometrice şi valorile spectrelor obținute prin analizarea aceleiaşi probe sunt comparabile. În continuare sunt prezentate spectrele de masă, valorile de masă (Da) şi picurile caracteristice ale tulpinii *Bacillus anthracis* luate în studiu.

Din analiza spectrului de masă şi a greutăților moleculare ale fragmentelor polipeptidice caracteristice identificate se observă că valorile de masă pentru tulpinile de *Bacillus anthracis* 1190 R sunt cuprise în domeniul 1 272-11 534 Da.

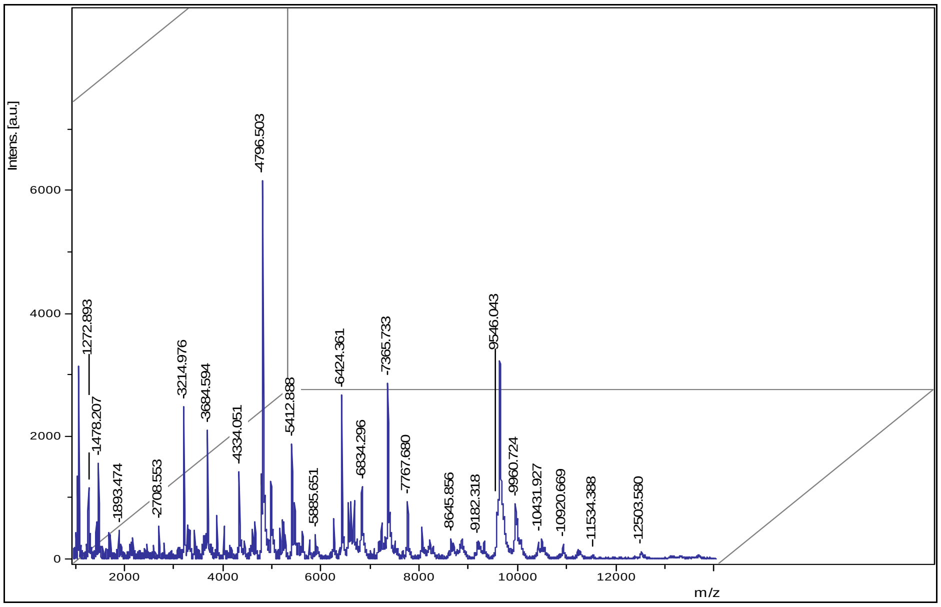

Fig. 1. Reprezentare grafică a spectrului de masă *Bacillus anthracis* 1190 R

Totodată, aşa cum se observă în reprezentările grafice ale spectrelor proteinelor ribozomale nr. 2 şi 3, au fost identificate picurile caracteristice de tulpină, care sunt cuprinse în domeniul 9 643,48 Da şi 9 960,72 Da pentru tulpina *Bacillus anthracis* 1190 R. În figura numărul 3, picul haşurat, cu greutatea moleculară de 9 643,48 Da, reprezintă semnătura proteică de specie pentru agentul biologic *Bacillus anthracis* 1190 R, tulpina utilizată pentru prepararea vaccinului anticărbunos destinat imunizării animalelor, dar şi ca model experimental în cercetarea bacilului cărbunos, fiind cunoscut faptul că tulpina vaccinală nu este patogenă.

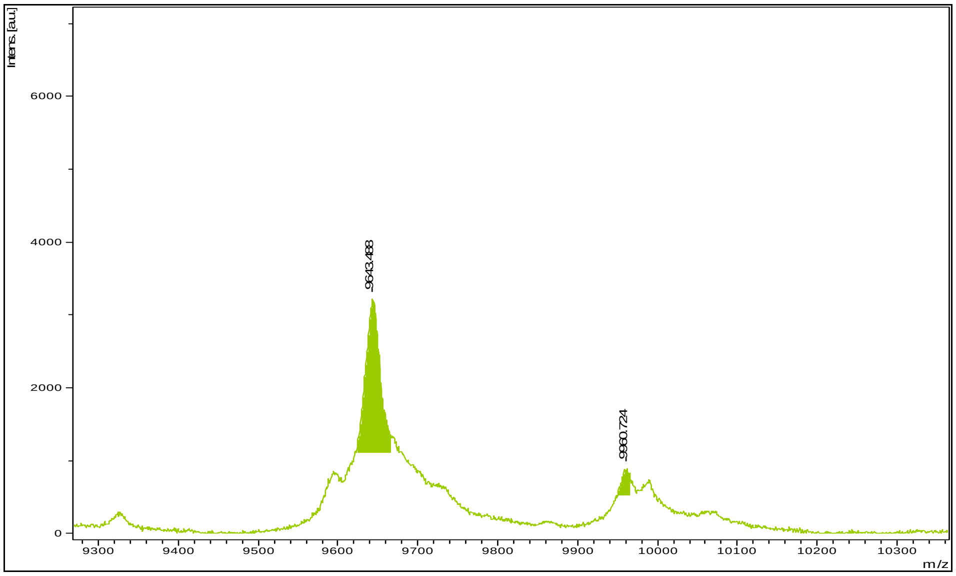

Fig. 2. Reprezentare grafică analiza spectru *Bacillus anthracis* 1190 R
"picuri" caracteristice de tulpină (9.643,48/9.960,72 Da).

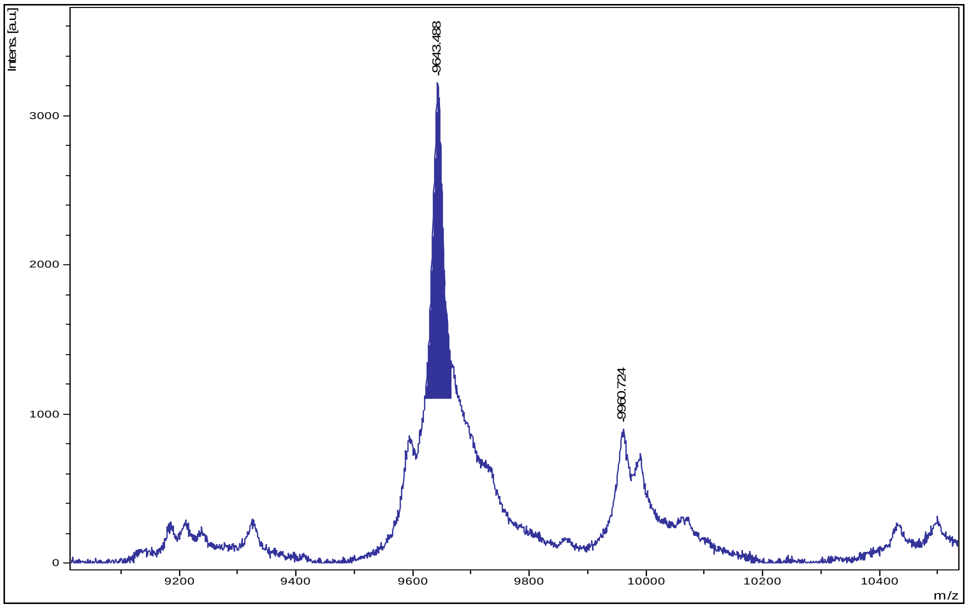

Fig. 3. Reprezentare grafică analiza spectru *Bacillus anthracis* 1190 R “pic” caracteristic de specie (9.643,48 Da).

De aici o importantă utilizare rezultată ar fi validarea identității şi purității tulpinii vaccinale din inoculul bacterian folosită în procesul de preparare a vaccinului anticărbunos. Luând ca model experimental specia *Bacillus anthracis,* pot fi optimizate tehnicile de identificare a agenților biologici prin spectrometrie de masă MALDI-TOF, pentru completarea Bazei Naționale de spectre ale tulpinilor bacteriene din genul Bacillus spp. Baza de date poate fi utilizată atunci când este nevoie să fie identificați rapid şi precis agenții biologici izolați de pe teritoriul României, dar şi din altă proveniență.

Concluzii

Tulpina *Bacillus anthracis* 1190 R. provenită din colecția INCDMI Cantacuzino, identificată prin metode bacteriologice clasice, a fost luată în studiu pentru confirmare şi caracterizare proteomică prin spectrometrie de masă, tehnologia Maldi Tof.

A fost optimizată o metodă de extracție a proteinelor ribozomale 16S derivată din metoda „Microorganisme Profiling" extracție TFA 80%, în vederea analizei şi identificării tulpinilor bacteriene din genul Bacillus spp, prin spectrometrie de masă.

Scorul mediu luat în calcul pentru introducerea spectrelor în baza de date a fost de ≥80, un scor reprezentativ, care dă certitudinea unei identificări corecte.

Media picurilor caracteristice genului a fost cuprinsă în următoarele domenii de masă: 1 100-1 300 Da; 3 550-3 650 Da; 4 600-4 950 Da; 5 400-5 900 Da; 6 300-7 300 Da; 9 300- 10 300 Da; 11 500-12 000 Da; 10 500-11 500 Da.

Importanța relativă a picurilor individuale (9 643,48 Da şi 9 960,72 Da, pentru tulpina *Bacillus anthracis* 1190R Stamatin) este reproductibilitatea (frecvența apariției) spectrelor, replici folosite pentru a construi semnătura de referință.

Tulpinile din genul *Bacillus anthracis* 1190R testate pentru confirmare cu sistemul Microflex LT 20 au fost validate în procent de 100%.

Sistemul Microflex LT 20 permite identificarea rapidă şi precisă a agenților biologici, fiind un mijloc important de confirmare/validare pentru alte metode de diagnostic microbiologic.

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Andrei Oişteanu:

Narcotice în cultura română. Istorie, religie şi literatură – o carte problematică?

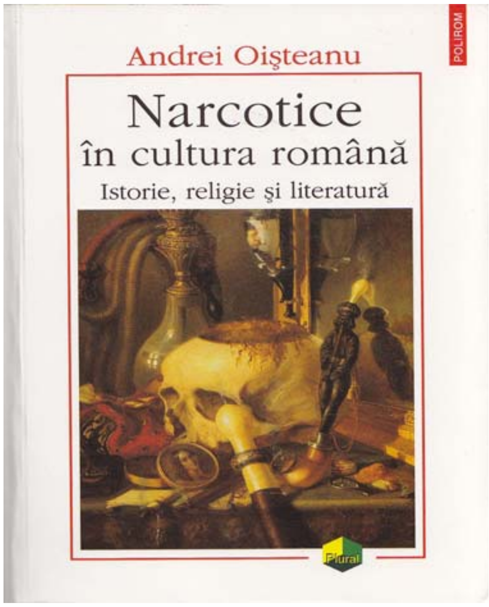

Apariţia unei astfel de lucrări în peisajul editorial românesc este o întreprindere îndrăzneaţă chiar şi după libertăţile de exprimare câştigate după momentul decembrie `89. De altfel, o atitudine definită a autorului sau a editorului, de întâmpinare a unor reacţii din partea societăţii în momentul când sunt abordate astfel de teme întâlnim şi în alte ţări. Aşa se face că nu de puţine ori prefaţa unui volum care îşi propune să abordeze subiectul narcoticelor cuprinde atenţionări referitoare la legislaţia prohibitivă în domeniu. Iată, spre exemplu, un astfel de text, poziţionat de un editor francez la începutul unei antologii literar artistice referitoare la fumat: “Prezenta antologie nu constituie nici o apologie a tutunului, nici o incitare la folosirea lui. În realitate, utilizarea tutunului dăunează sănătăţii fumătorilor şi a celor din jurul acestora etc.”.

În mod evident o astfel de menţiune este valabilă şi pentru prezenta lucrare, chiar dacă autorul nu o face în mod expres. Andrei Oişteanu este cât se poate de direct şi nu se sfiieşte să-şi afirme poziţia: “am urmărit fenomenul din perspectiva istoriei literaturii, culturii şi a mentalităţilor, şi nu din perspectiva morală. Nici perspectiva juridică a fenomenului nu a stat în centrul preocupărilor mele. Aspectul moral m-a interest în măsura în care perspectiva etică a scriitorilor despre care vorbesc s-a modificat în timp. Dar nu mi-am propus să incriminez sau să dezincriminez acest tip de năravuri. Este o carte de istorie a culturii, nu una care să fie distribuită prin licee pentru a descuraja flagelul toxicomaniei. Nu spun că astfel de cărţi nu ar fi necesare. Spun doar că volumul meu nu abordează subiectul anume din această perspectivă”.

Parcurgând cele aproape 500 de pagini ale cărţii, cititorul are prilejul să afle modul în care o serie de scriitori, artişti dar şi oameni de ştiinţă (este vorba în primul rând de medici) din epoca modernă şi cea contemporană s-au raportat la diferite plante sau substanţe psihotrope. Şi exemplele sunt cât se poate de numeroase, de la cei care au studiat folosirea remediilor în Orient (Nicolae Milescu Spătarul, Dimitrie Cantemir, farmacistul J. M. Honigberger – eroul nuvelei “Dispariţia doctorului Honigberger” a lui Mircea Eliade, preocupat şi el de această temă), până la scriitori care s-au sinucis folosind opiacee (Daniil Scavinschi, Alexandru Odobescu); de la scriitori care au folosit substanţele narcotice din raţiuni medicale (Eminescu, Maiorescu), până la cei care le-au utilizat în căutarea “paradisurilor artificiale”(Alexandru Macedonschi) sau pentru a-şi potenţa imaginaţia şi creativitatea (Tristan Tzara, Ion Barbu, Geo Bogza, Saşa Pană); de la oameni de ştiinţă care au experimentat efectele substanţelor psihedelice pe ei înşişi sau pe alţi subiecţi (Nicolae Leon, Eduard Pamfil, Gh. Marinescu), până la cei care au studiat utilizarea plantelor psihotrope în cadrul manifestărilor magico-rituale (Mircea Eliade, Petru Culianu); de la romancieri ale căror personaje îşi administrează substanţe stupefiante (Matei Caragiale, Henriette Yvonne Stahl, Mircea Cărtărescu ş.a.), până la scriitori care-şi analizează experienţele narcotice proprii (Andrei Codrescu, Alin Fumurescu, Dragoş Bucurenci).

Obişnuit cu biografiile destul de şablonizate ale scriitorilor, lectorul acestei lucrări va afla amănunte inedite din viaţa creatorilor, mici picanterii, atât de gustate de către cititori şi care de multe ori sunt trecute cu vederea în mod premeditat, amănunte ce se regăsesc şi în operele acestora. Reţin atenţia, prin noutatea informaţiei şi prin maniera în care este redată, paginile dedicate poetului-matematician Ion Barbu, poezia între matematică şi narcoză, cele care se referă la poetul Emil Botta, cele care îi vizează pe poeţii avangardişti sau pe cunoscuţii istorici ai religiilor Mircea Eliade şi I.P. Culianu ori pe scriitorii contemporani.

Nu mai puţin interesantă este descrierea experimentelor efectuate de oamenii de ştiinţă, de savanţi, cum îi numeşte autorul, doctori, farmacişti, psihiatri sau neurologi români care au experimentat pe ei sau pe alţii efectul substanţelor psihotrope. Nume precum Eduard Pamfil (1912-1994), psihiatrul care s-a intoxicat voluntar cu mescalină, în scopul cercetării psihozelor, dr. psihiatru Ion Vianu sau doctorul C. Stancu, voci pregnate în acuzarea regimului ceauşist pentru folosirea psihiatriei ca armă politică, dr. Nicolae Leon, profesor la Facultatea de medicină din Iaşi, preocupat de farmacopeea populară, care din curiozitate profesională şi-a administrat extract de seminţe de măselariţă, descriindu-şi ulterior halucinaţiile onirice, Nicolae Minovici, specializat în "cercetarea agoniei" pe sine însuşi prin strangulare voluntară, urmărind stările de ebrietate, anumite halucinaţii şi manifestări provocate de asfixie, neurologul Gh. Marinescu, care a efectuat cercetări aprofundate asupra acţiunii mescalinei (interesante sunt rezultatele obţinute prin testarea beţiei mescalinice pe nu mai puţin de şapte pictori), asupra rolului pe care îl au profesiunea şi cultura în manifestarea fenomenelor induse de intoxicarea cu mescalină etc.

Andrei Oişteanu (cercetător la *Institutul de istorie a religiilor* şi preşedinte al *Asociaţiei Române de Istorie a Religiilor*, autor al unor cărţi de reală valoare: *Grădina de dincolo. Zoosofia, Motive şi semnificaţii mito-simbolice, Studii de antropologie culturală, Mytos&Logos. Studii de antropologie culturală, Religie politică şi mit. Texte despre Mircea Eliade şi Ion Petru Culianu* ş.a.) reuşeşte şi de această dată să elaboreze o lucrare extreme de interesantă, cu tentă monografică, care neîndoielnic va rămâne un titlu de referinţă în domeniu. Impresionante sunt şi izvoarele la care recurge, fie ele autohtone, fie străine, dovadă certă a permanentei preocupări a autorului pentru finalizarea unei idei care l-a preocupat de mai multă vreme, încă din anii `80, idée antamată de altfel prin capitolul de început al lucrării.

Aminteam în primele rânduri ale acestei scurte intervenţii de intenţia mărturisită a autorului de a nu fi preocupat în prezenta lucrare de aspectul etic, social al temei abordate, motiv pentru care am considerat a fi utilă interpretarea acestei teme şi de către un medic. Am apelat în acest sens la doctorul psihiatru Octavian Vasiliu, colaborator preţuit al publicaţiei noastre şi autor, împreună cu col. conf. dr. Daniel Vasile, al unor studii şi lucrări în domeniu, care ne-a oferit un material pe care îl publicăm alăturat.

D. Nicolescu

Aspecte cultural-istorice ale consumului de substanțe psihoactive

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Rezumat. Consumul de substanțe psihoactive reprezintă o patologie frecvent întâlnită în clinica psihiatrică, asociată cu un procent înalt de complicații şi comorbidități somatice şi psihice. Acelaşi consum, privit din perspectiva istoriei culturii capătă însă multiple nuanțe, după cum drogul este folosit ca un mijloc de creştere a creativității, pentru funcția sa de facilitare a comunicării cu divinitatea, ca o metodă de explorare a inconştientului sau de ameliorare a unor simptome fizice ori psihice. Există şi multe puncte în care analiza psihopatologică şi cercetarea istoriei culturii se întrepătrund, de exemplu studiul biografiilor şi al operelor ai căror creatori au consumat substanțe psihoactive, studiul autoexperimentelor celebre cu astfel de substanțe în lumea ştiințifică ori studiul curentelor culturale care au avut la bază exploatarea efectelor substanțelor psihoactive.

Cuvinte cheie: tulburări legate de consumul de substanțe, antropologie medicală, stări de conştiință modificată, tehnici de meditatie, substanțe psihoactive

Abstract. The use of psychoactive agents is a frequent pathology in clinical psychiatric practice, associated with a high incidence of psychological or somatic comorbidities or complications. The same drugs use, when interpreted from the cultural history perspective, presents itself as multifaceted phenomenon: is the drug used as a mean to increase the power of creativity, facilitates this agent the communication with the divinity, is the drug considered a method of the subconscious exploration or is it used in order to alleviate physical or psychological discomfort? There are many points of convergence between the psychopathological analysis and the cultural history research. The study of famous biographies and artworks which are connected to drugs use, the study of self-experiments with psychoactive drugs in the scientific world, or the evaluation of cultural trends based upon the exploitation of drugs effects are some of these converging points.

1. Motivație şi vulnerabilitate

Consumul de substanțe psihoactive, capabile să modifice starea de conştiență, reprezintă un comportament observabil în cele mai variate civilizații, privite atât din perspectivă longitudinală (din preistorie până în contemporaneitate), cât şi transversală (la cele mai variate clase sociale, grupuri profesionale sau categorii de vârstă). Acest consum a făcut parte, de-a lungul istoriei civilizațiilor, dintr-un set de comportamente normate socio-cultural, cum ar fi ritualurile şamanice sau folosirea proprietăților analgetice sau relaxante ale unor substanțe, dar se regăseşte învestit cu valoare inițiatică şi în diferite *rites de passage* existente în cadrul civilizației contemporane. Putem întâlni astăzi consumul de substanțe psihoactive ca element constitutiv al ritualurilor de integrare a adolescentului în microgrupuri, de la relativ comunele "prima țigară" sau "prima sticlă de bere", până la "prima pastilă de ecstasy" sau "întâia injecție cu heroină". Motivația consumului de substanțe psihoactive poate fi, în prezent, extrem de variată, de la dorința obținerii unei stări de relaxare, pseudo-comunicarea cu partenerul sau cu membrii grupului de apartenență prin împărtăşirea unor experiențe psihedelice, “evadarea” dintr-un cotidian resimțit ca frustrant, compensarea unor trăiri disforice, reducerea disconfortului asociat unor experiențe psihotice, creşterea creativității, explorarea Sinelui, comunicarea cu Divinitatea etc.

Chiar dacă elementele motivaționale sunt modelate cultural, ele nu diferă semnificativ dacă sunt privite din perspectivă istorică. Opiul a fost utilizat pentru efectele sale euforice, anestezice, ca aliment sau în cadrul ritualurilor magice încă din perioada neolitică. Din *Papaver somniferum* se pregăteau anestezice folosite de sumerieni, egipteni, romani sau greci, după cum rezultă din cele mai vechi documente scrise ale umanității (papirusul Ebers, tablele de la Nippur, învățăturile vedantice) [1,2]. Consumul de substanțe psihoactive poate fi urmărit pe parcursul istoriei umanității, efectele sale fiind evaluate şi normate diferit de diferite civilizații, în unele momente ale istoriei (opiul a fost interzis în China în secolul XVIII, alcoolul în SUA între 1920 şi 1933 etc.).

Există numeroase cercetări privind influența factorilor psihologici (trăsături de personalitate, simptome sau tulburări psihice, nivelul de stres resimțit), biologici (dezechilibre în neurotransmisia dopaminergică, teren genetic favorizant) sau sociali (accesibilitate, politici economice de reglare a cererii de substanțe psihoactive) în crearea unei vulnerabilități a persoanelor față de droguri.

Conform analizei unor autori [3] semnificația experienței drogului se află în primul rând în persoană şi nu în substanță, care nu face decât să elibereze anumite tendințe latente ale individului. Manifestările acestei experiențe pot fi complet opuse la două persoane ce consumă aceeaşi substanță : de la eliberare, senzația de plutire, euforie, amintiri plăcute, până la confuzie, dezorientare, anxietate, halucinații terifiante; de la perspicacitate şi viziuni extatice la apatie, senzație de epuizare, stereotipii mentale. Importanța semnificației individuale în cazul experienței drogului a fost subliniată şi de scriitori precum Baudelaire, pentru care drogul nu revelează individului decât pe individul însuşi (« drogul va fi pentru impresiile şi gândurile familiare ale omului o oglindă care măreşte, dar o oglindă ») [4].

Conduitele adictive nu sunt rezultatul exclusiv al acțiunii factorilor externi subiectului (factori sociologici şi potențialul adictiv al substanței), fără a avea şi o legătură cu personalitatea lui. Pe de altă parte nu există o ’’personalitate toxicomană’’ în sensul unei structuri univoce, ci există un ansamblu de factori psihodinamici cu importanță în dobândirea dependenței. Unii toxicomani sunt caracterizați printr-o lipsă de autonomie, printr-o nevoie permanentă de o altă persoană care să ia deciziile în numele său, în timp ce alții prezintă o impulsivitate crescută, fiind predispuşi la acte antisociale sau la conduite autoagresive.

Toxicomanul poate recurge la drog pentru a-şi anula tensiunea internă, secundară unei frustrări, unei decepții, unei anxietăți, depresii, sau, în mod general, pentru a-şi anula o senzație dezagreabilă.

Drogul tinde să se substituie mecanismelor defensive nevrotice obişnuite, cum ar fi refularea, denegarea, izolarea şi de aceea drogul pentru toxicoman reprezintă o necesitate, o nevoie şi nu doar o dorinţă. Relaţia cu mecanismele defensive şi cu strategiile de coping trebuie atent investigată, iar profilul personalităţii evaluat în detaliu. Drogul poate fi substitut al unui obiect învestit afectiv, care a fost pierdut, după cum poate fi consumat doar pentru reducerea nivelului de angoasă ori ca mijloc de comunicare cu un anumit subgrup social.

Un studiu realizat de Agenţia Naţională Antidrog pe un eşantion de persoane din învăţământul preuniversitar arată că principalele motivaţii pentru care adolescenţii ar consuma substanţe psihoactive sunt influenţa anturajului/prietenilor (65.5%), dorinţa de senzaţii tari (21.3%), curiozitatea (8.6%), problemele personale (14.4%), altele - dorinţa de “a fi la modă”, “pentru a fi cool”, “pentru a părea interesant/important”, “dorinţa de a ieşi în evidenţă”, “imitaţia/dorinţa de a fi ca ceilalţi”, “obişnuinţa” [5].

Din perspectivă neurobiologică, este important de menţionat că sistemul nervos este capabil să-şi producă propria morfină, regăsită sub forma endorfinelor, propria nicotină (respectiv acetilcolina), propriul canabis (anandamida) şi chiar propria cocaină/amfetamină (sub forma dopaminei) [6]. Calea finală comună a recompensei, cea responsabilă de întărirea comportamentului de căutare şi autoadministrare a drogului, este presupusă a fi calea dopaminergică mezolimbică. Chiar şi alcoolul sau nicotina acţionează pe această cale, care face legătura dintre aria tegmentală ventrală şi nucleus accumbens, producând o senzaţie de plăcere. Consumul cronic de alcool determină creşterea producţiei de endomorfine şi a numărului de receptori opiacei, de aceea există fenomene de toleranţă încrucişată între alcool şi opiacee, unii heroinomani consumând cantităţi importante de alcool pentru a-şi limita simptomatologia sindromului de sevraj opiaceu.

Din perspectiva factorilor sociali, presiunea grupului social şi disponibilitatea drogului sunt factori determinanţi majori în iniţierea şi menţinerea consumului de droguri. În general utilizarea tutunului, alcoolului şi cannabisului precede uzul de cocaină şi opioide.

Factorii socio-culturali implicaţi în experienţa drogului sunt reprezentaţi de particularităţile specifice fiecărui popor sau grup de naţii în privinţa mijloacelor folosite pentru modificarea stării de conştiinţă [7]. Aceste particularităţi îşi au justificare în mentalitatea şi temperamentul acestuia (acestora): chinezii recurg la opiu, un drog care corespunde tendinţelor de izolare şi contemplare specifice acestei culturi; în India se foloseşte haşişul pentru că astfel se obţin vise şi viziuni necesare unei civilizaţii ce valorizează cunoaşterea extatică, în timp ce occidentalul recurge la alcool şi tonice sintetice, mai adecvate vieţii dinamice şi nevoii de succes în plan profesional şi social.

2. Incursiuni în istoria culturii române

Deoarece, aşa cum s-a menționat în capitolul precedent, factorii socio-culturali contribuie la modelarea motivațiilor şi a comportamentelor de consum al substanțelor psihoactive, o analiză din perspectiva istoriei culturii a fenomenologiei adictive poate fi interesantă atât pentru un medic, cât şi pentru un istoric, antropolog, sociolog, istoric al religiilor etc.

Într-o lucrare recent apărută [8], Andrei Oişteanu realizează un demers investigațional în premieră pentru cultura noastră, axat pe analiza utilizării substanțelor psihoactive de către scriitorii şi medicii români. Această carte, care tratează consumul de substanțe psihoactive din perspectivă culturală, are meritul de a nu aluneca în dileme axiologice sau etichetări ale personalităților studiate, ci abordează practic, cu multe exemple, efectele acestui consum în diferite momente ale istoriei culturii noastre. Ferindu-se de un reducționism tentant într-un astfel de domeniu, Andrei Oişteanu arată cum substanțele psihoactive, fie că au fost administrate în scop medical (probabil la Mihai Eminescu), pentru rațiuni artistice (Alexandru Macedonski, Tristan Tzara, Ion Barbu) sau ca experiment autoprovocat (medici - Eduard Pamfil, Gh. Marinescu, scriitori - Dragoş Bucurenci, Andrei Codrescu, Alin Fumurescu) îşi manifestă diferit acțiunea prin prisma factorilor psihologici individuali. Astfel, imaginea drogului ca oglindă sau ca mijloc de a revela subiectului pe sine însuşi, formulată de Baudelaire [4], capătă o susținere practică.

Este interesantă, în această lucrare a lui Andrei Oişteanu, abordarea metodică, aproape de “studiul de caz” din psihopatologie şi includerea în sfera conceptului de substanțe psihoactive a “drogurilor cotidiene”, respectiv cafeaua, nicotina şi alcoolul, abordare ce corespunde clasificărilor psihiatrice actuale (ICD-10, DSM IV TR).

Dintre personalitățile analizate în această carte, am sublinia cazul lui Ion Barbu, care declara că a consumat eter şi cocaină (prima substanță cu frecvență zilnică şi a doua o dată pe săptămână), în poeziile sale regăsindu-se manifestările clinice ale intoxicației cu astfel de agenți chimici. Consumul de cocaină l-a condus pe Ion Barbu la o tulburare depresivă, pentru care a fost internat într-un spital de psihiatrie de lângă Bucureşti, iar decesul scriitorului, prin insuficiență hepatică, nu pare a fi străin de abuzul de substanțe psihoactive [8].

Tristan Tzara este şi el menționat în literatura vremii ca folosindu-se de proprietățile psihoactive ale alcoolului, haşisului şi opiului, iar Mateiu Caragiale, cel puțin din analiza personajelor care populează lucrările sale, pare a fi trecut şi el prin experiențe narcotice [8]. Dacă Macedonski elogiază efectele tutunului de inducere a „extazului”, considerându-l un ingredient necesar actului de creație literară, Ion Pillat este autorul unui poem intitulat „Opium”, în care descrie o experiență alimentată de consumul acestui drog.

Există şi alte cazuri interesante din perspectivă medicală, respectiv sinuciderea cu opiacee la unii scriitori (Daniil Scavinschi, Alexandru Odobescu). În cazul lui Odobescu, acesta urma un tratament cu morfină pentru gută şi se pare că avea probleme financiare în procurarea drogului. În orice caz, el a încercat să se sinucidă de două ori, folosind o supradoză de morfină. Dacă prima dată el a fost descoperit în timp util şi doza utilizată a fost, se pare, destul de mică, a doua intoxicație a avut deznodământ fatal şi pentru faptul că scriitorul şi-a luat măsuri de protecție pentru a nu fi descoperit [8].

Din capitolul dedicat experimentelor narco-psihiatrice, aflăm despre cercetările lui Eduard Pamfil referitoare la efectele mescalinei (transpirații, tremor, iluzii şi halucinații vizuale, sinestezii, depersonalizare), pe care le-a descris în lucrările sale pornind de la autoexperimente, precum şi de experimentele realizate de Gheorghe Marinescu pe subiecți voluntari, folosind halucinogene (cu efecte precum euforie, dezinhibiție, delir oniric, perturbarea percepției timpului şi spațiului, sinestezii etc) [8].

O astfel de analiză a personalităților culturii româneşti se înscrie într-un demers care s-a realizat, fără false tabuuri, în numeroase alte culturi. Este cunoscută influența opiului, haşişului şi a alcoolului în crearea „Paradisurilor artificiale” la Baudelaire, consumul de opiu la de Quincey („Confesiunile unui opioman englez”), intoxicațiile cu LSD la Aldous Huxley („Porțile percepției”), călătoriile inițiatice ale lui Carlos Castaneda sub influența halucinogenelor („Cercul de foc al puterii”) sau cu mescalină la Henri Michaux („Mizerabilul miracol”).

3. Consumul de droguri în secolul XX: de la “modă culturală” la “realitatea clinică”

Un domeniu în care psihiatria se poate apropia de domeniul istoriei culturii este reprezentat de studierea biografiilor personalităților vieții literar-artistice, desigur fără a reduce operele de artă la epifenomene ale unei intoxicații cu substanță şi fără a atribui creațiile literar-artistice unor perioade de depresie sau psihoză induse de agenți chimici. Sunt necesare câteva precizări metodologice: în primul rând, după cum s-a mai menționat în această lucrare, experiența consumului de substanțe psihoactive este trăită diferit de fiecare consumator, astfel încât cunoaşterea personalității, a experienței sale de viață individuale sunt necesare pentru a putea înțelege de ce o persoană care consumă morfină, de exemplu, ajunge la suicid, în timp ce o alta poate să-şi sublimeze pulsiunile, producând opere de artă de o valoare inestimabilă; în al doilea rând, motivația consumului este, din perspectiva analizei medicale, deosebit de importantă, explicând traiectoriile diferite ale consumatorilor în plan existențial.

Un alt domeniu în care psihiatria se apropie de istoria culturii, privită în mod extins, este analiza curentelor de gândire care au valorificat proprietățile substanțelor psihoactive. Th.Fr.Leary, psiholog american din secolul XX, a introdus în practica psihoterapeutică substanțele psihedelice, susținând valoarea acestora în remodelarea comportamentală. Psilocibina a fost folosită de Leary în cadrul unor autoexperimente şi a unor experimente în care au fost implicați studenți în anii ´60, ulterior acesta preferând LSD-ul ca pe un "catalizator psihic". Stanislav Grof, unul dintre fondatorii psihologiei transpersonale, a utilizat stările de conştiință modificate pentru dezvoltarea personală, analiza psihologică şi creşterea insight-ului. Abordarea psihedelică încuraja intrarea persoanei într-o experiență indusă de drog, după administrarea a 300-1500 micrograme LSD [9].

Administrarea de MDMA ca adjuvant al psihoterapiei în depresie a fost utilizată [10], rezultatele raportate fiind pozitive. Astfel de protocoale includeau administrarea de doze relativ mici, 25-100 micrograme, în contextul psihoterapiei psihanalitice, metodă intitulată „psiholitică". LSD-ul şi antidepresivele au o acțiune comună de creştere a transmisiei serotoninergice, fapt care ar putea explica sinergia terapeutică a agenților [11]. De asemenea, au fost create scale de evaluare a efectelor halucinogenelor („Hallucinogen Rating Scale", „Linton-Langs Scale"), instrumente care pot fi utile monitorizării reacțiilor subiecților la efectele acestor droguri [12].

De asemenea, psihologia experimentală şi psihiatria care a inclus experimentele cu substanțele psihoactive au furnizat date necesare înțelegerii simptomatologiei, neurobiologiei şi, finalmente, abordării terapeutice a pacienților cu dependențe de droguri. Unele experimente în psihiatrie au arătat că amfetaminele şi cocaina pot mima o tulburare psihotică, fenomen care susține ipoteza dopaminergică în schizofrenie [13]. Important, din punct de vedere al istoriei psihofarmacologiei, este faptul că ipoteza dopaminergică a început să fie criticată şi pe baza observațiilor care au implicat efectele PCP. Atât PCP, cât şi ketamina sunt antagonişti ai receptorilor de N-metil-D-aspartat şi se asociază cu inducerea unor simptome pozitive şi negative psihotice, fapt care stă la baza formulării ipotezei glutamatergice a schizofreniei. Pornind de la astfel de observații experimentale, o întreagă generație de noi antipsihotice au început să fie create [14].

Din perspectiva psihiatriei clinice, deznodământul unor „mode culturale" sau curente de gândire care au implicat utilizarea drogurilor pentru tratamentul patologiei psihice sau pentru dezvoltarea personală nu a fost unul fericit. Experimentele lui Th. Leary au avut şi efecte secundare la unii subiecți (inclusiv sinuciderea uneia dintre studentele care a folosit halucinogene), iar rezultatele cercetărilor sale au fost considerate ca fiind „biasate" [15]. Alte curente psihoterapeutice au evoluat de la administrarea de halucinogene la folosirea unor tehnici de respirație pentru relaxare şi atingerea stărilor modificate de conştiință, precum în cazul şcolii lui Stanislav Grof.

Abordarea consumului de substanțe din perspectivă psihiatrică trebuie să țină cont de toți factorii de vulnerabilitate menționați în primul capitol. Dependența este considerată, actualmente, o maladie rezultată din interacțiunea vulnerabilității individuale cu un eveniment psihostresant şi o substanță psihoactivă cu proprietăți specifice. Datorită acestui triplu determinism, este important să se realizeze o evaluare cât mai detaliată a factorilor ce pot influenţa geneza şi, consecutiv, schimbarea terapeutică la o persoană afectată de acest gen de patologie. O abordare terapeutică adecvată a consumului se face pornind de la factorii psihologici, biologici şi sociali care compun profilul specific al toxicomanului. Analiza comorbidităţilor şi a complicaţiilor somatice şi psihice, frecvente la această categorie de pacienţi, reprezintă o necesitate. Procesul schimbării comportamentelor disfuncţionale ale pacientului în timpul terapiei, indiferent dacă aceasta este o psihoterapie, consiliere, farmacoterapie, management de caz sau orice altă formă specializată de intervenţie, reprezintă un demers colaborativ, în care obiectivele sunt stabilite de comun acord. Factorii schimbării pe care terapeutul îi foloseşte includ instrumente precum sugestia, încurajarea comunicării deschise, interpretarea materialului inconştient, modelarea comportamentală, restructurarea cognitivă. Alţi factori ai schimbării sunt prezentaţi de pacient : dorinţa de schimbare, încrederea în terapie, cunoştiinţele sale despre terapie. Existenţa unor factori extraterapeutici cu importanţă în obţinerea succesului terapeutic nu poate fi însă minimalizată : reuşite sau insuccese profesionale ori familiale, intervenţia « şansei » în împlinirea unor obiective propuse, schimbările mediului social de care aparţine subiectul etc.

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Aspecte etiologice şi clinice în pneumotoraxul spontan

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Rezumat. Afecțiune respiratorie acută, pneumotoraxul spontan este rezultatul acțiunii unor factori congenitali sau al unora dobândiți. Simptomatologia variază de la o simplă jenă respiratorie la insuficiență respiratorie acută. Autorii trec în revistă principalele cauze dar şi manifestările clinice în pneumotoraxul spontan.

Cuvinte cheie : pneumotorax, etiologie, semne clinice

Abstract. The acute respiratory disease, spontaneous pneumothorax is given by congenital acquired factors. The symptomatology varies from a simple respiratory embarrassement to acute respiratory failure. The autors review the main causes and the clinical manifestations of the spontaneous pneumothorax.

Pneumotoraxul (PNO) reprezintă un accident, de regulă acut, care constă în prezența aerului în cavitatea pleurală. Termenul de pneumotorax a fost folosit pentru prima dată, în 1803, de Itard, un student al lui Laennec. Însuşi Laennec a descris în 1819 tabloul clinic al pneumotoraxului la bolnavii cu tuberculoză, afirmând, în acelaşi timp, că el poate apărea şi la cei fără afecțiuni pulmonare.

Descrierea modernă a pneumotoraxului primar spontan îi aparține lui Kjaergard în 1932.

La noi în țară, primul caz de pneumotorax este publicat în 1925, la Cluj, de către Daniello. Interes deosebit pentru diagnosticul şi tratamentul pneumotoraxului a prezentat C. Coman, a cărui experiență vastă include peste 1 500 de cazuri de pneumotorax .

PNO spontan primar apare la persoanele fără boli pulmonare subiacente şi în absența unui eveniment declanşator, în timp ce pneumotoraxul secundar se dezvoltă la pacienții cu o mare varietate de boli ale parenchimului pulmonar.

PNO iatrogen poate surveni în timpul efectuării unor proceduri de diagnostic sau terapeutice.

Frecvența

Incidența PNO spontan primar la bărbați este de 7,4 cazuri la 100 000 de persoane/an, iar la femei de 1,2 cazuri la 100 000 persoane/an.

În PNO spontan secundar la bărbați există o incidență de 6,3 cazuri la 100 000 de persoane/an, în timp ce la femei este de 2 cazuri la 100 000 persoane /an. La pacienții cu BPOC, incidența este de 26 cazuri la 100 000 de pacienți /an.

Sexul

Pentru PNO spontan primar raportul bărbați / femei este de 6/1 față de PNO spontan secundar, unde raportul bărbați femei este de 3/1.

Vârsta

PNO spontan primar apare la persoane cu vârste cuprinse între 20 şi 30 de ani, vârful incidenței fiind atins la 20 de ani. Rareori PNO spontan primar se observă la persoanele în vârstă de 40 de ani. PNO spontan secundar se dezvoltă mai frecvent la pacienții cu vârste între 60 şi 65 de ani.

Elemente de anatomie, fiziologie şi patogenie

Suprafaţa interioară a cutiei toracice este învelită de pleura parietală, care este în contiguitate cu pleura viscerală ce acoperă suprafaţa plămânului. Cele două foiţe se întâlnesc la nivelul hilului pulmonar formând o seroasă neîntreruptă şi delimitând între ele o cavitate virtuală, închisă, numită cavitatea pleurală, în care se află o cantitate mică de lichid. În cavitatea pleurală este o presiune negativă.

Cauza pneumotoraxului primar este efracţia pleurei viscerale la nivelul unor mici spaţii aeriene cunoscute sub denumirea de „vezicule pulmonare (blebs)'', situate în straturile celulare ale pleurei , în contiguitate cu parenchimul pulmonar.

Mecanismul de formare a blebsurilor este neclar ; ele apar în special la fumători, prin degradarea fibrelor elastice din plămân producându-se un dezechilibru între protează-antiprotează şi oxidant-sistem antioxidant. După ce blebsul s-a format, inflamaţia indusă de obstrucţia căilor respiratorii mici creşte presiunea alveolară, ducând la pierdere de aer în interstiţiul pulmonar. Aerul se propagă spre hilul pulmonar, rezultând pneumomediastin, care rupe pleura mediastinală cu apariţia pneumotoraxului.

Două sunt condiţiile care contribuie la producerea unui pneumotorax spontan :

- un defect congenital sau dobândit al pleurei viscerale şi/sau al parenchimului pulmonar subpleural ;
- hiperpresiunea intrapulmonară, care apare la tuse, efort (emfizem obstructiv, zbor la presiuni subatmosferice, munca în chesoane).

Localizarea preferenţială a veziculelor aeriene, „blebsurile'', la vârfurile pulmonare a fost corelată cu tracţiunea gravitaţională care poate atinge niveluri maxime, exprimate prin valori negative, mai mari decât la baze, ale presiunii pleurale.

Pătrunderea aerului în cavitatea pleurală antrenează colabarea tridimensională a plămânului, care se retractă în virtutea tensiunii sale elastice fiziologice. De cele mai multe ori, colapsul pulmonar închide fistula pleuropulmonară şi opreşte pătrunderea aerului în pleură. În continuare plămânul se reexpansionează progresiv, prin resorbţia aerului.

Când pneumotoraxul se produce la persoane fără afecţiune pulmonară anterioară, are loc, relativ rapid, o reducere CV , CFR , CPT, proporţional cu mărimea pneumotoraxului.

Dacă acesta produce un colaps pulmonar de peste 20 %, iniţial survine o scădere a presiunii parţiale a oxigenului dar care revine la valori normale după câteva ore, chiar dacă plămânul rămâne colabat.

Restabilirea normoxemiei, în condiţiile persistenţei colapsului pulmonar, rezultă din vasoconstricţia compensatorie care aboleşte dezechilibrul ventilaţie-perfuzie în plămânul afectat.

În condiţiile în care fistula pleuropulmonară persistă şi acţionează ca un ventil care permite intrarea aerului în pleură în expir sau în tuse, pneumotoraxul se agravează progresiv şi devine pneumotorax « sufocant » . În acest tip de pneumotorax sub presiune, presiunea intrapulmonară creşte şi colapsul pulmonar se agravează nu numai la plămânul ipsilateral, ci şi la cel controlateral, mai ales în condiţiile deplasării mediastinului. Aceste modificări compresive explică hipoxemia persistentă, reducerea debitului cardiac şi hipotensiunea sau sincopa, care apar frecvent în pneumotoraxul sufocant.

Factorii de risc în pneumotoraxul spontan

- *fumatul*. 91% dintre pacienţii cu PNO spontan primar sunt fumători sau au fost fumători. Riscul de PNO este strâns legat de intensitatea fumatului, incidenţa fiind foarte mare la cei care fumează peste 20 ţigări/zi şi mai mică la cei care fumează 1-2 ţigări/zi ;
- *efortul fizic*. Deşi nu există dovezi clare că între efortul fizic şi debutul pneumotoraxului se află o legătură, este foarte posibil ca acesta să apară în timpul activităţii fizice. Totodată, este foarte probabil ca pneumotoraxul să se producă şi în repaus ;
- *înălţimea, talia subţire*, la o persoană aparent sănătoasă ;
- *sindromul Marfan* ;

- *sarcina.* Sarcina rămâne un factor de risc nerecunoscut, deşi au fost semnalate câteva cazuri ;
- *PNO familial*, descris ca o moştenire autozomal dominantă.

Boli şi condiții asociate cu PNO spontan secundar

- *boli cronice obstructive pulmonare*;
- *astmul*;
- *pneumonia necrozantă;*
- *carcinom bronşic;*
- *tuberculoza;*
- *fibroza chistică* : 18,9% dintre pacienții cu fibroză chistică au prezentat PNO spontan secundar ;
- *cancerul metastatic.* Bolnavii cu afecțiuni maligne, în special sarcoame, necesită supraveghere atentă. Medicamentele chimioterapice pot induce, ca răspuns la tratament PNO spontan secundar;
- *fibroza pulmonară idiopatică;*
- *limfangiomiomatoza*, boală prezentă la femeile de vârstă fertilă şi caracterizată prin chisturi cu pereți subțiri ;
- *sarcoidoza ;*
- *histiocitoza* ;
- *sindromul de detresă respiratorie acută*, 3% dintre pacienții tratați pe ventilator pot dezvolta un PNO ;
- *HIV /SIDA, infecția cu Pneumocystis carinii ;*
- *sindromul respirator acut sever* : la 1,7% dintre pacienții cu acest sindrom se observă PNO ;
- *inhalarea şi utilizarea drogurilor intravenos*, precum marijuana sau cocaina ;
- *endometrioza toracică.* PNO apare în timpul sau la scurt timp după menstruație, de obicei în dreapta, la femei cu vârsta cuprinsă între 30 şi 40 de ani.

Cauze de pneumotorax iatrogen

Apare ca o complicație a unor proceduri medicale sau chirurgicale, cum sunt :

- *aspirația cu ac transtoracic* - cea mai frecventă cauză de pneumotorax iatrogen ;
- *toracenteza terapeutică.*Toracenteza terapeutică se complică cu pneumotorax în 30% din cazuri dacă este efectuată de medici lipsiți de experiență şi în numai 4% din cazuri dacă este practicată de către medici experimentați.

Folosirea de rutina a ecografiei în timpul toracentezei se asociază cu rate mai mici ale pneumotoraxului iatrogen. În mod similar la pacienții ventilați mecanic, toracenteza ghidată prin ecografie fără sprijin radiologic oferă un risc mai mic de pneumotorax ;

- *biopsia pleurală;*
- *biopsia transtoracică;*
- *biopsia transbronhială;*
- *inserarea de cateter venos central;*
- *ventilație mecanică cu presiune pozitivă ;*
- *intubarea accidentală a bronşiei primitive drepte;*
- *traheostomia*;
- *acupunctura* - tehnică tradițională chineză folosită de medici din întreaga lume, cu o frecvență scăzută a pneumotoraxului;
- *introducerea tubului nazogastric;*
- *resuscitarea cardiopulmonară.*

Tabloul clinic al pneumotoraxului

Tabloul clinic al pneumotoraxului spontan variază în funcție de volumul şi de vechimea pungii de aer, ca şi de caracterul primar sau secundar al pneumotoraxului. Anamneza poate sugera existența unui pneumotorax.

În general, manifestările clinice asociate pneumotoraxului secundar sunt mai severe decât cele asociate pneumotoraxului primar, pacienții prezentând lipsă de aer.

În majoritatea cazurilor el se manifestă prin simptome acute, manifestările clinice nefiind indicatori fideli ai mărimii pneumotoraxului.

Mulți pacienți, în special cei cu pneumotorax primar, nu apelează imediat la medic, ci doar după 2-3 zile. Această perioadă mai lungă de timp creşte riscul de edem pulmonar de reexpansiune.

La o proporție redusă de bolnavi, pneumotoraxul se produce în condiții de latență clinică totală, fiind descoperit radiologic întâmplător.

Când simptomele severe sunt însoțite de semne de detresă cardio-respiratorie, trebuie luat în considerare pneumotoraxul sub tensiune (cianoză, transpirații, tahipnee severă, hipotensiune arterială).

Cele mai frecvente manifestări clinice sunt :

- *durerea toracică* (90% din cazuri). La aproximativ 2/3 dintre bolnavi, unicul simptom care semnalează accidentul pleural este durerea toracică acută, deseori cu caracter de „junghi toracic'', localizată de partea unde s-a produs pneumotoraxul. Durerea se poate simți şi în umăr, gât, abdomen. Durerea este accentuată de inspirul profund şi de modificările de postură ;
- *dispneea* (80 % din cazuri). Însoțeşte frecvent durerea toracică. Există cazuri însă în care dispneea lipseşte sau se rezumă la o jenă toracică surdă, pe care bolnavul o poate tolera câteva zile înainte de a se prezenta la medic. Astfel, relativ frecvent nu se poate stabili o relație între apariția durerii şi eforturile fizice.

Dispneea tinde să fie mai severă în pneumotoraxul secundar, datorită rezervelor pulmonare scăzute ;

- *scurtarea bruscă a respirației;*
- *anxietate;*
- *tuse uscată, apărută brusc;*
- *dureri acute epigastrice* (rar);
- *astenie fizică.*

Uneori, simptomele au caracter dramatic : durere intensă, dispnee severă, asfixie, agitație. Ocazional, ele pot avea caracter progresiv (sincopa) colaps cardiocirculator. Este cazul pneumotoraxului „sufocant''. Simptomele pot să fie mai puțin severe într-un pneumotorax lent, în curs de dezvoltare față de un pneumotorax apărut brusc, dezvoltat rapid.

Cu excepția pneumotoraxului foarte mare, simptomele au tendința să se atenueze pe măsură ce organismul se adaptează la colabarea pulmonară.

Semnele fizice în pneumotorax

Semnele fizice pot lipsi dacă volumul de aer pătruns în pleură este mic. Colecțiile aeriene destul de mari produc modificări caracteristice la nivelul aparatului respirator :

- *dilatarea şi mobilizarea hemitoracelui afectat;*
- *hipersonoritate percutorie;*
- *abolirea vibrațiilor vocale;*
- *respirație mult diminuată sau abolirea murmurului vezicular;*
- *suflu amforic.*

Se mai pot observa :

- *tahicardie* (frecvență crescută). La o tahicardie de peste 135 bătăi/' există mare probabilitate a unui pneumotorax sub tensiune ;
- *hipotensiune arterială* – frecventă în pneumotoraxul sufocant;
- *tahipnee;*

- *cianoză* – rar*;*
- *distensia venelor jugulare* – rar *;*
- *alterarea stării de conştienţă* – rar.

Ocazional se mai poate decela deplasarea controlaterală a mediastinului şi a inimii, precum şi împingerea în jos a ficatului.

Concluzii

1. Factori etiologici diverşi produc colabarea pulmonară.
2. Incidenţa pneumotoraxului este mai mare la sexul masculin.
3. Boli respiratorii cronice se pot complica cu pneumotorax.
4. Simptomatologia este dominată de durere sau de dispnee.
5. Semnele obiective pot fi minore.

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Integrarea managementului proiectelor de cercetare ştiinţifică medico-militară în sistemul managerial instituţional

Colonel (r.) dr. Viorel ORDEANU, Marioara CHIVU
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Rezumat. C.C.S.M.M. are în organigramă mai multe secţii şi compartimente, între care şi Secţia de Protecţie Medicală Antiinfecţioasă şi Urgenţe Epidemiologice, care funcţionează ca unitate specializată de *protecţie medicală contra armelor biologice*. Aceasta execută cercetare medicală fundamentală şi aplicativă în domeniul microbiologiei şi epidemiologiei, prin echipe interdisciplinare: bacteriologie, virusologie, micologie, toxinologie, farmacologie antiinfecţioasă, zooantroponoze, biologie moleculară şi genetică, epidemiologie specifică ş.a.

În ansamblu, situaţia de criză biologică impune valorificarea rezultatelor cercetării ştiinţifice în domeniu şi a expertizei fiecărui cercetător în domeniul său.

C.C.S.M.M. are capacitatea (forţe şi mijloace) şi expertiza pentru a se implica, la ordin, în managementul crizelor biologice, şi în general al evenimentelor CBRN.

Îmbunătăţirea capacităţii administrative permite dezvoltarea sistemului informatizat de management instituţional, cu efect în creşterea vizibilităţii rezultatelor cercetării şi implicit stimularea parteneriatului din domeniul public şi privat.

Cuvinte cheie: cercetare ştiinţifică, management instituţional, management de proiect, sistem informatizat, protecţie medicală

Abstract. The Army Center for Medical Research organization has several divisions and departments, including the Division of Protection and Emergency Medical Epidemiology operating as a specialized medical protection unit against biological weapons. It runs basic and applied medical research in microbiology and epidemiology, through interdisciplinary teams: bacteriology, virology, mycology, anti-infective pharmacology, zooantroponoses, molecular biology and genetics, epidemiology etc.

Overall, the crisis requires biological recovery to scientific research results and expertise of each researcher in his field.

The Army Center for Medical Research has the capability (forces and means) and expertise to get involved into the biological crisis management and general CBRN events, on request.

Improving the administrative capacity computerized system allows the development of institutional management, increasing the visibility of research results and thus stimulates the public and private partnership.

Centrul de Cercetări Ştiinţifice Medico-Militare (C.C.S.M.M.) este o instituţie de cercetare ştiinţifică medicală unicat în ţară, organizată ca unitate militară subordonată Direcţiei Medicale a Ministerului Apărării Naţionale.

C.C.S.M.M. face parte din Sistemul naţional de cercetare-dezvoltare care este constituit din ansamblul unităţilor şi instituţiilor de drept public şi de drept privat cu personalitate juridică ce au în obiectul lor de activitate cercetarea-dezvoltarea (cod CAEN 72), conform art.7-8 din Ordonanţa nr. 57 din 2002 privind cercetarea ştiinţifică şi dezvoltarea tehnologică, cu modificările şi completările ulterioare, publicată în Monitorul Oficial nr. 643 din 2002.

Principala misiune a C.C.S.M.M. este activitatea de CDI pentru protecţia medicală contra noxelor specifice activităţilor militare, în special CBRN (diagnostic, profilaxie şi tratament). Managementul instituţiei este asigurat de director – General maior (r.) medic Acad.Prof. Dr. Victor Voicu, personalitate cu înaltă competenţă ştiinţifică şi militară, şi de Consiliul Ştiinţific constituit în conformitate cu prevederile OG.57/2002.

Analiza internă a sistemului managerial al instituţiei şi sesizarea nevoii de îmbunătăţire a început odată cu punerea în aplicare a ordinelor şi instrucţiunilor din M.Ap.N: Ordinul M.S.113/26.10.2009 - *Norme metodologice privind elaborarea şi dezvoltarea sistemului de control managerial în M.Ap.N*; Instrucţiunea I.1000.5 v.2 - *privind managementul activităţilor de cercetare-dezvoltare şi inovare în cadrul M.Ap.N.*, care stabileşte cadrul general pentru managementul activităţilor de cercetare, furnizând reglementările generale şi atribuind responsabilităţile structurilor implicate în această activitate.

Implementarea proiectului derulat în cadrul Programului Operaţional Sectorial Creşterea Competitivităţii Economice, Axa prioritară 2. CD&I, operaţiunea 2.2.4, ***Creşterea capacităţii administrative prin integrarea sistemului de management al activităţii de CD cu managementul activităţii în situaţii de criză,*** acronim MAPROMED - finanţatat din fonduri structurale, constituie o etapă nouă de *reproiectare a sistemului de management al organizaţiei, aplicând diverse metodologii manageriale.*

Factorii principali care generează schimbarea managementului sunt: dezvoltarea cunoştinţelor, progresul tehnic şi economic, apariţia de noi valori, globalizarea sistemului economic, iar atitudinea pozitivă a managerilor trebuie să fie factorul de susţinere a schimbării. Orice modificare, transformare sau transpunere în formă/conţinut a unui proces, activitate, acţiune, sistem etc. reprezintă o schimbare. Schimbarea managementului este definită ca adaptarea managementului unei societăţi, organizaţii *sau proiect, la schimbările intervenite în structura socio-economică, urmare a unor factori interni sau externi structurii* (Opran C., Stan S., 2008).

Kenneth, citat de Opran, prezintă *demersul strategic de amploare, de reproiectare a managementului organizaţiei ca activitate absolut necesară, prin introducerea unor noi metode administrative şi de conducere la nivelul unei societăţi, pentru asigurarea unor parametri calitativi superiori sistemelor microeconomice şi macroeconomice de management, care au drept rezultat o creştere a competitivităţii organizaţiei respective.* Odată diagnosticate nevoile de schimbare, este necesară remodelarea managerială şi proiecţia viitorului organizaţiei, respectiv ***fundamentarea strategiei*** acesteia, ţinând cont de faptul că reprezintă un demers complex şi dificil, având în vedere că implică şi resursa umană. Organizaţiile implică oameni şi depind de efortul oamenilor, esenţa fiecărei organizaţii fiind factorul uman, eficacitatea şi eficienţa fiind influenţate în cea mai mare măsură de comportamentul lor; este ştiut că *succesul, performanţa şi competitivitatea unei organizaţii rezidă în oamenii săi, căci altfel, ce este o organizaţie fără angajaţii săi? Nu este nimic în absenţa resurselor umane, eventual doar o mulţime de echipamente scumpe…*(Lock D., 2001)

Dincolo de strategii şi tehnici de management folosite în instituţii, este evident necesar un nivel ridicat de încredere în rândul angajaţilor, pentru că cei care fac parte din instituţie trebuie să-şi demonstreze loialitatea şi ataşamentul faţă de ea. Aspectele legate de managementul resurselor umane ale activităţii pe bază de proiecte se află printre problemelele care au o importanţă strategică hotărâtoare pentru organizaţii. Turner şi Keegan (2004) identifică trei probleme esenţiale de care depinde gestionarea cu succes a resurselor umane din aceste organizaţii şi anume: selecţia personalului în organizaţia profilată pe proiecte; dezvoltarea carierei pentru un mediu aflat în schimbare; managementul cunoştinţelor şi învăţarea.

În mod concret, pentru îmbunătăţirea capacităţii administrative a C.C.S.M.M. obiectivul principal este realizarea unui sistem integrat de management care să coreleze activitatea derulată în diferitele proiecte, prin dezvoltarea sistemului informatizat, cu efect în creşterea vizibilităţii rezultatelor cercetării şi implicit stimularea parteneriatului public-privat. Implementarea proiectului vine în sprijinul deciziilor manageriale la nivelul instituţiei, atât din punctul de vedere al gestionării resurselor financiare şi umane, cât şi din punctul de vedere al unei intervenţii rapide în situaţii de criză.

Conform specialiştilor, reproiectarea sistemului de management al organizaţiei se poate realiza în concordanţă cu un scenariu metodologic (Verboncu I., Nicolescu O., 2008) structurat în următoarele etape:

a). Diagnosticarea viabilităţii economice manageriale (analiza diagnostic). Aceasta poate fi o ***metodă*** de management componentă a sistemului metodologico-managerial, o ***etapă*** de debut într-un studiu complex de reproiectare managerială şi o ***modalitate de fundamentare a strategiei.*** **C**a metodă managerială, constă în analiza organizaţiei utilizând instrumentar specific, adecvat pentru identificarea punctelor tari şi slabe şi formulării unor recomandări pentru amplificarea potenţialului organizaţiei;

b). Realizarea strategiei organizaţiei (proiecţia viitorului organizaţiei);

c).Reproiectarea propriu-zisă: reproiectarea sistemului metodologico-managerial; reproiectarea sistemului organizatoric; reproiectarea sistemului decizional şi reproiectarea sistemului informaţional;

d). Implementarea sistemului managerial perfecţionat;

e). Controlul şi evaluarea noului sistem managerial.

Comparativ, **managementul proiectelor** presupune aplicarea unui set coerent de principii, reguli, cunoştinţe, metode, tehnici, instrumente utilizate în planificarea, organizarea, conducerea şi controlul unui proiect. El este definit ca fiind *un concept managerial, care poate susţine în mod deosebit din punct de vedere metodic (organizare procesuală), structural (organizare structurală) şi al managementului resurselor umane, o activitate complexă, într-un mediu dinamic* (Hinescu A., 2007)

Proiectul poate fi descris ca fiind *un mod de a lucra, de a-i organiza pe oameni şi de a gestiona activităţi; este o manieră de organizare şi coordonare a muncii. Ceea ce îl deosebeşte de alte genuri de management este faptul că se concentrează în totalitate pe un anume rezultat final şi că, în momentul în care se obţine acest rezultat, proiectul se încheie şi i se pune capăt* (Newton R., 2005) .

Realizarea oricărui proiect se face prin descompunerea în activităţi după metoda *WBS – Work Breakdown Structure*: activităţile se grupează în mulţimi logice pe pachete de lucru, cu subdiviziunea fiecăruia în activităţi; fiecare activitate are caracteristici: rol bine determinat, consumă resurse fizice şi umane în timp bine determinat şi are un moment de început şi un moment de încheiere. La elaborarea proiectului, pe lângă instrumentele curente pentru colectarea, sistematizarea şi analiza informaţiilor privind organizaţia solicitantă şi mediul economico-social în care aceasta funcţionează – bilanţ, cont de profit şi pierderi, alte evidenţe financiar-contabile ale organizaţiei, studii de piaţă etc., managementul proiectelor utilizează o serie de instrumente şi tehnici specifice cum sunt: metoda cadrului logic, analizele SWOT, diagramele GANTT sau PERT.

În concepţia integrată asupra managementului proiectelor, ansamblul de metode şi tehnici utilizate trebuie să realizeze echilibrul între principalii factori: ***timp*** (termenele de execuţie); ***buget*** (bani şi resurse – manoperă, materiale şi tehnologii); ***performanţă*** (calitate, o anumită tehnologie, anumite specificaţii, caracteristici ale unui produs impuse de legislaţie), dar iau în calcul şi ***aşteptările generate de proiect*** (finanţatori, membrii echipei de proiect, managerul de proiect, beneficiari, stakeholderi - angajaţi, acţionari, furnizori, organisme de interes public etc.).

Scopul managementului proiectelor de CDI. Scopul principal al managementului proiectelor este elaborarea propunerii de proiect, câştigarea finanţării, executarea planului de realizare la termenul stabilit şi cu volumul de cheltuieli estimat, asigurarea calităţii şi obţinerii rezultatelor şi indicatorilor de eficienţă planificaţi, şi de a atinge obiectivele planificate prin transferarea rezultatelor cercetării în economia reală.

Funcţiile managementului proiectelor de CDI. Funcţiile managementului de proiect sunt următoarele: stabilirea şi formularea clară şi precisă a obiectivelor proiectului; organizarea activităţilor de cercetare şi a celor conexe în scopul bunei lor derulări, activităţi cuprinse în Planul de realizare a proiectului; asigurarea resurselor necesare, a logisticii şi infrastructurii necesare implementării proiectului; controlul şi monitorizarea realizării obiectivelor etapelor şi ale proiectului, inclusiv indicatorii economici; asigurarea calităţii; realizarea valorificării rezultatelor prin transfer tehnologic (Niculiţă L., 2007).

Deoarece şi-a dovedit capacitatea de a face faţă noilor tendinţe manifestate pe plan mondial, managementul proiectelor s-a extins la nivelul tuturor activităţilor din economie, având în vedere creşterea accelerată a volumului de informaţie şi a cunoştinţelor, creşterea cererii pentru bunuri şi servicii tot mai complexe, creşterea competiţiei pe piaţă şi dzvoltarea economiei bazată pe cunoaştere, extinderea tipului de organizaţie modernă inteligentă. Indiferent de sursă, finanţarea prin proiecte este cel mai modern instrument, permițând o selecție obiectivă a parteneriatelor şi echipelor de realizare a proiectului, cât şi un mod judicios de utilizare a fondurilor de finanțare.

Prin programele componente ale Planului Național pentru CDI se finanţează proiecte şi activități de cercetare științifică, dezvoltare tehnologică, transfer tehnologic, valorificare şi alte activități cu rol de sprijin pentru stimularea CDI. La realizarea proiectelor pot să participe persoanele juridice române legal constituite şi persoane fizice autorizate, care îndeplinesc condițiile de eligibilitate, în conformitate cu prevederile OG nr. 57/2002, cu modificările şi completările ulterioare. În funcție de tipul de proiect la care se face referire, există cerințe specifice privind categoriile de participanți la acest program. Participanți la proiect pot fi instituțiile care îndeplinesc condițiile de participare, formulează propunerea de proiect şi contribuie efectiv la realizarea proiectului. În funcție de natura activităților de cercetare-dezvoltare şi profilul economic al participanților, realizarea proiectului poate fi susținută fie integral din bugetul programului, fie prin finanțare din surse complementare (alte surse interne, fonduri ale participanților surse externe, inclusiv fonduri PC7 sau fonduri structurale) sau prin cofinanțare.

Finanțarea proiectelor se face în sistem competițional prin proceduri bine puse la punct şi legiferate. În funcție de tipul de competiție, obiectivele proiectului de CDI sunt fixate de către Autoritatea Contractantă (respectiv MEdCI prin ANCS) şi de către ofertantul propunerii de proiect prin propunerea întocmită. În Pachetele de informații sunt prezentate toate elementele necesare elaborării unei propuneri de proiect şi participării la competiție (condiții, termene, eligibilitate, criterii de evaluare şi selecție etc). De aici rezultă că este obligatoriu ca o propunere de proiect elaborată şi depusă pentru solicitarea finanțării să aibă obiective care să fie în concordanță cu obiectivele programului în cadrul căruia se solicită finanțarea şi să contribuie la rezolvarea problemei enunțate.

Având în vedere experiența acumulată de cercetătorii din C.C.S.M.M în implementarea proiectelor de cercetare din Programul Național de Cercetare-Dezvoltare-Inovare, Programul de Cercetare de Excelență CEEX, Programe sectoriale, Granturi ale Academiei Române, Programele europene COST B15 şi B25, ş.a., într-o primă etapă se va trece la reorganizarea administrativă (reflectarea în evidența contabilă a alocării de fonduri, a costurilor consumurilor materiale, a consumului de manoperă, a achizițiilor) direct pentru fiecare proiect în parte, indiferent de sursa de finanțare.

Prezentăm integrarea managementului proiectelor de cercetare științifică medicală în sistemul managerial instituțional aplicat la protecția medicală antiinfecțioasă şi urgențele epidemiologice din Centrul de Cercetări Ştiinifice Medico-Militare.

C.C.S.M.M. are în organigramă mai multe secții şi compartimente, între care şi o secție care funcționează ca unitate specializată de *protecție medicală contra armelor biologice*. Aceasta execută cercetare medicală fundamentală şi aplicativă în domeniul *microbiologiei şi epidemiologiei*, prin echipe interdisciplinare: bacteriologie, virusologie, micologie, toxinologie, farmacologie antiinfecțioasă, zooantroponoze, biologie moleculară şi genetică, epidemiologie specifică ş.a.

Secția este formată din două laboratoare şi are ca atribuțiuni efectuarea de cercetări științifice pentru protecția trupelor şi a populației civile contra armelor biologice şi cu toxine, pentru situații de război biologic, atac bioterorist sau accident biologic. Activitatea de cercetare în domeniu vizează în principal agenții biologici ca bacterii, virusuri, fungi etc. şi toxinele acestora, pentru a stabili procedurile de protecție medicală, şi se realizează prin colaborare între diferite specialități medicale precum: microbiologie, epidemiologie, boli infecțioase, medicină veterinară etc. Cercetarea se desfăşoară prin proiecte anuale/multianuale cuprinse în Planul intern de cercetare medico-militară şi, după caz, prin proiecte din Planul Sectorial al M.Ap.N, din Planul Național de CDI sau Programe europene etc.

Cercetarea de microbiologie stabileşte şi aplică experimental metode şi tehnici de diagnostic, profilaxie şi tratament în condiții de laborator, iar cercetarea de epidemiologie aplică practic tehnicile de diagnostic, profilaxie şi tratament, în condiții de laborator şi în teren, prin Laboratorul de Epidemiologie şi Urgențe Epidemiologice. Activitatea de cercetare se poate extinde la nevoie şi la alte boli infecţioase cu impact major asupra trupelor sau a populaţiei, în colaborare cu formaţiunile specializate, militare şi civile.

Secţia are competenţa să organizeze şi să instruiască echipa mobilă de intervenţie în caz de atac biologic EMI-BIO (care acţionează la ordinul eşalonului superior, în proximitatea focarului de atac biologic şi asigură rezerva de personal şi materiale de protecţie pentru echipă, inclusiv rezervele de materiale pentru diagnosticul de laborator al principalilor agenţi biologici şi agenţi patogeni infecţioşi (reactivi specifici, tulpini de referinţă, materiale de laborator ş.a.) pentru situaţii de criză.

Cercetătorii secţiei depun eforturi pentru a fi în măsură să evacueze la nevoie, la ordinul eşalonului superior, personalul, aparatura şi materialele de laborator strict necesare, în locurile stabilite şi să intre în acţiune în cel mai scurt timp posibil, pentru a primi şi îndeplini la ordin şi alte atribuţiuni sau misiuni specifice.

Pentru efectuarea cercetărilor privind diagnosticul microbiologic, unitatea dispune de personal calificat şi specializat (dar care în prezent este redus la strictul necesar), de spaţii adecvate izolate în afara perimetrului urban (pentru evitarea cazurilor de contaminare) şi la INCDMI Cantacuzino (pentru cooperarea în timp real cu specialiştii civili). Dispune de dotare corespunzătoare cu laboratoare, biobază, tehnică de calcul, mijloace fixe, obiecte de inventar, materiale consumabile etc. şi facilităţi adecvate. Aplică proceduri de operare conform instrucţiunilor Ministerului Sănătăţii şi Academiei de Ştiinţe Medicale (care sunt conforme cu recomandările OMS şi UE), şi participă la ratificarea unor STANAG-uri (standarde NATO) în domeniul CBRN.

Pentru desfăşurarea activităţii de cercetare, unitatea dispune de o serie de echipamente, cum sunt: echipamente de spectrometrie de masă cu laser pentru detecţie şi identificare de agenţi biologici; linie computerizată MiniApi pentru identificare microbiană şi antibiograme; confirmă diagnosticul microbiologic pentru bacterii şi fungi (agenţi biologici, flora de spital, flora polifarmacorezistentă sau precizare de diagnostic pentru tulpini primite din teritoriu) şi execută teste de sensibilitate, pentru instituirea şi controlul antibioterapiei; linie PCR computerizată pentru diagnostic microbiologic de confirmare prin biologie moleculară din cantităţi foarte mici (agenţi biologici bacterieni şi virali, precizare de diagnostic, genetică); linie ELISA computerizată, pentru diagnostic de virusuri şi toxine (agenţi biologici, floră de spital, precizare de diagnostic); linie TLC pentru cromatografie cu densitometrie computerizată, pentru diagnostic de toxine şi toxice (agenţi biologici, precizare de diagnostic); linie de electroforeză cu densitometrie computerizată; microscop optic computerizat, cu prelucrare de imagine, pentru diagnostic microbiologic; linie computerizată pentru culturi celulare, pentru virusologie şi teste de citotoxicologie; computere compatibile, cu configuraţie standard; hote de biosiguranţă microbiologică: hote cu flux laminar BSC clasa III, II şi I, hota cu atmosfera controlată *glove box*, hota chimică etc.; dotare curentă pentru laborator de microbiologie şi de epidemiologie.

Proiectele în derulare acoperă cele mai importante direcţii din domeniu: protecţia medicală contra armelor biologice, agenţi biologici bacterieni, virali, fungici şi toxine ale acestora, biologie moleculară şi epidemiologie. Subliniem faptul că prin natura domeniului de cercetare ştiinţifică de microbiologie şi epidemiologie, precum şi de protecţie medicală contra armelor biologice, în practică nu se poate face o diferenţiere netă între activităţile laboratorului de microbiologie şi cel de epidemiologie, activităţile fiind complementare.

Personalul de cercetare este angrenat şi în Programele Naţionale de Cercetare fundamentală, Dezvoltare tehnologică şi Inovare (PNCDI), Cercetare de Excelenţă (CEEX) prin contracte finanţate de Ministerul Educaţiei şi Cercetării: BIOTECH, VIASAN, SECURITATE, INVENT, INFOSOC, AEROSPATIAL, CAPACITATI, precum şi în FP6. În paralel se lucrează şi la proiectele de cercetare din Planul Sectorial de Cercetare Ştiinţifică al Ministerului Apărării Naţionale, în colaborare cu Centrul de Cercetare Ştiinţifică pentru Apărare CBRN şi Ecologie, şi cu Centrul de Medicină Preventivă al Direcţiei Medicale al M.Ap.N.

Activitatea de protecţie a mediului. Mişcarea de lobby în favoarea mediului a devenit foarte puternică, iar cei care nu ţin seama de acest lucru îşi asumă riscuri serioase; poluarea *clasică* - atmosferică, a apei, cu deşeuri şi fonică - este în tot mai mare măsură reglementată; prin actualitatea temei, reprezintă o mare responsabilitate şi solicită de multe ori prezenţa în echipele de proiect a unor consultanţi în materie de mediu. Este o preocupare permanentă a personalului din laborator, acesta fiind instruit pentru protecţia muncii şi PSI, la care se insistă în mod deosebit pe aspectele de biosecuritate şi protecţie a mediului**.** Deşi se lucrează permanent cu material infectant şi/sau toxic, nu am avut nici un fel de incidente sau accidente şi nu există nici un risc pentru protecţia mediului.

Activitatea medico-militară. Pentru situaţiile de criză biologică**,** secţia dispune de un laborator cu nivel de biosecuritate P2+ pentru Diagnostic de Agenţi Biologici**,** care execută inclusiv expertiza pentru corespondenţă şi obiectele suspecte de a fi arme biologice sau contaminate cu agenţi biologici, la solicitarea diverselor eşaloane. Acest Laborator P2+ a fost operaţionalizat încă din anul 2001, ca urmare a atacurilor bioteroriste din SUA şi UE. Laboratorul are o suprafaţă de 52 mp şi este raportat anual ca P2 (pentru agenţi patogeni în general) pe formularul MAE care se transmite la ONU. Prin utilizarea echipamentelor NBC individuale din dotare, nivelul de biosecuritate creşte la P3- (pentru agenţi biologici), dar este necesară reabilitarea acestuia pentru a deveni un adevărat laborator P3, conform normelor europene. Până atunci funcţionează cu dotarea existentă.

Din anul 2002 fost constituită Echipa Mobilă de Intervenţie Biologică EMI-BIO, formată din echipa principală şi cea de rezervă, echipament de protecţie NBC şi medical, materiale specifice pentru intervenţie, pentru recoltarea şi transportul probelor la laborator şi un minimum de mijloace de diagnostic în teren. Se lucrează la constituirea unei rezerve antiepidemice pentru situaţii de urgenţă: criză biologică, atac biologic, accident biologic, atac bioterorist sau atac *bio-chem*. În prezent se lucrează şi la documentaţia pentru un Laborator biologic mobil şi a unui Autolaborator biologic echipate după standardele militare. După intrarea în dotare a acestor capacităţi specifice, M.Ap.N. va dispune de echipament modern şi eficient pentru prevenirea şi combaterea atacurilor biologice şi combinate, a atacurilor teroriste *bio-chem* în timp real, precum şi pentru expertize în domeniul CBRN şi al agenţilor biologici. Acesta poate fi utilizat oriunde în ţară, acolo unde este nevoie să se intervină pentru protecţia trupelor sau a populaţiei, inclusiv în cadrul Alianţei NATO, UE etc. Din păcate, în fiecare an nu au fost alocate fondurile necesare, aşa că până în prezent nu avem decât planurile, machetele şi modelele experimentale; în prezent, *echipa mobilă* nu este mobilă, pentru că îi lipseşte mijlocul specific de deplasare.

În anul 2007 au fost câştigate prin competiţie naţională două proiecte la Programul PNCDI Capacităţi (pentru perioada 2007-2009), respectiv *BSL 4* pentru amenajarea unui laborator de microbiologie înalt securizat cu nivel de biosecuritate 4 (maximal) pentru diagnosticul *in vitro* şi cercetarea agenţilor biologici de război şi *Biobaza 2* pentru amenajarea unei biobaze securizate pentru diagnosticul *in vivo* şi cercetarea agenţilor biologici de război. Finanţarea de 4 milioane lei a fost asigurată de Autoritatea Naţională pentru Cercetare Ştiinţifică din Ministerul Educaţiei Cercetării şi Inovării.

În acelaşi scop, am dotat şi operaţionalizat în anul 2008 un *Laborator pentru Detecţie şi Identificare de Agenţi Biologici.* În prezent laboratorul funcţionează la nivel de biosecuritate P2+ (pentru agenţi patogeni) şi se are în vedere extinderea şi ridicarea nivelului la P3 (pentru agenţi biologici).

Activitatea de cercetare ştiinţifică a secţiei este implementată în practica medico-militară prin capacitatea de intervenţie de specialitate pentru protecţia medicală contra armelor biologice şi cu toxine şi contra atacurilor *bio-chem* alături de celelalte forţe ale sistemului naţional de apărare. Prin implementare creşte vizibilitatea rezultatelor cercetării şi implicit se stimulează parteneriatul public-privat. La aceasta poate să contribuie şi diseminarea cunoştinţelor nesecrete către public şi specialiştii civili, a celor secrete către eşaloanele abilitate, dar contribuie în mod hotărâtor şi la îmbunătăţirea capacităţii administrative care să permită dezvoltarea sistemului informatizat de management instituţional, care va contribui la creşterea performanţei instituţionale.

Finalitatea şi scopul cercetării medico-militare a secţiei este, aşa cum am menţionat, protecţia medicală contra armelor biologice şi a bioterorismului, precum şi participarea efectivă la acţiuni, antrenamente şi aplicaţii NBC.

Contextul internaţional actual de ameninţări şi atacuri bioteroriste readuce în actualitate riscurile legate de războiul biologic, arme biologice şi cu toxine, terorismul *bio-chem* şi conexiunea cu problemele epidemiologice actuale (pandemii, panzootii), care pot să culmineze cu criză biologică. Având în vedere faptul că România este la graniţa de est a NATO şi a UE, fiind considerată de preşedintele SUA ca fiind *vârful de lance spre Est al Alianţei*, riscurile biologice trebuie să fie luate în calcul.

În anul 2010, la Prima Ediţie a Târgului Est-European de Proiecte în Domeniul Energiei, Mediului şi Securităţii, 20-22 Octombrie 2010, desfăşurată la Universitatea Naţională de Apărare „Carol I" Bucureşti, a fost propus pentru finanţarea NATO proiectul de cercetare *Platformă integrată de cercetare ştiinţifică şi expertiză pentru protecţie medicală faţă de agenţi biologici, arme biologice şi bioterorism,* ce se va realiza în cadrul unui consorţiu naţional, avându-l ca director de proiect pe General maior (r.) medic Acad.Prof. Dr. Victor Voicu. Importanţa proiectului rezidă din faptul că serviciul medical militar trebuie să poată monitoriza şi să fie în măsură să intervină pentru protecţia medicală contra agenţilor biologici, în primul rând pentru protecţia forţei dar şi pentru protecţia populaţiei din zona afectată. Pentru managementul crizei biologice, cercetarea ştiinţifică medico-militară stabileşte metodele de diagnostic, profilaxie şi tratament aplicabile în diferite cazuri, pe care decidenţii le ordonă în funcţie de situaţie.

Componenţa Platformei integrate de cercetare ştiinţifică şi expertiză pentru protecţie medicală faţă de agenţi biologici, arme biologice şi bioterorism va fi formată din mai multe obiective, unele existente dar nefuncţionale din diferite motive (sau care nu îndeplinesc în totalitate normele actuale de dotare, de biosiguranţă, biosecuritate şi bioprotecţie).

Pentru eficientizarea protecţiei medicale contra armelor biologice, este necesar ca facilităţile complementare, care se găsesc în aceeaşi cazarmă, să fie completate, modernizate, reunite într-o Platformă integrată, care să fie funcţională, autorizată, acreditată, securizată şi recunoscută ca un Laborator naţional de referinţă pentru agenţi biologici. Această entitate este utilă interdepartamental pentru M.Ap.N, M.A.I, M.S.P, M.Ed.C.I, S.R.I ş.a., dar şi pentru UE, NATO, OMS etc., având importanţă naţională şi regională; în acelaşi timp, răspunde cerinţei UE de a completa reţeaua de laboratoare BSL4 funcţionale.

În prezent se lucrează la conceperea şi realizarea unui *Sistem Informatic de Supraveghere a Bolilor Infecţioase cu impact medico-militar*, respectiv cele produse de germeni listaţi ca agenţi biologici şi de flora nosocomială poli-farmacorezistentă. Pentru aceasta, armata va accesa datele din medicina militară, medicina umană şi medicina veterinară, pentru monitorizarea epidemiologică specifică. Considerăm că ameninţările reprezentate de bioterorism sunt reale şi că este necesar să fim pregătiţi în orice moment pentru prevenirea, combaterea şi lichidarea urmărilor atacurilor *bio-chem*.

Specialiştii laboratorului au fost şi sunt angrenaţi (prin cumul de sarcini) în mod activ în activităţi antibioteroriste şi contrabioteroriste de combatere a bioterorismului, implementând şi validând rezultatele cercetării ştiinţifice. Putem exemplifica participarea la formaţiuni antiteroriste ale M.Ap.N şi MAI, Grupul român de combatere a bioterorismului, asigurarea antibioteroristă a Olimpiadei de la Atena 2004, colaborarea cu MAE pentru evaluarea ameninţărilor biologice, parteneriatul NATO-Rusia pentru combaterea bioterorismului, Comisia de rezistenţă microbiană a Ministerului Sănătăţii etc. Specialiştii Secţiei au participat activ la diferite aplicaţii specifice la baza de antrenament pentru apărare CBRN, la Exerciţiul naţional BIOEX'09, Republica Moldova 2010, diferite activităţi NATO etc. Au fost expertizate scrisori suspecte de a fi contaminate cu agenţi biologici sau obiecte suspecte, dar nu s-a confirmat prezenţa de agenţi CBRN în niciunul din cazuri. Nu în ultimul rând, specialiştii secţiei participă la manifestări ştiinţifice naţionale şi internaţionale în domeniu, publică articole (inclusiv cotate ISI), redactează materiale documentare (caiete, ghiduri, instrucţiuni) şi au publicat cărţi şi manuale de specialitate. Cerinţa de a respecta informaţia clasificată în privința unor capacități şi a unor acțiuni specifice face ca activitatea instituției în general şi a laboratorului în special să fie mai puțin cunoscută în afara acestuia.

Pentru implementarea în practica medico-militară a studiilor şi cercetărilor efectuate, participăm activ la alarmele epidemiologice care interesează România: antrax, gripa aviară, gripa porcină, febra West Nile, în perspectiva riscului de epidemie/pandemie luat în calcul de Ministerul Sănătății Publice sau Organizația Mondială a Sănătății. Dacă în România ar exista premise pentru izbucnirea unei epizootii dublate de epidemie, din aceste cazuri ar putea să apară simultan câteva zeci sau sute de complicații ale bolii, care vor necesita internare în secții de boli infecțioase, recoltări de prelevate şi diagnostic virusologic sau bacteriologic. Capacitățile secției noastre sunt utile în orice situație de criză biologică, pentru întărirea sistemului național de răspuns.

Este cunoscut de noi toți faptul că motorul dezvoltării economice şi sociale din orice țară este susținut de activitatea de cercetare-dezvoltare-inovare, motiv pentru care țările au alocate în bugetul de stat fonduri speciale pentru cercetare; aceste fonduri, mari sau mici, reflectă gradul de dezvoltare al respectivei țări. Din păcate, finanțarea noastră de la bugetul M.Ap.N. a fost deficitară în ultimii ani, dar cu eforturi am compensat prin obținerea unor contracte de cercetare de la M.Ed.C.I.

Concluzii

- În ansamblu, situația de criză biologică impune valorificarea rezultatelor cercetării ştiințifice în domeniu şi a experienței fiecărui cercetător în domeniul său.

- C.C.S.M.M. are capacitatea (forțe şi mijloace) şi expertiza pentru a se implica, la ordin, în managementul crizelor biologice, şi în general al evenimentelor CBRN.

- Îmbunătățirea capacității administrative permite dezvoltarea sistemului informatizat de management instituțional, cu efect în creşterea vizibilității rezultatelor cercetării şi implicit stimularea parteneriatului public-privat.

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Malpraxisul medical

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Malpraxisul medical, practica greşită medicală, poate îmbrăca forme multiple, de la neglijenţă până la ignorare sau depăşire de competenţe şi poate fi cu sau fără intenţie. *Eroarea este omenească şi cine nu munceşte nu greşeşte* spune un proverb pe care îl întâlnim la multe popoare. Totuşi greşeala este un fenomen care nu trebuie tolerat, cu atât mai puţin într-un domeniu cum este medicina, unde practicanţii sunt chemaţi să vindece şi să salveze vieţi, nu să le distrugă sau suprime. De la personalul medical sunt aşteptări pozitive din partea societăţii iar legile morale sancţionează cazurile de eroare medicală şi nu numai ele. Încă din Evul Mediu, cazurile de malpraxis au fost pedepsite , astfel că în zilele noastre în cele mai multe ţări există legi distincte în cadrul Codurilor penale, civile sau al legilor speciale care sancţionează această abatere.

Este şi cazul României, unde pe lângă Codul Penal, care incriminează uciderea din culpă, lovirea, sluţirea sau alte vătămări corporale, există şi pedeapsa complementară, extrem de aspră, care constă în *interzicerea exercitării unor profesii,* ca măsură asiguratorie, cu consecinţe pozitive asupra societăţii şi extrem de motivante asupra personalului aflat în situaţia de a putea cădea în aceste culpe, în cazul nostru personalul medical. Din articolele 998-999 Cod civil rezultă că atât greşeala cât şi culpa pot îmbrăca forma dolului, a intenţiei directe, imprudenţei, neglijenţei şi *atrag, totodată, răspunderea juridică.*

La nivel naţional avem şi *Legea nr. 95/2006 privind reforma în domeniul sănătăţii,* care în Cap. I defineşte *Răspunderea civilă a personalului medical.*

Art. 642. (1) În sensul prezentului titlu se definesc astfel:

a) personalul medical este medicul, medicul dentist, farmacistul,asistentul medical şi moaşa care acordă servicii medicale;

b) malpraxisul este eroarea profesională săvârşită în exercitarea actului medical sau medico-farmaceutic generatoare de prejudicii asupra pacientului, implicând răspunderea civilă a personalului medical şi a furnizorului de produse şi servicii medicale, sanitare şi farmaceutice;

(2) Personalul medical răspunde civil pentru prejudiciile produse din eroare, care includ şi neglijenţa, imprudenţa sau cunoştinţe medicale insuficiente în exercitarea profesiunii, prin acte individuale în cadrul procedurilor de prevenţie, diagnostic sau tratament.

(3) Personalul medical răspunde civil şi pentru prejudiciile ce decurg din nerespectarea reglementărilor prezentului titlu privind confidenţialitatea, consimţământul informat şi obligativitatea acordării asistenţei medicale.

Personalul răspunde şi pentru nerespectarea confidenţialităţii, depăşirea competenţelor, acţiunea sau inacţiunea sa. *Personalul medical răspunde civil pentru prejudiciile produse în exercitarea profesiei şi atunci când îşi depăşeşte limitele competenţei, cu excepţia cazurilor de urgenţă în care nu este disponibil personal medical ce are competenţa necesară.*

(5) Răspunderea civilă reglementată prin prezenta lege nu înlătură angajarea răspunderii penale dacă fapta care a cauzat prejudiciul constituie infracţiune conform legii.

Mai există şi excepţia răspunderii unităţii spitaliceşti, în situaţiile următoare:

Art. 643. – (1) Toate persoanele implicate în actul medical vor răspunde proporţional cu gradul de vinovăţie al fiecăreia.

(2) Personalul medical nu este răspunzător pentru daunele şi prejudicii produse în exercitarea profesiunii:

a) când acestea se datorează condiţiilor de lucru, dotării insuficiente cu echipament de diagnostic şi tratament, infecţiilor nosocomiale, efectelor adverse, complicaţiilor şi riscurilor în general acceptate ale *metodelor de investigație şi tratament, viciilor ascunse ale materialelor sanitare, echipamentelor şi dispozitivelor medicale, substanțelor medicale şi sanitare folosite;*

b) când acționează cu bună-credință în situații de urgență cu respectarea competenței acordate….

Capitolul II. *Răspunderea civilă a furnizorilor de servicii medicale, materiale sanitare, aparatură, dispozitive medicale şi medicamente.*

În Capitolul III - *Acordul pacientului informat, Art. 649. – (1). Pentru a fi supus la metode de prevenție, diagnostic şi tratament, cu potențial de risc pentru pacient, după explicarea lor de către medic, medic dentist, asistent medical/moaşă, conform prevederilor alin. (2) si (3), pacientului i se solicită acordul scris;* nu este suficient pentru a exclude răspunderea în caz de malpraxis, şi nici măcar asigurarea de malpraxis, obligatorie conform aceleiaşi legi. *Capitolul V.* Totodată, *asigurarea obligatorie de răspundere civilă profesională pentru medici, farmacişti şi alte persoane din domeniul asistenței medicale* nu exonerează răspunderea personalului medical, ci doar acoperă eventuale prejudicii convertibile în bani.

Pentru celeritatea actului medical, *Capitolul VI* specifică: *Procedura de stabilire a cazurilor de răspundere civilă profesională pentru medici, farmacişti şi alte persoane din domeniul asistenței medicale:*

Art. 668. – (1) La nivelul autorităților de sănătate publică județene şi a Municipiului Bucureşti se constituie Comisia de monitorizare şi competență profesională pentru cazurile de malpraxis, numită, în continuare, Comisia.

(2) Comisia are în componență reprezentanți ai autorităților de sănătate publică județene şi respectiv ai municipiului Bucureşti, Casei județene de asigurări de sănătate, Colegiului Județean al Medicilor, Colegiului Județean al Medicilor Dentişti, Colegiului Județean al Farmaciştilor, Ordinului Județean al Asistenților şi Moaşelor din România, un expert medico-legal, sub conducerea unui director adjunct al autorității de sănătate publică județene, respectiv a Municipiului Bucureşti.

Cu toate că intenția legii este să protejeze personalul medical în fața abuzurilor acuzațiilor de malpraxis, există posibilitatea şi obligativitatea asigurării de răspundere civilă, există principiul răspunderii mai multe persoane şi sau instituții în solidar, ajungându-se până la instituțiile publice şi implicit a contribuabilului, cazurile de malpraxis trebuie evitate iar cel care are posibilitatea de a-l comite trebuie să gândească atent la această situație şi să nu uite că există şi sancțiunea interzicerii dreptului de a mai practica profesia.

Directiva 2005/36/CE are ca principiu şi interzicerea dreptului de a practica o anumită profesie în caz de eroare profesională gravă, malpraxis de exemplu. În ceea ce priveşte pedeapsa complementară de interzicere a unor drepturi, conform raportului congresului RL 34175 din 17 septembrie 2007, Statele Unite ale Americii sunt pe locul 3 din 26 de țări, după cum indică o statistică din anul 2006 a Organizației de cooperare economică şi dezvoltare, OECD, cu sediul la Paris, după Grecia şi Austria, dar cu mult înaintea Japoniei sau a Finlandei, cu un scor de 0,7 la 1 000 față de 0,4 la 1000, procent mediu. Totuşi SUA stau pe un fruntaş loc 3 la prevenție, într-un eşalon de țări din care România nu a făcut parte.

Pentru prevenirea suprasolicitării cu reclamații de malpraxis, Codul Civil californian limitează suma de despăgubire la două sute cinci zeci de dolari pentru daune nemateriale. Totuşi, în sistemul de drept american, decizia este luată de către un comitet de jurați, în coordonarea judecătorului, la fel şi suma ce urmează să fie acordată reclamantului de către cel găsit vinovat.

Organizația Mondială a Sănătății face toate demersurile pentru promovarea drepturilor pacienților, inclusiv o serie de seminarii sau concursuri, dar cu toate că se promovează protecția pacientului, este aproape imposibil de găsit resurse legislative on-line, fără taxe, ci doar link-uri către firme specializate de avocatură.

Conform Codului de procedură civilă al Germaniei, reclamantul cazului de malpraxis trebuie să aducă dovezi de cauzalitate între acțiunea personalului medical şi urmările asupra sănătății sale, motiv pentru care există o mare dificultate în a se demonstra cauzalitatea. Cu toate acestea, dacă pacientul nu dezvăluie toate detaliile la consult, există o comunicare asimetrică între acesta şi doctor, motiv pentru care acest fenomen a stat la baza celor mai multe erori medicale în această țară. De exemplu, o persoană de 30 de ani care aleargă la maraton şi nu declară la controlul medical că uneori are dureri în piept poate să fie uşor victima unei erori medicale de tratament în caz de colaps. În această situație, în caz de infarct miocardic de exemplu, un tratament întârziat duce frecvent la decesul pacientului iar medicul poate fi găsit vinovat de malpraxis.

Principiile care stau la baza medicinii militare a NATO şi SUA conțin elemente asemănătoare, bazate pe asigurarea capacității de luptă a forțelor. Nici cele ale celorlalte state membre nu sunt diferite. Este universal valabil recunoscut faptul că un soldat sănătos este eficient şi nu implică costuri, îşi poate îndeplini misiunile şi se poate alinia statutului său în organizația militară şi în societate. Nu degeaba în cel de-al Doilea Război Mondial trupele aliate au avut în vedere sabotarea capacității de luptă a inamicului prin difuzarea de broşuri care descriau amănunţit cum se pot simula cu succes anumite boli, stări diareice, gripale sau alte boli, pentru a se putea obţine scutiri medicale.

Planificarea medicală este parte integrantă şi obligatorie a planificării de luptă, iar implicarea medicului şef sau a echipei medicale este obligatorie, fiind o condiţie esenţială a succesului. Aici comandanţii şi echipele de planificare sunt obligate să respecte opinia specialistului medical, să lase de o parte toate orgoliile legate de grad, funcţie, vechimea în structura militară şi să acorde medicului sau asistentului medical al echipei tot sprijinul în asigurarea medicală a forţelor, pentru că în mod special în sistemul militar este mult mai eficient să previi decât să tratezi, nu numai pentru costuri directe , ci şi pentru costurile indirecte.

Mă refer, în primul rând, la elementul timp de pregătire al unui soldat profesionist, nu neapărat la costuri, dar trebuie să ţinem seama şi de valoarea umană şi a dreptului omului la tratament medical şi îngrijire. Nici elementul economic nu este de neglijat, dar nu poate fi contrapus dreptului omului la un tratament medical corect sau necesităţii şi obligativităţii de a asigura capacitatea de luptă a forţelor militare care implică direct siguranţa naţională. Tot aici trebuie să menţionez necesitatea asigurării unui climat de încredere a luptătorului faţă de sistemul militar în general, acea încredere care îi dă capacitatea de a-şi asuma riscuri peste medie, cu uşurinţă şi fără ezitare, pentru a nu fi supus stresului negativ ce induce eşecul.

Dacă există încredere în echipa medicală, în sistemul medical militar, în sistemul de protecţie socială, prin instituţia militară şi societate în general, vor exista tot timpul motivaţii pozitive pentru formarea, menţinerea şi perfecţionarea personalului militar, cu implicaţii directe ale creşterii încrederii societăţii în armată în general şi nu acea teamă care ar putea fi exprimată prin cuvintele ”...dacă te accidentezi, nu poţi fi salvat, eşti eliminat din sistem ca un adevărat rebut”. Vor veni curând vremurile când recruţii voluntari vor întreba: *„şi dacă mă accidentez la instrucţie sau în luptă, ce se întâmplă cu mine? Care e rata malpraxisului în spitalele militare? Îi controlează cineva? Răspund pentru ceea ce fac*?”

Bineînţeles, există o greutate care apasă pe umerii fiecărui ofiţer/militar specialist. Obligaţia dată de statutul militar şi cea dată de profesia pe care o practică implică, nu de puţine ori, dublă sau triplă constrângere. Aici, tot timpul trebuie să fie prezentă în prim plan omenia şi specialitatea, în limitele respectării statutului de cadru militar. Dar dacă ne întoarecem la lege, tot ceea ce este moral este implicit legal, tot ceea ce este uman este implicit legal şi nu poate fi contrar statutului de cadru militar. Acest lucru uneori nu este înţeles de o parte dintre militarii de carieră, hotărâţi să facă orice pentru a-şi aduna rapid laurii, chiar dacă îmbolnăvesc cel puţin o companie sau un batalion de gripă sau dacă produc disstres asupra întregii trupe, conform dictonului *„Cei care nu rezistă să plece”,* omiţându-se faptul că şi inacţiunea poate fi infracţiune sau că neimplicarea medicului în prevenţie poate fi considerată sabotaj faţă de capacitatea de luptă a unităţii.

În politica NATO un rol important este acordat asigurării medicale şi interoperativităţii. În acest sens, a fost elaborată o doctrină bazată pe principii simple, eficiente, cum ar fi:

- menţinerea sănătăţii personalului şi implicit a capacităţii de luptă;
- salvarea vieţii, prevenţia, inclusiv a disabilităţilor, revenirea rapidă în luptă;
- eficienţa maximă a sistemului medical, acordarea suportului medical în luptă.

Totul trebuie să plece de la acordarea corectă a primului ajutor, evacuarea medicală, resuscitarea şi tratamentul la locul accidentului, intervenţia chirurgicală promptă, corectă, tratament adecvat, recuperarea, convalescenţa şi implicit reintegrarea în sistemul militar. Niciun pas nu trebuie să fie neglijat, totul este interdependent şi este menit să asigure o revenire rapidă a luptătorului la locul său de muncă. Orice eroare în acest lanţ poate produce efecte în cascadă, nefaste.

Documentele cheie ale planificării medicale NATO includ *Military Committee Document 326/2 (NATO Medical Support, Principles and Policies), Allied Joint Publication 4A (Allied Joint Logistics Doctrine), and Allied Joint Publication 4.10 (Medical) dar şi "Abbreviations available in military and military-medical terminology of NATO".The "Essential List of STANAGS" includes two NATO agreements of this kind: (1) STANAG 2131 – "Multilingual Phrase Book for the use by the NATO Medical Services (AMEDP-5) a 2) STANAG 2409 – "NATO Glossary of Medical Terms and Definitions (AMEDP-13)".*

Experienţa exerciţiilor şi misiunilor din teatrele de operaţiuni a demonstrat că medicina militară românească de campanie este la nivelul standardelor NATO, dar nu trebuie să se uite faptul că de cele mai multe ori a fost folosit pentru îndeplinirea acestor misiuni personal medical înalt calificat din spitalele militare, civili sau militari, şi nu numai personal medical de la nivelul unităţilor luptătoare care în condiţiile desfăşurării activităţii strict la acest nivel se poate plafona din punct de vedere profesional.

În SUA, dar şi în celelate state NATO, legile malpraxisului medical au aceleaşi principii, sunt bazate pe eroarea de fapt, neglijenţă, acţiune greşită sau inacţiune a personalului medical, acoperite sau nu de un ordin, verbal, scris, legal, ilegal sau la limita legii. Cazurile de malpraxis medical sunt reclamate în instanţă, civilă sau militară, judecate de către judecători şi/ sau-şi juraţi, existenţa lor este confirmată de experţi medicali sau cercetări tehnico-ştiinţifice care de obicei au rolul hotărâtor în stabilirea vinovăţiei. Dacă omenia nu îşi mai are loc în personalitatea şi acţiunile celor care au depus jurământul lui Hipocrate, trebuie să se ţină cont de faptul că în general erorile medicale sunt extrem de uşor de detectat, lasă întotdeauna urme şi sechele care sunt lesne de investigat şi folosit având în vedere evoluţia tehnologiei actuale. Cu toate că legislaţia malpraxis-lui este greu de abordat, bine ascunsă şi de cele mai multe ori greu de accesat în link-uri şi site-uri care cer parolă şi plata online cel puţin, motivaţia constrângerii trebuie luată în seamă. Aproape la fiecare *click* găseşti o firmă de avocatură gata să ajute victima şi, din nefericire, şi presupusa victimă, care chiar dacă nu va reuşi să obţină o despăgubire sau ordinul de ridicare a dreptului de liberă practică a profesiei medicale va reuşi cel puţin să producă acel disconfort moral şi/sau psihic deloc de neglijat.

Se pune problema într-un mod foarte delicat în cazurile de malpraxis asupra membrilor forţelor armate străine, membre NATO sau partenere PfP. Pentru prevenirea situaţiilor există deja STANAG-urile şi SOP-urile, multe dintre ele clasificate şi care nu pot fi publicate aici dar care sunt perfect cunoscute şi implementate la nivelul fiecărei structuri militare. De fapt, documentele cu caracter medical deja completate sunt protejate atât de legea protecţiei informaţiilor clasificate 182 din 2002 dar şi de *Legea drepturilor pacientului* (legea nr. 46 din 21.01.2003, MO Partea I nr. 51 din 29/01/2003). Repet, nici consimţământul pacientului, nici asigurarea medicală nu exonerează personalul medical de răspundere în caz de malpraxis, şi nu trebuie să se uite de măsura asiguratorie de interzicere a dreptului de practică, de graniţa ascuţită a răspunderii penale, care efectiv încetează cariera celui vinovat.

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8. *Statutul OAMMR*
9. *Codul de etică şi deontologie profesională al asistentului medical şi al moaşei din România.*
10. *Legea nr. 95 / aprilie 2006 privind răspunderea civilă profesională a personalului medical.*
11. *Legea nr. 46/ 2003 privind drepturile pacientului*
12. *Cod penal*
13. *Cod civil*
14. *Declaraţia universală a drepturilor omului*
15. *Convenţia europeană asupra drepturilor omului şi libertăţilor fundamentale*
16. *Charta socială europeană*
17. *Medical malpractice law USA,NY*

Hemoragie digestivă superioară severă prin esofagită peptică şi gastrită hemoragică secundare unei hernii hiatale prin alunecare

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Rezumat. Se prezintă cazul unui pacient diagnosticat cu hernie hiatală prin alunecare, cu esofagită de reflux şi gastrită hemoragică, la care simptomatologia dominantă a fost determinată de hemoragia cronică persistentă, cu sindrom anemic sever. Cura chirurgicală a herniei hiatale a avut rezultate bune în ceea ce privește vindecarea gastritei, cu oprirea hemoragiei și amendarea refluxului gastro-esofagian. Se evidențiază o posibilitate evolutivă mai rară a herniei hiatale, cu hemoragie digestivă superioară cronică gravă.

Cuvinte cheie: hernie hiatală, esofagită de reflux, gastrită hemoragică, hemoragie digestivă superioară.

Abstract: The author presents the case of a patient diagnosed with sliding hiatal hernia, including reflux esophagitis and hemorrhagic gastritis, whose dominant symptomatology was determined by the persisting chronic bleeding, with severe anemic syndrome.

The surgical treatment of the hiatal hernia was effective in healing the gastritis, as it stopped the hemorrhage and improved the acid reflux.

The author emphasizes the rare evolution of hiatal hernia, which would present severe and chronic bleeding of the upper-digestive tract.

Prezentarea cazului

Bolnavul C.I., în vârstă de 69 ani, pensionar, cu origine în mediul rural, se internează în Secția Chirurgie II a Spitalului Universitar de Urgență Militar Central pentru dureri retrosternale postprandiale precoce, cu remisiune spontană la o oră, regurgitații alimentare, scaune melenice, sindrom anemic, scădere ponderală (aproximativ 6 kg în două luni). Debutul simptomatologiei este situat în urmă cu 3 ani. Anterior internării, pacientul a fost investigat în altă unitate sanitară, fiind suspectat diagnosticul de neoplasm gastric. Radiografia după ingestie de pastă baritată evidenția: esofag cudat şi stomac cu pungă de hernie transhiatală de 6/6 cm, ireductibilă, prin alunecare.

La internare, pacientul prezenta: paloare intensă cu tentă subicterică, astenie fizică cu tendință de lipotimie, abdomen dureros în epigastru cu iradiere retrosternală, meteorizat, fără alte modificări la examenul obiectiv. Melena este obiectivată prin tact rectal. TA=90/50 mmHg, AV=100/min. Probele biologice evidențiază anemie hipocromă severă: Ht 20%, Hb 6,80 g%, frotiu sangvin periferic: anizocitoză, poikilocitoză, hipocromie ++. Celelalte investigații de laborator şi cardio-pulmonare se situează în limitele normalului.

Se instituie terapie intensivă de reechilibrare hidro-electrolitică, hematologică şi metabolică, antisecretorie gastrică, având ca rezultat ameliorarea stării clinice în primele 24 ore, cu îmbunătățirea probelor biologice.

Pentru precizarea diagnosticului se indică repetarea pasajului baritat, care evidențiază imagine de hernie transhiatală prin alunecare, de dimensiuni mari şi ascensionarea intratoracică a corpului gastric (fig.1). Fibroendoscopia digestivă superioară decelează leziuni de esofagită gr. II-III în 1/3 inferioară, interesând întreaga circumferință, cu mici ulcerații superficiale pe fond de mucoasă hiperemică, cu false membrane, care sângerează la detaşare. Stomac cu pliuri îngroşate, cu leziuni de gastrită fundică – peteşii şi ulceraţii superficiale multiple. Defileu antro-piloro-duodenal normal.

Diagnosticul pozitiv fiind stabilit şi clasificate problemele de diagnostic diferenţial, se continuă terapia intensivă şi pacientul este pregătit pentru operaţie. Indicaţia chirurgicală este dată de diagnosticul: hernie hiatală prin alunecare cu gastrită hemoragică şi esofagită de reflux gr. II-III. Obiectivele intervenţiei sunt: explorarea cavităţii peritoneale, reducerea herniei, hemostază (dacă există leziuni abordabile chirurgical) şi suprimarea refluxului prin recalibrarea hiatusului şi corectarea geometriei joncţiunii eso-gastrice.

După preanestezie şi anestezie generală cu I.O.T. se efectuează celiotomie mediană supraombilicală. Intraperitoneal: stomac cu polul superior ascensionat intratoracic transhiatal (marea şi mica curbură în 1/3 superioară), cu un fund de sac peritoneal preesofagian. Hiatusul esofagian lărgit, cu diametrul de circa 6 cm. Reducerea herniei se face cu uşurinţă, constatându-se laxitatea mijloacelor de contenţie esogastrică. Stomacul prezintă un perete îngroşat în 1/3 superioară, fără leziuni ischemice sau ulceraţii profunde. Se infirmă suspiciunea de neoplasm gastric. Controlul cavităţii peritoneale nu evidenţiază alte aspecte patologice. Se practică recalibrarea hiatusului esofagian prin sutura pilierilor şi se efectuează fundoplicatura după procedeul Nissen.

Postoperator se continuă tratamentul de reechilibrare şi susţinere a funcţiilor vitale în Secţia A.T.I., evoluţia postoperatorie fiind favorabilă. Nu am înregistrat complicaţii chirurgicale sau de altă natură, pacientul reluând alimentaţia orală, cu tranzit digestiv normal la 5 zile, şi fiind externat după 8 zile, vindecat chirurgical.

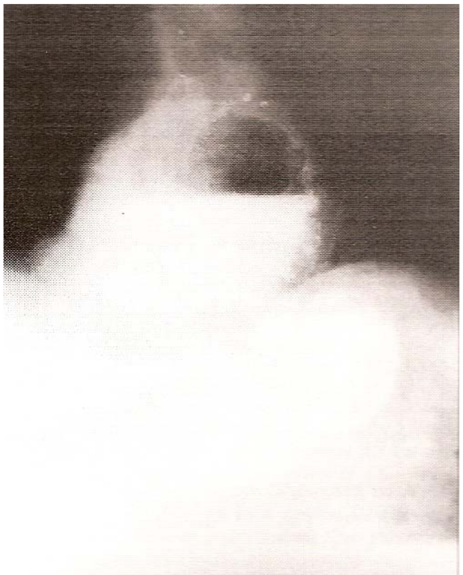

Fig. 1. Pasaj baritat preoperator: hernie transhiatală prin alunecare

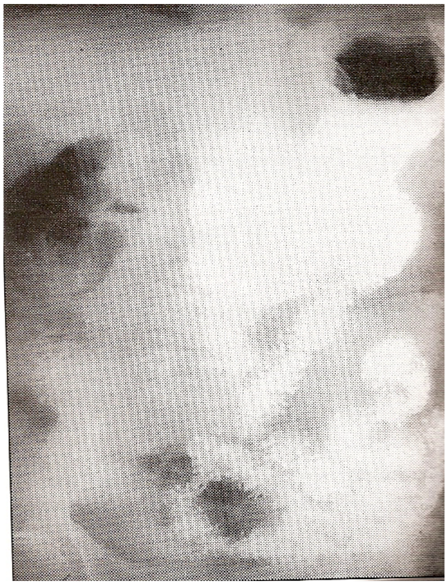

Fig. 2. Control radiologic la 30 zile postoperator: poziţie şi configuraţie normale ale joncţiunii eso-gastrice

Controlul radiologic la 30 de zile postoperator arată poziţie şi configuraţie normale ale joncţiunii eso-gastrice şi a fornixului, fără reflux în poziţie Trendelenburg (fig. 2). Controlul clinic la 3 şi la 6 luni atestă evoluţia postoperatorie favorabilă, cu toleranța alimentară bună, dispariția sindromului anemic, creştere ponderală semnificativă.

Concluzii

Hernia hiatală este o afecțiune ce beneficiază de corecție chirurgicală, indicația fiind absolută în cazul apariției complicațiilor, dintre care cele mai redutabile sunt hemoragia la nivelul leziunilor esofagiene sau gastrice, strangularea gastrică, ulcerul esofagian sau gastric şi, nu în ultimul rând, malignizarea în zonele de esofagită sau gastrită.

Reducerea chirurgicală a herniei hiatale trebuie completată de un procedeu antireflux, prevenind astfel majoritatea complicațiilor grave ale unei entități clinice cu evoluție aparent benignă.

Cazul prezentat este de dată mai veche. Utilizare, în prezent, a chirurgiei laparoscopice face ca operația să fie mult mai rapid acceptată de către pacient, şi astfel de cazuri să fie mai rar întâlnite.

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PRIMA EDIȚIE A
ZILELOR SPITALULUI UNIVERSITAR DE URGENȚĂ MILITAR CENTRAL "DR. CAROL DAVILA"

7-9 SEPTEMBRIE 2011
CERCUL MILITAR NAȚIONAL, BUCUREŞTI

SECȚIUNI ŞTIINȚIFICE: MEDICALE, CHIRURGICALE, VARIA, NURSING
REZUMATELE VOR FI PUBLICATE ÎNTR-UN SUPLIMENT AL *REVISTEI DE MEDICINĂ MILITARĂ*, PUBLICAȚIE COTATĂ CNCSIS "C"
LUCRĂRILE VOR FI SUSȚINUTE SUB FORMĂ DE POSTERE SAU PREZENTĂRI ORALE ŞI POT FI PUBLICATE ÎN REVISTA MENȚIONATĂ

VĂ AŞTEPTĂM CU INTERES PARTICIPAREA!

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4 – probleme nerezolvate, întrebări la care nu s-a răspuns şi care rămân deschise în viitor pentru studiile ce vor urma, eventuale direcţii propuse pentru investigare în domeniul respectiv.

CONCLUZII

La nevoie, capitolele mari pot fi împărţite în subcapitole, după necesităţi.

Se recomandă utilizarea precaută a *abrevierilor* şi menţionarea numelui complet la prima apariţie în text a abrevierii respective. Toate referinţele bibliografice vor fi citate în text cu cifre arabe, la exponent, fără paranteze, în ordinea apariţiei (de exemplu: Un alt studiu[18] arată...). Toate figurile şi tabelele vor fi citate în text în locul în care ele urmează să apară (de exemplu: *Fig. 3*, *Tab. 2*). Datele care apar în tabele, legendele tabelelor şi figurilor trebuie să fie rezumate, nu duplicate în text.

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Tabelele trebuie să fie procesate la două rânduri, pe o pagină separată, *numerotate* consecutiv (cu referire în text în locul unde ele trebuie să apară) şi *cu un titlu* (exemplu: *Tabelul 1.* Distribuţia pe vârste şi sex a pacienţilor). Capetele de tabel se scriu cu caractere *italice.* Nu trimiteţi fotografii sau imagini ale tabelelor. Utilizaţi tasta „Tab” pentru a separa coloanele (nu bara de spaţiu) şi utilizaţi tasta „Enter” la terminarea liniei. Toate căsuţele goale din tabel trebuie să aibă o liniuţă. Dacă este nevoie, pentru cuvintele sau frazele lungi din căsuţe se vor utiliza abrevieri, însă cu explicarea acestora în subsolul tabelului. Asiguraţi-vă că toate tabelele sunt citate în text.

5. FIGURILE

Toate figurile (ilustraţiile), fie fotografii, diagrame sau grafice, trebuie să fie de calitate. Ele se *numerotează* în ordinea apariţiei în text (cu referire în text la numărul figurii în locul în care aceasta ar trebui să apară) şi *vor purta un titlu* (de exemplu: *Figura 1.* Distribuţia pe vârste şi sexe a pacienţilor).

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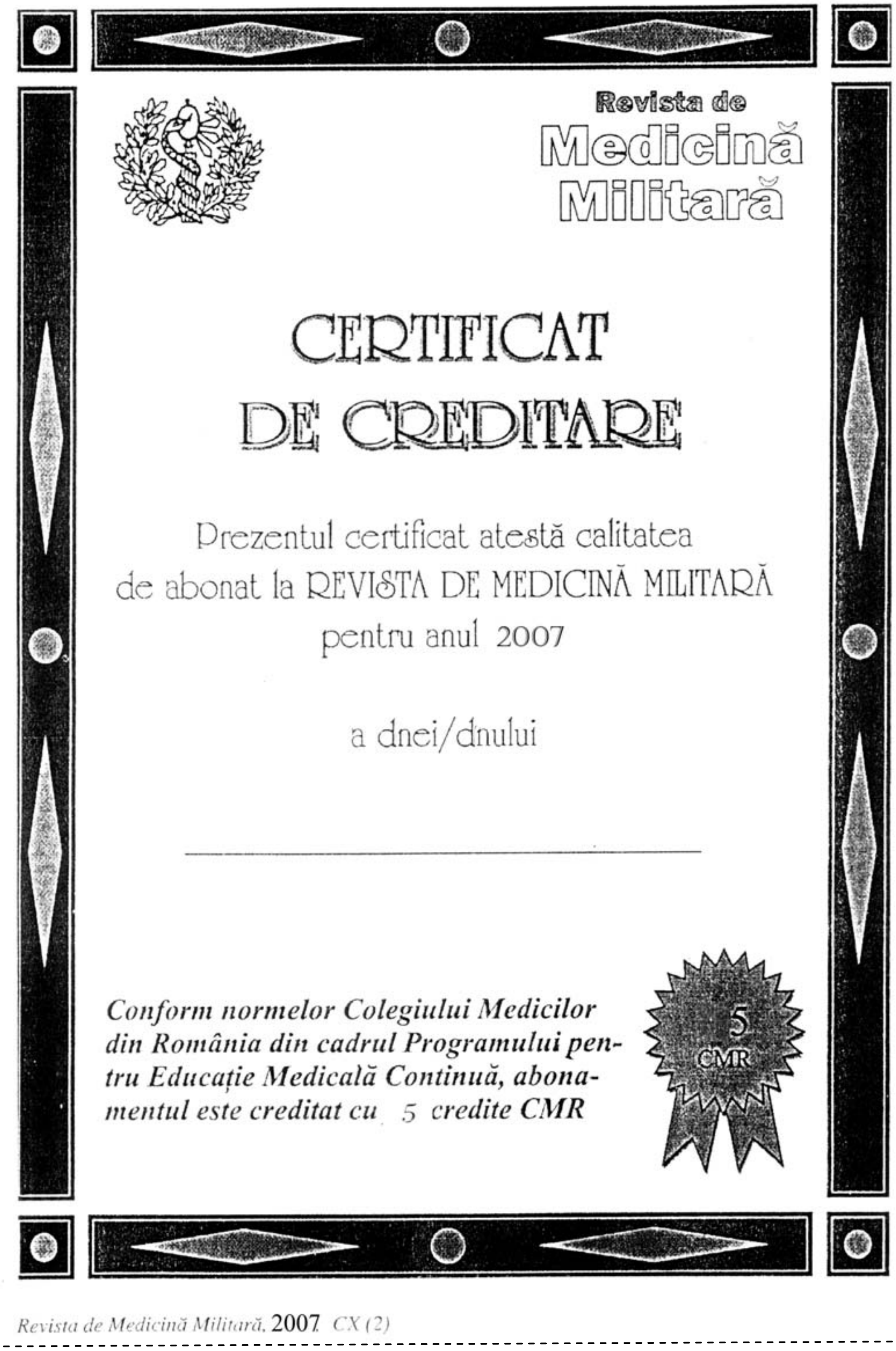

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MESAJUL
MINISTRULUI APĂRĂRII NAȚIONALE GABRIEL OPREA CU OCAZIA SĂRBĂTORIRII A 180 DE ANI DE LA ÎNFIINȚAREA SPITALULUI UNIVERSITAR DE URGENȚĂ MILITAR CENTRAL "DR. CAROL DAVILA"

La 13 septembrie, sărbătorim 180 de ani de la înființarea *Spitalului Oştirii*, ale cărui tradiții sunt duse astăzi mai departe de Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila".

Vă felicit pentru întreaga activitate desfăşurată şi vă transmit gândurile mele de apreciere şi prețuire dumneavoastră, celor aproape 1 500 de angajați care contribuiți zilnic la asigurarea asistenței medicale de specialitate în această instituție de prestigiu a Armatei României.

Doresc, de asemenea, să aduc un călduros omagiu tuturor celor care şi-au respectat, de-a lungul a aproape două secole, în cadrul celui mai important spital militar al țării, jurământul de credință față de Patrie în acelaşi timp cu jurământul lui Hipocrate.

Este un fapt binecunoscut că Spitalul Militar Central, aşa cum a fost numit în cea mai mare parte a existenței sale, a dat învățământului medical românesc profesori şi specialişti de excepție, întemeietori de şcoală şi pionieri în domenii medicale de vârf, profesionişti desăvârşiți care, cu credință şi tenacitate, şi-au onorat atât statutul de medic, cât şi pe cel de militar chemat să-şi slujească țara sub Drapelul național.

Întemeietorul învățământului medical românesc, doctorul Carol Davila, a contribuit la formarea unor structuri medicale fundamentale în țara noastră, între care şi *Serviciul Sanitar al Armatei*. De asemenea, Carol Davila, al cărui bust realizat de Constantin Brâncuşi este expus, în semn de recunoştință şi aleasă cinstire, în curtea spitalului, a contribuit şi la dezvoltarea Spitalului Oştirii, pe care l-a comandat între anii 1853 şi 1882.

Spitalul Universitar de Urgență Militar Central, care funcționează în actuala locație din anul 1889, s-a dezvoltat treptat pentru a răspunde cererilor crescânde de asistență medicală, ajungând în prezent să aibă 10 secții chirurgicale şi 14 secții medicale.

Aflat în permanență în avangarda medicinei româneşti, spitalul dispune astăzi de laboratoare şi aparatură imagistică moderne, tomograful computerizat *multislice* cu multiple aplicații clinice, liniile noi de endoscopie şi bronhoscopie sau aparatul de rezonanță magnetică nucleară fiind doar câteva exemple.

În ultimii ani a fost renovat şi dat în folosință Ambulatoriul de Specialitate al SUUMC care asigură, prin munca dumneavoastră susținută, consultații de specialitate pentru pacienții ambulatori.

Recent, în cadrul procesului de modernizare a spitalului a fost renovat pavilionul de neurologie şi psihiatrie, au fost îmbunătățite condițiile de cazare din secțiile medicale şi chirurgicale, a fost refăcută stația de sterilizare şi a fost ridicată o biserică, pentru nevoile liturgice ale pacienților şi ale cadrelor medicale.

Asistența medicală pe care o asigură astăzi Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila" permite tratarea, în fiecare an, a peste 175 000 de pacienți internați sau în regim ambulatoriu, acoperind o gamă largă de persoane, de la militarii întorşi din teatrele de operații la membrii familiilor militarilor sau pacienți civili.

Înființarea, în cadrul spitalului, a Serviciului de asistență medicală spitalicească pentru militarii care au participat la misiuni operative, dotat cu paturi, aparatură medicală şi încadrat cu personal sanitar calificat propriu, este încă o expresie a grijii manifestate de conducerea Ministerului Apărării Naționale pentru cadrele militare care au participat la misiuni externe şi care au suferit traume.

De asemenea, SUUMC a contribuit esențial la înființarea şi funcționarea Serviciului Medical Militar de Urgență, pentru a răspunde unei nevoi reale de realizare şi funcționare a unei structuri specializate în anunțarea rapidă, intervenția, acordarea primului ajutor calificat la fața locului, transportul şi asistența medicală de urgență pentru personalul Ministerului Apărării Naționale aflat în misiune pe teritoriul național şi în marea liberă, în limita razei tactice de acțiune a navelor militare.

O altă dimensiune importantă a activității dumneavoastră este cea universitară, în cadrul spitalului desfăşurându-şi activitatea de pregătire profesională aproximativ 1 500 de rezidenți militari şi civili în fiecare an, sub îndrumarea medicilor şi cadrelor universitare din secțiile spitalului, la care se adaugă un număr important de studenți ai Institutului Medico-Militar şi ai facultăților de medicină din Bucureşti.

În cadrul spitalului se desfăşoară şi o intensă activitate de cercetare ştiințifică, reflectată în numărul important de lucrări publicate de medici la congresele şi conferințele de specialitate.

Dragi camarazi,

Spitalul Universitar de Urgență Militar Central „Dr. Carol Davila", pe care îl recunoaştem cu mândrie drept *motorul* medicinei militare româneşti, se poate mândri, la împlinirea a 180 de ani de existență, cu îndeplinirea în excelente condiții a tuturor misiunilor încredințate şi, mai ales, cu realizări de excepție în nobilul domeniu al medicinei.

Doresc să vă mulțumesc tuturor pentru modul exemplar în care v-ați îndeplinit sarcinile profesionale, să vă felicit şi să vă urez multă sănătate, putere de muncă, satisfacții profesionale şi personale alături de cei dragi.

Aşa să ne ajute Dumnezeu!

GABRIEL OPREA

MINISTRUL APĂRĂRII NAȚIONALE

O sută optzeci de ani…“Timpul este darul veşniciei”

> *“Vreme trece, vreme vine,*
> *Toate-s vechi şi nouă toate;*
> *Ce e rău şi ce e bine*
> *Tu te-ntreabă şi socoate”*

Râul timpului în care noi călătorim ne lasă să răsuflăm şi să admirăm un clişeu al nemuririi.

Aşa cum marile suflete nu pier odată cu timpul, nici marile instituții, edificii ale umanității, probabil, nu vor pieri.

În contemplarea scurtei noastre călătorii, în relativitatea timpului, devenim, din viitor, acum, prezent şi, peste o clipă, trecut.

Spitalul Militar Central din Bucureşti reprezintă o parte a eternității medicinei militare româneşti şi are vârsta, practic, a statului român modern.

Profesia de medic este una aparte, iar, alături de cariera militară, creează simbioza care generează apariția unei profesii distincte şi distinse – cea de medic militar.

După cum lesne se poate observa, medicul militar reprezintă o parte a echipei, a armatei, a țării.

Istoria Spitalului Militar Central se suprapune cu istoria medicinei militare, cu năzuințele a mii de medici militari, a ostaşilor români şi chiar a tuturor românilor. Aceste ziduri reprezintă istorie, sunt încărcate de istoria noastră.

În obținerea structurilor fundamentale, în orizontul temporalității şi sălăşluind mereu în grija pentru ceilalți, Spitalul Militar Central, prin medicii săi şi întreaga echipă, trebuie să răspundă provocărilor medicinei contemporane, a medicinei genomice şi moleculare.

Am încrederea că aceste generații vor răspunde acestor provocări, pentru a putea dovedi şi confirma valoarea şcolii medicinei militare româneşti şi, implicit, a Spitalului Militar Central.

Istoria acestui spital unic este legată atât de conflictele armate, de războaiele trecute, dar şi de cele prezente, întrucât, în procesul de integrare a României în structurile euro-atlantice de securitate militară, securitatea medicală reprezintă o prioritate.

Spiritul Părintelui Medicinei Militare Româneşti, Gl. dr. Carol Davila, trăieşte prin noi, prin generațiile care ne vor urma, indiferent de intemperiile cărora urmează să le facem față.

Spitalul Militar Central are atribuții noi, universitare, dar, de-a lungul istoriei, putem observa o continuitate a acestor preocupări, pentru perfecționarea medicilor, pentru cercetare.

Spitalul Militar Central, sanctuar al medicinei militare româneşti, reprezintă creația şi efortul a generații de medici militari, care s-au pus în slujba vieții camarazilor lor, a compatrioților şi a ştiinței medicale, de multe ori chiar cu prețul vieții.

“Spitalul Militar Central este o legendă, noi suntem parte a ei”. Să fim, deci, mândri şi responsabili !

COMANDANTUL SPITALULUI UNIVERSITAR DE URGENȚĂ
MILITAR CENTRAL “Dr. CAROL DAVILA”

Colonel medic,
Dr. Aurelian-Emil RANETTI

Spitalul Militar Central: istorie, destine...

Motto*: "Dacă vrei ca viitorul să arate credibil*
pune în el şi trecutul!"
Stanley Kubrick (1928-1999) - regizor american

Atunci când am fost solicitat să scriu acest Editorial pentru *Revista de Medicină Militară* (publicație tricentenară, cum puține reviste există în arealul nostru, lucru demn de reținut!) cu prilejul Jubileului a 180 de ani de la atestarea documentară a înființării "Lazaretului Regimentului 1 din Bucureşti"- nucleul formator a ceea ce a devenit mai apoi Spitalul Militar Central - am intrat, vă mărturisesc, într-o uşoară stare de alertă...

Îmi place istoria, citesc cu nesaț articole de sorginte istorică, atent fiind întotdeauna la aforismul lui Hegel*: "Atât experiența cât şi istoria ne arată însă că nicicând popoarele şi regimurile n-au învățat ceva din istorie şi n-au acționat potrivit preceptelor ce ar fi trebuit desprinse din ea"* sau, cum am parafrazat aceste înțelesuri într-un capitol de carte - *din istorie, vă rog să mă credeți, nimeni nu învață nimic! Cred că acesta este şi motivul pentru care ea* se *repetă!..."*

Jubileul unei instituții devenită "brand", cum se spune acum, sau "bun național", cum se zicea mai deunăzi, este un lucru demn de luat în seamă.

Academia Română (Societatea Academică Română) a împlinit de curând 145 de ani de la înființare iar Banca Națională a României, opera marelui om politic şi de stat Eugeniu Carada, despre care 99% dintre români nu ştiu mai nimic, a sărbătorit anul trecut 130 de ani de când a fost edificată. Răsfoiam, mai deunăzi, o carte superbă ***"1001 Days Shaped the World"*** produs ***Quintessence Book, 2008.*** Între zilele consemnate acolo nu am găsit, din păcate, alături de ziua de 4 iulie 1776 (Life and Liberty), 14 iulie 1789 (Rebels Storm the Bastile) sau de 25 decembrie 1989 (Death of a Despot) şi ziua de 13 septembrie 1831.

În accepțiunea publică românească ziua de 13 septembrie este asociată unui eveniment poate mult mai important: pe Dealul Spirii din Bucureşti căpitanul Pavel Zăgănescu a ținut piept la 5 000 de turci la 13.09.1848. Este data de naştere a armei Pompierilor din România. Dar tot în aceaşi zi, şeful oştirii - spătarul Alexandru Ghica – mustrat mai înainte de domnitor că întârzia să înființeze "Spitalul pentru oastea pământească" îl însărcinează pe Doctorul Johann Georg Andreas Grünau să întocmească proiectul Spitalului ostăşesc. Credem că aceasta este data oficială a înființării a ceea ce, mai târziu, va deveni Spitalul Universitar de Urgență Militar Central "Dr Carol Davila" şi merită consemnat că printre primii medici – cu diplomă – care au făcut parte din oştirea Munteniei s-au numărat Alcibiade Tavernier, Petre Ferrari, Ignat Navarra, Ştefan Episcopescu şi chirurgii Carol Vasarhely şi Alexandru Leding. Vă rog să reţineţi că totul era gândit de primul comandant al Spitalului Militar care se afla în Mânăstirea Mihai Vodă - colonelul dr. Johann Georg Andreas Grünau (1831- 1837). Generalul de divizie Prof. dr Carol Davila va fi cel de-al cincilea şi cel mai "longeviv " comandant al acestui aşezământ (1853-1882). El a fost, de fapt, şi *Reformatorul* acestui sistem...

De ce am fost totuşi uşor "alertat", cum scriam la început? Probabil fiindcă mi-am dat brusc seama că mă "poziţionez" printre cei mai cu experienţă (ca să nu folosesc un eufemism) membri ai acestui "areal" care dă sănătate semenilor de lângă Gara de Nord, şi nu numai... Îndrăznesc să fac o paranteză pentru a explica. Faptul că Spitalul Militar Central există de 128 de ani lângă Gara de Nord nu este întâmplător. Între 1859-1883 Spitalul a avut drept locaţie actuala clădire din strada Ştirbei Vodă a DNA, iar în 1883 Ministerul de Răsboiu alocă şi achiziţionează cu 1 370 000 lei suprafaţa de 82 802 m^2 de teren, diriguitorii acestei decizii gândind, probabil, că transportul vagoanelor cu răniţi este mai facil (lucru ce s-a întâmplat şi în primul şi în cel de-al doilea Război Mondial!)

Există păreri că pe aceste locuri ar fi existat o vie a boierilor Goleşti (idee generoasă, în sensul de "înmugurire"!), alţii spun că terenul ar fi aparţinut unui anume farmacist Witting! Cine mai ştie?...

Destinul meu a fost să păşesc în curtea Spitalului Militar Central exact în ziua de 4 iulie 1973, zi în care împlineam 19 ani. Terminasem liceul, Bacalaureatul nu era o problemă pentru cei de generaţia mea, urma să ni se facă vizita medicală tuturor celor care nădăjduiam să fim "apţi pace şi război" pentru a concura la admitere într-o instituţie militară de învăţământ – "Centrul de pregătire medico-militară" (aşa se şi chema atunci) şi care, accentuez acest lucru, şi-a desfăşurat totdeauna activitatea sub auspiciile şi sprijinul Universităţii de Medicină şi Farmacie "Carol Davila" (atunci se numea IMF Bucureşti). Instituţiile, fiţi siguri, au şi acum, ca şi atunci, precum oamenii, destine alambicate! Am intrat la Medicină, nici nu se pusese problema să apelez la vreun profesor meditator, învăţasem probabil ca pe vremea lui Aristotel, cu creionul şi cu foaia albă de hârtie în faţă…

Astăzi, "generaţia IT", cum îmi place să o numesc, învaţă mult mai uşor. Butonând, se pare…

Spitalul Militar Central (care, la jumătatea secolului XX s-a chemat "Regina Elisabeta") a devenit în 1996 prin atestarea Senatului Universităţii de Medicină şi Farmacie din Bucureşti (decizia Ministerului Învăţământului şi Ştiinţei, nr 11503/1996) Spitalul Clinic Militar Central. În perioada imediat postdecembristă s-au deschis larg porţile şi spitalul - în fond o fabrică cu foc continuu! – a început să redevină ceea ce a fost dintotdeauna – *un brand al aşezămintelor de acordare şi îngrijire a sănătăţii* de la noi. Mai apoi a fost numit întru cinstirea memoriei omului providențial care a așezat piatra de temelie a învățământului medical românesc şi a organizat sistemul sanitar conform normelor şi preceptelor în vigoare atunci – Spitalul Universitar de Urgență Militar Central “Dr. Carol Davila”.

Am să vă fac o confidență. În fiecare dimineață când intru în spital mă opresc în fața bustului strămoşului nostru comun, al tuturor celor ce vor citi aceste rânduri, Generalul Profesor dr. Carol Davila, bust realizat de inegalabilul Constantin Brâncuşi (originalul se află peste drum, la Muzeul Militar) în 1903 şi aşezat - acum 99 de ani (1912) - în curtea Spitalului şi îmi pun o întrebare: prin ceea ce realizăm, prin ceea ce întreprindem şi prin ceea ce înfăptuim răspundem aşteptărilor şi speranțelor pe care acest om le-a aşezat în opera Sa?

Hippocrate - patronul nostru universal (460-370 î. Ch) - credea cuvenit că medicii trebuie să aibă şapte calități pentru a putea profesa această nobilă meserie: să fie educați, să fie apți (în sensul de aptitudine pentru a profesa această meserie), să fie morali în relația cu pacienții, să aibă iubire sau dragoste de muncă, să aibă aplecare spre studiu permanent, să dorească a dărui sănătate şi, mai cu seamă, să aibă iubire de oameni. Fac o mărturisire: în fiecare seară, înainte de a pune capul pe pernă, gândesc şi iau seamă şi la aceste învățăminte…

Trăiesc, pe undeva, cu convingerea că şi antecesorii noştri, nume nobile ale medicinii militare din România, fie că s-au chemat Athanase Demosthen, Zaharia Petrescu, Ioan Vercescu, Iacob Potârcă, Mihail Butoianu, Constantin Zamfir, Bogdan Marinescu, Eugeniu Turcu, Gheorghe Niculescu, Traian Oancea, Iuliu Şuteu şi mulți, mulți alții, au fost animați de aceleaşi gânduri şi deziderate. Ei reprezintă popasuri obligatorii pentru orice medic militar român.

Probabil, cum spunea un înțelept, „principiile sunt luxul celor care stau pe margine!...”. Cred că nu este cazul celor numiți mai sus – oameni de mare şi intens angajament – care au făurit ceva!

Cu toții, proveniți din „***suşa***” numită „***Institutul Medico Militar***” au avut în minte drept far călăuzitor şi au acționat conform celor şapte precepte ale medicinii hipocratice.

Anii de după ’90 ne-au adus în atenție alți oameni, alte fapte, alte întâmplări, cum se spune.

Oamenii, ca şi timpurile, vin şi trec. Timpurile se schimbă chiar mai repede. Sub ochii mei s-au ***petrecut*** o seamă de oameni şi am constatat un număr de ***schimbări,*** bune sau mai puțin bune, legate toate de ceea ce înseamnă istoria Spitalului Militar Central...

Profesorul universitar dr. Victor Ciobanu, ministru al Sănătății între 1985-1989, a lucrat ca medic primar de boli interne în Spitalul Militar Central. A trecut în rezervă cu gradul de maior medic după ce a lucrat la Policlinica Cobălcescu (actualul Ambulatoriu „Academician Ştefan Marius Milcu”).

Academicianul dr. Eugeniu Gh. Proca (Profesor dr. docent al UMF „Carol Davila”, Rectorul Universității între 1976-1978, Ministrul Sănătății între 1978-1985), model al multor generații de urologi român, a fost trecut în rezervă când era locotenent dr. în 1950 pentru simplul fapt că a aprins focul în sobă cu ziarul partidului – „Scânteia”. Exact ca şi acum; alți oameni, alte timpuri.

Academicianul Gl.mr.(r) dr. Gheorghe Niculescu a fondat specialitatea de Ortopedie-Traumatologie în Spitalul Militar Central. A fost un şef de şcoală, un profesionist desăvârşit, un om care a influențat prin energie şi prin abnegație destinul multora. Chiar şi al meu! Îi rămân profund recunoscător!

Doresc să amintesc şi numele unor oameni dragi sufletului meu fără intenția de a transforma acest articol într-un panegiric. Cei despre care scriu sunt „***nume***” importante ale medicinii militare române, „formatate” în acelaşi laborator – Spitalul Militar Central.

Generalul de armată (r) Profesorul dr. Vasile Cândea, om cu mare responsabilitate sufletească de a ajuta pe cei în suferință, rămâne un spirit tenace, un fondator (a înființat în 1990 Centrul de Boli Cardiovasculare al Armatei) şi un om care a reaprins o lumină – Academia Oamenilor de Ştiință (AOŞ), existentă încă din 1936, uşor căzută în desuetudine sub guvernarea comunistă.

Academicianul General mr. (r) dr. Victor Voicu, Secretar General al Academiei Române (2000), este unul dintre templierii medicinii militare române. A condus Direcția Medicală a Armatei şi rămâne pentru noi toți acelaşi spirit viu, deschis la nou, abordabil şi analitic.

Academicianul Prof. dr. Ioanel Sinescu a absolvit Liceul Militar de la Câmpulung Moldovenesc şi a fost şef de promoție (1977) al Facultății de Medicină – secția medicină militară a Institutului de Medicină şi Farmacie Bucureşti (actual UMF „Carol Davila”). Este promotor al transplantului renal în România (peste 1100 de pacienți transplantați până acum), un spirit enciclopedic, Prorector al UMF „Carol Davila” şi un om cu înțelegere vastă asupra fenomenului şi problemelor medicinii militare de la noi.

În ceea ce priveşte „medicina militară română”, evoluția profesională sau „traseele” înaintaşilor noştri fie că s-au numit Carol Davila, C.D. Severeanu, Eugeniu Mareş, Constantin Zamfir, Bogdan Marinescu, Iuliu Şuteu sau cei anterior numiți reprezintă, cu siguranță, trasee fundamentale pentru profesia oricărui om care se identifică cu această „meserie”.

Editorii acestui număr omagial al Revistei de Medicină Militară ce prezintă şi „producțiile ştiințifice” ale medicilor militari de azi probabil că au crezut că le voi oferi un Editorial searbăd şi vetust...

Nu am avut în intenție a scrie un articol panegiric. Am dorit să arăt, în limita spațiului editorial pus la dispoziție, date remarcabile şi împliniri ale unor foşti medici militari (toți suntem la un moment dat nişte foşti!) al căror destin a fost, cândva, tangent cu istoria Spitalului Militar Central.

Mă simt dator, în sinea mea, ca unul dintre „ultimii mohicani” ai acestui Spital să îmi pun şi întrebări filozofice de genul celor puse de Apostolul Petru Mântuitorului :

“ Quo vadis Spitalul Militar Central ? ” Această „tranziţie” care nu se mai termină şi această „reformă” ce continuă la nesfârşit cred că nu au nici o noimă. Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila” este un spital etalon al României, nu numai al Armatei acestei ţări, un spital cu adresabilitate maximă şi, cu siguranţă, una dintre cele mai performante unităţi sanitare de la noi.

Citesc acum un articol al Academicianului Dinu C. Giurescu – apărut în Revista „Istorie şi Civilizaţie” din luna iulie 2011 şi care se intitulează „Industrie bucureşteană: ce a fost, câtă a mai rămas?” Toate „plăsmuirile” la care face referinţă autorul, vechi „*brand - uri*” româneşti au fost sau vor deveni „foste alcătuiri”: Postăvăria Băleanu, moara Assan, Platforma industrială 23 august – fostă Malaxa, Întreprinderea Republica, Întreprinderea de materiale de construcţii Granitul, Întreprinderea Optică Română (IOR), Spitalul Brâncovenesc ş.a...

Nu ştiu !?...

Acest articol a început cu un ***motto*** pe care, la un moment dat, l-am considerat că este inspirat. Poate ar fi fost mai nimerit, tocmai pentru a vă resuscita atenţia, un aforism al marelui om politic Winston Spencer Churchill „Priveşte înapoi, dacă vrei să vezi unde duce drumul! ”

Performanţele notabile ale tututor generaţiilor de medici militari ce au căutat să îmbogăţească realizările înaintaşilor, „***pepiniera***” de personalităţi medicale române care începe cu Generalul Prof. dr. Carol Davila şi continuă să împlinească opera tuturor celor neştiuţi mă fac să cred că cel mai fericit mod de a încheia acest articol este dictonul ce mă conduce zi de zi: „Respice, adspice, prospice”.

General Brigadă (r) Prof. Univ. Dr. Dan MISCHIANU

Inflamația şi restenoza în stentul coronarian

Dr. Blanca CĂLINESCU, Dr. Adela CÎRSTEA, Dr. Ambrose KIBOS SAMWEL, Dr. Daniel NIȚĂ,
Col. dr. Gabriel CRISTIAN, Dr. Florina PINTE, Dr. Silviu DUMITRESCU, Col. dr. Liviu CHIRIAC,
Col. dr. Vasile GREERE, Dr. Lavinia GRAMA, Gl. bg.(r) prof. dr. Ion ȚINTOIU

Centrul Clinic de Urgență de Boli Cardiovasculare al Armatei

Rezumat. **Revascularizația intervențională (PCI) este recunoscută ca tratament util în stenozele coronariene. Studii recente sugerează că răspunsul inflamator are un rol important în restenoză după angioplastia cu balon (PTCA) sau implantarea de stent. Aceste proceduri declanşează şi susțin un răspuns inflamator local şi sistemic[1]. Reacția vasului la leziunea produsă de stent poate fi proinflamatorie şi prin alte mecanisme[2]. Restenoza este consecința unor mecanisme ale inflamației[10] pentru că leziunile aterosclerotice sunt infiltrate cu leucocite înainte de revascularizație.**

Cuvinte cheie: inflamație, restenoză, stent coronarian

Abstract. **Interventional revascularisation (PCI) is well known as a useful treatment in coronary stenosis. Recent studies shows that the inflammatory answer has an important role in restenosis after baloon angioplasty (PTCA) or stent placement. These procedures starts and sustain a local and a systemic inflammatory response. The vessel reaction to the stent lesion can also be a proinflammatory and with other mechanisms. Restenosis is a consequence of inflammation mechanism because atheroscelrotic lesions are infiltrated by leucocytes before revascularisation.**

Introducere

Restenoza este un răspuns de vindecare a leziunii produse de implantarea stentului şi cuprinde patru etape: recoil elastic, formarea şi organizarea trombusului, hiperplazia neointimală şi remodelarea vasului.[3] Fiecare dintre aceste etape este influențată de elemente inflamatorii specializate şi etapizate în cascada vindecării.

Inflamația, fenomen local şi general, trebuie studiată în toate etapele de evoluție a restenozei de la statusul inflamator preexistent până la implicarea directă a mecanismelor inflamatorii în dezvoltarea restenozei.[4]

Dilatația arterei coronare prin PCI produce o creştere a markerilor inflamației, aceştia reprezentând diferența dintre componentele proinflamatorii şi antiinflamatorii pre şi postrevascularizație.

Determinarea markerilor inflamației constituie un element important în anticiparea restenozei şi impune soluții terapeutice noi.[5]

Hiperplazia neointimală are rol decisiv în dezvoltarea restenozei, acest proces începe cu depozitarea la nivelul leziunii stentate de trombocite şi fibrină care determină activarea genelor inflamatorii şi acumularea de leucocite în endoteliul lezat[6]. Studii experimentale au schimbat această concepție sugerând că derivatele de celule circulante progenitoare produse de măduva hematogenă sau alte organe pot pătrunde în vasul lezat, se transferă în SMC, contribuind direct la formarea neointimei.[7]

PATOGENIA RÃSPUNSULUI INFLAMATOR

Importanța leziunii arteriale în dezvoltarea hiperplaziei intimale este stabilită de studii experimentale.[4] Există corelație între răspunsul inflamator şi gradul de injurie arterială, acestea fiind *trigger* pentru cascada trombotică şi de hiperplazie.

Aceste mecanisme sunt inițiate în faza G1 a ciclului celular, controlată de complexul de fosforilare al CDK (*cycline dependent kinase*)[11]. Factorul de creştere celular este triggerul de activare al complexului CDK în proliferarea SMC[11]. Scăderea proliferării SMC de rapamicină se realizează prin inhibarea *mitogen-stimulated protein kinase*, iar tacrolimus acționează prin blocarea activării celulelor inflamatorii[11].

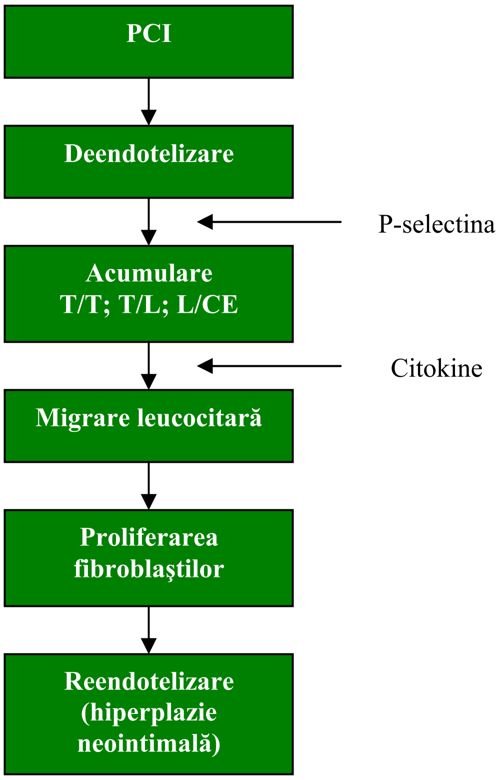

Fig. 1. PCI produce deendotelizarea care generează acumularea în teritoriul lezat al complexelor trombocite-trombocite (T/T); trombocite-leucocite (T/L) leucocite-celule endoteliale (L/CE). Acest proces este urmat de migrarea leucocitară prin stratul de trombocite şi fibrină producând proliferare fibroblastică şi reendotelizare.

Stent vs angioplastie cu balon. Există diferențe în mecanismele fiziopatologice între aceste proceduri intervenționale în ceea ce priveşte hiperplazia neointimală şi răspunsul inflamator, procese care în stentare sunt mai proeminente.[4] Studii experimentale sugerează că impactul acestor proceduri asupra arterei lezionale este diferit, în special prin inițierea inflamației.[4]

În angioplastia cu balon (PTCA) infiltrația cu neutrofile este importantă, pe când în stentare după acumularea de neutrofile urmează cea a macrofagelor care pătrund în neointimă.

P-selectina mediază adeziunea trombocitelor la monocite şi neutrofile, aceste complexe înlocuiesc endoteliul lezat de la nivelul injuriei vasculare, reprezentând cel mai important element fiziopatologic care asociază tromboza şi leziunea vasculară (endotelială) cu inflamația.

Citokinele stimulează migragrea leucocitelor prin stratul constituit de trombocite-fibrină, iar factorii de creştere eliberați de trombocite şi SMC produc proliferarea fibroblaştilor în 3-4 zile după intervenție.[9]

Proteazele produc degradarea proteinelor intracelulare care este reglată de mediatorii proliferării inflamației şi apoptozei[4]. În studii experimentale, blocarea proteazelor duce la reducerea hiperplaziei neointimale, dovedind contribuția importantă a inflamației în acest proces.[10] În stentare, faza inițială este formarea trombusului, urmată de invazia SMC, limfocitelelor T şi macrofagelor, formând neointima ce acoperă suprafața arterială a stentului[4].

Răspunsul perivascular al leziunii tratate intervențional cuprinde inflamația adventicială, prin acumularea de neutrofile la acest nivel în primele 3-4 zile, urmată de macrofage, producând remodelare negativă. Relația dintre inflamația adventicială şi hiperplazia intimală necesită studii viitoare.[12]

Există corelație între structura stentului, densitatea celulelor inflamatorii penetrante, grosimea neointimei şi dezvoltarea restenozei[12]. Stentarea coronariană se însoțeşte de leziuni ale mediei prin penetrarea stentului în stratul lipidic al leziunii, care pot induce creşterea markerilor inflamației, grosimii neointimei şi a leziunilor perivasculare.[13]

În studiile histologice după aterectomie s-a constatat o creştere a numărului de macrofage în leziunea din restenoză, ceea ce explică rolul activării macrofagelor în mecanismele de hiperplazie intimală din toate straturile peretelui coronarian.[14]

STATUS INFLAMATOR PRE PCI

Rolul inflamației din peretele arterial în patogenia aterotrombotică este bine definit şi necontroversat. Traumatismul coronarian realizat de PCI se produce într-un perete vascular ateromatos cu multiple elemente stenozante realizate prin unicitatea sau secvențialitatea plăcilor de aterom.

Considerând ateroscleroza ca o boală inflamatorie indusă de dezechilibrul lipidic, înțelegând placa de aterom ca o leziune endotelială și subendotelială, studiile recente acordă importanță prioritară inflamației în explicarea procesului aterogen şi după stentare[14]. Injuria produsă de PCI se adresează tocmai locului de stenoză coronariană la nivelul plăcii aterosclerotice unde există deja un fenomen inflamator (fig. 2).

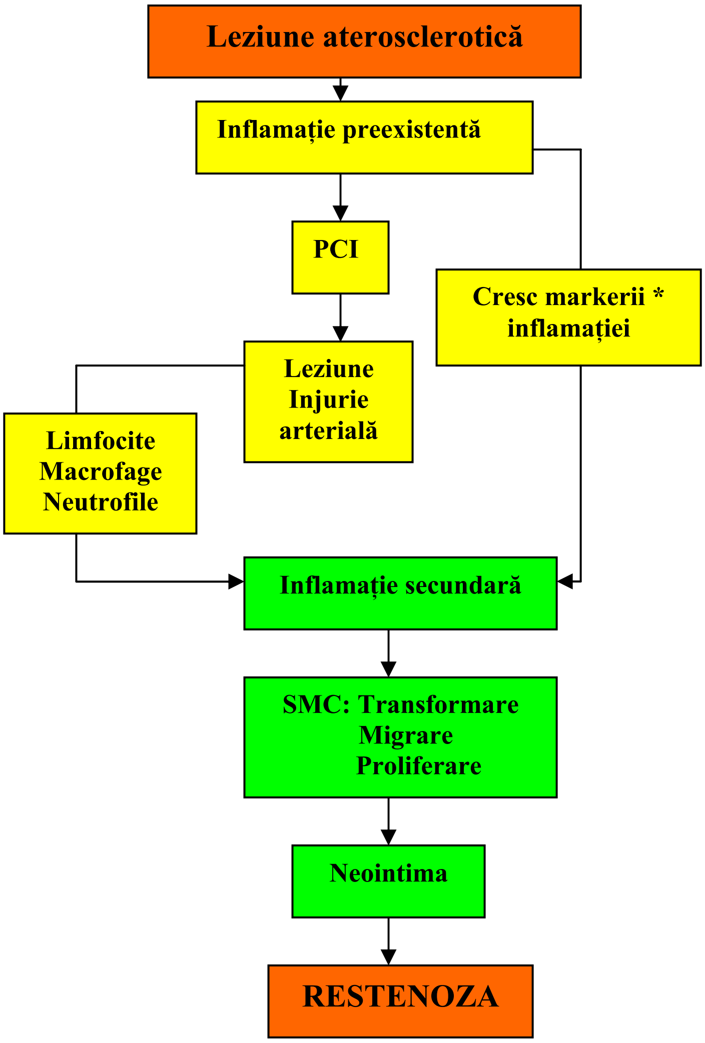
Leziune aterosclerotică
Inflamație preexistentă
PCI
Cresc markerii * inflamației
Leziune Injurie arterială
Limfocite Macrofage Neutrofile
Inflamație secundară
SMC: Transformare Migrare Proliferare
Neointima
RESTENOZA

Fig. 2. Mecanismul de activare inflamatorie pre şi post PCI
* (markeri ai inflamației:PCR, amiloid seric, interleukina-1)

Angina instabilă este mai frecventă la pacienții cu markeri inflamatori crescuți comparativ cu cei asimptomatici, prezența ei postprocedural a fost corelată cu inflamația preexistentă în placa de aterom, aşa cum dovedesc studiile histologice efectuate pe țesutul obținut din aterectomie.[17] Cercetările privind importanța statusului inflamator pre PCI având ca markeri : CRP, IL-1+Lp(a)+fibrinogen, CD66+IL-1, macrofage + limfocite T, IL-1 evaluați la diferite intervale de timp (4-18 luni) au dovedit că inflamația preexistentă este un predictor important în dezvoltarea restenozei (tabel 1).

Folosind markerii diferiți ai inflamației în cercetările prezentate în tabelul 1, se desprinde concluzia importanței selective a acestora în anticiparea restenozei.

Walter şi colab.[18], folosind ca marker PCR, stabileşte că nivelul seric al PCR se asociază cu risc mare de MACE şi dezvoltarea restenozei după stentare.

Patti şi colab.[19] constată că antagoniştii de IL-1 ß (IL-1Ra) la nivelul plasmei sunt predictori ai MACE în perioada de urmărire a apariției restenozei.

Tabel 1. Studii privind rolul inflamației pre PCI

Studiu	Model	Indici	Parametri	Rezultate
Rahel şi colab.[16]	Sânge periferic	CRP IL-6 Fibrinogen	Clinic(8 luni) Pacienți (n=600) MACE(n=54)	Valorile CRP au fost mai crescute la pacienții cu angină recurentă(p=0.0322). Concentrațiile de Lp(a) şi fibrinogen s-au corelat cu MACE (p=0.0337 şi respectiv p=0.0253)
Meuwissen şi colab.[17]	Artere coronare	Macrofage Limfocite T	Clinic(12 luni) Pacienți (n=110) MACE(n=37)	Celulele inflamatorii au fost prezente în număr mai mare la pacienții cu angină instabilă recurentă comparativ cu cei asimptomatici sau cu angina stabil recurentă
Walter şi colab.[18]	Sânge periferic	CRP	Angiografic (6 luni) Pacienți (n=276) MACE(n=112)	Restenoza a fost mai frecventă în cele două treimi cu CRP crescut comparativ cu cei cu CRP scăzut (45.5 vs. 38.3 vs. 18.5%, p=0.002)
Patti şi colab.[19]	Sânge periferic	Nivelul plasmatic de antagonist de receptor al IL-1	Clinic(18 luni) Pacienți (n=73) MACE(n=12)	Valorile de IL-1Ra în quartila superioară au fost singurul predictor independent al MACE (19% vs. 0%; p=0.032)
Pieterasmo şi colab.[20]	Sânge periferic	CRP	Angiografic (6 luni) Pacienți (n=1152) MACE(n=86)	Valorile crescute ale CRP au fost asociate cu o creştere a mortalității sau a infarctului miocardic (OR=1,8) diferența în rata evenimentelor a apărut în primele 30 de zile
Danenberg şi colab.[21]	Sânge periferic	CD66 IL-1ß	Angiografic (6 luni) Pacienți (n=32) MACE(n=6)	Expresia CD66 şi producția de IL-1ß au valoare predictivă de îngustare tardivă după PTCA (p<0.0001)
Stefanidis şi colab.[22]	Artere coronare	Temperatura leziunii implicate	Clinic(18 luni) Pacienți (n=86) MACE(n=21)	Incidența MACE a fost mai mare la pacienții cu temperatura crescută (41% vs. 7%, p<0.001)
Buffon şi colab.[23]	Sânge periferic	CRP	Clinic(12 luni) Pacienți (n=121) MACE(n=51)	Restenoza evaluată clinic a fost prezentă la 63% din pacienții vu valori crescute ale CRP vs. 27% din pacienții cu valori normale (p<0.001)
Chan şi colab.[24]	Sânge periferic	CRP	Clinic(12 luni) Pacienți (n=937) MACE(n=326)	Incidența MACE a fost mai mare la cei cu valori crescute ale CRP vs. cei cu valori scăzute ale CRP (hazard ratio=1.32,p=0.001)

CRP:Proteina C-reactivă; MACE: evenimente cardiace adverse majore; IL: interleukine

Estimarea statusului inflamator preprocedural prin studierea IL-1ß, produsă de monocite, stabileşte că există o corelație pozitivă cu dezvoltarea restenozei şi *late loss.* Expresia genică CD66 din granulocite s-a dovedit a avea acțiune preventivă[20].

Studiul unui lot de pacienți cu angină pectorală stabilă tratați prin stentare coronariană dovedeşte că nivelul crescut al PCR este asociat cu sporirea numărului de decese sau infarct miocardic acut după PCI. Cele mai multe complicații apar în primele 30 de zile, probabil datorită acțiunii protrombotice a PCR.[24] Markerii inflamației determinați pre PCI sunt considerați factori de risc ai restenozei.

Pacienții cu risc crescut de restenoză nu pot fi identificați printr-un singur marker al inflamației. Între nivelul crescut al PCR şi al monocitelor există o corelație de creştere a riscului de restenoză[22]. Alți markeri ai inflamației, cum sunt: SSA, IL-6, IL-1ß (IL-1Ra), Lp(a), fibrinogen pot aduce date suplimentare în anticiparea fenomenului de restenoză[16,19]. Sunt puține informații în privința statusului inflamației de la nivelul plăcii de aterom unde se va implanta stentul. Termografia coronariană este o metodă funcțională rar utilizată, dar studiile evidențiază corelația dintre temperatura locală crescută şi dezvoltarea restenozei.[22] Alte metode de evaluare a inflamației de la nivelul plăcii de aterom (elastografia, tomografia optică) nu au stabilit relații cu restenoza, probabil datorită imperfecțiunii acestora de apreciere a inflamației locale.[22] Folosind pentroxina (derivat sensibil al hsPCR) ca marker al inflamației şi concentrația acesteia în sinusul venos şi la rădăcina aortei, s-au stabilit diferențe între aceste determinări explicate prin agresivitatea fenomenului inflamator la nivelul local, pentru totalitatea leziunilor coronariene aterosclerotice. [24] Sunt necesare studii viitoare privind statusul inflamator de la nivelul plăcii aterosclerotice şi dezvoltarea restenozei.[24]

Răspunsul inflamator post PCI

Impactul injuriei arteriale produse de procedurile de revascularizație intervențională conduce la dezvoltarea şi hiperplazia neointimală la nivelul plăcii de aterom stenozate; acest subiect a fost studiat în multiple cercetări folosind markeri diferiți ai inflamației (tabel 2).

Tabel 2. Studii despre rolul inflamației post PCI

Studiu	Model	Indici	Parametri	Rezultate
Pieterasmo şi colab.[20]	Sânge periferic	IL-1;IL-6, fibrinogen, CRP şi lipoproteina(a)	Angiografic (6 luni) Pacienți (n=32) MACE(n=6)	Expresia CD66 la nivelul granulocitelor şi secreția de IL-1ß de către monocite se asociază cu restenoza (R^2=0,65:p<0.0001)
Stefanidis şi colab.[22]	Specimen de artere coronare	Macrofage Celule musculare netede	Angiografic (4 luni) Pacienți (n=30)	Numărul de macrofage a fost mai mare în leziunile cu restenoză (20.4±2%) vs cele fără restenoză (9,3±0.0007)
Kastrati şi colab.[25]	Sânge periferic	IL-1;IL-6, fibrinogen, CRP şi lipoproteina	Angiografic (6 luni) Pacienți (n=1850) MACE(n=506)	Prezența alelei 2 a IL-IRN se asociază cu un risc mai mic al restenozei (OR=0.78)
Gottsauner Wolf şi colab.[26]	Sânge periferic	CRP	Angiografic (6 luni) Pacienți (n=1850) MACE(n=506)	Valorile de IL-1Ra în quartila superioară au fost singurul predictor independent al MACE (19% vs. 0%; p=0.032)
Hakimoto şi colab.[27]	Sânge periferic	Nivelul plasmatic al antigenului MCP-1	Angiografic (3 luni) Pacienți (n=70) MACE(n=27)	Nivelul antigenului MCP-1 a fost mai crescut în aria cu restenoză (735±35 vs. 571±32 pg/ml,p<0.05)
Gaspardone şi colab.[28]	Sânge periferic	CRP	Clinic(12 luni) Pacienți (n=76) MACE(n=13)	Incidența MACE a fost mai mare la pacienții cu valori crescute ale CRP vs. cei cu valori scăzute ale CRP
Fukada şi colab.[29]	Sânge periferic	Monocite	IVUS (6 luni) Pacienți (n=107)	Volumul neointimei la nivelul restenozei a fost independent corelat cu numărul maxim de monocite (r=0.35, p<0.001)
Schillinger şi colab.[30]	Sânge periferic	CRP, SAA	Ultrasonografie (6 luni) Pacienți (n=108) MACE(n=15)	Valorile CRP la 48 de ore au fost în mod semnificativ asociate cu restenoza la 6 luni
Inoue şi colab.[31]	Sânge de la nivelul sinusului coronar	CDI 1b, 8B2, activarea Mac-1	Angiografic (6 luni) Pacienți (n=62)	Restenoza a fost corelată cu creşterea CDI1b (R=0.42;p<0.01) şi a 8B2 (R=0.55, p<0.001) după PTCA
Hokimoto şi colab.[32]	Specimen de artere coronare	MCP-1 Macrofage	Angiografic (6 luni) Pacienți (n=27)	Imunoreactivitatea MCP-1 a fost prezentă la toți pacienții cu restenoză
Antoniucci şi colab.[33]	Post-mortem	Macrofage Limfocite T Limfocite B	10 luni Specimene(n=116)	Prezența de celule inflamatorii în neointimă a fost de 2.4 ori mai mare în restenoză vs. non-restenoză

CRP : Proteina C-reactivă , MACE : evenimente cardiace adverse majore, IL-1 : interleukina-1 , IL-1RN : gena antagonistului receptorului interleukinei-1 , MCP-1 : proteina-1 chemoatractant a monocitului, SAA :amiloidul A seric.

Există o corelație semnificativă între răspunsul inflamator şi gradul injuriei arteriale, triggerii reacției inflamatorii fiind activați de cascada coagulării.[31,32,33,34] Dilatația prin PCI a leziunii aterosclerotice provoacă o deendotelizare care este acoperită de straturi trombocitare şi fibrină. Pe aceste depozite se creează complexe mixte, mediate de molecule de adeziune, formate din trombocite/trombocite, trombocite/leucocite, leucocite/celule endoteliale.[8,35] După formarea acestor complexe, urmează adeziunea trombocitelor activate la monocite şi neutrofile, fenomen activat de P-selectină şi E-selectină (fig. 3).

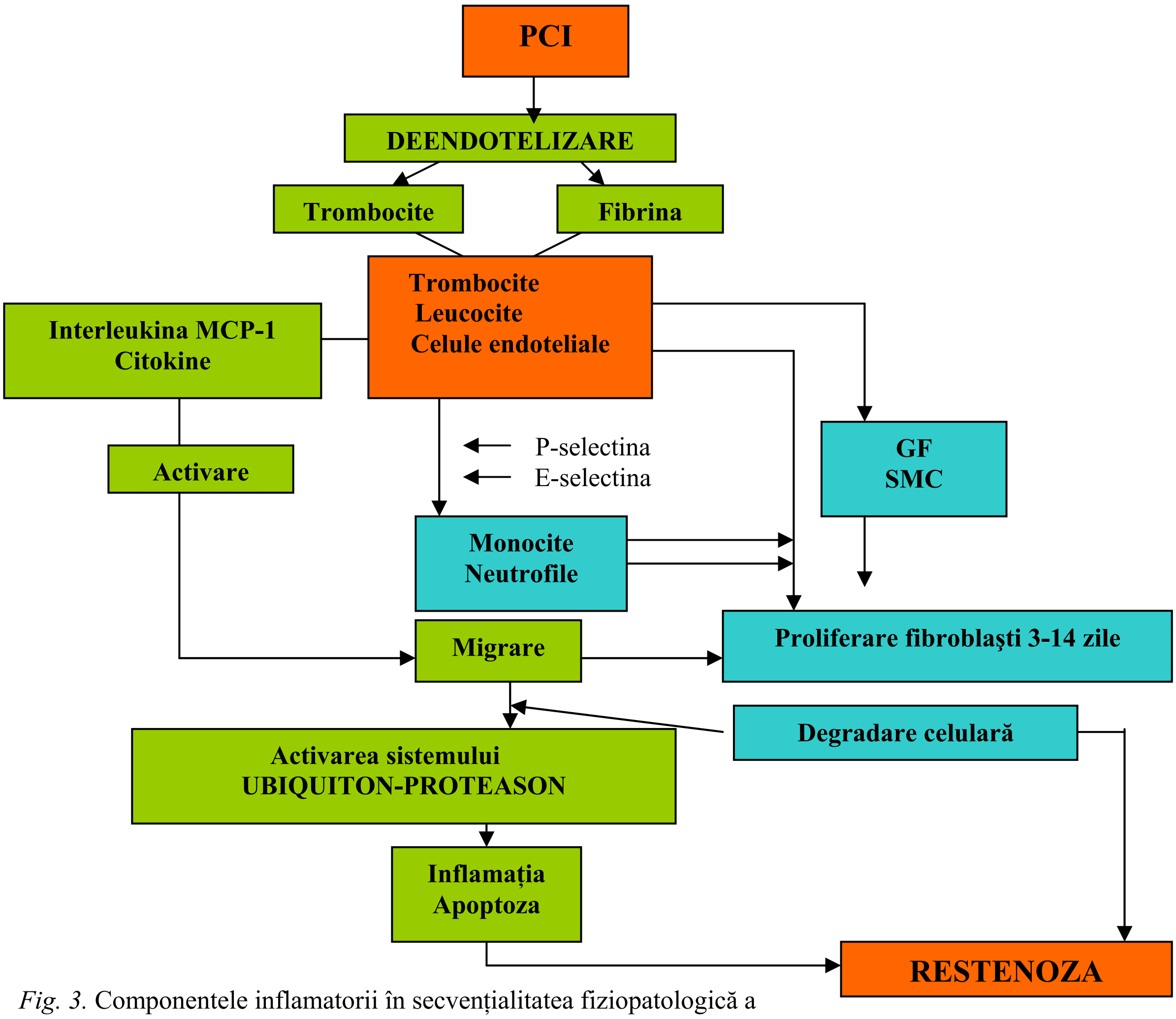
PCI
DEENDOTELIZARE
Trombocite
Fibrina
Trombocite
Leucocite
Celule endoteliale
Interleukina MCP-1
Citokine
Activare
P-selectina
E-selectina
GF
SMC
Monocite
Neutrofile
Migrare
Proliferare fibroblaşti 3-14 zile
Degradare celulară
Activarea sistemului
UBIQUITON-PROTEASON
Inflamația
Apoptoza
RESTENOZA

Fig. 3. Componentele inflamatorii în secvențialitatea fiziopatologică a restenozei post PCI (GF-growth factor, SMC-smooth muscle cells), MCP-monocyte

Growth factor (eliberat din trombocite), leucocitele şi SMC produc proliferarea fibroblaştilor şi transformarea lor în miofibroblaşti în 3-14 zile după intervenție.[9] Un rol important în restenoză îl are degradarea proteinelor intracelulare şi activarea sistemului *ubiquiton-proteasom* care prin mediatori specifici controlează proliferarea, inflamația şi apoptoza[10].

Proteasonul este produs prin activarea NF_kB_1 rezultat din degradarea proteinei IkB şi este controlat de genele inflamației.[10] Studiile experimentale au dovedit că blocarea sistemului proteonilor reduce hiperplazia intimală. [10]

Calea comună a acestor mecanisme este inițierea fazei G1 a ciclului celular controlată de fosforilarea cyclinei dependentă de complexul kinazelor (CDK).

Agenții utilizați în structura DES acționează diferit în etapele ciclului celular, având proprietăți antiinflamatorii (paclitaxel) şi citotoxice prin oprirea ciclului celular (sirolimus) (fig. 4).

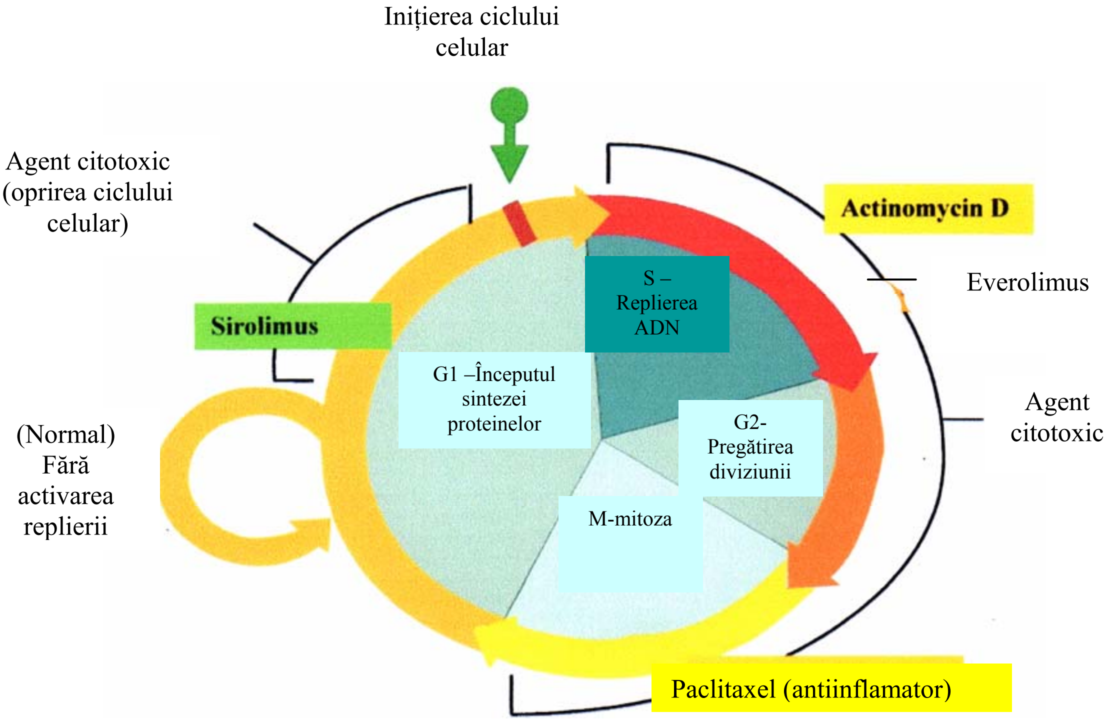
Inițierea ciclului celular
Agent citotoxic (oprirea ciclului celular)
Actinomycin D
Everolimus
Sirolimus
S – Replierea ADN
G1 –Începutul sintezei proteinelor
Agent citotoxic
(Normal) Fără activarea replierii
G2- Pregătirea diviziunii
M-mitoza
Paclitaxel (antiinflamator)

Fig. 4. Ciclul celular şi locul de acțiune al agenților farmacologici din structura diferitelor tipuri de stent (Actinomicina D, everolimus şi paclitaxel blochează ciclul celular în fazele S şi G_2 , iar sirolimus în etapa finală G_1).

Studii recente au demonstrat că inhibarea proliferării SMC prin rapamicină blochează stimularea proteonkinazelor responsabile de geneza restenozei,[11] iar tacrolimus acționează prin inhibarea activării celulelor endoteliale.[34]

Procesul inflamator peristent este inițiat de traumatismul vasului produs de PCI. În imediata vecinătate a stentului, deendotelizarea este redusă şi poate avea grade diferite de infiltrație inflamatorie.[34]

Intensitatea procesului inflamator în spațiul peristent constituie trigger pentru inițierea hiperplaziei neointimale sau pentru tromboza intrastent, mai ales pentru DES (*drug eluting stent*).[34]

Stent vs PTCA

Mecanismele patogenice ale PTCA şi stentării sunt asemănătoare, dar diferă prin intensitatea hiperplaziei intimale, răspunsului inflamator şi remodelarea negativă.[35]

Imediat după PTCA are loc procesul de infiltrare cu neutrofile, după stentare este mai importantă acumularea de macrofage în neointimă.[12] Aceste date sugerează că tipul şi gradul de leziune arterială sunt diferite şi au impact specific asupra vasului lezat în activarea inflamaţiei locale.

Analiza coronarografică din două trialuri importante STRESS (*Stent Restenosis Study*) şi BENESTENT (*Belgian Netherlunds Stent Study*) relevă prin analiza calitativă şi cantitativă diferenţele dintre PTCA şi implantarea de stent.[35]

Arterele stentate au lumen coronarian mai mare, datorită structurii stentului care previne remodelarea negativă acută. Această ipoteză este confirmată prin IVUS.[36]

Markeri predictivi

Determinarea exactă a markerilor inflamaţiei la nivelul stenozei coronariene dilatate este greu de realizat, dar sunt deducţii privind componenta inflamatorie a diferenţei dintre PTCA şi stent în revascularizaţia intervenţională.

Folosindu-se determinarea expresiei CD11b prin metoda *flow cytometry,* s-a observat că infiltraţia cu neutrofile la bolnavii cu implantare de stent este mult mai mare comparativ cu PTCA, ceea ce poate explica hiperplazia intimală mai importantă la bolnavii cu arterele stentate.[36]

Într-un studiu anatomo-patologic pe un lot de 87 pacienţi cu 116 stenturi implantate în urmă cu mai mult de 90 zile, Farb şi colab.[37] găsesc o corelaţie între leziunea din medie, inflamaţie şi restenoză.

Infiltraţia leucocitară, marker al inflamaţiei pentru restenoză, a fost studiată de Boisvert şi colab.[38] Folosind materialul obţinut prin cateterul de aterectomie direcţională, autorii au constatat că există o corelaţie între numărul de macrofage prezente în leziune şi elemente histologice predictive pentru restenoză. Identificând markerii inflamaţiei (L-selectina, CD-1b) în probele de sânge recoltate proximal şi distal de stent, Neuman şi colab.[39] constată o creştere a acestora după angioplastie.

La pacienţii care au dezvoltat restenoză, există o corelaţie între concentraţia de IL-1 şi activarea monocitelor, considerându-se aceşti markeri ca predictori ai restenozei.[28]

Activarea granulocitelor evaluată prin nivelul CDLL a fost invers corelată cu *late loss*[40].

În studiul privind importanţa CD40 în anticiparea restenozei (prin evaluare pre şi post PCI), Cipollone F.C. şi colab.[41] au constatat o corelaţie între acest marker şi restenoză ($p<0.01$).

CD40 sub formă activată (CD402), cunoscut ca CD154, este o proteină transmembranară cu originea în celulele T, întâlnită în trombocitele activate.[8] Atât forma legată de membrană, cât şi cea solubilă (sCD40) pot interacţiona cu CD40.[8]

Legarea CD40 de celula endotelială (EC_s) inhibă migrarea celulară după injuria coronariană, accentuează efectul MCP-1 (*monocyte chemoattractant protein*), E-selectinei, VCAM (*vascular cell adhesion molecule*) şi ICAM (*intercelular adhesion molecule)* în cascada vindecării.

Pe un lot de 70 pacienţi trataţi prin PTCA, la care s-a efectuat controlul angiografic la 1,5,15 şi 180 zile postprocedural, s-au analizat markerii inflamatori (SCD40L, MCP-1, VCAM, ICAM, E-selectina). Se concluzionează că aceştia reprezintă predictori ai restenozei.[10]

Există corelaţie între CRP (marker nespecific al inflamaţiei) evaluată post revascularizaţie intervenţională şi perspectiva restenozei.[42,43]

Bazat pe biologia vasculară, Forester şi colab.[44] propun patru etape în secvenţialitatea fenomenului de restenoză: faza inflamatorie, granularea, proliferarea celulară şi remodelarea. Din această secvenţialitate se desprinde rolul iniţial al inflamaţiei şi participarea ei în etapele următoare.

Elementele principale în restenoză sunt factorul de creştere (*growth factor*) şi citokinele. Ele pot fi localizate în trombocite, celule endoteliale, SMC, fibroblaşti, macrofage etc., putând avea acţiuni diferite asupra inflamaţiei. [35]

Consecinţe terapeutice

Din datele prezentate anterior rezultă că terapia antiinflamatorie are un rol important şi efecte benefice asupra proliferării neointimale.

Sirolimus, cu efecte remarcabile antirestenotice, este folosit în stenturile acoperite (DES), fiind cunoscut efectul inhibitor în reglarea ciclului celular şi cu proprietăţi antinflamatorii. Efectul antiinflamator al rapamicinei se realizează prin reducerea citokinelor MCP-1 şi IL-6, aşa cum dovedesc studiile experimentale.[45]

Paclitaxel este agent cu proprietăţi antiinflamatorii şi antiproliferative, utilizarea lui în stenturile acoperite (DES) a dovedit proprietăţile antirestenotice în multiple trialuri.[45]

Everolimus reduce răspunsul hiperplazic după stentare.[45]

Inflamaţia este parte importantă în geneza restenozei şi după brahiterapie. Studiile experimentale au dovedit că densitatea macrofagelor este crescută în peretele arterial şi după brahiterapie.[46]

Concluzii

Din datele experimentale şi clinice privind restenoza, rezultă importanța inflamației, existând rezerve de interpretare datorită diferitelor metode de evaluare.

Relația dintre fenomenul local şi general privind inflamația pare a acorda mai multă importanță celui local, dar şi elementelor inflamatorii preprocedurale.

Inflamația preexistentă procedurii intervenționale este element important în geneza restenozei şi poate fi accentuată de această procedură.

Markerii predictivi ai inflamației sunt multipli, au metode diferite de cuantificare şi nu sunt utilizați în practica clinică. Cel mai folosit pare a fi determinarea plasmatică a CPR.

Secvențialitatea genezei restenozei cuprinde etape multiple şi poate oferi soluții terapeutice variabile, dar structura stenturilor şi medicația preventivă sunt prioritare.

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Dicționar de psihiatrie

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Anhedonia

Termenul îşi are originea în limba greacă, *αν* însemnând “fără”, iar *ηδονή* “plăcere”, şi a fost utilizat pentru prima dată în literatura de specialitate de Th.Ribot [1], cel care a descris la unii pacienți lipsa de interes şi retragerea din activitățile plăcute pe care aceştia le desfăşurau anterior. Simptomul se defineşte prin incapacitatea persoanei de a simți emoțiile agreabile şi prin pierderea plăcerii de a trăi, manifestate în plan comportamental prin reducerea sferei intereselor proprii şi neimplicarea în activități care altădată îi făceau plăcere persoanei. Sfera pulsională este afectată şi ea, prin scăderea dorinței sexuale sau reducerea frecvenței şi inițiativei în raporturile sexuale. Pacientul se simte incapabil să mai aibă sentimente față de partener, copii, părinți, persoane pe care altădată le iubea.

Anhedonia este asociată cu diferite afecțiuni psihice, cum ar fi tulburările depresive, tulburarea schizoafectivă, schizofrenia, tulburarea delirantă, tulburări de personalitate etc. Astfel, la pacientul depresiv, cognițiile automate negative induc anhedonia, iar aceasta, la rândul ei, scade inițiativa subiectului. Subiectul anhedonic nu mai poate formula un plan de acțiune, deoarece toate variantele pe care le anticipează au acelaşi deznodământ, respectiv lipsa oricărei bucurii sau satisfacții. În depresiile severe, pacienții se plâng de detaşare emoțională, incapacitate de a se relaționa cu ceilalți, putând aparea depersonalizarea şi derealizarea, fenomene care cresc senzația de stranietate şi desprindere de mediul înconjurător. Depresivul suferă intens din cauza pierderii capacității de trăire a emoțiilor şi acest simptom trebuie evaluat ca un indicator al riscului suicidar.

Anhedonia poate fi observată şi la pacienții cu schizofrenie, acolo unde este inclusă printre simptomele negative, alături de alogie, aplatizare afectivă, apatie etc. Din perspectivă istorică, atât Bleuler, cât şi Kraepelin au descris anhedonia ca pe un simptom tipic schizofreniei [2,3]. Alți autori, cum ar fi Meehl, au considerat anhedonia ca fiind o trăsătură, un simptom fundamental, care precedă sau chiar determină schizofrenia [4]. Unele studii arată că anhedonia nu are o specificitate prea mare pentru sindromul de deficit din schizofrenie, dar în schimb covariază cu alte simptome depresive la pacienții cu schizofrenie (inhibiție, distorsiuni cognitive) [5].

Chapman consideră că există două tipuri de anhedonie - fizică şi socială, prima referindu-se la o reducere a abilității de a simți plăcerea fizică (alimentație, sex), în timp ce a doua este un deficit al capacității de a resimți plăcere din comunicarea cu alte persoane [6].

Din punct de vedere neurobiologic, anhedonia pare a fi asociată cu un deficit al activității din aria tegmentală ventrală (inclusiv nucleul accumbens) şi un exces al activității din regiunea ventrală a cortexului prefrontal (cortexul prefrontal ventromedial şi orbitofrontal), un rol important avându-l dopamina [7].

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Aspecte ale managementului pneumotoraxului spontan

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Rezumat. Afecțiune respiratorie cu potențial letal, pneumotoraxul se tratează, de obicei, în clinicile şi secțiile de chirurgie toracică. Aspectele managementului pneumotoraxului spontan depind de starea clinică a bolnavului, existența chirurgului toracic, asocierea cu alte afecțiuni, posibilitățile unității sanitare. Sunt descrise principalele metode de tratament aplicate la noi în țară în pneumotoraxul spontan.

Cuvinte cheie : pneumotorax, pleurotomie, toracotomie, chirurgia toracică videoasistată

Abstract. Respiratory disease with lethal potential, the pneumothorax is usualy treated in the clinics or departments of thoracic surgery.The aspects of the management of the spontaneous pneumothorax depend on the clinical status of the patient, the existence of a thoracic surgeon, the association with other disease and the equipment of the hospital. They describe the main methods of treatment of the spontaneous pneumothorax applied in our country.

Pneumotoraxul spontan reprezintă o problemă semnificativă de sănătate pentru pneumolog, chirurgul toracic şi specialistul în urgențe medico-chirurgicale. Deşi simptomele pot fi alarmante, pneumotoraxul spontan poate amenința rareori viața în cazul abordării unui algoritm terapeutic corespunzător. Tratamentul variază considerabil de la un centru medical la altul, în funcție de disponibilitatea locală a tehnicilor chirurgicale şi chiar de existența chirurgiei video-toracoscopice.

În cele mai multe cazuri, colaborarea multidisciplinară permite alegerea celor mai bune metode de tratament în această afecțiune. *Obiectivul principal al tratamentului în pneumotorax este eliminarea aerului din spațiul pleural şi diminuarea la maximum a riscului de recidive.*

Nu există un consens în ceea ce priveşte gestionarea pneumotoraxului inițial sau a celui recidivant. Decizia de a observa sau de a interveni chirurgical imediat depinde de aprecierea riscului la prezentarea pacientului şi posibilitatea de vindecare şi de apariție ale recidivei.

British Thoracic Society pledează pentru aspirație simplă şi pentru amânarea spitalizării în pneumotoraxul spontan primar.

În Statele Unite ale Americii chirurgii toracici recomandă plasarea de tub toracic.

În România, majoritatea chirurgilor toracici opiniază pentru efectuarea pleurotomiei minime.

Tratamentul pneumotoraxului spontan are drept obiective :

- combaterea simptomelor acute (dispnee, durere) prin decomprimarea plămânului şi prin medicație simptomatică;
- restabilirea condițiilor anatomice normale prin suprimarea colecției aeriene şi reexpansionarea plămânului;
- prevenirea recidivelor şi a cronicizării prin simfiza pleurală;
- eliminarea cauzei primare a accidentului pleural prin tratament medical şi/sau chirurgical al bolii de fond.

Alegerea metodei de tratament este influențată de o serie de parametri:

- ***mărimea pneumotoraxului.*** Măsurarea exactă a volumului pneumotoraxului este dificilă. Radiografia clasică nu este un mijloc bun de a calcula mărimea exactă a pneumotoraxului în comparație cu tomografia computerizată. Deşi clasificarea pneumotoraxului în „mic'' şi „mare'' nu este agreată, ea este practică. „Mic'' defineşte pneumotoraxul parţial, deseori apical, iar „mare'' semnifică colapsul complet;

- ***severitatea simptomelor.*** Pentru decizia terapeutică este importantă nu numai mărimea pneumotoraxului, ci şi simptomatologia pacientului ;
- ***momentul primului episod de PNO sau de recidivă***;
- ***preferinţa pacientului;***
- ***problema medico-legală.***

Variantele practice curente în managementul pneumotoraxului de care dispune un specialist competent în domeniu sunt:

- simpla observaţie;
- aspiraţia simplă;
- drenajul pleural;
- drenajul pleural cu pleurodeză;
- drenajul pleural şi toracoscopia;
- drenajul pleural şi toracoscopia videoasistată.

La prezentarea la camera de gardă pacientul poate fi :

- *asimptomatic:* descoperire întâmplătoare, iar metodele de tratament se stabilesc în funcţie de riscul pe termen lung al reapariţiei pneumotoraxului;
- *simptomatic* - dar stabil clinic. Tratamentul va fi ghidat în funcţie de posibilitatea unităţii sanitare la care se prezintă bolnavul, respectiv aspiraţie sau tub de dren intrapleural;
- *instabil clinic* : pneumotoraxul provoacă instabilitate hemodinamică, ceea ce pune în pericol viaţa bolnavului. Necesită intervenţie chirurgicală de urgenţă.

Monitorizarea în timp a dinamicii pneumotoraxului este foarte importantă:

- *intervalul 0-6 ore*: se poate realiza observarea timp de 6 ore. Într-un serviciu de urgenţă, se repetă radiografia pulmonară şi dacă nu se constată extinderea pneumotoraxului, pacientul poate merge la domiciliu;
- *intervalul 24-48 ore* : se urmăreşte bolnavul. Se repetă radiografia pulmonară. Se poate efectua CT, pentru a face distincţie între un pneumotorax spontan primar şi unul secundar;
- *la 1 lună* - recidiva pneumotoraxului ; poate apărea la 3 săptămâni după evenimentul iniţial.

Opţiuni pentru decomprimarea plămânului

Simpla observaţie, fără administrare de oxigen, este adecvată pentru pacienţii asimptomatici cu un pneumotorax minim (<15-20%). Aerul se poate resorbi spontan.

Aspiraţia simplă ; oferă o durată mai mică de spitalizare faţă de inserarea de tub pleural. Procedura aspiraţiei este următoarea:

- dezinfecţia pielii cu soluţie Betadine; acoperirea cu câmp steril;
- reperarea spaţiului intercostal 2-3 pe linia medioclaviculară sau spaţiul intercostal 4-5 pe linia axilară medie;
- anestezie locală cu xilină 1%, 10 ml;
- introducerea acului cu trocar în spaţiul pleural;
- ataşarea robinetului cu 3 căi şi apoi a unei seringi mari cu care se aspiră aerul;
- se scoate acul, se pansează steril;
- se efectuează radiografie toracică.

Pleurotomia (drenajul pleural). Reprezintă inserarea unui tub de dren la nivelul cavităţii pleurale prin care se evacuează aer sau lichide.

Indicaţiile drenajului pleural în pneumotoraxul spontan:

- pneumotoraxul spontan, primar sau secundar;
- pneumotoraxul iatrogen;
- hipoxemia moderată sau severă.

În cele mai multe cazuri, pentru identificarea pneumotoraxului este necesară radiografia toracică. De asemenea, pneumotoraxul poate fi localizat rapid prin ultrasonografie. Există şi situaţii în care starea bolnavului impune plasarea unui tub în cavitatea pleurală, fără a mai aştepta aportul imagisticii. Este cazul bolnavilor cu pneumotorax bilateral, a celor cu hipoxemie severă.

Tehnica drenajului pleural. Chiar dacă se efectuează în condiţii de urgenţă, se recomandă atenţie mare pentru evitarea complicaţiilor.Tubul de dren se plasează pe linia axilară medie, în spaţiul 5 intercostal. Pleurotomia este o tehnică dureroasă, mai ales pentru persoanele musculoase. Combinarea analgeziei intravenoase cu anestezia locală oferă confortul operator necesar.

Pentru anestezia locală se folosesc 10-20 ml anestezic. Acesta este infiltrat sub piele, de-a lungul liniei de incizie. Apoi acul este îndreptat perpendicular pe piele, infiltrând peretele toracic strat cu strat până se ajunge la spaţiul intercostal respectiv (fig. 4).

Incizia se face de-a lungul marginii superioare a coastei inferioare a spaţiului intercostal (fig. 5). Se introduce cateterul în cavitatea pleurală (fig. 6). Tubul de dren se fixează cu fire de aţă la tegument şi apoi se conectează la un sistem de drenaj toracic. O radiografie pulmonară poate confirma ulterior poziţia tubului de dren.Tubul de dren fiind prevăzut cu orificii, este optim ca ultima gaură a tubului să se afle în interiorul cavităţii pleurale. Dacă tubul de dren este introdus prea adânc, poate provoca dureri severe.

Drenurile sunt confecţionate din silicon, au diametrul variabil, unele fiind prevăzute cu mandren. Ele favorizează crearea unei simfize pleurale între foiţele parietale şi viscerale ale pleurei.

Sistemul de drenaj subacvatic permite ieşirea aerului, dar nu şi reintrarea în cavitatea pleurală (fig.1,7). Recipientul de colectare trebuie să fie întotdeauna menţinut sub nivelul pacientului, altfel conţinutul său se va înapoia în cavitatea pleurală.Tubul de dren nu trebuie niciodată să fie comprimat deoarece se va dezvolta un pneumotorax sub presiune.

Tubul de dren poate fi conectat la valva Heimlich, pacientul putând fi transportat între unităţile spitaliceşti sau se poate întoarce la domiciliu, reducându-se cheltuielile de spitalizare (fig. 2,3).

La sistemul de drenaj subacvatic se poate conecta o aspiraţie moderată (-20 cm de apă) pentru a ajuta reexpansiunea pulmonară.

Pierderile de aer pe tubul de dren se observă aproximativ 7 zile la 80% dintre pacienţi, iar media spitalizării este de 5 zile. Tubul de dren se va menţine încă 24 de ore de la încetarea eliminărilor de aer. Dacă pierderea de aer persistă mai mult de 4 zile, se va interveni chirurgical, prin toracotomie.

Tubul de dren toracic se poate elimina atunci când nu mai sunt drenate lichide sau aer. Înainte de suprimarea drenajului se clampează tubul de dren pentru 24 de ore, iar după repetarea radiografiei pulmonare se decide extragerea sau menţinerea drenului. Îndepărtarea tubului de dren se efectuează de către doi oameni, unul elimină tubul şi celălalt blochează locul tubului de dren.Tubul trebuie eliminat fie la sfârşitul expirului, fie la inspirul maxim, pentru a evita intrarea aerului în cavitatea pleurală. Zona respectivă se dezinfectează şi apoi se foloseşte un pansament ocluziv. Nu este obligatorie instituirea antibioterapiei sistemice la pacienţii cu drenaj pleural.

Incidente şi accidente posibile ale drenajului pleural:

- lezarea vaselor intercostale;
- lezarea parenchimului pulmonar;
- lezarea diafragmului;
- lezarea organelor mediastinale;
- introducerea tubului de dren subcutan sau submuscular;
- introducerea tubului de dren insuficient, cu apariţia emfizemului subcutanat.

Şocul anafilactic în alergia la anestezic

Şocul pleural

Complicaţiile drenajului pleural:

- hemotoraxul, poate apărea atunci când sunt lezate vase intercostale sau plămânul ;
- durerea toracică, atunci când tubul e plasat prea adânc;
- alunecarea tubului în afara cavităţii pleurale;
- blocarea tubului cu cheag;
- reapariţia pneumotoraxului;

- edemul pulmonar de reexpansiune;
- emfizem subcutanat.

În cazul eşuării metodelor de tratament anterior prezentate, se recurge la tratamentul chirurgical.

Indicațiile tratamentului chirurgical în pneumotoraxul spontan:

- pierderi de aer peste 4-5 zile;
- pneumotorax recidivat ipsilateral;
- pneumotorax controlateral;
- pneumotorax bilateral;
- pacienți cu ocupații cu risc crescut de pneumotorax (scafandri, piloți);
- pacienți cu SIDA;
- limfangiomiomatoza.

Metodele de tratament ale pneumotoraxului spontan se adresează atât pleurei, cât şi parenchimului. Există două obiective în gestionarea chirurgicală a unui pneumotorax spontan:

- rezecția blebsurilor ;
- crearea unei simfize pleurale pentru prevenirea recurențelor.

În decursul anilor, tehnicile chirurgicale s-au modificat, astfel încât la începutul anilor 1990 toracoscopia câstigă teren în fața toracotomiei clasice.

Toracoscopia reprezintă examinarea cavității toracice prin introducerea unui endoscop în spațiul pleural. Toracoscopia poate fi folosită pentru vizualizare sau pentru efectuarea unor proceduri chirurgicale. Pleuroscopia se poate efectua cu pacientul sedat, în timp ce chirurgia video asistată presupune anestezie generală.

În pneumotoraxul spontan, toracoscopic se poate realiza pleurodeza chimică, pleurectomia, decorticarea, evacuarea colecțiilor lichidiene, rezecția blebsurilor.

Chirurgia toracică video asistată (CTVA) necesită minitoracotomie de acces.

Rezecția bulelor însoțită de pleurectomie apicală este metoda optimă pentru a obține cele mai bune rezultate pe termen lung.

Avantajele videotoracoscopiei :

- durata mică a intervenției;
- durere postoperatorie diminuată;
- spitalizare redusă;
- costuri diminuate;
- permite conversia la toracotomia clasică;
- numărul pacienților care beneficiază de intervenție chirurgicală la primul episod a crescut (piloții de avion, scafandri).

Toracotomia clasică permite un acces larg asupra întregii cavități toracice. Rămâne procedura cu cea mai scăzută rată a recurenței.

Momentul intervenției chirurgicale este încă controversat. Prelungirea unui drenaj pleural nu este în beneficiul bolnavului şi de aceea se recomandă asocierea unei alte intervenții chirurgicale.

În anul 1970 Becker şi Munro folosesc pentru prima dată minitoracotomia transaxilară, procedură minim invazivă, incizia fiind de 5-6 cm. Astfel, se putea efectua pleurectomia apicală şi ligatura belbsurilor. Durata de spitalizare este scăzută, de aproximativ 6 zile, iar complicațiile sunt minore.

Toracotomia axilară cu o incizie mai mare de 5-6 cm se practică astăzi în mod frecvent în serviciile de chirurgie toracică în pneumotoraxul spontan.

Concluzii

1. Pneumotoraxul spontan necesită tratament medical şi/sau chirurgical
2. Drenajul pleural este metoda de elecție pentru tratamentul chirurgical
3. Tratamentul chirurgical al pneumotoraxului este efectuat de către chirurgul toracic, care alege procedurile chirurgicale optime fiecărui bolnav.

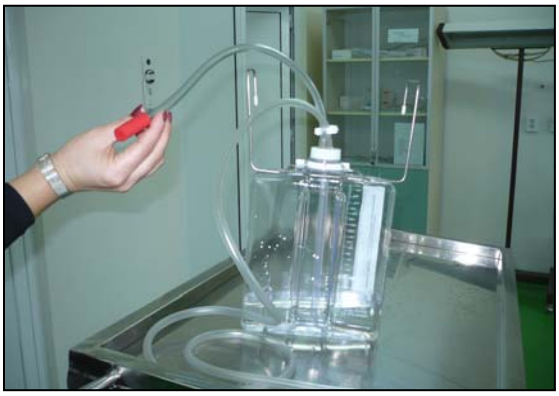

Fig. 1. Sistem de drenaj toracic.

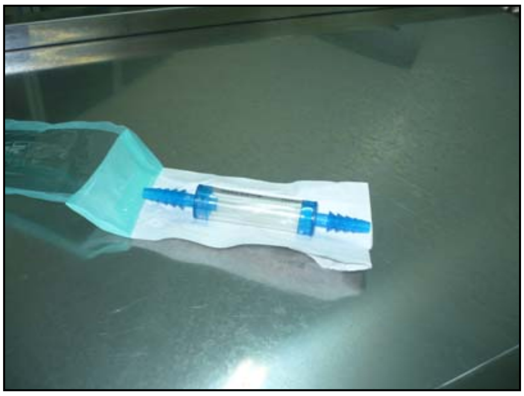

Fig. 2. Valva Heimlich

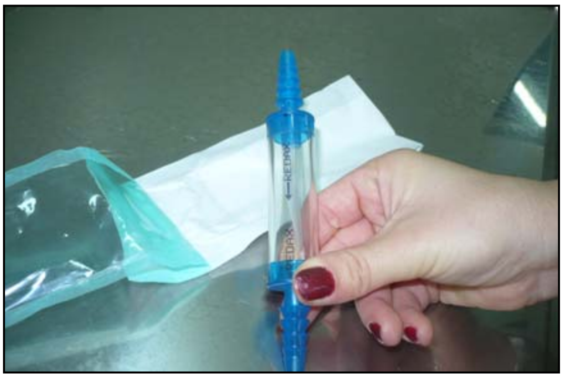

Fig. 3. Valva Heimlich (sensul de ataşare la tubul de dren toracic)

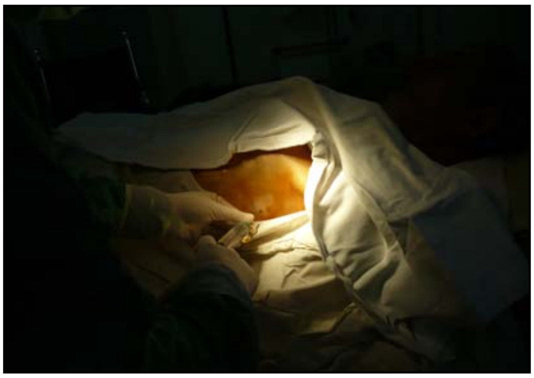

Fig. 4. Anestezia locală.

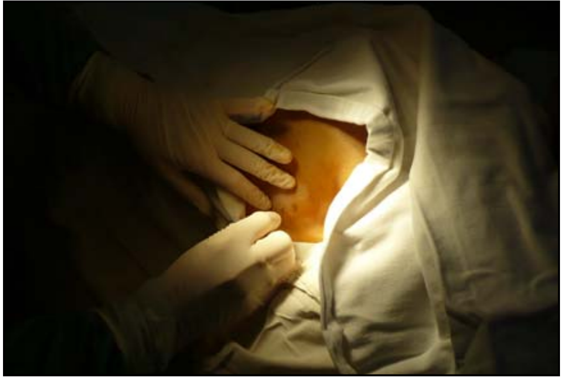

Fig. 5. Efectuarea inciziei

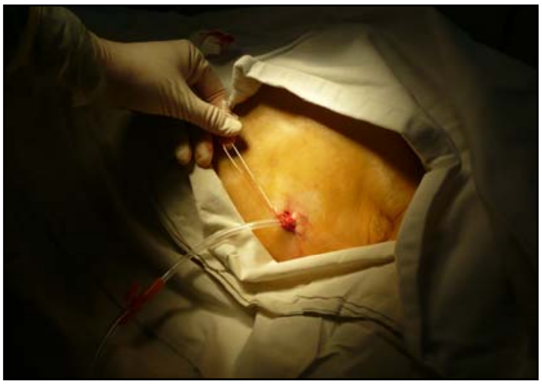

Fig.6. Plasarea şi fixarea tubului de dren toracic

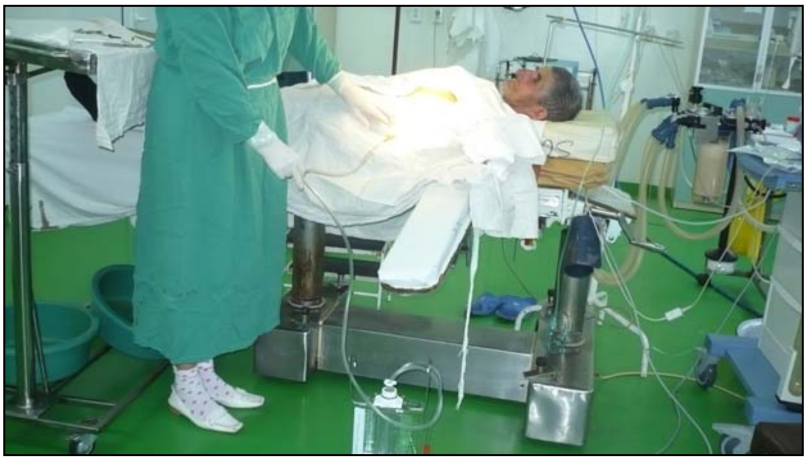

Fig. 7. Conectarea tubului de dren toracic la sistemul de drenaj subacvatic.

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Analiza eficacității medicației psihotrope în coşmarurile asociate tulburării de stres posttraumatic

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Rezumat. Această analiză sistematică a literaturii a inclus studii publicate până în iunie 2011, identificate în baza de date PubMed, utilizând următoarele cuvinte cheie : « tulburare de stres posttraumatic (PTSD) », « coşmaruri », « tratament farmacologic » şi denumirile internaționale ale substanțelor psihotrope utilizate în tratamentul stresului posttraumatic. Căutarea inițială a detectat un număr de 59 de studii. Au fost selectate, conform criteriilor de includere şi excludere, doar 16 de articole, care au rămas în analiză. Aproximativ 1 480 de pacienți au fost tratați cu diferite tipuri de agenți psihotropi. Cele mai multe date au provenit din studii nerandomizate, analize retrospective ale foilor de observație şi studii deschise. Venlafaxina a fost evaluată în două studii randomizate, controlate placebo şi nu a fost asociată cu rezultate superioare placebo. Alte antidepresive - duloxetina, mirtazapina, trazodona, nefazodona, fluvoxamina, triciclicele - au fost asociate cu date din studii de caz, analize retrospective şi studii prospective deschise, care au fost favorabile (scăderea frecvenței coşmarurilor şi a altor tulburări hipnice). Un studiu deschis, pe un lot redus de subiecți şi două serii de cazuri au asociat antipsihoticele cu un răspuns pozitiv, ceea ce permite încadrarea acestor substanțe în clasa C de recomandări terapeutice. Date pozitive au fost obținute în ceea ce priveşte rezultatele topiramatului. Referitor la buspironă, fenelzină şi clonazepam, rezultatele nu sunt satisfăcătoare. Concluzii: Deoarece recomandări de nivel A şi B nu pot fi susținute în prezent pentru medicația psihotropă, iar recomandările de nivel C pot fi formulate doar pentru unii agenți farmacologici, nevoia de studii clinice care să evalueze noi terapii în coşmarurile asociate stresului posttraumatic este semnificativă.

Cuvinte cheie : tulburarea de stres posttraumatic, coşmaruri, antidepresive, antipsihotice, anxiolitice

Abstract. We conducted a systematic literature review of published studies identified in PubMed database, through february 2011, using several keywords - "posttraumatic stress disorder (PTSD)", "nightmare", "drug treatment", and individual drug names used for PTSD treatment. The initial search returned 59 results and, after the application of inclusion and exclusion criteria, only 16 articles remained to be analyzed. About 1480 patients have been treated with different types of drugs. Most of the data retrieved consisted in low quality case reports, chart reviews, retrospective and open label trials. Venlafaxine has been evaluated in 2 randomized, placebo controlled clinical trials and was found to be no better than placebo. Other antidepressants, like duloxetine, mirtazapine, trazodone, nefazodone, fluvoxamine, tricyclics were associated with data from case reports, chart reviews and open label trials that usually found a positive response (decreased nightmare frequency and other sleep disorders). A small open label trial and 2 case report series associated antipsychotics with positive response, therefore the grade of evidence is considered to be C in this case. Positive data was obtained for topiramate. Clonazepam, phenelzine and buspirone was found to be ineffective in PTSD associated nightmares. Conclusions: Because level A and B evidence does not exist for psychotropics and level C recommendation could be formulated only for a few agents, further exploration of new treatments in PTSD related nightmares should be conducted.

1. INTRODUCERE

Tulburarea de stres posttraumatic (TSPT) reprezintă, conform criteriilor DSM IV TR, o formă de patologie complexă, instalată în urma expunerii persoanei la un eveniment care a implicat o amenințare la adresa integrității sale fizice sau după ce persoana a asistat sau a aflat de o situație traumatică în cadrul căreia altcineva a fost rănit grav sau a decedat [1]. În cadrul clusterului simptomatic intitulat „retrăirea persistentă a evenimentului traumatic" este inclusă apariția viselor recurente asociate cu disconfort, cu tematică legată de evenimentul traumatic. De asemenea, în TSPT pot exista şi alte simptome aflate în legătură cu perturbarea somnului, respectiv dificultățile de adormire sau menținere a somnului.

Investigarea terapiilor farmacologice capabile să reducă frecvența coşmarurilor reprezintă un obiectiv important în abordarea pacientului cu TSPT deoarece acest tip de simptome se asociază cu disconfort şi afectează calitatea vieţii pacientului într-o măsură semnificativă. De altfel, coşmarurile reprezintă unul dintre simptomele cele mai uşor de sesizat de către pacient şi, în consecinţă, cele mai des raportate, spre deosebire de evitarea unor idei sau situaţii care pot aminti de evenimentul traumatic, restrângerea gamei de emoţii ori incapacitatea de reamintire a unor momente din desfăşurarea evenimentului respectiv. Multiple investigaţii clinice, care au inclus atât populaţia civilă, cât şi veterani, au arătat că tulburările hipnice şi simptomele care implică retrăirea evenimentului traumatic sunt întâlnite la peste 40% din persoanele care au experimentat un eveniment traumatic major [2].

În terapia coşmarurilor asociate TSPT au fost folosiţi numeroşi agenţi farmacologici, de la alfa1-blocante la antihistaminice şi de la antidepresive serotoninergice la antipsihotice atipice. Cel mai investigat medicament pentru coşmarurile din TSPT este prazosinul, care beneficiază de susţinerea mai multor studii clinice, metaanalize şi consensuri ale experţilor [2,3,4]. Medicaţia psihotropă, deşi este administrată curent în TSPT, a fost mai puţin investigată din punctul de vedere al eficacităţii pe acest simptom specific. În prezenta lucrare ne-am propus să realizăm o analiză sistematică a literaturii pentru a detecta posibilele strategii terapeutice eficiente în abordarea coşmarurilor din stresul posttraumatic, luând în considerare acele studii care şi-au fixat ca obiectiv ameliorarea acestui simptom.

2. ANALIZA SISTEMATICĂ A LITERATURII

Pentru a evalua nivelul de recomandare care poate fi formulat pentru diferite forme de terapie farmacologică în TSPT am inclus în această analiză sistematică a literaturii studii publicate până în iunie 2011, identificate în baza de date PubMed. Studiile au fost selectate utilizând următoarele cuvinte cheie : « tulburare de stres posttraumatic (PTSD) », « coşmaruri », « tratament farmacologic » şi denumirile internaţionale ale substanţelor psihotrope utilizate în tratamentul stresului posttraumatic.

Căutarea iniţială a detectat un număr de 59 de studii. Au fost selectate, conform criteriilor de includere şi excludere (vezi tabelul 1), doar 16 articole, care au rămas în analiză. În cadrul studiilor care au întrunit criteriile menţionate au fost incluse 1 480 de persoane, care au fost tratate cu diferite tipuri de agenţi psihotropi. Cele mai multe date au provenit din studii nerandomizate, deschise, analize retrospective ale foilor de observaţie şi studii de caz.

Subiecţii incluşi în studiile selectate au fost atât de veterani, cât şi civili (refugiaţi, victime ale accidentelor rutiere, ale traumelor sexuale, ale violenţei domestice etc.), care aveau vârsta minimă de 18 ani. Pacienţii puteau fi spitalizaţi sau monitorizaţi în regim ambulatoriu.

Metodele de intervenţie selectate pentru această analiză sistematică au fost terapiile farmacologice psihotrope, aplicate singure, ori în combinaţie cu psihoterapia. Am inclus atât studiile clinice în care s-a utilizat un singur agent farmacologic, cât şi studiile în care s-au folosit combinaţii terapeutice. În schimb, acele cercetări în care singura metodă de intervenţie pentru ameliorarea simptomatologiei TSPT a fost psihoterapia nu au fost incluse în această analiză. Nu am luat în considerare studiile care au utilizat alte medicamente decât cele psihotrope - antipsihotice, antidepresive, anticonvulsivante, anxiolitice.

Tabel 1. Criteriile de selectare a studiilor incluse în analiză

Criterii de includere	**Criterii de excludere**
Studii clinice, studii de caz, serii de cazuri , analize retrospective Subiecţii cu vârste de peste 18 ani Cercetările în care coşmarurile sunt menţionate ca un obiectiv al monitorizării Cercetările clinice în care sunt folosite medicamente psihotrope sau combinaţii psihotrope-psihoterapie	Metaanalize şi analize sistematice ale literaturii Studii clinice în care coşmarurile nu erau formulate explicit ca obiectiv al terapiei Comorbidităţi psihiatrice (altele decât depresia majoră) sau somatice Cercetările clinice în care se foloseşte medicaţie non-psihotropă (de exemplu, alfa1-blocante, antialergice etc.) Cercetările care au folosit ca metodă intervenţională unică psihoterapia

Au fost incluse în analiză studii randomizate sau nerandomizate, prospective, studii retrospective, studii de follow-up, studii de caz, serii de cazuri. Am exclus alte analize sistematice ale literaturii şi metaanalize cu obiective similare cu cele ale lucrării de față, pentru a evita redundanța şi supra-reprezentarea în analiză a unor studii clinice.

Variabilele urmărite au fost coşmarurile şi, în mod secundar, alte tulburări hipnice asociate coşmarurilor din TSPT. Coşmarurile trebuiau menționate în cadrul protocolului studiului ca variabilă primară, monitorizate printr-o metodă standardiată (scale clinice) sau prin interviu psihiatric, pentru ca respectivul studiu să poată fi inclus în analiză.

Rezultatele analizei sistematice

În urma aplicării criteriilor de includere şi excludere au rezultat 16 studii (tabelul 2), în care au fost folosiți multipli agenți psihotropi: antidepresive (n=8), antipsihotice (n=3), anxiolitice (n=2) şi anticovulsivante (n=3).

Tabel 2. Studiile incluse în analiză

DCI/doză		Obiective	Design	Populație	Variabile	Rezultate
Duloxetina 60-120mg/day	[5]	Eficacitatea şi tolerabilitatea duloxetinei la pacienții cu TSPT şi TDM comorbidă	naturalistic, 8 săptămâni	21 pacienți de sex masculin; cazuri de PTSD refractare la tratament; TDM comorbidă	Simptomele de TSPT; frecvența şi intensitatea coşmarurilor	Ameliorarea semnificativă a TSPT şi a simptomelor comorbide de TSPT; reducerea eficientă a coşmarurilor
Venlafaxina ER	[6]	Debutul activității şi timpul până la răspunsul terapeutic, monitorizat pe itemii individuali ai scalei CAPS-SX17, la pacienții tratați cu venlafaxină ER pentru TSPT	analiza datelor provenite din două studii randomizate, controlate placebo	687 pacienți	CAPS-SX17	Nu au fost observate diferențe semnificative între venlafaxina ER şi placebo pe parcursul perioadei de tratament şi la săptămâna 12 în privința variabilelor primare (itemii 2-vise neplăcute, 7- evitarea activităților, locurilor sau persoanelor, 8-incapacitatea de a-şi reaminti aspecte importante, 13-dificultate în reamintirea unor aspecte importante ale traumei, 13- dificultăți în menținerea sau instalarea somnului)
Risperidona doză flexibilă	[7]	(1) evaluarea preliminară a efectelor asupra somnului induse de terapia adjuvantă cu risperidonă (2) evaluarea prin ținerea unui jurnal de somn versus metode mai standardizate, retrospective	12 săptămâni, studiu deschis	17 pacienți, studiu de 6 săptămâni	Interviuri standardizate diagnostice pentru TSPT Automonitorizarea somnului, inclusiv prin mențiuni matinale în "jurnalele somnului"	Scalările globale au arătat ameliorări ale somnului, schimbări în frecvența trezirilor, reducerea coşmarurilor legate de traumă Frecvența trezirilor nocturne rezultate din jurnalele pacienților (dar nu şi din PSQI) au scăzut semnificativ Nu există schimbări la nivelul itemului "coşmaruri" din PSQI Datele din jurnalul pacientului indică o reducere a procentului de vise cu tematică traumatică la 6 săptămâni

Risperidona 2-3 mg	[8]	Risperidona plus timostabilizatoare, antidepresive, benzodiazepine	studii de caz	4 cazuri	Evaluarea simptomatologiei prin interviu psihiatric	Reducerea semnificativă a coşmarurilor şi flashback-urilor în toate cele 4 cazuri
Clonazepam 1 mg	[9]	Administrare a clonazepamului în tratamentul tulburărilor de somn asociate cu TSPT legată de război	randomizat, simplu-orb, cu design crossover	6 pacienţi cu TSPT legată de război	Jurnalul somnului, cantitatea de somn, calitatea somnului, frecvenţa şi intensitatea dificultăţilor de adormire, frecvenţa şi intensitatea viselor recurente neplăcute	Nu există diferenţe semnificative statistice între clonazepam şi placebo în nicio dimensiune evaluată
Olanzapina	[10]	Olanzapina în TSPT rezistentă la tratament, legată de război	Serie de cazuri	5 pacienţi, rezistenţi la tratament (multiple tratamente)	Intensitatea şi frecvenţa coşmarurilor	Atât coşmarurile, cât şi insomnia se ameliorează rapid după tratamentul cu olanzapină la toţi pacienţii
Mirtazapina	[11]	Mirtazapina pentru coşmarurile din TSPT	Studii de caz Scrisori către redacţie	300 refugiaţi	Frecvenţa şi intensitatea coşmarurilor	Aproximativ 75% din pacienţi au avut o reducere a frecvenţei şi intensităţii coşmarurilor
Topiramat 12.5 - 500 mg/zi, supraadăugat (N = 28) sau în monoterapie (N = 7)	[12]	Terapia cu topiramat în cazurile de TSPT cronic la civili	Studii deschise, naturalistic 33 săptămâni	9 bărbaţi, 26 femei cu simptome prezente de 18 +/- 15 ani cu TSPT (conform DSM-IV) la populaţia civilă	PCL-C	Topiramatul scade incidenţa coşmarurilor în 79% din cazuri (19/24) şi a flashback-urilor în 86% (30/35), cu suprimarea completă a coşmarurilor în 50% din cazuri şi a intruziunilor la 54% din pacienţii cu aceste simptome. Coşmarurile sau intruziunile s-au ameliorat parţial în 4 zile (11 +/- 13 zile) şi au dispărut complet în 8 zile (35 +/- 49 zile). Răspunsul a fost observat la 95% din respondenţii parţial la o doză de 75 mg/zi sau mai puţin şi în 91% din respondenţii complet la doze de 100 mg/zi sau mai puţin. Reducerile medii ale scorului PCL-C de la scorul iniţial la săptămâna 4 au fost semnificative (scor iniţial = 60 vs. scorul din săptămâna 4 = 39, p < .001), cu scăderi similare ale retrăirii, evitării, şi evitării şi hiperexcitabilităţii

Nefazodona Doza medie zilnică a fost 441 mg	[13]	Efectul nefazodonei asupra somnului şi EEG la pacienții cu TSPT cronică, cazuri instalate în legătură cu conflicte armate	Studiu deschis, cu durata de două săptămâni	12 veterani de sex masculin, (vârsta medie = 54 ani), care au îndeplinit criteriile DSM-IV pentru TSPT (durata medie = 30 ani). Toți, cu excepția unui pacient, au întrunit şi criteriile DSM-IV de TDM	Polisomnografie şi raportări subiective ale calității somnului şi coşmarurilor; CGI, CAPS, HAM-D, BDI	Pacienții au raportat semnificativ mai puține coşmaruri şi probleme legate de somn pe durata tratamentului. Spre deosebire de studiile cu pacienți depresivi, nefazodona nu afectează semnificativ măsurătorile polisomnografice, comparativ cu nivelul de pornire. În plus, pacienții arată o ameliorare semnificativă pe scala CGI şi CAPS (scor global, hiperexcitabilitate, intruziuni), HAM-D, BDI.
Fluvoxamina	[14]	Eficacitatea fluvoxaminei în diferite domenii ale calității autoevaluate a somnului la pacienții cu TSPT cronică, veterani de război	Studiu deschis, cu durata de 10 săptămâni	Persoane de sex masculin, veterani din Vietnam (N = 21)	Simptomele legate de calitatea somnului din TSPT (subiectiv)	Fluvoxamina conduce la reduceri ale simptomatologiei TSPT şi ale tuturor domeniilor de evaluare subiectivă a calității somnului. Efectele cele mai importante ale tratamentului au fost observate asupra viselor care relau tematica traumatică. Din contra, visele neplăcute fără legătură cu evenimentul traumatic au avut doar o reducere modestă a frecvenței apariției sub tratament. Insomnia de menținere şi itemul "somn perturbat" s-au ameliorat semnificativ, în timp ce insomnia de inducție s-a ameliorat mai puțin substanțial. Aceste beneficii terapeutice contrastează cu alte date publicate care au arătat efecte activatorii ale ISRS asupra somnului.
Trazodona	[15]	Efectele trazodonei la pacienții cu TSPT cu insomnie sau coşmaruri	8 săptămâni, pacienți spitalizați	74 veterani cu TSPT	Intensitatea şi frecvența coşmarurilor	60 pacienți au fost în stare să mențină o doză eficientă de trazodonă, iar ceilalți 14 pacienți nu au reuşit să respecte recomandările terapeutice. 72% din cei 60 de pacienți au considerat că trazodona a fost utilă în scăderea incidenței coşmarurilor, de la o medie de 3.3 la 1.3 nopți pe săptămână ($p<.005$). 92% din pacienți au evaluat ca eficient tratamentul pentru instalarea somnului, iar 78% au raportat ameliorări ale menținerii somnului. A existat o corelare semnificativă a eficacității în privința reducerii coşmarurilor şi ameliorarea globală a somnului ($r= .57$, $p < .005$). Doza eficientă de trazodonă pentru 70% din pacienți se află între 50 şi 200 mg/zi, administrată noaptea.

Topiramat doza medie 103 mg/zi	[16]	Efectele topiramatului vs. placebo în TSPT	Studiu dublu orb, randomizat, 12 săptămâni	35 pacienți cu TSPT, civili	Scorul total şi analiza itemilor CAPS, BDI şi scorul CGI	82.35% pacienții tratați cu topiramat au prezentat ameliorări ale simptomelor de tip retrăire a traumei, inclusiv a coşmarurilor ; CAPS a scăzut cu 57.78 (topiramat) vs. 32.41 (placebo)
Topiramat	[17]	Efectele topiramatului în TSPT cronic	Studii de caz	3 pacienți cu TSPT	Amintiri intruzive şi coşmaruri	Reducerea sau chiar eliminarea amintirilor intruzive şi a coşmarurilor, normalizarea dispoziției depresive.
Buspirona 35-60mg/zi	[18]	Buspirona în terapia TSPT	Studii de caz	3 pacienți	Simptome de anxietate, insomnie, flashback-uri, coşmaruri, dispoziție depresivă	Debutul efectului clinic a variat de la 5 la 29 de zile. Simptome ameliorate: anxietatea, insomnia, flashback-urile, dispoziția depresivă
Fenelzina 30-90mg/zi	[19]	Efectul tratamentului fenelzinei	Studiu deschis, prospectiv	25 veterani	HAM-D, o scală nouă pentru TSPT, HAMA	6 pacienți au urmat 4-8 săptămâni tratament cu fenelzină, 7 pacienți între 9 şi 13 săptămâni, 9 pacienți între 14 şi 18 săptămâni; perturbarea somnului a fost singurul item care a arătat o ameliorare semnificativă
ADTC	[20]	Efectul medicației psihotrope şi a psihoterapiei suportive	Studiu de follow-up	12 refugiați din Cambogia	Simptomele de tip intruziv, hiperreactivitatea şi evitarea	5 din 12 pacienți au avut o scădere semnificativă a coşmarurilor, altor tulburări hipnice şi racției de tresărire exagerată

TSPT-tulburare de stres posttraumatic
TDM-tulburare depresivă majoră
CAPS-SX17- Scala Clinică pentru TSPT Administrată de Clinician - versiunea cu 17 itemi
PSQI- Indexul Pittsburgh pentru Calitatea Somnulu
PCL-C – Inventarul pentru TSPT- Versiunea pentru Civili
DSM IV TR- Manualul de Diagnostic şi Statistică a Tulburărilor Mentale Ediția IV Revizuită
CGI- Impresia Clinică Globală
HAMD- Scala Hamilton pentru Depresie
BDI- Inventarul pentru Depresie Beck
ISRS- Inhibitori Selectivi ai Recaptării Serotoninei
HAMA- Scala Hamilton pentru Anxietate
ADTC- antidepresive triciclice

Referitor la clasa antidepresivelor, venlafaxina a fost evaluată în două studii randomizate, controlate placebo, care au inclus un număr important de subiecți (n=687) şi nu a fost asociată cu rezultate superioare placebo. Duloxetina, nefazodona şi fluvoxamina au fost investigate fiecare în câte un studiu deschis, pe un număr mic de pacienți (12-21), rezultatele fiind favorabile. În ceea ce priveşte mirtazapina, deşi există raportări de cazuri din partea unor autori, lipsa unor studii randomizate face dificilă recomandarea acestui agent farmacologic în coşmarurile asociate TSPT. Trazodona şi antidepresivele triciclice au fost evaluate în cadrul unor studii clinice deschise, pe loturi relativ mici de subiecți (12-74), asociindu-se cu rezultate pozitive. Monitorizarea efectelor fenelzinei asupra coşmarurilor din TSPT nu a arătat o ameliorare a acestui tip de simptome, în cadrul unui studiu prospectiv, deschis.

Studii deschise şi studii de caz susțin utilitatea risperidonei, singură sau în asociere cu alți agenți farmacologici (timostabilizatoare, antidepresive sau benzodiazepine), în terapia coşmarurilor din TSPT. O serie de 5 cazuri a analizat eficacitatea olanzapinei pentru tulburările hipnice din TSPT rezistentă la tratament, agentul antipsihotic determinând ameliorarea rapidă a simptomelor țintă.

Topiramatul a fost evaluat în două studii de calitate bună, unul deschis şi unul dublu orb, precum şi în studii de caz, iar rezultatele sunt încurajatoare.

Benzodiazepinele sunt reprezentate în această analiză de clonazepam, a cărui eficacitate asupra coşmarurilor din TSPT a fost investigată într-un studiu randomizat, dublu orb, rezultatele fiind negative. Totuşi, trebuie menționat numărul redus de subiecți din acest studiu, ceea ce face dificilă interpretarea şi generalizarea rezultatelor.

Buspirona este un alt anxiolitic investigat pentru această indicație. Din rezultatele unor studii de caz rezultă că buspirona poate ameliora insomnia şi alte simptome anxioase, dar mai puțin coşmarurile. În plus trebuie menționat timpul crescut de latență al efectului terapeutic (până la 29 de zile).

3. CONCLUZII

Recomandările care pot fi formulate în urma analizei datelor colectate pornesc de la calitatea datelor (studii randomizate vs. nerandomizate, dublu orb/simplu orb/deschise, prospective/retrospective, studii de caz, serii de cazuri, studii de follow up şi analize controlate), numărul subiecților incluşi, calitatea instrumentelor folosite pentru monitorizarea simptomatologiei (scale clinice, interviu structurat, interviu psihiatric nestandardizat).

Pentru evaluarea calității datelor din literatură şi formularea criteriilor de recomandare au fost utilizate criteriile Centrului Oxford pentru Medicină Bazată pe Dovezi [21].

În urma analizei datelor din literatura de specialitate, recomandările de tratament psihotrop pentru coşmarurile din TSPT au fost formulate astfel:

Tabel 3. Gradul de recomandare

Clasa de substanțe/agenții farmacologici individuali (număr de studii)	**Gradul de recomandare (clasificarea dovezilor conform Oxford CEBM)**
Antidepresive (8)	C (3a, 3b, 4)
Venlafaxină (1)	- (3a, rezultatele nu arată efect favorabil)
ADTC (1)	C (3a)
Fluvoxamină (1)	C (3a)
Trazodonă (1)	C (3a)
Duloxetină (1)	C (3a)
Mirtazapină (1)	C (3b)
Nefazodonă (1)	C (3a)
Fenelzină (1)	-(3b, rezultatele nu arată efecte favorabile)
Antipsihotice (3)	C (3a, 3b, 4)
Risperidonă (2)	C (3a, 3b)
Olanzapină (1)	C (3b)
Anxiolitice (2)	- (3a, 4, rezultatele nu sunt favorabile)
Clonazepam (1)	- (3a, rezultatele nu sunt favorabile)
Buspirona (1)	- (4, rezultatele nu sunt favorabile)
Anticonvulsivante/timostabilizatoare (3)	C (3a, 3b)
Topiramat (3)	C (3a, 3b)

CEBM=Center for Evidence-Based Medicine levels of evidence

Deoarece recomandări de nivel A sau B nu pot fi susținute în prezent pentru medicația psihotropă, iar recomandările de nivel C pot fi formulate doar pentru unii agenți farmacologici, nevoia de studii clinice care să evalueze alte noi terapii în coşmarurile asociate stresului posttraumatic este semnificativă.

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Managementul medical al situațiilor cu risc chimic

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Rezumat. În prezent, folosirea armelor de distrugere în masă este puțin probabilă, cu excepția actelor teroriste. Pregătirea sistemului medical pentru a face față unor asemenea situații are totuşi un loc important. Substanțele chimice care pot fi folosite ca arme de distrugere în masă sunt accesibile, ele fiind utilizate atât în industrie, cât şi mediul domestic. Agenții utilizați pot fi letali sau neletali şi pot avea acțiune la diferite niveluri ale organismului uman. Managementul medical al situațiilor cu risc chimic este influențat de o serie de factori care țin de agentul chimic folosit (tip, toxicitate, timp de înjumătățire, persistență, existența antidotului), de tipul expunerii (lichid, vapori sau amândouă), de modul expunerii (inhalație sau transcutanat), de prezența sau absența manifestărilor clinice şi timpul scurs de la expunere, precum şi de numărul victimelor şi existența resurselor.

Cuvinte cheie: armă chimică, management medical, facilități medicale de ROL 1 şi 2

Summary. Weapons of mass destruction were used from the ancient history, but nowadays their use is less probable, excepting the terrorist acts. A great importance has the medical system preparedness so that it can cope with that kind of situations. The chemical substances that can be used as weapons of mass destruction are very accesible being used in industry as at home. The chemical agents that are used can be lethal or unlethal and can action at different levels of the human organism. Medical management of the chemical risk situations is influenced by several factors related to: the chemical agent used (type, toxicity, half life, persistence, antidote), the type of exposure (liquid, vapors or both), the route of exposure (inhalation or transcutaneous), the presence or absence of clinical signs, the time from exposure, and also by the number of victims and the available resources.

A. GENERALITĂȚI

Întrebuințarea armelor de distrugere în masă, respectiv a armelor chimice (A.C.), într-un conflict militar, şi nu numai, chiar dacă este interzisă prin convenții internaționale, poate avea consecințe deosebit de grave asupra populației, motiv pentru care pregătirea sistemului medical pentru a face față unei astfel de situații are o importanță deosebită. De reținut că agenții chimici ce pot fi folosiți ca arme pot fi prezenți şi în accidente industriale, alții pot apărea în urma combustiei materialelor plastice sau se află în componența unor substanțe utilizate în domenii diverse; spre exemplu, insecticidele organofosforice au acelaşi mecanism de acțiune ca agenții chimici militari, afectând în mod direct sistemul nervos. Nu trebuie însă uitat nici riscul substanțial al unui act terorist care să implice arme chimice.

Se poate aprecia că forme incipiente ale A.C. datează încă din antichitate. Aş menționa în acest context „focul grecesc”, prezent în războaiele dintre Atena şi Sparta (428 î.e.n), apoi, în Evul Mediu, otrăvirea fântânilor etc. Evident, de o armă chimică în adevăratul sens al cuvântului putem vorbi în prima conflagrație mondială, când germanii au utilizat clorul, determinând moartea a peste 7% din militarii expuşi. Convenția de la Geneva din 1925 a interzis folosirea gazelor toxice în conflictele militare, dar A.C. s-au mai folosit în Etiopia, în 1930, când italienii au utilizat gazul mustard, în Irak, în războaiele din perioada 1980 - 1990 (agenți cu acțiune SNC şi gazul mustard) şi într-un act terorist, în 1995, în metroul din Tokio, Japonia, când s-a utilizat gazul sarin, rezultând 5 000 de răniți şi 12 morți. Pentru a reduce posibilitatea folosirii A.C., în 1993 a fost semnată *„Convenția privind interzicerea dezvoltării, producerii, stocării şi folosirii armelor chimice şi distrugerea acestora (CWC)”*, la care până în prezent au aderat 165 de state.

Substanțe toxice posibil a fi folosite ca A.C. care se utilizează în industrie sunt următoarele:

- cianurile (placarea metalelor);
- izocianatul (cauza dezastrului de la Bhopal, India);
- fosgenul;
- clorul (se eliberează şi în mediul domestic, atunci când hipocloritul este întrebuințat ca înălbitor şi intră în contact cu un acid);
- arsenicul (tratarea sub presiune a lemnului);
- organofosforicele.

B. MANAGEMENTUL SITUAȚIILOR CU RISC CHIMIC (SRC)

Pentru a putea realiza un management eficient al SRC este necesar să cunoaştem ce diferențe există între armele chimice şi cele biologice:

- **armele chimice -** instalare rapidă a simptomelor şi efectelor, locul evenimentului este clar, există antidot doar pentru organofosforice, cianuri şi levizită;
- **toxinele biologice:** perioadă de latență - 6-24 ore; dacă intoxicația apare prin intermediul apei, nu există un loc evident de producere; există antidot doar la toxina botulinică;
- **agenții infecțioşi biologici -** latență mare, greu de localizat originea, pentru unii agenți biologici există vaccinuri.

a. Factorii care influențează managementul medical al situațiilor cu risc chimic (MMSRC)

Factori care pot influența MMSRC:

- tipul agentului (toxicitate, T de ½, persistență, existența antidotului);
- tipul expunerii (lichid, vapori sau amândouă);
- modul expunerii (inhalare sau transcutanat);
- prezența sau absența manifestărilor clinice şi timpul scurs de la expunere;
- numărul victimelor şi existența resurselor.

b. Tipuri de agenți chimici folosiți ca arme chimice:

- ***agenți letali***: cu acțiune nervos centrală (SNC), vezicanți, methemoglobinizanți şi respectiv sufocanți;
- ***agenți neletali***: care provoacă vărsături, lacrimogeni şi spray-ul piper.

1. Agenții letali

1.1. Agenții neurotoxici – tabun (GA), soman (GD), sarin (GB, ciclosarin (GF)) şi VX. Au fost dezvoltați de germani în al Doilea Război Mondial, sunt numiți agenți "G" în codul aliaților. Au efecte asemănătoare cu insecticidele civile. Cel mai toxic este VX-ul, care se absoarbe prin mucoase şi prin tegumente.

1.1.1. Mecanism de acțiune: sunt inhibitori potenți ai acetilcolinesterazei, care determină acumularea în exces a acetilcolinei la nivelul sinapselor colinergice şi paralizarea transmisiei sinaptice subsecvente. Efectele sunt de stimulare excesivă parasimpatică, cu efecte muscarinice, nicotinice şi nervos centrale.

1.1.2. Efecte inițiale. După expunerea la nivelul pielii apar transpirații şi fasciculații localizate, iar după expunerea oculară, mioză şi vedere dublă.

*1.1.3. Sindromul SLUDGE: S*alivație, *L*ăcrimare, *U*rinare, *D*efecație, crampe *G*astrointestinale şi respectiv *E*meză.

1.1.4. Alte efecte după o expunere masivă: tulburarea vederii, mioză, dispnee datorită bronhospasmului, mioclonii, cefalee, confuzie, comă, convulsii, apnee şi moarte.

1.1.5. Managementul intoxicației la nivelul spitalelor de ROL 1 şi 2:

- protejaţi-vă singuri folosind mănuşi din butil sau costum de protecţie individuală şi mască la nevoie (PPE);
- scoateţi cazul din zona roşie (dacă aveţi costum de protecţie, dacă nu, lăsaţi trupele speciale să o facă);
- ventilaţi pacientul (nu se face respiraţie gură la gură);
- se administrează atropină - 2 mg im iniţial;
- decontaminaţi (în zona de decontaminare de către trupele speciale);
- administraţi oximă im sau iv (obidoximă sau pralidoximă – 2-PAM 1 g la 8/12 ore);
- confirmaţi intoxicaţia (determinaţi colinesteraza serică şi din hematii).

1.1.6. Complicaţii: insuficienţă respiratorie, acidoză, hipertermie, hipotermie, neuropatie periferică, edem cerebral, insuficienţă cardiacă acută; tardiv apar convulsii şi sindrom psihiatric.

1.2. Agenţii vezicanţi. Aceşti agenţi au capacitatea de a produce diferite leziuni, în funcţie de concentraţie. Cei mai importanţi reprezentanţi sunt gazul mustard sau sulf iperita (HD), levizita sau arsina (L), azot iperita (HN-1,2,3), oxima fosgenului (CX), fenil dicloroarsina (PD), etil dicloroarsina (ED) etc. Agenţii au fost folosiţi în Primul Război Mondial, înregistrându-se şi 600 de morţi, expunerea determinând leziuni dermice şi oculare în câteva minute.

1.2.1. Mecanism de acţiune. Sunt agenţi alchilanţi potenţi (asemănători celor folosiţi în oncologie) care determină apariţia de vezicule pe epidermă, iar la doze mari au efecte citotoxice sistemice, mai ales asupra celulelor sistemelor hematologic şi gastrointestinal. Au efecte mutagene şi carcinogene, inhibă glicoliza celulară şi determină în final necroză celulară.

1.2.2. Efecte clinice imediate. Perioada de latenţă este doză-dependentă (1-4 ore), după care apar următoarele manifestări:

- *la nivelul pielii*: eritem, edem, vezicule şi apoi necroză;
- *la nivelul ochilor*: durere, fotofobie, lăcrimare, vedere în ceaţă, edem conjunctival, iridociclită, leziuni corneene şi cecitate;
- *respiratorii**:*** traheobronşită, edem pulmonar acut hemoragic (noncardiogen) şi insuficienţă respiratorie.

1.2.3. Efecte tardive. Sunt determinate de toxicitatea sistemică şi se referă la:

- leziuni GI – greaţă, vărsături, diaree, hemoragii digestive;
- efectele hematologice: supresia măduvei hematogene cu prognostic prost;
- carcinogeneză: creşterea incidenţei cancerului pulmonar.

1.2.4. Expunerea prelungită (30-60 minute) determină leziuni respiratorii severe, intoxicaţie sistemică şi moarte. Decesele ajung până la 3-4%. Moartea apare prin insuficienţă respiratorie şi supresie medulară.

1.2.5. Managementul intoxicaţiei la nivelul spitalelor de ROL 1 şi 2:

- protejarea personalului – gazul mustard poate traversa hainele standard, necesită costum special cu strat de cărbune absorbant;
- îndepărtarea rapidă a reziduului cutanat este critică, deoarece odată agentul legat de ţesuturi efectele sunt ireversibile;
- nu există antidot recunoscut, se va face tratament suportiv şi decontaminare;
- se va proceda la irigarea copioasă a ochilor cu soluţie 0,5% de hipoclorit, care inactivează mustardul (gazul muştar). Se pot folosi unguente oftalmice cu antibiotic;
- **nu** se freacă pielea;
- pentru PD şi ED se decontaminează cu soluţie de înălbitor (hipoclorit);
- dacă există arsuri se foloseşte crema cu sulfadiazină argentică;
- se administrează oxigen la nevoie +/- ventilaţie mecanică la răniţii cu insuficienţă respiratorie acută;
- administrarea steroizilor este pusă sub semnul întrebării.

1.3. Agenţii hemotoxici şi toxici tisulari. Au potenţă relativ mică în comparaţie cu ceilalţi agenţi militari. Cei mai importanţi reprezentanţi sunt: acidul cianhidric (cod AC), acidul hidrocianic

(Cyclon), cianogenii (eliberează HCN, HCl, I_2 şi Br_2). Pot avea miros de migdale amare, detectabil de unele persoane.

1.3.1. Mecanism de acţiune. Acest tip de agent chimic inhibă respiraţia celulară prin blocarea activităţii citocrom oxidazei. Poate avea şi efecte iritante ale mucoaselor prin eliberarea de acid clorhidric, iod sau brom. Determină decesul pacientului în secunde la minute. Se descompun la lumină şi sunt neutralizate de alcalii.

Important de reţinut – în mediu deschis au tendinţa de a se dispersa rapid.

1.3.2. Efecte clinice. Efectele sunt rapide şi iniţial apare senzaţiile de gât uscat şi de arsură, urmate de “foame” de aer, polipnee, cefalee, greaţă, vărsături, vertij, agitaţie şi comportament agresiv. În stadiile avansate ale intoxicaţiei apar convulsiile, coma, paralizia, depresia respiratorie, apneea, aritmiile cardiace şi colapsul cardiovascular.

1.3.3. Diagnostic. Aproximativ jumătate din cei intoxicaţi percep un miros slab de migdale amare, marea majoritate au tahipnee. Pacienţii internaţi în spital prezintă acidoză metabolică inexplicabilă, PvO_2 (presiunea parţială venoasă a oxigenului) crescută, iar sângele venos are aceeaşi culoare cu cel arterial.

1.3.4. Managementul intoxicaţiei la nivelul spitalelor de ROL 1 şi 2:

- se administrează 100% oxigen şi suport respirator (VM) la nevoie;
- se decontaminează pielea şi ochii prin irigare cu ser fiziologic/apă;
- se administrează bicarbonat de sodium iv dacă există acidoză severă;
- se poate folosi kitul “Lilly Cyanide Antidote Kit”:
  - se inhalează o fiolă de nitrat de amil (max. 3);
  - se administrează iv nitrit de sodiu (sol. 3%) 10 ml iv (0,15 - 0,33 ml/kg);
  - se menţine nivelul methemoglobinei < 30%;
  - se administrează iv tiosulfat de sodiu (sol. 25%) 50 ml IV (1,65 ml/kg);
  - se repetă ½ din doză după 30 de minute la nevoie;
- Consideraţi administrarea de O_2 hiperbar sau a 5 grame de hidroxicobalamină iv.

1.4. Agenţi sufocanţi. Cei mai importanţi reprezentanţi ai acestei clase sunt fosgenul (CG), difosgenul (DP), oxima fosgenului (CX) şi clorul (Cl). Fosgenul a fost folosit în Primul Război Mondial şi a determinat 70% din decesele cauzate de armele chimice. Expunerea la acest tip de A.C. determină apariţia cu întârziere a simptomelor. Nu există antidot.

1.4.1. Prezentare clinică. Sunt marea majoritate simptome respiratorii şi apar la 2-24 de ore (fosgen) şi la peste 4 ore (clor). Iniţial apar odinofagie, lăcrimare, tuse, greaţă, vărsături, cefalee şi senzaţie de constricţie toracică. Mai târziu apar dispneea severă, edemul pulmonar acut noncardiogen, hemoptizia şi cianoza. Mult mai târziu apare nefrotoxicitatea.

1.4.2. Managementul intoxicaţiei la nivelul spitalelor de ROL 1 şi 2:

Intoxicaţie cu fosgen şi difosgen:

- se administrează oxigen şi se realizează suportul respirator (VM) pentru ARDS;
- administrarea steroizilor este cu semnul întrebării,
- se administrează fluide pentru a compensa hipovolemia, atunci când există.

Intoxicaţia cu clor:

- se administrează oxigen;
- se decontaminează cu atenţie ochii şi pielea;
- se intubează şi ventilează mecanic pacientul (cu PEEP), dacă există ARDS;
- se administrează β2 agonişti adrenergici, dacă există bronhospasm;
- administrarea de steroizi, diuretice şi antibiotice **nu** îmbunătăţeşte prognosticul.

2. Agenţii neletali

2.1. Agenţi care provoacă iritanţi. Reprezentativi sunt adamsita (DM) şi cloropicrina – triclornitrometanul (PS). Pot acţiona ca agenţi iritanţi care determină greaţă (DM şi PS), dar şi ca agenţi sufocanţi, lacrimogeni şi vezicanţi – PS.

2.1.1. Efecte clinice:

- iritant ocular puternic - determină lăcrimare şi durere;
- tuse, odinofagie, rinoree;

- poate apărea edem pulmonar noncardiogen fatal;
- iritaţia pielii, cefalee, vărsături.

2.1.2. Management intoxicaţiei în campanie. Nu există antidot. Se decontaminează şi se asigură îngrijire de suport.

2.2. Agenţi chimici iritant-lacrimogeni. Cei mai folosiţi sunt: gazul lacrimogen al poliţiei - cloracetofenona (CN), gazul lacrimogen britanic (CR), gazul lacrimogen militar pentru controlul revoltelor – o-benzilmalonitrilul (CS), cloracetofenona în cloroform - CNC (mace) şi respectiv sprayul piper. Cel mai toxic este CN, care are doza letală/min/m^3 cea mai mică.

2.2.1. Efecte clinice

- *oculare* – sunt cele mai frecvente simptome: lăcrimare, edem palpebral, dureri oculare, blefarospasm, fotofobie şi rinoree. Pot apărea şi ulcere corneene. De obicei, simptomele dispar în 1-2 ore;
- *pulmonare* – concentraţiile ridicate determină edem pulmonar acut noncardiogen şi wheezing la astmatici;
- *tegumentare* – arsuri ale pielii;
- *digestive* – ingestia determină greaţă, vărsături şi diaree.

2.2.2. Managementul intoxicaţiei la nivelul spitalelor de ROL 1 şi 2:

- expunere la aer curat;
- se administrează oxigen la nevoie;
- se decontaminează ochii, gura, pielea cu apă din abundenţă;
- se administrează un unguent cu antibiotic dacă există leziuni corneene sau tegumentare;
- veziculele tegumentare se tratează la fel ca arsurile clasice de gradul II;
- tratament simptomatic - se administrează AINS pentru eventualele dureri (oculare, cefalee etc.), tratament antiemetic etc.;
- se administrează agenţi Beta-2 dacă există bronhospasm.

2.3. Agenţi chimici incapacitanţi (psihochimici). Cel mai folosit este benzilatul de 3-quinuclidinil (BZ); produce victimei incapacitate, prin blocarea acţiunii acetilcolinei la nivelul sistemului nervos (central şi periferic), având acţiune asemănătoare cu a atropinei, dar cu durată mult mai mare. Este extrem de persistent în sol şi apă şi pe majoritatea suprafeţelor. Este periculos prin contact direct. Alţi agenţi incapacitanţi care pot fi folosiţi ca A.C. sunt fentanilul, remifentanilul, dimetiltriptamina (DMT), LSD-ul etc.

2.3.1. Efectele clinice ale BZ:

- *neurologice* – ataxie;
- *psihice* – halucinaţii, stupoare, stare confuzională;
- *oculare* – midriază, paralizia muşchilor intraoculari cu imposibilitatea vederii;
- *cardiace* – tahicardie;
- *respiratorii* – tahipnee şi polipnee;
- *tegumentare şi mucoase* – piele uscată şi roşie, uscăciunea gurii;
- *modificări de temperatură* – hipertermie;

Iniţial, după expunerea acută apar toate simptomele şi semnele de mai sus, pentru ca după 1-4 ore de la expunere să se menţină mai ales semnele cutanate şi cardiace. Mai târziu, la 3-5 ore, reapar manifestările secundare, care sunt reprezentate de nelinişte, fasciculaţii musculare, afectarea vederii de aproape şi incapacitare totală. La 6-10 ore de la expunere se menţin semnele psihice, care dispar total în 3-4 zile.

2.2.2. Managementul intoxicaţiei la nivelul spitalelor de ROL 1 şi 2:

- expunere la aer curat;
- se administrează oxigen la nevoie;
- tratament simptomatic – repleţie volemică şi menţinerea funcţiilor vitale;
- tratamentul hipertermiei;
- tratamentul tulburărilor psihice (sedative, antipsihotice etc.).

C. MANAGEMENTUL MEDICAL AL SITUAȚIILOR CU RISC CHIMIC LA CAMPANIE

Managementul medical al acestor situații depinde foarte mult de tipul de expunere, de tipul agentului, de prezența sau absența simptomelor. Astfel, pacienții care au fost expuşi unor agenți chimici, dar care sunt asimptomatici trebuie ținuți sub observație perioade de timp diferite, în funcție de tipul de agent şi modul de expunere, astfel: agenți hemotoxici (cianuri) – 0, vapori de agent neurotoxic – 0-1 oră, agent neurotoxic lichid – 18-24 ore, agenți vezicanți (mustard) – 12-24 ore, agenți sufocanți (fosgen) – 6-24 ore şi respectiv agenți sufocanți (clor) – 6-24 ore.

a. Planificarea managementul medical al situațiilor cu risc chimic (MMSRC). Pentru a asigura un management adecvat al pacienților la nivelul facilităților medicale de ROL 1 şi 2 personalul acestora, şi nu numai, trebuie să se pregătească. De aceea trebuie luate în considerare o serie de principii pentru pregătirea spitalelor, astfel:

- alcătuirea unui plan de răspuns pentru astfel de situații de risc;
- includerea întregului personal în stagiul de planificare, inclusiv a personalului care lucrează în sistemul de urgență pe teren (pe ambulanțe);
- răspândirea planului la toate categoriile de personal;
- asigurați-vă că personalul a studiat planul;
- asigurați-vă că există cursuri pentru reținerea şi reîmprospătarea planului;
- revederea periodică a planului atunci când există schimbări ale riscurilor, modificări de mediu, de personal sau ale capacităților spitalului;

1. Planul de MMSRC va include cel puțin următoarele componente:

- listă de control cu toate acțiunile (checklist);
- determinarea clară a rolului şi responsabilităților fiecăruia dintre angajații cuprinşi în plan;
- rezumat executiv pentru nou veniții în spital şi pentru aducere aminte;
- opțiuni de rezervă în cazul în care planul principal eşuează sau când situația se schimbă.

2. Pregătirea spitalelor. Angajamentul personal este una dintre cele mai importante resurse pe termen lung. La aceasta se adaugă identificarea resurselor, achiziționarea şi menținerea lor în activitate. Odată rezolvate aceste probleme trebuie pregătită deplasarea lor la locurile stabilite în plan. Nu vor fi neglijate resurse cum sunt: personalul (antrenat şi disponibil), existența PPE (echipament personal de protecție) şi a celui de decontaminare, a antidoturilor (incluzând accesul la depozitele regionale), a aparatelor de ventilație mecanică şi a paturilor de terapie intensivă.

2.1. Dificultățile de trafic rutier. În pregătirea facilităților medicale trebuie luate în considerare şi dificultățile care pot apărea în trafic datorită închiderii planificate a drumurilor contaminate/ suspecte de a fi contaminate. La acestea se adaugă şi blocajul în trafic datorat populației care vrea să scape din aria contaminată. Aceste blocaje vor împiedica personalul spitalului (staff-ul) să sosească în timp util la spital, dar pot împiedica şi afluxul masiv de pacienți mai ales în primele ore după eveniment, menținând un raport acceptabil între pacienți şi staff.

2.2. Pregătirea zonelor specifice. Această activitate presupune desemnarea spațiilor, amplasarea aparaturii medicale şi alocarea de resurse umane şi materiale (medicamente, materiale sanitare etc.). În cadrul facilităților medicale de ROL 1 şi 2 următoarele spații există din construcție (spații modulare), dar trebuie realizată amplasarea pe teren:

- spații de triaj - pentru pacienții contaminați, în afara UPU, bine demarcate;
- spațiul de decontaminare (Decon) – pentru pacienții contaminați, în afara UPU, bine demarcate;
- acces unic printr-un punct de control în interiorul UPU şi în spital;
- spațiu pentru triajul secundar - pentru pacienții necontaminați, în interiorul UPU, bine demarcat;
- spațiu de tratament - pentru pacienții necontaminați, spațios, aerisit.

La nivelul spitalelor civile zonele sunt identice cu cele de la nivelul spitalelor de ROL 1 şi 2, dar ele vor fi stabilite de către persoana desemnată de conducerea spitalului (şef secţie UPU/altă persoană) în funcţie de spaţiile existente.

3. Diferenţele majore dintre pregătirea unui MCI (eveniment cu cazuri multiple) convenţional şi respectiv unul neconvenţional sunt determinate de:

- numărul de cazuri;
- tipul de eveniment (deschis sau acoperit);
- dificultăţile majore logistice şi discontinuităţi datorate contaminării drumurilor, echipamentului şi personalului;
- necesitatea de a decontamina şi/sau izola cazurile;
- efectul psihologic asupra staff-ului;
- efectul psihologic asupra cazurilor şi publicului;
- riscul de contaminare a facilităţii de tratament.

3.1. Numărul cazurilor. Majoritatea evenimentelor convenţionale, cu excepţia cutremurelor, implică sute de cazuri pentru câteva ore, dar un eveniment neconvenţional de tipul atac chimic terorist/atac nuclear cu o armă de mici dimensiuni poate produce mii de cazuri în câteva ore sau mii/zeci de mii de cazuri în zile sau săptămâni în cazul unui atac biologic terorist sau a unui atac nuclear major.

3.2. Tipul de eveniment (deschis sau acoperit). Atacurile de tip acoperit permit o contaminare mult mai mare anterior diagnosticului. Incertitudinea expunerii, perioada de incubaţie şi posibilitatea expunerii personalului fac strategiile de management mult mai complexe şi necesită luarea de decizii pe măsură ce evenimentul este în desfăşurare, în contradicţie cu protocoalele relativ rigide ale managementului MCI convenţionale.

3.3. Necesitatea de a decontamina şi/sau izola cazurile. Nevoia de decontaminare a pacienţilor înainte de administrarea tratamentului întârzie terapia şi scade performanţa personalului care poate purta şi el un PPE. De aceea este necesar antrenamentul specializat al tuturor celor implicaţi. Acceptarea antrenamentului şi rămânerea după program sunt adesea greu de obţinut în prezent, mai ales înaintea apariţiei primului eveniment.

3.4. Efectele psihologice asupra personalului. Sunt greu de precizat. Aceste efecte depind şi de nivelul şi extinderea contaminării, implicarea zonelor din jurul spitalului etc. Sunt reduse prin antrenament, activitate în care este indicat să fie implicaţi şi membrii de familie, mai ales atunci când facilităţile medicale sunt în locaţii apropiate de domiciliul de reşedinţă. Antrenamentul trebuie luat în considerare atunci când se cere mobilizarea personalului, mai ales în primele 24 h.

3.5. Efectele psihologice asupra victimelor. În asemenea situaţii de risc chimic apare un procent mare de cazuri psihologice, iar efectele psihologice asupra victimelor sunt deosebit de complexe. Uneori, simptomele complexe sunt greu de diferenţiat de intoxicaţia adevărată sau de alte boli. MMSRC este dificil deoarece există posibilitatea de apariţie a unei presiunii extreme exercitate de către pacienţi asupra punctelor de acces în spitale. Alături de pacienţi se găseşte frecvent şi un număr mare de membri de familie care cer să-i însoţească.

3.6. Dificultăţile de trafic rutier. Datorită închiderii planificate a drumurilor contaminate sau suspecte de a fi contaminate, la care se adaugă blocajul în trafic datorat populaţiei care vrea să scape din aria contaminată pot apărea dificultăţi în sosirea personalului în timp util la spital. Blocajele pot împiedica fluxul de pacienţi mai ales în primele ore după eveniment, menţinând astfel un raport acceptabil între cerere (pacienţii sosiţi) şi ofertă (personalul prezent la program).

3.7. Contaminarea potenţială a facilităţii de tratament. Contaminarea poate apărea primar (spital în zona afectată) sau secundar (datorită pacienţilor contaminaţi din UPU), ceea ce reprezintă un risc pentru personalul propriu. Se va evita acordarea oricărei forme de îngrijire medicală, cu excepţia măsurilor de salvare a vieţii până când:

- se desemnează o arie adecvată pentru decontaminare şi tratament;
- se stabileşte şi se completează protecţia personalului;
- echipamentul specific este deplasat la locul adecvat;
- personalul specific şi cel adiţional (de suport) au sosit.

Principiile de decontaminare care trebuie respectate sunt următoarele:

- se îndepărtează hainele, ceea ce reprezintă ≈ 80% din decontaminare. Acestea se vor pune în saci de plastic pentru a fi analizaţi de laboratorul de toxicologie /de medicină legală;
- pacienţii vor fi introduşi într-o incintă special amenajată (chiar mobilă) unde pacienţii cod verde vor face singuri un duş cu săpun şi apă călduţă. Pacienţilor cod roşu şi galben (transportaţi cu targa) li se va face decontaminarea de către personalul special desemnat. Tuturor pacienţilor li se vor înmâna halate curate de unică folosinţă. Acest tip de decontaminare reduce fizic încărcarea chimică lichidă şi produce hidroliză alcalină.

b. Modul de desfăşurare a acţiunilor cu privire la MMSRC la nivelul UPU/CPU al spitalelor de ROL 1 şi 2.

Eveniment chimic acoperit (ECA). În cazul unui eveniment chimic acoperit există o serie de principii care trebuie urmate pentru a putea răspunde adecvat unui asemenea risc, astfel:

- detectarea şi apoi declararea evenimentului chimic;
- activarea planului de acţiune;
- identificarea agentului;
- oprirea accesul la nivelul UPU, cu excepţia pacienţilor critici şi a personalului specializat;
- determinarea şi asigurarea nevoii de protecţie a personalului;
- desemnarea unei echipe cu PPE pentru a asigura triajul şi îngrijirea conform algoritmului ABC a pacienţilor nedecontaminaţi;
- izolarea victimelor nedecontaminate până la decontaminare;
- contactarea rapidă a specialiştilor în arme chimice.

1. Detectarea şi apoi declararea evenimentului chimic acoperit (ECA). Acest lucru presupune recunoaşterea agentului chimic, iniţial clinic, apoi de către personalul specializat şi cu ajutorul detectoarelor. Următorul pas este obţinerea de antidoturi din depozite (pentru agenţii SNC şi cianuri), atunci când există. Se iniţiază terapia pe baza recunoaşterii agentului.

1.1. Situaţii epidemiologice (evenimente santinelă) care pot atrage atenţia asupra existenţei unui ECA:

- accident industrial, în agricultură, de transport;
- apariţia de nori de vapori;
- apariţia de animale şi peşti morţi;
- salvarea victimelor dintr-un spaţiu închis;
- victime multiple cu acuze similare;

Victimele implicate în ECA prezintă următoarele caracteristici:

- miros puternic şi lichide neidentificate pe corp;
- acuze respiratorii asociate cu simptome oculare şi la nivelul membranelor mucoase;
- arsuri chimice şi /sau acuze neurologice;
- acuze cardiace şi chiar stop cardiorespirator - SCR (în descreştere rapidă).

1.2. Declararea evenimentului. Evenimentul santinelă va avea probabil un impact public uriaş, de aceea ECA trebuie declarat formal cu implicarea rapidă a factorilor responsabili de MMSRC.

2. Activarea planului de acţiune. Planul va fi simplu şi cunoscut de tot personalul spitalului de ROL 1 şi 2. Se impun şedinţe de pregătire în care vor fi subliniate punctele importante, precum şi modul de folosire a listelor de control (checklists). Planul va fi repetat cel puţin o dată pe an.

3. Identificarea agentului. Iniţial, diagnosticul se va face clinic, avându-se în vedere şi caracteristicile victimelor, după care se vor consulta experţii toxicologi. Este important să fie determinate riscul la care este supus personalul şi disponibilitatea unui antidot. Ideal ar fi să existe posibilitatea de determinare automată a agentului chimic implicat.

4. Oprirea accesului la nivelul UPU, cu excepţia pacienţilor critici şi a personalului specializat. Această măsură se impune pentru a controla fluxul de victime nedecontaminate, cu excepţia cazurilor în care agentul chimic este benign pentru mediu (clor). Va fi însă permis accesul pacienţilor critici în zona de tratament, unde se va administra doar tratament de salvare a vieţii (atropină, IOT etc.)

5. Determinarea şi asigurarea nevoii de protecție a personalului. Agenții foarte toxici persistenți (VX) necesită costum de protecție individuală întreg tip C, iar cei nepersistenți (sarin, fosgen) impun un PPE parțial (mănuşi din butil şi mască tip M-15). În cazul agenților slab toxici nepersistenți este suficientă o protecție uşoară (mască) sau chiar deloc (în cazul clorului).

6. Asigurarea unei echipe cu PPE pentru a asigura triajul şi îngrijirea pacienților nedecontaminați conform algoritmului ABCDE. O echipă cu PPE trebuie să fie în standby pentru a acorda primul ajutor la locul de decontaminare (la duşuri), unde va realiza managementul CAS. Ariile contaminate vor fi izolate şi marcate corespunzător.

6.1.Principii de decontaminare. Zona de decontaminare (Decon) va fi amplasată în apropierea UPU (spațiul de triaj de la nivelul facilității de ROL 1 sau 2). Personalul pentru Decon va fi desemnat din timp, antrenat şi pus la dispoziție permanent. Echipamentul de Decon trebuie să fie la îndemână şi gata de utilizare, iar aria pentru Decon va deveni operativă în 20-30 de minute. Pacienții se vor deplasa pe fluxul medical într-un singur sens.

Ariile contaminate sunt demarcate clar, cu delimitări fizice în jur şi cu posibilitatea de defluire. În aceste zone accesul pacienților, al personalului şi al mulțimii trebuie strict controlat.

6.2. Procedura de decontaminare. La intrarea în aria Decon se va face triajul inițial de către o echipă (cei mai experimentați) cu PPE complet şi care va stabili categoriile de triaj următoare:

- ***cod negru*** – decedați;
- ***cod roşu*** – necesită de urgență suport ventilator (VM);
- ***cod galben*** – necesită targă, dar nu VM;
- ***cod verde*** – se deplasează singur.

6.3. Pacienții cod roşu

6.3.1. Pacienții care necesită VM imediată. Dacă pacienții sunt intoxicați cu agenți neurotoxici, prima doză de atropină şi oximă se va administra pe seringă automată. Personalul care intubează traheal pacientul (medici ATI/medicină de urgență) vor fi îmbrăcați în PPE complet. Echipa de transport va duce pacientul IOT la aria de Decon, unde va fi decontaminat în timp ce este ventilat manual de salvator. După Decon, pacientul se va transfera în aria decontaminată.

6.3.2. VM în cazul cazurilor multiple. Când se anticipează un număr mare de pacienți care necesită IOT şi VM, se vor desemna arii alternative de îngrijire a pacienților cod roşu şi se va asigura aportul de oxigen (butelii de oxigen). Se va asigura şi o rezervă de aparate de VM şi de personal antrenat, precum şi de baloane autogonflabile care să poată fi uşor decontaminate.

6.4. Pacienții cod galben - care nu necesită VM imediată. Se vor plasa pe tărgi simple, li se va administra antidotul (în cazul agenților SNC), vor fi decontaminați în aria decon, după care vor fi predați celor din aria de tratament.

6.5. Pacienții cod verde (care merg). Vor fi escortați spre duşuri, unde se vor autodecontamina, după care vor fi îndrumați spre ariile de tratament prestabilite pentru acest tip de victimă. Se va administra antidotul, dacă este nevoie, după care se va face consilierea psihologică în cazurile cu reacție anxioasă (PTSD). Vor fi internați în saloane speciale sub observație.

6.6. Tehnica decontaminării pentru agenții SNC. Se va folosi apa şi săpunul şi cu ajutorul unui burete se va freca pielea timp de 5-6 minute cu blândețe. Victimele se vor dezbrăca complet, cu păstrarea intimității sexelor. Personalul de Decon va purta PPE complete şi va fi instruit pentru a recunoaşte alterarea stării pacientului pe parcursul Decon sau va fi paramedic/asistent medical cu specializare în medicină de urgență pentru a putea acorda primul ajutor rapid şi eficace.

6.7. Tehnica decontaminării pentru agenți vezicanți. Inițial se va face o decontaminare "uscată", cu dezbrăcarea completă a victimelor, după care se va trece la decontaminarea umedă la duşuri frecând pielea cu blândețe. Se va insista la irigarea abundentă a ochilor cu apă curată. Se poate folosi soluție 0,5% de hipoclorit (înălbitor) în cazul expunerilor severe ale pielii la nivelul zonelor de Decon.

D. CONCLUZII

În cazul unui atac cu A.C. se impun mai multe măsuri care urmăresc creşterea şanselor de supravieţuire a victimelor şi, totodată, împiedică afectarea personalului medical care intervine la nivelul câmpului de luptă sau pe parcursul diferitelor facilităţi medicale. Principiile sunt următoarele:

- protejarea personalul medical şi a echipamentului;
- îndepărtarea victimelor din mediul contaminat (folosind echipament individual de protecţie);
- desfăşurarea îngrijirii respectând algoritmul ATLS - ABCDE;
- decontaminarea victimei;
- administrarea antidotului, dacă există;
- asigurarea terapiei de suport a funcţiilor vitale;
- monitorizarea apariţiei complicaţiilor;
- evacuarea victimelor (la nevoie) la o facilitate medicală cu competenţe mai mari;
- planificarea şi antrenarea după un plan care să fie parte din planul general al spitalului.
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Determinarea cantitativă a focarelor histonei γH2AX în celulele mononucleare din sângele periferic - biomarker sensibil al leziunilor ADN şi al proceselor de reparare consecutiv expunerilor la radiaţii ionizante

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Rezumat. A fost evaluată fosforilarea histonei H2AX în celulele mononucleare din sânge periferic supus iradierii cu scopul de a determina dacă numărul focarelor histonei γH2AX poate fi utilizat ca o metodă sensibilă de dozimetrie biologică pentru expunerile umane la radiaţii ionizante. În contrast cu majoritatea investigaţiilor radiologice, radioterapia este ideală pentru acest tip de testări deoarece expunerile sunt planificate, bine definite în spaţiu şi timp, şi de scurtă durată iar dozele sunt suficient de mari pentru a putea fi înregistrate prin analiza γH2AX.

În experimentele *in vitro* numărul de focare γH2AX creşte liniar cu doza de iradiere în celulele mononucleare din sângele periferic iradiat *in vitro*. Rezultatele obţinute prin determinări cantitative în celule sanguine mononucleare expuse la radiaţii ionizante *in vitro* indică faptul că focarele γH2AX reprezintă rupturile bicatenare şi pot fi utilizate pentru a determina răspunsul ADN la leziunea radică şi pentru a estima dozele de iradiere. Valori similare de focare γH2AX au fost observate şi consecutiv expunerilor la radiaţii X cu scop diagnostic, sugerând că mulţimea focarelor este independentă de calitatea radiaţiei în domeniul energiilor utilizate în practica medicală. Numărul focarelor γH2AX în sângele periferic poate fi utilizat ca un biomarker sensibil al leziunilor ADN şi al proceselor de reparare consecutiv expunerilor medicale, ambientale, ocupaţionale şi accidentale la radiaţii ionizante atât timp cât dispunem de informaţia privind momentul iradierii.

Cuvinte cheie: radiaţii ionizante, histona γH2AX, dozimetrie biologică

Abstract. Histone H2AX phosphorylation was assessed in peripheral blood mononuclear cells subjected to irradiation in order to determine if the number of histone γH2AX foci can be used as a sensitive method for biological dosimetry in human exposure to ionizing radiation. In contrast to most radiological investigations, radiation therapy is ideal for this type of testing because the exposures are planned, well defined in space and time, and the doses are large enough to be recorded by γH2AX analysis. During *in vitro* experiments the number of γH2AX foci increases linearly with the dose of radiation in irradiated peripheral blood mononuclear cells. Results from quantitative determination of γH2AX foci in blood mononuclear cells exposed to ionizing radiation *in vitro* indicate that γH2AX foci signal double strand fractures and can be used to determine the response to DNA damage and to estimate the radiation doses. Similar values of γH2AX foci were observed in exposure to diagnostic X-rays suggesting that the set of foci is independent of radiation quality and the energy used in medical practice. The number of γH2AX foci in the peripheral blood can be used as a sensitive biomarker of DNA damage and repair processes running medical exposures, environmental, occupational and accidental release of ionizing radiation as long as we have information on irradiation time.

Consideraţii asupra proceselor declanşate de radiaţiile ionizante la nivel molecular

O mare varietate de agenţi lezanţi ai ADN-ului, printre care şi radiaţiile ionizante, este cunoscută ca afectând stabilitatea genomului prin modificarea paternului metilării ADN. Hipometilarea globală a ADN-ului genomic este legată de instabilitatea genomului la nivelul ţesuturilor ţintă. Există o strânsă legătură între metilarea ADN-ului şi modificările histonelor. De aceea, modificările metilării ADN în celulele expuse nu constituie evenimente izolate, ci survin în contextul unor dereglări globale ale cromatinei. Deşi s-a acordat atenţie modificărilor radioinduse ale metilării ADN, histonele, "piese" importante, au fost în general omise. Printre modificările histonelor, fosforilarea histonei γH2AX, o variantă a histonei H2A, în cursul iradierii, este cel mai mult studiată. Histona γH2AX este fosforilată rapid la serina din poziţia 139 în momentul inducerii rupturii bicatenare a ADN-ului şi poate fi efectiv vizualizată la nivelul locusurilor de reparare utilizând anticorpi fosfospecifici[1]. Analiza fosoforilării histonei γH2AX este larg folosită pentru aprecierea amploarei leziunilor ADN-ului celular.

Studiile modificărilor histonelor induse de stresul genotoxic şi implicarea metilării histonelor în producerea transformării neoplazice şi progresiunea cancerului sunt în plină dezvoltare. Fraga şi colaboratorii[2] au arătat că tumorile suferă o pierdere masivă a trimetilării histonei H4-Lys20. Această pierdere, împreună cu hipometilarea AD sunt considerate a fi un marker universal pentru transformarea malignă. Mai mult, trimetilarea histonei H4-Lys20 este importantă pentru coordonarea răspunsului la apariţia leziunilor punctelor de control ale ADN la genul mucegaiurilor. Rolul modificărilor histonelor în transformările radioinduse *in vivo* sunt încă în curs de a fi definite ca şi relaţia dintre modificările radioinduse ale metilării ADN şi modificările histonelor.

Din cauza volumului mic de date din acest domeniu, în prezenta lucrare am investigat efectul expunerilor la radiaţii ionizante asupra acumulării leziunilor ADN şi al alterărilor ADN şi al metilării histonelor utilizând un model experimental clinic.

S-a demonstrat faptul că expunerea fracţionată corporală totală la doze mici conduce la scăderea mai accentuată a trimetilării histonei H4-Lys20 comparativ cu expunerea acută la doză unică[3]. Pierderea metilării histonei H4-Lys20 este însoţită de o scădere semnificativă a metilării globale a ADN, iar acumularea leziunilor ADN este însoţită de persistenţa focarelor de histonă γH2AX la subiecţii supuşi iradierii fracţionate.

Evaluarea *in vivo* a răspunsului biologic la expunerile la radiaţii ionizante constituie o abordare originală a dozimetriei. Radiaţia ionizantă produce un spectru larg de leziuni la nivelul genelor. Printre aceste forme de leziuni se numără: rupturile bicatenare (considerate a fi leziunile cele mai grave ale genomului), formarea aberaţiilor cromozomale şi inducerea apariţiei cancerelor. Unul din stadiile cele mai timpurii ale răspunsului celular la apariţia rupturilor bicatenare este fosforilarea unei variante de histonă, şi anume γH2AX. Prin utilizarea unui anticorp marcat fluorescent, specific pentru forma fosforilată γH2AX şi microscopie de fluorescenţă, se pot vizualiza focare discrete în nuclei, la nivelul rupturilor bicatenare. Formarea şi dispariţia focarelor γH2AX după iradiere au fost măsurate pentru prima dată pe culturi de fibroblaşti umani expuse la doze de radiaţii X de nivelul 1 mGy, iar apariţia focarelor s-a observat a creşte liniar cu doza. Această metodă bazată pe formarea γH2AX poate fi utilizată ca dozimetru biologic pentru diagnosticul expunerilor la radiaţii ionizante.

SCOPUL LUCRĂRII : măsurarea variaţiilor cantitative ale leziunilor ADN pe baza numărului de focare γH2AX care apar în celulele mononucleare din sângele periferic şi elaborarea, pe această bază, a unei metode de estimare a dozei de iradiere primite de pacienţii adulţi supuşi radioterapiei.

MATERIAL ŞI METODĂ

Au fost recoltate 20 de eşantioane a 5 ml de sânge venos din regiunea antecubitală, după obţinerea consimţământului scris al pacienţilor. Pe 10 eşantioane s-au efectuat determinări ale numărului de focare γH2AX fără a fi expuse la iradiere, pe celelalte 10 s-au efectuat determinări ale numărului de focare γH2AX la circa 1 oră de la expunerea la radiaţii.

A fost constituit un lot de 20 de pacienţi (6 bărbaţi şi 14 femei cu vârsta medie de 59,6 ani) diagnosticaţi cu diferite forme de cancer, care urmau să fie supuşi radioterapiei pe câmpuri toracale şi abdominale mari. 3 pacienţi sufereau de cancer colo-rectal, 6 de cancer ovarian, 10 de cancer de sân, 1 de cancer pulmonar. Toţi pacienţii aveau indicaţie terapeutică pentru iradiere. Dintre aceştia, 8 pacienţi au avut câmpuri abdominale mari şi 2 - câmpuri toracale.

Un număr de 8 pacienţi au efectuat tratamente cu citostatice cu aproximativ 3 săptămâni anterior determinărilor.

Sângele venos a fost recoltat înaintea expunerii la radiaţii şi a fost incubat timp de 1 oră pentru obţinerea valorilor de referinţă ale focarelor de γH2AX.

10 probe au fost expuse la un fascicul de radiații gama cu intensitatea de 10 MeV, la o doză absorbită de 1 Gy, utilizându-se acceleratorul liniar Varian Clinac 2300 C/D, aflat în dotarea laboratorului de terapie cu energii înalte.

Leucocitele au fost separate prin centrifugare, fixate şi colorate pentru evidențierea expresiei γH2AX. Focarele γH2AX au fost vizualizate prin imunofluorescență. Datele au fost analizate utilizând regresia liniară şi testul indicelui de dispersie.

După confirmarea unei creşteri marcate a focarelor γH2AX în aceste probe pilot, am colectat probe de la ceilalți 9 pacienți.

Mononuclearele din sângele periferic au fost izolate prin centrifugare în gradient de Ficoll cu Histopaque 107, întinse pe frotiuri şi lăsate la uscat 2 minute.

Colorația imunohistochimică şi microscopia în fluorescență

Frotiul cu celule mononucleare a fost fixat 10 minute în paraformaldehidă la temperatura camerei în metanol 100 % la -20$^{o.}$. Frotiurile au fost blocate în soluție fosfat salină cu albumină timp de 2 minute, fiecare la temperatura camerei. Probele au fost incubate cu anticorp murin anti γH2AX. Celulele au fost apoi spălate cu soluție salină tamponată cu fosfat timp de 2 minute. Imaginile în fluorescență au fost obținute utilizând microscopul cu fluorescență Nikon TE3000. Pentru analiza cantitativă focarele au fost numărate cu ochiul liber, utilizând un obiectiv cu magnitudinea 100x, observându-se numai celulele cu aspect de limfocit.

Valorile de referință ale numărului de focare γH2AX per unitate de doză de iradiere din fiecare celulă au fost calculate pentru probele de sânge iradiate *in vitro* la 1 oră şi la 24 de ore după expunere prin calcularea mediei numărului de focare ale tuturor pacienților. Numărul de focare din fiecare probă recoltată după expunere a fost împărțit la valoarea de referință pentru intervalul de timp corespunzător, cu scopul determinării dozei de iradiere primite în timpul şedinței de radioterapie.

Rezultate şi discuții

După expunere *in vitro* a limfocitelor recoltate, nivelul histonei γH2AX a crescut semnificativ. Acest fenomen poate fi observat după expunerea *in vitro* a limfocitelor recoltate de la pacienți expuşi la radiație ionizantă, când nivelul histonei γH2AX a crescut semnificativ. Acest fenomen poate fi observat prin creşterea intensității intranucleare a semnalului de fluorescență emis de histona γH2AX (fig.1). Prezența γH2AX din figura 1 (centru), evidențiată prin cuplarea cu anticorpul monoclonal primar şi secundar cuplat cu ITCF (izotiocianat de fluoresceină) evidențiază un număr de peste 100 de focare γH2AX.

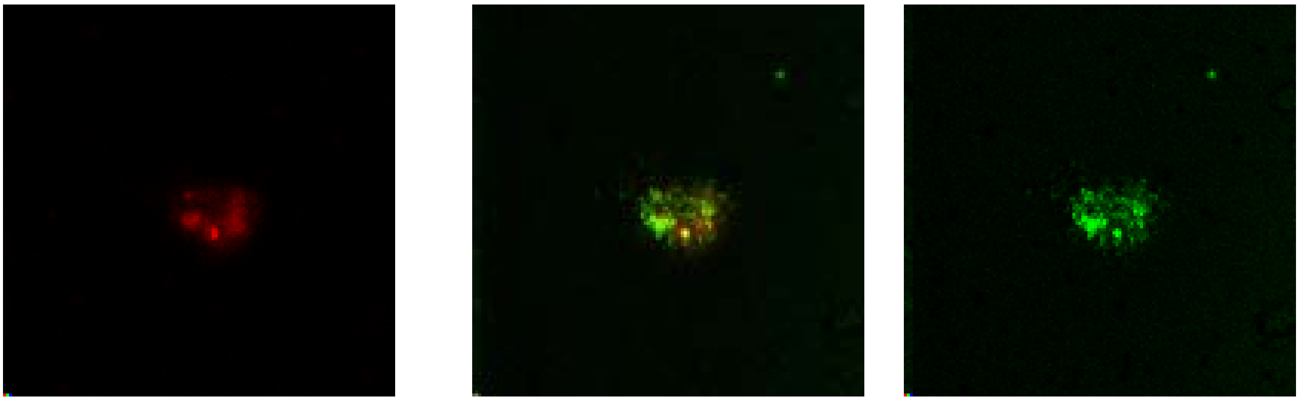

Contracolorație PI (propidium iodide) — Colorație pt. γH2AX — Imagine suprapusă

Fig. 1. Creşterea intensității intranucleare a semnalului de fluorescență emis de histona γH2AX după expunerea probei la radiație γ cu energia de 10 MeV, la doza de 1 Gy.

După tratament, limfocitele au fost resuspendate în mediul de cultură pentru încă 24 de ore. La 24 de ore după expunere, nivelul de γH2AX a scăzut semnificativ, nefiind observate decât câteva celule γH2AX pozitive. Fenomenul se datorează intervenţiei mecanismelor de reparare a rupturilor ADN (fig. 2).

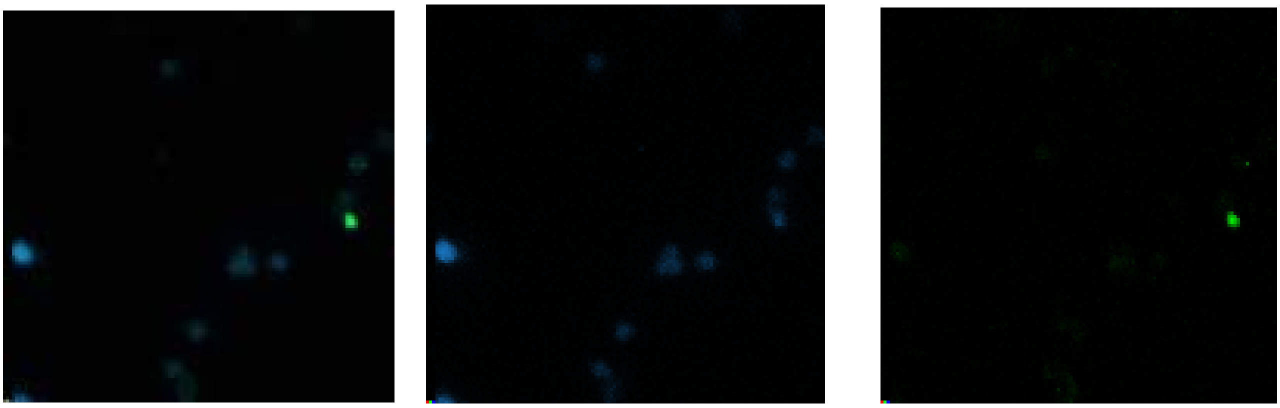

Coloraţie DAPI (4',6-diamidino-2-phenilindol) (specifică pentru nucleu)

Coloraţie pentru γH2AX

Imagine suprapusă

Fig. 2. Limfocite marcate pentru γH2AX, resuspendate, la 24 de ore după iradiere.

Rezultatele evidenţiază faptul că determinarea cantitativă a focarelor γH2AX la nivelul limfocitelor circulante reprezintă o metodă sensibilă de detecţie a inducerii şi reparării rupturilor ADN bicatenare la doze de iradiere cu relevanţă clinică. Cinetica focarelor γH2AX demonstrează că datele obţinute pe probele de sânge periferic pot fi utilizate pentru screening-ul deficienţelor de reparare a rupturilor ADN bicatenare şi poate constitui un factor predictiv al radiosensibilităţii.

Cinetica scăderii numărului focarelor γH2AX în diferite ţesuturi este similară, în pofida răspunsurilor clinice clar diferite între ţesuturi. Radiosensibilitatea diferită între ţesuturi nu are la bază diferenţe cu specificitate de ţesut în ceea ce priveşte repararea leziunilor bicatenare. Mai degrabă ar fi diferenţe în cascada evenimentelor ulterioare procesului de reparare.

Numărul focarelor γH2AX după iradierea *in vitro* a probei a crescut liniar cu doza de radiaţii în celulele mononucleare din sângele periferic. Numărul mediu de focare γH2AX a fost de 29,3 focare/celulă/Gy ± 0,80 (eroarea standard a mediei) şi de 28,2 focare/celulă/Gy ± 0,9, şi a fost determinat prin utilizarea analizei regresiei liniare a probelor de sânge expuse la fotoni gama cu energia de 10 MeV. Numărul mediu de focare γH2AX la pacienţii netrataţi a fost de 0,06 focare/celulă/Gy ± în celulele mononucleare din sângele periferic.

Numărul focarelor γH2AX după expunerea la radiaţii ionizante

Focarele γH2AX radioinduse au fost detectate în probele iradiate după circa 1 oră de la expunerea la radiaţii. Toate focarele γH2AX coexistau cu prezenţa altor proteine de răspuns al ADN la iradiere. Numărul de focare/celulă a fost de 8-10 ori mai mare în probele recoltate la 5 minute (0,52 focare/celulă ± 0,02) comparativ cu cele recoltate înaintea expunerii (0,06 focare/celulă ± 0,01).

Numărul focarelor a scăzut după 2-3 ore de la expunere, indicând iniţierea proceselor de reparare. Numărul mediu al focarelor normalizat la 5 minute, în probele de sânge expuse, a scăzut similar, într-o manieră dependentă de doză. În toate cazurile, aproximativ jumătate din focarele induse au dispărut în decurs de aproximativ 3 ore de la expunerea la radiaţii.

Nu s-au observat variaţii mari între indivizi în ceea ce priveşte valorile de fond ale numărul iniţial al focarelor. Numărul de focare γH2AX în probele neiradiate a fost semnificativ mai mic faţă de cele iradiate, ceea ce indică faptul că numărul de focare γH2AX reflectă expunerea la radiaţii.

Comparativ cu numărul focarelor γH2AX din probele neexpuse la iradiere, numărul focarelor evidenţiate în probele de sânge iradiate a fost de aproximativ 2-3 ori mai mare, factor ce ar corespunde unei doze de 200 cGy, adică valoarea fracțiunii de doză utilizată în protocoalele de tratament uzuale. Media a fost de 0,10 focare/celulă.

Răspunsul inițial la formarea rupturilor catenare, în special a celor bicatenare, este fosforilarea rapidă a serinei din poziția 139 a histonei γH2AX în mediul cromatinian care înconjoară ruptura ADN bicatenară. În câteva minute după expunerea la radiație fosforilarea γH2AX poate fi observată la nivelul focarelor de reparare utilizând anticorpi fosfospecifici. Determinarea cantitativă a focarelor în care apare histona γH2AX fosforilată constituie o metodă sensibilă şi sigură de măsurare a leziunilor ADN radioinduse. Am aplicat metoda pentru estimarea gradului de lezare a ADN în limfocitele martor şi cele expuse. Expunerea acută la 1 Gy a condus la o creştere semnificativă de 3,3 ori a focarelor γH2AX per celulă ($P<0,05$).

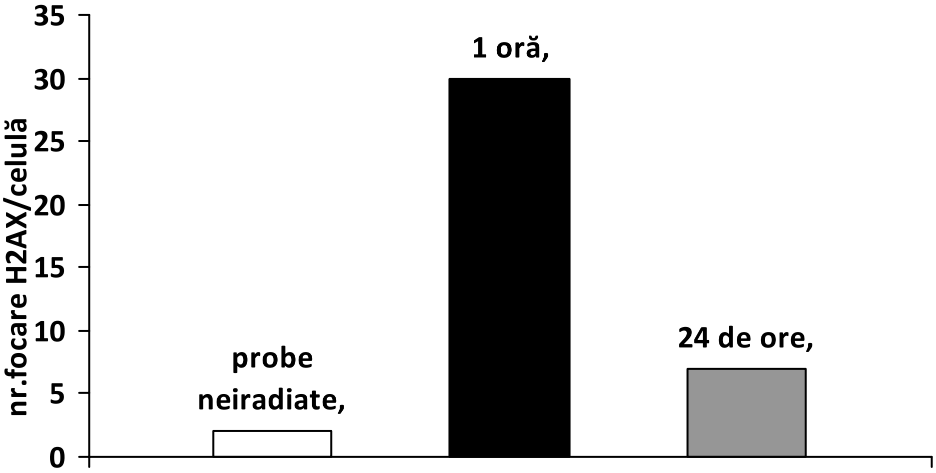

Fig. 3. Acumularea focarelor γH2AX în limfocitele expuse la radiații ionizante.

Lucrarea sprijină ipoteza conform căreia numărul focarelor γH2AX în sângele periferic poate fi utilizat ca un biomarker sensibil al leziunilor ADN şi al proceselor de reparare consecutiv expunerilor medicale, ambientale, ocupaționale şi accidentale la radiații ionizante atât timp cât dispunem de informația privind momentul iradierii. Analiza poate fi utilă ca dozimetru biologic în cazuri de expuneri medicale în care dozimetria fizică nu reuşeşte să determine nivelul de expunere. Analiza focarelor γH2AX pe probe prelevate de la pacienți neoplazici poate furniza informații despre capacitatea lor sistemică de reparare a leziunilor bicatenare ale ADN, care poate constitui un indicator util al răspunsului la radioterapie.

CONCLUZII

Numărul de focare γH2AX creşte semnificativ în celulele mononucleare din sângele periferic după expunerea la radiații ionizante.

Focarele γH2AX din celulele mononucleare periferice reprezintă rupturile bicatenare şi pot fi utilizate pentru a determina răspunsul ADN la leziunea radică şi pentru a estima dozele de iradiere.

Mulțimea focarelor este independentă de calitatea radiației în domeniul energiilor utilizate în practica medicală.

Restrângerea analizei la o perioadă scurtă de timp acoperă în principal componenta rapidă a procesului bifazic de reparare, ceea ce poate să împiedice decelarea diferențelor în privința componentei lente şi a numărului focarelor reziduale.

Rămâne de studiat dacă numărul focarelor reziduale poate fi utilizat ca o metodă de evaluare a capacității de reparare a rupturilor bicatenare.

Tehnica posedă o înaltă sensibilitate comparativ cu metodele biodozimetrice standard: pot fi detectate doze mici de 0,6 cGy.

Testul prezintă o serie de limitări: acoperă o perioadă scurtă de timp de la momentul iradierii, nu poate fi automatizat, poate fi influențat de expunerea prealabilă la alți agenți genotoxici (radioterapie, chimioterapie pentru boli neoplazice).

Numărul focarelor γH2AX în sângele periferic poate fi utilizat ca un biomarker sensibil al leziunilor ADN şi al proceselor de reparare consecutiv expunerilor medicale, ambientale, ocupaționale şi accidentale la radiații ionizante atât timp cât dispunem de informația privind momentul iradierii.

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Identificarea unor tulpini de *Bacillus anthracis* prin tehnica Real Time PCR

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Rezumat. Bacteriile sunt agenţi biologici care pot fi uşor multiplicaţi, stocaţi, condiţionaţi ca arme şi diseminaţi. *Bacillus anthracis*, alături de alţi agenţi biologici identificaţi ca reprezentând un potenţial ridicat de ameninţare pentru trupe sau populaţia civilă, reprezintă una dintre speciile de microorganisme favorite utilizate pentru fabricarea de arme biologice sau în scopuri bioteroriste. *Bacillus anthracis* este o bacterie de formă bacilară, care se colorează Gram pozitiv, este virulentă, toxigenă şi sporogenă, extrem de rezistentă în mediul extern, şi care poate fi cu uşurinţă multiplicată, calităţi care îl clasează în topul agenţilor microbiologici utilizaţi în bioterorism. Metodele de studiu molecular folosite pentru identificarea microorganismului *Bacillus anthracis* ţintesc fragmente din genele ce codifică sinteza capsulei şi a toxinei.

Pentru identificarea rapidă a tulpinilor de *Bacillus anthracis* s-au folosite tehnici moleculare (extracţii ADN, amplificare Real Time PCR - Polymerase Chain Reaction). Au fost optimizate metodele de extracţie a ADN-ului bacterian folosind tulpina bacteriană vaccinală *Bacillus anthracis* 1190 R. Tulpinile de *Bacillus anthracis* au fost cultivate pe medii uzuale (geloză nutritivă, geloză sânge). După cultivare au fost prelevate 1-2 colonii, din care a fost extras ADN-ului bacterian. ADN-ul obţinut a fost utilizat pentru amplificarea prin Real Time PCR a unui fragment ce aparţine genei ce codifică pentru exotoxină.

Cuvinte cheie: agenţi biologici, *Bacillus anthracis*, Real-Time PCR

Abstract. Bacteria are biological agents that can be easily multiplied, stored and conditioned like weapons and disseminated. *Bacillus anthracis*, represents, one of the favorite species of microorganisms for biological attacks and bioterrorism together with other biological agents identified like having high threat potential.

***Bacillus anthracis* it is a virulent, toxic and sporegen bacteria, it is also very resistant in to the extern environment, being so easy to multiplied. All those strength points is classifying this bacteria in to the top of the microbiological agents used in bio terrorism.**

Molecular research methods used to identify *Bacillus anthracis* are targeting fragments of genes that are responsible in codify the synthesis of the capsule and toxin.

For fast identification of *Bacillus anthracis* stem are used molecular techniques (DNA extractions, Real Time PCR - Polymerase Chain Reaction) amplification Have bean optimized DNA bacterian extraction methods using vaccinal bacteria strains. *Bacillus anthracis* strains are cultivated in usual environment (nutritive gels, blood gels). After cultivation, are prevailed 1-2 colonies used for DNA bacterial extraction. The DNA obtained is used for Real Time PCR amplification fragment belonging to the gene that codifies for exotoxin.

Introducere

Bacteriile sunt agenţi biologici care pot fi relativ uşor multiplicaţi, stocaţi, condiţionaţi ca arme biologice şi diseminaţi. *Bacillus anthracis* reprezintă una dintre speciile de microorganisme favorite pentru atacuri cu arma biologică şi pentru bioterorism. Bioterorismul este o componentă a terorismului şi poate fi definit ca folosirea sau ameninţarea cu folosirea a agenţilor biologici (etiologici) ai bolilor infecţioase (virusuri, bacterii, paraziţi şi fungi, ai toxinelor acestora, toxine vegetale, bioregulatori – compuşi organici naturali ai celulelor organismelor vii care reglează diverse procese naturale), fapt care poate induce şi răspândi neliniştea, teama, frica, teroarea în populaţia unei ţări, urmărind scopuri politice, ideologice, religioase, financiare sau interese personale.

Agenţii microbieni care pot fi utilizaţi în atacurile teroriste au fost grupaţi în funcţie de posibilităţile de folosire, precum şi de potenţialul impactului asupra sănătăţii publice, şi anume:

- uşurinţa de a fi folosiţi ca armă biologică şi uşurinţa de stocare şi diseminare;
- transmisibilitatea de la om la om şi mortalitatea;
- impactul asupra sistemului de servicii de sănătate publică;
- potenţialul de a produce panică publică şi perturbări sociale;
- pregătirile speciale necesare pentru a realiza un răspuns efectiv al asistenţei publice.

Bazat pe aceste criterii, *Bacillus anthracis* este considerat a fi cea mai importantă ameninţare ca armă biologică. Armele biologice şi cu toxine, deşi sunt formal interzise prin legislaţia internaţională (Biological and Toxin Weapons Convention BTWC 1972), reprezintă un pericol real, nu numai din perspectiva unui război biologic, ci şi a atacurilor teroriste „bio-chem”. *Bacillus anthracis* este o bacterie patogenă, virulentă, toxigenă şi sporogenă. Rezistenţa în mediul extern precum şi uşurinţa cu care poate fi multiplicată sunt principalele atuuri care o clasează în topul agenţilor microbiologici utilizaţi în scopuri bioteroriste. Convenţia confirmă faptul că *Bacillus anthracis,* virusul variolic şi toxina botulinică sunt agenţii biologici cei mai agresivi şi cu cea mai mare probabilitate de a fi întrebuinţaţi ca armă biologică sau în scopul unor atacuri bioteroriste. Antraxul a şi fost implicat în recentele evenimente internaţionale de după atacul terorist din 11 septembrie 2001 (în SUA şi în UE).

Medicina militară se află în prima linie de apărare contra armelor biologice, cu forţe şi mijloace specifice, în vederea stabilirii diagnosticului, prevenirii contaminărilor, elaborării tratamentului şi recuperarea bolnavilor. Cercetarea ştiinţifică medico-militară în domeniul protecţiei contra armelor biologice trebuie să orienteze şi să coordoneze efortul naţional pentru menţinerea sanătăţii trupelor, populaţiei, animalelor, plantelor şi a mediului înconjurător.

Lista agenţilor biologici cu posibile implicaţii în bioterorism cuprinde un număr mare de germeni, dintre care amintim: *Poxvirus variolae, Yersinia pestis*, toxina B de la *Clostridium botulinum*, *Francisella tularensis,* Filovirusurile (Febra Hemoragică - Ebola şi Marburg), Adenovirusurile (Lassa şi Juniu) etc.

Infecţia cu *Bacillus anthracis* produce antraxul, o boală infecţioasă comună mai multor specii de animale şi transmisibilă la om. Clinic se caracterizează prin evoluţie acută, febră, tulburări circulatorii şi digestive, urmate de moarte. Recunoscut de oamenii de ştiinţă de foarte mult timp ca posibilă armă biologică, antraxul nu este şi o armă ideală. *Bacillus anthracis* nu este foarte înalt patogenic, fiind necesară o doză infectantă minimală de spori (circa 8 000 per om) pentru a produce infecţia. În mod obişnuit, infecţia cărbunoasă nu se transmite de la persoană la persoană decât în circumstanţe excepţionale.

Bacillus anthracis are o structură antigenică complexă, posedând un antigen somatic polizaharidic cuplat cu un peptid, iar formele capsulate conţin un antigen capsulat polipeptidic, compus din acid poli d-glutamic, acesta reprezentând un factor de virulenţă prin activitatea antifagocitară. Capsula, cel mai important factor al virulenţei la *Bacillus anthracis,* are o acţiune antifagocitară, dar asigură totodată o protecţie relativă şi faţă de factorii umorali. La virulenţa germenului aduc o contribuţie importantă lecitinaza, proteazele, colagenaza şi hemolozina.

Bacillus anthracis are un genom de tip ADN, fiind alcătuit din două plasmide, pXO1 şi pXO2. Plasmida *pXO1* este purtătoare de toxină, are dimensiunea de 184,5 kpb şi codifică genele care produc exotoxinele secretate. Complexul genă-toxină este compus din antigenul protectiv, factorul letal, factorul edemaţiant. Cele 3 componente ale exotoxinei se combină pentru a forma toxine binare.

Plasmida cea mai mică purtătoare de capsulă, *pXO2*, are dimensiunea de 95,3kpb şi codifică 3 gene (*capB, capA, capC*) implicate în sinteza capsulei poliglutamidice. Exotoxinele inhibă răspunsul imun împotriva infecţiei, în timp ce capsula inhibă fagocitoza la formele vegetative de *Bacillus anthracis*. Metodele de studiu molecular folosite pentru identificarea agentului *Bacillus anthracis* ţintesc fragmente din genele ce codifică sinteza capsulei şi a toxinei.

Material şi metodă

Tulpini bacteriene. În acest studiu a fost utilizată tulpina vaccinală *Bacillus anthracis* 1190R. Aceasta a fost însămânțată pe medii de cultură uzuale (geloză-sânge) şi incubată la temperatura de 37°C, timp de 36 ore, până la atingerea fazei staționare de creştere (coloniile se prezintă nehemolizate, dar după 24 de ore se poate produce o zonă de hemoliză beta).

Extracția ADN plasmidial. Pentru extracția ADN-ului bacterian au fost folosite două metode:

a. inactivare pe thermoblock la 100°C, timp de 15 minute, cu volumul de lucru cuprins între 1-3 ml cultură bacteriană;

b. extracția cu kitul SNAP MiniPrep Kit (Invitrogen), folosind acelaşi volum de cultură bacteriană la care au fost adăugate buffere din kit, coloanele SNAP MiniPrep, protocolul suferind mici modificări. ADN-ul bacterian obținut a fost eluat în 50µl buffer TE sau apă distilată sterilă. Acesta poate fi păstrat astfel la -80ºC.

Electroforeza orizontală în gel de agaroză 2% în tampon de elecroforeză - Tris Acetat EDTA (TAE) 1X, *în* sistem submers pentru vizualizarea şi verificarea integrității ADN bacterian.

Amplificarea prin Real Time PCR. Pentru amplificarea ADN-ului extras a fost utilizat kitul Quantification of Bacillus anthracis advanced kit (*Primerdesing) folosind o* sondă de tip „TaqMan“, detecția amplificării realizându-se pe canalul FAM. Mix-ul a conținut: 2x Precision MasterMix - 10µl, Endogenous ACTB primer/probe mix - 1µl, RNAse/ DNAse free water - 4µl, volumul final fiind de 15µl. La mix-ul PCR se adaugă 5µl de ADN. Amplificarea probelor a fost realizată pe un SmartCycler (Cepheid). Condițiile de amplificare au fost în acord cu indicațiile kit-ului: PrimerDesing 2x Precision MasterMix: UNG tratament -15min / 37 ºC, activarea enzimei - 10 min / 95 ºC, denaturarea 10s/ 95 ºC, colectarea datelor 60s/ 60 ºC pentru 50 cicluri.

Spectrometrie de masă. Tulpina bacteriană *Bacillus anthracis* 1190R a fost cultivată pe medii uzuale (geloză-sânge) 24 ore. A fost prelevată o colonie (5-10 mg material biologic) şi suspendată într-un volum de 300 µl apă distilată sterilă. Pentru delipidare s-a folosit etanol 70%, iar pentru extracția proteinelor ribozomale 16S s-a adăugat acid formic 70%. Resuspendarea peletului obținut s-a făcut în acetonitril. Proba astfel prelucrată a fost etalată în godeuri pe placa de analiză, peste care s-a adăugat matrix-ul şi a fost analizată cu spectrometrul de masă MALDI TOF Microflex LT 20 Bruker.

Rezultate şi discuții

Prima etapă pentru utilizarea tehnicilor moleculare în identificarea tulpinilor de *Bacillus anthracis* a fost optimizarea tehnicii de lucru. Tulpina *Bacillus anthracis* 1190R a fost însămânțată pe medii de cultură bacteriologice uzuale, în vederea obținerii culturilor utilizate în tehnicile moleculare. Pentru extracția ADN-ului bacterian s-au folosit două metode, utilizând atât kitul de extracție (Invitrogen), cât şi metoda prin inactivare (fig.1).

Menționăm faptul că în procedurile NATO de identificare a agenților biologici se prevede ca detecția să fie confirmată şi prin tehnici moderne, PCR, ELISA etc., pentru evitarea unui diagnostic microbiologic fals-negativ sau fals-pozitiv.

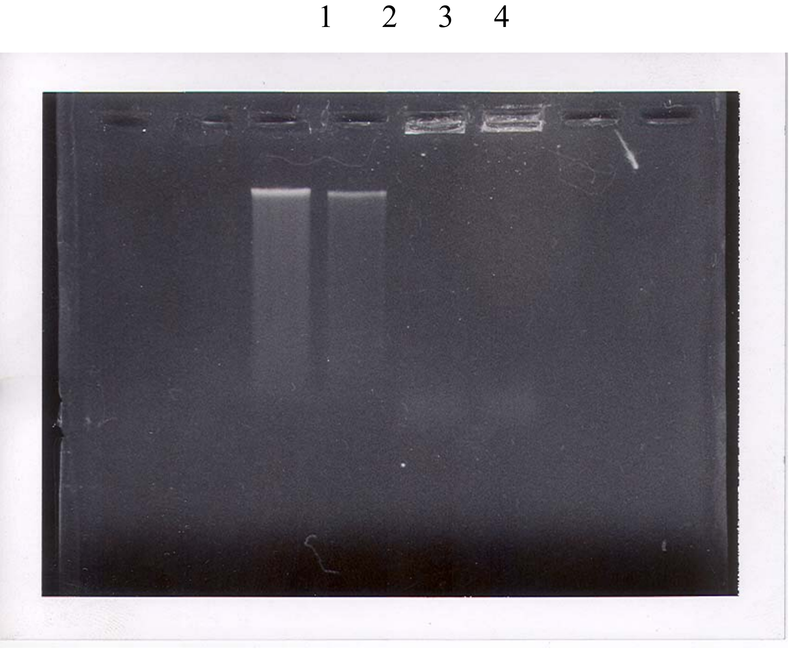

Fig. 1. Electroforeză în gel de agaroză (2 %) a ADN-ului bacterian
Linia 1- 4 : probă *Bacillus anthracis* 1190R.

ADN-ul bacterian obținut a fost amplificat prin tehnica Real Time PCR, utilizând primerii specifici genomului de *Bacillus anthracis*, ce amplifică un fragment care aparține genei care codifică exotoxina. Rezultatul reacției Real Time PCR a fost vizualizat în timp real cu ajutorul software-ului care însoțeşte sistemul de amplificare al aparatului, prin monitorizarea evoluției fluorescenței după fiecare ciclu de amplificare (figurile 2 şi 3).

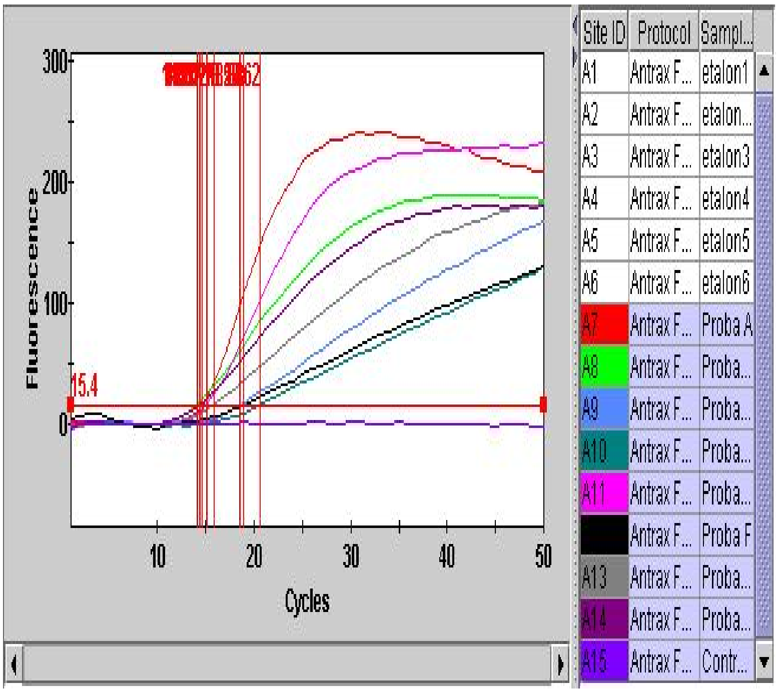

Site ID	Protocol	Sampl...
A1	Antrax F...	etalon1
A2	Antrax F...	etalon...
A3	Antrax F...	etalon3
A4	Antrax F...	etalon4
A5	Antrax F...	etalon5
A6	Antrax F...	etalon6
A7	Antrax F...	Proba A
A8	Antrax F...	Proba...
A9	Antrax F...	Proba...
A10	Antrax F...	Proba...
A11	Antrax F...	Proba...
	Antrax F...	Proba F
A13	Antrax F...	Proba...
A14	Antrax F...	Proba...
A15	Antrax F...	Contr...

Fig. 2. Plot-urile de amplificare (nr.cicluri vs.unități fluorescență) - *Bacillus anthracis* tulpina 1190R.

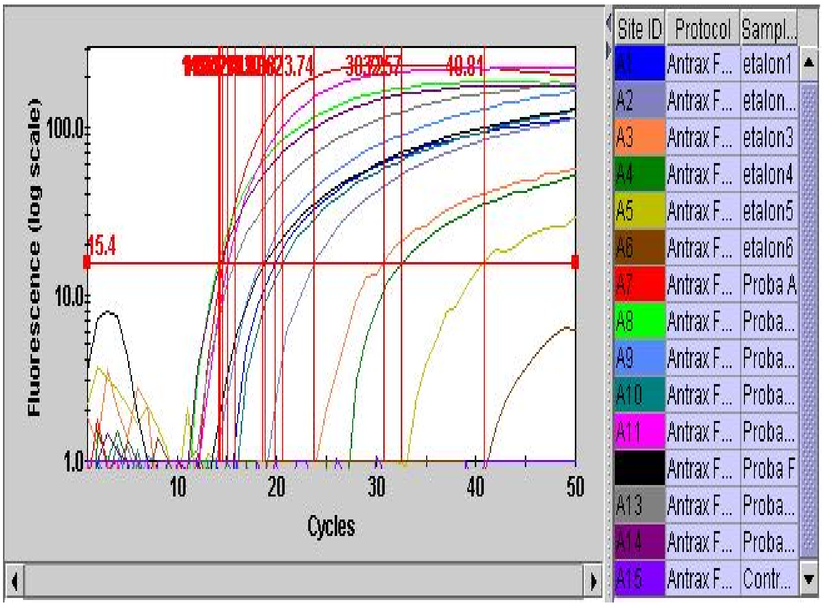

Site ID	Protocol	Sampl...
A1	Antrax F...	etalon1
A2	Antrax F...	etalon...
A3	Antrax F...	etalon3
A4	Antrax F...	etalon4
A5	Antrax F...	etalon5
A6	Antrax F...	etalon6
A7	Antrax F...	Proba A
A8	Antrax F...	Proba...
A9	Antrax F...	Proba...
A10	Antrax F...	Proba...
A11	Antrax F...	Proba...
	Antrax F...	Proba F
A13	Antrax F...	Proba...
A14	Antrax F...	Proba...
A15	Antrax F...	Contr...

Fig. 3. Plot-urile de amplificare (nr.cicluri vs. unități de fluorescență) în format logaritmic *Bacillus anthracis* tulpina 1190R etalon şi probe.

Pentru validarea rezultatelor obținute prin metode moleculare, au fost realizate analize prin spectrometrie de masă tehnologia MALDI-TO. Spectrele proteinelor ribizomale 16S (extrase din probele luate în studiu) au fost comparate cu spectrele stocate în banca de date a aparatului Microflex LT 20, rezultatele obținute demonstrând faptul că tulpina analizată aparține genului *Bacillus,* specia *Bacillus anthracis* (fig. 4).

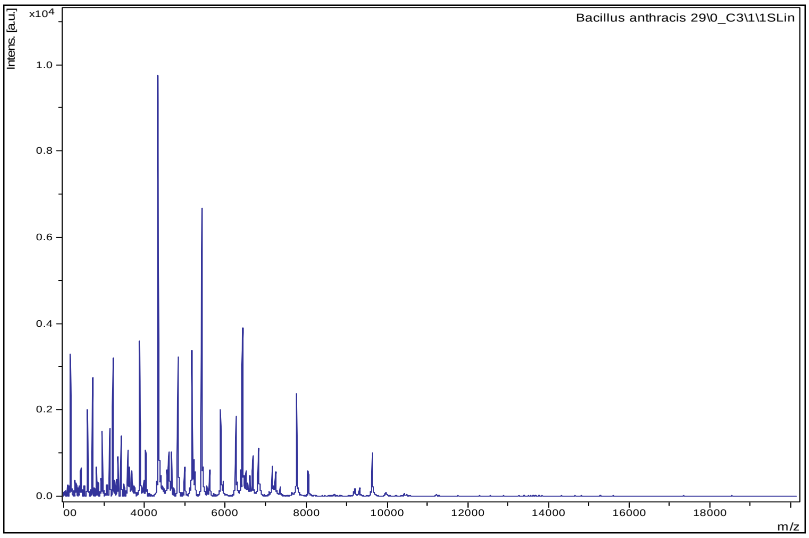

Fig. 4. Spectrul de masă la *Bacillus anthracis* – tulpina 1190R (Microflex LT20).

Concluzii

Tehnicile de biologie moleculară sunt folosite în prezent pentru caracterizarea izolatelor bacteriene în diagnosticul microbiologic de laborator, în cercetare, precum şi în domeniul protecției medicale contra agenților biologici.

Tehnicile moleculare constituie în prezent un procedeu rapid şi specific de identificare şi diagnostic a infecțiilor bacteriene, fungice şi virale, revoluționând metodologiile de laborator utilizate în detecția şi identificarea agenților biologici.

Capacitatea de discriminare intraspecifică crescută a tehnicilor de diagnostic moleculare poate furniza informații prețioase despre circulația anumitor tulpini în contexte epidemiologice particulare.

Analiza moleculară a genomului bacterian, folosind reacții de tip Real Time PCR a fost utilizată în ultimul timp de specialişti pentru caracterizarea tulpinilor bacteriene de *Bacillus anthracis* izolate de la om sau animale în timpul evoluției diverselor focare epidemice.

Extracția ADN-ului bacterian de *Bacillus anthracis* s-a realizat folosind atât kit-ul SNAP MiniPrep Kit (Invitrogen), cât şi prin izolare directă (fără purificare) a ADN prin inactivarea bacteriei pe thermoblock.

Analizele efectuate prin tehnici moleculare Real Time PCR şi prin spectrometrie de masă tehnologia MALDI-TOF au confirmat că tulpina bacteriană studiată a fost *Bacillus anthracis* 1190R.

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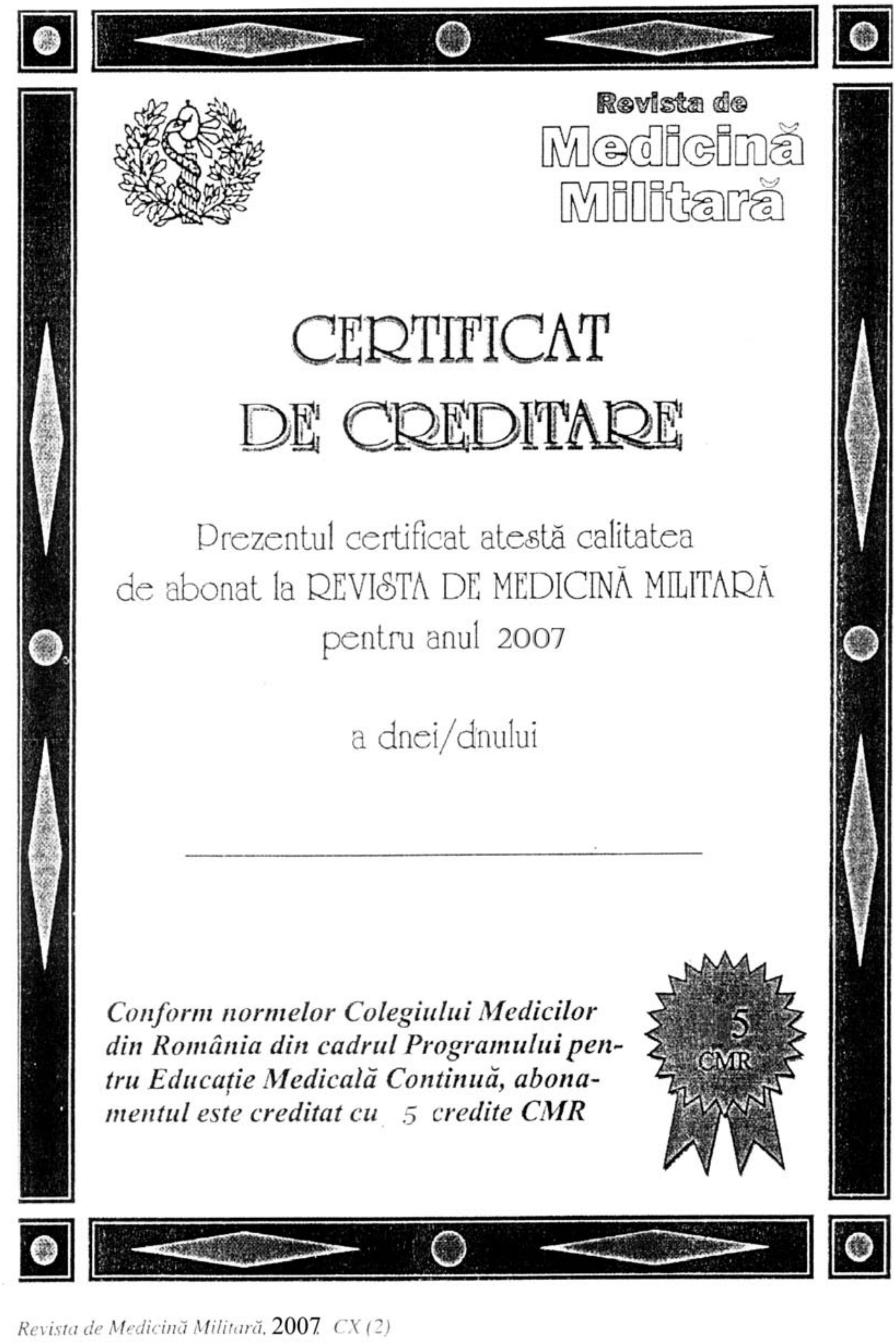

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Stresul de forfecare endotelial al peretelui vascular

("shear stress")

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Rezumat. Rolul principal al peretelui arterial este de a rezista presiunilor hemodinamice: presiunea transversală, dată de presiunea sangvină, şi presiunile tangențiale, produse de forțele de forfecare ale sângelui asupra endoteliului. O tensiune de forfecare notată este definită ca un stres care se aplică paralel sau tangențial la un substrat, spre deosebire de o tensiune normală, care este aplicată perpendicular. Endoteliul vascular este expus la un stres hemodinamic generat de fluxul sangvin cunoscut sub numele de stres de forfecare al peretelui vascular. Stresul de forfecare este definit ca forța exercitată pe unitatea de suprafață a peretelui vascular de către fluxul sangvin şi depinde de vâscozitatea sângelui şi de velocitatatea fluxului sangvin. Valoarea stresului de forfecare al peretelui vascular este exprimată în dynes/cm^2, valorile fiziologice fiind în sistemul venos în intervalul 1-6 dynes/cm^2, în timp ce în sistemul arterial sunt cel puțin de 10 la 15 dynes/cm^2.

Cuvinte cheie: placă de aterom, endoteliu, leziune aterosclerotică, stenoză, stent endovascular

Abstract. The main role of the arterial wall is to resist haemodynamic pressures: transverse pressure, due to blood pressure and tangential pressures, produced by shear forces of blood on the endothelium. A shear pressure, observed, is defined as a stress applied - parallel or tangential - to a material, unlike a normal pressure, that is applied perpendicular. Vascular endothelium is exposed to hemodynamic stress generated by blood flow known as the vascular wall shear stress. Shear stress is defined as the force exerted per unit area of the vessel wall by blood flow and depends on the blood viscosity velocity and blood flow. The amount of vessel wall shear stress is expressed in dynes/cm^2, physiological values being between 1-6 dynes/cm^2 in the venous system, while in the arterial system these values are at least 10 to 15 dynes/cm^2.

Keywords: atheroma plaque, endothelium, atherosclerotic lesions, stenosis, endovascular stent

Date generale

Circulația sângelui în sistemul vascular poate fi înțeleasă mai bine pe baza legilor fizice ale mecanicii fluidelor, care explică curgerea unui fluid cu proprietăți caracteristice într-un sistem de conducte. Parametrii hemodinamici care trebuie măsurați şi caracterizați din punct de vedere fizic țin atât de fluidul circulant (densitate, vâscozitate, presiune, viteza de circulație, flux sangvin), cât şi de vasele sangvine (diametrul vascular, elasticitate, complianța vasculară). Aplicarea modelelor matematice asupra curgerii sangvine este însă limitată de faptul că parametrii care caracterizează cele două componente sunt în permanență modificați prin intervenția mecanismelor de reglare ale organismului.

La curgerea unui lichid printr-un tub se constată că straturile nu se deplasează cu aceeaşi viteză. Stratul de lângă perete nu se mişcă, deci are viteza zero, pe când cel din centru (mijlocul tubului) are viteza maximă. Între margine şi mijloc vitezele cresc printr-o serie de valori intermediare. Fluxul laminar apare la viteze mici de circulație, cum sunt cele obținute în condiții fiziologice. Curgerea se face în straturi paralele subțiri, care alunecă unul peste altul, având viteza maximă în axul curentului şi minimă la perete; distribuția vitezelor are o curbă parabolică, fiind determinată, în primul rând, de vâscozitate. Fluxul turbulent apare atunci când viteza de circulație depăşeşte o valoare critică, situație în care regimul laminar este înlocuit cu unul dezordonat, în care translația în axul vasului se suprapune cu deplasarea transversală a straturilor de fluid, cu apariția de vârtejuri care îngreunează avansarea lichidului pe direcția de curgere. Turbulența este mai accentuată în partea inițială a aortei şi a arterei pulmonare. În vasele mari apare o microturbulență, deci o curgere intermediară între regimul laminar şi cel turbulent. În vasele mici curgerea poate fi considerată laminară, iar în capilare se produce o deformare a hematiilor, ele curgând una câte una. Viteza sângelui este determinată în special de ramificarea vaselor, trecând de la vasele mari (40 cm/s) la cele mici, unde viteza scade (în capilare ea este de 1 mm/s) (este vorba de viteza medie). În curgere poate fi socotită o deformare prin forfecare a corpului fluid.

Forța de frecare internă prin care lichidul se opune curgerii este tangentă la suprafața de forfecare, ca forță deformatoare, şi de sens contrar acesteia. Ea este proporțională cu gradientul de viteză şi cu aria *S* a straturilor glisante, printr-un factor de proporționalitate numit coeficient de vâscozitate. Pereții vaselor de sânge au în structura lor patru tipuri importante de țesuturi: endoteliul, fibrele de elastină, fibrele de colagen şi fibrele muşchilor netezi. Endoteliul asigură o față internă netedă şi permite o permeabilitate selectivă față de apă, electroliți, glucide etc. Fibrele de elastină se află în toate vasele de sânge, cu excepția capilarelor şi anastomozelor arteriovenoase. Ele sunt foarte uşor extensibile (de aproape şase ori mai elastice decât cauciucul). Fibrele de elastină produc în mod automat o tensiune elastică pasivă, conferind vasului o rezistență la forța de distensie datorată presiunii laterale a sângelui, fără cheltuială de energie. Ele împart acest rol cu fibrele de colagen, care formează o rețea spațială, au o structură pliată şi rezistă la întinderi mult mai bine decât elastina. Ambele au rol preponderent în artere. Un număr mic de fibre de colagen din peretele unei artere este suficient pentru a-i conferi acesteia rezistența necesară la întinderi mari. Fibrele muşchilor netezi produc o tensiune activă prin contracția lor sub control fiziologic, modificând astfel diametrul vaselor de sânge şi implicit debitul sangvin prin aceste vase. Controlul exercitat de muşchii netezi asupra calibrului vascular se manifestă cel mai pregnant la nivelul arteriolelor, unde se află o mare cantitate de muşchi netezi. Datorită structurii neomogene a peretelui vascular, curba tensiune-extensie a acestuia este neliniară. Curba este neliniară şi se observă în prima parte a ei o extensibilitate mare la forțe mici, după care, pentru aceeaşi alungire, este nevoie de forțe din ce în ce mai mari. Deci, peretele vascular rezistă la tensiuni cu atât mai mari, cu cât este mai bine întins. Vasele sangvine se pot întinde atât longitudinal, cât şi transversal, modulul de elasticitate transversal fiind de circa 3 ori mai mare.

Rolul principal al peretelui arterial este de a rezista presiunilor hemodinamice: presiunea transversală, dată de presiunea sangvină, şi presiunile tangențiale, produse de forțele de forfecare ale sângelui asupra endoteliului. O tensiune de forfecare, notată, este definită ca un stres care se aplică paralel sau tangențial la un substrat, spre deosebire de o tensiune normală, care este aplicată perpendicular.

Endoteliul vascular este expus la un stres hemodinamic generat de fluxul sangvin cunoscut sub numele de stres de forfecare al peretelui vascular. Stresul de forfecare este definit ca forța exercitată pe unitatea de suprafață a peretelui vascular de către fluxul sangvin şi depinde de vâscozitatea sângelui şi de velocitatatea fluxului sangvin. Valoarea stresului de forfecare al peretelui vascular este exprimată în dynes/cm^2, valorile fiziologice fiind în sistemul venos în intervalul 1-6 dynes/cm^2, în timp ce în sistemul arterial sunt cel puțin de 10 la 15 dynes/cm^2.

Efectul protector al stresului de forfecare al peretelui vascular

Deşi ateroscleroza este o boală care afectează sistemul vascular ca un întreg, are o distribuție inegală, cu diferențe substanțiale în localizarea şi progresia diferitelor plăci de aterom. La nivelul arterei carotide, de exemplu, există o predispoziție bine cunoscută pentru dezvoltarea plăcilor de aterom la nivelul bifurcației arterei carotide comune[1,2], în timp ce la nivelul arterelor coronare s-a demonstrat că există o progresie preferențială a plăcilor de aterom în segmente cu bifurcații[3,4]. Pe de altă parte, unele plăci de aterom rămân nemodificate pentru mulți ani, în timp ce altele progresează rapid.

Aceste constatări sugerează că, independent de factorii sistemici, sunt prezenți şi factori locali hemodinamici, cum ar fi stresul de forfecare a peretelui vascular, cu un rol major în generarea, progresia şi destabilizarea plăcilor aterosclerotice. Diferite studii au arătat că prezenţa unui stres normal sau crescut de forfecare a peretelui vascular ($\geq$ 10-15 dyne / cm ^{2}) are un efect protector asupra endoteliului mediat prin inhibarea proliferării endoteliale, are un efect local antiinflamator, de prevenire a apoptozei celulelor endoteliale şi de creştere a activităţii enzimelor antioxidante (superoxid dismutaza şi oxid nitric sintetaza) a celulelor endoteliale[5]. Reducerea stresului de forfecare a peretelui vascular, aşa cum se întâmplă, de exemplu, în anumite segmente ale bifurcaţiilor arteriale, favorizează oxidarea şi acumularea lipidelor în intimă. O creştere în activitatea enzimei de conversie a angiotensinei a fost demonstrată în zonele cu stres de forfecare a peretelui vascular redus, care determină o mărire a nivelului de angiotensină II şi, în consecinţă, o creştere a stresului oxidativ[5,6]. De asemenea, un stres de forfecare a peretelui vascular scăzut reduce producţia de oxid nitric endotelial şi de proteine antioxidante, şi creşte sinteza de E-selectină.

Stresul de forfecare a peretelui vascular şi ateroscleroza

Progresia plăcii de aterom poate fi rapidă, la pacienţii cu mai mulţi factori de risc, şi lentă. Ambele variante pot prezenta complicaţii trombotice acute. Existenţa disfuncţiei endoteliale facilitează pătrunderea lipidelor plasmatice şi a monocitelor. Perpetuarea acestui proces duce la formarea celulelor spumoase (“foam cells”), la eliberarea de factori de creştere şi mitogeni, şi la sinteza crescută de ţesut conjunctiv prin proliferarea celulelor musculare netede (C.M.N.). Cantităţile crescute de lipide din aceste celule duc la moartea lor cu dezintegrarea ulterioară, eliberând conţinutul de lipide şi formând conglomerate lipidice extracelulare (“lipid core”). De obicei, aceste plăci au o progresie clinică lentă şi silenţioasă, permiţând dezvoltarea circulaţiei colaterale.

Progresia rapidă este mediată de trombi. Formarea acută de trombi în plăcile fisurate sau rupte are expresie clinică în evenimentele ischemice acute[27,28]. Stresul de forfecare a peretelui vascular este o condiţie esenţială în leziunea aterosclerotică şi în vulnerabilitatea plăcii de aterom. Factorii locali reologici contribuie la dezvoltarea trombului fixat de placa ruptă. Experimental s-a demonstrat că formarea trombului trombocitar creşte proporţional cu accentuarea stenozei, existând o corelaţie importantă între formarea trombusului şi fenomenul de forfecare. O reducere mică a lumenului vascular este asociată cu creşterea importantă a ratei de forfecare.

La nivelul stenozei, curentul sangvin prezintă accelerare înainte şi decelerare după stenoză, fapt ce determină separarea în diferite curente şi recircularea unor zone poststenoză[29].

Studii cu fibrinogen marcat cu I-125 au demonstrat că depunerea fibrinogenului este determinată de fenomenul de forfecare cu turbulenţă. El este predominant în trombul adiacent stenozei. Această asociere de evenimente duce la formarea trombilor arteriali cu următoarea configuraţie: capul alb (bogat în trombocite) şi corpul roşu (bogat în fibrină şi hematii).

În 1969, Caro şi colab. au demonstrat o corelaţie între tensiunea de forfecare redusă de la nivelul peretelui vascular şi ateroscleroză. Ulterior, numeroase studii care au urmărit vasele coronare, carotide şi aorta au confirmat faptul că producerea şi progresia plăcii aterosclerotice tind să apară în zonele cu SFPV redus[1-4,8,9]. Stone şi colab.[10] au studiat efectele SFPV asupra evoluţiei plăcilor aterosclerotice şi a remodelării vasculare în arterele coronare. În toate cazurile au fost efectuate angiografia coronariană, ecografia intravasculară, măsurarea fluxului sangvin coronarian şi reconstrucţia 3D a arterei cu determinarea SFPV. La nivelul zonelor cu SFPV redus ($<$12.6 dyne/cm^2) s-a observat o creştere semnificativă în grosime a plăcii aterosclerotice şi a peretelui vasului prin remodelare, în timp ce în zonele cu SFPV fiziologic (12.6-26.9 dyne/cm^2) nu s-au evidenţiat schimbări semnificative, iar în zonele expuse la un mare stres de forfecare ($\geq$ 27 dyne/cm^2) s-a determinat o remodelare pozitivă a arterei fără modificări în placa de aterom.

Cu toate acestea, relaţia dintre SFPV şi plăcile aterosclerotice de la nivelul arterelor coronare pare să fie limitată la leziuni nesemnificative, deoarece printr-un fenomen de remodelare arterială pozitivă s-a evitat stenoza lumenului arterial[11]. Odată cu progresia stenozelor aterosclerotice coronariene, creşte SFPV, în special în partea proximală a plăcii, la intrarea în regiunea stenotică. S-a sugerat că această creştere a SFPV ar putea da naştere la o reducere a capsulei fibroase a plăcii printr-un proces de apoptoză şi, prin urmare, ar favoriza destabilizarea şi ruptura plăcii vulnerabile[12]. Într-adevăr, zonele în care ulcerația şi ruptura plăcii sunt cele mai comune în arterele coronare şi carotide sunt proximal de punctul de stenoză maximă şi coincid cu locul în care placa aterosclerotică este supusă la cel mai înalt SFPV[12].

În anii 1990 au fost publicate primele studii în care SFPV a fost măsurat cu ajutorul imagisticii prin rezonanță magnetică (IRM) şi corelat cu ateroscleroza de la nivelul aortei[8,9]. Wu şi colab.[13] au reuşit calcularea acestui parametru hemodinamic la nivelul arterelor carotidă, femurală şi brahială utilizând IRM. Wentzel şi colab.[14] au făcut un pas mai departe prin utilizarea IRM de înaltă rezoluție pentru a studia interacțiunea dintre SFPV şi progresia plăcii aterosclerotice în aorta toracică descendentă.

Stresul de forfecare a peretelui vascular şi restenoza intrastent

Stenturile endovasculare sunt implantate de rutină pentru a îmbunătăți sau restabili perfuzia distal, la nivelul arterelor coronariene şi periferice. Din păcate, hiperplazia neointimală se produce în 15-20% din implantările de stent BMS. Tulburările hemodinamice şi leziunile endovasculare produse prin stentare produc restenoza intrastent.

Restenoza intrastent intracoronarian se datorează în principal unui proces de proliferare neointimal în interiorul şi la marginile stentului. Variabilele care au fost asociate cu acest proces includ factori sistemici, cum ar fi diabetul zaharat, şi caracteristici relevate angiografic, cum ar fi lungimea şi diametrul stenozei tratate. Sanmartín şi colab.[15] au evaluat rolul SFPV în proliferarea neointimală postimplantare de stent coronarian. Calculul SFPV a fost efectuat utilizând o reconstrucție 3D geometrică a arterei coronare, bazată pe o combinație a angiografiei coronariane şi a ecografiei intravasculare coronariene. SFPV redus a fost corelat cu o creştere a proliferării neointimale, cu diferențe în grosime neointimală la 6 luni de urmărire de până la 20% dintre rezultatele asociate cu cel mai mare şi cea mai mică valoare a SFPV.

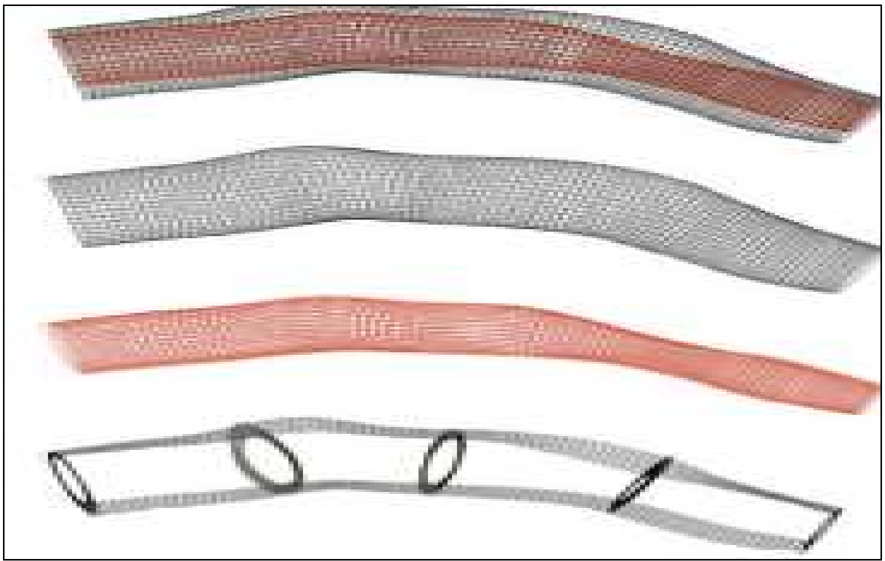

Fig. 1. Reconstrucția 3D a arterei coronare[15]

Această corelație inversă între proliferarea neointimală şi SFPV a fost observată în ambele segmente ale stentului precum şi la marginile acestuia. Rezultatele studiului sunt în concordanță cu cele ale lui Wentzel şi colab.[16], care au demonstrat, de asemenea, o corelație semnificativă între SFPV şi gradul de proliferare neointimală la 14 pacienți după implantarea de stenturi coronare (Wallstent, Schneider AG). De remarcat că SFPV este uniform în întreaga suprafață vasculară acoperită de stent[17] (maximă pe firele stentului şi minimă la marginile acestuia).

De asemenea, s-a sugerat că anumite caracteristici de proiectare şi de implantare a stenturilor au o influență semnificativă asupra hemodinamicii locale şi pot juca un rol decisiv în gradul de proliferare neointimală intrastent[19]. Astfel, o reducere a grosimii şi a numărului de fire ale stentului creşte semnificativ (87%, respectiv până la 2,75 ori) în stent SFPV şi poate avea un efect de protecție împotriva restenozei în stent. Aceasta poate explica parțial diferențele ratelor de restenoză observate între stenturi cu desene sau geometrie diferită[20-22]. Pe de altă parte, implantarea de stenturi, care sunt supradimensionate în raport cu dimensiunea arterei coronariene (raportul stent-arteră ≥ 1.2), a fost, de asemenea, legată de o reducere semnificativă (până la 12 ori) a SFPV[19]. În acord cu aceste concluzii, unele studii clinice au arătat o corelație semnificativă între implantarea de stenturi supradimensionate şi incidența restenozei în stent[23,24]. Deşi s-a sugerat că prejudiciul ar fi declanşat de răspunsul exagerat al intimei în cazul de stenturi supradimensionate, modificările hemodinamice locale ar putea fi, de asemenea, un factor care favorizează restenoza în respectivele situații.

Reconstrucția tridimensională a unui stent folosind o simulare numerică a arătat distribuția SFPV. Utilizarea de stenturi active farmacologic (DES) a condus la o reducere semnificativă a incidenței restenozei intrastent[25]. Gijsen şi colab.[26] au raportat că relația inversă între tensiunea SFPV şi proliferarea neointimală este menținută în stenturile acoperite cu sirolimus, în ciuda faptului că proliferarea neointimală este minimă. Rămâne de văzut dacă tehnica de proiectare şi de implantare a stentului DES are implicații clinice în aceste cazuri.

Stresul de forfecare a peretelui vascular, determinant al funcției endoteliale

Relaxarea musculaturii netede arteriale este parțial mediată prin mecanisme endoteliale-dependente[30], care implică eliberarea de oxid nitric[31]. *In vitro*, factorul determinant principal hemodinamic endotelial de eliberare de oxid nitric şi vasodilatație ulterioară este de SFPV[32,33]. S-a demonstrat că SFPV determină vasodilatație la şobolan în arteriolele muşchiului cremasteric (3) prin eliberare endotelială de oxid nitric şi vasodilatație în microcirculația coronariană la câine[34]. La om, o creştere a fluxului sangvin după o scurtă perioadă de ischemie a musculaturii scheletice este însoțită de dilatarea arterelor[35,36].

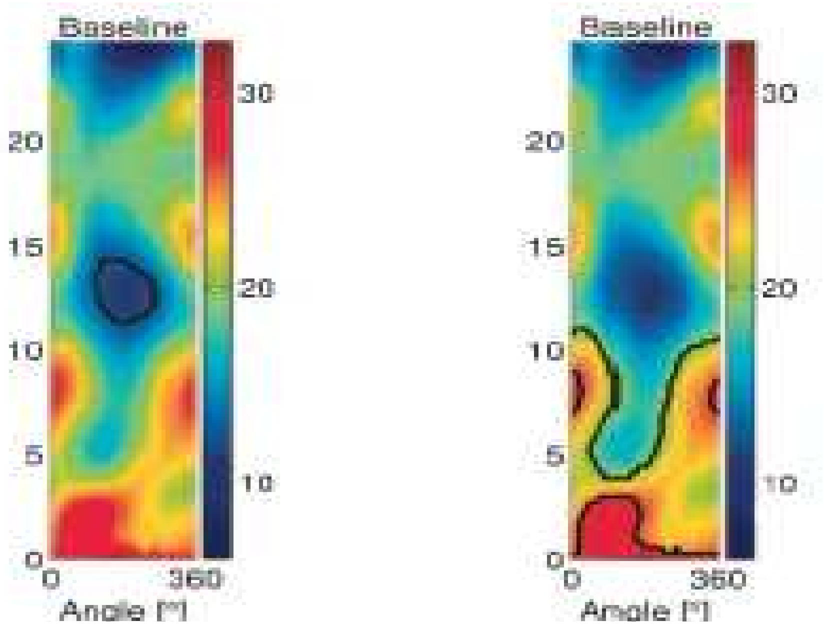

Fig 2. Harta topografică a SFPV la nivelul arterei coronare – utilizând o scală color; remarcăm zona cu SFPV scăzut (<9,1 dyne/cm^2) şi cu SFPV crescut (>27 dyne/cm^2).

În plus, o creştere a magnitudinii sau a duratei hiperemiei duce la amplificarea vasodilatației.[37,38] Dilatarea flux-mediată a fost măsurată la nivelul membrelor folosind ultrasunete de înaltă frecvență. Cu toate acestea, calculul SFPV necesită achiziționarea simultană a dimensiunii şi a velocității sangvine. În plus, ecografia este critic dependentă de operator, care poate reduce reproductibilitatea metodei. Măsurătorile ecografice ale dilatației mediate de velocitate au fost utilizate pe scară largă pentru a studia funcția endotelială la pacienții cu boli cardiace şi factori de risc cunoscuți[39,40] .

SFPV reglementează diametrul arterial, prin modificarea producției de mediatori vasoactivi de către celule endoteliale[41,42], în timp ce ambele SFPV şi deformarea circumferențială arterial sunt factorii determinanți ai expresiei genelor endoteliale[43]. Evaluate *in vitro*, genele endoteliale reglate de SFPV includ: factori de transcripție, factori de creştere, molecule de adeziune şi de enzime; acestea pot fi crescute tranzitor sau permanent[44]. Este de subliniat că SFPV crescută măreşte concentrația de moleculă de adeziune intercelulară-1 (ICAM-1)[45,46], dar că efectul asupra moleculei de adeziune a celulelor vasculare-1 (VCAM-1) este dependent de tipul de SFPV aplicate. Creşterea concentrației de VCAM-1 se observă atunci când celulele sunt expuse la SFPV constantă[47], iar scăderea nivelului de VCAM-1, atunci când sunt expuse la SFPV oscilatorie[48]. Walpola şi colab.[49] au arătat că, *in vivo*, efectul SFPV asupra ICAM-1 şi VCAM-1 este dependent de nivelul SFPV. În experimentele lor, SFPV redus creşte nivelul de VCAM-1 în mod substanțial şi suprimă ICAM-1, în timp ce SFPV crescut ridică nivelul CAM-1 în mod substanțial, iar VCAM-1 doar moderat. SFPV diminuează nivelul endotelinei-1[50] şi thrombomodulina[51], aceasta din urmă fiind complet reversibilă[52]. Aceste descoperiri indică faptul că celulele endoteliale pot reacționa diferit, având variații subtile în condiții diferite temporale şi spațiale ale SFPV[52] .

In vitro, nivelul de SFPV de 1.0[53] până la 1.5 Pa[54] induce un profil endotelial ateroprotectiv, în timp ce un SFPV de 0.4 Pa stimulează un fenotip aterogenic[54]. În ciuda creşterii dovezilor că forțele dinamice joacă un rol în expresia genelor endoteliale care sunt probabil implicate în aterogeneză, sunt necesare investigații suplimentare pentru a evalua aceste constatări la adevărata lor valoare. Mai multe cercetări trebuie efectuate asupra nivelului şi tipului de SFPV responsabil de exprimarea unui anumit model specific.

Forțele biomecanice afectează nu numai funcția celulelor endoteliale, ci şi structura acestora. Celulele endoteliale tind să se alinieze cu SFPV – cu cât SFPV este mai intens, cu atât celulele endoteliale sunt mai alungite[55,56,57]. Aceste modificări sunt asociate cu redistribuirea şi rearanjarea fibrelor intracelulare şi cu numărul lor[58] . Schimbarea modelului arterial poate depinde de tipul de SFPV aplicat. Sub SFPV oscilatorie, celulele endoteliale ale pertelui aortic bovin îşi mențin forma poligonală, la fel ca în cultura statică, în timp ce celulele nu prezintă fibre de actină de stres .

Forțele mecanice care acționează pe peretele luminal al celulelor endoteliale, deformând aceste celule, sunt transmise prin intermediul scheletului celular şi în alte locuri intracelulare[59]. Aceste forțe sunt în mod special resimțite la punctele de aderență bazale, în cazul în care celule endoteliale se află la nivelul matricei extracelulare şi membranei nucleare, care să conducă la redistribuirea forțelor asupra celulelor endoteliale[60]. Forțele biomecanice pot fi resimțite de către factorul de creştere derivat din trombocite, activatorul tisular al plasminogenului, factorul de creştere-β, endotelina-1, o formă endotelială a sintetazei oxidului nitric (NOS3) şi ICAM-1 [61].

În ultimii 30 de ani s-au înregistrat progrese considerabile în înțelegerea multor aspecte privind funcția endotelială şi alterările ei în condiții patologice; totuşi puțini clinicieni iau în considerație sănătatea endoteliului, atât pentru că endoteliul nu poate fi explorat convenabil până în prezent, cât şi datorită complexității şi adaptabilității sale. Discrepanța între cunoaştere şi aplicația curentă este vizibilă şi când se compară incidența cuvintelor cheie legate de endoteliu în lucrările ştiințifice față de cărțile medicale. Disfuncția endotelială este un eveniment cheie în fiziopatologia bolilor cardiovasculare şi apare ca un element predictiv independent şi puternic al evenimentelor cardiovasculare.

Angiografia prin rezonanță magnetică (ARM) poate spori capacitatea de investigare a mecanismelor de vasodilatație arterială endotelial-dependente. ARM oferă posibilitatea de evaluare a velocității fluxului sangvin mediu, precum şi a secțiunii transversal arteriale. Această tehnică a fost bine validată *in vivo*[62,63,64]. Mai mult chiar, un plan fix transversal poate fi proiectat în mod repetat, reducându-se astfel dependența de investigator. ARM a fost utilizată pentru a măsura SFPV în diferite artere[65,66].

Fiecare imagine constă dintr-o componentă anatomică şi o componentă de velocitate. Imaginile au fost achiziționate la momentul inițial bazal, în timpul ocluziei arteriale determinate de inflație (componenta anatomică prezintă aplatizare în secțiune transversală; componenta de velocitate arată lipsa de flux), în timpul hiperemiei, imediat şi la 1 minut după eliberarea manşetei, atunci când dilatarea mediată de flux a avut loc. Imaginile sunt uşor neclare, dar datele utilizate pentru măsurători şi calcule nu sunt afectate.

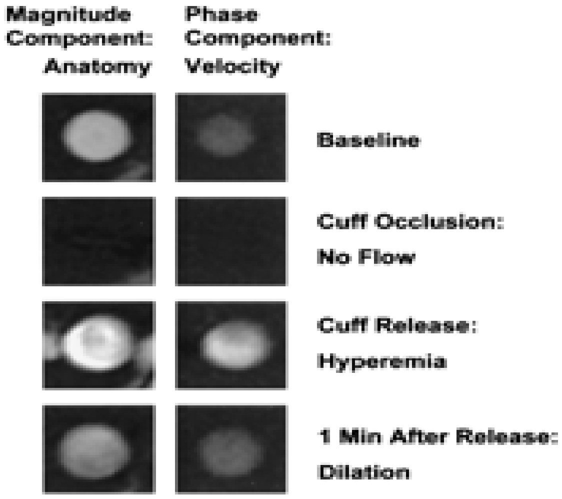
Magnitude Component: Anatomy
Phase Component: Velocity
Baseline
Cuff Occlusion: No Flow
Cuff Release: Hyperemia
1 Min After Release: Dilation

Fig. 3. ARM a arterei brahiale în secțiune transversală la un pacient sănătos.

Au existat diferențe în diametrul arterial obținut folosind ARM vs ecografie. Ecografic, măsurarea diametrului arterial nu poate fi uşor de programat la vârful debitului şi, de obicei, se face în concordanță cu unda R. Cu ARM, măsurătoarea a fost realizată la debitul de vârf, pentru că este momentul de vârf al semnalului între lumenul şi peretele arterial. Acest aspect explică, cel puțin în parte, de ce diametrul vasului este mai mare în evaluarea ARM. În protocolul ARM, manşeta a fost plasată pe partea superioară a brațului, pentru a maximiza sensibilitatea în măsurarea dilatației mediate de flux. Acest lucru se datorează faptului că hiperemia şi dilatația mediată de flux sunt mai mari după ocluzia părții superioare a brațului decât după ocluzia părții inferioare[67]. Manşeta a fost plasată pe brațul inferior în protocolul ecografic, pentru a maximiza stabilitatea în imagistică la nivelul arterei brahiale.

Concluzii

Progresia bolii cardiac ischemice este legată de progresia plăcii aterosclerotice şi de remodelarea vasculară. Factorii hemodinamici locali, în special SFPV scăzut, sunt deosebit de importanți în procesul de progresie a plăcii de aterom şi al remodelării vasculare. Conceptul Glagov explică implicarea membranei externe elastice în procesul de remodelare, ocluzia arterelor coronariene producându-se atunci când placa de aterom depăşeşte abilitatea membranei externe elastice de a se remodela[68]. Pe baza reconstrucției geometrice 3D a arterelor coronariene cu un lumen coronarian < 50%, realizat pe baza imaginilor ecografiei intracoronariene şi angiografice, au fost evaluate aria plăcii de aterom în secțiune transversală, lumenul coronarian, membranele elastic externe şi fluxul coronarian. Corelând modificările apărute în aria plăcii, aria membranei externe şi aria lumenului, s-a ajuns la concluzia că placa de aterom progresează în segmentele coronariene cu SFPV scăzut asociat cu remodelarea vasculară de tip constrictiv sau expansiv[69,70].

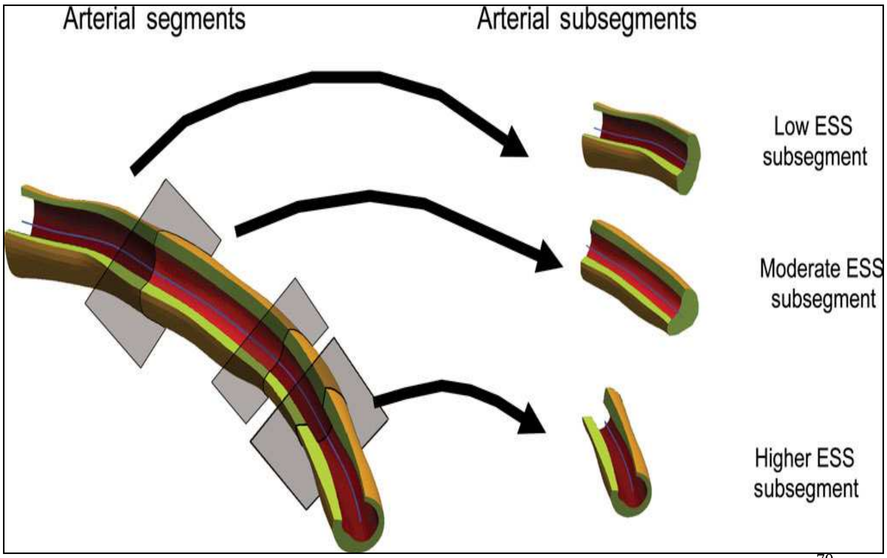

Fig. 4. Remodelarea membranei elastice externe corelată cu SFPV[70]

Studii bazate pe reconstrucția arterei coronariene în timp real şi pe simularea fluxului sangvin intra-stent au evidențiat că procesul de restenoză intrastent prin hiperplazie neointimală este strâns legat de scăderea SFPV. Astfel, segmentele coronariene cu SFPV moderat sau crescut au un flux sangvin unidirecțional şi aliniat axial, în timp ce leziunile focale s-au dezvoltat doar în aria cu flux scăzut sau cu pattern de recirculare.

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Tehnica DTI („diffusion tensor imaging") – prezentare şi aplicaţii neurologice

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Rezumat. Tehnica DTI ("Magnetic Resonance Diffusion Tensor Imaging") este o metodă neuroimagistică utilă pentru detectarea şi reprezentarea proprietăţilor de difuzie ale apei în ţesutul nervos. Tehnica de tractografie foloseşte datele DTI şi reconstruieşte tracturile de substanţă albă nervoasă contribuind în mod semnificativ la cunoaşterea configuraţiei in vivo a substanţei albe. Folosind algoritmi sofisticaţi şi software specializat, DTI şi tractografia evidenţiază modificările patologice ale sistemului nervos. Tracturile de substanţă albă pot fi investigate nu numai prin simpla observaţie, ci şi prin analizarea coeficienţilor de difuzie ai apei, magnitudinea anizotropiei şi parametrilor fibrelor.

Cuvinte cheie: DTI ("diffusion tensor imaging"), tractografie, neuroimagistică

Abstract. Magnetic Resonance Diffusion Tensor Imaging (DTI) is a neuroimaging method for detecting and representation of water diffusion properties of nervous tissues. Fiber tracking technique uses DTI data and reconstructs nervous fibers and significantly enhances the knowledge about the in vivo configuration of white matter. Using sophisticated algorithms and specialized software, DTI and fiber tracking detect in patients pathological changes of nervous system. The white matter tracts can be investigated not only by simple observation, but also through analyzing water diffusion coefficients, magnitudes of anisotropy and fibers parameters.

Key words: diffusion tensor imaging, DTI, fiber tracking, neuroimaging

Introducere

„Diffusion tensor imaging" (DTI) este o tehnică de imagistică a sistemului nervos ce permite reprezentarea tracturilor nervoase folosind cuantificarea difuziei apei (mişcarea moleculelor de apă) în ţesutul cerebral. Măsurând proprietăţile de difuzie ale apei într-un anumit ţesut, se poate descrie spaţial mediul studiat.

Difuzia poate fi :

- *izotropică* (la fel în toate direcţiile spaţiului), când mediul nu restricţionează mişcarea moleculelor sau
- *anizotropică* (inegală pe toate direcţiile), aşa cum se întâmplă la nivelul substanţei albe cerebrale datorită restricţionării difuziei apei pe direcţie perpendiculară axonilor din lungul tracturilor nervoase. (1)

Difuzia anizotropică la nivelul sistemului nervos se datorează dispunerii concentrice a straturilor de mielină şi a membranelor axonilor din fibrele nervoase. Prezenţa mielinei nu este obligatorie pentru anizotropia difuziei în tracturile nervoase, dar aceasta modulează gradul de anizotropie.

Tractografia (reconstrucţia de fibre nervoase cu ajutorul unor softuri speciale ce folosesc imaginile de difuzie) reprezintă metoda noninvazivă de studiere in vivo a arhitecturii substanţei albe cerebrale.

Difuzia apei în ţesuturi (sistemul nervos central)

Într-un lichid unde nu există bariere, moleculele de apă difuzează izotrop (la fel în toate direcţiile), iar dacă există bariere în calea difuziei (moleculele sunt respinse când ating aceste graniţe), acestea difuzează anizotrop. În ţesuturile biologice, barierele fizice pot fi membrane sau organite celulare, care sunt în general parţial permeabile, şi teci de mielină, în cazul axonilor sistemului nervos.

Difuzia anizotropică în substanţa albă îşi are originea în organizarea specifică acestui ţesut în tracturi (mănunchiuri de fibre axonale) mai mult sau mai puţin mielinizate ce au traiecte paralele. Difuzia în direcţia fibrelor este de câteva ori mai rapidă decât în direcţia perpendiculară.

Anizotropia a putut fi exploatată pentru a crea o hartă a orientării în spaţiu a tracturilor de substanţă albă la nivel cerebral, considerând că direcţia cu cea mai mare difuzie este direcţia generală de orientare a fibrelor.

Dezvoltarea subsanţei albe şi aspectul acesteia au putut fi cercetate doar prin studii anatomo-patologice şi tehnici convenţionale neuroimagistice. Teaca de mielină este o structură stratificată, având în componenţă lipide şi proteine (colesterol 28%, galacto-cerebrozide 22%, sfingomielina 8%, fosfatidil-serina 5%, fosfatidil-etanolamina 12%, fosfatidil-colina 11%, sulfatide 4%, fosfatidil-inozitol 1%, acizi graşi). Mielina nu este obligatorie pentru generarea difuziei anizotrope (preferenţiale pe o singură direcţie) la nivelul substanţei albe cerebrale, dar modulează gradul de anizotropie, acesta crescând odată cu mielinizarea. Astfel, anizotropia în creierul nou-născuţilor, ce au axoni mielinizaţi incomplet, este mai scăzută comparativ cu cea din ţesutul cerebral adult, ultimele fibre mielinizate fiind cele de asociaţie inter-corticală.

Numeroasele straturi lipidice ale mielinei limitează permeabilitatea apei şi e de aşteptat să scadă difuzia perpendicular pe fibre, mai mult decât în direcţia paralelă fibrelor. Dacă mielina ar fi singura sursă de anizotropie, atunci ar fi de aşteptat ca difuzia să fie mai mult izotropică (egală în toate direcţiile) în fibrele normale nemielinizate. Pentru două tracturi de mărimi şi densităţi axonale egale, unul mielinizat şi celălalt nemielinizat, se poate spune totuşi că mielina va creşte anizotropia datorită creşterii difuziei intraaxonale şi a creşterii tortuozităţii spaţiului extraaxonal şi a difuziei extraaxonale.

În interiorul axonului există un citoschelet complex şi dens, fiind compus din neurofibrile, orientate longitudinal, alcătuite din microtubuli (implicaţi şi în transportul axonal rapid) şi neurofilamente interconectate de mici microfilamente. Deoarece neurofibrilele numeroase prezintă suficiente bariere fizice pentru a obstrucţiona difuzia apei pe direcţie perpendiculară într-un grad mai mare decât pe direcţie paralelă, aceste structuri ar putea explica difuzia anizotropică. A fost demonstrat că microtubulii şi transportul axonal rapid facilitează, dar nu sunt cei mai importanți determinanți ai anizotropiei. (2)

ADC (coeficientul aparent de difuzie) a scăzut atât în direcție paralelă, cât şi perpendiculară dacă apare depolimerizarea microtubulilor in vitro. Membranele axonale intacte sunt determinantul primar al anizotropiei difuziei apei din fibrele neurale din substanța albă din creier şi măduva spinării. Deficitul de mielină alterează în schimb valorile ADC (coeficientul aparent de difuzie) măsurate cu ajutorul „Diffusion Tensor Imaging”.

Tehnica Diffusion Tensor Imaging

Tehnica imagistică a fost dezvoltată de Basser (1994), după ce în anul 1986 Le Bihan introducea tehnica Diffusion Weighted Imaging (DWI). Prin combinarea principiilor de rezonanță magnetică cu cele ale efectelor difuziei moleculelor a fost posibilă caracterizarea difuziei apei in vivo la nivelul creierului. Semnalele de rezonanță magnetică pot fi „sensibilizate” la difuzie prin utilizarea unor perechi de pulsuri - gradienți de câmp magnetic cu anumită durată şi la un anumit interval de timp.

Într-un câmp magnetic omogen, primul puls magnetic ținteşte nucleii de hidrogen (protonii) ai apei în funcție de localizarea spațială a acestora, iar pentru un scurt timp câmpul magnetic variază încet în altă direcție.

Al doilea puls este introdus ulterior, pentru a detecta modificările localizării spațiale a nucleilor şi astfel se poate determina deplasarea nucleilor în acel interval de timp dintre cele două pulsuri (timp de difuzie). O modificare a localizării spațiale a nucleului de hidrogen pentru un anumit gradient este proporțională cu mişcarea moleculelor de apă ce difuzează. Atenuarea semnalului este legată cantitativ de amplitudinea variației difuziei: difuzia rapidă va determina o distribuție largă şi o atenuare mare a semnalului, iar difuzia încetinită va determina o atenuare slabă a semnalului.

Datele DTI pot fi analizate în 3 moduri pentru a oferi informații despre microstructura şi arhitectura țesutului pentru fiecare voxel (unitate de volum). Prima metodă utilizează difuzivitatea medie - MD, ce caracterizează deplasarea medie a moleculelor şi prezența obstacolelor în calea difuziei. A doua metodă urmăreşte gradul de anizotropie, descriind gradul de variație a deplasărilor moleculare în spațiu şi este legată de prezența şi orientarea unor anumite structuri tisulare şi celulare. A treia metoda urmăreşte direcția principală de difuzie. Pentru a estima difuzia şi tensorul de difuzie (DTI), sunt necesare cel puțin şase măsurători (din cel puțin şase direcții diferite ale gradienților) față de imaginea obişnuită de rezonanță magnetică.

Tensorul folosit pentru descrierea difuziei poate fi vizualizat (conceptualizat) ca un elipsoid, iar cele trei axe principale ale elipsoidului descriu un sistem ortogonal de coordonate. (3) Direcția axelor principale ale elipsoidului reprezintă vectorii, iar lungimea lor reprezintă valorile tensorului. În tehnica Diffusion Tensor Imaging tensorul care descrie difuzia în toate direcțiile spațiale este calculat pentru fiecare voxel (unitate de volum).

Pentru interpretarea tensorului de difuzie şi determinarea direcțiilor principale de difuzie în fiecare voxel, acesta trebuie calculat pe trei direcții perpendiculare din spațiu. Se obțin trei vectori cu trei valori corespunzătoare: λ1, λ2, λ3, văzute ca trei direcții principale ale procesului de difuzie. Axa cea mai lungă a elipsoidului de difuzie reprezintă valoarea şi direcția maximă de difuzie, iar axa cea mai scurtă reprezintă direcția de difuzie minimă. Dacă cele trei valori ale vectorului sunt egale, difuzia este izotropică (la fel în toate direcțiile), iar tensorul de difuzie poate fi vizualizat ca o sferă (aşa cum e cazul lichidului cefalo-rahidian). Dacă aceste valori nu sunt egale, difuzia este anizotropică şi tensorul de difuzie este vizualizat ca un elipsoid, aşa cum e cazul cu restricționarea difuziei de către straturile de mielină din substanța albă.

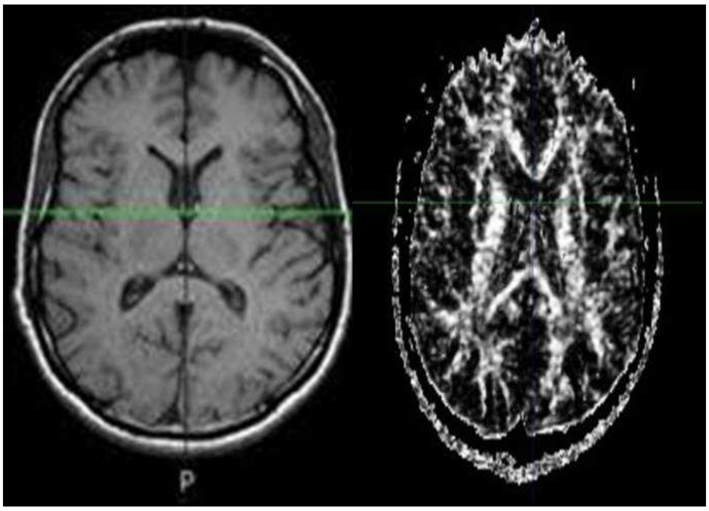

Fig. 1. RMN clasic (stânga) şi reprezentarea difuziei apei (dreapta)

Datele obținute prin tehnica diffusion tensor imaging – rezonanță magnetică pot fi analizate în mai multe moduri. Cea mai folosită metodă pentru caracterizarea difuziei generale a moleculelor (mărimea medie a elipsoidului de difuzie) este determinarea *MD (mean diffusivity – difuzivitate medie).* MD (difuzivitatea medie) este eficientă ca indicator în distingerea între lichidul cefalorahidian ce are o difuzivitate medie ridicată şi țesutul cerebral cu difuzivitate mai scăzută, dar eşuează în diferențierea diferitelor tipuri de țesut cerebral. MD poate fi calculată făcând media elementelor diagonalei tensorului de difuzie.

Câteva măsurători au fost introduse pentru descrierea difuziei anizotrope, independente de orientarea elipsoidului de difuzie şi astfel oferă informație specifică fiecărui țesut: RA (anizotropia relativă) şi FA (fracția de anizotropie). O valoare datorită difuziei crescute în lungul fibrelor neuronale față de direcția perpendiculară. FA este maxim la nivelul zonelor de difuzie anizotropică uniformă, cum ar fi corpul calos.

Direcția cu difuzibilitatea maximă poate fi reprezentată în spațiu folosind un cod de culori: roşu (stânga-dreapta), verde (anterior-posterior), albastru (superior-inferior), în funcție de FA, şi astfel poate fi vizualizat gradul de anizotropie şi direcția locală a fibrelor.

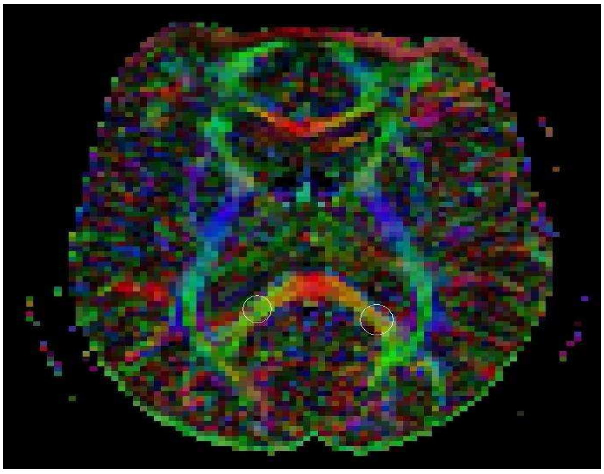

Fig. 2. Harta de difuzie a apei, folosind codul de culori

Există şi alți parametri ai Diffusion Tensor Imaging ce pot fi utilizați în studii clinice (caracterizează difuzia din fiecare voxel separat sau corelează difuzia din voxeli vecini, putând astfel caracteriza mai bine organizarea şi orientarea fibrelor la nivel macroscopic).

Reconstrucția tracturilor nervoase (tractografia)

Aplicațiile tractografiei sunt multiple. Interesul pentru reprezentarea tracturilor nervoase există de mult timp, iar metoda Diffusion Tensor Imaging (DTI) a permis modelarea acestora in vivo la pacienți. Fiecare voxel din DTI conține informația ce reflectă direcția şi magnitudinea difuziei apei în țesutul studiat. Tractografia foloseşte algoritmi pentru a determina dacă vectorul de difuzie efectivă dintr-un voxel este conectat cu voxelul vecin. Se construiesc apoi reprezentări vizuale ale fibrelor ce trec prin voxeli vecini. Tracturile se pot reconstrui din voxel în voxel, pornind de la un punct de strat sau se pot desena ținându-se cont de o minimă angulare a fibrelor. Rezultatul final produce imagini ce corespund cu anatomia respectivei zone.(5,6) Există totuşi limitări, de exemplu tractografia se poate efectua foarte bine în materia albă profundă cu fracție de anizotropie mare (cum ar fi corpul calos), dar nu este foarte eficientă în regiunile periferice ale substanței albe, unde fibrele au direcții variate, cum ar fi joncțiunea cu substanța cenuşie.

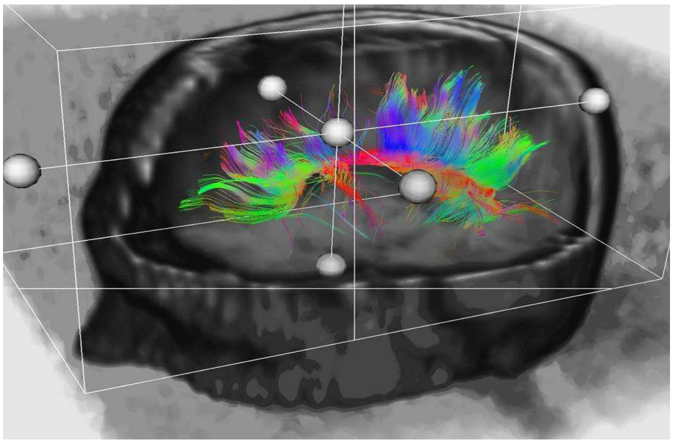

Fig. 3. Reconstrucția corpului calos

Metoda trebuie aplicată cu grijă pentru că pot apărea erori de genul unor conexiuni „fantomă” care nu există anatomic. (7)

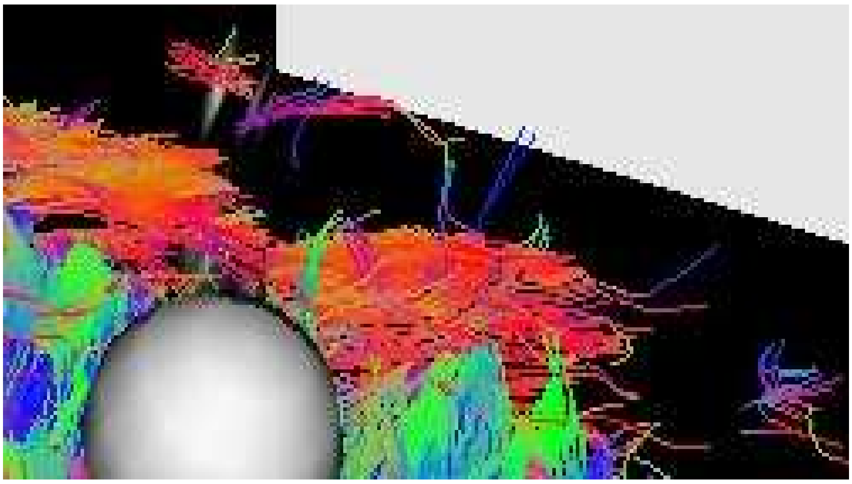

Fig. 4. Exemple de erori: fibre reconstruite în afara extremității cefalice

Pentru reprezentarea tracturilor nervoase şi construcția grafică a acestora, traiectoria difuziei este urmărită într-un câmp de vectori.

Există 3 moduri folosite pentru inițierea tractografiei:

1) Definirea manuală a regiunii de interes sau a punctelor de interes de unde pleacă fibrele;

2) Inițierea tractografiei în toți voxelii, iar apoi doar tracturile care intră într-o anumită regiune de interes sunt selectate;

3) Inițierea tractografiei în două regiuni de interes şi doar tracturile care intersectează ambele regiuni de interes sunt selectate.

Există mai multe criterii de oprire a desenării tracturilor: 1) în regiunile cu anizotropie scăzută, acolo unde vectorul principal nu este bine definit (aceasta se poate face utilizând indici ca FA - *fractional anisotropy* şi RA - anizotropie relativă); 2) definirea unghiului maxim între direcția fibrelor din 2 voxeli vecini (nu este permisă desenarea unor tracturi ce îşi schimbă brusc şi de multe ori direcția – nu ar corespunde cu realitatea anatomică). În general, mănunchiurile de fibre sunt mai importante decât fibrele individuale şi au fost propuse metode pentru identificarea mănunchiurilor de fibre.

APLICAȚII CLINICE ALE DTI ŞI ALE TRACTOGRAFIEI

Până în prezent, variate studii neuroimagistice au arătat o gamă largă de aplicații ale DTI:

- în *ischemia cerebrală*: DTI este sensibil la modificările tracturilor determinate de degenerarea axonală după accidente vasculare cerebrale (AVC). Se pot detecta modificări ale parametrilor de difuzie – de exemplu, scăderea magnitudinii anizotropiei fracționale post AVC acut, dar şi la un timp după AVC (8);

- în *scleroza multiplă*: apar procese de demielinizare pe care DTI le poate detecta precoce în substanța albă (scade FA şi creşte MD) atât la nivelul leziunilor acute, cât şi global (9);

- în *tumorile cerebrale*: există multe cauze de afectare a fibrelor de substanță albă. Când tumora ditruge complet fibrele de substanță albă, difuzia este aproape izotropică. Atunci când tumora deplasează numai fibrele de substanță albă, nu există alterări ale parametrilor de difuzie. Cea de a treia posibilitate apare cu o scădere a FA, dar tracturile au direcție normală. Aceste cunoştințe sunt utile în pregătirea preoperatorie (10);

- în *malformațiile cerebrale*: acestea pot fi detectate cu mare acuratețe. În plus, în timpul dezvoltării normale a creierului, FA corespunzătoare substanței albe a lobului frontal este mai redusă la copii decât la adulți (11);

- în *boala Alzheimer*: DTI a evidențiat precoce în cursul bolii întreruperi ale conexiunilor substanței albe.

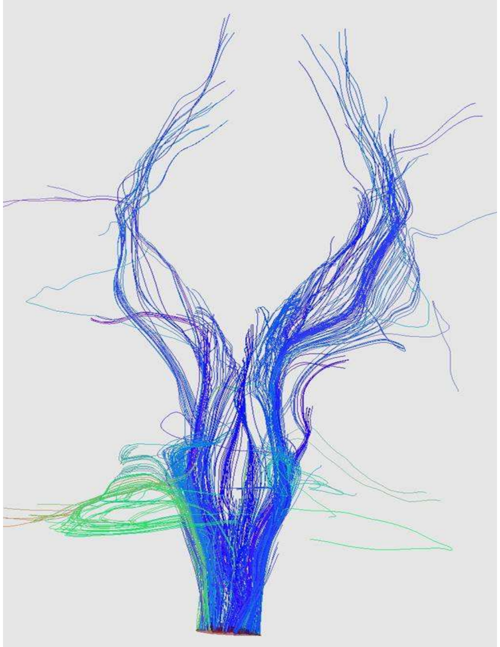

Fig. 5. Tracturile cortico-spinale

Traumatisme cerebrale, epilepsia, scleroza laterală amiotrofică - ca şi alte patologii neurologice - pot fi explorate cu ajutorul DTI (12).

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Explorarea IRM a leziunilor lojei muşchilor rotatori şi a celorlalte structuri ale articulației umărului

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Rezumat. Imagistica prin Rezonanță Magnetică are o senzitivitate şi sensibilitate de aproximativ 95% pentru leziunile complete şi de aproximativ 85% pentru leziunile incomplete sau tendinite. Artrografia RM creşte acuratețea, dar necesită o procedură mai complicată. IRM oferă de asemenea acuratețe şi informații diagnostice excelente despre spațiul subacromial şi despre acromion, precum şi despre loja muşchilor rotatori şi despre relațiile articulare.

Cuvinte cheie: IRM, acromion, loja muşchilor rotatori.

Abstract.The shoulder MRI has approximately 95% sensitivity and specificity for complete tears and 85% accuracy for incomplete tears and tendinitis. MRI arthrography increases the accuracy but requires a procedure. MRI also provides an excellent demonstration of the acromion and subacromial space allowing visualisation of the rotator cuff and its relationship to the acromion.

Key words : MRI, acromion, rotator cuff

Scopul examenului IRM constă în identificarea sediului leziunilor în vederea stabilirii conduitei terapeutice adecvate, analiza articulației gleno-humerale şi a structurilor sale componente, a fiecărui tendon, precum şi în excluderea unui alt tip de patologie decât cel traumatic sau degenerativ.

Tehnica şi protocolul de examinare. După reperajul axial se realizează secvențe în plan sagital şi coronal oblic, paralele sau perpendiculare pe planul scapulei. Umărul trebuie poziționat în uşoară rotație externă. Artrografia RM de tip direct sau indirect este indicată în cazul instabilității, respectiv a evaluării labrumului. Injectarea i.v. a substanței de contrast paramagnetice în cazul artrografiei RM este urmată de realizarea secvențelor artrografice după circa 15 minute, în scopul creşterii specificității, sensibilității şi a acurateței. Ideală este utilizarea bobinelor dedicate, capabile să ofere imagini de calitate foarte bună, datorită imobilizării mai bune a umărului, şi care permit reducerea artefactelor de mişcare şi poziționarea pacientului cât mai aproape de izocentru. Secvențele adecvate pot fi: o secvență în plan axial, de obicei PD cu supresie de grăsime, care poate evalua de la început şi starea labrumului glenoidal, urmată de secvențe T1 şi T2 cu şi fără supresie de grăsime, cu rol complementar şi poziționate oblic, paralel sau perpendicular pe scapulă.

Obiective

Analiza structurilor osoase, respectiv a măduvei roşii, frecvent vizibilă în epifiza şi diafiza humerală proximală.

Analiza cartilajului articular, care poate fi efectuată direct sau indirect prin artrografie RM, precum şi a remanierilor subcondrale şi a osteofitelor.

În cazul rupturilor tendinoase, leziunea este evidențiată printr-o zonă de hipersemnal dificil de diferențiat fără injectarea substanței de contrast paramagnetice.

Evaluarea celor mai importanți muşchi ai umărului:

- *subscapularul,* cu originea pe fosa subscapulară şi cu inserție pe tuberozitate mică a humerusului, are ca acțiune rotația internă şi adducția;
- *supraspinosul,* cu origine în fosa supraspinoasă şi cu inserție pe marea tuberozitate humerală, are ca acțiune abducția şi rotația externă;
- *infraspinosul,* cu origine în fosa infraspinoasă şi inserție pe porțiunea inferioară a marei tuberozități, are ca acțiune abducția şi rotația externă;
- *rotundul mic* are originea în porțiunea laterală a fosei infraspinoase, se inseră pe partea inferioară a tuberozității mari şi produce rotația externă;
- *rotundul mare* are originea pe marginea infero-laterală a scapulei, se inseră pe marginea micii tuberozități şi produce adducție şi rotație internă**;**
- *bicepsul brahial* are originea capului lung direct deasupra labrumului glenoidal sau medial de tuberculul supraglenoidal. Tendonul ajunge până lateral de capsula articulară şi şanțul bicipital, unde este acoperit de ligamentul transversal. Originea capului scurt este pe vârful procesului coracoid, se inseră pe tuberozitatea radială iar acțiunea sa este de flexie a cotului şi de supinație.

Tendinopatiile se exprimă din punct de vedere imagistic prin zone de hipersemnal în T2 sau în secvențe cu supresie de grăsime care pot asocia şi zone de lipsă de semnal datorate calcificărilor apărute prin depozite de hidroxi-apatită.

Rupturile muşchilor lojei rotatorii a umărului pot fi parțiale ale muşchilor în contact cu suprafața articulară, mai frecvente decât cele ale structurilor musculare în contact cu suprafața burselor articulare. Mai pot fi intratendinoase sau complete. În vederea stabilirii conduitei terapeutice adecvate este importantă identificarea şi descrierea amănunțită a extensiei leziunilor.

Cea mai frecventă leziune a muşchilor rotatori este considerată cea a tendonului supraspinos, la 1-2 cm de inserția pe tuberozitatea mare. Rupturi ale muşchilor infraspinos sau rotund mic pot fi observate în asociere cu cea a muşchiului supraspinos, dar foarte rar izolate.

Tenosinovita capului lung al muşchiului biceps brahial apare sub forma unei zone de hipersemnal vizibilă în T2. Este rareori izolată şi apare de cele mai multe ori în asociere cu tendinopatii ale celorlalți muşchi ai lojei rotatorii a umărului**.**

Evaluarea atrofiei musculare se face în T1 prin aprecierea cantității de grăsime conținută intramuscular. Cea mai frecventă este clasificarea Goutallier cu gradul 0 - fără grăsime, 1 - zone adipoase dispuse aleator, 2 - cantitatea de grăsime este mai mică decât cea de muşchi, 3 - cantitatea de muşchi este egală cu cea de grăsime, 4 - grăsimea este mai voluminoasă decât masa musculară.

Sindromul de conflict subacromial apare atunci când părțile moi subacromiale - tendonul supraspinos, structurile ce mărginesc bursa subacromială sau subdeltoidiană, capul lung al tendonului bicipital - sunt lezate de arcul coracoacromial în timpul abducției. Din punctul de vedere al modificărilor morfologice, acestea sunt de tipul osteoartritei asociate cu osteofit inferior, variantelor anatomice de localizare a acromionului, post-traumatice, ligamente coracohumerale îngroşate, tendinopatii, rupturi ale capului lung ale muşchiului biceps brahial, bursită subdeltoidiană sau subacromială.

Sindromul de conflict subcoracoid apare atunci când mica tuberozitate este presată de procesul coracoid în poziție de adducție, flexie şi rotație internă.

Sindromul de conflict posterosuperior rezultă în urma degenerării posterosuperioare a labrumului, precum şi a tendonului muşchiului infraspinos sau supraspinos. Clinic se prezintă sub forma durerii posterosuperioare şi instabilității anterioare.

Leziunile labrumului glenoidal şi instabilitatea articulară. Modificările degenerative ale cartilajului de acoperire se evidențiază prin zone de hipersemnal T2. Forma labrumului în secțiune axială depinde de poziția brațului şi, de obicei, labrumul posterior este mai mic decât cel anterior. Majoritatea leziunilor apar anteroinferior. Leziunile de tip SLAP (superior labrum anterior către posterior) prezintă 4 grade asociate cu leziunea muşchiului biceps brahial.

Există două forme de instabilitate:

- cea produsă de laxitatea capsulei sau de hipoplazia glenoidiană care, de obicei, este bilaterală;

- cea post-traumatică, produsă de leziuni ale ligamentelor articulare sau de leziunile labrumului sau ale cavității glenoide.

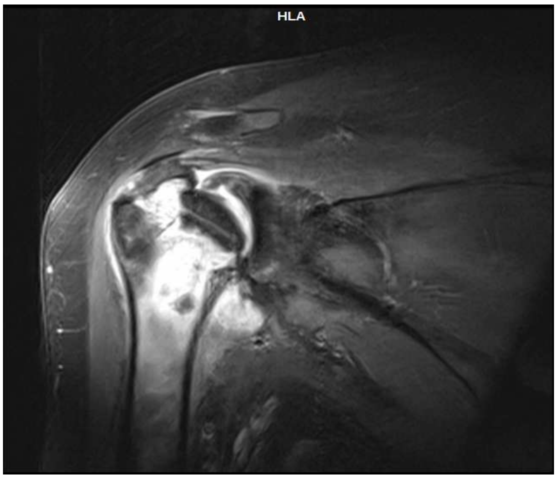

Fig. 1. Secțiune în plan coronal oblic. T1 FS după injectare de substanță de contrast paramagnetică. Tumoră infiltrativă în canalul medular humeral asociată cu fractură de cap humeral.

Evaluarea burselor articulare

Bursa subacromială/ subdeltoidiană este situată medial de procesul coracoid, lateral şi la 3 cm sub marea tuberozitate, iar anterior, caudal până la şanțul bicipital. Descoperirea fluidului în această bursă este un semn indirect de leziune a lojei muşchilor rotatori ai umărului.

Bursa subscapulară este situată între tendonul muşchiului subscapular şi capsula articulară**.**

Recesul subcoracoid este o extensie a capsulei articulare dincolo de tendonul subscapular.

Bursa subcoracoidă este localizată anterior de tendonul subscapularului şi are formă longitudinală în plan sagital. Acumularea fluidă la acest nivel se datorează leziunilor de la nivelul porțiunii anterioare a muşchilor lojei rotatori ai umărului.

Bursa coracoclaviculară sau supracoracoidă este localizată deasupra procesului coracoid, între porțiunea conoidă şi cea trapezoidă ale ligamentului coracoclavicular.

Bursa supraacromială este localizată superficial şi situată deasupra acromionului.

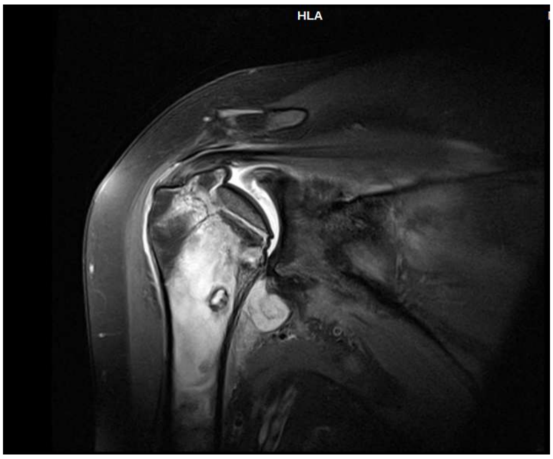

Fig. 2. Secțiune coronală PD FS. Se vizualizează semnalul neomogen osos, infiltrarea musculaturii adiacente şi traiectele multiple de fractură.

Evaluarea leziunilor nervoase

Leziunea nervului suprascapular poate produce edem nespecific muscular în etapa precoce şi ulterior atrofie musculară a supra- şi infraspinosului. Cea mai frecventă cauză de compresie a nervului o constituie chisturile paralabrale**.**

Compresia nervului axilar, delimitat între humerus lateral, tricepsul brahial medial, rotundul mic superior şi rotundul mare inferior produce atrofia muşchiului rotund şi parțial deltoid. Cea mai frecventă cauză a compresiei o constituie tumorile.

Aspectul postoperator al umărului

Cel mai util examen este cel preoperator, pentru comparație.

Postartroscopie, o leziune a muşchilor rotatori poate fi vizibilă prin o zonă de hipersemnal T2 sau STIR şi reprezintă traiectul de abord.

În intervenția pentru *leziuni de continuitate* musculară, semnalul reconstrucției trebuie să fie diferit de cel fluid, iar evoluția favorabilă presupune şi absența fluidului în burse prin apariția de țesut cicatriceal.

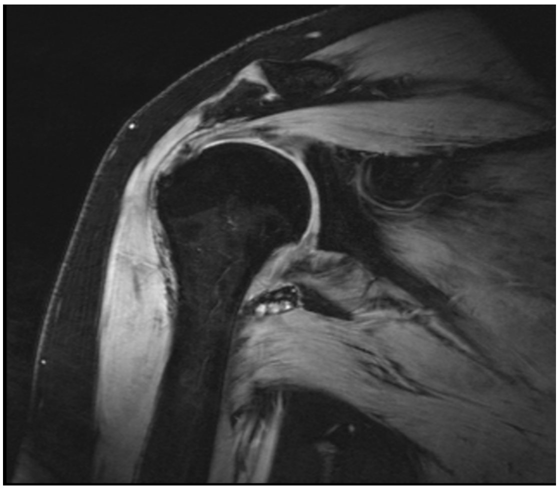

Fig. 3. Sindromul de „impingement” acromio-clavicular. Reducere a calibrului tendonului de inserție al muşchiului supraspinos. Secvență PD FS în plan coronal.

Intervenția chirurgicală pentru instabilitate are ca aspect de reuşită fragmentul desprins reataşat labrumului şi îngroşat. Apariția unei cicatrice fibroase se exprimă imagistic printr-o zonă de hiposemnal T1 şi T2.

Concluzii

IRM standard poate evalua eficient leziunile coafei muşchilor rotatori. Artrografia IRM directă sau indirectă, precum şi examinarea dinamică IRM evidențiază anumite leziuni care nu sunt sesizabile printr-o evaluare standard, aşa cum se întâmplă în cazul inserțiilor capsulare. IRM cu câmp de intensitate crescută poate evalua mai eficient şi cu acuratețe mai mare leziunile articulației şi ale structurilor musculare din această regiune.

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Ecografia de contrast – aplicații clinice

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Rezumat. Substanțele de contrast ecografice au apărut din nevoia de a ameliora limitele explorării ecografice convenționale şi Doppler, mai ales în ceea ce priveşte evaluarea microvascularizației. Tehnicile ecografice specifice de contrast se bazează astfel pe anularea şi/sau separarea semnalelor generate de țesuturi şi de către microbule, iar modificările ecogenității în timpul administrării substanțelor de contrast urmează o dinamică specifică descrisă prin utilizarea unor timpi vasculari bine stabiliți. Urmărirea timpilor vasculari şi a dinamicii substanței de contrast ecografice permite o evaluare mai completă a leziunii suspecte, cu creşterea specificității şi sensibilității diagnostice. În general, substanțele de contrast ecografic au o siguranță sporită, cu o incidență scăzută a efectelor secundare, ceea ce face ca în prezent ecografia cu substanțe de contrast să capete un rol din ce în ce mai bine definit în algoritmul de investigații al formațiunilor nodulare, în principal hepatice, studiile viitoare având misiunea de a o evalua în caracterizarea altor entități patologice.

Cuvinte cheie: ecografie de contrast, CEUS (Contrast-Enhanced Ultrasound), microbule, captare

Abstract. Ultrasound contrast agents are used to improve the limits of the conventional and Doppler ultrasound, especially in the evaluation of the microvascularization. Contrast enhanced ultrasound stand on the cancellation and/or the separation of the signals generated by the tissues and the microbubbles and the modification of the echogenicity using an ultrasound contrast agent has a specific dynamics described by well established vascular times. By following the vascular times and the dynamics of the contrast agent it will allow a more complete evaluation of the suspect lesion enhancing the sensibility and the specificity. Generally the ultrasound contrast agents are safety with a low incidence of the adverse reactions that’s why nowadays contrast enhanced ultrasound is getting a more and more important place in the algorithm of investigation of the nodular lesions especially of the liver and the future studies have the mission to evaluate this technique in characterization of other pathologies.

Keywords: contrast ultrasound, CEUS (Contrast-Enhanced Ultrasound), microbubbles, uptake

Apărută din încercarea de a depăşi limitelele metodei convenționale, ecografia cu substanțe de contrast (CEUS) se constituie azi ca o nouă metoda de investigație cu un potențial imens în ceea ce priveşte aplicațiile clinice.

Substanțele de contrast ultrasonografic sunt în realitate microbule de gaz puțin solubil (aer, azot, perfluorocarbon) stabilizate de un înveliş lipidofil (albumină, galactoză, lipide, polimeri).

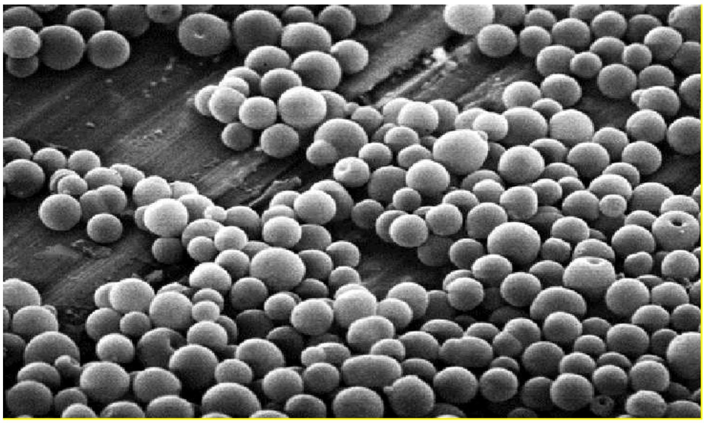

Fig. 1. Aspectul electronomicroscopic al microbulelor

Având diametrul de 1-4 microni (comparabil cu cel al unei hematii), aceste substanțe prezintă o farmacokinetică specifică, distribuindu-se strict intravascular şi oferind astfel o evaluare în timp real a fluxului sangvin din zona de interes în trei faze distincte .

	Vizualizare Timp post administrare (în secunde)	
Faza	Start	Sfârşit
Arterială	10 – 20	25 – 35
Portal venoasă	30 – 45	120
Tardivă	> 120	Dispariția microbulelor (aprox 240 – 360)

În prezent, în Europa se utilizează mai multe tipuri de substanțe de contrast ultrasonografice, fiecare cu indicații specifice: Levovist (1996) – cord, abdomen, reflux vezicoureteral, transcranial; Optison (1998) – cord; SonoVue (2001) – cord, macrovascular (artere cerebrale şi periferice, vena portă), microvascular (ficat, sân); Luminity (2006) – cord.

Tehnica ecografiei de contrast necesită utilizarea unui soft specific care realizează anularea şi/sau separarea semnalelor lineare generate de țesuturi şi utilizarea răspunsurilor nonlineare generate de microbule produse prin două mecanisme:

- răspuns nonlinear generat de oscilațiile microbulelor la presiuni acustice mici (index mecanic redus);
- răspuns nonlinear de înaltă energie produs de distrugerea microbulelor.

În general, substanțele de contrast ecografic au o siguranță sporită, cu o incidență scăzută a efectelor secundare, nu sunt nefrotoxice şi nu interacționează cu glanda tiroidă. Reacțiile adverse cele mai frecvente au fost: cefaleea (2.3%), durerea la locul inoculării (1,4%) sau alte reacții locale

(1,7%). Incidența fenomenelor alergice severe este comparativ egală cu cea a substanțelor de contrast CT, şocul anafilactic fiind raportat în 0.001%

Se recomandă aprecierea raportului beneficiu-risc la evaluarea anumitor țesuturi în care alterarea microvascularizației ar avea implicații clinice importante (ochi, creier, nou-născut, boala coronariană severă). Ecografia de contrast este contraindicată în: sindromul coronarian acut recent, ICC clasa III-IV NYHA, HTP severă, HTA necontrolată, insuficiență cardiacă/respiratorie acută, sarcină şi în alăptare.

În ceea ce priveşte indicațiile utilizării ecografiei cu substanțe de contrast în prezent, această metodă ecografică este folosită pentru explorarea următoarelor organe:

1. **FICAT:**
   Identificarea şi caracterizarea leziunilor hepatice:
   - descoperite accidental;
   - la pacienții cu hepatită cronică sau ciroză hepatică;
   - la pacienții cu antecedente de malignitate;
   - la pacienții cu rezultate CT/RMN, anatomopatologice şi histologice neconcludente;
   - în caracterizarea trombozei de venă portă;
   - în monitorizarea tehnicilor ablative percutane ale leziunilor maligne hepatice.

2. **RINICHI:**
   - evaluarea variantelor anatomice mimând o tumoră renală (pseudotumoare);
   - caracterizarea leziunilor chistice complexe şi a carcinomului chistic renal;
   - caracterizarea trombozei de venă renală şi venă cavă;
   - evaluarea afecțiunilor vasculare renale, incluzând infarctul renal şi necroza corticală;
   - evaluarea şi monitorizarea traumatismelor renale;
   - la pacienții cu contraindicație de utilizare a substanțelor de contrast CT şi RMN.

3. **REFLUX VEZICO-URETERAL:**
   - evaluarea refluxului după tratamentul conservator sau chirurgical;
   - evaluarea inițială a refluxului la fete;
   - screening pentru reflux (transplant, etc.).

4. **PANCREAS:**
   - caracterizarea leziunilor pancreatice, a dimensiunilor, marginilor şi a relațiilor cu sistemul vascular;
   - diferențierea psudochistelor de tumorile chistice;
   - diferențierea componentelor vasculare (solide) şi avasculare (lichidiene/necroză) ale unei leziuni pancreatice.

5. **TRAUMATISME ABDOMINALE:**
    - evaluarea leziunilor traumatice hepatice, splenice şi renale.

6. **CRANIU:**
    - evaluarea circulației cervico-craniene şi identificarea ocluziilor arteriale.

Constituirea substanței de contrast este foarte uşoară şi rapidă utilizând trusa de preparare a fiecărui produs, permițând astfel trecerea în câteva minute de la ecografia convenționala la cea cu contrast, ceea ce în anumite situații clinice echivalează cu trecerea de la a şti ceva la a şti (aproape) totul.

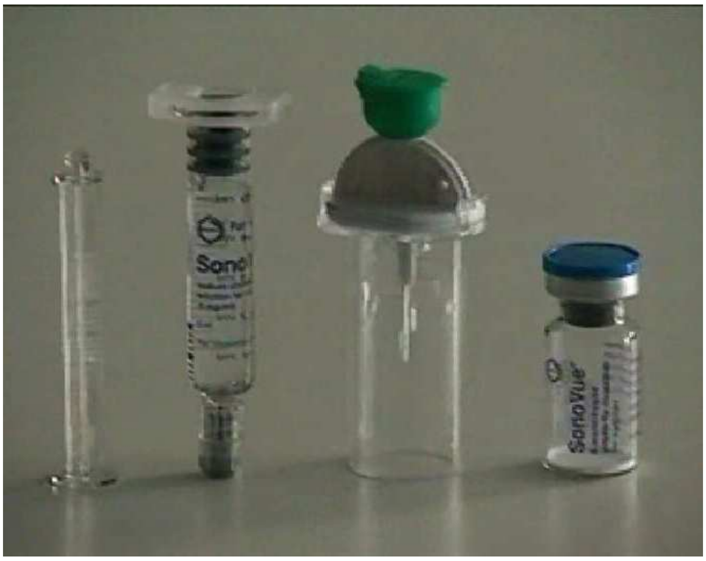

Fig.2. Trusa preparare Sonovue (Bracco) utilizată în SUUMC

În ceea ce priveşte posologia pentru preparatul SonoVue pe care noi îl utilizăm, se recomandă pentru ecocardiografie - 2 ml bolus, pentru ecografie Doppler - 2,4 ml bolus sau PEV 1ml/min, iar pentru ecografia intravezicală 1 - 10% vol. VzU.

Leziunile focale hepatice au devenit cea mai importanta aplicație a substanțelor de contrast (cu excepția aplicațiilor în ecocardiografie), datorită îmbunătățirii marcate a detecției şi caracterizării acestora prin utilizarea substanțelor de contrast. În prezent, ecografia cu substanțe de contrast egalează şi chiar depăşeşte acuratețea CT în examinarea dinamică a repartiției substanțelor de contrast, permițând o evaluare optimă a leziunii suspecte, iar locul ei în algoritmul de investigații al formațiunilor nodulare este din ce în ce mai bine definit, studiile viitoare având misiunea de a evalua tehnica ecografică cu substanțe de contrast în afectările difuze parenchimatoase sau în caracterizarea imunologică a tumorilor (tabelele 1,2).

Tabel 1. Caracteristicile CEUS ale leziunilor benigne hepatice

Leziuni benigne hepatice			
tumoră	Faza arterială	Faza porto-venoasă	Faza tardivă
Hemangiom			
caracteristici tipice	Captare periferică nodulară. Fără captare centrală, captare inelară.	Umplere parțială /completă centripetă	Captare completă
caracteristici suplimentare	Leziuni mici: captare completă centripetal, rapidă		Arii centrale necaptante (tromboză parțială, fibroză)
Hiperplazie focală nodulară			
caracteristici tipice	Hipercaptare completă şi precoce.	Hipercaptare	Izo sau hipercaptare
caracteristici suplimentare	Artere stelate cu umplere centrifugă	Hipocaptare –cicatrice centrală	Hipocaptare –cicatrice centrală
Steatoza focală			
caracteristici tipice	Izocaptare	Izocaptare	Izocaptare
Chiste			
caracteristici tipice	Fără captare	Fără captare	Fără captare
Adenom			
caracteristici tipice	Hipercaptare completă	Izocaptare	Izocaptare
caracteristici suplimentare	Arii necaptante (hemoragice)	Hipercaptare Arii necaptante (hemoragice)	Arii necaptante (hemoragice)
Abces			
caracteristici tipice	Captare inelară, fără captare centrală	Hiper/izocaptare inelară, fără captare centrală. Hipocaptare inelară.	Hipocaptare inelară, fără captare centrală.
caracteristici suplimentare	Sept hipercaptant Hipercaptare segmentară		

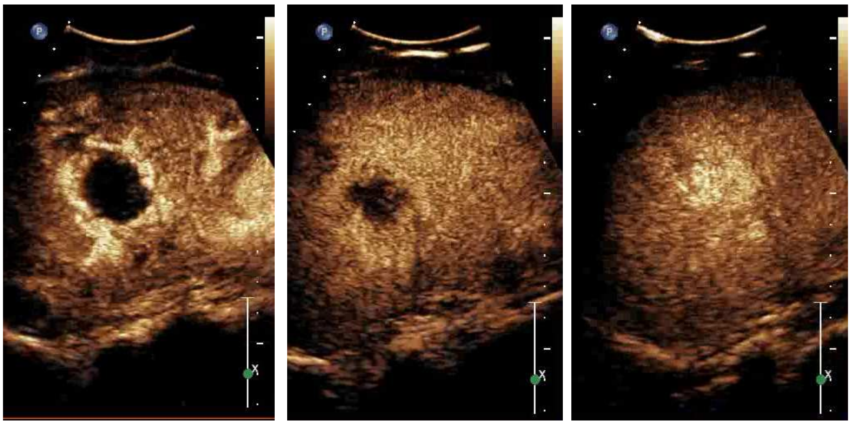

Fig. 3. Hemangiom hepatic: faza arterială - captare inelară; faza portală – umplere centripetă; faza tardivă – captare completă.

Tabel 2. Caracteristicile CEUS ale leziunilor maligne hepatice

Leziuni maligne hepatice			
HCC caracteristici tipice caracteristici suplimentare	Hipercaptare completă. Arii necaptante (necroză). Vascularizație haotică, captare tromb tumoral în VPo.	Izo/hipocaptare Arii necaptante(necroză)	Hipocaptare
Metastaze hipovasculare caracteristici tipice caracteristici suplimentare	Captare inelară. Hipercaptare completă. Arii necaptante (necroză)	Hipocaptare Arii necaptante (necroză)	Hipo/fără captare
Metastaze hipervasculare caracteristici tipice caracteristici suplimentare	Hipercaptare completă Vascularizație haotică	Hipocaptare	Hipo/fără captare
Colangiocarcinom caracteristici tipice caracteristici suplimentare	Captare inelară Fără captare	Hipo/ fără captare	Hipo/ fără captare

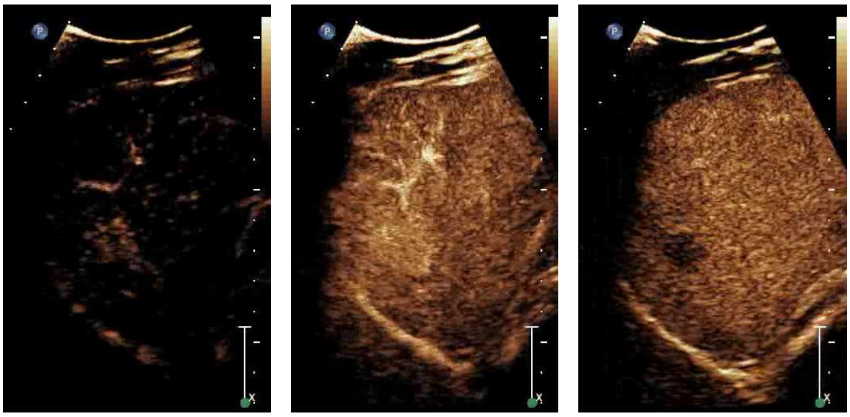

Fig. 4. Metastază hepatică: faza arterială – hipercaptare completă; faza portală şi tardivă -hipocaptare şi wash out.

În laboratoarele de cercetare, şi sperăm ca în viitor şi în practica clinică, se utilizează microbule de gaz cu liganzi specifici pentru inflamație (Boala Crohn, sindroame coronariene acute, ATS), boala neoplazică (angiogeneză), administrarea țintită a medicației sau inginerie genetică.

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Evaluarea răspunsului hemodinamic prin bioimpedanță toracică al pacienților la medicamentele antihipertensive (studiu pilot)

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Rezumat. Medicamentele antihipertensive prezintă efecte diferite asupra funcției cardiace, rezistenței periferice totale, rezistenței vasculare sistemice şi a volumului plasmatic. Acest studiu evaluează prin bioimpedanță răspunsul hemodinamic sistemic la 21 de pacienți hipertensivi la administrarea medicamentelor antihipertensive. Datele hemodinamice sunt rezultatul utilizării valorilor obținute cu ajutorul electrozilor ECG şi calcularea parametrilor pe baza acestor valori. În lotul analizat, pentru valorile inițiale ale indexului cardiac, nu am identificat nici un pacient cu valori crescute ale acestuia, 6 pacienți aveau un index cardiac scăzut, iar la 15 pacienți valorile erau în limite normale (2.5-4.7). De asemenea, valorile indexului rezistenței vasculare sistemice au fost normale la 2 dintre pacienți, restul având valori crescute. Am observat că evoluția indexului cardiac reprezintă un parametru pentru urmărirea răspunsului la medicamente. Bioimpedanța toracică poate fi folosită ca o alternativă neinvazivă de evaluare a performanței cardiace la pacienții hipertensivi în Unitatea de Primiri Urgențe.

Cuvinte cheie: bioimpedanță, hipertensiune, medicamente antihipertensive

Abstract. Antihypertensive drugs have different effects on cardiac function, total peripheral resistance, systemic vascular resistance and plasma volume. This study evaluates the systemic haemodynamic response by bioimpedance in 21 hypertensive patients taking antihypertensive drugs. Hemodynamic data are obtained by using values obtained with ECG electrodes and calculating parameters based on these values. In the group analyzed for baseline cardiac index, we have not identified any patient with elevated it, 6 patients had a low cardiac index, and 15 patients were within normal values (2.5-4.7). Also, the values of systemic vascular resistance index were normal in two patients, the rest having elevated. We noticed that the evolution of cardiac index parameter is a measure of medication. Bioimpedanța chest can be used as an alternative noninvasive cardiac evaluation of performance in hypertensive patients in the emergency department.

Key words: bioimpedance, hypertension, antihypertensive drugs

INTRODUCERE

Fiziopatologia urgențelor hipertensive nu este pe deplin elucidată. Afectarea autoreglării normale şi creşterea bruscă a rezistenței vasculare sistemice (*RVS*) sunt în mod tipic etapele inițiale în declanşarea acestei patologii. Creşterea RVS este determinată de eliberarea substanțelor vasoconstrictoare umorale de către celulele peretelui vascular. Ulterior, creşterea presiunii vasculare determină un ciclu care cuprinde afectare endotelială, activarea locală intravasculară a cascadei coagulării, necroză fibrinoidă a vaselor sangvine de calibru mic şi eliberarea altor substanțe vasoconstrictoare. Dacă acest proces nu este oprit, se formează un ciclu ulterior determinat de leziunea vasculară, ischemie tisulară şi disfuncția autoreglării [1,2].

Urgențele hipertensive cuprind un spectru larg de manifestări clinice determinate de valorile necontrolate ale tensiunii arteriale (*TA*) care determină disfuncții progresive sau rapide de sisteme de organe. Pentru pacienții care se prezintă cu urgențe hipertensive afectarea unui singur sistem este identificată la 83% dintre aceştia, afectarea a două sisteme la 14% dintre pacienți, iar afectarea multisistemică (mai mult de 3 sisteme) la aproximativ 3% dintre aceştia [3].

Cele mai comune forme clinice de prezentare ale urgențelor hipertensive sunt reprezentate de accidentele vasculare (24.5%), edemul pulmonar acut (22.5%), encefalopatia hipertensivă (16.3%) şi insuficiența cardiacă congestivă (12%). Forme clinice de prezentare mai puțin frecvente sunt reprezentate de hemoragia intracraniană, disecția de aortă şi eclampsia [3, 4].

Odată cu dezvoltarea medicamentației antihipertensive utilizate în tratamentul urgențelor hipertensive (*tabel 1*), incidența acestora a scăzut de la 7% la aproximativ 1% la pacienții hipertensivi [**5**]. De asemenea, ratele de supraviețuire la 1 an ale pacienților au crescut de la doar 20% (în anii 50) la mai mult de 90% la cei cu tratament antihipertensiv adecvat [6].

Tabel 1. Medicamente antihipertensive utilizate în funcție de tipul urgenței hipertensive
(prelucrare după: Winshall şi Lederman [**7**])

Tipuri de urgențe hipertensive	**Ținta tratamentului**	**Prima linie de medicamente**	**Observații**
Encefalopatia hipertensivă	Reducerea cu 20-25% a TAM în 2-3 ore (păstrarea TAD> 100mmHg)	Nitroprusiat Fenoldapam Labetolol Nicardipină	Tratamentul poate agrava disfuncția neurologică; A se evita clonidina, betablocantele
Accidentul vascular ischemic	Scăderea TA lent în primele 24 de ore	Labetolol Nicardipină	Nitroprusiatul, fenoldapamul şi nitroglicerina pot creşte presiunea intracraniană
Hemoragia intracraniană	TAS 140-160 mmHg (sau la nivelul dinaintea AVC)	Labetolol Nitroprusiat Nicardipină	Monitorizarea agravării disfuncțiilor neurologice după scăderea TA
Hemoragia subarahnoidiană	Dacă pacientul este alert, se discută terapia de scădere a riscului de resângerare	Nimodipină ± Labetolol	Nitroprusiatul, Fenoldapamul şi Nitroglicerina pot creşte presiunea intracraniană
Edemul pulmonar acut	TAD < 100 mmHg sau rezoluția simptomatologiei	Nitroprusiat + NTG + diuretic	Se evită medicația inotrop pozitivă în disfuncția de VS Se identifică semnele de ischemie miocardică
Infarctul de miocard	TAD < 100 mmHg sau rezoluția simptomatologiei	NTG Betablocante	
Disecția de aortă	TAS 100-120 mmHg sau TAM 80 mmHg	Nitroprusiat sau Fenoldapam + Betablocante sau Labetolol	Se monitorizează diureza Se evită monoterapia cu vasodilatatoare
Eclampsia	TAD 90-105 mmHg sau TAM < 125 mmHg	Hidralazină Labetolol Nifedipină	Se poate administra per os metildopa Se evită nitroprusiatul şi inhibitorii enzimei de conversie

Obs: TAS – tensiunea arterială sistolică; TAD – tensiunea arterială diastolică; TAM – tensiunea arterială medie.

În practica medicală, metoda clasică de măsurare a parametrilor hemodinamici în unitățile de terapie intensivă a fost reprezentată de cateterul Swan-Ganz, termodiluția fiind metoda standard de evaluare a modificărilor hemodinamice şi metoda de referință pentru compararea sensibilității şi specificității altor metode [8, 9].

Bioimpedanța toracică reprezintă o metodă relativ nouă de monitorizare hemodinamică cardiacă neinvazivă [10, 11]. Această metodă permite calcularea volumului bătaie, a debitului cardiac şi a volumului fluidului toracic, indici importanți pentru determinarea modificărilor hemodinamice, care pot fi utilizați pentru selectarea medicamentelor şi dozelor pentru tratamentul hipertensiunii arteriale cronice [12, 13].

În studiul nostru am evaluat efectele hemodinamice ale antihipertensivelor în tratamentul puseului de hipertensiune arterială la pacienții monitorizați în Unitatea de Primiri Urgențe (UPU).

MATERIALE ŞI METODĂ

Pacienții cu puseu de hipertensiune arterială au fost selectați pentru investigații de-a lungul anului 2010 în cadrul Unității Primire Urgențe a Spitalului Universitar de Urgență Militar Central.

Criteriile de includere au fost: semne clinice de encefalopatie hipertensivă, TAM ≥ 120 mmHg, mai puțin de două ore de la debutul simptomatologiei.

Criteriile de excludere au fost: regurgitare aortică mai mare de gradul II, fibrilație atrială, pacemaker, tahicardia mai mare de 120 bătăi/min, existența unui anevrism ventricular.

Măsurătorile hemodinamice au fost obținute prin bioimpedanță electrică toracică folosind monitorul BeneView T6/T8 (*figura 1*). Instrumentul foloseşte electrozi de suprafață aplicați la baza gâtului şi la baza toracelui. Un curent alternativ de joasă tensiune şi mare amplitudine este introdus prin intermediul senzorilor periferici externi şi sesizat prin intermediul senzorilor interni. Diferența de voltaj dintre curentul introdus şi cel sesizat este invers proporțională cu cantitatea de sânge toracic.

Figura 1. Monitorul BeneView T6/T8

Volumul bătaie a fost calculat prin algoritmi care utilizează modificările impedanței în funcție de timp de-a lungul sistolei electrice. Debitul cardiac a fost determinat ca produs al volumului bătaie şi al frecvenței cardiace. Indicele de rezistență vasculară sistemică a fost calculat din determinarea indexului cardiac şi a tensiunii arteriale.

Prin implementarea unui algoritm de monitorizare prin impedanță toracică, am folosit 3 etape standard de tratament pentru fiecare dintre subiecți (inhibitori ai enzimei de conversie a angiotensinei, diuretice tiazidice şi un vasodilatator). După 5, 10, 20 şi respectiv 30 de minute am evaluat efectele medicamentelor utilizate asupra debitului cardiac, a valorii totale a rezistenței periferice, a rezistenței vasculare sistemice şi a volumului plasmatic.

REZULTATE ŞI DISCUȚII

Între iunie şi decembrie 2010, în acest studiu pilot am inclus 21 de pacienți (12 bărbați şi 9 femei). Au fost investigați un număr total de 28 de pacienți cu puseu hipertensiv. În conformitate cu protocolul de includere / excludere nu au fost analizați 7 pacienți (25%).

Vârsta medie a fost de 63.85±10.4 ani, iar indexul de masă corporală 29.6±3.8. La pacienții incluşi în studiu, dintre comorbiditățile cardiovasculare au fost prezente: diabetul zaharat (23.8%), angina pectorală (38.1%), hiperlipemia (33%).

După 30 de minute, am observat că valoarea medie a valorilor TAS a scăzut cu 22.7%, iar cea pentru TAD cu 14.5%. De asemenea, valoarea medie a TAM a scăzut cu 18.6% (*figura 2*).

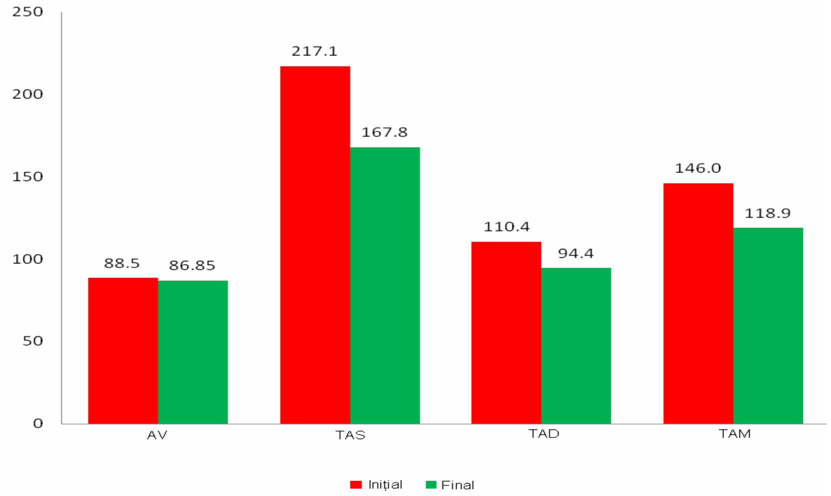

Figura 2. Variația valorilor medii ale parametrilor hemodinamici
Obs: AV – alura ventriculară; TAS – tensiunea arterială sistolică; TAD – tensiunea arterială diastolică; TAM – tensiunea arterială medie.

În *figura 3* sunt reprezentate variațiile temporale ale debitului cardiac şi indexului cardiac pentru pacienții analizați. Se observă că evoluția indexului cardiac reprezintă un parametru pentru urmărirea răspunsului la medicamente.

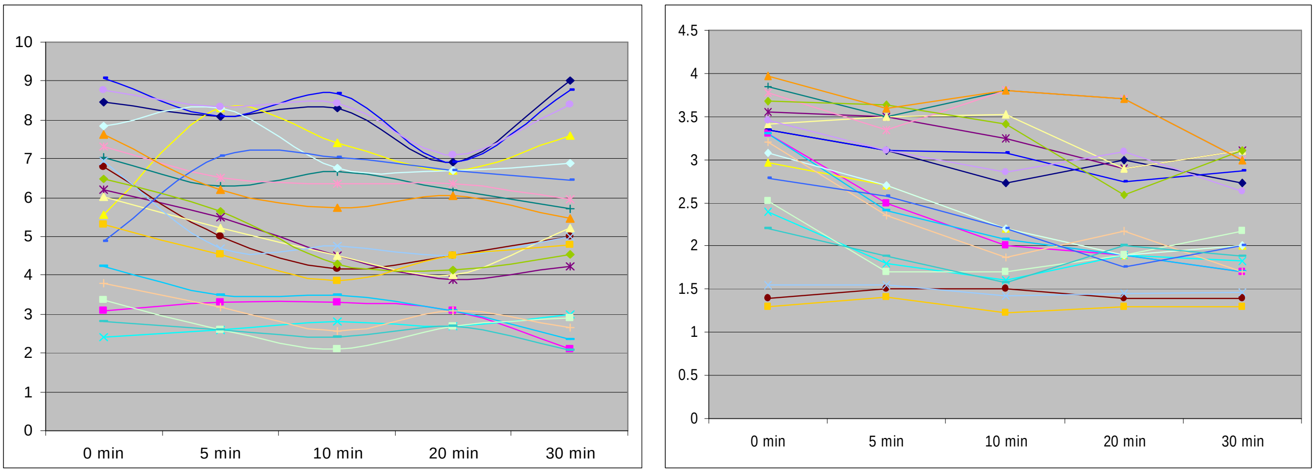

Figura 3. Variațiile temporale ale debitului cardiac şi indexului cardiac

În lotul analizat, pentru valorile inițiale ale indexului cardiac, nu am identificat nici un pacient cu valori crescute ale acestuia, 6 pacienți aveau un index cardiac scăzut, iar la 15 pacienți valorile erau în limite normale (2.5-4.7). De asemenea, valorile indexului rezistenței vasculare sistemice au fost normale la 2 dintre pacienți, la restul fiind crescute.

Studiile anterioare au demonstrat o îmbunătățire, în controlul tensiunii arteriale folosindu-se măsurători neinvazive hemodinamice, cum ar fi bioimpedanța toracică [14, 15].

Într-un studiu recent, Smith şi colaboratorii au evaluat utilizarea acestei metode la pacienții cu hipertensiune necontrolată. Studiul a înrolat 164 pacienți, care au întrerupt toate medicamentele antihipertensive, şi apoi au fost randomizați în două grupuri: primul grup la care a fost utilizată selecția empirică de medicamente antihipertensive, şi grupul al doilea la care a fost utilizată administrarea hipertensivelor în funcție de monitorizarea hemodinamică. La cel de-al doilea grup a fost observat un răspuns mai bun la terapia antihipertensivă.

Folosirea măsurătorilor prin bioimpedanță pentru ghidarea terapiei antihipertensive este un concept relativ nou. Rezultatele studiilor publicate demonstrează beneficiul reprezentat de utilizarea măsurătorilor seriate hemodinamice prin bioimpedanță pentru tratamentul antihipertensiv cronic în ambulator, dar şi pentru tratamentul de urgență.

Prin ajustarea dozelor de medicamente antihipertensive administrate utilizând ghiduri terapeutice se va obține o reducere semnificativă a tensiunii arteriale şi o îmbunătățire a controlului acesteia în UPU. Astfel, bioimpedanța toracică poate fi folosită ca o alternativă neinvazivă de evaluare a performanței cardiace la pacienții hipertensivi în urgență.

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Markeri tumorali serici în ginecologie

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Rezumat. Instrumentele de screening utilizate în identificarea cancerelor, indiferent de localizarea acestora, au suferit modificări majore în ultimii douăzeci de ani. Cancerele ginecologice beneficiază de un spectru restrâns de markeri serici, iar până în prezent ne mulțumeam cu un singur reprezentant utilizat intensiv – CA 125. Noile tehnologii au permis introducerea, inițial în studii clinice şi ulterior în practica clinică, a unor molecule (hCG hiperglicozilat, CA 72-4, CYFRA 21.1, SCC , HE4) utilizate singular sau grupate sub formă de algoritmi a căror capacitate de discriminare a diferitelor patologii tumorale este din ce în ce mai crescută.

Cuvinte cheie: marker tumoral, cancer ovarian, boală trofoblastică gestațională, cancer cervical, ROMA

Abstract. The last 20 years have seen major changes in cancer screening tools regardless their location. Gynecological cancers have a low range of serum markers, CA-125 being the only one extensively used in clinical practice. New molecules (hyperglicosilated hCG, CA 72-4, CYFRA 21.1, SCC, HE4) were used at first in clinical studies and then either single or grouped as algorithms, allowing higher sensitivity rates in differentiating cancer or other conditions.

Key words: tumor marker, ovarian cancer, trophoblastic gestational disease, cervical cancer, ROMA

Instrumentele de screening utilizate în identificarea cancerelor, indiferent de localizarea acestora, au suferit modificări majore în ultimii douăzeci de ani. Dacă până în prezent ne mulțumeam cu un singur marker - utilizat intensiv în practica ginecologică – CA-125, noile tehnologii au permis introducerea, inițial în studiile clinice şi ulterior în practica clinică, a diferiților indicatori, întrebuințați singular sau grupați sub formă de algoritmi. Nu toate cancerele ginecologice beneficiază de markeri. Cancerele vulvar şi endometrial nu se află nici măcar la nivel de studii clinice din acest punct de vedere, iar în ceea ce priveşte cancerul cervical, trendul actual îl reprezintă testarea HPV mai degrabă decât utilizarea unor markeri serici. Cancerul ovarian este marele “câştigător” al competiției, fiind cel mai studiat, însă până în prezent niciuna dintre metodele de screening nu se apropie de ideal. O altă patologie caracteristică sarcinii - boala trofoblastică gestațională - şi-a îmbogățit spectrul serologic de identificare şi diferențiere cu recunoaşterea oficială şi încercarea de a introduce în practica curentă a unei forme hiperglicozilate de hCG - moleculă diferită de hCG-ul folosit până în prezent pentru evaluarea diferitelor forme şi etape ale stării de gestație.

Abilitatea de a detecta tumorile maligne cu ajutorul unui test de sânge a fost unul dintre dezideratele majore în screeningul medical. Biomarkerul tumoral este o moleculă asociată unui anumit tip de procese tumorale, produs în exces de către celula tumorală, ale cărei modificări cantitative se corelează cu geneza şi dezvoltarea tumorii. În practica clinică termenul este folosit pentru moleculele care pot fi detectate în serul plasmatic, lichidele corporale, tumori solide, celule tumorale circulante, noduli limfatici şi măduva osoasă. Orice proteină tumorală este un potențial antigen. Markerii tumorali sunt utili în determinarea prognosticului bolii, detectarea recurențelor tumorale, monitorizarea şi stabilirea tratamentului şi, în anumite cancere, pot fi utilizați pentru screening. Marker-ul ideal ar trebui să aibă 100% sensibilitate, specificitate şi valoare predictivă pozitivă. Mai mult, ar trebui să prezinte specificitate tumorală şi să fie produs în cantități suficiente pentru a permite detectarea bolii minimale, astfel încât să reflecte cantitativ amploarea tumorii.

Principalul factor limitativ îl constituie absența specificității, markerii tumorali utilizați astăzi în practica curentă fiind crescuți atât în neoplazii, cât şi în diferite condiții fiziologice şi patologice. Din acest motiv, cu mici excepții, markerii tumorali sunt utilizați în principiu pentru monitorizarea răspunsului terapeutic şi al evoluției bolii. Singura moleculă care se apropie de caracteristicile markerului ideal este PSA.

Markerii tumorali se clasifică, în funcție de specificitate, în markeri tumorali specifici pentru țesut (tisulari) şi markeri specifici unui anumit tip de cancer. Criteriile biochimice împart aceste molecule în antigene de suprafață celulară, proteine citoplasmatice, enzime şi hormoni. După greutatea moleculară se pot împărți în proteine cu greutate moleculară mică (<10.000 Daltoni) şi antigene moleculare macrotumorale. Markerii tisulari se detectează prin tehnici de imunohistochimie, imunofluorescența şi ELISA.

PROTEINE	**CA-19.9/ CA-125/ CA72-4/ CA15-3**
HORMONI	hCG/ hCG-H
ANTIGENE ONOCOFETALE	CEA, CA 72-4

Cancerul de sân	**CA-15.3, CEA, Her-2/neu, CA19.9**
Cancerul de col uterin	CYFRA 21.1, SCC
Cancerul ovarian şi tubar	**CA125**, HE4, **CEA**, AFP, CA72.4, BhCG
Boala trofoblastică gestațională	**hCG, h-hCG**

Cancerul ovarian şi cel tubar

La naştere, o femeie din şapte este predispusă la cancer de sân, în timp ce doar una din 68 poate dezvolta de-a lungul vieții o formă de cancer ovarian. Cu toate acestea, principala cauză de deces prin cancere genitale este cancerul ovarian. Se explică astfel necesitatea identificării cât mai precoce a acestei forme de neoplazii, cu atât mai mult cu cât simptomatologia în cancerele ovariene este nespecifică în stadiile incipiente, iar depistarea sa se face în stadii avansate, când posibilitățile terapeutice sunt nu de puține ori depăşite.

Aproximativ 90% dintre cancerele ovariene sunt carcinoame epiteliale celomice şi exprimă o glicoproteină numită CA-125. CA-125 este un antigen solubil cu greutate moleculară mare exprimat în toate țesuturile cu aceeaşi origine embriologică provenite fie din epiteliul celomic (pleură, pericard, peritoneu), fie mülerian (trompe uterine, endometru, endocervix), dar nu şi în epiteliul de suprafață ovarian normal al adultului şi fătului. Se regăseşte în schimb în tumorile papilare seroase şi mucinoase. Este cel mai investigat marker tumoral utilizat în practică. Valorile CA-125 sunt crescute în 50% din cancerele ovariene aflate în stadiul I şi în 90% în cele din stadiul II. Pragul utilizat de 30 UI/mL este lipsit de specificitate, valorile fiind crescute într-o multitudine de patologii. În ciuda acestor limitări, CA-125 a fost utilizat în screening-ul cancerului ovarian, singur sau în combinație cu ecografia.

De vreme ce toate țesuturile derivate din epiteliul celomic exprimă CA-125, valorile acestuia cresc în toate patologiile cu iritație a seroaselor. CA-125 se sintetizează intracelular şi este secretat activ în lumen. În cazul tumorilor benigne, CA-125 poate fi excretat în lichidul chistic. În tumorile invazive se crede că CA-125 este eliberat în ser prin dezorganizarea arhitecturală tumorală. CA-125 poate creşte şi în patologiile benigne caracterizate prin modificări arhitecturale tisulare.

Datorită absenței specificității, CA-125 nu poate fi folosit ca marker singular în screening-ul precoce al cancerului ovarian. Mai mult, la aproape jumătate dintre pacienții cu stadii incipiente ale CA-125, valorile CA-125 rămân în limite normale.

Valoarea predictivă preterapeutică a CA-125 este controversată, însă nivelurile acestui marker se corelează cu regresia, stabilitatea şi progresia bolii în 87-94% dintre cazuri.

Patologii caracterizate prin valori crescute ale CA 125	
Patologie ginecologică	**Patologie non-ginecologică**
Boala inflamatorie pelvină	Hepatita activă
Adenomioza	Pancreatita acută
Tumori ovariene benigne	Hepatopatie cronică
Endometrioza	Ciroza
Chist ovarian funcțional	Colita
Sindrom Meigs	Insuficiența cardiacă congestivă
Menstruație	Diabet zaharat (slab controlat)
Sindrom de hiperstimulare ovariană	Diverticulita
Infertilitate inexplicabilă	Mezoteliom
Fibrom uterin	Ascita de etiologie non-neoplazică
	Pericardită
	Pneumonie
	Poliarterita nodoasă
	Convalescență post intervenție chirurgicală
	Boala renală
	Lupus eritematos sistemic

CA 19-9 (antigen monosialogangliozidic utilizat frecvent în diagnosticul adenocarcinoamelor gastrointestinale) este crescut în 68-83% dintre cancerele ovariene mucinoase şi în doar 28% dintre cancerele non-mucinoase ovariene comparativ cu CA-125, care creşte în 80% dintre formele non-mucinoase.

CA72-4 sau TAG 72 este o glicoproteină mucinoasă specific tumorală, un antigen al glicoproteinelor asociate tumorilor umane mucinoase. Este utilizat frecvent în monitorizarea pacienților cu carcinoame gastrointestinale şi pancreatice. În tumorile mucinoase, CA-125, spre deosebire de CA 72-4, nu are valori crescute. S-au realizat mai multe studii în care s-au evaluat sensibilitatea şi specificitatea celor doi markeri separat şi împreună în detecția tumorilor ovariene iar concluziile au arătat într-adevăr că utilizarea combinată a CA-125, CA 72-4 şi a ecografiei transvaginale au crescut rata sensibilității la 87% în diferențierea tumorilor benigne de cele maligne.

Din necesitatea unei diagnosticări cât mai precoce a cancerului ovarian s-a încercat crearea unui algoritm, bazat pe vârsta pacientei şi pe evoluția valorilor CA-125, numit *ROCA* (Risk Of Ovarian Cancer Algorithm). În studiile efectuate utilizând acest algoritm au fost înregistrate foarte puține valori fals pozitive, iar specificitatea a ajuns la 90%. Utilizarea ROCA oferă trei posibilități de manageriere a pacientelor: cele cu risc scăzut vor monitoriza anual CA-125, cele cu risc intermediar vor repeta CA-125 la 3 luni, iar cele cu risc înalt vor fi evaluate clinic şi ecografic. Valoarea predictivă pozitivă a fost de 37.5% (CI 95%) iar specificitatea combinată a ROCA, urmată de ecografie transvaginală pentru identificarea cazurilor ce au necesitat intervenție chirurgicală, a fost de 99.7% (95%CI). ROCA este un model de medicină personalizată ce aplică mult mai bine tehnologiile disponibile la momentul actual.

HE4 este un marker biologic utilizat pentru detectarea cancerului ovarian având o expresie scăzută în țesutul ovarian normal. Nivelurile crescute de HE4 din serul pacientelor cu cancer ovarian se corelează cu expresia sa la nivel celular, sensibilitatea şi specificitatea fiind de 70-80%, respectiv 95-96%. Determinările repetate şi estimarea creşterii procentuale ale HE4 sunt utile în monitorizarea bolii şi a recurențelor la pacientele cu cancer epitelial. În prezent nu se utilizează ca test de screening, el nefiind specific pentru tumorile mucinoase cu celule germinale. Totuşi sensibilitatea HE4 pentru cancerele ovariene o depăşeşte pe cea a CA-125. Sensibilitatea HE4 a fost crescută atât în stadiile incipiente (62.4-82.7%), cât şi în cele avansate (74.6-92.5%). Huhtinen şi co. au analizat concentrațiile serice ale HE4 şi CA-125 la 225 de paciente cu cancer ovarian, cancer endometrial şi endometrioză. Combinația HE4-CA 125 s-a dovedit a fi superioară atât ca sensibilitate (92.9%), cât şi ca specificitate (95%) comparativ cu HE4 (sensibilitate 78.6%) şi CA-125(78.6%) utilizate singular. Mai mult, nivelurile HE4 au fost crescute atât în cancerul ovarian, cât şi în cel endometrial, dar nu şi în leziunile endometriozice (precum CA-125). Astfel, valorile crescute ale CA-125 combinate cu HE4 în limite normale sunt indicative pentru leziuni benigne sau endometriozice. Însă atât CA-125, cât şi HE4 crescute indică prezența unei forme de cancer fie ovarian, fie endometrial.

Odată cu identificarea HE4 (sensibilitate 72.9%, specificitate 95%) şi demonstrarea sensibilității şi specificității extrem de scăzute ale CA-125 în premenopauză s-a luat în considerare combinarea celor doi markeri pentru obținerea unei rate de discriminare superioare în identificarea neoplasmelor ovariene. Din acest motiv s-a încercat utilizarea combinată a celor doi markeri şi s-a stabilit un algoritm pentru obținerea unui index predictiv-*ROMA* (Risk Of Ovarian Malignancy Algorithm).

ROMA	**Risc scăzut**	**Risc crescut**
Premenopauză	<7.4%	≥7.4%
Postmenopauză	<25.3%	≥25.3%

Jacobs şi co. au dezvoltat un index preoperator al riscului de malignitate (*RMI*) prin evaluarea combinată a CA-125, ecografiei transvaginale (U) şi a statusului menopauzal (M). Valorile scorului astfel calculat (RMI= U x M x CA125) care depăşesc 200 sunt un indice serios de malignitate.

RMI = U x M x CA-125	
Ecografic	1 punct pentru fiecare dintre următoarele: chisturi multiloculare/ arii solide/ metastaze/ ascită/ leziuni bilateral U=0 (scor 0) U=1 (scor 1) U=2 (scor 2-5)
Menopauză	1= premenopauză 3= postmenopauză (definită ca menstră absentă>1 an sau histerectomie la pacientele peste 50 de ani)
CA-125	Valoarea serică măsurată în IU/mL

S-a încercat şi testarea altor markeri - CA 72-4, inhibina, osteopontina, receptorul EGF, oncogena HER2, MMP7, SLPI, PAI-1, fără însă a se obține rezultate semnificative.

Boala trofoblastică gestațională

Boala trofoblastică gestațională (BTG) este o afecțiune rară, asociată sarcinii, care are la bază anomalii ale proliferării trofoblastului. Spectrul de manifestări variază de la patologii benigne (mola hidatiformă incompletă şi completă) până la cele maligne (coriocarcinomul). Proliferarea anormală a trofoblastului se evaluează prin monitorizarea nivelurilor de hCG, în BTG nivelul seric al hCG fiind persistent crescut.

hCG (gonadotrofina corionică umană) a fost mult timp considerată ca fiind o moleculă unică produsă de trofoblast în timpul sarcinii, cu rol în promovarea producției de progesteron de către corpul galben. În ultimii 20 de ani acest concept a suferit modificări radicale prin demonstrarea existenței a trei molecule independente, cu o structură peptidică beta comună. Una dintre acestea - hCG hiperglicozilat (hCG-H) - diferă de hCG, utilizat în practica comună, prin structura carbohidrată. Diferitele izoforme ale hCG se exprimă diferit în sarcină şi în coriocarcinom, iar pe măsură ce sarcina normală avansează, glicozilarea hCG se modifică, permițând, prin existența unor anticorpi specifici, diferențierea celor două stări. Majoritatea testelor utilizate pentru cuantificarea hCG se bazează pe anticorpi direcționați împotriva epitopilor peptidici ai hCG. Însă, prin identificarea anticorpului B152, specific porțiunii glicozilate a hCG, s-a putut realiza profilul producției diferitelor forme de hCG de-a lungul sarcinii şi identificarea patologiilor asociate. Astfel, în sarcina normală, la sfârşitul celei de-a treia săptămâni, hCG-H reprezintă 90±11% din hCG total, în timp ce la sfârşitul celei de-a patra săptămâni scade la 54±27% din hCG total.

	hCG	**hCG-H**
Produs de către…	Sinciţiotrofoblast	citotrofoblast
Roluri…	Producţia Pg de către celulele luteale	**Promovează invazia** trofoblastică în sarcină şi în coriocarcinom
Promovarea angiogenezei în vasele uterine	Promovează creşterea celulelor trofoblastice	
Promovează creşterea uterina	Promovează implantarea placentei	
Rol în creşterea şi dezvoltarea organelor fetale		
Previne rejecţia placentei de către făt		
Mecanism…		Blocarea apoptozei în celulele trofoblastului cu creştere celulară secundară

Dacă în diferențierea sarcinii normale de cea patologică hCG-H este util, el fiind promotorul invaziei fiziologice a placentei în primele săptămâni de sarcină prin aceeaşi capacitate de invazie, în manifestările neoplazice este implicat prin acelaşi mecanism, de data aceasta necontrolat. Coriocarcinomul este cea mai agresivă neoplazie cunoscută, fiind indusă în totalitate de hCG-H, cu un tip de invazie asemănător placentației. Practic, hCG-H este promotorul fizic al bolii trofoblastice gestaționale şi un adevărat marker al acesteia, fiind singurul care poate identifica cazurile agresive sau responsive la chimioterapie, minim agresive sau ne-responsive la chimioterapie, precum şi BTG inactivă. Aceasta din urmă se caracterizează prin valori persistent (cel puțin trei luni) scăzute ale hCG, sub 207 mUI/mL, şi urmează, de regulă, evacuării unei mole complete. Histologic se caracterizează prin prezența de sincițiotrofoblast înalt diferențiat şi număr scăzut, până la absența totală, a celulelor citotrofoblastului. În lipsa citotrofoblastului (care produce hCG-H) nu există promotorul invaziei celulare. Întrucât în BTG inactivă nu există creştere celulară, chimioterapia este ineficientă. În majoritatea cazurilor, BTG inactivă regresează spontan în 6 luni, la câteva paciente fiind constatate niveluri persistente de hCG timp de 9 ani. La 10% dintre pacientele cu molă hidatiformă sau avort spontan progresia s-a făcut către boala trofoblastică invazivă, nivelurile de hCG dublându-se la intervale de timp variate. La 25% din cazurile de neoplasm trofoblastic gestațional sau coriocarcinom s-au înregistrat recurențe.

Absența hCG-H (hCG-H <1%) în coriocarcinom sau în neoplasmul TG indică BTG inactivă. Invers, nivelul crescut (>40% hCG-H) relevă o formă foarte agresivă de boală. Între cele două praguri valorice se înscrie boala trofoblastică minim agresivă, definită ca neoplasm gestațional lent progresiv (timp de dublare a valorilor hCG mai mare de două săptămâni), cu o producție de hCG-H scăzută (<40% din hCG total), fiind refractară la chimioterapie. Decesul în cazul acestor paciente nu se datorează agresivității bolii, ci mai degrabă utilizării chimioterapiei agresive în cazuri rezistente. Alte utilizări ale hCG-H au fost studiate în sarcină pentru screening-ul sindromului Down, în predicția bolii hipertensive în sarcină şi în identificarea avorturilor spontane cauzate de invazie anormală.

Cancerul de col uterin

Cancerul de col se plasează pe locul doi în lume în cadrul cancerelor prezente la femei, după cancerul de sân constituind o cauză majoră de morbiditate şi mortalitate. Rata de supraviețuire variază între 95%, pentru pacientele cu tumori mici, localizate la nivelul cervixului, la 47%, la cele cu stadii avansate de boală, şi 21 % în cazurile cu metastaze la momentul diagnosticului.

Tratamentul în stadiile incipiente depinde în mare măsură de dimensiunile tumorii şi de amploarea adenopatiilor, acestea din urmă constituind un factor prognostic important.

Screeningul citologic al cancerului cervical a redus incidența apariției cancerului cervical în Statele Unite în ultimii 30 de ani cu peste 50%. Cu toate acestea, 50% dintre pacientele diagnosticate anual nu au efectuat niciodată acest test. Incidența şi mortalitatea prin această formă de neoplazie ar putea fi scăzute fie prin creşterea ratei de screening la femeile aflate la risc, fie prin identificarea unor markeri serici specifici stadiilor incipiente, preclinice. În acest scop s-a identificat antigenul tumoral celular scuamos SCC (din familia inhibitorilor de proteaze serice), utilizat pentru formele scuamoase de cancer cervical, care reprezintă 85-90% dintre toate formele de cancer de col uterin. Nivelul de referință pentru femeile sănătoase este sub 1.9 µg/L, *cut-off-ul* adoptat în studiile clinice fiind 2.0-2.5 µg/L. Markerul a fost identificat în diferite neoplazii (vulvare, vaginale, cap şi gât, esofag, pulmonare), precum şi în alte stări non-neoplazice (psoriazis, eczemă, insuficiență renală, sarcoidoză), pentru neoplasmul cervical nefiind stabilit un interval valoric de referință. Niveluri ridicate de SCC au fost detectate la 28-88% dintre cancerele scuamoase de col uterin, iar nivelurile SCC preterapeutice s-au corelat cu stadiul bolii, dimensiunile tumorale, adâncimea invaziei stromale, afectarea limfatică şi metastazele limfatice. Câteva studii au identificat şi o valoare predictivă a nivelurilor ridicate ale SCC în ceea ce priveşte prognosticul. Nivelurile SCC au urmat aceeaşi curbă cu răspunsul la radio- şi chimioterapie. Mai mult, au reprezentat un predictor independent al răspunsului la chimioterapia neoadjuvantă în cancerul cervical avansat şi în chirurgia radicală. Recurențele tumorale au fost precedate de creşteri ale valorilor SCC.

CYFRA 21-1 (fragmentele serice ale citocheratinei 19) este cel mai sensibil marker tumoral pentru tumorile cu celule spinocelulare (scuamoase), fiind un alt marker utilizat timid în cancerul cervical, mai ales în formele scuamoase. 45-52% dintre pacientele cu carcinom scuamos de col uterin au avut niveluri crescute ale acestui marker şi, similar cu SCC, nivelurile preterapeutice s-au corelat cu stadiul, dimensiunile tumorale, adâncimea invaziei stromale, afectarea şi metastazarea limfatică. Au acelaşi rol în monitorizarea răspunsului la radio- şi chimioterapie, precum şi în identificarea recurențelor în stadiul preclinic.

Aflat încă în studiu, dar cu un potențial serios, YKL-40 (CHI3L1 sau glicoproteina cartilaginoasă umană 39) a avut valori crescute în 75% dintre cazurile de carcinom cervical scuamos şi în 78% dintre adenocarcinoame. Mai mult, nivelurile crescute preterapeutice au avut valoare predictivă pozitivă în ceea ce priveşte prognosticul nefavorabil, independent de stadiul bolii.

La momentul actual nu există recomandări în ghidurile de diagnostic şi tratament în ceea ce priveşte utilizarea acestor markeri în mod obligatoriu în managementul cazurilor, însă, în baza studiilor şi a trialurilor clinice, se pot lua în considerare aceste rezultate în planificarea terapeutică individuală.

Cancerul endometrial nu beneficiază de un test seric specific, deşi s-a încercat stabilirea unei corelații între CA-125 şi acest tip de neoplasm. Trebuie să reținem însă că la pacienții care au urmat şedințe de radioterapie la nivel abdominal nivelurile CA-125 sunt crescute în absența unei patologii endometriale propriu-zise. Alți markeri “de împrumut” studiați au fost CA 19-9, CA 15-3, CA 72-4 şi CEA, fără a se stabili asocieri semnificative.

Cancerele ginecologice beneficiază de un spectru restrâns de markeri serici, iar dintre aceştia doar câțiva sunt determinați frecvent în practica clinică. Utilitatea unora dintre aceştia nu constă doar în identificarea cât mai precoce a diferitelor patologii, ci constituie veritabili markeri predictivi ai prognosticului şi evoluției postterapeutice. Câțiva dintre aceştia - HE4 şi YKL 40 - sunt promițători în ceea ce priveşte utilitatea lor clinică.

La momentul actual majoritatea markerilor studiați evaluează mai degrabă progresia leziunilor deja inițiate şi sunt lipsiți de specificitate. Valoarea diagnostică clinică nu este susținută prin dovezi clare, fiind necesare studii de cohortă. Trendul actual îl constituie încă necesitatea utilizării markerilor serici (fie singulari, fie grupați) în discriminarea cât mai precoce a neoplaziilor.

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Invitație la lectură

Motto:
,, Istoria este o ficțiune care s-a întâmplat,
în timp ce ficțiunea este o istorie care ar fi putut să se întâmple''
André (Paul Guillame) Gide

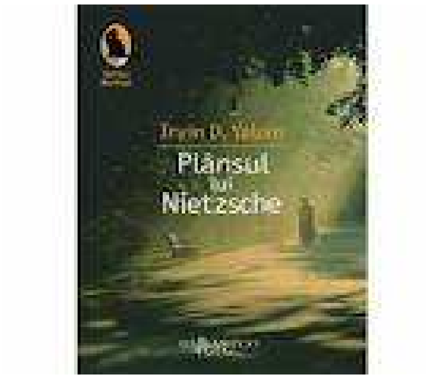

Întâmplarea face ca, în timp ce parcurgeam ultimele file din romanul ,,Viața lui Freud'' a lui Irving Stone, o distinsă colegă neurolog să-mi recomande o carte din colecția ,,Raftul Denisei'', a Editurii Humanitas, ,,Plânsul lui Nietzsche''.

Autorul, Irving D.Yalom, s-a născut în 1931 la Washington, având origine rusă. Este un eminent psihiatru şi psihoterapeut care publică numeroase lucrări de specialitate dar şi literatură de bună calitate. La 61 de ani, în plină maturitate profesională şi spirituală îşi face debutul de romancier cu ficțiunea ,, When Nietzsche wept'', apărută la noi în 2010.

După un succes teribil şi premii binemeritate, cartea are parte şi de o ecranizare de excepție în 2007, având ca protagonişti pe Armand Assante şi Ben Cross, sub regia lui Pinchas Perry.

Cum aveam plăcerea să descopăr, romanele s-au înlănțuit într-un mod natural, ținând cont că ambele relevă epoca Vienei *fin de siecle*, în care personaje reale binecunoscute îşi au propriile roluri: Josef Breuer, Sigmund Freud, Richard Wagner, Friedrich Nietzsche, Bertha Pappenheim, Lou Andreas-Salome. Paradoxul acestei ficțiuni este că deşi subiectul principal îl constituie întâlnirea dintre Breuer şi Nietzsche, întâlnire care cunoaşte subtile răsturnări de situații şi în care pe rând cei doi devin când terapeuți, când pacienți, într-o serie de sedințe de psihoterapie, presărate din belşug cu întrebări existențiale şi situații limită, cei doi nu s-au întâlnit niciodată.

În realitate însă, puțin a lipsit ca aceşti doi contemporani să se cunoască în rolurile de pacient şi respectiv medic, aşa cum reiese dintr-o scrisoare găsită de Renate Muller-Buck în arhiva din Weimar, din care aflăm că filozoful era prea bolnav pentru a suporta drumul la Viena.

Lou Salome, scriitoare strălucită şi psihanalist practician, a fost într-adevăr prietenă cu Nietzsche, Paul Ree, Sigmund Freud şi Rainer Maria Rilke.

Bertha Pappenheim a avut o carieră importantă în asistența socială, domeniu de avangardă pe atunci.

Josef Breuer – important fiziolog şi clinician, medic al elitei vieneze, părinte al psihanalizei, a fost mentorul şi prietenul lui Freud.

Nu vă dezvălui mai mult, lăsându-vă plăcerea să trăiți această lectură. Aşa cum spunea cineva: ,,aventura cititului este la fel de palpitantă precum cea a trăitului, evident, pentru cei care ştiu să citească''.

Lt. col. dr. Carmen-Adella SÎRBU

Consecințele medicale ale consumului de “produse etnobotanice”

Theodor JIQUIDI*

Rezumat. În ultimul timp, în țara noastră, şi nu numai, a avut loc o explozie a utilizării non-medicale (ilicite) a unor aşa-zise preparate etnobotanice, care, din punct de vedere al compoziției, sunt foarte heterogene, nefiind constituite exclusiv din componente vegetale, ci conținând adaosuri adesea neidentificate. Au fost identificați totuşi compuşi cu efect psihodisleptic, majoritatea nefiind de origine naturală, vegetală, ci de origine sintetică. Se poate afirma aşadar că o astfel de utilizare a *etnobotanicelor* nu are nicio fundamentare derivată din etnobotanică sau etnofarmacologie.

Consumul ilicit actual al produselor inadecvat clasate în categoria celor *etnobotanice* a produs numeroase drame şi tragedii individuale, cu victime umane şi cheltuieli materiale, cu implicații majore asupra criminalității, accidentelor de circulație etc. (Shbair *et al*, 2010). Chiar dacă consumul de etnobotanice psihoactive autentice poate fi periculos în sine, prin psihotoxicitatea acestora, în cazurile menționate avem de-a face cu consumul unor substanțe psihoactive sintetice, de mare toxicitate, multe fără caracterizare psihofarmacologică sau toxicologică adecvată. De aici rezultă şi riscurile grave pentru sănătatea consumatorilor şi pentru societate în general.

Cuvinte cheie: medicamente de origine vegetală, psihotomimetice, psihodisleptice, intoxicații acute

Abstract. Lately, in our country and beyond, there was an explosion of non-medical use (illicit) of so-called ethnobotanical agents, which, in terms of composition, are very heterogeneous, consisting not only of plant components, but containing often unidentified additives. Compounds with psihodisleptic effect have been identified, most of which are not naturally occurring, instead they are synthetic. It can be said therefore that such use of the “ethnobotanical products” has no foundation derived from ethnobotanical or ethnopharmacology. Inadequate current illicit products, classified in the category of ethnobotanical, produced numerous individual dramas and tragedies, with human victims and material expenses, with major implications for crime, traffic accidents etc. (Shbair et al, 2010). Even if the consumption of authentic psychoactive ethnobotanical can be dangerous in itself, by their psihotoxicity, in the cases referred we have to deal with the consumption of synthetic psychoactive substances, with high toxicity, many of them without adequate toxicological or psychopharmacological characterization. Hence the serious risks to consumer health and society in general.

Keywords: drugs of plant origin, psychotomymetics, psychodysleptics, acute poisoning

1. Definiții, domeniul de studiu

Etnobotanica este o ştiință ce are ca obiect relația dintre plante şi om. Mai exact, aceasta descrie cum au fost utilizate plantele în cadrul diferitelor societăți umane, ca hrană, în scop medical, în scop ritual, în cosmetică, pentru vopsele, ca fibre textile, în construcții, ca unelte, ca monedă, în literatură şi în cadrul vieții sociale (C. Rätsch, 1998). Cuvântul *etnobotanică* s-a constituit prin contragerea cuvântului grec *ethnos* (cultură populară) şi *botanică*.

2. Scurt istoric

Prima mențiune conexă cu viitorul acestui domeniu ştiințific este în opera lui Dioscorides, un chirurg antic grec, intitulată *De materia medica*, publicată în anul 77 e.n., care descrie 600 de plante mediteraneene, accentuând pe utilizarea de către greci a plantelor respective în scop medical. Interesant este faptul că Dioscorides subliniază efectul toxic sau netoxic al acestor plante, insistând asupra potențialului lor economic. Până după evul mediu, a fost o operă de bază pentru studiul domeniului.

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[*] Theodor Jiquidi este inginer horticol şi student medicinist în anul VI.

În 1542, un artist al Renaşterii, al cărui nume era Leonard Fuchs, reia tema şi publică *De historia stirpium*, în care descrie 400 de plante care creşteau în Germania şi Austria. În secolul 17, John Ray (1686-1704) definea noţiunea de *specie* în opera sa *Historia Plantarum.* Un moment important în istoria ştiinţei a fost publicarea operei lui Carl Linne, *Species Plantarum,* în 1753, care descria 5 900 de plante. În acest context, Linne prezenta metoda sa *nomenclatura binominală*, în care speciile sunt denumite cu două cuvinte, după gen şi specie.

Secolul 19 se caracterizează prin ample explorări botanice ale unor personalităţi din domeniu : Alexander von Humboldt aduce date din *Lumea nouă,* iar John Cook aduce colecţii şi informaţii din Pacificul de Sud. Se înfiinţează primele grădini botanice în Europa şi în alte regiuni ale lumii.

Prima lucrare etnobotanică modernă (1896) este cea a medicului german Leopold Glueck, în care descrie utilizarea medicală a plantelor în zonele rurale ale Bosniei.

Regnul vegetal a constituit în lunga istorie evolutivă a speţei umane o sursă complexă pentru supravieţuire, furnizându-i alimente, combustibil, fibre, materiale de construcţie, arme şi, nu în ultimul rând, medicamente (M. Thomas, *Ethnobotany research and applications,* 2003, 1, 65-73).

Termenul de *etnobotanică* a fost avansat, în 1895, de botanistul american John W. Harshberger, în contextul descrierii plantelor utilizate de popoarele primitive şi aborigene. Lucrarea *The purpose of ethnobotany* este considerată ca debut al acestui domeniu ştiinţific.

Etnobotanica şi cei implicaţi în cercetarea acestui domeniu presupun conexiuni complexe interdisciplinare, din care menţionăm : antropologia, arheologia, botanica, chimia, lingvistica, psihologia, psihiatria, ecologia, farmacologia, toxicologia, reglementări legale etc.

Pe măsura acumulării informaţiilor complexe din domeniu, s-au constituit colecţii etnobotanice care sunt distribuite în diverse instituţii şi locaţii geografice, apărând necesitatea unor baze de date care permit atât depozitarea electronică, cât şi accesarea la nivel global. Se defineşte astfel un nou domeniu, foarte cuprinzător, bio-informatica, cu un adevărat forum de schimb de informaţii în biologia moleculară şi genetică, cu destinaţia finală aprofundarea înţelegerii şi cunoaşterii vieţii terestre.

La ora actuală, sunt accesibile multe zeci de baze de date etnobotanice. Aceste baze de date sunt cu atât mai necesare dacă estimăm că, actualmente, există circa 300 000 de specii de plante, din care peste 30% au fost folosite ca bază pentru obţinerea de remedii la un moment dat. Trierea acestor plante, istoric vorbind, s-a făcut prin încercări succesive, succese şi erori de-a lungul secolelor, omul învăţând empiric care plante sunt remedii şi care sunt otrăvuri (Etkin *et al*, 2005).

În ceea ce priveşte domeniul utilizării medicale tradiţionale a plantelor, au apărut probleme de preluare corectă a informaţiilor sub aspect lingvistic şi semantic (M. Thomas, Nan Lin, H. W. Beck – *Pharmaceutical Biology*, 2001, vol. 37, 1-12). În sens medical, se analizează, de exemplu, aspectul lingvistic şi semantic al sintagmei *durere de cap*. Atât în limba engleză şi, interesant, şi în limba română, expresia are sens direct de durere la nivelul capului, datorată *dilatării vaselor cerebrale*, spre exemplu, dar şi un sens care descrie o stare psihologică de preocupare, anxietate etc. La rândul său, durerea de cap în sens somatic, senzorial, poate include: migrena, durerea sinusală, durerea dentară difuză etc., toate sinonime cu cefalgia sau cefaleea. Aşa cum vom vedea, acest model se aplică şi în alte domenii, descrierea spre exemplu a efectelor farmaco-toxicologice la nivelul SNC presupunând nuanţe deosebit de complexe (Heinrich Michael, 2003).

3. Utilizări medicale ale resurselor etnobotanice

Medicamentele de origine vegetală vizează cele mai diverse domenii ale terapeuticii, din care am putea menţiona:

- farmacoterapia sistemului cardiovascular (digitalice, antihipertensive, vasodilatatoare);
- farmacoterapia bolilor SNC şi a durerii (serpentina – *Rauwolfia Serpentina*, opiaceele – *Papaver Somniferum*, metilxantinele etc.);
- terapia bolilor neoplazice (taxonii – *Taxus Bacatta* ; vincristina, vinblastina – *Vinca Minor*)

- terapii cu ținte diverse (anticolinergice – atropina – *Atropa Belladona*, scopolamina – *Hyosciamus Niger*);
- domeniul curarizantelor s-a deschis odată cu cercetarea ştiințifică a d-tubocurarinei – extrasă din *Chondondendron Tomentosum*.

Cele enumerate mai sus fac parte din arsenalul modern al farmacoterapiei, dar nu vom detalia aici lunga serie de exemple de surse vegetale ale medicamentelor moderne.

Este de remarcat faptul că utilizarea medicală a preparatelor medicamentoase din plante presupune o indicație medicală, respectiv un uz terapeutic, care se disociază de utilizarea nemedicală în alte scopuri, cu consecințe nefaste pentru om. Este suficient să amintim aici opiaceele, naturale sau semisintetice (morfina, heroina), a căror utilizare medicală inițială a fost analgezia (combaterea durerii intense de diverse origini), la polul opus situându-se utilizarea nemedicală, ilicită, în scop de abuz (în special heroina). Un alt exemplu interesant este cocaina, extrasă din *Erythroxilon coca*, ale cărei caracteristici farmacotoxicologice deosebit de interesante au determinat utilizarea ca anestezic local, deschizând un nou domeniu al anesteziologiei. Descrierea efectelor sale adverse la nivelul SNC şi al sistemului cardiovascular a dus la reducerea majoră a utilizării sale medicale, rămânând, în schimb, în mod paradoxal, utilizarea sa non-medicală, ca substață de abuz.

4. Utilizări non-medicale, în scop recreativ sau de abuz; aspecte medicale, exemple

Domeniul substaților cu acțiune asupra SNC este foarte amplu şi ne vom referi mai ales la cele care produc perturbări ale funcțiilor SNC, denumite, printr-un termen foarte sugestiv, *psihotomimetice* sau *psihodisleptice*. În fapt, substanțele de origine vegetală sau de sinteză sunt cuprinse în sintagma de *substanțe psihochimice* cu conotație de substanțe toxice de luptă.

Dintre substanțele psihotomimetice menționăm halucinogenele, LSD, derivat al acidului lisergic, alți compuşi de tip indolalchilamine (psilocina, psilocibina), feniletilaminele (mescalina naturală), derivații de amfetamină, inclusiv Ecstasy.

Halucinogenele induc geneza unor trăiri senzoriale fără suport real, cu alterări psihedelice sau psihotomimetice, inclusiv alterări ale percepției timpului şi spațiului (Voicu 2005 ; Halberstadt *et al* 2011).

Canabisul (marijuana), obținut din diverse specii de Canabis, are ca principiu activ tetrahidrocanabinolul şi substanțe înrudite. Abuzul de canabis determină delir, ideație paranoidă, tulburări de tip anxios, sindrom amotivațional (Voicu 2005 ; Ben Amer 2006).

În ultimul timp, în țara noastră s-a produs o explozie a utilizării non-medicale (ilicite) a unor aşa-zise preparate etnobotanice, care, din punct de vedere al compoziției, sunt foarte heterogene, nefiind constituite exclusiv din componente vegetale, ci conținând adaosuri adesea neidentificate. Au fost identificați totuşi compuşi cu efect psihodisleptic, majoritatea nefiind de origine naturală, vegetală, ci de origine sintetică. Se poate afirma aşadar că o astfel de utilizare a *etnobotanicelor* nu are nicio fundamentare derivată din etnobotanică sau etnofarmacologie.

Cazurile de intoxicații acute sau sindroamele de dependență, sindroamele de întrerupere, au fost caracterizate, din punct de vedere diagnostic, drept encefalopatie toxică, indusă de substanțe psihotrope sau psihodisleptice, caracterizate simptomatic prin agitație psihomotorie extremă, tulburări motorii, dizartrie, insomnie sau somnolență.

Determinările analitice toxicologice din laboratorul de profil al Centrului de Cercetări Ştiințifice Medico-Militare, cu sediul în Spitalul Clinic de Urgență Floreasca, au evidențiat, în cadrul intoxicațiilor cu etnobotanice, prezența unor compuşi de sinteză, psihoactivi, de tipul mefedronă, piperonal, pirovaleronă, vanilonă, prolintan etc. Nu toți compuşii incriminați au putut fi caracterizați şi definiți, negăsindu-se în baza de date a analizoarelor utilizate (lichid cromatograf sau gaz cromatograf cuplate cu spectrometru de masă).

Consumul ilicit actual al produselor inadecvat clasate în categoria celor *etnobotanice* a produs numeroase drame şi tragedii individuale, cu victime umane şi cheltuieli materiale, cu implicații majore asupra criminalității, accidentelor de circulație etc. (Shbair *et al*, 2010). Chiar dacă consumul de etnobotanice psihoactive autentice poate fi periculos în sine, prin psihotoxicitatea acestora, în cazurile menționate avem de-a face cu consumul unor substanțe psihoactive sintetice, de mare toxicitate, multe fără caracterizare psihofarmacologică sau toxicologică adecvată. De aici rezultă şi riscurile grave pentru sănătatea consumatorilor şi pentru societate în general.

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Metodă de monitorizare a concentrației plasmatice a platinei la bolnavi oncologici tratați cu săruri de platină

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Rezumat. Medicamentele citostatice din grupul sărurilor de platină intră în schemele terapeutice care se adresează mai multor tipuri de cancer: sarcoame, carcinoame (pulmonar microcelular şi non-micro celular, ovarian), limfoame, tumori ale celulelor germinale. Compuşii cu platină îşi exercită acțiunea antitumorală prin crearea de punți intracatenare, intercatenare şi prin formare de compuşi inerți la nivelul lanțurilor ADN, fenomene ce interferă cu diviziunea celulară mitotică. Acest grup de medicamente determină numeroase efecte secundare: nefrotoxicitate, neurotoxicitate, toxicitate digestivă (grețuri, vărsături), ototoxicitate şi tulburări hidro-electrolitice. În clinică se utilizează mai multe medicamente citostatice care conțin ioni de platină: cisplatin, carboplatin, oxaliplatin. În scopul monitorizării eficacității administrării sărurilor de platină autorii prezintă o metodă de măsurare a nivelurilor sangvin şi urinar ale platinei. A fost utilizată o instalație AAS (spectrometrie de absorbție atomică) cu atomizare în cuptor de grafit Varian. Sunt prezentate rezultatele obținute de la un lot de pacienți tratați cu cisplatin și carboplatin în Secția de Radiobiologie a Spitalului Clinic Fundeni. Metoda este utilă pentru atingerea unui index terapeutic optim în condițiile minimizării reacțiilor adverse.

Cuvinte cheie: cisplatin, carboplatin, spectrometrie de absorbție atomică (AAS).

Abstract. Cytostatics in the platinum salts group enter into regimens that address several types of cancer: sarcomas, carcinomas (lung small cell and non-small cell, ovarian cancer), lymphomas, germ cell tumors. Platinum compounds have antitumoral activity by creating intrastrand and interstrand bridges and non-functional adducts in the DNA chains, acts that interfere with mitotic cell division. This group of drugs cause many side effects: nephrotoxicity, neurotoxicity, gastrointestinal toxicity (nausea, vomiting), ototoxicity and fluid and electrolyte disorders. In clinical use multiple chemotherapy regimens include drugs containing platinum ions: cisplatin, carboplatin, oxaliplatin. To monitor the efficacy of platinum salts authors present a method for measuring blood and urine levels of platinum. It was a facility used AAS (atomic absorption spectrometry) with Varian graphite furnace atomisation. Are presented the results obtained from a batch of patients treated with cisplatin and carboplatin at the Radiobiology Department of Fundeni Clinical Hospital. The method is useful for achieving an optimal therapeutic index in terms of minimizing adverse reactions.

Keywords: cisplatin, carboplatin, atomic absorption spectrometry (AAS).

Cisplatinul [*cis*-diaminodichloroplatinum(II) (CDDP)] este un medicament antineoplazic şi radiosensibilizant indicat în tratamentul tumorilor maligne ale aparatului uro-genital masculin şi feminin, al tumorilor esofagiene, din sfera ORL, şi al osteosarcoamelor la copii.

Carboplatinul [cis-diamino(1,1-cyclobutanedicarboxylato)platinum(II)] este o sare de platină utilizată în mod curent în tratamentul multor tipuri de tumori solide (cancere genitale feminine, ale celulelor germinale, ale capului şi gâtului, neuroblastoame, rabdominosarcoame, cancer pulmonar micro- şi non-microcelular).

Oxaliplatinul [(1*R*,2*R*)-ciclohexan-1,2-diamino](ethandioato-*O*,*O'*)platinum(II)], deşi este un citostatic activ în neoplasmele cu localizările menționate, este indicat preponderent în tratamentul cancerelor colo-rectale.

Cisplatinul se cuplează cu ADN-ul în mai multe moduri, interferând cu diviziunea celulară prin mitoză. ADN-ul deteriorat declanşează mecanisme de reparare a ADN, care, la rândul lor, activează apoptoza atunci când se dovedeşte imposibil procesul de reparare. Recent s-a demonstrat că apoptoza indusă de cisplatin asupra celulelor unor linii tumorale umane de cancer de colon depinde de serin-proteaza serică mitocondrială Omi/Htra2[1]. Din moment ce acest lucru a fost demonstrat numai pentru celulele de carcinom de colon, rămâne o întrebare deschisă dacă proteina Omi/Htra2 participă la apoptoza cisplatin - indusă în carcinoamele din alte țesuturi.

Măsurarea fracțiunii libere plasmatice a platinei a stat la baza studierii farmacocineticii şi farmacodinamiei acestor medicamente.

Având în vedere că reacțiile secundare ale acestui grup de citostatice sunt multiple şi severe, mai ales în cazul cisplatinului, lucrarea de față îşi propune să investigheze posibilitățile de monitorizare terapeutică a concentrațiilor plasmatice ale platinei la bolnavi cu diferite forme de cancer, în scopul atingerii indexului terapeutic optim în condițiile minimizării efectelor nedorite.

Utilizarea dozelor calculate pe baza acestei monitorizări poate conduce la diminuarea incidenței reacțiilor toxice severe care adesea limitează sau duc la amânarea administrării la intervale optime a ciclurilor de chimioterapie, determinând un prognostic nefavorabil al bolnavului de cancer. Farmacocinetica sărurilor de platină este în mare măsură determinată de funcția renală a pacientului[2], fiind stabilite formule pentru atingerea concentrațiilor plasmatice terapeutice. Aceste metode au dus la obținerea unei dozări mai eficiente a carboplatinului şi a cisplatinului, comparativ cu administrarea în funcție de suprafața corporală. Stabilirea dozei pe baza funcției renale a devenit o metodă larg acceptată. Majoritatea protocoalelor au la bază atingerea unei arii de sub curbă (*area under curve* - AUC) țintă. Această abordare a dus la o administrare mai eficace a acestor medicamente față de exprimarea dozei în mg/suprafață corporală, atât sub raportul toxicității, cât şi al răspunsului terapeutic.

Deşi metoda este utilizată pentru administrarea dozelor convenționale, saturarea proteinelor transportatoare sau metabolismul compusului poate duce la variații imprevizibile ale farmacocineticii. De aici ipoteza conform căreia monitorizarea concentrației platinei poate duce la atingerea dozelor eficace individualizate pentru fiecare pacient şi la reducerea riscurilor supra- sau subdozării.

Ne propunem să stabilim dacă adaptarea dozelor va permite atingerea valorilor AUC țintă şi reducerea variabilității concentrațiilor plasmatice comparativ cu dozarea care ține cont exclusiv de funcția renală şi suprafața corporală.

Protocoalele terapeutice indicate pentru cancerele genitale feminine şi cele ale regiunii cervico-cefalice oferă oportunitatea investigării expunerii pacienților la carboplatin sau cisplatin.

Scopul studiului este de a optimiza doza de carboplatin, urmărindu-se valori țintă de 6 - 8 µg/ml, în doză unică repetată la intervale de 21 zile.

Material şi metodă

A fost constituit un lot de 17 pacienți cu vârste cuprinse între 36 şi 68 ani, 13 urmând să fie supuşi chimioterapiei cu carboplatin şi patru terapiei cu cisplatin, ca parte a protocoalelor terapeutice standard.

Carboplatinul a fost administrat diluat în soluție de glucoză 5 % , timp de 60 de minute. Doza de carboplatin a fost stabilită pe baza ratei filtrării glomerulare (RFG) (a clearance-ului la creatinină) cu ajutorul formulei Cockcroft şi Gault:

$$Cl_{cr} = \frac{140 - vârsta}{72 * creatininã\_serică} * Greutatea$$

şi a formulei Calvert [3]:

$$Doza = ASC * (RFG + 25)$$

Cisplatinul a fost administrat în soluție de ser fiziologic, perfuzie intravenoasă cu durata de 60 minute.

Concentrațiile plasmatice de platină au fost utilizate pentru calcularea AUC actuale.

După obținerea consimțământului în cunoştință de cauză al pacienților, probele de sânge au fost recoltate dintr-o venă periferică, alta decât cea prin care a fost administrat medicamentul, la sfârşitul perfuziei (la 60 de minute de la începerea perfuziei).

Determinarea platinei din sângele sau urina pacienților s-a efectuat prin metoda AAS (spectrometrie cu absorbție atomică) cu atomizare în cuptor de grafit (GF)[4,5].

Deproteinizarea:

- pentru urină s-a realizat prin adăugarea în 10 ml a 1 ml HNO_3;
- pentru sânge s-a realizat prin adăugarea a 200 μl în 800 μl antispumant B şi 1000 μl apă acidulată cu HNO_3 1.6 N.

Pentru măsurarea concentrației de platină a fost utilizată o instalație Varian, cu următoarele componente: spectrometrul de absorbție atomică model SectrAA 880, atomizorul în cuptor de grafit model – GTA 100, programator automat pentru probe model PSD, răcitor de apă model Neslab CFT 33, generator de azot model Dominick Hunter, argon analitic. Etalon de platină Aldricht 1g/l.

Metoda AAS

Instalația GF-AAS are stabiliți următorii parametri:
-mod de măsurare: înălțime peak;
-netezirea semnalului: 9 puncte;
-număr de replicate: 2;
-lungime de undă: 265.9 nm;
-curentul lămpii: 10 mA;
-slit: 0.2 nm;
-background: lampă cu D2;
-cantitate de probă: 20 μl;
-cantitate totală: 25 μl;
-temperatura apei de răcire: 20.5^{0}C;
-presiunea apei de răcire: 2 atm;
-azot: debit maxim 4l/min la 4.5 atm., puritate 99.999%;
-argon: debit maxim 3.1 l/min la 4.5 atm, puritate 99.999%;
-calibrare în trei puncte: 0, 250 şi 500 μg/l. Curba de calibrare este prezentată în figura 1;
-factor automat de diluție: 2;
-control de calitate automat.

În tabelul nr. 1 sunt date programul de temperaturi ale cuptorului de grafit şi debitele gazelor de lucru.

Tabelul 1. Programul de lucru al cuptorului de grafit.

Pas	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
T (^{0}C)	40	85	85	95	120	1000	1000	1000	2700	2700	2700	40	40
t (s)	2	1	5	40	10	5	1	2	1.3	2	2	21.7	10
D l/min	3	3	3	3	3	3	3	0	0	0	3	3	0

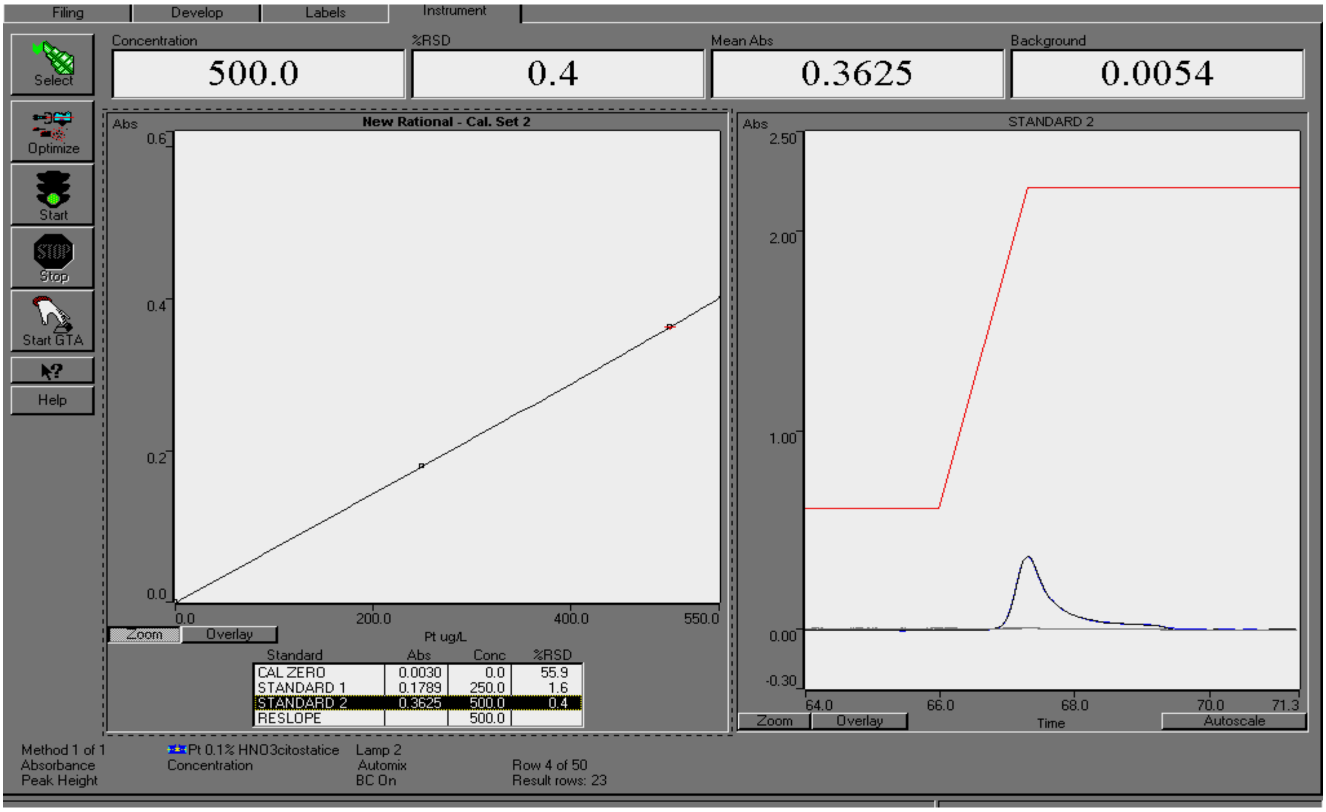

Concentration 500.0
%RSD 0.4
Mean Abs 0.3625
Background 0.0054

Standard	Abs	Conc	%RSD
CAL ZERO	0.0030	0.0	55.9
STANDARD 1	0.1789	250.0	1.6
STANDARD 2	0.3625	500.0	0.4
RESLOPE		500.0	

Method 1 of 1
Absorbance
Peak Height
Pt 0.1% HNO3citostatice
Concentration
Lamp 2
Automix
BC On
Row 4 of 50
Result rows: 23

Fig. 1. Curba de calibrare a platinei.

Rezultate şi discuții

Concentrațiile de platină din probele biologice recoltate de la loturile de pacienți sunt prezentate în tabelul nr. 2.

După cum se poate observa în tabel, concentrațiile platinei s-au situat în intervalul 170,1-544,1 µg/dl (1,7 - 5,4 µg/ml).

Se poate observa că deşi dozele de carboplatin s-au situat între 300 şi 600 mg, concentrațiile sanguine au fost cuprinse între 0,52 şi 5,44 µg/ml. La doza de carboplatin de 450 mg, concentrația sanguină a fost cuprinsă între 0,32 şi 2,90 µg/ml.

La lotul cu cisplatin, doza a fost aceeaşi pentru cei patru pacienți – 100 mg/m^2. Concentrația sanguină a fost cuprinsă între 0,33 şi 3,00 µg/ml.

Concentrațiile plasmatice ale platinei la pacienții tratați cu cisplatin s-au situat la un nivel mai scăzut, în general cu un ordin de mărime, față de pacienții tratați cu carboplatin. Se poate lua în considerare drept cauză a acestei valori mai scăzute volumul mare de soluții electrolitice necesar ca prehidratare la administrarea curei, cu scopul reducerii toxicității renale a medicamentului.

Considerăm că monitorizarea concentrațiilor platinei prin acest procedeu permite stabilirea unor doze optime care să conducă la realizarea concentrațiilor terapeutice optime, constituind concomitent un procedeu de verificare a modului de administrare.

Din motive obiective, nu a putut fi recoltată urină de la toți pacienții care au făcut parte din lot. Prin studierea concentrației urinare ne-am propus să apreciem capacitatea de excreție a compuşilor utilizați, funcția renală a pacienților condiționând viteza de eliminare urinară a platinei.

Concentrațiile plasmatice de platină au fost utilizate pentru calcularea AUC la administrările ulterioare.

Tabelul 2. Concentrațiile de platină din sângele şi urina pacienților tratați cu cisplatin sau carboplatin.

Pacient	**Vârstă**	**Doză carboplatin (mg)**	**Doză cisplatin (mg)**	**Concentrație plasmatică (μg/dl)**	**Concentrație urinară (μg/dl)**
M.P.	59	450	-	202.1	-
Z.I.	59	450	-	170.1	-
B.P.	36	600	-	198.8	-
G.M.	55	450	-	259.4	-
C.A.	56	450	-	268.8	-
B.M.	58	600	-	544.1	82
A.G.	68	300	-	276.0	-
S.A.	49	450	-	217.9	-
C.D.	70	450	-	52.0	-
M.M.	56	450	-	32.2	-
N.L.	58	450	-	136.9	-
B.M.	58	450	-	290.9	54.2
I.M.	52	450	-	290.0	11.9
N.E.	65	-	100	50.9	-
G.M.	42	-	100	33.9	2.01
F.M.	50	-	100	48.0	12.5
P.A.	39	-	100	300	27.8

Concluzii

Metoda AAS poate determina cantitatea de platină totală existentă în probele biologice.

Metoda este rapidă, reproductibilă, cu o sensibilitate foarte bună şi un preț de cost acceptabil.

Monitorizarea în timp real a concentrației de platină din sângele total şi/sau urina pacienților tratați cu carboplatin sau cisplatin este utilă pentru optimizarea farmacoterapiei acestor medicamente.

Stabilirea concentrației sangvine are avantajul că oferă medicului posibilitatea adaptării dozelor la ciclurile ulterioare de chimioterapie, în funcție de caracteristicile individuale ale fiecărui pacient, în vederea atingerii nivelurilor eficace.

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Revista de Medicină Militară este o publicație de prestigiu, cu o vechime de peste 100 de ani. În scopul integrării revistei în circuitul internațional al publicațiilor ştiințifice, autorii sunt rugați să respecte *Cerințele uniforme pentru manuscrisele trimise jurnalelor biomedicale* (stilul Vancouver de prezentare) (vezi: *New England Journal of Medicine* 1991, 324 : 424 – 428; *British Medical Journal*, 1991, 302; *Romanian Journal of Gastroenterology* 1992, 1/4, 1996, 5/1).

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Studiile pe pacienți umani sau studiile experimentale trebuie să respecte reglementările internaționale de etică. Sursele de finanțare sau suportul financiar se vor menționa cu mulțumirile de rigoare.

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Lucrările vor fi examinate de către doi revizori de specialitate din comitetul editorial. Dacă opiniile lor diferă, articolul este analizat de un alt revizor. Evaluarea articolelor se face fără ca revizorii să cunoască numele autorilor lucrării.

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Manuscrisele trimise spre publicare nu pot fi returnate. Când se primeşte un manuscris, autorii vor fi informați în scris, într-un interval de cinci-şase săptămâni, cu privire la acceptarea, nevoia de revizuire sau respingerea articolului.

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MANUSCRISUL VA FI PREGĂTIT ASTFEL:

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- Titlul lucrării (nu mai mult de 100 de caractere cu spații), fără abrevieri;
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c. **Referat general –** cel mult 2 000 de cuvinte (10 pagini).

- **INTRODUCERE**: prezentarea problemei, plasarea în context, scopul şi obiectivele studiului, ipoteza de lucru.
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- **REZULTATE**: fără interpretări.
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1 – analiza rezultatelor prin prisma obiectivelor propuse ale studiului cu comentarii cu privire la aspectele lămurite (pozitive sau negative) sau nelămurite prin acest studiu;

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3 – comentarii privind importanța studiului de față, precum şi o punere la punct a situației actuale a subiectului investigat atât în literatură, cât şi în practică; eventual se vor prezenta aici cele mai importante studii sau aspecte din domeniu;

4 – probleme nerezolvate, întrebări la care nu s-a răspuns şi care rămân deschise în viitor pentru studiile ce vor urma, eventuale direcții propuse pentru investigare în domeniul respectiv.

CONCLUZII

La nevoie, capitolele mari pot fi împărțite în subcapitole, după necesități.

Se recomandă utilizarea precaută a *abrevierilor* şi menționarea numelui complet la prima apariție în text a abrevierii respective. Toate referințele bibliografice vor fi citate în text cu cifre arabe, la exponent, fără paranteze, în ordinea apariției (de exemplu: Un alt studiu^{18} arată...). Toate figurile şi tabelele vor fi citate în text în locul în care ele urmează să apară (de exemplu: *Fig. 3*, *Tab. 2*). Datele care apar în tabele, legendele tabelelor şi figurilor trebuie să fie rezumate, nu duplicate în text.

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Tabelele trebuie să fie procesate la două rânduri, pe o pagină separată, *numerotate* consecutiv (cu referire în text în locul unde ele trebuie să apară) şi *cu un titlu* (exemplu: *Tabelul 1*. Distribuția pe vârste şi sex a pacienților). Capetele de tabel se scriu cu caractere *italice.* Nu trimiteți fotografii sau imagini ale tabelelor. Utilizați tasta „Tab” pentru a separa coloanele (nu bara de spațiu) şi utilizați tasta „Enter” la terminarea liniei. Toate căsuțele goale din tabel trebuie să aibă o liniuță. Dacă este nevoie, pentru cuvintele sau frazele lungi din căsuțe se vor utiliza abrevieri, însă cu explicarea acestora în subsolul tabelului. Asigurați-vă că toate tabelele sunt citate în text.

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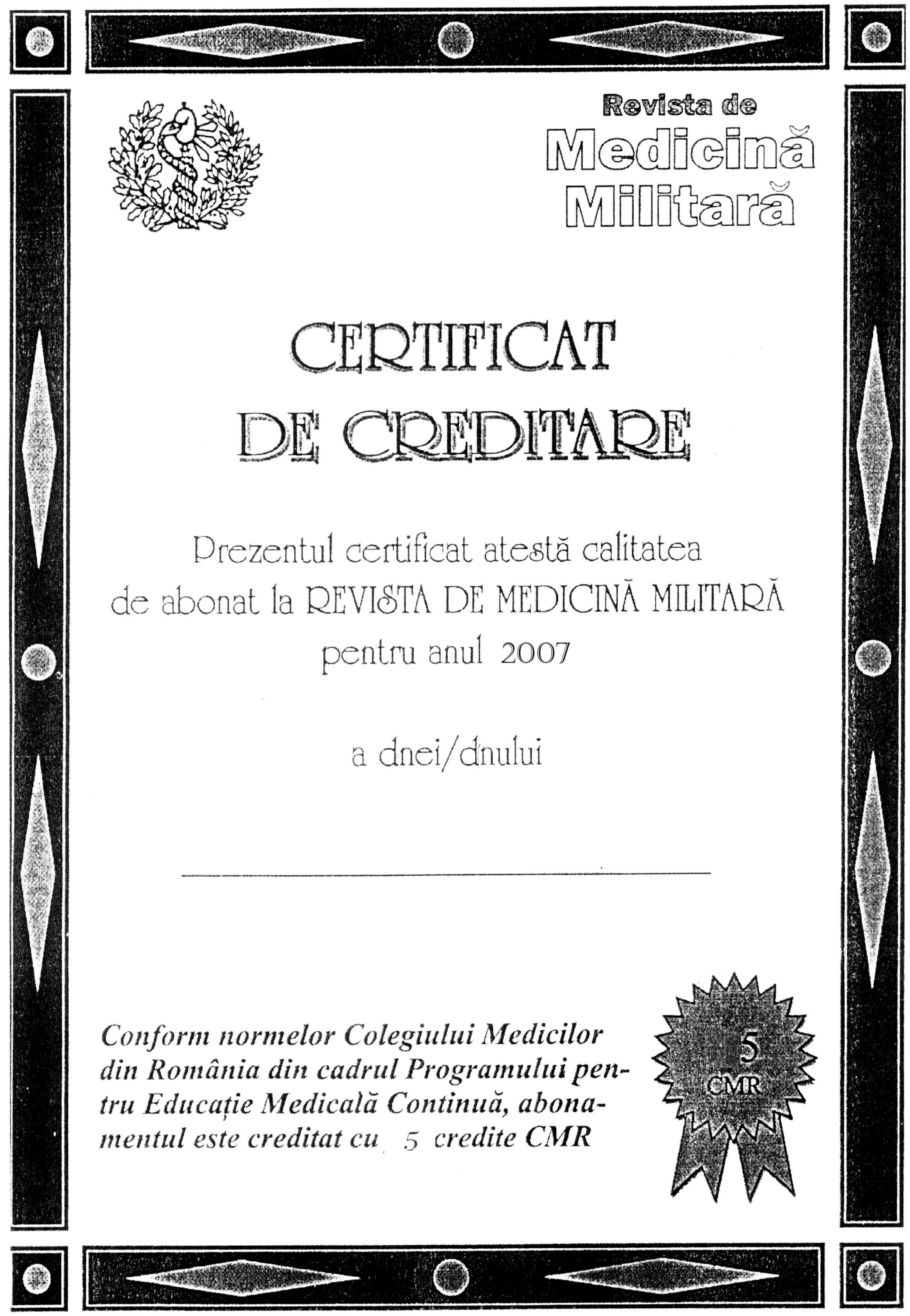

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Lt. col. Dr.
Silviu Stanciu

Cel care speră fără temei, deznădăjduiește fără temei ***(Seneca)***

Medicina militară românească a constituit de-a lungul timpului motorul stiințific și inovativ al medicinei românești atât prin logistică și dotarea de vârf, dar mai ales prin medicii pe care i-a generat, mulți dintre ei adevărate elite în diverse domenii medicale.

Prezentul este cel în care încercăm să supraviețuim, în care normalitatea medicală se atenuează treptat, iar speranța că viitorul ne va salva pâlpâie ca o flacără ce trage să se stingă. De multe ori mă uit în trecut pentru a căpăta puteri și a putea trece peste prezent, amăgindu-mă că o să renaștem și o să clădim la moștenirea lăsată de înaintași. Cred că prezentul trebuie reconsiderat, aici trebuie să ne concentrăm resursele, chiar dacă ele sunt din ce în ce mai mici, mai ales cele umane, care de la an la an se reduc, mirajul atracției pentru a practica în UE nu a iertat nici sistemul medical militar.

Avem nevoie de repere medicale pentru a clădi și a merge mai departe, repere care la rândul lor au dispărut și trebuiesc reconstruite. Un medic bun, un profesor, o elită științifică nu apare peste noapte, crește în timp, ceea ce mă face să percep renașterea medico-militară ca pe un fenomen de lungă durată.

Ceea ce putem face în momentul de față este să începem de undeva. Am început astfel cu revista noastră de Medicină Militară pe care am schimbat-o ca imagine, am reconsiderat formula editorială și calitatea articolelor, dar mai ales am facilitat accesul și contribuția dumneavoastră la dezvoltarea ei, cu publicarea pe un nou site – www.amfmr.ro. Și nu ne oprim aici.

Ne propunem ca în 2014 să publicăm revista în limba engleză, să lărgim comitetul științific și contribuția editorială, astfel încât să depășim barierele medico-militare și naționale, pentru a o putea indexa în 2015 cel puțin în PubMed.

Trăiesc, muncesc, aștept nerăbdător această renaștere pe care o doresc din tot sufletul ca un adevărat cadou de la Moș Crăciun și sunt conștient că pleacă de la oameni, de la colegi, de la conducători și are șanse să se înfăptuiască dacă ne vom așeza la aceeași masă pentru a gândi împreună.

Vă urez un 2014 cu multe împliniri, sănatate și La Mulți Ani!

Cu multă speranță,
Editor-șef
Silviu STANCIU

Investigaţii imunologice pentru evaluarea protecţiei postvaccinale la militarii din teatrele de operaţiuni

Diana Popescu*, Viorel Ordeanu*, Radu Hertzog*, Gheorghe Cristian Iordache,**
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Rezumat

Introducere *Obiectivul studiului este evaluarea statusului imun postvaccinal la militarii români care participă la misiuni în diferite teatre de operaţii în afara graniţelor ţării. Imunizarea activă s-a efectuat conform schemei de vaccinare prevăzută în Dispoziţia tehnică a Şefului Direcţiei Medicale a M.Ap.N. nr. A-3555 din 01.07.2004, cu vaccinuri de import. Scopul a fost de a determina protecţia imunologică a militarilor faţă de hepatita A şi B, tetanos şi febră galbenă.*

Material şi metodă *Pentru analiza statusului imun la personalul militar care participă la misiuni în Teatrele de Operaţii au fost recoltate la Centrul Medical de Diagnostic şi Tratament Ambulatoriu „Academician Ştefan Milcu“ (cu sprijinul Centrului de Medicină Preventivă) probe de sânge de la 168 de subiecţi. Pentru efectuarea analizelor imunologice pentru anticorpii de interes, stabiliţi de Direcţia Medicală şi de Centrul de Medicină Preventivă al M.Ap.N., s-au folosit kit-uri imunoenzimatice ELISA pentru hepatitele A şi B, pentru toxină tetanică şi kit-uri de imunofluorescenţă pentru febra galbenă.*

Rezultate şi discuţii *Prin cele patru tipuri de teste efectuate pe un lot de 168 de subiecţi am realizat o cercetare retrospectivă şi am putut evidenţia o imunitate reziduală înregistrată la diferite intervale de timp (ani) după vaccinare. Am urmărit dinamica descreşterii titrului de anticorpi până la un nivel minim detectabil. Perioada de timp luată în studiu a fost între 1991 şi 2013.*

Concluzii *Rezultatele investigaţiilor imunologice, efectuate de noi, pentru evaluarea protecţiei postvaccinale la militarii din T.O., confirmă eficienţa profilactică a schemei de vaccinare prevăzută în documentele în vigoare.*

Cuvinte cheie*: agenţi biologici, protecţie antiinfecţioasă, status imunitar, vaccinologie*

Abstract

Introduction *The objective of the study is to assess the immune status after vaccination of the Romanian troops that are participating in different missions abroad. Active immunization was performed according to the schedule privided in the Technical Provision MND Head of Medical Department No. A-3555 of 01.07.2004, with imported vaccines. The aim was to determine the immunological protection of the military against hepatitis A and B, tetanus and yellow fever.*

Material and methods *For the analysis of immune status in military personnel participating in missions in theaters, blood samples from 168 subjects were collected to Ambulatory Diagnostic and Treatment „Academician Stefan Milcu“ Medical Center Medical Center (with support of Preventive Medical Center). For the immunological analysis of the antibodies of interest, determined by the Preventive Medical Center of the Ministry of Defence, we used ELISA kits for hepatitis A and B, for tetanus toxin and immunoflorescence kits for yellow fever .*

Results and discussion *In order to highlight the residual immunity at different timelines (years) after vaccination, a study was performed on a 168 subjects lot using the four types of tests. We followed the antibody dynamics decrease to a minimum detectable level. The time period of study was between 1991 and 2013.*

Conclusions *The results of immunological investigations, that we performed to assess protection after vaccination in the military from TO, confirm the effectiveness of prophylactic vaccination schedule as specified in documentation.*

Keywords: *biological agents, antiinfection protection, immune status, vaccinology*

Introducere

Vaccinoprofilaxia se poate realiza: în cadru organizat, prin campanii de vaccinare înscrise în programele obligatorii de imunizare din fiecare țară; în situația declanșării unor focare infecto-contagioase ale căror boli beneficiază de un vaccin corespunzător; opțional, pentru profilaxia bolilor pentru care există vaccin specific, dar care nu este inclus în programele obligatorii de imunizare.

Eficiența individuală a profilaxiei prin vaccinare este variabilă. Din punct de vedere statistic, dacă vaccinul este eficient pentru peste 80% din populația expusă, eficiența epidemiologică a vaccinării este bună, deoarece nu se mai poate produce o epidemie. În această situație vom avea doar cazuri izolate (1-2) sau focare (3-10) și nu vor apare epidemii/epizootii (peste 10 cazuri în aceeași colectivitate) iar riscul de evoluție spre pandemie/panzootie nu mai există. Nu trebuie uitat că vaccinarea nu este doar o metodă de protecție a individului, ci și a colectivității. Persoana vaccinată nu are garanția absolută că nu va face boala respectivă, dar avem garanția că nu va izbucni o epidemie (sau epizootie) în colectivitatea vaccinată. Testări efectuate la INCDMI Cantacuzino pe personalul propriu vaccinat pentru hepatită A și B au arătat că răspunsul individual poate fi imprevizibil: titru mic de anticorpi vaccinali, urmat tardiv de o creștere, de menținere sau de scădere; titru mare de anticorpi vaccinali, urmat tardiv de menținere sau de scădere; lipsa anticorpilor vaccinali, dar și imunitate față de boală la unele persoane expuse. Dar, în ansamblu, rezultatele vaccinării hepatitice au fost bune, deoarece nu au existat cazuri de boli profesionale sau focare de hepatită virală. Acest studiu pilot, coroborat cu absența de metode și date din literatura de specialitate (Epidemia din SUA, etc) ar putea fi extrapolat și la alte vaccinuri.

Obiectivul studiului nostru este evaluarea statusului imun postvaccinal la militarii români care participă la misiuni în diferite teatre de operații în afara granițelor țării. Imunizarea activă a militarilor români se efectuează după următoarea schemă: cu 60 de zile înainte de plecarea în teatrul de operații (T.O.) (vaccin tifoidic, vaccin tetanic, vaccin poliomielitic); cu 30 de zile înainte de plecarea în T.O. (vaccin hepatitic A și B – doza I -, vaccin amarilic); cu 2 de zile înainte de plecarea în T.O. (vaccin hepatitic A și B – doza II -, vaccin meningitic A-C în zona ecuatorială endemo-epidemică pentru meningită); la întoarcerea din T.O. (vaccin hepatitic A și B – doza III- concomitent cu vizita medicală). Vaccinarea antitifoidică se administrează militarilor români din T.O. din Irak, iar pentru alte zone numai la ordinul șefului Direcției Medicale, în funcție de situația epidemiologică. Vaccinarea antiamarilică, antipoliomielitică și antimeningitică se administrează numai militarilor români care execută misiuni în Africa, în funcție de situația epidemiologică din zonă. Scopul a fost de a determina protecția imunologică a militarilor față de hepatita A și B, tetanos și febră galbenă.

Material și metodă

Am efectuat un studiu privind apariția și persistența răspunsului imun după administrarea unor vaccinuri personalului militar care participă la misiuni în T.O., pentru actualizarea și optimizarea schemelor de profilaxie antiinfecțioasă la militari.

Pentru analiza statusului imun la personalul militar care participă la misiuni în Teatrele de Operații au fost recoltate la Centrul Medical de Diagnostic și Tratament Ambulatoriu „Academician Ștefan

Milcu" (cu sprijinul Centrului de Medicină Preventivă) probe de sânge de la 168 de subiecți. Sângele a fost lăsat să coaguleze timp de 2h la temperatura camerei, înainte de centrifugare timp de 15 min la 1000×G, pentru separarea serului sanguin care a fost prelevat și folosit pentru analize.

Pentru efectuarea analizelor imunologice pentru anticorpii de interes, stabiliți de Direcția Medicală și de Centrul de Medicină Preventivă al M.Ap.N., au fost achiziționate kit-uri ELISA (enzyme-linked immunosorbent assay) pentru hepatita A și B, pentru toxină tetanică și kit-uri de imunofluorescență pentru febra galbenă.

Testele imunoenzimatice utilizate pentru probele de ser sanguin au fost: Anti-HAV ELISA (DIAsource, Belgia), Anti-HBs ELISA (DIAsource, Belgia), *Clostridium tetani* toxin IgG-ELISA (NovaTec, Germania) și testul de imunofluorescență Anti-Yellow fever virus IIFT IgG (Euroimmun, Germania).

Tehnica de imunofluorescență s-a realizat folosind un microscop confocal Zeiss 510 (Germania) păstrând parametri de scanare laser pentru fiecare probă evaluată: pinhole: 210 µm, Obiective Plan-Nofluor 20 x 0,5, intensitate 30%, Laser Argon filtru ex:488nm/em:505nm (semnal verde). Contracolorarea citoplasmei s-a realizat cu EvB și s-a evidențiat folosind o sursa de laser He/Ne ex:543nm /em : 585nm, intensitate 12%. (semnal rosu).

Am efectuat dozarea de anticorpi pentru evaluarea statusului imunitar și a protecției postvaccinale la hepatita A și B, tetanos și febra galbenă la subiecți sănătoși, vaccinați conform schemei M.Ap.N. pentru militarii care pleacă în misiuni externe, în diferite teatre de operații. Prin cele 4 teste efectuate pe un lot de 168 de subiecți am putut realiza o cercetare retrospectivă. Astfel, am putut evidenția o imunitate reziduală înregistrată la diferiți ani după vaccinare. Am urmărit dinamica descreșterii titrului de anticorpi până la un nivel minim detectabil. Perioada de timp luată în studiu a început cu anul 1991 și s-a continuat până în anul 2013.

Controlul intern de calitate, pentru dozarea anticorpilor vaccinali pentru hepatita virală A și B, a fost efectuat cu colaborarea INCDMI Cantacuzino. Rezultatele au fost foarte bune, deci trusele de reagenți, linia ELISA și tehnica de lucru sunt corespunzătoare pentru testări.

Rezultate și discuții

Testul Anti-HAV ELISA a evidențiat la 95,23% dintre subiecții luați în studiu prezența unui nivel protector de anticorpi, 4 cazuri nu au fost determinate și 7 cazuri au avut rezultat negativ. În urma calculelor, am constatat că probele cu valoarea ab-

Tabel nr. 1 Interpretarea rezultatelor și recomandări

Valoare D.O.	Nivel protector	Recomandări	Total rezultate
< 0,01	fără nivel protector de anticorpi	imunizare imediată	0
0,01 – 0,1	fără nivel protector de încredere	injecție de rapel și control al concentrației anticorpilor la 4 - 6 săptămâni	0
0,11 – 0,5	nivel protector de încredere	injecție de rapel și control al concentrației anticorpilor la 4 - 6 săptămâni	2
0.51 - 1.0	nivel protector de încredere	control al concentrației anticorpilor după aproximativ 2 ani, nu este necesar rapel	3
1.1 -5.0	nivel protector pe termen lung	control după 5 – 10 ani	163
> 5.0	nivel protector pe termen lung	control după 10 ani	0

sorbanței mai mare decât 0,99 nm sunt considerate non-reactive pentru Anti-HAV, iar probele cu valoarea absorbanței mai mică sau egală cu valoarea 0,99 nm sunt considerate reactive pentru Anti-HAV. Din cei 168 de subiecți folosiți în studiu, 157 au prezentat nivel protector de anticorpi pentru hepatita A, 4 probe s-au încadrat în domeniul de retestare și doar 7 nu sunt protejați față de hepatita A.

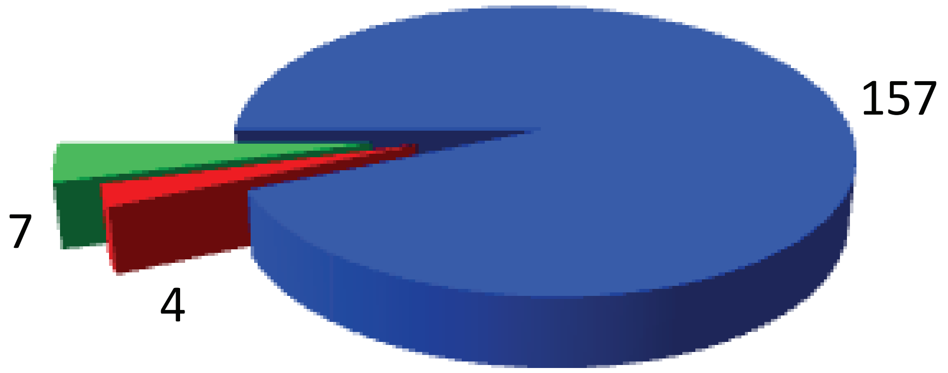

Grafic nr.1 Nivelul protector al anticorpilor pentru hepatita A

Testul Anti-HBs ELISA a evidențiat la 86,31% dintre subiecții luați în studiu prezența unui nivel protector de anticorpi, 2 cazuri nu au fost determinate și 23 subiecți nu au prezentat nivel protector de anticorpi. Domeniul de retestare este cuprins între 0,0315 și 0,0385.

În urma calculelor, am constatat că probele cu valori de absorbție mai mici de 0,0315 nm sunt considerate negative pentru anticorpii anti-HBs, iar probele cu o valoare de absorbție mai mare de 0,0385 nm sunt considerate reactive și sunt pozitive pentru anti-HBs. Din cei 168 de subiecți luați în studiu, 143 au prezentat nivel protector de anticorpi pentru hepatita B, 23 subiecți nu sunt protejați față de hepatita B și 2 subiect, se situează în domeniul de retestare.

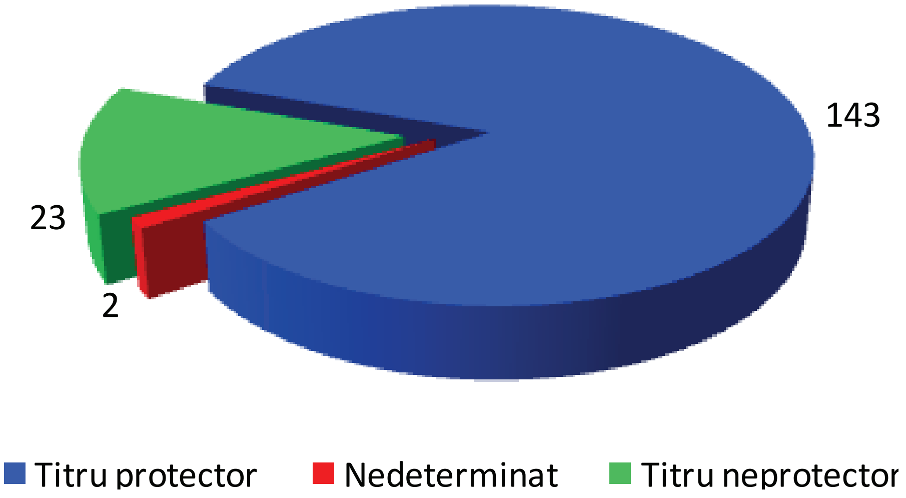

Grafic nr.2 Nivelul protector al anticorpilor pentru hepatita B

Testul *Clostridium tetani* toxin IgG ELISA a evidențiat la 2,98% dintre subiecții luați în studiu prezența unui nivel protector de încredere, iar la restul de 97,02% prezența unui nivel protector pe termen lung.

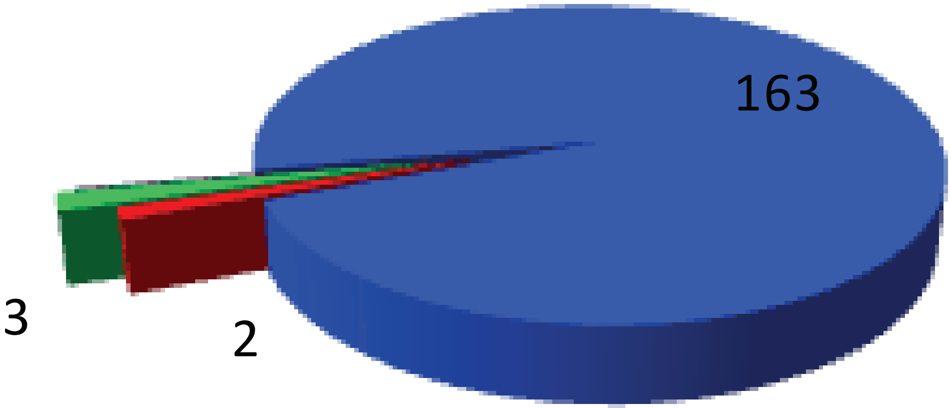

Grafic nr.3 Nivelul protector al anticorpilor pentru toxină tetanică

Testul Anti-Yellow fever virus IIFT descris aici este un test de imunofluorescență care folosește tehnologia cu BIOCHIP-uri pentru a detecta anticorpii IgG anti-YFV, testul este simplu, are o redresare rapidă și se dovedește util în special pentru screening-ul de probe de ser numeroase, cum ar fi ar fi necesar în cercetări epidemiologice la fața locului (în focar).

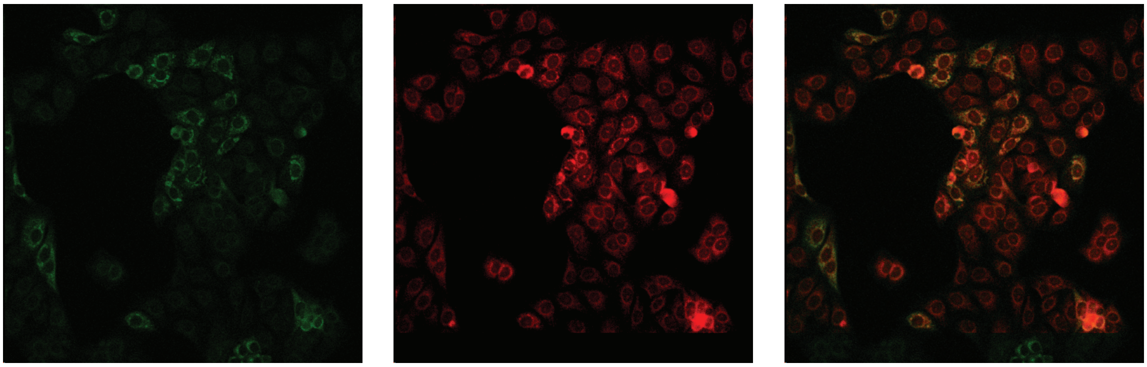

Fig.1 Anti-Yellow fever virus IIFT, proba 104, diluţie 1:10 (semnal fluorescent moderat - verde)

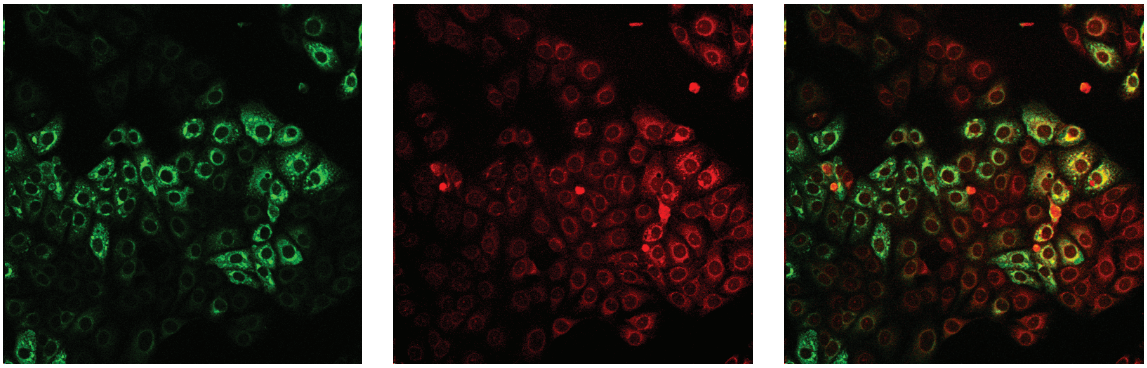

Fig.2 Anti-Yellow fever virus IIFT, proba 105, diluţie 1:10 (semnal fluorescent puternic - verde)

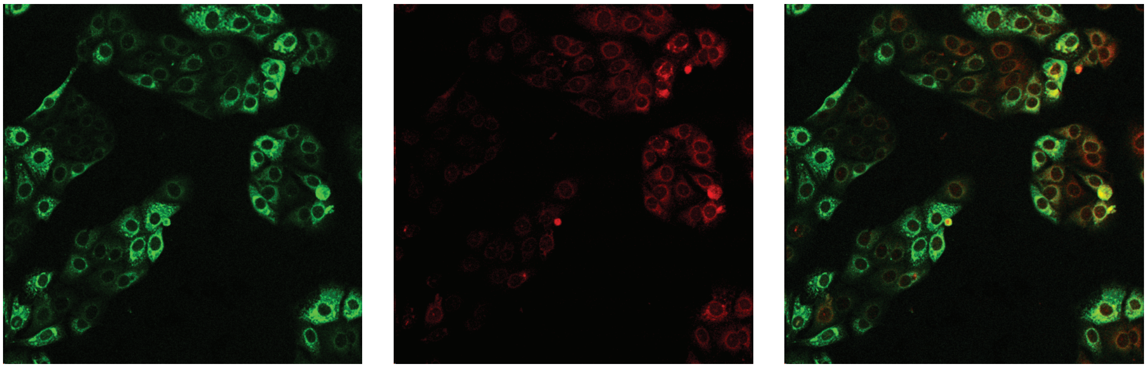

Fig.3 Anti-Yellow fever virus IIFT, proba 106, diluţie 1:10 (semnal fluorescent puternic - verde)

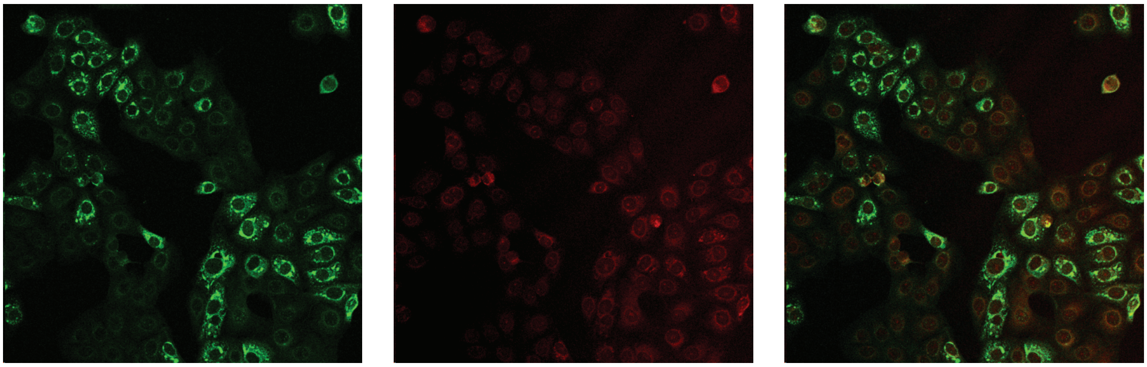

Fig.4 Anti-Yellow fever virus IIFT, proba 107, diluţie 1:10 (semnal fluorescent moderat)

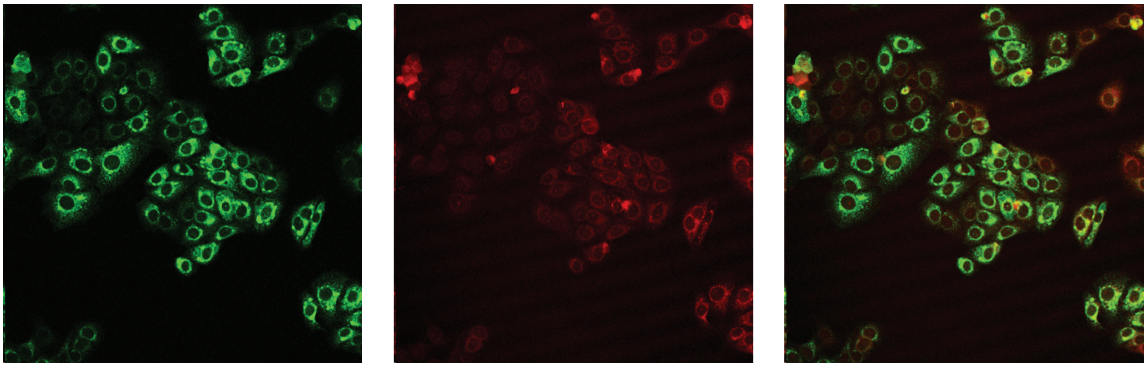

Fig.5 Anti-Yellow fever virus IIFT, proba 121, diluţie 1:10 (semnal fluorescent puternic - verde)

Interpretarea rezultatelor pentru testul Anti-Yellow fever virus IIFT se bazează pe faptul că anticorpii împotriva virusului febrei galbene prin cuplarea cu anticorpi secundari specifici marcarti cu un fluorocrom, determina aparitia unui semnal de fluorescenţă a celulelor infectate. Citoplasma conţine structuri fine sau granulare şi incluzii colorate fluorescent. Unele dintre celulele din domeniul vizibil nu sunt infectate si nu prezintă nici fluorescenţă specifică. Dacă proba unui subiect conţine anticorpi împotriva virusului febrei galbene, imaginea obţinută la microscopul cu fluorescenţă trebuie să fie asemănătoare cu a controlului pozitiv. Titrul este definit ca factorul de diluție a probei pentru care fluorescența specifică este slab vizibilă. Din cei 168 de subiecți luați în studiu, pentru acest test am selectat cinci probe (fig.1-5), la subiecți care s-au întors din Africa, deoarece vaccinarea anti-virus febră galbenă se efectuează doar pentru cei care pleacă în misiuni în această zonă endemică. Testarea a demonstrat că în toate cele cinci cazuri nivelul protector de anticorpi a fost atins.

Concluzii

Dozarea de anticorpi pentru evaluarea statusului imunitar și a protecției postvaccinale la hepatita A și B, tetanos și febra galbenă la subiecți sănătoși, vaccinați conform schemei M.Ap.N. pentru militarii care pleacă sau se întorc din misiuni externe în diferite teatre de operații, este necesară pentru studiul privind apariția și persistența răspunsului imun postvaccinal.

Cercetarea retrospectivă, prin cele patru tipuri de teste efectuate pe un lot de 168 de subiecți, certifică dinamica statusului postvaccinal.

Evidențierea imunității reziduale înregistrată la diferiți ani după vaccinare și descreșterea titrului de anticorpi până la un nivel minim detectabil, pentru subiecții vaccinați în intervalul 1991 – 2013, pot contribui la actualizarea și optimizarea schemelor de vaccinare conform principiilor de farmacovigilență și farmacoeconomie, precum și la creșterea eficienței protecției prin imunizare artificială la principalele boli infecțioase (stabilite de epidemiologi) și economii prin imunizarea judicioasă a militarilor.

Protecția imunologică față de infecția cu virusul hepatitei A este prezentă la peste 95% din militarii testați. Protecția imunologică față de infecția cu virusul hepatitei B este prezentă la peste 86% din militarii testați, fiind posibil ca nu toți subiecții să fi primit corect cele trei doze de vaccin necesare. Toți militarii testați prezintă protecție imunologică față de toxina tetanică, deci rănile contaminate cu pământ nu prezintă risc tetanigen major. Militarii vaccinați contra febrei galbene au un nivel de protecție imunologică eficient, dar lotul testat nu este suficient pentru o prelucrare statistică, fiind necesară continuarea studiului.

Considerăm că nivelul de protecție imunologică al militarilor din T.O., care au fost vaccinați conform schemei de vaccinare prevăzută în Dispoziția tehnică a șefului Direcției Medicale, este eficient, dar vaccinarea contra hepatitei B va trebui monitorizată în continuare, pentru validarea nivelului de protecție.

Rezultatele investigațiilor imunologice, efectuate de noi pentru evaluarea protecției postvaccinale la militarii din T.O., confirmă eficiența profilactică a schemei de vaccinare prevăzută în documentele în vigoare.

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Riscul cardiovascular la populaţia militară activă

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Rezumat

Au fost luați în studiu un număr de 920 de militari activi cu vârsta cuprinsă între 20 și 55 de ani, la care s-a investigat prevalența factorilor de risc cardiovascular.

Hipertensiunea arterială are o prevalență de 20,76% din populația militară activă și apare la vârste mai tinere. TA „normal înaltă" a avut de asemenea o prevalență crescută, atingând 28,26% pentru întregul lot de studiu.

Hipercolesterolemia s-a înregistrat la 58,48% dintre subiecți.

Prevalența fumatului a fost foarte crescută la populația militară activă, atingând cifra de 61,2%.

Prevalența diabetului zaharat a fost inferioară (2,39%) celei din populația generală, care după Studiul SEPHAR fost de 5% (1), dar prevalența supragreutății și obezității (52,6%) a fost similară.

Prevalența stresului este foarte ridicată în rândul populației militare active, atingând cifra de 89,67%, prin aceasta stresul devenind un factor de risc cardiovascular specific pentru acest grup populațional.

Cuvinte cheie: *factori de risc cardiovascular, populație militară activă, patologie cardiovasculară*

Introducere

Patologia cardiovasculară în general, și cea aterosclerotică în special, reprezintă un subiect important de cercetare clinică și epidemiologică, datorită nivelurilor înalte de morbiditate și mortalitate pe care le determină. Numeroase studii epidemiologice populaționale asociate asociate cercetărilor fundamentale și clinice, au dus la elaborarea unui concept profilactic unitar care are la bază existența factorilor de risc cardiovascular(2). Factorii de risc cardiovascular definesc elemente care caracterizează individul sănătos, dar care sunt asociate statistic cu posibilitatea apariției în viitor a bolilor cardiovasculare aterosclerotice(2,3).

Plecând de la această idee, activitatea de prevenire a patologiei cardiovasculare (aterosclerotice în special) implică 3 genuri de strategii:

- *strategia populațională*, care implică controlul factorilor de risc la nivelul întregii populații (profilaxia primară populațională, constând în influiențarea stilului de viață și a factorilor de mediu);
- *strategia grupurilor aflate la risc* reprezintă identificarea factorilor de risc la anumite grupe populaționale și apoi controlul acestora (profilaxie primară individuală);
- *strategia profilaxiei secundare* reprezintă controlul factorilor de risc la grupuri care prezintă deja patologie cardiovasculară aterosclerotică(3).

Studierea prevalenței factorilor de risc la un grup populațional are o importanță deosebită pentru evaluarea riscului cardiovascular global și a celui relativ și, în ultimă instanță, a probabilității de apariție a patologiei aterosclerotice, comparativ cu alte categorii profesionale(2,3,4).

Prin specificul activității profesionale, este de așteptat ca populația militară activă să prezinte unele particularități ale expunerii la factorii de risc cardiovascular. Scopul acestui studiu este acela de a identifica aceste particularități, precum și răsunetul lor asupra patologiei cardiovasculare aterosclerotice.

Material și metodă

Au fost luați în studiu un număr de 920 de angajați ai unităților militare din Cluj-Napoca , cu profil terestru din majoritatea specialităților, precum și din domeniul forțelor aeriene, acestea la rândul lor cuprinzând piloți de pe avioanele militare, piloți de pe elicoptere, parașutiști, precum și personal de la sol, specializat în activitatea de control și dirijare a zborului („personal navigant”).

Din cei 920 de membri ai lotului 868 (94,3 %) au fost bărbați, cu vârsta cuprinsă între 21 și 55 ani, cu toții militari activi (cadre militare active sau militari angajați pe bază de contract, categorie care face parte de asemenea din rândul personalului militar activ).

Sexul feminin a fost reprezentat de un număr de 52 de angajate ale unităților militare, între care au fost atât cadre militare active, cât și personal civil cu activități asemănătoare în foarte mare măsură (administrație, personal medical sau din domeniul logistic), aceasta ținînd cont de faptul că personalul strict militar de sex feminin este încă reprezentat într-o pondere mică.

Din totalul de 52 de femei, 17(32,69 %) au fost cadre militare propriu zise, iar 35 (67,31 %) fac parte din categoria salariaților civili, a căror activitate permanentă în domeniul militar nu diferă foarte mult de cea a cadrelor militare femei.

Credem că este important de subliniat faptul că din totalul de 920 de membrii ai lotului de studiu, majoritatea au desfășurat misiuni în cadrul forțelor NATO, în special în Afganistan și Irak, dar și în alte zone, durata acestor misiuni fiind de cel puțin 6 luni.

Astfel, 740 de militari (80,43%), din întregul lot au participat la misiuni internaționale, iar 180 de subiecți (19,57%) și-au desfășurat activitatea în unitatea în care sunt angajați. Din totalul de 740 de militari participanți la misiuni internaționale, doar 3 au fost femei (0,40%).

Tuturor membrilor lotului laut în studiu li s-a efectuat examen clinic general, determinarea greutății, calcularea IMC, determinarea valorilor TA, determinarea colesterolului total (CT), a trigliceridelor (TG), a glicemiei.

De asemenea subiecții au completat câte un chestionar din care să rezulte și alți factori de risc cardiovascular sau comportamente cu risc cardiovascular.

Astfel, întrebările din chestionar s-au referit la fumat (în cazul fumătorilor s-a cerut să se precizeze și numărul de țigări fumate pe zi), consumul de alcool, cafea, regim alimentar (consumul de grăsimi, sare și dulciuri), sedentarism (activitate fizică în afara celei legate de programul zilnic de la locul de muncă și pregătire), gradul de expunere la stresul psihic, inclusiv referiri la calitatea și durata somnului.

S-au înregistrat, de asemeni relații despre antecedentele personale și heredocolaterale pe linia bolilor cardiovasculare, precum și cele referitoare la tulburările metabolismului lipidc sau de glicoreglare.

În ceea ce privește evaluarea gradului de stres psihic, am folosit un chestionar propriu, adaptat după chestionarele de specialitate, bazat pe 2 întrebări: calitatea somnului și o autoevaluare a stării de anxietate (și/sau depresie), folosind o scală formată din 3 trepte: 0 - fără stres; 1- stres ușor sau moderat; 2- stres crescut.

Perioada de efectuare a studiului a fost de cca. 2 ani, de la sfârșitul anului 2003 până la sfârșitul anului 2005 (luna noiembrie).

Rezultate

Caracteristicile lotului studiat sunt expuse în Figura 1, iar principalele rezultate obținute sunt expuse în Tabelul 1.

Hipertensiunea arterială a fost prezentă la 20,76% dintre subiecți (20,39% pentru bărbați și 26,92% pentru femei).

Un procent semnificativ din populația militară activă a prezentat TA normal înaltă – 28,26% (28,92% bărbați, 17,31% femei).

Prevalența **hipercolesterolemiei** a fost de 58,48% (59,56% bărbați și 40,38% femei). De asemenea, prevalența **hipertrigliceridemiei** a fost ridicată, fiind notată la 42,28% dintre subiecţi (43,78% bărbaţi; 17,31% femei).

Prevalenţa **fumatului** a fost extrem de înaltă, fiind notată la 61,2% dintre subiecţi (61,8% bărbaţi şi 50,1% femei), procent aproape dublu faţă de cel înregistrat în populaţia generală.

Alterarea metabolismului glucidic reperezentată de prezenţa **diabetului zaharat** sau de **glicemia bazală alterată** („impaired fasting glucose" – IFG) a fost înregistrată la 12,93% dintre subiecţi. În schimb diabetul zaharat propriu zis a fost notat într-un procent redus, de numai 2,39% dintre subiecţi).

Supraponderea şi obezitatea au fost surprinzător de frecvente la populaţia militară activă, fiind notate împreună într-un procent de 52,60%. De asemenea, supraponderea a fost întâlnită în procent de 45,43%, obezitatea fiind înregistrată în doar 7,17% din cazuri.

Stresul – considerat oarecum specific activităţii militare, a fost „autoraportat" de 89,66% dintre subiecţii lotului, 90,55% bărbaţi şi 75% femei.

Sedentarismul s-a înregistrat într-un procent surprinzător de mare pentru această categorie, fiind autoraportat de 37,5% dintre subiecţi (35,48% bărbaţi; 71,15% femei).

Discuţii

Evaluarea factorilor de risc cardiovascular la populaţia militară activă reprezintă un demers clinic şi epidemiologic care se justifică prin faptul că acest segment populaţional, deşi este expus la o întreagă pleiadă de comportamente şi factori de risc particulari, nu a fost suficient explorat din acest punct de vedere.

Explicaţia constă, cel puţin în parte, în faptul că această categorie populaţională este considerată din start sănătoasă şi se ignoră faptul că este vorba despre un gen de activitate care implică un grad mare de solicitare fizică şi psihică, la care se adaugă o serie de privaţiuni care se constituie în adevăraţi factori de risc cardiovascular.

Factorii de risc cardiovascular apar la vârste mai tinere decât în populaţia generală şi sunt corelaţi cu un stres profesional crescut, specific acestei profesii(5).

Hipertensiunea arterială are o prevalenţă ridicată la populaţia militară activă (20,76%) şi apare la vârste mai tinere. Desigur, pentru populaţia generală din ţara noastră prevalenţa HTA este de 40% (1), dar la o medie de vârstă net superioară celei a lotului studiat de noi. TA„normal înaltă" are de asemenea o prevalenţă semnificativ crescută, (21,3%) faţă de populaţia generală. Prevalenţa HTA este crescută şi la militarii altor state. Astfel, la un lot de piloţi militari tineri din Ungaria s-a decelat în 2007 o prevalenţă a HTA de 14,7%(6), explicată prin consumul de sare, fumat şi stres. În Israel, un studiu din 2006, raportează la militarii de vârstă tânără o prevalenţă a prehipertensiunii arteriale de 50,6% la bărbaţi şi de 35,9% la femei, cauzele incriminate fiind creşterea BMI, dislipidemiile şi stilul de viaţă(7). Valori ale prevalenţei HTA, oarecum asemănătoare cu cele decelate de către noi au fost raportate la militarii francezi(21,7%, în Studiul EPIMIL, publicat în 2007(8), iar în Grecia (2005) se estimează o prevalenţă a HTA de 78% la cei 23,6% dintre militarii care prezentau sindrom metabolic.(9)

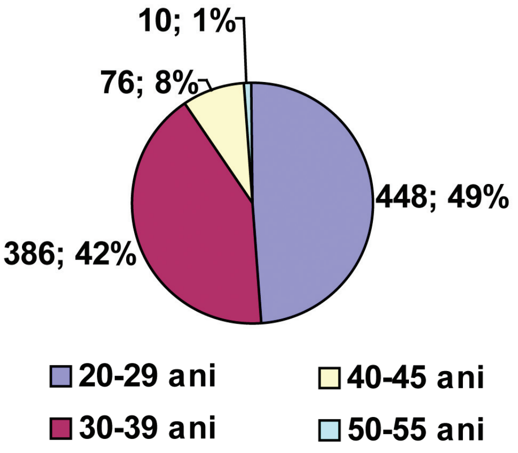

Figura 1 - Grafic cu lotul de studiu pe limite de vârstă

Hipercolesterolemia are o prevalenţă mult crescută, net superioară celei din populaţia generală a ţării noastre, care se situează la 24%(1). Prevalenţa hipercolesterolemiei este crescută de la vârste tinere (decadele a 2-a şi a 3-a) şi creşte semnificativ ($p<0,01$), o dată cu decadele de vârstă. Într-un studiu efectuat pe un lot de militari din marina SUA, prevalenţa hipercolesterolemiei a oscilat între 11,3% şi 34,6%(10). Un studiu efectuat pe o populaţie tânără militară (17-35 de ani) din USA , decelează faptul că 32% dintre aceştia prezentau valori ale $CT\geq200$ mg%, la care se adăuga obezitatea şi fumatul(11).

Studiul AFCAPS a demonstrat în SUA, în 2000 pe un lot de militari, că tratamentul cu lovastatin, alături de schimbarea stilului de viață au determinat o scădere cu 37% a incidenței primului eveniment coronarian major, la subiecți cu valori mediu crescute ale CT (12), aspect susținut și de către Clearfield M.și col. pe un eșantion de piloți militari(13). O prevalență înaltă a hipercolesterolemiei (53,9) a fost decelată de către Grosz C. la tineri militari unguri (6).

Hipertrigliceridemia are o prevalență de 42,28% în lotul luat în studiu, fiind net superioară celei întâlnite pentru populația generală (23%, după STUDIUL SEPHAR)(1).

Un studiu recent efectuat asupra piloților militari din Arabia Saudită (în 2007), arată că 100% dintre subiecții cu sindrom metabolic au valori ale TG serice≥150mg/dl(14). Într-un alt studiu efectuat în 2005 de către Al-Qahtani și col.asupra unui lot de militari saudiți cu vârsta cuprinsă între 20 și 60 ani, prevalența hipertrigliceridemiei a fost de 32,2%.(15).

Prevalența fumatului a fost extrem de crescută pentru populația militară activă, atingând cifra de 61,2%, mult superioară față de media pe țară (29%, după Studiul SEPHAR)(1).

Prevalența este superioară celei înregistrate de Patton JF. și col. la tineri piloți militari americani (36%) (11). Un alt studiu efectuat în Marea Britanie în 1981 constată o prevalență semnificativ crescută a cardiopatiei ischemice la militari comparativ cu populația civilă și explică acest fenomen prin ponderea mult mai mare a fumatului la populația militară(16).

Supraponderea și obezitatea au o pondere globală de 52,60%, cifră asemănătoare cu cea înregistrată la populația generală, de Studiul SEPHAR (1), dar crescută pentru specificul grupului de studiu. Spre deosebire de populația generală însă, domină supraponderea (45,43%), obezitatea propriu zisă fiind rar notată (7%).

Datele din literatura de specialitate referitoare la supraponedere și obezitate la populația militară din armatele altor națiuni, arată că acestea au de asemenea o prevalență neașteptat de mare pentru această categorie profesională. Astfel, pentru o populație militară tânără, formată din bărbați cu vârsta cuprinsă între 17 și 35 de ani din SUA, în 1980 se descrie o prevalență a obezității de 29%(11). Un studiu efectuat pe un lot de militari din Singapore de către Gan SK. și col. (2003), arată o asociere puternică între HTA și obezitate (17).

Tabelul 1. Prevalenţa factorilor de risc cardiovascular la lotul de studiu

Factor de risc cardiovascular	**Lot global**	**Lot masculin**	**Lot feminin**
HTA	20,76%	20,39%	26,92%
TA „normal înaltă”	28,26%	28,92%	17,31%
Hipercolesterolemia	58,48%	59,56%	40,38%
Hipertrigliceridemia	42,28%	43,78%	17,31%
Fumatul	61,2%	61,8%	50,1%
Alterarea metabolismului glucidic	12,93%	13,36%	5,77%
Diabetul zaharat	2,39%	2,3%	3,85%
Glicemia bazală alterată	10,54%	11,06%	1,92%
Supraponderea și obezitatea	52,60%	52,76%	50%
Supraponderea	45,43%	46,65%	25%
Obezitatea	7,17%	6,10%	25%
Stresul	89,66%	90,55%	75%
Sedentarismul	37,5%	35,48%	71,15%

Într-un alt studiu efecuat în SUA (2005) pe un grup de militari în retragere și familiile lor, cu vârsta cuprinsă între 38-64 de ani, se arată că supraponderea și obezitatea împreună au o prevalență de 80% la bărbați și 60% la femei, iar obezitatea singură atinge 33% la bărbați și 29% la femei(18).

Tulburările de glicoreglare au avut o prevalență de 12,93%, asemănătoare celor întâlnite în populația generală, dar net inferioare pentru prevalența diabetului zaharat (2,39). După Studiul SEPHAR, procentul diabetului în populația generală din țara noastră este de 5%, iar a toleranței alterate la glucoză de 13%(1).

Chen CH. și col. decelează pe un eșantion de populație militară și civilă o prevalență a diabetului zaharat de 6,7% (19). Un alt studiu efectuat de către Jiang BQ. și col. (în 2007), pe o populație urbană chineză (militari și civili), de vârstă medie și adultă, decelează o prevalență a diabetului zaharat de 11,2% (20).

Sedentarismul înregistrat la peste 1/3 din grupul studiat este ridicat pentru specificul acestuia și ar putea contribui la creșterea riscului cardiovascular.

Un studiu efectuat pe un grup de militari pakistanezi de către Gandapur AS.și col, evidențiază, în 2006, faptul că exercițiul fizic aerobic, constant, determină o înbunătățire a profilului lipidic (scăderea Apoproteinei B, a LDL-col. și a raportului CT/HDL), comparativ cu sedentarii(21).

Prevalența stresului în lotul studiat atinge cifra de 89,67 %. Prin prevalența sa crescută, stresul este un adevărat factor de risc cardiovascular, specific pentru populația militară activă.

În studiul publicat în 1995, în Taiwan de către Chen CH.și col, referitor la populația militară și civilă din provincia Kin-Chen, Kinmen, aflată peste 40 de ani sub control militar străin, se arată că stresul determină o paletă largă de factori de risc cardiovascular și patologie cardiovasculară. În consecință prevalența HTA (valori ≥160/95 mm Hg) a fost de 25,2% la bărbați, fumatul s-a extins la 41,5% dintre bărbați, iar boala ischemică coronariană posibilă a avut o prevalență de 21,4% la femei și de 17,6% la bărbați(19).

Un raport publicat în 1993 de către Nanda A., asupra stării de sănătate a populației militare și civile din țările est-europene, confirmă că bolile cardiovasculare reprezintă principala cauză de mortalitate și aceasta se datorează în mare măsură stresului(22). Nisara S. și col. , demonstrează în 2009 pe un lot de militari americani faptul că există o relație directă între stres (forțele combatante desfășurate) și prevalența HTA. Astfel, s-a constatat că la forțele combatante, desfășurate în dispozitiv, după 3 ani de monitorizare, s-au identificat cu 6,9% mai multe cazuri de HTA, comparativ cu forțele neangrenate în acțiuni combatante. Dintre membrii lotului de studiu, o mare parte au particitat la acțiunile din Irak și Afganistan. La militarii mobilizați, probabilitatea de a dezvolta HTA a fost de 1,33 de ori mai mare, demonstându-se rolul stresului ca unic factor de risc pentru apariția de noi cazuri de HTA(23). Mullie P. și col. demonstrează în august 2009 pe un eșantion de 5000 de militari , bărbați, belgieni, că obiceiurile alimentare sănătoase depind de factori culturali, socioeconomici și nutriționali(tradiționali). Astfel, margarina cu fitosteroli sau fitostanol este utilizată zilnic de către 26,3% dintre militari, 10,2% consumă un pahar de vin roșu zilnic, 19,1% consumă mai multe porții de fructe pe zi și 26,6% consumă mai multe porții de vegetale. Doar 12,3% dintre militari consumă o porție de pește gras pe săptămână. Consumul de lactate fermentate este crescut proporțional cu activitatea fizică și cu utilizarea de suplimente vitaminice(24).

Concluzii

1. Factorii de risc cardiovascular majori (HTA, dislipidemia, fumatul), au o prevalență mai mare la populația militară activă, comparativ cu populația generală și apar la vârste mai tinere.
2. Prevalența fumatului este dublă față de populația generală, iar hipercolesterolemia și hipertrigliceridemia au de asemenea o prevalență net superioară față de populația generală.
3. Supraponderea și obezitatea au o pondere surprinzător de mare pentru acest segment populațional, chiar dacă nu este mult diferită față de cea din populația generală.

Situația este asemănătoare și în ceea ce privește tulburările de glicoreglare.

4. Surprinzător de crescută este prevalența sedentarismului la populația militară activă.
5. Stresul are o pondere foarte ridicată în rândul populației militare active, reprezentând un adevărat factor de risc particular, specific pentru acest segment de populație.

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Hipoplazie pulmonară unilaterală

– *prezentare de caz*

Sindromul Swyer-James MacLeod (SSJM) sau sindromul de hipertransparență pulmonară unilaterală este o entitate rară ce se asociază cu un istoric al pacientului de episoade repetate de bronșiolită obliterantă în copilărie [1]. Swyer și James au descris pentru prima oară acest sindrom în 1953, la un copil [2], pentru ca mai apoi, un an mai târziu, MacLeod să descrie 9 cazuri cu același sindrom [3].

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INTRODUCTION

Pulmonary hypoplasia is defined as an incomplete development of the lungs; a broncho-pulmonary anomaly characterized by a decreased number of alveoli and decreased alveolar air space. This congenital malformation most often occurs secondary to fetal abnormalities that interfere with normal development of the lungs. Primary pulmonary hypoplasia is very rare and usually is not associated with other abnormalities.

MATERIAL AND METHOD

We discuss the case of a 35 years-old male, ex-smoker, admitted in our service with complaints of recurrent chest infections since childhood. Clinical examination showed diminished respiratory movements of left hemithorax and left diffuse crepitation on auscultation. The rest of the systems were within normal limits. Because of these severe, recurrent chest infections, not responsive to antibiotic medication, after several investigations (CT aspect of small, polycystic left lung) we proposed a surgical intervention – left pneumonectomy.

RESULTS

The patient underwent thoracic surgery, removing the hypoplastic left lung, which was the cause of his recurrent pneumonia. Intraoperative we observed a small left pleural cavity with a hypoplastic, polycystic, not ventilated left lung. After surgery, the patient had a favorable outcome, with no postoperative chest infection. Six months after surgery the patient is still negative for lung bacterial infection, with good effort tolerance.

CONCLUSION

The surgical intervention was the most appropriate treatment for this patient. We appreciate that these recurrent pulmonary infections on the hypoplastic lung lead to an irreversible evolution to not functional, destroyed left lung.

Introducere

Acest sindrom se caracterizează printr-o hipoplazie sau agenezie a vaselor pulmonare, rezultând o hipoperfuzie pulmonară cu o dezvoltare normală a sistemului bronșic, ceea ce duce la un aspect radiologic de plămân hipertransparent, mic sau de dimensiuni normale [4].

Deși este cunoscut faptul că această afecțiune este rezultatul unor episoade repetate de bronșiolită obliterantă în copilărie(afecțiune virală) [5], recent s-a stabilit că acest sindrom poate apărea și în urma infecțiilor cu bacilul tuberculos sau Mycoplasma Pneumoniae [6, 7]. Cei mai mulți pacienți sunt diagnosticați încă din copilărie, dar, uneori, aceștia pot rămâne asimptomatici până la vârsta adultă [8].

Prezentăm în cele ce urmează cazul unui pacient în vârstă de 34 de ani, diagnosticat cu SSJM în urma prezentării la spital pentru un episod de hemoptizii reduse cantitativ.

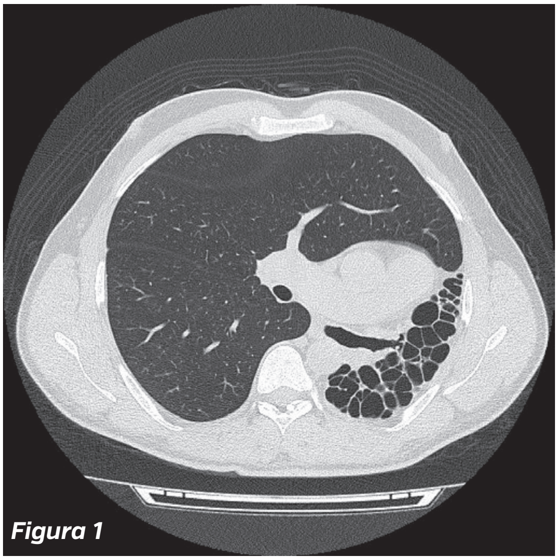

Figura 1

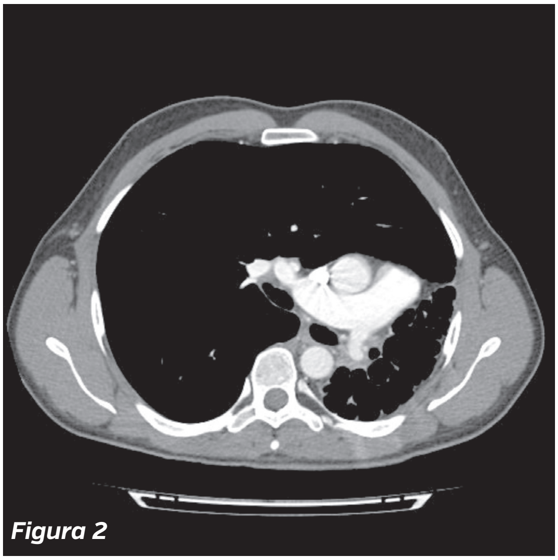

Figura 2

Prezentare de caz

Pacient în vârstă de 34 de ani, fumător 3 PA, se prezintă în serviciul de Pneumologie acuzând un episod de hemoptizii reduse cantitativ. Din istoricul medical al acestuia reținem un episod infecțios respirator la vârsta de 4 ani pentru care a fost internat în spital aproximativ 10 luni (pacientul afirmă că a fost vorba de o pneumonie de aspirație), urmată de un episod de pleurezie stângă la vârsta de 20 de ani.

La prezentarea în serviciul de Pneumologie starea generală a pacientului era bună, murmurul vezicular ușor diminuat la nivelul hemitoracelui stâng, cu raluri ronflante la acest nivel. SpO2 la prezentare era de 98%, cu o TA=110/60 mmHg și o AV=89 bpm.

Biologic, starea pacientului nu prezenta nici o modificare, cu un nivel al leucocitelor în limite normale, precum și o hemoglobină de 13.7 g/dl, de asemenea în limite normale. Nici partea de biochimie sau coagulare nu prezenta nicio modificare notabilă.

Radiografiile toracice postero-anterioară și de profil efectuate arată structuri osoase fără modificări, hemidiafragm stâng ascensionat, sinusuri costo-diafragmatice libere. La nivelul întregului câmp pulmonar stâng se observă o opacitate întinsă, de opacitate subcostală, cu multiple zone de hipertransparență în interior, imprecis delimitată și cu elementele mediastinale tracționate spre stânga.

Pletismografia efectuată arată capacități, volume și debite pulmonare în limite normale, rezistență centrală la flux normală, factor de transfer gazos prin membrana alveolară normal (CPT=5.72 L, 97.5% din prezis, FEV1=79.1% și DLCO SB 77.2% din valorile prezise). Testul de mers 6 minute efectuat s-a situat de asemenea în limite normale, fără agravarea semnificativă a oboselii, dispneei pe scara BORG.

Examenul bronhoscopic efectuat arată un aspect bronșitic difuz bilateral, sânge vechi redus cantitativ la nivelul întregului sistem bronșic, dar mai ales pe stânga. Se efectuează toaletă bronșică. Nu se găsesc surse active de sângerare la momentul investigării, iar segmentarele bronșice sunt libere până în distal.

Examenul microbiologic al sputei pentru BK este negativ, iar la examenul pentru flora bacteriană banală se identifică mucus și detritus celular în care se mai văd și resturi de PMN, foarte rari cocobacili și bacili scurți Gram-negativi extracelulari, foarte rari coci Gram-pozitivi în perechi extracelulari. Pe mediile de cultură nu s-au dezvoltat bacterii potențial patogene. În schimb, la examenul microbiologic al aspiratului bronșic s-au identificat frecvente hematii, foarte rare PMN, rari bacili Gram-negativi extracelulari, iar pe culturile efectuate s-a identificat un Paeudomonas aeruginosa (++) sensibil la ciprofloxacină, levofloxacină și colistin, rezistent la ceftriaxon.

Examenul CT toracic cu substanță de contrast efectuat arată o hipoplazie pulmonară stângă cu bronșiectazii chistice la acest nivel, precum și o arteră pulmonară stângă de calibru redus (figurile 1 și 2)

În urma investigațiilor efectuate se evidențiază hipoplazia arterei pulmonare stângi și a plămânului stâng, care are aspect chistic (bronșiectazii chistice),

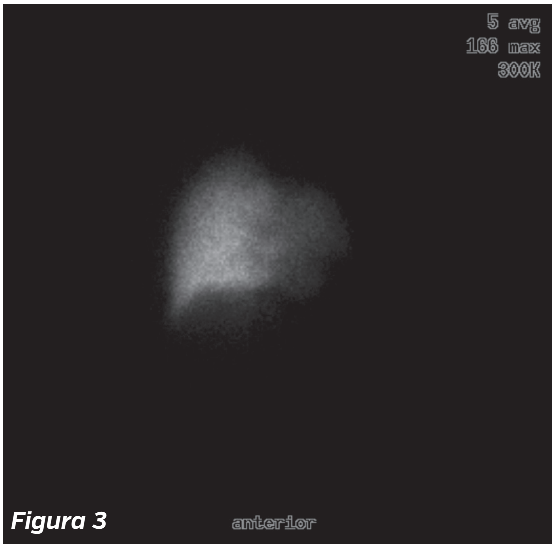

Figura 3

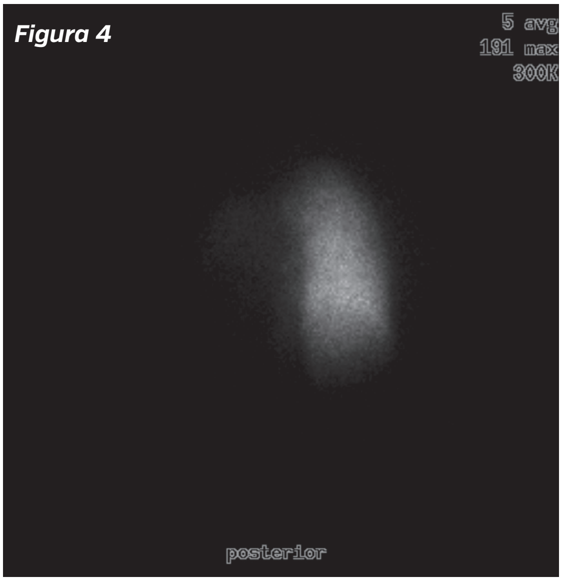

Figura 4

dar este dificil de precizat dacă hipoplazia pulmonară este congenitală sau este consecința unei bronșiolite infecțioase din copilărie (SSJM). Cea de a doua variantă pare mai plauzibilă, având în vedere istoricul pacientului. Pe parcursul a aproximativ un an, pacientul urmează tratament medicamentos antibiotic intermitent, cu reapariția intermitentă a hemoptiziilor și menținerea colonizării cu Pseudomona aeruginosa. Având în vedere această evoluție nefavorabilă și pentru stabilirea diagnosticului de certitudine și a unei atitudini terapeutice adecvate, pacientul este îndrumat către serviciul nostru de Chirurgie toracică.

În urma unei scurte reevaluări, în cursul căreia s-a efectuat și o scintigrafie pulmonară de perfuzie (figura 3, 4), se stabilește indicația chirurgicală a cazului și se decide intervenția chirurgicală.

Intraoperator, se constată un plămân stâng hipoplazic, degenerat chistic (figura 5), vase pulmonare hipoplazice (figura 6), dar o bronșie primitiva stângă de calibru aparent normal (figura 7). Se disecă, ligaturează și se secționeaza vasele pulmonare stângi, urmate de disecția și sutura și secționarea mecanică a bronșiei primitive stângi (figura 8). Bontul bronșic este întărit cu un fir de sutură monofilament resorbabil din polydioxanonă și acoperit cu un fragment de grăsime pericardică pediculată pentru o mai bună protecție a acestuia (figura 9). Drenajul postoperator al cavității toracice s-a efectuat cu un singur tub de dren cuplat la o baterie de drenaj de tip Becler. Evoluția postoperatorie a pacientului a fost favorabilă, cu suprimarea drenajului a treia zi postoperator. Controlul radiologic postoperator arată un aspect în limite normale (figura 10).

Discuții

SSJM este considerat ca fiind o boală relativ rară și complexă, caracterizată print hipertransparența unilaterală a unei părți sau a unui întreg plămân. În prezent, este considerată o boală secundară unor episoade de bronșiolită virală sau pneumonie în copilărie cu microorganisme precum Paramyxovirus Morbillivirus, Bordetella pertussis, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Influenza A și adenovirus tipul 3, 7 și 21. Pacientul nostru a suferit multiple episoade infecțioase pulmonare, se pare cu Pseudomonas aeruginosa, asimptomatic până la vârsta de 34 de ani, când s-a prezentat cu dispnee de efort și hemoptizii în cantitate redusă. Alți pacienți pot rămâne asimptomatici până la vârste mai înaintate [9]. Diagnosticul de SSJM se bazează pe aspectul radiologic [10] de hipertransparență pulmonară sau lobară, asociată cu o sechestrare a aerului în timpul expirului. Parenchimul pulmonar afectat prezintă grade variabile de destrucție și poate asocia bronșiectazii. Aceste aspecte se asociază de cele mai multe ori cu un aspect de disfuncție ventilatorie mixtă pe probele de spirometrie. De asemenea, la examinarea CT cu substanță de contrast intravenoasă se observă, în mod tipic, o arteră pulmonară cu calibru redus de partea afectată. În cele din urmă, o altă caracteristică descrisă a acestei afecțiuni este hipoperfuzia plămânului afectat, evidențiată scintigrafic [11].

Figura 5

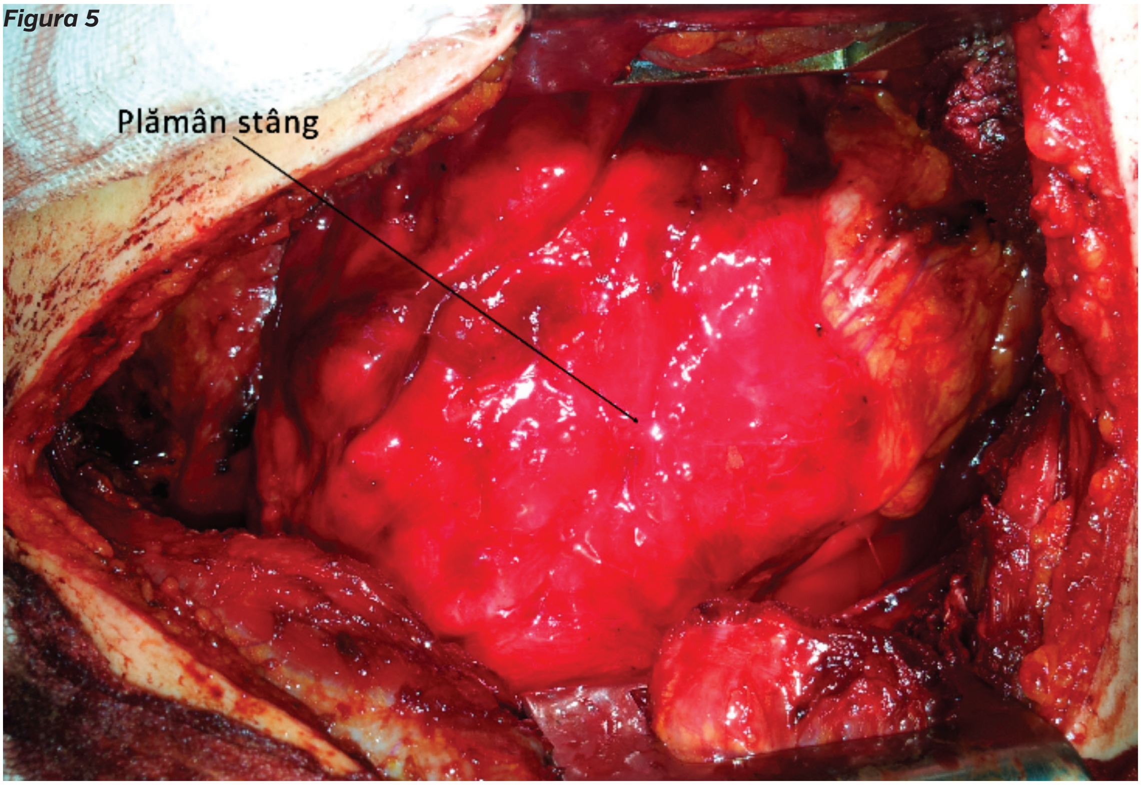
Plămân stâng

Figura 6

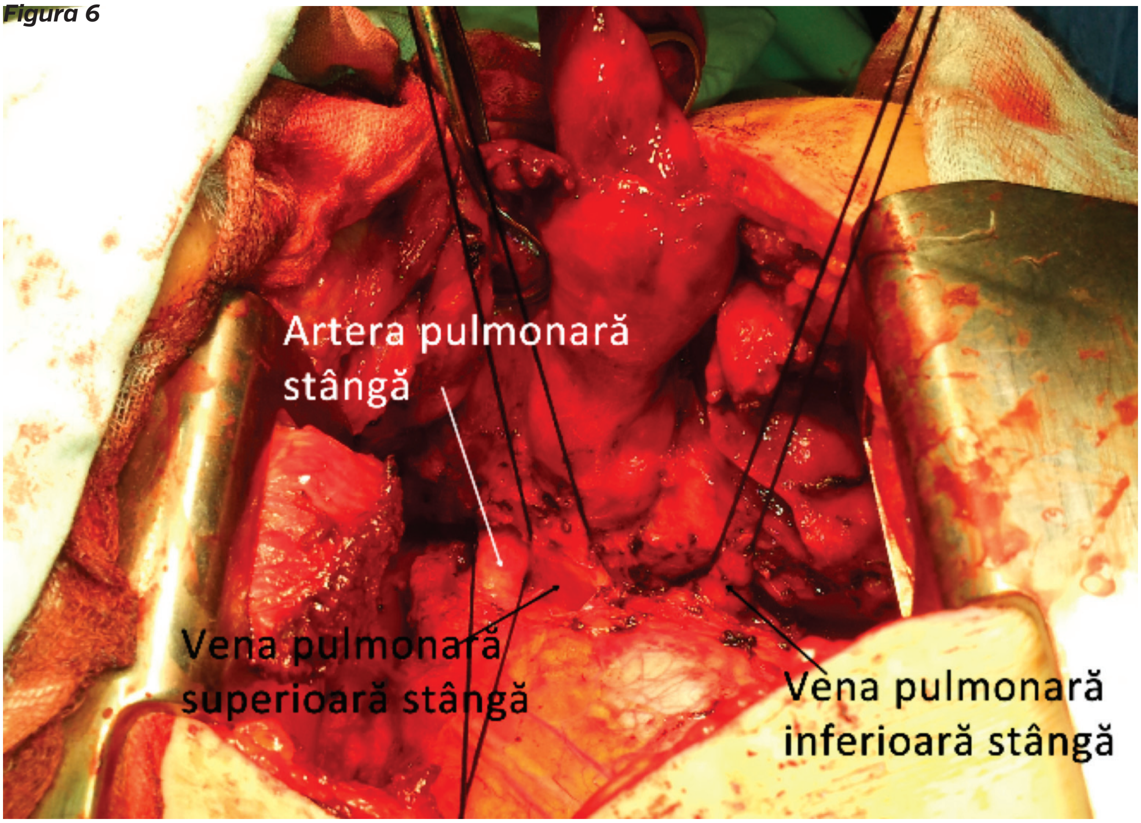
Artera pulmonară stângă
Vena pulmonară superioară stângă
Vena pulmonară inferioară stângă

Diagnosticul acestui sindrom este cel mai bine stabilit prin coroborarea unei examinări CT de înaltă rezoluție, cu substanță de contrast, cu o scintigrafie de perfuzie pulmonară [12]. Tratamentul SSJM se compune din controlul timpuriu al infecției pulmonare, vaccinări împotriva virusului gripal și a pnemococului [4, 10], precum și rezecția chirurgicală a plămânului afectat [4].

Studii efectuate de-a lungul timpului au arătat o predilecție a hipoplaziei pulmonare stângi. Lucaya et al. au arătat că numai 2 pacienți dintr-o serie de 13 aveau afectare pulmonară dreaptă [13]. Predilecția afectării plămânului stâng poate fi explicată prin factori anatomici și hemodinamici [14]. Astfel, lungimea mai mare, diametrul mai mic și o angulație mai apropiată de orizontală a bronșiei primitive stângi sau traiectul său prin tunel sub-aortic, pot explica predilecția obstrucției și a infecțiilor la acest nivel.

Diagnosticul diferențial al SSJM se face în special cu hipoplazia pulmonară unilaterală congenitală. Pacienții cu hipoplazie pulmonară unilaterală congenitală nu prezintă sechestrarea aerului la nivelul plămânului hipoplazic, astfel, este ușor de diferențiat în urma unui examen CT de înaltă rezoluție [15]. De asemenea, în cazul plămânului hipoplazic congenital, examenul bronhoscopic arată și o hipoplazie a sistemului bronșic de partea afectată [15]. Este important de diferențiat SSJM de hipoplazia pulmonară unilaterală congenitală și din punct de vedere al tratamentului: dacă hipoplazia pulmonară congenitală este diagnosticată în copilă-

Figura 7

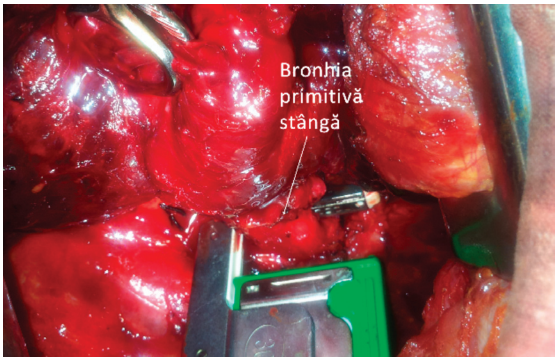
Bronhia
primitivă
stângă

Figura 8

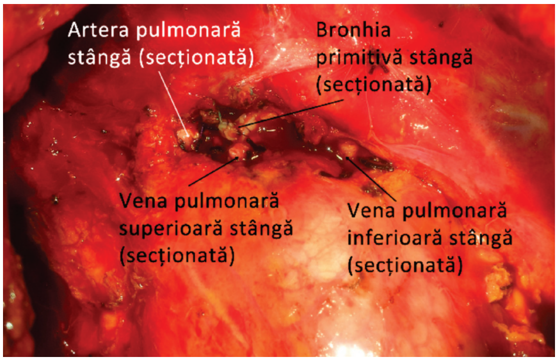
Artera pulmonară
stângă (secționată)
Bronhia
primitivă stângă
(secționată)
Vena pulmonară
superioară stângă
(secționată)
Vena pulmonară
inferioară stângă
(secționată)

Figura 9

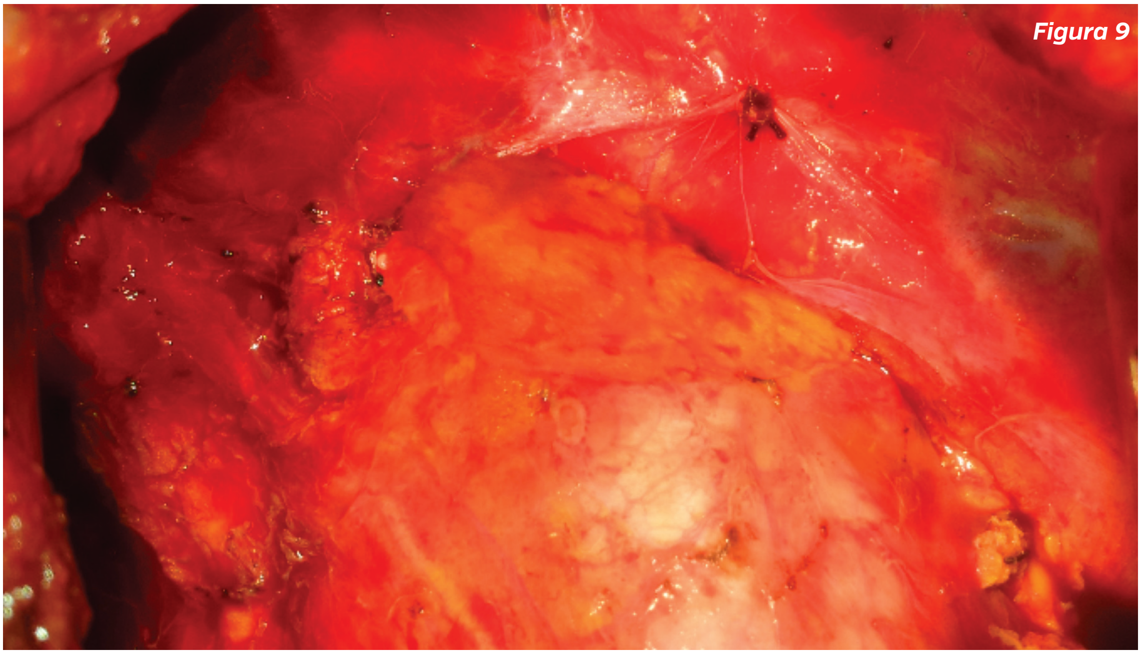

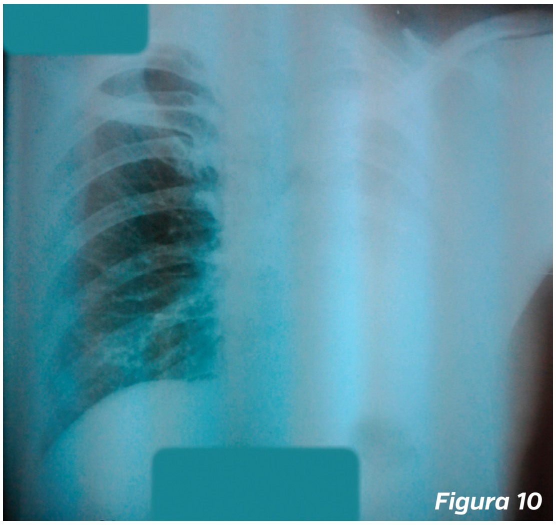
Figura 10

rie, pe lângă tratamentul antibiotic, vaccinările recomandate și tratamentul chirurgical – comune atât pentru aceasta cât și pentru SSJM, administrarea de surfactant pulmonar, expectoranți și substanțe bronhodilatatoare cresc rata supraviețuirii acestor pacienți [15]. Nu am găsit efectuate studii specifice de mortalitate și morbiditate la pacienții cu SSJM [4], dar se pare că evoluția postoperatorie a acestora este foarte bună.

Concluzii

Motivul principal pentru care am ales această prezentare de caz este numărul mic al pacienților adulți cu SSJM raportat la nivel mondial. Prezentul caz își propune, de asemenea, să sublinieze câteva aspecte care diferențiază acest sindrom de hipoplazia pulmonară unilaterală congenitală. Este important de menționat și de reținut că, cu toate că diagnosticul este stabilit în principal imagistic, o simplă radiografie toracică nu poate evidenția în mod clar această afecțiune, fiind nevoie de investigații imagistice de amploare mai mare: examinare CT de înaltă rezoluție, studii scintigrafice de perfuzie pulmonară etc.

Tratamentul SSJM este atât medical (tratamentul antibiotic al infecției pulmonare), cât și chirurgical (îndepărtarea plămânului afectat, distrus chistic). Evoluția postoperatorie a pacienților este favorabilă, totuși studii ample cu privire la această problemă (a evoluției postoperatorii) nu au fost efectuate la nivel mondial, în primul rând din cauza rarității sindromului Swyer-James-MacLeod.

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Eozinofilia esofagiană și esofagita eozinofilică – coordonate diagnostice și terapeutice în prezent

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Rezumat

Eozinofilia esofagiană și esofagita eozinofilică sunt tot mai frecvent recunoscute în practica clinică, constituind probleme de intersecție între competențele medicului gastroenterolog, alergolog și anatomopatolog. Studierea EoE a devenit un domeniu dinamic, cu aprofundarea continuă a diagnosticului, patogenezei și tratamentului. Articolul de față abordează cele mai importante aspecte ale managementului acestei patologii. Este prezentată noua terminologie punându-se accent pe diagnosticul diferențial între boala de reflux gastroesofagian, eozinofilia esofagienă responsivă la tratamentul cu inhibitori de pompă de protoni (PPI-REE) și EoE, prognosticul și tratamentul acestora fiind senibil diferit. Sunt prezentate rolul și beneficiile terapiei dietetice, piatra unghiulară a tratamentului pe termen lung al EoE, considerată a fi o suferință cu patogeneza alergologică. Endoscopia digestivă superioară joacă un rol important atât în diagnostic cât și în tratamentul stricturilor esofagiene frecvent întalnite la pacienții cu EoE.

Cuvinte cheie: *eosinofilia, esofagită, stricturi esofagiene*

Abstract

Esophageal eosinophilia and eosinophilic esophagitis(EoE) are increasingly being recognized in clinical practice at the crossingroad between the three specialities: gastroenterology, allergology and pathology. The study of EoE became a dynamic field of continuous research in diagnosis, patogenesis and treatment. The article deals with important aspects of the management of this ailment. We present the new terminology including the differential diagnosis between reflux disease, proton pump inhibitors responsive esophageal esosinophilia (PPI-REE) and EoE, ailments with very different prognosis and treatments. We made also coments on the importance of the diet, considering the allergic pathogenesis of the disease. Digestive endoscopy plays an important role in diagnosis as well in the treatment of esophageal strictures which a very often encountered in EoE pacients.

Key word: *eosinophilia, esophagitis, esophageal strictures*

Cunoștințele noastre despre mucoasa esofagiană sunt limitate la informațiile furnizate de biopsiile endoscopice. Acestea documentează prezența unui epiteliu scuamos stratificat relativ neted. În mod normal, epiteliul esofagian conține un număr redus de limfocite dar fără alte tipuri celulare leucocitare. În cazul inflamației alergice sau peptice, suprafața epiteliului devine hiperplastică și acumulează eozinofile. Din acest motiv, eozinofilia esofagiană se definește curent prin prezența eozinofilelor la nivelul epiteliului esofagian indiferent de numărul acestora.

Este important de reținut că eozinofilia esofagiană este definită histologic, necesită o interpretare și corelare în contextul clinic în care s-a obținut materialul bioptic și că nu se poate pune diagnosticul de esofagită eozinofilică numai pe seama prezenței sale izolate. Considerată izolat, eozinofilia esofagiană este cel mai adesea întâlnită în trei situații clinice: boala de reflux gastroesofagian (GERD), eosofagita eozinofilică (EoE) și eozinofilia esofagiană responsivă la inhibitorii pompei de protoni (PPI-REE). Aceste entități clinice nu pot fi diferențiate între ele numai prin cuantificarea eozinofilelor sau a altor aspecte morfologice asociate. În plus, un număr de alte afecțiuni cu aspecte clinice și histologice diferite se pot asocia și ele cu eozinofilia esofagiană ca de exemplu: gastroenteritele eozinofilice, maladia celiacă, boala Crohn, sindromul hipereozinofilic, achalazia, hipersensibilitatea la medicamente, vasculitele, pemfigusul, bolile de colagen, boala grefă contra gazdă. În practica clinică curentă, eozinofilia esofagiană izolată trebuie diferențiată în primul rând față de gastroenteritele eozinofilice și de sindromul hipereozinofilic.

Ca maladie alergică, EoE a fost raportată pentru prima dată în 1995. Inițial s-a crezut că este vorba despre o boală rară dar, în ultimii 15 ani, prevalența și interesul pentru ea au crescut mult. Fundamentul alergenic al EoE se bazează pe studiile care au demonstrat că substratul său etiologic este foarte probabil un răspuns aberant „antigenic“ sau „imun“ asociat unor anomalii clinice și histologice consistente.

Ghidurile clinice pentru aceasta afecțiune au apărut abia în 2007, după care au fost actualizate în 2011 și 2013. Conform acestor ghiduri, EoE este definită ca o afecțiune esofagiană cronică, mediată imunologic/antigenic, caracterizată clinic prin simptome legate de disfuncția esofagiană, iar histologic prin inflamația predominant eozinofilică. Este o entitate clinico-histopatologică, ceea ce semnifică faptul că datele clinice și histologice vor fi considerate numai împreună și niciodată izolat.

Caracteristicele clinice ale EoE

Deși mai multe studii au descris aspectele clinice relevante pentru EoE, ele nu sunt totuși patognomonice. Pacientul tipic, cu EoE este de sex masculin (raportul M/F=3:1), aflat la vârsta copilăriei sau în decada a 3-4-a de viață. EoE apare la multe grupuri etnice sau rasiale , deși s-a raportat o predominanță la albii non-hispanici. La copil, manifestările clinice sunt nespecifice și variază cu vârsta. Copii mici prezintă de obicei dificultăți de alimentare iar copii de vârstă școlară prezintă mai ales vomă și dureri retrosternale. EoE se asociază frecvent cu diateze atopice ca alergia alimentară, astmul, dermatita atopică , rinita alergică etc.. La adulți, simptomul cel mai frecvent este reprezentat de disfagia la alimente solide la care se adaugă impactul esofagian al bolului alimentar, necesitând extragerea endoscopică în 35-54% din cazuri. Alte simptome cu care se confruntă pacientul adult sunt: durerea toracică, pirozisul și durerea abdominală superioară. Deși examenul clinic obiectiv este util atât la copii, pentru identificarea anomaliilor de creștere cât și la copii si adulți pentru depistarea comorbidităților alergice, nici una dintre modificările observate nu sunt specifice diagnosticului de EoE.

Examinarea endoscopică

Modificările patologice depistabile endoscopic la pacienții cu EoE sunt: inelele esofagiene (denumite și – trahealizarea esofagului – figura 1), exudatele sau plăcile albe de pe suprafața mucoasei esofagului, cutele/pliurile longitudinale, edemul mucoasei (relevat de paloarea și vascularitatea redusă), reducerea lumenului esofagian și friabilitatea/fragilitatea crescută a mucoasei, cu dilacerări induse de trecerea endoscopului. Cu toate aceste aspecte multiple regăsite la examenul endoscopic esofagian, deoarece ele pot fi regăsite și în alte afecțiuni esofagiene, nici unul dintre ele nu poate fi considerat patognomonic pentru EoE. Meta-analizele datelor endoscopice care au reunit un număr semnificativ de pacienți (4.678) cu EoE, au demonstrat că sensibilitatea, specificitatea și valoarea predictivă a modificărilor endoscopice, considerate izolat, sunt insuficiente pentru diagnosticul EoE. Mai mult, examenul endoscopic poate fi normal în 10-25% din cazurile cu EoE. De aceea, în cazurile cu suspiciune clinică crescută de EoE, se vor preleva biopsii din mucoasa esofagiană chiar dacă examenul endoscopic este normal. Utilizarea noului sistem de clasificare și gradare a datelor endoscopice (tabelul 1) facilitează compararea severității modificărilor semnalate de către clinicieni și oferă informații despre complicațiile fibrostenotice ale EoE.

Examinarea radiologică

Stricturile, inele persistente, cutele/pliurile longitudinale difuze și mai rar diverticuloza intramurală esofagiană au fost descrise și radiologic, dar sunt la fel de nespecifice. De aceea, esofagogramele nu sunt recomandate ca test diagnostic de rutină în EoE. În

MODIFICĂRI MAJORE:	
Edemul (definit şi ca desen vascular redus, paloarea mucoasei):	Gradul 0: Absent. Vascularizaţie distinctă Gradul 1: Pierderea clarităţii sau absenţa desenului vascular
Inele fixate (sau inele concentrice, inele suprapuse, esofag inelat sau trahealizat):	Gradul 0: Absente Gradul 1: Inele circumferenţiale schiţate Gradul 2: Inele moderate care nu afectează trecerea endoscopului standard pentru adulţi (8-9,5 mm) Gradul 3: Inele care nu permit trecerea endoscopului diagnostic
Exsudate (sau spoturi albe sau plăci):	Gradul 0: Absente Gradul 1: Leziuni uşoare care se extind pe maximum 10% din suprafaţa esofagului Gradul 2: Leziuni severe, extinse pe mai mult de 11% din suprafaţa esofagului
Linii verticale (linii/pliuri longitudinale):	Gradul 0: Absente Gradul 1: Prezente
Stricturi:	Gradul 0: Absente Gradul 1: Prezente (se estimează diametrul lumenului esofagian)
MODIFICĂRI MINORE:	
Esofag creponat (mucoasă fragilă sau lacerată la trecerea endoscopului diagnostic dar nu după dilatarea esofagiană):	Gradul 0: Absent Gradul 1: Prezent
Esofag cu calibru îngustat (diametrul luminal redus în majoritatea esofagului tubular):	Gradul 0: Absente Gradul 1: Prezente

Tabel 1 ***Clasificarea și sistemul de gradare propuse pentru evaluarea endoscopica a modificărilor esofagiene din esofagită eozinofilică*** *(Hirano I, Moy N, Heckman MG et al. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system . Gut 2013 ; 62 : 489 – 95)*

situaţii selectate, pot fi utile pentru caracterizarea anomaliilor anatomice cum sunt stricturile subtile sau calibrul esofagian redus, care sunt dificil de apreciat endoscopic sau pot oferi informaţii despre lungimea şi diametrul stricturilor esofagiene atunci când urmează să se efectueze dilatarea endoscopică.

Strategia prelevărilor endoscopice de mucoasă esofagiană

Având în vedere fapul că modificările inflamatorii din EoE sunt frecvent parcelare şi prin urmare pot să nu fie prezente în toate prelevările bioptice,

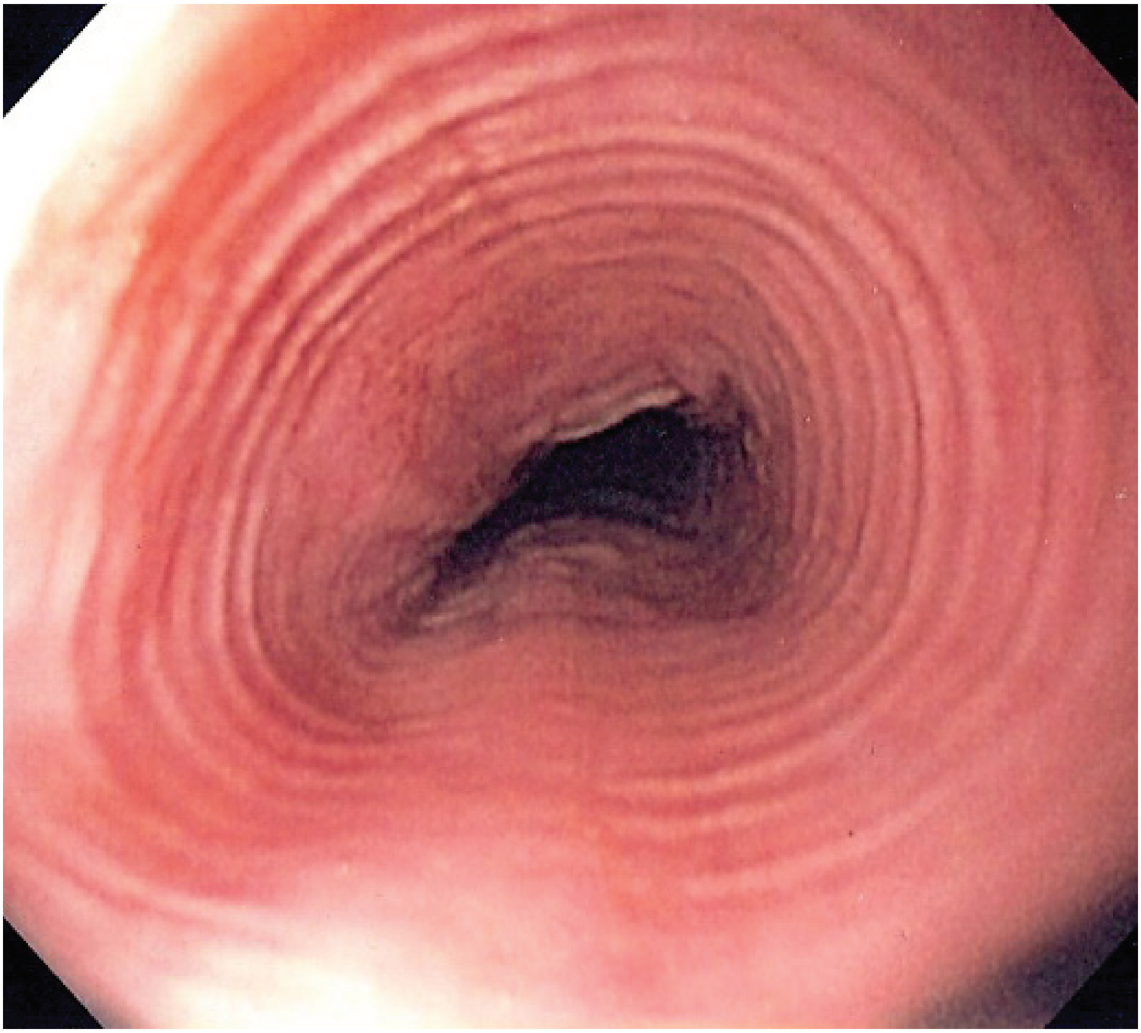

Figura 1: Trahealizarea esofagului.

se recomandă obținerea a cel puțin 2-4 biopsii din minimum două zone diferite, de regulă din jumătatea proximală respectiv cea distală a esofagului. De asemenea este recomandabil să se preleveze biopsii țintite și din zonele care prezintă modificări endoscopice, respectiv inele, plăci, pliuri etc. Cu privire la numărul biopsiilor necesare pentru maximizarea șanselor diagnosticului, concluzia general acceptată este că numărul mai mare de biopsii crește randamentul diagnostic. Astfel, dacă numărul de biopsii ajunge la 6-9, sensibilitatea diagnostică poate ajunge la 100%! Prelevarea de material biopsic din două zone diferite permite aprecierea gradului de extensie a inflamației dar și a eozinofiliei care poate fi diferită în esofagul distal și proximal. Pe lângă biopsiile esofagiene se vor preleva în decursul aceleiași examinări endoscopice și biopsii din antrul gastric și din duoden la toți copii, pentru excluderea altor cauze potențiale de eozinofilie esofagiană. În cazul adulților, se vor preleva de asemenea biospii gastrice și duodenale dar numai dacă endoscopic, au fost depistate modificări patologice sau dacă sunt prezente și alte simptome gastrointestinale ori se suspicionează clinic coexistența unor afecțiuni ca boala celiacă sau gastroenterita eozinofilică. În absența simptomelor sau anomaliilor endoscopice, nu există date suficiente în sprijinul efectuării de rutină a biopsiilor gastrice și duodenale, la pacientul adult.

Caracteristicile histopatologice și limitele diagnostice

Deoarece încă nu există studii care să precizeze cu exactitate numărul minim de eozinofile necesar pentru diagnosticarea EoE, există în schimb, un consens care statuează că dacă într-un specimen bioptic de mucoasă esofagiană, recoltat după un tratament prealabil cu inhibitor de pompă de protoni (PPI) sunt numărate minimum 15 eozinofile pe cel puțin un câmp microscopic de putere înaltă, atunci se poate stabili diagnosticul de EoE, în context clinic adecvat. Deoarece există unele limite în cuantificarea numărului eozinofilelor, inclusiv lipsa standardizării mărimii câmpului microscopic de putere înaltă precum și variabile în definirea eozinofilului intraepitelial în secțiunile tisulare colorate cu hematoxilină-eozină, se recomandă o bună colaborare cu specialistul anatomopatolog mai ales atunci când există rezerve cu privire la rezultate. La fel ca și în cazul simptomelor și datelor de endoscopie, numărul crescut al eozinofilelor, considerat izolat, nu este patognomonic pentru EoE.

Este important ca raportul anatomopatologic să notifice, pe lângă numărul absolut de eozinofile, și alte aspecte de interes precum existența microabceselor eozinofilice, stratificarea superficială a eozinofilelor, granulele eozinofilice extracelulare, hiperplazia celulelor bazale, fibroza laminei proprii subepiteliale sau creșterea numărului altor tipuri de celule, ca de exemplu, limfocitele. Deși aceste aspecte nu sunt specifice pentru EoE, ele sunt importante pentru evaluarea clinicopatologică generală a pacientului. În plus, chiar dacă date preliminare sugerează că prezența granulelor eozinofilice extracelulare (peroxidaza eozinofilică, proteina bazică majoră și neurotoxina de origine eozinofilică, constituie un element util pentru diferențierea histologică dintre EoE și Boala de reflux gastroesofagian (GERD), colorațiile speciale care le evidențiază nu au intrat încă în practica clinică curentă.

Provocarea diagnostică: Eozinofilia esofagiană responsivă la PPI și GERD

Există două situații clinice în care pacienții cu eozinofiie esofagiană răspund la terapia cu inhibitori de pompă de protoni (PPI). Prima situație este aceea în care pacienții cu simptome clasice de reflux și cu esofagită erozivă sau esofag Barrett la endoscopie, au și eozinofilie esofagiană la biopsie. Acești pacienți au GERD care a indus eozinofilia esofagiană iar tratamentul pentru GERD va avea drept consecință și reducerea numărului de eozinofile din mucoasa esofagiană. A doua situație se referă la pacienții cu simptome sugestive de EoE, care pot avea și modificări endoscopice compatibile cu EoE și la care atât simptomele cât și eozinofilia esofagiană se remit în urma tratamentului cu PPI. Această situație a fost etichetată ca esofagită eozinofilică responsivă la inhibitorii pompei protonice (PPI-REE). Studii clinice multicentrice au arătat că peste 1/3 dintre toți pacienții cu eozinofilie esofagiană diagnosticată histologic răspund la PPI și că, prin urmare, aceștia nu vor fi diagnosticați cu EoE. Ei formează categoria denumită esofagită eozinofilică responsivă la inhibitori de protoni (PPI-REE).

Motivul acestui răspuns la PPI este doar parțial elucidat și, foarte probabil, angajează un complex multifactorial. Este posibil ca în GERD, expunerea epiteliului esofagian la acidul gastric să lezeze joncțiunile strânse intercelulare iar acest fapt să determine creșterea permeabilității epiteliale și dilatarea spațiilor intercelulare, facilitând astfel penetrarea alergenilor. Aceștia, la rândul lor, vor recruta eozinofilele la nivelul epiteliului esofagian. O altă ipoteză sugerează un efect direct antiinflamator al PPI la nivelul epiteliului esofagian. Astfel, un studiu recent arată că în liniile celulare esofagiene, expunerea la omeprazol a celulelor stimulate cu citokine de tipul IL-13 și IL-4 a blocat secreția de eotaxină-3, considerată ca având un rol major în apariția EoE.

În prezent nu se știe dacă pacienții cu PPI-REE reprezintă o variantă a GERD, o variantă a EoE sau un proces patologic separat complet. PPI-REE nu s-a dovedit asociată cu vreo cauză antigenică sau imunologică documentate în esofagita eozinofilică și, prin urmare, nu poate fi etichetată drept variantă fenotipică a EoE. După ce unui pacient a fost diagnosticat cu PPI-REE, clinicianul ar trebui să continue evaluarea pentru a se stabili dacă avem de a face cu GERD, dat fiind că răspunsul pozitiv la PPI nu este patognomonic pentru refluxul gastroesofagian. Evaluarea poate fi direcționată prin monitorizare ambulatorie a pH-ului esofagian. Efectuarea acestei investigații de la început, poate avea valoare predictivă cu privire la răspunsul esofagitei eozinofilice la tratamentul de probă cu PPI. Recomandările specifice cu privire la durata și dozele tratamentului cu

EoE	BRGE
Clinică:	**Clinică:**
Vomă/epigastralgii	Vomă/epigastralgii
Impact alimentar la copii mari și adulți	Impact alimentar rar
Raport masculin/feminin - 3/1	Raport masculin/feminin – 1/1
Asociere obișnuită cu atopia și sensibilizarea la alimente	Poate exista asociere cu atopia și sensibilizarea la alimente
Impedanța/pHmetria esofagiană:	**Impedanța/pHmetria esofagiană:**
Normală (sau RGE ușor)	Identifică RGE
Endoscopie:	**Endoscopie:**
Esofagită, trahealizare, papule albe	Esofagită (distală)
Histopatologie:	**Histopatologie:**
Inflamație proximală și distală	Inflamație distală
Hiperplazie epitelială	Oarecare hiperplazie epitelială
Minimum 15 eozinofile pe câmpul microscopic de putere mare	Tipic- rare eozinofile (ocazional mai multe în atopie)

Elemente similare și discriminatorii între esofagita eozinofilică și esofagita de reflux;
EoE=esofagita eoziinogilică; **RGE**=reflux gastroesofagian, **BRGE**=boală de reflux gastroesofagian.

PPI ca terapie inițială pentru esofagita eozinofilică sunt puține. Deși date retrospective sprijină ideea administrării PPI în 1-2 doze/zi, majoritatea studiilor recomandă administrarea a 20-40 mg x 2/zi, din oricare dintre PPI existente pe piață. În general este recomandabil ca posologia să fie cel puțin similară celei utilizate în tratarea esofagitei erozive din GERD, minimum 8 săptămâni, până în momentul repetării endoscopiei și a biopsiilor de control.

Obiectivele terapeutice în esofagita eozinofilică

Puține date avem până în prezent care să specifice nivelul până la care trebuie redusă densitatea eozinofilelor pentru a se obține reversibilitatea sau stoparea injuriei esofagului. Țintele terapeutice din literatură sunt variabile și oarecum arbitrare, cu privire la stabilirea limitei superioare a numărului de eozinofile pe câmpul microscopic de mare putere, care să indice răspunsul complet la tratament. Țintele histologice variază de la 0-1 eoz./hpf la 0-6 eoz. hpf sau o reducere cu peste 90% a numărului inițial de eozinofile, rezultate combinate cu alți parametri histologici cum ar fi hiperplazia stratului celular bazal. Chiar și conceptul de răspuns histologic parțial este definit variabil.

Chiar și definirea parametrilor simptomatologici modificați terapeutic este dificilă deoarece simptomele din EoE sunt nespecifice și pot fi mimate de modificările dietei care sunt dificil de cuantificat. Mai multe studii clinice au evaluat budesonidul, fluticazona sau prednisonul demonstrând existența unei corelații generale a răspunsului histologic și simptomatologic la tratament. Alte studii terapeutice ale EoE nu au găsit însă o corelare a răspunsului histologic cu cel al simptomatologiei. Definirea bazată pe dovezi a obiectivelor terapeutice in EoE constituie în prezent, o arie de cercetări intense cu rezultate palpabile în etapa următoare.

Tratamentul farmacologic al EoE

Corticosteroizii topici și-au dovedit eficiența terapeutică în EoE și, ca urmare, fac parte din prima linie

Medicație:	Grup de vârstă:	Posologie:
Fluticasonă	-Copii: -Adulți:	-88-440mcg/zi, divizat; -880-1760mcg/zi, divizat.
Budesonid	-Copii: -Adulți:	-1 mg/zi -2 mg/zi, divizat.

Tabel 2 Tratament steroid topic iniţial în esofagita eozinofilică

terapeutică a acesteia. Medicația (Tabel 2), disponibilă sub formă de inhalatoare multi-doză sau ca soluții apoase nebulizatoare pentru astmatici, sunt mai ales înghițite pentru a acoperi cât mai mult din suprafața mucoasei esofagului având, astfel, acțiune locală.

La copii, studii randomizate, comparând fluticasona cu prednisonul și placebo, pe o durată de 1-3 luni, au demonstrat un răspuns complet în 50% cazuri și unul parțial în 95% din cazurile tratate. Răspunsul simptomatic și endoscopic au fost de asemenea semnificative. Doar într-un singur studiu, controlat placebo, răspunsul histologic la fluticasonă a fost remarcat la 62% din cazuri, fără ameliorare simptomatologică semnificativă. Doza de fluticasonă este eliberată din inhalator în gură, în momentul opririi respirației, apoi este înghițită iar pacientul nu mai bea și nici nu mănâncă în următoarele 30-60 minute.

Budesonidul și-a dovedit la fel eficiența terapeutică în EoE. La copii, soluția apoasă de budesonid este mixată cu un îndulcitor (1mg/2ml budesonid cu 5 mg sucraloză) pentru obținerea budesonidului vâscos oral. Utilizat astfel, budesonidul ameliorează semnificativ simptomele, modificările endoscopice și eozinofilia esofagiană comparativ cu placebo. Rezultate similare cu budesonid nebulizat și apoi înghițit, s-au raportat și la pacienții adulți. Nu există studii comparative ale eficienței fluticasonei și budesonidului, dar un studiu care a folosit budesonid oral vâscos comparativ cu budesonidul nebulizat și apoi înghițit, a demonstrat că formula orală vâscoasă a fost mai eficientă în reducerea numărului de eozinofile esofagiene, probabil datorită unui contact mai prelungit cu mucoasa esofagiană.

Deși, în general, terapia topică steroidiană este sigură, candidoza esofagiană poate apare la 5-30% din pacienții tratați astfel. Candidoza orală a fost raportată la doar 1% dintre pacienții tratați cu steroizi topici, indiferent de formula medicamentoasă, de doză sau dacă după administrarea medicamentului s-au folosit spălături oro-faringiene. Nu s-a observat inhibiția glandelor suprarenale după două luni de tratament iar datele despre siguranța administrării pe termen lung asupra creșterii și densității osoase nu sunt încă disponibile.

Corticoterapia sistemică

Un singur studiu randomizat, compară prednisonul cu fluticasonul administrat topic, în tratamentul EoE la copii. Deși rezultatele sunt similare între cei doi agenți terapeutici, prednisonul ameliorează mai pregnant histologia cu costul mai multor reacții adverse (40% dintre pacienții tratați cu prednison au dezvoltat efecte secundare ca hiperfagia, creștere ponderala și/sau manifestări cushingoide). La un singur grup de 39 copii cu EoE tratați cu prednison, s-a obținut o reducere a numărului de eozinofile de la 34 la 1 eoz./hpf, după 4 săptămâni de tratament. Datorită efectelor adverse potenţiale, prednisonul este rezervat cazurilor în care steroizii topici nu mai sunt eficienți sau este nevoie de o ameliorare rapidă a simptomatologiei.

Alți agenți terapeutici în EoE

Dacă pacientul nu răspunde la corticoterapia topică sau sistemică după creșterea dozelor sau prelungirea duratei tratamentului, se recomandă instituirea unui tratament non-farmacologic și anume dieta restrictivă sau dilatarea esofagiană endoscopică.

Alți agenți terapeutici au fost studiați limitat în EoE, cu eficiență insuficient precizată și, de aceea, nu sunt recomandați. Astfel, un studiu non placebo controlat cu inhibitori de leucotriene la 12 pacienți adulți cu EoE, a utilizat doze mari de montelukast, obținând ameliorarea simptomatologiei dar fără răspuns histologic. Într-un alt studiu pe 11 adulți, montelukast s-a dovedit ineficient în menținerea remisiunii induse de corticoizi.

Deşi inhibitorii mastocitelor ar avea o justificare teoretică în tratamentul EoE, nu există studii controlate în acest sens. Astfel, cromolinul sodic folosit 4 săptămâni la 14 copii, nu a demonstrat nicio ameliorare simptomatologică sau histologică.

Imunomodulatoarele **6-mercaptopurina** şi **azathioprina** au fost încercate la trei pacienţi cu eozinofilie esofagiană cortico-dependentă, cu remisiune simptomatică şi histologică dar datorită efectelor adverse potenţiale şi a datelor clinice insuficiente, nu pot fi recomandate în terapia de rutină.

Interleukina-5 (IL-5) este o citokină-cheie în fiziologia eozinofilului şi, de aceea, o ţintă atractivă pentru terapia anti-eozinofilică. Anticorpii anti-IL-5 sunt în curs de studiere dar indisponibili în prezent pentru practica clinică. Mepolizumabul a fost utilizat în două studii, la copii şi adulţi. În ambele studii s-a obţinut reducerea numărului de eozinofile la majoritatea pacienţilor, dar rezoluţia histologică a fost remarcată la un procent redus de pacienţi. În lotul adulţilor, simptomele au rămas neschimbate. Prin urmare, este necesară continuarea cercetăriilor clinice pentru precizarea rolului acestor medicamente în tratamentul EoE.

Omalizumab, un anticorp anti-IgE, s-a dovedit ineficient într-un studiu de caz efectuat pe doi pacienţi.

Tratamentul dietetic. Strategiile dietei restrictive

În prezent sunt configurate trei strategii de terapie dietetică.

Prima strategie constă în eliminarea totală a tuturor alergenilor alimentari şi folosirea unei formule alimentare elementale sau bazată pe aminoacizi (dieta elementală).

A doua strategie are la bază eliminarea ţintită din dietă ghidată pe testarea alergologică, apelând la testul cutanat (prick test – sau la testul patch).

A treia strategie constă în eliminarea empirică din dietă a 6 alimente cunoscute ca fiind implicate în etiologia EoE: soia, ouăle, laptele, grâul, nucile şi fructele de mare. În studii necontrolate, toate cele trei strategii enumerate au demonstrat rezoluţie simptomatologică şi histologică la pacienţi pediatrici. De obicei, durata tratamentului este de 4-8 săptămâni, urmată de o perioadă de reintroducere, după obţinerea remisiunii. Deoarece dieta restrictivă şi identificarea alergenilor alimentari este laborioasă, costisitoare şi consumatoare de timp, decizia abordării acestei terapii trebuie individualizată în funcţie de resursele disponibile precum şi de preferinţele pacientului şi familiei.

Formula dietetică elementală s-a dovedit cea mai eficientă metodă, realizând o rezoluţie simptomatologică şi histologică în 95-98% din cazuri, după 4 săptămâni de tratament. Cu toate acestea, există unele limitări în practică şi anume: este costisitoare, necesită adesea plasarea unui tub de alimentare şi poate avea impact negativ asupra calităţii vieţii pacientului. Soluţia alternativă mai practică recurge la dieta din care se elimină 6 tipuri de alimente care conţin cei mai frecvenţi alergeni alimentari cunoscuţi, fără să se mai efectueze testele alergologice cutanate. La populaţia pediatrică, metoda a obţinut rezoluţie simptomatologică şi histologică în 74% din cazuri. Studii ulterioare pe aceleaşi loturi de pacienţi, au demonstrat că cei mai frecvenţi factori alimentari care au declanşat EoE la reintroducerea lor în dietă au fost: laptele, grâul, ouăle şi soia.

Dieta restrictivă nu a fost studiată extensiv până în prezent. Unele studii, folosind eliminarea ţintită din dietă în funcţie de sensibilizare la grâu şi secară, documentată prin creşterea IgE, nu au obţinut răspuns simptomatologic sau histologic. Într-un studiu din 2011 pe 64 pacienţi, cu dietă restrictivă pentru grâu, porumb, lapte, ouă, nuci/alune, legume, peşti, crustacee, orez şi soia, 73% din pacienţi au avut sub 5 eoz./hpf. Reintroducerea alimentelor a dovedit că factorii declanşatori ai EoE cei mai implicaţi au fost: laptele, grâul şi legumele.

Scopul terapiei prin dietă nu este numai acela de a induce remisiunea clinică şi histologică dar, cel puţin la fel de important, de a menţine remisiunea, o dată obţinută, prin eliminarea definitivă a factorilor alimentari declanşatori ai EoE. Pacienţii pot trece pe terapia dietetică sub formă de alternativă la tratamentul cronic farmacologic dacă sunt motivaţi în depistarea alimentelor cu rol etiopatogenic în EoE, pentru a le elimina din dietă. Iniţierea în terapia dietetică a EoE apare adesea cu o consultaţie la un specialist dietetician familiarizat cu alergiile alimentare şi cu afecţiunile eozinofilice gastrointestinale. Implicarea dieteticianului este importantă mai ales dacă se are în vedere introducerea formulei elementale pentru asigurarea necesarului caloric şi pentru evitarea diselectrolitemiilor.

După eliminarea alergenilor din dietă se vor reintroduce alimentele suspendate pentru a se depista declanşatorii specifici ai EoE. Astfel, se poate reintroduce câte un aliment la fiecare 2 săptămâni iar endoscopia de control să se repete după 2 alimente (deci lunar). Factorul alimentar declanşator este identificat pe baza recurenţei simptomatologiei şi a eozinofiliei esofagiene (peste 15 eoz./hpf) după reintroducerea grupului respectiv de alimente. Deoarece pacienţii au de obicei mai mulţi factori alimentari incriminaţi, demersul este continuat până ce toate grupurile de alimente au fost reintroduse sau până când se atinge un nivel al dietei acceptabil. După identificarea factorului alimentar incriminat, pacienţilor li se recomandă să-l elimine complet din dietă. Factorii practici consideraţi pentru o asemenea abordare au în vedere costul şi riscul asociat endoscopiilor repetate. Pentru documentarea eficienţei terapiei dietetice sunt necesare studii pe termen lung după cum sunt necesare şi studii de perspectivă pentru determinarea strategiei optime de reintroducere a alimentelor restricţionate iniţial.

Rolul alergologului

Utilizarea testelor alergologice pentru identificarea alimentelor implicate în declanşarea EoE este controversată. Există trei tipuri majore de teste alergologice: testul imunologic pentru depistarea IgE seric specific alimentului, testul cutanat prin înţepare (skin prick test) şi testul plasturelui (atopy patch test). Nici una dintre metode nu este universal adoptată datorită variabilităţii în selecţia subiecţilor studiaţi, a antigenelor cercetate şi a diferenţelor de tehnică şi interpretare. De aceea sunt necesar studii multicentrice pentru standardizarea şi validarea acestor teste.

Rolurile importante pe care medicul specialist alergolog le are în tratarea EoE includ identificarea, tratamentul şi evaluarea testelor alegice la pacienţii cu EoE, paralel cu evaluarea afecţiunilor alergice asociate precum dermatita atopică, astmul şi rinita respectiv conjunctivita alergică. Colaborarea dintre medicul alergolog şi gastroenterolog poate fi şi este, cheia succesului terapeutic. În final şi dieteticienii au un rol bine definit. Cunoştinţele lor cu privire la compoziţia şi amestecul alimentelor precum şi expertiza lor cu privire la dieta restrictivă, mai ales pe termen lung, chiar nelimitat, din punctul de vedere al complianţei, al suportului social şi economic pot fi de un real folos.

Tratamentul endoscopic

Complicaţiile fibrostenotice ale EoE includ stricturile esofagiene focale şi esofagul cu calibrul îngustat. Dilataţia endoscopică esofagiană este eficientă în asemenea cazuri şi a constituit una din primele modalităţi terapeutice utilizate pentru pacienţii adulţi cu EoE. Disfagia este ameliorată în majoritatea cazurilor dilatate endoscopic, durata medie a răspunsului la dilataţie fiind de peste un an. Se remarcă un grad mare de acceptare de către pacienţi a dilataţiei esofagiene ca primă modalitate terapeutică, în faţa acestor complicaţii toţi pacienţii dilataţi preferând repetarea procedurii ori de câte ori este nevoie. În general, este preferabil să se recurgă la dilataţia esofagiană după evaluarea eficienţei terapiei medicale şi dietetice. Însă, dacă la endoscopia iniţială se depistează o stenoză critică sau dacă suntem în faţa unui episod de impactare alimentară la nivelul esofagului, dilataţia se va efectua înaintea altor modalităţi terapeutice. Rolul dilataţiei ca monoterapie primară în EoE este încă controversat şi trebuie individualizat până la obţinerea unor date suplimentare.

Dilataţia esofagiană apelează la două tehnici şi anume dilatatorul cu balon hidrostatic introdus pe canalul endoscopului, respectiv dilataţia cu bujii. Dilatarea cu bujii folosind un sistem de ghidare cu fir, oferă posibilitatea dilatărilor stricturilor multiple dar şi a stricturilor mai lungi din esofagul îngustat difuz din EoE. Este, în prezent, forma preferată în EoE.

Se preferă o abordare conservatoare a dilataţiei, creşteri mici în diametru în sesiuni multiple. De asemenea, alegerea diametrului dilatatorului iniţial este foarte importantă. Astfel, dacă gastroscopul obişnuit pentru adulţi (cu diametrul tipic de 9-11 mm) trece fără să i se opună rezistenţă, atunci mărimea dilatatorului iniţial selectat va fi imediat superioară acestuia. Rezistenţa la trecerea bujiei poate estima gradul stenozei lumenului chiar mai bine decât endoscopul. Scopul final al dilatărilor este obţinerea unui diametru esofagian de 15-18 mm, ceea ce ar ameliora semnificativ şi de durată disfagia, rezultat la care se poate ajunge după mai multe sesiuni.

RISCURILE DILATAŢIEI ESOFAGIENE DIN EOE. Complicaţiile severe relatate în primele studii au redus entuziasmul gastroenterologilor. Fisurile esofagiene şi perforaţiile survenite în timpul tratamentului endoscopic al alimentelor impactate sau al endoscopiilor diagnostice sugerau o fragilitate marcată esofagiană în EoE. Un factor important care a influențat probabil frecvența ridicată inițială a acestor complicații a fost lipsa datelor suficiente despre această afecțiune și tehnicile mai agresive de dilatație folosite. Astfel, multe din raportările inițiale despre perforația esofagiană se refereau la pacienți la care EoE nu a fost recunoscută de la început și înainte ca publicațiile de specialitate să descrie riscurile potențiale ale dilatației esofagiene.

În două meta-analize recente despre dilatația esofagiană din EoE, perforația datorată dilatației esofagului a fost notată la 0,3% din cazuri, similară celei observate în cazul dilatației efectuată pentru alte afecțiuni esofagiene benigne. De remarcat că, riscul cel mai frecvent al dilatării în EoE este durerea pectorală post-procedurală, raportată la ¾ dintre pacienții intervievați prospectiv. Hemoragia majoră definită prin necesarul hemostazei endoscopice sau al administrării de sânge este rară (apărută la un singur pacient). Probabil că, siguranța sporită semnalată în studiile recente, reflectă adoptarea unei abordări mai conservatoare de către gastroenterologii tot mai avertizați cu privire la riscurile potențiale ale dilatărilor din EoE.

Istoria naturală a EoE

Cu toate că datele disponibile cu privire la istoria naturală a EoE sunt încă limitate, este clar că EoE este o afecțiune cronică și că în multe cazuri, mai ales la adulți, diagnosticul este stabilit după cel puțin o decadă de la apariția inițială a simptomatologiei. În studii randomizate și controlate- placebo, modificările endoscopice și histologice (eozinofilia esofagiană) au persistat în lotul tratat placebo. De reținut de asemenea că nici un caz de EoE nu a evoluat spre sindrom hipereozinofilic sau spre transformare malignă.

Studiile endoscopice demonstrează faptul că unele cazuri de EoE pot progresa de la un stadiu inflamator la un proces fibrotic, ceea ce ar putea explica diferențele clinico-patologice la prezentarea inițială dintre copii și adulți.

EoE reapare după oprirea tratamentului (dietetic si medicamentos) în aproape toate cazurile, dar intervalul de timp diferă de la un pacient la altul. Rezultatele studiilor prospective și retrospective despre dieta restrictivă sugerează că la reintroducerea alergenilor incriminați sau la oprirea dietei restrictive sau elementale reapar și simptomele împreună cu modificările endoscopice și cu eozinofilia esofagiană.

Terapia de menținerea remisiunii EoE

Deoarece EoE este o afecțiune cronică în care simptomele reapar frecvent după oprirea terapiei, este necesară educarea pacienților despre opțiunile terapeutice necesare pentru menținerea remisiunii EoE.

Indicațiile potențiale pentru terapia cronică, de menținere a remisiunii EoE sunt: esofagul cu calibrul îngustat, stricturile esofagiene antecedente care au necesitat dilatări frecvente, endoscopie antecedentă de urgență pentru bol alimentar impactat, perforație esofagiană antecedentă, istoric de sindrom Boerhaave, simptome severe sau persistente și preferința pacientului.

Rezultatele terapiei farmacologice de menținere a remisiunii EoE au fost publicate intr-un singur studiu, cu 28 pacienți adulți cu EoE care au răspuns la tratamentul de 2 săptămni cu budesonid nebulizat sau deglutit și care ulterior au fost randomizați pentru a primi tratament de menținere cu budesonid 0,25 mg x 2/zi, fie cu placebo pe o perioadă de 50 de săptămâni. Pacienții menținuti pe budesonid au avut o reducere modestă a eozinofiliei esofagiene și mai puține simptome recurente comparativ cu lotul de control. Nici un pacient nu a fost diagnosticat cu infecții virale sau fungice și nici nu s-a semnalat atrofia de mucoasă. O posibilă doză inițială pentru terapia de menținere la adult ar putea fi de 1 mg/zi de budesonid sau 880 mcg/zi de fluticasonă, cu posologie mai redusă la copii și cu titrarea dozelor pentru obținerea răspunsului clinic optim cu dozaj minimal.

Terapia dietetică restrictivă de lungă durată constituie o strategie frecventă mai ales la copii dar și la adulți. Dilatația esofagiană intermitentă, „la cerere“, efectuată pentru simptomele recurente de disfagie, este posibilă de asemenea ca strategie de tratament de menținere a remisiunii EoE pe termen lung. Această strategie, utilizată singular, nu influențează eozinofilia esofagiană dar s-a dovedit eficientă pentru tratarea simptomelor disfagiei.

Concluzii.

Cunoştinţele medicale cu privire la eozinofilia esofagiană şi EoE au avansat substanţial în ultimele două decade. EoE este în prezent notificată ca o cauză frecventă a simptomatologiei gastrointestinale şi a morbidităţii, atât la copii cât şi la adulţi. Merită reţinut faptul că, notificarea unor aspecte izolate compatibile cu EoE, nu echivalează cu diagnosticul de EoE. Din punct de vedere diagnostic, depistarea EoE necesită evaluarea cauzei sale. Deşi diagnosticul diferenţial este larg, în discuţie intră GERD, PPI-REE şi EoE. Pentru a face diferenţierea dintre EoE şi PPI-REE este necesar un tratament de probă cu PPI, înaintea diagnosticării EoE. După precizarea diagnosticului de EoE opţiunile pentru terapia de linia I sunt corticosteroizii topici deglutiţi sau dieta restrictivă. Alegerea terapiei depinde de preferinţa pacientului, experienţa medicului curant şi de expertiza instituţiei medicale respective. Dilataţia endoscopică este terapia eficientă pentru stricturile şi esofagul cu calibru îngustat, mai ales dacă simptomele disfagiei s-au dovedit refractare la terapia medicală şi/sau restricţia dietetică. Deoarece EoE este o afecţiune cronică iar simptomatologia reapare la întreruperea tratamentului, se va lua în considerare, la toţi pacienţii, terapia de menţinere a remisiunii şi mai ales la pacienţii având complicaţii fibrostenotice sau simptome severe. Deoarece cercetările ştiinţifice în legătură cu subiectul prezentat în acest material sunt în plin progres, se aşteaptă ca în următorii ani, ghidurile şi recomandările specifice să conţină noi elemente cu privire la strategiile de diagnostic neinvaziv, cu folosirea de biomarkeri şi cu analize genetice şi de asemenea să abordeze noi modalităţi terapeutice şi metode non-endoscopice de monitorizare a răspunsului la tratament şi evoluţiei pe termen lung.

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Aspecte practice în explorarea IRM a meniscurilor

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Rezumat

Imagistica prin Rezonanţă Magnetică (IRM) este una dintre cele mai eficiente şi utile metode de diagnosticare a leziunilor meniscale. Metoda este utilă pentru că elimină artroscopiile inutile şi poate fi utilă ortopedului în adoptarea celei mai adecvate decizii terapeutice. Scopul prezentării noastre este acela de a familiariza cu anatomia normală şi a explica terminologia care descrie aspectele patologice precum şi modul de structurare a unei interpretări a examinării prin IRM a patologiei meniscale pentru a asigura un diagnostic clar şi scurt.

Abstract

MRI (Magnetic Resonance Imaging) is one of the most efficient, powerful and accurate methods for diagnosing meniscal lesions. This method is also useful because it can eliminate unnecessary diagnostic arthroscopy and help orthopedist in taking the most appropriate therapeutic decision. In our presentation, our purpose is to become familiar with normal anatomy, to review the imaging appearances of common abnormalities and to understand the use of terminology to describe pathological findings and how to structure a radiological report concerning meniscal lesions for being able to ensure clarity and brevity.

IRM este cea mai eficientă metodă neinvazivă de explorare a meniscurilor din articulaţia genunchiului. Rolul metodei este acela de a completa cu elemente de diagnostic examinarea clinică, a evita artroscopiile diagnostice inutile şi a exclude diagnosticele care au alt substrat anatomo-patologic.

Clinic nu pot fi evaluate exact topografia, întinderea şi amploarea leziunilor meniscale, în special când sunt prezente şi leziunile ligamentare. Radiologul care interpretează examinarea IRM are un rol foarte important, deoarece orientează pacientul către conduita terapeutică adecvată. Este foarte importantă stabilirea stadiului în care se află leziunile meniscale pentru prevenirea evoluției către complicațiile asociate şi gonartroză.

Elemente de anatomie a meniscurilor articulației genunchiului

Meniscurile sunt structuri anatomice interpuse între suprafețele articulare, care au formă de litera C sau semilunară şi sunt situate între condili şi platourile tibiale. Meniscurile acționează ca amortizoare între două suprafețe, îmbunătățesc stabilitatea şi lubrifierea şi permit flexia şi extensia. Au rol important în biomecanica articulației genunchiului, absorbția şi distribuția şocurilor. Meniscurile prezintă o structura organizată dintr-o zonă circumferențială externă şi o zonă transversală împărțită de un fascicul de colagen în două porțiuni una superioară şi alta inferioară. Meniscurile sunt slab vascularizate şi vase sunt prezente doar în treimea externă printr-un plex derivat din ramurile laterale şi mediale ale arterelor geniculate. Consecința acestui tip de vascularizaţie constă în vindecarea cu dificultate a leziunilor meniscale.

Meniscul medial are aspect de litera C deschisă și este fixat regiunii intercondiliene a tibiei atât anterior cât și posterior și atașat meniscului lateral în 40% dintre cazuri precum și capsulei posterioare și ligamentului colateral medial. Meniscul lateral este mai ovoid ca formă și prezintă fixare anterioară și posterioară fiind fixat de ligamentul meniscofemural Wrisberg posterior și Humphrey anterior. În extensie femurul se rotește intern și se fixează ca un mecanism de încuietoare cu șurub iar în flexie intervine pentru fixare tendonul mușchiului popliteu. Acesta se contractă iar cornul posterior este împins posterior.

Leziunile meniscale

Prin IRM pot fi evaluate criteriile de apreciere a morfologiei normale a meniscurilor. Meniscurile normale apar cu hiposemnal T1 și T2. Planul de evaluare inițial din algoritmul de lucru cuprinde pentru început secvențe sagitale. În acestea, cornul posterior al meniscului medial are volum mai mare decât cel anterior. În cazul meniscului lateral dimensiunile cornului anterior și cel posterior sunt egale.

În planul coronal coarnele posterioare acoperă în întregime platourile tibiale.

Meniscul medial este atașat de capsula articulară și nu există fluid între aceste două structuri.

MENISCUL DISCOID este o variantă anatomică întâlnită cel mai frecvent în compartimentul lateral și se poate aprecia prezența acestuia dacă apare pe mai mult de 2-3 secțiuni adiacente cu grosime de 4 mm. sau de 3 mm. În secvențele standard, dar meniscurile discoide pot fi evaluate eficient în secvențele 3D atunci când acestea sunt disponibile în protocolul de lucru. Această variantă anatomică predispune meniscul la leziuni. Clinic, pacientul cu o astfel de variantă poate prezenta simptomatologie dureroasă asociată cu zgomote intra-articulare sub forma unor pocnituri sau cracmente.

Leziunile pot apărea ca urmare a unui traumatism acut și puternic sau minor suprapus unei degenerescențe meniscale.

Leziunile meniscale se prezintă sub formă de traiecte cu semnal intermediar în T1 și hipersemnal T2 sau în secvența PD cu supresie de grăsime PD FS. Aspectul leziunilor meniscale poate fi vizibil astfel:

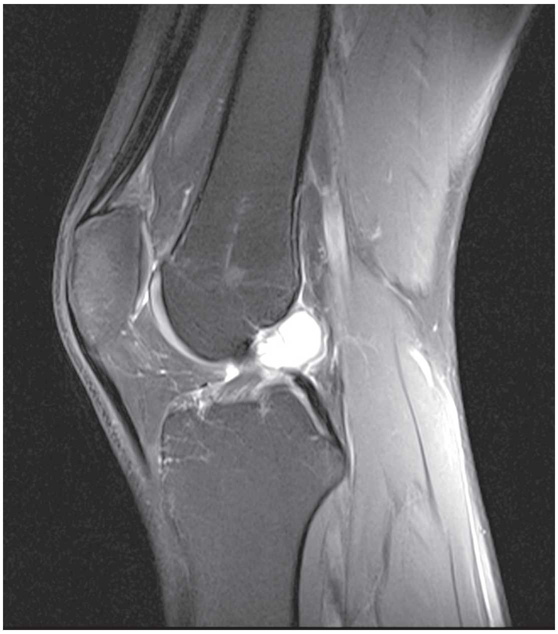

Figura 1. Secțiune sagitală de tip PD cu supresie de grăsime-FS. Imagine chistică perimeniscală asociată unei leziuni meniscale de tip III și leziuni fibrilare difuze ale ligamentului încrucișat anterior. Chistul perimeniscal este neomogen multiseptat și este situat intra-articular.

- **Leziunile globuloase, difuze sau orizontale care apar mai frecvent în cornul posterior la vârstnici și sunt degenerative.**
- **Leziunile longitudinale sunt cele care apar de obicei post-traumatic.**
- **Leziunile radiare sunt de obicei post-traumatice și aspectul particular este de „cioc de papagal“ sau „toartă de găleată“ la care există o asociere între traiectul vertical, de la radiar la cel longitudinal trecând prin un traiect curbiliniu.**

Dovezile instabilității meniscale sunt fragmentarea discală, leziunile vizibile pe o distanță mai mare de 1cm. asociate cu hipersemnal T2.

Consecințele traumatismelor repetate constau în leziuni ale porțiunii centrale cu aspect globulos și apoi progresiv, liniar sau mucoid.

Leziunile meniscului lateral sunt mai puțin frecvente decât ale celui medial, deoarece meniscul extern este mai mobil și prezintă mijloace de fixare mai puține față de cel medial.

Un aspect particular este acela al diferențierii leziunilor vechi meniscale de cele noi, aspect care se realizează prin artrografie IRM metodă în care în leziunile vechi nu pătrunde substanța de contrast introdusă intra-articular.

„Unhappy triad“ – descrisă de O`Donoghue este o leziune apărută în special după antrenamentele sau meciurile de fotbal sau după ski ca rezultat al stressului traumatic asupra articulației surprinsă în poziția valgus.

Stadializarea leziunilor meniscale se face ținând cont de aspectele corelative cu cele ale clasificării histologice.

GRADUL 1 este leziunea focală sau difuză care apare IRM ca o creștere difuză de semnal a substanței meniscale. Aceasta se corelează cu degenerescența meniscală precoce. Sunt utilizați termeni de tipul aspect mucinos, mixoid și de degenerescență mixoidă și hialină, cu rolul de a explica producerea și acumularea unei cantități crescute de mucopolizaharide în ariile stresate ca urmare a microleziunilor cu aspect repetitiv.

GRADUL 2 este leziunea meniscală liniară care nu afectează conturul meniscal. Gradul 2 al leziunilor meniscale este dat și de de leziunile care pot progresa din leziunile de tip 1 și sunt de obicei asimptomatice. Pot fi de trei tipuri: de tip 2 A cu aspect liniar care nu vine în contact cu suprafața articular, tipul 2B vine în contact doar pe o singură imagine cu suprafața articulară iar de tip 2C are formă de pană și semnal anormal dar nu vine în contact cu suprafețele articulare. Histologic se evidențiază fragmentarea colagenului și a matricei fibrocartilaginoase. Porțiunea mijlocie de colagen care divizează în cele două jumătăți superioară și inferioară este sediul acumulării preferențiale al substanței mucinoase. Aceasta reprezintă și originea leziunilor de acest tip. Cornul posterior al meniscului medial este cea mai frecventă regiune în care se evidențiază o leziune a meniscului.

GRADUL 3 este echivalentul unui aspect anormal al intensității meniscale care se extinde și comunică cel puțin cu una dintre suprafețele articulare. Pot fi prezente mai multe focare lezionale la nivel meniscal. Aceste leziuni pot fi prezente în regiuni greu accesibile artroscopiei de rutină.

GRADUL 4 apare atunci când leziunea meniscală atinge suprafața meniscală și separă fragmente meniscale.

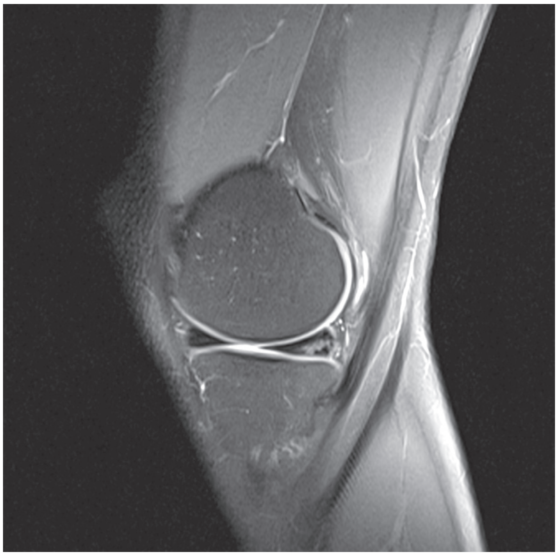

Figura 2. Secţiune sagitală PD FS. Traiectul leziunii meniscale atinge suprafaţa articulară inferioară. Leziune de tip III. Leziunea este oblică şi situată la nivelul cornului posterior al meniscului intern.

CRITERIILE PENTRU EVALUAREA LEZIUNILOR MENISCALE CONSTAU ÎN EVALUAREA LEZIUNILOR și semnalului meniscal atunci când traiectele meniscale sunt prezente în două secțiuni consecutive. Imaginile sunt mai vizibile în secvențele cu TE scurt. În leziunile meniscale lichidul este absorbit la nivelul marginilor iar schimbările care apar la nivel meniscal se explică prin creșterea densitații evaluată de predilecție prin secvența PD la nivelul traiectului lezional. Ideală este evaluarea leziunilor în incidențe perpendiculare, adică vizibile în plan coronal și sagital completate și cu planul axial dacă instalația oferă imagini cu rezoluție corespunzătoare. În buletinul de interpretare a leziunilor, radiologul trebuie să descrie numărul traiectelor lezionale, unghiurile în care sunt vizibile, forma lor și planurile în care sunt descrise.

Pentru a fi util ortopedului care realizează artroscopia atunci când instalația IRM o permite, se realizează și o achiziție de tip 3 D urmată de prelucrare multiplanară MPR. Leziunile meniscale pot avea traiect vertical sau orizontal, iar leziunile mai pot fi împărțite în longitudinale, radiale și orizontale.

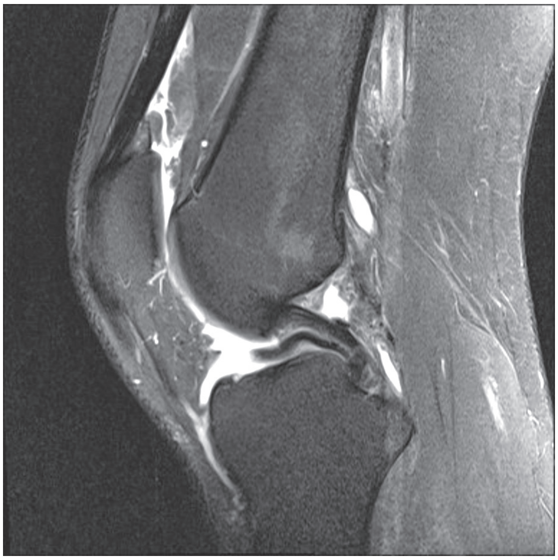

Figura 3. Secţiune sagitală PD FS . Leziune meniscală cu aspect care simulează semnul de ligament încrucişat dublu. Fragmentul meniscal este deplasat în regiunea intercondiliană.

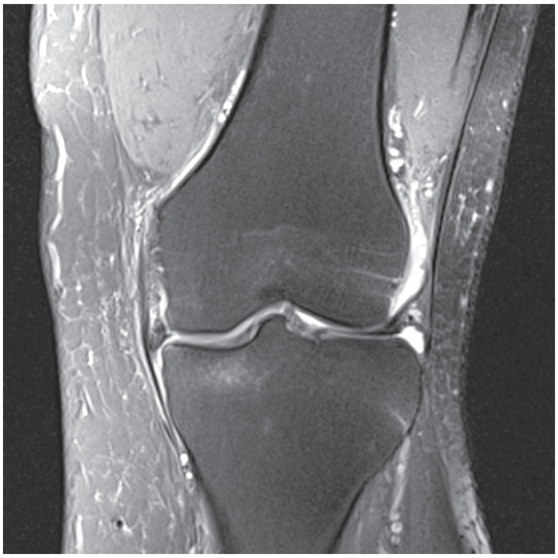

Figura 4. Imagine de tip PD FS în plan coronal. Se vizualizează leziuni meniscale complexe atât la nivelul cornului posterior intern cât şi celui extern şi leziuni parţiale asociate la nivelul ligamentelor colaterale şi edem osos de platou tibial.

LEZIUNILE VERTICALE pot fi aliniate perpendicular pe planul coronal al meniscului şi pot fi subdivizate în longitudinale sau radiale. Leziunile complexe apar post-traumatic la pacienţi tineri şi pot evolua către aspectul de toartă de găleată. Leziunile longitudinale separă meniscul în fragmente externe şi interne şi se asociază cu leziuni ale ligamentului încrucişat anterior. Cel mai frecvent sunt situate în treimea medie sau posterioară a meniscului şi se propagă către porţiunea sa anterioară. O leziune parţială longitudinală vine în contact cu suprafaţa articulară meniscală superioară sau inferioară, dar nu cu amândouă în acelaţi timp astfel încât fragmentul este încă stabil.

LEZIUNILE RADIALE SAU TRANSVERSALE sunt tot leziuni verticale care se propagă perpendicular pe axa principală a meniscului şi au consecinţe grave asupra integrităţii meniscale. Forţa se distribuie asupra fibrelor de colagen pe o direcţie diferită de cea obişnuită de distribuţie şi de conducere a forţelor astfel încât traiectul leziunii avansează.

LEZIUNILE ORIZONTALE sunt în marea lor majoritate consecinţa evoluţiei unor modificări degenerative.

LEZIUNILE COMPLEXE MENISCALE asociază leziuni cu diverse traiecte, uneori cu leziuni degenerative sau traiecte apărute în moment diferite, şi pot lua forme diferite în funcţie intensitatea şi vechimea traumatismului până la avulsia meniscală sau fragmente meniscale deplasate liber intra-articular.

LEZIUNILE MENISCALE POT FI STABILE SAU INSTABILE iar criteriile de stabilitate depind de un număr de criterii ce pot fi verificate artroscopic. Oricare leziune meniscală cu un fragment detaşat este instabilă.

Disocierea capsulo-meniscală

În cazul secvenţelor sensibile la fluide este vizibilă acumulare fluidă între menisc şi capsula articulară.

Periferia meniscală este neregulată, iar marginea platoului tibial este descoperită de platoul tibial pe o suprafaţă mai mare de 5mm. Atât fascicolul menisco – femural cît şi cel menisco-tibial apar neregulate.

Chisturile meniscale

Sunt colecţii de fluid adiacente periferiei meniscale şi probabil leziunilor meniscale. Pot fi perimeniscale, intrameniscale sau parameniscale în

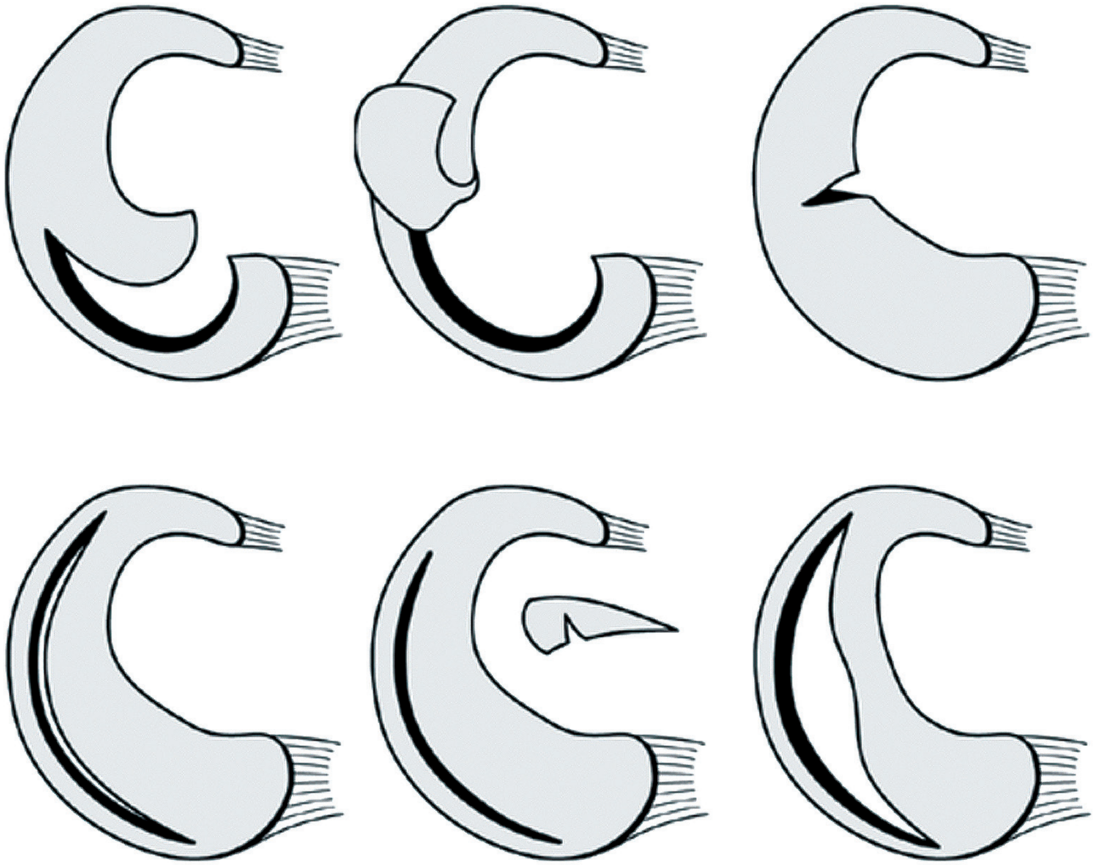

Figura 1. Schemă cu principalele tipuri de leziuni mensicale. Pe rândul de sus sunt vizibile leziuni longitudinale și în toartă de găleată iar pe rândul de jos cu aspect de fald sau radiale.

Adaptare după Orthopedics Stoller Tirmann Bredella 2005 capitolul V.

funcție de localizare. Pot apărea și secundar meniscectomiei dar, de obicei, sunt secundare traumatismelor.

Protocol și planuri de secțiune

Genunchiul este de obicei poziționat în ușoară extensie și rotație externă pentru a facilita explorarea ligamentului încrucișat anterior. Pentru evaluarea leziunilor subtile este nevoie de rezoluție crescută. Pentru aceasta este nevoie de un câmp de explorare- (Field of View- FOV) de 16 cm sau mai puțin și de secțiuni cu grosime de 5mm sau mai puțin – (3-4 mm de preferință) și cu o matrice de cel puțin 256 în direcția de fază și în cea de codare în direcție. Un „gap“ (un spațiu) între secțiuni de 0,5-1 mm este necesar sau se pot folosi achiziții 3D izotropice. Acești parametri pot fi obținuți cu bobine de suprafață sau de tip solenoid. O bobină pentru extremități este folosită pentru optimizarea raportului semnal/zgomot. Imaginile sunt obținute în plan sagital în T1 și coronal și sagital în PD FS, precum și axial PD FS dar și secvențe dedicate de tip 3D cu particularități în funcție de tipul de instalație.

Diferențe de diagnostic dintre IRM și artroscopie.

Cauzele variațiilor de diagnostic sunt datorate curbelor de învățare diferite precum și a nivelurilor diferite de experiență. Acuratețea de diagnostic artroscopic a fost raportată în diferite studii comaprative ca fiind cuprinsă între 68-98% în funcție de experiența examinatorului și localizarea leziunii. Descrierea leziunii este diferită astfel încât o anomalie a marginii libere poate fi diferit interpretată de către radiolog și de medicul care realizează artroscopia. Fiecare dintre metodele comparate respectiv IRM și artroscopia pot prezenta avantaje și dezavantaje, astfel încât prin valoarea complementară a metodelor poate fi stabilit diagnosticul și tipul de leziune. Prin artroscopie nu poate fi vizualizat aspectul degenerativ al fibrelor din interiorul meniscului precum și marginea internă a coarnelor meniscale iar prin IRM pot apărea aspecte fals pozitive.

Concluzii:

În funcție de diagnosticul stabilit sunt disponibile diferite metode de tratament pentru reconstrucție și reconstituire sau refacere meniscală. Tratamentul optim al leziunilor meniscale constituie o entitate controversată și în continuă evoluție. Tratamentul este influențat de gradul de cunoaștere al leziunilor meniscale, iar acesta depinde de descrierea leziunilor, respectiv a tipului de leziune, a localizării și a extinderii acesteia. Absența conduitei terapeutice adecvate în cazul leziunilor severe poate conduce la instabilitate meniscală și progresiv la gonartroză urmată de grade tot mai avansate de dizabilitate, care în final necesită protezare articulară. Există și factori asociați care pot produce agravare și evoluție accelerată degenerativă a bolii sau evoluție negativă post-meniscectomie cum este cazul în cadrul asocierii cu cartilajul de acoperire sau fragmentare meniscală.

După leziunea meniscală există riscul dezvoltării într-o etapă ulterioară de leziuni cartilaginoase. Leziunile meniscale mediale sunt de obicei asociate cu defecte cartilaginoase difuze, iar cele laterale de obicei sunt focale. Aceste leziuni din gradele inferioare sunt de obicei însoțite de simptomatologie dureroasă doar atunci când asociază și edem osos sau alte tipuri de leziuni intra-articulare. Patologia post-traumatică meniscală este întâlnită frecvent în rândul persoanelor adulte active astfel încât numărul de examinări prin IRM este foarte important în ponderea leziunilor traumatice ale regiunii osteoarticulare. Asocierea frecventă și cu alte tipuri de leziuni intra-articulare necesită studii atente și fie protocoale dedicate și particularizate pentru fiecare pacient atunci când timpul alocat examinării permite, fie protocoale cu secvențe care să acopere global patologia.

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Terapia cu antidiabetice orale în diabetul zaharat tip 2

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Rezumat

Terapia cu antidiabetice orale în diabetul zaharat este o opțiune de tratament, bine fundamentată în practica clinică prin cercetare avansată, structurată, atât de ordin academic, dar și de ordin comercial, mult diversificată în ultimii 20 ani.

În mod curent sunt folosite 7 clase de medicație antidiabetică orală (ordinea menționării lor fiind aleatorie): biguanidele, sulfoniluree, tiazolidindione, glinide, gliptine, inhibitorii de alfa-glucozidază, sechestranții de acizi biliari. Mai sunt: derivații de amilină, analogii de glucose-like peptid și insulina, dar acestea se administrează injectabil, existând însă tentative de a găsi o formă orală a administrării lor. Având in vedere potențialul uriaș al pieței de desfacere (sunt actual în lume peste 370 de milioane pacienți diagnosticați cu diabet zaharat tip 2) există un efort asiduu în acest domeniu, în încercarea de a găsi remediul ideal oral al acestei boli.

Abstract

Therapy with oral agents for type 2 diabetes mellitus is an well grounded option in clinical practice through advanced research, academicaly and comercially structered. The therapy has been more diverse in the last 20 years.

There are currently 7 classes of oral medication (mentioned randomly): biguanides, sulfonylureas, thiazolidinediones, glinides, gliptine (dipeptidyl peptidase-4 inhibitors), alpha-glucosidase inhibitors, bile acid sequestrants. In the same time, there are other classes such as amylin derivatives, analogs glucose-like peptide and insulin, but they must to be injected. There are attempts to find an orally administering form for these classes. Given the huge potential market outlets (more than 370 million type 2 diabetes patients in the world) an assiduous effort in this area, trying to find the ideal remedy oral remedy for this disease.

Key words: *biguanides, sulfonylureas, thiazolidinediones, glinides, gliptine (dipeptidyl peptidase-4 inhibitors), alpha-glucosidase inhibitors, bile acid sequestrants.*

Introducere

Există perspectiva sumbră a Organizației Mondiale a Sănătății ca în perioada imediat următoare (2050) să existe în lume peste 370 milioane de pacienți diagnosticați cu diabet zaharat tip 2 (DZ2). Pentru fiecare dintre aceștia, în conformitate cu ghidurile terapeutice actuale, ar fi necesară intervenția terapeutică. Este clar cunoscut că intervenție în diabetul zaharat înseamna modificarea stilului de viață (dietă/terapie medicală nutrițională, efort fizic) și terapie medicală (antidiabetice orale +/- insulină).

Medicamentul ideal poate că ar trebui să se administreze cât mai rar, să fie unic dozat, să nu aibă efecte toxice, să fie indicat în orice stadiu al bolii – indiferent de complicații, să substituie dieta și, eventual, să acționeze și în cazul altor boli concomitente.

Depășind aceasta introducere, vom încerca în paginile ce urmează să prezentam medicația antidiabetică orală punctând pentru fiecare clasă elementele importante, astfel încât un medic de altă specialitate să poată folosi aceste pagini drept mic îndreptar de tratament al DZ2 folosind medicația orală disponibilă actual, cu precizarea că – totuși – prin legislație medicul specialist de diabet, nutriție și boli metabolice are dreptul inițierii și schimbării acestui tratament, urmărind toți factorii implicați în prevenția evenimentelor cardiovasculare, dar și a complicațiilor microvasculare ale DZ2. Astfel că tratamentul DZ2 poate însemna în fapt prevenția complicațiilor acestei boli, nu scăderea glicemiei, așa cum este perceput superficial acum tratamentul.

Există medicație la acest moment care este aprobată în România pentru a fi folosită, dar care nu are susținerea statului. Există și versiunea mai puțin fericită în care există medicație antidiabetică aprobată în lume și pentru care nu există un importator/distribuitor. Articolul evaluează medicația orală care există în țară, este aprobată și care poate fi folosită la momentul scrierii articolului. Studiile clinice pentru inhibitorii de SGLT2 au fost expuse încercând a da o imagine asupra fenomenului cercetării la acest moment.

Biguanidele

Liliacul francez (*Galega officinalis*) a fost utilizat pentru tratamentul diabetului zaharat în medicina tradițională. În 1920 a fost sintetizat și principiul activ al acestei plante: guanidina. Acest compus scade glicemia, dar derivate mai puțin toxice (Syntalina-A și Syntalina-B) au fost folosite imediat în tratamentul diabetului zaharat. Unul dintre mecanismele principale ale DZ2 este insulinorezistența, apărând imediat disfuncționalități la nivelul țesutului adipos și țesutului muscular. În cadrul medicației utilizate pentru creșterea sensibilității la insulină, clasa biguanidelor are unul dintre cele mai importante roluri. Fenforminul a fost cel mai utilizat inițial, dar în anii următori ai punerii pe piață, din cauza incidenței crescute a acidozei lactice fatale, a fost retras de pe piață (SUA-1977). Metforminul are un profil de siguranța mult mai bun și este astăzi principalul reprezentant al biguanidelor utilizat în tratamentul DZ2.

Actual mai mulți compuși fac parte din această clasă:

1. Biguanida – altfel numită guanidil-guanidina – compusul de bază al clasei.
2. Metforminul (dimetilbiguanida) cea mai folosită biguanidă actual.
3. Buforminul (butilbiguanida) interzisă în unele țări (SUA, Canada, Germania etc.), constituie alternativa pentru metformin în cadrul tratamentului DZ2, acolo unde este acceptat (România, Spania).
4. Fenforminul (feniletil biguanida) retras de pe piață ca urmare a acidozei lactice metabolice, mortale în peste 50% dintre cazuri.
5. Alte biguanide – proguanilul – medicament antimalaric, nu face obiectul indicațiilor pentru DZ2.

Medicina bazată pe dovezi este clar în favoarea utilizării metforminului ca primă linie farmacologică în prevenția și tratamentul DZ2, alături de celelalte metode: terapia medicală nutrițională, efortul fizic terapeutic ș.a., asfel încât acest reprezentant este astăzi liderul clasei.

Metforminul

Comercializarea lui se realizează din 1957. Metforminul (MTF) este cel mai important medicament antidiabetic, se absoarbe în intestinul subțire, are nivelul maxim al concentrației sangvine la 1,5-2 ore, absorbția fiind completă la 6 ore. Durata medie de absorbție este de 0,9-2,6 ore, biodisponibilitatea este de 50-60%, timpul de înjumătățire plasmatic este de 1,5-4,9 ore. În proporție de 90% este eliminat pe cale renală, asfel încât afectarea renală importantă contraindică administrarea acestui medicament.

Administrarea MTF-ului se face mai ales postprandial, din cauza efectelor secundare digestive. Administrarea preprandială (conform indicatiilor ghidului de tratament al Asociației Americane de Diabet) crește aria de sub curbă a medicamentului cu 25% (absorbția) și concentrația sangvină maximă cu 40%, în condițiile tolerabilității lui. După absorbție, MTF-ul nu se leagă de proteinele plasmatice și distribuția în țesuturi este în concentrație similară cu cea plasmatică.

Mecanismul de acțiune al MTF-lui a fost studiat extensiv, biochimia acțiunii acestuia încă nu au fost elucidate. MTF-ul exercită câteva efecte care contribuie la scăderea glicemiei prin: combaterea insulinorezistenței și ameliorarea sensibilității la acțiunea periferică a insulinei, prin prezervarea funcției betacelulare, potențarea primei faze de secreție a insulinei (efect controversat), creșterea captării de glucoză mediată de insulină la nivelul musculaturii, creșterea activității tirozinkinazei la nivelul receptorilor de insulină, creșterea legării insulinei de receptori, amplificarea sintezei de glicogen la nivel hepatic, scăderea glicogenolizei, creșterea activității transportorului GLUT 4, descreșterea apetitului cu o scădere consecutivă în greutate (2-3 kg) și reducerea țesutului adipos visceral, ameliorează starea proinflamatorie și starea protrombotică, prin scăderea PAI-1, a proteinei C reactive, a factorului de necroză tumorală alfa, etc.

Datorită acestor mecanisme de acțiune, ameliorarea glicemică determinată de MTF nu se asociază cu o creștere a insulinemiei, iar riscul de hipoglicemiei este minin. MTF-ul scade hiperglicemia bazală reducând și excursiile glicemice postprandiale. Scăderea glicemiei este de aproximativ 50mg/dl, (HbA1c scade în medie cu 1,5%); clasic nu dă hipoglicemie și e numit medicament antihiperglicemiant. MTF-ul nu crește insulinemia, dimpotrivă o scade, probându-se astfel ipoteza creșterii sensibilității celulelor la insulina atât endogenă, cât și exogenă. Aceste elemente sunt probate și cu ajutorul clampului euglicemic-hiperinsulinemic.

Conform ghidurilor actuale de tratament, metforminul constituie prima indicație de tratament în DZ2. Utilizarea în practică a MTF-ului este postulată de ghidul de management clinic al DZ2, realizat în consens de Asociația Europeană de Studiu a Diabetului (EASD) și Asociația Americană de Diabet (ADA). Acest ghid, republicat în 2008, recomandă MTF-ul ca primă alegere în terapia antihiperglicemiantă a DZ2. MTF-ul este recomandat pentru pacienții cu DZ2 nou descoperit și/sau pentru pacienții pentru care terapia medicală nutrițională devine ineficientă. MTF-ul va fi menținut pe parcursul tuturor etapelor de tratament.

Conform recomandărilor societăților profesionale ADA și EASD, administrarea MTF-lui se face inițiindu-se terapia cu 1-2X500mg, seara și dimineața la mese sau numai la prânz. Ulterior se poate ajunge, titrându-se doza în funcție de glicemiile preprandiale, până la 3000mg/zi. Doza optimă recomandată variază între 2000-2500mg/zi, în trei prinze, cu un maxim de eficiență și reacții adverse intestinale minime la 2000mg/zi. Dozarea MTF-ului, pentru preparatele existente pe piața din România este 500mg, 850mg, 1000mg.

MTF-ul a dovedit efecte pozitive și în prevenția DZ2 la persoanele cu risc crescut. Noțiunea de „risc crescut" este definită de ADA în 2010 și se confundă des în clinică cu noțiunea de „prediabet", definită de aceeași asociație profesională în 2009. Asocierea MTF-lui cu optimizarea stilului de viață la acești pacienți s-a dovedit superioară intervenției nutriționale singulare, cu o reducere a progresiei spre DZ2 de aproximativ 31%. MTF-ul a produs o scădere ponderală de aproximativ 2kg, asociată cu efecte pozitive asupra sensibilității la insulină. O metaanaliză care strânge peste 30 de studii cu MTF la pacienții aflați la risc pentru DZ2 concluzionează: reduce incidența diabetului zaharat cu 40% și riscul absolut pentru mortalitate cu 6%, reduce IMC-ul cu 5,3%, reduce glicemia bazală cu peste 4%, reduce insulino-rezistența cu peste 22%.

MTF se poate utiliza în asociere cu toate clasele de antidiabetice orale sau insulină, atât în combinații fixe puse la dispoziție de către producători, cât și în cadrul unor scheme terapeutice dictate de medicul specialist pe baza ghidurilor actuale. Beneficiile asocierii cu MTF sunt constatate o dată cu îmbunătățirea controlului glicemic, reducerea dozelor de medicație/insulină, reducerea efectelor adverse ale terapiei maximale singulare, controlul ponderal și/sau limitarea creșterii ponderale. Studiile actuale dovedesc uneori superioritatea combinațiilor fixe comparativ cu monoterapia sau cu administrarea separat a medicației (exemple de combinații fixe metformin-rosiglitazonă, metformin-glibenclamid s.a).

Contraindicațiile administrării metfominului sunt clare și evaluarea pacientului cu DZ2 le evidențiază cu ușurință: hipersensibilitate la MTF sau la excipienții folosiți, ciroza hepatică, insuficiența hepatică sau hepatopatiile severe cu un nivel al citolizei hepatice ce depășeste de 3X valoarea normalului, insuficiența renală cu un nivel al creatininei serice peste 1,4mg/dl la femei și 1,5mg/dl la bărbați și un clearance al creatininei calculat mai mic de 60ml/min, insuficiența cardiacă de clasă III/IV NYHA și boli obstructive severe ale arterelor periferice, insuficiența respiratorie, retinopatie diabetică medie/severă, efectuarea de investigații cu substanțe iodate.

Contraindicații relative pot constitui următoarele situații, când se recomandă precauție în administrare: stări hipoxice, stări cu catabolism crescut, consum de alcool, deficit de vitamina B12 etc.

Reacții adverse. La începutul tratamentului, la aproximativ 5-20% dintre pacienții tratați, apar reacții adverse gastro-intestinale, cum sunt greața, balonările, vărsăturile, dureri abdominale, flatulența, scăderea ponderală, diaree, disgeuzia (gust metalic). În general nu este necesară întreruperea tratamentului, deoarece aceste tulburări dispar chiar dacă doza nu este modificată. Diareea persistentă dispare la întreruperea tratamentului. Prin creșterea progresivă a dozelor și administrarea de MTF în timpul/după mese frecvența și severitatea tulburărilor gastro-intestinale pot fi diminuate. Ocazional pot apărea: cefalee, vertij, fatigabilitate. În cazuri foarte rare se produce o reacție de hipersensibilitate cutanată și din cauza scăderii absorbției vitaminei B12 și a acidului folic pot apărea tulburări ale hematopoezei (anemie megaloblastică).

Acidoza lactică determinată de MTF are o incidență rarisimă 1:100.000 cazuri tratate, este diagnosticată în condițiile unui pH<7,35 și o concentrație a lactatului arterial mai mare de 5mmol/l, soldându-se la peste 50% dintre cazurile identificate cu deces. Tabloul clinic cuprinzând dureri si crampe musculare, hiperventilație, obnubilare și comă se poate instala în câteva ore. În cazuri excepționale tratamentul concomitent cu alte antidiabetice orale sau cu insulină poate duce și la hipoglicemie cu manifestările clinice caracteristice.

Sulfonilureicele

Sulfonilureicele (SU) sunt printre primele medicamente antidiabetice orale folosite în tratamentul DZ2, efectul lor hipoglicemiant fiind descoperit întâmplător la jumătatea secolului XX. În ultimii 50 de ani au fost dezvoltate numeroase molecule noi din aceasta clasă, toate având un mecanism de acțiune similar; ele sunt clasificate în SU de generația întâi, a doua și a treia. Primii reprezentanti ai clasei, SU de generația I (acetohexamida, chlorpropamida, tolazamida si tolbutamida), nu se mai folosesc în practica clinică curentă, fiind inlocuiți de generațiile noi, cu o eficacitate superioară și efecte adverse mult mai reduse.

Conform consensului ADA/EASD 2012, care accentuează importanța alegerii medicației centrate pe pacient, SU reprezintă o medicație de linia a doua în DZ2, când MTF-ul, dieta și exercițiul fizic nu sunt suficiente pentru a controla profilul glicemic sau de primă linie în cazul în care MTF-ul este contraindicat.

Mecanismul de acțiune este reprezentat de stimularea producției de insulină de către pancreas, astfel fiind utile numai la pacientii cu DZ2 la care celulele pancreatice au prezervată capacitatea de a secreta insulina. Practic, stimulează insulinosecreția, fără a influența semnificativ insulinorezistența, motivând selectarea atentă a pacienților.

Efectul hipoglicemiant al clasei este substanțial, reducând HbA1c în medie cu 1,5%, atât prin reducerea valorilor glicemiei a jeun cu 60-70 mg/dl, cât și a glicemiei post-prandiale. În cazul unui dezechilibru glicemic major cu hiperglicemie severă, efectul inițierii terapiei cu SU este însă redus, astfel încât se recomandă utilizarea acestei clase numai pentru glicemii <250-300 mg/dl, important este însă ca și alți factori să fie luați în calculul alegerii acestei medicații. Studiile prospective au demonstrat în plus faptul că după mai mulți ani de tratament cu substante secretagoge, intensitatea efectului hipoglicemiant scade, mecanismele propuse fiind multiple (desensibilizarea receptorilor specifici, regresia funcției beta-celulare în istoria naturală a bolii, fiind propusă și o terorie legată de o posibilă accelerare a apoptozei beta-celulare în contextul supra-stimulării).

Instalarea efectului SU, vârful de acțiune și durata, diferă în functie de generația și tipul acestora și sunt exemplificate mai jos. Farmacocinetica este importantă, atât în privința ratei de absorbtie diferită de la o substanță la alta (fiind mai mare pentru glibenclamid decât pentru gliclazid si glimepirid), a metabolizării (majoritatea metaboliților sunt inactivi, cu excepția glimepiridei – de unde efectul hipoglicemiant mai crescut al acestuia), cât și a eliminării (majoritatea SU se elimină predominant renal, excepție face qliquidona cu eliminare pedominant biliară, iar glimepirida în proporții apropiate renal si biliar). Astfel, qliquidona (și în anumite condiții glimepirida) poate fi administrată și în condițiile unei insuficiențe renale, cu monitorizarea atentă a parametrilor metabolici, în timp ce restul SU nu pot fi folosite la pacienții cu insuficiență renală. Mai nou, gliclazidul își câștigă un rol în administrarea în DZ2 complicat renal. Terapia cu SU se ințiază plecând de la doza minimă și se titrează treptat până la la obținerea efectului metabolic scontat; totuși, se recomandă ca doza finală să nu depăsească 1/2-2/3 din doza maximă admisă, caz în care se reia reevaluarea schemei terapeutice, alegându-se o terapie combinată.

Efecte secundare/Reactii adverse: Hipoglicemia este frecvent întâlnită și poate fi în unele situații severă și prelungită, mai ales la pacienții în vârstă sau cu insuficiență renală, necesitând asistență medicală specializată de urgență. În scopul prevenirii hipoglicemiilor, atenționări sunt necesare pentru următoarele situații: supradozarea medicației, riscul de funcție renală și hepatică afectate, consum de alcool, omiterea meselor, efort fizic excesiv, interacțiuni medicamentoase. Riscul hipoglicemic diferă în funcție de tipul de SU, cel mai scăzut pare a fi pentru gliclazid. Creșterea ponderală în medie de 3-4 kg este de asemenea frecvent întalnită și poate fi explicată atât pe baza efectului anabolic al insulinei, cât și pe baza faptului ca pacienții au tendința de a crește aportul de glucide compensator în cazul hipoglicemiilor frecvente, în locul titrării corecte a dozei optime terapeutice de ADO. Studii recente arată însă că o educație nutrițională optimală reduce la minim acest risc, cu menținerea relativ în limite a greutății corporale. Efecte adverse mai rare pot fi dermatologice, hematologice și gastrintestinale. Unele studii au adus în discuție un risc cardio-vascular crescut în cazul terapiei cu SU, aspect aflat încă în dezbatere.

Contraindicații: decompensarea metabolică acidozică, coma diabetica cetoacidozică, boli infecțioase, intervenții chirurgicale, tulburări hepatice grave, clearance renal <30 ml/min, sarcina și alăptarea (in lipsa unor dovezi certe).

În ciuda faptului că în ultimii 50 de ani au fost descoperite multiple terapii inovatoare în DZ, MTF-ul și SU reprezintă încă terapia de elecție la debut, datorită eficacității acestora, dar și a prețului relativ redus. Alegere unui reprezentant din clasa SU se face ținând cont de aspectele enumerate. Tabelul de mai jos ilustrează schematic principalii reprezentanți ai SU de generația a doua și a treia, și modul de administrare al acestora:

Tabel 1. **Suldonilureice – reprezentanți**

DCI	Varf acțiune	T ½ ore	Număr doze/zi	Doza min-max (mg)
Glibenclamid		4-11	1-2	2,5-15
Glipizid	2-3 h	2-4	1-2	2,5-40
Gliclazid		10	1-2	40-320
Gliquidona		1,4	2-3	15-120
Glimepirid	2-4 h	7	1-2	1-6

Inhibitorii de alfa-glucozidază

Inhibitorii de alfa-glucozidază reprezintă o clasă de antidiabetice orale care acționează prin modificarea absorbției și a digestiei glucidelor, determinând astfel un efect semnificativ de reducere a excursiei glicemice post-prandiale.

Dintre cei trei reprezentanți ai clasei (acarboza, vogliboza și miglitol) în țara noastră este disponibilă clinic numai acarboza sub forma de comprimate de 50 si 100 mg. În monoterapie, au dovedit un efect de scădere a glucozei prandiale între 30 si 70 mg/dl, scăderea glicemiei bazale cu 20-30 mg/dl, și o scădere a HbA1c cu 0,7 până la 1 %. Sunt utilizați în principal ca terapie combinată în DZ2, dar mai nou și în prevenția DZ2. În pofida eficacității acestora, trebuie însă să se țină cont de o serie de efecte secundare și contraindicații care le pot limita utilizarea.

Efectele adverse principale sunt cele abdominale, incluzând: flatulența, durere abdominală și accelerarea tranzitului intestinal, care, deși nu sunt periculoase, sunt deranjante, motivând întreruperea terapiei de către un număr semnificativ de pacienți. Pentru a scădea intensitatea acestor simptome, se recomandă inițierea terapiei cu o doza minimă și ajustare lent-progresivă în funcție de toleranța individuală și de profilul glicemic, doza de menținere fiind de 3x50 sau 3x100 mg. În cazul nerespectării dietei antidiabetice, simptomele de asemenea se pot intensifica. În cazul unei exacerbări a simptomelor digestive, în ciuda respectării dietei, se recomandă reducerea temporară a dozelor.

Contraindicații: afecțiuni gastrointestinale (precum boli inflamatorii cronice, boli de maldigestie și malabsorbtie), insuficiență hepatică, insuficiența renală severă. Nu există date privind siguranța la femeile însărcinate, în alăptare și la copii. Au fost raportate cazuri rare de toxicitate hepatică, necesitând o monitorizare periodică a enzimelor hepatice. Având în vedere mecanismul de acțiune, se va evita administrarea concomitentă de suplimente enzimatice pancreatice. Dacă se asociază unui secretagog (SU), în cazul apariției unui episod hipoglicemic a se avea în vedere faptul că reversia hipoglicemiei este posibilă numai cu glucoza, uneori numai cu glucagon.

Tiazolidindionele (glitazonele)

În prezent din această clasă terapeutică se folosesc pioglitazona și rosiglitazona (cu restricție în România pentru rosiglitazonă).

Mecanism de acțiune

Tiazolidindionele sunt utilizate în practică, deoarece induc reducerea insulinorezistenței prin creșterea insulinosensibilității periferice (efect primar) la nivelul țesutului adipos, tesutului muscular și ficatului și prezervarea functiei β-pancreatice. Acestea nu afectează insulinosecreția.

Efectul hipoglicemiant se instalează progresiv și este însotit de reducerea nivelurilor serice ale acizilor grași liberi și ale insulinemiei, de asemenea și trigliceridele circulante pot să scadă, în timp ce fracțiunile de colesterol HDL si LDL rămân nemodificate sau cresc ușor, fără ca raportul dintre ele să se modifice semnificativ.

Cele două TZD-uri se folosesc în monoterapie sau în asociere cu metforminul, sulfonilureicele sau insulina. TZD sunt agoniști ai receptorilor nucleari, receptori PPAR gama (peroxizom proliferators activator receptor gamma) la nivel muscular, adipos și hepatic și astfel ameliorează sensibilitatea la insulina endogenă și exogenă. PPAR aparțin unei familii de receptori nucleari hormonali de tip steroidal și joacă un rol de factori de transcripție.

TZD-le scad semnificativ hiperglicemia la pacienți cu DZ2 cu insulinorezistență periferică din mușchi și țesut adipos, cu aproximativ 40mg/dl la o doză medie, efect lent, cu maxim de eficacitate la 2-3 luni. Cresc prelucrarea și utilizarea de glucoză mediată de insulina. TZD – urile au efecte benefice asupra profilului lipidic.

Terapia cu TZD se asociază cu o scădere a nivelurilor acizilor grași liberi și a trigliceridelor plasmatice, dacă sunt crescute (≥200g/dl), și cu o creștere a colesterolului total, în paralel cu o creștere a HDL- si LDL-colesterolului. De asemenea, TZD cresc adipogeneza. Pacienții prezintă o tendință la creștere ponderală de 2-5 kg observată ulterior. În asociere cu MTF-ul creșterea în greutate este mai redusă.

O parte din surplusul ponderal poate să rezulte și din retenția hidrosalină (0.5kg creștere în greutate, în absența insuficienței cardiace). Acestea exercită și alte efecte: scad tensiunea arterială prin scăderea insulinorezistenței și a contractilității musculare, reduc rezistența vasculară periferică, îmbunătățesc statusul procoagulant, reduc microalbuminuria.

Anemia diluțională, rezultat al retenției hidrosaline este ușoară ca magnitudine (cu maxim 1gr/dl). Timpul de înjumătățire a celor două tiazolidindione este nemodificat la pacienții cu insuficiență renală moderată sau severă.

***Tabel 2*. Tiazolidindione – date actuale**

Substanta activă	Priza de administrare	Doza totală	Absorbție	Timp înjumătățire	Vârf plasmatic	Eliminare
Pioglitazona	Doza unică, neinfluențată de mese	Monoterapie 15-45mg/zi	Rapidă	3-7 ore	<2 ore	Biliară
Rosiglitazona (restrictie temporara)	1-2 prize	4-8mg/zi	Rapidă	3-4 ore	1 ora	Renală

Indicații. TZD sunt indicate la pacienți cu DZ2 și insulinorezistență, ca primă intenție terapeutică, sau secundar la cei care nu tolerează metforminul. Mai pot fi recomandate pacienților cu obezitate abdominală și dislipidemie asociată. Ca și precauții, sunt menționate monitorizarea enzimelor hepatice lunar inițial, apoi la 2 luni pe parcursul tratamentului.

Contraindicații: DZ1, sarcina și alăptarea, consum cronic de alcool, la pacienții cu valori crescute ale transaminazelor (>2-3 ori N), insuficiența cardiacă clasa III-IV NYHA, boli hepatice, pacienți cu risc cardiovascular crescut, hipersensibilitate la produs.

Reacțiile adverse sunt prezentate mai jos, în tabel:

***Tabel 3.* Reacții adverse tiazolidindione**

Creștere ponderală (2-8% pac)	Retentia hidrica	Scădere ușoara a Hb	Insuficiență cardiaca	Hepatotoxicitatea	Fracturile osoase
1,5kg - la doze mici. 3,5-4kg – la doze mari Creșterea adipozitatii subcutanate si scaderea grasimii viscerale.	Edeme ușoare la 2-3% din pac.	Datorită hemodilutie se stabilizează după 8-12 sapt.	1,5-1,7% la pacientii tratati cu rosiglitazona	0,25% pentru pioglitazona 0,2% pentru rosiglitazona.	Risc adițional crescut de producerea fracturilor prin reducerea ratei de formare a tesutului osos si cresterea apoptozei osteoblastelor si osteocitelor.

În concluzie, actual am putea spune (pentru România) că TZD-urile sunt o clasă de medicație cu un singur reprezentant și că scad HbA1c cu 1,5-1,6%, cresc sensibilitatea țesuturilor hepatic, adipos și muscular pentru insulina endogenă și exogenă, scad atât glicemia bazală, cât și pe cea postprandială. Ameliorează profilul lipidic, nu determina hipoglicemie. Nu s-au demonstrat interacțiuni medicamentoase în pofida unei legări plasmatice de 99%. TZD-urile au un cost ridicat.

Glinidele

În practica medicală se folosesc repaglinida și nateglinida, acestea au efect secretagog betacelular rapid și durată scurtă de acțiune.

Mecanism de actiune:

Glinidele sau analogii de meglitinide (repaglinida și nateglinida) stimulează secreția de insulină prin mecanisme similare cu sulfonilureicele. Diferența între cele 2 clase constă în rapiditatea și durata stimulării insulinosecreției. Pot fi asociate cu biguanidele, tiazolidindionele sau insulina bazală. Datorită timpului scurt de acțiune, reduc mai ales glicemia postprandială și ar trebui administrate înainte de fiecare masă sau mai ales înainte de masa principală.

Glinidele stimulează secreția betapancreatică de insulină printr-un mecanism dependent de nivelul glucozei, închid canalele de potasiu ATP-dependente, determinând depolarizarea membranei, deschiderea canalelor de calciu voltaj dependente și creşterea influxului de calciu intracelular, urmată de eliberarea de insulină. Se elimină la nivel urinar în proporţie de 10%, metabolizându-se preponderant hepatic. Reduc HbA1c cu 0,5-1,5%, stimulează eliberarea de insulină şi scad glicemia postprandială, efectul este rapid determinând câştig ponderal. Fixarea pe receptorul betacelular este diferită, ceea ce pledează pentru un mecanism de acţiune diferit al celor doi compuşi. Secreţia de insulină indusă de glinide apare precoce dupa administrarea tratamentului şi revine la nivelul bazal iniţial înainte de următoarea administrare.

***Tabel 4.* Glinidele – date generale**

Substanța activă	Structura	Clasa	Debutul acțiunii	Concentrația plasmatică maximă	Timp de înjumătățire	Eliminare
Nateglinida	Sulfonilureica	Derivat al aminoacidului D-fenilalanina	<1 oră	În 30 min	60 min	Rapidă Renală
Repaglinida	Benzamidica	Derivat de meglitinida Primul reglator prandial al gliemiei	10 min	În 50 min	60 min	Rapidă Renală

Cele două interacționează nesemnificativ cu canalele de K+ dependente de ATP de la nivelul miocardului și de la nivelul musculaturii netede vasculare, astfel că nu interferă cu procesul de precondiționare ischemică. Din date clinice s-a demonstrat că repaglinida are o eficiență hipoglicemiantă apropiată de cea sulfonilureicelor din generația a doua putând contribui la reducerea cu 1,5-2% a HbA1c în tratamentul pacienţilor cu T2DM. Medicamentul se administrează după un program, ţinând cont de mese, omiterea unei mese este asociată cu omiterea prizei respective de repaglinida. Nateglinida stimulează secreţia de insulină pe durată scurtă, dependent de prezenţa glucozei şi induce o creştere rapidă a insulinemiei plasmatice.

***Tabel 5*. Glinidele – date generale - continuare**

Substanța	Mod de administrare	Doza totală	Nr de prize	Toleranta	Crestere in greutate	Risc hipoglicemie	Efecte secundare digestive
Repaglinid	0-30 min inainte de masa	1,5-16 mg	3 asociata meselor principale	Buna	Usoara	Redus	Reduse
Nateglinid	1-30 min inainte de masa	180 -360 mg	3 la mesele principale	Buna	Ușoara	Redus	Reduse

***Tabel 6*. Glinidele - date farmacologice**

Substanta activa	Varf de actiune (ore)	Timp de injumatatire (ore)	Durata maxima de actiune(ore)	Scaderea HbA1c
Repaglinid	1	1	4-6	1,5-2%
Nateglinid	0,5	1	4	0,51%

Indicații: la pacienții cu DZ2 insuficient controlat prin dieta, exercițiu fizic si/sau metformin.

Contraindicații: DZ1, cetoacidoza diabetică, sarcina și alăptarea, insuficiența hepatică și renală, hiperglicemii majore (HbA1c>10%), traumatisme severe, intervenții chirurgicale majore, hipersensibilitatea la produs.

Reacții adverse: hipoglicemia, în special la vârstnici și cei cu afectare hepatică sau renală, creștere în greutate, tulburări gastrointestinale, hipersensibilitate cutanată, creșterea enzimelor hepatice. Preparatele nu formează metaboliți activi și nici nu determină interacțiuni medicamentoase prin intermediul citocromului P-450. C. Costul tratamentului cu glinide este unul intermediar.

Inhibitorii de SGLT2

Inhibarea co-transportorilor de glucoză-sodiu (SGLTs), care au un rol esențial în reabsorbția glucozei la nivel relal, a fost propusă ca o strategie terapeutică pentru DZ2. Pragul pentru reabsorbție este 180mg/dl.

Mutații genetice în SGLT2 (izoforma rinichi-specifică), care duce la glicozurie renală benignă, precum și studiile preclinice și clinice cu inhibitori de SGLT2 în DZ2 susțin potențialul acestei abordări. Aceste investigații arată că o crestere a excreției renale a glucozei prin suprimarea SGLT2 poate reduce nivelul glucozei plasmatice și determină scăderea în greutate. Cu toate că datele din studiile fază III, studii în curs de desfășurare ale acestor agenți sunt necesare pentru a evalua mai bine profilul de siguranță, rezultatele actuale sugerează că efectele benefice ale inhibării SGLT2 pot fi realizate fără a exercita efecte secundare semnificative.

Glucoza circulă în întregul flux sangvin, este filtrată de către rinichi și revine în organism prin intermediul acestor transportori glucozo–specifici la nivel renal. Prin blocarea reabsorbției glucozei, aceasta este excretată în urină. În ciuda respingerii inițiale a dapagliflozinului și a asocierii (efect de clasă) cu infecțiile urinare, mai multe companii sunt în curs de dezvoltare a acestor inhibitori de SGLT2 – exemplu Astelas Pharma INC care a depus recent aprobare pentru ipragliflozin si Boehringer Ingelheim impreuna cu Eli Lilly Company care a depus aprobare pentru empagliflozin.

***Tabel 7*. Studii pentru inhibitorii de SGLT2.**

Status	**ClinicalTrials.gov identifier**		
Dapagliflozin (BMS-512148	Bristol-Myers Squibb/AstraZeneca	Phase III	NCT01055691; NCT00643851; NCT00528372; NCT01095666; NCT01095653; NCT00673231; NCT00528879; NCT00660907; NCT00680745
Canagliflozin (TA-7284, JNJ-2843175	Johnson & Johnson/Mitsubishi Tanabe Pharma	Phase III	NCT01064414; NCT01081834; NCT01106625; NCT01106677; NCT01106690; NCT01106651; NCT01032629; NCT00968812
ASP-1941	Astellas/Kotobuki	Phase III	NCT01054092; NCT01057628
BI-10773	Boehringer Ingelheim	Phase II	NCT01011868
BI-44847	Boehringer Ingelheim (under license from Ajinomoto)	Phase II	Not applicable
TS-071	Taisho Pharmaceutical	Phase II	Not applicable
CSG-452 (R-7201, RG-7201)	Roche/Chugai Pharmaceutical	Phase II	Not applicable
LX-4211	Lexicon Pharmaceuticals	Phase II	NCT00962065
DSP-3235 (KGA-3235, GSK-1614235, 1614235)*	GlaxoSmithKline/Dainippon Sumitomo (under license from Kissei Pharmaceuticals)	Phase I	NCT00976261
ISIS-SGLT2Rx (ISIS-388626	Isis Pharmaceuticals	Phase I	NCT00836225
Unknown	Sirona Biochem (under license from TFChem)	Discovery	Not applicable

Sechestranții de acizi biliari

Colesevelamul este deja prezent în piața internațională din 2008. A fost indicat inițial în scăderea LDL-colesterolului, apoi s-a observat că scade foarte mult HbA1c, realizându-se studii și în această direcție. Nu este medicație de primă linie, este asociat medicației insuficiente (MTF și SU) cu un impact de 0,5 puncte procentuale pe scăderea HbA1c. Glicemia scade cu aproximativ 13-15% după folosirea timp de 24 săptămâni a acestui tip de medicație, fiind adresat pacientului cu DZ2 și cu LDL colesterol ridicat.

Mecanismul prin care acționează nu este clar cunoscut, teoretizându-se că sechestranții de acizi biliari intervin în calea metabolismului entero-hepatic al bilei, plauzibil fiind deoarece se consideră că pacienții cu DZ2 au un metabolism deficient al acesteia. Al doilea mecanism propus este creșterea indirectă a eliberării de incretine, cu acțiune imediată pe ambele faze ale eliberării de insulină. Efectele secundare ale aceste medicații (constipația, greața, voma) susțin cele două mecanisme propuse.

Medicația este dozată sub forma de capsule (625mg) administrându-se de 3 ori/zi, până la 6 capsule. În România nu este încă disponibil acest tip de medicație.

Alte clase de medicație în DZ2

Derivații de amilină (pramlintid)

Această clasă de medicamente a fost aprobată de FDA în 2005. Amilina este un hormon co-secretat cu insulina, absent în DZ1 și mult redus în DZ2. Pramlintid-ul este un medicament asemănător amilinei, având substituiți mai mulți aminoacizi. Pramlintid-ul are o acțiune similară cu a amilinei, având trei efecte majore: încetinește golirea gastrică, reduce eliberarea de glucagon din pancreas, acționează central stimulând centrii sațietății. Se administrează injectabil și de aceea nu face obiectul acestei expuneri.

Analogii de GLP1

Incretina (glucagon like peptid 1=GLP1) este un hormon sintetizat și secretat de celulele L ale ileonului si colonului. GLP1 endogen este degradat în câteva minute de către dipeptidil peptidaza 4 (DPP4-). Analogii de GLP1 sunt derivați din saliva unei șopârle (Gila Monster) și rezistă mult mai bine în fața enzimei DPP-4. Are multiple locații pentru acțiune: încetinește golirea gastrică, acționează central asupra centrilor sațietății, scade eliberarea de glucagon, ducând în final la scădere ponderală. Se administrează injectabil și de aceea nu face obiectul acestei expuneri.

Fitoterapia – în tratarea DZ2

Există unele plante cu acțiune hipoglicemiantă certă pentru care nu s-au facut studii clinice să se încerce dovedirea eficacității de-a lungul timpului sau impactul asupra factorilor de risc cardiovasculari, dar acestea sunt folosite ca extracte, decocturi, ceai-uri fiind promovate ca agenți hipoglicemianți cu o putere de scădere a glicemiei de 10-15mg/dl și a Hb1c de 0.2-0.5%. Acestea nu se încadrează în noțiunea de medicație orală, dar sunt preferate de pacienți și de unii dintre medici, fără a avea un suport științific solid.

Se cunosc următoarele plante folosite în terapia inițială sau concomitentă a DZ2: rădăcina proaspătă de brusture (decoct preparat din 60 g rădăcină la un litru de apă), frunzele de afin (conțin mirtyllina), frunzele de nuc (diminuează glicozuria și scad setea; infuzie), frunzele de dud negru (diminuează glicemia), frunzele de anghinare (infuzie), rădacina și frunzele de cicoare (stimulează depozitarea glicogenului la nivel hepatic, infuzie), tecile de fasole (decoct), scaiul (hipoglicemiant ușor, suc proaspăt), frunzele de salvie (macerat la rece).

Preparatele fitoterapice nu sunt recunoscute ca fiind eficiente în lupta cu complicațiile DZ2, astfel că – pentru moment – acestea constituie în fapt opțiuni ale pacientului în tratarea diabetului zaharat.

Concluzie

Tratamentul diabetului zaharat dispune astăzi de o multitudine de medicamente/clase terapeutice care acționează optim atunci când are loc o evaluare inițială a situației pacientului prezentat. Eșecul terapeutic al unei clase deschide calea folosirii unei alteia sau dechide calea pentru insulină. Este relativ dificil ca pacientul să înțeleagă un algoritm prezentat și corect aplicat, dar este de dorit ca peste 20 de ani să nu prezinte complicații majore ale diabetului zaharat deja instituite. Prezentarea unui raționament relativ simplu pentru alegerea acestor grupe de medicație va fi făcută într-un număr viitor al revistei.

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Medicii militari – în elita ştiinţifică a României

Military Physicians – in Romanian scientific elite

Dan Mischianu, Robert Popescu
Clinica de Urologie, Spitalul Universitar de Urgenţă Militar Central „Dr Carol Davila”,
Universitatea de Medicină şi Farmacie „Carol Davila” Bucureşti

Motto: ***„Spiritus flat ubi vult”,***
Evanghelia lui Ioan 3,8.

Rezumat

Articolul prezintă succint biografiile unor medici militari români a căror instrucție universitară a fost desăvârșită în cadrul Universității de Medicină și Farmacie București iar cea militară în cadrul Institutului Medico-Militar.

Aceștia au ajuns, mai apoi, membri ai Academiei Române, cel mai înalt for științific din România.

Se doreşte, în acest fel, o resuscitare a memoriei comunității medico-universitare române cu scopul de a nu-i uita pe cei care, cu adevărat, au impulsionat știința și cercetarea medicală și medico-militară română.

Cuvinte cheie: Medici militari, UMF „Carol Davila“, SUUMC „Dr. Carol Davila“, Elita științifică, Elita academică, Institutul Medico-Militar, Academia Română.

Abstract

This article presents brief biographies of Romanian military physicians whose academic training was completed at the University of Medicine and Pharmacy in Bucharest and military training at the Military Medical Institute.

They later became members of the Romanian Academy, the highest science forum in Romania.

This article aims at the refreshing of the memory of the Romanian academic medical community in order to never forget those who have truly assisted in the development of the Romanian medical and military medical science and research.

Keywords: Military physicians, UMF „Carol Davila“, SUUMC „Dr. Carol Davila“, Scientific elite, Academic elite, Military Medical Institute, Romanian Academy

Introducere

Dicționarul explicativ al limbii române (DEX) precizează pentru noțiunea de elită următoarele: grup de persoane care reprezintă ceea ce este mai bun, mai valoros într-o comunitate, într-o societate; ceea ce este mai bun, mai demn de a fi ales.

Problema elitelor din România a făcut obiectul unor prezentări recente începând cu tendința de globalizare a acestui fenomen [1], continuând cu studiul ei aprofundat [2] și ajungând până la prospecțiuni asupra viitorului [3].

Din informațiile ce ne-au stat la dispoziție [4] considerăm că este nimerit să prezentăm o situație de fapt: de-a lungul a 147 ani de existență cât numără Academia Română, „organismul“ științific cel mai reprezentativ al națiunii noastre și, de fapt, al oricărei entități naționale, sub cupola sa de nemuritori au fost aleși și ne-au reprezentat pe noi, ca breaslă, cu munca, cu realizările și cu beneficiul gândirii lor un număr de 22 de absolvenți ai Institutului Medico-Militar și de „foști“ medici militari. Trebuie să recunoaștem, cu absolută înțelegere, că, la un moment dat, toți vom deveni niște „foști“!

Medici militari academicieni

Medicii militari români care au înscrise numele în elita ştiinţifică a ţării noastre sunt:

1. **Col.Dr. Iacob Cihac (1800-1887)** – membru corespondent al Academiei Române din 1872
2. **Gral. Prof.Dr. Zaharia Petrescu (1841-1901)** - membru corespondent al Academiei Române din 1885
3. **Prof.Dr. Constantin C. Istrati (1850-1918)** – membru corespondent din 1889, membru titular 1899, Președinte al Academiei Române 1913-1916
4. **Lt.Col. Prof.Dr. Dimitrie Grecescu (1841-1910)** – membru corespondent 1902, membru titular 1907
5. **Col. Prof.Dr. docent Mihai Ciucă (1883-1962)** – membru titular 1938
6. **Dr. veterinar George N. Udrițchi (1867-1958)** – membru de onoare din 1946
7. **Lt.Col. Prof.Dr. docent Dumitru Combiescu (1887-1961)** – membru corespondent din 1946, membru titular 1948
8. **Mr. Prof.Dr. Dumitru Bagdasar** – membru postmortem din 1948
9. **Col. Prof.Dr.docent Ștefan Milcu (1903-1997)** – membru titular al Academiei din 1948, vicepreședinte al Academiei Române
10. **Prof.Dr Radu Vlădescu** – membru titular din 1955
11. **Lt.Col. Prof.Dr.docent Ion Țurai (1907-1970)** – membru corespondent din 1963
12. **Prof.Dr. Vancea Petre** – membru corespondent din 1963
13. **Gral. Prof. Dr. Scarlat Longhin (1889-1979)**, membru corespondent din 1963
14. **Col. Prof.Dr.docent Nicolae Nestorescu (1901-1969)** – membru corespondent din 1963
15. **Lt.Col. Dr. Ștefan Odobleja** – membru postmortem 1990
16. **Col. Prof.Dr. docent Gheorghe Zarnea** – membru corespondent 1991,
17. **Gral. Mr. Prof. Dr. Victor Voicu** – membru corespondent 1991, membru titular 2001, secretar general al Academiei Române.
18. **Gral.Bg. Prof.Dr. docent Mircea Olteanu** – membru de onoare 1992
19. **Gral.Mr. Prof. Dr. docent Gheorghe Niculescu** – membru de onoare 1992
20. **Lt.col. Prof.Dr. docent Eugeniu Proca** – membru de onoare 1992
21. **Gral. Prof.Dr. Carol Davila** – membru postmortem din 2003
22. **Prof.Dr. Ioanel Sinescu** – membru corespondent din 2009, membru titular din 2011.

Considerăm că este bine meritat să adăugăm la această listă și numele unui „constructor“ și totodată vizionar în ceea ce privește specialitatea căreia i s-a dedicat cu pasiune:

23. **Gral. Prof.Dr. Vasile Cândea** – membru titular fondator și Președinte al Academiei Oamenilor de Știință din România și membru titular al Academiei de Științe Medicale.

Aproape toți au câteva date evolutive comune: s-au făcut remarcați de către maeștrii lor încă din studenție, probând mai apoi, de-a lungul timpului, interes constant pentru specialitățile lor medicale. Cu toți au parcurs treptele firești ale ierarhiei universitar-academice, și-au susținut onorante doctorate (originale și serioase prin investigațiile întreprinse), au făcut cunoscute inițiative personale inedite, abordând, cel mai adesea, aspecte îndelung exersate, care cu siguranță nu au fost sesizate de confrații din domeniul lor. S-au impus exemplar la Catedră, la Clinică, prin ceea ce au scris și publicat, precum și la Academia Română.

Scurte date biografice
Vom face scurte referiri privind câțiva dintre aceștia:

Gral. Prof. Dr. Academician p.m. Carol Davila

Despre Carol Davila s-a scris o întreagă literatură. El este părintele fondator al medicinei militare și civile românești, „Întemeietorul" **!** S-a născut în anul 1828 în Parma, Italia primind numele de Carlo Antonio Francesco d'Avila (în franceză Charles d'Avila). A studiat la Paris absolvind Facultatea de Medicină în februarie 1853. În ziua de 13 martie 1853 ajunge în Țara Românească la invitația Domnitorului Barbu Știrbei cu scopul de a organiza serviciul sanitar român. Cândva chiar a afirmat: „Găsind pe meleagurile Dunării o nouă patrie, România, o Franță a orientului, cu ideile, tradițiile și aspirațiile ei de civilizațiune ale surorii celei mari, am devenit cu inima și în faptă cetățean român cu îndoită iubire pentru patria de origine" [5].

În anul 1855 înființează o școală de felceri și în 1856 școala de chirurgie care la scurt timp se transformă în „Școala națională de medicină și farmacie"[6].

În 1860 a fost ridicat la gradul de General de către Domnitorul Alexandru Ioan Cuza.

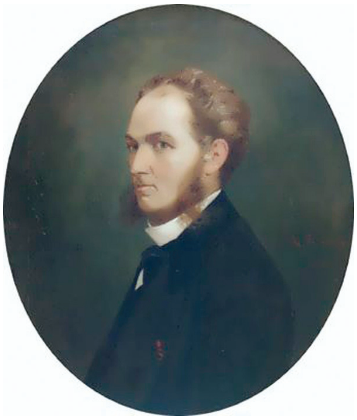

În 1861 împreună cu iubita sa soție Anica Davila organizează primul azil pentru 40 de orfane urmat de un orfelinat de băieți și de o școală de surdo-muți ce cuprindea și un atelier de tâmplărie și de sculptură [7].

În anul 1869 înființează Facultatea de Medicină. Întemeiază învățământul farmaceutic (ajutat de farmacistul Hepites) și împreună cu horticultorul austriac Ülrich Hoffmann pune bazele Grădinii Botanice din București.

A fost fondatorul a numeroase reviste și societăți din care enumerăm *Asociația medicilor români, Monitorul medical, Gazeta spitalelor*, organizatorul unor conferințe și cel ce a introdus concursurile medicale și stagiile obligatorii în spitale.

Pe 31 mai 1868 printr-un decret-lege semnat de regele Carol I primește cetățenia română [7].

A avut, de asemenea, o activitate masonică deplin recunoscută și apreciată. A fost inițiat în ziua de 4 iunie 1874 în Loja „Înțelepții din Heliopolis" (București) pentru ca, la scurt timp, să fie ales Maestru Venerabil al acestui atelier.

Între 1879-1884 a fost primul Pro Mare Maestru al Marelui Orient al României [8].

A încetat din viață în anul 1884.

În anul 2003 a devenit post-mortem membru al Academiei Române.

Maior. Acad. Dimitrie Bagdasar

S-a născut la 17 decembrie 1893 în satul Idrici de Jos, comuna Roșiești-Fălciu (jud. Vaslui) într-o familie cu 12 copii. După ce a terminat școala primară în satul natal a urmat cursurile Liceului „Gh. Roșca Codreanu" din Bârlad iar în 1913 este admis la Facultatea de Medicină din București. Din anul al III-lea de facultate, îndemnat de Gral.dr. Mihail Butoianu - distins chirurg militar și șef al Secției Chirurgie I - intră la Institutul Medico-Militar, devenind locotenent-medic în 1922. În perioada următoare lucrează ca intern și secundar în Clinica de Chirurgie a SMC dar și în cea de Neurologie, condusă de viitorul Prof.dr. Dimitrie Noica. Între anii 1919 și 1927 a aprofundat pregătirea neurologică în Spitalul Colentina alături de Prof. Dr. Gheorghe Marinescu. A părăsit cadrele militare active în noiembrie 1926. În anul 1927, cu ajutorul unei burse J.D. Rockefeller a efectuat un stagiu de pregătire de doi ani la Boston, Statele Unite ale Americii alături de fondatorul specialității neurochirurgicale, Profesorul Dr. Harvey William Cushing [9]. Reîntors în țară efectuează primele operații neurochirurgicale la Spitalul Jimbolia unde a profesat între anii 1929 și 1931. Dimitrie Bagdasar va conduce între 1931 și 1933 serviciul de neurologie din Cernăuți pentru ca în anul 1934 să revină în București, la Spitalul de Urgență. În 1935 cu sprijinul profesorilor Ion Iacobovici, Nicolae Minovici și Ion T. Niculescu înființează la Spitalul Central de Boli Nervoase și Mintale un mic serviciu de neurochirurgie cu opt paturi și o singură sală de operație, serviciu care va deveni nucleul Clinicii de Neurochirurgie a Facultății de Medicină și unde se va dezvolta școala românească de neurochirurgie.

Devine Conferențiar (1937-1945) și mai apoi primul Profesor de neurochirurgie din țara noastră (1945).

A ajuns Ministrul Sănătății la 6 martie 1945, în guvernul Petru Groza.

S-a stins din viață prematur, în ziua de 15 iulie 1946, la 53 de ani.

Este membru post-mortem al Academiei Române din anul 1948.

Col. Acad. Ștefan Marius Milcu

Ștefan Marius Milcu s-a născut în 15 august 1903 la Craiova

A învățat la școala primară din orașul Calafat între anii 1910-1914. Cu siguranță, absolut din întâmplare, unul din autorii acestui articol a locuit în aceiași casă din orașul în care a învățat și viitorul Academician.

Urmează cursurile Liceului Traian din Turnu Severin și ale Colegiului Carol I, astăzi Liceul „Nicolae Bălcescu“, iar în iunie 1922 își susține examenul de bacalaureat cu dizertația *Originea omului după Darwin.*

A intrat la Institutul Medico-Militar (1922) și fiind remarcat de profesorul de anatomie Francisc Rainer ajunge preparator voluntar (1923-1927) și asistent în anul 1935 la catedra de embriologie, catedră nou înființată.

În 1928 își susține examenul de doctorat în medicină și chirurgie.

În anul 1929 își dă demisia din armată. Evoluția sa pe linie universitară s-a desfășurat astfel: în anul 1940 devine Șef de lucrări, în 1946 Conferențiar și în 1948 Profesor. Este numit Decan al Facultății de Pediatrie, iar în perioada 1953-1955 este Rector al Facultății de Medicină. A deținut și funcția de director adjunct (1946-1949) și apoi de director (1969-1978) al Institutului de Endocrinologie.

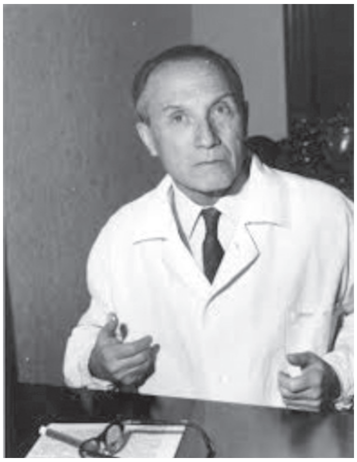

Elev și colaborator apropiat al lui Constantin I. Parhon, a devenit un reprezentant de frunte al școlii românești de endocrinologie. S-a dedicat cu pasiune studiului endocrinologiei publicând monografii recunoscute privind glandele epifiză și timus.

A adus contribuții importante la studiul gușii endemice fiind organizatorul programului de protecție în această patologie. A pus bazele teoretice și organizatorice ale chirurgiei endocrine și a întreprins cercetări în domeniul antropologiei, fiind un activ continuator al maestrului său, profesorul Francisc Rainer.

Secretar general al Academiei Române (1955-1963), devine în 1964 vicepreședinte al acestei

prestigioase instituții, funcție pe care o exercită până în 1971. În 1971 este ales Președinte al Secției de Științe Medicale a Academiei.
În 1964 devine membru al Academiei de Științe Medicale, pentru ca în 1990 să devină președinte al acestei Instituții. Devine Președinte al Comitetului de Istoria și Filosofia Științei din Academia Română, funcție pe care o exercită în perioadele 1973-1982 și 1994-1997.
A decedat la 1 decembrie 1997.

Lt.Col. Acad. Eugeniu Proca

Academicianul Eugeniu Proca s-a născut la 12 ianuarie 1927, la Godeni județul Argeș. A absolvit Liceul Dinicu Golescu din Câmpulung-Muscel în 1945 și a urmat cursurile Facultății de Medicină Militară din București (1945-1949). În anul 1950 a fost exclus din Institutul Medico-Militar pentru că a aprins focul din sobă cu ziarul „Scânteia“ – ziarul Partidului Comunist. Delatori existau și atunci!... A continuat studiile la Facultatea de Medicină din București fiind șef de promoție (1951). La terminarea facultății, deoarece își dorea foarte mult să fie chirurg, a ales singurul post cu specific chirurgical, la Spitalul din Comănești. În 1953 intră ca asistent universitar prin concurs, la Spitalul Panduri din București, devenind astfel elev al profesorului dr. Theodor Burghele, cel care i-a influențat hotărâtor cariera.
În 1957 își susține teza de doctorat cu titlul *Insuficiența Renală Acută.*
În perioada 1953-1975 își desfășoară activitatea în cadrul Clinicii Chirurgicale și de Urologie din Spitalul Panduri. Parcurge etapele carierei universitare ca asistent, șef de lucrări, conferențiar și, mai apoi, profesor universitar (1973).
Efectuează prima hemodializă din România (7 aprilie 1959).
Urmează un stagiu de pregătire în chirurgia de transplant renal la Institutul de Urologie din Londra (1963-1964) și un stagiu de 6 luni la Harvard Medical School, Boston, Statele Unite ale Americii în 1967.

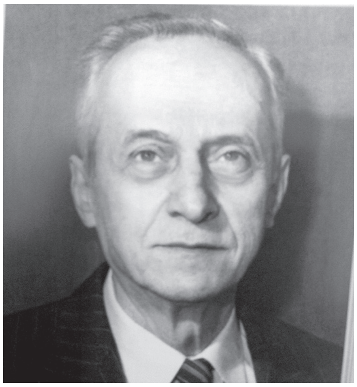

În 1975, după decesul Profesorului Dr. Gh. Olănescu, devine șeful Clinicii de Urologie a Spitalului Fundeni. Activitatea de la Fundeni este încununată de o premieră absolută pentru medicina din România – primul transplant reușit al unui organ parenchimatos la om – rinichi de la donator în viață (mama receptorului), transplant efectuat în februarie 1980.
A fost Rector al Institutului de Medicină și Farmacie din București între anii 1976-1978, Ministru al Sănătății timp de șapte ani, între 1978 și 1985, și Presedintele Comisiei de Urologie a Ministerului Sănătății (1990-1995).
În anul 1995 reînființează Societatea Română de Urologie, care fusese desființată în 1945, al cărei președinte a fost până în anul 2000, când a devenit președinte de onoare. A reînființat și Revista Română de Urologie.
Profesorul Proca a participat la realizarea a 19 monografii, a fost redactor al Tratatului de Patologie Chirurgicală și autor a două volume de Urologie din Tratatul de Chirurgie.
A fost membru titular al Academiei de Științe Medicale și membru de onoare al Academiei Române.
A încetat din viață la 7 martie 2004.

Gl. lt. Acad. Gheorghe Niculescu

Doctorul Gheorghe Niculescu s-a născut la 28 aprilie 1923 la Craiova și a trecut în neființă la 21 mai 1995 la București. Nu îi plăcea și nu dorea să fie numit decât cu acest titlu: „Doctorul“!
A înființat Secția de Ortopedie și Traumatologie din Spitalul Militar Central în 1959 prin trans-

formarea secției Chirurgie III, i-a dat statutul de Clinică universitară în 1984 și a condus-o cu profesionalism și devoțiune până în anul 1991. A fost un spirit constructiv și un vizionar.

Avea o ușurință în scris absolut admirabilă, ușor contrastantă cu exprimarea verbală. Printre multe cărți și tratate pe care le-a scris și publicat rămâne de referință prin originalitatea abordării și înțelesurile practice ce decurg din lectura ei *„Traumatismele membrelor. Atlas schematic de tehnici operatorii“* (1973), lucrare distinsă cu Premiul „Gheorghe Marinescu“ al Academiei Române.

Nu trecea o săptămână ca să nu ne îndemne, pe cei care îi simțea apropiați și capabili întru aceasta „să punem pe hârtie ceva!“ Era adeptul absolut al dictonului : „Nulla dies sine linea!“

Practic, a fost un om vertical și dotat cu o smerenie constituțională fără de care, o viață bogată în realizări și considerată a fi împlinită, nu e completă.

Gral. Acad. Vasile Cândea

S-a născut la 24 mai 1932, în comuna Lisa-Vânători (acum Viișoara), județul Teleorman. După ce a absolvit cursurile Facultății de Medicină Generală ale Universității de Medicină și Farmacie „Carol Davila“ din București în 1957 a profesat ca medic de medicină generală până în 1959. Următorii doi ani a fost medic militar de unitate la trupele de grăniceri; între 1961 și 1971 a practicat chirurgia generală la Spitalul Militar Central.

A ocupat următoarele funcții: Șef secție Chirurgie Cardiovasculară la Spitalul Militar Central (1976-1990); Director al Centrului de Boli Cardiovasculare al Armatei (1990-1995); Director al Institutului de Boli Cardiovasculare „Prof.Dr. C.C. Iliescu“ București și Șef Clincă II Chirurgie Cardiacă.

În anul 1994, devine profesor titular, prin concurs. Din 2002 este profesor consultant la UMF Carol Davila.

A participat la redactarea a peste 750 de lucrări științifice ce au fost comunicate în reviste de specialitate din țară și de peste hotare, la congrese și simpozioane naționale sau internaționale.

A fost inițiatorul unor noi tehnici și procedee în chirurgia cardiovasculară. A întreprins o serie de cercetări științifice legate de șocul traumatic și contribuția sistemului limfatic în șoc. A efectuat primele limfografii cervicale în România.

În urma colaborării cu Prof. Noirhomme de la Spitalul Saint Luc din Bruxelles a efectuat cu succes implantarea primei inimi artificiale autonome de tip NOVACOR din Sud-Estul Europei.

Este membru titular fondator și Președinte al Academiei Oamenilor de Știință din România și membru titular al Academiei de Științe Medicale. Este de asemenea Fondatorul Centrului de Boli Cardiovasculare al Armatei „Acad. Vasile Cândea“

Gral.mr. Acad. Victor Voicu

S-a născut la 29 iunie 1939 în comuna Bolovani, județul Dâmbovița. Este medic și farmacolog, Profesor Universitar, Șeful Catedrei de Farmacologie Clinică, Toxicologie și Psihofarmacologie

a Universității de Medicină și Farmacie „Carol Davila“, București din 1992.
A ocupat următoarele funcții: Director al Centrului de Cercetări Stiințifice Medico-Militare, Președintele Consiliului Științific al Agenției Naționale a Medicamentului, Președintele Societății Române de Farmacologie, Terapeutică și Toxicologie Clinică (1998).
A fost Şef al Direcției Medicale a Armatei (1990-1995), într-o perioadă de schimbări semnificative pentru breasla medicilor militari români.
A efectuat numeroase cercetări științifice cu predilecție în domeniul compușilor organofosforici și ai antidotului acestora. A studiat și farmacologia radioprotectorilor chimici, farmacologia experimentală și clinică a antihipertensivelor și antiartimicelor, psihofarmacologia psihotomimeticelor și a drogurilor de abuz.
Este coordonator pentru numeroase studii clinice de fază I și studii de bioechivalență.
În ceeace privește cariera editorială este autorul a peste 20 de volume având și nenumărate lucrări științifice publicate și comunicate (peste 290).
Este posesorul a 18 brevete de invenție.
General maior și membru titular al Academiei Române din 2001.

Acad. Prof. Dr. Sinescu C.Ioanel

S-a născut la 8 decembrie 1951 în comuna Movileni, județul Iași. A urmat cursurile Institutului Medico-Militar (promoția 1977, șef de promoție). Între anii 1976 și 1979 a fost intern în grupa specialităților chirurgicale dupa care asistent universitar stagiar-preparator (1979-1983) și asistent universitar medic specialist - 1983.
Şef de lucrări și medic primar urolog - 1990, Conferențiar universitar - 1993, Şef Clinică Urologie - 1997, Profesor universitar - 1998, Prorector al Universității de Medicină și Farmacie „Carol Davila“, București (2004-2012), Rector al aceleiași Universități din 2012, membru corespondent al Academiei Române - 2009 și membru titular al aceluiași for suprem din 2011.
Academicianul Ioanel Sinescu este președinte al Asociației Române de Urologie din 1999.
Locotenent medic în 1975, locotenent major medic în anul 1980, a trecut în rezervă la cerere în 1983.
Liderul de necontestat al medicinii clinice din România actuală, continuator la nivel mondial al predecesorului său - Academicianul Eugeniu Proca în activitatea de transplant renal de la noi (peste 1300 de cazuri în 16 ani), având în CV-ul său peste 600 de lucrări științifice, numeroase monografii și tratate din care este de remarcat Tratatul de Urologie – 2008 - adevărat „*Campbell românesc*“, adică „Biblia“ urologilor români.

Academicianul Ioanel Sinescu este un exemplu de muncă neostoită, de putere de luptă, de profesionalism și înțelegere în a aborda întotdeauna corect greutățile și provocările vieții.

În loc de încheiere

Am dorit să prezentăm câte ceva din puținul pe care îl cunoaștem privind biografiile unor oameni pe care noi îi considerăm emblematici pentru breasla medicală militară și care au un numitor comun - provin din aceeași „sușă“ numită Institutul Medico-Militar.

Am întreprins acest demers cu respect față de dascălii noștri, față de toți cei de la care am învățat ceva privind meșteșugul meseriei noastre, considerând că respectul față de *elita medicinii militare române* nu trebuie să aibă limită și acest lucru trebuie exprimat în modul cel mai clar cu putiință.

Având, aproape toți, o ascendență ce nu prevestea poziția științifică înaltă la care au ajuns, aceasta este încă un argument că *motto*-ul are perfectă dreptate: „apariția spiritului, talentului și imaginației nu depinde de nicio lege!“ [10]

Am reținut atenția cititorului cu biografiile acestor iluștri medici militari ajunși în poziții înalte ale cercetării și științei în țara noastră, nutrind speranța că având exemplul lor, noi, cei de azi, vom fi mai motivați, mai energici, mai productivi, mai buni.

Dar cum se zice: *speranța moare ultima!.....*

„Fiecare loc are o istorie a sa, dar îți trebuie puțin timp ca să o cunoști și puțină dragoste ca s-o înțelegi“ spunea Nicolae Iorga. Cam așa este și cu Spitalul Militar Central. Când este vorba despre oamenii eminenți care și-au început desăvârșirea în acest așezământ de sănătate însărcinarea este foarte grea, fiindcă nu dorești să neîndreptățești pe nimeni.
Sperăm că demersul nostru va fi înțeles pe deplin.

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[1] Petre T. Frangopol, *Globalizarea formării elitelor, combaterea provincialismului*, în Mediocritate și Excelență, o Radiografie a Științei și Învățământului din România, vol. 3, Casa Cărții de Știință, Cluj-Napoca, 77 (2008).

[2] Petre T. Frangopol, *Prestigiul IFA în știința și cultura românească*, în Mediocritate și Excelență, o Radiografie a Științei și Învățământului din România, vol. 4, Casa Cărții de Știință, Cluj-Napoca, 129 (2011).

[3] Petre T. Frangopol, *Horia Hulubei,* Revista de Politica Științei și Scientometrie – Serie nouă-, **1**(3), 178 (2012).

[4] Gral. brigadă (r) dr. Mircea Diaconescu (coordonator), *Oameni și fapte din istoria medicinii militare românești*, Editura Pro Transilvania, București, 2005, vol II, pg 154-155.

[5] Maria Manu Bădescu, *Călătoriile lui Carol Davila*, Editura Sport Turist, București, 1985, pg. 26

[6] Gheorghe Brătescu, *Tinerețea lui Carol Davila* editura Albatros, București, 1979

[7] Carol I al României, *Jurnal*, Volumul I, Editura Polirom, București, 2007, p. 573

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[10] Virgil Matei, *Dicționar de maxime, reflecții, expresii latine comentate*, Ed. Didactică și pedagogică R.A., București, 2007, pg.374.

Autor corespondent: danmischianu@yahoo.co.uk

45 de ani de activitate a Centrului de Cercetări Ştiinţifice Medico-Militare

ISTORIC ŞI REALIZĂRI

Colonel medic dr. Gabriel POPESCU
Centrul de Cercetări Științifice Medico-Militare, București

Centrul de Cercetări Ştiinţifice Medico-Militare (C.C.S.M.M.) a fost fondat la data de 31 octombrie 1968 când Consiliul de Miniştri a hotărât crearea unei instituţii noi sub autoritatea Ministerului Apărării, prin transformarea Centrului de radiobiologie şi biologie moleculară aflat în coordonarea Ministerului sănătăţii.

La înființare, personalul C.C.S.M.M. era constituit dintr-un nucleu de cercetători (printre care și medici militari) care activau în cadrul diferitor institute naționale de cercetare precum și din cercetători preluați din Centrul de radiobiologie.

Instituția a luat naștere într-un context politico-militar tensionat, începând de la criza rachetelor din Cuba (1961) și marcat de invazia Cehoslovaciei de către U.R.S.S. în august 1968, eveniment care a adus în centrul atenției problemele legate de protecția medicală a personalului militar și a populației civile împotriva efectelor armelor chimice, biologice și nucleare.

Organizarea C.C.S.M.M.

La înființare, centrul purta denumirea de „*Centrul de Radiobiologie*“ iar primul comandant a fost col. dr. Alexandru Popescu.

Structura organizatorică inițială cuprindea următoarele laboratoare: chimie organică, sinteze de compuşi organici, biochimie, bacteriologie şi inframicrobiologie, farmacologie şi fiziopatologie, explorări funcţionale, medicină nucleară, fiziopatologia bolii de iradiere, anatomie patologică.

Începând din februarie 1969 Centrul de radiobiologie a devenit Centrul de Cercetări Ştiinţifice Medico-Militare în cadrul căruia au fost constituite următoarele secţii şi laboratoare:

SECŢII ŞI LABORATOARE	ŞEF DE LABORATOR
Secția de radiopatologie clinică	**Lt.col.dr.Cioba Gh.**
Radiobiologie experimentală	**Lt.col.dr. vet. Cavulea Octavian**
Radiochimie	**Lt.col.farm.Bergheanu Sandrin-Seneca**
Virusologie	**Lt.col.dr. Strati Ion**
Epidemiologie	**Lt.col.farm.Grigoriu Celus**
Toxicologie şi farmacologie	**Lt.col.dr. Andrieş Aurel**
Farmacodinamică şi fiziopatologie	**Lt.col.dr. Mihăilescu C-tin**
Explorări psiho-fiziologice	**Lt.col.dr.Gheorghiu Dumitru**
Biochimie şi chimie fizică	**Col.farm. Bruja Nicolae**
Chimie biologică	**Lt.col.farm. Correni Eugen**
Sinteze de compuşi organici	**Lt.col.farm.Miu Constantin**
Hematologie şi citologie	**Lt.col.dr. Teodosiu Traian**

Conceput astfel încât să acopere problematica protecţei împotriva atacurilor cu agenţi CBRN, C.C.S.M.M. a suferit de-a lungul timpului mai multe reorganizări care au condus la o mai bună definire a componentei militare, la extinderea atribuţiilor la pace şi război şi la realizarea unei mai bune complementarităţi profesionale prin organizarea secţiilor şi laboratoarelor pe domenii care necesită abordări interdisciplinare**:** radiobiologie, microbiologie, toxicologie, psiho-fiziologie.

Prima reorganizare a dus la definirea principalelor laboratoare şi colective axate pe domeniile: chimic, biologic, radiologic şi nuclear, astfel:

- ***Laboratorul de toxicologie:***
  - Toxicologie
  - Sinteze chimice
  - Farmacodinamie

- ***Laboratorul de microbiologie:***
  - Bacteriologie
  - Epidemiologie
  - Virusologie şi imunologie
  - Biochimie

- ***Laboratorul de radiobiologie:***
  - Radiopatologie clinică
  - Dozimetrie biologică
  - Hematolgie şi anatomie patologică
  - Radiochimie

La acestea se adaugă Laboratorul de psihofiziologie, centrul de documentare ştiinţifică şi atelierul de prototipuri şi mentenanţă pentru echipamente medicale de cercetare.

Reorganizările structurale ulterioare au dus la o mai bună conturare a domeniilor de competenţe şi au pus bazele unor cooperări şi parteneriate cu instituţii semnificative militare şi civile din ţară şi străinătate.

Structură, atribuţii şi misiuni actuale

Secţia de protecţie antiinfecţioasă şi urgenţe epidemiologice:

- efectuează cercetări ştiinţifice în vederea protecţiei medicale a militarilor şi populaţiei civile împotriva armelor biologice, acţiunilor bioteroriste sau incidentelor şi accidentelor biologice;
- elaborează metode şi mijloace de diagnostic precoce, profilaxie şi principii de tratament;
- facilităţile de diagnostic sunt găzduite într-o

incintă cu nivel de biosecuritate P2+. Se află în curs de amenajare încă 2 laboratoare cu nivel P3.

Secția de toxicologie, farmacologie si protectie medicala impotriva STL are ca obiective:
- screeningul farmacologic pentru determinarea toxicității acute, subacute și cronice a agenților chimici;
- elaborarea de noi tehnici și metode de evaluare a antidoturilor potențiale, cu scopul determinării cu acuratețe a eficacității;
- elaborarea unor metode de evaluare a eficacității terapeutice și a toxicității potențiale a compușilor (evaluarea preclinică în vederea înregistrării);
- elaborarea unor tehnici și modele experimentale în domeniul toxicității acute a agenților chimici susceptibili a fi utilizați ca arme chimice;
- diagnosticul clinic și de laborator al intoxicațiilor acute la om în condițiile unei unități specializate de terapie intensivă în cadrul Spitalului de Urgență Floreasca din București;
- cercetări științifice în domeniul toxicologiei analitice;
- determinări cantitative și calitative a unor compuși considerați potențiale amenințări;
- studii de farmacodinamie.

Secția de radiobiologie și protecție împotriva radiațiilor ionizante

Obiective:
- elaborarea de metode și mijloace de intervenție în incidente sau accidente nucleare (inclusiv în caz de atac nuclear);
- metode de diagnostic și tratament al bolii de iradiere, a leziunilor combinate și/sau reanimare hematologică;
- algoritmi și tehnici standardizate de dozimetrie fizică, radiochimie și dozimetrie biologică în situații de accident/incident nuclear;
- radiobiologie experimentală;
- elaborarea de mijloace de radioprotecție cu scopul reducerii fixării și decorporării radionuclizilor;
- actualizarea metodelor de diagnostic și tratament al bolii de iradiere.

Secția de neuropsihofarmacologie experimentala. Obiective:
- abordarea la nivel celular și molecular a mecanismelor care pot influența statusul fizic și psihic al militarilor;
- identificarea mecanismelor și elaborarea de metode de prevenție și tratament împotriva acțiunii unor factori de stres cu efect asupra organismului uman care pot afecta factorul uman în diferite circumstanțe;
- cercetări privind efectul dozelor mici de compuți organofosforici.

Laboratorul de tehnică farmaceutică și biofarmacie:
- efectuează cercetări biofarmaceutice în vederea condiționării și stabilizării compușilor sau combinațiilor medicamentoase susceptibile a fi utilizate ca antidoturi.

Subordonarea și structura de personal

C.C.S.M.M. este subordonat ierarhic Direcției medicale a M.Ap.N. Structura cuprinde laboratoare de diagnostic și cercetare, subunități independente și formațiuni de servicii (resurse umane, activitate financiară).

În cadrul a două sectoare clinice (toxicologie și radiobiologie), concomitent cu activitățile de cercetare, se desfășoară activități medicale în parteneriat cu formațiuni spitalicești aparținând Ministerului Sănătății (Spitalul clinic de Urgență Floreasca și Institutul clinic Fundeni).

Secția de toxicologie clinică asigură pregătire universitară și postuniversitară de profil, personalul acestei secții constituind și nucleul *Catedrei de farmacologie, toxicologie și psihofarmacologie* din cadrul U.M.F. Carol Davila București.

În cadrul C.C.S.M.M. activează sau au activat o serie de cercetători prestigioși care desfășoară activitate didactică în cadrul universităților de medicină și farmacie:
- G-ral.mr.(r.) Prof. Victor Voicu, membru al Academiei Române
- Col. (r.) Prof. Farm. Constantin Mircioiu
- Prof.univ.dr. Radu Macovei
- Col. Prof. Mihai Hinescu
- Col.(r) Prof. Gheorghe Țărălungă

- Col. (r.) Viorel Ordeanu
- Col. șef de lucrări dr. Mihai Tudosie
- Șef de lucrări Dr. Dida Ardeleanu

De asemenea foști cercetători din cadrul C.C.S.M.M. activează în instituții medicale de prestigiu: Prof. Dr. Cezar Macarie, Prof. Dan-Dominic Ionescu, Col.dr.Meclea Gheorghe, Col. Dr. Mihai Ciochinaru, Col. Prof. Dr. Adrian Barbilian.

Personalități care au îndeplinit funcția de comandant al C.C.S.M.M.

1 decembrie 1968 – 1977	**Col. Dr. Alexandru Popescu**
1977 – 1978	**Col. Dr. Laurenţiu Dragomirescu**
1978 – 1981	**Col. Dr. Aurel Andrieş**
1982 – 1985	**Col. Dr. Tudor Toma**
1985 – 1987	**Col. Dr. Ion Urseanu**
1987 – 31 mai 2013	**G-ral. mr. (r) Prof. Dr. Victor Voicu,** membru al Academiei Române

REALIZĂRI ALE C.C.S.M.M.

Rezultatele activităţii de cercetare Ştiinţifică s-au materializat în procedee, metode de tratament sau investigare şi în realizarea de noi produse şi compuşi cu rol protector.

Realizări. Brevete:

- **Produs medicamentos cu acțiune eficace împotriva compușilor organofosforici**. Brevet OSIM 79914/1974 - V. A. VOICU, C. Miu, M. Jiquidi, C. Furnica, I. Elian.
- **Dixidextracaine 70, medicament destinat anesteziei hiperbare subarahnoidiene**. Brevet OSIM 81100/1975 - I. Curelaru, C. Furnica, V. A. VOICU, E. Ionescu-Visan, C. Miu, P. Nitescu, L. Ivanescu, I. Manolache.
- **Combinații farmaceutice cu efecte antiseptice**. Brevet OSIM 65237/1977 - Al. Bors, V.A.VOICU, C. Rizescu.
- **Soluție de repolarizare dinamică**. Brevet OSIM 65235/1977 - Al. Bors, V .A. VOICU.
- **Compus antiaritmic**. Brevet OSIM 65236/1977 - Al. Bors, V .A. VOICU
- Echipament pentru obiectivarea interacțiunilor medicamentoase. Brevet OSIM 74803/1980 - Al. Ionescu-Stoian, V. A. VOICU, M. Jiquidi.
- **Compus antiaritmic și procedeu de sinteză**. Brevet OSIM 69027/1978 - Al. Bors, V. A. VOICU, V. Aldea, E. Olteanu.
- **Compus radioprotector**. Brevet OSIM 3198/1970 - Al. Bors, V. A. VOICU, C. Paun, Gh. Dumitrescu.
- **Compus antihipertensiv nou**. Brevet OSIM 76911/1980 - V. A. VOICU, St. Muresan, Gh. Radu, M. Jiquidi.
- **Compus antihipertensiv nou**. Brevet OSIM 76912/1980 - V. A. VOICU, St. Muresan, Gh. Radu, M. Jiquidi.
- **Compus antiseptic nou**. Brevet OSIM 80051/ 1979 - Al. Bors, V. A. VOICU, A. Karassi.
- **Compus anticholinergic**. Brevet OSIM 87010/1985 - V. A. VOICU, Al. Ionescu-Stoian, L. Antoniu, M. Jiquidi.

- **Antidot complex pentru absorbția, neutralizarea și inactivarea toxicelor ingerate**. Brevet OSIM 87147/1985 - Al. Ionescu-Stoian, L. Antoniu, V. A. VOICU, V. Florea.
- **Compus vegetal cu acțiune antiinflamatorie, analgezică și vasoprotectoare**. Brevet OSIM 99921/1989 - V. Mihaila , V. A. VOICU .
- **Medicament cu efect antiinflamator, analgezic și descongestionant**. Brevet OSIM 102954/1991 - V. A. VOICU , C. Mircioiu, Al. Ionescu-Stoian, M. Jiquidi, E. Rolea.
- **Bandaj cu colagen pentru tratamentul arsurilor**. Brevet OSIM 103550/1991 - V. A.VOICU, V. Trandafir, T. Giurgiu, T. Safta, D. Burlacu, A. Nica , L. Dragoescu.
- **Medicament cu efect antiinflamator, analgezic și descongestionant**. Brevet OSIM 106333/1996 - V. A. VOICU, M. Jiquidi, C. Mircioiu.
- **Metodă și compuși pentru determinarea simultană a acetilcolinesterazei și pseudocolinesterazei în sânge** – Brevet 67856/19.10.1976
- **Antidot polivalent împotriva agenților chimici, cu efect asupra leziunilor cutanate și ale mucoaselor** – Brevet 7959/23.06.1978.
- **Dispozitiv pentru măsurarea răspunsului caloric.**
- **Amestec protector pentru impregnarea țesăturilor.**
- **Dispozitiv pentru măsurarea contaminării cu izotopi radioactivi a organismului uman.**
- Dispozitiv pentru desicarea rapidă a compușilor biologici sau chimio-farmaceutici.
- **Produs medicamentos cu actiune specifica antistres, antiuzura si cu efecte sinergice, polivalente, de revitalizare neuropsihobiologica** - Brevet de inventie Nr 87905/2005;
- **Test imuno-enzimatic pentru detectarea microorganismelor** – Brevet 91566/1984/09.01.1987.
- **Procedeu de conjugare enzimatică** – Brevet 90105/1984.
- **Compus vegetal cu efect antiinflamator, analgezic și vasculo-protector** - Brevet 9992/30.10.1989.
- **Bandaj colagenic pentru leziuni cutanate** – Brevet 103550/20.02.1989.

C.C.S.M.M.a participat in cadrul planului național de cercetare-dezvoltare I (2000-2006) și II (2007-2013), la competiții naționale și a obținut finanțarea unor proiecte de cercetare științifică prin următoarele programe: CEEX/VIASAN, CEEX/AEROSPAȚIAL, CEEX/INFOSOC, CEEX/BIOTECH.

Merită amintită și participarea specialiștilor la manifestări științifice internaționale precum *Congresele de Farmacologie și Toxicologie, Congresele Cercetătorilor și Inventatorilor din România, Târgurile Internaționale de Invenții și Idei Practice „Tesla“* și *„Invest-Invent“* (activitate apreciată cu o medalie de aur, o diplomă de excelență și o diplomă de onoare), *EXPOMIL 2011 și 2013, Congresele Anuale de Medicină Militară Balcanică, Simpozionul internațional NATO-STO pe tema efectelor biologice ale expunerii la radiații ionizante și contramăsuri - Lubliana, 2012 și 2013*), dar și la manifestări naționale precum *Congresul de medicină veterinară - Timișoara, 2012.*

Tematica abordată în lucrări face referire la contramăsurile medicale și capabilitățile necesare pentru protecția medicală a trupelor și populației în cazul amenințărilor sau atacurilor biologice și chimice, problematică de mare actualitate la nivel mondial.

Participări la misiuni ale armatei române în teatrele de operații

Specialiștii CCSMM au participat la misiuni naționale și internaționale, fiind prezenți în teren/teatre de operații în Irak, Somalia și Angola, sau furnizând produse de protecție împotriva efectelor STL (ex. produsul Dostirav, pentru misiunea din Irak); astfel, CCSMM a participat la organizarea primului Spitalul Militar din Golf, fiind prima prezență medico-militară românească participantă la misiuni în afara țării.

Implicări în cadrul reacțiilor de răspuns la amenințări cu agenți CBRN pe teritoriul țării

Pe plan național, personalul CCSMM a participat la:

- monitorizarea a unor grupe de populație, consecutiv accidentului nuclear de la Cernobîl;
- supravegherea epidemiologică și intervenția cu scop diagnostic în focare de holeră pe teritoriul României;
- diminuarea efectelor unor accidente (Fabrica de apă grea Halânga);
- precizarea cauzei epidemiei de meningo-encefalită cu virus West Nile, valorificând expertiza în arboviroze (1996) (diagnosticul a fost confirmat de către Institutul Pasteur de la Paris și de către un grup de cercetători din cadrul Centrului pentru controlul bolilor din Atlanta, Fort Collins - SUA, în colaborare cu Institutul Cantacuzino din București); expertiza medico-militară în epidemiile de antrax (2000 și 2005);
- identificarea virusului gripei aviare în țara noastră (laboratorul de biologie moleculară a fost primul care a identificat agentul viral, utilizând analizorul genetic de diagnostic ABI PRISM 310, diagnostic confirmat și la Londra);
- colaborarea cu SRI/FBI pentru expertizarea unor potențiali agenți CBRN la ambasade, verificarea corespondenței și a unor colete suspecte după atacurile teroriste cu antrax din anul 2001;
- exercițiul de antrenament de la Centrala Nucleară Cernavodă;
- exerciții comune cu structuri din SNAOPSN, dintre care le amintim pe cele de la Brașov și Câmpulung Muscel – 2008, BIOEX – Tulcea 2009, TTX – Chișinău, R. Moldova 2010, etc.

C.C.S.M.M. - element al sistemului național de protecție împotriva amenințărilor C.B.R.N

- Centru metodologic de referință care furnizează expertiză medicală în caz de accidente sau incidente cauzate de agenți C.B.R.N.
- C.C.S.M.M. poate furniza expertiză privind soluțiile medicale în amenințările C.B.R.N. atât în teatre de operații cât și în viața civilă.
- Structurile M.Ap.N. dar și armatele NATO pot beneficia de această expertiză, CCSMM posedând un semnificativ potențial uman și un bun suport tehnic, în special în domeniile toxicologiei și microbiologiei;
- Misiunile centrului ar putea fi îndeplinite la un nivel superior dacă s-ar reuși realizarea unui laborator de microproducție pentru antidoturi, atât pentru necesitățile proprii cât și ale alianței NATO.

C.C.S.M.M. – direcții majore de cercetare:

- Noi metode de diagnostic rapid și dezvoltarea unor noi strategii de prevenție și tratament al îmbolnăvirilor cauzate de agenții C.B.R.N.;
- Tratament medical specializat în intoxicațiile acute, accidente/incidente chimice și nucleare, supravegherea personalului expus;
- Sinteza și producerea de compuși radioprotectori, antidoturi, seruri, vaccinuri și medicamente;
- Dezvoltarea unor echipamente specializate, inclusiv autolaboratoare, pentru lucrul în mediul contaminat cu agenți C.B.R.N.;
- Experții din diferite domenii din cadrul C.C.S.M.M. au participat, la ordinul ministrului apărării, la diferite acțiuni ale alianței NATO sau ale altor organizații internaționale;

Concluzii

- În decursul celor 45 de ani de istorie, uneori dificili în special din cauza restructurărilor, C.C.S.M.M. a rămas o instituție matură și posedă în prezent un nucleu valoros de cercetători (medici, biologi, chimiști, fizicieni, ingineri, personal didactic);
- Cercetarea științifică medico-militară își poate realiza sarcinile specifice și posedă experții necesari în diferitele domenii ale protecției medicale împotriva efectelor agenților C.B.R.N.;
- Activitatea de cercetare în domeniu (cu resurse umane de calitate și echipament adecvat) rămâne una dintre condițiile „sine qua non" ale existenței unui sistem medical performant, capabil să facă față amenințărilor prezente.

MEDICINA operațională

În prezent, este unanim recunoscut faptul că principala resursă a oricărui stat o constituie chiar membrii acestuia. Starea de sănătate a membrilor unei societăți are implicații directe asupra bunăstării, competitivității și eficienței acesteia. Toate amenințările la adresa securității naționale implică și sectorul de sănătate, ca actor și / sau ca victimă, de la război, invazie, ocupație, criza biologică (declanșată de atacul biologic, bioterorism, urgențe epidemiologice, epidemii, epizootii, pandemii, panzootii, de scăderea drastică a nivelului de trai sau de prăbușirea sistemului de sănătate) până la atacul informatic sau criza financiară.

Sistemul medical militar trebuie să-și aducă propria contribuție la constituirea unei capacități de apărare a țării suficientă pentru a permite protejarea intereselor naționale și, în același timp, îndeplinirea angajamentelor asumate ca stat membru NATO sau UE. În acest context, medicina militară trebuie să găsească cele mai potrivite mijloace de adaptare și reacție la noile provocări ale lumii contemporane.

Asigurarea medicală operațională, ca o caracteristică specifică mediului militar, trebuie construită astfel încât să constituie un sprijin real pentru militarii dislocați în misiuni de luptă în Teatre de Operații. Activitățile specifice medicinii operaționale trebuie să acopere tot parcursul unei misiuni, începând cu etapa pre-dislocare, în timpul dislocării și etapa post-dislocare. Aceste acțiuni trebuie să fie coordonate și armonizate, sub o comandă unică, indiferent de componentele sistemului medical militar care le execută, astfel încât să-și atingă scopul final: menținerea sănătății militarilor și îngrijirea bolnavilor și răniților din timpul misiunilor în T.O.

În atingerea acestui scop, trebuie avut în vedere faptul că operațiile viitoare nu trebuie doar să fie desfășurate în comun, ci și integrate. Sistemul medical militar va trebui să interacționeze și să coopereze cu alte structuri ale Armatei, dar și cu entități aparținând altor ministere și agenții sau organe neguvernamentale, organizații publice și companii comerciale. Deoarece este foarte probabil ca operațiunile viitoare de apărare și securitate să nu fie desfășurate individual, ci ca parte a unei coaliții, medicina militară trebuie să se alăture obiectivului principal al transformării militare și anume realizarea de capacități cu un grad ridicat de sustenabilitate și interoperabilitate, flexibile, mobile, ușor de desfășurat în zone de operații, capabile să participe la întreaga gamă de operații și misiuni naționale, dar și ale ONU, NATO, UE sau ale altor organizații.

Pentru îngrijirea sănătății întregului personal al Armatei, militarilor în rezervă și a oricărei persoane aflate în suferință, componentele sistemului medical militar trebuie să acționeze unitar și eficient, indiferent de tipul de activitate medicală pe care o desfășoară sau de locul unde se desfășoară această activitate.

Astfel, în ce privește asistența medicală spitalicească, spitalele militare trebuie să-și diversifice serviciile pe care le oferă, în vederea integrării lor ca unități de elită în sistemul de sănătate publică național, dar și pentru consolidarea poziției lor în relație cu casele de asigurări de sănătate, esențială pentru funcționarea lor. În același timp însă, trebuie acordată o atenție sporită aspectelor specific militare din activitatea spitalelor (cum ar fi, serviciile medicale pentru supravegherea stării de sănătate a personalului armatei sau asigurarea de specialiști pentru sprijinul medical operațional la ordin). Trebuie menționat că, în condițiile în care spitalele militare sunt unități sanitare publice, pentru care contractele cu casele de asigurări de sănătate sunt vitale, precum și în condițiile lipsei acute de personal din prezent, activitățile specific militare se desfășoară uneori cu dificultăți.

Referitor la asistența medicală primară, este necesară o colaborare mai bună între medicii din unitățile militare și unitățile sanitare militare care le coordonează această activitate, pentru creșterea eficienței acestui tip de asistență medicală de prim contact, mai ales în condițiile în care spitalele militare deservesc teritorii care cuprind mai multe județe.

Pentru îmbunătățirea asistenței medicale de urgență, trebuie continuată dezvoltarea Sistemului Medical Militar de Urgență, pentru constituirea unităților (compartimentelor) de primiri urgențe (UPU/CPU) din spitalele militare în componente cu adevărat funcționale, în condițiile în care toate spitalele militare au titulatura de spitale de urgență. În acest context, este necesară întărirea colaborării cu structurile din domeniu ale Ministerului Sănătății, pornind de la realitățile și posibilitățile fiecărui sistem în parte. De asemenea, trebuie asigurat un echilibru între necesitatea funcționării continue a UPU/CPU și asigurarea asistenței medicale de urgență pentru activități și misiuni militare pentru perioade de timp îndelungate.

În același timp, se impune ca activitatea de medicină preventivă să fie înțeleasă la adevărata valoare și sprijinită, având în vedere că un număr mic de cadre medicale din cinci unități sanitare militare, cu susținere logistică foarte slabă, se străduiesc să asigure supravegherea și inspecția de sănătate publică și sanitară-veterinară pentru toate unitățile militare ale Armatei, răspândite pe tot teritoriul țării, uneori chiar în zone cu acces foarte dificil.

Nu în ultimul rând, trebuie subliniat că în domeniul medico-militar apar permanent noi provocări specifice mediului militar. Dintre acestea se impune a fi menționată recuperarea medicală și integrarea în viața activă a militarilor răniți în misiuni în T.O., care a necesitat, și va necesita și în viitor, mari eforturi profesionale și financiare legate atât de asistența medicală specifică, corectarea sechelelor fizice și psihice, cât și de găsirea unor soluții viabile de integrare socială a acestor persoane.

Ca un aspect uneori trecut cu vederea poate prea repede, sistemul medico-militar trebuie să ia în considerare cu seriozitate rolul său în gestionarea situațiilor de urgență. Ministerele și celelalte organe centrale și organizațiile guvernamentale din cadrul Sistemului Național de Management al Situațiilor de Urgență au repartizate funcții de sprijin, din care derivă atribuții obligatorii în toate domeniile, care să asigure în mod unitar și profesionist, apărarea vieții și sănătății populației, a mediului înconjurător, a valorilor materiale și culturale importante, pe timpul producerii unor situații de urgență, care să permită restabilirea rapidă a stării de normalitate.

În acest context, se cunoaște că persistă riscul apariției unor situații de criză generate de utilizarea armelor de distrugere în masă (chimice, biologice și nucleare) sau generate de eliberarea (accidentală sau intenționată) în mediu a agenților chimici, biologici, radiologici și nucleari. Medicina militară are tradiție și expertiză legată de protecția medicală împotriva agresiunilor cu astfel de agenți, care trebuie menținută și dezvoltată, mai ales în condițiile în care această problematică este insuficient abordată de alte instituții din cadrul sistemului național de sănătate publică.

După această scurtă trecere în revistă a câtorva aspecte de actualitate, se poate concluziona că atât noi, cei care lucrăm direct în domeniu, cât și factorii de decizie din Ministerul Apărării Naționale, trebuie să acționăm, fiecare la locul său de muncă, pentru ca medicina militară să devină mai profesionistă și mai performantă în beneficiul direct al pacienților noștri, și în același timp să ofere un sistem medical flexibil, modern, capabil să își asigure o integrare cât mai eficientă în sistemul național de sănătate publică, dar și în structurile similare NATO și UE.

Gral. Brigadă medic,
*Dr. **Mihai-Marius MUREȘAN***

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EDITORIAL

Reconsidering the importanceof Military Medicine at the levelof National Ministry of Defenseand into society

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This goal can be achieved through the implementation of a modern management bothof the Medical Directorate and of the subordinate units, which basically involves areform of these institutions, keeping them out of the incertitude of annual budgetaryprovision, looking for contractual stability with National Defence, Internal Affaires,National Security and Justice Authority Health Insurance Company (C.A.S.A.O.P.S.N.A.J) andplacing them on the right path for institutional performance.

The reform of the military medical institution is an act of courage that has to be accomplishedwith great determination.

The first step would be submitting to the Supreme Council of National Defence (CSAT)the concept of the modernization of the military medical system including elements ofinnovative vision on the military medical phenomenon. One of these issues is the needfor modernisation of medical equipment and hospital infrastructure in order to allow thetreatment in national military hospitals of soldiers injured in combat theaters and of civiliansafter natural disasters or industrial accidents with multiple victims.

Another goal is to create a fully equipped base for physical and neuropsychological recoveryof military personnel executing missions in operation theaters and of their family members,allowing a fast and complete social and family reintegration.

A particular attention should be paid to hero combatants, wounded in combat theaters; theywill take benefit from the best specialists in health care in the new social-medical edificewhich will be available since 2015.

In 2015 also, the construction of the new Emergency Universitary Central Military Hospitalwill begin in Bucharest in Garrison 867, part of the Ministry of National Defense estatepatrimony, under Government Decision no 1594/04.12.2008.

This investment is the natural consequence of the need to be aligned with the realities ofmodern medicine promoted by North Atlantic Alliance through ROL 4 level hospital units,covering all medical and surgical specialties required for complete rehabilitation andrecovery of military personnel wounded in the operation theaters or in the current trainingactivities, on national territory.

In the same time with updating of the clinical basis, we should concentrate our attention onimproving the continuous training of our healthcare staff regarding the achievement of newtreatment techniques and solutions. Continuous professional training is an asset that ourphysicians are providing to the military personnel in distress.

From the experience of some colleagues, who have chosen to leave the system, the mainreasons for discontent were: the working environment and the lack of modern investigationmeans; to the surprise of many, the financial reason was the last one.

Promoting team spirit and preserving the values in the system has been a permanentconcern of the Medical Directorate Management.

All these contribute to achieving outstanding results in our activity, leading to increasingsatisfaction for patients and doctors.

REVIEW ARTICLE

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Clostridium difficile – emergent hospital flora

Gabriela V. Dumitrescu, Viorel Ordeanu, Simona Bicheru, Lucia Ionescu,Diana Popescu, Marius Necşulescu[1]

Abstract: *Clostridium difficile (C. difficile) is a Gram-positive sporogenous bacillus strictly anaerobic, which in the last decade has became the most important anaerobic bacterium in nosocomial human pathology. Cl.dificile is the etiological agent of more than 20% of diarrhea postantibiotics, over 95% of pseudomembranous colitis and the first cause of nosocomial infectious diarrhea in adults.*

Although this bacterium usually colonizes the intestine of vertebrates (the normal microbiota), the toxinogenic strains (tcdA and tcdB) are pathogenic in the digestive tract. Given the excessive use of antibiotics and the increased spores resistance, it is possible an environment contamination, with strains which may already be resistant to antibiotics. The main causes of this infection are decreased resistance to antibiotic-induced colonization, contamination with a pathogenic strain of Cl.difficile, secretion of A and/or B toxins and deficient immune response.

Due to the increasing worldwide incidence of infections with C. difficile on one hand and to the discovery of new ways of transmitting the infection according with some studies regarding the genetic diversity of bacterium strains on the other hand, a new approach is necessary for C. difficile related topics..

Keywords: *antibiotics, Clostridium difficile, epidemiology, nosocomial infection, toxins*.

INTRODUCTION

Clostridium difficile (C. difficile) is a Grampositivesporogenous bacillus strictly anaerobic,which in the last decade has became the mostimportant anaerobic bacterium in nosocomialhuman pathology. Cl.dificile is the etiological agentof more than 20% of diarrhea postantibiotics, over95% of pseudo-membranous colitis and the firstcause of nosocomial infectious diarrhea in adults.

Although this bacterium usually colonizes theintestine of vertebrates (the normal microbiota),the toxinogenic strains (tcdA and tcdB) are pathogenicin the digestive tract. Given the excessive useof antibiotics and the increased spores resistance,it is possible an environment contamination, withstrains which may already be resistant to antibiotics.

The main causes of this infection are decreasedresistance to antibiotic-induced colonization, contaminationwith a pathogenic strain of Cl.difficile,secretion of A and/or B toxins and deficient immuneresponse.

Microbiological diagnosis is made by severalmethods and techniques for bacteria or toxins identification.

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Cytotoxicity test reveals the cytopathiceffect of fecal filtrate with pg sensitivity. Immunoenzymaticassay enables a rapid diagnosis, firstgeneration with ELISA, the second generation byimmuno-enzymatic or immuno-chromatographycassette. Molecular biology techniques based onquantitative real-time PCR detect tcdA and tcdBgenes in stool, responsible for toxigenesis with verygood sensitivity and specificity. Through cultivationand microscopy Cl. difficile can be revealed inthe stool or on contaminated surfaces; spores areresistant in the environment and are found in nosocomialflora. A characteristic enzyme, glutamate dehydrogenase (GDH) can be revealed in stool byimmuno-enzymatic assay correlated with the outcomeof cultivation, or latex agglutination test withantiGDH antibody.

Due to the increasing worldwide incidence ofinfections with C. difficile on one hand and to thediscovery of new ways of transmitting the infectionaccording with some studies regarding the geneticdiversity of bacterium strains on the other hand,a new approach is necessary for C. difficile relatedtopics.

CLINICAL

Clostridium difficile (C. difficile) is a Grampositive,spore forming bacteria, spread by thefecal-oral route. It is non-invasive, producestoxins A and B, which cause disease, ranging fromasymptomatic carriage, to mild diarrhea, to colitis,or pseudo-membranous colitis. Clostridium difficileinfection (CDI) is defined as the acute onset ofdiarrhea with toxigenic C. difficile or its toxin andno other cause for diarrhea.

Since 2000 the rate of CDI has been increasing,especially in the elderly with a recent hospitalizationor residing in long-term care facility (LTCF).

Carriage of C. difficile occurs in 5– 15% of healthyadults, up to 57 % in residents in LTCF and canreach 84.4 % in newborns and healthy infants.

In simple diarrhea cases, the classic symptomsmay not occur and the endoscopic examinationshows normal or ulcerated mucous; in 25% of casesending the antibiotic therapy was followed by clinicalrecovery in 2-3 days. Further on antibiotheraphyis a prolonging factor of diarrhea relapse.

Pseudomembranous colitis represent up to 9%of CDI and starts with abundant watery diarrhea,over 7 stools a day, with heterogenic no bleedingaspect. They are accompanied by fever in 75%of cases and abdominal pains in 70% cases. Thesymptoms are non-specific, leukocytolysis up to ex80.000 PMN/l¼l, extracellular dehydrating caused by exudative enteropathy.

Digestive endoscopyconfirms the diagnosis, allowing canker yellowishsores visualization, named pseudomembrane, onmucous colon membrane. In the first stage theyare isolated, afterwards they come together. In CDIforms with severe onset and no obvious etiology of diarrhea an endoscopy is recommended, butthis test is difficult to perform on aged and fragilepatients. Complications such as septic shock andtoxic megacolon may occur, septic shock and toxicmegacolon occur and provoke the colon perforation(colectomy required) and even death.

The ratio of severe forms differs (7-18%),depending on the studies we consider. Consecutivemortality with C. difficile varies 0,6-3% and whencomplications occur is 35-50%. Some studies showincreased mortality in North America, a doubleno. of cases in EU, heading to 24/milion, C. difficilebeing involved in death cases three times morefrequent than Staphilococcus aureus MRSA.In 20% of cases, relapses appear in the firsttwo months after the initial episode. In over 50%of cases they are connected with the persistenceof pathogen strain (spores) inside digestive tract;a new stain could appear and provoke reinfectionespecially during hospital admission. Multiple strains have been identified during one episode ofinfection. Approximately 3% of adults are asymptomaticcarriers and often with toxin-free strainsand sometimes specific toxins may be identified insome asymptomatic patients stool. The asymptomatictransmission of toxinogen strains in neonatesis 5-70%, but there is no explanation what so ever.Although nosocomial infections are the mostfrequent, some of them could be communal. Thereare recorded 17.5% postantibiotics diarrhea inEU, from which 66% have one day manifestation.After two weeks antibiotherapy, the frequency becomes 3.8%, from which 70% are toxic. In NorthAmerica were identified a lot of cases but no strainhigh pathogen 027 had been isolated in communalinfectious. Differential diagnosis will be made withother infectious diarrhea: bacterial, viral, fungusand parasitic or non-infectious causes; for example,the outcome of some ”cool” drugs is in realitylaxative ones (supplements for straitening theimmunity, sugar free sweets, food with magnesiumand decaf products) with no connection with CDIetiology. [Duker Freuman T., 2014]

Figure 1. Pathogenesis of Clostridium difficile –associated disease (http://bioweb.uwlax.edu/bio203/s2009/kumm_jakl/pathology.htm)

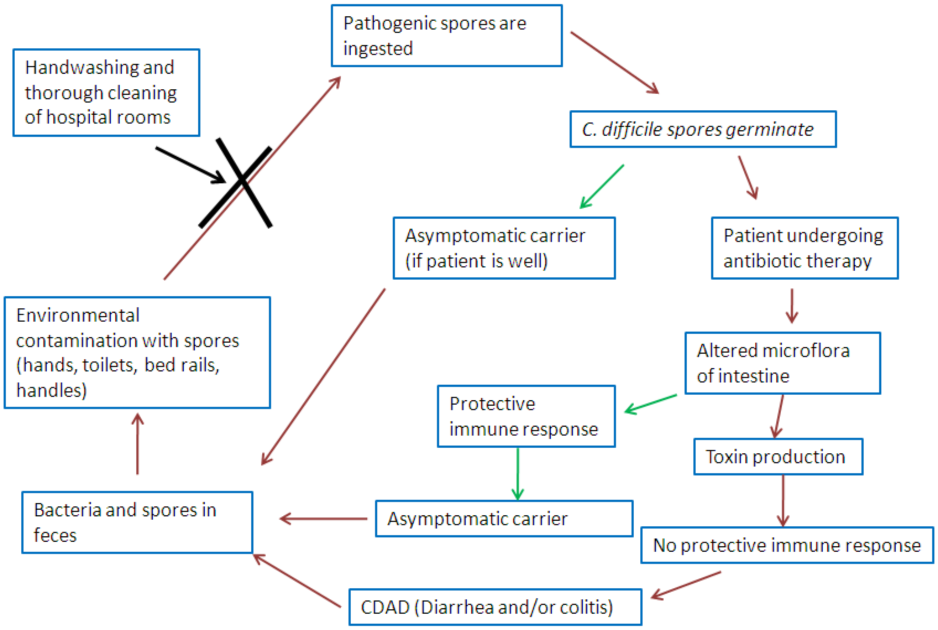
Pathogenic spores are ingested
C. difficile spores germinate
Handwashing and thorough cleaning of hospital rooms
Asymptomatic carrier (if patient is well)
Patient undergoing antibiotic therapy
Environmental contamination with spores (hands, toilets, bed rails, handles)
Altered microflora of intestine
Protective immune response
Toxin production
Bacteria and spores in feces
Asymptomatic carrier
No protective immune response
CDAD (Diarrhea and/or colitis)

MICROBIOLOGICAL DIAGNOSTIC

CDI diagnostic is based on revealing thetoxins in stool or isolating a toxinogenic strain ofCl.difficile, this being the only pathogenic strain.Diagnostic testing for C. difficile has rapidlyevolved in the past decade. Previously, toxin A +B EIAs were the most widely used diagnostic testsbecause of ease of use and objective interpretation.

However, EIA tests have substantially reduced sensitivitiescompared with reference standards. Moreover,toxin A immunoassays (without toxin B) lackdetecting the small number of pathogenic strainsthat only produce toxin B. Two major advances inthe laboratory diagnosis are the use of GDH detection in stools as a means of screening for CDI andthe development of Nucleic acid amplification tests(NAATs) such as PCR to detect toxigenic strainsof C. difficile. Glutamate dehydrogenase (GDH)screening tests for C.difficile can be used in two- orthree-step algorithms with subsequent toxin A + BEIA testing, but the sensitivity of such strategies islower than NAATs [Surawicz et al., 2013] (fig 2).

Testing the toxigenic C. difficile shouldbe limited to patients with > 3 nonformedstool specimens per 24 hr period, unless ileus(obstruction) is suspected. Repeat testing following apositive test (test of cure) is not recommended sincepatients may carry toxigenic C. difficile for monthsafter clinical cure. Repeated testing following apositive test is appropriate if the patient improveswith therapy and relapses after the completion ofa treatment regimen (clinical relapse). Testing asecond specimen from a negative patient is morelikely to be a false positive [American Society forMicrobiology, 2010].

The optical microscopy swab is pathognomonic,revealing long gram-positive bacilli with a bulgeat terminal ends, with long terminal and isolatedspores, visible with Gray coloration. While thepresence of C. difficile can be suspected, we cannotdifferentiate the pathogencal strains from the nonpathogencalones, therefore the examination shouldbe supplemented with toxigenical and molecularbiology tests. In the last years, a very pathogenicaland virulent strain, C. difficile 027, has beenidentified, that causes severe epidemic episodes (Fig 3).

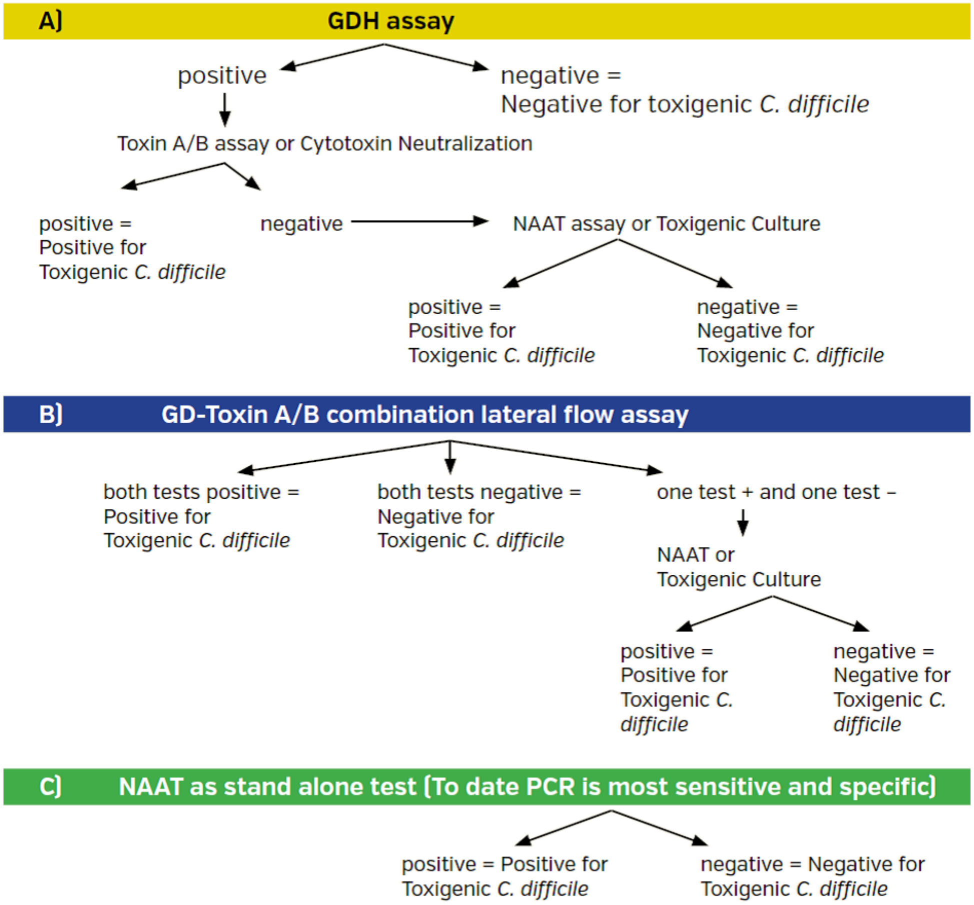
A)
GDH assay
positive
negative =
Negative for toxigenic *C. difficile*
Toxin A/B assay or Cytotoxin Neutralization
positive =
Positive for
Toxigenic *C. difficile*
negative
NAAT assay or Toxigenic Culture
positive =
Positive for
Toxigenic *C. difficile*
negative =
Negative for
Toxigenic *C. difficile*
B)
GD-Toxin A/B combination lateral flow assay
both tests positive =
Positive for
Toxigenic *C. difficile*
both tests negative =
Negative for
Toxigenic *C. difficile*
one test + and one test –
NAAT or
Toxigenic Culture
positive =
Positive for
Toxigenic *C. difficile*
negative =
Negative for
Toxigenic *C. difficile*
C)
NAAT as stand alone test (To date PCR is most sensitive and specific)
positive = Positive for
Toxigenic *C. difficile*
negative = Negative for
Toxigenic *C. difficile*

Figure 2. Diagnostic algorithm of Clostridium difficile (Surawicz et al., 2013)

The epidemic strain currently describedin North America and EU, has the followingfeatures: PCR ribotype 027 in accordance withAnaerobe Reference Laboratory surveillance data[ECDC, 2006], pulsotype NAP 1 on pulsed-fieldelectrophoresis, enzymatic restriction-profile BI,toxinotype III by Rupnik toxinotyping method,positive for binary toxin actinia-specific ADPribozyltransferase,deletion of 18 bp in tcdCgene controlling the expression of toxins A andB, hyperproduction of toxins A and B (Ax16and Bx23) in comparison with strains of othergenotypes, resistant to macrolides (erythromycin)and la flororquinolones (moxifloxacin, gatifloxacinand levofloxacin).

Only specialised laboratoriesare able to perform the techniques for identifyingthese features and a two weeks period is requiredfor confirmation [INVS, 2006].

In practice, CDI diagnostic is based on toxinB detection in stool or revealing the toxigen strain.A- and B+ strains cannot be detected by currentimunoenzymatic assays which detect only A stain.

Figure 3. Analysis of anaerobic bacterial isolates in the microbiology laboratory of CCSMM

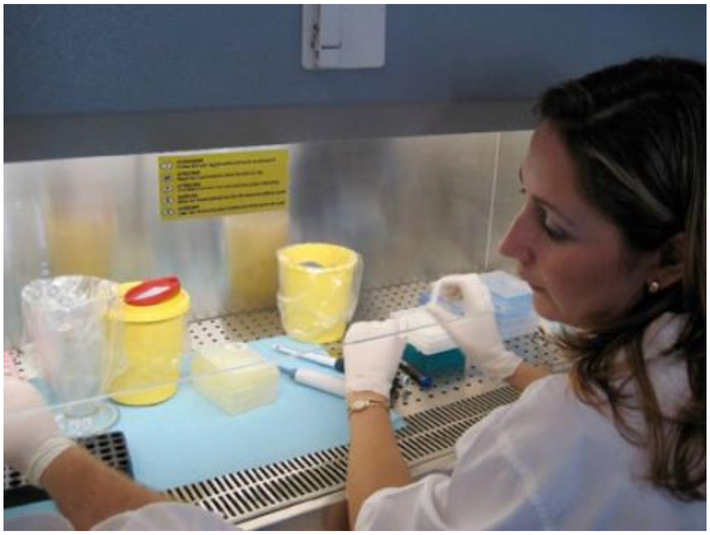

The strain isolation through culture is a necessarystage for epidemic clone 027 characterisation;PCR profile identification provides the certaintydiagnosis.

This clone presence is clinically suspectedif a severe form of the disease is diagnosed,epidemiologically suspected if several cases occur,or microbiologically suspected if the isolated strainis resistant to new fluoroquinolones (moxifloxacinCMI > 4 mg/l) or to erythromycin (CMI > 256mg/l).

These characteristics are not specific to clone027, but justify the stool culture in anaerobiosis inorder to isolate the responsible stain and to sendit to a specialised reference laboratory for furtherexamination.

The genes encoding TcdA and TcdB, tcdA andtcdB, respectively, have been sequenced and arefound in single open reading frames located withina 19.6-kb pathogenicity locus (8, 38).

As expected,both open reading frames are large, with tcdAfound within an 8,133-nucleotide region and tcdB is 7,098 nucleotides in length (fig.4).

Both tcdA and tcdB are low-G C (28%) genes,which are comparable to the G C content (29%)of the C. difficile genome, and the toxins exhibita high degree of overall similarity (66%).

Giventhe proximal locations of tcdA and tcdB and thehigh sequence and functional homology betweenthe two proteins, it has been proposed that thetwo genes may have arisen as the result of a geneduplication event.

Furthermore, the similarity inthe biochemical activity of TcdA and TcdB, whereinboth toxins use a highly conserved N-terminaldomain to modify identical substrates, supportsthe notion of gene duplication. The major regionsof homology between TcdA and TcdB fall withinthe enzymatic and receptor-binding domains of thetwo toxins. The N-terminal domains of TcdA andTcdB show 74% homology, and this homology providesa basis for the similar substrate specificity ofthese two toxins.

The C terminus of TcdA and TcdB show a numberof short, homologous regions termed combined repetitive oligopeptides (CROPs). TcdA encodesfive groups of CROPs, which range in size from 21to 50 residues and can be repeated throughout theC terminus of the protein. TcdB also encodes fivegroups of CROPs, four of which show homology tothe CROPs of TcdA.

Yet the CROPs found in TcdBare more divergent and less frequent than thosefound in TcdA. CROPs appear to play a putativerole in initial target cell interaction and receptorbinding, but the mechanism explaining the necessityfor these repeats in cell binding remains unclear[Daniel E. Voth, 2005].

Figure 4. Genetic arrangement of the C. difficile pathogenicity locus and proposed protein domain structures of TcdA and TcdB. Both TcdA and TcdB are encoded on the 19.6-kb pathogenicity locus. In addition to the two toxin genes tcdA and tcdB, three additional regulatory open reading frames are located on this island. tcdD is a proposed positive regulator, tcdE is a putative holing protein, and tcdC is a proposed negative regulator of toxin gene expression. Through deletion mutagenesis, research combined from multiple research groups has revealed a three-domain structure of the large clostridial toxins. The glycosyltransferase activity is located at the N terminus of the protein, and the C terminus is involved in receptor binding. Located in the middle domain of the protein is a putative transmembrane segment that is thought to be involved in membrane translocation. [Daniel E. Voth, 2005]

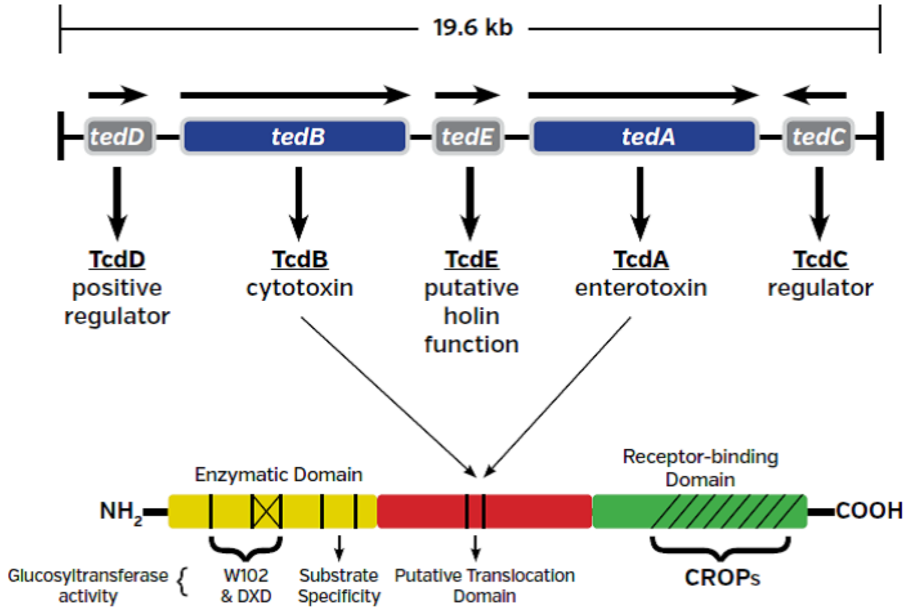

EPIDEMIOLOGY

C. difficile transmission is made by fecal-oralroute, by hands and contaminated objects orenvironment. The fast transmission in healthcareenvironments is a result of several factors: straindissemination in CDI patients, half of samples frompatients rooms being positive; high resistance ofspores on inert supports for several months; toomany patients crowded in common healthcaresettings; numerous healthcare maneuvers creatinga high possibility of contamination by the medicalpersonnel hands; inadequate usage of antibiotics which diminishes the resistance to colonization andfacilitates C. difficile development.

The main individual risk factors are the advancedage and antibiotherapy. There are severalstudies which correlate the consumption of someclasses of antibiotics with CDI incidence: clindamicyn,3-rd generation cephalosporins, macrolides,and amoxicillin with clavulanic acid, 1-st generationcephalosporins and fluoroquinolones. It seamsthat the role of fluoroquinolones in C. difficile 027strains emergence and spreading is connected tothe resistance level towards them [INVS, 2006].

All factors stimulating the digestive ecosystemalteration, like laxatives, antacids, antisecretors,transit retarders, baritosis transit, gastrointestinalsurgery, etc. may facilitate this infection [DukerFreuman, 2014].

In March 2014, an epidemic episode with 31cases of postantibiotic C. difficile infection wasrecorded in Ploiesti Emergency Hospital (Romania)and the patients were isolated and treated. Mostof them were aged people from Neurology,Nephrology and Intensive Care Unit [Libertateanewspaper, 2014].

In May 2014 the Ministry of Health of Romaniagave the alert for C.difficile in Vaslui and Bucharesthospitals. The beginning of the year is worrying,in only 4 months, in Bucharest health facilitieswere registered 462 infected patients [Pro

TV, 22Mai 2014].

In accordance with Annual epidemiologicalreport: Reporting on 2011 surveillance data and2012 epidemic intelligence data, 2013, uttered byEuropean Centre for Disease Prevention and Control(ECDC), 48% cases of HAI (Healthcare-AssociatedInfections) associated with gastro-intestinalinfections were connected with C.difficile, andfrom all HAI (15.000 cases) in 3 only 5,4% of casesthe Clostridium difficile has been isolated. Takinginto consideration that in Romania over 92.3% ofpatients were the beneficiary of an antimicrobialprophylaxis during more than a day surgeries, theHAI risk associated with C. difficile is very high[ECDC, 2013]

TREATMENT

There is worldwide observed natural resistanceand/or acquired to the medicines of the quinolonegroup.

A mild CDI can usually be controlled bywithdrawing treatment with the antibiotics causingthe infection (25% of patients could recover in2-3 days). More severe cases can be treated usingan oral specific treatment with metronidazole (1g/day) or vancomycin (1-2g/day) for 10 days. Themetronizadole is a better choice, being a less expensivetreatment with no risk of selecting glycopeptidesresisting germs like golden enterococcus andstaphylococcus.

Failure to respond to metronidazole therapywithin 5 – 7 days should prompt consideration of achange in therapy to vancomycin at standard dosing.For mild-to-moderate CDI in patients who are intolerant/allergic to metronidazole and for pregnant/ breastfeeding women, vancomycin should be usedat standard dosing. In patients in whom oral antibioticscannot reach a segment of the colon, such aswith Hartman's pouch, ileostomy, or colon diversion,vancomycin therapy delivered via enema should beadded to treatments (500 mg in 100 – 500 ml of normalsaline every 6 h) until the patient improves.

However, relapse is common and requires furthertreatment with repeated series of metronidazoleor vancomycin, in high doses first and smallerdoses associated with probiotics (i.e. Saccharomycesboulardii) after improvement. Severe cases mayneed intensive care for maintaining the vital functionsand even surgical treatment for colectomy(in case of toxic megacolon or colon perforation).

CT scanning is an important technique for perforationdiagnosis in comparison with colonoscopytechnique which presents a perforation risk due togas inflation. The antibiotic treatment for healthyindividuals colonized with C.difficile is not recommended,being inefficient for eradicating for goodthis bacteria in digestive tract.[Ordeanu, 2010; Ordeanu 2012]

Considering the antibiotherapy limitations,there has been designed the fecal bacteriotheraphy,known as “stool transplant”/fecal microbiotatransplant (FMT) of bacterial flora acquired fromthe feces of a healthy donor to reverse the bacterialimbalance responsible for the recurring natureof the infection, with good results [ASGE, 2013].

This “synthetic stool” is a super-biotic obtained usingseveral cultures of saprophyte intestinal culture[Allen-Vercoe, 2013]. Studies show that patientswith recurrent CDI (RCDI) have abnormally proportioned colon microbiota, and that reintroductionof normal bacteria via donor feces correctsthis imbalance, restoring phylogenetic richness andcolonization resistance.

There is no international consensus for definingand surveillance CDI, but we have to consider local(regional and national) epidemiology conditionsand possibilities. ECDC created a working groupfor early detection and monitoring the CDI. Theyhave suggested recorded signals criteria for severeand grouping cases of CDI.

C. difficile infectious can usually be preventedby practicing good hygiene in healthcare environments,such as: individual bed space, washinghands regularly (mechanical action of washing aftergloves removal), using gloves, protection mask,glasses and gown in bed space area and in contactwith patients, using medical supplies for one usageonly, cleaning surfaces using bleach wipes of sodiumhypochlorite containing 0.5 % active chlorine,and patient removal limitation. [CCLIN, 2013]

COMMENT

Due to the increasing worldwide incidence ofinfections with C. difficile on one hand, and to thediscovery of new ways of transmitting the infectionaccording with some studies regarding the genetic diversityof C. difficile strains on the other hand, (http://www.pharmacypracticenews.com) a new approach isnecessary for C. difficile related topicsIt is important to adopted NAAT testing aloneor a 2 or 3 step algorithm for CDI diagnosis.

If the C. difficile is confirmed and classified asa severe form or in an epidemic context it should bereported to Public Health Territorial Authorities andto The Anaerobe Reference Laboratory from INCDMICantacuzino, for a clear diagnosis and adequatemeasures.

CONCLUSION

Due to the increasing worldwide incidence of infections with C. difficile on one hand and to thediscovery of new ways of transmitting the infection according with some studies regarding the geneticdiversity of bacterium strains on the other hand, a new approach is necessary for C. difficile related topics.

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SYSTEMATIC REVIEW

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Stress managementfor optimization oforganizational activity

Iuliana Guiţă – Alexandru[1,2]

Abstract: *Stress is a constant presence in our lives, whether we analyze it in professional, social or family terms. This daily reality creates a state of tension, strain and discomfort, causing significant changes in physical and mental health. Stress at work can affect anyone, at any level, in any sector and in organizations of any size. Stress affects health and safety of individuals and also organizations' welfare and national economies. There is a definite correlation between the level of stress at work and the changes in organization's productivity.*

Keywords: *stress management, organizational optimization, occupational stress, organizational culture, psychological contract*

STRESS: DEFINITION, TYPES AND CAUSESOF ITS OCCURRENCE

We meet every day people who are overworked, overwhelmed or underpaid. We wonder what to do to cope with the stress in our life. Often we forget to put on paper all the endless tasks we have to do during the day, and paradoxically we receive other new tasks to fulfill.

According to Peter Drucker, we have to make a clear distinction regarding those who are accomplishing an intellectual work today, the situation being more complicated for them than for production line workers of the past, who knew what they have to do. Instead, those involved in intellectual work should not only execute the plan, but also to conceive it. This puts an important pressure on the human brain so in case you see a doctor, one of the question is: "How stressed are you?" Certainly, stress at work can affect anyone at any level. It is produced in no matter what domain and in organizations of any size. Stress affects the health and safety of individuals and also welfare of organizations and national economies.

Stress was introduced by Hans Hugo Bruno Selye, professor of histology, who sets up the foundation of International Institute of Stress with Alvin Tofler in 1977. He believes that stress is related to adaptation syndrome as a reaction of individual against environmental aggressions. Hans Selye defines stress as "the set of reactions of the body to the external action of the causative agents (physical, chemical, biological and psychological) consisting in morphofunctional changes, most often endocrine".

According to the Romanian Language ExplanatoryDictionary (1998), stress is "the name given toany environmental factor (or set of factors) causingan abnormal reaction to human body; adverse effecton the human body produced by an environmentalfactor."

According to Terry Looker, Olga Gregson(2009, p.31),

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“Stress is seen as a reaction of theorganism against changes occured in our environment.”

All mentioned authors consider that “stresscan be defined as the condition that we feel when weperceive a discrepancy between perceived demandsand ability to cope with them.”

Lazarus and Folkman (1984) define stress as“a cognitive and behavioral effort (with obviousemotional expression) to reduce, control or tolerateexternal or internal demands that exceed personalresources.”

Ioan Bratu Iamandescu (2002) believes thatmental stress is a syndrome consisting of exacerbationbeyond the level of simple homeostaticadjustments, certain psychic reactions and theirsomatic connections (affecting almost all bodycomponents), in connection with external andinternal excitement exerted by triggering factors(stressor factors) acting intense, surprisingly, suddenand/or persistent and having a symbolic nature, “threating”, or, othertime, extremely favorableto the subject (perceived or anticipated by thesubject). In other cases, stressor factors could bepsychical excitants with major affective resonance(positive – eustress or negative – distress) or overloadingfactors of cognitive (attention, thinking,etc) and volitional process, mentioning that mentalstress is mainly based on a major emotional involvement.”

The definition given by Golu M. (1981) shouldalso be taken into consideration: “state of tension,tightness, discomfort, caused by affective agentswith negative significance (or positive, in case ofeustress), frustration or repression of some motivations(needs, desires, aspirations – including underloading),the difficulty or impossibility of solvingproblems”.

However, Paul Popescu Neveanu, in the “Dictionaryof Psychology “ (1978), defines stress interms of two meanings: “a) situation, stimulus whichputs the body into a state of tension ; b) the specialtension state, itself, of the body as an activation ofall its resources to cope with physical or psychicalaggression (strong emotion)”.

“Stress is an individual reaction and the resultof interaction between environmental demands onone hand and the resources, capabilities and opportunitiesof the individual on the other hand.”

It is certain that stress is characterized byintense hormonal changes, massive secretionof adrenaline. There are also morbid changes(hypertension, gastric ulcer etc). Psychical stressis caused by prolonged emotions primarily mainlydue to frustration, conflict and anxiety. There isstress of overload and also stress of underload. Amoderate stress boosts and stimulates the bodyvitality. The harmful feature of stress occurs whenthe damage is too large, exceeding the individualadaptive capacity.”

Doctor Ioan Bratu Iamandescu, in his book“Psychical stress and internal diseases” (1993), believesthat stress can be positive (eustress) or negative(distress).

Eustress (“positive stress“) has beneficial effectson the human body, occurring when thestress agents have positive significance for the individual.

These triggers positive emotions (intensejoy, ecstasy, triumph, laugh out loud), and positivelyaffect the body’s organs and apparatus. Eustressappears in the course of positive emotionalstates or coupled with moderate exercise (eg sexor jogging). Eustress is essentially acute. Frequentrepetition of eustress contributes to increase antiinfectionand antitumor immunity, becoming alongevity premise.

Distress (“negative mental stress“) is widelyrecognized as pathological. It usually produces painand inadaptation as a result of contact with a stressoragent.

Situations generating mental stress:

- the existence of unusual circumstances forthe individual, which find him unpreparedto deal with them
- the significance of an event
- engaging the individual into an exaggeratedaction or relation
- peculiarities of the social context
- lack of internal conditions
- subjective way of perceiving environmentaldemands

- underload / overload
- existing conflictual situations in the family,profession or intellectually related
- lack of time
- isolation
- the emergence of a physical or mentalobstacle in the way of a goal that leads tofrustration
- disturbing circumstances arising fromphysical agents (noise, vibration,temperature fluctuations).

STRESS AND HEALTH AT WORKPLACE

MANIFESTATIONS OF STRESS AT WORKPLACE

Stress at work can affect anyone, at any level.

It is produced in every sector and in organizationsof any size. Stress affects the health and safety ofindividuals and also welfare of organizations andnational economies.

Workplace stress occurs when job demandsexceed the available resources of human beings.Stress is not only the result of major adverse events,but also is the result of stress and daily pressures.The latter, by their frequency, have an importantrole in the professional environment and affectmore individuals than major adverse events, whichare rare.

Andreescu Anghel, Liţă Ştefan (2006), statesthat "sometimes, professional stress is consideredas a positive, beneficial factor on performance.

Thisrefers to eustress which means activation, mobilizationof individual resources. It is important todistinguish between eustress and distress, one as astate of stress with beneficial effects, the other withadverse health effects."

Today's world, more dynamic in work fieldperspective, brings in the foreground workers, personnelreductions and services externalization, increasedflexibility in terms of position and competencies,the growth in number of determined timejob contracts, growing uncertainty of jobs and intensity of work (overload and higher pressure), andunstable balance of life and work.

That is why stresscan bring disease and pain to individuals, both atwork and at home.

Stress can compromise safety at work too, thuscontributing to other health issues related to work,such as musculoskeletal disorders.

Stress greatly affects the image of an organization.

Reducing stress associated with work andpsychosocial risks is not only a moral imperativebut also a legal one. It is also an important problemof economic efficiency. The good news is thatstress associated with work can be approached inthe same logical and systematic way as other healthand safety issues.

There is an abundance of practicalexamples to cope this problem in the area ofEurpean Union countries. Using the appropriatemethod, workers may be stress protected.

Considering the example of a person who feelsgreat pressure meaning job demands (work time, liability)larger than individual capabilities and corroboratedby conflicts with colleagues/leaders, frequentchanges or threats on job security – such asthe possible personnel downsizing; all these couldbe perceived as a stressful situation by someone orchallenging by another. What makes the differencein perception depends on job nature, the psychologicalprofile of individuals, as well as physicalhealth or health, in general.

Manifestations of stress at workplace canlead to: depression, anxiety, feelings of being overwhelmedand unable to cope with them, a decreasein professional performance, an increase in thenumber of days for sickness treatment, absenteeism,insomnia, cognitive difficulties (reducedability to concentrate or make decisions), fatigue,headache, palpitations, gastrointestinal problems, increased aggressivity, etc.

All these lead to the followingconsequences:
- a decrease in productivity
- an increased risk of having an accident atwork
- damage in personal relationships
- an increased risk of health problems(cardiovascular disease, digestive problems,cervical pain, etc.)

STRATEGIES TO ELIMINATE AND

PREVENTSTRESS AT WORK

Recent studies of the so-called "healthy organizations"suggest that policies for employee healthlead to benefits for organizations.

A healthy organizationis defined as an organization with a low rateof illness or disability in working personnel.

Thatmeans an increased competitivity. Researchers haveidentified that a minimum stress at work leads toa higher level of productivity as definition for ahealthy organization.

These relate to:
- Recognizing employee's performance
- Opportunities for career development
- Organizational culture that values employee
- Managerial actions in concordance with thevalues of the organization.

Strategies to reduce stress at workinclude:
a) psychological assistance for employees isan occupational health service; that is provided byorganization in order to reduce or eliminate the decreasingperformance at workplace;
b) training services for employees: performancemanagement, time management, stress management,career management, etc. ;
c) educational workshops: parenting,work lifebalance, time management, stress management,emotional control, anger management, decisionmaking and problem solving, alertness, communicationin couple, smoking quittance, weight control, etc.;
d) online educational resources: newsletter, accessto educational information for the employeeand his family (web resources, brochures);
e) career management services;
f) services for managers and professionals inhuman resources departments are:
- management of critical incidents ;
- assistance for enhancing skills in control ofinadequate behaviors of employees;
- assistance and training in management ofemployees capabilities;
- development and implementation of employeeassistance programs in the company.

STRESS PREVENTION AT WORKPLACE

There is no standardized approach or a manualfor developing stress prevention programs. Designprograms and solutions will be influenced by manyfactors: the size and complexity of the organization,available resources, and in particular the typeof problems the organization faces.

For example, insome companies the main problem is overloadingemployees and others, an inflexible program or lackof communication with the public. In other words,it is not possible to find a universal prescription forstress prevention in the workplace, but it is possibleto provide some guide lines for the prevention ofstress in organizations.

In all cases, the process of stress preventionprograms involves three distinct approaches: problemidentification, intervention and evaluation. Organizationsmust be prepared properly to lead thisprocess successfully.

A minimum level in preparing a stress preventionprogram should include the following:
- workplace stress awareness (causes, costs,control);
- ensuring a quality management and supportfor the program;
- employee involvement in all phases of theprogram;
- establishing the technical capability ofprogram management (specific training formembers or consultants involvement).

Assembling employees or employers and managersin the same committee or "solving problemgroup" can be a very useful approach for developinga stress prevention program.

Researches show that these participatory effortswere successfully achieved on ergonomic issuesin the workplace, partialy due to the capitalizationof direct knowledge of employees about theproblems encountered in their workplace.

The "psychological contract" as a mean of preventingand combating stress is today a frequentapproach. In the literature the term "psychologicalcontract" is commonly used in the sense of mutuallyshared set of expectations between the employeeand the organization.

The author Denise Rousseau defines the psychologicalcontract as “individual beliefs shaped byan organization, in terms of exchange between theindividual and the organization”.

Psychological contractcan be accepted as a mental model that employeesuse to inframe and interpret organizationalphenomena. Its terms shall constitute a referencesystem to which employees report their work andthe attitude of employers towards employees is alsovery important.

Characteristics of the psychologicalcontract:
- represents, essentially, a subjectiveperception that differs from one individualto another;
- psychological contract is dynamic, thatmeans it is changing over time, duringthe relationship between employer andemployee;
- refers to the mutual obligations basedon investment in promises made by bothparties, with the hope of a positive outcomefor each party;
- is closely linked to the context of laborrelationship, the psychological contract cannot be created by individual or organizationonly.

The main functions of thepsychological contract:
- reduces incertitude, because not all possibleaspects of the employment relationship maybe covered by a formal written contractbetween the two parts: employer andemployee;
- dictates employee behaviors - like a system,the employee weighs their commitmentto the organization and the organization’sobligations to themselves and change theirbehavior in relatation with critical companyoutcomes;
- the third function of psychological contract,gives to employee a sense of influence onwhat happens to him, in the organization.

To conclude, stress is a “disease” of our time, affecting people no matter of their lifestyle.

Stress is found everywhere, more obvious and more frequent in advanced countries. To find theway to manage stressful situations is up to each of us.

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SYSTEMATIC REVIEW

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New quaternaryammonium salts baseddecontaminants

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Abstract: *Decontamination after terrorist attacks or industrial accidents with biological and/or chemical agents („bio-chem“) must be fast and efficient, in order to reduce the number of victims and to eliminate the consequent damages. The decontamination of living biological agents (bacteria, viruses) or nonliving ones (toxins, regulators) and toxic chemicals could be accomplished by reactions of hydrolysis in various experimental conditions, in particular in alkaline medium, reactions with amines or ammonia, alcohols, phenols etc. and by their transformation into less toxic degradation products.*

"Bio-chem" intentional or unintentional contamination is a real risk, towards which an effective management must be available to prevent and control it. Decontamination is an essential measure to protect the personnel and the environment.

Synthesis and testing of new „bio-chem" decontaminants, based on quaternary ammonium salts, complete the arsenal of protection against chemical and biological agents.

The most effective selected substances could be produced and used for decontamination in accordance with legal procedures.

Keywords: *„bio-chem" contamination, decontaminants, disinfectant, quaternary ammonium salts, chemical synthesis*

DECONTAMINATION

After terrorist attacks, biologicaland/or chemical attacks or industrial hazardswith bio-chem agents, the decontaminationmust be prompt and efficient, in order to reducethe number of victims and to eliminate the consequentdamages. The decontamination of the livingbiological agents (bacteria, viruses, fungus, andparasites) or of the nonliving agents (toxins, regulators)and of the toxic chemicals could be accomplishedby hydrolytic reactions in various experimentalconditions (especially in alkaline medium),reactions with amines or ammonia, with alcoholsand phenols etc. or by their transformation in lesstoxic degradation products.

The biological contamination refers to bacteriaand viruses generating diseases as anthrax,plague, smallpox, botulism etc. From the chemicalstructure point of view, the range of the productsused in microbial decontamination is wide butvery few fulfill the conditions of a good decontaminant.

In this paper there are taken into accountthe quaternary ammonia salts obtained by treatingthe tertiary amines with alkyl halogen, particularlyalkyl chlorides or benzyl chlorides. These compoundsare the most important agents having abiocide action.

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Synthesis and conditioning decontaminant byMechanical Engineering and Research Institute,Bucharest (ICTCM) in different forms with thechemical structure of quaternary ammonium saltsoluble in water was carried out in accordance withnational and European Union legislation related toecology and environmental protection.

Figure 1. Microbiological testing of decontaminating substances, in vitro, in CCSMM microbiology laboratory

Figure 2. Microbiological testing of decontaminating substances in various materials, CCSMM minipolygon biological testing

Spectrum of activity and penetrating ability ofdecontaminating substance is enhanced by a surfactantthat is designed to reduce surface tensionthus facilitating contact between the microbiancell and decontaminant compound.

Decontaminant qualities of each product aredetermined by the choice of the active substanceand should take account of its antimicrobial qualities,the purpose, and the conditions that will beused in.

Decontamination efficiency is determinedby the contact time of each decontaminant product,decontaminating solution concentration, theamount of solution used per unit area and, not thelast, by the decontaminant agent application mode.

SCREENING TEST

Screening of potential decontaminatingsubstances was performed in the Laboratoryof Microbiology-Epidemiology, in diagnosislaboratory for biological agents, ranked by P2+biosafety level for the pathogen microorganisms.

Bacterial sensitivity assays were performed (theantibiotic disc diffusion method on several lots),according to the following parameters: cultivationon solid culture medium Mueller-Hinton, 72hours aerobic incubation at 37°C, with daily reading.

The following bacterial species were tested:Staphylococcusaureas, Bacillus anthracis, Escherichiacoli, Pseudomonas aeruginosa, Vibrio cholerae.

Results were quantified by measuring with 0.1mmof accuracy (caliper and magnifier), comparatively,on many tests, to calculate the average of each substanceon each species.

Product code DC-3 and code DC-7 was carriedout on quaternary ammonium salts and Nalkylpyridiniumbasis. Their testing revealed thatthe best microbiological activity was recorded inthe product code DC-7. The possible synergisticeffect with oxidizing compounds was tested in orderto achieve possible decontaminating mixtures.

The bactericidal effect of the substance wastracked for a set of standard bacterial (gram-positiveand gram-negative, anaerobic bacterial and spores)and other pathogenic microorganisms; bacterialsensitivity results were read after 24 and 48 hours ofincubation and then, after storage at room temperature48 hours, to watch the effect in time.

The antimicrobial effect was quantified by calculatingthe average diameter of bacterial inhibitionzone and bactericidal concentration (g/liter),calculated as the quantity of substance (20 mg)divided by the volume in which the antimicrobialdiffusion was effective. (figure 1, 2).

Figure 3. Microbiological testing of decontaminating substances in various materials, CCSMM minipolygon biological testing

TOXICOLOGICAL TESTING

Toxicological screeningfor acute toxicity aimed to confirm that thesesubstances are not highly toxic and are not dangerousto operators.

To demonstrate, 0.5 ml ofeach substance was injected in mice (approx. 20gweight, young adult), tracking morbidity and mortalityfor three days. The test results have enabledexperiment achievements under conditions of aspecially arranged experimental minipolygon.

Experiencing decontaminating products

Representative microbial strains were used for themain groups of pathogenic bacteria: gram-positivecocci: Staphylococcus aureus, Streptococcuspneumoniae, Enterococcus fecalis; gram-positivebacilli: Bacillus anthracis (vaccine strain), Bacilluscereus, Bacillus subtilis; gram-negative bacilli:Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae,Pseudomonas aeruginosa; vibrio: Vibriocholerae.

The experimental contamination and decontaminationhave been carried out on an out ofservice military vehicle, representing the "target"(figure 3, 4) marked with numbered areas of approximately0.1 square meters on which have beenimplemented operating procedures for CBRN(chemical, biological, radiological and nuclear)contamination control.

Areas were chosen as follows: vertical paintedmetal sheet, for aqueous solution decontaminants;glass window for aqueous solution decontaminants;rubber tires for aqueous solution decontaminantsand horizontal painted metal sheet for suppliedpowder decontaminants as a positive control.

Microbial contamination was done by sprayingthe allocated surface, separately, with every microbial strain and with a mixture of them also. Amicrobial suspension culture in a liquid mediumwas used, with approximately 1 million live bacteriaper ml. Also, it was sprayed 1 ml of suspensionper square decimeter, enough to create a uniformcontaminant film. For liquid decontaminatingsubstances, the aqueous solution (conc.10%) wassprayed to cover the contaminated area until theexcess liquid begun to tear, about, 10ml/sqdm onsmooth surfaces (glass, metal sheets) and about20ml/sqdm on rough surfaces (peeling metalsheet, tires).

Figure 4. Microbiological testing of decontaminating substances on combat vehicle with Biological Mobile Intervention Team of CCSMM in CBRN training area from Campulung

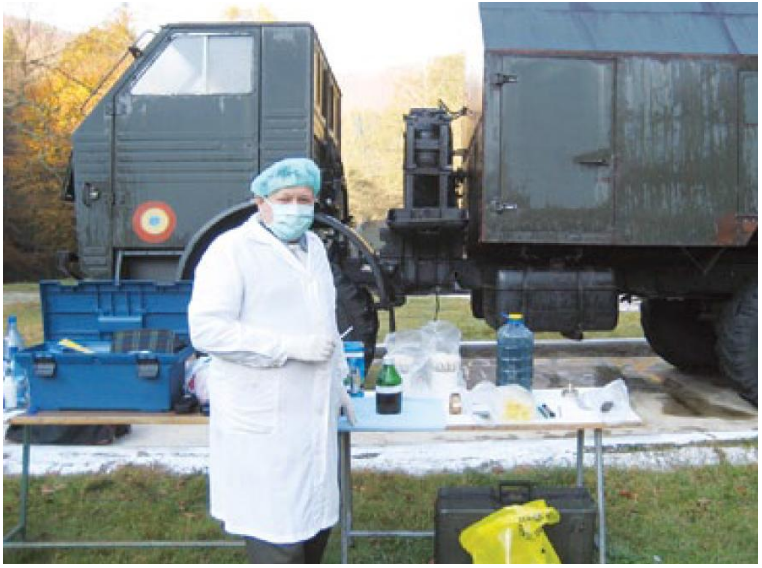

Microbiological samples were collected bymeans of hygienic-sanitary pad, as follows:

0. before contamination (to establish a baselinelevel of natural contamination);

1. immediately after contamination (tocheck the level of contamination experiment);

2. after decontamination for each decontaminantupon each biological agent within 10 min(as required for military using decontaminant);

3. at every 45 min (as for general householddisinfectant).

In addition, at the end of the experiment,samples were collected from different parts of theoperator's protective equipment and from the environmentto detect any residual contamination. Allsamples were immediately transported under biosafetyconditions and tested in the microbiologyP2+ laboratory.

Decontamination achieved resultsafter 45 min are shown in Table 1.

Table 1. Contamination level after decontamination by 10% aqueous solution

No.	Decontaminant	Biological agent	Surface	Time (min.)	Growth (tube)	Growth (pane)	Remarks
1	DC 17 ICTCM	Mixture	Glass	45	-	++hem.	Contaminated
2	DC 17 ICTCM	B. cereus	Glass	45	-	-	Uncontaminated
3	DC 17 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
4	DC 17 ICTCM	B. cereus	vertical metal sheet	45	+/-	-	Uncontaminated
5	DC 18 ICTCM	Mixture	Glass	45	-	-	Uncontaminated
6	DC 18 ICTCM	B. cereus	Glass	45	+/-	-	Uncontaminated
7	DC 18 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
8	DC 18 ICTCM	B. cereus	vertical metal sheet	45	+dep.	-	Contaminated
9	DC 19 ICTCM	Mixture	Glass	45	-	-	Uncontaminated
10	DC 19 ICTCM	B. cereus	Glass	45	+/-	-	Uncontaminated
11	DC 19 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
12	DC 19 ICTCM	B. cereus	vertical metal sheet	45	+veil, dep.	+	Contaminated

- or +/-: no microbial growth; dep.: bacterial deposit at medium bottom;
veil: bacterial population at medium top; hem.: hemolytic colonies.

COMMENTS

The antimicrobial effect remains and is moreobvious after 45 min, suggesting that these productscan be proposed as potential disinfectants formedical or hygienic-sanitary use, according withDrug Law.

The decontamination with powder is less effectivein all cases, compared with the laboratorycontrol sample, because of weak contact betweenmicro-organism and decontaminant, so a residualcontamination remains on surfaces.

In all cases, the final stage of decontaminationshould be washing with water because the substancesused can be corrosive upon certain materialsor irritating for personnel.

After the final washingaction, a microbiological analyze was madeto determine residual contamination of surfaces,waste water or protection equipment, but a significantcontamination was not recorded, so it can beconcluded that there is no risk to the environment.

CONCLUSIONS

A number of potential decontaminating substanceswere synthesized and tested, eight of whichwere selected and recommended as decontaminatingagents because these can be characterized byhigh chemical stability. These products are solublein water and various organic solvents. These biocidalproducts have bactericidal and fungicidal capabilities,with a large spectrum of usability. Thesubstances are characterized by low toxicity onanimals.

Theoretical and experimental scientific researchhas been made to design and implementpolyvalent “bio-chem” decontaminants for destructionof biological and toxic chemical agents,hazardous to health. Technologies have been developedfor obtaining polyvalent decontaminants,selected by efficiency assays, in laboratory. The experimentalpolyvalent model for chemical and biologicaldecontamination with decontaminants ofquaternary ammonium salts types was developed.

Among the tested products, the best antimicrobialactivity is achieved by products based onmixture of oxidizing compounds and quaternaryammonium salts, which also have a synergic effectwhen used, expanding the action spectrum.

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ORIGINAL ARTICLES

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News and Perspectiveson Treatment of NormalPressure InternalHydrocephalus

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Abstract: *Many patients, usually over 60 years old, presenting presenile dementia associated with marked gait disorders, impaired balance, urinary incontinence, have been shown to have enlarged ventricles associated with relatively small cortical atrophy. Intracranial pressure monitoring indicates normal values, or subject to only minor peaks, usually at night. Because some of these patients improve markedly after ventricular shunting procedures it has been suggested that their neurological dysfunction may be caused by a pressure effect on the brain from the increased internal surface of the ventricles. Many of these patients do benefit from surgery, and a lot of them have a history of subarachnoid hemorrhage, traumatic brain injury or meningitis which might have impaired the CSF absorption.*

INTRODUCTION

We would like to present the experienceof our clinic over the last fiveyears regarding the treatment ofnormal pressure internal hydrocephalus(28 patients operated between January2009 and December 2013), to report our results andcompare them with the statistics and results fromthe international literature. Normal pressure internalhydrocephalus (NPIH) represents an increase ofCSF volume, with different etiology, that causes anenlargement of the ventricular system as a consequenceof the hydrodynamic CSF circulation disorders.

The cause of this disease cannot be identifiedin 60% of cases. It was described in 1965 with theHakim & Adams triad: gait disorders and impairedbalance, cognitive disorders (progressive dementia),sphincter disorders (8). The imagistic explorations(CT scan) indicate the size of hydrocephalus; ICP< 15 mmHg and the pressure gradient between theventricles and subarachnoid space is very low. Fortreatment there are extrathecal shunts (particularlyventriculo-peritoneal shunt) and intrathecal shunts(particularly endoscopic ventriculocisternostomy).

Both methods have been practiced successfully inour clinic.

The ideal treatment method for hydrocephalusstill does not exist. None of the surgical techniquesis perfect and no shunting device gave total satisfaction(4, 7).

Hydrocephalus represents the enlargement ofone or more parts of the CSF containing anatomicstructures. The CSF total volume is about 150 ml inadult; the ventricular system contains 25-30 ml, thespinal subarachnoid space contains 30 ml and therest of CSF is contained by the cranial subarachnoidspace and basal cisterns (1).

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Figure 1. The ventricular enlargement shown on CT scan

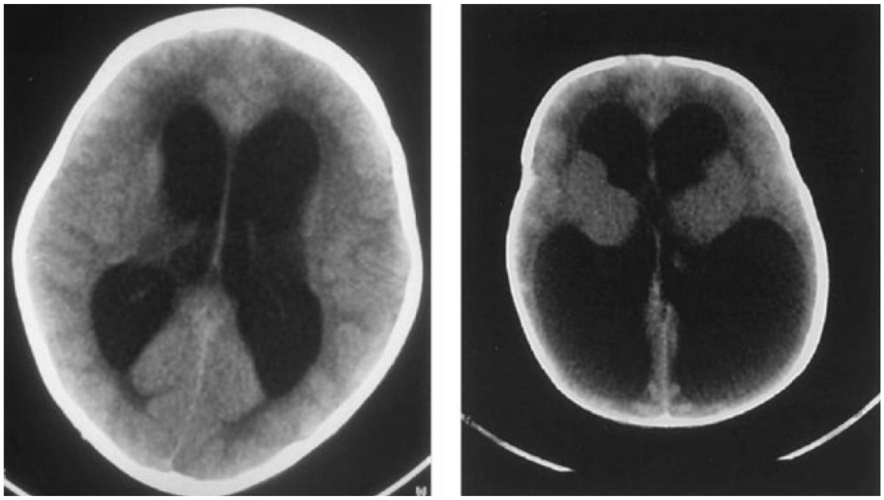

The total production ofCSF is about 600 – 700 ml/24 hours, approximately0,35 ml/min. The CSF participates in maintainingthe endocranial volume constant by adjusting theproduction/absorption ratio depending on the cerebral parenchyma volume and the intracranial bloodvolume variations according to the Monro-Kellierelationship. Changing the volume of one of thethree intracranial components (brain tissue, blood,CSF) is followed by a compensatory reaction fromthe other two components in order to maintain insidean inextensible space the endocranial volumeconstant (9).

Based on its underlying mechanisms, hydrocephaluscan be classified into obstructive and communicating.

The obstructive hydrocephalus is definedby any condition that restricts the CSF flowto and from the ventricular system. Any CSF flowinterruption outside the ventricular system definesthe communicating hydrocephalus (5).

Figure 2. Lateral ventricles puncture

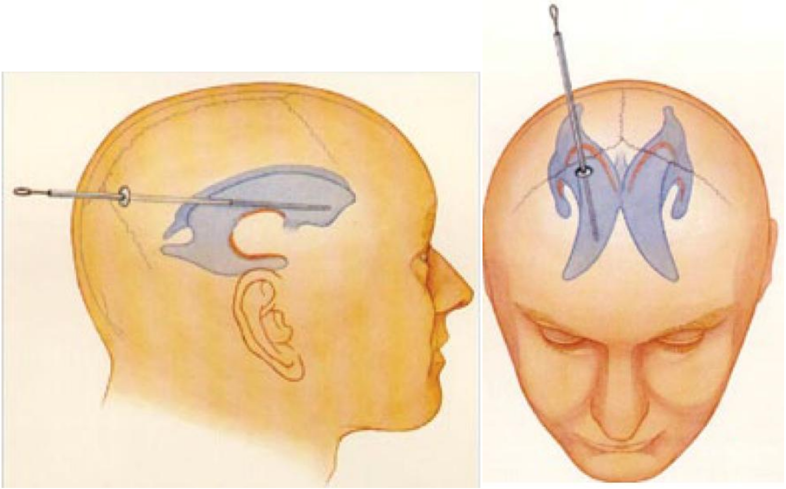

The NPIH etiology is not fully known. Thereare many possible congenital or acquired causes, butthe most important are the subarachnoid hemorrhages(20%), meningitis (1%), parasite infections,traumatic brain injuries, neurosurgical procedureswith open ventricular system, intoxications, Alzheimerdisease (15%).

Hydrocephalus of the adult patients is a communicating,chronic and normal pressure hydrocephalus(3).

DIAGNOSTIC

The CT scan and the MRI revolutionized thediagnosis and the postoperative follow-up of thehydrocephalus. The CT scan is the first stage of diagnosis.

It highlights an obstructive cause, evaluatesthe ventricular enlargement, appreciates the cerebralparenchyma condition (periventricular hypodensity)and the subarachnoid spaces condition (basal cisterns,sylvian fissures, interhaemispheric fissure, corticalsulci).

Bifrontal index measuring (the distancebetween frontal horns / intracranial distance ratio, onthe same CT slice) > 50% is suggestive for a possibledecompensation of hydrocephalus.

The Evans index> 30% has the same meaning (the frontal horns size/ the maximum biparietal diameter ratio). It suggestsan active hydrocephalus. The MRI completes the CTscan by describing the obstructive lesions accuratelyand obtaining dynamic information over the CSFflow (the absence of CSF flow through Sylvius aqueduct).

The preventing treatment is important becauseof the existing risk of postoperative hydrocephalus afterany neurosurgical procedure. Cisternograms withradioactive markers (99Tc-DTPA) remain controversialand rarely used.

The radionuclide is injectedinto the subarachnoid space by a lumbar punctureand serial images are taken by planar scintigraphy 3,6 and 24 hours after the injection. In case of NPIHintraventricular radioactivity can be obtained even48 hours after the injection. Serial lumbar punctureswith repeated evacuation of 15 – 30 ml CSF associatedwith clinical improvement can predict a favorableresponse to shunting procedures. Patients withinitial measured CSF pressure >15 mmHg respondedfavorably after ventriculo-peritoneal shunting (6, 8, 9).

TREATMENT

The treatment of hydrocephalus depends onthe moment of diagnosis, etiology, age and clinicalcondition of the patient (particularly the acuteform) and the complementary investigations results.

The treatment with acetazolamide, a carbonic anhydraseinhibitor has favorable effects predominantlyby inhibiting the chorioid plexus secretion and lessby the diuretic effect.

The acetazolamide dose is 25mg/kg/day with simultaneously administration offurosemide 1 mg/kg/day.

The treatment of obstructive hydrocephalus isremoving the obstacle (excision of tumors). The surgicaltreatment seeks not returning to normal size ofventricles but regaining most of the lost neurological functions.

The diuretic and corticosteroid therapiescomplete the CSF evacuation by lumbar punctions.The CSF lumbar drainage will be performed onlyafter the confirmation of communicating chronichydrocephalus by imagistic methods.

There are several types of extrathecal derivationsof CSF: controlled external ventricular drainage,ventriculoperitoneal drainage, ventriculoatrialdrainage, ventriculopleural drainage.

The currentlyused valves are predetermined opening pressurevalves (low, medium and high pressure), modularopening valves and programmable valves with variableresistances, self-regulating valves etc.

The intrathecal derivation (particularly theendoscopic ventricular cysternostomy) representsan alternative treatment method. Both extra- andintrathecal derivations have been successfully performedin our clinic.

The intrathecal CSF derivations consist of endoscopicventriculostomy through the 3rd ventriclefloor aiming to restore the communication betweenintraventricular and subarachnoid liquidian compartments.

MATERIAL AND METHODS

We retrospectively studied 28 patients admittedto our clinic between January 2009 and December 2013. The patients were all neurologicaland imagistic diagnosed with normal pressure internalhydrocephalus.

Only in 18 cases, a cause forimpaired CSF absorption and ventricular enlargementcould be detected. 8 patients had a historyof traumatic brain injury, 4 patients had a historyof subarachnoid hemorrhage (First and secondgrade on Hunt and Hess scale) with normal “4vessels” cerebral angiogram, 2 patients were diagnosedwith Alzheimer disease prior to admissionand 4 patients had a history of ischemic stroke.

3 of the patients with NPIH after subarachnoidhemorrhage underwent endoscopic procedureswith intrathecal derivations (ventriculocisternostomy).

On the other 15 patients were performedventriculo-peritoneal shunts using various valves(most of them were low pressure valves). The mostfrequently used were the Delta (Medtronic), Spitz – Holter, Heyer – Schulte, Pudenz, Cordis – Hakimvalves. In 10 cases, a cause for NPIH was not revealedand these patients were diagnosed withidiopathic NPIH. They have also been performedventriculoperitoneal shunts with low pressurevalves.

Clinical improvement was significant inmost patients, but only partial in the patients diagnosedwith Alzheimer disease.

According to statistics from literature, urinaryincontinence is the main symptom that resolves aftershunting procedures.

Gait disorders and impairedbalance are subsequently remitted and dementia isthe last that improves (3, 4, 8).
Black and collaboratorsestablished few criteria that can predict thefavorable clinical course after shunting procedures:
- clinical: the presence of symptomatic triad;approximately 77% of the patients presenting gaitdisorders as primary symptom improve their locomotorfunction after shunting; the patients withdementia without gait disorders rarely improve afterdrainage;
- patients with CSF pressure >18 mmHgon lumbar puncture or continuous monitoring improvetheir neurological status after ventriculoperitonealdrainage;
- patients with CT or MRI showing largeventricles with minimal cortical atrophy have favorableevolution after shunting procedures.

The response to drainage is especially good asthe symptoms started recently (1, 5).

ILLUSTRATIVE CASES

Case 1.

66 year old male patient complaining ofgait disorders, impaired balance, cognitive impairment(occasionally) and sphincter disturbances(imperious need to urinate) which started abouta year ago, slowly progressive despite of conservativetreatment. We decided to install ventriculoperitonealdrainage with self-regulating valve. Postoperativeevolution was favorable, symptoms therebyimproving considerably about 3 months after theprocedure.

Case 2.

59 year old male patient complaining of headache, nocturnal insomnia, depressive syndrome,cognitive impairment, gait disorders and locomotor’s instability.

Case 3.

68 year old female patient hospitalized for memory disorders, sphincter disturbances (imperiousneed to urinate), gait disorders, vertigo, occasional headaches and depressive syndrome. The CT scan

revealsenlarged ventricular system. It was decided to install VP drainage with Delta low pressure valve. The symptomsimproved partially after one month with significant improvement 6 months after the procedure.

Figure 3. The ventricular catheter placed inside the right lateral ventricle and the selfregulating valve placed in the right parietal region, under the scalp.

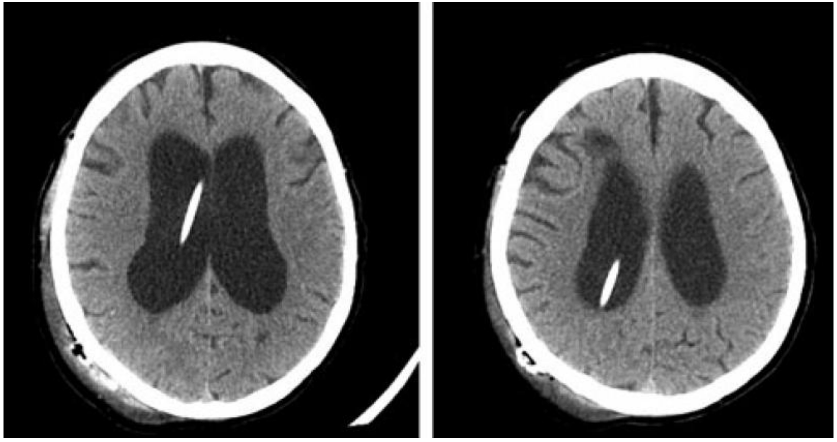

Figure 4. The ventricular catheter placed inside the frontal horn of the right lateral ventricle, near septum pellucid; VP drainage with Delta (Medtronic) low pressure valve.

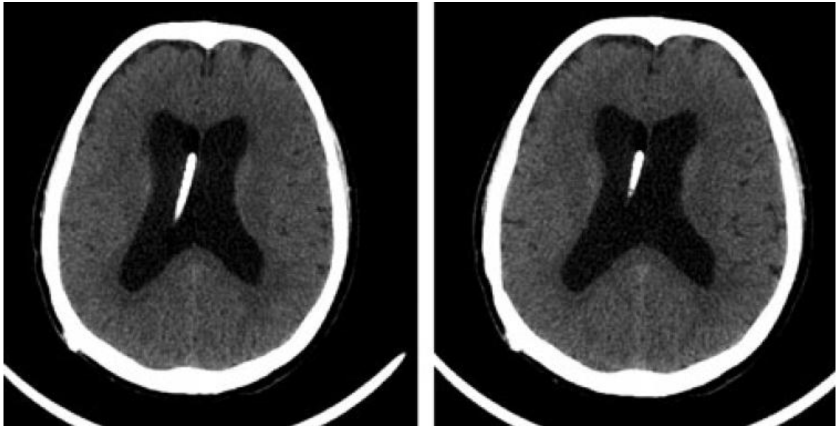

Case 4.

64 year old male patient facing important balance disorders, persistent vertigo, extremely difficultgait, urinary incontinence (and incipient stercoral), onset of Alzheimer disease (after neurological and psychiatricexaminations). It was inserted a Delta low pressure valve. The symptoms improved partially after3-4 months; the gait has become easier, sphincter disturbances have improved and the cognitive impairmentstill exist, but more tolerable.

Case 5.

72 year old female patient hospitalized for walking difficulties, balance disorders, persistent vertigoand vomiting, dehydration. A central venous catheter was inserted into the right subclavian vein andVP drainage with a Delta low-pressure valve was inserted on the left side; intraoperative CSF pressure was15 mmHg.

Case 6.

66 year old male patient with memory disorders, impaired balance and gait disorders, cognitiveimpairment, urinary incontinence (occasionally), started over a year ago, with progressive evolution. Intraoperativemeasured ICP was 18 mmHg. Ventriculoperitoneal drainage with Holter low pressure valve wasinstalled. The

symptoms improved after one month with the complete remission of gait disorders, impairedbalance and sphincter disturbances.

Figure 5. The ventricular catheter placed inside right lateral ventricle (functional drainage).

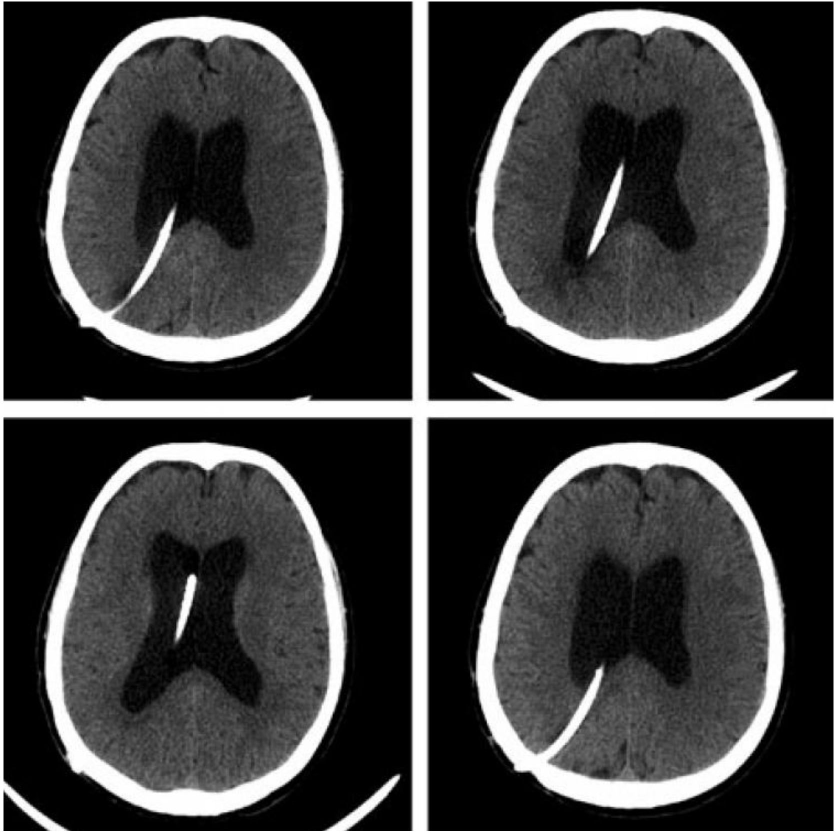

CONCLUSIONS

The ventricular enlargement and the pressureon the frontal lobes are probably responsible for theoccurrence of cognitive disorders and dementia.

The pressure on the regulator centers of the sphincterfunctions located in the paracentral lobule maybe responsible for the urinary incontinence. Theventricular dilatation may compress the internalcapsule and secondary the pyramidal tract, responsiblefor the gait disorders, impaired balance and pyramidalsyndrome (4, 5, and 9).

To determine the surgical indication and to anticipatethe subsequent postoperative evolution, severalclinical and imaging criteria must be established:

- the obstructive causes for hydrocephalus must be excluded;it must be a communicating form;
- a periventricular hypodensity on CT scan or hyperintensityon T2 sequence (MRI) might be transependimar CSFresorption and might anticipate a favorable evolutionafter the shunt procedure;
- the rounding and symmetrical ballooning of the frontalhorns;
- dilated focal of convexity sulci may be revealed by imagingstudies and they are atypical CSF reservoirs whichsubsequently decrease after drainage and they should notbe confused with cortical atrophy; typical cortical atrophy(ex vacuo hydrocephalus) occurs frequently with Alzheimerdisease, and there is a limited response to ventriculoperitonealdrainage (11).

Figure 6. Preoperative MRI (T2 sequence) and postoperative CT scan; right ventricular catheter;prominent convexity sulci; incipient cortical atrophy

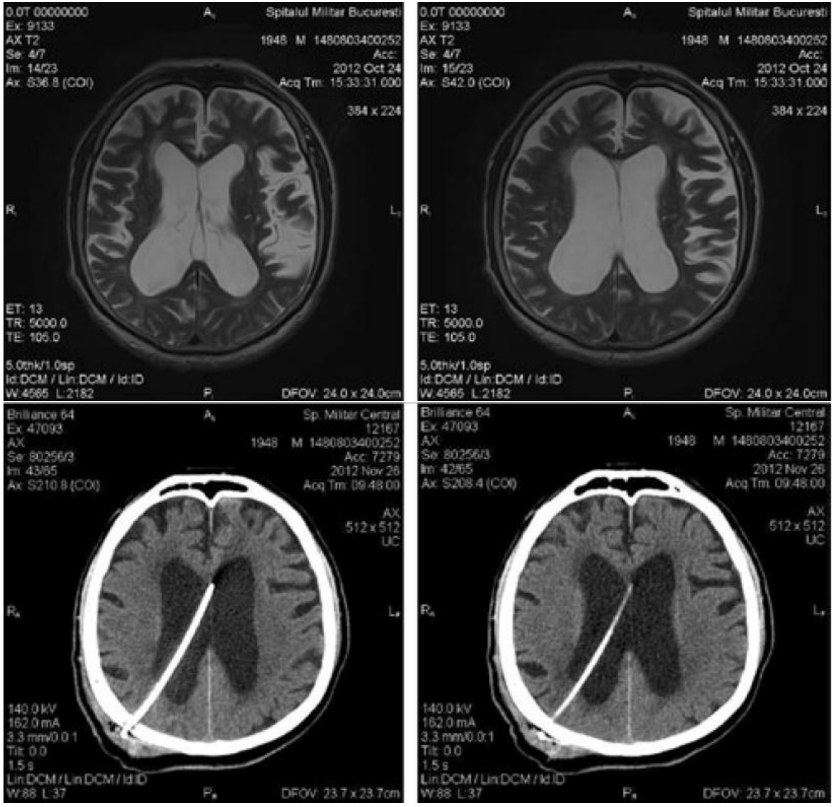

Figure 7. Preoperative and postoperative CT images; the catheter has been placed inside the left lateral ventricle because a central venous catheter was inserted into the right subclavian vein (functional drainage).

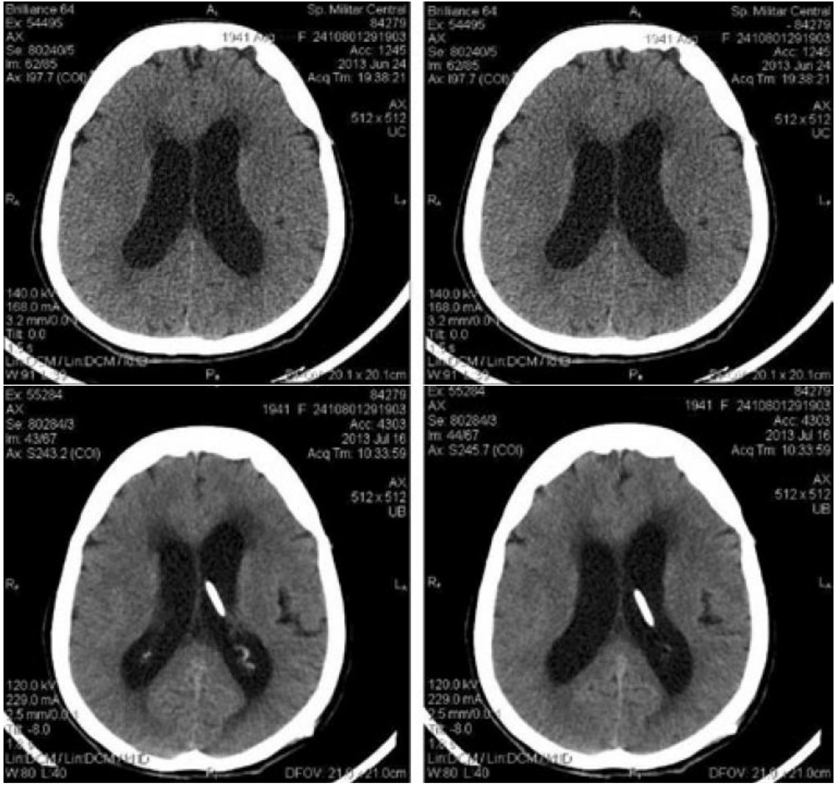

Figure 8. Important hydrocephalus with cortical sulcus persistent; catheter placed inside the left lateral ventricle (coronal section).

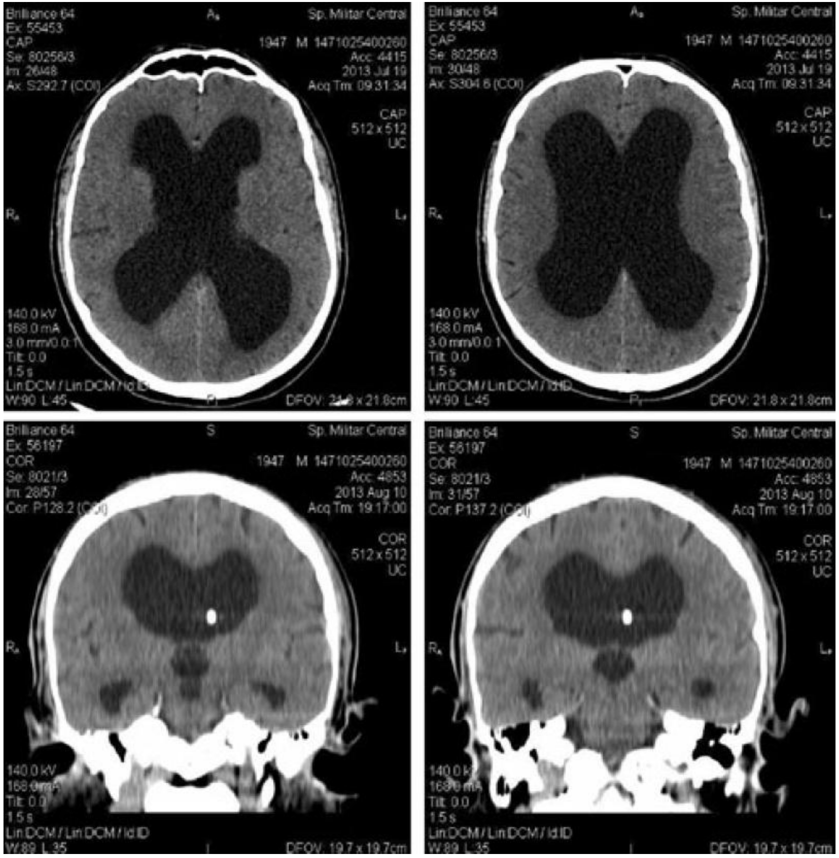

Maintaining good results depends on periodicmedical checks, immediate recognition and treatmentof complications (insufficient drainage shouldbe suspected first). There is no ideal method for thetreatment of hydrocephalus. None of the surgicaltechniques is perfect and no device gave total satisfaction.

The accelerated development of technology andthe practical experience in CSF intra- and extrathecaldrainage allow overcoming the current difficulties.

The world population undergoes a pronounced agingprocess, the number of people aged over 60, increasingto 400 million over the last 40 years. The ratio ofthe elderly population has been modified, representingover 50% in developed countries, with the Europeanzone being the aged (10).

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CLINICAL PRACTICE

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Nonalcoholic fatty liverdisease – an etiologicalapproach

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Abstract: *Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of fat in the liver (hepatic steatosis) either on imaging or on liver histology only after the exclusion of secondary causes of fat accumulation in the liver (e.g. high alcohol drinking, drugs and other medical ailments). Considering the fact that there are many causes of hepatic steatosis, the term NAFLD is reserved for the liver disease that is predominantly associated with obesity and metabolic syndrome. The presence of inflammation and cell injury defines steatohepatitis (NASH) which has the potential to evolve into cirrhosis and hepatocarcinoma, being, therefore, the stage of NAFLD most amenable to treatment. Among the treatments available, the most important are: weight loss, vitamin E and, last but not least, probiotics.*

INTRODUCTION

Excessive alcohol consumption must beexcluded (>21 drinks per week in menand >14 drinks per week in women overa 2-year period before the baseline liverbiopsy).

Insulin resistance (figure 1) is, therefore,central to the development of NAFLD, as it is centralto metabolic syndrome (MS). The Adult TreatmentPanel III defines MS as the presence of three or moreof the following features:

1. Waist circumference greater than 102 cm inmen or greater than 88 cm in women,
2. Triglyceride level greater than or equal to 150mg/dL,
3. High-density lipoprotein cholesterol level lessthan 40 mg/dL in men and less than 50 mg/dLin women,
4. Systolic blood pressure greater than or equalto 130 mm Hg or a diastolic pressure greaterthan or equal to 85 mm Hg, and
5. Fasting plasma glucose level greater than or equal to 110 mg/dL.

Patients with features of MS are at high risk forNAFLD.

The gold standard for the diagnosis of NAFLDis hepatic biopsy which further characterizesthe ailment deriving two stages of histologicalevolution: nonalcoholic fatty liver (NAFL) (thismay be the plain hepatic steatosis) and nonalcoholicsteatohepatitis (NASH).

NAFL is defined as hepaticsteatosis with no evidence of hepatocellular injuryin the form of hepatocyte ballooning. NASH isdefined as the presence of hepatic steatosis andinflammation with hepatocyte injury (ballooning)with or without fibrosis. Although NAFL may beproportionally more common than NASH, onlypatients with NASH have the potential to progressto cirrhosis. The presence of the characteristicballooning injury is considered to be the key tothe diagnosis.

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Figure 1: Insulin resistance – central to metabolic syndrome

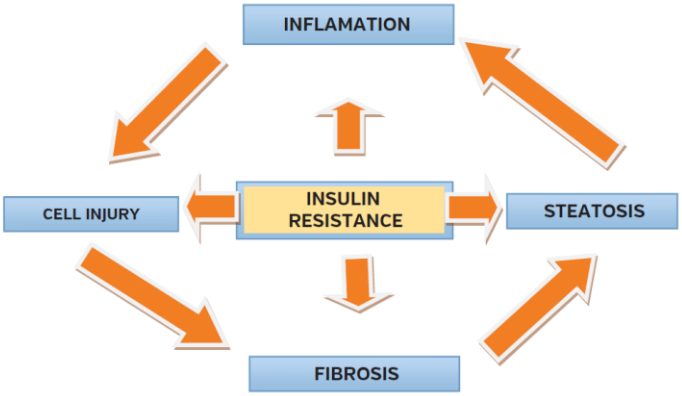

Ballooning injury results in enlargedvacuolated cells, classically containing Mallory-Denk bodies, which are eosinophilic cytoplasmicinclusions near the nucleus. The most importantinjury may be identified in zone 3 (around thecentral venule of the hepatic lobule) and this patternof distribution is also characteristic of NAFLD.

Thecardinal histologic feature of NAFLD is the presenceof an excessive accumulation of triacylglycerols(TAG) and in hepatocytes. The presence of obesityand insulin resistance lead to an increased hepaticfreefatty acid (FFA) flux creating an environmentappropriate for the development of NAFLD/NASH.

The resultant net increase in hepatic FFAis hepatotoxic unless it is converted to nontoxicintracellular triglyceride (TG). When the synthesisof TG is impaired, the level of FFA in the liver isincreased with subsequent augmentation of hepaticfatty acid oxidation resulting in the overproductionof reactive oxygen species (ROS) also known as freeradicals causing hepatocellular injury.

Based on this biochemical knowledge, a two-hithypothesis for the pathogenesis of NASH has beenproposed. The first hit involves the accumulationof excess triglyceride and particularly FFA inhepatocytes. The second hit is the generation oftoxic reactive oxygen species with the production ofhepatic injury and inflammation as a consequenceof FFA oxidation which ultimately leads to theinitiation and progression of fibrosis [1].

Hence, steatosis is mandatory for the diagnosisof NAFLD but alcohol consumption and chronichepatitis C should be taken into account as two ofthe most important alternative causes amenable todifferent treatments.

The diagnosis of NAFLD requires the following:

(1) Hepatic steatosis according to imaging or histology

(2) No significant alcohol consumption

(3)No competing etiologies for hepatic steatosis (table 1)

and

(4) No coexisting causes for chronic liver disease.

NAFLD is the most common cause of abnormalliver chemistry, so other causes, like those in table 1,should be ruled out. The majority of patients withNAFLD are asymptomatic. The most frequentlyencountered symptoms are: vague right upperquadrant dull ache or discomfort. Hepatomegaly isthe most common physical finding. Other clinicalsymptoms and physical findings are, also, nonspecific:general malaise, abdominal discomfort, nausea.

Celiac disease always should be ruled out insuspected individuals considering the fact that thisdisease is often underdiagnosed and seldom to betaken into account as a differential diagnosis ofhipertransaminasemia.

Table 1. Conditions associated with the risk of hepatic steatosis

STEATOSIS
• Insulin resistance
• Obesity
• Type 2 diabetes mellitus
• Dyslipidemia
• Hypertension
• Sedentary lifestyle
• Corticosteroids
• Estrogens
• Amiodarone
• Antiretroviral medications
• Obesity surgery (e.g., jejunoilealbypass)
• Rapid weight loss
• Carbohydrate excess (e.g., diet andtotal parenteral nutrition)
• Chronic hepatitis C virus, mainlygenotype 3
• Hypothyroidism
• Polycystic ovarian syndrome

Once NAFLD is diagnosed, the next step is todetermine the severity as it is necessary to establish theprognosis. Clinical examinations and laboratory andimaging studies in combination lack the sensitivityand specificity for distinguishing NAFL from NASHand for determining the presence and stage offibrosis, which is the most important determinant forthe severity and progression of disease.

Circulating levels of cytokeratin 18 fragmentshave been investigated extensively as novelbiomarkers for the presence of steatohepatitisin patients with NAFLD, but this testing is notroutinely recommended. Other noninvasive testsare emerging; however, these are not yet ready forprime time. [1,2, 3]

Liver biopsy still remains the most reliable approachfor identifying the presence of steatohepatitisand fibrosis in patients with NAFLD.

The recommendations for liver biopsy areas follows (figure 2):

1. Patients at increased risk for steatohepatitisand advanced fibrosis according to the presenceof features of MS and possibly the NAFLD fibrosisscore.
2. Patients with suspected NAFLD for whomcompeting etiologies of hepatic steatosis andcoexisting chronic liver diseases cannot be excludedwithout liver biopsy.

There is a general consensus that patients withNAFL have a very slow progression (if any). Onthe other hand, patients with NASH can exhibithistological progression and can develop fibrosis(37%-41%) and cirrhosis (Approximately 5%) [3].Importantly, hepatic cancer can occur in NASH inthe absence of cirrhosis. This is why every effortshould be made to identify patients with NASH asthey are the ones to progress to more severe formsof disease. The presence of NASH can be associatedwith higher liver-specific mortality in comparisonwith the general population. Cardiovascularailments associated with NASH (as metabolicsyndrome) contribute significantly to mortalityand morbidity. Patients with NAFLD are also atincreased risk for hepatocellular carcinoma, butthis risk is likely limited to those with advancedfibrosis and cirrhosis (1%-42%) [2]. Furthermore, acomparison of the natural history of NASH cirrhosiswith hepatitis C cirrhosis reveals that patients withNASH cirrhosis have a significantly lower risk ofhepatocellular carcinoma. [2]

NAFLD is typically characterized by ahepatocellular pattern of liver-related enzymeswith mild elevations (1-2 times the upper limit ofnormal) in serum alanine aminotransferase (ALT)and aspartate aminotransferase (AST). Up to 50%of NAFLD patients have normal liver biochemistry.Therefore, several biomarkers may aid in thediagnosis. The diagnosis of NASH without a liverbiopsy remains the most significant clinical challengein the evaluation of a patient with hepatic steatosis.Several biomarkers may distinguish between simplesteatosis and NASH. Some of the inflammatorymarkers include serum C-reactive protein,interleukin-6, ferritin, hyaluronic acid (HA), tumornecrosis factor a, leptin, adiponectin, and resistin.

Apoptosis plays a key role in the pathogenesis ofNASH. Among the markers of apoptosis, plasmacytokeratin 18 (CK-18) is emerging as one ofthe promising biomarkers for the noninvasivedetection of NASH. Since oxidative stress also playsan important role in the pathogenesis of NASH,several biomarkers of oxidative stress have beeninvestigated. Among these, oxidized low-densitylipoprotein, thiobarbituric acidreacting substances,superoxide dismutase, and glutathione peroxidasedismutase have been examined. The NASH testcombined 13 variables [age, sex, height, weight, andserum levels of triglycerides (TGs), cholesterol, a2-macroglobulin, apolipoprotein A1, haptoglobin,gammaglutamyl transpeptidase (GGT), ALT, AST,and total bilirubin] to achieve positive predictivevalue, and negative predictive value of 66%, and81%, respectively. [2, 3, 4]

Figure 2: Algorithm for liver biopsy in NAFLD

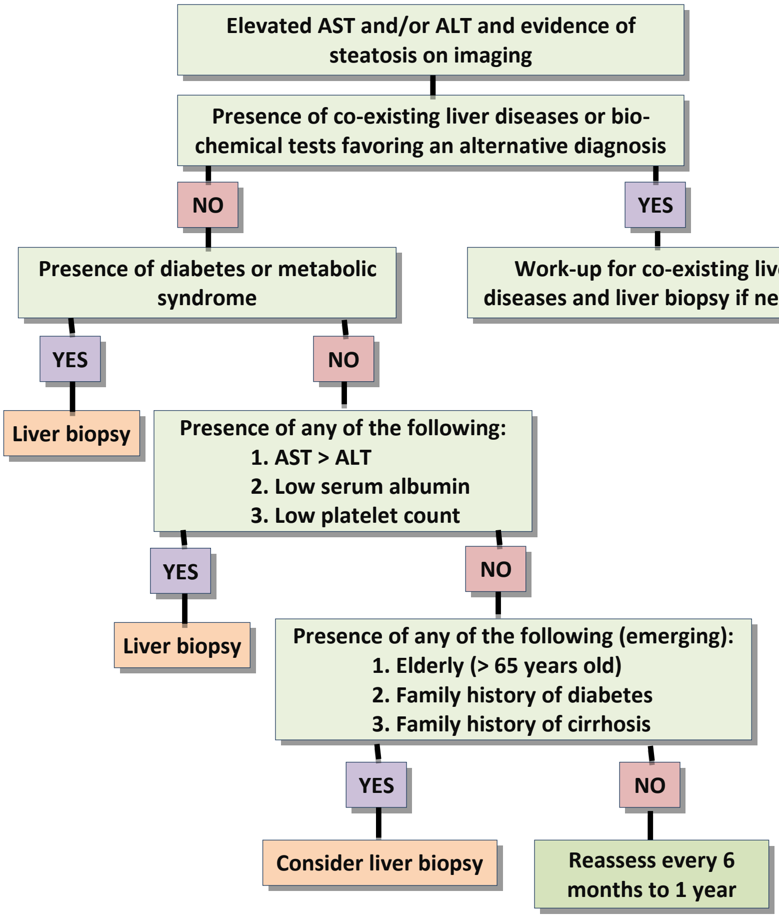

Elevated AST and/or ALT and evidence of steatosis on imaging
Presence of co-existing liver diseases or bio-chemical tests favoring an alternative diagnosis
NO
YES
Presence of diabetes or metabolic syndrome
Work-up for co-existing liver diseases and liver biopsy if needed
YES
NO
Liver biopsy
Presence of any of the following:
1. AST > ALT
2. Low serum albumin
3. Low platelet count
YES
NO
Liver biopsy
Presence of any of the following (emerging):
1. Elderly (> 65 years old)
2. Family history of diabetes
3. Family history of cirrhosis
YES
NO
Consider liver biopsy
Reassess every 6 months to 1 year

Two of the most promising tests for diagnosingadvanced fibrosis in NAFLD are the EuropeanLiver Fibrosis (ELF) score and the NAFLD fibrosisscore. The ELF score includes HA, tissue inhibitorof metalloproteinase 1 (TIMP1), aminoterminalpeptide of procollagen 3, and age. The NAFLDfibrosis score is helpful in the clinical setting becauseit uses routinely available variables in the clinicalsetting, including age, BMI, hyperglycemia, plateletcount, serum albumin, and AST/ALT ratio. We useroutinely available models or markers that increasethe pretest likelihood of finding more advancedliver disease on liver biopsy. These tests can aid inclinical decision making for patients with NAFLD.

Some of these markers are a high AST/ALT ratio, ahigh AST/platelet ratio, low albumin levels, and lowplatelet levels. [2, 3]

TREATMENT LANDMARKS

Among patients suffering from NAFLD (morethan 50% of them being asymptomatic) treatmentis mandatory only in NASH patients because onlythose have the potential to evolve into more severediseases (cirrhosis, hepatocarcinoma) [5].

Because NASH is linked to excess body weightand resulting insulin resistance, diet and lifestylemeasures are the recommended first-line therapy.

Optimal treatment begins with weight loss andphysical exercise. A tangible target for patientswith NAFLD is a weight loss of 5% to 10% of totalbody weight over a 6- to 12-month period [6]. Thosemeasures may improve insulin sensitivity, increaseadiponectin expression, lipid profiles, and liverbiochemistry. The improvement in liver enzymedoes not always correlate with improvement inhepatic histology, unfortunately. As it is alreadydemonstrated, weight loss by dietary changesmay be beneficial even without physical exercise;although physical exercise may lead to furtherimprovement in insulin sensitivity. The initiationof increased physical activity must be the first stepto the treatment of NAFLD; vigorous exercise andresistance training are more helpful than aerobicexercises. The intensity may be more important thanthe duration or total volume of exercise. [7,8, 9]

Current guidelines do not recommend theuse of hepaticopharmacological therapy in patientswith steatosis alone. Instead, patients with NASHand significant liver disease (bridging fibrosis) aregood candidates for this type of therapy. Accordingto the clinical practice guidelines of the AmericanAssociation for the Study of Liver Diseases, the firstchoice of therapy is vitamin E (preferably 800 IU/day). A 2-year treatment in the PIVENS trial (800IU/day) reversed steatohepatitis and improved allhistological features of NASH (except fibrosis) incomparison with a placebo. This beneficial effect ofvitamin E was not associated with an improvement ininsulin sensitivity. Recent studies and meta-analysesshowed increased mortality, a risk of hemorrhagicstroke, and a risk of prostate in long term vitamin Etreatments. [10, 11]

Animal studies have shown that omega-3polyunsaturated fatty acids promote insulinsensitivity, reduce intrahepatic triglyceride content,and ameliorate steatohepatitis. [10, 11]

The drugs to increase insulin sensitivity (glitazone,metformin) may be indicated as a treatmentalternative in NASH 16. Ursodesoxicholic acid andpentoxifilin, which may benefit marginally. [6]

Probiotics and NAFLD

The newest topic in treatment of NAFLD isthat of the involvement of gut microbiota in thepathogenesis of liver steatosis and inflammation.

Intestinal microbiota plays an important rolein health and disease. The gut-liver axis involvesan interaction between bacterial components likelipopolysaccharide and hepatic receptors (Toll-likereceptors). Our gut has approximately 100 trillion(1014) microbes, which make up approximately 1 to2 kilograms of our weight. Gut microbiota performdiverse immunologic, digestive, and metabolicfunctions. [11]

Changes in microbiota may be involved invarious disease pathogenesis (nonalcoholic fattyliver disease (NAFLD), hepatic encephalopathy,alcohol-related liver disease, and hepatocellularcarcinoma). Gut microbiota may cause NAFLDby luminal ethanol production by metabolizationof carbohydrates, causing an increased intestinalpermeability (“leaky gut”) just like in alcoholassociated steatohepatitis (ASH). [12, 13]

In 2009 Miele was the first author to provideevidence of increased intestinal permeability inpatients suffering from NAFLD and this fact wasassociated with increased prevalence of small bowelbacterial overgrowth (SIBO) in those patients [14]. Theincreased permeability appears to play an importantrole in the pathogenesis of NAFLD. Loguerciodemonstrated in 2005 that probiotics may improveNAFLD histology and biochemistry [15]. In October2013 Yan-Yan Ma et al published a meta-analysisin World Journal of Gastroenterology to concludethat the treatment with probiotics and prebioticsmay definitely benefit patients with NASH [11].

Probiotics can inhibit the proliferation of harmfulbacteria, reduce SIBO, restore gastrointestinalbarrier function and modulate the immune system,all of which contribute to the improvement ofNAFLD. This meta-analysis showed that probioticssignificantly reduced ALT, AST, T-chol, TNF-αand insulin resis-tance, which are all related to theprocess and con-sequences of NAFLD. Regularconsumption of probi-otics reduced, also, cholesterollevels which is part of metabolic disturbances inNAFLD patients. [11]

A high fat diet that induces obesity, insulinresistance and hepatic steatosis also leads tohepatic NKT cell depletion. The hepatic NKT cellis the key mediator of HF diet-induced metabolicabnormalities. Moreover, recently, Cani andcolleagues reported that a high-fat diet increasesplasma lipopolysaccharide (LPS) level, which alsocontributes to the pathogenesis of insulin resistanceand increased liver triglyceride content. It is possiblethat this bacterial endotoxinemia caused by highfat diet reduces intrahepatic NKT cells and leadsto worsened or amplified insulin resistance. Theability of probiotics to restore hepatic NKT cellsand improve HF diet-induced insulin resistance andfatty liver are novel findings and intriguing. [12, 13]

These data suggest that strategies designedto down regulate inflammatory mediators withprobiotics have promising potential in patients withNAFLD.

CONCLUSIONS

NAFLD/NASH is a prevalent health problemin general population in close proximity with thesame increased rate of obesity, diabetus mellitus andmetabolic syndrome to which it is pathogenicallyrelated. Proper management of insulin resistance bydiet, weight loss and physical exercise may providethe patients with strong tools to fight the disease.

The increasing evidence of the role of gut microbiotain disease pathogenesis and the role of probioticsin decreasing hepatic steatosis and inflammationpoints firmly towards new and handy solutions inthe nearest future.

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CLINICAL PRACTICE

An unusual cause for cerebellar syndrome – case report

Carmen A. Sîrbu[1], OctavianM. Sîrbu[2], AncaM. Sandu[2],Cristina P. Sandu[1], Marian Ștefănescu[1]

Abstract: *A male from rural area, S.M., aged 77 years, was admitted in our department for discontinuous headache. His medical history was irrelevant. He has been experiencing intermittent right parietal-occipital headaches during the last 3 months. Neurologic exam revealed a slight right limb ataxia. Initial laboratory findings revealed a white blood cell count of 6500/mm^3 with 75% polymorphonuclear leukocytes, 15% lymphocytes and 8% monocytes. His serum glucose was 90 mg/dL. Non Gadolinium CT scan shows rounded, inhomogenous spontaneous hyperdense area (40-45 UH) between 5-12 mm diameter, localized frontal, temporal, occipital and cerebellar bilaterally.*

The question was whether the lesions were metastasis or parasitic infection?

Cerebral MRI showed unenhanced,well defined,multiple lesions between3-17 mm, with iso-hyperintensityT1, T2, and FLAIR, spreadout periventricular, subcortically, infrontal, temporal, parietal lobes andsubtentorially, right and left cerebellum(figure 2). After serological testsfrom blood and CSF the diagnosticof neurocysticercosis was certified(an enzyme-linked immunosorbentassay of the CSF was positivefor immunoglobulin G cysticercosisantibody, with 1.32 optical densityunits (OD) (positive result > 0.50OD); his serum IgG cysticercosis antibodywas positive with 5.12 OD).

CT findings are depending on the stageof evolution:

- Vesicular stage (viable larva): hypodense, nonenhancinglesions
- Colloidal stage (larval degeneration): hypodense/isodense lesions with peripheral enhancementand edema
- Nodular-granular stage: nodular-enhancinglesions
- Cysticercotic encephalitis: diffuse edema,collapsed ventricles, and multiple enhancingparenchymal lesions
- Active parenchymal stage: the scolex within acyst may appear as a hyperdense dot
- Calcified stage: when the parasite dies, nodularparenchymal calcifications are seen.

Our patient has multiple lesions in differentphases of evolution (active and calcified).

DISCUSSIONS

Neurocysticercosis is a parasitic brain infection,caused by larval cysts of the tapeworm Taenia soliumby accidental ingestion of eggs.

It is the most common parasitic disease of the nervous system and it is themain cause of acquired epilepsy mainly in developingcountries. Once in the human intestine, Taenia eggsevolve to oncospheres

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that cross the intestinal walland lodge in the brain where cysticerci develop.

Time from infestation until first symptoms isbetween days and many years (30).This extremelylong incubation is due to hipnobiosis. They may belocated in subarachnoid space, ventricular system,or spinal cord too, causing a clinical heterogeneity.

Figure 1. Non Gadolinium CT scan shows rounded, inhomogenous spontaneous hyperdense area (40-45 UH) between 5-12 mm diameter, localized frontal, temporal, occipital and cerebellar bilaterally.

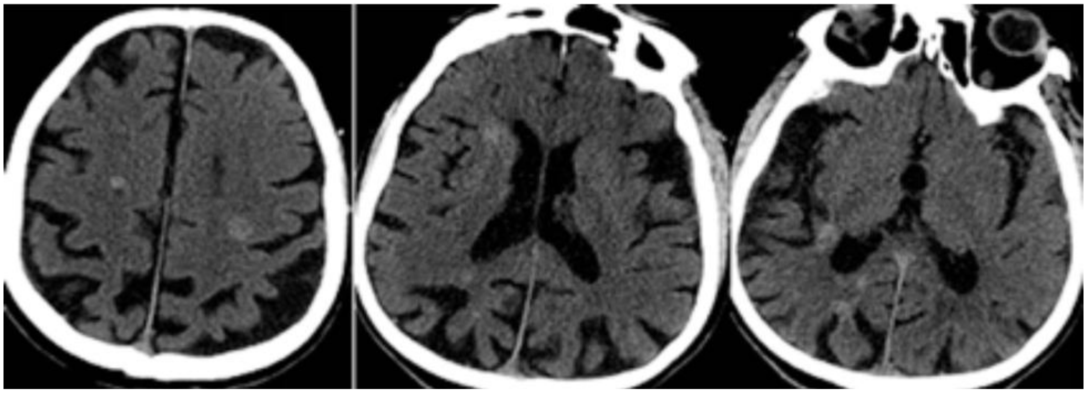

Figure 2. Cerebral MRI showed unenhanced, well defined, multiple lesions between 3-17 mm, with iso-hyperintensity T1, T2, and FLAIR, spread out periventriculary, subcortically, in frontal, temporal, parietal lobes and subtentorially, right and left cerebellum

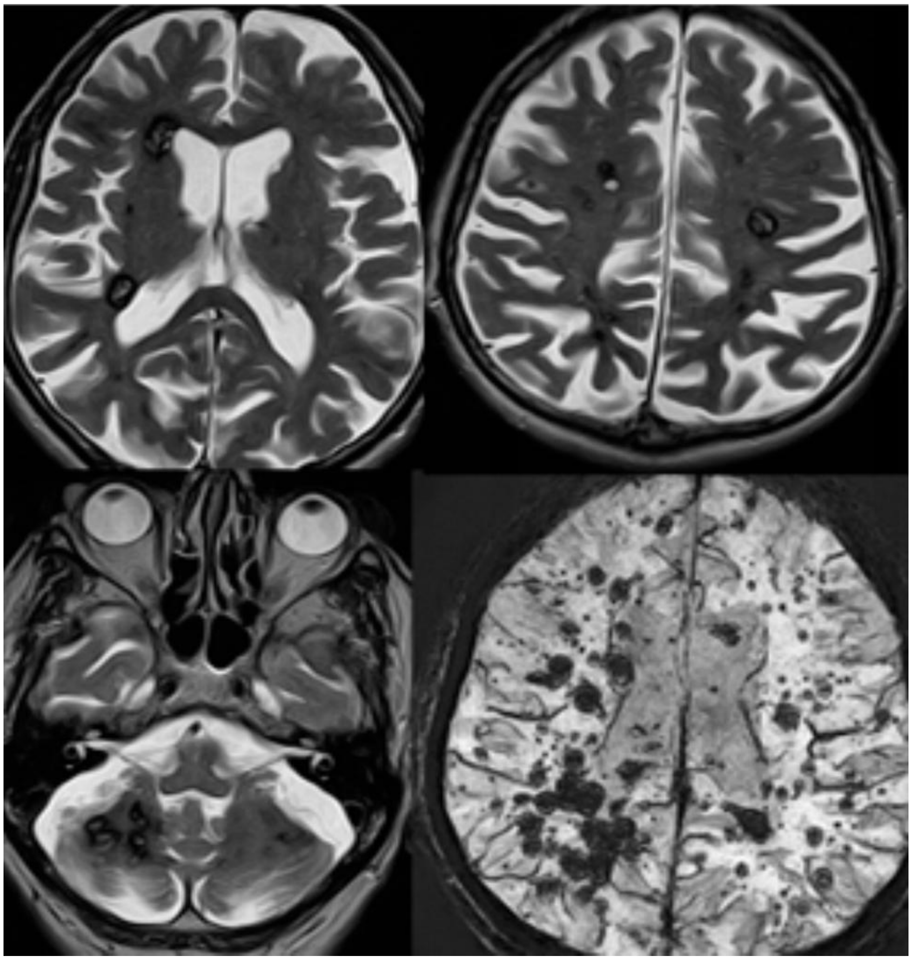

Onset of most symptoms is usually insidiousto chronic, with seizures (most common presentation),headache, dizziness, stroke, neuropsychiatricdysfunctions. Almost every neurological sign orsymptom may be present but physical findings occurin less than 20% of the cases.

There is a pleomorphism of the immune responseagainst Taenia solium. In some cases,cysticerci are destroyed by immunological attack,while in others, parasites may live unchanged foryears. CT scan shows a rounded, homogeneous hyperdensearea with no enhancement with contrastmedium. This phase corresponds to the inactive parenchymalform of the disease.

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Past and future

Andrei Necşulescu[1]

Abstract: *I was thinking a lot about choosing this title which at first glance doesn't seem not to be related to the main topic of this article. However, I think these two opposites have the ability to describe in the most subtle form my journey in Istanbul, the city on two continents. The streets covered with history invites you to discover them step by step and the congress showed me a picture of my future when I will have the opportunity to present important projects in front of a selected audience.*

The 18th edition of the Balkan MilitaryMedical Committee Congress was the startpoint. I can say that it remains, so far, themost important scientific event I have participated.

I also had the opportunity to visit Istanbul,a city like a story that I truly recommend everyone tovisit. Novelty began for me at the military airport Baneasawhen, in a cold morning, I had the chance tosee a C17 Spartan military transport aircraft for thefirst time. I was curious because that type of militaryaircraft had to take me to destination offering me aunique experience. First flight was quiet and we weregreeted in Istanbul by friendly hosts and a beautifulweather. Coach riding to the hotel in the bustling metropolisgave me a chance to admire the panorama forthe first time especially because there was quite a distancebetween the airport and our hotel, the complexwhere we were accommodated being in Asia. When Ireached the destination I had the honor to admire oneof the most beautiful hotels in Istanbul, a true pride ofthe Turkish army and also a proof of their elite role insociety. Beyond the luxury and good taste I noticedagain the perfect planning of every detail, startingwith accommodation. The Congress began. In the firstevening I started meeting my colleagues from othercountries during an elegant and discreet reception.

Beyond the speeches from heads of delegations I was very happy to see that the prize for the best project was awarded to Romania. It was a pride for the whole delegation and we returned to the hotel very happy.

That was the first time I changed impressions with myfellow military students from the other participatingcountries. We linked friendships that I'm sure will belong-lasting and we set up a future collaboration forthe upcoming congresses, everything in a festive andfriendly atmosphere. Beyond the congress I venturedfor the first time into crowded Istanbul with my colleagueRobert Popescu.

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In the second day of the congress I went for thefirst time at the Military Museum from Istanbul whereI attended the opening ceremony. After visiting themuseum, my colleague and I were invited in the hallwhere the presentations were kept. In front of a hugepicture of Mustafa Kemal Ataturk I attended militarymedical exposures from Serbia, Greece, Turkey, Bulgariaand Romania. I started to get ready for my firstspeech in front of an audiencecomposed mostly of doctors.

Day three was the highlightof the congress for me. AlthoughI knew Robert Popescuwas going to present the projectwith doctors and universitystudents, I was told that myposter will be exhibited in the museum lobby where Ishould provide clarifications for the numerous questionsthat might be asked. I admit that things for mewere much more effortless but I was kept up-to-datewith the events of the congress thank to my colleaguewho was getting ready to present his project. I appreciatedthe presentations of my colleagues from othercountries and together we agreed to collaborate in thefuture and gather together at these conferences withbigger projects that will integrate more teamwork. Ileft there convinced that at the next congress I willcome with an oral presentation. I gathered the minimumnecessary experience to deal with future congresswork and I hope I managed to fit in that greatatmosphere provided by the hosts. The presentationof my colleague was praised; his experience at previouscongresses he attended made a difference. I leftthere thinking that I fulfilled the task of coping withchallenges and building the idea that I can come upwith a lot more and improve myself.

Day four was great. It was time to visit the impressiveDolmabahce, a true monument and one ofthe most spectacular buildings in Istanbul. That day itwas warm, summer was definitely approaching. Manypeople were gathered in front of the palace, a sign ofthe importance of that particular place. Huge halls,sophisticated architecture, historical significance andopulence made me think about what many years agowas considered absolute luxury. I recommend visitingthe palace along with other major sights of Istanbul.

The evening was one of the most important momentsof the congress due to the cruise on the Bosphorus.

The boat that was waiting for us was state-of-the-art,impressive and equipped with a luxury dining room.

As already expected, we were welcomed aboard withfriendship. It was the last moment when I changedimpressions about congress with my colleagues fromother countries. There were five delegations in thedebut of the party but at the end we were a singlegroup with many things in common. That was themoment when we had the chance to know us betterthan we did before. Seenfrom the

Bosphorus, Istanbulwas like a fairytale. I think oneof the most spectacular city'soverall images can be seen atnight. Picture of opulence wasreflected in every detail whenI saw the most famous clubsfrom the city during the cruise.

I went back to the hotel thinking that an experiencelike that was unique and I rarely had the opportunityto see so much luxury gathered in a single place. Lastday brought me back at the military museum whichhosted the closing ceremony. Beyond the speechesfrom heads of delegations I was very happy to see thatthe prize for the best project was awarded to Romania.

It was a pride for the whole delegation and we returnedto the hotel very happy. Those were already thelast hours in Turkey. We started preparing to go backhome so we had lunch and then we started packingthe luggage. At the airport our plane was waiting forus. After a turbulent flight we arrived back in Romaniaon a cold and rainy weather, a lot different than thesun and the heat from Turkey.

The experience was unforgettable. I recommendeveryone to take part in this kind of event even if theyhave to deal with oral presentations. I think that whatmatters the most is participation and the numerousthings that you can learn. Since little, brick by brick,experience accumulates and allows overcoming newlimits. I hope I gave a good read and I want to thankyou for the time you have given me.

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EDITORIAL

Reconsidering the importanceof Military Medicine at the levelof National Ministry of Defenseand into society

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Major General IOAN SÎRBU

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This goal can be achieved through the implementation of a modern management bothof the Medical Directorate and of the subordinate units, which basically involves areform of these institutions, keeping them out of the incertitude of annual budgetaryprovision, looking for contractual stability with National Defence, Internal Affaires,National Security and Justice Authority Health Insurance Company (C.A.S.A.O.P.S.N.A.J) andplacing them on the right path for institutional performance.

The reform of the military medical institution is an act of courage that has to be accomplishedwith great determination.

The first step would be submitting to the Supreme Council of National Defence (CSAT)the concept of the modernization of the military medical system including elements ofinnovative vision on the military medical phenomenon. One of these issues is the needfor modernisation of medical equipment and hospital infrastructure in order to allow thetreatment in national military hospitals of soldiers injured in combat theaters and of civiliansafter natural disasters or industrial accidents with multiple victims.

Another goal is to create a fully equipped base for physical and neuropsychological recoveryof military personnel executing missions in operation theaters and of their family members,allowing a fast and complete social and family reintegration.

A particular attention should be paid to hero combatants, wounded in combat theaters; theywill take benefit from the best specialists in health care in the new social-medical edificewhich will be available since 2015.

In 2015 also, the construction of the new Emergency Universitary Central Military Hospitalwill begin in Bucharest in Garrison 867, part of the Ministry of National Defense estatepatrimony, under Government Decision no 1594/04.12.2008.

This investment is the natural consequence of the need to be aligned with the realities ofmodern medicine promoted by North Atlantic Alliance through ROL 4 level hospital units,covering all medical and surgical specialties required for complete rehabilitation andrecovery of military personnel wounded in the operation theaters or in the current trainingactivities, on national territory.

In the same time with updating of the clinical basis, we should concentrate our attention onimproving the continuous training of our healthcare staff regarding the achievement of newtreatment techniques and solutions. Continuous professional training is an asset that ourphysicians are providing to the military personnel in distress.

From the experience of some colleagues, who have chosen to leave the system, the mainreasons for discontent were: the working environment and the lack of modern investigationmeans; to the surprise of many, the financial reason was the last one.

Promoting team spirit and preserving the values in the system has been a permanentconcern of the Medical Directorate Management.

All these contribute to achieving outstanding results in our activity, leading to increasingsatisfaction for patients and doctors.

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REVIEW ARTICLE

Article received on October 1, 2013 and accepted for publishing on December 13 2013.

Clostridium difficile – emergent hospital flora

Gabriela V. Dumitrescu, Viorel Ordeanu, Simona Bicheru, Lucia Ionescu,Diana Popescu, Marius Necşulescu[1]

Abstract: *Clostridium difficile (C. difficile) is a Gram-positive sporogenous bacillus strictly anaerobic, which in the last decade has became the most important anaerobic bacterium in nosocomial human pathology. Cl.dificile is the etiological agent of more than 20% of diarrhea postantibiotics, over 95% of pseudomembranous colitis and the first cause of nosocomial infectious diarrhea in adults.*

Although this bacterium usually colonizes the intestine of vertebrates (the normal microbiota), the toxinogenic strains (tcdA and tcdB) are pathogenic in the digestive tract. Given the excessive use of antibiotics and the increased spores resistance, it is possible an environment contamination, with strains which may already be resistant to antibiotics. The main causes of this infection are decreased resistance to antibiotic-induced colonization, contamination with a pathogenic strain of Cl.difficile, secretion of A and/or B toxins and deficient immune response.

Due to the increasing worldwide incidence of infections with C. difficile on one hand and to the discovery of new ways of transmitting the infection according with some studies regarding the genetic diversity of bacterium strains on the other hand, a new approach is necessary for C. difficile related topics..

Keywords: *antibiotics, Clostridium difficile, epidemiology, nosocomial infection, toxins*.

INTRODUCTION

Clostridium difficile (C. difficile) is a Grampositivesporogenous bacillus strictly anaerobic,which in the last decade has became the mostimportant anaerobic bacterium in nosocomialhuman pathology. Cl.dificile is the etiological agentof more than 20% of diarrhea postantibiotics, over95% of pseudo-membranous colitis and the firstcause of nosocomial infectious diarrhea in adults.

Although this bacterium usually colonizes theintestine of vertebrates (the normal microbiota),the toxinogenic strains (tcdA and tcdB) are pathogenicin the digestive tract. Given the excessive useof antibiotics and the increased spores resistance,it is possible an environment contamination, withstrains which may already be resistant to antibiotics.

The main causes of this infection are decreasedresistance to antibiotic-induced colonization, contaminationwith a pathogenic strain of Cl.difficile,secretion of A and/or B toxins and deficient immuneresponse.

Microbiological diagnosis is made by severalmethods and techniques for bacteria or toxins identification.

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Cytotoxicity test reveals the cytopathiceffect of fecal filtrate with pg sensitivity. Immunoenzymaticassay enables a rapid diagnosis, firstgeneration with ELISA, the second generation byimmuno-enzymatic or immuno-chromatographycassette. Molecular biology techniques based onquantitative real-time PCR detect tcdA and tcdBgenes in stool, responsible for toxigenesis with verygood sensitivity and specificity. Through cultivationand microscopy Cl. difficile can be revealed inthe stool or on contaminated surfaces; spores areresistant in the environment and are found in nosocomialflora. A characteristic enzyme, glutamate dehydrogenase (GDH) can be revealed in stool byimmuno-enzymatic assay correlated with the outcomeof cultivation, or latex agglutination test withantiGDH antibody.

Due to the increasing worldwide incidence ofinfections with C. difficile on one hand and to thediscovery of new ways of transmitting the infectionaccording with some studies regarding the geneticdiversity of bacterium strains on the other hand,a new approach is necessary for C. difficile relatedtopics.

CLINICAL

Clostridium difficile (C. difficile) is a Grampositive,spore forming bacteria, spread by thefecal-oral route. It is non-invasive, producestoxins A and B, which cause disease, ranging fromasymptomatic carriage, to mild diarrhea, to colitis,or pseudo-membranous colitis. Clostridium difficileinfection (CDI) is defined as the acute onset ofdiarrhea with toxigenic C. difficile or its toxin andno other cause for diarrhea.

Since 2000 the rate of CDI has been increasing,especially in the elderly with a recent hospitalizationor residing in long-term care facility (LTCF).

Carriage of C. difficile occurs in 5– 15% of healthyadults, up to 57 % in residents in LTCF and canreach 84.4 % in newborns and healthy infants.

In simple diarrhea cases, the classic symptomsmay not occur and the endoscopic examinationshows normal or ulcerated mucous; in 25% of casesending the antibiotic therapy was followed by clinicalrecovery in 2-3 days. Further on antibiotheraphyis a prolonging factor of diarrhea relapse.

Pseudomembranous colitis represent up to 9%of CDI and starts with abundant watery diarrhea,over 7 stools a day, with heterogenic no bleedingaspect. They are accompanied by fever in 75%of cases and abdominal pains in 70% cases. Thesymptoms are non-specific, leukocytolysis up to ex80.000 PMN/Ι¼l, extracellular dehydrating caused by exudative enteropathy.

Digestive endoscopyconfirms the diagnosis, allowing canker yellowishsores visualization, named pseudomembrane, onmucous colon membrane. In the first stage theyare isolated, afterwards they come together. In CDIforms with severe onset and no obvious etiology of diarrhea an endoscopy is recommended, butthis test is difficult to perform on aged and fragilepatients. Complications such as septic shock andtoxic megacolon may occur, septic shock and toxicmegacolon occur and provoke the colon perforation(colectomy required) and even death.

The ratio of severe forms differs (7-18%),depending on the studies we consider. Consecutivemortality with C. difficile varies 0,6-3% and whencomplications occur is 35-50%. Some studies showincreased mortality in North America, a doubleno. of cases in EU, heading to 24/milion, C. difficilebeing involved in death cases three times morefrequent than Staphilococcus aureus MRSA.In 20% of cases, relapses appear in the firsttwo months after the initial episode. In over 50%of cases they are connected with the persistenceof pathogen strain (spores) inside digestive tract;a new stain could appear and provoke reinfectionespecially during hospital admission. Multiple strains have been identified during one episode ofinfection. Approximately 3% of adults are asymptomaticcarriers and often with toxin-free strainsand sometimes specific toxins may be identified insome asymptomatic patients stool. The asymptomatictransmission of toxinogen strains in neonatesis 5-70%, but there is no explanation what so ever.Although nosocomial infections are the mostfrequent, some of them could be communal. Thereare recorded 17.5% postantibiotics diarrhea inEU, from which 66% have one day manifestation.After two weeks antibiotherapy, the frequency becomes 3.8%, from which 70% are toxic. In NorthAmerica were identified a lot of cases but no strainhigh pathogen 027 had been isolated in communalinfectious. Differential diagnosis will be made withother infectious diarrhea: bacterial, viral, fungusand parasitic or non-infectious causes; for example,the outcome of some ”cool” drugs is in realitylaxative ones (supplements for straitening theimmunity, sugar free sweets, food with magnesiumand decaf products) with no connection with CDIetiology. [Duker Freuman T., 2014]

Figure 1. Pathogenesis of Clostridium difficile –associated disease (http://bioweb.uwlax.edu/bio203/s2009/kumm_jakl/pathology.htm)

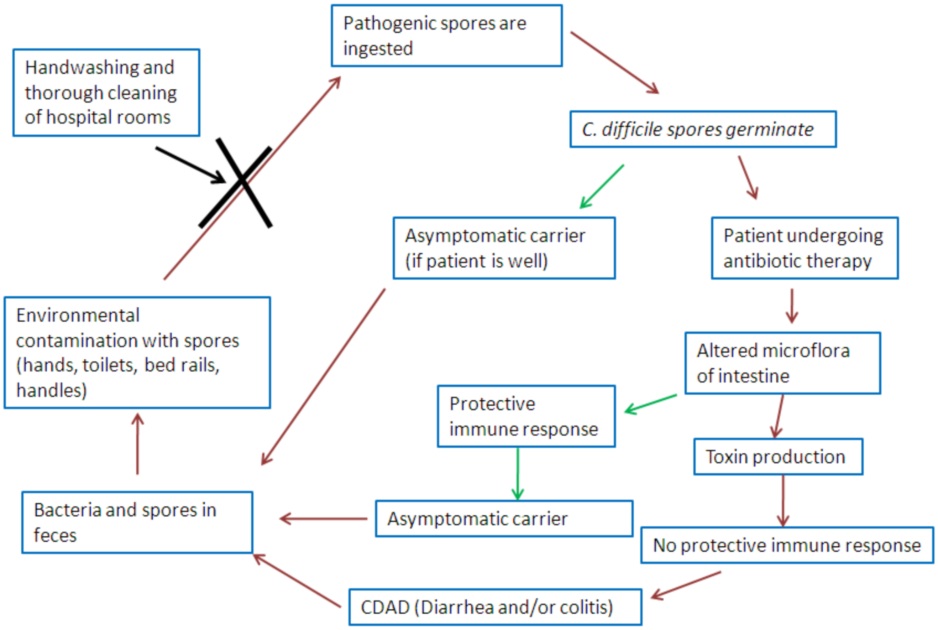
Pathogenic spores are ingested
Handwashing and thorough cleaning of hospital rooms
C. difficile spores germinate
Asymptomatic carrier (if patient is well)
Patient undergoing antibiotic therapy
Environmental contamination with spores (hands, toilets, bed rails, handles)
Altered microflora of intestine
Protective immune response
Toxin production
Bacteria and spores in feces
Asymptomatic carrier
No protective immune response
CDAD (Diarrhea and/or colitis)

MICROBIOLOGICAL DIAGNOSTIC

CDI diagnostic is based on revealing thetoxins in stool or isolating a toxinogenic strain ofCl.difficile, this being the only pathogenic strain.Diagnostic testing for C. difficile has rapidlyevolved in the past decade. Previously, toxin A +B EIAs were the most widely used diagnostic testsbecause of ease of use and objective interpretation.

However, EIA tests have substantially reduced sensitivitiescompared with reference standards. Moreover,toxin A immunoassays (without toxin B) lackdetecting the small number of pathogenic strainsthat only produce toxin B. Two major advances inthe laboratory diagnosis are the use of GDH detection in stools as a means of screening for CDI andthe development of Nucleic acid amplification tests(NAATs) such as PCR to detect toxigenic strainsof C. difficile. Glutamate dehydrogenase (GDH)screening tests for C.difficile can be used in two- orthree-step algorithms with subsequent toxin A + BEIA testing, but the sensitivity of such strategies islower than NAATs [Surawicz et al., 2013] (fig 2).

Testing the toxigenic C. difficile shouldbe limited to patients with > 3 nonformedstool specimens per 24 hr period, unless ileus(obstruction) is suspected. Repeat testing following apositive test (test of cure) is not recommended sincepatients may carry toxigenic C. difficile for monthsafter clinical cure. Repeated testing following apositive test is appropriate if the patient improveswith therapy and relapses after the completion ofa treatment regimen (clinical relapse). Testing asecond specimen from a negative patient is morelikely to be a false positive [American Society forMicrobiology, 2010].

The optical microscopy swab is pathognomonic,revealing long gram-positive bacilli with a bulgeat terminal ends, with long terminal and isolatedspores, visible with Gray coloration. While thepresence of C. difficile can be suspected, we cannotdifferentiate the pathogencal strains from the nonpathogencalones, therefore the examination shouldbe supplemented with toxigenical and molecularbiology tests. In the last years, a very pathogenicaland virulent strain, C. difficile 027, has beenidentified, that causes severe epidemic episodes (Fig 3).

Figure 2. Diagnostic algorithm of Clostridium difficile (Surawicz et al., 2013)

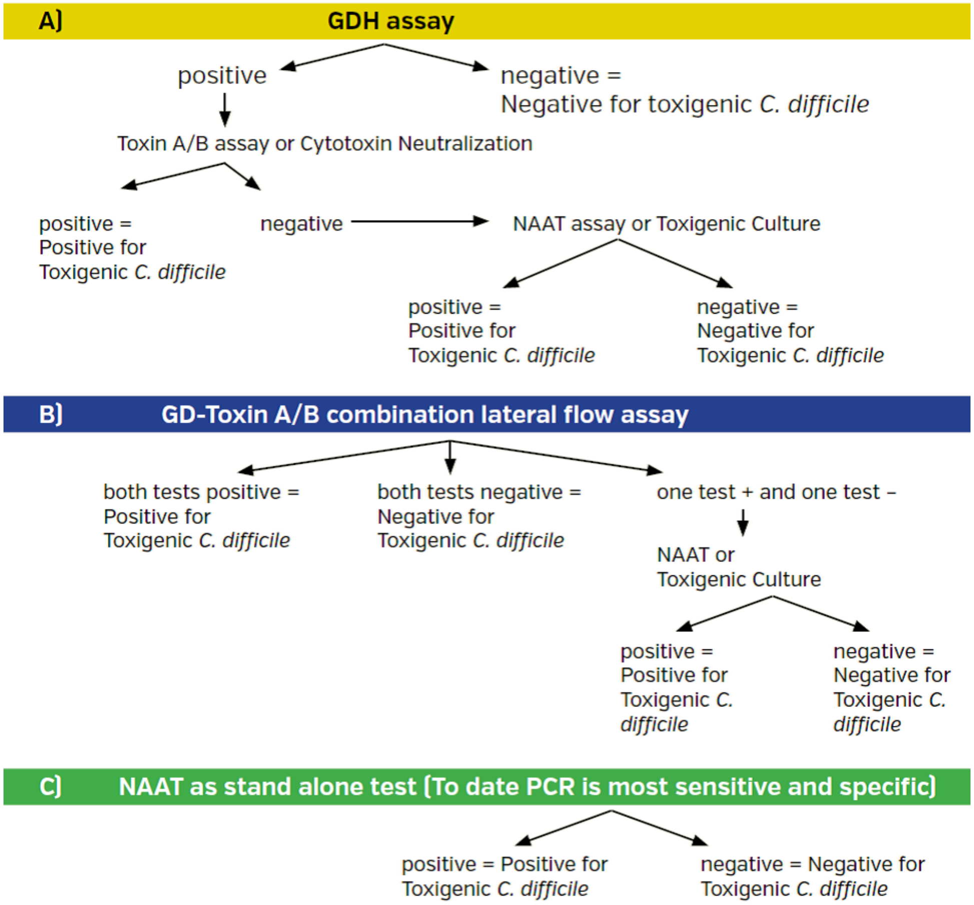
A)
GDH assay
positive
negative =
Negative for toxigenic *C. difficile*
Toxin A/B assay or Cytotoxin Neutralization
positive =
Positive for
Toxigenic *C. difficile*
negative
NAAT assay or Toxigenic Culture
positive =
Positive for
Toxigenic *C. difficile*
negative =
Negative for
Toxigenic *C. difficile*
B)
GD-Toxin A/B combination lateral flow assay
both tests positive =
Positive for
Toxigenic *C. difficile*
both tests negative =
Negative for
Toxigenic *C. difficile*
one test + and one test –
NAAT or
Toxigenic Culture
positive =
Positive for
Toxigenic *C. difficile*
negative =
Negative for
Toxigenic *C. difficile*
C)
NAAT as stand alone test (To date PCR is most sensitive and specific)
positive = Positive for
Toxigenic *C. difficile*
negative = Negative for
Toxigenic *C. difficile*

The epidemic strain currently describedin North America and EU, has the followingfeatures: PCR ribotype 027 in accordance withAnaerobe Reference Laboratory surveillance data[ECDC, 2006], pulsotype NAP 1 on pulsed-fieldelectrophoresis, enzymatic restriction-profile BI,toxinotype III by Rupnik toxinotyping method,positive for binary toxin actinia-specific ADPribozyltransferase,deletion of 18 bp in tcdCgene controlling the expression of toxins A andB, hyperproduction of toxins A and B (Ax16and Bx23) in comparison with strains of othergenotypes, resistant to macrolides (erythromycin)and la flororquinolones (moxifloxacin, gatifloxacinand levofloxacin).

Only specialised laboratoriesare able to perform the techniques for identifyingthese features and a two weeks period is requiredfor confirmation [INVS, 2006].

In practice, CDI diagnostic is based on toxinB detection in stool or revealing the toxigen strain.A- and B+ strains cannot be detected by currentimunoenzymatic assays which detect only A stain.

Figure 3. Analysis of anaerobic bacterial isolates in the microbiology laboratory of CCSMM

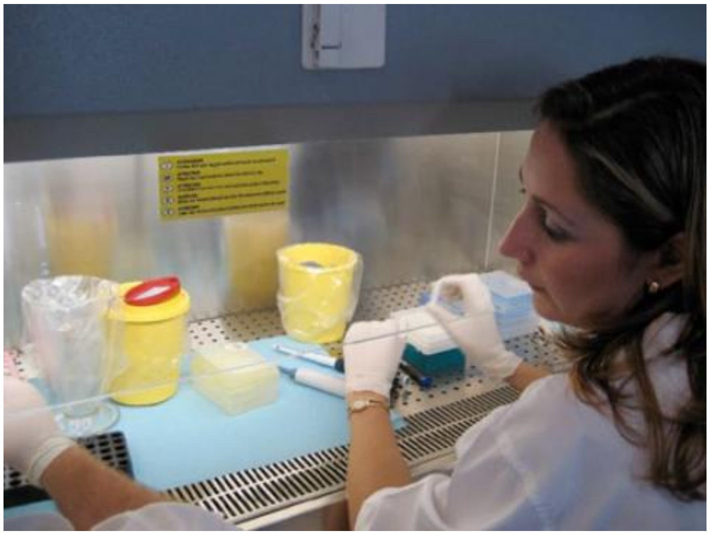

The strain isolation through culture is a necessarystage for epidemic clone 027 characterisation;PCR profile identification provides the certaintydiagnosis.

This clone presence is clinically suspectedif a severe form of the disease is diagnosed,epidemiologically suspected if several cases occur,or microbiologically suspected if the isolated strainis resistant to new fluoroquinolones (moxifloxacinCMI > 4 mg/l) or to erythromycin (CMI > 256mg/l).

These characteristics are not specific to clone027, but justify the stool culture in anaerobiosis inorder to isolate the responsible stain and to sendit to a specialised reference laboratory for furtherexamination.

The genes encoding TcdA and TcdB, tcdA andtcdB, respectively, have been sequenced and arefound in single open reading frames located withina 19.6-kb pathogenicity locus (8, 38).

As expected,both open reading frames are large, with tcdAfound within an 8,133-nucleotide region and tcdB is 7,098 nucleotides in length (fig.4).

Both tcdA and tcdB are low-G C (28%) genes,which are comparable to the G C content (29%)of the C. difficile genome, and the toxins exhibita high degree of overall similarity (66%).

Giventhe proximal locations of tcdA and tcdB and thehigh sequence and functional homology betweenthe two proteins, it has been proposed that thetwo genes may have arisen as the result of a geneduplication event.

Furthermore, the similarity inthe biochemical activity of TcdA and TcdB, whereinboth toxins use a highly conserved N-terminaldomain to modify identical substrates, supportsthe notion of gene duplication. The major regionsof homology between TcdA and TcdB fall withinthe enzymatic and receptor-binding domains of thetwo toxins. The N-terminal domains of TcdA andTcdB show 74% homology, and this homology providesa basis for the similar substrate specificity ofthese two toxins.

The C terminus of TcdA and TcdB show a numberof short, homologous regions termed combined repetitive oligopeptides (CROPs). TcdA encodesfive groups of CROPs, which range in size from 21to 50 residues and can be repeated throughout theC terminus of the protein. TcdB also encodes fivegroups of CROPs, four of which show homology tothe CROPs of TcdA.

Yet the CROPs found in TcdBare more divergent and less frequent than thosefound in TcdA. CROPs appear to play a putativerole in initial target cell interaction and receptorbinding, but the mechanism explaining the necessityfor these repeats in cell binding remains unclear[Daniel E. Voth, 2005].

Figure 4. Genetic arrangement of the C. difficile pathogenicity locus and proposed protein domain structures of TcdA and TcdB. Both TcdA and TcdB are encoded on the 19.6-kb pathogenicity locus. In addition to the two toxin genes tcdA and tcdB, three additional regulatory open reading frames are located on this island. tcdD is a proposed positive regulator, tcdE is a putative holing protein, and tcdC is a proposed negative regulator of toxin gene expression. Through deletion mutagenesis, research combined from multiple research groups has revealed a three-domain structure of the large clostridial toxins. The glycosyltransferase activity is located at the N terminus of the protein, and the C terminus is involved in receptor binding. Located in the middle domain of the protein is a putative transmembrane segment that is thought to be involved in membrane translocation. [Daniel E. Voth, 2005]

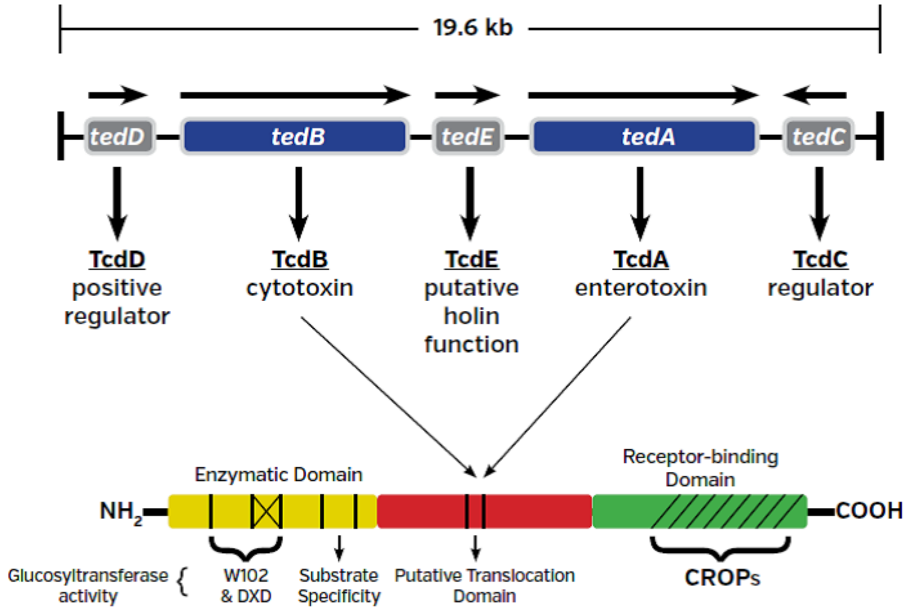

EPIDEMIOLOGY

C. difficile transmission is made by fecal-oralroute, by hands and contaminated objects orenvironment. The fast transmission in healthcareenvironments is a result of several factors: straindissemination in CDI patients, half of samples frompatients rooms being positive; high resistance ofspores on inert supports for several months; toomany patients crowded in common healthcaresettings; numerous healthcare maneuvers creatinga high possibility of contamination by the medicalpersonnel hands; inadequate usage of antibiotics which diminishes the resistance to colonization andfacilitates C. difficile development.

The main individual risk factors are the advancedage and antibiotherapy. There are severalstudies which correlate the consumption of someclasses of antibiotics with CDI incidence: clindamicyn,3-rd generation cephalosporins, macrolides,and amoxicillin with clavulanic acid, 1-st generationcephalosporins and fluoroquinolones. It seamsthat the role of fluoroquinolones in C. difficile 027strains emergence and spreading is connected tothe resistance level towards them [INVS, 2006].

All factors stimulating the digestive ecosystemalteration, like laxatives, antacids, antisecretors,transit retarders, baritosis transit, gastrointestinalsurgery, etc. may facilitate this infection [DukerFreuman, 2014].

In March 2014, an epidemic episode with 31cases of postantibiotic C. difficile infection wasrecorded in Ploiesti Emergency Hospital (Romania)and the patients were isolated and treated. Mostof them were aged people from Neurology,Nephrology and Intensive Care Unit [Libertateanewspaper, 2014].

In May 2014 the Ministry of Health of Romaniagave the alert for C.difficile in Vaslui and Bucharesthospitals. The beginning of the year is worrying,in only 4 months, in Bucharest health facilitieswere registered 462 infected patients [Pro

TV, 22Mai 2014].

In accordance with Annual epidemiologicalreport: Reporting on 2011 surveillance data and2012 epidemic intelligence data, 2013, uttered byEuropean Centre for Disease Prevention and Control(ECDC), 48% cases of HAI (Healthcare-AssociatedInfections) associated with gastro-intestinalinfections were connected with C.difficile, andfrom all HAI (15.000 cases) in 3 only 5,4% of casesthe Clostridium difficile has been isolated. Takinginto consideration that in Romania over 92.3% ofpatients were the beneficiary of an antimicrobialprophylaxis during more than a day surgeries, theHAI risk associated with C. difficile is very high[ECDC, 2013]

TREATMENT

There is worldwide observed natural resistanceand/or acquired to the medicines of the quinolonegroup.

A mild CDI can usually be controlled bywithdrawing treatment with the antibiotics causingthe infection (25% of patients could recover in2-3 days). More severe cases can be treated usingan oral specific treatment with metronidazole (1g/day) or vancomycin (1-2g/day) for 10 days. Themetronizadole is a better choice, being a less expensivetreatment with no risk of selecting glycopeptidesresisting germs like golden enterococcus andstaphylococcus.

Failure to respond to metronidazole therapywithin 5 – 7 days should prompt consideration of achange in therapy to vancomycin at standard dosing.For mild-to-moderate CDI in patients who are intolerant/allergic to metronidazole and for pregnant/ breastfeeding women, vancomycin should be usedat standard dosing. In patients in whom oral antibioticscannot reach a segment of the colon, such aswith Hartman’s pouch, ileostomy, or colon diversion,vancomycin therapy delivered via enema should beadded to treatments (500 mg in 100 – 500 ml of normalsaline every 6 h) until the patient improves.

However, relapse is common and requires furthertreatment with repeated series of metronidazoleor vancomycin, in high doses first and smallerdoses associated with probiotics (i.e. Saccharomycesboulardii) after improvement. Severe cases mayneed intensive care for maintaining the vital functionsand even surgical treatment for colectomy(in case of toxic megacolon or colon perforation).

CT scanning is an important technique for perforationdiagnosis in comparison with colonoscopytechnique which presents a perforation risk due togas inflation. The antibiotic treatment for healthyindividuals colonized with C.difficile is not recommended,being inefficient for eradicating for goodthis bacteria in digestive tract.[Ordeanu, 2010; Ordeanu 2012]

Considering the antibiotherapy limitations,there has been designed the fecal bacteriotheraphy,known as “stool transplant”/fecal microbiotatransplant (FMT) of bacterial flora acquired fromthe feces of a healthy donor to reverse the bacterialimbalance responsible for the recurring natureof the infection, with good results [ASGE, 2013].

This “synthetic stool” is a super-biotic obtained usingseveral cultures of saprophyte intestinal culture[Allen-Vercoe, 2013]. Studies show that patientswith recurrent CDI (RCDI) have abnormally proportioned colon microbiota, and that reintroductionof normal bacteria via donor feces correctsthis imbalance, restoring phylogenetic richness andcolonization resistance.

There is no international consensus for definingand surveillance CDI, but we have to consider local(regional and national) epidemiology conditionsand possibilities. ECDC created a working groupfor early detection and monitoring the CDI. Theyhave suggested recorded signals criteria for severeand grouping cases of CDI.

C. difficile infectious can usually be preventedby practicing good hygiene in healthcare environments,such as: individual bed space, washinghands regularly (mechanical action of washing aftergloves removal), using gloves, protection mask,glasses and gown in bed space area and in contactwith patients, using medical supplies for one usageonly, cleaning surfaces using bleach wipes of sodiumhypochlorite containing 0.5 % active chlorine,and patient removal limitation. [CCLIN, 2013]

COMMENT

Due to the increasing worldwide incidence ofinfections with C. difficile on one hand, and to thediscovery of new ways of transmitting the infectionaccording with some studies regarding the genetic diversityof C. difficile strains on the other hand, (http://www.pharmacypracticenews.com) a new approach isnecessary for C. difficile related topicsIt is important to adopted NAAT testing aloneor a 2 or 3 step algorithm for CDI diagnosis.

If the C. difficile is confirmed and classified asa severe form or in an epidemic context it should bereported to Public Health Territorial Authorities andto The Anaerobe Reference Laboratory from INCDMICantacuzino, for a clear diagnosis and adequatemeasures.

CONCLUSION

Due to the increasing worldwide incidence of infections with C. difficile on one hand and to thediscovery of new ways of transmitting the infection according with some studies regarding the geneticdiversity of bacterium strains on the other hand, a new approach is necessary for C. difficile related topics.

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SYSTEMATIC REVIEW

Article received on August 12, 2013 and accepted for publishing on October 9 2013.

Stress managementfor optimization oforganizational activity

Iuliana Guiţă – Alexandru[1,2]

Abstract: *Stress is a constant presence in our lives, whether we analyze it in professional, social or family terms. This daily reality creates a state of tension, strain and discomfort, causing significant changes in physical and mental health. Stress at work can affect anyone, at any level, in any sector and in organizations of any size. Stress affects health and safety of individuals and also organizations' welfare and national economies. There is a definite correlation between the level of stress at work and the changes in organization's productivity.*

Keywords: *stress management, organizational optimization, occupational stress, organizational culture, psychological contract*

STRESS: DEFINITION, TYPES AND CAUSESOF ITS OCCURRENCE

We meet every day people who are overworked, overwhelmed or underpaid. We wonder what to do to cope with the stress in our life. Often we forget to put on paper all the endless tasks we have to do during the day, and paradoxically we receive other new tasks to fulfill.

According to Peter Drucker, we have to make a clear distinction regarding those who are accomplishing an intellectual work today, the situation being more complicated for them than for production line workers of the past, who knew what they have to do. Instead, those involved in intellectual work should not only execute the plan, but also to conceive it. This puts an important pressure on the human brain so in case you see a doctor, one of the question is: "How stressed are you?" Certainly, stress at work can affect anyone at any level. It is produced in no matter what domain and in organizations of any size. Stress affects the health and safety of individuals and also welfare of organizations and national economies.

Stress was introduced by Hans Hugo Bruno Selye, professor of histology, who sets up the foundation of International Institute of Stress with Alvin Tofler in 1977. He believes that stress is related to adaptation syndrome as a reaction of individual against environmental aggressions. Hans Selye defines stress as "the set of reactions of the body to the external action of the causative agents (physical, chemical, biological and psychological) consisting in morphofunctional changes, most often endocrine".

According to the Romanian Language ExplanatoryDictionary (1998), stress is "the name given toany environmental factor (or set of factors) causingan abnormal reaction to human body; adverse effecton the human body produced by an environmentalfactor."

According to Terry Looker, Olga Gregson(2009, p.31),

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[2] Researcher in the Research Center for the Promotion of Excellence in Training - University of Pitești

“Stress is seen as a reaction of theorganism against changes occured in our environment.”

All mentioned authors consider that “stresscan be defined as the condition that we feel when weperceive a discrepancy between perceived demandsand ability to cope with them.”

Lazarus and Folkman (1984) define stress as“a cognitive and behavioral effort (with obviousemotional expression) to reduce, control or tolerateexternal or internal demands that exceed personalresources.”

Ioan Bratu Iamandescu (2002) believes thatmental stress is a syndrome consisting of exacerbationbeyond the level of simple homeostaticadjustments, certain psychic reactions and theirsomatic connections (affecting almost all bodycomponents), in connection with external andinternal excitement exerted by triggering factors(stressor factors) acting intense, surprisingly, suddenand/or persistent and having a symbolic nature, “threating”, or, othertime, extremely favorableto the subject (perceived or anticipated by thesubject). In other cases, stressor factors could bepsychical excitants with major affective resonance(positive – eustress or negative – distress) or overloadingfactors of cognitive (attention, thinking,etc) and volitional process, mentioning that mentalstress is mainly based on a major emotional involvement.”

The definition given by Golu M. (1981) shouldalso be taken into consideration: “state of tension,tightness, discomfort, caused by affective agentswith negative significance (or positive, in case ofeustress), frustration or repression of some motivations(needs, desires, aspirations – including underloading),the difficulty or impossibility of solvingproblems”.

However, Paul Popescu Neveanu, in the “Dictionaryof Psychology “ (1978), defines stress interms of two meanings: “a) situation, stimulus whichputs the body into a state of tension ; b) the specialtension state, itself, of the body as an activation ofall its resources to cope with physical or psychicalaggression (strong emotion)”.

“Stress is an individual reaction and the resultof interaction between environmental demands onone hand and the resources, capabilities and opportunitiesof the individual on the other hand.”

It is certain that stress is characterized byintense hormonal changes, massive secretionof adrenaline. There are also morbid changes(hypertension, gastric ulcer etc). Psychical stressis caused by prolonged emotions primarily mainlydue to frustration, conflict and anxiety. There isstress of overload and also stress of underload. Amoderate stress boosts and stimulates the bodyvitality. The harmful feature of stress occurs whenthe damage is too large, exceeding the individualadaptive capacity.”

Doctor Ioan Bratu Iamandescu, in his book“Psychical stress and internal diseases” (1993), believesthat stress can be positive (eustress) or negative(distress).

Eustress (“positive stress“) has beneficial effectson the human body, occurring when thestress agents have positive significance for the individual.

These triggers positive emotions (intensejoy, ecstasy, triumph, laugh out loud), and positivelyaffect the body’s organs and apparatus. Eustressappears in the course of positive emotionalstates or coupled with moderate exercise (eg sexor jogging). Eustress is essentially acute. Frequentrepetition of eustress contributes to increase antiinfectionand antitumor immunity, becoming alongevity premise.

Distress (“negative mental stress“) is widelyrecognized as pathological. It usually produces painand inadaptation as a result of contact with a stressoragent.

Situations generating mental stress:

- the existence of unusual circumstances forthe individual, which find him unpreparedto deal with them
- the significance of an event
- engaging the individual into an exaggeratedaction or relation
- peculiarities of the social context
- lack of internal conditions
- subjective way of perceiving environmentaldemands

- underload / overload
- existing conflictual situations in the family,profession or intellectually related
- lack of time
- isolation
- the emergence of a physical or mentalobstacle in the way of a goal that leads tofrustration
- disturbing circumstances arising fromphysical agents (noise, vibration,temperature fluctuations).

STRESS AND HEALTH AT WORKPLACE

MANIFESTATIONS OF STRESS AT WORKPLACE

Stress at work can affect anyone, at any level.

It is produced in every sector and in organizationsof any size. Stress affects the health and safety ofindividuals and also welfare of organizations andnational economies.

Workplace stress occurs when job demandsexceed the available resources of human beings.Stress is not only the result of major adverse events,but also is the result of stress and daily pressures.The latter, by their frequency, have an importantrole in the professional environment and affectmore individuals than major adverse events, whichare rare.

Andreescu Anghel, Liţă Ştefan (2006), statesthat "sometimes, professional stress is consideredas a positive, beneficial factor on performance.

Thisrefers to eustress which means activation, mobilizationof individual resources. It is important todistinguish between eustress and distress, one as astate of stress with beneficial effects, the other withadverse health effects."

Today's world, more dynamic in work fieldperspective, brings in the foreground workers, personnelreductions and services externalization, increasedflexibility in terms of position and competencies,the growth in number of determined timejob contracts, growing uncertainty of jobs and intensity of work (overload and higher pressure), andunstable balance of life and work.

That is why stresscan bring disease and pain to individuals, both atwork and at home.

Stress can compromise safety at work too, thuscontributing to other health issues related to work,such as musculoskeletal disorders.

Stress greatly affects the image of an organization.

Reducing stress associated with work andpsychosocial risks is not only a moral imperativebut also a legal one. It is also an important problemof economic efficiency. The good news is thatstress associated with work can be approached inthe same logical and systematic way as other healthand safety issues.

There is an abundance of practicalexamples to cope this problem in the area ofEurpean Union countries. Using the appropriatemethod, workers may be stress protected.

Considering the example of a person who feelsgreat pressure meaning job demands (work time, liability)larger than individual capabilities and corroboratedby conflicts with colleagues/leaders, frequentchanges or threats on job security – such asthe possible personnel downsizing; all these couldbe perceived as a stressful situation by someone orchallenging by another. What makes the differencein perception depends on job nature, the psychologicalprofile of individuals, as well as physicalhealth or health, in general.

Manifestations of stress at workplace canlead to: depression, anxiety, feelings of being overwhelmedand unable to cope with them, a decreasein professional performance, an increase in thenumber of days for sickness treatment, absenteeism,insomnia, cognitive difficulties (reducedability to concentrate or make decisions), fatigue,headache, palpitations, gastrointestinal problems, increased aggressivity, etc.

All these lead to the followingconsequences:
- a decrease in productivity
- an increased risk of having an accident atwork
- damage in personal relationships
- an increased risk of health problems(cardiovascular disease, digestive problems,cervical pain, etc.)

STRATEGIES TO ELIMINATE AND

PREVENTSTRESS AT WORK

Recent studies of the so-called “healthy organizations”suggest that policies for employee healthlead to benefits for organizations.

A healthy organizationis defined as an organization with a low rateof illness or disability in working personnel.

Thatmeans an increased competitivity. Researchers haveidentified that a minimum stress at work leads toa higher level of productivity as definition for ahealthy organization.

These relate to:
- Recognizing employee’s performance
- Opportunities for career development
- Organizational culture that values employee
- Managerial actions in concordance with thevalues of the organization.

Strategies to reduce stress at workinclude:
a) psychological assistance for employees isan occupational health service; that is provided byorganization in order to reduce or eliminate the decreasingperformance at workplace;
b) training services for employees: performancemanagement, time management, stress management,career management, etc. ;
c) educational workshops: parenting,work lifebalance, time management, stress management,emotional control, anger management, decisionmaking and problem solving, alertness, communicationin couple, smoking quittance, weight control, etc.;
d) online educational resources: newsletter, accessto educational information for the employeeand his family (web resources, brochures);
e) career management services;
f) services for managers and professionals inhuman resources departments are:
- management of critical incidents ;
- assistance for enhancing skills in control ofinadequate behaviors of employees;
- assistance and training in management ofemployees capabilities;
- development and implementation of employeeassistance programs in the company.

STRESS PREVENTION AT WORKPLACE

There is no standardized approach or a manualfor developing stress prevention programs. Designprograms and solutions will be influenced by manyfactors: the size and complexity of the organization,available resources, and in particular the typeof problems the organization faces.

For example, insome companies the main problem is overloadingemployees and others, an inflexible program or lackof communication with the public. In other words,it is not possible to find a universal prescription forstress prevention in the workplace, but it is possibleto provide some guide lines for the prevention ofstress in organizations.

In all cases, the process of stress preventionprograms involves three distinct approaches: problemidentification, intervention and evaluation. Organizationsmust be prepared properly to lead thisprocess successfully.

A minimum level in preparing a stress preventionprogram should include the following:
- workplace stress awareness (causes, costs,control);
- ensuring a quality management and supportfor the program;
- employee involvement in all phases of theprogram;
- establishing the technical capability ofprogram management (specific training formembers or consultants involvement).

Assembling employees or employers and managersin the same committee or “solving problemgroup” can be a very useful approach for developinga stress prevention program.

Researches show that these participatory effortswere successfully achieved on ergonomic issuesin the workplace, partialy due to the capitalizationof direct knowledge of employees about theproblems encountered in their workplace.

The “psychological contract” as a mean of preventingand combating stress is today a frequentapproach. In the literature the term “psychologicalcontract” is commonly used in the sense of mutuallyshared set of expectations between the employeeand the organization.

The author Denise Rousseau defines the psychologicalcontract as “individual beliefs shaped byan organization, in terms of exchange between theindividual and the organization”.

Psychological contractcan be accepted as a mental model that employeesuse to inframe and interpret organizationalphenomena. Its terms shall constitute a referencesystem to which employees report their work andthe attitude of employers towards employees is alsovery important.

Characteristics of the psychologicalcontract:
- represents, essentially, a subjectiveperception that differs from one individualto another;
- psychological contract is dynamic, thatmeans it is changing over time, duringthe relationship between employer andemployee;
- refers to the mutual obligations basedon investment in promises made by bothparties, with the hope of a positive outcomefor each party;
- is closely linked to the context of laborrelationship, the psychological contract cannot be created by individual or organizationonly.

The main functions of thepsychological contract:
- reduces incertitude, because not all possibleaspects of the employment relationship maybe covered by a formal written contractbetween the two parts: employer andemployee;
- dictates employee behaviors - like a system,the employee weighs their commitmentto the organization and the organization’sobligations to themselves and change theirbehavior in relatation with critical companyoutcomes;
- the third function of psychological contract,gives to employee a sense of influence onwhat happens to him, in the organization.

To conclude, stress is a “disease” of our time, affecting people no matter of their lifestyle.

Stress is found everywhere, more obvious and more frequent in advanced countries. To find theway to manage stressful situations is up to each of us.

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SYSTEMATIC REVIEW

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New quaternaryammonium salts baseddecontaminants

Diana M. Popescu[1], Viorel Ordeanu[1], Lucia E. Ionescu[1], Gabriela V. Dumitrescu[1],Simona N. Bicheru[1], Marius Necşulescu[1]

Abstract: *Decontamination after terrorist attacks or industrial accidents with biological and/or chemical agents („bio-chem“) must be fast and efficient, in order to reduce the number of victims and to eliminate the consequent damages. The decontamination of living biological agents (bacteria, viruses) or nonliving ones (toxins, regulators) and toxic chemicals could be accomplished by reactions of hydrolysis in various experimental conditions, in particular in alkaline medium, reactions with amines or ammonia, alcohols, phenols etc. and by their transformation into less toxic degradation products.*

"Bio-chem" intentional or unintentional contamination is a real risk, towards which an effective management must be available to prevent and control it. Decontamination is an essential measure to protect the personnel and the environment.

Synthesis and testing of new „bio-chem“ decontaminants, based on quaternary ammonium salts, complete the arsenal of protection against chemical and biological agents.

The most effective selected substances could be produced and used for decontamination in accordance with legal procedures.

Keywords: *„bio-chem“ contamination, decontaminants, disinfectant, quaternary ammonium salts, chemical synthesis*

DECONTAMINATION

After terrorist attacks, biologicaland/or chemical attacks or industrial hazardswith bio-chem agents, the decontaminationmust be prompt and efficient, in order to reducethe number of victims and to eliminate the consequentdamages. The decontamination of the livingbiological agents (bacteria, viruses, fungus, andparasites) or of the nonliving agents (toxins, regulators)and of the toxic chemicals could be accomplishedby hydrolytic reactions in various experimentalconditions (especially in alkaline medium),reactions with amines or ammonia, with alcoholsand phenols etc. or by their transformation in lesstoxic degradation products.

The biological contamination refers to bacteriaand viruses generating diseases as anthrax,plague, smallpox, botulism etc. From the chemicalstructure point of view, the range of the productsused in microbial decontamination is wide butvery few fulfill the conditions of a good decontaminant.

In this paper there are taken into accountthe quaternary ammonia salts obtained by treatingthe tertiary amines with alkyl halogen, particularlyalkyl chlorides or benzyl chlorides. These compoundsare

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the most important agents having abiocide action.

Synthesis and conditioning decontaminant byMechanical Engineering and Research Institute,Bucharest (ICTCM) in different forms with thechemical structure of quaternary ammonium saltsoluble in water was carried out in accordance withnational and European Union legislation related toecology and environmental protection.

Figure 1. Microbiological testing of decontaminating substances, in vitro, in CCSMM microbiology laboratory

Figure 2. Microbiological testing of decontaminating substances in various materials, CCSMM minipolygon biological testing

Spectrum of activity and penetrating ability ofdecontaminating substance is enhanced by a surfactantthat is designed to reduce surface tensionthus facilitating contact between the microbiancell and decontaminant compound.

Decontaminant qualities of each product aredetermined by the choice of the active substanceand should take account of its antimicrobial qualities,the purpose, and the conditions that will beused in.

Decontamination efficiency is determinedby the contact time of each decontaminant product,decontaminating solution concentration, theamount of solution used per unit area and, not thelast, by the decontaminant agent application mode.

SCREENING TEST

Screening of potential decontaminatingsubstances was performed in the Laboratoryof Microbiology-Epidemiology, in diagnosislaboratory for biological agents, ranked by P2+biosafety level for the pathogen microorganisms.

Bacterial sensitivity assays were performed (theantibiotic disc diffusion method on several lots),according to the following parameters: cultivationon solid culture medium Mueller-Hinton, 72hours aerobic incubation at 37°C, with daily reading.

The following bacterial species were tested:Staphylococcusaureas, Bacillus anthracis, Escherichiacoli, Pseudomonas aeruginosa, Vibrio cholerae.

Results were quantified by measuring with 0.1mmof accuracy (caliper and magnifier), comparatively,on many tests, to calculate the average of each substanceon each species.

Product code DC-3 and code DC-7 was carriedout on quaternary ammonium salts and Nalkylpyridiniumbasis. Their testing revealed thatthe best microbiological activity was recorded inthe product code DC-7. The possible synergisticeffect with oxidizing compounds was tested in orderto achieve possible decontaminating mixtures.

The bactericidal effect of the substance wastracked for a set of standard bacterial (gram-positiveand gram-negative, anaerobic bacterial and spores)and other pathogenic microorganisms; bacterialsensitivity results were read after 24 and 48 hours ofincubation and then, after storage at room temperature48 hours, to watch the effect in time.

The antimicrobial effect was quantified by calculatingthe average diameter of bacterial inhibitionzone and bactericidal concentration (g/liter),calculated as the quantity of substance (20 mg)divided by the volume in which the antimicrobialdiffusion was effective. (figure 1, 2).

Figure 3. Microbiological testing of decontaminating substances in various materials, CCSMM minipolygon biological testing

TOXICOLOGICAL TESTING

Toxicological screeningfor acute toxicity aimed to confirm that thesesubstances are not highly toxic and are not dangerousto operators.

To demonstrate, 0.5 ml ofeach substance was injected in mice (approx. 20gweight, young adult), tracking morbidity and mortalityfor three days. The test results have enabledexperiment achievements under conditions of aspecially arranged experimental minipolygon.

Experiencing decontaminating products

Representative microbial strains were used for themain groups of pathogenic bacteria: gram-positivecocci: Staphylococcus aureus, Streptococcuspneumoniae, Enterococcus fecalis; gram-positivebacilli: Bacillus anthracis (vaccine strain), Bacilluscereus, Bacillus subtilis; gram-negative bacilli:Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae,Pseudomonas aeruginosa; vibrio: Vibriocholerae.

The experimental contamination and decontaminationhave been carried out on an out ofservice military vehicle, representing the "target"(figure 3, 4) marked with numbered areas of approximately0.1 square meters on which have beenimplemented operating procedures for CBRN(chemical, biological, radiological and nuclear)contamination control.

Areas were chosen as follows: vertical paintedmetal sheet, for aqueous solution decontaminants;glass window for aqueous solution decontaminants;rubber tires for aqueous solution decontaminantsand horizontal painted metal sheet for suppliedpowder decontaminants as a positive control.

Microbial contamination was done by sprayingthe allocated surface, separately, with every microbial strain and with a mixture of them also. Amicrobial suspension culture in a liquid mediumwas used, with approximately 1 million live bacteriaper ml. Also, it was sprayed 1 ml of suspensionper square decimeter, enough to create a uniformcontaminant film. For liquid decontaminatingsubstances, the aqueous solution (conc.10%) wassprayed to cover the contaminated area until theexcess liquid begun to tear, about, 10ml/sqdm onsmooth surfaces (glass, metal sheets) and about20ml/sqdm on rough surfaces (peeling metalsheet, tires).

Figure 4. Microbiological testing of decontaminating substances on combat vehicle with Biological Mobile Intervention Team of CCSMM in CBRN training area from Campulung

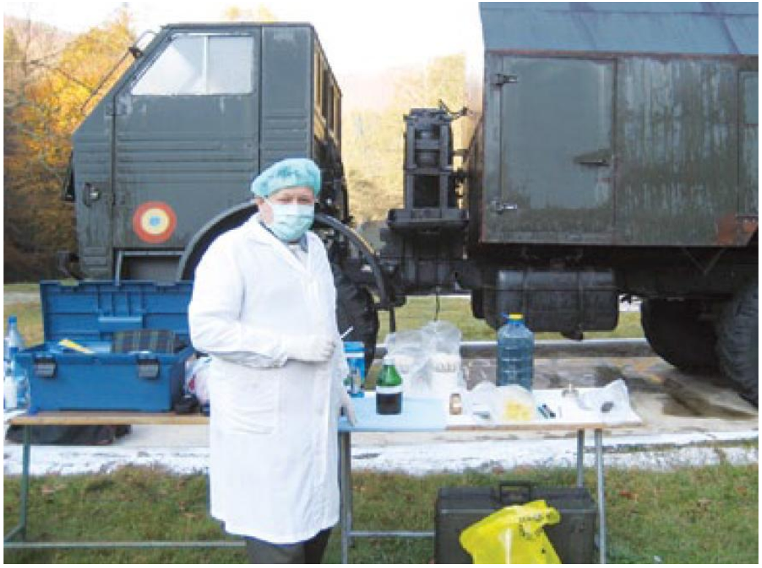

Microbiological samples were collected bymeans of hygienic-sanitary pad, as follows:

0. before contamination (to establish a baselinelevel of natural contamination);
1. immediately after contamination (tocheck the level of contamination experiment);
2. after decontamination for each decontaminantupon each biological agent within 10 min(as required for military using decontaminant);
3. at every 45 min (as for general householddisinfectant).

In addition, at the end of the experiment,samples were collected from different parts of theoperator's protective equipment and from the environmentto detect any residual contamination. Allsamples were immediately transported under biosafetyconditions and tested in the microbiologyP2+ laboratory.

Decontamination achieved resultsafter 45 min are shown in Table 1.

Table 1. Contamination level after decontamination by 10% aqueous solution

No.	Decontaminant	Biological agent	Surface	Time (min.)	Growth (tube)	Growth (pane)	Remarks
1	DC 17 ICTCM	Mixture	Glass	45	-	++hem.	Contaminated
2	DC 17 ICTCM	B. cereus	Glass	45	-	-	Uncontaminated
3	DC 17 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
4	DC 17 ICTCM	B. cereus	vertical metal sheet	45	+/-	-	Uncontaminated
5	DC 18 ICTCM	Mixture	Glass	45	-	-	Uncontaminated
6	DC 18 ICTCM	B. cereus	Glass	45	+/-	-	Uncontaminated
7	DC 18 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
8	DC 18 ICTCM	B. cereus	vertical metal sheet	45	+dep.	-	Contaminated
9	DC 19 ICTCM	Mixture	Glass	45	-	-	Uncontaminated
10	DC 19 ICTCM	B. cereus	Glass	45	+/-	-	Uncontaminated
11	DC 19 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
12	DC 19 ICTCM	B. cereus	vertical metal sheet	45	+veil, dep.	+	Contaminated

- or +/-: no microbial growth; dep.: bacterial deposit at medium bottom;
veil: bacterial population at medium top; hem.: hemolytic colonies.

COMMENTS

The antimicrobial effect remains and is moreobvious after 45 min, suggesting that these productscan be proposed as potential disinfectants formedical or hygienic-sanitary use, according withDrug Law.

The decontamination with powder is less effectivein all cases, compared with the laboratorycontrol sample, because of weak contact betweenmicro-organism and decontaminant, so a residualcontamination remains on surfaces.

In all cases, the final stage of decontaminationshould be washing with water because the substancesused can be corrosive upon certain materialsor irritating for personnel.

After the final washingaction, a microbiological analyze was madeto determine residual contamination of surfaces,waste water or protection equipment, but a significantcontamination was not recorded, so it can beconcluded that there is no risk to the environment.

CONCLUSIONS

A number of potential decontaminating substanceswere synthesized and tested, eight of whichwere selected and recommended as decontaminatingagents because these can be characterized byhigh chemical stability. These products are solublein water and various organic solvents. These biocidalproducts have bactericidal and fungicidal capabilities,with a large spectrum of usability. Thesubstances are characterized by low toxicity onanimals.

Theoretical and experimental scientific researchhas been made to design and implementpolyvalent “bio-chem” decontaminants for destructionof biological and toxic chemical agents,hazardous to health. Technologies have been developedfor obtaining polyvalent decontaminants,selected by efficiency assays, in laboratory. The experimentalpolyvalent model for chemical and biologicaldecontamination with decontaminants ofquaternary ammonium salts types was developed.

Among the tested products, the best antimicrobialactivity is achieved by products based onmixture of oxidizing compounds and quaternaryammonium salts, which also have a synergic effectwhen used, expanding the action spectrum.

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News and Perspectiveson Treatment of NormalPressure InternalHydrocephalus

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Abstract: *Many patients, usually over 60 years old, presenting presenile dementia associated with marked gait disorders, impaired balance, urinary incontinence, have been shown to have enlarged ventricles associated with relatively small cortical atrophy. Intracranial pressure monitoring indicates normal values, or subject to only minor peaks, usually at night. Because some of these patients improve markedly after ventricular shunting procedures it has been suggested that their neurological dysfunction may be caused by a pressure effect on the brain from the increased internal surface of the ventricles. Many of these patients do benefit from surgery, and a lot of them have a history of subarachnoid hemorrhage, traumatic brain injury or meningitis which might have impaired the CSF absorption.*

INTRODUCTION

We would like to present the experienceof our clinic over the last fiveyears regarding the treatment ofnormal pressure internal hydrocephalus(28 patients operated between January2009 and December 2013), to report our results andcompare them with the statistics and results fromthe international literature. Normal pressure internalhydrocephalus (NPIH) represents an increase ofCSF volume, with different etiology, that causes anenlargement of the ventricular system as a consequenceof the hydrodynamic CSF circulation disorders.

The cause of this disease cannot be identifiedin 60% of cases. It was described in 1965 with theHakim & Adams triad: gait disorders and impairedbalance, cognitive disorders (progressive dementia),sphincter disorders (8). The imagistic explorations(CT scan) indicate the size of hydrocephalus; ICP< 15 mmHg and the pressure gradient between theventricles and subarachnoid space is very low. Fortreatment there are extrathecal shunts (particularlyventriculo-peritoneal shunt) and intrathecal shunts(particularly endoscopic ventriculocisternostomy).

Both methods have been practiced successfully inour clinic.

The ideal treatment method for hydrocephalusstill does not exist. None of the surgical techniquesis perfect and no shunting device gave total satisfaction(4, 7).

Hydrocephalus represents the enlargement ofone or more parts of the CSF containing anatomicstructures. The CSF total volume is about 150 ml inadult; the ventricular system contains 25-30 ml, thespinal subarachnoid space contains 30 ml and therest of CSF is contained by the cranial subarachnoidspace and basal cisterns (1).

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Figure 1. The ventricular enlargement shown on CT scan

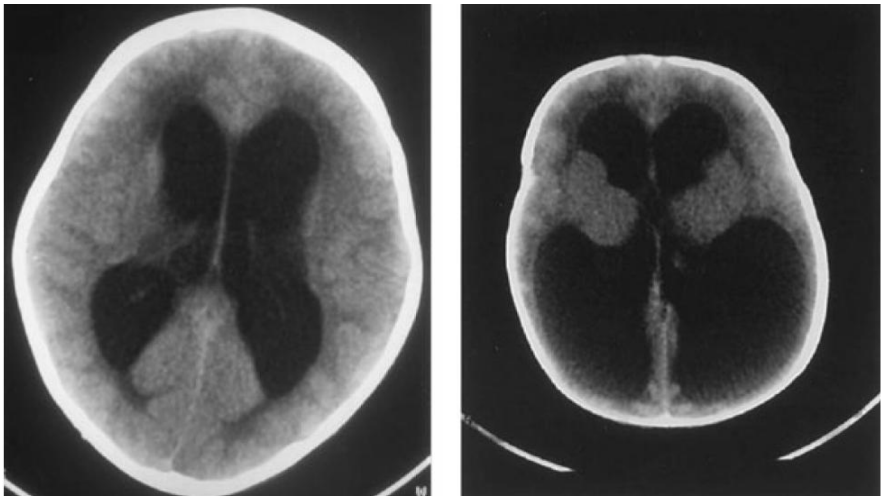

The total production ofCSF is about 600 – 700 ml/24 hours, approximately0,35 ml/min. The CSF participates in maintainingthe endocranial volume constant by adjusting theproduction/absorption ratio depending on the cerebral parenchyma volume and the intracranial bloodvolume variations according to the Monro-Kellierelationship. Changing the volume of one of thethree intracranial components (brain tissue, blood,CSF) is followed by a compensatory reaction fromthe other two components in order to maintain insidean inextensible space the endocranial volumeconstant (9).

Based on its underlying mechanisms, hydrocephaluscan be classified into obstructive and communicating.

The obstructive hydrocephalus is definedby any condition that restricts the CSF flowto and from the ventricular system. Any CSF flowinterruption outside the ventricular system definesthe communicating hydrocephalus (5).

Figure 2. Lateral ventricles puncture

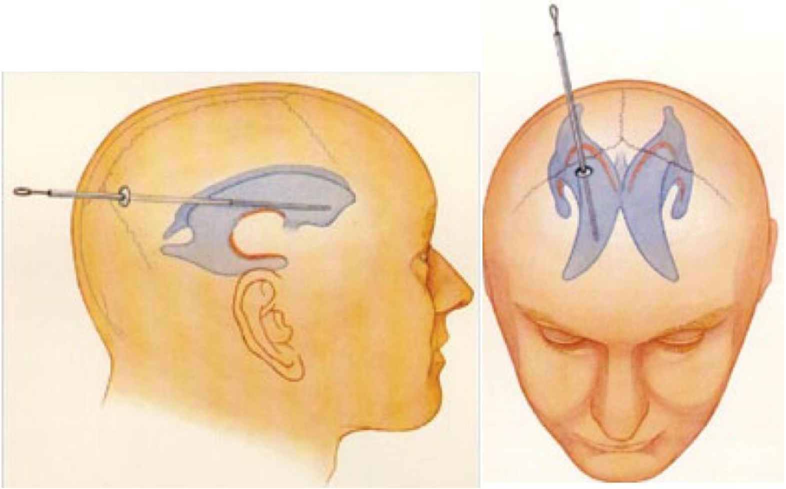

The NPIH etiology is not fully known. Thereare many possible congenital or acquired causes, butthe most important are the subarachnoid hemorrhages(20%), meningitis (1%), parasite infections,traumatic brain injuries, neurosurgical procedureswith open ventricular system, intoxications, Alzheimerdisease (15%).

Hydrocephalus of the adult patients is a communicating,chronic and normal pressure hydrocephalus(3).

DIAGNOSTIC

The CT scan and the MRI revolutionized thediagnosis and the postoperative follow-up of thehydrocephalus. The CT scan is the first stage of diagnosis.

It highlights an obstructive cause, evaluatesthe ventricular enlargement, appreciates the cerebralparenchyma condition (periventricular hypodensity)and the subarachnoid spaces condition (basal cisterns,sylvian fissures, interhaemispheric fissure, corticalsulci).

Bifrontal index measuring (the distancebetween frontal horns / intracranial distance ratio, onthe same CT slice) > 50% is suggestive for a possibledecompensation of hydrocephalus.

The Evans index> 30% has the same meaning (the frontal horns size/ the maximum biparietal diameter ratio). It suggestsan active hydrocephalus. The MRI completes the CTscan by describing the obstructive lesions accuratelyand obtaining dynamic information over the CSFflow (the absence of CSF flow through Sylvius aqueduct).

The preventing treatment is important becauseof the existing risk of postoperative hydrocephalus afterany neurosurgical procedure. Cisternograms withradioactive markers (99Tc-DTPA) remain controversialand rarely used.

The radionuclide is injectedinto the subarachnoid space by a lumbar punctureand serial images are taken by planar scintigraphy 3,6 and 24 hours after the injection. In case of NPIHintraventricular radioactivity can be obtained even48 hours after the injection. Serial lumbar punctureswith repeated evacuation of 15 – 30 ml CSF associatedwith clinical improvement can predict a favorableresponse to shunting procedures. Patients withinitial measured CSF pressure >15 mmHg respondedfavorably after ventriculo-peritoneal shunting (6, 8, 9).

TREATMENT

The treatment of hydrocephalus depends onthe moment of diagnosis, etiology, age and clinicalcondition of the patient (particularly the acuteform) and the complementary investigations results.

The treatment with acetazolamide, a carbonic anhydraseinhibitor has favorable effects predominantlyby inhibiting the chorioid plexus secretion and lessby the diuretic effect.

The acetazolamide dose is 25mg/kg/day with simultaneously administration offurosemide 1 mg/kg/day.

The treatment of obstructive hydrocephalus isremoving the obstacle (excision of tumors). The surgicaltreatment seeks not returning to normal size ofventricles but regaining most of the lost neurological functions.

The diuretic and corticosteroid therapiescomplete the CSF evacuation by lumbar punctions.The CSF lumbar drainage will be performed onlyafter the confirmation of communicating chronichydrocephalus by imagistic methods.

There are several types of extrathecal derivationsof CSF: controlled external ventricular drainage,ventriculoperitoneal drainage, ventriculoatrialdrainage, ventriculopleural drainage.

The currentlyused valves are predetermined opening pressurevalves (low, medium and high pressure), modularopening valves and programmable valves with variableresistances, self-regulating valves etc.

The intrathecal derivation (particularly theendoscopic ventricular cysternostomy) representsan alternative treatment method. Both extra- andintrathecal derivations have been successfully performedin our clinic.

The intrathecal CSF derivations consist of endoscopicventriculostomy through the 3rd ventriclefloor aiming to restore the communication betweenintraventricular and subarachnoid liquidian compartments.

MATERIAL AND METHODS

We retrospectively studied 28 patients admittedto our clinic between January 2009 and December 2013. The patients were all neurologicaland imagistic diagnosed with normal pressure internalhydrocephalus.

Only in 18 cases, a cause forimpaired CSF absorption and ventricular enlargementcould be detected. 8 patients had a historyof traumatic brain injury, 4 patients had a historyof subarachnoid hemorrhage (First and secondgrade on Hunt and Hess scale) with normal “4vessels” cerebral angiogram, 2 patients were diagnosedwith Alzheimer disease prior to admissionand 4 patients had a history of ischemic stroke.

3 of the patients with NPIH after subarachnoidhemorrhage underwent endoscopic procedureswith intrathecal derivations (ventriculocisternostomy).

On the other 15 patients were performedventriculo-peritoneal shunts using various valves(most of them were low pressure valves). The mostfrequently used were the Delta (Medtronic), Spitz – Holter, Heyer – Schulte, Pudenz, Cordis – Hakimvalves. In 10 cases, a cause for NPIH was not revealedand these patients were diagnosed withidiopathic NPIH. They have also been performedventriculoperitoneal shunts with low pressurevalves.

Clinical improvement was significant inmost patients, but only partial in the patients diagnosedwith Alzheimer disease.

According to statistics from literature, urinaryincontinence is the main symptom that resolves aftershunting procedures.

Gait disorders and impairedbalance are subsequently remitted and dementia isthe last that improves (3, 4, 8).

Black and collaboratorsestablished few criteria that can predict thefavorable clinical course after shunting procedures:

- clinical: the presence of symptomatic triad;approximately 77% of the patients presenting gaitdisorders as primary symptom improve their locomotorfunction after shunting; the patients withdementia without gait disorders rarely improve afterdrainage;
- patients with CSF pressure >18 mmHgon lumbar puncture or continuous monitoring improvetheir neurological status after ventriculoperitonealdrainage;
- patients with CT or MRI showing largeventricles with minimal cortical atrophy have favorableevolution after shunting procedures.

The response to drainage is especially good asthe symptoms started recently (1, 5).

ILLUSTRATIVE CASES

Case 1.

66 year old male patient complaining ofgait disorders, impaired balance, cognitive impairment(occasionally) and sphincter disturbances(imperious need to urinate) which started abouta year ago, slowly progressive despite of conservativetreatment. We decided to install ventriculoperitonealdrainage with self-regulating valve. Postoperativeevolution was favorable, symptoms therebyimproving considerably about 3 months after theprocedure.

Case 2.

59 year old male patient complaining of headache, nocturnal insomnia, depressive syndrome,cognitive impairment, gait disorders and locomotor’s instability.

Case 3.

68 year old female patient hospitalized for memory disorders, sphincter disturbances (imperiousneed to urinate), gait disorders, vertigo, occasional headaches and depressive syndrome. The CT scan

revealsenlarged ventricular system. It was decided to install VP drainage with Delta low pressure valve. The symptomsimproved partially after one month with significant improvement 6 months after the procedure.

Figure 3. The ventricular catheter placed inside the right lateral ventricle and the selfregulating valve placed in the right parietal region, under the scalp.

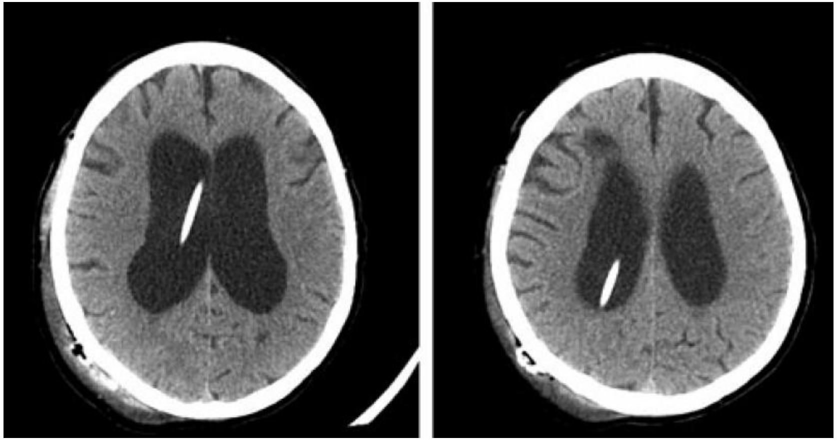

Figure 4. The ventricular catheter placed inside the frontal horn of the right lateral ventricle, near septum pellucid; VP drainage with Delta (Medtronic) low pressure valve.

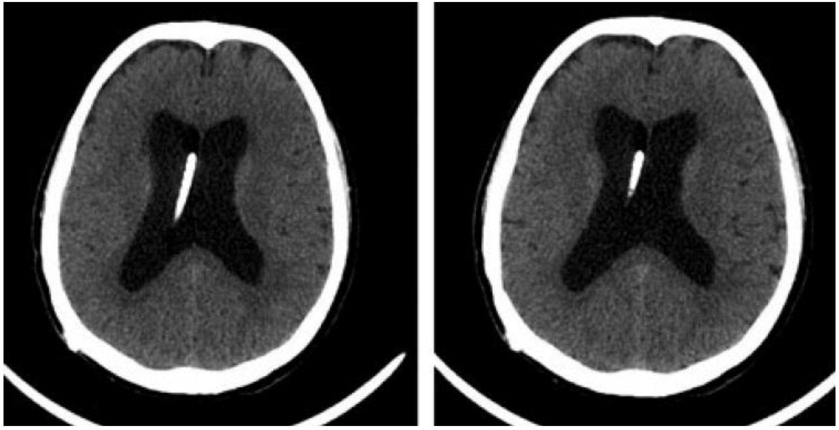

Case 4.

64 year old male patient facing important balance disorders, persistent vertigo, extremely difficultgait, urinary incontinence (and incipient stercoral), onset of Alzheimer disease (after neurological and psychiatricexaminations). It was inserted a Delta low pressure valve. The symptoms improved partially after3-4 months; the gait has become easier, sphincter disturbances have improved and the cognitive impairmentstill exist, but more tolerable.

Case 5.

72 year old female patient hospitalized for walking difficulties, balance disorders, persistent vertigoand vomiting, dehydration. A central venous catheter was inserted into the right subclavian vein andVP drainage with a Delta low-pressure valve was inserted on the left side; intraoperative CSF pressure was15 mmHg.

Case 6.

66 year old male patient with memory disorders, impaired balance and gait disorders, cognitiveimpairment, urinary incontinence (occasionally), started over a year ago, with progressive evolution. Intraoperativemeasured ICP was 18 mmHg. Ventriculoperitoneal drainage with Holter low pressure valve wasinstalled. The

symptoms improved after one month with the complete remission of gait disorders, impairedbalance and sphincter disturbances.

Figure 5. The ventricular catheter placed inside right lateral ventricle (functional drainage).

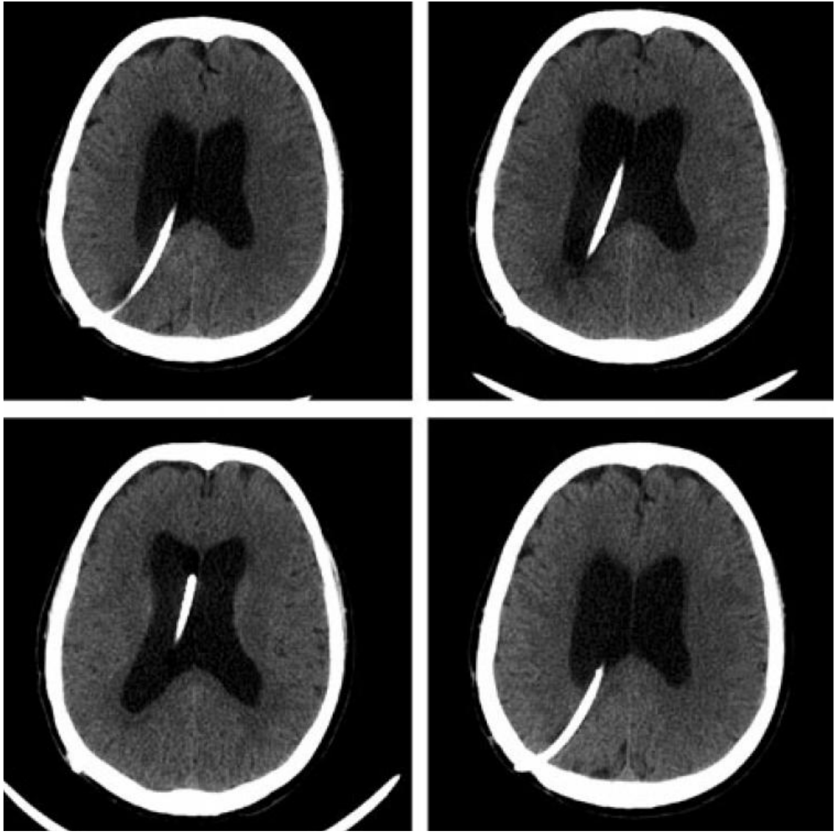

CONCLUSIONS

The ventricular enlargement and the pressureon the frontal lobes are probably responsible for theoccurrence of cognitive disorders and dementia.

The pressure on the regulator centers of the sphincterfunctions located in the paracentral lobule maybe responsible for the urinary incontinence. Theventricular dilatation may compress the internalcapsule and secondary the pyramidal tract, responsiblefor the gait disorders, impaired balance and pyramidalsyndrome (4, 5, and 9).

To determine the surgical indication and to anticipatethe subsequent postoperative evolution, severalclinical and imaging criteria must be established:

- the obstructive causes for hydrocephalus must be excluded;it must be a communicating form;
- a periventricular hypodensity on CT scan or hyperintensityon T2 sequence (MRI) might be transependimar CSFresorption and might anticipate a favorable evolutionafter the shunt procedure;
- the rounding and symmetrical ballooning of the frontalhorns;
- dilated focal of convexity sulci may be revealed by imagingstudies and they are atypical CSF reservoirs whichsubsequently decrease after drainage and they should notbe confused with cortical atrophy; typical cortical atrophy(ex vacuo hydrocephalus) occurs frequently with Alzheimerdisease, and there is a limited response to ventriculoperitonealdrainage (11).

Figure 6. Preoperative MRI (T2 sequence) and postoperative CT scan; right ventricular catheter;prominent convexity sulci; incipient cortical atrophy

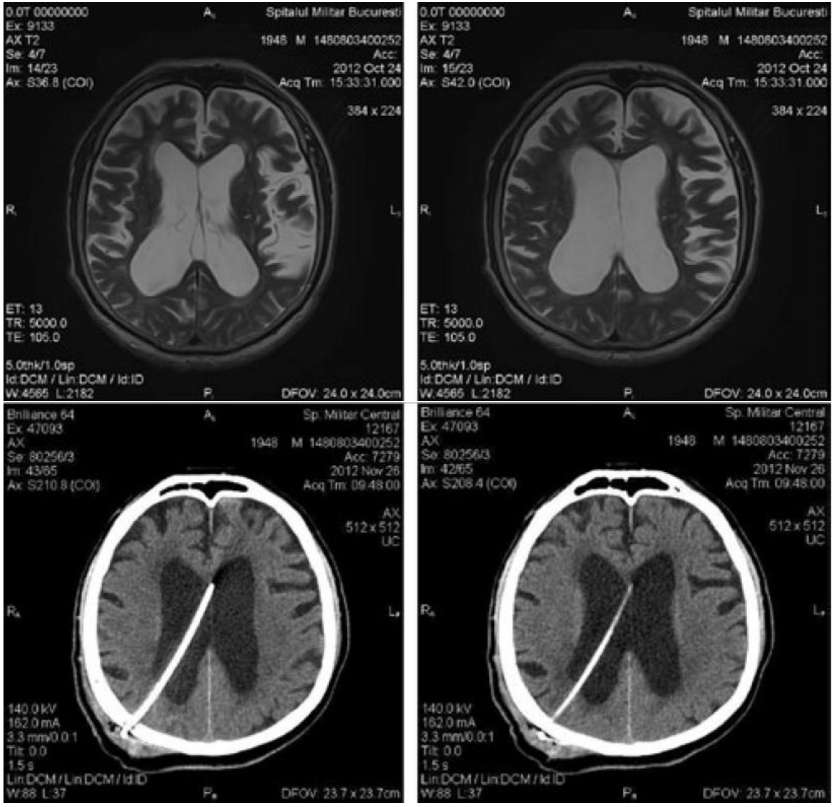

Figure 7. Preoperative and postoperative CT images; the catheter has been placed inside the left lateral ventricle because a central venous catheter was inserted into the right subclavian vein (functional drainage).

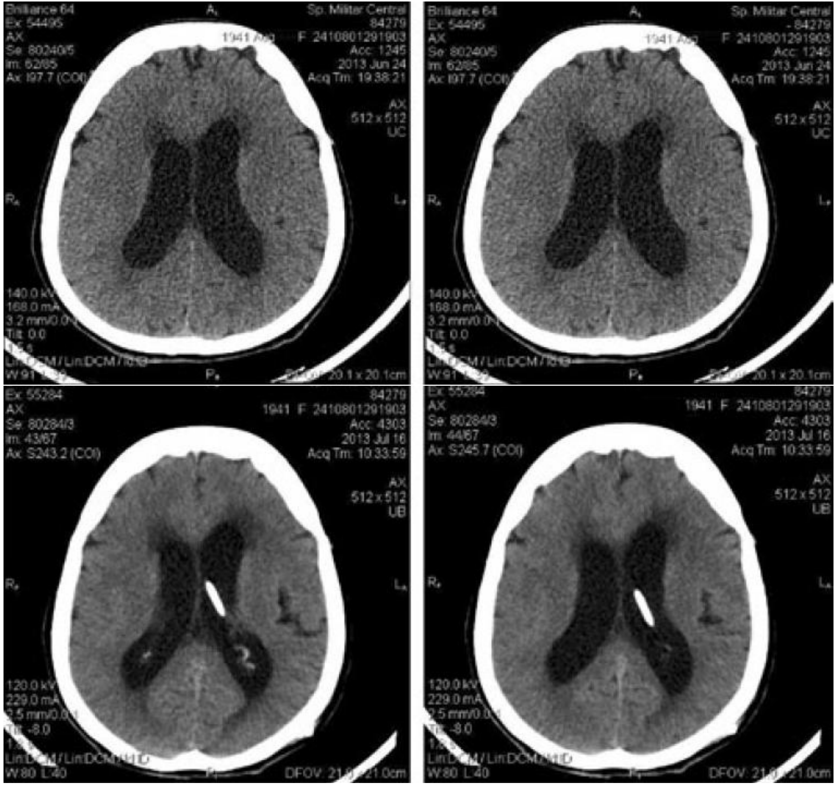

Figure 8. Important hydrocephalus with cortical sulcus persistent; catheter placed inside the left lateral ventricle (coronal section).

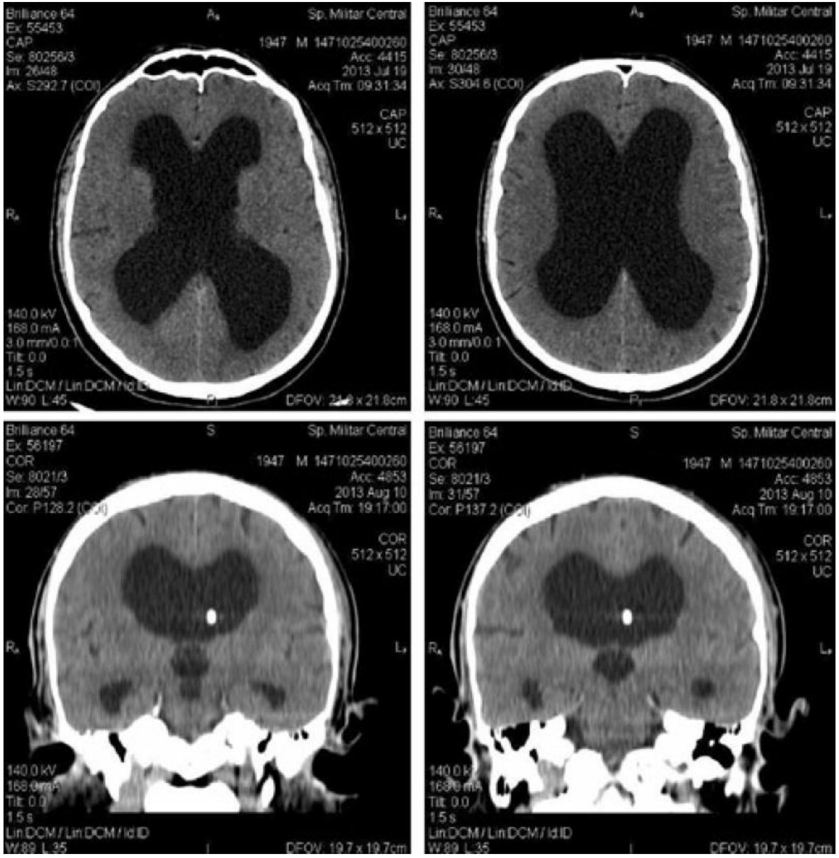

Maintaining good results depends on periodicmedical checks, immediate recognition and treatmentof complications (insufficient drainage shouldbe suspected first). There is no ideal method for thetreatment of hydrocephalus. None of the surgicaltechniques is perfect and no device gave total satisfaction.

The accelerated development of technology andthe practical experience in CSF intra- and extrathecaldrainage allow overcoming the current difficulties.

The world population undergoes a pronounced agingprocess, the number of people aged over 60, increasingto 400 million over the last 40 years. The ratio ofthe elderly population has been modified, representingover 50% in developed countries, with the Europeanzone being the aged (10).

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CLINICAL PRACTICE

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Nonalcoholic fatty liverdisease – an etiologicalapproach

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Abstract: *Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of fat in the liver (hepatic steatosis) either on imaging or on liver histology only after the exclusion of secondary causes of fat accumulation in the liver (e.g. high alcohol drinking, drugs and other medical ailments). Considering the fact that there are many causes of hepatic steatosis, the term NAFLD is reserved for the liver disease that is predominantly associated with obesity and metabolic syndrome. The presence of inflammation and cell injury defines steatohepatitis (NASH) which has the potential to evolve into cirrhosis and hepatocarcinoma, being, therefore, the stage of NAFLD most amenable to treatment. Among the treatments available, the most important are: weight loss, vitamin E and, last but not least, probiotics.*

INTRODUCTION

Excessive alcohol consumption must beexcluded (>21 drinks per week in menand >14 drinks per week in women overa 2-year period before the baseline liverbiopsy).

Insulin resistance (figure 1) is, therefore,central to the development of NAFLD, as it is centralto metabolic syndrome (MS). The Adult TreatmentPanel III defines MS as the presence of three or moreof the following features:

1. Waist circumference greater than 102 cm inmen or greater than 88 cm in women,
2. Triglyceride level greater than or equal to 150mg/dL,
3. High-density lipoprotein cholesterol level lessthan 40 mg/dL in men and less than 50 mg/dLin women,
4. Systolic blood pressure greater than or equalto 130 mm Hg or a diastolic pressure greaterthan or equal to 85 mm Hg, and
5. Fasting plasma glucose level greater than or equal to 110 mg/dL.

Patients with features of MS are at high risk forNAFLD.

The gold standard for the diagnosis of NAFLDis hepatic biopsy which further characterizesthe ailment deriving two stages of histologicalevolution: nonalcoholic fatty liver (NAFL) (thismay be the plain hepatic steatosis) and nonalcoholicsteatohepatitis (NASH).

NAFL is defined as hepaticsteatosis with no evidence of hepatocellular injuryin the form of hepatocyte ballooning. NASH isdefined as the presence of hepatic steatosis andinflammation with hepatocyte injury (ballooning)with or without fibrosis. Although NAFL may beproportionally more common than NASH,

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onlypatients with NASH have the potential to progressto cirrhosis. The presence of the characteristicballooning injury is considered to be the key tothe diagnosis.

Figure 1: Insulin resistance – central to metabolic syndrome

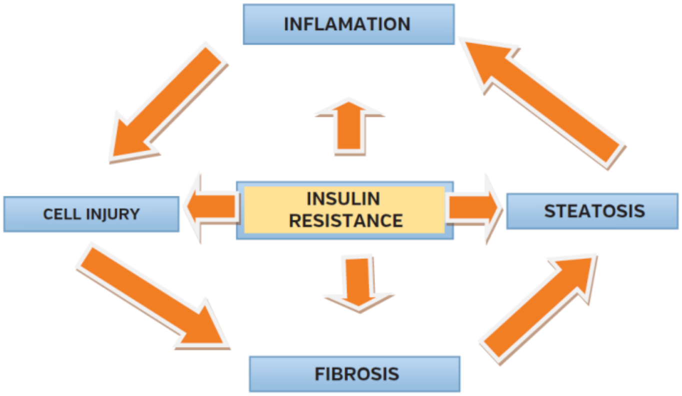

Ballooning injury results in enlargedvacuolated cells, classically containing Mallory-Denk bodies, which are eosinophilic cytoplasmicinclusions near the nucleus. The most importantinjury may be identified in zone 3 (around thecentral venule of the hepatic lobule) and this patternof distribution is also characteristic of NAFLD.

Thecardinal histologic feature of NAFLD is the presenceof an excessive accumulation of triacylglycerols(TAG) and in hepatocytes. The presence of obesityand insulin resistance lead to an increased hepaticfreefatty acid (FFA) flux creating an environmentappropriate for the development of NAFLD/NASH.

The resultant net increase in hepatic FFAis hepatotoxic unless it is converted to nontoxicintracellular triglyceride (TG). When the synthesisof TG is impaired, the level of FFA in the liver isincreased with subsequent augmentation of hepaticfatty acid oxidation resulting in the overproductionof reactive oxygen species (ROS) also known as freeradicals causing hepatocellular injury.

Based on this biochemical knowledge, a two-hithypothesis for the pathogenesis of NASH has beenproposed. The first hit involves the accumulationof excess triglyceride and particularly FFA inhepatocytes. The second hit is the generation oftoxic reactive oxygen species with the production ofhepatic injury and inflammation as a consequenceof FFA oxidation which ultimately leads to theinitiation and progression of fibrosis [1].

Hence, steatosis is mandatory for the diagnosisof NAFLD but alcohol consumption and chronichepatitis C should be taken into account as two ofthe most important alternative causes amenable todifferent treatments.

The diagnosis of NAFLD requires the following:
(1) Hepatic steatosis according to imaging or histology
(2) No significant alcohol consumption
(3)No competing etiologies for hepatic steatosis (table 1)
and
(4) No coexisting causes for chronic liver disease.

NAFLD is the most common cause of abnormalliver chemistry, so other causes, like those in table 1,should be ruled out. The majority of patients withNAFLD are asymptomatic. The most frequentlyencountered symptoms are: vague right upperquadrant dull ache or discomfort. Hepatomegaly isthe most common physical finding. Other clinicalsymptoms and physical findings are, also, nonspecific:general malaise, abdominal discomfort, nausea.

Celiac disease always should be ruled out insuspected individuals considering the fact that thisdisease is often underdiagnosed and seldom to betaken into account as a differential diagnosis ofhipertransaminasemia.

Table 1. Conditions associated with the risk of hepatic steatosis

STEATOSIS
• Insulin resistance
• Obesity
• Type 2 diabetes mellitus
• Dyslipidemia
• Hypertension
• Sedentary lifestyle
• Corticosteroids
• Estrogens
• Amiodarone
• Antiretroviral medications
• Obesity surgery (e.g., jejunoilealbypass)
• Rapid weight loss
• Carbohydrate excess (e.g., diet andtotal parenteral nutrition)
• Chronic hepatitis C virus, mainlygenotype 3
• Hypothyroidism
• Polycystic ovarian syndrome

Once NAFLD is diagnosed, the next step is todetermine the severity as it is necessary to establish theprognosis. Clinical examinations and laboratory andimaging studies in combination lack the sensitivityand specificity for distinguishing NAFL from NASHand for determining the presence and stage offibrosis, which is the most important determinant forthe severity and progression of disease.

Circulating levels of cytokeratin 18 fragmentshave been investigated extensively as novelbiomarkers for the presence of steatohepatitisin patients with NAFLD, but this testing is notroutinely recommended. Other noninvasive testsare emerging; however, these are not yet ready forprime time. [1,2, 3]

Liver biopsy still remains the most reliable approachfor identifying the presence of steatohepatitisand fibrosis in patients with NAFLD.

The recommendations for liver biopsy areas follows (figure 2):

1. Patients at increased risk for steatohepatitisand advanced fibrosis according to the presenceof features of MS and possibly the NAFLD fibrosisscore.
2. Patients with suspected NAFLD for whomcompeting etiologies of hepatic steatosis andcoexisting chronic liver diseases cannot be excludedwithout liver biopsy.

There is a general consensus that patients withNAFL have a very slow progression (if any). Onthe other hand, patients with NASH can exhibithistological progression and can develop fibrosis(37%-41%) and cirrhosis (Approximately 5%) [3].Importantly, hepatic cancer can occur in NASH inthe absence of cirrhosis. This is why every effortshould be made to identify patients with NASH asthey are the ones to progress to more severe formsof disease. The presence of NASH can be associatedwith higher liver-specific mortality in comparisonwith the general population. Cardiovascularailments associated with NASH (as metabolicsyndrome) contribute significantly to mortalityand morbidity. Patients with NAFLD are also atincreased risk for hepatocellular carcinoma, butthis risk is likely limited to those with advancedfibrosis and cirrhosis (1%-42%) [2]. Furthermore, acomparison of the natural history of NASH cirrhosiswith hepatitis C cirrhosis reveals that patients withNASH cirrhosis have a significantly lower risk ofhepatocellular carcinoma. [2]

NAFLD is typically characterized by ahepatocellular pattern of liver-related enzymeswith mild elevations (1-2 times the upper limit ofnormal) in serum alanine aminotransferase (ALT)and aspartate aminotransferase (AST). Up to 50%of NAFLD patients have normal liver biochemistry.Therefore, several biomarkers may aid in thediagnosis. The diagnosis of NASH without a liverbiopsy remains the most significant clinical challengein the evaluation of a patient with hepatic steatosis.Several biomarkers may distinguish between simplesteatosis and NASH. Some of the inflammatorymarkers include serum C-reactive protein,interleukin-6, ferritin, hyaluronic acid (HA), tumornecrosis factor a, leptin, adiponectin, and resistin.

Apoptosis plays a key role in the pathogenesis ofNASH. Among the markers of apoptosis, plasmacytokeratin 18 (CK-18) is emerging as one ofthe promising biomarkers for the noninvasivedetection of NASH. Since oxidative stress also playsan important role in the pathogenesis of NASH,several biomarkers of oxidative stress have beeninvestigated. Among these, oxidized low-densitylipoprotein, thiobarbituric acidreacting substances,superoxide dismutase, and glutathione peroxidasedismutase have been examined. The NASH testcombined 13 variables [age, sex, height, weight, andserum levels of triglycerides (TGs), cholesterol, a2-macroglobulin, apolipoprotein A1, haptoglobin,gammaglutamyl transpeptidase (GGT), ALT, AST,and total bilirubin] to achieve positive predictivevalue, and negative predictive value of 66%, and81%, respectively. [2, 3, 4]

Figure 2: Algorithm for liver biopsy in NAFLD

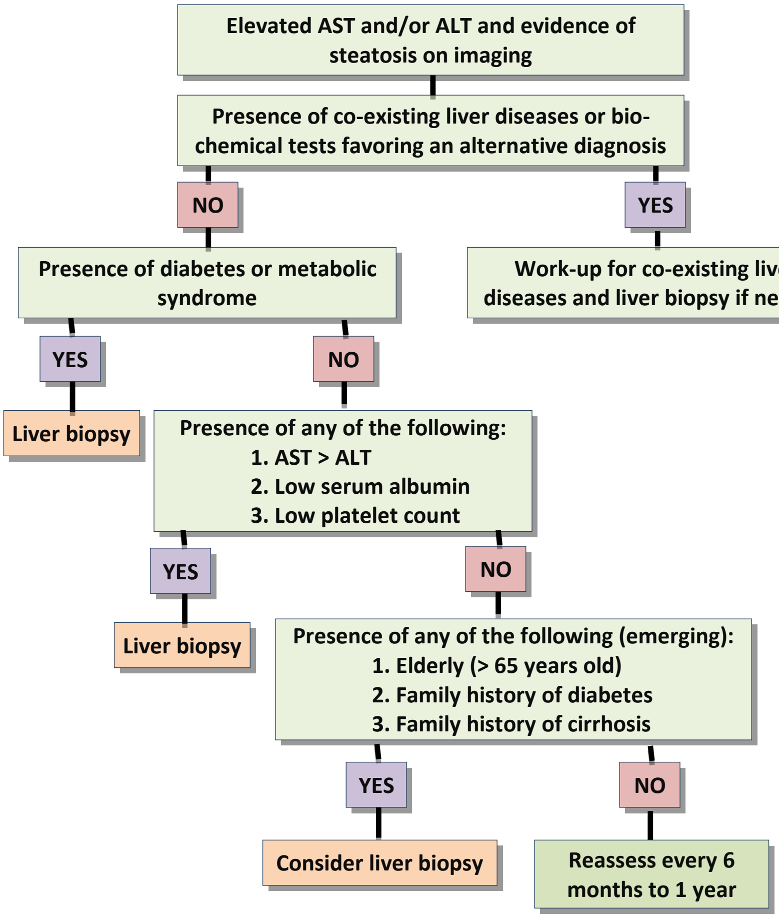
Elevated AST and/or ALT and evidence of steatosis on imaging
Presence of co-existing liver diseases or bio-chemical tests favoring an alternative diagnosis
NO
YES
Presence of diabetes or metabolic syndrome
Work-up for co-existing liver diseases and liver biopsy if needed
YES
NO
Liver biopsy
Presence of any of the following:
1. AST > ALT
2. Low serum albumin
3. Low platelet count
YES
NO
Liver biopsy
Presence of any of the following (emerging):
1. Elderly (> 65 years old)
2. Family history of diabetes
3. Family history of cirrhosis
YES
NO
Consider liver biopsy
Reassess every 6 months to 1 year

Two of the most promising tests for diagnosingadvanced fibrosis in NAFLD are the EuropeanLiver Fibrosis (ELF) score and the NAFLD fibrosisscore. The ELF score includes HA, tissue inhibitorof metalloproteinase 1 (TIMP1), aminoterminalpeptide of procollagen 3, and age. The NAFLDfibrosis score is helpful in the clinical setting becauseit uses routinely available variables in the clinicalsetting, including age, BMI, hyperglycemia, plateletcount, serum albumin, and AST/ALT ratio. We useroutinely available models or markers that increasethe pretest likelihood of finding more advancedliver disease on liver biopsy. These tests can aid inclinical decision making for patients with NAFLD.

Some of these markers are a high AST/ALT ratio, ahigh AST/platelet ratio, low albumin levels, and lowplatelet levels. [2, 3]

TREATMENT LANDMARKS

Among patients suffering from NAFLD (morethan 50% of them being asymptomatic) treatmentis mandatory only in NASH patients because onlythose have the potential to evolve into more severediseases (cirrhosis, hepatocarcinoma) [5].

Because NASH is linked to excess body weightand resulting insulin resistance, diet and lifestylemeasures are the recommended first-line therapy.

Optimal treatment begins with weight loss andphysical exercise. A tangible target for patientswith NAFLD is a weight loss of 5% to 10% of totalbody weight over a 6- to 12-month period [6]. Thosemeasures may improve insulin sensitivity, increaseadiponectin expression, lipid profiles, and liverbiochemistry. The improvement in liver enzymedoes not always correlate with improvement inhepatic histology, unfortunately. As it is alreadydemonstrated, weight loss by dietary changesmay be beneficial even without physical exercise;although physical exercise may lead to furtherimprovement in insulin sensitivity. The initiationof increased physical activity must be the first stepto the treatment of NAFLD; vigorous exercise andresistance training are more helpful than aerobicexercises. The intensity may be more important thanthe duration or total volume of exercise. [7,8, 9]

Current guidelines do not recommend theuse of hepaticopharmacological therapy in patientswith steatosis alone. Instead, patients with NASHand significant liver disease (bridging fibrosis) aregood candidates for this type of therapy. Accordingto the clinical practice guidelines of the AmericanAssociation for the Study of Liver Diseases, the firstchoice of therapy is vitamin E (preferably 800 IU/day). A 2-year treatment in the PIVENS trial (800IU/day) reversed steatohepatitis and improved allhistological features of NASH (except fibrosis) incomparison with a placebo. This beneficial effect ofvitamin E was not associated with an improvement ininsulin sensitivity. Recent studies and meta-analysesshowed increased mortality, a risk of hemorrhagicstroke, and a risk of prostate in long term vitamin Etreatments. [10, 11]

Animal studies have shown that omega-3polyunsaturated fatty acids promote insulinsensitivity, reduce intrahepatic triglyceride content,and ameliorate steatohepatitis. [10, 11]

The drugs to increase insulin sensitivity (glitazone,metformin) may be indicated as a treatmentalternative in NASH 16. Ursodesoxicholic acid andpentoxifilin, which may benefit marginally. [6]

Probiotics and NAFLD

The newest topic in treatment of NAFLD isthat of the involvement of gut microbiota in thepathogenesis of liver steatosis and inflammation.

Intestinal microbiota plays an important rolein health and disease. The gut-liver axis involvesan interaction between bacterial components likelipopolysaccharide and hepatic receptors (Toll-likereceptors). Our gut has approximately 100 trillion(1014) microbes, which make up approximately 1 to2 kilograms of our weight. Gut microbiota performdiverse immunologic, digestive, and metabolicfunctions. [11]

Changes in microbiota may be involved invarious disease pathogenesis (nonalcoholic fattyliver disease (NAFLD), hepatic encephalopathy,alcohol-related liver disease, and hepatocellularcarcinoma). Gut microbiota may cause NAFLDby luminal ethanol production by metabolizationof carbohydrates, causing an increased intestinalpermeability (“leaky gut”) just like in alcoholassociated steatohepatitis (ASH). [12, 13]

In 2009 Miele was the first author to provideevidence of increased intestinal permeability inpatients suffering from NAFLD and this fact wasassociated with increased prevalence of small bowelbacterial overgrowth (SIBO) in those patients [14]. Theincreased permeability appears to play an importantrole in the pathogenesis of NAFLD. Loguerciodemonstrated in 2005 that probiotics may improveNAFLD histology and biochemistry [15]. In October2013 Yan-Yan Ma et al published a meta-analysisin World Journal of Gastroenterology to concludethat the treatment with probiotics and prebioticsmay definitely benefit patients with NASH [11].

Probiotics can inhibit the proliferation of harmfulbacteria, reduce SIBO, restore gastrointestinalbarrier function and modulate the immune system,all of which contribute to the improvement ofNAFLD. This meta-analysis showed that probioticssignificantly reduced ALT, AST, T-chol, TNF-αand insulin resis-tance, which are all related to theprocess and con-sequences of NAFLD. Regularconsumption of probi-otics reduced, also, cholesterollevels which is part of metabolic disturbances inNAFLD patients. [11]

A high fat diet that induces obesity, insulinresistance and hepatic steatosis also leads tohepatic NKT cell depletion. The hepatic NKT cellis the key mediator of HF diet-induced metabolicabnormalities. Moreover, recently, Cani andcolleagues reported that a high-fat diet increasesplasma lipopolysaccharide (LPS) level, which alsocontributes to the pathogenesis of insulin resistanceand increased liver triglyceride content. It is possiblethat this bacterial endotoxinemia caused by highfat diet reduces intrahepatic NKT cells and leadsto worsened or amplified insulin resistance. Theability of probiotics to restore hepatic NKT cellsand improve HF diet-induced insulin resistance andfatty liver are novel findings and intriguing. [12, 13]

These data suggest that strategies designedto down regulate inflammatory mediators withprobiotics have promising potential in patients withNAFLD.

CONCLUSIONS

NAFLD/NASH is a prevalent health problemin general population in close proximity with thesame increased rate of obesity, diabetus mellitus andmetabolic syndrome to which it is pathogenicallyrelated. Proper management of insulin resistance bydiet, weight loss and physical exercise may providethe patients with strong tools to fight the disease.

The increasing evidence of the role of gut microbiotain disease pathogenesis and the role of probioticsin decreasing hepatic steatosis and inflammationpoints firmly towards new and handy solutions inthe nearest future.

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CLINICAL PRACTICE

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An unusual cause for cerebellar syndrome – case report

Carmen A. Sîrbu[1], OctavianM. Sîrbu[2], AncaM. Sandu[2],Cristina P. Sandu[1], Marian Ștefănescu[1]

Abstract: *A male from rural area, S.M., aged 77 years, was admitted in our department for discontinuous headache. His medical history was irrelevant. He has been experiencing intermittent right parietal-occipital headaches during the last 3 months. Neurologic exam revealed a slight right limb ataxia. Initial laboratory findings revealed a white blood cell count of 6500/mm^3 with 75% polymorphonuclear leukocytes, 15% lymphocytes and 8% monocytes. His serum glucose was 90 mg/dL. Non Gadolinium CT scan shows rounded, inhomogenous spontaneous hyperdense area (40-45 UH) between 5-12 mm diameter, localized frontal, temporal, occipital and cerebellar bilaterally.*

The question was whether the lesions were metastasis or parasitic infection?

Cerebral MRI showed unenhanced,well defined,multiple lesions between3-17 mm, with iso-hyperintensityT1, T2, and FLAIR, spreadout periventriculary, subcortically, infrontal, temporal, parietal lobes andsubtentorially, right and left cerebellum(figure 2). After serological testsfrom blood and CSF the diagnosticof neurocysticercosis was certified(an enzyme-linked immunosorbentassay of the CSF was positivefor immunoglobulin G cysticercosisantibody, with 1.32 optical densityunits (OD) (positive result > 0.50OD); his serum IgG cysticercosis antibodywas positive with 5.12 OD).

CT findings are depending on the stageof evolution:

- Vesicular stage (viable larva): hypodense, nonenhancinglesions
- Colloidal stage (larval degeneration): hypodense/isodense lesions with peripheral enhancementand edema
- Nodular-granular stage: nodular-enhancinglesions
- Cysticercotic encephalitis: diffuse edema,collapsed ventricles, and multiple enhancingparenchymal lesions
- Active parenchymal stage: the scolex within acyst may appear as a hyperdense dot
- Calcified stage: when the parasite dies, nodularparenchymal calcifications are seen.

Our patient has multiple lesions in differentphases of evolution (active and calcified).

DISCUSSIONS

Neurocysticercosis is a parasitic brain infection,caused by larval cysts of the tapeworm Taenia soliumby accidental ingestion of eggs.

It is the most common parasitic disease of the nervous system and it is themain cause of acquired epilepsy mainly in developingcountries. Once in the human intestine, Taenia eggsevolve to oncospheres that cross the intestinal walland lodge in the brain where cysticerci develop.

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Time from infestation until first symptoms isbetween days and many years (30).This extremelylong incubation is due to hipnobiosis. They may belocated in subarachnoid space, ventricular system,or spinal cord too, causing a clinical heterogeneity.

Figure 1. Non Gadolinium CT scan shows rounded, inhomogenous spontaneous hyperdense area (40-45 UH) between 5-12 mm diameter, localized frontal, temporal, occipital and cerebellar bilaterally.

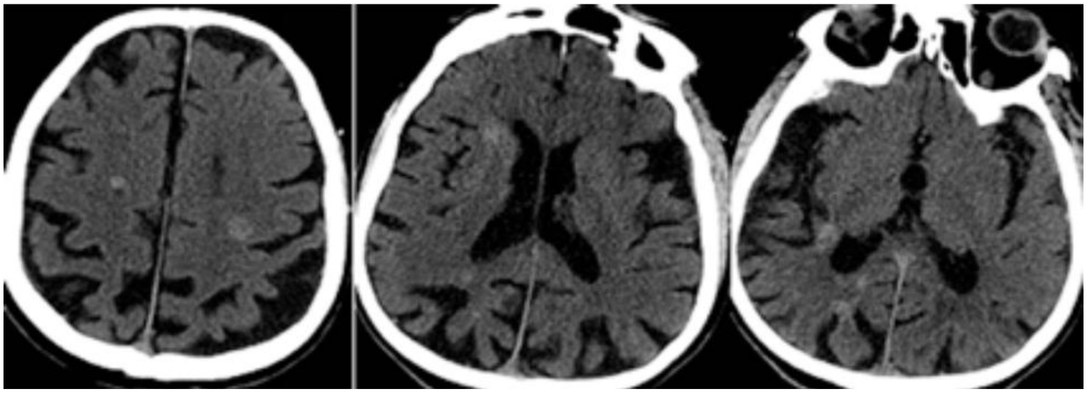

Figure 2. Cerebral MRI showed unenhanced, well defined, multiple lesions between 3-17 mm, with iso-hyperintensity T1, T2, and FLAIR, spread out periventriculary, subcortically, in frontal, temporal, parietal lobes and subtentorially, right and left cerebellum

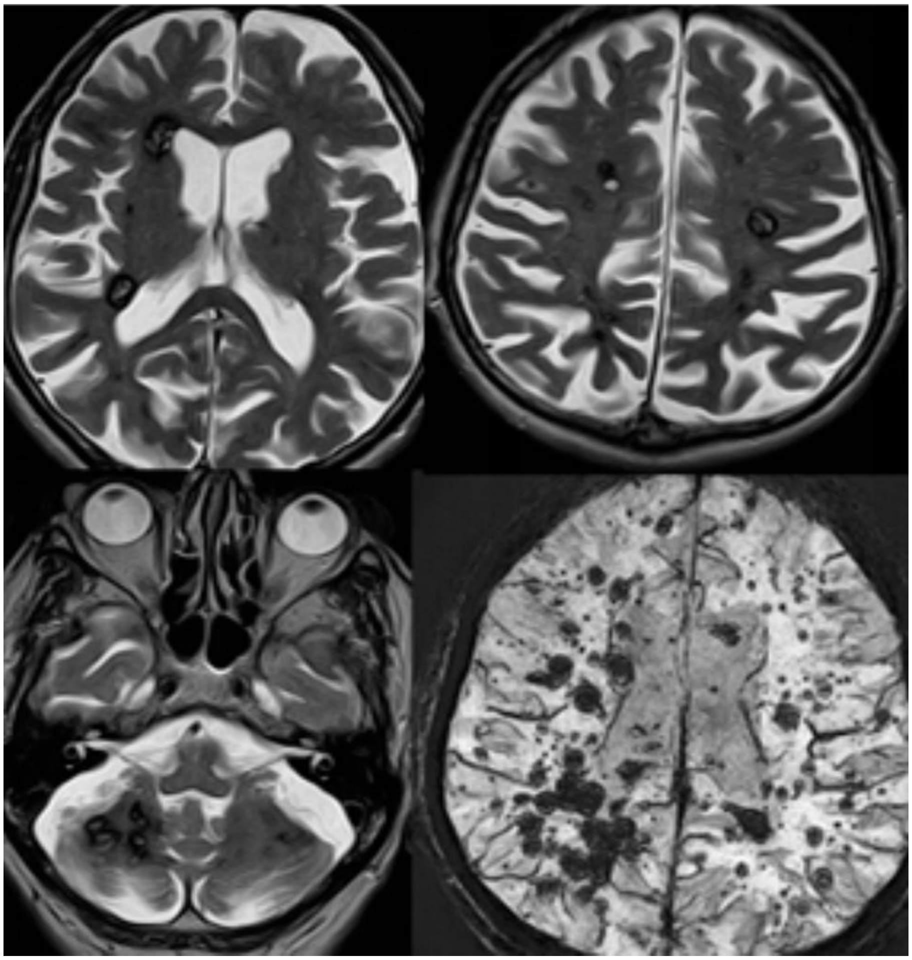

Onset of most symptoms is usually insidiousto chronic, with seizures (most common presentation),headache, dizziness, stroke, neuropsychiatricdysfunctions. Almost every neurological sign orsymptom may be present but physical findings occurin less than 20% of the cases.

There is a pleomorphism of the immune responseagainst Taenia solium. In some cases,cysticerci are destroyed by immunological attack,while in others, parasites may live unchanged foryears. CT scan shows a rounded, homogeneous hyperdensearea with no enhancement with contrastmedium. This phase corresponds to the inactive parenchymalform of the disease.

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Past and future

Andrei Necşulescu[1]

Abstract: *I was thinking a lot about choosing this title which at first glance doesn't seem not to be related to the main topic of this article. However, I think these two opposites have the ability to describe in the most subtle form my journey in Istanbul, the city on two continents. The streets covered with history invites you to discover them step by step and the congress showed me a picture of my future when I will have the opportunity to present important projects in front of a selected audience.*

The 18th edition of the Balkan MilitaryMedical Committee Congress was the startpoint. I can say that it remains, so far, themost important scientific event I have participated.

I also had the opportunity to visit Istanbul,a city like a story that I truly recommend everyone tovisit. Novelty began for me at the military airport Baneasawhen, in a cold morning, I had the chance tosee a C17 Spartan military transport aircraft for thefirst time. I was curious because that type of militaryaircraft had to take me to destination offering me aunique experience. First flight was quiet and we weregreeted in Istanbul by friendly hosts and a beautifulweather. Coach riding to the hotel in the bustling metropolisgave me a chance to admire the panorama forthe first time especially because there was quite a distancebetween the airport and our hotel, the complexwhere we were accommodated being in Asia. When Ireached the destination I had the honor to admire oneof the most beautiful hotels in Istanbul, a true pride ofthe Turkish army and also a proof of their elite role insociety. Beyond the luxury and good taste I noticedagain the perfect planning of every detail, startingwith accommodation. The Congress began. In the firstevening I started meeting my colleagues from othercountries during an elegant and discreet reception.

Beyond the speeches from heads of delegations I was very happy to see that the prize for the best project was awarded to Romania. It was a pride for the whole delegation and we returned to the hotel very happy.

That was the first time I changed impressions with myfellow military students from the other participatingcountries. We linked friendships that I'm sure will belong-lasting and we set up a future collaboration forthe upcoming congresses, everything in a festive andfriendly atmosphere. Beyond the congress I venturedfor the first time into crowded Istanbul with my colleagueRobert Popescu.

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In the second day of the congress I went for thefirst time at the Military Museum from Istanbul whereI attended the opening ceremony. After visiting themuseum, my colleague and I were invited in the hallwhere the presentations were kept. In front of a hugepicture of Mustafa Kemal Ataturk I attended militarymedical exposures from Serbia, Greece, Turkey, Bulgariaand Romania. I started to get ready for my firstspeech in front of an audiencecomposed mostly of doctors.

Day three was the highlightof the congress for me. AlthoughI knew Robert Popescuwas going to present the projectwith doctors and universitystudents, I was told that myposter will be exhibited in the museum lobby where Ishould provide clarifications for the numerous questionsthat might be asked. I admit that things for mewere much more effortless but I was kept up-to-datewith the events of the congress thank to my colleaguewho was getting ready to present his project. I appreciatedthe presentations of my colleagues from othercountries and together we agreed to collaborate in thefuture and gather together at these conferences withbigger projects that will integrate more teamwork. Ileft there convinced that at the next congress I willcome with an oral presentation. I gathered the minimumnecessary experience to deal with future congresswork and I hope I managed to fit in that greatatmosphere provided by the hosts. The presentationof my colleague was praised; his experience at previouscongresses he attended made a difference. I leftthere thinking that I fulfilled the task of coping withchallenges and building the idea that I can come upwith a lot more and improve myself.

Day four was great. It was time to visit the impressiveDolmabahce, a true monument and one ofthe most spectacular buildings in Istanbul. That day itwas warm, summer was definitely approaching. Manypeople were gathered in front of the palace, a sign ofthe importance of that particular place. Huge halls,sophisticated architecture, historical significance andopulence made me think about what many years agowas considered absolute luxury. I recommend visitingthe palace along with other major sights of Istanbul.

The evening was one of the most important momentsof the congress due to the cruise on the Bosphorus.

The boat that was waiting for us was state-of-the-art,impressive and equipped with a luxury dining room.

As already expected, we were welcomed aboard withfriendship. It was the last moment when I changedimpressions about congress with my colleagues fromother countries. There were five delegations in thedebut of the party but at the end we were a singlegroup with many things in common. That was themoment when we had the chance to know us betterthan we did before. Seenfrom the

Bosphorus, Istanbulwas like a fairytale. I think oneof the most spectacular city'soverall images can be seen atnight. Picture of opulence wasreflected in every detail whenI saw the most famous clubsfrom the city during the cruise.

I went back to the hotel thinking that an experiencelike that was unique and I rarely had the opportunityto see so much luxury gathered in a single place. Lastday brought me back at the military museum whichhosted the closing ceremony. Beyond the speechesfrom heads of delegations I was very happy to see thatthe prize for the best project was awarded to Romania.

It was a pride for the whole delegation and we returnedto the hotel very happy. Those were already thelast hours in Turkey. We started preparing to go backhome so we had lunch and then we started packingthe luggage. At the airport our plane was waiting forus. After a turbulent flight we arrived back in Romaniaon a cold and rainy weather, a lot different than thesun and the heat from Turkey.

The experience was unforgettable. I recommendeveryone to take part in this kind of event even if theyhave to deal with oral presentations. I think that whatmatters the most is participation and the numerousthings that you can learn. Since little, brick by brick,experience accumulates and allows overcoming newlimits. I hope I gave a good read and I want to thankyou for the time you have given me.

ADMINISTRATIVE ISSUES

Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine.* Please read the complete Author Guidelines carefully prior to submission, including the section on copyright. To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

ETHICAL CONSIDERATIONS

Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html.

It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome.

Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) accessible to the public at no charge;

(2) searchable by standard, electronic (Internet-based) methods;

(3) open to all prospective registrants free of charge or at minimal cost;

(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

Plagiarism Detection

The journal employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

Committee on Publication Ethics

The journal subscribes to the principles of the Committee on Publication Ethics (COPE).

MANUSCRIPT CATEGORIES AND SPECIFICATIONS

All articles, with the exception of Editorials, must contain an abstract of no more than 250 words. Abstracts for original articles should be formatted into subheadings, as detailed below. Titles must not be longer than 120 characters (including spaces).

Editorials

These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

Review Articles

RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

Meta-Analyses or Systematic Reviews

RJMM particularly welcomes submission of Meta-Analyses and Systematic Reviews, which underpin evidence-based medicine. Guidelines for preparation of Meta-Analysis and Systematic Reviews are similar to other reviews, and articles are subject to the usual peer review process. Meta-Analyses and Systematic Reviews have a word limit of 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

Original Articles (including clinical trials)

RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

Education and Imaging

The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as ‘What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?’ Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

MANUSCRIPT PREPARATION

Style

Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors’ revised ‘Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication’, as presented athttp://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster’s Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader’s task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

Text

Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion, Acknowledgments and References*.

References

The Vancouver system of referencing should be used. In the text, references should be cited using superscript Arabic numerals in the order in which they appear. If cited only in tables or figure legends, number them according to the first identification of the table or figure in the text. In the reference list, the references should be numbered and listed in order of appearance in the text. Cite the names of all authors when there are six or less; when seven or more list the first three followed by et al. Names of journals should be abbreviated in the style used in MEDLINE. Reference to unpublished data and personal communications should appear in the text only.

References should be listed in the following form:

Number references in the order cited as Arabic numerals in parentheses on the line. Only literature that is published or in press (with the name of the publication known) may be numbered and listed; abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Refer to only in the text, in parentheses, other material (manuscripts submitted, unpublished data, personal communications, and the like) as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication. Use Index Medicus as the style guide for references and other journal abbreviations. List all authors up to six, using six and "et al." when the number is greater than six.

Tables

Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure legends

Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

Figures

All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals, and cited in consecutive order in the text. Each figure should be supplied as a separate file, with the figure number incorporated in the file name.

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, publication requires high quality images to prevent the final product being blurred or fuzzy.

SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at daniel_costache@yahoo.com

A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies

involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.

4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.
5. For clinical trials, that these have been registered in a publically accessible database (see more under 'ETHICAL CONSIDERATIONS (Further Information)' later in these guidelines).

If the above items are not included in the cover letter, manuscripts cannot be sent for review.

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Two Word-files need to be included upon submission: A title page file and a main text file that includes all parts of the text in the sequence indicated in the section 'Parts of the manuscript', including tables and figure legends but excluding figures which should be supplied separately.

The main text file should be prepared using Microsoft Word, doubled-spaced. The top, bottom and side margins should be 30 mm. All pages should be numbered consecutively in the top right-hand corner, beginning with the first page of the main text file.

Each figure should be supplied as a separate file, with the figure number incorporated in the file name. For submission, low-resolution figures saved as .jpg or .bmp files should be uploaded, for ease of transmission during the review process. Upon acceptance of the article, high-resolution figures (at least 300 d.p.i.) saved as .eps or .tif files will be required.

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EDITORIAL

Ethical principles of health care in emergencies

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Civilian and military health-care organizations share the common goal of improving the safety of their personnel and other health assets and the delivery of impartial and efficient health care in armed conflicts and other emergencies,

Referring to the principles of humanity, whereby human suffering shall be prevented and alleviated wherever it may be found and impartiality, whereby health care shall be provided with no discrimination;

Bearing in mind the standards of international humanitarian law, in particular the 1949 Geneva Conventions and their 1977 Additional Protocols, and of international human rights law, specifically the Universal Declaration of Human Rights (1948) and the International Covenants on Civil and Political Rights and on Economic, Social and Cultural Rights (1966);

Considering the principles of professional ethics adopted by health-care professional associations, including the WMA Regulations in Times of Armed Conflict and Other Situations of Violence;

Endorse the following ethical principles of health care:

General principles

1. Ethical principles of health care do not change in times of armed conflict and other emergencies and are the same as the ethical principles of health care in times of peace.

2. Health-care personnel shall at all times act in accordance with relevant international and national law, ethical principles of health care and their conscience. In providing the best available care, they shall take into consideration the equitable use of resources.

3. The primary task of health-care personnel is to preserve human physical and mental health and to alleviate suffering. They shall provide the necessary care with humanity, while respecting the dignity of the person concerned, with no discrimination of any kind, whether in times of peace or of armed conflict or other emergencies.

4. Privileges and facilities afforded to health-care personnel in times of armed conflict and other emergencies are never to be used for purposes other than for health-care needs.

5. No matter what arguments may be put forward, health-care personnel never accept acts of torture or any other form of cruel, inhuman or degrading treatment under any circumstances, including armed conflict or other emergencies. They must never be present at and may never take part in such acts.

Relations with patients

6. Health-care personnel act in the best interest of their patients and whenever possible with their explicit consent. If, in performing their professional duties, they have conflicting loyalties, their primary obligation, in terms of their ethical principles, is to their patients.

7. In armed conflict or other emergencies, health-care personnel are required to render immediate

attention and requisite care to the best of their ability. No distinction is made between patients, except in respect of decisions based upon clinical need and available resources.

8. Health-care personnel respect patients' right to confidentiality. It is ethical for health-care personnel to disclose confidential information only with the patient's consent or when there is a real and imminent threat of harm to the patient or to others

9. Health-care personnel make their best efforts to ensure respect for the privacy of the wounded, sick and deceased, including avoiding the use of health care for the wounded and sick, whether civilian or military, for publicity or political purposes.

Protection of health-care personnel

10. Health-care personnel, as well as health-care facilities and medical transports, whether military or civilian, must be respected by all. They are protected while performing their duties and the safest possible working environment shall be provided to them.

11. Safe access by health-care personnel to patients, health-care facilities and equipment shall not be unduly impeded, nor shall patients' access to health-care facilities and health-care personnel be unduly impeded.

12. In fulfilling their duties and where they have the legal right, health-care personnel are identified by internationally recognized symbols such as the Red Cross, Red Crescent or Red Crystal as a visible manifestation of their protection under applicable international law.

13. Health-care personnel shall never be punished for executing their duties in compliance with legal and ethical norms.

Final

14. By endorsing these ethical principles of health care, the signatory organizations commit themselves to work for the promotion and implementation thereof wherever possible, including by appropriate dissemination amongst their members.

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REVIEW ARTICLE

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Bio-terrorism: still interesting or concerning nowadays?

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Abstract: *Bio-terrorism involves using biological agents/toxins with the intent to intimidate or coerce a government or civilian population to further political or social objectives, usually leading to deaths or illnesses of humans but also of plants/animals.*

Their use would probably initially be considered as a natural or unintentional event, especially in case of live biological agents. Moreover, a natural occurring disease outbreak could have the same destructive outcome as an efficient biological weapon.

There is a need for proper differentiation between natural and intentional events although in the first stages the medical response should be similar; however, the course of incident management would take different paths later on.

Biological agents' investigation of dangerous pathogens, from natural unusual outbreaks or bio-terrorist attacks/other intentional use, imply the collaboration of different institutions with responsibilities in public health but also in national security and defense. The National Security and the Defense System institutions think mainly in security terms while national health care system institutions think principally` in medical care/prevention terms. These two ways of acting have to be combined in order to deal properly with hazardous biological agents.

Keywords: *bio-terrorism; disease outbreak; biological agents*.

INTRODUCTION

"Bio-terrorism refers to the intentional release of biological agents or toxins for the purpose of harming or killing humans, animals or plants with the intent to intimidate or coerce a government or civilian population to further political or social objectives" as suggested by Interpol. (1)

Deliberate or accidental release of a biological agent, chemical agent, or radio-nuclear materials (CBRN agents), could potentially cause severe harm and pose a huge burden on public health systems (1).

Such a release would probably initially be considered as a natural or unintentional event (2).

From all the CBRN agents, biological agents can cause the widest range of effects, being as dangerous as nuclear weapons and as easily available as industrial/domestic chemical agents (1). Moreover, a natural event such as an emerging disease outbreak could have the same destructive outcome as an efficient biological weapon. But there are some important differences between intentional and unintentional events involving biological agents and these differences raise the main topic discussed

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below.

GENERAL SITUATION

The threat of natural epidemics is always present, no matter the level of awareness of the public health systems, authorities, or populations. A convenient example is of course the 2014 Ebola epidemic: so far it is the largest of its kind in history, affecting multiple countries in West Africa and even producing few secondary cases in USA and EU (Spain).

On February 15, 2015, according with USA Centers for Disease Control and Prevention website, (CDC Atlanta), the overall situation was the following (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html) (accessed on February 19, 2015): Countries with widespread transmission (Guinea, Liberia, Sierra Leone): total cases* – 23218; Laboratory-confirmed cases – 14088; total deaths – 9365. Countries with an initial case or cases and/or localized transmission (United Kingdom): 1 case, Laboratory-confirmed cases – 1; no death. Previously affected countries** (Nigeria, Senegal, Spain, United States, Mali): total cases* – 34; Laboratory confirmed cases – 32; Total deaths – 15. (*Total case counts include probable, suspected, and confirmed cases. **The outbreaks of Ebola virus disease (EVD) in Senegal, Nigeria, Spain, the United States, and Mali have ended. A national EVD outbreak is considered to be over when 42 days, double the 21-day incubation period of the Ebola virus, has elapsed since the last patient in isolation became laboratory negative for EVD.)

In December 2014 and January 2015, some reports suggested that the "Islamic State in Iraq and Syria" (ISIS) terrorist organization, operating in Iraq, would try to use Ebola infected militants as perpetrators, spreading the disease (i.e. by sending volunteers to the afflicted African areas to get infected on purpose, then sending them to Europe or USA to spread the disease). The World Health Organization (WHO) investigation did not confirm this information (http://www.emro.who.int/irq/iraq-news/no-ebola-cases-detected-in-iraq.html). (Accessed on February 19, 2015)

Moreover, let's not forget that the 1918 flu pandemic – the "Spanish flu" – caused between 50 and 100 million deaths, making it one of the deadliest natural disasters in human history. By comparison, the estimated death toll of First World War ranges "only" between 15 and 35 million deaths and even the deadliest conflict, Second World War ranges between 40 and 72 million deaths.

The more robust a nation's preparedness, however, the more reduced will be the consequences of any outbreak whether natural or man-made and the likelihood of being attacked. Every state should improve his bio-defense capabilities in order to avoid the development of an incident involving biological agents into a catastrophe. Yet, there is no perfect national bio-defense system, regardless the wealth and power of the country. Cooperation among relevant institutions is crucial in this critical area; especially cooperation between the public health system and the institutions involved in homeland security is needed in order to achieve a better level of preparedness.

However, there is no such thing as a standard, "universal" response algorithm, given the fact that the actual response process depends on a multitude of factors, such as: national institutional specificity (number of institutions involved in response, their responsibilities and competencies, equipment and resources employed etc.), specific national policies in place, specific social-economic issues and so on.

In Romania the relevant legislative framework has been updated according to European and international laws and regulations (under BTWC – Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction and IHR – International Health Regulations).

Also, the responsible institutions have specific procedures in place for different parts of the hazardous agent investigation.

However, up to date, in Romania there is no overall coherent algorithm of response to incidents involving biological agents (detailed and adapted both for known and for potentially unknown or emergent agents) with the subsequent SOPs (Standard Operational Procedures) well synchronized, efficient and agreed by all responsible institutions.

Moreover, for some key components of the response chain of actions there are no updated SOPs, especially in the detection/identification/typing of the hazardous agent component. Therefore, in case of a real incident, the overall response speed will be slowed and important errors might impair the efficacy of the procedures and strategies selected for mitigation/containment of the biological threat. And this, of course, could lead to severe consequences.

Since the beginning of the current Ebola epidemic efforts have been made in Romania for planning, preparedness and developing structures and procedures to be followed for defending, minimizing the consequences and recovering after this outbreak and any other similar (http://goo.gl/nsyOg2).

And for sure, this will result in a higher capacity to deal with an unusual outbreak, being an important and necessary part of the "bio-defense" system.

But is this covering for deliberate induced incidents, outbreaks or epidemics, or for accidental release from research laboratories? We shall investigate if there are some characteristics of the "artificial" outbreaks that result in the need for a different approach of the investigation and reaction compared with natural ones. Although by no means exhaustive, a list of patterns that may give some indication of a deliberate release would include (1;2;3,4):

» sudden increase in patient with similar symptoms/ multiple cases of unexplained disease, syndrome or death, especially if having common work location or activities/"point source outbreak"/multiple cases with similar disease or syndrome presenting around the same time; »» simultaneous outbreaks of similar illness in noncontiguous areas;
» similar typing of agents isolated from temporally or spatially distinct sources;
» disease with an unusual geographic or seasonal distribution;
» recognized illness occurring in an unusual setting within a community;
» single case of disease caused by uncommon agent;
» multiple unusual or unexplained disease entities coexisting in the same patient without other explanation;
» unusual form of infection e.g. inhalational anthrax, pneumonic plague, mediastinitis/recognition of atypical transmission routes e.g. by aerosol, food or water;
» unexpected degree or speed of onset;
» failure of a common disease to respond to usual therapy;
» intelligence of a credible threat;
» heightened terrorist alert level (severe, critical);
» suspected or known deliberate or accidental release in another country;
» illness affecting a key sector of the community/ attenuated morbidity in protected professionals groups;
» illness only among people in proximity to common ventilation systems;
» "downwind plume pattern": cases concentration found downwind from a source point;
» death or illness among animals preceding, accompanying or especially succeeding illness in humans;
» unusual, atypical, genetically engineered, or antiquated (or "extinct") strain of agent, authors' "signature" (known past researches either civil or military);
» diagnosis of a disease generated by an agent from the lists of potential useable in a terrorist attack.

These "indicators" are especially useful when we are dealing with a covert (hidden) attack and the actual disease cases or intelligence gained represents the first "piece of information" of the intended release of a harmful biological agent. In order to be recognized and to trigger a specific chain of action all the actors involved have to be aware of the potential meaning of these indicators: medical staff, public health authorities, law enforcement agencies, and so on.. (1, 2, 3)

When dealing with an overt attack (at least law enforcement and/or other responders are informed about it), the awareness of it occurs earlier than in covert ones but this doesn't mean that it is an easier situation to deal with. A well prepared system can gain most advantage from the time given by knowing from the onset that a bio-terrorist incident is developing. Otherwise, only the early psycho-social effect is different, knowing that the main levels of intended epidemics effects are (2):

» physical – actual diseases;
» psychological – fear, mass panic;
» economic – travel restrictions, business affected;
» environmental – affecting humans, animals, plants.

These effects can be recognized in natural outbreaks as well, but at least the second and the third kind will be far more intense in an intentional (or accidental release) event, if not all. The aim of such an incident management is to partially minimize its' consequences if not to avoid them altogether.

This requires some mechanisms "in place" to defend from the threat and others to detect, investigate and react to it. And the intentional biological agent release needs some specific investigation and solving components. Both the health and the criminal sides of the situation have to be tackled simultaneously (1,3).

The investigation proceeds within the frame of the national legislation on: working/securing/developing/acquiring/possessing/transferring/transporting biological agents (2). In overt attacks, usually an incident response team has to be deployed, with multidisciplinary approach: first responders,

CBRN experts, medical personnel, risk assessment specialists, decision makers, etc. Good decisions made at this point can lower the consequences significantly (2,3).

In both overt and covert attacks, a chain of actions has to be accomplished by a partnership of institutions mainly from law enforcement and public health systems, with some either common or specific goals: protect health and safety of public/determine cause of bio-terrorism attack/outbreak, stop further crimes/stop further cases of disease and outbreaks, apprehend and convict criminals/build science base for future prevention (1,3).

Sharing information between law enforcement and medical or public health components is crucial both for better timing and for more efficient decisions and actions (1). Some joint investigations and operations are essential for a good coordination between agencies. (3) Main priorities in biological incidents are: preservation of life, safety of personnel involved, investigation (microbiological, epidemiological, criminal etc.), intelligence to prevent further attacks, public reassurance and return to normality (1).

Another special concern in intentional (or accidental) biological agents release incidents is related to genetically modified organisms. These pathogens can pose any imaginable problem from the investigation stage up to the afterward recovery.

From biological agents expressing other species characters and virulence factors up to special "powers" like multi-drug resistance and high resilience in the environment or resistance to different decontamination methods, an GMO (genetically modified organism) can be anything (4,5).

Intentionally released biological agents are most likely to be delivered by aerosols (1). Therefore the most probable syndrome resulted will be a respiratory one, despite the pattern of the usual naturally occurring disease caused by the biological agent. The optimum particle size is 0.3-5.0 µ in diameter, which is small enough to reach the alveoli when it is inhaled (1, 4,5). This can be achieved by aerosol generators mounted in fixed locations or on trucks, cars, or boats as well as from cruise missiles and planes equipped with tanks and spray nozzles (1,2). Numerous climatic factors affect the efficiency of such methods including wind velocity and direction, humidity, degree of cloud protection from direct sunlight, and rainfall (1,4,5,6).

Aerosols could be disseminated by direct delivery into ventilation or air conditioning systems or via letters or parcels (1,2). Suicide attacks would be extremely effective for disseminating diseases (1,2).

Food and water are also suitable vehicles for local delivery of pathogens, especially when the goal of the attacker is more social disturbance and panic than actual killings (1,2,3).

DISCUSSION

The main question is: presuming that facing the threat of 2014 Ebola epidemics the medical community and the public health system will improve their capacity to prevent/investigate/react and recover in natural epidemics, is this enough to reduce to an acceptable level the risk posed by a potential bioterrorist attack?

Hazardous biological agents investigation for rapid detection and identification of novel, emerging and dangerous pathogens, from natural unusual outbreaks or bio-terrorist attacks/other intentional use, imply the collaboration of different institutions with responsibilities in public health but also institutions with responsibilities in national security and defense, especially in the case of "unnatural" events.

Each of these institutions has specific responsibilities and a specific set of procedures for their own part of the action. But until now, in Romania there is no agreed, detailed and acknowledged chain of action.

In every step of the investigation/reaction: on site detection, sampling, transport, laboratory investigation (screening, investigation, in depth analysis), case management, public communication, criminal investigation, epidemiological investigation, public health decision, political and economic decisions, there are several institutions involved, depending on the actual situation and the legal frame (e.g. General Inspectorate for Emergency Situations, Romanian Intelligence Service, the NBC department of Romanian Police, other departments from the Ministry of Internal Affairs, Ministry of Defense – in some special cases).

These response units would be involved in site response, including sampling and transport and in other law enforcement actions and in recovery and return to normality; Ministry of Health, National Institute for Public Health, Local Public Health Authorities, Infectious Disease Hospitals, National Institute for Research and Development for Microbiology and Immunology "Cantacuzino" etc. will be involved in microbiological, epidemiological and clinical investigation, in case management/recovery. Even if the legal frame defines the role and responsibilities of each of these organizations, their action procedures are not properly linked nor their collaboration entirely formalized. Some of the specific SOPs are adapted from international guidelines, some designed to relate to Romanian conditions. Some aspects remain uncovered because not all institutional procedures relate to the others.

On European and international level there are several action plans, recommendations and guidelines in place. The major international organisms (e.g. EU, NATO, etc.) have their algorithms and SOPs. Also this is true for the most important institutions from developed countries which are involved in hazardous biological agents' investigation and response (e.g. Health Protection Agency, UK; "Robert Koch" Institute, Germany; Centers for Disease Control and Prevention, USA, and so on).

These procedures cannot be applied in Romania exactly as they are. Therefore, a thorough assessment of the entire biological agents investigation and response to biological threats process has to be done taking into consideration Romanian specificity and the SOPs have to be optimized accordingly, up to an efficient algorithm usable by relevant national institutions, followed by improving the logistics where needed and within the financial possibilities of Romania.

One of the problems is the difference between the mind settings of the major actors. The National Security and Defense System institutions think mainly in security terms and the National Health Care System institutions (including public health authorities) think mainly in medical care/prevention or usual outbreaks terms. These two ways of understanding/acting have to be combined in order to deal properly with hazardous agents either from unusual natural outbreaks and especially bio-terrorist attacks.

CONCLUSION

Our opinion is that bio-terrorism is (or should be) still a matter of concern in Romania (and elsewhere in EU or worldwide). This should trigger a process of increasing the awareness about intentional outbreaks and epidemics and as a consequence begin to enhance the capacity of medical community and public health system to proper manage the risk of a bio-terrorist attack or an accidental release of an unusual biological agent. And last but not least, the cooperation between institutions involved in health care and those responsible for security and defense should be improved up to the proper level of efficiency.

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SYSTEMATIC REVIEW

Practical aspects of MRI of the prostate

Dragoș Cuzino[1], Oana M. Baston[1], Cătălin Blaj[1]

Abstract: *The article presents the main aspects of sectional anatomy, lymph nodes and adjacent structures as well as MRI examination standard protocol for prostate cancer diagnosis. Using MRI multiparametric examination we succeed in classifying efficiently the malignant prostatic tumors using PI- RADS system.*

Also, using MRI multiparametric examination we can evaluate the effectiveness of prostate cancer treatment.

Keywords: *multiparametric MRI, prostate cancer, PIRADS, PSA, benign hypertrophic hyperplasia (BPH)*

ANATOMY OF THE PROSTATE GLAND

The prostate gland is the largest male accessory reproductive gland. The prostate gland looks like a flattened cone or inverted pyramid. The base of the prostate is in continuity with the bladder.

It ends at the apex at the urogenital diaphragm. It typically weights between 20-40 grams. The prostate normal size is 3 x 4 x 2cm. The prostate is comprised of 70% glandular tissue and 30% fibromuscular or stromal tissue. The prostate has four zones. The peripheral zone is located under the capsule and formed of glandular tissue. The central zone is deep to the peripheral zone and formed of stroma. The transitional zone surrounds the prostatic urethra.

The periureteral zone is formed of short ducts. The central and transitional zones are found more superiorly within the gland. The gland tapers inferiorly.

The central gland located anterior and superior, is the portion of the prostate most intimate to the urethra. The peripheral gland comprises the posterior and inferior parts of the prostate. It is statistically proved that most of the cancers of the prostate are located in the peripheral gland, but cancer can occur in the central gland also. The cranial part of the gland is called the base and the more caudal portion is called the apex. (1)

Considering prostate a flattened cone, the tip of the cone represents the apex of the gland and is situated more caudally. The flattened portion of the cone is analogous to the base of the prostate and is situated more cranially. In young men, the peripheral zone occupies approximately 70% of the total volume of the prostate, the central part 24% and the transitional zone about 5% but the percentage is changing with age. (2) Using MRI we manage to see the described zonal anatomy with the four zones of the prostate. On CT we can do it only on occasion. (2)

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In T2 we may see the peripheral zone with high signal intensity and the transitional zone with low signal intensity. In T1 we can assess to demonstrate areas of post-biopsy hemorrhage and the status of the neurovascular bundles.

The prostate capsule appears as a continuous dark area around the prostate in T2. The DWI sequence may show restricted diffusion in prostate cancer.

T1 Dynamic contrast/DCE sequence could show enhancement but it is unsepcific because it may be difficult to distinguish from prostatitis or benign prostatic hyperplasia.

Figure 1. Axial T2 TSE high resolution. Peripherical lesion with intermediary irregular zone located inferior and posterior in the left zone.

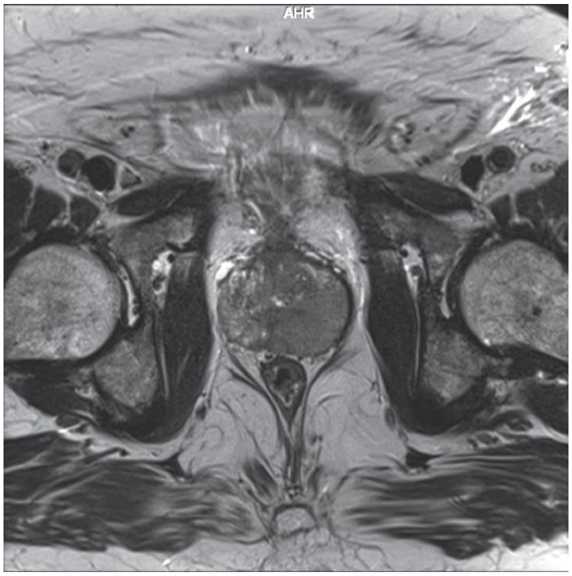

LYMPH NODES

In assessing prostate anatomy it is very important to evaluate the lymphatic drainage. The main lymph nodes are obturator and internal iliac; secondary, the drainage is made into the external iliac, presacral and para-aortic nodes.

The lymphatic spread of the disease in unspecific and only the evaluation of the lymph nodes dimensions is a value indicator. The seminal vesicles appear with low signal intensity in T1 and high homogeneous intensity in T2.

The normal size of the prostate is compared with the one obtained when we measure the dimensions of the prostate, when we estimate the volume by multiplying antero-posterior, tranverse and longitudinal measurements by one another and then multiplying that result by 0,52 which is π/6. The expected prostate volume increases with advancing age and a prostate with a volume greater than 40 ml is considered to be enlarged. The normal volume of the prostate in milliliters is approximately equal to the weight of the prostate in grams. (2)

A normal PSA value is less than 4 and an abnormal value is greater than 10. Values vary between 4 and 10 which are considered boundaries.

Prostatitis, BPH, prostate infarcts and biopsy could cause elevated PSA levels.

Benign prostatic hyperplasia (BPH) usually starts in the transitional zone of the central gland and is more heterogeneous in T2 than the normal gland. When it grows, it can indent the bladder (1). Stromal BPH appears dark on T2 and glandular BPH appears bright. A low signal intensity rim may be seen surrounding these nodules. We see often a combination of these findings in BPH.

The diagnosis of prostate cancer is usually made by biopsy of the prostate which is often accomplished transrectally with ultrasound guidance.

Figure 2. Axial STIR. The zone is located subcapsular posterior and inferior.

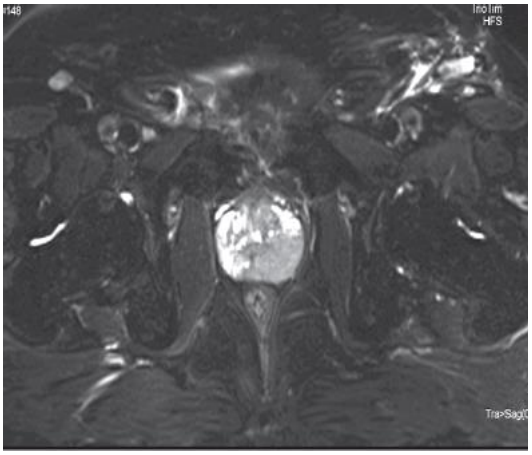

The percentage of the prostate cancer arising within each of the three zones is: peripheral zone 70%, central zone 10% and transitional zone 20%.

Most of prostate cancers are adenocarcinomas which develop from the acini of the prostatic ducts. Pathologic specimens are graded using the Gleason score which is the sum of the most prevalent and second most prevalent types of dysplasia, each on a scale of 1 to 5, with 5 being the most dysplastic.

We also use a TNM staging system for classification of the prostate cancer and also the PI-RADS system (Prostate Imaging Reporting and Data System).

Figure 4. T1 axial FSE post-contrast examination. The lesion has unhomogenous enhancement.

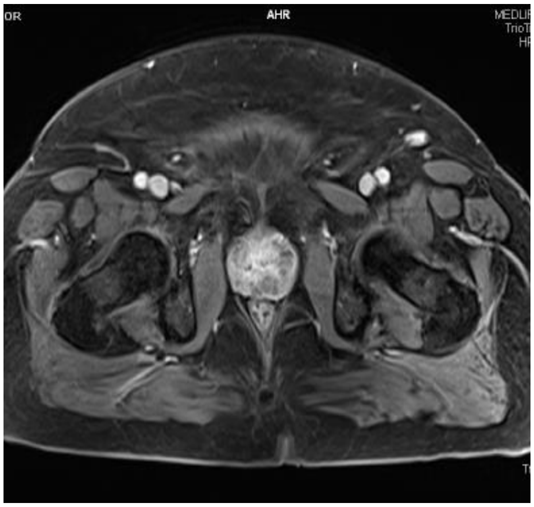

Figure 3. Axial DWI. Area of restriction of diffusion located inferior posterior on the left side.

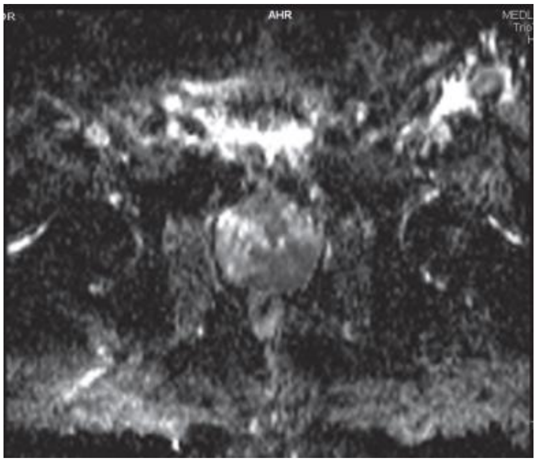

There is no consensus about the use of imaging for evaluating prostate cancer. MRI is the method of choice for local staging, to demonstrate tumor location, extracapsular extension and seminal vesicle invasion. The most efficient method for evaluating prostate tumor and the aggressiveness of the tumor is multiparametric MRI.

Biopsy is essential for the diagnosis. MRI should be performed about 3 weeks after biopsy because at that time about 50% of patients have residual hemorrhage. Hemorrhage after about 3 weeks post biopsy is different from tumour to tumour. T1 hypertintense and T2 hypointense areas correspond to postbiopsy hemorrhage and T1 – izointense and T2 hyperintense areas are more likely to correspond with tumour. There is also disruption of the prostate capsule which may mimic the invasion into the prostatic fat.

Figure 5. Axial T2. Benign prostate hypertrophy and a prominent nodule.

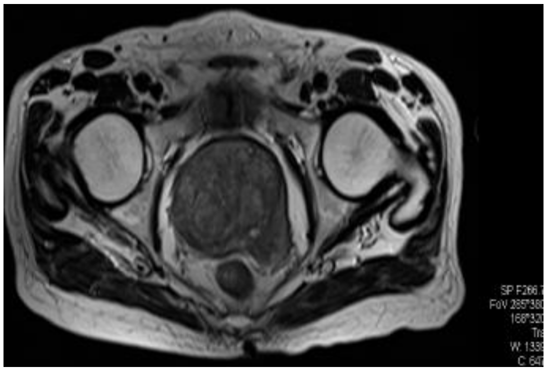

Figure 6. Coronal STIR. Protrusion of the cranial large nodule with extrinsic compression of the urinary vesicle.

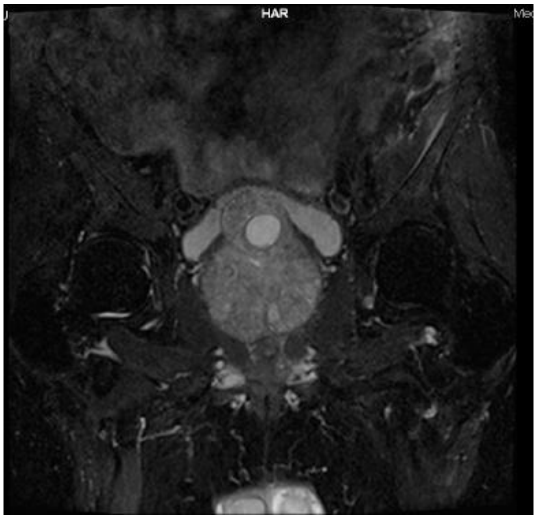

Typically prostate cancer is isointense to gland in T1 and hypointense to the normally high intensity peripheral zone on T2. Central gland malignancy is difficult to be identified because of BPH which always distorts normal anatomy. There are other diseases that may cause low signal intensity areas within the peripheral zone on T2 weighted image like prostatitis, granulomatous disease stromal disease within the peripheral zone and postbiopsy blood or scars.

MRI is very important because it can reveal the real extent of the tumour and can show its spreading beyond the prostate capsule. The tumour can spread into periprostatic fat or neurovascular bundle, ejaculatory ducts, seminal vesicles, bladder, lymph nodes or we can assess the osseous metastatic disease. The capsular penetration occurs most commonly at the prostatic apex (1). We use multiple criteria to detect extracapsular extension of prostate cancer. We estimate the possible irregular bulge in capsule, changes of the rectoprostatic angle, asymmetry of the neurovascular bundle and capsular retraction or local scar. MRI must assess capsular tumour contact and visualize contigous tumour local extension. Seminal vesicular extension has to evaluate direct tumour extension into the seminal vesicles. MRI should evaluate tumour extension in other direction. Contigous spread of the tumour must also be assessed along the ejaculatory ducts.

The MRI semiology consists in seminal vesicles of hypo- intensity in T2WI and non visualisation of the ejaculatory duct. Also, after dynamic T1 evaluation of the contrast enhancement we can predict malignant involvement of the tissue.

The lymphatic drainage of the prostate is made into the internal and external iliac lymph nodes including the obturator nodes (2). Lymph nodes with a diameter greater than 1 cm are suspicious for metastasis disease.

On T2–weighted images with low signal intensity areas within the seminal vesicles and asymmetry in size may indicate tumour invasion. However, blood, changes secondary to radiation therapy and other benign processes may appear as low intensity areas inside the seminal vesicles.

Key Points

Prostatic cancer usually occurs in the peripheral zone. Bone metastases are very unlikely to appear when the PSA is less than 10. The extracapsular spread indicates a nonsurgical treatment of the disease. (1)

MRI is the preferred imaging modality in evaluating prostate cancer and prostate lesions. The aspect of the normal prostate can be described as follows: in T1 the prostate tissue has homogenous intermediate signal intensity; in T2 the anterior fibromuscular stroma is low T1W and T2W signal.

Figure 7. Sagital T2 TSE. Multiple nodules but the prostate is still well delineated.

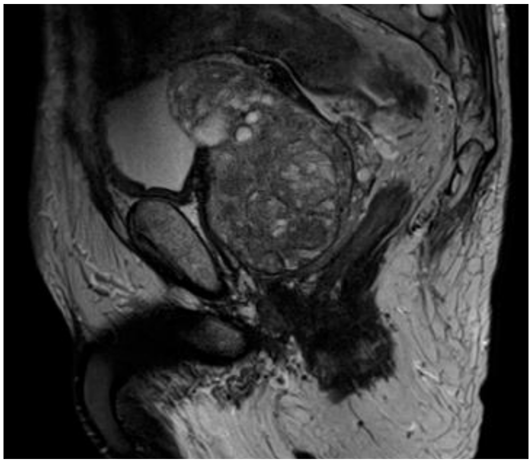

The peripheral zone has high T2W signal, similar to or greater than adjacent fat. The central and transitional zones are lower T2W signals than peripheral zone. The true capsule is a thin rim of low signal intensity. The distal urethra is a low intensity ring in T1 and T2.

Figure 8. T1 TSE axial. Presence of blood at the seminal vescicles (hypersignal corresponding to methemoglobine inside seminal left vesicle)

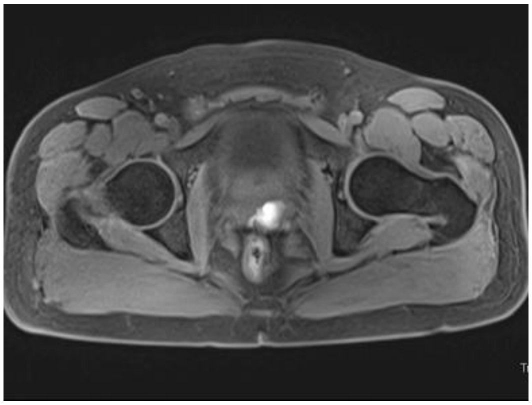

In the last years, MRI became a common procedure in the detection of prostate cancer. The main indication is to evaluate patients with increased PSA and with prior negative biopsies. The best MRI procedure is multiparametric MRI. Multiparametric MRI consists of high resolution T2 axial sequence, as morphological study, T1 dynamic contrast examination and spectroscopy.

The standard MRI examination recommended was published in the interest of improving the quality of procedure and reporting. It was improved by a group of experts of the European Society of Urogenital Radiology (ESUR). In addition to the standard minimum examination protocol, the guideline describes also a structured reporting scheme. This scheme is called PI-RADS based on the BI-RADS. The score varies from 1-5. For a correct estimation of the score we have to choose the most adequate images and to add a scoring table that combines the aggregated multiparametric score with a total PI-RADS score.

In practical clinical activity, we also use Gleason score to determine the histological grading of the prostate malignant involvement. The name of the score is given after Donald Gleason – an US pathologist.

A score of 1 to 5 is assigned to each of the two largest areas of tumor involvement in the samples obtained, based on the worst feature with 1 least aggressive and 5 most aggressive.

PI-RADS classification includes several criteria.

The 3 Tesla magnets is the hardware tool necessary for obtaining best images in prostate assessment but we can also use other variants.

In T2 with high resolution quality images we grade from I to V for the peripheral zone:
I – normal aspect with hyperintense homogenous peripheral zone
II – discrete hypointense focal lesion wedge or bandshaped ill defined
III – more advanced lesion and different from 1,2,4,5 classification
IV – severe hypointense lesion well defined without extracapsular extension
V – hypointense mass round and bulging with capsular involvement or seminal vesicle invasion.

Figure 9. DWI axial. Diffusion restriction area.

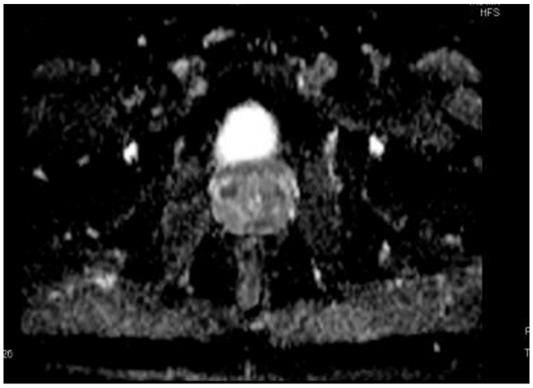

For example, according to the German inter-disciplinary guideline of prostatic cancer for a correct imaging diagnosis we should perform MRI with a minimum strength of 1, 5 Tesla (T) using a combined endorectal body phased – array coil or a 3 Tesla machine and a combination of multiparametric sequences. When we use a 3 Tesla machine an endorectal coil is not mandatory.

Also in T2 for the central zone, we grade the lesion from I to V:
I – the central zone has stromal and glandular hyperplasia without focal hypointense nodular or oval – shaped

II – round hypointense lesion with signs of well defined capsule or band shaped hypointense region.
III – changes not falling into category 1,2 or 4,5
IV – oval shaped anterior hypointense lesion without evidence of capsular involvement – homogenous hypointense lesions with loss of matrix ill defined margins
V – oval shaped with compression, retraction, extension of the anterior capsule irregular infiltrating mass with architectural disintegration and invasion into adjacent structures.

Figure 10. T2 TSE axial. Low signal intensity area corresponding to a prostate tumour.

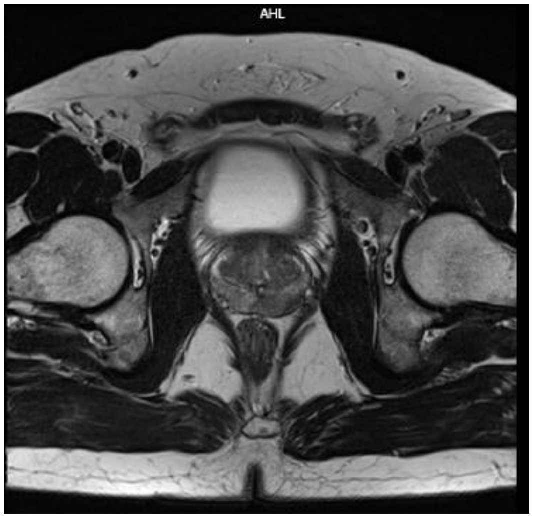

MRI is very efficient in differentiating lesions with almost similar morphological appearance like post-biopsy scars, atrophic changes, prostatitis, post-treatment lesions, and intraepithelial neoplasias.

In diffusion weighted imaging the analysis of the movement (diffusion) of the water molecules are expressed by the ADC parameter (apparent diffusion coefficient).The molecular diffusion in tissues is generally restricted by cell structures and membranes.

Intracellular edemas or higher cell densities lead to the reduction of the free molecular movement. In tissues with higher cell densities, like prostate carcinoma, ADC has reduced values. The diffusion sequence use upper values of 800-1000s/mm but there are studies that prove that there is a benefit in improving the diagnostic value of the sequence with a value of 2000 and at 3 Tesla magnetic fields. The orientation of the sequence must be in the same plane like T2 axial and with same FOV for efficient multiparametric comparaison. We can also grade prostate lesions using DWI:
I – no reduction in ADC compared to normal tissues
II – diffuse hyperintensity image with low ADC and no focal lesions
III – unilateral hyperintensity with diffuse reduced ADC (no focal lesion)
IV – focal area with reduced ADC and isointense on the diffusion sequence
V – focal hyperintese mass or focal lesion with a reduced ADC.

T1 with dynamic contrast – DCE – MRI (dynamic contrast enhancement – MRI) is a very efficient method to obtain data about vascularisation and neoangiogensis of the prostate. When we perform DCE – MRI, we evaluate the curves of enhancement after a bolus injection of a gadolinium based contrast medium (the injection rate for a correct evaluation must be 2.5 ml/sec and a we must use a subsequent injection of 20 ml of isotonic saline solution).

Dynamic contrast-enhanced magnetic resonance imaging strategies vary, but, in general, three types of imaging data are acquired. Initial images localise the tumour and provide anatomical information.

Sequences that allow calculation of baseline tissue T1-values before contrast agent administration are acquired to enable analysis of blood flow into the tumour. The dynamic data are acquired every few seconds in T1-weighted images over a period of around 5–10 min depending on the soft and power MRI machien gradients. We use T1-weighted spoiled gradient echo sequences are, as they allow good contrast medium sensitivity, high signal-to-noise ratio, adequate anatomical coverage and rapid data acquisition (Parker and Buckley, 2005). The Signal Intensity curves measured by DCE – MRI correspond to three maintypes of curves. Curve type I – the signal intensity continue to increase. Type II is characterized by a progression and a slight decression of the Signal Intensity. The type III curve is defined by the immediate wash out after reaching the peak enhancement. We add point to the scoring system if there is a focal lesion, another one is added if there are asymmetric lesions or unsually located lesions (anterior parts of the transition zone and anterior horns of the peripheral zone).

Magnetic Resonance Spectroscopy Imaging (MRSI) allows the measurements of the relative concentration and distribuiton of the metabolites like citrate, creatine and choline in the prostate. The metabolic information can increase the specificity of the morphogical value of the MRI examination and evaluate the progression or the involution after different kind of therapy. The quality of the MRSI is dependent on the physical and technical support and also of the local and particular condition like post-biopsy hemorrhage, regional prosthetic or metal clips and artifacts.

PI-RADS Clasiffication	Definition	Protocol T2, DWI, DCE	Protocol T2, DWI, DCE, MRS
1	most probably benign	3, 4	4 ,5
2	probably benign	5, 6	6 – 8
3	indeterminate	7 – 9	9 – 12
4	probably malignant	10 – 12	13 – 16
5	highly suspicious of malignancy	13 – 15	17 – 20

Citrate (C) is synthesized, secreted and stored in large quantities in normal glandular tissue of the prostate and is therefore used as an organmarker for healthy prostate tissue.

Creatine plays an important role in the cells' energy metabolism and serves as an internal reference of intensity. Choline (Cho) refers to the sum of choline-containing compounds, which includes various free choline compounds such as phosphocholine, glycerophosphocholine, free choline, CDPcholine, acetylcholine and choline plasmalogen. The intensity of the choline resonance reflects the extent of membrane turnover and is significantly elevated in cancerous tissue.

- Type 1: Cho is significantly lower than citrate.
- Type 2: Cho is elevated but still lower than citrate .
- Type 3: Cho is approx- imately on the same level as citrate.
- Type 4: Cho is elevated compared to citrate.
- Type 5: Cho is significantly elevated compared to citrate.

In analogy to the BI-RADS, the PI-RADS system offers the advantage of a standardized and easy communication of findings to other professional colleagues. Every lesion should be evaluated using a standardized graphic prostate scheme with at least 16, better 27, sectors. For each lesion, a point score between 1 and 5 is to be assigned per method. This is used to calculate the total score, which reflects the probability of the presence of clinically relevant cancer.

The total score is then converted to the relevant PI-RADS score, providing the advantage that the final PI-RADS score is independent of the number of techniques used and can, thus, be easily communicated.

Since the conversion of point scores is not explicitly explained in the ESUR guidelines, the authors recommend using the algorithm given in. For routine clinical work, the authors further suggest that a diagnosis of suspected prostate cancer should be made if the PI-RADS score is 4.

CONCLUSION

In summary, structured reporting of a lesion, using the multi-parametric approach, increase the quality and diagnostic value of prostate MRI.

Therefore, application of the PI-RADS scheme based on the representative images provided here is recommended for clinical routine. The standardized graphic reporting scheme facilitates the communication with referring colleagues. Moreover, a standardized reporting system not only contributes to quality assurance, but also promotes widespread use of the method and implementation of large-scale multicenter studies, which are needed for further evaluation of the PI-RADS system, in analogy to the BI-RADS system used in breast imaging.

The therapeutic approach varies widely and includes also the watchful waiting, androgen ablation, hormone therapy radical surgery, various forms of radiation therapy.

Multiparametric MRI is the most efficient method in providing information about the size, location and extent of the prostate cancer. These useful information allow the surgeon to choose the best way to resect the tumor, to perform optimal resection and also, to plan radiation therapy.

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SYSTEMATIC REVIEW

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Clinical aspects of hearing loss associated with cisplatin therapy – a review

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Abstract:
Introduction: Cisplatin is a well known platinum-based chemotherapeutic agent used for the treatment of diverse malignant tumours.
A frequent side effect of cisplatin therapy is ototoxicity.
Material and methods: Experimental, clinical studies and reviews from the English language medical literature concerning ototoxicity were selected either from PubMed or printed medical journals, pointing out cisplatin-induced ototoxicity. Molecular mechanisms, clinical audiological and histological marks of cisplatin-induced ototoxicity as well as current experimental and clinical strategies for prevention or treatment of hearing loss were reviewed.
Results and discussion: A wide range of otoprotective molecules and efficient strategies against cisplatin-induced hearing loss proved to be helpful in experimental studies whereas only dexamethasone settled a slight otoprotective effect in a clinical study.
Conclusion: There is no currently available treatment for cisplatin associated hearing loss and further research is required for future therapeutic options.

Keywords: *cisplatin-induced hearing loss, otoprotection, intratympanic therapy, systemic therapy, gene therapy.*

INTRODUCTION

Although synthesized already since 1845 by Peyrone and named Peyrone's salt, cisplatin was used clinically in oncologic therapy for head and neck, lung, bladder, cervical, ovarian, testicular, gastrointestinal cancers, as well as malignant gliomas and metastatic cancers such as melanoma, mesothelioma, and those of the prostate and breast [1] only in the late 1960s. Irreversible neurosensorial hearing loss which affects both ears symmetrically, high frequencies in the first place followed by low, speech range frequencies in a dose related, cumulative fashion is one of its major side effects.

Pre-existing afflictions like hypoalbuminemia, anaemia, renal failure, noise-induced hearing loss or by risk factors like therapy with loop diuretics, aminoglycoside antibiotics, radiotherapy fields which includes the inner ear, extreme ages (very young or very old), duration and dose schedule of cisplatin infusion, genetic factors can favour cisplatin-induced hearing loss. The average incidence of cisplatin-induced hearing loss is about 62% [2]. Cisplatin-induced tinnitus is another frequent side effect. These phenomena may occur within hours to days after cisplatin administration. As subsequent multiple cisplatin regimens for the control of cancer may be necessary, side effects should be prevented or treated without reducing the efficacy of the antitumoral mechanisms.

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MATERIAL AND METHODS

Outer hair cells are the main target for cisplatin ototoxicity which progresses from the third to the first row, and to some extent, to inner hair cells of the or gan of Corti in the basal turn of the cochlea followed by alterations in sensorial cells situated in the apex.

Supporting cells, marginal cells of the stria vascularis and the spiral ligament are also afflicted by cisplatin ototoxicity [3]. Vestibular organs and spiral ganglion cells may also suffer from cisplatin ototoxicity, especially in experimental conditions [4]. In patients with cancers of the head and neck whose communication is already impaired, the ensuing hearing loss is very disabling and cause for depression. [5].

The creation of reactive oxygen species and depletion of antioxidant glutathione and its regenerating enzymes as well as increased rate of lipid peroxidation, oxidative modifications of proteins, nucleic acids damaged by Caspase system activation [2] and S-nitrosylation of cochlear proteins and resulting apoptosis of inner ear cells stand for the molecular mechanisms of cisplatin-induced hearing [6].

Cochlear tissues fight the oxidative stress by means of antioxidant defence systems including glutathione, glutathione reductase, superoxide dismutase and catalase. Potassium uptake and secretion in the stria vascularis also might be disturbed by cisplatin administration, leading to impairment of the function of outer and inner hair cells in the organ of Corti, with alteration of the endocochlear potential and subsequent hearing loss [7].

Measurements of hearing loss by means of pre-treatment and follow-up serial audiologic tests are used to assess the extension of ototoxicity due to cisplatin therapy. Experimentally, histological examinations could point out injuries due to cisplatin administration [8]. Audiologic tests include pure tone (normal frequency and extended high frequency range), speech and impedance audiometry, auditory brainstem responses and Distortion Product Otoacustic Emissions (DPOAEs) testing.

DPOAEs reflect early injuries to outer hair cells in the organ of Corti thereby allowing monitoring and early detection of cisplatin ototoxicity during cancer treatment [9,10]. Cisplatin early ototoxic effects cause hearing impairment in the high frequencies at 6 kHz, 8 kHz and above as measured by conventional or extended high-frequency pure tone audiometry [11]. Multiple doses of cisplatin worsen hearing, ultimately affecting the speech frequency range (500 - 4000 Hz). Since DPOAEs measurement is based on the integrity of outer hair cells which is affected by cisplatin therapy before elevation of auditory threshold as measured by pure tone audiometry.

DPOAE testing is more sensitive than the latter for the detection of cisplatin-induced ototoxicity.

Moreover, the results provided by DPOAEs, as an objective testing, are not influenced by the ability of the deteriorating cancerous patient to respond to the sound stimulus [9]. In small children who undergo cisplatin administration for cancer treatment and who cannot cooperate for pure-tone testing because of their younger age and poor cognitive ability, either soundfield behavioural testing or DPOAEs could be performed [12].

In experimental animals, auditory brainstem response recordings mirror hearing loss after cisplatin treatment [3].

Histological examinations of inner ear after cisplatin administration reveals destruction primarily of outer hair cells and to some extent inner hair cells and associated nerves, degeneration of the vestibular organs, stria vascularis edema and detachment of the myelin sheath of the spiral ganglion cells [4]. The damage is especially noticed in the high frequency region of the cochlea (i.e. the basal turn) probably due to a base to apex gradient of the cisplatin ototoxicity [14]. Immunostaining detected tumour necrosis factor-alpha (TNF-α) and other inflammatory cytokines (IL-8, IL-6) in the outer hair cells, stria vascularis, spiral ligament and spiral ganglion neurons in cisplatin exposed cochlea [3].

Intravenous injection of cisplatin is followed by strong binding of the alkylating agent to the plasma proteins rendering a large non-active part of it.

The barrier systems in the cochlea, the blood-perilymph barrier, separating blood from perilymph, and the intrastrial fluid-blood barrier, separating blood from endolymph also influence the pharmacokinetics of cisplatin therapy [15]. The amount of free chemotherapeutic agent reaching targets in the cochlea is responsible for the ototoxic effect and consequent hearing loss. High frequency audiometric thresholds are initially affected. When doses in excess of 100 mg/m^2 are used, the hearing impairment may progress from high frequencies to involve the middle frequencies. Reducing the total amount of cisplatin by limitation of the total dose per cycle, dose intensity and the cumulative dose would diminish the antitumour effect, which is not desirable.

Various otoprotective compounds have been tested both in experimental animals and humans. If given systemically, they should be non-toxic, must attain efficient concentrations in the inner ear to protect labyrinthine tissues from cisplatin ototoxicity and should not hamper the anti-tumour effect of cisplatin. Higher concentrations of otoprotective molecules can be achieved by intratympanic administration.

This latter route provides direct access of the protective agents to inner ear structures while avoiding systemic side effects and interference with the anti-neoplastic activity of cisplatin [16].

Experimental studies

Several otoprotective molecules and strategies against cisplatin-induced ototoxicity have been tested in experimental animals.

Hyperbaric oxygen therapy consisting of intermittent inhalations of 100% oxygen at a pressure higher than 1 atm is used as adjuvant therapy in pathological processes like soft tissue infections, radiation injury, gas gangrene and decompressive disease.

Tissue hyperoxygenation through plasma dissolved oxygen was succesfully tested in guinea pigs as a protective agent against cisplatin ototoxicity.

Otoacoustic emissions recordings following hyperbaric treatment and scanning electron microscopy examination proved the efficacy of otoprotection by hyperbaric oxygen therapy after cisplatin administration.

The study confirmed that cisplatin induces dose-dependent cochlear alterations consisting of cellular lesions and significant hair damage in outer hair cells. Analysis of anatomical changes in cochlear outer hair cells indicated signs of otoprotection against cisplatin in animals treated with hyperbaric oxygen therapy although in functional studies the distorsion product otoacoustic emissions were absent reflecting a certain degree of hearing loss, most probably reversible and related to experimental artifacts.

The study concluded that hyperbaric oxygen therapy has otoprotective effects against cisplatin induced ototoxicity. However, further studies are needed to test other effects of high pressure oxygen on the cochlea [17].

The effect of epigallocatechin gallate (EGCG) on the transcription factor STAT1, an important mediator of cell death was investigated by another study. STAT1 phosphorylation is involved in both hair cells and support cells transformation after experimental exposure of mouse utricle to cisplatin.

EGCG proved its efficiency as an otoprotective agent against cisplatin ototoxicity due to its inhibition of STAT1. The failure of EGCG to provide protection against cisplatin in STAT1-deficient mice also supported the hypothesis [18].

Near total preservation of otoacoustic emissions after cisplatin therapy was possible when lactate was injected intratympanically in guinea pigs treated with ototoxic levels of cisplatin, as shown in former studies [19]. A further study was conducted to prove the Ringer solution otoprotective effect against cisplatin ototoxicity when injected intratympanically, before intraperitoneal cisplatin administration since lactate is a part of Ringer solution and it has the smallest molecular weight among other antioxidants which facilitates transport across the round window membrane. The molecular protective mechanism is based on the enzyme lactate dehydrogenise located in the mitochondria of outer hair cells. The conversion of lactate to pyruvate in the presence of the enzyme leads to the formation of nicotinamide adenine dinucleotide (NADH) which is a natural antioxidant that may be involved in fighting against oxygen reactive species resulted at cellular level after cisplatin therapy. Electron microscopy examinations of guinea pig cisplatin-insulted inner ears pre-treated with lactate showed partial preservation of outer hair cells stereocilia, more significant at mid frequencies (2000-4000 HZ) but not statistically significant at higher frequencies. The study used auditory brainstem responses recordings which is a more sensible method than otoacoustic emissions testing, a fact that explains the differences in otoprotective effect reported by previous studies.

The same study investigated the otoprotective effect of intratympanic N-acetylcysteine injections as well as its systemic diffusion following the administration.

The results showed that high concentration of intratympanic N-acetylcysteine is not reliable for otoprotection against cisplatin ototoxicity since it caused more middle and inner ear damage than cisplatin alone. Yet, N-acetylcysteine did not diffuse systemically when applied to the middle ear. This was confirmed by high-performance liquid chromatography testing of blood samples taken from the venous system of the experimental animals after intratympanic injections of N-acetylcysteine. This latter outcome proves that the intratympanic route of administration would be safe and prevent inactivation of antitumoral effect of cisplatin by binding between the thiol moiety of N-acetylcysteine and the platinum-containing molecule of the chemotherapeutic drug [20].

Most of the existing studies deal with exogenous administration of antioxidants. Pharmacological activation of intrinsic defence mechanisms against oxidative stress in the inner ear caused by cisplatin therapy also proved helpful as showed by an experimental animal study using systemic administration of thiamine pyrophosphate (TPP). Thiamine pyrophosphate functions as coenzyme for peroxisomes, being a crucial factor for energy metabolism, antioxidation and myelinization of nerve cells. Its intraperitoneal injection increased the level of natural antioxidants like glutathione and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and glutathione reductase) and reduced the content of malonildialdehyde, an indicator of lipid peroxidation following increased levels of oxygen reactive species resulted from cisplatin toxicity. The histological evaluation of cochleas harvested from TPP treated animals showed preservation of the morphology of the organ of Corti and outer hair cells, and no destruction of spiral ganglion cells and stria vascularis following cisplatin therapy [4].

Sertraline, an antidepressant with neuroprotective effects in rats was also studied for its presumed otoprotective effect against cisplatin-induced ototoxicity [6]. The selective serotonin reuptake inhibitor has antioxidant effects, stimulates neurogenesis and increases antiapoptotic protein levels [21].

Oral administration of sertraline in cisplatin-treated rats has prevented hearing loss above 5000 Hz, in a statistically significant manner, as documented by distortion product otoacoustic emissions recordings.

Besides, sertraline would be beneficial to depressed patients due to their cancerous disease and their deteriorated communication abilities [5].

The otoprotective effect of systemic histone deacetylase inhibitor sodium butyrate was proved in an experimental single dose model of cisplatin ototoxicity.

A sensitive assay of the functional state of outer hair cells after systemic cisplatin insult on the cochlea was provided by distortion product otoacoustic emissions testing. The systemic administration of the otoprotective agent avoided the side effects of the more invasive tympanical local route of administration.

Moreover, sodium butyrate did not interfere with the tumoricidal effect of cisplatin providing both protections from reactive oxygen species and a certain degree of antitumor activity according to previous reports. The protective effect against oxidative stress and the cell division inhibition and subsequent anticancer activity follow acetylation of different cell proteins, including histones. The experiment's weak point is the single dose of cisplatin model whereas in clinical practice cisplatine in multiple doses [22].

One animal study showed the efficacy of short interfering RNA in preventing cisplatin ototoxicity by reducing the expression of NOX3, the unique isoforme of NADPH oxidase involved in the generation of reactive oxygen species, in outer hair cells, spiral ganglion cells and stria vascularis in the rat. Auditory brainstem responses certified reduced threshold shifts in cisplatin treated animals who received transtympanic NOX3 and RNA. Since cisplatin administration has been previously associated with upregulation of NOX3 in the inner ear, Nox3 is thought to be a major source of free radicals in the cochlea following cisplatin exposure. The resulting free radicals initiated the inflammatory process in the cochlea by activating signal transducer and activator of transcription-1 (STAT1), followed by activation of p53 and the increase in inflammatory mediators like TNF-alpha and interleukin-1ß [23]. A single transtympanic injection of RNA has atenuated cisplatin ototoxicity by suppressing inflammation in a dose-related manner. It hampered cisplatin-induced auditory brainstem responses threshold shift and higher doses allowed for complete morphological preservation of outer hair cells as proven by scanning electron microscopy examinations of the rat cochleas [24].

Minocycline is a tetracycline derivative that proved its partial efficacy among various strategies that have been devised in experimental settings to prevent cisplatin ototoxicity. The anti-inflammatory and neuroprotective properties of minocycline have been previously reported. The biochemical mechanism involves caspase-1 and caspase-3 inhibition, which decreases the amount of interleukin-1 and prevents apoptosis. The protective effect of minocycline has been tested both in cisplatin treated cell cultures and in experimental animals which underwent cisplatin intraperitoneal therapy after systemic administration of the otoprotective agent.

Cell viability assays showed that minocycline had a protective effect against cisplatin toxic action. Yet, minocycline failed to protect cells at higher concentrations of cisplatin. Recordings of auditory brainstem responses and evaluation of the scanning electron microscopy sections of inner ears harves ted from minocycline plus cisplatin treated animals indicated a partial preservation of the function and morphology of the outer hair cells as compared to those from animals treated with cisplatin alone [25].

Intraperitoneal erdosteine effect on cisplatin-induced ototoxicity in a guinea pig model was also studied. Erdosteine is a thiol derivative with well-known antioxidant properties due to its active sulfhydryl groups following liver first-pass metabolism.

Living animals underwent pre and posttreatment auditory brainstem responses measurements while outer hair cell counts were analyzed by scanning electron microscopy of the cochlea removed from euthanized animals. Despite the study's limitations (i.e. minimal number of animals included, lack of enzymatic activity detection for the main antioxidant enzymatic systems of the inner ear), the results outlined the systemic administration of erdosteine as a promising therapeutic strategy for cisplatin-induced ototoxicity [26].

Intratympanic dexamethasone prevents hearing loss in a frequency related manner as reflected by a first murine model for cisplatin-induced ototoxicity.

Evoked brainstem responses audiometry indicated that 8 kHz and 16 kHz stimulus elicited responses in cisplatin plus dexamethasone treated mice while high frequency stimulus (32 kHz) perception was affected. Apparently, cisplatin had deleterious effects on outer and inner ear cells situated in the basal turn of the cochlea despite intratympanic administration of protective dexamethasone [27].

Further experimental studies supported the finding that cisplatin exerts its damaging effect in a base to apex gradient, lower frequencies being spared for long. Higher doses of dexamethasone also seem to be more protective than lower doses. Moreover, lower doses of cisplatin allow the naturally present antioxidants to annihilate the resulting reactive oxygen species, explaining the spontaneous hearing threshold recovery even in the absence of protective dexamethasone administration [28].

Dexamethasone diminishes the tumoricidal activity of cisplatin, hencing the preference for intratympanic administration which avoids this unwanted effect. Down-regulating apoptosis genes in tumor cells are responsible for this common side effect of systemic steroid therapy [29]. An experimental study conducted on cisplatin treated guinea pigs asserted the safety of intratympanic dexamethasone based on audiologic and histologic results.

Auditory brainstem responses testing, optic microscopic and scanning electron microscopic examinations of cochlea showed no significant differences between intratympanic dexamethasone-treated animals and saline-treated controls. Dexamethasone administered intratympanicaly proved efficacious in protecting the labyrinth against cisplatin-induced ototoxicity as shown by reduced auditory brainstem responses threshold shifts and unaltered histological inner ear structures. The molecular mechanisms involve increased expression of Na/K channels and aquaporins in the endolymphatic sac and the tissues around the endolymphatic spaces. The study's results also suggest that giving the intratympanic dexamethasone one hour before the cisplatin administration provides the best protection (total protection) against the ototoxic insult by the alkylating agent compared to dexamethasone injections one day prior to cisplatin administration (partial protection) [14].

In order for the dexamethasone to exert a protective effect against cisplatin ototoxicity, the timing of administration of the two drugs should be highly synchronized so that the peak concentration of dexamethasone in the perilymph should be correlated with the peak concentration of the chemotherapeutic agent, according to another study [2].

Otoprotection with dexamethasone against age-related cisplatin-induced hearing loss was investigated in a guinea pig model following observations that persons older than 65 account for more than half of the newly diagnosed malignancies.

Hearing loss due to the aging process shares the same cause (i.e. oxidative stress) with hearing loss due to ototoxic chemotherapeutic agents.

A single dose of cisplatin was administered intraperitoneally in old mice preceded and followed by dexamethasone injected intratympanically to counteract the cisplatin toxic effect on inner ear hair cells. Pre and post treatment auditory brainstem responses were recorded to evaluate hair cell function. The results of the study pointed out that no synergistic action between age related hearing loss and cisplatin-induced hearing loss exists since threshold shifts were smaller in older animals than those in young mice.

Another finding of the study was that the protective effect of dexamethasone against cisplatin-induced ototoxicity was a function of stimulus frequency in old mice. Susceptibility to otoprotective effect of dexamethasone was higher in mid to basal cochlear regions (at and above 24 kHz) in old mice , whereas in young mice, dexamethasone bestowed more protection in apical regions of the cochlea (at 16 kHz and below). Age-related changes of the mechanism of distribution of dexamethasone in scala tympani perlymph after round window membrane application in guinea pigs seem to account for the frequency dependent otoprotective effect [30].

Intratympanic dexamethasone did not protect against cisplatin-induced ototoxicity in a multidose cisplatin ototoxicity mouse model. The study was prompted by typical clinical protocols of cancer treatments which require administration of multiple, smaller cisplatin doses, exerting their curative effect through cumulative dosing. Contrary to previous experimental studies, the mice received five doses of cisplatin throughout five days, mimicking the cumulative exposure seen in malignant tumours treatment.

Intratympanic dexamethasone was administered on the same days as the intraperitoneal cisplatin. The results mirrored by auditory brainstem responses threshold measurements demonstrated continued change in hearing thresholds several weeks after cisplatin exposure and no protective effect of intratympanic dexamethasone against cisplatin ototoxicity [31].

An experimental study focusing on systemic administration of steroid for protection against cisplatin-induced ototoxicity showed no otoprotection following several days' prophylaxis with a high dose dexamethasone treatment. Only a slight decrease of TNF-alpha expression in the cochlea was demonstrated by imunohistochemical staining of anatomical samples harvested from systemic cisplatin plus dexamethasone treated animals. Dexamethasone also seemed to protect stria vascularis from morphological alterations, probably owing this effect to higher concentrations of steroid in the lateral cochlear wall following increased cochlear flow and a naturally highly vascularised stria vascularis.

Still, a functional otoprotective effect of systemic dexamethasone against cisplatin-induced hearing loss was not observed [3].

Among naturally occurring molecules, Rosmarinic acid, a water-soluble polyphenolic compound extracted from Dansam-Eum, was tested for its protective effect against cisplatin-induced ototoxicity in laboratory settings. The results of the study showed that Rosmarinic acid inhibited cisplatin induced caspase-1 activation providing protection against stereocilia loss in the primary organ of Corti explants [32].

Another natural remedy, the Maytenus ilicifolia aqueous extract, was evaluated for its possible otoprotection in guinea pigs. Despite the well-known South America plant's antioxidant effects (due to the presence of flavonoids and alkaloids), functional tests did not demonstrate any protective action on the cisplatin exposed cochlea. Yet, the extract improved the clinical status and weight of guinea pigs and diminished mortality after cisplatin exposure [33].

Resveratrol, a polyphenol found in grape skin and seed, has anti-oxidant, neuroprotective and dose dependent antiapoptotic properties [34]. Recent experimental research pointed the preventive effect of resveratrol against cisplatin induced ototoxicity.

An in vitro study on House Ear Institute-Organ of Corti 1 cell line showed that resveratrol in low doses prevented ototoxicity mainly influencing apoptotic gene expression but proved cytotoxic effect in high doses [34]. Two other studies showed conflicting results. Thus, high doses of oral resveratrol administered to mice seem to enhance cisplatin ototoxicity[35] whereas systemic administration of lower doses of resveratrol provided significant protection to the cochlea against cisplatin [36].

Clinical studies

Intratympanic dexamethasone was clinically tested for its otoprotective effect in patients afflicted with neoplastic diseases for which the treatment protocol included cisplatin. Intratympanic dexamethasone is a current remedy for idiopathic sudden sensorineural hearing loss and Meniere disease [37].

Intratympanic administration of drugs avoids significant systemic side effects. The intratympanic route also provides higher concentrations of drug in the inner ear fluids and prevents significant interference between dexamethasone, which is known to reduce efficacy of chemotherapeutic agents, and cisplatin.

Patients enrolled in the study underwent unilateral intratympanic dexamethasone administration prior to every cisplatin treatment session, with the contralateral ear used as a control. Serial follow-up audiometry and distortion product otoacoustic emissions testing were performed to check the functional state of both study and control ears. The statistically significant results showed that intratympanic dexamethasone is slightly protective against cisplatin-induced hearing loss at 6000 Hz and decreases the outer hair cells dysfunction in the frequency range of 4000 to 8000 Hz. The conclusion of the study is that intratympanic dexamethasone has minimal effect towards reducing cisplatin ototoxicity. Optimal concentrations of dexamethasone and a perfect timing of administration would be necessary to prevent hearing loss after cisplatin therapy [2].

Patients with head and neck cancer undergoing cisplatin therapy were given transtympanic L-N-Acetylcysteine to check its otoprotective effect.

Thiol compounds either directly bind cisplatin or act as free radical scavengers. Based on that, their intratympanic administration was suggested to avoid the decrease of oncologic effectiveness of cisplatin and to reduce the oxidative stress caused by it.

Intratympanic L-N-Acetylcysteine was well tolerated by patients receiving multiple doses of cisplatin as part of their oncologic treatment. The relation between dose and otoprotection was not taken into account. Higher concentrations may have yielded better otoprotection. The study protocol required the L-NAC injection to be approximately 1 hour before systemic administration of cisplatin, for the sake of better timing. The outcome of the pure tone audiometry testing at 1 and 2 months after the last cycle of cisplatin showed that L-N-Acetylcysteine was overall not significantly otoprotective. Still, hearing loss was reduced in two patients out of eleven who completed the study. The study protocol had several challenges like the difficulty in maintaining high enough concentrations of aqueous solution of L-N-Acetylcysteine in the middle ear due to the technique of administration and the different initial hearing thresholds due to preexisting hearing loss [38].

Among systemic otoprotective molecules for preventing cisplatin-induced hearing loss, amifostine, a phosphorylated aminothiol designed to protect against radiation damage, was known to counteract the toxic effect of different anticancer treatments without interfering with the tumoricidal effect. Although significant protection of amifostine against haematological toxicity after high dose carboplatin therapy in a child with medulloblastoma was reported [39], a clinical study considering systemic administration of amifostine failed to prove any otoprotective effect against cisplatin-induced hearing loss in a group of paediatric patients treated with cisplatin associated with other chemotherapeutic agents [40].

OTOPHARMACOGENETICS

The well-isolated inner ear organ makes it prone to targeted genetic therapies. Viral or non-viral gene vectors can be delivered through a transtympanic route without the risk of dispersing them and reaching other tissues with subsequent undesirable genetic alteration. Long term effects after single administration, cellular selectivity and replacement of genetically flawed nucleic acid sequences are the main benefits of gene therapy. Common viral vectors include herpes simplex virus, recombinant adenoassociated virus, recombinant adenovirus, adenovirus, used to amplify the expression of targeted genes. Cells are infected with the vectors (transfection) which transfer genes whose expressed proteins influence important processes like growth, oxidative stress and apoptosis. Chemical transfection can also be achieved with plasmid vectors. Short interfering RNA can be used to shut down target genes.

Among inner ear target genes dealt with by the gene therapy studies are ATOH1 (Math1), CAT (catalase), SOD1 (Cu/Zn superoxidedismutase), SOD2 (Mn superoxide dismutase), BDNF (brain-derived neurotrophic factor), HGF (hepatocyte growth factor), GJB2 (gap junction protein), BclxL (B-cell lymphoma-extra large), FGF2 (basic fibroblast growth factor). The gene therapy modifies the synthesis of a wide range of proteins including neurotrophic factors (NTF3, GDNF), apoptosis mediators (XIAP,BCL2), oxidases (NADPH, NOX1, NOX3, NOX4), an antioxidant response regulator (Nfe2l2), a cytoprotective enzyme (HO-1), a copper transportes (Ctr1), a nonselective cation channel (Trpv1) and a protein Otospiralin (Otos).

Cisplatin-exposed tissues can benefit from genetically induced up-regulation of neurotrophic factors, inhibition of apoptosis and generation of endogenous antioxidant enzymes.

Experimental animal studies and in vitro experiments show the efficacy of gene therapy for cisplatin-induced ototoxicity. Clinical applications require further studies regarding safety, immunogenicity and consequences of genetic manipulation [41].

Another strategy to avoid cisplatin-induced hearing loss would be the pretreatment genotyping to find out patients at risk for the ototoxic effect of cisplatin [42]. Genetic variants (polymorphism) of different protein systems (Tiopurine S-methyltransferase, Catechol-O-methyl transferase, Glutathione-S-transferase with its subclasses M1/T1/ P1, Megalin) can stand for the interindividual variability in cisplatin ototoxicity [43].

RESULTS AND DISCUSSION

43 publications were reviewed concerning prevention or treatment of cisplatin induced ototoxicity.

Publications were devised in either experimental or clinical studies. All researches emphasize the importance of reactive oxygen species in cisplatin induced ototoxicity. The hearing loss following cisplatin treatment affects principally the high frequencies, extending to speech range frequencies as multiple doses of cisplatin worsen hearing.

Experimental studies supported the efficiency of hyperbaric oxygen therapy, epigallocatechin therapy and intratympanic lactate. The latter two therapies provide exogenous antioxidants while pharmacologic activation of endogenous antioxidants by means of intratympanic thiamine pyrophosphate was consistent with higher levels of natural antioxidants.

Oral sertraline, besides its otoprotective effect against cisplatin induced ototoxicity, also has therapeutic value concerning the depression occurring frequently in oncologic patients. Sodium butyrate proved its efficiency against cisplatin induced hearing loss in a monodose cisplatin model. Yet, in clinical practice the patient receives multiple doses of ciplatin. The production of endogenous radicals of oxygen species was reduced after intratympanic administration of short interfering RNA which reduces the expression of NOX3 in the cochlea. Minocycline appeared to be efficient only at low doses of cisplatin, while systemic erdosteine showed promising results. Dexamethasone in experimental studies combined efficiency against cisplatin induced ototoxicity with preservation of the tumoricidal activity of cisplatin. When older mice were treated, the dexamethasone was more otoprotective at higher frequencies compared to experiments including younger subjects. Rosmarinic acid also proved to be otoprotective while the Maytenus ilicifolia aqueous extract was not. Resveratrol had contradictory effects, systemic low doses and in vitro administration preventing ototoxicity, whereas high oral doses seem to enhance cisplatin ototoxicity.

Also, there were some experimental studies which showed inefficiency of intratympanic NAcetylcysteine and intratympanic and systemic dexamethasone. N-Acetylcysteine, in addition having a damaging effect on middle and inner ear structures.

Clinical studies proved a minor otoprotective effect of intratympanic dexamethasone and no effect of systemic amifostine and intratympanic LN-Acetylcysteine.

New perspectives are brought about by genetic therapy using viral vectors and genotyping to anticipate interindividual variability in cisplatin ototoxicity.

CONCLUSION

New strategies are required for hearing loss prevention and treatment during cisplatin therapy for cancer besides optimization of old ones. The intratympanic route of administration and the gene therapy are among the most promising perspectives in further experimental and clinical studies.

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Imaging in the diagnosis of chronic pancreatitis

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Abstract: *Chronic pancreatitis is characterised by progressive and irreversible damage of the pancreatic parenchyma and ductal system, which leads to chronic pain, loss of endocrine and exocrine functions. Clinically, pancreatic exocrine insufficiency becomes apparent only after 90% of the parenchima has been lost. Despite the simple definition, diagnosing chronic pancreatitis remains a challenge, especially for early stage disease. Because pancreatic function tests can be normal until late stages and have significant limitations, there is an incresing interest in the role of imaging techniques for the diagnosis of chronic pancreatitis. In this article we review the utility and accuracy of different imaging methods in the diagnosis of chronic pancreatitis, focusing on the role of advanced imaging (magnetic resonance imaging, endoscopic retrograde cholangiopancreatography and endoscopic ultrasound).*

INTRODUCTION

Chronic pancreatitis is represented by a progressive inflammation and fibrosis of the pancreas, resulting in permanent structural damage and loss of function. Regarding its epidemiology, incidence and prevalence worldwide varies depending on the frequency of risk factors (rates mainly paralelling alcohol consumption) and on the diagnostic method used (with higher incidence and prevalance rates where diagnosis was based on advanced imaging techniques – a study in Japan using advanced diagnostic tools reported a prevalence of 45.4 and incidence of 12.4/100.000, compared to 10-15 and respectively 3.5-4 in western countries). Etiology is represented by heavy alcohol consumption, ductal obstruction (post-traumatic fibrosis, stones, pseudocyst, tumors, pancreas divisium, Oddi sphicter dysfunction), genetic causes (hereditary pancreatitis), tropical pancreatitis, autoimmune pancreatitis or systemic disease (lupus, hyperparathyroidism).

The natural history of c hronic pancreatitis evolves with a slowly progressing subclinical phase, followed by recurrent episodes of abdominal pain and finally, when over 90% of the pancreatic parenchyma is lost, exocrine insufficiency appears. Although it is not yet known if early diagnosis of chronic pancreatitis can alter its natural history and change the outcomes of this disease, efforts should be made for earlier detection of the disease.

In real-life there are two main diagnostic issues regarding this disease: on one hand it's over-diagnosed by considering a hyperechoic pancreas on

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ultrasound as chronic pancreatitis, and on the other hand it's under-diagnosed in early stages when patients don't have exocrine insufficiency but present with recurrent abdominal pain and abdominal distention, symptoms which are frequently attributed to irritable bowel syndrome (IBS).

On clinical grounds, early chronic pancreatitis can be asymptomatic or paucisymptomatic; moreover, early symptoms are nonspecific, of low intensity and often attributed to IBS. The classic steathoreea appears late in the evolution of the disease, but our aim is to detect chronic pancreatitis before overt exocrine insufficiency develops.

Diagnostic tests for chronic pancreatitis are separated in two: those that evaluate pancreatic function and those that detect structural changes[2].

Pancreatic function tests (PFT) can be classified as direct (which directly measure pancreatic secretions) and indirect (which measure either the pancreatic enzymes in blood or stool or the effect/lack of effect of the pancreatic enzymes, by administering a substrate which requires pancreatic enzymes for digestion and measuring the metabolites of the substrate in stool, urine, blood or breath). Direct PFT is done by collecting and measuring intraduodenal bicarbonate and lipase levels after stimulation with a secretagogue (secretin-cholecystokinin); this method has a sensitivity of 96% (which means a normal result can accurately rule out chronic pancreatitis) and specificity of 37%, but it has major limitations – it's not standardized, not widely available, it depends on the patient to tolerate the oroduodenal tube/endoscope for an hour (here we add the costs and risks of prolonged sedation) and it has many false positive results (in Billroth II anastomosis, diabetes mellitus, celiac disease, cirrhosis and recent acute pancreatitis)[2]. Other PFT such as fecal elastase, fecal chymotrypsin or serum trypsinogen have proven useful only for advanced disease[2].

Histopathological examination of the pancreas could be diagnostic in the early stages, however tissue sampling in chronic pancreatitis is rarely used because of many reasons: histopathological changes are non-specific (chronic inflammation and fibrosis), sampling can be nonrepresentative in focal disease, normal aging can produce changes similar to those seen in chronic pancreatitis and pancreatic biopsy has a high risk of complications (acute pancreatitis, fistula, pseudocyst).

In this setting, imagery has emerged as a very valuable tool for evaluation of chronic pancreatitis.

Imaging methods used for the diagnosis and staging (with regard to severity and complications) consist of: plain abdominal x-ray, transabdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreatography (MRCP) and secretin stimulation (S-MRCP), endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasound (EUS).

Plain abdominal X-Ray

Plain abdominal radiography can reveal diffuse calcifications on the projection area of the pancreas, which are considered pathognomonic of chronic pancreatitis[3].

Abdominal ultrasound (US)

Abdominal ultrasound is a simple, non-invasive, widely available imaging tool. Its limitations are represented by the fact that it's highly examiner-dependent and patient-dependent (excessive adipose tissue, history of upper GI tract surgery or overlying gas can lead to a poor view of the pancreas).

Examination is done using a convex probe, with a frequency of 3.5-5 Mhz, using epigastric and left subcostal approaches. To optimise visualisation of the pancreas, some recommend drinking 500 ml of water before the examination, to make the stomach an acoustic window.

Ultrasound can show atrophy/enlargement of the pancreas, inhomogenous echostructure (by hyperechoic areas corresponding to fibrosis), irregular gland border, pancreatic duct changes (dilatation, irregularity or stones), cysts and pseudocysts. US has a sensitivity of 60-70% and specificity of 80-90% in diagnosing chronic pancreatitis3. Although its role in positive diagnosis is limited, US is very useful in follow-up: it can assess position of intraductal or pseudocystogastric stent and it can evaluate the efficacy of extracorporeal lithotripsy by showing the disappearance of stones and reduction in pancreatic duct diameter.

Computed tomography (CT)

From its first use in chronic pancreatitis back in 1976, CT is now widely used for the evaluation of pancreatic pathology. Computed tomography has a reported sensitivity of 75-90% and specificity of 85% in the diagnosis of chronic pancreatitis, but it can be normal in patients with early stage disease[3,6].

CT scan can reveal chages in size (enlargement, atrophy) and contour (irregularity of the anterior border) of the pancreas, calcifications (with higher detection rates than ultrasound, being the imaging method of choice for calcifications), dilatation of the main pancreatic duct or common bile duct, alterations in peripancreatic fat – see figures 1, 2. It can also detect complications such as pseudocysts, arterial pseudoaneuyrisms or splenic vein thrombosis[6].

Contrast-enhanced computed tomography

(CECT) is also useful in differentiating pseudotumoral chronic pancreatitis from pancreatic cancer[6].

The disadvantages of the CT scan consist of irradiation, risk of contrast-induced nephropathy and its contraindication in patients with iodine-allergy[6].

Endoscopic retrograde cholangiopancreatography (ERCP)

ERCP is a very useful tool for detecting morphological changes in the pancreatic ductal system. It is done using a lateral-view endoscope by cannulating the main pancreatic duct from the duodenum and injecting contrast to create a pancreatogram. The pancreatogram can show structural changes (dilatation, narrowing, irregularity, stones, leaks) of the pancreatic duct, abnormal side branches, communicating pseudocysts, which are reported in a standardized manner, using the Cambridge criteria, (Table 1). In interpreting the pancreatogram, one should keep in mind that focal ductal changes can follow an episode of severe acute pancreatitis,. Also a normal pancreatogram does not exclude early chronic pancreatitis.

Table 1 Cambridge Classification for ERCP
(Banks PA. J Gastroenterol 2007)

Grade	Main duct	Abnormal side branches
Normal	Normal	None
Equivocal	Normal	< 3
Mild	Normal	> 3
Moderate	Abnormal	> 3
Marked	Abnormal with at least one additional feature: large cavity (>10 mm), obstruction, filling defects, severe dilatation or irregularity	> 3

The main disavantages of ERCP are represented by the fact that it's strongly operator dependent, it's an invasive technique with potential serious complications (pancreatitis, hemorrhage, infection) and it only reveals ductal changes[6]. Over the last years, ERCP has become a primarily therapeutic tool, so that diagnostic studies have been abandoned in favor of other imaging methods such as MRI/MRCP or EUS. However, ERCP remains of great importance in pancreatic endotherapy (pancreatic sphincterotomy, stenting and cyst drainage).

Magnetic resonance imaging (MRI). Magnetic resonance cholangiopancreatography (MRCP)

Due to its noninvasivity and lack of irradiation, MRI with MRCP, gadolinium enhancement and secretin stimulation is being increasingly seen as the imaging method of choice in the evaluation of pancreatic disease. MRI can be used in renal impairment and in patients with iodine allergy, in whom CECT cannot be performed[7]. One of the drawbacks of MRI is represented by the artefacts caused by respiratory movements, but this has improved with the latest technology.

Pancreatic parenchyma can be best examined on T1-weighted, fat-suppressed sequences, where it reveals a high signal due to the high-protein content (enzymes, hormones). In chronic pancreatitis, the pancreatic signal decreases (due to loss of exocrine function) and there is a delayed enhancement after gadolinium administration (due to fibrosis, blood flow inside the pancreas is altered and the gland peaks in the venous phase instead of the arterial one)6. MRI is inferior to CT in detecting calcifications, because they are seen as signal void.

Pancreatic ductal system is best evaluated by magnetic resonance cholangiopancreatography.

MRCP images are obtained without injecting contrast medium, by using heavily T2-weighted sequences.

On T2-weighted images, fluid from the bilio-pancreatic system has a bright signal and by adding fat-supression, the signal of fluid within the ducts becomes more pronounced[6]. MRCP can show dilatation, strictures, irregularities in the Wirsung duct or similar changes in the side-branch ducts – see figures 3,4.

The advantages of MRCP over ERCP are lack of instrumentation of the Wirsung duct and lack of need for sedation during the procedure; however, MRCP is only diagnostic, whereas ERCP is therapeutic. Another difference between the two is that while during ERCP the collapsed secondary branches are distended by the contrast agent, MRCP visualizes the ductal system in a physiologic state, thus being less acurate for small duct disease. To overcome this difference a combined functional-structural test has been developped, called MRCP-S (magnetic resonance cholangiopancreatography with secretin stimulation): secretin stimulates secretion in the ductal system and it also increases the tonus of the Oddi sphincter (preventing the release of secretion in the duodenum), thus filling in the collapsed branches and better delineating minimal duct changes in patients in whom MRCP showed no abnormalities under physiological conditions; the study of Manfredi et al. showed that visualization of the secondary branches improved significantly in both patients with severe disease (from 71% to 100%) and those with a mild-moderate disease (from 4% to 63%). MRCP-S also allows for calculation of the change in pancreatic duct size before and after secretin stimulation (index called pancreatic duct compliance, PDC), which is reduced in chronic pancreatitis[6].

Table 2. Rosemont criteria for chronic pancreatitis

	Rank	Criteria	Feature	Definition
Parenchymal features	1	Major A	Hyperechoic foci with shadowing	Echogenic structures ≥ 2 mm in length and width that shadow
	2	Major B	Lobularity with honeycombing	Well-circumscribed, ≥ 5 mm structures with enhancing rim and relatively echo-poor center, contiguous ≥ 3 lobules
		Minor	Lobularity without honeycombing	Same as above, noncontiguous lobules
	3	Minor	Hyperechoic foci without shadowing	Echogenic structures foci ≥ 2 mm in both length and width with no shadowing
	4	Minor	Cysts	Anechoic, rounded/elliptical structures with or without septations
	5	Minor	Stranding	Hyperechoic lines of ≥ 3 mm in length in at least 2 different directions with respect to the imaged plane
Ductal features	1	Major A	MPD calculi	Echogenic structure(s) within MPD with acoustic shadowing
	2	Minor	Irregular MPD contour	Uneven or irregularoutline and ectatic course
	3	Minor	Dilated side branches	3 or more tubular anechoic structure seach measuring ≥1 mm in width,budding from the MPD
	4	Minor	MPD dilation	≥3.5-mm body or >1.5-mm tail
	5	Minor	Hyperechoic MPD margin	Echogenic, distinct structure greater than 50% of entire MPD inthe body and tail

Endoscopic ultrasound (EUS)

Endoscopic ultrasound is done using a linear/radial transducer integrated into the tip of the scope, using 7.5-12 Mhz frequencies. It is a great method of evaluating the pancreas, considering that the transducer gets in very close range of the gland, from which is separated only by the digestive tract wall.

EUS is especially useful for early chronic pancreatitis, being able to reveal small structural changes. Its advantage over ERCP is that it detects both parenchymal and ductal changes and doesn't use ionizing radiation. One of the disadvantages of EUS is that it's operator-dependent.

Add-on modules such as elastography and contrast-enhanced EUS (CEEUS) provide further details in evaluating pancreatic disease. Moreover, linear EUS can also provide tissue sampling (to differentiate an inflammatory mass from adenocarcinoma) and can guide therapy in chronic pancreatitis (celiac plexus neurolysis for unresponsive pain, pseudocyst drainage, main pancreatic duct drainage).

EUS characteristics of chronic pancreatitis have been grouped into ductal and parenchymal, focusing on 9 criteria which correspond to histopathological changes: 5 of them are parenchymal (hyperechoic foci, hyperechoic strands, lobularity, cysts/ pseudocysts, calcifications) and 4 are ductal (dilated pancreatic duct, irregular pancreatic duct, hyperechoic pancreatic duct walls, visible side branches) – see figures 5,6,7,8. Because not all of these criteria have the same importance in the diagnosis of chronic pancreatitis, these sonographic features have been redefined as major and minor features in a consensus classification called Rosemont.

According to the Rosemont criteria, EUS result can be23: consistent with chronic pancreatitis (1 major A feature + ≥3 minor features, or 1 major A feature + major B feature, or 2 major A features), suggestive of chronic pancreatitis (1 major A feature + <3 minor features, or 1 major B feature + ≥3 minor features, or ≥5 minor features), indeterminate for chronic pancreatitis (3 to 4 minor features, no major features or major B feature alone or with <3 minor features) or normal (no major features, ≤ 2 minor features – excluding cysts, dilated MPD, hyperechoic non-shadowing foci, dilated side branch).

Table 3. Sensitivity and specificity of imaging studies for the diagnosis of chronic pancreatitis[10]

	Sensitivity (%)	Specificity (%)
Ultrasound	60-70	80-90
CT	75-90	85
MRI/MRCP	85	100
ERCP	75-95	90
EUS	97	60

Figures 1,2: CT scan of a 40 years-old male, smoker, with chronic alcohol intake and history of multiple episodes of acute pancreatitis: Enlarged head of the pancreas with multiple cystic structures (up to 3 cm, compressing the CBD) and calcifications; large pseudocyst (8/7 cm) in the body-tail.

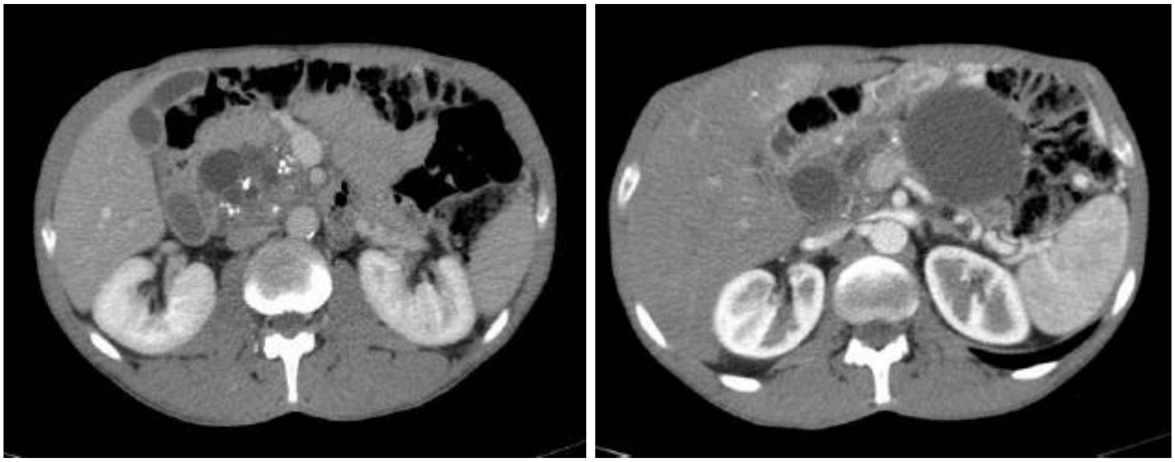

Figures 3,4: MRI and MRCP, same patient: Pseudotumoral chronic pancreatitis, 9/7 cm pseudocyst in the bodytail with splenic vein occlusion

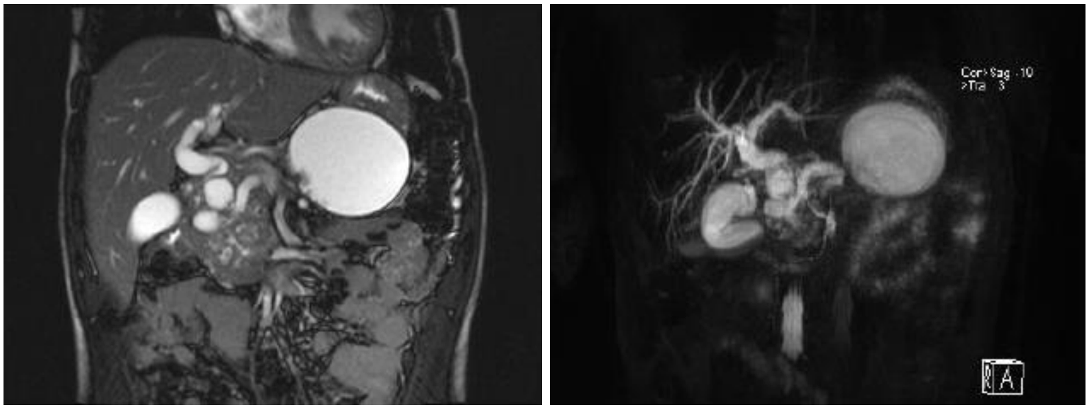

Figures 5-8: EUS examination, same patient: Inhomogeneous head of the pancreas with multiple calcifications (up to 6 mm) and cystic structures; large cystic lesion in the body of the pancreas with hyperechoic debris

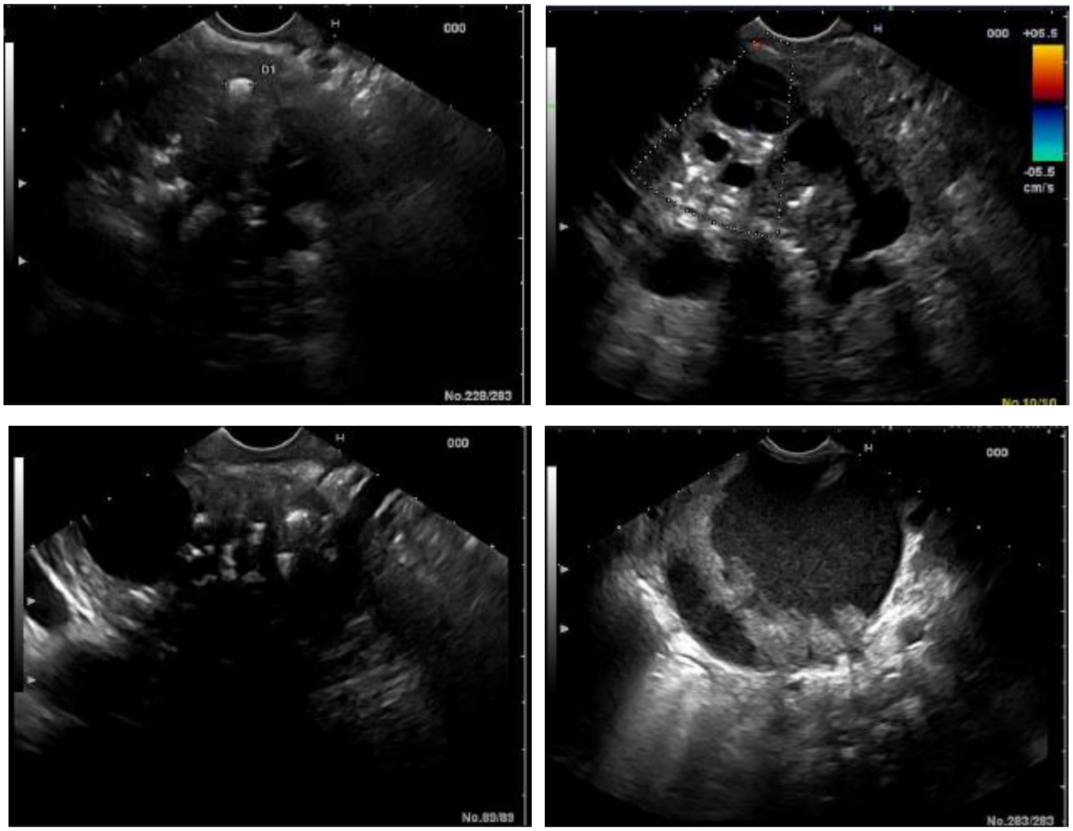

CONCLUSIONS

Imaging is becoming more and more important in the evaluation of chronic pancreatitis.

Among the imaging methods, plain abdominal radiograph is useful when calcifications are present and abdominal ultrasound is valuable for follow-up.

CT is best for identifying pancreatic calcifications and for evaluating complications of chronic pancreatitis.

ERCP can accurately detect changes in the ductal system, but it’s nowadays accepted only for therapeutic purposes. MRI with MRCP and EUS are currently the imaging methods of choice for the evaluation of chronic pancreatitis.

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CLINICAL PRACTICE

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Re-emerging infectious diseases: Ebola hemorrhagic fever

Lucia E. Ionescu[1], Gabriela V. Dumitrescu[1], Viorel Ordeanu[1]

Abstract: *The spring of 2014 has brought a new calamity, the exotic infectious disease: Ebola Hemorrhagic Fever, which is caused by a highly contagious and pathogenic virus, transmitted directly by interpersonal contact or indirectly by common usage of objects. The epidemic which occurred in Guinea tended to expand to neighboring countries; 83 deaths have been reported on April 1st 2014. Genetic analysis have revealed that the virus that causes this epidemic is similar in a proportion of 98% to Ebolavirus Zaire (EBOV) species that were responsible for the epidemic in Democratic Republic of Congo, in 2008.*

The Ebola virus belongs to the Filoviridae family, Ebolavirus genus and causes Ebola Hemorrhagic Fever, with a rate of fatality of up to 90% in humans. There are five distinct species: Bundibugyo Ebolavirus (BDBV), Ebolavirus Zaire (EBOV), Reston Ebolavirus (RESTV), Sudan Ebolavirus (SUDV) and Taï Forest Ebolavirus (TAFV).

Keywords: *Ebola, Hemorrhagic Fever, exotic diseases, epidemic*

INTRODUCTION

The spring of 2014 has brought a new calamity, an exotic infectious disease – Ebola Hemorrhagic Fever, which is caused by a highly pathogenic and virulent virus, highly contagious by interpersonal contact or through objects. Epidemic which first occurred in Guinea tended to expand to neighboring countries and beyond, on April 1st 2014, 83 deaths cases being reported. Genetic tests have revealed that the virus that causes this epidemic is similar in a proportion of 98% with Ebolavirus Zaire (EBOV) species, which were responsible for the epidemic in the Democratic Republic of Congo in 2008.

Ebola virus belongs to the Filoviridae family, Ebolavirus genus and causes Ebola Hemorrhagic Fever, a fatal disease to humans in more than 90% of cases. Five distrinct species have been identified: Bundibugyo Ebolavirus (BDBV) Ebolavirus Zaire (EBOV), Reston Ebolavirus (RESTV), Sudan Ebolavirus (SUDV), Tai Forest Ebolavirus (TAFV) by now (http://ecdc.europa.eu).

The virions have the characteristic appearance of rod or filaments in the form of U or 6, or entangled branched. Type of genome is RNA (ribonucleic acid) linear, single-stranded, negative-sense (1) (Figure 1).

Ebola Hemorrhagic Fever is an acute viral infection characterized by an incubation period of about 8 days, with extremes of 2-21 days. Onset occurs suddenly, with high fever, chills, headache, myalgia, anorexia associated with rash. In the next 2-3 days appear nausea, vomiting, pharyngitis and hemorrhagic phenomena, haematemesis, melaena, bruising and petechiae. Nervous disorders appear, accompanied by prostration, anxiety, confusion, and

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memory loss and balance disorders. Laboratory analyses show a low number of leukocytes and platelets and an elevated liver enzymes. Abortion in pregnant women occurs in 66% of cases and newborns of infected mothers fatally infected. Mortality in pregnant women is 95.5%.

Figure 1. Virus Ebola – electronic microscope image (Centers for Disease Control and Prevention, USA)

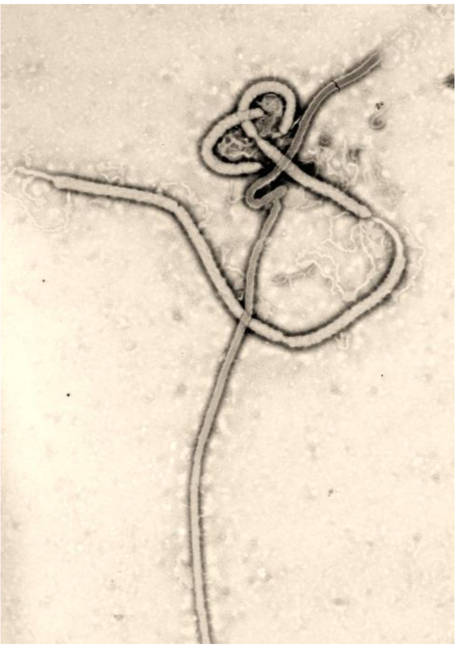

Death may occur, in 6-9 days after the illness debut, by hemorrhagic shock with massive and generalized bleedings. Virus and viral antigen are present at high levels in the blood and organs. In case of illness remission, convalescence is long and is accompanied by intensive hair fall for about 3 month, extreme fatigue, anorexia, abdominal pain, myalgia and arthralgia, hearing loss in 6 months after acute phase.

Myalgias and arthropathies are described even after 21 months. Infected people are contagious as long as their blood and secretions contain the virus which could be isolated from semen until day 61 after debut and persists for patients in recovering.

Viral RNA was revealed in vaginal, rectal and conjunctival samplings after 33 days, and presence of viral RNA in semen was demonstrated for 61, 82 and 91 days after illness onset (1).

EPIDEMIOLOGY

History. The Ebola virus was first identified in 1976 during the epidemic in Zaire (Democratic Republic of Congo) and Sudan. Natural infection cases were reported in Africa: Democratic Republic of Congo (1976, 1977, 1995, 2007, 2008, 2012), Sudan (1976, 1979, 2004), Gabon (1994, 1996, 2001, 2002), Ivory Coast (1994), Uganda (2000, 2001, 2007, 2011, 2012), Congo Republic (2001-2003, 2005), Guinea and Liberia (2014). In England (1976) was reported an accidental infection in a laboratory. Ebola was introduced into quarantine facilities in the USA (1989, 1990, 1996), in Italy (1992) and the Philippines (1996) (www.who.int, www.ecdc.europa.eu).

Ebola in Uganda/2012

On July 29, 2012 Ministry of Health of Uganda has notified the World Health Organisation (WHO) on an outbreak of hemorrhagic fever in a western area of the country, Kibaale, with 20 cases and 14 deaths. Since the beginning of the epidemic was reported a total of 24 human cases (confirmed or probable) and 17 deaths. Laboratory tests conducted by Uganda Virus Research Institute (UVRI) and Centres for Disease Control and Prevention (CDC) confirmed the presence of Ebola Sudan virus in 5 patients, from which 2 died. All reported infection cases and infected people contacts were investigated (www.cdc.gov). On October 4, 2012 Ministry of Health of Uganda said that the epidemic ended.

Ebola in Democratic Republic of Congo/2012

On August 17 2012, Ministry of Health of Democratic Republic of Congo reported an outbreak of Ebola hemorrhagic fever with 10 suspected cases and 6 deaths. Laboratory tests conducted by Uganda Virus Research Institute (UVRI) and Centres for Disease Control and Prevention (CDC) confirmed the presence of Bundibugyo Ebola virus in 2 patients. However, epidemic in the Democratic Republic of Congo it is not epidemiologically linked to the one in Uganda. Final Report of November 26 shows a total of 77 cases of which 36 were confirmed by laboratory tests, 17 were probable, 24 were suspicious and 36 deaths were reported (www.cdc.gov).

Ebola in Guinea and Liberia/2014

On March 22, 2014, the Ministry of Health of Guinea notified WHO about an outbreak of Ebola Hemorrhagic Fever in the forested southeast areas, rapidly evolving, with a total of 49 cases, including 29 deaths (fatality rate: 59%). On March 30, 2014 a total number of 112 suspected cases of Ebola virus infection were reported, from which 70 were deaths and on April 12; Centres for Disease Control announced 83 deaths; 35 of these cases were confirmed by laboratory tests carried out in several laboratories, including Pasteur Institute, Lyon in France, Institute Pasteur, Dakar in Senegal and Bernhard Nocht Institute for Tropical Medicine, Hamburg in Germany, using PCR (Polymerase Chain Reaction).

Liberia has reported 8 suspect cases, including 5 deaths, and two laboratory confirmed cases in people who recently travelled in Guinea. Ministry of Health of Guinea and WHO have implemented measures to control the spread of the epidemic and to restrict travels and trade till the problem is solved (www.cdc.gov).

The natural infection

Source of the virus still remains unknown, but it is suspected that virulent strains used as hosts could be species which come less in contact with people. The transmission is achieved by direct contact with sick animals or humans through blood, organs, secretions or excretions or by sexual contact. Contamination by aerosols (6), although it is not regarded as the main route of transmission, has been incriminated in the 1989 epizootic in Reston (the virus was isolated from monkeys).

The nosocomial transmission (by linen, syringes, needles and other contaminated material) should be considered to impose preventive measures. The susceptibility related to natural infection is generalized and the resistance to infection or re-infection is not known (1).

Biological weapon

The Ebola virus is a potential biological agent to be used in bioterrorism because it causes a highly severe disease and the means of defence against this virus are extremely poor for the moment. Russian researchers have shown that low doses of Ebola virus administered by aerosols could cause infections in monkeys, implying mortality.

There is no effective therapy for infection prevention, nor vaccines are available so this virus may be considered as a potential bio-terrorist biological agent (4). We should not forget that at the first outbreak of Ebola in Africa, among the international volunteers, representatives of the AUM sect from Japan also came whose they real target was to take with them virus strain samples, possible in intention to cause biological attacks. Fortunately, Interpol has prevented on time that fact, counteracting in an antiterrorist manner. There are suspicions that some armies would have developed an Ebola-Smallpox hybrid virus as a biological agent, hybrid which would benefit from a maximum of pathogenicity, virulence and infectiousness and for which there is no effective treatment.

LABORATORY DIAGNOSIS

Necessary samples for analysis in the acute fever stage are: serum, heparinied plasma, nasopharyngeal washings, urine, soft tissues blood samples, skin biopsies.

Necessary samples for analysis in the recovering stage are: semen, eye discharge.

Necessary samples for postmortem analysis are: fragments of organs (spleen, lymphatic nodes, liver, and kidneys).

After sampling, the pathological materials are going to be maintained in triple layered packaging, according to WHO recommendations (perfect sealed plastic bags with clear inscriptions, in resistant containers, protected against leakage).

These containers are to be proofed against outside surface contamination. Samples will be stored frozen in liquid nitrogen or dry ice. Due to the high contamination hazard, handling of infectious materials will be made with special precautions in laboratories that have an appropriate grade of biosafety, level 4, regarding personnel and technical equipment (1).

According to CDC recommendations, the virological diagnosis is presented in Table 1.

In Romania, suspicious samples could be analyzed for laboratory diagnosis at the National Institute of Research and Development for Microbiology-Immunology Cantacuzino (NIRDMI Cantacuzino) and the Institute of Virology "Stefan Nicolau" in Bucharest, but there are no facilities BSL4 (Bio-safety Level 4 Laboratory highly security).

In veterinarian medicine, the sample analysis may be performed at Institute for Diagnosis and Animal Health Bucharest (IDAH), where two laboratories BSL3 are functional. In Medical Center of Military Scientific Research in Bucharest (MCMSR) there is a highly secure microbiological laboratory, but is still under construction.

Table 1. Laboratory diagnosis schema for Ebola, according CDC

Timeline of Infection	Diagnostic tests available
Within a few days after symptoms begin	Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing IgM ELISA Polymerase chain reaction (PCR) Virus isolation
Later in disease course or after recovery	IgM and IgG antibodies
Retrospectively in deceased patients	Immunohistochemistry testing PCR Virus isolation

TREATMENT

The European Medicines Agency confirms that there is no specific treatment for Ebola Hemorrhagic Fever (www.ema.europa.eu). When cases of infection with Ebola virus occur, the sole treatment is the supportive one.

Patients are usually dehydrated and, therefore, require administration of fluids intravenous or oral with solutions that are composed of electrolytes (www.who.int).

Effective antiviral compounds do not exist yet and ribavirin has no effect on the Ebola virus. Administration of interferon has neither given satisfactory results nor in experimental treatments of disease neither in monkeys nor in patients.

Some promising results were obtained when 3-deazadenosine carbo-cyclic was used to treat experimental infection with lethal doses of Ebola virus in mice: survival rates up to 90% when treatment was started after 2 days from the infestation, and 40% after 3 days (1) .

PROPHILAXY

Since there is no clear data on the reservoir of infection, we are not able to speak about a classical approach for infections control. It may take measures of putting animals under quarantine at most, and in case of death of those animals or illness of carers, with fever over 38.5°C for a period longer than two days, it could be considered as a suspected Ebola virus infection and, accordingly, action would be taken.

Patients will be kept in strict isolation, possibly with bed isolation devices. All materials, objects, tools etc. which came in contact with the patient should be transported in a separate facility and sterilized. Health care personnel will be equipped with protective clothing (possibly sealed, with positive pressure), masks, gloves, shoes. It is desirable to prevent contamination by the aerosol, the air is recommended to be filtered through HEPA type filters (1). Patients' transportation is recommended to be made by sealed stretchers equipped with filtered ventilation system (www.karcher-futuretech.com), like that one presented in Figure 2.

Figure 2. Transportation system provided with patient decontamination facility (www.karcher-futuretech.com)

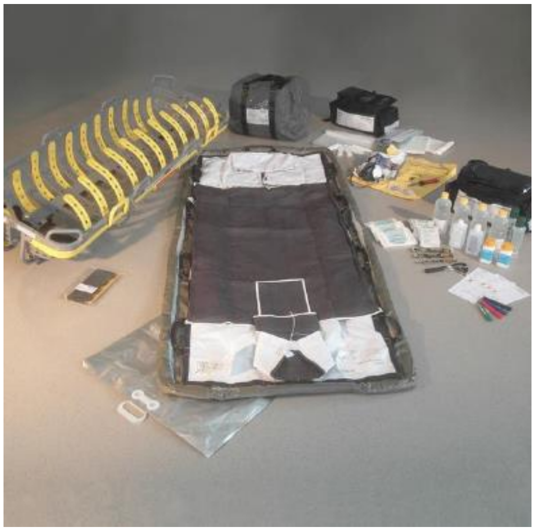

Vaccination

No approved vaccine is currently against Ebola virus currently available on market. However, laboratory tests are promising and their results are being evaluated (www.who.int). For example, a team of researchers from Arizona, is studying a vaccine that protects mice against infection with Ebola virus.

This vaccine includes a surface glycoprotein of the virus, combined with a monoclonal antibody and a chemical substance which activates biochemical cascade involved in natural immunity. More than 80% of the mice receiving this treatment, survived after administration of high doses of Ebola virus (5).

RNA experimental therapy has revealed satisfactory results in experiments on monkeys, researchers at Boston University School of Medicine suggesting that this therapy could induce protection in humans, providing hope for the future (3).

COMMENTS

According with World Health Organisation, the last Ebola Situation Report, from 11 February 2015 presents the following: “There have been almost 23 000 reported confirmed, probable, and suspected cases of EVD in Guinea, Liberia and Sierra Leone, with almost 9000 reported deaths (outcomes for many cases are unknown).

A total of 65 new confirmed cases were reported in Guinea, 3 in Liberia, and 76 in Sierra Leone in the 7 days to 8 February.

Six countries (Mali, Nigeria, Senegal, Spain, the United Kingdom and the United States of America) have reported a case or cases imported from a country with widespread and intense transmission.

The introduction of a case into unaffected countries remains a risk for as long as cases are reported in any country. With adequate levels of preparation, however, such introductions of the disease can be contained with a rapid and adequate response.

The response to the EVD epidemic has now moved to a second phase, as the focus shifts from slowing transmission to ending the epidemic. To achieve this goal as quickly as possible, efforts have moved from rapidly building infrastructure to ensuring that capacity for case finding, case management, safe burials, and community engagement is used as effectively as possible.”

CONCLUSIONS

Origin and virus reservoir remain unknown and have to be pursued by:

- development of new systems for early diagnosis of Ebola virus infection, which could help epidemiological investigations;
- monitoring of suspicious areas in order to determine the incidence of the disease;
- acquiring extensive knowledge on natural virus reservoir and mode of transmission, which could be useful in preventing epidemics.

Epidemics in Uganda and the Democratic Republic of Congo have been monitored by the World Health Organization (WHO) and were limited.

Currently, they are trying to limit the epidemic in Guinea, which is under observation WHO.

It is necessary to establish anti-epidemic measures in relation to the affected countries to prevent a possible import of Ebola virus. It is highly recommended to avoid travelling to epidemic areas.

Furthermore, this epidemic should be a lesson -

learned for the upcoming epidemics.

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CLINICAL PRACTICE

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Gallbladder strangulation within a recurrent incisional hernia: An unique cause of acute cholecystitis

A. Popențiu[1], D. Moga[1], C. Weber-Lauer[2], C. Nieman[3], D. S. Kauvar[4], D. Sabău[5]

Abstract: *We present a report of a unique surgical entity: acute cholecystitis caused by the gallbladder strangulation within a recurrent right upper quadrant incisional hernia. A previously healthy 39 year-old male patient presented to our Emergency Department with abdominal pain, nausea, vomiting, and a tender mass in the right upper quadrant of the abdomen, where an incisional hernia was palpable. He had a history of a gunshot wound to the right upper quadrant and multiple operations to include repairing mesh of the incisional hernia in that area. Abdominal computed tomography demonstrated an acutely inflamed gallbladder within the recurrent hernia. Open cholecystectomy and primary hernia repair were performed. Intraoperatively, the gallbladder appeared being strangled within the hernia. The patient recovered uneventfully and no cholelithiasis was observed on gross examination of the gallbladder. Though there have been a few reports of gallbladder strangulation within primary incisional hernia, ours is the first to describe this phenomenon in a recurrent hernia.*

INTRODUCTION

Neither incisional hernia nor acute cholecystitis is uncommonly encountered in surgical practice. While the presence of an abdominal wall hernia in the right upper quadrant (RUQ) might be complicated by the presence of a herniated gallbladder, strangulation of the gallbladder within an incisional hernia is an exceedingly rare cause of cholecystitis, with only a handful of cases reported in the literature. We are presenting such a case, the first reported with a recurrent incisional hernia as the cause of cholecystitis.

The presence of an incisional hernia in the RUQ might be complicated by the presence of the herniated gallbladder. This situation is facilitated by anatomic variations of the gallbladder, such as the presence of a complete gallbladder mesentery, and also by the location and the size of the abdominal wall defect.

The acute cholecystitis of a herniated gallbladder can be triggered by the presence of the stones (obstructive mechanism), or by strangulation or torsion.

CASE REPORT

A previously healthy 39 year-old male with a history of a gunshot wound to the RUQ with injury to the colon, six years prior to presentation, had undergone

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multiple abdominal operations concluding with mesh repair of midline and RUQ incisional hernia, two years prior to presentation to our Emergency Department, complaining of a 48-hour history of severe and unremitting RUQ abdominal pain, nausea, and vomiting. The patient reported no changes in his bowel habits, melena, or hematochezia.

Physical examination revealed a 5cm tender mass in the RUQ, underlying a 12cm subcostal incision through which a recurrent incisional hernia was palpable. The mass was clearly within the hernia and was irreducible. The patient reported that the mass had been present and slowly enlarging for about eighteen months. The patient's other abdominal incisions in the midline and the right lower quadrant were well-healed and without indication of hernia. Vital signs and laboratory evaluation were normal with the exception of a mild leukocytosis (11.6 x 1000/mm^3).

The abdominal CT scan demonstrated the fundus of the distended, inflamed gallbladder herniated into a 8 cm. AP / 10 cm. CC fascial defect, distended to 10 cm. long. The gallbladder wall is thickened to 4 mm. and there is hyperenhancing mucosa and extensive pericholecystic inflammation. No gallstones are seen. There was no dilatation of billiary duct. The muscles of the abdominal wall are atrophied, and the right incisional hernia has been previously closed with mesh. An injury of the caecum or the ascending colon was presumed as a result of previous abdominal gunshot wound. A previous diverting ileostomy was closed, but the loop of terminal ileum remains adhered to the right abdominal wall.

Into OR, a right subcostal incision was performed through the previous incision. The gallbladder fundus wall, edematous and under tension, was exposed in the subcutaneous fat, under the previously placed prosthetic mesh. The hernia defect was exposed under the mesh, with a hernia sac filled with the gallbladder fundus between the muscle and the inferior aspect of the prosthesis. After opening of the abdominal wall, the distended gallbladder was exposed with the lysis of multiple cholecysto-enteral and cholecysto-epiploic adhesions. Cholecystectomy was performed in a top-down fashion, with drainage of the sub-hepatic space. The existing mesh was not explanted and we closed the abdomen primarily, suturing muscle to muscle and mesh to mesh where appropriate.

Figure1. Postcontrast CT. Distended and inflamed gallbladder herniated. The gallbladder wall is thickened to 4 mm. and there is hyperenhancing mucosa and extensive pericholecystic inflammation.

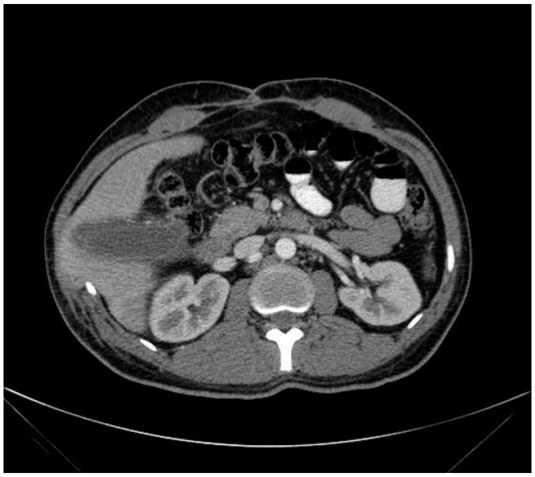

Figure 2. No gallstones are seen. There is no billiary duct dilatation. The muscles of the abdominal wall are atrophied, and the right incisional hernia has been previously closed with mesh.

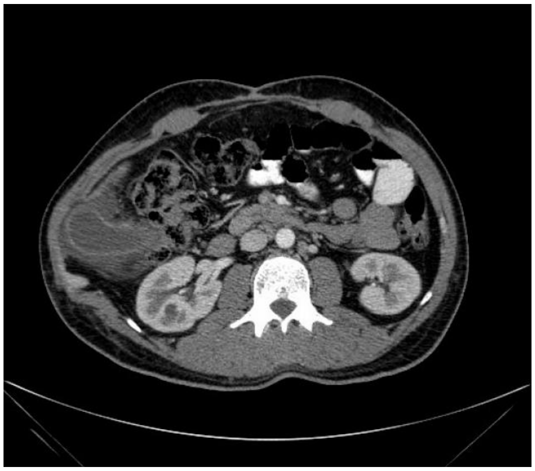

DISCUSSION

The incidence of incisional hernia is between 2% for clean, elective surgeries, and 20% for contaminated cases (Hodgson, Hoer).

Suture repair of incisional hernia results in recurrence rates of 12% to 54% (Anthony, Luijdenijk), while mesh repair is followed by a rate of 2% to 36% (Korenkov, Mc Lanahan)

Gunshot wounds, considering the high velocity of projectiles, is the most common cause (64%) of penetrating abdominal trauma, followed by stab wounds (31%) and shotgun blast wounds (5%). The injury patterns differ, depending on the weapon. Low-velocity stab wounds are, generally, less destructive and have a lower degree of morbidity and mortality than gunshot wounds and shotgun blasts. Gunshot wounds and other projectiles have a higher degree of energy and produce fragmentation and cavitation, resulting in greater morbidity.

The most common morbidities following penetrating abdominal trauma are wound infection (2- 8%) and intra-abdominal abscess with or without sepsis (10-80%, depending on presence or absence of bowel injury in combination with major vascular injury). The wound infection, together with the extensive destruction of the abdominal wall, leaded in our case to the recurrence of the incisional hernia.

The presence of an incisional hernia in the RUQ might be complicated by the presence of the herniated gallbladder. In order for the gallbladder to herniate through the defect, it needs to have some particular anatomic predisposing factors, same factors that led to gallbladder volvulus: the presence of a gallbladder mesentery, long longitudinal axis. Both of these feature were present in our case.

The acute cholecystitis of a herniated gallbladder can be triggered by the presence of the stones (obstructive mechanism), or by the strangulation or torsion. Since there was no obstruction, and the intraoperative findings demonstrated the herniated gallbladder through the abdominal wall hernia, we can conclude that the mechanism involved in our case was the strangulation.

There were only two previous cases reported in the literature to describe a gallbladder strangulation within primary incisional hernia (Benzoni, Herich). This is the first case ever reported involving a recurrent incisional hernia. The clinical examination offered enough data to suggest the diagnosis, but the CT scan and the supplementary US examination confirmed and documented the findings.

CONCLUSIONS

The GSW of the abdominal wall have a great risk of developing incisional hernias, and the recurrence rate after the repair, even with mesh, is significantly high. In the rare event of an incisional hernia in the RUQ, and a gallbladder with long mesentery, the strangulation within the hernia sac hernia is possible.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Competing interests. The authors declare that they have no competing interests.

Authors' contribution. A. Popențiu, D. Moga, C. Weber-Lauer, C. Nieman conceived the study, carried out a detailed literature review, collected and presented the pertinent data. D. S. Kauvar and D. Sabău participated in the study design and coordination and helped to draft the final manuscript. All authors read and approved the final manuscript.

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CLINICAL PRACTICE

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The surgical treatment of decubitus lesions

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Abstract: *Several methods have been exercised during the past years, thus offering the surgeon the possibility to choose when planning reconstructive surgery. Among these methods, we recall: the dislocation of a large surface of skin and fasciocutaneous flaps, the transposition of the muscle and its covering with a simple cutaneous flap or a cutaneo-adipose flap, musculocutaneous pediculate flaps, neurovascular flaps.*

The study was conducted on 292 patients with 3rd and 4th degree decubitus lesions admitted into and operated at the Plastic Surgery and Reconstructive Microsurgery ward during 10 years. Pediculated flaps on septofasciocutaneous perforating arteries or on musculocutaneous perforating arteries are more difficult to harvest (they require a precise knowledge about the localization of the perforating artery) but provide a better post-op result.

INTRODUCTION

The surgical approach of decubitus lesions has changed greatly during the last decade. Some methods were used predominantly at the beginning of the last century, other just in the past ten years. Anyhow, a classical method can be used at any time, and a modern method may be too much for a patient at a certain moment during his/her evolution. As such, depending on the therapeutic prescription, methods may be interposed.

Several methods have been exercised during the past years, thus offering the surgeon the possibility to choose when planning reconstructive surgery. Among these methods, we recall: the dislocation of a large surface of skin and fasciocutaneous flaps, the transposition of the muscle and its covering with a simple cutaneous flap or a cutaneo-adipose flap, musculocutaneous pediculate flaps, neurovascular flaps.

All these are selective methods for the treatment of complications arising from previous surgical interventions.

Tobin and Brown recommend the following for the treatment of complicated decubitus lesions:

- For good quality tissue but with a large ulceration (more than 10 cm), they recommend tissue dislocation, free flap or pediculate flap:
- For afflicted tissue with a large ulceration (more than 10 cm), they recommend flaps on perforating arteries or pediculate flaps.

We should give some examples of a few closure methods for defects of soft tissue in areas where usually decubitus lesions appear.

PREREQUISITES OF THE STUDY

In complementing the previous study, we turned to compare two surgical treatment methods for

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decubitus lesions. One of these methods consisted in applying flaps on random circulation, and the other one in harvesting and applying flaps that contain a vascular network capable of ensuring its survival, developed from the perforating artery or arteries at the level of the muscle mass directly towards the tegument (musculocutaneous) or from the level of the muscle mass towards the tegument via the fasciae or the septal formations (128). These perforating arteries may be anatomized or dissected along with a portion of the muscle wherefrom they emerge or along with the septum or fascia fragment.

OBJECTIVES

Starting from data from the literature, we strived to demonstrate that the number of surgical reinterventions (immediate relapse) in the case of the batch submitted to the surgical treatment with flaps on perforating arteries is more reduced than for the batch of patients submitted to surgical treatment with flaps on random arteries or free skin grafts.

MATERIAL AND METHODS

The study was conducted on 292 patients with 3rd and 4th degree decubitus lesions admitted into and operated at the Plastic Surgery and Reconstructive Microsurgery ward during 10 years.

The age distribution of patients reveals a maximum incidence of bed-sores between the ages of 25 to 45; 135 cases (figure 1), predominantly for males.

Half of the cases included in the study (146 cases) presented lesions localized at the level of the sacral region. The greatest number of cases, both with trochanteric localization as well as with sacral localization, was recorded in 2004.

Figure 1. Graphical representation for the distribution of the number of decubitus lesions depending on their localization

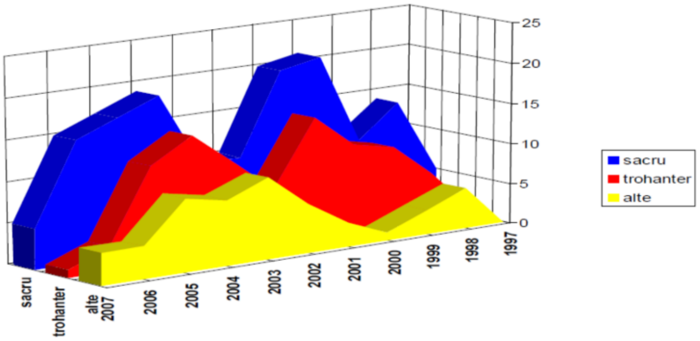

Group no. 1: tegument flaps on fasciocutaneous or musculocutaneous perforating arteries.

Patient selection criteria:
98 patients who were submitted to surgical treatment with tegument flaps on fasciocutaneous or musculocutaneous perforating arteries;

The candidates for surgical interventions are patients for whom conservative treatment and chemical or physical debridement was performed with the same methods: lavage with Betadine solution and mechanical debridement using the scalpel.

Exclusion criteria:
Patients whose biological status did not allow for the execution of the surgical intervention;
Patients diagnosed with neoplasm.

Group no. 2: tegument flaps on random circulation or skin graft.

192 patients with 3rd and 4th degree decubitus lesions who were submitted to surgical treatment with a prescription for tegument flaps on random circulation or free skin graft.

The work method used within the batch with the application of flaps on perforating arteries

The distinctiveness of this work method for this batch consisted in the specific harvesting of flaps with the anatomization of one or two perforating arteries. These were identified using the Doppler method thereafter the tegument flap was designed based on the localization of the perforating artery and on the surface of the defect remaining after the excision of detritus from the decubitus lesion.

In the case of septofasciocutaneous perforating arteries, the tegument flap or cutaneous-adipose flap was harvested with a portion of the septum or fascia that the perforating artery crossed (figure 2).

Figure 2. Musculocutaneous perforating arteries depending on their localization

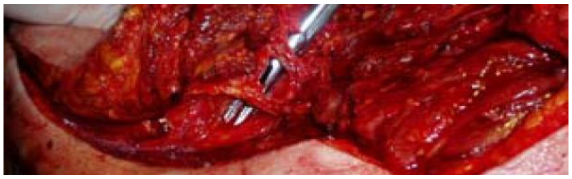

CONCLUSIONS

Although the vascularization of the areas with the highest frequency of development for decubitus lesions presents right networks of anastomosis, results indicate that the number of surgical reinterventions is higher for the batch submitted to treatment with flaps on random circulation.

Pediculated flaps on septofasciocutaneous perforating arteries or on musculocutaneous perforating arteries are more difficult to harvest (they require a precise knowledge about the localization of the perforating artery) but provide a better post-op result. This is owed to the fact that they have a better vascularization which contributes to the draining of the infection remaining at the level of the apparently healthy tissue, as well as in the formation of fibrous scar tissue through the coalescence of the wound.

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Communication barriers in therapist – patient – caregiver relationship

Iuliana Guiță – Alexandru[1]

Abstract: *The success or failure of a therapeutic approach, medical or psychological, are influenced by many factors. Among them we can mention the patient's insufficient knowledge about the disease, preconceptions of patient or caregiver's fear of stigmatization, the issue regarding duration and side effects of treatment, cost of medicines and so on. Last but not least, the therapist's ability to communicate accordingly to the beneficiaries' comprehension and barriers encountered in the communication between actors directly involved in the therapeutic process (patient caregiver, psychologist, and doctor) can change the opportunity to obtain a suitable therapeutic benefit yet from beginning.*

Materials and methods: In order to assess the concordance between the messages sent by doctors and how they are received by patients and caregivers, we have applied a different questionnaire for the three categories of respondents mentioned above. These three distinct sets of questions were completed during 100 psychiatric consultations, in ambulatory regimen.

Results: Comparative analysis of the questionnaire results has shown major differences between the information that doctors thought they had sent and what was actually received by patients and caregivers. Paradoxically, the more medical explanations were elaborated and detailed, the less volume of adequate information was taken home by beneficiaries.

Conclusions: It is necessary for each of us to have a self-assessment of how we communicate with patients and which is the real benefit that we offer through our words. It is also mandatory to adapt the „language of medicine” to the common people understanding abilities (without medical training).

Keywords: *communication, relationship therapy, case management, psycho-education, occupational health, therapeutic benefit, therapeutic alliance.*

ADHERENCE TO TREATMENT

1 of 2 patients does not follow the therapeutic/ psychotherapeutic prescribed program.

Theoretically, the psychotherapeutic process should be simple, direct and fast, so that the service beneficiaries to have access as early as possible to a specialized consultation, after which to establish an optimal therapeutic conduct in order to provide significant improvement or even cure the health issues.

Everyone involved in this process of health (re) generating are convinced that everything is impeccable in respect of themselves: conforming to Oath of Hippocrates, the physician ensures that “... as long as my strength and my reason will help me, my prescriptions will be made only against sickness and for the benefit and good condition of the sick people" and provides a correct diagnostic (psycho-diagnosis) and an appropriate treatment, the patients and their relatives understand the information provided about the disease and the importance of following a correct conduct, the patient strictly follows the treatment schema and returns to periodical control and psychological advising, ideally considering that the psychologist and the psychiatrist complement each other no matter the case case.

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Comparative assessment of the amount of prescription drugs in medical offices and pharmacies obtained (EFPIA - European Federation of Pharmaceutical Industries and Associations) shows that only 50% of prescriptions are being released from pharmacies. In Europe, the non-adherence to treatment "costs" annually state budgets for health about 125 billion euros and contributes to 200,000 premature deaths recorded per year.

Poor therapeutic adherence can have an immediate negative impact on the clinical benefits of treatment (impact felt by the doctor, the patient and the patient's relatives) and also a negative social echo, more extensive on medium and long term because of sub-optimal health problems solving, lack of quality remission, incomplete recovery or when the certain diseases become chronic.

To improve this alarming situation that 1 of 2 patients simply do not succeed in adhering to the prescribed treatment, increasing collaboration among all involved factors in the therapeutic process is more than evident. Especially, the interaction between health professionals and patients should be improved in order to ensure not only to diagnose and treat properly, but also that the therapeutic recommendations are eventually complied with. Most often the subject „passes over" the psychological counseling, which is absolutely necessary because, during the specialized clinical interview, helpful elements are revealed in order to achieve a correct approach.

TYPES AND CAUSES OF NON-ADHERENCE

The problem of non-adherence to intervention of any kind is not new. Hippocrates (460-370 BC), the most famous physician of ancient Greece considered to be the "father of medicine", recommend that the physicians "to be aware of the reasons why patients tend to lie about how they take the prescribed medicines". More recently, Charles Everett Koop (1916 - 2013), vice-admiral of the US Public Health Service Commissioned Corps, chief-physician of the US from 1982 to 1989, summarized this problem in the dictum "Medicines do not work in patients who do not take them!"

Sokol et al. (2005) and Ho et al. (2009) have mentioned two types of non-adherence to the intervention, everyone having a different point of view regarding the patient.

The first way of seeing the patient presents a patient "guilty" of unwillingness, uninterested in his health, lazy, disobedient, who simply does not make a minimal effort in taking prescribed medications.

The second pattern highlights the non-adherence caused by a rational, intentional response of the patient facing with some disagreed recommendations. In this situation the patient, who, after leaving the cabinet or the hospital, never completes the recipe, raises medicines from the pharmacy or takes these, even if purchased on his behalf by someone else.

The fact that the patient does not comply with treatment recommendations, usually generates revolt and frustration for therapist. Basically, the therapist believes that he consumes his time, energy and skills to act in the interest of a person who seems not to do the same for himself. However, in real life, the events do not evolve so simple and linear as we have described in the earlier theoretical model.

The therapist (clinical psychologist) has not always have the necessary time, patience, understanding, empathy or absolutely necessary communication skills for a discussion with beneficiaries. It happens sometimes that the patient does not get to have a face-to-face contact with the therapist, so a conversation about diagnosis, disease severity or efficacy and safety of treatment is even less probable. Studies have shown that 50% of patients do not agree with the posed diagnosis or recommended treatment and the lack of proper communication with mental health professionals, reach either at self-psycho-diagnosis or even at self-treatment, either to seek pseudo-medical aid (provided by relatives, friends or internet). That issue touch a relatively new domain in Romania, namely the concept of Occupational Health obviously correlated with the education of population in that domain. Occupational health psychology has earned a special place in psychological theory and practice, being related to health psychology and derived from organizational psychology. The objective of this sub-domain of psychology is to study healthy work environments, defined as those in which individuals, using their skills and talents, achieve high performance, high satisfaction and psychological well-being.

This context in which the pro-intervention information is not consistent enough to counter the avalanche of counter-intervention arguments (generated by fear of disease or treatment, feeling of shame or dysfunctional emotions, financial reasons, fear of stigma, preconceived ideas and so on), pops up an issue: patients do not come to specialist!

COMMUNICATION – AN ESSENTIAL FACTOR IN THERAPEUTIC SUCCESS

In nowadays medical world when the professional activity is exercised more and more along the lines of “poor funding - lack of time - Increased request” is harder to remember that we are treating people, but not medical cases. The image of therapist today is different of patient expectations: instead of a benevolent advisory actor, a counselor of the patient concerning what is good or bad for him, the pattern of common doctor is rather of an exhausted and annoyed person, in a permanent rush between ambulatory cabinet and inmate patients in the ward and patients in the emergency room.

In the preface of his book “A Curious Calling: Unconscious Motivations for Practicing Psychotherapy” (M. Sussman - Ed. Three, 2011), the author says that “narcissistic and exaggerated aspirations as well as overestimation seem to be almost universal among the beginner therapists (Sharaf and Levinson, 1964). What are their aspirations? Maltsberger and Buie (1974) believe that the three most common traps are narcissistic aspirations to cure everyone, to know everything and to love everyone - all doomed to failure, leading therapists to magical and destructive answers.” Thus, the lack of communication with the beneficiary can conduct to a slightly defensive behavior of the therapist meant to build a protective shield against questions, indecision or doubts related to his clinical approach, coming from the patient or his caregivers.

O. Sacks, neurologist and psychiatrist, in his book “A Leg to Stand On” (Humanitas Ed., 2013) describes the personal experience of a doctor who, after an accident, pass to the other side of “the fence” and becomes a patient. On this occasion is analyzed, initially with surprise, then with consternation and anger, finally with comprehension, the distant and professional attitude of the doctor which is in conflict with almost desperate need for information and ensuring of the patient.

LIMITS OF COMMUNICATION WITH THE PATIENT (BENEFICIARY) AND THE CAREGIVERS

In accordance with current legal provisions, the duration of medical consultation should be limited to a maximum of 15, 20 or 30 minutes, depending on the specialty. In that time the specialist, psychologist or psychiatrist, should receive the patient, introduce himself, find out the reasons of the visit, obtain as complete history of the problem, listen carefully to establish one or more diagnoses, debate an intervention scheme with the patient and caregivers, provide information about intervention (benefits, side effects, duration, risks, warnings, others) and regimen, register data in registry and computer, release documents as appropriate (electronic prescriptions, treatment regimens, medical letters, sick leave etc.).

In this context, verbal communication is strictly reduced and, instead of empathy, ensuring and benevolent neutrality, a non-verbal recommendation appears: “you have to do as I say, because I know better what disease you have and what treatment do you need"!

The patient perceives such behavior as mandatory and discretionary, which further increase his previous resistance in coming to specialist as a defense mechanism against unpleasant feelings.

The problem of time allocated to the communication with patient is widely considered worldwide.

At a minimum, the patient should be informed, has to understand and must accept that:
1. He has a problem;
2. The problem may present health risks if not treated;
3. The recommended treatment, if necessary, is appropriate, safe and effective.

Prelipceanu et al. (2011) has suggested that all patients visiting a psychiatrist should be informed in detail about the therapeutic effects and side effects of any pharmacological agent that they receive. From the psychodynamic perspective, the notions of transfer, counter-transference, resistance and therapeutic alliance are equally important when prescribing medication as when psychotherapy is performed. A positive transfer and a therapeutic alliance could have a positive influence on adherence to treatment. The benefits of combining psychotherapy with drug therapy are supported by numerous studies and consist in improving compliance and ensuring a complex care of the patient, more efficient.

A DETAILED COMMUNICATION IS NECESSARILY BETTER?

In a symposium held at the National Conference of Psychiatry in 2013 (Targu Mures) Michael Davidson, professor of psychiatry at the Sackler School of Medicine Tel Aviv University and Mount Sinai School of Medicine NY, launched a provoking debate on consistency between messages that the therapist believes them transmitted to the patient and what the patient actually received.

The subject is too large to be analyzed in depth without an enlarged study, well structured, multi-centered and extremely well balanced between the various medical specialties. However, to evaluate the messages sent by therapists and how they are perceived by patients and caregivers, we have designed and implemented a simple questionnaire with similar questions for the three categories of respondents. The three distinct sets of questions were completed for 50 psychiatric or psychological counseling sessions (both initial consults as well as control) under specialty ambulatory (each lasting 30 minutes).

Subjects and their caregivers were randomly selected, with the only conditions of being sure they can understand the questionnaire requirements and they can freely provide required answers. The questionnaire included both open-response questions and closed-answer questions, as follows:
1. Please note, with a note between 0 to 10, the allocated time for information about the diagnosis and treatment (0 = totally insufficient; 10 = extremely high);
2. Please note, with a note between 0 and 10, availability of necessary information provided to patient/received from psychologist/psychiatrist (0 = very difficult to understand; 10 = very easy to assimilate);
3. Please note, with a note between 0 and 10, specialized information relevance provided to patient/received by the psychologist/psychiatrist (0 = completely irrelevant; 10 = highly relevant);
4. Please indicate the most important three (3) peaces of information that you have transmitted to patient/you have received from specialist, related to diagnosis and/or to received treatment;
5. Please indicate what other information you wish you had transmitted to patient/you wish you had received from the therapist, related to the diagnosis and/or to the received treatment.

For the questions from 1 to 3 differences between the scores given by the therapist and those of recipients were not significant: an average score of self-assessment of therapists 8.4 versus 7.8 of the patients and 8.0 of caregivers. Given the open nature of the questionnaire and respondents awareness that the evaluation will take place, it is expected that health professionals manifest a little more than usual the interest to communicate and the politeness of beneficiaries may have result in sparing pride of the therapist.

Surprises however occurred at comparative analysis of open responses, which showed significant differences between what information therapist thought he had sent and what was actually received by the patients and caregivers.

Frequently encountered types of answers to the question referring to messages sent, in descending order of frequency of occurrence, were related to questionnaire.

They may say that if specialist considered important messages related to objective reasons to be followed for effective treatment, patients and caregivers were more interested in the subjective aspects of the disease and treatment.

Questionnaire:

Therapist	Patient	Caregiver
Information transmitted to the patient/caregiver by therapist		
1. risks of untreated disease	1. disease severity	1. side effects of treatment
2. efficacy of the treatment	2. risks of untreated disease	2. risks of untreated disease
3. disease severity	3. side effects of treatment	3. efficacy of the treatment
Information that would have been transmitted		
	• causes of disease • duration of treatment • impact on family life, professional, social • risk of transmission to others	

For me, the need of some additional information regarding these differences between disease and intervention is directly and indirectly involving: the psychologist/psychiatrist believes that said everything there is to say, while the beneficiaries want to know what triggered the disease, how long therapeutic approach must be followed and what to do once he is at home.

Another aspect worth to be mentioned is that, paradoxically, the more space for the therapist was filled with information complete and complex, the less was completed by beneficiaries. In other words, the more explanations were elaborated and over-detailed, the less information beneficiaries took home.

DISCUSSIONS

The quality of “public employee” of health professionals brings not only the attribute of service provider, but also a bit of self-protective and annoyed attitude of one who does not want to be excessively strained with others problems. So, if I do my job well in the profession what I learned and I perform it with so many sacrifices, the patient is obliged to follow my decisions without comment. If he does not, then you are fully entitled to revolt, refusing other consults and even being a little more incisive when he will ask for help.

From another point of view, the patient knows that he depends on the help of the therapist, the skill and his dedication for the work, but is scared that the disease is his (not of the therapist), with major implications on his life and intimates and approach to be followed is his and not of someone else (good or bad, well tolerated or not accessible or very expensive). And all these worries are multiplied by stories published by media with doctors not very helpful or not very competent.

Between these two points of view a binder, a solid bridge must be found. This may be the communication between all involved factors, most reliable basis for the concept of “therapeutic alliance”.

In conclusion, it is necessary, for each of us, an objective and rigorous self-evaluation of how we communicate with patients and of the real benefit we offer by words.

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EDITORIAL

Ethical principles of health care in emergencies

Dragoș Cuzino

Colonel DRAGOȘ CUZINO

Commander of the Institute of Military Medicine, Ministry of National Defence, Romania Lecturer, Department of Radiology, Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Civilian and military health-care organizations share the common goal of improving the safety of their personnel and other health assets and the delivery of impartial and efficient health care in armed conflicts and other emergencies,

Referring to the principles of humanity, whereby human suffering shall be prevented and alleviated wherever it may be found and impartiality, whereby health care shall be provided with no discrimination;

Bearing in mind the standards of international humanitarian law, in particular the 1949 Geneva Conventions and their 1977 Additional Protocols, and of international human rights law, specifically the Universal Declaration of Human Rights (1948) and the International Covenants on Civil and Political Rights and on Economic, Social and Cultural Rights (1966);

Considering the principles of professional ethics adopted by health-care professional associations, including the WMA Regulations in Times of Armed Conflict and Other Situations of Violence;

Endorse the following ethical principles of health care:

General principles

1. Ethical principles of health care do not change in times of armed conflict and other emergencies and are the same as the ethical principles of health care in times of peace.

2. Health-care personnel shall at all times act in accordance with relevant international and national law, ethical principles of health care and their conscience. In providing the best available care, they shall take into consideration the equitable use of resources.

3. The primary task of health-care personnel is to preserve human physical and mental health and to alleviate suffering. They shall provide the necessary care with humanity, while respecting the dignity of the person concerned, with no discrimination of any kind, whether in times of peace or of armed conflict or other emergencies.

4. Privileges and facilities afforded to health-care personnel in times of armed conflict and other emergencies are never to be used for purposes other than for health-care needs.

5. No matter what arguments may be put forward, health-care personnel never accept acts of torture or any other form of cruel, inhuman or degrading treatment under any circumstances, including armed conflict or other emergencies. They must never be present at and may never take part in such acts.

Relations with patients

6. Health-care personnel act in the best interest of their patients and whenever possible with their explicit consent. If, in performing their professional duties, they have conflicting loyalties, their primary obligation, in terms of their ethical principles, is to their patients.

7. In armed conflict or other emergencies, health-care personnel are required to render immediate

attention and requisite care to the best of their ability. No distinction is made between patients, except in respect of decisions based upon clinical need and available resources.

8. Health-care personnel respect patients' right to confidentiality. It is ethical for health-care personnel to disclose confidential information only with the patient's consent or when there is a real and imminent threat of harm to the patient or to others

9. Health-care personnel make their best efforts to ensure respect for the privacy of the wounded, sick and deceased, including avoiding the use of health care for the wounded and sick, whether civilian or military, for publicity or political purposes.

Protection of health-care personnel

10. Health-care personnel, as well as health-care facilities and medical transports, whether military or civilian, must be respected by all. They are protected while performing their duties and the safest possible working environment shall be provided to them.

11. Safe access by health-care personnel to patients, health-care facilities and equipment shall not be unduly impeded, nor shall patients' access to health-care facilities and health-care personnel be unduly impeded.

12. In fulfilling their duties and where they have the legal right, health-care personnel are identified by internationally recognized symbols such as the Red Cross, Red Crescent or Red Crystal as a visible manifestation of their protection under applicable international law.

13. Health-care personnel shall never be punished for executing their duties in compliance with legal and ethical norms.

Final

14. By endorsing these ethical principles of health care, the signatory organizations commit themselves to work for the promotion and implementation thereof wherever possible, including by appropriate dissemination amongst their members.

Article received on March 12, 2014 and accepted for publishing on May 13 2014.

REVIEW ARTICLE

Bio-terrorism: still interesting or concerning nowadays?

Claudiu E. Sbârcea[1], Lucian Lerescu[1], Mariana Jinga[2,3]

Abstract: *Bio-terrorism involves using biological agents/toxins with the intent to intimidate or coerce a government or civilian population to further political or social objectives, usually leading to deaths or illnesses of humans but also of plants/animals.*

Their use would probably initially be considered as a natural or unintentional event, especially in case of live biological agents. Moreover, a natural occurring disease outbreak could have the same destructive outcome as an efficient biological weapon.

There is a need for proper differentiation between natural and intentional events although in the first stages the medical response should be similar; however, the course of incident management would take different paths later on.

Biological agents' investigation of dangerous pathogens, from natural unusual outbreaks or bio-terrorist attacks/other intentional use, imply the collaboration of different institutions with responsibilities in public health but also in national security and defense. The National Security and the Defense System institutions think mainly in security terms while national health care system institutions think principally` in medical care/prevention terms. These two ways of acting have to be combined in order to deal properly with hazardous biological agents.

Keywords: *bio-terrorism; disease outbreak; biological agents*.

INTRODUCTION

"Bio-terrorism refers to the intentional release of biological agents or toxins for the purpose of harming or killing humans, animals or plants with the intent to intimidate or coerce a government or civilian population to further political or social objectives" as suggested by Interpol. (1)

Deliberate or accidental release of a biological agent, chemical agent, or radio-nuclear materials (CBRN agents), could potentially cause severe harm and pose a huge burden on public health systems (1).

Such a release would probably initially be considered as a natural or unintentional event (2).

From all the CBRN agents, biological agents can cause the widest range of effects, being as dangerous as nuclear weapons and as easily available as industrial/domestic chemical agents (1). Moreover, a natural event such as an emerging disease outbreak could have the same destructive outcome as an efficient biological weapon. But there are some important differences between intentional and unintentional events involving biological agents and these differences raise the main topic discussed

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below.

GENERAL SITUATION

The threat of natural epidemics is always present, no matter the level of awareness of the public health systems, authorities, or populations. A convenient example is of course the 2014 Ebola epidemic: so far it is the largest of its kind in history, affecting multiple countries in West Africa and even producing few secondary cases in USA and EU (Spain).

On February 15, 2015, according with USA Centers for Disease Control and Prevention website, (CDC Atlanta), the overall situation was the following (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html) (accessed on February 19, 2015): Countries with widespread transmission (Guinea, Liberia, Sierra Leone): total cases* – 23218; Laboratory-confirmed cases – 14088; total deaths – 9365. Countries with an initial case or cases and/or localized transmission (United Kingdom): 1 case, Laboratory-confirmed cases – 1; no death. Previously affected countries** (Nigeria, Senegal, Spain, United States, Mali): total cases* – 34; Laboratory confirmed cases – 32; Total deaths – 15. (*Total case counts include probable, suspected, and confirmed cases. **The outbreaks of Ebola virus disease (EVD) in Senegal, Nigeria, Spain, the United States, and Mali have ended. A national EVD outbreak is considered to be over when 42 days, double the 21-day incubation period of the Ebola virus, has elapsed since the last patient in isolation became laboratory negative for EVD.)

In December 2014 and January 2015, some reports suggested that the “Islamic State in Iraq and Syria” (ISIS) terrorist organization, operating in Iraq, would try to use Ebola infected militants as perpetrators, spreading the disease (i.e. by sending volunteers to the afflicted African areas to get infected on purpose, then sending them to Europe or USA to spread the disease). The World Health Organization (WHO) investigation did not confirm this information (http://www.emro.who.int/irq/iraq-news/no-ebola-cases-detected-in-iraq.html). (Accessed on February 19, 2015)

Moreover, let’s not forget that the 1918 flu pandemic – the “Spanish flu” – caused between 50 and 100 million deaths, making it one of the deadliest natural disasters in human history. By comparison, the estimated death toll of First World War ranges “only” between 15 and 35 million deaths and even the deadliest conflict, Second World War ranges between 40 and 72 million deaths.

The more robust a nation’s preparedness, however, the more reduced will be the consequences of any outbreak whether natural or man-made and the likelihood of being attacked. Every state should improve his bio-defense capabilities in order to avoid the development of an incident involving biological agents into a catastrophe. Yet, there is no perfect national bio-defense system, regardless the wealth and power of the country. Cooperation among relevant institutions is crucial in this critical area; especially cooperation between the public health system and the institutions involved in homeland security is needed in order to achieve a better level of preparedness.

However, there is no such thing as a standard, “universal” response algorithm, given the fact that the actual response process depends on a multitude of factors, such as: national institutional specificity (number of institutions involved in response, their responsibilities and competencies, equipment and resources employed etc.), specific national policies in place, specific social-economic issues and so on.

In Romania the relevant legislative framework has been updated according to European and international laws and regulations (under BTWC – Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction and IHR – International Health Regulations).

Also, the responsible institutions have specific procedures in place for different parts of the hazardous agent investigation.

However, up to date, in Romania there is no overall coherent algorithm of response to incidents involving biological agents (detailed and adapted both for known and for potentially unknown or emergent agents) with the subsequent SOPs (Standard Operational Procedures) well synchronized, efficient and agreed by all responsible institutions.

Moreover, for some key components of the response chain of actions there are no updated SOPs, especially in the detection/identification/typing of the hazardous agent component. Therefore, in case of a real incident, the overall response speed will be slowed and important errors might impair the efficacy of the procedures and strategies selected for mitigation/containment of the biological threat. And this, of course, could lead to severe consequences.

Since the beginning of the current Ebola epidemic efforts have been made in Romania for planning, preparedness and developing structures and procedures to be followed for defending, minimizing the consequences and recovering after this outbreak and any other similar (http://goo.gl/nsyOg2).

And for sure, this will result in a higher capacity to deal with an unusual outbreak, being an important and necessary part of the "bio-defense" system.

But is this covering for deliberate induced incidents, outbreaks or epidemics, or for accidental release from research laboratories? We shall investigate if there are some characteristics of the "artificial" outbreaks that result in the need for a different approach of the investigation and reaction compared with natural ones. Although by no means exhaustive, a list of patterns that may give some indication of a deliberate release would include (1;2;3,4):
» sudden increase in patient with similar symptoms/ multiple cases of unexplained disease, syndrome or death, especially if having common work location or activities/"point source outbreak"/multiple cases with similar disease or syndrome presenting around the same time; »» simultaneous outbreaks of similar illness in noncontiguous areas;
» similar typing of agents isolated from temporally or spatially distinct sources;
» disease with an unusual geographic or seasonal distribution;
» recognized illness occurring in an unusual setting within a community;
» single case of disease caused by uncommon agent;
» multiple unusual or unexplained disease entities coexisting in the same patient without other explanation;
» unusual form of infection e.g. inhalational anthrax, pneumonic plague, mediastinitis/recognition of atypical transmission routes e.g. by aerosol, food or water;
» unexpected degree or speed of onset;
» failure of a common disease to respond to usual therapy;
» intelligence of a credible threat;
» heightened terrorist alert level (severe, critical);
» suspected or known deliberate or accidental release in another country;
» illness affecting a key sector of the community/ attenuated morbidity in protected professionals groups;
» illness only among people in proximity to common ventilation systems;
» "downwind plume pattern": cases concentration found downwind from a source point;
» death or illness among animals preceding, accompanying or especially succeeding illness in humans;
» unusual, atypical, genetically engineered, or antiquated (or "extinct") strain of agent, authors' "signature" (known past researches either civil or military);
» diagnosis of a disease generated by an agent from the lists of potential useable in a terrorist attack.

These "indicators" are especially useful when we are dealing with a covert (hidden) attack and the actual disease cases or intelligence gained represents the first "piece of information" of the intended release of a harmful biological agent. In order to be recognized and to trigger a specific chain of action all the actors involved have to be aware of the potential meaning of these indicators: medical staff, public health authorities, law enforcement agencies, and so on.. (1, 2, 3)

When dealing with an overt attack (at least law enforcement and/or other responders are informed about it), the awareness of it occurs earlier than in covert ones but this doesn't mean that it is an easier situation to deal with. A well prepared system can gain most advantage from the time given by knowing from the onset that a bio-terrorist incident is developing. Otherwise, only the early psycho-social effect is different, knowing that the main levels of intended epidemics effects are (2):

» physical – actual diseases;
» psychological – fear, mass panic;
» economic – travel restrictions, business affected;
» environmental – affecting humans, animals, plants.

These effects can be recognized in natural outbreaks as well, but at least the second and the third kind will be far more intense in an intentional (or accidental release) event, if not all. The aim of such an incident management is to partially minimize its' consequences if not to avoid them altogether.

This requires some mechanisms "in place" to defend from the threat and others to detect, investigate and react to it. And the intentional biological agent release needs some specific investigation and solving components. Both the health and the criminal sides of the situation have to be tackled simultaneously (1,3).

The investigation proceeds within the frame of the national legislation on: working/securing/developing/ acquiring/possessing/transferring/transporting biological agents (2). In overt attacks, usually an incident response team has to be deployed, with multidisciplinary approach: first responders,

CBRN experts, medical personnel, risk assessment specialists, decision makers, etc. Good decisions made at this point can lower the consequences significantly (2,3).

In both overt and covert attacks, a chain of actions has to be accomplished by a partnership of institutions mainly from law enforcement and public health systems, with some either common or specific goals: protect health and safety of public/determine cause of bio-terrorism attack/outbreak, stop further crimes/stop further cases of disease and outbreaks, apprehend and convict criminals/build science base for future prevention (1,3).

Sharing information between law enforcement and medical or public health components is crucial both for better timing and for more efficient decisions and actions (1). Some joint investigations and operations are essential for a good coordination between agencies. (3) Main priorities in biological incidents are: preservation of life, safety of personnel involved, investigation (microbiological, epidemiological, criminal etc.), intelligence to prevent further attacks, public reassurance and return to normality (1).

Another special concern in intentional (or accidental) biological agents release incidents is related to genetically modified organisms. These pathogens can pose any imaginable problem from the investigation stage up to the afterward recovery.

From biological agents expressing other species characters and virulence factors up to special "powers" like multi-drug resistance and high resilience in the environment or resistance to different decontamination methods, an GMO (genetically modified organism) can be anything (4,5).

Intentionally released biological agents are most likely to be delivered by aerosols (1). Therefore the most probable syndrome resulted will be a respiratory one, despite the pattern of the usual naturally occurring disease caused by the biological agent. The optimum particle size is 0.3-5.0 µ in diameter, which is small enough to reach the alveoli when it is inhaled (1, 4,5). This can be achieved by aerosol generators mounted in fixed locations or on trucks, cars, or boats as well as from cruise missiles and planes equipped with tanks and spray nozzles (1,2). Numerous climatic factors affect the efficiency of such methods including wind velocity and direction, humidity, degree of cloud protection from direct sunlight, and rainfall (1,4,5,6).

Aerosols could be disseminated by direct delivery into ventilation or air conditioning systems or via letters or parcels (1,2). Suicide attacks would be extremely effective for disseminating diseases (1,2).

Food and water are also suitable vehicles for local delivery of pathogens, especially when the goal of the attacker is more social disturbance and panic than actual killings (1,2,3).

DISCUSSION

The main question is: presuming that facing the threat of 2014 Ebola epidemics the medical community and the public health system will improve their capacity to prevent/investigate/react and recover in natural epidemics, is this enough to reduce to an acceptable level the risk posed by a potential bioterrorist attack?

Hazardous biological agents investigation for rapid detection and identification of novel, emerging and dangerous pathogens, from natural unusual outbreaks or bio-terrorist attacks/other intentional use, imply the collaboration of different institutions with responsibilities in public health but also institutions with responsibilities in national security and defense, especially in the case of "unnatural" events.

Each of these institutions has specific responsibilities and a specific set of procedures for their own part of the action. But until now, in Romania there is no agreed, detailed and acknowledged chain of action.

In every step of the investigation/reaction: on site detection, sampling, transport, laboratory investigation (screening, investigation, in depth analysis), case management, public communication, criminal investigation, epidemiological investigation, public health decision, political and economic decisions, there are several institutions involved, depending on the actual situation and the legal frame (e.g. General Inspectorate for Emergency Situations, Romanian Intelligence Service, the NBC department of Romanian Police, other departments from the Ministry of Internal Affairs, Ministry of Defense – in some special cases).

These response units would be involved in site response, including sampling and transport and in other law enforcement actions and in recovery and return to normality; Ministry of Health, National Institute for Public Health, Local Public Health Authorities, Infectious Disease Hospitals, National Institute for Research and Development for Microbiology and Immunology "Cantacuzino" etc. will be involved in microbiological, epidemiological and clinical investigation, in case management/recovery. Even if the legal frame defines the role and responsibilities of each of these organizations, their action procedures are not properly linked nor their collaboration entirely formalized. Some of the specific SOPs are adapted from international guidelines, some designed to relate to Romanian conditions. Some aspects remain uncovered because not all institutional procedures relate to the others.

On European and international level there are several action plans, recommendations and guidelines in place. The major international organisms (e.g. EU, NATO, etc.) have their algorithms and SOPs. Also this is true for the most important institutions from developed countries which are involved in hazardous biological agents' investigation and response (e.g. Health Protection Agency, UK; "Robert Koch" Institute, Germany; Centers for Disease Control and Prevention, USA, and so on).

These procedures cannot be applied in Romania exactly as they are. Therefore, a thorough assessment of the entire biological agents investigation and response to biological threats process has to be done taking into consideration Romanian specificity and the SOPs have to be optimized accordingly, up to an efficient algorithm usable by relevant national institutions, followed by improving the logistics where needed and within the financial possibilities of Romania.

One of the problems is the difference between the mind settings of the major actors. The National Security and Defense System institutions think mainly in security terms and the National Health Care System institutions (including public health authorities) think mainly in medical care/prevention or usual outbreaks terms. These two ways of understanding/acting have to be combined in order to deal properly with hazardous agents either from unusual natural outbreaks and especially bio-terrorist attacks.

CONCLUSION

Our opinion is that bio-terrorism is (or should be) still a matter of concern in Romania (and elsewhere in EU or worldwide). This should trigger a process of increasing the awareness about intentional outbreaks and epidemics and as a consequence begin to enhance the capacity of medical community and public health system to proper manage the risk of a bio-terrorist attack or an accidental release of an unusual biological agent. And last but not least, the cooperation between institutions involved in health care and those responsible for security and defense should be improved up to the proper level of efficiency.

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SYSTEMATIC REVIEW

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Practical aspects of MRI of the prostate

Dragoș Cuzino[1], Oana M. Baston[1], Cătălin Blaj[1]

Abstract: *The article presents the main aspects of sectional anatomy, lymph nodes and adjacent structures as well as MRI examination standard protocol for prostate cancer diagnosis. Using MRI multiparametric examination we succeed in classifying efficiently the malignant prostatic tumors using PI- RADS system.*

Also, using MRI multiparametric examination we can evaluate the effectiveness of prostate cancer treatment.

Keywords: *multiparametric MRI, prostate cancer, PIRADS, PSA, benign hypertrophic hyperplasia (BPH)*

ANATOMY OF THE PROSTATE GLAND

The prostate gland is the largest male accessory reproductive gland. The prostate gland looks like a flattened cone or inverted pyramid. The base of the prostate is in continuity with the bladder.

It ends at the apex at the urogenital diaphragm. It typically weights between 20-40 grams. The prostate normal size is 3 x 4 x 2cm. The prostate is comprised of 70% glandular tissue and 30% fibromuscular or stromal tissue. The prostate has four zones. The peripheral zone is located under the capsule and formed of glandular tissue. The central zone is deep to the peripheral zone and formed of stroma. The transitional zone surrounds the prostatic urethra.

The periureteral zone is formed of short ducts. The central and transitional zones are found more superiorly within the gland. The gland tapers inferiorly.

The central gland located anterior and superior, is the portion of the prostate most intimate to the urethra. The peripheral gland comprises the posterior and inferior parts of the prostate. It is statistically proved that most of the cancers of the prostate are located in the peripheral gland, but cancer can occur in the central gland also. The cranial part of the gland is called the base and the more caudal portion is called the apex. (1)

Considering prostate a flattened cone, the tip of the cone represents the apex of the gland and is situated more caudally. The flattened portion of the cone is analogous to the base of the prostate and is situated more cranially. In young men, the peripheral zone occupies approximately 70% of the total volume of the prostate, the central part 24% and the transitional zone about 5% but the percentage is changing with age. (2) Using MRI we manage to see the described zonal anatomy with the four zones of the prostate. On CT we can do it only on occasion. (2)

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In T2 we may see the peripheral zone with high signal intensity and the transitional zone with low signal intensity. In T1 we can assess to demonstrate areas of post-biopsy hemorrhage and the status of the neurovascular bundles.

The prostate capsule appears as a continuous dark area around the prostate in T2. The DWI sequence may show restricted diffusion in prostate cancer.

T1 Dynamic contrast/DCE sequence could show enhancement but it is unsepcific because it may be difficult to distinguish from prostatitis or benign prostatic hyperplasia.

Figure 1. Axial T2 TSE high resolution. Peripherical lesion with intermediary irregular zone located inferior and posterior in the left zone.

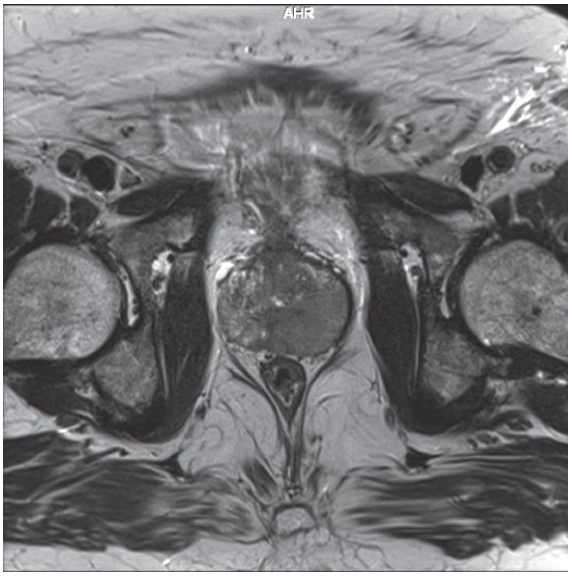

LYMPH NODES

In assessing prostate anatomy it is very important to evaluate the lymphatic drainage. The main lymph nodes are obturator and internal iliac; secondary, the drainage is made into the external iliac, presacral and para-aortic nodes.

The lymphatic spread of the disease in unspecific and only the evaluation of the lymph nodes dimensions is a value indicator. The seminal vesicles appear with low signal intensity in T1 and high homogeneous intensity in T2.

The normal size of the prostate is compared with the one obtained when we measure the dimensions of the prostate, when we estimate the volume by multiplying antero-posterior, tranverse and longitudinal measurements by one another and then multiplying that result by 0,52 which is π/6. The expected prostate volume increases with advancing age and a prostate with a volume greater than 40 ml is considered to be enlarged. The normal volume of the prostate in milliliters is approximately equal to the weight of the prostate in grams. (2)

A normal PSA value is less than 4 and an abnormal value is greater than 10. Values vary between 4 and 10 which are considered boundaries.

Prostatitis, BPH, prostate infarcts and biopsy could cause elevated PSA levels.

Benign prostatic hyperplasia (BPH) usually starts in the transitional zone of the central gland and is more heterogeneous in T2 than the normal gland. When it grows, it can indent the bladder (1). Stromal BPH appears dark on T2 and glandular BPH appears bright. A low signal intensity rim may be seen surrounding these nodules. We see often a combination of these findings in BPH.

The diagnosis of prostate cancer is usually made by biopsy of the prostate which is often accomplished transrectally with ultrasound guidance.

Figure 2. Axial STIR. The zone is located subcapsular posterior and inferior.

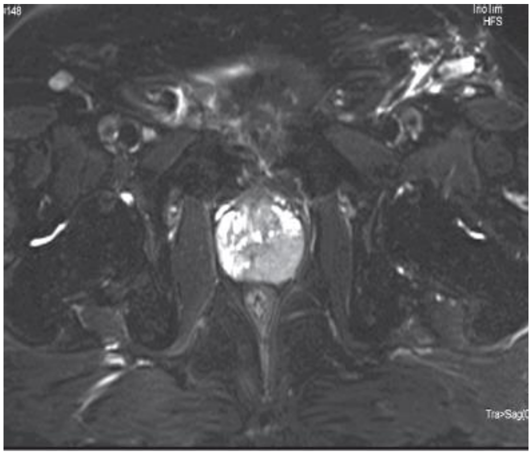

The percentage of the prostate cancer arising within each of the three zones is: peripheral zone 70%, central zone 10% and transitional zone 20%.

Most of prostate cancers are adenocarcinomas which develop from the acini of the prostatic ducts. Pathologic specimens are graded using the Gleason score which is the sum of the most prevalent and second most prevalent types of dysplasia, each on a scale of 1 to 5, with 5 being the most dysplastic.

We also use a TNM staging system for classification of the prostate cancer and also the PI-RADS system (Prostate Imaging Reporting and Data System).

Figure 4. T1 axial FSE post-contrast examination. The lesion has unhomogenous enhancement.

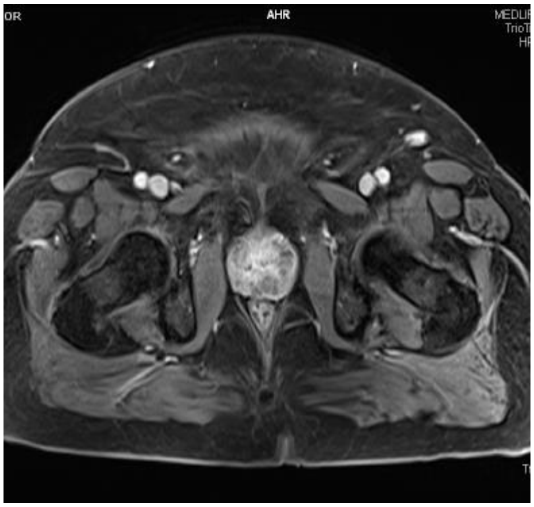

Figure 3. Axial DWI. Area of restriction of diffusion located inferior posterior on the left side.

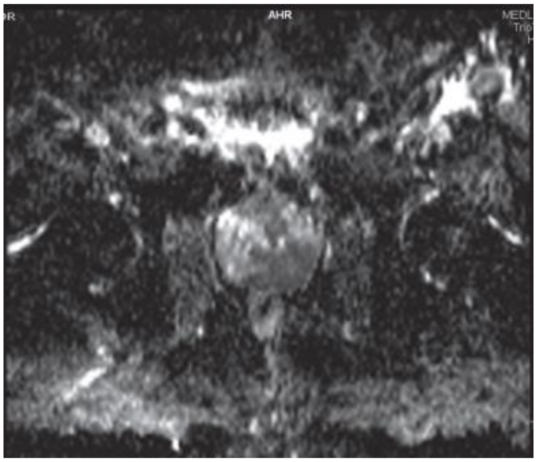

There is no consensus about the use of imaging for evaluating prostate cancer. MRI is the method of choice for local staging, to demonstrate tumor location, extracapsular extension and seminal vesicle invasion. The most efficient method for evaluating prostate tumor and the aggressiveness of the tumor is multiparametric MRI.

Biopsy is essential for the diagnosis. MRI should be performed about 3 weeks after biopsy because at that time about 50% of patients have residual hemorrhage. Hemorrhage after about 3 weeks post biopsy is different from tumour to tumour. T1 hypertintense and T2 hypointense areas correspond to postbiopsy hemorrhage and T1 – izointense and T2 hyperintense areas are more likely to correspond with tumour. There is also disruption of the prostate capsule which may mimic the invasion into the prostatic fat.

Figure 5. Axial T2. Benign prostate hypertrophy and a prominent nodule.

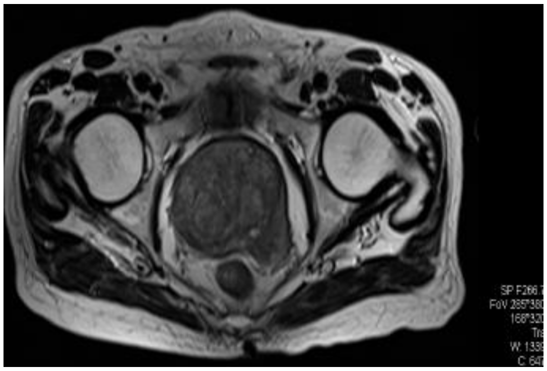

Figure 6. Coronal STIR. Protrusion of the cranial large nodule with extrinsic compression of the urinary vesicle.

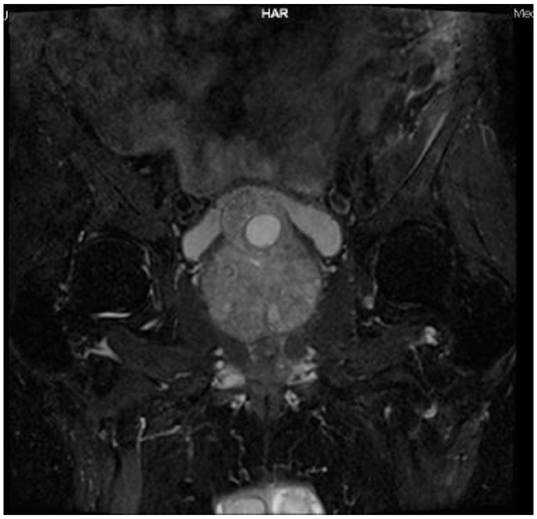

Typically prostate cancer is isointense to gland in T1 and hypointense to the normally high intensity peripheral zone on T2. Central gland malignancy is difficult to be identified because of BPH which always distorts normal anatomy. There are other diseases that may cause low signal intensity areas within the peripheral zone on T2 weighted image like prostatitis, granulomatous disease stromal disease within the peripheral zone and postbiopsy blood or scars.

MRI is very important because it can reveal the real extent of the tumour and can show its spreading beyond the prostate capsule. The tumour can spread into periprostatic fat or neurovascular bundle, ejaculatory ducts, seminal vesicles, bladder, lymph nodes or we can assess the osseous metastatic disease. The capsular penetration occurs most commonly at the prostatic apex (1). We use multiple criteria to detect extracapsular extension of prostate cancer. We estimate the possible irregular bulge in capsule, changes of the rectoprostatic angle, asymmetry of the neurovascular bundle and capsular retraction or local scar. MRI must assess capsular tumour contact and visualize contigous tumour local extension. Seminal vesicular extension has to evaluate direct tumour extension into the seminal vesicles. MRI should evaluate tumour extension in other direction. Contigous spread of the tumour must also be assessed along the ejaculatory ducts.

The MRI semiology consists in seminal vesicles of hypo- intensity in T2WI and non visualisation of the ejaculatory duct. Also, after dynamic T1 evaluation of the contrast enhancement we can predict malignant involvement of the tissue.

The lymphatic drainage of the prostate is made into the internal and external iliac lymph nodes including the obturator nodes (2). Lymph nodes with a diameter greater than 1 cm are suspicious for metastasis disease.

On T2–weighted images with low signal intensity areas within the seminal vesicles and asymmetry in size may indicate tumour invasion. However, blood, changes secondary to radiation therapy and other benign processes may appear as low intensity areas inside the seminal vesicles.

Key Points

Prostatic cancer usually occurs in the peripheral zone. Bone metastases are very unlikely to appear when the PSA is less than 10. The extracapsular spread indicates a nonsurgical treatment of the disease. (1)

MRI is the preferred imaging modality in evaluating prostate cancer and prostate lesions. The aspect of the normal prostate can be described as follows: in T1 the prostate tissue has homogenous intermediate signal intensity; in T2 the anterior fibromuscular stroma is low T1W and T2W signal.

Figure 7. Sagital T2 TSE. Multiple nodules but the prostate is still well delineated.

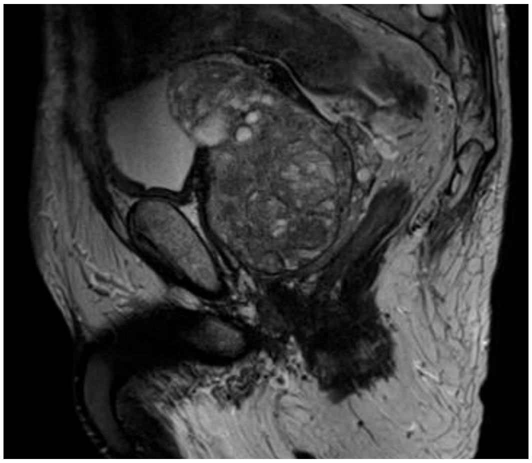

The peripheral zone has high T2W signal, similar to or greater than adjacent fat. The central and transitional zones are lower T2W signals than peripheral zone. The true capsule is a thin rim of low signal intensity. The distal urethra is a low intensity ring in T1 and T2.

Figure 8. T1 TSE axial. Presence of blood at the seminal vescicles (hypersignal corresponding to methemoglobine inside seminal left vesicle)

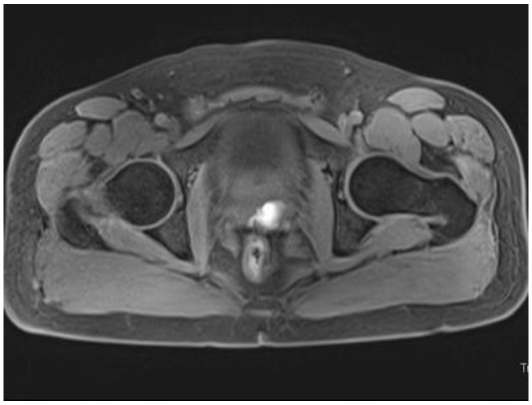

In the last years, MRI became a common procedure in the detection of prostate cancer. The main indication is to evaluate patients with increased PSA and with prior negative biopsies. The best MRI procedure is multiparametric MRI. Multiparametric MRI consists of high resolution T2 axial sequence, as morphological study, T1 dynamic contrast examination and spectroscopy.

The standard MRI examination recommended was published in the interest of improving the quality of procedure and reporting. It was improved by a group of experts of the European Society of Urogenital Radiology (ESUR). In addition to the standard minimum examination protocol, the guideline describes also a structured reporting scheme. This scheme is called PI-RADS based on the BI-RADS. The score varies from 1-5. For a correct estimation of the score we have to choose the most adequate images and to add a scoring table that combines the aggregated multiparametric score with a total PI-RADS score.

In practical clinical activity, we also use Gleason score to determine the histological grading of the prostate malignant involvement. The name of the score is given after Donald Gleason – an US pathologist.

A score of 1 to 5 is assigned to each of the two largest areas of tumor involvement in the samples obtained, based on the worst feature with 1 least aggressive and 5 most aggressive.

PI-RADS classification includes several criteria.

The 3 Tesla magnets is the hardware tool necessary for obtaining best images in prostate assessment but we can also use other variants.

In T2 with high resolution quality images we grade from I to V for the peripheral zone:
I – normal aspect with hyperintense homogenous peripheral zone
II – discrete hypointense focal lesion wedge or bandshaped ill defined
III – more advanced lesion and different from 1,2,4,5 classification
IV – severe hypointense lesion well defined without extracapsular extension
V – hypointense mass round and bulging with capsular involvement or seminal vesicle invasion.

Figure 9. DWI axial. Diffusion restriction area.

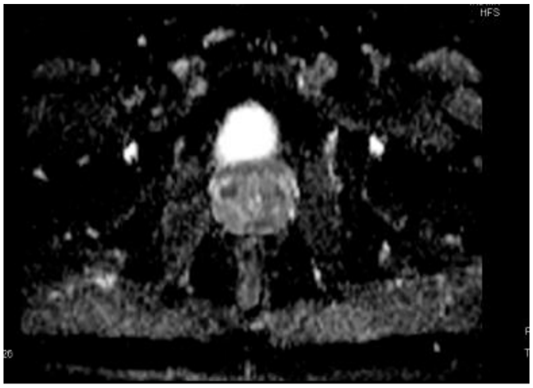

For example, according to the German inter-disciplinary guideline of prostatic cancer for a correct imaging diagnosis we should perform MRI with a minimum strength of 1, 5 Tesla (T) using a combined endorectal body phased – array coil or a 3 Tesla machine and a combination of multiparametric sequences. When we use a 3 Tesla machine an endorectal coil is not mandatory.

Also in T2 for the central zone, we grade the lesion from I to V:
I – the central zone has stromal and glandular hyperplasia without focal hypointense nodular or oval – shaped

II – round hypointense lesion with signs of well defined capsule or band shaped hypointense region.
III – changes not falling into category 1,2 or 4,5
IV – oval shaped anterior hypointense lesion without evidence of capsular involvement – homogenous hypointense lesions with loss of matrix ill defined margins
V – oval shaped with compression, retraction, extension of the anterior capsule irregular infiltrating mass with architectural disintegration and invasion into adjacent structures.

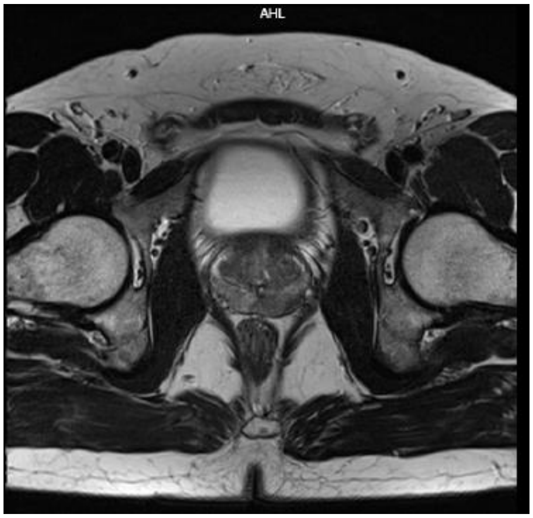

Figure 10. T2 TSE axial. Low signal intensity area corresponding to a prostate tumour.

MRI is very efficient in differentiating lesions with almost similar morphological appearance like post-biopsy scars, atrophic changes, prostatitis, post-treatment lesions, and intraepithelial neoplasias.

In diffusion weighted imaging the analysis of the movement (diffusion) of the water molecules are expressed by the ADC parameter (apparent diffusion coefficient).The molecular diffusion in tissues is generally restricted by cell structures and membranes.

Intracellular edemas or higher cell densities lead to the reduction of the free molecular movement. In tissues with higher cell densities, like prostate carcinoma, ADC has reduced values. The diffusion sequence use upper values of 800-1000s/mm but there are studies that prove that there is a benefit in improving the diagnostic value of the sequence with a value of 2000 and at 3 Tesla magnetic fields. The orientation of the sequence must be in the same plane like T2 axial and with same FOV for efficient multiparametric comparaison. We can also grade prostate lesions using DWI:
I – no reduction in ADC compared to normal tissues
II – diffuse hyperintensity image with low ADC and no focal lesions
III – unilateral hyperintensity with diffuse reduced ADC (no focal lesion)
IV – focal area with reduced ADC and isointense on the diffusion sequence
V – focal hyperintese mass or focal lesion with a reduced ADC.

T1 with dynamic contrast – DCE – MRI (dynamic contrast enhancement – MRI) is a very efficient method to obtain data about vascularisation and neoangiogensis of the prostate. When we perform DCE – MRI, we evaluate the curves of enhancement after a bolus injection of a gadolinium based contrast medium (the injection rate for a correct evaluation must be 2.5 ml/sec and a we must use a subsequent injection of 20 ml of isotonic saline solution).

Dynamic contrast-enhanced magnetic resonance imaging strategies vary, but, in general, three types of imaging data are acquired. Initial images localise the tumour and provide anatomical information.

Sequences that allow calculation of baseline tissue T1-values before contrast agent administration are acquired to enable analysis of blood flow into the tumour. The dynamic data are acquired every few seconds in T1-weighted images over a period of around 5–10 min depending on the soft and power MRI machien gradients. We use T1-weighted spoiled gradient echo sequences are, as they allow good contrast medium sensitivity, high signal-to-noise ratio, adequate anatomical coverage and rapid data acquisition (Parker and Buckley, 2005). The Signal Intensity curves measured by DCE – MRI correspond to three maintypes of curves. Curve type I – the signal intensity continue to increase. Type II is characterized by a progression and a slight decression of the Signal Intensity. The type III curve is defined by the immediate wash out after reaching the peak enhancement. We add point to the scoring system if there is a focal lesion, another one is added if there are asymmetric lesions or unsually located lesions (anterior parts of the transition zone and anterior horns of the peripheral zone).

Magnetic Resonance Spectroscopy Imaging (MRSI) allows the measurements of the relative concentration and distribuiton of the metabolites like citrate, creatine and choline in the prostate. The metabolic information can increase the specificity of the morphogical value of the MRI examination and evaluate the progression or the involution after different kind of therapy. The quality of the MRSI is dependent on the physical and technical support and also of the local and particular condition like post-biopsy hemorrhage, regional prosthetic or metal clips and artifacts.

PI-RADS Clasiffication	Definition	Protocol T2, DWI, DCE	Protocol T2, DWI, DCE, MRS
1	most probably benign	3, 4	4 ,5
2	probably benign	5, 6	6 – 8
3	indeterminate	7 – 9	9 – 12
4	probably malignant	10 – 12	13 – 16
5	highly suspicious of malignancy	13 – 15	17 – 20

Citrate (C) is synthesized, secreted and stored in large quantities in normal glandular tissue of the prostate and is therefore used as an organmarker for healthy prostate tissue.

Creatine plays an important role in the cells' energy metabolism and serves as an internal reference of intensity. Choline (Cho) refers to the sum of choline-containing compounds, which includes various free choline compounds such as phosphocholine, glycerophosphocholine, free choline, CDPcholine, acetylcholine and choline plasmalogen. The intensity of the choline resonance reflects the extent of membrane turnover and is significantly elevated in cancerous tissue.

- Type 1: Cho is significantly lower than citrate.
- Type 2: Cho is elevated but still lower than citrate .
- Type 3: Cho is approx- imately on the same level as citrate.
- Type 4: Cho is elevated compared to citrate.
- Type 5: Cho is significantly elevated compared to citrate.

In analogy to the BI-RADS, the PI-RADS system offers the advantage of a standardized and easy communication of findings to other professional colleagues. Every lesion should be evaluated using a standardized graphic prostate scheme with at least 16, better 27, sectors. For each lesion, a point score between 1 and 5 is to be assigned per method. This is used to calculate the total score, which reflects the probability of the presence of clinically relevant cancer.

The total score is then converted to the relevant PI-RADS score, providing the advantage that the final PI-RADS score is independent of the number of techniques used and can, thus, be easily communicated.

Since the conversion of point scores is not explicitly explained in the ESUR guidelines, the authors recommend using the algorithm given in. For routine clinical work, the authors further suggest that a diagnosis of suspected prostate cancer should be made if the PI-RADS score is 4.

CONCLUSION

In summary, structured reporting of a lesion, using the multi-parametric approach, increase the quality and diagnostic value of prostate MRI.

Therefore, application of the PI-RADS scheme based on the representative images provided here is recommended for clinical routine. The standardized graphic reporting scheme facilitates the communication with referring colleagues. Moreover, a standardized reporting system not only contributes to quality assurance, but also promotes widespread use of the method and implementation of large-scale multicenter studies, which are needed for further evaluation of the PI-RADS system, in analogy to the BI-RADS system used in breast imaging.

The therapeutic approach varies widely and includes also the watchful waiting, androgen ablation, hormone therapy radical surgery, various forms of radiation therapy.

Multiparametric MRI is the most efficient method in providing information about the size, location and extent of the prostate cancer. These useful information allow the surgeon to choose the best way to resect the tumor, to perform optimal resection and also, to plan radiation therapy.

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SYSTEMATIC REVIEW

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Clinical aspects of hearing loss associated with cisplatin therapy – a review

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Abstract:
Introduction: Cisplatin is a well known platinum-based chemotherapeutic agent used for the treatment of diverse malignant tumours.
A frequent side effect of cisplatin therapy is ototoxicity.
Material and methods: Experimental, clinical studies and reviews from the English language medical literature concerning ototoxicity were selected either from PubMed or printed medical journals, pointing out cisplatin-induced ototoxicity. Molecular mechanisms, clinical audiological and histological marks of cisplatin-induced ototoxicity as well as current experimental and clinical strategies for prevention or treatment of hearing loss were reviewed.
Results and discussion: A wide range of otoprotective molecules and efficient strategies against cisplatin-induced hearing loss proved to be helpful in experimental studies whereas only dexamethasone settled a slight otoprotective effect in a clinical study.
Conclusion: There is no currently available treatment for cisplatin associated hearing loss and further research is required for future therapeutic options.

Keywords: *cisplatin-induced hearing loss, otoprotection, intratympanic therapy, systemic therapy, gene therapy.*

INTRODUCTION

Although synthesized already since 1845 by Peyrone and named Peyrone's salt, cisplatin was used clinically in oncologic therapy for head and neck, lung, bladder, cervical, ovarian, testicular, gastrointestinal cancers, as well as malignant gliomas and metastatic cancers such as melanoma, mesothelioma, and those of the prostate and breast [1] only in the late 1960s. Irreversible neurosensorial hearing loss which affects both ears symmetrically, high frequencies in the first place followed by low, speech range frequencies in a dose related, cumulative fashion is one of its major side effects.

Pre-existing afflictions like hypoalbuminemia, anaemia, renal failure, noise-induced hearing loss or by risk factors like therapy with loop diuretics, aminoglycoside antibiotics, radiotherapy fields which includes the inner ear, extreme ages (very young or very old), duration and dose schedule of cisplatin infusion, genetic factors can favour cisplatin-induced hearing loss. The average incidence of cisplatin-induced hearing loss is about 62% [2]. Cisplatin-induced tinnitus is another frequent side effect. These phenomena may occur within hours to days after cisplatin administration. As subsequent multiple cisplatin regimens for the control of cancer may be necessary, side effects should be prevented or treated without reducing the efficacy of the antitumoral mechanisms.

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MATERIAL AND METHODS

Outer hair cells are the main target for cisplatin ototoxicity which progresses from the third to the first row, and to some extent, to inner hair cells of the or gan of Corti in the basal turn of the cochlea followed by alterations in sensorial cells situated in the apex.

Supporting cells, marginal cells of the stria vascularis and the spiral ligament are also afflicted by cisplatin ototoxicity [3]. Vestibular organs and spiral ganglion cells may also suffer from cisplatin ototoxicity, especially in experimental conditions [4]. In patients with cancers of the head and neck whose communication is already impaired, the ensuing hearing loss is very disabling and cause for depression. [5].

The creation of reactive oxygen species and depletion of antioxidant glutathione and its regenerating enzymes as well as increased rate of lipid peroxidation, oxidative modifications of proteins, nucleic acids damaged by Caspase system activation [2] and S-nitrosylation of cochlear proteins and resulting apoptosis of inner ear cells stand for the molecular mechanisms of cisplatin-induced hearing [6].

Cochlear tissues fight the oxidative stress by means of antioxidant defence systems including glutathione, glutathione reductase, superoxide dismutase and catalase. Potassium uptake and secretion in the stria vascularis also might be disturbed by cisplatin administration, leading to impairment of the function of outer and inner hair cells in the organ of Corti, with alteration of the endocochlear potential and subsequent hearing loss [7].

Measurements of hearing loss by means of pre-treatment and follow-up serial audiologic tests are used to assess the extension of ototoxicity due to cisplatin therapy. Experimentally, histological examinations could point out injuries due to cisplatin administration [8]. Audiologic tests include pure tone (normal frequency and extended high frequency range), speech and impedance audiometry, auditory brainstem responses and Distortion Product Otoacustic Emissions (DPOAEs) testing.

DPOAEs reflect early injuries to outer hair cells in the organ of Corti thereby allowing monitoring and early detection of cisplatin ototoxicity during cancer treatment [9,10]. Cisplatin early ototoxic effects cause hearing impairment in the high frequencies at 6 kHz, 8 kHz and above as measured by conventional or extended high-frequency pure tone audiometry [11]. Multiple doses of cisplatin worsen hearing, ultimately affecting the speech frequency range (500 - 4000 Hz). Since DPOAEs measurement is based on the integrity of outer hair cells which is affected by cisplatin therapy before elevation of auditory threshold as measured by pure tone audiometry.

DPOAE testing is more sensitive than the latter for the detection of cisplatin-induced ototoxicity.

Moreover, the results provided by DPOAEs, as an objective testing, are not influenced by the ability of the deteriorating cancerous patient to respond to the sound stimulus [9]. In small children who undergo cisplatin administration for cancer treatment and who cannot cooperate for pure-tone testing because of their younger age and poor cognitive ability, either soundfield behavioural testing or DPOAEs could be performed [12].

In experimental animals, auditory brainstem response recordings mirror hearing loss after cisplatin treatment [3].

Histological examinations of inner ear after cisplatin administration reveals destruction primarily of outer hair cells and to some extent inner hair cells and associated nerves, degeneration of the vestibular organs, stria vascularis edema and detachment of the myelin sheath of the spiral ganglion cells [4]. The damage is especially noticed in the high frequency region of the cochlea (i.e. the basal turn) probably due to a base to apex gradient of the cisplatin ototoxicity [14]. Immunostaining detected tumour necrosis factor-alpha (TNF-α) and other inflammatory cytokines (IL-8, IL-6) in the outer hair cells, stria vascularis, spiral ligament and spiral ganglion neurons in cisplatin exposed cochlea [3].

Intravenous injection of cisplatin is followed by strong binding of the alkylating agent to the plasma proteins rendering a large non-active part of it.

The barrier systems in the cochlea, the blood-perilymph barrier, separating blood from perilymph, and the intrastrial fluid-blood barrier, separating blood from endolymph also influence the pharmacokinetics of cisplatin therapy [15]. The amount of free chemotherapeutic agent reaching targets in the cochlea is responsible for the ototoxic effect and consequent hearing loss. High frequency audiometric thresholds are initially affected. When doses in excess of 100 mg/m^2 are used, the hearing impairment may progress from high frequencies to involve the middle frequencies. Reducing the total amount of cisplatin by limitation of the total dose per cycle, dose intensity and the cumulative dose would diminish the antitumour effect, which is not desirable.

Various otoprotective compounds have been tested both in experimental animals and humans. If given systemically, they should be non-toxic, must attain efficient concentrations in the inner ear to protect labyrinthine tissues from cisplatin ototoxicity and should not hamper the anti-tumour effect of cisplatin. Higher concentrations of otoprotective molecules can be achieved by intratympanic administration.

This latter route provides direct access of the protective agents to inner ear structures while avoiding systemic side effects and interference with the anti-neoplastic activity of cisplatin [16].

Experimental studies

Several otoprotective molecules and strategies against cisplatin-induced ototoxicity have been tested in experimental animals.

Hyperbaric oxygen therapy consisting of intermittent inhalations of 100% oxygen at a pressure higher than 1 atm is used as adjuvant therapy in pathological processes like soft tissue infections, radiation injury, gas gangrene and decompressive disease.

Tissue hyperoxygenation through plasma dissolved oxygen was succesfully tested in guinea pigs as a protective agent against cisplatin ototoxicity.

Otoacoustic emissions recordings following hyperbaric treatment and scanning electron microscopy examination proved the efficacy of otoprotection by hyperbaric oxygen therapy after cisplatin administration.

The study confirmed that cisplatin induces dose-dependent cochlear alterations consisting of cellular lesions and significant hair damage in outer hair cells. Analysis of anatomical changes in cochlear outer hair cells indicated signs of otoprotection against cisplatin in animals treated with hyperbaric oxygen therapy although in functional studies the distorsion product otoacoustic emissions were absent reflecting a certain degree of hearing loss, most probably reversible and related to experimental artifacts.

The study concluded that hyperbaric oxygen therapy has otoprotective effects against cisplatin induced ototoxicity. However, further studies are needed to test other effects of high pressure oxygen on the cochlea [17].

The effect of epigallocatechin gallate (EGCG) on the transcription factor STAT1, an important mediator of cell death was investigated by another study. STAT1 phosphorylation is involved in both hair cells and support cells transformation after experimental exposure of mouse utricle to cisplatin.

EGCG proved its efficiency as an otoprotective agent against cisplatin ototoxicity due to its inhibition of STAT1. The failure of EGCG to provide protection against cisplatin in STAT1-deficient mice also supported the hypothesis [18].

Near total preservation of otoacoustic emissions after cisplatin therapy was possible when lactate was injected intratympanically in guinea pigs treated with ototoxic levels of cisplatin, as shown in former studies [19]. A further study was conducted to prove the Ringer solution otoprotective effect against cisplatin ototoxicity when injected intratympanically, before intraperitoneal cisplatin administration since lactate is a part of Ringer solution and it has the smallest molecular weight among other antioxidants which facilitates transport across the round window membrane. The molecular protective mechanism is based on the enzyme lactate dehydrogenise located in the mitochondria of outer hair cells. The conversion of lactate to pyruvate in the presence of the enzyme leads to the formation of nicotinamide adenine dinucleotide (NADH) which is a natural antioxidant that may be involved in fighting against oxygen reactive species resulted at cellular level after cisplatin therapy. Electron microscopy examinations of guinea pig cisplatin-insulted inner ears pre-treated with lactate showed partial preservation of outer hair cells stereocilia, more significant at mid frequencies (2000-4000 HZ) but not statistically significant at higher frequencies. The study used auditory brainstem responses recordings which is a more sensible method than otoacoustic emissions testing, a fact that explains the differences in otoprotective effect reported by previous studies.

The same study investigated the otoprotective effect of intratympanic N-acetylcysteine injections as well as its systemic diffusion following the administration.

The results showed that high concentration of intratympanic N-acetylcysteine is not reliable for otoprotection against cisplatin ototoxicity since it caused more middle and inner ear damage than cisplatin alone. Yet, N-acetylcysteine did not diffuse systemically when applied to the middle ear. This was confirmed by high-performance liquid chromatography testing of blood samples taken from the venous system of the experimental animals after intratympanic injections of N-acetylcysteine. This latter outcome proves that the intratympanic route of administration would be safe and prevent inactivation of antitumoral effect of cisplatin by binding between the thiol moiety of N-acetylcysteine and the platinum-containing molecule of the chemotherapeutic drug [20].

Most of the existing studies deal with exogenous administration of antioxidants. Pharmacological activation of intrinsic defence mechanisms against oxidative stress in the inner ear caused by cisplatin therapy also proved helpful as showed by an experimental animal study using systemic administration of thiamine pyrophosphate (TPP). Thiamine pyrophosphate functions as coenzyme for peroxisomes, being a crucial factor for energy metabolism, antioxidation and myelinization of nerve cells. Its intraperitoneal injection increased the level of natural antioxidants like glutathione and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and glutathione reductase) and reduced the content of malonildialdehyde, an indicator of lipid peroxidation following increased levels of oxygen reactive species resulted from cisplatin toxicity. The histological evaluation of cochleas harvested from TPP treated animals showed preservation of the morphology of the organ of Corti and outer hair cells, and no destruction of spiral ganglion cells and stria vascularis following cisplatin therapy [4].

Sertraline, an antidepressant with neuroprotective effects in rats was also studied for its presumed otoprotective effect against cisplatin-induced ototoxicity [6]. The selective serotonin reuptake inhibitor has antioxidant effects, stimulates neurogenesis and increases antiapoptotic protein levels [21].

Oral administration of sertraline in cisplatin-treated rats has prevented hearing loss above 5000 Hz, in a statistically significant manner, as documented by distortion product otoacoustic emissions recordings.

Besides, sertraline would be beneficial to depressed patients due to their cancerous disease and their deteriorated communication abilities [5].

The otoprotective effect of systemic histone deacetylase inhibitor sodium butyrate was proved in an experimental single dose model of cisplatin ototoxicity.

A sensitive assay of the functional state of outer hair cells after systemic cisplatin insult on the cochlea was provided by distortion product otoacoustic emissions testing. The systemic administration of the otoprotective agent avoided the side effects of the more invasive tympanical local route of administration.

Moreover, sodium butyrate did not interfere with the tumoricidal effect of cisplatin providing both protections from reactive oxygen species and a certain degree of antitumor activity according to previous reports. The protective effect against oxidative stress and the cell division inhibition and subsequent anticancer activity follow acetylation of different cell proteins, including histones. The experiment's weak point is the single dose of cisplatin model whereas in clinical practice cisplatine in multiple doses [22].

One animal study showed the efficacy of short interfering RNA in preventing cisplatin ototoxicity by reducing the expression of NOX3, the unique isoforme of NADPH oxidase involved in the generation of reactive oxygen species, in outer hair cells, spiral ganglion cells and stria vascularis in the rat. Auditory brainstem responses certified reduced threshold shifts in cisplatin treated animals who received transtympanic NOX3 and RNA. Since cisplatin administration has been previously associated with upregulation of NOX3 in the inner ear, Nox3 is thought to be a major source of free radicals in the cochlea following cisplatin exposure. The resulting free radicals initiated the inflammatory process in the cochlea by activating signal transducer and activator of transcription-1 (STAT1), followed by activation of p53 and the increase in inflammatory mediators like TNF-alpha and interleukin-1ß [23]. A single transtympanic injection of RNA has atenuated cisplatin ototoxicity by suppressing inflammation in a dose-related manner. It hampered cisplatin-induced auditory brainstem responses threshold shift and higher doses allowed for complete morphological preservation of outer hair cells as proven by scanning electron microscopy examinations of the rat cochleas [24].

Minocycline is a tetracycline derivative that proved its partial efficacy among various strategies that have been devised in experimental settings to prevent cisplatin ototoxicity. The anti-inflammatory and neuroprotective properties of minocycline have been previously reported. The biochemical mechanism involves caspase-1 and caspase-3 inhibition, which decreases the amount of interleukin-1 and prevents apoptosis. The protective effect of minocycline has been tested both in cisplatin treated cell cultures and in experimental animals which underwent cisplatin intraperitoneal therapy after systemic administration of the otoprotective agent.

Cell viability assays showed that minocycline had a protective effect against cisplatin toxic action. Yet, minocycline failed to protect cells at higher concentrations of cisplatin. Recordings of auditory brainstem responses and evaluation of the scanning electron microscopy sections of inner ears harves ted from minocycline plus cisplatin treated animals indicated a partial preservation of the function and morphology of the outer hair cells as compared to those from animals treated with cisplatin alone [25].

Intraperitoneal erdosteine effect on cisplatin-induced ototoxicity in a guinea pig model was also studied. Erdosteine is a thiol derivative with well-known antioxidant properties due to its active sulfhydryl groups following liver first-pass metabolism.

Living animals underwent pre and posttreatment auditory brainstem responses measurements while outer hair cell counts were analyzed by scanning electron microscopy of the cochlea removed from euthanized animals. Despite the study's limitations (i.e. minimal number of animals included, lack of enzymatic activity detection for the main antioxidant enzymatic systems of the inner ear), the results outlined the systemic administration of erdosteine as a promising therapeutic strategy for cisplatin-induced ototoxicity [26].

Intratympanic dexamethasone prevents hearing loss in a frequency related manner as reflected by a first murine model for cisplatin-induced ototoxicity.

Evoked brainstem responses audiometry indicated that 8 kHz and 16 kHz stimulus elicited responses in cisplatin plus dexamethasone treated mice while high frequency stimulus (32 kHz) perception was affected. Apparently, cisplatin had deleterious effects on outer and inner ear cells situated in the basal turn of the cochlea despite intratympanic administration of protective dexamethasone [27].

Further experimental studies supported the finding that cisplatin exerts its damaging effect in a base to apex gradient, lower frequencies being spared for long. Higher doses of dexamethasone also seem to be more protective than lower doses. Moreover, lower doses of cisplatin allow the naturally present antioxidants to annihilate the resulting reactive oxygen species, explaining the spontaneous hearing threshold recovery even in the absence of protective dexamethasone administration [28].

Dexamethasone diminishes the tumoricidal activity of cisplatin, hencing the preference for intratympanic administration which avoids this unwanted effect. Down-regulating apoptosis genes in tumor cells are responsible for this common side effect of systemic steroid therapy [29]. An experimental study conducted on cisplatin treated guinea pigs asserted the safety of intratympanic dexamethasone based on audiologic and histologic results.

Auditory brainstem responses testing, optic microscopic and scanning electron microscopic examinations of cochlea showed no significant differences between intratympanic dexamethasone-treated animals and saline-treated controls. Dexamethasone administered intratympanicaly proved efficacious in protecting the labyrinth against cisplatin-induced ototoxicity as shown by reduced auditory brainstem responses threshold shifts and unaltered histological inner ear structures. The molecular mechanisms involve increased expression of Na/K channels and aquaporins in the endolymphatic sac and the tissues around the endolymphatic spaces. The study's results also suggest that giving the intratympanic dexamethasone one hour before the cisplatin administration provides the best protection (total protection) against the ototoxic insult by the alkylating agent compared to dexamethasone injections one day prior to cisplatin administration (partial protection) [14].

In order for the dexamethasone to exert a protective effect against cisplatin ototoxicity, the timing of administration of the two drugs should be highly synchronized so that the peak concentration of dexamethasone in the perilymph should be correlated with the peak concentration of the chemotherapeutic agent, according to another study [2].

Otoprotection with dexamethasone against age-related cisplatin-induced hearing loss was investigated in a guinea pig model following observations that persons older than 65 account for more than half of the newly diagnosed malignancies.

Hearing loss due to the aging process shares the same cause (i.e. oxidative stress) with hearing loss due to ototoxic chemotherapeutic agents.

A single dose of cisplatin was administered intraperitoneally in old mice preceded and followed by dexamethasone injected intratympanically to counteract the cisplatin toxic effect on inner ear hair cells. Pre and post treatment auditory brainstem responses were recorded to evaluate hair cell function. The results of the study pointed out that no synergistic action between age related hearing loss and cisplatin-induced hearing loss exists since threshold shifts were smaller in older animals than those in young mice.

Another finding of the study was that the protective effect of dexamethasone against cisplatin-induced ototoxicity was a function of stimulus frequency in old mice. Susceptibility to otoprotective effect of dexamethasone was higher in mid to basal cochlear regions (at and above 24 kHz) in old mice , whereas in young mice, dexamethasone bestowed more protection in apical regions of the cochlea (at 16 kHz and below). Age-related changes of the mechanism of distribution of dexamethasone in scala tympani perlymph after round window membrane application in guinea pigs seem to account for the frequency dependent otoprotective effect [30].

Intratympanic dexamethasone did not protect against cisplatin-induced ototoxicity in a multidose cisplatin ototoxicity mouse model. The study was prompted by typical clinical protocols of cancer treatments which require administration of multiple, smaller cisplatin doses, exerting their curative effect through cumulative dosing. Contrary to previous experimental studies, the mice received five doses of cisplatin throughout five days, mimicking the cumulative exposure seen in malignant tumours treatment.

Intratympanic dexamethasone was administered on the same days as the intraperitoneal cisplatin. The results mirrored by auditory brainstem responses threshold measurements demonstrated continued change in hearing thresholds several weeks after cisplatin exposure and no protective effect of intratympanic dexamethasone against cisplatin ototoxicity [31].

An experimental study focusing on systemic administration of steroid for protection against cisplatin-induced ototoxicity showed no otoprotection following several days' prophylaxis with a high dose dexamethasone treatment. Only a slight decrease of TNF-alpha expression in the cochlea was demonstrated by imunohistochemical staining of anatomical samples harvested from systemic cisplatin plus dexamethasone treated animals. Dexamethasone also seemed to protect stria vascularis from morphological alterations, probably owing this effect to higher concentrations of steroid in the lateral cochlear wall following increased cochlear flow and a naturally highly vascularised stria vascularis.

Still, a functional otoprotective effect of systemic dexamethasone against cisplatin-induced hearing loss was not observed [3].

Among naturally occurring molecules, Rosmarinic acid, a water-soluble polyphenolic compound extracted from Dansam-Eum, was tested for its protective effect against cisplatin-induced ototoxicity in laboratory settings. The results of the study showed that Rosmarinic acid inhibited cisplatin induced caspase-1 activation providing protection against stereocilia loss in the primary organ of Corti explants [32].

Another natural remedy, the Maytenus ilicifolia aqueous extract, was evaluated for its possible otoprotection in guinea pigs. Despite the well-known South America plant's antioxidant effects (due to the presence of flavonoids and alkaloids), functional tests did not demonstrate any protective action on the cisplatin exposed cochlea. Yet, the extract improved the clinical status and weight of guinea pigs and diminished mortality after cisplatin exposure [33].

Resveratrol, a polyphenol found in grape skin and seed, has anti-oxidant, neuroprotective and dose dependent antiapoptotic properties [34]. Recent experimental research pointed the preventive effect of resveratrol against cisplatin induced ototoxicity.

An in vitro study on House Ear Institute-Organ of Corti 1 cell line showed that resveratrol in low doses prevented ototoxicity mainly influencing apoptotic gene expression but proved cytotoxic effect in high doses [34]. Two other studies showed conflicting results. Thus, high doses of oral resveratrol administered to mice seem to enhance cisplatin ototoxicity[35] whereas systemic administration of lower doses of resveratrol provided significant protection to the cochlea against cisplatin [36].

Clinical studies

Intratympanic dexamethasone was clinically tested for its otoprotective effect in patients afflicted with neoplastic diseases for which the treatment protocol included cisplatin. Intratympanic dexamethasone is a current remedy for idiopathic sudden sensorineural hearing loss and Meniere disease [37].

Intratympanic administration of drugs avoids significant systemic side effects. The intratympanic route also provides higher concentrations of drug in the inner ear fluids and prevents significant interference between dexamethasone, which is known to reduce efficacy of chemotherapeutic agents, and cisplatin.

Patients enrolled in the study underwent unilateral intratympanic dexamethasone administration prior to every cisplatin treatment session, with the contralateral ear used as a control. Serial follow-up audiometry and distortion product otoacoustic emissions testing were performed to check the functional state of both study and control ears. The statistically significant results showed that intratympanic dexamethasone is slightly protective against cisplatin-induced hearing loss at 6000 Hz and decreases the outer hair cells dysfunction in the frequency range of 4000 to 8000 Hz. The conclusion of the study is that intratympanic dexamethasone has minimal effect towards reducing cisplatin ototoxicity. Optimal concentrations of dexamethasone and a perfect timing of administration would be necessary to prevent hearing loss after cisplatin therapy [2].

Patients with head and neck cancer undergoing cisplatin therapy were given transtympanic L-N-Acetylcysteine to check its otoprotective effect.

Thiol compounds either directly bind cisplatin or act as free radical scavengers. Based on that, their intratympanic administration was suggested to avoid the decrease of oncologic effectiveness of cisplatin and to reduce the oxidative stress caused by it.

Intratympanic L-N-Acetylcysteine was well tolerated by patients receiving multiple doses of cisplatin as part of their oncologic treatment. The relation between dose and otoprotection was not taken into account. Higher concentrations may have yielded better otoprotection. The study protocol required the L-NAC injection to be approximately 1 hour before systemic administration of cisplatin, for the sake of better timing. The outcome of the pure tone audiometry testing at 1 and 2 months after the last cycle of cisplatin showed that L-N-Acetylcysteine was overall not significantly otoprotective. Still, hearing loss was reduced in two patients out of eleven who completed the study. The study protocol had several challenges like the difficulty in maintaining high enough concentrations of aqueous solution of L-N-Acetylcysteine in the middle ear due to the technique of administration and the different initial hearing thresholds due to preexisting hearing loss [38].

Among systemic otoprotective molecules for preventing cisplatin-induced hearing loss, amifostine, a phosphorylated aminothiol designed to protect against radiation damage, was known to counteract the toxic effect of different anticancer treatments without interfering with the tumoricidal effect. Although significant protection of amifostine against haematological toxicity after high dose carboplatin therapy in a child with medulloblastoma was reported [39], a clinical study considering systemic administration of amifostine failed to prove any otoprotective effect against cisplatin-induced hearing loss in a group of paediatric patients treated with cisplatin associated with other chemotherapeutic agents [40].

OTOPHARMACOGENETICS

The well-isolated inner ear organ makes it prone to targeted genetic therapies. Viral or non-viral gene vectors can be delivered through a transtympanic route without the risk of dispersing them and reaching other tissues with subsequent undesirable genetic alteration. Long term effects after single administration, cellular selectivity and replacement of genetically flawed nucleic acid sequences are the main benefits of gene therapy. Common viral vectors include herpes simplex virus, recombinant adenoassociated virus, recombinant adenovirus, adenovirus, used to amplify the expression of targeted genes. Cells are infected with the vectors (transfection) which transfer genes whose expressed proteins influence important processes like growth, oxidative stress and apoptosis. Chemical transfection can also be achieved with plasmid vectors. Short interfering RNA can be used to shut down target genes.

Among inner ear target genes dealt with by the gene therapy studies are ATOH1 (Math1), CAT (catalase), SOD1 (Cu/Zn superoxidedismutase), SOD2 (Mn superoxide dismutase), BDNF (brain-derived neurotrophic factor), HGF (hepatocyte growth factor), GJB2 (gap junction protein), BclxL (B-cell lymphoma-extra large), FGF2 (basic fibroblast growth factor). The gene therapy modifies the synthesis of a wide range of proteins including neurotrophic factors (NTF3, GDNF), apoptosis mediators (XIAP,BCL2), oxidases (NADPH, NOX1, NOX3, NOX4), an antioxidant response regulator (Nfe2l2), a cytoprotective enzyme (HO-1), a copper transportes (Ctr1), a nonselective cation channel (Trpv1) and a protein Otospiralin (Otos).

Cisplatin-exposed tissues can benefit from genetically induced up-regulation of neurotrophic factors, inhibition of apoptosis and generation of endogenous antioxidant enzymes.

Experimental animal studies and in vitro experiments show the efficacy of gene therapy for cisplatin-induced ototoxicity. Clinical applications require further studies regarding safety, immunogenicity and consequences of genetic manipulation [41].

Another strategy to avoid cisplatin-induced hearing loss would be the pretreatment genotyping to find out patients at risk for the ototoxic effect of cisplatin [42]. Genetic variants (polymorphism) of different protein systems (Tiopurine S-methyltransferase, Catechol-O-methyl transferase, Glutathione-S-transferase with its subclasses M1/T1/ P1, Megalin) can stand for the interindividual variability in cisplatin ototoxicity [43].

RESULTS AND DISCUSSION

43 publications were reviewed concerning prevention or treatment of cisplatin induced ototoxicity.

Publications were devised in either experimental or clinical studies. All researches emphasize the importance of reactive oxygen species in cisplatin induced ototoxicity. The hearing loss following cisplatin treatment affects principally the high frequencies, extending to speech range frequencies as multiple doses of cisplatin worsen hearing.

Experimental studies supported the efficiency of hyperbaric oxygen therapy, epigallocatechin therapy and intratympanic lactate. The latter two therapies provide exogenous antioxidants while pharmacologic activation of endogenous antioxidants by means of intratympanic thiamine pyrophosphate was consistent with higher levels of natural antioxidants.

Oral sertraline, besides its otoprotective effect against cisplatin induced ototoxicity, also has therapeutic value concerning the depression occurring frequently in oncologic patients. Sodium butyrate proved its efficiency against cisplatin induced hearing loss in a monodose cisplatin model. Yet, in clinical practice the patient receives multiple doses of ciplatin. The production of endogenous radicals of oxygen species was reduced after intratympanic administration of short interfering RNA which reduces the expression of NOX3 in the cochlea. Minocycline appeared to be efficient only at low doses of cisplatin, while systemic erdosteine showed promising results. Dexamethasone in experimental studies combined efficiency against cisplatin induced ototoxicity with preservation of the tumoricidal activity of cisplatin. When older mice were treated, the dexamethasone was more otoprotective at higher frequencies compared to experiments including younger subjects. Rosmarinic acid also proved to be otoprotective while the Maytenus ilicifolia aqueous extract was not. Resveratrol had contradictory effects, systemic low doses and in vitro administration preventing ototoxicity, whereas high oral doses seem to enhance cisplatin ototoxicity.

Also, there were some experimental studies which showed inefficiency of intratympanic NAcetylcysteine and intratympanic and systemic dexamethasone. N-Acetylcysteine, in addition having a damaging effect on middle and inner ear structures.

Clinical studies proved a minor otoprotective effect of intratympanic dexamethasone and no effect of systemic amifostine and intratympanic LN-Acetylcysteine.

New perspectives are brought about by genetic therapy using viral vectors and genotyping to anticipate interindividual variability in cisplatin ototoxicity.

CONCLUSION

New strategies are required for hearing loss prevention and treatment during cisplatin therapy for cancer besides optimization of old ones. The intratympanic route of administration and the gene therapy are among the most promising perspectives in further experimental and clinical studies.

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Imaging in the diagnosis of chronic pancreatitis

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Abstract: *Chronic pancreatitis is characterised by progressive and irreversible damage of the pancreatic parenchyma and ductal system, which leads to chronic pain, loss of endocrine and exocrine functions. Clinically, pancreatic exocrine insufficiency becomes apparent only after 90% of the parenchima has been lost. Despite the simple definition, diagnosing chronic pancreatitis remains a challenge, especially for early stage disease. Because pancreatic function tests can be normal until late stages and have significant limitations, there is an incresing interest in the role of imaging techniques for the diagnosis of chronic pancreatitis. In this article we review the utility and accuracy of different imaging methods in the diagnosis of chronic pancreatitis, focusing on the role of advanced imaging (magnetic resonance imaging, endoscopic retrograde cholangiopancreatography and endoscopic ultrasound).*

INTRODUCTION

Chronic pancreatitis is represented by a progressive inflammation and fibrosis of the pancreas, resulting in permanent structural damage and loss of function. Regarding its epidemiology, incidence and prevalence worldwide varies depending on the frequency of risk factors (rates mainly paralelling alcohol consumption) and on the diagnostic method used (with higher incidence and prevalance rates where diagnosis was based on advanced imaging techniques – a study in Japan using advanced diagnostic tools reported a prevalence of 45.4 and incidence of 12.4/100.000, compared to 10-15 and respectively 3.5-4 in western countries). Etiology is represented by heavy alcohol consumption, ductal obstruction (post-traumatic fibrosis, stones, pseudocyst, tumors, pancreas divisium, Oddi sphicter dysfunction), genetic causes (hereditary pancreatitis), tropical pancreatitis, autoimmune pancreatitis or systemic disease (lupus, hyperparathyroidism).

The natural history of chronic pancreatitis evolves with a slowly progressing subclinical phase, followed by recurrent episodes of abdominal pain and finally, when over 90% of the pancreatic parenchyma is lost, exocrine insufficiency appears. Although it is not yet known if early diagnosis of chronic pancreatitis can alter its natural history and change the outcomes of this disease, efforts should be made for earlier detection of the disease.

In real-life there are two main diagnostic issues regarding this disease: on one hand it's over-diagnosed by considering a hyperechoic pancreas on

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ultrasound as chronic pancreatitis, and on the other hand it's under-diagnosed in early stages when patients don't have exocrine insufficiency but present with recurrent abdominal pain and abdominal dis tention, symptoms which are frequently attributed to irritable bowel syndrome (IBS).

On clinical grounds, early chronic pancreatitis can be asymptomatic or paucisymptomatic; moreover, early symptoms are nonspecific, of low intensity and often attributed to IBS. The classic steathoreea appears late in the evolution of the disease, but our aim is to detect chronic pancreatitis before overt exocrine insufficiency develops.

Diagnostic tests for chronic pancreatitis are separated in two: those that evaluate pancreatic function and those that detect structural changes[2].

Pancreatic function tests (PFT) can be classified as direct (which directly measure pancreatic secretions) and indirect (which measure either the pancreatic enzymes in blood or stool or the effect/lack of effect of the pancreatic enzymes, by administering a substrate which requires pancreatic enzymes for digestion and measuring the metabolites of the substrate in stool, urine, blood or breath). Direct PFT is done by collecting and measuring intraduodenal bicarbonate and lipase levels after stimulation with a secretagogue (secretin-cholecystokinin); this method has a sensitivity of 96% (which means a normal result can accurately rule out chronic pancreatitis) and specificity of 37%, but it has major limitations – it's not standardized, not widely available, it depends on the patient to tolerate the oroduodenal tube/endoscope for an hour (here we add the costs and risks of prolonged sedation) and it has many false positive results (in Billroth II anastomosis, diabetes mellitus, celiac disease, cirrhosis and recent acute pancreatitis)[2]. Other PFT such as fecal elastase, fecal chymotrypsin or serum trypsinogen have proven useful only for advanced disease[2].

Histopathological examination of the pancreas could be diagnostic in the early stages, however tissue sampling in chronic pancreatitis is rarely used because of many reasons: histopathological changes are non-specific (chronic inflammation and fibrosis), sampling can be nonrepresentative in focal disease, normal aging can produce changes similar to those seen in chronic pancreatitis and pancreatic biopsy has a high risk of complications (acute pancreatitis, fistula, pseudocyst).

In this setting, imagery has emerged as a very valuable tool for evaluation of chronic pancreatitis.

Imaging methods used for the diagnosis and staging (with regard to severity and complications) consist of: plain abdominal x-ray, transabdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreatography (MRCP) and secretin stimulation (S-MRCP), endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasound (EUS).

Plain abdominal X-Ray

Plain abdominal radiography can reveal diffuse calcifications on the projection area of the pancreas, which are considered pathognomonic of chronic pancreatitis[3].

Abdominal ultrasound (US)

Abdominal ultrasound is a simple, non-invasive, widely available imaging tool. Its limitations are represented by the fact that it's highly examiner-dependent and patient-dependent (excessive adipose tissue, history of upper GI tract surgery or overlying gas can lead to a poor view of the pancreas).

Examination is done using a convex probe, with a frequency of 3.5-5 Mhz, using epigastric and left subcostal approaches. To optimise visualisation of the pancreas, some recommend drinking 500 ml of water before the examination, to make the stomach an acoustic window.

Ultrasound can show atrophy/enlargement of the pancreas, inhomogenous echostructure (by hyperechoic areas corresponding to fibrosis), irregular gland border, pancreatic duct changes (dilatation, irregularity or stones), cysts and pseudocysts. US has a sensitivity of 60-70% and specificity of 80-90% in diagnosing chronic pancreatitis3. Although its role in positive diagnosis is limited, US is very useful in follow-up: it can assess position of intraductal or pseudocystogastric stent and it can evaluate the efficacy of extracorporeal lithotripsy by showing the disappearance of stones and reduction in pancreatic duct diameter.

Computed tomography (CT)

From its first use in chronic pancreatitis back in 1976, CT is now widely used for the evaluation of pancreatic pathology. Computed tomography has a reported sensitivity of 75-90% and specificity of 85% in the diagnosis of chronic pancreatitis, but it can be normal in patients with early stage disease[3,6].

CT scan can reveal chages in size (enlargement, atrophy) and contour (irregularity of the anterior border) of the pancreas, calcifications (with higher detection rates than ultrasound, being the imaging method of choice for calcifications), dilatation of the main pancreatic duct or common bile duct, alterations in peripancreatic fat – see figures 1, 2. It can also detect complications such as pseudocysts, arterial pseudoaneuyrisms or splenic vein thrombosis[6].

Contrast-enhanced computed tomography

(CECT) is also useful in differentiating pseudotumoral chronic pancreatitis from pancreatic cancer[6].

The disadvantages of the CT scan consist of irradiation, risk of contrast-induced nephropathy and its contraindication in patients with iodine-allergy[6].

Endoscopic retrograde cholangiopancreatography (ERCP)

ERCP is a very useful tool for detecting morphological changes in the pancreatic ductal system. It is done using a lateral-view endoscope by cannulating the main pancreatic duct from the duodenum and injecting contrast to create a pancreatogram. The pancreatogram can show structural changes (dilatation, narrowing, irregularity, stones, leaks) of the pancreatic duct, abnormal side branches, communicating pseudocysts, which are reported in a standardized manner, using the Cambridge criteria, (Table 1). In interpreting the pancreatogram, one should keep in mind that focal ductal changes can follow an episode of severe acute pancreatitis,. Also a normal pancreatogram does not exclude early chronic pancreatitis.

Table 1 Cambridge Classification for ERCP
(Banks PA. J Gastroenterol 2007)

Grade	Main duct	Abnormal side branches
Normal	Normal	None
Equivocal	Normal	< 3
Mild	Normal	> 3
Moderate	Abnormal	> 3
Marked	Abnormal with at least one additional feature: large cavity (>10 mm), obstruction, filling defects, severe dilatation or irregularity	> 3

The main disavantages of ERCP are represented by the fact that it's strongly operator dependent, it's an invasive technique with potential serious complications (pancreatitis, hemorrhage, infection) and it only reveals ductal changes[6]. Over the last years, ERCP has become a primarily therapeutic tool, so that diagnostic studies have been abandoned in favor of other imaging methods such as MRI/MRCP or EUS. However, ERCP remains of great importance in pancreatic endotherapy (pancreatic sphincterotomy, stenting and cyst drainage).

Magnetic resonance imaging (MRI). Magnetic resonance cholangiopancreatography (MRCP)

Due to its noninvasivity and lack of irradiation, MRI with MRCP, gadolinium enhancement and secretin stimulation is being increasingly seen as the imaging method of choice in the evaluation of pancreatic disease. MRI can be used in renal impairment and in patients with iodine allergy, in whom CECT cannot be performed[7]. One of the drawbacks of MRI is represented by the artefacts caused by respiratory movements, but this has improved with the latest technology.

Pancreatic parenchyma can be best examined on T1-weighted, fat-suppressed sequences, where it reveals a high signal due to the high-protein content (enzymes, hormones). In chronic pancreatitis, the pancreatic signal decreases (due to loss of exocrine function) and there is a delayed enhancement after gadolinium administration (due to fibrosis, blood flow inside the pancreas is altered and the gland peaks in the venous phase instead of the arterial one)6. MRI is inferior to CT in detecting calcifications, because they are seen as signal void.

Pancreatic ductal system is best evaluated by magnetic resonance cholangiopancreatography.

MRCP images are obtained without injecting contrast medium, by using heavily T2-weighted sequences.

On T2-weighted images, fluid from the bilio-pancreatic system has a bright signal and by adding fat-supression, the signal of fluid within the ducts becomes more pronounced[6]. MRCP can show dilatation, strictures, irregularities in the Wirsung duct or similar changes in the side-branch ducts – see figures 3,4.

The advantages of MRCP over ERCP are lack of instrumentation of the Wirsung duct and lack of need for sedation during the procedure; however, MRCP is only diagnostic, whereas ERCP is therapeutic. Another difference between the two is that while during ERCP the collapsed secondary branches are distended by the contrast agent, MRCP visualizes the ductal system in a physiologic state, thus being less acurate for small duct disease. To overcome this difference a combined functional-structural test has been developped, called MRCP-S (magnetic resonance cholangiopancreatography with secretin stimulation): secretin stimulates secretion in the ductal system and it also increases the tonus of the Oddi sphincter (preventing the release of secretion in the duodenum), thus filling in the collapsed branches and better delineating minimal duct changes in patients in whom MRCP showed no abnormalities under physiological conditions; the study of Manfredi et al. showed that visualization of the secondary branches improved significantly in both patients with severe disease (from 71% to 100%) and those with a mild-moderate disease (from 4% to 63%). MRCP-S also allows for calculation of the change in pancreatic duct size before and after secretin stimulation (index called pancreatic duct compliance, PDC), which is reduced in chronic pancreatitis[6].

Table 2. Rosemont criteria for chronic pancreatitis

	Rank	Criteria	Feature	Definition
Parenchymal features	1	Major A	Hyperechoic foci with shadowing	Echogenic structures ≥ 2 mm in length and width that shadow
	2	Major B	Lobularity with honeycombing	Well-circumscribed, ≥ 5 mm structures with enhancing rim and relatively echo-poor center, contiguous ≥ 3 lobules
		Minor	Lobularity without honeycombing	Same as above, noncontiguous lobules
	3	Minor	Hyperechoic foci without shadowing	Echogenic structures foci ≥ 2 mm in both length and width with no shadowing
	4	Minor	Cysts	Anechoic, rounded/elliptical structures with or without septations
	5	Minor	Stranding	Hyperechoic lines of ≥ 3 mm in length in at least 2 different directions with respect to the imaged plane
Ductal features	1	Major A	MPD calculi	Echogenic structure(s) within MPD with acoustic shadowing
	2	Minor	Irregular MPD contour	Uneven or irregularoutline and ectatic course
	3	Minor	Dilated side branches	3 or more tubular anechoic structure seach measuring ≥1 mm in width,budding from the MPD
	4	Minor	MPD dilation	≥3.5-mm body or >1.5-mm tail
	5	Minor	Hyperechoic MPD margin	Echogenic, distinct structure greater than 50% of entire MPD inthe body and tail

Endoscopic ultrasound (EUS)

Endoscopic ultrasound is done using a linear/radial transducer integrated into the tip of the scope, using 7.5-12 Mhz frequencies. It is a great method of evaluating the pancreas, considering that the transducer gets in very close range of the gland, from which is separated only by the digestive tract wall.

EUS is especially useful for early chronic pancreatitis, being able to reveal small structural changes. Its advantage over ERCP is that it detects both parenchymal and ductal changes and doesn't use ionizing radiation. One of the disadvantages of EUS is that it's operator-dependent.

Add-on modules such as elastography and contrast-enhanced EUS (CEEUS) provide further details in evaluating pancreatic disease. Moreover, linear EUS can also provide tissue sampling (to differentiate an inflammatory mass from adenocarcinoma) and can guide therapy in chronic pancreatitis (celiac plexus neurolysis for unresponsive pain, pseudocyst drainage, main pancreatic duct drainage).

EUS characteristics of chronic pancreatitis have been grouped into ductal and parenchymal, focusing on 9 criteria which correspond to histopathological changes: 5 of them are parenchymal (hyperechoic foci, hyperechoic strands, lobularity, cysts/ pseudocysts, calcifications) and 4 are ductal (dilated pancreatic duct, irregular pancreatic duct, hyperechoic pancreatic duct walls, visible side branches) – see figures 5,6,7,8. Because not all of these criteria have the same importance in the diagnosis of chronic pancreatitis, these sonographic features have been redefined as major and minor features in a consensus classification called Rosemont.

According to the Rosemont criteria, EUS result can be23: consistent with chronic pancreatitis (1 major A feature + ≥3 minor features, or 1 major A feature + major B feature, or 2 major A features), suggestive of chronic pancreatitis (1 major A feature + <3 minor features, or 1 major B feature + ≥3 minor features, or ≥5 minor features), indeterminate for chronic pancreatitis (3 to 4 minor features, no major features or major B feature alone or with <3 minor features) or normal (no major features, ≤ 2 minor features – excluding cysts, dilated MPD, hyperechoic non-shadowing foci, dilated side branch).

Table 3. Sensitivity and specificity of imaging studies for the diagnosis of chronic pancreatitis[10]

	Sensitivity (%)	Specificity (%)
Ultrasound	60-70	80-90
CT	75-90	85
MRI/MRCP	85	100
ERCP	75-95	90
EUS	97	60

Figures 1,2: CT scan of a 40 years-old male, smoker, with chronic alcohol intake and history of multiple episodes of acute pancreatitis: Enlarged head of the pancreas with multiple cystic structures (up to 3 cm, compressing the CBD) and calcifications; large pseudocyst (8/7 cm) in the body-tail.

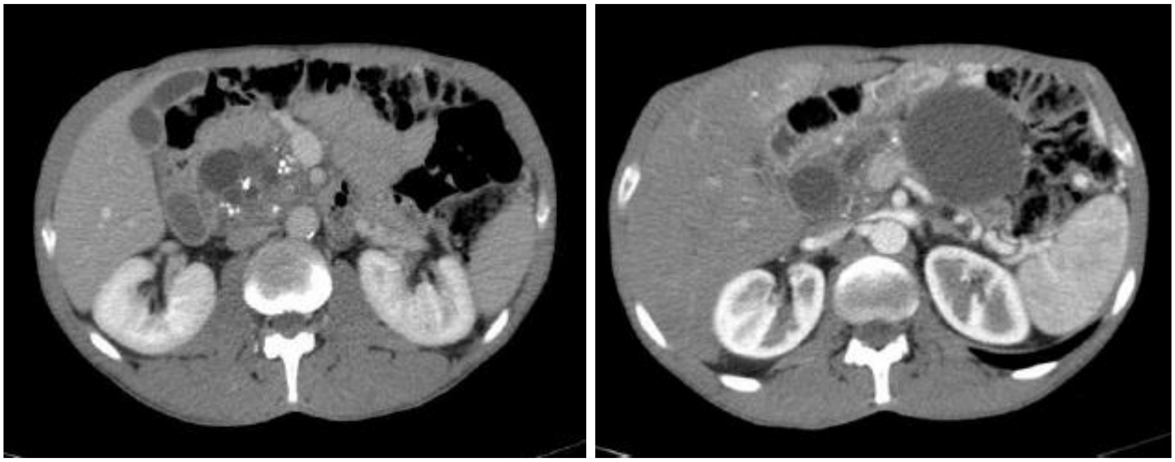

Figures 3,4: MRI and MRCP, same patient: Pseudotumoral chronic pancreatitis, 9/7 cm pseudocyst in the bodytail with splenic vein occlusion

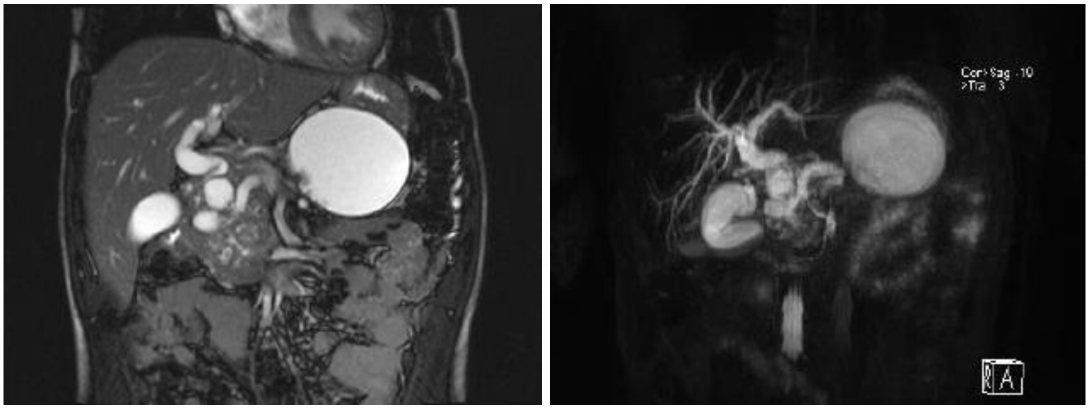

Figures 5-8: EUS examination, same patient: Inhomogeneous head of the pancreas with multiple calcifications (up to 6 mm) and cystic structures; large cystic lesion in the body of the pancreas with hyperechoic debris

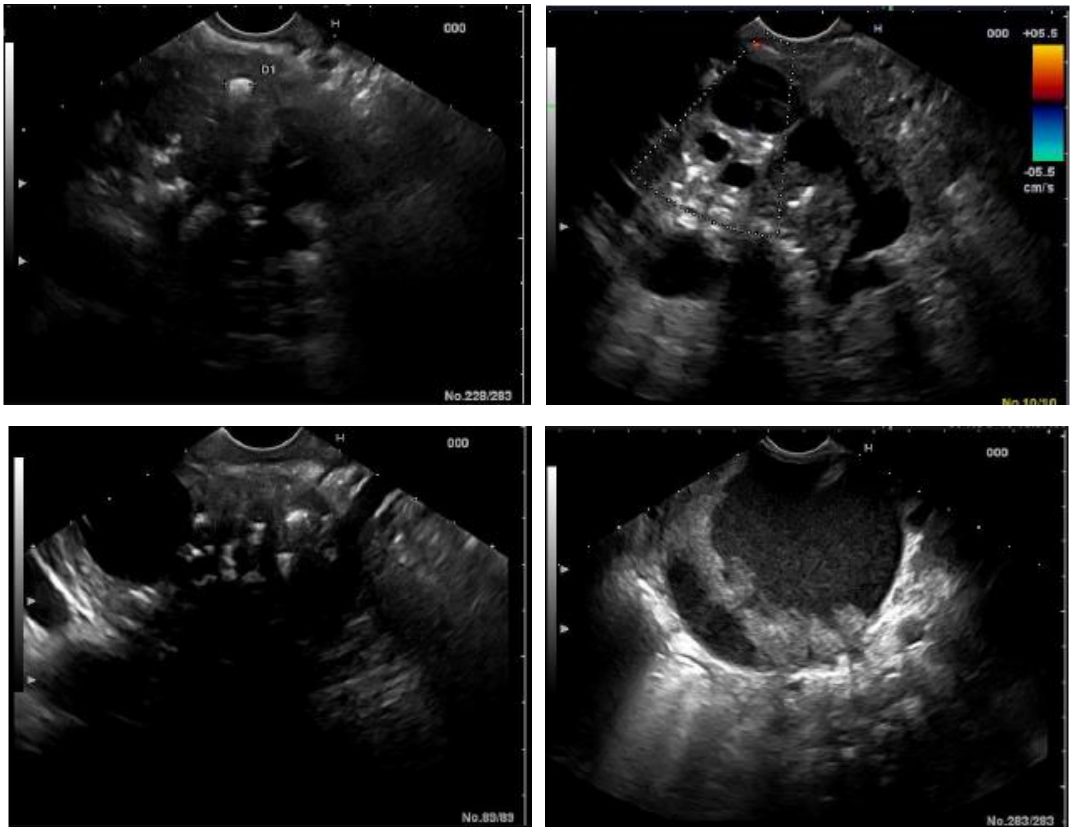

CONCLUSIONS

Imaging is becoming more and more important in the evaluation of chronic pancreatitis.

Among the imaging methods, plain abdominal radiograph is useful when calcifications are present and abdominal ultrasound is valuable for follow-up.

CT is best for identifying pancreatic calcifications and for evaluating complications of chronic pancreatitis.

ERCP can accurately detect changes in the ductal system, but it’s nowadays accepted only for therapeutic purposes. MRI with MRCP and EUS are currently the imaging methods of choice for the evaluation of chronic pancreatitis.

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CLINICAL PRACTICE

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Re-emerging infectious diseases: Ebola hemorrhagic fever

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Abstract: *The spring of 2014 has brought a new calamity, the exotic infectious disease: Ebola Hemorrhagic Fever, which is caused by a highly contagious and pathogenic virus, transmitted directly by interpersonal contact or indirectly by common usage of objects. The epidemic which occurred in Guinea tended to expand to neighboring countries; 83 deaths have been reported on April 1st 2014. Genetic analysis have revealed that the virus that causes this epidemic is similar in a proportion of 98% to Ebolavirus Zaire (EBOV) species that were responsible for the epidemic in Democratic Republic of Congo, in 2008.*

The Ebola virus belongs to the Filoviridae family, Ebolavirus genus and causes Ebola Hemorrhagic Fever, with a rate of fatality of up to 90% in humans. There are five distinct species: Bundibugyo Ebolavirus (BDBV), Ebolavirus Zaire (EBOV), Reston Ebolavirus (RESTV), Sudan Ebolavirus (SUDV) and Taï Forest Ebolavirus (TAFV).

Keywords: *Ebola, Hemorrhagic Fever, exotic diseases, epidemic*

INTRODUCTION

The spring of 2014 has brought a new calamity, an exotic infectious disease – Ebola Hemorrhagic Fever, which is caused by a highly pathogenic and virulent virus, highly contagious by interpersonal contact or through objects. Epidemic which first occurred in Guinea tended to expand to neighboring countries and beyond, on April 1st 2014, 83 deaths cases being reported. Genetic tests have revealed that the virus that causes this epidemic is similar in a proportion of 98% with Ebolavirus Zaire (EBOV) species, which were responsible for the epidemic in the Democratic Republic of Congo in 2008.

Ebola virus belongs to the Filoviridae family, Ebolavirus genus and causes Ebola Hemorrhagic Fever, a fatal disease to humans in more than 90% of cases. Five distrinct species have been identified: Bundibugyo Ebolavirus (BDBV) Ebolavirus Zaire (EBOV), Reston Ebolavirus (RESTV), Sudan Ebolavirus (SUDV), Tai Forest Ebolavirus (TAFV) by now (http://ecdc.europa.eu).

The virions have the characteristic appearance of rod or filaments in the form of U or 6, or entangled branched. Type of genome is RNA (ribonucleic acid) linear, single-stranded, negative-sense (1) (Figure 1).

Ebola Hemorrhagic Fever is an acute viral infection characterized by an incubation period of about 8 days, with extremes of 2-21 days. Onset occurs suddenly, with high fever, chills, headache, myalgia, anorexia associated with rash. In the next 2-3 days appear nausea, vomiting, pharyngitis and hemorrhagic phenomena, haematemesis, melaena, bruising and petechiae. Nervous disorders appear, accompanied by prostration, anxiety, confusion, and

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memory loss and balance disorders. Laboratory analyses show a low number of leukocytes and platelets and an elevated liver enzymes. Abortion in pregnant women occurs in 66% of cases and newborns of infected mothers fatally infected. Mortality in pregnant women is 95.5%.

Figure 1. Virus Ebola – electronic microscope image (Centers for Disease Control and Prevention, USA)

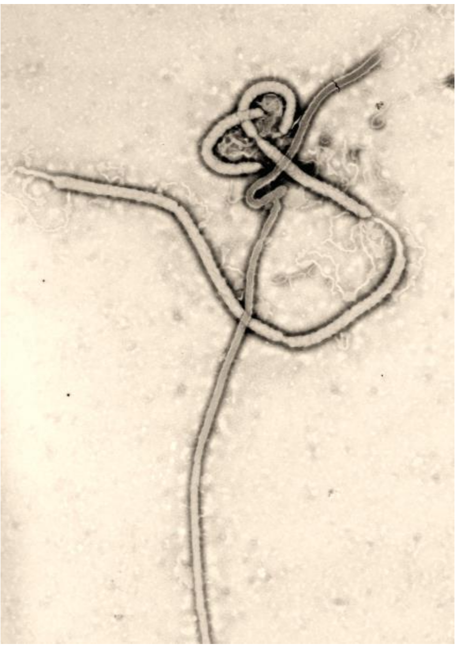

Death may occur, in 6-9 days after the illness debut, by hemorrhagic shock with massive and generalized bleedings. Virus and viral antigen are present at high levels in the blood and organs. In case of illness remission, convalescence is long and is accompanied by intensive hair fall for about 3 month, extreme fatigue, anorexia, abdominal pain, myalgia and arthralgia, hearing loss in 6 months after acute phase.

Myalgias and arthropathies are described even after 21 months. Infected people are contagious as long as their blood and secretions contain the virus which could be isolated from semen until day 61 after debut and persists for patients in recovering.

Viral RNA was revealed in vaginal, rectal and conjunctival samplings after 33 days, and presence of viral RNA in semen was demonstrated for 61, 82 and 91 days after illness onset (1).

EPIDEMIOLOGY

History. The Ebola virus was first identified in 1976 during the epidemic in Zaire (Democratic Republic of Congo) and Sudan. Natural infection cases were reported in Africa: Democratic Republic of Congo (1976, 1977, 1995, 2007, 2008, 2012), Sudan (1976, 1979, 2004), Gabon (1994, 1996, 2001, 2002), Ivory Coast (1994), Uganda (2000, 2001, 2007, 2011, 2012), Congo Republic (2001-2003, 2005), Guinea and Liberia (2014). In England (1976) was reported an accidental infection in a laboratory. Ebola was introduced into quarantine facilities in the USA (1989, 1990, 1996), in Italy (1992) and the Philippines (1996) (www.who.int, www.ecdc.europa.eu).

Ebola in Uganda/2012

On July 29, 2012 Ministry of Health of Uganda has notified the World Health Organisation (WHO) on an outbreak of hemorrhagic fever in a western area of the country, Kibaale, with 20 cases and 14 deaths. Since the beginning of the epidemic was reported a total of 24 human cases (confirmed or probable) and 17 deaths. Laboratory tests conducted by Uganda Virus Research Institute (UVRI) and Centres for Disease Control and Prevention (CDC) confirmed the presence of Ebola Sudan virus in 5 patients, from which 2 died. All reported infection cases and infected people contacts were investigated (www.cdc.gov). On October 4, 2012 Ministry of Health of Uganda said that the epidemic ended.

Ebola in Democratic Republic of Congo/2012

On August 17 2012, Ministry of Health of Democratic Republic of Congo reported an outbreak of Ebola hemorrhagic fever with 10 suspected cases and 6 deaths. Laboratory tests conducted by Uganda Virus Research Institute (UVRI) and Centres for Disease Control and Prevention (CDC) confirmed the presence of Bundibugyo Ebola virus in 2 patients. However, epidemic in the Democratic Republic of Congo it is not epidemiologically linked to the one in Uganda. Final Report of November 26 shows a total of 77 cases of which 36 were confirmed by laboratory tests, 17 were probable, 24 were suspicious and 36 deaths were reported (www.cdc.gov).

Ebola in Guinea and Liberia/2014

On March 22, 2014, the Ministry of Health of Guinea notified WHO about an outbreak of Ebola Hemorrhagic Fever in the forested southeast areas, rapidly evolving, with a total of 49 cases, including 29 deaths (fatality rate: 59%). On March 30, 2014 a total number of 112 suspected cases of Ebola virus infection were reported, from which 70 were deaths and on April 12; Centres for Disease Control announced 83 deaths; 35 of these cases were confirmed by laboratory tests carried out in several laboratories, including Pasteur Institute, Lyon in France, Institute Pasteur, Dakar in Senegal and Bernhard Nocht Institute for Tropical Medicine, Hamburg in Germany, using PCR (Polymerase Chain Reaction).

Liberia has reported 8 suspect cases, including 5 deaths, and two laboratory confirmed cases in people who recently travelled in Guinea. Ministry of Health of Guinea and WHO have implemented measures to control the spread of the epidemic and to restrict travels and trade till the problem is solved (www.cdc.gov).

The natural infection

Source of the virus still remains unknown, but it is suspected that virulent strains used as hosts could be species which come less in contact with people. The transmission is achieved by direct contact with sick animals or humans through blood, organs, secretions or excretions or by sexual contact. Contamination by aerosols (6), although it is not regarded as the main route of transmission, has been incriminated in the 1989 epizootic in Reston (the virus was isolated from monkeys).

The nosocomial transmission (by linen, syringes, needles and other contaminated material) should be considered to impose preventive measures. The susceptibility related to natural infection is generalized and the resistance to infection or re-infection is not known (1).

Biological weapon

The Ebola virus is a potential biological agent to be used in bioterrorism because it causes a highly severe disease and the means of defence against this virus are extremely poor for the moment. Russian researchers have shown that low doses of Ebola virus administered by aerosols could cause infections in monkeys, implying mortality.

There is no effective therapy for infection prevention, nor vaccines are available so this virus may be considered as a potential bio-terrorist biological agent (4). We should not forget that at the first outbreak of Ebola in Africa, among the international volunteers, representatives of the AUM sect from Japan also came whose they real target was to take with them virus strain samples, possible in intention to cause biological attacks. Fortunately, Interpol has prevented on time that fact, counteracting in an antiterrorist manner. There are suspicions that some armies would have developed an Ebola-Smallpox hybrid virus as a biological agent, hybrid which would benefit from a maximum of pathogenicity, virulence and infectiousness and for which there is no effective treatment.

LABORATORY DIAGNOSIS

Necessary samples for analysis in the acute fever stage are: serum, heparinied plasma, nasopharyngeal washings, urine, soft tissues blood samples, skin biopsies.

Necessary samples for analysis in the recovering stage are: semen, eye discharge.

Necessary samples for postmortem analysis are: fragments of organs (spleen, lymphatic nodes, liver, and kidneys).

After sampling, the pathological materials are going to be maintained in triple layered packaging, according to WHO recommendations (perfect sealed plastic bags with clear inscriptions, in resistant containers, protected against leakage).

These containers are to be proofed against outside surface contamination. Samples will be stored frozen in liquid nitrogen or dry ice. Due to the high contamination hazard, handling of infectious materials will be made with special precautions in laboratories that have an appropriate grade of biosafety, level 4, regarding personnel and technical equipment (1).

According to CDC recommendations, the virological diagnosis is presented in Table 1.

In Romania, suspicious samples could be analyzed for laboratory diagnosis at the National Institute of Research and Development for Microbiology-Immunology Cantacuzino (NIRDMI Cantacuzino) and the Institute of Virology "Stefan Nicolau" in Bucharest, but there are no facilities BSL4 (Bio-safety Level 4 Laboratory highly security).

In veterinarian medicine, the sample analysis may be performed at Institute for Diagnosis and Animal Health Bucharest (IDAH), where two laboratories BSL3 are functional. In Medical Center of Military Scientific Research in Bucharest (MCMSR) there is a highly secure microbiological laboratory, but is still under construction.

Table 1. Laboratory diagnosis schema for Ebola, according CDC

Timeline of Infection	Diagnostic tests available
Within a few days after symptoms begin	Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing IgM ELISA Polymerase chain reaction (PCR) Virus isolation
Later in disease course or after recovery	IgM and IgG antibodies
Retrospectively in deceased patients	Immunohistochemistry testing PCR Virus isolation

TREATMENT

The European Medicines Agency confirms that there is no specific treatment for Ebola Hemorrhagic Fever (www.ema.europa.eu). When cases of infection with Ebola virus occur, the sole treatment is the supportive one.

Patients are usually dehydrated and, therefore, require administration of fluids intravenous or oral with solutions that are composed of electrolytes (www.who.int).

Effective antiviral compounds do not exist yet and ribavirin has no effect on the Ebola virus. Administration of interferon has neither given satisfactory results nor in experimental treatments of disease neither in monkeys nor in patients.

Some promising results were obtained when 3-deazadenosine carbo-cyclic was used to treat experimental infection with lethal doses of Ebola virus in mice: survival rates up to 90% when treatment was started after 2 days from the infestation, and 40% after 3 days (1) .

PROPHILAXY

Since there is no clear data on the reservoir of infection, we are not able to speak about a classical approach for infections control. It may take measures of putting animals under quarantine at most, and in case of death of those animals or illness of carers, with fever over 38.5°C for a period longer than two days, it could be considered as a suspected Ebola virus infection and, accordingly, action would be taken.

Patients will be kept in strict isolation, possibly with bed isolation devices. All materials, objects, tools etc. which came in contact with the patient should be transported in a separate facility and sterilized. Health care personnel will be equipped with protective clothing (possibly sealed, with positive pressure), masks, gloves, shoes. It is desirable to prevent contamination by the aerosol, the air is recommended to be filtered through HEPA type filters (1). Patients' transportation is recommended to be made by sealed stretchers equipped with filtered ventilation system (www.karcher-futuretech.com), like that one presented in Figure 2.

Figure 2. Transportation system provided with patient decontamination facility (www.karcher-futuretech.com)

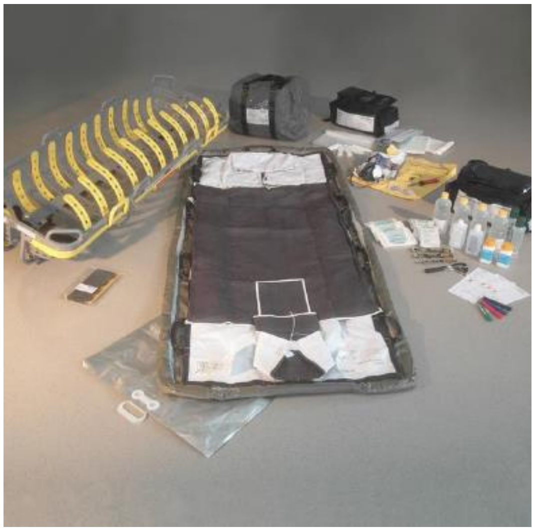

Vaccination

No approved vaccine is currently against Ebola virus currently available on market. However, laboratory tests are promising and their results are being evaluated (www.who.int). For example, a team of researchers from Arizona, is studying a vaccine that protects mice against infection with Ebola virus.

This vaccine includes a surface glycoprotein of the virus, combined with a monoclonal antibody and a chemical substance which activates biochemical cascade involved in natural immunity. More than 80% of the mice receiving this treatment, survived after administration of high doses of Ebola virus (5).

RNA experimental therapy has revealed satisfactory results in experiments on monkeys, researchers at Boston University School of Medicine suggesting that this therapy could induce protection in humans, providing hope for the future (3).

COMMENTS

According with World Health Organisation, the last Ebola Situation Report, from 11 February 2015 presents the following: “There have been almost 23 000 reported confirmed, probable, and suspected cases of EVD in Guinea, Liberia and Sierra Leone, with almost 9000 reported deaths (outcomes for many cases are unknown).

A total of 65 new confirmed cases were reported in Guinea, 3 in Liberia, and 76 in Sierra Leone in the 7 days to 8 February.

Six countries (Mali, Nigeria, Senegal, Spain, the United Kingdom and the United States of America) have reported a case or cases imported from a country with widespread and intense transmission.

The introduction of a case into unaffected countries remains a risk for as long as cases are reported in any country. With adequate levels of preparation, however, such introductions of the disease can be contained with a rapid and adequate response.

The response to the EVD epidemic has now moved to a second phase, as the focus shifts from slowing transmission to ending the epidemic. To achieve this goal as quickly as possible, efforts have moved from rapidly building infrastructure to ensuring that capacity for case finding, case management, safe burials, and community engagement is used as effectively as possible.”

CONCLUSIONS

Origin and virus reservoir remain unknown and have to be pursued by:
- development of new systems for early diagnosis of Ebola virus infection, which could help epidemiological investigations;
- monitoring of suspicious areas in order to determine the incidence of the disease;
- acquiring extensive knowledge on natural virus reservoir and mode of transmission, which could be useful in preventing epidemics.

Epidemics in Uganda and the Democratic Republic of Congo have been monitored by the World Health Organization (WHO) and were limited.

Currently, they are trying to limit the epidemic in Guinea, which is under observation WHO.

It is necessary to establish anti-epidemic measures in relation to the affected countries to prevent a possible import of Ebola virus. It is highly recommended to avoid travelling to epidemic areas.

Furthermore, this epidemic should be a lesson -

learned for the upcoming epidemics.

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CLINICAL PRACTICE

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Gallbladder strangulation within a recurrent incisional hernia: An unique cause of acute cholecystitis

A. Popențiu[1], D. Moga[1], C. Weber-Lauer[2], C. Nieman[3], D. S. Kauvar[4], D. Sabău[5]

Abstract: *We present a report of a unique surgical entity: acute cholecystitis caused by the gallbladder strangulation within a recurrent right upper quadrant incisional hernia. A previously healthy 39 year-old male patient presented to our Emergency Department with abdominal pain, nausea, vomiting, and a tender mass in the right upper quadrant of the abdomen, where an incisional hernia was palpable. He had a history of a gunshot wound to the right upper quadrant and multiple operations to include repairing mesh of the incisional hernia in that area. Abdominal computed tomography demonstrated an acutely inflamed gallbladder within the recurrent hernia. Open cholecystectomy and primary hernia repair were performed. Intraoperatively, the gallbladder appeared being strangled within the hernia. The patient recovered uneventfully and no cholelithiasis was observed on gross examination of the gallbladder. Though there have been a few reports of gallbladder strangulation within primary incisional hernia, ours is the first to describe this phenomenon in a recurrent hernia.*

INTRODUCTION

Neither incisional hernia nor acute cholecystitis is uncommonly encountered in surgical practice. While the presence of an abdominal wall hernia in the right upper quadrant (RUQ) might be complicated by the presence of a herniated gallbladder, strangulation of the gallbladder within an incisional hernia is an exceedingly rare cause of cholecystitis, with only a handful of cases reported in the literature. We are presenting such a case, the first reported with a recurrent incisional hernia as the cause of cholecystitis.

The presence of an incisional hernia in the RUQ might be complicated by the presence of the herniated gallbladder. This situation is facilitated by anatomic variations of the gallbladder, such as the presence of a complete gallbladder mesentery, and also by the location and the size of the abdominal wall defect.

The acute cholecystitis of a herniated gallbladder can be triggered by the presence of the stones (obstructive mechanism), or by strangulation or torsion.

CASE REPORT

A previously healthy 39 year-old male with a history of a gunshot wound to the RUQ with injury to the colon, six years prior to presentation, had undergone

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multiple abdominal operations concluding with mesh repair of midline and RUQ incisional hernia, two years prior to presentation to our Emergency Department, complaining of a 48-hour history of severe and unremitting RUQ abdominal pain, nausea, and vomiting. The patient reported no changes in his bowel habits, melena, or hematochezia.

Physical examination revealed a 5cm tender mass in the RUQ, underlying a 12cm subcostal incision through which a recurrent incisional hernia was palpable. The mass was clearly within the hernia and was irreducible. The patient reported that the mass had been present and slowly enlarging for about eighteen months. The patient's other abdominal incisions in the midline and the right lower quadrant were well-healed and without indication of hernia. Vital signs and laboratory evaluation were normal with the exception of a mild leukocytosis (11.6 x 1000/mm^3).

The abdominal CT scan demonstrated the fundus of the distended, inflamed gallbladder herniated into a 8 cm. AP / 10 cm. CC fascial defect, distended to 10 cm. long. The gallbladder wall is thickened to 4 mm. and there is hyperenhancing mucosa and extensive pericholecystic inflammation. No gallstones are seen. There was no dilatation of billiary duct. The muscles of the abdominal wall are atrophied, and the right incisional hernia has been previously closed with mesh. An injury of the caecum or the ascending colon was presumed as a result of previous abdominal gunshot wound. A previous diverting ileostomy was closed, but the loop of terminal ileum remains adhered to the right abdominal wall.

Into OR, a right subcostal incision was performed through the previous incision. The gallbladder fundus wall, edematous and under tension, was exposed in the subcutaneous fat, under the previously placed prosthetic mesh. The hernia defect was exposed under the mesh, with a hernia sac filled with the gallbladder fundus between the muscle and the inferior aspect of the prosthesis. After opening of the abdominal wall, the distended gallbladder was exposed with the lysis of multiple cholecysto-enteral and cholecysto-epiploic adhesions. Cholecystectomy was performed in a top-down fashion, with drainage of the sub-hepatic space. The existing mesh was not explanted and we closed the abdomen primarily, suturing muscle to muscle and mesh to mesh where appropriate.

Figure1. Postcontrast CT. Distended and inflamed gallbladder herniated. The gallbladder wall is thickened to 4 mm. and there is hyperenhancing mucosa and extensive pericholecystic inflammation.

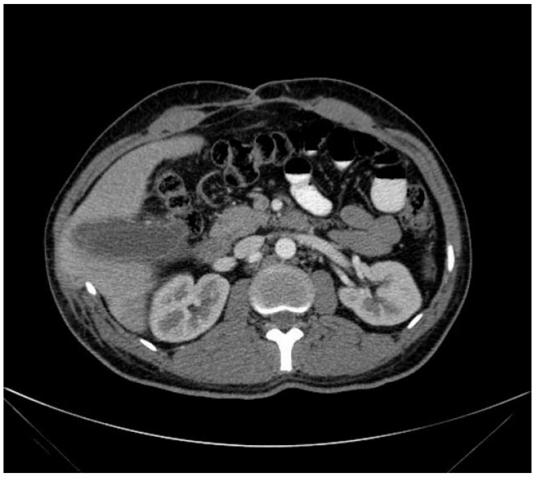

Figure 2. No gallstones are seen. There is no billiary duct dilatation. The muscles of the abdominal wall are atrophied, and the right incisional hernia has been previously closed with mesh.

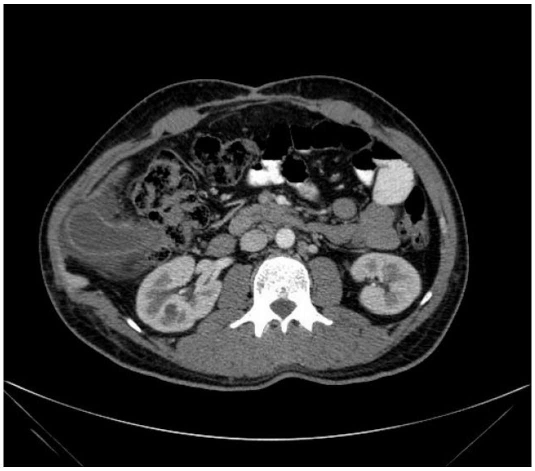

DISCUSSION

The incidence of incisional hernia is between 2% for clean, elective surgeries, and 20% for contaminated cases (Hodgson, Hoer).

Suture repair of incisional hernia results in recurrence rates of 12% to 54% (Anthony, Luijdenijk), while mesh repair is followed by a rate of 2% to 36% (Korenkov, Mc Lanahan)

Gunshot wounds, considering the high velocity of projectiles, is the most common cause (64%) of penetrating abdominal trauma, followed by stab wounds (31%) and shotgun blast wounds (5%). The injury patterns differ, depending on the weapon. Low-velocity stab wounds are, generally, less destructive and have a lower degree of morbidity and mortality than gunshot wounds and shotgun blasts. Gunshot wounds and other projectiles have a higher degree of energy and produce fragmentation and cavitation, resulting in greater morbidity.

The most common morbidities following penetrating abdominal trauma are wound infection (2- 8%) and intra-abdominal abscess with or without sepsis (10-80%, depending on presence or absence of bowel injury in combination with major vascular injury). The wound infection, together with the extensive destruction of the abdominal wall, leaded in our case to the recurrence of the incisional hernia.

The presence of an incisional hernia in the RUQ might be complicated by the presence of the herniated gallbladder. In order for the gallbladder to herniate through the defect, it needs to have some particular anatomic predisposing factors, same factors that led to gallbladder volvulus: the presence of a gallbladder mesentery, long longitudinal axis. Both of these feature were present in our case.

The acute cholecystitis of a herniated gallbladder can be triggered by the presence of the stones (obstructive mechanism), or by the strangulation or torsion. Since there was no obstruction, and the intraoperative findings demonstrated the herniated gallbladder through the abdominal wall hernia, we can conclude that the mechanism involved in our case was the strangulation.

There were only two previous cases reported in the literature to describe a gallbladder strangulation within primary incisional hernia (Benzoni, Herich). This is the first case ever reported involving a recurrent incisional hernia. The clinical examination offered enough data to suggest the diagnosis, but the CT scan and the supplementary US examination confirmed and documented the findings.

CONCLUSIONS

The GSW of the abdominal wall have a great risk of developing incisional hernias, and the recurrence rate after the repair, even with mesh, is significantly high. In the rare event of an incisional hernia in the RUQ, and a gallbladder with long mesentery, the strangulation within the hernia sac hernia is possible.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Competing interests. The authors declare that they have no competing interests.

Authors' contribution. A. Popențiu, D. Moga, C. Weber-Lauer, C. Nieman conceived the study, carried out a detailed literature review, collected and presented the pertinent data. D. S. Kauvar and D. Sabău participated in the study design and coordination and helped to draft the final manuscript. All authors read and approved the final manuscript.

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CLINICAL PRACTICE

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The surgical treatment of decubitus lesions

Argentina Vidrașcu[1]

Abstract: *Several methods have been exercised during the past years, thus offering the surgeon the possibility to choose when planning reconstructive surgery. Among these methods, we recall: the dislocation of a large surface of skin and fasciocutaneous flaps, the transposition of the muscle and its covering with a simple cutaneous flap or a cutaneo-adipose flap, musculocutaneous pediculate flaps, neurovascular flaps.*

The study was conducted on 292 patients with 3rd and 4th degree decubitus lesions admitted into and operated at the Plastic Surgery and Reconstructive Microsurgery ward during 10 years. Pediculated flaps on septofasciocutaneous perforating arteries or on musculocutaneous perforating arteries are more difficult to harvest (they require a precise knowledge about the localization of the perforating artery) but provide a better post-op result.

INTRODUCTION

The surgical approach of decubitus lesions has changed greatly during the last decade. Some methods were used predominantly at the beginning of the last century, other just in the past ten years. Anyhow, a classical method can be used at any time, and a modern method may be too much for a patient at a certain moment during his/her evolution. As such, depending on the therapeutic prescription, methods may be interposed.

Several methods have been exercised during the past years, thus offering the surgeon the possibility to choose when planning reconstructive surgery. Among these methods, we recall: the dislocation of a large surface of skin and fasciocutaneous flaps, the transposition of the muscle and its covering with a simple cutaneous flap or a cutaneo-adipose flap, musculocutaneous pediculate flaps, neurovascular flaps.

All these are selective methods for the treatment of complications arising from previous surgical interventions.

Tobin and Brown recommend the following for the treatment of complicated decubitus lesions:

- For good quality tissue but with a large ulceration (more than 10 cm), they recommend tissue dislocation, free flap or pediculate flap:
- For afflicted tissue with a large ulceration (more than 10 cm), they recommend flaps on perforating arteries or pediculate flaps.

We should give some examples of a few closure methods for defects of soft tissue in areas where usually decubitus lesions appear.

PREREQUISITES OF THE STUDY

In complementing the previous study, we turned to compare two surgical treatment methods for decubitus lesions. One of these methods consisted in applying flaps on random circulation, and the other one in harvesting and applying flaps that contain a vascular network capable of ensuring its survival, developed from the perforating artery or arteries at the level of the muscle mass directly towards the tegument (musculocutaneous) or from the level of the muscle mass towards the tegument via the fasciae or the septal formations (128). These perforating arteries may be anatomized or dissected along with a portion of the muscle wherefrom they emerge or along with the septum or fascia fragment.

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OBJECTIVES

Starting from data from the literature, we strived to demonstrate that the number of surgical reinterventions (immediate relapse) in the case of the batch submitted to the surgical treatment with flaps on perforating arteries is more reduced than for the batch of patients submitted to surgical treatment with flaps on random arteries or free skin grafts.

MATERIAL AND METHODS

The study was conducted on 292 patients with 3rd and 4th degree decubitus lesions admitted into and operated at the Plastic Surgery and Reconstructive Microsurgery ward during 10 years.

The age distribution of patients reveals a maximum incidence of bed-sores between the ages of 25 to 45; 135 cases (figure 1), predominantly for males.

Half of the cases included in the study (146 cases) presented lesions localized at the level of the sacral region. The greatest number of cases, both with trochanteric localization as well as with sacral localization, was recorded in 2004.

Figure 1. Graphical representation for the distribution of the number of decubitus lesions depending on their localization

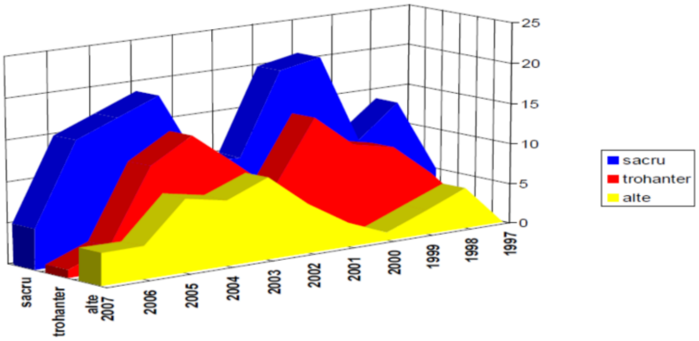

Group no. 1: tegument flaps on fasciocutaneous or musculocutaneous perforating arteries.

Patient selection criteria:
98 patients who were submitted to surgical treatment with tegument flaps on fasciocutaneous or musculocutaneous perforating arteries;

The candidates for surgical interventions are patients for whom conservative treatment and chemical or physical debridement was performed with the same methods: lavage with Betadine solution and mechanical debridement using the scalpel.

Exclusion criteria:
Patients whose biological status did not allow for the execution of the surgical intervention;
Patients diagnosed with neoplasm.

Group no. 2: tegument flaps on random circulation or skin graft.

192 patients with 3rd and 4th degree decubitus lesions who were submitted to surgical treatment with a prescription for tegument flaps on random circulation or free skin graft.

The work method used within the batch with the application of flaps on perforating arteries

The distinctiveness of this work method for this batch consisted in the specific harvesting of flaps with the anatomization of one or two perforating arteries. These were identified using the Doppler method thereafter the tegument flap was designed based on the localization of the perforating artery and on the surface of the defect remaining after the excision of detritus from the decubitus lesion.

In the case of septofasciocutaneous perforating arteries, the tegument flap or cutaneous-adipose flap was harvested with a portion of the septum or fascia that the perforating artery crossed (figure 2).

Figure 2. Musculocutaneous perforating arteries depending on their localization

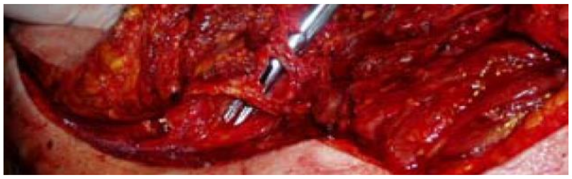

CONCLUSIONS

Although the vascularization of the areas with the highest frequency of development for decubitus lesions presents right networks of anastomosis, results indicate that the number of surgical reinterventions is higher for the batch submitted to treatment with flaps on random circulation.

Pediculated flaps on septofasciocutaneous perforating arteries or on musculocutaneous perforating arteries are more difficult to harvest (they require a precise knowledge about the localization of the perforating artery) but provide a better post-op result. This is owed to the fact that they have a better vascularization which contributes to the draining of the infection remaining at the level of the apparently healthy tissue, as well as in the formation of fibrous scar tissue through the coalescence of the wound.

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Communication barriers in therapist – patient – caregiver relationship

Iuliana Guiță – Alexandru[1]

Abstract: *The success or failure of a therapeutic approach, medical or psychological, are influenced by many factors. Among them we can mention the patient's insufficient knowledge about the disease, preconceptions of patient or caregiver's fear of stigmatization, the issue regarding duration and side effects of treatment, cost of medicines and so on. Last but not least, the therapist's ability to communicate accordingly to the beneficiaries' comprehension and barriers encountered in the communication between actors directly involved in the therapeutic process (patient caregiver, psychologist, and doctor) can change the opportunity to obtain a suitable therapeutic benefit yet from beginning.*

Materials and methods: In order to assess the concordance between the messages sent by doctors and how they are received by patients and caregivers, we have applied a different questionnaire for the three categories of respondents mentioned above. These three distinct sets of questions were completed during 100 psychiatric consultations, in ambulatory regimen.

Results: Comparative analysis of the questionnaire results has shown major differences between the information that doctors thought they had sent and what was actually received by patients and caregivers. Paradoxically, the more medical explanations were elaborated and detailed, the less volume of adequate information was taken home by beneficiaries.

Conclusions: It is necessary for each of us to have a self-assessment of how we communicate with patients and which is the real benefit that we offer through our words. It is also mandatory to adapt the „language of medicine" to the common people understanding abilities (without medical training).

Keywords: *communication, relationship therapy, case management, psycho-education, occupational health, therapeutic benefit, therapeutic alliance.*

ADHERENCE TO TREATMENT

1 of 2 patients does not follow the therapeutic/ psychotherapeutic prescribed program.

Theoretically, the psychotherapeutic process should be simple, direct and fast, so that the service beneficiaries to have access as early as possible to a specialized consultation, after which to establish an optimal therapeutic conduct in order to provide significant improvement or even cure the health issues.

Everyone involved in this process of health (re) generating are convinced that everything is impeccable in respect of themselves: conforming to Oath of Hippocrates, the physician ensures that "... as long as my strength and my reason will help me, my prescriptions will be made only against sickness and for the benefit and good condition of the sick people" and provides a correct diagnostic (psycho-diagnosis) and an appropriate treatment, the patients and their relatives understand the information provided about the disease and the importance of following a correct conduct, the patient strictly follows the treatment schema and returns to periodical control and psychological advising, ideally considering that the psychologist and the psychiatrist complement each other no matter the case case.

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Comparative assessment of the amount of prescription drugs in medical offices and pharmacies obtained (EFPIA - European Federation of Pharmaceutical Industries and Associations) shows that only 50% of prescriptions are being released from pharmacies. In Europe, the non-adherence to treatment "costs" annually state budgets for health about 125 billion euros and contributes to 200,000 premature deaths recorded per year.

Poor therapeutic adherence can have an immediate negative impact on the clinical benefits of treatment (impact felt by the doctor, the patient and the patient's relatives) and also a negative social echo, more extensive on medium and long term because of sub-optimal health problems solving, lack of quality remission, incomplete recovery or when the certain diseases become chronic.

To improve this alarming situation that 1 of 2 patients simply do not succeed in adhering to the prescribed treatment, increasing collaboration among all involved factors in the therapeutic process is more than evident. Especially, the interaction between health professionals and patients should be improved in order to ensure not only to diagnose and treat properly, but also that the therapeutic recommendations are eventually complied with. Most often the subject „passes over" the psychological counseling, which is absolutely necessary because, during the specialized clinical interview, helpful elements are revealed in order to achieve a correct approach.

TYPES AND CAUSES OF NON-ADHERENCE

The problem of non-adherence to intervention of any kind is not new. Hippocrates (460-370 BC), the most famous physician of ancient Greece considered to be the "father of medicine", recommend that the physicians "to be aware of the reasons why patients tend to lie about how they take the prescribed medicines". More recently, Charles Everett Koop (1916 - 2013), vice-admiral of the US Public Health Service Commissioned Corps, chief-physician of the US from 1982 to 1989, summarized this problem in the dictum "Medicines do not work in patients who do not take them!"

Sokol et al. (2005) and Ho et al. (2009) have mentioned two types of non-adherence to the intervention, everyone having a different point of view regarding the patient.

The first way of seeing the patient presents a patient "guilty" of unwillingness, uninterested in his health, lazy, disobedient, who simply does not make a minimal effort in taking prescribed medications.

The second pattern highlights the non-adherence caused by a rational, intentional response of the patient facing with some disagreed recommendations. In this situation the patient, who, after leaving the cabinet or the hospital, never completes the recipe, raises medicines from the pharmacy or takes these, even if purchased on his behalf by someone else.

The fact that the patient does not comply with treatment recommendations, usually generates revolt and frustration for therapist. Basically, the therapist believes that he consumes his time, energy and skills to act in the interest of a person who seems not to do the same for himself. However, in real life, the events do not evolve so simple and linear as we have described in the earlier theoretical model.

The therapist (clinical psychologist) has not always have the necessary time, patience, understanding, empathy or absolutely necessary communication skills for a discussion with beneficiaries. It happens sometimes that the patient does not get to have a face-to-face contact with the therapist, so a conversation about diagnosis, disease severity or efficacy and safety of treatment is even less probable. Studies have shown that 50% of patients do not agree with the posed diagnosis or recommended treatment and the lack of proper communication with mental health professionals, reach either at self-psycho-diagnosis or even at self-treatment, either to seek pseudo-medical aid (provided by relatives, friends or internet). That issue touch a relatively new domain in Romania, namely the concept of Occupational Health obviously correlated with the education of population in that domain. Occupational health psychology has earned a special place in psychological theory and practice, being related to health psychology and derived from organizational psychology. The objective of this sub-domain of psychology is to study healthy work environments, defined as those in which individuals, using their skills and talents, achieve high performance, high satisfaction and psychological well-being.

This context in which the pro-intervention information is not consistent enough to counter the avalanche of counter-intervention arguments (generated by fear of disease or treatment, feeling of shame or dysfunctional emotions, financial reasons, fear of stigma, preconceived ideas and so on), pops up an issue: patients do not come to specialist!

COMMUNICATION – AN ESSENTIAL FACTOR IN THERAPEUTIC SUCCESS

In nowadays medical world when the professional activity is exercised more and more along the lines of "poor funding - lack of time - Increased request" is harder to remember that we are treating people, but not medical cases. The image of therapist today is different of patient expectations: instead of a benevolent advisory actor, a counselor of the patient concerning what is good or bad for him, the pattern of common doctor is rather of an exhausted and annoyed person, in a permanent rush between ambulatory cabinet and inmate patients in the ward and patients in the emergency room.

In the preface of his book "A Curious Calling: Unconscious Motivations for Practicing Psycho-therapy" (M. Sussman - Ed. Three, 2011), the author says that "narcissistic and exaggerated aspirations as well as overestimation seem to be almost universal among the beginner therapists (Sharaf and Levinson, 1964). What are their aspirations? Maltsberger and Buie (1974) believe that the three most common traps are narcissistic aspirations to cure everyone, to know everything and to love everyone - all doomed to failure, leading therapists to magical and destructive answers." Thus, the lack of communication with the beneficiary can conduct to a slightly defensive behavior of the therapist meant to build a protective shield against questions, indecision or doubts related to his clinical approach, coming from the patient or his caregivers.

O. Sacks, neurologist and psychiatrist, in his book "A Leg to Stand On" (Humanitas Ed., 2013) describes the personal experience of a doctor who, after an accident, pass to the other side of "the fence" and becomes a patient. On this occasion is analyzed, initially with surprise, then with consternation and anger, finally with comprehension, the distant and professional attitude of the doctor which is in conflict with almost desperate need for information and ensuring of the patient.

LIMITS OF COMMUNICATION WITH THE PATIENT (BENEFICIARY) AND THE CAREGIVERS

In accordance with current legal provisions, the duration of medical consultation should be limited to a maximum of 15, 20 or 30 minutes, depending on the specialty. In that time the specialist, psychologist or psychiatrist, should receive the patient, introduce himself, find out the reasons of the visit, obtain as complete history of the problem, listen carefully to establish one or more diagnoses, debate an intervention scheme with the patient and caregivers, provide information about intervention (benefits, side effects, duration, risks, warnings, others) and regimen, register data in registry and computer, release documents as appropriate (electronic prescriptions, treatment regimens, medical letters, sick leave etc.).

In this context, verbal communication is strictly reduced and, instead of empathy, ensuring and benevolent neutrality, a non-verbal recommendation appears: "you have to do as I say, because I know better what disease you have and what treatment do you need"!

The patient perceives such behavior as mandatory and discretionary, which further increase his previous resistance in coming to specialist as a defense mechanism against unpleasant feelings.

The problem of time allocated to the communication with patient is widely considered worldwide.

At a minimum, the patient should be informed, has to understand and must accept that:
1. He has a problem;
2. The problem may present health risks if not treated;
3. The recommended treatment, if necessary, is appropriate, safe and effective.

Prelipceanu et al. (2011) has suggested that all patients visiting a psychiatrist should be informed in detail about the therapeutic effects and side effects of any pharmacological agent that they receive. From the psychodynamic perspective, the notions of transfer, counter-transference, resistance and therapeutic alliance are equally important when prescribing medication as when psychotherapy is performed. A positive transfer and a therapeutic alliance could have a positive influence on adherence to treatment. The benefits of combining psychotherapy with drug therapy are supported by numerous studies and consist in improving compliance and ensuring a complex care of the patient, more efficient.

A DETAILED COMMUNICATION IS NECESSARILY BETTER?

In a symposium held at the National Conference of Psychiatry in 2013 (Targu Mures) Michael Davidson, professor of psychiatry at the Sackler School of Medicine Tel Aviv University and Mount Sinai School of Medicine NY, launched a provoking debate on consistency between messages that the therapist believes them transmitted to the patient and what the patient actually received.

The subject is too large to be analyzed in depth without an enlarged study, well structured, multi-centered and extremely well balanced between the various medical specialties. However, to evaluate the messages sent by therapists and how they are perceived by patients and caregivers, we have designed and implemented a simple questionnaire with similar questions for the three categories of respondents. The three distinct sets of questions were completed for 50 psychiatric or psychological counseling sessions (both initial consults as well as control) under specialty ambulatory (each lasting 30 minutes).

Subjects and their caregivers were randomly selected, with the only conditions of being sure they can understand the questionnaire requirements and they can freely provide required answers. The questionnaire included both open-response questions and closed-answer questions, as follows:
1. Please note, with a note between 0 to 10, the allocated time for information about the diagnosis and treatment (0 = totally insufficient; 10 = extremely high);
2. Please note, with a note between 0 and 10, availability of necessary information provided to patient/received from psychologist/psychiatrist (0 = very difficult to understand; 10 = very easy to assimilate);
3. Please note, with a note between 0 and 10, specialized information relevance provided to patient/received by the psychologist/psychiatrist (0 = completely irrelevant; 10 = highly relevant);
4. Please indicate the most important three (3) peaces of information that you have transmitted to patient/you have received from specialist, related to diagnosis and/or to received treatment;
5. Please indicate what other information you wish you had transmitted to patient/you wish you had received from the therapist, related to the diagnosis and/or to the received treatment.

For the questions from 1 to 3 differences between the scores given by the therapist and those of recipients were not significant: an average score of self-assessment of therapists 8.4 versus 7.8 of the patients and 8.0 of caregivers. Given the open nature of the questionnaire and respondents awareness that the evaluation will take place, it is expected that health professionals manifest a little more than usual the interest to communicate and the politeness of beneficiaries may have result in sparing pride of the therapist.

Surprises however occurred at comparative analysis of open responses, which showed significant differences between what information therapist thought he had sent and what was actually received by the patients and caregivers.

Frequently encountered types of answers to the question referring to messages sent, in descending order of frequency of occurrence, were related to questionnaire.

They may say that if specialist considered important messages related to objective reasons to be followed for effective treatment, patients and caregivers were more interested in the subjective aspects of the disease and treatment.

Questionnaire:

Therapist	Patient	Caregiver
Information transmitted to the patient/caregiver by therapist		
1. risks of untreated disease	1. disease severity	1. side effects of treatment
2. efficacy of the treatment	2. risks of untreated disease	2. risks of untreated disease
3. disease severity	3. side effects of treatment	3. efficacy of the treatment
Information that would have been transmitted		
	• causes of disease • duration of treatment • impact on family life, professional, social • risk of transmission to others	

For me, the need of some additional information regarding these differences between disease and intervention is directly and indirectly involving: the psychologist/psychiatrist believes that said everything there is to say, while the beneficiaries want to know what triggered the disease, how long therapeutic approach must be followed and what to do once he is at home.

Another aspect worth to be mentioned is that, paradoxically, the more space for the therapist was filled with information complete and complex, the less was completed by beneficiaries. In other words, the more explanations were elaborated and over-detailed, the less information beneficiaries took home.

DISCUSSIONS

The quality of "public employee" of health professionals brings not only the attribute of service provider, but also a bit of self-protective and annoyed attitude of one who does not want to be excessively strained with others problems. So, if I do my job well in the profession what I learned and I perform it with so many sacrifices, the patient is obliged to follow my decisions without comment. If he does not, then you are fully entitled to revolt, refusing other consults and even being a little more incisive when he will ask for help.

From another point of view, the patient knows that he depends on the help of the therapist, the skill and his dedication for the work, but is scared that the disease is his (not of the therapist), with major implications on his life and intimates and approach to be followed is his and not of someone else (good or bad, well tolerated or not accessible or very expensive). And all these worries are multiplied by stories published by media with doctors not very helpful or not very competent.

Between these two points of view a binder, a solid bridge must be found. This may be the communication between all involved factors, most reliable basis for the concept of "therapeutic alliance".

In conclusion, it is necessary, for each of us, an objective and rigorous self-evaluation of how we communicate with patients and of the real benefit we offer by words.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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Acknowledgments

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been

obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.

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EDITORIAL

The Romanian Military Medical System transformation strategy

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Facing the Romanian military sistem changeover determined by the affiliation to international security alliances and by the necessity to adapt it to global and regional security environment in order to be able to cope with actual threats and risks, the military medical system should be adapted in order to become efficient in providing medical support in operations and in the same time to provide medical care for soldiers and their families.

The military medical system transformation strategy represents the basic document which reflects the picture of desired end state showing the roads to get there.

The major challenge is represented by the harmonizing process between national and operational NATO laws and rules, because the interoperability is one of the main tasks in National Security Strategy.

Excellent trained medical personnel, establishing the standards of medical logistic support and procedures, the adapting process of the medical structures are the key points of this strategy, being in the same time the main courses of action.

Having the medical military system as a picture on the wall, it can be described as a dual functional mechanism, depending on its acting conditions: operational medicine providing medical support in operations, and stationary medicine providing medical care in peace time. In order to be efficient, the military medical system should have the following features: to be complete from the battlefield through all stage off care, to be self-sustained, to be deployable with troops, and to be able for integration in multinational operational medical system and in the national civilian medical system as well.

The two main missions of the medical military system are preservation of health and the second, treating casualties and healing ill soldiers and their families. During all the actions, in order to accomplish both previous missions, the following general principles will be applied: the best medical practice, continuity of care, the interest of patient and the importance of the mission. This complex system works with excellent trained medical personnel in military medical educational system and combat medical training centers, having a proper medical logistics. For that, beside regulation domain, a significant financial effort will be needed. Moreover, a coordinated long time term financial strategic program to provide proper medical supplies will solve logistical issues in medical military system. Medical research and standardization processes are the engines to progress of all parts of the military medicine. A dedicated and efficient command-control system, working under well defined procedures will integrate medical considerations in the complex military mechanism.

The implementation plan will have the Medical Directorate in the center of the process, being involved from the highest to the smallest level of the medical support, using the military hospitals and the others subordinate structures to coordinate medical activities and to optimize the whole process.

Eventually, in the operational medicine domain every battalion level unit will be supported by a ROL 1 medical facility, every brigade level unit by a ROL 2 medical facility, all military hospitals except Central Universitary Emergency Military Hospital will fulfill the ROL 3 medical facility criteria, and Central Universitary Emergency Military Hospital will be the Romanian ROL 4 medical facility.

An important goal in the development of military hospitals will be represented by involving them in the training activities in their area of responsibility, so six military hospitals near the main ranges will have specific capabilities in order to be able to solve all medical situations with can be a result of high risk activities.

The medical military system will be fully integrated in the operations at all level by a professional and dedicated medical planning system which is the key of an effective medical support.

In the stationary medical domain, the target is to grow up the quality of the medical act from primary medicine to hospitals, in the mean time to prioritize the access of soldiers and their families to military medical system. Another important goal is to optimize the procedures for soldiers to get their legal rights regarding the payment for medical services and pharmaceutically services.

Last but not least, the military medical system should become the most available medical solution for soldiers and their families on the free medical services market.

The military medical system is a special medical system serving a special structure which is Romanian Military Force.

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REVIEW ARTICLE

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The diagnosis of sepsis using POCT in the Emergency Department – Medical and legal implications

Bogdan C. Teușdea[1], Sebastian Dogaru[1], Florentina Ioniță Radu[1,2]

Abstract: *Sepsis, severe sepsis and septic shock are some of the most serious affections which threaten the lives of the patients who come to the Emergency Department and which require fast treatment because the more severe the sepsis was, the higher the mortality, up to 50% higher in severe sepsis. That is why, at present, the 2013 Guides of Surviving Sepsis Campaign recommend that the potential source of infection should be confirmed as soon as possible, in the first 6 hours since the patient arrived in the Emergency Unit if possible, moreover the large spectrum antibiotics therapy must be administered in one hour after the severe sepsis or the septic shock were identified. That is why the identification of these patients at risk is very important and this identification can only be made using POCT type devices.*

This type of devices has the capacity to make precise determinations, in a short time (15-17 minutes), using minimum quantities of integral blood, without using test tubes, sepsis biomarkers and other additional material. The possibility to fast diagnose sepsis, offers the doctors from the Emergency Department, the capacity to fast initiate an antibiotic treatment, to hospitalize the patient and at the same time, it gives them the certainty that they did not miss the sepsis diagnosis, thus avoiding the situation of malpractice. A preliminary study, regarding the sepsis biomarkers, which took place in the Emergency Unit of University Central Emergency Military Hospital, is also presented within this article.

Keywords: *sepsis, POCT, Emergency Department*.

INTRODUCTION

Sepsis, severe sepsis and septic shock are some of the most common affections which are handled in the Emergency Departments and in the intensive therapy sections and they still represent a major cause of morbidity and mortality for critic patients despite the use of respiratory and cardiovascular support, modern antibiotics and resuscitation therapy. In compliance with the newest guides published, fast identification and the speed of implementation of an adequate treatment in the first hours after the patient came to the Emergency Department can influence radically the prognostic of septic patients, facts which determined the concentration on biomarkers for precocious diagnosis, risk stratification and the assessment of these patients' prognostic.[1]

Sepsis is an exacerbated systemic reaction at

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something which would normally be a common infection. Although it was identified from the oldest times, the sepsis is still a challenge for clinicians and it remains, most of the times, a lethal complication. During the last 10 years, in compliance with the Protocol initiated by Surviving Sepsis Campaign for the management of patients with sepsis, the mortality was reduced from 37% to 30%, nevertheless its percentage is still unacceptably high.[2]

The legal framework for the management of patients who come to the Emergency Unit is provided in the Order of the Health Ministry no. 1706/2007 regarding the management and organization of the emergency receiving units and compartments.

The patients who come to the Emergency Department are taken over by the employees of the department and they are selected according to the emergency degree in compliance with the Order of the Health Ministry no. 48/2009 – National Protocol for the Selection of Patients from the Emergency Receiving Structures.[33]Patients' selection is defined by law as follows: the mechanism or procedure by means of which the patients who come to the Emergency Receiving Unit (ERU) or to the Emergency Receiving Department (ERD) are assessed and classified, upon arrival in ERU or ERD, by a competent person (physician or average sanitary employee), taking into consideration their clinical state and the symptoms they declare, correlated with their age and medical history, how stable their vital functions are, the potential of exacerbation of their medical state, the necessity to implement a treatment or to perform some investigations, as well as other information which are considered to be relevant, so that it can be decided which is the priority for each patient to be assisted and the level of assistance necessary for each of them.[33]

Patients' selection within the Emergency Department represents the medical procedure used to assess and categorize the patients arrived within the ERU by a nurse/physician, in order to decide their priority for medical care and its level. The nurse responsible with the patients' selection procedure is the nurse with specific preparation and with appropriate experience and abilities. The recommendation provided by the law is that the time allowed for the patients' selection should not be longer than 2 minutes.[33]

The level of patients' selection refers to all patients who have the same priority degree according to the severity and/or critical state of their pathology and to the necessary resources. Priority levels are the following:[33]

- Level I – *resuscitation* (Red Code): The patient who requires an intervention to save his/her life NOW. Maximum time allowed for the patient to be taken over in the treatment area: 0 minutes.
- Level II – *critical* (Yellow Code): The patient who is in a situation with major risk or altered mental status (acute modification) or any intense pain or major discomfort. Maximum time allowed for the patient to be taken over in the treatment area: 10 minutes.
- Level III – *urgent* (Green Code): The patient with stable vital functions who requires however, two or more medical laboratory or paraclinical investigations. Maximum time allowed for the patient to be taken over in the treatment area: 30 minutes.
- Level IV – *nonurgent* (Blue Code): The patient with stable vital functions who requires only one laboratory or paraclinical investigation. Maximum time allowed for the patient to be taken over in the treatment area: 60 minutes.
- Level V – *examination* (White Code). The patient who requires neither emergency medical assistance nor laboratory or paraclinical investigations. This category includes people who come for one of the following reasons: vaccination; social case without clinical symptoms; clinical and administrative issues (medical certificates, prescriptions, etc.). Maximum time allowed for the patient to be taken over in the treatment area: 120 minutes.

The patient has the right to have his health state periodically reevaluated if he has been taken over in the treatment area after more than 30 minutes or if there are significant modifications in his/her state, which means that patients' selection procedure within the Emergency Department is periodically resumed.[33] More than once, this task is a difficult task to be performed, taking into account the big number of patients, insufficient personnel and the permanent stress the personnel from the Emergency Department has to deal with. In addition to these, the risk of bad evolution for septic patients and not only for them is quite high, therefore it is necessary that the diagnosis methods in this department are fast, precise and trustworthy.

We debate the issue of septic patients in this article because this kind of patients have a rather unpredictable evolution if they are not diagnosed correctly and in due time. More than often, old patients who also suffer from diseases in different stages are neglected or superficially treated at home, fact which makes the sepsis evolve frequently up to advanced stages until patients come to the hospital. This kind of patient is brought to the hospital with the ambulances of the national system 112 (SMURD or Ambulance Service) or by his/her relatives, being already in a critical state, dehydrated, with high temperature or with neurological signs.

Diagnosis of sepsis is not easy, it involves special issues, because at present, the available methods are related to the performance of the following laboratory analyses: the number of leukocytes (WBC) in the patient's blood resulting from a complete hemogram with leukocytes formula, the number of germs, sepsis biomarkers, CRP (reactive protein C) and last but not least the performance of bacterial cultures from blood (hemocultures) and from other fluids. Moreover when the physician from the Emergency Department is under time pressure and under pressure from the patients' relatives as well as from the other patients who do not understand the patients' selection Protocol mentioned above, a fast diagnosis has an even greater importance. The main topic of discussions is the fact that some patients have higher priority than others, meaning that some have to wait longer and others, who have just arrived, are immediately taken over and treated in the Emergency Department. The duty to introduce the patients in the 5 levels of selection belongs to the nurse from the patients' selection and eventually to the physician on duty; the two decide which patients have a higher level of priority.

Therefore, the physician from the Emergency Department should have available all necessary resources in order to make a fast, correct and hard to question diagnosis in the prospect of any malpractice accusations. Sometimes, there are patients who arrive in a state of septic shock and they have a fast evolution to decease because of multisystem organ failure (MSOF), so it is difficult for the patients' relatives to understand the reason why a patient having an apparently good state during the day, dies in hospital in less than 24 hours. That is why the physician from the Emergency Department (emergency medicine physician or intensive care physician) must make the correct diagnosis: SIRS, sepsis, severe sepsis or septic shock, so that a fast, appropriate and complex treatment can be initiated.

According to the law, the physician from the Emergency Department has the possibility to require all necessary examinations from different specialties in order to make a diagnosis, the period of time allowed before the patient is examined varies between 10 and 60 minutes according to the level of emergency – 10 minutes for red code and maximum 60 minutes for green code.(32)

It is important to have in mind that patients must be subjected to a series of laboratory and paraclinical investigations, as soon as possible upon their arrival, especially in the case of patients belonging to the first 2 levels of priority. In view of a better understanding of the phenomenon, we shall make a review of the definitions of the Systemic Inflammatory Response Syndrome (SIRS) and of the sepsis with its stages.

DEFINITIONS

Systemic Inflammatory Response Syndrome (SIRS) is the clinical syndrome which results as a consequence of an inadequate inflammatory response of the body at a noninfectious origin lesion, such as: pancreatitis, vasculitis, autoimmune affection, thrombembolism, burns or surgery interventions.(3) SIRS diagnosis is made in the presence of 2 or more of the following criteria, as follows: temperature >38,5°C or <36°C; ventricular rate >90/min; respiratory frequency >20/min or $PaCO_2<32$ mmHg; leukocytes $>12000/mm^3$ or $<4000/mm^3$;

Sepsisis the clinical syndrome which results as a consequence of an inadequate inflammatory response of the body at an infection. Sepsis can be presumed if 2 or more of the conditions mentioned above are present and the infection is identified either through germs culture or visually.[1]

Severe sepsis: sepsis associated with the organ dysfunction/insufficiency, impercipient perfusion or hypotension.[1]

Septic shock: hypotension within sepsis, with all appropriate replenishment.[1]

Multisystem organ dysfunction – MSOD: organ dysfunction at a patient with an acute pathological state so that homeostasis cannot be maintained without medical intervention.[1]

WHY „POINT OF CARE TESTING – POCT" DEVICES FOR EMERGENCY DEPARTMENTS?

Point-of-care testing (POCT) is defined as a medical testing at or near the site of patient care.[21,22] Another definition would be the following: a group of investigation, diagnosis or screening technologies, which require neither personnel nor laboratory conditions, performed on spot where medical assistance is provided, within or outside a medical unit.The purpose of POCT is to provide immediate, convenient, and easy-to-use diagnostic testing that shortens the therapeutic turnaround time when providing care for a patient.

The objective is to provide rapid diagnostic information that permits immediate clinical management decisions to be made that will improve patient safety and clinical outcomes, not to mention patient satisfaction.It is important to be noticed that these technologies do not require special spaces or additional personnel, other than those that can be found in any hospital/physician office. POCT can be found in more environments: hospital bedside, ambulatory care settings (clinics or physician offices), alternate care (skilled nursing facilities), and home settings.

In Romania, medical devices are governed by Law no. 176/2000 regarding medical devices, within this law, in article 2 point a) and b) we find their definition as follows[29]:

a) Medical device – any instrument, device, mechanism, material or another article used alone or in combination with others, including the necessary software for its correct use, designed by the manufacturer for human use and which does not fulfill the main action for which it was designed in/or on the human body by pharmacological, immunological or metabolic means, but which can be helped in its function by such means, with the purpose of: diagnosis, prevention, monitoring, treatment…;

b) Active medical device – any medical device whose functioning is based on another source of power or of energy than the one generated by the human body or by gravity;

POCT benefits are numerous, below we are presenting the most important of them[21,22]:

- Positive patient identification;
- Immediate diagnostic test results (maximum 15-17 minutes) – reduced test and therapeutic turnaround time, 24/24 hours;
- Reduction and/or elimination of specimen/sample transport;
- Elimination of blood collection tubes and centrifugation with fresh whole blood specimen;
- Reduced blood specimen volume;
- Reduced volume of reagents – POCT is a less invasive method from the clinical point of view;
- Data management and connectivity – POCT systems can be connected to the informatics systems of the hospitals having as result: less transcription errors, immediate data analysis – utilization, quality control, compliance and data mining, development of disease specific algorithms;
- Good cost-benefits report – although generally the tests are more expensive, they can offer large economical benefits, by reducing the number of visits in hospital, days spent in hospital and repeated hospitalizations.

Potential disadvantages[21,22]:

- Weak analyses quality;
- Lack of results interpretation;
- Wrong results which are difficult to trace;
- The possibility to make a battery of tests can lead to the performance of unnecessary and inadequate

analyses;

- Lack of alignment with laboratory results – reference intervals and results can be different to those issued from the classical laboratory which makes the comparison difficult.

The current diagnostic laboratory system has been slow to change and is in need of change to a more patient-centered system. Thus, today we need personalized medicine and a patient-centered medical system.

The model of centralized lab testing was developed in the late 1960s but the new technologies and the evolution of the health system demanded more rapid testing which led to a significant increase of POCT. The development of new technologies, such as „lab on a chip"[23,24]and DNA/RNA-based molecular diagnostic tests[25,26, 27,28], will expand the menu of POCT tests and will lead to an increase in the use of this device.

Taking into consideration all the benefits mentioned above, POCT improves the diagnostic activity; the most important aspects are immediate and precise diagnostic, the use of whole blood specimen in an extremely small quantity and reduction of specimen/sample transport to the laboratory. Due to the fact that it provides information to the Emergency Department physician upon diagnosis and patient's critical state, it also offers him/her a certain safety which, at the same time, eliminates the stress related to malpractice.

The physician from the Emergency Department can ask for interdisciplinary examinations, as already mentioned, however, if he/she does not have any investigations to show to his colleagues in order to make at least a presumptive diagnosis, the physician extends patient's hospitalization time in the Emergency Department waiting for the laboratory analyses made in the central laboratory.

In addition to that, the Romanian legislation, more precisely, the Order of the Health Ministry no. 1706/2007 – which regulates the activities from the Emergency Departments, in Annex 1 – stipulates a series of minimum mandatory paraclinical and laboratory investigations which should always be available for the patients from the Emergency Departments.

These investigations complexity depends on the type of the Emergency Department (I or II), as related to imaging, but if we refer to basic analyses (hemogram, blood glucose meter, electrolytes, sanguineous gases), these are mandatory and it is preferable for them to be made in the Emergency Department in order to reduce the time until a diagnosis is made.

High level Emergency Departments – type I, must also have the possibility to make toxicology analyses. All these mandatory analyses, but also other analyses which can lead to an immediate and precise diagnosis, can only be made with the help of POCT technology.

SEPSIS DIAGNOSIS

Biomarkers

Biomarkers can have an important role in proving the presence, absence or stringency of sepsis and they can make the difference between fungal and viral infections, between systemic sepsis and local infection.Other potential roles of the biomarkers include prognostic, antibiotic therapy, evaluation of treatment answer and postsepsis recovery, differentiation between gram-negative and gram-positive germs, the possibility to predict sepsis complications and the development of organ malfunctions (heart, kidneys, liver or multiple organ malfunctions)[4]

Biomarkers definition. At present, the accepted definitions for biomarkers are in compliance with the studies made by U.S. National Institute of Health (NIH) and by European Medicines Agency. A biomarker is a "biological characteristic, objectively measured (with acceptable accuracy and reproducibility) and used as an indicator for a physiological or pathological process or for the activity of a medicine".

In compliance with NIH standards, biomarkers can be classified in two categories: *prognostic markers* – which allow for the patients to be classified according to the individual risk to have a specific prognostic, regardless of the treatment (or lack of treatment) and *predictive markers* – which allow the forecast of the potential benefit (efficacy) and/or the risks (toxicity) of a treatment according to the status of a biomarker (absent/present)[5].

The ideal biomarker in infectious diseases is used to identify a high risk group or predisposing factors, as a tool for disease identification or for treatment prescription and classification of patients according to their specific factors as well as/or as indicator of the therapeutic management in order to avoid reinfection.An ideal marker for infections would combine the diagnostic, prognostic and treatment follow-up characteristics and it should be easily and fast available for clinic use[6].Biomarkers should evaluate the severity of an infection or predict an evolution excluding complications to help the clinician make a decision regarding the best therapeutic approach in the most appropriate environment (hospital or specialty ambulatory, intensive therapy or hospital section). Furthermore, it should help the clinician decide if it is necessary to introduce or to continue antibiotic therapy.

Concluding, the “ideal” biomarker for sepsis diagnosis should make different diagnosis between SIRS and sepsis, between viral infections and bacterial ones, it should also “detect” sepsis fast, reflect de severity of the disease so that therapy can be monitored, have a high predictive value, be stable in different samples and eventually be quantified fast using “Point of Care” devices.

During the last few years, more potential biomarkers for infection were suggested and their analysis is a complex task. The present tendency is to use biomarkers – especially cytokine – in correlation with multiple tests which measure simultaneously more biomarkers from only one biological sample. The main purpose is to examine if the clinical performance and utility can be transposed in every day clinical situations, taking into account the great number of patients which come to the Emergency Departments and the necessity to make a diagnosis fast.

A fast diagnosis allows physicians from the Emergency Department to implement a precocious treatment which increases the patient’s survival rate and which, at the same time, offers them the certainty that they are not wrong and that they have not passed by an infectious affection with lethal potential for that patient.Thus, the malpractice risk for the physicians from the Emergency Department is lower, since it is known that they have to deal with a great number of patients every day.

Available routinebiomarkers

Three biomarkers fulfill the criteria mentioned above and are available at all times: C reactive protein (CRP), procalcitonine (PCT) and presepsin (PSEP).

CRP was tested in different studies but only some of these studies focused on its use for the improvement of antibiotic therapy. Further to these studies – completed or ongoing – the use of CRP cannot be recommended at present as a criterion for the initiation or end of antibiotic therapy in adults; however, for children, CRP can be probably used as an indicator to end the antibiotic treatment although the proofs obtained up to present are limited[5].

Procalcitonine – PCT was testedon a larger scale for the improvement of antibiotic therapy, both for adults and for children. The conclusion of more studies completed recently, which involved a significant number of patients, is that the introduction of procalcitonine values in the decision algorithms for infection management in specific infections is most likely adequate. It is however necessary to continue researches for specific infections which have not been examined enough up to the present time, for a more precise definition of procalcitonine role in the management of antibiotic therapy[5].

Presepsin – PSEP. In this article, we shall present a new biomarker, which is a viable option for sepsis precocious diagnosis – **presepsin *(sCD14-ST)*** and we shall present its correlation with a score (MEDS score) to supply the gaps which are related to this biomarker, in comparison with what is used in present in clinical practice. The biomarker was used for the first time in 1993[32] and then in 1994 (*Durieux et al. Eur J Immunol 1994;24:2006-12*). Presepsin was studied starting with 2005 and it became an important new marker for the diagnosis and prognostic of sepsis in the last years.[8,9,10,11,12].

CD14 is a glycoprotein expressed on the surface of the monocytes/macrophages membrane (mCD14) and it serves as receptor for lipopolysaccharides (LPS) and for the protein which ties the LPS (LPBP). CD14 co-locates using a receptor 4, Toll-like type (TLR4). When tying LPBP complex, CD14 activates the specific pro-inflammatory signalizing cascade TLR4, thus initiating the host inflammatory reaction against any type of infectious agents.

LPS-LPBP-CD14 complex is released in circulation, canceling CD14 from the cellular membrane, thus soluble CD14 (sCD14) is produced. Nevertheless the activity of the proteose from the plasma generates another molecule sCD14, referred to as subtype sCD14 (sCD14-ST) or presepsin – a protein of 13 kDa which is actually a part of CD14, lysated at N-terminal head (*Durieux et al. Eur J Immunol 1994;24:2006-12*).

PSEP levels were significantly higher at septic patients than at those with SIRS or at those apparently healthy. The increase of PSEP levels was more precocious than the increase of IL-6 and D-dymers levels in a study which created a model of bacteremia on animals (cecal ligation and puncture on rabbit – CLP). The determination of presepsin concentration can be used not only for the diagnosis and prognostic of sepsis, but also to monitor disease evolution and feedback at therapeutical interventions.[8,9,10,11,12]

Recently discovered biomarkers of potential interest in the near future

At present, intensive efforts are being made in the research field of some new prognostic and diagnostic biomarkers which can be useful in antibiotics therapy management from acute infections. For adults, three of these seem promising: sTREM-1 (soluble Triggering Receptor Expressed on Myeloid cells-1), suPAR (Soluble urokinase-type Plasminogen receptor) and ProADM (proadrenomeduline). These biomarkers are accessible, they have proved sensitivity and/or specificity and they were studied on a significant number of patients so that they are worth to be taken into consideration further on. For children and babies other studies are also necessary.[5, 13]

STREM-1 is a member of immunoglobulins big family, surface receptor which appears at mature monocites and polimorphonuclears, they contribute at native immunity. Its expression is upregulated when phagocytes are exposed to bacterial fungic pathogens but not during other noninflammatory processes. TREM-1 amplifies the inflammatory response by increasing the proinflammatory cytokines production, inhibiting the synthesis of IL10. During the up-adjustment of the surface receptor TREM-1, the soluble form sTREM-1 increases in biological fluids (blood, bronchoalveolar lavage fluid, cerebrospinal fluid) and it can be determined with ELISA commercial kits. According to some recent studies, the dosage from the infection spot seems to be more significant than the measurements made from plasma.[5,13]

SuPAR (Soluble urokinase-type Plasminogen Activator Receptor) or CD87 is a receptor for inflammatory response spread on a large scale. It appears only on the surface of a few cell types, such as: endothelial cells and leukocytes (monocytes/ macrophages,polymorphonuclears).

Expression of its gene is under the control of immune and inflammatory effectors, such as bacterial products (LPS), cytokines (IFN-γ, TNF-α, IL-1β) and growth factors (FGF-2, VEGF, TGF-β, EGF). During the inflammatory and immune response, suPAR expression is upregulated by epithelial cells, white blood cells (lymphocytes), smooth muscle cells and fibroblasts. Expression is also upregulated during tumor growth and metastatic spread. The dosage may be achieved using commercial ELISA kits available on the market, but also as part of the multiplex kit with multiple cytokines. However, suPAR seems to be of limited value as a diagnostic test in specific pathologies (patients at risk, HIV patients on antiretroviral therapy, monitoring patients with non-pulmonary bacterial infection and children with malaria with Plasmodium falciparum).[5,13]

Pro-ADM Adrenomedulinis a52amino acidpeptide, a marker ofCALCgene familythat worksasa mediator ofcell proliferation, hormonalregulationandembryogenesis. ADMis produced by theendothelial cellswhere itinducesvasodilationand maintainshomeostasis. Pro-hormonal fragments(pro-ADM) are more stable thanthe complete peptideandtheir levelsin biological fluidscan be measuredbyautomated methodsTRACE(TimeResolvedAmplifiedCrypt-Emission) after the capture of immunoassays. The secretion ofproADMincreases duringan immune response againstviral or bacterialproductsaccording to the sizeof the stimulus. Pro-ADM is avaluableprognosticmarker. Aspart of anevaluationscore ofpneumonia severity, it can identifypatients in rather critical state which would requiremonitoring/hospitalization inanintensive care unit.[5,13]

Future Biomarkers

Micro-RNA (miR)are newly discovered potential biomarkers. miR are small molecules (approximately 20 nucleotides) present in eukaryotic cells, which act as biological regulators by modulating post-translational regulation. They are ubiquitous and present in abundance in the lungs, liver and kidneys. After binding to the appropriate smRNA sequence, they regulate the expression of the gene by a repressor effect or by altering the target sequence. A fragment of miR can bind several smRNA. Their expression can be measured by RT-PCR and quantitative PCR. miR are potential candidates for early diagnosis and/or prognostic markers in sepsis, but other numerous studies are necessary to understand their role in biochemical and immunobiological processes before it can be used to stratify, to make prognosis or therapeutic decision in septic patients.[5,13]

The diagnostic and prognostic evaluation of***presepsin*** in sepsis in the Emergency Department was studied in comparison to other available routine biomarkers, in a study involving 859 patients, conducted by an university hospital which has between 240,000 and 260,000 hospitalizations per year.[10] These findings were published recently, and the conclusions are consistent both with literature data available in today specialty literature and with personal observations generated during similar studies in our hospital which has 25,000 presentations through the Emergency Department per year.

The efficiency of using presepsin to diagnose cases of sepsis, severe sepsis and septic shock was significantly increased by using MEDS score in evaluating these patients as it will be shown below. It is also worth mentioning that although PCT (procalcitonin) can be used as a biomarker for the diagnosis of sepsis, it increases in other situations, such as: multiple injury, extensive burns, pancreatitis, organ transplantation, major surgery and SIRS – not only in infections, therefore, only the positive and negative predictive values are not enough to exclude or confirm sepsis.[10] A recent meta-analysis showed that the diagnostic performance of PCT was reduced, with 71% (95% confidence interval 67-76%) sensitivity and specificity for serum PCT, as a biomarker for sepsis. In conclusion PCT can not clearly differentiate sepsis from other diseases in critic adult patients.[10,14,14,16,17,18] Thus, to diagnose sepsis, severe sepsis and septic shock, PSEP proved to be superior to PCT, moreover, PSEP together with the assessment by MEDS score was superior to PSEP taken as sole indicator.[10,19]

In the 28-day mortality prognosis PSEP was inferior to PCT, but these were lower to the correlated interpretation and MEDS score. For septic patients and prognostication of mortality after 28 days, PSEP, MEDS and APACHE II score proved to be independent predictors unlike PCT which did not have this capacity. The mentioned study showed that plasma levels of PSEP were a parameter closely related to the severity of sepsis.[10,19,20]

Compared to the PCT, PSEP is a highly specific biomarker for the diagnosis of a bacterial infection because it is produced in conjunction with bacterial phagocytosis.

PSEP was higher than PCT and had greater sensitivity, specificity, positive predictive value, negative predictive value and accuracy of prognosis in early stages of sepsis which is consistent with the data from the updated literature.

The more severe the sepsis was, mortality also increased with over 50%, that is: mortality in severe sepsis. Dellinger et all., in Early-Goal Directed Therapy from 2013 guides of the Surviving Sepsis Campaign, recommends that the potential source of infection should be confirmed as quickly as possible, if possible within 6 hours since presentation and that large spectrum antibiotic therapy should be administered within one hour from the identification of severe sepsis or septic shock. Therefore identifying these patients at risk is very important.

Table 1. MEDS Score (Mortality in Emergency Department Sepsis Score)[30,31]

Variables for MEDS Score	Points
Comorbidityfastterminal– fast terminalassociated disease(metastatic canceroranothercondition that cancause deathin30 days)	6
Age >65 years	3
Septic shock (PAs<90 mmHg with all volemic repletion)	3
Number of plates <150000/mm^3	3
Leukocytesprematureunsegmented-bands(>5% of totalleukocytes)	3
Lowerrespiratory infection(clinical infiltratedpneumoniaor chestRxG)	2
Altered mental status(level ofalert or another change in the levelof consciousness)	2
Chronic patients treated at home	2

STUDY ON THE IMPORTANCE OF PRESEPSIN IN ASSOCIATION WITH MEDS SCORE, SEPSIS PRECOCIOUS DIAGNOSIS. PRELIMINARY DATA

In the Emergency Department of the Universitary Central Emergency Military Hospital, a study was made during a year, involving 300 patients suspected of sepsis that came to the Emergency Department, patients brought by their relatives or by the ambulances from the national emergency system.

From these, 32 patients were introduced in the study (19 men and 13 women), for which the following inclusion and exclusion criteria were used:

Criteria for inclusionin the study:

•Age ≥18yearswith clinical signs ofsevereinfectionsrequiringbloodsampling;

•The presence of2 of 4SIRScriteria: fever > 38^{0}C<36^0 C; heartrate>90/ min; respiratory rate >20/minor existence of hyperventilation($PaCO_2$<4.3 kPa/ 32mmHgin arterial blood); leukocytosis>12,000/ml, leukopenia<4000/mlor >10%prematureunsegmentedgranulocytes(bands);

Exclusion criteria: age under 18andrefusal to sign theconsent.

All patients were examined by doctors of emergency medicine and ATI employees of the Emergency Department and after the former signed the informed consent, venous peripheral approach was made, blood was collected for laboratory testing and they were subjected to other paraclinical investigations (abdominal ultrasound, CT in different regions, x-rays) in order to make a diagnosis and start the appropriate treatment immediately.

The following laboratory tests were made: complete blood count with differential, coagulation (Quick time, fibrinogen), biochemistry (glucose, urea, creatinine, ionogram, samples of liver, etc.), presepsin, procalcitonin.

Analyses were performed in the Central Laboratory of SUUMC, and the Emergency Department's own laboratory, using "Point of care testing - POCT" and PATHFAST® device type.

Evaluation of patients admitted to the Emergency Department was made with the score MEDS - Sepsis Mortality in Emergency Department[19, 30, 31]. The score was developed to predict mortality for patients with SIRS hospitalized in the Emergency Department. The maximum score is 27 points, score variables and the score awarded to each variable can be found in Table 1.

For all these variables, a score are awarded, score which is summed up for each patient, the sum of the obtained points offers a prognostic for mortality at 28 days.

The determination of presepsin was made using a POCT type device, more precisely PATHFAST®, and the evaluation of the patient's state according to the values/concentration of presepsin obtained was made using the correlations from Table 3.

The patients included in the study were aged between 18 and 92 and they were selected using the inclusion and exclusion criteria mentioned above. The existence of SIRS criteria was considered, the results are those from Figure 1 (14 patients with fever, 25 with RR>20/minute, 22 with leucocytosis, 22 with HR> 90/minute).

Table 2. Correlation between MEDS score and mortality at 28 days as percentage[30,31]

MEDS Score range	Mortality at 28 days (95% CI)
0-4	0,6% (0-3%)
5-7	5% (1-13%)
8-12	19% (11-29%)
13-15	32% (15-54%)
> 15	40% (12-74%)

Table 3. Correlation between PSEP values, diagnosis and measures to be taken [14,15,16,17,18,19]

PSEP (pg/mL)	Diagnosis	Case management
Under 200	Sepsis absence	It is not necessary to sample hemocultures
200-300	Small probability of systemic infection	Other investigations are also necessary among which bacterial cultures (hemocultures etc.)
300-499	Possible systemic infection (SEPSIS)	Therapy with medicines starts after samples were taken for bacterial cultures
500-999	High risk of infection progression in the body (SEVERE SEPSIS) High risk of unfavorable prognostic	Surgery treatment must also be taken into consideration
Over 1000	Major risk of systemic infection progression (SEVERE SEPSIS/ SEPTIC SHOCK), major risk of mortality at 30 days – comparable with score 25 on APACHE II	„Maximal” therapy

Further to patients' anamnesis, we obtained the data represented in Figure 2, in which we resumed the types of affections (number of cases): neurological – 9, neoplastic – 7, cardiac – 1, global cardiac insufficiency – 5, coronary artery diseases – 5, valvular heart diseases – 3), chronic renal diseases – 4 , diabetes – 4, pulmonary diseases (pneumonia – 5, BPOC/asthma – 5), thyroid diseases – 2, others – 27 (HTA, digestive diseases etc).

Patients were evaluated using MEDS score which varied between 3 – 21 points according to the health state of each patient. Some of the laboratory analyses were made in the Central Laboratory, that is the blood cultures and other cultures sampled from 15 patients, out of which only 3 were positive. POCT (Point of Care Testing) devices were used to determine the biomarkers, in the case of PSEP; presepsin had values between 102 – 7129 pg/mL, as it can be seen in figure 3. In the case of procalcitonine, the results were negative or they reached the maximum value of >10 ng/mL, and for PCT, the determination method that was used, was a semiquantitative one, THERMO SCIENTIFIC BRAHMS, which has the following intervals as possibilities:< 0,5; ≥0,5-≤2,0; ≥ 2,0-10; >10 ng/mL. Regarding the diagnosis of sepsis in its different stages, there were 13 patients with SIRS, 8 patients with sepsis, 6 patients with severe sepsis and 5 patients with septic shock who also had the highest values of presepsin.

Considering the affections which led to sepsis (Figure 4), the patients from the study can be divided as follows: pulmonary diseases (COPD, asthma, pneumonia) – 12 cases (5 ♀, 7 ♂), urinary infections – 9 cases (5 ♀, 4 ♂), digestive infections (acute gangrenous appendicitis with generalized peritonitis – 2, gangrenous acute cholecystitis – 4, bowel obstruction – 1, diarrhea – 1, acute angiocholitis – 1) – total 9 cases (3 ♀, 6 ♂), gynaecologycal infections – 1 case and acute endocarditis – 1 case (♂).

Figure 1. The correlation between the number of cases and SIRS criteria

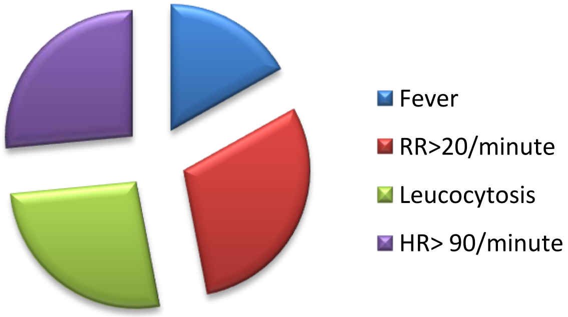

Figure 2. Anamnesis of the patients from the study
– number of cases according to the type of affection –

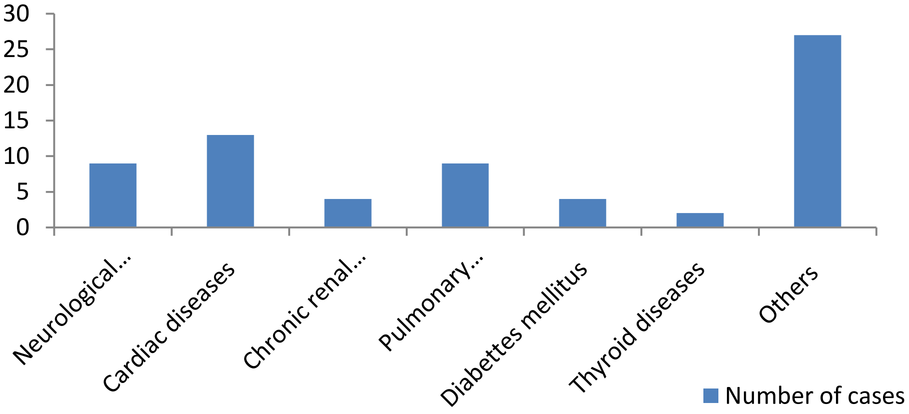

A total of 26 cases were hospitalized in Universitary Central Emergency Military Hospital (81.25%), out of which: 15 cases in medical wards, pulmonology – 2, gastroenterology – 3, internal diseases – 4, oncology – 1, neurology – 2, cardiology – 2 and dermatology – 1 case) and 11 cases in surgical departments (gynecology – 1, urology – 2, general surgery – 8). Out of the total of hospitalized patients, 11 were admitted to ICU and 7 were mechanically ventilated.

Out of the 32 patients included in the study, 8 died in hospital (25%) who had MEDS with values ranging between 8-21 points, the values obtained for presepsin were between 593-6745 pg/mL. 3 cases were diagnosed with sepsis, 2 cases were diagnosed with severe sepsis and 3 cases were diagnosed with septic shock. The affections which were the starting point for the development of sepsis were the following types of infections: respiratory – 5 cases, endocarditis – 1 case, diabetic ketoacidosis coma – 1 case, bowel obstruction – 1 case. Patients who died had a number of associated diseases, some of which were extremely serious, namely: neurological diseases – 4 cases, malignancies – 2 cases, ischemic heart disease – 3 cases.

Figure 3. The evolution of presepsin values for the patients involved in the study

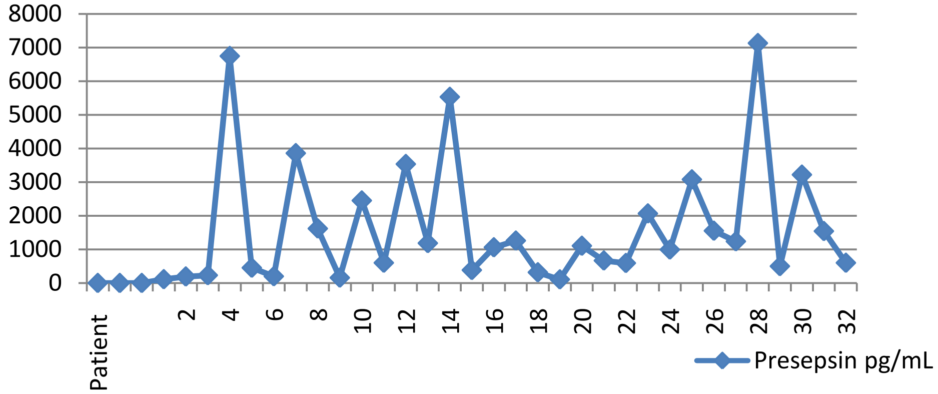

Figure 4. Correlation between the number of cases and the affections which led to sepsis

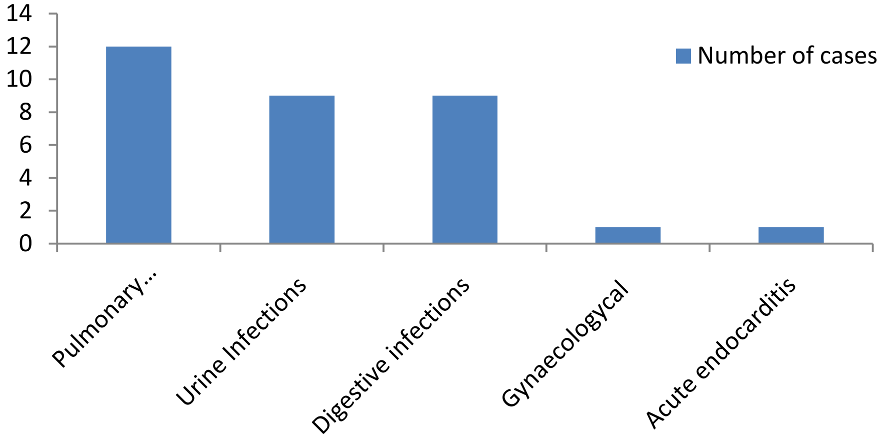

Preliminary conclusions of the study

Presepsinul is an important biomarker having a role in rapid diagnosis of sepsis (17 minutes) to 30 minutes for PCT (on the type of test that we had available), it allows the patient evaluation and classification in a risk category, and its association with MEDS score increased the possibility to provide a correct evaluation of the patients. Rapid diagnosis allows to early start any kind of treatment, primarily the antibiotic, right from the Emergency Department, and in the unclear cases, the physician from the Emergency Department requires further investigations to elucidate the diagnosis.

In selected cases - patients with unrecognized sepsis, the determination of PSEP allows the physician from the Emergency Department, to present their situation to the doctors within Universitary Central Emergency Military Hospital and to suggest and if necessary to hospitalize the patient in cases with diagnostic on the edge according to legal provisions.[32] In our experience related to the sepsis cases presented to the Emergency Department, PSEP determination had a special importance because we could demonstrate the presence of sepsis and not of SIRS as it would have been diagnosed unless for this biomarker. Diagnosing sepsis favored the decision of hospitalizing the patient, his/her close supervision, the commencement of antibiotic treatment in the Emergency Department, in some cases earlier surgical intervention. All patients hospitalized in the Emergency Department and then in the hospital, were investigated using the imaging tools available in the hospital (ultrasound, X-rays, CT with or without contrast, etc.).

In addition to that, determination of the dynamic values of PSEP in hospitalized patients with sepsis allowed monitoring the effectiveness of the implemented treatment, the cost-effectiveness ratio was very good, given the early start of antibiotic treatment and beyond.

In our study, we used the device type PATHFAST® of the company Mitsubishi Chemical that has all the advantages of POCT, namely: the results are quantitative (using a chemiluminescence method type), fast (between 17 minutes), they can be obtained in our own laboratory at local level - from the Emergency Department, can be printed easily, there is a unit memory to store patient data, it uses whole blood – 150 µL (no spin) as sampled from the patient without the need for tube collection.

Moreover, the device is extremely precise (CV <5%), quantitative results are obtained from the blood tests carried out at the same time, because it results in the same test / or up to 6 different assays for six patients at the same time. Such determinations can be made for 1 to 6 patients, making any combination of 1 to 6 tests required.

Presepsin determination by this method allowed risk stratification in criticc patients, monitoring of disease progression and very important, monitoring of the response to drug therapy and other measures (surgery, supportive treatment in the ICU, etc.).

As for forensic results by POCT, given that the sheets can be printed and attached to Emergency Department presentation sheets and general clinical observation sheets, these are of particular importance. It certifies permanent care of the patient under close monitoring of blood different parameters and not only those. With this type of equipment, we can quickly modify patient treatment in real time to correct the dysfunctions and inadequacies thus arisen.

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SYSTEMATIC REVIEW

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Romanian sanitary system assisted by knowledge management

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Abstract: *As the medical sciences advances, so does the volume of information which becomes more and more consistent.*

The health care system is one of the most complex systems encountered in our society. Today, knowledge management practices have been adopted in many Romanian business sectors. However, Romanian health care system is slowly adopting such principles and concepts. This fact is created mainly because of the organizational culture. In the sanitary industry, this barrier is composed both from an organizational perspective and also, from an individual perspective.

Through the knowledge management practices, doctors could benefit from the amount of data spread in different geographical regions.

Keywords: *sanitary system, knowledge management, quality*.

INTRODUCTION

In a normal health care system, the patient must be in the center of all activities and any medical error should be avoided, in order to protect the patient from a fatal result. For this reason, a complex source of knowledge could be of great help for doctors.

Health care delivery involves more than one could know, more than a simple relation between doctor and patient. Health care delivery covers health care professionals like physicians, specialists, nurses, lab technicians, social workers, counselors, etc. Also, it includes further parties like hospital/clinic administrators, managers in finances/accounting/human resources/drug companies/ health care insurance companies etc. What is more, health care partners are dispersed around many areas, even if they are acting on the same patient.

Therefore, it is well understood that the amount of knowledge is extremely relevant and that any data knowledge, created by one of the partners, is of great importance to the others in order to deliver quality care.

The term of "knowledge management" (KM) appeared in the 20th century, from fields like management, cognitive sciences and psychology. The present activity of KM started in the 1980s along with the wide use of information technologies in enterprises. There, the main focus was on what

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knowledge represented as an intangible asset.

Basically, the word KM was revealed in the 80s and the academic field was created in 1995 (*Stankosky, 2005*).

WHAT EXACTLY IS “KNOWLEDGE”?

Basically, there are 3 major elements often used together: data, information and knowledge. Still, there are some differences between them, as specified below:

- Data: represents a specific tact or figure, without a specified context.
- Information: represents a data that is organized.
- Knowledge: built on the information, in order to define a proper context.

The main difference between knowledge and information is that knowledge lets us the power to take action. Therefore, we are able to use it.

According to Bryan Duhon (1998), knowledge management represents a discipline that promotes an integrated approach in order to identify, capture, evaluate, and share the amount of an enterprise's information assets. These assets may include databases, documents, policies, procedures, and previously un-captured expertise and experience in individual workers.

Basically, Knowledge management is essentially especially for getting the right knowledge to the right person at the right time.The main objective is to create value and to leverage, improve, and refine the firm's competences and knowledge assets in order to meet organizational goals and targets.

When we look to implement knowledge management, we have to take into consideration several dimensions like:

- KM Strategy: Knowledge management strategy must present a certain dependence on corporate strategy. Its objective is to manage, share, and create relevant knowledge assets that will help meet tactical and strategic requirements
- Organizational Culture: The way people interact, the context where knowledge is created, people’s resistance towards change and also the way they share (or not) knowledge are easily influenced by the organizational culture.
- Organizational Processes: Are represented by the right processes, environments, and systems that enable knowledge management to be implemented in a certain organization.
- Management & Leadership: KM needs competent and experienced leadership at all levels. There are a wide variety of KM-related roles that an organization may or may not need to implement.
- Technology: Represents by the assembly of systems, tools, and technologies that fit the organization's requirements - properly designed and implemented.
- Politics: The long-term support in order to implement and sustain initiatives that involve virtually all organizational functions, which may require a higher cost for implementation (both from the perspective of time and money), and which often do not have a directly visible return on investment.

Knowledge management could be described in 3 ways:

- Explicit: represented by the information or knowledge set out in a tangible form. Also, is represented by the information that is easy to capture, to structure or share with other people. For example, in a hospital, an explicit knowledge could be represented by the hospital’s documentation, like internal policies and procedures, clinic methodologies etc.
- Implicit: represented by the information or knowledge that is not set out in tangible form but could be made explicit.
- Tacit: represented by the information or knowledge that would have extreme difficulty in an operational process setting out in a tangible form. What is more, this kind of knowledge is composed of the amount of experience and skills that a doctor can acquire overtime and apply to problems.

Unlike the explicit knowledge, tacit knowledge is sometimes difficult to capture, to structure and/or transferto other individuals[5].

Figure 1. Key elements that constitute the knowledge management infrastructure (*from Wickramasinghe and Sharma, 2004*)

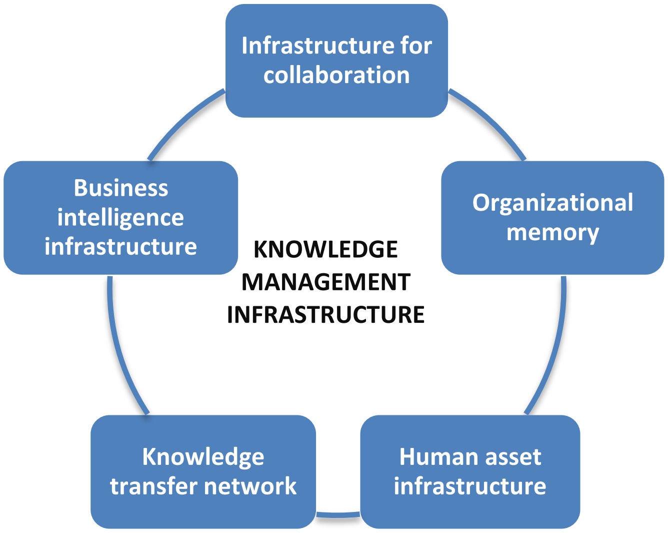
Infrastructure for collaboration
Organizational memory
Human asset infrastructure
Knowledge transfer network
Business intelligence infrastructure
KNOWLEDGE MANAGEMENT INFRASTRUCTURE

TYPES OF HEALTHCARE KNOWLEDGE

a. Provider knowledge – also called "practitioner knowledge". This type of knowledge is the most obvious one especially due to the fact that medical professionals in this capacity have both explicit and tacit knowledge.

An example is sustained by the fact that doctors have to know standard medical information easily comprehended from different reference materials such as text books. Still, some may consider that one of the most important types of providers' knowledge is of tacit form.

b. Patient knowledge – this type of knowledge consists also in tacit knowledge. Nowadays, patients have complex knowledge in past and current medical conditions that doctors may not know about. However, such knowledge is mandatory for doctors to know.

c. Organizational knowledge – this type of knowledge consists in sum of knowledge elements like medical diagnostic systems, text-based materials, etc. What is more, this field contains medical land treatment that is strongly recommended by an institution or medical society.

Why Romania needs to adopt knowledge management practices in its clinics and hospitals?

According to a report from the World Health Organization in 2014, in Romania there are almost 40,000 doctors with free practice. This number is relatively small, with a position that represents a total of 2,5 physicians reported at 1000 individuals.

As we can observe in the above figure, in 2014 Romania was occupying the last position regarding the number of medical personnel. Countries like United Kingdom, France, Germany and even Bulgaria, were in front of us.

Even if, in Romania there is not a huge number of physicians, the information needs to circulate as well as possible. That is why, every doctor has to have knowledge of every new information that appears regarding a medicine, a treatment, a diagnostic, etc.

Here are several reasons why KM needs to be adopted by the Romanian sanitary system:

1. A significant amount of data is in multiple places. Data collected from healthcare tend to be stored in multiple places-from different systems like HR software, to different departments, like radiology or cardiology. Practically, healthcare data comes from the entire hospital/clinic.

Another example is that healthcare data is under multiple forms (like text, numeric, paper, digital, etc). There are times when the same data exists in different formats and in different systems

What sanitary institution could do, is to aggregate this data into a single and central system, accessible to every physician.

2. Healthcare data could be structured or unstructured. In present, Romanian sanitary system is strong related to the National Healthcare Insurance House (NHIH), which created a software that is able to capture consistent data from patients. This would be helpful, but the software appeared only 3 years ago, and before it, all the information regarding the patients' state were wrote down on a paper folder. This is way, hospitals and clinics need KM – to gather all the structured and unstructured data in the same place.

3. Variable definitions; new research practices are coming out daily. Often, healthcare data have variable definitions (regarding a certain treatment, diagnostic etc). There are cases when there may not be a level of consensus about a particular treatment or definition. What is more, the parts that are conducting a certain discussion about a medicine, treatment, etc. are constantly discovering new data knowledge.

Figure 2. World Health Organization, Medical personnel per 1,000 people

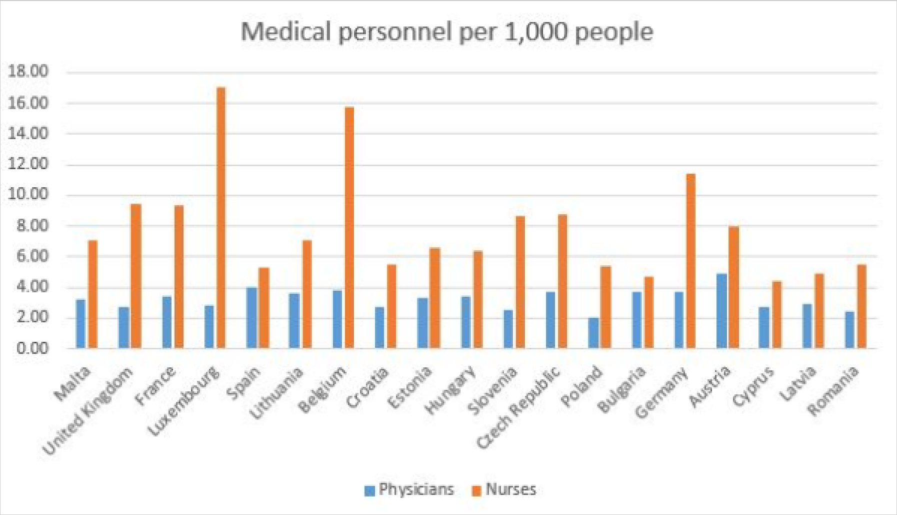

4. The complexity of data. Searching for different developing standard processes that are able to improve quality has always been a main objective for health care providers. Still, the number of data variables involved makes this process more challenging. In health care system, doctors are not working with machines or computer; instead, they work with individuals, more precisely, with human body, which is an amalgam of complex information in constant changing.

Seeing these reasons, we can be sure that healthcare data will not get simpler in the future. On the contrary, it will advance; it will need more than simple software and will present challenges that only a complex and well organized sanitary system could cope.

A POSSIBLE EXAMPLE FOR APPLYING KM IN ROMANIAN SANITARY SYSTEM

From the beginning of 2015, in Romania has been implemented the so called "national health care card", which is nominative for each patient. Its role is to show the doctor what medical treatment has the patient followed, what was his diagnostic and at what medical sections the patient went. In this way, if a patient has firstly went to his family doctor, who diagnosed him and gave him a certain treatment, the second doctor (let's say for example, the dermatologist) would be able to see (with the help of this new system by card) the day when the patient was at his family doctor, the medicines prescribed and the exact diagnose. So far, everything is in order.

Issue that needs to be solved through KM: The new system shows the information only beginning with the date that the card was introduced. In other words, if a 40 years old patient comes for 10 years (since 2005) at the same family doctor, and only this year he had the card (2015), that means that the information area from 2005 till 2015 (regarding his treatments, illness, diagnostics, lab tests etc) is missing on the software program (the doctor is able to see the medical history from a patient only starting with 2015).

Practically, this represents a gap both for physician and also for the patient (of course that the information from 2005 exists, written in the patient's medical record – a notebook, in most of the cases- but is not inserted in the medical informatics system).

Solution of KM: If KM practices would be applied, the present informatics system could be extended, in a manner that would include the whole patient's history from the beginning. Also, the system could specify, for example, if a patient is allergic to some substances (when the doctor prescribes a medicine, if the patient is allergic to a certain substance from that medicine, the program would alert the doctor and in this way, the doctor would prescribe something else – it is also a way of avoiding medical errors).

MAIN BENEFITS FOR IMPLEMENTING KNOWLEDGE MANAGEMENT

The main advantage of introducing knowledge management in Romanian sanitary system is that the information between doctors is easily shared, and most of all, that information is not lost if one of the doctors is sick or has another reason for leaving the cabinet earlier.

In this way, hospitals could be able to have substantial savings. Doctors are brought up to speed, and valuable knowledge assets are not lost (this translates into fact that the hospital or clinic do not lose time and money when doctors need to learn new information quickly).

In a hospital, knowledge management has the power to increase innovation and also to create better patient relationship. For example, if a certain clinic has a global team, knowledge management can create a more powerful workforce when all cultures are brought together in order to share assets (in Romanian clinics, this example is more suitable for private sanitary system).

Another important advantage is that KM could reduce the medical errors and their cost, by providing a decision support.

Next, we will present another KM advantages for healthcare:

- Cooperation between different health care providers;
- Innovation;
- Quality of care;
- Efficiency of work;
- Cost reduction.

All in all, knowledge management gives doctors the capacity of knowledge they need to do their jobs better. In this way, they become more productive.

CONCLUSION

The adoption of knowledge management practices into the Romanian sanitary system could become either a success or a failure. Like any other project, just because a system has been put in place, with the help of the appropriate methodologies or practices, this does not mean that it will become successful in terms of adoption and use.

Because healthcare data is so complex, it's more than relevant that a traditional approach to manage it will not be able to succeed.

This is why, a different approach is needed. The new approach needs to handle multiple sources, structured and unstructured data, variability, the complexity within a constantly changing environment.

The use of KM in sanitary industry promises to enhance the quality of care for patients. Its implementation will allow healthcare partners to conduct evidence based practice, and also to collaborate relying on the best knowledge available.

To sum up, the implementation of knowledge management practices and systems is a topic of great interest for the healthcare community around the world.

A complex KM system for health care industry would represent a huge step, both for doctors (preventing medical errors) and for patients.

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SYSTEMATIC REVIEW

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Spondylodiskitis, etiology, diagnosis and treatment

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Spondylodiskitis is a spinal infection comprising vertebral osteomyelitis and intervertebral disc. Diagnosis can be difficult considering that the symptomatology is not specific, low back pain is common in people over 50 years.

The etiology of the disease includes pyogenic germs, tuberculous, parasitic and fungal etiology. It is known that staphylococcal and tuberculous etiology are responsible for most cases in clinical practice, being the main issue of differential etiological diagnosis. Gram-negative germs are a rarer etiology, being involved in secondary spinal infections as a consequence of dissemination of retroperitoneal or intra-abdominal collections. Other etiologies are very rare: parasitic, fungal or Brucella.

Spinal infection has, in most cases, marrow dissemination as pathogen mechanism; it is recorded in staphylococcal sepsis and secondary determinations of tuberculosis (Pott morbidity).

Contiguous infection from septic foci nearby is a rare complication due to esophageal ruptures or retropharyngeal abscess or aortic vascular prosthesis infections. Iatrogenic, postoperative, postpuncture infection is also recognized.

Due to the particularities of spine vasculature, marrow infection includes intervertebral disc and vertebral body; septic emboli cause infarcts within bones, leading to osteolysis, mechanical deformations, cavitation, mechanical instability. Paravertebral abscesses could occur, the infection can spread into the spinal canal.

The location of these spinal infections is more common in the lower back, however, thoracic and cervical locations are also common.

Initial infectious outbreaks are often difficult to identify, less than 50% of cases are recognized as the source of spondylodiskitis, skin and soft tissue infections, genitourinary infections, respiratory infections, endocarditis, ORL infections. Predisposing factors are recognized and represented by comorbidities, diabetes, rheumatic diseases, cirrhosis, malignancy, immunosuppressive treatments.

Staphylococcus aureus is the most common non-tuberculous etiology, methicillin-resistant Staphylococcus is more and more common in both community infections and in hospital infections. Staphylococcal spondylodiskitis has as starting point, infections of the soft tissues, infections of intravascular devices, infectious endorcaditis and iatrogenic infections: postpuncture, postoperative. MRSA staphylococcus spectrum of resistance comprises lately, besides penicillin, aminopenicillins, 3rd generation cephalosporins and other classes of antibiotics: macrolides, clindamycin, aminoglycosides, florochinolone rifampicin, moreover, the rate of resistant strains is increasing.

Viridans streptococci represent less than 10% of the etiology of spondylodiskitis, their association with bacterial endocarditis in sepsis is known.

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Pneumococcus, anaerobic bacteria represent a rare etiology.

Tuberculosis is a constant presence in the range of spinal infections. As a form of bone tuberculosis, bone cold abscess has a slow, insidious, soundless development and it is sometimes associated with other bacillary determinations: psoas abscess, renal, prostate, lung or even brain abscess.

Gram negative bacteria represent 10-30% of the etiology of spinal infections, the most common germ is Escherichia coli, followed by Proteus, Klebsiella and Enterobater.

Rarely, spondylodiskitis etiology may be represented by other bacteria: Brucella, Bartonella or fungi: candida albicans (more frequently), Cryptococcus, Coccidioides, blastomices. As an exception, cases of spinal infection with Echinococcus granulosus are described.

The difficulty of diagnosis lies in the poor symptoms; lumbar spine pains are common in the general population. Sometimes, the presence of low grade fever or febrile episodes draws attention. Neurologic deficit may occur in complicated cases with radiculopathy and spinal cord compression. During anamnesis, recent history of skin or soft tissue infections, presence of an intravascular device or surgery in the spine is presented. Patients with comorbidities and immunosuppressive treatments are more exposed to the risk of sepsis with secondary determinations. Bacillary etiology is insidious, diagnosis is retroactive.

Laboratory examination raises suspicion of infection when neutrophilic leukocytosis is present, high ESR, CRP is often higher in spondylodiskitis. Alkaline phosphatase may be increased.

Radiological examination and magnetic resonance imaging of the spine brings valuable proofs for diagnosis, showing inflammatory modifications and bone structure modifications of the vertebrae and intervertebral disc, which also appear in degenerative lesions and can therefore be difficult to interpret. Technetium 99 scintigraphy and the one with Gallium 67 can bring sensitive data to support the diagnosis. Computed tomography has good resolution for changes in bone structure, it highlights destruction of vertebral plateaus, bone formation seizure, but MRI is superior in viewing abscesses and lesions in nervous tissue. From the imaging point of view, RM lesions TB are different because the intervertebral disc is not affected but paravertebral abscesses are present, bone destruction.

Proof of infectious etiology can be obtained by biopsy-puncture and culture for aerobic bacteria, anaerobes, mycobacteria and fungi. Blood cultures can have a positive result in about 50% of cases, infection is frequently marrow. Histopathological examination of biopsies can guide diagnosis in bacillary infections but also the differential diagnosis of vertebral bone tumors and metastases.

Complications of the disease are caused by damage to the spinal architecture and mechanics, with neurologic and infectious complications.

Neurological complications can be significant, from the root syndrome up to paralysis. Infectious complications are: paravertebral abscesses, epidural abscesses, secondary meningitis, but also sepsis complications , in the context of which spondylodiskitis was diagnosed.

Treatment is anti-infective, analgesic, immobilization and surgery.

Antimicrobial treatment is injected on the basis of probability criteria or after the results of biopsy or blood culture. Antistaphylococcal antibiotics with broad-spectrum are used depending on the etiological suspicion. In the context of increasing frequency of MRSA staphylococcus, Vancomycin, Linezolid or Targocid is used for minimum 6 weeks. Injectable antibiotic therapy may be continued orally up to 3 months, depending on the disease evolution. For bacillary etiology, tritherapy for 3 months, in the 4th month, the 4th antituberculosis antibiotic is associated, then, the therapy continues with two antibiotics up to 12 months.

The favorable evolution is clinically assessed, especially by clinical evidence of inflammation in normal limits, C-reactive protein and VSH within normal limits, as well as the course of radiologic imaging, magnetic resonance. If paraspinal, epidural abscesses or neurological injuries secondary to compression appear, surgery is required.

The prognosis is favorable by antimicrobial fair treatment and surgery if necessary, spondylodiskitis mortality is less than 5%. Prompt diagnosis and antimicrobial treatment are essential.

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ORIGINAL ARTICLES

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A cholestatic syndrome may be a surprising cause of medical error

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Abstract: *Autoimmune cholangitis defines a spectrum of cholestatic liver diseases that are characterized by inflammation of bile ducts and a reasonable response to immunosuppressive therapy. The two most common diseases associated with this term in the literature are: an overlap syndrome of primary biliary cirrhosis and autoimmune hepatitis and a form of hyper IgG4 syndrome (currently associated with autoimmune pancreatitis). Liver biopsy is mandatory for the diagnosis. There are, whatsoever, in clinical practice, many cases that do not meet current diagnostic criteria but that have a good response to corticosteroid treatment.*

Keywords: *autoimmune cholangitis, primary biliary cirrhosis, hyper IgG4 syndrome*.

Cholestatic syndromes are very often encountered in gastroenterology practice. The causes may be quite different but there is a largely accepted consensus of classification into two categories: extrahepatic and intrahepatic, rendering very different treatment and prognosis. A proper diagnosis is mandatory for a proper treatment. Conversely, a failure of a correct diagnosis may result in wrong treatment and medico-legal issues. The subject of malpraxis legal actions is often considered to be associated with an embarrassing professional failure with only punitive results. In fact, the conclusions of malpraxis cases may, as well, give very documented information to healthcare professionals in order to better improve medical care.

The differential diagnosis of the causes of cholestatic syndromes is quite challenging. An extrahepatic cause is an obstructive one, may it be acute (billiary colic) or slowly developing (benign or malign strictures, pancreatic head tumor, ampuloma). The imaging workup (CT scan, cholangioMRI) are sufficiently revealing for an extrahepatic obstructive cause in order to conduct a proper treatment. The intrahepatic cholestatic syndromes are even more challenging as there are a quite large array of very different causes with very different treatment and prognosis spanning from viral hepatitis to primary billiary cirrhosis and primitive sclerosing cholangitis. The rare the cause the frequent misdiagnosis and medico-legal consequences.

Among rare intrahepatic causes, autoimmune cholangitis is a term recently coined for a spectrum of

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cholestatic liver diseases where an immunologic pathological mechanism is highly suspected with regard to the cause of inflammation of bile ducts. This term implies also a fare response to immunosuppressive therapy. There is, though, some confusion in literature regarding this disease as it may refer to several ailments: an overlap syndrome between primary biliary cirrhosis AMA (antimithocondrial antibodies) negative and autoimmune hepatitis, a form of hyper IgG4 syndrome (associated with autoimmune pancreatitis), a separate entity as a transition form in spectrum of cholestatic disorders. This confounding data makes the diagnosis in this cases very difficult and, also, a potential source of medico-legal issues

The name autoimmune cholangitis was coined for the first time be Brunner et al[1] to describe the situation of three patients suffering of primary biliary cirrhosis AMA negative; all those patients had antinuclear antibody (ANA) and cholangiopancreatography showed no abnormality. The therapy with prednisolone and azathioprine was successful.

The antigen specificity for AMA was demonstrated to be an inner membrane mithocondrial antigen, a 74 kDa E2 subunit of pyruvate dehidrogenase complex (PDC-E2). The most sensitive lab test to assess the presence of these antibodies is an ELISA using recombinant or purified antigens.

5 to 8% of patients suffering of primary biliary cirrhosis lack AMA in spite of having typical clinical and histological features of this disease.[2] All of these patients had ANA (antinuclear antibodies), highly suggestive of autoimmune hepatitis. Moreover the level of serum IgM was significantly lower in these patients then AMA positive counterparts.

Considering the fact that AMA may have a role in the pathogenesis of the disease, a study by Kitami et al of patients AMA negative and AMA positive demonstrated that there is not truly AMA negative primary biliary cirrhosis. Kitami performed an exhaustive immunoblotting studies of various inner mithocondrial membrane antigens that are not currently looked for.[3]

From the pathologist's point of view AMA positive patients have an increased risk of cirrhosis comparing with AMA negative counterparts. Among histological features, the granulomatous destruction of bile ducts is the only histological marker highly specific for primary biliary cirrhosis AMA negative or positive, but this lacks sensitivity. These data further increases the difficulty of diagnosis.[4]

What about the treatment: ursodeoxycholic acid is the treatment of choice in both forms of primary biliary cirrhosis with equal efficiency.

Autoimmune cholangitis may also be a manifestation of immunoglobulin G4 associated systemic disease, most commonly encountered in patients with autoimmune pancreatitis. Autoimmune pancreatitis (AIP) was first described by Yoshida and colleagues[5] in 1995 referring to a type of chronic pancreatitis with certain histopathologic and imaging features. Early or atypical manifestations of autoimmune cholangitis may not involve the pancreas, thus making the diagnosis even more challenging. There are no definitive diagnosis criteria so far, also some have been proposed like Mayo criteria (HISORt) and Asian Consensus Criteria. Two types of AIP have been described: type I which is a pancreatitis associated with immunoglobulin G4 systemic disease characterized by lymphoplasmocitic infiltration and elevated serum IgG4 levels; type II which is less commonly associated with elevated serum IgG4 levels and involves a granulocytic infiltration. 44% of all patients do not have hyper IgG4 serum levels.[6] The cholangitis is a sclerosing stricturing inflammation of bile ducts. The occasional absence of pancreatic involvement have been described. The natural course of AIP involves relapses. Low dose steroid treatment is very effective treatment.

Besides those two types of autoimmune cholangitis, the clinical practice may come along different situations where an intrahepatic cholestatic syndrome may not have sufficient diagnostic criteria to render a proper diagnosis also the response to corticosteroids is straight forward. These situations may be prevalent.

CASE PRESENTATION

Clinical data

We present the case of a male patient aged 48, living in country area, driver, who is admitted in an emergency department for biliary colic comprising of Charcot triad (colicky pain, fever, jaundice), intense itching, acolic stools and hyperchrome urine. This clinical picture is elicited by a fatty meal. The patient does not smoke cigarettes or drink alcohol and personal as well as family disease history are unremarkable.

Labs data

Labs indicates a cholestatic syndrome (increased total bilirubine – 7.5 mg/dL, direct bilirubine – 5.3 mg/dL, alkaline phosphatase – 190 UI/L, gammaglutamyl transpeptidase – 130 UI/L), hepatocytolitic syndrome (increased alanine aminotransferase ALAT – 300 UI/L, aspartate aminotransferase ASAT – 120 UI/L), minimum pancreatic reaction (increased serum amylase – 200 UI/L).

Abdominal US indicated an increased common bile duct (8 mm) with normal sized intrahepatic ducts, thickened gall bladder walls (3 mm) with multiple small (less than 1 cm) calculi. The clinical picture subsides progressively due to a treatment with: antispastics, antibiotics and proton pump inhibitors. The itching and jaundice didn't subside, though. The total bilirubine level reaches 5 mg/dL and direct bilirubine 2.7 mg/dL. In this particular moment the patient is referred to our clinic with the diagnosis of remitted biliary colic and with indication of elective endoscopic biliary sphincterotomy.

Clinical exam was remarkable for intense sclerotegumentary jaundice, pruritus, dark urine and white chalky stools.

Otherwise the patients had no complains.

Labs indicated normal CBC, cholestatic syndrome (increased total bilirubine – 7.7 mg/dL, direct bilirubine – 4.3 mg/dL, alkaline phosphatase – 199 UI/L, gammaglutamyl transpeptidase – 133 UI/L), hepatocytolitic syndrome (increased ALAT – 307 UI/L, aspartate aminotransferase ASAT – 107 UI/L), no pancreatic reaction (serum amylase – 49 UI/L), normal serum albumin, normal INR.

Imaging and and endoscopy data

Abdominal US indicated: normal size and ecogenicity of the liver, normal sized common bile duct (6 mm), normal gall bladder with minimal biliary sludge. We performed superior digestive endoscopy and side-view duodenoscopy which were unremarkable for pathologic changes, but indicated increased amount of intraluminal bile and a normal papilla major. CT scan with radiocontrast media was unremarkable. Colangio MRI was, also, unremarkable.

Immunology data

In the meanwhile the level of total bilirubine continued to increase to 8.5 mg/dL without the sharp predominance of direct fraction. An extended lab workup indicated: normal level of gammaglobulin, AgHBs negative, Ab antiHBc negative, Ab anti HCV negative, Ab anti HAV negative, CA 19-9 negative, alpha fetoprotein normal, CEA negative, AMA negative, Ab anti LKM1 negative, ANA negative, ASMA negative, pANCA negative, cANCA negative, Ab anti Ro negative, Ab anti La negative, Ab anti HIV negative.

Histopathology data

Liver biopsy was performed: liver tissue sample with marked biliary stasis predominantly intraductular, biliary clots, areas of hepatocellular steatosis, chronic inflammatory infiltrate of portal areas and intralobular areas, hepatocellular regeneration, interface hepatitis with bridging necrosis. (Figure 1, Figure 2).

This description fits into pathology diagnosis of autoimmune cholangitis.

Diagnosis and differential diagnosis

We agreed upon the final diagnosis: autoimmune cholangitis; remitted biliary colic by passage of microcalculi. Hence, it has been concluded that the patient had two different causes of jaundice (acute extrahepatic cholestasis and chronic intrahepatic cholestasis), segregated by quite different prognosis and treatment. Considering the presence of interface hepatitis with chronic inflammatory infiltrate it had suggested that future evolution of clinical, serological, immunological and histopathology data may be in the direction of an overlap syndrome between AMA negative PBC and autoimmune hepatitis. The differential diagnosis included: primitive and secondary sclerosing cholangitis, primitive biliary cirrhosis, HIV cholangitis, Sjogren syndrome, non Hodgkin lymphoma.

The treatment was highly effective (32 mg of methylprednisolone daily – 0.5 mg/kg prednisolon equivalent – with tapering to 8 mg daily in one month and ursodeoxicholic acid 10 mg/kg daily), resulting in disappearance of cholestasis and hepatocitolysis after 2 month. The dosage of Medrol reached 4 mg per day in the 4th month of treatment, the current clinical and biological status of the patient being excellent.

Figure 1. Biliary clots (hematoxilin-eosine dye)

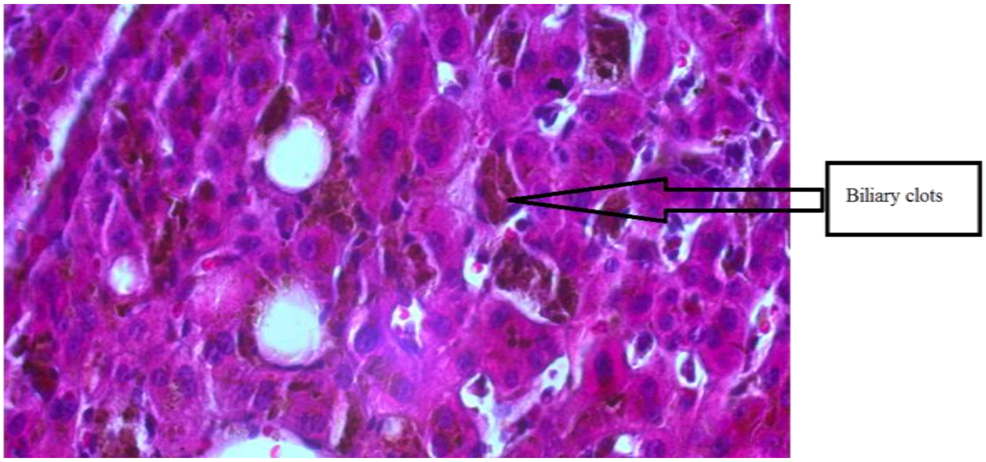

Figure 2. Interface hepatitis and bridging necrosis (hematoxilin-eosine dye)

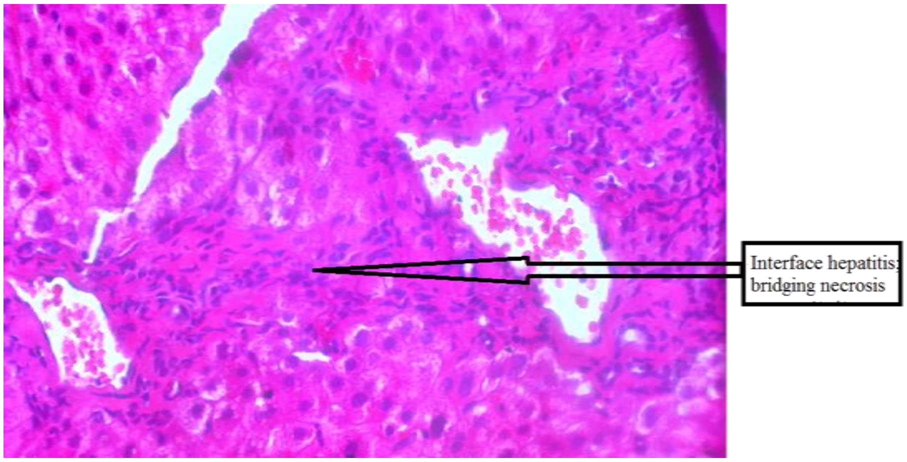

DISCUSSION AND CONCLUSION

Autoimmune cholangitis is a very challenging diagnosis in the face of a lack of international consensus on terminology used in the literature. This very particular confounding situation may come in defense of the practitioner in case of a malpraxis legal action as it may occur. On the other hand should the diagnosis be late the disease may progress to complications such as cirrhosis, liver failure and, even, death. Referral of patient to a tertiary center of hepatology may be a reasonable course of action to fully benefit medical care act. This is not always very handy.

A diagnosis workup involves various differential diagnosis of cholestasis syndrome; among these diagnosis AMA negative primary biliary cirrhosis, primitive sclerosing cholangitis of small ductules and hyperIgG4 cholangitis are the most important. Liver biopsy, high definition imaging workup and immunology panels are mandatory for a proper diagnosis.

The response to corticosteroid treatment is highly suggestive of immunologic process involved in the pathogenesis of liver ailment but it is not, whatsoever, pathognomonic as some are tempted to consider. The diagnostic criteria may uncover progressively in time, endorsing the idea of a continuum in a spectrum of autoimmune cholestatic diseases.

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EDUCATION AND IMAGING

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Capsule endoscopy

Raluca S. Costache[1,2]

We present the case of a 42 years old, female, diagnosed with chronic hepatitis C viral infection treated with peginterferon and ribavirin.

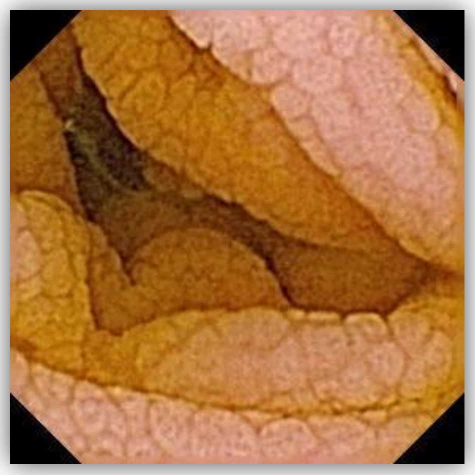

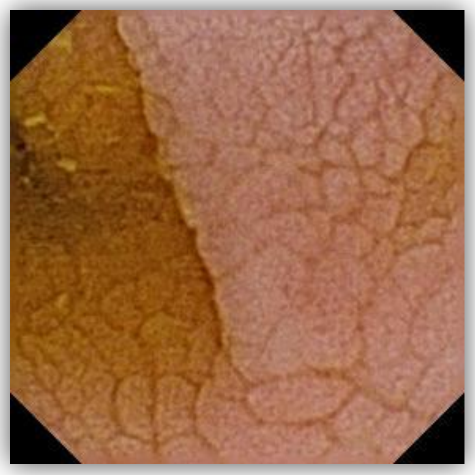

During the treatment she was admitted in the hospital with persistent diarrhoea, weight loss, and anemia. The serologic tests for celiac disease were positive together with upper endoscopy.

We performed small bowel investigation with Endocapsule® in order to determine the extent of disease.

The images (figures 1, 2 and 3) show typical aspects of celiac disease: absence of normal villi, nodularity of the jejunal mucosa with absent folds, fissuring and mild nodularity of the valvulae coniventes.

The gluten free diet was beneficent for the patient which continue the antiviral treatment with sustained viral response.

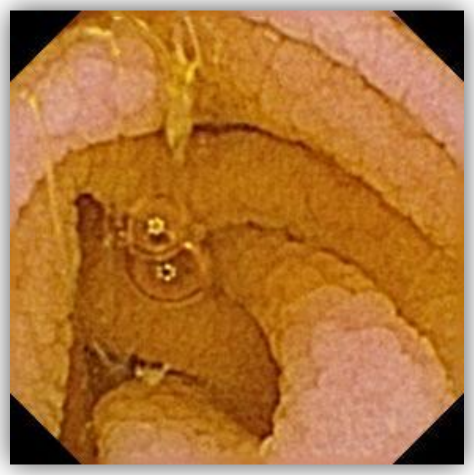

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Paraneoplastic Cushing's Syndrome in a patient with multiple tumors – case report

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INTRODUCTION

Paraneoplastic Cushing syndrome represents 5-10% of all Cushing syndrome and has a severe prognosis due to severe metabolic imbalance, denutrition, associated infections and progression of tumoral underlying pathology. The death is a rule in more than 50% of cases. Some medication used to treat it – Metirapone – is not available in Romania. Ketoconazole was recently approved by CHMP for treatment of paraneoplastic Cushing's only in November 2014.

CLINICAL CASE

A 67 years old woman presented on the 21st of November with mental confusion, progressive weight loss, severe edema and kypokalemia, without typical features of Cushing or hyperpigmentation. Patient's behavior changed in the last 5 months, she was nasty with her daughter, bickering, while diabetes and hypertension aggravated in the last 3 months.

The electrolytic imbalance was severe at admission K 1.65 mmol/l, in spite of multiple attempts to correct it with 150 mmol/day KCl on peripheral i.v. line, 40 mmol/day of KCl orally and 200 mg/day of Spironolactone, treatment used initially in "C. I. Parhon" National Institute of Endocrinology. Patient was transferred in the I. C. U. of "Dr. Carol Davila" Central Military Emergency Hospital for the weekend, in order to obtain a better control using a central intravenous catheter.

Laboratory work and imaging

One month prior to admittance patient had hypercortisolism, with normal hepatic citolytic enzymes and normal TSH, free T4 and calcitonin values. Investigations at admittance revealed paraneoplastic Cushing's with ACTH 82.5 pg/ml, cortisol levels more than 63 mcg/dl, UFC (urinary free cortisol) 2,866 mcg/24 h (21-111); DHEA-sulfate 230.5 mcg/24 h; 2 mg Dexametasone suppression test showed unsuppressed cortisol 59.17 mcg/dl, ACTH 123.8 pg/ml.

Patient also associated empty sella syndrome with thyrotrophic and gonadotrophic insufficiency, normal prolactin and IGF-1, normal mineralocorticoid hormones, cathecolamines, serotonin and 5HIAA, slightly enlarged cromogranine A – 148 ng/ml (upper limit 125 ng/ml).

Patient had also left breast tumor, Helicobacter pylori gastritis, polinodular goiter, denutrition and hepatic dysfunction.

Imaging techniques: 99m Tc Tektrotyd scintigraphy

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showed uptake at 10 minute in left breast and jejunal loop; upper and lower endoscopy, echo endoscopy – revealed no tumors; bronchoscopy – no visible tumor (after procedure, patient suffered a syncope that lasted 2 minutes); thyroid ultrasound – found nodule of 15/18/22 mm located in the lower part of left lobe – 15/18/22 mm, with low peripheral vascularisation, uptakes iodine at CT scan and has a peripheral calcification.

Figure 1. Abdominal CT-showed minimally enlarged right adrenal gland, with nodule 0.88/0.88 cm; enlarged left adrenal gland, with nodule of 1.03/1.24 cm.

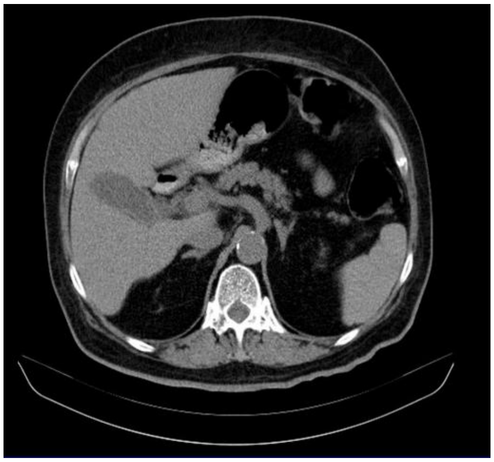

Figure 2. Thoracic CT revealed pulmonary tumor located in Fowler segment of left superior lung lobe.

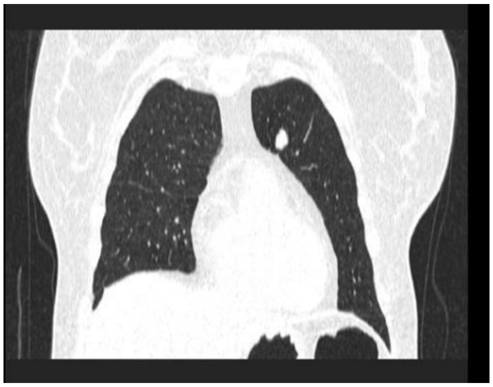

Treatment

We initiated treatment with Ketoconazole 400 mg, 1 day, and then 600 mg, for 2 days, but with inadequate correction of alkalosis and kypokalemia – pH was 7.54-7.59, BE 5.7-9.8 mmol/l, K 3.16 mmol/l. The third day patient became septic (probably MRSA Staphylococcus) due to central catheter and interstitial pneumonia – fibrinogen 660 mg/dl, with high liver enzymes – AST 87-160 UI/l, ALT 95-103 UI/L, GGT 348-365 UI/l, total bilirubine 2.44 mg/dl, leucocytes 13,400/mm^3, granulocytes 8,500/mm^3. Cortisol levels were 26.3-29.2 mcg/dl and Ketoconazole was increased to 1,200 mg/day, also associating Tavanic 500 mg initially, then Tigecycline 100 mg/day. The high values of ALT and AST were due to sepsis and did not increase after doubling Ketoconazole dosage. After 1 day of high dose Ketoconazole, K was 4.7 mmol/l, allowing introduction of Mifepristone 200 mg/day. The seventh day after Mifepristone was introduced, cortisol levels were 18.7 mcg/dl (4.2-38.4), allowing surgery.

Due to denutrition, pulmonary sepsis, lack of localization of tumor – lung/thyroid/ileum, recent syncope, severe brain atrophy with cognitive impairment, we decided to perform left adrenal gland resection.

The adrenal resection was difficult due to diffuse bleeding and lack of tissue elasticity.

Hepatic biopsy showed periportal fibrosis, but no necrosis of hepatocytes, probably due to toxic substances used at work; left adrenal was 7/3/1.5 cm in diameters, with focal hemorrhage. Imunochemistry – Ck7, Ck20, CEA, TF1, ER – negative, MELAN A positive – suggested diffuse hyperplasia of left adrenal gland.

Evolution of patient

One hour afterleft adrenalectomy – cortisol was 18.2 mcg/dl, ACTH 42.3 pg/ml, patient needed inotrop support with Noradrenaline, hydrocortisone 75 mg 1 day, 50 mg in the second day.

The third day cortisol desupressed to 51.25 mcg/dl, ACTH 43 pg/ml (3-66), K decreased again to 2.9 mmol/l, Hb was 8.4 g/dl. Ketoconazole 600 mg/day was started again. Patient had fever, delirium, pulmonary riles, so Meronem was initiated for 2 days, then Tigecycline 3 days, then 7 days of Klacid at home, also Calcium 1 g/day, 1,000 UI D3, 0.5 mcg of 1 alfa-calcidol/day, hepatic protection, vitamins, basal insulin.

Figure 3.Cromogranine staining – magnification 20X – proves neuroendocrine tumor.

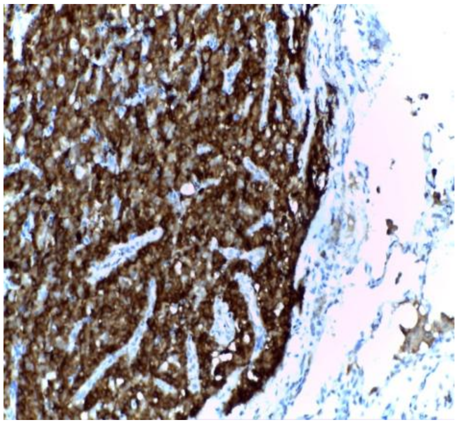

Figure 4.Ki67 staining – magnification 40X – reveals Ki67 of 3% in pulmonary nodule (well-differentiated tumor).

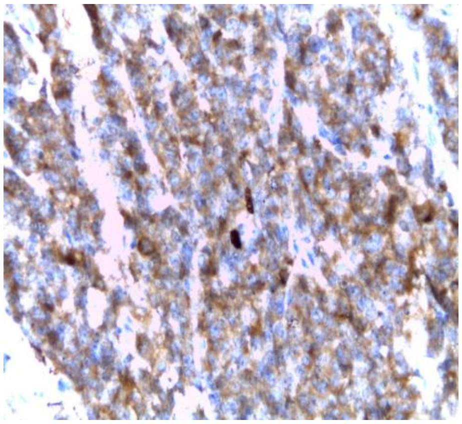

Figure 5.ACTH staining – magnification 20X – ACTH receptor is present in carcinoid tumor.

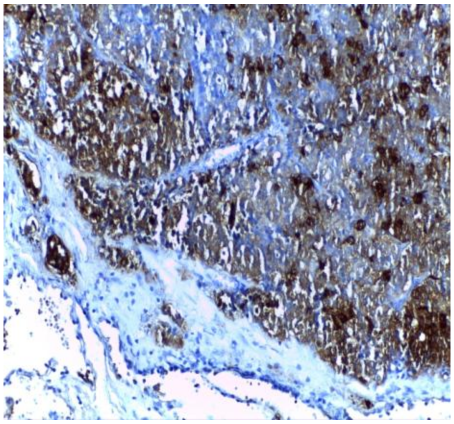

10 days after adrenal resection cortisol was 26.6 mcg/dl, K 3.9 mmol/l, calcium was normal, Mg was 1.57 mg/dl, allowing second operation – resection of lung tumor – proved to be typical carcinoid with ki-67 3%, ACTH, synaptophysin and cromograninepositive.

Evolution after second surgery: 1 day after carcinoid resection ACTH was 5.95 pg/ml (3-66), cortisol 17.38 mcg/dl – labs were done at „C. I. Parhon"Institute.

12 days after carcinoid resection: ACTH 16.56 pg/ml (7.2-63); Cortisol 10.72 mcg/dl (6.2-19.4).

At 22 days: she lost 8 kg, ACTH 19.1 pg/ml, normal ALT and AST, GGT 236 UI/l, Mg 1.39 mg/dl, Ca 9.96 mg/dl.

At 47 days: she lost 15 kg, ACTH 16.5 pg/ml; cortisol 9 mcg/dl, Mg low even with supplementation.

At 78 days: GGT 259 UI/l (5-36), Mg 1.45 mg/dl, K 3.83 mmol/l, glucose 142 mg/dl, cortisol 20.87 mcg/dl, TSH 2 microUi/ml.

At 3 months: basal cortisol 11.2 mcg/dl (6.2-19.4), basal ACTH 17.46 pg/ml (3-88), cortisol 24 EET – 3.24 mcg/dl, cortisol during suppression test with Dexamethasone 1 mg overnight – 0.48 mcg/dl, PTH 12.32 pg/ml (15-165), low 25OH–D 12.8 ng/ml (30-100), UFC 40.3 mcg/24 h (21-111).

We also performed control thoracic CT – revealed left breast tumor of 0.76/1.21 cm, right adrenal with stationary aspect; portal vein was enlarged 14.5 mm; patient performed FNAB of left thyroid nodule on 20 April 2015, showing benign adenoma.

Mild kypokaliemia and hypomagnesemia, even with oral supplementation, sartan therapy and normal levels of cortisol and ACTH persisted after surgery, probably due to severe deficit of intracellular compartment, even at 3 months after carcinoid resection. Patient does not remember the 2 months prior to surgery, even if cognitive impairment is mild now.

COMMENTS

This case was difficult due to metabolic challenges, multiple associated pathology, lack of SSTR2 and SSTR5 receptors on lung carcinoid with negative scan, mild elevation of cromogranine A levels despite a typical bronchial carcinoid. Patient's sister was operated for adrenal adenoma confirmed on histology exam, her daughter had papillary thyroid cancer, but no MEN association was proven in this family. Patient needed more than 30 days of hospital admittance in two different hospitals and five clinics in order to obtain a good clinical result. The vital risk was high due to sepsis, denutrition, metabolic and ionic imbalance, hepatic lesions, anesthesia, brain atrophy, relative adrenal insufficiency after surgery.

There are no guidelines that state the adequate cortisol levels to be reached before surgery, nor the duration of Ketoconazole wash-out to prevent adrenal insufficiency.

Recently the patient discovered ductal invasive left breast carcinoma, operated at 6 months after thoracic surgery and will soon start chemotherapy.

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Median arcuate ligament syndrome (Dunbar Syndrome)

Crina Laslo[1],Anamaria Puiu[1], Ștefan Mardale[2], Iulian Raus[2], Cosmin Căpușan[2]

Thisrare conditionis caused byextrinsiccompression, given the low abnormalinsertion of medianarcuateligamentorfibrousbands and ganglionperiaortitis tissue from the celiac plexus. The ligamentiscomposed oftendonmedialedgesof the twopolesof the diaphragmwhichmeetin the median planeto forman archbefore the aorta.

Figure 1. Graphic image of the arcuate ligament compressing the celiac trunk
Case courtesy of Dr Matt Skalski, Radiopaedia.org, rID: 36837

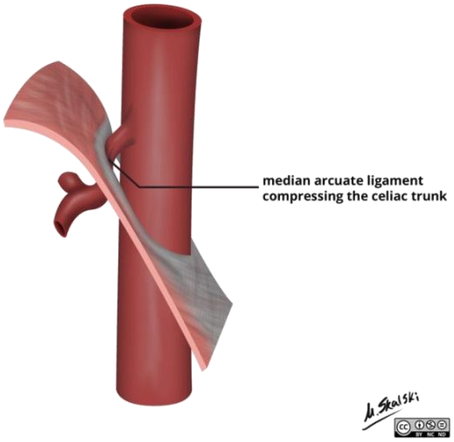

The clinical presentation is variable:
- In most cases asymptomatic
- Weight loss
- Chronic abdominal pain
- Postprandial abdominal discomfort
- Hypertension and tachycardia in the case of renal artery compression

The diagnosis is one of exclusion. Symptomatic patients have numerous investigations for most cases of abdominal pain and underwent cholecystectomies and appendectomies, many unnecessary in order to alleviate the pain. Diagnosis methods: Doppler, angio-CT, angiography or MRI. The gold standard is the angioCT.

Angio-CT: it is highlighted, on sagittal reconstruction, a focal stenosis in the proximal portion of the celiac trunk, bent (hooked appearance). 3D reconstructions can be helpful in identifying stenosis. Gastro-duodenal and hepatic arteries can often be seen as prominent with multiple collaterals.

Differential diagnosis based on imaging is made with atherosclerotic disease.

Our patient D.G., male, aged 53, known with hypertension responsive to treatment and diabetes type II non-insulin requiring, with no clinical complaints, came to the Nephrology Department where an abdominal ultrasound was performed and a pancreatic formation was detected. He came to our department for abdominopelvic CT with contrast iv.

During examination, multiple arterial branches arising from the pancreatic duodenal artery and superior mesenteric artery (arch pancreatitis) are observed, which include pancreatic duodenal region; filiform stenosis in the aortic origin of the celiac trunk, through the median arcuate ligament.

Figure 2. Axial section acquired during early arterial time that highlightsarterialtypecollateral circulationbetweenAMSandtheceliactrunk at the level of the pancreaticduodenalarch.

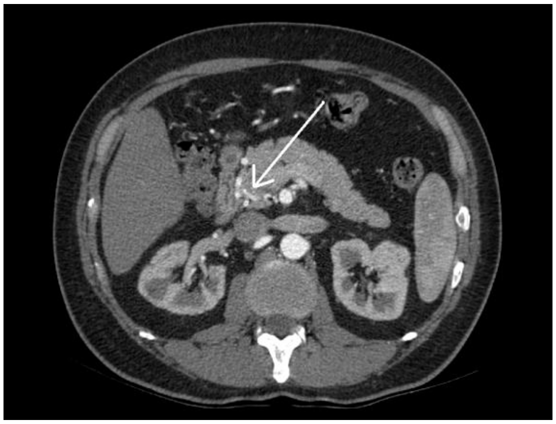

Figure 3. SagittalMPRhighlightingthe trunkceliacfiliformstenosis..

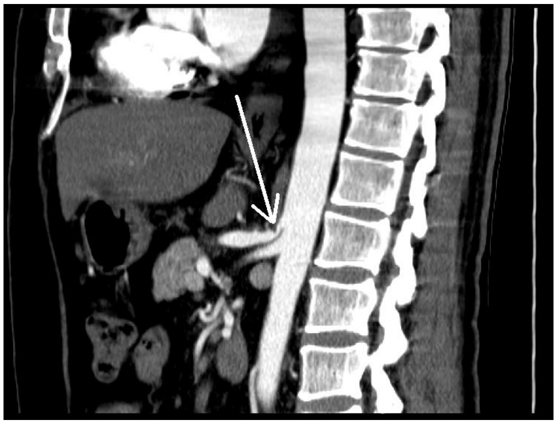

Treatment

The goal of the treatmentis todecompressand obtain celiacarteryrevascularization.

Treatment optionsare quite limitedandthe revascularization surgery has a riskof morbidity and mortalityof5% to15%.

Astreatment methodsmay be used: conventionallaparotomy, laparoscopyorendovascularmethods(percutaneous transluminalangioplasty, stent implantation).

Laparotomy, by retroperitoneal approach via left subcostal incision or by transabdominal approach via midline incision, allows surgical separation of the median arcuate ligament fibers to decompress the celiac artery. Decompression is completed by celiac ganglion resection and evaluation of celiac artery blood flow using a Doppler ultrasound intraoperatively. In case of low blood flow, celiac artery revascularization is performed.

Figure 4.3DVRImagehighlightingthe filiformstenosis at the origin of theceliac trunk

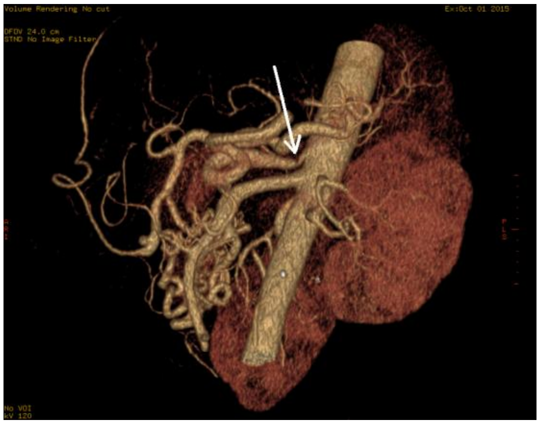

Revascularization methods include aorto-celiac bypass, angioplasty patch.

Literature studies show that classical laparotomy is no longer prefered, due to an increased risk of morbidity and mortality, especially in patients with associated pathologies.

Laparoscopy is used more frequently compared to laparotomy because it is less invasive presenting a lower risk of morbidity and mortality, with low cost due to short hospitalization.

Laparoscopic intervention also allows decompression of celiac artery, but if the celiac artery requires revascularization, surgical management should be changed and the classic approach should be used (laparotomy).

Percutaneous balloonangioplastyis anattractive methodbecause it isa minimally invasive procedure, ideal forpatients with associated comorbidities. This methoddeliverssuboptimal results because mostlesionsdevelop in theostium. Percutaneous balloonangioplastyinostiallesionsis associated withmore severeresidualstenosisdue tointenseelastic recoilcaused bya large number ofcircularelasticfibersfrom theostium. Because of this,the rateof complications(acute occlusion) and restenosis increases.

Stent implantationoffersa metallic supportthat preventselasticrecoilandthusdecreasesthe complicationsof this type.The risksof this methodincludedistalembolism with secondary ischemia, fat embolism, aortic dissection.

Percutaneous angioplastyandstentimplantation,accordingto a 2009 study,arecomplementary methods to laparoscopyafterdecompression of celiacartery was performed.

DISCUSSION

The peculiarity of thiscaseis thatthe patient did notpresentanysymptoms.Thismay be due tothe development of arichcollateralblood supplythat compensates forceliacartery stenosis.

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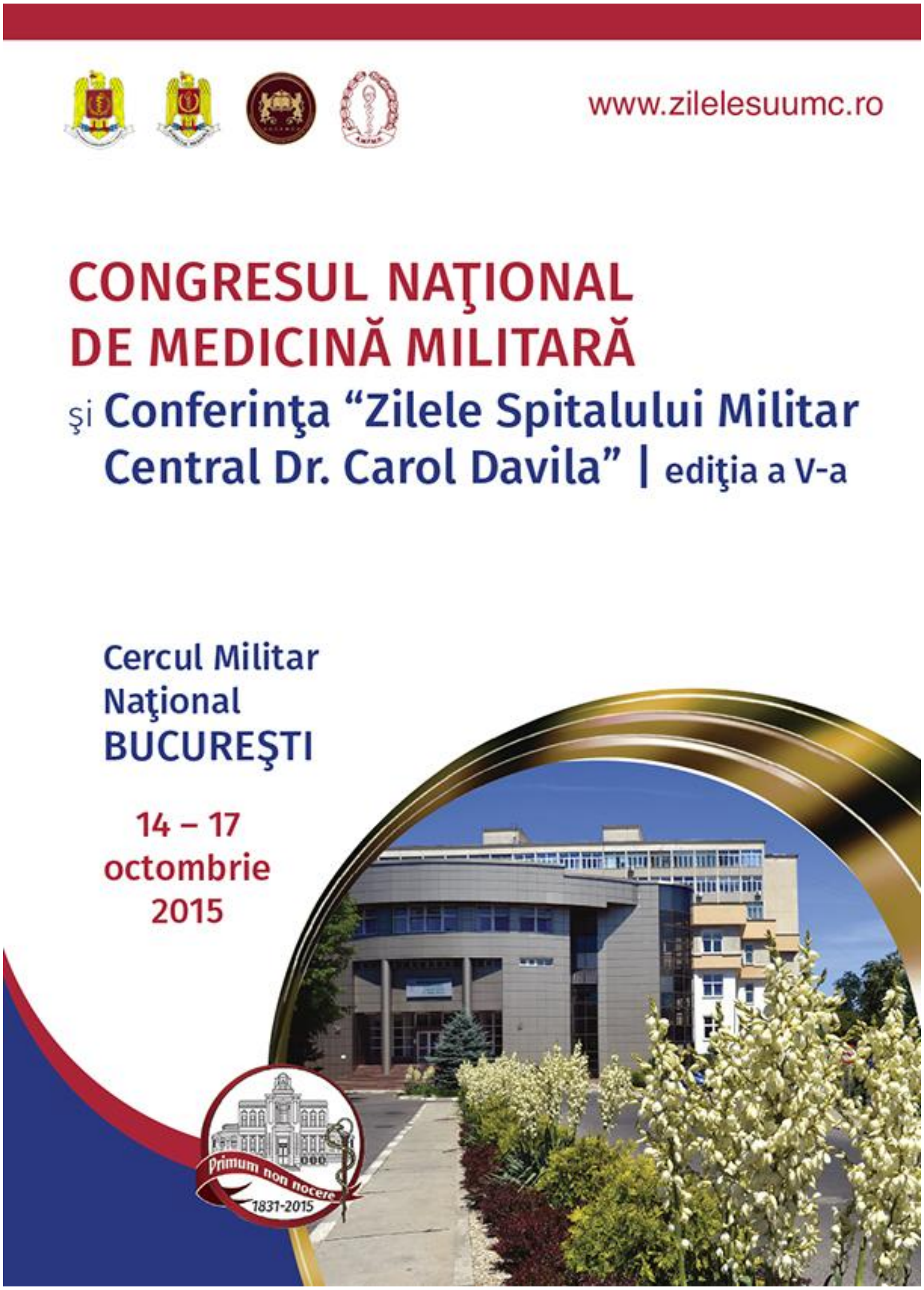

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- *The diagnosis of sepsis using POCT in the Emergency Department. Medical and legal implications*
- *Romanian sanitary system assisted by knowledge management*
- *Spondylodiskitis, etiology, diagnosis and treatment*
- *A cholestatic syndrome may be a surprising cause of medical error*
- *Capsule endoscopy*
- *Paraneoplastic Cushing’s syndrome in a patient with multiple tumors. Case report*
- *Median arcuate ligament syndrome (Dunbar syndrome). Case report*
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EDITORIAL

The Romanian Military Medical System transformation strategy

Constantin Ștefani, Daniel Aron

Colonel CONSTANTIN ŞTEFANI

Chief of MedicalDirectorate, Ministry ofNational Defence, Romania

Facing the Romanian military sistem changeover determined by the affiliation to international security alliances and by the necessity to adapt it to global and regional security environment in order to be able to cope with actual threats and risks, the military medical system should be adapted in order to become efficient in providing medical support in operations and in the same time to provide medical care for soldiers and their families.

The military medical system transformation strategy represents the basic document which reflects the picture of desired end state showing the roads to get there.

The major challenge is represented by the harmonizing process between national and operational NATO laws and rules, because the interoperability is one of the main tasks in National Security Strategy.

Excellent trained medical personnel, establishing the standards of medical logistic support and procedures, the adapting process of the medical structures are the key points of this strategy, being in the same time the main courses of action.

Having the medical military system as a picture on the wall, it can be described as a dual functional mechanism, depending on its acting conditions: operational medicine providing medical support in operations, and stationary medicine providing medical care in peace time. In order to be efficient, the military medical system should have the following features: to be complete from the battlefield through all stage off care, to be self-sustained, to be deployable with troops, and to be able for integration in multinational operational medical system and in the national civilian medical system as well.

The two main missions of the medical military system are preservation of health and the second, treating casualties and healing ill soldiers and their families. During all the actions, in order to accomplish both previous missions, the following general principles will be applied: the best medical practice, continuity of care, the interest of patient and the importance of the mission. This complex system works with excellent trained medical personnel in military medical educational system and combat medical training centers, having a proper medical logistics. For that, beside regulation domain, a significant financial effort will be needed. Moreover, a coordinated long time term financial strategic program to provide proper medical supplies will solve logistical issues in medical military system. Medical research and standardization processes are the engines to progress of all parts of the military medicine. A dedicated and efficient command-control system, working under well defined procedures will integrate medical considerations in the complex military mechanism.

The implementation plan will have the Medical Directorate in the center of the process, being involved from the highest to the smallest level of the medical support, using the military hospitals and the others subordinate structures to coordinate medical activities and to optimize the whole process.

Eventually, in the operational medicine domain every battalion level unit will be supported by a ROL 1 medical facility, every brigade level unit by a ROL 2 medical facility, all military hospitals except Central Universitary Emergency Military Hospital will fulfill the ROL 3 medical facility criteria, and Central Universitary Emergency Military Hospital will be the Romanian ROL 4 medical facility.

An important goal in the development of military hospitals will be represented by involving them in the training activities in their area of responsibility, so six military hospitals near the main ranges will have specific capabilities in order to be able to solve all medical situations with can be a result of high risk activities.

The medical military system will be fully integrated in the operations at all level by a professional and dedicated medical planning system which is the key of an effective medical support.

In the stationary medical domain, the target is to grow up the quality of the medical act from primary medicine to hospitals, in the mean time to prioritize the access of soldiers and their families to military medical system. Another important goal is to optimize the procedures for soldiers to get their legal rights regarding the payment for medical services and pharmaceutically services.

Last but not least, the military medical system should become the most available medical solution for soldiers and their families on the free medical services market.

The military medical system is a special medical system serving a special structure which is Romanian Military Force.

REVIEW ARTICLE

Article received on October 12, 2014 and accepted for publishing on November 21 2014.

The diagnosis of sepsis using POCT in the Emergency Department – Medical and legal implications

Bogdan C. Teușdea[1], Sebastian Dogaru[1], Florentina Ioniță Radu[1,2]

Abstract: *Sepsis, severe sepsis and septic shock are some of the most serious affections which threaten the lives of the patients who come to the Emergency Department and which require fast treatment because the more severe the sepsis was, the higher the mortality, up to 50% higher in severe sepsis. That is why, at present, the 2013 Guides of Surviving Sepsis Campaign recommend that the potential source of infection should be confirmed as soon as possible, in the first 6 hours since the patient arrived in the Emergency Unit if possible, moreover the large spectrum antibiotics therapy must be administered in one hour after the severe sepsis or the septic shock were identified. That is why the identification of these patients at risk is very important and this identification can only be made using POCT type devices.*

This type of devices has the capacity to make precise determinations, in a short time (15-17 minutes), using minimum quantities of integral blood, without using test tubes, sepsis biomarkers and other additional material. The possibility to fast diagnose sepsis, offers the doctors from the Emergency Department, the capacity to fast initiate an antibiotic treatment, to hospitalize the patient and at the same time, it gives them the certainty that they did not miss the sepsis diagnosis, thus avoiding the situation of malpractice. A preliminary study, regarding the sepsis biomarkers, which took place in the Emergency Unit of University Central Emergency Military Hospital, is also presented within this article.

Keywords: *sepsis, POCT, Emergency Department*.

INTRODUCTION

Sepsis, severe sepsis and septic shock are some of the most common affections which are handled in the Emergency Departments and in the intensive therapy sections and they still represent a major cause of morbidity and mortality for critic patients despite the use of respiratory and cardiovascular support, modern antibiotics and resuscitation therapy. In compliance with the newest guides published, fast identification and the speed of implementation of an adequate treatment in the first hours after the patient came to the Emergency Department can influence radically the prognostic of septic patients, facts which determined the concentration on biomarkers for precocious diagnosis, risk stratification and the assessment of these patients' prognostic.[1]

Sepsis is an exacerbated systemic reaction at

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something which would normally be a common infection. Although it was identified from the oldest times, the sepsis is still a challenge for clinicians and it remains, most of the times, a lethal complication. During the last 10 years, in compliance with the Protocol initiated by Surviving Sepsis Campaign for the management of patients with sepsis, the mortality was reduced from 37% to 30%, nevertheless its percentage is still unacceptably high.[2]

The legal framework for the management of patients who come to the Emergency Unit is provided in the Order of the Health Ministry no. 1706/2007 regarding the management and organization of the emergency receiving units and compartments.

The patients who come to the Emergency Department are taken over by the employees of the department and they are selected according to the emergency degree in compliance with the Order of the Health Ministry no. 48/2009 – National Protocol for the Selection of Patients from the Emergency Receiving Structures.[33]Patients' selection is defined by law as follows: the mechanism or procedure by means of which the patients who come to the Emergency Receiving Unit (ERU) or to the Emergency Receiving Department (ERD) are assessed and classified, upon arrival in ERU or ERD, by a competent person (physician or average sanitary employee), taking into consideration their clinical state and the symptoms they declare, correlated with their age and medical history, how stable their vital functions are, the potential of exacerbation of their medical state, the necessity to implement a treatment or to perform some investigations, as well as other information which are considered to be relevant, so that it can be decided which is the priority for each patient to be assisted and the level of assistance necessary for each of them.[33]

Patients' selection within the Emergency Department represents the medical procedure used to assess and categorize the patients arrived within the ERU by a nurse/physician, in order to decide their priority for medical care and its level. The nurse responsible with the patients' selection procedure is the nurse with specific preparation and with appropriate experience and abilities. The recommendation provided by the law is that the time allowed for the patients' selection should not be longer than 2 minutes.[33]

The level of patients' selection refers to all patients who have the same priority degree according to the severity and/or critical state of their pathology and to the necessary resources. Priority levels are the following:[33]

- Level I – *resuscitation* (Red Code): The patient who requires an intervention to save his/her life NOW. Maximum time allowed for the patient to be taken over in the treatment area: 0 minutes.
- Level II – *critical* (Yellow Code): The patient who is in a situation with major risk or altered mental status (acute modification) or any intense pain or major discomfort. Maximum time allowed for the patient to be taken over in the treatment area: 10 minutes.
- Level III – *urgent* (Green Code): The patient with stable vital functions who requires however, two or more medical laboratory or paraclinical investigations. Maximum time allowed for the patient to be taken over in the treatment area: 30 minutes.
- Level IV – *nonurgent* (Blue Code): The patient with stable vital functions who requires only one laboratory or paraclinical investigation. Maximum time allowed for the patient to be taken over in the treatment area: 60 minutes.
- Level V – *examination* (White Code). The patient who requires neither emergency medical assistance nor laboratory or paraclinical investigations. This category includes people who come for one of the following reasons: vaccination; social case without clinical symptoms; clinical and administrative issues (medical certificates, prescriptions, etc.). Maximum time allowed for the patient to be taken over in the treatment area: 120 minutes.

The patient has the right to have his health state periodically reevaluated if he has been taken over in the treatment area after more than 30 minutes or if there are significant modifications in his/her state, which means that patients' selection procedure within the Emergency Department is periodically resumed.[33] More than once, this task is a difficult task to be performed, taking into account the big number of patients, insufficient personnel and the permanent stress the personnel from the Emergency Department has to deal with. In addition to these, the risk of bad evolution for septic patients and not only for them is quite high, therefore it is necessary that the diagnosis methods in this department are fast, precise and trustworthy.

We debate the issue of septic patients in this article because this kind of patients have a rather unpredictable evolution if they are not diagnosed correctly and in due time. More than often, old patients who also suffer from diseases in different stages are neglected or superficially treated at home, fact which makes the sepsis evolve frequently up to advanced stages until patients come to the hospital. This kind of patient is brought to the hospital with the ambulances of the national system 112 (SMURD or Ambulance Service) or by his/her relatives, being already in a critical state, dehydrated, with high temperature or with neurological signs.

Diagnosis of sepsis is not easy, it involves special issues, because at present, the available methods are related to the performance of the following laboratory analyses: the number of leukocytes (WBC) in the patient's blood resulting from a complete hemogram with leukocytes formula, the number of germs, sepsis biomarkers, CRP (reactive protein C) and last but not least the performance of bacterial cultures from blood (hemocultures) and from other fluids. Moreover when the physician from the Emergency Department is under time pressure and under pressure from the patients' relatives as well as from the other patients who do not understand the patients' selection Protocol mentioned above, a fast diagnosis has an even greater importance. The main topic of discussions is the fact that some patients have higher priority than others, meaning that some have to wait longer and others, who have just arrived, are immediately taken over and treated in the Emergency Department. The duty to introduce the patients in the 5 levels of selection belongs to the nurse from the patients' selection and eventually to the physician on duty; the two decide which patients have a higher level of priority.

Therefore, the physician from the Emergency Department should have available all necessary resources in order to make a fast, correct and hard to question diagnosis in the prospect of any malpractice accusations. Sometimes, there are patients who arrive in a state of septic shock and they have a fast evolution to decease because of multisystem organ failure (MSOF), so it is difficult for the patients' relatives to understand the reason why a patient having an apparently good state during the day, dies in hospital in less than 24 hours. That is why the physician from the Emergency Department (emergency medicine physician or intensive care physician) must make the correct diagnosis: SIRS, sepsis, severe sepsis or septic shock, so that a fast, appropriate and complex treatment can be initiated.

According to the law, the physician from the Emergency Department has the possibility to require all necessary examinations from different specialties in order to make a diagnosis, the period of time allowed before the patient is examined varies between 10 and 60 minutes according to the level of emergency – 10 minutes for red code and maximum 60 minutes for green code.(32)

It is important to have in mind that patients must be subjected to a series of laboratory and paraclinical investigations, as soon as possible upon their arrival, especially in the case of patients belonging to the first 2 levels of priority. In view of a better understanding of the phenomenon, we shall make a review of the definitions of the Systemic Inflammatory Response Syndrome (SIRS) and of the sepsis with its stages.

DEFINITIONS

Systemic Inflammatory Response Syndrome (SIRS) is the clinical syndrome which results as a consequence of an inadequate inflammatory response of the body at a noninfectious origin lesion, such as: pancreatitis, vasculitis, autoimmune affection, thrombembolism, burns or surgery interventions.(3) SIRS diagnosis is made in the presence of 2 or more of the following criteria, as follows: temperature $>38,5^{o}C$ or $<36°C$; ventricular rate >90/min; respiratory frequency >20/min or $PaCO_2<32$ mmHg; leukocytes $>12000/mm^3$ or $<4000/mm^3$;

Sepsisis the clinical syndrome which results as a consequence of an inadequate inflammatory response of the body at an infection. Sepsis can be presumed if 2 or more of the conditions mentioned above are present and the infection is identified either through germs culture or visually.[1]

Severe sepsis: sepsis associated with the organ dysfunction/insufficiency, impercipient perfusion or hypotension.[1]

Septic shock: hypotension within sepsis, with all appropriate replenishment.[1]

Multisystem organ dysfunction – MSOD: organ dysfunction at a patient with an acute pathological state so that homeostasis cannot be maintained without medical intervention.[1]

WHY „POINT OF CARE TESTING – POCT" DEVICES FOR EMERGENCY DEPARTMENTS?

Point-of-care testing (POCT) is defined as a medical testing at or near the site of patient care.[21,22] Another definition would be the following: a group of investigation, diagnosis or screening technologies, which require neither personnel nor laboratory conditions, performed on spot where medical assistance is provided, within or outside a medical unit.The purpose of POCT is to provide immediate, convenient, and easy-to-use diagnostic testing that shortens the therapeutic turnaround time when providing care for a patient.

The objective is to provide rapid diagnostic information that permits immediate clinical management decisions to be made that will improve patient safety and clinical outcomes, not to mention patient satisfaction.It is important to be noticed that these technologies do not require special spaces or additional personnel, other than those that can be found in any hospital/physician office. POCT can be found in more environments: hospital bedside, ambulatory care settings (clinics or physician offices), alternate care (skilled nursing facilities), and home settings.

In Romania, medical devices are governed by Law no. 176/2000 regarding medical devices, within this law, in article 2 point a) and b) we find their definition as follows[29]:

a) Medical device – any instrument, device, mechanism, material or another article used alone or in combination with others, including the necessary software for its correct use, designed by the manufacturer for human use and which does not fulfill the main action for which it was designed in/or on the human body by pharmacological, immunological or metabolic means, but which can be helped in its function by such means, with the purpose of: diagnosis, prevention, monitoring, treatment…;
b) Active medical device – any medical device whose functioning is based on another source of power or of energy than the one generated by the human body or by gravity;

POCT benefits are numerous, below we are presenting the most important of them[21,22]:

- Positive patient identification;
- Immediate diagnostic test results (maximum 15-17 minutes) – reduced test and therapeutic turnaround time, 24/24 hours;
- Reduction and/or elimination of specimen/sample transport;
- Elimination of blood collection tubes and centrifugation with fresh whole blood specimen;
- Reduced blood specimen volume;
- Reduced volume of reagents – POCT is a less invasive method from the clinical point of view;
- Data management and connectivity – POCT systems can be connected to the informatics systems of the hospitals having as result: less transcription errors, immediate data analysis – utilization, quality control, compliance and data mining, development of disease specific algorithms;
- Good cost-benefits report – although generally the tests are more expensive, they can offer large economical benefits, by reducing the number of visits in hospital, days spent in hospital and repeated hospitalizations.

Potential disadvantages[21,22]:

- Weak analyses quality;
- Lack of results interpretation;
- Wrong results which are difficult to trace;
- The possibility to make a battery of tests can lead to the performance of unnecessary and inadequate

analyses;

- Lack of alignment with laboratory results – reference intervals and results can be different to those issued from the classical laboratory which makes the comparison difficult.

The current diagnostic laboratory system has been slow to change and is in need of change to a more patient-centered system. Thus, today we need personalized medicine and a patient-centered medical system.

The model of centralized lab testing was developed in the late 1960s but the new technologies and the evolution of the health system demanded more rapid testing which led to a significant increase of POCT. The development of new technologies, such as „lab on a chip"[23,24]and DNA/RNA-based molecular diagnostic tests[25,26, 27,28], will expand the menu of POCT tests and will lead to an increase in the use of this device.

Taking into consideration all the benefits mentioned above, POCT improves the diagnostic activity; the most important aspects are immediate and precise diagnostic, the use of whole blood specimen in an extremely small quantity and reduction of specimen/sample transport to the laboratory. Due to the fact that it provides information to the Emergency Department physician upon diagnosis and patient's critical state, it also offers him/her a certain safety which, at the same time, eliminates the stress related to malpractice.

The physician from the Emergency Department can ask for interdisciplinary examinations, as already mentioned, however, if he/she does not have any investigations to show to his colleagues in order to make at least a presumptive diagnosis, the physician extends patient's hospitalization time in the Emergency Department waiting for the laboratory analyses made in the central laboratory.

In addition to that, the Romanian legislation, more precisely, the Order of the Health Ministry no. 1706/2007 – which regulates the activities from the Emergency Departments, in Annex 1 – stipulates a series of minimum mandatory paraclinical and laboratory investigations which should always be available for the patients from the Emergency Departments.

These investigations complexity depends on the type of the Emergency Department (I or II), as related to imaging, but if we refer to basic analyses (hemogram, blood glucose meter, electrolytes, sanguineous gases), these are mandatory and it is preferable for them to be made in the Emergency Department in order to reduce the time until a diagnosis is made.

High level Emergency Departments – type I, must also have the possibility to make toxicology analyses. All these mandatory analyses, but also other analyses which can lead to an immediate and precise diagnosis, can only be made with the help of POCT technology.

SEPSIS DIAGNOSIS

Biomarkers

Biomarkers can have an important role in proving the presence, absence or stringency of sepsis and they can make the difference between fungal and viral infections, between systemic sepsis and local infection.Other potential roles of the biomarkers include prognostic, antibiotic therapy, evaluation of treatment answer and postsepsis recovery, differentiation between gram-negative and gram-positive germs, the possibility to predict sepsis complications and the development of organ malfunctions (heart, kidneys, liver or multiple organ malfunctions)[4]

Biomarkers definition. At present, the accepted definitions for biomarkers are in compliance with the studies made by U.S. National Institute of Health (NIH) and by European Medicines Agency. A biomarker is a "biological characteristic, objectively measured (with acceptable accuracy and reproducibility) and used as an indicator for a physiological or pathological process or for the activity of a medicine".

In compliance with NIH standards, biomarkers can be classified in two categories: *prognostic markers* – which allow for the patients to be classified according to the individual risk to have a specific prognostic, regardless of the treatment (or lack of treatment) and *predictive markers* – which allow the forecast of the potential benefit (efficacy) and/or the risks (toxicity) of a treatment according to the status of a biomarker (absent/present)[5].

The ideal biomarker in infectious diseases is used to identify a high risk group or predisposing factors, as a tool for disease identification or for treatment prescription and classification of patients according to their specific factors as well as/or as indicator of the therapeutic management in order to avoid reinfection.An ideal marker for infections would combine the diagnostic, prognostic and treatment follow-up characteristics and it should be easily and fast available for clinic use[6].Biomarkers should evaluate the severity of an infection or predict an evolution excluding complications to help the clinician make a decision regarding the best therapeutic approach in the most appropriate environment (hospital or specialty ambulatory, intensive therapy or hospital section). Furthermore, it should help the clinician decide if it is necessary to introduce or to continue antibiotic therapy.

Concluding, the “ideal” biomarker for sepsis diagnosis should make different diagnosis between SIRS and sepsis, between viral infections and bacterial ones, it should also “detect” sepsis fast, reflect de severity of the disease so that therapy can be monitored, have a high predictive value, be stable in different samples and eventually be quantified fast using “Point of Care” devices.

During the last few years, more potential biomarkers for infection were suggested and their analysis is a complex task. The present tendency is to use biomarkers – especially cytokine – in correlation with multiple tests which measure simultaneously more biomarkers from only one biological sample. The main purpose is to examine if the clinical performance and utility can be transposed in every day clinical situations, taking into account the great number of patients which come to the Emergency Departments and the necessity to make a diagnosis fast.

A fast diagnosis allows physicians from the Emergency Department to implement a precocious treatment which increases the patient’s survival rate and which, at the same time, offers them the certainty that they are not wrong and that they have not passed by an infectious affection with lethal potential for that patient.Thus, the malpractice risk for the physicians from the Emergency Department is lower, since it is known that they have to deal with a great number of patients every day.

Available routinebiomarkers

Three biomarkers fulfill the criteria mentioned above and are available at all times: C reactive protein (CRP), procalcitonine (PCT) and presepsin (PSEP).

CRP was tested in different studies but only some of these studies focused on its use for the improvement of antibiotic therapy. Further to these studies – completed or ongoing – the use of CRP cannot be recommended at present as a criterion for the initiation or end of antibiotic therapy in adults; however, for children, CRP can be probably used as an indicator to end the antibiotic treatment although the proofs obtained up to present are limited[5].

Procalcitonine – PCT was testedon a larger scale for the improvement of antibiotic therapy, both for adults and for children. The conclusion of more studies completed recently, which involved a significant number of patients, is that the introduction of procalcitonine values in the decision algorithms for infection management in specific infections is most likely adequate. It is however necessary to continue researches for specific infections which have not been examined enough up to the present time, for a more precise definition of procalcitonine role in the management of antibiotic therapy[5].

Presepsin – PSEP. In this article, we shall present a new biomarker, which is a viable option for sepsis precocious diagnosis – **presepsin *(sCD14-ST)*** and we shall present its correlation with a score (MEDS score) to supply the gaps which are related to this biomarker, in comparison with what is used in present in clinical practice. The biomarker was used for the first time in 1993[32] and then in 1994 (*Durieux et al. Eur J Immunol 1994;24:2006-12*). Presepsin was studied starting with 2005 and it became an important new marker for the diagnosis and prognostic of sepsis in the last years.[8,9,10,11,12].

CD14 is a glycoprotein expressed on the surface of the monocytes/macrophages membrane (mCD14) and it serves as receptor for lipopolysaccharides (LPS) and for the protein which ties the LPS (LPBP). CD14 co-locates using a receptor 4, Toll-like type (TLR4). When tying LPBP complex, CD14 activates the specific pro-inflammatory signalizing cascade TLR4, thus initiating the host inflammatory reaction against any type of infectious agents.

LPS-LPBP-CD14 complex is released in circulation, canceling CD14 from the cellular membrane, thus soluble CD14 (sCD14) is produced. Nevertheless the activity of the proteose from the plasma generates another molecule sCD14, referred to as subtype sCD14 (sCD14-ST) or presepsin – a protein of 13 kDa which is actually a part of CD14, lysated at N-terminal head (*Durieux et al. Eur J Immunol 1994;24:2006-12*).

PSEP levels were significantly higher at septic patients than at those with SIRS or at those apparently healthy. The increase of PSEP levels was more precocious than the increase of IL-6 and D-dymers levels in a study which created a model of bacteremia on animals (cecal ligation and puncture on rabbit – CLP). The determination of presepsin concentration can be used not only for the diagnosis and prognostic of sepsis, but also to monitor disease evolution and feedback at therapeutical interventions.[8,9,10,11,12]

Recently discovered biomarkers of potential interest in the near future

At present, intensive efforts are being made in the research field of some new prognostic and diagnostic biomarkers which can be useful in antibiotics therapy management from acute infections. For adults, three of these seem promising: sTREM-1 (soluble Triggering Receptor Expressed on Myeloid cells-1), suPAR (Soluble urokinase-type Plasminogen receptor) and ProADM (proadrenomeduline). These biomarkers are accessible, they have proved sensitivity and/or specificity and they were studied on a significant number of patients so that they are worth to be taken into consideration further on. For children and babies other studies are also necessary.[5, 13]

STREM-1 is a member of immunoglobulins big family, surface receptor which appears at mature monocites and polimorphonuclears, they contribute at native immunity. Its expression is upregulated when phagocytes are exposed to bacterial fungic pathogens but not during other noninflammatory processes. TREM-1 amplifies the inflammatory response by increasing the proinflammatory cytokines production, inhibiting the synthesis of IL10. During the up-adjustment of the surface receptor TREM-1, the soluble form sTREM-1 increases in biological fluids (blood, bronchoalveolar lavage fluid, cerebrospinal fluid) and it can be determined with ELISA commercial kits. According to some recent studies, the dosage from the infection spot seems to be more significant than the measurements made from plasma.[5,13]

SuPAR (Soluble urokinase-type Plasminogen Activator Receptor) or CD87 is a receptor for inflammatory response spread on a large scale. It appears only on the surface of a few cell types, such as: endothelial cells and leukocytes (monocytes/ macrophages,polymorphonuclears).

Expression of its gene is under the control of immune and inflammatory effectors, such as bacterial products (LPS), cytokines (IFN-γ, TNF-α, IL-1β) and growth factors (FGF-2, VEGF, TGF-β, EGF). During the inflammatory and immune response, suPAR expression is upregulated by epithelial cells, white blood cells (lymphocytes), smooth muscle cells and fibroblasts. Expression is also upregulated during tumor growth and metastatic spread. The dosage may be achieved using commercial ELISA kits available on the market, but also as part of the multiplex kit with multiple cytokines. However, suPAR seems to be of limited value as a diagnostic test in specific pathologies (patients at risk, HIV patients on antiretroviral therapy, monitoring patients with non-pulmonary bacterial infection and children with malaria with Plasmodium falciparum).[5,13]

Pro-ADM Adrenomedulinis a52amino acidpeptide, a marker ofCALCgene familythat worksasa mediator ofcell proliferation, hormonalregulationandembryogenesis. ADMis produced by theendothelial cellswhere itinducesvasodilationand maintainshomeostasis. Pro-hormonal fragments(pro-ADM) are more stable thanthe complete peptideandtheir levelsin biological fluidscan be measuredbyautomated methodsTRACE(TimeResolvedAmplifiedCrypt-Emission) after the capture of immunoassays. The secretion ofproADMincreases duringan immune response againstviral or bacterialproductsaccording to the sizeof the stimulus. Pro-ADM is avaluableprognosticmarker. Aspart of anevaluationscore ofpneumonia severity, it can identifypatients in rather critical state which would requiremonitoring/hospitalization inanintensive care unit.[5,13]

Future Biomarkers

Micro-RNA (miR)are newly discovered potential biomarkers. miR are small molecules (approximately 20 nucleotides) present in eukaryotic cells, which act as biological regulators by modulating post-translational regulation. They are ubiquitous and present in abundance in the lungs, liver and kidneys. After binding to the appropriate smRNA sequence, they regulate the expression of the gene by a repressor effect or by altering the target sequence. A fragment of miR can bind several smRNA. Their expression can be measured by RT-PCR and quantitative PCR. miR are potential candidates for early diagnosis and/or prognostic markers in sepsis, but other numerous studies are necessary to understand their role in biochemical and immunobiological processes before it can be used to stratify, to make prognosis or therapeutic decision in septic patients.[5,13]

The diagnostic and prognostic evaluation of***presepsin*** in sepsis in the Emergency Department was studied in comparison to other available routine biomarkers, in a study involving 859 patients, conducted by an university hospital which has between 240,000 and 260,000 hospitalizations per year.[10] These findings were published recently, and the conclusions are consistent both with literature data available in today specialty literature and with personal observations generated during similar studies in our hospital which has 25,000 presentations through the Emergency Department per year.

The efficiency of using presepsin to diagnose cases of sepsis, severe sepsis and septic shock was significantly increased by using MEDS score in evaluating these patients as it will be shown below. It is also worth mentioning that although PCT (procalcitonin) can be used as a biomarker for the diagnosis of sepsis, it increases in other situations, such as: multiple injury, extensive burns, pancreatitis, organ transplantation, major surgery and SIRS – not only in infections, therefore, only the positive and negative predictive values are not enough to exclude or confirm sepsis.[10] A recent meta-analysis showed that the diagnostic performance of PCT was reduced, with 71% (95% confidence interval 67-76%) sensitivity and specificity for serum PCT, as a biomarker for sepsis. In conclusion PCT can not clearly differentiate sepsis from other diseases in critic adult patients.[10,14,14,16,17,18] Thus, to diagnose sepsis, severe sepsis and septic shock, PSEP proved to be superior to PCT, moreover, PSEP together with the assessment by MEDS score was superior to PSEP taken as sole indicator.[10,19]

In the 28-day mortality prognosis PSEP was inferior to PCT, but these were lower to the correlated interpretation and MEDS score. For septic patients and prognostication of mortality after 28 days, PSEP, MEDS and APACHE II score proved to be independent predictors unlike PCT which did not have this capacity. The mentioned study showed that plasma levels of PSEP were a parameter closely related to the severity of sepsis.[10,19,20]

Compared to the PCT, PSEP is a highly specific biomarker for the diagnosis of a bacterial infection because it is produced in conjunction with bacterial phagocytosis.

PSEP was higher than PCT and had greater sensitivity, specificity, positive predictive value, negative predictive value and accuracy of prognosis in early stages of sepsis which is consistent with the data from the updated literature.

The more severe the sepsis was, mortality also increased with over 50%, that is: mortality in severe sepsis. Dellinger et all., in Early-Goal Directed Therapy from 2013 guides of the Surviving Sepsis Campaign, recommends that the potential source of infection should be confirmed as quickly as possible, if possible within 6 hours since presentation and that large spectrum antibiotic therapy should be administered within one hour from the identification of severe sepsis or septic shock. Therefore identifying these patients at risk is very important.

Table 1. MEDS Score (Mortality in Emergency Department Sepsis Score)[30,31]

Variables for MEDS Score	Points
Comorbidityfastterminal– fast terminalassociated disease(metastatic canceroranothercondition that cancause deathin30 days)	6
Age >65 years	3
Septic shock (PAs<90 mmHg with all volemic repletion)	3
Number of plates <150000/mm^3	3
Leukocytesprematureunsegmented-bands(>5% of totalleukocytes)	3
Lowerrespiratory infection(clinical infiltratedpneumoniaor chestRxG)	2
Altered mental status(level ofalert or another change in the levelof consciousness)	2
Chronic patients treated at home	2

STUDY ON THE IMPORTANCE OF PRESEPSIN IN ASSOCIATION WITH MEDS SCORE, SEPSIS PRECOCIOUS DIAGNOSIS. PRELIMINARY DATA

In the Emergency Department of the Universitary Central Emergency Military Hospital, a study was made during a year, involving 300 patients suspected of sepsis that came to the Emergency Department, patients brought by their relatives or by the ambulances from the national emergency system.

From these, 32 patients were introduced in the study (19 men and 13 women), for which the following inclusion and exclusion criteria were used:

Criteria for inclusionin the study:
•Age ≥18yearswith clinical signs ofsevereinfectionsrequiringbloodsampling;
•The presence of2 of 4SIRScriteria: fever > 38^{0}C<36^0 C; heartrate>90/ min; respiratory rate >20/minor existence of hyperventilation(PaCO$_2$<4.3 kPa/ 32mmHgin arterial blood); leukocytosis>12,000/ml, leukopenia<4000/mlor >10%prematureunsegmentedgranulocytes(bands);

Exclusion criteria: age under 18andrefusal to sign theconsent.

All patients were examined by doctors of emergency medicine and ATI employees of the Emergency Department and after the former signed the informed consent, venous peripheral approach was made, blood was collected for laboratory testing and they were subjected to other paraclinical investigations (abdominal ultrasound, CT in different regions, x-rays) in order to make a diagnosis and start the appropriate treatment immediately.

The following laboratory tests were made: complete blood count with differential, coagulation (Quick time, fibrinogen), biochemistry (glucose, urea, creatinine, ionogram, samples of liver, etc.), presepsin, procalcitonin.

Analyses were performed in the Central Laboratory of SUUMC, and the Emergency Department's own laboratory, using "Point of care testing - POCT" and PATHFAST® device type.

Evaluation of patients admitted to the Emergency Department was made with the score MEDS - Sepsis Mortality in Emergency Department[19, 30, 31]. The score was developed to predict mortality for patients with SIRS hospitalized in the Emergency Department. The maximum score is 27 points, score variables and the score awarded to each variable can be found in Table 1.

For all these variables, a score are awarded, score which is summed up for each patient, the sum of the obtained points offers a prognostic for mortality at 28 days.

The determination of presepsin was made using a POCT type device, more precisely PATHFAST®, and the evaluation of the patient's state according to the values/concentration of presepsin obtained was made using the correlations from Table 3.

The patients included in the study were aged between 18 and 92 and they were selected using the inclusion and exclusion criteria mentioned above. The existence of SIRS criteria was considered, the results are those from Figure 1 (14 patients with fever, 25 with RR>20/minute, 22 with leucocytosis, 22 with HR> 90/minute).

Table 2. Correlation between MEDS score and mortality at 28 days as percentage[30,31]

MEDS Score range	Mortality at 28 days (95% CI)
0-4	0,6% (0-3%)
5-7	5% (1-13%)
8-12	19% (11-29%)
13-15	32% (15-54%)
> 15	40% (12-74%)

Table 3. Correlation between PSEP values, diagnosis and measures to be taken [14,15,16,17,18,19]

PSEP (pg/mL)	Diagnosis	Case management
Under 200	Sepsis absence	It is not necessary to sample hemocultures
200-300	Small probability of systemic infection	Other investigations are also necessary among which bacterial cultures (hemocultures etc.)
300-499	Possible systemic infection (SEPSIS)	Therapy with medicines starts after samples were taken for bacterial cultures
500-999	High risk of infection progression in the body (SEVERE SEPSIS) High risk of unfavorable prognostic	Surgery treatment must also be taken into consideration
Over 1000	Major risk of systemic infection progression (SEVERE SEPSIS/ SEPTIC SHOCK), major risk of mortality at 30 days – comparable with score 25 on APACHE II	„Maximal" therapy

Further to patients' anamnesis, we obtained the data represented in Figure 2, in which we resumed the types of affections (number of cases): neurological – 9, neoplastic – 7, cardiac – 1, global cardiac insufficiency – 5, coronary artery diseases – 5, valvular heart diseases – 3), chronic renal diseases – 4 , diabetes – 4, pulmonary diseases (pneumonia – 5, BPOC/asthma – 5), thyroid diseases – 2, others – 27 (HTA, digestive diseases etc).

Patients were evaluated using MEDS score which varied between 3 – 21 points according to the health state of each patient. Some of the laboratory analyses were made in the Central Laboratory, that is the blood cultures and other cultures sampled from 15 patients, out of which only 3 were positive. POCT (Point of Care Testing) devices were used to determine the biomarkers, in the case of PSEP; presepsin had values between 102 – 7129 pg/mL, as it can be seen in figure 3. In the case of procalcitonine, the results were negative or they reached the maximum value of >10 ng/mL, and for PCT, the determination method that was used, was a semiquantitative one, THERMO SCIENTIFIC BRAHMS, which has the following intervals as possibilities:< 0,5; ≥0,5-≤2,0; ≥ 2,0-10; >10 ng/mL. Regarding the diagnosis of sepsis in its different stages, there were 13 patients with SIRS, 8 patients with sepsis, 6 patients with severe sepsis and 5 patients with septic shock who also had the highest values of presepsin.

Considering the affections which led to sepsis (Figure 4), the patients from the study can be divided as follows: pulmonary diseases (COPD, asthma, pneumonia) – 12 cases (5 ♀, 7 ♂), urinary infections – 9 cases (5 ♀, 4 ♂), digestive infections (acute gangrenous appendicitis with generalized peritonitis – 2, gangrenous acute cholecystitis – 4, bowel obstruction – 1, diarrhea – 1, acute angiocholitis – 1) – total 9 cases (3 ♀, 6 ♂), gynaecologycal infections – 1 case and acute endocarditis – 1 case (♂).

Figure 1. The correlation between the number of cases and SIRS criteria

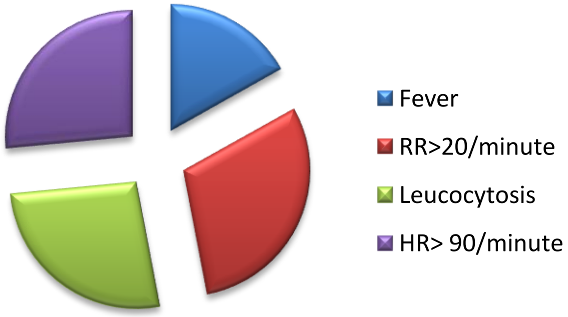

Figure 2. Anamnesis of the patients from the study
– number of cases according to the type of affection –

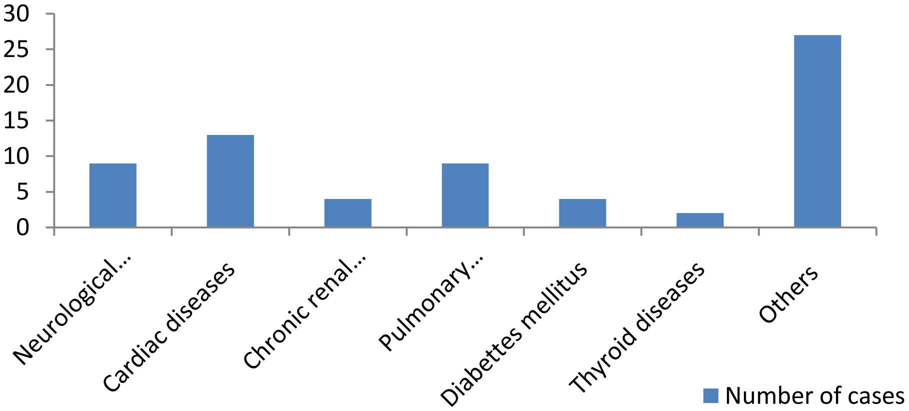

A total of 26 cases were hospitalized in Universitary Central Emergency Military Hospital (81.25%), out of which: 15 cases in medical wards, pulmonology – 2, gastroenterology – 3, internal diseases – 4, oncology – 1, neurology – 2, cardiology – 2 and dermatology – 1 case) and 11 cases in surgical departments (gynecology – 1, urology – 2, general surgery – 8). Out of the total of hospitalized patients, 11 were admitted to ICU and 7 were mechanically ventilated.

Out of the 32 patients included in the study, 8 died in hospital (25%) who had MEDS with values ranging between 8-21 points, the values obtained for presepsin were between 593-6745 pg/mL. 3 cases were diagnosed with sepsis, 2 cases were diagnosed with severe sepsis and 3 cases were diagnosed with septic shock. The affections which were the starting point for the development of sepsis were the following types of infections: respiratory – 5 cases, endocarditis – 1 case, diabetic ketoacidosis coma – 1 case, bowel obstruction – 1 case. Patients who died had a number of associated diseases, some of which were extremely serious, namely: neurological diseases – 4 cases, malignancies – 2 cases, ischemic heart disease – 3 cases.

Figure 3. The evolution of presepsin values for the patients involved in the study

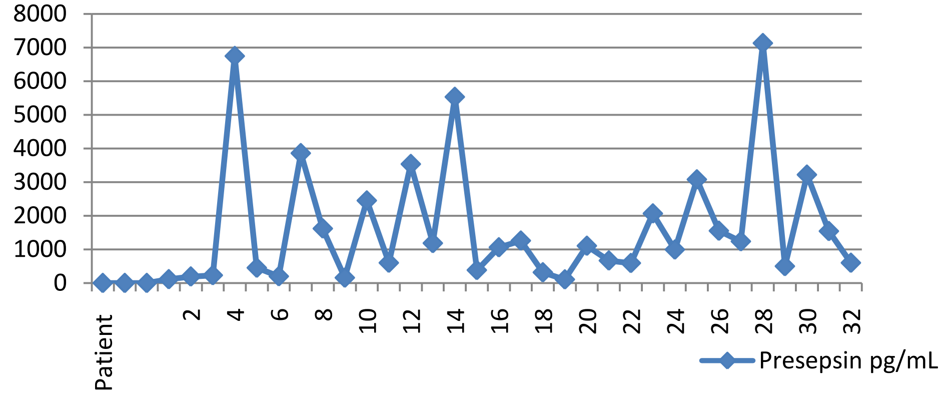

Figure 4. Correlation between the number of cases and the affections which led to sepsis

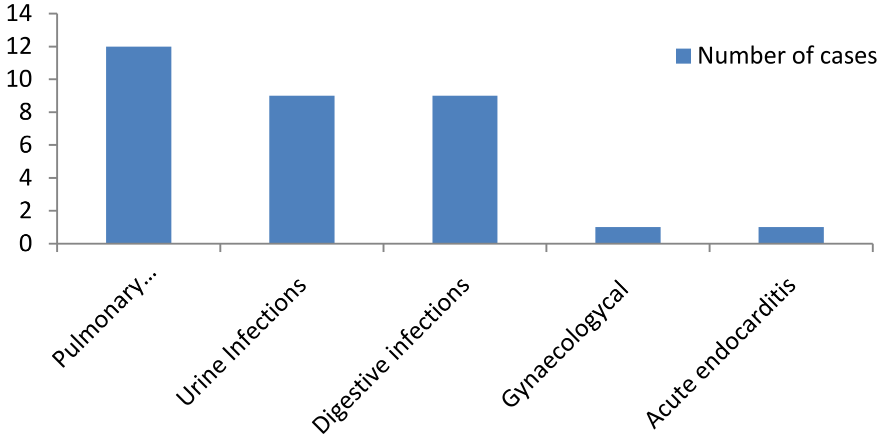

Preliminary conclusions of the study

Presepsinul is an important biomarker having a role in rapid diagnosis of sepsis (17 minutes) to 30 minutes for PCT (on the type of test that we had available), it allows the patient evaluation and classification in a risk category, and its association with MEDS score increased the possibility to provide a correct evaluation of the patients. Rapid diagnosis allows to early start any kind of treatment, primarily the antibiotic, right from the Emergency Department, and in the unclear cases, the physician from the Emergency Department requires further investigations to elucidate the diagnosis.

In selected cases - patients with unrecognized sepsis, the determination of PSEP allows the physician from the Emergency Department, to present their situation to the doctors within Universitary Central Emergency Military Hospital and to suggest and if necessary to hospitalize the patient in cases with diagnostic on the edge according to legal provisions.[32] In our experience related to the sepsis cases presented to the Emergency Department, PSEP determination had a special importance because we could demonstrate the presence of sepsis and not of SIRS as it would have been diagnosed unless for this biomarker. Diagnosing sepsis favored the decision of hospitalizing the patient, his/her close supervision, the commencement of antibiotic treatment in the Emergency Department, in some cases earlier surgical intervention. All patients hospitalized in the Emergency Department and then in the hospital, were investigated using the imaging tools available in the hospital (ultrasound, X-rays, CT with or without contrast, etc.).

In addition to that, determination of the dynamic values of PSEP in hospitalized patients with sepsis allowed monitoring the effectiveness of the implemented treatment, the cost-effectiveness ratio was very good, given the early start of antibiotic treatment and beyond.

In our study, we used the device type PATHFAST® of the company Mitsubishi Chemical that has all the advantages of POCT, namely: the results are quantitative (using a chemiluminescence method type), fast (between 17 minutes), they can be obtained in our own laboratory at local level - from the Emergency Department, can be printed easily, there is a unit memory to store patient data, it uses whole blood – 150 µL (no spin) as sampled from the patient without the need for tube collection.

Moreover, the device is extremely precise (CV <5%), quantitative results are obtained from the blood tests carried out at the same time, because it results in the same test / or up to 6 different assays for six patients at the same time. Such determinations can be made for 1 to 6 patients, making any combination of 1 to 6 tests required.

Presepsin determination by this method allowed risk stratification in criticc patients, monitoring of disease progression and very important, monitoring of the response to drug therapy and other measures (surgery, supportive treatment in the ICU, etc.).

As for forensic results by POCT, given that the sheets can be printed and attached to Emergency Department presentation sheets and general clinical observation sheets, these are of particular importance. It certifies permanent care of the patient under close monitoring of blood different parameters and not only those. With this type of equipment, we can quickly modify patient treatment in real time to correct the dysfunctions and inadequacies thus arisen.

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SYSTEMATIC REVIEW

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Romanian sanitary system assisted by knowledge management

Daniel. O. Costache[1], Cosmin Dobrin[2], Ruxandra Dinulescu[2], Laura Voicu[1], Raluca S. Costache[1,3]

Abstract: *As the medical sciences advances, so does the volume of information which becomes more and more consistent.*

The health care system is one of the most complex systems encountered in our society. Today, knowledge management practices have been adopted in many Romanian business sectors. However, Romanian health care system is slowly adopting such principles and concepts. This fact is created mainly because of the organizational culture. In the sanitary industry, this barrier is composed both from an organizational perspective and also, from an individual perspective.

Through the knowledge management practices, doctors could benefit from the amount of data spread in different geographical regions.

Keywords: *sanitary system, knowledge management, quality*.

INTRODUCTION

In a normal health care system, the patient must be in the center of all activities and any medical error should be avoided, in order to protect the patient from a fatal result. For this reason, a complex source of knowledge could be of great help for doctors.

Health care delivery involves more than one could know, more than a simple relation between doctor and patient. Health care delivery covers health care professionals like physicians, specialists, nurses, lab technicians, social workers, counselors, etc. Also, it includes further parties like hospital/clinic administrators, managers in finances/accounting/human resources/drug companies/ health care insurance companies etc. What is more, health care partners are dispersed around many areas, even if they are acting on the same patient.

Therefore, it is well understood that the amount of knowledge is extremely relevant and that any data knowledge, created by one of the partners, is of great importance to the others in order to deliver quality care.

The term of “knowledge management” (KM) appeared in the 20th century, from fields like management, cognitive sciences and psychology. The present activity of KM started in the 1980s along with the wide use of information technologies in enterprises. There, the main focus was on what

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knowledge represented as an intangible asset.

Basically, the word KM was revealed in the 80s and the academic field was created in 1995 (*Stankosky, 2005*).

WHAT EXACTLY IS “KNOWLEDGE”?

Basically, there are 3 major elements often used together: data, information and knowledge. Still, there are some differences between them, as specified below:

- Data: represents a specific tact or figure, without a specified context.
- Information: represents a data that is organized.
- Knowledge: built on the information, in order to define a proper context.

The main difference between knowledge and information is that knowledge lets us the power to take action. Therefore, we are able to use it.

According to Bryan Duhon (1998), knowledge management represents a discipline that promotes an integrated approach in order to identify, capture, evaluate, and share the amount of an enterprise's information assets. These assets may include databases, documents, policies, procedures, and previously un-captured expertise and experience in individual workers.

Basically, Knowledge management is essentially especially for getting the right knowledge to the right person at the right time.The main objective is to create value and to leverage, improve, and refine the firm's competences and knowledge assets in order to meet organizational goals and targets.

When we look to implement knowledge management, we have to take into consideration several dimensions like:

- KM Strategy: Knowledge management strategy must present a certain dependence on corporate strategy. Its objective is to manage, share, and create relevant knowledge assets that will help meet tactical and strategic requirements
- Organizational Culture: The way people interact, the context where knowledge is created, people’s resistance towards change and also the way they share (or not) knowledge are easily influenced by the organizational culture.
- Organizational Processes: Are represented by the right processes, environments, and systems that enable knowledge management to be implemented in a certain organization.
- Management & Leadership: KM needs competent and experienced leadership at all levels. There are a wide variety of KM-related roles that an organization may or may not need to implement.
- Technology: Represents by the assembly of systems, tools, and technologies that fit the organization's requirements - properly designed and implemented.
- Politics: The long-term support in order to implement and sustain initiatives that involve virtually all organizational functions, which may require a higher cost for implementation (both from the perspective of time and money), and which often do not have a directly visible return on investment.

Knowledge management could be described in 3 ways:

- Explicit: represented by the information or knowledge set out in a tangible form. Also, is represented by the information that is easy to capture, to structure or share with other people. For example, in a hospital, an explicit knowledge could be represented by the hospital’s documentation, like internal policies and procedures, clinic methodologies etc.
- Implicit: represented by the information or knowledge that is not set out in tangible form but could be made explicit.
- Tacit: represented by the information or knowledge that would have extreme difficulty in an operational process setting out in a tangible form. What is more, this kind of knowledge is composed of the amount of experience and skills that a doctor can acquire overtime and apply to problems.

Unlike the explicit knowledge, tacit knowledge is sometimes difficult to capture, to structure and/or transferto other individuals[5].

Figure 1. Key elements that constitute the knowledge management infrastructure (*from Wickramasinghe and Sharma, 2004*)

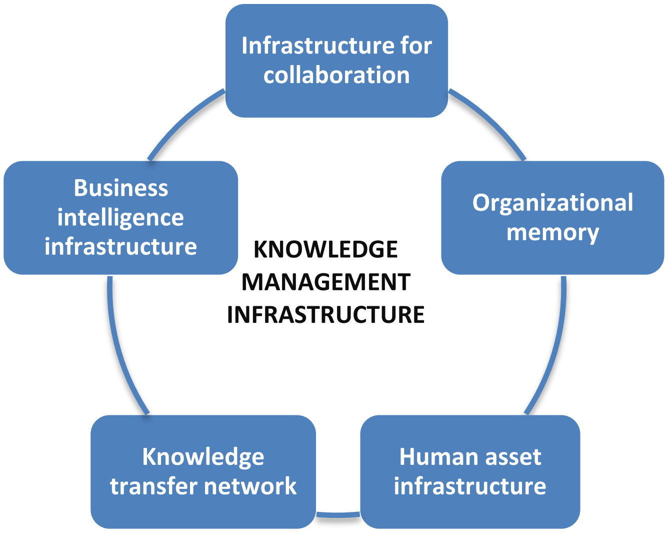
Infrastructure for collaboration
Organizational memory
Human asset infrastructure
Knowledge transfer network
Business intelligence infrastructure
KNOWLEDGE MANAGEMENT INFRASTRUCTURE

TYPES OF HEALTHCARE KNOWLEDGE

a. Provider knowledge – also called "practitioner knowledge". This type of knowledge is the most obvious one especially due to the fact that medical professionals in this capacity have both explicit and tacit knowledge.

An example is sustained by the fact that doctors have to know standard medical information easily comprehended from different reference materials such as text books. Still, some may consider that one of the most important types of providers' knowledge is of tacit form.

b. Patient knowledge – this type of knowledge consists also in tacit knowledge. Nowadays, patients have complex knowledge in past and current medical conditions that doctors may not know about. However, such knowledge is mandatory for doctors to know.

c. Organizational knowledge – this type of knowledge consists in sum of knowledge elements like medical diagnostic systems, text-based materials, etc. What is more, this field contains medical land treatment that is strongly recommended by an institution or medical society.

Why Romania needs to adopt knowledge management practices in its clinics and hospitals?

According to a report from the World Health Organization in 2014, in Romania there are almost 40,000 doctors with free practice. This number is relatively small, with a position that represents a total of 2,5 physicians reported at 1000 individuals.

As we can observe in the above figure, in 2014 Romania was occupying the last position regarding the number of medical personnel. Countries like United Kingdom, France, Germany and even Bulgaria, were in front of us.

Even if, in Romania there is not a huge number of physicians, the information needs to circulate as well as possible. That is why, every doctor has to have knowledge of every new information that appears regarding a medicine, a treatment, a diagnostic, etc.

Here are several reasons why KM needs to be adopted by the Romanian sanitary system:

1. A significant amount of data is in multiple places. Data collected from healthcare tend to be stored in multiple places-from different systems like HR software, to different departments, like radiology or cardiology. Practically, healthcare data comes from the entire hospital/clinic.

Another example is that healthcare data is under multiple forms (like text, numeric, paper, digital, etc). There are times when the same data exists in different formats and in different systems

What sanitary institution could do, is to aggregate this data into a single and central system, accessible to every physician.

2. Healthcare data could be structured or unstructured. In present, Romanian sanitary system is strong related to the National Healthcare Insurance House (NHIH), which created a software that is able to capture consistent data from patients. This would be helpful, but the software appeared only 3 years ago, and before it, all the information regarding the patients' state were wrote down on a paper folder. This is way, hospitals and clinics need KM – to gather all the structured and unstructured data in the same place.

3. Variable definitions; new research practices are coming out daily. Often, healthcare data have variable definitions (regarding a certain treatment, diagnostic etc). There are cases when there may not be a level of consensus about a particular treatment or definition. What is more, the parts that are conducting a certain discussion about a medicine, treatment, etc. are constantly discovering new data knowledge.

Figure 2. World Health Organization, Medical personnel per 1,000 people

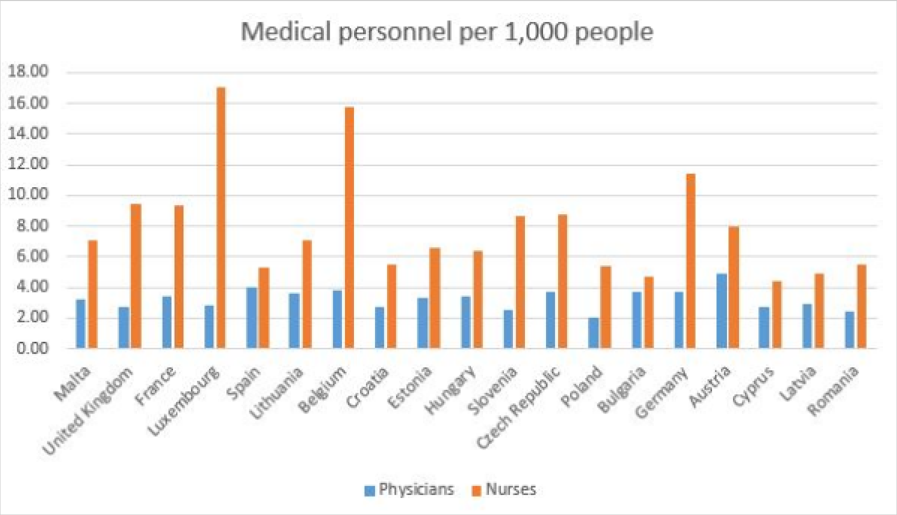

4. The complexity of data. Searching for different developing standard processes that are able to improve quality has always been a main objective for health care providers. Still, the number of data variables involved makes this process more challenging. In health care system, doctors are not working with machines or computer; instead, they work with individuals, more precisely, with human body, which is an amalgam of complex information in constant changing.

Seeing these reasons, we can be sure that healthcare data will not get simpler in the future. On the contrary, it will advance; it will need more than simple software and will present challenges that only a complex and well organized sanitary system could cope.

A POSSIBLE EXAMPLE FOR APPLYING KM IN ROMANIAN SANITARY SYSTEM

From the beginning of 2015, in Romania has been implemented the so called "national health care card", which is nominative for each patient. Its role is to show the doctor what medical treatment has the patient followed, what was his diagnostic and at what medical sections the patient went. In this way, if a patient has firstly went to his family doctor, who diagnosed him and gave him a certain treatment, the second doctor (let's say for example, the dermatologist) would be able to see (with the help of this new system by card) the day when the patient was at his family doctor, the medicines prescribed and the exact diagnose. So far, everything is in order.

Issue that needs to be solved through KM: The new system shows the information only beginning with the date that the card was introduced. In other words, if a 40 years old patient comes for 10 years (since 2005) at the same family doctor, and only this year he had the card (2015), that means that the information area from 2005 till 2015 (regarding his treatments, illness, diagnostics, lab tests etc) is missing on the software program (the doctor is able to see the medical history from a patient only starting with 2015).

Practically, this represents a gap both for physician and also for the patient (of course that the information from 2005 exists, written in the patient's medical record – a notebook, in most of the cases- but is not inserted in the medical informatics system).

Solution of KM: If KM practices would be applied, the present informatics system could be extended, in a manner that would include the whole patient's history from the beginning. Also, the system could specify, for example, if a patient is allergic to some substances (when the doctor prescribes a medicine, if the patient is allergic to a certain substance from that medicine, the program would alert the doctor and in this way, the doctor would prescribe something else – it is also a way of avoiding medical errors).

MAIN BENEFITS FOR IMPLEMENTING KNOWLEDGE MANAGEMENT

The main advantage of introducing knowledge management in Romanian sanitary system is that the information between doctors is easily shared, and most of all, that information is not lost if one of the doctors is sick or has another reason for leaving the cabinet earlier.

In this way, hospitals could be able to have substantial savings. Doctors are brought up to speed, and valuable knowledge assets are not lost (this translates into fact that the hospital or clinic do not lose time and money when doctors need to learn new information quickly).

In a hospital, knowledge management has the power to increase innovation and also to create better patient relationship. For example, if a certain clinic has a global team, knowledge management can create a more powerful workforce when all cultures are brought together in order to share assets (in Romanian clinics, this example is more suitable for private sanitary system).

Another important advantage is that KM could reduce the medical errors and their cost, by providing a decision support.

Next, we will present another KM advantages for healthcare:

- Cooperation between different health care providers;
- Innovation;
- Quality of care;
- Efficiency of work;
- Cost reduction.

All in all, knowledge management gives doctors the capacity of knowledge they need to do their jobs better. In this way, they become more productive.

CONCLUSION

The adoption of knowledge management practices into the Romanian sanitary system could become either a success or a failure. Like any other project, just because a system has been put in place, with the help of the appropriate methodologies or practices, this does not mean that it will become successful in terms of adoption and use.

Because healthcare data is so complex, it's more than relevant that a traditional approach to manage it will not be able to succeed.

This is why, a different approach is needed. The new approach needs to handle multiple sources, structured and unstructured data, variability, the complexity within a constantly changing environment.

The use of KM in sanitary industry promises to enhance the quality of care for patients. Its implementation will allow healthcare partners to conduct evidence based practice, and also to collaborate relying on the best knowledge available.

To sum up, the implementation of knowledge management practices and systems is a topic of great interest for the healthcare community around the world.

A complex KM system for health care industry would represent a huge step, both for doctors (preventing medical errors) and for patients.

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SYSTEMATIC REVIEW

Spondylodiskitis, etiology, diagnosis and treatment

Cristian Bănică[1], IonŞtefan[1]

Spondylodiskitis is a spinal infection comprising vertebral osteomyelitis and intervertebral disc. Diagnosis can be difficult considering that the symptomatology is not specific, low back pain is common in people over 50 years.

The etiology of the disease includes pyogenic germs, tuberculous, parasitic and fungal etiology. It is known that staphylococcal and tuberculous etiology are responsible for most cases in clinical practice, being the main issue of differential etiological diagnosis. Gram-negative germs are a rarer etiology, being involved in secondary spinal infections as a consequence of dissemination of retroperitoneal or intra-abdominal collections. Other etiologies are very rare: parasitic, fungal or Brucella.

Spinal infection has, in most cases, marrow dissemination as pathogen mechanism; it is recorded in staphylococcal sepsis and secondary determinations of tuberculosis (Pott morbidity).

Contiguous infection from septic foci nearby is a rare complication due to esophageal ruptures or retropharyngeal abscess or aortic vascular prosthesis infections. Iatrogenic, postoperative, postpuncture infection is also recognized.

Due to the particularities of spine vasculature, marrow infection includes intervertebral disc and vertebral body; septic emboli cause infarcts within bones, leading to osteolysis, mechanical deformations, cavitation, mechanical instability. Paravertebral abscesses could occur, the infection can spread into the spinal canal.

The location of these spinal infections is more common in the lower back, however, thoracic and cervical locations are also common.

Initial infectious outbreaks are often difficult to identify, less than 50% of cases are recognized as the source of spondylodiskitis, skin and soft tissue infections, genitourinary infections, respiratory infections, endocarditis, ORL infections. Predisposing factors are recognized and represented by comorbidities, diabetes, rheumatic diseases, cirrhosis, malignancy, immunosuppressive treatments.

Staphylococcus aureus is the most common non-tuberculous etiology, methicillin-resistant Staphylococcus is more and more common in both community infections and in hospital infections. Staphylococcal spondylodiskitis has as starting point, infections of the soft tissues, infections of intravascular devices, infectious endorcaditis and iatrogenic infections: postpuncture, postoperative. MRSA staphylococcus spectrum of resistance comprises lately, besides penicillin, aminopenicillins, 3rd generation cephalosporins and other classes of antibiotics: macrolides, clindamycin, aminoglycosides, florochinolone rifampicin, moreover, the rate of resistant strains is increasing.

Viridans streptococci represent less than 10% of the etiology of spondylodiskitis, their association with bacterial endocarditis in sepsis is known.

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Pneumococcus, anaerobic bacteria represent a rare etiology.

Tuberculosis is a constant presence in the range of spinal infections. As a form of bone tuberculosis, bone cold abscess has a slow, insidious, soundless development and it is sometimes associated with other bacillary determinations: psoas abscess, renal, prostate, lung or even brain abscess.

Gram negative bacteria represent 10-30% of the etiology of spinal infections, the most common germ is Escherichia coli, followed by Proteus, Klebsiella and Enterobater.

Rarely, spondylodiskitis etiology may be represented by other bacteria: Brucella, Bartonella or fungi: candida albicans (more frequently), Cryptococcus, Coccidioides, blastomices. As an exception, cases of spinal infection with Echinococcus granulosus are described.

The difficulty of diagnosis lies in the poor symptoms; lumbar spine pains are common in the general population. Sometimes, the presence of low grade fever or febrile episodes draws attention. Neurologic deficit may occur in complicated cases with radiculopathy and spinal cord compression. During anamnesis, recent history of skin or soft tissue infections, presence of an intravascular device or surgery in the spine is presented. Patients with comorbidities and immunosuppressive treatments are more exposed to the risk of sepsis with secondary determinations. Bacillary etiology is insidious, diagnosis is retroactive.

Laboratory examination raises suspicion of infection when neutrophilic leukocytosis is present, high ESR, CRP is often higher in spondylodiskitis. Alkaline phosphatase may be increased.

Radiological examination and magnetic resonance imaging of the spine brings valuable proofs for diagnosis, showing inflammatory modifications and bone structure modifications of the vertebrae and intervertebral disc, which also appear in degenerative lesions and can therefore be difficult to interpret. Technetium 99 scintigraphy and the one with Gallium 67 can bring sensitive data to support the diagnosis. Computed tomography has good resolution for changes in bone structure, it highlights destruction of vertebral plateaus, bone formation seizure, but MRI is superior in viewing abscesses and lesions in nervous tissue. From the imaging point of view, RM lesions TB are different because the intervertebral disc is not affected but paravertebral abscesses are present, bone destruction.

Proof of infectious etiology can be obtained by biopsy-puncture and culture for aerobic bacteria, anaerobes, mycobacteria and fungi. Blood cultures can have a positive result in about 50% of cases, infection is frequently marrow. Histopathological examination of biopsies can guide diagnosis in bacillary infections but also the differential diagnosis of vertebral bone tumors and metastases.

Complications of the disease are caused by damage to the spinal architecture and mechanics, with neurologic and infectious complications.

Neurological complications can be significant, from the root syndrome up to paralysis. Infectious complications are: paravertebral abscesses, epidural abscesses, secondary meningitis, but also sepsis complications , in the context of which spondylodiskitis was diagnosed.

Treatment is anti-infective, analgesic, immobilization and surgery.

Antimicrobial treatment is injected on the basis of probability criteria or after the results of biopsy or blood culture. Antistaphylococcal antibiotics with broad-spectrum are used depending on the etiological suspicion. In the context of increasing frequency of MRSA staphylococcus, Vancomycin, Linezolid or Targocid is used for minimum 6 weeks. Injectable antibiotic therapy may be continued orally up to 3 months, depending on the disease evolution. For bacillary etiology, tritherapy for 3 months, in the 4th month, the 4th antituberculosis antibiotic is associated, then, the therapy continues with two antibiotics up to 12 months.

The favorable evolution is clinically assessed, especially by clinical evidence of inflammation in normal limits, C-reactive protein and VSH within normal limits, as well as the course of radiologic imaging, magnetic resonance. If paraspinal, epidural abscesses or neurological injuries secondary to compression appear, surgery is required.

The prognosis is favorable by antimicrobial fair treatment and surgery if necessary, spondylodiskitis mortality is less than 5%. Prompt diagnosis and antimicrobial treatment are essential.

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A cholestatic syndrome may be a surprising cause of medical error

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Abstract: *Autoimmune cholangitis defines a spectrum of cholestatic liver diseases that are characterized by inflammation of bile ducts and a reasonable response to immunosuppressive therapy. The two most common diseases associated with this term in the literature are: an overlap syndrome of primary biliary cirrhosis and autoimmune hepatitis and a form of hyper IgG4 syndrome (currently associated with autoimmune pancreatitis). Liver biopsy is mandatory for the diagnosis. There are, whatsoever, in clinical practice, many cases that do not meet current diagnostic criteria but that have a good response to corticosteroid treatment.*

Keywords: *autoimmune cholangitis, primary biliary cirrhosis, hyper IgG4 syndrome*.

Cholestatic syndromes are very often encountered in gastroenterology practice. The causes may be quite different but there is a largely accepted consensus of classification into two categories: extrahepatic and intrahepatic, rendering very different treatment and prognosis. A proper diagnosis is mandatory for a proper treatment. Conversely, a failure of a correct diagnosis may result in wrong treatment and medico-legal issues. The subject of malpraxis legal actions is often considered to be associated with an embarrassing professional failure with only punitive results. In fact, the conclusions of malpraxis cases may, as well, give very documented information to healthcare professionals in order to better improve medical care.

The differential diagnosis of the causes of cholestatic syndromes is quite challenging. An extrahepatic cause is an obstructive one, may it be acute (billiary colic) or slowly developing (benign or malign strictures, pancreatic head tumor, ampuloma). The imaging workup (CT scan, cholangioMRI) are sufficiently revealing for an extrahepatic obstructive cause in order to conduct a proper treatment. The intrahepatic cholestatic syndromes are even more challenging as there are a quite large array of very different causes with very different treatment and prognosis spanning from viral hepatitis to primary billiary cirrhosis and primitive sclerosing cholangitis. The rare the cause the frequent misdiagnosis and medico-legal consequences.

Among rare intrahepatic causes, autoimmune cholangitis is a term recently coined for a spectrum of

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cholestatic liver diseases where an immunologic pathological mechanism is highly suspected with regard to the cause of inflammation of bile ducts. This term implies also a fare response to immunosuppressive therapy. There is, though, some confusion in literature regarding this disease as it may refer to several ailments: an overlap syndrome between primary biliary cirrhosis AMA (antimithocondrial antibodies) negative and autoimmune hepatitis, a form of hyper IgG4 syndrome (associated with autoimmune pancreatitis), a separate entity as a transition form in spectrum of cholestatic disorders. This confounding data makes the diagnosis in this cases very difficult and, also, a potential source of medico-legal issues

The name autoimmune cholangitis was coined for the first time be Brunner et al[1] to describe the situation of three patients suffering of primary biliary cirrhosis AMA negative; all those patients had antinuclear antibody (ANA) and cholangiopancreatography showed no abnormality. The therapy with prednisolone and azathioprine was successful.

The antigen specificity for AMA was demonstrated to be an inner membrane mithocondrial antigen, a 74 kDa E2 subunit of pyruvate dehidrogenase complex (PDC-E2). The most sensitive lab test to assess the presence of these antibodies is an ELISA using recombinant or purified antigens.

5 to 8% of patients suffering of primary biliary cirrhosis lack AMA in spite of having typical clinical and histological features of this disease.[2] All of these patients had ANA (antinuclear antibodies), highly suggestive of autoimmune hepatitis. Moreover the level of serum IgM was significantly lower in these patients then AMA positive counterparts.

Considering the fact that AMA may have a role in the pathogenesis of the disease, a study by Kitami et al of patients AMA negative and AMA positive demonstrated that there is not truly AMA negative primary biliary cirrhosis. Kitami performed an exhaustive immunoblotting studies of various inner mithocondrial membrane antigens that are not currently looked for.[3]

From the pathologist's point of view AMA positive patients have an increased risk of cirrhosis comparing with AMA negative counterparts. Among histological features, the granulomatous destruction of bile ducts is the only histological marker highly specific for primary biliary cirrhosis AMA negative or positive, but this lacks sensitivity. These data further increases the difficulty of diagnosis.[4]

What about the treatment: ursodeoxycholic acid is the treatment of choice in both forms of primary biliary cirrhosis with equal efficiency.

Autoimmune cholangitis may also be a manifestation of immunoglobulin G4 associated systemic disease, most commonly encountered in patients with autoimmune pancreatitis. Autoimmune pancreatitis (AIP) was first described by Yoshida and colleagues[5] in 1995 referring to a type of chronic pancreatitis with certain histopathologic and imaging features. Early or atypical manifestations of autoimmune cholangitis may not involve the pancreas, thus making the diagnosis even more challenging. There are no definitive diagnosis criteria so far, also some have been proposed like Mayo criteria (HISORt) and Asian Consensus Criteria. Two types of AIP have been described: type I which is a pancreatitis associated with immunoglobulin G4 systemic disease characterized by lymphoplasmocitic infiltration and elevated serum IgG4 levels; type II which is less commonly associated with elevated serum IgG4 levels and involves a granulocytic infiltration. 44% of all patients do not have hyper IgG4 serum levels.[6] The cholangitis is a sclerosing stricturing inflammation of bile ducts. The occasional absence of pancreatic involvement have been described. The natural course of AIP involves relapses. Low dose steroid treatment is very effective treatment.

Besides those two types of autoimmune cholangitis, the clinical practice may come along different situations where an intrahepatic cholestatic syndrome may not have sufficient diagnostic criteria to render a proper diagnosis also the response to corticosteroids is straight forward. These situations may be prevalent.

CASE PRESENTATION

Clinical data

We present the case of a male patient aged 48, living in country area, driver, who is admitted in an emergency department for biliary colic comprising of Charcot triad (colicky pain, fever, jaundice), intense itching, acolic stools and hyperchrome urine. This clinical picture is elicited by a fatty meal. The patient does not smoke cigarettes or drink alcohol and personal as well as family disease history are unremarkable.

Labs data

Labs indicates a cholestatic syndrome (increased total bilirubine – 7.5 mg/dL, direct bilirubine – 5.3 mg/dL, alkaline phosphatase – 190 UI/L, gammaglutamyl transpeptidase – 130 UI/L), hepatocytolitic syndrome (increased alanine aminotransferase ALAT – 300 UI/L, aspartate aminotransferase ASAT – 120 UI/L), minimum pancreatic reaction (increased serum amylase – 200 UI/L).

Abdominal US indicated an increased common bile duct (8 mm) with normal sized intrahepatic ducts, thickened gall bladder walls (3 mm) with multiple small (less than 1 cm) calculi. The clinical picture subsides progressively due to a treatment with: antispastics, antibiotics and proton pump inhibitors. The itching and jaundice didn't subside, though. The total bilirubine level reaches 5 mg/dL and direct bilirubine 2.7 mg/dL. In this particular moment the patient is referred to our clinic with the diagnosis of remitted biliary colic and with indication of elective endoscopic biliary sphincterotomy.

Clinical exam was remarkable for intense sclerotegumentary jaundice, pruritus, dark urine and white chalky stools.

Otherwise the patients had no complains.

Labs indicated normal CBC, cholestatic syndrome (increased total bilirubine – 7.7 mg/dL, direct bilirubine – 4.3 mg/dL, alkaline phosphatase – 199 UI/L, gammaglutamyl transpeptidase – 133 UI/L), hepatocytolitic syndrome (increased ALAT – 307 UI/L, aspartate aminotransferase ASAT – 107 UI/L), no pancreatic reaction (serum amylase – 49 UI/L), normal serum albumin, normal INR.

Imaging and and endoscopy data

Abdominal US indicated: normal size and ecogenicity of the liver, normal sized common bile duct (6 mm), normal gall bladder with minimal biliary sludge. We performed superior digestive endoscopy and side-view duodenoscopy which were unremarkable for pathologic changes, but indicated increased amount of intraluminal bile and a normal papilla major. CT scan with radiocontrast media was unremarkable. Colangio MRI was, also, unremarkable.

Immunology data

In the meanwhile the level of total bilirubine continued to increase to 8.5 mg/dL without the sharp predominance of direct fraction. An extended lab workup indicated: normal level of gammaglobulin, AgHBs negative, Ab antiHBc negative, Ab anti HCV negative, Ab anti HAV negative, CA 19-9 negative, alpha fetoprotein normal, CEA negative, AMA negative, Ab anti LKM1 negative, ANA negative, ASMA negative, pANCA negative, cANCA negative, Ab anti Ro negative, Ab anti La negative, Ab anti HIV negative.

Histopathology data

Liver biopsy was performed: liver tissue sample with marked biliary stasis predominantly intraductular, biliary clots, areas of hepatocellular steatosis, chronic inflammatory infiltrate of portal areas and intralobular areas, hepatocellular regeneration, interface hepatitis with bridging necrosis. (Figure 1, Figure 2).

This description fits into pathology diagnosis of autoimmune cholangitis.

Diagnosis and differential diagnosis

We agreed upon the final diagnosis: autoimmune cholangitis; remitted biliary colic by passage of microcalculi. Hence, it has been concluded that the patient had two different causes of jaundice (acute extrahepatic cholestasis and chronic intrahepatic cholestasis), segregated by quite different prognosis and treatment. Considering the presence of interface hepatitis with chronic inflammatory infiltrate it had suggested that future evolution of clinical, serological, immunological and histopathology data may be in the direction of an overlap syndrome between AMA negative PBC and autoimmune hepatitis. The differential diagnosis included: primitive and secondary sclerosing cholangitis, primitive biliary cirrhosis, HIV cholangitis, Sjogren syndrome, non Hodgkin lymphoma.

The treatment was highly effective (32 mg of methylprednisolone daily – 0.5 mg/kg prednisolon equivalent – with tapering to 8 mg daily in one month and ursodeoxicholic acid 10 mg/kg daily), resulting in disappearance of cholestasis and hepatocitolysis after 2 month. The dosage of Medrol reached 4 mg per day in the 4th month of treatment, the current clinical and biological status of the patient being excellent.

Figure 1. Biliary clots (hematoxilin-eosine dye)

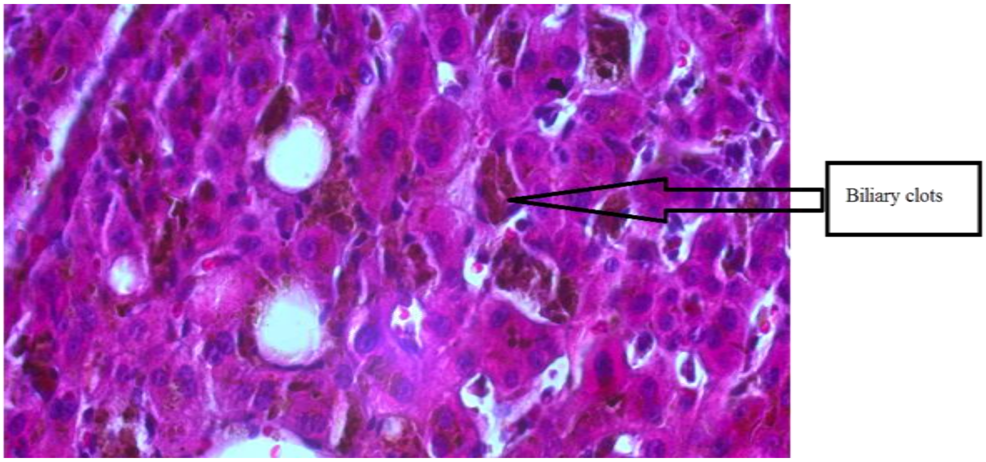

Figure 2. Interface hepatitis and bridging necrosis (hematoxilin-eosine dye)

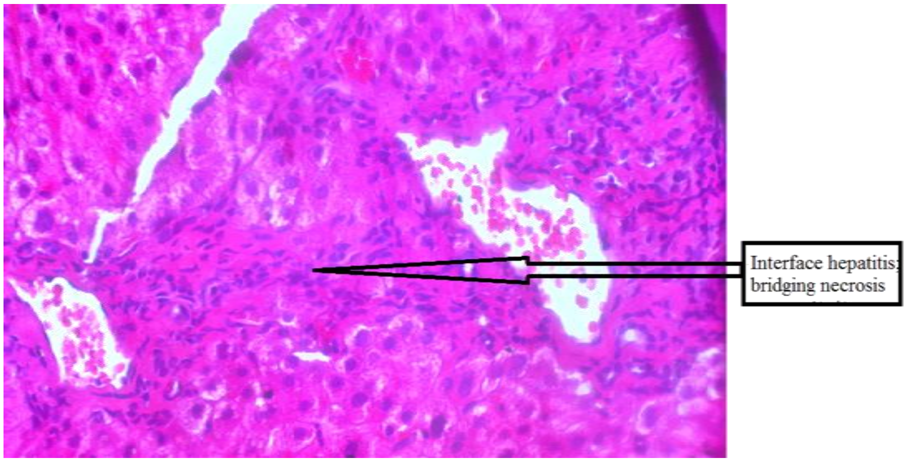

DISCUSSION AND CONCLUSION

Autoimmune cholangitis is a very challenging diagnosis in the face of a lack of international consensus on terminology used in the literature. This very particular confounding situation may come in defense of the practitioner in case of a malpraxis legal action as it may occur. On the other hand should the diagnosis be late the disease may progress to complications such as cirrhosis, liver failure and, even, death. Referral of patient to a tertiary center of hepatology may be a reasonable course of action to fully benefit medical care act. This is not always very handy.

A diagnosis workup involves various differential diagnosis of cholestasis syndrome; among these diagnosis AMA negative primary biliary cirrhosis, primitive sclerosing cholangitis of small ductules and hyperIgG4 cholangitis are the most important. Liver biopsy, high definition imaging workup and immunology panels are mandatory for a proper diagnosis.

The response to corticosteroid treatment is highly suggestive of immunologic process involved in the pathogenesis of liver ailment but it is not, whatsoever, pathognomonic as some are tempted to consider. The diagnostic criteria may uncover progressively in time, endorsing the idea of a continuum in a spectrum of autoimmune cholestatic diseases.

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EDUCATION AND IMAGING

Capsule endoscopy

Raluca S. Costache[1,2]

We present the case of a 42 years old, female, diagnosed with chronic hepatitis C viral infection treated with peginterferon and ribavirin.

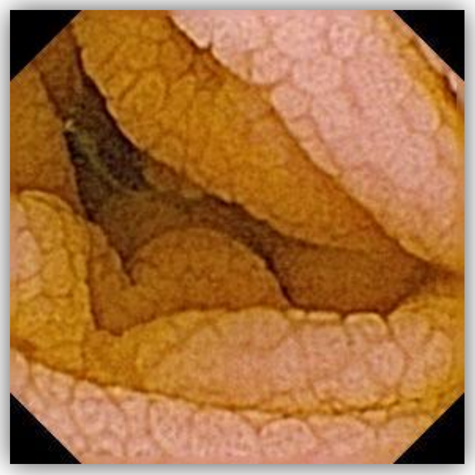

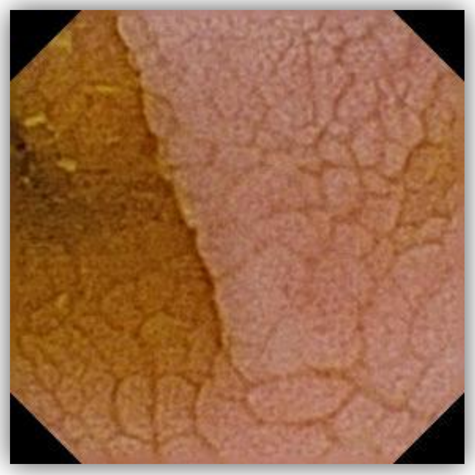

During the treatment she was admitted in the hospital with persistent diarrhoea, weight loss, and anemia. The serologic tests for celiac disease were positive together with upper endoscopy.

We performed small bowel investigation with Endocapsule® in order to determine the extent of disease.

The images (figures 1, 2 and 3) show typical aspects of celiac disease: absence of normal villi, nodularity of the jejunal mucosa with absent folds, fissuring and mild nodularity of the valvulae coniventes.

The gluten free diet was beneficent for the patient which continue the antiviral treatment with sustained viral response.

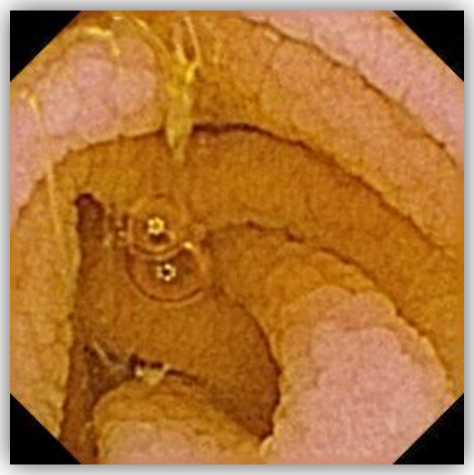

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Paraneoplastic Cushing's Syndrome in a patient with multiple tumors – case report

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INTRODUCTION

Paraneoplastic Cushing syndrome represents 5-10% of all Cushing syndrome and has a severe prognosis due to severe metabolic imbalance, denutrition, associated infections and progression of tumoral underlying pathology. The death is a rule in more than 50% of cases. Some medication used to treat it – Metirapone – is not available in Romania. Ketoconazole was recently approved by CHMP for treatment of paraneoplastic Cushing's only in November 2014.

CLINICAL CASE

A 67 years old woman presented on the 21st of November with mental confusion, progressive weight loss, severe edema and kypokalemia, without typical features of Cushing or hyperpigmentation. Patient's behavior changed in the last 5 months, she was nasty with her daughter, bickering, while diabetes and hypertension aggravated in the last 3 months.

The electrolytic imbalance was severe at admission K 1.65 mmol/l, in spite of multiple attempts to correct it with 150 mmol/day KCl on peripheral i.v. line, 40 mmol/day of KCl orally and 200 mg/day of Spironolactone, treatment used initially in "C. I. Parhon" National Institute of Endocrinology. Patient was transferred in the I. C. U. of "Dr. Carol Davila" Central Military Emergency Hospital for the weekend, in order to obtain a better control using a central intravenous catheter.

Laboratory work and imaging

One month prior to admittance patient had hypercortisolism, with normal hepatic citolytic enzymes and normal TSH, free T4 and calcitonin values. Investigations at admittance revealed paraneoplastic Cushing's with ACTH 82.5 pg/ml, cortisol levels more than 63 mcg/dl, UFC (urinary free cortisol) 2,866 mcg/24 h (21-111); DHEA-sulfate 230.5 mcg/24 h; 2 mg Dexametasone suppression test showed unsuppressed cortisol 59.17 mcg/dl, ACTH 123.8 pg/ml.

Patient also associated empty sella syndrome with thyrotrophic and gonadotrophic insufficiency, normal prolactin and IGF-1, normal mineralocorticoid hormones, cathecolamines, serotonin and 5HIAA, slightly enlarged cromogranine A – 148 ng/ml (upper limit 125 ng/ml).

Patient had also left breast tumor, Helicobacter pylori gastritis, polinodular goiter, denutrition and hepatic dysfunction.

Imaging techniques: 99m Tc Tektrotyd scintigraphy showed uptake at 10 minute in left breast and jejunal loop; upper and lower endoscopy, echo endoscopy – revealed no tumors; bronchoscopy – no visible tumor (after procedure, patient suffered a syncope that lasted 2 minutes); thyroid ultrasound – found nodule of 15/18/22 mm located in the lower part of left lobe – 15/18/22 mm, with low peripheral vascularisation, uptakes iodine at CT scan and has a peripheral calcification.

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Figure 1. Abdominal CT-showed minimally enlarged right adrenal gland, with nodule 0.88/0.88 cm; enlarged left adrenal gland, with nodule of 1.03/1.24 cm.

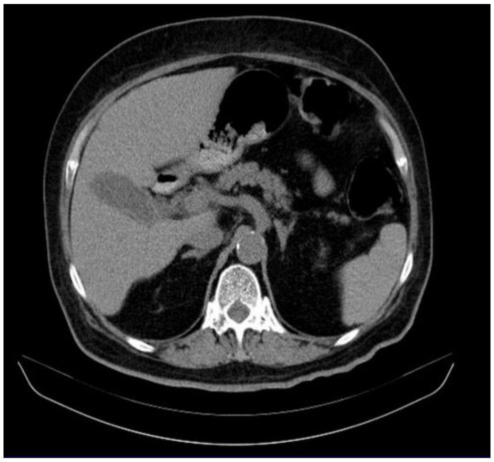

Figure 2. Thoracic CT revealed pulmonary tumor located in Fowler segment of left superior lung lobe.

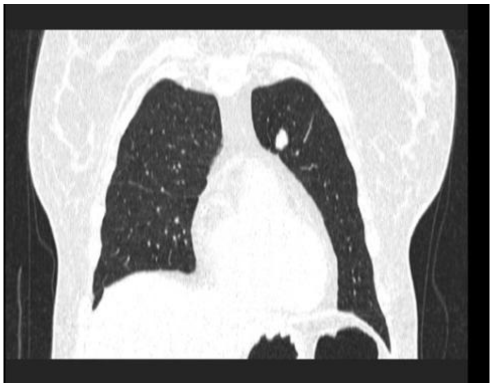

Treatment

We initiated treatment with Ketoconazole 400 mg, 1 day, and then 600 mg, for 2 days, but with inadequate correction of alkalosis and kypokalemia – pH was 7.54-7.59, BE 5.7-9.8 mmol/l, K 3.16 mmol/l. The third day patient became septic (probably MRSA Staphylococcus) due to central catheter and interstitial pneumonia – fibrinogen 660 mg/dl, with high liver enzymes – AST 87-160 UI/l, ALT 95-103 UI/L, GGT 348-365 UI/l, total bilirubine 2.44 mg/dl, leucocytes 13,400/mm^3, granulocytes 8,500/mm^3. Cortisol levels were 26.3-29.2 mcg/dl and Ketoconazole was increased to 1,200 mg/day, also associating Tavanic 500 mg initially, then Tigecycline 100 mg/day. The high values of ALT and AST were due to sepsis and did not increase after doubling Ketoconazole dosage. After 1 day of high dose Ketoconazole, K was 4.7 mmol/l, allowing introduction of Mifepristone 200 mg/day. The seventh day after Mifepristone was introduced, cortisol levels were 18.7 mcg/dl (4.2-38.4), allowing surgery.

Due to denutrition, pulmonary sepsis, lack of localization of tumor – lung/thyroid/ileum, recent syncope, severe brain atrophy with cognitive impairment, we decided to perform left adrenal gland resection.

The adrenal resection was difficult due to diffuse bleeding and lack of tissue elasticity.

Hepatic biopsy showed periportal fibrosis, but no necrosis of hepatocytes, probably due to toxic substances used at work; left adrenal was 7/3/1.5 cm in diameters, with focal hemorrhage. Imunochemistry – Ck7, Ck20, CEA, TF1, ER – negative, MELAN A positive – suggested diffuse hyperplasia of left adrenal gland.

Evolution of patient

One hour afterleft adrenalectomy – cortisol was 18.2 mcg/dl, ACTH 42.3 pg/ml, patient needed inotrop support with Noradrenaline, hydrocortisone 75 mg 1 day, 50 mg in the second day.

The third day cortisol desupressed to 51.25 mcg/dl, ACTH 43 pg/ml (3-66), K decreased again to 2.9 mmol/l, Hb was 8.4 g/dl. Ketoconazole 600 mg/day was started again. Patient had fever, delirium, pulmonary riles, so Meronem was initiated for 2 days, then Tigecycline 3 days, then 7 days of Klacid at home, also Calcium 1 g/day, 1,000 UI D3, 0.5 mcg of 1 alfa-calcidol/day, hepatic protection, vitamins, basal insulin.

Figure 3.Cromogranine staining – magnification 20X – proves neuroendocrine tumor.

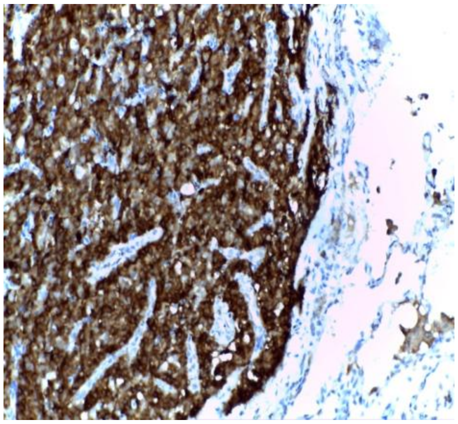

Figure 4.Ki67 staining – magnification 40X – reveals Ki67 of 3% in pulmonary nodule (well-differentiated tumor).

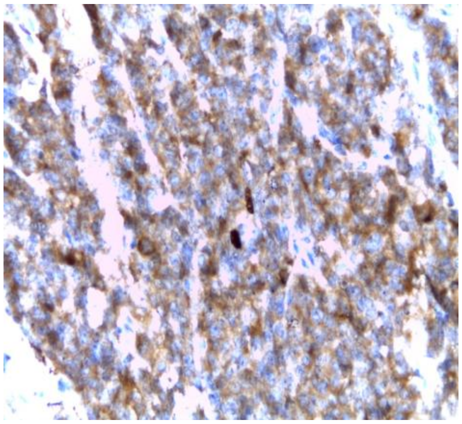

Figure 5.ACTH staining – magnification 20X – ACTH receptor is present in carcinoid tumor.

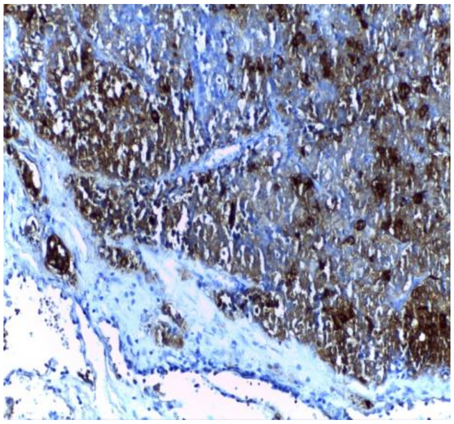

10 days after adrenal resection cortisol was 26.6 mcg/dl, K 3.9 mmol/l, calcium was normal, Mg was 1.57 mg/dl, allowing second operation – resection of lung tumor – proved to be typical carcinoid with ki-67 3%, ACTH, synaptophysin and cromograninepositive.

Evolution after second surgery: 1 day after carcinoid resection ACTH was 5.95 pg/ml (3-66), cortisol 17.38 mcg/dl – labs were done at „C. I. Parhon"Institute.

12 days after carcinoid resection: ACTH 16.56 pg/ml (7.2-63); Cortisol 10.72 mcg/dl (6.2-19.4).

At 22 days: she lost 8 kg, ACTH 19.1 pg/ml, normal ALT and AST, GGT 236 UI/l, Mg 1.39 mg/dl, Ca 9.96 mg/dl.

At 47 days: she lost 15 kg, ACTH 16.5 pg/ml; cortisol 9 mcg/dl, Mg low even with supplementation.

At 78 days: GGT 259 UI/l (5-36), Mg 1.45 mg/dl, K 3.83 mmol/l, glucose 142 mg/dl, cortisol 20.87 mcg/dl, TSH 2 microUi/ml.

At 3 months: basal cortisol 11.2 mcg/dl (6.2-19.4), basal ACTH 17.46 pg/ml (3-88), cortisol 24 EET – 3.24 mcg/dl, cortisol during suppression test with Dexamethasone 1 mg overnight – 0.48 mcg/dl, PTH 12.32 pg/ml (15-165), low 25OH–D 12.8 ng/ml (30-100), UFC 40.3 mcg/24 h (21-111).

We also performed control thoracic CT – revealed left breast tumor of 0.76/1.21 cm, right adrenal with stationary aspect; portal vein was enlarged 14.5 mm; patient performed FNAB of left thyroid nodule on 20 April 2015, showing benign adenoma.

Mild kypokaliemia and hypomagnesemia, even with oral supplementation, sartan therapy and normal levels of cortisol and ACTH persisted after surgery, probably due to severe deficit of intracellular compartment, even at 3 months after carcinoid resection. Patient does not remember the 2 months prior to surgery, even if cognitive impairment is mild now.

COMMENTS

This case was difficult due to metabolic challenges, multiple associated pathology, lack of SSTR2 and SSTR5 receptors on lung carcinoid with negative scan, mild elevation of cromogranine A levels despite a typical bronchial carcinoid. Patient's sister was operated for adrenal adenoma confirmed on histology exam, her daughter had papillary thyroid cancer, but no MEN association was proven in this family. Patient needed more than 30 days of hospital admittance in two different hospitals and five clinics in order to obtain a good clinical result. The vital risk was high due to sepsis, denutrition, metabolic and ionic imbalance, hepatic lesions, anesthesia, brain atrophy, relative adrenal insufficiency after surgery.

There are no guidelines that state the adequate cortisol levels to be reached before surgery, nor the duration of Ketoconazole wash-out to prevent adrenal insufficiency.

Recently the patient discovered ductal invasive left breast carcinoma, operated at 6 months after thoracic surgery and will soon start chemotherapy.

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CLINICAL PRACTICE

Article received on October12, 2014 and accepted for publishing on November 15 2014.

Median arcuate ligament syndrome (Dunbar Syndrome)

Crina Laslo[1],Anamaria Puiu[1], Ștefan Mardale[2], Iulian Raus[2], Cosmin Căpușan[2]

Thisrare conditionis caused byextrinsiccompression, given the low abnormalinsertion of medianarcuateligamentorfibrousbands and ganglionperiaortitis tissue from the celiac plexus. The ligamentiscomposed oftendonmedialedgesof the twopolesof the diaphragmwhichmeetin the median planeto forman archbefore the aorta.

Figure 1. Graphic image of the arcuate ligament compressing the celiac trunk
Case courtesy of Dr Matt Skalski, Radiopaedia.org, rID: 36837

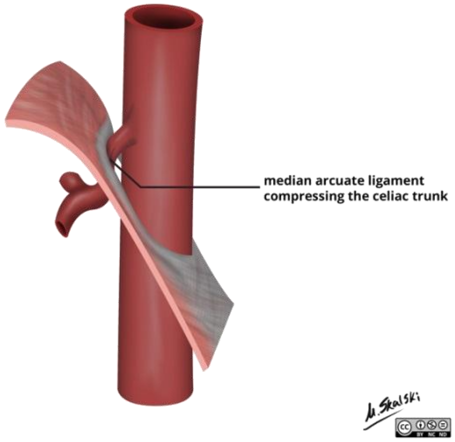

The clinical presentation is variable:

- In most cases asymptomatic
- Weight loss
- Chronic abdominal pain
- Postprandial abdominal discomfort
- Hypertension and tachycardia in the case of renal artery compression

The diagnosis is one of exclusion. Symptomatic patients have numerous investigations for most cases of abdominal pain and underwent cholecystectomies and appendectomies, many unnecessary in order to alleviate the pain. Diagnosis methods: Doppler, angio-CT, angiography or MRI. The gold standard is the angioCT.

Angio-CT: it is highlighted, on sagittal reconstruction, a focal stenosis in the proximal portion of the celiac trunk, bent (hooked appearance). 3D reconstructions can be helpful in identifying stenosis. Gastro-duodenal and hepatic arteries can often be seen as prominent with multiple collaterals.

Differential diagnosis based on imaging is made with atherosclerotic disease.

Our patient D.G., male, aged 53, known with hypertension responsive to treatment and diabetes type II non-insulin requiring, with no clinical complaints, came to the Nephrology Department where an abdominal ultrasound was performed and a pancreatic formation was detected. He came to our department for abdominopelvic CT with contrast iv.

During examination, multiple arterial branches arising from the pancreatic duodenal artery and superior mesenteric artery (arch pancreatitis) are observed, which include pancreatic duodenal region; filiform stenosis in the aortic origin of the celiac trunk, through the median arcuate ligament.

Figure 2. Axial section acquired during early arterial time that highlightsarterialtypecollateral circulationbetweenAMSandtheceliactrunk at the level of the pancreaticduodenalarch.

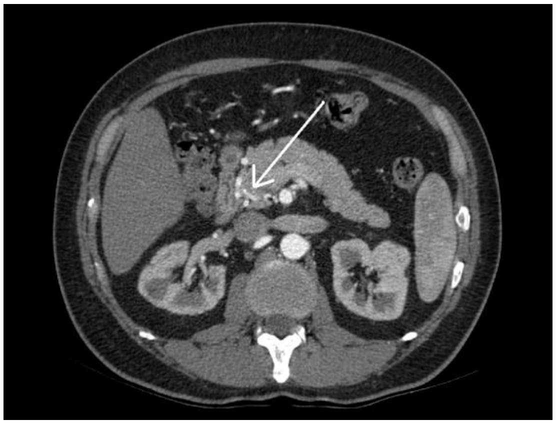

Figure 3. SagittalMPRhighlightingthe trunkceliacfiliformstenosis..

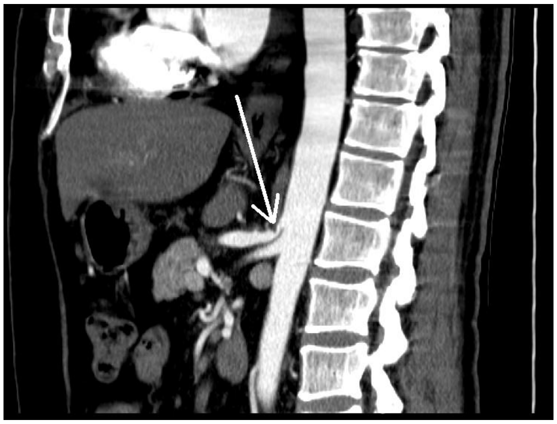

Treatment

The goal of the treatmentis todecompressand obtain celiacarteryrevascularization.

Treatment optionsare quite limitedandthe revascularization surgery has a riskof morbidity and mortalityof5% to15%.

Astreatment methodsmay be used: conventionallaparotomy, laparoscopyorendovascularmethods(percutaneous transluminalangioplasty, stent implantation).

Laparotomy, by retroperitoneal approach via left subcostal incision or by transabdominal approach via midline incision, allows surgical separation of the median arcuate ligament fibers to decompress the celiac artery. Decompression is completed by celiac ganglion resection and evaluation of celiac artery blood flow using a Doppler ultrasound intraoperatively. In case of low blood flow, celiac artery revascularization is performed.

Figure 4.3DVRImagehighlightingthe filiformstenosis at the origin of theceliac trunk

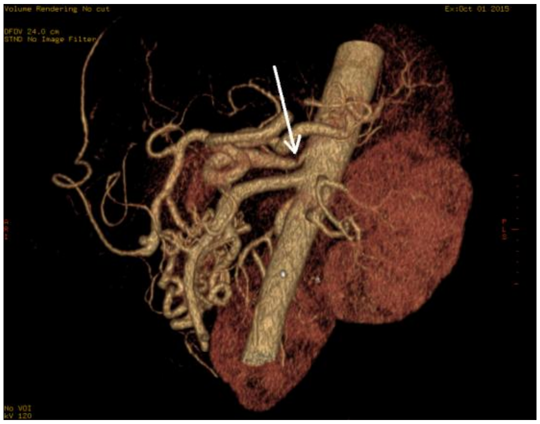

Revascularization methods include aorto-celiac bypass, angioplasty patch.

Literature studies show that classical laparotomy is no longer prefered, due to an increased risk of morbidity and mortality, especially in patients with associated pathologies.

Laparoscopy is used more frequently compared to laparotomy because it is less invasive presenting a lower risk of morbidity and mortality, with low cost due to short hospitalization.

Laparoscopic intervention also allows decompression of celiac artery, but if the celiac artery requires revascularization, surgical management should be changed and the classic approach should be used (laparotomy).

Percutaneous balloonangioplastyis anattractive methodbecause it isa minimally invasive procedure, ideal forpatients with associated comorbidities. This methoddeliverssuboptimal results because mostlesionsdevelop in theostium. Percutaneous balloonangioplastyinostiallesionsis associated withmore severeresidualstenosisdue tointenseelastic recoilcaused bya large number ofcircularelasticfibersfrom theostium. Because of this,the rateof complications(acute occlusion) and restenosis increases.

Stent implantationoffersa metallic supportthat preventselasticrecoilandthusdecreasesthe complicationsof this type.The risksof this methodincludedistalembolism with secondary ischemia, fat embolism, aortic dissection.

Percutaneous angioplastyandstentimplantation,accordingto a 2009 study,arecomplementary methods to laparoscopyafterdecompression of celiacartery was performed.

DISCUSSION

The peculiarity of thiscaseis thatthe patient did notpresentanysymptoms.Thismay be due tothe development of arichcollateralblood supplythat compensates forceliacartery stenosis.

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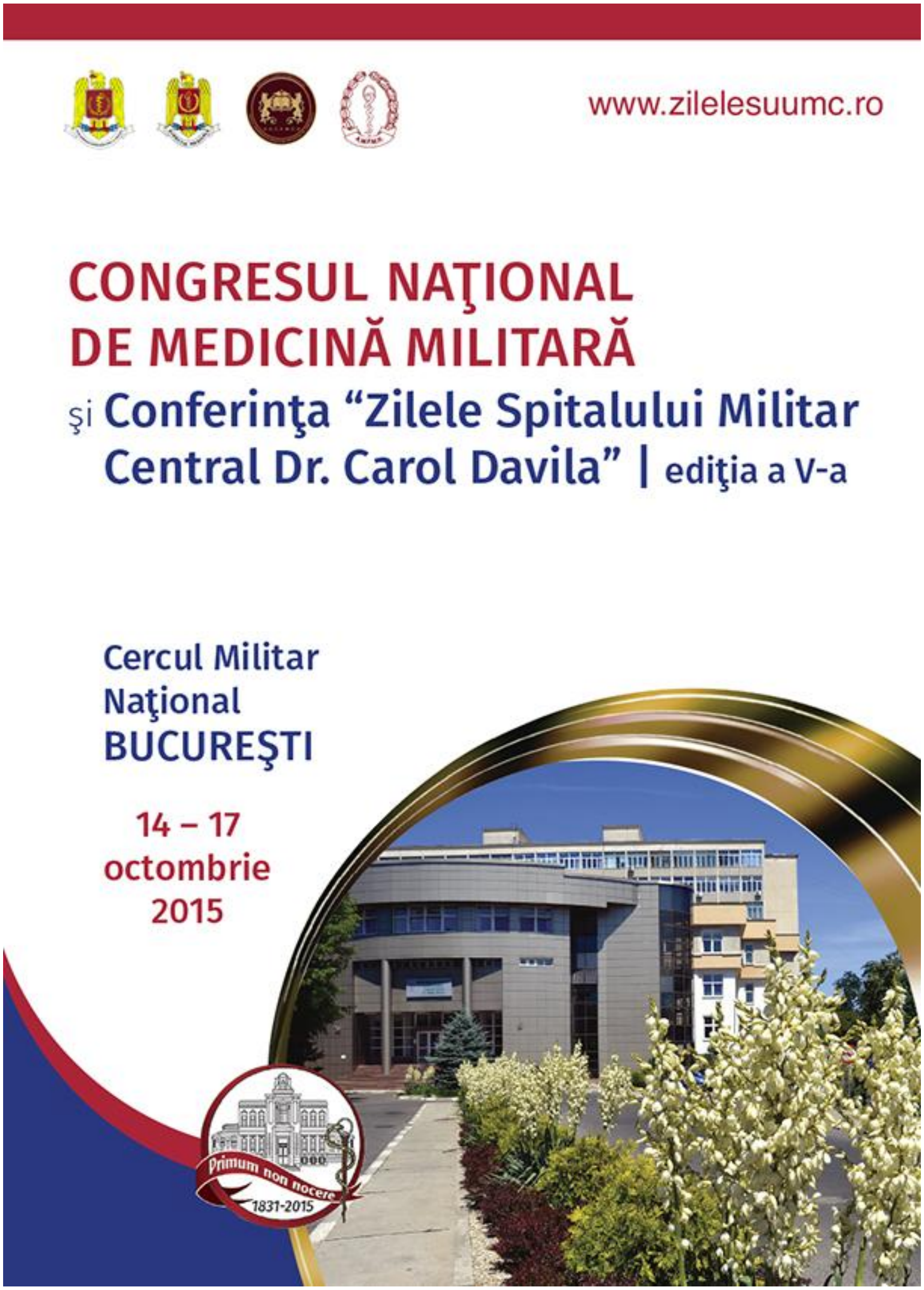

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The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’

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Acknowledgments

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As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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If the above items are not included in the cover letter, manuscripts cannot be sent for review.

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EDITORIAL

PancreaticFest 2015, annual EUS international workshop

Mariana Jinga, Vasile Balaban

Colonel MARIANA JINGA

Deputy Commander, Central Emergency Military Hospital, Bucharest
Associate Professor, Carol Davila University of Medicine and Pharmacy, Bucharest

Although neglected in the past, pancreatic pathology (comprising acute and chronic pancreatitis and the fearful pancreatic cancer) has been in the spotlight in the last decades both in gastroenterology and surgery. This growing interest for pancreatology has led to funding of innovative research, publishing of highly accessed papers, and development of tertiary centers, with solid teams of gastroenterologists, radiologists, pathologists and surgeons; this has also led to a need for better risk stratification and for improvement in both early diagnosis and treatment of pancreatic diseases.

While death rates for many other cancers are declining, mortality for pancreatic cancer is increasing. The incidence of acute pancreatitis is also on the rise, and the disabling symptoms of chronic pancreatitis can greatly alter quality of life and limit work.

Despite the fact that these pancreatic diseases carry significant burden for society and medical systems, there are still many unmet needs in managing patients suffering from these illnesses, especially in Romania – lack of awareness in primary care, few dedicated centers and difficult access to novel diagnostic and treatment methods, which are not reimbursed by policy-makers.

In this setting, the Internal Medicine and Gastroenterology Clinic at “Dr. Carol Davila” Central Military Emergency University Hospital has proved a special interest for the study and management of pancreatic pathology. Back in 1998, the hospital was the first in Romania to have a linear endoscopic ultrasound (EUS) system. A few years later, EUS guided fine needle aspiration was also performed in our clinic in national premiere. Since then, EUS has evolved into a therapeutic instrument, especially for the pancreas and biliary tree, and research to extend its indications is ongoing.

To promote the interest for pancreatology, our clinic has been hosting an annual EUS workshop, with a first edition in 1999, which has grown into a tradition in the fall of the last few years. Since 2013, the workshop is held together with the International Pancreatic Disease Course, coordinated by Professor Cristian Gheorghe. The “Pancreatic Fest” is organized by the Association for Pancreatic Pathology Romania (APPR), which is part of the European Pancreatic Club. Over the years, world renowned experts have lectured and performed live demonstrations at the combined pancreas meeting, setting high standards for Romanian doctors and motivating young gastroenterologists to get training in this field. This up-to-date meeting in pancreatology reunites more and more attendees each year, creating opportunities for exchange programs abroad, developing multicenter research projects and promoting excellence care for patients.

This year's edition of the Pancreatic Fest, will take place on September 11-12th in Bucharest. The first day, dedicated to the EUS Workshop, will bring the participants complex cases, difficult techniques, tips and tricks, and cutting-edge EUS technology, all in HD live demo transmission from the Endoscopy Unit of the "Dr. Carol Davila" Central Military Emergency University Hospital. The second day will offer the participants a great educational programme, with state of the art lectures from international expert pancreatologists and interactive talks. We aim to bring together gastroenterologists, surgeons, pathologists, oncologists, radiologists, intensive care specialists and basic scientists, to create multidisciplinary teams with expertise in diagnosing and treating patients with pancreatic diseases. The message we are trying to convey is that partnering of specialties is the key to the successful management of these patients. We look forward to seeing you in Bucharest for a valuable scientific experience in the start of the fall!

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REVIEW ARTICLE

Article received on May 12, 2015 and accepted for publishing on June 21 2015.

From liver biopsy to non-invasive markers in evaluating fibrosis in chronic liver disease

Cătălina Diaconu[1], Florentina Ioniță Radu[1,3], Mariana Jinga[1,2], Petruț Nuță[1], Săndica Bucurică[1,2], Bogdan Macadon[1], Mihăiță Pătrășescu[1], Andrada Popescu[1], Vasile Balaban[1,2], Laura Voicu[1], Daniel O. Costache[1], Raluca S. Costache[1,2]

Abstract: *Chronic liver disease is a late stage of progressive hepatic fibrosis. It consists of functional and structural disruptions in most chronic liver diseases. An accurate diagnosis allows us to establish the degree of fibrosis and the stage of the disease, the prognosis of the patient and to predict a treatment response. Despite the fact that liver biopsy is considered a gold standard, non-invasive methods for diagnosing liver fibrosis have gained more and more importance. Whether we talk about serum biomarkers or imagistic methods from transient elastography to 3-D magnetic resonance elastography, the question remains: are these useful or useless? Serum biomarkers represent blood components that can reflect liver histological changes, thus they can monitor the continuous process of fibrosis. These can be subcategorized in direct (that show extracellular matrix turnover) and indirect markers (that reflect disturbances in the hepatic function). However these markers alone are not as accurate in the staging of fibrosis, only help differentiate patients without or with low grade of fibrosis from those with significant fibrosis and cannot be considered alone in the diagnosis of liver fibrosis. Imagistic methods include: ultrasound-based transient elastography, magnetic resonance elastography (MRE), 2D-shear wave elastography, acoustic radiation impulse imaging (ARFI) and cross sectional imaging, the first being the most used. Using a combination of non-invasive tools allows us to diminish the number of patients in need of liver biopsy. However, the patient must always be informed of the advantages and disadvantages of each method and its limitations.*

Keywords: *fibrosis, biopsy, elastography, biomarkers*.

INTRODUCTION

Liver fibrosis remains an important problem worldwide. At first liver fibrosis was considered irreversible, but now scientists show that it is a dynamic process [1]. Advanced chronic liver disease, also known as cirrhosis is defined by the replacement of normal liver tissue with fibrotic one, leading to the disruption of the normal architecture of the liver and formation of regenerative nodules. It is usually considered to be irreversible, eventually leading to the need of liver transplantation. Many liver diseases can lead to cirrhosis. Most encountered causes are:

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[2] Titu Maiorescu University, Faculty of Medicine, Bucharest

viral hepatitis (mostly B and C hepatic virus), alcoholic liver disease and nonalcoholic liver disease, hemochromatosis. One must bear in mind less common causes of advanced end stage liver disease such as: autoimmune hepatitis, primary sclerosing cholangitis, primary and secondary biliary cirrhosis, Wilson disease, veno-occlusive disease, alpha-1 antitrypsin deficiency or right sided heart failure [2].

In normal hepatic tissue there is a balance between the production and degradation of extracellular matrix components. In liver fibrosis the balance is skewed in favor of the latter [3]. There is a high number of cells involved in the process of fibrogenesis. However, the hepatic stellate cell is the main factor involved. Chronic liver injury leads to the activation of hepatic stellate cells that transforms into myofibroblasts. These are contractile cells that secrete extracellular matrix components, pro-inflammatory citokines and chemokines. The first such protein produced in liver fibrosis is fibronectin, leading to the formation of excess collagen type 1. This process is cyclic since the production of extracellular matrix activates other stellate cells, which produce more proteins and finally change the hepatic architecture. Portal fibroblasts are also involved in the process of fibrosis, after stellate cells, tuning into myofibroflasts. Since these are closer to the portal tract they have a more important role in cholestatic injury from liver fibrosis, encountered in primary biliary cirrhosis and primary sclerosing cholangitis [4].

Liver biopsy is considered the golden standard in evaluating the degree of fibrosis. However it is limited by sampling errors, intra- and interobserver variability in histological interpretation and by the possible complications that may be encountered. There are many histologic systems that can appreciate fibrosis, but the one mostly used is semi-quantitative METAVIR score [5].

There are two categories of non-invasive methods in evaluating chronic liver disease: serologic panels of tests and radiologic tests. First ones quantify the levels of biomarkers in the serum samples and are more available. The latter consists of tests that measures liver stiffness using a “physical” approach. The two methods are complementary. Serum biomarkers are less accurate in detecting intermediate stages of fibrosis that in identifying cirrhosis, but present many advantages: high applicability, low costs, wide availability, and the possibility to be performed as outpatient [6]. Radiologic tests measure liver stiffness and are very useful in detecting cirrhosis. Combination between serum biomarkers and imagistic methods are very useful, slowly becoming a new golden standard in evaluating fibrosis in advanced liver disease [7].

Genetic risk factors for fibrosis are only now starting to gain importance. Literature shows that external factors such as alcohol and coinfections accelerate fibrogenesis. Studies now report variants in several genes that become associated with higher risk of fibrosis in chronic hepatitis C, NAFLD or AFLD [3].

LIVER BIOPSY

Considered the gold standard for the evaluation of tissue damage, including end-stage liver disease, liver biopsy is slowly falling behind non-invasive methods. Despite the fact that a high range of sensitive and accurate blood and imagistic tests can now be used to detect and even diagnose liver diseases, liver biopsy remains a valuable diagnosis tool, especially in difficult cases[8]. However the well-recognised limitations of this procedure have fuelled discussions regarding the correct diagnosis pathway in advanced chronic liver disease. The major problems of liver biopsy remain sampling and observational errors. Studies have shown that the larger the piece is, the lowest the risk of sampling errors is. The quality of the piece is in the length and the number of portal fields contained. A 20-25mm biopsy with more than 11 portal tracts is considered optimal specimen, even though some studies show that even a 15mm piece can be useful [3.9]. Even though we can obtain a large size biopsy, unfortunately it only reflects 1:50000 of the entire liver. Not only the length, but also the caliper of the biopsy needle is essential, a 16 gauge needle being the most appropriate to use [6]. Intra- or interobserver variability can also limit the usefulness of liver biopsy, leading to discrepancies in up to 20% of cases. Using histopathological scoring systems diminished this problem [3].

Histologic scoring systems in chronic liver disease are of great importance to the progression of the disease, prognosis and helping us establish appropriate treatments. In *chronic hepatitis* the most encountered systems are: the Knodell score (histology activity index), METAVIR score, Ishak score (modified Knodell score). Other less used scoring systems are: the Scheurer system, the Batts-Ludwig system and Laennec system. *Nonalcoholic fatty liver disease* (NAFLD) can be divided in nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFLD can be evaluated by nonalcoholic fatty liver activity score (NAS). It sums up the degree of steatosis, lobular inflammation and ballooning of the liver cells, ranging from 0-8. Fibrosis is not included in NAS. *Primary biliary cirrhosis* uses two scoring systems: Scheuer or Ludwing, based on: the degree of fibrosis, bile duct loss, copper deposition and the degree of cholangitis and hepatitis. Even though liver biopsy in *primary sclerosing cholangitis* is uncommon, if it is performed then a histologic scoring system can be used. It allows us to define 4 stages based on the degree of inflammation and fibrosis [10].

Complication rates related to liver puncture range from 0.75% to 13.6% [3]. Minor complications after liver biopsy include transient discomfort, moderate pain and mild transient hypotension. Bleeding remains the most important complication of liver biopsy, along biliary peritonitis, perforation and pneumothorax [11]. Biopsy through transjugular route can reduce the risks in patients with coagulation disorders or advanced liver disease [9]. The number of complications is also diminished if we choose ultrasound-guided biopsies [12].

Liver biopsies should be performed as in-patient procedure since 60% of complications occur in the first 2 hours and more than 90% of complications are prone to happen during the first 24 hours[3,11].

SERUM BIOMARKERS

The "biological" approach of evaluating end stage liver disease is represented by serum biomarkers. Even though these parameters may not be liver-specific, they have been associated with fibrosis stage [6]. These so-called surrogate markers can be direct and indirect. The changes in the content of extracellular matrix are quantified by direct markers, whilst indirect markers show hepatic function disturbances. These markers can be used alone or combined, leading to different scores, which can be simple (AST to platelet ratio index (APRI), FIB-4 index, FORNS) or calculated after a formula (Fibrotest, Fibroscore, Fibromax) [3]. Simple scores might have lower diagnosis accuracy, but they have greater advantages: lower price, easy to calculate and widely available [6].

Indirect markers

AST to platelet ration index (APRI) is easy to calculate based on the platelet count and AST level. APRI was first used in patients with hepatitis C virus (HCV), human immunodeficiency virus and HCV coinfection and alcoholic liver disease [13,14].

Table 1. The correlation between AST to platelet ratio index and the probability of cirrhosis.

APRI <=0.3:	Unlikely cirrhosis or significant fibrosis
APRI >0.3 and <=0.5:	Unlikely cirrhosis, significant fibrosis possible
APRI >0.5 and <=1.5:	Significant fibrosis or cirrhosis possible
APRI >1.5 and <=2:	Likely significant fibrosis, cirrhosis possible
APRI >2:	Likely cirrhosis

FibroTest and *ActiTest* were first used in patients with hepatitis B and C. *Fibrotest* uses alpha-2-macroglobulin, gamma globulin, apolipoprotein A1, alpha-2-globulin (haptoglobin), gamma-glutamyl-transpeptidase and total bilirubin. It takes into consideration patients' age and sex [1]. Even though there are advantages of FibroTest such as: high availability and applicability (>95%), there are many drawbacks: lack of specificity for liver disease, interference with other comorbidities that can change the result (Gilbert's syndrome or hemolysis) or the difficulty to differentiate intermediate stages of fibrosis [15]. ActiTest is a modified FibroTest that involves ALT, and evaluates both fibrosis and necroinflamatory activity of the liver [1].

Hepascore involves assessment of bilirubin, GGT, alpha-2-macroglobulin, hyaluronic acid, age and sex. A metaanalysis showed that Hepascore did not bring more information than FibroTest in patients with alcoholic liver disease. However, other studies showed its accuracy in predicting fibrosis in patients with chronic HC [1,9].

AST/ALT ratio in normal patients is around 0.8. Some studies show that a value over 1 suggests cirrhosis, but its utility is not certified [1].

Other indirect markers that are not worldwide used include: FIB-4 index, NAFLD fibrosis score (cutoff higher than 0.676 is associated with positive predictive value of fibrosis and a value under -1.455 is associated with a negative predictive value of 88 percent), PGA index (alcoholic liver disease with a detection range 66-72%), Forns (similar performance with APRI), BARD score (predictive in patients with NAFLD)[1,6].

Direct markers are used in forming panels to predict fibrosis, but none are available in clinical practice. (Table 2).

Table 2. Direct markers of fibrogenesis and fibrolysis

Matrix deposition	Procollagen I peptide
	Procollagen III peptide
	Type I collagen
	Type IV collagen
	YKL-40 (chondrex)
	Laminin
	Hyaluronic acid
Matrix degradation	MMP-2
	TIMP-1, -2
Cytokines	TGF-beta
	TGF-alpha
	PDGF

Serum biomarkers aren’t useful in distinguishing between intermediate stages of fibrosis, but are more accurate in detecting fibrosis. Moreover careful consideration is entitled when talking about patients with HCV-HIV coinfection since false positive results can appear. For example: APRI, FIB-4, FibroTest (due to hyperbilirubinemia induced by atazanavir), Forns Index (related to GGT increase from nevirapine) and HepaScore. Despite the fact that bioseric markers can be used for detecting cirrhosis, their utility is reduced in diagnosing significant fibrosis [6]. Combination of different scores can be effective in avoiding biopsies, but a single surrogate marker cannot replace liver biopsy [3].

RADIOLOGIC TESTS

Radiologic tests represent the “physical” approach in evaluating fibrosis in chronic liver disease. The methods include: ultrasound-based elastography, magnetic resonance elastography (MRE), acoustic radiation force impulse imaging (ARFI), 2D-shear wave elastography (2D-SWE) and cross sectional imaging [3].

Trasient elastography was first evaluated as valid marker for fibrosis in a clinical study in 2002, therefore it remains rather new in the diagnostic pathway. It assesses the liver fibrosis by calculating the velocity of a low-frequency shear wave (50Hz) produced by a mechanical probe placed directly on the skin. The wave penetrates the liver tissue depending on the stiffness of the liver, correlating to the extent of liver fibrosis. The technique goes as following:

- the transductor is placed in contact with the skin with the tip covered in coupling gel;
- the position is in the 9th to 11th intercostal space (where liver biopsy is performed),
- we select an area of liver at a maximum of 6 cm deep, free of vessels;
- we gather 10 consecutive successful measurements. If one value is not valid, the entire set must be repeated [16].

There are several markers that evaluate if the result is valid: 10 consecutive valid results, a success rate (valid shots/total number of shots) above 60%, interquartile range lower than 30% of the median liver stiffness measurements, serum aminotransferase levels less than 5 times the normal value. Liver stiffness is expressed in kilo Pascal (kPa), ranging from 1.5 to 75kPa. A three category system is used: very reliable (IQR/M≤0.10), reliable (0.10<IQR/M≤0.30 or IQR/M>0.30 and liver stiffness evaluation (LSE) <7.1kPa) and poorly reliable (IQR/M>0.30 and LSE≥7.1kPa) [16,17].

Figure 1. The accuracy of transient elastography is based on a system made from: interquartile range/median liver stiffness measurements (IQR/M) and LSE (liver stifness evaluation) [3].

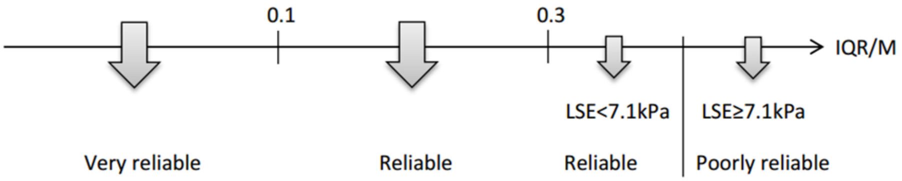

The largest series report evaluating TE show that in 3.1% of cases the measurement was impossible and in 15.8% of cases the results were unreliable (mostly due to patient obesity). Therefore a new probe was developed: XL, 2.5MHZ transducer. This allows us to measure liver stiffness from 35 to 75mm depth [3,6]. In their study Myers et al concluded that in 276 patients with chronic liver disease (42% viral hepatitis, 46% NAFLD) and a BMI>28 kg/m^2, using an XL probe was more successful than using M probe. The number of unreliable results was lower when using XL probe (25%) than with M probe (50%). Stiffness values calculated with XL probe are lower by a median of 1.4kPa than when obtained with M probe [18,19].

Other limitations in using transient elastography are: narrow intercostal spaces, ascites, cardiac failure, high necroinflammatory activity (quantified by a high AST/ALT ratio), steatosis and cholestasis [20]. Excessive alcohol ingestion or food intake might modify the results of TE, therefore a fasting period prior to the investigation in recommended, 3-6 hours [6,21].

A special S probe was introduced for evaluating liver stiffness in children or patients with small intercostal space [22].

A meta-analysis of 50 studies comparing transient elastography and liver biopsy showed that we can choose an imagistic method in distinguishing cirrhosis from lesser degrees of fibrosis, but it is less reliable in detecting lower grades of fibrosis thus it has moderate negative predictive values. Moreover the study shows that transient elastography is better in diagnosing nonalchoholic steatohepatitis and alcohol-related disease than in chronic viral hepatitis. Transient elastography is useful in detecting cirrhosis with an optimal cut-off value of 13.01 kPa[3]. However one must take into consideration that the target population might have different characteristics and it should be pre-tested [6]. TE should not be used alone in evaluation on liver fibrosis in some patients, but if it predicts significant fibrosis there is no need for biopsy [3,4]

The performance of TE in hepatitis B and C is similar if we compare the two. When evaluating hepatitis e antigen (HBeAg-) negative patients with normal ALT values, non-invasive methods, especially TE could be used to estimate levels of HBV-DNA and identify patients in need of liver biopsy [6].

TE might be used in monitoring liver fibrosis: either after or during treatment or to evaluate the natural progression of the disease. A most discussed topic in the world of hepatology is the role of TE in predicting portal hypertension. A cut-off value of 27.5kPa can predict the presence esophageal varices and the need of endoscopy to evaluate the extent of the disease and the need to start primary prophylaxis with non-selective β-Blockers, whilst a cut-off value of 62.7kPa can suggest a high risk of esophageal bleeding. Studies show that a cut-off value >25kPa is associated with > 45-fold risk to develop hepatocellular carcinoma in patients known with viral hepatitis [3,23]

Shear wave elastography (SWE) is divided in: point shear wave elastography (pSWE), also known as acoustic radiation force impulse imaging (ARFI) and 2D-shear wave elastography (2D-SWE). ARFI excites liver tissue by using short-duration acoustic pulses (~262µsec) that propagate shear waves and lead to localized µ-scale displacement in the tissue. The target zone (10x15mm) is set 10-20mm under the liver capsule. Ten consecutive valid measurements are required and the accuracy is evaluated with the interquartile range. The failure rate is lower when using ARFI to evaluate fibrosis than TE (2.9% vs 6.4%, p<0.001), especially in patients who suffer from obesity or associate ascites. Food intake, necroinflammatory activity and serum levels of aminotransferases might influence the results. ARFI is better in evaluating fibrosis in chronic hepatitis C than in chronic hepatitis B, NAFLD, HIV-HCV coinfection and other liver diseases. *2D-SWE* uses a combination of radiation force from focused ultrasonic beams and a very high frame rate ultrasound imaging sequence. Failure rate is lower than TE, especially in patients with ascites, but not in patients with obesity where XL probe is more accurate. However quality criteria in SWE are not yet well defined [6, 24].

Table 3. The correlation between the number of cases and SIRS criteria

Cirrhosis caused by:	Trasient elastography evaluation cut-off value (kPa)
Hepatitis C	13.01
Hepatitis B	11.7
Alcoholic fatty liver disease if drinking	22.7
Alcoholic fatty liver disease if abstinent	12.5
Biliary liver disease	17.9
Non-alcoholic fatty liver disease	10.3

Magnetic resonance elastography (MRE) involes placing a probe on the patient's back that emits low-frequency vibrations that pass through the liver and can be measured. With an optimal cut-off of 4.71 it can suggest cirrhosis with a sensitivity of 91% and a specificity of 81%[1]. Some studies show a better diagnostic accuracy and technical success than TE[25]. Moreover, MRE has the advantage of scanning the entire liver tissue, thus it cannot miss areas of fibrosis, hepatocellular carcinoma, focal liver lesions, and it is independent to an acoustic window and is operator independent [1]. However due to the costs and excess of time MRE requires, it is not used for routine clinical practice use [6].

Other imagistic methods such as computerized tomography are not currently in use for evaluating fibrosis in chronic liver disease, due to low sensitivity and specificity and high costs [3].

We now know that non-invasive tests have striking advantages against liver biopsy when talking about patient safety. Therefore by combining the serologic biomarkers with imagistic methods (preferably) or even different bio-markers we can limit using liver biopsy only in case of doubt or discordance between serological biomarkers and imagistic method. The combination of non-invasive markers allows rapid staging of the liver without the need for liver biopsy for hepatitis C. The current gold standard in hepatitis C is to associate TE and serum biomarkers [6].

In hepatitis B, TE is superior to serologic biomarkers and it is best used to detect the stage of fibrosis in patients with active viremia (DNA-HBV>2000 IU/ml) and normal ALT. TE is not indicated in hepatitis with very high levels of ALT >10 times the normal value. Liver biopsy should be used when in doubt [6].

Non-invasive methods in evaluating fibrosis in NAFLD have a lower accuracy compared to liver biopsy and in time patients might require histological confirmation. However, follow-up can be performed with serum biomarkers or TE once every 3 years to evaluate the progression of the disease [6].

Despite the fact that non-invasive tests cannot replace hepatic venous pressure gradient (HVPG) in evaluating portal hypertension or endoscopy that reveals clinical implication of portal hypertension (oesophageal varices), these might be useful in selecting patients that require these procedures. HVPG over 10mmHg is predictive of varices and decompensation, whilst values under 10 mmHg has a 90% negative predictive value for the development of clinical decompensation in 4 years. However measuring HVPG is both expensive and invasive therefore repeating measurements is impractical [4,6].

CONCLUSION

Non-invasive markers do not abolish the need for liver biopsy. They should be used to select the patients in need of liver biopsy more carefully.

Smart combinations of non-invasive markers can greatly reduce the number of liver biopsies performed and the risks associated, but when the diagnosis is unclear one must use liver biopsy.

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SYSTEMATIC REVIEW

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Diabetes mellitus and colorectal cancer – a revealed connection

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Abstract: *The burden of colorectal cancer (CRC) is increasing all over the world. The prevalence of diabetes mellitus is increasing. It is estimated that diabetes affects 387 million people worldwide. It is predicted that 552 million people worldwide will develop diabetes by 2030. A large pool of data indicate that DM increases by 2 fold the risk of CRC. This is the reason to firmly suggest the inclusion of DM in the criteria for CRC screening as an important measure to decrease the mortality of this ailment.*

INTRODUCTION

Type 2 diabetes mellitus and colorectal cancer are major causes of morbidity and mortality in the United States and other Western countries.

The burden of colorectal cancer (CRC) is increasing all over the world. It is recognized as the second cause of cancer death in USA and in Western Europe. Also, it is a third cause of cancer in men and a second cause of cancer in women. Every year there are 1,2 million new cases in the world. The highest incidence is encountered in Australia (75/100000), New Zeeland, North America and Europe[1]. In Romania the incidence has doubled from 1994 (13/100000) to 2005 (23/100000)[2]. The lifetime risk of CRC is 5%. 90% of cases appear after the age of 50; beneath this age threshold is rare encountered but the current trend indicates an increasing prevalence in young[3].

It is estimated that diabetes affects 387 million people worldwide. It is predicted that 552 million people worldwide will develop diabetes by 2030. In the United States, about 23.6 million people or 7.8% of adults have DM and by 2050, the number of people in the United States with diagnosed DM is estimated to grow to 48.3 million[4]. The prevalence of diabetes mellitus in Romania is 11.6% as PREDATTOR Study has concluded in 2014[5]. Even those figures are very high it is appreciated that almost one-third of diabetics is undiagnosed[6].

STUDIES THAT CONFIRM THE RELATION BETWEEN DM AND CRC

In 1910, Maynard[7] provided the earliest reports on the association between DM and cancer of any type. In 1984, Williams et al[8] published a retrospective study that documented a statistically significant, over 2-fold increase in the prevalence of DM in CRC

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patients compared with age-matched controls. The search for a pathological explanation for this connection has led to the so-called hyperinsulinemia hypothesis. Giovannucci[9] hypothesized that insulin, an important growth factor, may at high serum concentrations increase the risk of CRC by promoting growth of colon tumors, and acting as a cell mitogen. Other proposed explanations for the increased risk include decreased bowel transit time and elevated fecal concentrations of bile acids[10]. Yang et al.[11] showed that insulin treatment may further elevate risk for CRC among patients with type 2 DM.

Figure 1: Risk factors for DM and CRC

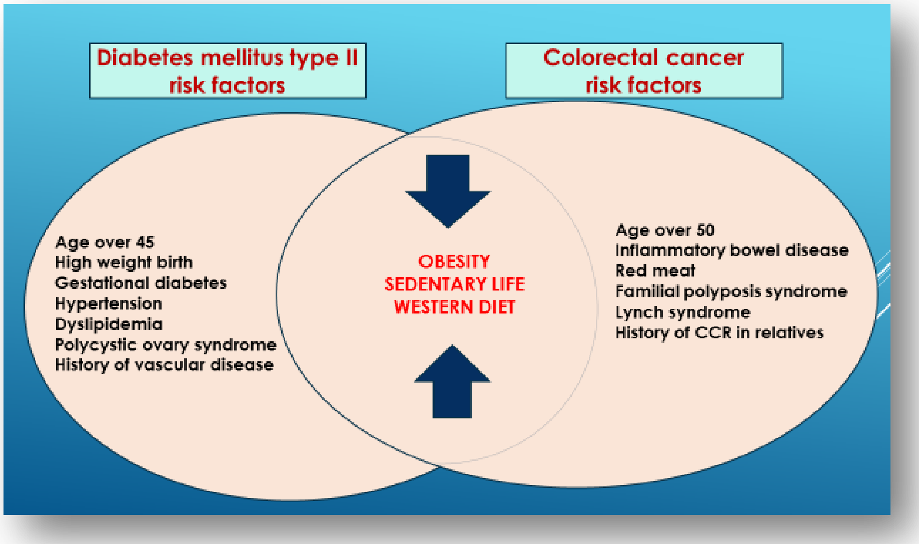
Diabetes mellitus type II risk factors
Colorectal cancer risk factors
Age over 45
High weight birth
Gestational diabetes
Hypertension
Dyslipidemia
Polycystic ovary syndrome
History of vascular disease
OBESITY
SEDENTARY LIFE
WESTERN DIET
Age over 50
Inflammatory bowel disease
Red meat
Familial polyposis syndrome
Lynch syndrome
History of CCR in relatives

Overall, studies indicate that diabetes risk increases twofold for liver, pancreatic, and endometrial cancer and 1.2- to 1.5-fold for colorectal, breast, and bladder cancer, but is associated with decreased risk for prostate cancer[12]. Diabetes is an independent risk factor for liver cancer. Cancer patients with diabetes relapse, and die, sooner than those without the disease.

Although proving a causal connection in observational human studies is uncertain and may take decades, preclinical research is revealing possible biochemical links between cancer and diabetes, which also share many risk factors.

The American College of Gastroenterology Guidelines for Colorectal Cancer Screening 2008 recommend that clinicians be aware of an increased risk of CRC in cigarette smokers and obese patients[13], but do not highlight the increased risk in patients with DM. Obesity and smoking are associated with the incidence of both type 2 DM and CRC[14]; thus, they also could be important positive confounders of the association between DM and CRC.

In July 2010, the American Cancer Society and the American Diabetes Association released a consensus report that reviewed the science behind the link and evidence for whether diabetes treatments influence cancer risk or prognosis. Published in the July August 2010 *CA: A Cancer Journal of Clinicians*, the report recommended regular cancer screening for diabetics. Preliminary evidence that metformin is associated with lower cancer risk, and exogenous insulin with higher risk[12].

Diabetes is characterized by high levels of both insulin and blood sugar, and both promote cancer cell proliferation in preclinical models In a Nov. 29, 2011, *British Journal of Cancer* study, he examined levels of the two in nearly 5,000 women from the Women's Health Initiative over 12 years. Elevated glucose levels were associated with a nearly doubled risk of colorectal cancer[12].

A recent meta-analysis on 41 studies associated DM with an increased incidence of colorectal cancer [relative risk, 1.27; 95% confidence interval (CI), 1.21–1.34][15]. No associations between DM and colon cancer were evident among African Americans[16]. A large-scale, population-based study in Japan suggested that diabetic men carried a higher risk for colorectal cancer than diabetic women did. The incidence of colorectal cancer was significantly higher in DM patients than in control patients (HR, 2.10; 95% CI, 1.82–2.42. The incidence of colorectal cancer is higher in males than in females (adjusted HR, 1.34; 95% CI, 1.18–1.53)[17].

CRC risk was higher among diabetics with longer duration of disease[18].

Metanalysis [19] of 15 studies (six case – control and nine cohort studies), including 2 593 935 participants, found that diabetes was associated with an increased risk of colorectal cancer, compared with no diabetes (summary RR of colorectal cancer incidence = 1.30, 95% CI = 1.20 to 1.40), without heterogeneity between studies (*P* heterogeneity = 0.21). The association between diabetes and colorectal cancer incidence did not differ statistically significantly by sex (summary RR among women = 1.33, 95% CI = 1.23 to 1.44; summary RR among men = 1.29, 95% CI = 1.15 to 1.44; *P* heterogeneity = .26) or by cancer subsite. Diabetes was positively associated with colorectal cancer mortality (summary RR = 1.26, 95% CI = 1.05 to 1.50), but there was evidence for heterogeneity between studies (*P* heterogeneity = 0.04). Findings from this meta-analysis indicate that individuals with diabetes have an approximately 30% increased relative risk of developing colorectal cancer compared with nondiabetic individuals.

Risk factors such as obesity, physical inactivity, smoking, Western diet and metabolic syndrome are common in both DM and colorectal cancer patients.

THE PROBLEM OF CONFOUNDERS AS RISK FACTORS

The first metaanalysis to quantitatively assess the effect of certain potentially important confounding variables including obesity, smoking, and physical exercise was published in 2008. It showed a statistically significant association between DM and risks of colon cancer and rectal cancer separately after controlling for obesity, smoking, and physical exercise. The association of DM and cancer risk was stronger for colon cancer than for rectal cancer (summary RR 1.38, 95 % CI 1.26 – 1.51 vs. summary RR 1.20, 95 % CI 1.09–1.31, respectively). This difference may be because the proximal colon, distal colon, and rectum have different embryological origins[20].

The positive association of DM with colon cancer and rectal cancer risk did not decrease when the metaanalysis was limited to studies that controlled for smoking and BMI (summary RR for colon 1.34, 95 % CI 1.17–1.52, and summary RR for rectum 1.28, 95% CI 1.06–1.54). This suggests that the confounding effect of obesity and smoking is relatively weak, and that DM appears to be an independent risk factor for colon cancer and rectal cancer.

Furthermore, as a recent study indicated, persons with DM and CRC may be at increased risk for CRC recurrence, non-response to chemo and radiotherapy treatment, and treatment-related complications. CRC deaths may be reduced through CRC screening programs.[21]

PROPOSED MECHANISMS

Several mechanisms have been proposed to explain the potential relationship between DM and colorectal cancer. Hyperinsulinemia, insulin-like growth factor-1, glucagon-like peptide-1 and the relative binding proteins each play an important role in metabolism, cell growth, proliferation and the regulation of the apoptotic process in colon cells[22].

Mechanistically, insulin stimulates cell proliferation through two pathways. One pathway involves direct binding of insulin to insulin or insulin-like growth factor-1 (IGF-1) receptors, and the other pathway is via inhibition of IGF binding proteins and the resultant increase in IGF-1 availability to the IGF receptor. The IGF system is a potent growth regulator closely linked with carcinogenesis[23]. These data support the notion that IGF-1 has a role in the biological pathway of colorectal neoplasia, beginning at the adenoma stage.

CONCLUSION

The increasing prevalence of DM may, significantly, be responsible for the increasing prevalence of CRC and its mortality. A great pool of data may suggest a need for more intensive CRC screening program in patients with type 2 DM, especially those who receive chronic insulin therapy.

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Culprits in non-celiac gluten-sensitivity

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INTRODUCTION

The growing interest for gluten-free diet (GFD) in the last decades has lead to a significant number of people going for this diet, in the absence of a diagnosis of celiac disease (CD). Owing to the perception that dietary gluten could be responsible for some of the symptoms in patients with irritable bowel syndrome (IBS)[1,2,3] (*Figure 1*), a new concept emerged, that of non-celiac gluten-sensitivity (NCGS), as it was named in an international consensus report[4]. NCGS describes the individuals whose symptoms get better on a GFD, in the absence of CD or wheat allergy[5]. The first reports about this condition date back to the late 1970s (two case reports) and the early 1980s (the Birmingham study)[6,7,8], and recently, some authors consider more appropriately to term it "people who avoid gluten"[9]. Biesiekierski et al.[10] were the first to publish a randomized clinical trial that proved the existence of NCGS; confirmation later came from the italian study of Di Sabatino[11], who showed in a randomized, placebo-controlled, cross-over design, a small, but statistically significant, alteration of symptoms in NCGS patients when they were gluten challenged.

The main issues of NCGS are[12,13,14]:

1. most of the times, NCGS is self-diagnosed of diagnosed by alternative health care practitioners
2. people who decide to go gluten-free rarely exclude CD before starting the diet, and when they address the doctor for evaluation, alternative diagnoses such as bacterial overgrowth or food intolerances (lactose, fructose) are found
3. aproximately one in four people with NCGS have persistent symptoms despite dietary gluten restriction

Figure 1: Spectrum of IBS-like syndromes (modified after Verdu AF, Am J Gastro 2009)

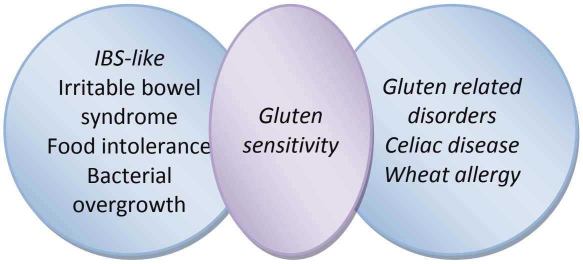

The prevalence of NCGS is variable, with reported values between 0.5 and 13%[15]. It is estimated that nowadays more people are following a GFD after a self-diagnosis of gluten-sensitivity than patients with CD[16]. Unlike the other gluten-related disorders, the diagnosis of NCGS is purely clinical, considering that there is no specific biomarker or characteristic histopathological changes on enterobiopsy. A diagnostic algorithm was recently proposed, which includes proof of both gluten responsiveness and

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relapse on gluten challenge (Salerno criteria)[17].

ROLE OF GLUTEN AND OTHER TRIGGERS IN NCGS

The pathogenesis of NCGS is not completeley understood. The name NCGS implies that gluten is the culprit for the symptoms associated with this condition. However, recently there has been some questioning about gluten being the real trigger in NCGS, while considering other components which are also found in the cereals avoided in the GFD[18] – Figure 2.

Figure 2. Wheat components as possible triggers for NCGS

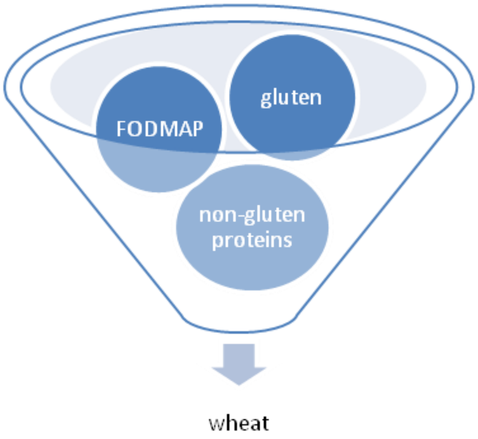

In this setting a "gluten conspiracy"[19] was described, which comprises two main ideas: if wheat is causing the symptoms, it must be the gluten in wheat responsible for them, and if the GFD improves the symptoms, this benefit is due to withdrawal of gluten. The pitfall of proving this misconception, that gluten is not the (only) one responsible, is that most of the challenges in clinical trials are performed with wheat (slices of bread, wheat flour capsules), and wheat contains not only gluten, but non-gluten proteins and non-protein components such as FODMAPs.

Among the possible non-gluten triggers, FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) and α-amylase/trypsin inhibitors (ATIs) have been studied in the literature.

The wheat kernel contains many components, among which the following are of interst for NCGS:
i) Proteins
- Storage proteins – represented by gluten (glutenins and prolamins – gliadin in wheat, and the equivalents secalin in barley and hordein in rye)
- Metabolic proteins – enzymes, enzyme inhibitors (eg. ATI – amylase-trypsin inhibitor), lectins (eg. wheat germ aglutinin – WGA)

ii) *Carbohydrates*
- Oligosaccharides, lactose, fructose, polyols (sorbitol, mannitol) – they osmotically draw water and are easly fermented by colonic bacteria (leading to gas formation in the gut lumen), which clinically translates into diarrhea, bloating and flatulence

FODMAPs

FODMAPs, an acronym for some osmotically-active, easily fermented carbohidrates, which are found also in gluten-containing cereals, have been described as a possible culprit of digestive symptoms in patients with NCGS[20,21,22,23]. A diet with reduced intake of FODMAPs (low-FODMAP), which has proven beneficial for IBS[24], has also been theoretized as being useful for NCGS patients.

In 2011, Biesiekierski showed that among cereal foods there is an overlap between the gluten-free and low-FODMAP ones, which could represent a confounder regarding the real culprit in NCGS: thus, the misconception that gluten is responsible for the digestive symptoms could be in fact explained by the presence of FODMAPs in the diet[25].

Another study performed by Biesiekierski on 37 NCGS patients (normal duodenal histology, negative HLA DQ 2/8) revealed that after 2 weeks on low-FODMAP (which lead to a significant relief of symptoms), there were no specific or dose-dependent effects upon dietary challenge in 3 randomized groups – high gluten (16 g gluten/day), low gluten (2 g gluten/day + 14 g whey protein/day) or control (16 g whey protein/day) diet for 1 week[26]. Gluten-specific effects were observed in only 3 out of 37 patients (8.1%). After a washout period of at least 2 weeks, 22 of the 37 patients crossed over to 3 groups which were given gluten (16 g/day), whey (16 g/day) or control (no additional protein) diet for 3 days. After the 3-day rechallenge, there were no differences between the three groups. Moreover, none of the 3 individuals who had a previous gluten-specific response in the first part of the trial reported such a response upon crossover. However, this short exposure to gluten in the second part of the study was associated with higher depression scores, which could explain why NCGS patients feel better on a GFD despite the persistence of gastrointestinal symptoms.

In conclusion, this study proved that gluten had no effect in patients with NCGS placed on low FODMAPs diets, and these carbohydrates could be the true cause of symptoms. Thus, we can state that people who eat gluten-free could be in fact sensitive to FODMAP and not to gluten, and the benefits of the GFD could be explained by its overlap with the low-FODMAP diet.

Among the limits of this trial, we should consider the possible nocebo effect on reintroduction of gluten/whey in the study groups.

The questionable role of gluten in NCGS is also supported by the double blind, cross-over trial of Zanini, who showed that gluten challenge induces symptom recurrence in only one third of NCGS patients[27].

Amylase-trypsin inhibitors (ATIs)

Beside FODMAPs, Schuppan's group identified some non-gluten proteins as a possible trigger for NCGS, specifically amylase-trypsin inhibitors (ATIs)[28,29]. These have been shown to induce intestinal inflammation by activation of TLR4 (toll-like receptor 4), effect limited to gluten-containing cereals only (wheat,barley, rye)[18].

In addition to ATIs, other non-gluten proteins such as WGA (wheat germ aglutinin) are considered as possible triggers for NCGS-like symptoms[30]. Current data has shown that these lectins can increase intestinal permeability, through mechanisms still unknown.[31]

CONCLUSIONS

Current knowledge suggests that the symptomatic benefit of a GFD in NCGS patients could be in fact not the consequence of gluten elimination, but avoidance of other compounds found in cereals such as FODMAPs or ATIs. Considering the evidences that question the causative role of gluten in NCGS, some authors have proposed renaming of this condition to non-celiac wheat sensitivity[32].

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ORIGINAL ARTICLES

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Diagnostic and treatment difficulties in insulinomas

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Abstract: *Background: Neuroendocrine tumors of the pancreas (NTP) comprise a unique and relatively rare group of tumors, of which gastrinoma and insulinoma are the most common types. Insulinomas tend to be small, solitary and benign, with surgical resection curable in most cases.*
Introduction: Insulinomas are localized preoperatively using conventional imaging studies as transabdominal ultrasonography (US), computed tomography (CT), and/or magnetic resonance imaging (MRI).
Purpose: Endoscopic ultrasound (EUS) is a valuable tool in the diagnosis of insulinomas.
Goals & methods: We performed a retrospective study on 21 patients with insulinoma (6 male and 15 female, 25 to 73 years of age), who were hospitalized and operated on between 2003 and 2012 at "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest.
Results: US view was positive in 10% of patients (2 of 20), that presented proximal location. The sensitivity of CT was unsatisfactory, 21.05% (4 positive results of 19). CT failed to detect liver metastases, but identified nodal metastasis in one patient. MRI was performed in 18 patients and was diagnostic in 11 of them, recording a detection sensitivity of 61.11%, including infra-centimetric tumor size. EUS has a high resolution which allows detection of lesions with very small diameter is safe and minimally invasive. EUS was performed in all patients, being able to identify formations in 17, was inconclusive in 3, showing a diagnosis sensitivity of 81%. Liver metastases were demonstrated in 3 patients, one by US and all 3 by MRI.
Conclusions:
- CT with intravenous iodinated contrast agent had a poor sensitivity in detecting the primary tumors, was insensitive in detecting liver metastases, but showed metastases in lymph nodes.
- MRI has higher sensitivity than CT in detecting primary tumors, including insulinomas with infracentimetric size, and is the imaging test of choice for possible liver metastases.
- EUS is the preoperative imaging test of choice.

Keywords: *insulinoma, neuroendocrine tumors of the pancreas, endoscopic ultrasound (EUS), preoperative localization*

INTRODUCTION

Neuroendocrine tumors of the pancreas (NTP) are rare, but increasingly recognized entities. Tumors may be functional or nonfunctional, which are frequently detected incidentally or with symptoms related to mass effect of the tumor or its metastases (1). NTP have been considered rare, with an estimated incidence of less than 1 per 100000 individuals (2). Insulinoma is the most common

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neuroendocrine tumor of the pancreas and cause of hypoglycaemia related to endogenous hyperinsulinism with an annual incidence of four in every 1 million persons (3, 4, 5). As many as 90% of insulinomas have been reported to be benign, 90% are solitary, >90% occur in intrapancreatic sites, and 90% are < 2 cm in diameter. Regarding the location of pancreatic tumors, 50% are in the head, 25% in the tail, and 25% caudally or permeates the entire pancreas (5, 6).

Most of these tumors occur sporadically, but they can also be associated with multiple endocrine neoplasia (MEN)-1 syndrome. Approximately 4% of patients with insulinoma have MEN-1 (3, 4, 6). In sporadic forms, the highest incidence is considered in the age group 40-60 years (mean age 50 years), with a slight predominance of females (sex ratio 3:2 for women). (3). The diagnosis of insulinoma can be established by determining plasma proinsulin, insulin, C-peptide, and glucose levels, which are usually performed during a 72-hour fast (4). Insulinomas classically present with "Whipple's triad:" a combination of symptoms of hypoglycaemia, inappropriately high insulin levels with associated documented blood glucose levels of <50 mg/dL, and symptom relief with administration of glucose (2).

Non-invasive imaging procedures, such as CT and MRI, are used when a diagnosis of insulinoma has been made to localize the source of pathological insulin secretion. Invasive modalities, such as endoscopic ultrasound (EUS) and arterial stimulation venous sampling, are highly accurate in the preoperative localization of insulinomas and have frequently been shown to be superior to noninvasive localization techniques. Topographic preoperative diagnosis is difficult due to small tumor size; EUS is the most sensitive diagnostic method for this purpose. High resolution of EUS allows detection of lesions with very small diameter, is safe and minimally invasive (5, 7, 8). In the table below, we are presenting data from studies showing the accuracy of imaging methods in diagnosing insulinomas (Table 1).

Table 1. Accuracy of imaging methods in diagnosing insulinomas (5, 9, 10, 11, 12, 13, 14, 15, 16)

	US	CT scan	MRI	EUS	Intraoperative US	Intraoperative palpation
Pitre J, 1996				90%		
Angeli E, 1997	79.3%	44.8%	65.5%			
Ardengh JC, 2000		16.7%		83.3%		
Machado MCC, 2001	30%	25%	17%	27%		
Mirallié E, 2002				85%		
Jyotsna VP, 2006		57-83%	31%		92%	76%
Sotoudehmanesh R, 2007				89.5%		
Joseph AJ, 2013		69%		89%		
Takehiro O, 2013		33-64%	40-90%		80-100%	75-95%

There are still great difficulties in correct positive and topographic diagnosis of insulinomas. Advances in the pre-and intraoperative diagnosis allow precise localization and excision of the tumors, avoiding blind pancreatic resections (5, 17). Objectives of treatment are: symptomatic treatment of the tumor hypersecretion syndrome and surgical treatment which consists in enucleation of benign tumor or extensive resections. The surgery may use laparoscopic or open techniques. It is essential to perform intraoperative ultrasound, which will have an important role in choosing the optimal resection technique. Enucleation is the excision method of choice, whenever it is technically possible. Currently, resection of insulinomas can be successful with the techniques of minimally invasive surgery - laparoscopy or robotics, combined with intraoperative ultrasound performance. Medical treatment addresses to control symptoms or complications, for surgery preparation or as palliative treatment in cases where it can be done. When technically feasible, tumor enucleation is the procedure of choice; however, a more formal resection may be necessary for certain tumors (3, 5, 7, 14, 18, 19).

GOALS

Given the low incidence of pancreatic neuroendocrine tumors and the relatively limited medical experience regarding this group of tumors, there is concern in the medical world to find methods of diagnosis and appropriate treatment.

This study aims to identify characteristic features of pancreatic neuroendocrine tumors, especially of insulinoma, the most frequently diagnosed of them. We analyzed in terms of demographics, clinical, laboratory, imaging, pathology and treatment 21 cases of pancreatic insulinoma, all diagnosed and treated at “Dr. Carol Davila” Central Military Emergency University Hospital”, Bucharest, highlighting the best diagnostic and therapeutic attitude and the comparison with literature data.

We will try to determine which are most sensitive and specific methods applied at this time to establish early diagnosis and what treatment is recommended considering the particular features of the disease, the low incidence, appropriate index of suspicion, non-specificity of symptoms with often delayed diagnosis, involving a complex team (endocrinology, internal medicine, gastroenterology, oncology, intensive care, oncology, surgery) and the difficulties of this pathology.

MATERIAL AND METHODS

This article aims to develop a retrospective study to track the best diagnosis and treatment for insulinomas. In the period 25.01.2003 – 26.11.2012, 21 patients diagnosed with insulinoma were hospitalized and/or operated at the “Dr. Carol Davila” Central Military Emergency University Hospital in Bucharest. Medical records of patients with insulinoma seen in the period 2003-2012 were retrieved from the hospital’s medical records department. From these, data on clinical and diagnostic features, localization and surgical outcome were extracted. All mathematical analysis was performed using Microsoft Excel 2007.

The study analyzes the following data: epidemiology (distribution by sex and age), clinical (symptoms), laboratory (laboratory diagnosis, topography, location, locoregional extension and/or distant metastases), histological (macroscopic, microscopic and immunohistochemical) and treatment (surgery, medical), postoperative evolution, complications and prognosis. Features of patients were highlighted and analyzed by comparison with the data currently available in the literature. Biological, imaging and histological methods of diagnosis and techniques and results of surgical treatment and care were analyzed in particular.

RESULTS

Over a period of nine years, between January 2003 and November 2012, 21 patients diagnosed with pancreatic insulinoma were admitted to “Dr. Carol Davila” Central Military Emergency University Hospital, Bucharest.

Gender distribution was 15 women (71.4%) and 6 men (28.6%) and sex ratio women: men of 2.5:1. A particular case was the association with Wermer syndrome (MEN I) found in a patient of 36 years, who was hospitalized for symptoms of insulin hypersecretion. The mean age at diagnosis of insulinoma was 46.66 years (25-73 years), being lower for women than men (41.06 vs. 53.66). Distribution by age showed a peak between 40 and 49 years.

Positive diagnosis is suggested by clinical manifestations. In 1938, Whipple described a triad with a high specificity: 1) neurological crises that occur on an empty stomach or after exercise 2) decrease in blood glucose below 50 mg% during the crisis, 3) disappearance of neuropsychiatric disorders following administration of glucose orally or intravenously.

The diagnosis is based on simultaneous measurement of blood glucose and insulin levels (5, 6, 7). In our cases, positive diagnosis was suggested by Whipple triad. All patients had presented with fasting hypoglycemia and fulfilled the triad to suspect a diagnosis of insulinoma.

CLINICAL PRESENTATION

Patients had various associations of symptoms, shown after prolonged overnight fasting and physical activity or stress. The fact that the symptoms occur early in the morning, on waking, and disappear after ingestion of carbohydrates or glucose administration oriented the diagnosis (7). Delays in the diagnosis of insulinoma are common because the symptoms usually precede detection of a tumor and there may be misattribution of the symptoms to psychiatric, cardiac, or neurological disorders and, on average, it takes up to 2 years before the correct diagnosis is made (5, 7, 8). Neuroglicopenic symptoms delayed diagnosis in 8 patients (38%), as they were hospitalized in the neurology and psychiatry wards and mistreated. Of them, four were diagnosed with epilepsy and received anticonvulsant treatment, and the others were labeled with neurological disorders, anxiety disorders or thyroid dysfunction. Finally, the diagnosis was correct, the time period between onset of symptoms and diagnosis ranging from several days to 10 years. The symptomatic evolution of the disease before diagnosis had a mean of 25.14 months. More than half of patients in the study (61.9%) were diagnosed as having insulinoma after less than a year of evolution.

Table 2. Frequency of symptoms and signs that patients presented with in the study

Symptoms	Number of patients (%)
Preprandial hypoglycaemia	21 (100)
Loss of consciousness	13 (62)
Dizziness	12 (57)
Diaphoresis	12 (57)
Tremor	10 (48)
Obesity	8 (38)
Fatigue / asthenia	8 (38)
Increased appetite / hunger	7 (33)
Palpitations	7 (33)
Headache	6 (28)
Loss of balance	6 (28)
Diplopia, blurred vision	6 (28)
Seizures	5 (24)
Extremity paresthesia	5 (24)
Impaired memory or concentration	5 (24)
Dysarthria	5 (24)
Confusion	4 (19)
Abnormal behavior	4 (19)
Anxiety, irritability, psychomotor agitation	4 (19)
Difficulty in waking up in the morning	4 (19)
Amnesia	3 (14)
Hypoglycemic coma	3 (14)
Temporo-spatial disorientation disorder	2 (9)
Affective disorder	2 (9)
Distal neuropathy associated with hypoglycaemia	1 (5)

Positive biological diagnosis

Insulinomas are the most common cause of hypoglycemia resulting from endogenous hyperinsulinism. B cell insulin secretion does not decrease in the presence of hypoglycemia, and so plasma insulin greater than or equal to 3µU/ml along with a plasma glucose less than or equal to 50 mg/dL suggest an insulinoma. The diagnosis is made by simultaneous measurements of blood sugar and insulin levels in the blood (5).

The 72-h fasting test is a demonstration of Whipple's triad of symptoms, which is considered as the gold standard for the diagnosis. This test is conducted under supervised conditions, which requires hospitalisation of the patient. During the fasting period, the patient is allowed to drink calorie-free fluids and physical activity is encouraged. Blood glucose should be measured every 6 hours until it reduces to 60 mg/dL or less, when the interval is reduced to every 1–2 h. The fast is terminated when the plasma glucose level is 45mg/dl or less and the patient has symptoms and signs of hypoglycemia.

When symptoms of hypoglycemia appear, blood should be sampled for measurement of glucose, insulin, C-peptide, and proinsulin. Insulinoma is diagnosed when the following criteria are fulfilled: plasma concentrations of glucose less than 55 mg/dL (3.0 mmol/L), insulin of at least 3.0 μU/mL (18 pmol/L), C-peptide of at least 0.6 ng/mL (0.2 nmol/L), proinsulin of at least 5.0 pmol/L. Some authors described modified fasting test where duration of the fasting period is reduced to 48 h (3, 5, 7, 8, 21).

Glycemia during the crisis was on average 30.37 mg/dl (range 20-45 mg/dl). Plasma insulin measured was increased, the average being 25.9 µU/ml (6 to 79.2 µU/ml). A supervised fasting test was performed in 11 patients and was positive in 10 of them. Median time to a positive test was 15.5 hours (4-28 hours). In 3 patients (30%) the test was positive in the first 10 hours (after 4 hours fastest in 1 patient), in 6 patients (60%) was positive within 24 hours and in all patients (100%) was positive within 48 hours. No patient passed the boundary of 48 hours.

Differential diagnosis

Hypoglycemia due to excessive endogenous insulin secretion can be caused by a primary pancreatic beta cell disorder including an insulinoma, beta cell hyperplasia or nesidioblastosis (presence of diffuse microadenomatosis, in which multiple small non-encapsulated tumors or nodules are distributed throughout the pancreas), a beta cell secretagogue including a sulphonylurea or a beta cell stimulating antibody, or an antibody to insulin. The differential diagnosis of hypoglycaemia includes hormonal deficiencies, hepatic insufficiency, exogenous hyperinsulinism, medication, drugs and enzyme defects. Occasionally differentiating insulinoma from these other causes of hypoglycaemia can be quite difficult (7).

Differentiation for nondiabetic-associated hypoglycemia is broad and includes insulinoma, drugs, hormone deficiencies, and critical illness. For stratification, the causes of hypoglycemia can be divided into those associated with diabetes mellitus and those who do not suffer from this condition, as the pathophysiology of each is distinct. Drugs are the most common cause of hypoglycemia (antidiabetic agents – insulin, sulfonylurea and meglitinides, salicylates, quinine derivatives, disopyramide, pentamidine, ethanol). Hypoglycemia is not an infrequent finding in the critically ill patient and may be related to sepsis, hepatic or renal failure, or to general malnourishment. Hepatic or renal failure, deficiency of cortisol and/or growth hormone can lead to hypoglycemia, leads to an inability to maintain adequate fasting glucose levels despite adaptive renal gluconeogenesis. Hypoglycemia associated with endogenous hyperinsulinaemia is very rare. Differential diagnosis of fasting hypoglycemia in adults should also include nonislet tumor (mesenchymal tumors, hepatocellular carcinoma or hematological malignancies) and autoimmune hypoglycemia (insulin receptor autoantibodies, insulin autoantibodies) (22, 23, 24, 25).

Topographic diagnosis

Transabdominal ultrasonography (US) was performed in 20 of the 21 patients and was positive in two of them (10%), which shows no accuracy of topographic diagnosis. A positive US result was obtained in a patient with proximal insulinoma (the pancreatic region most accessible for abdominal ultrasound exploration) – at the pancreatic head-body junction, and the other in a patient with caudally located tumor, but large (63/43 mm).

In one patient with a history of metastatic insulinoma, previously operated, followed by a second surgical intervention for hepatic metastases, US still showed liver metastases. US showed a tumor with regular contour, hyperechoic, homogeneous, with a diameter of 12 mm, in contact with the left suprahepatic vein.

Computed tomography (CT) could not be performed in one patient because she exceeded allowable weight, so of the 20 patients undergoing this investigation, one result was inconclusive, and from the remaining 19, 4 results were positive, with tumor size over 1 cm.

The sensitivity of CT was unsatisfactory: 4 positive results out of 19 (21.05%). CT failed to detect liver metastasis, but identified lymph node metastasis in one patient.

Magnetic resonance imaging (MRI) was performed in 18 patients and was diagnostic in 11 of them, recording a detection sensitivity of 61.11%, including infracentimetric tumor size.

Figure 1. Sensitivity of imaging methods (%)

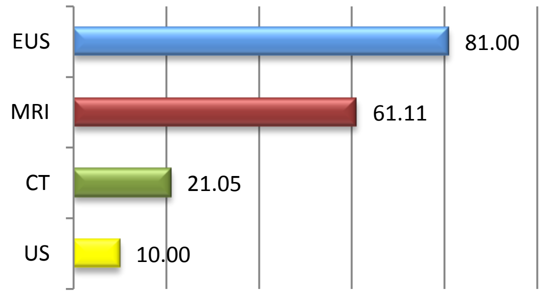

Endoscopic ultrasound (EUS) was performed in all patients, being able to identify formations in 17 out of 21, showing a diagnostic sensitivity of 81%.

In two patients, the results were inconclusive, as inhomogenous, poorly delineated areas were seen in the head and tail of the pancreas, but without clear identification of a tumor.

Excluding the other two patients in whom EUS failure was caused by lack of cooperation or poor conditions related to patient preparation, reporting the results of the remaining 19 patients who underwent EUS, we can say that its sensitivity was 89.47%.

Regarding EUS location of insulinomas, in our study, 44.44% were proximal (head and uncinate process), 27.77% in the body and the same percentage in the tail. The mean size of insulinomas was 1.958 cm (range 0.91 cm - 5.37cm). Most of them were between 1 and 3 cm (13 out of 18, meaning 72.2%).

Preoperative diagnosis and treatment

The preoperative topographic diagnosis was possible in 18 (86%) of the 21 patients with insulinoma. It was not possible in 3 patients, in whom all localization imaging investigations were negative, but in the presence of biological and symptomatic confirmed insulinoma.

In these patients surgical intervention was not performed, the solution consisting of reevaluation, follow-up and palliative treatment.

17 of 21 patients with insulinoma were referred to surgery. In addition to the 3 patients where the tumor could not be located, another patient with rare symptoms, drug controlled, was not operated because he did not accept surgery; he is being monitored clinically and by imaging methods.

At surgical exploration, the abdomen is initially investigated for evidence of metastatic disease. The pancreas is then completely exposed, allowing palpation of the entire pancreas.

Intraoperative ultrasonography and palpation can be performed at this time in order to effectively localize and guide in the dissection of the tumor. Since most insulinomas are benign and solitary, tumor enucleation is the procedure of choice, when is technically feasible (3).

In patients undergoing surgery, intraoperative diagnosis was made by intraoperative palpation of the tumor and/or intraoperative ultrasound. In 8 patients the intraoperative diagnosis was established by manual palpation of the pancreas and in 8 patients the two techniques were combined (pancreas palpation and intraoperative ultrasound).

In our cases, intraoperative ultrasound combined with a manual exploration of the pancreas showed a diagnostic sensitivity of 94.11%. The tumor could not be identified at the first surgery on a patient, but intraoperative ultrasound managed to locate it at the second intervention.

Table 3. Imaging methods and EUS location of insulinomas

	Patient	Gender	Age	US	CT	MRI	EUS tumor localization within the pancreas
1	PA	F	43	negative	negative	negative	between the body and the tail, near the posterior part of the gland
2	CC	F	37	negative	negative	negative	neck
3	ATI	F	26	negative	patient's weight was above the upper limits accepted for CT	patient's weight was above the upper limits accepted for MRI	located in the tail, posterior, near the left kidney
4	ME	F	46	positive, after EUS	negative	positive, after EUS	near the confluence, posterior
5	TC	M	53	negative	negative	negative	head
6	BM	M	41	negative	negative	positive, after EUS	head (uncinate)
7	AA	F	27	negative	negative	negative	fare tail, posterior, between spleen, left kidney and adrenal gland
8	SG	M	75	liver metastatis	negative	positive	head area
9	AZA	F	52	negative	negative	positive	border between body and tail, posterior
10	RL	F	50	negative	positive	positive	inhomogeneous head; cystic tumor in the tail
11	MNC	F	30	negative	positive	positive, after EUS	neck, posterior, near the confluence
12	SM	F	61	negative	negative	positive	neck
13	MI	M	72	not performed	negative	positive	body
14	LM	F	63	negative	negative	negative	tumor not located
15	GC	M	45	negative	negative	not performed	inhomogeneous head
16	VM	F	52	negative	negative	negative	head (uncinate), posterior
17	AD	F	38	negative	inconclusive	not performed	inhomogeneous tail
18	UV	M	43	negative	positive	negative	head (uncinate)
19	ELE	F	36	positive	negative	positive	tail
20	CG	F	38	negative	positive	positive	tumor not located
21	GC	F	43	negative	negative	positive, after EUS	between body and tail

Grade and differentiation

According to European Neuroendocrine Tumors Society (ENETS), detailed description of macroscopic, microscopic and immunohistochemical features is absolutely necessary to confirm the diagnosis of NTP and allow correct classification. The grade of a tumor refers to its biologic aggressiveness. For NTP, the grading system is based on the rate of proliferation, which is defined by the number of mitoses per 10 high-power microscopic fields or per 2 mm^2 (mitotic rate), or as the percentage of tumor cells that immunolabel positively for the Ki-67 antigen (Ki-67 index). An important role in insulinoma histological diagnosis is played by immunohistochemical analysis. NTP can also be classified based on differentiation, which refers to the extent to which cancerous, or neoplastic, cells resemble normal cells. Well differentiated NET cells produce large amounts of secretory granules with diffuse immunoexpression of neuroendocrine markers. In contrast, poorly differentiated NTP have atypical, sheet-like, diffuse and irregular nuclei, less cytoplasmic secretory granules, and limited biomarker immunoexpression. General markers of well-differentiated NET include cytosolic markers (neuron-specific enolase and PGP-9.5), markers associated with small vesicles (synaptophysin and synaptotagmin), markers associated with secretory granules (chromogranin A and HISL-19). Specific cell markers include insulin and proinsulin hormones. Poorly differentiated tumors do not express cell markers but can be positive for some of general markers. Endocrine differentiation may be confirmed at immunohistochemical analysis with neuroendocrine markers such as chromogranin A or synaptophysin. In well-differentiated NTP, determination of malignancy is straightforward only if the tumor invades adjacent organs or a distant metastasis is present. Histologic confirmation of complete excision and the benign nature of the insulinoma are essential (7).

Histological findings were available for our study in 7 of the 17 operated patients (tests were not available for the patients that were included in the first years of the study and we did not manage to recover the paraffin blocks to make them new tests). In 6 of these, the neuroendocrine tumor was identified; in one patient the tumor was not found and the absence of exact location of reccurent insulinoma led to a palliative treatment.

Table 4. Surgical interventions performed in patients from the study

	Patient	Previous surgery for insulinoma	Surgical intervention	Type of intervention
1	PA	no	yes	Surgical enucleating
2	CC	yes	yes	Surgical enucleating
3	ATI	no	yes	Surgical enucleating
4	ME	no	yes	Surgical enucleating
5	TC	yes	Patient refused surgical intervention	Patient refused surgical intervention
6	BM	no	yes	Tumor resection
7	AA	no	yes	Tumor resection
8	SG	yes	yes	Follow up (liver metastasis)
9	AZA	no	yes	Tumor resection
10	RL	no	yes	Tumor resection
11	MNC	no	yes	Duodenopancreatectomy
12	SM	no	yes	Laparoscopic enucleation
13	MI	no	yes	Tumor resection
14	LM	no	no	Follow up
15	GC	no	no	Follow up
16	VM	no	yes	Surgical enucleating
17	AD	no	no	Follow up
18	UV	no	yes	Tumor resection
19	ELE	no	yes	Tumor resection
20	CG	yes	yes	Tumor resection
21	GC	yes	yes	Surgical enucleating

Dietary treatment and medication were recommended especially for preoperative preparation of patients, aiming to reduce the frequency and severity of hypoglycemic symptoms and consisted of administration of frequent meals rich in carbohydrates and possibly an intravenous glucose solution and Verapamil.

In patients in whom the tumor could not be located with any imaging technique and for the patient who refused the surgery because of light and rare symptoms, and last but not least, in patients whose surgery was not curative, treatment was palliative. Drugs used for this purpose were: Verapamil tablets 80 mg or 40 mg 3 times daily, Octreotide (Sandostatin 0.1 mg every 8 hours subcutaneously) and 1 mg Glucagon kit (glucagon) intramuscular injection (the thigh or deltoid) in case of impaired consciousness (the latter was used in one case). These were indicated by the severity of symptoms. General instructions were: diet, frequent small meals rich in carbohydrates, avoidance of high physical exertion, and also periodic medical examination. Their combination succeeded to control the clinical manifestations in patients in whom surgery was not possible as a solution.

Surgical treatment was applied to 17 of 21 patients with insulinoma. In all 17 patients the tumor was localized by EUS. Some patients had two or even three surgical interventions. In 4 of them, EUS was performed before the second intervention, in another EUS was diagnostic and performed several times, but it did not provide an exact location of reccurent insulinoma. One patient underwent two surgeries, the first following suspicion of insulinoma, so partial pancreatic resection was performed, but no evidence of neuroendocrine tumor was found in the excised pancreatic tissue (blind partial pancreatic resection did not include tumor). Operative failures were due to lack of precise localization of the tumor and presence of metastases at EUS (inhomogeneous head/tail), but there were only 3 such cases. Regarding the 4 patients with negative EUS results, 3 of them were not operated because of failure to locate the tumor, therefore they are followed up. The fourth patient underwent surgery (tumor resection) with a good subsequent recovery. Among patients with positive EUS diagnosis surgery was successful, intraoperative diagnosis being made by intraoperative palpation of the tumor and/or intraoperative ultrasound showing the same location of the tumor as in EUS. There was one exception in a patient with metastasis that was followed up.

Recurrence of symptoms has led, ultimately, to a secondary surgical intervention (resection of pancreatic head and duodenum); this time intraoperative ultrasound confirmed location of the tumor and its excision was performed successfully. It is important to note that EUS was not performed in this patient. Another case is of a patient who underwent corporeo-caudal pancreatectomy without remission of hypoglycemic syndrome even if the tumor was localized preoperatively by EUS. Three years later EUS revealed tumor recurrence in the remaining parenchyma, which was enucleated with good recovery. Of the 7 enucleations, 3 (43%) were for tumors located in the pancreatic head.

Complications

The immediate postoperative complications occurred in 8 of the 17 patients who had surgical intervention, so we can say that postoperative morbidity was 47.05%. The complications are presented in table 5.

Table 5. Postoperative complications

Complication	Number
Transient hyperglycemia	4
Late postoperative pancreatic pseudocyst	2
Postoperative anemic syndrome	1
Transient pancreatitis	1
Postoperative eventration	1
Permanent diabetes mellitus	1
Exocrine pancreatic insufficiency	1
Malabsorption	1

The most common complication was transient hyperglycemia that occurred in 4 patients (23.52%), ranging between 3 and 15 days, with values between 130-170 mg/dl.

Diabetes mellitus occurred in a patient who underwent cephalic duodeno-pancreatectomy and exocrine pancreatic insufficiency in a patient who underwent a corporeo-caudal pancreatectomy followed by enucleation after three years.

Postoperative eventration and pancreatic pseudocysts were resolved surgically.

Metastases and recurrence

Malignancy is suggested by large tumor size, presence of metastases or vascular invasion. Tumors larger than 3 cm raise the suspicion of a malignant insulinoma. Other biological explorations advocating for malignancy are: high insulinemia, proinsulin large proportion (90%), elevated C-peptide. Ki-67 index is also a predictive factor for malignancy. Necrosis and high mitotic rate also correlate with malignancy. (5, 6, 7)

Concerning metastasis, ultrasound identified a hyperechoic area, with regular contour, on hepatic segment IV. This patient was previously operated for insulinoma with liver metastases: corporeo-caudal pancreatectomy and splenectomy were performed and liver metastases were resected in segments V, VI, VII of the liver. The therapeutic approach involves close patient monitoring and guidance for oncology service for cancer therapy. Liver metastases were demonstrated in 2 more patients by MRI. MRI confirmed the one found at US too. 5 patients had postoperative recurrences at varying intervals of time. In one patient in whom symptoms reappeared after seven years, imaging methods could not identify the tumor so he received palliative treatment (Verapamil 240 mg per day orally, Sandostatin 0.1 mg every 8 hours subcutaneously, diet, avoiding intense exercise). In another case, 3 years after surgery, at the EUS reevaluation a tumor was observed suggesting a possible recurrence; the patient refused surgical intervention. Excluding those 2 patients with liver metastases and the 5 with recurrence, we can say that the remaining 10 received curative surgery. And out of the 21 patients with insulinoma in the study, the remaining 4 underwent a palliative treatment, with periodic evaluation to locate the tumor. Where recurrent tumor could be identified, but the patient refused surgical intervention, anatomical location was the same as the primary tumor.

Evolution and mortality

Postoperative evolution was generally favorable, with 0% mortality. Curative surgery was followed by the disappearance of hypoglycemic symptoms, good clinical condition and weight loss.

DISCUSSION

Patients with insulinoma have symptoms of excessive insulin release with consecutive hypoglycemia, namely neuroglicopenic and adrenergic symptoms. The delay in establishing the diagnosis is explained by misleading and nonspecific clinical presentation (5, 7, 8). A high index of clinical suspicion is therefore essential. Eight of our patients were hospitalized in the neurology and psychiatry clinics and mistreated. Of them, four patients were diagnosed with epilepsy and received anticonvulsant treatment, and the other four patients were labeled with neurological disorders, anxiety disorders or thyroid dysfunction.

Between the onset of symptoms and diagnosis days, months, years or even decades can pass. This situation is found in our patients, the correct diagnosis being established over a period of symptomatic evolution averaged 25.14 months, but there are delays that have reached 10 years.

Diagnosis is based on Whipple's triad. The biochemical diagnosis of insulinoma is supported by the presence of hypoglycemia and inappropriately elevated insulin levels during prolonged fasting. In most patients (90%), insulinomas appear as single pancreatic tumors. In our study all patients were classified in this category – single pancreatic tumor – and the most common location was the proximal one (head and uncinate process) - 44.44%.

Most insulin-secreting tumors are small, so they are a diagnostic challenge for physicians, endocrinologists, gastroenterologists, surgeons and radiologists. In our case, most of them were between 1 and 3 cm (average size 1.9 cm). The role of imaging is to detect and provide precise information on anatomical location and staging before surgery.

Ultrasound, CT and MRI showed poor sensitivity. In our case it was 10% for US (literature 9-79.3% (6, 10)), positive results being obtained in patients with tumor located proximally (pancreatic region most accessible for abdominal US exploration), at the junction head – pancreatic body, and in only one tumor located caudally, but large (63/43 mm).

The diagnostic utility of somatostatin receptor scintigraphy is limited by the fact that only about 40-50% of them have somatostatin receptors, so that a negative scan cannot exclude the presence of an insulinoma. Octreoscan was performed in two patients; in one was not conclusive, in the other one revealed metastatis.

Studies show that PET (Positron Emission Tomography) is a sensitive imaging method to locate insulinoma with higher sensitivity than CT or MRI (26). PET was performed in three patients in our study, identifying metastatic insulinoma in only one of them. It should be noted that both Octreoscan and PET-CT became available later; however none of them have helped us greatly in clarifying the diagnosis.

EUS is particularly useful. EUS detection rates vary in different studies from 40% to 93% (6). EUS located the tumor in a patient where US and CT were negative. The typical lesion at EUS is round, smooth, hypoechoic, located mostly in the pancreas. Neuroendocrine tumors of the pancreas are usually slow growing, indolent, and growth is still a major determinant of survival in these patients. Because they usually grow slowly, their metastatic potential is relatively low and there are specific criteria to predict their behavior (the distinction between benign and malignant tumors is based on the presence of metastasis), so long-term monitoring is necessary. Currently, the most important prognostic factor is the presence or absence of metastases. Even metastatic tumors grow slowly, the prognosis in these patients being relatively good compared with non-endocrine pancreatic neoplasms (27, 28).

10% (5.8-15%) of insulinomas are malignant and already present liver and locoregional lymph node metastases at diagnosis. In our study three patients with insulinoma had liver metastases (14.28%).

Treatment options for pancreatic neuroendocrine tumors require a multidisciplinary approach and depend on the presence or absence of metastases. Surgical resection is the only potentially curative approach. (29)

The main technical options available are: enucleation and pancreatic resections, the choice of either method being determined by location, size and number of tumors. Enucleation is generally preferred because it strictly removes the lesion without unnecessary sacrifice of healthy pancreatic tissue; in our study was performed in 7 of the 19 surgeries (30).

Intraoperative diagnosis was made by intraoperative palpation of tumor and/or intraoperative ultrasound. Intraoperative ultrasonography is valuable for locating small lesions and has an overall sensitivity of 80-100% (5), being more sensitive than all other preoperative imaging techniques and better than physical examination performed intraoperatively by the surgeon. In our case, intraoperative ultrasound combined with a manual exploration of the pancreas showed a diagnostic sensitivity of 94.11%.

Postoperative complications – transient hyperglycemia, anemia, transient pancreatitis, pancreatic pseudocyst, diabetes mellitus, exocrine pancreatic insufficiency, malabsorption, eventration – occurred in 8 of 17 patients (postoperative morbidity 47.05%).

In one case, the tumor was not found by intraoperative palpation, probably due to its deep location, but was easily detected by intraoperative ultrasound evaluation.

In metastatic disease the most important therapeutic goal is to reduce the life-threatening hypoglycemic syndrome. This can be achieved by surgical removal of metastases or a variety of other cytoreductive techniques, radiofrequency ablation, with good results. An active approach to patients with advanced disease may result in longer survival and prevention of hypoglycemia.

Medical treatment was used to prepare the surgical one aiming at reducing the frequency and severity of hypoglycemic symptoms in patients whose tumor could not be located with any imaging techniques and in the patient who refused surgery because of mild and rare symptoms and, not least, in patients whose surgery was not curative (palliative treatment).

Drugs used for this purpose were: Verapamil, Octreotide (Sandostatin) and Glucagon (glucagon). They were indicated by the severity of symptoms. General instructions were: diet, frequent small meals rich in carbohydrates, avoidance of high physical exertion, and also periodic medical examination. Their combination succeeded to control the clinical manifestations in patients in whom curative surgery was not possible as a solution (31).

Evolution was generally good, with 0% mortality, and curative interventions were followed by the disappearance of hypoglycemic symptoms, good clinical condition and weight loss. Unfortunately, there were some failures of surgery, especially when the location of the tumor has not exactly been made preoperatively or intraoperatively, more likely associated with metastatic disease.

CONCLUSIONS

The challenging aspect of treating insulinomas is localizing the tumors. Diagnosis has three steps: identifying the clinical syndrome, biological diagnosis with hormonal determination and topographic diagnosis. Due to nonspecific symptoms, a high index of suspicion is required. The supervised fast is the classic diagnostic test for insulinoma. Histopathological diagnosis is essential for diagnosis and therapeutic management. Native CT or CT with intravenous iodinated contrast material had low sensitivity in detecting insulinomas and liver metastases, but identified in our study, lymph node metastases. MRI is more sensitive than CT and can diagnose insulinomas with infracentimetric size; MRI is also a technique of choice for highlighting possible metastases in the liver. EUS can locate the tumors and describes the size, shape, contour, echostructure and relationship with adjacent structures. High resolution of EUS allows detection of lesions with very small diameter, is safe and minimally invasive. EUS is the imaging investigation of choice in preoperative diagnosis of insulinomas. Surgical resection remains the mainstay of treatment for patients with localized disease. Future studies must lead to further improving therapeutic options for patients with this disease.

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Endoscopic faces of Helicobacter Pylori infection

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Abstract: *Introduction: The infection caused by H. pylori appears secondary after a bacterial colonization of the stomach and the initial portion of the small bowel. H. pylori –infected patients can develop gastritis, peptic ulcer, stomach cancer or MALT lymphoma. H. pylori infection is defined by WHO like a type I carcinogen, its role in gastric carcinogenesis being supported by the greatest researchers.*

Objectives: In this study our purpose was to determine the endoscopic appearances in H. pylori infection quoted in medical literature until now and the frequency of their appearance in our group of interest.

Materials and methods: In this study it was made an analytic study in which it was realized a retrospective cohort investigation at the Emergency Central Military and University Hospital "Dr. Carol Davila" Bucharest, gastroenterology branch –endoscopic department between 18.12.2012-21.08.2013 on 1694 patients between 18 and 92 years old, with the medium age of 55 years old.

As a diagnostic method for H. pylori infection we used superior digestive endoscopy during which were taken biopsies and it was made a fast urease test.

Results: Regarding the variation of the endoscopic aspects at the population of study, we have found gastritis with all its aspects (which was Sidney classified) in the biggest percentage meaning 59.3% of the cases, followed with a percentage of 18.8% by those without any endoscopic abnormality, and then in 10,33% of the cases we have found peptic ulcer. With a smaller percentage, under 10%, we have found duodenitis at 8.67% of this patients, and finally the most severe lesions represented by gastric cancer and lymphoma were found at 2,7% of the H.pylori infected patients.

INTRODUCTION

H. pylori is the most common infection found in humans, is a transmissible chronic infectious disease in which clinical manifestations are preceded by a long asymptomatic period. Helicobacter pylori is a gram-negative bacterium, the infection occurs due to bacterial colonization of the stomach and the initial portion of the small intestine and it is found on the luminal surface of the gastric epithelium. Was first isolated by Warren and Marshall in 1983.(1) In the entire world more than 50 % of the population have the infection into their upper gastrointestinal tract, the infection is found more often in the developing countries and with a significantly low percent in industrialized countries (2).

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The transmission routes in developing countries are largely represented by water, while in industrialized countries the transmission routes are directly, and the persons are infected from saliva, vomitus and others fluids.(3)(4)

Helicobacter pylori infection leads to development of gastrointestinal pathologies such as duodenal or gastric ulcers, many forms of gastritis, gastric cancers, and gastric mucosa-associated lymphoid-tissue (MALT) lymphoma. At the patients infected with Helicobacter pylori the risk of developing one of this pathologies varies widely in population, but a lot of the patients will not have any of this complications. (5)

By altering the normal gastric secretion, the bacterium produces local injury of the gastric mucosa especially at the antrum and distal part of the stomach. The most common pathology is gastritis and ulcers. At patients with adenocarcinoma, a known complication of Helicobacter pylori, the patients suffer a pangastritis with highly secretion of the body and antrum of stomach.(6)

The standard treatment for Helicobacter pylori includes PPI and three antibiotics which involves taking for 7 to 14 days and no single drug cures the infection. The proton pump inhibitors are decreasing the production of acid from the stomach and which allows the tissues to heal. Beside PPI also are recommended two antibiotics such as clarithromycin (500 mg, bid), amoxicillin (1 g, bid) and metronidazole. (7)

Unfortunately several clinical studies have shown that the triple therapy scheme from Europe the success rates are only 20-30% (10)(11). And therefore the detection of this bacteria can be realized in early stages and we can prevent various complications determined by it (especially gastric cancer) and use the treatment guides which were very well established within years has declined to the limit of 80% or less (8)(9) and in some countries

In this study our purpose was to determine the types of endoscopic aspects found in H. pylori infection quoted by now in medical literature and the frequency of their appearance in the group of study.

Materials and method

This study was realized between 18.12.2012-21.08.2013 on 1694 patients between 18 and 92 years old, with the medium age of 55 years old.

Inclusion criteria of endoscopic examined patients were represented by: patients with age over 18 years, unspecific digestive symptomatology to whom can associate alarm signals, the endoscopy indication made by the doctor, presence of a suggestive symptomatology for gastric or duodenal pathology, pain in the upper abdominal region or retrosternal, postprandial discomfort, distended bowel, early satiety, abdominal discomfort, pirosis, nausea, vomiting, anorexia, weight loss.

Exclusion criteria for this patients were similar with those for superior digestive endoscopy and are represented by: patients under 18 years , shock, acute myocardial infarction, peritonitis, acute perforation, fulminate colitis, recent colonic surgery, patient’s refuse, low cooperation of the patient, coma (except intubated patient), arrhythmias, acute myocardial ischemia.

In this study were included 1964 adults with age between 18-92 years old, as it is shown in table 1.

Table 1. Gender distribution of patients

Male	Female
866	828

As a diagnostic method of H. pylori infected patients, we used superior digestive endoscopy. Within the intervention were taken biopsies and were realized fast urease tests.

Fast urease test is based on the principle that excessive urease produced by H. pylori hydrolyses urea in ammonia. Therefore we have an increase of the pH of the environment in which we have introduced the bioptic fragment, determined with a colour indicator.

Although this test has a high specificity and sensibility, in general over 90%, the result seems to be influenced by certain drugs such as: PPI (proton pump inhibitors), antibiotics, H2 receptor antagonists and bismuth.

According to the large amount of data given by the medical literature, H. pylori infection produces various gastric and duodenal lesions with a low to high severity, and here we can mention gastritis, duodenitis, gastric and duodenal ulcer, gastric cancer and lymphoma.

In the group of patients with indication for superior digestive endoscopy, we have managed to visualize the endoscopic aspect mentioned up above, with a small mention that some of this patients had a normal endoscopic aspect.

Regarding those varied endoscopic aspects found in our group, we have determined that gastritis with all its aspects according to Sidney classification (described below) has the highest percentage of 59.3%, followed by normal endoscopic aspect with 18.8%, then we found ulcer in 10.33% of cases, duodenitis 8.67%, and finally the most severe- gastric cancer and lymphoma – only 2.7%, respectively 0.18% of the total examined group.

Figure 1. Distribution of endoscopic aspects found at endoscopic examined patients

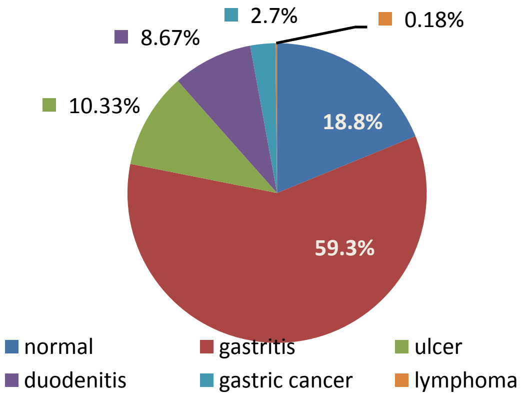

According to Sidney classification we determined the following types of gastritis: exudative-erythematous gastritis, macular-erosive gastritis, papular-erosive gastritis, atrophic gastritis, haemorrhaging gastritis, entero-gastric reflux gastritis and hypertrophic folds gastritis.

In all the endoscopic examined patients, from the total percentage of 59.3% that was represented by gastritis we have determined that the most frequent is exudative-erythematous gastritis, which represents better than a half with 57.8%.

The following as a frequency is represented by entero-gastric reflux gastritis with a percentage of 14% which was followed by papular-erosive gastritis, haemorrhaging gastritis, atrophic gastritis, and macular-erosive gastritis, with small differences between them, these representing 8%, 7, 27%, 6, 15%, respectively 5.35%.

Finally the less frequent was hypertrophic folds gastritis with a percentage of 14.45%.

Figure 2. Gastritis distribution according to Sidney classification of endoscopic examined patients

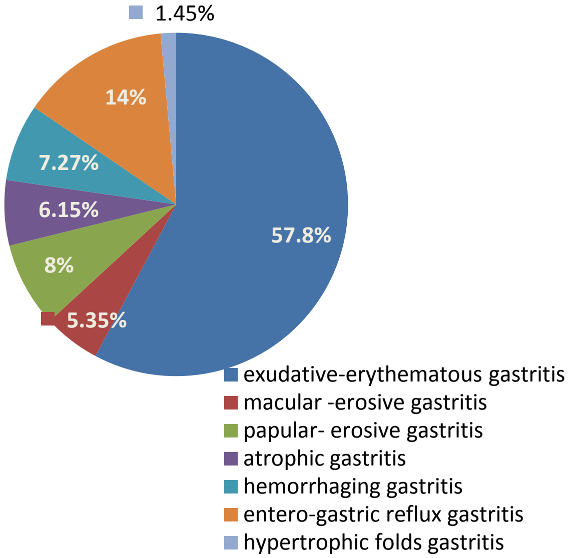

In the following figure we have presented gastritis distribution according to Sidney classification at the H. pylori-infected patients, and we determined that from all the cases with gastritis the most frequent was exudative-erythematous gastritis with a share of 61%. Must be mentioned here that 21.43% from all the cases with gastritis were infected with H.pylori.

The following places as a frequency, but still at pretty big difference from the first mentioned, were occupied by papular-erosive gastritis with 11.8%, macular-erosive gastritis with 10.62%, and entero-gastric with 6.7%. We can mention here that the percentage of these three determinations of gastritis are at the opposite pole from their situation in general examined population, where entero-gastric reflux gastritis has a bigger percentage than the others two. Now if we make a parallel between the occurrence of those gastritis types and the rate of occurrence in general population we can tell that 40.3% from the total macular-erosive gastritis cases and 30% from all the total popular-erosive gastritis found in general population appear in H. pylori infection, in contrast to entero-gastric reflux gastritis where only 9.7% from the cases occur in H. pylori infection.

Regarding haemorrhaging gastritis and atrophic gastritis, these have a smaller frequency of occurrence within the pale of infected patients, with a percentage of 4.72%, respectively 4.33%.

If we relate again at their distribution in general population we can observe that 14.3% from the total number of cases with atrophic gastritis is found in H. pylori infection. Haemorrhaging gastritis occurs in 13.2% of all the total haemorrhaging gastritis found in general population.

The smallest value as a frequency of occurrence in the H. pylori infected patients belongs to gastritis with hypertrophic folds with a percentage of 0.8% and we mention here that this value represents 11.11% from all the total cases of hypertrophic folds gastritis found at general population that was endoscopic examined.

Figure 3. Gastritis distribution according to Sidney classification of positive fast urease test patients.

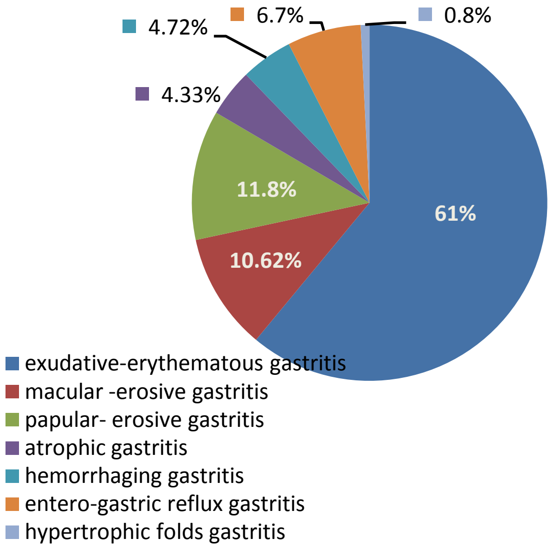

We observed at the persons in which was determined a positive fast urease test that the most frequent determination of H. pylori infection is represented by gastritis, which was found in 73.62% of the cases, a percentage up high as against general population where gastritis was determined in 59.3% of the cases. If we make a parallel of gastritis cases found at H. pylori infected patients and the cases found in general population we can observe that 20.3% of the gastritis cases occurred secondary to H. pylori infection.

The following two places as a frequency are occupied by ulcers and duodenitis with a small difference between them of only 0.3%, these two having the percentage of 11%, respectively 11.3%.

Figure 4. Distribution of endoscopic aspects found at patients with positive fast urease test.

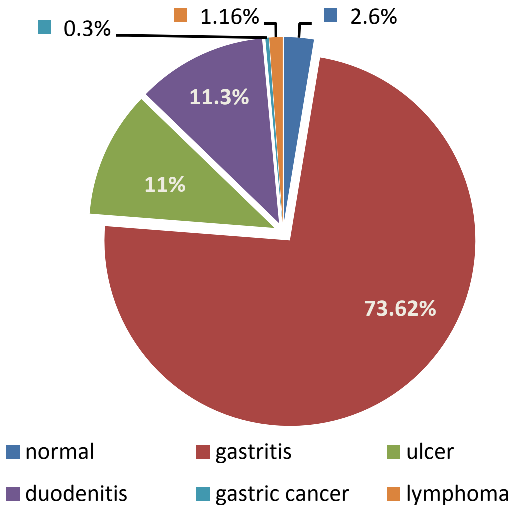

It is known that H. pylori infection can determine ulcerated lesions with gastric and duodenal localization.

From all the ulcerated lesions that were endoscopic discovered at the general examined population we determined that is a significant difference between these two types of localization, the one with the biggest percentage being the duodenal ulcer meaning 64.2%, in comparison with gastric ulcer which represents 35.8%.

In figure 6 was determined the distribution of ulcerated lesions at male gender with H. pylori infection where we can observe that three quarters from all the ulcer cases found at this group belongs to duodenal ulcer and the rest of 25% belongs to gastric ulcer.

Figure 5. Distribution of ulcer types according to its localization at endoscopic examined patients.

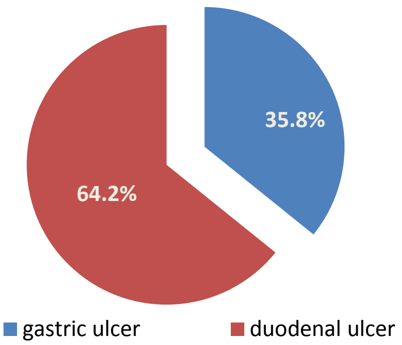

Figure 6. Distribution of ulcer types according to its localization at male gender patients with positive urease test

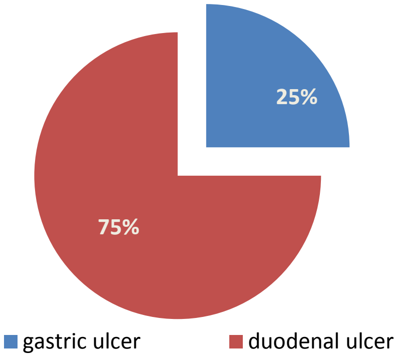

CONCLUSIONS

1. Helicobacter pylori was found in 52.22% of the cases tested for this infection and in 15.23% of the general population with superior digestive symptomatology.
2. Helicobacter pylori infection doesn't have gender predominance, being equivalent at both sexes.
3. In 97, 4% from Helicobacter pylori infected cases was determined a pathologic endoscopic aspect, the most frequent being gastritis with all its forms.
4. The most frequent gastritis type met in Helicobacter pylori infection was exudative-erythematous gastritis.
5. From all the ulcerated lesions met in Helicobacter pylori infection, the most frequent we have determined was at duodenal level.
6. Almost 20% from all the gastritis cases, ulcer and duodenitis were found in Helicobacter pylori infection.
7. All the lymphoma cases were found at Helicobacter pylori infected patients.

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CLINICAL PRACTICE

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Multidisciplinary approach to anemia

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Abstract: *Introduction: We present the case of a 65 years- old woman who was admitted with a severe macrocytic anemia Hb= 5.7g/dl and diffuse bone pain. Biologically she has moderate thrombocytopenia 35 000/µl, a hepatic cytolysis and cholestatic syndrome.*

Material and method: The patient was extensively evaluated before presentation for a mild iron - deficiency anemia for which she underwent endoscopic examination of the upper and lower gastrointestinal tract- normal. The bone marrow aspiration on admission revealed a marked hyperplasia of the erythroblastic line with ~50% basophilic erythroblasts suggesting a regenerative erythroid hyperplasia. These changes along with the marked reticulocytosis on the peripheral blood smear oriented us towards a hemolytic anemia; Folic acid, vitamin B12, autoimmune tests and hemolytic tests were all normal. We continued the investigations with a thoraco-abdomino-pelvic computed tomography which identified diffuse demineralization, vertebral compactation and pelvic stress fractures. The breast examination revealed a right breast nodule, but the breast ultrasonography pleaded for benignity. Lacking a clear definitive diagnosis we decided to perform a bone marrow biopsy.

Results: The osteo- medullary biopsy pointed towards a medullar invasion from a lobular mammary carcinoma; In these circumstances we performed an ultrasound guided biopsy of the right mammary lump thus histologically confirming a tumoral invasion of the bone marrow with subsequent anemia. The patient started chemotherapy in the Oncology ward.

Conclusion: The particularity of this case consists in the pattern of anemia, which initially seemed iron deficient and afterwards macrocytic – apparently hemolytic and was actually due to the tumoral medullar invasion and also the nonspecific ultrasonographic appearance of the breast tumor.

Keywords: *anemia, breast cancer, Evans syndrome*

INTRODUCTION

Anemia is a clinical feature in many emergency admissions to hospital. It is associated with increased mortality, morbidity and length of hospital stay in patients presenting with a range of conditions. If discovered in primary care it is seldom incompletely investigated. Although the most frequent cause of anemia is blood loss, as clinicians, we often find ourselves in front of complex and intricate causes which lead to a severe deficit. Therefore the management of such patients remains problematic. The prevalence of anemia under widely different conditions and the fact that anemia is managed by clinicians with a broad variety of backgrounds have fostered the need of multidisciplinary teams of experts (primary care physician, gastroenterologist,

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hematologist) to manage such cases.

The most important aspect of treating anemia lies not in correcting it and discharging the patient when stabile, but in identifying the cause and reversing it in order to prevent recurrences.

CASE PRESENTATION

Disease history

We present the case of a female patient aged 65, living in the country area, whose history began in January 2015. She consulted her family physician for fatigability and after having a series of blood test taken was prescribed iron substitution for 4 months. She had microcytic hypochromic anemia – her hemoglobin was 9.1g/dl, MCV = 72.5 fL, MCH = 24.3 pg and low blood level of iron = 39 ug/dl. Her family physician also recommended some tumor markers (aFP, CEA, CA 19.9) which turned out to be negative.

The patient thoroughly followed the treatment, but at the end of the treatment, when she performed a control full blood count her hemoglobin level was lower. This is the moment when she was referred to us.

Gastroenterology admission

On admission she was pale, stable hemo- dynamically BP = 120/70 mmHg and HR = 90 bpm and alert, asthenic . She reported not loosing any blood and on rectal examination there were no signs of fresh blood or melena.

Her laboratory data showed anemia with hemoglobin 7.1 g/dl, MCV 110 fl, MCH 35.6pg, blood iron 155 micrograms/dl, platelets 39.000/µl. So we are now facing a macrocytic anemia with a normal iron level and associated thrombocytopenia. Peripheral smear shows macrocytosis, numerous polycromatophylic red cells agglutination, erythroblasts 8% and 7.9% reticulocytes, in conclusion hyperplasic marrow with pronounced erythropoiesis – regenerative erythroid hyperplasia.

Endoscopic examination were performed: upper endoscopy showed duodeno-gastric reflux and small diaphragmatic hernia and colonoscopy revealed hemmorhoidal disease with absent signs of bleeding.

After excluding a bleeding site and because the anemic syndrome was now macrocytic protein electrophoresis, folic acid and vitamin B12 levels were also performed and both were normal.

Because the patient was feeling worse and the hemoglobin was dropping despite blood transfusion and iron supplementation she was transferred to Hematology for further investigation.

Hematology admission

In addition to fatigability, after 4 days in the gastroenterology ward, the patient now reports bone pain, without loss of range of motion. The pain responds to usual analgesics. On examination she is stable and on physical examination we discover superficial bilateral axillary lymph nodes 1.5 cm diameter, mobile and non tender and a right mammary nodule on breast examination .

Laboratory test now shows a worsening anemia and thrombocytopenia with a hemoglobin of 5.7 g/dl, MCV 114 fl, platelets 35.000/µl, Iron 304 micrograms/dl and hepatocytolytic and cholestatic syndrome: AST 108 UI/l, ALT 45 UI/l, GGT 228 UI/l (N 7-50), alkaline phosphatase 180 UI/l (N 38-91 UI/l), LDH 1081 (N 266-500). Hepatic viral serology was negative for hepatic viruses B and C. Coombs test and cold agglutinins test were also negative.

Ultrasonography was also performed and showed fatty liver and normal sized spleen without abdominal adenopathy, the heart was normal. Soft tissue ultrasonography shows bilateral axillary lymph nodes with apossible non-inflammatory character and 1.5 cm diameter. We also identified smaller lymphadenopathy bilaterally in the cervical area, resembling the appearance. On breast examination we described fibromatous placard in the supero-external area of the right breast with the long axis parallel with the skin, 4a BIRADS classification.

The biological panel strongly resembled Evans syndrome on admission, so we immediately decided to start treatment with high doses of dexamethasone while continuing investigations.

The non-inflammatory character of the axillary adenopathies prompts searching for additional lymph nodes. Therefore tomography of the thorax and abdomen was performed and it identified diffuse demineralization of the scanned bones, numerous vertebral compression fractures and stress fractures of both iliac wings, during consolidation.

Figure 1. Right axillary lymph node, 1.48 cm diameter

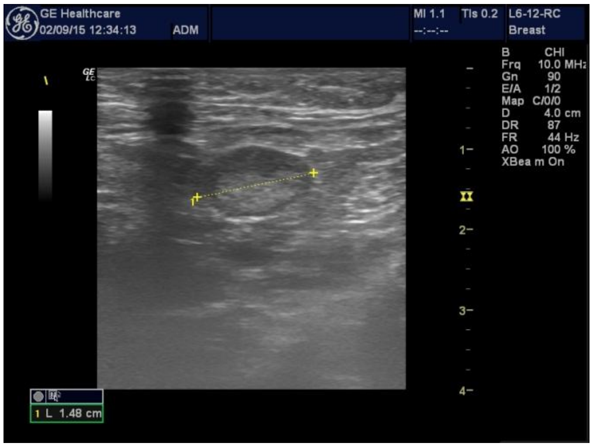
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Figure 2. Right breast nodule, 3.5 cm diameter

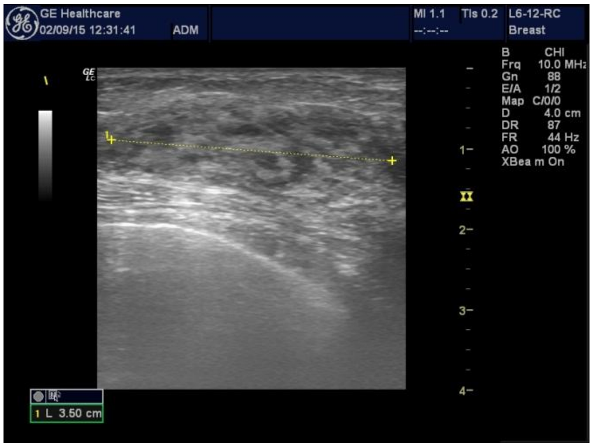
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Not having a definite diagnosis and because our patient was feeling worse and her hemoglobin was stationary despite treatment, we performed a diagnostic test.

A bone marrow biopsy was performed. Histologically and imuno-histochemically a metastatic lobular carcinoma of the breast was identified. Faced with a metastasis from a still not identified tumor, we returned to the fibromatous placard we saw in the right breast and an echo-guided biopsy of the nodule was performed. The biopsy revealed a lobular carcinoma strongly positive for estrogen receptors (ER) and negative for progesterone receptors (PR) and E-cadherin by immunohistochemical staining. The tumor marker was intensely elevated – CA 15-3 530 mg/dl.

Figure 3. Thoraco-abdominal CT showing diffuse demineralization and vertebral compression fracture

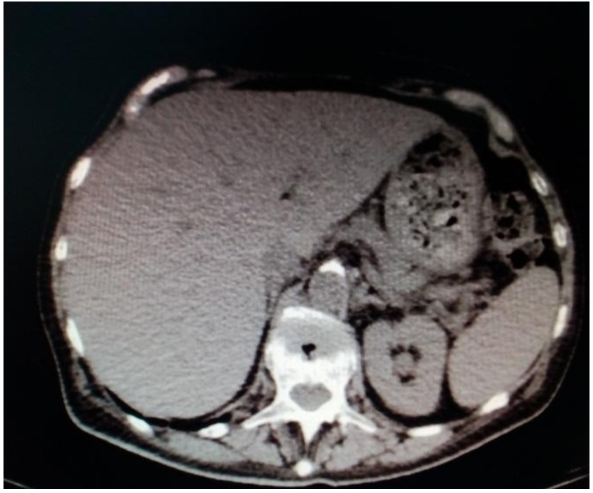

Figure 4: Whole body bone scintigraphy: low visualization of kidneys>> "super-scan" pattern pleading for secondary bone determinations with a possible paraneoplastic medullary impregnation syndrome

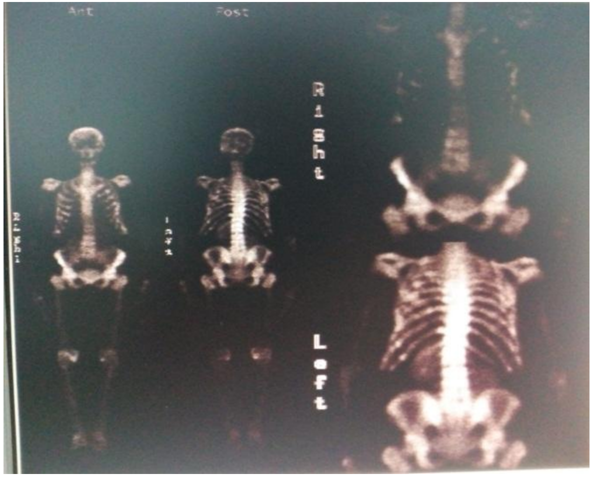
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Oncology admission

The patient was referred to oncology and she started chemotherapy with capecitabine and zoledronic acid after stabilization. The oncologist further investigated the implication of the bone system through a whole body bone scintigraphy. The result pleaded for multiple secondary bone determinations with a possible paraneoplastic medullary impregnation syndrome. Three months after initiation of appropriate systemic treatment for metastatic disease we see a partial recovery of hematopoietic function – her hemoglobin rose to 9 g/dl and platelets are around 90.000/µl .

COMMENTS

In conclusion, in front of a typical anemia that doesn't respond to typical treatment, as clinicians, we are obliged to search for additional causes and "cut the weed at the root".

The particularities of this case reside in the biochemical profile of the anemia – iron deficient at first encounter, then suggestive of Evans syndrome which delayed the diagnosis. In addition the ultrasonographic appearance of the breast tumor – more typical of a fibromatous degeneration did not raise suspicion at the first glance.

Breast cancer is the most common malignancy in women. Approximately 80% of women with metastatic breast cancer have skeletal metastases, which are often the result of bone marrow infiltration of malignant cells with subsequent progression and invasion of the skeletal cortex [1][2][3][4][5]. The most common complications of skeletal metastases are pathologic fractures, spinal cord compression as the result of vertebral compression fracture or extension of the tumor beyond the epidural space, and hypercalcemia [3][4][5]. Furthermore, skeletal metastases sometimes require surgery or radiation therapy to treat pain or an impending fracture. Bone marrow metastases result in the invasion and destruction of the bone tissue matrix by tumor cells [2]. Although bone marrow infiltration with micro metastasis is commonly present among breast cancer patients, total bone marrow infiltration – bone marrow carcinomatosis, BMC – resulting in profound pancytopenia is extremely rare.[2][6][7]

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Case report – hepatocytolytic syndrome hiding mesenteric venous ischemia

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BACKGROUND

Acute mesenteric ischemia (AMI) is a syndrome caused by inadequate blood flow through the mesenteric vessels, resulting in ischemia and eventual gangrene of the bowel wall. AMI may be classified as arterial or venous, non-occlusive or occlusive. The overall prevalence of AMI is 0.1% of all hospital admissions [1]. The exact prevalence of mesenteric venous ischemia (MVT) is not known, because many cases are presumed to be limited in symptomatology and to resolve spontaneously.

CASE PRESENTATION

A 70 years old male patient presented to our gastroenterology department for investigating an acute hepatocytolytic syndrome (AST 297 UI/ml, ALT 616 UI/ml). He was non-smoker, with no history of hepatotoxic treatment or other toxic substance abuse. One month before he had normal liver enzymes at clinical tests (AST 50 UI/). He was asymptomatic on admission, with unremarkable physical exam: except sensitive abdomen in the right hypochondrium, moderate hepatomegaly (left liver lobe hypertrophy) and abdominal postsurgical scars. His blood pressure was 110/70 mmHg with no orthostatic changes. A regular heart rate of 90 beats/min and respirations of 18-20/min were observed.

From the patient history: he was operated six years ago for abdominal aortic aneurysm with reimplantation of both renal artery after aortic graft, plus aorto-iliac bilateral stent, he has diabetes mellitus type 2 (on oral antidiabetic drugs from 2008), hypertension, ischaemic heart disease, systemic atheromatosis, laparoscopic colecistectomy in 2011 and right coxartrosis. A control angiogram and Doppler ultrasound was performed six months ago, with no evidence of occlusion or blockage of the mesenteric arteries.

At presentation the patient had hepatocitolytic syndrome – elevated serum transaminases ALT 537 UI/L ; AST 297 UI/L, cholestatic syndrome – elevated alkaline phosphatase ALP 124 UI/L, no modification on hemograme, hyperglycemia 190 mg/dl with HbA1c 6.8 %, serum creatinine 1.3 mg/dl. We tested the patient for virus B and C infection, and we found Ac anti HVC positive. Then we performed HCV RNA viral load 115 UI/ml (low viral load) and supportive hepatic treatment was immediate conducted. On abdominal ultrasound we found: mild hepatomegaly, post-colecistectomy status, normal CBP and a left renal

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cyst.

After a few days the patient accused upper abdominal pain, nausea, fatigue, vomiting, constipation and oliguria. The blood pressure was 140/80 mmHg, the abdomen was symmetrically distended, dull to percution with absent bowel sounds with firm and tender to palpation in the mid-epigastrium. We did an upper GI endoscopy that revealed hiatal hernia, diabetic gastroparesis and duodenitis (figures 1a, 1b)

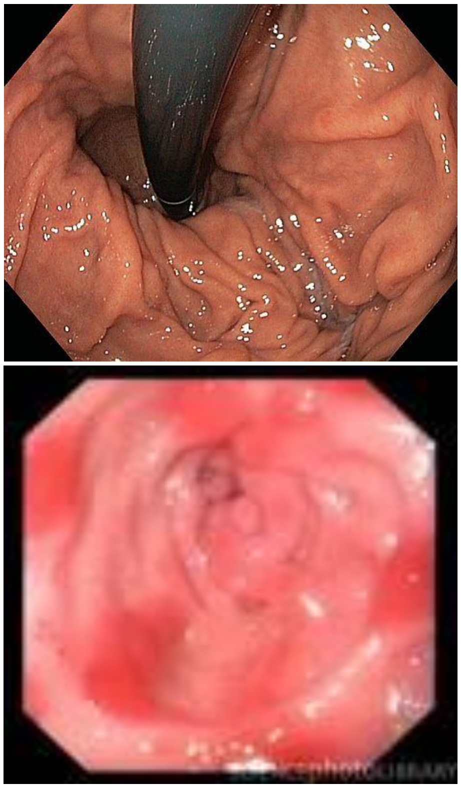

Figures 1 (a, b).

The laboratory tests showed normalization of liver enzymes, but rapid increase of urea 270 mg/dl and creatinine 4.88 mg/dl needing temporary hemodialysis in ICU, by femoral venous catheter placement. On abdominal examination, the patient had persistent abdominal pain, tenderness to palpation and increased abdominal wall rigidity. The native CT scan performed immediately (due to renal function impairment) did not confirmed arterial mesenteric ischemia suspicion, and also the biological markers D-dimers, CK, CK-MB where negative, with no signs of hypovolemia or hypotension.

After the temporary hemodialysis, another CT scan was performed, this time with contrast, because the suspicion of mesenteric ischemia remains, the patient had signs of peritonitis (right inguinal fossa pain and tenderness, exacerbated by moving the peritoneum, coughing and the Blumberg sign positive) and on ultrasound he has periappendiceal fluid collection and intestinal wall thickening. The contrast enhanced CT scan revealed: terminal ileum wall oedema, suggestive for acute mesenteric vein ischaemia, without lacunar imagine of a possible thrombus (arterial or venous) – figures 2a, 2b.

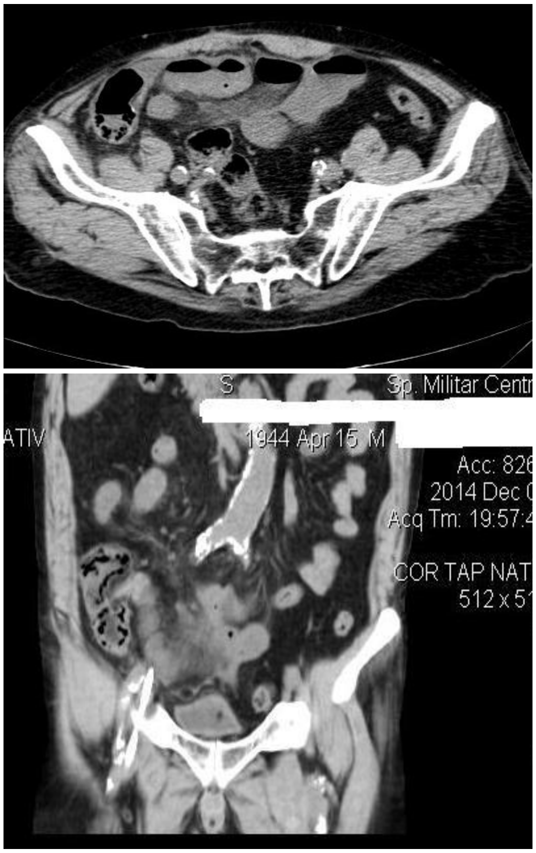

Figures 2 (a, b).

Initially the surgical intervention was temporized and the patient had conservatory heparinotherapy for 10 days (taking into account the patient comorbidities, history and risk factors for thrombosis), but the patient general status did not improved, he had nausea and vomiting, also persistent abdominal pain, fever and leucocytosis. We took blood cultures, remove central venous catheter and femoral venous catheters and continued antibiotherapy.

We did another CT scan with contrast one week later that showed distal ileum thickening with iodophile walls in arterial phase and necrosis, plus entero-enteral fistula localized in the right inguinal fossa (figures 3a, 3b, 4a, 4b).

Figures 3 (a, b).

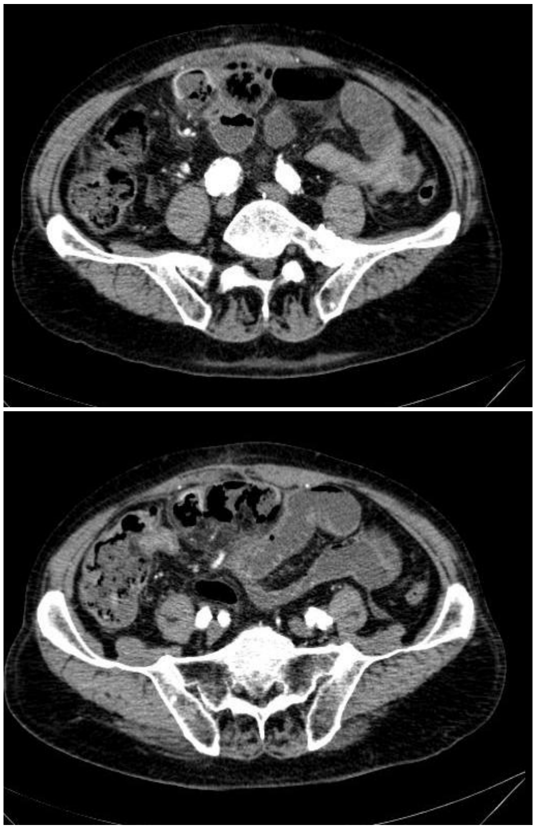

The treatment included both surgical and anti-thrombotic therapy and we transferred the patient to the surgical department. A median laparotomy was performed, and mesenteric venous ischemia modifications were found at 5 cm from ileocecal valve, with entero-enteral fistula and cecal abscess. A large enterectomy was made, with resection of approximately 150 cm of the ileum (figure 5), followed by peritoneal cavity lavage and drainage, then protective right ileostomy. The postoperative evolution was favorable, and the patient was released with normal liver and renal function, with protective ileostomy. At three months the patient came in good clinical status, and ileostomy reversal was made. Further investigations are needed for this patient regarding hypercoagulability status or identifying other MVT risk factors, and of course close follow up for prompt diagnosis and treatment of recurrence.

Figures 4 (a, b).

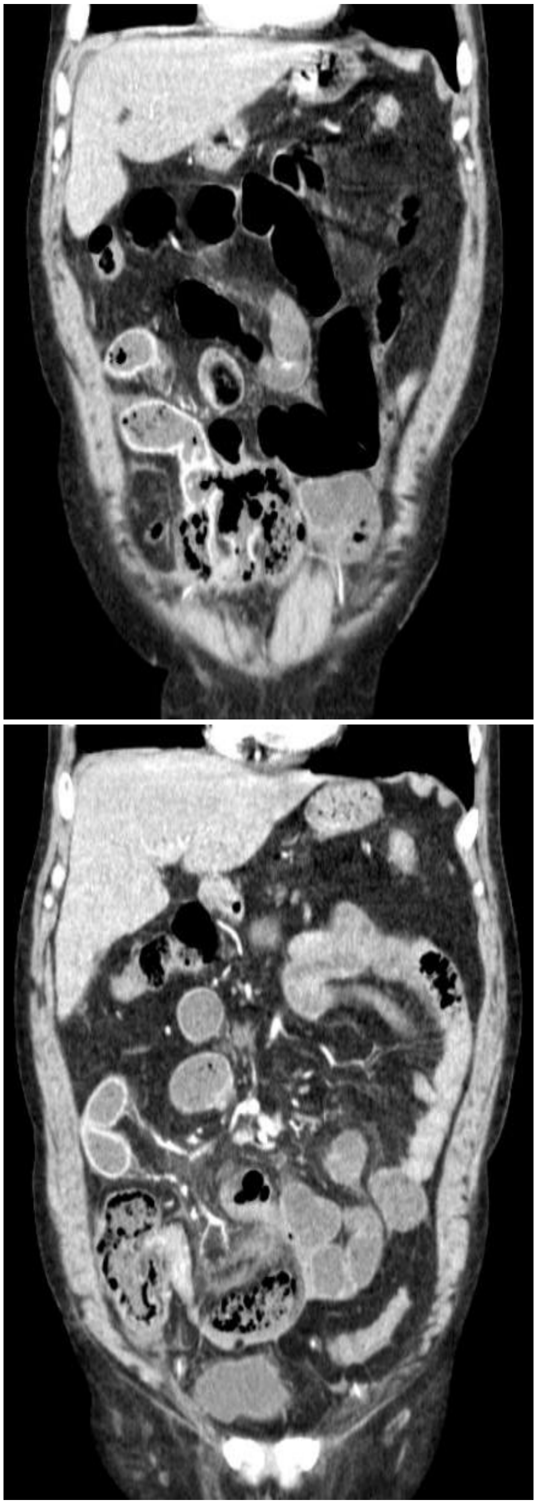

Figures 5.

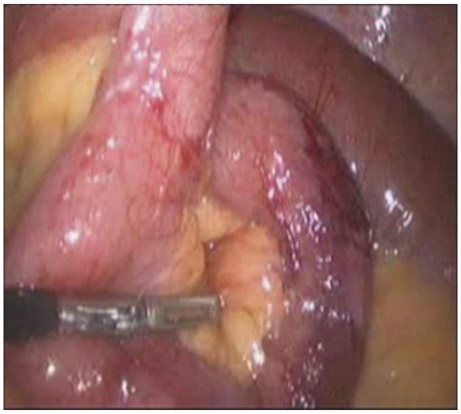

DISCUSSION

Mesenteric ischemia is caused by a reduction in intestinal blood flow, due to occlusion, vasospasm and/or hypoperfusion of the mesenteric vessels. The clinical consequences can be catastrophic, including sepsis, bowel infarction, and death, making rapid diagnosis and treatment imperative.

Injury severity is inversely proportional to the mesenteric blood flow and is influenced by the number of vessels involved, systemic mean blood pressure, duration of ischemia, and collateral circulation [1]. Mesenteric ischemia may be classified acute and chronic, based upon the rapidity and the degree to which blood flow is compromised.

Acute mesenteric ischemia refers to the sudden onset of intestinal hypoperfusion, which can be due to occlusive or nonocclusive obstruction of arterial or venous blood flow. Nonocclusive arterial hypoperfusion is most commonly due to primary splanchnic vasoconstriction [2].

Chronic mesenteric ischemia (also called intestinal angina) refers to episodic or constant intestinal hypoperfusion, which usually develops in patients with mesenteric atherosclerotic disease [2].

Venous thrombosis is predominantly a result of stagnation of blood flow, vascular injury, and hypercoagulability (ie, Virchow’s triad) [3]. First differentiated from arterial causes of acute mesenteric ischemia 75 years ago, acute mesenteric venous thrombosis (MVT) is an uncommon disorder with non-specific signs and symptoms, the diagnosis of which requires a high suspicion rate [4].

Mesenteric vein thrombosis almost always involves the distal small intestine (superior mesenteric venous drainage) and rarely involves the colon (inferior mesenteric venous drainage) [5]. The anatomic site of involvement in acute mesenteric venous thrombosis is most often ileum or jejunum, followed by colon and duodenum [6].

Acute thrombotic occlusion of one or more mesenteric veins reduces perfusion pressure due to increased resistance in the mesenteric venous bed. As flow stagnates, increased venous pressure leads to efflux of fluid into the tissues, causing profound bowel-wall edema, which can lead to submucosal hemorrhage. If the venous arcades and vasa recta are involved and venous return from the bowel wall is completely occluded, bowel infarction will occur [4].

Causes of MVT include the following (>80% of patients with MVT are found to have predisposing conditions):

- Hypercoagulability [7] from protein C and S deficiency [8, 9] antithrombin III deficiency, dysfibrinogenemia, abnormal plasminogen, polycythemia vera (most common), thrombocytosis, sickle cell disease, factor V Leiden mutation, pregnancy, and oral contraceptive use [10, 11]
- Tumor causing venous compression or hypercoagulability (paraneoplastic syndrome)
- Infection, usually intra-abdominal (eg, appendicitis, diverticulitis, or abscess)
- Venous congestion from cirrhosis (portal hypertension)
- Venous trauma from accidents or surgery, [12, 13] especially portacaval surgery
- Increased intra-abdominal pressure from pneumoperitoneum during laparoscopic surgery [12]
- Pancreatitis
- Decompression sickness

The diagnosis is most often made by contrast-enhanced computed tomography, though angiography and exploratory surgery still have important diagnostic as well as therapeutic roles [4]. CT findings of intestinal ischemia due to acute MVT include wall thickening > 3 mm in an adequately distended segment, abnormal enhancement patterns, distended luminal diameter, thickened mesentery, indistinct bowel wall margins, and the presence of new or unexplained ascites [14].

Thus, abdominal CT with adequate venous phase contrast should be used as the initial diagnostic test of choice for MVT, with angiography reserved for patients with non-diagnostic CT results yet a reasonable clinical suspicion for MVT (i.e. no alternate explanation for symptoms and either a known hypercoagulable risk factor or family history of venous thrombosis)[4].

Anticoagulation prevents clot propagation and is associated with decreased recurrence and mortality [4]. Immediate heparinization is currently accepted as standard therapy. Heparin has been shown to prevent recurrence of thrombosis after intestinal resection and to be associated with lower mortality when recurrence does occur [4].

When intestinal infarction is suspected by virtue of clinical decompensation or radiologic/CT findings, immediate exploration of the abdomen – either by laparotomy or laparoscopy is needed and non-viable segments of intestine are resected.

CONCLUSION

MVT is associated with a 30-day mortality of 13-15%. Without anticoagulant therapy, mortality increases, with a 25% recurrence rate. The combination of anticoagulant therapy with surgery is associated with the lowest recurrence rate (~3-5%)[1]. Although its mortality rate has fallen over time, acute MVT remains a life-threatening condition requiring rapid diagnosis and aggressive management.

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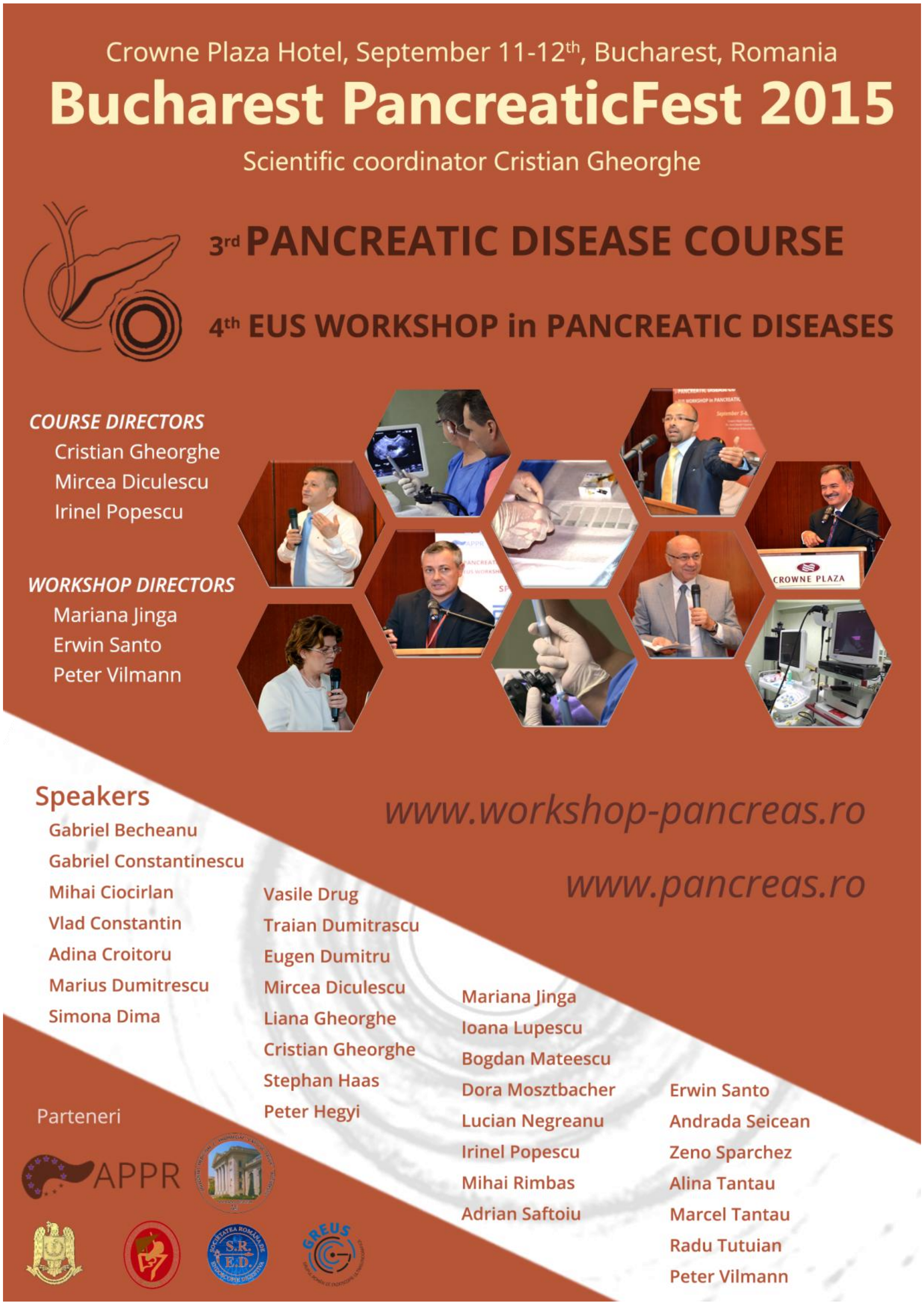

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EDITORIAL

PancreaticFest 2015, annual EUS international workshop

Mariana Jinga, Vasile Balaban

Colonel MARIANA JINGA

Deputy Commander, Central Emergency Military Hospital, Bucharest
Associate Professor, Carol Davila University of Medicine and Pharmacy, Bucharest

Although neglected in the past, pancreatic pathology (comprising acute and chronic pancreatitis and the fearful pancreatic cancer) has been in the spotlight in the last decades both in gastroenterology and surgery. This growing interest for pancreatology has led to funding of innovative research, publishing of highly accessed papers, and development of tertiary centers, with solid teams of gastroenterologists, radiologists, pathologists and surgeons; this has also led to a need for better risk stratification and for improvement in both early diagnosis and treatment of pancreatic diseases.

While death rates for many other cancers are declining, mortality for pancreatic cancer is increasing. The incidence of acute pancreatitis is also on the rise, and the disabling symptoms of chronic pancreatitis can greatly alter quality of life and limit work.

Despite the fact that these pancreatic diseases carry significant burden for society and medical systems, there are still many unmet needs in managing patients suffering from these illnesses, especially in Romania – lack of awareness in primary care, few dedicated centers and difficult access to novel diagnostic and treatment methods, which are not reimbursed by policy-makers.

In this setting, the Internal Medicine and Gastroenterology Clinic at “Dr. Carol Davila” Central Military Emergency University Hospital has proved a special interest for the study and management of pancreatic pathology. Back in 1998, the hospital was the first in Romania to have a linear endoscopic ultrasound (EUS) system. A few years later, EUS guided fine needle aspiration was also performed in our clinic in national premiere. Since then, EUS has evolved into a therapeutic instrument, especially for the pancreas and biliary tree, and research to extend its indications is ongoing.

To promote the interest for pancreatology, our clinic has been hosting an annual EUS workshop, with a first edition in 1999, which has grown into a tradition in the fall of the last few years. Since 2013, the workshop is held together with the International Pancreatic Disease Course, coordinated by Professor Cristian Gheorghe. The “Pancreatic Fest” is organized by the Association for Pancreatic Pathology Romania (APPR), which is part of the European Pancreatic Club. Over the years, world renowned experts have lectured and performed live demonstrations at the combined pancreas meeting, setting high standards for Romanian doctors and motivating young gastroenterologists to get training in this field. This up-to-date meeting in pancreatology reunites more and more attendees each year, creating opportunities for exchange programs abroad, developing multicenter research projects and promoting excellence care for patients.

This year's edition of the Pancreatic Fest, will take place on September 11-12th in Bucharest. The first day, dedicated to the EUS Workshop, will bring the participants complex cases, difficult techniques, tips and tricks, and cutting-edge EUS technology, all in HD live demo transmission from the Endoscopy Unit of the "Dr. Carol Davila" Central Military Emergency University Hospital. The second day will offer the participants a great educational programme, with state of the art lectures from international expert pancreatologists and interactive talks. We aim to bring together gastroenterologists, surgeons, pathologists, oncologists, radiologists, intensive care specialists and basic scientists, to create multidisciplinary teams with expertise in diagnosing and treating patients with pancreatic diseases. The message we are trying to convey is that partnering of specialties is the key to the successful management of these patients. We look forward to seeing you in Bucharest for a valuable scientific experience in the start of the fall!

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REVIEW ARTICLE

From liver biopsy to non-invasive markers in evaluating fibrosis in chronic liver disease

Cătălina Diaconu[1], Florentina Ioniță Radu[1,3], Mariana Jinga[1,2], Petruț Nuță[1], Săndica Bucurică[1,2], Bogdan Macadon[1], Mihăiță Pătrășescu[1], Andrada Popescu[1], Vasile Balaban[1,2], Laura Voicu[1], Daniel O. Costache[1], Raluca S. Costache[1,2]

Abstract: *Chronic liver disease is a late stage of progressive hepatic fibrosis. It consists of functional and structural disruptions in most chronic liver diseases. An accurate diagnosis allows us to establish the degree of fibrosis and the stage of the disease, the prognosis of the patient and to predict a treatment response. Despite the fact that liver biopsy is considered a gold standard, non-invasive methods for diagnosing liver fibrosis have gained more and more importance. Whether we talk about serum biomarkers or imagistic methods from transient elastography to 3-D magnetic resonance elastography, the question remains: are these useful or useless? Serum biomarkers represent blood components that can reflect liver histological changes, thus they can monitor the continuous process of fibrosis. These can be subcategorized in direct (that show extracellular matrix turnover) and indirect markers (that reflect disturbances in the hepatic function). However these markers alone are not as accurate in the staging of fibrosis, only help differentiate patients without or with low grade of fibrosis from those with significant fibrosis and cannot be considered alone in the diagnosis of liver fibrosis. Imagistic methods include: ultrasound-based transient elastography, magnetic resonance elastography (MRE), 2D-shear wave elastography, acoustic radiation impulse imaging (ARFI) and cross sectional imaging, the first being the most used. Using a combination of non-invasive tools allows us to diminish the number of patients in need of liver biopsy. However, the patient must always be informed of the advantages and disadvantages of each method and its limitations.*

Keywords: *fibrosis, biopsy, elastography, biomarkers*.

INTRODUCTION

Liver fibrosis remains an important problem worldwide. At first liver fibrosis was considered irreversible, but now scientists show that it is a dynamic process [1]. Advanced chronic liver disease, also known as cirrhosis is defined by the replacement of normal liver tissue with fibrotic one, leading to the disruption of the normal architecture of the liver and formation of regenerative nodules. It is usually considered to be irreversible, eventually leading to the need of liver transplantation. Many liver diseases can lead to cirrhosis. Most encountered causes are:

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viral hepatitis (mostly B and C hepatic virus), alcoholic liver disease and nonalcoholic liver disease, hemochromatosis. One must bear in mind less common causes of advanced end stage liver disease such as: autoimmune hepatitis, primary sclerosing cholangitis, primary and secondary biliary cirrhosis, Wilson disease, veno-occlusive disease, alpha-1 antitrypsin deficiency or right sided heart failure [2].

In normal hepatic tissue there is a balance between the production and degradation of extracellular matrix components. In liver fibrosis the balance is skewed in favor of the latter [3]. There is a high number of cells involved in the process of fibrogenesis. However, the hepatic stellate cell is the main factor involved. Chronic liver injury leads to the activation of hepatic stellate cells that transforms into myofibroblasts. These are contractile cells that secrete extracellular matrix components, pro-inflammatory citokines and chemokines. The first such protein produced in liver fibrosis is fibronectin, leading to the formation of excess collagen type 1. This process is cyclic since the production of extracellular matrix activates other stellate cells, which produce more proteins and finally change the hepatic architecture. Portal fibroblasts are also involved in the process of fibrosis, after stellate cells, tuning into myofibroflasts. Since these are closer to the portal tract they have a more important role in cholestatic injury from liver fibrosis, encountered in primary biliary cirrhosis and primary sclerosing cholangitis [4].

Liver biopsy is considered the golden standard in evaluating the degree of fibrosis. However it is limited by sampling errors, intra- and interobserver variability in histological interpretation and by the possible complications that may be encountered. There are many histologic systems that can appreciate fibrosis, but the one mostly used is semi-quantitative METAVIR score [5].

There are two categories of non-invasive methods in evaluating chronic liver disease: serologic panels of tests and radiologic tests. First ones quantify the levels of biomarkers in the serum samples and are more available. The latter consists of tests that measures liver stiffness using a "physical" approach. The two methods are complementary. Serum biomarkers are less accurate in detecting intermediate stages of fibrosis that in identifying cirrhosis, but present many advantages: high applicability, low costs, wide availability, and the possibility to be performed as outpatient [6]. Radiologic tests measure liver stiffness and are very useful in detecting cirrhosis. Combination between serum biomarkers and imagistic methods are very useful, slowly becoming a new golden standard in evaluating fibrosis in advanced liver disease [7].

Genetic risk factors for fibrosis are only now starting to gain importance. Literature shows that external factors such as alcohol and coinfections accelerate fibrogenesis. Studies now report variants in several genes that become associated with higher risk of fibrosis in chronic hepatitis C, NAFLD or AFLD [3].

LIVER BIOPSY

Considered the gold standard for the evaluation of tissue damage, including end-stage liver disease, liver biopsy is slowly falling behind non-invasive methods. Despite the fact that a high range of sensitive and accurate blood and imagistic tests can now be used to detect and even diagnose liver diseases, liver biopsy remains a valuable diagnosis tool, especially in difficult cases[8]. However the well-recognised limitations of this procedure have fuelled discussions regarding the correct diagnosis pathway in advanced chronic liver disease. The major problems of liver biopsy remain sampling and observational errors. Studies have shown that the larger the piece is, the lowest the risk of sampling errors is. The quality of the piece is in the length and the number of portal fields contained. A 20-25mm biopsy with more than 11 portal tracts is considered optimal specimen, even though some studies show that even a 15mm piece can be useful [3.9]. Even though we can obtain a large size biopsy, unfortunately it only reflects 1:50000 of the entire liver. Not only the length, but also the caliper of the biopsy needle is essential, a 16 gauge needle being the most appropriate to use [6]. Intra- or interobserver variability can also limit the usefulness of liver biopsy, leading to discrepancies in up to 20% of cases. Using histopathological scoring systems diminished this problem [3].

Histologic scoring systems in chronic liver disease are of great importance to the progression of the disease, prognosis and helping us establish appropriate treatments. In *chronic hepatitis* the most encountered systems are: the Knodell score (histology activity index), METAVIR score, Ishak score (modified Knodell score). Other less used scoring systems are: the Scheurer system, the Batts-Ludwig system and Laennec system. *Nonalcoholic fatty liver disease* (NAFLD) can be divided in nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFLD can be evaluated by nonalcoholic fatty liver activity score (NAS). It sums up the degree of steatosis, lobular inflammation and ballooning of the liver cells, ranging from 0-8. Fibrosis is not included in NAS. *Primary biliary cirrhosis* uses two scoring systems: Scheuer or Ludwing, based on: the degree of fibrosis, bile duct loss, copper deposition and the degree of cholangitis and hepatitis. Even though liver biopsy in *primary sclerosing cholangitis* is uncommon, if it is performed then a histologic scoring system can be used. It allows us to define 4 stages based on the degree of inflammation and fibrosis [10].

Complication rates related to liver puncture range from 0.75% to 13.6% [3]. Minor complications after liver biopsy include transient discomfort, moderate pain and mild transient hypotension. Bleeding remains the most important complication of liver biopsy, along biliary peritonitis, perforation and pneumothorax [11]. Biopsy through transjugular route can reduce the risks in patients with coagulation disorders or advanced liver disease [9]. The number of complications is also diminished if we choose ultrasound-guided biopsies [12].

Liver biopsies should be performed as in-patient procedure since 60% of complications occur in the first 2 hours and more than 90% of complications are prone to happen during the first 24 hours[3,11].

SERUM BIOMARKERS

The "biological" approach of evaluating end stage liver disease is represented by serum biomarkers. Even though these parameters may not be liver-specific, they have been associated with fibrosis stage [6]. These so-called surrogate markers can be direct and indirect. The changes in the content of extracellular matrix are quantified by direct markers, whilst indirect markers show hepatic function disturbances. These markers can be used alone or combined, leading to different scores, which can be simple (AST to platelet ratio index (APRI), FIB-4 index, FORNS) or calculated after a formula (Fibrotest, Fibroscore, Fibromax) [3]. Simple scores might have lower diagnosis accuracy, but they have greater advantages: lower price, easy to calculate and widely available [6].

Indirect markers

AST to platelet ration index (APRI) is easy to calculate based on the platelet count and AST level. APRI was first used in patients with hepatitis C virus (HCV), human immunodeficiency virus and HCV coinfection and alcoholic liver disease [13,14].

Table 1. The correlation between AST to platelet ratio index and the probability of cirrhosis.

APRI <=0.3:	Unlikely cirrhosis or significant fibrosis
APRI >0.3 and <=0.5:	Unlikely cirrhosis, significant fibrosis possible
APRI >0.5 and <=1.5:	Significant fibrosis or cirrhosis possible
APRI >1.5 and <=2:	Likely significant fibrosis, cirrhosis possible
APRI >2:	Likely cirrhosis

FibroTest and *ActiTest* were first used in patients with hepatitis B and C. *Fibrotest* uses alpha-2-macroglobulin, gamma globulin, apolipoprotein A1, alpha-2-globulin (haptoglobin), gamma-glutamyl-transpeptidase and total bilirubin. It takes into consideration patients' age and sex [1]. Even though there are advantages of FibroTest such as: high availability and applicability (>95%), there are many drawbacks: lack of specificity for liver disease, interference with other comorbidities that can change the result (Gilbert's syndrome or hemolysis) or the difficulty to differentiate intermediate stages of fibrosis [15]. ActiTest is a modified FibroTest that involves ALT, and evaluates both fibrosis and necroinflamatory activity of the liver [1].

Hepascore involves assessment of bilirubin, GGT, alpha-2-macroglobulin, hyaluronic acid, age and sex. A metaanalysis showed that Hepascore did not bring more information than FibroTest in patients with alcoholic liver disease. However, other studies showed its accuracy in predicting fibrosis in patients with chronic HC [1,9].

AST/ALT ratio in normal patients is around 0.8. Some studies show that a value over 1 suggests cirrhosis, but its utility is not certified [1].

Other indirect markers that are not worldwide used include: FIB-4 index, NAFLD fibrosis score (cutoff higher than 0.676 is associated with positive predictive value of fibrosis and a value under -1.455 is associated with a negative predictive value of 88 percent), PGA index (alcoholic liver disease with a detection range 66-72%), Forns (similar performance with APRI), BARD score (predictive in patients with NAFLD)[1,6].

Direct markers are used in forming panels to predict fibrosis, but none are available in clinical practice. (Table 2).

Table 2. Direct markers of fibrogenesis and fibrolysis

Matrix deposition	Procollagen I peptide
	Procollagen III peptide
	Type I collagen
	Type IV collagen
	YKL-40 (chondrex)
	Laminin
	Hyaluronic acid
Matrix degradation	MMP-2
	TIMP-1, -2
Cytokines	TGF-beta
	TGF-alpha
	PDGF

Serum biomarkers aren't useful in distinguishing between intermediate stages of fibrosis, but are more accurate in detecting fibrosis. Moreover careful consideration is entitled when talking about patients with HCV-HIV coinfection since false positive results can appear. For example: APRI, FIB-4, FibroTest (due to hyperbilirubinemia induced by atazanavir), Forns Index (related to GGT increase from nevirapine) and HepaScore. Despite the fact that bioseric markers can be used for detecting cirrhosis, their utility is reduced in diagnosing significant fibrosis [6]. Combination of different scores can be effective in avoiding biopsies, but a single surrogate marker cannot replace liver biopsy [3].

RADIOLOGIC TESTS

Radiologic tests represent the "physical" approach in evaluating fibrosis in chronic liver disease. The methods include: ultrasound-based elastography, magnetic resonance elastography (MRE), acoustic radiation force impulse imaging (ARFI), 2D-shear wave elastography (2D-SWE) and cross sectional imaging [3].

Trasient elastography was first evaluated as valid marker for fibrosis in a clinical study in 2002, therefore it remains rather new in the diagnostic pathway. It assesses the liver fibrosis by calculating the velocity of a low-frequency shear wave (50Hz) produced by a mechanical probe placed directly on the skin. The wave penetrates the liver tissue depending on the stiffness of the liver, correlating to the extent of liver fibrosis. The technique goes as following:

- the transductor is placed in contact with the skin with the tip covered in coupling gel;
- the position is in the 9th to 11th intercostal space (where liver biopsy is performed),
- we select an area of liver at a maximum of 6 cm deep, free of vessels;
- we gather 10 consecutive successful measurements. If one value is not valid, the entire set must be repeated [16].

There are several markers that evaluate if the result is valid: 10 consecutive valid results, a success rate (valid shots/total number of shots) above 60%, interquartile range lower than 30% of the median liver stiffness measurements, serum aminotransferase levels less than 5 times the normal value. Liver stiffness is expressed in kilo Pascal (kPa), ranging from 1.5 to 75kPa. A three category system is used: very reliable (IQR/M≤0.10), reliable (0.10<IQR/M≤0.30 or IQR/M>0.30 and liver stiffness evaluation (LSE) <7.1kPa) and poorly reliable (IQR/M>0.30 and LSE≥7.1kPa) [16,17].

Figure 1. The accuracy of transient elastography is based on a system made from: interquartile range/median liver stiffness measurements (IQR/M) and LSE (liver stifness evaluation) [3].

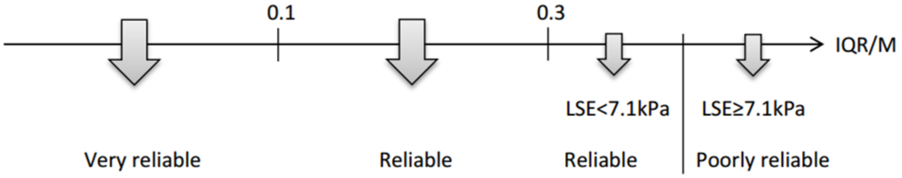

The largest series report evaluating TE show that in 3.1% of cases the measurement was impossible and in 15.8% of cases the results were unreliable (mostly due to patient obesity). Therefore a new probe was developed: XL, 2.5MHZ transducer. This allows us to measure liver stiffness from 35 to 75mm depth [3,6]. In their study Myers et al concluded that in 276 patients with chronic liver disease (42% viral hepatitis, 46% NAFLD) and a BMI>28 kg/m^2, using an XL probe was more successful than using M probe. The number of unreliable results was lower when using XL probe (25%) than with M probe (50%). Stiffness values calculated with XL probe are lower by a median of 1.4kPa than when obtained with M probe [18,19].

Other limitations in using transient elastography are: narrow intercostal spaces, ascites, cardiac failure, high necroinflammatory activity (quantified by a high AST/ALT ratio), steatosis and cholestasis [20]. Excessive alcohol ingestion or food intake might modify the results of TE, therefore a fasting period prior to the investigation in recommended, 3-6 hours [6,21].

A special S probe was introduced for evaluating liver stiffness in children or patients with small intercostal space [22].

A meta-analysis of 50 studies comparing transient elastography and liver biopsy showed that we can choose an imagistic method in distinguishing cirrhosis from lesser degrees of fibrosis, but it is less reliable in detecting lower grades of fibrosis thus it has moderate negative predictive values. Moreover the study shows that transient elastography is better in diagnosing nonalchoholic steatohepatitis and alcohol-related disease than in chronic viral hepatitis. Transient elastography is useful in detecting cirrhosis with an optimal cut-off value of 13.01 kPa[3]. However one must take into consideration that the target population might have different characteristics and it should be pre-tested [6]. TE should not be used alone in evaluation on liver fibrosis in some patients, but if it predicts significant fibrosis there is no need for biopsy [3,4]

The performance of TE in hepatitis B and C is similar if we compare the two. When evaluating hepatitis e antigen (HBeAg-) negative patients with normal ALT values, non-invasive methods, especially TE could be used to estimate levels of HBV-DNA and identify patients in need of liver biopsy [6].

TE might be used in monitoring liver fibrosis: either after or during treatment or to evaluate the natural progression of the disease. A most discussed topic in the world of hepatology is the role of TE in predicting portal hypertension. A cut-off value of 27.5kPa can predict the presence esophageal varices and the need of endoscopy to evaluate the extent of the disease and the need to start primary prophylaxis with non-selective β-Blockers, whilst a cut-off value of 62.7kPa can suggest a high risk of esophageal bleeding. Studies show that a cut-off value >25kPa is associated with > 45-fold risk to develop hepatocellular carcinoma in patients known with viral hepatitis [3,23]

Shear wave elastography (SWE) is divided in: point shear wave elastography (pSWE), also known as acoustic radiation force impulse imaging (ARFI) and 2D-shear wave elastography (2D-SWE). ARFI excites liver tissue by using short-duration acoustic pulses (~262µsec) that propagate shear waves and lead to localized µ-scale displacement in the tissue. The target zone (10x15mm) is set 10-20mm under the liver capsule. Ten consecutive valid measurements are required and the accuracy is evaluated with the interquartile range. The failure rate is lower when using ARFI to evaluate fibrosis than TE (2.9% vs 6.4%, p<0.001), especially in patients who suffer from obesity or associate ascites. Food intake, necroinflammatory activity and serum levels of aminotransferases might influence the results. ARFI is better in evaluating fibrosis in chronic hepatitis C than in chronic hepatitis B, NAFLD, HIV-HCV coinfection and other liver diseases. *2D-SWE* uses a combination of radiation force from focused ultrasonic beams and a very high frame rate ultrasound imaging sequence. Failure rate is lower than TE, especially in patients with ascites, but not in patients with obesity where XL probe is more accurate. However quality criteria in SWE are not yet well defined [6, 24].

Table 3. The correlation between the number of cases and SIRS criteria

Cirrhosis caused by:	Trasient elastography evaluation cut-off value (kPa)
Hepatitis C	13.01
Hepatitis B	11.7
Alcoholic fatty liver disease if drinking	22.7
Alcoholic fatty liver disease if abstinent	12.5
Biliary liver disease	17.9
Non-alcoholic fatty liver disease	10.3

Magnetic resonance elastography (MRE) involes placing a probe on the patient’s back that emits low-frequency vibrations that pass through the liver and can be measured. With an optimal cut-off of 4.71 it can suggest cirrhosis with a sensitivity of 91% and a specificity of 81%[1]. Some studies show a better diagnostic accuracy and technical success than TE[25]. Moreover, MRE has the advantage of scanning the entire liver tissue, thus it cannot miss areas of fibrosis, hepatocellular carcinoma, focal liver lesions, and it is independent to an acoustic window and is operator independent [1]. However due to the costs and excess of time MRE requires, it is not used for routine clinical practice use [6].

Other imagistic methods such as computerized tomography are not currently in use for evaluating fibrosis in chronic liver disease, due to low sensitivity and specificity and high costs [3].

We now know that non-invasive tests have striking advantages against liver biopsy when talking about patient safety. Therefore by combining the serologic biomarkers with imagistic methods (preferably) or even different bio-markers we can limit using liver biopsy only in case of doubt or discordance between serological biomarkers and imagistic method. The combination of non-invasive markers allows rapid staging of the liver without the need for liver biopsy for hepatitis C. The current gold standard in hepatitis C is to associate TE and serum biomarkers [6].

In hepatitis B, TE is superior to serologic biomarkers and it is best used to detect the stage of fibrosis in patients with active viremia (DNA-HBV>2000 IU/ml) and normal ALT. TE is not indicated in hepatitis with very high levels of ALT >10 times the normal value. Liver biopsy should be used when in doubt [6].

Non-invasive methods in evaluating fibrosis in NAFLD have a lower accuracy compared to liver biopsy and in time patients might require histological confirmation. However, follow-up can be performed with serum biomarkers or TE once every 3 years to evaluate the progression of the disease [6].

Despite the fact that non-invasive tests cannot replace hepatic venous pressure gradient (HVPG) in evaluating portal hypertension or endoscopy that reveals clinical implication of portal hypertension (oesophageal varices), these might be useful in selecting patients that require these procedures. HVPG over 10mmHg is predictive of varices and decompensation, whilst values under 10 mmHg has a 90% negative predictive value for the development of clinical decompensation in 4 years. However measuring HVPG is both expensive and invasive therefore repeating measurements is impractical [4,6].

CONCLUSION

Non-invasive markers do not abolish the need for liver biopsy. They should be used to select the patients in need of liver biopsy more carefully.

Smart combinations of non-invasive markers can greatly reduce the number of liver biopsies performed and the risks associated, but when the diagnosis is unclear one must use liver biopsy.

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SYSTEMATIC REVIEW

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Diabetes mellitus and colorectal cancer – a revealed connection

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Abstract: *The burden of colorectal cancer (CRC) is increasing all over the world. The prevalence of diabetes mellitus is increasing. It is estimated that diabetes affects 387 million people worldwide. It is predicted that 552 million people worldwide will develop diabetes by 2030. A large pool of data indicate that DM increases by 2 fold the risk of CRC. This is the reason to firmly suggest the inclusion of DM in the criteria for CRC screening as an important measure to decrease the mortality of this ailment.*

INTRODUCTION

Type 2 diabetes mellitus and colorectal cancer are major causes of morbidity and mortality in the United States and other Western countries.

The burden of colorectal cancer (CRC) is increasing all over the world. It is recognized as the second cause of cancer death in USA and in Western Europe. Also, it is a third cause of cancer in men and a second cause of cancer in women. Every year there are 1,2 million new cases in the world. The highest incidence is encountered in Australia (75/100000), New Zeeland, North America and Europe[1]. In Romania the incidence has doubled from 1994 (13/100000) to 2005 (23/100000)[2]. The lifetime risk of CRC is 5%. 90% of cases appear after the age of 50; beneath this age threshold is rare encountered but the current trend indicates an increasing prevalence in young[3].

It is estimated that diabetes affects 387 million people worldwide. It is predicted that 552 million people worldwide will develop diabetes by 2030. In the United States, about 23.6 million people or 7.8% of adults have DM and by 2050, the number of people in the United States with diagnosed DM is estimated to grow to 48.3 million[4]. The prevalence of diabetes mellitus in Romania is 11.6% as PREDATTOR Study has concluded in 2014[5]. Even those figures are very high it is appreciated that almost one-third of diabetics is undiagnosed[6].

STUDIES THAT CONFIRM THE RELATION BETWEEN DM AND CRC

In 1910, Maynard[7] provided the earliest reports on the association between DM and cancer of any type. In 1984, Williams et al[8] published a retrospective study that documented a statistically significant, over 2-fold increase in the prevalence of DM in CRC

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patients compared with age-matched controls. The search for a pathological explanation for this connection has led to the so-called hyperinsulinemia hypothesis. Giovannucci[9] hypothesized that insulin, an important growth factor, may at high serum concentrations increase the risk of CRC by promoting growth of colon tumors, and acting as a cell mitogen. Other proposed explanations for the increased risk include decreased bowel transit time and elevated fecal concentrations of bile acids[10]. Yang et al.[11] showed that insulin treatment may further elevate risk for CRC among patients with type 2 DM.

Figure 1: Risk factors for DM and CRC

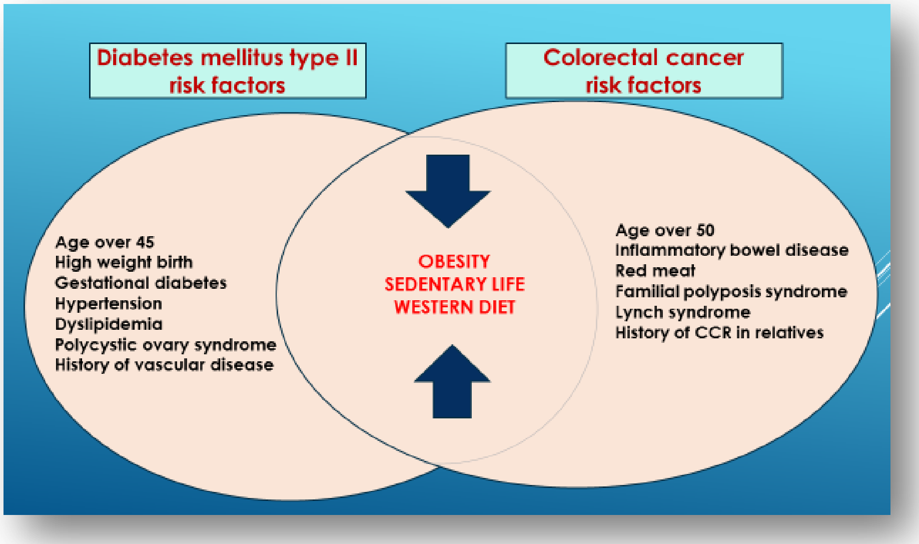
Diabetes mellitus type II risk factors
Colorectal cancer risk factors
Age over 45
High weight birth
Gestational diabetes
Hypertension
Dyslipidemia
Polycystic ovary syndrome
History of vascular disease
OBESITY
SEDENTARY LIFE
WESTERN DIET
Age over 50
Inflammatory bowel disease
Red meat
Familial polyposis syndrome
Lynch syndrome
History of CCR in relatives

Overall, studies indicate that diabetes risk increases twofold for liver, pancreatic, and endometrial cancer and 1.2- to 1.5-fold for colorectal, breast, and bladder cancer, but is associated with decreased risk for prostate cancer[12]. Diabetes is an independent risk factor for liver cancer. Cancer patients with diabetes relapse, and die, sooner than those without the disease.

Although proving a causal connection in observational human studies is uncertain and may take decades, preclinical research is revealing possible biochemical links between cancer and diabetes, which also share many risk factors.

The American College of Gastroenterology Guidelines for Colorectal Cancer Screening 2008 recommend that clinicians be aware of an increased risk of CRC in cigarette smokers and obese patients[13], but do not highlight the increased risk in patients with DM. Obesity and smoking are associated with the incidence of both type 2 DM and CRC[14]; thus, they also could be important positive confounders of the association between DM and CRC.

In July 2010, the American Cancer Society and the American Diabetes Association released a consensus report that reviewed the science behind the link and evidence for whether diabetes treatments influence cancer risk or prognosis. Published in the July August 2010 *CA: A Cancer Journal of Clinicians*, the report recommended regular cancer screening for diabetics. Preliminary evidence that metformin is associated with lower cancer risk, and exogenous insulin with higher risk[12].

Diabetes is characterized by high levels of both insulin and blood sugar, and both promote cancer cell proliferation in preclinical models In a Nov. 29, 2011, *British Journal of Cancer* study, he examined levels of the two in nearly 5,000 women from the Women's Health Initiative over 12 years. Elevated glucose levels were associated with a nearly doubled risk of colorectal cancer[12].

A recent meta-analysis on 41 studies associated DM with an increased incidence of colorectal cancer [relative risk, 1.27; 95% confidence interval (CI), 1.21–1.34][15]. No associations between DM and colon cancer were evident among African Americans[16]. A large-scale, population-based study in Japan suggested that diabetic men carried a higher risk for colorectal cancer than diabetic women did. The incidence of colorectal cancer was significantly higher in DM patients than in control patients (HR, 2.10; 95% CI, 1.82–2.42. The incidence of colorectal cancer is higher in males than in females (adjusted HR, 1.34; 95% CI, 1.18–1.53)[17].

CRC risk was higher among diabetics with longer duration of disease[18].

Metanalysis [19] of 15 studies (six case – control and nine cohort studies), including 2 593 935 participants, found that diabetes was associated with an increased risk of colorectal cancer, compared with no diabetes (summary RR of colorectal cancer incidence = 1.30, 95% CI = 1.20 to 1.40), without heterogeneity between studies (*P* heterogeneity = 0.21). The association between diabetes and colorectal cancer incidence did not differ statistically significantly by sex (summary RR among women = 1.33, 95% CI = 1.23 to 1.44; summary RR among men = 1.29, 95% CI = 1.15 to 1.44; *P* heterogeneity = .26) or by cancer subsite. Diabetes was positively associated with colorectal cancer mortality (summary RR = 1.26, 95% CI = 1.05 to 1.50), but there was evidence for heterogeneity between studies (*P* heterogeneity = 0.04). Findings from this meta-analysis indicate that individuals with diabetes have an approximately 30% increased relative risk of developing colorectal cancer compared with nondiabetic individuals.

Risk factors such as obesity, physical inactivity, smoking, Western diet and metabolic syndrome are common in both DM and colorectal cancer patients.

THE PROBLEM OF CONFOUNDERS AS RISK FACTORS

The first metaanalysis to quantitatively assess the effect of certain potentially important confounding variables including obesity, smoking, and physical exercise was published in 2008. It showed a statistically significant association between DM and risks of colon cancer and rectal cancer separately after controlling for obesity, smoking, and physical exercise. The association of DM and cancer risk was stronger for colon cancer than for rectal cancer (summary RR 1.38, 95 % CI 1.26 – 1.51 vs. summary RR 1.20, 95 % CI 1.09–1.31, respectively). This difference may be because the proximal colon, distal colon, and rectum have different embryological origins[20].

The positive association of DM with colon cancer and rectal cancer risk did not decrease when the metaanalysis was limited to studies that controlled for smoking and BMI (summary RR for colon 1.34, 95 % CI 1.17–1.52, and summary RR for rectum 1.28, 95% CI 1.06–1.54). This suggests that the confounding effect of obesity and smoking is relatively weak, and that DM appears to be an independent risk factor for colon cancer and rectal cancer.

Furthermore, as a recent study indicated, persons with DM and CRC may be at increased risk for CRC recurrence, non-response to chemo and radiotherapy treatment, and treatment-related complications. CRC deaths may be reduced through CRC screening programs.[21]

PROPOSED MECHANISMS

Several mechanisms have been proposed to explain the potential relationship between DM and colorectal cancer. Hyperinsulinemia, insulin-like growth factor-1, glucagon-like peptide-1 and the relative binding proteins each play an important role in metabolism, cell growth, proliferation and the regulation of the apoptotic process in colon cells[22].

Mechanistically, insulin stimulates cell proliferation through two pathways. One pathway involves direct binding of insulin to insulin or insulin-like growth factor-1 (IGF-1) receptors, and the other pathway is via inhibition of IGF binding proteins and the resultant increase in IGF-1 availability to the IGF receptor. The IGF system is a potent growth regulator closely linked with carcinogenesis[23]. These data support the notion that IGF-1 has a role in the biological pathway of colorectal neoplasia, beginning at the adenoma stage.

CONCLUSION

The increasing prevalence of DM may, significantly, be responsible for the increasing prevalence of CRC and its mortality. A great pool of data may suggest a need for more intensive CRC screening program in patients with type 2 DM, especially those who receive chronic insulin therapy.

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ORIGINAL ARTICLES

Culprits in non-celiac gluten-sensitivity

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INTRODUCTION

The growing interest for gluten-free diet (GFD) in the last decades has lead to a significant number of people going for this diet, in the absence of a diagnosis of celiac disease (CD). Owing to the perception that dietary gluten could be responsible for some of the symptoms in patients with irritable bowel syndrome (IBS)[1,2,3] (*Figure 1*), a new concept emerged, that of non-celiac gluten-sensitivity (NCGS), as it was named in an international consensus report[4]. NCGS describes the individuals whose symptoms get better on a GFD, in the absence of CD or wheat allergy[5]. The first reports about this condition date back to the late 1970s (two case reports) and the early 1980s (the Birmingham study)[6,7,8], and recently, some authors consider more appropriately to term it "people who avoid gluten"[9]. Biesiekierski et al.[10] were the first to publish a randomized clinical trial that proved the existence of NCGS; confirmation later came from the italian study of Di Sabatino[11], who showed in a randomized, placebo-controlled, cross-over design, a small, but statistically significant, alteration of symptoms in NCGS patients when they were gluten challenged.

The main issues of NCGS are[12,13,14]:

1. most of the times, NCGS is self-diagnosed of diagnosed by alternative health care practitioners
2. people who decide to go gluten-free rarely exclude CD before starting the diet, and when they address the doctor for evaluation, alternative diagnoses such as bacterial overgrowth or food intolerances (lactose, fructose) are found
3. aproximately one in four people with NCGS have persistent symptoms despite dietary gluten restriction

Figure 1: Spectrum of IBS-like syndromes (modified after Verdu AF, Am J Gastro 2009)

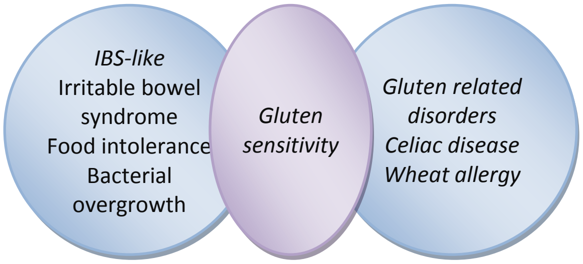

The prevalence of NCGS is variable, with reported values between 0.5 and 13%[15]. It is estimated that nowadays more people are following a GFD after a self-diagnosis of gluten-sensitivity than patients with CD[16]. Unlike the other gluten-related disorders, the diagnosis of NCGS is purely clinical, considering that there is no specific biomarker or characteristic histopathological changes on enterobiopsy. A diagnostic algorithm was recently proposed, which includes proof of both gluten responsiveness and relapse on gluten challenge (Salerno criteria)[17].

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ROLE OF GLUTEN AND OTHER TRIGGERS IN NCGS

The pathogenesis of NCGS is not completeley understood. The name NCGS implies that gluten is the culprit for the symptoms associated with this condition. However, recently there has been some questioning about gluten being the real trigger in NCGS, while considering other components which are also found in the cereals avoided in the GFD[18] – Figure 2.

Figure 2. Wheat components as possible triggers for NCGS

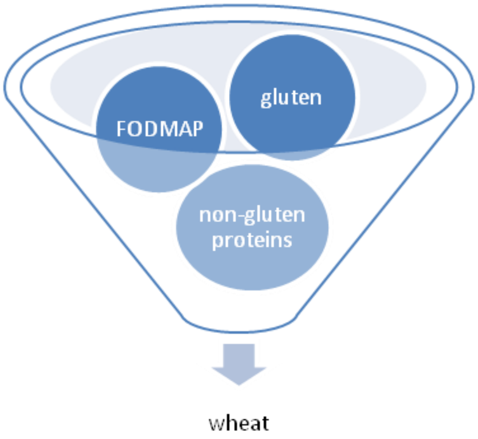

In this setting a “gluten conspiracy”[19] was described, which comprises two main ideas: if wheat is causing the symptoms, it must be the gluten in wheat responsible for them, and if the GFD improves the symptoms, this benefit is due to withdrawal of gluten. The pitfall of proving this misconception, that gluten is not the (only) one responsible, is that most of the challenges in clinical trials are performed with wheat (slices of bread, wheat flour capsules), and wheat contains not only gluten, but non-gluten proteins and non-protein components such as FODMAPs.

Among the possible non-gluten triggers, FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) and α-amylase/trypsin inhibitors (ATIs) have been studied in the literature.

The wheat kernel contains many components, among which the following are of interst for NCGS:
i) Proteins
- Storage proteins – represented by gluten (glutenins and prolamins – gliadin in wheat, and the equivalents secalin in barley and hordein in rye)
- Metabolic proteins – enzymes, enzyme inhibitors (eg. ATI – amylase-trypsin inhibitor), lectins (eg. wheat germ aglutinin – WGA)

ii) *Carbohydrates*
- Oligosaccharides, lactose, fructose, polyols (sorbitol, mannitol) – they osmotically draw water and are easly fermented by colonic bacteria (leading to gas formation in the gut lumen), which clinically translates into diarrhea, bloating and flatulence

FODMAPs

FODMAPs, an acronym for some osmotically-active, easily fermented carbohidrates, which are found also in gluten-containing cereals, have been described as a possible culprit of digestive symptoms in patients with NCGS[20,21,22,23]. A diet with reduced intake of FODMAPs (low-FODMAP), which has proven beneficial for IBS[24], has also been theoretized as being useful for NCGS patients.

In 2011, Biesiekierski showed that among cereal foods there is an overlap between the gluten-free and low-FODMAP ones, which could represent a confounder regarding the real culprit in NCGS: thus, the misconception that gluten is responsible for the digestive symptoms could be in fact explained by the presence of FODMAPs in the diet[25].

Another study performed by Biesiekierski on 37 NCGS patients (normal duodenal histology, negative HLA DQ 2/8) revealed that after 2 weeks on low-FODMAP (which lead to a significant relief of symptoms), there were no specific or dose-dependent effects upon dietary challenge in 3 randomized groups – high gluten (16 g gluten/day), low gluten (2 g gluten/day + 14 g whey protein/day) or control (16 g whey protein/day) diet for 1 week[26]. Gluten-specific effects were observed in only 3 out of 37 patients (8.1%). After a washout period of at least 2 weeks, 22 of the 37 patients crossed over to 3 groups which were given gluten (16 g/day), whey (16 g/day) or control (no additional protein) diet for 3 days. After the 3-day rechallenge, there were no differences between the three groups. Moreover, none of the 3 individuals who had a previous gluten-specific response in the first part of the trial reported such a response upon crossover. However, this short exposure to gluten in the second part of the study was associated with higher depression scores, which could explain why NCGS patients feel better on a GFD despite the persistence of gastrointestinal symptoms.

In conclusion, this study proved that gluten had no effect in patients with NCGS placed on low FODMAPs diets, and these carbohydrates could be the true cause of symptoms. Thus, we can state that people who eat gluten-free could be in fact sensitive to FODMAP and not to gluten, and the benefits of the GFD could be explained by its overlap with the low-FODMAP diet.

Among the limits of this trial, we should consider the possible nocebo effect on reintroduction of gluten/whey in the study groups.

The questionable role of gluten in NCGS is also supported by the double blind, cross-over trial of Zanini, who showed that gluten challenge induces symptom recurrence in only one third of NCGS patients[27].

Amylase-trypsin inhibitors (ATIs)

Beside FODMAPs, Schuppan's group identified some non-gluten proteins as a possible trigger for NCGS, specifically amylase-trypsin inhibitors (ATIs)[28,29]. These have been shown to induce intestinal inflammation by activation of TLR4 (toll-like receptor 4), effect limited to gluten-containing cereals only (wheat,barley, rye)[18].

In addition to ATIs, other non-gluten proteins such as WGA (wheat germ aglutinin) are considered as possible triggers for NCGS-like symptoms[30]. Current data has shown that these lectins can increase intestinal permeability, through mechanisms still unknown.[31]

CONCLUSIONS

Current knowledge suggests that the symptomatic benefit of a GFD in NCGS patients could be in fact not the consequence of gluten elimination, but avoidance of other compounds found in cereals such as FODMAPs or ATIs. Considering the evidences that question the causative role of gluten in NCGS, some authors have proposed renaming of this condition to non-celiac wheat sensitivity[32].

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Diagnostic and treatment difficulties in insulinomas

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Abstract: *Background: Neuroendocrine tumors of the pancreas (NTP) comprise a unique and relatively rare group of tumors, of which gastrinoma and insulinoma are the most common types. Insulinomas tend to be small, solitary and benign, with surgical resection curable in most cases.*

Introduction: Insulinomas are localized preoperatively using conventional imaging studies as transabdominal ultrasonography (US), computed tomography (CT), and/or magnetic resonance imaging (MRI).

Purpose: Endoscopic ultrasound (EUS) is a valuable tool in the diagnosis of insulinomas.

Goals & methods: We performed a retrospective study on 21 patients with insulinoma (6 male and 15 female, 25 to 73 years of age), who were hospitalized and operated on between 2003 and 2012 at "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest.

Results: US view was positive in 10% of patients (2 of 20), that presented proximal location. The sensitivity of CT was unsatisfactory, 21.05% (4 positive results of 19). CT failed to detect liver metastases, but identified nodal metastasis in one patient. MRI was performed in 18 patients and was diagnostic in 11 of them, recording a detection sensitivity of 61.11%, including infra-centimetric tumor size. EUS has a high resolution which allows detection of lesions with very small diameter is safe and minimally invasive. EUS was performed in all patients, being able to identify formations in 17, was inconclusive in 3, showing a diagnosis sensitivity of 81%. Liver metastases were demonstrated in 3 patients, one by US and all 3 by MRI.

Conclusions:

- CT with intravenous iodinated contrast agent had a poor sensitivity in detecting the primary tumors, was insensitive in detecting liver metastases, but showed metastases in lymph nodes.

- MRI has higher sensitivity than CT in detecting primary tumors, including insulinomas with infracentimetric size, and is the imaging test of choice for possible liver metastases.

- EUS is the preoperative imaging test of choice.

Keywords: *insulinoma, neuroendocrine tumors of the pancreas, endoscopic ultrasound (EUS), preoperative localization*

INTRODUCTION

Neuroendocrine tumors of the pancreas (NTP) are rare, but increasingly recognized entities. Tumors may be functional or nonfunctional, which are frequently detected incidentally or with symptoms related to mass effect of the tumor or its metastases (1). NTP have been considered rare, with an estimated incidence of less than 1 per 100000 individuals (2). Insulinoma is the most common

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neuroendocrine tumor of the pancreas and cause of hypoglycaemia related to endogenous hyperinsulinism with an annual incidence of four in every 1 million persons (3, 4, 5). As many as 90% of insulinomas have been reported to be benign, 90% are solitary, >90% occur in intrapancreatic sites, and 90% are < 2 cm in diameter. Regarding the location of pancreatic tumors, 50% are in the head, 25% in the tail, and 25% caudally or permeates the entire pancreas (5, 6).

Most of these tumors occur sporadically, but they can also be associated with multiple endocrine neoplasia (MEN)-1 syndrome. Approximately 4% of patients with insulinoma have MEN-1 (3, 4, 6). In sporadic forms, the highest incidence is considered in the age group 40-60 years (mean age 50 years), with a slight predominance of females (sex ratio 3:2 for women). (3). The diagnosis of insulinoma can be established by determining plasma proinsulin, insulin, C-peptide, and glucose levels, which are usually performed during a 72-hour fast (4). Insulinomas classically present with "Whipple's triad:" a combination of symptoms of hypoglycaemia, inappropriately high insulin levels with associated documented blood glucose levels of <50 mg/dL, and symptom relief with administration of glucose (2).

Non-invasive imaging procedures, such as CT and MRI, are used when a diagnosis of insulinoma has been made to localize the source of pathological insulin secretion. Invasive modalities, such as endoscopic ultrasound (EUS) and arterial stimulation venous sampling, are highly accurate in the preoperative localization of insulinomas and have frequently been shown to be superior to noninvasive localization techniques. Topographic preoperative diagnosis is difficult due to small tumor size; EUS is the most sensitive diagnostic method for this purpose. High resolution of EUS allows detection of lesions with very small diameter, is safe and minimally invasive (5, 7, 8). In the table below, we are presenting data from studies showing the accuracy of imaging methods in diagnosing insulinomas (Table 1).

Table 1. Accuracy of imaging methods in diagnosing insulinomas (5, 9, 10, 11, 12, 13, 14, 15, 16)

	US	CT scan	MRI	EUS	Intraoperative US	Intraoperative palpation
Pitre J, 1996				90%		
Angeli E, 1997	79.3%	44.8%	65.5%			
Ardengh JC, 2000		16.7%		83.3%		
Machado MCC, 2001	30%	25%	17%	27%		
Miralliè E, 2002				85%		
Jyotsna VP, 2006		57-83%	31%		92%	76%
Sotoudehmanesh R, 2007				89.5%		
Joseph AJ, 2013		69%		89%		
Takehiro O, 2013		33-64%	40-90%		80-100%	75-95%

There are still great difficulties in correct positive and topographic diagnosis of insulinomas. Advances in the pre-and intraoperative diagnosis allow precise localization and excision of the tumors, avoiding blind pancreatic resections (5, 17). Objectives of treatment are: symptomatic treatment of the tumor hypersecretion syndrome and surgical treatment which consists in enucleation of benign tumor or extensive resections. The surgery may use laparoscopic or open techniques. It is essential to perform intraoperative ultrasound, which will have an important role in choosing the optimal resection technique. Enucleation is the excision method of choice, whenever it is technically possible. Currently, resection of insulinomas can be successful with the techniques of minimally invasive surgery - laparoscopy or robotics, combined with intraoperative ultrasound performance. Medical treatment addresses to control symptoms or complications, for surgery preparation or as palliative treatment in cases where it can be done. When technically feasible, tumor enucleation is the procedure of choice; however, a more formal resection may be necessary for certain tumors (3, 5, 7, 14, 18, 19).

GOALS

Given the low incidence of pancreatic neuroendocrine tumors and the relatively limited medical experience regarding this group of tumors, there is concern in the medical world to find methods of diagnosis and appropriate treatment.

This study aims to identify characteristic features of pancreatic neuroendocrine tumors, especially of insulinoma, the most frequently diagnosed of them. We analyzed in terms of demographics, clinical, laboratory, imaging, pathology and treatment 21 cases of pancreatic insulinoma, all diagnosed and treated at “Dr. Carol Davila” Central Military Emergency University Hospital”, Bucharest, highlighting the best diagnostic and therapeutic attitude and the comparison with literature data.

We will try to determine which are most sensitive and specific methods applied at this time to establish early diagnosis and what treatment is recommended considering the particular features of the disease, the low incidence, appropriate index of suspicion, non-specificity of symptoms with often delayed diagnosis, involving a complex team (endocrinology, internal medicine, gastroenterology, oncology, intensive care, oncology, surgery) and the difficulties of this pathology.

MATERIAL AND METHODS

This article aims to develop a retrospective study to track the best diagnosis and treatment for insulinomas. In the period 25.01.2003 – 26.11.2012, 21 patients diagnosed with insulinoma were hospitalized and/or operated at the “Dr. Carol Davila” Central Military Emergency University Hospital in Bucharest. Medical records of patients with insulinoma seen in the period 2003-2012 were retrieved from the hospital’s medical records department. From these, data on clinical and diagnostic features, localization and surgical outcome were extracted. All mathematical analysis was performed using Microsoft Excel 2007.

The study analyzes the following data: epidemiology (distribution by sex and age), clinical (symptoms), laboratory (laboratory diagnosis, topography, location, locoregional extension and/or distant metastases), histological (macroscopic, microscopic and immunohistochemical) and treatment (surgery, medical), postoperative evolution, complications and prognosis. Features of patients were highlighted and analyzed by comparison with the data currently available in the literature. Biological, imaging and histological methods of diagnosis and techniques and results of surgical treatment and care were analyzed in particular.

RESULTS

Over a period of nine years, between January 2003 and November 2012, 21 patients diagnosed with pancreatic insulinoma were admitted to “Dr. Carol Davila” Central Military Emergency University Hospital, Bucharest.

Gender distribution was 15 women (71.4%) and 6 men (28.6%) and sex ratio women: men of 2.5:1. A particular case was the association with Wermer syndrome (MEN I) found in a patient of 36 years, who was hospitalized for symptoms of insulin hypersecretion. The mean age at diagnosis of insulinoma was 46.66 years (25-73 years), being lower for women than men (41.06 vs. 53.66). Distribution by age showed a peak between 40 and 49 years.

Positive diagnosis is suggested by clinical manifestations. In 1938, Whipple described a triad with a high specificity: 1) neurological crises that occur on an empty stomach or after exercise 2) decrease in blood glucose below 50 mg% during the crisis, 3) disappearance of neuropsychiatric disorders following administration of glucose orally or intravenously.

The diagnosis is based on simultaneous measurement of blood glucose and insulin levels (5, 6, 7). In our cases, positive diagnosis was suggested by Whipple triad. All patients had presented with fasting hypoglycemia and fulfilled the triad to suspect a diagnosis of insulinoma.

CLINICAL PRESENTATION

Patients had various associations of symptoms, shown after prolonged overnight fasting and physical activity or stress. The fact that the symptoms occur early in the morning, on waking, and disappear after ingestion of carbohydrates or glucose administration oriented the diagnosis (7). Delays in the diagnosis of insulinoma are common because the symptoms usually precede detection of a tumor and there may be misattribution of the symptoms to psychiatric, cardiac, or neurological disorders and, on average, it takes up to 2 years before the correct diagnosis is made (5, 7, 8). Neuroglicopenic symptoms delayed diagnosis in 8 patients (38%), as they were hospitalized in the neurology and psychiatry wards and mistreated. Of them, four were diagnosed with epilepsy and received anticonvulsant treatment, and the others were labeled with neurological disorders, anxiety disorders or thyroid dysfunction. Finally, the diagnosis was correct, the time period between onset of symptoms and diagnosis ranging from several days to 10 years. The symptomatic evolution of the disease before diagnosis had a mean of 25.14 months. More than half of patients in the study (61.9%) were diagnosed as having insulinoma after less than a year of evolution.

Table 2. Frequency of symptoms and signs that patients presented with in the study

Symptoms	Number of patients (%)
Preprandial hypoglycaemia	21 (100)
Loss of consciousness	13 (62)
Dizziness	12 (57)
Diaphoresis	12 (57)
Tremor	10 (48)
Obesity	8 (38)
Fatigue / asthenia	8 (38)
Increased appetite / hunger	7 (33)
Palpitations	7 (33)
Headache	6 (28)
Loss of balance	6 (28)
Diplopia, blurred vision	6 (28)
Seizures	5 (24)
Extremity paresthesia	5 (24)
Impaired memory or concentration	5 (24)
Dysarthria	5 (24)
Confusion	4 (19)
Abnormal behavior	4 (19)
Anxiety, irritability, psychomotor agitation	4 (19)
Difficulty in waking up in the morning	4 (19)
Amnesia	3 (14)
Hypoglycemic coma	3 (14)
Temporo-spatial disorientation disorder	2 (9)
Affective disorder	2 (9)
Distal neuropathy associated with hypoglycaemia	1 (5)

Positive biological diagnosis

Insulinomas are the most common cause of hypoglycemia resulting from endogenous hyperinsulinism. B cell insulin secretion does not decrease in the presence of hypoglycemia, and so plasma insulin greater than or equal to 3µU/ml along with a plasma glucose less than or equal to 50 mg/dL suggest an insulinoma. The diagnosis is made by simultaneous measurements of blood sugar and insulin levels in the blood (5).

The 72-h fasting test is a demonstration of Whipple's triad of symptoms, which is considered as the gold standard for the diagnosis. This test is conducted under supervised conditions, which requires hospitalisation of the patient. During the fasting period, the patient is allowed to drink calorie-free fluids and physical activity is encouraged. Blood glucose should be measured every 6 hours until it reduces to 60 mg/dL or less, when the interval is reduced to every 1–2 h. The fast is terminated when the plasma glucose level is 45mg/dl or less and the patient has symptoms and signs of hypoglycemia.

When symptoms of hypoglycemia appear, blood should be sampled for measurement of glucose, insulin, C-peptide, and proinsulin. Insulinoma is diagnosed when the following criteria are fulfilled: plasma concentrations of glucose less than 55 mg/dL (3.0 mmol/L), insulin of at least 3.0 µU/mL (18 pmol/L), C-peptide of at least 0.6 ng/mL (0.2 nmol/L), proinsulin of at least 5.0 pmol/L. Some authors described modified fasting test where duration of the fasting period is reduced to 48 h (3, 5, 7, 8, 21).

Glycemia during the crisis was on average 30.37 mg/dl (range 20-45 mg/dl). Plasma insulin measured was increased, the average being 25.9 µU/ml (6 to 79.2 µU/ml). A supervised fasting test was performed in 11 patients and was positive in 10 of them. Median time to a positive test was 15.5 hours (4-28 hours). In 3 patients (30%) the test was positive in the first 10 hours (after 4 hours fastest in 1 patient), in 6 patients (60%) was positive within 24 hours and in all patients (100%) was positive within 48 hours. No patient passed the boundary of 48 hours.

Differential diagnosis

Hypoglycemia due to excessive endogenous insulin secretion can be caused by a primary pancreatic beta cell disorder including an insulinoma, beta cell hyperplasia or nesidioblastosis (presence of diffuse microadenomatosis, in which multiple small non-encapsulated tumors or nodules are distributed throughout the pancreas), a beta cell secretagogue including a sulphonylurea or a beta cell stimulating antibody, or an antibody to insulin. The differential diagnosis of hypoglycaemia includes hormonal deficiencies, hepatic insufficiency, exogenous hyperinsulinism, medication, drugs and enzyme defects. Occasionally differentiating insulinoma from these other causes of hypoglycaemia can be quite difficult (7).

Differentiation for nondiabetic-associated hypoglycemia is broad and includes insulinoma, drugs, hormone deficiencies, and critical illness. For stratification, the causes of hypoglycemia can be divided into those associated with diabetes mellitus and those who do not suffer from this condition, as the pathophysiology of each is distinct. Drugs are the most common cause of hypoglycemia (antidiabetic agents – insulin, sulfonylurea and meglitinides, salicylates, quinine derivatives, disopyramide, pentamidine, ethanol). Hypoglycemia is not an infrequent finding in the critically ill patient and may be related to sepsis, hepatic or renal failure, or to general malnourishment. Hepatic or renal failure, deficiency of cortisol and/or growth hormone can lead to hypoglycemia, leads to an inability to maintain adequate fasting glucose levels despite adaptive renal gluconeogenesis. Hypoglycemia associated with endogenous hyperinsulinaemia is very rare. Differential diagnosis of fasting hypoglycemia in adults should also include nonislet tumor (mesenchymal tumors, hepatocellular carcinoma or hematological malignancies) and autoimmune hypoglycemia (insulin receptor autoantibodies, insulin autoantibodies) (22, 23, 24, 25).

Topographic diagnosis

Transabdominal ultrasonography (US) was performed in 20 of the 21 patients and was positive in two of them (10%), which shows no accuracy of topographic diagnosis. A positive US result was obtained in a patient with proximal insulinoma (the pancreatic region most accessible for abdominal ultrasound exploration) – at the pancreatic head-body junction, and the other in a patient with caudally located tumor, but large (63/43 mm).

In one patient with a history of metastatic insulinoma, previously operated, followed by a second surgical intervention for hepatic metastases, US still showed liver metastases. US showed a tumor with regular contour, hyperechoic, homogeneous, with a diameter of 12 mm, in contact with the left suprahepatic vein.

Computed tomography (CT) could not be performed in one patient because she exceeded allowable weight, so of the 20 patients undergoing this investigation, one result was inconclusive, and from the remaining 19, 4 results were positive, with tumor size over 1 cm.

The sensitivity of CT was unsatisfactory: 4 positive results out of 19 (21.05%). CT failed to detect liver metastasis, but identified lymph node metastasis in one patient.

Magnetic resonance imaging (MRI) was performed in 18 patients and was diagnostic in 11 of them, recording a detection sensitivity of 61.11%, including infracentimetric tumor size.

Figure 1. Sensitivity of imaging methods (%)

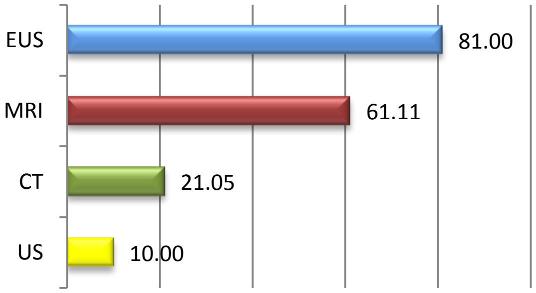

Endoscopic ultrasound (EUS) was performed in all patients, being able to identify formations in 17 out of 21, showing a diagnostic sensitivity of 81%.

In two patients, the results were inconclusive, as inhomogenous, poorly delineated areas were seen in the head and tail of the pancreas, but without clear identification of a tumor.

Excluding the other two patients in whom EUS failure was caused by lack of cooperation or poor conditions related to patient preparation, reporting the results of the remaining 19 patients who underwent EUS, we can say that its sensitivity was 89.47%.

Regarding EUS location of insulinomas, in our study, 44.44% were proximal (head and uncinate process), 27.77% in the body and the same percentage in the tail. The mean size of insulinomas was 1.958 cm (range 0.91 cm - 5.37cm). Most of them were between 1 and 3 cm (13 out of 18, meaning 72.2%).

Preoperative diagnosis and treatment

The preoperative topographic diagnosis was possible in 18 (86%) of the 21 patients with insulinoma. It was not possible in 3 patients, in whom all localization imaging investigations were negative, but in the presence of biological and symptomatic confirmed insulinoma.

In these patients surgical intervention was not performed, the solution consisting of reevaluation, follow-up and palliative treatment.

17 of 21 patients with insulinoma were referred to surgery. In addition to the 3 patients where the tumor could not be located, another patient with rare symptoms, drug controlled, was not operated because he did not accept surgery; he is being monitored clinically and by imaging methods.

At surgical exploration, the abdomen is initially investigated for evidence of metastatic disease. The pancreas is then completely exposed, allowing palpation of the entire pancreas.

Intraoperative ultrasonography and palpation can be performed at this time in order to effectively localize and guide in the dissection of the tumor. Since most insulinomas are benign and solitary, tumor enucleation is the procedure of choice, when is technically feasible (3).

In patients undergoing surgery, intraoperative diagnosis was made by intraoperative palpation of the tumor and/or intraoperative ultrasound. In 8 patients the intraoperative diagnosis was established by manual palpation of the pancreas and in 8 patients the two techniques were combined (pancreas palpation and intraoperative ultrasound).

In our cases, intraoperative ultrasound combined with a manual exploration of the pancreas showed a diagnostic sensitivity of 94.11%. The tumor could not be identified at the first surgery on a patient, but intraoperative ultrasound managed to locate it at the second intervention.

Table 3. Imaging methods and EUS location of insulinomas

	Patient	Gender	Age	US	CT	MRI	EUS tumor localization within the pancreas
1	PA	F	43	negative	negative	negative	between the body and the tail, near the posterior part of the gland
2	CC	F	37	negative	negative	negative	neck
3	ATI	F	26	negative	patient's weight was above the upper limits accepted for CT	patient's weight was above the upper limits accepted for MRI	located in the tail, posterior, near the left kidney
4	ME	F	46	positive, after EUS	negative	positive, after EUS	near the confluence, posterior
5	TC	M	53	negative	negative	negative	head
6	BM	M	41	negative	negative	positive, after EUS	head (uncinate)
7	AA	F	27	negative	negative	negative	fare tail, posterior, between spleen, left kidney and adrenal gland
8	SG	M	75	liver metastatis	negative	positive	head area
9	AZA	F	52	negative	negative	positive	border between body and tail, posterior
10	RL	F	50	negative	positive	positive	inhomogeneous head; cystic tumor in the tail
11	MNC	F	30	negative	positive	positive, after EUS	neck, posterior, near the confluence
12	SM	F	61	negative	negative	positive	neck
13	MI	M	72	not performed	negative	positive	body
14	LM	F	63	negative	negative	negative	tumor not located
15	GC	M	45	negative	negative	not performed	inhomogeneous head
16	VM	F	52	negative	negative	negative	head (uncinate), posterior
17	AD	F	38	negative	inconclusive	not performed	inhomogeneous tail
18	UV	M	43	negative	positive	negative	head (uncinate)
19	ELE	F	36	positive	negative	positive	tail
20	CG	F	38	negative	positive	positive	tumor not located
21	GC	F	43	negative	negative	positive, after EUS	between body and tail

Grade and differentiation

According to European Neuroendocrine Tumors Society (ENETS), detailed description of macroscopic, microscopic and immunohistochemical features is absolutely necessary to confirm the diagnosis of NTP and allow correct classification. The grade of a tumor refers to its biologic aggressiveness. For NTP, the grading system is based on the rate of proliferation, which is defined by the number of mitoses per 10 high-power microscopic fields or per 2 mm^2 (mitotic rate), or as the percentage of tumor cells that immunolabel positively for the Ki-67 antigen (Ki-67 index). An important role in insulinoma histological diagnosis is played by immunohistochemical analysis. NTP can also be classified based on differentiation, which refers to the extent to which cancerous, or neoplastic, cells resemble normal cells. Well differentiated NET cells produce large amounts of secretory granules with diffuse immunoexpression of neuroendocrine markers. In contrast, poorly differentiated NTP have atypical, sheet-like, diffuse and irregular nuclei, less cytoplasmic secretory granules, and limited biomarker immunoexpression. General markers of well-differentiated NET include cytosolic markers (neuron-specific enolase and PGP-9.5), markers associated with small vesicles (synaptophysin and synaptotagmin), markers associated with secretory granules (chromogranin A and HISL-19). Specific cell markers include insulin and proinsulin hormones. Poorly differentiated tumors do not express cell markers but can be positive for some of general markers. Endocrine differentiation may be confirmed at immunohistochemical analysis with neuroendocrine markers such as chromogranin A or synaptophysin. In well-differentiated NTP, determination of malignancy is straightforward only if the tumor invades adjacent organs or a distant metastasis is present. Histologic confirmation of complete excision and the benign nature of the insulinoma are essential (7).

Histological findings were available for our study in 7 of the 17 operated patients (tests were not available for the patients that were included in the first years of the study and we did not manage to recover the paraffin blocks to make them new tests). In 6 of these, the neuroendocrine tumor was identified; in one patient the tumor was not found and the absence of exact location of reccurent insulinoma led to a palliative treatment.

Table 4. Surgical interventions performed in patients from the study

	Patient	Previous surgery for insulinoma	Surgical intervention	Type of intervention
1	PA	no	yes	Surgical enucleating
2	CC	yes	yes	Surgical enucleating
3	ATI	no	yes	Surgical enucleating
4	ME	no	yes	Surgical enucleating
5	TC	yes	Patient refused surgical intervention	Patient refused surgical intervention
6	BM	no	yes	Tumor resection
7	AA	no	yes	Tumor resection
8	SG	yes	yes	Follow up (liver metastasis)
9	AZA	no	yes	Tumor resection
10	RL	no	yes	Tumor resection
11	MNC	no	yes	Duodenopancreatectomy
12	SM	no	yes	Laparoscopic enucleation
13	MI	no	yes	Tumor resection
14	LM	no	no	Follow up
15	GC	no	no	Follow up
16	VM	no	yes	Surgical enucleating
17	AD	no	no	Follow up
18	UV	no	yes	Tumor resection
19	ELE	no	yes	Tumor resection
20	CG	yes	yes	Tumor resection
21	GC	yes	yes	Surgical enucleating

Dietary treatment and medication were recommended especially for preoperative preparation of patients, aiming to reduce the frequency and severity of hypoglycemic symptoms and consisted of administration of frequent meals rich in carbohydrates and possibly an intravenous glucose solution and Verapamil.

In patients in whom the tumor could not be located with any imaging technique and for the patient who refused the surgery because of light and rare symptoms, and last but not least, in patients whose surgery was not curative, treatment was palliative. Drugs used for this purpose were: Verapamil tablets 80 mg or 40 mg 3 times daily, Octreotide (Sandostatin 0.1 mg every 8 hours subcutaneously) and 1 mg Glucagon kit (glucagon) intramuscular injection (the thigh or deltoid) in case of impaired consciousness (the latter was used in one case). These were indicated by the severity of symptoms. General instructions were: diet, frequent small meals rich in carbohydrates, avoidance of high physical exertion, and also periodic medical examination. Their combination succeeded to control the clinical manifestations in patients in whom surgery was not possible as a solution.

Surgical treatment was applied to 17 of 21 patients with insulinoma. In all 17 patients the tumor was localized by EUS. Some patients had two or even three surgical interventions. In 4 of them, EUS was performed before the second intervention, in another EUS was diagnostic and performed several times, but it did not provide an exact location of reccurent insulinoma. One patient underwent two surgeries, the first following suspicion of insulinoma, so partial pancreatic resection was performed, but no evidence of neuroendocrine tumor was found in the excised pancreatic tissue (blind partial pancreatic resection did not include tumor). Operative failures were due to lack of precise localization of the tumor and presence of metastases at EUS (inhomogeneous head/tail), but there were only 3 such cases. Regarding the 4 patients with negative EUS results, 3 of them were not operated because of failure to locate the tumor, therefore they are followed up. The fourth patient underwent surgery (tumor resection) with a good subsequent recovery. Among patients with positive EUS diagnosis surgery was successful, intraoperative diagnosis being made by intraoperative palpation of the tumor and/or intraoperative ultrasound showing the same location of the tumor as in EUS. There was one exception in a patient with metastasis that was followed up.

Recurrence of symptoms has led, ultimately, to a secondary surgical intervention (resection of pancreatic head and duodenum); this time intraoperative ultrasound confirmed location of the tumor and its excision was performed successfully. It is important to note that EUS was not performed in this patient. Another case is of a patient who underwent corporeo-caudal pancreatectomy without remission of hypoglycemic syndrome even if the tumor was localized preoperatively by EUS. Three years later EUS revealed tumor recurrence in the remaining parenchyma, which was enucleated with good recovery. Of the 7 enucleations, 3 (43%) were for tumors located in the pancreatic head.

Complications

The immediate postoperative complications occurred in 8 of the 17 patients who had surgical intervention, so we can say that postoperative morbidity was 47.05%. The complications are presented in table 5.

Table 5. Postoperative complications

Complication	Number
Transient hyperglycemia	4
Late postoperative pancreatic pseudocyst	2
Postoperative anemic syndrome	1
Transient pancreatitis	1
Postoperative eventration	1
Permanent diabetes mellitus	1
Exocrine pancreatic insufficiency	1
Malabsorption	1

The most common complication was transient hyperglycemia that occurred in 4 patients (23.52%), ranging between 3 and 15 days, with values between 130-170 mg/dl.

Diabetes mellitus occurred in a patient who underwent cephalic duodeno-pancreatectomy and exocrine pancreatic insufficiency in a patient who underwent a corporeo-caudal pancreatectomy followed by enucleation after three years.

Postoperative eventration and pancreatic pseudocysts were resolved surgically.

Metastases and recurrence

Malignancy is suggested by large tumor size, presence of metastases or vascular invasion. Tumors larger than 3 cm raise the suspicion of a malignant insulinoma. Other biological explorations advocating for malignancy are: high insulinemia, proinsulin large proportion (90%), elevated C-peptide. Ki-67 index is also a predictive factor for malignancy. Necrosis and high mitotic rate also correlate with malignancy. (5, 6, 7)

Concerning metastasis, ultrasound identified a hyperechoic area, with regular contour, on hepatic segment IV. This patient was previously operated for insulinoma with liver metastases: corporeo-caudal pancreatectomy and splenectomy were performed and liver metastases were resected in segments V, VI, VII of the liver. The therapeutic approach involves close patient monitoring and guidance for oncology service for cancer therapy. Liver metastases were demonstrated in 2 more patients by MRI. MRI confirmed the one found at US too. 5 patients had postoperative recurrences at varying intervals of time. In one patient in whom symptoms reappeared after seven years, imaging methods could not identify the tumor so he received palliative treatment (Verapamil 240 mg per day orally, Sandostatin 0.1 mg every 8 hours subcutaneously, diet, avoiding intense exercise). In another case, 3 years after surgery, at the EUS reevaluation a tumor was observed suggesting a possible recurrence; the patient refused surgical intervention. Excluding those 2 patients with liver metastases and the 5 with recurrence, we can say that the remaining 10 received curative surgery. And out of the 21 patients with insulinoma in the study, the remaining 4 underwent a palliative treatment, with periodic evaluation to locate the tumor. Where recurrent tumor could be identified, but the patient refused surgical intervention, anatomical location was the same as the primary tumor.

Evolution and mortality

Postoperative evolution was generally favorable, with 0% mortality. Curative surgery was followed by the disappearance of hypoglycemic symptoms, good clinical condition and weight loss.

DISCUSSION

Patients with insulinoma have symptoms of excessive insulin release with consecutive hypoglycemia, namely neuroglicopenic and adrenergic symptoms. The delay in establishing the diagnosis is explained by misleading and nonspecific clinical presentation (5, 7, 8). A high index of clinical suspicion is therefore essential. Eight of our patients were hospitalized in the neurology and psychiatry clinics and mistreated. Of them, four patients were diagnosed with epilepsy and received anticonvulsant treatment, and the other four patients were labeled with neurological disorders, anxiety disorders or thyroid dysfunction.

Between the onset of symptoms and diagnosis days, months, years or even decades can pass. This situation is found in our patients, the correct diagnosis being established over a period of symptomatic evolution averaged 25.14 months, but there are delays that have reached 10 years.

Diagnosis is based on Whipple’s triad. The biochemical diagnosis of insulinoma is supported by the presence of hypoglycemia and inappropriately elevated insulin levels during prolonged fasting. In most patients (90%), insulinomas appear as single pancreatic tumors. In our study all patients were classified in this category – single pancreatic tumor – and the most common location was the proximal one (head and uncinate process) - 44.44%.

Most insulin-secreting tumors are small, so they are a diagnostic challenge for physicians, endocrinologists, gastroenterologists, surgeons and radiologists. In our case, most of them were between 1 and 3 cm (average size 1.9 cm). The role of imaging is to detect and provide precise information on anatomical location and staging before surgery.

Ultrasound, CT and MRI showed poor sensitivity. In our case it was 10% for US (literature 9-79.3% (6, 10)), positive results being obtained in patients with tumor located proximally (pancreatic region most accessible for abdominal US exploration), at the junction head – pancreatic body, and in only one tumor located caudally, but large (63/43 mm).

The diagnostic utility of somatostatin receptor scintigraphy is limited by the fact that only about 40-50% of them have somatostatin receptors, so that a negative scan cannot exclude the presence of an insulinoma. Octreoscan was performed in two patients; in one was not conclusive, in the other one revealed metastatis.

Studies show that PET (Positron Emission Tomography) is a sensitive imaging method to locate insulinoma with higher sensitivity than CT or MRI (26). PET was performed in three patients in our study, identifying metastatic insulinoma in only one of them. It should be noted that both Octreoscan and PET-CT became available later; however none of them have helped us greatly in clarifying the diagnosis.

EUS is particularly useful. EUS detection rates vary in different studies from 40% to 93% (6). EUS located the tumor in a patient where US and CT were negative. The typical lesion at EUS is round, smooth, hypoechoic, located mostly in the pancreas. Neuroendocrine tumors of the pancreas are usually slow growing, indolent, and growth is still a major determinant of survival in these patients. Because they usually grow slowly, their metastatic potential is relatively low and there are specific criteria to predict their behavior (the distinction between benign and malignant tumors is based on the presence of metastasis), so long-term monitoring is necessary. Currently, the most important prognostic factor is the presence or absence of metastases. Even metastatic tumors grow slowly, the prognosis in these patients being relatively good compared with non-endocrine pancreatic neoplasms (27, 28).

10% (5.8-15%) of insulinomas are malignant and already present liver and locoregional lymph node metastases at diagnosis. In our study three patients with insulinoma had liver metastases (14.28%).

Treatment options for pancreatic neuroendocrine tumors require a multidisciplinary approach and depend on the presence or absence of metastases. Surgical resection is the only potentially curative approach. (29)

The main technical options available are: enucleation and pancreatic resections, the choice of either method being determined by location, size and number of tumors. Enucleation is generally preferred because it strictly removes the lesion without unnecessary sacrifice of healthy pancreatic tissue; in our study was performed in 7 of the 19 surgeries (30).

Intraoperative diagnosis was made by intraoperative palpation of tumor and/or intraoperative ultrasound. Intraoperative ultrasonography is valuable for locating small lesions and has an overall sensitivity of 80-100% (5), being more sensitive than all other preoperative imaging techniques and better than physical examination performed intraoperatively by the surgeon. In our case, intraoperative ultrasound combined with a manual exploration of the pancreas showed a diagnostic sensitivity of 94.11%.

Postoperative complications – transient hyperglycemia, anemia, transient pancreatitis, pancreatic pseudocyst, diabetes mellitus, exocrine pancreatic insufficiency, malabsorption, eventration – occurred in 8 of 17 patients (postoperative morbidity 47.05%).

In one case, the tumor was not found by intraoperative palpation, probably due to its deep location, but was easily detected by intraoperative ultrasound evaluation.

In metastatic disease the most important therapeutic goal is to reduce the life-threatening hypoglycemic syndrome. This can be achieved by surgical removal of metastases or a variety of other cytoreductive techniques, radiofrequency ablation, with good results. An active approach to patients with advanced disease may result in longer survival and prevention of hypoglycemia.

Medical treatment was used to prepare the surgical one aiming at reducing the frequency and severity of hypoglycemic symptoms in patients whose tumor could not be located with any imaging techniques and in the patient who refused surgery because of mild and rare symptoms and, not least, in patients whose surgery was not curative (palliative treatment).

Drugs used for this purpose were: Verapamil, Octreotide (Sandostatin) and Glucagon (glucagon). They were indicated by the severity of symptoms. General instructions were: diet, frequent small meals rich in carbohydrates, avoidance of high physical exertion, and also periodic medical examination. Their combination succeeded to control the clinical manifestations in patients in whom curative surgery was not possible as a solution (31).

Evolution was generally good, with 0% mortality, and curative interventions were followed by the disappearance of hypoglycemic symptoms, good clinical condition and weight loss. Unfortunately, there were some failures of surgery, especially when the location of the tumor has not exactly been made preoperatively or intraoperatively, more likely associated with metastatic disease.

CONCLUSIONS

The challenging aspect of treating insulinomas is localizing the tumors. Diagnosis has three steps: identifying the clinical syndrome, biological diagnosis with hormonal determination and topographic diagnosis. Due to nonspecific symptoms, a high index of suspicion is required. The supervised fast is the classic diagnostic test for insulinoma. Histopathological diagnosis is essential for diagnosis and therapeutic management. Native CT or CT with intravenous iodinated contrast material had low sensitivity in detecting insulinomas and liver metastases, but identified in our study, lymph node metastases. MRI is more sensitive than CT and can diagnose insulinomas with infracentimetric size; MRI is also a technique of choice for highlighting possible metastases in the liver. EUS can locate the tumors and describes the size, shape, contour, echostructure and relationship with adjacent structures. High resolution of EUS allows detection of lesions with very small diameter, is safe and minimally invasive. EUS is the imaging investigation of choice in preoperative diagnosis of insulinomas. Surgical resection remains the mainstay of treatment for patients with localized disease. Future studies must lead to further improving therapeutic options for patients with this disease.

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ORIGINAL ARTICLES

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Endoscopic faces of Helicobacter Pylori infection

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Abstract: *Introduction: The infection caused by H. pylori appears secondary after a bacterial colonization of the stomach and the initial portion of the small bowel. H. pylori –infected patients can develop gastritis, peptic ulcer, stomach cancer or MALT lymphoma. H. pylori infection is defined by WHO like a type I carcinogen, its role in gastric carcinogenesis being supported by the greatest researchers.*

Objectives: In this study our purpose was to determine the endoscopic appearances in H. pylori infection quoted in medical literature until now and the frequency of their appearance in our group of interest.

Materials and methods: In this study it was made an analytic study in which it was realized a retrospective cohort investigation at the Emergency Central Military and University Hospital "Dr. Carol Davila" Bucharest, gastroenterology branch –endoscopic department between 18.12.2012-21.08.2013 on 1694 patients between 18 and 92 years old, with the medium age of 55 years old.

As a diagnostic method for H. pylori infection we used superior digestive endoscopy during which were taken biopsies and it was made a fast urease test.

Results: Regarding the variation of the endoscopic aspects at the population of study, we have found gastritis with all its aspects (which was Sidney classified) in the biggest percentage meaning 59.3% of the cases, followed with a percentage of 18.8% by those without any endoscopic abnormality, and then in 10,33% of the cases we have found peptic ulcer. With a smaller percentage, under 10%, we have found duodenitis at 8.67% of this patients, and finally the most severe lesions represented by gastric cancer and lymphoma were found at 2,7% of the H.pylori infected patients.

INTRODUCTION

H. pylori is the most common infection found in humans, is a transmissible chronic infectious disease in which clinical manifestations are preceded by a long asymptomatic period. Helicobacter pylori is a gram-negative bacterium, the infection occurs due to bacterial colonization of the stomach and the initial portion of the small intestine and it is found on the luminal surface of the gastric epithelium. Was first isolated by Warren and Marshall in 1983.(1) In the entire world more than 50 % of the population have the infection into their upper gastrointestinal tract, the infection is found more often in the developing countries and with a significantly low percent in industrialized countries (2).

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The transmission routes in developing countries are largely represented by water, while in industrialized countries the transmission routes are directly, and the persons are infected from saliva, vomitus and others fluids.(3)(4)

Helicobacter pylori infection leads to development of gastrointestinal pathologies such as duodenal or gastric ulcers, many forms of gastritis, gastric cancers, and gastric mucosa-associated lymphoid-tissue (MALT) lymphoma. At the patients infected with Helicobacter pylori the risk of developing one of this pathologies varies widely in population, but a lot of the patients will not have any of this complications. (5)

By altering the normal gastric secretion, the bacterium produces local injury of the gastric mucosa especially at the antrum and distal part of the stomach. The most common pathology is gastritis and ulcers. At patients with adenocarcinoma, a known complication of Helicobacter pylori, the patients suffer a pangastritis with highly secretion of the body and antrum of stomach.(6)

The standard treatment for Helicobacter pylori includes PPI and three antibiotics which involves taking for 7 to 14 days and no single drug cures the infection. The proton pump inhibitors are decreasing the production of acid from the stomach and which allows the tissues to heal. Beside PPI also are recommended two antibiotics such as clarithromycin (500 mg, bid), amoxicillin (1 g, bid) and metronidazole. (7)

Unfortunately several clinical studies have shown that the triple therapy scheme from Europe the success rates are only 20-30% (10)(11). And therefore the detection of this bacteria can be realized in early stages and we can prevent various complications determined by it (especially gastric cancer) and use the treatment guides which were very well established within years has declined to the limit of 80% or less (8)(9) and in some countries

In this study our purpose was to determine the types of endoscopic aspects found in H. pylori infection quoted by now in medical literature and the frequency of their appearance in the group of study.

Materials and method

This study was realized between 18.12.2012-21.08.2013 on 1694 patients between 18 and 92 years old, with the medium age of 55 years old.

Inclusion criteria of endoscopic examined patients were represented by: patients with age over 18 years, unspecific digestive symptomatology to whom can associate alarm signals, the endoscopy indication made by the doctor, presence of a suggestive symptomatology for gastric or duodenal pathology, pain in the upper abdominal region or retrosternal, postprandial discomfort, distended bowel, early satiety, abdominal discomfort, pirosis, nausea, vomiting, anorexia, weight loss.

Exclusion criteria for this patients were similar with those for superior digestive endoscopy and are represented by: patients under 18 years , shock, acute myocardial infarction, peritonitis, acute perforation, fulminate colitis, recent colonic surgery, patient’s refuse, low cooperation of the patient, coma (except intubated patient), arrhythmias, acute myocardial ischemia.

In this study were included 1964 adults with age between 18-92 years old, as it is shown in table 1.

Table 1. Gender distribution of patients

Male	Female
866	828

As a diagnostic method of H. pylori infected patients, we used superior digestive endoscopy. Within the intervention were taken biopsies and were realized fast urease tests.

Fast urease test is based on the principle that excessive urease produced by H. pylori hydrolyses urea in ammonia. Therefore we have an increase of the pH of the environment in which we have introduced the bioptic fragment, determined with a colour indicator.

Although this test has a high specificity and sensibility, in general over 90%, the result seems to be influenced by certain drugs such as: PPI (proton pump inhibitors), antibiotics, H2 receptor antagonists and bismuth.

According to the large amount of data given by the medical literature, H. pylori infection produces various gastric and duodenal lesions with a low to high severity, and here we can mention gastritis, duodenitis, gastric and duodenal ulcer, gastric cancer and lymphoma.

In the group of patients with indication for superior digestive endoscopy, we have managed to visualize the endoscopic aspect mentioned up above, with a small mention that some of this patients had a normal endoscopic aspect.

Regarding those varied endoscopic aspects found in our group, we have determined that gastritis with all its aspects according to Sidney classification (described below) has the highest percentage of 59.3%, followed by normal endoscopic aspect with 18.8%, then we found ulcer in 10.33% of cases, duodenitis 8.67%, and finally the most severe- gastric cancer and lymphoma – only 2.7%, respectively 0.18% of the total examined group.

Figure 1. Distribution of endoscopic aspects found at endoscopic examined patients

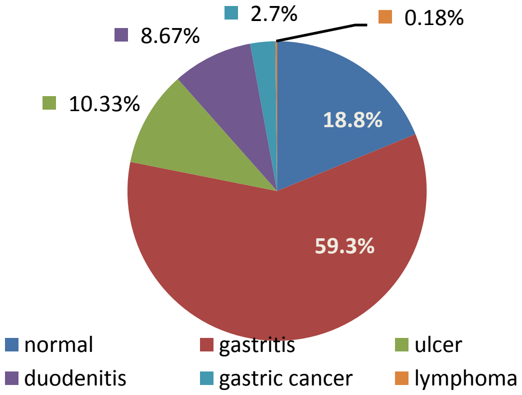

According to Sidney classification we determined the following types of gastritis: exudative-erythematous gastritis, macular-erosive gastritis, papular-erosive gastritis, atrophic gastritis, haemorrhaging gastritis, entero-gastric reflux gastritis and hypertrophic folds gastritis.

In all the endoscopic examined patients, from the total percentage of 59.3% that was represented by gastritis we have determined that the most frequent is exudative-erythematous gastritis, which represents better than a half with 57.8%.

The following as a frequency is represented by entero-gastric reflux gastritis with a percentage of 14% which was followed by papular-erosive gastritis, haemorrhaging gastritis, atrophic gastritis, and macular-erosive gastritis, with small differences between them, these representing 8%, 7, 27%, 6, 15%, respectively 5.35%.

Finally the less frequent was hypertrophic folds gastritis with a percentage of 14.45%.

Figure 2. Gastritis distribution according to Sidney classification of endoscopic examined patients

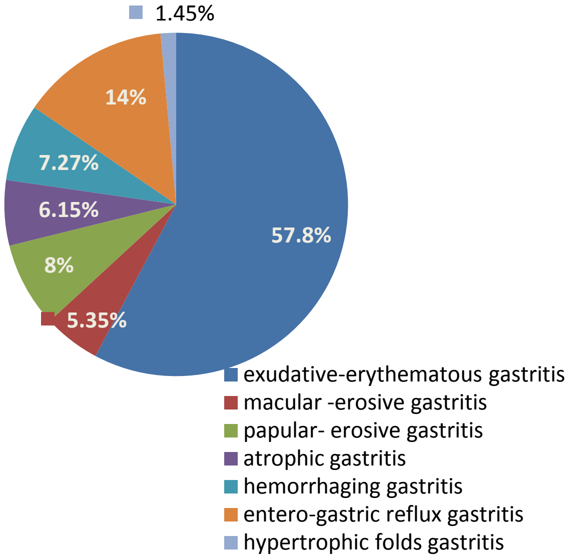

In the following figure we have presented gastritis distribution according to Sidney classification at the H. pylori-infected patients, and we determined that from all the cases with gastritis the most frequent was exudative-erythematous gastritis with a share of 61%. Must be mentioned here that 21.43% from all the cases with gastritis were infected with H.pylori.

The following places as a frequency, but still at pretty big difference from the first mentioned, were occupied by papular-erosive gastritis with 11.8%, macular-erosive gastritis with 10.62%, and entero-gastric with 6.7%. We can mention here that the percentage of these three determinations of gastritis are at the opposite pole from their situation in general examined population, where entero-gastric reflux gastritis has a bigger percentage than the others two. Now if we make a parallel between the occurrence of those gastritis types and the rate of occurrence in general population we can tell that 40.3% from the total macular-erosive gastritis cases and 30% from all the total popular-erosive gastritis found in general population appear in H. pylori infection, in contrast to entero-gastric reflux gastritis where only 9.7% from the cases occur in H. pylori infection.

Regarding haemorrhaging gastritis and atrophic gastritis, these have a smaller frequency of occurrence within the pale of infected patients, with a percentage of 4.72%, respectively 4.33%.

If we relate again at their distribution in general population we can observe that 14.3% from the total number of cases with atrophic gastritis is found in H. pylori infection. Haemorrhaging gastritis occurs in 13.2% of all the total haemorrhaging gastritis found in general population.

The smallest value as a frequency of occurrence in the H. pylori infected patients belongs to gastritis with hypertrophic folds with a percentage of 0.8% and we mention here that this value represents 11.11% from all the total cases of hypertrophic folds gastritis found at general population that was endoscopic examined.

Figure 3. Gastritis distribution according to Sidney classification of positive fast urease test patients.

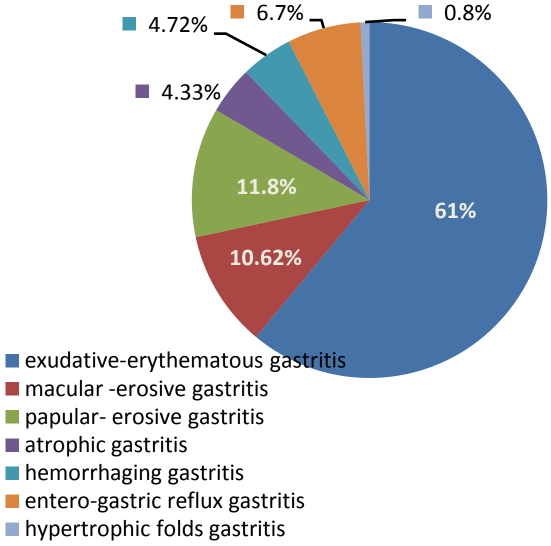

We observed at the persons in which was determined a positive fast urease test that the most frequent determination of H. pylori infection is represented by gastritis, which was found in 73.62% of the cases, a percentage up high as against general population where gastritis was determined in 59.3% of the cases. If we make a parallel of gastritis cases found at H. pylori infected patients and the cases found in general population we can observe that 20.3% of the gastritis cases occurred secondary to H. pylori infection.

The following two places as a frequency are occupied by ulcers and duodenitis with a small difference between them of only 0.3%, these two having the percentage of 11%, respectively 11.3%.

Figure 4. Distribution of endoscopic aspects found at patients with positive fast urease test.

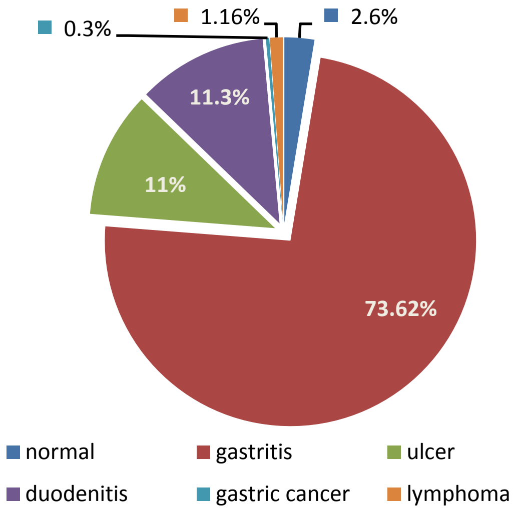

It is known that H. pylori infection can determine ulcerated lesions with gastric and duodenal localization.

From all the ulcerated lesions that were endoscopic discovered at the general examined population we determined that is a significant difference between these two types of localization, the one with the biggest percentage being the duodenal ulcer meaning 64.2%, in comparison with gastric ulcer which represents 35.8%.

In figure 6 was determined the distribution of ulcerated lesions at male gender with H. pylori infection where we can observe that three quarters from all the ulcer cases found at this group belongs to duodenal ulcer and the rest of 25% belongs to gastric ulcer.

Figure 5. Distribution of ulcer types according to its localization at endoscopic examined patients.

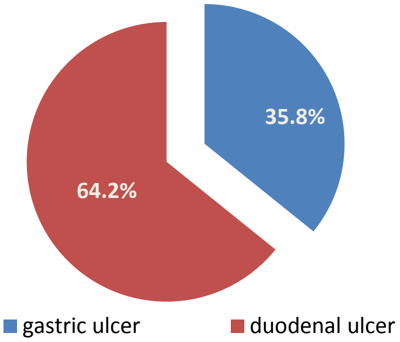

Figure 6. Distribution of ulcer types according to its localization at male gender patients with positive urease test

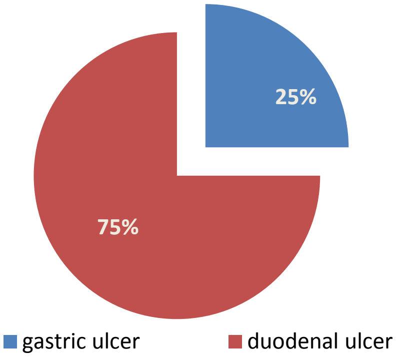

CONCLUSIONS

1. Helicobacter pylori was found in 52.22% of the cases tested for this infection and in 15.23% of the general population with superior digestive symptomatology.
2. Helicobacter pylori infection doesn't have gender predominance, being equivalent at both sexes.
3. In 97, 4% from Helicobacter pylori infected cases was determined a pathologic endoscopic aspect, the most frequent being gastritis with all its forms.
4. The most frequent gastritis type met in Helicobacter pylori infection was exudative-erythematous gastritis.
5. From all the ulcerated lesions met in Helicobacter pylori infection, the most frequent we have determined was at duodenal level.
6. Almost 20% from all the gastritis cases, ulcer and duodenitis were found in Helicobacter pylori infection.
7. All the lymphoma cases were found at Helicobacter pylori infected patients.

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CLINICAL PRACTICE

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Multidisciplinary approach to anemia

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Abstract: *Introduction: We present the case of a 65 years- old woman who was admitted with a severe macrocytic anemia Hb= 5.7g/dl and diffuse bone pain. Biologically she has moderate thrombocytopenia 35 000/µl, a hepatic cytolysis and cholestatic syndrome.*

Material and method: The patient was extensively evaluated before presentation for a mild iron - deficiency anemia for which she underwent endoscopic examination of the upper and lower gastrointestinal tract- normal. The bone marrow aspiration on admission revealed a marked hyperplasia of the erythroblastic line with ~50% basophilic erythroblasts suggesting a regenerative erythroid hyperplasia. These changes along with the marked reticulocytosis on the peripheral blood smear oriented us towards a hemolytic anemia; Folic acid, vitamin B12, autoimmune tests and hemolytic tests were all normal. We continued the investigations with a thoraco-abdomino-pelvic computed tomography which identified diffuse demineralization, vertebral compactation and pelvic stress fractures. The breast examination revealed a right breast nodule, but the breast ultrasonography pleaded for benignity. Lacking a clear definitive diagnosis we decided to perform a bone marrow biopsy.

Results: The osteo- medullary biopsy pointed towards a medullar invasion from a lobular mammary carcinoma; In these circumstances we performed an ultrasound guided biopsy of the right mammary lump thus histologically confirming a tumoral invasion of the bone marrow with subsequent anemia. The patient started chemotherapy in the Oncology ward.

Conclusion: The particularity of this case consists in the pattern of anemia, which initially seemed iron deficient and afterwards macrocytic – apparently hemolytic and was actually due to the tumoral medullar invasion and also the nonspecific ultrasonographic appearance of the breast tumor.

Keywords: *anemia, breast cancer, Evans syndrome*

INTRODUCTION

Anemia is a clinical feature in many emergency admissions to hospital. It is associated with increased mortality, morbidity and length of hospital stay in patients presenting with a range of conditions. If discovered in primary care it is seldom incompletely investigated. Although the most frequent cause of anemia is blood loss, as clinicians, we often find ourselves in front of complex and intricate causes which lead to a severe deficit. Therefore the management of such patients remains problematic. The prevalence of anemia under widely different conditions and the fact that anemia is managed by clinicians with a broad variety of backgrounds have fostered the need of multidisciplinary teams of experts (primary care physician, gastroenterologist, hematologist) to manage such cases.

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The most important aspect of treating anemia lies not in correcting it and discharging the patient when stabile, but in identifying the cause and reversing it in order to prevent recurrences.

CASE PRESENTATION

Disease history

We present the case of a female patient aged 65, living in the country area, whose history began in January 2015. She consulted her family physician for fatigability and after having a series of blood test taken was prescribed iron substitution for 4 months. She had microcytic hypochromic anemia – her hemoglobin was 9.1g/dl, MCV = 72.5 fL, MCH = 24.3 pg and low blood level of iron = 39 ug/dl. Her family physician also recommended some tumor markers (aFP, CEA, CA 19.9) which turned out to be negative.

The patient thoroughly followed the treatment, but at the end of the treatment, when she performed a control full blood count her hemoglobin level was lower. This is the moment when she was referred to us.

Gastroenterology admission

On admission she was pale, stable hemo- dynamically BP = 120/70 mmHg and HR = 90 bpm and alert, asthenic . She reported not loosing any blood and on rectal examination there were no signs of fresh blood or melena.

Her laboratory data showed anemia with hemoglobin 7.1 g/dl, MCV 110 fl, MCH 35.6pg, blood iron 155 micrograms/dl, platelets 39.000/µl. So we are now facing a macrocytic anemia with a normal iron level and associated thrombocytopenia. Peripheral smear shows macrocytosis, numerous polycromatophylic red cells agglutination, erythroblasts 8% and 7.9% reticulocytes, in conclusion hyperplasic marrow with pronounced erythropoiesis – regenerative erythroid hyperplasia.

Endoscopic examination were performed: upper endoscopy showed duodeno-gastric reflux and small diaphragmatic hernia and colonoscopy revealed hemmorhoidal disease with absent signs of bleeding.

After excluding a bleeding site and because the anemic syndrome was now macrocytic protein electrophoresis, folic acid and vitamin B12 levels were also performed and both were normal.

Because the patient was feeling worse and the hemoglobin was dropping despite blood transfusion and iron supplementation she was transferred to Hematology for further investigation.

Hematology admission

In addition to fatigability, after 4 days in the gastroenterology ward, the patient now reports bone pain, without loss of range of motion. The pain responds to usual analgesics. On examination she is stable and on physical examination we discover superficial bilateral axillary lymph nodes 1.5 cm diameter, mobile and non tender and a right mammary nodule on breast examination .

Laboratory test now shows a worsening anemia and thrombocytopenia with a hemoglobin of 5.7 g/dl, MCV 114 fl, platelets 35.000/µl, Iron 304 micrograms/dl and hepatocytolytic and cholestatic syndrome: AST 108 UI/l, ALT 45 UI/l, GGT 228 UI/l (N 7-50), alkaline phosphatase 180 UI/l (N 38-91 UI/l), LDH 1081 (N 266-500). Hepatic viral serology was negative for hepatic viruses B and C. Coombs test and cold agglutinins test were also negative.

Ultrasonography was also performed and showed fatty liver and normal sized spleen without abdominal adenopathy, the heart was normal. Soft tissue ultrasonography shows bilateral axillary lymph nodes with apossible non-inflammatory character and 1.5 cm diameter. We also identified smaller lymphadenopathy bilaterally in the cervical area, resembling the appearance. On breast examination we described fibromatous placard in the supero-external area of the right breast with the long axis parallel with the skin, 4a BIRADS classification.

The biological panel strongly resembled Evans syndrome on admission, so we immediately decided to start treatment with high doses of dexamethasone while continuing investigations.

The non-inflammatory character of the axillary adenopathies prompts searching for additional lymph nodes. Therefore tomography of the thorax and abdomen was performed and it identified diffuse demineralization of the scanned bones, numerous vertebral compression fractures and stress fractures of both iliac wings, during consolidation.

Figure 1. Right axillary lymph node, 1.48 cm diameter

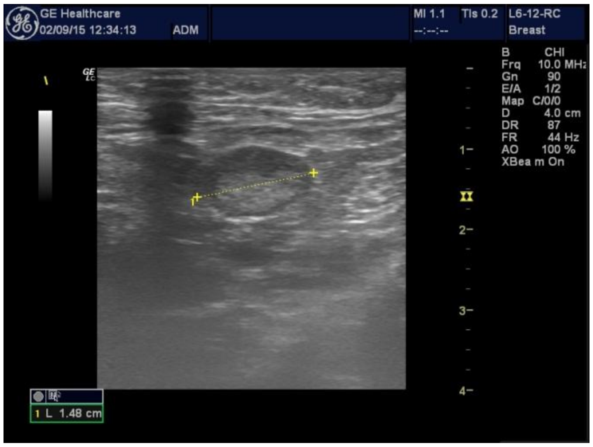
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Figure 2. Right breast nodule, 3.5 cm diameter

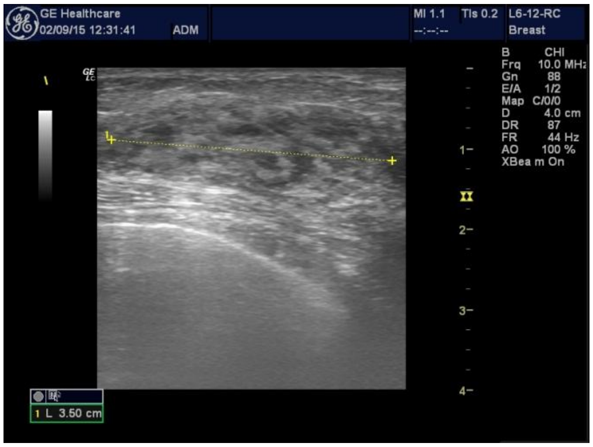
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Not having a definite diagnosis and because our patient was feeling worse and her hemoglobin was stationary despite treatment, we performed a diagnostic test.

A bone marrow biopsy was performed. Histologically and imuno-histochemically a metastatic lobular carcinoma of the breast was identified. Faced with a metastasis from a still not identified tumor, we returned to the fibromatous placard we saw in the right breast and an echo-guided biopsy of the nodule was performed. The biopsy revealed a lobular carcinoma strongly positive for estrogen receptors (ER) and negative for progesterone receptors (PR) and E-cadherin by immunohistochemical staining. The tumor marker was intensely elevated – CA 15-3 530 mg/dl.

Figure 3. Thoraco-abdominal CT showing diffuse demineralization and vertebral compression fracture

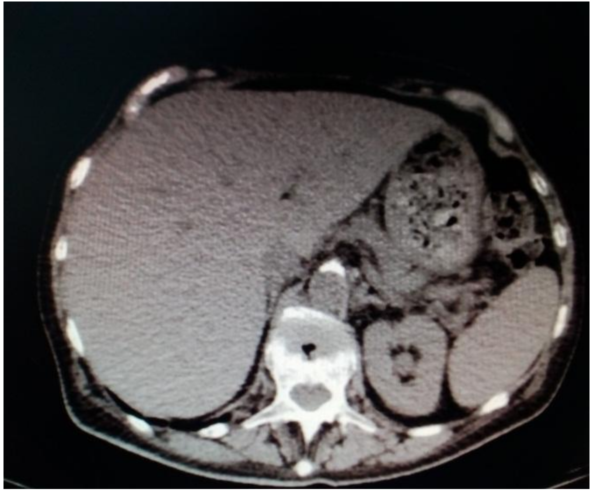

Figure 4: Whole body bone scintigraphy: low visualization of kidneys>> "super-scan" pattern pleading for secondary bone determinations with a possible paraneoplastic medullary impregnation syndrome

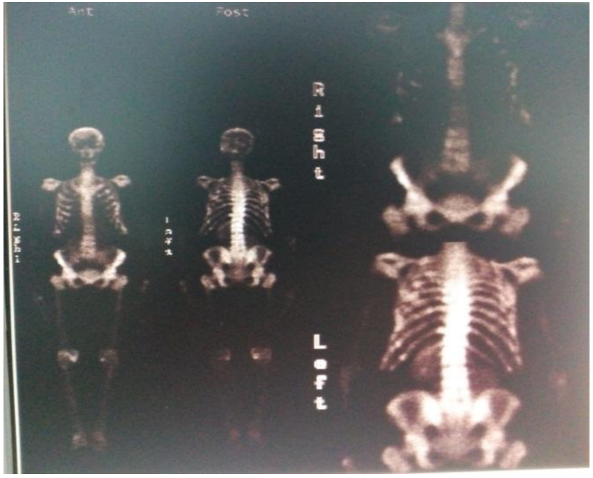
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Oncology admission

The patient was referred to oncology and she started chemotherapy with capecitabine and zoledronic acid after stabilization. The oncologist further investigated the implication of the bone system through a whole body bone scintigraphy. The result pleaded for multiple secondary bone determinations with a possible paraneoplastic medullary impregnation syndrome. Three months after initiation of appropriate systemic treatment for metastatic disease we see a partial recovery of hematopoietic function – her hemoglobin rose to 9 g/dl and platelets are around 90.000/µl .

COMMENTS

In conclusion, in front of a typical anemia that doesn't respond to typical treatment, as clinicians, we are obliged to search for additional causes and "cut the weed at the root".

The particularities of this case reside in the biochemical profile of the anemia – iron deficient at first encounter, then suggestive of Evans syndrome which delayed the diagnosis. In addition the ultrasonographic appearance of the breast tumor – more typical of a fibromatous degeneration did not raise suspicion at the first glance.

Breast cancer is the most common malignancy in women. Approximately 80% of women with metastatic breast cancer have skeletal metastases, which are often the result of bone marrow infiltration of malignant cells with subsequent progression and invasion of the skeletal cortex [1][2][3][4][5]. The most common complications of skeletal metastases are pathologic fractures, spinal cord compression as the result of vertebral compression fracture or extension of the tumor beyond the epidural space, and hypercalcemia [3][4][5]. Furthermore, skeletal metastases sometimes require surgery or radiation therapy to treat pain or an impending fracture. Bone marrow metastases result in the invasion and destruction of the bone tissue matrix by tumor cells [2]. Although bone marrow infiltration with micro metastasis is commonly present among breast cancer patients, total bone marrow infiltration – bone marrow carcinomatosis, BMC – resulting in profound pancytopenia is extremely rare.[2][6][7]

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Case report – hepatocytolytic syndrome hiding mesenteric venous ischemia

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BACKGROUND

Acute mesenteric ischemia (AMI) is a syndrome caused by inadequate blood flow through the mesenteric vessels, resulting in ischemia and eventual gangrene of the bowel wall. AMI may be classified as arterial or venous, non-occlusive or occlusive. The overall prevalence of AMI is 0.1% of all hospital admissions [1]. The exact prevalence of mesenteric venous ischemia (MVT) is not known, because many cases are presumed to be limited in symptomatology and to resolve spontaneously.

CASE PRESENTATION

A 70 years old male patient presented to our gastroenterology department for investigating an acute hepatocytolytic syndrome (AST 297 UI/ml, ALT 616 UI/ml). He was non-smoker, with no history of hepatotoxic treatment or other toxic substance abuse. One month before he had normal liver enzymes at clinical tests (AST 50 UI/). He was asymptomatic on admission, with unremarkable physical exam: except sensitive abdomen in the right hypochondrium, moderate hepatomegaly (left liver lobe hypertrophy) and abdominal postsurgical scars. His blood pressure was 110/70 mmHg with no orthostatic changes. A regular heart rate of 90 beats/min and respirations of 18-20/min were observed.

From the patient history: he was operated six years ago for abdominal aortic aneurysm with reimplantation of both renal artery after aortic graft, plus aorto-iliac bilateral stent, he has diabetes mellitus type 2 (on oral antidiabetic drugs from 2008), hypertension, ischaemic heart disease, systemic atheromatosis, laparoscopic colecistectomy in 2011 and right coxartrosis. A control angiogram and Doppler ultrasound was performed six months ago, with no evidence of occlusion or blockage of the mesenteric arteries.

At presentation the patient had hepatocitolytic syndrome – elevated serum transaminases ALT 537 UI/L ; AST 297 UI/L, cholestatic syndrome – elevated alkaline phosphatase ALP 124 UI/L, no modification on hemograme, hyperglycemia 190 mg/dl with HbA1c 6.8 %, serum creatinine 1.3 mg/dl. We tested the patient for virus B and C infection, and we found Ac anti HVC positive. Then we performed HCV RNA viral load 115 UI/ml (low viral load) and supportive hepatic treatment was immediate conducted. On abdominal ultrasound we found: mild hepatomegaly, post-colecistectomy status, normal CBP and a left renal

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cyst.

After a few days the patient accused upper abdominal pain, nausea, fatigue, vomiting, constipation and oliguria. The blood pressure was 140/80 mmHg, the abdomen was symmetrically distended, dull to percution with absent bowel sounds with firm and tender to palpation in the mid-epigastrium. We did an upper GI endoscopy that revealed hiatal hernia, diabetic gastroparesis and duodenitis (figures 1a, 1b)

Figures 1 (a, b).

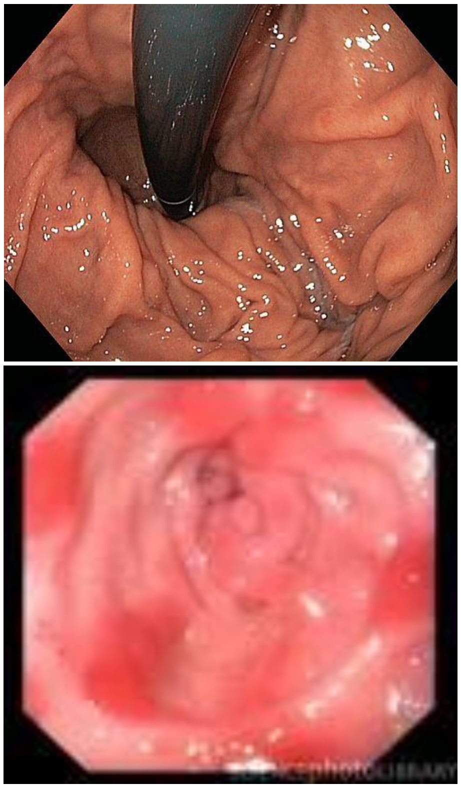

The laboratory tests showed normalization of liver enzymes, but rapid increase of urea 270 mg/dl and creatinine 4.88 mg/dl needing temporary hemodialysis in ICU, by femoral venous catheter placement. On abdominal examination, the patient had persistent abdominal pain, tenderness to palpation and increased abdominal wall rigidity. The native CT scan performed immediately (due to renal function impairment) did not confirmed arterial mesenteric ischemia suspicion, and also the biological markers D-dimers, CK, CK-MB where negative, with no signs of hypovolemia or hypotension.

After the temporary hemodialysis, another CT scan was performed, this time with contrast, because the suspicion of mesenteric ischemia remains, the patient had signs of peritonitis (right inguinal fossa pain and tenderness, exacerbated by moving the peritoneum, coughing and the Blumberg sign positive) and on ultrasound he has periappendiceal fluid collection and intestinal wall thickening. The contrast enhanced CT scan revealed: terminal ileum wall oedema, suggestive for acute mesenteric vein ischaemia, without lacunar imagine of a possible thrombus (arterial or venous) – figures 2a, 2b.

Figures 2 (a, b).

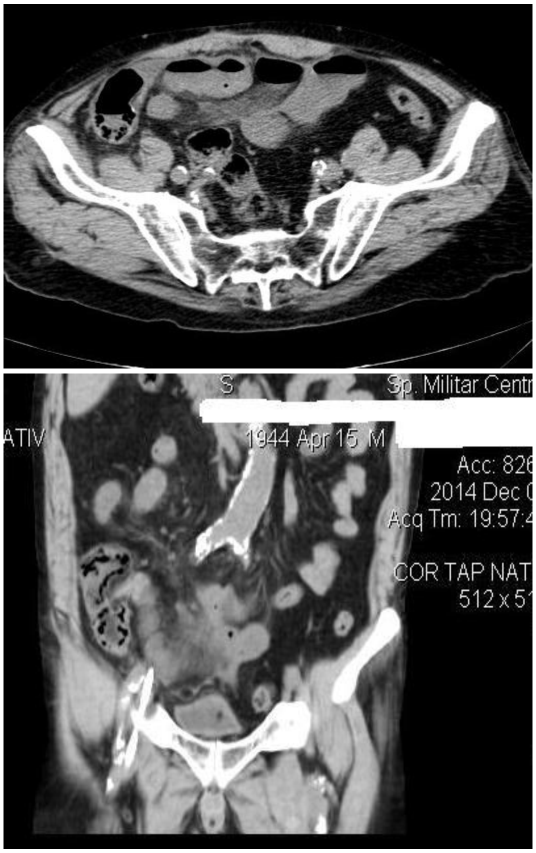

Initially the surgical intervention was temporized and the patient had conservatory heparinotherapy for 10 days (taking into account the patient comorbidities, history and risk factors for thrombosis), but the patient general status did not improved, he had nausea and vomiting, also persistent abdominal pain, fever and leucocytosis. We took blood cultures, remove central venous catheter and femoral venous catheters and continued antibiotherapy.

We did another CT scan with contrast one week later that showed distal ileum thickening with iodophile walls in arterial phase and necrosis, plus entero-enteral fistula localized in the right inguinal fossa (figures 3a, 3b, 4a, 4b).

Figures 3 (a, b).

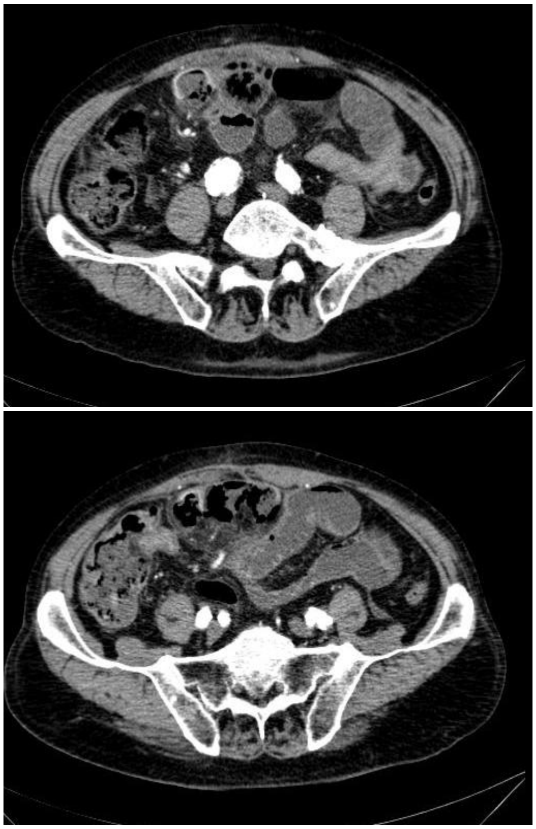

The treatment included both surgical and anti-thrombotic therapy and we transferred the patient to the surgical department. A median laparotomy was performed, and mesenteric venous ischemia modifications were found at 5 cm from ileocecal valve, with entero-enteral fistula and cecal abscess. A large enterectomy was made, with resection of approximately 150 cm of the ileum (figure 5), followed by peritoneal cavity lavage and drainage, then protective right ileostomy. The postoperative evolution was favorable, and the patient was released with normal liver and renal function, with protective ileostomy. At three months the patient came in good clinical status, and ileostomy reversal was made. Further investigations are needed for this patient regarding hypercoagulability status or identifying other MVT risk factors, and of course close follow up for prompt diagnosis and treatment of recurrence.

Figures 4 (a, b).

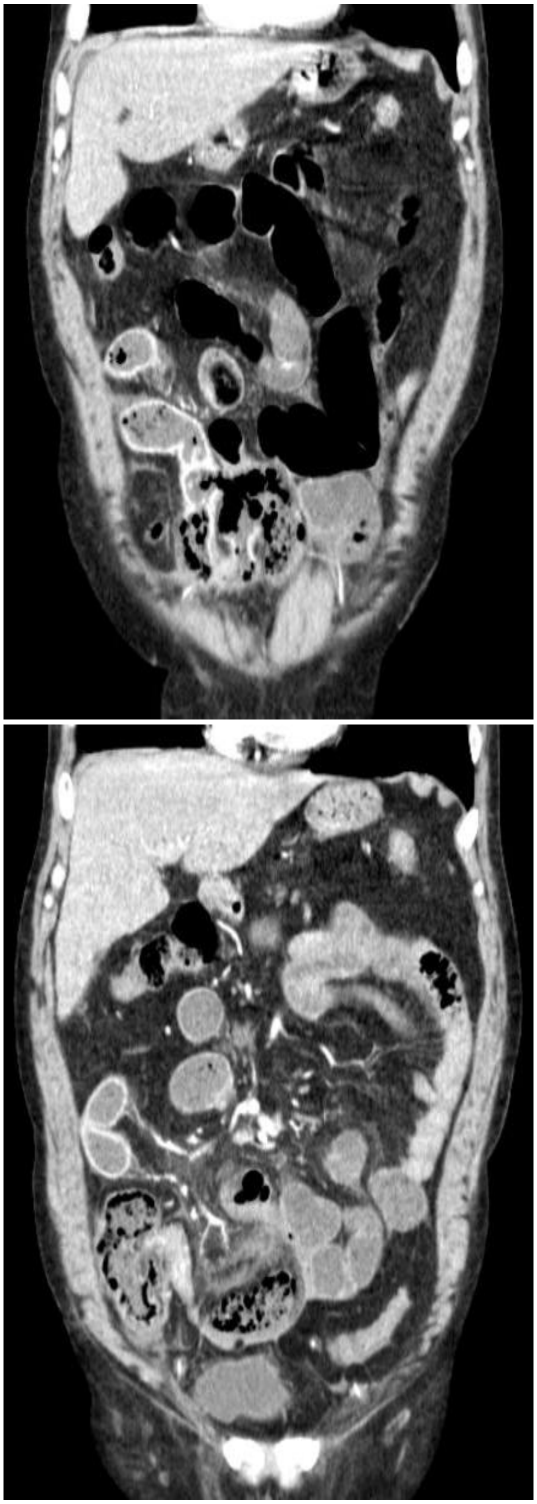

Figures 5.

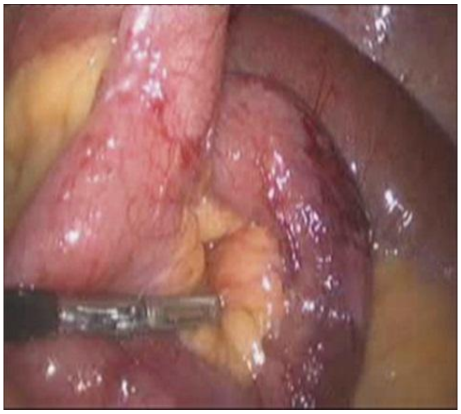

DISCUSSION

Mesenteric ischemia is caused by a reduction in intestinal blood flow, due to occlusion, vasospasm and/or hypoperfusion of the mesenteric vessels. The clinical consequences can be catastrophic, including sepsis, bowel infarction, and death, making rapid diagnosis and treatment imperative.

Injury severity is inversely proportional to the mesenteric blood flow and is influenced by the number of vessels involved, systemic mean blood pressure, duration of ischemia, and collateral circulation [1]. Mesenteric ischemia may be classified acute and chronic, based upon the rapidity and the degree to which blood flow is compromised.

Acute mesenteric ischemia refers to the sudden onset of intestinal hypoperfusion, which can be due to occlusive or nonocclusive obstruction of arterial or venous blood flow. Nonocclusive arterial hypoperfusion is most commonly due to primary splanchnic vasoconstriction [2].

Chronic mesenteric ischemia (also called intestinal angina) refers to episodic or constant intestinal hypoperfusion, which usually develops in patients with mesenteric atherosclerotic disease [2].

Venous thrombosis is predominantly a result of stagnation of blood flow, vascular injury, and hypercoagulability (ie, Virchow's triad) [3]. First differentiated from arterial causes of acute mesenteric ischemia 75 years ago, acute mesenteric venous thrombosis (MVT) is an uncommon disorder with non-specific signs and symptoms, the diagnosis of which requires a high suspicion rate [4].

Mesenteric vein thrombosis almost always involves the distal small intestine (superior mesenteric venous drainage) and rarely involves the colon (inferior mesenteric venous drainage) [5]. The anatomic site of involvement in acute mesenteric venous thrombosis is most often ileum or jejunum, followed by colon and duodenum [6].

Acute thrombotic occlusion of one or more mesenteric veins reduces perfusion pressure due to increased resistance in the mesenteric venous bed. As flow stagnates, increased venous pressure leads to efflux of fluid into the tissues, causing profound bowel-wall edema, which can lead to submucosal hemorrhage. If the venous arcades and vasa recta are involved and venous return from the bowel wall is completely occluded, bowel infarction will occur [4].

Causes of MVT include the following (>80% of patients with MVT are found to have predisposing conditions):

- Hypercoagulability [7] from protein C and S deficiency [8, 9] antithrombin III deficiency, dysfibrinogenemia, abnormal plasminogen, polycythemia vera (most common), thrombocytosis, sickle cell disease, factor V Leiden mutation, pregnancy, and oral contraceptive use [10, 11]
- Tumor causing venous compression or hypercoagulability (paraneoplastic syndrome)
- Infection, usually intra-abdominal (eg, appendicitis, diverticulitis, or abscess)
- Venous congestion from cirrhosis (portal hypertension)
- Venous trauma from accidents or surgery, [12, 13] especially portacaval surgery
- Increased intra-abdominal pressure from pneumoperitoneum during laparoscopic surgery [12]
- Pancreatitis
- Decompression sickness

The diagnosis is most often made by contrast-enhanced computed tomography, though angiography and exploratory surgery still have important diagnostic as well as therapeutic roles [4]. CT findings of intestinal ischemia due to acute MVT include wall thickening > 3 mm in an adequately distended segment, abnormal enhancement patterns, distended luminal diameter, thickened mesentery, indistinct bowel wall margins, and the presence of new or unexplained ascites [14].

Thus, abdominal CT with adequate venous phase contrast should be used as the initial diagnostic test of choice for MVT, with angiography reserved for patients with non-diagnostic CT results yet a reasonable clinical suspicion for MVT (i.e. no alternate explanation for symptoms and either a known hypercoagulable risk factor or family history of venous thrombosis)[4].

Anticoagulation prevents clot propagation and is associated with decreased recurrence and mortality [4]. Immediate heparinization is currently accepted as standard therapy. Heparin has been shown to prevent recurrence of thrombosis after intestinal resection and to be associated with lower mortality when recurrence does occur [4].

When intestinal infarction is suspected by virtue of clinical decompensation or radiologic/CT findings, immediate exploration of the abdomen – either by laparotomy or laparoscopy is needed and non-viable segments of intestine are resected.

CONCLUSION

MVT is associated with a 30-day mortality of 13-15%. Without anticoagulant therapy, mortality increases, with a 25% recurrence rate. The combination of anticoagulant therapy with surgery is associated with the lowest recurrence rate (~3-5%)[1]. Although its mortality rate has fallen over time, acute MVT remains a life-threatening condition requiring rapid diagnosis and aggressive management.

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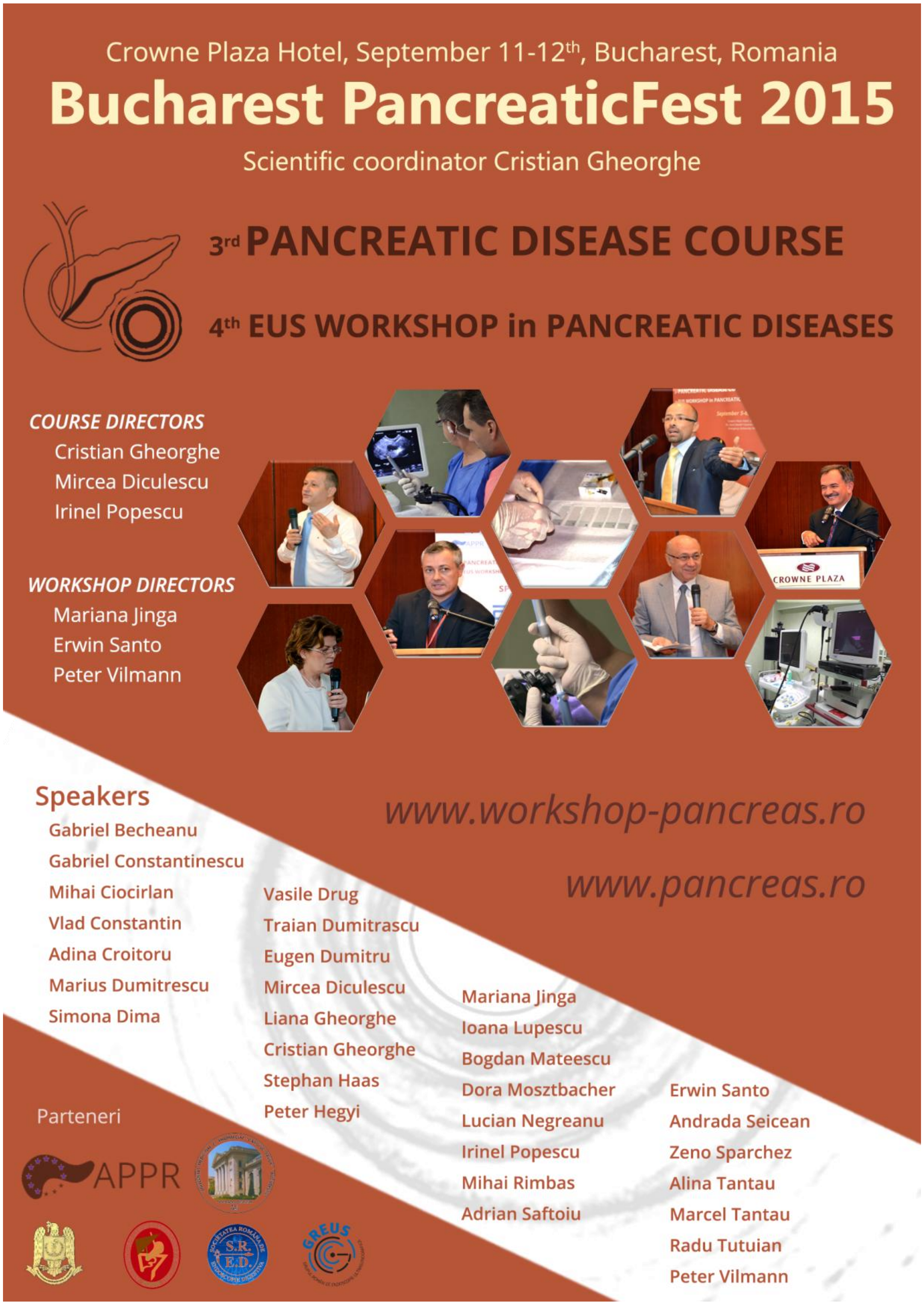

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We look forward to your submission.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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ETHICAL CONSIDERATIONS

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

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Acknowledgments

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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EDITORIAL

About the beauty of urology…

Dan Mischianu

Gral (R) Prof DAN MISCHIANU

Chief of 3rd Clinical Department,
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Medicine and Pharmacy,
Bucharest, Romania

Urologic Surgery is one of the most beautiful, exciting, spectacular and unpredictable surgical specialties.

From 1907 it stands as a specialty of it's own, breaking free from general surgery, as other surgical specialties, Professor Felix Guyon being the godfather of this specialty. In the last 20 years, urology itself has become a "tree" of sub-specialties, from which medical branches such as andrology, urogynecology, oncological urology have developed. These medical strains tend to become independent.

From 1909 urology begins to be recognized in Romania, as a specialty of it's own, time when Professor Petre Herescu, Guyon's student, establishes this speciality within Coltea Hospital. Over time it has experienced a steady development as few specialties have had. Over the last century, romanian urology has given world renowned characters, which have settled romanian urology in a place of honor.

We have the strength to affirm that urology is "a friendly specialty", not only with the surgical specialties, but also with other medical specialties. There are many surgical and non-surgical specialties, whom we as urologists can offer our help, or an advise, or a surgical procedure. Urology can have harmonious collaborations with many other specialties, and I give as example, the selective arterial embolization after PNL or arterial stenting in erectile dysfunction, procedures which are performed by interventional radiologist.

Like in any other surgical specialty, the urologist has two important "friends", with whom the collaboration must be at the highest level. I refer of course to the anesthesiologist and to the pathologist.

Urologic surgery faces or may face urgent situations, emergencies which if not noticed in time and skillfully managed it could have a poorly outcome for the patient. Among these situations, we give as an example just a few: obstructive anuric acute renal failure, urosepsis, urological trauma with hemodynamic instability etc.

We haven't taken into account the postoperative hematuria, on one hand due to urological diseases, but also secondary to various other conditions, such as an eventual hepatic cirrhosis or secondary to an overdose or misuse of anticoagulants, out of which we are almost convinced that it is a practice at "national" level at the indications of the anesthesiologists.

These pathologies mentioned above are just a few aspects of what it represents the "salt and pepper", but also the challenges of an urologist's life.

While the general surgeon performs, with difficulty, for example a retrocecal appendectomy and waits with some concern for the 5th day peritonitis, the urologist may be in the situation, in which he must promptly intervene at 2-3 hours after the patients admission or after a correctly performed intervention, to stop a bleeding which may occur without any explanation or for an obstructed drainage.

I have discovered slowly and quite late the charm of urology. Later I have learned that it is a surgical specialty much better encoded than abdominal surgery. Urology has an ethiopathogenic and topographic diagnosis more accurate and much more faster. I have later discovered it's minimally invasive valences regarding diagnosis and treatment, for example the ability to perform endoscopic surgery, laparoscopic and robotic procedures, with the possibility of "one day surgery" hospitalization and with maximum results for the patient. In this context it should be noted that robotic surgery, very popular nowadays, was initially born in the specialty of urology, for one purely urologic intervention, the radical prostatectomy.

Certainly the technological developments have greatly influenced the evolution of urology. There have been no new surgical devices that couldn't be used in urology. Nowadays the classical interventions, have come to have a smaller proportion compared with those listed above. For instance, regarding urinary lithiasis, only 1% of cases are treated using open surgery.

I do not know how urology will look over a few decades. Perhaps in the future and one not so distant, the young residents that I try to teach and mold, will operate Earthlings on another planets, using consoles such as the "Da Vinci " from now...

Finally, without having a morbid curiosity, I am satisfied with what I have seen, realized and undertook during my active urological lifetime!

REVIEW ARTICLE

Article received on September 12, 2015 and accepted for publishing on October 21 2015.

Minimally invasive surgical techniques – robotic surgery

Florin Săvulescu, Costin Duţu, Cristian Cârlan

Da Vinci robotic surgical platform is the result of many years of research of the military physicians from United States Army. This platform was initially intended to operate in close proximity to the battlefield, it was supposed to allow for complex surgeries without threatening medical personnel, but also to ensure a high survival rate among the wounded. Basically, after a soldier was wounded, he was supposed to be taken away from the front line, transported to the first sanitary structure which had this platform available and a surgical team from long distance, satellite connected, performed the surgery. The advantages of this course of action are related to the fact that the team is permanently safe and the injured person benefits from the experience of a well-trained surgical team.

Due to the large amount of information transmitted between platform components, which means millions of operations performed each second, and the impossibility to secure a channel for information dissemination, even more difficult in a hostile environment such as in the vicinity of the front line, and the long preparation of a patient to be subjected to a robotic surgery, the platform is not in current use of military doctors, the way it was originally intended. So far, only one surgery was performed in which the operator was far away from the patient. The two surgical platform elements were arranged one in the United States and one in France. During the surgery, the transfer of information was made by satellite, with great efforts to secure the information channel, as well as with the suspension of any satellite phone links between the two countries.

The advantages desired by military doctors have proved to be far from being put into practice, however, their research work led to the development of the best technology that allows for minimally invasive surgery. In 2015, worldwide, about 3,400 platforms daVinci were installed: 2295 in the United States of America, 573 in Europe and 7 in Romania.

Robotic surgical platform consists of several components which will be described below. Using the system is only possible if all three components are available, none of them is optional. Although there are three components, they are interconnected and have the same purpose: to allow for a minimally invasive surgery in maximum security conditions for the patient. The system combines technological advantages: high resolution three-dimensional image, video image stability and filtering of surgeons' hands unwanted movements, with the surgeons' experience and ability to make decisions in real time.

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Operator surgeon’s console consists of three parts. The video system allows viewing intracorporeal images in a tridimensional manner thanks to the two images submitted separately for each eye; here, there is also a number of sensors that detect the presence of the surgeon at the console, this thing is necessary as a safety measure to block chaotic motion of the device arms inside the body if the operator is absent. The second component of the console is represented by the console commands for each hand, called "master controls" and a series of commands for adjusting an ergonomic position. This component records the surgeon's hand movements allowing this information to go to the working forceps. The pedals system is the last component of the console, here it is possible to activate several functions for the working tools: electric coagulation or sectioning, handling video camera, change the working arm: the surgical system has three arms and it is necessary to change one tool with another for a certain hand. The whole assembly allows adjustment on more axes of all the components, depending on the preferences of the operator, the scope is to obtain an ergonomic position. If more surgeons use the equipment, the system stores each surgeon’s preference, and when the personal account is activated, it recreates the ergonomic position preferred by each surgeon.

Figure 1. The working console on which one can notice the three subcomponents from top to bottom: video playback system, master control, crank

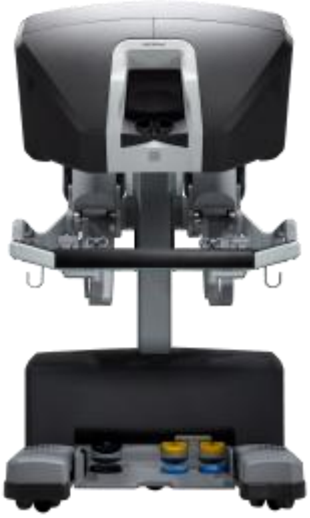

Patient's charter is like a tower which has three working arms attached and a video camera arm. This charter, as its name implies, is beside the operating table. On each working arm, a certain tool is fixed and it is activated by a pulley system that allows movements in the seven degrees of freedom. Patient's charter only performs commands coming from the surgeon’s console, it has no autonomy to make decisions, it can not be programmed to execute movements without commands in real time. Fixing / replacing instruments is performed by a surgeon who is next to the patient, he is also the one who places all working cannula into the patient’s body. The working tools have a diameter of 8 mm and reproduce, in miniature, the instruments used in open surgery.

Figure 2. Patient's charter with the three working arms for tools and the video camera arm.

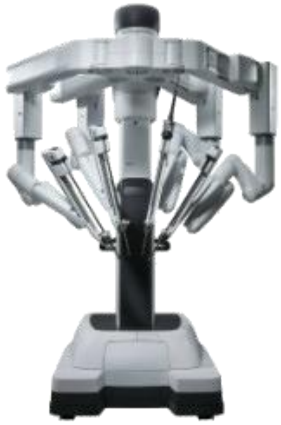

Figure 3. Working tools

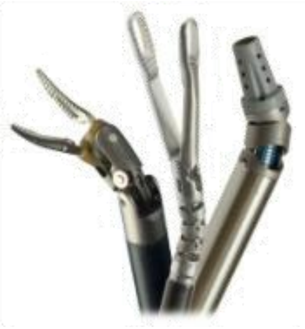

The video system displays intracorporeal images on bidimensional monitors in the operating room. This system allows recording of images and setting of certain parameters. Here lies the source of light transmitted to the intracorporeal video camera through an optical fiber. All components described above are dependent on the presence of at least two surgeons, the system is not designed to fulfill the role of the surgeon but to support surgery by involving the present technological advantages.

Figure 4. Video tower and bidimensional monitor of high resolution

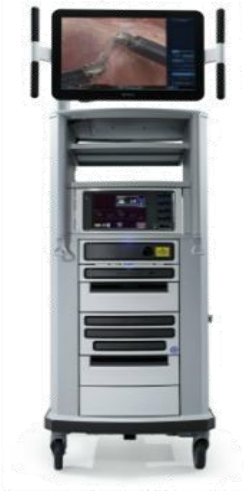

The three systems are interconnected by optical fiber since the information flow is very high.

Robotics is a minimally invasive technique, similar to laparoscopic surgery, in which working tools are inserted through small incisions of 5-15 mm in the peritoneal cavity through which cannula are introduced to work. Comparative studies between open surgery and robotic surgery have highlighted the following advantages in favor of robotic surgery: shorter stay in hospital, reduced bleeding, less postoperative pain, faster social reinsertion, small incisions that allow a much quicker recovery and lower risk of developing eventrations. Patients who have no indication for minimally invasive surgery, do not have indication for robotic surgery either.

The recommendations of robotic surgery are the surgeries that require access to limited spaces, inaccessible for open surgery and restricted by the small maneuverability of laparoscopy instruments. In urology, robotic prostatectomy is considered as the "gold standard". As a general idea, robotic surgery approach is indicated for the organs situated at the top and bottom pole of the peritoneal cavity, where limited space and difficult access are not impediments for daVinci surgical system instruments.

Complications can occur in robotic surgery just as it happens with any surgery, the maximum risk is death. Complications that may arise during surgery are: intraperitoneal organs injury, intraoperative bleeding, septic complications. Another factor to be taken into consideration is the human error, system failures or potential complications of anesthesia.

At this time, da Vinci surgical platform is the most advanced tool that allows a minimally invasive surgery. A very important element about the functioning of this system is that it can not "operate" independently; it cannot be programmed to carry out independent actions. It must be seen only as an extension of the arms of the surgical team members, executing commands coming from the surgeon at the working console, under the supervision of at least one surgeon.

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SYSTEMATIC REVIEW

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Surgical team and surgical communication

Costin Duţu[1], A. Luchian[1], Elena Stârcu[1], Florin Săvulescu[1]

A significant percentage (43%) of medical errors is caused by errors in communication between healthcare professionals or between them and the patient. Today the surgeon has a multiple role (leader of the surgical team, member of the medical team, scientific role, management role)(1).

The surgical team has the duty to ensure and promote a positive work environment that improves team performance and maximizes outcomes for patient's safety (2).

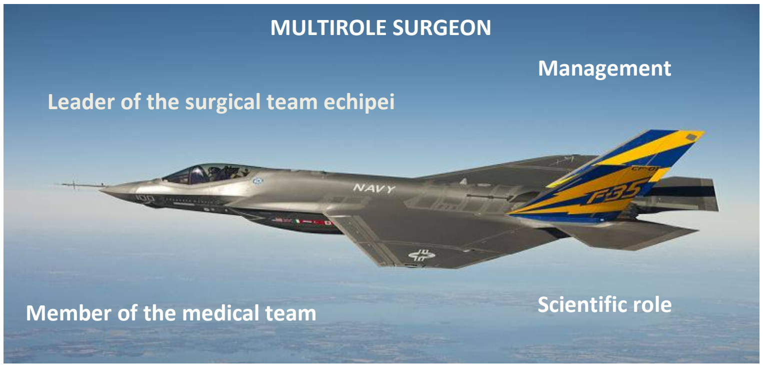
MULTIROLE SURGEON
Management
Leader of the surgical team echipei
Member of the medical team
Scientific role

The most important attributes, that are critical to the development of high performance teams, are membership and leadership.

Membership presupposes the following (6):

- To understand the role and responsibilities of the teammates
- To encourage input from all members (young people!)
- To respect the role and competence of other specialties colleagues
- To respect team leaders
- To share the objective of high quality in patient treatment
- To trust the right to speak and to intervene
- Commitment to teamwork for the patient's interest

Leadership comes with a clearly defined role, especially in critical situations, which implies a style that minimizes the difference in status and power, suitable under particular conditions, designed to seek permanent information from team members and make decisions based on these (6).

Effective communication in the surgical team should be:

- Complete
- Clear – fully understood information
- Short – concise and organized information
- Relevant (timely)

In order of most important characteristics, surgical team members should feel engaged and involved in a common goal, together with mutual trust and respect (2).

In a safe interpersonal environment, team members should not hesitate to express their views, to challenge each other positively, to raise certain issues without fear of ridiculous or subtle attacks. They must also feel safe to discuss errors and mistakes (5).

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Table 1. Behavior strategies which influence the performance of the surgical team (7)

Positive attitudes	Negative attitudes
Listening to and exploring others' opinions	Aggressive challenges
Clarification of ideas; Requests for alternative explanations, thoughts or opinions	Distrust of alternative ideas
Supporting and encouraging members of the surgical team	Sarcastic behavior
Challenge of colleagues in a respectful manner regardless of their status	Ignore, dismiss, mock at other points of view
Disputes resolution in a constructive way by explaining the decisions that were made in a fair manner	Assuming to know the absolute truth due to position or knowledge
Appreciation, recognition of merits, contributions from others	Lack of appreciation and regular criticism without recognition of positive contributions
Search and accept feedback (positive or negative) regarding one's contribution and performance	Feedback ignorance and disregard
Own assessment and evaluation of the performance and contribution within the team	

Team STEPPS - team actions (4).

1. BRIEF: short session before surgery, discussing the team members, the role of each team member, expectations setting, discussing potential problems, anticipate unexpected things

2. HUDDLE: ad hoc session to restore certain conditions, situations, plans

3. DEBRIEF: Discussion performed after surgery, meant to improve team performance - aiming at understanding the role and responsibilities of each team member; surgical equipment problems; other improvements to the surgical procedure; lessons to be learned further.

Within a team, an important thing is the coordination of tasks leading to a better awareness of each individual performance and also of the team performance, meant to encourage the members' desire to offer support to the others, thereby increasing the ability of the team to remain flexible and adaptable to changing circumstances (3).

A simple way to review and reflect on performance is to answer the following questions:
- What did I do well?
- What could be done better?
- What should we not do anymore?
- What should we continue to do?

Table 2. Errors observed in operating rooms classified by behavior categories (8)

Behavior category	Errors
Communications/decisions	The surgeon does not provide enough information to the anesthetist
Preparation/planning/ vigilance	The inability to predict certain events during a complex procedure; Failure to monitor other activities of the team
Information dissemination	Failure to inform own team
Inquiries/statements	Failure to comply with the conclusion of a discussion on an alternative procedure
Interpersonal relations/ group climate	Hostility and frustration due to poor team coordination
Auto criticism	Inability to learn from certain situations
Disputes management	Disputes between doctors from the surgery team and anesthetists not solved.

Figure 1. The structure of a performing team (9)

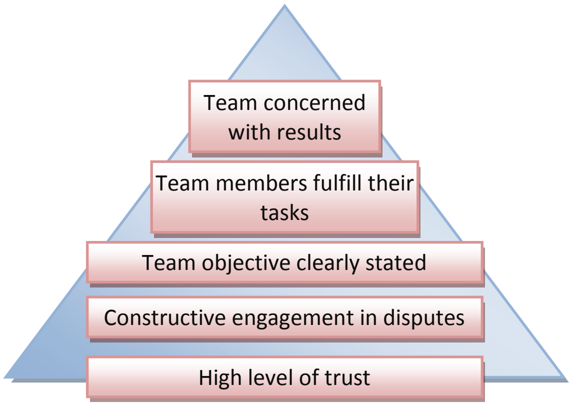
Team concerned with results
Team members fulfill their tasks
Team objective clearly stated
Constructive engagement in disputes
High level of trust

CONCLUSION

Communication skills are a key component in surgical practice, which can be taught, learned, improved and this will maximize performance and enjoyment in clinical practice.

Interprofessional communication problems are the leading cause of many medical errors.

Structured communication protocols such as SBAR are designed for effective and complete communication (4).

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SYSTEMATIC REVIEW

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Clostridium difficile – an emerging plague

D. Spînu[1], Ovidiu Bratu[1,2], R. Popescu[1], D. Marcu[1], A. Rădulescu[1], Dan Mischianu[1,2]

Abstract: Introduction: Clostridium difficile infection stands nowadays as one of the major emerging health problems still underestimated. Only in 2009 the European Society of Clinical Microbiology and Infection (ESCMID) was able to publish guidelines for this serious disease. Actually the guidelines were updated in 2013 so we can speak of a united action towards the resolution of this healthcare problem.

Methods: This is a review article aiming to shed light in various aspects of clostridium difficile infection as clinical presentation, symptoms and signs, therapeutic options and risk factors with a focus on urologic pathology.

Conclusions: This type of infection represents a challenge from many points of view like treatment, relapse approach, mortality, morbidity, germ' s adaptability to treatment and last but not the least the outbreak of different aggressive strains like B1,NAP1 or ribotype 027 toxinotype III.

Keywords: Clostridium difficile infection, treatment, diarrhea

INTRODUCTION

C difficile was first described in 1935 by Hall and O'Toole as a Gram positive anaerobic commensal rod[1]. It took 43 years to discover the C difficile toxin in patients with pseudomembranous colitis.

The classic strains release two toxins A and B both of them monoglycosyltransferases causing colonic tissue damage. The name Difficile was given because of the the difficulty they had culturing this anaerobic bacterium on conventional media.

The interesting part is that from a nosocomial infection nowadays it appears to have spread to communities and what is more problematic to antepartum period, pregnant women and children[2].

The problem with this pathogen was that it evolved. In the early 2000 there was an outbreak in North America and Europe with a new strain, the ribotype 027 strain which has evolved and adapted. Metronidazole resistance was higher; the strain had modified genetic structure permitting to produce much more toxins than the classic types and incorporated another binary toxin which role is still unknown[3,4]. First time discovered in 1980s it presented high resistance to fluoroquinolones.

METHODS

A comprehensive search using PubMed, Medline and Google Scholar without publication status or other restrictions was conducted.

Studies using comparaisons between different antibiotics, reviews of risk factors, case presentations and all sorts of guidelines were included. The main

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objective of this paper was to review the main features and also to raise awareness of this health problem.

RESULTS

Diagnosis

Diagnosis is based on: clinical presentation, diagnostic tests, imaging and miniinvasive surgery.

The classical clinical exam reveals usually diarrhea in a patient with current or recent antibiotic use, frequent loose or watery stools. Occult blood or mucus may appear but melena or hematochezia are rare. Sometimes patients can develop colonic ileus or toxic megacolon. Both of them are very serious and unfortunately because of the ileus there can be no diarrhea so there can be a fatal delay in diagnosis. In this case diffuse abdominal pain, fever, abdominal distension are precious clinical signs. Finally there are those patients with very serious signs like septic shock or multiple organ failure.

All diagnostic tests have their strengths and weaknesses, the most used are EIA (enzyme immunoassays) for toxins A and B and then we have PCR, tissue culture cytotoxicity and assays for clostridial glutamate dehydrogenase (as a first line test).

Imaging unfortunately it is not specific for this kind of disease; still an abdominal X-ray or a computed tomography is of great value when we have to differentiate for other cases of occlusion or ileus.

There is another way of diagnosing the infection; still it holds a higher degree of invasiveness with all the secondary aspects.

We are talking about colonoscopies which can biopsy the membranes and solve the diagnosis in an ironclad manner.

Risk factors

Antibiotics

Multiple studies, some of them multicentric focused on what classes of antibiotics are involved in this disease. Most of them imply clindamycin, fluoroquinolones, cephalosporines(generations II, III, IV but not first generation). Rarely are cited also carbapenems and trimethoprim/sulphonamides, also peniclins seem to be involved[5].

Proton pump inhibitors and H2 antagonists

Normally they should be in same group but this affiliation is a bit forced. Proton pomp inhibitors are one of the most used therapies worldwide instead of H2 antagonists. Also there are some studies that recommend the use of H2 antagonists instead of proton pump inhibitors. Their role as a risk factor or not is not so clear. Some of them say that more than 2 days of proton pump inhibitor greatly increases the risk to develop C Difficile infection[6,7].

There are studies that demonstrated that one of those two protective agents can be administred concurentially with C Difficile treatment may it be metronidazole / vancomycin / fidaxomicin[8]. There are also studies that could not find any connection between the infection and the use of PPI[9].

Age> 65 years

This is one of the risk factors almost universally recognized. Almost all organisms at this age present somehow reduced turn-over, their innate resources are more limited so the immune response in this case is not so good, and the antibodies production rate is not so high so the host has lesser chances to suppress the infection.

Underlying illness

It is pure logic to assume that a weakened body would be more presumptuous to an infection. There are studies that associate this factor to the development of severe C. Difficile infections. In fact most of the studies at least agree to two of these factors respectively age and underlying illness.

In this category we include also neoplasia, gastro-intestinal surgery, naso-gastric tubes and gastro-intestinal disorders.

Long term hospitalization

This is also true for nursing homes. Both types of facilities present a high risk of infection given the high concentration of resistant germs and the difficulty to identify and isolate the infected persons. C Difficile is a spore type germ so the isolation is a must for the infected, and in this dormant form it can stay alive for months, so it is easy to assume the worst has passed[10].

The European Society of Clinical Microbiology and Infectious Diseases established a biochemical set of risk factors to predict an increased risk of developing severe disease[11].

1. Marked leucocytosis (leucocyte count >15 9 109/L)

2. Decreased blood albumin (<30 g/L)

3. Rise in serum creatinine level (≥133 lM or ≥1.5 times the premorbid level)

Treatment

For decades the only recognized and valuable tools were metronidazole and vancomycin. Both of them still stand as valuable medication.

A new antibiotic appeared a few years ago the fidaxomicin. As a last resort the infectionists use last tier drugs like Tygecycline.

Until 2009 there were no regulations for the treatment of this infection so there were a lot of medication schemes, some of them more or less efficient.

Fortunately nowadays there are international guidelines for the treatment of this infection. Less used substances have been tried some of them using great promise (Teicoplanin, Nitazoxanide, Rifaximin).

Tygecycline is a new glycylcyline from the tetracycline drug class. It has been shown to have activity against a wide variety of bacteria, including the antimicrobial-resistant strains. As with all tetracycline drugs, it is not recommended for pregnant or nursing women. One of the most important aspect especially for the urologic patient is that renal function monitoring is not necessary.

There are studies which compare vancomycin with fidaxomicin. Cure status are similar, however, fidaxomicin is associated with significantly lower recurrence rates than vancomycin for patients infected with non-NAP1 strains of C. difficile. For patients with the NAP1 strain, recurrence rates did not differ by treatment.

Emerging therapies

Toxin-binding resins and polymers and probiotics are two of the novel therapies which have been used in the treatment of this infection.

Unfortunatelly the results are still inconclusive, they are included in guidelines but not as a stand alone treatment but more as a adjuvant therapy[12].

As for immunotherapy, human monoclonal antibodies have been used with success, the main shortcoming is that the studies included few patients and we can not generalise the results. Still they show promising results some of them being in phase III trial.

In one of those studies at about 4 weeks after the resolution of symptoms the diarrhea reappeared. The question asked was if it was a relapse or the lifetime of antibodies just ran out and the organism was left defenseless[13].

European Society of Clinical Microbiology and Infectious Diseases

In case of non-severe infection (no signs of severe colitis) in non-epidemic situations and with CDI infection clearly induced by the use of antibiotics, it may be acceptable to stop the inducing antibiotic and observe the clinical response for 48 h, but patients must be followed very closely for any signs of clinical deterioration and placed on therapy immediately if this occurs.

Metronidazole is recommended as oral antibiotic treatment of initial CDI in mild/moderate disease.

There is insufficient evidence to support administration of probiotics, toxin-binding resins and polymers, or monoclonal antibodies.

Based on its pharmacokinetic properties vancomycin is considered superior to metronidazole in severe C. difficile disease.The use of high doses of vancomycin (500 mg orally four times daily) was included in the Infectious Diseases Society of America/Society for Healthcare Epidemiology of America treatment guidelines for management of severe complicated CDI as defined by the treating physician. However, there is insufficient evidence to support the use of doses >125 mg four times daily in the absence of ileus.

Fidaxomicin was not inferior to vancomycin for initial cure of CDI, but there are no data available on the efficacy of this drug in severe life-threatening disease.

Total abdominal colectomy should be performed to treat CDI in case of:
_ Perforation of the colon
_ Systemic inflammation and deteriorating clinical condition despite maximal antibiotic therapy; this includes the clinical diagnoses of toxic megacolon, acute abdomen and severe ileus. Colectomy should preferably be performed before colitis becomes very severe. Serum lactate may, inter alia, serve as a marker for severity (operate before lactate exceeds 5.0 mM).

A future alternative to colectomy may be diverting loop ileostomy and colonic lavage, combined with antibiotic treatment (intracolonic antegrade vancomycin and intravenous metronidazole).

Treatment of the relapse

There is evidence that suggests that vancomycin and fidaxomicin can be equally used in case of relapse. What is to be observed is that metronidazole is considered as a second line for the treatment of relapse.

In case of a secondary relapse or multiple relapses the medication conduit remains basically the same with some alterations of the alternative therapies.

DISCUSSIONS

This type of infection is usually an underestimated one. From the surgeon's point of view it may mean less but in our case the urologist must be very careful when dealing with this type of disease.

Not only most of the patients are > 65 years old but we must not forget that urinary sepsis is one of the most insidious ones, usually presenting with few symptoms and signs.

Being unable to diagnose fast enough the Clostridium Difficile infection usually is fatal for an old already surgically manipulated patient with lots of co morbidities.

Another problem luckily rare is the time to operate a patient with toxic colon. It is difficult to differentiate between the underlying surgical problem of the patient (may it be kidney, urether, prostate or bladder) and the acute onset of a colonic disease.

More, in these cases we find an ileus that is masking the underlying diarheea, making the moment to intervene very hard to choose.

It is very important for the urologists to be aware of this type of disease, to be capable of dealing with it and most important to recognize it fast enough.

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SYSTEMATIC REVIEW

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Neuroendocrine differentiation in prostate cancer – a review

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Abstract: Objectives: This review aims to provide practicing clinicians with the most recent knowledge of the biological nature of prostate cancer especially the information regarding neuroendocrine differentiation.

Methods: Review of the literature using PubMed search and scientific journal publications.

Results: Much progress has been made towards an understanding of the development and progression of prostate cancer.

The prostate is a male accessory sex gland which produces a fraction of seminal fluid. The normal human prostate is composed of a stromal compartment (which contains: nerves, fibroblast, smooth muscle cells, macrophages) surrounding glandular acins – epithelial cells.

Neuroendocrine cells are one of the epithelial populations in the normal prostate and are believed to provide trophic signals trough the secretion of neuropeptides that diffuse and influence surrounding epithelial cells.

Prostate cancer is the most frequently diagnosed malignancy in men. In prostate cancer, neuroendocrine cells can stimulate growth of surrounding prostate adenocarcinoma cells (proliferation of neighboring cancer cells in a paracrine manner by secretion of neuroendocrine products).

Neuroendocrine prostate cancer is an aggressive variant of prostate cancer that commonly arises in later stages of castration resistant prostate cancer.

The detection of neuroendocrine prostate cancer has clinical implications. These patients are often treated with platinum chemotherapy rather than with androgen receptor targeted therapies.

Conclusion: This review shows the need to improve our knowledge regarding diagnostic and treatment methods of the Prostate Cancer, especially cancer cells with neuroendocrine phenotype.

Keywords: prostate cancer, neuroendocrine cells, neuroendocrine differentiation

INTRODUCTION

Prostate cancer is the most frequently diagnosed malignancy in men, majority of them are adenocarcinoma (derived from transformation of the glandular cells) [1]. The prostatic glands are under the influence of androgens. Prostatic adenocarcinoma cells present androgen receptors and relies on androgens for development and progression. That's why standard therapies for advanced and recurrent prostate cancer is based on two principles: block androgen synthesis (chemical or surgical castration)

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and inhibit androgen receptors [2][3].

Usually, this treatment provide clinical responses, but rarely eradicate all prostatic cancer cell populations and most patients after months/years develop resistance to these treatment – that means progression to castrate – resistant adenocarcinoma.

Neuroendocrine differentiation for most of the clinicians is associated with progression of prostate cancer to castrate – resistant disease.

NEUROENDOCRINE CELLS IN THE NORMAL PROSTATE

Neuroendocrine cells are found in many types of tissues, including in the normal prostate [4]. This cells secrete neuropeptides such as: serotonin, histamine, chromogranin A, calcitonin, bombesin, neuropeptide Y, vasoactive intestinal peptide, adrenomodulin [5],[6],[7],[8] and also growth factors (VEGF) [9]. The principal role is to maintain local homeostasis of the surrounding epithelial cells. Some of these neuropeptides have been found in seminal fluid, raising the idea that they may regulate sperm function [10][11].

Studies shows that neuroendocrine cells are present in all anatomic zones of the prostate and comprise less then 1% of benign prostatic glandular epithelium [12].

Neuroendocrine cells cannot be recognized on routine Hematoxilyn-Eosine staining. Ussualy this cells are found using immunohistochemistry with specific markers: chromogranin, synaptophysin, neuron specific enolase and CD 56 [13][14][15][16]. Other neuroendocrine cells markers reported in literature but not ussualy used in clinical practice include: cytochrome b561 [17], synaptic vesicle protein 2 [18][19], vesicular monoamine transporters [20].

NEUROENDOCRINE DIFFERENTIATION IN PROSTATE CANCER

Neuroendocrine prostatic carcinomas are often diagnosed on primary prostate needle biopsy with negative or low PSA level.

Neuroendocrine carcinoma of the prostate may be linked to resistance to androgen receptor signaling inhibition. Some prostatic tumors such as carcinoid tumor and small cell carcinomas are entirely composed by this type of cells with neuroendocrine differentiation. These tumors are aggressive and often present locally advanced or metastatic diseases with poor survival prognosis [21]. In general, these cells are negative for androgen receptor and PSA. Hormonal therapy is not effective while chemotherapy may have some value [22].

More frequently, prostatic tumors are composed by conventional adenocarcinoma with a small component of cells with neuroendocrine differentiation.

The neuroendocrine cells are indistinguishable from the normal adenocarcinoma cells on Hematoxilin-Eosine stained section under light microscope, and can be identified only by immunohistochemical stained using specific markers. Chromogranin A is the most used marker in clinical practice and is considered to be sensitive and specific [23].

Prostatic tumors with high grade or high stage and particularly in hormonal treated or hormone-refractory tumors, neuroendocrine differentiation is increased [24][25][26]. Also the level of serum chromogranin A are increased and is associated with tumor resistence to hormonal therapy [27]. In patients with hormone-refractory tumors, elevated serum chromogranin A level is predictor of poor prognosis, independent of PSA level [28].

NEUROENDOCRINE DIFFERENTIATION AND HORMONE-REFRACTORY PROSTATE CANCER

If the local curative treatments (radical prostatectomy, brachytherapy, radiotherapy) are no longer indicate for the patients with prostate cancer, androgen deprivation therapy is an effective therapy with significant symptomatic relief, suggesting that tumor growth is dependent on androgen receptors signaling. It is known that prostatic stromal cells support survival and proliferation of epithelial cells (paracrine secretion), that’s why, it is possible that neuroendocrine cells (who do not express androgen receptors and so are resistant to androgen deprivation) being capable to secrete neuropeptides and cytokines and so stimulate proliferation of adenocarcinomas cells. This situation suggest that androgen deprivation generally works, but eventually fails because neuroendocrine cells gradually substitute the function of stromal cells and so continued to stimulate the proliferation of prostate cancer cells.

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Antibiotic resistant urinary tract infections in an urology ward

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Abstract: Introduction: UTI (urinary tract infections) represent a central pathology for a urological service. Antibiotic resistance is growing at a steady and alarming rate worldwide and especially in Romania.

Method and materials: We have analyzed all the patients that were admitted to our clinic for continuous hospitalization between January 2015 and October 2015. All patients undergone urine culture and all cultures positive had an antibiogram worked up. We have selected all patients that had antibiotic resistance to at least an antibiotic.

Results: From 1745 patients admitted for continuous hospitalization, we had 180 positive urine cultures at admission from which 125 had at least an antibiotic resistance.

Conclusions: Antibiotic resistance is a serious phenomenon, with potential lethal complications, which we encounter daily in urological practice

INTRODUCTION

Uncomplicated UTI are probably the most common urological condition. It is estimated that at least 1 out of 3 females will develop an UTI during their lifetime. UTI frequency at young females is associated with sexual intercourse. When urological malformations, pregnancy, menopause, urolithiasis occur conditions for recurrence of the disease are met. Recurrences are defined as at least 2 episodes of UTI in 6 months or 3 episodes a year.

Male patients develop UTI due to different mechanisms and at an elderly age. The most common mechanism that facilitates the development of UTI in men is bladder outlet obstruction caused mainly by prostatic enlargement.

UTI are caused mainly by Gram negative *enterobacteriacea*. Escherichia Coli is the cause for about 80-90 % of community acquired UTI's. In healthcare related infections E. Coli is still the leading cause but with a more modest percentage. Other pathogens that cause UTI are: Proteus mirabilis, Enterococous, Klebsiella pneumonie, Pseudomonas aeruginosa.

METHOD AND MATERIALS

Between January and October 2015, 1745 patients were admitted for continuous hospitalization in our Clinic. At admission all of these patients had their urine sent for culture.

The urine culture was tested for antibiotic response for the following antibiotics:

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Trimethoprime-sulfamethoxazole, cefuroxime, cefotaxime, ceftriaxone, ciprofloxacine, ofloxacine, levofloxacine, gentamicine, ampiciline, oral penicillin, nitrofurantoine, imipenemcilastatin, ertapenem, meropenem and tigeciclin.

We had 180 positive urine cultures from which 125 were antibiotic resistant at at least one tested antibiotic.

We have analyzed the relationship between the germ and antibiotic resistance and also the sex and age of patients.

RESULTS

We had 180 positive urine cultures representing 10.31% out of all patients. 7.16% of all patients had at least one antibiotic resistance. 70% of the patients with positive urine cultures had at least one resistance.

Out of the 180 patients 104 were females (57,7%) and 76 were males (42,3%). Resistance was encountered in 56 females (44,8%) and 69 male patients (55,2%)

Out of all UTI's we had 143 E. Coli cases (79%), 25 Klebsiella pneumoniae (13,88%), 12 Proteus mirabilis (6,66%), 8 Enterococus (4,44%) faecalis and 2 cases with Pseudomonas aeruginosa (1,11%).

In the antibiotic resistant group we encountered the following bacterial involvement: 88 patients had E. coli, 19 had Klebsiella pneumoniae, 10 had Proteus mirabilis, 6 had Enterococus faecalis and 2 had Pseudomonas aeruginosa.

The decreasing percentage of antibiotic resistance was the following: Psedomonas aeruginosa cases had 100% resistance at at least one antibiotic, 83% of Proteus mirabilis cases had resistance, 76% of Klebsiella cases had resistance, 75% of Enterococus faecalis had resistance while the least resistant germ was E. Coli with 61,53%.

There were no significant differences between sexes except the fact that all the 2 cases with Pseudomonas aeruginosa were registered at male patients with long time users of indwelling ureteral catheters.

Regarding antibiotic resistance we had only 2 cases(1 case with Pseudomonas and 1 case with Klebsiella) which had no sensibility to any of the tested antibiotics. The strongest resistance was registered to ampicllin meaning that 115 (92%) cases with resistance had no sensibility to the drug. Resistance to penicillin was counted at 97 (77%). Third highest resistance was encountered to Thimetoprim with 90 patients (72%). The lowest resistance was registered for imipenem cilastatin, ertapenem, meropenem and tigeciclin. With only 2 cases (1,6%). Levofloxacin had also low resistance with 25 cases meaning 20%.

CONCLUSIONS

Antibiotic resistance is real threat to the general health of humanity, being more and more encountered especially in hospital related infections but also in community acquired infections. UTIs make no exceptions. Romania is especially at high risk with reported resistance to quinolones exceeding 30%.

The data we obtained from patients admitted for continuous hospitalization in our Clinic is similar to data in international literature reports concerning antibiotic resistance. It is important to realize the fact that urological patients are at high risk of developing antibiotic resistant infections due to the age profile, the pathology and the increasing number of indwelling catheters.

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CLINICAL PRACTICE

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Complications of radical retropubic prostatectomy – our experience

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Abstract: Introduction: Radical retropubic prostatectomy represents in its various forms (open, laparoscopic and robotic) the "gold standard" treatment for adenocarcinoma of the prostate. Unfortunately like al treatment solutions it has its shortcomings.

Retropubic radical prostatectomy, external beam radiation therapy and brachytherapy are the curative options.

Materials and Method: The experience of Clinic of Urology from Central Military Hospital representing more than 100 cases was analyzed . Classical open retropubic prostatectomy was performed in all cases.

We focused in this paper on intraoperative complications and also precocious and late postoperative complications.

Results: Our results are matching the other centers in terms of intraoperative complications (blood loss), early postoperative complications (hematuria, urinary tract infection, lymphatic drainage) or late postoperative complications (erectile dysfunction and urinary incontinence). However none of these complications are to be underestimated

Conclusions: The limits of this intervention can be pushed a litle bit further, in our opinion the age factor is a relative one, some of these patients having a longer than 10 years life expectancy. It provides good oncological outcome with manageable complications most of the times.

Keywords: radical retropubic prostatectomy, complications, nerve sparing

INTRODUCTION

Prostate cancer is nowadays the second most frequent neoplasia in men. Most probably the reason for this frequency is the presence of PSA as a valuable diagnosis tool and also the existence of the guidelines.

Also the very aggressive policy of controls for all men after 50-55 years has begun to show its results. Although radical prostatectomy, radiotherapy and brachytherapy are almost equal in terms of oncological results, in psychological, financial, and why not life quality radical prostatectomy is represented better.

MATERIALS AND METHOD

We have analyzed our experience at the Central Military Hospital regarding radical prostatectomy between January 2009 and February 2015. Our surgical teams carried on during these period more

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than one hundred radical prostatectomies for patients with localized or advanced prostate cancer. This paper focuses on intra and post - operative complications we encountered[1,2].

Patients mean age was 63.09 years ranging between 51 to 78 years of age. We haven't taken into consideration the general life expectancy for men in Romania, moreover the general biologic status was considered as inclusion criteria.

Major diagnosis criteria for prostate adenocarcinoma were: anamnesis, total PSA and free PSA levels, digital rectal examination and transrectal ultrasonography, which was usually performed when prostatic biopsies were obtained[3,4].

Medium prostatic volume of 45 cc without taking into consideration patients that prior have benefited of endoscopic surgery.

PSA levels varied between 0.5 ng/ml and 24 ng/ml. Two cases presented with PSA levels above 20 ng/ml. Those two patients were classified postoperative as pT3b – locally advanced.

Mean Gleason score, which is an important prognosis factor, was between 5-7 at 84 patients. Low risk prostate adenocarcinoma was recorded in six cases, ten patients with Gleason 8 were included in high risk and very high risk groups Patients diagnosed with Gleason 9 score were unsuitable for surgery.

In accordance with European Guidelines surgery was performed at six to eight weeks after the transrectal biopsy and 12 weeks after the transurethral resection of the prostate in order to to reduce the prostatic inflammation. General anesthesia was chosen for all patients.

Pelvic lymph node dissection was performed at almost all patients. We used extended lymph node dissection when patients were included in high or very high risk groups.

Urethro-vesical anastomosis was realized using four to eight resorbable sutures excepting six cases when no sutures could be placed due to the short urethra left after the prostatic excision. Those six cases were managed by gentle traction of the urethro-vesical catheter and maintaining the catheter for 14 days compared to 7 to 10 days as we did on the other cases.

RESULTS

The most redutable intraopearatory complication we encountered was blood loss which ranged between 400 milliliters and 2500 milliliters. Twenty - two patients needed intraoperatory blood transfusions.

Early complications included: gross hematuria (eight cases) which spontaneously remitted in 24 to 48 hours, wound infection (six cases), prolonged lymphatic pelvic drainage (sixteen cases), urinary tract infection (twenty cases), pulmonary embolism with subsequent death of the patient (one case).

Eighteen patients developed moderate or severe LUTS caused by bladder neck sclerosis or urethral stricture which was managed trough endoscopical procedures in the first year post-op. Four cases developed urinary fistula during the first 72 hours after prostatectomy three needing surgery in order to perform a new urethro-vesical anastomosis and one being resolved by placing a gentle traction on the urethro-vesical catheter for 24 hours.

Late complications recorded were urinary incontinence and erectile dysfunction. Following data were analyzed at one year after the surgery, only 78 patients being included in the evaluation. Urinary incontinence was evaluated by the number of pads used by the patient.

Almost 80% confirmed that they do not use any incontinence pads. Ten patients use only one pad a day and lose urine only when changing positions or when sneezing or coughing. Three patients affirmed moderate urinary incontinence using 2-3 pads a day[5].

We used a similar technique like the TOT used for women to place a suburethral transobturatory mesh for eleven ot if the thirteen patients with urinary incontinence with very good results and lack of symptoms in nine cases.

Using Walsh technique neurovascular bundles were spared in 62 patients. Erectile function, a very important part in quality of life was assessed using anamnesis and standardized questionnaires. 68.42%

(26 out of 38 patients) of the patients with surgical bilateral neurovascular bundles preservation affirmed satisfactory erections while only 33% (eight out of 24 patients) with unilateral bundle preservation affirmed satisfactory errection[6].

In sixteen cases due to oncological reasons or intraoperative bleeding neurovascular bundles were not preserved. All patients that benefited from the nerve sparing technique surgery were set on low dose daily 5 PDE inhibitors.

CONCLUSIONS

Radical prostatectomy is the standard treatment for localized and in some cases of locally advanced disease. We consider biologic status to be a more reliable indicator than age alone. That being said we consider far better to evaluate the patient before judging only the age.

Surprisingly one of the most bothering complications we encountered was bladder neck sclerosis; in this case one of the patients had undergone endoscopic surgery for three consecutive times. One of the hypotheses is the using of tension on the urethro-vesical catheter consequent bladder neck ischemia and in a secondary time sclerosis. This was not the case of this patient.

Intraoperative bleeding remains one of the most serious problems also because of the underlying co morbidities which may be aggravated during surgery.

Incontinence is a very bothersome late complication. Psychologically it has a great impact on the patient quality of life; actually along with erectile dysfunction those two are considered to be the highest burden from the patient's point of view. Still the existence of TOT like procedure for men offers an efficient solution to this problem.

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Scintigraphic evaluation of the kidney

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Abstract: *There are more than one technique used to evaluate the kidney, besides the standard ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI), there is also renal scintigraphy. The renal nuclear medicine procedures are grouped as in vitro (urine counting wells, basic probe detectors for clearance studies) and in vivo procedures (static and/or dynamic examinations done with planar gamma cameras, and single-photon emission computed tomography (SPECT) to determine kidney parameters or for cortical imaging). Renal scintigraphy has been a useful tool, since the early 1950s, in the diagnosis and management of many pathological changes in the kidney, especially in measuring renal function (e.g. obstructive/nonobstructive uropathies, renal inflammatory diseases, tumours, renal hypertension, and renal transplant viability). [1]*

INTRODUCTION

The radionuclide investigation of the kidney includes detection of renal afflictions and measurements of quantitative indices that estimate the renal perfusion and function. Ultrasound and computed tomography are commonly used for the evaluation of renal structural anatomy, and the role of nuclear renal imaging is more for functional analysis, less in anatomical imaging (e.g. cortical imaging).[1,2]

Renal scintigraphy, also known as a "renal scan" or "renal radionuclide imaging" or “renography” includes various investigations that use different radioisotopes to evaluate renal blood flow, renal split function, and the renal excretion performance of both. It yields specific and often unachievable information by using other imaging procedures. [2]

Clinical indications for renal scintigraphy (adapted from *Nuclear Medicine: The Requisites*, 4th ed)**:** [3-5]

1. Blood flow abnormalities
2. Function quantification (reduced performance of one or both kidneys)
   a. Differential function
   b. Glomerular filtration rate (GFR), effective renal plasma flow (EPRF)
3. Cirrhosis of the kidney(s)
4. Differentiation between a mass lesion and a column of Bertin
5. In infants with abnormalities of the urinary tract to study the urinary flow
6. Obstruction: ureteropelvic junction, ureteral
7. Pyelonephritis: both acute and chronic tubule-interstitial nephritis and parenchymal scarring
8. Renal failure: acute and chronic
9. Renal artery stenosis with/without renovascular hypertension
10. Renal vein thrombosis
11. Surgical:

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a. performance assessment before operations in the case of chronic diseases (e.g. diabetes)
b. Renal allographs: for clinical monitoring of renal function, transplant rejection or other complications, transplant anastomosis assessment
12. Trauma

PURPOSE

The purpose of this paper is to provide a better understanding about dynamic renal imaging in several kidney pathologies, with an emphasis on obstructive renal pathology and to clarify the meaning of some quantitative parameters used in renal scintigraphy.

MATERIAL AND METHODS

Nuclear renal scans are various, complex and also a subject to institutional preferences, that is why a standardization of the techniques is hard to achieve. All nuclear renal procedures have some basic common conditions that must be taken into account before, during and after patient examination. First of them involves the intravenous bolus injection of a renotrophic radiopharmaceutical (a medicine marked with a radioisotope) that emits a small amount of radioactivity into the patient. Usually, the radiotracer administered to the patient is well tolerated, with no systemic toxic effects. Some mild-moderate allergies or adverse reactions (such as dizziness, headache, metal taste, flushing) are extremely rare reported. The radiation dose is relatively low, less than the standard chest x-ray. The examination is performed with the patient either lying on the examination table, in the supine position, or standing. [3-7]

The pre-scan preparatory measures that are common are hydration, about 30 minutes prior to the actual examination, the patient should drink about 1 litre of fluid, and an empty bladder, the patient should void before examination. [3-7]

It is of great importance that in the last 3-6 months prior to the renal scintigraphy, no high doses of iodine substances to be taken, like x-ray and CT contrast agents, or certain medicines (e.g. Amiodarone) because the results could otherwise be distorted. [3-7]

In general, chronic therapy medications should not be discontinued prior to the exam. For specific problems, specific blood pressure lowering drugs (ACE inhibitors, diuretics) must be mentioned to the nuclear medicine physician as there is a period of drug abstinence in some cases. [3-7]

Different scanning protocols with various radiopharmaceuticals are available for scanning the kidneys. Choosing the right technique and optimal radiopharmaceutical depends on the patients' medical history, clinical setting and indication. [3-5, 8]

Plenty and divers radiopharmaceuticals have been produced in the last 60 years to assess renal function; some are used in laboratory assays by measuring blood samples and determining the renal clearance of the radiopharmaceutical and others for dynamic or static studies with a gamma camera or a SPECT system. The gamma cameras and SPECT systems monitor the passage of radiopharmaceuticals through the kidney and urinary tract by registering and processing of the emitted gamma radiation. [9, 10]

The radionuclide agents usually used for determining renal function and anatomy can be grouped into three main categories [5,8]: those filtered by glomerular filtration, those excreted by tubular secretion via proximal tubule receptor-mediated endocytosis from the glomerular filtrate, and those retained in the renal tubules for long periods, useful for cortical imaging. [8]

The most frequently used radiopharmaceuticals in renal scintigraphy are illustrated in Table 1.

Table 1. Most frequently used renal radiotracers

Glomerular Filtration (GF)	• Tc-99m diethylenetnamine pentaacetic acid (Tc-99m DTPA) • 99mTc-mercaptoacetyltriglycine (Tc-99m MAG3) • I-131/I-123 orthoiodohippurate (OIH)
Tubular Secretion (TS)	• I-131 OIH • Tc-99m MAG3 • Tc-99m ethylenedicisteine (Tc-99m EC)
Tubular Filtration (TF)	• Tc-99m dimercaptosuccinic acid (Tc-99m DMSA) • Tc-99m glucoheptonate (Tc-99m GHA)

Table 2. Renal radiotracers excretion mechanisms (adapted from *Semin NM Apr.92*)

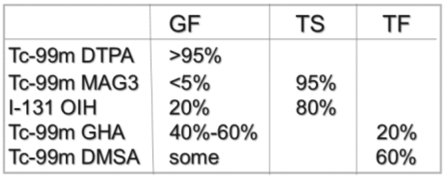

	GF	TS	TF
Tc-99m DTPA	>95%		
Tc-99m MAG3	<5%	95%	
I-131 OIH	20%	80%	
Tc-99m GHA	40%-60%		20%
Tc-99m DMSA	some		60%

There are four important types of renal imaging methods obtained by using a planar gamma-camera or a SPECT system in order to evaluate whether the kidneys are working normally or abnormally:

- Renal perfusion and renal function imaging are dynamic studies with images taken in series, over a period of 30 minutes immediately after the bolus radiotracer injection and determines the blood flow distributed to the kidneys, recognizes a potential narrowing of the renal arteries, and helps in determining the kidneys functioning. [2, 11]

- Diuretic renal scintigraphy is used to detect kidney obstruction. The procedure is similar to the renal perfusion, and seriated images are taken before and after the introduction of a diuretic (in the 15th minute of acquisition) to help urine elimination from the kidneys. [2, 11]

- ACE-inhibitor renal scintigraphy utility is in detecting renovascular hypertension, not renal artery stenosis, by comparing renographic images before and after taking an ACE-inhibitor. [2, 11] It is also a dynamic study and the image acquisition process begins when the radiotracer is injected intravenously in bolus. [3-5, 8, 10]

- Cortical renal scintigraphy is a static study and detects the amount of normal functioning kidney tissue. [2, 11] After the tracer administration, there is a three hour delay before the imaging acquisition can begin. [3-5, 8, 10]

Generally the dynamic renal functional studies are acquired in two parts. The first part evaluates the renal blood flow, which is calculated in the first pass of the radiopharmaceutical bolus through the abdominal aorta and renal arteries. The second part assess the kidney uptake and clearance function over the next 25 to 30 minutes of acquisition. [3-5, 8, 10]

GFR and EPRF are important kidney function markers evaluated by dynamic renal scintigraphy. Normal GFR varies in accordance with age, sex, weight (nutritional status), diet, race, and kidney size, which is proportional to body surface area. [12, 13, 14] The estimated GFR is calculated using these factors and the serum creatinine value, but it is not helpful when there are unilateral changes or when kidney function is very abnormal. Unilateral renal function changes are difficult to identify with other imaging techniques, but easy to determine with camera-based renal scan split functions. The percent differential function (split function) calculates the contribution of each kidney to the renal function and can be applied to GFR or ERPF data. [3-4, 15]

Nuclear techniques are not 100% "accurate" in measuring kidney function (GFR, ERPF), but are superior to investigations based on the serum creatinine value. [3, 14] Using renal scintigraphy patients can be followed over a long period of time, because this technique is highly reproducible in any one patient. [3, 13, 14]

Table 3. Renal radiotracers used for renal function measures
(adapted from *Requisites of Nuclear Medicine 4th ed*)

Effective Renal Plasma Flow (ERPF)	Tc-99m MAG3 I-123 / I-131 OIH H-3 or C-14 paraamino-hippurate (PAH) I-125 or I-131 iodopyracet
Glomerular Filtration Rate (GFR)	Tc-99m DTPA Cr-51 EDTA I-125 diatrizoate I-125 iothalamate C-14 or H-3 inulin In-111 or Yb-169 DTPA Co-57 vitamin B12

The Tc-99m DMSA cortical scan is used to evaluate renal cortical lesions in patients with suspected pyelonephritis, to detect renal scarring in a patient with reflux, and, in some occasions, to differentiate a prominent column of Bertin from a tumorous formation. Renal cortical scintigraphy provides additional information by showing changes and improvements over time, that are impossible to evaluate with conventional structural and anatomical imaging modalities, like ultrasound, CT, and MRI. [3, 13, 14]

RESULTS

The images obtained with the gamma camera should be inspected after acquisition in order to evaluate if the examination was done in proper conditions. After visual inspection the image data is processed and regions of interest (ROIs) are defined over the kidney and surrounding background so that the renogram curves can be generated. [5, 7]

The time-activity curve (TAC) represents a graphic illustration of the renal function and is composed of 3 parts: initial rise, upslope and downslope. The first part reflects the radioactivity that arrives via the renal artery to the kidney. The upslope (ascending limb) reflects kidney uptake before the radiopharmaceutical begins to be excreted by the kidney (the descending limb). The peak time accurately indicates the point at which the extraction and accumulation trend is reversed to the evacuation process. [5, 7]

This curve is important in diuretic renal scintigraphy. Diuresis renography helps to discern between obstructive (calculus) and nonobstructed dilated urinary tract, and in the postsurgical evaluation of the renal system function and urodynamic. Acute obstructive uropathy is a commonly encountered condition. When unilateral obstruction occurs the changes in the measured renal function are a little decrease or imperceptible, but the bilateral form can result in significant kidney function losses. [5, 16]

The radionuclide renogram TAC provides an accurate graphic illustration of the dynamics of urinary excretion. In obstructive uropathy a calculus can give various degrees of hydronephrosis, depending on the site in which it is lodged. In the case of acute obstruction due to a renal calculus, pressures increase fast in the pyelocaliceal system and in the ureters above the point of obstruction. The affected kidney has a characteristic aspect: a dilated urinary tract with a thin cortex, and it shows on the TAC a slow continuous accumulation of tracer in the collecting system, a slow increasing ascending curve with no downslope. In contrast, the opposite kidney, which has a normal function and no obstruction, will show a good uptake and excretion, and the three part TAC. [7, 17]

In some cases the calculus produces a partial obstruction of the urinary excretion pathways. In order to demonstrate this a diuretic like Furosemide is administrated at various times during the course of the renal scan. The decreasing aspect of the curve after the administration of the diuretic indicates an incomplete obstructive pattern (as in Curve 3), and as for the rising curve a complete obstruction (as in Curve 2). [7]

Figure 1. Time activity curves
(adapted from *EANM: Dynamic renal imaging in obstructive renal pathology. A Technologist's Guide*)
Curve 1- normal kidney function; Curve 2- complete obstruction; Curve 3 - incomplete obstructive pattern after the administration of Furosemide at minute 15.

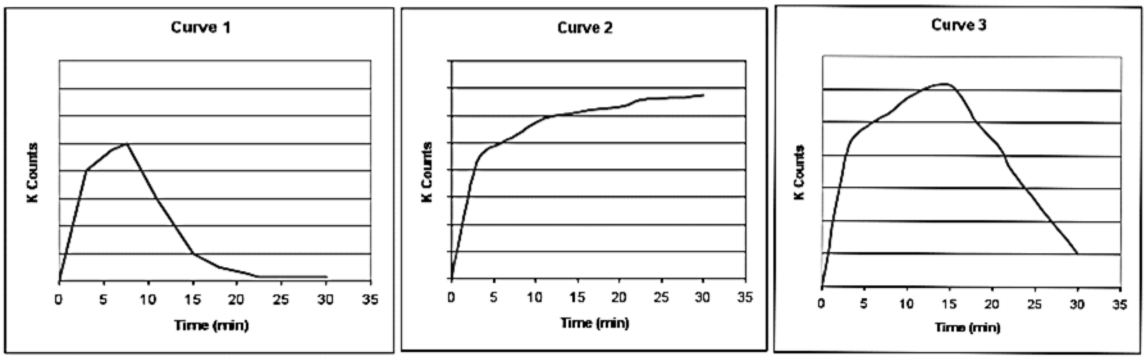

QUANTITATIVE INDICES

Renal perfusion is evaluated by visual and quantitative analysis (1- to 3-second images) of the initial bolus as it transits the abdominal aorta and enters the renal arteries (used in renal number anomalies, renal transplant). [10, 11]

Relative Function represents the relative uptake of the radiopharmaceutical for the evaluation of uni-/ bilaterally impaired kidneys. [10] The split function is particularly useful because estimated GFR and serum creatinine may not identify unilateral lesions. [3]

Renal Size. Several chronic renal diseases will result in bilaterally small kidneys, whereas the kidneys may be bilaterally enlarged in early diabetic renal disease, acute interstitial nephritis, HIV nephropathy, and amyloidosis. The resolution of structures of the renal parenchyma with nuclear medicine may not be as clear as with other imaging techniques, such as CT or MRI that is why nuclear renal images are not usually used to differentiate between cysts and tumours. [10, 11, 18]

The time to peak, or T max, refers to the time from radiopharmaceutical intravenous administration to the peak height of the renogram curve. 99mTc-MAG3, 99mTc-DTPA and OIH renograms normally peak by 5 min and drop to half-peak value until the 15th minute after injection; however, in some cases, physiologic retention of the radiotracer in the renal calyces or pelvis can alter the aspect of the TAC in normal kidneys and lead to prolonged values for the time to peak, 20-min/ maximum count ratio, and T½. [8, 10, 19, 20]

Figure 2. Normal right kidney renogram. Specific scintigraphic aspect of left kidney obstruction (kidney calculus).

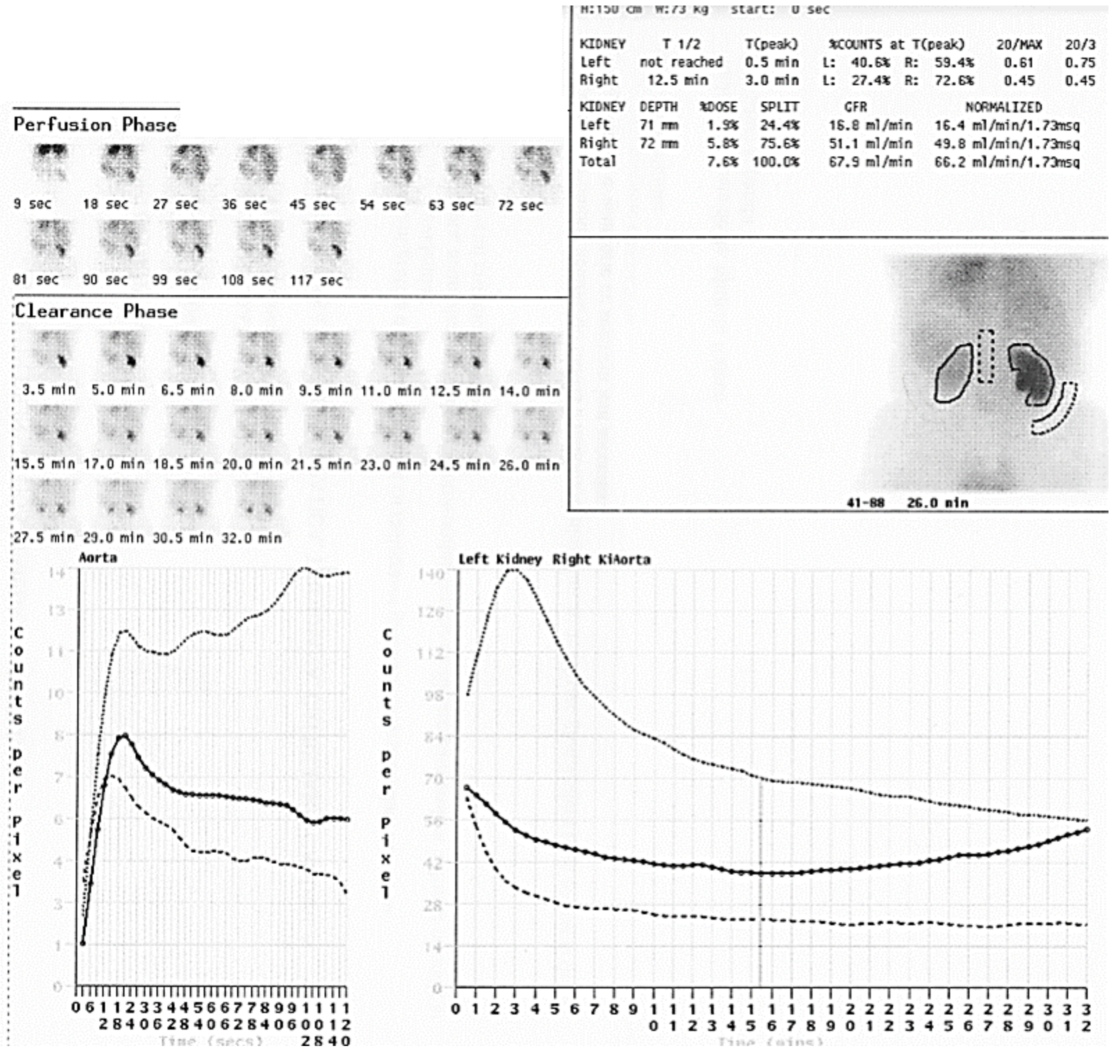

H:150 cm W:73 kg start: 0 sec

KIDNEY	T 1/2	T(peak)	%COUNTS at T(peak)	20/MAX	20/3
Left	not reached	0.5 min	L: 40.6% R: 59.4%	0.61	0.75
Right	12.5 min	3.0 min	L: 27.4% R: 72.6%	0.45	0.45

KIDNEY	DEPTH	%DOSE	SPLIT	GFR	NORMALIZED
Left	71 mm	1.9%	24.4%	16.8 ml/min	16.4 ml/min/1.73msq
Right	72 mm	5.8%	75.6%	51.1 ml/min	49.8 ml/min/1.73msq
Total		7.6%	100.0%	67.9 ml/min	66.2 ml/min/1.73msq

Perfusion Phase
9 sec 18 sec 27 sec 36 sec 45 sec 54 sec 63 sec 72 sec
81 sec 90 sec 99 sec 108 sec 117 sec
Clearance Phase
3.5 min 5.0 min 6.5 min 8.0 min 9.5 min 11.0 min 12.5 min 14.0 min
15.5 min 17.0 min 18.5 min 20.0 min 21.5 min 23.0 min 24.5 min 26.0 min
27.5 min 29.0 min 30.5 min 32.0 min
41-88 26.0 min
Aorta
Counts per pixel
Time (secs)
Left Kidney Right KiAorta
Counts per pixel
0 14 28 42 56 70 84 98 112 126 140
Time (mins)

Figure 3. Renal scintigraphic appearance of chronic renal failure; hypoplasic left kidney.

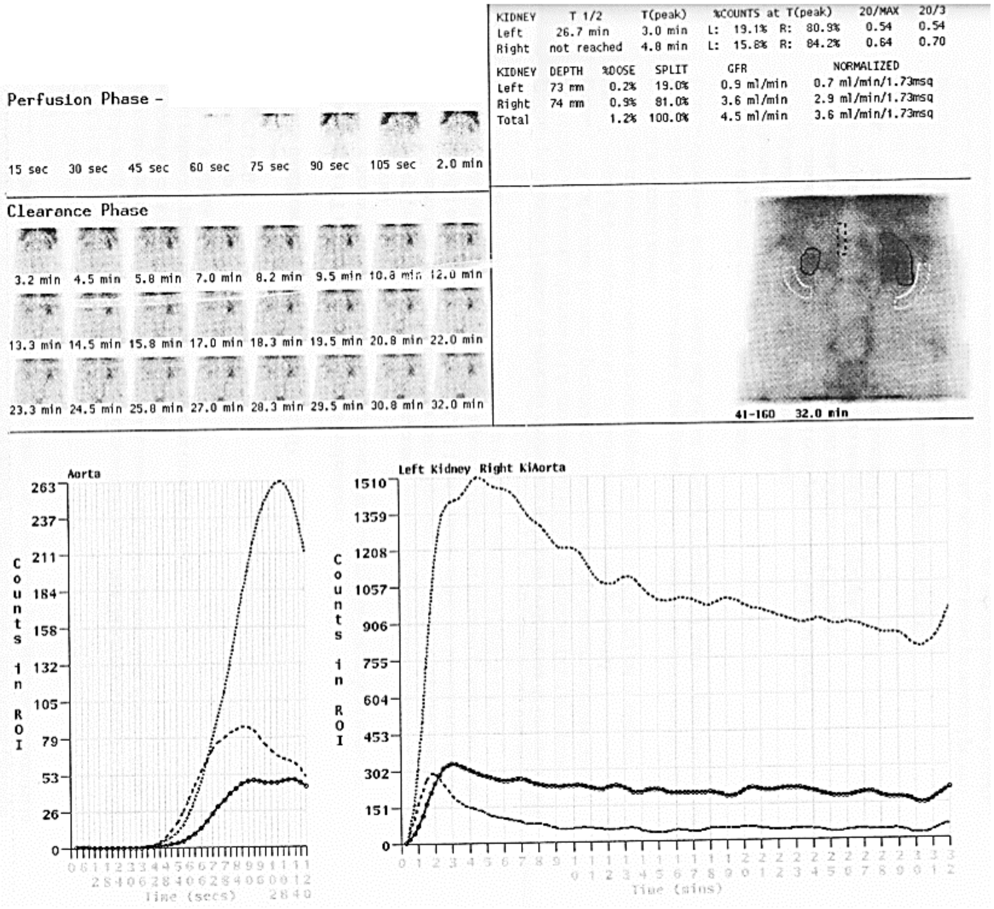

KIDNEY	T 1/2	T(peak)	%COUNTS at T(peak)	20/MAX	20/3
Left	26.7 min	3.0 min	L: 19.1% R: 80.9%	0.54	0.54
Right	not reached	4.8 min	L: 15.8% R: 84.2%	0.64	0.70

KIDNEY	DEPTH	%DOSE	SPLIT	GFR	NORMALIZED
Left	73 mm	0.2%	19.0%	0.9 ml/min	0.7 ml/min/1.73msq
Right	74 mm	0.9%	81.0%	3.6 ml/min	2.9 ml/min/1.73msq
Total		1.2%	100.0%	4.5 ml/min	3.6 ml/min/1.73msq

Perfusion Phase -
15 sec
30 sec
45 sec
60 sec
75 sec
90 sec
105 sec
2.0 min
Clearance Phase
3.2 min
4.5 min
5.8 min
7.0 min
8.2 min
9.5 min
10.8 min
12.0 min
13.3 min
14.5 min
15.8 min
17.0 min
18.3 min
19.5 min
20.8 min
22.0 min
23.3 min
24.5 min
25.8 min
27.0 min
28.3 min
29.5 min
30.8 min
32.0 min
41-160
32.0 min
Aorta
Counts in ROI
263
237
211
184
158
132
105
79
53
26
0
Time (secs)
Left Kidney
Right Ki
Aorta
1510
1359
1208
1057
906
755
604
453
302
151
0
Time (mins)

The T½ represents the time is necessary for the radioactivity in the kidney to drop to 50% of the maximum value (time to peak, or T max); this index is important in diuretic renography for patients with suspected urinary tract obstruction. [8, 10, 19]

The 20-min/maximum count ratio is an index of the transit time and a measurement of residual cortical activity, the ratio between the kidney counts at 20 min to the maximum (peak) radioactivity; it is useful in monitoring patients with suspected urinary tract obstruction and for detecting renovascular hypertension. [8, 10, 19]

CONCLUSION

Renal radionuclide studies are versatile procedures and vary depending on institutional preference, clinical setting of the patient and medical indication.

Renal scintigraphy is a complex subject and understanding some basic principles, the use of radiopharmaceuticals available to image the kidney and monitor its function, the quantitative indices that can be generated and protocols can be useful for the physicians specialized in nephrology and urology in evaluating patients with diseases of the urinary tract and renal physiology.

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CLINICAL PRACTICE

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Penile prosthesis – a viable solution for erectile dysfunction refractory to conservative therapy

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Abstract: Introduction: Erectile dysfunction(ED) is a serious condition which can affect men of all ages, with an important impact over the quality of life. When conservative therapy fails, a viable solution is the penile prosthesis implantation (PPI).
Objective: The purpose of this paper is to present data from recent literature regarding the satisfaction rates of men who have undergone penile PPI and as well as the partners satisfaction rates, ease of use, informations regarding long term survival of these prosthesis, postoperative complications and long distance complications, the effectiveness and the way that penile prosthesis have influenced the quality of life of the men who have opted for this solution.
Material and methods: We have analyzed recent long term studies concerning the outcomes of the PPI, studies which were made retrospectively, over a period of time of 10 to 15 years.
Results: Compared with the conservative treatment for ED, the satisfaction rates achieved after penile prosthesis implantation are higher. According to these studies, the overall patient's satisfaction is 70-90%. The difference between patient and their partners' satisfaction rate is negligible, this difference ranging between 2 to 8%. The satisfaction rate regarding the adequate erection for sexual intercourse is 80-90%. The overall satisfaction rates for the malleable prostheses are lower compared with inflatable devices, 30-75%, respectively 75-90% for the inflatable prosthesis. The mechanical and overall survival rates for the malleable prosthesis range between 65-80% at 10 years, and 58-75% for the inflatable devices. Overall ease of use is rated as 78%. Complications after implantation can be encountered in less than 5% of cases and infections in less than 2%. The likelihood of continued use is higher in the group of the patients with the inflatable prosthesis compared with the malleable ones 70-80%, respectively 50-60%. Up to 85-90% of patients with inflatable prosthesis would recommend them.
Conclusions: PPI is a high effective treatment for erectile dysfunction, refractory to pharmacological treatment. The inflatable penile prosthesis (IPP) provides more overall satisfaction than the malleable ones. The patients with IPP are more likely to continue using their devices than those with the malleable prosthesis. PPI improves significantly the quality of life for patients with erectile dysfunction.

INTRODUCTION

Erectile dysfunction is defined as the inability to obtain and maintain an erection with sufficient penis rigidity, adequate for successful sexual contact. Although ED decreases significantly the quality of life for both men and their partners, in many countries

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ED is understated because of cultural aspects.

ED can affect men of all ages, but as life expectancy continues to increase, so does its prevalence. It is estimated that the prevalence of ED in men over the age of 50 years is higher than 50 percent, and this prevalence grows as the age increases. This condition is not exclusively encountered in men over the age of 50. It is estimated 3-5 percent of young men with ages between 15 and 25 years may suffer from this condition and 7 to 10 percent of those with ages between 25-45 [1].

Despite initial beliefs that the most common cause for ED was the psychological factor, especially in young men, it is now proven that organic causes are responsible for 70 to 90 % of ED. There are several proven pathologies proven in ED [2]:

a) Diabetes Mellitus-is a frequent cause of ED. Men with DM compared with general population may have ED at an earlier age. The prevalence of ED in the population of men with DM can be as high as 70-80%. The peripheral polineuropathy, microangiopathy and arterial insufficiency encountered in DM evolution can explain the earlier age of ED appearance and a higher prevalence than individuals without DM [3].

b) Vascular causes-atherosclerosis plays an important role in ED physiopathology. The presence of aortic or iliac atheromatosis can decrease the blood inflow, thus explaining the ED prevalence of up to 50 % in patients with atheromatosis. Another vascular cause may be the increased venous outflow, which depends on the trabecular smooth muscle relaxation and on the erectile tissue's capacity of expandability.

c) Iatrogenic causes responsible for ED- renal transplantation, aortic or peripheral surgery, abdominal perineal resection of the rectum, perineal irradiation, cystoprostatectomy or radical prostatectomy (RP). Erectile dysfunction secondary to cystoprostatectomy and radical prostatectomy may be encountered in up to 80% of cases. Since the nerve sparing RP was introduced by Walsh over years ago, the incidence of ED was decreased to 40-60% [4]. The incidence of ED post RP depends on patient age, comorbidities, preoperative potency status, type of surgery, surgical technique (open, laparoscopic or robot assisted RP), surgical experience, extensive nerve sparing (unilateral or bilateral) [5]. Although some patients believe that radiotherapy may be associated with a lower risk of ED, it has been proven that ED may be encountered in 20-80% of patient who has undergone radiotherapy. Studies have shown that the incidence of cavernosal fibrosis and ED are similar between RP and radiotherapy [6,7].

d) Trauma – pelvic fractures may affect the internal pudendal or common penile artery or the neurovascular bundle.

e) Endocrinological causes such as: hypogonadotripic hypogonadism, hypergonadotropic hypogonadism, hyperprolactinemia and hyperthyroidism.

It is known that testosterone increases sexual interest and the frequency of sexual acts, as well as the frequency of nocturnal erections, but it's role on visual or fantasy induced erections is little to none.

f) Neurological causes:
- CNS: Parkinson's disease, encephalitis, temporal lobe epilepsy, stroke, multiple sclerosis (50-70% of patients with MS may have ED), tumors, myelodisplasia;
- periphereal nerves (trauma, diabetic polineuropahty, alcoholic neurophathy and radiopelvic surgery).

g) Penile diseases: Peyronie's disease, trauma, priapism (vascular lesions).
h) Drugs: Marijuana, Opioids, Ethanol, Antimuscarinic drugs, Antihistamine, Tricyclic antidepressants, Estrogens, Spironolactones, Ketoconazole, and many other drugs.
i) Renal failure-up to 40-50% patients on dialysis suffer from ED.

TREATMENT

Currently there are three lines of treatment for ED. The first line and the most used one is oral medication, especially phosphodiesterase 5 inhibitors. Since 1998, when the first PDE-5 inhibitor was introduced, Sildenafil (Viagra), the management of ED was revolutionized due to its high efficacy and few adverse effects. As time passed other drugs with higher efficiency and superior safety profiles became available, such as: Vardenafil (Levitra) and Tadalafil (Cialis). These drugs block the hydrolysis of cyclic GMP, increasing the accumulation of GMPc and potentiating the effect of NO [2]. For many men the treatment with PDE-5 inhibitors has allowed them to regain normal sexual function and regain self-confidence.

The second line treatment is the intracavernous injection of vasoactive drugs such as papaverine, prostaglandin E1 or phentolamine. The efficacy of this therapy is high, but the pain and the risk of ischaemic priapism limit its utilization [8]. The third line of treatment is the surgical implantation of a penile prosthesis. When the conservative therapy fails(PDE 5 inhibitors, intracavernosal injections, vacuum erection device, intraurethral suppositories with alprostadil – Is a PGE1 which increases corporeal oxygenation by promoting blood inflow) or when the patients are reluctant to any form of conservative therapy, the solution is the penile prosthesis implantation. The first penile prosthesis were introduced at the beginning of the 1970s . Since then they have been greatly improved and the surgical techniques for placement have evolved and made penile prosthesis implant a very effective treatment for ED [9]. This kind of treatment is effective and safe, and it also offers high satisfaction rates for patients and for their partners. With the technical improvement the number of complications and revision surgeries decreased, thereby the popularity and use of penile prosthesis for the treatment of ED increased.

The penile prosthesis available on the market include the one piece malleable and the two or three piece inflatable versions, each type of prosthesis having its own advantages and disadvantages. Malleable prosthesis is easier to use and have fewer mechanical problems compared with the inflatable prosthesis and they are also less expensive. The disadvantage of malleable implants is that complete penile detumescence cannot be achieved and this may be an important factor for patient unsatisfaction [10]. The inflatable prosthesis permit flaccidity and have a better functional result, but not all patients have the dexterity to use them, especially older men. Despite the implantation of penile prosthesis has been well documented, many urologists are reluctant to this form of treatment in older patients, due to the concern that older men have less dexterity and ability to operate an inflatable device [11]. The patients' satisfaction regarding the penile prosthesis implant is associated with their expectations and the performance of the implanted prosthesis [12]. Therefore, the decision regarding the type of prosthesis should be made by the doctor and by the patient, during a discussion where the doctor explains the advantages and disadvantages of every type of prosthesis, the risk of complications, the rate of satisfaction and unsatisfaction that both patient and his partner may have from this type of treatment, thus making sure that the patients will have expectations in line with reality.

The three piece inflatable penile implants are currently the most used prosthesis and their results regarding patient satisfaction are very good. The malleable and two piece penile prosthesis can be used in selected cases, where the three piece prosthesis has questionable indications (like in the case of elderly men who may have low dexterity and ability to operate such devices) [13,14].

Partners' satisfaction is questionable because few studies have investigated this problem during a long term follow-up period; although in literature the rate of partners' satisfaction is similar with men satisfaction. For more realistic results regarding this problem, evaluating the two partners separately during the follow-up period should bring out interesting results (in line with reality) [15].

Hakan Kilicarslan et. al in a recent study regarding the comparison of patient satisfaction for the malleable prosthesis (AMS 600-650) and two piece inflatable penile prosthesis (AMS Ambicor) concluded that the two piece inflatable prosthesis was found to be more successful and more likely for continous use when compared to the malleable device. A total of 72 patients had either AMS 600-650 or AMS Ambicor, but only 46 patients were reviewed during a long time follow-up. The percentages of patients with AMS 600-650 who reported to be satisfied, very satisfied and unsatisfied after the implant were 34,78% (8 patients out of the total of 23 questioned after the operation), 30.43% (7 patients out of 23) and 34,78% (8 patients out of 23). For the patients with the two piece inflatable penile prosthesis the results were: 73.91% (17 out of 23), 13.04% (3 out 23) and 13.04%. The percentages of patients who reported to be very likely, unlikely or very unlikely to continue using the malleable implants were: 30.43% (7 patients), 34.78% (8 patients) and 34.78%; in the case of the patients with the two piece inflatable devices the results were: 65.21%, 21.33% and 13.04% [16].

A study made by Natali et. al revealed that the likelihood of continuous for the two piece device AMS Ambicor was 89% (59 patients) and for the malleable device AMS 600-650 was 56% (9 patients) [13].

In another comparative study Lux et. al reported that the partners satisfaction rated 79% with the two piece prosthesis [17].

Levine related an overall satisfaction rate of 90% for men and the 82% for their partners, of a total of 131 patients, patients which benefited from the AMS two piece Ambicor implant [18].

Minervini reported that the 71% of patients using the malleable AMS 600-650 were satisfied with the implant [19].

Yoon Seob Ji in a study made on 74 patients who underwent implantation of inflatable penile prosthesis reported that the overall satisfaction was 86,8% and that 81,1% said that the prosthesis improved their sexual life. 83% of the patients in the study would undergo surgery again and 88.7% would recommend it to a friend. Only 60.4% of the patients interviewed during the follow-up period reported that they had an orgasm [20].

Mario Paranhos and collaborators established that 86.3% of patients (120 patients questioned out of 249 operated) rated their satisfaction after the implant as good, excellent or very good. The partners' satisfaction percentages was similar. The follow-up period was 40 months [21].

Humberto G. Villarreal and LeRoy Jones, in a study about penile prosthesis in the elderly men, reported that overall satisfaction was 4.3 (from a scale of 1 to 5, 5 meaning very satisfied), the ease of use was 4.1 (from a scale of 1 to 5.5 meaning very easy) and the mean use was 3,3 per month (0 to 7 times a month) [11].

Carson and associates evaluated satisfaction rate in 372 patients, group in which was used the AMS 700 CX prosthesis and it was stated that 79% used this prosthesis at least twice a month and that 88% would recommend the prosthesis for a friend [22].

There was little data regarding implantation of penile prosthesis in elderly people. As the life expectancy and longevity continuous to increase so does the ED problem refractory to first line treatment. Accordingly to Lindau, elderly couples remain sexual active even into the eight decade of life [23]. So the number of elderly men seeking penile prosthesis implantation will have a significantly increase. There are numerous potential complications of the inflatable penile prosthesis such as surgery related complications (haematoma, infections, erosion of the corpus cavernosum and even perforation) and mechanical failure (reservoir leakage, cylinder leakage, pump failure) [14,24].

The incidence of these complications depends on the follow-up period. In the immediate postoperative period, Paranhos stated that 24.5% patients reported pain, 7,9% had local infections and 8,6% had other complications such as wound dehiscence, difficulty voiding or local secretions without infection. In regards to late complications 8.6% of patients had to undergo revision surgery (12 patients out of 139 operated and followed up), of whom 4 the prosthesis had removed due to inefficacy and 8 had them exchanged. 2 of the 8 patients who had them exchanged had prosthesis fractured, 4 had inadequate size and the other 2 had extrusion of the cylinder. He remarked in his study that the risks of complication for the patients that have had radical prostatectomy previously was higher, 3,3 times higher. The rate of postoperative infections in this study was 7.9%. Paranhos explains that this high rate of infections compared to other studies is due to the experience of medical trainees, the high number of surgery in the operating rooms and the economic and social conditions of the patients [21]. The rigorous preoperatory asepsy has shown to reduce the incidence of infection to 4% from initial overall rate of 12% [25].

Jarow et. al [26] reported an infection rate of 1,8% and Gower a rate of 2.1% [27].

Merino in a meta-analysis related a lower infection rate in the case of malleable prosthesis compared to inflatable prosthesis (1.3% vs 3.5%) [28].

Menard in a study which examined over 400 patients with penile prosthesis after radical prostatectomy showed that the rate of complications for infection, revision or mechanical failure was less 5% and that the satisfaction rate was 86.1% [29]. The use of antibiotic impregnated penile prosthesis could significantly reduce the postoperative infection incidence and as well as the number of revision surgeries related to infectious complications. The overall postoperative complications are less than 5% and for infections is less than 2%. The incidence for mechanical failure is related of the follow-up period [30] and the overall incidence is around 5% [31]. Mechanical failure occurs more frequently in a case of multipiece inflatable prosthesis, due to their complexity. Malleable prosthesis due to the simple design are associated with a lower risk of mechanical problems. Lotan et al. related that the rate of survival (without technical problems) for malleable prosthesis compared to the inflatable ones is 87% vs 50% [32]. Paranhos encountered during his study a rate of mechanical problems in 3.59% of cases [21].

Atienza, in a meta-analysis regarding this problem found that the overall rate of mechanical failure was 1.4% [33]. He also found a rate of prosthesis exchange or withdrawal of 7.1%, which was similar to the rate that Paranhos described in his study of 8,68% [21].The causes of exchange or withdrawal were inadequate cylinder size, fracture of bioprosthesis (very rare, Parahos [21] reported 2 cases of patients with prosthesis fracture that didn't know of their problem and they had a normal sexual activity, this issue was found during check-ups).

Yoon Seeob Ji [20] related that 21.6% of patients (16 patients) experienced mechanical problems and 5.4% (4 patients) mechanical problems. The mean follow-up period was 98 months. The mechanical problems encountered were caused by cylinder leakage 8.1% (6 patients), reservoir leakage 1.4% (1 patient out of 16) and pump failure 1.4%. In the case of the other 8 patients with mechanical failure the cause was not found because they didn't undergo reoperation. The nonmechanical failure was due to erosion, infection of the prosthesis and other medical problems. All the patients with non mechanical failure underwent implant removal and surgical exploration. The survival rate of the inflatable prosthesis during the follow-ups over 5.10 and 15 years were 67.6%, 43.2% and 23%.

RESULTS

Compared with the conservative treatment for ED, the satisfaction rates achieved after penile prosthesis implantation are higher. According to the studies that we have analyzed, the overall patients satisfaction is 70-90%. The difference between pacient and their partners satisfaction rate is negligible, this difference ranging between 2 to 8%. The satisfaction rate regarding the adequate erection for sexual intercourse is 80-90%. The overall satisfaction rates for the malleable prostheses are lower compared with inflatable devices, 30-75%, respectively 75-90% for the inflatable prosthesis. The mechanical and overall survival rates for the malleable prosthesis range between 65-80% at 10 years, and 58-75% for the inflatable devices. Overall ease of use is rated as 78%.

Complications after implantation can be encountered in less than 5% of cases and infections in less than 2%. The likelihood of continued use is higher in the group of the patients with the inflatable prosthesis compared with the malleable ones 70-80%, respectively 50-60%. Up to 85-90% of patients with inflatable prosthesis would recommend them.

CONCLUSIONS

PPI is a high effective treatment for erectile dysfunction, refractory to pharmacological treatment. The technological improvements have decreased the rate of complications and revision surgeries which may appear after implantation, making these devices a safe solution and with great results for the men who choose penile prosthesis implantation as an answer to their problem. Many studies have related that the satisfaction rate after the operation was high, both for the patients and their partners [20]. The patients unsatisfaction after the implantation, was related to the unnatural feel of the sexual relations, due to the delayed ejaculation which was encountered in few cases and also because of the partners dissatisfaction, situation that may be related to the fact that the expectations were higher than the results. Thus before the operation, the doctor should make sure that the patient and his partners will have expectations in line with reality. Today, the three piece inflatable prosthesis is the most preferred one. Regarding the long term survival and complications of the penile prosthesis, the malleable prosthesis have a lower rate of mechanical problems than the inflatable ones, due to their less complex technological design, but patients satisfaction is higher in the group of inflatable prosthesis because they permit flaccidity and they have a better functional result. Patients who undergo implantation for the first time have a lower risk of postoperative complications compared with the patients who have had several interventions for different complications related to the penile prosthesis implant. The likelihood of continued use is higher in the group of the patients with the inflatable prosthesis compared with the malleable ones. The success of the implantation is very important, because the surgical revision is associated with a worse prognosis and a higher rate of complications. Regarding diabetes, several studies have shown a small correlation between diabetes mellitus and postoperatory infections [34,35]. Diabetes mellitus and radical prostatectomy are associated with a higher of postoperative complications. PPI enhances sexual capacity and self-confidence, therefore it should be considered as a final choice for patients with ED refractory to drugs.

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TOT versus TVT – mesh surgical treatment in stress urinary incontinence

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Abstract: *Introduction: Stress urinary incontinence is a highly debilitating condition, with an important impact over the quality of life. When the conservative treatment fails, the surgical treatment is a viable solution. Minimally invasive sling procedures have become the gold standard of surgical management for stress urinary incontinence (SUI) in women.*

Material and methods: The study was conducted on 68 patients with stress urinary incontinence, 52 have undergone the retropubic tension-free vaginal tape and 16 patients were operated using the transobturator tape procedure. All the patients were evaluated before the operation and the indication of the surgical treatment was established on the basis of physical examination (all the patients had positive cough test), abdominal ultrasound (to determine postvoid residual urine volume), urinalysis and urine culture (the majority of the patients have had before the operation recurrent urinary tract infections).

Results: The satisfaction was similar in both groups of patients, with an average satisfaction rate of 91% for TVT and 86% for the patients who have undergone TOT procedure. The patients were in a proportion of 78,9% at menopause, with an average number of natural births of 1.9 and a mean BMI of 28.2. Regarding the surgical duration, this has varied between 20 and 40 minutes, being higher in the TVT cases (up to 5-7 minutes longer than TOT), because cystoscopy was performed during the operation to verify if the bladder was perforated or not. The urethral catheter was removed immediately after the operation in the case of the TOT procedure and in the day after the operation for the patients who have undergone TVT procedures (all of these patients associated genital prolapse). The hospitalization time was 3 days. We haven't encountered significant intraoperative and postoperative complications.

Conclusions: The TOT and TVT procedures have proven to be highly effective and safe methods in the treatment of SUI. There are no significant differences regarding the efficacy of these two surgical methods, but the risk of intraoperative complications is a little higher for the TVT procedure. The mid urethral sling procedures improve significantly the quality of life for patients with stress urinary incontinence.

INTRODUCTION

Female stress urinary incontinence is a major public health problem, with psychological and social effects and with an important impact over the quality of life. Stress urinary incontinence is defined as involuntary leakage of urine through the urethra during physical activities which increase the intra-abdominal

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pressure, like laughter, coughing and sneezing, climbing stairs. There are numerous factors which contribute to the appearance of SUI: muscle weakness of pelvic diaphragm secondary to vaginal births, gynecological interventions, overweight and obesity, hormonal disorders. When the conservative treatment (bladder control exercises and pelvic floor muscle training, lifestyle modifications such as dietary habits, weight loss and physical activities) fails, the surgical treatment is a viable solution for SUI.

The first surgical treatment for stress urinary incontinence was described by Burch in the early 1960s and for almost 30 years, till the mid 1990s, it has remained the standard treatment for stress urinary incontinence. The long term follow-ups have suggested that this type of surgical treatment is effective, with a satisfaction rate at 5 years after the operation of nearly 80% and at 10 years of 70%. At the beginning of the 1990s, DeLancey established that the physiopathology of the SUI is associated with a defect at level of the bladder neck and urethra, related with the laxity of the surrounding tissue and as well as with the insufficiency of the urethral sphincter. He reported that the urethra lays on a layer of the endopelvic fascia and anterior vaginal wall and it is stabilized to the lateral by attachements of the levator ani muscle and arcus tendinous fascia. The urethral closure is conditioned by the urethral compression against the support offered by the anterior vaginal wall and the pubocervical fascia. This was the beginning of a new era in the treatment of SUI, the minimal surgical invasive treatment which would prove to be very effective.

In 1995, Ulmsten and Petros described the minimal invasive technique of retropubic tension-free vaginal tape. It was the first generation of sling operations, which used a polypropylene mesh. This procedure involves the blind penetration of the paraurethral and the retropubic space with a needle and the operator must exit with the needle behind the pubic symphysis. Because of its good results and low complication rate, it has become the solution for SUI. It is cited in the specialty literature that the rate of success ranges from 80 to 95%, after more than 5 years after the operation. Although the effective rate was high, it exist the intraoperative risk of complications such as bladder and bowel perforation and vascular injuries. As a solution to these concerns, Delorme described in 2001 another minimal invasive technique, the transobturator tape procedure. As well as the TVT the TOT is also a blind procedure. During the TOT surgery the puncture is performed at the clitoral level of the inferior ramus of the pubic bone and the operator must pass with the needle through the obturator foramen. This is the second generation of sling operations for SUI. It has been proven that the efficacy of the TOT procedure is similar to the TVT and with this type of procedure the risks of perforating the bladder or the bowels have disappeared, but more patients have groin pain postoperatively and vaginal or urethral erosion due to the polypropyline mesh. Both procedures are in current use for the SUI treatment, but because of its lower complication risks and similar efficacy, the TOT procedure is more popular.

MATERIALS AND METHODS

The purpose of this paper is to present our experience at the Urology Clinic of the Central Military Emergency University Hospital “Dr. Carol Davila” regarding the minimally invasive surgical treatment of stress urinary incontinence, using the vaginal sling procedures of TVT and TOT. We have analyzed several variables like: patient age, the number of vaginal births, body mass index, and gynecological interventions like hysterectomy. We present our cases and experience regarding perioperative complications and their management and as well as the efficacy rates, compared with other studies.

Between March 2010 and November 2015, in the Urology Clinic of the Central Military Emergency University Hospital “Dr. Carol Davila”, a number of 68 patients with stress urinary incontinence have been operated, using the minimally invasive technique of retropubic tension-free vaginal tape or the transobturator tape procedure. Out of the 68 patients who were admitted in our clinic for the surgical treatment of SUI, a number of 52 patients have undergone the transobturator tape procedure and only 16 patients were operated using the tension free vaginal tape technique. The TVT procedure was used only in the cases of the patients who associated genital prolapse. TVT has the same assembly principle as the TOT, but the incisions are made in the suprapubic region. The intraoperative complication risks, like bladder or bowel perforation, are higher in the case of the TVT compared with TOT. Therefore cystoscopy is essential after puncture. In all the cases with TVT we have performed cystoscopy to exclude a potential bladder perforation. Before the operation, all the patients were examined and the indication of the surgical treatment was established on the basis of physical examination (all the patients had positive cough test), abdominal ultrasound (to determine postvoid residual urine volume), urodynamic studies (testing was not performed in all the cases), urinalysis and urine culture (the majority of the patients have had before the operation recurrent urinary tract infections).

RESULTS

The mean age of the patients was 60.9 years. The patients were in a proportion of 78.9% at menopause and the average number of natural births was 1.9 ranging between zero and five. Mean BMI was 28.2, ranging from 23.3 to 37.4. Regarding the surgical duration this has varied between 20 and 40 minutes, being higher in TVT cases (up to 5-7 minutes longer than TOT), because cystoscopy was performed during the operation to verify if the bladder was perforated or not. The mean operative time was 27 minutes. Both surgeries required a 3 day hospitalization, the patients being discharged in all the cases the day following the surgery. The urethral catheter was removed immediately after the operation in the case of the TOT procedure and in the day following the operation for the patients who have undergone TVT procedures (all of these patients associated genital prolapse). In what concerns the urinary residual volume in the first days after the operation, we haven't noticed significant differences between the two types of procedures (the patients didn't accuse incomplete bladder voiding). All the patients were reevaluated at one month after the operation using the cough test, abdominal ultrasonography to determine the postvoid residual urine volume and to find potential complications secondary to the procedure. The long-term treatment outcomes were evaluated after 3, 6 and 12 months. Regarding the patients satisfaction, it was considered after the evaluation that the SUI was cured if the patient was completely continent, improved if partial urinary incontinence remained after the surgery and failed if the urinary incontinence remained the same or got worse after the surgery. The patient satisfaction was similar in both groups of patients, with an average satisfaction rate of 91% for TVT and 86% for patients who had undergone TOT procedure. Our results are in line with data from specialty literature.

J. Y. Han et all in a study about the effectiveness of TVT and TOT procedures in women with overactive bladder and stress urinary incontinence, stated that TVT and TOT procedures have decreased the proportion of the daily incontinence in these patients, with a slightly higher efficacy in the TVT group. In a study published in 2013 the ”Scandinavian Journal of Urology”, V. Nyyssonen concluded that 70% of the patients that were included in the study, with symptoms of detrusor over activity, declared significant improvement after the mid urethral tape surgery. There was no significant difference between the two operating techniques.

Cerniauskiene and collaborators in a comparative study regarding TOT and TVT outcomes reported that the satisfaction rate for the patients who have undergone the TOT procedure was 81,3%, meanwhile the satisfaction rate for the TVT group was 90%.

Zyckowsci et al stated in their comparative study regarding the TVT and TOT surgery, that the patient satisfaction rate at 6 months after surgery was 96,4% for TVT patients and 96.1% for TOT patients. Regarding the cough test and pad test, they have stated that it was negative in 91.5% and 94.4% in TVT cases, and in 93% and 93% TOT cases. The findings in our study were similar to general literature. We have observed in our study that the cough test was negative for 91.2% of TVT cases and 92% of TOT cases at 6 months, and the pad test was negative for 94% of TVT cases and 92.8% of TOT cases at 6 months postoperative. We haven't encountered significant intraoperative and postoperative complications. We haven't had significant bleeding during the interventions. The mean blood loss was under 25 ml. Local hematoma was encountered in 5 TOT cases and in only one TVT case and conservative treatment was effective. We haven't had any bladder or bowel perforation complications. We have encountered one case of relapse of incontinence due to the exaggerated physical activities, at two and a half years after the operation. In this case, we have performed a second intervention. We have removed the mid urethral portion of the tape and we have performed the tension-free vaginal tape procedure. The results at 3 and 6 months after the operation were good, although it is mentioned in general literature that in the case of reintervention for relapse of SUI the outcomes may not be as good as in the case of the first operation.

De novo overactive bladder symptoms ranged between 3 and 4.5% without differences between TOT and TVT patients. The pharmacological treatment which was applied in these cases has had good results. There are studies in which it is mentioned that the incidence of the overactive bladder symptoms ranges between 4 and 30 % after the operation.

Bladder botulinum toxin A injection and percutaneous sacral nerve modulation have proven to be good alternatives for the TOT and TVT surgery. Since its introduction (2005-2006) the number of patients who were admitted for bladder botulinum toxin A injections has increased constantly, overtaking in 2010 the number of TVT/TOT procedures. So far we haven't applied this method of treatment for the SUI.

With the passing of the time, the number of patients who have undergone TOT/TVT surgery and have chosen to remove their tapes grows. Most tape removals are performed at a relatively short time after the procedure, the main cause being urinary retention or tape extrusion. We haven't encountered such situations in our study.

CONCLUSIONS

The minimally invasive surgical treatment of SUI (TOT/TVT) has become the gold standard for patients with this debilitating condition. Mid-urethral sling surgery is performed to fix the urethra and involves few anatomic changings in the surrounding tissues.

These procedures have proven to be highly effective, increasing the quality of life for the patients who suffer from SUI. Regarding the efficacy rate of this two type of procedures it hasn't been noticed significant differences in the study that we have done, our results being in line with specialty data.

In terms of complications the risks are higher for the TVT procedure, due to the risk of bowel or bladder perforation. To exclude this possible accident, which may happen during TVT, cystoscopy is essential. Therefore, the TOT procedure has become more popular.

Till the introduction of TVT and TOT, SUI was either simply accepted or required invasive surgery.

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CLINICAL PRACTICE

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Penile fracture – clinical case presentation

C. Farcaș[1], V. Botea[1], R. Popescu[1], Marius Dinu[1], Ovidiu Bratu[1,2], D. Spînu[1], Dan Mischianu[1,2]

INTRODUCTION

Fracture of the penis is a rupture of the tunica albuginea of the corpus cavernosum, usually while the penis is rigid and erect. When erect, the albuginea layer stretches so far until its thickness reaches approximately 0.25 – 0.5 mm, compared with a 2 mm thick when flaccid. Thus the erect penis is much more vulnerable to serious damage from blunt trauma than the pendulous, flaccid penis. The most common cause of penile fracture is vigorous vaginal intercourse, more likely when woman is on top, but it can also occur during masturbation, forced bending of the erect penis or any mechanical trauma to the erect penis. The classic presentation described by the patient cracking or popping sound, followed by rapid detumescence, severe pain, ecchymosis of the penile skin, and swelling of the affected side with deviation of the penis shaft away from the affected side. In most cases, the penile fracture is a transverse corporeal tear close to the corpus spongiosum on the ventral aspect of penis shaft or on the lateral aspect and the tunical tear is usually 1 to 2 cm in length. The rupture can involve one or both corpora cavernosa as well as corpus spongiosum with the contained urethra therefore urethral rupture can also occur in. Most urethral injuries are associated with gross hematuria, blood at the meatus or inability to void, although the absence of these findings does not definitively rule aut urethral injury.

DIAGNOSIS AND IMAGING

The penile fracture is considered by the most to be a clinical diagnosis, with little need for imaging if the patient presents with a convincing history and physical examination. However given that urethral injury occurs not infrequently, preoperative exploration of the urethra should be considered when urethral injury is suspected (urethrography or flexible/rigid cystoscopy). There are numerous reports of the accuracy of cavernosography and magnetic resonance imaging (MRI), inspite of their accurate results, they are sometimes too invasive, time consuming or costly to justify, particularly when the diagnosis can be established by clinical examination. Ultrasonography although noninvasive and easy to perform is also associated with significant false-negative studies. Another condition that may mimic penile fracture is rupture of the dorsal penile artery or vein during sexual intercourse, in these circumstances physical examination may not be adequate for definitive diagnosis of a corporeal tear and further investigations should be performed like MRI, ultrasonography or even surgical exploration.

MANAGEMENT OF PENILE FRACTURE

Multiple publications indicate that suspected penile fractures should be promptly investigated and surgically repaired. Many reports show low complication rate and good outcomes as a result of immediate surgical repair, (less than 5% penile curvature after immediate surgical repair – El Atat et al, 2008) as the favorite approach for treatment of this urological emergency. Conservative management can also be an option in patients with uncomplicated albugineal tears, without extensive hematoma or urethral injury, but most untreated fractures usually develop delayed and persistent penile pain or curvature with erection. Conservative management of penile fracture has been associated with penile curvature in more than 10% of patients, abscess or debilitating plaques in 25% to 30%, and significantly longer hospitalization times and recovery (Meares,1971; Nicolaisen et al, 1983; Kalash and Young, 1984; Orvis and McAninch, 1989).

Although small lateral incisions may be used for localized hematomas or palpable tunical defects, a distal circumcising incision is the best option in most cases thus providing exposure to all three penile compartments. For the closing of the tunical defect is recommended to use interrupted absorbable suture 2-0 or 3-0. Deep corporeal vascular ligation or extensive debridement of the underlying erectile delicate tissue should be avoided. Timing of the intervention may also influence the long-term outcome of erectile function or postoperative complications in the way that the patient undergoing surgical repair intervention in the first 8 h after injury has significantly better long-term results than those having surgery delayed 24-36 h after fracture occurrence (Asgari et al, 1996; Karadeniz et al, 1996).

PATIENT, METHOD AND RESULTS

A 31 years old patient presents in the emergency department in our hospital at approximately 03:15 a.m., this being the third medical unit he was attending that day, accusing pain in the external genital area associated with extended hematoma of the penis and scrotum with the deviation of the penis on the left side (Figure 1A). Further anamnesis discovers posttraumatic injury of the penis while sexual intercourse that happened at approximately 01:00 a.m. The patient describes powerful bending of the penile shaft, and at the same time strong local pain, accompanied by a snapping sound and after that immediate detumescence of the penis with decrease in pain intensity. Patient describes urine voiding normal and no bleeding out of the urethra.

Figure 1. Extended hematoma of the penis and scrotum and pathological deviation of the penis shaft on the left side (A)

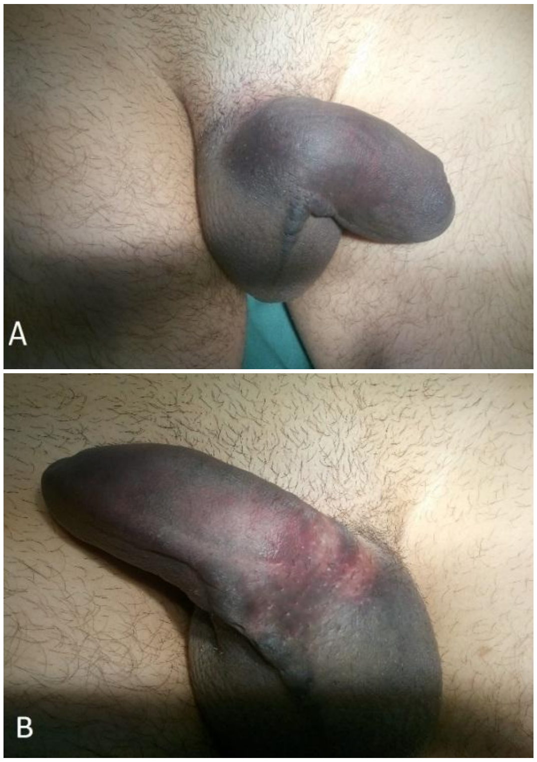

The patient is admitted in our department and undergoes a soft tissue ultrasound that reveals a local hematoma of the penis and a small breach of approximately 3-4 mm in diameter which is situated on the ventral aspect of the right cavernous body, of the proximal third of penis shaft.

Emergency surgery is decided, with spinal anesthesia at 08:00 in the morning (7 hours after the injury occurrence).

After local antiseptic measures, a 14 Fr. Foley catheter was placed before the beginning of the intervention. A distal circumcising incision is made at approximately one cm proximal of the coronal sulcus, followed by degloving the penis, and exposing the tunical fracture site in the albuginea.

The tunical tear was fond on the ventral aspect of the corpora cavernosum at a distance of 3-4 cm from the base of the penis.

Figure 2. A: albugineal tear on the left cavernous body. B: albugineal tear on the right cavernous body.

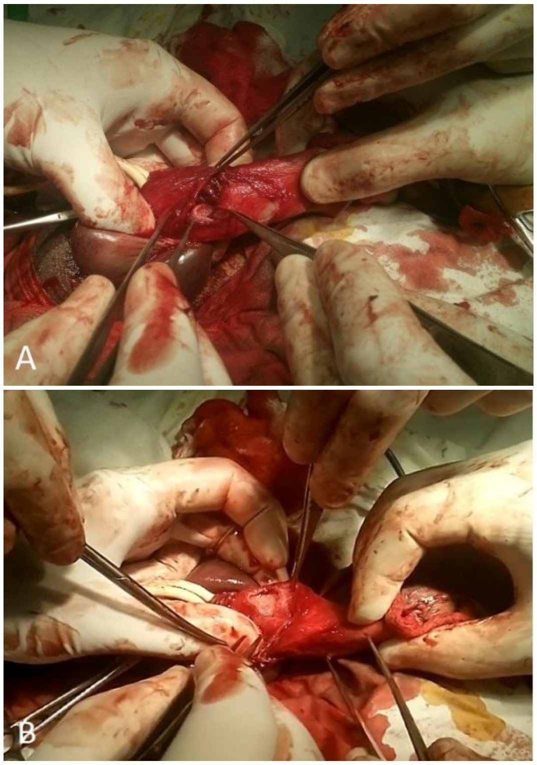

The rupture site was much greater than the one observed on the ultrasound scan. The real size of the tear was about 3 cm and involved both cavernous body on their anterior aspect, as well as the septum of the albuginea underneath the spongy body (Figure 2, A and B).

The spongy body was carefully dissected from the corpora cavernosa and so the entire length of the transversal rupture was individualized.

A temporary elastic Tourniquet at the base of the penis was necessary for local control of bleeding.

Defect repair was performed with separate absorbable 3-0 USP sutures. For the full length of the breach 14 to 15 separate sutures were needed. Final aspect of the repaired defect of the tunica albuginea can be seen in both pictures below (Figure 3, A-B).

The patient's evolution was favorable under antibiotic therapy for 5 days with no infectious complications. Second day postop he mentioned a mild morning erection although he was under treatment with Cyproterone acetate 50 mg twice a day and Diazepamum 10 mg a day in two doses.

Figure 3. A: ventral aspect of penis with the spongy body mounted on a lasso and the albugineal surure. B: final aspect after suture of the skin.

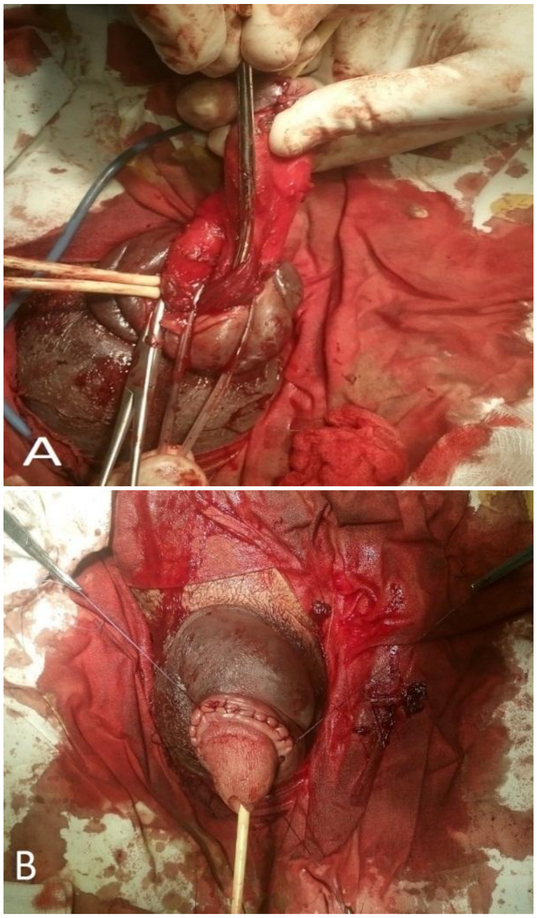

DISCUSSION AND CONCLUSION

Penile fracture is a rare case of urological emergency that should undergo in most of cases emergency surgical treatment.

The immediate surgical repair attitude (<8 h) is the preferred approach for treatment of penile fracture as reported in numerous centers around the world due to their low complication rates.

While the conservative approach is described in patients with small and uncomplicated tears, most unrepaired fractures develop late penile curvature or pain with erection.

Figure 4. Aspect of the penis and scrotum the second day postoperatively while patient still carries a Foley catheter.

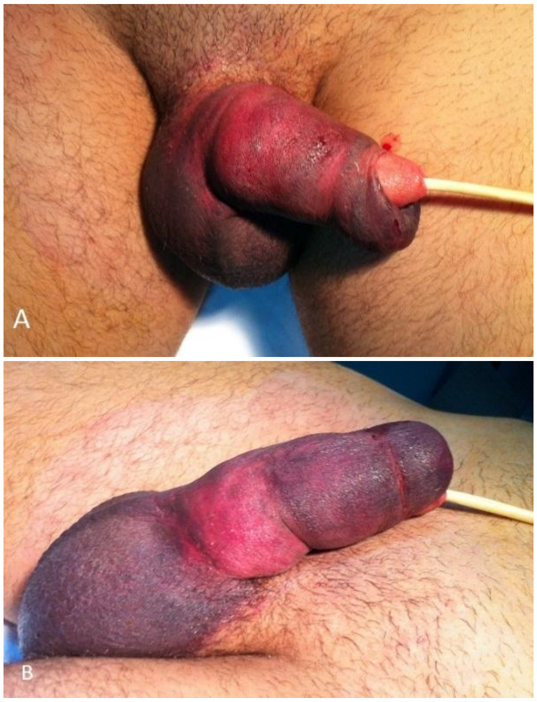

The urgency of surgical exploration justified in our case the discrepancy between imaging findings and the reality found during the surgical exploration.

Albugineal tear that involves the both corpora cavernosum is extremely rare and also usually is associated with urethral injury, which in this patient, the last one, was not the case.

The incidence of concomitant urethral injury is approximately 20 % in Europe and the USA. Most urethral injuries are associated with urethral bleeding or gross hematuria or even inability to void, but also, the absence of these signs does not exclude a potential urethral injury.

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EDITORIAL

About the beauty of urology…

Dan Mischianu

Gral (R) Prof DAN MISCHIANU

Chief of 3rd Clinical Department,
Faculty of General Medicine,
Carol Davila University of
Medicine and Pharmacy,
Bucharest, Romania

Urologic Surgery is one of the most beautiful, exciting, spectacular and unpredictable surgical specialties.

From 1907 it stands as a specialty of it's own, breaking free from general surgery, as other surgical specialties, Professor Felix Guyon being the godfather of this specialty. In the last 20 years, urology itself has become a "tree" of sub-specialties, from which medical branches such as andrology, urogynecology, oncological urology have developed. These medical strains tend to become independent.

From 1909 urology begins to be recognized in Romania, as a specialty of it's own, time when Professor Petre Herescu, Guyon's student, establishes this speciality within Coltea Hospital. Over time it has experienced a steady development as few specialties have had. Over the last century, romanian urology has given world renowned characters, which have settled romanian urology in a place of honor.

We have the strength to affirm that urology is "a friendly specialty", not only with the surgical specialties, but also with other medical specialties. There are many surgical and non-surgical specialties, whom we as urologists can offer our help, or an advise, or a surgical procedure. Urology can have harmonious collaborations with many other specialties, and I give as example, the selective arterial embolization after PNL or arterial stenting in erectile dysfunction, procedures which are performed by interventional radiologist.

Like in any other surgical specialty, the urologist has two important "friends", with whom the collaboration must be at the highest level. I refer of course to the anesthesiologist and to the pathologist.

Urologic surgery faces or may face urgent situations, emergencies which if not noticed in time and skillfully managed it could have a poorly outcome for the patient. Among these situations, we give as an example just a few: obstructive anuric acute renal failure, urosepsis, urological trauma with hemodynamic instability etc.

We haven't taken into account the postoperative hematuria, on one hand due to urological diseases, but also secondary to various other conditions, such as an eventual hepatic cirrhosis or secondary to an overdose or misuse of anticoagulants, out of which we are almost convinced that it is a practice at "national" level at the indications of the anesthesiologists.

These pathologies mentioned above are just a few aspects of what it represents the "salt and pepper", but also the challenges of an urologist's life.

While the general surgeon performs, with difficulty, for example a retrocecal appendectomy and waits with some concern for the 5th day peritonitis, the urologist may be in the situation, in which he must promptly intervene at 2-3 hours after the patients admission or after a correctly performed intervention, to stop a bleeding which may occur without any explanation or for an obstructed drainage.

I have discovered slowly and quite late the charm of urology. Later I have learned that it is a surgical specialty much better encoded than abdominal surgery. Urology has an ethiopathogenic and topographic diagnosis more accurate and much more faster. I have later discovered it's minimally invasive valences regarding diagnosis and treatment, for example the ability to perform endoscopic surgery, laparoscopic and robotic procedures, with the possibility of "one day surgery" hospitalization and with maximum results for the patient. In this context it should be noted that robotic surgery, very popular nowadays, was initially born in the specialty of urology, for one purely urologic intervention, the radical prostatectomy.

Certainly the technological developments have greatly influenced the evolution of urology. There have been no new surgical devices that couldn't be used in urology. Nowadays the classical interventions, have come to have a smaller proportion compared with those listed above. For instance, regarding urinary lithiasis, only 1% of cases are treated using open surgery.

I do not know how urology will look over a few decades. Perhaps in the future and one not so distant, the young residents that I try to teach and mold, will operate Earthlings on another planets, using consoles such as the "Da Vinci " from now...

Finally, without having a morbid curiosity, I am satisfied with what I have seen, realized and undertook during my active urological lifetime!

REVIEW ARTICLE

Article received on September 12, 2015 and accepted for publishing on October 21 2015.

Minimally invasive surgical techniques – robotic surgery

Florin Săvulescu, Costin Duţu, Cristian Cârlan

Da Vinci robotic surgical platform is the result of many years of research of the military physicians from United States Army. This platform was initially intended to operate in close proximity to the battlefield, it was supposed to allow for complex surgeries without threatening medical personnel, but also to ensure a high survival rate among the wounded. Basically, after a soldier was wounded, he was supposed to be taken away from the front line, transported to the first sanitary structure which had this platform available and a surgical team from long distance, satellite connected, performed the surgery. The advantages of this course of action are related to the fact that the team is permanently safe and the injured person benefits from the experience of a well-trained surgical team.

Due to the large amount of information transmitted between platform components, which means millions of operations performed each second, and the impossibility to secure a channel for information dissemination, even more difficult in a hostile environment such as in the vicinity of the front line, and the long preparation of a patient to be subjected to a robotic surgery, the platform is not in current use of military doctors, the way it was originally intended. So far, only one surgery was performed in which the operator was far away from the patient. The two surgical platform elements were arranged one in the United States and one in France. During the surgery, the transfer of information was made by satellite, with great efforts to secure the information channel, as well as with the suspension of any satellite phone links between the two countries.

The advantages desired by military doctors have proved to be far from being put into practice, however, their research work led to the development of the best technology that allows for minimally invasive surgery. In 2015, worldwide, about 3,400 platforms daVinci were installed: 2295 in the United States of America, 573 in Europe and 7 in Romania.

Robotic surgical platform consists of several components which will be described below. Using the system is only possible if all three components are available, none of them is optional. Although there are three components, they are interconnected and have the same purpose: to allow for a minimally invasive surgery in maximum security conditions for the patient. The system combines technological advantages: high resolution three-dimensional image, video image stability and filtering of surgeons' hands unwanted movements, with the surgeons' experience and ability to make decisions in real time.

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Operator surgeon’s console consists of three parts. The video system allows viewing intracorporeal images in a tridimensional manner thanks to the two images submitted separately for each eye; here, there is also a number of sensors that detect the presence of the surgeon at the console, this thing is necessary as a safety measure to block chaotic motion of the device arms inside the body if the operator is absent. The second component of the console is represented by the console commands for each hand, called "master controls" and a series of commands for adjusting an ergonomic position. This component records the surgeon's hand movements allowing this information to go to the working forceps. The pedals system is the last component of the console, here it is possible to activate several functions for the working tools: electric coagulation or sectioning, handling video camera, change the working arm: the surgical system has three arms and it is necessary to change one tool with another for a certain hand. The whole assembly allows adjustment on more axes of all the components, depending on the preferences of the operator, the scope is to obtain an ergonomic position. If more surgeons use the equipment, the system stores each surgeon’s preference, and when the personal account is activated, it recreates the ergonomic position preferred by each surgeon.

Figure 1. The working console on which one can notice the three subcomponents from top to bottom: video playback system, master control, crank

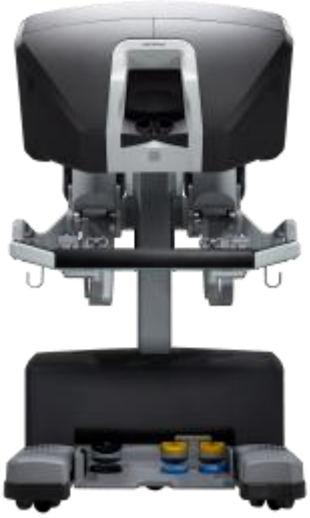

Patient's charter is like a tower which has three working arms attached and a video camera arm. This charter, as its name implies, is beside the operating table. On each working arm, a certain tool is fixed and it is activated by a pulley system that allows movements in the seven degrees of freedom. Patient's charter only performs commands coming from the surgeon’s console, it has no autonomy to make decisions, it can not be programmed to execute movements without commands in real time. Fixing / replacing instruments is performed by a surgeon who is next to the patient, he is also the one who places all working cannula into the patient’s body. The working tools have a diameter of 8 mm and reproduce, in miniature, the instruments used in open surgery.

Figure 2. Patient's charter with the three working arms for tools and the video camera arm.

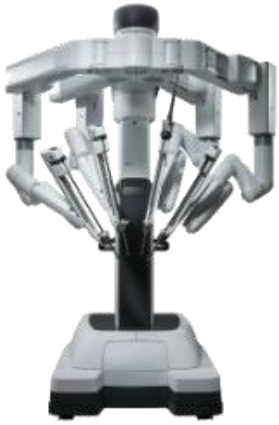

Figure 3. Working tools

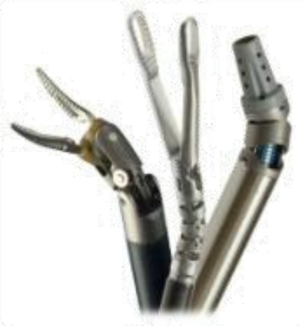

The video system displays intracorporeal images on bidimensional monitors in the operating room. This system allows recording of images and setting of certain parameters. Here lies the source of light transmitted to the intracorporeal video camera through an optical fiber. All components described above are dependent on the presence of at least two surgeons, the system is not designed to fulfill the role of the surgeon but to support surgery by involving the present technological advantages.

Figure 4. Video tower and bidimensional monitor of high resolution

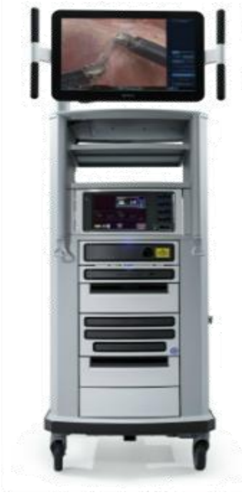

The three systems are interconnected by optical fiber since the information flow is very high.

Robotics is a minimally invasive technique, similar to laparoscopic surgery, in which working tools are inserted through small incisions of 5-15 mm in the peritoneal cavity through which cannula are introduced to work. Comparative studies between open surgery and robotic surgery have highlighted the following advantages in favor of robotic surgery: shorter stay in hospital, reduced bleeding, less postoperative pain, faster social reinsertion, small incisions that allow a much quicker recovery and lower risk of developing eventrations. Patients who have no indication for minimally invasive surgery, do not have indication for robotic surgery either.

The recommendations of robotic surgery are the surgeries that require access to limited spaces, inaccessible for open surgery and restricted by the small maneuverability of laparoscopy instruments. In urology, robotic prostatectomy is considered as the "gold standard". As a general idea, robotic surgery approach is indicated for the organs situated at the top and bottom pole of the peritoneal cavity, where limited space and difficult access are not impediments for daVinci surgical system instruments.

Complications can occur in robotic surgery just as it happens with any surgery, the maximum risk is death. Complications that may arise during surgery are: intraperitoneal organs injury, intraoperative bleeding, septic complications. Another factor to be taken into consideration is the human error, system failures or potential complications of anesthesia.

At this time, da Vinci surgical platform is the most advanced tool that allows a minimally invasive surgery. A very important element about the functioning of this system is that it can not "operate" independently; it cannot be programmed to carry out independent actions. It must be seen only as an extension of the arms of the surgical team members, executing commands coming from the surgeon at the working console, under the supervision of at least one surgeon.

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SYSTEMATIC REVIEW

Surgical team and surgical communication

Costin Duţu[1], A. Luchian[1], Elena Stârcu[1], Florin Săvulescu[1]

A significant percentage (43%) of medical errors is caused by errors in communication between healthcare professionals or between them and the patient. Today the surgeon has a multiple role (leader of the surgical team, member of the medical team, scientific role, management role)(1).

The surgical team has the duty to ensure and promote a positive work environment that improves team performance and maximizes outcomes for patient's safety (2).

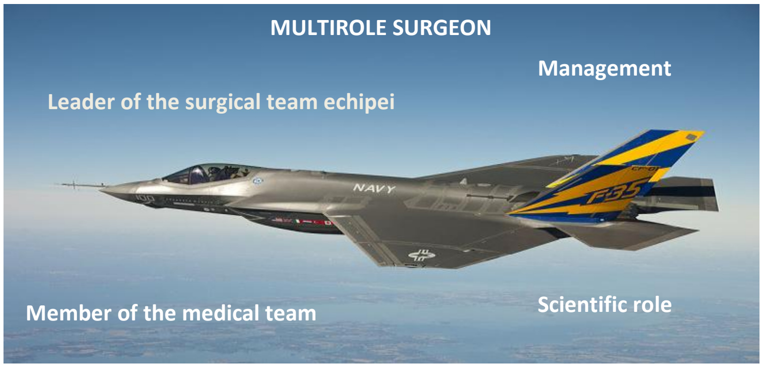
MULTIROLE SURGEON
Management
Leader of the surgical team echipei
Member of the medical team
Scientific role

The most important attributes, that are critical to the development of high performance teams, are membership and leadership.

Membership presupposes the following (6):
- To understand the role and responsibilities of the teammates
- To encourage input from all members (young people!)
- To respect the role and competence of other specialties colleagues
- To respect team leaders
- To share the objective of high quality in patient treatment
- To trust the right to speak and to intervene
- Commitment to teamwork for the patient's interest

Leadership comes with a clearly defined role, especially in critical situations, which implies a style that minimizes the difference in status and power, suitable under particular conditions, designed to seek permanent information from team members and make decisions based on these (6).

Effective communication in the surgical team should be:
- Complete
- Clear – fully understood information
- Short – concise and organized information
- Relevant (timely)

In order of most important characteristics, surgical team members should feel engaged and involved in a common goal, together with mutual trust and respect (2).

In a safe interpersonal environment, team members

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should not hesitate to express their views, to challenge each other positively, to raise certain issues without fear of ridiculous or subtle attacks. They must also feel safe to discuss errors and mistakes (5).

Table 1. Behavior strategies which influence the performance of the surgical team (7)

Positive attitudes	Negative attitudes
Listening to and exploring others' opinions	Aggressive challenges
Clarification of ideas; Requests for alternative explanations, thoughts or opinions	Distrust of alternative ideas
Supporting and encouraging members of the surgical team	Sarcastic behavior
Challenge of colleagues in a respectful manner regardless of their status	Ignore, dismiss, mock at other points of view
Disputes resolution in a constructive way by explaining the decisions that were made in a fair manner	Assuming to know the absolute truth due to position or knowledge
Appreciation, recognition of merits, contributions from others	Lack of appreciation and regular criticism without recognition of positive contributions
Search and accept feedback (positive or negative) regarding one's contribution and performance	Feedback ignorance and disregard
Own assessment and evaluation of the performance and contribution within the team	

Team STEPPS - team actions (4).

1. BRIEF: short session before surgery, discussing the team members, the role of each team member, expectations setting, discussing potential problems, anticipate unexpected things

2. HUDDLE: ad hoc session to restore certain conditions, situations, plans

3. DEBRIEF: Discussion performed after surgery, meant to improve team performance - aiming at understanding the role and responsibilities of each team member; surgical equipment problems; other improvements to the surgical procedure; lessons to be learned further.

Within a team, an important thing is the coordination of tasks leading to a better awareness of each individual performance and also of the team performance, meant to encourage the members' desire to offer support to the others, thereby increasing the ability of the team to remain flexible and adaptable to changing circumstances (3).

A simple way to review and reflect on performance is to answer the following questions:
- What did I do well?
- What could be done better?
- What should we not do anymore?
- What should we continue to do?

Table 2. Errors observed in operating rooms classified by behavior categories (8)

Behavior category	Errors
Communications/decisions	The surgeon does not provide enough information to the anesthetist
Preparation/planning/ vigilance	The inability to predict certain events during a complex procedure; Failure to monitor other activities of the team
Information dissemination	Failure to inform own team
Inquiries/statements	Failure to comply with the conclusion of a discussion on an alternative procedure
Interpersonal relations/ group climate	Hostility and frustration due to poor team coordination
Auto criticism	Inability to learn from certain situations
Disputes management	Disputes between doctors from the surgery team and anesthetists not solved.

Figure 1. The structure of a performing team (9)

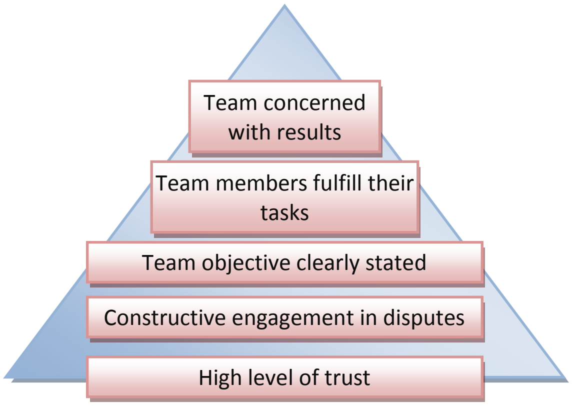
Team concerned with results
Team members fulfill their tasks
Team objective clearly stated
Constructive engagement in disputes
High level of trust

CONCLUSION

Communication skills are a key component in surgical practice, which can be taught, learned, improved and this will maximize performance and enjoyment in clinical practice.

Interprofessional communication problems are the leading cause of many medical errors.

Structured communication protocols such as SBAR are designed for effective and complete communication (4).

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SYSTEMATIC REVIEW

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Clostridium difficile – an emerging plague

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Abstract: Introduction: Clostridium difficile infection stands nowadays as one of the major emerging health problems still underestimated. Only in 2009 the European Society of Clinical Microbiology and Infection (ESCMID) was able to publish guidelines for this serious disease. Actually the guidelines were updated in 2013 so we can speak of a united action towards the resolution of this healthcare problem.

Methods: This is a review article aiming to shed light in various aspects of clostridium difficile infection as clinical presentation, symptoms and signs, therapeutic options and risk factors with a focus on urologic pathology.

Conclusions: This type of infection represents a challenge from many points of view like treatment, relapse approach, mortality, morbidity, germ' s adaptability to treatment and last but not the least the outbreak of different aggressive strains like B1,NAP1 or ribotype 027 toxinotype III.

Keywords: Clostridium difficile infection, treatment, diarrhea

INTRODUCTION

C difficile was first described in 1935 by Hall and O'Toole as a Gram positive anaerobic commensal rod[1]. It took 43 years to discover the C difficile toxin in patients with pseudomembranous colitis.

The classic strains release two toxins A and B both of them monoglycosyltransferases causing colonic tissue damage. The name Difficile was given because of the the difficulty they had culturing this anaerobic bacterium on conventional media.

The interesting part is that from a nosocomial infection nowadays it appears to have spread to communities and what is more problematic to antepartum period, pregnant women and children[2].

The problem with this pathogen was that it evolved. In the early 2000 there was an outbreak in North America and Europe with a new strain, the ribotype 027 strain which has evolved and adapted. Metronidazole resistance was higher; the strain had modified genetic structure permitting to produce much more toxins than the classic types and incorporated another binary toxin which role is still unknown[3,4]. First time discovered in 1980s it presented high resistance to fluoroquinolones.

METHODS

A comprehensive search using PubMed, Medline and Google Scholar without publication status or other restrictions was conducted.

Studies using comparaisons between different antibiotics, reviews of risk factors, case presentations and all sorts of guidelines were included. The main

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objective of this paper was to review the main features and also to raise awareness of this health problem.

RESULTS

Diagnosis

Diagnosis is based on: clinical presentation, diagnostic tests, imaging and miniinvasive surgery.

The classical clinical exam reveals usually diarrhea in a patient with current or recent antibiotic use, frequent loose or watery stools. Occult blood or mucus may appear but melena or hematochezia are rare. Sometimes patients can develop colonic ileus or toxic megacolon. Both of them are very serious and unfortunately because of the ileus there can be no diarrhea so there can be a fatal delay in diagnosis. In this case diffuse abdominal pain, fever, abdominal distension are precious clinical signs. Finally there are those patients with very serious signs like septic shock or multiple organ failure.

All diagnostic tests have their strengths and weaknesses, the most used are EIA (enzyme immunoassays) for toxins A and B and then we have PCR, tissue culture cytotoxicity and assays for clostridial glutamate dehydrogenase (as a first line test).

Imaging unfortunately it is not specific for this kind of disease; still an abdominal X-ray or a computed tomography is of great value when we have to differentiate for other cases of occlusion or ileus.

There is another way of diagnosing the infection; still it holds a higher degree of invasiveness with all the secondary aspects.

We are talking about colonoscopies which can biopsy the membranes and solve the diagnosis in an ironclad manner.

Risk factors

Antibiotics

Multiple studies, some of them multicentric focused on what classes of antibiotics are involved in this disease. Most of them imply clindamycin, fluoroquinolones, cephalosporines(generations II, III, IV but not first generation). Rarely are cited also carbapenems and trimethoprim/sulphonamides, also peniclins seem to be involved[5].

Proton pump inhibitors and H2 antagonists

Normally they should be in same group but this affiliation is a bit forced. Proton pomp inhibitors are one of the most used therapies worldwide instead of H2 antagonists. Also there are some studies that recommend the use of H2 antagonists instead of proton pump inhibitors. Their role as a risk factor or not is not so clear. Some of them say that more than 2 days of proton pump inhibitor greatly increases the risk to develop C Difficile infection[6,7].

There are studies that demonstrated that one of those two protective agents can be administred concurentially with C Difficile treatment may it be metronidazole / vancomycin / fidaxomicin[8]. There are also studies that could not find any connection between the infection and the use of PPI[9].

Age> 65 years

This is one of the risk factors almost universally recognized. Almost all organisms at this age present somehow reduced turn-over, their innate resources are more limited so the immune response in this case is not so good, and the antibodies production rate is not so high so the host has lesser chances to suppress the infection.

Underlying illness

It is pure logic to assume that a weakened body would be more presumptuous to an infection. There are studies that associate this factor to the development of severe C. Difficile infections. In fact most of the studies at least agree to two of these factors respectively age and underlying illness.

In this category we include also neoplasia, gastro-intestinal surgery, naso-gastric tubes and gastro-intestinal disorders.

Long term hospitalization

This is also true for nursing homes. Both types of facilities present a high risk of infection given the high concentration of resistant germs and the difficulty to identify and isolate the infected persons. C Difficile is a spore type germ so the isolation is a must for the infected, and in this dormant form it can stay alive for months, so it is easy to assume the worst has passed[10].

The European Society of Clinical Microbiology and Infectious Diseases established a biochemical set of risk factors to predict an increased risk of developing severe disease[11].

1. Marked leucocytosis (leucocyte count >15 9 109/L)

2. Decreased blood albumin (<30 g/L)

3. Rise in serum creatinine level (≥133 lM or ≥1.5 times the premorbid level)

Treatment

For decades the only recognized and valuable tools were metronidazole and vancomycin. Both of them still stand as valuable medication.

A new antibiotic appeared a few years ago the fidaxomicin. As a last resort the infectionists use last tier drugs like Tygecycline.

Until 2009 there were no regulations for the treatment of this infection so there were a lot of medication schemes, some of them more or less efficient.

Fortunately nowadays there are international guidelines for the treatment of this infection. Less used substances have been tried some of them using great promise (Teicoplanin, Nitazoxanide, Rifaximin).

Tygecycline is a new glycylcyline from the tetracycline drug class. It has been shown to have activity against a wide variety of bacteria, including the antimicrobial-resistant strains. As with all tetracycline drugs, it is not recommended for pregnant or nursing women. One of the most important aspect especially for the urologic patient is that renal function monitoring is not necessary.

There are studies which compare vancomycin with fidaxomicin. Cure status are similar, however, fidaxomicin is associated with significantly lower recurrence rates than vancomycin for patients infected with non-NAP1 strains of C. difficile. For patients with the NAP1 strain, recurrence rates did not differ by treatment.

Emerging therapies

Toxin-binding resins and polymers and probiotics are two of the novel therapies which have been used in the treatment of this infection.

Unfortunatelly the results are still inconclusive, they are included in guidelines but not as a stand alone treatment but more as a adjuvant therapy[12].

As for immunotherapy, human monoclonal antibodies have been used with success, the main shortcoming is that the studies included few patients and we can not generalise the results. Still they show promising results some of them being in phase III trial.

In one of those studies at about 4 weeks after the resolution of symptoms the diarrhea reappeared. The question asked was if it was a relapse or the lifetime of antibodies just ran out and the organism was left defenseless[13].

European Society of Clinical Microbiology and Infectious Diseases

In case of non-severe infection (no signs of severe colitis) in non-epidemic situations and with CDI infection clearly induced by the use of antibiotics, it may be acceptable to stop the inducing antibiotic and observe the clinical response for 48 h, but patients must be followed very closely for any signs of clinical deterioration and placed on therapy immediately if this occurs.

Metronidazole is recommended as oral antibiotic treatment of initial CDI in mild/moderate disease.

There is insufficient evidence to support administration of probiotics, toxin-binding resins and polymers, or monoclonal antibodies.

Based on its pharmacokinetic properties vancomycin is considered superior to metronidazole in severe C. difficile disease.The use of high doses of vancomycin (500 mg orally four times daily) was included in the Infectious Diseases Society of America/Society for Healthcare Epidemiology of America treatment guidelines for management of severe complicated CDI as defined by the treating physician. However, there is insufficient evidence to support the use of doses >125 mg four times daily in the absence of ileus.

Fidaxomicin was not inferior to vancomycin for initial cure of CDI, but there are no data available on the efficacy of this drug in severe life-threatening disease.

Total abdominal colectomy should be performed to treat CDI in case of:
_ Perforation of the colon
_ Systemic inflammation and deteriorating clinical condition despite maximal antibiotic therapy; this includes the clinical diagnoses of toxic megacolon, acute abdomen and severe ileus. Colectomy should preferably be performed before colitis becomes very severe. Serum lactate may, inter alia, serve as a marker for severity (operate before lactate exceeds 5.0 mM).

A future alternative to colectomy may be diverting loop ileostomy and colonic lavage, combined with antibiotic treatment (intracolonic antegrade vancomycin and intravenous metronidazole).

Treatment of the relapse

There is evidence that suggests that vancomycin and fidaxomicin can be equally used in case of relapse. What is to be observed is that metronidazole is considered as a second line for the treatment of relapse.

In case of a secondary relapse or multiple relapses the medication conduit remains basically the same with some alterations of the alternative therapies.

DISCUSSIONS

This type of infection is usually an underestimated one. From the surgeon's point of view it may mean less but in our case the urologist must be very careful when dealing with this type of disease.

Not only most of the patients are > 65 years old but we must not forget that urinary sepsis is one of the most insidious ones, usually presenting with few symptoms and signs.

Being unable to diagnose fast enough the Clostridium Difficile infection usually is fatal for an old already surgically manipulated patient with lots of co morbidities.

Another problem luckily rare is the time to operate a patient with toxic colon. It is difficult to differentiate between the underlying surgical problem of the patient (may it be kidney, urether, prostate or bladder) and the acute onset of a colonic disease.

More, in these cases we find an ileus that is masking the underlying diarheea, making the moment to intervene very hard to choose.

It is very important for the urologists to be aware of this type of disease, to be capable of dealing with it and most important to recognize it fast enough.

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SYSTEMATIC REVIEW

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Neuroendocrine differentiation in prostate cancer – a review

R. Popescu[1], Ovidiu Bratu[1,2], D. Spînu[1], D. Marcu[1], C. Farcaș[1], Marius Dinu[1], Dan Mischianu[1,2]

Abstract: Objectives: This review aims to provide practicing clinicians with the most recent knowledge of the biological nature of prostate cancer especially the information regarding neuroendocrine differentiation.

Methods: Review of the literature using PubMed search and scientific journal publications.

Results: Much progress has been made towards an understanding of the development and progression of prostate cancer.

The prostate is a male accessory sex gland which produces a fraction of seminal fluid. The normal human prostate is composed of a stromal compartment (which contains: nerves, fibroblast, smooth muscle cells, macrophages) surrounding glandular acins – epithelial cells.

Neuroendocrine cells are one of the epithelial populations in the normal prostate and are believed to provide trophic signals trough the secretion of neuropeptides that diffuse and influence surrounding epithelial cells.

Prostate cancer is the most frequently diagnosed malignancy in men. In prostate cancer, neuroendocrine cells can stimulate growth of surrounding prostate adenocarcinoma cells (proliferation of neighboring cancer cells in a paracrine manner by secretion of neuroendocrine products).

Neuroendocrine prostate cancer is an aggressive variant of prostate cancer that commonly arises in later stages of castration resistant prostate cancer.

The detection of neuroendocrine prostate cancer has clinical implications. These patients are often treated with platinum chemotherapy rather than with androgen receptor targeted therapies.

Conclusion: This review shows the need to improve our knowledge regarding diagnostic and treatment methods of the Prostate Cancer, especially cancer cells with neuroendocrine phenotype.

Keywords: prostate cancer, neuroendocrine cells, neuroendocrine differentiation

INTRODUCTION

Prostate cancer is the most frequently diagnosed malignancy in men, majority of them are adenocarcinoma (derived from transformation of the glandular cells) [1]. The prostatic glands are under the influence of androgens. Prostatic adenocarcinoma cells present androgen receptors and relies on androgens for development and progression. That's why standard therapies for advanced and recurrent prostate cancer is based on two principles: block androgen synthesis (chemical or surgical castration)

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and inhibit androgen receptors [2][3].

Usually, this treatment provide clinical responses, but rarely eradicate all prostatic cancer cell populations and most patients after months/years develop resistance to these treatment – that means progression to castrate – resistant adenocarcinoma.

Neuroendocrine differentiation for most of the clinicians is associated with progression of prostate cancer to castrate – resistant disease.

NEUROENDOCRINE CELLS IN THE NORMAL PROSTATE

Neuroendocrine cells are found in many types of tissues, including in the normal prostate [4]. This cells secrete neuropeptides such as: serotonin, histamine, chromogranin A, calcitonin, bombesin, neuropeptide Y, vasoactive intestinal peptide, adrenomodulin [5],[6],[7],[8] and also growth factors (VEGF) [9]. The principal role is to maintain local homeostasis of the surrounding epithelial cells. Some of these neuropeptides have been found in seminal fluid, raising the idea that they may regulate sperm function [10][11].

Studies shows that neuroendocrine cells are present in all anatomic zones of the prostate and comprise less then 1% of benign prostatic glandular epithelium [12].

Neuroendocrine cells cannot be recognized on routine Hematoxilyn-Eosine staining. Ussualy this cells are found using immunohistochemistry with specific markers: chromogranin, synaptophysin, neuron specific enolase and CD 56 [13][14][15][16]. Other neuroendocrine cells markers reported in literature but not ussualy used in clinical practice include: cytochrome b561 [17], synaptic vesicle protein 2 [18][19], vesicular monoamine transporters [20].

NEUROENDOCRINE DIFFERENTIATION IN PROSTATE CANCER

Neuroendocrine prostatic carcinomas are often diagnosed on primary prostate needle biopsy with negative or low PSA level.

Neuroendocrine carcinoma of the prostate may be linked to resistance to androgen receptor signaling inhibition. Some prostatic tumors such as carcinoid tumor and small cell carcinomas are entirely composed by this type of cells with neuroendocrine differentiation. These tumors are aggressive and often present locally advanced or metastatic diseases with poor survival prognosis [21]. In general, these cells are negative for androgen receptor and PSA. Hormonal therapy is not effective while chemotherapy may have some value [22].

More frequently, prostatic tumors are composed by conventional adenocarcinoma with a small component of cells with neuroendocrine differentiation.

The neuroendocrine cells are indistinguishable from the normal adenocarcinoma cells on Hematoxilin-Eosine stained section under light microscope, and can be identified only by immunohistochemical stained using specific markers. Chromogranin A is the most used marker in clinical practice and is considered to be sensitive and specific [23].

Prostatic tumors with high grade or high stage and particularly in hormonal treated or hormone-refractory tumors, neuroendocrine differentiation is increased [24][25][26]. Also the level of serum chromogranin A are increased and is associated with tumor resistence to hormonal therapy [27]. In patients with hormone-refractory tumors, elevated serum chromogranin A level is predictor of poor prognosis, independent of PSA level [28].

NEUROENDOCRINE DIFFERENTIATION AND HORMONE-REFRACTORY PROSTATE CANCER

If the local curative treatments (radical prostatectomy, brachytherapy, radiotherapy) are no longer indicate for the patients with prostate cancer, androgen deprivation therapy is an effective therapy with significant symptomatic relief, suggesting that tumor growth is dependent on androgen receptors signaling. It is known that prostatic stromal cells support survival and proliferation of epithelial cells (paracrine secretion), that’s why, it is possible that neuroendocrine cells (who do not express androgen receptors and so are resistant to androgen deprivation) being capable to secrete neuropeptides and cytokines and so stimulate proliferation of adenocarcinomas cells. This situation suggest that androgen deprivation generally works, but eventually fails because neuroendocrine cells gradually substitute the function of stromal cells and so continued to stimulate the proliferation of prostate cancer cells.

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Antibiotic resistant urinary tract infections in an urology ward

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Abstract: Introduction: UTI (urinary tract infections) represent a central pathology for a urological service. Antibiotic resistance is growing at a steady and alarming rate worldwide and especially in Romania.

Method and materials: We have analyzed all the patients that were admitted to our clinic for continuous hospitalization between January 2015 and October 2015. All patients undergone urine culture and all cultures positive had an antibiogram worked up. We have selected all patients that had antibiotic resistance to at least an antibiotic.

Results: From 1745 patients admitted for continuous hospitalization, we had 180 positive urine cultures at admission from which 125 had at least an antibiotic resistance.

Conclusions: Antibiotic resistance is a serious phenomenon, with potential lethal complications, which we encounter daily in urological practice

INTRODUCTION

Uncomplicated UTI are probably the most common urological condition. It is estimated that at least 1 out of 3 females will develop an UTI during their lifetime. UTI frequency at young females is associated with sexual intercourse. When urological malformations, pregnancy, menopause, urolithiasis occur conditions for recurrence of the disease are met. Recurrences are defined as at least 2 episodes of UTI in 6 months or 3 episodes a year.

Male patients develop UTI due to different mechanisms and at an elderly age. The most common mechanism that facilitates the development of UTI in men is bladder outlet obstruction caused mainly by prostatic enlargement.

UTI are caused mainly by Gram negative *enterobacteriacea*. Escherichia Coli is the cause for about 80-90 % of community acquired UTI's. In healthcare related infections E. Coli is still the leading cause but with a more modest percentage. Other pathogens that cause UTI are: Proteus mirabilis, Enterococous, Klebsiella pneumonie, Pseudomonas aeruginosa.

METHOD AND MATERIALS

Between January and October 2015, 1745 patients were admitted for continuous hospitalization in our Clinic. At admission all of these patients had their urine sent for culture.

The urine culture was tested for antibiotic response for the following antibiotics:

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Trimethoprime-sulfamethoxazole, cefuroxime, cefotaxime, ceftriaxone, ciprofloxacine, ofloxacine, levofloxacine, gentamicine, ampiciline, oral penicillin, nitrofurantoine, imipenemcilastatin, ertapenem, meropenem and tigeciclin.

We had 180 positive urine cultures from which 125 were antibiotic resistant at at least one tested antibiotic.

We have analyzed the relationship between the germ and antibiotic resistance and also the sex and age of patients.

RESULTS

We had 180 positive urine cultures representing 10.31% out of all patients. 7.16% of all patients had at least one antibiotic resistance. 70% of the patients with positive urine cultures had at least one resistance.

Out of the 180 patients 104 were females (57,7%) and 76 were males (42,3%). Resistance was encountered in 56 females (44,8%) and 69 male patients (55,2%)

Out of all UTI's we had 143 E. Coli cases (79%), 25 Klebsiella pneumoniae (13,88%), 12 Proteus mirabilis (6,66%), 8 Enterococus (4,44%) faecalis and 2 cases with Pseudomonas aeruginosa (1,11%).

In the antibiotic resistant group we encountered the following bacterial involvement: 88 patients had E. coli, 19 had Klebsiella pneumoniae, 10 had Proteus mirabilis, 6 had Enterococus faecalis and 2 had Pseudomonas aeruginosa.

The decreasing percentage of antibiotic resistance was the following: Psedomonas aeruginosa cases had 100% resistance at at least one antibiotic, 83% of Proteus mirabilis cases had resistance, 76% of Klebsiella cases had resistance, 75% of Enterococus faecalis had resistance while the least resistant germ was E. Coli with 61,53%.

There were no significant differences between sexes except the fact that all the 2 cases with Pseudomonas aeruginosa were registered at male patients with long time users of indwelling ureteral catheters.

Regarding antibiotic resistance we had only 2 cases(1 case with Pseudomonas and 1 case with Klebsiella) which had no sensibility to any of the tested antibiotics. The strongest resistance was registered to ampicllin meaning that 115 (92%) cases with resistance had no sensibility to the drug. Resistance to penicillin was counted at 97 (77%). Third highest resistance was encountered to Thimetoprim with 90 patients (72%). The lowest resistance was registered for imipenem cilastatin, ertapenem, meropenem and tigeciclin. With only 2 cases (1,6%). Levofloxacin had also low resistance with 25 cases meaning 20%.

CONCLUSIONS

Antibiotic resistance is real threat to the general health of humanity, being more and more encountered especially in hospital related infections but also in community acquired infections. UTIs make no exceptions. Romania is especially at high risk with reported resistance to quinolones exceeding 30%.

The data we obtained from patients admitted for continuous hospitalization in our Clinic is similar to data in international literature reports concerning antibiotic resistance. It is important to realize the fact that urological patients are at high risk of developing antibiotic resistant infections due to the age profile, the pathology and the increasing number of indwelling catheters.

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CLINICAL PRACTICE

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Complications of radical retropubic prostatectomy – our experience

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Abstract: Introduction: Radical retropubic prostatectomy represents in its various forms (open, laparoscopic and robotic) the "gold standard" treatment for adenocarcinoma of the prostate. Unfortunately like al treatment solutions it has its shortcomings.

Retropubic radical prostatectomy, external beam radiation therapy and brachytherapy are the curative options.

Materials and Method: The experience of Clinic of Urology from Central Military Hospital representing more than 100 cases was analyzed . Classical open retropubic prostatectomy was performed in all cases.

We focused in this paper on intraoperative complications and also precocious and late postoperative complications.

Results: Our results are matching the other centers in terms of intraoperative complications (blood loss), early postoperative complications (hematuria, urinary tract infection, lymphatic drainage) or late postoperative complications (erectile dysfunction and urinary incontinence). However none of these complications are to be underestimated

Conclusions: The limits of this intervention can be pushed a litle bit further, in our opinion the age factor is a relative one, some of these patients having a longer than 10 years life expectancy. It provides good oncological outcome with manageable complications most of the times.

Keywords: radical retropubic prostatectomy, complications, nerve sparing

INTRODUCTION

Prostate cancer is nowadays the second most frequent neoplasia in men. Most probably the reason for this frequency is the presence of PSA as a valuable diagnosis tool and also the existence of the guidelines.

Also the very aggressive policy of controls for all men after 50-55 years has begun to show its results. Although radical prostatectomy, radiotherapy and brachytherapy are almost equal in terms of oncological results, in psychological, financial, and why not life quality radical prostatectomy is represented better.

MATERIALS AND METHOD

We have analyzed our experience at the Central Military Hospital regarding radical prostatectomy between January 2009 and February 2015. Our surgical teams carried on during these period more

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than one hundred radical prostatectomies for patients with localized or advanced prostate cancer. This paper focuses on intra and post - operative complications we encountered[1,2].

Patients mean age was 63.09 years ranging between 51 to 78 years of age. We haven't taken into consideration the general life expectancy for men in Romania, moreover the general biologic status was considered as inclusion criteria.

Major diagnosis criteria for prostate adenocarcinoma were: anamnesis, total PSA and free PSA levels, digital rectal examination and transrectal ultrasonography, which was usually performed when prostatic biopsies were obtained[3,4].

Medium prostatic volume of 45 cc without taking into consideration patients that prior have benefited of endoscopic surgery.

PSA levels varied between 0.5 ng/ml and 24 ng/ml. Two cases presented with PSA levels above 20 ng/ml. Those two patients were classified postoperative as pT3b – locally advanced.

Mean Gleason score, which is an important prognosis factor, was between 5-7 at 84 patients. Low risk prostate adenocarcinoma was recorded in six cases, ten patients with Gleason 8 were included in high risk and very high risk groups Patients diagnosed with Gleason 9 score were unsuitable for surgery.

In accordance with European Guidelines surgery was performed at six to eight weeks after the transrectal biopsy and 12 weeks after the transurethral resection of the prostate in order to to reduce the prostatic inflammation. General anesthesia was chosen for all patients.

Pelvic lymph node dissection was performed at almost all patients. We used extended lymph node dissection when patients were included in high or very high risk groups.

Urethro-vesical anastomosis was realized using four to eight resorbable sutures excepting six cases when no sutures could be placed due to the short urethra left after the prostatic excision. Those six cases were managed by gentle traction of the urethro-vesical catheter and maintaining the catheter for 14 days compared to 7 to 10 days as we did on the other cases.

RESULTS

The most redutable intraopearatory complication we encountered was blood loss which ranged between 400 milliliters and 2500 milliliters. Twenty - two patients needed intraoperatory blood transfusions.

Early complications included: gross hematuria (eight cases) which spontaneously remitted in 24 to 48 hours, wound infection (six cases), prolonged lymphatic pelvic drainage (sixteen cases), urinary tract infection (twenty cases), pulmonary embolism with subsequent death of the patient (one case).

Eighteen patients developed moderate or severe LUTS caused by bladder neck sclerosis or urethral stricture which was managed trough endoscopical procedures in the first year post-op. Four cases developed urinary fistula during the first 72 hours after prostatectomy three needing surgery in order to perform a new urethro-vesical anastomosis and one being resolved by placing a gentle traction on the urethro-vesical catheter for 24 hours.

Late complications recorded were urinary incontinence and erectile dysfunction. Following data were analyzed at one year after the surgery, only 78 patients being included in the evaluation. Urinary incontinence was evaluated by the number of pads used by the patient.

Almost 80% confirmed that they do not use any incontinence pads. Ten patients use only one pad a day and lose urine only when changing positions or when sneezing or coughing. Three patients affirmed moderate urinary incontinence using 2-3 pads a day[5].

We used a similar technique like the TOT used for women to place a suburethral transobturatory mesh for eleven ot if the thirteen patients with urinary incontinence with very good results and lack of symptoms in nine cases.

Using Walsh technique neurovascular bundles were spared in 62 patients. Erectile function, a very important part in quality of life was assessed using anamnesis and standardized questionnaires. 68.42%

(26 out of 38 patients) of the patients with surgical bilateral neurovascular bundles preservation affirmed satisfactory erections while only 33% (eight out of 24 patients) with unilateral bundle preservation affirmed satisfactory errection[6].

In sixteen cases due to oncological reasons or intraoperative bleeding neurovascular bundles were not preserved. All patients that benefited from the nerve sparing technique surgery were set on low dose daily 5 PDE inhibitors.

CONCLUSIONS

Radical prostatectomy is the standard treatment for localized and in some cases of locally advanced disease. We consider biologic status to be a more reliable indicator than age alone. That being said we consider far better to evaluate the patient before judging only the age.

Surprisingly one of the most bothering complications we encountered was bladder neck sclerosis; in this case one of the patients had undergone endoscopic surgery for three consecutive times. One of the hypotheses is the using of tension on the urethro-vesical catheter consequent bladder neck ischemia and in a secondary time sclerosis. This was not the case of this patient.

Intraoperative bleeding remains one of the most serious problems also because of the underlying co morbidities which may be aggravated during surgery.

Incontinence is a very bothersome late complication. Psychologically it has a great impact on the patient quality of life; actually along with erectile dysfunction those two are considered to be the highest burden from the patient's point of view. Still the existence of TOT like procedure for men offers an efficient solution to this problem.

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Scintigraphic evaluation of the kidney

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Abstract: *There are more than one technique used to evaluate the kidney, besides the standard ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI), there is also renal scintigraphy. The renal nuclear medicine procedures are grouped as in vitro (urine counting wells, basic probe detectors for clearance studies) and in vivo procedures (static and/or dynamic examinations done with planar gamma cameras, and single-photon emission computed tomography (SPECT) to determine kidney parameters or for cortical imaging). Renal scintigraphy has been a useful tool, since the early 1950s, in the diagnosis and management of many pathological changes in the kidney, especially in measuring renal function (e.g. obstructive/nonobstructive uropathies, renal inflammatory diseases, tumours, renal hypertension, and renal transplant viability). [1]*

INTRODUCTION

The radionuclide investigation of the kidney includes detection of renal afflictions and measurements of quantitative indices that estimate the renal perfusion and function. Ultrasound and computed tomography are commonly used for the evaluation of renal structural anatomy, and the role of nuclear renal imaging is more for functional analysis, less in anatomical imaging (e.g. cortical imaging).[1,2]

Renal scintigraphy, also known as a "renal scan" or "renal radionuclide imaging" or “renography” includes various investigations that use different radioisotopes to evaluate renal blood flow, renal split function, and the renal excretion performance of both. It yields specific and often unachievable information by using other imaging procedures. [2]

Clinical indications for renal scintigraphy (adapted from *Nuclear Medicine: The Requisites*, 4th ed)**:** [3-5]

1. Blood flow abnormalities
2. Function quantification (reduced performance of one or both kidneys)
   a. Differential function
   b. Glomerular filtration rate (GFR), effective renal plasma flow (EPRF)
3. Cirrhosis of the kidney(s)
4. Differentiation between a mass lesion and a column of Bertin
5. In infants with abnormalities of the urinary tract to study the urinary flow
6. Obstruction: ureteropelvic junction, ureteral
7. Pyelonephritis: both acute and chronic tubule-interstitial nephritis and parenchymal scarring
8. Renal failure: acute and chronic
9. Renal artery stenosis with/without renovascular hypertension
10. Renal vein thrombosis
11. Surgical:

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a. performance assessment before operations in the case of chronic diseases (e.g. diabetes)
b. Renal allographs: for clinical monitoring of renal function, transplant rejection or other complications, transplant anastomosis assessment
12. Trauma

PURPOSE

The purpose of this paper is to provide a better understanding about dynamic renal imaging in several kidney pathologies, with an emphasis on obstructive renal pathology and to clarify the meaning of some quantitative parameters used in renal scintigraphy.

MATERIAL AND METHODS

Nuclear renal scans are various, complex and also a subject to institutional preferences, that is why a standardization of the techniques is hard to achieve. All nuclear renal procedures have some basic common conditions that must be taken into account before, during and after patient examination. First of them involves the intravenous bolus injection of a renotrophic radiopharmaceutical (a medicine marked with a radioisotope) that emits a small amount of radioactivity into the patient. Usually, the radiotracer administered to the patient is well tolerated, with no systemic toxic effects. Some mild-moderate allergies or adverse reactions (such as dizziness, headache, metal taste, flushing) are extremely rare reported. The radiation dose is relatively low, less than the standard chest x-ray. The examination is performed with the patient either lying on the examination table, in the supine position, or standing. [3-7]

The pre-scan preparatory measures that are common are hydration, about 30 minutes prior to the actual examination, the patient should drink about 1 litre of fluid, and an empty bladder, the patient should void before examination. [3-7]

It is of great importance that in the last 3-6 months prior to the renal scintigraphy, no high doses of iodine substances to be taken, like x-ray and CT contrast agents, or certain medicines (e.g. Amiodarone) because the results could otherwise be distorted. [3-7]

In general, chronic therapy medications should not be discontinued prior to the exam. For specific problems, specific blood pressure lowering drugs (ACE inhibitors, diuretics) must be mentioned to the nuclear medicine physician as there is a period of drug abstinence in some cases. [3-7]

Different scanning protocols with various radiopharmaceuticals are available for scanning the kidneys. Choosing the right technique and optimal radiopharmaceutical depends on the patients' medical history, clinical setting and indication. [3-5, 8]

Plenty and divers radiopharmaceuticals have been produced in the last 60 years to assess renal function; some are used in laboratory assays by measuring blood samples and determining the renal clearance of the radiopharmaceutical and others for dynamic or static studies with a gamma camera or a SPECT system. The gamma cameras and SPECT systems monitor the passage of radiopharmaceuticals through the kidney and urinary tract by registering and processing of the emitted gamma radiation. [9, 10]

The radionuclide agents usually used for determining renal function and anatomy can be grouped into three main categories [5,8]: those filtered by glomerular filtration, those excreted by tubular secretion via proximal tubule receptor-mediated endocytosis from the glomerular filtrate, and those retained in the renal tubules for long periods, useful for cortical imaging. [8]

The most frequently used radiopharmaceuticals in renal scintigraphy are illustrated in Table 1.

Table 1. Most frequently used renal radiotracers

Glomerular Filtration (GF)	• Tc-99m diethylenetnamine pentaacetic acid (Tc-99m DTPA) • 99mTc-mercaptoacetyltriglycine (Tc-99m MAG3) • I-131/I-123 orthoiodohippurate (OIH)
Tubular Secretion (TS)	• I-131 OIH • Tc-99m MAG3 • Tc-99m ethylenedicisteine (Tc-99m EC)
Tubular Filtration (TF)	• Tc-99m dimercaptosuccinic acid (Tc-99m DMSA) • Tc-99m glucoheptonate (Tc-99m GHA)

Table 2. Renal radiotracers excretion mechanisms (adapted from *Semin NM Apr.92*)

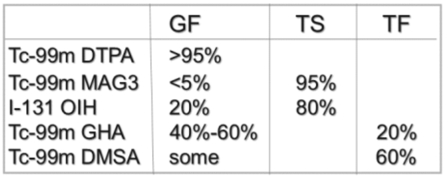

	GF	TS	TF
Tc-99m DTPA	>95%		
Tc-99m MAG3	<5%	95%	
I-131 OIH	20%	80%	
Tc-99m GHA	40%-60%		20%
Tc-99m DMSA	some		60%

There are four important types of renal imaging methods obtained by using a planar gamma-camera or a SPECT system in order to evaluate whether the kidneys are working normally or abnormally:

- Renal perfusion and renal function imaging are dynamic studies with images taken in series, over a period of 30 minutes immediately after the bolus radiotracer injection and determines the blood flow distributed to the kidneys, recognizes a potential narrowing of the renal arteries, and helps in determining the kidneys functioning. [2, 11]

- Diuretic renal scintigraphy is used to detect kidney obstruction. The procedure is similar to the renal perfusion, and seriated images are taken before and after the introduction of a diuretic (in the 15th minute of acquisition) to help urine elimination from the kidneys. [2, 11]

- ACE-inhibitor renal scintigraphy utility is in detecting renovascular hypertension, not renal artery stenosis, by comparing renographic images before and after taking an ACE-inhibitor. [2, 11] It is also a dynamic study and the image acquisition process begins when the radiotracer is injected intravenously in bolus. [3-5, 8, 10]

- Cortical renal scintigraphy is a static study and detects the amount of normal functioning kidney tissue. [2, 11] After the tracer administration, there is a three hour delay before the imaging acquisition can begin. [3-5, 8, 10]

Generally the dynamic renal functional studies are acquired in two parts. The first part evaluates the renal blood flow, which is calculated in the first pass of the radiopharmaceutical bolus through the abdominal aorta and renal arteries. The second part assess the kidney uptake and clearance function over the next 25 to 30 minutes of acquisition. [3-5, 8, 10]

GFR and EPRF are important kidney function markers evaluated by dynamic renal scintigraphy. Normal GFR varies in accordance with age, sex, weight (nutritional status), diet, race, and kidney size, which is proportional to body surface area. [12, 13, 14] The estimated GFR is calculated using these factors and the serum creatinine value, but it is not helpful when there are unilateral changes or when kidney function is very abnormal. Unilateral renal function changes are difficult to identify with other imaging techniques, but easy to determine with camera-based renal scan split functions. The percent differential function (split function) calculates the contribution of each kidney to the renal function and can be applied to GFR or ERPF data. [3-4, 15]

Nuclear techniques are not 100% “accurate” in measuring kidney function (GFR, ERPF), but are superior to investigations based on the serum creatinine value. [3, 14] Using renal scintigraphy patients can be followed over a long period of time, because this technique is highly reproducible in any one patient. [3, 13, 14]

Table 3. Renal radiotracers used for renal function measures
(adapted from *Requisites of Nuclear Medicine 4th ed*)

Effective Renal Plasma Flow (ERPF)	Tc-99m MAG3 I-123 / I-131 OIH H-3 or C-14 paraamino-hippurate (PAH) I-125 or I-131 iodopyracet
Glomerular Filtration Rate (GFR)	Tc-99m DTPA Cr-51 EDTA I-125 diatrizoate I-125 iothalamate C-14 or H-3 inulin In-111 or Yb-169 DTPA Co-57 vitamin B12

The Tc-99m DMSA cortical scan is used to evaluate renal cortical lesions in patients with suspected pyelonephritis, to detect renal scarring in a patient with reflux, and, in some occasions, to differentiate a prominent column of Bertin from a tumorous formation. Renal cortical scintigraphy provides additional information by showing changes and improvements over time, that are impossible to evaluate with conventional structural and anatomical imaging modalities, like ultrasound, CT, and MRI. [3, 13, 14]

RESULTS

The images obtained with the gamma camera should be inspected after acquisition in order to evaluate if the examination was done in proper conditions. After visual inspection the image data is processed and regions of interest (ROIs) are defined over the kidney and surrounding background so that the renogram curves can be generated. [5, 7]

The time-activity curve (TAC) represents a graphic illustration of the renal function and is composed of 3 parts: initial rise, upslope and downslope. The first part reflects the radioactivity that arrives via the renal artery to the kidney. The upslope (ascending limb) reflects kidney uptake before the radiopharmaceutical begins to be excreted by the kidney (the descending limb). The peak time accurately indicates the point at which the extraction and accumulation trend is reversed to the evacuation process. [5, 7]

This curve is important in diuretic renal scintigraphy. Diuresis renography helps to discern between obstructive (calculus) and nonobstructed dilated urinary tract, and in the postsurgical evaluation of the renal system function and urodynamic. Acute obstructive uropathy is a commonly encountered condition. When unilateral obstruction occurs the changes in the measured renal function are a little decrease or imperceptible, but the bilateral form can result in significant kidney function losses. [5, 16]

The radionuclide renogram TAC provides an accurate graphic illustration of the dynamics of urinary excretion. In obstructive uropathy a calculus can give various degrees of hydronephrosis, depending on the site in which it is lodged. In the case of acute obstruction due to a renal calculus, pressures increase fast in the pyelocaliceal system and in the ureters above the point of obstruction. The affected kidney has a characteristic aspect: a dilated urinary tract with a thin cortex, and it shows on the TAC a slow continuous accumulation of tracer in the collecting system, a slow increasing ascending curve with no downslope. In contrast, the opposite kidney, which has a normal function and no obstruction, will show a good uptake and excretion, and the three part TAC. [7, 17]

In some cases the calculus produces a partial obstruction of the urinary excretion pathways. In order to demonstrate this a diuretic like Furosemide is administrated at various times during the course of the renal scan. The decreasing aspect of the curve after the administration of the diuretic indicates an incomplete obstructive pattern (as in Curve 3), and as for the rising curve a complete obstruction (as in Curve 2). [7]

Figure 1. Time activity curves
(adapted from *EANM: Dynamic renal imaging in obstructive renal pathology. A Technologist's Guide*)
Curve 1- normal kidney function; Curve 2- complete obstruction; Curve 3 - incomplete obstructive pattern after the administration of Furosemide at minute 15.

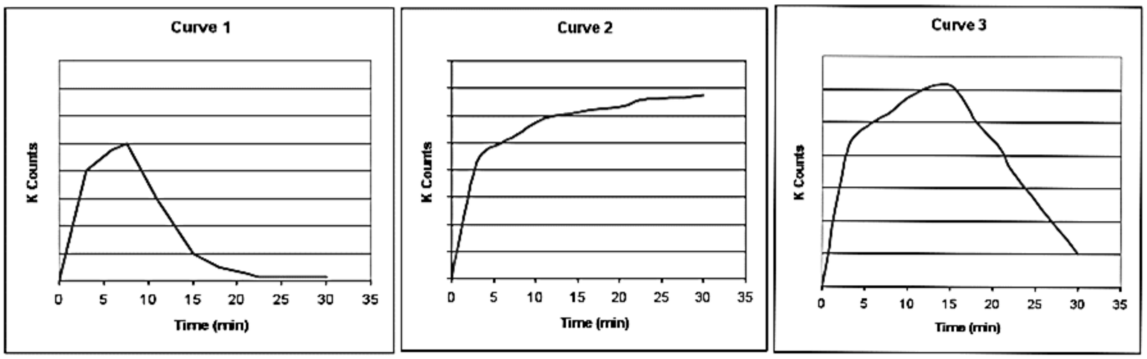

QUANTITATIVE INDICES

Renal perfusion is evaluated by visual and quantitative analysis (1- to 3-second images) of the initial bolus as it transits the abdominal aorta and enters the renal arteries (used in renal number anomalies, renal transplant). [10, 11]

Relative Function represents the relative uptake of the radiopharmaceutical for the evaluation of uni-/ bilaterally impaired kidneys. [10] The split function is particularly useful because estimated GFR and serum creatinine may not identify unilateral lesions. [3]

Renal Size. Several chronic renal diseases will result in bilaterally small kidneys, whereas the kidneys may be bilaterally enlarged in early diabetic renal disease, acute interstitial nephritis, HIV nephropathy, and amyloidosis. The resolution of structures of the renal parenchyma with nuclear medicine may not be as clear as with other imaging techniques, such as CT or MRI that is why nuclear renal images are not usually used to differentiate between cysts and tumours. [10, 11, 18]

The time to peak, or T max, refers to the time from radiopharmaceutical intravenous administration to the peak height of the renogram curve. 99mTc-MAG3, 99mTc-DTPA and OIH renograms normally peak by 5 min and drop to half-peak value until the 15th minute after injection; however, in some cases, physiologic retention of the radiotracer in the renal calyces or pelvis can alter the aspect of the TAC in normal kidneys and lead to prolonged values for the time to peak, 20-min/ maximum count ratio, and T½. [8, 10, 19, 20]

Figure 2. Normal right kidney renogram. Specific scintigraphic aspect of left kidney obstruction (kidney calculus).

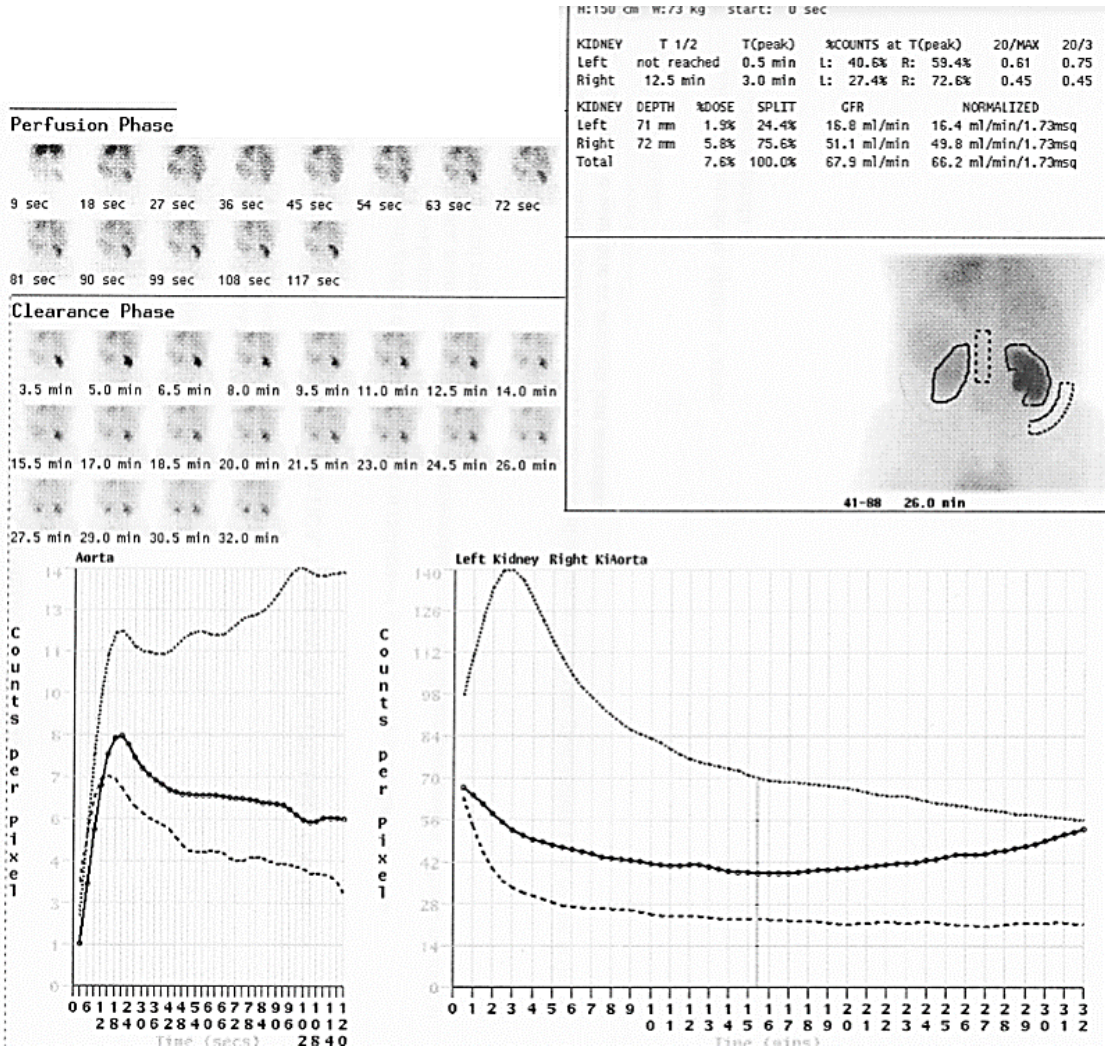

H:150 cm W:73 kg start: 0 sec

KIDNEY	T 1/2	T(peak)	%COUNTS at T(peak)		20/MAX	20/3
Left	not reached	0.5 min	L: 40.6%	R: 59.4%	0.61	0.75
Right	12.5 min	3.0 min	L: 27.4%	R: 72.6%	0.45	0.45

KIDNEY	DEPTH	%DOSE	SPLIT	GFR	NORMALIZED
Left	71 mm	1.9%	24.4%	16.8 ml/min	16.4 ml/min/1.73msq
Right	72 mm	5.8%	75.6%	51.1 ml/min	49.8 ml/min/1.73msq
Total		7.6%	100.0%	67.9 ml/min	66.2 ml/min/1.73msq

Perfusion Phase
9 sec
18 sec
27 sec
36 sec
45 sec
54 sec
63 sec
72 sec
81 sec
90 sec
99 sec
108 sec
117 sec
Clearance Phase
3.5 min
5.0 min
6.5 min
8.0 min
9.5 min
11.0 min
12.5 min
14.0 min
15.5 min
17.0 min
18.5 min
20.0 min
21.5 min
23.0 min
24.5 min
26.0 min
27.5 min
29.0 min
30.5 min
32.0 min
41-88 26.0 min
Aorta
Counts per Pixel
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 100 104 108 112
Time (secs)
Left Kidney Right KiAorta
Counts per Pixel
0 14 28 42 56 70 84 98 112 126 140
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Time (mins)

Figure 3. Renal scintigraphic appearance of chronic renal failure; hypoplasic left kidney.

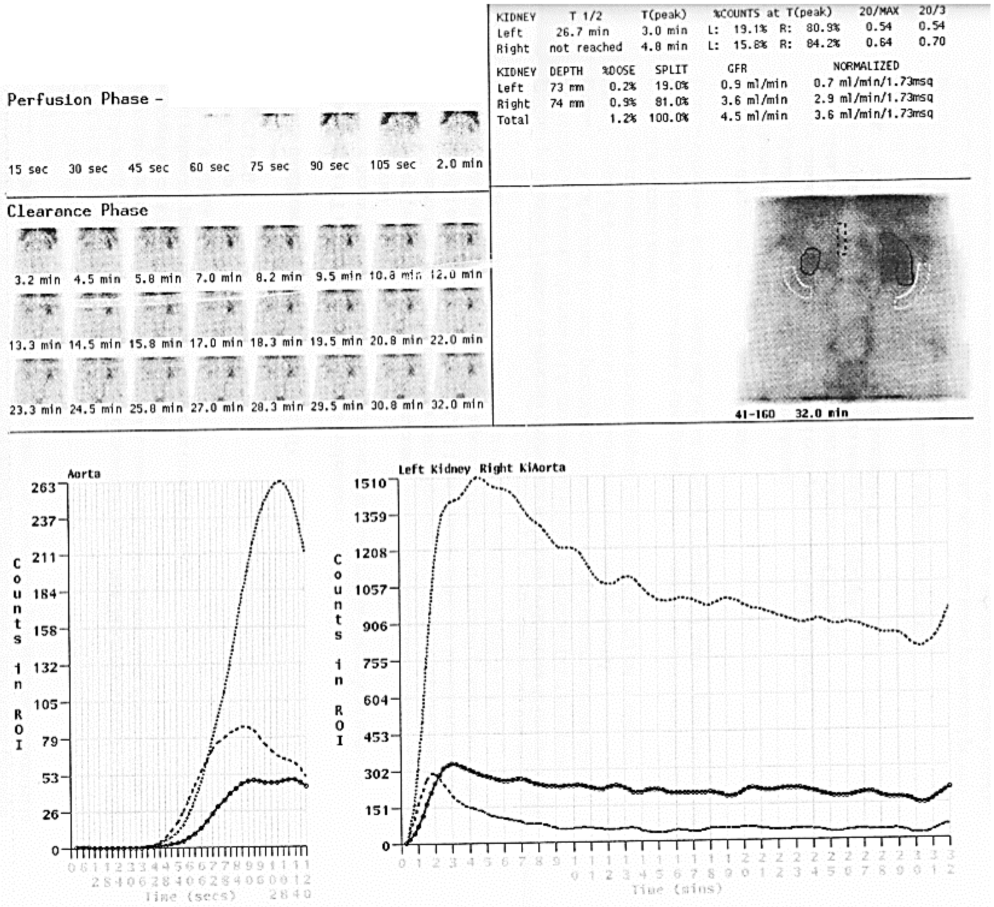

KIDNEY	T 1/2	T(peak)	%COUNTS at T(peak)	20/MAX	20/3
Left	26.7 min	3.0 min	L: 19.1% R: 80.9%	0.54	0.54
Right	not reached	4.8 min	L: 15.8% R: 84.2%	0.64	0.70

KIDNEY	DEPTH	%DOSE	SPLIT	GFR	NORMALIZED
Left	73 mm	0.2%	19.0%	0.9 ml/min	0.7 ml/min/1.73msq
Right	74 mm	0.9%	81.0%	3.6 ml/min	2.9 ml/min/1.73msq
Total		1.2%	100.0%	4.5 ml/min	3.6 ml/min/1.73msq

Perfusion Phase -
15 sec
30 sec
45 sec
60 sec
75 sec
90 sec
105 sec
2.0 min
Clearance Phase
3.2 min
4.5 min
5.8 min
7.0 min
8.2 min
9.5 min
10.8 min
12.0 min
13.3 min
14.5 min
15.8 min
17.0 min
18.3 min
19.5 min
20.8 min
22.0 min
23.3 min
24.5 min
25.8 min
27.0 min
28.3 min
29.5 min
30.8 min
32.0 min
41-160
32.0 min
Aorta
Counts in ROI
263
237
211
184
158
132
105
79
53
26
0
Time (secs)
Left Kidney
Right KiAorta
Counts in ROI
1510
1359
1208
1057
906
755
604
453
302
151
0
Time (mins)

The T½ represents the time is necessary for the radioactivity in the kidney to drop to 50% of the maximum value (time to peak, or T max); this index is important in diuretic renography for patients with suspected urinary tract obstruction. [8, 10, 19]

The 20-min/maximum count ratio is an index of the transit time and a measurement of residual cortical activity, the ratio between the kidney counts at 20 min to the maximum (peak) radioactivity; it is useful in monitoring patients with suspected urinary tract obstruction and for detecting renovascular hypertension. [8, 10, 19]

CONCLUSION

Renal radionuclide studies are versatile procedures and vary depending on institutional preference, clinical setting of the patient and medical indication.

Renal scintigraphy is a complex subject and understanding some basic principles, the use of radiopharmaceuticals available to image the kidney and monitor its function, the quantitative indices that can be generated and protocols can be useful for the physicians specialized in nephrology and urology in evaluating patients with diseases of the urinary tract and renal physiology.

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CLINICAL PRACTICE

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Penile prosthesis – a viable solution for erectile dysfunction refractory to conservative therapy

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Abstract: Introduction: Erectile dysfunction(ED) is a serious condition which can affect men of all ages, with an important impact over the quality of life. When conservative therapy fails, a viable solution is the penile prosthesis implantation (PPI).

Objective: The purpose of this paper is to present data from recent literature regarding the satisfaction rates of men who have undergone penile PPI and as well as the partners satisfaction rates, ease of use, informations regarding long term survival of these prosthesis, postoperative complications and long distance complications, the effectiveness and the way that penile prosthesis have influenced the quality of life of the men who have opted for this solution.

Material and methods: We have analyzed recent long term studies concerning the outcomes of the PPI, studies which were made retrospectively, over a period of time of 10 to 15 years.

Results: Compared with the conservative treatment for ED, the satisfaction rates achieved after penile prosthesis implantation are higher. According to these studies, the overall patient's satisfaction is 70-90%. The difference between patient and their partners' satisfaction rate is negligible, this difference ranging between 2 to 8%. The satisfaction rate regarding the adequate erection for sexual intercourse is 80-90%. The overall satisfaction rates for the malleable prostheses are lower compared with inflatable devices, 30-75%, respectively 75-90% for the inflatable prosthesis. The mechanical and overall survival rates for the malleable prosthesis range between 65-80% at 10 years, and 58-75% for the inflatable devices. Overall ease of use is rated as 78%. Complications after implantation can be encountered in less than 5% of cases and infections in less than 2%. The likelihood of continued use is higher in the group of the patients with the inflatable prosthesis compared with the malleable ones 70-80%, respectively 50-60%. Up to 85-90% of patients with inflatable prosthesis would recommend them.

Conclusions: PPI is a high effective treatment for erectile dysfunction, refractory to pharmacological treatment. The inflatable penile prosthesis (IPP) provides more overall satisfaction than the malleable ones. The patients with IPP are more likely to continue using their devices than those with the malleable prosthesis. PPI improves significantly the quality of life for patients with erectile dysfunction.

INTRODUCTION

Erectile dysfunction is defined as the inability to obtain and maintain an erection with sufficient penis rigidity, adequate for successful sexual contact. Although ED decreases significantly the quality of life for both men and their partners, in many countries

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ED is understated because of cultural aspects.

ED can affect men of all ages, but as life expectancy continues to increase, so does its prevalence. It is estimated that the prevalence of ED in men over the age of 50 years is higher than 50 percent, and this prevalence grows as the age increases. This condition is not exclusively encountered in men over the age of 50. It is estimated 3-5 percent of young men with ages between 15 and 25 years may suffer from this condition and 7 to 10 percent of those with ages between 25-45 [1].

Despite initial beliefs that the most common cause for ED was the psychological factor, especially in young men, it is now proven that organic causes are responsible for 70 to 90 % of ED. There are several proven pathologies proven in ED [2]:

a) Diabetes Mellitus-is a frequent cause of ED. Men with DM compared with general population may have ED at an earlier age. The prevalence of ED in the population of men with DM can be as high as 70-80%. The peripheral polineuropathy, microangiopathy and arterial insufficiency encountered in DM evolution can explain the earlier age of ED appearance and a higher prevalence than individuals without DM [3].

b) Vascular causes-atherosclerosis plays an important role in ED physiopathology. The presence of aortic or iliac atheromatosis can decrease the blood inflow, thus explaining the ED prevalence of up to 50 % in patients with atheromatosis. Another vascular cause may be the increased venous outflow, which depends on the trabecular smooth muscle relaxation and on the erectile tissue’s capacity of expandability.

c) Iatrogenic causes responsible for ED- renal transplantation, aortic or peripheral surgery, abdominal perineal resection of the rectum, perineal irradiation, cystoprostatectomy or radical prostatectomy (RP). Erectile dysfunction secondary to cystoprostatectomy and radical prostatectomy may be encountered in up to 80% of cases. Since the nerve sparing RP was introduced by Walsh over years ago, the incidence of ED was decreased to 40-60% [4]. The incidence of ED post RP depends on patient age, comorbidities, preoperative potency status, type of surgery, surgical technique (open, laparoscopic or robot assisted RP), surgical experience, extensive nerve sparing (unilateral or bilateral) [5]. Although some patients believe that radiotherapy may be associated with a lower risk of ED, it has been proven that ED may be encountered in 20-80% of patient who has undergone radiotherapy. Studies have shown that the incidence of cavernosal fibrosis and ED are similar between RP and radiotherapy [6,7].

d) Trauma – pelvic fractures may affect the internal pudendal or common penile artery or the neurovascular bundle.

e) Endocrinological causes such as: hypogonadotripic hypogonadism, hypergonadotropic hypogonadism, hyperprolactinemia and hyperthyroidism.

It is known that testosterone increases sexual interest and the frequency of sexual acts, as well as the frequency of nocturnal erections, but it’s role on visual or fantasy induced erections is little to none.

f) Neurological causes:
- CNS: Parkinson’s disease, encephalitis, temporal lobe epilepsy, stroke, multiple sclerosis (50-70% of patients with MS may have ED), tumors, myelodisplasia;
- periphereal nerves (trauma, diabetic polineuropahty, alcoholic neurophathy and radiopelvic surgery).

g) Penile diseases: Peyronie’s disease, trauma, priapism (vascular lesions).
h) Drugs: Marijuana, Opioids, Ethanol, Antimuscarinic drugs, Antihistamine, Tricyclic antidepressants, Estrogens, Spironolactones, Ketoconazole, and many other drugs.
i) Renal failure-up to 40-50% patients on dialysis suffer from ED.

TREATMENT

Currently there are three lines of treatment for ED. The first line and the most used one is oral medication, especially phosphodiesterase 5 inhibitors. Since 1998, when the first PDE-5 inhibitor was introduced, Sildenafil (Viagra), the management of ED was revolutionized due to its high efficacy and few adverse effects. As time passed other drugs with higher efficiency and superior safety profiles became available, such as: Vardenafil (Levitra) and Tadalafil (Cialis). These drugs block the hydrolysis of cyclic GMP, increasing the accumulation of GMPc and potentiating the effect of NO [2]. For many men the treatment with PDE-5 inhibitors has allowed them to regain normal sexual function and regain self-confidence.

The second line treatment is the intracavernous injection of vasoactive drugs such as papaverine, prostaglandin E1 or phentolamine. The efficacy of this therapy is high, but the pain and the risk of ischaemic priapism limit its utilization [8]. The third line of treatment is the surgical implantation of a penile prosthesis. When the conservative therapy fails(PDE 5 inhibitors, intracavernosal injections, vacuum erection device, intraurethral suppositories with alprostadil – Is a PGE1 which increases corporeal oxygenation by promoting blood inflow) or when the patients are reluctant to any form of conservative therapy, the solution is the penile prosthesis implantation. The first penile prosthesis were introduced at the beginning of the 1970s . Since then they have been greatly improved and the surgical techniques for placement have evolved and made penile prosthesis implant a very effective treatment for ED [9]. This kind of treatment is effective and safe, and it also offers high satisfaction rates for patients and for their partners. With the technical improvement the number of complications and revision surgeries decreased, thereby the popularity and use of penile prosthesis for the treatment of ED increased.

The penile prosthesis available on the market include the one piece malleable and the two or three piece inflatable versions, each type of prosthesis having its own advantages and disadvantages. Malleable prosthesis is easier to use and have fewer mechanical problems compared with the inflatable prosthesis and they are also less expensive. The disadvantage of malleable implants is that complete penile detumescence cannot be achieved and this may be an important factor for patient unsatisfaction [10]. The inflatable prosthesis permit flaccidity and have a better functional result, but not all patients have the dexterity to use them, especially older men. Despite the implantation of penile prosthesis has been well documented, many urologists are reluctant to this form of treatment in older patients, due to the concern that older men have less dexterity and ability to operate an inflatable device [11]. The patients' satisfaction regarding the penile prosthesis implant is associated with their expectations and the performance of the implanted prosthesis [12]. Therefore, the decision regarding the type of prosthesis should be made by the doctor and by the patient, during a discussion where the doctor explains the advantages and disadvantages of every type of prosthesis, the risk of complications, the rate of satisfaction and unsatisfaction that both patient and his partner may have from this type of treatment, thus making sure that the patients will have expectations in line with reality.

The three piece inflatable penile implants are currently the most used prosthesis and their results regarding patient satisfaction are very good. The malleable and two piece penile prosthesis can be used in selected cases, where the three piece prosthesis has questionable indications (like in the case of elderly men who may have low dexterity and ability to operate such devices) [13,14].

Partners' satisfaction is questionable because few studies have investigated this problem during a long term follow-up period; although in literature the rate of partners' satisfaction is similar with men satisfaction. For more realistic results regarding this problem, evaluating the two partners separately during the follow-up period should bring out interesting results (in line with reality) [15].

Hakan Kilicarslan et. al in a recent study regarding the comparison of patient satisfaction for the malleable prosthesis (AMS 600-650) and two piece inflatable penile prosthesis (AMS Ambicor) concluded that the two piece inflatable prosthesis was found to be more successful and more likely for continous use when compared to the malleable device. A total of 72 patients had either AMS 600-650 or AMS Ambicor, but only 46 patients were reviewed during a long time follow-up. The percentages of patients with AMS 600-650 who reported to be satisfied, very satisfied and unsatisfied after the implant were 34,78% (8 patients out of the total of 23 questioned after the operation), 30.43% (7 patients out of 23) and 34,78% (8 patients out of 23). For the patients with the two piece inflatable penile prosthesis the results were: 73.91% (17 out of 23), 13.04% (3 out 23) and 13.04%. The percentages of patients who reported to be very likely, unlikely or very unlikely to continue using the malleable implants were: 30.43% (7 patients), 34.78% (8 patients) and 34.78%; in the case of the patients with the two piece inflatable devices the results were: 65.21%, 21.33% and 13.04% [16].

A study made by Natali et. al revealed that the likelihood of continuous for the two piece device AMS Ambicor was 89% (59 patients) and for the malleable device AMS 600-650 was 56% (9 patients) [13].

In another comparative study Lux et. al reported that the partners satisfaction rated 79% with the two piece prosthesis [17].

Levine related an overall satisfaction rate of 90% for men and the 82% for their partners, of a total of 131 patients, patients which benefited from the AMS two piece Ambicor implant [18].

Minervini reported that the 71% of patients using the malleable AMS 600-650 were satisfied with the implant [19].

Yoon Seob Ji in a study made on 74 patients who underwent implantation of inflatable penile prosthesis reported that the overall satisfaction was 86,8% and that 81,1% said that the prosthesis improved their sexual life. 83% of the patients in the study would undergo surgery again and 88.7% would recommend it to a friend. Only 60.4% of the patients interviewed during the follow-up period reported that they had an orgasm [20].

Mario Paranhos and collaborators established that 86.3% of patients (120 patients questioned out of 249 operated) rated their satisfaction after the implant as good, excellent or very good. The partners' satisfaction percentages was similar. The follow-up period was 40 months [21].

Humberto G. Villarreal and LeRoy Jones, in a study about penile prosthesis in the elderly men, reported that overall satisfaction was 4.3 (from a scale of 1 to 5, 5 meaning very satisfied), the ease of use was 4.1 (from a scale of 1 to 5.5 meaning very easy) and the mean use was 3,3 per month (0 to 7 times a month) [11].

Carson and associates evaluated satisfaction rate in 372 patients, group in which was used the AMS 700 CX prosthesis and it was stated that 79% used this prosthesis at least twice a month and that 88% would recommend the prosthesis for a friend [22].

There was little data regarding implantation of penile prosthesis in elderly people. As the life expectancy and longevity continuous to increase so does the ED problem refractory to first line treatment. Accordingly to Lindau, elderly couples remain sexual active even into the eight decade of life [23]. So the number of elderly men seeking penile prosthesis implantation will have a significantly increase. There are numerous potential complications of the inflatable penile prosthesis such as surgery related complications (haematoma, infections, erosion of the corpus cavernosum and even perforation) and mechanical failure (reservoir leakage, cylinder leakage, pump failure) [14,24].

The incidence of these complications depends on the follow-up period. In the immediate postoperative period, Paranhos stated that 24.5% patients reported pain, 7,9% had local infections and 8,6% had other complications such as wound dehiscence, difficulty voiding or local secretions without infection. In regards to late complications 8.6% of patients had to undergo revision surgery (12 patients out of 139 operated and followed up), of whom 4 the prosthesis had removed due to inefficacy and 8 had them exchanged. 2 of the 8 patients who had them exchanged had prosthesis fractured, 4 had inadequate size and the other 2 had extrusion of the cylinder. He remarked in his study that the risks of complication for the patients that have had radical prostatectomy previously was higher, 3,3 times higher. The rate of postoperative infections in this study was 7.9%. Paranhos explains that this high rate of infections compared to other studies is due to the experience of medical trainees, the high number of surgery in the operating rooms and the economic and social conditions of the patients [21]. The rigorous preoperatory asepsy has shown to reduce the incidence of infection to 4% from initial overall rate of 12% [25].

Jarow et. al [26] reported an infection rate of 1,8% and Gower a rate of 2.1% [27].

Merino in a meta-analysis related a lower infection rate in the case of malleable prosthesis compared to inflatable prosthesis (1.3% vs 3.5%) [28].

Menard in a study which examined over 400 patients with penile prosthesis after radical prostatectomy showed that the rate of complications for infection, revision or mechanical failure was less 5% and that the satisfaction rate was 86.1% [29]. The use of antibiotic impregnated penile prosthesis could significantly reduce the postoperative infection incidence and as well as the number of revision surgeries related to infectious complications. The overall postoperative complications are less than 5% and for infections is less than 2%. The incidence for mechanical failure is related of the follow-up period [30] and the overall incidence is around 5% [31]. Mechanical failure occurs more frequently in a case of multipiece inflatable prosthesis, due to their complexity. Malleable prosthesis due to the simple design are associated with a lower risk of mechanical problems. Lotan et al. related that the rate of survival (without technical problems) for malleable prosthesis compared to the inflatable ones is 87% vs 50% [32]. Paranhos encountered during his study a rate of mechanical problems in 3.59% of cases [21].

Atienza, in a meta-analysis regarding this problem found that the overall rate of mechanical failure was 1.4% [33]. He also found a rate of prosthesis exchange or withdrawal of 7.1%, which was similar to the rate that Paranhos described in his study of 8,68% [21].The causes of exchange or withdrawal were inadequate cylinder size, fracture of bioprosthesis (very rare, Parahos [21] reported 2 cases of patients with prosthesis fracture that didn’t know of their problem and they had a normal sexual activity, this issue was found during check-ups).

Yoon Seeob Ji [20] related that 21.6% of patients (16 patients) experienced mechanical problems and 5.4% (4 patients) mechanical problems. The mean follow-up period was 98 months. The mechanical problems encountered were caused by cylinder leakage 8.1% (6 patients), reservoir leakage 1.4% (1 patient out of 16) and pump failure 1.4%. In the case of the other 8 patients with mechanical failure the cause was not found because they didn’t undergo reoperation. The nonmechanical failure was due to erosion, infection of the prosthesis and other medical problems. All the patients with non mechanical failure underwent implant removal and surgical exploration. The survival rate of the inflatable prosthesis during the follow-ups over 5.10 and 15 years were 67.6%, 43.2% and 23%.

RESULTS

Compared with the conservative treatment for ED, the satisfaction rates achieved after penile prosthesis implantation are higher. According to the studies that we have analyzed, the overall patients satisfaction is 70-90%. The difference between pacient and their partners satisfaction rate is negligible, this difference ranging between 2 to 8%. The satisfaction rate regarding the adequate erection for sexual intercourse is 80-90%. The overall satisfaction rates for the malleable prostheses are lower compared with inflatable devices, 30-75%, respectively 75-90% for the inflatable prosthesis. The mechanical and overall survival rates for the malleable prosthesis range between 65-80% at 10 years, and 58-75% for the inflatable devices. Overall ease of use is rated as 78%.

Complications after implantation can be encountered in less than 5% of cases and infections in less than 2%. The likelihood of continued use is higher in the group of the patients with the inflatable prosthesis compared with the malleable ones 70-80%, respectively 50-60%. Up to 85-90% of patients with inflatable prosthesis would recommend them.

CONCLUSIONS

PPI is a high effective treatment for erectile dysfunction, refractory to pharmacological treatment. The technological improvements have decreased the rate of complications and revision surgeries which may appear after implantation, making these devices a safe solution and with great results for the men who choose penile prosthesis implantation as an answer to their problem. Many studies have related that the satisfaction rate after the operation was high, both for the patients and their partners [20]. The patients unsatisfaction after the implantation, was related to the unnatural feel of the sexual relations, due to the delayed ejaculation which was encountered in few cases and also because of the partners dissatisfaction, situation that may be related to the fact that the expectations were higher than the results. Thus before the operation, the doctor should make sure that the patient and his partners will have expectations in line with reality. Today, the three piece inflatable prosthesis is the most preferred one. Regarding the long term survival and complications of the penile prosthesis, the malleable prosthesis have a lower rate of mechanical problems than the inflatable ones, due to their less complex technological design, but patients satisfaction is higher in the group of inflatable prosthesis because they permit flaccidity and they have a better functional result. Patients who undergo implantation for the first time have a lower risk of postoperative complications compared with the patients who have had several interventions for different complications related to the penile prosthesis implant. The likelihood of continued use is higher in the group of the patients with the inflatable prosthesis compared with the malleable ones. The success of the implantation is very important, because the surgical revision is associated with a worse prognosis and a higher rate of complications. Regarding diabetes, several studies have shown a small correlation between diabetes mellitus and postoperatory infections [34,35]. Diabetes mellitus and radical prostatectomy are associated with a higher of postoperative complications. PPI enhances sexual capacity and self-confidence, therefore it should be considered as a final choice for patients with ED refractory to drugs.

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TOT versus TVT – mesh surgical treatment in stress urinary incontinence

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Abstract: *Introduction: Stress urinary incontinence is a highly debilitating condition, with an important impact over the quality of life. When the conservative treatment fails, the surgical treatment is a viable solution. Minimally invasive sling procedures have become the gold standard of surgical management for stress urinary incontinence (SUI) in women.*

Material and methods: The study was conducted on 68 patients with stress urinary incontinence, 52 have undergone the retropubic tension-free vaginal tape and 16 patients were operated using the transobturator tape procedure. All the patients were evaluated before the operation and the indication of the surgical treatment was established on the basis of physical examination (all the patients had positive cough test), abdominal ultrasound (to determine postvoid residual urine volume), urinalysis and urine culture (the majority of the patients have had before the operation recurrent urinary tract infections).

Results: The satisfaction was similar in both groups of patients, with an average satisfaction rate of 91% for TVT and 86% for the patients who have undergone TOT procedure. The patients were in a proportion of 78,9% at menopause, with an average number of natural births of 1.9 and a mean BMI of 28.2. Regarding the surgical duration, this has varied between 20 and 40 minutes, being higher in the TVT cases (up to 5-7 minutes longer than TOT), because cystoscopy was performed during the operation to verify if the bladder was perforated or not. The urethral catheter was removed immediately after the operation in the case of the TOT procedure and in the day after the operation for the patients who have undergone TVT procedures (all of these patients associated genital prolapse). The hospitalization time was 3 days. We haven't encountered significant intraoperative and postoperative complications.

Conclusions: The TOT and TVT procedures have proven to be highly effective and safe methods in the treatment of SUI. There are no significant differences regarding the efficacy of these two surgical methods, but the risk of intraoperative complications is a little higher for the TVT procedure. The mid urethral sling procedures improve significantly the quality of life for patients with stress urinary incontinence.

INTRODUCTION

Female stress urinary incontinence is a major public health problem, with psychological and social effects and with an important impact over the quality of life. Stress urinary incontinence is defined as involuntary leakage of urine through the urethra during physical activities which increase the intra-abdominal

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pressure, like laughter, coughing and sneezing, climbing stairs. There are numerous factors which contribute to the appearance of SUI: muscle weakness of pelvic diaphragm secondary to vaginal births, gynecological interventions, overweight and obesity, hormonal disorders. When the conservative treatment (bladder control exercises and pelvic floor muscle training, lifestyle modifications such as dietary habits, weight loss and physical activities) fails, the surgical treatment is a viable solution for SUI.

The first surgical treatment for stress urinary incontinence was described by Burch in the early 1960s and for almost 30 years, till the mid 1990s, it has remained the standard treatment for stress urinary incontinence. The long term follow-ups have suggested that this type of surgical treatment is effective, with a satisfaction rate at 5 years after the operation of nearly 80% and at 10 years of 70%. At the beginning of the 1990s, DeLancey established that the physiopathology of the SUI is associated with a defect at level of the bladder neck and urethra, related with the laxity of the surrounding tissue and as well as with the insufficiency of the urethral sphincter. He reported that the urethra lays on a layer of the endopelvic fascia and anterior vaginal wall and it is stabilized to the lateral by attachements of the levator ani muscle and arcus tendinous fascia. The urethral closure is conditioned by the urethral compression against the support offered by the anterior vaginal wall and the pubocervical fascia. This was the beginning of a new era in the treatment of SUI, the minimal surgical invasive treatment which would prove to be very effective.

In 1995, Ulmsten and Petros described the minimal invasive technique of retropubic tension-free vaginal tape. It was the first generation of sling operations, which used a polypropylene mesh. This procedure involves the blind penetration of the paraurethral and the retropubic space with a needle and the operator must exit with the needle behind the pubic symphysis. Because of its good results and low complication rate, it has become the solution for SUI. It is cited in the specialty literature that the rate of success ranges from 80 to 95%, after more than 5 years after the operation. Although the effective rate was high, it exist the intraoperative risk of complications such as bladder and bowel perforation and vascular injuries. As a solution to these concerns, Delorme described in 2001 another minimal invasive technique, the transobturator tape procedure. As well as the TVT the TOT is also a blind procedure. During the TOT surgery the puncture is performed at the clitoral level of the inferior ramus of the pubic bone and the operator must pass with the needle through the obturator foramen. This is the second generation of sling operations for SUI. It has been proven that the efficacy of the TOT procedure is similar to the TVT and with this type of procedure the risks of perforating the bladder or the bowels have disappeared, but more patients have groin pain postoperatively and vaginal or urethral erosion due to the polypropyline mesh. Both procedures are in current use for the SUI treatment, but because of its lower complication risks and similar efficacy, the TOT procedure is more popular.

MATERIALS AND METHODS

The purpose of this paper is to present our experience at the Urology Clinic of the Central Military Emergency University Hospital “Dr. Carol Davila” regarding the minimally invasive surgical treatment of stress urinary incontinence, using the vaginal sling procedures of TVT and TOT. We have analyzed several variables like: patient age, the number of vaginal births, body mass index, and gynecological interventions like hysterectomy. We present our cases and experience regarding perioperative complications and their management and as well as the efficacy rates, compared with other studies.

Between March 2010 and November 2015, in the Urology Clinic of the Central Military Emergency University Hospital “Dr. Carol Davila”, a number of 68 patients with stress urinary incontinence have been operated, using the minimally invasive technique of retropubic tension-free vaginal tape or the transobturator tape procedure. Out of the 68 patients who were admitted in our clinic for the surgical treatment of SUI, a number of 52 patients have undergone the transobturator tape procedure and only 16 patients were operated using the tension free vaginal tape technique. The TVT procedure was used only in the cases of the patients who associated genital prolapse. TVT has the same assembly principle as the TOT, but the incisions are made in the suprapubic region. The intraoperative complication risks, like bladder or bowel perforation, are higher in the case of the TVT compared with TOT. Therefore cystoscopy is essential after puncture. In all the cases with TVT we have performed cystoscopy to exclude a potential bladder perforation. Before the operation, all the patients were examined and the indication of the surgical treatment was established on the basis of physical examination (all the patients had positive cough test), abdominal ultrasound (to determine postvoid residual urine volume), urodynamic studies (testing was not performed in all the cases), urinalysis and urine culture (the majority of the patients have had before the operation recurrent urinary tract infections).

RESULTS

The mean age of the patients was 60.9 years. The patients were in a proportion of 78.9% at menopause and the average number of natural births was 1.9 ranging between zero and five. Mean BMI was 28.2, ranging from 23.3 to 37.4. Regarding the surgical duration this has varied between 20 and 40 minutes, being higher in TVT cases (up to 5-7 minutes longer than TOT), because cystoscopy was performed during the operation to verify if the bladder was perforated or not. The mean operative time was 27 minutes. Both surgeries required a 3 day hospitalization, the patients being discharged in all the cases the day following the surgery. The urethral catheter was removed immediately after the operation in the case of the TOT procedure and in the day following the operation for the patients who have undergone TVT procedures (all of these patients associated genital prolapse). In what concerns the urinary residual volume in the first days after the operation, we haven't noticed significant differences between the two types of procedures (the patients didn't accuse incomplete bladder voiding). All the patients were reevaluated at one month after the operation using the cough test, abdominal ultrasonography to determine the postvoid residual urine volume and to find potential complications secondary to the procedure. The long-term treatment outcomes were evaluated after 3, 6 and 12 months. Regarding the patients satisfaction, it was considered after the evaluation that the SUI was cured if the patient was completely continent, improved if partial urinary incontinence remained after the surgery and failed if the urinary incontinence remained the same or got worse after the surgery. The patient satisfaction was similar in both groups of patients, with an average satisfaction rate of 91% for TVT and 86% for patients who had undergone TOT procedure. Our results are in line with data from specialty literature.

J. Y. Han et all in a study about the effectiveness of TVT and TOT procedures in women with overactive bladder and stress urinary incontinence, stated that TVT and TOT procedures have decreased the proportion of the daily incontinence in these patients, with a slightly higher efficacy in the TVT group. In a study published in 2013 the ”Scandinavian Journal of Urology”, V. Nyyssonen concluded that 70% of the patients that were included in the study, with symptoms of detrusor over activity, declared significant improvement after the mid urethral tape surgery. There was no significant difference between the two operating techniques.

Cerniauskiene and collaborators in a comparative study regarding TOT and TVT outcomes reported that the satisfaction rate for the patients who have undergone the TOT procedure was 81,3%, meanwhile the satisfaction rate for the TVT group was 90%.

Zyckowsci et al stated in their comparative study regarding the TVT and TOT surgery, that the patient satisfaction rate at 6 months after surgery was 96,4% for TVT patients and 96.1% for TOT patients. Regarding the cough test and pad test, they have stated that it was negative in 91.5% and 94.4% in TVT cases, and in 93% and 93% TOT cases. The findings in our study were similar to general literature. We have observed in our study that the cough test was negative for 91.2% of TVT cases and 92% of TOT cases at 6 months, and the pad test was negative for 94% of TVT cases and 92.8% of TOT cases at 6 months postoperative. We haven't encountered significant intraoperative and postoperative complications. We haven't had significant bleeding during the interventions. The mean blood loss was under 25 ml. Local hematoma was encountered in 5 TOT cases and in only one TVT case and conservative treatment was effective. We haven't had any bladder or bowel perforation complications. We have encountered one case of relapse of incontinence due to the exaggerated physical activities, at two and a half years after the operation. In this case, we have performed a second intervention. We have removed the mid urethral portion of the tape and we have performed the tension-free vaginal tape procedure. The results at 3 and 6 months after the operation were good, although it is mentioned in general literature that in the case of reintervention for relapse of SUI the outcomes may not be as good as in the case of the first operation.

De novo overactive bladder symptoms ranged between 3 and 4.5% without differences between TOT and TVT patients. The pharmacological treatment which was applied in these cases has had good results. There are studies in which it is mentioned that the incidence of the overactive bladder symptoms ranges between 4 and 30 % after the operation.

Bladder botulinum toxin A injection and percutaneous sacral nerve modulation have proven to be good alternatives for the TOT and TVT surgery. Since its introduction (2005-2006) the number of patients who were admitted for bladder botulinum toxin A injections has increased constantly, overtaking in 2010 the number of TVT/TOT procedures. So far we haven't applied this method of treatment for the SUI.

With the passing of the time, the number of patients who have undergone TOT/TVT surgery and have chosen to remove their tapes grows. Most tape removals are performed at a relatively short time after the procedure, the main cause being urinary retention or tape extrusion. We haven't encountered such situations in our study.

CONCLUSIONS

The minimally invasive surgical treatment of SUI (TOT/TVT) has become the gold standard for patients with this debilitating condition. Mid-urethral sling surgery is performed to fix the urethra and involves few anatomic changings in the surrounding tissues.

These procedures have proven to be highly effective, increasing the quality of life for the patients who suffer from SUI. Regarding the efficacy rate of this two type of procedures it hasn't been noticed significant differences in the study that we have done, our results being in line with specialty data.

In terms of complications the risks are higher for the TVT procedure, due to the risk of bowel or bladder perforation. To exclude this possible accident, which may happen during TVT, cystoscopy is essential. Therefore, the TOT procedure has become more popular.

Till the introduction of TVT and TOT, SUI was either simply accepted or required invasive surgery.

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CLINICAL PRACTICE

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Penile fracture – clinical case presentation

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INTRODUCTION

Fracture of the penis is a rupture of the tunica albuginea of the corpus cavernosum, usually while the penis is rigid and erect. When erect, the albuginea layer stretches so far until its thickness reaches approximately 0.25 – 0.5 mm, compared with a 2 mm thick when flaccid. Thus the erect penis is much more vulnerable to serious damage from blunt trauma than the pendulous, flaccid penis. The most common cause of penile fracture is vigorous vaginal intercourse, more likely when woman is on top, but it can also occur during masturbation, forced bending of the erect penis or any mechanical trauma to the erect penis. The classic presentation described by the patient cracking or popping sound, followed by rapid detumescence, severe pain, ecchymosis of the penile skin, and swelling of the affected side with deviation of the penis shaft away from the affected side. In most cases, the penile fracture is a transverse corporeal tear close to the corpus spongiosum on the ventral aspect of penis shaft or on the lateral aspect and the tunical tear is usually 1 to 2 cm in length. The rupture can involve one or both corpora cavernosa as well as corpus spongiosum with the contained urethra therefore urethral rupture can also occur in. Most urethral injuries are associated with gross hematuria, blood at the meatus or inability to void, although the absence of these findings does not definitively rule aut urethral injury.

DIAGNOSIS AND IMAGING

The penile fracture is considered by the most to be a clinical diagnosis, with little need for imaging if the patient presents with a convincing history and physical examination. However given that urethral injury occurs not infrequently, preoperative exploration of the urethra should be considered when urethral injury is suspected (urethrography or flexible/rigid cystoscopy). There are numerous reports of the accuracy of cavernosography and magnetic resonance imaging (MRI), inspite of their accurate results, they are sometimes too invasive, time consuming or costly to justify, particularly when the diagnosis can be established by clinical examination. Ultrasonography although noninvasive and easy to perform is also associated with significant false-negative studies. Another condition that may mimic penile fracture is rupture of the dorsal penile artery or vein during sexual intercourse, in these circumstances physical examination may not be adequate for definitive diagnosis of a corporeal tear and further investigations should be performed like MRI, ultrasonography or even surgical exploration.

MANAGEMENT OF PENILE FRACTURE

Multiple publications indicate that suspected penile fractures should be promptly investigated and surgically repaired. Many reports show low complication rate and good outcomes as a result of immediate surgical repair, (less than 5% penile curvature after immediate surgical repair – El Atat et al, 2008) as the favorite approach for treatment of this urological emergency. Conservative management can also be an option in patients with uncomplicated albugineal tears, without extensive hematoma or urethral injury, but most untreated fractures usually develop delayed and persistent penile pain or curvature with erection. Conservative management of penile fracture has been associated with penile curvature in more than 10% of patients, abscess or debilitating plaques in 25% to 30%, and significantly longer hospitalization times and recovery (Meares,1971; Nicolaisen et al, 1983; Kalash and Young, 1984; Orvis and McAninch, 1989).

Although small lateral incisions may be used for localized hematomas or palpable tunical defects, a distal circumcising incision is the best option in most cases thus providing exposure to all three penile compartments. For the closing of the tunical defect is recommended to use interrupted absorbable suture 2-0 or 3-0. Deep corporeal vascular ligation or extensive debridement of the underlying erectile delicate tissue should be avoided. Timing of the intervention may also influence the long-term outcome of erectile function or postoperative complications in the way that the patient undergoing surgical repair intervention in the first 8 h after injury has significantly better long-term results than those having surgery delayed 24-36 h after fracture occurrence (Asgari et al, 1996; Karadeniz et al, 1996).

PATIENT, METHOD AND RESULTS

A 31 years old patient presents in the emergency department in our hospital at approximately 03:15 a.m., this being the third medical unit he was attending that day, accusing pain in the external genital area associated with extended hematoma of the penis and scrotum with the deviation of the penis on the left side (Figure 1A). Further anamnesis discovers posttraumatic injury of the penis while sexual intercourse that happened at approximately 01:00 a.m. The patient describes powerful bending of the penile shaft, and at the same time strong local pain, accompanied by a snapping sound and after that immediate detumescence of the penis with decrease in pain intensity. Patient describes urine voiding normal and no bleeding out of the urethra.

Figure 1. Extended hematoma of the penis and scrotum and pathological deviation of the penis shaft on the left side (A)

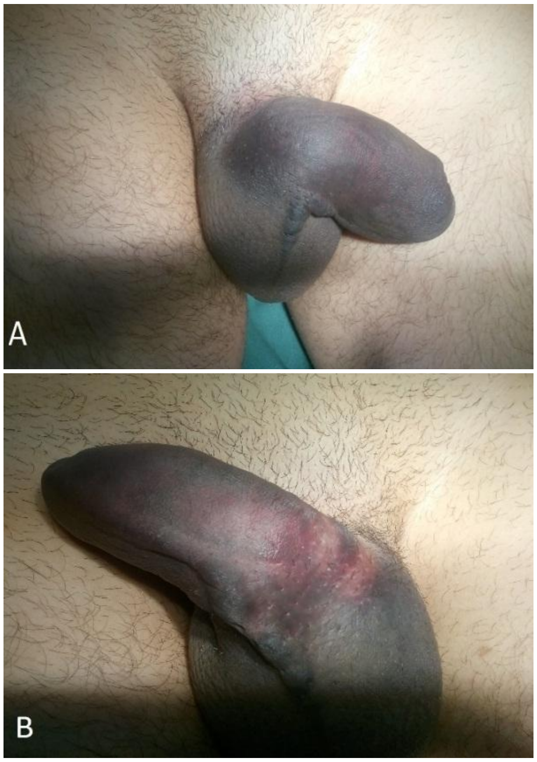

The patient is admitted in our department and undergoes a soft tissue ultrasound that reveals a local hematoma of the penis and a small breach of approximately 3-4 mm in diameter which is situated on the ventral aspect of the right cavernous body, of the proximal third of penis shaft.

Emergency surgery is decided, with spinal anesthesia at 08:00 in the morning (7 hours after the injury occurrence).

After local antiseptic measures, a 14 Fr. Foley catheter was placed before the beginning of the intervention. A distal circumcising incision is made at approximately one cm proximal of the coronal sulcus, followed by degloving the penis, and exposing the tunical fracture site in the albuginea.

The tunical tear was fond on the ventral aspect of the corpora cavernosum at a distance of 3-4 cm from the base of the penis.

Figure 2. A: albugineal tear on the left cavernous body. B: albugineal tear on the right cavernous body.

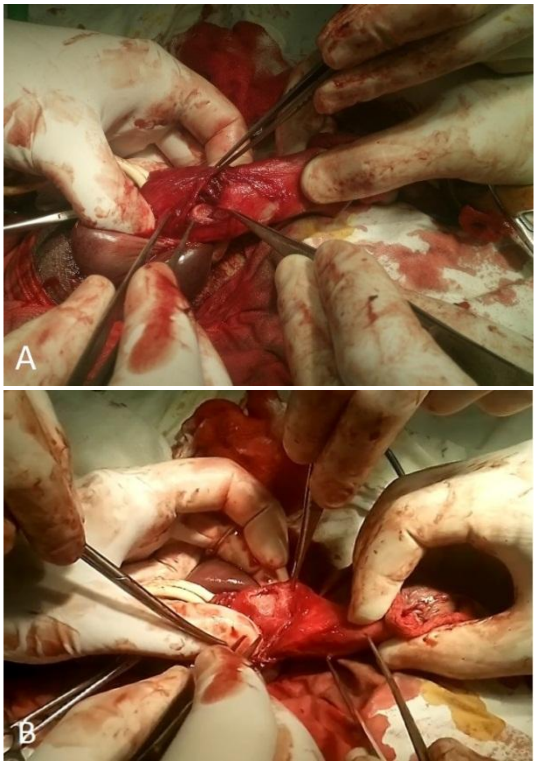

The rupture site was much greater than the one observed on the ultrasound scan. The real size of the tear was about 3 cm and involved both cavernous body on their anterior aspect, as well as the septum of the albuginea underneath the spongy body (Figure 2, A and B).

The spongy body was carefully dissected from the corpora cavernosa and so the entire length of the transversal rupture was individualized.

A temporary elastic Tourniquet at the base of the penis was necessary for local control of bleeding.

Defect repair was performed with separate absorbable 3-0 USP sutures. For the full length of the breach 14 to 15 separate sutures were needed. Final aspect of the repaired defect of the tunica albuginea can be seen in both pictures below (Figure 3, A-B).

The patient's evolution was favorable under antibiotic therapy for 5 days with no infectious complications. Second day postop he mentioned a mild morning erection although he was under treatment with Cyproterone acetate 50 mg twice a day and Diazepamum 10 mg a day in two doses.

Figure 3. A: ventral aspect of penis with the spongy body mounted on a lasso and the albugineal surure. B: final aspect after suture of the skin.

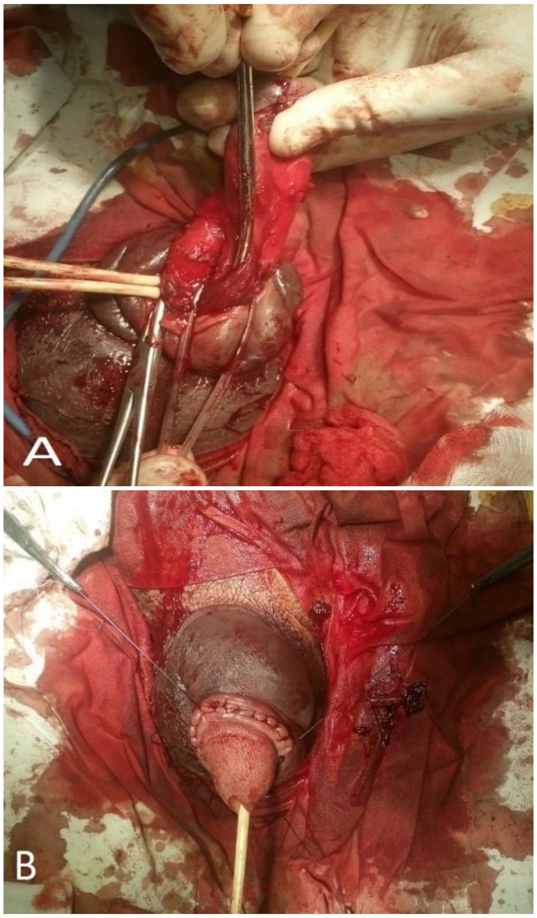

DISCUSSION AND CONCLUSION

Penile fracture is a rare case of urological emergency that should undergo in most of cases emergency surgical treatment.

The immediate surgical repair attitude (<8 h) is the preferred approach for treatment of penile fracture as reported in numerous centers around the world due to their low complication rates.

While the conservative approach is described in patients with small and uncomplicated tears, most unrepaired fractures develop late penile curvature or pain with erection.

Figure 4. Aspect of the penis and scrotum the second day postoperatively while patient still carries a Foley catheter.

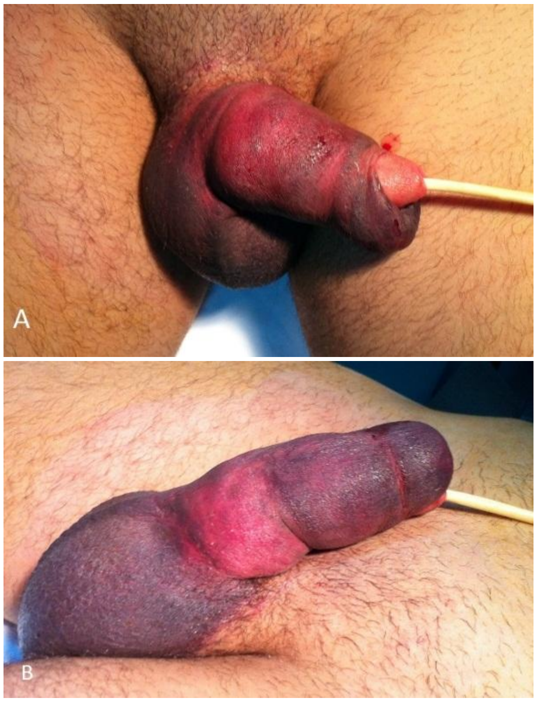

The urgency of surgical exploration justified in our case the discrepancy between imaging findings and the reality found during the surgical exploration.

Albugineal tear that involves the both corpora cavernosum is extremely rare and also usually is associated with urethral injury, which in this patient, the last one, was not the case.

The incidence of concomitant urethral injury is approximately 20 % in Europe and the USA. Most urethral injuries are associated with urethral bleeding or gross hematuria or even inability to void, but also, the absence of these signs does not exclude a potential urethral injury.

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We look forward to your submission.

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Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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Acknowledgments

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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Each figure should be supplied as a separate file, with the figure number incorporated in the file name. For submission, low-resolution figures saved as .jpg or .bmp files should be uploaded, for ease of transmission during the review process. Upon acceptance of the article, high-resolution figures (at least 300 d.p.i.) saved as .eps or .tif files will be required.

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ORIGINAL ARTICLE

Climbing – between athletic performance and psychological performance

Antonia I. Vasile[1], Florin Pelin[1], Monica I. Stănescu[1]

Abstract: *The aim of this research is to investigate different aspects of the functioning of the psychological system of performance climbers in Romania. We look for correlations between the concepts of competitive anxiety, self-esteem, motivation, willingness to take sports risks and age, sport experience and competitive experience, performance level. We also try to find out if the first contacts with the climbing world resulted in a favouring of the environment in which the athlete chooses his/her performance goals. Specifically, we aim to divide climbers into two categories, those with a passion for panel climbing (indoor) and those with a passion for rock climbing (outdoor). We did not distinguish between the bouldering challenge and the difficult challenge. In addition, we want to analyse the level of competitive anxiety of athletes in national climbing in order to objectify again the idea that the coach and sport psychologist must be able to identify, in the crucial moments before and during the competition, which athletes need motivation, which ones are anxious, which ones feel good and which ones can be left alone. We can classify athletes who need relaxation, activation or mindfulness sessions.*

Objectives: • Identifying the performance level of Romanian climbing athletes at the moment; • Highlighting the motivational dimensions of performance climbers in relation to the practice of the sport; • Investigating the passion for indoor versus outdoor climbing, with implications on the level of intrinsic and extrinsic motivation and self-esteem; • Knowing the level of competitive anxiety, with a focus on the fine delineation between being debilitating or facilitating for athletes.

Methodology: The research method used was the application of instruments (psychological tests and an opinion questionnaire). The test was administered between January-March 2020, online via google docs, on 28 performance climbers, aged between 13 and 48 years, female and male, juniors and seniors.

Results: The climber's personality shines through on the rock (outdoors), where the desirability, and if it exists, is not a social one, but is a genuine desire to push your own limits, to face difficult challenges, to feel danger, to develop your wits and to know where to stop. These concepts correspond entirely to intrinsic motivation and a more mature personality, both in social and sporting terms, from this maturity a fundamental dimension of which is the match between real possibilities and aspirations.

Conclusion: Rock climbing involves bringing out the authentic self when you are with yourself, compared to a sports competition held at the panel that highlights a socially constructed self, adapted to the fans, an image and smiles to support your image in the sporting world. In other words, avid rock athletes will train at the panel with low anxiety looking at these workouts only as technical drills for the future challenges they will face at the cliff. They are less reliant on extrinsic motivation when conducting their training on the panel, keeping it somewhere in the background for what they consider their own goals. For those for whom the stakes are to increase the difficulty of their rock routes, those will be the situations where they will have higher anxiety and a higher need to rely on extrinsic motivation as well until they reach the maturity where performance is staked by intrinsic determination.

Keywords: *indoor climbing, outdoor climbing, competitive anxiety, performance, motivation, sport experience*

INTRODUCTION

Performance can be defined as the outcome of an individual as a result of performing a task that can be measured and evaluated by an observer or by him/her (the referee or him/her as an athlete). This evaluation in climbing is quantitative – number of routes completed, number of holds achieved,

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time taken to complete the route.

Sporting performance is the athlete's bio-psycho-social value, expressed in the context of official competitions, which is assessed according to rigorously established scales and is due to a multiply determined capacity (the sum of all abilities).

Sports performance is the result of factors that depend on the athlete's personality, attitudinal orientation and motivation to perform. The limit activity is at the choice of the athlete who wants to reach a stage of excellence, break a personal or world record, win a major competition. Maximising performance can only be achieved after maximising the athlete's personality. Unfortunately, many sports trainers see the athlete as a biological machine programmed to perform certain motor actions in a repetitive number, placing little emphasis on what athletes think and feel.

It all starts with the genetic make-up of each athlete. Any subject can be distinguished: biological traits (arm span, height, weight etc – there is no typical top climber (there are very tall champions like Adam Ondra, but also short ones like Juliane Wurm – each has advantages and disadvantages for the movements in the trails that he/she has to know how to use and be aware of), physiological traits (heart rate, respiratory capacity etc), psychological traits (the mental state of the athlete) and metabolic traits (aerobic, anaerobic processes, lactic acid capacity etc) [1,2].

There are four types of trainings to be developed:

1) Physical training – there are two types: general and specific physical training. The specific one involves exercises on specialised training boards (pulls on slipped holds, on spiked holds, for example), specific exercises for body-tension (a muscle tension that cumulates several muscle groups at the same time). What is specific to climbing is working against gravity and working with your own body weight.

2) Technical training which involves three components: Initially, specific training – this refers to learning general training rules (such as to perform a route you need to double the last top hold or for the starting position you need to have all four limbs in certain holds); learning to fall on the mat for the bouldering event (teaching the athlete to jump on the mat by bending the knees, for example, to avoid injury); learning how to belay for the climbing event (type of cuff, type of rope-head, use of the spinning device, tying the safety knot); basic technical training – learning, reinforcing and perfecting technical procedures, followed by their application on routes of increased difficulty; specialised kinaesthetic system – developing techniques for holding various holds

3) Tactical training – tactical training is not similar to that in combat or opponent sports. We discuss the athletes' pattern of approaching routes and what strategies they can use to achieve a complete ascent: Route visualization – a correct and efficient visualization of the route the athlete has to achieve will save him/her many attempts: route approach strategy (e.g. the three attempts method), creative imaginationthe idea that routes differ from competition to competition (except for the speed event) is emphasized and therefore the athlete must allow his/her imagination to find variations and alternatives to achieve the route [3].

4) Psychological training which can be defined on three levels: basic psychological training – involves the formation of athletes' personality, character, specific attitudes and the skill to carry out sports activities systematically and organized. It also focuses on physical hygiene, social skills, nutrition and sleep hygiene, and the importance of invisible training; specific psychological training – aims to develop the physical, psychomotor and psychological skills needed for the sport branch. The importance of shaping the competition in training is stressed so that the athlete perceives the stakes and the tension created by the competition.

HYPOTHESES

1) There are significant differences between the group of rock enthusiasts and panel enthusiasts in terms of personality questionnaire scores and level of motivation.

2) There are significant differences between juniors and seniors in terms of motivation level.

3) There are significant differences between the group of first mountain experience and first panel experience in terms of anxiety and motivation.

4) There are significant differences in competitive anxiety by level of competitive experience and level of sport experience.

5) There is a statistically significant correlation between competitive experience and level of competitive anxiety.

6) There is a statistically significant correlation between the level of extrinsic motivation and the level of intrinsic motivation.

7) There is a statistically significant correlation between the level of self-esteem and the level of intrinsic motivation.

8) There is a statistically significant correlation between the

level of performance and the level of extrinsic motivation.

METHODOLOGY

The methodology of this study is based on the following steps:

- choice of the problem to be researched;
- determining the group of athletes to which we will apply the research instruments;
- developing the research questionnaire and its rating;
- entering the questionnaire in google docs;
- creating the database with the athletes' answers in google docs;
- analysing the database using descriptive and inferential statistics;
- analysing the confirmation or refutation of working hypotheses;
- making psychological assumptions and interpretations of the results based on statistical analysis and with reference to the literature;
- outlining the conclusions of the present research;
- highlighting possible directions for further research.

1. Participants

The study was carried out on 28 participants. We considered only performance climbers who regularly participate in internal and/or external competitions. The ages of the subjects ranged from 13 to 48 years. There are both female (11) and male (17) participants. The study group can be divided into seniors (19) and juniors, up to 20 years old (9), according to the type of competitions in which they can participate internationally (seniors are considered to be at least 20 years old, and the categories for juniors are 14-15 years old, 16-17 years old, 18-19 years old – but in senior competitions, competitors can participate from the age of 16). Criteria for inclusion in the study include: a training frequency of at least two training sessions per week and active participation in national and international competitions during 2018-2019 (competitors in National Climbing Championships, National Bouldering Championships, Boulder Games, World or European Cup stages). Athletes fall only in the Masters category (female or male) [4].

2. Tools

- Introductory questionnaire: to record participants' factual data (age, gender, sporting experience, competitive experience, maximum performance level);

- the Impulsive Sensation Seeking and Social Desirability scales of the Zuckerman – Kuhlman Personality Questionnaire, ZKPQ

The Impulsive Sensation Seeking scale contains two clusters of items, which measure two constructs: the Impulsivity construct (which refers to the lack of planning and the tendency to act quickly, on impulse, without prior reflection) and the Sensation Seeking construct (which describes the general need for excitement and agitation, the preference for unexpected situations and friends with unpredictable behaviour, and the need for change and novelty).

The Social Desirability Scale expresses the subject's need/ desire to present him/herself in a particular way, as he/she believes at that moment in time that the entourage expects of him/her. It expresses a kind of poikilotimia, moulding on the mood and expectations of the entourage, the unconscious aim being to be liked, accepted and to build and sustain a self-image slightly higher than the subject concerned knows he/she deserves;

- competitive anxiety questionnaire taken from the research literature (“Psihologia sportului. Ghid pentru optimizarea performanțelor”, Stephen Bull, pp. 64-67)

It is a questionnaire that measures competitive anxiety as a trait. The first part (with the answers Rarely/Sometimes/ Often) indicates how often symptoms occur before competition. The higher the score (values between 10 and 30), the more prone the athlete is to competitive anxiety. The second part involves measuring the score that represents how facilitating or debilitating the anxiety is for the athlete. It attempts to separate positive (facilitating, enabling, constructive) and negative (debilitating, anxiety-provoking, destructive) emotions;

- Rosenberg's self-esteem scale (1965)

This scale is used to measure the athlete's overall sense of self-worth and self-acceptance. It is a four-choice scale (1-4 items), scores range from 10-40. It divides subjects as having: low, medium and high self-esteem.

- Sport motivation scale taken from the research literature) [5], translated from English into Romanian, preserving the meaning of the items and adapting them for the Romanian sport culture.

This scale is divided into several concepts: intrinsic motivation, extrinsic motivation and amotivation. Intrinsic motivation refers to the desire to engage in an activity simply for the pleasure and satisfaction that comes from the action [6]. If a person is internally motivated, he or she will want to perform for the self, not staked by material rewards or external constraints [7]. Intrinsic motivation involves: intrinsic motivation to know, intrinsic motivation to achieve, and intrinsic motivation to have stimulating experiences.

Knowledge motivation involves curiosity, exploration, a wish to learn and a genuine wish to understand. It can be seen as driving the athlete to act for the pleasure and satisfaction of learning a new experience, of trying to understand something previously unheard of.

Achievement motivation is a concept that in psychology is also referred to as self-improvement motivation, efficacy motivation or goal orientation. It can be defined as engaging in an activity because of the pleasure and satisfaction you get from the attempt, from the idea of creating something. An example from sport might be: the attempt to perfect a difficult technique to gain personal satisfaction.

Motivation towards stimulating experiences refers to the athlete's wish to try an activity for the stimulating sensation it generates (example: sensory stimulation, aesthetic experience, amusement, pleasure).

Extrinsic motivation involves engaging in a behaviour that has a purpose, a purpose that is not for personal development [6]. Grolnick [8] in 1990 investigated extrinsic motivation beyond external conditioning and postulated three dimensions of extrinsic motivation: unconscious extrinsic motivation, identification extrinsic motivation and regulatory extrinsic motivation.

Regulatory motivation is the one most often found in the research literature. It explains why certain behaviours are driven by external sources, such as material rewards, money prizes, trophies, medals, diplomas or constraints imposed by others [7]. An example from sport psychology might be competitors expecting rewards from parents or coaches. In this case, sport is not practised for pleasure or wish, but for rewards or to avoid negative consequences (criticism, arguments, etc.).

Unconscious motivation involves an internalisation of external rewards and therefore they are no longer necessary. In contrast, positive sporting behaviour is reinforced by internal pressures such as anxiety or guilt. Such examples might be athletes who engage in physical activity because they do not feel good in their bodies, they do not feel they are in good physical shape.

Identifying motivation refers to the moment when the athlete becomes autonomous and is able to value and judge his/her own behaviour. It is still an external, material goal, but it is internally regulated and self-determined (e.g. to achieve their own goals).

Amotivation is found when the athlete can no longer specify the reason for practising sport, he/she does not know why he/she should continue or whether he/she should continue practising. Eventually, they may decide to withdraw from the activity;

- opinion questionnaire on rock climbing, a questionnaire specially developed for the purpose of this research, on the interest of Romanian high-performance athletes in rock climbing and the differentiation of climbers into the two categories – rock enthusiasts and panel enthusiasts.

3. Experimental model

Variables are: age, performance level, competitive experience, sport experience, level of competitive anxiety, level of self-esteem, intrinsic motivation, extrinsic motivation, impulsive sensation seeking scale, social desirability scale, passion. Independent variables are: age, passion, competitive experience, sport experience, personality scales (impulsive sensation seeking and social desirability). Dependent variables are: competitive anxiety, performance level, motivation scales, self-esteem.

4. Data collection procedure

The research method used was the application of instruments (psychological tests and an opinion questionnaire). The test was administered during January-March 2020, in an online environment, through google docs.

The tests applied are:

- ZKPQ test, of which we used only two scales (impulsive sensation seeking and social desirability) – 28 questions. The answers are True/False. The questionnaire is calibrated and validated on the Romanian population by Test Central (Cronbach's alpha coefficient of internal validity is explained in the test manual).

- Competitive anxiety test taken from the book „Psihologia sportului- Ghid pentru optimizarea performanțelor”/"Sport Psychology – A Guide to Optimising Performance", Stephen Bull, pages 64-67 – 15 questions. It has three response options (Rarely/Sometimes/Often) and a Likert scale from 1 to 7 (from very debilitating to very facilitating).

- Rosenberg Self-Esteem Scale – 10 questions. Items are scored with four response possibilities between strongly disagree (1 point) and strongly agree (4 points).

- Sport Motivation Scale (SMS) (taken from Journal of Sport & Exercise Psychology, volume 15, pages 35-53) – 28 questions, translated from English into Romanian, keeping the meaning of the items and adapting them for the Romanian sport culture. It is scored from 1 to 7 (1, 2 = does not correspond at all; 3, 4, 5 = corresponds moderately; 6, 7 = corresponds very much). The scoring is as follows: Amotivation corresponds to items 3, 5, 19, 28; extrinsic motivation consists of items 6, 10, 16, 22; intrinsic motivation has items 1, 2, 4, 8, 12, 13, 15, 18, 20, 23, 25, 27; identified motivation consists of items 7, 11, 17, 24; and unconscious motivation corresponds to the sum of items 9, 14, 21, 26.

A total of 104 questions were asked. Completion of the questionnaire takes approximately 8 minutes.

RESULTS

Data analysis and interpretation

The following figures illustrate classifications of athletes according to an independent variable.

The graph from Figure 1 shows the distribution of the sample according to performance level.

Figure 1: Performance level of Romanian climbers

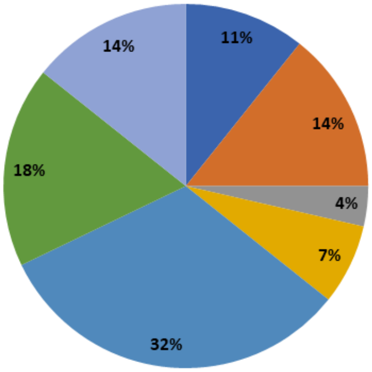

(Performance level: Balkan champion, national champion, I participated in a final/semi-final in European competition, I participated in a Balkan final, national final, participation in a world competition, I never participated in a final)

The graph from Figure 2 shows the distribution of the group according to the passion for rock or panel.

Figure 2: The passion of the athletes in the group

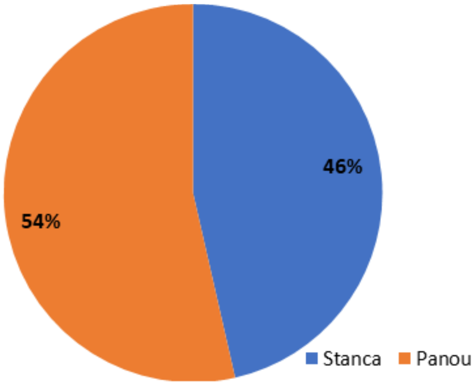

The graph from Figure 3 shows the group of athletes who had first contact with rock climbing versus those who had first contact with panel climbing.

Figure 3: First experience of athletes in the group

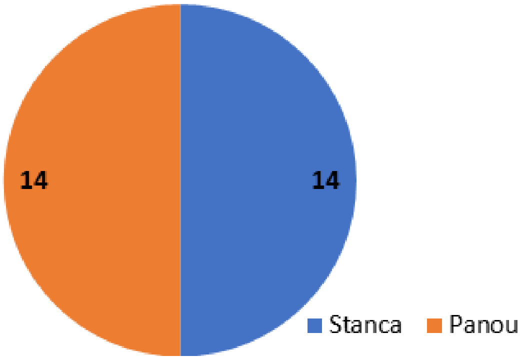

Following the achievement of the database after completing the questionnaire, 16 variables were identified: gender, age, sport experience (how many years of climbing), competitive experience (how long they have been participating in Masters competitions), performance level, impulsive sensation seeking score, social desirability score, self-esteem score, competitive anxiety score, competitive anxiety facilitation score, motivation scores (intrinsic, extrinsic, unconscious, identified motivation and amotivation), passion (rock or panel) and first experience (first contact with climbing was at panel or rock).

To decide the passion score, each question had a panel or rock response for which a score was given (panel=0, rock=1). Questions 1, 2, 4, 5, 6, 8, 10, 12, 13 (see Annex 1) were used to determine the passion scale score. Question 11 was used to determine the first experience. Of the 13 questions, 9 were with an exact response (rock or panel) to discover the athlete's passion, so the maximum possible score was 9. The remaining items were considered distracting and are not summed to determine the final score. Those scoring 0-4 were framed as having passion for the panel and those scoring 5-9 were framed as having passion for the rock.

In order to classify the level of performance, taking into account the general levels of Romanian climbers in relation to external competitions (championships or world cups), we ranked the athletes according to the following score: 1 point = Balkan champion, 2 points = national champion, 3 points = participated in a European final/semi-final, 4 points = participated in a Balkan final, 5 points = national final, 6 points = participated in a world competition, 7 points = participated in European competition, 8 points = participated in a Balkan competition, 9 points = never participated in a final

The lower the score, the higher the performance level of the athlete. Of course, it is a question about the highest level of performance perceived by him (for example, if an athlete is a national champion and has participated in European competition but came 44/49, he/she will not consider the highest level to be worth 7 points).

For the other scales, the score was calculated according to the scoring mode in the inventory.

Descriptive analysis

The descriptive statistics for the sample/group researched are illustrated in Table 1 and Table 2.

Table 1: Descriptive analysis

	Age	Exp-sport	Exp-comp	Sensation seeking	Desirability	Anxiety	Facilitation
Mean	27.07	12.02	8.64	11.04	1.82	20.96	30.04
Standard Error	1.92	1.34	1.18	0.52	0.28	0.92	2.15
Median	26.00	10.00	7.00	11.00	2.00	20.50	31.00
Mode	17.00	9.00	7.00	9.00	1.00	17.00	16.00
Standard Deviation	10.18	7.09	6.23	2.73	1.47	4.89	11.36
Sample Variance	103.70	50.23	38.85	7.44	2.15	23.89	129.15
Kurtosis	-0.77	5.70	3.99	-0.96	1.00	-0.77	-0.68
Skewness	0.46	2.02	1.74	-0.05	0.94	-0.10	0.33
Range	35.00	34.00	28.50	10.00	6.00	19.00	42.00
Minimum	13.00	4.00	1.50	6.00	0.00	10.00	12.00
Maximum	48.00	38.00	30.00	16.00	6.00	29.00	54.00
Sum	758.00	336.50	242.00	309.00	51.00	587.00	841.00
Count	28.00	28.00	28.00	28.00	28.00	28.00	28.00

Table 2: Descriptive analysis

	Amotivation	Extrinsic	Intrinsic	Identified	Unconscious	Self-esteem
Mean	8.07	11.71	69.21	19.61	18.32	26.29
Standard Error	0.91	1.03	1.89	0.81	0.90	0.41
Median	6.50	10.00	73.00	20.00	18.00	26.00
Mode	4.00	9.00	73.00	21.00	18.00	25.00
Standard Deviation	4.80	5.45	9.98	4.30	4.75	2.19
Sample Variance	23.03	29.69	99.51	18.47	22.52	4.80
Kurtosis	3.15	-0.24	0.83	2.72	-0.18	0.37
Skewness	1.63	0.75	-1.18	-0.93	-0.16	0.13
Range	20.00	19.00	39.00	22.00	20.00	10.00
Minimum	4.00	4.00	43.00	6.00	8.00	21.00
Maximum	24.00	23.00	82.00	28.00	28.00	31.00
Sum	226.00	328.00	1938.00	549.00	513.00	736.00
Count	28.00	28.00	28.00	28.00	28.00	28.00

One of the objectives of the present work was to determine the anxiety dimension of the athletes in the chosen group, demonstrating the importance of knowing each athlete individually, so that the coach can figure out if the athlete needs activation, relaxation, mindfulness or to be left alone [9]. There were two dimensions investigated: the level of competitive anxiety and the anxiety facilitation dimension which was intended to indicate whether the emotions felt by the athlete push him/her towards activation or inhibition. The results obtained are:

- 5 of the athletes recorded a low level of competitive anxiety (below 16) – meaning that they rarely have anxious moments during competition.

However:

O 2 of them score low on the facilitation dimension (below 25) – meaning that these two athletes do not often have feelings of anxiety, but when they do, these emotions are debilitating. Therefore, anxiety management sessions would be needed for these athletes for situations where anxiety may arise.

O None of the athletes scored on the facilitation size above 55, but if they had, they would have had positive, facilitating emotions. It follows that these athletes would only need activation to release facilitative anxiety for better performance in the competition.

- 12 of the athletes had a moderate level of anxiety (17-22), which means that they control their emotions well during a competition, but the level of facilitation needs to be checked to look for imbalances. And therefore:

O 5 of them scored low on the facilitation dimension (below 25), which means that if their anxiety increases in competition, the resulting emotions would be debilitating. Therefore, they would need negative emotion control sessions and anxiety control sessions.

O One athlete was at the threshold of having facilitating emotions (54) which may categorize him/her as the most mentally prepared athlete in the group since he/she has a normal level of competitive anxiety and is facilitating

O The remaining 6 athletes have a normal level of anxiety and know how to control it

- 11 athletes have a high level of anxiety (over 24), and of these:

O None had an increased facilitation level (over 55) to have constructive emotions

O 4 of them had a low facilitation level (below 25), which categorizes them as anxious athletes with debilitating, negative emotions. But we know that they need anxiety-reducing interventions.

O The remaining 7 athletes have a high level of anxiety, but normal facilitation without being debilitating or facilitating.

The results of the competitive anxiety scale compared to the level of facilitation can be better explained in Table 3.

Table 3: Level of anxiety and facilitation

Negative anxiety (5)		Normal anxiety (12)			Positive anxiety (11)	
Nevative emotions (2)	Normal emotions (3)	Nevative emotions (5)	Normal emotions (6)	Positive emotions (1)	Nevative emotions (4)	Normal emotions (7)

(Negative anxiety: negative emotions, normal emotions, Normal anxiety: negative emotions, normal emotions, positive emotions, Positive anxiety: negative emotions, normal emotions)

Non-parametric tests

Next, we will use statistical procedures to confirm/infirm the hypotheses of this paper. We will use the correlational study (Spearman correlation) to see if there are significant correlations between certain dependent and independent variables.

We will also divide the analyzed group into groups according to a criterion (age, performance level, passion, competitive experience, and first experience) in order to see if there are significant differences between them using the Mann-Whitney, U test.

To test Hypothesis 1 which seeks to see if there are statistically significant differences between the rock enthusiast group and the panel enthusiast group, the U-test was run and the results are defined in Table 4.

Table 4: Hypothesis 1

	Extrinsic motivation	Social desirability
U value	53.500	51.500
z score	2.003	-2.095
p value	0.046	0.035
Critical value of U	54.000	54.000
Effect size	0.379	0.396

The table wants to explain that by means of the Mann-Whitney test we tested whether there are significant differences between the panel enthusiasts group and the rock enthusiasts group in terms of extrinsic motivation and social desirability. The U-test value for extrinsic motivation is 53.500 and for social desirability is 51.500. The critical value for the α threshold of 0.05 and n=28 participants (the rock enthusiasts group has 13 and the panel enthusiasts group has 15) is 54. Since the calculated U-test value is lower than the critical value (53.5<54 and 51.5<54), and p<0.05 (=0.046 and 0.035, respectively), it can be concluded that there are significant differences between the two groups in terms of extrinsic motivation and social desirability. The null hypothesis is rejected. The effect size is 0.379 for extrinsic motivation and 0.396 for social desirability. Thus, hypothesis 1 is confirmed. There is a strong mean difference in intensity (r=0.379) between the two samples on extrinsic motivation. There is a strong mean intensity difference (r=0.396) between the two samples on social desirability. To test Hypothesis 2 which seeks to see if there are statistically significant differences in motivation between the junior and senior groups, the U-test was run, the results being defined in Table 5.

Table 5: Hypothesis 2

	Extrinsic motivation
U value	43.000
z score	-2.066
p value	0.038
Critical value of U	45.000
Effect size	0.390

The table explains that using the Mann-Whitney test we tested for significant differences between juniors and seniors in terms of motivation. The U-test value for extrinsic motivation is 43.000. The critical value for the α threshold of 0.05 and n=28 participants (juniors are 9 and seniors are 19) is 45. Since the calculated U-test value is lower than the critical value (43.00<45), and p<0.05 (=0.038), it can be concluded that there are significant differences between the two groups in terms of extrinsic motivation. The null hypothesis is rejected. The effect size is 0.390 for extrinsic motivation. Thus, hypothesis 2 is confirmed. There is a strong-mean intensity difference (r=0.390) between the two samples on extrinsic motivation.

To test hypothesis 3 which seeks to see if there are statistically significant differences between the first mountain experience group and the first-panel experience group, the U-test was run, the results being defined in Table 6.

The table shows that using the Mann-Whitney test we tested whether there are significant differences between the group with the first experience in the artificial environment and the group with the first experience in the natural environment in terms of extrinsic motivation and competitive anxiety. The U-test value for extrinsic motivation is 26.5 and for competitive anxiety is 45.5.

Table 6: Hypothesis 3

	Competitive anxiety	Extrinsic motivation
U value	45.500	26.5
z score	2.389	3.262
p value	0.017	0.001
Critical value of U	55.000	55
Effect size	0.451	0.616

The critical value for the α threshold of 0.05 and n=28 participants (first rock experience group is 14 and first panel experience group is 14) is 55. Since the calculated U-test value is lower than the critical value (45.5<55 and 26.5<55), and p<0.05 (=0.017, respectively 0.001), it can be concluded that there are significant differences in terms of extrinsic motivation and competitive anxiety between the two groups. The null hypothesis is rejected. The effect size is 0.451 for competitive anxiety and 0.616 for extrinsic motivation. Thus, hypothesis 3 is confirmed. There is a strong-mean intensity difference (r=0.451) between the two groups on competitive anxiety. There is a strong-very strong intensity difference (r=0.616) between the two groups on extrinsic motivation.

To test Hypothesis 4 which seeks to see if there are statistically significant differences in the level of competitive anxiety between the lower competitive experience group (under 10 years) and the higher competitive experience group (over 10 years), the U-test was run.

The results of the test are shown in Table 7.

Table 7: Hypothesis 4

	Competitive anxiety
U value	30.000
z score	2.517
p value	0.012
Critical value of U	41.000
Effect size	0.476

The table demonstrates by means of the Mann-Whitney test the existence of significant differences between the group with less competitive experience (under 10 years) and the group with more competitive experience (over 10 years) in terms of competitive anxiety. The value of the U-test is 30. The critical value for the α threshold of 0.05 and n=28 participants (those with lower competitive experience (under 10 years) has 20 subjects and the group with higher competitive experience (over 10 years) has 8 subjects) is 41. Since the calculated U-test value is lower than the critical value (30<41, and p<0.05 (=0.012), it can be concluded that there are significant differences between the two groups in terms of competitive anxiety. The null hypothesis is rejected. The difference in competitive anxiety according to sport experience, is illustrated in Table 8.

Table 8: Hypothesis 4

	Competitive anxiety
U value	50.000
z score	2.022
p value	0.043
Critical value of U	51.000
Effect size	0.382

The table demonstrates by means of the Mann-Whitney test the existence of significant differences between the group of those with below average sport experience (<=10 years) and the group of those with above average sport experience (>10 years) in terms of competitive anxiety. The U-test value is 50. The critical value for the α threshold of 0.05 and n=28 participants (the group with people below average (<10 years) sports experience has 17 subjects and the group with people above average (>10 years) sports experience has 11 subjects) is 51. Since the calculated U-test value is lower than the critical value (50<51, and p<0.05 (=0.043), it can be concluded that there are significant differences between the two groups in terms of competitive anxiety. The null hypothesis is rejected.

The effect size is 0.476 for competitive anxiety depending on competitive experience. Whereas, according to sport experience, the effect size is 0.382 for competitive anxiety. Thus, hypothesis 4 is confirmed. There is an medium intensity difference (r=0.382) according to sport experience and a strong-medium intensity difference (r=0.476) according to competition experience between the two samples on competitive anxiety [10].

Given the small group of respondents, which is due to the fact that in Romania, the community of performance climbers is small, the number of athletes and sportswomen who frequently participate in internal competitions is small, and even smaller in external competitions (for example, at the 2019 national championship in the senior category, 9 girls participated, and 6 entered the final), we will use for the correlational study, the Spearman correlation.

For testing hypotheses 5, 6, 7, 8 we used Spearman correlation, and the results are highlighted in Table 9.

The analysis of the results presented in the above table shows the confirmation of hypotheses 5, 6, 7, 8, which can be detailed as follows:

Table 9: Hypotheses 5, 6, 7, 8

	rs	p	Size effect
Anxiety - competitional experience	-0.437	0.020	0.191
Extrinsic motivation - unconscious motivation	0.434	0.021	0.188
Self-esteem - unconscious motivation	-0.504	0.006	0.254
Performance level- extrinsic motivation	-0.446	0.017	0.199

- There is a statistically significant negative correlation (with r = -0.437) between the level of competitive experience and the level of competitive anxiety (p<0.05). It follows that athletes with more competitive experience will have lower levels of competitive anxiety. The effect size is 0.191, showing a statistically significant correlation of very low to low strength [11].

- There is a statistically significant positive correlation (r=0.434) between the level of extrinsic motivation and the level of unconscious motivation (p<0.05). It thus follows that athletes with higher extrinsic motivation will have a higher level of unconscious motivation. The effect size is 0.188, showing a very low-low intensity statistically significant correlation.

- There is a statistically significant negative correlation (r=-0.504) between the level of self-esteem and the level of unconscious motivation (p<0.05). It follows that athletes with higher self-esteem will have a higher level of unconscious motivation. The effect size is 0.254, showing a statistically significant correlation of low intensity.

- There is a statistically significant negative correlation (r=-0.504) between athletes' performance level and level of extrinsic motivation (p<0.05). It thus follows that athletes with higher levels of extrinsic motivation will have better sports performance. The effect size is 0.199, showing a statistically significant correlation of low intensity [12,13].

CONCLUSION

Concerning the first hypothesis, we issue the following psychological assumptions. We would dare to say, despite some terrible accidents that have taken place on the cliff and in which prominent athletes have been involved, that this form of training in solitude, for balanced athletes, is more valuable, developing their caution and teaching them to know their limits. Conversely, on the panel, social desirability can bring with it the risk of showmanship and extravagance, when an athlete, although intuitively sensing correctly where his or her limits and capabilities lie to cross a threshold at a given point in time, may choose the opposite in the sense of skirting risk, out of demonstrativeness, extrinsic motivation and the desire to please supporters [14].

As a conclusion, we could say that passion for rock is positively correlated with intrinsic motivation, a balanced personality and authentic knowledge of physical and psychological resources at that moment of time, while passion for panel is more likely to be related to extrinsic motivation, social desirability, maintaining an image, the fact being also supported by a more vivid communication and in a here and now with the audience [15].

- Thought of in a long-term dynamic, things are not so exclusivist, as genuine athletes train on the panel for long periods in order to accumulate sufficient "psychological nourishment" provided by the admiration of others for the moments when they are about to climb the cliff and when the reminiscences of success on the panel will help them to surpass their limits [16,17].

- The results of the second hypothesis, which emphasizes the shift from junior to senior of motivation from extrinsic to intrinsic, appeals to several psychological concepts. Starting from Piaget, who describes the stages of intelligence development, the shift from childhood to adolescence and adulthood follows the same path from externalisation to internalisation of the desire for knowledge, of the perception and internalisation of the sense of danger, of the balance in the judicious allocation of resources. Juniors are dependent on the admiration of parents and coaches, on the public which in itself is happy to create young images, especially in a less developed sport in a country like ours [18]. Any junior considered a hope in Romanian climbing is greeted with joy, climbing being a sport aimed at an audience mature enough to overcome envy, malice, lack of opportunities for collaboration, promotion of non-values as is perhaps the case in other team sports or with a longer tradition and many followers.

- Climbing is a sport that is easy to enter, the environment and the circle of seniors who are frequently attached to competitions (direct participants, supporters, coaches or sponsors), capable of emotionally supporting juniors, promoting them, ranking them without them feeling blamed, pushed aside, criticised, rejected [9]. Currently, in Romanian climbing, for ages between 4 and 16 years old, perhaps extending to 18, we consider extrinsic motivation important because it supports the inner construction of the athlete, helps him/her reach and maintain the state of readiness, creates a sense of a continuum between him/her and the audience. Let's not forget those key moments when the junior is majorly dependent on the ideal match between the shouts and support in the gym and his/her internal moment when he/she is ready to step off the track. Seniors, however, are mature people who bring together multiple competitive experiences with personal life experiences from the various fields in which they are still active, and from the questionnaires administered they emerge as authentic personalities with no false selves, no false modesty, no hiding or desire to present themselves in a more favourable light [19].

- For seniors, intrinsic motivation requires dedication, composure, balance, responsibility, the ability to know how to lose, and the ability to recognize a new value emerging in your shadow or next to you, long-term planning of resources so that competition matches peak performance. Intrinsic motivation is one that is underpinned by good self-knowledge, with pluses and minuses, strengths and weaknesses both in terms of techniques used and psychological background and the ability to induce and maintain the state of readiness [20].

- Many juniors owe their success on extrinsic motivation, namely in the junior period for two athletes who have similar technical and physical competitive qualities, a higher extrinsic motivation of one (more encouragement from the coach, more involved parents, more admirers from the public on the model of sympathy) can make a significant difference in terms of a competitive result. Among seniors, intrinsic motivation is much correlated with technical development, with a balanced psyche, with a more cautious calculation of what the individual can or cannot do, each new competition being a dynamic putting the individual's personality in competition not only with others but also with his/her own expectations, in a continuum of sports training. We thus encourage the maintenance of extrinsic motivation as a stimulus up to a certain age in junior sport, which proves its usefulness up to the point where it would become an intrusion into the athlete's inner space (the athlete would be conditioned, perhaps even threatened, blackmailed, compared to others, accused of having his/her admiration withdrawn, or the situation where significant discrepancies between likes and dislikes would affect the fragile psyche of a budding athlete) [21].

- An important variable we wanted to consider in this study was the first experience of contact with the sport, as from the constructed questionnaire it appeared that for some of the athletes the first experience corresponded with their subsequent passion for rock versus panel, but for others this was not the case. The first experience, especially if it took place at an early age correlates with extrinsic motivation, support from the entourage, praise and encouragement from others, especially if there are coaches, friends maybe even parents in the vicinity involved and dedicated to the passion for rock versus passion for panel. First experience and extrinsic motivation become important when the person in question joins a lineage. Introducing anxiety as a variable in relation to first experience and extrinsic motivation could be explained as follows: when first experience encourages passion in one direction or another, extrinsic motivation supports the direction (rock versus panel), while anxiety tends to decrease when it is not consistent with passion [22].

- It is well known that accumulated competitive experience develops not only athletic techniques, but also mental skills of concentration, stability, mobility and distributivity of attention, motor memory, memory of a chain of sequenced movements belonging to a technique, as well as the ease of habituation to get into the competitive mood, to activate on command one's readiness and flow state, to make up through technique and experience the deficit of athletic performance (if that competition does not overlap with the athlete's sports form). The more complete, valuable and comprehensive this competition is, both from a tactical and strategic point of view and in terms of the development of the athlete's personality milestones (motivation, willpower, determination, preparation), the better prepared the athlete will be and will consider tenacity superior to anxiety. In other words, competitive anxiety decreases as experience in the field is gained [17].

- The hypothesis confirming the positive correlation between extrinsic motivation and unconscious motivation is based on the fact that as the athlete progresses from childhood to junior and adulthood, he/she internalizes his/her rewards and does not need to represent them in an external plane (promises, support, cups, medals, praise), but he/she has taken within him/herself an amount of pressure "sufficiently big and at the same time sufficiently little" to be able to filter between eustress and distress so as to keep his/her anxiety within limits consistent with eustress and with the possibility of physiologically determining his/her state of alertness and vigilance as an important area of the construction of the state of flow [23].

- The fine transition from extrinsic to unconscious motivation is natural in conjunction with the development of personality, as one of the coach's desires should be a filter as careful as possible in terms of the athlete's experience of feelings of guilt in the event of failure. As it is known from the research literature, the concept of extrinsic motivation can include reprimand or punishment from secondary sources when the athlete has not brought them the expected results. Conversely, unconscious motivation, in the absence of sound principles to help develop a balanced and normal personality of the athlete, can contribute to increased feelings of guilt, anxiety, low self-esteem, subjective feelings of not being good enough, which can ultimately lead to failure. This is where we should find the great art of coaches and sports psychologists in helping juniors to put a filter when they internalize aspects of extrinsic motivation unconsciously [24].

- The following hypothesis confirms the negative correlation between self-esteem and unconscious motivation. Turning to the psychological explication, we could consider as follows: athletes able to maintain high self-esteem are those less concerned with being in touch with the unconscious strand of their own motivational resources. They are those who have strengthened their facade, their competitive selves and the imago with which they interact in the sporting world, calling on the unconscious reservoir only when they wish to make contact with what once represented praise, encouragement, admiration, resource enhancement. The lower the self-esteem of a competitive athlete, the more we are dealing with an unconscious motivation in which the athlete has a severe over-ego, punishes him/herself, blames him/herself, criticizes him/herself, exacerbates his/her guilt, considers that he/she has not done enough, self-stigmatizes him/herself and can no longer find the way to reactivate his/her beneficial resources, the techniques that once proved successful, the positive mental states. Athletes with low self-esteem also lose patience and forget to think by steps, little by little, expecting the coach or psychologist to provide miraculous solutions that will free them from a new circuit of effort in order to recover both their self-esteem and their high position in the competitive rankings [25].

- As the athlete and competitive years progress, extrinsic motivation loses ground to intrinsic motivation, so that a high level of performance can only be based on strong intrinsic motivation, which in turn is the axis of the personality structure of the competitive athlete [26]. Intrinsic motivation is that which is less dependent on circumstances, it is based on the concepts of effort and patience, on the adaptation of wishes to the possibilities of the body, while extrinsic motivation is fuelled by the reservoir of wishes, hopes and expectations in someone else's mind, and from one level onwards, the performance athlete tries to create their own inner world, their own competitive 'battle' space, in which both their physical and psychological attributes and qualities are brought together.

From this point, extrinsic motivation and the mind of an outsider can give errors in terms of correctly assessing the level of performance an athlete may find him/herself at a given point in time, thus asking more or less of him/her than he/she him/herself knows he/she can give.

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ORIGINAL ARTICLES

Clinical significance of p16INK4a and p53 and their involvement in penile cancer development. A literature review

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Abstract: *(1) Background: Human papillomavirus (HPV) E6 and E7 oncoproteins are vital for developing HPV induced penile carcinoma. The viral oncoproteins play a central role in oncogenesis by interacting with several cellular regulatory proteins, such as p16^{INK4a} and p53. Many studies suggest that these proteins showed clinical utility in predicting nodal disease, cancer specific survival, overall survival and even tumor grade. Understanding the molecular mechanism involved in the carcinogenesis of penile cancer could offer biomarkers for disease progression, treatment response and potential targeted therapies; (2) Methods: This paper is a prospective study on a group of 100 patients who underwent prostate surgery during 2013 and 2014 in the Urology Clinic of "Carol Davila" Central Military Emergency University Hospital Bucharest. They were tested for HPV by PCR and IHC (p16) methods; (3) Results: 11 cases (22%) of HPV were found in the cluster of patients tested. PCR and P16 were the HPV diagnostic tests used. In order to determine the consistency of the 2 tests, the Cohen's kappa coefficient was used at a p level < 0.05. The PCR method had a sensitivity of 81.8% and a specificity of 94.9%. The P16 method had a sensitivity of 63.6% and a specificity of 89.7%.*

Keywords: *penile cancer, p53, p16^{INK4a}, prognostic, potential therapies*

INTRODUCTION

Penile cancer accounts for less than 1% of cancers in men in developed countries, being more common in less developed areas of the world such as parts of Africa, Asia, and South America (up to 20%). Beingusually an elderly disease, the mean age of diagnosis for penile carcinoma is 60 years old. Early diagnosis is of utmost importance, this disease leading to a devastating disfigurement and advanced disease being associated with poor outcomes. Two major etiologies have been proposed for the development of penile carcinoma: chronic inflammation secondary to chronic balanitis due to phimosis and HPV-induced disease. In literature,basaloid and warty subtypes are almost always associated with HPV. Studies have demonstrated that the most common type of HPV infection is HPV 16 and 18, which occur in 60–75% of in situ and invasive tumors. In terms of HPV-induced penile carcinoma, E6 and E7 viral oncoproteins are essential for developing HPV-induced penile cancer, disrupting various cell-cycle checkpoints and apoptosis. Viral oncoproteins release the cell from p53 control, thus surviving the S phase of cellular cycle with DNA damage. Furthermore, E7 overexpression is followed by the overexpression of cyclin-dependent kinase inhibitor p16INK4a, which can be used as a biomarker for HPV-transformed epithelial cells. Studies have shown that p16INK4a overexpression is only seen in high-risk HPV genotypes. Mannweiler et al. found that in the absence of HPV infection, penile carcinoma still presented p53 mutations, indicating high proliferative activity of the cells, thus disease progression [1-4].

Researching the role that p16 and p53 have in the development of penile cancer could give somemuch-needed biomarkers for disease progression and treatment response for this aggressive disease. Furthermore, understanding the molecular mechanisms involved in the pathogenesis of

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penile carcinoma can offer potential targeted therapies [5].

MATERIALS AND METHODS

Markers in penile carcinoma

Role of p53 in penile carcinoma

Encoded by TP53 gene located on chromosome 17, which is the most frequent mutated gene in human cancer, p53 is a nuclear transcription factor, having a pro-apoptotic function. Named "guardian of the genome", p53 transactivates several genes implicated in the induction of cell cycle arrest and apoptosis [6,7]. Under normal circumstances, the levels of p53 are extremely low. However, in the presence of DNA damage, p53 accumulates in the nucleus through post-translational modifications like phosphorylation or acetylation.

Depending on the extent of DNA damage, p53 can either stop the cell cycle, allowing cells to repair the DNA and then letting the cells re-enter the normal cell cycle, or if the DNA damage is beyond repair it will exert its pro-apoptotic functions inhibiting the transfer of damaged DNA to daughter cells. Through these actions p53 maintains genomic integrity.

Certain pathogens, including HPV, can affect p53 protein. HPV interacts with p53 through E6, marking the protein for proteasomal degradation by transferring ubiquitin peptides from E6AP complex (E6 binds to E6-associated protein) to p53 tumor suppressor protein. Furthermore, by biding itself to pRb, which is a cell cycle regulator, and inactivating it, E7 complements the action of E6 oncoprotein. Consequently, by releasing the cell from p53 control, damaged DNA will be transferred to daughter cells [8].

As stated above, several studies have found that HPV-negative penile squamous cell carcinomas express p53 linked to TP53 mutations [4,9,10]. Although TP53 was initially described as a tumor suppressor gene disabled by loss-of-function mutations, recently it was discovered that it harbors oncogenic potential [11]. Multiple mutations of TP53 have been discovered over the years. R175H, which is the most common mutation, leads to cell cycle progression and initiation of genes that promote cell survival.

Yoshikawa et al. found that, when overexpressed, R248Q mutation offer cells an invasive ability [12]. Other mutations found are R273C, implicated in proliferation and inhibition of apoptosis, and R282, associated with higher activity of drug-metabolizing enzymes [13,14]. Even though newly pathways and mutations are being discovered, HPV-negative penile carcinogenesis is still poorly understood [15].

Role of p16 in penile carcinoma

Encoded by CDKN2A gene located on chromosome 9 (9p21.3), p16 (also known as p16^{INK4a}) is an inhibitor of cyclin-dependent kinases (CDK) that impede cell division by slowing the progression of the cell cycle from the G1 phase to the S phase, therefore acting as a tumor suppressor. p16^{INK4a} induces cell cycle arrest and prevents cell division by inhibition of CDK4 and CDK6 and inhibition of CDK-mediated phosphorylation ofRb (retinoblastoma) protein, whose expression increases as the cell cycle progresses. Phosphorylated (inactivated) pRb dissociates from the transcription factor E2F1, allowing it to enter the nucleus, thus promoting the transcription of target genes that are crucial for transition from G1 to S phase. Through its actions, p16INK4a maintains pRb bound to E2F, denying the cell progression to S phase, thus preventing excessive cell growth.

The HPV-E7 protein, which is an oncogenic protein of HPV, can bind and inactivate Rb protein causing carcinogenesis with promotion of cell-cycle progression. Therefore,high-risk HPV positive penile carcinomas have increased cell proliferation with p16INK4a overexpression, allowing p16 to be used as a surrogate marker for HPV-E7 expression. Furthermore, it has been observed that in HPV negative penile carcinomas p16 overexpression is absent, strengthening the idea that p16INK4a can be used as a biomarker for HPV related cancers [16,17].

DISCUSSION

Several papers studied the presence of HPV and its viral oncoproteins as a prognostic factor for penile carcinoma. A mutated TP53 gene usually leads to a mutated P53 protein, rather than the absence of it. The regulatory protein MDM2 (the human homologue to the murine double minute 2 oncogene) controls the intracellular p53 concentration through a negative feedback loop. Mutant p53 protein cannot bind to MDM2, thus leading to an overexpression of p53. Studies have shown that p53 overexpression is an independent predictor of lymph node metastases. In their paper, Lopes et al. observed that patients who were HPV and p53 positive had a worse overall survival than those negative for p53, with a mean follow-up of 88.7 months. Furthermore, p53 immunoreactivity and p53 grade were associated with a higher incidence of lymph node metastasis [18-20]. Martins et al. stated in their study that p53 overexpression was associated with regional lymphatic spread, recurrence, and disease specific mortality [21]. The same results regarding overall survival of patients with HPV-induced penile cancer and positive for p53 expression were found by many authors. In their paper, Prapiska et al. observed that the survival rate over 36 months in the p53 positive group was 18%, compared with a survival rate of 60% in the p53 negative group [22]. Strengthening the idea that p53 overexpression is associated with poor survival, Gunia et al. found that the 5-year cancer-specific survival was 84% in p53-negative and 51% in p53-positive PC [23].

The role of p53 was studied in several other aspects regarding its involvement in penile carcinoma. For example, Marinescu et al. conducted a research on the association of p53 and the grading of penile carcinoma specimens. They concluded that all the poorly differentiated penile cancer cases had a positive expression of p53 oncoprotein, no matter the tumor stage. Moreover, as the tumor grading improves the expression of p53 decreases, thus, p53 was found in 91.2% of the moderately-differentiated tumors a and 72.2% of the well-differentiated tumors [24]. As stated above, in HPV-induced penile carcinoma, TP53 tumor suppressor function is suppressed by E6 oncoprotein, while simultaneous activation of pRB by E7 initiates epithelial neoplasia. Multiple studies in literature found that TP53 can have mutations in the absence of HPV infection. In HPV-induced carcinogenesis, it has been observed that TP53 function can be restored via radiation and chemotherapy. Furthermore, chemotherapy and radiation therapy resistance are mediated by permanent inactivation of TP53, namely, by mutations of the TP53 coding region, since the effectiveness chemotherapeutic agents, such as 5-fluorouracil, doxorubicin, bleomycin, and cisplatin, and radiation depend partially on p53-mediated responses. This can explain the worse prognosis regarding the overall survival of patients with HPV-negative penile carcinoma after radiation and chemotherapy [15].

Zargar-Shoshtari et al. conducted a study on the clinical significance of p53 and p16 status of patients with penile carcinoma. They found that the cancer specific survival (CSS) and overall survival rate (OS) were improved in p16-positive patients. Furthermore, they observed an association with p53 overexpression and nodal disease in p16 negative patients. They also concluded in their paper that patients who had positive lymph nodes and a double p16 and p53 negative status had a significantly worse CSS. It is known that E6 oncoprotein activation leads to a reduction in host cell p53 expression, thus cells expressing only E7 could be positive for p53, whereas cells expressing both E6 and E7 are negative for p53. Because multiple molecular and genetic pathways can lead to an overexpression of p16 and p53, it is possible to detect both in the same cancer cell [25].

It has been established that pRb is a target for E7 oncoprotein leading to an overexpression of p16^{INK4a}. Because of this, p16INK4a can be used as a marker for HPV infection. Mannweiler et al. reported in their study that lesions which were p16 negative had very low rates of HPV detection. Moreover, they stated that all p16-negative cases were positive for p53 nuclear expression and that out of 72 patients with p16 overexpression none was positive for p53(4). Senba et al. observed that in the HPV-positive penile carcinoma cases, p16^{INK4a} was positive in 61.5%, compared to 25% in the HPV-negative group, strengthening the idea that p16 can be used as a surrogate marker for HPV infection [26]. Several other studies suggested that p16^{INK4a} positivity in penile carcinoma is strongly related to the presence of high-risk oncogenic HPV genotype and that negativity can indicate an infection with a low-risk genotype or the absence of HPV infection [27-29]. In their paper, Steinestel et al. found that the sensitivity and specificity of p16^{INK4a} staining to predict the presence of high-risk HPV was 100% and 57%, respectively [30].

Regarding prognostic significance of p16INK4a expression in penile carcinoma, a meta-analysis on 323 cases was performed by Zhang et al. They concluded that p16INK4a expression is independently associated with improved CSS for patients with penile carcinoma [31]. Another study found that high-risk HPV status and p16 positivity, independent of histological tumor grade, indicated a less aggressive local behavior [29]. Sand et al. observed that patients with HPV induced penile cancer with negative p16 staining had a worse disease specific survival than men with p16-positive penile carcinoma [32].

In their paper, Martínez-Bailón et al. declared that all-cancers with p16^{INK4a} overexpression were positive for HPV DNA, but, interestingly, not all HPV-positive patients were positive for p16^{INK4a}. Several papers in literature suggested that the detection of HPV DNA is not sufficient for the attribution of HPV infection to the etiology of the cancer, transcriptional activity or the molecular signature of HPV-induced carcinogenesis needed to be proven [33-35].

The report from the International Society of Urological Pathology (ISUP) consultation conference on molecular pathology of urogenital cancers recommended the use of p16 staining in cases in which the histological features of the squamous cell carcinoma are not classical for HPV-induced cancers, especially in poorly differentiated tumors. The consensus was that p16 can be used as a surrogate of polymerase chain reaction for the identification of high-risk HPV genotypes [36].

As seen so far, p16^{INK4a} is overexpressed as an early consequence of high-risk HPV-mediated carcinogenesis. Furthermore, it has been noticed that humoral and cellular immune responses occur spontaneously against p16. This observation is the basis for creating a therapeutic vaccine using a synthetic p16 peptide to prevent the outgrowth or destroy the affected cells. Two trials tested the vaccine to asses safety and immune response (ClinicalTrials.gov Identifier: NCT01462838 and ClinicalTrials.gov Identifier: NCT01462838). The conclusion was that the vaccine was safe and that it showed strong immune responses against p16, strongly induced T cell CD4+ and humoral immune responses in all patients. Moreover, it is safe to use in combination with standard cisplatin-based chemotherapy with excellent safety and tolerability [37-40].

CONCLUSION

More common in less developed countries of the world and usually an elderly disease, penile carcinoma leads to important disfigurement and poor outcome if not diagnosed in its early stages. It is well-known that HPV induced penile cancer patients have a better outcome than HPV-negative ones. Furthermore, HPV-induced penile carcinoma with p16 positive staining have better overall survival. These findings point to the possible clinical value of HPV and p16 testing when planning the most optimal management and follow-up strategy. The gold standard for HPV detection is polymerase chain reaction, but this technique is expensive and not always available, thus, since p16 protein was found to be associated with the presence of high-risk HPV, it can be used as an alternative marker. Several studies concluded that p53 overexpression was associated with poor survival and an independent predictor of lymph node metastases. Moreover, p53 positivity was strongly associated with poorly differentiated penile carcinomas. Early results of the p16 vaccine showed that it is safe and that it can trigger a good immune response, but further studies on larger patient groups are needed.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.
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Authors' contribution

ADS and DRM collected, analyzed, and interpreted the patient data. DM, RAA, DLI, DOC, RSC and AC had substantial contribution to the conception of the work and interpretation of data; also, they drafted the manuscript and contributed substantially to writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies, is in accordance with the tenets of the Helsinki Declaration, and has been approved by the review board of "Carol Davila" Central Military Emergency University Hospital Bucharest, Romania.

Patient consent for publication

Not applicable.

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ORIGINAL ARTICLE

Evaluation of children with ADHD and their psychiatric symptoms

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Abstract: *Background: Mental disorders, especially in children, can be manifested in different ways, which is of great importance to them, so the purpose of this evaluation was to evaluate the changes in psychiatric symptoms in children with ADHD.*

Method: This evaluation was performed as a descriptive-analytic study on 141 children with ADHD who were followed up for 6 months. At the beginning of the study and the third and sixth months after, patients' symptoms were assessed with the CBCL Behavior Checklist and the ADHD rating scale questionnaire, as well as the course of illness and association with other disorders. Finally, the data were entered into SPSS statistical software and analyzed.

Results: The mean age of the children was 5.89±0.81 years and the prevalence of males was 68.8%. Among the two treatment groups, only the mean score below the ADHD-RS questionnaire score in the non-Ritalin group at the end of the third and sixth months was significantly lower than the Ritalin group. During the study in both treatment groups, the overall ADHD-RS questionnaire and both subscales of attention deficit hyperactivity disorder (ADHD) had a significant decrease from baseline to the end of the sixth month. In relation to the CBCL behavioral checklist, the mean score of the total checklist except for anxiety/depression in the non-Ritalin group and the somatic complaints subscale in the Ritalin group decreased significantly from baseline to the end of the sixth month.

Conclusion: Based on the results observed in this evaluation, Ritalin had a significant effect on the symptoms of patients with ADHD, although these symptoms improved in both groups, however, further studies are needed.

Keywords: *attention deficit hyperactivity disorder, Ritalin, children*

INTRODUCTION

Attention Deficit Hyperactivity Disorder is a persistent pattern of inattention and hyperactivity, which is more common and severe than typical of children with similar developmental levels. While in the past hyperactivity was thought to be a devastating underlying symptom of the disorder, the general view today is that hyperactivity is often secondary to poor impulse control. Impulsivity and hyperactivity are common in today's diagnostic criteria for ADHD [1]. At present, the diagnosis of ADHD has based on the agreement of experts that three observable types include: inattentive, hyperactive/impulsive, or mixed, all manifestations of a disorder. To meet the diagnostic criteria for ADHD according to the DSM-IV-TR criteria, symptoms must be present before the age of 7 (and, according to the DSM-V, before the age of 12) [2,3]. However, the diagnosis of ADHD in many children is not made until after the age of 7, when their behaviors cause problems at school and elsewhere. To confirm the diagnosis of ADHD, impairment due to inattention or hyperactivity/impulsivity must exist in at least two areas and interfere with proper social, educational, and extracurricular activities. This disorder should not occur during pervasive developmental disorder, schizophrenia, or another psychotic disorder, and no other mental disorder is a better explanation for it [4].

Attention Deficit Hyperactivity Disorder is one of the most common psychiatric disorders in children with a prevalence of 3-7% and is 3-5 times more common in boys than girls [5, 6]. Symptoms are a persistent pattern of inattention and/or

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hyperactivity and impulsive behavior that is more severe than expected for the child's age and level of development. Some children may have more active symptoms and impulsive behaviors, and some may have more inattention symptoms. According to the DSM-V criteria, the symptoms of this disease start before the age of 12 and usually exist from the age of 3. But serious problems often arise during school [7]. The course of the disease is variable and most patients recover partially, and inattention is often the last symptom to improve. However, these patients are prone to antisocial behavior, substance abuse and mood disorders, etc. [8,9].

Based on the cases stated in this section, the importance of paying attention to the association of other disorders with ADHD and evaluating the effectiveness of treatment methods on these symptoms is determined. Has suffered from ADHD.

METHOD

This evaluation was a cross-sectional-analytical and longitudinal study that was performed on children with attention deficit hyperactivity disorder, in which psychiatric changes and disease course were evaluated in this group of patients.

For this purpose, 141 children aged 5 to 7 years with ADHD were included in this study by available sampling. Patients were followed up for 6 months and every 3 months their symptoms were assessed using the CBCL Behavioral Checklist and the ADHD-RS (ADHD rating scale) questionnaire filled out by parents. ADHD Rating Scale is an 18-item questionnaire for children 5 to 17 years old. This questionnaire examines the child's behavior over the past 6 months and is completed by their parents or teacher. The overall score of this questionnaire is between zero and 54 and each item is graded from zero to 3. The questionnaire also has two subscales: signs of hyperactivity / impulsivity (including 9 questions) and attention disorders (including 9 questions). This scale has two forms of school and home, the first form is filled out by school teachers and the second form by parents. In validated studies, high validity and reliability have been mentioned for it and its psychometric properties have been comparable with other valid scales for measuring the severity of ADHD (10).

The CBCL Behavioral Checklist is also a general diagnostic method for identifying behavioral problems in children. This checklist is classified into 4 forms based on different ages that examine different aspects of behavior. Similar questions are usually categorized into syndromic subscales. Accordingly, 8 syndromes include Aggressive behavior, Anxious/depressed, Attention problems, Rule-breaking behavior, Somatic complaints, Social disorders. problems), Thought problems, Withdrawn/depressed. The two general subgroups in the CBCL Behavioral Checklist also include internalization disorders (sum of scores of anxiety/ depression subscales, deprivation/depression, and somatic complaints) and extrinsic disorders (total scores of normative and aggressive behavior subscales). In this study, children aged 5 to 7 years were included in the study who were normal in terms of IQ and otherwise did not meet the inclusion criteria. Finally, the information of all patients was entered into SPSS software version 23 for further analysis and evaluation.

Ethical Considerations

This study was approved by the ethics committee of Mashhad University of Medical Sciences. Written consent was obtained from all patients' parents to participate in this study. Participants' information was kept confidential and none of their personal details, including their first and last names, were entered into the software. All individuals were given a project-specific code and analyzed accordingly. The initial information will remain with the main executor until the completion of the project and the publication of the articles. This research was presented in the Organizational Ethics Committee of Mashhad University of Medical Sciences on 12/4/1695 under the title "Study of changes in accompanying psychiatric symptoms in children with ADHD" and No. 951040 with the code IR.MUMS.fm.REC.1395.578 has been approved.

RESULTS

A total of 141 children aged 5 to 7 years with ADHD were studied in this study. The mean age of children was 5.89 ± 0.81 years. The minimum and maximum ages of children included in the study were 5 and 7 years, respectively. The frequency of boys in the studied patients was 68.8% and the frequency of girls was 31.2%. In terms of parents' education level, most fathers with a frequency of 6.2% in the diploma and bachelor's group and most mothers with a frequency of 68.3% in the same group. Also, the mean age variable in the Ritalin treatment group was 5.86 82 0.82 and in the non-Ritalin treatment group was 5.99 ± 0.77 years. There was no significant difference between the mean age in the two general groups of drug treatment (P = 0.41) and all 4 groups of drug treatment (P = 0.67). The frequency of boys in the treatment group with Ritalin and the non-Ritalin treatment group was 67% and 73.7%, respectively, but again there was no significant difference in the distribution of sex in the two groups of drug treatment (P = 0.45). Regarding the variables of father's education level (P = 0.35) and mother's education

(P = 0.13), no significant difference was observed between the two groups of drug treatment (Table 1).

Table 1: Comparison of demographic variables in groups based on the type of drug treatment

Variables		Ritalin treatment (n=103)	Non-Ritalin treatment (n=6)	P value
Gender	Boy	69	28	0.45
	Girl	34	10	
Fathers education	Less than a diploma	42	12	0.35
	Diploma and Bachelor	60	25	
	Master's degree and higher	0	0	
Mothers education	Less than a diploma	35	7	0.13
	Diploma and Bachelor	65	30	
	Master's degree and higher	2	0	

Initially, at the end of the third month and the end of the sixth month of the follow-up of children with ADHD, the ADHD-RS questionnaire score was measured for all patients. This index between the two groups of treatment with Ritalin and non-Ritalin treatment, in none of the stages of the study, there was no significant difference between the mean score of the ADHD-RS questionnaire. However, in the Ritalin and non-Ritalin groups, the mean total ADHD-RS score and both the Attention Deficit and Hyperactivity Disorder subscales had significant changes from the beginning of the study to the end of the sixth month and the trend of these changes was decreasing (Table 2).

Table 2: Examining the trend of changes in the score of the questionnaire ADHD-RS in groups based on drug treatment

Variables	At the beginning of the study	At the end of the third month	At the end of the sixth month	P Value
Overall score ADHD-RS				
Ritalin	92/53±39/62	78/10±34/45	67/75±43/40	0/003*
Non-Ritalin	92/0±51/59	71/74±47/73	55/87±49/62	0/3
Attention Deficit				
Ritalin	31/17±13/09	32/06±12/29	28/47±11/62	0/019*
Non-Ritalin	26/71±14/26	24/58±13/93	20/74±13/91	0/003*
Hyperactivity/Impulsivitiy				
Ritalin	57/93±27/68	46/89±25/74	38/46±35/72	<0.001*
Non-Ritalin	63/57±40/20	49/86±36/79	31/05±32/23	<0.001*

The mean total score of the ADHD-RS questionnaire was significantly different between the beginning of the study and the third month, between the beginning of the study and the sixth month and also between the end of the third month and the end of the sixth month. Only the mean score of the Attention Deficit Scale was significantly different between the third and sixth months. However, no significant difference was observed in this scale between the beginning of the study and the third month, as well as between the third month and the sixth month. As can be seen in the table below, the mean score of the hyperactivity/impulsivity subscale was significantly different between the beginning of the study and the third month, as well as between the beginning of the study and the sixth month. Also, the mean score of the hyperactivity/impulsivity subscale was significantly different between the beginning of the study and the third month, between the beginning of the study and the sixth month, and also between the end of the third month and the end of the sixth month (Table 3).

In evaluating the indicators related to the CBCL questionnaire, in the subgroup of internalization disorders, which includes the subscales of anxiety/depression, deprivation/depression, and somatic complaints, the average score of all these subscales except anxiety/ depression in the non-Ritalin group and the subscales of somatic complaints In the Ritalin group, it decreased significantly from the beginning of the study to the end of the sixth month.

Table 3: Difference in Ritalin treatment group measured each time

Variables	The beginning of the study	The end of the third month	The end of the sixth month
The overall score of ADHD-RS is the Ritalin Group			
The beginning of the study	-	0.02	0.011
The end of the third month	0.02	-	0.11
The end of the sixth month	0.011	0.11	-
The overall score of ADHD-RS is the Non- Ritalin Group			
The beginning of the study	-	0.001	<0.001
The end of the third month	0.001	-	0.017
The end of the sixth month	<0.001	0.017	-
Ritalin Groups Attiude Disorder			
The beginning of the study	-	>0.99	0.256
The end of the third month	>0.99	-	0.012
The end of the sixth month	0.256	0.012	-
Ritalin Groups Attiude Disorder			
The beginning of the study	-	0.558	0.012
The end of the third month	0.558	-	0.059
The end of the sixth month	0.012	0.059	-
Hyperactivity/Impulsivitiy of the Ritalin Group			
The beginning of the study	-	0.001	0.005
The end of the third month	0.001	-	0.177
The end of the sixth month	0.005	0.177	-
Hyperactivity/Impulsivitiy of the Non- Ritalin Group			
The beginning of the study	-	0.002	<0.001
The end of the third month	0.002	-	0.03
The end of the sixth month	<0.001	0.03	-

The mean score of total internalization disorders also decreased significantly in both the Ritalin treatment group and the non-Ritalin treatment group during the study. In the subgroup of extrinsic disorders, which includes subscales of normative and aggressive behavior, the mean score of all these subscales significantly decreased from the beginning of the study to the end of the sixth month in both study groups. The mean score of total extrinsic disorders during the study was significantly reduced in both the Ritalin treatment group and the non-Ritalin treatment group. Among the remaining subscales, the mean score of attention and social disorders in both groups (either Ritalin treatment or non-Ritalin treatment) decreased during the study, which was also statistically significant. But in the subspecies of thinking disorders, only in the non-Ritalin treatment group, the decrease was significant during the study. However, the same decrease in the mean subscale score of thinking disorders in the Ritalin treatment group was not significant. The overall score of the CBCL Behavioral Checklist in the Ritalin and non-Ritalin treatment groups decreased significantly during the study (Table 4).

Based on the mean and standard deviation of sub-indices related to and comparison of subgroups related to CBCL behavioral checklist, most indices had a statistically significant relationship between the time of the beginning of the study, 3 and 6 months after the intervention. There was a statistically significant difference between Ritalin and non-Ritalin groups, so both groups had an improvement over time (Table 5).

DISCUSSIONS

In the present evaluation, it was observed that the results of the evaluation of patients using the ADHD-RS questionnaire and the CBCL behavioral checklist in different treatment groups were not statistically significant, however, these cases need to be compared with studies. Accordingly, this has been evaluated in continuation of other studies conducted in this field. In a study of 154 children with ADHD treated with Ritalin, Wang et al. Observed children for two years and ADHD-RS questionnaire symptoms at the beginning, end of month 6, end of month 12, end 18 months and the last two years have been measured. They observed that the mean age of patients in this study was 6.87. 1.04 years. 72% of the patients in this study were boys and the rest were girls. In this study, for a total of two years, the mean score of the ADHD-RS questionnaire was significantly reduced (P = 0.02). The interesting finding of this study was that the decrease in the mean score of ADHD-RS questionnaire for girls was more than boys and this difference was also significant (P = 0.01). This study ultimately concludes that changes in the signs and symptoms of the disease in children with ADHD and treated with Ritalin can vary over time depending on gender [11]. Accordingly, in this evaluation, as in the present study, it was observed that the difference between the treatment groups was not significant, although these symptoms decreased in all treatment groups during treatment. In another study with a descriptive and longitudinal structure on 79 children with ADHD, Liang et al. Observed that after 12 months of follow-up, the mean score of the ADHD-RS questionnaire and both subscales decreased significantly (P = 0.001). However, the mean score of the CBCL behavioral checklist was not significantly different [12].

Table 4: Checking the process of changes in size scores of questionnaires CBCL

Variables	The beginning of the study	The end of the third month	The end of the sixth month	P Value
Attention disorder				
Ritalin	8/19±4/24	5/90±3/57	3/72±3/40	<0.001*
Non-Ritalin	7/84±4/44	5/52±3/54	3/59±3/30	<0.001*
Social disorders				
Ritalin	6/75±3/78	5/0±3/10	4/0±3/68	<0.001*
Non-Ritalin	6/88±4/46	4/83±3/87	3/78±4/33	<0.001*
Thinking disorder				
Ritalin	5/28±4/13	4/65±3/67	4/45±5/13	0/64
Non-Ritalin	6/92±5/52	5/39±5/09	4/0±4/66	0/001*
Abnormal behavior				
Ritalin	6/09±4/29	4/78±3/40	4/47±5/52	<0.001*
Non-Ritalin	7/20±5/29	5/43±4/33	3/57±4/27	<0.001*
Aggressive behavior				
Ritalin	14/23±7/43	10/41±5/52	7/54±6/89	<0.001*
Non-Ritalin	13/76±8/40	9/39±6/39	6/23±5/61	<0.001*
Extrinsic disorders				
Ritalin	20/43±10/50	15/20±8/38	12/0±11/90	<0.001*
Non-Ritalin	20/96±13/25	14/83±10/42	9/95±9/53	<0.001*
CBCL				
Ritalin	116/88±24/44	106/17±23/43	98/12±31/83	<0.001*
Non-Ritalin	120/75±37/50	110/40±33/78	93/05±31/60	<0.001*
Anxiety/Depression				
Ritalin	7/0±4/51	5/14±3/2	4/98±4/36	0/006*
Non-Ritalin	6/84±4/62	5/70±4/61	4/91±5/44	0/071
Deprivation/Depression				
Ritalin	4/41±3/63	3/08±2/57	2/49±2/79	0/001*
Non-Ritalin	4/68±3/17	3/61±2/89	2/44±2/97	0/001*
Somatic complaints				
Ritalin	67/12±2/84	66/44±2/06	67/41±4/81	0/33
Non-Ritalin	67/96±4/44	67/39±4/92	66/74±4/58	0/007*
Internalization				
Ritalin	78/69±9/22	74/65±6/76	74/88±10/97	0/001*
Non-Ritalin	79/48±10/99	76/70±11/57	74/09±12/18	0/003*

The results of this study are somewhat consistent with the present study. Similar to Liang's study, in our study, both ADHD-RS and Attention Deficit/Hyperactivity Disorder subscale had a significant decrease from the beginning of the study to the end of the sixth month. However, contrary to the results of Mr. Liang's study, in this study, in relation to the CBCL Behavioral Checklist, the mean score of all its subscales, except for somatic complaints and thinking disorders, in patients treated with Ritalin significantly decreased from the beginning of the study to the end of the sixth month.

Table 5: Difference of total CBCL Score and its subgroups in Ritalin and Non-Ritalin group

Variables	The beginning of the study	The end of the third month	The end of the sixth month
Overall rating CBCL Ritalin			
The beginning of the study	-	0.001	0.004
The end of the third month	0.001	-	0.171
The end of the sixth month	0.004	0.171	-
Overall rating CBCL Non-Ritalin			
The beginning of the study	-	0.003	<0.001
The end of the third month	0.003	-	0.032
The end of the sixth month	<0.001	0.032	-
Ritalin internalization			
The beginning of the study	-	<0.001	0.002
The end of the third month	<0.001	-	0.154
The end of the sixth month	0.002	0.154	-
Non-Ritalin internalization			
The beginning of the study	-	0.002	<0.001
The end of the third month	0.002	-	0.003
The end of the sixth month	<0.001	0.003	-
Ritalin exogenous			
The beginning of the study	-	<0.001	<0.001
The end of the third month	<0.001	-	<0.001
The end of the sixth month	<0.001	<0.001	-
Non-Ritalin exogenous			
The beginning of the study	-	<0.001	<0.001
The end of the third month	<0.001	-	<0.001
The end of the sixth month	<0.001	<0.001	-

Bloom et al. also evaluated the effect of Ritalin on behavioral changes and working memory of children with ADHD and observed that after 3 weeks of intervention, children in the drug group treated with Ritalin had an average score of their ADHD-RS questionnaire. It was significantly improved, but there was no significant difference in working memory performance between the two groups [13]. Based on this, it can be said that the results of this study are similar to the present study because during the current study, both in the Ritalin group and in the non-Ritalin group, both subscales of attention deficit and hyperactivity/impulsivity of the ADHD-RS questionnaire significantly decreased from the beginning. The study lasted until the end of the sixth month.

In a systematic review and meta-analysis, Strobo et al. Found that in ADHD patients, the ratio of male to female patients in all studies was 5 to 1, and the mean age of patients in all studies was 9.7 years. The researchers found that treatment with methylphenidate reduced the mean score of the ADHD-RS questionnaire filled out by teachers. The difference between the mean scores of this variable in the Ritalin treatment group and the placebo treatment group was about 9.6 points lower (CI-6.38 to C-11.75). Finally, this systematic review has concluded that treatment with methylphenidate improves ADHD-RS questionnaire scores [14]. Accordingly, these cases differed from the present study. In the present study, in comparison between the two treatment groups, the mean score of the ADHD-RS questionnaire subscales in the non-Ritalin group at the end of the third and sixth months was significantly lower than the same scale in The group has been Ritalin. While the mean subscale of hyperactivity/impulsivity at the beginning, end of the third month and end of the sixth month was not significantly different between the two groups, this difference may be due to differences in the groups evaluated in the two studies. Finally, in another study, Nasiri et al., using the Connors Parent Questionnaire, evaluated children's behavioral records and children's continuous functioning test.

Before and after the implementation of this method, there was a significant difference in 95% confidence level. While in the case of attention deficit, impulsivity and reaction time before and after the implementation of the combined method and perceptual-motor exercises, there was a significant difference in 95% confidence level, and this result means that all methods focus on the components of attention deficit. Impulsivity and reaction time are effective that these results are almost consistent with the findings of our study [15]. Accordingly, in most studies in this field, it has been observed that there is no statistically significant difference between treatment groups, however, more studies are needed in this field to confirm these cases.

CONCLUSIONS

Based on the results observed in this evaluation, the treatment groups did not notice a significant difference in the symptoms of patients with ADHD, although these symptoms improved in both groups, however, more studies are needed in this area.

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ORIGINAL ARTICLE

Evaluation of the effect of drug on physiotherapy methods on cytokines and chemokines of Covid-19 patients

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Abstract: *Previous investigations have demonstrated that interleukin-6 (IL-6), C reactive protein (CRP), tumor necrosis factor-alpha (TNF-alpha), in Coronavirus disease 2019 (COVID-19) patients are considerably increased, and their progressive elevation are clinical threat indicators of disease severity. The purpose of this systematic review is to evaluate the efficacy of physiotherapy, specifically chest physiotherapy, on different cytokines in Covid-19 and non-covid-19 patients. The main complications and symptoms of this virus are as follows; a dry cough, fever, and progressive dyspnea. Quickly, the coronavirus, which is named SARS-CoV-21, has spread worldwide, causing severe lung inflammation, respiratory distress syndrome, cardiac and renal injury, especially in comorbidities patients. Approximately 96% of the cases experience mild respiratory symptoms; some progress to pneumonia, respiratory insufficiency, acute respiratory distress syndrome, and multiorgan failure. The overall mortality rate per number of diagnosed cases is 4.6%; it can range from 0.2% to 15% according to age and health problems. Combination MeSH and text terms were used to perform the search strategy. Interventions in RCTs and clinical trials with or without comparison were assessed. Six studies met the inclusion criteria. Studies demonstrated that physiotherapy could have an effect on TNF-alpha, IL-6, IL-10, IL-1β and CRP.*

Keywords: *COVID-19, MeSH, CRP, TNF-alpha, SARS-CoV-21*

INTRODUCTION

Severe cases of Covid-19 have been shown to cause a mysterious condition that disrupts the immune system; Instead of attacking only infected cells, the immune system attacks healthy cells as well. This condition, known as the cytokine storm, can be fatal, but researchers have been trying to figure out how the storm works and how it can be treated. A cytokine storm is an abnormal immune response (Figure 1). This reaction is a type of systemic inflammatory response syndrome and can be accompanied by symptoms such as fever, fatigue, anorexia, joint pain, nausea, vomiting, diarrhea, skin complications, rapid breathing, palpitations, hypotension, seizures, headache, hallucinations and tremors. Researchers have now discovered in a new study to look at the progression of coronavirus infection in mice that the cytokine storm is a vicious cycle caused by the overproduction of two signaling proteins called "tumor necrosis factor alpha" (TNF) - "interferon gamma". (INF) is caused by th e body's immune system.

Together, these two proteins lead to serious conditions that can eventually lead to inflammation, organ damage, and even death. But this study in mice showed that using pre-existing antibodies to fight these proteins is an effective way to prevent death in cases of severe Covid-19 infection. The researchers noted that knowing these pathways and the mechanisms that stimulate this inflammation are very important for devising effective treatment strategies. The researchers also identified specific cytokines that activate

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inflammatory cell death pathways and have significant potential for treating Covid-19 and other deadly diseases, including sepsis. Sepsis is a dangerous disease that is caused by an overreaction of the immune system to infection and gradually spreads throughout the body. Cytokines are small signaling proteins that enter the body by immune system cells to tell other cells what to do. These proteins are vital for the immune response because they can limit the spread of the virus, but some of them also stimulate inflammation. Inflammation is a natural part of the immune response in which white blood cells rush to the site of infection to fight infection.

Figure 1: Hypoxia, HIF-1α, and COVID-19: from pathogenic factors to potential therapeutic targets

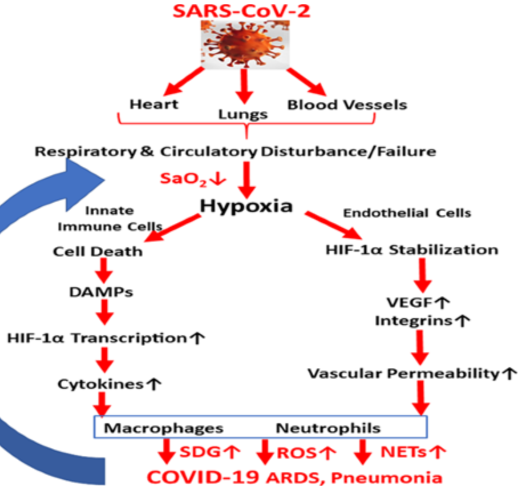
SARS-CoV-2
Heart
Lungs
Blood Vessels
Respiratory & Circulatory Disturbance/Failure
SaO₂↓
Hypoxia
Innate Immune Cells
Endothelial Cells
Cell Death
HIF-1α Stabilization
DAMPs
VEGF↑
Integrins↑
HIF-1α Transcription↑
Cytokines↑
Vascular Permeability↑
Macrophages
Neutrophils
SDG↑
ROS↑
NETs↑
COVID-19 ARDS, Pneumonia

But inflammation can also cause some autoimmune disorders, such as osteoarthritis, in which the body despite the absence of the cause of inflammation causes it. The findings of this study link the inflammatory cell death induced by the "tumor necrosis factor-alpha" (TNF)-"interferon-gamma" (INF) proteins to Covid-19. The results of this study also show that treatment Targets of this cytokine compound could be candidates for rapid clinical trials to treat Covid-19 and other often fatal disorders associated with cytokine storms. [1,2]. The coronavirus family is single-stranded RNA viruses with an approximate diameter of 80 ~120 nm. It includes 4 types: α-CoV, β-CoV, δ-CoV, and γ-CoV. Bats are the primary critical natural hosts. About 35% of their carried viruses are coronaviruses [3]. The COVID-19 is a novel coronavirus with a circular shape and approximately 60 to 140 nm [3].

In most of cases, Coronaviruses (CoVs) targeting the human respiratory system, which are the leading cause of Middle East respiratory syndrome, severe acute respiratory syndrome (SARS), and lastly, coronavirus disease 2019 (COVID-19) [4-6]. Complementary treatments, including; chest physiotherapy and therapeutic massage, are safe and noninvasive strategies that decrease the adverse side effects of disease [8]. Physiotherapy and Massage are restorative therapy involving the systematic application of pressure with the aim of improving blood and lymph circulation [7-9]. Until now, there is some lack of medical evidence for the treatment of Covid-19 in patients, and it is critical to find a low-risk and effective way to manage the Covid-19. In this investigation, we aimed to assess the effect of chest physiotherapy on the inflammatory cytokines of Covid-19 patients (Figure 2).

Figure 2: The signal pathways and treatment of cytokine storm in COVID-19

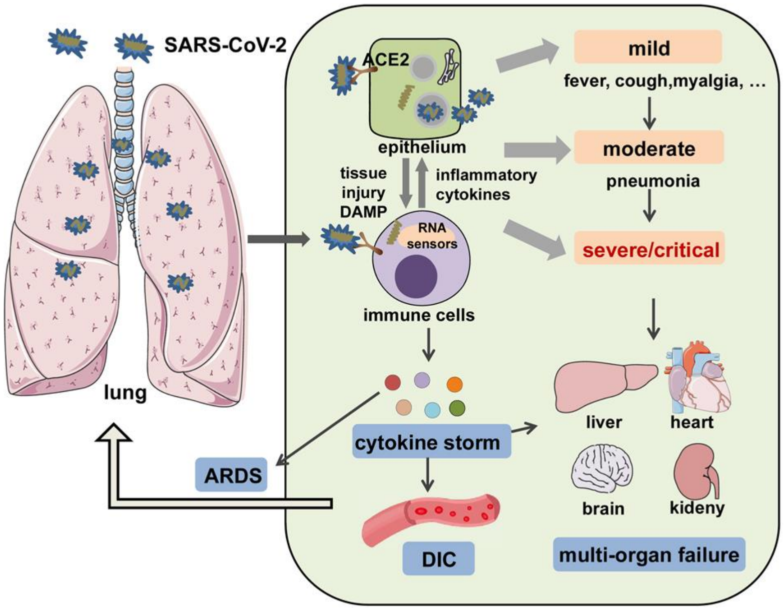
SARS-CoV-2
ACE2
epithelium
tissue injury DAMP
inflammatory cytokines
RNA sensors
immune cells
mild
fever, cough,myalgia, ...
moderate
pneumonia
severe/critical
lung
ARDS
cytokine storm
DIC
liver
heart
brain
kideny
multi-organ failure

METHODS

Inclusion criteria for study selection

Type of study

We will include investigations related to chest physiotherapy in patients with COVID-19. we will search for investigations in English; all articles included are clinical trial and randomized controlled trial (RCT) type articles.

Type of participant

All patients with COVID-19 are included regardless of sex, age, economic status, race, and education. Children, Pregnant women, postoperative infections, and patients with severe cardiovascular and other complications are not included in this systematic review.

Type of outcome measure

The primary outcome was the influence of chest physiotherapy on the cytokines of patients.

Data collection and analysis

Selection of studies

Our investigation is in line with the Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) guidelines flow chart to demonstrate the process of selecting literature for the entire study. Before searching the articles, all reviewers debated and determined the screening criteria. After that, the two reviewers separately reviewed and screened the articles. They screened the titles and abstracts of the articles to get related articles and then removed some duplications and articles with incomplete information. We also tried to obtain the full text, and the articles were managed using EndNote software. By the end, Discrepancies were resolved by consensus. The whole process is shown in Figure 3.

RESULTS

Six investigations which included in our study, two of them assessed the effect of Tai chi in the level of insulin, insulin-like growth factor (IGF)-1, insulin growth factor-like binding protein (IGFBP)-1, IGFBP-3, and cytokines interleukin (IL)-6, IL-2, and interferon (IFN)-γ and other investigation evaluate the effect of physical therapy modalities on inflammatory cytokines and chemokines. These studies demonstrated that physical therapy modalities could significantly diminish the monocyte production of proinflammatory cytokines and proinflammatory gene expression. However, physical therapy modalities like tai chi may increase the production of anti-inflammatory cytokines.

Macrophage activation syndrome is characterized by a marked increase in proinflammatory cytokines called storm cytokines. This severe inflammation, without any therapeutic intervention, leads to severe tissue damage and eventually death of the patient. Research has shown that specific cytokines, especially TNF-α, IL-6, and IL-1β, are involved in this phenomenon. Macrophages produce large amounts of these proinflammatory cytokines when stimulated by macrophage activation syndrome. Inflammation upsets the exact balance between coagulation and fibrinolysis.

Certain inflammatory cytokines, such as TNF-α and IL-1, initiate the production of tissue factors from monocytes and macrophages, which leads to the activation of coagulation. IL-1 and IL-6, on the other hand, increase the production of plasminogen activator inhibitors. Therefore, the overproduction of inflammatory cytokines causes intravascular coagulation. Cytokine storm is a condition of the immune system in which various immune cells are highly activated and produce large amounts of cytokines; This in turn leads to excessive inflammation, which is often associated with multiple organ failure and high mortality. Inflammatory cytokines or various chemokines such as tumor necrosis factor (TNF-α), type I and II interferons, interleukins (IL-1, IL-6, CCL2 or monocyte-chemotactic protein (MCP-1), as well as cytokines Immunosuppressants such as IL-10 or β-TGF- have been implicated.

Cytokine storm has been observed and discussed in various clinical conditions such as rheumatic or blood disorders. In addition, it sometimes occurs in infectious diseases and makes the patient resistant to treatment. This is associated with the development of acute respiratory distress syndrome (ARDS), which is one of the most severe pathogenic disorders of the respiratory system and causes pulmonary edema, decreased gas exchange and a lack of lethal oxygen. It has recently been suggested that cytokine storms, particularly acute respiratory distress syndrome, are involved in and exacerbate pneumonia associated with the Covid-9 virus. Although the body of most patients with Covid-19 does not show any symptoms of pulmonary disorders, even mild ones, about 20% of patients develop severe pulmonary dysfunction. It is not clear why only a fraction of patients with Covid-19 develop severe inflammation, but the Covid-19 virus appears to infect certain types of cells, such as endothelial vessels in the lungs or cells in the alveolar wall or macrophages.

Infection of cell types may induce immune responses that trigger a cytokine storm, including macrophage activation syndrome. Severe inflammation associated with Covid-19 disease, which can be detected by coronary artery bypass grafting in Ahvaz, is clinically similar to macrophage activation syndrome. There have been reports of severe increases in IL-1β, IL-2, IL-6, IL-7, IL-17 and TNF-α in patients with severe pneumonia caused by Covid-19. It is noteworthy that the levels of IL-2, IL-7, TNF-α, G-CSF, CXCL10, CCL3 and MCP1 were higher in the plasma of patients treated in the ICU than in patients who were not admitted to the ICU.

In addition to elevated cytokines, elevated serum levels of ferritin, CRP, and D-dimer also indicate severe inflammation similar to macrophage activation syndrome and fibrinolysis in Covid-19 patients. Immunological and pathological manifestations of this disease are mainly observed in the lung, which is associated with acute respiratory distress syndrome (ARDS). Clinically, acute respiratory distress syndrome is associated with hypoxia, extensive pulmonary infiltration, pulmonary edema, and decreased lung adaptation. Which in general leads to respiratory failure. Plasma levels of TNF-α, IL-1β, IL-6 and IL-8 are elevated, indicating severe acute respiratory distress syndrome in the deceased compared to survivors. It is reported that about 20% of patients develop acute respiratory distress syndrome during Covid-19 disease, and some patients deteriorate rapidly and die.

Recent genomic studies have shown that the enzyme converter angiotensin-2 (ACE2) and serine membrane protease (TMPRSS2) are expressed at the level of type II pneumocytes and cause SARS-CoV-2 virus to enter target cells. The onset of local inflammation due to SARS-CoV-2 infection activates macrophages at that site and spreads rapidly throughout the lung. This is likely due to the abundant expression of virus receptors, angiotensin-converting enzyme 2 (ACE2) and serine membrane protease (TMPRSS2). The accumulation of immune cells accelerates the rate of pneumonia to acute respiratory distress syndrome (ARDS). In severe cases, local inflammation inside the lungs cannot be relieved, resulting in multiple organ failure and death. The result is shown in Table1

Table 1: The results of physical therapy modalities

First author	Published	Population	Intervention	Cytokines	
Janelsins et al. [10]	2011	Breast cancer	Tai chi	IL-6 , IL-2	The effect of Tai chi in the level of insulin, insulin-like growth factor (IGF)-1, insulin growth factor-like binding protein (IGFBP)-1, IGFBP-3, and cytokines interleukin (IL)-6, IL-2, and interferon (IFN)-γ
Irwin et al. [11]	2012	Adult	Tai chi	IL-6	significantly diminished the monocyte production of proinflammatory cytokines and proinflammatory gene expression
Cetisli-Korkmaz et al. [12]	2021	ICU patients	Whole body vibration (WBV) exercise	IL-10	Whole body vibration (WBV) exercise increases the production of anti-inflammatory cytokines
Parry et al. [13]	2012	Individuals with sepsis and covid-19	functional electrical muscle stimulation-assisted supine cycling	TNF-α and IL-1β	may drive mitochondrial oxidative stress and increase intracellular calcium
Adly et al. [14]	2020	Patients with stage one pneumonia	thoracic lymphatic pump techniques and thoracic inlet	Cytokine storm	the thoracic lymphatic pump techniques and thoracic inlet release have been shown to increase chemokines and cytokines in the thoracic
Lavretsky et al. [15]	2012	Depressed patients	Tai chi	The decline in the inflammatory marker, C-reactive protein	This study reported the tai chi effect on the level of CRP and inflammatory markers

DISCUSSION

With the quick enhancement in the number of COVID-19 patients, the number of cured COVID-19 patients is increasing, and the number of patients recovering from COVID-19 is also rising. Patients with COVID-19 often experience the series of respiratory symptoms and complications. There are a few evidence that the physical therapy modalities can have an effect on the level of cytokines and chemokines through the body. All in all, chest physiotherapy or other physical modalities are noninvasive methods, which can affect Covid-19 management, especially patients in the recovery period. However, a few numbers of studies worked on the effect of chest physiotherapy on cytokine and chemokines in Covid-19 patients. Moreover, further investigations are required to evaluate the exact effect and mechanism of these therapies on Covid-19 patients to better manage this outbreak.

The researchers found that the coronavirus behaves differently, turning off genes that prevent the virus from replicating, but does not affect immune cell-calling genes, and that these genes can function normally.

This causes the virus to multiply and overproduce immune cells that invade the lungs and other organs, resulting in excessive inflammation. The team at Icon Medical School in New York says early treatment of patients should focus more on repairing the pathway blocked by the coronavirus than on inflammation. "One thing is to tell all the cells around to be amplified, and the second thing is to send the cell," Dr. Benjamin Tanveer, a virologist, and professor of microbiology at Aikan Medical School, told DailyMail.com "More professional immunity to the site of infection." Cells in our bodies usually have two groups of antiviral genes: interferons and chemokines. Interferons are messaging proteins that are released by infected cells and are named because of their ability to prevent the virus from multiplying. Chemokines are small molecules that attach immune cells to the site of infection so that they can target and kill the virus. According to Tanveer, the first set of genes control the virus for about 7 to 10 days, so the set second has enough time to prepare the immune cells for the attack. "Interferons are called 'defense's' genes, and chemokines are called 'support's' genes,".

They are neutralized and destroyed." "Even the first line of defense, the 'defense call', is often enough to completely stop the proliferation and neutralize the infection even without a second response." However, unlike the flu or severe acute respiratory syndrome (SARS), the coronavirus shuts down a number of genes and activates other genes. An animal model of a date rat was examined. In the case of coronavirus, there was a very mild response to the "defense call" genes - which prevent the virus from replicating - and a significant response from the "support call" genes.

Figure 3: Flow Chart of this study

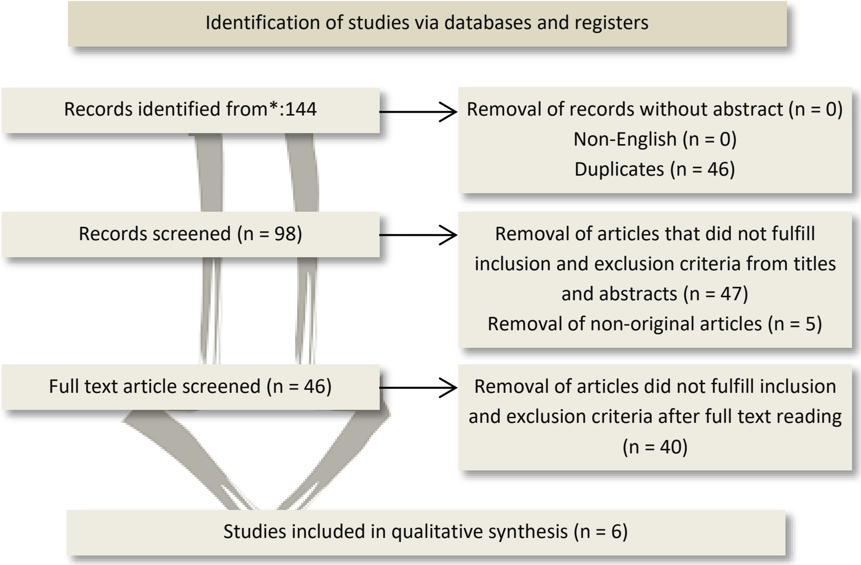
Identification of studies via databases and registers
Records identified from*:144
Removal of records without abstract (n = 0)
Non-English (n = 0)
Duplicates (n = 46)
Records screened (n = 98)
Removal of articles that did not fulfill inclusion and exclusion criteria from titles and abstracts (n = 47)
Removal of non-original articles (n = 5)
Full text article screened (n = 46)
Removal of articles did not fulfill inclusion and exclusion criteria after full text reading (n = 40)
Studies included in qualitative synthesis (n = 6)

"The combination of the two is a bad combination," Tanvir said. When they examined the lung cells of two patients with Covid 19 who had lost their lives, they found that they were giving exactly the same answer. "It begins to multiply, and the infected cells at the site of proliferation do not report the infection well, and as a result, the infection spreads to the lungs." This means that the virus is still multiplying because there are no interferons, but those cells still need to call for support. So different types of immune system cells - neutrophils, macrophages and lymphocytes - come into play, but when they arrive, there is nothing to control the viruses. "And more has reached the lungs, but the cells are calling for support."

Now, the lungs have immune cells such as macrophages and neutrophils that lead to more inflammation. Basically, the body's immune system revolves around itself. This is likely to cause cytokine storms, which occur when the body fights its own cells and tissues instead of fighting the virus. "The behavior of this virus is such that I have not seen in 20 years of studying how cells respond to viral infections.

There are two ways to treat patients. The first group consists of people who have just shown symptoms," says Tanveer.

And their test results are positive. "They can take drugs that inhibit the defense genes so that the virus can behave more like the flu,".

For those hospitalized and intubated, it is too late to use this treatment, so they can benefit from a group of drugs called interleukin-6 and interleukin-1 inhibitors. Help reduce cytokine storms and inflammation throughout the body.

Modulation of biological reactions plays an important role in improving body function following immunological challenges.

Echinacea is one of the best and most effective plants for modulating the activity of the immune system, and the results of laboratory studies and clinical experiments have confirmed this claim.

The effects of this plant on stimulating the immune system and promoting the body's defense are manifested by increasing the activation of phagocytosis, stimulating fibroblasts, increasing the activity of leukocytes and increasing the production of cytokines.

The anti-inflammatory effects of the plant are also effective in regulating the immune response in suppressing infection and preventing cytokine storms.

CONCLUSIONS

Physical therapy modalities like chest physiotherapy or tai chi can be beneficial in managing covid-19 because of their effects on cytokines and chemokines through the body. Nonetheless, further evidence is needed to evaluate the exact effect of these interventions. What is certain is that the SARS-CoV-2 virus threatens the lives of millions of people around the world. By better understanding the nature of the virus, experts have been able to prevent its widespread spread in certain parts of the world. However, no effective drug or vaccine has yet been developed. Severe cases of Covid-19 disease are often seen with acute respiratory distress syndrome, with clinical and laboratory features similar to macrophage activation syndrome. Previous studies have shown that the symptoms of macrophage activation syndrome improve with anti-cytokine therapy. So far, anti-IL-6, IL-1 and TNF therapy have shown promising results in the treatment of macrophage activation syndrome. Based on these findings, treatment with anti-IL-6 or IL-1 drugs has been performed in Covid-19 disease and has caused significant improvement in patients so far! There are currently more than 1,900 clinical trials worldwide, and it looks like these studies will soon provide a new and effective treatment for Covid-19.

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ORIGINAL ARTICLE

Evaluation of the effects of dexamethasone in modulating breathing pattern decomplexification in rats with 2-chloroethyl ethyl sulfide induced lung injury

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Abstract: *Background and Objectives: Sulfur mustard is a functional alkylating chemical warfare agent that gives rise to appalling lung injury. In people with pulmonary diseases, including asthma, the complication of respirational dynamics is reduced. However, the complexity of breathing patterns in lung injury caused by chemical agents is not clear. In the current study, the outcome of 2-chloroethyl ethyl sulfide (CEES), and mustard analogue, upon breathing pattern of rats without or with treatment were reviewed.*

Methods: The interbeat interval (IBI) and respiratory volume (RV) data have been acquired from spontaneous respiration rats with lung injury by CEES using a whole-body plethysmograph. We calculated mean and coefficient of variation, alpha exponent derived from detrended fluctuation analysis (DFA), and sample entropy of IBI and RV.

Finding: Entropy examination of respiratory variation displayed reduced inconsistency (less complication) in the breathing pattern of this rat model of lung injury. The mustard analogue also led to increased lung inflammation in damaged rats. However, treatment by NAC and dexamethasone had a compelling impact on the complication of the breathing rhythm and lung inflammation of rats with lung injury.

Conclusion: Our findings show that inflammation could be the possible origin of respiratory dynamics shifting apart from the normal variation in CEES-induced lung injury.

Keywords: *2-chloroethyl ethyl sulfide, breathing pattern, dexamethasone, Inflammation, modulates, rat model*

INTRODUCTION

Mustard agents have been used in various battles since the First World War and are responsible for more than 80% of all chemical war gas victims [1]. Severe lung damage is recognized to happen in different conditions, encompassing exposure to mustard gas and its subordinates, such as 2-chloroethyl ethyl sulfide (CEES) [2]. Acute lung injury is defined as necrosis in the pharynx and epithelial detachment, bronchioles and, trachea, while long-term effects comprise of fibrosis and, in some cases, cancer [3]. Also, both animal models and human exposure data indicate the inclusion of mediators and inflammatory cells in SM-generated lung injury. After exposure of rodents to SM and CEES by intratracheal instillation or inhalation, inflammatory cells have been spotted in the higher, and lower inhaling tract [4]. Mustard sulfur causes changes in respiratory parameters and inspiratory airflow limitation [5]. However, the mechanisms involved in changing respiratory parameters are not clear. Previous studies have shown therapeutic effects of anti-inflammatory agents, including corticoste roids, in SM-induced lung injury. Wigenstam and colleagues reported that anti-inflammatory steroids, such as dexamethasone reduce the inflammation of passageway for air, lymphocyte activity, and collagen deposition within an hour following the exposure by nitrogen mustard [6]. Furthermore, short-term studies using intravenous and oral N-acetylcysteine (NAC) in patients with chemical lung injury have revealed the potency of this compound to restore pulmonary function tests with a low incidence of adverse

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effects [7]. Normal respiration demonstrates fluctuating dynamics in respiratory volume and rate, arising from the complex relationship between various internal and external climate factors. This system continuously fluctuates under fragile stability to maintain adjustability to stimuli [8]. Pulmonary diseases have a variable medical disorder and show complex behavior, which might be related to a change in the dynamics of the respiratory system toward either too irregular or too regular [9]. Investigating breathing pattern complexity is beneficial to the determination of disease control, severity, blockage, and response to treatment in pulmonary patients. Therefore, understanding the changes in breathing pattern can be contributive to our understanding of the pathophysiology of lung injury and might provide new perception to establish new interventions and curing strategies [10]. In this study, we hypothesized that the complexity of the respiratory pattern is decreased in rats with CEES-Induced lung injury, and since these animals had inflammation, the respiratory pattern could be brought close to natural conditions using an anti-inflammatory agent. To assess this, we compared the complexity of the respiratory dynamics among control and CEES-induced lung injury rats and also reviewed the outcome of N-acetylcysteine and dexamethasone on treating rat models of pulmonary injury.

METHODS

Experimental groups

The Ethical Committee of Baqiyatallah University approved all the protocols.

Specific pathogen-free male Wistar rats, weighing 250 ± 20 g, were randomly splitted into five groups (n = 6 for each group): (i) Vehicle group, which received a single intratracheal dose of vehicle (60 µL of ethanol + 340 µL phosphate-buffered saline (PBS)); (ii) CEES group, a single dose of CEES was intratracheally instilled to the second group (Sigma Chemicals, MO) (2 µL/rat or 6 mg/kg in 58 µL of ethanol + 340 µL PBS) [11] (iii) CEES+PBS group, during intraperitoneally handeling of PBS was (i.p.) one hour after instillation of CEES; (iv) CEES+NAC group, to which NAC was handeled intraperitoneally (i.p.) 1 hour after instillation of CEES (20 mg/kg body weight of NAC in in 400 µl PBS) [12] and (v) CEES+Dex group, in which Dexamethasone was administered intraperitoneally (i.p.) 1 hour after instillation of CEES (10 mg/kg body weight of Dexamethasone in 400 µl PBS [6] Intraperitoneal ketamine (100 mg/kg body weight) was used for intraperitoneal xylazine and anesthesia (13 mg/kg body weight) was used for sedation when required [12].

Recording of respiration

In all groups, respiration was recorded a day before administrating the drugs and 24 h after the challenge. Conscious rats respiration was recorded by a purpose-built whole-body plethysmograph [13]. In short, this comprises of an animal chamber made of a clear Plexiglas cylinder (wall thickness 5 mm, volume 1.32 L, length 300 mm, i.d. 75 mm) with inversely straighten outlet and inlet ports in each wall. To avoid any increase of CO_2 in the plethysmograph, the air in the room was repeatedly pumped into the chamber at a controlled flow rate (4 L/min). A plastic Yconnector was placed at a distance of 10 cm from the outlet of the chamber, and one of the exit ports was linked to one input of a differential pressure probe (MPXV7002DP, Freescale Semiconductor Inc.), while the other input exposed to room air. The plethysmograph chamber was also connected to one input of a CO_2 sensor (MG811, Sandbox Electronics Inc.), through polyethylene tubing connected to a second exit port of the Y-connector, and another input of the CO_2 sensor was exposed to room air. Each test incorporates approximately 15 min early recording period (as the acclimatization time) succeeding by a 60-min main recording period.

Breathing Rythm examination

By using a PowerLab A/D converter (ADInstruments, Sydney, Australia), the breathing signals were digitized at a 10 KHz sampling rate and exhibited on ChartPro 7.0 software. For breathing pattern analysis, a minimum artifact with 20 minutes of data was selected. For calculation of the time-series of respiratory volume (RV) and inner breath interval (IBI), a custom program written in MATLAB software was adapted. Identification and verification of the peaks for each respiratory signal were carried out. Subsequently, the amplitude of peaks and the peak-to-peak time intervals were considered as the RV and IBI series, respectively. Irregularity of breathing pattern was quantified by calculating the sample entropy (SampEn) as an index displaying the degree of complexity. The negative natural logarithm of the conditional eventuality is SampEn that periods within a physiological-series and match within tolerance r for m points will also match for m+1 points [14]. Our calculation showed that SampEn of RV and IBI series are equivalent to 2 for m and 0.2 for r, using MATLAB code available from the physionet (http://www.physionet.org). DFA method can be used for evaluating fractal-like temporal structure of a time series. In this technique, the root mean square of the variation of the combined and detrended data is evaluated within time windows of different sizes and then plotted against window size on a log±log scale. A lineal relationship among log (window size) and log (oscillation) show the attendance of self-similar time-series [15]. The alpha (α) scaling exponent is the slope of this line. This parameter demonstrates the relationship within the time-series inconsiderate of the scale.

Bronchoalveolar lavage cells and Histological assessment

At 2 h after respiration recording, rats were anesthetized and 3 ml of sterile physiological saline was slowly instilled and considerately pulled out into the syringe (3 times) through the endotracheal. Samples were centrifuged, and total cell count was applied on cytocentrifugation prepared stained with Giemsa stain and, BAL cytospin samples (cytospin 3, Shandon Instruments, Pittsburgh, PA) [13].

After BALF collection, animal lungs, which had been removed earlier, were settled in formalin solution, embedded in paraffin, and slashed into 5-lm-thick slices. For identification of airway infiltration of inflammatory cells, the animal lung slices were stained with haematoxylin and eosin (H&E).

For lung inflammation measurement, the percentage of inflamed area to total lung area was considered in H&E stained lung sections [16].

Statistical analysis

For statistical analysis of data, GraphPad Prism V6.0 (GraphPad Software, San Diego, CA) was used. The significance of changes in BALF cells, the breathing pattern, and histological among groups were compared with a one-way ANOVA with a Bonferroni post-test. Results have been introduced as mean ± standard error of the mean (SEM). P-values less than 0.05 were contemplated statistically important.

RESULTS

Lung inflammation analysis

Rats with lung injury caused by CEES (CEES group) had significantly higher levels of total BAL inflammatory cells and airway inflammation ($p < 0.001$) (Figure 1) compared to control group. However, airway inflammatory cells and total inflammation were significantly decreased in CEES group compared to CEES+NAC group ($p<0.01$ and $p<0.001$, respectively) (Figure 1). These parameters also displayed significantly lower measurements in rats of CEES+Dex group compared to CEES group ($p<0.001$) (Figure 1).

Figure 1: Breathing pattern in a representative rat. (a), Representative pieces of the breathing signal exhibit the inter-breath interval (IBI) and respiratory volume (RV), as well as deep inspiration. Original ("raw") IBI (b) and RV (c) time series during 20 min of respiration in a representative rat.

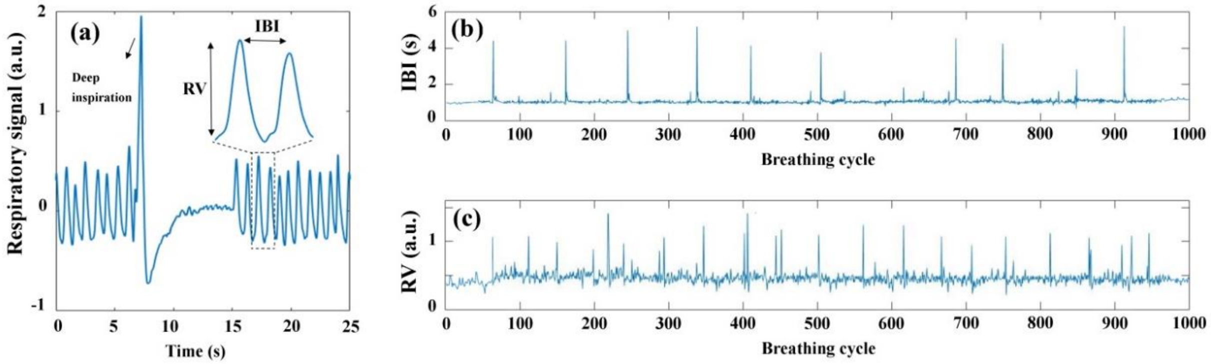

Breathing pattern analysis

The mean respiratory rate (RR) of CEES group was considerably higher compared to the vehicle group ($p < 0.001$). However, there was no significant difference in the coefficient of variation (CV) of the RR (CVRR) between the groups ($p > 0.05$). The mean of RR in CEES+NAC and CEES+Dex groups had significantly decreased compared to CEES group ($p < 0.001$) (Figures 2a and 2b). The mean of respiratory volume (RV) and coefficient of variation of the RV (CVRV) significantly decreased and increased in rats with lung injury by CEES as compared with the vehicle group, respectively ($p < 0.001$ and $p < 0.05$). However, RV and CVRV partially decreased in rats treated with NAC and dexamethasone compared to CEES rats ($p < 0.05$) (Figures 2e and 2f). The index of the irregularity for the Sample Entropy of IBI (SampEnIBI) indicated a significant decrease in CEES group compared with the vehicle group ($p < 0.001$). Similarly, this index has decreased in treatment groups ($p<0.05$). The SampEnIBI of CEES+Dex group significantly increased compared to other groups that received CEES ($p < 0.05$) (Figure 2c). Nevertheless, no notable discrepancies were discovered in SampEnRV among the experimental groups ($p > 0.05$) (Figure 2g). In the CEES group, an index of self-similarity for the slope of detrended fluctuation analysis of RV (DFARV), was greater than the vehicle group ($P < 0.01$).

This index of self-similarity had significantly decreased in animals of CEES+NAC and CEES+Dex groups as compared with CEES rats ($p < 0.05$) (Figure 2h).

Nonetheless, among the experimental groups ($p > 0.05$) (Figure 2d), DFAIBI time-series disclose no significant difference.

Figure 2: Effect of administration of NAC and dexamethasone on lung inflammation and bronchoalveolar lavage (BAL) inflammatory cells in CEES-induced lung injury Hematoxylin and eosin (H & E) staining to identify the lung inflammation (Scale bar 50 µm). Data were analyzed by a one-way ANOVA, with the Bonferroni post-test. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ compared to vehicle, \$\$ $p<0.01$, and \$\$\$ $p<0.001$ compared to CEES, # $p<0.05$, and ### $p<0.001$ compared to Dex. (a) vehicle group, (b) CEES group, (c) CEES+PBS group, (d) CEES+Dex group, (e) CEES+NAC group, (f) graph of lung inflammation, (g) total inflammatory cell.

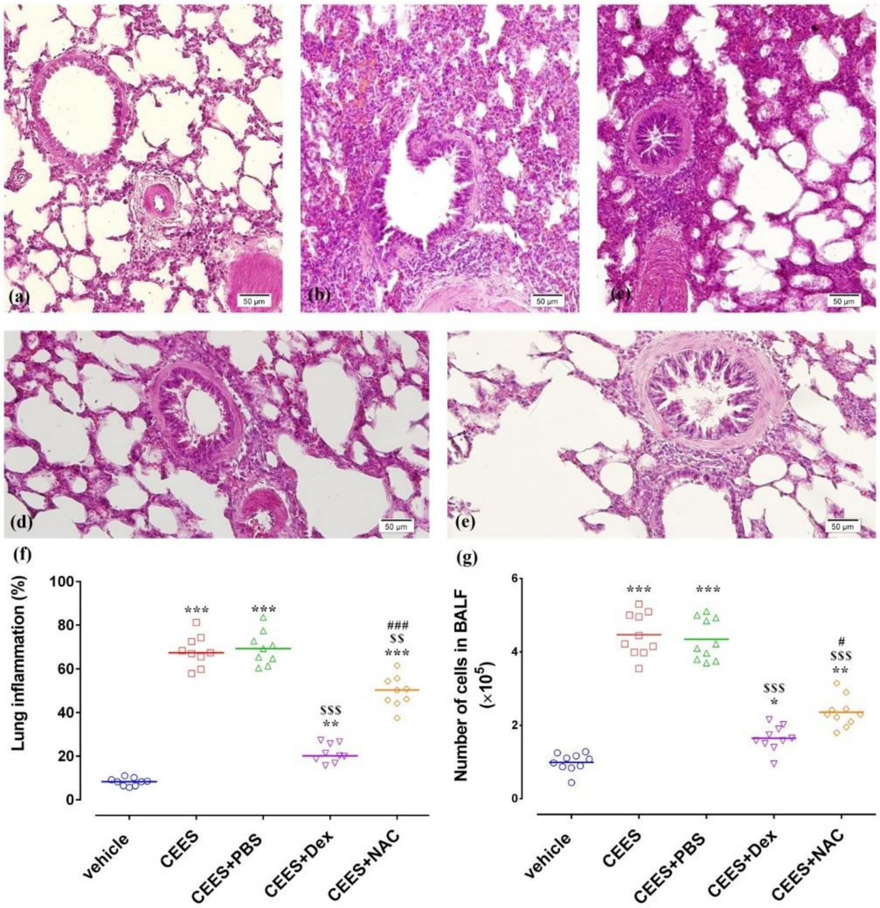

DISCUSSION

Biological functions are intricate and based on a complex feedbacks and network of nonlinear dynamics [9,16]. Physiological variables fluctuate consistently under imbalanced steady-state conditions to retain compatibility to internal or external stimuli [10]. Similar to other physiological systems, the respiratory system is stable and fluctuates under normal conditions. A change in dynamics of a system towards either too irregular or regular may be associated with diseases such as asthma and COPD [10]. Analysis of breathing fluctuations complexity is non-invasive technique and permit us to scrutinize the integrity and adaptability of the respiratory control system. Moreover, this method of analysis has the potential to predict prognosis, severity, and controllability of lung diseases [17].

Evaluation of respiratory dynamics in an animal model of CEES-induced lung injury by complexity analysis of both volume and respiratory rate was carried out. In this animal model, respiratory rate and respiratory volume increased and decreased, respectively. These results are consistent with the previous study where chemical war victims experience cough, dyspnea, tachypnea, and chest pressure after several hours of SM exposure [18]. Some researcher demonstrated that minute volume decreased following sulfur mustard inhalation in guinea pigs [4]. Reduced SampEn values in the IBI time series of rats with lung injury shows higher regularity or low complexity in respiratory dynamics. These findings are consistent with the improved regularity of respiratory pattern observed in other pulmonary diseases such as asthma [19,20]. Previous studies have reported increased variability and α exponent of peak expiratory flow affiliated with the inadequacy of treatment in asthmatic and COPD patients [21,22]. These findings raise the possibility that higher α exponent delineates a firm and deterministic use in lung function [10]. Higher respiratory volume variability (CVRV) corroborates with the reports of respiratory volume variability in other pulmonary diseases such as asthma [16]. Nevertheless the specific pathophysiological mechanisms underlying the reduced complexity of respiratory dynamics in CEES-induced lung injury is not still thoroughly unveiled. Understanding the basis of such variable manner may lead to developing treatment strategies and better-targeted intervention in lung injury by CEES and Sulfur Mustard.

Sulfur mustard (SM) is highly poisonous to the pulmonary system, causing both chronic and acute effects encompassing airway inflammation, obstructive disease, bronchitis, and acute respiratory distress syndrome. Therapeutic Strategies for mitigating SM-induced lung Injury include antioxidants, anti-inflammatory agents, surfactants, and protease inhibitors [24]. In the present study, we reduced lung inflammation and the fluctuation of the breathing pattern by applying dexamethasone and n-acetylcysteine, where glucocorticoid proved more effective than NAC. In other words, inhibiting the inflammation process brings the complexity of respiration closer to normal conditions. It has been assumed that sulphur mustard toxicity is related to lung inflammation, and few of their symptoms may be owing to the permeation of inflammation on the nervous system. In addition, in inflammatory pulmonary diseases such as asthma, it has been shown that influenced inflammation in the nervous system [25,26]. There is some evidence to suggest that peripheral inflammation related to airway allergy may vary the immunologic state of the central nervous system (CNS), such as increased intracerebral levels of IgE and IgG. Furthermore, there is experimental evidence of neuroplasticity and brain inflammation in the nucleus tractus solitaries in animal models [25]. Therefore, regarding these changes in inflammation, the inherent properties of the central nervous system could change and causes the alteration of the respiratory system towards reduced irregularity [16]. However, we did not examine peripheral or central neural activity in the present study, and these subjects can be regarded in next studies.

CONCLUSIONS

The cycle-by-cycle changing of respiratory pattern and volume in rats were examined, and reduced complexity of breathing rhythm in a rat model of CEES-induced lung injury was revealed. According to the results of the respiratory complexity analysis, among the different treatments, dexamethasone had a greater therapeutic effect on breathing pattern decomplexification than NAC. It appears that inflammation processes may be the cause of shifting the respiratory dynamics in this model of lung injury. Future studies are needed to characterize the role of inflammation in decomplexification of breathing pattern and determine the central or environmental factors involved in inflammation in chemical-induced lung injury.

Acknowledgments

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Conflict of interest

The authors report no conflicts of interest.

Funding Statement

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Ethical Statement

The study protocol was reviewed and approved by the Ethics Committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1396.330).

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ORIGINAL ARTICLES

Evaluation the impact of occupational anchoring and organizational commitment on improving efficiency

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Abstract: *Background and Aim: One of the most effective ways to improve efficiency in organizations is human resources management. Job Anchors and Organizational Commitment are constructs having important implications for human resources management practices, especially in efficiency. The purpose of this perusal was to study a structural equation model (SEM) of the impact of job anchors and organizational commitment on efficiency in a military hospital.*

Methods: The statistical population of this study was the personnel of a military hospital, which gathered accomplishing simple random sampling method. As a result, the research used two hundred and thirty questionnaires so to analyse, accomplishing as survey was developmental-applied, that was, requirement data with questionnaires. To measure efficiency, organizational commitment and job anchors variables respectively the Persian version of Hersey & Goldsmith, Meyer & Allen, and Schein questionnaires standard were used. In order to test the research conceptual model, structural equations modelling based on partial least squares method was used, and the model was analysed using AMOS statistical software.

Results: The results of data analysis demonstrated that hidden variables such as organizational commitment, job anchors, and efficiency had acceptable convergent validity, and the fitness of the conceptual model was confirmed. Based on the research model analysis, organizational commitment did not have impact on efficiency, and job anchors had a positive and significant impact on efficiency. Eventually, the modified model showed that organizational commitment variable moderates the connection between job anchors and efficiency.

Conclusion: In light of the findings of this research, managers of military organizations should develop programs to create and improve organizational commitment in personnel. Furthermore, they should pay attention to employees' characteristics and the proportion of their occupation with job anchors in cases of changing and promoting employees. Implementing aforementioned items will have a positive impact on improving the efficiency of military organizations.

Keywords: *job anchors, organizational commitment, efficiency, SEM, military hospital*

INTRODUCTION

Nowadays, human resources are the most valuable asset of any organization and a factor of survival in competitive levels of business, helping organizations achieve their goals. Thus, human resources management are considered a challenging task whose main goal are to improve employees’ efficiency and increase organizational performance in the long run. Efficiency as a philosophy of life and improvement strategy is considered as improvement, prevalent goal, and important organizations. In fact, improving efficiency is the main goal of any organization so that its promotion strategy encompasses all units and levels of the organization. Efficiency, mentioned in the literature, is defined as "the amount of services or products that an employee produces over a period of time with an emphasis on quality. The National Efficiency Organization of Iran defines efficiency as the culture of a rational attitude towards a better and transcendent life and work. Employees’ efficiency, ultimately leading to desirable organizational performance, is a key index of organizational success, and since performance measures an organization's competitiveness, Emphasis on efficiency seems to be a requirement for management. In addition, efficiency is an important factor in identifying profits, for recognizing potential opportunities

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for organizational success in business, with determining the index for employees' reward and a way to appreciate hard work. Efficiency measures the efficiency of using organizational resources in order to obtain specific outputs.

In this research, the "ACHIEVE" model has been used to measure efficiency. Hersey and Blanchard, i.e., help managers to determine efficiency problems and edit strategy to solve them, developed this model. This model consists the main and influential factors in manpower performance including seven variables: ability, realization, organizational support, motivation, environmental compromise, validity, and evaluation and feedback. Organizational commitment: Organizational commitment has attracted considerable attention in the literature since various studies have emphasized the ramifications of organizational commitment, including occupational behavior and performance. A number of behavioral cues such as learning, thinking, perception, motivation, and organizational commitment are considered as the most important criteria for evaluating employees' behavior in the organization. Luthans stated that employees' commitment to an organization can be measured by their positive desire to remain an active member of the organization, their willingness to sacrifice responsibilities, and tasks at their best to do, and their acceptance reflected on the value of organizational goals. Past studies have shown that organizational commitment plays a conspicuous role in influencing employee efficiency, That is, researchers examined the connection between organizational commitment and employees' performance and found a positive and significant connection between the two variables. IN view of these studies, high levels of organizational commitment prove the success of an organization in human resources management, ultimately leading to improved performance and high employees' efficiency. Allen and Meyer model [11] asserts that despite of the extensive research being on organizational commitment, there is no consensus among management experts on the concept of organizational commitment and its focal points, having led to the emergence of different models of organizational commitment. In their view, organizational commitment has three distinct dimensions, including emotional commitment, continuous commitment, and normative commitment. Career anchors: The concept of career path as a link between organizations and the people working for them, examined from both internal and external psychological aspects. External career path includes jobs and situations through that a person progresses and is defined as organizational indicators of career path, whereas internal career path is the tendency of employees to orient or career anchor. Actually, just as sailors anchor to keep a ship afloat, so do people anchor to stay afloat.Bidabadi et al. [13] take for instance, anchor in their study shows that oxidizer and fuel are distinctly injected into a system, and before the reaction area are not mixed, in counter flow of combustion.Bidabadi et al. [14] take for example, anchor is in the burning lycopodium particle involving vaporization and drying processes in non-premixed flames of non-adiabatic combustion.Bidabadi et al. [15] another example of anchor is that single-fuel case operated by combustion of lycopodium whereas dual-combustion case is initial level. Jahromi et al. [16] final example of anchor is that this research down and up drafts gasifiers have been simulated by Computational Fluid Dynamics models to expand the process of gasification in two-dimension.Therefore, people try to establish their talents, abilities, and requirements and attitudes by making changes in their career path. Career anchors or career aspirations are specific patterns of talents, capacities, motivations, attitudes, and values imagined by the individual that guide and establish a person's progress after several years of experience and feedback from the real world. These career aspirations are aspects of a job or career path that motivate people and make them reluctant to quit when they accept a job. In addition, job anchors put forward the idea that most people do not want to limit themselves to a particular field [17]. Based on previous studies, career path anchors explain the fit between employees' job orientations and work environment, which will lead to job satisfaction, increase employee commitment and performance [18-20]. Nowadays, efficiency and identifying the effective factors is one of the management concerns in any organization, so that experts in this field consider improving efficiency as one of the vital goals of any organization [21]. This is important in healthcare organizations, such as hospitals, for the use of specialist human capital. Because governments spend significant amounts of GDP annually on defense and military organizations protect a country's national interests, independence, and security, being at the forefront in times of crisis, efficiency in military organizations is more important than in other organizations [22]. A review of the subject literature showed that efficiency studies are interesting topics for managers. In the study of Bahadori et al. [23], the factors affecting efficiency were extracted by reviewing previous studies and they present a model with 10 components, including organizational culture, mental growth, intellectual, and personality, organizational support, motivation, education, personal health, performance appraisal, job clarity, ability, and environment. Jafari and Tehran [22] identified the effective factors on efficiency in a review study whom they evaluated. They identified effective factors on efficiency including individual factors consisting motivation, mental development, personal health, ability and organizational environmental factors containing organizational support, job clarity, environment, management style, education, culture, and performance appraisal. Ali Akbar Esfahan et al. [24] in their study on the connection between intellectual capital and efficiency of human resources and occupational performance from the perspective of military hospital staff showed that intellectual capital has a positive and significant effect on efficiency.

Given that thus far limited studies have been conducted on the effect of organizational commitment variables and job anchors on efficiency, especially in military organizations, this study tried to investigate this issue (Figure 1).

Figure 1: Conceptual model of research

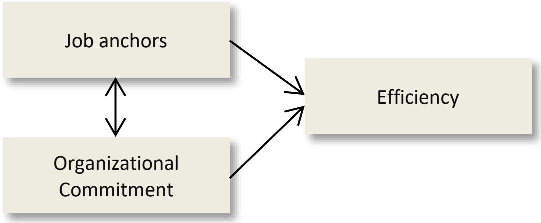

METHODS

The present study was conducted as a cross-sectional survey study in a military hospital. The target population of this study consists of all hospital staff with more than 3 years of work experience. Due to the limited population of the study, the sample size was determined to be 225 based on Morgan table and Cochran's formula. Owing to the possibility of non-return of a number of questionnaires or their incompleteness, a drop of 10% was considered for the sample size. Finally, 248 questionnaires were distributed among the samples, completed at the end of 230 questionnaires and provided to the research team. The criterion for entering the samples was more than 3 years of work experience and the criterion for leaving was the incomplete or unconventional answers of the distributed questionnaire. A simple random method was used for sampling, so the instrument used in this research was a four-part questionnaire. The first part included demographic information including gender, age, marital status, education, occupation, employment status, and work experience. The second part included the efficiency questionnaire, the third part included the organizational commitment questionnaire, and the fourth part included the job anchor questionnaire.

Hersey & Goldsmith Efficiency Questionnaire: this Persian and standardized questionnaire [25] contains twenty-six questions. The scale of answering the questions was in the form of a five choice spectrum very low, low, medium, high, and very high and the whole questionnaire included seven constructs ability, comprehension, organizational support, motivation, feedback, credibility, and adaptability. The reliability of this questionnaire in the present study was obtained by Cronbach's alpha index of point eight nine one.

Meyer & Allen Organizational Commitment Questionnaire: this Persian and standardized questionnaire [26] contains twenty-four questions. The scale of answering the questions was in the form of a seven choice spectrum strongly disagree, relatively disagree, slightly disagree, have no opinion, slightly agree, partially agree, and strongly agree. The whole questionnaire included three being a structure emotional commitment, normative commitment, and continuous commitment. The reliability of this questionnaire in the present study was obtained by Cronbach's alpha index of zero point eight zero one.

Schein Job Anchor Questionnaire: this Persian and standardized questionnaire [27] contains forty questions. The scale of answering the questions was in the form of a four choice spectrum never, rarely, often, and always. The whole questionnaire consisted of eight structures technical-applied, general management, autonomy-independence, security-stability, Creativity-entrepreneurship, service and self-sacrifice, and pure challenges and lifestyle. The reliability of this questionnaire in the present study was obtained by Cronbach's alpha index of zero point eight six-four.

Statistical methods and structural equations modelling were used to analyse the data in this study. Quantitative indices, percentage, and mean indices, standard deviation, were used for descriptive tables and reporting of qualitative variables.

Given the normality of the data distribution, assessed by the Kolmogorov-Smirnov (K-S) test, Pearson correlation coefficient index was used to determine the correlation among quantitative variables.

Structural equation modelling was used to evaluate the research model and the connection between the main research variables. The level of statistical significance in this study was considered zero point zero five,

That was, SPSS software version twenty first was involved to prepare descriptive tables and statistical analyses and AMOS software version twenty first was used to analyse structural equations modelling.

Observance of ethics standards in research

All individuals were reassured about being free to participate in the research and not being forced to continue participating in the research**.**

RESULTS

In this study, two hundred and thirty personnel of a military hospital participated. Forty eight point thirty percent of the participants were male and fifty one point seventy percent were female. Seventy one point seven percent of the participants were married, sixty three point nine percent of all participants had a master's degree, and-six point one percent had a bachelor's degree or lower. The average age of participants was thirty-two point twenty two years with a standard deviation of four point sixty seven years and the average age of work experience was eleven point eighty one years with a standard deviation of four point three years (Table 1).

Table 1: Summary of demographic information

Variable		Number	Percentage
Sex	Male	111	48.3
	Female	119	51.7
Marital Status	Bachelor	65	28.3
	Married	165	71.7
Education	Bachelor of Science and Lower	83	36.1
	Master of Science	147	63.9
		Average	**Standard Deviation**
Age		32.29	4.67
Occupation history		11.81	4.30

The results of this study showed that the score of job anchors and organizational commitment do not have significant connection with demographic variables, P >0.05. In this study, there is a significant connection between efficiency score and demographic variables of age and work experience, P <0.05. Therefore, that with increasing age and work experience, the efficiency of individuals for the organization decreases significantly (Table 2).

Structural equations modelling was used to answer the question whether there is a significant connection between job anchor variables and organizational commitment and if these two variables affect the efficiency score of individuals in the organization (Figure 2).

Figure 2: Connection of structural model among organizational commitment, job anchors, and efficiency

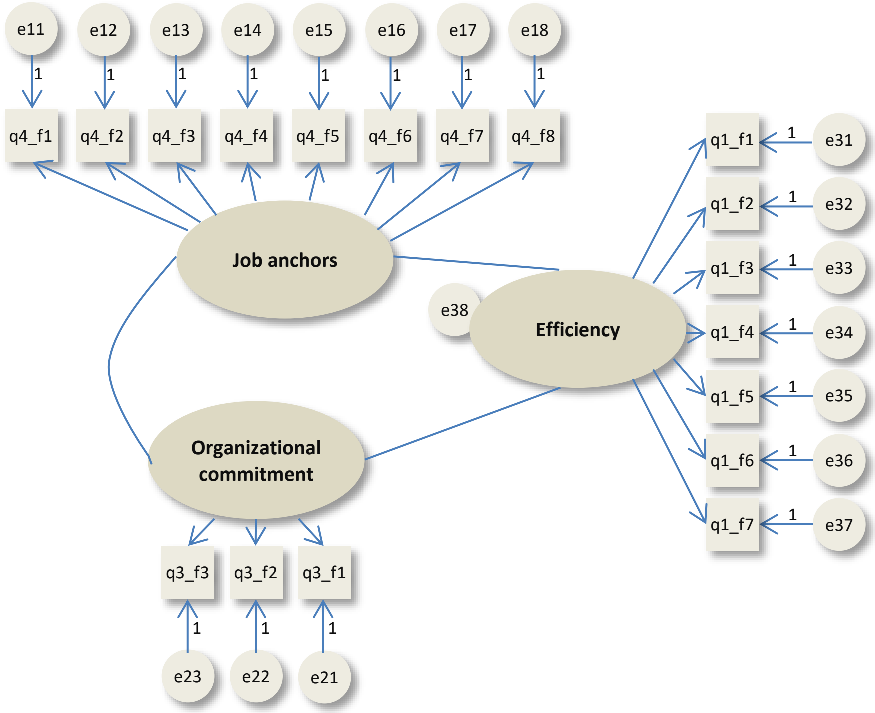
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1
q4_f1
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q4_f6
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q4_f8
Job anchors
Efficiency
e38
Organizational commitment
q3_f3
q3_f2
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Figure 3: Structural model of final modified (connection between job anchors and efficiency with the effect of variable moderating of organizational commitment

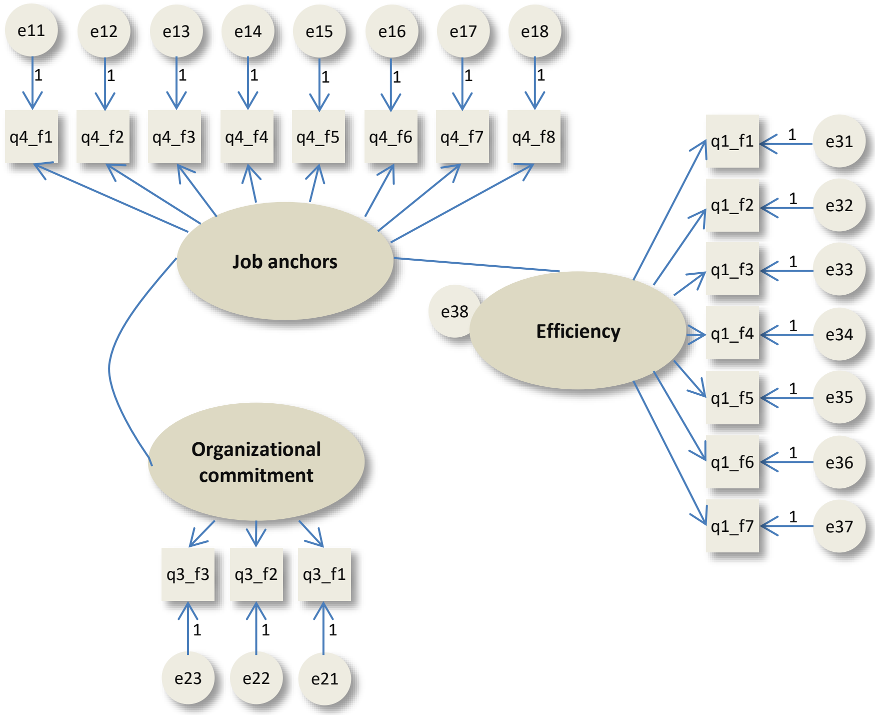

Table 2: Commitment connection of organizational and efficiency with age and occupation precedent

Variable	Efficiency	
	Pearson correlation	P significant
Age	-0.371	0.033
Occupation history	-0.303	0.046

Structural model studies (Figure 1) show that the model is significant, P <0.05. Considering that the Chi-Sq./df index is a value between two to five and the GFI, AGFI, CFI and NFI indices are greater than zero point nine and the RMSEA index is less than zero point one, indicating a good fit of the model has it (Table 3).

Table 3: Summary of fitting indicators of structural model of connection between organizational commitment, job anchors, and efficiency

Index RMSEA	Index NFI	Index CFI	Index AGFI	Index GFI	Index Chi-Sq/df	P Significant	Degrees of Freedom df	Ki Do Chi-Sq
0.066	0.924	0.941	0.900	0.912	3.85	0.008	133	512.58

Considering the significance of the coefficients of each questionnaire and their structures and the connections among variables, the results showed that job anchors separately have a significant effect on efficiency, P <0.05, but organizational commitment alone on Efficiency do not have significant effect, P >0.05. Considering the significant correlation between organizational commitment and job anchors, P <0.05, it can be seen that organizational commitment acts as a moderating variable. In other words, the connection between job anchor and efficiency can be justified when the effect of organizational commitment variable is applied to the model (Table 4). Finally, Figure 1) can be modified and explained as Figure 2 and Figure 3.

DISCUSSION

Efficiency and effectiveness are economic requirements for every country whose goal is economic development, such as Iran. Hospitals in all countries account for a significant share of resource consumption in the health sector. Because resources are limited, maximizing efficiency in military hospitals allows resources to be best distributed in this area. As problems increase and organizations become more complex, the need for better solutions based on the role of job anchors and organizational commitment to efficiency in military organizations is becoming increasingly important. Therefore, in the present study, the researcher decided to provide a model with a broad perspective on the impact of job anchors on job satisfaction, organizational commitment, and efficiency in military hospitals from the perspective of a military hospital staff. The research findings based on structural equations modeling indicated that the research variables such as: organizational commitment, job anchors, and efficiency had acceptable convergent validity.

Table 4: Studying coefficients significant of hidden variables and observed in the structural model

Time	Connections	Coefficients Estimation	Standard Deviation	P Significant
q1_f1	Efficiency → Ability	1.000		-
q1_f2	Efficiency → Understanding and Cognition	0.302	0.043	0.001
q1_f3	Efficiency → Organizational Support	0.472-	0.014	0.001
q1_f4	Efficiency → Motivation	0.094	0.052	0.070
q1_f5	Efficiency → Feedback	0.481	0.036	0.001
q1_f6	Efficiency → Validity	0.312	0.026	0.001
q1_f7	Efficiency → Compatibility	0.184	0.034	0.001
q4_f1	Job Anchors → Applied Technical Major	0.001	-	-
q4_f2	Job Anchors → General Management Major	-2.757	0.163	0.001
q4_f3	Job Anchors → Autonomy Independence Major	-0.643	0.048	0.001
q4_f4	Job Anchors → Security-Stability Major	-0.601	0.060	0.001
q4_f5	Job Anchors → Creativity - Entrepreneurship Major	-0.336	0.025	0.001
q4_f6	Job Anchors → Service, Self-sacrifice, and Dedication Major	-0.432	0.037	0.001
q4_f7	Job Anchors → Pure Challenges Major	-0.232	0.040	0.001
q4_f8	Job Anchors → Life Style	0.148	0.113	0.189
q3_f1	Job Anchors → Emotional Commitment	1.000	-	-
q3_f2	Job Anchors → Norm Commitment	-0.399	0.113	0.001
q3_f3	Job Anchors → Continuous Commitment	-0.328	0.079	0.001
	Job Anchors → Efficiency	0.101	0.029	0.003
	Organizational Commitment → Efficiency	-0.006	0.004	0.457
		Correlation		**P Significant**
Job Anchors ↔ Organizational Commitment		-0.202		0.038

The model is acceptable since the fit indices of the model were generally proportional; in other words, based on the available indicators, the fit of the model was confirmed. According to the results, there is a significant correlation between age and work experience with efficiency. Since this coefficient is negative, with increasing age, work experience, and efficiency decreases significantly. Organizational commitment did not show a significant connection with demographic variables; also, there is a significant correlation between age and work experience with organizational commitment; in other words, with increasing age, work experience, and organizational commitment decreases significantly. Analysis of the research model showed that organizational commitment do not have significant effect on efficiency; thus, this result is similar to a number of previous studies [28,29] and contradicts some others [30].

On the other hand, the results showed that job anchors would have a great impact on human efficiency; the results indicate a positive and significant correlation between organizational commitment and job anchors, having been obtained in other studies.It can be concluded that job anchors will have a beneficial effect on efficiency by synergizing the effect of organizational commitment; however, none of the structures showed a significant connection with demographic variables. Hence, no significant correlation was found between age and work experience variables with job anchors, none of its structures. According to the results of this study, every organization should pay special attention to job anchors, in particular, when it seeks change and needs to evaluate the strengths and weaknesses of its manpower. Furthermore, it can be said that differences in job anchors will lead to diversifications in the likelihood of career advancement. By increasing information about these non-financial incentives, organizations can explain effective approaches to their employees' career development.Job anchors show the reasons why people have progressed in their careers as a pattern. This model explains why and how an individual's interactions with the organization's job anchors affect work and job satisfaction, and job orientations provide a way to understand motivational factors in job decisions. Analyzing the job anchors of individuals helps organizations redesign jobs (to meet the needs of individuals) and plan job changes. Finally, in light of the evidence of this study, committed employees help organizations to improve efficiency by choosing the appropriate job anchors.

This study is one of the first studies on the impact of job anchors and organizational commitment on efficiency and since the statistical population is limited, the generalization of results should be done with caution. Moreover, some results are inconsistent with other previous studies conducted abroad, so it is suggested that the model proposed in this study be examined in other studies for greater validity.

CONCLUSION

In view of the general overview of the results of the present study, designing the knowledge structure at all, levels of military hospitals in the country will be effective in improving and efficiency of these organizations and in this regard, staff managers as producers and national knowledge capitals have important role. Therefore, it is necessary for senior managers of military hospitals in the country to focus more on these components: namely organizational commitment, efficiency, and job anchors in order to implement this system in their organization and take full advantage of it.One of the first activities that should be considered by the managers of this organization and emphasized in different sectors, educational needs assessments with emphasis on job anchors and organizational commitment for greater efficiency and determination and development of continuous training programs for different parts of the organization. In this way, empowering employees, organizational efficiency is improved.

Acknowledgments

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Conflict of interest

The authors of this study state that there is not conflict of interest.

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ORIGINAL ARTICLE

Introducing a new medical method innovative suture technique to reduce scars in central lip lift medical surgery

Seid R. Hosaini[1], **Kamyar Azhdari**[2]

Abstract: *The central lip lift surgery with a new suture technique called; continuous locking suture in three cases is reported in this article. In this novel method, we used a 5.0 chromic suture by applying a 30-degree angle of the entrance to the skin semi-deeply. We start suturing at the middle of the wound,3-4 mm from the edges of the skin. The needle should be way out at the exact point of entrance, and this process is repeated to reach out to the two sides of the wound. More than 90% of patients (in more than 100 cases) were satisfied with the cosmetic results. Moreover, 90% of cases did not demonstrate any scar after three months without laser or reconstructive ointments. We evaluate many patients who undergo central lip lift surgery with this novel technique, and the scar problem was resolved in more than 90 % of cases with no supportive treatment.*

Keywords: *scar, central lip lift surgery, cosmetic results, patients*

INTRODUCTION

The central lip lifts

The first doctor that performed central lip lift surgery was Dr. Austin in 1980 and presented 83 cases in 1986. The lip lift surgery is performed to make the long lip of aging shorter and is designed to excise the wavy ellipse skin that follows the contour of the nostril crease below the sill. However, the nostril sill is not degraded. No attempt is needed to hide the scar in the nose; the reason is that the scar is well-hidden in the fold and is usually undetectable [1-3].

The continuous locking suture is an innovative method in central lip lift surgery that can be applied for at least six possible goals in closing the wounds made by the scalpel and are full-thickness. These goals are as follows: 1) reduce tension in the dermis, 2) reduce scarring after the surgery, 3) reduce suture marks to get an excellent cosmetic result, 4) Reduce the wound healing process to 5-6 days, 5) It is not visible in the surface of the skin. However, none of the other suture techniques fulfills all five above criteria. Those techniques that achieve good epidermal wound apposition will not give dermal support, may cause suture marks, or require wound care such as laser or medications. Those techniques that reduce tension in the dermis will not lead to precise epidermal wound edge apposition. The locking suture is a continuous suture. Three cases are presented in which the continuous locking suture was used in central lip lift surgery. The details of this technique are reported in this article. Finally, the advantages of the continuous locking intradermal suture will be summarized.

CASE SERIES

Case 1

A 40-year-old man clinically had a long distance between his nose and mouth (Figure 1). The area was disinfected with chlorhexidine and draped in the usual manner at the surgery. The lower incision is applied first via a 15-blade scalpel to the skin perpendicularly, reaching out to the fat and muscle junction. After that, the upper incision is made parallel to the lower incision; after finishing the surgery, suspension and closure must be performed accurately. In our novel technique, we start from the middle of the wound, insert the needle via a 30-degree angle for about 3-4 mm to the upper edge of the wound, and then take out the needle at the exact

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entrance point. Then, we perform the exact procedure on the lower edge of the wound and continue the process by the end of the incision. The wound was lastly dressed with transparent dressing and Bacitracin ointment. The patient came back for a follow-up examination in 30 days (Figure 2). At the first postoperative examination, no obvious scar was detected, and the patient was fully satisfied.

Figure 1: The 40-year-old man before the surgery

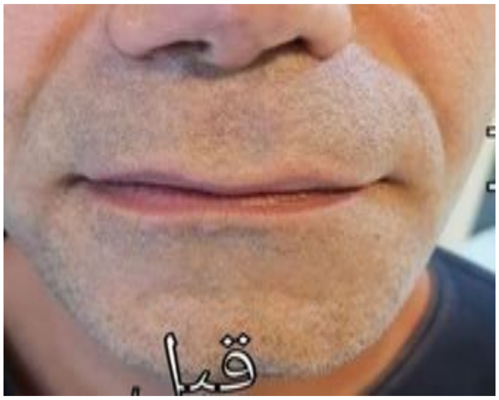

Figure 2: The 40-year-old man 30 days after the surgery

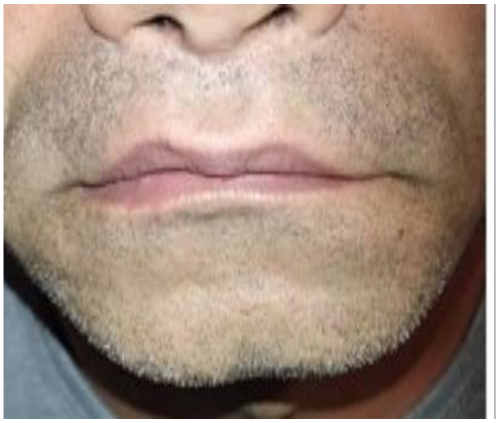

Case 2

Second case was a 40-year-old white woman. At central lip lift surgery, the goal area was cleansed by chlorhexidine. A superficial, vertical incision was made, and the skin and the subcutaneous flap were then excised over the orbicularis, remaining a thin glossy layer of fat intact. The edges of the skin were undermined to reduce the tension. Finally, The wound was closed via a continuous locking suture using 5-0 Vicryl (Figure 3). Bacitracin ointment was administered to the surgical wound. At the follow-up visit 30 days later, the wound edges were fully approximated. No redness or irritation was detected at the surgical site, and the wound was healed completely (Figure 4).

Figure 3: The 40-year-old woman before the surgery

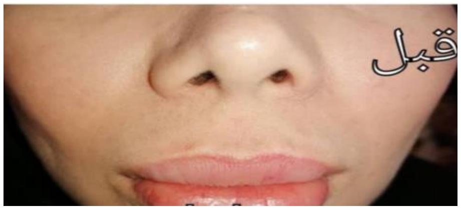

Figure 4: The 40-year-old woman 30 days after the surgery

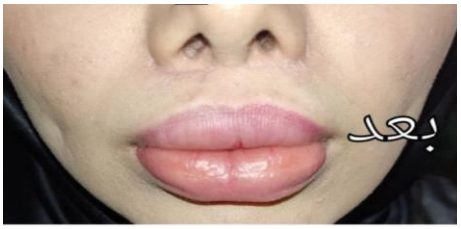

Case 3

A 20-year-old woman underwent central lip lift surgery, the area of the incision was cleaned with chlorhexidine. A superficial, vertical incision was made by the scalpel. The edges of the wound were undermined to decrease the tension. In the end, the wound was closed with 5-0 Vicryl with a continuous locked suture (Figure 5). Bacitracin ointment was administered to the surgical wound. At the patient's first postoperative visit, the wound was closed five days later without any scar. The wound edges were greatly approximated. A follow-up examination at one year demonstrated that the wound healed completely (Figure 6).

Figure 5: The 20-year-old woman before the surgery

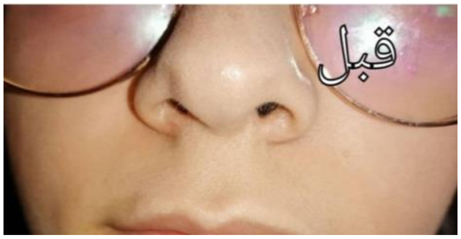

Figure 6: The 20-year-old woman after the surgery

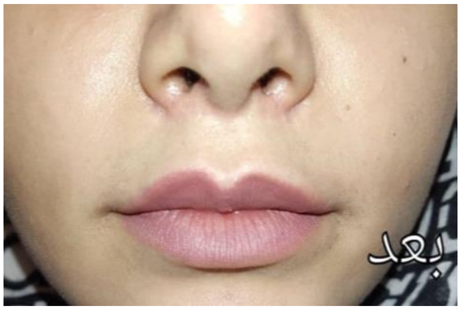

THE CONTINUOUS LOCKING SUTURE

Excision

The procedure is done using local anesthesia, and there is a surgeon at the head of the bed. The lower incision is performed first via a scalpel (15 blades) perpendicular to the skin, reaching out to the junction of the fat and the muscle. The upper incision is made parallel to the lower one in the next step. There is a thin glossy layer of fat which is left after excision of the skin and subcutaneous flap. This intact glossy layer is where vasculature lays, which is deep to the superficial muscular aponeurotic system (SMAS).

Dissection

After the excision process, we elevate the labial flap in a deep sub-SMAS plane. This dissection makes the labial SMAS free from the underlying orbicularis oris. The area of dissection is at the surgeon's discretion, as a larger dissection area may mitigate tension; however, it causes a considerable increase in postoperative swelling. The effacement of the Cupid's bow is probable in full central dissection. In most patients, the dissection extends to the vermillion, lateral to the philtral columns, and just finished before the nasolabial fold.

Suspension and Closure

Simple dermal closure is performed by most practitioners, which is the prevalent mistake [4]. The best lift can be obtained by doing an adequate release of tethering and then suspense the dense tissue upward in a fixed area. When this is done in the upper lip, the skin/SMAS flap released can roll over above the contracted orbicularis.

The continuous locking suture technique

In our novel technique, we start from the middle of the wound, insert the needle via a 30-degree angle for about 3-4 mm to the upper edge of the wound, and then take out the needle at the exact entrance point. Then, we perform the exact procedure on the lower edge of the wound and continue the process by the end of the incision. This technique is called locking because the 30 degrees of entrance and back and force repeatition, let the suture be locked in the skin. This provides a great advantage to push the dermal edges together. In the end, the incision must be closed from the center to the lateral. Then, the skin edges are closely approximated. At the lateral most side, a 4-0 Vicryl suture is passed from inside the pyriform, emerging radially to obtain the SMAS and then come back into the nose to complete a mattress suture. It is better to identify and protect the inferior alar artery.

RESULTS AND DISCUSSION

More than 100 cases had great cosmetic results with very few consequences. In 3 patients which were reported, any slight inflammation resolved within a few days of the first follow-up visit. None of the cases experienced significant discomfort or wound infection. The continuous locking suture combines beneficial features of other common techniques. It is locked for reducing any tendency to loosen. As a result, the apposition of wound edges is better.

CONCLUSION

There are five benefits for continuous locking suture: 1) reduce tension in the dermis, 2) reduce scar after the surgery(more than 90 % of patients did not demonstrate any scar after surgery without laser or other medication), 3)this technique is so effective in central lip lift surgery, 4) Reduce the wound healing process to 5-6 days, 5) It is not visible in the surface of the skin. In this technique, if the applied pressure to the needle increases, it can reduce the area's blood supply when the needle is excised from the nose. This reduction of blood supply can lead to small necrosis. All in all, we should regulate the applied pressure to prevent necrosis in the affected area.

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ORIGINAL ARTICLES

Medical and chemical efficacy of respiratory physiotherapy and Remdesivir in patients with COVID-19 pneumonia: A systematic review and meta-analysis

Alireza Bahmani[1], Zahra Mollashahi[2], Negar Shahkarami[3], Elahe Delavar[4], Hooman Esfahani[5]

Abstract: *Evaluated efficacy of Respiratory Physiotherapy and Remdesivir on patients with COVID-19 pneumonia. In current systematic review and meta-analysis study, articles published January 2019 to December 1, 2021 were reviewed in the databases of PubMed, Scopus, Web of Science, and EBSCO. Risk ratio and mean differences with 95% confidence interval (CI), fixed effect model and Mantel–Haenszel or Inverse-variance formula were calculated. The Meta analysis have been evaluated with the statistical software Stata/MP v.16 (The fastest version of Stata). Mean differences of PaO2/FiO2 ratio at 6h after chest Respiratory Physiotherapy was (MD, 66 mmHg 95 % CI 64.71 mmHg, 67.28 mmHg; p=0.0007). Risk ratio of recovery rate between experimental and control group was 0.20 (RR, 0.20 95 % CI 0.15, 0.25) with high heterogeneity (I2 =78.84%; p=0.00). Risk ratio of mortality rate between experimental and control group was -0.34 (RR, -0.34 95 % CI -0.65, -0.03) with low heterogeneity (I2<0%; p=0.51). Based on the findings of meta-analysis, Respiratory Physiotherapy can play an effective role in respiratory therapy and rehabilitation of patients admitted to the ICU with COVID-19. A meta-analysis showed that treatment with Remdesivir could increase the recovery rate, especially in the early days of COVID-19; also reduces the mortality rate.*

Keywords: *COVID-19, respiratory physiotherapy, Remdesivir*

INTRODUCTION

Coronaviruses are a large family of viruses. From this collection, seven species transmitted to humans have been discovered so far, which cause diseases such as colds in humans [1]. Coronaviruses often attack the respiratory tract and sometimes show symptoms in the intestines and stomach [2,3]. Coronaviruses usually first infect the mucous membranes of the respiratory tract in the throat and nose, causing symptoms similar to the common cold [4]. Coronaviruses usually first infect the mucous membranes of the respiratory tract in the throat and nose, causing symptoms similar to the common cold [5]. Sometimes can cause more serious illnesses such as bronchitis and exacerbation of asthma and chronic bronchitis in adults and even lung infection (pneumonia) in adults, the elderly and people with weakened immune systems [6]. Covid-19 is an airway infection caused by a mutated version of the coronavirus and was first reported in Wuhan, China in December 2019 [7]. Initially, the mortality rate of patients with Covid-19 was announced at 3 to 5 percent, but recent reports have shown a reduction in mortality [8]. Due to the fact that this disease is very new and emerging, so scientific and documented findings in this area are very few. Therefore, considering the secondary effects that this disease has on the pulmonary system and affects the pulmonary system, so specialized findings on the improvement of pulmonary patients and their conclusion are very helpful in advancing the goals [9]. Physiotherapists, as a member of the multiple treatment team in the intensive care unit, play an effective role in reducing respiratory complications and preventing weakness due to hospitalization in the intensive care unit [10]. Since one of the most important organs involved in Covid-19 disease is

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the respiratory system.

Therefore, the role of physiotherapists, especially in the field of respiratory physiotherapy, along with other health professionals, plays an important role in the treatment and care of this group of patients and overcoming the respiratory symptoms of patients with Covid-19.

In addition to medical treatment, rehabilitation techniques and pulmonary physiotherapy are essential for the treatment and improvement of patients' respiratory function. These measures are also recommended as self-care training and breathing exercises for quarantine patients at home [11]. Pulmonary rehabilitation has a variety of techniques, including breathing exercises and chest physiotherapy that help clear the airways, create a deep breathing pattern, increase arterial oxygen saturation and improve pulmonary volume, and increase voluntary expiratory volume in the first second [12]. Due to the increase in hospitalization costs, reducing the risk of nosocomial infections, reducing the number of hospitalizations, pulmonary rehabilitation programs for empowerment and also improving patients' self-care have been emphasized more than before; Therefore, the main role of physiotherapists to start patients' pulmonary rehabilitation programs at the time of hospitalization and the emphasis on continuing and following it at home becomes more apparent. Therefore, the aim of current study was evaluated efficacy of Respiratory Physiotherapy and Remdesivir on patients with COVID-19 pneumonia.

METHOD

In this systematic review study, Science direct, Google scholar, PubMed, Scopus, Web of Science, and EBSCO databases were used to search for articles. The present study is a systematic review and meta-analysis. In current study review of previous studies, the Preferred Reporting Items for Systematic Reviews (PRISMA) checklist was used to search for the studies [13].

Figure 1: Flowchart of the literature search and selection criteria

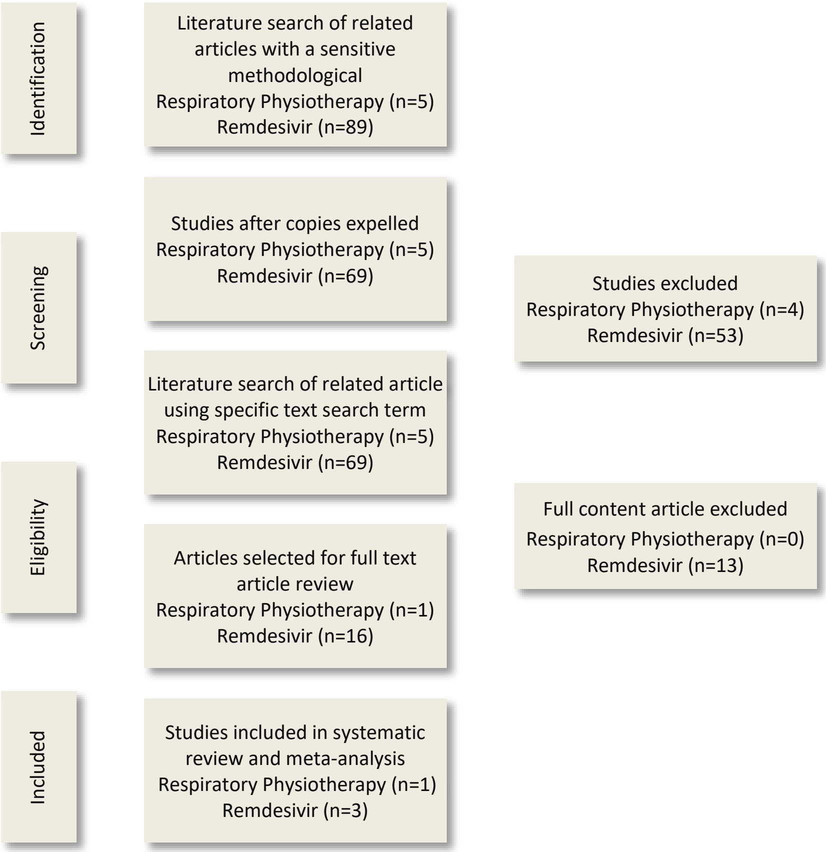
Identification
Literature search of related articles with a sensitive methodological
Respiratory Physiotherapy (n=5)
Remdesivir (n=89)
Screening
Studies after copies expelled
Respiratory Physiotherapy (n=5)
Remdesivir (n=69)
Studies excluded
Respiratory Physiotherapy (n=4)
Remdesivir (n=53)
Literature search of related article using specific text search term
Respiratory Physiotherapy (n=5)
Remdesivir (n=69)
Eligibility
Full content article excluded
Respiratory Physiotherapy (n=0)
Remdesivir (n=13)
Articles selected for full text article review
Respiratory Physiotherapy (n=1)
Remdesivir (n=16)
Included
Studies included in systematic review and meta-analysis
Respiratory Physiotherapy (n=1)
Remdesivir (n=3)

Data Extraction and analysis method

The data were extracted from the research included years, study design, number of patients, mean of age. To extract the data, two blind and independent reviewers extracted the data from the abstract and full text of the studies. Prior to screening, kappa statistics were performed to confirm the level of agreement between the reviewers. Kappa values were higher than 0.80.

Risk ratio and odds ratio with 95% confidence interval (CI), fixed effect model and Mantel–Haenszel formula; mean differences with 95% CI, fixed effect model and Inverse-variance formula were calculated. Random effects were used to deal with potential heterogeneity and I2 showed heterogeneity. I2 values above 50% signified moderate-to-high heterogeneity. The Meta analysis have been evaluated with the statistical software Stata/MP v.16.

RESULT

Respiratory physiotherapy: A total of 5 articles were found in the initial search. After removing duplicates, entry criteria for the titles were applied to the remaining 5 articles, and a summary of the remaining articles was reviewed. In this step, 4 articles were excluded. In the next, full text of 1 article were reviewed, finally one study was selected.

Remdesivir: A total of 89 articles were found in the initial search. After removing duplicates, entry criteria for the titles were applied to the remaining 69 articles, and a summary of the remaining articles was reviewed. In this step, 53 articles were excluded. In the next, full text of 16 articles were reviewed and 13 articles were excluded, finally three studies were selected.

Characteristics the studies included for evaluated efficacy of respiratory physiotherapy

One study (observational study) has been included in present article. The number of patients a total was 20 with mean age 63 years. Table 1 showed summary of studies for patient demographics, comorbidities, and clinical manifestations. Only one studies was found that evaluate efficacy of respiratory physiotherapy in COVID-19 patients [14].

Table 1: Summary of characteristics of included studies

Study. Years	Study design	Number of patients	Mean of age	Type of ventilation during RPT	ICU discharge characteristics
Battaglini et al., 2021 [14]	observational study	20	63	45% PSV 55% COT	% 5 Dead 95% Alive

PaO_2/FiO_2 ratio

Battaglini et al., 2021 [14] reported that PaO2/FiO2 ratio improved at T1 (RPT) compared to T0 (baseline) (MD, 63 mmHg 95 % CI 61.83 mmHg, 64.16 mmHg; p=0.00), the mean differences of PaO_2/FiO_2 ratio at 6h after chest RPT was (MD, 66 mmHg 95 % CI 64.71 mmHg, 67.28 mmHg; p=0.0007), this result showed PaO_2/FiO_2 ratio improved at 6h after chest RPT than baseline (Table 2).

Lung ultrasound score (LUS)

According to Battaglini et al.,2021 [14] study, the mean difference of LUS score between baseline and immediately after RPT was (MD, -4.95 % CI -4.26, -3.75; p=0.00).

Battaglini et al., 2021 [14] reported Correlation between LUS and CT scan, volume of gas and hyperaeration at CT scan between baseline, immediately after RPT and 6h after chest RPT, the result showed there was statistically significant association between LUS score and lung gas volume.

Characteristics the studies included for evaluated efficacy of Remdesivir

Three studies have been included in present article. The number of patients in experimental and control group was 1,125 with mean age 60.55 years and 799 with mean age 60 years, respectively. In all studies used 200 mg loading dose (IV), 100 mg maintenance dose from Second to Tenth day. The follow up period in two studies was 28 days and in one study was 29 days (Table 3).

Table 2: Meta-analysis of PaO_2/FiO_2 ratio

Group	Study	Mean Diff.	[95% Conf. Interval]		% Weight
Baseline – Immediately after RPT	Battaglini et al., 2021 [14]	63.000	61.838	64.162	54.92
Baseline – 6h after chest RPT	Battaglini et al., 2021 [14]	66.000	64.717	67.283	45.08

Meta-analysis summary Fixed-effects model Method: Inverse-variance Test of theta = 0: z = 146.42 Test of homogeneity: Q = chi2(1) = 11.54	Number of studies = 2 Heterogeneity: I^2 (%) = 91.33 H^2 = 11.54 Prob > |z| = 0.0000 Prob > Q = 0.0007

Figure 2: The Forest plot showed mean differences of lung ultrasound score

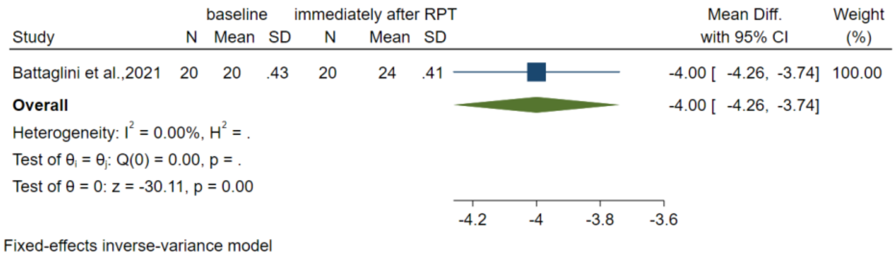

Table 3: Summary of characteristics of included studies

Study. Years	Number of patients		Mean of age		Remdesivir arm	Follow up period (day)
	Treatment	Control	Treatment	Control		
Spinner et al., 2002 [15]	384	200	58	57	200 mg loading dose (IV), 100 mg maintenance dose/2, 5 and 10 days	28
Wang et al., 2020 [16]	158	78	66	64	200 mg loading dose (IV), 100 mg maintenance dose/2-10 days	28
Beigel et al., 2020 [17]	583	521	58	59	200 mg loading dose (IV), 100 mg maintenance dose/2-10 days	29

Recovery rate

Risk ratio of recovery rate between experimental and control group was 0.20 (RR, 0.20 95 % CI 0.15, 0.25) with high heterogeneity (I2 =78.84%; p=0.00) (Figure 3). Subgroup meta-analysis showed Risk ratio of recovery rate on 7th day between experimental and control group was 0.19 (RR, 0.19 95 % CI 0.06, 0.33) with moderate heterogeneity (I2 =52.24%; p=0.12) (Figure 3).

Risk ratio of recovery rate on 14th day between experimental and control group was 0.29 (RR, 0.29 95 % CI 0.20, 0.38) with high heterogeneity (I2 =90.51%; p=0.00); Risk ratio of recovery rate on 28th day between experimental and control group was 0.13 (RR, 0.13 95 % CI 0.06, 0.20) with low heterogeneity (I2 =49.21%; p=0.14).

Remdesivir treatment significantly increased the recovery rate.

Mortality rate

Risk ratio of mortality rate between experimental and control group was -0.34 (RR, -0.34 95 % CI -0.65, -0.03) with low heterogeneity (I2<0%; p=0.51) (Figure 4).

Subgroup meta-analysis showed Risk ratio of mortality rate on 14th day between experimental and control group was -0.44 (RR, -0.44 95 % CI -0.82, -0.10) with low heterogeneity (I2<0%; p=0.52); Risk ratio of Mortality rate on 28th day between experimental and control group was 0.04 (RR, 0.04 95 % CI -0.59, 0.67) with low heterogeneity (I2<0%; p=0.67); Remdesivir treatment significantly reduction the mortality rate.

Adverse effects

Risk difference of adverse effects between experimental and control group was -0.01 (RD, -0.01 95 % CI -0.02, -0.00) with low heterogeneity (I2=27.92%; p=0.17) (Figure5).

Subgroup meta-analysis

Increased serum alanine aminotransferase

Risk difference of Serum alanine aminotransferase between experimental and control group was -0.01 (RD, -0.01 95 % CI -0.02, -0.00) with high heterogeneity (I2=79.26%; p=0.01).

Increased aspartate aminotransferase

Risk difference of aspartate aminotransferase between experimental and control group was -0.02 (RD, -0.02 95 % CI -0.03, -0.00) with low heterogeneity (I2=34.50%; p=0.22).

Increased bilirubin

Risk difference of bilirubin between experimental and control group was -0.00 (RD, -0.00 95 % CI 0.01, -0.01) with low heterogeneity (I2<0%; p=0.96).

Decreased creatinine clearance

Risk difference of Creatinine clearance between experimental and control group was -0.01 (RD, -0.01 95 % CI -0.02, 0.01) with low heterogeneity (I2=33.40%; p=0.20).

Vomiting

Risk difference of Vomiting between experimental and control group was 0.02 (RD, 0.02 95 % CI -0.02, 0.07) with low heterogeneity (I2<0%; p=0.33).

Figure 3: The Forest plot showed recovery rate on 7, 14 and 28 days

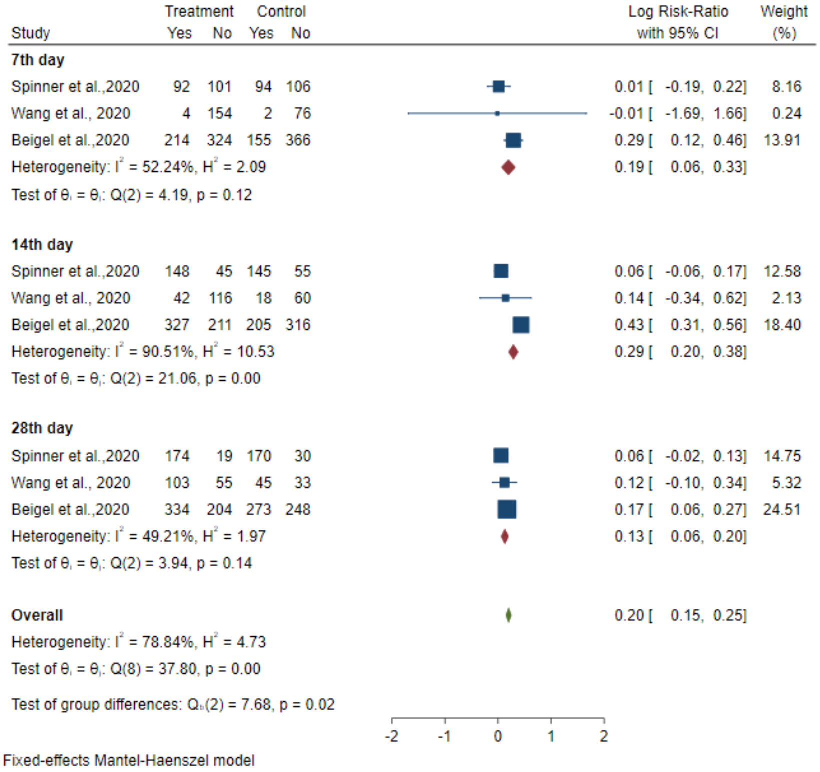

Figure 4: The Forest plot showed mortality rate on 14 and 28 days

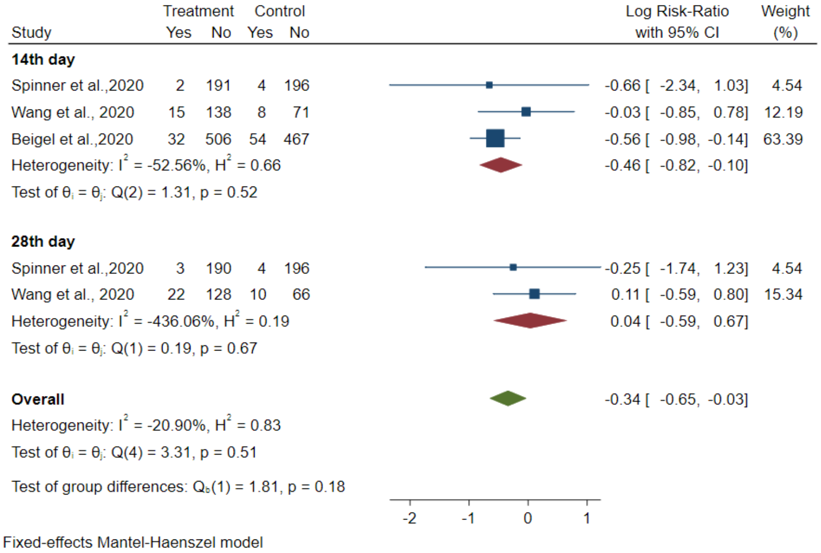

DISCUSSION

The aim of current systematic review and meta-analysis study was evaluating the efficacy of Respiratory Physiotherapy and Remdesivir on patients with COVID-19 pneumonia.

Meta-analysis showed respiratory physiotherapy improves oxygenation in critically ill COVID-19 patients. Only one study [14] was found to investigate the role of respiratory physiotherapy in patients with COVID-19. Hence, further studies are needed to emphasize the findings.

However, the findings showed that respiratory physiotherapy plays an important role in the rehabilitation of patients admitted to the ICU [18].

Figure 5: The Forest plot showed adverse effects

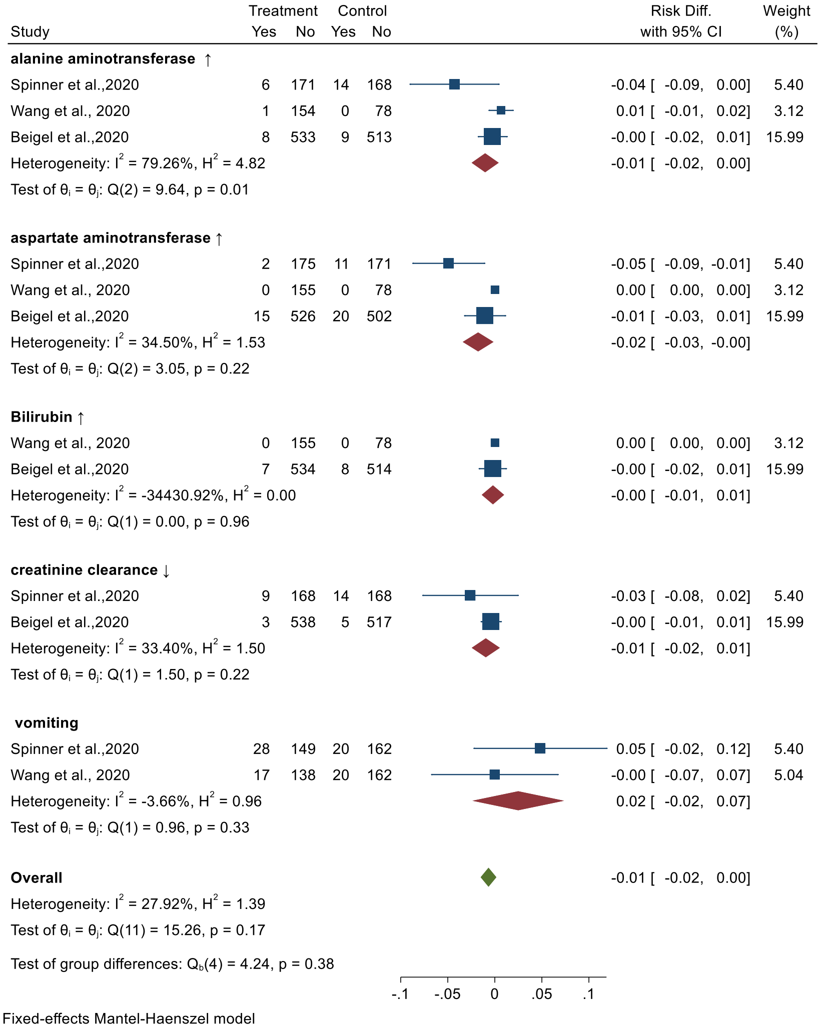

A study did not find a role for respiratory physiotherapy in patients with different respiratory patterns. As the meta-analysis shows, the LUS score improved, but these findings were not statistically significant. LUS is a diagnostic tool in the prognosis of COVID-19 [19]. To confirm this evidence, studies with larger sample sizes are needed to investigate the role of respiratory physiotherapy in patients with COVID-19 [14]. Limitations of the present study on the role of respiratory physiotherapy are: the existence of only one study, small sample size, and observational study (retrospective), RCT studies were not found, the study was conducted at the peak of COVID-19 epidemic, no comparison was found with the control group to evaluate the effectiveness of respiratory physiotherapy. Therefore, summarizing the practical and effective techniques of pulmonary physiotherapy is valuable to increase the awareness of all audiences, including physiotherapists, nurses and patient caregivers. Most review studies indicate that pulmonary physiotherapy is a safe and effective intervention, especially in stable conditions, to clear the lungs and improve the patient's respiratory function. Of course, there are a number of precautions and time limits for performing these techniques that should be considered. According to the Guideline published in March 2020 for physiotherapists in the treatment of patients with COVID-19, cardiopulmonary physiotherapy focuses on the treatment and rehabilitation of acute and chronic respiratory conditions in patients and aims to improve the physical fitness of individuals seeking the disease. Accordingly, physiotherapy can play an effective role in respiratory therapy and physical rehabilitation of patients with COVID-19.

To evaluated effectiveness of remdesivir, only three studies were found to be included in the meta-analysis. In these studies, the role of remdesivir on days 7, 14 and 28 in patients with COVID-19 was investigated. Meta-analysis showed that the effectiveness of remdesivir in these patients increases the recovery rate and reduces the mortality rate. Studies show that Remdesivir is more effective on the seventh day in the early stages of the disease, and patients who do not require mechanical ventilation respond better to Remdesivir [20]. According to the guidelines of the national institute of health, remdesivir works best in hospitalized patients with COVID-19 who require less supplemental oxygen [21]. However, further studies are needed to confirm these findings and provide sufficient and stronger evidence, as there is insufficient evidence to confirm the effectiveness of remdesivir in mild to moderate patients, and further studies are needed. Meta-analysis showed that the mortality rate on the 14th day of hospitalization was lower than on the 28th day, further studies are needed to confirm these findings [15,22].

The findings of this study are consistent with the results of previous meta-analysis studies [23,24]. However, the experimental studies reviewed in the current and previous meta-analysis were very few and, in most studies, the same, more RCT studies are needed in this area. Limitations of the study on the effectiveness of remdesivir in the treatment of patients with COVID-19 include: High heterogeneity in study results, time difference between results, age difference of patients, and low number of studies, low sample size and risk of bias not evaluated.

CONCLUSIONS

Meta-analysis showed that respiratory physiotherapy improves oxygen delivery in ICU patients with COVID-19, especially in less severe patients. It is recommended to review various respiratory physiotherapy techniques and their effectiveness in future studies. All respiratory physiotherapy interventions should be carefully considered to reach a complete conclusion in this regard. Unfortunately, so far only one study has been performed to evaluate the effectiveness of respiratory physiotherapy as a retrospective observational study (without control group); RCT and intervention studies are needed to confirm the effectiveness of respiratory physiotherapy techniques in patients with COVID-19. Based on the findings of meta-analysis, respiratory physiotherapy can play an effective role in respiratory therapy and rehabilitation of patients admitted to the ICU with COVID-19.

In the present study, a meta-analysis showed that treatment with Remdesivir could increase the recovery rate, especially in the early days of COVID-19; also reduces the mortality rate, although there is insufficient evidence in this regard because high heterogeneity was found between studies, so the reduction in mortality has not been proven. The efficacy of Remdesivir in patients with severe COVID-19 who do not require mechanical ventilation has been clearly demonstrated.

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ORIGINAL ARTICLE

Medicinal plants effective in the treatment of glaucoma from the perspective of traditional Persian medicine

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Abstract: *Traditional Persian medicine (TPM) is one of the richest medical schools in the world, which has nurtured valuable books. From Ibn Sina's law to Heravi's successful medicinal facts, all emphasize the treatment of various diseases, especially eye diseases based on herbal medicines, as well as their prevention. In this study, by reviewing the authoritative books of Traditional Persian Medicine, we are looking for medicinal plants so that they can be used in the treatment of glaucoma or glaucoma. the purpose of this study is not to express the complete cure of glaucoma, but this article seeks to classify plants that from the perspective of Traditional Persian Medicine can be used as complementary medicine alongside conventional medical treatments.*

Methods and Materials: This is a summative qualitative content analysis that focused on Traditional Persian Medicine (TPM) texts from 2th to 13th AH centuries. Literature was searched during centuries 4th to 13th AH, and after described of glaucoma, extracting and classifying plants.

Results: The scholars of TPM have opinions in the field of recognizing, describing and treating eye diseases and defining practical words. In almost all books of general Persian PM, the diagnosis and treatment of eye diseases have been discussed. Glaucoma in Traditional Persian Medicine is called "black water".

Conclusion: Unlike other medical schools in the world, in addition to treating diseases, Persian medicine has a strong recommendation to prevent the occurrence of disease and also to strengthen the body's visual powers by using 6 principles of maintaining health along with herbal medicines.

Keywords: *traditional medicine, traditional Persian medicine, glaucoma, classify plants*

INTRODUCTION

The discovery of drugs in the modern era began in the nineteenth century with the separation of active compounds from plants, such as morphine, from opium [1]. Initial treatment for glaucoma relied on herbal remedies, but the method of preparation of these drugs was raw and experimental [2]. One of the first herbs used for glaucoma was pilocarpine - a parasympathetic alkaloid extracted from a South American shrub, Pilocarpusmicrophyllus [3]. Pilocarpine causes the ciliary muscles to contract, which facilitates the outflow of fluid from the trabecular meshwork into the iridocorneal angle of the eye [4]. Although pilocarpine is still available for glaucoma management, its use has declined due to the availability of modern anti-glaucoma drugs with safe side effect profiles [5]. Glaucoma is a major cause of irreversible vision disorders and blindness worldwide [6]. The current standard of glaucoma care is intraocular pressure (IOP) reduction through medical, laser, or surgical treatment [7]. However, the use of alternative

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therapies such as herbs, vitamins and minerals has become more common. Alternative therapies (methods other than conventional medical therapies) have a global market of $ 109 billion and are widely used by 52% of the general population [8,9].

In the authorities of Traditional Persian Medicine, visual impairment has been considered as "impairment of vision" and it has been defined as any impairment in the practice of seeing. In this disease, even with effort, objects can not be seen as they are and in practice, error occurs [10]. The approach of Traditional Persian Medicine in the treatment of eye diseases such as glaucoma is to protect health based on lifestyle modification and emphasizes the importance of prevention. In the field of modern therapy, although drug therapy and other invasive therapies are used to treat patients, but lifestyle modification, especially the emphasis on nutrition, has broadened the horizons of researchers in a variety of diseases, including eye diseases [11,12]. Ancient Persian physicians specialized in the diagnosis, description, and treatment of eye diseases, as well as in the definition of practical terms. Almost all books of Traditional Persian Medicine have diagnosed and treated eye diseases. In addition, several specialized ophthalmology books have been written from the perspective Traditional Persian Medicine [13]. About 25% of all medicines prescribed are made from herbs. Medicinal plants are less known for their low toxicity, effectiveness and side effects. A wide range of herbs have been found to be effective in treating eye diseases such as cataracts and glaucoma in complementary medicine schools, such as Ayurveda and Chinese medicine [14]. Studies have shown that the probability of discovering a new effective drug using traditional medical experiments increases by up to 40% versus random examination of drugs experimentally.

Therefore, searching for traditional reference books may be an effective way to find new drugs [15]. The purpose of this study is to provide a classified list of medicinal plants mentioned in Persian traditional medicine sources that after clinical studies can be used to enhance vision or treat poor vision.

It is worth noting that the vast majority of herbs in this study have not been used in clinical trial studies, so readers should refrain from any arbitrary use of these compounds if they do not have access to a competent physician.

METHODS AND MATERIALS

This research is based on the medicinal plants mentioned in the books Traditional Persian Medicine that somehow affect the improvement of visual function. As described, the purpose of this study is not to express the complete cure of glaucoma, but this article seeks to classify plants that from the perspective of Persian Traditional Persian Medicine can be used as complementary medicine alongside conventional medical treatments. Thus, from the books of the truths of medicine, the gift of the believers (Momen Tonekaboni), was used. The keywords "Ein", "Basar", "Basra" and "Magnifying" were used to evaluate the plants effective in increasing visual acuity and glaucoma. In such a way that any plant that has more than one of the above keywords, was selected as a plant that affects the visual status and increase the power of vision. The results of the studies can be seen briefly in Table 1.

Methods

This article is a summative qualitative content analysis that processed in seven classic steps:

The research questions to be answered: “What is glaucoma in the Persian medicine texts, we don’t find any disease that called as “Glaucoma”; To find the name of “glaucoma” disease in the context of Persian medicine, we tried to collect all data’s about glaucoma in the context of up to date modern medical texts; This information includes etiology, symptoms, signs, risk factor and treatment of glaucoma. And then we tried to find one disease have most overlap with glaucoma. As a result, glaucoma in Persian Medicine texts is known as “Ab e siyah”or black water” [1].

1. Selecting the sample to be analyzed: In this study, all of available Persian Medicine books and texts. These texts included manuscripts (the book itself or its electronic file) in different centuries from 11 to 20 A.D. we studied and searched historical books are in Table 1.

In the second step, the databases were searched by using key words of “glaucoma’s etiology, prevention and treatment” in PubMed, PMC, Google Scholar and Scopus.

2. To identify explicit versus euphemistic terms: Word frequency counts for each identified term calculated with source, to contextualize the codes. Modern equivalent terms were identified.

3. Coding process: three titles: definition and Name (Nam e Bīmārī); ethology (Asbāb va ʿElal); And treatment (Darmān). According to Persian Medicine text, we coded and categorized all data under these titles. We tried to translate these finding from Arabic & Ancient Persian to modern medicine language [1].

4. Defining the categories: All data was classified in three categories: definition, etiology and treatment of “Ab e siyah” [1].

5. Trustworthiness: Credibility, conformability & dependability: Given the explanation of different stage of methods such as key words, codding process, and etc. Anyone who has the ability to read and understand Arabic & Ancient Persian language & texts, can derive similar results.

6. Analyzing the result [26,27]: According to summative qualitative content analysis and given the skill of the authors of this article, we tried to collect all data about the prevention and treatment of glaucoma and introduced several plants as one of complementary treatment for effective treatment for glaucoma.

Table 1: Historical books which are used

Author	Title of the Book	Date Written	Subject
Ibn Sina	Book of law		
Ibn Nafis Gharshi	al-Shamil fi al-Sinaatah al-Tabiyyah		
Ali Ibn Hussein Ansari Shirazi	The authority of badi'i		
Mohammad Hussein Khorasani	The reservoir of medicine		
Ḥakīm Muʾmin M.	Tuḥfat al Muʾminīn	17th century	Traditional Medicine and Pharmacology
Razī M/Rhazes/Rasis	Al Ḥāvī fi al Ṭibb	10th century	Medicine
ʾAhwāzī A./Haly Abbas	Complete book of the medical art (Kāmil al-Ṣināʿa al-Ṭibbīya or Royal Medicine)	10th century	Medicine
Bīrūnī M.	Al Ṣaydana	11th century	Pharmacology
Avicenna/Ibn Sīnā H	Canon on Medicine (Al Qānūn fi al Ṭibb)	11th century	Medicine
Ğurğānī SI.	Ḏaḫīra ḫārazmšāhīi & Al ʿAġrāḍ al-Ṭibbīya wa al mabāhiṯ al ʿAlāīya	12th century	Medicine
Ibn Bayṭār	Tafsīr-u Kitāb discoridus	13th century	Medicinal Herbs

RESULTS

The scholars of traditional medicine have opinions in the field of recognizing, describing and treating eye diseases and defining practical words. In almost all books of general Persian medicine, the diagnosis and treatment of eye diseases have been discussed. Glaucoma Traditional Persian Medicine is called black water. This disease occurs in the moisture of the testicles (clear fluid), the causes of which are not out of two cases; It is either in quantity or in quality of testicular moisture [16]. This disease, ie glaucoma, has not been given special attention in Persian medicine textbooks and has been treated more under the title of eye diseases, and some reports have been recorded from them. Identified a specific disease as glaucoma; However, according to the anatomy mentioned in Persian books and the study of eye diseases in Persian medicine sources, it seems that glaucoma may be one of the diseases of testicular moisture. In Akbari Medicine, in the discussion of eye diseases, Volume 1, in the topic of testicular moisture, there are topics that seem to be compatible with glaucoma. This moisture is considered similar to the whiteness of the testicles in terms of consistency, purity and color, and the benefit of this moisture in front of the glide (eye lens) is that the strong lights are gradually placed on the glide and because of this, the glide is irritated and constipated. Preserved [17]. One of the diseases of this moisture is considered to be its large volume and it continues that if it was a small increase due to the lack of transparency that makes the majority of the necessary components, it would fail to adapt the ghosts to Jalidiyah and the radius would reach the natural duct. The volume should be more than that, in which case, the insight shakes itself, imagining in front of itself that the sea is standing and in the state of full stomach and back sleep, weakness in insight prevails and also when hunger in the middle of the day is not so weak. The patient sees the unlikely things better than the near objects, and the mutation is that the soul becomes dirty due to the abundance of this moisture, and because they do not move farther, its concentration is softened and its consistency is not moderated. And effort) can not be seen; Because in Absar, the relative sensations of the soul do not move so much that its concentration becomes soft [17].

Remedy

The first purification of the body should be done in the kitchen of Sajj and then with the purification of Sarhabn[17]. The body and the nose [18,19]. In removing the blockage and reducing the moisture of the testicles, drugs containing patience and halilah are used in the sources of traditional medicine [16]. In general, the symptoms mentioned in the books of Persian medicine, testicular hyperhidrosis seems to be consistent with the symptoms of chronic glaucoma. Visual impairment in different forms that exists in chronic glaucoma, in the increase of testicular moisture, which is the moisture between the cornea and the lens, these symptoms are also present in Persian books.

Elliptical moisture

From the point of view of traditional medicine, aqueous humor is behind the iris and anterior arachnoid. Aqueous humor has several roles, which is to protect the lens, to protect it from both natural internal heat and heat from strong light coming from outside, and to be a barrier between the lens and the iris so that the two do not intersect [18,20-22].

A. Quantity of testicular moisture

It should be less or more and smaller or bigger, and whenever it is more or greater than the natural amount, it should be veiled between the moisture of the jelly and between the outside light. His sign is that hunger and austerity are beneficial, and in the heat, it is better for half a day, and in the morning and evening it is worse, and the heat is harmful, and whenever it is less or less, it is due to the deterioration of the testicles. Analysis is repelled and dryness prevails. His sign is that the blackness of the eyes becomes smaller and is worse when he is hungry and in the heat for half a day, and mathematics is harmful, and baths and more food are increasingly beneficial [18].

B: Quality of testicular moisture

What is in quality or is a plague in consistency or is in decline in Loon or plague in wetness and dryness and what is in consistency is such that some moderate consistency is a little thicker and God cannot see this cause as far as he is and What is close, as it should be, cannot be seen by the good, and some of the temperate textures turn a lot and become hard and thick, and this is of two kinds, or some of its components become hard, or all of it, and if all of it becomes hard, it thickens the vision. And his ruling is the ruling of water is the same, and if some of his components are concentrated, it will reduce vision [18].

Maintaining health (maintaining health) in the eyes

A study in the sources of Iranian Persian medicine shows that the sages believed that the prevalence of eye diseases in winter is lower than in other seasons. In summer, due to the frequency of ascents, and in Rabi, due to the flow of substances, and in the opponent, due to the corruption of substances, it is more, and it is difficult to diagnose eye diseases, whether it is in the layers or moisture of the eyes, or in the nerves or partners. The temperament of the eye is a function of the temperament of the brain, on the other hand, because the eye has many arteries, most of it is temperate; because it requires a lot of movements and movements of the solution, and it is not necessary to make everyone's temperament hot. In traditional medicine for the treatment of eye diseases, the three principles of causal action, strengthening all the powers of the eye and purifying the eye from wastes have been mentioned as general rules. Eye diseases are some external, such as remedies, and some esoteric ones are easily defined, such as the state of the soul of Basra in its abundance and tenderness, and the like, and some esoteric ones are defined as pests that occur in moisture, and some esoteric ones are estimated, such as pests in the inner classes or The optic nerve is caused by mood swings and the like, eye diseases. Constipation and violence need to be corrected with violets and the addition of a little bit of sage. Iarj fiqra removes substances from the head if it is concentrated. The important point is that most eye diseases are caused by the organ of participation and it must be the members of the head, and most of this is when it occurs.

Harms of the eye

Lots of things may harm the eyes: dust and smoke, very hot and cold weather, wind, toxins, all kinds of things like too much sex, too much salt, long-sightedness towards shiny and bright things, excessive white like lightning and bright white, sun, snow, durability One thing, the setting of precise lines to extremes, too much incarceration, the frequency of crying, the intense sadness, the delusions and the rage, the study between evening and dusk, all the good news like garlic, beans, beans, onions, lentils, eggplant, mustard and blackberries, multiplicity strong laxatives, including abdominal astringents. But diarrhea is sometimes beneficial to drinking medicine and sometimes to congestion, and this is more beneficial in ingesting substances, and injections are beneficial to all material diseases of the eye, and the most agreeable laxatives in eye diseases are Halilah; Because it strengthens the stomach and prevents the excretion of substances, but due to the treatment of the eyes in a way before purification is not permissible [23]. In the book of Akbari medicine, he writes: The cure for eye diseases is of four genders, one is to change the simple temperament, the second is to purify the substance, the third is to manage the division of the joints, and the fourth is to correct the heat of the eye and reduce it. There are seven ways to make the eye vomit. Second, if the body is smooth, first clean the body from bad sputum. Thirdly, it is useful to clean the nose with meningeal sprays and to gather in the jugular vein of the veins that are inside the head. The use of sneezes has an effect on enemaing the eyes, but they should not cleanse the body until the substance is applied, which should not be done. It is harmful. The purity of the blood vessels of the eye is also conditioned by the priority of purification, so that the harm is safe and there is no harm where the body is clean. Among the drugs used in Persian medicine to empty the feces of the head, it seems that the drug of patience is a suitable drug to achieve the above goal. Other drugs include Halilah, which is frequently mentioned in the sources. Herbal medicine is the oldest form of treatment, known to mankind and has been used for a long time. The use of this method of treatment has a history in all civilizations and is an important component in the development of common medical science. The important point in all eye diseases mentioned in the sources is that the elimination of feces and its emptying and softening of nature are the general principles of treatment in the majority, and for this purpose, in most of the above cases, the love of patience and reason has been suggested.

Table 2: Plants identified from sources and references, in order from right to left, including Persian name, scientific name of the plant, method of consumption and references

Used reviews	How to use	Temperament	Scientific name of the plant	Persian name of the plant
[24-29]	Food	Warm and dry	Thymus capitatus	Thumon
[24-29]	It's my head.	Warm and dry	Peganumharmala L.	Espand
[24-29]	It's my head.	Warm and dry	Commiphoragileadensis	Arabic Bilal
[24-29]	Eye drops	Warm and dry	Marrubium vulgare L.	Mountain mess
[24,25, 27-29]	Food	Warm and dry	Rutagraveolens L.	Ruta graveolens
[24-29]	It's my head.	Warm and dry	Foeniculum vulgare L.	Foeniculum vulgare
[24-27, 29]	Food	Warm and dry	Allium cepa L.	Piyaaz
[25-29]	Food	Cold and dry	Terminalia bellerica	Belleric myrobalan
[26-29]	It's my head.	Warm and dry	Ferula assa-foetida L.	Ferula assa-foetida
[25-29]	It's my head.	Cold and dry	Solanum nigrum L.	Solanum nigrum
[24-29]	It's my head.	Warm and dry	Cinnamomumzeylanicum	Cinnamon
[25-29]	It's my head.	Cold and dry	Rheum ribes L	Rheum ribes
[25-29]	It's my head.	Warm and dry	Calotropisproceradryand	Calotropis procera
[24, 26-29]	It's my head.	Warm and dry	Aloe vera L	Aloe vera
[24-27]	It's my head.	Warm and dry	Zingiberofficinaleroscore	Zingiber officinale

As can be seen from the plants mentioned in Table 1, the vast majority of plants affecting the visual acuity and treatment of eye diseases had a dry temperament and most of them were hot and dry. Most of the plants are extracted from the medicine repository and the least of them are extracted from the medicinal plants and facts. It seems that according to the texts of medicine books, most plants were effective in strengthening vision and a few were effective in visual impairment. Meanwhile, most herbs have been shown to be effective in treating cataracts compared to other ocular disorders such as glaucoma. The method of consumption for most plants, like the common method of medicine for the treatment of eye diseases, is kohlrabi, but in the meantime, oral methods are also found to a lesser extent. Aqueous extract of fennel seed (Foeniculum vulgare) has protective and therapeutic properties against cataracts caused by sodium selenite in rabbit eyes. The possible mechanism of this extract is antioxidant activity. Various phytochemical studies have shown the presence of compounds such as flavonoids, phenolic compounds and volatile compounds in this plant. It has several other medicinal properties such as anti-inflammatory and anti-hypertensive effects. Lens opacity was lower in rabbits that received eye drops of aqueous extract of this plant twice a day for 5 days before cataract induction and 21 days after cataract induction [30]. Considering the antioxidant role of this drug in the treatment of cataracts, it seems that it can play a similar role in relieving the symptoms of glaucoma. In addition to its antioxidant properties, this plant also seems to be effective in reducing intraocular pressure. And can be used specifically to treat glaucoma. Another study by Agarwal et al. Showed that aqueous extract of fennel seed reduced intraocular pressure and its maximum pressure reduction was comparable to that of thymolol. The mechanism of action is through the effect of anti-cholinesterase, so its anti-glaucoma property is proven [31]. In the following clinical trial results, we will present several studies that prove the antioxidant properties of certain plants, since the herbal treatment of eye diseases such as glaucoma and cataracts is jointly based on its antioxidant properties, so the result can be It is likely that these drugs, which are effective against cataracts, will also improve eye conditions in glaucoma. Javadzadeh et al. Stated that administration of 1 drop of onion extract in the eyes of mice for 14 days every 8 hours prevents the formation of cataracts caused by selenite [32]. In 2010, a study by Gupta et al. On 9-day-old mouse puppies showed that intraperitoneal injection of aqueous extracts of Trifala (Emblica officinalis), Terminalia Bellerica and Terminaliachebula at 25 mg/d. Each kilogram of body weight prevents the formation of cataracts caused by selenite and the formation of selenite. In addition, the activity of antioxidant enzymes increases glutathione and reduces the amount of lipid peroxide in contrast to selenite-induced oxidative stress [33]. Another case-control study has shown the role of oxidative stress as the main mechanism of eye damage in cataracts, glaucoma and ARMD, which shows the role of plasma antioxidant capacity in the prevention of these eye diseases. Plasma antioxidant capacity is increased by foods rich in antioxidants such as fruits and vegetables [34]. Despite extensive research, there is insufficient clinical trial evidence of the effectiveness of all plants on visual impairment extracted from sources, but the antioxidant properties of many of these plants indicate their potency in treating or enhancing vision impairment. These include Aloe vera (Solanum nigrum) [35], Peganum Harmala [36], Zingiber officinalis [37], Ferula assafoetida [38], Rheum ribes [39] and Belila (Terminalia bellerica) [40].

DISCUSSION

Visual disturbances generally occur for a variety of reasons, including disorders of the interpretive part of the vision in the brain, disorders of the eyeball such as the layers of the eye (choroid, retina, and sclera), and eventually disorders of the optic nerves. In glaucoma or glaucoma, the disorder occurs due to increased pressure inside the eyeball for a variety of reasons, of which there are many types. Doctors of IranianPersian medicine attribute the causes of all diseases to not paying attention to the 6 principles of health (climatic and environmental conditions, food and drink, physical activity and rest, excretion status, mental condition and sleep and wakefulness). For this reason, they first try to treat the disease by regulating the above conditions, and then turn to the use of drugs and invasive measures such as surgery. In other words, Iranian Persian physicians believed that in the early stages of the disease, the disease can be controlled by maintaining and regulating the six principles of health, and only in advanced conditions of the disease should aggressive measures be taken. For example, in the treatment of cataracts (descent of water), Persian physicians initially sought to treat the disease with nutrition and herbs, and only in advanced conditions did they perform aggressive measures such as surgery. Also in glaucoma and cataracts, they advise not to eat meat, fish and fruits and also recommend to use dry foods [41]. Regarding the recommendation of medicine not to rush into initiating aggressive measures, Ibn Sina's law states that one should not rush to start cataract surgery and should wait until its sufficient progress [42]. In general, the opinion of Persian medicine in the field of eye diseases such as glaucoma is that some environmental conditions such as dust, very hot or very cold weather, smoke and looking at small objects too much to do too much Activities such as crying and sexual intercourse, eating too much salt, lentils, cabbage, garlic, sugar and alcohol eventually cause these diseases. On the other hand, it is highly recommended to eat foods such as saffron and turnips to improve eyesight [41]. The general mechanism of eye damage in diseases such as cataracts, glaucoma and age-related retinal detachment (ARMD), from the perspective of biochemistry, is oxidative stress. Oxidative stress leads to the formation of free radicals that can cause severe damage to the ocular and lens layers. Therefore, in the treatment and prevention of these diseases, the best way is to use antioxidants as complementary medicine. Table 1, which is mentioned in the results section, shows a group of plants with strong antioxidant properties, which seems to be the recommendation of Persian physicians to use these substances in the treatment of eye diseases [43]. Although some medical schools, such as traditional Chinese medicine, seek to treat glaucoma with the use of herbal medicines, it seems that the main recommendation of Persian medicine is to regulate the six conditions of maintaining health as well as the use of herbal medicines. In the form of kohl, drops and even oral, seeks to prevent the disease and increase the body's resistance to the disease. In fact, in the treatment of glaucoma, the herbal medicines mentioned in traditional medicine can be used as a complementary treatment to the main treatment, because in the acute stages of the disease, the use of invasive treatments such as surgery instead of herbal medicines, it seems inevitable.

CONCLUSIONS

Unlike other medical schools in the world, in addition to treating diseases, Persian medicine has a strong recommendation to prevent the occurrence of disease and also to strengthen the body's visual powers by using 6 principles of maintaining health along with herbal medicines. Finally, it is inferred from this study that despite the many benefits of using herbal medicines mentioned in Persian medicine books, but due to the lack of sufficient clinical evidence, until the effectiveness of these herbal medicines is proven, it is better to use them. Complementary therapy should be used alongside the main treatment (modern medicine). Despite the mention of numerous herbs for the treatment of eye diseases such as glaucoma and cataracts, as well as the strengthening of eyesight, but it seems because so far no proper clinical trials have been conducted in the field of trying these herbal medicines. This rich capacity of Persian medical experiences has been neglected. Therefore, it is recommended that Iranian Persian medical researchers in their future studies diligently test plant compounds using appropriate and valid research methods for the treatment of eye diseases.

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Conflict of interest

The authors declare that they are no conflicts of interest.

Compliance with Ethical Standards

This article does not contain any studies with human participants performed by any of the authors.

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ORIGINAL ARTICLES

New pathophysiological aspects in chronic venous disease

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Abstract: *Chronic venous disease has been pathology of great interest over time. With its large prevalence among the world population (10-33% adult woman and 10-20% of men) through profound socio-professional implications that affect the quality of life of patients, chronic venous disease is a real public health problem, being an important cause of morbidity and disability, a permanent area of research in the medical-pharmaceutical field. Special attention is now given to identifying the mechanisms and stages of disease development. From studies that have highlighted the importance of venous hypertension as an essential element in the development of the disease, to this day it has come up to demonstrating the fundamental role of the venous endothelium and glycocalyx in triggering and progressing of chronic venous disease. Biochemical, immunohistochemical, and functional investigations have allowed the evaluation of changes that occur in cellular metabolism and evidence of some immune-vascular factors responsible for vascular alterations in chronic venous disease: specialized receptors, adhesion molecules, cytokines, matrix and metalloproteinases.*

The analysis of the pathophysiology of chronic venous disease is still a vast area open to scientific research.

Keywords: *chronic venous disease, venous insufficiency, endothelium, glycocallix, matrix metalloproteinases*

INTRODUCTION

Chronic venous disease (CVD) is being defined as a long term decompensation of the venous circulation, with the occurrence of venectasia, reticular veins and hydrostatic varicose (secondary to venous stasis), stasis in the lymphatic system and various skin changes: vesperal or permanent edema, eczematous dermoepidermitis (stasis chronic eczema), pigmented purpuric dermatitis, dermohypodermitis with sclerous development (lipodermatosclerosis), Milian's white atrophy and leg chronic ulcer. [1]

The first information about CVD was suggested by prehistoric drawings and paintings, papyrus writings and hieroglyphic and cuneiform descriptions engraved in stone. Chronic venous hypertension (VH) and chronic venous insufficiency (CVI) may be secondary to venous pump failure due to the reflux of the varicosities of the superficial venous network or secondary to prior thrombosis of the veins (incompetence and/ or obstruction due to poor reperfusion of deep vein branches). Deep venous disease is associated thromboembolism. Rarely, CVI is due to primitive valvular insufficiency. VH will lead to permanent dilatation of the dermal veins visible through the epidermis. VH is responsible for capillary distention with extravasation of plasma, red blood cells, fibrinogen, α2-macroglobulin and other macromolecules, perivascular diapedesis of leukocytes, into the interstitium. Absorption capacity of the lymphatic vessels is exceeded resulting edema and chronic inflammatory changes. Edema compresses and deforms capillaries with microcirculation disorders. Fibrin deposits form sleeves around the capillaries that block nutritional changes. The slowing of blood flow in the capillaries favors the aggregation of the red blood cells. Small thrombus can obstruct the capillary lumen and leukocytes adhere to the vessel endothelium. The macromolecules trap growth and repair factors so that minor traumatic wounds cannot be

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repaired and an ulcer develops. The activation of leukocytes release free radicals, proteolytic enzymes, cytokines (TNFα), leukotrienes, matrix and metalloproteinases responsible for capillary hyperpermeability and capillary and tissue injury.

ENDOTHELIUM AND GLYCOCALLIX

Endothelium is a key element in regulating vascular tonus, hemostasis and coagulation. Due to its structure the endothelial glycolalysis prevents leukocyte adhesion, inflammation and thrombosis. [2] Secondary to dilatation, increased blood flow and venous pressure, the endothelial function is negatively affected in CVI.

Through specific receptors the endothelium and the glycocallix detect the changes produced by the shear stress, with the stimulation of the chemoattractants (MCP-1, VCAM-1, ICAM-1) and nitric oxide activation, amplifying the inflammatory response with subsequent release of inflammatory mediators (chemokines, cytokines, growth factors and metalloproteinases), attraction of leukocytes (macrophages, monocytes, T lymphocytes) and their attachment and transmigration in venous and interstitial walls, inducing cutaneous modifications specific for CVI. [2,3] The changes in venous pressure and wall tension are perceived by the endothelial cells through ICAM-1 molecules (differentiation cluster 54-CD54-) that have an increased expression in patients with CVI. ICAM-1 molecules have a specific role in activation, adhesion of endothelial cells, leucocyte's diapedesis and transmigration and through transient receptor potential vanilloid channels (TRPVs). [4] They induce disruptions in the production of NO–, the release of vasoactive substances, the extravasation of macromolecules and erythrocytes that degrades in fibrin and hemosiderin with leucocyte activation, in the expression of MCP-1, MIP-1 and vascular cell adhesion molecule-1 (VCAM-1, CD-106) and in the expression and release of L-selectin and E-selectin. [5]

The adherence and transmigration of leukocytes in venous valves and venous walls triggers an inflammatory cascade, stimulating the production of tumor growth factor-β1 (TGF-β1) by the activated endothelial cells and smooth muscle cells [6] and the production of TNF-α and interleukin-1 (IL-1) factor with subsequent increased expression of matrix metalloproteinases (MMPs). [4,7,8]

VH determines the stretch of the venous wall and an increased venous wall tension, followed by an increased expression of hypoxia inducing factors (HIF-α1, HIF-α2) that further on induces an increase in the level of MMP-2 and MMP-9 in order to relax the venous wall, with the consequently development of the venous dilatations. [2]

THE ROLE OF MATRIX METALLOPROTEINASES, THEIR TISSUE INHIBITORS AND CYTOKINES

The mechanisms involved in the development of venous ulcer (VU) and the factors that intervene in the slowhealing process are not fully elucidated. The healing of chronic ulcers is a complex process involving inflammation, reepithelization, matrix deposits and tissue remodeling. These processes are controlled by MMPs and their tissue inhibitors (TIMP). In the case of VU, MMPs, zinc-binding endopeptidases, are synthesized by local proliferating fibroblasts, their activity being inversely proportional to the degree of healing. Generally, MMPs and TIMP are temporarily induced in response to exogenous signals, such as different proteases, cytokines or growth factors, cell-matrix interactions and modified cell contacts. Gelatinases MMP2 and MMP-9, as well as TIMP-1 appear to be elevated in exudates of VU compared to acute wounds. [6,9]

However, biopsy sample analyzes have shown variable results. MMP-1, MMP-2 and TIMP-1 levels were elevated in patients with lipodermatosclerosis compared to normal skin. [10] In a later investigation, biopsies from patients with VU were found to have elevated levels of active MMP-2 compared to normal skin.

MMPs production and their function is regulated by cytokines, urokinase plasminogen activator (uPa), extracellular matrix metalloproteinase inducer (EMMPRIN-CD147), AA platelet growth factor (PDGF-AA) and mitogen-activating protein kinases (MAPK). [11,12] Excessive MMP activity in the tissue is controlled by their endogenous inhibitors, tissue metabolic inhibitors (TIMP). In UV matrix metalloproteinase synthesis outweighs the effect of their tissue inhibitors, the imbalance resulting in the degradation of elastin and collagen. [13]

At the level of VU fibroblasts, MAPK pathways over-express p38, a negative modulator of cell proliferation, stopping the healing.

VH induces MMP activators which increase MMP expression and activity, extracellular matrix (ECM) degradation, relaxation of venous smooth muscle (VSM) and venous dilatation. [14,15] Inflammation of the venous wall causes additional increase in MMP levels, vein dilatation and valve dysfunction. [16] The result consists in remodeling and fibrosis of the venous wall causing progressive venous dilation and varicose vein formation. [14] The ECM composition is dependent on MMP and can affect the venous wall structure. In CVI there is an imbalance of the venous wall collagen types. The type III collagen responsible for tissue distension is low, whereas type I collagen – which determines rigidity – is high, leading to a reduction in the elasticity of the varicose veins. [16]

Imbalance between MMPs and TIMP activity increases MMP tisular levels resulting in degradation of elastin and collagen [13] with pathological changes of the venous wall and installation of CVI. Thus hypertrophic and atrophic vein segments develop in the varicose veins and damage the venous valves. The remodeling of the venous wall and abnormal venous distention restrains the valve's closure and therefore initiate the venous reflux. [6]

MMP-2 (gelatinase A) may induce venous relaxation or inhibition of venous contraction [17], affecting ECM remodeling in vascular structures and playing an important role in the development of the VU. Healing of VU is impaired in patients with high levels of MMP-2 and low levels of TIMP-2 in the ulcer's exudate. [16]

MMP-9 (gelatinase B) is considered to be the major protease in ECM degradation in chronic wounds. MMP-9 levels are much higher in cases of varicose veins and VU. [18] The MMP-2 and MMP-9 are up-regulated in experimentally induced VH, [19] and MMP-9 has been shown to influence remodeling of the vein by modulating the collagen content of the vein wall, thus contributing to inflammation and fibrosis. [16]

MMP-12 (macrophage metallomelastase) allows macrophage migration by degradation of several ECM components. MMP-12 can block angiogenesis, acting as a potent inhibitor of angiogenesis. MMP-12 concentration is increased in VU's exudate in the inflammatory phase compared to the granulation phase. [20]

In addition, MMP extracellular inducer (EMMPRIN) and membrane metalloproteinases type 1 and 2 (MT1-MMP and MT2-MMP) were detected in the dermal and perivascular regions of VU. [21] There are no differences in overall MMP-1, 2, 9 and TIMP-1 protein levels in patients with CEAP (Clinical, Etiology, Anatomical, Pathophysiology) class C2 to C6 CVD compared to normal controls. [6] However, in C6 CEAP class level, MMP-2 levels were increased compared to MMP-1, 9 and TIMP1 in patients with C4 and C5 CEAP class disease.

These data indicate that active tissue remodeling occurs in CVD patients. It is currently unclear which MMPs are involved and how are they activated and regulated. It appears that MMP-2 may be activated by uPa.

mRNA, uPa and urokinase plasminogen activator receptor have increased levels of in VU compared to normal skin. Elevated levels of active TGFβ-1 in the dermis of CVI patients suggest a regulatory role for TGFβ-1 in the synthesis and activity of MMP and TIMP. [6,22]

The regulatory role of TGFβ-1 in the synthesis and activity of MMP and TIMP is supposed by TGFβ-1 increased levels in the dermis of CVI patient as well as during the healing process of VU. TGFβ-1 is secreted by interstitial leukocytes and binds to dermal fibroblasts and ECM proteins, also increases with the severity of the CVD, being a risk factor. [23] The cytokines interfere with CVI pathophysiology in its various stages starting with inflammation. In the untreated VU there are elevated levels of pro-inflammatory cytokines (IL, TNF-α, IFN-γ).

Along with VH and inflammation, the cytokines interfere in post-capillary venules where they form asheath which includes collagen I and III, fibronectin, vitronectin, laminin, tenascin, fibrin, TGF-β1 and α2-macroglobulin. [7] Although the causes and mechanisms of fibrin sheath production are not fully elucidated, chemical and structural changes from dermal microcirculation will lead to the progressive installation of cutaneous trophic disorders and ulceration. [2,24- 26]

Red blood cell degradation products and interstitial protein extravasation are potent chemoattractants that represent the initial inflammatory signal at the base of responsibility for the recruitment of leukocytes. These cytochemical events are responsible for increasing intracellular adhesion molecule-1 (ICAM-1) expression on endothelial cells of microcirculatory vessels observed in dermal biopsies in patients with CVD.

ICAM-1 is the activation-dependent adhesion molecule used by macrophages, lymphocytes and mast cells for diapedesis. α and β platelet-derived growth factor receptors (PDGFR-α and PDGFR-β) and vascular endothelial growth factor (VEGF) have increased levels in CVD patients dermis. They are regulating upward adhesion molecules leading to leucocyte recruitment in CVD. [6]

CONCLUSIONS

Along with VH (key element), endothelium and glycocallix, MMPs and cytokines play an important role in the CVD pathophysiology. Understanding the physopathological mechanisms involved in triggering and evolving CVD, knowing the aetiological factors and the facilitating factors are an essential stage in the management of the disease, allowing the development of a complete therapeutic plan adapted to each patient.

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ORIGINAL ARTICLE

Open treatment options in massive rotator cuff tears associated with acromion non-union

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Abstract: *We evaluated the available English literature for cases of acromion non-union and rotator cuff tear, looking at surgical methods of treatment as well as at functional results. As expected, the literature regarding rotator cuff pathology was found to be abundant, and a good number of articles were found regarding acromion non-union, but articles concerning the association of the two were scarce. Treatment recommendations varied, and were made on a case-to-case fashion. The accepted treatment methods for acromion non-union consist of non-surgical management, open or arthroscopic fragment removal, open or arthroscopic reduction and fixation (bone grafting may be used), while the rotator cuff witll be reinserted with anchors. In this rare occurence, a large rotator cuff tear with retracted tendons combined with an acromion non-union are usually repaired by an open technique in an one-stage surgery, with good results. In order to chose the right treatment option scapular kinematics must be taken into consideration especially when acromial fractures are associated with rotator cuff tears.*

Keywords: *shoulder, rotator cuff tear, acromion, non-union*

INTRODUCTION

Fractures of the scapula range from 0.4 to 1% of all fractures, with acromion fractures representing 7% of all scapular fractures [1]. Usually though acromial fractures are rarely reported due to their low incidence [2]. Lesions associated with acromial fractures such as rotator cuff tear are even more rarely described [3]. An MRI performed for a rotator cuff lesion (Figure 1) may show an associated acromial fracture, whereas a CT performed to describe an acromial fracture/non-union will not show a rotator cuff tear [4].

Treatment methods

A direct consequence of low acromial fracture incidence is the fact that there is no clear consensus on repair options resulting, more often than not, in surgeons choosing treatment options which they have the most experience withat the same time without overseeing patients'demand levels [5,6]. There are many reported methods for acromial fracture fixation a variety of them being presented in the literature such as tension band wiring, screw and plate fixation techniques, Kirschner wire fixation and in each of them patient specific factors and associated lesions are always taken into consideration when choosing a treatment option [7-11].

Figure 1: T2W_TSE coronal MRI image showing a massive tear in the supraspinatus tendon (arrow). This image is from the author's personal database

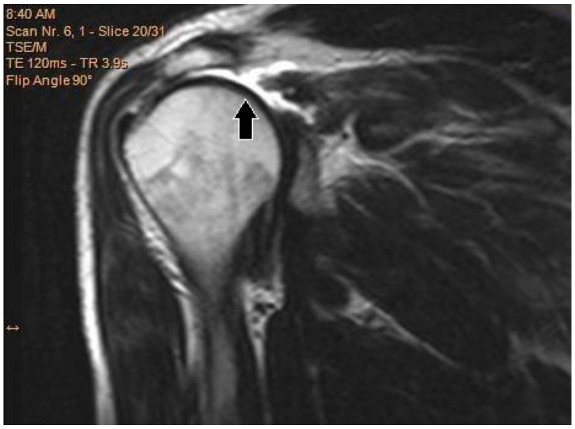

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The surgical treatment has several complications, mostly locoregional, ranging from hematoma and infection to persistent pain irradiating to the distal arm and forearm, posing a problem especially in patients with associated ipsilateral medical conditions of the forearm or of the arm [12-14]. Letting surgical treatment aside there are at least two more important factors that need be considered when dealing with an acromial fracture and these are the complex3D anatomy of the acromion and the so called superior shoulder suspensory complex (SSSC) which is an important factor in the aftercare postoperative prognosis of acromial fractures [2,8,15-17]. The SSSC is not the only factor to consider, as a system of bursae and the deltoid muscle allows for the possibility of pain irradiating in the distal arm and forearm, making for a difficult differential diagnosis [18].

Figure 2: Intraoperative image showing the non union fragment of acromion being held with a Kocher clamp. In the secondary plane of view we can see the torn supraspinatus tendon and the humeral head. This image is from the author's personal database.

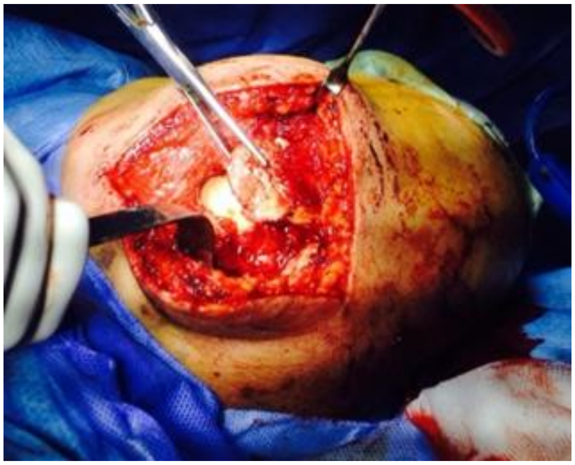

Figure 3: Intraoperative image showing the torn supraspinatus tendon being pulled into place with traction sutures, before reinsertion. This image is from the author's personal database.

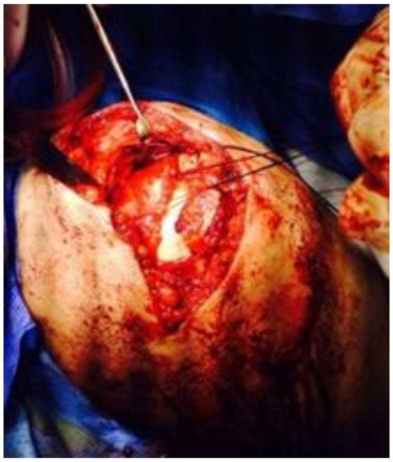

The complex 3D anatomy of the acromion makes it hard to fit any available plate system, most acromial plates requiring intraoperative bending in order to fit the required anatomy without excessive soft-tissue debridement while at the same time trying to maintain rigid fixation in an area of multiple pull vectors mainly arising from the deltoid and trapezius muscles [19-21]. When considering the complete management of acromial fracture non-union associated with rotator cuff tear (Figures 2, 3) one must take into consideration complex subjects like shoulder force vectors and SSSC implications in patient expected outcome.

DISCUSSIONS

As expected, the literature regarding rotator cuff pathology was found to be abundant, and a good number of articles were found regarding acromion non-union, but articles concerning the association of the two were scarce. Treatment recommendations varied, and were made on a case-to-case fashion.

The non-unions of the acromion can be treated in a variety of ways. In their 2006 article, Lumbreras et al describe the case of 56 year old male patient treated surgically with plating andautologus iliac crest bone grafting, with excellent clinical and radiologic results [23]. Liodakis et al report using the same method, but with an adapted distal radius plate; they offer a long follow-up of two years with measurable improvement in both range of motion as well as in the SSRS (Subjective Shoulder Rating System) Score [24].

Os acromiale

A differential diagnosis can be made with os acromiale, an unusual anatomic variant usually discovered incidentally [25]. In their 2016 article, Garcia et al define the os acromiale as a failure in the fusion of an accessory ossification nucleus of the acromion, and propose an arthroscopic method of fixation with cannulated screws, as opposed to the open technique [26]. Hurst claims an 8% incidence rate in his 2019 article, and advises for the careful diagnosis of this variant in all of the patients that are about to undergo shoulder surgery [27].

The accepted treatment methods consist of non-surgical management, open or arthroscopic fragment removal, open or arthroscopic reduction and fixation (bone grafting may be used) and arthroscopic subacromial decompression [28]. Failure to recognize an incidence of os acromiale may lead to confusion with an acromial non-union, as the imaging may be similar, but the patient's history and previous investigations may help with the differential diagnosis.

When looking for os acromiale, MRI findings on axial images generally report a relatively triangular unfused ossicle with an almost transverse interface with the acromion and irregular margins, with interface edema and marrow edema of the opposite bony structures [29]. At the same time, acromial non-unions possess similar characteristics on MRI (Figure 4), but key finding during history taking are crucial for diagnostic orientation, for example the lack of patient-reported shoulder trauma or bone disease like osteoporosis may incline the diagnosis towards os acromiale rather than acromial non-union [30]. In a symptomatic patient it is our opinion that the differential diagnosis has a theoretical purpose, as the treatment methods are similar.

CONCLUSION

In this rare occurence, a large rotator cuff tear with retracted tendons combined with an acromion non-union are usually repaired by an open technique in an one-stage surgery, with good results. In order to chose the right treatment option scapular kinematics must be taken into consideration especially when acromial fractures are associated with rotator cuff tears.

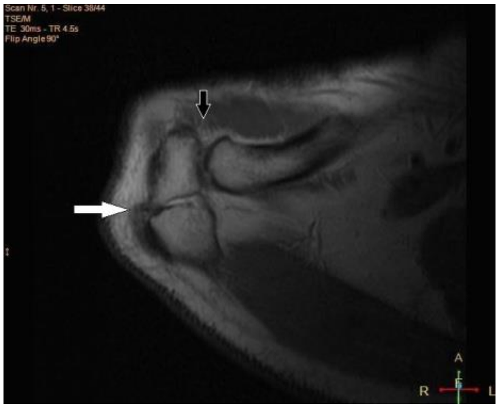

Figure 4: PDW_aTSE axial MRI image showing acromial non union (white arrow), easily confused with os acromiale. The acromioclavicular joint is indicated by the black arrow. This image is from the author's personal database

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ORIGINAL ARTICLE

Outcomes of frozen embryo transfer in patients with and without ovarian hyperstimulation syndrome

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Abstract: *Introduction: One of the methods utilized to treat infertility is the use of frozen embryos. This technique is particularly employed in patients with ovarian hyperstimulation syndrome (OHSS), which leads to heightened blood estrogen levels. The purpose of this study was to compare the percentage of pregnancy and abortion between patients who used the frozen embryo method due to OHSS and others who practiced the same method for other reasons.*

Materials and Methods: This retrospective, cohort study was conducted on a total of 338 patients who visited in vitro fertilization (IVF) section of Kowsar Hospital of Urmia-Iran to employ the frozen embryo method (May 2013 to December 2015). The patients were classified into two groups, i.e. the OHSS group (N=150) and the non-OHSS group (N=188). Data were obtained and examined by evaluating the files in a questionnaire.

Results: The two groups did not differ significantly with regard to their mean age (p = 0.57). There was also no statistically significant difference between the OHSS and non-OHSS groups regarding the quality of frozen embryo transferred (P = 0.17). Also, there was also no statistically significant difference between the two groups in terms of their pregnancy rate (OHSS = 30.0% vs. non-OHSS = 25.0%) and miscarriage rate (OHSS = 31.11% vs. non-OHSS = 24.44%) (p = 0.32 and p = 0.31, respectively).

Conclusion: Ovum exposure to high estrogen during ovulation stimulation does not affect embryo implantation and miscarriage in patients with OHSS.

Keywords: *abortion, embryo, estrogen, frozen-thawed embryo transfer (FET), Ovarian hyperstimulation syndrome*

BACKGROUND

Infertility is characterized as the inability to conceive after 12 months of regular unprotected sexual intercourse in women aged <35 years and 6 months of regular unprotected sexual intercourse in women aged >35 years [1]. Studies have revealed that 8 to 12% of couples worldwide are facing fertility problems [2], accounting for nearly 72.4 million people in the world who are affected by infertility [3]. The World Health Organization asserts that infertility will become the world's third most prevalent disorder in the next few years preceded by cardiovascular diseases [4]. Study by Akhondi et al. showed the primary infertility rate to be 20.2% among Iranians [5].

There are various methods of infertility treatment including change of lifestyle, medications or surgery, and assisted reproductive technology (ART) [6]. Overall, as a costly assisted reproduction method employed in various countries, IVF (in vitro fertilization) is utilized by couples in a limited way on the other hand, the results of these methods have not been that fruitful [7].

The duration of uterine receptivity to blastocyst acceptance is highly dependent on estrogen levels such that this process lasts longer at low estrogen levels than at high estrogen levels [8]. That the uterine environment is not conducive to blastocyst acceptance occurring at high estrogen levels is the result of improper expression of the genes responsible for embryo acceptance to the uterus. With the use of the FET

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(frozen embryo transfer) method, endometrial receptivity can be increased as the method eliminates the effects of estrogen on the endometrium produced by ovulation stimulation [9].

Nowadays, FET is regarded as one of the most prominent techniques of infertility treatment that enhances the cumulative rate of pregnancy. Additionally, FET lessens the cost of treatment, the rate of multiple pregnancies, and OHSS (ovarian hyperstimulation syndrome) [9]. In this method, upon stimulation of ovulation, removing oocytes, and insemination (i.e. embryo formation) in vitro, the formed embryos are frozen.

Despite the fact that the use of frozen embryo and its transfer to following cycles, in which the patient does not undergo ovulation stimulation and the estrogen level is not high, has removed the effect of estrogen on implantation in these cycles, whether or not the high rate of estrogen impacts ovulation stimulation, embryo quality, implantation, and embryonic growth and development is to be understood. The results of the study by Weinerman R et al. revealed that if an embryo is exposed to high estradiol levels, its development, and attachment to the endometrium are undermined [10]. Estradiol can influence the proliferation of human embryonic cells to the extent that the physiological levels of estradiol make it possible for stem cells to proliferate. However, estradiol at levels higher than physiological ones impairs stem cell proliferation, occurring through the lowered expression of the HDACI gene, which contributes to embryonic development [10,11].

This study was conducted to assess and compare the rate of implantation and abortion in patients who used frozen embryo transfer either due to OHSS syndrome or other reasons such as inadequate endometrial thickness, uterine bleeding, etc. Their embryos were frozen and transferred in subsequent cycles after endometrium was prepared. Then, we investigated the impacts of oocytes exposure to high estrogen on ovulation stimulation and its effects on the quality, implantation, and growth of the embryo in the uterine.

MATERIAL AND METHODS

The present study was implemented on patients who visited the IVF section of Kowsar Hospital in Urmia, Iran (May 2013 to December 2015). The data were accumulated by reviewing the files and in cases where patients' records were not complete, their data was collected through phone calls and face-to-face visits in the form of a questionnaire. This project was approved by the Ethic Committee of Urmia University of Medical Sciences, and was in accordance with the Helsinki Declaration.

The patients were divided into two groups in terms of why they wished to use embryo freezing. The first group comprised participants whose embryos were frozen due to OHSS (OHSS group), and the second group included participants who used the frozen embryo transfer method for reasons other than OHSS (non-OHSS group). One or two days after their second period time, patients referred from the first or second day after the second period of egg retrieval to prepare the uterus. From the second day of the cycle, they were treated with 2 mg Valerate Estradiol every 8 hours. If their endometrium was more than 8 mm thick and three-linear, they received 50 mg progesterone intramuscularly every 12 hours, along with estradiol at the same time. On the fourth day of their progesterone, their embryos were transferred, followed by estradiol, and progesterone. After 14 to 15 days, patients' β-HCG levels were checked. If positive, they visited the hospital for an ultrasound two weeks later. Also, patients were evaluated for miscarriages up to 20 weeks of gestation.

Inclusion criteria comprised: all patients who volunteered to undergo IVF, yet after ovulation and embryo formation, their embryo transfer was canceled and it was instead frozen due to reasons such as OHSS, uterine bleeding, inadequate endometrial thickness for embryo transfer, presence of polyps, or any uterine space lesion or cervical stenosis, etc., were included in the study. Exclusion criteria also were <8mm endometrial thickness on day 13, consumption of Valerate Estradiol or hyperechogenic, and homogenous endometrium on vaginal ultrasound on this day.

Statistical analysis

The statistical analyses were performed using the SPSS software version 16.0 for Windows (SPSS Inc., Chicago, IL). Categorical variables were summarized as numbers (percentages). The variables were compared between two groups by the Chi-square/Fisher’s Exact test. $p < .05$ were considered statistically significant.

RESULTS

Of the 338 patients analyzed, 150 were patients with OHSS, and 188 were non-OHSS. Out of 150 OHSS patients, 46 had positive pregnancy, one of which one patient was excluded due to chemical pregnancy and 45 had positive pregnancy results (30.20%). Of 188 non-OHSS patients, 48 had positive pregnancy, of which 2 and 3 patients were excluded due to chemical pregnancy and EP, respectively, and 45 had positive pregnancy (25.13%). Nonetheless, no EP incidence was reported in OHSS patients. Of 45 patients with OHSS, 11 had a miscarriage (24.44%) and 34 continued their pregnancy after 20 weeks (75.55%). Of 45 non-OHSS patients, 14 had miscarriages (31.11%) and 31 had continued pregnancies after 20 weeks (65.95%) (Figure 1).

Figure 1: Flow chart of included subjects

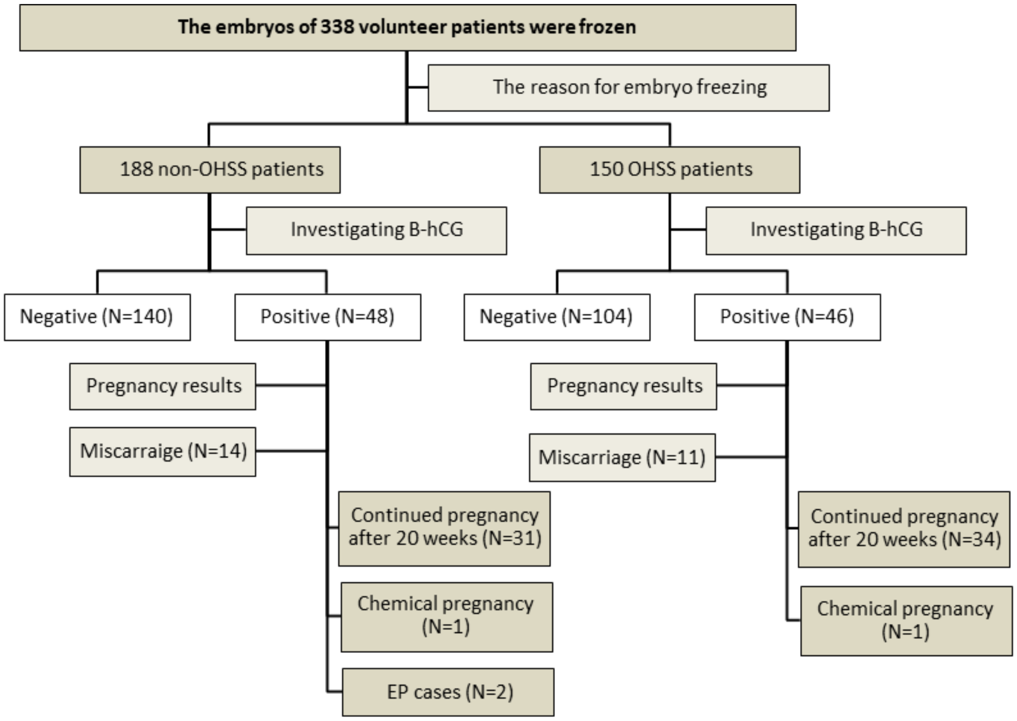
The embryos of 338 volunteer patients were frozen
The reason for embryo freezing
188 non-OHSS patients
150 OHSS patients
Investigating B-hCG
Investigating B-hCG
Negative (N=140)
Positive (N=48)
Negative (N=104)
Positive (N=46)
Pregnancy results
Miscarraige (N=14)
Continued pregnancy after 20 weeks (N=31)
Chemical pregnancy (N=1)
EP cases (N=2)
Pregnancy results
Miscarriage (N=11)
Continued pregnancy after 20 weeks (N=34)
Chemical pregnancy (N=1)

The mean age of the research population was 30.13 ± 5.5 years (OHSS = 29.91 ± 5.67 years and control = 30.35 ± 5.4 years (p = 0.57)).

A total of 338 patients experienced frozen embryo transfer, 2 embryos transferred in the OHSS and the non-OHSS groups, respectively. When assessing the quality of transferred embryos, 66.86% and 69.07% of embryos belonged to patients with OHSS and non-OHSS groups, which had embryos A and B, respectively. This finding was not statistically significant (p = 0.17).

Concerning the pregnancy rates from transferred embryos in patients between the two groups, 45 out of 150 OHSS patients (30%) had positive pregnancies, while 47 out of 188 (25%) non-OHSS patients had positive pregnancies, suggesting no significant difference between the two groups in terms of pregnancy rates (P = 0.32). Regarding the rate of miscarriage in patients with positive pregnancy, there was no statistically significant difference between non-OHSS patients (14 cases out of 45 cases, 31.11%) and the OHSS group (11 cases out of 45 cases, 24.44%) (P = 0.312). There was no significant difference between the two groups in terms of chemical pregnancy rate (0.66% in OHSS group versus 0.5% in the non-OHSS group) (p = 0.85). Two ectopic pregnancies were found in non-OHSS patients, whereas ectopic pregnancy did not occur in OHSS patients (p = 0.50).

DISCUSSIONS

Despite substantial efforts made to prevent it, OHSS remains an iatrogenic disorder with potentially life-threatening complications [12]. The most significant way to stop OHSS is prevention, and one of the major ways to prevent it is avoiding embryo transfer in these patients, and instead, freezing and transferring the embryo in future cycles with no ovulation stimulation is an important method in preventing this major problem. Using FET in these cycles makes a great contribution to reduced incidence or severity of OHSS. Moreover, it decreases the adverse effects of high estrogen on embryo implantation [6]. The purpose of this study was to assess and compare the pregnancy and miscarriage rates among OHSS and non-OHSS patients in order to indirectly examine the effects of high estrogen on embryo implantation and growth in the patients. In the present study, an examination of the percentage of pregnancies in both groups revealed that high estrogen during ovulation stimulation has no impact on oocyte and embryos formed for implantation and early embryo growth and development in the uterus. On the other hand, it did not affect miscarriage either.

Studies have revealed that endometrial receptivity is highly reliant on estrogen levels since, at high estrogen levels, the uterus is not conducive to embryo implantation. Nevertheless, the impacts of high estrogen on embryo implantation and growth in OHSS cycles are yet to be known [13,14]. In a study, it was confirmed that high estrogen levels can undermine the proliferation of embryonic stem cells, hence its adverse effects on embryonic growth and development [10]. Meanwhile, the differentiation of the embryo for the implantation process is an important factor in the occurrence of pregnancy [13].

Check et al. examined the patients whose fresh embryo transfer was canceled due to OHSS or deficient endometrial thickness, and instead underwent the frozen embryo method. The rates of clinical pregnancy and live birth in OHSS patients were 42.8% and 31.9%, respectively, whereas the values were 28.7% and 21.8% for non-OHSS patients (insufficient endometrial thickness), respectively. As is shown, the rate of pregnancy and live birth in the non-OHSS group is significantly lower than that of the OHSS group ($P = 0.01$ and $P = 0.07$, respectively) [15].

Raziel et al. assessed the rate of premature miscarriage in OHSS patients undergoing IVF. Conducted on 104 patients with severe OHSS, this study revealed that 58% of these patients were pregnant, of which 38% had miscarriages. Compared to non-OHSS patients who practiced IVF, the pregnancy rate among OHSS patients was significantly higher, which was seemingly due to the non-OHSS patients' higher mean age. in this study, the rate of miscarriage in patients with OHSS was higher than the control group, i.e. non-OHSS group, which was not consistent with findings of our study [16].

With regard to the outcome of pregnancy in Check et. al, the live birth rate in the OHSS group was 31.9% compared to 21.8% in patients with less endometrial thickness, which was also statistically significant [15]. In the study by Howarth and et al., the rate of live births in the first and fourth groups was lower than in the second, third, and fifth groups, which was also statistically significant [17]. Nonetheless, in our study, which assessed pregnancies up until the 20th week of pregnancy, the groups were compared only in terms of their rate of miscarriage such that in the second non-OHSS group's patients, the rate of miscarriage was higher than in OHSS patients, yet there was no statistically significant difference between the two groups. The present study also had its own limitations. Due to the lack of information in the files and unavailability of some patients, which decreased the number of samples in this study, more comprehensive and longer-term prospective studies are required to increase the power and reliability by expanding the number of samples and controlling the study as much as possible.

CONCLUSIONS

Accordingly, the results of our study potentially show that oocytes exposure to high estrogen during ovulation stimulation has no impact on oval and embryos formed for implantation, growth, and development in utero.

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ORIGINAL ARTICLE

Pharmacological evaluation of glomerulonephritis in Covid-19 patients

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Abstract: *Acute kidney injury (AKI) and glomerulonephritis can be complications in COVID-19 patients, which is announced with widely incidence rates in different investigations and is determined to have a major effect on the prognosis of the disease. There are considerable variations in AKI and glomerulonephritis rates between other countries. The rate is generally lower than in Western Europe and the United States in China. Heterogeneity in different racial and ethnic lines can be a potential explanation. This investigation systematically reviews the scientific resources regarding AKI and glomerulonephritis among hospitalized COVID-19 patients. Both observational and interventional investigations(including case reports) with English full-text provide essential data, with no limitation in release or peer-review. More evidence is required to assess the AKI, especially glomerulonephritis in Covid-19 patients, for better management of Covid-19.*

Keywords: *COVID-19, AKI, patient, glomerulonephritis*

INTRODUCTION

The kidneys play an important role in regulating acid-base balance, water and electrolyte balance, nitrogen balance, and calcium and phosphorus metabolism. Chronic Kidney Disease (CKD) is a disease that is caused by the progressive and irreversible destruction of the number and function of nephrons by various factors. Among the most important of these factors are diabetes, hypertension, glomerulonephritis, and polycystic kidney disease, respectively. Causes of chronic kidney disease reduce the number of nephrons through progressive destruction of nephrons, in which case the amount of blood flow and pressure in the remaining healthy nephrons increases, and thus the remaining nephrons increase their function (Figure 1). Try to keep the Glomerular Filtration Rate (GFR) within the normal range; For this reason, after a while, the remaining nephrons become hypertrophic due to overactivity, and eventually, this compensatory mechanism causes glomerular sclerosis, resulting in the destruction of glomeruli and the disappearance of the remaining nephrons.

In patients with chronic kidney disease, especially in its advanced stages, transient complications such as nausea, vomiting, anorexia, hyperkalemia, metabolic acidosis, hyperphosphatemia, fluid retention, hyperparathyroidism, bone disorders, cardiovascular and pulmonary disorders, neuromuscular disorders, normochromic normocystic anemia, hypersensitivity to infection, gastrointestinal disorders, metabolic-endocrine disorders (lipid abnormalities), Inflammation and oxidative stress occur. Kidney problems are directly related to the nutritional status of patients.

The prevalence of energy-protein malnutrition in these patients is very high. Numerous studies have shown that the life expectancy of these patients increases with the improvement of nutritional status. According to various studies, the COVID-19 infects all groups of different ages include: newborns, pediatrics, young people, elderly, but some groups are more vulnerable to this infection.

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Autoimmune patients are one such group [1,2].

Figure 1: Test your knowledge on glomerulonephritis

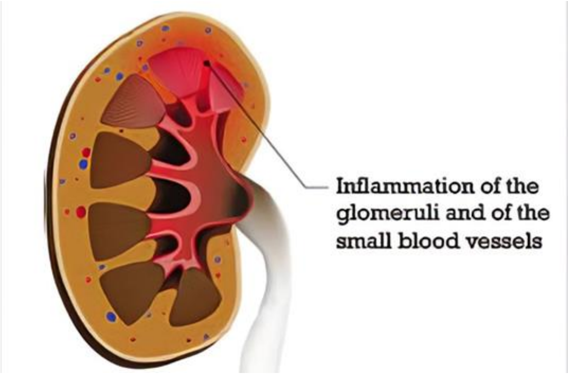
Inflammation of the glomeruli and of the small blood vessels

Despite most of the cases presented with mild symptoms so far, many of them experienced lethal complications, including organ failures, acute respiratory distress syndrome (ARDS septic shock, and pulmonary edema. Acute kidney injury (AKI) is one of the complications in patients with COVID-19, which is detected as a widely varied incidence rate in various investigations and is observed to have a significant effect on the prognosis and outcome of the disease [3-9]. Many reports from China, Europe, and the United States expressed the characteristics and outcomes of acute kidney injury among hospitalized patients with COVID-19 [8,9]. The pathophysiology of COVID-19 related to glomerulonephritis is not determined by a specific theory up to date. It is recommended that the cause of kidney injury be multifactorial; for example, cardiovascular and sepsis, hypovolemia, and nephrotoxins are critical contributors [10]. The study described the incidence of acute kidney injury as a feature of thrombotic microangiopathy (TMA)-like phenomenon, secondary to infection of SARS-CoV-2 [11]. Moreover, viruses particles in renal endothelial cells lead to viremia as a possible reason for endothelial damage in the kidney and a potential contributor to glomerulonephritis and acute kidney disease. Other mechanisms of action may be the direct infection of the renal tubular epithelium and podocytes via an angiotensin-converting enzyme 2 (ACE2)-related pathway in patients with Covid-19 (Figure 2), which can lead to the formation of protein reabsorption vacuoles, mitochondrial dysfunction, acute tubular necrosis, protein leakage in Bowman's capsule, and collapsing glomerulopathy [12].

Figure 2: Glomerulonephritis in body

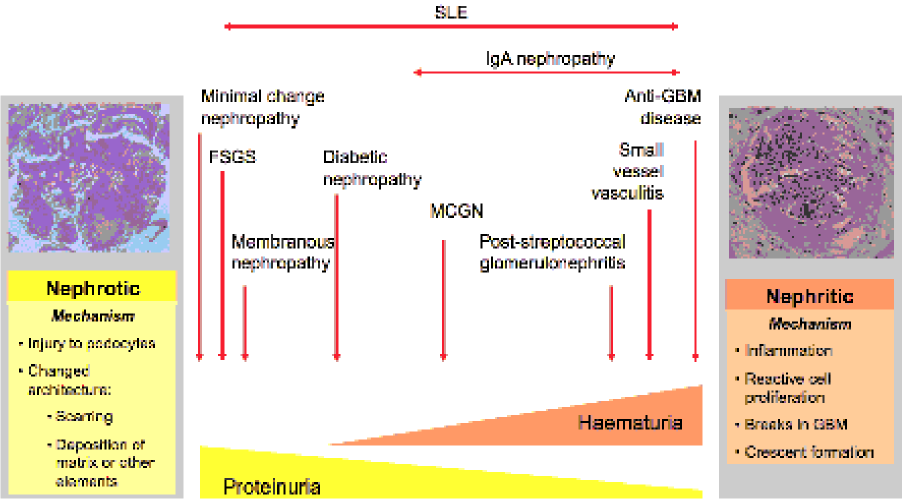
SLE
IgA nephropathy
Minimal change nephropathy
Anti-GBM disease
FSGS
Diabetic nephropathy
Small vessel vasculitis
MCGN
Membranous nephropathy
Post-streptococcal glomerulonephritis
Haematuria
Proteinuria
Nephrotic
Mechanism
• Injury to podocytes
• Changed architecture:
• Scarring
• Deposition of matrix or other elements
Nephritic
Mechanism
• Inflammation
• Reactive cell proliferation
• Breaks in GBM
• Crescent formation

METHODS

Inclusion and exclusion criteria

Observational and interventional investigations (e.g., case reports) with English full-text with presenting data for analysis were included in our study with no limitation in time of publication. Exclusion criteria are review articles and other types of publications with no patient enrollment, articles that specifically focus on the pediatric population, pregnant women, and studies with insufficient evidence on glomerulonephritis occurrence.

Protein catabolism and nitrogen balance in patients with CKD are directly related to energy intake. Inadequate energy intake increases the process of protein catabolism and causes a negative nitrogen balance. Many studies have shown that basal metabolic rate in CKD patients is lower than in others. In dialysis patients, the basal metabolic rate increases by 12-20%, so adequate energy intake in these patients is very important because low carbohydrate (glucose) intake in the diet puts the body in the phase of gluconeogenesis and depletes glycogen stores. Liver and conversion of muscle proteins to glucose, the body is in an unfavorable metabolic state. In other words, proteolysis increases the catabolism of proteins by causing metabolic acidosis and causes the breakdown of muscle mass. Lack of adequate physical activity is also effective in muscle atrophy. In addition, increased production of nitrogen products in the blood causes symptoms such as anorexia, nausea, which is effective in reducing food intake by the patient and maintaining the unfavorable conditions of catabolism. In patients with chronic renal failure, impaired renal function also increases blood triglycerides, impaired carbohydrate metabolism, insulin resistance, and diabetes by increasing the production of inflammatory cytokines and altering the secretion of endocrine hormones. Sometimes severe dietary restrictions in these patients can also lead to malnutrition. Establishing a proper diet is essential to prevent malnutrition in patients with kidney failure. These patients usually need 30-35 cal/kg/IBW of energy per day. The ratio of macronutrients in the diet of these patients is usually as follows: The need for protein in patients with chronic renal failure depends on the function of their kidneys. For example, in patients with 50% renal function and blood creatine levels less than 2 mg/dl or GFR above 70 ml/min, the need for protein is 0.8-1 g/kg/d and in patients with 20-50% Renal function and blood creatine level 2-5 mg/dl or 25-70 ml/min GFR protein requirement 0.6 g/kg/d in hemodialysis 1-2 g/kg/d and in peritoneal dialysis 1-1.5 g/kg/ d, carbohydrates are considered 55-70% and fats are considered 30-30%. Usually the need for fluids in these patients is 600-500 ml + volume of excreted urine + abnormal loss of water from non-renal routes. However, in patients with chronic renal failure in the pre-dialysis phase, there is no need to calculate the volume of fluid intake unless the patient has severe edema. The amount of electrolytes and minerals required depends on the patient's nutritional status and the extent of kidney damage. For example, in an oligarchy patient (urinary excretion less than 500 cc per day), severe sodium, potassium, magnesium, and phosphorus restrictions should be imposed on the diet. In some of these patients, the use of commercial formulas is recommended to meet their maximum nutritional needs. Typically, commercial formulas for kidney patients with high calorie density (2.8-8 kcal/ml), low protein (81-70 g/l), sodium (700-1000 mg/l), potassium (1600 mg/l) - 1000) and phosphorus (600-700 mg/l) are considered. The need for vitamins in patients with kidney failure depends on the type of diet. Patients with severe dietary restrictions need water-soluble vitamins. Normally, the levels of vitamin B1, folic acid and vitamin C in these patients are lower than normal. Hemodialysis patients are usually deficient in vitamins A, vitamin D and B12, and it is recommended to take these vitamins as supplements. Also, when the kidneys fail, the synthesis of 1 and 25 dihydroxycholecalciferol in the kidneys decreases and this vitamin needs to be prescribed. Iron is one of the minerals that is essential in these patients. It is usually recommended to take 10-15 mg/d of iron, but in kidney patients with ferritin levels below 100 mg, it is necessary to take at least 60 mg of ferrosulfate. It is recommended to take iron supplementation with citrus juice in these patients to increase iron absorption. One of the most common problems in kidney patients is metabolic acidosis, which requires oral bicarbonate to maintain blood pH in these patients. In addition to using a proper diet, encouraging the patient to increase physical activity is effective in increasing the expression of anti-inflammatory proteins, improving the sensitivity of insulin-like growth factor, increasing the production of muscle fibers and preventing the breakdown of muscle mass.

Data extraction

Search results were assessed by two independent authors, and study selection was performed assessing the title and the abstract. Among selected publications, full-text articles were evaluated to determine the eligibility for inclusion. . In the end, Discrepancies were resolved by consensus. The following information was extracted from the articles using an organized checklist: name of the first author, date of the publication, name of the journal, study location, study designs, the definition of, age range, gender ratio, and proportion of glomerulonephritis occurrence. General information of the included articles was reviewed precisely to prevent duplication and data overlap.

RESULTS

A total of 289 articles were identified through the systematic search of the databases, as previously mentioned. After removing all duplications, 259 records remained and were carefully examined, which returned an Evaluation of the full-texts to check the eligibility was performed, and 198 publications were excluded due to either not meeting the inclusion or exclusion criteria. From 61 remaining articles, ten were excluded due to data overlap. Finally, 13 studies were selected and included in the review. The process and results are outlined in Figure 3. Some articles defined AKI according to KDIGO criteria as an increase in serum creatinine by 0.3 mg/dL or a 50% level rise in serum creatinine from baseline within seven days. AKI was defined roughly by serum creatinine cut-off if specified in other studies. The diagnosis of glomerulonephritis in Covid-19 patients was confirmed via biopsy tests. The included studies are demonstrated in Table 1 and Table 2.

Table 1: The glomerulonephritis patients with Covid-19

GN definition	Population	Sample Size	Age	Sex	Glomerulo-nephritis	Results
Waldman et al. 2020 (20 April 2020 to 20 August 2020)						
Biopsy-proven	Patients with glomerulonephritis and COVID-19	120	Control 62.5 ± 15.6	Female/ Male 30 (37.5)/ 50 (62.5)	40/120	The majority (70%) of the patients with glomerulonephritis and all the controls were hospitalized. Patients with glomerulonephritis had significantly higher mortality (15% vs. 5%, respectively) and acute kidney injury (39% vs. 14%) than controls, while the need for kidney replacement therapy was not statistically different between the two groups
Xingguo et al, 2020 (February 3 to 16, 2020/Wuhan Fourth Hospital)						
Biopsy-proven	Patients with COVID-19	32	58.5 (36–83)	Male 23 (71.9%)	12/32	Out of the 32 patients, 23 were male, and the median age was 58 years; the median dialysis vintage was 33 months. Two groups were divided according to the patient's primary renal disease: group 1 (16 patients with diabetic nephropathy), group 2 (12 patients with primary glomerulonephritis, 2 with obstructive kidney disease, 1 with hypertensive renal damage, and 1 with polycystic kidney)

Table 2: The case reports of Covid-19 patients with glomerulonephritis

First Author	Country	Age	Disease duration	SLE system involvement	Severity of COVID-19
Hakroush et al. [14]	United Kingdom	79	2 weeks	Kidney	-
Nagai et al. [15]		70	4 month	Left finger	Mild
Raeeskarami et al. [16]	Iran	14	3 weeks	Respiratory tract infection	Severe
Wu et al. [17]	China	46		Kidney	Severe
Winkler et al. [18]		31		Kidney	Severe
Basiratnia et al. [19]		17	2 weeks	Kidney	Mild
Dehmukh et al. [20]	USA	42	1 month		Mild
Tan et al. [21]	Singapore	60	6 weeks		-
Lazareth et al. [22]	France	29	1month	Kidney	-
Perez et al. [23]	Spain	88	33 days	Respiratory insufficiency	
Laboux et al. [24]		57	4 month	Kidney	Mild

What are the symptoms of urinary tract infections in children?

"Fever in young children may be the only sign of a urinary tract infection,". Due to the lack of more obvious symptoms, when girls younger than 2 years old or uncircumcised boys younger than 1 year old have a fever, many medical associations recommend urine culture. "Babies may not eat well, vomit, sleep more, or show signs of jaundice,". In older children, urinary incontinence (loss of bladder control) may occur again; symptoms of a urinary tract infection are often not seen in the elderly; these symptoms may be vague and accompanied by malaise, and the only symptoms present are a change in mental state or fatigue. Bagheri added: "Although in some cases, the first sign that a health care provider encounters is a blood infection or infection in the blood; The fact that many older people already have impotence or dementia may complicate the diagnosis.

During the recovery from acute kidney failure, your gastroenterologist may prescribe special meals to help support and limit your kidney function. Your doctor may refer you to a nutritionist who can analyze your current meals and suggest ways to prepare meals that put less strain on your kidneys. Depending on your situation, your nutritionist may suggest the following: Choose foods with less potassium. Your dietitian may suggest that you choose low-potassium foods. Foods high in potassium include bananas, oranges, potatoes, spinach, and tomatoes. Examples of low-potassium foods include apples, cabbage, green beans, grapes and strawberries. Avoid products with added salt. Get less sodium by avoiding products with extra salt every day. These foods include many ready-to-eat foods, such as frozen foods, canned soups, and fast foods. Other foods with added salt include salty snacks, canned vegetables, and processed meats and cheeses. Limit phosphorus intake. Phosphorus is a mineral found in foods such as milk, cheese, nuts, nuts and peanut oil. High levels of phosphorus in the blood can weaken bones and cause itchy skin. Your dietitian can give you tips on how to limit phosphorus in your specific situation. As your kidneys improve, you may no longer need to follow a specific diet, although a healthy diet is still important.

Figure 3: Flow chart of the study

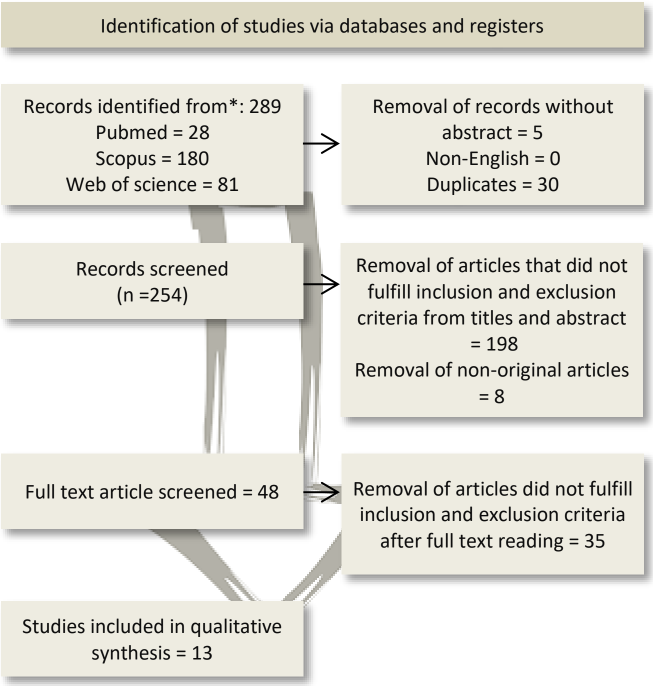
Identification of studies via databases and registers
Records identified from*: 289
Pubmed = 28
Scopus = 180
Web of science = 81
Removal of records without abstract = 5
Non-English = 0
Duplicates = 30
Records screened (n =254)
Removal of articles that did not fulfill inclusion and exclusion criteria from titles and abstract = 198
Removal of non-original articles = 8
Full text article screened = 48
Removal of articles did not fulfill inclusion and exclusion criteria after full text reading = 35
Studies included in qualitative synthesis = 13

DISCUSSION

Early diagnosis of MGN is desirable because it is different from other nephropathies, especially secondary nephropathy, which is caused by causes such as tumor, infection, drug toxicity, or other autoimmune diseases such as systemic lupus erythematosus. Of all cases, 20-30% are MGN due to secondary factors and the remaining 70-80% are classified as primary. Reliable diagnosis of both forms of the disease is important because of differences in treatment: primary MGN is treated with immunosuppressive drugs, while secondary MGN is treated by targeting the causative agent. MGN is detected by a kidney hole followed by a histological test or electron microscopy (to identify immune complexes in the glomeruli). In order to make a final diagnosis of primary MGN, secondary factors must be eliminated, which saves time in the diagnosis process, for example, tumor screening must be performed. In some patients, MGN can be identified as a secondary cause before it is created, which complicates diagnosis and treatment decisions. Primary MGN should be distinguished from other autoimmune diseases involving the kidney, such as lupus nephritis, vasculitis associated with antibodies against neutrophil cytoplasm (ANCA), and Goodpasture syndrome.

In December 2019, Since the COVID-19 emerged in Wuhan city of China and quickly spread throughout the world, it has remained the most prominent health problem all over the world [5]. Our knowledge about this disease is still evolving, and the combination of studies and reports yields a more understanding as time goes on. We investigated a systematic review of the studies that reported glomerulonephritis in hospitalized COVID-19 patients. Treatment of glomerulonephritis include:

1- If your blood pressure is high, your blood pressure will drop with limited water and sodium.

2- Antihypertensive drugs are also used if the blood pressure is high.

3- Proteins of high biological value should be given to improve the nutritional status of the patient.

4- Treat a urinary tract infection immediately.

5- Use diuretics to reduce fluid load.

6- In case of severe edema, raise the head of the patient's bed for more comfort and better urination.

7- Weigh the patient daily.

8- Adjust the patient's sodium and water intake based on the patient's kidneys' ability to excrete water and sodium.

9- Iron and vitamin supplements are given if there is anemia.

10- Cortisone or cytotoxic drugs if the disease is severe.

11- In the early stages of the disease, dialysis is performed in order to improve the patient's physical condition, prevent water and electrolyte imbalance and reduce the risk of complications of renal failure.

CONCLUSION

A report (from China) had even concluded that acute kidney injury (AKI) is not a complication of COVID-19. However, the following evidence modified this perspective. Although, many researchers announced the lower rates of AKI (which may lead to glomerulonephritis) from China compared with elsewhere. These evidence indicated that kidney damages like glomerulonephritis could be a common complication of the Covid-19. Compared to previous reports, our investigation found many case reports of Covid-19 patients with glomerulonephritis, which is demonstrated in table 2. This systematic review was conducted to evaluate glomerulonephritis in Covid-19 patients. Further investigations are warranted to precisely determine whether glomerulonephritis is a complication of COVID-19 or not. However, according to present studies, Covid-19 may lead to several kidney injuries, including glomerulonephritis.

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ORIGINAL ARTICLES

Pre-surgical management of primary leiomyosarcoma of the bone

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Abstract: *Leiomyosarcoma is a rare type of smooth muscle cell tumor, with an incidence of 7-10% compared to other types of sarcomas. It develops primarily in the retroperitoneal cavity, the genital tract and extremities. Leiomyosarcoma can also affect the bone, either as a primary tumor or as metastasis. Primary osseus leiomyosarcoma is a rare finding in patients with radiologically identified osteolytic lesions having an incidence of <1% of total primary bone tumors.Due to its low incidence patients with primary osseous leiomyosarcoma are never diagnosed before immunohistochemical analysis and case management is difficult because of the lack of specific indications pertaining to primary osseus leiomyosarcoma. Data on age specific incidence is scarce but there seems to be a tendency towards a higher incidence with advanced age.*

The purpose of this work is to discuss the complete management of primary osseus leiomyosarcomas based on interdisciplinary medical teams' indications in a Romanian hospital setting.

Keywords: *primary leiomyosarcoma of bone, immunohistochemical analysis, case management*

INTRODUCTION

Primary osseous leiomyosarcoma was first described in 1965 as a malignant tumour arising in the proximal left tibia of a 73 year old patient [1].

An exact reported incidence is unknown due to the scarcity of cases but out of the total amount of primary malignant tumours of the bone, primary leiomyosarcoma is listed together with Ewing and other sarcomas as having a <1% annual incidence [2]. Twenty-eight case have been reported in the USA between 1965 and 1990, however the number rose to a total of 120 cases between 1990 and 2005 and by 2020 many more authors reported their experience with this type of tumour as well as outcomes and prognostic factors [3,4,5,6,7]. Rising reported cases are most likely due to the widespread use of immunohistochemistry.

To date, there is no clearly defined prevalent location of primary osseous leiomyosarcoma but a nationwide study of 104 patients diagnosed with leiomyosarcoma during 1975 and 2009 reported the femur, pelvis and tibia as the first three most common locations [5]. Regarding age and gender distribution of leiomyosarcoma, no clear pattern was identified, although a nationwide study based on the French network of cancer registries reported an ascending incidence rate up to 35% incidence in the 40-64 years age group declining thereafter to 27% in the 75+ years group while gender distribution seems to incline to a higher reported incidence in females [7].

Certain diagnostic is based on combined histopathological and immune histochemistry exams with histological grading and surrounding tissue invasion being the key elements for long-term prognosis [3,4].

Surgical options range from fine needle biopsy to locoregional excision and limb amputation. Function is severely compromised by amputation, although there are on-going efforts to develop artificial limbs [8] and to integrate them with the remaining limb stump [9,10].

A patient will present for an orthopaedic examination with

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pain in a localised area, for example the proximal tibia, with no or scarce other local or systemic clinical signs, except for a painful elevation gradually appearing on the proximal tibia. Physical examination may show a tumorous formation located at the proximal tibial level presenting with renitent consistency and adherence to adjacent tissue.

Radiological findings

Physical examination would be followed by standard anteroposterior and latero-lateralradiographic exam of the affected segment, in our example, the knee and proximal calf (Figure 1). Radiographic results may reveal an osteolytic lesion localized at the transition zone between the proximal metaphysis and epiphysis of the tibia, with a mild extension towards the tibial shaft and with a taller than wider aspect. The margins may not be clearly defined and there may not be peripheral sclerosis. Erosion and narrowing of the medial cortex may be shown. Bone marrow may have a lobulated aspect but no extra-axial marrow growth. No periosteal reaction nor other bone structural changes may be found.

Figure 1: Right knee anteroposterior and lateral radiographs, suggestive of osteolytic lesion on the proximal metaphysis of the tibia.

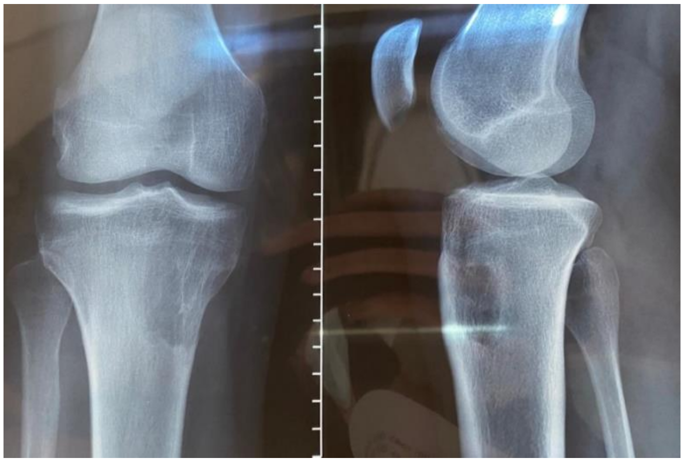

Solely based on the radiographic examination, reported results may reveal both benign and malignant features. The benign feature would be represented by the lobulated structure of the bone marrow. The malignant features would be represented by the absence of peripheral sclerosis which would be suggestive of a rapidly evolving lesion and by the absence of periosteal reaction which would signify a fast growing mass.

Figure 2: A – axial sequence of the proximal tibial metaphysis showing osteolysis and extraosseous invasion; B – coronal sequence of the proximal tibial metaphysis; C – sagittal sequence of the proximal tibial metaphysis

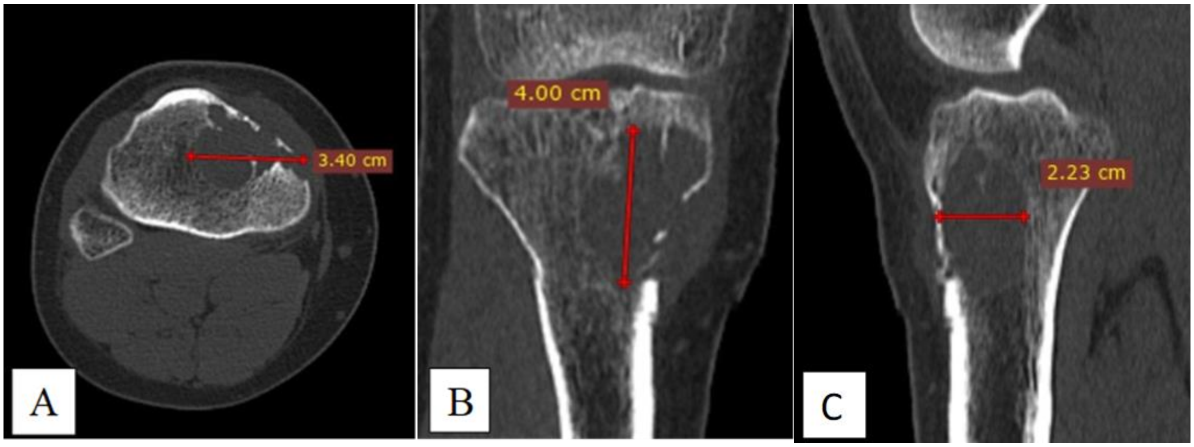

Following the radiographic examination a native CT scan (Figure 2) would be performed, that may be describing an osteolytic lesion, with no clear outline and irregular shape, localised at the proximal metaphyseal-epiphyseal junction of the tibia. The lesion may interrupt the medial cortex and may not affect the articular outline. Based on compared densities, tibial intra-axial trabecular bone signal may be identified within the ingrown mass. The left over trabeculae would be a result of a fast growing osteolytic lesion. Infiltration of the soft tissue, absence of periosteal reaction, as well as the absence of adenopathy in the popliteal fossa may be observed.

Such CT findings strongly would suggest the presence of an aggressive osteolytic tumour.

Histopathological exam

In order to confirm the diagnosis, an incisional biopsy will be performed and the sampled tissue will be sent for histological examination (Figure 3). Results may reveal a sarcomatous proliferation composed of fusiform, elongated cells, arranged in a storiform fashion, forming fascicles that intersect at sharp angle; hyperchromatic ovoid, elongated nuclei, that present abnormal variations and frequent mitoses (7-8 mitoses/10 HPF) and extended necrotic areas; absence of osteoid matrix may also be noticed. Following histopathological examination a immunohistochemical exam will be recommended.

Figure 3: A – histopathological analysis with haematoxylin and eosin stain, x200, with typical aspects for sarcoma; B –histopathological analysis with haematoxylin and eosin stain, x200, showing multiple faults and mitoses; C – immunohistochemical analysis, diffuse positive for desmin; D – diffuse positive for caldesmon and E – diffuse positive for smooth muscle actin (SMA).

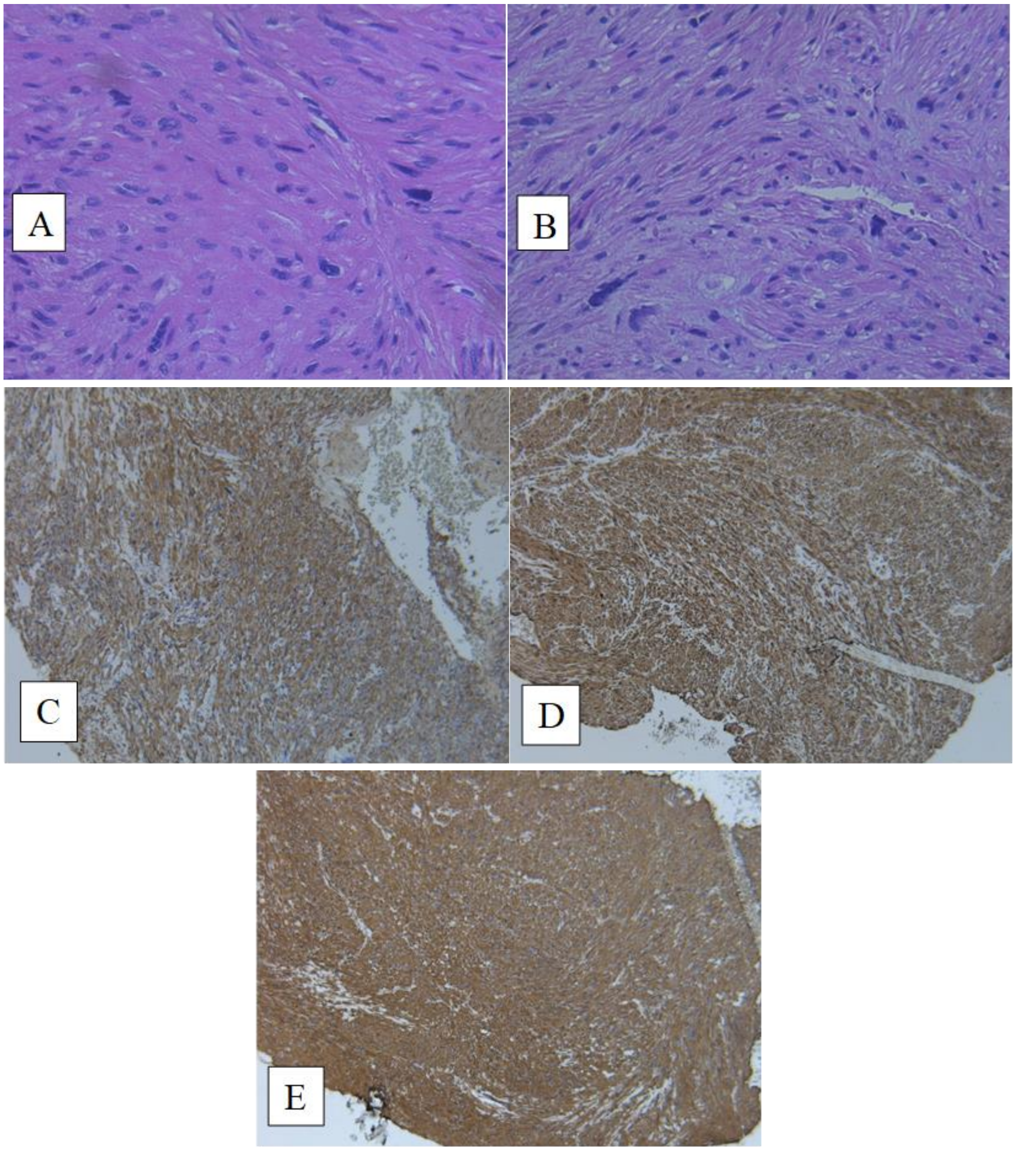

Following immunohistochemistry examination (Figure 3) the tissue sample may be found to be positive to the following immunohistochemical markers: smooth muscle actin (SMA), caldesmon (+), desmin (+), special AT-rich sequence-binding protein 2 (SATB2) (+), 60% (+) ki67, (-) CD34, (-)transducin-like enhancer of split 1 (TLE1), (-) S100.

Immunohistochemistry tests will be correlated with the histopathological exam and together sugget the final diagnostic of leiomyosarcoma of bone, G2 stage–moderately differentiated. Staging is based on the Fédération Nationale des Centres de Lutte Contre le Cancer indications (FNCLCC)[11].

The correlation of imaging, histopathological and immunohistochemical results would lead to the formation of a interdisciplinary team of medical experts for the management of this case. The next step would be to perform a full-body native CT scan in order to rule out the presence of other primary site leiomyosarcoma locations that could bea source of metastases and could reclassify the proximal tibial lesion as not a primary osseous leiomyosarcoma but rather a metastases from a leiomyosarcoma formed elsewhere. After a full-body native CT scan would be performed, results may report no other identified lesions effectively establishing the final diagnosis of primary leiomyosarcoma of bone.

Because leiomyosarcomas have such a low incidence and difficult to interpret radiological and histological results, together with a poorly established prognosis, treatment options that best fit these types of cases would be dependent on the surgical and oncological team's experience. A multidisciplinary team is needed in order to synchronize radiotherapy and chemotherapy with surgery, as well as for establishing the proper conditions for psychological support. Also, as an attempt to preserve the affected limb, a tumoral reconstruction prosthesis would be indicated. In order to diminish the risk of local recurrence, the multidisciplinary team would also recommend one session of external neoadjuvant radiotherapy of 50Gy followed by surgical excision, prosthesis implantation and administration of adjuvant chemotherapy consisting of a dose of 5000mg/ m2ifosfamide (IFO) associated with intra-venous (i.v.)mesna for 24h infusion and doxorubicin (DOX) 60mg/m2i.v. (short infusion). Six sessions of adjuvant chemotherapy would be recommended by the oncological team to be administered once every 3 weeks (DOX + IFO x 6, q3w). Filgrastim would also be recommended by the oncological team in order to stimulate granulocyte colony-forming cells as a single administration of 5 mg/mL dose at least 24 hours after chemotherapy. The purpose of these recommendations is to reach local cancer margin control after neoadjuvant radiotherapy and to eradicate any minimal residual disease with adjuvant chemotherapy and to diminish the risk of metastases.

DISCUSSIONS

Numerous studies report variable results of leiomyosarcoma treated with chemotherapy and radiotherapy [12-15]. Radiotherapy was found to have useful results in controlling tumour margins especially in R0 and R1 reported margins [13,16,17]. This being said the multidisciplinary team may consider appropriate to recommend neoadjuvant radiotherapy of up to 50Gy in order to account for any possible microscopic tumoral cells that might remain. Together with neoadjuvant radiotherapy good results were obtained by adding surgery and adjuvant chemotherapy consisting of doxorubicin and ifosfamide [5,18,19]. In order to obtain the best possible long-term outcome a tumoral prosthesis would be recommended by the team for these cases.

Among all the treatment protocols with good outcomes that have been reported in literature, we should bring to mind the association of ifosfamide and doxorubicin with filgrastim 24 hours laterfor stimulating granulocyte colonies in order to permit chemotherapy amplification [15,20].

At the same time it is worth mentioning that the chemotherapeutical doses are not standardized and, thus, we would like to remark the possibility of lowering the dose of ifosfamide in order to raise the dose of doxorubicin, and at the same time to maintain the myelosuppression within the desired limits, and to offer a better response in correlation with dosage [18,21]. For leiomyosarcoma cases we could take into consideration other therapies, such as gemcitabine or gemcitabine-docetaxel association, mitomycin-doxorubicin-cisplatin triple therapy or administration of epirubicin in association with ifosfamide [14,22]. Regardless of the chosen therapy, it is important to mention the fact that the complete response to chemotherapy has not exceeded 8% of the total amount of patients who were treated with a known therapy plan and similar results have also been obtained for partial response to chemotherapy [22,23].

CONCLUSIONS

In conclusion, complicated, rare primary bone leiomyosarcoma cases do not have a stable management recommendation as of yet and are best treated by a multidisciplinary team which, most often than not, bases its recommendations on the established leiomyosarcoma treatment. The scope of this paper was achieved, by presenting the full pre-surgical management of such a tumour, with a focus on the importance of immunohistochemical exam, full-body CT for metastases exclusion and oncological treatment which may be doxorubicin and ifosfamide combined with mesna and Filgrastim.

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ORIGINAL ARTICLES

Predictive value of serum level of B type Natriuretic Peptide (BNP) in neonatal sepsis

Mirhadi Mousavy[1], Ahmad J. Khosroshahi[2], Sepideh Shadravan[3]

Abstract: *Background: NT-proB-type Natriuretic Peptide (BNP) (NT-pro BNP) is one of the biomarkers that has been studied in recent decades in connection with pediatric heart failure (HF) and congenital heart disease (CHD). Recently, infants hypothesized that serum. NT-pro BNP levels might be a good predictor of septicemia severity and response to treatment and prognosis in these patients. This study investigated the relationship between serum natriuretic type B peptide level and neonatal sepsis.*

Materials and Method: In this case-control study, all full-term and pre-term neonates admitted to children's Hospital, Tabriz-Iran(2021), with sepsis and a septicemia diagnosis were confirmed after obtaining consent. The study was deliberately entered by the patient's parents or legal guardian. Complete Blood Count with Differential (CBC/Diff), C - reactive protein (CRP) , Bacterial Culture (B/C) , Urine Analyze(U/A),Urine Culture (U/C) ,NT-pro BNP were checked and finally compared with laboratory results of the same number of infants who did not have clinical and laboratory symptoms of sepsis. After collecting samples, laboratory results, including serum levels of NT-pro BNP, were compared in case and control groups.

Results: One hundred patients were studied in two groups. Thirty-eight patients (38%) were boys, and 62 patients (62%) were girls. The mean age of the patients was 7.58±7.46 days. The mean weight of the studied patients was 2811.80±620.33 grams, with a median of 2855 grams. The most common clinical symptom observed in patients in the case group was fever (100%) followed by Poor feeding (84%). In the control group, all patients had jaundice. Neonates with sepsis had significantly higher initial pro-BNP values than the control group (10023.80 vs. 2247.20; p=0.001). The NT-pro BNP level cut-off point in predicting the final treatment status and mortality of neonates with sepsis was 9583 pg/ml with 97.7% sensitivity and 93.6% specificity.

Conclusion: Measurement of serum level of NT-Pro BNP in neonates with sepsis at the time of clinical signs with 97.7% sensitivity and 93.6% specificity with a cut-off point of 9583 is an important prognostic factor in the therapeutic management of patients.

Keywords: *septicemia, neonates, sepsis, mortality, natriuretic brain peptide*

INTRODUCTION

B-type natriuretic peptide(BNP is a natriuretic peptide with 32 amino acids and a ring structure, which is secreted from the atria and ventricles of the heart in response to changes in pressure or overload of heart chambers. NT-pro BNP (N terminal of the prohormone brain natriuretic peptide) is a marker of BNP in the blood. The mechanism of action of BNP is by activating Natriuretic peptide receptor-A (NPR-A) and increasing the production of Cyclic guanosine monophosphate (cGMP), which has a diuretic effect and dilation of pulmonary arteries [1,2]. BNP is a rapid and highly sensitive marker in myocardial disorders. It also plays an essential role in the organogenesis of the fetal heart and the stage of fetal transfer to the uterus. About 20% of pediatric intensive care units hospitalizations include sepsis, the most common cause of morbidity and mortality, and information from Italian pediatric intensive care units provides 50% mortality in children with septic shock. In adults, there is evidence that increased BNP is associated with heart failure (HF) and increased mortality in cases of severe sepsis and

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septic shock. Sepsis is a systemic response to life-threatening infection, including symptoms of fever, increased heart (HR) rate, and increased respiratory rate (RR). The patient's life is endangered if the diagnosis is delayed. BNP is a useful diagnostic marker in patients with pulmonary hypertension. Increased BNP is associated with hemodynamic compromise, congenital heart disease (CHD), and severe clinical conditions [1-3]. The study of 73 patients by Gisela L et al. (2017) found that BNP can predict hemodynamic changes and prognosis and response to treatment in infants with septicemia [1]. The study of Wang et al. (2015) on 38 patients reported that BNP could be used as a routine hospital marker to predict sepsis in patients [2]. Astudy by Samransamruajkit et al. (2014) on 43 patients with high BNP levels, the mortality rate of children with septicemia was strongly correlated [3]. In another study conducted by Okur et al. (2019) on 87 patients and 35 controls showed that BNP has a high sensitivity (72%) and specificity (86%) for predicting sepsis [4]. According to studies of various biomarkers used to diagnose sepsis, none have sufficient predictive value to confirm or rule out sepsis. However, it has recently been hypothesized in infants that serum NT-pro BNP levels may be a good predictor of the severity of sepsis and the response rate to treatment and prognosis of these patients.

This study aimed was to investigate the relationship between serum natriuretic level of peptide type B and neonatal sepsis.

MATERIALS AND METHODS

In this case-control study, all term and preterm infants were admitted to Tabriz Children's Hospital, Tabriz – Iran, with sepsis from May 2021 to September 2021. Their septicemia diagnosis was confirmed after obtaining informed consent. According to the sample size formula [3] and placing it in the procedure related to the sample size (X = Z2 * p * (1-q)) in intervention studies with a small variable, this number has been obtained and considered as 100 patients; that neonates in the study group (Case) was 50 patients and the number of control patients (Control) was 50 patients. Neonates with cardiovascular disease (CVD), other non-cardiac conditions that involve myocardium, and neonates with pulmonary disease were excluded. Inclusion criteria were term and preterm neonates with symptoms and paraclinical signs of sepsis and approved diagnosis of sepsis by neonatal specialist.

Complete Blood Count with Differential (CBC-Diff), C-reactive protein (CRP), Blood culture (B/C), Urine culture (U/C), Urine microscopic analysis, and NT-pro BNP were checked as study nodes, and finally with laboratory results of the control group with the same number of infants who did not have clinical and laboratory symptoms of sepsis only because of high bilirubin was hospitalized and compared with phototherapy.

Criteria for diagnosing sepsis included clinical signs of seizures, fever, vomiting, anorexia, hypothermia, cyanosis, respiratory distress, and hyperreflexia.

To measure NT-pro BNP, onecc of blood was taken from the patient and examined in the laboratory by ELISA method with a Siemens kit made in Germany. Sampling was performed for Urine Analyze (U/A) and U/C after washing and drying the site using a catheter. U/A results whit white blood cells (WBCs) greater than five or Red blood cells (RBCs) greater than five and positive nitrite test or observation of bacteria and yeast if epithelial cells were low were considered active urinalysis. For CBC- diff, 2 ccs of blood were collected in an EDTA test tube and sent to the laboratory. Also, for CRP, half a cc of blood was centrifuged in the laboratory, and its serum was isolated, and then measured by ELISA (Scaleo CRP kit is made in Italy). Toperform blood culture, 2 ccs of whole blood were obtained under sterile conditions in a two-phase culture medium (Iranian manufacturing company), sent to the laboratory, and performed manually. After collecting samples, laboratory results, including serum levels of NT-pro BNP, were compared in case and control groups.

The data obtained from the study were analyzed using descriptive statistical methods (mean±SD and frequency(%)) and mean difference test for independent groups and chi-square test and analyzed using SPSS/WIN statistical software. Furthermore, statistical analysis was performed. P values less than 0.05 were considered significant.

RESULTS

Thirty-eight patients (38%) were boys, and 62 patients (62%) were girls. The mean age of the patients was 7.58±7.46 days, with a median of 4 days. The minimum age of patients was 3.5 days, and the maximum was 29 days. The mean weight of the studied patients was 2811.80±620.33 grams, with a median of 2855 grams. The minimum weight of patients was 1190 grams, and the maximum was 4400 grams. The demographic findings and clinical symptoms of patients are shown in Table 1. The most common clinical symptom observed in patients in the case group was fever (100%) followed by poor feeding (84%). In the control group, all patients had jaundice.

The laboratory findings of the studied patients by study groups are shown in Table 2. The mortality rate in the study group was 4%. Evaluation of the relationship between primary NT-pro BNP and response to treatment and survival of patients showed that NT-pro BNP levels were significantly higher in infants who did not respond to treatment and died.

Table 1: Demographic findings and clinical symptoms of patients

Variable	Case (N=50)	Control (N=50)
Demographics		
Boy	24 (48%)	14 (28%)
Girl	26 (52%)	36 (72%)
Age (days)	10.90±9.23	4.12±1.45
Weight (grams)	2644.80±598.52	2978.80±601.93
Clinical Symptoms		
Fever	50 (100%)	0
Weakness	40 (80%)	0
Shock State	12 (24%)	0
Vomiting	8 (16%)	0
Poor feeding	42 (84%)	0
Icter	0	50 (100%)

** The results are presented as mean±SD and frequency (%).*

The NT-pro BNP level cut-off point in predicting the final treatment status and mortality of neonates with sepsis was 9583 with a sensitivity of 97.7% and specificity of 93.6% (Figure 1).

Figure 1: Evaluation of diagnostic value of NT-pro BNP measurement in predicting final treatment status and mortality of neonates with sepsis

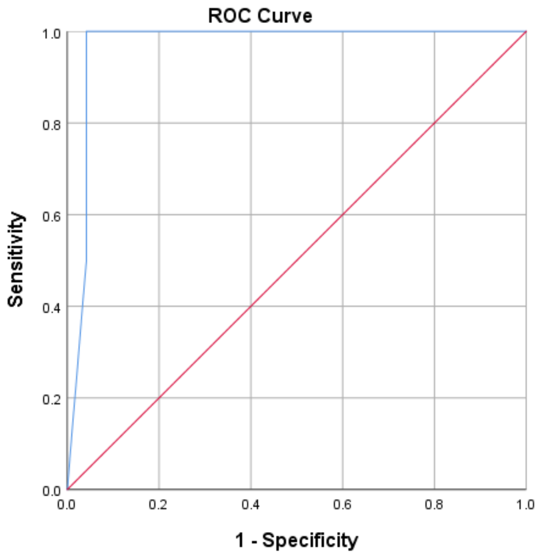

Diagonal segments are produced by ties.

Table 2: Laboratory findings of patients studied by study groups

Variable	Case (N=50)	Control (N=50)	p-value
CRP Negative	0	50 (100%)	
CRP 1+	22 (44%)	0	
CRP 2+	12 (24%)	0	
CRP 3+	16 (32%)	0	
WBC (cells*10^3/ml)	10.75±4.37	10.04±2.05	0.299
Neut (cells*10^3/ml)	5.27±3.44	4.07±1.57	0.029
Lymph (cells*10^3/ml)	3.91±1.87	4.55±.126	0.048
NT-pro BNP			
Basic	10023.80±10689.77	2247.20±2076.99	0.001
After Treatment	2378.56±2004.48	-	-
Positive Blood Culture	6 (12%)	0	0.013
Gram negative Bacillus	2	0	-
Yeast	2	0	-
Coagolase-negative Staff	2	0	-
Positive Urine Analysis	30 (60%)	0	-
Positive Urine Culture	0	0	-
Mortality	2 (4%)	0	0.231

** The results are presented as mean±SD and frequency (%).*

DISCUSSION

In this study, a case-control analysis was performed to evaluate the predictive value of serum natriuretic type B peptide level in neonatal sepsis. Fifty infants were also studied as a control group, 38% of whom were boys, and their mean age was 7.58±7.46 days with a median of 4 days.. Sepsis is one of the standard clinical conditions that directly and significantly affect infant mortality and morbidity. According to World health organization (WHO) estimates, sepsis accounts for about 60 to 80% of infant mortality cases per year [5]. Sepsis also accounts for nearly 20% of intensive care unit admissions and is the leading cause of mortality and morbidity in the Pediatric Intensive Care Unit (PICU). The results of the surviving sepsis campaign show mortality of 34.8%, and this number is higher in developing countries [6, 7]. Results from Italy also show a mortality rate of nearly 50% in the PICU of neonates admitted with septic shock [7]. The results show that the hospitalization rate of neonates with sepsis is higher than controls, and most studies have emphasized the importance of rapid diagnosis and treatment. A recent study reported that rapid diagnosis and intervention in infants with septic shock can be highly life-saving and that the outcome of patients can be more favorable [8]. Rapid diagnosis of sepsis and balancing hemodynamics of patients are the main factors involved in reducing patient mortality. Intravenous antibiotics are also a key to treating patients [9].

The results of the present study showed that the most common clinical symptoms in neonates with sepsis include fever (100%), weakness (80%), shock state (24%), and vomiting (16%). In the control group, all patients had jaundice. Evaluation of patients' laboratory findings showed that neonates with sepsis had significantly higher initial NT-pro BNP values than the control group (10,023.80 vs. 2,247.20; p = 0.001). Numerous biomarkers have been evaluated to evaluate the status of patients with severe sepsis and septic shock. For example, procalcitonin has recently been directly associated with the severity of bacterial infections [10]. In addition, cardiovascular disease is more common in children than in adults with severe sepsis or septic shock, so that in 58% of cases, low cardiac output with high peripheral vascular resistance, and in 22% of cases, they have low cardiac output with low peripheral vascular resistance [11,12]. Therefore, biomarkers associated with cardiovascular disorders in infants and children can benefit infants with sepsis. NT-pro BNP is a biomarker synthesized and secreted by myocytes and atrial and ventricular fibroblasts and is also associated with cardiovascular dysfunction [13,14]. As mentioned in the present study, neonates with sepsis had significantly higher serum NT-pro BNP levels than the control group. Also, after treatment, the level of this biomarker in patients in the affected group was reduced considerably (23,786.56 vs. 10,023.80; p = 0.001). Recently, researchers found that measurement of primary NT-pro BNP levels in neonates with sepsis was significantly associated with patient mortality and lays the groundwork for fluid therapy decision-making for fluid therapy. This study indicated that the volume of positive fluid balance in patients was directly related to increased serum BNP levels [15]. The above research results are consistent with our study It is better to evaluate the level of electrolyte balance in patients with sepsis and its relationship with the serum level of NT-pro BNP in future studies.

In the present study, in terms of the outcome, 96% of patients with sepsis had successful treatment, and 4% died. The results of evaluating the value of pro-BNP diagnosis in predicting the final treatment status of neonates with sepsis with a 9,583 cut-off point had a sensitivity of 97.7% and specificity of 93.6% (p = 0.002; AUC = 0.969). Similarly, a good prognosis has been reported for PICU mortality [3]. In recent years, several studies have been performed that show a significant and traceable increase in serum BNP levels in patients with sepsisthTherise in serum BNP and NT-pro BNP levels havebeen directly related to increased mortality in patients [3,16,17]. The increase in BNP is high in patients with sepsis, but ventricular dysfunction in these patients is not easily measured.

On the other hand, several factors such as lipopolysaccharides (LPS) [18], inflammatory cytokines like Tumour Necrosis Factor - alpha (TNF alpha) and interleukin-1 beta (IL-1 beta) [19], and catecholamines [20] also increase BNP. Recent studies have shown that BNP levels in neonates with sepsis caused by gram-negative bacteria were higher, indicating the effect of the type of bacterial strain, so that the bacterial polysaccharide may have led to a more severe immune response. Therefore, it is suggested to be considered in future studies [21]. In neonates with sepsis, other mediators, including endotoxins, inflammatory cytokines, and myocardial depressant factors, increase NT-pro BNP levels through myocardial damage and dysfunction [22]. Therefore, it is better to perform Doppler echocardiography in these patients to evaluate cardiac function after the initial critical procedures. In this study, negative urine culture results despite active urinalysis may be due to the early initiation of antibiotic treatment in the first venipuncture.

Limitations of study

One of the limitations of this study is the lack of data on the effect of serum bilirubin on BNP. The second limitation of this study is the lack of information on the effect of electrolytic disorders on serum BNP levels.

CONCLUSIONS

Overall, according to the results obtained in this study, it can be concluded that measuring the serum level of NT-pro BNP in neonates with sepsis at the time of observation of clinical symptoms with a sensitivity of 97.7% and specificity of 93.6% with a cut-off point of 9,583 is an important prognostic factor. In the medical management of patients. In future studies, the relationship between the type of bacterial strain leading to sepsis and the level of electrolyte balance in patients with sepsis be compared with the serum levels of NT-pro BNP and BNP.

Ethical Approve

The ethics committee approved the present study with the code (IR.TBZMED.REC.1400.141) of Tabriz University of Medical Sciences. Because all diagnostic procedures have been performed for the diagnosis and treatment of patients and in addition to the treatment process of patients, no additional blood sampling and no cost for measuring serum NT-pro BNP level wastaken from patients, so no ethical case does not exist.

Authors' Contribution

Study concept and design: MH. M. and A. G Kh. Acquisition of data: S. Sh; Analysis and interpretation of data: R.P; Drafting of the manuscript: MH. M. and A. G Kh; Critical revision of the manuscript for important intellectual content: R.P; Statistical analysis: A. Kh and S.Sh

Conflict of Interest

There is no conflict of interest.

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ORIGINAL ARTICLES

Quality of life assessment for rectal cancer patients: A comparison between sphincter preservation and permanent colostomy

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Abstract: *This study aims to compare the quality of life in patients with rectal cancer between sphincter preservation and permanent colostomy. The present study is a cross-sectional study that was performed on 120 patients (60 cases in the sphincter maintenance group and 60 cases in the permanent colostomy group) who underwent surgery from February 2017 to November 2016. Quality of life assessment was provided with QLQ-C30 and QLQ-CR29 questionnaires. Finally, the collected data were analyzed using SPSS23 software. According to the results of the QLQ-C30 questionnaire, there was no statistically significant difference in patients' quality of life in terms of performance in the study groups (P>0.05). Symptoms such as nausea, pain, shortness of breath, sleep disturbance, loss of appetite, constipation and economic problems in the study groups were not statistically significant (P>0.05). But the mean symptoms of fatigue (P=0.038) and diarrhea (P=0.037) were significantly higher in the sphincter maintenance group than the permanent colostomy group. The mean score of patients' quality of life was 53.43 ± 8.40 in the sphincter retention group and 52.23 ± 11.45 in the permanent colostomy group (P = 0.556). According to the results, the quality of life in the two surgical methods of sphincter preservation and permanent colostomy was not statistically significant. Therefore, treatment decisions should be made by informing patients, both based on their preferences and the physician's clinical judgment.*

Keywords: *rectal cancer, colostomy, sphincter*

INTRODUCTION

In most countries, especially developed countries, cancers are the second leading cause of death after heart disease [1]. After lung and prostate cancer, colorectal cancer (CRC) is the third most common cancer in the world and among men, and after breast cancer it is the second most common cancer among women (Figure 1) [2]. In Iran, after heart disease and accidents, cancers are the third leading cause of death [3]. In the United States, it is the fourth most common cancer in men after skin, prostate and lung cancers, and the most common cancer in women after skin, lung and breast cancers [4]. In fact, colorectal cancer is caused by the uncontrolled growth of the inner layer of the organs of the colon and rectum, which increases with age [4].

Figure 1: Under endoscopic visualization

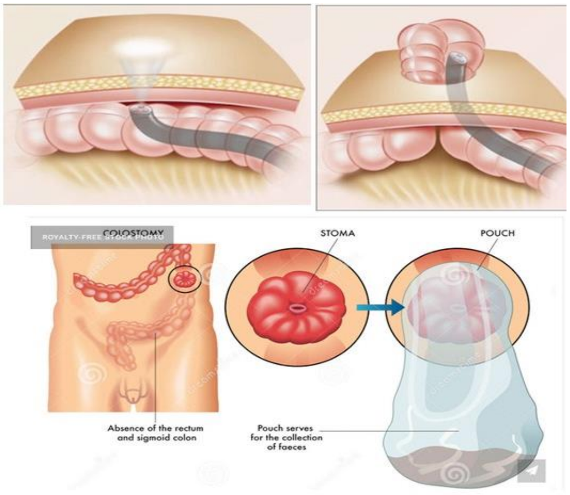
COLOSTOMY
STOMA
POUCH
Absence of the rectum and sigmoid colon
Pouch serves for the collection of faeces

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The exact cause of colorectal cancer is not known, however, the disease is not contagious and no one gets the disease from another person [5]. Research has shown that people with certain risk factors, such as aging, family history, genetic disorders, smoking, colorectal polyps, and poor diet, are more likely suffering colorectal cancer than other people [6]. The first line of treatment is surgery to remove the tumor. Surgical methods include: anterior resection with reconstructive anastomosis and tumor removal, and surgery to remove the rectum from the perineum and abdomen with a permanent ostomy [7].

In case of metastasis, supportive surgery is performed to eliminate the symptoms, prevent obstruction and improve the patient's quality of life. In some cases, an ostomy may be needed to treat cancer during bowel resection [8]. The choice of treatment is based on the staging of the disease and surgery is the basis of definitive treatment in the early stages. Today, chemotherapy and sometimes radiotherapy (in rectal cancers) are used in the form of adjuvant in certain stages of the disease [9]. An ostomy (ileostomy or colostomy) is the creation of a wall on the abdomen that is used to help the bowel or bladder drain. Depending on the type of surgery used and the extent of the disease, a colostomy may be temporary or permanent [10]. Most patients with colorectal cancer survive for at least 5 years after diagnosis, so quality of life is an important issue in these patients [11].

Understanding the quality of life experienced by patients with colorectal cancer is essential to assessing the impact of the disease on individuals, their families, and their communities [12]. Cancers can cause a change in the patient's assessment of their health and quality of life by causing physical, social, economic, and etc. disorders. Awareness of patients' quality of life can be considered as an effective factor in making clinical decisions and managing the problems of these people and can also be useful in guiding and accompanying these patients in better use of support and rehabilitation facilities [13]. Abdominal Perineal Resection (APR) surgery, which involves a permanent colostomy, is performed for patients with lower rectal cancer with sphincter involvement or those who are more likely have a malfunctioning sphincter after sphincter retention. Numerous studies have suggested that patients with colostomy experience have significant changes in various aspects of quality of life, including the physical, social, and physiological dimensions, compared with those undergoing anatomical preservation of the sphincter [14]. Therefore, in the present study, we aimed to compare the quality of life in patients with rectal cancer between sphincter preservation and permanent colostomy.

MATERIALS AND METHODS

This study is a cross-sectional study and was performed in colorectal surgery clinic or ostomy care clinic of Ghaem Hospital in Mashhad from February 2017 to November 2016. This research was presented in the organizational ethics committee of the university which was approved with the code IR.MUMS.MEDICAL.REC.1399.457. Inclusion criterias were patients over 18 years of age who presented for follow-up after previous treatment for rectal cancer, including radical surgery with Total Mesorectal Excision (TME), in the colorectal ward of Ghaem Hospital with or without sphincter retention. At least 12 months after APR or aberrant ostomy closure, if they agreed to participate in the study, they entered the project. Exclusion criterias were patients with ostomy with a different histology from adenocarcinoma and intraperitoneal rectal tumor, patients with peritoneal carcinomatosis, and patients who had not received neoadjuvant before surgery were excluded from the study. Patients were divided into two groups: 1) the sphincter retention group (SPG), which included patients who underwent a lower colorectal or coloanal anastomosis; And 2) permanent colostomy (OCG) group, which included patients who underwent permanent APR and terminal colostomy. In this study, 120 patients (60 cases in the sphincter maintenance group and 60 cases in the permanent colostomy group) were included in the study.

QLQ-C30 Questionnaire

A multidimensional questionnaire consisting of 30 questions that quality of life in 5 functional areas (physical function, role-playing, emotional, cognitive and social) and 9 areas of symptoms (fatigue, pain, nausea, vomiting, shortness of breath, diarrhea, constipation examines, sleep deprivation, loss of appetite, and economic problems caused by illness) and one general area of quality of life.

QLQ-CR29 Questionnaire

This questionnaire is designed to assess the quality of life of patients with colorectal cancer and includes 29 questions that describe the symptoms (gastrointestinal, urinary and pain symptoms) and functional status (such as sexual function and body image) of patients with colorectal cancer. This questionnaire has a separate questionnaire to measure the sexual function of men and women.

Quality of life assessment

Quality of life assessment was performed with QLQ-C30 and QLQ-CR29 questionnaires. In this way, patients were informed about the design and nature of the questionnaires; They filled out the questionnaires independently and if they had any problems, they were given a proper explanation by the project implementers.

Statistical Analysis

After completing the checklists, the information was analyzed by entering it in SPSS23 software. Data description was expressed with tables and graphs and central indicators and dispersion. Mann-Whitney test was used to compare quantitative data between the two groups due to abnormal data distribution. Chi-square test was used to compare qualitative data between the two groups. Significance level in the tests was considered P>0.05.

RESULTS

Average age of patients

The mean age of patients in the sphincter maintenance group was 57.80 ± 9.96 years and in the permanent colostomy group was 59.73 ± 9.31 years (P=0.196) (Figure 2).

Gender of patients

60% of the participants in this study were male and 40% were female. The frequency of patients related to gender in each of the study groups can be seen in Table 1. The results of Chi-square test showed that the sex of patients in the study groups was not meaninful (P=0.456).

Figure 2: Mean age of patients in the studied groups

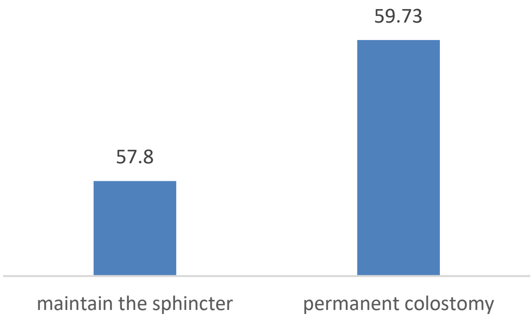

Other variables

The results of Chi-square test showed that marital status (P=0.283), level of education (P=0.6), place of residence (P=0.224) and family history of colorectal cancer (P=0.409) were not significant in the study groups (Table 2).

Table 1: Evaluation and sex determination of patients in the study groups

Variables		Groups			p-value
		SPG Frequency (percentage)	OCG Frequency (percentage)	Total Frequency (percentage)	
Gender	Male	38 (63.3)	34 (56.7)	72 (60)	0.456
	Female	22 (36.7)	26 (43.3)	48 (40)	
	Total	60 (100)	60 (100)	120 (100)	

Table 2: Frequency of other variables in the study groups

Variables		Groups			p-value
		SPG Frequency (percentage)	OCG Frequency (percentage)	Total Frequency (percentage)	
Marital status	Married	50 (83.3)	54 (90.0)	104 (86.7)	0.283
	Single	10 (16.7)	6 (10)	16 (13.3)	
	Total	60 (100)	60 (100)	120 (100)	
Education level	High School	44 (73.3)	48 (80.0)	92 (76.7)	0.600
	Diploma	12 (20.0)	10 (16.7)	22 (18.3)	
	Above the diploma	4 (6.7)	2 (3.3)	6 (5.0)	
	Total	60 (100)	60 (100)	120 (100)	
Place of life	City	40 (66.7)	46 (76.7)	86 (71.7)	0.224
	Village	20 (33.3)	14 (23.3)	34 (28.3)	
	Total	60 (100)	60 (100)	120 (100)	
Family history of colorectal cance	Yes	18 (30.0)	18 (30.0)	32 (26.7)	0.409
	No	42 (70.0)	42 (70.0)	88 (73.3)	
	Total	60 (100)	60 (100)	120 (100)	

Quality of Life Assessment Questionnaire for cancer patients (QLQ-C30 Questionnaire)

According to the results of QLQ-C30 questionnaire in Table 3, patients' quality of life in the field of performance including physical function (P=0.103), role playing (P=0.162), emotional (P=0.050), cognitive (P=0.801) and social (P=0.713) There was no significant difference in the study groups. Symptoms include nausea (P=0.535), pain (P=0.298), shortness of breath (P=0.513), sleep disturbance (P=0.231), loss of appetite (P=0.3609), constipation (P=0.303), and economic problems (P= 0.584) in the study groups were not meaninful. But the mean symptoms of fatigue (P=0.038) and diarrhea (P=0.037) were significantly higher in the sphincter retention group than the permanent colostomy group. The mean score of patients' quality of life was 53.43 ± 8.40 in the sphincter retention group and 52.23 ±11.45 in the permanent colostomy group. The results of Mann-Whitney test showed that the quality of life of patients based on the QLQ-C30 questionnaire in the study groups was not significant (P=0.556).

Table 3: Evaluation the average life quality of patients based on the QLQ-C30 questionnaire in the study groups

QLQ-C30 questionnaire			Average	Standard deviation	Middle	At Least	At Most	p-value
Function	Physical status	SPG	59.83	8.14	59.50	40.00	76.00	0.103
		OCG	57.80	6.97	57.50	44.00	72.00	
	Play role	SPG	51.10	6.67	51.50	40.00	65.00	0.162
		OCG	49.83	8.15	50.00	38.00	72.00	
	Emotional	SPG	48.86	5.91	50.00	38.00	62.00	0.050
		OCG	47.23	8.93	43.00	37.00	72.00	
	Cognitive	SPG	67.16	6.71	69.50	50.00	78.00	0.801
		OCG	67.40	6.97	68.50	53.00	78.00	
	Social	SPG	42.36	5.84	41.00	33.00	53.00	0.713
		OCG	42.76	9.97	40.00	21.00	72.00	
Signs	Fatigue	SPG	48.10	5.92	49.00	38.00	60.00	0.038
		OCG	46.30	9.22	42.00	33.0	69.00	
	Nausea	SPG	38.36	9.08	38.50	22.00	53.00	0.535
		OCG	39.60	9.13	39.00	21.00	60.00	
	Pain	SPG	44.40	5.75	43.00	34.00	58.00	0.298
		OCG	44.33	11.05	41.00	21.00	72.00	
	Shortness of reath	SPG	22.00	3.55	21.00	17.00	31.00	0.513
		OCG	21.80	4.32	21.00	16.00	34.00	
	sleep disorder	SPG	45.73	5.81	46.00	34.00	57.00	0.231
		OCG	44.80	11.20	41.00	21.00	72.00	
	Decreased appetite	SPG	48.40	5.55	48.00	38.00	59.00	0.360
		OCG	49.16	11.49	50.00	21.00	68.00	
	Constipation	SPG	45.96	6.34	45.50	32.00	58.00	0.303
		OCG	44.86	11.26	42.00	21.00	72.00	
	Diarrhea	SPG	18.66	4.15	19.00	11.00	27.00	0.037
		OCG	17.40	5.67	15.00	11.00	31.00	
	Financial problems	SPG	65.43	11.47	69.00	38.00	82.00	0.584
		OCG	64.00	11.64	68.50	40.00	79.00	
Quality of Life		SPG	53.43	8.40	52.00	38.00	68.00	0.556
		OCG	52.23	11.45	52.00	37.00	72.00	

According to the results of the QLQ-C29 questionnaire in Table 4, the quality of life of patients in the areas of functions including anxiety (P=0.171) and female sexual function (P=0.0725) in the study groups were not significantly different. But the quality of life of patients in the areas of functions such as body image (P=0.021), weight (P=0.032) and male sexual function (P= 0.016) in the sphincter maintenance group was significantly higher than the permanent colostomy group. There were no significant differences in the mean symptoms such as hip pain (P= 0.125), hair loss (P = 0.265), taste disturbance (P=0.070) and skin ulcers (P= 0.100). But the mean of symptoms such as frequent urination (P =0.018), blood in the stool (P = 0.013), stool frequency (P=0.044), urinary incontinence (P=0.01), heartburn (P= 0.01), abdominal pain (P=0.01), bloating (P=0.01), dry mouth (P=0.01), excessive bowel movements (P= 0.01), fecal incontinence (P= 0.01), and embarrassment sense (P=0.01), impaired. There was a meaningful difference between erection (P=0.01) and dyspareunia (P=0.001) in the study groups.

Table 4: Evaluation the average life quality of patients based on the QLQ-C29 questionnaire in the study groups

QLQ-C29 questionnaire			**Average**	**Standard deviation**	**Middle**	**At Least**	**At Most**	**p-value**
Function	Body imagination	SPG	65.20	8.12	68.00	40.00	76.00	0.021
		OCG	63.23	5.82	63.00	52.0	74.00	
	Anxiety	SPG	67.63	6.51	70.00	50.00	78.00	0.171
		OCG	65.56	7.86	68.00	40.00	76.00	
	Weigth	SPG	70.40	6.65	70.00	54.00	84.00	0.032
		OCG	67.00	8.29	68.00	40.00	80.00	
	Male sexual function	SPG	76.06	7.85	77.00	54.00	89.00	0.016
		OCG	72.96	6.61	73.50	54.00	83.00	
	Female sexual function	SPG	73.43	6.73	74.00	54.00	87.00	0.072
		OCG	70.83	7.28	72.00	52.00	83.00	
Signs	Frequent urination	SPG	27.30	4.50	27.50	18.00	37.00	0.018
		OCG	29.70	5.97	29.00	18.00	39.00	
	Blood in the stool	SPG	23.43	5.49	22.50	14.00	37.00	0.013
		OCG	26.13	5.45	25.50	18.00	37.00	
	Frequency of stool	SPG	27.20	4.66	28.00	18.00	37.00	0.044
		OCG	25.46	5.20	25.00	17.00	37.00	
	urinary incontinence	SPG	22.03	5.19	21.00	14.00	37.00	0.01
		OCG	27.10	4.52	28.00	18.00	37.00	
	Urinary incontinence	SPG	22.50	4.97	21.00	14.00	37.00	0.01
		OCG	27.93	4.07	28.00	19.00	37.00	
	stomach ache	SPG	25.03	4.99	25.50	18.00	35.00	0.01
		OCG	29.73	7.31	29.00	15.00	42.00	
	Hip Pain	SPG	26.86	6.23	27.50	18.00	40.00	0.125
		OCG	28.93	7.53	29.52	15.00	42.00	
	Feeling bloated	SPG	27.33	5.89	28.00	18.00	40.00	0.01
		OCG	31.96	7.69	32.50	15.00	45.00	
	Dry Mouth	SPG	25.60	6.20	24.50	18.00	40.00	0.01
		OCG	30.80	8.23	30.50	15.00	43.00	
	Hair Loss	SPG	32.06	7.04	32.50	19.00	45.00	0.265
		OCG	30.73	6.21	30.00	20.00	41.00	
	Taste Disorder	SPG	19.10	4.00	20.00	11.00	27.00	0.070
		OCG	18.26	6.43	15.50	11.00	37.00	
	Excessive excretion of intestinal gas	SPG	26.20	4.48	26.00	18.00	37.00	0.01
		OCG	35.46	4.02	36.00	26.00	42.00	

QLQ-C29 questionnaire			Average	Standard deviation	Middle	At Least	At Most	p-value
Signs	Stool incontinence	SPG	23.53	4.64	22.00	15.00	37.00	0.01
		OCG	34.86	4.69	35.00	26.00	41.00	
	Skin Wounds	SPG	25.76	5.96	25.50	16.00	38.00	0.100
		OCG	27.40	4.86	28.00	18.00	37.00	
	Embarrassment	SPG	24.30	5.04	23.50	16.00	37.00	0.01
		OCG	29.16	5.28	28.00	18.00	39.00	
	Erectile dysfunction	SPG	28.90	6.34	28.00	18.00	40.00	0.01
		OCG	32.03	6.13	33.00	20.00	42.00	
	Disparony	SPG	29.93	5.83	28.00	20.00	40.00	0.001
		OCG	33.70	6.24	35.00	20.00	42.00	

DISCUSSION

Annual colorectal cancer, with nearly one million new cases and the death of about 50% of patients in the first five years of disease onset, is a major public health problem of worldwide [15]. This is necessary to study the quality of life in patients with CRC. However, according to some reports, the cases of CRC in our society are younger than the cases in developed societies [16]. This indicates the patient's level of involvement in the disease in terms of working age, and social activities. The mean age of patients in our study is 58.76 ± 9.65 years old, although the mean age of colorectal cancer patients has been reported differently in several studies [17, 18]. The results of our study demonestrated a higher prevalence of the disease among men (60%). Previous studies have shown that the disease is more common in men than women and occurs more at older ages [19, 20]. Studies in Australia, Italy, and the United Kingdom have found that men are more prevalent than women, but American studies have shown that the prevalence is equal in both sexes [17, 21]. The above comparison indicates that the prevalence rates of sex as well as age of disease onset vary in different societies. In our study, most of the patients lived in the city (71.7%). A study in China noted a very rapid and significant increase in the incidence of colorectal cancer in Shanghai, the most industrial city in China, showing a relationship between the incidence of colorectal cancer and industrial life [22]. The results of our study showed no significant difference in the quality-of-life score between the two methods of sphincter maintenance and permanent colostomy. To confirm our results, we can refer to the cross-sectional cohort study conducted by Trenti et al. 2018, noted that 385 patients with middle and lower rectal cancer underwent APR, cancer risk assessment (CRA) and colon anastomosis (CAA) were compared. The results showed that the quality of life of the patients was generally similar in the 3 groups [23]. In 2012, How et al. compared 32 patients with low anterior resection (LAR) and 30 patients with APR in the United Kingdom, and concluded that the APR method was not necessarily associated with a lower quality of life than LAR [24]. Another study conducted by Mariand Sias et al. in the colorectal tumor department of the Camargo Cancer Center in Brazil, they compared 83 patients who underwent inferior or coloanal anastomosis and 42 patients who underwent APR. Quality of life scores did not differ between the two groups, but patients who underwent permanent colostomy had significantly better scores in terms of function and symptoms [25]. In addition, at the University of Aldomord in Italy, Dignar et al. examined 26 patients who underwent APR and 34 patients who underwent CAA colloidal anastomosis, and the quality of life in patients with permanent ostomy compared to patients who underwent CAA. There was no significant difference. However, APR patients had poorer sexual function, while most CAA patients had fecal incontinence [26]. The results of their study are consistent with the results of our study in terms of no significant difference in quality of life between the two groups, but in our study, fecal incontinence as well as male sexual function in patients in sphincter maintenance surgery group compared to patients in permanent colostomy group was better evaluated. The reasons for this discrepancy are the low sample size in these studies and the demographic and age differences between patients in the two studies. Recent study, disclosed differences in quality of life between patients with sexual dysfunction and patients without sexual dysfunction. Sexual dysfunction may be due to pelvic nerve damage or, indirectly, to vascular injury produced by radiotherapy or surgery. In addition, psychological factors can affect these disorders [27, 28]. Similarly, it has been demonstrated that women's sexual function and sexual arousal ability are complex and can easily be affected by factors such as pain [28]. In our study, patients' sexual performance in both men and women after permanent colostomy was worse than sphincter preservation surgery, but this difference was not\significant in women. Based on a review study by Fernández-Martínez et al. loss of normal rectal function, urinary problems, sexual dysfunction, or pelvic pain are frequently reported in patients after rectal surgery, and these abnormalities significantly affect the quality of life [29].

CONCLUSIONS

According to the results of our study and the findings of other studies, the general conclusion that can be drawn from this study is that the quality of life in the two methods of sphincter maintenance surgery and permanent colostomy was not statistically significant. Therefore, treatment decisions should be made by informing patients, both based on their preferences and the physician's clinical judgment, to ensure that patients enjoy an optimal quality of life after treatment. However, due to study limitations (sample size and short-term follow-up), longer follow-up studies with more patients are needed.

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The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Retinal migraine, a contentious clinical entity

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Abstract: *Retinal migraine (ophthalmic migraine, ocular migraine, anterior visual pathway migraine) is a rarely encountered medical condition, but with an interesting and complex clinical presentation. The goal of this article is to compile the literature on retinal migraine, detailing nomenclature, pathophysiology, differential diagnosis, and medical investigations including newer imaging modalities, like optical coherence tomography (OCT). We will approach topics of great concern such as risk factors, complications, treatment, and management of this condition. Retinal migraine is mainly a diagnosis of exclusion and it must be isolated from transient monocular vision loss (TMVL) developed from other causes, for instance, papilledema, giant cell arteritis, carotid artery disease (amaurosis fugax), intermittent angle closure glaucoma, and others. Its treatment is similar to migraine treatment; topiramate, amitriptyline, and nortriptyline are some of the pharmacological drugs successfully used.*

Keywords: *ocular migraine, arterial vasospasm, retinal migraine, monocular vision loss, visual phenomena, ophthalmic migraine, anterior visual pathway migraine, scotoma, scintillation, headache*

INTRODUCTION

Retinal migraine is a debatable disorder, that was firstly introduced by Galezowski in 1882 who used the term ‘‘ophthalmic megrim’’ to describe a permanent unilateral loss of vision associated with headache [1]. The official term, “retinal migraine” was introduced in 1970 by Carroll [2] and the authors have used it to describe a multitude of monocular visual symptoms, including events without the associated headache and those resulting in persistent visual loss. The evolution of terminology accepts retinal migraine, ophthalmic migraine, ocular migraine, and anterior visual pathway migraine, as the same notion, but all of these should not be confused with ophthalmoplegic migraine, which is manifest by ophthalmoplegia and unilateral headache [3].

The International Classification of Headache Disorders (ICHD) published 1988 an international definition for this condition [4] and following the 2018 ICHD-3 updates, ocular migraine is defined as fully reversible and repeated attacks of unilateral visual manifestations, such as scotoma, scintillation, or even blindness, associated with headache [5].

Furthermore, because it is not a common entity and there are many other potential causes of monocular visual loss, retinal migraine is considered a diagnosis of exclusion [6] and a careful evaluation is needed because it can share characteristics with migraine with aura and acephalgic migraine, which can create confusion in their differentiation [7]. In some articles was also suggested the term “migraine associated with monocular visual symptoms” was to differentiate it between the loss of vision in one hemifield and that of one eye which includes sites other than the

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retina, such as the choroid or the optic nerve [8].

RETINAL MIGRAINE-WERE WE ARE?

Epidemiology

The lack of reported cases, the paucity of existing literature, and the continuously evolving diagnostic criteria made the understanding of this condition's epidemiology challenging, moreover, some authors suggested that there's no epidemiologic data specific to ocular migraine [7]. While migraine is a common disorder, with a prevalence of 18.2% in female patients and 6.5% in male patients, with a higher prevalence in whites (20%) than blacks (16%) and Asian Americans (9%) [9], there were indicated only 12 cases of retinal migraine, that followed the ICHD diagnosis criteria in the last 15 years [6], between 2006 and 2020. Of 12 patients, 10 were women with an average age of 39.5 years, 11 out of 12 had a previous history of migraine headaches, 4 patients reported a history of migraine with aura and a positive family history of migraine was present in 4 of the 8 patients for which that information is available [6].

Even though based on the statistics, retinal migraine tends to affect women more so than men [6] and begins in the second to third decades, with peak prevalence in the fourth and fifth decades [10], it can also affect children, in which 7% of migraines are thought to be ocular [11] and the onset of ocular migraine may occur as late as the fifth decade, including patients with no previous history of migraine [12]. It is crucial for this last age group (>50 years of age) to consider retinal migraine a diagnosis of exclusion and make a careful cardiac and carotid evaluation, because atherosclerosis, collagen vascular disease, and hypercoagulable states are just a few examples of diseases that can explain the visual disturbances on elderly patients. A genetic predisposition is suggested by the strong family history of ocular migraine, but no clear inheritance patterns have been delineated, however, 50% of patients have a family history of some type of migraine [13,14]. In patients with familial hemiplegic migraine have been found calcium channel mutations such as 19p13 CACNA1A, ATP1A2, SCN1A, mutation appeared in siblings with migraine with aura at a statistically significant occurrence [15]. Moreover, some patients, during and between migraine episodes have been shown high levels of endothelin-1, a potent vasoconstrictor peptide, which in these cases, had significant polymorphism for a receptor found to mediate vasoconstriction [16]. However, its effect on ocular migraine is considered to be minimal because the blood-brain barrier prevents endothelin entry into the retinal circulation [7]. Finally, it is considered that retinal migraine is a less benign disorder than migraine with aura due to the higher risk for patients with transient monocular visual loss to develop permanent monocular visual loss [8].

Pathophysiology

Despite that multiple theories have been proposed, the pathophysiology of ocular migraine remains controversial because this complex disorder which associates headache and monocular visual disturbances is not fully understood. In first, the cephalgia that also appears in common migraine and in migraine with aura, is considered as trigeminal nerve activation by an unknown trigger [17]. Even if, in migraine patients, the trigeminal nerve seems to produce higher levels of glutamate onto the circle of Willis, the primary event remains unclear [18]. It is also suggested that the potential cause of inflammation and pain may be represented by a series of substances and peptides, such as bradykinin, substance P, calcitonin gene-related peptide (CGRP), and nitric oxide [19]. Secondly, to explain the monocular visual loss, we will mention the vasospastic theory that was firstly introduced by Carroll in 1970 when he exposed the hypothesis that the mechanism of visual symptoms is due to "paroxysmal constriction or spasm in either the central retinal artery or ophthalmic artery" [2]. It seems that serotonin and stimuli such as food may induce the production of prostaglandin, causing disturbance of cerebral autoregulation and platelet aggregation [20]. Once axons are excited, neurogenic and vascular inflammation (especially the inflammation of the retinal and ophthalmic vessels) may cause vasospasm, obstruction, and ocular sympathetic dysfunction which can explain the visual symptoms [7]. Serotonin may have a major role in ocular migraine because it has been measured that it is produced by platelets in increased levels in aura phases of migraine, mediating intracranial vasoconstriction [21], and, furthermore, in patients with atherosclerosis, it reduces blood flow to the choroid and retina while the intracranial blood flow is not significantly affected [22]. However, other authors consider that the vascular theory is nowadays largely rejected by new cerebral blood flow imaging techniques [23] and it is indicated a series of potential mechanisms including cortical spreading depolarization, brainstem and hypothalamic activation, alteration in thalamo-cortical circuits or neuropeptide production (pituitary adenylate cyclase activating polypeptide and calcitonin gene-related peptide) [24]. None of these new hypotheses is confirmed. The last theory, cortical spreading depression (CSD) of retinal neurons, is indicated by several articles as a plausible explanation, but it hasn't been demonstrated in vivo or in any animal with retinal vasculature. The phenomenon can be exposed as an "excitation wave followed by depression of neuronal activity propagating through gray matter with a velocity of approximately 3 mm/min" [25], as it is described by Leao in 1944. Based on magneto-encephalographic studies, CSD is suggested to be the biological explanation for the occipital aura that affects both eyes and precedes headache in migraine with aura patients [26]. Retinal SD has only been studied on chick or frog retina, and never been demonstrated in mammals, furthermore, the isolated retina in animal experiments is not representative of the human eye in vivo, which is vascularized and supported by the vascular choroid [27].

Clinical presentation

According to literature, where has been reported a limited number of cases of retinal migraine that followed ICHD diagnosis criteria, the patients, which are usually females, in the second to third decade of life, with family history of migraine and previous history of migraine with aura, present to the specialist with unilateral visual loss that can last even few seconds or between 5 to 30 minutes, and can be preceded, accompanied or followed by ipsilateral headache [6]. The visual disturbances include positive or negative phenomena that are fully reversible, but a majority of patients (>70%) has repeated attacks [13]. There have been described positive phenomena such as zigzag lightning, diagonal lines, halos, and scintillation [7], while the negative ones are described by the patients as black dots or spots, blurring, blank areas, and complex patterns are less common [8]. It is also considered that benign episodic pupillary dilation, an under-reported ophthalmologic sign, might be connected with ocular migraine [7]. All these monocular visual symptoms can be caused by any process that affects the optic nerve or the ocular blood flow and therefore a normal neuro-ophthalmic exam is crucial to exclude any other causes of transient monocular visual loss [28]. The majority of the patients has a good prognosis with a full recovery and permanent visual loss is uncommon.

Differential diagnosis

The diagnosis of retinal migraine is by exclusion [29]. The patients accusing transient monocular vision loss (TMVL), who fits the criteria for retinal migraine according to ICHD-3 must undergo a series of further investigations in order to exclude alternative diagnoses such as: vascular disease, ocular disease or optic nerve disorders. ICHD-3 criteria require positive and/or negative visual phenomena. These are monocular, fully reversible and are confirmed by clinical visual field examination and the patient's drawing of a monocular field defect. Additionally, it should meet two out of three criteria of the following: spreading gradually over 5 minutes or more; symptoms lasting between 5 and 60 minutes; headache present simultaneously, or following the sympyoms within 60 minutes. Vascular diseases that mimic TMVL are carotid artery disease, central retinal artery occlusion, giant cell arteritis (affects large and medium sized arteries), choroidal ischemia (originating from posterior ciliary arteries or arteritis processes affecting the short posterior ciliary arteries). There are some conditions affecting the optic nerve that must be taken into consideration, for example, Uhtoff's phenomenon (can be induced by exercise) from demyelinating disease, papilledema, and compression of the optic nerve [30]. Amaurosis fugax appears as a result of the stenosis or occlusion of the internal carotid artery (caused by hypoperfusion from the stenosis or thromboembolism from the carotid circulation) [31]. Interrupted circulation to the ophthalmic artery or central retinal artery can manifest through a blackout in the entire or only half of the visual field. Patients can accuse a "curtain sweeping in from one side of the vision" and it usually takes between a few minutes and an hour [30]. Amaurosis lasting seconds is commonly found in disc edema (papiloedema), whereas painless amaurosis lasting less than 15 minutes occurs typically from an embolic source (cholesterol, platelets, calcium). Furthermore, dry eyes and intermittent angle closure glaucoma needs to be excluded since they are causes of TMVL as well [30]. To differentiate retinal migraine from other types of visual loss it is important to establish if the issue affects one or both eyes and to define whether the visual loss is monocular or in one hemifield. If the problem is binocular, it is likely a retrochiasmatic injury. In contrast, if the loss of vision concerns one eye it can be as a result of prechiasmatic lesions. It can be problematic for patients to recognize hemianopias from monocular vision loss, therefore the physician needs to guide them in this regard.

Investigations

The evaluation of a patient with acute monocular vision loss begins with a thoroughly medical history. Data including patient's age, history of vascular risk factors, cancer, autoimmune diseases is essential in establishing a proper differential diagnosis. Crucial information regarding the episode of vision loss is frequency, characteristics and duration [30]. It is important to check if the patient has a positive medical history for other types of migraine and if exercises precipitate the attacks [32]. Patients must complete formal visual field assessments in search of visual field defects [6] including negative symptoms, for instance, altitudinal vision loss, central scotoma and sectorial loss [30]. Other visual manifestations that may appear are positive symptoms like scintillations, flashes of color, light, stars, and heat waves [7]. A detailed retinal examination following pharmacologic pupillary dilation should be done, because information regarding the appearance of the retina and optic disc are fundamental to make an accurate diagnosis [33]. Retinal arterioles can be constricted and the fovea may become more distinct with surrounding macular retinal pallor. The optic disc may be pale early and hyperaemic later. Nerve fibre bundle defect can sometimes be a late finding [34]. Newer imaging modalities, such as optical coherence tomography (OCT), a non-invasive imaging test that uses light waves to take cross-section pictures of your retina may be helpful [30]. Ophthalmological examination is expected to be normal outside of attacks. Besides echocardiogram and auscultation of carotid arteries and heart, carotid Doppler is required to exclude vasculo-oclusive diseases [30], a Holter monitor if an arrhythmia is suspected [29] and transesophageal echocardiography to check all the valves and chambers in more detail [7]. Laboratory evaluation is required for diagnostic possibilities such as giant cell arteritis, collagen vascular disease, hypercoagulable states, vasculitis, and illicit drug use. Neuroimaging can exclude an orbital or intracranial mass [6].

Complications

Although it is uncommon [7], some of the complications of retinal migraine can lead to permanent loss of vision [35]. Complications reported in the literature include: “reversible and irreversible central retinal artery occlusion (CRAO), branch retinal artery occlusion (BRAO), retinal infarction” [36], retinal hemorrhages, vitreous hemorrhage, ischemia of choroid or optic nerve [35] dilatation of retinal veins and retinal pigmentary changes [3]. Optic nerve drusen are refractive, calcified nodules located within the optic disc [37]. They make the retinal vasculature more vulnerable to ischemia and lead to irreversible BRAO [38].

Risk factors

Similar to migraine, risk factors for retinal migraine are represented by stress, smoking, hypertension, and contraceptive pills. It can also be precipitated by exercise, bending over, high altitude, dehydration, hypoglycemia and excessive heat load, although dietary factors (caffeine, alcohol, tyramine, monosodium glutamate, and aspartame), traumas and hypoglycemic factors are not as influential [39]. Vascular instability plays a significant role in the pathogenesis of ocular migraine. This stands out through the co-occurrence of retinal migraine and vascular diseases such as Prinzmetal’s angina and Reynaud’s phenomenon. Moreover, it is documented that the comorbidity with lupus, sickle cell disease and atheromatous disease increases the risk of developing retinal migraine [35]. The risk for stoke in patients with atherosclerosis is 1% at 1 year; whereas patients with stenosis of internal carotid artery presents a risk of 16% of ipsilateral stroke after 3 years [40].

Prophylactic treatment

In the first place, it is essential to keep under control stress factors, hypertension and to reduce, or even quit smoking. Patients should avoid triggers like hormonal contraceptive pills or exercises (if they induce amaurosis). It helps if the patients keep a diary in order to keep track of all the factors who lead to an attack, in this way it is easier to avoid them. Pharmacological treatment is required if the life style changes are not effective. Prophylactic therapy consists in calcium channel blockers (nifedipine, verapamil), nonsteroidal anti-inflammatory drugs (aspirin, dipyridamole and sulfinpyrazone if aspirin intolerant), anticoagulants (coumadin, heparin), antiepileptics (divalproex sodium/ sodium valproate, gabapentin, topiramate), antidepressants (tricyclics- amitriptyline, nortriptyline, imipramine; selective serotonin reuptake inhibitors) [7,35]. Although nifedipine, and especially verapamil are the most effective drugs in this case, there are some contraindications to calcium channel blockers including congestive heart failure, hypotension, sick sinus syndrome, cardiac conductive defects, concomitant, and renal or hepatic failure [35]. Aspirin is used for its antiplatelet activity [8] and, along with nifedipine, it is used to prevent exercise induced retinal migraine [12]. Topiramate, amitriptyline, and nortriptyline are some agents used in classical migraine that have been demonstrated effective in retinal migraine as well [6]. It is usually recommended to avoid beta blockers in ocular migraine due to its properties of arteriolar constriction [31].

Abortive treatment

It is important to avoid medication overuse by matching treatment to patient needs. Given the unsufficient number of studied cases in literature, treatment options for ophthalmic migraine are limited. According to some authors, it is enough to manage the manifestations of retinal migraine by observations alone [41,42]. Due to the short duration of the attack, abortive treatment is uncommon and the main focus of treatment should be to reduce the recurrence of attacks [35]. Regarding management, triptans and calcium channel blockers can be used as abortive pharmacological treatment. Triptans are more effective on migraine without aura, but may be successful for patients in whom other therapies do not work and who have no other risk factors for ischemic heart disease or stroke [7].

Calcium channel blockers were demonstrated effective in both prophylactic and abortive situations [43]. There have been reported two cases in literature that advocated the use of sublingual nifedipine (10 mg) for treating attacks (both patients diagnosed with systemic lupus erythematosus) [44]. During attacks, inhaled amyl nitrate and isoproterenol showed “good efficacy”, while inhaled carbon dioxide improved vision ‘slightly’ in one patient [45]. Successful treatment has been reported for coumadin and heparin used in isolated cases of patients with antiphospholipid antibody syndrome and retinal migraine [35,46]. Other medications such acetazolamide (500 mg) and sublingual nitroglycerine (0.8 mg) were effective [47]. Systemic corticoid therapy was successfully used in patients with systemic lupus erythematosus [48].

CONCLUSIONS

Retinal migraine is a rare, but increasingly recognized clinical condition. The pathophysiological mechanisms are still unclear. It is a cause of TMVL and uncommon, during prolonged periods of hypoxia, it can lead to permanent loss of vision. Retinal infarction is more susceptible in patients with anomalous optic discs or intrapapillary drusen may render the patient more susceptible to retinal infarction Researches show that retinal migraine may successfully respond to usual migraine prophylactic medications, but a current guideline is missing. It is also important to attempt to change the lifestyle factors that precipitate this condition, before initiating medical treatment. Its prognosis is a good one overall. Unfortunately, there is not enough research in this distinct clinical entity, hence, it is difficult to determine an optimal treatment regimen.

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REVIEW

Analysis of neuroprotective medication in patients with neurocognitive disorders: The efficacy and tolerability of highly purified animal tissues extracts

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Abstract: *Neurocognitive disorders are extremely invalidating psychiatric disorders with chronic courses and significant negative impacts over all areas of cognitive functioning and behavioral activity. Although extensive research on these progressive neurodegenerative disorders has been conducted, pathogenetic treatments with long-term significant benefits are yet controversial. From a clinical perspective, there is an acute need to find therapeutic strategies that could delay cognitive impairment in patients diagnosed with Alzheimer's disease (AD), vascular dementia (VaD), Lewy body dementia (LBD), etc. Also, slowing the transition from mild cognitive impairment (MCI) to clinically significant AD is another important clinical aspect, with a major impact on the patient's daily functioning, quality of life, and caregivers' burden. Acetylcholinesterase inhibitors (AChEI) are still the first line of treatment in AD patients, and they are also administered in the case of VaD or Parkinson's dementia. Various nootropics have been studied in this population, as add-on agents. Highly purified animal tissue extracts (HPATE) are administered in patients with neurocognitive disorders due to their neurotrophic properties, but many questions remain unanswered regarding their pharmacodynamic characteristics. These extracts may be added to AChEI to enhance their pro-cognitive effect, but evidence to support the superior efficacy of this association versus AChEI monotherapy is mainly derived from low-to-medium quality clinical trials. In conclusion, HPATE may be a useful add-on to first-line pro-cognitive agents in AD and VaD, but larger trials with better methodology are needed. In particular cases, however, HPATE may be of significant interest for patients with mild-to-moderate AD, based on results from clinical practice.*

Keywords: *Alzheimer's disease, vascular dementia, ischemic stroke, Actovegin, Cerebrolysin, neurotrophic factors*

INTRODUCTION

Neurocognitive disorders are associated with significant impairment in daily functioning and a high degree of depression, anxiety, and negative symptoms, which decrease, even more, the overall level of functioning in these patients [1,2]. The management of the neurocognitive disorder and associated non-cognitive symptoms is a challenge for clinicians and caregivers, due to the relatively low number of available, evidence-based, efficient pharmacological agents [3-5]. Also, special care should be taken concerning adverse events in a population with possible organic comorbidities and multiple concomitant medications [3]. Another important aspect in this population is the low overall efficacy of acetylcholinesterase inhibitors (AChEIs), which may not even be clinically significant in long term [6,7]. Still, the differences in the effect of individual AChEIs over cognitive functioning might be significant, and these differences may be modulated by yet insufficiently described patients' characteristics [6,7].

Highly purified animal tissue extracts (HPATE), based on neurotrophic factors or blood components, are used to treat the consequences of acute cerebrovascular events or traumatic brain injury, but also for the therapeutic management of neurodegenerative disorders [8-10]. The prophylactic properties of such products in the domain of brain circulatory and trophic diseases, but also for peripheral vessels diseases, allow these products to be recommended for yet another indication, e.g. Actovegin is approved to be used for skin grafts, bedsores, skin burns, etc [10]. Several HPATE are currently marketed, but the most extensively

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researched agents for neurocognitive disorders treatment are Cerebrolysin and Actovegin. Although HPATE is not widely recognized as an efficient therapeutic intervention for dementias, multiple trials (reviewed below) support their efficacy in patients with mild cognitive impairment or clinically manifest dementia. Treatment with neurotrophic agents might enhance the effects of AChEIs, which are considered the first-line treatment for Alzheimer's disease (AD) [11].

In a comparative analysis of Cerebrolysin (1 or 3 mg/kg/day), Actovegin (200 mg/kg/day), and Cortexin (538 or 1614 mg/kg/day) in rodent models of acute and chronic brain ischemia, these agents were administered for 10 days [8]. Cortexin, Cerebrolysin, and Actovegin may be efficient in improving the recovery of neurological functions, by decreasing the severity of sensorimotor and cognitive impairments in preclinical models of brain ischemia [8].

OBJECTIVE

The main objective of this paper was to explore the available data in the literature regarding the efficacy and tolerability of Cerebrolysin and Actovegin in patients with neurocognitive disorders, mainly AD and vascular dementia (VaD). An inclusion criterion for the trials in this review was the existence of a structured approach for the monitoring of HPATE efficacy and/or tolerability.

The secondary objective of this review was the collection of data about the possible mechanisms of action of HPATE, based on preclinical models of AD, VaD, or acute cerebral ischemia.

Augmenting the effects of AChEIs with neurotrophic agents is frequently tried in clinical practice, because of their long-term potentially synergistic effects in the treatment of neurocognitive disorders. Therefore, illustrating the clinical use of these two products in a case series was yet another objective of this paper.

METHODS

A literature review was performed, using the search paradigm "Cerebrolysin" OR "Actovegin" OR "neuroprotective medication" AND "Alzheimer's disease" OR "Alzheimer dementia" OR "vascular dementia" OR "neurocognitive disorders". All papers published between January 1982 and February 2022 were included in the primary search. The databases included in this search were PubMed, Cochrane, Web of Science, EMBASE, and CINAHL.

A case series was presented in the second part of this article as a way to illustrate the possible pragmatic, clinical impact of HPATE on the evolution and prognosis of patients diagnosed with AD. These patients were randomly selected from our Psychiatry department database, and their evolution under treatment was described based on their chart review and post-discharge monitoring visits data.

RESULTS OF THE LITERATURE REVIEW

Some 145 papers were found during the primary search and 13 remained after de-duplication, elimination of papers which does not correspond to the main objective of this review, and of papers with insufficient accuracy of the methodology applied (i.e., not using validated instruments for efficacy monitoring, not specifying main outcomes or heterogenous population included form the principal diagnosis perspective). Five papers refer to the efficacy and/or tolerability of Actovegin in post-stroke cognitive impairment, AD, or organic brain syndrome, while 8 papers included data about Cerebrolysin effects in patients with AD, mild cognitive impairment, or VaD [11-21]. Out of these 13 papers, 12 included clinical trials, and one was a review.

Regarding the secondary objective of this review, 3 papers were found, one review (Actovegin), one animal study, and one in-vitro study (Cerebrolysin) [22-24].

Actovegin is a biological drug manufactured by ultrafiltration from calf blood, and the result is a deproteinated, pyrogen- and antigen-free hemodialysate [25]. The final product contains a mixture of natural substances, over 200 bioactive constituents with low molecular weight, both inorganic- e.g., blood electrolytes, and organic components- e.g., amino acids, oligopeptides, nucleosides, etc. [25,26]. Actovegin presents complex pharmacodynamics properties, as it may target the enhancement of cellular metabolism, with increased oxygen uptake and utilization, as well as glucose uptake in the cells [25]. This enhancement derives from an insulin-like activity mediated by inositol-phospho-oligosaccharides, therefore Actovegin is recommended for cerebral blood circulation dysfunctions and trophic disturbances, impairments of peripheral blood circulation and associated diseases, dermal transplants, and acute chronic wounds [25]. Actovegin also promotes oxidative metabolism and shifts the redox-balance of the cells into the direction of oxidized substrates, increasing the availability of energy-rich phosphates like ATP and creatine phosphate [25]. Preclinical data support a positive influence of Actovegin on the NF-κB pathway, but many molecular and cellular pathways remain unexplored [26].

According to a systematic review of Actovegin in ischemic stroke (n=5 articles), this drug acts over ischemic stroke lesions ensemble, issue oxidation, energy metabolism, and glucose availability through their augmentation, mitigation of the ischemic process and oxidative stress, and decreasing of inflammation and apoptosis-like processes [22]. Actovegin has anti-apoptotic effects by reducing the caspase-3 activation induced by amyloid β-peptides [22].

Actovegin has been used in clinical practice for more than six decades for various indications, and its safety and tolerability profile is considered very well [27]. Its clinical efficacy and favorable tolerability profile were proven in patients diagnosed with mixed dementia [14,27]. Patients aged 50-85, diagnosed with mild-to-moderate degenerative dementia or multi-infarct dementia received either Actovegin 250 ml 20% i.v. or a placebo for 4 weeks [14]. Actovegin had a significantly superior efficacy vs. placebo, according to the Clinical Global Impression (CGI) and Syndrome Short Test (SKT)- improvements with 32% vs. 4% (active drug vs. placebo) and -2.8 vs. -0.4 (active drug vs. placebo), respectively [14].

The efficacy of Actovegin in patients with poststroke cognitive impairment was evaluated in a 12-month, parallel-group, randomized, multicenter, double-blind, placebo-controlled study, which included patients ≥60 years old with a Montreal Cognitive Assessment test ≤25 points [12]. Actovegin was administered as 2000 mg/day for ≤20 intravenous infusions followed by 1200 mg/day orally or placebo for 6 months, in 503 patients [12]. The least squares mean change from baseline to month 6 in Alzheimer's Disease Assessment Scale- cognitive subscale (ADAS-Cog), extended version, was -6.8 for Actovegin vs. -4.6 for placebo, a significant difference [12]. The most frequently reported serious adverse event was a recurrent ischemic stroke, but without differences between active drug and placebo [12].

A double-blind, randomized clinical trial had as its main objective to compare the therapeutic effects of Actovegin vs. placebo in elderly patients with organic brain syndrome (N=60) [13]. Actovegin was administered as daily infusions of 250 ml 20% in 40 geriatric patients, and 20 patients received 250 ml 0.9% saline solution as a placebo, for 4 weeks [13]. These patients were diagnosed with simple dementia (58%) or senile dementia with depressive or paranoid symptoms (ICD-9) [13]. The mean Sandoz Clinical Assessment Geriatric Scale (SCAG) score in the drug group decreased from 56.3 at baseline to 36.3 points at the last trial visit in the Actovegin group, and from 61.2 to 52.0 ($p<0.01$) in the placebo group [13]. On the CGI, Actovegin administration led to "distinct improvement" or "improvement" in 70% of cases vs. only 35% in the placebo group [13]. SKT and subscales of SCAG scores supported the superior effects of Actovegin compared to the placebo, as did social behavior measurements [13].

A group of 43 patients with mild-moderate ischemic stroke received either Actovegin (N=32) or piracetam (N=11) and the analysis of their neuropsychological performances showed positive effects of the active drug over general cerebral and focal neurological symptoms after 30 days [15]. By the end of the study, the extent of recovery of impaired functions, assessed in terms of total ischemic points and cognitive functions, was significantly greater in patients treated with Actovegin than in those given piracetam [15]. Another study (N=104 patients with acute carotid ischemic stroke) evaluated comparatively the efficacy of (1) basic and reperfusion therapy without any cytoprotection, (2) citicoline 1000 mg/day, and (3) Actovegin 250 ml/day [16]. A significant decrease in neurological deficit and improvement of functional recovery was reported in patients treated both with citicoline and Actovegin on the 30th day vs. the control group [16].

In conclusion, the effect of Actovegin on cognitive function (either post-stroke cognitive impairment or AD) was evaluated in only two placebo-controlled trials and one piracetam-controlled trial [12,13,15], which included 606 patients. These trials used different scales for the quantification of the active drug's efficacy, but the results were favorable and the overall tolerability was good.

Cerebrolysin is a neuropeptide product mimicking the pharmacodynamics action of endogenous neurotrophic factors, and its positive effects on β-amyloid and τ-related pathologies have been demonstrated in experimental conditions [28]. It exerts nerve growth factor (NGF)-like activity on neurons and increases levels of NGF vs. controls in the hippocampus, in studies with animal models (mice) which overexpressed the human amyloid precursor protein (hAPP) [29]. Also, Cerebrolysin had a favorable impact on neuroinflammation, neurotrophic factors, oxidative stress, excitotoxicity, neurotransmission, brain metabolism, neuroplasticity, neuronal apoptosis and degeneration, neurogenesis, and cognition in animal models [28]. The presumed mechanism of Cerebrolysin in AD involves the activation of phosphatidylinositol 3-kinase, AKT, and glycogen synthase kinase-3β intracellular signaling pathway [28]. Cerebrolysin reduced amyloid plaque formation by regulating APP, and inhibits calpain, caspase, Bcl-2, and upregulates Bax, protecting neurons from apoptosis [20,30,31].

The clinical efficacy of Cerebrolysin was demonstrated over global functioning and cognition in several clinical trials, and improvements in behavior at high doses, or minor effects on daily living activities were also reported in these patients [28]. The most consistent benefit was observed in mild-to-moderate AD patients, but also several subgroups of moderate to moderate-severe patients [28]. Clinical benefits of Cerebrolysin were preserved for several months after the last dose administration, therefore the recommendation of a discontinuous administration regimen is supported by evidence [28].

Cerebrolysin reversed the reserpine-induced reduction in latency to immobility and prolongation of immobility time in the forced swimming test, decreased tumor necrosis factor-alpha (TNF-α) levels, elevated glutathione(GSH) levels, and BDNF cortical and hippocampal brain concentration, in animal models of depression [23]. Cerebrolysin elevated protein kinase A and nuclear factor kappa-B (NF-κB) cortical and hippocampal protein expression [23]. Other effects of Cerebrolysin detected on animals (rats) were: amelioration of mRNA expression alterations of protein-kinase B (AKT), CREB, and BDNF in the cortical and hippocampal tissues; elevation of norepinephrine, serotonin, and dopamine levels in cortical and hippocampal tissues; antidepressant effects, as well as attenuation of oxidative stress and inflammation [23].

A study that evaluated in-vitro human cerebral endothelial cell permeability during Cerebrolysin administration showed that tissue plasminogen activator (tPA)- and fibrin-impairments of endothelial permeability were significantly diminished and even reversed by this agent [24]. Cerebrolysin reduced tPA- and fibrin-augmented proinflammatory and pro-coagulation proteins, also [24]. Unlike the cerebroprotein hydrolysate, with a distinct peptide composition, Cerebrolysin had favorable effects on tPA- and fibrin-impaired permeability and elevated tight junction proteins, previously affected by experimental conditions [24].

In a dose-finding clinical trial, Cerebrolysin was administered in patients with moderate-to-moderately-severe (N=133, Mini Mental State Examination - MMSE=14-20) or mild (N=118, MMSE=21-25) AD [17]. Participants received Cerebrolysin, administered as 10, 30, or 60 ml diluted in saline solution, or placebo over 12 weeks (5 days per week for 4 weeks, and 2 days per week for another 8 weeks) and were monitored for 24 weeks [17]. At week 24, Cerebrolysin improved significantly the global clinical functioning with all 3 dosages and induced significant improvements in cognition, initiation of daily living activities, and neuropsychiatric symptoms [17]. However, treatment effects on total activities of daily living (ADL), and other secondary parameters (MMSE, Trail-making test) were not significant [17]. The overall treatment tolerability was good, and no safety issues have been reported [17].

A double-blind, randomized, placebo-controlled, multi-center, 4-week clinical trial enrolled 53 patients aged at least 50 years, admitted to a hospital with mild-to-moderate AD and no severe organic comorbidities [18]. The treatment group received Cerebrolysin 30 ml in 100 ml physiologic saline i.v. once daily 5 times/week, for 4 weeks [18]. After 4 weeks of treatment, Cerebrolysin-treated patients presented significant improvements in the ADAS-Cog, CGI-S, and MMSE scores vs. placebo-treated patients [18]. The rates of improvement were 82%, 62%, and 44% on ADAS-Cog, CGI-S, and MMSE in patients treated with Cerebrolysin, compared to 31.6%, 22%, and 17% in placebo-treated patients [18]. No significant improvement in the Cerebrolysin group was detected compared to the placebo group on the Geriatric Depression Scale (GDS), ADL (Activities of Daily Living), and IADL (Instrumental Activities of Daily Living) [18]. Only one case of febrile sensation was reported, but it was mild in severity and transient [18].

Another randomized, double-blind, placebo-controlled multicenter trial enrolled 242 patients diagnosed with VaD, and the primary outcome was the combined cognition and overall clinical functioning score (ADAS-Cog+ plus CIBIC+-Clinician's Interview-Based Impression of Change plus Caregiver Input) assessed after 24 weeks of treatment [19]. At the end of the study, ADAS-Cog+ improved with 10.6 points in the Cerebrolysin group vs. 4.4 points in the placebo group (significant difference, $p<0.0001$) [19]. CIBIC+ showed a mean improvement of 2.84 in the active treatment arm and 3.68 in the placebo arm, a statistically significant difference ($p<0.0001$ vs. placebo) [19]. Higher rates of responsiveness were detected in the Cerebrolysin group, as reflected by the combined ADAS-Cog+ and CIBIC+ (67.5% vs. 27.0%), and the odds ratio for achieving a favorable response using this combined score was 5.63 ($p<0.05$) [19].

According to a Cochrane systematic review dedicated to Cerebrolysin treatment in people with VaD (n=6 randomized controlled trials, N=597 participants), mainly mild to moderate forms (n=4 trials), cognitive function had a more favorable evolution during Cerebrolysin treatment (MMSE, ADAS-Cog+), although these observations were based on very low-quality evidence [20]. When response rates based on global function (CIBIC+, CGI) assessments were included, there was a beneficial effect of Cerebrolysin (n=2 studies, N=379 participants) [20]. Four trials reported adverse events, and data from two studies (N=379 participants) included in a meta-analytic format did not report any difference between rates of adverse events (very low quality of evidence) [20].

A prospective, open-label trial, a 2-year course of Cerebrolysin therapy, administered as repeated sessions every 6 months, in patients with a mild cognitive impairment-amnestic type (N=73 participants), had as its main objective to explore if this type of intervention may slow down or prevent the transition to clinically relevant dementia [21]. The results at 2 years suggest the existence of a slower cognitive deterioration in patients who received Cerebrolysin, compared to those who did not [21].

A comparison of the safety and efficacy of Cerebrolysin (10 ml), donepezil (10 mg), and a combination of both treatments (N=64, 66, and 67, respectively) in mild-to-moderate probable AD, in a randomized, double-blind trial, concluded there were no significant differences in cognitive, functional, and behavioral domains between interventions [11]. Improvements in the global outcome, however, favored Cerebrolysin and the combined therapy at week 28 [11]. Cognitive performance was also superior at the end-point in the combined treatment group more than in the other two groups, but the difference was not statistically significant [11]. The overall tolerability of the combined treatment was good [11].

Administration of Cerebrolysin i.v. infusions (10 or 30 ml) or placebo (normal saline) 5 days/week for 4 weeks in patients with mild-to-moderate probable VaD (N=41 participants) led to significant improvements of cognitive performance at 10 ml dose and diminished the EEG slowing with both dose regimes [32]. Also, a significant correlation between fast EEG power ratio and ADAS-Cog scores change from baseline to endpoint was observed in Cerebrolysin-treated patients [32]. The extension of this 4-week trial, which enrolled 33 out of the 41 initial participants, showed that at week 16, Cerebrolysin improved significantly cognitive performance at 10 and 30 ml, and also reduced qEEG significantly at 30 ml dose [33]. These results suggest the persistence of beneficial effects of Cerebrolysin on cognition and qEEG activity in VaD patients for at least 12 weeks after treatment administration [33].

In conclusion, most of the reviewed data on clinical trials supported the efficacy of Cerebrolysin (n=6 trials, N=699 patients) in AD and VaD, but their efficacy was not consistent across all the evaluated cognitive or functioning domains [11,17-19,32,33]. However, the cognitive function was consistently favorably influenced by Cerebrolysin, and a systematic review confirmed this effect [11,17-20,32,33]. Although the rate of conversion from mild cognitive impairment to AD under Cerebrolysin decreased in one trial, there is an obvious need to replicate these data, and the open-label design of this trial raises supplementary questions about the generalizability of its results [21].

Regarding the secondary objective of the review, preclinical studies indicate multiple hypotheses about the mechanisms of action for HPATE, mostly based on mitigation of the ischemic process and oxidative stress, a decrease of inflammation and apoptosis-like processes in case of stroke (Actovegin); also, HPATE may increase BDNF cortical and hippocampal brain concentration, while decreasing the oxidative stress and inflammation (Cerebrolysin) [22-24].

Table 1: Case series – demographics, baseline values of structured instruments administration, and treatment schedule

Patient no.	Age	Gender	Severity (baseline)	Treatment
1	68	Male	ADAS-Cog: 51 MMSE: 16 CDT: 4 NPI total score: 14 NPI distress score: 12 CGI-S: 3 GAF: 35	Donepezil 10 mg/day 12 months + Cerebrolysin 20 ml/day 10 days/month, 3 months, repeated after 3 months free of HPATE
2	72	Male	ADAS-Cog: 40 MMSE: 19 CDT: 5 NPI total score: 22 NPI distress score: 16 CGI-S: 4 GAF: 40	Galantamine 16 mg/day 12 months+ Actovegin 10 ml/day 10 days, followed by 400 mg q.d. orally 6 weeks, repeated after 3 months free of HPATE
3	80	Female	ADAS-Cog: 58 MMSE: 14 CDT: 1 NPI total score: 20 NPI distress score: 14 CGI-S: 3 GAF: 30	Rivastigmine 13.3 mg/day 12 months + Cerebrolysin 20 ml/day 10 days/month, 3 months, repeated after 3 months free of HPATE

ADAS-Cog = Alzheimer's Disease Assessment Scale - cognitive subscale, MMSE = Mini Mental State Examination, NPI= Neuropsychiatric Inventory, CGI-S = Clinical Global Impression - Severity, GAF = Global Assessment of Functioning, CDT = Clock drawing Test

CASE SERIES

This case series is based on repeated evaluations of 3 patients (two male, one female, mean age 73.3 years), all of them being diagnosed with Major Neurocognitive Disorder due to AD (probable), according to the DSM-5 criteria [34]. These patients presented memory, learning, and executive dysfunctions, accompanied by the gradual decline of these cognitive functions for at least one year before their baseline psychiatric evaluation, and no evidence of mixed etiology.

All patients were evaluated using brain MRI/CT scans, blood analyses, neurological exams, and psychological evaluations, to quantify the level of brain atrophy, and to exclude other possible diagnoses, e.g. VaD, dementia due to normal pressure hydrocephalus, etc. In all these patients frontal atrophy (mild to moderate), inferolateral regions of the atrophy of the temporal lobe (moderate), and medial parietal lobes atrophy were detected. These structural changes are consistent with data in the literature regarding AD neuro-imagistic parameters evolution [35,36].

The absence of evidence for folate or vitamin B12 deficiency, thyroid disorders, Parkinson's disease, Huntington's disease, HIV/prionic disease, head trauma, demyelinating disorders, and brain tumors was confirmed by the results of laboratory analyses and clinical examination.

Personal history or current diagnoses of abusive use or dependence on alcohol/sedative, hypnotic, or anxiolytic drugs/inhalants were investigated, and no significant data were found [37,38]. Patients were screened negative for drugs with potential negative effects on cognitive functioning (first-generation antipsychotics, antihistamine drugs, antidepressants with anticholinergic properties, etc.) [39].

The change in cognitive functioning during one year was the primary outcome of these case series, and it was based on two widely-used and validated instruments – ADAS-Cog and MMSE. Secondary outcomes were the Clock-drawing-test (CDT), the Neuropsychiatric Inventory (NPI), CGI-S, and the Global Assessment of Functioning (GAF) score changes from baseline to month 12, during the same period.

The interpretation of the CDT (which measures executive function, visual-spatial abilities, abstractization capacity, and attention) was based on a 0 to 10 scale, with higher scores reflecting the higher severity of dementia symptoms (Figures 1-3) [40].

At baseline, ADAS-Cog total score reflected low functioning on multiple domains in all three cases. The mean baseline MMSE score was 16.3, the ADAS-Cog score was 49.6, and the CDT score was 3.3. The Neuropsychiatric Inventory (NPI) reflected at baseline high scores on anxiety, irritability, and depression, with a mean overall score of 18.6, and a distress score of 14. The clinical global impression corresponded at baseline to a mean score of 3.33, and the GAF score was 35. All these instruments were administered at every 3 to 4 months (flexible visit regimen), for one year.

Figure 1: Clock test for patient no. 1

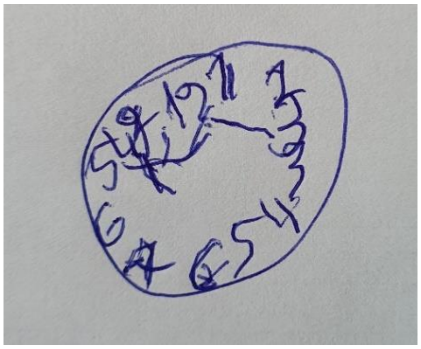

Figure 2: Clock test for patient no. 2

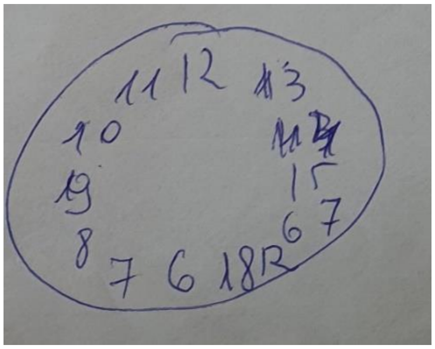

Figure 3: Clock test for patient no. 3

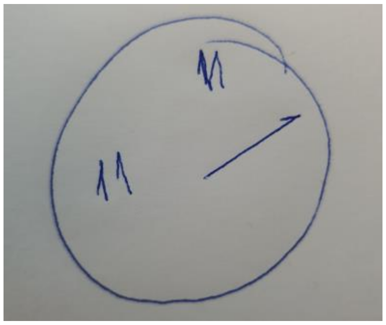

The therapeutic intervention consisted of HPATE, which was added to rivastigmine patches (n=1), oral galantamine (n=1), or oral donepezil (n=1). Actovegin was administered in one case and Cerebrolysin in two cases. The treatment schedule is represented in Table 1.

MMSE scores showed only a very small decrease during the 12 months of active monitoring (-0.33 points compared to baseline), and ADAS-Cog scores increased by 0.66 points (higher scores reflect more severe deterioration) (Figure 4). NPI total scores decreased with a mean of 7.6 points compared to baseline, and the overall distress with 4 points. The mean CDT score did not change after 12 months.

CGI-S and GAF scores also showed relative stability (no change for CGI-S, and -5 points on GAF).

Figure 4: Evolution of ADAS-Cog and MMSE scores from baseline to week 52

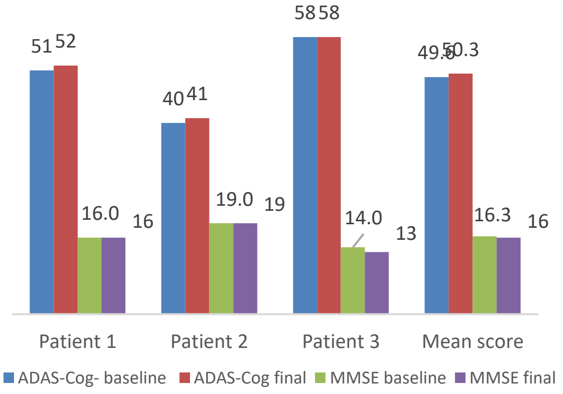

Both explored HPATE, Cerebrolysin and Actovegin, were well tolerated and they may be administered together with AChEIs because of their presumed synergistic effects.

This combination may be useful in stabilizing the overall cognitive status in moderate AD and in reducing the associated non-cognitive symptoms.

CONCLUSION

HPATE has been associated with potential benefits in patients diagnosed with AD and VaD as add-ons to AChEIs, or mild cognitive impairment as monotherapy. HPATE has a good safety and tolerability profile in AD or VaD patients. These conclusions are based on moderate and low-quality studies [11-15,17-20,32,33], therefore it may be considered that more clinical trials, with a randomized, double-blind design and a larger number of subjects are needed, to support HPATE positive effects on cognition. Regarding the impact of delaying the cognitive deterioration from mild cognitive impairment to clinically relevant AD using Cerebrolysin as monotherapy, data are even more inconclusive (one open-label study was found in the literature) [21].

AChEIs are associated with modest, but persistent cognitive benefits and these drugs may also reduce the mortality risk, a phenomenon which could be related to their cognitive favorable effects [41-43]. To augment the AChEIs effect, the administration of HPATE as an add-on may be useful for preserving the cognitive status or slowing the cognitive decline in patients with dementia. Therefore, HPATE may represent a useful and well-tolerated therapeutic option, and it may also favorably influence the evolution of non-cognitive symptoms in AD.

Disclaimer

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REVIEW

Individualized surgical antibiotic prophylaxis – why, when, and how?

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Abstract: *Surgical site infections (SSI) greatly concern clinicians, as they are associated with significant morbidity and mortality, prolonged hospitalization, and costs. Antibiotic prophylaxis plays a pivotal role among the procedures that are usually employed for the prevention of surgical-related infections. This narrative review aims to cover some of the particular situations when the clinician might consider individualizing antibiotic prophylaxis for a patient. With the rising incidence of multi-drug resistant bacteria carriage among not only hospitalized or institutionalized patients but also patients from the community, there might be a tendency to use extended-spectrum antibiotics for longer periods for surgical infection prevention. However, the inappropriate use of antibiotics increases the selection pressure, thus favoring the spreading of resistant bacteria. Moreover, specific patient characteristics or pathologies might need to be considered to customize the type, dose, or length of administration of an antibiotic as surgical prophylaxis. Using prosthetic material or prolonged surgeries with large fluid shifts are other situations when individualized antibiotic prophylaxis might be thought of. Keeping in mind that it is of utmost importance that everyone adheres to the current guidelines for surgical antibiotic prophylaxis, customization of local protocols according to well-thought-out strategies might prove beneficial in SSI prevention.*

Keywords: *surgical site infection; antibiotic prophylaxis; multi-drug resistance; personalized medicine*

INTRODUCTION

Current surgical antibiotic prophylaxis (SAP) is based on guidelines' recommendations that rely on solid evidence-based studies and, where data are lacking, on expert opinion. However, in practice, clinicians might encounter some particular situations that need to be addressed by tailoring antibiotic prophylaxis or choosing alternative strategies to prevent surgical site infections (SSI). At the same time, doctors must remember that a permissive large-spectrum antibiotic use or an unnecessary prolongation of antibiotic prophylaxis can lead to increased antibiotic resistance. Thus, a prudent decision must be made when choosing the right type and dose of antibiotic and the proper duration of SAP.

This literature review highlights the exceptions that may need a personalized approach regarding SAP, particularly the choice of antibiotics for patients colonized with multi-drug resistant bacteria (MDR), the length of antibiotic prophylaxis for specific surgeries, and the choice and dosing of antibiotics for special populations. In addition, other therapeutic options for lowering SSI are addressed.

SURGICAL ANTIBIOTIC PROPHYLAXIS GUIDELINES AND GUIDELINE COMPLIANCE

In France, Germany, and the USA, studies report overall compliance to guideline recommendations of about two-thirds when it comes to antibiotic choice for SAP and timing of administration [1–3], but the adherence rate is much lower in developing countries, varying widely between 18% and 50% [4–8]. Surprisingly, even in Italy, only 18% of cases in a small cohort study received appropriate SAP [9]. Table 1 summarises the doses and redosing intervals for the main antibiotics used in practice, according to guideline recommendations.

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The most commonly reported deviation from the guideline was an unnecessary prolongation of SAP. For example, SAP was prolonged in France for more than 24 hours in 45.2% of surgical interventions [1], but their national guidelines allow 48 hours of antibiotic prophylaxis for certain types of surgery [10]. In Germany, unnecessary prolongation of SAP occurred in 30% of cases [3]. Lack of intra-operative redosing when needed is another deviation frequently encountered, being reported in around 20% of cases in developed countries [1,2].

Table 1: Dosage and redosing intervals for the main antibiotics used in surgical prophylaxis

Antibiotic	Pre-incision dose	First redosing interval	Subsequent redosing intervals
Ampicillin	2 g	+ 2h	every 6h
Ampicillin-Sulbactam	3 g	+ 2h	every 6h
Cefazolin	2 g (3g [1)])	+ 4h	every 8h
Cefuroxime	1.5 g	+ 4h	every 8h
Ceftriaxone	2 g	-	-
Ciprofloxacin	400 mg	+ 8h	-
Gentamicin	5 mg/kg [2)]	-	-
Metronidazole	500 mg	+ 8h	every 8h
Clindamycin	900 mg	+ 6h	every 8h
Piperacillin-Tazobactam	3.375 g	+ 2 h	every 6h
Vancomycin	15 mg/kg	-	-

[1)] Obese patients with weight>120 kg; [2)] adjusted body weight for obese patients

A qualitative study that interviewed surgeons and anesthetists in a tertiary hospital in Australia concluded that improvisation with novel SAP strategies is ubiquitous and confers a sense of reassurance for surgeons. However, these are not following guidelines or have no clinically demonstrated efficacy [11]. Lack of knowledge of guideline content, reliance on old habits and practices, lack of communication between surgeons and anesthetists, fear of medical malpractice linked to SSIs, environmental context and resources, or personal beliefs are some of the factors related to low adherence to guideline recommendations [12].

However, the good news is that concentrated efforts can improve guideline adherence [13–15]. Probably one solution would be to create local guidelines that follow international recommendations and adapt to particular contexts and local specificities.

MULTI-DRUG-RESISTANT BACTERIA ARE A CONCERNING PROBLEM

Globally, over 700,000 people die yearly from drug-resistant infections, and the predictions are that over 10 million people might die annually by 2050 if no preventive measures are taken [16]. Furthermore, a European prevalence study analyzing data from 2015 discovered that infections with MDR are on the rise, causing 6.44 deaths and 170 DALYs per 100000 population, with community-associated MDR infections representing a substantial proportion of the burden [17].

SSI makes no exception when talking about the burden of healthcare systems. SSIs have a pooled cumulative incidence of 5.6/100 surgical procedures in low and mid-dle-income countries (LMICs), strikingly higher when com-pared to the pooled cumulative incidence of 2.6 SSI/100 surgical procedures in the USA and 2.9 in Europe [18]. As the prevention of SSI supposes an antibiotic coverage of possible pathogens, it comes into question if patients with a high risk of being carriers of MDR might need an individualized approach to SAP.

A helpful model of adapting SAP comes from methicil-lin-resistant S. aureus (MRSA) SSIs. The incidence of SSIs caused by MRSA in the USA increased from 16% in 2003 to 21% in 2007, increasing mortality, hospital length of stay, and costs [19]. A meta-analysis confirmed that bundle measures for the prevention of MRSA SSIs in cardiac and orthopedic surgery, including nasal decontamination and glycopeptide antibiotic prophylaxis, decreased with 70% the risk of infection [20]. However, vancomycin is less effective than beta-lactam antibiotics for preventing SSIs caused by methicillin-susceptible S. aureus (MSSA) [20,21]. Thus, guidelines have rapidly adapted and recommend vancomycin use, in addition to routine prophylaxis, in patients who are colonized with MRSA or are at high risk of colonization [10,21,22].

Although data on colonization with antibiotic-resistant bacteria are scarce when talking about Gram-negative bacteria [23], more than 1 in 10 persons might be gut carriers of MDR in some countries [24]. Moreover, it is known that LMICs present a high incidence of infections with MDR [16]. Crowding, homelessness, low income, sanitation, and water sources are some factors associated with antimicrobial resistance in LMICs [25]. Comorbidities (diabetes, cancer, inflammatory diseases), extremes of age, recent hospitalization or antibiotic treatment, residency in a nursing home, and occupational risks, such as health workers or farmers, or veterinarians who frequently use antibiotics, or travel to LMICs are other factors associated with the acquisition of MDR infections [26]. An interesting study examined the dynamics of gut bacteria colonization of 20 European volunteer visitors to Laos. They registered a 70% colonization rate after 22 days of surveillance, but daily sampling revealed that all participants had acquired an extend-ed-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) at some point [27].

Infections following trans-rectal prostate biopsy (TRPB) are an actual problem for urologists. Guidelines generally recommend the utilization of fluoroquinolones as SAP for TRPB [10,21,22], but there is a rising concern that these antibiotics might be ineffective for the novel antimicrobial-resistance patterns [28]. The prevalence of resistant strains of E. coli for patients undergoing PB varies between 13 and 25% for stains resistant to fluoroquinolones, and 8% and 13% for strains resistant to cephalosporins and, more concerning, with ESBL carriage on the rise [29–33]. Furthermore, ESBL carriers have a higher risk of infections post-TRPB [34]. Thus, introducing rectal swabs that could detect potential carriers and tailored antibiotic prophylaxis before TRPB might be a cost-efficient measure that might lower infectious complication rates after TRPB. Data from a meta-analysis [35] and other studies support this strategy [36]. Moreover, studies identified some risk factors associated with re-sistant-stains colonization, such as the history of the use of fluoroquinolones, previous hospitalization [37], travel his-tory [29], obesity or diabetes mellitus [34,38]; patients could be selected based on questionnaire to be further investigated in a cost-efficient manner for colonization.

In a cohort of patients with cancer undergoing gastrointestinal and gynecologic surgery, 17.5% were colonized with ESBL-E at admission. The risk of developing an SSI was twice higher in ESBL carriers versus non-carriers (RR, 2.16; 95% CI, 1.2-3.89)[39]. Similarly, in a prospective study including 3700 patients undergoing colorectal surgery from 3 hospitals in Israel, Switzerland, and Serbia, ESBL-E carriage more than doubled the risk of SSI (OR, 2.36; 95% CI, 1.50–3.71). Interestingly, the prevalence of ESBL-E carriers differed significantly between countries: 9.4% in Serbia, 12.0% in Switzerland, and 28.8% in Israel [40].

Among 360 patients who underwent abdominal surgery in a university hospital in Thailand, 36% had preoperative enteric colonization with an ESBL-E, and 14.1% developed an SSI. Although colonization with ESBL-E represented an independent risk factor for SSI (aOR 2.4; 95% CI, 1.19–19.91), the study found no association between carbapenem surgical prophylaxis and reduction in SSIs. However, it must be kept in mind that the study was single-centered, included a small study group, the incidence of SSI reported was relatively high, the majority of SSIs were superficial, and the authors did not explain the choice of antibiotic prophylaxis [41].

The number needed to treat (NNT) is an important concept when analyzing the impact of SAP. A higher risk of SSIs would lead to a lower NNT regarding SAP, and thus a more significant impact on its incidence of SAP. Nutman et al. analyzed the effect of changing the standard cephalosporin-in-metronidazole prophylaxis for colorectal surgeries to individualized prophylaxis with ertapenem for ESBL-E carriers. SSI rates were 22.7% for non-carriers versus 15.8% for ESBL-E carriers (ARD, –7.7%, 95% CI, –14.6% to –0.8%), with a number needed to treat to prevent one SSI in ESBL-E carriers of 13 [42]. Starting from this study and considering the prevalence of MDR, Temkin et al. analyzed different clinical scenarios for SAP. Thus, if a broad-spectrum SAP were used only for MDR carriers, for a prevalence of 5% carriers, 260 patients would need to be screened to avoid one SSI. If the prevalence of 50% carriers, this number would drop to 26. However, if a broad-spectrum SAP were used for all patients, no matter if they are carriers, there would be 247 patients who would receive unnecessary broad-spectrum antibiotics for a real prevalence of 5% MDR carriers. In comparison, the number would drop to 13 patients if the real prevalence of MDR carriers is 50% [43].

Hence, there is a great need for MDR prevalence studies. These would offer the possibility to adapt local guidelines of SAP while lowering MDR-SSI and improving, at the same time, the costs and antibiotic stewardship programs.

Some centers from high-income countries have already adopted a personalized management pathway for patients undergoing elective interventions in visceral surgery [44]. The algorithm encompasses screening in patients at risk, very strict basic hygiene, targeted isolation who are MDR carriers or at risk of being carriers, and targeted surgical antibiotic prophylaxis [44]. This section may be divided into subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

LESS IS MORE. ARE THERE EXCEPTIONS?

There is a bundle of risk factors associated with SSI (figure 1). The consensus recommendation for antibiotic prophylaxis is to administer a single dose for the majority of surgeries with clean or clean-contaminated surgical wounds. The exceptions that can receive a 24-hours postoperative antibiotic regimen include cardiac surgeries, head and neck surgery that involve the oral cavity, or some procedures that involve prosthetic materials (joint replacement, internal fixation of fractures, breast augmentation) [10,21,22]. The French guidelines are more permissive and accept a 48-hour postoperative antibiotic regimen for limb amputations, grade II open fractures, or craniocerebral surgical wounds [10].

During cardiopulmonary bypass (CPB), needed in most cardiac surgeries, an important increase in circulating volume due to pump priming can lead to a decreased concentration of the prophylactic antibiotic. A pharmacokinetic model study that included pediatric patients analyzed the serum levels of cefazolin before, during, and after cardiopulmonary bypass. Considering the higher ratio of priming volume needed for children during CPB, an additional bolus dose at the start of CPB improved the probability of target attainment by more than 90% [45]. When comparing bolus infusion versus continuous infusion of cefuroxime for the first 24 hours following CPB, plasma concentrations of cefuroxime were higher in patients receiving the continuous infusion [46]. An undergoing clinical trial that compares short-course and long-course SAP with either cefazolin monotherapy or combination therapy with cefazolin and vancomycin might shed some light on which strategy is best for CPB [47].

Regarding intraoperative antibiotic redosing, there is limited clinical evidence, but there are expert opinions that sustained a more aggressive redosing based on pharmacokinetic data. In a recent meta-analysis, Wolfhagen et al. ad-dress this question and the clinical utility of intraoperative re-dosing; the findings attest to the beneficial results of redosing, but the certainty of the evidence was low [48]. The question of redosing will persist in literature until some results of well-powered RCTs will arise.

Figure 1: Risk factors associated with surgical site infections

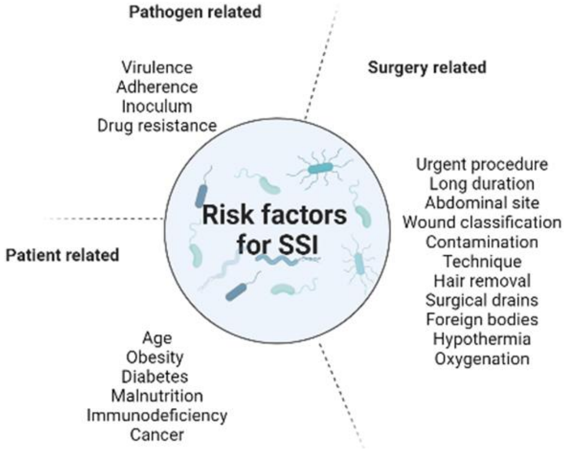

Orthopedic surgery is another field of interest when talking about SAP. A retrospective cohort study analyzed standard SAP versus a one-week course of antibiotic prophylaxis after total joint arthroplasties. Infection rates dropped from 2.1% to 0.4% for total knee arthroplasties and from 4.3% to 1.1% for total hip arthroplasties, respectively, for patients that received seven days of antibiotics [49].

Another similar study proposed the use of extended postoperative oral antibiotic prophylaxis for seven days for patients with a high risk of infection after total joint arthroplasties – are diabetic, active smokers, obese, or have other conditions associated with risk for infections. High-risk patients that received extended antibiotic prophylaxis had similar infection rates to low-risk patients, with a number of needed to treat 57 patients to prevent one prosthetic infection [50].

Although using an extended course of antibiotic prophylaxis may decrease infection rates, further studies need to weigh the risk of increasing antimicrobial resistance, the costs, and the risk of developing Clostridium difficile infections before recommending the routine use of extended antibiotic strategy use.

PATIENT PARTICULARITIES

1. Allergic patients

Allergy to β-lactam antibiotics is one of the most commonly cited adverse drug reactions, with a reported prevalence of around 10% in western countries [51,52]. But among inpatients with an alleged penicillin allergy, only 5% had a positive penicillin skin test [53]. Moreover, cross-reactivity rates of allergic reactions between penicillins and 1st generation cephalosporins are 1% and even smaller when using 3rd generation cephalosporins [54]. Still, when it comes to SAP choice, many clinicians opt for using alternative antibiotics, such as clindamycin, vancomycin, or gentamicin, when a patient claims to have an allergic reaction to penicillin [55].

There is a four-fold increased risk of SSI (95% CI 1.37-10.47) when clindamycin was used instead of the standard cephalosporin SAP for patients with declared allergy to penicillin that underwent free tissue transfer in head and neck surgery [56]. Furthermore, a meta-analysis that investigated SSIs after cardiac surgery discovered that β-lactam is superior to glycopeptides for the prevention of chest SSI (RR, 1.47; 95% CI 1.11–1.95). Blumenthal et al. also concluded that second-line antibiotic use raises the odds of SSI by 50% [55]. Besides SSI, alternative regimens used for patients labeled allergic to penicillin may lead to increased antibiotic resistance, hospital length of stay, and higher costs [57].

Clinicians should develop strategies to rule out spurious β-lactam allergies. A first step may include a thorough his-tory investigation or a standardized questionnaire that could rule out falsely labeled patients and select high-risk patients for further investigation. Further, selected patients may follow a classical allergy diagnosis (skin prick or patch test or a specific IgE test) or be subjected to an intradermic test done by properly instructed personnel [57,58]. Thus, labeling patients with reported penicillin allergies could lower SSIs and improve antibiotic stewardship.

2. Obese patients

Obese patients may present a challenge when it comes to adequate antibiotic dosing. Guidelines consider that serum and tissue concentrations of certain drugs vary in obese and non-obese populations. It is recommended that the dosing of vancomycin and aminoglycosides be based on total body weight and adjusted body weight, while the dose of cefazolin be increased from 2 g to 3 g for patients over 120 kg [21,22], or even to 4 grams for bariatric patients with a BMI over 35kg/m2 [10]. Although this is backed up by pharmacokinetic studies [59], some opinions do not support the increased dose of cefazolin in obese patients [60].

Microdialysis studies are contradictory. Palma et al. concluded that 2 g of cefazolin, reduced at 4 hours intervals, is enough and effective to prevent infections caused by bacteria with a MIC up to 2 mg/l, even in obese patients [61]. On the contrary, Brill et al. support higher dosages of cefazolin in obese patients, arguing that distribution to the subcutaneous adipose tissue is reduced and the probability of target attainment is decreased for MIC of 2mg/l or higher [62].

Another study that included 12 obese pregnant women undergoing cesarean section suggested redosing cefazolin after 2 hours, following either a 2 or 3 g initial bolus [63]. Finally, a swine model that targeted a plasma and tissue concentration of 4 mg/l proposes a weight-based cefuroxime dosage of 20 mg/kg [64].

Until future pharmacokinetic studies and controlled trials bring hard evidence regarding the right dose of antibiotic for obese patients in SAP, it may be more prudent to follow guideline recommendations, even though they are based on expert opinion.

3. Organ recipients

In solid organ transplantation, numerous factors contribute to increased rates of infections: prolonged pre-transplant hospitalization, exposure to antibiotic treatment, immuno-suppressive therapies, donor-derived infections, or the rising number of MDR portages [65].

One strategy for lowering SSIs associated with MDR is screening recipients and using an extended-spectrum anti-biotic as prophylaxis for MDR carriers.

For example, in one study, out of 61 patients screened for VRE colonization before liver transplant, 27 (44%) were carriers. All carriers received daptomycin in addition to GNB antibiotic as SAP, and only 3 developed postoperative VRE infections [66]. In another cohort of patients who under-went liver transplantation, 13.3% were ESBL-E carriers. Known carriers received a carbapenem as SAP. In ESBL-E carriers the risk factors associated with ESBL-E infection were colonization with K. pneumoniae, long-term spontaneous bacterial peritonitis prophylaxis with quinolones, antimicrobial treatment >3 days within the month before LT, and MELD ≥25. In the early postoperative period, ESBL-E infections were significantly lower in patients who received tailored SAP versus those who received standard SAP (29.8% vs 63.6%, p=0.04) [67]. Similarly, ESBL-E stool carriage before liver transplant in a pediatric population was associated with the emergence of ESBL-E-caused infections (OR = 4.5, 95% CI 1.4–17.4), although ESBL-

E carriers received imipenem as SAP, while non-carriers receives cefoxitin or piperacillin-tazobactam [68].

Another strategy is to broaden the spectrum of antibiotic prophylaxis for all solid-organ transplantation. A single dose of ertapenem prophylaxis instead of cefazolin reduced by a third of early infections caused by ESBL-E in kidney transplant recipients in a Spanish university hospital. Moreover, ESBL-E infection was associated with cefazolin prophylaxis and acute rejection, while ertapenem did not seem to increase the incidence of Clostridium difficile or other MDR infections [69].

Donor respiratory cultures do not influence post-transplant infections in non-lung recipients [70]. Still, prophylactic antibiotics must provide coverage for pathogens isolated from the donor lung or the recipient before lung transplantation [22,71].

Spanish Society of Transplantation recommends for patients colonized with ESBL-E a large-spectrum antibiotic prophylaxis regimen before solid-organ transplantation, avoiding, as possible, carbapenem use [71]. Infectious Disease Socie-ty of America also mentions that prophylactic antibiotics may need to be altered according to the recipient's pathogen isolation before liver transplant [22].

In conclusion, data support as a strategy for solid-organ receivers pre-transplant surveillance of MDR carriage and tailored SAP. Another strategy might be the use of broad-spectrum antibiotic prophylaxis for all solid-organ recipients, but this might favor the development of resistance.

4. Extremes of age

Particularities in the pharmacokinetics of antibiotics emerge not only in neonates but also in elderlies. Membrane permeability, plasma protein binding, and total body water, which vary significantly at the extremes of age compared to healthy adults, determine the final plasma concentration and tissue concentration of antibiotics. Moreover, the metabolism and elimination of antibiotics are also different from adults, as neonates have immature metabolic pathways, and the elderly might suffer from liver or renal failure. Furthermore, the elderly are at risk of adverse effects due to polypharmacy and potential drug interactions [72,73].

Although therapeutic drug monitoring might be the correct way of dosing the antibiotics in these two age groups, it is too sophisticated and expensive to be applied in the real world just for surgical antibiotic prophylaxis.

Thus, a correct antibiotic dose calculation for both groups based on guide-line recommendations is essential.

COMPLEMENTARY MEASURES FOR PREVENTION OF SSI

In addition to classical antibiotic prophylaxis, novel complementary measures rise to prevent surgical site infection, including local or topical measures and systemic decontamination.

For example, a local prolonged release of antibiotic containing a matrix of polymers and lipids doxycycline was tested for the prevention of sternal wound infections after cardiac surgery [73]. The prospective, single-blind randomized controlled multicentre study, counting 81 patients, proved that this novel compound, D-PLEX, was safe for cardiac surgery patients. There were zero sternal wound infections in D-PLEX treated patients, but larger scale studies are needed to prove its efficiency versus standard care [74].

Another study that tested dual-topic antibiotic application before sternotomy wound closure showed encouraging results [75]. The observational study analyzed over 2500 patients after cardiac surgery, revealing that dual antibiotic topical prophylaxis had the lowest rate (1.1%) of sternal wound infection versus the group treated with topical vancomycin (2.2%) and the control group (4.5%).

Furthermore, there was a higher rate of occurrence of gram-negative bacteria in the control group (3.4%) versus the vancomycin (1.2%) and the dual antibiotic group (0.6%) [75].

Although digestive tract decontamination was greatly debated, its application might prove beneficial in SSI prevention [76]. A retrospective study from the Netherlands evaluated the strategy of selective oral digestive tract decontamination with the use of an oral solution with amphotericin B 2%, colistin 2% and tobramycin 2% in patients with oesophageal carcinoma who underwent minimally invasive esophagectomy. The study's conclusions revealed a lower rate of fistulas and postoperative pneumonia (36.9% vs. 20.1%) in the group of patients with selective digestive decontamination versus the control group, but without an impact on mortality rates [77]. A similar regimen for digestive tract decontamination was used in a RCT that included patients with colorectal cancer scheduled for elective surgery with a primary anastomosis. Compared to the control group, the patients randomized to the oral decontamination group had similar anastomotic leakage rates but significantly lower rates of infectious complications [78].

Faecal microbiota transplantation arises as a novel method of SSI prevention. It is a proven way of gastrointestinal decolonization of multidrug-resistant bacteria, and it is beneficial for the treatment of refractory Clostridium difficile infection, with success rates close to 90% [79]. A review of the literature showed that more than half of the patients achieve digestive decontamination for multidrug-resistant bacteria when treated with faecal microbiota transplantation [80].

Bacteriophage use might be another novel therapy to help prevent SSIs. However, there is only limited evidence from small case studies and experimental trials. The bacteriophages are virus-like structures with bacterial cell lysis activity. A series of patients from Finland with multi-drug-resistant bacterial infection after cardiothoracic surgery received conventional targeted antibiotics and bacteriophages, successfully treating infections in 87% of patients. The literature cites applications of bacteriophages in the surgical setting with topical application of phage solutions to a surgical wound before closure [81-83].

CONCLUSION

All things considered, surgical antibiotic prophylaxis remains a vital tool in the fight against SSI. Following the general rules of good practice, antibiotic prophylaxis could be individualized when clinicians face MDR carriers, allergic, obese, immunosuppressed or elderly patients or when the surgical intervention calls for prosthetic material insertion, large fluid shifts or prolonged duration. There is, of course, a need to develop evidence-based local SAP policies in accordance with the local prevalence of MDR and the incidence of SSIs.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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REVIEW

Medical and chemical evaluation of robotic surgery methods; A review study

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Abstract: *Along with the advances in medical science, surgical methods have also undergone many advances. Today, with the advancement of technology in all fields, including medicine, robots have entered medical science. These robots have many uses as well as advantages and disadvantages that they enjoy in Iran and other countries. In this study, it was addressed. This study is a review of robotic surgery methods in Iran and other countries in the form of a review study. This study is a conceptual review. The steps performed are 1- Designing a research question, 2- Searching and extracting research-related studies, 3- Selecting related studies, 4- Tabulating and summarizing information and data, and 5- Reporting results. The results showed that robotic surgery in operations such as brain, kidney, open heart, liver, eye, laparoscopy, dental surgery, coronary artery surgery, hysterectomy, lymphadenectomy, general surgery, obstetrics, head and neck, shelf Chest, urology, endoscopy, colonoscopy, ear, nose, and throat are used and have advantages such as three-dimensional vision, flexible rotation of the instrument, reduction of surgeon hand vibration with vibration filter, ease of surgery and the ability to create the required patterns It reduces the volume of blood lost during surgery, the length of stay in the hospital, the amount of pain, and subsequently the number of analgesic doses consumed in the postoperative ward. Disadvantages include the relative increase in operating time, the high cost of robots, and the physician's lack of sensory perception of the patient's environment.*

Keywords: *robotic surgery, surgery, robotics*

INTRODUCTION

From the past until now, surgery has been known as a treatment for some diseases, but it was performed due to its many limited complications. Along with the advances in medical science, surgical methods have also undergone many advances [1]. In traditional surgery, the surgeon has to make larger incisions to perform the surgery and damage a large amount of tissue in the patient's body. This increases the likelihood of surgical complications such as pain, bleeding, and infection, and prolongs the patient's recovery time [2-5]. Given the problems that exist in open surgery, surgeons have sought to minimize harm to the patient by creating new tools. This led to the emergence of a new approach called minimally invasive surgery (Figure 1) [6-7].

Figure 1: Robotic surgery

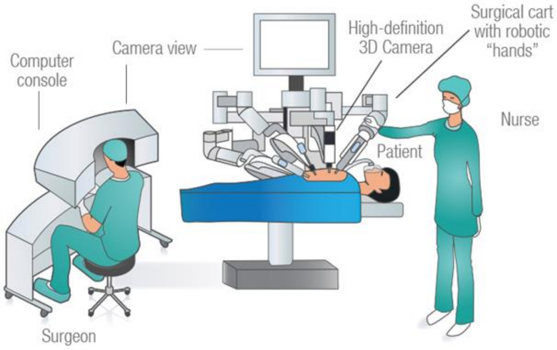

Today, with the advancement of technology in all fields, including medicine, robots have entered medical science. In medical science, surgery and operations in which robots are directly or indirectly involved are called "robotic surgery" [8].

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Robotic surgery is performed by making several small incisions in which the surgeon can be present without the patient's bedside and remotely while having three-dimensional vision by controlling the robot arms that have a range of motion similar to human hands and wrists and around Are placed on the operating table to perform surgery (Figure 2) [9]. Today, robots have become a very useful tool to help doctors, and their irreplaceable role in medical science is increasing every day. That's why the US National Institutes of Health has significantly increased research funding. The NIH budget for this project for 5 years is $ 2.3 million [10]. The difference between a robot and a human is not negligible in many ways. Robotic surgery, compared to traditional surgery, results in smaller incisions, increased accuracy, flexibility, better visual control, and reduced pain and wounds, which leads to less time spent in the hospital. And recovery time is faster [11].

Figure 2: A review of camera viewpoint automation in robotic and laparoscopic.

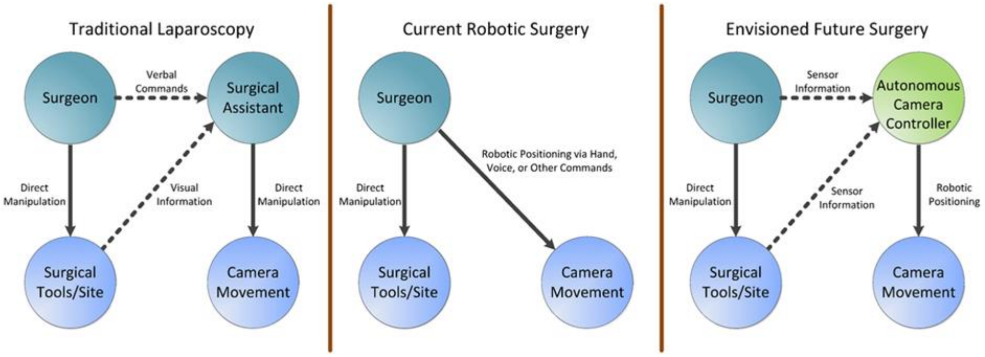
Traditional Laparoscopy
Surgeon
Verbal Commands
Surgical Assistant
Direct Manipulation
Visual Information
Direct Manipulation
Surgical Tools/Site
Camera Movement
Current Robotic Surgery
Surgeon
Robotic Positioning via Hand, Voice, or Other Commands
Direct Manipulation
Surgical Tools/Site
Camera Movement
Envisioned Future Surgery
Surgeon
Sensor Information
Autonomous Camera Controller
Direct Manipulation
Sensor Information
Robotic Positioning
Surgical Tools/Site
Camera Movement

Among the benefits of robotic surgery can be; Reduction of the long recovery period after surgery, reduction of postoperative pain, reduction of the possibility of postoperative infection, reduction of hospitalization time, no need to break the sternum and open the chest in open surgeries, rapid healing of wounds, in the availability of certified surgeons with unique capabilities in more locations, greater accuracy, elimination of surgeon hand vibration, continuous and 24-hour operation 24 hours a day without fatigue. In addition to having many benefits, according to robotic surgery studies (Figure 3).

Figure 3: The evolution of robotic surgery: surgical and anesthetic aspects

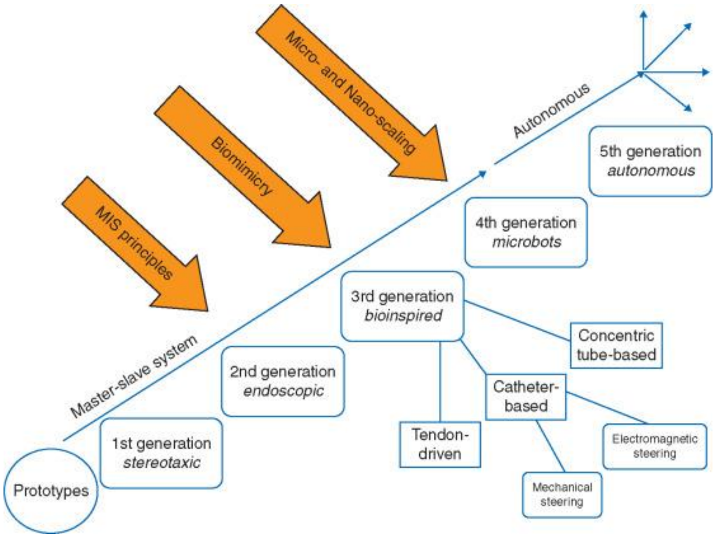
Micro- and Nano-scaling
Biomimicry
MIS principles
Autonomous
Master-slave system
5th generation autonomous
4th generation microbots
3rd generation bioinspired
Concentric tube-based
2nd generation endoscopic
Catheter-based
Tendon-driven
Electromagnetic steering
1st generation stereotaxic
Mechanical steering
Prototypes

Disadvantages are including: increasing the distance between doctor and patient, reducing the speed of communication with equipment and monitors, increasing the possibility of cutting the wrong place, the possibility of disconnecting the surgeon from the operating room, low network security, and the possibility of hacking communication, patients' reluctance to use this equipment and technology, Expensive robots, lack of assistance for the doctor, lack of sensory perception of the patient's environment by the doctor. Many operations by surgical robots such as brain surgery, kidney, open heart, liver, eye, laparoscopy, dental surgery, Coronary artery surgery, hysterectomy, lymphadenectomy, general surgery, obstetrics, head and neck, chest, urology, endoscopy, colonoscopy, ear, throat, and nose have been successfully performed [10]. The high accuracy of some surgical robots makes it possible to perform very difficult surgeries such as vitreoretinal surgery and eye vitrectomy in a favorable manner [12].

There are several types of surgical robots. One of them is Heartlander, which is known as the smallest surgical robot in the world. Zeus robot is another robot. The other robot is the Da Vinci robot, which is known as the best robotic surgery system in the world and is made by a single commanding company. The system was approved by the US food and drug administration in 2000. In the meantime, other countries have also started working.

In Iran, the Sina robot surgeon (Da Vinci's rival), was developed by researchers at the Tehran University of Science in collaboration with researchers at the Sharif University of Technology. Its unique feature compared to the Da Vinci robot is that it measures the force applied to the patient's body and transmits it to the surgeon through the surgical console, and its price is estimated to be one-third of the American version [10]. Although we are facing the development of surgical robots around the world, a review of scientific products in the field of surgical robots shows that the United States, China, and Japan are the three leading countries in this field, respectively, and Iran is in 15th place.

Figure 4: Robot-assisted kidney surgery results similar to those of traditional methods

The trend of scientific production in this field started in 1990 and has been moving forward with a gentle slope and has gone through an upward trend since 2001. This trend has continued upward with fluctuations in different years. The results indicate that. There are few scientific developments in this field and few countries, universities, authors, and publications have addressed this issue [13] (Figure 4). According to studies, efforts should be made to recognize The right requirements to make it possible to use robotic surgery worldwide [14]. Therefore, this study aims to investigate robotic surgery methods in Iran and other countries in the form of a review study.

METHOD

This study is a conceptual review. The steps performed are 1- designing a research question, 2- searching and extracting research-related studies, 3- selecting related studies, 4- tabulating and summarizing information and data, and 5- reporting its results.

SEARCH AND EXTRACTION OF RESEARCH-RELATED STUDIES

First, articles were researched by researchers. Researchers first extracted related keywords using the MeSH strategy. Search for resources using the keywords robotic surgery, surgery, robotics, robot surgeon in Persian databases Barekat, Iran Medex, Irandoc, Magiran, SID and using the English keywords telesurgery, Robotic surgery, in Google scholar databases, Scopus, science direct, PubMed, web of science were conducted from 2013 to 2021 in October and November 1400. These words were searched separately and also as a combination of two words together using Boolean operators. To increase the validity of the study findings and control the bias in data entry, the search process, selection of studies, and decision-making on entering or constructing the study and evaluation of the full text of the articles were performed by two independent browsers. This process was overseen by a third party.

Inclusion criteria include all articles that have examined robotic surgery and their full text is available and written in Persian and English. Exclusion criteria were studies unrelated to the title

EXTRACTING ARTICLES ACCORDING TO INCLUSION CRITERIA

Using the above keywords at the end of the search, including 37 articles from Persian databases and 105718 articles from international databases and a total of 105755 studies were collected. The studies were reviewed based on inclusion criteria. The selection of related studies was done in such a way that first a list of titles and abstracts of all articles in databases is prepared by researchers. And if after reading the title and abstract, it was not possible to decide on the study, the full text was studied. Then, the related articles entered the research process independently, and finally, 43 articles were included in the study.

TABULATING AND SUMMARIZING INFORMATION AND DATA

At this stage, after the data has been extracted, all study data will be summarized based on the name of the first author, year, place, title, tools, or research elements in the relevant table.

REPORTING THE RESULTS

After reading the summary and full text of the articles related to the research topic, the desired information was extracted for writing. Information required for each study included author name, year, place, type of study, sampling method, data collection tool, and results. Finally, the information obtained from the articles was categorized and finally reported in the full text of a review article.

Figure 5: The schematic method

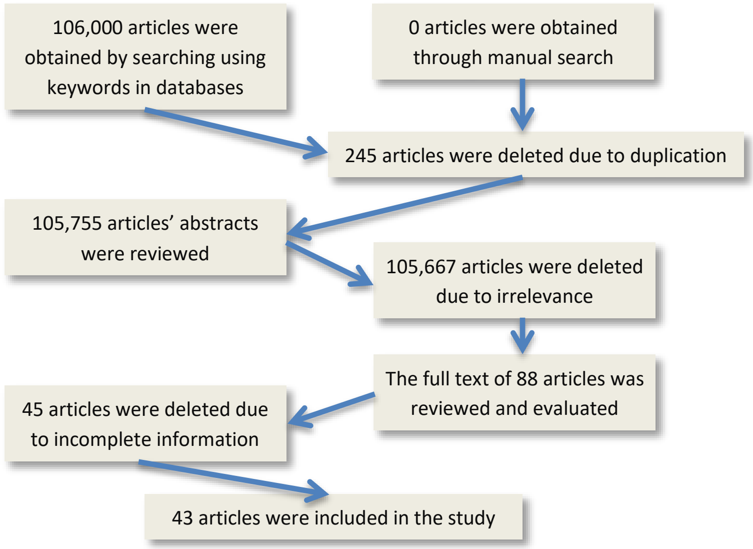
106,000 articles were obtained by searching using keywords in databases
0 articles were obtained through manual search
245 articles were deleted due to duplication
105,755 articles' abstracts were reviewed
105,667 articles were deleted due to irrelevance
The full text of 88 articles was reviewed and evaluated
45 articles were deleted due to incomplete information
43 articles were included in the study

Table 1: Studies related to the research topic

Findings	Research tools	Type of study	Title	Place	Year	Author
The results of measuring and recording the time required to start the robot, the ease of working with the robot, the quality of the robot in response to the surgeon's instructions, the accuracy of the robot's movements, the robot's ability to provide full image coverage, no shaking or shaking image, indefatigability Robot in long surgeries, lack of unwanted movements and lack of errors in movements indicate the safety and efficiency of the robot and its satisfactory performance during surgery.	Intervention	Experimental	Clinical evaluation of a new camera-preserving robot in laparoscopic surgery preliminary results	Iran	2019	Karimian
Studies have shown that three-dimensional vision, flexible rotation of the instrument, reduction of surgeon hand vibration with the help of a vibration filter, ease of surgery, and the ability to create the required patterns are the characteristics of surgeon robots.	Review of studies	Review	Comparison of robotic technique with standard laparoscopy in abdominal and visceral surgeries	Iran	2017	Theoretical

There are many surgical robots or intelligent medical assistants in the field of medicine, but to conclude which of these robots is better, you must first assess the time and place conditions and services, because each of these robots is in a field of activity and it must be admitted that they also have limitations [15,16].

Robotic technology allows general surgeons to operate with great precision, and areas that are difficult or even impossible to reach by humans can be accessed using a robotic system. The robotic surgeon's arms can rotate up to 280 degrees. All this brings better results for the patient. Iranian specialists have succeeded in gaining the technical knowledge of this robot surgeon and the technical knowledge of the mechanism design process, hardware, and software Robots have also been achieved [17]. The results of studies conducted in Iran in the field of robotic surgery are as follows.

The results of measuring and recording the time required to start the robot, the ease of working with the robot, the quality of the robot in response to the surgeon's instructions, the accuracy of the robot movements, the ability of the robot to provide full image coverage, no shaking or shaking image, The indefatigability of the robot in prolonged surgeries, the absence of unwanted movements and the lack of errors in movements indicate the safety and efficiency of the robot and its satisfactory performance during surgeries. Although launching a robot requires time and initial work, the results show a reduction in total operation time in most cases. The smoothness of motion, stability and no-image shake are among the advantages of the robot holding the laparoscope over the human hand, so this robot can be a good alternative for the person holding the camera.

Table 2: Another review of the method on 2021

Findings	Research tools	Type of study	Title	Place	Year	Author
Mitral valve robotic surgery is well possible through a limited surgical procedure Mortality around the operation seems very acceptable and possibly even lower than a sternotomy.	Review of studies	Review	Mitral valve robotic surgery: Methods and results in a 20-year review	Australia	2021	Monati
Robotic head and neck surgery are now regularly used in specialized centers for head and neck surgery.	Review of studies	Review	Current Advances in Robotic Head and Neck Surgery	Germany	2021	Their boe
A review of studies showed that robotic surgery had better overall survival than non-robotic resection of oral squamous cell carcinoma, and this method is recommended.	Review of studies	Review	Robotic surgery versus non-robotic resection of oral squamous cell carcinoma	USA	2021	Chilacula
The process of industrialization of surgical robots in several developing countries, such as the United States, must be accompanied by the removal of technical barriers and increased international cooperation	Review of studies	Review	analyze 100Most Read About Robotic surgery versus open surgery	China	2021	Chow
Robotic systems are widely accepted in the U.S and various surgical specialties.	Studies	Review	Robotics in Spine Surgery	USA	2021	Farber
Robotic surgery in a rural head and neck center is a safe and appropriate treatment option and has very few side effects and this method is recommended.	Questionnaire	Retrospective	Oral robotic surgery: a safe and effective tool for the head and neck Surgery	Australia	2021	Huang
Robotic surgical systems were divided into open/closed, image-based/imageless, and active/inactive/systems. The main goal is to improve the accuracy of operations.	Descriptive	Descriptive	Robotics in Orthopedic Surgery: Why, what, and how?	Belgium	2021	Innocent
Robotic spine surgery was introduced in 2004.	Descriptive	Descriptive	The evolution of robotic surgery in the spine	USA	2021	Because
Robotic surgery is a good option for adult spinal deformity surgery.	Questionnaire	A cohort	Have robotic methods improved postoperative results?	USA	2021	Croll
Robotic surgery can significantly reduce blood loss, intraoperative complications, and length of hospital stay, but increase readmission and surgery time. Robotic surgery in the treatment of endometrial cancer showed better results.	Review of studies	Review	The effectiveness of robotic surgery for endometrial cancer		2021	Hafng
Robotic-assisted radical prostatectomy was less recurrent and had lower pain scores up to 6 weeks postoperatively compared to open radical prostatectomy. Compared to laparoscopic prostatectomy, robotic surgery improves urinary and sexual function.	Review of studies	Review	Clinical results of robotic surgery compared to conventional surgical procedures (laparoscopic or open)	USA	2021	Hala

The next step will be to simplify work with the device by upgrading the software and conducting more clinical studies to evaluate robot performance comparisons [18]. Studies have shown that three-dimensional vision, flexible rotation of the instrument, reduction of surgeon hand vibration with the help of a vibration filter, ease of surgery, and the ability to create the required patterns are the characteristics of surgeon robots. Also, in general, the volume of blood lost during surgery, the length of hospital stay, and the amount of pain, followed by a reduction in the number of analgesic doses consumed in the postoperative ward and the time to return to a normal diet and normal activity compared to the laparoscopic procedure is reduced.

On the other hand, the results of the research have shown that the duration of the operation by the robot and the cost of surgery are longer than the laparoscopic method.

DISCUSSION

Robot surgery is not necessarily more expensive than laparoscopic surgery. Evidence now suggests that robotic surgery has a permanent role to play in the future of surgery. Therefore, surgeons should be actively diligent in the development of robotic surgery and begin preliminary and randomized studies with high prognosis and quality in this field [19]. The results showed that the use of robot traps in hip arthroplasty, in addition to providing more accurate 3D images for the surgeon to assess the depth and size of the acetabular cavity, reduces the risk of displacement or dislocation of the replaced joint. Reduces the risk of complications such as infection and site bleeding for the patient as well as readmission to the hospital. The results of studies show that robotic surgeries have effective applications as a minimally invasive method. Therefore, they can be gradually replaced by other surgical methods, but what is important is to pay attention to improving skills in the field of relevant new inventions, strengthening infrastructure, and allocating sufficient money to be able to use advanced technologies desirably and efficiently. Used for the benefit of the patient and the surgeon and other members of the treatment team [20]. It is true that the elimination of the natural vibration of the surgeon's hand, reducing the possibility of infection and postoperative infections, the possibility of a deeper vision of the operating field, and the creation of small and delicate incisions instead of large incisions by microscopic robots are the advantages of using robotic surgery. However, it is possible to reduce the feeling of tissue specificity under the operation by the surgeon, reduce the speed of communication with equipment and monitors and the possibility of a twenty-five-second delay on the monitors can also be considered as disadvantages of remote surgery. Remote surgery is performed by robots and advanced medical systems, but due to its cost and lack of strong technological and communication infrastructure, this method has not yet been properly transferred to developing countries and is limited to developed countries [21].

Limitation of tactile data Using graphic data It was thought that robotic surgery would be used more, but this did not happen. Reasons for this include lack of public awareness of the benefits of this method, patients 'refusal to have robotic surgery, and surgeons' desire for traditional surgery [22]. A study showed that the use of viewfinders will be effective in reducing the cost, weight, and dimensions of equipment and also simplifying some medical operations. It should be noted that the use of observers strongly depends on the type of operation used and not all of these methods are yet clinically applicable because there are many challenges in ensuring the performance and safety of these methods. However, the multidisciplinary nature of this subject provides a wide field for study and research [23]. Robotic surgery has made slow progress due to cost and patent protection. But now we are seeing an increase in the number of new robotic surgery systems. Several systems are CE-certified, while in the EU alone TransEnterix Senhence has received FDA approval, and several more are currently being tested, which will increase competition and reduce costs by approving these new robots. In the future, robotic surgery will be more common than manual surgery [24]. A "driver's license" is seriously required for robotic surgery. Ideally, a certified training program is required to define a robotic surgeon and ensure a high level of work. And the training pyramid must start from the ground up, so that, with the effectiveness of the training, robotic surgery is safe for the patient and the surgeon [25]. Robotic surgical systems were divided into open/closed, image-based/imageless, and active/inactive/ systems. The main goal is to improve the accuracy of operations. It should be noted that regardless of the typology of the robotic system, the surgeon is responsible for planning and approving the use of the type of operation. Accurate and consistent execution of instructions is entrusted to robots. Every surgeon, when considering which robotic system to use, must correctly evaluate their differences [26].

One of the most important issues in remote surgery is the touch feedback system. Although surgeon robots increase the surgeon's agility, have less vibration of the surgeon's hand, and have better vision, they reduce their ability to understand. Other disadvantages of robotic surgery include the possibility of a twenty-five-second delay on monitors. Depending on the doctor's condition, the delay between when the surgeon sees the knife moving and the incision is less than one-fifth of a second. It is performed and increases the probability of cutting in the wrong place and the risk of surgery and the cost of the required equipment [27]. The amount of bleeding after robotic surgery is low and severe bleeding, even if it is small, requires embolization or tracheotomy to control the bleeding. Large tumors, preoperative anticoagulant use, and a history of previous radiation are associated with an increased risk of bleeding after robotic surgery [28]. In comparison with immediate and long-term results, no difference was observed in the duration of the operation, the number of times the blood transfusion was required, and the occurrence of severe postoperative complications. Compared to traditional surgical methods, robotic surgery reduces the number and severity of postoperative complications and improves treatment outcomes [29]. In a study in the robotic surgery group, the rate of blood loss, length of hospital stay, and complications were lower, but the mean time of operation was longer compared to open surgery [30]. Operating times for robotic surgery are time-consuming due to the connection and stabilization of the instrument. The results of 260 cases of robotic surgery showed no increase in postoperative death [31]. Over time, with experience and innovation in platform design (Da Vinci), operating time is likely to decrease further [32,33]. However, a study showed that the benefits of robotic surgery have yet to be explored. Although the initial results are promising [34].

Also, studies conducted in other countries regarding robotic surgery are as follows. One study found no major complications during surgery. Kidney surgery with the help of robots by experienced robotic surgeons is a safe and repeatable surgery in selected cases and the result is excellent connective tissue function. The use of robotic technology can provide low complication rates, rapid recovery, and better connective tissue function [35]. Robotics in spine surgery is an emerging technology that promises plans. The accuracy of surgery in instruments implanted using robotics seems to be high. However, the effect of robotics on radiation levels is unclear and seems to depend on the technique and type of robot [36]. Robotic surgery greatly affects spinal surgery and is still safer and more efficient. Finally, it offers a better 3D image [37]. Robotic spine surgery was introduced in 2004. Robotic spine surgery has made great strides. This surgery is a reliable and accurate tool for spine surgery and has grown rapidly in recent decades and is still on the path of development and progress [38]. Artificial intelligence has tremendous potential to revolutionize the comprehensive care of the spine. It can assist surgeons in selecting preoperative patients, and surgical symptoms, and improving individual postoperative care. Robotic surgery, while still in its infancy, has a very clear vision for robotic spine surgery. Robotic surgery can reduce surgeon fatigue and improve surgeon technical accuracy [39]. Robotic systems are widely accepted in the United States and various surgical specialties. This innovative technology is still relevant in the field of spine surgery. These systems have potential benefits such as increased screw placement accuracy, reduced operating time, and reduced radiation. However, the various technical factors of these systems are constantly being re-evaluated to improve performance and achieve these goals [40]. Robotic surgery is also very effective in adult spinal deformity surgery [41].

Robotic hip arthroplasty was associated with a reduced length of hospital stay and reduced costs compared to conventional methods. However, robotic surgery was associated with readmission and the need for more blood transfusions [42].

Robotic surgery is an innovation in thyroid surgery that compensates for the disadvantages of conventional endoscopy. Robotic surgery has many advantages, including 3D imaging, motion scaling, and vibration elimination. Although robotic thyroid surgery is often associated with longer operating times than conventional open surgery, more robotic techniques are safer. Robotic surgery is compared to conventional open surgery or endoscopy. It is associated with several benefits such as quality of life, aesthetic results, reduction of neck pain and sensory changes, and reduction of discomfort in swallowing after surgery. According to surgeons, robotic surgery improves the patient's ergonomics. The time and cost of surgery in robotic surgery have been longer. Patient mortality was not different. The benefits of robotic thyroid surgery With conventional surgery, it is shown that robotic surgery can be an advanced surgical option for thyroid disease. Robotic thyroid surgery should be further developed as a surgical procedure for endocrinology, and head and neck surgeons have received this training, and robotic thyroid surgery is a surgical option [43].

Robotic surgery for the treatment of rectal cancer appears to be oncologically safe and appears to be better in terms of short-term complications and pathological quality indicators. The potential and proven benefits of robotic surgery should be considered. The benefits of robotic surgery for rectal cancer include improved three-dimensional vision, ease of use of the tool, improved surgeon ergonomics, and better access to narrow spaces such as the pelvis. However, studies have clearly shown the value of robotic surgery for patients and surgeons and need further studies [44].

Mesorectal robotics is a safe and sufficient technology for the treatment of rectal cancer and helps to maintain better urinary and sexual functions. Robotic surgery due to better wrist movements and better use of tools that facilitate autopsy, especially in narrow spaces, facilitates the operation by minimizing damage to the surrounding tissues as well as due to the magnified view [45].

Robotic surgery seems to be an effective method in terms of oncological and functional results and is effective in patient survival and its use is recommended in tumor staging and HPV status [46].

Robotic-assisted radical prostatectomy was less recurrent and had lower pain scores up to 6 weeks postoperatively compared to open radical prostatectomy. Compared to laparoscopic prostatectomy, robotic surgery improves urinary and sexual function. Robotic surgery had fewer complications for endometrial cancer than laparoscopy [47].

Mitral valve robotic surgery is well possible through a limited surgical procedure. Mortality around surgery seems very acceptable and possible. It is even lower than sternotomy. The rate of stroke was higher than sternotomy in the early stages of mitral valve robotic surgery but now appears to be at least the same. After robotic surgery, 1 in 4 people needed a blood transfusion. The rate of renal failure around surgery is low. One in 200 patients develops a wound infection. The length of hospital stay did not decrease. Due to the huge level of innovation in mitral valve robotic surgery, this method seems very satisfactory [48].

Robotic head and neck surgery are now regularly used in specialized centers for head and neck surgery. It is used in simple surgeries and easily accessible anatomical areas such as the mouth and throat. Disadvantages of this method include increasing the time required for the operation and increasing costs. For basal cranial, nasopharyngeal, or laryngeal surgery in patients with difficult anatomical conditions, systems with special surgical instruments for head and neck surgery should be developed. Despite the obstacles, we strongly believe that robotic systems will have a major impact on how we perform head and neck surgery over the next decade. Robotic head and neck surgery are recommended for better surgery in difficult areas, to reduce surgical complications, and to increase patient safety [49].

Robotic surgery in a rural head and neck center is a safe and appropriate treatment option and has very few side effects and this method is recommended [50].

A review of studies showed that robotic surgery has better overall survival than non-robotic resection of oral squamous cell carcinoma, and this method is recommended [51].

The number of female robotic surgeries has increased dramatically since the introduction of robotic surgery systems in Korea. Based on these data, it is expected that the continuous use of robotic surgery is widely used for both benign and malignant types of gynecological diseases. Many traditional surgeries are expected to be replaced by robotic surgeries [52].

A study on the application of robotic surgery in gynecological diseases showed that robotic surgery can significantly reduce blood loss, intraoperative complications, and length of hospital stay, but the rate of readmission and operation time Increasing. Robotic surgery has shown better results in the treatment of endometrial cancer [53].

The robotic system has overcome some of the shortcomings of laparoscopy, including three-dimensional vision limitation and hand tremors. Therefore, robotic surgery has taken many steps toward further progress in the field of general surgery. Although the safety and effectiveness of robotic surgery systems have been confirmed in most methods, in some methods robotic surgery has not been widely performed and is limited to a few highly specialized centers. Robotic surgery in hepatectomy, gastrectomy, esophageal surgery, colectomy, and pancreatectomy is performed unequally in the world, which may be related to the economic power of countries and the preference of surgeons, with the gradual reduction of the cost of robotic surgery systems and its standardization, we believe that robotic surgery will become gold and will become a standard method in general surgery, including surgical procedures as well as health insurance coverage [54].

The operating time for robotic surgery was 30% longer compared to laparoscopy. The rate of re-admission to robotic surgery is higher. Compared to laparoscopic patients, robotic surgery patients were hospitalized longer. There was no evidence of improved robotic surgery performance over surgery or clinical outcomes [55].

The rate of robotic segmentectomy has increased, and according to our study on robotic surgery, the operation time was longer, the blood loss rate was lower, and the hospital stay time was shorter. Our results show that robotic surgery can facilitate the function of complex anatomical parts [56].

Robotic surgery has become the preferred method for abdominal surgery. Robotic surgery is recommended when vaginal surgery is not possible. So far, gynecological robotic surgery has shown better safety and clinical results than laparoscopy. Robotics has been developed to overcome laparoscopic challenges and has led to technological benefits such as improved ergonomics, 3D visualization capabilities, skill and range of motion with the tool joint, and elimination of hand vibration. To date, robotic applications in benign obstetrics and gynecology include hysterectomy, myomectomy, endometriosis surgery, sacrocopopexy, adnexal surgery, tube re-anastomosis, and cerclage.

Robotic surgery may offer greater benefits than other methods in the obese patient population and more complex cases. Advances in robotic technology, such as telemedicine surgery, could revolutionize gynecological surgery. Finally, this robotic device is an additional minimally invasive surgical instrument that can help minimize laparotomy in women [57].

The use of robotic substrates in liver and bile duct surgery is growing rapidly. There is evidence that robotic surgery results are good. Robotic surgery is useful for managing complex cases such as iatrogenic bile duct injury, hepatic autopsy, or complex autopsy due to difficult anatomical positions.

We believe that, for selected patients, robotic surgery should be part of the treatment of complex liver surgeries [58].

According to studies, there is still room for the development of robotic surgery. Lack of close cooperation between scientists, institutions, and countries may have affected this issue. The process of industrialization of surgical robots in several developing countries, such as the United States, must be accompanied by the removal of technical barriers and increased international cooperation and exchange.

Removing barriers and developing global surgical relationships should seize the opportunity to improve the situation [59].

On the other hand, there is considerable variation in US hospital accreditation policies. However, the purpose of performing performance appraisals and monitoring is to improve patient outcomes. Extensive development and implementation of validation guidelines are recommended to optimize patient safety and outcomes [60]. The subject of training in robotic surgery is also very important.

The study showed that a lack of technical skills affects the results of robotic surgery. Therefore, physician education is very important [61}. The use of a standard curriculum for robotic surgery remains unclear. The choice of training methods and evaluation tools should be based on validity and application based on performance data. Clinical education seems to simulate effective education [62].

Also, in studies, the lack of clarity in the roles and responsibilities of nurses in robotic surgery and the lack of coordination for working conditions cause professional dissatisfaction and dismissal in nurses [63], which should also be considered.

CONCLUSION

Robotic surgery is one of the new and diverse technologies that the medical community at the national and international levels will have to use and enter this path. According to research, the results showed that robots can not be used in any way and relied only on manpower, because manpower, ie the same surgeons has mistakes, so these robots can not be left out.

Among the benefits of robotic surgery can be; Reduction of the long recovery period after surgery, reduction of postoperative pain, reduction of the possibility of postoperative infection, reduction of hospitalization time, no need to break the sternum and open the chest in open surgeries, rapid healing of wounds, in the availability of certified surgeons with unique capabilities in more locations, greater accuracy, elimination of surgeon hand vibration, continuous and 24-hour operation 24 hours a day without fatigue.

In addition to having many advantages, according to robotic surgery studies, it has disadvantages such as; Increasing the distance between doctor and patient, reducing the speed of communication with equipment and monitors, increasing the possibility of cutting the wrong place, the possibility of disconnecting the surgeon from the operating room, low network security and the possibility of hacking communication, patients' reluctance to use this equipment and technology,

The robots are expensive, the doctor does not have an assistant, and the doctor does not have a sensory understanding of the patient's environment. It must be adapted to other technologies and used to serve the people. Although we are facing the development of surgical robots around the world, a review of scientific products in the field of surgical robots shows that the United States, China, and Japan are the three leading countries in this field, respectively, and Iran is in 15th place.

The trend of scientific production in this field started in 1990 and has been moving forward with a gentle slope and has gone through an upward trend since 2001. This trend has continued upward with fluctuations in different years. The results indicate that there are few worldwide available scientific developments in this field and few countries, universities, authors, and publications have addressed this issue [13].

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REVIEW

Risk factors for abdominal compartment syndrome in trauma – A review

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Abstract: *Background and Aim: Abdominal compartment syndrome is a life-threatening complication that can occur in trauma patients and greatly increase their mortality. Although there is a better scientific understanding of the general phenomena involved in the pathogenesis of this complication, the particular risk factors and their implications in the trauma patient population are yet to be deciphered.*

*Methods: The authors conducted research through 3 electronic databases (PubMed, Scopus, and ScienceDirect) using the following search formula: "(ACS OR abdominal compartment syndrome) AND (*trauma*) AND (risk factor)". Subsequently, additional search formulas were used, including the risk factors taken into consideration (i.e. "shock", "hypotension", "acidosis", "base deficit", "coagulopathy", "retroperitoneal hematoma", "HOB elevation", "fluid resuscitation", "damage control laparotomy").*

Results: Throughout the 41 articles analyzed in this paper, 7 risk factors transcended and were further discussed: head of bed elevation/patient positioning, fluid resuscitation, the "lethal triad" of acidosis hypothermia and coagulopathy, Damage Control Laparotomy, shock/hypotension, retroperitoneal hematoma and demographics (age, gender, and race).

Conclusions: To summarize, many potential risk factors were evaluated for the envisagement of the present paper, but the ones that prevailed the most were excessive fluid resuscitation, shock/hypotension, retroperitoneal hematomas, and the lethal triad. Consistent with other studies, no connection was found between age, gender, or race and the development of ACS. Further studies should focus more on the likely involvement of damage control laparotomy and patient positioning, as well as hypocalcemia, in the unfolding of ACS in trauma patients.

Keywords: *abdominal compartment syndrome, trauma, risk factors*

INTRODUCTION

Trauma is considered to be the primary cause of death for people aged 1-44 years old in the United States [1,2], with similar demographics in the European Union [2]. There are two types of trauma, regarding the mechanism of injury: blunt and penetrating. Blunt trauma defines any injury caused by an impact with a dull surface or object and is most frequently encountered in motor-vehicle accidents, falls and assaults, whereas penetrating trauma represents an injury caused by a foreign object piercing the skin and creating an open wound, generally involved being firearms and stabbing [1]. Overall, blunt trauma accounts for 70% of all trauma cases [2].

Abdominal compartment syndrome (ACS) is a life-threatening complication that can occur in trauma patients and greatly increase their mortality. In 2006, the World Society of Abdominal Compartment Syndrome (WSACS) proposed a series of universal terms to use when referring to this intricate disease [3], which were later revised in a 2013 conference [4]. Throughout their research, major advances have been made toward a superior comprehension of the diagnostic criteria for ACS [5]. The prevalence of ACS in major trauma has been previously reported higher than 30% and the mortality rate greater than 60% [6].

Although there is a better scientific understanding of the general phenomena involved in the pathogenesis of ACS, the particular risk factors and their implications in specific

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patient populations are yet to be deciphered. This article aims to address the particular occurrence of ACS in trauma patients, while also reiterating the information currently available in the literature to a greater extent.

The abdomen can be considered a relatively incompressible enclosed space and the intra-abdominal pressure (IAP) represents the steady-state pressure concealed within this cavity. IAP normally ranges between subatmospheric and 0 mmHg and changes slightly with respiratory movements. Pathologically, IAP rises with the accumulation of fluid in the abdominal cavity, space-occupying lesions, changes in the volume of its viscera, or conditions that limit the expansion capacity of the abdominal wall [3]. IAP is particularly elevated in critically ill adults, with a typical range of 5 to 7 mmHg [3].

Intra-abdominal hypertension (IAH) refers to a prolonged or repeated pathological elevation of IAP ≥ 12 mmHg [3,4]. Both IAH and ACS have been determined to be important independent predictors of mortality [7,8]. According to WSACS [3], IAH can be classified into 4 grades, as follows:

- Grade I: IAP 12-15 mmHg.
- Grade II: IAP 16-20 mmHg.
- Grade III: IAP 21-25 mmHg.
- Grade IV: IAP > 25 mmHg.

Malbrain et al. [9] suggested 4 subdivisions of IAH that were based on the time-length of symptoms:

- Hyperacute IAH defines a short-term increase in IAP over a couple of seconds-minutes, without pathological significance, due to sneezing, coughing, straining, or physical activity.
- Acute IAH is most commonly seen in surgical patients suffering from trauma or intra-abdominal hemorrhage. IAP rises abruptly over hours and imposes a high risk of producing new-onset organ failure, thus becoming a new clinical entity: ACS.
- Subacute IAH is a consequence of the interaction between casual and predisposing factors and develops over days.
- Chronic IAH progresses for months (i.e. pregnancy) or years (i.e. tumors, morbid obesity, cirrhosis with ascites), allowing the abdominal wall to accommodate the excess pressure through distension without decompensation.

A consensual research-based definition of the ACS was implemented by WSACS in 2013: ACS represents a sustained IAP > 20 mmHg, with or without an APP ≤ 60 mmHg, correlated mandatory with a new organ impairment/failure [4]. Abdominal perfusion pressure (APP) is the difference between mean arterial pressure (MAP) and IAP [3] and its relevance will be discussed within the pathophysiology of ACS.

Moreover, ACS can be classified into 3 types, depending on the duration and mechanism [3]:

- Primary ACS – involves acute or subacute IAH due to an intra-abdominal cause, most commonly trauma or surgery.
- Secondary ACS – subacute or chronic IAH originated from an extra-abdominal cause, most commonly burns or sepsis.
- Recurrent ACS – formerly labeled as Tertiary ACS, implies that a patient treated for primary or secondary ACS redevelops symptoms, most commonly acute IAH (“second-hit” phenomenon).

Given the fact that direct measurements are too invasive to be widely applicable in all patients [10], there have been numerous discussions regarding the indirect technique which most accurately estimates IAP. In 2006, the WSACS reached a consensus: IAP should be expressed in mmHg, measured via the bladder with a maximal instillation volume of 25 ml sterile saline solution, with the patient in a supine position at end-expiration, without abdominal muscle contraction and with the transducer zeroed at the mid-axillary line [3]. The standardized protocol reinforces that intermittent measurements should be conducted every 4-6 hours in critically ill patients with at least one risk factor for ACS [11].

METHODS

The authors conducted research through 3 electronic databases (PubMed, Scopus, and ScienceDirect) using the following search formula: “(ACS OR abdominal compartment syndrome) AND (*trauma*) AND (risk factor)”.

Subsequently, additional search formulas were used, including the risk factors taken into consideration (i.e. “shock”, “hypotension”, “acidosis”, “base deficit”, ”coagulopathy”, “retroperitoneal hematoma”, “HOB elevation”, “fluid resuscitation”, “damage control laparotomy”) and the terms “ACS” and “trauma”.

Three inclusion criteria were applied: the articles must be available in full text, written in English, and published no more than 10 years ago. The references of the selected papers were also checked for other relevant material. The resulting references were included in the current review, focusing on the pathophysiology of the abdominal compartment syndrome and its risk factors, particularly in the adult trauma patient population. In the end, a total number of 41 articles were included in this research.

RESULTS AND DISCUSSIONS

For a better understanding of the implications of various risk factors in the pathogenesis of ACS, a brief description of the key pathophysiological concepts is needed.

The abdominal cavity is a well-defined space limited cranially by the diaphragm, caudally by the pelvic floor, anteriorly and posteriorly by the abdominal wall, and contains numerous parenchymatous viscera such as the liver, spleen, pancreas or kidneys, as well as hollow organs such as the stomach, small intestine, colon, cholecyst or urinary bladder. Given the fact that this cavity represents a closed compartment, one can easily understand that the ACS behaves similarly to any other compartment syndrome in general – an increase in intra-compartmental pressure generates a compression over the blood vessels, initially resulting in a restricted venous and lymphatic flow, followed by an arterial blood supply impairment and subsequently entering a vicious cycle which generates and maintains cellular hypoxia [11,12,13].

The cause of elevated intra-abdominal pressure can differ between trauma, burns, or sepsis, but they all result in a molecular cascade that ultimately determines capillary leak syndrome, fluid extravasation, and interstitial edema [14]. Capillary leak refers to undesirable extravasation of fluid and electrolytes +/- protein followed by edema [15]. In sepsis, interleukin-6, interleukin-8, and tumor necrosis factor-α have been found to interact with the capillary endothelial cells and trigger edema of the bowel wall. When hypotension occurs due to reflex vasodilation and fluid retention in the third compartment, physicians use fluid resuscitation to compensate for the fluid sequestration. This, in turn, aggravates the changes in hydrostatic and oncotic pressures, worsening the interstitial fluid extravasation, while the upward compression of the diaphragm limits respiratory movements, increases central venous pressure, and finally, contributes to the potential risk of developing ACS. This sequence is not solely applicable to sepsis originating from a non-traumatic medical condition but should be kept in mind when caring for a trauma patient with a penetrating abdominal wound, which perforated a hollow organ such as the digestive tract and spread germs in the abdominal cavity
[14].

Otherwise, the typical polytrauma patient presents with one or multiple bleeding sources, potentially developing hemorrhagic shock within minutes-hours of the traumatic event. In this situation, a massive amount of fluid resuscitation may be necessary for the survival of the patient, either by blood transfusions or crystalloid resuscitation. Apart from the capillary leak syndrome and interstitial edema, the attempt to restore the effective circulating volume after an important ischemia-inducing blood loss will result in an ischemia-reperfusion injury to the bowel wall and increased capillary permeability. Crystalloid solutions will decrease the oncotic pressure, further aggravating the pressure imbalance and increasing the fluid extravasation and ultimately, IAP [14]. A trauma patient who, besides the initial injury, develops a condition that further aggravates his state, is a patient who suffers from the "second-hit" phenomenon. This phenomenon corresponds to neutrophil priming, which means that activated neutrophils (from the traumatic injury mechanism) are overstimulated by a subsequent mechanism, most often inadequate fluid resuscitation, and therefore have an exaggerated immune response and determines a massive cytokine release, which leads to ACS and MOF (multiple organ failure). The most vulnerable window of time for this phenomenon also matches the most frequent timeframe of occurrence of the ACS, the first 3-6 hours after the traumatic event [6].

Nevertheless, elevated IAP induces cardiovascular, neurological, hepatic, and renal dysfunction [6,10-14,16]. An increase in IAP exerts pressure on the inferior vena cava, resulting in a decrease in venous return to the heart and a reduced preload. By reducing the preload of the heart, the cardiac output is also decreased aggravating cellular hypoxia and generating crucial amounts of lactic acid which, combined with the respiratory acidosis induced by the restricted movement of the diaphragm and the ventilatory impairment caused by atelectasis, result in worsened cellular function. The respiratory function is also hindered by increased intra-thoracic pressure, increased pleural pressure, and decreased dynamic and static compliance [10]. Moreover, the decrease in blood flow translates into reduced renal perfusion and potentially acute kidney injury (AKI). The renin-angiotensin-aldosterone hormone system (RAAS) is activated when renal hypoperfusion stimulates renin secretion by the juxtaglomerular cells. Angiotensin II is a potent vasoconstrictor that increases peripheral vascular resistance and activates aldosterone secretion from the adrenal glands. Aldosterone, in turn, induces sodium and water retention, intending to increase renal perfusion, but fails to stabilize the mean arterial pressure (MAP) [11]. One study reports that the onset of oliguria may announce the debut of ACS [17]. The liver is also affected by increased IAP, even at a moderate elevation of 10 mmHg [10], and impaired liver function translates into a decreased serum lactate clearance [12]. Finally, elevated IAP increases intracranial pressure and therefore decreases cerebral perfusion pressure due to a domino mechanism initiated by the cephalad displacement of the diaphragm and elevation of the intra-thoracic pressure, accompanied by a reduced cardiac output.

Risk factors

Throughout time, the scientific world has shown great interest in the most important risk factors for IAH/ACS. Finally, in 2013, the WSACS [4] summarized them and created 4 categories divided by mechanism and a 5th additional miscellaneous group: diminished abdominal wall compliance, increased intra-luminal contents, increased intra-abdominal contents, capillary leak/fluid resuscitation, and others. In the first group, identified risk factors were found to be major trauma, major burns, abdominal surgery, and prone positioning among several studies. An increase in intra-luminal contents was suggested particularly in gastroparesis and ileus, while fluid collections in the abdominal cavity, acute pancreatitis, and sepsis were responsible for an increase in intra-abdominal contents. Acidosis was prevalent among risk factors in multiple studies, as well as massive fluid resuscitation, while others suggested hypothermia, damage control laparotomy, or poly-transfusion as a possible mechanism of capillary leak. An increased APACHE-II or SOFA score was also associated with a risk of developing ACS, as they are typically present in a critically ill patient. Other factors taken under consideration were age, increased HOB angle, obesity and increased BMI, mechanical ventilation, and positive end-expiratory pressure (PEEP) > 10. Shock, hypotension, and sepsis were also proposed.

In comparison, Holodisnky et al. [18] observed a noteworthy clinical heterogeneity and ambiguity between the studies that reported risk factors in ACS and aimed to address this crucial impediment by identifying evidence-based risk factors for particular patient populations. Therefore, the authors conducted a systematic review and meta-analysis in which potential risk factors were investigated (38 for IAH and 24 for ACS) and separated into three categories: patient characteristics, systemic physiology, and fluid resuscitation. In addition to those mentioned by Kirkpatrick et al. [4], this systematic review and meta-analysis included, among others: gender, white blood cell count, amylase, calcium, C-reactive protein and albumin level, Glasgow Coma Scale score, Ranson score for acute pancreatitis, cardiac index, serum creatinine level and urine output, blood glucose, base deficit, hemoglobin and hematocrit, pre-ICU and pre-hospital crystalloid, crystalloid to packed red blood cell ratio. Nevertheless, this study concluded that large-volume crystalloid resuscitation and the presence of shock/ hypotension were commonly found in all patient populations, while others were specific to a particular group. The trauma patient population associated a higher prevalence of pre-ICU crystalloid resuscitation, base deficit ≥12, and abdominal surgery as risk factors. In addition, hypothermia (temperature ≤ 34°), urine output ≤ 150 ml in 24 hours, hemoglobin ≤ 80 g/L, and $GAPCO_2 \geq 16$ were strongly associated with the development of ACS in the trauma patient group. Another relevant finding was that, as the volume of crystalloid used for resuscitation increased (≥ 7.5 L), the risk of ACS increased as well, with an Odds Ratio of 166.2 (CI 95%).

Head of bed elevation/patient positioning

Concerning the head of bed angle, a study published in 2009 by De Keulenaer et al. [19] raises the concern of a patient's position interfering with IAP measurement. The patients in the ICU may need to be nursed at a head-of-bed elevation of 30-45° to reduce the risk of ventilator-associated pneumonia and pressure ulcers, and this semi-recumbent position has been found to increase IAP by up to 4-9 mmHg, due to the viscera descending and exerting external pressure over the bladder. Furthermore, it has also been suggested that keeping a patient with a higher risk of developing IAH/ACS in a semi-recumbent position might trigger ACS [19]. In comparison, prone positioning has been demonstrated to elevate IAP, although the exact effects imposed on systemic and hepato-splanchnic blood flow or gastric-mucosal arterial PCO2 gradient have not been consensually established. This might be due to inaccuracies regarding the patient population taken under consideration and the type of mattress used for prone positioning [19]. Lastly, lateral positioning was analyzed in a small trial conducted by the same authors [20], in which De Keulenaer et al. concluded that out of the 10 patients included, the occurrence of IAP was 3.3% in the supine position and 26.6% in the lateral semi-recumbent position, which suggested that lateral decubitus may increase IAP by the same mechanism of visceral-induced external pressure on the bladder. A noteworthy limitation, besides the small study group, is that the trial only addressed patients with a low baseline IAP level, deeming it unreliable for the trauma patient population, in which a higher IAP level may be expected. Therefore, while the head-of-bed elevation effect on IAP has been established, further research on prone and lateral positioning is due. These findings are consistent with a study published by McBeth et al. [21] in 2007, in which a statistically significant relationship between a head of bed elevation of 30-45° and increased IAP was found.

Smit et al. [11] address these concerns in their study, proposing a reference table that can be used to correct IAP values depending on the head of a bed elevation. Their paper suggested that, for a head of bed angle between 0-20°, the measured IAP is correct and does not need any adjustment. Otherwise, for a head of bed elevation of 20-40°, the correct pressure is obtained by subtracting 4 mmHg from the measured IAP, while a head of bed angle greater than 40° requires subtraction of 9 mmHg from the measured value.

Fluid resuscitation

Fluid resuscitation is a vital part of therapy for trauma patients, given the fact that the most prevalent cause of death in the Early stage (first 72 hours after the traumatic injury) is hypovolemic shock due to blood loss [22,23]. Post-injury ACS is most frequently associated with inadequate fluid resuscitation of trauma patients [6]. Parenteral solutions used for fluid resuscitation are classified according to their molecular volume in crystalloids and colloids. Crystalloids include isotonic saline solution, Ringer's lactate, and hypertonic saline solution (contains larger quantities of sodium than the physiological concentration). Colloids, such as albumin, hydroxyethyl starch, and dextrans, have higher molecular weight and therefore generate an osmotic effect. Although rapid fluid resuscitation was widely considered the standard approach to hypovolemic shock in trauma patients [22], significant concern has been raised by some clinicians regarding the safety of the fluid resuscitation protocols. Hence, several studies addressed the issue: in a 2007 retrospective study, Madigan et al. [24] evaluated 48 patients with severe extremity injury who developed secondary ACS after trauma and compared them to a control group of patients who did not develop ACS. The authors concluded that the ACS group had received significantly higher amounts of crystalloid and blood products during all stages of care: pre-hospital, Emergency Department resuscitation, and post-admission hospital course. Therefore, early aggressive resuscitation by large volumes of crystalloids was found to be an important predictor of secondary ACS in the trauma patient population. This conclusion adheres to the reasoning of Balogh et al. [25] who suggested in 2003 that supranormal resuscitation (oxygen delivery index > 600 ml/m² achieved by a greater amount of crystalloid and packed red blood cell transfusions) was associated with an increased risk of IAH, ACS, MOF, and death.

In a 2012 multicenter prospective cohort study, Neal et al. [26] analyzed the crystalloid to packed red blood cell ratio (C:PRBC ratio) in a 452 patients population, only including those who needed ≥10 units of PRBC in the first 24 hours and survived past the initial 24 hours post-injury. The study population was divided into 2 groups by the C:PRBC ratio median: HIGH C:PRBC and LOW C:PRBC. The authors discovered that high ratios of C:PRBC (> 1.5:1 crystalloid to PRBC) were linked to a greater risk of MOF, ACS, and ARDS (Acute Respiratory Distress Syndrome) and this relationship was dose-dependent.

In 2015 Kaplan et al. [15] noted that excessive use of crystalloids causes dilution coagulopathy and hypothermia (if used un-warmed) and is therefore involved in triggering the "deadly triad" of acidosis, hypothermia, and coagulopathy. Hypertonic saline was found to be a valid alternative to colloids for increasing plasma osmolarity and also a better option than isotonic saline because of the ability to rapidly raise blood pressure without the need for a large volume of administration. Colloids, such as hydroxyethyl starch (HES), although useful for their osmotic effect of keeping fluid in the vessel, could be dangerous because of adverse effects and should only be used for a short time (< 24 hours) and respecting the resuscitation endpoints. The results of this study were that aggressive fluid resuscitation with isotonic crystalloid solutions may exacerbate the capillary leak syndrome and generate peripheral tissue edema, ascites, and IAH/ACS and that a valuable alternative could be hypertonic saline. HES should be avoided in the event of burns or sepsis. Finally, the implication of exaggerated fluid resuscitation in the pathogenesis of post-injury ACS was sustained by various other studies [8,27-30] and further emphasizes the importance of resuscitation endpoint criteria, awareness, and early recognition of IAH and ACS.

The "lethal triad" of acidosis, hypothermia, and coagulopathy

The "lethal triad" of acidosis, hypothermia, and coagulopathy is a catastrophic appearance in the management of trauma and is a result of fluid sequestration, hypoperfusion of vital organs, development of ischemia and lactate release in the bloodstream, along with systemic inflammatory response syndrome and the association of supranormal fluid resuscitation [31]. Stawicki et al. [32] note that ACS is a consequence of the "lethal triad" and should be prevented by Damage Control Laparotomy (DCL), which consists of a planned surgical approach with 3 steps: firstly, an abbreviated surgery should be implemented to control the active bleeding and limit the risk of sepsis. The abdomen should be partially covered and not closed, for example using a Bogota bag. Additional re-explorations may be necessary to drain potentially forming fluid collections (continued bleeding, pus, bile). The second step comprises ICU management with physiological restoration by correcting base deficit and coagulopathy, warming-up procedures, and continuing fluid resuscitation. When the patient is stable enough, the third step is implemented: definite surgical treatment which addresses, for example, bowel continuity restoration, and finally, the abdomen is closed.

Ditzel et al. [33] recently suggested that hypocalcemia is also an important predictor of mortality among trauma patients and should be included among the "lethal triad", forming a "lethal diamond". The authors described an intricate mechanism in which hypocalcemia is both a consequence and a promoting factor in the development of coagulopathy and acidosis: as serum calcium decreases, the calcium-dependent clotting factors become unfunctional and coagulopathy occurs; lower levels of serum calcium are linked to metabolic acidosis; hydrogen can competitively block ionized calcium receptors necessary for clotting; hypothermia decreases the liver function and therefore the liver is deemed unable to metabolize the citrate that is derived from transfusions; finally, ionized calcium is necessary for the proper function of cardiac myocytes and the lack of calcium is associated with significantly reduced cardiac contractility and decreased cardiac output, hypoperfusion, ischemia, and lactate production by hypoxic cells. Since the majority of trauma patients are hypocalcemic before receiving blood products and transfusions exacerbate the ionized calcium deficit, the authors concluded that calcium product administration for trauma patients requiring transfusions should be taken under consideration for further studies.

Damage Control Laparotomy

A mixed, retrospective, and prospective study conducted at a level 1 trauma center by Ertel et al. [34] demonstrated a clear association between Damage Control Laparotomy (DCL) and ACS. 311 patients with severe abdominal or pelvic trauma were included in this study and needed DCL for their injuries and 17 of them developed ACS, resulting in a 5.5% incidence, compared to the 14% obtained by Meldrum et al. [30]. Ertel et al. further noted that the association of both pelvic and abdominal trauma was remarkably more often linked to the development of ACS at a higher speed (the first 4-5 hours after trauma) compared to those who only presented abdominal or pelvic trauma [34]. The crucial discovery of this study is the fact that all the patients who developed ACS had had primary closure of the abdomen after the initial exploratory laparotomy. Set site by side, the patients that were treated with mesh closure of the abdomen did not develop ACS. This discovery suggests that primary fascial closure of the abdomen is an important risk factor for ACS in the surgically managed trauma patient population.

2 years later, Gracias et al. [35] analyzed this issue to a greater extent and conducted a retrospective review to discover whether or not the management of the open abdomen with temporary abdominal closure could prevent ACS and if there were any predictors of ACS in the patients treated with an open abdomen. A comparison was made between the 5 patients treated with temporary abdominal closure by vacuum pack who developed ACS and the other 15 patients who underwent the same treatment but did not develop ACS. Although base deficit levels, pH, and lactate levels on surgical ICU admission notably deteriorated in the ACS group, systolic blood pressure, prothrombin time, and temperature were similar between the 2 groups. Regrettably, no statistically significant relation between these potential predictors and the development of ACS could be made. Nevertheless, the mortality rate was remarkably higher in the ACS group (60%) compared to the non-ACS group (7%), further supporting the theory that ACS is an important predictor of mortality.

Shock/hypotension

Hypovolemic shock is most commonly seen in trauma patients as a result of acute hemorrhage, both internal and external. It can also be derived from massive fluid sequestration in the abdominal cavity, bowel wall, and interstitium due to increased hydrostatic pressure and decreased oncotic pressure in plasma, as seen in SIRS. The consequences are diminished effective circulatory volume, decreased cardiac output, and reduced perfusion to vital organs, including the kidneys and brain. The severity of hypovolemic shock varies from mild (less than 20% of total blood is lost) to severe (>40%) and depends not only on the volume of the blood loss but also the rapidity of which it is produced and the age and comorbidities of the patient [22]. Hypovolemic shock requires fluid resuscitation and blood products, but the endpoint of resuscitation is vital for avoiding a catastrophic complication: ACS. One must be aware of the hemodynamic criteria [36]: central venous pressure (CVP) between 8 to 12 mmHg in the first 6 hours of resuscitation, mean arterial pressure (MAP) > 65 mmHg, central venous oxygen saturation ($ScvO_2$ > 70%) and transthoracic echocardiography (TTE) inferior vena cava diameter > 20 mm.

Interestingly, Schreiber et al. [36] proposed, in a prospective randomized pilot trial that permissive hypotension might be beneficial for traumatic injury survivors. Eligible patients (out-of-hospital systolic blood pressure SBP ≤ 90 mmHg) were randomized and assigned into 2 groups: those who received controlled resuscitation, meaning 250 ccs of crystalloid in the absence of radial pulse or SBP < 70 mmHg and additional 250 cc boluses to maintain radial pulse or SBP ≥ 70 mmHg and those who received standard resuscitation, which consisted in 2 liters of fluid initially and additional fluid as needed to maintain SBP ≥ 110 mmHg. The controlled resuscitation strategy ultimately associated a decrease in early crystalloid use by 1 liter and an increase in early blood products use, adhering to the Damage Control Resuscitation (DCR) philosophy of permissive hypotension until hemorrhage control and a therapeutic plasma:platelets: PRBC ratio of 1:1:1. Although there were no statistically significant differences regarding mortality rate at 24 hours between standard and controlled resuscitation groups, improved mortality in blunt trauma patients was detected.

Duchesne et al. [15] also evaluated whether or not permissive hypotension was ultimately helpful for trauma patients who suffered a massive hemorrhage. The potential benefit suggested by former articles was that a slightly reduced arterial pressure could limit the amount of ongoing hemorrhage and therefore buy some time to allow surgical control of the bleeding. The authors raised concerns regarding the appropriateness of this practice in the general trauma population, the absence of a lower systolic or mean arterial pressure safety limit and the disastrous effects of severe traumatic brain injury. Hence, permissive hypotension is not currently recommended by the guidelines and should be further analyzed.

Retroperitoneal hematoma

In 2009, He et al. [37] suggested an increased prevalence of secondary ACS after pelvic fractures, due to an expanding retroperitoneal hematoma or the fluid resuscitation needed to compensate for the blood loss associated with this particularly severe type of fracture. Although the multivariable regression model showed that for each 1L of resuscitation fluid administered over 24hrs there was an expected 5 mmHg elevation of IAP ($p = 0.002$), no statistically significant correlation was found between pelvic fractures and IAP ≥ 12mmHg. Cook et al. [38] also approached the subject in 2002 and consequently demonstrated that the patients who suffered a rotationally or vertically unstable pelvic fracture were at a higher risk of developing resistant hypotension that could only be resolved by angiographic embolization. Agri et al. [39] later indicated that type C pelvic fractures were associated with a higher mortality rate and a greater rate of packed red blood cell (PRBC) transfusion than type B and A fractures. More research is necessary for the future to inform a possible correlation between pelvic fractures and ACS.

Although retroperitoneal hematomas are most frequently encountered after pelvic fractures, there are other possible causes. Beckman et al. [40] mentioned the implication of ruptured abdominal aortic aneurysms in the occurrence of retroperitoneal hematomas. The ongoing hemorrhage expands the hematoma and increases IAH, resulting in the development of primary ACS, hence this is considered a surgical emergency. The surgical approach to a ruptured abdominal aortic aneurysm can be endovascular (EVAR) or open (laparotomy). Dayama et al. [41] analyzed 352 patients who suffered from a ruptured abdominal aortic aneurysm following polytrauma and compared the efficiency of two different treatment options. 91 patients underwent endovascular repair and the remaining 234 patients were treated with an open aortic repair. Albeit the overall mortality rate was remarkably high (up to 51.7% during admission), the authors discovered that endovascular repair was associated with a decreased mortality rate (20.9%, p-value < 0.001) compared to the open abdominal approach (63.7%). An important downside of the endovascular approach is the possibility of post-repair ACS. In a systematic review and meta-analysis, Oliveira-Pinto et al. [42] reported a 9% incidence of ACS after endovascular repair of ruptured abdominal aortic aneurysms, as a result of an important retroperitoneal hemorrhage that could not be properly addressed without open surgery.

Demographics: age, gender, and race

None of the studies taken under consideration demonstrated any statistically significant relationship between demographic factors such as age, gender, or race and the development of IAH/ACS.

CONCLUSION

ACS is a significantly threatening condition for the trauma patient population. To summarize, many potential risk factors were evaluated for the envisagement of the present paper, but the ones that prevailed the most were excessive fluid resuscitation, shock/hypotension, retroperitoneal hematomas, and the “lethal triad”. Consistent with other studies, no connection was found between age, gender, or race and the development of ACS. Further studies should focus more on the likely involvement of damage control laparotomy and patient positioning, as well as hypocalcemia, in the unfolding of ACS in trauma patients.

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Social and psychological predictors of alcohol-related incidents involving servicemen

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Abstract: *The aim of the research was to determine the social and psychological predictors of alcohol-related incidents involving servicemen. The research involved 310 servicemen, who were subjected to disciplinary and administrative penalties within 2016-2021 years for the offence commitment under the influence of alcohol. The control group included 650 servicemen who joined the service within this period. The analysis of psychological features of servicemen was carried out with the help of the following methods: "Determination of Type Accentuation of Character Traits and Temper" Questionnaire, "Self-Esteem Structures of Temper Questionnaire", "Multilevel Personality 'Adaptability' Questionnaire", "Progressive Matrix", "16 PF Questionnaire", and "Self-Esteem Structures of Temper Questionnaire". Cluster analysis was used to differentiate servicemen who committed incidents under the influence of alcohol. The determined typology was valuable for the organization of preventive works, events dedicated to the improvement of socialization by recruits, adequate formation of servicemen image as well as their identification in accordance with it, and events dedicated to the prevention of suicidal behaviour.*

Keywords: *incidents under the influence of alcohol, servicemen, adequate behaviour, preventive psychology*

INTRODUCTION

The use of alcohol by servicemen was a problem for numerous armies all over the world. Unpleasant statistics concerning the alcohol abuse was highlighted by the researchers from Australia [1], Great Britain [2-3], France [4], and the USA [5]. They proved that alcohol abuse did not only have negative effect on the servicemen's health, wellbeing, moral state, the effectiveness of their military activity, and on readiness to complete missions, but also had financial losses because of alcohol-related accidents (ARIs). The researchers proposed the program of alcoholism prevention for army in general as well as for communities where the military units were located. Other researchers such as Grattan, Mengistu, Bullock, Santo, & Jackson [6] determined that the prohibition to sell alcohol beverages to servicemen at night had positive influence on the statistics of the crime level in definite community.

The research of recent years demonstrated that the military service was joined mostly by school leavers who were drinking alcohol beverages but not by those who did not have such obsession [7]. Other researchers such as Shirvani, Reed, Clingan [8], and Lamb, & Baron [9] distinguished the connection between the alcohol abuse by youth from 24 to 32 years old and military personnel. In accordance with their works servicemen drank alcohol because that was the part of military culture.

Another group of scientists Hunter, Talcott, Klesget, Lando, & Haddock [10], Jeffry, & Mattiko [11] pointed out that there was a close link between the alcohol abuse and tendency to risky behaviour by male servicemen as well as by female ones.

Traditionally, high level of alcohol abuse by servicemen was caused by experienced stress during the participation in combat operations and their negative consequences

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including post-traumatic stress disorder (PTSD) [12]. In accordance with the research conducted by Jones and Fear [13] the increased number of cases of alcohol abuse related to negative experience which was caused by transition from combat operations to civil life.

Ong and Joseph [14] pointed out that unexperienced servicemen of junior management who served abroad on long-term business trips (they used to long separation from their families, friends, resources' limitation, high level of combat readiness, and limited local jurisdiction, etc.) were the most vulnerable part. They chose negative copings including alcohol abuse.

Bartone, Hystad, Eid, & Brevik [15] studied psychological hardiness and avoidance of coping as predictors of risk for alcohol abuse in military personnel following war-zone deployment. Their results showed that low level of psychological hardiness, more combat exposures, younger age, and lower rank were associated with increased risk for alcohol abuse soon after the return from deployment. At follow-up, avoidance of coping was a significant predictor of risky alcohol abuse.

Skipper, Forsten, Kim, Wilk, & Hoge [16] examined the association between specific combat experience and post-deployment hazardous drinking patterns of selected military personnel that was considered to pose high risk, such as personnel belonging to the U.S. Army Special Operations Forces. In their study, combat experience was divided into the following categories: (1) Fighting, (2) Killing, (3) Threat to oneself, (4) Death/Injury of others, and (5) Atrocities. They determined, that combat experience related to fighting, threat to oneself, and atrocities was significantly connected with alcohol abuse being analyzed individually. However, when the factors were examined simultaneously, combat experience in the fighting category was significantly associated with a positive screen of alcohol abuse. In conclusion, soldiers who belonged to certain elite combat units were more likely to screen positive of alcohol abuse if they were exposed to specific types of combat experience versus any other type of combat exposure.

Other researchers such as Russell, Russell, Riviere, Thomas, Wilk, & Bliese [17] distinguished that among the participants of the research there increased the quantity of use of alcohol up to 80.5% while alcohol abuse increased from 8.51% to 19.15%. However, alcohol abuse among those who killed enemy during the combat significantly decreased.

Hoopsick, Homish, Collins, Nochajski, Read, & Homish [18] having compared servicemen of the National Guard of the USA who took part in combat operations with those who did not have such experience distinguished that low resilience and existence of psychological problems due to this situation became the reason of addictive behaviour of servicemen, but not the participation in combat operations.

Hanwella, de Silva, & Jayasekera [19] compared the reasons of alcohol abuse by servicemen of Great Britain and the USA with servicemen of Sri Lanka Navy's Special Forces where the tradition of alcohol abuse among the citizens in general significantly differed from the traditions of alcohol abuse in Great Britain and the USA. They pointed out that the high level of alcohol abuse during combat operations was connected with worsened psychological health and functional disorders of servicemen of Great Britain and the USA, but that was not diagnosed with servicemen of Sri Lanka. The authors concluded that the alcohol abuse was significantly influenced by national traditions of the country where they served which included the features related to alcohol accessibility and attitude to alcohol.

McCabe, Watrous, & Galarneau [20] said that 49% of servicemen who were wounded in past even 10 years later had positive screening on PTSD and depression. The participants of this research had high level of alcohol abuse, problems of tobacco and cigarette use, lack of sleep as well as physical activity.

Other researchers such as Boscarino, Hoffman, Pitcavage, & Thomas [21] figured out that group of veterans who were on outpatient care where the majority were veterans of the Vietnam War (72%) among this group 9.6% had eternal PTSD, 18.4% had eternal depression, and 50.1% had eternal consultations with the service of psychological aid. Among the key factors which were connected with PTSD, depression, and a need to use the service of psychological aid they pointed out low self-esteem, alcohol or drug abuse to overcome histories of childhood trauma, high level of combat influence, and low psychological resilience.

Angkaw, Haller, Pittman, Nunnink, Norman, Lemmer, McLay, & Baker [22] distinguished that the veterans who returned from Operation Enduring Freedom and Operation Iraqi Freedom (OEF/OIF) were found to be at increased risk for post-traumatic stress disorder (PTSD) and alcohol abuse disorders, leading to negative mental health–related quality of life (MHRQoL).

Recent research conducted by Derefinko, Hallsell, Isaacs, Salgado Garcia, Colvin, Bursac, McDevitt-Murphy, Murphy, Little, Talcott, & Klesges [23] demonstrated that the separated transition from active duty to civilian life could become critical in terms of the future functioning of the veteran. Repeated estimating tests indicated that endorsement of alcohol abuse, cigarette abuse, and prescription of drug abuse was similar during active duty and dismissal; marijuana and hard drugs abuse significantly increased in the 6-month period of dismissal. Active-duty of anxiety/depression and aggressive feelings were maintained from active-duty to dismissal, loneliness and military-based trauma symptoms increased significantly as well.

Kintzle, Oh, Wilcox, Hassan, Ell, & Castro [24] figured out that post-deployment civilian unemployment became a common problem and source of additional stress for the National Guard (NG) personnel. Alcohol abuse was concluded to moderate the relationship between civilian unemployment and mental health symptoms.

Since the problem of alcohol abuse happened to servicemen within the whole period of service starting with the entrance and finishing with dismissal (even in civil life after the dismissal), modern research was aimed at the search of predictors which could give possibility to decrease the quantity of servicemen who had potential tendency to alcohol abuse during their service including combat operations.

Hunter et alia [10] examined demographic, lifestyle, and psychosocial variables to determine predictors of the Air Forces recruits who were likely to have alcohol-related problems. The study involved the recruits of the Air Forces (N = 32 144), who underwent basic training from August 1995 to September 1996. They determined that psychosocial predictors included positive rebellious attitudes, decreased seatbelt use, and positive risk-taking attitudes.

The research conducted by Sarramon, Vendoux, Schmitt, & Bourgeois [25] demonstrated that susceptibility to boredom, impulsiveness, and search of thrills as well as adventures could increase the possibility to gain adequate behaviour.

Gutierrez, Blume, Schmaling, Stoever, Fonseca, & Russell [26] considered that identifying the predictors of alcohol consequences which occurred upon demobilization could help in determining which soldiers were at risk for such consequences before deployment and could help to maintain military readiness. They identified that significant predictors of greater alcohol-related incidents, included less quantity of years of formal education, male gender, non-engagement in an intimate relationship, racial/ethnic minority status, enlisted rank, deployment to the continental United States, and greater stress, whereas significant predictors of drinking and driving included male gender, non-engagement in an intimate relationship, and greater stress.

There was a need to point out that researchers Selman et alia [1], Henderson et alia [2], Iversen et alia [3], Ong et alia [14], and Bartone et alia [15] determined that young age as well as low rank were social and demographic predictors of alcohol abuse of servicemen. That, in turn, turned urgency to the topics such as victimization and unfair attitude as the cases of alcohol abuse.

Other researchers Ames, Moore, Cunradi, Duke, & Galvin [27] identified interrelationships of unfair treatment at work, stress, and problem drinking among a sample of the U.S. In their research, it was found that more women than men (51.4% versus 16.2%) reported gender unfair treatment; approximately 20% of respondents reported ethnic/racial unfair treatment. The researchers concluded that unfair treatment was associated with likelihood of problem drinking, but associations were attenuated after adjusting for frequency of work problems and expecting alcohol to alleviate stress.

Kim Y, Kim J, Park, & Fear [28] investigated workplace victimization as a cause of alcohol abuse in junior military personnel (JMP). Workplace victimization in JMP was prevalent (17.7%). Victims in the workplace showed higher level of anger and alcohol abuse. In mediation analyses, workplace victimization was related to higher levels of anger, which, in turn, were associated with greater alcohol abuse.

In addition to the interrelationships and mediators the researchers Benjamin, Bell, and Hollander [29] resorted to the determination of the typology in the process of examining the problems of alcohol abuse by servicemen. This allocation of subgroups gave the possibility to figure out the tendencies for drinking alcohol which were inherent in servicemen. It could help to prevent alcohol abuse [4] or could become additional factor to diagnose suicidal risks of servicemen [5].

MATERIALS AND METHODS

Participants and Procedure

This research determined the social and psychological predictors which were based on the generalization of data concerning the professional psychological selection of servicemen who committed ARIs. This research had retrospective character. Received results could help to improve professional psychological selection for service and could help to differentiate the approaches for the events based on prevention of alcohol abuse.

This research involved the servicemen who were prosecuted to disciplinary and administrative penalties for committed offences under the influence of alcohol (310 individuals). As the control group there were presented the servicemen who joined the military service within this period of time (650 individuals).

Instruments and Measures

The results of psychological selection of candidates for the service were used to determine the types of servicemen who committed alcohol-related incidents. A range of psychological tests included several questionnaires implemented in electronic form for testing and processing the results obtained in the Automated Psychodiagnostic Complex (APC), called "Psychodiagnostics" in short form [30].

To determine the characteristics of personality temper there was used the "Self-Esteem Structures of Temperament Questionnaire" (Smirnov, as cited by Vorobyova et alia) [30].

Determination of character traits which worsened the process of adaptation to new conditions and reasons of life was formed with the questionnaire "Determination of Type Accentuation of Character Traits and Temperament" (DTACTT; Schmieschek, as cited by Vorobyova et alia) [30]. In the basis of this questionnaire there was the theory of character accentuation developed by Leonhard (as cited by Vorobyova et alia) [30]. In accordance with this conception specific character traits were divided into two groups such as main and additional. The core of individual was in the basis of main traits of a person. These traits were: individuality, development, adaptation, and psychological health. Excessive expression of these traits led to structural psychological changes and in hostile environment to the development of individual's structure.

General skills of servicemen were figured out with the "Progressive Matrix" test (Raven and Raven) [31]. Results based on this test provided us with information on the abilities of servicemen to get definite forms, to take their personalities, temper and mutual relations or ensemble as well as combination of relations.

The specificity of motivation of choosing the profession of a serviceman was determined with the "Questionnaire on Examination of Professional Selection Motivation" (Moskalenko, Kobzin, & Starodubtsev, as cited by Vorobyova et alia) [30].

General information about character traits which were formed throughout the candidates' life was obtained with the "Sixteen Personality Factor Questionnaire" (16-PF Questionnaire, as cited by Vorobyova et alia) [30].

Among the examined group of servicemen who committed ARIs there were distinguished the groups for comparison with the help of cluster analysis. As a criterium of segmenting there were used the scales of the method "Multilevel Personality 'Adaptability' Questionnaire" (MPAQ; Maklakov and Chermyanin) [32] that gave the possibility to provide the analysis with the summarized estimation of the specificity of the individual's adaptation to new conditions and of the tolerance to its changes.

Data Analysis

A cluster analysis was used to compare the data of psychodiagnostic scales in studied groups. To represent the data, we used the main descriptive statistics (arithmetic mean – M – and standard deviation – SD). To reliably detect significant differences between comparative groups, there was used the t-Student criterion. Mathematical data were processed through SPSS 17.0.

RESULTS

In comparison with the control group servicemen who committed ARIs were less motivated, active, and capable to rapid mobilization of strength. The indicators of personal peculiarities of servicemen who committed ARIs were proposed in Table 1.

The indicators of peculiarities of servicemen who committed ARIs in comparison with the control group were proposed in Table 2.

The indicators of distinctions' significance of servicemen who committed ARIs in comparison with the control group were proposed in Table 3.

In accordance with almost all the scales of close questionnaire "Studying the Motivation of Professional Choice by Applicants of Higher Educational Institutions of the Minister of Internal Affairs of Ukraine" servicemen who committed ARIs had the lowest indicators of the "Motives Connected with Objective Socially Set Aims and Content of the Profession" ($t= 5.18$, $p \leq .01$), "Motives Connected with Personal and Professional Development" ($t= 5.67$, $p \leq .01$), "Independent Professional Choice" ($t= 3.23$, $p \leq .01$), "Motives Connected with External Prestige of Profession and Material Wellbeing" ($t= 5.71$, $p \leq .01$), "Motives Connected with Romantic Appeal of Profession" ($t= 4.97$, $p \leq .01$), "Motives Connected with Eagerness to Compensate the Characterological Weaknesses" ($t= 7.31$, $p \leq .01$), "Antisocial Motives" ($t= 4.67$, $p \leq .01$). Lower indicators of Types of Accentuation were distinguished due to the method "Determination of Type Accentuation of Character Traits and Temper" – "Hyperthymic" ($t= 5.39$, $p \leq .01$) and "Exalted" ($t= 2.75$, $p \leq .01$). Servicemen who committed ARIs, due to the method "Self-Esteem Structures of Temper Questionnaire" were less extroverted ($t= 1.96$, $p \leq .1$). Due to the indicators of method "16 PF Questionnaire" they had the lowest indicators of conformism (t= 3.97, p ≤.01), practicality (t= 4.20, p ≤.01), sociability (t= 3.59, p ≤0.01), and intellect. These distinctions were expressed for intellect as general awareness (t= 3.40, p ≤.01) but not for general ability to study due to the method "Raven's Progressive Matrices" (t= 2.81, p ≤.01).

Table 1: Personal peculiarities of servicemen who committed alcohol-related incidents (in Standard Points)

	A	**B**	**C**	**t A–B**	**t A–C**	**t B–C**
"Determination of Type Accentuation of Character Traits and Temper" Questionnaire						
Hyperthymic	17.17±3.82	17.07±4.10	14.63±5.88	0.10	1.12	1.16
Stuck	11.67±2.50	11.05±2.62	12.75±3.37	0.97	0.82	1.40
Emotive	8.67±4.59	8.88±4.50	12.00±6.41	0.18	1.33	1.36
Pedantic	10.56±4.33	9.84±4.18	13.00±7.17	0.66	0.89	1.23
Anxious (fearful)	4.67±5.37	3.05±3.56	7.88±4.52	1.24	1.57	2.96**
Cyclothimic	10.33±3.12	8.88±3.02	13.88±4.79	1.85^0	1.92^0	2.91**
Demonstrative	14.11±5.02	14.20±4.00	11.25±2.38	0.07	1.97^0	3.22**
Excitable	7.50±4.96	5.24±3.46	12.38±3.74	1.87^0	2.76*	5.25**
Disthymic	9.33±3.07	9.32±2.93	9.75±4.74	0.02	0.23	0.25
Exalted	9.67±3.65	9.93±4.35	14.25±5.50	0.28	2.16*	2.18*
"Multilevel Personality 'Adaptability' Questionnaire"						
Probability	1.56±1.29	6.07±2.56	4.50±1.20	11.78**	5.65**	3.25**
Adaptivity	38.28±7.53	24.84±7.20	59.00±5.40	7.11**	7.95**	16.94**
Neuropsychological resilience	15.67±6.22	10.33±4.77	33.00±4.14	3.49**	8.37**	14.85**
Communication	12.72±2.24	8.35±2.63	17.38±3.58	7.53**	3.39**	7.00**
Morality	9.89±2.17	6.16±2.38	8.63±1.19	6.74**	1.91^0	5.23**
"Progressive Matrix" Test						
IQ (general indicator)	104.44±14.40	102.07±13.03	95.13±11.70	0.66	1.74^0	1.61
"Studying the Motivation of Professional Choice Questionnaire"						
Professional motives	19.22±6.00	21.12±5.40	20.75±5.12	1.27	0.67	0.20
Motives for improvement	17.94±5.40	19.03±4.88	18.50±7.15	0.81	0.20	0.21
Independent profession choice	19.50±5.52	19.09±5.04	16.63±7.65	0.30	0.96	0.90
Non-independent profession choice	10.89±5.11	12.25±5.06	12.63±5.01	1.06	0.81	0.20
Prestige Motives	18.06±4.63	17.93±4.95	18.38±4.21	0.10	0.17	0.28
Romantic motives	14.28±5.91	15.75±4.73	14.50±6.59	1.01	0.08	0.53
Compensatory motives	17.17±5.06	18.57±4.87	19.88±7.06	1.11	0.98	0.51
Antisocial motives	10.78±4.68	12.30±5.12	14.13±4.39	1.27	1.76^0	1.12
"16 PF Questionnaire"						
Md (low – high self-esteem)	7.50±1.95	9.87±2.03	9.13±1.46	4.79**	2.35*	1.36
A (introversion-fellowship)	8.06±2.39	8.89±1.70	7.13±1.89	1.42	1.07	2.57*
B (low-high intellect)	3.39±1.38	3.84±1.58	3.00±1.51	1.28	0.62	1.53
C (emotional instability - stability)	8.89±1.68	9.84±1.54	7.50±1.60	2.28*	2.01^0	4.01**
E (subordination-dominance)	5.39±1.42	5.89±1.71	6.38±2.77	1.37	0.95	0.48
F (restraint-expressivity)	5.00±2.17	5.39±1.89	6.00±1.77	0.73	1.24	0.93
G (low-high normativity)	8.72±1.90	8.39±1.99	8.38±1.19	0.68	0.56	0.04
H (timidity (fearfulness)-bravery)	8.50±1.92	8.57±1.79	7.38±2.20	0.14	1.25	1.50
I (toughness-sensitivity)	4.78±2.32	4.63±1.88	6.13±0.83	0.26	2.17*	4.39**
L (credulity-suspicion)	4.67±2.40	4.48±1.89	4.00±1.77	0.31	0.79	0.74

	A	B	C	t A–B	t A–C	t B–C
M (practicality-reverie)	5.28±2.32	5.02±1.70	6.00±1.85	0.45	0.85	1.45
N (straightforwardness-diplomacy)	5.78±1.66	5.25±1.80	6.63±1.92	1.25	1.08	1.97^{0}
O (calmness-anxiety)	4.56±2.41	3.72±2.11	5.75±0.71	1.39	1.93^{0}	6.44**
Q$_1$ (conservatism-radicalism)	5.28±2.37	6.30±2.01	5.50±1.77	1.74^{0}	0.26	1.23
Q$_2$ (conformity-non-conformity)	5.39±1.79	4.50±1.68	5.50±2.39	1.98*	0.12	1.16
Q$_3$ (low-high self-control)	7.17±1.42	7.68±1.51	7.88±1.96	1.42	0.92	0.28
Q$_4$ (relaxation-tension)	3.56±1.76	3.47±1.79	4.75±1.83	0.20	1.55	1.92^{0}
“Self-Esteem Structures of Temper Questionnaire”						
Extraversion-introversion	16.44±3.65	17.88±3.93	14.13±6.22	1.54	0.98	1.68^{0}
Rigidity-flexibility	9.11±4.32	8.25±3.75	10.50±2.39	0.81	1.05	2.47*
Emotional anxiety-poise	4.06±2.44	3.06±3.18	11.50±4.44	1.55	4.45**	5.29**
Reaction rate	13.17±3.00	12.65±3.65	10.88±2.36	0.67	2.10*	1.98*
Activity	17.44±7.11	19.81±5.26	16.13±2.85	1.36	0.67	3.31**
Sincerity scale	13.00±3.40	7.14±4.56	10.63±4.31	6.51**	1.38	2.21**

*Notes: A= Group A; B= Group B; C= Group C; 0p≤ .1; *p≤ .05; **p≤ .01*

Table 2: Personal peculiarities of servicemen who committed alcohol-related incidents in comparison with the control group (in Standard Points)

	CG	ARIs	A	B	C
“Determination of Type Accentuation of Character Traits and Temper” Questionnaire					
Hyperthymic	18.89±3.84	16.92±4.16	17.17±3.82	17.07±4.10	14.63±5.88
Stuck	11.71±2.62	11.16±2.64	11.67±2.50	11.05±2.62	12.75±3.37
Emotive	9.42±4.96	9.10±4.62	8.67±4.59	8.88±4.50	12.00±6.41
Pedantic	10.52±4.46	9.90±4.45	10.56±4.33	9.84±4.18	13.00±7.17
Anxious (fearful)	3.45±3.75	3.50±4.07	4.67±5.37	3.05±3.56	7.88±4.52
Cyclothimic	9.26±3.24	9.21±3.35	10.33±3.12	8.88±3.02	13.88±4.79
Demonstrative	15.04±3.40	14.00±4.05	14.11±5.02	14.20±4.00	11.25±2.38
Excitable	6.09±3.89	5.86±4.09	7.50±4.96	5.24±3.46	12.38±3.74
Disthymic	8.96±2.80	9.39±3.15	9.33±3.07	9.32±2.93	9.75±4.74
Exalted	11.21±3.32	10.18±4.40	9.67±3.65	9.93±4.35	14.25±5.50
“Multilevel Personality ‘Adaptability’ Questionnaire”					
Probability	5.39±2.92	5.57±2.87	1.56±1.29	6.07±2.56	4.50±1.20
Adaptivity	27.84±9.91	28.81±11.95	38.28±7.53	24.84±7.20	59.00±5.40
Neuropsychological resilience	12.01±6.14	12.50±7.86	15.67±6.22	10.33±4.77	33.00±4.14
Communication	8.96±3.34	9.44±3.71	12.72±2.24	8.35±2.63	17.38±3.58
Morality	6.87±2.69	6.87±2.69	9.89±2.17	6.16±2.38	8.63±1.19
“Progressive Matrix” Test					
IQ (general indicator)	104.89±10.54	101.69±13.23	104.44±14.40	102.07±13.03	95.13±11.70
“Studying the Motivation of Professional Choice Questionnaire”					
Professional motives	23.27±2.67	20.97±5.36	19.22±6.00	21.12±5.40	20.75±5.12
Motives for improvement	21.21±2.87	18.86±4.97	17.94±5.40	19.03±4.88	18.50±7.15
Independent profession choice	20.45±3.14	19.06±5.16	19.50±5.52	19.09±5.04	16.63±7.65
Non-independent profession choice	12.51±4.84	12.21±5.01	10.89±5.11	12.25±5.06	12.63±5.01
Prestige Motives	20.32±3.04	18.03±4.76	18.06±4.63	17.93±4.95	18.38±4.21
Romantic motives	17.68±3.91	15.57±4.91	14.28±5.91	15.75±4.73	14.50±6.59

	CG	ARIs	A	B	C
Compensatory motives	21.56±3.22	18.52±4.94	17.17±5.06	18.57±4.87	19.88±7.06
Antisocial motives	14.46±4.59	12.38±5.07	10.78±4.68	12.30±5.12	14.13±4.39
"16 PF Questionnaire"					
Md (low – high self-esteem)	9.17±2.53	9.57±2.20	7.50±1.95	9.87±2.03	9.13±1.46
A (introversion-fellowship)	9.31±1.87	8.72±1.85	8.06±2.39	8.89±1.70	7.13±1.89
B (low-high intellect)	4.21±1.46	3.74±1.57	3.39±1.38	3.84±1.58	3.00±1.51
C (emotional instability - stability)	9.61±1.59	9.59±1.69	8.89±1.68	9.84±1.54	7.50±1.60
E (subordination-dominance)	6.18±2.07	5.88±1.73	5.39±1.42	5.89±1.71	6.38±2.77
F (restraint-expressivity)	5.57±1.87	5.40±1.90	5.00±2.17	5.39±1.89	6.00±1.77
G (low-high normativity)	8.52±1.95	8.42±1.94	8.72±1.90	8.39±1.99	8.38±1.19
H (timidity (fearfulness)-bravery)	8.56±1.79	8.41±1.87	8.50±1.92	8.57±1.79	7.38±2.20
I (toughness-sensitivity)	4.59±1.96	4.74±1.90	4.78±2.32	4.63±1.88	6.13±0.83
L (credulity-suspicion)	4.16±1.85	4.46±1.99	4.67±2.40	4.48±1.89	4.00±1.77
M (practicality-reverie)	4.44±2.07	5.14±1.81	5.28±2.32	5.02±1.70	6.00±1.85
N (straightforwardness-diplomacy)	5.22±1.89	5.35±1.78	5.78±1.66	5.25±1.80	6.63±1.92
O (calmness-anxiety)	4.04±2.48	3.93±2.20	4.56±2.41	3.72±2.11	5.75±0.71
Q_1 (conservatism-radicalism)	6.31±2.24	6.12±2.06	5.28±2.37	6.30±2.01	5.50±1.77
Q_2 (conformity-non-conformity)	3.97±1.88	4.61±1.80	5.39±1.79	4.50±1.68	5.50±2.39
Q_3 (low-high self-control)	7.31±1.77	7.66±1.51	7.17±1.42	7.68±1.51	7.88±1.96
Q_4 (relaxation-tension)	3.33±1.78	3.52±1.83	3.56±1.76	3.47±1.79	4.75±1.83
"Self-Esteem Structures of Temper Questionnaire"					
Extraversion-introversion	18.05±4.03	17.31±4.25	16.44±3.65	17.88±3.93	14.13±6.22
Rigidity-flexibility	9.50±3.78	8.44±3.96	9.11±4.32	8.25±3.75	10.50±2.39
Emotional anxiety-poise	3.18±3.20	3.59±3.73	4.06±2.44	3.06±3.18	11.50±4.44
Reaction rate	12.79±3.57	12.43±3.66	13.17±3.00	12.65±3.65	10.88±2.36
Activity	18.87±5.01	19.10±5.68	17.44±7.11	19.81±5.26	16.13±2.85
Sincerity scale	7.56±5.07	7.97±4.92	13.00±3.40	7.14±4.56	10.63±4.31

Notes: CG= Control Group; ARIs= Alcohol-Related Accidents; A= Group A; B= Group B; C= Group C

Table 3: The indicators of significance of distinctions between the control group and the groups of servicemen who committed alcohol-related incidents (in Standard Points)

	t CG – ARIs	t CG – A	t CG –B	t CG – C
"Determination of Type Accentuation of Character Traits and Temper" Questionnaire				
Hyperthymic	5.39**	1.89^0	4.56**	2.05*
Stuck	2.33*	0.07	2.55*	0.87
Emotive	0.76	0.68	1.19	1.14
Pedantic	1.58	0.03	1.65^0	0.97
Anxious (fearful)	0.15	0.96	1.13	2.76**
Cyclothimic	0.15	1.44	1.27	2.72**
Demonstrative	2.95**	0.78	2.17*	4.45**
Excitable	0.63	1.19	2.45*	4.72**
Disthymic	1.54	0.51	1.24	0.47
Exalted	2.75**	1.78^0	3.09**	1.56
"Multilevel Personality 'Adaptability' Questionnaire"				
Probability	0.71	11.78**	2.61**	2.04*

	t CG – ARIs	**t CG – A**	**t CG –B**	**t CG – C**
Adaptivity	0.94	5.75**	3.96**	16.00**
Neuropsychological resilience	0.73	2.46*	3.40**	14.15**
Communication	1.48	6.91**	2.22*	6.61**
Morality	0.00	5.79**	2.99**	4.05**
“Progressive Matrix” Test				
IQ (general indicator)	2.81**	0.13	2.25*	2.35*
“Studying the Motivation of Professional Choice Questionnaire”				
Professional motives	5.18**	2.86**	4.30**	1.39
Motives for improvement	5.67**	2.56*	4.78**	1.07
Independent profession choice	3.23**	0.73	2.88**	1.41
Non-independent profession choice	0.68	1.33	0.51	0.07
Prestige Motives	5.71**	2.06*	5.14**	1.30
Romantic motives	4.97**	2.43*	4.23**	1.36
Compensatory motives	7.31**	3.66**	6.50**	0.67
Antisocial motives	4.67**	3.29**	4.34**	0.21
“16 PF Questionnaire”				
Md (low – high self-esteem)	1.94^0	3.56**	3.32**	0.09
A (introversion-fellowship)	3.59**	2.21*	2.50*	3.26**
B (low-high intellect)	3.40**	2.48*	2.35*	2.24*
C (emotional instability - stability)	0.12	1.79^0	1.56	3.69**
E (subordination-dominance)	1.86^0	2.29*	1.62	0.20
F (restraint-expressivity)	0.98	1.09	0.92	0.69
G (low-high normativity)	0.56	0.45	0.63	0.33
H (timidity (fearfulness)-bravery)	0.90	0.14	0.02	1.52
I (toughness-sensitivity)	0.88	0.35	0.24	5.05**
L (credulity-suspicion)	1.67^0	0.88	1.71^0	0.26
M (practicality-reverie)	4.20**	1.51	3.34**	2.36*
N (straightforwardness-diplomacy)	0.84	1.40	0.15	2.06*
O (calmness-anxiety)	0.54	0.90	1.47	6.38**
Q_1 (conservatism-radicalism)	1.01	1.82^0	0.01	1.27
Q_2 (conformity-non-conformity)	3.97**	3.32**	3.13**	1.80^0
Q_3 (low-high self-control)	2.50*	0.41	2.43*	0.81
Q_4 (relaxation-tension)	1.17	0.53	0.76	2.18*
“Self-Esteem Structures of Temper Questionnaire”				
Extraversion-introversion	1.96^0	1.83^0	0.44	1.78^0
Rigidity-flexibility	3.02**	0.38	3.38**	1.17
Emotional anxiety-poise	1.27	1.50	0.38	5.29**
Reaction rate	1.09	0.53	0.39	2.26*
Activity	0.46	0.84	1.83^0	2.67**
Sincerity scale	0.93	6.59**	0.92	1.99*

*Notes: CG= Control Group; ARIs= Alcohol-Related Accidents; A= Group A; B= Group B; C= Group C; 0 p ≤.1; * p ≤.05; ** p ≤.01; *** p ≤.001*

The majority of servicemen who committed ARIs were 50.32% of servicemen of enlisted personnel, 38.71% of non-commissioned and petty officers, and 10.32% of junior officers. The minority of servicemen of this group were senior officers – 0.65%. 2/3 of this group (66.45) were unmarried or divorced. 53.55% had experience of service which was not longer than 3 years, 29.68% had experience of service which was from 4 to 10 years, and 16.77% had experience of service which was longer than 10 years. 50.32% of servicemen had the status of participants in hostilities.

Even though all the servicemen who committed ARIs were characterized as those who had the decrease of the quality of interaction with surrounding substantive and social world (decrease of activity, adequacy, etc.) this group was not homogenous. There were determined the following groups A, B, and C after the use of cluster analysis (as a criterium of segmenting there were used the scales of the method "Multilevel Personality 'Adaptability' Questionnaire") among the servicemen who committed ARIs. The increase of the quantity of clusters of analysis gave the possibility to figure out the subgroups A1, A2, B1, B2, B3, and B4 within the groups A and B.

Despite other typical distinctions for servicemen who committed ARIs (the quantity was 12.16% from the analyzed group of servicemen who committed ARIs) group A differed from the control group with high level of sincerity due to the method "Multilevel Personality 'Adaptability' Questionnaire" – "Credibility" (t= 11.76, p ≤.01) and due to the method "Self-Esteem Structures of Temper Questionnaire" – "Sincerity" (t= 6.59, p ≤.01) and significantly higher indicators of disadaptivity on all the scales of the method "Multilevel Personality 'Adaptability' Questionnaire" – "Neuropsychological Resilience" (t= 2.46, p ≤.05), "Communication" (t= 6.91, p ≤.01), "Morality" (t= 5.79, p ≤.01), general indicator of "Adaptivity" (t= 5.75, p ≤.01). Due to the method "16 PF Questionnaire" the representators of group A in comparison with the control group had significantly lower level of self-esteem (t= 3.56, p ≤.01), emotional stability (t= 1.79, p ≤.1), and significantly higher level of conservatism (t= 1.82, p ≤.1), and subordination (t= 2.29, p ≤ .05).

Servicemen of groups B and C differed from group A due to the method "Multilevel Personality 'Adaptability' Questionnaire" with higher level of credibility (tA-B= 11.78, tA-C= 5.65, p ≤.01 for both values) and scales of the method "Self-Esteem Structures of Temper Questionnaire" – "Sincerity", (tA-B= 6.51, p ≤.01), "Morality" (tA-C= 1.91, p ≤.1). Due to the method "16 PF Questionnaire" they had the lowest indicators of Antisocial Motives (tA-C= 1.76, p ≤.1) and self-esteem (tA-B= 4.79, p ≤.01 and tA-C= 2.35, p ≤.05). However, group A due to the method "Progressive Matrix" had the highest indicators of IQ among the distinguished groups B and C (tA-C= 1.74, p ≤.1), which brought representators of group A closer to the control group.

Social and demographic data of group A was determined by the fact that 60% of this group had experience of service not longer than 3 years, they were the part of enlisted personnel and were divorced. A half of servicemen of this group had the status of participants in hostilities. About 40% of servicemen had experience from 10 to 25 years and were the participants in hostilities. A half of servicemen of group A were divorced.

The analysis of results gave the possibility to conclude that servicemen of group A experienced crisis of values, they highly regarded norms, rules of behaviour and understood their inconsistency. Such situation was a result of getting into new surrounding or of overthinking the values.

The increase of the quantity of clusters gave the possibility to determine two subgroups in group A (subgroup A1 – 72.22% and subgroup A2 – 27.78%). The indicators of significance of distinctions among the determined subgroups in group A were presented in Table 4.

Table 4: The indicators of significance of distinctions among the determined subgroups of group A (in Standard Points)

	A	A1	A2	T A1 – A2
"Determination of Type Accentuation of Character Traits and Temper" Questionnaire				
Hyperthymic	17.17±3.82	17.31±4.27	16.80±2.68	0.30
Stuck	11.67±2.50	11.08±2.40	13.20±2.28	1.74
Emotive	8.67±4.59	8.08±4.13	10.20±5.85	0.74
Pedantic	10.56±4.33	10.77±3.79	10.00±6.00	0.27
Anxious (fearful)	4.67±5.37	4.15±5.68	6.00±4.74	0.70
Cyclothimic	10.33±3.12	10.15±2.61	10.80±4.55	0.30
Demonstrative	14.11±5.02	13.54±5.04	15.60±5.18	0.76
Excitable	7.50±4.96	6.46±4.56	10.20±5.45	1.36
Disthymic	9.33±3.07	9.69±2.50	8.40±4.45	0.61

	A	A1	A2	T A1 – A2
Exalted	9.67±3.65	10.15±3.78	8.40±3.29	0.97
“Multilevel Personality ‘Adaptability’ Questionnaire”				
Probability	1.56±1.29	1.08±0.95	2.80±1.30	2.69*
Adaptivity	38.28±7.53	34.69±4.79	47.60±4.67	5.22**
Neuropsychological resilience	15.67±6.22	13.54±5.24	21.20±5.40	2.72*
Communication	12.72±2.24	12.31±1.97	13.80±2.77	1.10
Morality	9.89±2.17	8.85±1.46	12.60±0.89	6.59**
“Progressive Matrix” Test				
IQ (general indicator)	104.44±14.40	106.15±15.18	100.00±12.49	0.88
“Studying the Motivation of Professional Choice Questionnaire”				
Professional motives	19.22±6.00	20.31±4.91	16.40±8.17	1.00
Motives for improvement	17.94±5.40	18.31±4.42	17.00±7.97	0.35
Independent profession choice	19.50±5.52	20.31±4.37	17.40±8.05	0.77
Non-independent profession choice	10.89±5.11	11.31±5.15	9.80±5.40	0.54
Prestige Motives	18.06±4.63	18.54±4.39	16.80±5.54	0.63
Romantic motives	14.28±5.91	14.00±6.12	15.00±5.92	0.32
Compensatory motives	17.17±5.06	18.00±4.32	15.00±6.67	0.93
Antisocial motives	10.78±4.68	10.54±4.33	11.40±6.02	0.29
“16 PF Questionnaire”				
Md (low – high self-esteem)	7.50±1.95	7.62±1.66	7.20±2.77	0.31
A (introversion-fellowship)	8.06±2.39	8.46±1.94	7.00±3.32	0.93
B (low-high intellect)	3.39±1.38	3.62±1.39	2.80±1.30	1.17
C (emotional instability - stability)	8.89±1.68	8.92±1.75	8.80±1.64	0.14
E (subordination-dominance)	5.39±1.42	5.46±1.45	5.20±1.48	0.34
F (restraint-expressivity)	5.00±2.17	4.62±2.06	6.00±2.35	1.16
G (low-high normativity)	8.72±1.90	9.31±1.60	7.20±1.92	2.18*
H (timidity (fearfulness)-bravery)	8.50±1.92	8.08±1.66	9.60±2.30	1.35
I (toughness-sensitivity)	4.78±2.32	4.85±2.48	4.60±2.07	0.21
L (credulity-suspicion)	4.67±2.40	4.00±2.35	6.40±1.67	2.42*
M (practicality-reverie)	5.28±2.32	5.46±2.60	4.80±1.48	0.67
N (straightforwardness-diplomacy)	5.78±1.66	6.00±1.73	5.20±1.48	0.98
O (calmness-anxiety)	4.56±2.41	4.38±2.79	5.00±1.00	0.69
Q_1 (conservatism-radicalism)	5.28±2.37	4.54±2.18	7.20±1.79	2.65*
Q_2 (conformity-non-conformity)	5.39±1.79	5.23±1.64	5.80±2.28	0.51
Q_3 (low-high self-control)	7.17±1.42	7.54±1.13	6.20±1.79	1.56
Q_4 (relaxation-tension)	3.56±1.76	3.38±1.56	4.00±2.35	0.54
“Self-Esteem Structures of Temper Questionnaire”				
Extraversion-introversion	16.44±3.65	15.54±3.71	18.80±2.39	2.20*
Rigidity-flexibility	9.11±4.32	9.54±4.39	8.00±4.42	0.66
Emotional anxiety-poise	4.06±2.44	3.38±2.10	5.80±2.59	1.86^0
Reaction rate	13.17±3.00	13.46±3.18	12.40±2.61	0.73
Activity	17.44±7.11	18.31±5.76	15.20±10.33	0.64
Sincerity scale	13.00±3.40	13.23±3.92	12.40±1.52	0.65

*Notes: A= Group A; A1= Subgroup A1; A2= Subgroup A2; 0 p ≤.1; * p ≤.05; ** p ≤.01; *** p ≤.001*

Subgroups A1 and A2 differed with their level of the crisis of values. Thus, servicemen of subgroup A2 had significantly higher indicators of answers' sincerity – "Credibility" (t= 2.69, p ≤.05). There were higher indicators of disadaptivity due to the method "Multilevel Personality 'Adaptability' Questionnaire" – "Neuropsychological Resilience" (t= 2.72, p ≤.05), "Morality" (t= 6.59, p ≤.01), and "Adaptivity" (t= 5.22, p ≤.01). Results received due to the method "16 PF Questionnaire" demonstrated higher indicators of suspicion (t= 2.42, p ≤.05), radicalism (t= 2.65, p ≤.05) and lower indicators of normativity (t= 2.18, p ≤.05). Moreover, subgroup A2 in comparison with subgroup A1 due to the method "Self-Esteem Structures of Temper Questionnaire" had higher indicators of extraversion (t= 2.20, p ≤.05) and anxiety (t= 1.86, p ≤.1). Furthermore, representators of subgroup A1 due to the method "Progressive Matrix" were diagnosed with the higher indicators of IQ; however, those distinctions did not reach the level of statistical significance.

The analysis of the results gave the possibility to conclude that the problems of disadaptivity of subgroup A1 were connected with the process of understanding their own inconsistency to the norms which were unacceptable for the surrounding. Servicemen of subgroup A2 had a decreased ability to be led by norms, to control their reactions in accordance with them, had lower ability to entrust themselves to those rules. These peculiarities were caused by the age and lack of experience of self-regulation. Servicemen of subgroup A1 were enlisted personnel who had little experience of service (up to 3 years), moreover, 80% of them were divorced.

The indicators of personal peculiarities of servicemen who committed ARIs from group B were presented in Table 5.

Table 5: The indicators of personal peculiarities of servicemen who committed alcohol-related incidents within the subgroups of group B (in Standard Points)

	B	B1	B2	B3	B4
"Determination of Type Accentuation of Character Traits and Temper" Questionnaire					
Hyperthymic	17.07±4.10	18.67±3.79	13.96±5.32	17.25±3.49	17.82±3.21
Stuck	11.05±2.62	11.56±2.23	11.13±3.45	10.81±2.32	10.98±2.55
Emotive	8.88±4.50	9.83±4.22	9.65±4.33	8.06±4.41	8.69±4.75
Pedantic	9.84±4.18	8.89±3.83	10.26±4.23	9.44±4.52	10.24±4.10
Anxious (fearful)	3.05±3.56	2.50±2.77	4.96±4.30	1.69±2.74	3.24±3.56
Cyclothimic	8.88±3.02	9.33±2.50	9.65±3.94	7.69±2.15	9.12±3.06
Demonstrative	14.20±4.00	15.33±3.56	11.91±5.24	14.94±3.33	14.37±3.57
Excitable	5.24±3.46	5.83±3.33	6.65±3.94	3.75±2.53	5.33±3.53
Disthymic	9.32±2.93	9.83±3.22	10.43±3.60	9.19±2.84	8.69±2.39
Exalted	9.93±4.35	11.67±3.83	8.09±5.89	9.94±3.27	10.16±4.10
"Multilevel Personality 'Adaptability' Questionnaire"					
Probability	6.07±2.56	3.11±1.84	6.83±1.87	8.38±2.11	5.29±1.76
Adaptivity	24.84±7.20	19.89±3.38	35.35±3.81	18.56±4.10	25.82±4.45
Neuropsychological resilience	10.33±4.77	7.67±2.30	17.87±3.27	7.50±2.13	9.61±3.46
Communication	8.35±2.63	7.94±2.34	11.09±2.41	6.81±2.58	8.22±1.83
Morality	6.16±2.38	4.28±1.99	6.39±1.78	4.25±1.63	7.98±1.59
"Progressive Matrix" Test					
IQ (general indicator)	102.07±13.03	107.56±11.71	100.65±13.15	100.25±12.55	101.92±13.56
"Studying the Motivation of Professional Choice Questionnaire"					
Professional motives	21.12±5.40	21.00±5.58	18.96±6.32	21.34±5.87	22.04±4.33
Motives for improvement	19.03±4.88	19.61±4.72	17.78±5.33	18.91±5.34	19.49±4.44
Independent profession choice	19.09±5.04	19.78±4.82	16.09±5.81	19.50±4.93	19.98±4.39
Non-independent profession choice	12.25±5.06	11.61±4.10	11.78±6.21	12.59±3.95	12.49±5.53
Prestige Motives	17.93±4.95	17.72±5.31	16.22±5.66	18.38±5.12	18.53±4.26
Romantic motives	15.75±4.73	14.33±4.58	14.30±5.79	16.13±3.92	16.71±4.57

	B	B1	B2	B3	B4
Compensatory motives	18.57±4.87	18.72±5.43	17.26±5.68	17.47±5.22	19.86±3.70
Antisocial motives	12.30±5.12	10.94±4.73	11.96±5.81	11.69±4.21	13.37±5.38
“16 PF Questionnaire”					
Md (low – high self-esteem)	9.87±2.03	9.94±2.01	9.26±1.86	11.19±1.65	9.27±1.98
A (introversion-fellowship)	8.89±1.70	9.39±1.75	8.30±1.89	9.25±1.72	8.73±1.50
B (low-high intellect)	3.84±1.58	4.33±1.50	3.48±1.59	3.88±1.83	3.82±1.42
C (emotional instability - stability)	9.84±1.54	10.22±1.40	8.78±1.91	10.41±1.04	9.84±1.46
E (subordination-dominance)	5.89±1.71	5.28±1.49	6.17±1.92	5.50±1.52	6.24±1.73
F (restraint-expressivity)	5.39±1.89	5.33±1.61	5.91±1.88	4.75±1.83	5.59±1.96
G (low-high normativity)	8.39±1.99	8.83±1.47	7.48±2.78	8.88±1.70	8.35±1.80
H (timidity (fearfulness)-bravery)	8.57±1.79	8.94±1.63	7.74±1.81	8.75±1.81	8.69±1.76
I (toughness-sensitivity)	4.63±1.88	4.17±1.95	4.96±1.85	4.69±1.86	4.61±1.90
L (credulity-suspicion)	4.48±1.89	4.28±1.87	4.96±2.48	3.84±1.76	4.76±1.57
M (practicality-reverie)	5.02±1.70	5.00±1.37	5.30±1.26	4.44±2.05	5.29±1.70
N (straightforwardness-diplomacy)	5.25±1.80	5.06±1.55	5.65±1.99	5.75±1.87	4.80±1.66
O (calmness-anxiety)	3.72±2.11	3.89±1.81	4.70±2.20	2.63±1.68	3.92±2.18
Q_1 (conservatism-radicalism)	6.30±2.01	6.72±1.78	6.48±2.43	6.00±1.85	6.27±2.00
Q_2 (conformity-non-conformity)	4.50±1.68	3.83±1.50	5.35±1.82	4.16±1.80	4.57±1.46
Q_3 (low-high self-control)	7.68±1.51	7.89±1.49	7.61±1.75	8.25±1.24	7.27±1.45
Q_4 (relaxation-tension)	3.47±1.79	3.72±1.18	3.83±2.25	2.78±1.79	3.65±1.65
“Self-Esteem Structures of Temper Questionnaire”					
Extraversion-introversion	17.88±3.93	18.83±3.71	16.30±4.81	16.88±3.33	18.92±3.59
Rigidity-flexibility	8.25±3.75	8.61±4.19	8.57±4.13	8.00±3.25	8.12±3.81
Emotional anxiety-poise	3.06±3.18	1.89±2.05	3.91±3.93	2.28±2.68	3.59±3.27
Reaction rate	12.65±3.65	12.56±3.05	11.04±4.54	12.81±3.31	13.33±3.47
Activity	19.81±5.26	19.89±3.97	16.13±8.04	21.25±4.22	20.57±3.80
Sincerity scale	7.14±4.56	9.17±4.08	7.17±4.38	3.78±3.46	8.57±4.35

Notes: B= Group B; B1= Subgroup B1; B2= Subgroup B2; B3= Subgroup B3; B4= Subgroup B4

Servicemen from group B (82.43% from the general quantity of servicemen who committed ARIs) differed from other groups of research with the lowest level of sincere questions due to the method “Multilevel Personality ‘Adaptability’ Questionnaire”. With their answers they tried to create socially approved image. Servicemen from group B in comparison with the control group had better indicators of adaptivity – “Neuropsychological Resilience” (t= 3.40, p ≤.01), “Communication” (t= 2.22, p ≤.05), “Morality” (t= 2.99, p ≤.01), and general indicators of “Adaptivity” (t= 3.96, p ≤.01). Besides the eagerness to gain social approval servicemen of group B as well as other representators who committed ARIs had decreased quantity of interaction with surrounding substantive and social world.

Group B included 4 subgroups: subgroup B1 constituted 14.75%, subgroup B2 constituted 18.85%, subgroup B3 constituted 26.22%, subgroup B4 constituted 40.16%. Indicators of significance of distinctions between the determined subgroups of Group B which were presented in Table 6.

Servicemen of subgroup B1 differed from other subgroups B2, B3 and B4 with the highest level of sincerity due to the method “Multilevel Personality ‘Adaptability’ Questionnaire” – “Credibility” (3.11±1.84), due to the method “Self-Esteem Structures of Temper Questionnaire” – “Sincerity” (9.17±4.08) and the highest level of intellect due to the method “Progressive Matrix” – “IQ” (107.56±11.71), “16 PF Questionnaire” – “Low-high intellect” (4.33±1.50). Besides that, those servicemen differed due to the method “Determination of Type Accentuation of Character Traits and Temper” with indicators such as “Hyperthymic” (18.67±3.79) and “Exalted” (11.67±3.83). Due to the data received with the method “16 PF Questionnaire” they were less predisposed to dominance

(5.28±1.49) and non-conformity (3.83±1.50). The results of the method “Self-Esteem Structures of Temper Questionnaire” showed that servicemen had high level of emotional stability (1.89±2.05).

Table 6: The indicators of significance of distinctions among the determined subgroups of group B (in Standard Points)

	T B1 – B2	T B1 – B3	T B1 –B4	T B2 – B3	T B2 – B4	T B3 – B4
“Determination of Type Accentuation of Character Traits and Temper” Questionnaire						
Hyperthymic	3.31**	1.31	0.85	2.59*	3.22**	0.74
Stuck	0.48	1.11	0.90	0.38	0.19	0.30
Emotive	0.13	1.40	0.95	1.33	0.85	0.61
Pedantic	1.09	0.45	1.26	0.69	0.02	0.82
Anxious (fearful)	2.21*	1.00	0.90	3.21**	1.66	2.22*
Cyclothimic	0.32	2.35*	0.29	2.17*	0.57	2.48*
Demonstrative	2.48*	0.39	0.98	2.44*	2.04*	0.73
Excitable	0.72	2.31*	0.54	3.10**	1.38	2.34*
Disthymic	0.56	0.71	1.37	1.38	2.11*	0.81
Exalted	2.35*	1.61	1.40	1.36	1.53	0.27
“Multilevel Personality ‘Adaptability’ Questionnaire”						
Probability	6.36**	9.20**	4.33**	2.87**	3.32**	6.88**
Adaptivity	13.75**	1.23	5.82**	15.62**	9.37**	7.53**
Neuropsychological resilience	11.72**	0.25	2.65**	13.33**	9.82**	3.40**
Communication	4.21**	1.58	0.46	6.29**	5.05**	2.68**
Morality	3.53**	0.05	7.09**	4.57**	3.66**	10.18**
“Progressive Matrix” Test						
IQ (general indicator)	1.77^{0}	2.06*	1.67^{0}	0.11	0.38	0.57
“Studying the Motivation of Professional Choice Questionnaire”						
Professional motives	1.10	0.21	0.72	1.42	2.12*	0.58
Motives for improvement	1.16	0.48	0.09	0.77	1.34	0.51
Independent profession choice	2.22*	0.19	0.16	2.29*	2.85**	0.45
Non-independent profession choice	0.11	0.82	0.70	0.55	0.47	0.10
Prestige Motives	0.87	0.42	0.58	1.45	1.74^{0}	0.14
Romantic motives	0.02	1.40	1.89	1.31	1.76^{0}	0.62
Compensatory motives	0.84	0.79	0.82	0.14	2.00*	2.25**
Antisocial motives	0.61	0.55	1.79^{0}	0.19	0.98	1.57
“16 PF Questionnaire”						
Md (low – high self-esteem)	1.11	2.23*	1.23	3.96**	0.01	4.73**
A (introversion-fellowship)	1.90^{0}	0.27	1.41	1.90^{0}	0.96	1.38
B (low-high intellect)	1.77^{0}	0.96	1.27	0.86	0.87	0.15
C (emotional instability - stability)	2.79**	0.49	0.99	3.71**	2.35*	2.04*
E (subordination-dominance)	1.68	0.50	2.26*	1.40	0.15	2.04*
F (restraint-expressivity)	1.06	1.17	0.55	2.29*	0.67	1.97^{0}
G (low-high normativity)	2.01^{0}	0.09	1.13	2.14*	1.37	1.34
H (timidity (fearfulness)-bravery)	2.24*	0.39	0.55	2.04*	2.10*	0.14
I (toughness-sensitivity)	1.32	0.92	0.84	0.53	0.73	0.18
L (credulity-suspicion)	1.00	0.80	0.96	1.85^{0}	0.36	2.37*
M (practicality-reverie)	0.73	1.16	0.71	1.94^{0}	0.05	1.95^{0}

N (straightforwardness-diplomacy)	1.08	1.41	0.60	0.18	1.79^0	2.35*
O (calmness-anxiety)	1.29	2.43*	0.06	3.78**	1.40	3.01**
Q_1 (conservatism-radicalism)	0.37	1.36	0.90	0.79	0.37	0.61
Q_2 (conformity-non-conformity)	2.91**	0.68	1.79^0	2.40*	1.79^0	1.09
Q_3 (low-high self-control)	0.55	0.87	1.53	1.50	0.82	3.25**
Q_4 (relaxation-tension)	0.19	2.23*	0.19	1.85^0	0.33	2.21*
“Self-Esteem Structures of Temper Questionnaire”						
Extraversion-introversion	1.90	1.86^0	0.08	0.49	2.32*	2.62*
Rigidity-flexibility	0.04	0.53	0.43	0.55	0.43	0.15
Emotional anxiety-poise	2.13*	0.58	2.53*	1.72^0	0.34	1.97^0
Reaction rate	1.27	0.28	0.88	1.59	2.14*	0.67
Activity	1.96^0	1.14	0.63	2.79**	2.52*	0.74
Sincerity scale	1.50	4.73**	0.52	3.09**	1.27	5.49**

*Notes: B1= Subgroup B1; B2= Subgroup B2; B3= Subgroup B3; B4= Subgroup B4; 0 $p \leq .1$; * $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$*

The representators of subgroup B1 in comparison with the control group due to the method “Multilevel Personality ‘Adaptability’ Questionnaire” had better indicators of adaptive abilities: “Neuropsychological Resilience” (7.67±2.30), “Communication” (7.94±2.34), “Morality” (4.28±1.99), and general scale of “Adaptivity” (19.89±3.38). Due to the method “16 PF Questionnaire” they had higher level of self-esteem (9.94±2.01) and due to the method “Determination of Type Accentuation of Character Traits and Temper” they had high level of general activity (“Hyperthymic” – 18.67±3.79).

2/3 of the subgroup B1 had the status of participants in hostilities, 2/3 had experience of service from 3 to 5 years, a half of the subgroup was not married. Enlisted personnel, non-commissioned and senior officers were presented almost equally, and their quantity was about 45%, junior officers constituted about 10%.

Received results proved that servicemen of subgroups 2.1 were intellectually developed, active, and adaptive, they had professional and combat experience; however, they were not ambitious, did not see the possibility to realize themselves in profession as a serviceman and, probably, regretted their professional selection.

Servicemen of subgroup B2 among all the representators of group B due to the method “Multilevel Personality ‘Adaptability’ Questionnaire” had the highest level of disadaptivity: “Adaptivity” (35.35±3.81), “Neuropsychological Resilience” (17.87±3.27), “Communication” (11.09±2.41), except for “Morality” (6.39±1.78) which were the highest indicators among the representators of subgroup B4.

They differed from other subgroups of group B and the control group with more expressed indicators due to the method “Determination of Type Accentuation of Character Traits and Temper” – “Anxious-Fearful” (4.96±4.30), “Excitable” (6.65±3.94), and “Disthymic” (10.43±3.60), also they had lower indicators of “Hyperthymic” (13.96±5.32), “Demonstrative” (11.91±5.24), and “Exalted” (8.09±5.89). The representators of this group had the lowest indicators due to the scales of close questionnaire “Studying the Motivation of Professional Choice by Applicants of Higher Educational Institutions of the Minister of Internal Affairs of Ukraine” – “Motives Connected with Objective Socially Set Aims and Content of the Profession” (18.96±6.32), “Independent Professional Choice” (16.09±5.81), “Motives Connected with External Prestige of Profession and Material Wellbeing” (16.22±5.66), and “Motives Connected with Romantic Appeal of Profession” (14.30±5.79). Servicemen of subgroup B2 due to the method “16 PF Questionnaire” were more reserved (8.30±1.89), emotionally instable (8.78±1.91), anxious (4.70±2.20), had lower indicators of conformity (5.35±1.82) and normativity (7.48±2.78). Due to the method “Self-Esteem Structures of Temper Questionnaire” they had the highest indicators of emotional anxiety (3.91±3.93) and the lowest indicators of “Reaction Pace” (11.04±4.54) and “Activity” (16.13±8.04) which were the highest indicators among all other servicemen of group B.

Among the servicemen of subgroup B2 the majority (52%) was constituted by enlisted personnel with experience from 1 to 2 years. The quarter of servicemen had experience longer than 15 years. 2/3 of the representators were unmarried or divorced. More than a half of representators (56.52%) participated in hostilities. The officers of this subgroup constituted not more than 5%.

Received results gave the possibility to conclude that the representators of subgroup B2 tried to create the image of individual who due to the suffering could control themselves not so efficiently and had lower level of productivity. These servicemen tended to appeal to their negative experience as to excuses of their impertinence, unproductivity, and alcohol abuse.

The representators of subgroup B3 in comparison with all the groups and subgroups due to the results of the method "Self-Esteem Structures of Temper Questionnaire" were less sincere in their answers – "Sincerity" (3.78±3.46) and "Multilevel Personality 'Adaptability' Questionnaire" – (8.38±2.11). They described themselves as those who had the best indicators of adaptivity due to the method "Multilevel Personality 'Adaptability' Questionnaire" – "Adaptivity" (18.56±4.10), "Neuropsychological Resilience" (7.50±2.13), "Communication" (6.81±2.58), and "Morality" (4.25±1.63). Moreover, they had the lowest indicators of emphasizing due to the types such as: "Anxious-fearful" (1.69±2.74), "Cyclothymic" (7.69±2.15), and "Excitable" (3.75±2.53). They were extremely self-confident, had high level of self-esteem (11.19±1.65), calm (2.63±1.68), relaxed (2.78±1.79), self-controlled (8.25±1.24), and restrained (4.75±1.83). They trusted to the surrounding world (3.84±1.76), they were practical (4.44±2.05) and active (21.25±4.22).

About 60% of those servicemen had experience of service up to 3 years. 47% did not have any experience of participation in combat operation. 62.5% of servicemen of this subgroup were unmarried or divorced.

Therefore, servicemen of subgroup B3 abused alcohol because they demonstrated the role of experienced professional.

The representators of subgroup B4 differed from other subgroups with expressed disadaptivity related to the attitude to norms which regulated relations. They used the profession of serviceman to gain priority over other people. The representators had higher indicators due to the scales of questionnaire "Studying the Motivation of Professional Choice by Applicants of Higher Educational Institutions of the Minister of Internal Affairs of Ukraine" among which there were "Motives Connected with Objective Socially Set Aims and Content of the Profession" (22.04±4.33), "Motives Connected with External Prestige of Profession and Material Wellbeing" (18.53±4.26), "Motives Connected with Romantic Appeal of Profession" (16.71±4.57), "Motives Connected with Eagerness to Compensate the Characterological Weaknesses" (19.86±3.70), and "Antisocial Motives" (13.37±5.38). They had higher indicators of dominance (6.24±1.73). The representators of subgroup B4 due to the method "Self-Esteem Structures of Temper Questionnaire" had highly expressed extraversion (18.92±3.59) and higher indicators of reaction pace (13.33±3,47) than any other subgroup.

Only a quarter of servicemen of subgroup B4 was married. Almost a half of them had experience of service longer than 3 years. A quarter of servicemen had experience of service not less than 10 years. 47% of servicemen had experience of combat operations. Almost 43% of representators of this group were enlisted personnel, 45% were non-commissioned and petty officers, 12% were officers.

Servicemen of subgroup B4 wanted to dominate, to assert themselves at the expense of others, to achieve that they did not want to use physical strength, but their status and belonging to social group which was empowered. They wanted to become respectful people.

Group C (that was 5.4% from the general selection of servicemen with alcohol dependence) differed from other groups who committed ARIs and the control one with the highest indicators of general disadaptivity (59.00±5.40) which were mostly connected with low level of neuropsychological resilience (33.00±4.14) and communication (17.38±3.58), also they had slightly lower indicators of morality (8.63±1.19). Besides that, due to the method "Type of Accentuation of Character Traits and Temper" among all other groups they had the lowest indicators of "Hyperthymic Type" (14.63±5.88) and the highest indicators of "Anxious-Fearful" (7.88±4.52), and "Exalted Type" (14.25±5.50). Due to the method "16 PF Questionnaire" the representators of the third group had the highest level of excitability (7.50±1.60), anxiety (5.75±0.71), sensitivity (6.13±0.83), reverie (6.00±1.85), and diplomacy (6.63±1.92). They were marked by the highest indicators of tension (4.75±1.83). Due to the data received from the method "Self-Esteem Structures of Temper Questionnaire" the representators of the third group differed from others with the highest levels of introversion (14.13±6.22), emotional anxiety (11.50±4.44), and the lowest reaction pace (10.88±2.36). The representators of this group due to the method "Progressive Matrix" were diagnosed with the lowest level of IQ (95.13±11.70).

Everyone from group C with no exceptions were enlisted personnel with experience of service from 1 to 2 years, unmarried, a quarter of them had experience of participation in combat operations.

Such results gave the possibility to conclude that due to certain constitutional peculiarities (low neuropsychological resilience, emotional anxiety, and decreased intellectual abilities) these servicemen had difficulties with adaptation in new military surrounding and tried to decrease the emerged anxiety with typical for them way which was alcohol abuse.

DISCUSSION

The results which were received in this research proved one more time that ARIs were mostly committed by servicemen with lower military rank. The conclusion was considered to be typical, and it pointed out that servicemen who committed ARIs were characterized by the decreased quality of interaction with the surrounding substantive and social world (were less motivated, less active and less capable to rapid mobilization). They were characterized by lower level of extraversion, sociability, conformity, and practicality; moreover, they had lower indicators of intellectual abilities and awareness.

Combat operations in Eastern Ukraine has been already carrying out for 8 years already [33], thus a half of servicemen (50.32%) who took part in the research had the status of participants in hostilities. This fact could not be the indication that participation in combat operations increased the quantity of those who committed ARIs.

The research proved that the group of servicemen who committed ARIs was homogeneous and due to the specificity of adaptation it could be divided into three more groups. The most interesting fact was that a significant criterium of segmenting was the sincerity of answering the questions by servicemen. Thus, the biggest group of servicemen who committed ARIs in comparison with the control group was characterized by low level of sincerity of answers. They wanted to demonstrate their socially approved image. The same tendency was revealed in the process of analyzing the cadets from Higher Educational Military Institutions who were known to buy forbidden substances [34]. However, among the servicemen who committed ARIs the eagerness to demonstrate socially approved image was far more spread (there were 67.74% from the general group of analyzed servicemen who committed ARIs and 42.11% from the general group of analyzed military cadets who were known to use forbidden substances).

The determination of groups in this research was quite important to figure out the reasons of ARIs and their spreading. The representators of group A, in particular, understood the importance of rules and norms of behavioral regulation; however, they experienced the crisis connected with realization of their own inconsistency to norms and values which regulated the activity of servicemen. Servicemen from subgroup A1 had such crisis due to the process of overthinking of values, while servicemen from subgroup A2 were characterized not only by inconsistency to norms, but also by low possibility to control their reactions and believe to existing rules. The vast majority of subgroup A2 were enlisted personnel of the 1st year of service. Specifically, the problem of the secondary socialization was the reason of ARIs' commitment by those servicemen. Group A was not so widespread as it was generally considered. Thus, about 12% of the group of servicemen who committed ARIs had the problems of the secondary socialization (problems of accepting the norms of new social group) and the crisis of values.

Among the representators of group B there was distinguished subgroup B1. The representators of subgroup were sincere, active, and intellectually developed, they could rapidly mobilize their strength, but they were not ambitious and were unmotivated while executing their duties of low enforcement. Received results concerning this group proved the conclusions made by Kintzle at alia [24] that unemployment was the reason of stress and alcohol abuse. The case was not in unemployment but in absence of favourite work which could provide them with self-realization; namely that turned to be the reason of ARIs' commitment. Moreover, the research conducted by Hoopsick, Homish, Vest, & Homish [35] pointed out that servicemen of the US Army and the US National Guard who did not participate in hostilities had negative concerns on the fact of being unrealized that became the predictor of addictive behaviour.

The representators of subgroup B2 had tendency to negative concerns which disorganized their activity. However, taking into account the high level of their insincerity it was possible to conclude that they tended to use references to their miseries as excuses of their impertinence and alcohol abuse. These servicemen demonstrated victim-like behaviour and according to Kim et alia [28] that was one of the reasons of alcohol abuse in junior military personnel. However, another research by Hoopsick, Homish, Vest, & Homish G [36] distinguished that the negative social relations such as problems of perceptions and social victimization were connected with higher chances of forbidden substances abuse and non-medical use of prescribed medications among the servicemen of the US National Guard. However, those problems did not lead to the increase of alcohol abuse.

Subgroups B3 and B4 were the biggest ones and they both wanted to increase their status. Thus, the servicemen of subgroup B3 while abusing alcohol tried to add themselves the traits of proficiency and to develop their image of experienced servicemen. The servicemen of subgroup B4 wanted to become important individuals. Both subgroups abused alcohol and that gave them the possibility not only to feel themselves more confident and independent but also it was a significant component for their image of successful individuals. According to the research by Barry et alia [7] and Shirvani et alia [8] such reasons of alcohol abuse explained why among those who wanted to enter the military service there were more school-leavers and youth who abused alcohol than those who did not have such habit.

Connection between alcohol abuse and problems of identification was examined in the work by Lostutter, Neighbors, Simpson, & Larimer [37]. The researchers distinguished that identifying themselves as a veteran and/ or a student at the college could influence the connection between the status of veteran and alcohol as well as psychological symptoms. They figured out that when veterans who were studying in college started identifying themselves as typical students at the college it decreased the symptoms of PTSD.

The features of group C of servicemen who committed ARIs turned out to be significantly close to those which were distinguished in our previous works devoted to the analysis of constitutionally weak subtype among the servicemen who committed suicide [38]. The comparison of groups such as "the control group" (1), "constitutionally weak who committed suicide" (2), "constitutionally weak individuals who committed alcohol-related incidents" (3) demonstrated that the last group had worse or even the worst nature to confront all the life failures as well as "constitutionally weak individuals who committed suicide" (2). Furthermore, earlier Prykhodko I, Matsehora, Kolesnichenko, Pasichnik, Kuruch, Yurieva, Kravchenko, Radko, & Prikhodko D [39] determined that constitutionally weak individuals did not tend to commit suicide while being drunk because they reacted on alcohol abuse as on absolute relaxation. This information gave the possibility to regard alcohol as if it was a coping which protected from suicide commitment.

Received results from group C proved the determined by Herberman Mash et alia [5] thesis that understanding of the reasons of alcohol abuse helped to distinguish the subgroups of servicemen who either tended to risky suicidal behaviour or were stable concerning suicide.

All the servicemen who committed ARIs were characterized by the decrease of the quality of interaction with the surrounding substantive and social world; however, the reasons of their alcohol abuse significantly differed. As a criterium for segmenting in the research there were used indicators of (dis)adaptivity which transferred the review of the topic concerning alcohol abuse to the plane of unproductive copings and relevant description of the groups such as "inconsistency to the norms" (group A), "desire to demonstrate socially approved image" (group B), "protection from suicide commitment" (group C).

Among the servicemen who committed ARIs there were distinguished three groups such as A, B, and C which differed with their peculiarities of (dis)adaptation. That was valuable to organize preventive works, events dedicated to the improvement of socialization by recruits, adequate formation of servicemen image as well as their identification in accordance with it, and events dedicated to the prevention of suicidal behaviour.

The results of this research will be used to specify the norms in accordance with psychodiagnostic methods due to which the psychological and professional selection to the military service are carried out.

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ORIGINAL ARTICLES

Typology and structure of servicemen motivation

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Abstract: *The results of the conducted research gave the possibility to distinguish the structure of the motivation which was suitable for the distinguished types of motivation of servicemen of the National Guard of Ukraine with different levels of effectiveness of their professional activity including motivation based on public service, stagnation, prosaically, romantic, deficient, and dependent. 2435 male servicemen participated in the research. They equally represented all the operational and territorial reunifications and military units of the National Guard of Ukraine. Every unit equally presented the officers, servicemen under the contract including the participants in hostilities as well as those who did not have such experience. The research was conducted with the help of the methods which were standardized by the Ukrainian language: "Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs", "Methods of Studying the Motivational Profile of Personality", and "Motivation Sources Inventory – MSI". The conducted research posed the possibility to describe not only the determined types of professional motivation of servicemen, but also specific phenomena such as self-motivation of the representatives of a type which was run by public service motivation; "rest on the laurels" for the type of stagnation; problems related to choice-making for the prosocial type for whom profession, family, and friends had equal meaning; the tendency for manipulation to gain access to desirable aspects of professional activity of the type which struggled for adventures; lack of sincere interest in the military profession for the deficient type which could change it for more prospective having the same requests for the candidates; attitude to the profession as if it was a means of satisfaction of the needs of their emotional life for the dependent type.*

Keywords: *motive, motivational sphere, servicemen, the effectiveness of the professional activity*

INTRODUCTION

With the beginning of combat operations in Eastern Ukraine, it turned out to be obvious that the estimation system of motivation of servicemen of NGU did not satisfy all the requirements related to the profession [1]. The number of those who wanted to sign a contract or to prolong it had significantly decreased. However, there increased staff turnover, the number of violations of discipline related to alcohol and drug abuse [2], spontaneous unit leaving, ignoring and defying orders, and other issues which were closely connected with discrepancy of servicemen motivation to profession requirements. It turned out to be urgent to determine the adequate motivation of professional activity for servicemen of NGU and to select and develop new psychodiagnostic methods to estimate it.

Having analyzed the world experience of studying the motivation of servicemen and law enforcement there was a need to point out that these days it was implemented in the frames of the theory of public service motivation (PSM), self-determination theory (SDT), and terror management theory

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(TMT) [3]. They were used to explain the selection of serviceman profession, the effectiveness (productivity, success) of professional activity, achievement of aims related to the activity, satisfaction with professional activity, professional burnout, exhaustion, and staff turnover.

Israeli researchers Taubman-Ben-Ari and Findler [3] studied the conscripts by the terror management theory (TMT). It was distinguished that the main psychological conflict occurred due to the existence of self-preservation instinct through understandability that death was irreversible and unpredictable. They concluded that the significance of death rate related to higher level of motivation directed to public service and higher expectations of physical difficulties in the army.

According to Griffith and Perry [4] the motives of military service enlistment before as well as after the operation "Desert Storm" was explained by the following factors: "desire to perform military service", "personal development", "salary (benefits)", and "career development". However, after the operation "Desert Storm" there significantly decreased the number of recruits entering military colleges due to three first factors from the list.

Like-Jensen and Glad [5] studied the motives of entering the missions of preservation and implementation of peace. They determined three types of servicemen motives among them there were: challenge, personal well-being, and loyalty. Challenge was the motivation directed to the profession. Personal well-being was a desire to get adventures (romantic motivation). Loyalty was the institutional motivation. Motives before as well as after the participation in hostilities significantly differed in dependence on the type of mission.

Siryi [6] pointed out that among the servicemen who served under the contract in the Armed Forces of Ukraine there were spread three types of motivation "motivation of activity" (36.6%), "motivation of pragmatics" (58.6%), and "motivation of reward" (4.8%).

Chambel, Castanheira, Oliveira-Cruz, and Lopes [7] having compared the studies carried out by Portuguese servicemen distinguished that motivation directed to autonomous work was positively connected with the involvement into the work as well as negatively connected with burnout. Controlled motivation, in turn, demonstrated an opposite picture. Moreover, autonomous motivation was a mechanism that explained the interrelation between contextual factors and well-being at workplaces.

Gillet, Becker, Lafrenière, Huart, and Fouquereau [8] distinguished that servicemen with high indicators of autonomous motivation showed the highest level of well-being including the support from the side of leaders, positive worries, communication, and a high level of organizational support.

Based on the carried out studies Breaugh, Ritz, and Alfes [9] pointed out the interconnection between the theory of public service motivation (RSM) and the theory of self-determination (STD). Having learned their relations with work satisfaction gave the possibility to figure out that the motivation of self-determination was significantly bound with the satisfaction at work-position. However, the co-workers with a high level of PSM had more stable indicators of work satisfaction in comparison with their colloquies who had a low level of PSM.

Schott, Neumann, Baertschi, and Ritz [10] conducted the comparison of prosocial motivation as well as public service motivation and studied the peculiarities of Swiss police officers. These researchers determined that despite PSM and prosocial motivation Swiss police officers were motivated with various tasks and excitement (for example, car chases). In daily routine, they were motivated by complicated tasks, by the way, they were estimated by the public and their colleagues.

The research conducted by Holton, Smith, Lindsay, and Burton [11] distinguished that a desire of cadets of the US Air Force Academy to voluntarily resign was negatively connected with work satisfaction, emotional commitment, work engagement, and individual compliance with professional requirements. The researchers figured out that the strongest predictor of staff turnover was individual compliance with the profession.

Meyer, Kam, Goldenberg, and Bremner [12] determined that Canadian servicemen with high indicators of affectivity and normative commitment of organization reported about the most favorable conditions at work, desire to continue their service and well-being. Servicemen who were focused on the consequences of their actions (understandability of the "price" of the resign consequences) and informed about less favorable consequences were more active in their search for work and had the highest level of anxiety and depression.

Lakhani and Fugita [13] studied the problem of resignment among the US Army and the National Guard reservists after the "Desert Storm" from the side of income and patriotism. They determined that the decrease of those who resigned from service could happen due to the increase of salary for reservists or due to the encouragement of wife/husband for a more positive attitude to reservists. However, the results of this research turned out to be weak support of the income theory but gave significant support for the patriotism theory.

The presented analysis showed that there was a need to consider heterogeneous motives of professional activity of servicemen as well as of law enforcement officials. Moreover, the search manner of this research proved the necessity to decrease the number of analyzed variables to increase the accuracy of the perception of the received results. By the highlighted information, the empirical data received in the research proceeded to factorial analysis.

METHOD

Participants and Procedure

The research was conducted throughout the 2019-2021 years. 2435 servicemen of the National Guard of Ukraine participated in the research. Those servicemen equally represented all the operational and territorial reunifications as well as military units of the National Guard of Ukraine. Every unit equally presented the officers – 29.84% (from the second lieutenants to colonels), servicemen who served under the contract – 70.16% (from soldiers to senior warrant officers), participants in hostilities, and those who did not have such experience. The age of all the participants was from 20 to 55. All procedures carried out in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants gave consent for their data to be used in this research.

Instruments and Measures

To determine the structure of motivation which was typical for the distinguished type of NGU servicemen motivation with various levels of effectiveness of professional activity there were used the methods standardized by the Ukrainian selection:

1. “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” by Moskalienko [14]. This questionnaire was developed by the Ukrainian researcher for professional psychological selection for future policemen education; it gave the possibility to evaluate “Motives Related to Objective Socially Posed Tasks and Content of Profession”, “Motives Related to Personal and Professional Development”, “Independent Profession Selection”, “Dependent Profession Selection”, “Motives Related to External Prestigious Professions and Financial Welfare”, “Motives Related to Romanticization of Profession”, “Motives Related to Eagerness of Compensation of Temper Flaws”, “Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)”. The instruction of this method proposed to answer the question concerning the reason for choosing this profession where the respondents using a scale made of 5 points could estimate 40 statements that described the profession of law enforcement.

2. “Methods of Studying the Motivational Profile of Personality” by Ritchie and Martin [15]. The use of this method gave the possibility to determine the urgent motivation of professional activity. It had the following scales: “Need of High Salary and Financial Benefits”, “Need of High Working Conditions and Comfortable Surrounding”, “Need of Accurate Structurization of Work and Feedback”, “Need of Social Relations”, “Need of Forming and Supporting Long-Term Stable Relations”, “Need of Obtaining the Recognition by Other People”, “Need of Posing Brave and Tough Goals and of Achieving Them”, “Need of Interesting and Socially Beneficial Work”, “Need of Self-Improvement and Development as Personality”, “Need of Being Creative, Thoughtful, and Open for further Ideas Employee”, “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine”, “Need of Manipulation and Power, Eagerness to Control the Other People”. This questionnaire included 33 questions with 4 variants of answers where the respondents had to place 11 points by the importance of the answers.

3. “Motivation Sources Inventory – MSI” by Barbuto and Scholl [16]. The use of this method gave the possibility to determine: “Internal Processes” (a desire to get satisfaction and pleasure from the process of activity), “Instrumental Motivation” (desire to valuable external benefits such as benefits, etc.), “External conception – I” (desire to except and support personal traits, competencies, and values from the side of other individuals or referential group), and “Internal competence – I” (desire to achieve aims and relevant internalized values). Respondents were proposed to answer 30 questions related to profession using a 7-point scale (from complete “no” to complete “yes”).

Data Analysis

To determine the structure of motivation which was typical for every distinguished type there were used the extraction method: principal component analysis and rotation method: varimax with Kaiser normalization. As variables, there were represented the indicators by scales of three psychodiagnostic methods. Mathematical data was processed through SPSS 17.0.

RESULTS

According to previously carried out research there were distinguished the following types of NGU servicemen motivation with different levels of effectiveness of their professional activity: motivated by public service, stagnated, prosocial, romantic, deficient, and dependent [17].

The first type of motivation of NGU servicemen (about 35% from general selection) was marked as one which was run by public service motivation. Servicemen of this type chose the military profession due to its content, goals, and desire to develop. They had expressed orientation directed to socially beneficial work as well as professional development. The motives which described the conditions, working hours, and social interaction were significantly lower and subcontract. Those servicemen had high indicators of a need for high financial benefits and recognition which demonstrated the importance of the high social status of the chosen profession. It was typical for the representation of this group to have significant time prospects, distinct internal locus control, and developed strong-willed qualities.

Used factors of the analysis showed that resistance and mentioned peculiarities of professional motivation ensured specific structure for the representation of this type. The motivational structure of this type is represented in Table 1.

Table 1: The structure of motivation of NGU servicemen who were guided by PSM

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Objective Socially Posed Tasks and Content of Profession	0.73	-0.01	0.13	-0.21	-0.13	-0.30
Motives Related to Personal and Professional Development	0.81	0.12	-0.01	-0.13	-0.05	0.14
Independent Profession Selection	0.73	-0.01	-0.29	0.04	-0.04	-0.13
Motives Related to External Prestigious Professions and Financial Welfare	0.75	0.05	-0.07	0.33	0.08	0.12
Motives Related to Eagerness of Compensation of Temper Flaws	0.82	0.03	0.10	-0.05	-0.14	0.06
Need of High Working Conditions and Comfortable Surrounding	-0.05	0.07	0.07	0.91	-0.15	-0.11
Need of Accurate Structurization of Work and Feedback	0.07	0.87	-0.05	-0.13	-0.16	-0.14
Need of Obtaining the Recognition by Other People	-0.14	-0.01	0.02	-0.15	0.93	-0.04
Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine	-0.06	-0.85	-0.03	-0.25	-0.16	-0.01
Need of Self-Improvement and Development as Personality	0.02	-0.12	0.07	-0.10	-0.05	0.93
Internal Processes	0.03	-0.05	0.83	-0.10	-0.20	0.14
Instrumental Motivation	-0.09	0.04	0.81	0.20	0.28	-0.08
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 6 iterations.						

This factor structure explained 77.59% of total dispersive features. The first and the most significant factor (24.90%) was distinguished with the help of the following variables: “Motives Related to Eagerness of Compensation of Temper Flaws” (0.82), “Motives Related to Personal and Professional Development” (0.81), “Motives Related to External Prestigious Professions and Financial Welfare” (0.75), and “Motives Related to Objective Socially Posed Tasks and to Content of Profession” (0.73). This factor was marked as a desire to gain professional dignity – the desire for personal, professional, and social development turned to the professional requirements.

The second and the third factors had almost the same value in this structure (12.64% and 12.32%) and were part of the mechanism of self-motivation. Therefore, the second factor demonstrated a desire to produce and practice the professional algorithm of actions: “Need of Accurate Structurization of Work and Feedback” (0.87) and “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine” (-0.85). The third factor showed the process of enjoying professional activity (process as well as result). It was described with the help of the variables such as “Internal Processes” (0.83) and “Instrumental Motivation” (0.81).

Such a combination of the second and the third factors gave the possibility to highly effective servicemen to support their high level of professional activity, accuracy, and intensity of the professional activity.

The three last factors also had almost identical values (9.61%, 9.15%, and 8.96%, accordingly) and they described the peculiarities of needs’ satisfaction. Thus, the content of the fourth factor was connected with the conviction that the financial benefits received due to hard work related to the objective content of the profession. The fourth factor was characterized with “Need of High Working Conditions and Comfortable Surrounding” (0.91). Having clarified that the content of this factor had positive correlations with the variable such as “Motives Related to External Prestigious Professions and Financial Welfare” (0.33) and negative correlations with the variable such as “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine” (-0.25) and of “Motives Related to Objective Socially Posed Tasks and to Content of Profession” (-0.21). It was figured out that namely, the achievement of professional aims and the performance of professional duties were the source of financial well-being.

The content of the fifth factor was in the fact that favorite activity was the source of social approvement. The fifth factor was described with the help of the variable such as “Need of Obtaining the Recognition by Other People” (0.93). Its content was clarified with the direct connection with the variable “Instrumental Motivation” (0.28) and with the resource-based connection with the variable such as “Internal Processes” (-0.20).

The sixth factor correlated with the variable “Need of Self-Improvement and Development as Personality” (0.93). Negative connection correlated with the variable such as “Motives Related to Objective Socially Posed Tasks and Content of Profession” (-0.30). It was determined that namely the profession, its aims, and content were the resource of personal development for servicemen of this type. Thus, the reference point of the acquisition of professional dignity by the first type of NGU servicemen was the combination of a desire for professional, personal, and social development with a desire of meeting the requirement of the profession and running its aims. In this structure of the motivation, there was determined the mechanism of self-motivation and support of activity on a high level which was foreseen by the process of producing and practicing professional algorithm of actions as well as by the satisfaction of working processes and its results. Such a structure of motivation of servicemen provided them with a high level of effectiveness in their profession, with a desire to follow the rules, and with close affection to their profession. It provided them with the prevention from professional stagnation and gave them the possibility to support the accuracy and intensity of their professional activity.

Servicemen of the stagnated type (it compiled about 15% from a general selection of this research) also chose the military profession due to its content. They had significant achievements in professional activity, recognition of their success, but they did not want any professional development, challenges, and responsibility. They tried to distance themselves from their colleagues in service. The structure of motivation of this type is presented in Table 2.

Table 2: The structure of motivation of NGU Servicemen who experiences stagnation

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Objective Socially Posed Tasks and Content of Profession	0.26	-0.01	0.03	0.79	0.01	-0.09
Dependent Profession Selection	-0.08	0.86	0.04	-0.07	-0.05	-0.02
Motives Related to Eagerness of Compensation of Temper Flaws	-0.15	0.18	-0.10	0.86	-0.03	0.16
Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)	-0.03	0.77	0.09	0.29	0.01	0.04
Need of High Salary and Financial Benefits	-0.16	0.06	-0.88	-0.06	-0.08	0.14
Need of High Working Conditions and Comfortable Surrounding	-0.09	0.05	0.09	-0.08	0.87	0.03
Need of Accurate Structurization of Work and Feedback	0.05	-0.29	0.04	-0.03	0.15	-0.76
Need of Social Relations	-0.24	0.21	0.87	-0.15	0.01	0.06
Need of Obtaining the Recognition by Other People	-0.11	0.10	0.01	-0.06	-0.85	0.10
Internal Processes	0.17	-0.34	-0.06	0.05	0.08	0.80
Instrumental Motivation	0.77	0.24	-0.17	-0.16	0.14	0.23
Internal Conception – I	0.81	-0.28	0.20	0.15	-0.16	0.12
Internalization of the Aim	0.81	-0.11	-0.06	0.12	0.05	-0.14
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 6 iterations.						

This factor structure included 77.12% of the total dispersion of the peculiarities.

The structure of servicemen motivation who experienced stagnation, the first and the most valuable factor (16.50%) demonstrated an expressed individualism in the process of achieving professionally important aims based on interiorized professional values. This factor was marked with the variables such as “Internal Conception – I” (0.81). “Internalization of the Aim” (0.81), and “Instrumental Motivation” (0.77). There was clarified the content of the variables of this type’s factor “Motives Related to Objective Socially Posed Tasks and Content of Profession” (0.26) and “Need of Accurate Structurization of Work and Feedback” (-0.24).

The content of the second factor (13.70%) was highlighted due to such variables as “Dependent Profession Selection” (0.86) and “Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)” (0.77). This factor was clarified with the help of the positive poles of the variables such as “Instrumental Motivation” (0.24) and “Need of Social Relations” (0.21) as well as negative poles of the variables such as “Internal Processes” (-0.34), “Need of Accurate Structurization of Work and Feedback” (-0.29), and “Internal Conception – I” (-0.28). This factor was described as a forced compromise. It was quite important for servicemen to gain financial benefits, but it did not provide them with any kind of satisfaction.

The next three factors had almost identical values (12.48%, 12.03%, and 11.85%, accordingly). These factors developed the mechanism to support positive self-relationship which preserved their achievements. The content of the third factor was expressing themselves as role model for other servicemen. The positive pole of the third factor was marked with the variable such as “Need of Social Relations” (0.87) and clarified positive poles with the variable such as “Internal competence – I” (0.20). The negative pole of the third factor was marked with the variable “Need of High Salary and Financial Benefits” (-0.88). Some clarifications were conducted with “Instrumental Motivation” (-0.17). Thus, it was very important for servicemen of the stagnated type to demonstrate their principles to the surrounding in opposition to their financial benefits.

The fourth factor was marked with the variables such as “Motives Related to Eagerness of Compensation of Temper Flaws” (0.86) and “Motives Related to Objective Socially Posed Tasks and to Content of Profession” (0.79). It was clarified the variable “Antisocial Motives” (0.29). Such a combination of the variables of the fourth factor described a desire of servicemen to gain importance at the expense of using all the possibilities given by the profession for their purpose.

The fifth factor had significantly marked poles which provided a positive description to the variables such as “Need of High Working Conditions and Comfortable Surrounding” (0.87) and the reversed was “Need of Obtaining the Recognition by Other People” (-0.85). This factor marked the refusal from professional ambitions of servicemen to the benefit of peaceful life till the end of their contract. In other words, they wanted to rest on laurels.

The sixth factor (10.57%) also had marked poles. The positive pole was “Internal Processes” (0.80). Its content was clarified with the variables such as “Instrumental Motivation” (0.23) and “Motives Related to Eagerness of Compensation of Temper Flaws” (0.16). The negative pole was marked with a variable such as “Need of Accurate Structurization of Work and Feedback” (-0.76). This factor was marked as being satisfied at the expense of demonstrating their honed professional skills.

Having summed up the results of servicemen who were included in the stagnated type there was a need to point out that they were characterized with marked individualism and perception of the service continuation as a forced compromise. The structure of their motivation had a formation due to which they supported high positive self-relationship, demonstrated their integrity and success at the expense of carrying out the improvement of professional skills, and used the position of “rest on laurels”.

Servicemen of the prosocial type (15%) even if they considered their military service to be temporal work which gave them the possibility to make living for their families, however, they made huge efforts to acquire military profession to realize its sense and content. They were responsible performers and did not require additional control from the side of their commanders. It was important for them to support friendly relations with colleagues in service and recognition of their combat achievements by society including financial benefits (benefits). The structure of motivation of this type is presented in Table 3.

This factor structure explained 77.75% of the total dispersion of the peculiarities. The factors had almost equal values.

Thus, the first factor which compiled 15.03% was marked with the variables such as “External Conception – I” (0.87), “Instrumental Motivation” (0.78), and “Internalization of the Aim” (0.73). It was marked as a desire to be accepted by military colleagues and to be helpful to them.

Table 3: The structure of motivation of NGU Servicemen of prosocial type

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Objective Socially Posed Tasks and Content of Profession	0.12	-0.04	0.02	0.08	0.13	0.90
Dependent Profession Selection	0.01	0.11	0.89	0.06	0.07	0.03
Motives Related to Romanticization of Profession	0.22	0.08	0.74	0.22	0.05	0.33
Motives Related to Eagerness of Compensation of Temper Flaws	0.14	0.04	0.44	-0.19	0.08	0.74
Need of High Salary and Financial Benefits	0.07	0.72	0.30	0.06	-0.11	0.08
Need of Accurate Structurization of Work and Feedback	-0.14	0.85	-0.04	0.05	0.14	0.01
Need of Social Relations	0.11	-0.28	0.29	0.71	-0.30	-0.06
Need of Forming and Supporting Long-Term Stable Relations	-0.03	-0.77	-0.01	0.37	0.21	0.14
Need of Obtaining the Recognition by Other People	0.15	-0.21	-0.12	-0.13	0.87	0.08
Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine	0.16	-0.12	-0.32	0.02	-0.78	-0.14
Need of Interesting and Socially Beneficial Work	-0.18	-0.07	-0.02	-0.94	-0.01	-0.01
Instrumental Motivation	0.78	0.01	0.17	0.13	-0.11	0.15
External Conception – I	0.87	0.07	0.09	-0.02	-0.05	-0.01
Internalization of the Aim	0.73	-0.19	-0.08	0.21	0.20	0.14

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 6 iterations.

The positive pole of the second factor (14.62%) was marked with the variables such as “Need of Accurate Structurization of Work and Feedback” (0.85) and “Need of High Salary and Financial Benefits” (0.72). The reversed pole of a factor was marked with the variables such as “Need of Forming and Supporting Long-Term Stable Relations” (-0.77) and was clarified with the help of “Need of Obtaining the Recognition by Other People” (-0.21). The second factor testified that the representations of this group's military profession were temporal means of high income which they left their families for. Choosing this profession, the final step for servicemen was the possibility to acquire the profession and to earn with its help.

The content of the third factor (13.24%) was connected with the variables such as “Dependent Profession Selection” (0.89) and “Motives Related to Romanticization of Profession” (0.74). This factor clarified “Motives Related to Eagerness of Compensation of Temper Flaws” (0.44), “Need of High Salary and Financial Benefits” (0.30), and “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine” (-0.30). Such combination gave the possibility to conclude that the choice of military profession was made under the influence of family, friends, and familiar people who described this profession which was connected with adventurous (romantic), hard, sometimes routine-like but, at the same time, highly paid.

The positive pole of the fourth factor (12.12%) corresponded with variables such as “Need of Social Relations” (0.71). The clarified variables were “Need of Forming and Supporting Long-Term Stable Relations” (0.37), “Motives Related to Romanticization of Profession” (0.22), and “Internalization of the Aim” (0.21). The reversed pole was marked with a variable such as “Need of Interesting and Socially Beneficial Work” (-0.94). In the majority of cases, servicemen of the prosocial type were motivated with the accurate realization of military fellowship and friendship; they wanted to find real friends and to have identical aims with them.

The fifth factor (11.53%) also had both distinguished poles. The positive features mostly correlated with the variable such as “Need of Obtaining the Recognition by Other People” (0.87). The clarified variable was “Internalization of the Aim” (0.20). The reversed pole was introduced in “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine” (-0.78) and “Need of Social Relations” (-0.30). This factor was marked as a desire to get recognition of achievements gained with the help of hard work and tasks performance but not at the expense of friendly relations.

The sixth factor (11.21%) was correlated with the variables such as “Motives Related to Objective Socially Posed Tasks and Content of Profession” (0.90) and “Motives Related to Eagerness of Compensation of Temper Flaws” (0.74). The clarification of its content was done due to the variable

“Motives Related to Romanticization of Profession” (0.33). For servicemen of the prosocial type, the abstract content of professional activity “Serve and Protect” gained completely accurate meaning including such features as coping with their fears and flaws, presenting their desires, learning how to use the weapon, improving their physical strength, being good colleague, etc.

It was important for servicemen of this type to be the good breadwinner for their families, to be helpful in their professional activity, and to be reliable for their colleagues in service. They seemed to be more relatives-centered than socially centered.

Servicemen who were focused on the satisfaction of their personal needs (6%) were the only ones from the whole range of distinguished types who chose the profession due to mercenary interests and not due to the objective content of the profession. These servicemen wanted to beneficially exchange their professional skills for their financial well-being. They used social relations because they got satisfaction from communication. They did not want to be responsible, did not want to realize their volitional qualities. These servicemen demonstrated indifference to the problems of their colleagues in service. They were not active because it did not bring them any additional income. Almost 75% of servicemen of this type had the status of the participants in hostilities. These servicemen admired the adventures and neglected the content of the military profession. Their actions were constantly controlled by their commanders. This type was marked as one motivated by a desire for adventures. The structure of motivation of this type is presented in Table 4.

Table 4: The structure of motivation of NGU Servicemen who were motivated with their desire to have adventures

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Objective Socially Posed Tasks and Content of Profession	0.05	0.15	-0.01	-0.12	0.87	-0.09
Motives Related to Personal and Professional Development	0.82	-0.17	-0.15	0.07	-0.04	0.07
Dependent Profession Selection	0.82	-0.22	0.26	0.01	-0.15	-0.24
Motives Related to Romanticization of Profession	0.73	-0.04	0.28	0.27	0.34	0.01
Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)	0.79	0.26	-0.10	-0.08	0.10	0.39
Need of High Salary and Financial Benefits	-0.07	-0.36	-0.72	0.27	-0.01	-0.35
Need of Accurate Structurization of Work and Feedback	0.06	-0.02	0.09	-0.08	-0.08	0.94
Need of Social Relations	-0.17	0.81	0.02	-0.05	0.16	0.08
Need of Forming and Supporting Long-Term Stable Relations	0.05	-0.03	0.93	-0.02	0.02	-0.04
Need of Posing Brave and Tough Goals and of Achieving Them	0.08	0.06	-0.12	0.70	-0.46	0.01
Need of Being Creative, Thoughtful, and Open for further Ideas Employee	0.07	-0.70	0.20	0.23	0.38	0.27
Need of Self-Improvement and Development as Personality	-0.08	0.16	0.08	-0.89	-0.09	0.11
Need of Interesting and Socially Beneficial Work	-0.06	0.75	0.31	-0.14	0.02	0.04
Internal Conception – I	0.12	0.71	-0.03	0.29	0.45	0.07

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 9 iterations.

The presented factor structure explained 80.65% of the total dispersion of the peculiarities.

The first factor (18.45%) characterizes military personnel as prone to gambling to achieve their interests due to the awareness of their lack of existing skills and personal qualities. This factor included such variables as "Motives related to personal and professional development" (0.82), "Dependent choice of a profession" (0.82), "Asocial motives (attitude towards the profession as a way to satisfy one's own asocial needs)", (0.79) and "Motives associated with the romanticization of the profession" (0.73).

The second factor (18.24%) described the search for the surrounding which could make the lives of servicemen more interesting and eventful. The positive pole of this factor was connected with the variables such as “Need of Social Relations” (0.81), “Need of Interesting and Socially Beneficial

Work" (0.75), and "Internal Conception – I" (0.71). Somewhat specific meaning to this factor was given with the variable such as "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.26). The reversed pole was marked with a variable such as "Need of Being Creative, Thoughtful, and Open for further Ideas Employee" (-0.70). There were clarified the variables of the reserved pole such as "Need of High Salary and Financial Benefits" (-0.36) and "Dependent Profession Selection" (-0.22). Thus, these servicemen did not tend to perform something new, creative. They searched for interesting surroundings that made their lives eventful.

The third and the fourth factors were similar in their values. The third factor (12.24%) characterized a desire of servicemen to use financial resources and gained stable social relations. That could diversify their professional activity and made it more romantic. The fourth factor (11.58%) described servicemen which wanted to get superiority over other people, to self-realize at the expense of desperate actions as opposed to a desire of self-development as well as of development of personal individuality. Servicemen of this type with the help of all possible means wanted the profession to correlate with their "romantic" expectation; however, they did not even try to comply with all the real requirements of the military profession.

Thus, the positive pole of the third factor (12.24%) was connected with variables such as "Need of Forming and Supporting Long-Term Stable Relations" (0.93). It was clarified with the help of the variables such as "Need of Interesting and Socially Beneficial Work" (0.31), "Motives Related to Romanticization of Profession" (0.28), and "Dependent Profession Selection" (0.26). The negative pole was described with variables such as "Need of High Salary and Financial Benefits" (-0.72). The content of this factor indicated that servicemen wanted some adventures and refused financial benefits.

The positive pole of the fourth factor was connected with the variable such as "Need of Posing Brave and Tough Goals and of Achieving Them" (0.70). The content of this pole was clarified with the help of the variables such as "Internal Conception – I" (0.29), "Motives Related to Romanticization of Profession" (0.27), "Need of High Salary and Financial Benefits" (0.27), and "Need of Being Creative, Thoughtful, and Open for further Ideas Employee" (0.23). The reversed pole was determined with a variable such as "Need of Self-Improvement and Development as Personality" (-0.89). According to the content of this factor servicemen of this type tended to desperate acts to build self-esteem and to experience the emotions of priority over other people.

The fifth factor (10.66%) described servicemen who used a lot of creativity to avoid dangerous tasks to perform professional duties. It was correlated with variables such as "Motives Related to Objective Socially Posed Tasks and Content of Profession" (0.87). Its content was clarified with the variables such as "Internal Conception – I" (0.45), "Need of Being Creative, Thoughtful, and Open for further Ideas Employee" (0.38), and "Motives Related to Romanticization of Profession" (0.34). The reversed pole was marked with variables such as "Need of Posing Brave and Tough Goals and of Achieving Them" (-0.46). Servicemen of this type put huge efforts to shift the accents of their profession from dangerous features to adventurous ones. They mostly used the tasks which were attractive for them, and which met their romantic impressions related to the military profession.

The sixth factor (9.47%) was connected with the variable such as "Need of Accurate Structurization of Work and Feedback" (0.94). Its content was clarified with the variables such as "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.39), "Need of Being Creative, Thoughtful, and Open for further Ideas Employee" (0.27), "Need of High Salary and Financial Benefits" (-0.35), and "Dependent Profession Selection" (-0.24). This factor described the servicemen who wanted to get work with definite support (personal interests) even if there was a need to use financial benefits.

Servicemen of the deficient type (25%) chose the military profession because of its content and wanted to change their lives. It was important for them to have a profession with a high salary and social status. Servicemen who wanted to work put huge efforts; however, their motivation turned out to be "blocked" with unsatisfied deficient needs. It negatively reflected on their effectiveness. The structure of motivation of this type is presented in Table 5.

This factor structure explained 77.73% of total dispersal peculiarities.

The most valuable first factor (21.40%) was marked with the variables such as "Motives Related to Eagerness of Compensation of Temper Flaws" (0.90), "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.82), "Motives Related to Personal and Professional Development" (0.81), and "Motives Related to External Prestigious Professions and Financial Welfare" (0.76). It was marked as a desire to change themselves as well as to change their lives for the better, to go beyond their old way of living at the expense of their chosen military profession.

Table 5: The structure of motivation of NGU Servicemen of deficient type

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Personal and Professional Development	0.81	-0.02	0.17	0.08	-0.27	0.09
Motives Related to External Prestigious Professions and Financial Welfare	0.76	0.09	0.06	-0.33	0.02	-0.04
Motives Related to Eagerness of Compensation of Temper Flaws	0.90	0.04	0.03	-0.01	0.12	-0.04
Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)	0.82	0.01	-0.11	-0.02	0.07	0.08
Need of High Working Conditions and Comfortable Surrounding	-0.12	-0.05	-0.01	0.92	0.06	0.03
Need of Social Relations	-0.02	0.03	0.84	-0.23	0.12	-0.13
Need of Forming and Supporting Long-Term Stable Relations	0.10	0.06	0.82	0.21	-0.14	-0.05
Need of Obtaining the Recognition by Other People	0.04	-0.08	-0.01	0.06	0.95	0.03
Need of Interesting and Socially Beneficial Work	0.07	-0.10	-0.15	0.03	0.03	0.96
Internal Processes	-0.11	0.78	-0.19	-0.18	-0.01	0.22
Instrumental Motivation	-0.05	0.75	0.23	0.23	0.11	-0.15
Internal Conception – I	0.20	0.79	0.09	0.06	-0.28	-0.09
Internalization of the Aim	0.07	0.78	0.02	-0.15	0.01	-0.13
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 5 iterations.						

The second factor (18.76%) was marked with the variables such as “Internal Conception – I” (0.79), “Internal Processes” (0.78), “Internalization of the Aim” (0.78), and “Instrumental Motivation” (0.75). This factor was described as a desire to experience the feeling of their value.

The content of the third factor (11.81%) was distinguished with the variables such as “Need of Social Relations” (0.84) and “Need of Forming and Supporting Long-Term Stable Relations” (0.82). The content of this factor was clarified with the variables such as “Instrumental Motivation” (0.23), “Motives Related to Personal and Professional Development” (0.17), and “Internal Processes” (-0.19). This factor described servicemen who wanted to get new social surroundings, new social surroundings, a new social status which could change them as well as their financial situation due to their efforts.

The fourth factor (8.97%) correlated with “Need of High Working Conditions and Comfortable Surrounding” (0.92). Its content was clarified with the positive connections with the variables such as “Instrumental Motivation” (0.23) and “Need of Forming and Supporting Long-Term Stable Relations” (0.21). The reversed pole was marked with the variables such as “Motives Related to External Prestigious Professions and Financial Welfare” (-0.33) and “Need of Social Relations” (-0.23). Accordingly, the fourth factor was interpreted as an expressed need of servicemen to get welfare (good conditions and surrounding, competitive salary) without paying attention to the number of effort they put in.

The fifth factor (8.71%) correlated with the variable such as “Need of Obtaining the Recognition by Other People” (0.95). Its reversed pole gave the possibility to mark the connections with the variables such as “Internal Conception – I” (-0.28) and “Motives Related to Personal and Professional Development” (-0.27). Thus, this factor was marked as “Need of Obtaining the Recognition by Other People” which servicemen wanted to satisfy at the expense of personal development and professional improvement for what they could change their old persuasions and refuse from their old lives.

The sixth factor (8.08%) correlated with the variable such as “Need of Interesting and Socially Beneficial Work” (0.96). The content of this factor was clarified with the variables such as “Internal Processes” (0.22), “Instrumental Motivation” (-0.15), “Internalization of the Aim” (0.13), and “Need of Social Relations” (-0.13). This factor was marked as a desire for interesting and useful actions achieved through professional socialization.

Thus, servicemen of the deficient type had a marked desire to change themselves and their lives for the better, to go beyond the limits of the previous way of living, to experience the feeling of their value, and to get a new status. Their motivation was weakly connected with the direct content of the military profession despite a desire to work putting huge efforts and changing everything. Profession for them was a kind of means, not an individual value. Thus, the military profession could be substituted with any other which was more prospective having identical requirements for their candidates.

Servicemen of the dependent type (about 4% from a general selection of the research) were affective, weak-willed, dependent on other people, as well they expressed a desire for definite emotional life and had a low level of internality. According to the self-determination theory (SDT), there was traditionally used term "autonomous and controlled motivation". However, that type except "autonomous and controlled motivation" had some traits suitable for addictive behavior. The structure of motivation of this type is presented in Table 6.

Table 6: The structure of motivation of NGU Servicemen of dependent type

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Personal and Professional Development	0.94	0.02	-0.11	0.12	0.05	0.08
Dependent Profession Selection	0.90	0.08	-0.15	0.31	-0.18	0.03
Motives Related to External Prestigious Professions and Financial Welfare	0.88	0.26	-0.30	-0.05	0.11	-0.01
Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)	0.86	0.29	0.16	-0.02	0.07	-0.24
Need of High Salary and Financial Benefits	0.24	0.10	0.10	0.93	-0.01	-0.09
Need of High Working Conditions and Comfortable Surrounding	0.04	-0.01	0.02	-0.03	0.96	0.14
Need of Accurate Structurization of Work and Feedback	-0.04	-0.01	-0.04	-0.10	0.15	0.98
Need of Obtaining the Recognition by Other People	0.18	-0.14	-0.87	-0.24	-0.28	-0.14
Need of Interesting and Socially Beneficial Work	-0.11	0.05	0.93	-0.10	-0.17	-0.17
Internal Processes	0.23	0.71	0.28	-0.46	0.24	0.20
External Conception – I	0.37	0.74	0.07	0.25	0.12	-0.06
Internal Conception – I	0.36	0.80	-0.04	0.09	-0.26	-0.14
Internalization of the Aim	-0.09	0.93	0.06	-0.01	-0.01	0.04
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 6 iterations.						

This factor structure described 92.32% of total dispersal peculiarities.

The first two factors (28.01% and 21.16% accordingly) described the dependency on antisocial needs. The first factor pressed to the process of profession selection and the second factor was marked with specifically realized aims of professional activity.

The content of the first factor (28.01%) was marked with the variables such as "Motives Related to Personal and Professional Development" (0.94), "Dependent Profession Selection" (0.90), "Motives Related to External Prestigious Professions and Financial Welfare" (0.88), and "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.86). This factor was marked as a forced selection made under pressure from their own needs including even antisocial personal ones (development, self-affirmation) and financial benefits.

The second factor (21.16%) was described through such variables as "Internalization of the Aim" (0.93), "Internal Conception – I" (0.80), "External Conception – I" (0.74), and "Internal Processes" (0.71). The content of this factor had the almost complete coincidence of the concept internalization of the aim with the situation when the achievement of the aim of valuable features of others was understood as self-realization and satisfaction of their activity. The content of this factor was clarified with the variables such as "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.29) and "Motives Related to External Prestigious Professions and Financial Welfare" (0.26).

The third factor (14.45%) described a desire of servicemen to fill their personal life with a positive experience at the expense of their being a part of the interesting surroundings. Thus, usually dependent personalities had difficulties with the process of finding satisfaction inside themselves. To experience positive emotions, they needed external stimulation. The positive pole of the third factor was determined with “Need of Interesting and Socially Beneficial Work” (0.93). Definite clarifications were made with the variable such as “Internal Processes” (0.28). The reversed pole was marked with variables such as “Need of Obtaining the Recognition by Other People” (0.87). The clarification was made with the variable such as “Motives Related to External Prestigious Professions and Financial Welfare” (-0.30).

The last three factors had almost identical values – 10.27%, 9.49%, and 8.93% accordingly. The fourth factor described the specific attitude to the process of professional activity. This factor did not bring any satisfaction. Servicemen had to perform professional activities to get financial benefits (which was their main aim). This factor was formed with variables such as “Need of High Salary and Financial Benefits” (0.93). The content was clarified with a variable such as “Dependent Profession Selection” (0.31). The reversed pole was marked with a variable such as “Internal Processes” (-0.46).

The fifth factor was marked with a variables such as “Need of High Working Conditions and Comfortable Surrounding” (0.96). It was clarified with variables such as “Internal Processes” (0.24). The reversed pole was weakly marked with “Need of Obtaining the Recognition by Other People” (-0.28) and “Internal Conception – I” (-0.26). This factor was described as a desire to get comfort and hedonism.

The sixth factor (8.93%) was developed with variables such as “Need of Accurate Structurization of Work and Feedback” (0.98). The content of this factor was clarified with the variables such as “Internal Processes” (0.20) and “Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)” (-0.24). This factor was described as a desire to avoid problems related to their activity, desire to obtain easy work.

Thus, the results of the conducted research gave the possibility to determine a range of peculiarities of the structure of motivation of servicemen of dependent type. Firstly, it provided the relations of dependency where personal development and well-being depended on the realization of their antisocial needs. Secondly, their activity was directed to the fulfillment of their interesting internal emotional life at the expense of their belonging to the interesting surroundings. Thirdly, their motivation was directed to the organization of the professional activity which did not disturb their internal emotional life, did not bring any troubles, and provided them with comfortable conditions.

DISCUSSION

In this research there were determined 6 types of professional motivation with various levels of effectiveness of professional activity (the list was provided by the indicators of their effectiveness): 1) motivated with a desire of public service, 2) stagnated, 3) prosocial, 4) adventures-centered, 5) deficient, 6) dependent [17]. Despite similar typologies, there was a broad list of the types of negative motivation. Moreover, there were determined the types such as stagnated, adventures-centered, deficient, and dependent. However, the stagnated type occupied medium step between highly effective and medium effective types but in the developed typology it was included in the type with negative motivation. It was connected with the fact that servicemen who were not satisfied with their profession did not prolong their contract. Thus, the research by Harrington, Bean, Pintello, and Mathews (2001) distinguished that the personnel of military sphere were more likely to change their work if they were emotionally exhausted, had a lower level of internal satisfaction with their work, satisfaction with their salary as well as possibilities to be promoted at service.

Distinguished types, their descriptions, and structure of motivation did not contradict the regulations related to the relations between public service motivation (PSM) with effective work and its average self-efficiency. In the conclusion, there were added existing relations of autonomous and controlled motivation with productive activity. The received results would be used for further development of those regulations.

By the results presented in the works by Miao, Eva, Newman, and Schwarz [18]; van der Voet, Steijn, and Kuipers [19], Breaugh J, Ritz A, and Alfes K [9] the public service motivation (PSM) was considered as one connected with a high level of effectiveness with a desire to be developed, to get satisfaction with their work, with a decrease of task complications up to the level which correlated with their ability to live with their values and believes. However, the received structural motivation of servicemen related to PSM gave the possibility to determine existing mechanisms of self-motivation of those who had PSM which connected and described those earlier distinguished regulations. Thus, firstly, there is the structural motivation was conducted through the process of connection of algorithmization of their work (easiness and generalization) and satisfaction with work. Secondly, the structure of motivation of this type of satisfaction with main needs was performed not only at the expense of chosen profession but also by its content. It could prevent corruption – situations when the end justified the means and when their priorities were set above the priorities of others. Personal and professional development, obtaining the social status also happened by the content and aims of professional law enforcement officers. Thus, namely the content of the profession which was connected with public service provided the limits of development and the search of resources which made it impossible to refuse this way of development. Such a structure of motivation supported self-realization and a high level of activity.

The structure of motivation of the stagnated type gave the possibility to broaden the view concerning average relations of PSM and productivity of activity with the psychological mechanism of self-efficiency. Thus, Schott, Neumann, Baertschi, and Ritz [10] pointed out that policemen who did not get any feedback lost their confidence in their ability to realize the motives of PSM. Because of the feeling of disability (for example, when individuals who committed crimes are set free in the courtroom) PSM is considered to be a general type of motivation related to work with limited behavioral consequences. Other than that, the received data related to the structure of motivation of the stagnated type proved the necessity of paying attention to another side of such averaging. When previous achievements which reflected on self-perception and sometimes even ruined it (for example, formed inadequately overstated self-estimation) prevented them from development and were potentially dangerous for law enforcement officers' productivity.

The research by Schott, Neumann, Baertschi, and Ritz [10] also proved that prosocial motivation differed from PSM with their orientation on the individuals and groups which were involved in the direct contact, or on the organization-employer. It lived on the feedback of those who were helped with senses of gratitude as well as with emotional appeal. Presented research gave the possibility to form the conclusion that those peculiarities of motivation had their specific reflection and the structure of motivation the prosocial type differed from the type with PSM with a lower level of subordinate structure of motivation and a higher level of concreteness of motivational factors. The structure of motivation of servicemen of the prosocial type had an almost identical value of factors which described the importance of being useful in professional activity, being good at breadwinning their families, being a good colleague in service. According to the content of factors, servicemen of the prosocial type were mostly orientated on the ability not to fail their families but not on abstracted social benefit. The protection of the motherland was for them a way of overcoming their fears, etc. Such structure developed a complicated process of selection and decision-making when the situation dealt with the interests of several sides which were important for servicemen of the NGU.

It was quite interesting to compare the received results related to the adventure-centered type with the data by Lyk-Jensen and Glad [5] who determined that servicemen who were motivated with a desire to get adventures finished only secondary school and, usually, served only one year contract. The received structure gave the possibility to conclude that servicemen of this type were not self-sufficient and thus, they required the surrounding which made their lives eventful. They did not want to change by the requirements of the profession, instead, by any means (manipulations), they wanted to join the process of performing those aspects of professional activity which gave the possibility to satisfy their desire to work.

American researchers figured out that military service was mostly joint by Afro-Americans, indigenous Latin Americans, and rarely those people who had good school grades, planned to enter college, and whose parents had higher education [2]. This tendency was strengthened after the “Desert Storm”. Thus, the candidate for lower positions who entered the military service was motivated with salary (benefits), was married, had basic education, and did not get secondary education [4]. However, Asoni, Gilli, Gilli, and Sanandaji [20] denied the theses that the American Army was hired by the Army of poor people which was used by the critics of the US external policy. They determined that despite generally accepted ideas American servicemen did not recruit primarily people with socially and economically dysfunctional layers of society. Technological, tactical, operational, and doctrinal changes led to the changes in personnel demand. As a result, according to various indicators such as family income, family wellbeing, as well as cognitive abilities servicemen on average, had identical or better indicators as civilians had. In this research the deficient type included a quarter of respondents, and, at first sight, the problem was not in the fact that they had lower education and were less confident in their abilities and skills, the problem was in the fact that even having a desire to work, to put huge efforts, and to change their motivation was weakly connected with direct content of military profession. For them, this profession was a kind of means but not an independent value.

Thus, the military profession could be substituted with any other profession which was more prospective having identical requirements for the candidates. Accordingly, they wasted efforts in the process of acquiring a profession, were not confident that servicemen would not substitute their military profession as soon as possible with the profession which was considered to provide them with better prospects.

A range of studies which included the comparison of autonomous and controlled motivation related to work satisfaction and desire to dismiss [21] being exhausted and belonging [22], being at the workplace [7], having the feeling of organizational support, positive worries, and level of communication [8].

The conducted research demonstrated that the type with the lowest level of activity effectiveness had features of dependent and controlled motivation.

The analysis of the structure of its motivation showed that profession selection and realization of the aims of professional activity of the representatives of that group were dependent on the satisfaction of personal including antisocial requirements.

They wanted to fulfill their internal lives with positive worries at the expense of external stimulation, so-called "belonging to the interesting surrounding".

The process of professional activity did not provide them with satisfaction, however, they had to deal with it to get financial benefits.

Their motivation was directed to the organization of the professional activity which did not disturb their internal emotional lives, did not bring them problems, and provided them with a comfortable environment.

CONCLUSION

The conducted research proved that motivation of servicemen could be appropriate to learn connecting the theory of public service motivation (PSM) with self-determination theory (SDT). That gave the possibility to describe not only the distinguished types of professional motivation of servicemen but also specific peculiarities including self-motivation of the representatives of this type which was run with public service motivation; "rest on laurels" of the stagnated type; problems with selection of prosocial type for whom profession, motherland, and friends had big value; tendency to manipulate to get an access to desired aspects of professional activity of the type which was adventure-centered; absence of sincere interest in military profession of the deficient type which could substitute it with more prospective work having identical requirements for the candidates; attitude to profession as if it was a kind of means of satisfaction of demands of their emotional lives of the dependent type.

The prospects of further studies

It was distinguished to be prospective to develop or standardize existing methods which correlated with the theory of public service motivation (PSM) and self-determination theory (SDT) to estimate the motivation of servicemen.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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ORIGINAL ARTICLE

Assessment of Low-Doses Doxorubicin Effects in Mice Using Chromosomal Aberration Assay

Raluca S. Costache[1*], Remus Nica[2*], Silvia Nica[3], Robert M. Sionel[4,5], Sonia Spandole-Dinu[4,6], Oana Costianu[2], Irina Radu[4], Mirela Oancea[2], Matei I. Nica[3], Danut Cimponeriu[4], Lavinia Berca[5]

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Abstract: *The cytogenetic aberrations induced by doxorubicin (DOX) may be influenced by diet. Investigation regarding effects of low doses of DOX on chromosomal aberrations frequency in mice bone marrow. The study explored a possible relationship between diet, follow-up period, and cytostatic doses. Four mice groups received normal or high-fat (pork) diet. At the beginning of the 8th day mice from each group received a single intraperitoneal injection with DOX (dose range: 1.7-7.7 mg/kg b.w.) or isotonic saline solution (0.02 mL/g control mice). In the 10th (groups I, II) and 14th (groups III, IV) days from the beginning of the experiment, the mice were euthanized and chromosome aberrations were tested in bone marrow cells. The body weight of all mice who received DOX decreases, especially at higher doses. In the first three days post-injection, weight loss percentage was significantly influenced by DOX doses and/or type of diet ($p<0.003$). The interaction between the type of diet and DOX doses was not statistically significant in the follow-up period (p interactions >0.05). DOX doses were able to increase the frequency of chromosomal aberrations. DOX acts as a potent inductor of cytogenetic aberrations in bone marrow cells, regardless of the type of diet.*

Keywords: *mice, animals, injections, intraperitoneal, cytostatic agents, Doxorubicin, chromosome aberrations*

INTRODUCTION

Doxorubicin (Adriamycin, DOX) belongs to the anthracycline family of antitumor antibiotics which is prescribed for several decades in a variety of tumors [1-3]. Doses used for chemotherapy can determine serious, multiorgan or systemic, side effects in many patients [1,4] and in animal models of chemotherapy [5,6]. Most specialists consider that its antineoplastic and cytotoxic effects are the consequence of the drug's insertion between DNA strands base pairs (thus interfering with replication and gene transcription), inhibition of topoisomerase II (which predisposes to single and double-strand breaks) [7,8] and generation of reactive oxygen species (ROS) [9-11].

The DNA damage induced by this drug seems to affect preferentially some tissues (e.g. cardiac myocytes) [10,12] and it can represent an early event in doxorubicin-induced death, at least for some types of cells [13,14]. All these effects can be influenced by different factors, doses, rhythms, and routes of DOX administration [15].

Currently, studies performed on animals explore the effects of diet on DOX-side effects. Thus, long-term milk or kefir ingestion performed on old male Sprague Dawley rats may be helpful before and during DOX treatment [16]. Moreover, creatine is involved in managing Dox-induced skeletal muscle side effects [17]. Regarding the fat diet, experimental results revealed that switching to a low-fat diet 1 week before and during DOX treatment, may attenuate the DOX-increased cardiotoxicity [18]. Instead, the high-fat diet may decrease the efficacy of treatment with DOX [19].

Moreover, a hypercaloric diet will not affect only the doxorubicin efficacy, but also has a negative impact on the human body, leading to various human and animal pathologies [20-25].

Taking all these aspects into consideration, our study aimed to test the effects of low-dose DOX on the frequency of chromosomal aberrations in mice bone marrow, and whether these are influenced by diet and follow-up period.

DOX as a chemotherapeutic agent has various side effects in human and animal models. Therefore, the main aim of our study was to explore the effects of low doses of DOX on male mice fed with normal food of pork cracklings (PC).

MATERIALS AND METHODS

Animal care and doxorubicin treatment

Eight to nine weeks old BALBc male mice weighing 21.33±2.19 g were included in this study. They were accustomed to their environment for at least 10 days before the initiation of the experiment. During the experiment, the mice were maintained in the National Institute of Research and Development for Food Bioresources facility, in rooms with 20–24°C, 55–65% humidity, on a 12:12-h dark/light cycle. Mice were housed 2 in plastic cages. They received ad libitum a commercial pelleted food and filtered drinking water. The food and water were changed every other day throughout the experiment. During the experimental period, the hygienic conditions were kept constant. All mice were weighed on the 1st, 8th, and 10th day of the experiment; the mice from groups III and IV were also weighed on the 14th day (Table 1).

Table 1: Study design. NF – normal food, PC – pork cracklings

Days	1-7	8	10	14
Intervention	**Diet**	**Injection**	**Euthanasia**	**Euthanasia**
Groups	NF	i.p.	Group I	Group III
	PC	i.p.	Group II	Group IV

On the first day of the experiment, mice were distributed into 2 groups that received a normal diet (groups NFI, NFIII) and 2 groups that received only pork cracklings – PC (groups PCII, PCIV). Subsequently, the type of diet did not change until the end of the experiment. At the beginning of the 8th day, mice received a single intraperitoneal (i.p.) injection with DOX (2 mg/ml, Actavis). Eight mice from each group received one of the following doses of DOX: 1.7; 1.9; 2.2; 2.6; 3.1; 3.8; 5.1; 7.7 mg/kg b.w. In addition, four mice from each group were considered controls (C) and received a single i.p. injection with isotonic saline solution (0.02 mL/g). On the 10th (groups I, II) and 14th (groups III, IV) days from the beginning of the experiment the mice were euthanized (Table 1).

Cytogenetic analysis

Mice were injected i.p. with an aqueous solution of colchicine (0.05 %) two hours before euthanasia. For each mouse, both femurs were removed and the medullary canal was washed with 1.5 ml of NaCl hypotonic solution (75 mM). Bone marrow cells were re-suspended with a Pasteur pipette and then were centrifuged at 1000 rpm/10 minutes. The supernatant was discarded and the resulting sediment was re-suspended in 1.5 ml NaCl (75 mM). Cells were fixed in a fresh fixative solution (methanol: acetic acid 3:1) and after 20 min storage at 4°C the fixation step was repeated. After fixation, cells were re-suspended in a small volume of the fixative solution and were dropped onto clean slides that were dried at room temperature for 24 h. Slides were stained by Giemsa stain (4%) for 5-10 min and were subsequently washed with tap water and air-dried. Stained slides were coded and scored by light microscopy (at X100 magnification).

The structural and numerical chromosomal aberrations were counted for 100 well-spread metaphase/animal. The numerical (polyploidy) and structural chromosomal aberrations (e.g., deletions, translocations, acentric fragments, gaps, and chromosome stickiness) were counted. Some of these cytogenetic abnormalities (e.g., gaps, stickiness) were scored but they were not included in the total number of aberrations or the subsequent statistical analyses because we considered that they are not good indicators of chromosome damage.

Statistical analysis

Results are reported as means ± SE. The percentage weight gain was calculated as (weight gain/previous weight) x 100. Statistical analysis was performed using R statistical software (version 4.2.0). The cutoff point for significance was Bonferroni/Yates corrected.

RESULTS

Body weight, diet, and treatment

The weight of mice from the four groups was similar at the beginning of the experiment (one-way ANOVA: p=0.23). The change in body weight during the experiment can be influenced by diet and the dose of cytostatic administrated

(Figure 1).

Figure 1: Average body weight of mice (g) for the groups who received DOX (DOX injection – continuous lines) or saline solution (controls – dotted lines). The type of diet is indicated by line color: red lines – NF; blue lines – PC. NF – normal food, PC – pork chops, C – control.

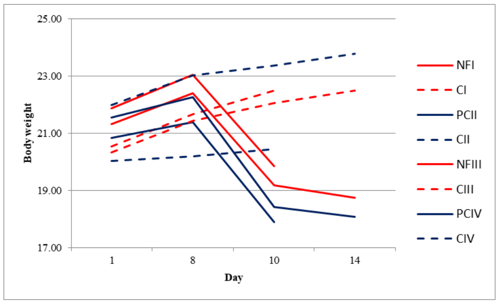

Thus, during the first 7 days of the experiment, the percentage of weight gain was positive but significantly lower, for mice who received PC compared with those who received NF (I, III vs. II, IV: 5.26±1.57% vs. 2.98±1.66%, $p<0.00001$). In the next three days, the percentage of weight gain for control mice remains positive and much lower in PC than in NF (3.36±0.56 vs. 1.41±0.51, $p=0.0009$).

In the interval between the 10th-14th day, this tendency was maintained, yet the differences were no longer significant (2.03±0.71% vs. 1.76±1.02%, $p>0.05$). The percentage of weight loss in the first three days after administration of DOX was influenced by either dose of cytostatic (one-way ANOVA: p=1.7E-28) (Figure 2 a) and type of diet (groups I, III vs. II, IV: -14.47±4.56 vs. -16.83±4.15, $p=0.003$) (Figure 1).

Post hoc test reveals significant differences in percentage weight loss (Tukey HSD $p<0.05$) when comparing different DOX doses, except for the two lowest doses (1.7 and 2.9 mg/kg b.w.). For the interval between days 10th-14th, this parameter was neither associated with the dose of cytostatic (groups III vs. IV: -2.25±2.88 vs. -1.83±0.98, $p=0.52$) nor with the type of diet (Figure 2 b).

The interaction between the type of diet and DOX doses was not significant neither in the first three days after DOX administration (groups I-IV two-ways ANOVA: p interactions = 0.68) nor the following four days (groups III and IV Two ways Anova: p interactions = 0.81).

Chromosomal aberrations

The number of cytogenetic changes and deletions identified on days three (-0.76 and -0.7 respectively) and seven (-0.66) post-injection of DOX has a negative linear correlation with weight change. The frequency of cytogenetic aberrations may be influenced by DOX doses or their interaction with the type of diet, or the post-administration follow-up interval.

Doxorubicin doses between 1.7–7.7 mg/kg b.w. were found to increase the frequency of polyploidy, chromatid deletion, acentric fragments, and Robertsonian translocations (Figures 3 and 4) ($p<0.05$).

Figure 2: Percentage of weight change post-injection of DOX at three (groups I-IV) (a) or seven (groups III-IV) (b) days post-injection. Data are presented as means ± SE for both charts. p< 0.05 for overall difference (one-way ANOVA). Significant differences ($p<0.05$) identified by post hoc Tukey HSD test are indicated by letters [compared with DOX dose 1.7 (a), 1.9 (b), 2.2 (c), 2.6 (d), 3.1 (e) or 3.8 (f) mg/kg b.w.].

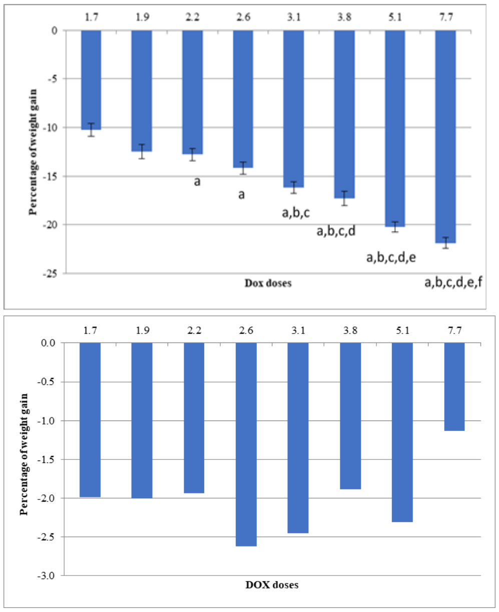

The total number of cytogenetic aberrations (Figure 3a), polyploidies (Figure 3b), and deletion (Figure 3c) showed significant differences between mice treated with different DOX doses (one-way ANOVA $p<0.0003$); the differences were more important when comparing values corresponding to lowest and highest DOX doses (5.1 or 7.7 mg/kg b.w., post hoc Tukey HSD $p<0.05$).

Interactions

A significant interaction between DOX dose and diet was found in the case of polyploidy (two ways Anova p= 4.5E-11); its frequency significantly increased in mice who received the highest DOX doses (5.1 or 7.7 mg/kg b.w.) and PC compared with those who received NF ($p<0.009$) (Figure 4). The interaction between DOX doses and the post-administration follow-up interval was found to not influence the frequency of cytogenetic aberration scored in this study ($p>0.05$).

DISCUSSION

Chromatin remodeling plays an essential role in cell proliferation, differentiation, and DNA repair as a consequence of gene expression regulation. Many studies on different biological models have revealed that chromatin remodeling complexes and enzymes that target histone modifications could sensitize cells to selective cytotoxic stress [26,27]. Investigation of epigenetic, cytotoxic, genotoxic, and/or mutagenic potential of cytostatics is useful for a better insight into the mechanisms of both drugs' actions and adverse effects. The reversal or accumulation of DNA lesions induced by cytostatic play an important role in cell survival and the efficiency of chemotherapy [28,29].

Figure 3: The distribution of an average number of cytogenetic aberrations according to DOX dose. Data are presented as means ± SE for all charts. p< 0.05 for overall difference (one-way ANOVA) were detected for the number of cytogenetic ab-errations (a), polyploidies (b), and deletion (c). Significant differences (p<0.05) identified by post hoc Tukey HSD test are indicated by letters (compared with 1.7 (a), 1.9 (b), 2.2 (c), 2.6 (d) 3.1 (e), 3.8 (f) or 5.1 (g) mg/kg b.w.).

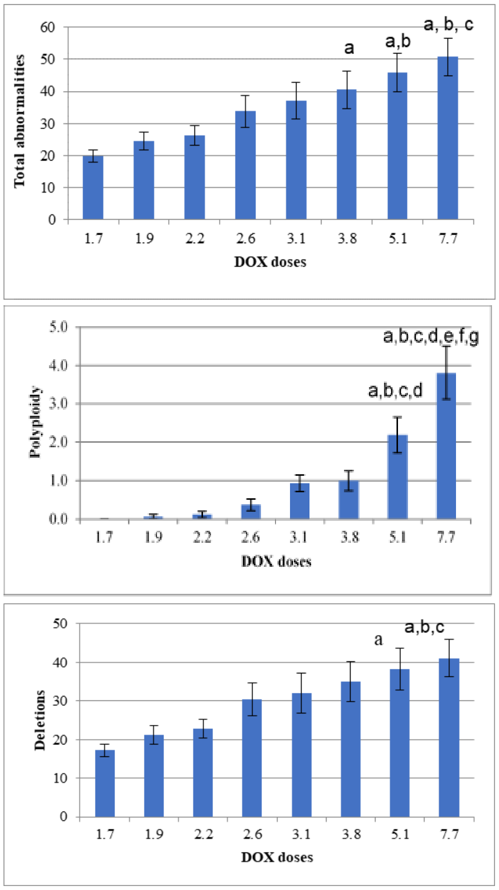

We set out to test the clastogenic effects of eight low doses of DOX in mice bone marrow cells detected after several rounds of cell cycles.

The LD50 is related to the particularities of both expo-sure and testing systems [15]. The LD50 values for adult mice treated i.p. with DOX were estimated to be in the range of 11.98 [30] and 56.87 mg/kg b.w. [31].

Figure 4: The distribution of an average number of polyploidies according to DOX dose and type of diet. Data are presented as means ± SE for all charts. p< 0.05 for overall difference (two-way ANOVA) were detected. NF – normal food, PC – pork chops

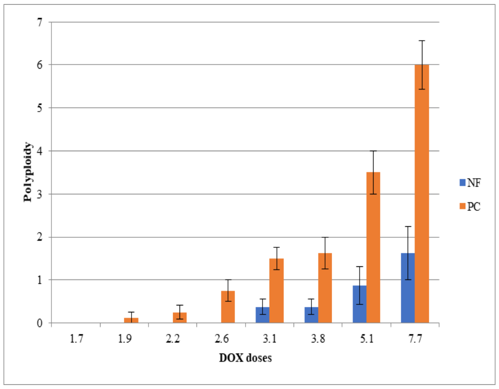

DOX can promote localized tissue damage at the site of administration that can interfere with the absorption of the subsequent doses of the drug [32]. After exposure to high doses of cytostatic, some animals died [31] or exhibited multiple tissue toxicity [33-35]. In an attempt to mitigate some of these effects, we decided to test the impact of a single dose of cytostatic administrated i.p. to each mouse. The tested doses (the range: 1.7-7.7 mg/kg b.w.) were comparable lower to those to which human patients [36] or mice [37] are frequently exposed (20 mg/kg b.w.).

The body weight change registered during the experiment in the four groups reflects the compliance of the mice with the type of diet and the effects of the cytostatic.

In the first week of the experiment, the percentage of weight gain was positive yet much lower in mice that received PC than in those who remained on a normal diet (p=0.00001). The control mice kept this trend in the interval between the 8th-10th (p=0.0009) and the 10th-14th day (p>0.05). These results suggested that PC was not well appreciated by mice, at least in the first 10 days of the experiment (Figure 1).

The body weight of all mice decreases after DOX administration, especially at higher doses of cytostatic. In the first three days post-injection, but not in the next four days, the percentage of weight loss was significantly influenced by doses of cytostatic (Figure 2a) and/or type of diet (p<0.003) (Figure 1). The interaction between the type of diet and DOX doses was not statistically significant in the follow-up period (p interactions >0.05).

Our results are in accordance with previous studies in which i.p. administration of DOX was associated with a decrease in food and water consumption [38] and a decline of mice body weight to over three days [39]. The impact of DOX on patients' body weight was also reported [40]. The effects of DOX at the nuclear level may reflect the ability of cytostatic to alkylate DNA, to inhibit DNA denaturation, replication, and topoisomerase II activity [41].

The aneugenic and clastogenic [42,43] potential of DOX was shown in different test systems (e.g., human lymphocytes, albino mice) [44,45].

Previous studies performed on mice found that the frequencies of chromatid and chromosome type aberrations detected during the first 24h post-administration were directly correlated with DOX doses (3, 12, or 24 mg/kg b.w.) [46,47]. Also, 24 h after administration of two injections with 0.8 mg/kg DOX on consecutive days, chromosome breaks (12.6%), acentric fragments (11.6%), ring chromosomes (8.3%), Robertsonian translocations (4%) and polyploidy (2%) were identified [48].

In our study, especially in the case of higher doses of cytostatic, the frequency of chromatids breaks, polyploidy, and Robertsonian translocations had a higher frequency. Doses tested in this study were associated with a low frequency of acentric fragments. Fragmented chromosomes may be attributed to the clastogenic effects of xenobiotics on DNA strands. Differences between our results and those previously reported can be explained by the comportment of acentric fragments during mitosis and by the number of cell cycles performed by the bone marrow cells during the post-administration follow-up period (1 day vs. 3- or 7- days post-administration).

In a previous experiment with 3 or 12 mg/kg DOX, similar results for breakage (20% or 26%) and cells with translocations (0% and 1%) were estimated [49].

Some cells can become giant, polyploid, and polymorphic after exposure to a low dose of DOX (e.g., HCT116, MCF7). [47,50,51]. Twenty-four hours after 0.8 mg/kg of DOX was injected i.p. in consecutive days, the frequency of polyploidy in the bone marrow cells of mice was 0.2% (47). We observed that polyploidy was more frequent in mice that received PC vs. NF and the differences are more evident at high DOX doses (Figure 4).

Division of polyploid cells may be relevant for the generation of cancer stem cells [52] and repopulation with tumoral cells after therapy [53].

Chromosome stickiness may reflect the highly toxic effects of xenobiotics and it can lead to cell death [54-56]. In our experiment, this abnormality increases with cytostatic doses. Even though the frequency of some aberrations like gaps, complex interchange, Robertsonian translocations, and chromosomal stickiness seems to increase when reaching the end range of dosage, there are no significant results that would imply a correlation between dose and frequency for aforementioned aberrations.

Limitations of the study

Some of the DNA lesions induced by mutagens can lead to apoptosis or can be fixed. It was estimated that in 12 h after DOX treatment, some bone-marrow cells went through two cell cycles [46]. Consequently, in our experiment during the post-administration period, some cells have come through a significant number of cell cycles (up to 28). During these cycles, the chromatid deletion could be converted into chromosomal deletion and some acentric fragments can be lost. Thus, the results we have obtained using conventional cytogenetic analysis highlight only a limited spectrum of cytogenetic modifications induced by DOX.

CONCLUSIONS

A brutal shift of diet from normal feed to high fat (pork cracklings) is associated with significant weight loss. The main conclusion which emerges from our study is that treatment of mice with low drug doses significantly increases the number of chromosomal aberrations compared with controls. These aberrations are an indication of DOX toxicity for the bone marrow cells and their clastogenic impact. This clastogenic potential of DOX was not influenced by diet.

The frequencies of polyploidy and deletions were significantly higher in mice who received 5.7-7.1 mg/kg and those treated with lower doses. In addition, our results suggested that some cytogenetic damage induced by low doses of DOX can be retained even three – or seven – days post-administration.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Acknowledgments

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Authors' contribution

Conceptualization: RSC, RN, SN, DC, and LB; methodology: RN, RMS, SSD, and OC; software: RMS and MIN; validation: SSD, IR, and MO; formal analysis: RMS, SSD, MIN, and DC; investigation: SSD, MIN, and IR; resources: RN, SN, and LB; data curation: RSC, RN, SN, and DC; writing: RN, RMS, MIN; writing- review and editing, RN, and SN; project administration, LB. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The animal study protocol was approved by the National Institute of Research and Development for Food Bioresources Ethics Committee with registration number 342/16.5.2014.

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ORIGINAL ARTICLE

Relationship between Glycemic Control and Triglyceride-Glucose Index in Patients with Type 2 Diabetes Mellitus

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Abstract: *The triglyceride-glucose index is a newly discovered parameter that indicates insulin resistance. This study aims to investigate the relationship between the triglyceride-glucose index and triglyceride/high-density lipoprotein ratio with glycemic control in patients with type 2 diabetes mellitus. This study was performed retrospectively in 296 patients with type 2 diabetes mellitus. Patients were divided into two equal groups according to their HbA1c levels as having good glycemic control (HbA1c 7 and below) and poor glycemic control (HbA1c above 7). Age, gender, body mass index, fasting blood glucose, HbA1c, total cholesterol, serum triglyceride level, serum HDL level, serum LDL level, triglyceride-glucose index, and triglyceride/HDL ratio were compared between the groups. Fasting blood glucose, HbA1c, triglyceride, HDL, triglyceride-glucose index, and triglyceride/HDL ratio was significantly different between patient groups with good and poor blood glucose regulation ($p<0.05$). Patients with poor glycemic control had lower fasting blood glucose, HbA1c, triglycerides, triglyceride-glucose index, and HDL values compared to patients with high triglyceride/HDL ratio and good glycemic control. There was no difference between the groups in terms of other variables ($p>0.05$). Triglyceride-glucose index and triglyceride/HDL ratio may be indicators of poor glycemic control in diabetic individuals. These indices are cheaper, universal, and easy-to-measure parameters compared to HbA1c and can be used to predict glycemic regulation.*

Keywords: *type 2 diabetes mellitus, triglyceride-glucose index, HbA1c*

INTRODUCTION

Diabetes mellitus is a global health problem whose incidence and morbidity continue to increase significantly in all countries of the world.

The most important mechanism in pathogenesis is a decrease in the sensitivity of target tissues to insulin [1]. Persistent hyperglycemia and insulin resistance are associated with long-term damage to organs, particularly the eye, kidney, nervous system, and cardiovascular system [2].

Type 2 diabetic patients have atherogenic dyslipidemia and obesity, which markedly increase the risk of coronary artery disease. Lipid profile changes in these patients increase insulin resistance and insulin resistance-related complications [3].

Regulating glycemic control and reducing insulin resistance reduces diabetic complications. Glycated hemoglobin (HbA1c) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), which are predictors of microvascular and macrovascular complications, are the best-known parameters for retrospective long-term glycemic control and insulin resistance [4].

In several studies, indicators such as Triglyceride/Glucose index, Triglyceride/HDL ratio, body mass index (BMI), waist circumference (WC), and visceral adiposity index have shown promising results in the assessment of insulin resistance [5,6]. In addition, a recent study showed that the triglyceride-glucose index was correlated with HOMA-IR and hyperinsulinemic-euglycemic clamp test [7]. There are also studies showing the association of triglyceride-glucose index with cardiovascular diseases [8,9].

The present study aimed to examine the relationship between HbA1c, which is currently the most commonly used parameter that retrospectively shows glycemic blood control, and the triglyceride-glucose index. In addition to HbA1c, the relationship between the height-weight index and the triglyceride-glucose index was also examined.

MATERIALS AND METHODS

Study Design

The study was conducted retrospectively with a total of 296 patients with diabetes mellitus 2 who applied to the Internal Medicine outpatient clinic of Mersin Şehir Training and Research Hospital between 20.06.2021 and 20.06.2022. Patients with type 2 diabetes mellitus were selected from those who met the American Diabetes Association criteria. Patients with coronary artery disease, cancer, rheumatologic diseases, type 1 diabetes mellitus, pregnant women, patients with chronic kidney disease, patients with chronic liver disease, patients with autoimmune diseases, patients with hypothyroidism or hyperthyroidism, patients receiving anti-hyperlipidemic therapy, and patients with diabetic complications were excluded.

Demographic data of patients such as height, weight, duration of diabetes, age, and smoking status were obtained from the electronic information system. Body-mass index (BMI) was calculated by weight in kilograms divided by the square of height in meters. Regarding previous studies, the TyG index was calculated separately for each individual with the formula = ln [fasting TG (mg/dL) x fasting glucose (mg/dL)/2] [6,10]. Serum triglyceride level (mg/dL) was divided by serum HDL level (mg/dL) to obtain the Triglyceride/HDL ratio (THR). Fasting plasma glucose, HbA1c, and lipid profile were studied from venous blood samples obtained after fasting for at least 8 hours.

The 296 patients included in the study were divided into two equal groups: patients with good glycemic control (N=148, HbA1c:7 and below) and patients with poor glycemic control (N=148, HbA1c above 7). Age, gender, duration of diabetes, smoking status, body mass index, fasting blood glucose, HbA1c, total cholesterol, serum triglyceride level, serum HDL level, serum LDL level, triglyceride - glucose index, and triglyceride/HDL ratio were compared between the groups.

Ethics committee approval was obtained from Mersin University Ethics Committee before starting the study (dated 20.07.2022 and numbered 2022/491).

Statistical Analysis

Shapiro-Wilk test was used to test whether the variables conformed with normal distribution. Normally distributed variables were presented as mean±standard deviation and an independent sample t-test was used for comparisons between two independent groups. Non-normally distributed variables were presented as median (minimum-maximum). Mann-Whitney U test was used to compare non-normally distributed variables between two independent groups and the Kruskal Wallis H test was used for comparisons between three independent groups. Categorical variables were presented as frequency and percentage values (n(%)) and the Pearson chi-square test was used for intergroup comparisons.

Statistical analyses were performed in IBM SPSS Statistics 22.0 program. P<0.05 was accepted as statistically significant in all analyses.

RESULTS

A total of 298 patients were included in the study. The median age was 57 (33-75) years in the good glycemic control group and 56 (27-75) years in the poor glycemic control group. Basic demographic data and biochemical blood parameters of the patients included in the study are shown in Table 1.

Although the duration of diabetes was less than 20 years in both groups, there was a statistically significant difference between the groups (P <0.001). There was no statistically significant difference between the good and poor glycemic control groups in terms of gender and age (P= 0.201, 0.390). There was no statistically significant difference between the two groups in terms of smoking (P=0.118). No statistically significant difference was found between the two groups in terms of body mass index (P=0.118).

DM duration, fasting blood glucose, HbA1c, triglyceride, HDL, triglyceride-glucose index, and triglyceride/HDL ratio was significantly different between the patient groups with good and poor blood glucose regulation (p<0.05). DM duration, fasting blood glucose, HbA1c, triglycerides, triglyceride-glucose index, and triglyceride/HDL ratio was higher in patients with poor glycemic control while HDL values were lower compared to patients with good glycemic control. No difference was found in other variables between the groups (p>0.05). Statistical results showing the differences in biochemical parameters and triglyceride-glucose index and triglyceride-HDL ratio between the good and poor glycemic control groups are presented in Table 2.

When the relationship between triglyceride-glucose index, triglyceride/HDL ratio, and body mass index was analyzed, it was found that the TyG index and THR values did not differ between the groups concerning body mass index. (p>0,05). The results of the analysis are shown in Table 3**.**

DISCUSSION

Type 2 diabetes mellitus is a global health problem whose prevalence continues to rise. Dyslipidemia and insulin resistance are the main mechanisms involved in the development of microvascular and macrovascular complications. HbA1c is still the most widely used blood parameter that retrospectively shows blood glucose regulation over the longest period. The present study aimed to evaluate various metabolic indices that may be associated with HbA1c, which may indicate glycemic control, are economical and easily applicable.

Table 1: Anthropometric Data and Biochemical Blood Tests of the Groups with Good and Poor Diabetic Control

	Well glycemic controlled group (N=148)	**Poor glycemic controlled group (N=148)**
	$\bar{X}$+SS/Median (Min-Max)	**$\bar{X}$+SS/Median (Min-Max)**
Age (year)	57 (33-75)	56 (27-75)
Gender (n, %)	Female: 83 (56%) Male: 66 (44%)	Female: 77 (52%) Male: 71 (48%)
Smoking (n, %)	No (122, 82.4%) Yes (26, 17.6%)	No (111, 75%) Yes (37, 25%)
Diabetes duration(year)	6 (1-17)	8 (1-20)
Body Mass Index (kg/m^2)	Normal 33 (22.3%) Overweight 57 (38.5%) Obese 38 (29.2%)	Normal 49 (33.1%) Overweight 50 (33.8%) Obese 49 (33.1%)
Fasting blood sugar (mg/dL)	128 (71-303)	215 (98-445)
HbA1c (%)	6.8 (5.6-7)	9.4 (7.1-14.7)
Total cholesterol (mg/dL)	194±36.55	203.52±42.79
HDL (mg/dL)	45 (27-98)	43 (25-80)
LDL (mg/dL)	109.89±27.63	111.73±36.29
Triglyceride (mg/dL)	175 (61-780)	201.5 (72-982)
Triglyceride-glucose index (TGI)	9.28 (6.62-10.69)	9.95 (8.64-12)
Triglyceride/HDL ratio (THR)	3.76 (0.3-20.53)	4.57 (1.29-28.88)

Table 2: Comparison of Biochemical Parameters Between Groups

		N	**$\bar{x}$+SS/Median (Min-Max)**	**Z/2t**	**p**
Diabetes duration	Well Regulated group	148	6(1-17)	-4,022	<0,001
	Poorly Regulated group	148	8(1-20)		
Fasting blood sugar	Well Regulated group	148	128(71-303)	-12,811	<0,001
	Poorly Regulated group	148	215(98-445)		
HbA1c	Well Regulated group	148	6,8(5,6-7)	-14,928	<0,001
	Poorly Regulated group	148	9,4(7,1-14,7)		
Total cholesterol	Well Regulated group	148	194±36,55	-1,944	0,053
	Poorly Regulated group	148	203,52±42,79		
Triglyceride	Well Regulated group	148	175(61-780)	-3,101	0,002
	Poorly Regulated group	148	201,5(72-982)		
HDL	Well Regulated group	148	45(27-98)	-2,355	0,019
	Poorly Regulated group	148	43(25-80)		
LDL	Well Regulated group	148	109,89±27,63	-0,491	0,624
	Poorly Regulated group	148	111,73±36,29		
TGİ	Well Regulated group	148	9,28(6,62-10,69)	-9,079	<0,001
	Poorly Regulated group	148	9,95(8,64-12)		
THR	Well Regulated group	148	3,76(0,3-20,53)	-3,645	<0,001
	Poorly Regulated group	148	4,57(1,29-28,88)		

Table 3: Analysis Results Showing the Relationship Between Body Mass Index and TGI and THR

		N	Median (Min-Max)	Ki-Kare	P
TGI	Normal	82	9.76 (8.25-12)	4.88	0.087
	Overweight	107	9.6 (8.38-11.44)		
	Obese	107	9.46 (6.62-11.49)		
THR	Normal	82	4.79 (1.03-28.88)	3.729	0.155
	Overweight	107	4.22 (1.47-14)		
	Obese	107	3.77 (0.3-28.88)		

Good glycemic control, i.e. an HbA1c level below 7, plays a very important role in reducing the complications of diabetes mellitus [10]. In type 2 diabetes mellitus, hyperglycemia and decreased insulin sensitivity be associated with hypertriglyceridemia and low HDL levels when blood glucose regulation is impaired. Increased plasma cholesterol ester transfer protein (CETP) increases VLDL-C levels by facilitating the transfer of triacylglycerol and cholesterol esters between HDL and LDL particles [11,12]. Increased small dense LDL is associated with increased diabetic dyslipidemia and cardiovascular complications. Estimation of small dense LDL particles is not useful in clinical practice; the triglyceride/HDL ratio may be an indirect indicator of these LDL particles [6,13].

Previous studies have shown that the triglyceride/HDL ratio is correlated with HbA1c [13,14].

One study found higher triglyceride-glucose index and lower HDL levels in patients with poor glycemic control [14]. The result obtained in the present study is similar. The group with high HbA1c levels, the poor glycemic control group, had a higher Triglyceride-glucose index and higher triglyceride-HDL ratio compared to patients with good glycemic control (P<0.001). Similar to the other study, HDL level was significantly lower in patients with poor glycemic control in the present study [14]. Low HDL levels may be the result of increased oxidative stress associated with impaired glycemic control.

Poor glycemic control is known to increase microvascular and macrovascular complications of diabetes. Triglyceride-glucose index and triglyceride/HDL ratio are correlated with HbA1c and thus may be predictive markers of complications in diabetic patients. In a study on this subject, triglyceride/glucose index was shown to be associated with cardiac autonomic neuropathy [15]. Another study in hospitalized diabetic patients showed that nephrotic and vascular complications were associated with elevated triglyceride-glucose index [16]. Other studies have shown that the triglyceride-glucose index is higher in patients with diabetic nephropathy, retinopathy, and coronary artery disease [17,18]. Since patients with known diabetic complications (diabetic retinopathy, nephropathy, coronary artery disease, diabetic neuropathy) were excluded from the present study, it can be concluded that triglyceride-glucose index and triglyceride/HDL ratio in correlation with HbA1c is already high even before diabetic complications occur. This group of patients should be monitored more closely for the occurrence of diabetes complications. However, there may be possible micro-complications and atherosclerotic processes that have not been evaluated. Further studies may clarify this subject.

In the present study, the relationship between triglyceride-glucose index, triglyceride/HDL ratio, and body mass index was also evaluated. In both groups, patients were divided into three sub-groups according to body mass index: normal weight (body mass index of 18.5 and below), overweight (body mass index of 25-29.9), and obese (body mass index of 30 and above). When the relationship between triglyceride-glucose index, triglyceride/HDL ratio, and body mass index was analyzed, it was found that the TyG index and THR values did not differ between the groups concerning body mass index. (p>0,05). This result suggests that the triglyceride-glucose index and triglyceride/HDL ratio are not associated with obesity. That is, they are correlated with insulin resistance but not with body mass index. A previous cohort study has also shown that the triglyceride glucose index is associated with the development of diabetes in young, non-obese individuals [19]. Considering the relationship between the triglyceride-glucose index and insulin resistance, the TyG index is expected to be higher in obese individuals compared to non-obese individuals. Another study suggested that the TyG index in non-diabetic individuals can be used as a diagnostic criterion for identifying metabolically obese but normal-weight individuals [20]. In contrast to our results, another study in the literature showed that the triglyceride-glucose index was correlated with the body mass index in diabetic individuals [14]. Further investigations are needed to explain why the results are not similar in diabetic individuals.

There were two important limitations in this study. The study was conducted retrospectively and the number of patients was relatively small.

In conclusion, triglyceride-glucose index and triglyceride/HDL ratio may be indicators of poor glycemic control in diabetic individuals. These indices are cheap, universal, easily accessible parameters similar to HbA1c and can be used to predict blood glucose regulation. In addition, although these two indexes were associated with insulin resistance, they were not found to be associated with the body-mass index in diabetic individuals. Further studies are needed to clarify and validate the results. Since patients with known diabetic complications were excluded from the present study, it can be concluded that the triglyceride-glucose index and triglyceride/HDL ratio in correlation with HbA1c is already high before diabetic complications occur. This group of patients should be monitored more closely for the occurrence of diabetes complications.

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ORIGINAL ARTICLE

Value of Electrocardiogram Algorithms Predicting Right Ventricular Outflow Tract Origin in the Era of Catheter Mapping

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Abstract: *Background: The 12-lead electrocardiogram (ECG) is an extremely useful tool for analyzing cardiac arrhythmias and numerous ECG algorithms have been developed to predict the origin of right ventricular outflow tract (RVOT) arrhythmias. Aim: To determine if using complex, time-consuming analysis, and difficult-to-memorize ECG algorithms is feasible and preferable before the ablation procedure when compared with the use of multipolar mapping catheter use during the ablation procedure. Methods: 47 consecutive patients admitted for radiofrequency catheter ablation of idiopathic PVC were included prospectively in this study. Of the algorithms selected for comparison, three have a memorable design and easy applicability (Dixit, Joshi, Pytkowski) and the fourth has a complex, detailed design and is more difficult to memorize (Zhang). Results: The greatest accuracy was observed for the algorithms proposed by Dixit and Pytkowski – 92. 3%. However, when the algorithms were compared for their accuracy, specificity, and sensitivity, no significant differences were found (p=0. 96). Conclusions: 3-D guided mapping using a conventional ablation catheter in a narrower, precise area of interest in the RVOT, based on ECG algorithms is not inferior compared to the multielectrode catheter mapping in the whole RVOT, in terms of mapping time, total procedural time, and radiofrequency application time.*

Keywords: *electrocardiogram (ECG) algorithm; multielectrode mapping catheter; premature ventricular contraction (PVC) ablation; right ventricular outflow tract*

INTRODUCTION

Idiopathic ventricular arrhythmias originating in the right ventricular outflow tract (RVOT) represent 10% of all ventricular tachycardia (VT) admissions [1,2]. The most common presentation scenario is represented by frequent premature ventricular complexes (PVC) [3], but non-sustained or sustained ventricular tachycardias are other scenarios. Ventricular arrhythmias may cause heart failure and ventricular dysfunction [4]. PVCs originating from the RVOT pre-sent on 12 lead electrocardiogram (ECG) had a left bundle branch block (LBBB) morphology and an inferior axis. The exact cut-off of ventricular burden that causes PVC-induced cardiomyopathy is not yet known. Several studies showed a correlation between PVC burden and the development of cardiomyopathy [5,6]. Baman's study [6] revealed that in patients with normal LV dimensions and systolic left ventricular (LV), a PVC burden cut-off of 24% may identify the patients at risk to develop left ventricular (LV) dysfunction.

The most common site of origin of RVOT arrhythmias is below the pulmonary valve, in the anterior septal region [7]. Less common sites of PVC are found in the right ventricular free wall, posterior septal area, and other rare areas [8,9,10]. The current guidelines for symptomatic patients with RVOT arrhythmias, recommend catheter ablation as first-line therapy [11,12]. In experienced centers, for patients without structural heart disease, the acute success rate of catheter ablation is usually high, >95% [13,14,15]. It is paramount to correctly predict the precise origin of RVOT arrhythmias to establish the appropriate ablation approach before the procedure. As a 12-lead electrocardiogram (ECG) is an extremely useful tool for analyzing cardiac arrhythmias, numerous ECG algorithms have been developed to predict the origin of RVOT arrhythmias [16-27].

The major endpoint of this study was to evaluate the differences between mapping the RVOT arrhythmias using a 3-D electroanatomical system as Ensite (Abbott Laboratories, Abbott Park, IL, USA) and a quadripolar catheter (Abbott Laboratories, Abbott Park, IL, USA) only in the region of interest based on ECG algorithms shortens significantly the mapping time, the total procedural time, the radiofrequency application time,. when compared to the use of a multipolar mapping catheter (16 electrodes) (Abbott Laboratories, Abbott Park, IL, USA) in the entire RVOT.

Another endpoint was to determine if using complex, time-consuming analysis, and difficult-to-memorize is feasible and preferable instead of determining a wider location of RVOT ectopies (septal or free wall location) before the ablation procedure. Other objectives were to evaluate the concordance between the arrhythmia location based on each algorithm and the location obtained on the 3-D mapping system and to define the level of accuracy of each algorithm used in our study.

MATERIALS AND METHODS

Study population

Between January 2019 and October 2022, 47 consecutive patients admitted to two different centers for radiofrequency catheter ablation of idiopathic PVC were included prospectively in this study. PVCs with LBBB morphology and an inferior axis were documented for all patients. Usually, symptomatic patients have a high PVC burden (>10000 PVCs/24h). Because symptoms are not found exclusively in this category, patients with a reduced PVC burden (5000-10 000/24h) are frequently highly symptomatic and warrant ablation[28]. In this study, we included symptomatic with PVCs refractory or intolerant to an antiarrhythmic drug (beta-blocker and/or at least one class IC anti-arrhythmic agent), with a ventricular burden above 5% on a 24-hour Holter monitoring. All patients presented normal left ventricular ejection fraction.

Of the algorithms selected for comparison, three algorithms have a memorable design and easy applicability (Dixit [26], Joshi [3], Pytkowski [27]). The fourth algorithm has a complex, detailed design and is more difficult to memorize (Zhang [24]).

Evaluated algorithms

1. The Dixit algorithm (2003) evaluates the presence or the absence of “notching” in leads II, III, and aVF, and pre-cordial transition (R ≥ S at or beyond lead V4) to predict the arrhythmia origin. QRS polarity in lead aVL is further assessed to determine arrhythmia locations in the anterior to the posterior plane.

2. The Joshi algorithm (2005) is based on three criteria for differentiating the free wall from septal wall origin (presence or absence of R-wave “notching” in inferior leads, a QRS duration ≥140 ms, an R/S ratio ≤1 in lead V3). According to the QRS polarity in lead I, the anterior or posterior location of arrhythmias is determined. Next, the QRS polarity in aVL lead determines a cranial versus a caudal origin.

3. The Zhang algorithm (2009) allows the location of the ventricular arrhythmias in four different sites (RV septal, or free wall origin, near the His, LV). It begins with the determination of the QRS morphology and axis. LBBB morphology with the inferior axis suggests an RVOT origin. The transitional zone in V4, the R wave duration index, the R/S amplitude index, and RR’/RSR’ pattern in lead aVL are evaluated. The PVC-QRS duration/the QRS duration of the preceding sinus beat and QRS pattern in lead I am also evaluated. Arrhythmia origin is further described as anterior, posterior, and mid-region.

4. The Pytkowski algorithm (2013) uses RVOT arrhythmia location in one of the 9 sub-regions created by 3 vertical and 3 horizontal zones. It starts by evaluating the presence or the absence of “notching” in leads II, III, and aVF. A low R wave amplitude in the inferior leads suggests a free wall origin. RVOT is next divided into 3 vertical zones (posterior to anterior) based on the lead I polarity. Then, 3 horizontal zones are defined, depending on the transitional zone in the precordial leads, with the horizontal intermediate zone (R[r] = S[s] in V4) occupying a zone in the median part of the RVOT which delineates the superior and the inferior zones.

All the ECG tracings were analyzed at a paper speed of 25 mm/s and were reviewed by two experienced electrophysiologists to locate the origin of the arrhythmia based on the 4 presented algorithms. Each electrophysiologist used ECG assessment one consecutive algorithm for every consecutive patient. Furthermore, based on the precise or wider location obtained from ECG evaluation, the ablation procedure was performed using the same 3-D mapping system, mapping, and ablation catheter. The algorithm used for evaluation, the time from the beginning till the end of the mapping with the multielectrode catheter and next with the mapping and ablation catheter, the success rate, procedural time, and radiofrequency application time during ablation procedures were registered for each patient.

Based on Dixit and Zhang algorithm, a less precise sub-localization in the RVOT was performed – septal vs free wall and on the horizontal plane (posterior, median, and anterior zones). When Zhang or Pytkowski’s algorithms were used, the location was identified in one of the nine regions of the RVOT divided by 3 vertical and 3 horizontal lines.

Electrophysiological procedure

The anti-arrhythmic therapy was interrupted for all patients at least five half-lives before the procedure. Spontaneous PVC at baseline and during intravenous isoprenaline (1-4 µg/min) infusion were recorded. A multipolar (16 poles) electrode catheter was inserted into a femoral vein and positioned in the RVOT. The 3D geometry of RVOT was created by navigating the mapping catheter within the RVOT using the non-contact electro-anatomical mapping system. To identify the earliest activation (EA) site, a broad color setting with a high pass filter set at 2 Hz was used. The EA site was identified by stepping further back in time where the red color zone shrinks down to the blue. For each patient, the EA site obtained from the mapping with the multi-polar catheter was noted, as well as the time from the insertion of the mapping catheter in the RVOT until the final definition of the earliest activation zone. Next, an eight-French quadripolar catheter with a 3. 5 mm distal electrode, sensor-enabled interelectrode spacing of 1-4-1mm, and a flexible tip (Abbott Laboratories, Abbott Park, IL, USA) was placed in the RVOT for mapping the earliest activation zone in the region of interested obtained from the ECG algorithm. The time from the insertion of the ablation catheter in the RVOT until the final definition of the earliest activation zone was also noted. Unipolar electro-grams at this site were reconstructed. The presence of a QS morphology was used as an additional criterion for the EA site.

For the Ensite system, several modules can be used to facilitate the localization of ventricular arrhythmia origin - Template Score Matching (Figure 1, Figure 2) and Real Time PVC Map Score. Template Score Matching analyses the QRS morphology of the clinical PVC in all 12 leads ECG during mapping and creates a compatibility score for the PVC. This way, all PVCs that are not in the interest zone are eliminated, either non-clinical PVC or PVC induced by the catheter movement.

The filter settings for the intracardiac electrograms were set at 30 –500 Hz. The pacing was performed at twice the diastolic threshold with a programmable stimulator using stimuli with a duration of 2ms. Pace mapping for each of the targeted sites was also performed to ensure at least 11 of 12 matching on the 12-lead ECG.

Concordance between the earliest activation zone obtained from the multielectrode catheter and the mapping and ablation electrode for each patient was evaluated. In the case of concordance, the earliest activation site was targeted by radiofrequency catheter ablation. In case of mismatch, radiofrequency energy was delivered through the ablation catheter in temperature-control mode (Stockert, Biosense Webster, Diamond Bar, CA, USA) in the earliest activation zone obtained from the multielectrode catheter. The power output was titrated to as high as 40 W to achieve a target temperature of 48°C for 60 – 120 s.

Figure 1: 3-D reconstructed image of the right ventricular outflow tract with activation map revealing the origin of ventricular in the white zone (with the red dots added) in the septal area of RVOT. The compatibility score of 100% and the earliest activation time (LAT) of 24 ms (the time from the earliest ventricular EGM to the onset of QRS) are displayed in the inferior part of the image. In the right panel, the 12 lead ECG shows a PVC characterized by an LBBB morphology with late transition in V4 and R/S ratio > 1 in lead II, III, and AVF. The presence of QRS notching and the lower R wave amplitude in the inferior leads is highly suggestive of a free wall origin.

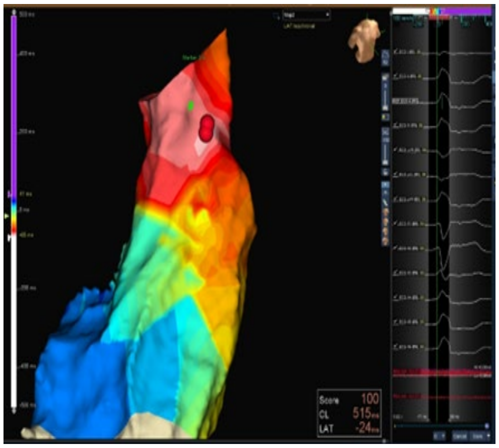

Figure 2: 3-D reconstructed image of the right ventricular outflow tract with activation map revealing the origin of ventricular ectopy in the white zone in the septal area of RVOT. The compatibility score of 99% and the earliest activation time (LAT) of 21 ms (the time from the earliest ventricular EGM to the onset of QRS) are displayed in the inferior part of the image. In the right panel, the 12 lead ECG shows a PVC characterized by an LBBB morphology with late transition in V4 and R/S ratio > 1 in lead II, III, and AVF. The lack of QRS notching and a higher R wave amplitude in the inferior leads suggest a septal origin.

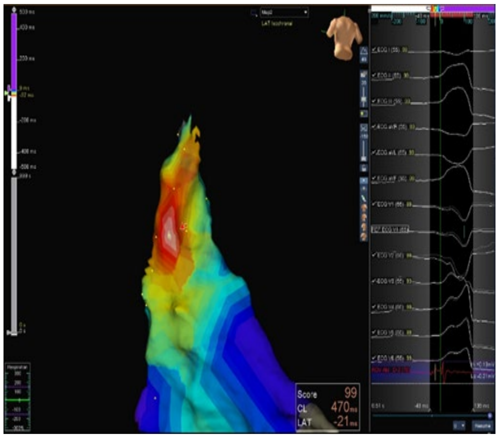

An observational period of 30 min was applied for all patients. In case of incomplete elimination of VAs, surrounding sites were further investigated by activation and pace map-ping to find alternative ablation sites. Next, subsequent ablations were applied to those sites. The ablation procedure was considered successful if clinical PVC disappeared during RF and didn't reoccur within 30 minutes after the last RF application, and if PVCs were non-inducible after Isoproterenol infusion (1-10 µg/min) [29,30].

Based on the 3D electroanatomical non-contact mapping, RVOT was divided through vertical and horizontal imaginary lines into nine distinct regions to facilitate the description of the origin of PVC based on the successful ablation site.

Statistical analysis

For data analysis, IBM SPSS Statistics for Windows (version 20. 0; IBM Corp. , Armonk, NY, USA) was used. Continuous variables are presented as median and mean ± standard deviation (SD). Categorical variables were presented as counts and percentages.

RESULTS

47 patients with RVOT arrhythmias were enrolled in this study. Patient clinical characteristics are presented in Table 1. Ablation was delivered at 14 free wall sites and 33 septum sites.

The 3D electroanatomic maps were created for all patients. The precise origin of RVOT was identified as it is presented in Table 2. The RVOT chamber was correctly identified by the evaluators in 47/47 (100%) patients. Furthermore, the concordance between the origin obtained from the multielectrode catheter also targeted for ablation, and the origin obtained by the mapping and ablation catheter guided by the ECG algorithm was evaluated. The results are presented in Table 3.

Table 1: Clinical characteristics (PVC, premature ventricular contraction; LVEF, left ventricular ejection fraction)

Age (years; median)	42. 76
Female/ Male (n)	27/20
PVC burden (%; median)	17. 95
LVEF (%; median)	62. 1
Use of antiarrhythmic drugs(%) - Beta-blockers - Class I C	76. 59% 44. 68% 31. 91%

Table 2: Localization of arrhythmia in RVOT based on mapping with the multielectrode catheter when performed by both electrophysiologists (EP1 and EP2).

Location in RVOT	EP1	EP2	Total
Free wall	6	8	14
Anterior	1	0	1
Mid	1	2	2
Posterior	4	6	11
Septal wall	17	16	33
Anterior	8	7	15
Mid	2	2	4
Posterior	7	7	14

Table 3: The concordance between the origin obtained from the multielectrode catheter, also targeted for ablation and the origin obtained by the mapping and ablation catheter guided by ECG algorithm.

Algorithm	Electrophysiologist 1 Concordance - number of patients/percentages	Electrophysiologist 2 Concordance - number of patients/percentages	Concordance - number and percentage in our study
Dixit	5/6 patients 83. 33%, P=0. 68	6/6 patients 100%, P=0. 54	11/12 patients 91. 6%, P=0. 77
Joshi	4/6 patients 66. 66%, P=0. 51	5/6 patients 83. 33%, P=0. 68	9/12 patients 75%, P=0. 75
Zhang	4/5 patients 80%, P=0. 42	3/6 patients 50%, P=0. 55	7/11 patients 63. 63%, P=0. 94
Pytkowski	5/6 patients 83. 33%, P=0. 51	6/6 patients 100%, P=0. 55	11/12 patients 91. 66%, P=0. 96
Total	18/23 patients	20/24 patients	38/47 patients 80. 85%, P=0. 88

Acute procedural success was achieved for 45 out of 47 patients (95. 74%). Failure of RF ablation was observed in 2 out of 47 patients, both patients in the concordance origin group. The diagnostic accuracy evaluations showed that the greatest accuracy was observed for the algorithms proposed by Dixit and Pytkowski – 92. 3%. However, no significant differences were found when the algorithms were compared for their accuracy, specificity, and sensitivity (p=0. 96). The diagnostic accuracy, sensitivity, and specificity of each algorithm are presented in Table 4.

Table 4: Accuracy, sensitivity, and specificity of each algorithm in our study

Algorithm	Sensitivity	Specificity	Accuracy
Dixit	100%	80%	92. 3%
Joshi	87. 5%	71. 43%	80%
Zhang	80%	60%	73. 33%
Pytkowski	100%	66. 67%	92. 3%

The mean time for mapping with the multielectrode catheter was 14. 89±8. 15 min, whereas the time used for mapping with the ablation catheter in the region of interest obtained from the ECG algorithms was. 12. 27±7. 70 min. The mean total procedural time was. 95. 5±24. 42 min. The mean total RF application time was 264. 46±134. 12 seconds. Ectopy recurrence during the waiting time occurred in 5 out of 47 patients (10. 63%).

DISCUSSION

The predicted accuracy of the ECG algorithms in our study varied from 73. 3% to 92. 3%. The accuracy of algorithms was similar to the results previously reported [9-27] despite the differences in the population, and the heterogeneity between the assessors in our study and the developers of algorithms, a narrower sub-localization of the arrhythmias compared with algorithms differentiating only septal from the free wall and a more complex design of the Zhang algorithm.

Though the diagnostic accuracy was similar for the algorithms proposed by Dixit and Pytkowski – 92. 3%, the Pytkowski scheme allows a more precise location, not only septal vs free wall location, but also a more exact location in the horizontal and vertical plane.

Several major issues were observed when comparing the Dixit algorithm from our study and the Dixit publication. Dixit algorithm predicts the arrhythmia origin based on pace mapping and not based on activation maps or a combination of activation and pace mapping as it is known that pace mapping in RVOT exhibits a lower spatial resolution (2 cm). Secondly, our study allowed arrhythmia localization in one of the 9 regions of RVOT, while the Dixit algorithm is refined to 6 zones.

One major issue was also detected when comparing our evaluation based on the Pytkowski algorithm with the one from the Pytkowski study In the Pytkowski study, the precise location of arrhythmia was fluoroscopy only based, without the use of 3-D mapping.

The analysis of mapping time, total procedural, time, and RF application time performed in the concordance and non-concordance origin groups of the multielectrode catheter and ablation catheter mapping showed no significant differences (p=0. 15) – Table 5. Next, the impact of ventricular burden during the procedure and especially during the mapping interval on the mapping time, total procedural time, and RF application time performed in the concordance and non-concordance origin groups of the multielectrode catheter and ablation catheter mapping, was evaluated and results are presented in Table 6.

Table 5: The impact of concordance or non-concordance origin of RVOT arrhythmia on mapping time with a multielectrode catheter, ablation catheter, total procedural time, and RF application time.

Mean time ± SD	Concordance group	Non-concordance group	P value
Multielectrode catheter mapping time	16. 29 ± 7. 97 min	11 ± 7. 46 min	0. 71
Ablation catheter mapping time	12. 94 ± 7. 08min	10. 53 ± 9. 2 min	0. 43
Total procedural time	94. 17 ± 25 min	99. 15 ± 23. 4 min	0. 67
RF application time	243. 08 ± 127. 14 sec	320. 38 ± 140. 53 sec	0. 23

The evaluation revealed that a low ventricular burden du-ring mapping is associated with a longer total procedural time (P=0. 04) and a longer RF application time (p=0. 01). These findings can be explained by a cumulation of factors. A low ventricular burden during mapping can prolong the mapping time (though statistical differences were not observed in our study). The suppression of clinical PVC during catheter manipulation during low ventricular burden can be more frequently unnoticed. The waiting period after RF application in these cases can also be prolonged, either due to early or late recurrence of PVC, either by transient suppression caused by an incomplete lesion or ablation in an adjacent zone near the real focus.

Based on these findings we can conclude that there are no significant differences in terms of mapping time, total procedural time, and RF application time when mapping is performed with a multielectrode catheter or a conventional ablation catheter in a narrower, precise area of interest in the RVOT, based on ECG algorithms.

The ECG-based algorithms for a narrower localization in the RVOT may exhibit acceptable to excellent accuracy, simplify the mapping process, reduce the procedural and RF application time, and improve clinical outcomes, resulting in greater clinical utility. Algorithms with a more elementary and easy-to-memorize design as Pytkowski can be routinely used to precisely localize the RVOT arrhythmia origin instead of other algorithms with a less precise sub-localization of arrhythmia origin.

Table 6: The impact of ventricular burden during the procedure on the total mapping time, total procedural time, and RF application time performed in the concordance and non-concordance origin groups of the multielectrode catheter and ablation catheter mapping.

Mean time	Concordance group	Non concordance group	P value
Multielectrode catheter mapping time PVC : 1- 5/ min PVC : 6-10/min PVC : >11/min	22. 14 min 15. 38 min 5. 5 min	19. 75 min 9. 75 min 5 min	0. 79
Ablation catheter mapping time PVC : 1- 5/ min PVC : 6-10/min PVC : >11/min	18. 5 min 11. 07 min 4. 2 min	19. 75 min 7. 75 min 5. 4 min	0. 7
Total procedural time PVC : 1- 5/ min PVC : 6-10/min PVC : >11/min	115. 15 min 87. 92 min 64. 66 min	105. 5 min 93. 25 min 98. 8 min	**0. 04**
RF application time PVC : 1- 5/ min PVC : 6-10/min PVC : >11/min	318. 92 sec 198. 14 sec 171 sec	362 sec 323. 5 sec 284. 6 sec	**0. 01**

Meanwhile, it is paramount to understand that the algorithms with a discriminating and stepwise analysis may lessen the inaccurate results. Detailed algorithms are not easy to remember, and the orientation of the subsequent steps can be frequently challenging. Time-consuming and complex analyses of such algorithms may be more helpful while predicting the arrhythmia location and may lead to better preparation before the procedure. Also, in our study, in case of erroneous predictions of the RVOT arrhythmia origin based on ECG algorithms, the effectiveness of ablation was not impacted, and no inappropriate approaches were detected.

Limitations

The number of patients in our study is relatively small, which may affect the results. Several limitations associated with the ECG algorithms were found both in our and other studies such as inconsistencies and heterogeneities between the assessors, the fact that some algorithms require an accurate measurement of many ECG parameters, and the lack of electronic calipers or wandering baselines [20-22]. Variable spatial relationships of the heart in the thorax and variable lead placements are prone to create inaccuracies in ECG algorithms [13,31,32]. In our study, we did not perform magnetic resonance imaging (MRI) for all patients so right ventricular dysplasia cannot be ruled out. The patients' age range was 24 to 69 years for this study, and we did not include more pediatric or older patients, thus the age-related changes of PVC morphology are not discussed.

CONCLUSION

3-D guided mapping using a conventional ablation catheter in a narrower, precise area of interest in the RVOT, based on ECG algorithms is not inferior compared to the multi-electrode catheter mapping in the whole RVOT, in terms of mapping time, total procedural time, and RF application time. In case of misleading predictions of the RVOT arrhythmia origin based on ECG algorithms, the effectiveness of ablation is not impacted.

In our study, although both Dixit and Pytkowski algorithms have the best accuracy among the evaluated algorithms, the Pytkowski scheme is more precise in localizing the RVOT arrhythmias origin, not only in septal vs free wall but also in the horizontal and the vertical plane, has a more basic and easy-to-memorize design.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.
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To the Cardiology teams of both hospitals

Authors' contribution
Conceptualization, R.S.P., and L.R.; methodology, R.S.P., and L.R.; software, M.A, O.P.; validation, R.S.P., L.R., and I.M.; formal analysis, R.S.P., and L.R.; investigation, R.S.P., and L.R.; resources, R.S.P., and L.R; data curation, R.S.P., and L.R.; writing - original draft preparation, R.S.P., and L.R.; writing - review and editing, R.S.P., and L.R.; visualization, I.M.; supervision, I.M.; project administration, R.S.P., and L.R. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The present study was approved by the hospital's ethics committee, number 11/21/11/2018, and is performed under the ethical standards laid down by the Declaration of Helsinki.

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Risk Factors for Urinary Colonization in Patients with Double J Catheters Implanted for Treating Obstructive Urolithiasis

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Abstract: *Background: The highlighting of possible risk factors for urinary colonization in patients with obstructive urolithiasis that needed double J catheters implanted to preserve renal function. Methods: We performed a descriptive, retrospective study, carried out in the Urology Department of the Bucharest Central Military Hospital, between January 2020 and January 2022 and included 168 patients with urolithiasis who required the insertion of double J catheters. We studied the bacteriological profile, using both urine and JJ catheter samples. Results: We obtained a double J catheter colonization rate of 32% (54 patients) and 29% of urinary colonization (49 patients). The rate of urinary colonization is higher in patients with colonized ureteral catheters regardless of sex, age, and associated comorbidities. At the same time, we noticed an increased rate of urinary colonization in patients associated with diabetes, hypertension, and chronic kidney disease. Conclusions: The prevalence of urinary colonization in patients with double J catheters was 29%. The colonization of the JJ catheters, as well as the association with chronic diseases, such as diabetes, hypertension, and CKD (Chronic Kidney Disease), show an increased risk of urinary colonization.*

Keywords: *double J catheters, obstructive urolithiasis, urinary colonization, risk factors*

INTRODUCTION AND OBJECTIVES

Urolithiasis is a frequent pathology; in developed countries, its prevalence in the general population has tripled in the last 40 years. Acute Renal Colic is common in the Emergency Department, and approximately 6% of these require hospitalization due to subsequent complications [4,8].

Today we have multiple urological treatments available: extracorporeal lithotripsy, rigid or flexible ureteroscopy, and percutaneous nephrolithotomy, and their results can be improved by expulsive medical therapy and posturotherapy. Minimally invasive procedures have become common practice in urology departments, being very effective and having the advantage of rare post-procedure complications.

The method of treatment of urolithiasis depends on multiple factors: the patient's morphology and comorbidities; the size, chemical composition, and location of the calculus, urinary tract anatomy, and/or other possible complications.

In complicated urolithiasis with complete obstruction and/or secondary infection, an endo-prosthesis may be required temporarily, whatever the surgical method subsequently approached.

An ultrasound-guided percutaneous nephrostomy would be performed in case of physiological urinary drainage failure [3,6].

The urinary tract is normally sterile, except for the distal portion of the urethra. Urinary tract infections can occur through different mechanisms, but the contamination is ofte006E endo or extra-luminal. The use of catheters favors the colonization process by the means of an extracellular polysaccharide matrix to which germs adhere, resulting in a biofilm that will protect them from both defense mechanisms and microbial agents [2,5].

New methods have been constantly seeking to decrease the colonization rate of ureteral catheters by using different materials (silicone, polyethylene, polyurethane, biodegradable materials) or by covering the catheters with thin protective films (heparin, silver, polytetrafluoroethylene, antibiotics) [7].

Urinary colonization, unlike bacterial urinary infections, does not indicate systematic antibiotic treatment, even if the patient has an inserted catheter or not, is diabetic, elderly, with a neurological bladder. In special situations, antibiotic treatment is justified:

- if urinary colonization is a risk factor for morbidity and mortality: neutropenia, immunosuppressed, pregnant women;

- pre-operative: surgery and urological examinations;

- patients with joint, vascular, or cardiac prostheses that require invasive investigations;

- epidemic with multiresistant bacteria in a hospital unit.

Over time, many studies have been conducted on urinary infection and colonization [1], without looking for their prevalence amongst JJ ureteral catheter carriers, regardless of indication, comorbidities, or complications.

The objectives of the study were to identify possible risk factors of urinary colonization in patients with double J catheters inserted for complicated urolithiasis.

MATERIALS AND METHODS

This descriptive, retrospective study, was carried out in the Urology Department of the "Dr. Carol Davila" University Central Emergency Military Hospital, between January 2020 and January 2022.

The batch included 168 patients with obstructive urolithiasis who were inserted with double J ureteral catheters, regardless of sex, age, comorbidities, and complications.

Patients who did not agree to be included in the study, pregnant women (there is no integrated Obstetrics - Neonatology department within the Central Military Emergency University Hospital "Dr. Carol Davila"), and patients for whom transfer to other departments was necessary were excluded.

Preoperative biochemical evaluation and bacteriological examinations (urinary and JJ catheter sampling) were performed on nonselective media (BCP = bromocresol purple lactose and CLED = cystine – lactose – electrolyte – deficient).

It has been used as the standard method of insertion of JJ ureteral catheters - retrograde endoscopically under spinal anesthesia.

The following parameters were studied:

- sex and age;

- laterality of the catheter: right/left, both;

- associated comorbidities: general (high blood pressure, diabetes), reno-urinary (renal malformations, adenoma/ prostate cancer, bladder cancer), or immunosuppressed (grafts, Covid 19, other cancers);

- type and location of urinary infection: asymptomatic/ symptomatic, low/high;

- type and conditions of insertion of the ureteral catheter: emergency/appointment;

- indwelling time of the catheter: under/over 30 days;

- occurrence of post-stenting complications: calcifications, hematuria.

RESULTS

The study group included 168 patients, of which 101 men (60%) and 67 women (40%) with a sex-ratio M/W of 1.50. Urinary colonization is more frequent in women, 46% (31 patients) compared to 31% in men (31 patients), possibly explained by the shorter urethra in women and the proximity of the digestive flora.

The average age of the patients was 59 years, with extremes of 19 and 85 years.

The indication for the double J catheter insertion was ureteral-hydronephrosis, bilateral in 39% of cases (66 patients) and unilateral in 61% of cases (102 patients).

The distribution, from the point of view of stone laterality, was: 28% on the right (47 patients) and 72% on the left (121 patients); 52% pelvic ureteral stones (87 patients) and 48% pyelitic lithiasis (81 patients).

Many patients associated other comorbidities too (Table 1).

Table 1: Associated comorbidities with obstructive urinary lithiasis

Comorbidities	Number of patients	Rate [%]
High Blood Pressure	55	33
Diabetes	39	23
Obesity	27	16
Covid 19	18	11
Adenoma/Prostate cancer	28	17
Bladder cancer	10	6
Other cancers	7	4
Recurrent Urinary Tract Infections	49	29
CKD	32	19

Monitoring the bacteriological profile of the urine for the group of 114 patients with non-colonized catheters revealed that only 19 patients (17%) presented subsequent urinary colonization, and 83% (95 patients) remained non-colonized.

Amongst patients with colonized catheters (54 patients), only 12 (22%) remained bacteriologically negative throughout the study, regardless of comorbidities or associated complications (Table 2 and Figure 1).

The ureteral catheters colonized have a primary contributor to the pathogenesis of urinary infections, increasing their occurrence, regardless of sex, age, and comorbidities of patients in our batch.

Table 2: Urinary bacteriological profile depending on the JJ catheter bacteriological profile

Group	Number of patients	
	Urinary colonized	Urinary non-colonized
Non-colonized JJ catheters	19 (17%)	95 (83%)
Colonized JJ catheters	42 (78%)	12 (22%)

Figure 1: (a) Distribution of urinary colonization in patients with colonized catheters; (b) Distribution of urinary colonization in patients with non-colonized catheters

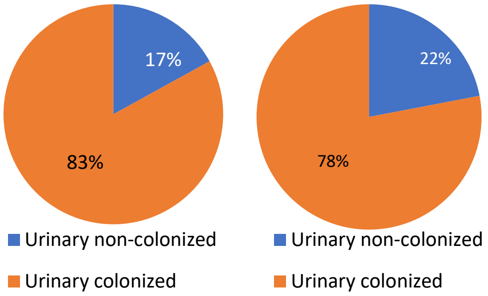

From a bacteriological point of view, different germs were identified, with the predominance of Escherichia Coli, and Klebsiella pneumoniae, both, at the urinary level and in the ureteral stents (Table 3).

We studied the urinary bacteriological profile in patients with urolithiasis and chronic diseases such as Diabetes, High Blood Pressure and CKD regardless of the ureteral catheters' bacteriological profile (Table 4).

Table 3: Germs identified at urinary and ureteral catheters level.

Bacterial germ	Number of patients with urinary colonized	Number of patients with JJ catheters colonized
Escherichia Coli	13	18
Klebsiella Pneumoniae	10	8
Enterococcus Faecalis	8	9
Enterobacter Cloaecae	1	2
Serratia Marcescens	1	0
Staphylococcus Hominis	3	0
Staphylococcus Aureus	1	6
Others	5	5

Table 4: Urinary profile of patients with associated chronic diseases

Associated chronic diseases	Number of patients	Urinary Colonization	
		Positive	Negative
Diabetes	39	22 (57%)	17
High Blood Pressure	55	26 (47%)	29
CKD	32	16 (50%)	16

In patients with chronic diseases such as diabetes, high blood pressure, and CKD we noticed an increased risk of urinary colonization regardless of the bacteriological profile of JJ catheters.

We noticed a higher rate of acute kidney failure in patients with obstructive urolithiasis associated with diabetes, high blood pressure, or chronic kidney failure, probably determined by the presence of secondary angionephrosclerosis.

It is already known that the immunosuppressed, pregnant women, alcoholics, and the elderly have a higher risk of bacterial colonization (urinary and catheters) regardless of associated comorbidities**.**

CONCLUSIONS

Double J (ureteral) catheter implants represent a minimally invasive alternative for deriving the upper urinary system to preserve the renal function, in case of acute or chronic, intrinsic or extrinsic obstruction, regardless of its type.

The study shows that 29% of the patients with urolithiasis carrying double J catheters have urinary colonization.

The colonization of double J catheters, regardless of the amount of time since they were inserted, plays an important role in the pathogenesis of urinary colonization.

The prevalence of urinary colonization increases with the indwelling time and especially in the case of emergency insertion of ureteral catheters.

Chronic diseases, such as Diabetes, High Blood Pressure, and CKD, are associated with increased risks of urinary colonization, requiring strict bacteriological monitoring.

Complications related to the indwelling of the double J catheter are rare and occur especially in the case of long-term indwelling (more than 30 days) and disappear with its ablation.

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ORIGINAL ARTICLE

Survey the Effect of Educational Intervention Based on Mobile Short Message Service (SMS) on Self-Care in Patients with Type 2 Diabetes Referring to the Diabetes Clinic in Khorram Abad City

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Abstract: *Background: Self-care behaviors are very important to control type 2 diabetes. The current study was conducted aiming at determining the effect of the educational intervention based on mobile short message service on the self-care behaviors in patients with type 2 diabetes in Khorram Abad. Methods: This study was a semi-experimental study on 191 patients with type 2 diabetes (45 cases and 45 control). Data collection tools included a demographic information form, International Physical Activity Questionnaire, Self-Care Scale, and Self-Efficacy Scale. Three to four educational messages were sent daily through mobile phones as an educational intervention. Data were analyzed with SPSS V24 at a significance level of 0.05. Results: The data analysis indicated that the average score of self-care, self-efficacy, and blood sugar was significantly higher in the case group than in the control group before and after the intervention (P<0.001). However, in terms of the physical activity variable, this relationship was not statistically significant in the case and control groups. Conclusion: Educational interventions to empower diabetic patients by strengthening their self-care can be an effective way to improve the health of diabetic patients. Therefore, it is suggested to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.*

Keywords: *self-care, self-efficacy, physical activity, blood sugar, type 2 diabetes*

INTRODUCTION

Diabetes is the most common disease caused by metabolic disorders, which is mainly characterized by a chronic increase in blood sugar and disturbed carbohydrate, fat, and protein metabolism. In general, diabetes is a multifactorial disorder diagnosed by a chronic increase in blood sugar or hyperglycemia (according to diagnostic criteria) and is caused by a disorder in insulin secretion or function or both [1]. Retinal damage and blindness, peripheral neuropathy, leg pain, stroke, peripheral vascular problems, end-stage renal disease, and limb amputation are among the most serious complications of diabetes [2]. The most common type of diabetes is type 2 diabetes, which accounts for approximately 90-95% of diabetes cases and is usually associated with metabolic disorders, weight gain, and physical inactivity [3]. About 422 million people worldwide suffer from diabetes, the majority of whom live in low- and middle-income countries, and 2019, diabetes was introduced as the direct cause of 1.5 million deaths [4]. According to the International Diabetes Federation (IDF), the number of diabetic patients will reach 439 million people in 2030, and the prevalence of diabetes in developing countries will be 69% [5]. More than 3 million people with diabetes live in Iran [6]. By 2030, Iran will be one of the most prevalent regions in the world in terms of diabetes [7]. Paying attention to the chronic and costly nature of this disease for the general health of society and the creation of a large financial burden, it is essential to pay serious attention to this problem and its consequences [8]. According to the results obtained from the studies, the long-term complications of this disease, including neuropathy, nephropathy, and retinopathy can be prevented or at least the occurrence of these complications can be postponed if blood sugar is controlled [9, 10]. Many studies have found physical activity and exercise to be effective in reducing blood sugar levels and controlling diabetes [11,12]. The World Health Organization considers education to be the basis of diabetes treatment. Therefore, self-care is one of the important topics of diabetes treatment. Self-care is a learned regulation function in humans, which is based on the ability of people to perform care actions on themselves [13]. It includes the behaviors of following a proper diet, taking medicine on time, self-monitoring blood sugar, performing regular physical activity, and physical care of the feet [14]. Studies show that 67% of patients with type 2 diabetes do not monitor their blood sugar according to the recommendations. Also, only 19-30% of diabetic patients use the recommended physical activities and compliance with nutritional behaviors in these patients is 65%, and only 7% of diabetic patients fully adhere to all aspects of self-care behaviors [15,16]. Self-efficacy is also known as a strategy to improve self-care in diabetic patients, which results in self-confidence and adherence to care recommendations to control blood sugar in these patients [17].

Nowadays, to follow up and educate diabetic people, new communication systems are required to direct the focus of care and treatment from the clinic to the patient's daily life. Researchers have pointed out the use of mobile phone service as a method of education and follow-up in chronic diseases, that short message is one of the main functions of mobile phone in the education of diabetic patients [18,19].

Considering the importance of self-care in controlling the blood sugar of diabetic patients and the value of self-efficacy as one of the self-care approaches, it seems necessary to design educational interventions on the platform of mobile phones and in the form of short messages as a relatively new educational method in controlling the disease of diabetics. Thus, the present study was conducted to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad.

MATERIALS AND METHODS

Population of study

The present study was a semi-experimental intervention research with a pre-test and post-test design. The sample size was specified as 90 people who were selected from the research population of patients with type 2 diabetes referring to the diabetes clinic in Khorramabad, Lorestan province. The participants were selected according to the inclusion criteria using random sampling. Then, they were divided into case and control groups using a simple randomization method (45 ones in each group).

Inclusion and Exclusion Criteria

The inclusion criteria include informed consent to participate in the research, having a minimum level of literacy and understanding of the content sent through text messages, having a fixed personal mobile number to receive educational SMS, lack of any serious diabetic complication, and not having special diets because of specific diseases or physical problems limiting physical activity. Exclusion criteria include suffering from serious complications of diabetes during the study, suffering from diseases that limit physical activity or nutrition, and the patient's unwillingness to receive educational messages.

Questionnaires

Data collection tools in this study included a demographic information form, International Physical Activity Questionnaire (IPAQ), Self-Care Scale developed by Tubert, and Self-Efficacy Scale. Demographic information included: age, height, weight, gender, educational level, employment status, marital status, and type of treatment. The IPAQ with seven items measured the duration of physical activity in the last week in four categories: moderate physical activity, intense physical activity, walking, and sitting activities. According to the scoring protocol in this questionnaire, the intensity of physical activity based on the ratio of energy consumption in terms of metabolic equivalent of task (MET) in the last seven days is classified into three levels: low, medium, and high. It was proposed by the World Health Organization and the Centers for Disease Control and Prevention in 1998 to measure the physical activity of people aged 15 to 69 years and its validity and reliability have been confirmed in 12 countries worldwide, including Iran [20,21]. A self-care questionnaire was used in this study that included 15 items in the areas of diet compliance, physical activity, blood sugar monitoring, insulin injection or diabetes pill consumption, foot care, and smoking, which was translated and standardized by Hamdzadeh [22,23]. The self-efficacy questionnaire, which is one of the constructs of the standard Health Belief Model Questionnaire, validity and reliability of which have been confirmed [24].

Data collection

After presenting a letter of introduction from the Iran University of Medical Sciences and obtaining the necessary permits, the researcher referred to a diabetes clinic in Khorram Abad and selected 90 people from among 200 eligible people using random sampling. People who were willing to participate in the study and met the inclusion criteria were assigned to the case and control groups (45 in each group) using a simple randomization method. After random allocation, blood sugar samples were taken in all groups four days before the start of the intervention. In the first session, the researcher explained the purpose of the meeting to the participants and written informed consent was obtained. Then, the personal information form and questionnaires were completed by the case and control groups. The case group received an educational program in the form of short educational messages via mobile phone, for four weeks and on average, at least three to four messages per day related to the educational content, including exercise, self-monitoring of blood sugar, diet, insulin therapy, medication use, and foot care. For example, short messages about physical activity and blood sugar monitoring were "for people with diabetes, walking is the best exercise" or "it is better for people with diabetes to measure their fasting blood sugar after waking up". To improve self-efficacy, encouraging messages about adherence to self-care were used. Finally, after two months, the same questionnaires (demographic, physical activity, self-care, and self-efficacy scales) were distributed and completed by the participants, and the blood sugar levels of the two groups were measured again. During this period, the control group did not receive any educational intervention. However, to comply with the ethical principles, an educational CD containing self-care training was given to the control group after completing the research.

Statistical Analysis

The data of the two groups before and after the intervention were entered into SPSS version 24 software and analyzed using Wilcoxon, Mann-Whitney, Chi-square test, independent t-test, and paired t-test at a significant level of 0.05.

RESULTS

The participants' average age was 51.01 ± 0.967, and the average height and weight were 164.57 ± 1.031 and 75.69 ± 1.101, respectively.

Before the implementation of the educational program, the case and control groups were matched in terms of demographic variables, including age, height, weight, gender, education, employment status, marital status, and type of treatment, and no statistically significant difference was observed between the two groups (Table 1).

Table 1: Comparison of the absolute and relative frequency distribution of demographic variables in case and control groups

Variable		Case		Control		P value
		No.	%	No.	%	
Gender	Female	31	69	26	57/7	0.796
	Male	14	31	19	42/2	
Employment	Unemployed	5	11	2	4	0.686
	Housewife	26	58	24	53	
	Self-employed	9	20	9	20	
	Public	5	11	10	22	
Education	Illiterate	5	11	4	9	0.479
	Primary school	17	38	10	22	
	Secondary school	9	20	5	11	
	High school	4	9	11	24	
	High school diploma	4	9	10	22	
	Higher education	6	9	5	11	
Marital status	Single	2	4	1	2	0.800
	Divorced	1	2	0	0	
	Widow	9	20	5	11	
	Married	33	73	39	87	
Type of treatment	Insulin	21	47	21	47	0.920
	Oral medication	20	45	22	49	
	Diet	4	9	2	4	
	Exercise	0	0	0	0	

There is no statistically significant difference in terms of the average score of physical activity, self-care, self-efficacy, and blood sugar between the case and control groups before the intervention, while after the intervention, the average scores of these variables, except for physical activity (P=0.8), was significantly higher in the case group compared to the control group (P<0.001). In the control group, before and after the intervention, no significant difference was observed in the average scores of physical activity, self-care, self-efficacy, and blood sugar (P>0.05) (Table 2).

The blood sugar level in all patients with different treatment methods (insulin, oral medication, diet) in the case group reduced after the educational intervention compared to before intervention, except for the patients who only used exercise to control their blood sugar (Table 3).

Table 2: Comparison of the average scores of physical activity, self-care, self-efficacy, and blood sugar, before and after the intervention in case and control groups

Scale	Group	Case	Control	p-value
		Mean ± SD	Mean ± SD	
Physical activity	Before intervention	677.5±954.938	307.5±461.75	0.6
	After intervention	903.75±973.125	655±925.625	0.8
	p-value	0.8	0.4	_
Self-care	Before intervention	2.31±0.813	2.38±0.844	0.12
	After intervention	3.38±0.688	2±0.906	0<0001
	p-value	0<001	0.22	_
Self-efficacy	Before intervention	2.7±0.4	2.7±0.3	0.15
	After intervention	3.7±0.7	2.7±0.7	0<0001
	p-value	0<001	0.286	_
Blood sugar	Before intervention	180±80.5	185±51	0.8
	After intervention	170±87.5	180±70.5	0<001
	p-value	0.009	0.491	_

Table 3: Comparison of blood sugar scores before and after the educational intervention in the case group

Method of treatment	Blood sugar level before the intervention	Blood sugar level after the intervention	Level of change
Insulin	227.09	217.92	-9.17
Oral medicine	173.48	170.65	-2.83
Diet	181	180.44	-0.56
Exercise	194	194.5	0.5
Medium	193.89	190.88	-3.02

DISCUSSION

The present study was conducted aiming to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad. The research findings suggest that the educational intervention through mobile phone short messages improved self-care in the case group, which is consistent with findings by Abaza et al. and Agbede et al. on the effect of the intervention based on mobile phone short messages in the self-care of diabetic patients and the findings by Yari et al. and Rahnavard et al. concerning SMS-based and self-care education of diabetic patients [25-28]. Krishna et al. reported that technologies such as mobile phones and text messages, which are currently part of people's daily lives, have the potential to influence self-care and improve people's health [29]. The approach of health education through SMS to promote self-care in diabetic patients is more welcomed than traditional approaches that require reading pamphlets or attending lectures [25,30].

In this study, self-efficacy also there was a significant difference in the case group in terms of self-efficacy after the educational intervention. Katz and Nordwall and Peymani et al. also found that interventions based on mobile phone messages improve self-efficacy and self-care [31,32]. Similar studies regarding the self-care of diabetic patients also indicate a significant difference in the average score of self-efficacy and self-care in the case group compared to the control group after the educational intervention. These studies are consistent with our work in that increasing self-efficacy causes the adoption of self-care behaviors in diabetic patients

[33,34]. Self-efficacy is an important prerequisite for behavior change [35]. An individual with low self-efficacy is less likely to make an effort to perform a new health behavior or to change the behavior he is used to [36]. Researchers believe that self-efficacy has a suitable framework for understanding and predicting and patient's commitment to self-care behavior in the treatment of diabetes [37].

The results of the present study showed that education through mobile phone short messages does not affect increasing physical activity in the case group, which is in line with the findings by Bakhshian et al regarding the effect of education through mobile phone short messages on self-care of patients with type 2 diabetes [38]. It is also in agreement with the findings by Kim et al. on adherence to diabetes control recommendations [39], while our findings are not consistent with the study of Paul et al. regarding the use of software that can be installed on mobile phones to improve the amount of daily walking in patients with a history of stroke [40] and the study Martha et al. concerning mobile phone SMS intervention on diabetes self-care activities, cardiovascular disease risk awareness, and food choices among type 2 diabetic patients [41]. This discrepancy can be due to the lack of sports facilities and parks in the current study or the addition of chronic diseases to the patients participating in this research, as well as social and cultural factors that prevent people from doing physical activities in public environments. In the present study, since the number of women is more than men, the problem of social and cultural factors could be more marked.

The use of mobile phone messages in the current study showed a significant positive effect on the reduction of blood sugar in the case group after the educational intervention, which is consistent with the findings of similar studies in this field [42,43,44]. In patients suffering from diabetes, education is as valuable as medicine, exercise, and diet, because the treatment will never be effective unless the patient is well aware of the nature of his disease and takes positive steps to deal with it.

CONCLUSIONS

The results of this study demonstrated that educational interventions based on mobile phone short messages can be effective in enhancing self-care in patients with diabetes. Educational interventions aimed at empowering diabetic patients by strengthening self-care in them can be an effective way to improve the health of patients with diabetes. Therefore, it is recommended to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research.

The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

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Authors' contribution

All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Vice President of Research and Technology of Iran University of Medical Sciences with code IR.IUMS.REC.1397.501. Informed consent was obtained from all individual participants included in the study before conducting.

Consent to publish

All participants consented verbally to the publication of the interview data.

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ORIGINAL ARTICLE

Peculiarities of Military Values of Combatants with Signs of Post-traumatic Stress Reactions and Moral Traumas

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Abstract: *According to the results of the study, there were determined the peculiarities of military values of servicemen who participated in hostilities and had signs of PTSD and moral trauma. The study included 397 servicemen (aged 20 to 55 years) with combat experience. The study showed that PTSD and violation of moral normativity were closely related to unfavorable social conditions of performing combat tasks. Unlike PTSD, the moral normativity indicator was less tightly related to the immediate threat to life. The structure of values of the servicemen had no signs of PTSD as well as no violation of moral normativity consisted of factors that indicated the value of teamwork, responsibilities for decisions and actions made based on ideas related to loyalty to the oath and the Motherland, resistance, and professionalism. For the servicemen who participated in hostilities who had violations of moral normativity, it was more important to have the ability to act following their conscience, not to lose dignity, and to remain honest in the faces of their brothers than to have professionalism, loyalty to the oath and the Motherland.*

Keywords: *values, violation of moral normativity, serviceman, combat experience, post-traumatic stress disorder*

INTRODUCTION

The topic of the moral suffering of a serviceman who participated in hostilities had always been acquiring relevance when there occurred armed conflicts. However, after the Second World War [1], Wars in Vietnam [2], Korea [3], Iraq, and Afghanistan [4-5], etc. the topic of moral suffering was largely replaced by the study of psychiatric disorders of servicemen and, in particular, the disorders of combatants. However, the practice of providing psychiatric and psychological assistance showed that the approaches and activities proposed to overcome the symptoms of PTSD were ineffective to overcome the moral injury of the combatant. Thus, Gray, Schorr, Nash, and others [6], Griffin, Purcell, Burkman, and others [7], Maguen, Lucenko, Reger, and others [8] indicated that there was not any confirmed treatment for mental health disorders for today.

Thus, the construct of moral trauma was offered to describe the suffering that some veterans experienced while carrying out the actions during the fight that violated their belief in their kindness and benevolence of the world [9], and could undermine confidence in themselves, others and the world in general [10]. It gave the possibility to identify them as potentially traumatic experiences which differed from the traumas which were based on fear and were related to post-traumatic stress disorder. Thus, Bryan, Roberge, Leifker, and Rozek [11] found out that the servicemen, who faced a life-threatening injury and developed post-traumatic stress disorder, had the profile of symptoms that primarily included loss of memory, nightmares, memories, and exaggerated reaction concerning fright. Griffin et al. and Williamson et al. suggested that the profile of symptoms of those who underwent moral damages more often included a higher level of guilt, anger, shame, depression, and social isolation [12-13].

Frankfurt and Frazier believed that the study of moral damage had to occur in three directions: a) the definition, prevalence, and potential correlation of actions (e.g., military training, leadership, combat actions, and personality); b) the relationship between actions and syndrome of moral trauma (e.g., demoralization and self-injuring), and c) mechanisms of the genesis of moral trauma (e.g., shame, guilt, social isolation, and self-disposal) [9].

Joseph Wiinikka-Lydon pointed out that the nature of moral damage would be misunderstood if the determination of moral injury did not take into account the relationship which provoked the appearance of damage, so due to his words it was advisable to determine moral injury as the injury that occurred among people within moral ecology of wartime considering it to be a form of intersubjectivity [10].

Shay highlighted that the army could be considered a moral structure. The general expectations and values of the army formed the moral universe, which servicemen considered "legitimate", "natural" and "obligatory for themselves" [14]. Thus, the morality of the servicemen was tightly related to the military organization and depended on it, close to the fact that the army could make a serviceman ready to risk his/her own life. Due to the specific and strong moral connection between the serviceman and the army, according to Shay, its violation could have catastrophic consequences.

Williamson, Greenberg, and Murphy in their study determined that moral injury had negative consequences for psychological well-being. Among the examples of potentially morally injurious events (PMIE), which caused moral trauma and were described by British military veterans in the course of their study – bad treatment of civilians or hostile combatants, obtaining an order to violate the rules of combat, and disrespect for corpses [15].

It was quite clear that having such reasons, veterans did not readily resort to the discussion of the experience, which was often used in PTSD. Thus, Shay pointed out that civilians would rather prefer outspoken stories about injuries, as that could lead to doubts related to their moral beliefs and "could bring them back and disorient them" [14]. Although according to Shay, this attitude was understandable, as it came from the moral construction of a "normal human life" [14], he emphasized that it could interfere with treatment.

British researchers [15] found out that even discussing PMIE with friends, colleagues, or family members was considered catharsis; veterans did not report that it helped to solve their moral dissonance. Veterans did not consider social support to be a way to solve problems. The study by Williamson with colleagues showed that the solution of moral dissonance that arose in the sphere of value was described by veterans as a key turning point and led to a decrease in their emotional stress and improvement of daily functioning [15]. These researchers considered it quite promising to overcome the consequences of moral injury by finding ways to resolve the conflict between systems of values for example, military values against civilians), as well as conflicts within the system of values (for example, the conflict between military moral obligations such as respect for civilian life and combatants of opponents and respect for the value of colleagues’ protection and successful mission performance.

Overcoming a moral conflict that arose was also important because the moral conflict, which was associated with a particular event, could generate fundamental moral conflicts that went beyond this event [16].

Describing existing studies of the category "moral trauma", it was necessary to note that from the very beginning of its research, the talk was about moral conflict, conflict in the sphere of values, but they were preceded by the studies conducted at the beginning of 2000s related to the peculiarities of value orientations of servicemen who had signs of PTSD after deployment.

Thus, almost decades ago, German researchers Zimmermann, Firnkes, Kowalski, Backus, Siegel, Willmund, and Maercer, worried that empirical studies of the peculiarities of valuable orientations of servicemen who had post-traumatic disorder after their deployment were very limited in time; the factors which were related to the deployment provided ideal scientific conditions” [17]. The studies which were available at that time concerning post-traumatic symptoms and personal values were devoted to the victims of crimes and people with adaptation disorders after critical life circumstances [18].

However, having formulated the topic of the study devoted to the values of the servicemen who had post-traumatic stress disorders due to deployment-related servicemen P. Zimmermann, Firnkes, Kowalski, Backus, Siegel, Willmund, and Maercer focused on personal values and did not pay attention to specific military values.

Almost at the same time as there was formulated the problem of value orientations by servicemen with signs of PTSD, there occurred studies were based on the study of values of servicemen who survived traumatic events. The studies were related to the differentiation between post-traumatic stress disorder and moral injury [16].

It could be confidently said that the study of the sphere of values and mental traumatization of servicemen became one of the reasons for reviving interest in the topic of moral injury of servicemen.

METHOD

Participants and Procedure

The presented article research had a fairly simple plan with a minimum of used psychodiagnostic techniques and groups, which was caused by the peculiarities of its conduct. The study was conducted within the frames of the program of restoration of servicemen who participated in hostilities. The examination was carried out before the rehabilitation to determine the depth of traumatization and peculiarities of the state of servicemen. The study had a searching basis and was intended to formulate problems important for the development of effective programmers related to the prevention of psychological traumatization of combatants.

The study was conducted in 2022. The study included 397 servicemen of combatants. The studied group consisted of officers – 29.84% (from a junior lieutenant to a colonel) and contract servicemen and mobilized – 70.16% (from a private to a senior warrant officer). The age of the studied participants ranged from 20 to 55 years.

Using the results obtained according to the methods "Maladjustment" and "Mississippi Scale for Combat-Related PTSD", a sample of studied servicemen who participated in hostilities was divided into three groups: group 1 – servicemen who did not have signs of maladjustment and PTSR – 277 individuals; group 2 – servicemen who had signs of violation of moral normativity (5 or more points according to the scale of VMN and more than 15 points according to the total indicator of maladjustment) – 59 individuals; group 3 – servicemen who had signs of post-traumatic stress reactions (78 or more points according to the Mississippi Scale) – 61 individuals. It had to be noted that all the servicemen, without any exceptions, who had signs of VMN servicemen also had signs of PTSD (78 or more points according to the Mississippi Scale). The third group included the servicemen who had signs of PTSD but did not have the peculiarities of VMN and maladjustment in general which exceeded the norms. Unfortunately, available tools did not give the possibility to divide the participants into groups without being specified in the second group "crossroad".

All procedures carried out in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants gave consent for their data to be used in this research.

Instruments and Measures

One of the reasons that there was a small number devoted to the studies of the "moral trauma" of servicemen was the lack of development of psychodiagnostic tools. In this study, as a criterion of moral trauma there was used a "violation of moral normativity" (VMN) – the scale of "maladjustment" techniques [19], which gave the possibility to differentiate individuals who had doubts about the expediency of using morals standards to regulate the relationships between individuals, as a result of which there was a loss of feeling of psychological well-being and signs of maladjustment arose.

The "maladjustment" technique was a rapid version of the "adaptability" technique [20], which had a long tradition of use in the Armed Forces of Ukraine. The "adaptability" technique was concluded by the Russian military psychologist Maklakov [20] to determine the adaptation potential of servicemen based on individual MMPI scales [21]. The developer reported the satisfactory psychometric characteristics of the concluded methodology. Since the beginning of hostilities in eastern Ukraine, the Maklakov methodology modification "Adaptability 200", formed by Chermyanin and Kondratiev, was widespread among military psychologists of Ukraine [22]. Unlike the previous methodology, it was supplemented by several issues that allowed us to distinguish "military-professional orientation", "tendency to deviant behaviors" and "suicidal risk" and to calculate "the level of resistance to combat stress". This technique consisted of 200 questions, so there was a need for a simpler and more reliable toolkit, which was the basis for the development of an express version of the methodology. The developed method of "maladjustment" [19] was aimed at the identification of specific and the most influential ability to adequately perform the tasks, and violations of adaptation potential. Thus, the technique had 4 scales, each with 10 issues – "violation of behavioral regulation", "probability of committing suicidal attempts", "violation of moral normativity", and "loss of communicative potential". There was the proposed calculation of the total indicator of "maladjustment". This calculation was simple and convenient and could be carried out in the field without any additional tools. Although the work with the technique involved the presence of trust in a psychologist, the technique had a scale of sincerity. The developers provided data on satisfactory psychometric indicators of the technique.

There was used the method "Mississippi Scale for Combat-Related PTSD" to determine the presence of post-traumatic stress reaction" [21]. At this time, it was one of the most common techniques used to measure PTSD symptoms of servicemen. The scale included 35 statements, each of which could be estimated under a five-point scale. The technique involved the process of distinguishing 3 levels – up to 77 points were normative indicators; 78-97 points were separate symptoms of PTSD; 98-145 points were the clinical variant of PTSD.

To determine the connection of violation of moral normativity with the action of combat stress factors, there was used the procedure of correlation analysis, and the method of "assessment of psychological trauma of combat experience of servicemen" by Doctor of Psychological Sciences Kolesnichenko [23] based on American methodology "Scales of assessment of combat experience" [24]. While developing the method there was taking into account the traumatic events and their intensity which was typical for the hostilities in Ukraine with the beginning of the Russian invasion in 2014. Unlike the "Scale of Evaluation of Combat Experience", this technique was a list of phenomena that reduced the trauma of the impact of combat stress factors. The technique had 5 scales that corresponded to 3 groups of combat stress factors and 2 factors that described internal and external resources to counteract stress factors. In the process of standardizing the technique, there was used regression analysis procedure, which gave the possibility to determine the contribution of each scale to acute stress disorder due to the action of combat stress factors. It was reported that the technique had satisfactory psychometric characteristics.

To determine the military values there was used a purposefully developed questionnaire.

At the end of 2021, there was developed a "questionnaire of determining the military values of servicemen of the National Guard of Ukraine" based on ideas related to the military values that existed in the armed formations of Ukraine, ethical codes of servicemen of European countries [25-26], historical information about the Cossack Ethical Code of the Warrior, availability of law enforcement and combat functions in the professional activity of NGU servicemen, a new experience of participating in the hybrid war and counteracting the numerically predominant opponent [27]. The questionnaire included a list of 11 generalized values including their content. The serviceman needed to evaluate the importance of values from this list using a scale in which 0 was the least important and necessary value for military service, and 10 was the most important and necessary value for military service. The scores had to be repeated.

In the proposed list for evaluation, there were generalized military values that united some values that described the experience of performing service and combat activity by servicemen:

1. To be physically brave – capable of controlling their fear, showing courage in situations that involved risks, injuries, and death; to be ready to give life for their Motherland as well as for their sworn brother.

2. To be morally brave – to find the strength to act correctly, and fairly, not to be indifferent to the injustice, grief, and sufferings of those whom they, being law enforcement, must protect; be human, humane, to be an example for imitation.

3. To be disciplined – to be guided by the legal requirements of the commander and the statutes; the execution of the commander's orders was the basis for the confidence of each member of the unit that would not be supplied, they would perform a part of their work accurately and in time, it was the basis for the coherence of the unit's actions, the basis for effective interaction with other units, and the basis for the unification of forces to achieve a common result.

4. To be self-disciplined – to perform their work without reminders, to meet high standards of professionalism and morality, so that their sworn brothers could trust them and rely on them, know that they would not fail, would not betray, and would not disappoint; to make the right choice and to be able to control their fear.

5. To be honest – one that could be trusted by fellow people; did not hide their shortcomings from sworn brothers, but corrected them, constantly professionally improving and developing. If necessary, they could be able to ask and receive help from their sworn brothers to get rid of defects, shortcomings in personal development, and vocational training. To report the conflicts of interest, about changes in their physical or mental state, so that the commander and sworn brothers could take this into account while performing the task, they had to know to what extent they could rely on the servicemen. Not to perform or hide the illegal and immoral actions of the sworn brothers, as it could potentially be the basis for blackmail, distrust, and a sense of guilt and, as a consequence, it could destroy the team, jeopardize the lives of other brothers and fail the accomplishment of the task.

6. To be worthy – to meet moral and professional requirements as well as to meet the standards of the profession. To follow their conscience and to follow the principles of Cossack chivalry. To improve constantly and professionally, and to acquire the necessary knowledge and skills required for the profession. To remain open to new professional experiences, not to dwell in their self-development, and to be self-critical.

7. To be a team member – to be able to rely on a sworn brother, to trust in a friend on weapons; be the one whom sworn brothers could rely on (competent, honest, faithful, disciplined, and predictable for a friend); respect sworn brothers and the commander, clearly obey the orders, as they were the basis for the unit control, the basis for concentrating the forces of the whole unit on the task and adjusting the forces of different units; to use humor to support and stimulate the development of sworn brothers, not to humiliate them; to prevent discrimination of comrades on weapons by nationality, race, and gender, etc.; to be an example and assistant for recruits. Being loyal to their unit did not mean

that they had to cover the illegal or immoral actions of the sworn brothers, as such actions made them and their unit open for manipulations and blackmail from the side of enemies. Their actions did not have to throw a shadow at their unit as well as at their sworn brothers. To be guided by the principle – "sworn brothers took the first place, then went the task (mission), and only after – their interests." Remember that military affairs were teamwork; it was effective when everyone did their part of the work completed.

8. To be faithful – to be faithful to the military oath – not to betray members of their unit; not to participate in any actions that could harm their weapons, not to provide any information related to the countries that were disloyal to Ukraine, not to express judgments that could devour the honor and dignity of the Ukrainian Army (National Guard), to harm its reputation; to be faithful to the Ukrainian people – to be faithful to the principles of independence, democracy, and protection of the rights and freedoms of the people of Ukraine; to be faithful to the traditions of the Ukrainian people, to imitate the traditions of Ukrainian Cossacks; to protect Ukraine and the way of life of the Ukrainians, to serve the interests of the Ukrainian people; to be faithful to themselves – not to betray themselves, their principles, to be reliable, integral, and predictable for sworn brothers (as a basis for effective interaction)

9. To be resistant – not to give up to the enemy, as long as there was strength and opportunities to resist themselves or as long as there was strength and opportunities to resist at least one of their companions on weapons; not to accept mercy from the enemy. Bravely face all the difficulties of service (not to complain, not to spread the information concerning household difficulties, and workload, as it could be used as a way of manipulation by opposing forces and of undermining the image of military service).

10. To be free – to take responsibility for their actions, to be capable of self-thinking and decision-making, to assert and maintain their point of view, to realize their values and principles, not to be manipulated, blackmailed, etc.

11. To be professional – to perform tasks at a high professional level, to constantly acquire and improve professional knowledge, skills, and possession of personal weapons; to maintain a physical form at a level that did not interfere with the tasks; to maintain their awareness of modern weapons, tactics, including those used by the enemy; to acquire and share professional experience with brothers, to be susceptible to the professional experience of others.

In 2022 there was published a study, which presented the peculiarities of the values of servicemen with different military-professional statuses [27].

Data Analysis

There was conducted the comparison of values in the studied groups using the Student's T-criterion. To determine the structure of the sphere of values there was used the procedure of factor analysis by Principal Components. The statistical analysis was performed using the SPSS 22 statistical package.

RESULTS

At the first stage of the study, it was necessary to consider the way how correlated psychological traumatization, the indicators of VMN (maladjustment), and PTSD with the factors of psychological traumatization of servicemen. The results of this stage of the study were presented in Table 1.

Table 1: Relationship between maladjustment and PTSD with the indicators according to the methodology "Evaluation of Psychological Traumatism of Combat Experience of Servicemen who participated in hostilities".

Scales	**Unfavorable social conditions for performing combat tasks**	**Specific combat conditions which become traumatic**	**The unfavorable social situation of participation in hostilities**	**Internal resources of resistance – professional and psychological training**	**External resources – friendship, respect**	**General indicator, traumatization of experience**
«Maladjustment»						
Violation of behavioral regulation	0.55***	0.29***	0.26***	-0.15**	-0.17**	0.40***
Likelihood of committing suicidal attempts	0.44***	0.13*	0.20***	-0.20***	-0.21***	0.41***
Violation of moral normativity	0.39***	0.16**	0.23***	-0.09	0.00	0.37***
Loss of communication potential	0.32***	0.14*	0.19***	-0.09	-0.27***	0.36***
General indicator of maladjustment	0.50***	0.22***	0.27***	-0.16**	-0.20***	0.42***
«Mississippi Scale»						
PTSD	0.57***	0.34***	0.26***	-0.18***	-0.26***	0.48***

*Note: *p≤ .05; **p≤ .01; ***p≤ .001*

As it could be seen from the proposed data, PTSD as well as violation of moral norms was the most closely related to "Unfavorable social conditions of performing combat tasks", such as the attitude of the population on the territories where there were the hostilities, media coverage of events, etc. However, unlike PTSD, VMN was weakly related to the direct threat to life – the number of shelling, bombings, encirclements, etc., which was described by the second scale of the methodology "Evaluation of Psychological Traumatism of Combat Experience of Servicemen who Participated in Hostilities" (r= 0.16, p≤ .01). Moreover, it was possible to highlight the data related to the relationship between the indicator of violation of moral norms (VMN) and the external resource that protected against traumatization – friendship, respect, attitude to the relatives – r=0.00. To some extent, they agreed with the presented data from a semi-structured interview with British researchers, who determined that social support was not crucial while overcoming moral trauma [15]. However, the relationship between the indicators of PTSD and "External resource", which was defined in this methodology as the support of family and friends, being statistically significant (r=-0.26; p≤ .001), was weak in terms of its density.

As could be seen, the VMN indicator was related to combat stress factors and factors that mitigated their impact, as well as moral trauma according to the existing descriptions of this psychological category. Therefore, it was considered to be possible to use the VMN indicator as one of the indicators of the presence of moral trauma in a serviceman, taking into account that currently there was practically no psychodiagnostic toolkit for its determination.

The next stage of the study was a comparison of military values and average ranks in selected research groups. These results were presented in Table 2.

As could be seen from the provided above data, the hierarchies of military values were quite similar in all three groups. The leading place in them was occupied by courage, and the least important was "Will" and "Resistance". However, each group had its characteristics.

Such peculiarities of the first group included the absence of expressed peaks and depreciations in the hierarchy – the gap between the largest ("Moral courage" (6.09±3.09)) and the smallest ("Will" (3.50±3.29)) indicators in the hierarchy of military values was minimal in comparison with others in the groups Moreover, in this group the indicators of courage ("Moral courage" (6.09±3.09), "Physical courage" (5.77±2.99)) and "Dignity" (4.68±2.89) had the lowest indicators among the compared groups (the latest feature also had the lowest rank in the hierarchy in comparison with other groups), and the indicators of "Professionalism" (5.03±3.47) and "Loyalty" (5.73±3.13) were the highest (the latest feature also had the highest ranks in the hierarchy of values in comparison with other groups). It was described in detail in comparison with other research groups.

Table 2: Indicators of military values among servicemen who participated in hostilities and had signs of VMN, PTSD, and those who did not have any disorders which occurred under the influence of combat stress factors

Military value	Studied individuals						The significance of difficulties		
	group 1		group 2		group 3		t_{1-2}	t_{1-3}	t_{2-3}
	without any disorders		with signs of VMN		with signs of PTSD				
	average	rank	average	rank	average	rank			
Physical courage	5.77±2.99	2	6.06±3.19	2	6.41±2.63	2	0.62	1.660	0.66
Moral courage	6.09±3.09	1	7.39±2.59	1	6.52±2.91	1	3.37**	1.03	1.730
Discipline	5.03±3.11	6.5	4.56±3.48	8	5.30±3.07	6	0.96	0.62	1.23
Self-discipline	4.90±2.89	8	4.72±3.49	6	4.44±3.00	9	0.37	1.08	0.47
Honesty	5.12±2.89	5	5.67±3.07	4	5.52±3.08	4	1.26	0.93	0.26
Dignity	4.68±2.89	9	4.78±2.56	5	6.19±3.22	3	0.27	3.37**	2.65*
Teamwork	5.59±2.91	4	5.83±2.71	3	5.41±2.93	5	0.63	0.43	0.83
Loyalty	5.73±3.13	3	4.67±2.06	7	4.85±3.00	8	3.25**	2.05*	0.40
Resistance	4.52±3.04	10	4.33±3.03	9.5	4.15±2.32	10	0.44	1.07	0.38
Will	3.50±3.29	11	4.33±3.51	9.5	3.15±3.07	11	1.660	0.81	1.96*
Professionalism	5.03±3.47	6.5	4.22±3.17	11	4.93±3.34	7	1.740	0.21	1.18

*Note: 0p≤ .1; *p≤ .05; **p≤ .01*

In the second group, such military value as "Moral Courage" (7.39±2.59) was emphasized and significantly elevated above the others. According to this indicator, servicemen of this group differed from the first group at a statistically significant level (t1-2=3.37, p≤ .01) and at the trend level from the group with signs of PTSD (t2-3=1.73, p≤ .1). Other most important values of servicemen with signs of VMN included "Physical courage" (6.06±3.19), "Teamwork" (5.83±2.71), and "Honesty" (5.67±3.07). In this group, there was no expressed depreciation of any values (average scores did not fall below 4). However, in contrast to other groups, the lowest scores, which occupied the last 11th position in the hierarchy of values, in this group belonged to such military value as "Professionalism" (4.22±3.17). The peculiarities of this group included significantly lower "Loyalty" indicators than in the first group (4.67±2.06; t1-2=3.25, p≤ .01) and slightly higher indicators of "Will" than in the other compared groups (4.33±3.51; t1-2=1.66, p≤ .1 and t2-3=1.96, p≤ .05), which only in this group did not occupy the last position in the hierarchy of values.

Comparing the ranks of servicemen who had signs of VMN (group 2) and servicemen who did not have signs of maladjustment and PTSD (group 1), the following information had to be noted: servicemen of group 2 had significantly higher ranks of "Dignity" (5 vs. 9). However, these ranks were lower than those of servicemen with signs of PTSD (group 3) and significantly lower ranks of "Loyalty" (7 vs. 3) and "Professionalism" (11 vs. 6.5).

Forming a general ethical portrait of this group, it was necessary to point out that for servicemen who had VMN, the ability to act following conscience, not to lose dignity, and to remain honest in the faces of their fellow men was more important than abstract ideas about professionalism, and loyalty to the oath and the Motherland. The typical peculiarity of this group was a higher level of personal responsibility for their actions, and reluctance to shift them on the actions posed by the orders or by the irresistible circumstances.

Although among servicemen with signs of PTSD (group 3), courage, as in other compared groups, occupied the highest position in the hierarchy of military values; however, in this group the indicators of "Physical courage" (6.41±2.63) were slightly higher than in the others. Thus, the differences with the group without any signs of VMN and PTSD occupied the level of the tendency to statistical significance – t1-3=1.66, p ≤ 0.1. Moreover, in this group, the differences between "Moral Courage" (6.52±2.91) and "Physical courage" (6.41±2.63) were minimal, which suggested that there was no expressed differentiation of these concepts in this group. Among the three most important military values of representatives of this group, in addition to "Moral" and "Physical courage", was "Dignity" (6.19±3.22). Such a high position of this value (rank 3) and significantly higher average values (t1-3=3.37, p ≤ .01 and t2-3=2.65, p ≤ .05) were a defining (significant) peculiarity of this group. Moreover, in this group, in comparison with all other groups, the indicators of "Will" were the lowest (3.15±3.07), according to which they differed at a statistically significant level from the group with signs of VMN – t2-3=1.96, p ≤ .05.

Comparing the ranks, it had to be pointed out that this group, in addition to the already indicated position of "Dignity", which differed from other groups, had a lower rank of "Loyalty" than in group 1 (8 against 3) and higher rank of "Professionalism" than in group 2 (7 against 11).

Thus, for the ethical portrait of group 3, it was typical not to allow oneself to be suspected of cowardice, they demonstrated courage that was not significantly guided by ideals – the protection of the Motherland or loyalty to the oath – but which was guided by the fear of not meeting the expectations of others, the fear of being accused of cowardice; for them, it was extremely important not to lose a sense of their dignity (respect of others and self-respect).

Perhaps the fact that servicemen without signs of maladjustment and PTSD were characterized by a lower level of "Courage" and "Dignity" and a greater orientation to "Professionalism" and "Loyalty" made their behavior more flexible and less dangerous – they were not so dependent on patterns of behavior, did not aspire to "heroism" and conflict, they focused on professional actions and loyalty to the oath and to the Motherland, which did not only reduce their victimhood but also made them more adequate concerning combat situations.

To clarify the obtained results, Table 3 there was presented the data of the factor analysis (with the procedure of simplifying the factor structure) for a better understanding of the structure of the sphere of values of servicemen who did not have signs of maladjustment and PTSD.

This factorial structure described 90.58% of dispersion peculiarities.

The first factor had a share of 21.91%. In other factors, the share was approximately the same and varied from 17.44 to 16.82%. The content of the first factor was determined by such variables as "teamwork" (0.819) and "loyalty" (0.794). This factor was marked as the conscious value of teamwork, belonging to the team, and brotherhood. The share of this factor reaffirmed that servicemen were aware of the importance and value of teamwork, and were aware of their strength as a group formation.

Table 3: The structure of military values of servicemen who did not have signs of maladjustment and PTSD

Variables	Factors				
	1	2	3	4	5
Dignity	-0.027	-0.010	-0.079	-0.080	0.984
Teamwork	0.819	-0.140	0.272	0.179	0.087
Loyalty	0.794	0.307	-0.130	-0.174	-0.141
Resistance	0.085	0.113	0.961	0.045	-0.083
Will	0.072	0.957	0.114	0.070	-0.006
Professionalism	0.010	0.065	0.046	0.977	-0.081

The second factor (17.44%) was closely linked to the variable "will" (0.957). It was interesting, that the content of this factor specified the variable "loyalty" (0.307). This factor was interpreted as their responsibility for the decisions made and actions taken based on their own beliefs about loyalty (oath, home, brothers, etc.).

The content of the third factor, the share of which was 17.26%, was correlated with the variable "resistance" (0.961). Some clarification of the content was made by the variable "teamwork" (0.272). This factor was marked as an awareness of the importance to keep up (being resistant) so as not to let their sworn brothers down.

The fourth factor (17.16%) was almost entirely determined by the variable "professionalism" (0.977), the reverse pole of this factor indicated the variable "loyalty" (-0.174). This factor was marked as the ability to act in the interests of the case, despite personal affection.

The fifth factor (16.82%) correlated with the variable "dignity" (0.984), such variables as "loyalty", "resistance", "will", and "professionalism" had weak feedback with this factor. This factor was marked as the ability to consciously comply with the given standards and standards of the profession and morality, which implied the ability to develop them, openness to the new, and the ability to self-criticism – "the ability to meet the changing situation". The presence of this factor in the structure of military values gave the possibility to be appropriate to changes that occurred during crucial events such as war.

There was developed a four-factorial structure of military values of servicemen with signs of VMN and PTSR (see Table 4).

Table 4: The structure of military values of servicemen who had signs of violation of moral normativity

Variables	Factors			
	1	2	3	4
Physical courage	-0.021	-0.100	-0.095	0.916
Discipline	0.915	-0.074	-0.007	0.030
Self-discipline	0.876	0.008	0.095	-0.271
Honesty	-0.210	0.870	0.138	0.101
Dignity	0.084	0.860	-0.162	-0.324
Loyalty	-0.713	0.078	0.043	-0.446
Resistance	-0.369	-0.386	-0.727	0.110
Will	-0.143	-0.178	0.927	-0.049

The factorial structure presented in Table 4 described 83.96% of dispersion peculiarities. The first factor had the highest share – 29.02%. Its content was determined by the direct connection between the values of "discipline" (0.915) and "self-discipline" (0.876). The reverse pole of the factor determined the value of "loyalty" (-0.713). The inverse relationship with “resistance” (-0.369) clarified the content of the factor. This factor was marked as the ability to comply with the requirement or internal conditions. This factor was not connected with beliefs or with endurance. The talk was mostly about the "habit" to be reliable for others to perform the assigned tasks, but not about the actions committed following abstract ideals – love for the Motherland, and loyalty to the oath.

The second factor (21.24%) was determined by the variables "honesty" (0.870) and "dignity" (0.860). Its content was clarified with the peculiarity "resistance" (-0,386). Unlike the fifth factor in the structure of servicemen who did not have signs of maladjustment and PTSD, this factor indicated not the correspondence of the current situation, but the correspondence of the existing relationships to the desire to meet the concept of decency in relations. It was the idea to preserve someone’s decency in the relationship that regulated their activity.

The positive pole of the third factor (18.17%) was almost entirely determined by the variable "will" (0.927), which described the importance of their responsibility for the decisions which were made as well as for the committed actions. The negative pole of the factor, like the first two, was associated with "resistance", but the third factor was much denser (-0.727). It was likely that servicemen, with the violated moral normativity, were aware that the maintenance of discipline and decent behavior as well as taking on full responsibility for their decisions and actions reduced their ability to be resistant which led to moral suffering.

The content of the fourth factor (15.53%) was interesting, which indicated that "physical courage" (0.916) was achieved by representatives of this group at the expense (resource of inverse relationship) of such values as "loyalty" (-0.446), "dignity” (-0.324), and “self-discipline” (0.271). Showing physical courage on the battlefield, these servicemen tried to maintain a previously created image of themselves, as decent, reliable people they used to consider themselves. It was possible that namely these resourceful connections could lead to exhaustion and moral crisis – a war that required brave actions, "squeezed" a drop of their usual ideas about themselves as reliable and decent people.

The structure of military values of servicemen who had signs of PTSD were given in Table 5.

Table 5: The structure of military values of servicemen who had signs of post-traumatic stressful reactions

Variables	Factors			
	1	2	3	4
Physical courage	0.782	-0.205	0.242	-0.090
Moral courage	0.887	-0.210	-0.046	0.028
Discipline	0.194	-0.196	0.194	-0.697
Self-discipline	-0.246	-0.268	0.772	0.055
Dignity	-0.763	-0.304	0.075	-0.091
Teamwork	0.255	-0.225	0.109	0.806
Honesty	-0.357	-0.032	-0.804	0.155
Resistance	-0.192	0.815	-0.066	0.173
Professionalism	0.067	0.810	-0.141	-0.179

DISCUSSION

The analysis of the results gave the possibility to describe servicemen who did not have signs of maladjustment and PTSR, as they were able to meet the difficult situation of participation in hostilities. These servicemen were not guided by the distorted ideas of courage and did not try to prove to anyone that they were not cowards, but instead, they acted being guided by their ideas related to professionalism and loyalty to the oath and the Motherland, due to that they were less victimized and more appropriate to the combat situations. Probably, such characteristics of the sphere of values of these servicemen could be reflected at the personal level of view of the army as a unique "moral structure" described by Shay [14], which gave it a possibility to be integrated into servicemen not only to maintain mental health but also to sacrifice their lives. These data confirmed previously made conclusions concerning the importance of relevant secondary socialization (professionalization) of servicemen, as prevention of their psychological traumatization under combat conditions [28] (p. 125).

Unlike the servicemen who were integrated into the moral construction of the army, combatants with signs of VMN had less abstract ideas about loyalty and become hostages to their image of a decent person, which could be counted by their sworn brothers. They were aware that keeping this image under the conditions of the war was the path of suffering and consciously they followed it. Every manifestation of physical courage for these servicemen became a step that took them away from themselves – from the ideas of their honesty and humanity. And despite this, they did not try to transfer responsibility for the actions taken into insurmountable circumstances and did not try to justify themselves with the need to act by order. These data expanded the idea of moral conflict, as the basis for moral trauma, described by Williamson with colleagues [15].

The obtained results also demonstrated that for servicemen with signs of VMN, as one of the manifestations of moral injury, social support was not a resource capable of solving their problems. These data agreed with the British researchers who determined that social support was not crucial in overcoming moral injury [15]. Moreover, the existing data showed that the construct of "moral injury" was more related to the social context of the situation, than the immediate threat to life.

The obtained results in the group of servicemen with signs of PTSD were quite interesting. Their traumatization, as expected, was related not only to the social context of participation in hostilities but also to the immediate threat to life. However, it was an unexpected assumption of the greater number of victims of these servicemen in comparison with those who had no signs of maladjustment and PTSD. It could be assumed that these servicemen could be hostage to their ideas about the need to manifest courage during hostilities, it was extremely important for them that others did not suspect them of cowardice.

For these servicemen, participation in hostilities was a life on the verge of their ideas about their capabilities, it was a test. It could be assumed that they had "excessive" expectations from their participation in hostilities, the requirements of the profession of a serviceman, which contributed to their exaggerated response to the situation of participation in hostilities (greater courage, aggressiveness, suspicion, etc.) and, accordingly, to the higher level of exhaustion. And although the assumption of "excessive expectations" as a peculiarity of their greater victimization was only an assumption related to the possible mechanism of formation of psychological traumatization of a military serviceman who participated in hostilities, its formulation, and further testing were important for the development of effective PTSD prevention programs.

Nowadays it was quite traditional to study the changes in the relationship under the influence of PTSD, but mainly they are related to the civilian life of veterans. Thus, in particular, Caska-Wallace, Smith, Renshaw, and Allen investigated the impact of PTSD on the marital relations of servicemen [29], Ellon, Colvonen, Haller, Norman, and Angkaw determined that the severity of PTSD was the only significant sign. The topic of the relationship between servicemen and the peculiarities of PTSD in the military team and with the commander was less studied. Moreover, these and other studies did not determine the role of values as regulators of relationships that were changed as a result of the psychological traumatization of servicemen.

Harper, Stanley, Exline, Pargament, Fletcher, and Teng found out that betrayal perception was positively linked to post-traumatic stress disorder. They suggested that the mechanism of communication of a potentially morally injurious event (PMIE) and symptoms of post-traumatic stress disorder was social separation (“promiscuity” from Russian). The mentioned researchers emphasized that the study of this mechanism was extremely limited. In the secondary analysis, they were able to distinguish that the interaction between betrayal and perceived social support (another significant issue and family) gave the possibility to predict the symptoms of post-traumatic stress disorder [31]. In the conducted research it was found that such values as "loyalty" of servicemen with signs of post-traumatic stressful reactions had the opposite position in the hierarchy of military values in comparison with the servicemen who had no signs of PTSD. And although the format of the study did not give the possibility to conclude whether the "loyalty" factor contributed to the development of PTSD or, conversely, the acquisition of signs of post-traumatic stress reactions led to the depreciation of "loyalty", it was also possible to assume that "social disconnection" was an important mechanism for the development of PTSD symptoms and there was a need in further study of the role of value regulation in the implementation of this mechanism.

Nowadays there were widespread studies that demonstrated that the cohesion of the military team was connected with a lower level of severity of post-traumatic stress disorder [32]. The conducted research gave the possibility to formulate another problem of the research – psychologically injured servicemen could reduce the coordination of the military team and the coordination of its actions.

The given data demonstrated that the PTSD indicator had a statistically significant, but the not very dense connection with social support as an external overcoming resource. These and other results of the study gave the possibility to suggest that more promising for determination of the ways of prevention of psychological traumatization of servicemen was not the study of social cohesion of military teams, but the study of the peculiarities of professional socialization of servicemen in which the cohesion of the military team was a derivative characteristic. In light of this, it was quite possible to assume that the study of the content and process of internalization of military values, as a means of professional socialization of servicemen, and their acquisition of professional identity, could expand the idea of ways of preventing psychological traumatization of servicemen who participated in hostilities.

CONCLUSIONS

The conducted study demonstrated that PTSD and VMN indicators were most closely related to adverse social conditions available for combat task performance. Unlike PTSD, VMN was less densely related to a direct threat to life. Social support had no preventive effect on VMN. The connection of a factor that described social support with PTSD was weak but statistically significant.

The servicemen who participated in hostilities and had no signs of PTSD and VMN had a slightly lower level of indicators of the value “bravery” in comparison with other studied groups, while they had a higher level of “professionalism” than in other groups of the study. Their structure of values consisted of the factors that testified to the value of teamwork; responsibilities for decisions and actions made based on ideas related to loyalty to the oath and the Motherland; resistance; professionalism as the ability to act in the interests of the case, despite personal affection; and the ability to be appropriate to the changing conditions. Such peculiarities made their pain integrated into the "moral construction of the army" and more appropriate (adaptive) to the combat situations, where dominated teamwork and professionalism as opposed to unjustified risks and excessive reflection.

For the servicemen who participated in hostilities and had VMN, it was more important to have the ability to act consciously, not to lose dignity, and to remain honest in the faces of their sworn brothers than to have professionalism and loyalty to the oath and the Motherland. The typical peculiarity of this group was a higher level of personal responsibility for their actions, an aversive to transfer it to the actions by the order or too insurmountable circumstances. In the structure of their values, the leading place was occupied by the factors that described the ability to comply with the standards; to remain honest in front of their sworn brothers; to be quite responsible for their actions. These factors in the group of servicemen who had VMNs were related to the realization that they made them vulnerable in combat. The process of following them led to suffering. The need to manifest physical courage (as the ability to be aggressive against another person) "squeezed" a drop of their usual ideas about themselves as reliable and decent people.

The "excessive" courage of servicemen who had signs of PTSD was a consequence not only of the desire to be guided by the ideals – the protection of the Motherland or faithfulness to oath, etc. – but the consequence of the fear not to meet the expectations of others, the desire not to lose their sense of dignity (respect of others and self-esteem). In the structure of their values, a leading place was occupied with courage and professionalism. Both of these factors in the group of servicemen who had the symptoms of PTSD involved the attitude toward them as if they required actions on the verge of their capabilities. Other factors in the structure of their military values indicated that they had a sense of unity with their team – leadership of their ideas concerning the relevance of actions, which made them less controlled and unreliable for brothers-in-arms as well as commanders under the combat conditions.

The presented study was mainly directed to theoretical research, and its main results were the formulation of research problems that were important for the development of an effective program for the prevention of psychological traumatization of servicemen who participated in hostilities. Unfortunately, the used format of the study did not give the possibility to state whether the peculiarities of the sphere of values made servicemen sensitive to the action of combat stress factors and to the specific reaction concerning them in the form of PTSD or VMN (moral injury), or vice versa, changes in the sphere of values were the results of the formation of signs of PTSD and VMN by servicemen who participated in hostilities. Moreover, there was a need for further research to clarify the role of military values in the mechanism of PTSD and VMN formation. Similarly, it was required to study and verify the suggestion related to the greater number of victims of servicemen who, participating in hostilities, acquired signs of psychological traumatization (maladjustment and PTSD). It was also advisable to study the impact of the peculiarity of secondary (professional) socialization of servicemen and their acquisition of military-professional identity as a factor in the prevention of psychological traumatization of a serviceman under combat conditions.

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Authors’ contribution
Conceptualization, YaM. and O.K; managed the project, I.P.; research, K.M.; writing – original draft preparation, Yu.R., I.T., and R.G.; methodology, D.P. All authors read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
All procedures followed were by the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

Consent to publish
Informed consent was obtained from all subjects for inclusion in the study while maintaining their confidentiality. Written informed consent was obtained from all subjects to publish this paper.

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ORIGINAL ARTICLE

Symptom Severity from Posttraumatic Stress is Not Correlated to C-reactive Protein Level or Altered by Ketone Salt Supplementation

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Abstract: *This study aimed to determine if: 1) C-reactive protein (CRP) level correlated with PTSD symptom severity as measured by PTSD Checklist-5 (PCL-5), and 2) ketone supplement reduced PTSD symptoms. Twenty participants with PTSD were randomly assigned to a ketone supplementation or placebo group and evaluated for 6-weeks for PTSD symptom severity and CRP levels. The Spearman Rank correlation between PCL-5 and CRP was positive, but not statistically significant. Mixed models on the ranks of the data were used to examine the difference in PCL-5 and CRP between ketone and control groups over time. Median PCL-5 at the pre-test was higher than at the post-test in the ketone group but not in the control. The reduction of PCL-5 medians between ketone and control groups at the post-test was not different. The median pre-test CRP was not different from than post-test in the ketone group or control group. This pilot study did not find a correlation between CRP levels and PTSD symptom severity but was limited by a lack of CRP data collection due to the loss of follow-up. PTSD symptom severity was not altered by 6 weeks of chronic ketone salt supplementation compared to the control group but should be examined in a larger sample size for further verification.*

Keywords: *PTSD; inflammation; PTSD Checklist-5; ketone salts; anxiety*

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a severe mental illness that can develop after an individual is exposed to a traumatic event. Traumatic events which can trigger PTSD include, but are not limited to, exposure to combat, sexual violence, interpersonal violence, and accidental violent death. The lifetime prevalence of PTSD ranges from 7-8% in the United States [1].

PTSD is associated with recurrent, involuntary, and intrusive memories and nightmares of the traumatic event. Individuals often feel that the traumatic event is recurring, which can be triggered by exposure to stimuli that evoke memories of the event. These symptoms are incredibly distressing and can promote avoidance of situations that involve triggers, potentially leading to impairment in daily activities [2]. PTSD is also associated with significant cognitive and mood dysfunction [3]. Furthermore, patients suffering from PTSD have higher rates of both psychiatric and physical comorbid disorders than the general population. Psychiatric comorbidities include substance use disorder, anxiety disorders, and depressive disorders [4]. Physical comorbidities found in veterans include greater incidences of cardiovascular, musculoskeletal, metabolic, and neurological diseases [5].

While the symptoms and morbidity associated with PTSD are well-documented, treatment continues to present a challenge, due to the lack of clear understanding of the pathophysiology of the disorder, and the barriers to access to treatments that have been proven to be effective. Current treatment guidelines vary, but generally recommend psychotherapy as a first-line therapy [6,7], with the addition of SSRIs or SNRIs if refractory, although some suggest that SSRIs should be part of initial therapy [8-12]. Prazosin has also been shown to be effective in reducing symptoms of PTSD, especially in individuals with elevated blood pressure [13]. However, all of these treatments have considerable drawbacks and barriers. Psychotherapy of any kind is time-consuming and often expensive, and many patients with PTSD do not have access to the consistent care necessary for improvement; even with improved access to care, many people do not utilize resources available due to internal factors, such as lack of motivation or fear of embarrassment due to stigma associated with the disorder and mental health in general [14]. SSRIs and SNRIs have a wide range of side effects, including weight gain and sexual dysfunction, and are sometimes discontinued for these reasons [15]. Additionally, because individuals with PTSD are at higher risk of comorbidities such as diabetes and obesity, these side effects are especially unfavorable. For these reasons, a nutritional supplement that could reduce the symptoms of PTSD in affected individuals would be of considerable value in reducing the burden of the disorder in many individuals.

Past research has shown that there are higher levels of pro-inflammatory cytokines in combat-exposed males with PTSD than in combat-exposed males without PTSD, suggesting that inflammation may play a role in PTSD symptomatology [16, 17]. Another study found that higher levels of C-reactive protein (CRP) were associated with increased PTSD symptom severity in African American women with Type 2 diabetes mellitus [18]. Several other studies have found that PTSD is correlated with higher levels of CRP, but the populations only included African Americans and veterans, respectively [19,20]. For the results to be generalizable, studies that identify the relationship of CRP (and inflammation) with a mixed demographic diagnosed with PTSD are needed.

Ketones are molecules naturally produced by the liver during limited carbohydrate availability that act as both a fuel source and signaling molecule. They are widely available as commercial supplements and are used for a variety of purposes. Ketones possess physiologic properties such as anti-inflammatory actions that may provide benefits for PTSD symptoms. Beta-hydroxybutyrate, a ketone, has been shown to inhibit NLRP3 inflammasome activity in human monocytes, displaying the potential anti-inflammatory effects of ketogenic diets/supplements [21]. In a case report, a ketogenic diet was shown to treat Crohn's disease and significantly reduce levels of CRP [22]. In addition, current evidence suggests that ketogenic diet therapies can be therapeutic in treating epilepsy, nonmotor Parkinson's disease, cognition and cerebral blood flow in Alzheimer's patients, and Type 2 diabetes [23]. However, there are no studies that investigate the effect of ketone supplementation on CRP levels in PTSD patients. Therefore, the questions posed by this preliminary report are:

(1) Do high levels of CRP—as an inflammatory blood marker—correlate with PTSD symptom severity (measured by PTSD Check List – 5 questionnaires)? We hypothesize that CRP levels positively correlate with PTSD symptom severity.

(2) Is there a difference between the level of CRP in blood in the group of participants receiving adjunct ketone supplements versus placebo? We hypothesize that patients on a ketone supplement have lower CRP levels compared to those on a placebo.

MATERIALS AND METHODS

Experimental Design

In this randomized, double-blinded, placebo-controlled clinical trial, participants were assigned to one of two groups—an experimental group receiving an adjunct ketone supplement to their usual treatment (or no treatment) and a control group receiving an adjunct placebo. The duration of the trial was 6 weeks, with either the ketone or placebo supplementation consumed twice daily. Phone calls, adherence logs, and text messages were used to monitor compliance with the regimen. Participants visited the laboratory for a familiarization visit which included an explanation of the study, proper completion of the informed consent, and collection of demographic and medical history information. Participants were also advised to fast for 10 hours (only water was allowed) and withhold from exercise, caffeine, and nicotine for 12 hours before the following data collection visits. Following familiarization, participants visited the laboratory twice for data collection: once at baseline and then again at the end of the 6-week intervention. The study was approved by the University's Institutional Review Board, and all procedures performed complied with the institutional guidelines. This study was registered as a clinical trial (ClinicalTrials.gov Identifier: NCT04083352) and a CONSORT 2010 Flow Diagram is provided in Figure 1.

Participants

A total of 20 participants were recruited. Participants were recruited via the Veteran Success department, paper flyers on the University's campus and in the local community, a local news interview, the Mood and Trauma Outpatient Clinical Program, Veteran organizational meetings, and the local Warrior Project. The study inclusion criteria were: (1) a diagnosis of PTSD and (2) men and non-pregnant women from 18-65 years old. No exclusion of PTSD patients who were taking medication for psychiatric comorbidities. Exclusion criteria were pregnancy, chronic smoking, and/or taking cardiovascular-related medications 12 hours before and during evaluations. Patients who were taking prescribed medication continued doing so at the same dosage throughout the trial.

Figure 1: CONSORT 2010 Flow Diagram.

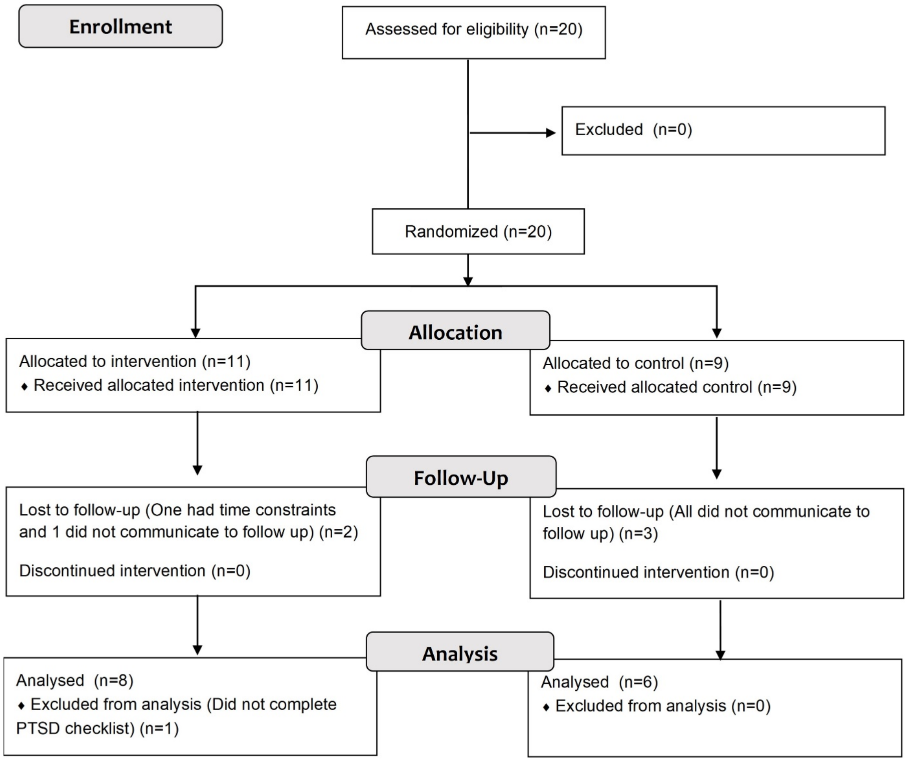
Enrollment
Assessed for eligibility (n=20)
Excluded (n=0)
Randomized (n=20)
Allocation
Allocated to intervention (n=11)
♦ Received allocated intervention (n=11)
Allocated to control (n=9)
♦ Received allocated control (n=9)
Follow-Up
Lost to follow-up (One had time constraints and 1 did not communicate to follow up) (n=2)
Discontinued intervention (n=0)
Lost to follow-up (All did not communicate to follow up) (n=3)
Discontinued intervention (n=0)
Analysis
Analysed (n=8)
♦ Excluded from analysis (Did not complete PTSD checklist) (n=1)
Analysed (n=6)
♦ Excluded from analysis (n=0)

Measures

To determine PTSD symptom severity, the PTSD checklist (PCL-5) was completed by each participant at the beginning and the end of the trial with an interviewer present for questions or clarification. C-Reactive Protein (CRP) was also measured for each participant at both the beginning and end of the trial. To measure CRP, venous blood samples were collected by an investigator and immediately transported to the Clinical Pathology Laboratory at the University's Hospital for subsequent analyses. The CRP analysis was performed on an automated chemistry analyzer (ADVIA 1800 Chemistry System; Siemens Healthineers, Erlangen, Germany).

Supplement

Participants were randomized to consume a ketone salt supplement or a placebo in conjunction with their normal diet and medication regimen. The ketone salt and placebo supplements were in powder form. The participants were instructed to take two packets a day mixed with water, once in the morning and once in the afternoon, for 6 weeks. The ketone salt supplement included 7 g of beta-hydroxybutyrate combined with erythritol, L-Taurine, fermented L-Leucine, citric acid, natural flavor, vegetable juice color, stevia, xanthan gum, beta carotene and approximately 920 mg of sodium (KETO//OS® MAX, Prüvit Ventures, Inc., Melissa, TX). The placebo supplement included maltodextrin and similar color, sodium, and flavoring, as the ketone salt supplement. Both supplements contained 41 kcal per serving.

Statistical Methods

All statistical analysis was performed using SAS 9.4 and statistical significance was assessed using an alpha level of 0.05. Due to the small sample size, rank-based and exact methods were used for all analyses. Descriptive statistics were determined by the ketone group and included frequencies and percentages for categorical variables and medians and interquartile ranges for continuous variables.

Fisher's Exact tests for categorical variables and Wilcoxon rank sum tests for continuous variables were used to examine differences by ketone groups for baseline values. Spearman Rank correlations between PCL-5 and CRP at baseline were determined.

Mixed models using the ranks of the data using the minimum variance quadratic unbiased estimation of covariance parameters method of estimation and assuming an unstructured covariance structure were used to examine changes over time between ketone groups for PCL-5 and CRP.

The denominator degrees of freedom were calculated using the Kenward-Roger method for factors in the model that did not involve time, and for factors that did involve time the denominator degrees of freedom were infinity. Each model included fixed effects of the ketone group and time as well as the two-factor interaction.

The F-test for the two-factor interaction was the statistical test of interest and the statistical significance of this F-test was determined using the Brunner, Domhof, and Langer (2002) method [24].

A Bonferroni adjustment to the overall alpha level was used to examine post hoc pairwise differences.

RESULTS

Table 1 gives the descriptive statistics by ketone status and the results for examining differences in baseline values. Briefly, 27% of those in the ketone group and 11% in the control group were male. The average age in the ketone group was 44 yrs and 50 yrs in the control group. There were no statistically significant differences between the ketone and control groups for gender, age, PCL-5, or CRP. The Spearman Rank correlation between PCL-5 and CRP was positive, but weak with a value of 0.21 (n=12, p=0.5149), and was not statistically different from zero.

Table 1: The descriptive statistics by ketone status and the results for examining differences in baseline values are shown in this table. *Abbreviations: CRP, c-reactive protein; n, number; PCL-5, PTSD Checklist 5; Q, quartile; SD, standard deviation*

Variable	Ketone N=11 (55%)	Control N=9 (45%)	P-value
Male Gender – n (%)	3 (27.3)	1 (11.1)	0.5913
Age – mean (SD)	44.8 (9.1)	40.0 (12.7)	0.3017
Baseline			
PCL-5 – median (Q1-Q3), n	58.5 (38.0-67.0) N=10	34.0 (26.0-60.0) N=9	0.1207
CRP – median (Q1-Q3), n	0.39 (0.30-0.73) N=6	0.47 (0.09-1.62) N=6	0.8099
Follow-Up			
PCL-5 – median (Q1-Q3)	54.0 (30.5-60.0) N=8	34.0 (19.0-36.0) N=6	
CRP – median (Q1-Q3)	0.62 (0.26-0.87) N=5	0.27 (0.09-0.46) N=2	

Table 2: The repeated measures non-parametric mixed model results examining differences in PCL-5 or CRP over time between groups on the ranks of the data are shown in this table. *Abbreviations: CRP, c-reactive protein; PCL-5, PTSD Checklist 5; Post, after the 6-week supplementation period; Pre, baseline; Q, quartile*

Outcome	Ketone Group	Time	Unadjusted Median	Unadjusted Q1-Q3	F	p-value
PCL-5	Ketone		55	38-67	2.77	0.1231
	Control		34	25-49		
		Pre	49	29-66	10.99	0.0009
		Post	38.5	20-57		
	Ketone	Pre	58.5	38-67	0.07	0.7988
		Post	54	30-60		
	Control	Pre	34	26-60		
		Post	34	19-36		
CRP	Ketone		0.47	0.26-0.87	0.15	0.7120
	Control		0.40	0.09-1.11		
		Pre	0.41	0.19-0.80	0.00	0.9580
		Post	0.46	0.09-0.87		
	Ketone	Pre	0.39	0.30-0.73	0.51	0.4520
		Post	0.62	0.26=0.87		
	Control	Pre	0.47	0.09-1.62		
		Post	0.27	0.09-0.46		

The results examining differences in PCL-5 or CRP over time between groups on the ranks of the data are given in Table 2.

The interaction between ketone and time was not statistically significant for PCL-5 indicating that the changes over time in the median PCL-5 scores in the ketone and control groups were not different.

The median PCL-5 at the pre-test was statistically, but not clinically, significantly higher than at the post-test (p=0.0003) in the ketone group but not in the control group (p=0.4418). There were no statistically significant differences in the PCL-5 medians between the ketone and control groups at the pre-test (p=1.0000) or post-test (p=0.6020). The interaction between ketone and time was not statistically significant for CRP indicating that the changes over time in the median PCL-5 scores in the ketone and control groups were not different. The median CRP at the pre-test was not significantly different from than post-test (p=1.0000) in the ketone group nor in the control group (p=1.0000). There were no statistically significant differences in CRP between the ketone and control groups at the pre-test (p=1.0000) or post-test (p=1.0000).

DISCUSSION

In this study, we did not find a statistically significant or clinically meaningful correlation between CRP levels and PTSD symptom severity. In addition, there was no significant difference in baseline CRP levels in patients in the ketone supplement group compared to the placebo group.

Several studies have examined the relationship between inflammation and PTSD. Lindqvist et al. examined the relationship between pro-inflammatory cytokines and PTSD in combat-exposed males. The study included 51 participants with PTSD and 51 participants without PTSD, comparing their "pro-inflammatory score", which consisted of summed standardized scores of Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), Tumor Necrosis Factor-alpha (TNFα), Interferon-gamma (IFNγ), and CRP. PTSD symptom severity was measured using the Clinician-Administered PTSD Scale (CAPS). The study found that subjects with PTSD had significantly higher pro-inflammatory scores compared to those without PTSD, with a p-value of 0.002 after controlling for early life trauma, depression diagnosis and severity, ethnicity, asthma/allergies, body mass index, education, possible confounding medications, and time since combat exposure. However, within the PTSD group, the pro-inflammatory scores were not significantly correlated with CAPS total score [16].

Lindqvist et al. conducted a replication study with a different cohort (31 combat-exposed participants with PTSD and 30 combat-exposed participants without PTSD). Again, they found that those with PTSD had higher total pro-inflammatory scores compared to those without PTSD. In addition, there was no significant correlation between pro-inflammatory score and PTSD symptom severity [17].

More recently, a study by Powers et al. examined the relationship between inflammation and PTSD. The study consisted of 55 traumatized African American women with type 2 diabetes mellitus. Participants were selected from a publicly funded hospital in Atlanta, Georgia, drawing from a low socioeconomic, urban minority population. The Clinician-Administered PTSD Scale (CAPS) was used to measure PTSD symptom severity, and CRP levels were taken as a measure of inflammation. The study found that CRP levels were positively correlated with PTSD symptom severity (p<0.01) [18].

Our study results indicated that CRP levels were not correlated with PTSD symptom severity. However, there are several differences to note. First, we measured PTSD symptom severity with PCL-5 rather than CAPS, which was the assessment used in previous studies. In addition, our study had a very small sample size—only 7 participants had data on both baseline and follow-up CRP levels. Because of this, it is difficult to draw any meaningful conclusions regarding CRP from the results obtained from this study.

One finding of note was that there was a statistically significant difference between pre-test and post-test PCL-5 scores in the ketone group, but not in the control group. However, because the difference was small and the difference in pre-test mean scores was large, this finding is not clinically significant. However, it does indicate that ketone supplementation should be further studied as a method of symptom reduction for PTSD patients. Future studies should aim to have a larger sample size to hold more statistical power, as the small sample size was a limitation of this pilot study.

CONCLUSIONS

This pilot study did not demonstrate a correlation between CRP level and PTSD severity; a statistically significant nor clinically meaningful correlation was not found between CRP levels and PTSD symptom severity measured by the PCL-5. In addition, there were no differences in CRP levels between the ketone salt supplementation and placebo groups, which suggests that ketone salt supplementation may not alter the levels of this inflammatory marker in individuals diagnosed with PTSD. However, as mentioned in the discussion, the study was limited by small sample size and ketones as a method for symptom reduction in patients with PTSD merit follow-up with a larger scale study due to their potential to provide more accessible relief if they are proven to be effective.

Conflicts of interest and sources of funding

The authors declare no conflicts of interest. Angelia Holland-Winkler received funding for this project from Augusta University's College of Education and Human Development and Prüvit Ventures, Inc. Ketone and placebo supplements were provided by Prüvit Ventures, Inc.

Authors' contribution

Authors include Langston Gilmore, Nagy Youssef, Jennifer Waller, Rebecca Nalloor, Max Wieder, and Angelia Maleah Holland-Winkler (PI). AMH-W designed the research, AMH-W and RIN collected the data, JLW analyzed the data, LBG, NAY, JLW, RIN, MAW, and AMH-W drafted and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by Augusta University's Institutional Review Board with approval number 907405. All subjects signed an approved and stamped informed consent document before participating.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Investigating the Relationship Between Social Responsibility and COVID-19 Morbidity in the Population Covered by Health Centers in Iran

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Abstract: *Introduction: Social responsibility and, is an indicator that is important in the epidemic of diseases with high prevalence and death rates. The current study aims at determining the relationship between social responsibility and COVID-19 in the population covered by health centers in Iran. Methods: 500 people participated in this descriptive-analytical study. The data was collected using a researcher-made social responsibility questionnaire in the context of the Covid-19 pandemic in three areas general, individual, and social responsibility. The quantitative content validity method was used to measure the validity of the questionnaire and Cronbach's alpha calculation method was used to measure its reliability. The data were analyzed using SPSS-21 software. Findings: Among the participants, 4.32% of them had a history of covid-19, and the history of complications was more in men (51.9%) and the mean social responsibility score of women was higher (P<0.001). The average responsibility score was higher in people without a history of Covid-19 than in people with a history of Covid-19 (P=0.006). Conclusion: Individuals with higher social responsibility observed more health protocols and had lower rates of covid-19 infection among them. Therefore, in the design of educational interventions, planning should be done to increase people's sense of social responsibility.*

Keywords: *social responsibility, health, COVID-19*

INTRODUCTION

The prevalence and incidence of various infectious diseases throughout history have been one of the most serious threats to human health; the most recent of which is the emerging COVID-19 virus from the coronavirus family [1]. COVID-19, which appeared in Johann, China in December 2019 [2] is considered a threat to the health and lives of millions of people worldwide [3]. This disease has changed the lifestyle of millions of people around the world [3], and in Iran, it is very prevalent, endangering the physical and mental health of people [4]. Individuals' responsibility for health is one of the most important aspects of a healthy lifestyle, with which a person chooses a lifestyle to maintain and promote his health and prevent diseases [5].

Since a human is a social being and lives in society from the moment of birth to the end of life and is in constant interaction with other human beings, he/she must always learn how to satisfy his/her life and needs in society. This requires learning social skills. One learns these skills within the socialization process, and if these skills are learned properly, it can lead to the formation of social responsibility [6].

According to Glaser, responsibility is formulated in the form of three components: first, learning to choose behaviors that meet our basic needs, second, accepting the consequences of our behavior, and third, it should not be an obstacle to meeting the needs of others [7]. Considering that social responsibility improves social abilities, academic motivation, attention to moral and social concepts, personal adaptation, sense of accountability to others, commitment to society, etc [8]. and given that responsibility is the outcome of three characteristics of recognition, decision, and creativity, so responsible individuals are conscientious, confident, serious, and possibly disciplined [6]. Besides, the goal of the health system is to promote the health of the community, and the factors that shape the behavior cause prevention and reduction of diseases and improvement of the community health, and the best platform and solution to achieve this goal are measures related to social responsibility [9]. Thus, considering previous research findings, and given the importance of the subject of social responsibility in the health of individuals, and since no study has been conducted on the relationship between social responsibility and COVID-19 in Iran, the present research was carried out for investigating the relationship between social responsibility and COVID-19 morbidity in the population covered by health centers of Iran in 2021.

MATERIALS AND METHODS

The present study is a cross-sectional research (descriptive-analytical) that was conducted on 500 individuals covered by Andimeshk city health centers in 2021. Andimeshk city is located in the north of Khuzestan province and southwest of Iran.

Inclusion criteria were having informed consent to participate in the study and literacy of completing the questionnaire, and exclusion criteria included the reluctance of the participant in each stage of the study and incomplete completion of the questionnaire.

The questionnaire used in this study is an author-made questionnaire on social responsibility during the COVID-19 pandemic. The designed questionnaire includes 13 demographic items and 32 items related to responsibility. With a review of related literature, two areas were considered including self-health responsibility for own health (individual area) and health responsibility for the health of others (social area).

Items of individual health responsibility area were designed according to the health responsibility dimension of the Health-Promoting Lifestyle Profile II (HPLP II) questionnaire established by Walker, which consisted of 12 items (items 1-12). The options are scored on a Likert scale ranging as strongly agree, agree, no opinion, disagree, and strongly disagree from 5 to 1, respectively. The range of scores in this area is between12-60. The items of the questionnaire related to the social responsibility area were designed according to the Social Responsibility Questionnaire developed by Harrison Goff, which included 20 items (items 13-32). To measure social responsibility in the social area, personality traits such as conscientiousness, sense of commitment, hardworking, seriousness, trustworthiness, discipline-based behavior, and behavior following the sense of responsibility towards family, society, and the world were considered. The items 19-20-25-27-28-29-30-31 were scored as strongly agree, agree, no opinion, disagree, and strongly disagree from 5 to 1, respectively. Items 16-17-18-21-22-23-24-26-32 were scored inversely, i.e., strongly agree, agree, no opinion, disagree, and strongly disagree from 1 to 5, respectively. Therefore, the range of scores in this area is between20-100. To assess the validity of the questionnaire, the quantitative content validity method was used by the panel of experts, and Cronbach's alpha calculation method was used to evaluate the reliability of the questionnaire, which was obtained as 0.78.

The sample size was calculated based on Cochran's sample size formula. To determine the sample size, by considering alpha as 0.05 accuracy as d = 0.4, and standard deviation as 6, the sample size was determined to be 484, which was increased to 500 for increasing the validity of the study, and 500 individuals completed the questionnaire as the research sample. Due to the problems related to the COVID-19 pandemic and the impossibility of face-to-face visits to collect information, the samples were selected using the convenience sampling method. So, to conduct the study, the questionnaire was initially developed The data were analyzed by SPSS software version 21. Mean and standard deviation as well as frequency distribution tables were used to describe the characteristics of the subjects. An Independent t-test was used for the comparison of responsibility scores in terms of the history of COVID-19 morbidity. To investigate the relationship between overall responsibility, as well as health responsibility for one's health (individual area) and health responsibility for the health of others (social area) with the chance of COVID-19 morbidity, Simple Logistic Regression was used at a significant level of 0.05. Then, the relationship between baseline characteristics variables and the chance of COVID-19 morbidity was measured at a significance level of 0.2. Variables that had a statistically significant relationship with the chance of COVID-19 morbidity were individually entered into the Multiple Logistic Regression model at a significance level of 0.2 to eliminate their confounding effect on the relationship between responsibility and history of COVID-19 morbidity. In case of the presence of variables with a confounding effect on the relationship between different areas of responsibility and the chance of COVID-19 morbidity (more than 10% change in the COVID-19 chance associated with responsibility), they are entered in the final model (simultaneously) at the significance level of 0.05. The variables of age and home space have entered the models as centering for interpretability of intercept of the regression model collected virtually through the questionnaire design website (https://porsline.ir/), and then the questionnaire link was sent to the participants through the largest virtual page of Andimeshk city news agency on Instagram. This page is mostly followed by residents of Andimeshk city, and it has about 70k members with a high rate of daily likes and visits. Following the completion of the questionnaire, it was available to us through the Porsline website.

RESULTS

Finally, 500 individuals were analyzed in this study, of which 162 (32.4%) had a history of COVID-19, and 338 (67.6%) did not report a history of COVID-19 morbidity. Of those with a history of COVID-19, 42 reported transmitting the disease to others.

Out of the total number of people surveyed, 321 (64.2%) reported that at least one of those around them had been infected by COVID-19 in the past. 210 (42%) were registered in the self-declaration system of the Ministry of Health (Table 1).

Table 1: Baseline characteristics of the participants in the study

Variable		No.	Percent
Incidence of COVID-19	No	338	67.6
	Yes	162	32.4
Gender	Male	240	48
	Female	260	52
Education	Illiterate	13	2.6
	Below high school degree	46	9.2
	High school diploma	95	19
	Higher education	346	69.2
Number of Household members	1	5	1
	2	50	10
	3	67	13.4
	Above 3	378	75.6
Proper ventilation of the residential house	No	124	24.8
	Yes	376	75.2
Registration in the COVID-19 self-declaration system	No	290	58
	Yes	210	42
Frequency of morbidity of COVID-19	No morbidity	340	68
	once	139	27.4
	Twice	19	3.8
	More than 2 times	4	0.8
Morbidity of COVID-19 in relatives	No	179	35.8
	Yes	321	64.2
Behavior leading to COVID-19	travel	4	2.5
	Going to a party	45	28
	Not wearing mask	38	23.6
	No hand sanitization	5	3.1
	Non-observance of social distance	18	11.2
	Contaminated work environment	35	21.7
	Caring for a patient with COVID-19	16	9.9
Transmitting your disease to others	No	379	75.8
	Yes	42	8.4
	I don’t know	79	15.8
Having underlying disease	No	56	11.2
	Yes	443	88.8

In the total subjects, the means of overall responsibility, responsibility in the individual area, and responsibility in the social area were equal to 126.17 ± 22.85, 44 ± 10.68, and 82.17 ± 14.77, respectively. In all areas, the mean score of responsibility of individuals with a history of COVID-19 was lower than those without a history of COVID-19, so that, the scores of overall responsibility, individual responsibility, and social responsibility in individuals with a history of COVID-19 were 5.93, 2.43, and 3.50 less than the scores of those without a history of COVID-19 (P <0.05) (Table 2).

Table 2: Comparison of the mean scores of different areas of responsibility in terms of the history of COVID-19 morbidity

Different areas	History of morbidity of COVID-19	No.	Mean ± SD	Difference of means	95% confidence interval		P-value*
					Lower limit	Upper limit	
Overall responsibility	Non-infected	338	128.09±22.23	5.93	1.67	10.20	0.008
	infected	162	122.15±23.67				
Individual responsibility	Non-infected	338	44.79±10.60	2.43	0.43	4.42	0.017
	infected	162	42.36±10.70				
Social responsibility	Non-infected	338	83.30±13.87	3.50	0.74	6.26	0.013
	infected	162	79.80±16.28				

** Independent Sample T Test*

As shown by the results of univariate analysis, for one unit increase in score of overall responsibility, individual responsibility, and social responsibility, the chances of morbidity of COVID-19 decreased by 2%, 3%, and 2%, respectively, which was statistically significant (P <0.05) (Table 3). There were statistically significant relationships between variables of literacy level, home space, history of COVID-19 in others, the transmission of COVID-19 to others, the presence of underlying disease, and age of individuals and the chance of morbidity of COVID-19 (P<0.20) (Table 3).

Table 3: Relationship between different areas of responsibility and baseline characteristics with the morbidity of COVID-19

Variable		OR (95% CI)	P-Value
Overall responsibility		0.98 (0.98, 0.99)	0.007
Individual responsibility		0.97 (0.96, 0.99)	0.018
Social responsibility		0.98 (0.97, 0.99)	0.013
Variable		**OR (80% CI)**	**P-Value**
Gender	Female (reference)	1	
	Male	1.25 (0.98, 1.60)	0.233
Level of literacy	Elementary school (ref.)	1	
	Below school	0.33 (0.14, 0.77)	0.093
	High school	0.41 (0.19, 0.89)	0.141
	Higher education	0.40 (0.19, 0.83)	0.111
Household dimension		1.04 (0.97, 1.11)	0.385
Ventilation of the residential place	No (ref.)	1	
	Yes	0.87 (0.65, 1.15)	0.532
House space		0.99 (0.99, 1.00)	0.134
Registration in the system of the Ministry of Health	No (ref.)	1	
	Yes	0.79 (0.62, 1.02)	0.243
History of morbidity of COVID-19 in others	No (ref.)	1	
	Yes	2.69 (2.03, 3.57)	< 0.001
Transmitting your disease to others	No (ref.)	1	
	I don't know	2.23 (1.60, 3.11)	0.002
	Yes	23.08 (12.29, 43.32)	< 0.001
Underlying disease	No (ref.)	1	
	Yes	1.74 (1.20, 2.51)	0.052
Age		1.02 (1.00, 1.03)	0.037

Table 4: Adjusting the confounding effect baseline characteristics variables on the relationship between different areas of responsibility and the morbidity of COVID-19

Adjusted variable	Overall responsibility			Individual responsibility			Social responsibility		
	aOR* (80% CI)	P-Value	% of change **	aOR* (80% CI)	P-Value	% of change **	aOR* (80% CI)	P-Value	% of change **
Level of literacy	0.98 (0.99, 0.98)	0.010	0%	0.97 (0.99, 0.96)	0.023	0%	0.98 (0.99, 0.97)	0.019	0%
Space of the house	0.98 (0.99, 0.98)	0.008	0%	0.98 (0.99, 0.96)	0.023	0.01%	0.98 (0.99, 0.97)	0.015	0%
History of coronary heart disease in those around	0.98 (0.99, 0.98)	<0.001	0%	0.97 (0.99, 0.96)	<0.001	0%	0.97 (0.99, 0.96)	<0.001	0.01%
Transmission of coronary heart disease to others	0.99 (0.98, 1)	0.170	0.01%	0.98 (0.97, 1)	0.186	0.01%	0.99 (0.98, 1)	0.245	0.01%
Underlying disease	0.99 (0.99, 0.98)	0.014	0.01%	0.98 (0.99, 0.96)	0.024	0.01%	0.98 (0.99, 0.97)	0.029	0%
Age	0.98	0.002	0%	0.97	0.007	0%	0.98	0.005	0%

** adjusted Odds Ratio for the relationship between responsibility and COVID-19 morbidity*

*** Percentage of OR change for relationship between responsibility and COVID-19 morbidity compared to its unadjusted state*

In the multiple logistic regression model, it was found that none of the variables of literacy level, home space, history of COVID-19 in others, the transmission of COVID-19 to others, presence of underlying disease, and age of individuals had a confounding effect on the relationship between overall responsibility, individuals responsibility, and social responsibility and morbidity of COVID-19 (Table 4).

DISCUSSION

The present study was conducted aiming at investigating the relationship between social responsibility and COVID-19 disease. A total of 500 residents of Andimeshk city in Khuzestan province in the southwest of Iran were surveyed.

The results of our study showed that individuals with a history of COVID-19 have lower scores of overall responsibility, individual responsibility, and social responsibility than those without a history of COVID-19 disease. Besides, the mean score of individuals' responsibilities for their health in the social area was higher than the individual area, and this finding is consistent with the study of Khajeh Nouri [8].

The mean score of responsibility in all areas surveyed was higher in individuals without a history of COVID-19 disease and those who showed better adherence to health protocols. In general, individual and social responsibility is a very important variable since it shows a person's commitment to his/her behavior, and the higher the commitment of individuals to an issue, its achievement would be easier realized through one's individual and social responsibility. Moreover, Thome maintains that social responsibility is based on a sense of commitment in individuals [10].

The results of the univariate analysis of our study showed that for one unit increase in score of overall responsibility, individual responsibility, or social responsibility, the chance of developing COVID-19 is decreased. The results of the present study also showed that men were more likely than women to get infected by COVID-19. This is in line with the findings of Brodin on the safety factors determining the manifestation and severity of COVID-19 disease. According to his results, he believes that men are more prone to COVID-19 disease than women [11]. Of course, the presence of men in crowded areas is a factor in increasing infection. Besides, men have poorer performance in terms of following health protocols than women [12], which increases the chances of getting the disease and this indicates less responsibility and commitment of men.

In this study, those registered in the COVID-19 self-declaration system of the Ministry of Health had a lower chance of getting infected by COVID-19, and this implies higher social commitment and responsibility of people to comply with health protocols and recognition of the infected cases and cutting the transmission chain. Because people are more committed to health, related behaviors are manifested in them earlier for anxiety management, and this suggests a responsibility to their health and those around them. It was also mentioned in the study of Fathi et al., and the author believes that individuals with higher responsibility experience less COVID-19 anxiety [13].

Our results demonstrated that for every year of age increase, the chances of getting infected by COVID-19 increase. The age variable itself is a determining factor in COVID-19 morbidity. This finding is in agreement with the findings of other studies [14-16].

In the present study, people who transmitted the disease to others had a higher chance of getting infected again because these people usually have a lower risk perception of the disease and also have lower health responsibility. This finding is consistent with the findings of Robinson's study [17].

In the present study, those with the underlying disease were more likely to develop COVID-19 disease, which is consistent with the study of Dadgari [14]. In our study, individuals with literacy levels below high school, high school, and higher education levels showed a lower chance of COVID-19 morbidity compared to those with an elementary school degree. They performed better following the health instructions and social responsibility, and this finding was in line with the study of Kharamin et al [12].

Usually, when people around you are not responsible, they create obstacles and problems for others that can increase their chances of risk. Our findings also indicated those with a history of COVID-19 morbidity among those around them are more prone to be infected, which this finding is consistent with the findings of Sahebi [7].

The results of this study demonstrated that in the case of COVID-19 morbidity, the most common causes of COVID-19 disease were related to behaviors, going to parties, not wearing a mask, and the contaminant work environment, respectively. Moreover, the chance of COVID-19 morbidity was less in less populated households and families with larger homes and more well-ventilated houses.

Consequently, given that the average score of social responsibility is higher in individuals without a history of COVID-19 disease and with better adherence to health protocols, research findings indicate that the more individuals take responsibility, the more attention they pay to the consequences of their actions, take their action and issues more seriously and try harder to solve them [18].

CONCLUSIONS

According to the results obtained in different studies, further research can be done to deepen the existing knowledge in the field of the correlation between social responsibility and health and different diseases and COVID-19 morbidity. In general, the component of responsibility should be institutionalized at different levels of society from the younger ages with different strategies, so that it can play an effective role in human health and the prevention of events and diseases.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research. The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

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Authors' contribution

All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee, Iran University of Medical Sciences with the code IR.IUMS.REC.1399.1199. Informed consent was obtained from all individual participants included in the study before conducting.

Patient consent for publication

All participants consented verbally to the publication of the interview data.

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ORIGINAL ARTICLE

A Family-based Association Test of the VDR Gene in Proximal Spinal Muscular Atrophy

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Abstract: *Different factors may be involved in the clinical heterogeneity of spinal muscular atrophy disease. The vitamin D receptor (VDR) might be a candidate gene for this disease. Our study aimed to assess the preferential transmission of VDR polymorphisms from parents to SMA children. We genotyped 261 subjects (87 SMA nuclear families) for VDR FokI, BsmI, ApaI, and TaqI polymorphisms. The transmission of the genetic marker was estimated with Plink and FBAT software. It detected a preferential transmission of the rs731236 and rs7975232 variants to SMA1 patients and of rs1544410 variants to SMA2 patients. The variants of rs2228570 were preferentially transmitted to parents of all SMA patients. Haplotype analysis identified that haplotypes C-C-G-A and T-A-A-G seem to be involved in the booth type of SMA whereas the impact of T-A-A-A seems to be limited only to SMA2. Strong linkage disequilibrium (LD) between rs7975232 and rs1544410 was detected in samples from parents. Even though we investigated a small number of nuclear families the results suggest a potential link between VDR polymorphisms and SMA disease.*

Keywords: *SMA, TDT analysis, VDR gene*

INTRODUCTION

Spinal muscular atrophy (SMA) refers to a group of hereditary recessive neuromuscular diseases leading to muscle weakness and infant mortality. The clinical onset of spinal muscular atrophy 1 (SMA1 or Werdnig Hoffmann disease) is evident in the first 6 months of life whereas for spinal muscular atrophy type II (SMA2 or Dubowitz disease) it is observed at ages 6 - 12 months.

The main symptoms of SMA1 and SMA2 are degeneration of anterior spinal cord motoneurons, muscular hypotonia, or atrophy. They are determined by mutations (e.g. homozygous deletion, punctiform mutations, or complex rearrangements) in the SMN1 locus (5q13.2) [1,2] but are influenced by other factors (e.g., mutations in SMN2, NAIP, PLS3) [3,4,5].

Vitamin D is involved in mineral metabolism and interferes with immune response, apoptotic and anti-proliferative processes, or neuromuscular function [6,7,8,9]. Vitamin D deficiency may predispose to reversible muscle weakness, wasting, and instability of gait [10,11], whereas supplementation with this vitamin may increase muscle strength in athletes [12] and reduce the risk of cancer death [13].

The vitamin D receptor (VDR), encoded by the VDR gene (12q.13.1), is a nuclear receptor belonging to class 2 of the family of steroid receptors. Several VDR polymorphisms (e.g. VDR FokI, BsmI, Apa, TaqI) have been associated or in linkage with different human diseases (e.g. diabetes mellitus type 1, osteoporosis, allergic diseases), including carcinomas and neurological disease (e.g. Parkinson's disease, Alzheimer's disease, multiple sclerosis, SMA1) [14,15,16,17,18,19]. However, the association between VDR polymorphisms and neurological diseases remains a subject of debate.

This study aimed to investigate the transmission of VDR polymorphisms in nuclear families with SMA1 or SMA2 patients.

MATERIALS AND METHODS

Subjects

For families with SMA type, 1 (n=43) or type 2 (n=44) patients who were referred to several hospitals from Bucharest (Romania), 24 of them were also included in a previous publication [19]. Diagnosis of SMA was based on age at clinical onset and of homozygous deletion of SMN1 exon 7. SMA1 patients never achieve sitting whereas those with SMA2 could sit but were not able to walk, according to classification criteria [20,21].

Genotyping

DNA was isolated using a commercial kit (The Wizard® Genomic DNA Purification Kit) from peripheral blood (0.5 ml) collected on EDTA anticoagulant tubes. The homozygous deletions of SMN1 exon 7 or 8 were tested by PCR -RFLP and the homozygous deletion of NAIP exon 5 was tested by PCR and electrophoresis, according to the previously published protocols [22,23,24]. VDR polymorphisms Taq (rs731236), Apa (rs7975232), Bsm (rs1544410), and Fok (rs2228570) were genotyped using PCR-RFLP method, as was previously described [19,25,26]. Restriction products were resolved in electrophoresis. The genotype of each sample was established independently by two investigators.

Statistical analysis

The frequencies of alleles and haplotypes, linkage disequilibrium (LD) between markers, and the pattern of genetic markers transmission from parents to affected offspring (Transmission disequilibrium test – TDT) was performed with Plink [27] and the FBAT (Family Based Association Test) software [28]. The linkage between VDR markers in samples from parents and the LD plot was estimated with Haploview 4.1 [29]. A p-value of 0.05 was considered statistically significant.

RESULTS

The gender distribution was similar in SMA1 and SMA2 patients (51.16% and 52.27%, respectively were boys).

The parents of children with SMA1 (25.76±3.42; 19-36 years old) or SMA2 (25.86±3.55; 19-35 years old) had similar ages. The age of patients with SMA1 was significantly lower than that of SMA2 (8.39±4.21 months vs. 13.33±3.54 months, $p < 0.0001$). The genetic test showed that all investigated parents have at least one copy of the SMN1 gene. The homozygous deletion of SMN1 exon 8 or NAIP exon 5 was detected more frequently in patients with SMA1 than in those with SMA2 (53.48% vs. 40.91%; and 25.58% vs. 20.45% of cases).

The distribution of all VDR genotypes in the sublot of parents and rs1544410 in the sublot of SMA patients are not in agreement with Hardy Weinberg's principle (Table 1). Only the Minor Allele Frequency (MAF) of rs1544410 is in the interval of values reported in 1000 Genomes for VDR polymorphisms (Table 2) [30].

The results obtained with FBAT and Plink software reveal a preferential transmission of the VDR alleles from parents to SMA children. The calculated statistical significance was much stronger for rs731236 and rs7975232 and more reduced for rs1544410 and rs2228570. These results depend on the type of disease; it was detected a preferential transmission of the rs731236 and rs7975232 variants to SMA1 patients and of rs1544410 variants to SMA2 patients (Table 3, Table 4).

Table 1: The distribution of VDR polymorphisms in sublots of SMA patients and parents

Polymorphisms	Lot of subjects	Genotypes		
rs731236		TT	TC	CC
	patients	18	43	26
	parents*	37	120	17
rs7975232		AA	AC	CC
	patients	18	39	30
	parents*	34	113	27
rs1544410		GG	GA	AA
	patients*	29	50	8
	parents*	32	127	15
rs2228570		GG	GA	AA
	patients	17	38	32
	parents*	29	112	33

** Distributions of genotypes that are not following HWE in sublot of parent*

Table 2: The MAF of VDR polymorphisms in sublots of patients and parents

VDR polymorphisms	Variants	1000 genomes MAF		SMA Patients	Parents
		Europeans	All samples		
rs731236	C	0.400	0.277	0.546	0.443
rs7975232	C	0.445	0.485	0.569	0.480
rs1544410	A	0.404	0.296	0.379	0.451
rs2228570	A	0.378	0.328	0.586	0.511

Table 3: TDT test calculated for VDR polymorphism (FBAT software)

SNPs	Allele	Families	afreq	Fam#	Z; p
rs731236	C	SMA all	0.547	78	3.31; 0.0009
		SMA1	0.570	39	2.78; 0.005
		SMA2	0.523	39	1.92; >0.05
rs7975232	C	SMA all	0.477	78	3.02; 0.003
		SMA1	0.483	36	2.66; 0.008
		SMA2	0.472	42	1.66; >0.05
rs1544410	A	SMA all	0.451-	78	2.34; 0.02
		SMA1	0.470	37	1.19; >0.05
		SMA2	0.433	41	2.077; 0.04
rs2228570	G	SMA all	0.491	77	2.46; 0.01
		SMA1	0.494	37	1.57; 0.11
		SMA2	0.489	40	1.89; >0.05

Note: # Fam: number of informative families, Z: standard normal variable. p: probability

Table 4: TDT test calculated for VDR polymorphism (Plink software).

SNPs	A1 allele	A2 allele	Families	Transmitted A1 allele count	Untransmitted A1 allele count	OR; p
rs731236	C	T	All	76	41	1.85; 0.001
			SMA1	38	16	2.38; 0.003
			SMA2	38	23	1.65; >0.05
rs7975232	C	A	All	71	40	1.78; 0.003
			SMA1	34	14	2.43; 0.004
			SMA2	37	24	1.54; >0.05
rs1544410	A	G	All	48	75	0.64; 0.02
			SMA1	23	31	0.74; >0.05
			SMA2	25	42	0.6; 0.04
rs2228570	G	A	All	42	69	0.61; 0.01
			SMA1	17	30	0.56; >0.05
			SMA2	24	39	0.62; >0.05

Note: The results of the TDT test performed for families with SMA cases (plink)

The frequency of haplotypes in SMA families was estimated with FBAT software (Table 5). The most frequent three (rs731236-rs7975232-rs1544410-rs2228570) haplotypes estimated for SMA1 and SMA2 families were C-C-G-A (0.120 vs. 0.147), T-A-G-A (0.116 vs. 0.145), T-A-A-G (0.115 vs. 0.124) and, the rarest was C-A-G-A (0.009 vs. 0.003). Statistically significant results were identified between C-C-G-A and both types of SMA, T-A-A-G haplotypes, and SMA1 disease and between T-A-A-A and SMA2 (Table 5).

We found a strong linkage disequilibrium (LD) measure between rs7975232 and rs1544410 and a less significant linkage between rs7975232 and rs731236 (Figure 1).

Table 5: VDR haplotype analysis in SMA families (FBAT software)

Haplotypes	Families	Frequency of haplotype	fam#	S-E(S)	Var(S)	Z;P
C-C-G-A	SMA All	0.133	36	12.589	10.339	3.91; 0.00009
	SMA 1	0.120	12	6.082	4.166	2.98; 0.003
	SMA2	0.147	21	7.464	6.647	2.89; 0.004
T-A-A-G	SMA All	0.101	34	10.205	7.728	3.67; 0.0002
	SMA 1	0.115	14	5.256	3.823	2.69; 0.007
	SMA2	0.124	19	1.808	5.08	0.80; 0.42
T-A-A-A	SMA All	0.068	28	4.713	4.886	2.13; 0.03
	SMA2	0.082	17	3.877	3.640	2.03; 0.04

Note: Haplotype: rs731236- rs7975232- rs1544410- rs2228570; # Fam: number of informative families, Z: standard normal variable. p: probability

Figure 1: VDR LD plot estimated for the four polymorphisms identified in samples from parents

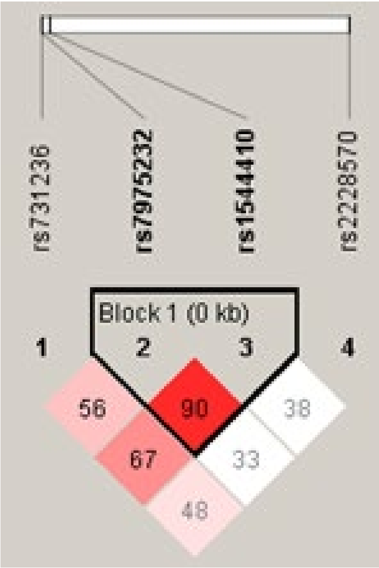

DISCUSSION

We performed a family-based association test, to bring new data regarding the relationship between VDR gene polymorphisms and SMA disease [19]. The transmission disequilibrium test, unlike the case-control association study, is not susceptible to population stratification. Eighty-seven families with children diagnosed with SMA1 or SMA2 were included in this study. The observed distribution of mutations may reflect some characteristics of families included in this study. Thus, the homozygous deletion of SMN1 exon 8 (53.48% and 40.91% respectively) in blood samples from our SMA1 and SMA2 patients was less frequent than in other populations (100% and 91.48%). These differences may be explained by the results of Vijzelaar R. and colleagues who considered that the distribution of SMN mutations is influenced by population background [31].

The homozygous deletion of NAIP exon 5 in SMA1 and SMA2 patients (25.58% and 20,45% respectively) was in the range of values reported in other studies (9.5%-82.85% respectively) [32,33,34].

The distribution of VDR polymorphisms in the sublot of parents is not in agreement with Hardy Weinberg equilibrium. In addition, the distribution of rs731236, rs1544410, and rs2228570 in a subgroup of parents differs from those reported in public databases [35]. However, the MAF values for rs2228570 estimated in this study and previous research performed in the population from South Romania are similar (0.378 vs 0.365) [36]. In this sublot of subjects, we also detected much stronger linkage disequilibrium (LD) between rs7975232 and rs1544410 than between rs7975232 and rs731236. This result is not following data from public databases [30]. These results could be explained, at least partially, by the size of this sublot of subjects and by the criteria used for selection.

In families investigated in this study, the C-C-G-A and T-A-A-G haplotypes were linked with booth types of SMA whereas T-A-A-A could be in relation only with SMA2 (Table 5). In a previous case-control study, we found an association between SMA1 and VDR haplotypes C-C-G-A (OR= 2.8, p=0.02) or T-A-A-A (OR= 0.27, p=0.03) [19].

The rs1544410, rs7975232, and rs731236, located near the 3'end of the VDR gene, were supposed to be non-functional. We detected a preferential transmission of the rs731236 and rs7975232 variants to SMA1 patients, and rs1544410 variants to SMA2 patients. These markers may be in linkage disequilibrium with a potential functional VDR polymorphism.

The rs2228570 was mapped in exon 2 of the VDR gene, in a potential translation initiation start site. Its variants were associated with two receptor variants: a long version of the protein (the T variant) and a shortened variant by three amino acids (the C variant). The VDR C variants seem to interact more efficiently with transcription factor TFIIB [37]. The variants of rs2228570 were preferentially transmitted to parents of all

SMA patients (Table 5).

This is the first report of a TDT test performed for VDR gene polymorphisms in SMA diseases. The results indicated that some VDR polymorphisms may be preferentially transmitted from parents to SMA patients. This result is completing the literature reports about the preferential transmission of VDR gene polymorphisms alleles in various diseases like diabetes [38, 39], rheumatoid arthritis [40], tuberculosis [41], or bone mineral density [42].

In conclusion, our data indicated a potential transmission of VDR variants from patients to SMA1 or SMA2 patients.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research. The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

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ORIGINAL ARTICLE

First Romanian Tele-Echography Network – Preliminary Results

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Abstract: *The evolution of society, the development of the Internet, and the increased need for medical services generated the emergence of telemedicine. The visual and auditory connection between the doctor and the patient allowed good communication, but the limitation of the clinical examination remained a problem. Tele-echography complements tele-consults and brings valuable information in many specialties. Our study is conducted on data obtained by organizing the first network of telemedicine with tele-echography in the country. The ultrasound examinations targeted only the abdomen, but the structure also allows tele-echocardiography as well as the extension to other services at distance. The purpose of the study is to objectively assess the ability of tele-echography to complete the teleconsultation with information on which we can rely. The national tele-echography network was created by distributing 40 tele-echographs in the country, one in each county, in the family doctors' offices. The obtained data were analyzed statistically. The study shows that tele-echography can be performed with very good accuracy in synchronous mode but low in asynchronous mode, which required re-examination or resumption of examination in the reference medical center. The widespread use of tele-echography associated with teleconsultations can have favorable consequences on the medical act, the quality of life of the patients and the doctors, and society by reducing pollution and urban agglomerations.*

Keywords: *tele-echography; teleconsultation; abdominal echography*

INTRODUCTION

Telemedicine is defined as a technique that comprises the usage of information and communication technologies (ICT) to enhance the access of patients to distant healthcare providers and technologies [1].

Tele-echography or tele-ultrasonography (Tele US) uses remote transmission to provide enhanced US diagnosis, based on the opinion of remote experts. TELUS is frequently performed at the point-of-care (PoC) by primary care doctors, with a profound impact on patients located at remote destinations [2].

The aim of the organization of the national network telemedicine with tele-echography is to provide patient-centered care, with enhancement in the clinical decision-making for individual cases. The procedures' results are amplified by the involvement of specialists as remote experts [3].

The results of previous studies have motivated the organization of the first tele-echography network in Romania [4,5,6].

In the studies carried out in the field of telemedicine with tele-echography, the following disadvantages are identified: the deficit of specialized medical staff, the difficulties of time management, and the costs of equipment and the internet [7,8,9].

The national telemedicine network with tele-echography is made up of 40 tele-echography centers organized as follows: In each county, the Lotus Image medical center from Targu Mures provided a tele-echography through which tele-consultations, including tele-echography, were performed with the specialists.

In this preliminary retrospective study, we present the details of this structure and the statistical analysis of the accumulated data from abdominal examinations. The presented data demonstrate the increase in the quality of the medical service when tele-echography is associated with teleconsultation and motivates the extension to the other specialties: cardiology, obstetrics and gynecology, pediatrics, endocrinology, and senology.

MATERIALS AND METHODS

The components of the tele-echography network are a doctor, portable POCUS device -Telemed LS64 FLT-1T with EchoWave II (EWII) software, a virtual private network (VPN)-secure, private, and encrypted online connection, remote expert. The two main parts of the process are the transmission and reception of ultrasound images. The transmission is done by the ultrasound device and the internet connection. The reception is made up of personal computers or smartphones with dedicated soft. In asynchronous mode, the third component intervenes, the server where echography films are stored and downloaded later by experts. In synchronous mode, the examinations are made at the same time and the result of the examination is issued immediately after its completion.

Figure 1: Tele-echography network

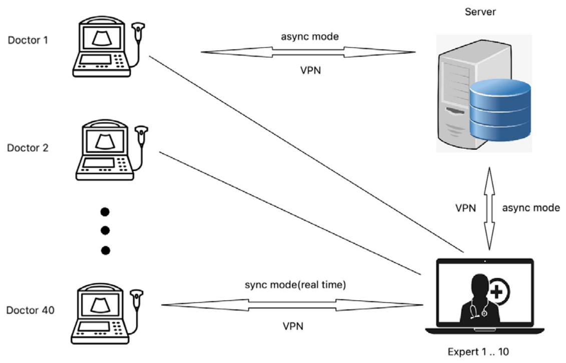

Synchronous examination or real-time is the most appreciated method of examination by patients, examining physicians, and experts because it alleviates the possibility of timely interaction between the three of them, the adjustment by verbal guidance of the examination to obtain the correct images and their interpretation in the clinical context and release the results immediately after the end of the examination.

Asynchronous examination or store-and-forward has the advantage that the videos and results are saved digitally and can constitute both an image database and forensic documentation in case of malpractice process. The second advantage is that it allows greater flexibility in the working time for the patient-examining physician and expert but is subject to the risk of incomplete or incorrect results with the possible consequence of requesting the resumption of the examination from the expert.

The national tele-echography network that was created within this project had both options. As a result, through the activity in the network, a database was established consisting of both the storage of echography videos and of the standardized results.

By entering the standardized elements in an excel database, a complex statistical analysis was possible, which included the following elements: the reasons for the patients' presentation for tele-echography, the number of organs completely or incorrectly reviewed, the ability to visualize the spleen and kidney, the collaboration with the patient to obtain and maintaining apnea necessary for a correct examination of the upper abdomen.

RESULTS

The study group resulted from the selection of approximately 3000 tele-echography abdominal examinations performed in synchronous and asynchronous modes within the created network. The results of the examinations were drafted and standardized, and the parameters were entered into the excel database. Incomplete results and re-examinations were excluded. The created database contains both medical documents and video recordings. The distributions of the ”reasons for presentation” for the tele-echography examination are shown in Table 1, Figure 2. A large proportion of persons presented themselves to the specialist for screening (30.3%). This parameter illustrates the awareness of the population for prevention and confirms the role of this method to meet the needs of the patients. Chronic abdominal pain syndrome and nonspecific syndromes were the reasons for teleconsultation with tele-echography in another third of the patients.

Figure 2: Reasons for the presentation at the tele-echography

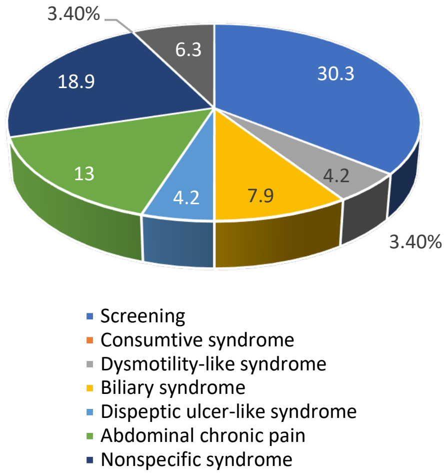

Table 1: The reasons for the presentation at the ultrasound

Reasons for the presentation	Number	Percentage
Screening	144	30.3
Consumptive Syndrome[1]	16	3.4
Dysmolity-Like Syndrome[2]	20	4.2
Biliary Syndrome[3]	38	7.9
Dyspeptic Ulcer-Like Syndrome[4]	60	12.6
Abdominal Pain Syndrome[5]	62	13
Nonspecific Syndrome[6]	90	18.9
High Urinary Syndrome[7]	16	3.4
Lower Urinary Syndrome[8]	30	6.3
Total	477	100

[1] *Consumptive syndrome – clinical syndrome characterized by consuming the body's reserves, secondary to the evolution of serious chronic diseases.*

[2] *Dysmotility-like dyspepsia – bloating, abdominal distention, flatulence, and prominent nausea.*

[3] *Biliary Syndrome – abdominal pain, especially in the upper right side of the abdomen under the rib cage, nausea or vomiting*

[4] *Dyspeptic Ulcer-Like Syndrome – discomfort or pain that occurs in the upper abdomen, often after eating or drinking.*

[5] *Abdominal Pain Syndrome – continuous or almost continuous abdominal pain, slightly related to intestinal function, associated with decreased daily tone, and which has been present for at least the last six months.*

[6] *Nonspecific Syndrome – Indigestion, abdominal pain with bowel movements, feeling full after eating a small amount of food, nausea, vomiting.*

[7] *High Urinary Syndrome – Painful urination (dysuria), frequent urination, especially during the day, pain in upper abdomen and back*

[8] *Lower Urinary Syndrome – blood in your urine (hematuria), difficulty urinating (urinary retention), feeling an immediate urge to urinate, frequent urination, especially during the day, painful intercourse (dyspareunia), painful urination (dysuria), pain in your genitals, lower abdomen and lower back.*

Correlation between the parameters in pathology where the ultrasound examination has a major role in the diagnosis such as lithiasis and proliferative disease

Correlations between biliary syndrome and cholelithiasis

Out of 38 patients who complained of biliary syndrome, only 4 were found biliary lithiasis ultrasound aspects, while out of 439 without the biliary syndrome, 25 (5.7%) had cholelithiasis Table 2. The differences are statistically insignificant (p -0.27). The absence of a syndrome in the anamnesis does not exclude a certain type of pathology. The usefulness of the ultrasound examination is obvious in this case.

Table 2: The relationship between biliary syndrome and cholelithiasis

P -0.27			Cholelithiasis		Total
			not	yes	
Biliary Syndrome[1]	not	number	414	25	439
		%	94.3%	5.7%	100.0%
	yes	number	34	4	38
		%	89.5%	10.5%	100.0%
Total		number	448	29	477
		%	93.9%	6.1%	100.0%

[1] *Biliary syndrome: Abdominal pain, especially in the upper right side of the abdomen under the rib cage. Nausea or vomiting with or without icter*

Correlations between high urinary syndrome and renal lithiasis

Of the 16 people who complained of a high urinary syndrome, only 2 were labeled with renal lithiasis. Of the 461 people who did not report a high urinary syndrome, only 18 were labeled with renal lithiasis. The differences are statistically insignificant (p -0.14), Table 3.

Table 3: The relationship between high urinary syndrome and renal lithiasis

P -0.14			Renal lithiasis		Total
			not	yes	
High urinary syndrome[1]	not	number	443	18	461
		%	96.1%	3.9%	100.0%
	yes	number	14	2	16
		%	87.5%	12.5%	100.0%
Total		number	448	457	20
		%	93.9%	95.8%	4.2%

[1] *High urinary syndrome: upper back pain, dysuria, polakiuria, with or without macroscopic hematuria, fever, chills*

Correlations between lower urinary syndrome – hypertrophy of the prostate

Of the 31 people who reported down urinary syndrome, 7 (22.6%) were labeled benign hypertrophy of the prostate (BPH). Of the 446 people who did not report a urinary syndrome down, only 43 (9.6%) were labeled with BPH. The differences are statistically significant (p -0.03), Table 4.

Table 4: The relationship between lower urinary syndrome and benign hypertrophy of the prostate (BPH)

P -0.03			**BPH**		**Total**
			not	**yes**	
Lower urinary syndrome[1]	not	number	403	43	446
		%	90.4%	9.6%	100.0%
	yes	number	24	7	31
		%	77.4%	22.6%	100.0%
Total		number	448	427	50
		%	93.9%	89.5%	10.5%

[1] Lower urinary syndrome: difficulty urinating (urinary retention), feeling an immediate urge to urinate, frequent urination, especially during the day, painful intercourse (dyspareunia), painful urination (dysuria), pain in your genitals, lower abdomen and lower back pain with or without hematuria, fever, chills

Tele-ultrasound examination skills

We analyzed the examiner's ability to highlight the abdominal organs that present difficulties of ultrasound examination at the classical, direct examination. One of the factors that makes ultrasound examination difficult is obesity. That's why we analyzed the number of unchecked organs and the body mass index (BMI).

Number of unvisualized organs and body mass index (BMI)

In 41 cases an organ was not revealed, in 5 situations two organs were not visualized, in 4 cases – 3 organs, in one case – 5, respectively 9 organs. In cases where an organ has not been visualized, 4 (13.3%) people with obesity grade II are noted, or 17 (10.4%) overweight people. Situations with two unvisualized organs were identified in normal or overweight people Table 5.

Table 5: Relationship between BMI and number of organs not viewed

P -0.98			**No invisible org**						**Total**
			0	**1**	**2**	**3**	**5**	**9**	
BMI[1]	under ponderal	number	18	0	0	0	0	0	18
		%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%
	normo ponderal	number	123	11	2	1	0	1	138
		%	89.1%	8.0%	1.4%	0.7%	0.0%	0.7%	100.0%
	Supra ponderal	number	144	17	2	1	0	0	164
		%	87.8%	10.4%	1.2%	0.6%	0.0%	0.0%	100.0%
	obesity grade I	number	103	8	1	2	1	0	115
		%	89.6%	7.0%	0.9%	1.7%	0.9%	0.0%	100.0%
	obesity grade II	number	26	4	0	0	0	0	30
		%	86.7%	13.3%	0.0%	0.0%	0.0%	0.0%	100.0%
	obesity degrees III	number	11	1	0	0	0	0	12
		%	91.7%	8.3%	0.0%	0.0%	0.0%	0.0%	100.0%
Total		number	425	41	5	4	1	1	477
		%	89.1%	8.6%	1.0%	0.8%	0.2%	0.2%	100.0%

[1] BMI = weight (kg)/height (cm^2)

Relationship between tele-ecographist and spleen visualization

The spleen has the same characteristics in ultrasound examinations as kidneys: intestinal gaseous overlaps and the chest cage make it difficult to examine these organs. Therefore, we evaluated the feasibility of the method by establishing the vision rate of the spleen at each of the examiners included in the study. The results, contained in the table below, demonstrate the uniformity of this parameter and support the promotion of the patient's examination method (Table 6).

Number of unidentified organs during an examination

The first condition for correct examination is the honesty of the examiner; declaring the parameters unidentified during the examination makes sure that they will be evaluated in other conditions (e.g. after the treatment of reducing bloating, examination of the bladder in the replete or gallbladder of fasting) or with other imaging means: retroperitoneum by CT or MRI, prostate by an endorectal probe, genital organs by an endovaginal probe (Table 7).

Table 6: Relationship between tele-ecographist and spleen visualization

P -0.96			View the spleen		Total
			not	yes	
Teleecographer	Other	number	0	19	19
		%	0.0%	100.0%	100.0%
	CJ	number	2	59	61
		%	3.3%	96.7%	100.0%
	PV	number	11	358	369
		%	3.0%	97.0%	100.0%
	RS	number	1	27	28
		%	3.6%	96.4%	100.0%
Total		number	14	463	477
		%	2.9%	97.1%	100.0%

Table 7: Relationship between the tele-ecographer and the number of unvisualized organs

P -0.38			No invisible organs						Total
			0	1	2	3	5	9	
Tele ecographer	Other	number	18	1	0	0	0	0	19
		%	94.7%	5.3%	0.0%	0.0%	0.0%	0.0%	100.0%
	CJ	number	57	4	0	0	0	0	61
		%	93.4%	6.6%	0.0%	0.0%	0.0%	0.0%	100.0%
	PV	number	325	35	3	4	1	1	369
		%	88.1%	9.5%	0.8%	1.1%	0.3%	0.3%	100.0%
	RS	number	25	1	2	0	0	0	28
		%	89.3%	3.6%	7.1%	0.0%	0.0%	0.0%	100.0%
Total		number	425	41	5	4	1	1	477
		%	89.1%	8.6%	1.0%	0.8%	0.2%	0.2%	100.0%

The two cases in which no organ could be visualized were correlated with very low acoustic impedance, increased BMI, and difficulties in maintaining clinostatism and apnea, due to the accusation of comorbidities, in conditions of postprandial examination and with the bladder in vacuity. In these cases, the limits of the method are declared and the patient was guided to imaging methods compatible with their particularities.

DISCUSSION

The terms used in this study are based on the recommendations of EFSUMB 2018 in which it is specified that the ultrasound examination suggests diagnostic hypotheses, but does not make a diagnosis [10]. To extend the accumulated experience to other specialties, we analyzed the studies published in the following areas: emergency medicine, obstetrics, gynecology, cardiology, pediatrics, musculo-skeletal, and pneumology.

Portable ultrasound units were used in the initial phase of trauma resuscitation for Focused Assessment with Sonography for Trauma, based on triage protocols for trauma patients by paramedics emergency physicians: infield FAST or e-FAST examinations. The study demonstrated similar quality with original movies; after 20 min lecture on FAST and US system presentation TeleUS (FAST) for US naive paramedics by emergency physicians was performed FAST on a patient with real-time EP connection. The result of the study was 100% complete views, with a mean time of 262 seconds (< 5 minutes) [11].

Obstetrics and gynecology: lack of fetal radiation exposure make teleUS the perfect tool for geographically isolated pregnant patients. Confirmation of intrauterine pregnancy, monitoring of fetal growth, and evaluation of pregnancy-related complications, with markedly reduced costs, were demonstrated in the studies [12,13,14].

Echocardiography is indispensable in clinical decision-making remote fetal echocardiography congenital prenatal heart disease left ventricular systolic function, LV ejection fraction [15].

Remote spatial applications International Space Station applications, 2h pre-flight training modules, 60 min in-flight session, e-learning system for US operators, no consistent significant effects of long-duration space flight on echocardiographic variables [16,17,18].

The use of ultrasound in the examination of musculoskeletal structures exceeded the limit of the penetrability of ultrasound through the bone and allowed the opening of new horizons both in direct examination and by tele-echography including self-examination [19].

In pediatrics, the use of point-of-care ultrasonography (POC US) is very useful [20].

During the COVID-19 pandemic, it was necessary to use all diagnostic means including ultrasound, which proved to be a low-cost tool very useful in remotely guided pulmonary resuscitation [21].

Tele-echography in addition to teleconsultations is a unanimously accepted aid.

The main problem is the number of specialists needed to solve the increasing demand for tele-echography services. The solution can be to accredit the overspecialization of the nurses in synchronous tele-echography, and the use of artificial intelligence in the field of tele-ultrasound.

On the existing structure, other types of ultrasound examinations are carried out: echo cardio, thyroid echo, and peripheral eco doppler, which is the subject of ongoing studies.

In the future, networks, such as those described, can be organized in human communities such as militia units, refugee camps, children's camps, and theaters of battle.

CONCLUSION

Portable hand-held ultrasound devices will move this valuable medical investigation near the patient and the medical society must prepare for this stage.

Future research directions may be a combination of tele-consultation with other investigations that can participate in the diagnosis of the remote patient: tele-EKG, tele EEG, tele-dermatoscopy, tele-otoscopy, telemedicine stethoscope.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Authors' contribution

Conceptualization, Nicolau Carmen Daniela and Luiza Enache methodology, Cormos Bogdan; software, Adrian Tarta validation, and formal analysis, Adrian Saftoiu, investigation, Remus Sipos resources, Nicolau Carmen Daniela; writing—Nicolau Carmen Daniela; writing—review and editing, Adrian Saftoiu.; project administration, Bogdan Cormos. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted following the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Lotus Image Medical Center (protocol code 10 / 22.10.2022)." for studies involving humans.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Assessment of Cerebral Tumors and Metastases by Two-Photon Excitation Microscopy

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Abstract: *Objective: The study subject approaches a topic of cerebral pathology, namely the development of a pathological diagnosis that is as accurate as possible for the cerebral tumors and the metastases, using a new method that complements the optical microscopic examination and immunohistochemistry to correctly diagnose cerebral malignancies. Methods: Two-photon excitation (TPE) microscopy techniques are non-linear optical imaging methods that are gaining momentum in the investigation of fixed tissue sections, fresh tissue, or even for in vivo experiments. This method can be used for offering complementary information on the tissue architecture. We argue that using non-linear optical microscopy (which includes TPE microscopy) images collected on this category of brain tissue samples, can facilitate the interpretation of brain tumors and metastases, thus it is a solution worthy to explore. Scanning large areas of the lesions is important to avoid false-negative diagnoses because lesions are often non-uniform. Finding potential invasion sites is also restricted when using a narrow field-of-view imaging method. Results: Our study demonstrated that TPE microscopy can detect specific cellular features of cerebral tumors and metastases in good correlation with histopathological results. This approach can improve the accuracy of the cerebral tumors diagnosis and possibly other neoplasms. Conclusion: TPE microscopy is very important for future research because it could prevent the false or inadequate diagnosis of specific lesions and differentiate a primary tumor from a metastasis. The significance of the findings is attributable to cross-disciplinary cooperation and the constant use of a working standard across all of the experiments in this study.*

Keywords: *Two-photon excitation (TPE) microscopy, cerebral tumors, metastases, non-linear optical microscopy*

INTRODUCTION

Cerebral tumors are common in the general population and can present with focal neurological symptoms, seizures, or headaches, while some are asymptomatic and found incidentally. Cerebral metastases, meningiomas, and gliomas, particularly glioblastoma, are the most frequent types of cerebral tumors. Complex coordination of treatment between medical oncologists, radiation oncologists, neurosurgeons, and primary care physicians is necessary for the management of brain tumors and their consequences.

The term "primary cerebral tumors" refers to primary intracranial tumors that originate from the meninges, neuroepithelial tissues, pituitary and associated structures, cranial nerves, germ cells, blood-forming organs, or a distant subclinical primary tumor. In the United States, with an estimated 23,380 new cases identified in 2014 and 14,320 fatalities, malignant tumors are uncommon in adults. They accounted for 1.4% of all new cases of cancer and 2.4% of all cancer deaths. New cerebral tumors occur at a rate of 6.4 per 100,000 people annually, and their overall five-year survival rate is 33.4%. The peak occurrence follows between the ages of 55 and 64, and men are slightly more likely than women to experience it [1].

Primary cerebral tumors have a complex etiology, and genetics undoubtedly play a part in how they arise. The only environmental risk factor for primary brain cancers that has been scientifically validated is high-dose ionizing radiation exposure. There is little evidence that additional toxins or exposures raise the risk [2,3].

The World Health Organization assigns malignancy-grade tumors based on a combination of morphological characteristics, growth patterns, and molecular profiles. Primary cerebral tumors are categorized based on histopathologic criteria and immunohistochemistry data [4].

Primary cerebral tumor clinical symptoms might be general or specific. The cause of general symptoms like headaches and seizures is elevated intracranial pressure [5]. The compression or tissue breakdown of specific regions causes focal symptoms, such as unilateral weakness or personality abnormalities [6]. Early signs of low-grade tumors or early stages of illness are frequently focal before becoming more generalized as the tumor grows and spreads [7].

The typical perception of a tumor headache is that it is intense, worse in the morning, and accompanied by nausea and vomiting. But bifrontal, tension-type headaches are more frequently reported in people with cerebral tumors [8].

There is no distinct pathognomonic marker on imaging that distinguishes between main cerebral tumors and metastatic or nonneoplastic disease, despite the fact that imaging considerably reduces the differential diagnosis of primary cerebral tumors. Chest and abdomen CT may be helpful in situations of suspected or pathologically confirmed metastatic cancer, but pinpointing the location of the source tumor is frequently challenging, particularly when there are no clinical cues from the history and physical examination [9].

A skilled interdisciplinary team made up of experts in neurosurgery, radiation oncology, and medical oncology makes customized treatment decisions for each patient. The nature and location of the tumor, its likelihood of being malignant, the patient's age, and general health all factor into treatment considerations. Although surgery, radiotherapy, chemotherapy, or a combination of these may be used as the sole form of treatment, registration in clinical trials should be made available as an option for some high-grade malignancies [10].

The most safe and effective surgical removal of the tumor, followed by radiotherapy and chemotherapy, is the preferred course of treatment for primary brain tumors. Although the size of the resection is a predictive factor, the size of a safe tumor resection depends on the location of the tumor, the patient's performance, and most crucially, the patient's age [11].

After surgical resection, radiotherapy may be administered as the main treatment or as an adjuvant. Although brachytherapy, fractional stereotactic radiation, and stereotactic radiosurgery are other alternatives, standard fractionated external beam radiotherapy is the most used method. In some circumstances, combining radiation and chemotherapy has been demonstrated to increase survival [12].

In addition, it was studied with two-photon excitation (TPE) microscopy on mouse brains, even in-vivo, which may have an important role in our study [19].

This microscopy technique can be applied in many fields including physiology, neurobiology, embryology, and tissue engineering. Thin, almost transparent tissues (such as tegument cells) can be visualized in sharp detail high-speed imaging capabilities can also be used in the non-invasive optical biopsy. In cell biology, TPE microscopy has been used to produce localized chemical reactions. To detect cells and collagen in the cornea using intrinsic fluorophores (autofluorescence and second harmonic generation) as well as using extrinsic fluorophores [18].

Fluorescence microscopy based on (TPE) is a fluorescence-based imaging technique that allows imaging of tissue up to about one millimeter in depth due to reduced absorption and much lower scattering of emitted light. It has recently been applied in pre-clinical studies [18].

Fluorescence microscopy based on TPE does not require a filter in the emission path because it minimizes the loss of emitted radiation. The attenuation of excitation radiation due to scattering is also reduced because the scattering cross-section decreases with increasing wavelength. Near-infrared radiation used in two-photon excitation has several orders of magnitude less absorption in biological tissue than UV or blue-green radiation. A clear separation is achieved between the excitation and emission spectrum, ensuring the removal of excitation radiation and Raman scattering [20].

The discovery of potential sites of invasion is restricted by a limited wide field of view. One option for increasing the field of view, to be used in future research, is the acquisition of adjacent images and stitching these by using mosaicking algorithms. High penetration depth is important for imaging abnormal cells in cerebral tissue samples [20].

Over the past two decades, significant efforts have been made to advance the state-of-the-art in the clinical diagnosis of malignant dermatological disorders (e.g., non-melanoma skin cancers) [18]. However, the gold standard for the identification of such disorders continues to be conventional histopathology assays that call for the removal of small tissue fragments, fixation, staining, and subsequent bright-field microscopy examination. However, it must be remembered that these methods have significant drawbacks, including sample error, sluggishness, high costs, and interpretation unpredictability [13]. Due to these restrictions, there is a significant demand for faster, less invasive alternative diagnosis approaches that maintain the benefits of traditional histopathology, particularly those that relate to simple picture interpretation. These would steadily enhance the diagnostic workflows, lower related expenses, and finally result in increased survival rates of the patients [14].

Magnetic resonance imaging, optical coherence tomography, and more recently non-linear optical microscopy are some of the most promising approaches that have been put out to date for brain diseases. TPE microscopy is a prominent non-linear optical technique that offers supplemental data that has been shown to date to be extremely beneficial for determining tissue conditions. TPE microscopy entails the simultaneous absorption of two photons whose combined energy is sufficient to cause a transition to an excited state and the ensuing emission of a single photon with an energy that is only marginally less than the combined energy of the two excitation photons as a result of non-radiative processes (e.g., vibrational relaxation) [15].

Optical microscopy is a technology that can collect size and shape details from human tissues, but when the cell structure becomes poorly differentiated, the features of this technique are limited. The main limitations of optical microscopy are related to resolution (implicit in light diffraction) and the wavelength of the radiation used. So, the modifications that can be made to improve the spatial resolution and image contrast are by using radiation with shorter wavelengths, increasing the numerical aperture, or using a medium with a high refractive index. Using even a microscope with very good quality lenses, a well-aligned system, and the largest aperture, the resolution remains limited to half the wavelength used. Confocal scanning microscopy and multi-photon microscopy bring an improvement in both spatial and axial resolution limits [20].

The TPE microscopy technique is a non-linear optical imaging method. It is based on the excitation of a fluorophore by the simultaneous absorption of two photons in a single event. This method can be used to provide complementary information on tissue architecture [16].

In the case of two photons excitation, it is necessary that the wavelength of the photons is twice the wavelength of the photons used in the case of excitation using a single photon to obtain the same excitation energy, which corresponds to the difference between the energy of the ground state of the molecule and the of the excited state. At the same time, the two photons must reach the sample in a very short time (less than 10-16 s).

Since the emission of fluorescence by two-photon excitation depends on the simultaneous absorption of two photons with infrared wavelengths, the probability of two-photon absorption by a fluorescent molecule is a quadratic function of the excitation radiance [17].

MATERIALS AND METHODS

The use of nonlinear optical microscopy images (which includes TPE microscopy) collected on this category of cerebral tissue samples can facilitate the interpretation of cerebral tumors and metastases and is thus a solution worth exploring. Scanning large areas of lesions is important to avoid false-negative diagnoses, as lesions are often non-uniform.

In order to achieve an excitation based on the coupling of two photons (which has a low probability), it is necessary to have a lot of photons (thus strong laser radiation) at the same time and in the same place. This thing is achieved using a Ti: Sapphire laser with femtosecond pulses, where basically the photons will be in these pulses, and placing them in the same place is done with high numerical aperture objective lenses that focus the radiation in very narrow areas. As an excitation source, we used a Ti: Sapphire laser (Chameleon Ultra II, Coherent) tuned at 870 nm, with ~140 fs pulses and a repetition rate of 80 MHz. A 10× magnification and 0.3 numerical aperture (NA) objective was used for focusing the laser beam on the sample and for collecting the two-photon excitation signals.

The material was collected from patients who had a histopathological diagnosis after surgery ("Dr. Carol Davila" Central Military Emergency Hospital Bucharest). Tissue fragments were fixed in 10% formalin, embedded in paraffin, with paraffin block sections – 4-5 microns thick. Images were acquired on labeled samples (Hematoxylin & Eosin stained) on the basis of which histopathological diagnoses were made under a conventional light microscope and fluorescence was from Eosin.

The macroscopy technique has been adapted to the particularities of each individual surgical specimen provided that the following requirements are:
- macroscopic description of the specimen and all macroscopic lesions;
- taking fragments of all macroscopically identified lesions, as far as possible including both macroscopically pathological and normal tissue in the same fragment;
- harvesting of fragments from the resection margin; if the macroscopically identified lesion was at a great distance from the resection margin (not being possible to include both lesion and resection margin in the same tissue fragment at the same time), the resection margin could be harvested parallel to the

plane of the incision; if the lesion was located close to the resection margin, harvesting perpendicular to the plane of the incision so that, after microscopic examination, the distance between the lesion and the resection margin could be measured. In the case of tumor pathology, this type of approach makes it possible to establish the completeness of the resection within oncological safety limits.

The final product is the permanent histopathological preparation which allows correct interpretation to establish the pathological diagnosis.

It worked according to the protocol of the Pathology Department of the Central University Emergency Military Hospital. For example, different reagents may be used, and the time of action and concentrations mentioned in the recipes are indicative and are adapted according to local conditions (ambient temperature, hardness, and pH of tap water). The technique used is considered correct if the results expected by the staining are obtained. Variations in the recommended staining recipes are allowed, depending on the structure to be revealed.

RESULTS

The standard of the histopathological technique requires the transformation of an organ or tissue specimen from an opaque tissue mass into a fine, translucent preparation that allows microscopic visualization of tissue structural details.

We made an analysis of the images acquired with fluorescence microscopy based on TPE and we'll try in future research to develop the earliest possible diagnosis of cerebral tumors based on distribution and tissue architecture. We acquired images for both a primary cerebral tumor and a secondary (metastatic) tumor.

Figure 1: Cerebral adenocarcinoma metastasis - (a), (b), (c) - Bright-field microscopy image of Hematoxilin&Eosin-stained section - low magnification: (a) cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (top left corner) (b) cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (in the center of the image), (c) cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (bottom left corner); (d) TPE microscopy image for stained section (a), which clearly shows the desmoplastic stroma with collagen fibers by the bright orange-yellow; (e) TPE microscopy image for stained section (b); (f) TPE microscopy image for stained section (c).

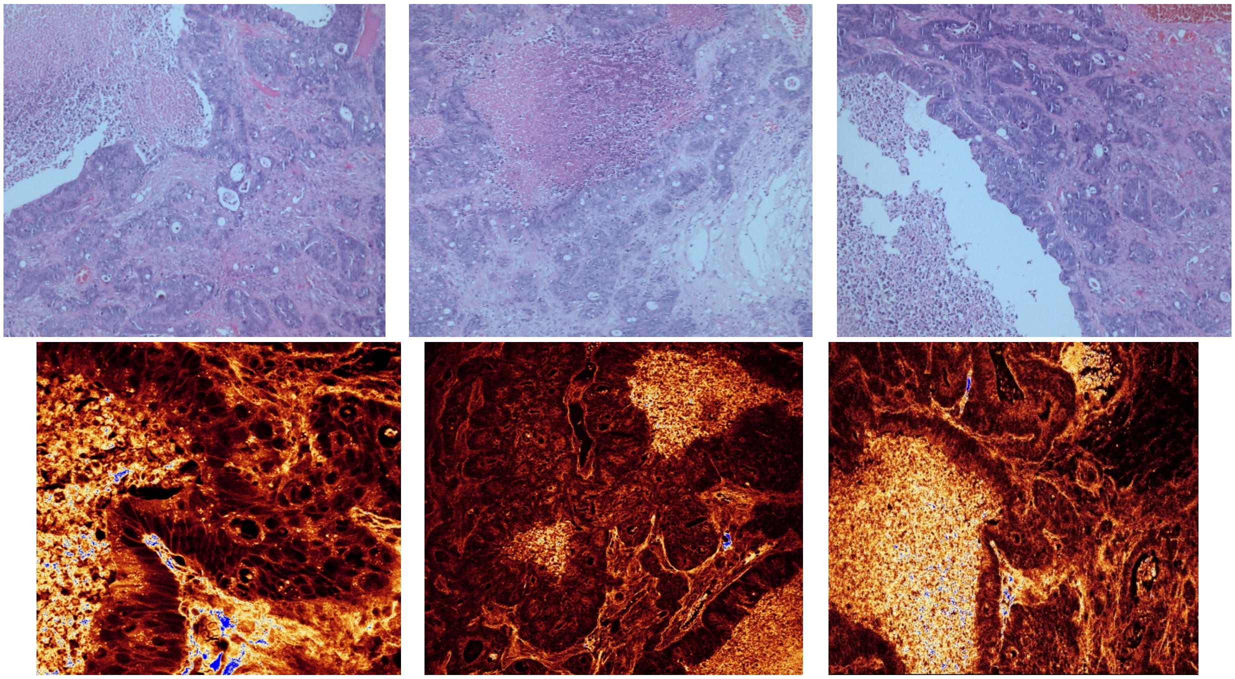

In Figure 1 (a) – cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (top left corner), 1(b) – cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (in the center of the image) and 1 (c) – cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (bottom left corner) acquired images, we can visualize tumor proliferation composed of medium-sized cells grouped in glandular structures with a cribriform, tubular and solid pattern, associated with extensive areas of necrotic debris and desmoplastic stroma – like a colonic adenocarcinoma metastasis in the right occipital lobe, with a history of a colon neoplasm.

In Figure 1 (d) (e) (f) acquired TPE microscopy images we can observe the glandular tumor structures represented in dark red, and the lakes of necrotic debris and desmoplastic stroma with collagen fibers in bright orange-yellow, with the mention that the areas of necrotic debris are punctate and brighter, and the collagen fibers in the desmoplastic stroma are represented by linear-wavy curves.

Figure 2: Atypical meningioma - (a)(b)(c) - Bright-field microscopy image of Hematoxilin&Eosin - stained section - low magnification; (a) Atypical meningioma with spindle cells with increased cell density areas; (b) Atypical meningioma with true psammoma body (black arrow); (c) Atypical meningioma with early ages psammoma bodies according to their level of calcification) and vascular spaces; (d) TPE microscopy image for stained section (a); (e) TPE microscopy image for stained section (b) – psammoma body (white arrow); (f) TPE microscopy image for stained section (c), but with high magnification to highlight the vascular spaces detectable on the Hematoxilin&Eosin-stained section (light blue).

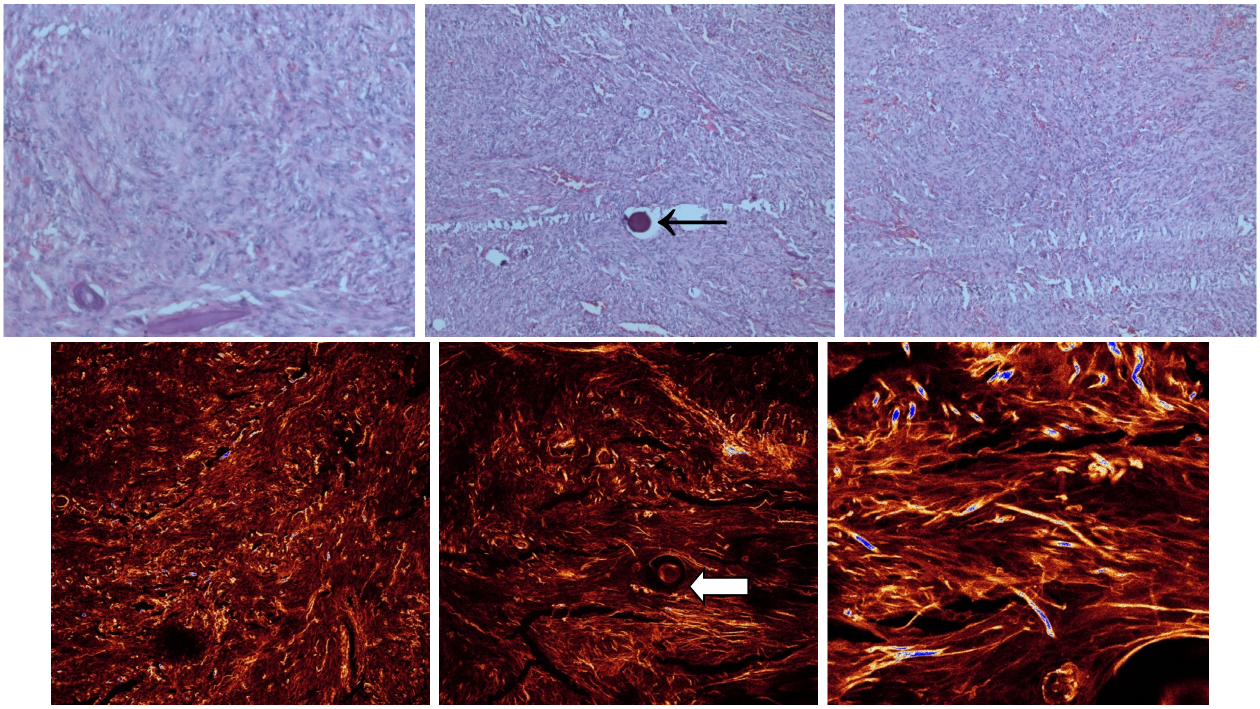

In Figure 2 (a), (b), (c) images, we detect a primary cerebral tumor in the left parietal lobe. We can observe on the Hematoxilin&Eosin-stained section, a tumor proliferation consisting of spindle cells with increased cell density, cellular atypia, and the presence of calcospherites well represented also in two-photon excitation microscopy from Figure 2 (d), (e), (f). Calcospherites or psamomma bodies are concentric calcifications of meningothelial cell nests (concentric laminated eosinophilic bodies) with different ages depending on their level of calcification. A true calcospheritis is well represented (black arrow) in a Bright-field microscopy image of Hematoxilin&Eosin – Figure 2(b). Histopathological features are consistent with atypical meningioma.

In Figure 2 (f) Corresponding two-photon microscopy image for stained section (c) – low magnification, we acquired two-photon excitation microscopy images at high magnification to highlight the vascular spaces (light blue) detectable in the standard Hematoxylin&Eosin-stained section.

DISCUSSION

Our study demonstrated that TPE microscopy can detect specific cellular features of atypical meningioma (primary cerebral tumor) and colonic adenocarcinoma (cerebral metastases) in good correlation with histopathological findings and even differentiate them.

Although such efforts are very valuable and play an important role in facilitating the interpretation of nonlinear optical data, it should be noted that a universal image processing or artificial intelligence method capable of generalizing and accurately addressing all tissue types is still lacking.

The use of this technique will also avoid the overuse of other relatively expensive complementary diagnostic techniques currently available (molecular biology techniques, genetic tests, IHC tests, electron microscopy, etc.).

The importance of the results obtained is due to interdisciplinary collaboration and the consistent application of a working standard throughout the experiments in this work. TPE microscopy has allowed us to obtain a global image of tissue because fluorescence has this advantage. Used as an additional technique with applicability in pathology could help to avoid underdiagnosis or overdiagnosis of some lesions, whose correct diagnosis is essential for proper and effective treatment.

CONCLUSION

Our research demonstrates that TPE microscopy can differentiate a cerebral metastasis from an atypical meningioma (primary cerebral tumor). Also with the help of two-photon excitation microscopy, it is possible to detect cellular, glandular, and vascular spaces, calcospherites in different stages, necrotic debris, and desmoplastic stroma structures. All of these are important tissue elements in diagnosing and differentiating certain tumor entities.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

All authors had equal contributions to this study elaboration.

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ORIGINAL ARTICLE

An Overview of Panniculitis – A Diagnostic Challenge for Dermatologists and Pathologists

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Abstract: *The term "panniculitis" refers to a heterogeneous group of conditions that involve inflammation of the subcutaneous cellular tissue. Although there is no universally accepted classification of panniculitis, to facilitate the diagnosis, they were divided into two large classes, based on the histopathological criteria: predominantly lobular and predominantly septal panniculitis. Depending on the presence or absence of vascular damage, each of the two classes mentioned above is divided in turn into panniculitis without vasculitis and panniculitis with vasculitis. The diagnosis of various types of panniculitis can sometimes be very difficult, due to the histopathological changes that occur in the various evolutionary stages of the lesions. Thus, the positive diagnosis relies on the corroboration of clinical data, histopathological examination, and ancillary laboratory tests. We provide an overview of the clinical and histopathological features of panniculitis, classified according to the histopathological criteria.*

Keywords: *panniculitis; inflammation; vasculitis; necrosis; subcutaneous tissue*

INTRODUCTION

The term "panniculitis" refers to a heterogeneous group of conditions that involve inflammation of the subcutaneous cellular tissue. The clinical and histopathological diagnosis of panniculitis is difficult to establish, this group of conditions being considered "the ugly duckling of dermatopathology", firstly because their deep localization does not allow the clinician to identify characteristic aspects for each type of panniculitis and secondly because of the inadequate sampling of the biopsy piece, which is often not deep enough.

Clinically, most panniculitis is characterized by the occurrence of erythematous nodules, that are not pathognomonic for a particular clinical subtype. A careful anamnesis, location, and evolution of the lesions, symmetry, age of onset, and association with systemic signs and symptoms must be taken into consideration for the clinical diagnosis.

Histopathological examination is a useful tool for a positive diagnosis. It is necessary to perform a deep incisional skin biopsy, which should also include subcutaneous cellular tissue, from an early-stage, evolving nodular lesion. Lesions in advanced stages usually show microscopic changes of lipoatrophy and sclerosis, leading to an inconclusive result of the histopathological examination [1,2].

Several histopathological features need to be considered in order to correctly classify panniculitis, mainly the location of the inflammatory infiltrate (septal, lobular) and the presence or absence of associated vasculitis. Based on these criteria, panniculitis is divided into septal and lobular panniculitis with or without vasculitis and if it exists, the size of the affected vessels can guide the diagnosis. Further details such as the type of adipocyte necrosis, the type of inflammatory cells, or the presence of granulomas may contribute to a more precise diagnosis. Although it is considered that all panniculitis is mixed panniculitis since the inflammatory infiltrate can be highlighted both in the lobules and the septa, based on where it predominates the condition may be classified into a predominantly lobular or a predominantly septal form [1,3].

Necrosis of adipocytes is another aspect that can be observed during the histopathological examination of panniculitis, these appearing either in the form of elements that have completely lost their cellular structure or in the form of anucleated cells, depending on the type of necrosis.

The most frequently encountered type of adipocyte necrosis is lipophagic necrosis, the so-called foamy macrophages, loaded with lipids, obtained by digesting dead adipocytes, being objectified during microscopic evaluation. Their cytoplasm can be granular or pale, with multiple microvacuoles. Lipodermatosclerosis and traumatic panniculitis are typical examples of this type of necrosis [1,3,4].

The hyalinizing necrosis of the adipose tissue consists of adipocytes with a homogeneous glassy protein material in the cytoplasm, altering the cellular structure. This type of necrosis is most often found in lupus panniculitis [1,3,4].

Another type of adipocyte necrosis is represented by liquefactive necrosis, which consists of granules formed by amphophilic detritus. This type of necrosis is frequently encountered in pancreatic panniculitis and in alpha-1-antitrypsin deficiency panniculitis. In pancreatic panniculitis, a subtype of liquefactive necrosis is objectified, the so-called enzymatic necrosis, which consists in the saponification of the cytoplasmic lipids in the adipocytes by the circulating pancreatic lipase [1,3,4].

Ischemic necrosis of the adipose tissue usually occurs in the center of the lobules, initially as anucleated adipocytes, but if the ischemia persists, lipophagic necrosis changes can also be observed, as seen in erythema induratum of Bazin [1,3,4].

Membranous necrosis of adipocytes represents an advanced stage of necrosis that consists of the appearance of an amphophilic or eosinophilic border of degraded cellular organelles. This type of necrosis is a histopathological change constantly encountered in sclerodermiform dermohypodermitis, but it is not specific to it, as it can be observed in almost all advanced stages of panniculitis [1,3,4].

The final aspect to take into account during the microscopic examination of the skin fragment is the type of cells in the inflammatory infiltrate [1,3,4].

In predominantly lobular panniculitis, in which the histiocytic inflammatory infiltrate predominates, septal sclerosis associated with a history of radiotherapy is suggestive of post-radiation panniculitis, while the microscopic objectification of crystals may be suggestive of panniculitis associated with gout, fat necrosis of the newborn, crystal storing panniculitis or panniculitis after administration of corticosteroids. Evidence of an inflammatory infiltrate rich in histiocytes, with no evidence of crystal, should direct the diagnosis towards posttraumatic panniculitis, lipoatrophy, or subcutaneous sarcoidosis, while cytophagocytosis suggests a possible cytophagic panniculitis [5].

In predominantly lobular panniculitis, in which an inflammatory infiltrate rich in neutrophils associated with foreign bodies is evident, we lean towards factitial panniculitis, while the presence of bacteria, fungi, or protozoa suggests infectious panniculitis. In the case of a predominantly lymphocytic infiltrate, the presence of lymphoid follicles and plasma cells is suggestive of the diagnosis of lupus panniculitis, while the location of infiltrate perivascularly in the deep dermis and subcutaneous adipose tissue tilts the scale towards the diagnosis of cold panniculitis. Last, but not least, the infiltrate without inflammatory cells leads to the diagnosis of sclerema neonatorum, if it is associated with needle-shaped crystals located in the center of fat cells, to calciphylaxis or oxalosis if calcifications are observed in the blood vessels or to sclerosing panniculitis if the microscopic examination shows necrosis in the center of the fat lobules [5].

In predominantly lobular panniculitis associated with vasculitis, the size of the affected blood vessels must be determined, venules damage suggesting either neutrophilic nodular panniculitis, Lucio phenomenon, or erythema nodosum leprosum, while damage of the arteries and veins is highly suggestive for erythema induratum, also known as nodular vasculitis [4,5].

In predominantly septal panniculitis that does not associate vascular damage, in which the histiocytic inflammatory infiltrate predominates, the radial granulomas in the septa are suggestive of erythema nodosum, while the microscopic objectification of cholesterol clefts may be suggestive of xanthogranulomatous necrobiosis. Evidence of an inflammatory infiltrate rich in histiocytes, associated with fibrin directs the diagnosis toward a rheumatoid nodule, while the association with mucin suggests a possible subcutaneous annular granuloma [5].

The histopathological examination of an inflammatory infiltrate made up of lymphocytes and plasma cells, associated or not with granulomatous infiltrate, in a patient with predominantly septal panniculitis tilts the scale towards the diagnosis of necrobiosis lipoidica, respectively deep morphea.

Predominantly lobular panniculitis is divided into forms with vasculitis of large vessels, such as nodular vasculitis, and forms without vasculitis, such as alpha-1-antitrypsin deficiency, cold panniculitis, sclerosing panniculitis, calciphylaxis, lupus panniculitis, pancreatic panniculitis, infectious panniculitis, traumatic panniculitis, polymeric microemboli from the coating of intravascular devices and histiocytic panniculitis with cytophagy and subcutaneous T-cell lymphoma.

Predominantly septal panniculitis without vasculitis may be found in erythema nodosum, rheumatoid nodule, lipoid necrobiosis, and eosinophilic fasciitis [6,7].

Predominantly septal panniculitis associated with vasculitis is divided according to the caliber of the affected vessels into panniculitis with vasculitis of large vessels and panniculitis with vasculitis of small vessels. If the affected vessels are represented by arteries, the suspected diagnosis is cutaneous polyarteritis nodosa, while the damage of veins suggests potential superficial thrombophlebitis. Venule involvement in a patient with panniculitis indicates leukocytoclastic vasculitis [8].

MOSTLY LOBULAR PANNICULITIS WITH VASCULITIS

Nodular vasculitis (Erythema induratum)

Nodular vasculitis is the most common form of predominantly lobular panniculitis with vasculitis, firstly described by Bazin in 1861, as firm, erythematous, indurated plaques and nodules, located predominantly on the dorsal side of the calves, more frequently in middle-aged obese women, with a history of thrombophlebitis, chronic venous stasis or cooling [9].

Although tuberculosis is the most frequent infectious triggering factor incriminated in the etiopathogenesis of nodular vasculitis, this being considered a "reactive disorder", there are also cases of the disease in which no trigger factor has been identified [10,11]. Many factors have been cited in the etiopathogenesis of erythema induratum, both non-infectious (hypothyroidism, chronic lymphocytic leukemia, superficial thrombophlebitis, rheumatoid arthritis, various drugs) and infectious (tuberculosis, group B and C hepatitis, Fusarium, Nocardia, Pseudomonas).

Clinically, erythema induratum appears as recurrent episodes of firm, indurated, erythematous nodules and plaques, located symmetrically on the dorsal side of the limbs, where the adipose tissue is well represented and the covering skin is cyanotic and cold. Nodules appear slowly, usually during winter and some of them ulcerate, discharge an oily material, then slowly heal, leaving atrophic scars.

Nodular vasculitis is characterized by a granulomatous type of inflammation that occurs as a specific response mediated by T lymphocytes to an antigen or as a type IV hypersensitivity reaction [12].

Acute cases of erythema induratum and infectious panniculitis present similar microscopic features, such as basophilic zonal necrosis and neutrophilic inflammatory infiltrate, which is why cultures and special stains for bacteria and other microorganisms are performed to exclude an infectious cause of panniculitis. In nodular vasculitis, microorganisms are never detected on special stains [13].

Histopathologically, nodular vasculitis is predominantly lobular panniculitis. In the early stages, a discrete inflammatory infiltrate with neutrophils can be detected in the lobules, but during the course of the disease, extensive areas of ischemic necrosis of the lobular adipocytes can be observed. The necrotic adipocytes are ingested by the histiocytes that later transform into foam cells. The histopathological examination of the nodular vasculitis lesions shows a characteristic aspect of granulomatous inflammation, the inflammatory infiltrate is composed of multinucleated giant cells, epithelioid histiocytes, and lymphocytes. In case of severe vascular damage, extensive areas of caseous necrosis can be noted, microscopically similar to tuberculosis lesions [14]. The type of inflammatory infiltrate also varies depending on the age of the lesions, so that in the early stages an inflammatory infiltrate predominantly composed of neutrophils is observed, while in the advanced stages of the disease, the inflammation is lymphocytic or granulomatous.

Once the histopathological diagnosis is established, it is necessary to evaluate a possible association with tuberculosis. In this sense, a chest x-ray, a Mantoux tuberculin test, and/or an interferon-gamma release assay test are performed. If one of the last two is positive, the patient will follow 9 months of triple tuberculosis therapy [15,16].

MOSTLY LOBULAR PANNICULITIS WITHOUT VASCULITIS

Panniculitis due to alpha-1-antitrypsin deficiency

Alpha-1-antitrypsin (AAT) is a circulating inhibitor of serine proteases. It inhibits the enzymes involved in the proteolytic degradation of tissues, has effects on the immune system, and inhibits complement activation. Severe deficiency can be associated with systemic diseases, such as pulmonary emphysema, pancreatitis, liver cirrhosis, glomerulonephritis, rheumatoid arthritis, vasculitis, and/or angioedema. Rarely, AAT deficiency is associated with recurrent lobular panniculitis and necrosis of the subcutaneous fat tissue. Patients with a partial deficiency may present panniculitis without systemic symptoms.

Clinically, panniculitis due to alpha-1-antitrypsin deficiency appears as recurrent, painful, erythematous plaques and nodules, with a proximal distribution on the hips, thighs, and buttocks, being most often induced by local trauma.

Characteristic of this type of panniculitis is the fact that the nodules ulcerate and suppurate, discharging an oily fluid, and heal with scarring.

The diagnosis is established on clinical, paraclinical, and histopathological criteria. The dosage of the serum AAT level and the evaluation of the AAT phenotype is indicated.

Histopathology shows in the early stages neutrophilic panniculitis in the reticular dermis and between the collagen bundles, followed by necrosis and destruction of adipocytes. Even though primary leukocytoclastic vasculitis is absent, secondary lymphocytic vasculitis may be found in areas with heavy neutrophilic infiltration and thrombosis [17].

Cold panniculitis

The cold panniculitis is a form of panniculitis that occurs most frequently in children exposed to extremely low temperatures or during winter, in people wearing tight pants that do not provide adequate thermal insulation and restrict blood supply to the subcutaneous fatty tissue [18,19].

Clinically, one can notice marbled, reddish-blue plaques similar to frostbite, located on the thighs and buttocks. If the affected skin areas are kept warm, the skin lesions disappear after a few days, without scars.

During the histopathological examination, an inflammatory infiltrate consisting of histiocytes and lymphocytes located in the adipose lobules can be noted, sometimes associated with edema in the papillary dermis and an extension of the inflammatory infiltrate in the dermis, the microscopic appearance being similar to that of perniosis [20].

Sclerosing panniculitis

Sclerosing panniculitis, also known as sclerodermiform dermohypodermitis or lipodermatosclerosis, is a complication of venous stasis, more common in women characterized by chronic inflammation, fibrosis, and progressive induration [21, 22].

Clinically, in the early stages, lipodermatosclerosis appears as erythematous-purple, indurated, imprecisely demarcated, painful plaques, similar to cellulitis, while in late stages, it appears as a woody plaque, with well-defined edges, hard on palpation. Fibrosis is accentuated in the subcutaneous tissue, and eventually, the leg takes on the appearance of an inverted champagne bottle.

The main etiological factor of lipodermatosclerosis is chronic venous insufficiency of the lower limbs, causing sludging in the lobular capillaries, with ischemia and necrosis of the central portion of the adipose lobules. [23]

Other factors involved in the etiopathogenesis of lipodermatosclerosis are represented by reduced plasma fibrinolytic activity, protein S and C deficiency, and excessive collagen production [24,25].

Histopathologically, the characteristic changes of stasis dermatitis are noticeable in the superficial dermis, while in the papillary dermis, there is a proliferation of capillaries and venules, fibrosis, and abundant deposition of hemosiderin. In the early stages, small amounts of inflammatory infiltrate, predominantly composed of lymphocytes, distributed among the collagen bundles of the septa are found, while in the center of the lobules, "ghost" outlines of the anucleated necrotic adipocytes can be observed. In the advanced stages of the disease, septal sclerosis is the main histopathological change. Other histopathological changes that can be objectified are represented by foci of necrotic keratinocytes, simultaneously with areas of extravasated erythrocytes and vascular congestion [24,25].

The diagnosis of lipodermatosclerosis is based on clinical criteria, the biopsy not be indicated due to the difficult healing given by the vascular damage and the risk of developing ulcers at this level. In the advanced stages of the disease, sclerosing panniculitis requires a differential diagnosis with conditions associated with diffuse induration in the lower limbs, such as scleromyxedema, morphea, erythema nodosum, myxedema, necrobiosis lipoidica, eosinophilic fasciitis, as well as another panniculitis.

Calciphylaxis

Calciphylaxis is a condition with a high mortality rate that occurs frequently in patients with chronic kidney disease undergoing dialysis. Calcium deposits in the middle tunic of the small arteries from the skin lead to thrombosis and ischemic necrosis with the appearance of refractory ulcers involving the lower extremities, the abdomen, and the gluteal region [26]. Major risk factors for calciphylaxis are end-stage renal disease, phospho-calcium metabolism imbalance, and primary and secondary hyperparathyroidism, while non-uraemic risk factors include female sex, obesity, diabetes, hypo-albuminemia, autoimmune diseases, warfarin use, protein C and S deficiency, active neoplasia or liver damage in a toxic-metabolic context [27].

Clinically, the lesions are similar to those in livedo reticularis, appearing as marbled, red-violet teguments, with reticular discoloration, reflecting the impaired vascular flow from the superficial dermis. Another variant of the appearance of calciphylaxis lesions can be painful erythema, with a marbled appearance and a sudden onset on the calves or on the feet, evolving into hardened plaques that later become necrotic and ulcerated. Other skin signs may include pathergia (ulceration triggered by minor trauma), recurrent digital ischemia, penile necrosis, and subcutaneous nodules on the breasts and abdomen. Extremely rare, patients can associate concomitant systemic damage such as proximal myopathy, aortic valve damage, or pulmonary calciphylaxis [26,27].

The main histopathological aspects objectified in patients with calciphylaxis are calcifications in small arteries, vascular thrombosis, and intimal hyperplasia (endarteritis obliterans) [28].

Lupus panniculitis

Lupus panniculitis is a chronic form of recurrent panniculitis, which affects women more frequently, the skin lesions being predominantly located on the buttocks, arms, shoulders and face [29].

Clinically, it presents itself as deeply located subcutaneous plaques or nodules, while the adjacent skin may present lesions of discoid lupus erythematosus. The regression of lupus panniculitis lesions can result in the loss of subcutaneous cellular tissue, with atrophy, this being a deforming complication. Subcutaneous nodules can calcify or ulcerate [30].

During the histopathological examination, approximately half of the cases of lupus panniculitis show changes in the epidermis and dermis similar to those in discoid lupus erythematosus, such as vacuolization at the dermo-epidermal junction, epidermal atrophy, mucin interposition between the collagen fibers in the dermis, thickening of the basement membrane or the presence of a lymphocytic inflammatory infiltrate in the dermis, while in the other half of the cases, the pathological changes of lobular panniculitis are located strictly in the subcutaneous adipose tissue [31,32]. A quite characteristic aspect of this type of panniculitis is represented by the presence of lymphoid follicles, with a germinal center and numerous plasma cells on the periphery and between the collagen fibers of the septa of the subcutaneous adipose tissue [33]. Although they are quite specific for lupus panniculitis, they can be objectified on microscopic examination in other conditions as well, such as erythema nodosum, erythema induratum of Bazin, morphea, subcutaneous panniculitis-like T-cell lymphoma or in panniculitis associated with dermatomyositis. In the late stages of this disease, hyaline fibrosis and calcification can be noticed. Membranous necrosis is also common in this stage of the disease. In the microscopic examination of the lesions collected from these patients, an inflammatory infiltrate rich in eosinophils can be objectified. The low titer of antinuclear antibodies does not exclude the diagnosis of lupus panniculitis [34,35].

Dermatomyositis is another systemic collagenosis that can be associated with panniculitis and requires a differential diagnosis with lupus panniculitis. Vasculitis with lymphocytic or neutrophilic infiltrate, the thickening of the walls of the subcutaneous vessels, and the presence of calcifications tip the scaling towards the diagnosis of panniculitis associated with dermatomyositis [36].

Eosinophilic fasciitis and deep morphea present histopathological diagnostic criteria (plasma cell-rich lymphoid infiltrates, fibrous thickening of the interlobular septa, eosinophils occasionally) and clinical (nodules and indurated plaques) are similar to lupus panniculitis. However, the absence of hyaline necrosis and the absence of dermo-epidermal changes (epidermal atrophy, thickening of the basal membrane, orthokeratosis, vacuolar degeneration of the basal layer, prominent dermal mucin) exclude the diagnosis of lupus panniculitis.

Subcutaneous panniculitis-like T-cell lymphoma is characterized by the "rimming" of adipocytes by atypical cytotoxic T cells, which get intensely colored when in contact with proliferation markers. The absence of lupus skin signs and nodular aggregates of lymphoid B cells excludes the diagnosis of lupus panniculitis. From a clinical point of view, there are no differences between lupus panniculitis and subcutaneous panniculitis-like T-cell lymphoma, but the persistence of the lesions, their rapid evolution, and absence of regression point the diagnosis towards lymphoma.

Immunofluorescence studies can be performed, objectifying the presence of linear deposits of C3 and IgM along the dermo-epidermal junction [31].

Pancreatic panniculitis

The pancreatic panniculitis is a form of panniculitis rarely associated with certain pancreatic diseases, such as pancreatic islet cell tumors, acinar cell carcinoma, pancreatic pseudocysts, acute or chronic pancreatitis, ductal adenocarcinoma or post-traumatic pancreatitis [38].

Clinically, pancreatic panniculitis appears as erythematous or red-brown subcutaneous nodules, painful or not, sometimes with a soft center, usually located on the lower limbs, but they can also appear on the arms, buttocks, or trunk. In mild cases of the disease, they heal with atrophic scars, while in severe cases, they can ulcerate and discharge a sterile, brownish, oily material, representing necrotic fat [39]. It can be associated with pleurisy or necrosis of the periarticular tissue [40]. Skin signs can appear a few months before the manifestation of the pancreatic disease. Circulating lipase and amylase are responsible for adipose tissue necrosis in pancreatic panniculitis. Disseminated necrosis of fatty tissue is associated with a high rate of mortality [41].

In pancreatic panniculitis, unlike other predominantly lobular panniculitis, it is preferable to take a skin biopsy from an established lesion. The histology of pancreatic panniculitis is characteristic, an important necrosis of adipose tissue being observed, as well as the appearance of the so-called "ghost cells", which are the former adipocytes that now present a thick, ghostly wall, the cell membrane not being destroyed by lipase. The latter present in the cytoplasm a granular, basophilic material, called calcium soap, which resulted from the combination of fatty acids with calcium ions [42,43].

In the early stages of pancreatic panniculitis, the characteristics of predominantly septal panniculitis determined by the pancreatic enzymes that reach this level through the permeable vascular walls are noted. Identifying the specific microscopic aspects of pancreatic panniculitis can be difficult also in the advanced stages of the disease, in which the structure of the fat lobule is completely replaced by a granulomatous inflammatory infiltrate, made up of multinucleated giant cells and foamy macrophages.

Severe cases of pancreatic panniculitis, associated with a history of pancreatic damage, are relatively easy to diagnose, but the same does not happen in mild cases. These lend themselves to differential diagnosis with erythema induratum of Bazin, infectious panniculitis, erythema nodosum, panniculitis associated with alpha-1 antitrypsin deficiency, aspergillosis, subcutaneous mucormycosis, and subcutaneous metastatic deposits [44,45].

MOSTLY SEPTAL PANNICULITIS WITH VASCULITIS OF THE LARGE VESSELS

Cutaneous polyarteritis nodosa

Cutaneous polyarteritis nodosa is a necrotizing vasculitis that affects the muscular layer of the small and medium arteries in the deep dermis and subcutaneous tissue. The vital organs are not involved, but the disease is recurrent and chronic [46].

Clinically, subcutaneous polyarteritis nodosa lesions manifest as painful skin nodules, associated with palpable purpura, ulcers, and livedo reticularis in the lower limbs. Usually, the presence of a pseudo-influenza syndrome characterized by low fever, unwell general condition, myalgia, and arthralgia is also observed [47].

During the histopathological examination, vasculitis lesions are observed in the small and medium-sized arteries and arterioles from the septa of the upper half of the subcutaneous adipose tissue. The affected vessels have thickened walls, presenting an inflammatory infiltrate composed predominantly of neutrophils. The intima of the affected vessels has a target appearance on the cross-section, presenting an eosinophilic ring of fibrinoid necrosis. Cutaneous polyarteritis nodosa is characterized by the segmental damage of the arterial vessels and by the simultaneous existence of lesions in different stages of evolution [46].

Direct immunofluorescence studies objectify the presence of complement and IgM deposits in the walls of the affected blood vessels [48].

MOSTLY SEPTAL PANNICULITIS WITHOUT VASCULITIS

Erythema nodosum

Erythema nodosum is the most common type of panniculitis in general and the main type of predominantly septal panniculitis without vasculitis. Most cases appear between the second and fourth decade of life, more frequently in women [49]. Erythema nodosum may be a delayed hypersensitivity response to infection or another inflammatory disease. In approximately one-third of the cases of erythema nodosum, a cause could not be identified, but in the rest of the cases, bacterial, viral, fungal, and protozoal infections, drugs, inflammatory bowel diseases, Behcet's disease, sarcoidosis, pregnancy, various neoplasms were blamed as triggering factors [50-52]. Streptococcal infection is the most common cause in children, while in adults different drugs, pregnancy, and sarcoidosis have been reported as the main triggering factors [53].

From a clinical point of view, erythema nodosum lesions appear abruptly as firm, tender, erythematous, non-purpuric, warm nodules, approximately 1-5 cm in diameter, symmetrically distributed on the calves, pretibial, ankles, and knees, after a prodromal period characterized by altered general condition, fever, headache, arthralgia, and myalgia. Rarely, nodules can also appear on the arms or trunk. The nodules from this pathology never ulcerate and they heal without scars. Sometimes, gastrointestinal symptoms can be associated, such as nausea, vomiting, abdominal pain, or diarrhea. In evolution, the nodular lesions in erythema nodosum flatten, leading to the appearance of hematoma-like lesions with a blue-green hue. The lesions fade within 2-6 weeks, even faster in children, leaving behind lesions with a purple appearance, similar to bruises, which disappear slowly [54].

A particular chronic form of erythema nodosum is represented by subacute migratory nodular panniculitis or erythema nodosum migrans, much less frequent, characterized by the appearance of a single firm, indurated, erythematous plaque, which slowly expands to the periphery while it heals centrally [55].

Histopathologically, in the early stages, the microscopic evaluation can objectify an abundant inflammatory infiltrate, rich in neutrophils, which could create diagnostic difficulties. The thickening of the septa is not very important, being caused by the accumulation of inflammatory infiltrate, edema and hemorrhage at this level, and cells around the cleft-like spaces in Miescher's radial granulomas are predominantly neutrophils. In the advanced stages, the inflammation is much reduced, being replaced by lipoatrophy and fibrosis [56].

Chronic erythema nodosum lesions are characterized by thickening of the septa and a slightly abundant inflammatory infiltrate, associating phlebitis and extravasation of erythrocytes, while in erythema nodosum migrans there is marked thickening of the septa from adipose tissue, the presence of multiple granulomas made up of granulation tissue, multinucleated giant cells, and an important capillary proliferation, but without signs of phlebitis [57]. Currently, most authors consider that chronic erythema nodosum and erythema nodosum migrans represent different stages of evolution of the same condition [3]. A quite specific histopathological aspect for chronic erythema nodosum is represented by Miescher's radial granulomas, which consist of small nodular aggregates, well delimited, of histiocytes, agglutinated around a banana- or stellate-shaped fissure, but it can also be identified in other conditions such as Behcet's disease, erythema induratum, Sweet syndrome, necrobiosis lipoidica or nephrogenic fibrosing dermopathy [58].

Necrobiosis lipoidica

Lipoid necrobiosis is a condition characterized by a granulomatous inflammatory process that extends from the deep dermis to the subcutaneous cellular tissue, leading to the appearance of predominantly septal panniculitis. There were no cases of lipoid necrobiosis affecting only the subcutaneous adipose tissue cited in the literature, its inflammation being always secondary to the inflammation of the deep dermis. The pathogenesis of granulomatous inflammation is not fully understood, but many cases of necrobiosis lipoidica have been associated with diabetes mellitus, although it does not occur exclusively in diabetic people [59].

The clinical picture consists in the appearance of oval, reddish-brown, well-demarcated papules and plaques, that may present telangiectasias on the surface, with clearly defined, erythematous, raised, shiny edges, usually located pretibial. The lesions progressively expand and they have a tendency to merge. In older plaques, the center is more yellow and can be atrophic, the subcutaneous vessels being visible through its transparency. The plaques can ulcerate, but the surrounding skin remains intact, showing, in general, some characteristics of necrobiosis lipoidica [59].

The histopathological features of necrobiosis lipoidica, which overlap with those of granuloma annulare, include palisading granulomas composed mainly of histiocytes, which surround the modified collagen fibers from the dermis, the inflammation being able to extend to the subcutaneous cellular tissue, affecting mainly the septa [59].

The immunofluorescence studies performed on lesions in the early stages of necrobiosis lipoidica objectify the presence of complement and IgM deposits in the walls of the vessels from the affected territories and sometimes even along the dermo-epidermal junction [60].

Eosinophilic fasciitis (Shulman’s syndrome)

Eosinophilic fasciitis is a form of localized scleroderma, which affects the deep fascia and sets in suddenly, most of the time being triggered by intense physical exercises. In the initial phase, it can mimic cellulite [61].

From a clinical point of view, eosinophilic fasciitis appears as symmetrical, edematous, reddish-brown plaques, which do not show sensitivity to palpation, usually located on the limbs, which evolve into brown, indurated plaques with a pale center. The extension of sclerosis in the subcutaneous adipose tissue restricts the movements of the tendons of the affected limb [62].

From a histopathological point of view, the lesions in eosinophilic fasciitis are similar to those in subcutaneous scleroderma. The microscopic examination shows thickening and edema of the interlobular fibrous septa and the deep fascia. Both in the septa and the fascia, an abundant inflammatory infiltrate is observed, consisting of eosinophils, lymphocytes, plasma cells, and histiocytes. Occasionally, lymphoid follicles are also present in the septa. The lesions extend deeply, to the underlying fascia, but unlike scleroderma, which usually does not present an inflammatory infiltrate, Shulman's syndrome is characterized by an abundant eosinophil infiltrate in the fascia [63,64].

CONCLUSION

One of the first steps that should be performed in the histopathological examination of lesions suspected of being panniculitis, is to establish whether it is primarily a panniculitis or if it is a dermatitis with extension to the subcutaneous fatty tissue, especially in the septa, as it happens in conditions such as sarcoidosis, lipoid necrobiosis or scleroderma.

Although there is no universally accepted classification of panniculitis, to facilitate the diagnosis, they were divided into two large classes, based on the histopathological criteria: predominantly lobular and predominantly septal panniculitis. Depending on the presence or absence of vascular damage, each of the two classes mentioned above is divided in turn into panniculitis without vasculitis and panniculitis with vasculitis.

Once these two criteria have been established, the type of inflammatory infiltrate in which histiocytes, neutrophils, lymphocytes, or no inflammatory cells may predominate, is also an important element in the diagnosis of panniculitis that does not associate vascular damage, but this may not bring additional information, or it can even delay the diagnosis, since, in this group of conditions, the histopathological picture varies according to the clinical age of the lesions. Most panniculitis lesions begin with an inflammatory infiltrate predominantly composed of neutrophils, after which they pass either through a granulomatous phase or through a phase in which the histiocytic inflammatory infiltrate predominates, while finally, in the late stages, the inflammation is reduced or even absent. Since it is a difficult mission for the clinician to identify with the naked eye the lesions of panniculitis in early stages, those that end up being biopsied are, most of the time, in advanced stages, which is why they do not provide specific information regarding the type of panniculitis.

The diagnosis of panniculitis is difficult, but there are clinical diagnostic keys (location of lesions, symmetry, age of onset) and histopathological ones (type and location of inflammatory infiltrate, presence or not of vasculitis, presence or not of liponecrosis, membranocystic changes, atypia or cellular necrosis). The corroboration of clinical data with histopathological ones and with the results of additional paraclinical explorations depending on the histological classification leads to a positive diagnosis.

The nosological classification of panniculitis is essential for the optimal therapeutic approach, with some cases only needing follow-up, and others having a severe, potentially fatal course in the context of systemic diseases, serious infectious diseases, or neoplasias.

Conflicts of interest and sources of funding

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Authors' contribution

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REVIEW

Pulmonary Hypertension in Autoimmune Diseases – A Review of New Concepts in Screening, Diagnosis and Therapeutic Management

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Abstract: *Pulmonary hypertension (PH) has gained more and more attention in the last years given the increased mortality and morbidity rate and in particular pulmonary arterial hypertension associated with autoimmune diseases (a specific subgroup of PH), entities with an increasing incidence and prevalence globally. Among these, systemic sclerosis is of particular interest, being the most common among connective tissue diseases (CTDs) with PH as a major complication. Other CTDs such as mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE), primary Sjogren's syndrome, and dermatomyositis may also develop PH as a complication of these severe and complex conditions, which can have a major impact on prognosis and quality of life. PH has also been reported in rheumatoid arthritis and ankylosing spondylitis but further studies are necessary. Several studies have shown that the 3-year survival rate for patients with systemic sclerosis-associated with pulmonary hypertension (SSc-PH) is between 31% and 52%, while the 5-year survival rate is below <50%. Therefore, an early and accurate diagnosis as well as subsequent management of PH in these conditions is mandatory, since survival remains suboptimal despite treatment advances. The aim of this article is to provide an overview of different autoimmune diseases associated with PH and to provide an easy reference source on current best practices.*

Keywords: *pulmonary hypertension; autoimmune diseases; connective tissue diseases; vasodilators; Raynaud phenomenon'*

INTRODUCTION

Pulmonary hypertension (PH) is a severe, progressive, and life-threatening disease that can often lead to premature death. Pulmonary hypertension (PH) describes a heterogeneous group of conditions that are defined by an increase in the mean pulmonary arterial pressure (mPAP) and pulmonary vascular resistance leading to an increase in the right ventricular (RV) afterload which results in RV overload and finally to right heart failure and even death. The most common causes of PH are left heart diseases and chronic lung diseases [1,2].

Patients with connective tissue diseases (CTDs) such as systemic sclerosis (SSc), mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE), primary Sjogren's syndrome, and dermatomyositis may develop PH as a complication of these complex conditions, which can have a major impact on prognosis and quality of life. In this review, PH is discussed among connective tissue diseases, with an emphasis on systemic sclerosis, which is the most common of all collagenoses that have PH as a complication. Therefore, new findings regarding epidemiology, early screening/ diagnosis, new therapeutic perspectives, and prognosis are brought to attention [3].

CLASSIFICATION

The current classifications of PH are based on clinical, physiopathological, and etiological similarities along with hemodynamic and treatment characteristics. PH is classified into five groups based on etiology: 1 – pulmonary arterial hypertension (PAH); 2 – pulmonary hypertension associated with left heart diseases; 3 – pulmonary hypertension associated with lung diseases; 4 – chronic thromboembolic pulmonary hypertension; 5 – pulmonary hypertension due to systemic or multifactorial conditions. The first group is also known as primary PH and the other four are under the common name of secondary PH [4].

Patients with CTDs such as SSc, MCTD, and SLE are at high risk of developing PH, especially with type 1-PAH (pulmonary arterial hypertension) as it is the most common and important due to its aggressiveness, short survival, and limited treatment options. In the last decades, the survival rate in this type of PH has improved, reaching 57% from the moment of diagnosis based on cardiac catheterization, but it is still suboptimal and continues to be a huge challenge [5]. At the same time, other forms of PH may appear in these patients, possibly an overlap of different forms of PH probably due to the autoimmune status of these conditions (Figure 1).

Figure 1: Different phenotypes of pulmonary hypertension in SSc-overlap. Abbreviations: CTEPH, chronic thromboembolic pulmonary hypertension; ILD, interstitial lung disease; LHD, left heart disease; PAH, pulmonary arterial hypertension, PVOD, pulmonary veno-occlusive disease; SSc, systemic sclerosis [Almaaitah, 2020].

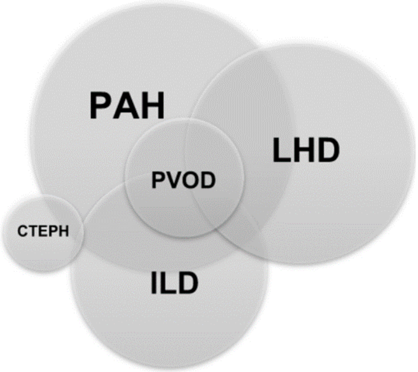

Connective tissue diseases represent the second major cause of PAH, after idiopathic ones, occupying about a quarter of the total population with PAH. Group 2 of PH, which is associated with left heart diseases can also occur in these collagenoses. Interstitial lung disease (pulmonary fibrosis) is another common complication of these autoimmune diseases, especially SSc and MCTD, so type 3 PH may be present to the same extent in these patients. Of note is pulmonary veno-occlusive disease which is associated with a worse prognosis due to the lack of response to PAH therapy and the risk of pulmonary edema due to these treatments. This form of PAH occurs especially in patients with SSc [3].

Another definition refers to the hemodynamic component, thus PH was defined in the past as mean pulmonary artery pressure (mPAP) of ≥25 mmHg, but recently it has been updated according to the 6th World Symposium on Pulmonary Hypertension (WSPH) to an mPAP value >20 mmHg. Studies have shown that normal mPAP in healthy individuals is approximately 14±3.3 mmHg and the upper limit of normal is 20 mmHg. This new definition appears to be less practical but may be useful in diagnosing more individuals with PH, as symptoms usually begin at a much higher value above the normal upper limit (i.e. >50mmHg). Nonetheless, there is a major concern regarding inclusion in this group of healthy individuals.

Another major hemodynamic change recommended by the 6th WSPH is the inclusion of pulmonary vascular resistance (PVR) in the classification and definition of PH. Thus, PH is further subclassified as pre-capillary PH (pre-PH), isolated post-capillary PH (IpcPH), or combined pre- and post-capillary PH (CpcPH) based on pulmonary arterial pressure and PVR [5].

EPIDEMIOLOGY

The lungs are often affected in connective tissue diseases (CTDs), because of the systemic nature of these autoimmune pathologies and due to the complex anatomic-functional patterns of the respiratory apparatus. Among the CTDs, systemic sclerosis (SSc) has the highest prevalence of pulmonary involvement (between 70% and 90% of patients with CTDs) and PAH is developed in 8-12% of patients with SSc. The incidence of PAH in SSc is about 0,61 cases per 100 patients per year. The prevalence of PAH among other CTDs remains to be determined due to the lack of large studies, but it is estimated to be lower than in patients with SSc. The prevalence of PAH in SLE is <4% and for the other diseases in this group is <1% [3,7].

PATHOGENESIS AND DIFFERENT FEATURES OF PULMONARY HYPERTENSION IN DIFFERENT AUTOIMMUNE DISEASES

Systemic sclerosis (SSc) or scleroderma is a rare and complex systemic autoimmune disease that affects the skin and the internal organs through multiple processes including fibrosis and thickening of these structures and vascular abnormalities [8]. The two major complications that might occur are pulmonary fibrosis and pulmonary arterial hypertension (PAH). The presence of both complications is not random since they have overlapping mechanisms. It seems to appear an abnormal fibroproliferation that results from a vascular injury such as endothelial cell injury and consecutive dysfunction [8]. Pulmonary fibrosis and PAH might appear as a consequence of an increased number of vasoconstrictors and proliferative mediators (endothelin-1) and a decrease in vasodilators (nitric oxide and prostacyclin), therefore leading to progressive pulmonary arterial remodeling and increase in pulmonary vascular resistance [6].

Systemic lupus erythematosus (SLE) is a chronic, autoimmune disorder that presents with a multitude of signs and symptoms and it affects multiple internal organs including the lungs. The most common lung manifestation is pleuritis but the parenchyma and the vasculature may also be involved causing thickening of the arterial media and subintima and immune complexes deposition in the arterial wall. PAH can rarely be present in SLE but it is a life-threatening pulmonary manifestation caused by left ventricular dysfunction or congestive cardiac failure and also by interstitial lung disease and chronic thromboembolic disease. Nevertheless, there is an increased risk of PAH and pulmonary venous thromboembolism (VTE) in SLE patients especially when it is associated with antiphospholipid antibody syndrome. Among SLE patients, most cases with PAH developed in the presence of Raynaud's phenomenon (60% of cases) and positive rheumatoid factor [9]. Another association was found between PAH and anti-RNP antibodies [7,9].

Primary Sjogren syndrome (pSS) is an autoimmune disease characterized by impairment of exocrine glands (lacrimal and salivary) due to inflammatory infiltrate but it can also affect extra glandular organs, including the respiratory tract and lung involvement which are one of the most common complications leading to increased mortality. PAH can rarely appear in pSS but can lead to right heart failure and death. The exact prevalence of PAH in pSS is difficult to estimate but one study has shown a value of 22% of patients based on echocardiographic evidence, although right heart catheterization is the gold standard for the diagnosis; There is an association between pSS-PAH and hypergamma-globulinemia, positive ANA and anti-RNP antibodies [7].

Rheumatoid arthritis (RA) associated pulmonary hypertension is very rare and can be attributed to parenchymal lesions, vascular disease such as rheumatoid vasculitis or it can develop due to chronic thromboembolic disease. But rheumatoid arthritis can also be associated with other autoimmune diseases such as scleroderma, SLE, and other CTDs in overlap syndromes, therefore the diagnosis of PH can be more relatable to these conditions.

Moreover, even though there is no disease-modifying treatment of RA (DMARDs or bDMARDs) suspected of causing PH, it is common knowledge that Methotrexate can cause pulmonary fibrosis that can lead to PH. The role of these drugs on PH can not be evaluated due to a lack of data. PH seems to be more common in patients with seropositive forms and advanced radiological signs. Systematic screening of PH is not necessary for RA patients but if is suspected further diagnostic testing should be pursued. In the case of RA, the prevalence of PH is identical to that seen in the general population [11,12].

Ankylosing spondylitis (AS) is a systemic inflammatory disease affecting the axial skeleton and/or peripheral joints and can be associated with extra-articular manifestations including uveitis, aortitis, and valvular heart disease. The association between PH and ankylosing spondylitis (AS) is not so common and a few case reports have described a high incidence based on echocardiography but without right heart catheterization and no other connective tissue disease was excluded [13]. Only one case from 2022 has confirmed the diagnosis of PAH based on transthoracic echocardiography and right heart catheterization [14]. In conclusion, further studies are needed to establish a causal relationship between PH and ankylosing spondylitis.

Mixed connective tissue disease and PH. It is very difficult to estimate the exact prevalence of PH in mixed CTD because of the very frequent overlap syndromes where SSc may contribute to the high rates of PH [15]. The estimated prevalence is somewhere around 8%.

CLINICAL PRESENTATION

Patients usually present at first with non-specific signs and symptoms such as fatigue, weakness, dyspnoea on exertion, and dizziness and are usually described to appear due to connective tissue diseases per se. Other symptoms such as syncope, angina, ankle edema, and abdominal distention appear only after the vasculopathy has advanced and are signs of right heart failure [3].

PH associated with left heart disease (type 2 of PH) may be present among patients with SSc since cardiac dysfunction can develop in more than 40% of patients with SSc leading to myocardial and conduction system fibrosis which determines arrhythmias and diastolic heart failure. Atherosclerotic coronary vessel disease and hypertensive crisis may also appear.

More often, interstitial lung disease (ILD) can be present as a complication of SSc (SSc-ILD) leading to PH in 31% of cases. It is associated with the diffuse cutaneous form of SSc and the presence of positive Scl-70 (anti-topoisomerase) antibodies. Although these antibodies seem to have a protective role against PAH, type 3 PH may still appear due to interstitial lung disease [6].

DIAGNOSIS

Screening procedures. Early diagnosis is essential to improve survival in PAH patients. Therefore, early screening for diagnosis leads to adequate treatment at the right moment. The proper screening for PAH in SSc with a disease duration of more than 3 years and a modified DLCO (diffusion capacity of the lung for carbon monoxide) value is by using the DETECT algorithm based on non-invasive clinical and laboratory tests in order to reduce the missed diagnosis of PAH. Among patients with concomitant pulmonary emphysema, the utility of lung function tests is reduced as it might increase FVC (forced vital capacity) and diminish DLCO. The DETECT algorithm is based on 2 steps diagnosis: in step 1 clinical status, laboratory findings, and lung function tests (FVC, telangiectasia, anticentromere antibody positivity, electrocardiogram right axis deviation, and serum levels of Nt-proBNP and urate) should be determined and based on the results, echocardiographic parameters might be recommended in the second step to generate a final score. Based on the results of these steps, a right heart catheterization might be performed. The sensitivity of this method is high (96%) but it has a low specificity for detecting PH in SSc patients (48%). Screening for PH should be performed at least once a year. For other CTDs, there is no current systematic screening for PAH because of its low prevalence, although careful monitoring of these patients is recommended [8,10].

Biomarkers such as NT-proBNP and uric acid, which reflect cardiac dysfunction, are used in the DETECT algorithm as part of a screening method for PAH in SSc patients or other CTDs although these are not specific. One study identified other circulating biomarkers that might have a future impact on the diagnosis of PH and reduce the necessity of invasive procedures. Besides NT-proBNP, other proteins such as collagen IV, endostatin, insulin-like growth factor binding protein (IGFBP)-2, IGFBP-7, matrix metallopeptidase-2, neuropilin-1 and RAGE (receptor for advanced glycation end products) could improve the DETECT algorithm for screening of PH and understanding of different mechanisms in the pathogenesis of PAH. These markers are known to be involved in the process of pulmonary vascular remodeling, angiogenesis, and cellular growth but further data is needed in order to establish the exact role of these markers in PH [16].

Spirometry with DLCO estimation is important to evaluate the state of pulmonary function since DLCO is reduced in PAH (<50%) but FVC has a normal value and they have a high specificity (90%) and a high positive predictive value for SSc-PAH. In pulmonary fibrosis, both parameters are low [10]. This occurs due to a low blood volume in the pulmonary capillary in PAH patients and a diminution in the transfer capacity of the alveolar-capillary membrane. The best screening method for PAH among lung function tests is the FVC (% predicted)/DLCO (% predicted) [17].

Autoantibodies: Several SSc-specific antibodies are thought to be associated with PAH such as high anti-centromere ab (ACA) titers and some of the ANA panel. One study reported a significant correlation between SSc-PAH and the positivity of anti-U1-RNP and Th/To antibodies. Although AECAs (anti-endothelial cell antibodies) are involved in the vascular pathology of SSc further data is needed to demonstrate the exact implications of these antibodies in SSc-PAH. Another study reported the presence of anti-endothelin receptor type A antibodies (ETAR) and anti-angiotensin receptor type I antibodies (AT1R) as being the main factor of pulmonary vascular inflammation. The level of these antibodies was higher in the SSc-PAH patients compared to other forms of PH.

Transthoracic echocardiography (TTE) is recommended in all SSc patients once a year and it is the most common tool for screening SSc-PAH although it has a low predictive value [10]. There are many parameters that can be modified on TTE in case of PH: 1. right ventricle/left ventricle basal diameter ratio >1.0 or flattening of the interventricular septum; 2. early diastolic pulmonary regurgitation velocity >2.2 m/sec or increase in the tricuspid regurgitant jet velocity (TRV) and/or systolic pulmonary artery pressures (sPAP) and/or pulmonary artery diameter > 25mm; 3. inferior cava diameter >21 mm with decreased inspiratory collapse (18 cm) [17, 18].

Right Heart Catheterization (RCH): with the help of this procedure the diagnosis of PAH is established with certainty and the measurements that can be performed are the mPAP and the pulmonary capillary wedge pressure (PCWP), PVR, right atrium (RA) volume, and transpulmonary pressure gradient (TPG), by using a catheter inserted into the right femoral vein. RHC is the most precise method for the diagnosis of PH and it can offer information about the severity of the disease and prognosis. RHC is necessary when there is a moderate or high risk of developing PH based on the results of TTE [10,19].

TREATMENT

Because pulmonary hypertension due to autoimmune disease is a relatively rare syndrome and because it is challenging to treat, patients are probably best served if they are referred to a center that has experience with treating pulmonary hypertension. Close communication should be maintained between the referring physician and the treating physician [20].

No standard guidelines exist for the treatment of pulmonary hypertension associated with connective tissue disease.

Treatment recommendations, however, are modeled from the available literature on patients with PPH for several reasons. First, the clinical presentations and histopathologic features of PPH and autoimmune disease–associated pulmonary hypertension may be quite similar. Second, some patients who are initially diagnosed with PPH later manifest symptoms and signs of systemic autoimmune disease [21]. A further link between PPH and collagen-vascular disease–associated pulmonary hypertension is suggested by Isern et al, who found that 9 of 31 patients (29%) with PPH had specific autoantibodies associated with connective tissue diseases and 39% had Raynaud's phenomenon or positive antinuclear antibody results. Anti-Ku antibodies were found in 23% of patients with PPH [22]. Although the outcome still remains poor, many advances have been made in the last 30 years in vasodilator therapy, supportive care, and, more recently, the use of lung transplantation. These advances have improved survival and quality of life for PPH patients to a considerable extent. Further study of these therapies' usefulness among patients with pulmonary hypertension associated with systemic autoimmune disease is forthcoming.

General Supportive Therapy

The treatment of pulmonary hypertension should begin with the reversal of any contributing factors, including hypoxemia, hypercarbia, and acidosis. Such treatment is intended to reduce right heart strain with hopes of increasing survival. Oxygen supplementation to maintain arterial oxygen tension above 60 mm Hg or oxyhemoglobin saturation over 90% reduces mortality from cor pulmonale and improves cognitive function and quality of life in patients with the severe chronic obstructive pulmonary disease [23,24,25]. Others have shown that in primary pulmonary hypertension, low arterial oxygen saturation is the single best predictor of poor survival, as it is associated with a higher incidence of sudden death.

Digitalis, diuretics, and sodium restriction should be considered when heart failure persists. Although the use of digitalis in right-heart failure has been controversial and the drug is known to increase pulmonary vascular resistance and has a high incidence of toxicity in patients with cor pulmonale, it is shown to improve right heart function when left heart failure is also present, and it may offset the negative inotropic effects of calcium-channel blockers when they are administered concomitantly. Diuretics are useful in improving peripheral edema and hepatic congestion. Cautious administration is warranted, because it may lead to metabolic alkalosis, which, in patients with derangements in ventilatory control, may depress ventilatory response to carbon dioxide and lead to hypercarbia. Aggressive diuresis may also reduce right ventricular blood volume and reduce cardiac output. Finally, blood viscosity may increase due to a rise in hematocrit associated with diuresis. Serial phlebotomy may be required to decrease blood volume and viscosity in patients who have polycythemia associated with cor pulmonale.

Anticoagulation has been shown to provide survival benefits in patients with PPH. In situ, thrombosis due to endothelial injury and venous stasis and, sometimes, due to the presence of anticardiolipin antibodies may contribute to pulmonary hypertension in some patients by reducing the cross-sectional area of the pulmonary vascular bed [26]. In situ, thrombosis in pulmonary vessels has been shown to occur in all forms of pulmonary hypertension. Moreover, right heart failure, by promoting venous stasis, may further increase the risk of pulmonary thromboembolism. As such, anticoagulation with warfarin or with adjusted-dose or low-molecular-weight subcutaneous heparin in patients at risk for bleeding may benefit patients with pulmonary hypertension resulting from systemic autoimmune disease. Alternatively, the use of antiplatelet drugs such as aspirin or dipyridamole may be helpful; this recommendation is based on the fact that levels of urinary thromboxane A2, indicating platelet activation, are increased in some patients with pulmonary hypertension. Speculation exists that the beneficial effects of chronic prostacyclin infusion in some patients with in situ thrombosis may occur because of its potent anti-platelet aggregating activity rather than through vasodilatation because improved outcomes are seen even in patients who do not experience substantial acute hemodynamic changes. The same theoretic supposition applies to the suspicion that heparin may be preferred to warfarin because it inhibits smooth muscle and endothelial cell proliferation. The risk of anticoagulation must be considered, particularly because more than half of all patients with severe pulmonary hypertension experience syncope, and 15% experience hemoptysis [27].

Vasodilators

Vasodilator therapy has been used for more than 30 years in patients with PPH under the premise that pulmonary vasoconstriction, at least in part, contributes to the pathogenesis of this disease. Although it is not clear whether the vasoconstriction is the primary cause of increased pulmonary vascular resistance or results from distension of pulmonary blood vessels by increased intraluminal pressures, the premise also exists that even a small reduction in right ventricular afterload leads to clinically significant increases in right ventricular output. Not all patients will respond to vasodilators, and trials of vasodilator therapy are indicated to distinguish those patients who have a reversible vasoconstrictive component of pulmonary hypertension from those who have fixed pulmonary hypertension. While we await the results of a forthcoming prospective trial investigating the use of long-term therapy with prostacyclin among patients with pulmonary hypertension from systemic autoimmune disease, we include a discussion of vasodilator therapy for two reasons: first, some patients diagnosed with PPH later will manifest specific evidence for systemic autoimmune disease; and second, some patients with autoimmune disease–associated pulmonary hypertension have histopathologic changes on lung biopsy that are identical to those of PPH. As such, the two forms of pulmonary hypertension may, in fact, represent different points along the same continuum of disease progression, or may represent a common final pathway of injury following some inciting stimulus. The optimal response of vasodilator therapy is a reduction in PA pressure without a change in pulmonary capillary wedge pressure, along with an increase in cardiac output and thus a decrease in pulmonary vascular resistance. These changes should occur without a decrease in systemic arterial pressure or oxygenation. Only 15% to 30% of patients will experience such a response, however [28].

A second pattern is exhibited by approximately half of all patients with PPH, who experience a reduction in pulmonary vascular resistance in association with an increase in cardiac output, without a change in PA pressure. Although these patients also have increased exercise tolerance and improved right ventricular function, the duration of these improvements is unknown, as is the presence of any long-term survival advantage. In some patients, these hemodynamic improvements are most pronounced during exercise rather than rest, and so clinical benefits may exceed those predicted by responses measured during the resting state. The remaining quarter of patients are those in whom vasodilator therapy is contraindicated: they suffer a reduction in systemic blood pressure with acute vasodilator challenge, whereas PA pressure and cardiac output remain unchanged or increase. In these patients, vasodilators affect the systemic vasculature more than they do the pulmonary vessels. In response to the decrease in systemic vascular resistance, cardiac output may increase or remain unchanged. If cardiac output increases in response to systemic vasodilation, the increased flow through the pulmonary vessels will achieve the undesirable effect of a further increase in PA pressure. If the cardiac output does not respond to systemic vasodilation, then systemic hypotension will result, and this outcome may be resistant to most therapies, including discontinuation of the vasodilator. Volume resuscitation is often not helpful, and vasopressors may worsen pulmonary hypertension and increase right ventricular afterload. Such systemic hypotension may also precipitate acute angina or heart failure. The patients who experience a reduction in systemic blood pressure without reduction in PA pressure on acute vasodilator challenge are classified as having fixed vascular disease. Many drugs have been used to evaluate pulmonary vascular responsiveness [28]. The ideal drug, however, is one that: (1) elicits pulmonary vasodilation at a dose that has little or no effect on the systemic circulation; (2) is short-acting and is rapidly cleared from the circulation, so that baseline hemodynamics are reestablished soon after the drug is discontinued; (3) is easy to administer accurately in an incremental dosing regimen; (4) has hemodynamic and other side effects that accurately predict the response to longer-acting agents; and (5) is able to identify those patients who will not respond to therapy. In summary, a screening agent must be able to accurately reproduce the risk-benefit profile a patient will experience with longer-acting agents. This means identifying patients who will benefit from further trials with long-acting agents, as well as delineating those who should be spared from further therapeutic trials because of potential adverse outcomes of therapy.

Several agents have been used for screening: prostacyclin and its analog iloprost, acetylcholine, adenosine, and nitric oxide. Nitric oxide is administered by the inhaled route, whereas the others are given intravenously. The most intensively studied of these agents is prostacyclin, a metabolite of arachidonic acid produced by endothelial cells. Although it has some effect on systemic vascular resistance, it has almost all of the properties of an ideal screening agent. Its half-life is only 2 to 3 minutes, it has a rapid onset of action, its effects resolve rapidly after it is discontinued, and it is amenable to incremental intravenous dosing. Acute administration of prostacyclin has been shown to predict pulmonary vasodilator response to traditional long-term agents and identify those patients with PPH in whom long-term therapy may not be useful [29].

The recent approval by the Food and Drug Administration (FDA) for continuous long-term intravenous infusion in primary pulmonary hypertension has resulted in increased use of this drug as an alternative to traditional oral medications in selected patients [29]. Iloprost is a short-acting, stable carbocyclic derivative of prostacyclin with a longer half-life of more than 10 minutes. It has similar pharmacologic effects on the pulmonary circulation of patients with PPH. Recently, it has been used in long-term therapy for patients with pulmonary hypertension in the setting of connective tissue diseases. Acetylcholine is also short-acting and well-tolerated but may be a less potent vasodilator than prostacyclin, although this observation may in part be explained by the use of lower doses of acetylcholine [30]. Adenosine is a very short-acting pulmonary vasodilator, with a half-life of less than 10 seconds. It has been shown to have acute hemodynamic effects similar to those of prostacyclin in PPH [31].

Inhaled nitric oxide has selective pulmonary vasodilatory properties, with little effect on systemic circulation. It has an interesting additional effect of reducing intrapulmonary shunt and increasing systemic arterial oxygen saturation.

Transplantation

Transplantation has generally been reserved for those patients whose symptoms progress despite aggressive medical management, and who have New York Heart Association functional class III or IV heart failure [32]. The presence of severe right-sided heart failure that is accompanied by hepatomegaly, hyperbilirubinemia, and ascites confers high mortality, however, and these patients are not suitable candidates for transplantation. Transplantation has received attention in the recent past as a potential panacea for patients with severe forms of pulmonary hypertension, as it produced a dramatic reduction in PA pressure and reversal of right heart failure [33]. As experience has accrued, however, many centers have found that the incidence of acute complications following lung transplantation is greater among patients with systemic autoimmune disease than those with PPH, and some have abandoned it as a therapeutic option. Moreover, the incidence of postoperative Raynaud's phenomenon may be higher in these patients, and the vasoconstrictive effects of cyclosporine, which is used in posttransplant immunosuppression, are evoked as a putative mechanism. Other potential complications include bronchiolitis obliterans, which occurs with an incidence of 25% to 40%, and opportunistic infection [34,35]. Other limitations of transplantation include its confinement to certain specialized centers, its expense, and, regrettably, the paucity of donor organs [36]. Treatment of underlying systemic autoimmune disease

When pulmonary hypertension occurs in the setting of significant parenchymal lung involvement with systemic autoimmune disease, or due to vasculitis, the response to therapies targeted toward the underlying autoimmune disorder has been variable [37,38,39]. Small-scale studies suggest that traditional vasodilators alone may be ineffective. Most patients do not respond to corticosteroids, but some may [39]. In one series of three patients who had pulmonary hypertension in the setting of vasculitis, pneumonitis, and thrombosis, an aggressive combination of corticosteroids, anticoagulation, and either cyclophosphamide or azathioprine appeared to be beneficial [40]. In patients with rheumatoid arthritis who exhibit pulmonary hypertension because of pulmonary arteritis, which is thought to be due to deposition of immune complexes in vessel walls, the use of high-dose corticosteroids or cytotoxic agents has been investigated. Evidence has been unclear on whether such therapy improves overall outcomes.

Calcium-Channel Blockers

Although there is a paucity of data regarding their use in autoimmune disease–associated pulmonary hypertension, nifedipine, verapamil, diltiazem, and recently, nicardipine have been used against PPH. They are thought to act by reducing calcium influx into pulmonary vascular smooth muscle cells, thereby blocking muscle contraction and promoting vasodilation. Nifedipine is the most frequently used of the four, although nicardipine and diltiazem are useful when adverse effects of nifedipine occur. Among 10 patients with diffuse systemic sclerosis, CREST syndrome, or mixed connective tissue disease, oral nifedipine has been shown to produce both acute and chronic reduction (at 3 to 6 months) in pulmonary vascular resistance [41].

Prostaglandins

Prostacyclin is now FDA-approved for continuous intravenous infusion for the treatment of PPH, and may soon be routinely used for pulmonary hypertension associated with collagen vascular disease if results of a proposed clinical trial show therapeutic efficacy in this setting. Although it was initially proposed for patients who had severe forms of pulmonary hypertension as a bridge to transplantation, it has since been continued in patients who exhibit pronounced response to prostacyclin (PGI2) infusion, for a duration exceeding 5 years, with consideration for transplantation only if symptoms recur or if complications occur. The drug must be delivered by an infusion pump through an indwelling central venous catheter. Frequent dosing increments are required to maintain responses; moreover, complications including death have occurred because of failures in the drug delivery system, including thrombosis or infection of the catheter, pump malfunction, or interruption in the infusion system due to accidental disconnection. Nevertheless, studies have shown that long-term prostacyclin infusion offers symptomatic and hemodynamic improvement [42,43] and confers a survival advantage in advanced PPH [44,45].

Iloprost is a stable prostacyclin analog that has been studied in a number of settings. In the acute situation, high doses have been shown to reduce pulmonary vascular resistance in healthy persons, patients with PPH, and those with toxic oil syndrome, which results from ingestion of contaminated rapeseed oil and resembles scleroderma. Recently, de la Mata et al have studied the effects of long-term iloprost treatment in patients with severe pulmonary hypertension due to systemic sclerosis and primary antiphospholipid syndrome. They followed prospectively three patients with systemic sclerosis and two with antiphospholipid syndrome who did not respond to oral vasodilator therapy for pulmonary hypertension. They found that acute infusion of maximum tolerated doses reduced pulmonary vascular resistance and raised cardiac index and pulmonary arterial oxygen saturation in a statistically significant manner. After 32 weeks, all patients had improved exercise tolerance and New York Heart Association functional class. This improvement in exercise tolerance, however, was not accompanied by improvements in resting hemodynamics nor in pulmonary oxygen saturation, and one patient died. Further studies are needed to determine whether iloprost is more beneficial for patients who have less severe degrees of pulmonary hypertension than those in this study. In addition, long-term intravenous therapy continues to pose problems associated with the drug delivery system—one patient in this study died of bacteremia [30].

Oral iloprost has also been studied as a potential long-term therapeutic alternative to intravenous administration. In a European multicenter, double-blind, placebo-controlled trial of 63 patients with Raynaud's phenomenon, iloprost given orally at the maximum tolerated dose for 10 days resulted in reduced duration and severity of attacks of vasospasm. The results did not reach statistical significance, however, and this study did not include any assessment of the effects of this therapy on pulmonary vascular disease [46].

CONCLUSION

PH can be caused by several conditions in patients with systemic sclerosis or other CTDs and it is an important cause of mortality and morbidity, therefore early diagnosis and annual screening in asymptomatic patients is mandatory in order to provide adequate treatment. Even though significant efforts are being made regarding treatment options, long-term survival remains an important issue. The mortality rate of PAH in SSc patients is worse than the one in idiopathic PH and other CTDs due to a lack of response to treatment and the severity of the disease. More studies are needed in order to see progress in elucidating the pathogenesis and treatment regimens, especially the immunosuppressive therapy, and new non-invasive tools are required to simplify the diagnosis of PH and therefore improve patients' survival rate and quality of life [18,47].

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution
All the authors contributed to the following aspects of this study: Conceptualization: D.A., I.N.N, and L.I.M; resources: D.A., R.S.C., and D.O.B.; writing- original draft preparation: D.A., I.N.N., and L.I.M.; writing – review and editing: I.N.N., O.G.P., V.C.B., D.O.B., and F.I.R.; writing- finalization: D.A., D.O.B., N.I.N, and L.I.M. The authors have given their approval for this article to be published. All of the authors have participated sufficiently in the work and take public responsibility for appropriate portions of the content. All of the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ORIGINAL ARTICLE

The Role of Chromogranin A Assay in the Diagnosis of Pheochromocytomas

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Abstract: *Background: Pheochromocytomas (Pheo) are rare neuroendocrine tumors with a suggestive clinical picture, characterized by hypersecretion of catecholamines and other neuroendocrine biomarkers. Methods: The purpose of the study was to analyze the diagnostic features of Pheo and investigate the role played by different neuroendocrine and hormonal markers in diagnosing Pheo.The retrospective study involved a group of 69 patients diagnosed and treated with Pheo, who had both urinary and plasma catecholamines and neuroendocrine markers measured pre- and postoperatively. Results: After comparing pre- and postoperative hormonal parameters and neuroendocrine biomarker changes, numerous statistically significant findings were found. The analysis of the relationships between chromogranin A (CgA) levels, plasma and urine metanephrines, and normetanephrine and Pheo tumor size was included in the study. Additionally, we evaluated Cg A's diagnostic efficacy in comparison to plasma metanephrines, normetanephrine, and neuron-specific enolase (NSE) for Pheo. Conclusions: We obtained statistically significant data on pre- and postoperative differences for plasma and urinary catecholamines, CgA and NSE. Pheo tumor size is interdependent with serum levels of Cg A, plasma and urinary metanephrines, and normetanephrine. The best diagnostic power for Pheo was plasma normetanephrine, followed by plasma metanephrines and CgA.*

Keywords: *pheochromocytomas, catecholamines, chromogranin A, neuron-specific enolase*

INTRODUCTION

The term "neuroendocrine tumors" (NETs) refers to a wide range of extremely rare neoplasms that fall under the category of borderline endocrinology. They have the capacity to secrete a wide variety of physiologically active peptides and amines, which may result in a variety of biological behaviors and, in turn, metabolic and systemic consequences.

Advances in genetic studies have had a major impact in characterizing the hereditary aetiopathogenesis of pheochromocytomas (Pheo). In the 2000s years, it was thought that only 10% of these tumors, were recognized with a genetic cause, but now more than 18 genes have been described, whose mutations lead to the development of these tumor types in more than 30-40% of cases. In addition, large-scale studies target the genetic fingerprint of these tumor types as a possible pathophysiological key to further developing personalized medical therapy [1].

Currently, Pheo is defined as a catecholamine-secreting tumor originating from neuroendocrine chromaffin cells of the adrenal gland. In terms of pathophysiological mechanisms, Pheo can synthesize, store, secrete and metabolize catecholamines (noradrenaline and adrenaline, respectively their metabolites normetanephrine and metanephrine), thanks to their enzymatic background, namely tyrosine hydroxylase and dopamine β-hydroxylase enzymes. These two enzymes are found in both the adrenal medulla and sympathetic nerve ganglia and are responsible for the production of catecholamines and their storage in dense secretory vesicles together with CgA, adenosine triphosphate, calcium, magnesium, adrenomedullin, and other neuropeptides. CgA was first described as originating from adrenal chromaffin cells. It is a member of the granin family together with chromogranin B, chromogranin C (secretogranin II), secretogranins III-VI and vascular endothelial growth factor (VEGF) and it is considered a pro-hormone that plays multiple biological roles, including calcium homeostasis and mediating the secretion of catecholamines and other hormones [2-4].

In addition, the adrenal medulla is uniquely capable of producing and secreting adrenaline (epinephrine) and its metabolite metanephrine, thanks to a specific glucocorticoid-dependent enzyme of its own called phenylethanolamineN-methyltransferase (PNMT), which converts noradrenaline (norepinephrine) to adrenaline by methylation [5,6].

Pheo is characterized by clinical signs and symptoms specific to catecholamine hypersecretion (paroxysmal hypertensive crisis, diaphoresis, palpitations, headache, weight loss, hyperglycemia, etc.). Moreover, for Pheo, the clinical picture may include the specific classic triad: headache, sweating, and palpitations. However, non-specific symptoms may also occur, such as anxiety, weight loss, insomnia, or life-threatening cardiovascular events: rupture of an aortic aneurysm or stroke during a hypertensive crisis, hypertensive encephalopathy, catecholamine cardiomyopathy, etc [7].

The general biochemical diagnosis of catecholamines secreting NETs is characterized by the determination of specific markers: catecholamines and their plasmatic and urinary metabolites, chromogranin A (CgA), neuron-specific enolase (NSE) as well as by identifying the immunohistochemical expression of synaptophysin, CgA, etc. [8].

Serum CgA dosing and monitoring in Pheo is a topic of great interest in the literature and is currently included in current diagnostic and treatment guidelines. According to the body's literature, CgA levels are correlating with tumor volume, disease recurrence, and the presence of secondary determinations, especially hepatic [9,10].

MATERIALS AND METHODS

The aim of the study was to analyze the diagnostic features of Pheo and to identify possible correlations between various clinical and hormonal factors with an impact on clinical outcome and prognosis. Subjects were diagnosed with Pheo based on the following data:

- Suggestive symptomatology (hypertensive crisis, palpitations, sweating, headache, weight loss)

- Values that exceeded the upper limit of the reference range of plasma or urinary metanephrines and normetanephrine at least four times were considered diagnostic values

- Identification and localization of adrenal tumors by imaging techniques

- Pathological examination confirmed the diagnosis of Pheo

The study involved data collection and inclusion of 69 patients evaluated, diagnosed, and treated for Pheo of sporadic or genetic etiology. We investigated hormonal levels pre and post-operative, neuroendocrine biomarkers, and their correlations. According to the inclusion and diagnostic criteria, after obtaining informed consent, we enrolled in the study 69 patients with clinical suspicion of Pheo supported on the basis of biological and hormonal evidence, as well as on the basis of imaging investigations in whom histopathological examination obtained postoperatively confirmed the diagnosis. Clinical, biochemical, and hormonal parameters were monitored dynamically, i.e. preoperatively (at the time of diagnosis) and immediately postoperatively (at 2-4 weeks, the date of the first endocrinological evaluation after surgery). Our study included subjects evaluated and diagnosed in C.I. Parhon National Institute of Endocrinology Bucharest, during the period 2000-2017. We performed retrospective data collection of patients diagnosed and treated by Pheo from clinical observation sheets and electronic patient files. The collected data were entered into a database. Statistical interpretation of the data obtained was performed using IBM SPSS Statistics 20.0 and MedCalc 12. Results were expressed as mean ± standard deviation for numerical variables and as the frequency for categorical variables. The statistical tests used to interpret the data were the usual ones (minimum, maximum, mean, median values) as well as complex ones such as t-test (with the assumption of equal and unequal variances, respectively) for comparing the variance of means and F-test for comparing standard deviations. For statistical hypothesis testing, a p-value < 0.05 was used to assert a statistical correlation. The diagnostic power of the parameters was tested using the ROC curve.

RESULTS

Comparative analysis of pre- and postoperative hormonal parameters and neuroendocrine biomarkers changes for the Pheo group revealed numerous statistically significant data. The results of the statistical analysis of the dependent sample T-test, taking into account pre and postoperative values are reported in Table 1. Thus, in the Pheo study group, the mean plasma CgA level preoperatively was 390.43±173.83 ng/ml, and postoperatively it decreased statistically significantly ($p<0.001$) to 56.43±63 ng/ml. The NSE concentration also showed a statistically significant change ($p=0.004$) decreasing from a preoperative mean value of 12.98±1.69 ng/ml to 10.3±3.01. Plasma metanephrines recorded a mean value at diagnosis of 937.82±1201.51 pg/ml and decreased statistically significantly ($p<0.001$) postoperatively to 41.2±22.7 pg/ml. Plasma normetanephrine ranged from a preoperative mean value of 1712.11±1893.39 pg/ml to a postoperative mean value of 94.94±205.85 pg/ml, the difference being statistically significant ($p<0.001$). Regarding urinary metanephrines, they ranged from a mean value at diagnosis of 1798.81±2259.44 µg/24 hours to mean postoperative values of 96.1±80.9 µg/24 hours, reaching the threshold of statistical significance. Similarly, urinary normetanephrine had preoperative mean values of 3212.8±3284.07 µg/24 hours and postoperative mean values of 178±188.01 µg/24 hours, the difference being statistically significant (p<0.001, n=50).

Table 1: T-test for paired samples – Evolution of pre- and postoperative parameters in the Pheo group.

Variable	Pheo		N	Sig.	Correlation	Mean Diff.	Sig (2 tailed)
	Preop. mean	Postop. mean					
CgA (ng/ml)	390.43±173.83	56.43±63	28	0.046	0.38	334±110.83	<0.001
NSE (ng/ml)	12.98±1.69	10.3±3.01	12	0.08	0.698	2.68±1.32	0.004
Plasma metanephrines (pg/ml)	937.82±1201.51	41.2±22.7	69	0.92	-0.12	896.62±1178.8	<0.001
Plasma normetanephrine (pg/ml)	1712.11±1893.39	94.94±205.85	69	0.017	0.286	1617.17±1687.5	<0.001
Urinary metanephrines (µg/24 ore)	1798.81±2259.44	96.1±80.9	52	0.218	0.151	1702.7±2178.54	<0.001
Urinary normetanephrine (µg/24 ore)	3212.8±3284.07	178±188.01	50	0.06	0.177	3034.8±3096.06	<0.001

We also statistically analyzed the correlations of CgA levels and plasma and urinary metanephrines and their metabolites with Pheo tumor size.

Regarding the correlation of preoperative serum Cg A levels with Pheo tumor size, the results of the statistical analysis showed a positive association, with high statistical significance (P<0.0001, r=0.86), being plotted in Figure 1.

Figure 1: Correlation of CgA and tumor size in the Pheo group

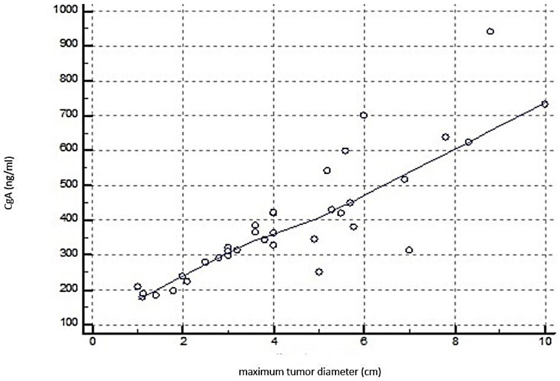

In addition, by studying the correlation of mean plasma metanephrines level with tumor size, we obtained a statistically significant positive correlation (P<0.0001, r=0.57), plotted in Figure 2.

In addition, serum plasma normetanephrine levels also showed statistically significant positive correlations with Pheo size (P=0.0002, r=0.43), plotted in Figure 3.

Similarly, regarding urinary metabolites of catecholamines, in Figure 4 it can be seen the presence of a positive association of urinary metanephrine level with Pheo maximum tumor diameter (P<0.0001, r=0.55).

In addition, urinary normetanephrine levels at the time of diagnosis showed a positive correlation as shown in Figure 5, with statistical significance (p<0.001, r=0.53).

Moreover, in terms of genetic or sporadic etiology in Pheo, the statistical data did not show a statistically significant difference (P=0.411, F-ratio=0.691) in plasma levels of Cg A. Thus, the mean serum CgA in genetic cases was 341.18±140.09 ng/ml (n=7), and in sporadic Pheo cases mean serum CgA was 402.32±181.15 ng/ml (n=29), without statistical significance.

Figure 2: Correlation of plasma metanephrines with tumor size in Pheo

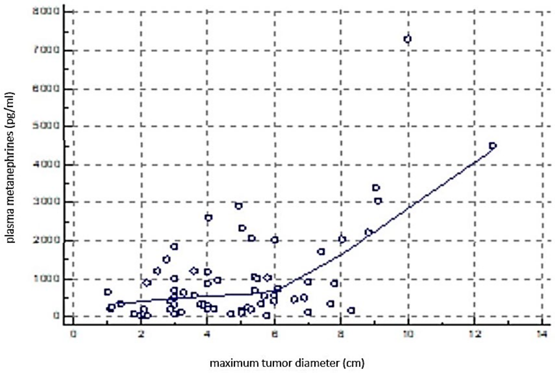

Figure 3: Correlation of plasma normetanephrine with tumor size in Pheo

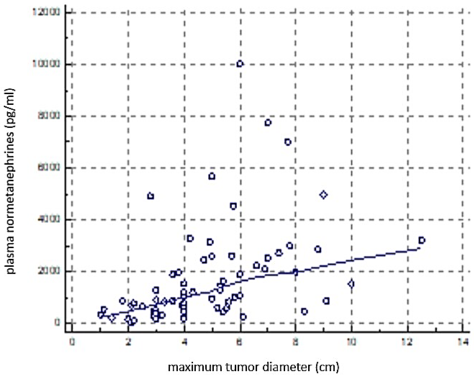

Figure 4: Correlation of urinary metanephrines with tumor size in Pheo

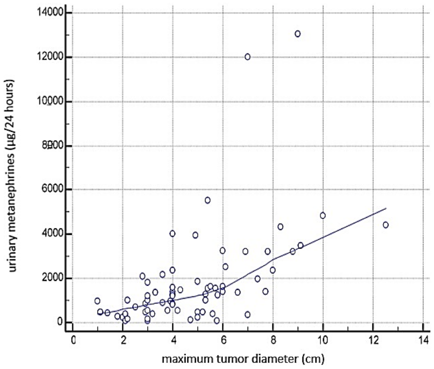

Figure 5: Correlation of urinary normetanephrine with tumor size in Pheo

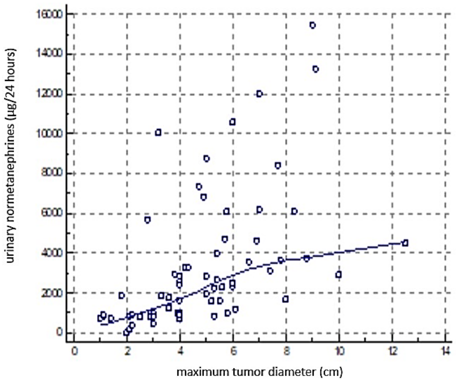

We also investigated correlations between CgA levels and hormone levels.

We identified a statistically significant positive correlation (p=0.02, r=0.37) between serum CgA and plasma metanephrine levels at the time of Pheo diagnosis, illustrated in Figure 6.

Figure 6: Correlation between preoperative plasma CgA and metanephrine levels in Pheo

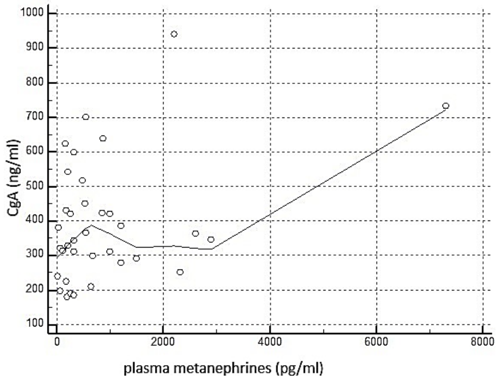

Similarly, regarding the correlation of urinary metanephrine levels with serum Cg A concentration, there was a statistically significant positive correlation (P=0.0001, r=0.62), as shown in Figure 7.

Figure 7: Correlation between serum CgA and preoperative urinary metanephrines in Pheo

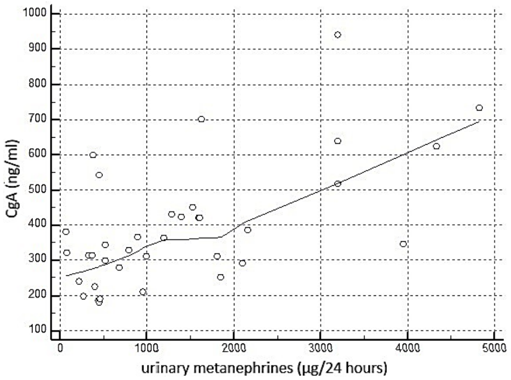

In contrast, we did not obtain statistically significant data implying correlations between preoperative serum CgA and plasma normetanephrine levels (p=0.18, r=0.22) nor urinary normetanephrine (p=0.12, r=0.26).

Regarding the correlations between serum NSE level, we identified a preoperative mean value of 12.98±1.7 ng/ml (n=18) which correlated positively, statistically significantly (p<0.0001, r=0.76) with Pheo tumor size, being illustrated in Figure 8.

Figure 8: Correlation between tumor diameter and serum NSE level in Pheo

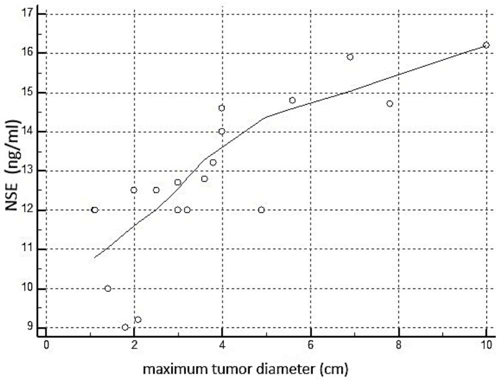

Similarly, we found no statistical significance for the correlation of serum NSE levels with plasma metanephrines at the time of Pheo diagnosis (p=0.10, r=0.35), urinary metanephrines (p=0.2, r=0.48) and urinary normetanephrine (p=0.23, r=0.26).

However, the correlation of preoperative serum NSE levels with plasma normetanephrine showed a positive line and was statistically significant (p=0.03, r=0.44), as shown in Figure 9.

Figure 9: Correlation of preoperative serum levels between serum NSE and plasma normetanephrine in Pheo

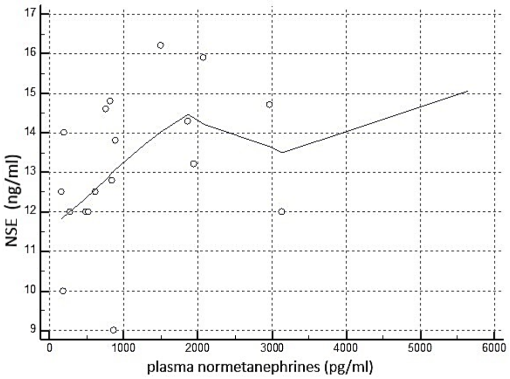

Regarding the analysis of NSE concentration according to Pheo etiopathogenesis, we found no statistically significant differences (p=0.74, r=1.332) between genetic Pheo whose mean serum NSE concentration was 12.75±1.19 ng/ml (n=4) and sporadic Peo whose mean NSE concentration was 13.06±2.22 ng/ml (n=14).

We performed also the analysis of the diagnostic power of Cg A versus plasma metanephrines and normetanephrine for Pheo.

Regarding the sensitivity and specificity of plasma metanephrines for Pheo diagnosis, being plotted in Figure 10, the results of statistical analysis indicated that a plasma metanephrines cut-off value >58 pg/ml has a sensitivity of 94.2% and a specificity of 100% for Pheo diagnosis, being highly statistically significant (p<0.0001) and AUC=0.970.

Figure 10: Sensitivity and specificity of metanephrines in the diagnosis of Pheo

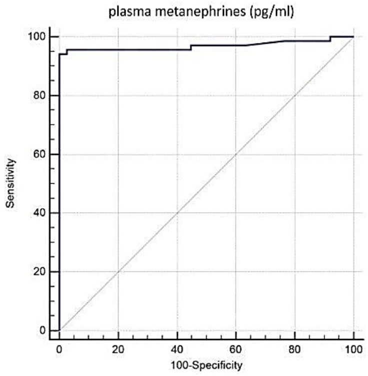

Similarly, analyzing the sensitivity and specificity of plasma normetanephrine for the diagnosis of Pheo, illustrated in Figure 11, the results indicated that a plasma normetanephrine cut-off value > 72 pg/ml has a sensitivity of 98.55% and a specificity of 100% for the diagnosis of Pheo, with high statistical significance (p<0.0001) and AUC=0.990.

Figure 11: Sensitivity and specificity of normetanephrine in the diagnosis of Pheo

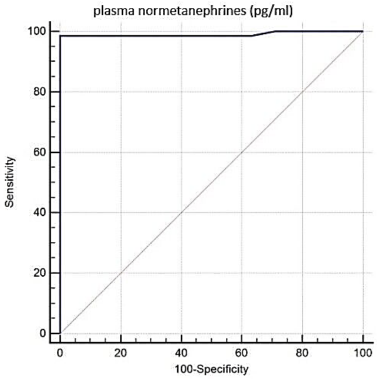

Additionally, combining plasma metanephrines and normetanephrine as a diagnostic element, there are no statistically significant differences between the two ROC curves (p=0.31), suggesting statistically similar diagnostic power, as shown in Figure 12.

Figure 12: Sensitivity and specificity of metanephrines and normetanephrine in the diagnosis of Pheo

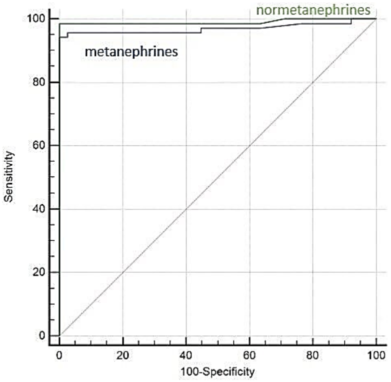

Figure 13: Sensitivity and specificity of CgA for the diagnosis of Pheo

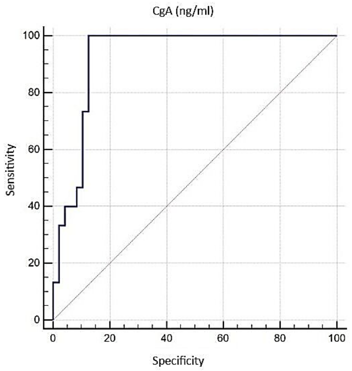

Analyzing the sensitivity and specificity of CgA for Pheo diagnosis, according to Figure 13, we obtained a sensitivity of 100% for cut-off values of CgA>197 ng/ml and a specificity of 87.5%, with high statistical significance (p<0.0001) and

AUC=0.926.

In Figure 14, it can be seen the sensitivity and specificity of CgA compared to that of plasma metanephrines and normetanephrine in the Pheo group at the time of diagnosis, but without a statistically significant difference (p=0.16, p=1) compared to the other two.

Figure 14: Sensitivity and specificity of plasma CgA, metanephrines, and normetanephrine in the diagnosis of Pheo

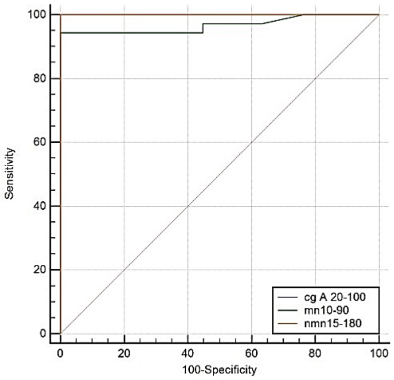

DISCUSSION

The biochemical diagnosis of Pheo is based on the determination of plasma and urinary metanephrines and normetanephrine and secondarily on the determination of neuroendocrine markers [11]. An important theme of our study was the dynamic (pre and postoperative) assessment of hormonal parameters. CgA is a glycoprotein that belongs to the granin family and Its dosing is recommended for the diagnosis and monitoring of NET [12]. Dynamic pre- and postoperative CgA assessment in Pheo was studied by others who reported a mean preoperative CgA ranging from 600- 632 ng/ml with a significant decrease postoperatively (p<0.001) [13-15]. Our data are consistent with the findings of the studies already mentioned, but the mean preoperative CgA value was 390.43±173.83 ng/ml, the difference may derive from different CgA assay methods (radioimmunoassay or ELISA technique), but the significant decrease postoperatively is conclusive. At the same time, the NSE dynamics recorded in our study (12.98±1.69 ng/ml preoperatively and 10.3±3.01 postoperatively) are within the literature studies [16]. Additionally, the course of plasma and urinary metanephrine and normetanephrine values showed significant decreases in the study, consistent with the data from the literature [17-19].

Analyzing the correlations between Pheo size and the amount of plasma and urinary metanephrines and normetanephrine, respectively neuroendocrine markers secreted at the time of diagnosis, we found statistically significant positive correlations for serum CgA levels (P<0. 0001, r=0.86), NSE (P<0.0001, r=0.76), as well as with plasma (P<0.0001, r=0.57); P=0.0002, r=0.4) and urinary (P<0.0001, r=0.55; p<0.001, r=0.5) metanephrines and normetanephrine levels. Our results correspond with data reported by the study of Unger N. et al. [18] which identified a positive correlation between CgA levels and tumor volume (p<0.001), as well as research of d'Herbomez et al. [20] that highlighted positive correlations between both preoperative CgA concentration and plasma and urinary fractional metanephrine levels with tumor volume. In contrast, the research of Guerrero M et al. [21] showed only a positive correlation between urinary normetanephrine levels with tumor size. In addition, similar to data confirmed in the literature, we obtained statistically significant data regarding positive correlations between preoperative CgA levels with plasma (p=0.02, r=0.37) and urinary (P=0.0001, r=0.62) metanephrines levels but we found no correlations with urinary and plasma normetanephrine. Similar to the study of Okamura T et al. [22] identified interdependence between CgA and urinary metanephrine levels (r= 0.80; P<0.0001), but as in our case, they did not reach the threshold of statistical significance for correlations of urinary and plasma normetanephrine with CgA.

Regarding the analysis of differences in biochemical parameters and genetic or sporadic etiopathogenesis of Pheo, we did not find statistically significant correlations for CgA (p=0.411, r=0.6) or NSE (p=0.74, r=0.1). In parallel, studies of Eisenhofer G et al. [23] and Zuber S et al. [24] reported a statistically significant difference in CgA values, whereas the research of d'Herbomez M et al. [14] reported a statistically insignificant difference (p=0.35) between CgA values between genetic and sporadic Pheo cases. We can assume that our data did not reach the threshold of statistical significance due to the small number of patients with Pheo of genetic cause in whom we had CgA and NSE assays available.

Regarding the sensitivity and specificity of plasma metanephrines for the diagnosis of Pheo, the results of our analysis indicated that a plasma metanephrine cut-off value >58 pg/ml has a sensitivity of 94.2% and a specificity of 100%, with AUC=0.970 for the diagnosis of Pheo. Similarly, the study of d'Herbomez M et al. [14] indicated a sensitivity of 71% and a specificity of 89.9%, and the study of Unger N et al. [18] indicated values of 70% respectively 90%, with AUC=0.8. Similarly, analyzing the sensitivity and specificity of plasma normetanephrine for Pheo diagnosis, our results indicated a sensitivity of 98.55% and a specificity of 100% for Pheo diagnosis, with AUC=0.990. Literature reports converge towards the same values, thus the study of d'Herbomez M et al. [14] reported a sensitivity of 95.2% and a specificity of 98.6% and the study of Unger N et al. [18] indicated a sensitivity of 89.5%, and a specificity of 98.3%, with AUC=0.91. Additionally, combining plasma metanephrines and normetanephrine as a diagnostic element, in our research we did not obtain statistically significant differences between the two ROC curves (p=0.31), suggesting statistically similar diagnostic power. Similarly, the research of Unger N et al. [18] reported a sensitivity of 89.5% and a specificity of 95% when combining the two diagnostic tests. Analyzing the sensitivity and specificity of CgA for Pheo diagnosis, we obtained a sensitivity of 100% and a specificity of 87.5%, with AUC=0.926. The study of Grossrubatscher E et al. [20] revealed a sensitivity of 91% and a specificity of 95% for CgA, while the study of d'Herbomez M et al. [14] reported a sensitivity of 98.3% and a specificity of 97% and the study of Unger N et al. [18] reported a sensitivity of 73.9% and a specificity of 74.2% with AUC=0.74.

CONCLUSIONS

We obtained statistically significant data on pre- and postoperative differences for the following hormonal and neuroendocrine parameters: CgA, plasma metanephrines and normetanephrine, urinary metanephrines, and normetanephrine. Pheo tumor sizes are interdependent with serum levels of Cg A, plasma and urinary metanephrines, and normetanephrine. In terms of inter-hormonal correlations, serum NSE levels correlate significantly with plasma normetanephrine levels and serum CgA levels correlate with plasma and urinary metanephrine levels. The best diagnostic power for Pheo was plasma normetanephrine (AUC=0.99), followed by plasma metanephrines (AUC=0.97) and CgA (AUC=0.92).

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ORIGINAL ARTICLE

Pitfalls of Ovarian Dysgerminoma

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Abstract: *This is a narrative review based on published articles, using the following criteria: key words "ovarian dysgerminoma" and "MRI (Magnetic Resonance Imaging)"; PubMed access; full-length English articles since inception to May 2023; original studies and case reports/series. Exclusion criteria were ovarian tumors other than dysgerminomas, non-English papers, non-human studies, and reviews. Dysgerminomas are the most common ovarian malignant germ cell tumors, accounting for 1-2% of all primary ovarian neoplasms.*

Keywords: *dysgerminomas, germ cell tumor, malignant tumor, MRI*

INTRODUCTION

The frame of ovarian dysgerminoma

Up to one-third of all ovarian malignancies are represented by germ cell tumors, but only 5% of these are malignant [1,2].

Ovarian germ cell tumors include, as proposed in the 2020 World Health Organization Classification of Tumors, teratomas, mature or immature, dysgerminomas, yolk sac tumors, embryonal carcinoma, non-gestational chorio-carcinoma, mixed germ cell tumors, mono dermal teratomas, and germ cell-sex cord-stromal tumors [3].

Dysgerminomas are the most common ovarian malignant germ cell tumors, accounting for 1-2% of all primary ovarian neoplasms [4,5]. In 75% of the cases, they occur after the onset of puberty [4]. Key features are the lack of precursor lesions and of specific differentiation patterns [5], a less invasive behavior with a good prognosis after surgical treatment, with a nearly 100% 5-year survival [6,7,8] and almost 90% at 10 years [9,10,11].

Although the age range reported for ovarian dysgerminoma is 7 months to 70 years old, their peak incidence occurs after puberty, more specifically in the second and third decades of life [12].

They are believed to develop from primordial germ cells that present spontaneous transmembrane tyrosine kinase receptor c-kit (CD 117) mutations. These mutations are usually observed in unilateral dysgerminomas, probably occurring after the cellular migration to the gonadal ridges [13,14].

Histologically, ovarian dysgerminomas, considered the microscopical female correspondent of the male testicular seminomas and extragonadal germinoma, are composed of undifferentiated germ cells with well-defined cytoplasmic borders, large nuclei, numerous mitoses, and clear cytoplasm, who are typically arranged in sheets or elongated nests with a palisaded peripheral cell arrangement, frequently infiltrating the stroma. The gross appearance of a well-encapsulated solid mass with an average diameter of 15 cm, but there are 50 cm cases reported, with a grey-white or light tan fleshy lobe-like pattern on section [2,3,6,7,14,15].

Immunohistochemistry shows positive testing for CD117, thus correlating with KIT mutations, while positive expression of organic cation/carnitine transporter 4 (OCT 4), spalt-like transcription factor 4, and D2–40 can also be determined. 30% of the tumours are present with vimentin (+), lactate dehydrogenase -LDH (+) and neuronal specific enolase- NSE (+). Although these tumors are not hormone-producing, still, human chorionic gonadotrophin (hCG) and ihibin are found positive in syncytiotrophoblastic cells [2,5,6,16,17].

These tumors have a propensity for bilateral involvement, with up to 5-10 % of the cases presenting as bilateral ovarian masses [18].

Their management involves surgery, and, in stages beyond IA chemo- and or radiotherapy are added as a mandatory therapeutic step [19,20].

Since these patients are usually under 30 years old and there is a time frame of at least 5 years between the diagnosis and the conception moment, optimal preoperative diagnosis is mandatory, and fertility preservation surgery becomes a key issue in their management [13,19,21,22]. 15%–20% are diagnosed during pregnancy or post-childbirth and are the most frequent tumors in pregnancy [23].

Diagnostic workup includes imaging studies, such as transabdominal ultrasound (in children), a transvaginal ultrasound, computed tomography scan (CT scan), and magnetic resonance imaging (MRI) studies, along with serum markers assay for differential diagnosis of the abdominal mass. Flourine-18 fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) is not a usual diagnostic tool, but rather used for relapse, or follow-up after chemotherapy or surgery [24, 25, 26]. Dysgerminomas are associated with various elevated serum markers. Elevated LDH, alkaline phosphatase is frequently found in these patients' assays [27].

Despite the progress of ultrasound imaging and the introduction of IOTA criteria for distinguishing between benign and malignant lesions, up to 25% of the lesions remain indeterminate, requiring further imaging investigation [28].

Clinical presentation of these rapid-growth abdominal masses ranges from incidental findings on imaging studies to the more common abdominal enlargement, associated pain, or menstrual abnormalities. Precocious puberty, metrorrhagia, and acute abdomen due to tumor rupture, torsion, or hemorrhage are also noted.

Differential diagnosis includes but is not limited to other histological types of ovarian tumors (benign or malignant), ovarian metastases, or ovarian myomas.

Ovarian dysgerminoma complicated with torsion, rupture can mimic other acute abdominal conditions, such as acute appendicitis, diverticulitis, tubo-ovarian abscess, ectopic pregnancy, or ruptured follicular cyst [29].

According to NCCN (National Comprehensive Cancer Network) Guidelines 2020, in patients who desire to preserve fertility, fertility-sparing surgery following comprehensive surgical staging is mandatory and consists of unilateral salpingo-oophorectomy along with all visible tumors' resection [30,31].

MATERIALS AND METHODS

This is a narrative review based on published articles, using the following criteria: keywords "ovarian dysgerminoma" and "MRI"; PubMed access; full-length English articles since inception to May 2023; original studies and case reports/ series. Exclusion criteria were ovarian tumors other than dysgerminomas, non-English papers, non-human studies, and reviews.

According to our four main criteria, we identified 40 papers that we manually checked and excluded 3 papers, 1 veterinary study, and 2 articles in another language than English. We considered the various imaging aspects with consideration to the clinical practice (Figure 1).

Figure 1: Flowchart according to our methodology (n = number of articles).

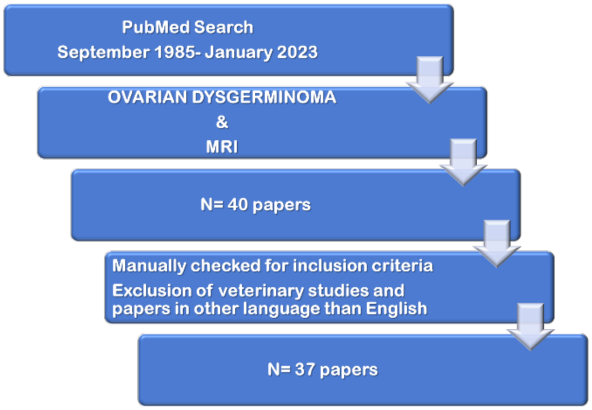
PubMed Search
September 1985- January 2023
OVARIAN DYSGERMINOMA
&
MRI
N= 40 papers
Manually checked for inclusion criteria
Exclusion of veterinary studies and
papers in other language than English
N= 37 papers

RESULTS

Ultrasound features

Ultrasound features involve a well-defined, highly vascularized, heterogenous multilobulated solid mass with significantly vascularized septa [32].

MRI features

MRI aspects include intermediate signal intensity on T2-weighted imaging (T2WI) and high the lobulated aspect seen on ultrasound is also found on CT scans, with enhanced septa. Cystic areas represent either hemorrhage or necrosis. Speckled calcification can be found, especially in hypercalcemia patients. MRI scan shows a similar solid lobulated mass with fibrovascular septa, with low intensity on T1-weighted images (T1WI) and intermediate signal intensity on T2-weighted imaging (T2WI). They usually have high signal intensity on DW imaging and low ADC value. Fibrovascular septa are hyperintense on T2W1 in the presence of edema [32-34].

Zhao et al. divided septa into four categories, according to the degree of edema: Type I consists of thin non-oedematous hypointense T2W1 septa, which are unrecognizable on pre-contrast CT scans. Type II are thin T2W1 hyperintense oedematous septa. Type III are included thick oedematous T2W1 hyperintense septae with hypoattenuation in pre-contrast CT scan. Type IV consists of map-like T2W1 oedematous morphous septae that are usually found in large tumors [35].

Multiplicity can be found as small nodules with high signals on DWI. Large tumor vessels can be found at the center of the lesions with radiant peripheral distribution through the vascular septae. In a case series, the multiplicity detected in the MRI studies is considered to be most likely a sign of the transformation of gonadoblastoma to germinoma [32-36].

According to the same authors, the central vasculature found in MRI studies is most likely a typical finding of dysgerminoma, but further studies are required [36].

18F-FDG PET/CT

The role of 18F-FDG PET/CT is not currently set for ovarian malignant germ cell tumors; it is usually used for the restaging of residual disease after chemotherapy, after surgery, or follow-up in apparently disease-free patients. A negative 18FDG PET/CT might be useful to reduce the number of excessive invasive procedures [37].

In a case of a 21-year-old female with ultrasound evidence of a large solid mass in the abdomen and elevated CA-125, right large pleural effusion on CT-scan, the 18F-fluoro-2-deoxy-D-glucose positron emission tomography maximum standardized uptake was 15,8. Areas of tumor mass reduced metabolism were secondary to tumor necrosis [38].

Associated conditions

Ovarian dysgerminoma can be associated with various other conditions. Patients with disorders of sex development or dysgenic gonads can associate malignant transformation. There are reported cases of Turner syndrome, triple X syndrome, or testicular feminization [39,40].

Patients with disorders of sex development, such as 46, XY with SRY mutations in the high mobility group domain have an increased risk of ovarian malignant transformation, requiring specific investigations [39,40].

Demon Meigs syndrome, consisting of co-occurrence of an ovarian mass, pleural effusion, and elevated serum cancer antigen CA-125 levels, can associate with ovarian dysgerminoma. In the case of a 19-year-old patient presenting with dyspnea and fever, a large right pleural effusion, a small number of ascites, and a huge (29.2 cm × 11.8 cm × 8.4 cm) abdominopelvic tumor were diagnosed. Elevated serum markers- CA-125, LDH, ROMA score suggested the presence of a malignant ovarian mass. The surgical specimen- solid, fleshy, cream-colored, creatine kinase (+), leukocyte common antigen (lymphocyte, +), placental alkaline phosphatase (+), CD30 (−), CD117 (+), α-fetoprotein (−), podoplanin (+), human chorionic gonadotropin (HCG) (+), and inhibin-α (−) was confirmed as dysgerminoma [41, 42].

Unlike seminoma in males, the association of Down syndrome with ovarian dysgerminoma is rather unusual, with only five cases being reported so far in the literature. There is no proven link so far between the two conditions, but the possible association might be secondary to an effect of genetic overexpression, hormonal imbalance, and a higher affinity of the trisomic cells to carcinogens [41,42].

Pregnancy-associated dysgerminoma

Pregnancy-associated dysgerminoma is an extremely rare presentation, and according to a literature review, only 21 cases were published in the last 7 decades. The symptoms are similar to non-pregnant patients, ranging from no symptoms to abdominal distension, and growing abdominal mass. In 2 cases, obstructed labor was reported leading to tumor incarceration. The diagnosis was made based on ultrasound or MRI reports along with elevated serum tumor markers- LDH, the most sensitive, AFP or CA-125. In pregnant patients, MRI studies are second-line imaging tools, used only for confirmation/further investigation of the cases where ultrasound was not sufficient for diagnosis. Several complications can occur during vaginal delivery- incarceration or a caesarean section- tumor rupture. Fetal death can occur in up to one-quarter of the cases [43,44].

Lymph node metastasis

The metastatic pattern in advanced dysgerminoma was only reported isolated in several studies, although this tumor is more metastatic than other histological types of ovarian cancer. It is more likely to develop para-aortic lymph node metastasis than in other stations.

FDG PET CT might help in the identification of these metastatic lymph nodes, reducing the burden associated with extensive tumor relapse treatment. A case series of two patients who presented with an initial FIGO IA stage showed how an improper initial surgery might lead to inadequate assessment and staging of the tumor. The first patient received surgery and lymph node sampling (who was negative) and had a subsequent nodal relapse. The second patient was offered surgery without lymph node sampling/lymphadenectomy and thirteen months later she presented with a para-aortic tumor that required extensive surgery and treatment [45,46].

A case of cervical lymph node metastasis was confirmed by fine needle aspiration after 18FDG PET-CT in a 20-year-old woman who presented with a large abdominal mass and cervical swelling [47].

Rare associations

A case of ovarian dysgerminoma was reported in association with Mullerian agenesis, in a 20-year-old woman who presented with symptoms of pregnancy, and a positive urinary pregnancy test. Clinical and ultrasound evaluation showed the lack of a right ovary and uterus with a normal-appearing left ovary. She confirmed primary amenorrhea. Seriated pelvic MRI initially showed a 2 cm low T1 and low T2 signal mass adjacent to the normal-appearing right ovary, corresponding to a 3/1.5 cm solid pink smooth tumor invading the ovarian hilum. The pathology report confirmed a dysgerminoma containing syncytiotrophoblast cells and microscopic tumoral foci away from the main tumor. Immunohistochemistry was positive for placental alkaline phosphatase and weekly positive for beta HCG [48].

A unicornuate uterus, non-communicating rudimentary horn, and right homogenous mass- dysgerminoma, was reported in an 18 -year- old woman [49].

Ovarian dysgerminoma complications

Dysgerminoma usually presents rapid growth with subsequent torsion and rupture of hemorrhage. Patients present abdominal pain with a sudden increase in intensity, abdominal distension, and a palpable mass revealed on clinical examination. Loss of appetite, nausea, or vomiting can be associated. The size of the tumor determines the intensity and the rapidity of the onset of the symptoms. In such emergency cases diagnosis is usually based on ultrasound and CT-scan, MRI requiring a longer acquisition time. A CT scan might show an area of necrosis or edema with low attenuation, the presence of pelvic free fluid, and the twisted vascular pedicle originating from the adnexa [50,51].

An unusual case of aggressive systemic mastocytosis was reported in a 16-years-old female who was diagnosed with ovarian dysgerminoma 2 years before and received 4 cycles of neoadjuvant chemotherapy (bleomycin, etoposide, and cisplatin) followed by surgery and adjuvant carboplatin and etoposide, with normalization of alpha-fetoprotein levels. 2 years later she complained of bone pain, skin rash, fever, and anaphylaxis and imaging studies revealed spine osteosclerosis and osteolysis, yet no tumor mass was present. AFP levels were normal and immunohistochemistry staining was positive for mast cell tryptase, CD25, CD33, and c-KIT. The serum tryptase level was elevated. Her symptoms were alleviated with prednisolone, but significant improvement was achieved with tyrosine kinase inhibitor – nilotinib [52].

Hypercalcemia can be found in 5% of gynecologic neoplasms, including dysgerminoma, with clinical symptoms depending on the baseline general status, level of calcemia, or rapidity of progression [53]. Two-thirds of ovarian cancer associated with hypercalcemia can be found in small cell carcinoma of the ovary, dysgerminoma being the second associated most common pediatric ovarian malignancy. Fifteen cases of dysgerminoma-associated hypercalcemia have been reported so far. Symptoms include the presence of tumoral mass, abdominal pain/distension, unintentional weight loss, dyspnoea on exertion, nausea constipation, lethargy, polydipsia, and polyuria [54].

Unlike hypercalcemia associated with breast cancer, where a local osteolytic mechanism is involved, in ovarian cancer associated with hypercalcemia, a systemic secretion of parathormone-related peptide (PTHrP) is the cause of the humoral abnormality. Abnormal tumoral expression of 1-alpha-hydroxylase can also be incriminated. 1,25 dihydroxy vitamin D might be involved in malignant-associated hypercalcemia by increasing both osteoclastic bone resorption and intestinal calcium absorption. Surgery is usually followed by the resolution of the symptoms [55,56].

DISCUSSION

Ovarian dysgerminoma is a rare malignant ovarian tumor, diagnosed in the first three decades of life, with a favorable prognosis if the appropriate treatment is given. According to FIGO staging, the initial treatment can either be surgery or neoadjuvant chemotherapy. Since patients are very young, fertility preservation is a major concern and current surgical options include fertility-sparing techniques [13,19,30,31,57].

Due to the scarcity of the literature data, imaging criteria are not very clear. The ultrasound IOTA criteria were introduced to distinguish between benign and malignant ovarian masses [28,31]. Additional imaging studies are further required in possible malignant tumors. CT scan is more likely to be used in emergency situations including acute abdomen due to rupture, hemorrhage, or ovarian torsion. In such cases, an MRI examination would be a time-consuming investigation and doesn’t really add supplementary information. Regarding the evaluation of lymph node status, both CT scan and MRI can give information on para-aortic nodes, as these are the most frequent metastatic site. 18FDG PET-CT might be helpful in identifying the metabolically active areas (lymph node stations) that should undergo surgery [37,38]. MRI studies are valuable for the evaluation of ovarian tissue and vascularization and can add important data on other abnormalities, such as disorders of sex development, and other associated soft tissue anomalies [39,40].

There are two specific aspects that are described in MRI scans. The first one, the multiplicity defined as the presence of small nodules that can be found in section studies, is indicative of early stages or slow-growing tumors. The central vascular vessels, the second characteristic, also seems to be kind of specific for these tumors, as it is usually not described in other histological types of ovarian malignancies, but further studies are required.

Considering the possibility of ovarian dysgerminoma-associated hypercalcemia, the measurement of serum levels of PTH, PTHrP, and 1,25-hydroxyvitamin D seem to be useful, as neurocognitive dysfunction, volume depletion, and renal failure might prove to be serious complications.

Given the reviewed data, the management algorithm in ovarian dysgerminoma patients should include several mandatory aspects. A careful history of all the symptoms is the first step in the evaluation of the patient, followed by ultrasound, abdominal or transvaginal. Serum markers assessment along with supplementary investigations, such as upper and lower GI endoscopy, and cystoscopy can be used in selected cases. A thorough description of the lesion, of the lymph nodes, and the evaluation of possible complications can be achieved through imaging studies – chest X-Ray for pleural effusions, CT/MR scan, and 18FDG PET-CT if available for lymph node evaluation. Neoadjuvant chemotherapy can be used in advanced metastatic disease, followed by surgery. According to current guidelines for non-epithelial ovarian tumors, in the early stages, initial minimally invasive fertility-sparing surgery is the treatment of choice [58,59].

Although ovarian dysgerminoma has a good prognosis with appropriate treatment, optimal diagnostic work-up is mandatory to achieve the best results in young women.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution
Conceptualization, N.I.A. and C.E.N.; methodology, C.A.P.; software, N.F. and S.N.M.E.D.; validation, N.I.A., C.A.P. and N.C.E.; formal analysis, N.F.; investigation, C.A.P.; resources S.N.M.E.D.; data curation N.F.; writing—original draft preparation, N.I.A., C.A.P. and C.E.N.; writing—review and editing, N.I.A, C.A.P. and N.C.E.; visualization, N.I.A.; supervision, N.C.E.; project administration, N.I.A and N.C.E.
All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Should Cervical Cancer Screening with Papanicolaou Smear be Recommended for Female Patients with Psoriasis before Biological Agent Treatment?

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Abstract: *Chronic inflammation and immunosuppressive treatment may play a role in the etiopathogenesis of cancer in psoriasis patients. We wanted to evaluate the Papanicolaou smear (Pap smear) results of female patients with psoriasis which were performed for cervical cancer screening before the initiation of biological agent treatment. Between April 2019 and November 2021, Pap smear results of female patients with psoriasis were reviewed retrospectively. This study included 70 female psoriasis patients with a mean age of 48.41±11.62 years. 49 (70%) patients were biologically naive, whereas 21 (30%) patients previously received biological agents. Obscuring inflammation was reported in 33 (47.1%) patients. Atrophy, intermediate cell predominance, reactive cellular changes due to inflammation, and both atrophy and reactive cellular changes were detected in 15 (21.4%), 7 (10%), 3 (4.3%) patients, and 1 (1.4%) patient, respectively. No malignancy or intraepithelial lesion was observed in 69 (98.6%) patients, whereas atypical squamous cells of undetermined significance were reported in 1 (1.4%) patient. Infections such as bacterial vaginosis, candidiasis, and actinomycosis were detected in 13 (18.6%) patients. Pap smear tests should be performed in female psoriasis patients to detect premalignant lesions of the cervix before the initiation of biological agent treatment. Moreover, psoriasis treatment guidelines should be updated accordingly.*

Keywords: *biological agents; cancer; cervical; psoriasis*

INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder that has been associated with systemic comorbidities such as psoriatic arthritis, cardiovascular disorders, and metabolic syndrome [1,2]. Furthermore, increased risk of developing cancer and cancer mortality have been reported in patients with psoriasis [3-6]. Lymphoma, lung, bladder, colorectal, liver, and kidney cancers have been related to psoriasis [4,7]. It has been suggested that predisposition to obesity, lifestyle factors such as smoking, chronic inflammation, and immunosuppressive drugs used for the treatment of psoriasis might play a role in the etiopathogenesis of cancer in patients with psoriasis [4,8]. Cancer risk increases with disease severity [9]. Patients with severe psoriasis are usually treated with systemic immunosuppressive drugs for a long period of time to control disease [8].

Biological agents such as tumor necrosis factor-α (TNF-α) inhibitors and interleukin (IL) 12/23, IL-23, and IL-17 inhibitors are choices of treatment in psoriasis [10]. TNF-α is an inflammatory cytokine that plays a role in the regulation of cell proliferation and apoptosis [11]. IL-12 and IL-23 show anti-tumor effects, whereas IL-17 has both anti-tumor and tumorigenic effects [10]. Therefore, concerns have been raised that treatment with biological agents might lead to increased cancer risk in psoriasis patients [10,12]. Joint American Academy of Dermatology-National Psoriasis Foundation guidelines recommends performing age-appropriate cancer screening tests in psoriasis patients treated with biological agents [13].

Cervical cancer is the third most common cancer in women globally. Moreover, cervical cancer is the second leading cause of cancer-related mortality in women. Papanicolaou (Pap) smear is the main test used to screen for cervical cancer. Colposcopy and biopsy may be performed in patients with atypical epithelial cells on Pap smear results [14].

Within this study, we evaluated Pap smear results of female patients with psoriasis which were performed to detect premalignant lesions of the cervix and cervical cancer before the initiation of biological agent treatment.

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained prior to this study (approval number: 2021-1067). Between April 2019 and November 2021, Pap smear results of female patients with psoriasis which were performed for cervical cancer screening before the initiation of biological agent treatment were reviewed retrospectively.

Specimen types such as liquid-based and conventional Pap smear, presence of endocervical transformation zone component, quality indicators such as obscuring inflammation and blood, non-neoplastic findings such as atrophy and reactive cellular changes, general categorization, and detected microorganisms were evaluated. Patients with inadequate Pap smear specimens for evaluation were excluded from the study.

Statistical analysis was performed using SPSS version 20.0. Continuous variables were defined as the mean (±) standard deviation and categorical variables were expressed as percentages. Differences between the two groups were detected with a chi-square test. P<0.05 was considered statistically significant.

RESULTS

This study included 70 female patients with psoriasis with a mean age of 48.41±11.62 years (range: 25-74 years). 54 (77.1%) patients had psoriasis vulgaris, 8 (11.4%) patients had generalized pustular psoriasis, 5 (7.1%) patients had palmoplantar psoriasis, 2 (2.9%) patients had palmoplantar pustular psoriasis and 1 (1.4%) patient had inverse psoriasis. The mean disease duration was 15.71±11.51 years (range: 1-50 years). 26 (37.1%) patients had psoriatic arthritis. Past medical history was unremarkable in 37 (52.9%) patients. Moreover, 24 (34.3%) patients had hypertension, 16 (22.9%) patients had diabetes, 5 (7.1%) patients had chronic hepatitis B virus infection, 4 (5.7%) patients had hypothyroidism, 4 (5.7%) patients had coronary artery disease, 3 (4.3%) patients had hyperlipidemia, 2 (2.9) patients had chronic obstructive pulmonary disease, 2 (2.9) patients had asthma and 1 (1.4%) patient had steatohepatitis. 69 (98.6%) patients were treated with conventional systemic treatments such as methotrexate, cyclosporine, and acitretin, and 18 (25.7%) patients were previously treated with phototherapy. 49 (70%) patients were biologic naive, however, 21 (30%) patients were previously treated with biological agents. Among them, 6 (8.6%) patients received infliximab, 5 (7.1%) patients received adalimumab, 3 (4.3%) patients received etanercept, 2 (2.9%) patients received ustekinumab, 1 (1.4%) patient received ixekizumab, 1 (1.4%) patient received secukinumab and 1 (1.4%) patient received certolizumab pegol. In addition, 1 (1.4%) patient was treated with both adalimumab and etanercept, and 1 (1.4%) patient was previously treated with both infliximab and etanercept.

Specimen type was liquid-based in 46 (65.7%) patients and conventional Pap smear in 24 (34.3%) patients (Table 1). The presence of endocervical transformation zone components was reported in 47 (67.1%) patients.

Table 1: Pap smear results of the patients with psoriasis before biological agent treatment

	n (%)			
Specimen type	Liquid-based 46 (65.7%)	Conventional 24 (34.3%)		
Endocervical transformation zone	Present 47 (67.1%)	Absent 23 (32.9%)		
The other quality indicators	Obscuring inflammation 33 (47.1%)	Obscuring blood 1 (1.4%)		
Non-neoplastic findings	Atrophy 15 (21.4%)	Intermediate cell predominance 7 (10%)	Reactive cellular changes due to inflammation 3 (4.3%)	Atrophy+reactive cellular changes 1 (1.4%)
General categorization	Negative for malignancy 69 (98.6%)	Atypical squamous cells 1 (1.4%)		
Microorganisms	None 57 (81.4%)	Bacterial vaginosis 6 (8.6%)	Candida 6 (8.6%)	Bacterial vaginosis+ Actinomyces 1 (1.4%)
3-dimensional cell groups	1 (1.4%)			

Atypical squamous cells of undetermined significance were reported in 1 (1.4%) patient. Fungal organisms consistent with Candida were encountered in 6 (8.6%) patients, 3 (4.3%) of them had fungal organisms morphologically consistent with Candida glabrata. In addition, three dimensional groups of endocervical cell shedding which might be associated with benign cervical lesions such as polyp or papillary cervicitis was reported in 1 (1.4%) patient.

However, the endocervical transformation zone component was absent and thus squamous epithelial cells were evaluated in 23 (32.9%) patients. Among other quality indicators, obscuring with inflammation was reported in 33 (47.1%) patients, and obscuring with blood was reported in 1 (1.4%) patient. As a non-neoplastic finding, atrophy was detected in 15 (21.4%) patients, intermediate cell predominance was detected in 7 (10%) patients, and reactive cellular changes due to inflammation were detected in 3 (4.3%) patients. In addition, 1 (1.4%) patient showed both atrophy and reactive cellular changes. No malignancy or intraepithelial lesion was observed in 69 (98.6%) patients, however, epithelial cell abnormality was detected in 1 (1.4%) patient. Atypical squamous cells of undetermined significance (ASC-US) [15] were reported in the latter. The patient with ASC-US was a 44-year-old female with a 36-year history of psoriasis vulgaris and psoriatic arthritis who had been previously treated with methotrexate and infliximab. No microorganism was detected in the Pap smear results of 57 (81.4%) patients. However, 6 (8.6%) patients showed flora change suggesting bacterial vaginosis. Fungal organisms consistent with Candida were encountered in 3 (4.3%) patients and fungal organisms morphologically consistent with Candida glabrata were encountered in 3 (4.3%) patients. Pap smear result of 1 (1.4%) patient revealed both flora changes suggesting bacterial vaginosis and microorganisms consistent with Actinomyces. Three-dimensional groups of endocervical cell shedding which might be associated with benign cervical lesions such as polyp or papillary cervicitis were reported in 1 (1.4%) patient.

Patients with atrophy on Pap smear were statistically significantly older than those who did not have atrophy on Pap smear (the mean age: 60.44±6.25 and 44.85±10.41 years, respectively) (p<0.001). On the other hand, the mean age of the patients with inflammation on Pap smear (44.88±8.98 years) was statistically significantly lower than the mean age of the patients without inflammation on Pap smear (51.57±12.87 years) (p=0.015). Inflammation on Pap smear was observed in 26 (37.1%) patients with psoriasis vulgaris, 3 (4.3%) patients with palmoplantar psoriasis, 2 (2.9%) patients with generalized pustular psoriasis, 1 (1.4%) patient with palmoplantar pustular psoriasis and 1 (1.4%) patient with inverse psoriasis. No statistically significant difference was detected between psoriasis type and inflammation on Pap smear (p=0.549). The mean disease duration with and without inflammation on Pap smear was 13.67±10.33 and 17.54±12.31 years, respectively (p=0.161). 13 (18.6%) patients with inflammation on Pap smear had psoriatic arthritis, and 20 (28.6%) patients with inflammation on Pap smear did not have psoriatic arthritis. No statistically significant difference was detected between psoriatic arthritis and inflammation on Pap smear (p=0.713). 11 (15.7%) patients with inflammation on Pap smear had accompanying comorbidities whereas 22 (31.4%) of the patients with inflammation had an unremarkable past medical history (p=0.029). 25 (35.7%) patients with inflammation on Pap smear were biologic naive, whereas 8 (11.4%) patients with inflammation on Pap smear received former biological agent treatment (p=0.321). Among 33 patients with inflammation, only 8 of them had Pap smear results suggesting infection such as bacterial vaginosis or candidiasis (p=0.249).

DISCUSSION

Cervical cancer screening aims for the early detection of premalignant and malignant lesions in order to reduce cervical cancer-related mortality [16]. US Preventive Services Task Force (USPSTF) recommends performing cervical cytology every 3 years in females between the ages of 21 and 29. Between the ages of 30 and 65, it is recommended to perform cervical cytology every 3 years, high-risk human papillomavirus (HPV) testing every 5 years, or both tests every 5 years. Cervical cancer screening is not recommended before 21 years of age and in females older than 65 without high-risk factors for cervical cancer.

According to USPSTF, immunocompromised individuals, patients with human immunodeficiency virus (HIV) infection, and individuals who were exposed in utero to diethylstilbestrol and previously treated for a high-grade precancerous lesion or cervical cancer are at high risk for cervical cancer. Individualized programs for cervical cancer screening have been recommended in the follow-up of these patients [17]. The American Cancer Society (ACS) recommends initiating cervical cancer screening at the age of 25. Between ages 25 and 65, primary HPV tests such as Onclarity HPV are preferred every 5 years. When primary HPV testing is unavailable, both Pap smear and HPV tests such as Digene hybrid capture 2 are recommended every 5 years, or Pap smear alone may be performed every 3 years. Cervical cancer screening should be ceased in women older than 65 with normal screening results for the last 10 years. However, these individuals should not have been diagnosed with cervical intraepithelial neoplasia 2 or more advanced disease for the last 25 years [18]. World Health Organization (WHO) recommends starting cervical cancer screening at the age of 30 in the general population and in women aged 25 with HIV infection. Primarily, HPV DNA detection is recommended every 5 to 10 years in the general population and every 3 to 5 years in women with HIV infection. If HPV-DNA testing is unavailable, either visual inspection with acetic acid (VIA) or cytology may be performed in both groups.

Screening is discontinued both in the general population and in women with HIV infection after 50 years of age [19]. According to the American College of Obstetricians and

Gynecologists (ACOG), cervical cancer screening should be started at 21 years of age. Cytology is preferred for cervical cancer screening in women aged 21-29 years. However, women aged 25-29 with an average risk for cervical cancer can also be screened by high-risk HPV testing every 5 years [20].

The age to start cervical cancer screening, primarily preferred screening tests, screening intervals, and age to stop screening differ in WHO and cervical cancer screening guidelines [17-19]. Moreover, recommendations about immunocompromised individuals such as patients with HIV infection and solid organ transplantation have not been detailed by USPSTF, whereas, in these patients, ACS recommends cytology annually between ages 21-29 and cytology as well as high-risk HPV testing every 3 years between ages 30-65 [21]. Therefore, we suggest that guidelines for the treatment of psoriasis with biological agents should include detailed information on cervical cancer screening in order to guide both physicians and female patients with psoriasis [19].

Studies investigating the risk of cervical cancer in women with psoriasis are inadequate and conflicting results have been reported on the risk of cervical cancer in patients with systemic inflammatory diseases [22,23]. For instance, Moscicki et al. reviewed the risk of cervical cancer in patients with immunosuppression for reasons other than HIV infection and in patients with autoimmune diseases. Moscicki et al. suggested that the risk for the development of cervical cancer increased in patients with inflammatory bowel disease and rheumatoid arthritis who were under immuno-suppressive treatment and in patients with systemic lupus erythematosus regardless of treatment [22]. On the other hand, Kim et al. reported that the risk of cervical dysplasia and cervical cancer were higher in patients with systemic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus but not different in patients with psoriasis and inflammatory bowel disease compared to women without systemic inflammatory diseases. Kim et al. suggested that the results might be affected by the variety in systemic inflammation levels between patient groups and low rates of administration of immunosuppressive medication in patients with psoriasis and inflammatory bowel disease [23].

Patients with psoriasis who are treated with biological agents may not undergo necessary cancer screening tests [24]. Kim et al. revealed that only 55% of the patients with psoriasis were admitted to a gynecologist at least once within an approximately two-year follow-up [23]. Silveira et al. suggested that 25.2% of the patients with psoriasis who were under biological agent treatment did not have necessary laboratory tests during the previous year [25]. Barbieri et al. reported that the rate of attendance of cervical cancer screening in psoriasis patients treated with biological agents was similar in patients with hypertension, however, 9% higher than in the general population [24].

CONCLUSION

Within this study, Pap smear results of 70 women with psoriasis were evaluated before the initiation of biological agent treatment. Atypical squamous cells were detected in 1 (1.4%) patient, whereas Pap smear results revealed infections such as bacterial vaginosis, candidiasis, and actinomycosis in 13 (18.6%) patients. Furthermore, obscuring with inflammation was reported in 33 (47.1%) patients. Inflammation on Pap smear was more commonly detected in younger patients, however, no statistically significant association was observed between inflammation on Pap smear and psoriasis type, disease duration, the existence of psoriatic arthritis, and previous biological agent treatment (p=0.549, p=0.161, p=0.713, p=0.321, respectively). Hence, we suggest that a Pap smear test should be performed in female psoriasis patients to detect premalignant lesions of the cervix before the initiation of biological agent treatment. Moreover, psoriasis treatment guidelines should be updated accordingly.

Conflicts of interest and sources of funding
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Authors' contribution
Conceptualization, F.T., I.O., A.G.; methodology, F.T.; software, F.T.; validation, F.T., I.O., A.G.; formal analysis, F.T.; investigation, F.T., I.O.; resources, F.T., I.O., A.G.; data curation, F.T., I.O., A.G.; writing—original draft preparation, F.T.; writing—review and editing, F.T., I.O., A.G.; visualization, F.T.; supervision, A.G.; project administration, F.T., I.O., A.G.
All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2021-1067).

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Haemodialysis Patients with Type 2 Diabetes Mellitus and COVID-19: An Observational Study

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Abstract: *Background and Objectives: Chronic Kidney Disease (CKD) affects 6,7% of the adult population in Romania and is associated with high morbidity. About one out of three adults with diabetes has kidney disease. According to current literature data, the prevalence of diabetes is very high, up to 11,6%, of whom 2,4% had undiagnosed diabetes, and is the leading cause of kidney damage and the need for renal replacement therapy (RRT). COVID-19 has brought with it a lot of unanswered questions, regarding the risk factors, the disease evolution, and the treatment possibilities. It became clear that diabetic kidney disease (DKD) is among the independent risk factors that predict unfavorable outcomes upon SARS-CoV-2 infection, so we aimed to evaluate the characteristics of diabetic and non-diabetic dialyzed patients, COVID-19 positive. Materials and Methods: It is an observational, single-center study that analyzed type 2 diabetes mellitus and non–diabetic patients in maintenance hemodialysis hospitalized for SARS CoV-2 infection. Results: A total of 101 adult dialyzed patients were admitted with a SARS-CoV-2 RT-PCR positive test, out of which 42 had a long history of diabetes mellitus type 2 and 59 of them have been known with other etiologies of CKD. Hypertension and heart disease were the most commonly associated comorbidities. Inflammatory markers and anemia were significantly increased in diabetic patients compared to non-diabetic. Conclusions: We found that anemia was more severe in patients COVID-19-positive MHD T2DM patients.*

Keywords: *COVID-19; diabetic nephropathy; diabetic kidney disease; mortality*

INTRODUCTION

The introduction of the term chronic kidney disease (CKD) was followed by the discovery of an unexpectedly high prevalence of CKD. According to PREDATORR study, the prevalence of CKD in Romania was estimated at 6.7% [1]. The real problem with this finding is the high morbidity and mortality burden associated with advanced stages of CKD. During COVID-19 pandemics, the mortality rates associated with SARS-CoV-2 infection were higher in maintenance hemodialysis patients (MHD) than in the non-renal population, varying between 27-41% [2], reaching 50% in other studies. [3]. The maintenance hemodialysis patients are even at a greater risk for death, as was observed in recent studies [4].

Another risk factor for unfavorable outcomes in COVID-19 infection is type 2 diabetes mellitus (T2DM) [5]. According to the International Diabetes Federation, there are 1.2 million patients with diabetes mellitus in Romania [6].

The association between diabetes and chronic kidney disease is extremely common, being the main cause of end-stage kidney disease (ESKD), in more than 40% of patients worldwide [7], even more than the traditional nephrological pathology, such as glomerular diseases. Poor glycemic control has been proven to be a risk factor for progression to ESKD, renal replacement therapy (RRT), and fatal cardiovascular events. During the COVID-19 pandemic, studies have reported diabetes mellitus (DM) to be associated with a two-fold increase in mortality as well as increased severity of COVID-19 disease, as compared to non-DM patients [8]. Maintaining optimal glycemic control is crucial for avoiding severe complications, and continuous glucose monitoring devices could improve glycemic control for patients with type 2 diabetes, thus decreasing the rates of high morbidity as seen in recent studies [9]. Given the fact that diabetes is a major concern worldwide, new biomarkers for glycemic control need to be found for better diagnosis and treatment [10]

The aim of the present study was to evaluate the characteristics of COVID-19 patients with DM as compared to non-DM COVID-19 population.

MATERIALS AND METHODS

It is an observational, single-center study conducted in the Nephrology Clinic of Saint John Emergency Clinical Hospital Bucharest and includes data on MHD patients admitted between November 2021-March 2022. COVID-19 disease was confirmed by positive real-time polymerase chain reaction (RT-PCR). The data was collected from the hospital's electronic medical database. The study was conducted according to the Declaration of Helsinki on human rights.

The protocol of the study was approved by the Institutional Ethics Committee. Considering the observational character of the study, the informed consent was waived by the Ethics Committee.

We collected data regarding age, gender, disease severity, oxygen supplementation need, etiology of chronic kidney disease (CKD), laboratory tests (hemoglobin levels, leukocyte, lymphocyte and platelet counts, serum albumin, C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6) and data about infectious and non-infectious complications. We divided the study population into two groups based on the T2DM status (T2DM vs. non-DM) and compared continuous variables from the two groups using the nonparametric Mann-Whitney U test and parametric t-test. We considered statistically significant a p-value of less than 0.05. The software used was Graph Pad Prism 6.0.

The limits of this study are represented by its retrospective character, including the small number of patients.

RESULTS

A complete physical examination was required for assessing the clinical status of patients and the performance status was evaluated according to the ECOG scale. Most patients (61.7%) presented with good performance status (ECOG 0), 35.6% with ECOG 1, and 0.7% with ECOG 2.

The study group consisted of 101 consecutive adults (>18 years of age) MHD patients who were admitted with a SARS-CoV-2 RT-PCR positive test, out of which 42 (48.5%) had a diagnosis of diabetic kidney disease (DKD), and 59 (51.5%) have been known with other etiologies of CKD, predominantly represented by hypertensive nephropathy (33.89%), chronic obstructive tubulointerstitial nephropathy (11.86%), and autosomal dominant polycystic kidney disease (8.47%).

For the study population, demographic and clinical characteristics are presented in Table 1.

In the T2DM group, the median age was 65.69±31.87 years (CI 95%) and the majority of the patients were men (66.6%). More than 50% of patients were obese and the most common comorbidities were represented by hypertension (100%), chronic coronary heart disease (61.9%), atherosclerosis (61.9%), followed by pulmonary disease (11.9%), and active malignancies (7.1%). In the T2DM study population, 42.8% of patients required noninvasive oxygen therapy at admission, out of which 11.1% needed positive pressure ventilation in evolution, while 11.9% needed positive pressure ventilation from admission and 23.8% required mechanical ventilation. In the T2DM group, hemorrhagic complications were observed in 16.6% of patients. We identified thrombosis in 4.76% of patients (arteriovenous fistula thrombosis, lower limb deep vein thrombosis, and splenic infarction). Sepsis was identified in 30.9% of the cases and one patient developed Clostridioides difficile colitis. The recorded mortality rate for the T2-DM patients was 42.5%, out of which 72.2% of the deaths were due to sepsis (Figure 1).

In the non-diabetes study group, the median age was 64.33 ±3.08 years (CI 95%), the majority being men (64.4%). The obesity rates were lower (30.5%) than those in the T2DM group and 89.8% were hypertensive. Chronic coronary heart disease (47.4%) and atherosclerosis (40.6%) were observed at lower rates, with higher rates of chronic pulmonary diseases (18.6%) and active malignancies (20.3%).

Regarding respiratory insufficiency management, we observed that when compared with T2DM patients, 49.1% of non-DM required non-invasive oxygen therapy at admission, with a statistically significant difference, but the rates for assisted ventilation (positive pressure ventilation (PPV) and mechanical ventilation (MV) were lower, 10.1% and 18.6%, respectively.

Table 1: Demographic and clinical characteristics and the significant differences

	Overall	T2DMgroup	Non-DM group	P value
Patient number (N)	101	42	59	
Demographic data				
Age (mean, years)	64.901 ±2.241	65.69 ±31.87	64.339 ±3.08	0.4111
Gender (%)				
Female (N, %)	35	14 (33%)	21 (35.6%)	0.6573
Male (N, %)	66	28 (67%)	38 (64.4%)	0.2390
Risk factors				
Obesity (N, %)	45	27 (64.2%)	18 (30.5%)	0.5796
Atherosclerosis (N, %)	50	26 (61.9%)	24 (40.6%)	0.3712
Comorbidities				
Hypertension (N, %)	95	42 (100%)	53 (89.8%)	0.8127
Ischemic heart disease (N, %)	54	26 (61.9%)	28 (47.4%)	0.2450
History of neoplasm (N, %)	15	3 (7.14%)	12 (20.3%)	0.7672
Pulmonary disease (N, %)	16	5 (11.9%)	11 (18.6%)	0.7732
Supplemental oxygen				
Nasal cannula (N, %)	47	18 (42.8%)	29 (49.1%)	0.0440
PPV (N, %)	13	7 (16.6%)	6 (10.1%)	0.7854
MV (N, %)	21	10 (23.8%)	11 (18.6%)	0.7310
Complications				
Hemorrhagic (N, %)	24	7 (16.6%)	17 (28.8%)	0.6509
Thrombosis (N, %)	4	2 (4.7%)	2 (3.3%)	0.8230
Infection (N, %)	23	13 (30.9%)	10 (25.4%)	0.7258
Laboratory testing				
Hemoglobin <12 g/dL (N,%)	85	34 (80.9%)	51 (86.4%)	0.0173
WBC > 11 x $10^3/mm^3$ (N, %)	21	11 (26.9%)	10 (16.9%)	0.0001
CRP > 5 ng/mL (N, %)	98	40 (95.2%)	58 (98.3%)	0.0001
Procalcitonin >0,05 ng/mL (N, %)	101	42 (100%)	59 (100%)	0.0001
Interleukin-6 > 7 pg/mL (N, %)	98	41 (97.6%)	57 (93.2%)	0.0001
Mortality (N, %)	39	19 (45.2%)	20 (33.8%)	0.5685

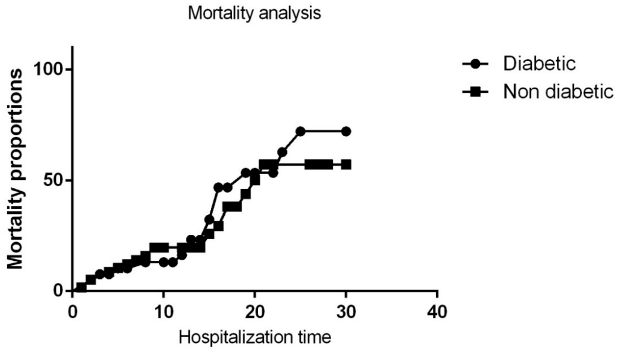

Figure 1: Mortality analysis between diabetic and non-diabetic patients

Hemorrhagic complications were observed in 28.8% of patients in the non-DM group, more frequently with soft tissue hematoma and only two cases of thrombosis. Infectious complications were observed in 25.4% of the non-DM patients (66.6% represented by sepsis and 33.3% by Clostridioides difficile colitis). The mortality rates for the non-DM group were lower (32.2%) when compared to the T2DM group.

We analyzed biological parameters in both groups. In the

T2DM group, 80.9% of patients had anemia (Hgb<12 g/dL), with a higher proportion in the non-DM group, accounting for 86.4%. Inflammatory status was assessed by leukocyte count, lymphocyte count, C-Reactive Protein level, procalcitonin, and Interleukin-6 (IL-6) levels. Leukocytosis (>11.000/mm^3) was present at admission in 26.9% of cases, while lymphopenia in 61.9% of cases.

C- Reactive Protein, procalcitonin, and IL-6 levels were increased at admission in almost all T2DM patients namely 95.2%, 100%, and 97.6% respectively. The non-DM-group also accounted for high inflammatory parameters, leukocytosis in 16.9%, C-Reactive Protein >5 mg/L in 98.3%, procalcitonin >0,05 ng/mL in 100%, and IL-6 >7 pg/mL in 93.2%. We found statistically significant differences when comparing the two groups ($p<0.001$). Hypoalbuminemia (serum albumin level <3.4 mg/dL) was noted in higher rates in the non-DM group (27.1%) as compared with T2DM patients (23.8%), with statistically significant differences (Table 1).

DISCUSSION

Diabetes is a major cause of chronic kidney disease, accounting for more than 50% of MHD patients all around the world, exceeding traditional kidney diseases [11,12]. Early COVID-19 reports mentioned DM among the most important risk factors for severe disease and increased mortality due to SARS-CoV-2 infection. The majority of COVID-19 reports stated that CKD is independently a risk factor for poor outcomes, with adjusted mortality in CKD and HD patients being described to be 2 to 3 times higher [4].

All of the 42 T2DM patients were known with hypertension. It is well known the equipotent relationship between CKD and hypertension: hypertension can cause CKD and CKD is a risk factor for hypertension. Also, DM and hypertension frequently occur together, with a substantial overlap between DM and hypertension in disease mechanisms. In the non-DM group, 89.8% were hypertensive, with lower rates when compared with the T2DM group, approaching the literature data that DM and CKD cause high blood pressure with poor control [13], and with a high mortality risk in SARS-CoV-2 infection [14,15].

Obesity rates were higher in the T2DM group, more than 50% vs 30.5%. In the first meta-analysis of obese patients and COVID-19, was stated that higher body mass index was associated with intensive care unit admission and critical disease [16], by decreasing expiratory reserve volume, diaphragm excursion, and restricting ventilation.

In DM patients, the risk of fatal cardiovascular events is much higher compared to the general population. A close link exists between diabetes and cardiovascular disease which is the most prevalent cause of morbidity and mortality in diabetic patients [17]. In the presented study, coronary heart disease was more frequently observed in T2DM patients as compared to non-DM patients. Also, atherosclerosis was seen with higher prevalence in the DM group, approaching the literature and the current evidence that diabetes dramatically accelerates atherosclerosis [18], all of this constellation increasing the COVID-19 mortality [19].

Some malignancies are shown to increase the risk of kidney failure [20,21,22] In our study, patients with active malignancies were in a higher proportion in the non-DM group.

Anemia is more commonly associated with diabetic kidney disease [23]. When comparing the two study groups, we found that anemia is a risk factor for poor outcomes in MHD T2DM patients.

In general, infectious diseases are more frequent and severe in patients with DM, due to the chronic hyperglycemic status that promotes immune dysfunction [24]. Studied T2DM patients presented with significantly altered inflammation markers compared to the non-DM group. Hypoalbuminemia has been observed in the T2DM group, to a lesser extent compared to non-DM, but the differences were statistically significant. T2DM status and impaired immune system are risk factors for poor outcomes in SARS-CoV-2 infected patients. The mortality rates were higher in the T2DM group, although the differences were not statistically significant.

CONCLUSION

In conclusion, we found that anemia is more severe in COVID-19 MHD T2DM patients. The severity of anemia in the T2DM group was consistent with significantly altered inflammatory markers. New biomarkers and studies are needed for better anemia management and more specific biomarkers that correlate the inflammatory status with the infectious disease.

We aim for a future in which we have as many studies as possible that include hemodialysis patients, a special category of patients due to the high morbidity and mortality associated with it, and also due to the important social impact.

The patient`s data used to support the findings of this study is available from the corresponding author upon request.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.
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Authors' contribution
Conceptualization: IAV, LFF; Methodology: IAV, LFF, EC and AEBS; Software: IAV and LFF; Validation: IAV, ALC. and DR; Formal analysis: IAV, EC, LR and CD; Investigation: EC, LFF and AEBS; Resources: IAV, DR and CD; Data curation: AEBS, ALC, MTG, SI and LR; Writing—original draft preparation, IAV and LFF; Writing—

review and editing, IAV, LFF, EC, and ALC; Visualization, CD, LR, MTG, SI, DR; Supervision EC, ALC; Project administration IAV, LFF

All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Descriptive Analysis of Internal Registry Data on Cemented and Uncemented Total Hip Arthroplasty

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Abstract: *Background: As total hip replacement cases increase year after year, registry-wide data becomes increasingly important in order to assess pathology distribution, reimbursement efficiency as well as physician performance. In order to assess all these factors patient evolution needs to be documented after surgery as well, but the main impediment to doing this is losing patients to follow-up. For this reason, the present study aims to perform a descriptive analysis of the hospital's internal registry data as well as to determine what percentage of patients can be expected to return to follow-up after total hip replacement. Methods: A query of the hospital's internal registry data in the last years was performed. Patient demographics, county distribution as well as ICD-10 code usage were documented. Data standardization was performed to determine the probability and cumulative distribution of the patient's age. The chi2 test of independence was used to assess whether there is a correlation between the patient's gender and ICD-10 codes. Pot hoc power analysis was performed to assess the accuracy of correlation analysis. Results: Only 29.6% of the patients undergoing total hip replacement are from the county within which the hospital is located. 25% of the patients were under 60 years old. Conclusions: Current registry data reveals that most patients undergoing total hip replacement are around 67.5 ± 11 years old, suffer from unilateral or bilateral primary or secondary hip osteoarthritis and only 29.6% of them are likely to return to follow-up.*

Keywords: *total hip replacement; ICD-10; registry; statistical analysis*

INTRODUCTION

Over 1 million total hip arthroplasty (THA) surgeries have been performed globally in the last year and this number is rising for both primary and revision procedures [1]. In the United States, the rate of primary total hip arthroplasties increased by 50%, from 47 per 100 000 people to 69 per 100 000 people, with a market size of nearly 2.30 billion USD [2]. With over 305 operations per 100,000 residents, Switzerland continues to lead the list of countries in Europe with the highest hip replacement rate, according to a 2016 report from the World Organization for Cooperation and Regional Development (OECD) [3].

In Romania, the annual rate of primary hip replacement has climbed dramatically during the previous 15 years, 51.8 out of every 100,000 people underwent a main hip replacement procedure in 2015, whereas 2.7 out of every 100,000 people required a hip revision surgery [4,5].

The demand for hip implants is expected to increase as a result of adequate health insurance coverage and reimbursement laws for joint replacement surgeries [6].

In our country, THAs were performed mostly on patients between 60 and 69 years old. Male patients are more prevalent in the 50-59 age range and younger age groups, while female patients are more likely to have THAs after the age of 59. With a total of 29,497 female patients and 19,675 male patients, women over the age of 59 make up the clear majority [5].

Although THA has demonstrated outstanding short and long-term results, the revision rate has remained constant over the past few decades despite improvements in surgical technique and implant design [7,8].

In the US the most common causes for re-intervention were: instability (22%), mechanical loosening (20%), infection (15%), implant failure (10%), osteolysis (7%), and periprosthetic fracture (6%) [9,10]. In Romania, the most common reasons for revision surgery are acetabular defects (mostly acetabular loosening and osteolysis), femoral defects, and other (un-standardized reasons for revision in 20%-25% of the cases), followed by were, luxation, infection, periprosthetic fracture and broken implants [11].

It has been established that primary surgeries use fewer financial resources than revision surgeries [12]. In order to maintain the sustainability of their finances, institutions must comprehend the costs and durability of these interventions [13]. Implants cost to make up a significant portion of the entire hospitalization bill (for revision surgery especially) and are a key area for cost-cutting overall which demands proper financial planning [14].

There is a wide range in reimbursements for both primary and revision surgeries, possibly in part because of the diagnosis-related group (DRG), intrinsic unpredictability, and complexity of these procedures with a rising need to reconcile the clinical challenges with the available technical stock [6,13]. That is why we consider that analyzing and supporting the cost-effectiveness of surveillance measures is a necessity. Orthopedic specialists generally concur that some follow-up after THA is important [11]. Nowadays there are no clear standardized instructions regarding the timing of the subsequent visits following hip replacement interventions [15]. The identification of asymptomatic patients who need revision surgery remains one of the primary justifications for early and late routine follow-ups [16].

There are only five recommended protocols for THA follow-up visits that are well described, according to a systematic review of the literature and the content of orthopedic associations' websites, with a lot of variation in the suggested follow-up frequency and duration [17]. The latter is simply mentioning the necessity of routine follow-up visits without defining their timing, duration, or their cost-effectiveness [18,19].

According to earlier research, patients who don't follow up with their surgeon regularly are more likely to experience worse outcomes and require additional surgical intervention at a different location [18,20]. Regardless of its importance, postoperative evaluation places a heavy load and financial burden on patients, families, and tertiary healthcare systems which have to manage a rising number of new patients [21,22].

This study's goal was to assess patient compliance with clinical follow-up in a line of patients who underwent hip surgery in different surgical centers in our country. In addition, we aim to analyze the positive and negative predictors for clinical follow-up compliance.

MATERIALS AND METHODS

Prior to the study's inception, ethical approval was sought and received. The aim of the study was to investigate the hospital's internal hip arthroplasty registry between 2018 and 2022 in order to determine patient gender and age distribution as well as how many patients would be likely to return to follow-up after surgery. An arbitrary distance boundary was set to only include the county in which the hospital is situated (B, Bucharest County). A descriptive analysis of ICD-10 codes frequency and gender distribution was also generated. Patients undergoing cemented and uncemented total hip arthroplasty were eligible for inclusion.

In order to plot an age frequency distribution, z-scores were generated and from them, the frequency distribution was plotted in Microsoft Excel.

A chi2 test of independence was performed to assess whether there is a gender propensity for any ICD-10 scores. Posthoc power analysis was also conducted to judge the appropriateness of the chi2 test results.

Further stratification based on relevant age groups has been performed in order to identify diagnostic patterns. Relevant age groups were constructed as follows: "Under 30", "30-39", "40-49", "50-59", and "60 and above". A histogram was used to present diagnostic patterns in each age group.

Data gathering, analysis, and storage were performed using SPSS v.26, Microsoft Excel, and Python programming language v.2.7.

RESULTS

After ethical approval, 929 patients were detected within the specified period. Patient age and gender distribution are presented in Table 1. Figure 1 presents the patient's distribution as a function of the county with 29.6% of the patients undergoing cemented or uncemented hip arthroplasty coming from the county in which the hospital is located, Bucharest. Figure 2 and Table 1 show that most often, patients 67.5 years or older undergo total hip arthroplasty. At the same time, a significant proportion of the included patients are under the age of 60, 25%. ICD-10 codes M16.1 "Unilateral primary osteoarthritis of the hip" and M16.7 "Other unilateral secondary osteoarthritis of the hip" were the most used, accounting for almost 45% of cases. ICD-10 codes M16.6 "Other bilateral secondary osteoarthritis of the hip" and M16.0 "Bilateral primary osteoarthritis of the hip" were the second most used codes accounting for 22.4% of cases.

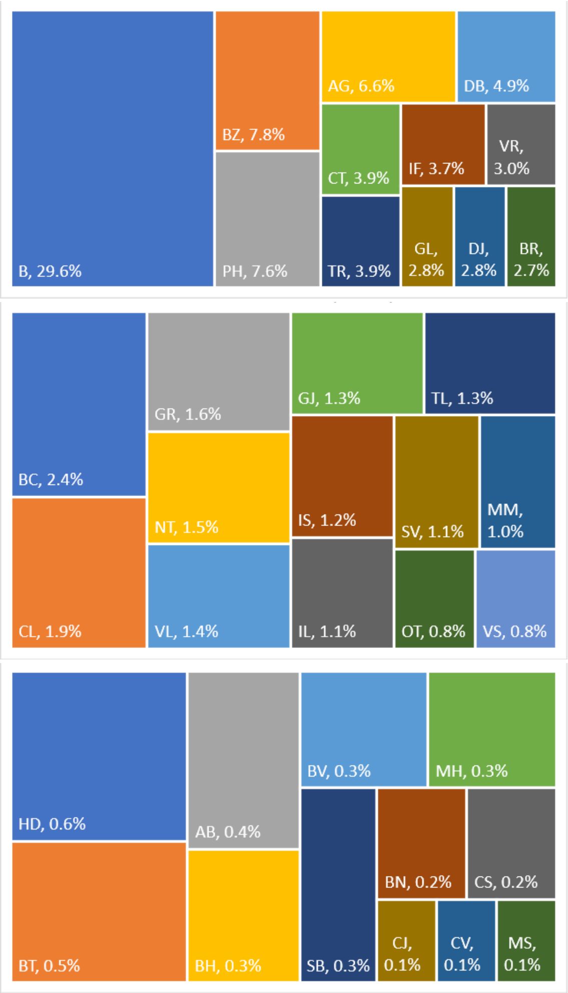

Figure 1: Visual representation of patient distribution by county. The figure is divided into three components for ease of interpretation. Each county name was abbreviated according to its national code

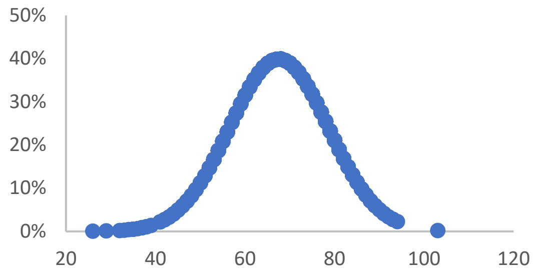

Figure 2: Patient age-standardized probability distribution

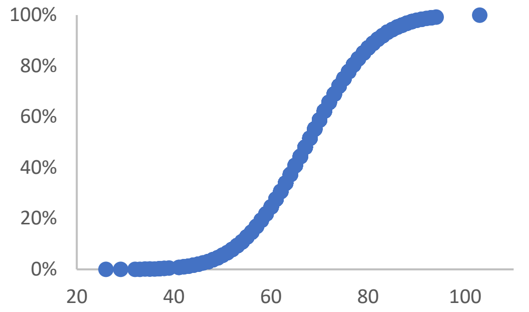

Figure 3: Patient age-standardized cumulative distribution

Figure 5 presents a descriptive analysis of each used ICD-10 code as a function of gender distribution. The chi2 test of independence has not identified any significant correlations between gender and any ICD-10 code. However, post hoc power analysis has shown that the sample size in each subgroup was insufficient to detect a meaningful effect. For this reason, we cannot draw any meaningful conclusions from the subgroup analysis. Age group diagnostic patterns reveal that the most often encountered patients are the ones at 60 years or above followed by the ones within the 50 to 59 age group. Diagnostic patterns do not reveal any significant differences between groups, M16.1 being the most often encountered diagnostic code as depicted in Figure 6.

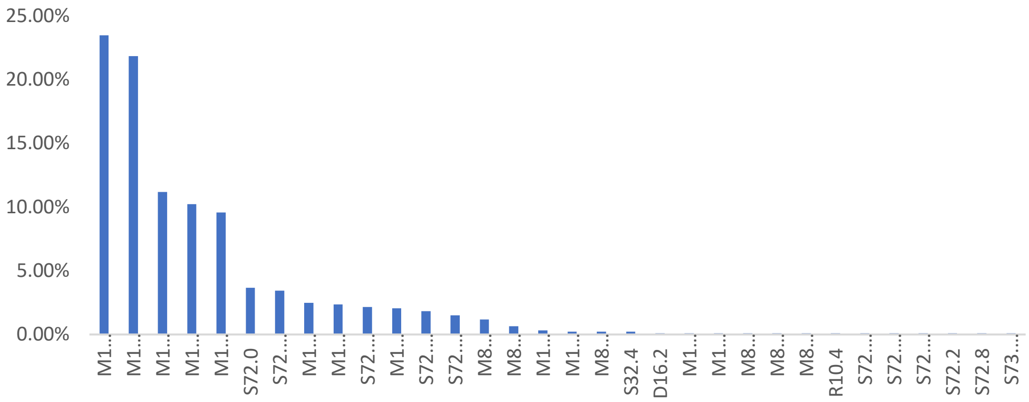

Figure 4: Frequency distribution of ICD-10 diagnostic codes used for patients undergoing cemented or uncemented total hip arthroplasty

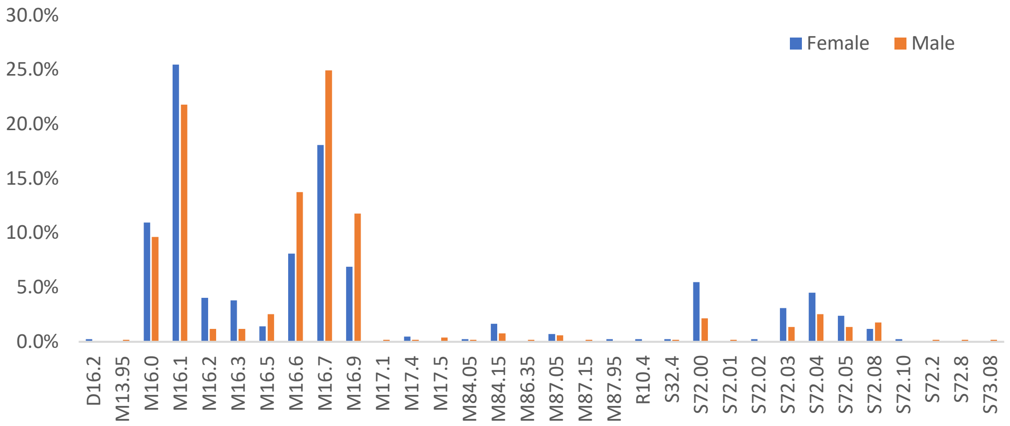

Figure 5: Frequency distribution of ICD-10 diagnostic codes as functions of patient gender

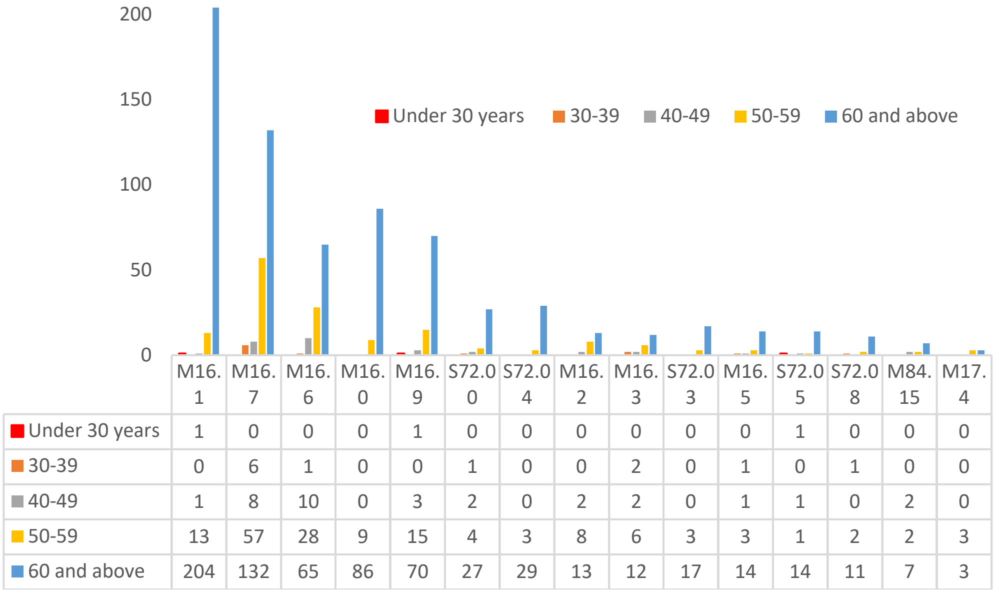

	M16.1	M16.7	M16.6	M16.0	M16.9	S72.00	S72.04	M16.2	M16.3	S72.03	M16.5	S72.05	S72.08	M84.15	M17.4
🟥 Under 30 years	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
🟧 30-39	0	6	1	0	0	1	0	0	2	0	1	0	1	0	0
▨ 40-49	1	8	10	0	3	2	0	2	2	0	1	1	0	2	0
🟨 50-59	13	57	28	9	15	4	3	8	6	3	3	1	2	2	3
🟦 60 and above	204	132	65	86	70	27	29	13	12	17	14	14	11	7	3

Figure 6: A brief descriptive analysis of ICD-10 code distribution in the 5 main patient age groups. Furthermore, a data table is provided with the main 15 ICD-10 codes for data transparency

Table 1: Patient demographics presenting gender frequency and age mean and standard deviation (SD).

	F, %	**M, %**
Gender	420, 45%	509, 55%
	Mean	**SD**
Age	67.5	11

DISCUSSION

The results presented indicate that patient follow-up poses significant challenges, as only 29.6% of the patients included were deemed suitable for returning to the surgical center for follow-up. In addition, it is anticipated that these patients will experience the typical follow-up loss rate of 10-20%, regardless of their proximity to the surgical center. The significance of this report lies in its importance for upcoming prospective studies at our hospital, which will enable the precise evaluation of implant durability and patient-reported outcome measures. It is crucial to monitor adverse events during follow-up, as changes in patient-reported outcomes may indicate the likelihood of such events occurring [23].

Tertiary hospitals often face a common issue where patients from various regions across the country are referred to them, making it challenging to monitor their symptoms and track their progress over an extended period. In light of this, remote reporting could serve as a viable solution for these hospitals to maintain contact with their patients and minimize follow-up loss rates, resulting in higher patient reporting rates during prospective studies [24,25].

The age probability and cumulative distributions provided in the report are extremely beneficial for the orthopedics department and healthcare providers, as they enable them to determine when patients are likely to require hip replacement surgery. Simultaneously, these distributions enable all concerned parties to anticipate future budget allocations based on patient age, as older patients are more susceptible to degenerative hip conditions, whereas younger patients are more prone to developmental hip diseases such as dysplasia. As a result, budget allocation can be optimized through informed decision-making, allowing for the advance procurement of specific resources to serve patients promptly after their initial consultation [26,27].

By implementing ICD-10 code reporting and rigorous oversight over its reporting, additional budget optimization can be achieved by providing decision-makers with information on which implants are likely to be required at each hospital [28,29]. Present results highlight the fact that in 45% of cases patients either suffer from “Unilateral primary osteoarthritis of the hip” or “Other unilateral secondary osteoarthritis of the hip”. Patients suffering from primary unilateral osteoarthritis who are 67 years or older most likely require standard uncemented total hip replacements for this reason at least 23.4% (Figure 4) of cases will require standard total hip implants. At the same time, patients suffering from unilateral secondary osteoarthritis most likely developed osteoarthritis secondary to aseptic osteonecrosis of the femoral head [30] while a smaller proportion of younger patients are expected to develop osteoarthritis secondary to acetabular or proximal femoral deformities like acetabular retroversion, excessive femoral anteversion with ace-tabular under coverage, CAM, pincer or mixed femoral-acetabular impingement (FAI) [31–33]. This kind of patient often requires specialized implants in order to accurately reconstruct hip offset and anatomical center of rotation or when associated with the need for femoral derotation or shortening osteotomy [34]. Furthermore, the following 22.4% of cases comprised of “Other bilateral secondary osteoarthritis of the hip”, and “Bilateral primary osteoarthritis of the hip”, signal an important need for sequential or single-step bilateral total hip replacement. Each option has to be considered against its risks. Sequential bilateral hip replacements can be done through any approach and are more suited for patients who do not have a reliable support network and for whom the disease process is not as debilitating [35,36]. Innovative anterior-based hip approaches have made single-step total hip replacement possible, enabling patients to be placed in a supine position while preserving their abductors and external hip rotators. As a result, rehabilitation can be easily carried out, even in patients with bilateral implants [35,36].

Unfortunately, the current employed ICD-10 codes do not allow for a more in-depth analysis of causative relations leading to hip degenerative disease. The prevalent problem is that "secondary" causes of hip osteoarthritis involve intricate clinical conditions that involve spinopelvic interactions, pelvic and femoral orientation, and their interrelationship. Thus, labeling a case as "secondary" hip osteoarthritis alone does not furnish healthcare providers and practitioners with sufficient information to formulate predictable models to attain economic and treatment efficiency [37].

The report emphasizes the significance of registry data while underscoring the current inadequacies in reporting standards that impede advanced clinical and economic planning. These inadequacies underscore the necessity for clinicians and healthcare providers to actively engage with technological advancements, particularly with respect to patient reporting standards and stringent follow-up [24,38–41].

Future research efforts should concentrate on enhancing ICD-10 reporting standards, devising innovative methods for maintaining contact with patients after an intervention, particularly in tertiary hospitals, and furnishing healthcare providers with reliable data that can facilitate efficient planning and procurement, ensuring the utmost standard of healthcare for patients.

CONCLUSION

Registry data provides valuable information to healthcare providers and is a key source of data for healthcare improvements. Healthcare practitioners will most of the time encounter patients 67 ± 11 years old most likely suffering from either unilateral primary or secondary osteoarthritis. Based on the available data surgeons could expect that standard total hip implants will most often than not meet patient needs and be sufficient to reconstruct the native hip anatomy.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.
This research received no external funding.

Authors’ contribution

Conceptualization, A.G.M.; methodology, A.G.M.; software, M.D.; validation, M.D., A.G.M., and L.T.M.; formal analysis, A.G.M.; investigation, A.G.M; data curation, A.S.; writing—original draft preparation, A.G.M.; writing—review and editing, A.G.M., L.T.M., A.S., M.D.; visualization, A.G.M., M.D.; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Prior to the study’s inception, a research protocol was submitted to and approved by the hospital’s ethics committee, registration number 521/05.05.2022.

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ORIGINAL ARTICLE

Multipurpose Hall for CBRNe Incidents

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Abstract: *A multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes plays a vital role in enhancing preparedness, response, and recovery capabilities in the face of CBRN incidents. A multipurpose hall for CBRN purposes provides a dedicated and specialized environment to support CBRN preparedness, response, and recovery efforts. It enhances coordination, facilitates training and exercises, ensures proper equipment storage, and fosters collaboration, ultimately strengthening the ability to protect personnel, mitigate risks, and minimize the impact of CBRN incidents. This study is qualitative research based on the content analysis method. To design and build multipurpose halls for CBRN events, this study can be used as a checklist. The purpose of this study is to collect important factors and sub-factors in the design, construction, and equipping of buildings and multi-purpose CBRN structures.*

Keywords: *Multipurpose Hall, CBRNe, Disaster, Emergency*

INTRODUCTION

A multi-purpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes plays an important role in handling CBRN incidents and supporting preparedness, response, and recovery efforts. Among the important reasons for the design and construction of such structures can be mentioned the centralization of operations, which specifies a multi-purpose hall provides a centralized place for CBRN operations, and the various resources, equipment, and personnel involved in the preparation, bring together CBRN response and recovery. It acts as a command center where decision-makers can effectively coordinate and manage activities and ensure a coordinated and effective response. In addition to training, the multi-purpose hall can serve as a dedicated place for CBRN training and exercises [1-4]. It provides a controlled environment where personnel can simulate and practice response procedures, test equipment and enhance their skills in handling CBRN incidents. Training and exercises conducted in the multi-purpose hall help ensure readiness and improve responsiveness. It can also be for proper storage, organization, and maintenance of CBRN equipment and supplies, which includes specialized protective equipment, detection and monitoring devices, disinfection equipment, and other essential tools used in CBRN incidents [5-7]. Having a designated place for equipment storage ensures quick access and proper care, minimizes response time, and optimizes equipment performance. Multipurpose halls for CBRN are equipped with decontamination facilities, including showers, washing stations, and appropriate disinfectant solutions. These facilities are critical for the rapid decontamination of people, equipment, and materials exposed to hazardous substances. Effective decontamination helps protect personnel, prevent cross-contamination, and maintain operational readiness. The multipurpose hall serves as a hub for communication and information sharing during CBRN incidents. It incorporates communication systems, command centers, and information management tools to facilitate real-time communication, coordination, and data exchange between response teams, external agencies, and other stakeholders. The important ones mentioned can support research and development efforts related to defense and CBRN technologies. They provide a controlled environment for testing and evaluating new equipment, protocols, and techniques for CBRN preparedness and response [8-10]. Research conducted at this center helps advance knowledge and improve CBRN capabilities. Also, its importance is in facilitating cooperation between different organizations involved in CBRN operations, where it brings together experts from different disciplines including emergency management, public health, law enforcement, and fire services, strengthening partnerships and increasing inter-agency coordination. This joint approach improves the effectiveness of response efforts and ensures a comprehensive and integrated response to CBRN incidents. Having a dedicated multi-purpose hall for CBRN operations demonstrates a commitment to public safety and preparedness. It instills confidence in the community by demonstrating the ability to effectively respond to CBRN incidents. The facility serves as a visible symbol of preparedness and preparedness, assuring the public that measures are in place to mitigate the risks associated with CBRN events [11-13].

Multipurpose halls are designed to accommodate a wide range of CBRN-related activities and can adapt to evolving needs and emerging threats. The versatility of the facility enables the integration of new technologies, the implementation of innovative approaches, and the ability to address a wide range of CBRN scenarios, ensuring the facility remains relevant and effective over time. The multipurpose hall provides a controlled environment for CBRN activities. It may have specialized ventilation and air filtration systems to prevent the release of hazardous agents, ensure personnel safety, and limit the impact of CBRN incidents. Facility design and infrastructure help contain CBRN materials and maintain a safe environment for operations [14-17].

MATERIALS AND METHODS

This research is qualitative research based on the content analysis method with a deductive and guided approach. A multipurpose hall for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes would need to be designed to accommodate various activities related to CBRN defense, response, and training. By studying and reviewing some sources including books, articles, and documents, some considerations for the design of such facilities were collected and reviewed as follows:

1. Location

The hall should be situated in a secure area away from densely populated areas, with easy access for emergency vehicles and personnel. The location of a multipurpose hall for CBRN (Chemical, Biological, Radiological, and Nuclear) activities is crucial and carries significant importance [18-20].

1.1. Safety and Security: The primary concern when dealing with CBRN hazards is the safety and security of personnel and the surrounding environment. Choosing an appropriate location away from densely populated areas helps minimize the risk of exposing a large number of people to potential CBRN incidents. It allows for better control, containment, and response to emergencies, reducing the potential for casualties and damage.

1.2. Proximity to Response Resources: The location of the hall should take into account its proximity to essential response resources, such as hospitals, emergency services, and specialized CBRN response teams. Being located within a reasonable distance of these resources enables faster and more efficient emergency response and medical assistance in case of an incident.

1.3. Access for Emergency Vehicles and Personnel: The hall should have suitable access points that allow easy entry and exit for emergency vehicles, such as ambulances and fire trucks. It should also provide convenient access for response personnel, enabling rapid deployment during emergencies. The location should consider factors like road infrastructure, transportation routes, and proximity to major highways or airports for logistical support.

1.4. Environmental Considerations: The location should take into account environmental factors that may affect the effectiveness of CBRN response operations. For instance, prevailing wind patterns, geographical features, and nearby bodies of water should be considered to assess the potential dispersion patterns of CBRN agents. This information helps in determining the best location to minimize the impact and spread of hazardous substances.

1.5. Zoning and Legal Requirements: Different jurisdictions may have specific zoning regulations and legal requirements for facilities handling CBRN materials. The location should comply with these regulations to ensure the facility's legality and appropriate adherence to safety protocols. Zoning restrictions may dictate areas suitable for CBRN-related activities, ensuring compatibility with neighboring properties and land use.

1.6. Training and Collaboration Opportunities: The location can also provide opportunities for collaboration and training with nearby CBRN response organizations, research institutions, or military installations. Proximity to such entities facilitates knowledge sharing, joint exercises, and the exchange of expertise, enhancing overall preparedness and response capabilities.

2. Structural Integrity

The building should be constructed to withstand potential CBRN incidents, including blasts and chemical or radiological hazards. Reinforced concrete walls and ceilings, as well as blast-resistant windows and doors, may be necessary. When designing a multipurpose hall for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes, ensuring the structural integrity of the facility is of utmost importance [21-24].

2.1. Robust Construction: The hall should be constructed using robust materials and techniques to withstand potential CBRN incidents. Reinforced concrete or other sturdy materials are commonly used for walls, floors, and ceilings. These materials provide strength and resistance to impacts, blasts, and structural damage.

2.2. Blast Resistance: The structure should be designed to resist the effects of explosions that may occur during CBRN incidents. This involves incorporating blast-resistant features, such as reinforced walls, windows, and doors. The design should consider blast loadings, shockwaves, and fragments, and ensure that the structure can withstand these forces without compromising its integrity.

2.3. Containment and Isolation: The hall should have the ability to contain and isolate CBRN hazards within designated areas. This can be achieved by using airtight seals, robust partitions, and barriers to prevent the spread of contaminants. The facility should also have separate zones to minimize cross-contamination and allow for efficient isolation of affected areas.

2.4. Protection from External Hazards: The design should consider potential external hazards that can impact the structural integrity, such as extreme weather events (e.g., hurricanes, earthquakes) or intentional attacks. Adequate measures, such as reinforced roofs, impact-resistant windows, and structural reinforcements, should be implemented to protect the facility from these external threats.

2.5. Structural Sealing: To prevent the ingress of CBRN agents, the hall should have a well-sealed structure. This includes ensuring tight seals around windows, doors, ventilation systems, and other openings. High-quality seals and gaskets should be used to maintain airtight integrity and prevent the infiltration of hazardous substances.

2.6. Access Points: The design should include controlled access points with appropriate security measures. These access points should be designed to withstand potential attacks or unauthorized entry attempts. Secure doors, locks, and surveillance systems should be employed to maintain the integrity of the facility.

2.7. Reinforced Utilities: Critical utilities, such as electrical systems, water supply, and HVAC (Heating, Ventilation, and Air Conditioning) systems, should be designed and positioned to withstand CBRN incidents. They may require additional reinforcement or protective measures to ensure continued operation during emergencies.

2.8. Structural Monitoring: Implementing structural monitoring systems can help detect and assess any potential structural vulnerabilities or damage. Structural sensors, such as strain gauges or vibration sensors, can be installed to monitor the health of the building and provide early warning of any compromised areas.

2.9. Compliance with Standards: The design should adhere to relevant building codes, safety regulations, and industry standards related to CBRN facilities. These standards ensure that the structural integrity meets specified requirements and guidelines for CBRN defense and response.

3. Ventilation and Filtration

The hall should have a robust ventilation system capable of providing a controlled airflow to prevent the spread of contaminants. High-efficiency air filtration systems, including HEPA filters, should be installed to remove CBRN agents from the air. Ventilation and filtration systems are critical components of a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. They play a vital role in maintaining a safe and controlled environment by minimizing the spread of CBRN agents. [25-29].

3.1. Controlled Airflow: The hall should have a well-designed ventilation system capable of providing controlled airflow. This ensures that fresh air is supplied to occupied areas while maintaining appropriate pressure differentials to prevent the migration of CBRN agents to unaffected areas. The ventilation system should be designed with adjustable airflow rates to accommodate different scenarios and response requirements.

3.2. Air Filtration Systems: High-efficiency air filtration systems, including High-Efficiency Particulate Air (HEPA) filters, should be an integral part of the ventilation system. HEPA filters are highly effective in removing particulates, including biological agents, radioactive particles, and many chemical contaminants, from the air. These filters should be properly sized, installed, and maintained to ensure optimal filtration efficiency.

3.3. Filtration System Redundancy: Redundancy is crucial in CBRN facilities to ensure the continuous operation of filtration systems. The inclusion of backup or redundant filtration units and provisions for rapid filter replacement or change-out are important considerations. This helps maintain filtration performance in the event of filter saturation or system malfunctions.

3.4. Air Pressurization: In CBRN scenarios, it may be necessary to create areas with positive or negative air pressure to prevent the migration of CBRN agents. Positive pressure areas ensure that air flows out of the controlled area, reducing the risk of contaminants entering. Negative pressure areas draw air into the controlled area, preventing the release of hazardous agents to the outside environment. The ventilation system should be capable of maintaining the desired pressure differentials between different zones within the facility.

3.5. Isolation and Containment: The ventilation system should support isolation and containment measures during CBRN incidents. This can include the ability to seal off affected areas, establish differential pressure zones, and control airflow between different sections of the hall. These features help contain and prevent the spread of CBRN agents to unaffected areas.

3.6. Air Monitoring and Detection: Integrating air monitoring and detection systems into the ventilation system allows for continuous monitoring of air quality and the presence of CBRN agents. These systems can provide early warnings and trigger appropriate response actions if hazardous substances are detected. Air monitoring devices, such as gas detectors or biological sensors, should be strategically placed throughout the facility.

3.7. Maintenance and Monitoring: Regular maintenance, testing, and inspection of the ventilation and filtration systems are crucial to ensure their continued effectiveness. Filters should be replaced or cleaned at recommended intervals, and the functionality of the ventilation system, including airflow rates, pressure differentials, and alarms, should be regularly tested and verified.

3.8. Emergency Shutdown Procedures: The ventilation system should have emergency shutdown procedures in place to quickly stop airflow in case of emergencies or incidents. These shutdown procedures should be easily accessible and understood by authorized personnel to minimize the spread of CBRN agents during critical situations.

4. Decontamination Facilities

Dedicated decontamination areas should be included within the hall. These areas would typically have showers, washbasins, and appropriate decontamination solutions for individuals or equipment exposed to CBRN agents. Decontamination facilities are a crucial component of a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. These facilities are necessary for the safe and effective decontamination of individuals and equipment that may have been exposed to CBRN agents [30, 31].

4.1. Showers and Washbasins: Dedicated areas with showers and washbasins should be included in the decontamination facility. These areas allow individuals to thoroughly wash their bodies, hair, and exposed skin to remove any contaminants. The showers should have an adequate water supply and temperature control to ensure effective decontamination.

4.2. Decontamination Solutions: The decontamination facility should provide appropriate decontamination solutions or agents to neutralize or remove CBRN agents from the skin, clothing, and equipment. These solutions should be selected based on the specific CBRN agents anticipated and their effectiveness in decontamination. Guidelines for the proper use and disposal of these solutions should be clearly provided.

4.3. Separate Areas for Contaminated and Clean Zones: The decontamination facility should have separate areas or designated zones for contaminated and clean areas to prevent cross-contamination. Individuals and equipment should enter the facility through the contaminated zone and proceed to the clean zone after decontamination.

4.4. Personal Protective Equipment (PPE): Adequate storage space should be provided within the decontamination facility for storing personal protective equipment (PPE) used during the decontamination process. This includes items such as gloves, masks, goggles, and disposable suits. The storage area should be easily accessible and well-organized for quick retrieval of PPE.

4.5. Waste Management: Proper waste management procedures should be in place to handle and dispose of contaminated waste generated during the decontamination process. This may include designated waste collection containers, hazardous waste disposal protocols, and compliance with local regulations and guidelines for waste management.

4.6. Water Drainage and Containment: The decontamination facility should have appropriate water drainage and containment systems to prevent the spread of contaminants. This includes the installation of floor drains, impervious flooring, and adequate water collection and containment mechanisms to prevent the runoff of contaminated water into other areas.

4.7. Accessibility and Safety: The decontamination facility should be designed to be accessible to individuals with disabilities and consider their specific needs during the decontamination process. Additionally, safety measures such as slip-resistant flooring, proper lighting, and emergency communication systems should be in place to ensure the safety of individuals using the facility.

4.8. Training and Procedures: Clear operating procedures and training materials should be developed and provided to personnel using the decontamination facility. This includes instructions on proper decontamination techniques, the sequence of actions, handling of equipment, and safety protocols to ensure effective decontamination and minimize the risk of recontamination.

4.9. Maintenance and Inspection: Regular maintenance, inspection, and testing of decontamination facilities are essential to ensure their continued functionality and compliance with safety standards. This includes routine checks of water supply, showers, drainage systems, PPE storage, and decontamination solutions to verify their effectiveness and readiness for use.

5. Equipment Storage

Adequate storage space should be provided to house CBRN-related equipment, such as personal protective equipment (PPE), detection devices, decontamination supplies, and specialized response tools. Equipment storage is a critical aspect of a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. Proper storage facilities are essential for organizing and safeguarding CBRN-related equipment, ensuring readiness and quick access when needed [32-35].

5.1. Dedicated Storage Areas: Designate specific areas within the multipurpose hall for equipment storage. These areas should be easily accessible and organized, allowing for efficient retrieval and inventory management. Depending on the size and quantity of equipment, different storage options such as cabinets, shelves, lockers, or specialized storage racks may be utilized.

5.2. Equipment Categorization: Categorize CBRN equipment based on its type, purpose, and level of protection. This helps in organizing and locating items quickly during emergencies or training exercises. For example, respiratory protection equipment, protective suits, detection devices, decontamination supplies, and specialized response tools may each have their designated storage area or section.

5.3. Secure Storage: Implement appropriate security measures to ensure the integrity and protection of stored equipment. This may include restricted access to storage areas, lockable cabinets or containers, surveillance systems, and inventory control protocols. Access should be limited to authorized personnel only, minimizing the risk of theft, tampering, or unauthorized use.

5.4. Equipment Maintenance: Provide space and resources for equipment maintenance, cleaning, and inspection within the storage area. This includes workbenches, tools, cleaning agents, and dedicated areas for equipment servicing. Regular maintenance ensures that the equipment remains in good working condition and is ready for use when needed.

5.5. Proper Ventilation: Ensure adequate ventilation within the storage area to prevent the buildup of moisture, humidity, or other environmental factors that may damage the equipment. Proper airflow helps maintain the longevity and functionality of sensitive equipment, especially when stored for extended periods.

5.6. Labeling and Inventory Management: Clearly label storage areas and equipment to facilitate easy identification. Implement an inventory management system that tracks the location, condition, and usage of equipment. This enables efficient inventory control, timely replacement of expired or damaged items, and ensures readiness during CBRN incidents or training exercises.

5.7. Compliance with Safety Regulations: Ensure compliance with safety regulations and guidelines for the storage of CBRN-related equipment. These regulations may include specific requirements for hazardous materials, controlled substances, or sensitive equipment. Adhering to these regulations promotes safety, minimizes risks, and maintains legal compliance.

5.8. Training and Familiarization: Provide appropriate training and familiarization to personnel on the location, access procedures, and handling protocols for stored CBRN equipment. This ensures that authorized personnel are knowledgeable about the equipment, understand its usage, and can retrieve or store it safely.

5.9. Emergency Preparedness: Develop emergency response plans that outline procedures for accessing and utilizing stored CBRN equipment during emergencies. This includes provisions for quick retrieval, distribution, and deployment of equipment to relevant personnel or response teams.

5.10. Regular Audits and Inspections: Conduct regular audits and inspections of the equipment storage areas to ensure compliance, proper organization, and readiness. This involves checking expiration dates, conducting inventory reconciliation, and assessing the overall condition of the stored equipment.

6. Training and Simulation

The hall should offer versatile training capabilities, including simulation scenarios for CBRN incidents. This may involve the use of specialized training props, mock environments, and immersive simulation technologies. Training and simulation capabilities are vital components of a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. These features allow personnel to acquire the necessary knowledge, skills, and experience in a controlled environment to effectively respond to CBRN incidents [32, 36-41].

6.1. Training Areas: Designate specific areas within the multipurpose hall for training exercises. These areas should be versatile and adaptable to simulate different CBRN scenarios, such as mock incident sites, contaminated zones, or confined spaces. The layout and features of these areas can be modified to replicate real-life scenarios and provide a realistic training environment.

6.2. Simulation Tools: Incorporate various simulation tools and equipment to enhance training effectiveness. This may include smoke generators, simulated hazardous substances, training mannequins, or specialized props that mimic CBRN hazards. The use of these tools helps create realistic scenarios, enabling trainees to practice response procedures and decision-making skills.

6.3. Training Props and Equipment: Provide training props and equipment that replicate actual CBRN response gear, including personal protective equipment (PPE), detection devices, decontamination equipment, and specialized response tools. These props should closely resemble the functionality and characteristics of real equipment, allowing trainees to become familiar with their use and limitations.

6.4. Emergency Communication Systems: Install reliable emergency communication systems throughout the training areas to facilitate coordination and communication between trainees, instructors, and control personnel. These systems may include intercoms, radios, or other communication devices that mimic real-world communication protocols used during CBRN incidents.

6.5. Monitoring and Feedback: Incorporate monitoring systems to capture trainees' actions, responses, and performance during training exercises. This may involve video recording, sensors, or observation booths for instructors to assess trainee actions and provide feedback. Monitoring and feedback play a crucial role in identifying areas for improvement and enhancing trainee proficiency.

6.6. Training Scenarios and Drills: Develop a variety of realistic training scenarios and drills that cover different aspects of CBRN incidents, such as detection, containment, decontamination, and response protocols. These scenarios should be designed to challenge trainees' knowledge and skills progressively, allowing them to practice different response strategies and experience various levels of complexity.

6.7. Safety Protocols: Implement strict safety protocols during training exercises to minimize risks to trainees and instructors. This includes setting clear safety guidelines, conducting risk assessments, and providing appropriate personal protective equipment. Safety measures should be strictly enforced to ensure the well-being of all participants during training activities.

6.8. Training Curriculum and Instructors: Develop a comprehensive training curriculum that covers theoretical knowledge, practical skills, and decision-making exercises related to CBRN incidents. The curriculum should be designed to cater to different levels of expertise, ranging from basic awareness to advanced response techniques. Qualified instructors with relevant expertise in CBRN defense and response should lead the training sessions.

6.9. After-Action Reviews: Conduct after-action reviews following each training exercise or drill to evaluate performance, identify strengths and areas for improvement, and facilitate continuous learning. These reviews should involve all participants, including trainees, instructors, and control personnel, to gather valuable feedback and insights for future training enhancements.

6.10. Integration with Emergency Response Plans: Ensure that the training and simulation activities align with established emergency response plans and procedures. This integration helps familiarize trainees with the actual protocols and coordination mechanisms that would be used during real CBRN incidents, enhancing overall preparedness and effectiveness.

7. Communication Systems

Robust communication infrastructure, including radio systems, internet connectivity, and intercoms, should be integrated into the facility to support coordination during emergencies. Effective communication systems are essential in a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. These systems enable efficient coordination, information sharing, and emergency response during CBRN incidents [42-44].

7.1. Intercom Systems: Install intercom systems throughout the multipurpose hall to facilitate direct communication between different areas, such as control rooms, training areas, storage facilities, and decontamination zones. Intercoms allow for quick and clear communication, enabling effective command and control during CBRN incidents.

7.2. Two-Way Radios: Equip personnel with two-way radios to enable real-time communication between individuals or teams within the multipurpose hall. Two-way radios provide mobile communication capabilities, allowing personnel to stay connected while moving within the facility. Ensure that the radio system has sufficient coverage within the hall and is compatible with existing communication infrastructure.

7.3. Emergency Broadcast Systems: Implement emergency broadcast systems that can disseminate critical announcements, instructions, or alerts to all personnel within the multipurpose hall. These systems can include loudspeakers strategically positioned throughout the facility, enabling mass notification and ensuring that important information reaches all occupants, even in noisy or crowded environments.

7.4. Digital Communication Platforms: Utilize digital communication platforms, such as email, instant messaging, or mobile applications, to enhance communication and information sharing among personnel. These platforms can be particularly useful for non-emergency situations, such as routine updates, scheduling, or sharing documents related to CBRN operations.

7.5. Video Conferencing: Integrate video conferencing capabilities into designated areas, such as command centers or control rooms, to facilitate remote communication and coordination with external stakeholders or off-site experts. Video conferencing allows for real-time visual communication, enabling efficient collaboration and decision-making during CBRN incidents.

7.6. Data and Network Connectivity: Ensure reliable data and network connectivity throughout the multipurpose hall to support effective communication. This includes providing sufficient Wi-Fi coverage, Ethernet ports, and network infrastructure to facilitate the use of communication devices, access to online resources, and data exchange among personnel.

7.7. Emergency Communication Protocols: Establish clear emergency communication protocols and procedures for CBRN incidents. These protocols should outline communication chains of command, designated emergency communication channels, and reporting mechanisms. Ensure that personnel are trained on these protocols and familiar with the appropriate use of communication systems during emergencies.

7.8. Integration with External Communication Networks: Coordinate with external emergency response agencies, such as fire departments, law enforcement, or medical services, to integrate the multipurpose hall's communication systems with their networks. This allows for seamless communication and coordination during joint response efforts or when seeking external assistance.

7.9. Backup Power and Redundancy: Implement backup power systems, such as uninterruptible power supplies (UPS) or emergency generators, to ensure communication systems remain operational during power outages or emergencies. Additionally, consider redundancy in communication systems to minimize the risk of single points of failure and maintain continuous communication capabilities.

7.10. Testing and Maintenance: Regularly test and maintain communication systems to ensure their reliability and functionality. Conduct periodic drills or simulations to validate the effectiveness of communication systems during various scenarios. Routinely check equipment, perform system checks, and update software or firmware to address any vulnerabilities or issues.

8. Safety Systems

The hall should be equipped with safety features, such as emergency alarms, evacuation routes, emergency lighting, and fire suppression systems, to ensure the safety of personnel during CBRN incidents. Safety systems play a crucial role in ensuring the protection of personnel and minimizing risks in a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes [42-50].

8.1. Fire Suppression Systems: Install fire suppression systems, such as sprinklers or specialized suppression agents, throughout the multipurpose hall. These systems should be designed to quickly detect and suppress fires, minimizing the risk of fire-related incidents. Ensure that the fire suppression systems are compatible with the CBRN activities and do not pose additional risks during CBRN incidents.

8.2. Emergency Lighting: Implement reliable emergency lighting systems that automatically activate during power outages or emergencies. Emergency lighting helps maintain visibility, especially in areas critical for evacuation or emergency response. Ensure that emergency lighting is strategically placed to illuminate evacuation routes, exits, and important safety equipment.

8.3. Emergency Power Backup: Provide emergency power backup systems, such as uninterruptible power supplies (UPS) or emergency generators, to ensure essential safety systems remain operational during power failures or emergencies. Critical systems, including emergency lighting, communication systems, fire suppression systems, and ventilation, should be connected to the emergency power backup.

8.4. Alarm Systems: Install comprehensive alarm systems that include audible and visual alarms throughout the multipurpose hall. These alarms should be triggered automatically in the event of CBRN incidents, fires, or other emergencies, alerting personnel to take appropriate actions. Consider integrating the alarm systems with the overall facility's alarm system to ensure a coordinated response.

8.5. Ventilation and Air Filtration Systems: Implement robust ventilation and air filtration systems designed to handle CBRN contaminants. These systems should be capable of providing a continuous supply of fresh air, removing airborne contaminants, and controlling the circulation of hazardous substances. Regular maintenance and filter replacement should be carried out to ensure their effectiveness.

8.6. Emergency Evacuation Plans: Develop and communicate clear emergency evacuation plans for the multipurpose hall. These plans should include designated evacuation routes, assembly points, and procedures for personnel to follow during CBRN incidents or other emergencies. Display evacuation maps prominently throughout the facility and conducts regular drills to familiarize personnel with evacuation procedures.

8.7. Personal Protective Equipment (PPE): Ensure that an adequate supply of appropriate personal protective equipment (PPE) is readily available throughout the multipurpose hall. This includes respiratory protection, protective clothing, gloves, goggles, and other necessary equipment for personnel to protect themselves during CBRN incidents or when handling hazardous substances.

8.8. Emergency Response Equipment: Equip the multipurpose hall with emergency response equipment such as first aid kits, stretchers, eye wash stations, and emergency showers. These provisions ensure that immediate medical attention can be provided in case of injuries or exposure to hazardous substances. Regularly check and replenish the emergency response equipment as needed.

8.9. Safety Signage: Install clear and visible safety signage throughout the multipurpose hall. This includes signs indicating the location of emergency exits, first aid stations, fire extinguishers, hazardous material storage areas, and other critical safety equipment. The signage should use internationally recognized symbols and be easily understandable in high-stress situations.

8.10. Safety Training and Education: Provide comprehensive safety training and education programs for all personnel working in the multipurpose hall. This includes CBRN safety protocols, proper use of safety equipment, evacuation procedures, and the recognition of potential hazards. Regularly refresh and update training programs to ensure personnel are well-prepared and informed.

9. Accessibility

The facility should be designed to accommodate individuals with disabilities and provide ease of access for emergency responders and equipment. Ensuring accessibility in a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes is important to accommodate individuals with disabilities or mobility challenges [51-54].

9.1. Accessibility Standards: Adhere to local accessibility standards and regulations when designing and constructing the multipurpose hall. These standards typically outline requirements for ramps, door widths, corridor widths, accessible parking spaces, and other accessibility-related features. Compliance with these standards promotes inclusivity and provides equal access for all individuals.

9.2. Ramps and Elevators: Install ramps or elevators at entrances and other areas with level changes to ensure wheelchair users and individuals with mobility aids can access different parts of the facility. Ramps should be designed with appropriate slopes, handrails, and tactile indicators to enhance safety and usability.

9.3. Accessible Parking Spaces: Designate accessible parking spaces near the entrance of the multipurpose hall. These spaces should be wide enough to accommodate accessible vehicles and provide sufficient clearance for wheelchair users to enter and exit the vehicles comfortably. Accessible parking spaces should also be conveniently located and clearly marked.

9.4. Wide Doorways and Corridors: Ensure that doorways and corridors within the multipurpose hall are wide enough to accommodate individuals using wheelchairs or mobility aids. The minimum width should comply with accessibility standards to allow for easy passage and maneuverability. Install automatic doors or door openers where feasible to facilitate independent access.

9.5. Accessible Restrooms: Provide accessible restrooms that meet the requirements of accessibility standards. These restrooms should have appropriate door widths, grab bars, accessible sinks, and other necessary features to ensure usability for individuals with disabilities. Signage indicating the location of accessible restrooms should be clearly visible.

9.6. Tactile and Visual Cues: Incorporate tactile and visual cues throughout the multipurpose hall to assist individuals with visual impairments or hearing impairments. This may include braille signage, tactile floor indicators, contrasting colors for wayfinding, and visual alarms or flashing lights for emergency notifications.

9.7. Seating Arrangements: Plan seating arrangements within the multipurpose hall to accommodate individuals with disabilities. Ensure that there are designated spaces for wheelchair users, as well as companion seating options for individuals with disabilities attending events or training sessions.

9.8. Assistive Listening Devices: Provide assistive listening devices, such as induction loop systems or FM systems, to assist individuals with hearing impairments during presentations, meetings, or training sessions. These devices transmit audio directly to compatible hearing aids or personal receivers, improving sound clarity and accessibility.

9.9. Training and Awareness: Train staff and personnel on disability awareness, including best practices for assisting individuals with disabilities and addressing their specific needs. Sensitize employees on the importance of inclusive practices and provide them with the knowledge and skills to ensure a welcoming and accessible environment.

9.10. Continuous Evaluation and Improvement: Regularly assess the accessibility features and gather feedback from individuals with disabilities or accessibility experts. Identify areas for improvement and implement necessary modifications to enhance accessibility in the multipurpose hall. This may include adjusting door widths, adding additional signage, or addressing any barriers that may impede accessibility.

10. Security Measures

Strict security measures should be implemented to prevent unauthorized access to CBRN materials and ensure the facility's integrity. Implementing robust security measures in a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes is crucial to protect personnel, prevent unauthorized access, and safeguard sensitive materials and equipment [51, 55-59].

10.1. Perimeter Security: Establish a secure perimeter around the multipurpose hall to control access and deter unauthorized entry. This may include fencing, gates, access control systems, surveillance cameras, and signage indicating restricted access. Implement measures to monitor and control vehicle and pedestrian access points.

10.2. Access Control Systems: Install access control systems at entry points to regulate and monitor personnel entering and exiting the multipurpose hall. This can include electronic key card systems, biometric readers, or other secure authentication methods. Access control systems enable authorized personnel to enter while restricting access to unauthorized individuals.

10.3. Video Surveillance: Deploy a comprehensive video surveillance system throughout the multipurpose hall. Install high-quality cameras in strategic locations, including entrances, exits, critical areas, storage facilities, and external perimeters. Monitor and record video footage to deter potential security breaches and assist in investigations if necessary.

10.4. Intrusion Detection Systems: Implement intrusion detection systems, including motion sensors, door/window sensors, and glass break detectors, to detect and alert security personnel about any unauthorized access attempts or suspicious activities within the multipurpose hall. Integrate these systems with the overall security infrastructure for a prompt response.

10.5. Security Personnel: Assign trained security personnel to monitor and patrol the multipurpose hall. They should be responsible for enforcing security policies, performing regular security checks, responding to alarms, and maintaining a visible security presence. Security personnel should be adequately trained in CBRN security protocols and emergency response procedures.

10.6. Restricted Areas: Designate specific areas within the multipurpose hall as restricted zones, accessible only to authorized personnel with appropriate security clearance. Use physical barriers, access control systems, or biometric authentication to ensure limited access to sensitive areas, such as storage facilities, control rooms, or laboratories.

10.7. Alarm and Alert Systems: Install audible and visual alarm systems throughout the multipurpose hall to notify personnel of emergencies, security breaches, or CBRN incidents. These systems should be interconnected and capable of providing specific alerts and instructions based on the nature of the incident.

10.8. Cybersecurity Measures: Implement robust cybersecurity measures to protect digital systems, networks, and sensitive information within the multipurpose hall. This includes firewalls, secure authentication protocols, regular software updates, data encryption, and employee awareness training on cybersecurity best practices. Protecting digital assets is crucial to prevent unauthorized access or manipulation of critical information.

10.9. Incident Response Plan: Develop a comprehensive incident response plan that outlines procedures and protocols to address security incidents, including CBRN-related events. The plan should cover roles and responsibilities, communication protocols, evacuation procedures, and coordination with external emergency response agencies. Regularly review and update the plan to reflect changing security needs and emerging threats.

10.10. Training and Awareness: Conduct regular security training sessions for all personnel working in the multipurpose hall. This should include CBRN security awareness, identification of suspicious activities, proper use of access control systems, and response to security incidents. Regularly reinforce security practices and encourage personnel to report any security concerns promptly.

RESULTS

The location of a multipurpose hall for CBRN activities significantly impacts the safety, response efficiency, accessibility, compliance with regulations, and collaboration opportunities. Careful consideration of these factors during the selection process ensures that the facility is strategically positioned to mitigate risks and effectively respond to CBRN incidents.

By incorporating these considerations, a multipurpose hall for CBRN purposes can be designed to provide a structurally sound and resilient environment that can withstand CBRN incidents and protect occupants from potential hazards. It is important to consult with structural engineers and professionals experienced in CBRN facility design to ensure compliance with safety standards and best practices.

Other important points are consulted with ventilation and filtration experts, as well as professionals with expertise in CBRN defense and response, to design and install appropriate systems that meet safety standards and requirements specific to your location and the intended use of the multi-purpose hall.

Experts have an important role in decontamination procedures, CBRN defense, and response when designing and implementing decontamination facilities. They can guide best practices, safety standards, and compliance with local regulations to ensure the decontamination facility meets the specific requirements for CBRN-related activities in the multipurpose hall.

The equipment storage in a multipurpose hall for CBRN purposes can be efficiently organized, secure, and readily accessible. Consulting with CBRN defense and response experts can provide valuable insights and guidance on specific storage requirements, safety protocols, and best practices.

A multipurpose hall for CBRN purposes can provide a realistic and effective training environment. It is crucial to collaborate with CBRN defense and response experts, experienced instructors, and relevant stakeholders to design and implement a comprehensive training and simulation program that meets specific training objectives and regulatory requirements.

A multipurpose hall for CBRN purposes can establish robust communication systems that enable effective coordination, information sharing, and emergency response. It is important to consult with communication experts and incorporate best practices specific to CBRN operations to ensure the implementation of reliable and efficient communication capabilities.

It is crucial to consult with safety experts, regulatory guidelines, and CBRN defense professionals to ensure the implementation of appropriate safety systems in the multipurpose hall. Regular inspections, maintenance, and adherence to safety regulations are essential to maintain a safe environment for personnel involved in CBRN activities.

A multipurpose hall for CBRN purposes can be designed to provide equal access and inclusion for individuals with disabilities or mobility challenges. Collaboration with accessibility experts and engaging individuals with disabilities during the design process can offer valuable insights and ensure that the facility meets its specific needs.

DISCUSSION

Creating a multi-purpose hall that is specifically designed for CBRNE incidents is necessary for several reasons, such as: having a dedicated facility, the possibility of a centralized response to CBRNE incidents, the need for CBRNE incidents to have specialized infrastructure and facilities to manage the unique challenges caused by Chemical, biological, radiological, nuclear and explosive risks.

Provide a dedicated controlled environment theater for training and preparedness activities where responders can simulate CBRNE incidents, practice response methods, test equipment, and coordinate between different agencies and organizations. improve Regular training exercises in a realistic environment to enhance preparedness and enable responders to develop the skills and knowledge necessary to effectively manage CBRNE incidents. Many other reasons are given in this study, in addition to the description of the necessity of building such structures, which deals with the detailed study of the required factors in order to be used as a minimum checklist for the establishment and creation of such multi-purpose halls.

Consequently, a multi-purpose theater designed specifically for CBRNE incidents, due to its ability to centralize response efforts, provide specialized infrastructure, facilitate training and preparedness, support decontamination and treatment, encourage research and development, enhance safety public, and promote cooperation between stakeholders is essential. Such facilities will significantly improve the overall response to CBRNE incidents and increase the resilience of communities to these complex and dangerous events.

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ORIGINAL ARTICLE

Validity of Photoplethysmography Mobile Analysis to Test the Autonomic Stress Status of Tactical Athletes

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Abstract: *Background: The effect of stress on sympathetic modulation measured by HRV has been studied in various population groups exposed to different stressors. HRV measurement tools are based on the electrocardiogram (ECG), portable tools such as POLAR V800, and recently photoplethysmography (PPG). The aim of this study was to analyze the validity of a PPG mobile app to measure HRV with a validated V800 system in a tactical athlete population. Methods HRV of 53 professional militaries were analyzed by POLAR V800 and an HRV Camera app with a smartphone during 5 min and 30 s protocol respectively. Results: The HRV values obtained by the PPG presented a significant negative significant correlation in the HRVI index, LF, HF, LF (n.u.), HF (n.u.), and a positive significant correlation in TINN index by the Polar V800 system with the application of a regression equation over the data obtained. Conclusions: HRV Camera PPG App 30s record analysis protocol could be applied using a regression formula depending on the HRV variables after the comparison with a standard 5-minute test conducted with a validated Polar V800. In future research, it is suggested to validate PPG protocols with longer durations.*

Keywords: *Heart rate variability, military, autonomic modulation, photoplethysmography*

INTRODUCTION

Heart rate variability (HRV) represents the physiological response of the variation in the interval of time between each heartbeat. It is measured by the variation in the interval from beat to beat [1]. This modulation is controlled by the balance between the activation of the Parasympathetic Nervous System (PNS), and Sympathetic Nervous System (SNS) which is named autonomic modulation [2]. This physiology response is related to the effect of the stress or to exposure to stressors agents due to experiences or the type of work performed, [3] showing a variation in HRV variables that is related to a low parasympathetic activity, which is characterized by an increased sympathetic activity [1].

The effect of stress on sympathetic modulation measured by HRV has been studied in various population groups exposed to different stressors as ultra-endurance athletes [4], skydivers [5], firefighters [6], policemen [7], soldiers [8], pilots [9], journalists [10], students [11], professors [12], and sanitary [13]. Independently of the population, an increased sympathetic modulation was observed when they were exposed to the stressor, a fact related to the activation of the fight-flight response [14]. The fight or flight response is a psychophysiological reaction that is produced by the presence of a threat, in which the body responds by activating the sympathetic nervous system (SNS) and the adren-al-cortical system [15]. Military personnel, due to their duty and the exigences of their operation are considered tactical athletes and are a clear example of a high-stress exposure population group. Previous research found a high activation of the SNS in melee combat simulations [16], as well as in asymmetrical [17] and urban combat [14]; the organic response was also evaluated in light infantry and heavy infantry in combat simulations [18].

Along the same line, HRV measurement can be an efficient device to understand the state of the autonomic nervous system in sports athletes, which showed changes in their HRV and stress level due to the stress that competition can produce [19]. In this way, HRV should be a tool to avoid the state of overload of the athlete, and at the same time provide us with how the state of an athlete and ad equating a control of the workout loads [20,21].

HRV measurement tools are based on the electrocardiogram (ECG) analysis which has proven effective for stress detection [22]. There are also portable tools such as the POLAR V800 heart rate monitor that showed its effectiveness to evaluate HRV and stress response [23,24]. Recently there are new tools such as photoplethysmography (PPG), which could be an alternative evaluation tool to the ECG and heart rate monitors. Previous studies showed how PPG could be an alternative to evaluated HRV signal in ECGs derivation and analysis in ambulatory cardiac monitoring of healthy individuals [25–27]. This kind of tool is proven in new technologies such as smartphones as fingertip recording mobile-app where there are some researchers that tested this kind of technology as a useful tool [28–31].

New technologies as smartphones or smartwatches, could be an interesting tool for the military who work in austere environments and are already familiar with the use of these devices. There are actually applications of weather impact in military corps [32], prototypes of military medical applications for combat medics and other health care providers [33], a behavioral health screening app [34] or tools for posttraumatic stress diseases [35], but not to analyze the acute autonomic status of this personnel. Then, the aim of this study was to analyze the validity of a PPG mobile app to measure HRV with a validated V800 system in a tactical athlete population. The initial hypothesis was that PPG mobile app would be a valid system to analyze HRV compared with a validated V800 system.

MATERIALS AND METHODS

53 professional military of the Spanish Army (age: M = 35.4 years, SD = 5.88 years; height: M = 174.89 cm, SD = 6.87 cm; body mass: M = 77.33 kg, SD = 11.95 kg; Military Duty= 14.44years, SD=6.43; Military Operation= 4 months, SD= 4.25 months) were analyzed. Procedures and protocols were explained to all the subjects who gave the voluntary written informed in accordance with the Declaration of Helsinki and approved by the European University Ethics Committee (CIPI/18/093).

HRV was measured by a validated Polar V800 heart rate monitor (Polar, Kempele)[36] and the PPGV mobile app for Android HRV by Camera. For this aim the Polar electrode belt was placed following Polar's guidelines in the middle of the chest. Then, participants remained seated for 5 minutes in a quiet room while the Polar V800 record the signal minutes before a maneuver meeting. After 2 minutes of starting recording, they place the index finger in the camera of a mobile device following the guideline of the HRV by Camera ECG Android App supported for BLE devices in order to analyze the HRV PPG based. The PPGV analysis lasted 30 seconds in which the participant remained sited, with the right arm flexed at 90º supported in an armrest.

The following HRV variables were obtained by the two devices:

Mean R-R; the standard deviation of successive differences (SDNN); mean HR; root mean square of successive differences (RMSSD); the number of adjacent intervals varying by more than 50ms (PNN50); HRV triangular index (HRVI); interval histogram (TINN); low-frequency power (LF); high-frequency power (HF); the low-frequency band in normalized units (LFun); the high-frequency band in normalized units (HFun); the sensitivity of the short-term variability (SD1) and the sensitivity long-term variability (SD2).

Statistical analysis

The SPSS statistical package (version 21.0; SPSS, Inc. Chica-go, Illinois) was used to analyze the data. Normality assumptions were checked with a Kolmogorov-Smirnov test. It evaluated an Intra-class Correlation (ICC) and 95% Confidence Interval (CI), Pearson correlation, and a regression equation. The level of significance for all the comparisons was set at $P < 0.05$.

RESULTS

The results are reported with their mean and standard deviation. Table 1 shows the results of HRV for each test. A negative significant correlation was found in HRVI, LF, HF, LF(n.u.), HF (n.u.) values. Also was found a significant positive correlation in TINN values. No correlations were found between other parameters.

DISCUSSION

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible.

Future research directions may also be highlighted.

CONCLUSION

The aim of this study was to analyze the validity of a PPG mobile app to measure HRV with a validated polar V800 system in the athlete population. The initial hypothesis was partially fulfilled since some PPG mobile app values presented positive and negative correlations than a validated V800 system.

HRV measurement is an efficient and important approach to assess autonomic modulation, which shows the stress state of the organism [37].

Table 1: This is a table.

	Polar		HRV Camera		ICC (CI 95%)	Pearson	Regression	p
	M	SD	M	SD				
MeanRR (ms)	916.72	136.10	941.37	165.15	.009 (-1.029, .519)	.005	0.003x+957.970 (p>0.05)	0.252
SDNN (ms)	101.54	45.96	416.79	1770.56	.002 (-1.071, .519)	-.173	0.000x+101.650 (p>0.05)	0.329
MeanHR (bpm)	68.06	9.88	64.58	12.50	-.401 (-1.967, .331)	.022	-0.126x+76.460 (p>0.05)	0.417
RMSSD (ms)	48.28	15.54	44.64	20.10	.252 (-.563, .640)	.148	0.130x + 43.057 (p>0.05)	0.204
pNN50 (%)	20.62	10.61	27.90	22.67	.126 (-.779, .574)	.091	0.041x+19.071 (p>0.05)	0.221
HRVI	17.43	4.07	3.90	1.94	-.024 (-.087, .095)	-.177	-0.382x+19.724 (p>0.05)	0.000
TINN	337.5	151.36	162.74	107.63	.063 (-.241, .378)	.082	0.142x+318.684 (p>0.05)	0.000
LF(ms2)	74.22	14.73	740.03	1207.76	-.006 (-.812, .487)	-.163	-0.002x+76.709 (p>0.05)	0.014
HF(ms2)	25.72	14.71	426.49	417.62	-.002 (-.444, .397)	-.023	-0.001x+24.775 (p>0.05)	0.000
LF (n.u.)	77.79	13.34	60.94	87.55	-.189 (-.664, .284)	-.224	-0.139x+83.575 (p>0.05)	0.000
HF (n.u.)	22.16	13.33	61.88	58.65	-.192 (-.674, .284)	-.226	-0.140x+30.308 (p>0.05)	0.000
SD1 (-)	34.20	11.01	43.20	52.76	.227 (-.630, .630)	.158	0.078x+31.195 (p>0.05)	0.562
SD2 (-)	138.65	65.35	225.45	476.18	-.093 (-.919, .567)	.150	0.025x+134.856 (p>0.05)	0.545

M: Mean. SD: Standard Deviation. F: Fisher-Snedecor test. ICC: Intraclass correlation. SDNN: Standard deviation of normal-to-normal R-R intervals. RMSSD: Square root of the mean of the sum of the squared differences between adjacent normal R-R intervals. pNN50: The percentage of differences between R-R intervals higher than 50 ms.HRVI: HRV triangular index. TINN: interval histogram. LF: Low frequency. HF: High frequency. n.u.: Normalised unit. SD1: Poincaré Plot index of instantaneous recording of the variability. SD2: Poincaré plot index of overall variability.

The benefits of evaluating heart rate variability have been observed in psychological disorder as depression and posttraumatic stress disorder [38] and other medical diseases that are accompanied by a dysregulation of the autonomic nervous system such as asthma, heart failure, or hypertension [39]. The utility in sports has been also demonstrated due to the organic alterations suffered by athletes performing physical training. In this line, HRV has been shown as an efficient tool to control the athlete's internal load as a stress marker [40], being useful in designing conditioning programs that ensure a sufficient load that corresponds to the specific capacity of the athlete [21] and prevent states of overload and fatigue of the athlete [21].

HRV measurements have been also used to evaluate the anxiety level of the military population[41,42]. In this line, we found similitudes between soldiers' RMSSD PPG smartphone app values and values of non-elite soldiers found in previous research (44.64 ± 20.10 ms and 41.82 ± 22.95 ms respectively) [43]. In this line, we found RMSSD similar values in military pilots in repose (44.28 ± 25.96 ms) where could be similar HRV response due to stressors before a military operative flight training [44]. Although, it is observed lower RMSSD results than helicopter flight-fighter before a night maneuver (93.4±71.1 ms) [9] and some higher than military corps before underwater evacuation training (33.11 ± 18.73 ms)[45]. Regarding the behavior of this parameter in other athletes. It is observed higher values in athletes in the competition period (61.8 ± 51.73 ms)[46] which indicate that the similarity of RMSSD values in different scenarios is not an exact predictive.

Regarding other PPG system values, we found similar results in the LF values (n.u.) with Special Operation soldiers population in basal conditions before a military operation maneuver (60.94 ± 87.55 vs 60.61 ± 6.66 respectively) [47]. Similar results were shown by a rescue helicopter crew before a flight maneuver (63.6 ± 21.5) [48]. In this line, it has found similarities as athletes in the competition period (64.2 ± 18.70) [46]. Although all cases showed a standard deviation minor than showed in this investigation it presented a similar response in basal conditions before exposure to stressors.

The great contrasts in the frequency values also are observed if we compare to previous literature in basal LF values (740.03 ± 1207.76 vs 64.44 ± 18.00) and basal HF values (426.49 ± 417.62 vs 35.49 ± 18.01) in pilots before a real and simulated flight maneuver [44]. This large discrepancy in the LF and HF values could be produced by an error in the measurement procedure of the mobile app.

In this line, other values in basal conditions show differences compared to previous research, such as the mean RR values, which we observed lower values than thirty-one male subjects from the Spanish Army prior to simulated combat (941.37±165.15 vs 872.8±150.4) [49]. This discrepancy of responses showed in the HRV values in basal conditions may not be produced by the stressor that the subject will face, if not by the difference of subject experience or research protocols.

A disagreement is also observed in the HRV values obtained by the PPG mobile application compared to those obtained with the validated Polar V800 tool. We found a discrepancy to the values obtained by the Polar V800 system where, there was a large discrepancy in the LF values (740.03 ± 1207.76 vs 74.22 ± 14.73 respectively), HF values (426.49 ± 417.62 vs 25.7± 214.71 respectively). The disparity in the HRV data analyzed in our investigation could be due to the APP's short-time analysis protocol, of 30 seconds. This duration was selected in order to find the protocol that would require the least time from the participants due to the low availability of time in the measured population, trying to find the most efficient protocol and enough time to provide HRV measurements as the application sentence. The protocol of the Polar V800 system was 5 minutes, which is more reliable due to the duration of the measurement, which is similar to those seen in the rest of the protocols in investigations with other PPG technology devices.

On the contrary, to solve the discrepancy in the data, the application of a regression equation produces the correlation of the values. Positive and negative correlations in frequency values, HRVI index, and TINN index have been observed between both devices. In this case, the PPG system could provide us with reliable results in a short time record.

This fact is related to previous research that validated different PPG tools with standard systems such as HRV4Training [31] which has been proven a systematic tool to measure to optimize individualized training in professional endurance runners [50] and cyclists [51]. This kind of app delivers efficient results comparable to the ECG for measuring HRV in basal conditions where measurements protocols consisted at least of a 1 min recording [52]. Therefore, there could be greater efficiency in terms of HRV recording as long as the measurement time is greater than that proposed in our research without the need to apply any type of equation.

The evaluation and measurement of HRV have also been evaluated in PPG technology in other devices such as wristband devices. It has been demonstrated satisfactory HRV-related mental stress prediction taking LF/HF feature and with a similar record protocol as our research, 30 seconds of registration, but in this case with more records, three times per day in wristband PPG device [53]. In addition, the Apple Watch showed a significant decrease in values related to increased stress, HF, and RMSSD, with a protocol of 2 sets of 5 minutes [54] and Wrist-Worn Polar Vantage V2 PPG which is regarded as an HRV PPG system record efficient in athletic and healthy population with long time period protocols,7 minutes in supine position in a laboratory and 4 hours recording period during nocturnal sleep time [55]. Showing in all the cases better results when the measurement time is longer in time or with several records in a period of time.

Practical application

Present results joining other research could help refine an exact protocol for using the PPG smartphone apps, and become an effective and easy-to-use tool in harsh conditions. And provide us with the correlation equation for the validity of the data obtained through this application with a protocol with a short time record.

Limitations of the study and future research lines

The main limitation of this study is the low sample of participants. Also, it could be recommended the use of ECG to compare values as in previous research. In future research, it is suggested to validate PPG protocols with longer durations.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution
Conceptualization, A.C.-R., V.J.C.-S and A.B.-S.; methodology and formal analysis A.C.-R., A.B.-S and V.J.C.-S investigation, A.C.-R., A.B.-S and V.J.C.-S.; data curation, V.J.C.-S.; Writing—Original draft preparation, , A.C.-R., A.B.-S and V.J.C.-S.; Writing—Review and Editing, A.C.-R., A.B.-S and V.J.C.-S.; visualization, LPA, A.C.-R., A.B.-S and V.J.C.-S.; supervision and funding acquisition, V.J.C.-S.

Ethics approval and consent to participate
Procedures and protocols were explained to all the subjects who gave the voluntary written informed in accordance with the Declaration of Helsinki and approved by the Euro-pean University Ethics Committee (CIPI/18/093).

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Predictive Factors for Death among Patients with Clostridium difficile Infection – A Single Center Experience Study

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Abstract: *Clostridium difficile infection (CDI) is one of the most common healthcare-associated infections and an important cause of morbidity and mortality worldwide. The objective of the study was to identify the predictive factors for unfavorable evolution and deaths among patients with CDI. Materials and methods: We conducted a retrospective, observational, non-randomized study on 202 patients diagnosed with CDI in the Clinical Emergency Hospital of Bucharest, Romania, for a period of two years. For the statistical analysis, the R program was used, with p-values <0.05 being considered statistically significant. Results: The average age of the patients included in the study was 70.8 years, the average duration of hospitalization was 18.4 days, and the mortality rate was 25.7%. During the follow-up period, only 5% of the patients presented a recurrence of CDI. Multiple binomial logistic regression defined as independent risk factors for death among CDI patients: advanced age, comorbidities such as chronic obstructive pulmonary disease (p=0.008), chronic kidney disease (p=0.007), or COVID-19 (p=0.036), leukocytes ≥ 16,755/mm^3 (p=0.009) and serum albumin < 2.83 g/dL (p<0.001). Conclusions: The identification of certain negative prognostic factors in patients with CDI requires a careful follow-up of the patients, for an early identification of complications and adaptation of therapeutic management.*

Keywords: *Clostridium difficile; chronic obstructive pulmonary disease; COVID-19; chronic kidney disease; prognosis*

INTRODUCTION

Clostridium difficile infection (CDI) is one of the most common healthcare-associated infections and an important cause of morbidity and mortality worldwide [1]. The latest epidemiological data provided by the European Center for Disease Prevention and Control (ECDC), in 2008, reported an incidence of approximately 4.2 cases of CDI per 10.000 patient-days per hospital, with a range of variation of 0.0-36.3 [2]. According to this report, the incidence of CDI increased dramatically compared to 2005 when an incidence of approximately 2.45 cases of CDI per 10,000 patient-days per hospital was reported, with a range of variation of 0.13-7.1 [3,4]. In the United States of America (USA), in 2012, approximately half a million cases of CDI and 29.000 deaths secondary to this disease were registered [5]. However, because of the implementation of a surveillance and infection prevention program in 10 USA states, the health-care-associated CDI decreased from 92.76 cases per 100,000 inhabitants in 2011, to 66.96 cases per 100,000 inhabitants in 2017 [6]. Regarding the incidence of community-associated CDI in the USA, it increased slightly between 2011 and 2017 (55.3 cases per 100,000 inhabitants in 2011 vs. 70.4 cases per 100,000 inhabitants in 2017) [6]. Another problem is represented by the important recurrence rate of CDI, respectively 20-30% [7]. Beyond the negative impact on the quality of life and life expectancy, the healthcare of these patients involves very high costs. For example, in the USA, 5.4-6.3 billion dollars per year are consumed by these patients [8].

It is also reported a Clostridium difficile carriage rate of 8-10% among hospitalized adults, compared to 3% among healthy adults [9]. Carriers, but asymptomatic patients, represent reservoirs of germs, which can eliminate spores and transmit the infection to other patients [10,11]. A study that followed the prevalence of colonization with Clostridium difficile, at admission, among 250 patients who did not present diarrhea, reported a percentage of 79% of patients colonized with this bacteria [12]. Among them, in 15% of cases, a toxigenic strain was identified, and in 6% of cases a non-toxigenic strain [12]. No differences were identified regarding recent antibiotic use or contact with medical care units between patients colonized with a toxigenic strain vs. uncolonized patients [12]. Also, the concentrations of toxins in stools have been shown to be similar in asymptomatic carriers vs. symptomatic patients [13].

CDI is a public health problem with a major impact on the healthcare system. This can be explained by the significant increase in the incidence after the year 2000, the existence of CDI outbreaks in some hospitals, but also by the appearance of hypervirulent strain NAP1/B1/027 [14,15].

The most common risk factors for CDI are represented by hospitalization, antibiotic use, and advanced age [16-18]. However, there are other risk factors that can modify the evolution and prognosis of these patients, respectively: obesity, severe comorbid illness, inflammatory bowel disease, chemotherapy, enteral feeding, hematopoietic stem cell transplantation, cirrhosis, and gastric acid suppression [19-23]. Regarding the risk of complications, a study that followed 1,380 patients with CDI identified a series of predictive factors for unfavorable evolution: older age, abnormal vital signs (respiratory rate over 20/minute and heart rate over 90 beats per minute), and abnormal blood tests (C-reactive protein ≥ 150 mg/L, albumin < 25 g/L, white blood cell count < 4,000/L or ≥ 20,000/L) [24]. According to data from the specialized literature, all cases' death rate at 30 days in patients with CDI varies between 9% and 38% [25].

Given the burden and the important death rates associated with CDI, we aimed to identify the predictive factors for an unfavorable evolution among patients with CDI. The primary endpoint of the study was the in-hospital mortality rate.

MATERIALS AND METHODS

We conducted a retrospective, observational, non-randomized study in which we evaluated the mortality rate associated with CDI and the risk factors for an unfavorable evolution. We followed 202 patients diagnosed with CDI between January 2020-January 2022 during hospitalization in the Clinical Emergency Hospital of Bucharest, Romania. The study was approved by the Ethics Committee of the Clinical Emergency Hospital of Bucharest (approval no. 10002/ 04.10.2022). The study respected the ethical standards of the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

The inclusion criteria consisted of the existence of clinical and paraclinical evidence attesting CDI. For the diagnosis, we applied the 2019 European Center for Disease Prevention and Control (ECDC) definition of CDI, respectively: diarrhea or toxic megacolon and a positive test for Clostridium difficile toxins A/B in stool samples or positive polymerase chain reaction (PCR) test for Clostridium difficile; colonoscopic examination that highlighted pseudomembranous colitis; histopathological examination that identified characteristic elements of CDI in a tissue sample obtained by endoscopy from a patient with or without diarrhea [4]. The exclusion criteria were represented by the absence of some clinical-paraclinical data that were followed in the study and the absence of the patient's informed consent. None of the patients initially included in the study were subsequently excluded.

The variables followed in the study are epidemiological data (age, sex), presence of comorbidities (chronic obstructive pulmonary disease, COVID-19, chronic kidney disease, heart failure, atrial fibrillation, diabetes mellitus, arterial hypertension, neoplasms), CDI recurrence, treatment with proton pump inhibitors, a series of biological variables (hemoglobin, number of leukocytes, C-reactive protein, erythrocyte sedimentation rate, fibrinogen, albumin), hospitalization length and death rate.

Statistical analysis

Patient data were obtained from the electronic medical records of the hospital and were centralized in an Excel database. For the statistical analysis, we used the R program, version 4.0.2 [26]. We used a simple univariate binomial logistic regression, the dependent variable being death during hospitalization, and the independent variables' demographic-clinical parameters monitored in the study. Later, the predictors that had statistical significance in the simple model were used in a multiple univariate binomial logistic regression, the model being built with the help of a retrograde selection algorithm of the predictors. We also performed a descriptive statistical analysis of the variables followed in the study. P-values lower than 0.05 were considered statistically significant.

RESULTS

The average age of the patients included in the study was 70.8 years, with a minimum value of 18 years and a maximum value of 95 years. Regarding gender, the proportion was approximately equal, respectively 47% women and 53% men. The average duration of hospitalization was 18.4 days, reaching up to 145 days in more severe cases. During the 2-year follow-up period, only 10 patients (5%) presented a recurrence of Clostridium difficile colitis. Regarding comorbidities, 58.4% of the patients had arterial hypertension (AH), 33.2% heart failure (HF), 29.7% chronic kidney disease (CKD), 27.7% diabetes mellitus, 26.2% atrial fibrillation (AF), 22.8% neoplasms, 22.3% COVID-19 and 3.96% chronic obstructive pulmonary disease (COPD). Among the patients included in the study, 45% were treated with proton pump inhibitors (PPI) (Table 1).

Table 1: Demographic, clinical, and paraclinical analysis of the study group

Variable	Number of patients (N=202)
Age	
Average (SD)	70.8 (15.7)
Median [Min, Max]	73.5 [18.0, 95.0]
Sex	
F	95 (47.0%)
M	107 (53.0%)
Length of hospital stay	
Average (SD)	18.4 (18.4)
Median [Min, Max]	14.0 [1.00, 145]
COPD	
Yes	8 (3.96%)
No	194 (96.04%)
Recurrence of CDI	
Yes	10 (5.0%)
No	192 (95.0%)
COVID-19	
Yes	45 (22.3%)
No	157 (77.7%)
PPI	
Yes	91 (45.0%)
No	111 (55.0%)
CKD	
Yes	60 (29.7%)
No	142 (70.3%)
HF	
Yes	67 (33.2%)
No	135 (66.8%)
AF	
Yes	53 (26.2%)
No	149 (73.8%)
DM	
Yes	56 (27.7%)
No	146 (72.3%)
AH	
Yes	118 (58.4%)
No	84 (41.6%)
Neoplasms	
Yes	46 (22.8%)
No	156 (77.2%)
Number of leukocytes	
Average (SD)	18600 (14000)
Median [Min, Max]	14500 [2800, 94000]
Fibrinogen (mg/dL)	
Average (SD)	498 (173)
Median [Min, Max]	469 [107, 1020]
CRP (mg/dL)	
Average (SD)	118 (73.8)
Median [Min, Max]	102 [0.710, 371]
ESR (mm/hr)	
Average (SD)	43.6 (22.0)
Median [Min, Max]	45.0 [2.00, 98.0]
Hemoglobin (g/dL)	
Average (SD)	10.6 (2.28)
Median [Min, Max]	10.4 [5.50, 18.3]
Albumin (g/dL)	
Average (SD)	2.91 (0.797)
Median [Min, Max]	2.90 [1.00, 5.10]
Death	
No	150 (74.3%)
Yes	52 (25.7%)

Legend: COPD = chronic obstructive pulmonary disease; CDI = Clostridium difficile infection; PPI = proton pump inhibitors; CKD = chronic kidney disease; HF = heart failure; AF = atrial fibrillation; DM = diabetes mellitus; AH = arterial hypertension; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

Also, a series of biological variables were analyzed, such as the number of leukocytes and the serum values of fibrinogen, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin, and albumin.

The average value of leukocytes was 18,600/mm^3, varying between 2,800/mm^3 and 94,000 mm^3. Also, increased values of inflammatory markers were identified, namely an average value of fibrinogen of 498 mg/dL (107-1,020 mg/dL), an average value of CRP of 118 mg/dL (0.710-371 mg/dL), and an average value of ESR of 43.6 mm/hr (2.00-98.00 mm/hr).

The mean serum hemoglobin was 10.6 g/dL and the mean serum albumin was 2.91 g/dL (Table 1). 52 patients died during follow-up, the death rate being 25.7% (Table 1).

Later, we performed a simple univariate binomial logistic to identify the risk factors for unfavorable prognosis and death (Table 2).

This statistical analysis identified a direct proportional relationship between the mortality rate and the following risk factors: advanced age, association with COVID-19, CKD or COPD, serum hemoglobin < 9.85 g/dL, leukocyte count ≥ 16,755/mm^3, CRP ≥ 104.02 mg/dL, serum albumin < 2.83 g/dL. Increasing age by 1 year was associated with a 2% increase in the death rate (p=0.048, 95% CI 1.02 (1.00-1.05)).

Table 2: Simple univariate binomial logistic regression to determine the risk factors for poor prognosis and death among patients with Clostridium difficile colitis.

Predictor	N	OR (95% CI)[1]	P value
Age	202	1.02 (1.00 la 1.05)	0.048
Sex	202		
F		—	
M		1.05 (0.56 la 1.98)	0.88
Recurrence of CDI	202		
Yes		—	
No		1.41 (0.34 la 9.54)	0.67
COVID-19	202		
Yes		—	
No		0.47 (0.23 la 0.97)	0.039
PPI	202		
Yes		—	
No		0.69 (0.36 la 1.30)	0.25
CKD	202		
Yes		—	
No		0.29 (0.15 la 0.57)	<0.001
HF	202		
Yes		—	
No		0.52 (0.27 la 1.01)	0.051
COPD	202		
Yes		—	
No		0.04 (0.00 la 0.25)	0.004
AF	202		
Yes		—	
No		0.57 (0.29 la 1.15)	0.11
DM	202		
Yes		—	
No		0.57 (0.29 la 1.13)	0.10
Neoplasms	202		
Yes		—	
No		0.49 (0.24 la 1.01)	0.050
Hemoglobin (g/dL)[2]	202		
< 9.85		—	
≥ 9.85		0.31 (0.16 la 0.60)	<0.001
AH	202		
Yes		—	
No		0.67 (0.34 la 1.29)	0.24

Predictor	N	OR (95% CI)[1]	P value
Number of leukocytes[2] (/mm³)	202		
< 16755		—	
≥ 16755		3.85 (2.01 la 7.56)	<0.001
Fibrinogen (mg/dL)	202	1.00 (1.00 la 1.00)	0.13
CRP[2] (mg/dL)	202		
< 104.02		—	
≥ 104.02		2.27 (1.20 la 4.39)	0.013
ESR (mm/hr)	202	1.01 (1.00 la 1.03)	0.17
Albumin[2] (g/dL)	191		
< 2.83		—	
≥ 2.83		0.21 (0.10 la 0.42)	<0.001

[1] OR = Odds Ratio, CI = Confidence Interval, [2] Cut-off determined by a ROC analysis

Legend: COPD = chronic obstructive pulmonary disease; CDI = Clostridium difficile infection; PPI = proton pump inhibitors; CKD = chronic kidney disease; HF = heart failure; AF = atrial fibrillation; DM = diabetes mellitus; AH = arterial hypertension; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

The predictors that had statistical significance in the simple model were later used in a multiple univariate binomial logistic regression, the model being built with the help of a retrograde selection algorithm of the predictors (Table 3). This statistical analysis identified COVID-19 as an independent risk factor, with an approximately 3-fold increase in the risk of death (95% CI 0.39 (0.16 – 0.94); p=0.036). Other factors associated with an independent increase in the risk of death, among the patients included in the study, were: the presence of CKD, with a 3-fold increase in the risk of death (95% CI 0.33 (0.15 – 0.73); P=0.007), the presence of COPD, with a 20-fold increase in the risk of death (95% CI 0.05 (0.00 – 0.33); p=0.008), the number of leukocytes ≥ 16,755/mm³, which led to an approximately 3-fold increase in the risk of death (95% CI 2.82 (1.31 – 6.22); p=0.009) and the value of serum albumin < 2.83 g/dL, which led to a 4-fold increase in the risk of death (95% CI 0.23 (0.09 – 0.50); p<0.001) (Table 3).

Table 3: Binomial univariate multiple logistic regression with predictors for death among patients with Clostridium difficile colitis

Predictors	OR (95% CI)[1]	P-value
COVID-19		
Yes	—	
No	0.39 (0.16 la 0.94)	0.036
CKD		
Yes	—	
No	0.33 (0.15 la 0.73)	0.007
COPD		
Yes	—	
No	0.05 (0.00 la 0.33)	0.008
Number of leukocytes[2] (/mm³)		
< 16755	—	
>= 16755	2.82 (1.31 la 6.22)	0.009
Albumin[2] (g/dL)		
< 2.83	—	
>= 2.83	0.23 (0.09 la 0.50)	<0.001

[1] OR = Odds Ratio, CI = Confidence Interval, [2] Cut-off determined by a ROC analysis

CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease

DISCUSSION AND CONCLUSIONS

Clostridium difficile colitis is one of the most common nosocomial infections in developed countries, for which higher morbidity and mortality rates were reported after 2000 [27]. In the USA, approximately half a million patients are diagnosed with CDI annually [28]. This phenomenon is partially explained by the spread of a more virulent strain, ribotype (RT) 027, after the year 2000, in the context of the increase in life expectancy and population aging [27,29]. However, the global burden associated with CDI decreased by 24% between 2011 and 2017 in the USA, due to the reduction of healthcare-associated infections [6]. The rates of reinfection with Clostridium difficile and mortality associated with this condition did not change significantly during this period [6, 30]. An explanation for the reduction of the global burden of CDI was represented by the natural decrease of RT 027 or as a result of the large-scale reduction in the use of fluoroquinolones [30-32]. Also, other strains that can be associated with a possible unfavorable evolution, such as RT 244 or 078, were rarely identified in the USA [33,34]. In 2016, ECDC reported 7711 cases of CDI in 20 member states of the European Union, 74.6% being associated with nursing [35]. The most frequent RT were 027 (22.9%), 001 (7.5%), 014 (6.7%), 078 (5.1%), 002 (4.2%), and 020 (4.2%) [35]. The all-cause mortality rate among these patients was 20.7% [35]. In Romania, the average prevalence of CDI is 5.2 cases per 10,000 patient days, increasing to approximately 25 cases per 10,000 patient days in the departments of gastroenterology or infectious diseases [36]. The dominant RT in Romania is 027. A study that followed 393 patients with CDI identified RT 027 in 82.6% of cases (95% CI: 73.6%-89%) [36]. Other identified RT were: 002, 018, 087, 014, 001, 011, 012, 017, 020, and 106 [36].

In our study, the average age of patients diagnosed with CDI was 70.8 years, and the death rate from all causes was 25.7. These results correspond to those reported in the literature. Redelings et al. reported an increase in the mortality rate associated with CDI in the USA from 5.7 deaths per 1 million individuals in 1999 to 23.7 deaths per 1 million individuals in 2004 [37]. The same authors reported an average age of patients whose death was associated with CDI of 82 years [37]. Another study that followed 66 patients with CDI, aged over 65 years, reported the following mortality rates: 11.5% at 7 days, 26.2% at 30 days, 38.1% at 90 days, and 41.4% at 180 days. Mitchell et al. highlighted a variability of all-cause mortality at 30 days in patients who had associated CDI between 9% and 38% and of in-hospital mortality between 8% and 37.2% [38]. The directly proportional relationship between advanced age and poor prognosis among patients diagnosed with CDI was also highlighted by a multicenter study that followed patients from hospitals in Quebec [39]. These authors highlighted the increase in the 1-year mortality rate among patients with CDI from 4.7% in the 18-64 years age group to 21.1% in the 65-74 years age group [39]. In our study, increasing age by one year was associated with increasing the risk of death by 2%.

Regarding the average length of hospitalization of the patients included in our study, it varied between one day and 145 days, with an average value of 18.4 days. This result corresponds to data from the literature [40-42]. Thus, Hebbinckuys et al. reported an average length of hospitalization of 18.5 days for patients with CDI, which can vary between 6 and 132 days [40]. Another study compared two groups of patients hospitalized for various ailments: the first group consisted of 38 patients who had associated CDI and the second group consisted of 76 patients who did not have associated CDI [41]. In the first batch, the average duration of hospitalization was significantly higher compared to the second batch (31.5 days vs. 5.5 days) [41]. The conclusion of this study was the increase of the average duration of hospitalization by approximately 4 days by the association of CDI ($p<0.001$) [41]. During the study, only 5% of the patients presented a recurrence of infection with Clostridium difficile. According to ECDC, the recurrence rate of CDI in Europe is approximately 7.9% [35]. Other studies reported higher rates of recurrence of CDI, respectively 15-30% [43,44]. The factors associated with the risk of CDI recurrence are advanced age, infection with hypervirulent strains, history of CDI and their severity, longer duration of hospitalization, use of antibiotics for non-CDI after establishing the diagnosis of CDI, use of gastric acid suppression drugs or renal failure [44,46,47]. An explanation for the lower recurrence rate of CDI in the population included in our study can be represented by the absence of an infectious disease department in the hospital where the study was carried out. Under these conditions, there is the probability that in case of relapse, some of the patients would have presented to other hospitals.

Among the comorbidities of patients included in our study, we list in order of their frequency: arterial hypertension, heart failure, CKD, diabetes mellitus, atrial fibrillation, neoplasms, COVID-19, and COPD. This wide range of comorbidities can be explained by the advanced age of the patients, the average age being approximately 71 years. Feuerstadt et al. identified among the most frequent comorbidities in CDI patients: HF, COPD, diabetes without complications, peripheral vascular disease, and renal disease ($p < 0.01$) [48]. Our study validates these results, adding to the comorbidities also COVID-19, being performed during the pandemic. According to data from the literature, the association of other diseases in patients with CDI leads to an increase in the disease burden, by increasing the risk of polypharmacy, compromising the immune status, and increasing the duration of hospitalization [49]. Almost half of the patients had treatment with proton pump inhibitors (PPI). A recent meta-analysis that included eight studies from the USA, Canada, Europe, and Asia highlighted a statistically significant association between PPI use and the risk of developing CDI [50]. Inghammar et al. also highlighted the fact that the risk of developing CDI remains high even one year after stopping PPI treatment [51]. Another meta-analysis that included 356,683 patients concluded that the use of PPIs leads to an increase in the risk of developing CDI in both groups, inpatients, and outpatients [52].

From a paraclinical point of view, the statistical analysis identified leukocytosis, inflammatory syndrome, mild anemia, and hypoalbuminemia among the patients included in our study. A prospective study that followed 60 patients with unexplained leukocytosis identified the presence of Clostridium difficile toxins in fecal specimens in 58% of them [53]. The data currently available suggest the need to perform paraclinical tests in order to identify CDI in hospitalized patients who develop leukocytosis [53,54]. A series of studies demonstrated the correlation between elevated CRP values, anemia, hypoalbuminemia, and the unfavorable prognosis of patients with CDI [55-60]. Ahmed et al. have highlighted the fact that the decrease in serum albumin values and the increase in the ATLAS score (which includes age, albumin and creatinine values, the number of leukocytes, and treatment with antibiotics during CDI therapy) among patients with Clostridium difficile colitis reflect the worsening of the disease [58].

The main objective of our study was to identify the predictive factors for unfavorable evolution and death among patients with CDI. The simple univariate binomial logistic regression identified the following risk factors for death: advanced age, comorbidities such as COVID-19, CKD, and COPD, but also the values of some paraclinical markers, such as serum hemoglobin < 9.85 g/dL, leukocyte count ≥ 16,755/mm^3, CRP ≥ 104.02 mg/dL and serum albumin < 2.83 g/dL. Later, the multiple binomial logistic regression analysis defined advanced age, the association of COPD, CKD, or COVID-19, the number of leukocytes ≥ 16,755/mm^3, and the serum albumin value < 2.83 g/dL as independent risk factors for death. A study carried out in Taiwan, which followed 401 patients with CDI, highlighted a 30-day mortality rate of 23.4%; among the predictive factors for death were age > 65 years, serum albumin value < 2.5 g/dL, and increased creatinine by 1.5 times [61]. De Miguel-Diez et al. highlighted a doubling of the incidence of CDI and an increase in mortality among patients with COPD compared to the general population [62].

Regarding the evolution of patients who associate CDI and COVID-19, our study validates other data from the literature [63,64]. Thus, a study that followed 2065 patients with COVID-19, of whom 40 had CDI, highlighted a more severe evolution among patients who combined both diseases, with 45% of them developing complications, and 22.5% dying in hospital [63]. The extensive use of antibiotics during the COVID-19 pandemic increased the risk of CDI development [63]. According to other studies, CKD increases both the prevalence and severity of Clostridium difficile colitis [65-67]. This phenomenon can be explained by the frequent hospitalizations of patients with CKD, but also by other factors such as the frequent use of antibiotics or PPIs, alteration of the immune system, or intestinal dysmotility [65]. Keddis et al. highlighted a higher prevalence of CDI among CKD patients compared to the general population (1.49% vs 0.70%) [66]. The same authors identified in the group of patients who had both CKD and CDI, compared to patients with CKD without CDI, longer average hospital stays (7 days vs. 4 days), higher rates of colectomy (0.67% vs. 0.28%) and higher death rates (6.62% vs. 3.99%) [66].

The peculiarity of our study consists of the follow-up of patients with CDI during the COVID-19 pandemic. The mortality rate did not show significant differences compared to the one recorded before the pandemic, but the CDI recurrence rate was slightly lower compared to that reported by the literature. This result can be explained by the patients' fear of infectious diseases and their presentation in case of relapse in infectious disease clinics. The main limitation of our study is the lack of differentiation between community cases of CDI and those associated with medical assistance.

In conclusion, CDI is a public health problem due to both high incidence rates and high mortality rates, especially among elderly patients and those with important comorbidities. To improve the therapeutic management and prognosis of these patients, more effective systems for risk identification are needed. In this regard, our study sought to identify a series of predictive factors of death among patients with CDI. Patients who present the clinical and paraclinical profiles described above as being at high risk of unfavorable evolution and death require more careful monitoring for the early identification of possible complications and optimal treatment. Also, these risk factors can contribute to the correct selection between ambulatory treatment, hospitalization, or the need for intensive therapy measures.

Author Contributions

Conceptualization, V.A.I. and G.G.; methodology, R.S.C. and V.A.I.; software, F.G.; validation, C.C.D.; formal analysis, A.G.A. and F.G; investigation, F.G.; resources, R.S.C; data curation, A.G.A.; writing—original draft preparation, G.G.; writing—review and editing, C.C.D.; visualization, C.C.D.; supervision, C.C.D. and R.S.C.; project administration, V.A.I..; funding acquisition, R.S.C. All authors have read and agreed to the published version of the manuscript.

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The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Clinical Emergency Hospital of Bucharest, Romania (approval no 10002/ 04.10.2022).

Informed Consent Statement

Not applicable.

Data Availability Statement
Not applicable.

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None.

Conflicts of Interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Self-esteem, Self-efficacy, and Smoking Prevalence: A Cross-sectional Study Among Military and Civilian Medical Students

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Abstract: *Background: The prevalence of smoking among students is a growing concern, particularly among medical students. Being associated with stress, competitiveness, and various social influences, smoking can have detrimental effects on self-esteem and self-efficacy and may create a vicious cycle that may contribute to student's well-being and academic performance outcomes. We aimed to investigate the relationship between self-esteem, self-efficacy, and smoking habits among medical students and their sociodemographic correlates. Methods: A cross-sectional study on 456 medical students was conducted via an online survey using structured questionnaires on socio-demographic characteristics, smoking habits, nicotine dependence, self-esteem, and self-efficacy. Results: The prevalence of smoking was 31.1% The scores of self-esteem and self-efficacy were positively correlated with smoking habits (W1=0.957, W2=0.975, P<0.05). Students presented rather low smoking addiction with male students having slightly higher scores than female students on self-esteem and self-efficacy, military students had higher self-esteem scores than civilians with physical activity having a positive correlation with non-smoking behavior. Conclusion: According to our results, self-esteem and self-efficacy were slightly positively correlated with smoking habits. Furthermore, there was no statistically significant association between other social factors and smoking. Our results may develop a theoretical basis for medical students' psychological variables studying for further implementation of university educational programs and preventive interventions empowering students towards an overall better quality of life, addressing, among others, smoking behaviors.*

Keywords: *prevalence, smoking, medical students, self-esteem, self-efficacy*

INTRODUCTION

Stress is a common experience for most medical students due to the rigorous demands of their training with medical education being challenging and students often facing heavy workloads and competition pressure situations. Having to absorb and retain a large volume of complex information, time pressure, and expecting to academically perform at a high level can create a significant amount of mental distress. Major years students can have even more challenging and stressful experiences as they are often exposed to the presentation of critically ill patients and life-threatening emergencies with supplementary emotional input. With less time for socializing, hobbies, or self-care and other socio-economic difficulties, medical students can develop various stages of burnout and use unhealthy coping mechanisms, especially after the COVID-19 lockdown when coping mechanisms deteriorated with smoking and drinking, among others, important factors of the stress perceived [1,2,3].

Smoking is one way, among others, that people usually cope with stress with nicotine providing a temporary sense of relaxation. However, smoking has been linked to a variety of negative health outcomes, including lung cancer, heart disease, and stroke, comprising 16% of non-communicable diseases, in Romania [4,5]. In addition, smoking can increase stress levels over the long term by disrupting sleep, reducing exercise tolerance, and impairing lung function [6,7].

There have been several studies that have looked at the link between smoking and stress in medical school. One study found that medical students who reported high levels of stress were more likely to smoke, with around 30% of students reporting smoking, and that smoking was more common among male students [8]. In Europe, the prevalence of smoking among medical students varies from 28.02% in Germany to 31.3% in Italy, which is higher than in the general population [9] and compared to students from other faculties [10]. Stress and symptoms of anxiety and depression increase in the first two years of medical studies but decrease towards their end [11]. In doctors, stress and burnout are rather typical. Both issues could start during medical school and continue during residency and then across a lifetime. Medical schools need to be informed about the persistent and important effects of stress and burnout among their students.

The effects of smoking on various psychological variables have been a topic of interest in recent years with studies investigating the relationship between smoking and mental health, including self-esteem and self-efficacy. Smoking was negatively associated with self-esteem and self-efficacy in medical students. This negative relationship is believed to be due to the harmful effects of smoking on both physical and mental health. Smoking may affect one's social and emotional well-being, leading to feelings of isolation and reduced self-worth. Smokers may also experience academic problems, which can further reduce their self-esteem and self-efficacy [12].

Unfortunately, smoking is not the only factor that comes along with stress. A vicious cycle is formed between smoking, low self-esteem, and low self-efficacy. A study published found that these factors were significant predictors of academic performance among medical students. Higher levels of stress were associated with lower academic performance, while higher levels of self-esteem were associated with better academic performance. The study also found that there was a significant negative correlation between stress and self-esteem [13]. Medical students who smoked had lower self-esteem than non-smoking students. Similarly, a study found that smoking was associated with lower self-esteem in medical students. The concerning part is that self-esteem and self-efficacy were negatively associated with smoking and that higher levels of self-efficacy were associated with a lower likelihood of smoking. [14].

The importance of self-esteem and self-efficacy was highlighted by their influence as mediating the relationship between motor proficiency and internalizing problems along with social support. This represents an important aspect of the concept of the environmental stress hypothesis which may also relate to unhealthy coping mechanisms such as smoking [15]. In recent years, there are no studies including all three variables as included in our research. This may represent a more complete understanding of how these psychological variables impact smoking behavior. Even if there are some studies regarding military medical students [16] we found no approaches concerning this type of students in aspects of self-esteem or self-efficacy. The social component is not discussed enough as loneliness and social circle may influence smoking behavior [17].

Thus, this study aimed to examine the relationship between smoking prevalence, self-esteem, and self-efficacy in medical students. Additionally, this study`s secondary aims were to compare these factors based on socio-demographic characteristics and investigate their potential correlation. Our hypothesis was based on correlations between variables but no presumptions as to whether they were negative or positive were made in advance. Also, there is likely that coping mechanisms may represent an additional factor for smoking behavior prevalence. Moreover, military students are expected to have lower self-esteem and self-efficacy scores as their military service goes along with their medical training with stress levels being considered higher.

MATERIALS AND METHODS

Study design and participants

The design of the study was cross-sectional, comprising a survey of 22 questions and three standardized psychometric instruments. The cross-sectional design was successfully used in previous literature on addictive behaviors [18,19], including in Romania [20,21].

456 medical students from the first three years of study (33.1% men, 65.7% women, 1,09% other; mean age = 20.8, SD = 2.24) agreed to participate in the study and answered the psychometric tests.

The inclusion criteria were as follows: (1) students currently studying at the university (2) obtained informed consent and willingness to participate in the study.

Context and Procedure

The data were gathered in April–May 2023 through the administration of an online survey containing the study instruments. Firstly, we informed the participants of the study’s aims. Participation was anonymous, and students’ responses did not affect the teacher’s evaluation of the student’s academic performance.

The study procedure was run via a SurveyMonkey® interface (One Curiosity Way, San Mateo, CA, USA). Online testing in university settings is reported to ensure the speed and representativeness of the collected data [22,23].

Methods in the study were designed according to the World Medical Association`s Declaration of Helsinki. The study protocol was approved by the UMFCD Ethics Review Board (no. 14873/2023). All responses were processed anonymously and a numerical code was assigned to each respondent. The data were accessible exclusively to study researchers (LM, CGI, MS). The interpretation of the questionnaires was performed independently by two researchers (MS, CGI). Results were included in a JASP version 0.10.2 statistical software (Department of Psychological Methods the University of Amsterdam, Amsterdam, The Netherlands, https://jasp-stats.org/) database.

Questionnaires

Three standardized psychometric instruments were used to measure their smoking addiction, self-esteem, and self-efficacy scales.

(1) The Fagerstrom Test for Nicotine Dependence (FTND) is a questionnaire used to measure the degree of nicotine dependence in cigarette smokers. The answers to the 6 questions are used to calculate a total score, with higher scores indicating greater nicotine dependence. Across literature studies, the scale has been shown to have good internal consistency, good validity, and a fairly stable factorial structure [24,25]. Even if sometimes it has a low consistency (0,4-0,6), it is rather acceptable in literature [26].

(2)The Rosenberg Self-Esteem Scale (RSES) is a commonly used scale for measuring self-esteem. It consists of ten statements, to which the respondent rates their level of agreement on a 4-point scale ranging from strongly agree to strongly disagree. The scale measures both positive and negative feelings about oneself, with higher scores indicating higher levels of self-esteem. Responses are scored from 0 to 3, with the total score ranging from 0 to 30. A higher score indicates higher self-esteem [27,28].

(3)The Schwarzer and Mathias self-efficacy scale, also known as the General Self-Efficacy Scale (GSE), is a psychometric tool designed to measure an individual's self-belief in their ability to handle difficult situations and achieve their goals. It consists of ten statements that participants rate on a four-point scale, with higher scores indicating greater self-efficacy. Scores are then calculated and interpreted to provide an overall measure of an individual's self-efficacy. [29,30].

In addition to these three psychometric tests, the participants provided information about their age, place of origin, current academic year, type of training-medical or civilian, current residence, specialty, and coping mechanisms.

Data analysis

We first described the demographics of our participants. The internal consistency of the smoking addiction, self-esteem, and self-efficacy scales was assessed using Cronbach's alpha coefficient and all instruments had coefficients above 0.7, which is considered to be the minimum acceptable value for proper reliability [31].

We made descriptive statistics using all the data depending on the smoker/non-smoker status. Afterward, we performed T-tests to compare various groups on the three main variables studied: smoking addiction, self-esteem, and self-efficacy. Furthermore, we used Chi-Squared tests to quantify differences in smoking/non-smoking depending on the place of living and gender. Other T-tests were used to compare the three scales depending on situations when smoking, coping mechanisms, and type of cigarettes smoked.

Data management and descriptive analyses were performed using JASP statistical software, version 0.17.2 statistical software [32].

Ethical Considerations

- Participants were appropriately informed about the nature of the research.
- Informed consent was taken from the participants.
- Anonymity of participants and confidentiality of the information was maintained.
- Participants were given the right to withdraw from research at any time.
- The responses were accurately represented

RESULTS

Sociodemographic data and smoking correlates

The response rate was 10.08% (456 out of 4521 medical students invited). Data was collected from 456 students who agreed to participate in the study (151 men, 300 women, 5 not specified gender; mean age = 20.88, SD = 2.24). A total of 86.8% of the participants were Romanian module students with 14.2% reported being Military Medicine students and only 27.4 % reporting living in a student campus (complete detailed results in Table S1. Supplementary Materials). Table 1 summarizes the descriptive indicators of the socio-demographic data.

The Fagerstrom nicotine dependence test showed a low mean level of nicotine addiction among medical students (m = 1.45, SD =1.406) [33]. The data reveal interesting patterns in smoking behavior across different sociodemographic categories.

As expected, the majority of both smokers and non-smokers are from Civil Medicine (being the majority), but the proportion of smokers in Military Medicine is noticeably higher than that met in Civil Medicine students. This could suggest a potential association between the stressors or lifestyle factors specific to Military Medicine and smoking behavior. In terms of residence, while the majority of both groups reside in personal properties, there is a slightly higher proportion of smokers residing on campus, probably due to frequent casual socialization. Details can be seen in Table 2.

Table 1: Sociodemographic characteristics of the sample

Variable	Condition	Participants (n)	Percent (%)
Gender	Female	300	65.79
	Male	151	33.11
	Other	5	1.10
Study module	Romanian module	396	86.84
	English module	56	12.28
	Missing	4	0.88
Specialty	Civil Medicine	391	85.75
	Military Medicine	65	14.25
Origin	Urban	371	81.36
	Rural	85	18.64
Current residence	Campus	125	27.41
	Personal propriety	331	72.59
Smoker	No	309	67.76
	Yes	142	31.14
	Missing	5	1.10
Smoking before college	No	30	6.58
	Yes	112	24.56
	Missing	314	68.86
Psychotropic treatment	No	431	94.52
	Yes	25	5.48
Psychological treatment	No	406	89.04
	Yes	50	10.97
Total	-	**456**	**100.00**

Table 2: Smoking and some of its sociodemographic correlates

Smoker	Categories	Participants (n)	Percent (%)
No	Civil Medicine	271	87.70
	Military Medicine	38	12.30
	Campus	85	27.51
	Personal propriety	224	72.49
	Total	**309**	100.00
Yes	Civil Medicine	115	80.99
	Military Medicine	27	19.01
	Campus	40	28.17
	Personal propriety	102	71.83
	Total	**142**	100.00

Patterns in smoking: risk situations and alternative coping

Next, we will deal with two opposing factors relevant to smoking: contexts that increase the risk of smoking behaviors and coping strategies that may lower its frequency or diminish its impact. Concerning the types of contexts that trigger smoking the results are presented in Table 3. The data reveal that the majority of smokers reported experiencing academic stress (78.17%) and social smoking (90.14%) as extremely common contexts for smoking. In contrast, emotional conflicts were reported by a smaller, yet still substantial, proportion of participants (66.90%).

Table 3: Types of situations for smoking

Situations	Present	Participants (n)	Percent (%)
Academic stress	Yes	111	78.17
	No	31	21.83
	Total	142	
Social smoking	Yes	128	90.14
	No	14	9.86
	Total	142	
Emotional conflicts	Yes	95	66.90
	No	47	33.10
	Total	142	

The alternative coping to smoking can be seen in Table 4. Not surprisingly, the mean score for smoking within the group engaged in physical activities is lower (1.260) compared to the group not engaged in physical activities, with a mean score of 1.774.

Table 4: Coping Strategies for Smoking

Strategy	Present	Participants (n)	Percent (%)
Mindfulness	Yes	43	30.28
	No	99	69.72
	Total	142	
Relaxation	Yes	89	62.68
	No	53	37.32
	Total	142	
Physical activity	Yes	66	46.48
	No	76	53.52
	Total	142	

Other methods for coping with stress reported verbatim by participants are music therapy, reading, and some negative methods such as alcohol, marijuana, and binge eating. There is also another series of apparently linked factors that are not actually related to smoking behavior. We found no significant correlation of Fagerstrom nicotine dependence results with gender, language module, military vs civilian module, area, living place, previous psychotropic treatment, previous psychotherapy, and starting before entering university (All the statistical analyses results are attached in Supplementary Materials).

There is no significant difference in smoking addiction scores between students who practice mindfulness and those who do not ($p>0.05$). Based on these p-values, there is no convincing evidence to suggest a significant difference in the measured variables between the relaxation and no-relaxation groups.

Self-Esteem, Self-Efficacy, and their socio-demographic correlates

The Self-Esteem assessment showed practically no difference between smokers/non-smokers (30.09 vs 30.98). The prototypical participant with high self-esteem is male, a Romanian module student, a Military Medicine student, from urban areas, living on campus, with no previous psychotropic treatment and no psychotherapy.

Overall, the differences in mean scores between individuals engaged in physical activities and those who are not are relatively small across the variables.

Differences in Self-Esteem and Self-Efficacy between Smokers and Non-Smokers

Significant differences were found between non-smokers and smokers in terms of self-efficacy and self-esteem. Smokers reported higher levels of self-efficacy ($p = .001$, $rpb = -0.198$) and self-esteem ($p = .044$, $rpb = -0.123$) compared to non-smokers. The effect sizes for the significant results were small to medium, suggesting the practical significance of the findings (Table 5).

The evaluation of the effort capacity from 0 initial value has reached 10 minutes without effort disorders so that later on the final evaluation on the best day of the patient will be 15 minutes without effort disorders. We mention that every day the patient's dynamics undergo changes from one hour to the next and the time of day matters a lot.

DISCUSSION

Smoking prevalence

Only one-third of the students responding to our survey were smokers, but rather low addiction scores were reported as confirmed by the Global Adult Tobacco Survey (GATS) 2018 study in a sample of the Romanian general population, remaining unchanged since 2011 [34]. There were no gender differences regarding smoking addiction with most of the smokers living in their own houses. Also, academic stressful situations and intense emotional conflicts were reported as the most frequent situations when students smoke as another study previously reported [35]. This may represent a norm for young adults as addictive behaviors were previously reported as adopted in various stressful situations, especially work and family-related ones [36].

Table 5: Comparison of Self-Efficacy, Rosenberg Self-Esteem, and Fagerstrom Test for Nicotine Dependence Scores Between Non-Smokers and Smokers

Dependent Variable	Non-Smokers			Smokers			U	p	r_{pb}
	N	M	SD	N	M	SD			
Self-Efficacy	271	30.705	4.155	132	32.265	4.608	14350.5	.001	-0.198
Self-Esteem	265	30.098	5.1	134	30.985	5.61	15571.5	.044	-0.123
Nicotine Dependence		-	-	81	1.457	1.406	-	-	-

*Note. M and SD represent the mean and standard deviation, respectively. U represents the Mann-Whitney U test statistic. p represents the p-value for the Mann-Whitney U test. rpb represents the rank biserial correlation, which is the effect size for the Mann-Whitney U test. All p-values are two-tailed. Effect sizes were calculated using the formula rpb = 1 - (2U / (n1 * n2)), where n1 and n2 are the sample sizes for the two groups*

Otherwise, our results may present an optimistic outcome concerning the relatively low degree of addiction even in those starting smoking before entering university and taking into consideration the growth in consuming heat-ed-tobacco products (HTP) as Romania is the second Euro-pean country reporting the use of HTPs [37,38]. This perspective is confirmed by studies including Romania as being the 9th EU member state on smoking prevalence and 10th on pack-year prevalence but persisting on a descendent trend in contrast to other Eastern European countries like Bulgaria or Greece [39,40].

Concerning coping mechanisms, only physical activity had a significant correlation to low smoking addiction scores. Physical activity, among others, was highlighted as an important predictor of health outcomes across life stages, including smoking [41,42].

Self-esteem, self-efficacy, and smoking prevalence

The present study aimed to investigate the relationship between smoking addiction with self-reported self-esteem and self-efficacy among medical students. The results showed that correlations between smoking addiction and self-esteem/ self-efficacy were positive, indicating that higher levels of smoking addiction were associated with higher levels of self-esteem and self-efficacy. However, it is important to note that the correlations were relatively weak and not statistically significant. It is important to retain that only one-third of the students declared smoking with 78.8% starting before entering university. The mean scores on the smoking scale were showing rather low addiction. This may represent a point of interest in discussing why the majority of the students did not develop a higher addiction since they began smoking a few years now. Based on the results of this study and the literature as well, it seems it is necessary to conduct more studies in this area, especially longitudinal and interventional studies to practically determine the effect of self-esteem and self-efficacy improvement programs on smoking prevention or cessation.

Most of the studies rank medical students among the students with low self-esteem and self-efficacy [43,44] this leads to various mental health issues like depression, anxiety, and burnout [45]. Also, most of the studies rank them lower than non-medical students regarding smoking [46,47,48]. Few studies tried to correlate the variables of self-esteem and self-efficacy with smoking addiction levels.

Our study found results that may explain the protective role high self-esteem and self-efficacy hay have in stopping students not to develop higher levels of addiction as the years go by. Also, the finding might be a result of cumulated factors including social interactions, breaks between learning sessions, but also other coping mechanisms that interfere with these results (mindfulness, relaxation, physical therapy, etc.)

With most of the studies focused on smoking cessation and self-esteem and self-efficacy [49-52] having predictor roles for successful abstinence and not advancing in higher stages of addiction, our study stays in line with those findings. Also, happiness was associated with lower scores of smoking in young adults [53] confirming our relatively low addiction scores in our research.

While few studies correlate high self-esteem and self-efficacy with habits like smoking [54] others show negative correlations, especially among young adults progressing in smoking [55-58] with negative social capital being also linked with smoking behavior [59]. In contrast, a study shows that self-esteem may not represent a predictor for smoking [60]. This may be considered important in the light of social, economic, and cultural characteristics of the Romanian population.

Gender differences

Gender differences were observed in the study, with males tending to have slightly higher self-efficacy and self-esteem scores compared to females which is supported by previous research [61]. Also, gender can influence social expectations and perceptions, which may further affect self-esteem and self-efficacy [62]. There was no significant difference in smoking addiction scores between genders. This suggests that gender alone may not be a significant factor influencing smoking addiction.

Other social correlates

The study found that individuals in the military group had higher self-esteem scores compared to the civilian group. This suggests that the context of military service may positively influence self-esteem.

Studies have shown that military medical students may vary in matters of well-being, depression, burnout, or the tendency to leave medical school while trying to adopt more coping strategies [63,64]. Military medical students are a unique population to study in relation to smoking, self-esteem, and self-efficacy, knowing that military service members and veterans have been found to have higher rates of smoking than the general population. Military medical students may face additional stressors related to their military obligations, such as deployments, training exercises, and other demands of military service. These stressors may impact their self-esteem and self-efficacy and may also influence their smoking behavior.

Students living on campus had higher self-esteem scores compared to those who lived alone. The social environment and support provided in the campus setting may contribute to higher self-esteem as highlighted before [65].

Individuals engaged in physical activities had slightly higher self-efficacy scores and lower smoking addiction scores. Physical activity and exercise can positively impact self-esteem and self-efficacy through improved physical well-being and feelings of accomplishment. Students engaged in physical activities had lower smoking addiction scores compared to those who were not engaged in physical activities. This suggests that being physically active may have a positive influence on reducing smoking addiction and improving overall quality of life as previous research showed [66,45,67].

CONCLUSIONS

In our study, self-esteem and self-efficacy were rather high among smokers and non-smokers as well. Our study suggests that there may be other psychological variables worth taking into consideration concerning smoking habits, like personality, cultural background, social attitudes, and mental individual variables. There is a need to investigate the cultural and social aspects of smoking in Eastern European countries like Romania where smoking policy and education still have a lot of space for improvement.

Self-esteem and self-efficacy were reported as high in most of our participants. Medical students are required to have higher intellectual effort and engagement than most of the superior education systems thus there is a need for confidence and self-enhancement to perform academically at the expected standards.

Smoking may not represent at the moment, in their first three years of study, a matter of concern regarding physical or mental aspects but the lifetime perspective needs to be addressed.

So, medical students need to be aware of the negative effects of smoking on their mental and physical health, as well as their academic performance. Quitting smoking and adopting healthy lifestyle habits can lead to improvements in self-esteem and self-efficacy, which can ultimately lead to better academic performance and overall well-being. Further research is needed to explore the specific mechanisms underlying the relationship between smoking and self-esteem/self-efficacy in medical students. It becomes crucial to address this issue through preventive interventions. By promoting smoking cessation and the adoption of healthy lifestyle habits, it is possible to break the cycle and potentially improve self-esteem and self-efficacy among students. These improvements, in turn, can lead to positive outcomes in academic performance and overall well-being.

Smoking is a major public health concern and understanding the factors that contribute to smoking behavior can help inform interventions and policies aimed at reducing smoking prevalence considering Romania already signed the WHO Framework Convention on Tobacco Control (FCTC), when a national campaign was launched as “the First Generation without Tobacco” endorses the WHO ‘tobacco end game’ by 2035 [68,69,70].

Understanding the relationship between smoking, self-esteem, and self-efficacy can help identify potential targets for intervention and provide insights into the psychological processes underlying smoking behavior. Medical students are at a critical point in their education and career development; addressing smoking behavior among medical students can have important implications for their future patients, as well as for the broader medical profession.

Limitations

This study has several limitations. The sample size was rather small, and the participation in the survey was based on self-selection. The sample was asymmetric in terms of gender and origin while the design of the study was cross-sectional, not allowing the assessment of the study variables in their long-term perspective. Also, the sample size only included the first three years of medical students mainly from Bucharest and Muntenia southern region of Romania. This may reduce the generalizability of the findings, taking into consideration cultural, social, and attitude aspects toward smoking.

Future Research

Next, studies on this subject could be run longitudinally and include other potentially relevant variables (e.g., pertaining to personality or other psychopathological variables). Moreover, a distinct line of development could be represented by the creative use of the qualitative methodology among medical students.

The in-depth evaluation of personality traits, mental and physical well-being, and social circle employing, for example, self-enhancement or counseling groups, could not only supplement the data, but also offer a valuable opportunity for students to reflect on the complex etiology of smoking and other addictive behaviors, and assess them in relation to their life and academic goals.

Author Contributions

M.L. and M.S. contributed to the design of the work. M.S. conducted the data collection. M.S. and C.G.I. conducted the data analyses. M.S. contributed to the interpretation of the results. C.G.I., S.P., M.S., and M.L. contributed to drafting the manuscript. M.L. and S.P. supervised the development of the work and helped with manuscript evaluation. M.S., S.P., and C.G.I. helped to evaluate and edit the manuscript.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the „Carol Davila" University of Medicine and Pharmacy — Bucharest, Romania (no. 7872/2021).

Informed Consent Statement

Written informed consent has been obtained from the participants to publish this paper" if applicable.

Data Availability Statement

The data presented in this study are available on reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare that there is no conflict of interest

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ORIGINAL ARTICLE

The Impact of Total Synovectomy on Blood Loss and Knee Function. A Prospective Randomized Study

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Abstract: *Synovial proliferation is a common intraoperative finding during total knee arthroplasty (TKA) and many studies have proposed synovectomy for the reduction of postoperative pain. We included 180 patients that were split into two groups, one which received a total synovectomy (TS) and the other with partial synovectomy (PS). We measured the amount of intra- and post-operative bleeding as well as perceived pain (using the Visual Analogue Scale) and knee function (using the Knee Society Knee Score) at 4 and 12 weeks. The blood loss during the surgical procedure was 367.77 ± 115.71 mL for TS, while the other group recorded 295.55 ± 106.17 mL ($p < 0.05$). Regarding postoperative bleeding, the TS group aspirated 533.77 ± 281.65 mL, which was significantly higher than the PS group (404.44 ± 211.55 mL, $p < 0.05$). No significant differences were recorded between the TS and PS groups regarding pain and knee function at 12 weeks. Total synovectomy demonstrated significantly higher blood loss and lower postoperative hemoglobin levels, even though knee function and pain level did not show improvements. We consider that the decision of performing synovectomy should rely on the clinical indication and, if conditions allow for it, a limited resection should be attempted***.**

Keywords: *synovectomy; total knee arthroplasty; osteoarthritis; therapeutic method; pathophysiology; surgical technique; joint replacement*

INTRODUCTION

Osteoarthritis is common in the elderly population and is the most frequent condition affecting the synovial joints, especially the knee. Restricted range of motion, discomfort, and local pain are the main symptoms deriving from progressive cartilage deterioration of the articular surface of the knee joint [1,2]. A decisive role in cartilage degrading is played by chronic inflammation of the synovial membrane, known as synovitis [3,4]. Furthermore, residual synovial tissue can be associated with postoperative impingement and decreased range of motion [4,5].

Total knee arthroplasty (TKA) is regarded as a radical effective treatment for patients with end-stage knee osteoarthritis [6,7]. This intervention is typically reserved for individuals who experience severe, persistent pain and mobility issues that cannot be adequately addressed through more conservative interventions such as anti-inflammatory medications or physical therapy. In advanced osteoarthritis, patients experience continuous pain that is unresponsive to typical anti-inflammatory medications, and radiographic imaging shows a narrowing of the joint space and the formation of bony growths known as osteophytes [8].

During total knee arthroplasty, surgeons often identify synovial proliferation in patients with osteoarthritis or other inflammatory conditions [3,9-11]. This proliferation of the synovial membrane can contribute to inflammation and cartilage degradation, leading to decreased joint function and increased pain. Surgical synovectomy involves excising the proliferated synovial membrane and was shown to improve joint function and reduce pain in patients with osteoarthritis [3,9,10]. However, while this procedure can be effective in reducing pain and inflammation, it also carries risks and potential complications that need to be carefully evaluated on a case-by-case basis.

Usually, the surgeon opts for the type of synovectomy depending on intraoperative assessment or personal preferences. Therefore, some surgeons perform total synovectomy as a mandatory step in the surgical technique of total knee arthroplasty, while other surgeons rather avoid performing total synovectomy because they consider it a substantial factor in postoperative bleeding [12-14]. Some researchers suggested that total synovectomy influences postoperative blood loss and operative time without being beneficial in terms of postoperative pain and knee function [12,15].

Decreasing postoperative discomfort and pain is essential to achieve a fast and efficient recovery and to improve the patient's living quality [16,17]. The primary objective of this study is to assess the influence of synovectomy performed during total knee arthroplasty for knee osteoarthritis on blood loss, postoperative pain, knee range of motion, and function.

MATERIALS AND METHODS

1. Study design

We conducted a prospective single-center randomized controlled trial between January 2022 and June 2022. A total of 180 patients were included and split into two even groups, one which received a total synovectomy (TS) and another with partial synovectomy during total knee replacement (PS). The randomization was performed by an independent doctor using sealed envelopes. The envelopes were stored in a box. The envelopes were drawn before surgery and contained instructions regarding the surgical technique.

The inclusion criteria were patients with late-stage knee arthritis. We excluded from our study patients with revisions and cases requiring a more significant constraint than a posterior stabilized TKA (constrained or hinged knee). We also excluded patients with acute coronary diseases, neurological diseases, infectious conditions, coagulation disorders, and a history of deep vein thrombosis or pulmonary embolism.

This paper was approved by the "Foisor" Orthopaedics Clinical Hospital Ethics Committee of Bucharest, Romania, approval number 6384/2021. Written informed consent was obtained from all patients included in this study. The procedures were conducted by international ethical standards and the 1964 Helsinki Declaration.

A radiological assessment was performed using plain X-rays, including an anteroposterior, lateral, and full weight-bearing lower limb, to develop proper preoperative digital planning.

2. Surgical Technique

All TKA procedures were performed according to a standard protocol by a single highly experienced orthopedic surgeon in our institution, specialized in TKA. The implant of choice was the Zimmer Biomet NexGen implant system. Preoperatively, 1 g of tranexamic acid was administered to all cases, and an additional 1 g after the procedure. All patients received spinal anesthetic with a femoral nerve block. A tourniquet was installed after three minutes of elevating the lower limb and was inflated up to 320 mm Hg before the incision and deflated after the implantation to perform hemostasis. We used a midvastus approach, an extramedullary tibial guide, an intramedullary femoral guide, and a measured resection technique. Wound closure was performed in a layered fashion and all patients received a deep drain. The drain was clamped for three hours and then released and removed 24 hours after surgery. The rehabilitation protocol was similar for both groups and began on the first day after surgery. Physical therapy was continued either at home or in a specialized clinic. All cases received Heparin for deep vein thrombosis prophylaxis for 30 days.

We recorded preoperative data, which included age, gender, knee range of motion, hemoglobin values, bleeding, and comorbidities. Knee function was measured using the Knee Society Knee Score (KSKS). The amount of bleeding was assessed by measuring the postoperative hemoglobin and bleeding during and after the surgery. Intraoperatively we measured the amount of blood aspiration by taking into account the amount of physiological serum used for lavage and the operative time. After the surgical procedure, we measured the amount of blood aspirated through the drains and hemoglobin. All complications were recorded throughout the study. For the functional evaluation, we measured flexion, extension, the Visual Analogue Scale (VAS), and KSKS score after 4 and 12 weeks. A blind independent observer evaluated all the measurements.

3. Outcome measures

The Visual Analogue Scale is a measure used to evaluate the patient's perspective of pain level. It may be dependent on the patient's mental status but is very frequently used to correlate with knee function evaluation [17,18].

The KSKS contains questions in 2 sections: knee score (pain, range of motion, stability) and function (walking distance, ability to climb stairs, walking aids) [19]. Both measures were provided to patients for answering in the hospital and during follow-up visits.

4. Statistical analysis

All values were calculated as mean values ± standard deviation. The statistical analysis was developed using the Student t-test and Mann-Whitney U test for comparison between groups. A p-value lower than 0.05 was considered statistically significant. The data was analyzed for Windows OS using SPSS version 28.0 (SPSS Inc., Chicago, IL).

RESULTS

We recorded 180 cases split into two even groups. The patient characteristics are displayed in Table 1.

The blood loss measured during the surgical procedure was 367.77 ± 115.71 mL for the total synovectomy, while the second group recorded 295.55 ± 106.17 mL of blood loss ($p < 0.05$). Regarding postoperative bleeding, the TS group aspirated 533.77 ± 281.65 mL, which was significantly higher than the PS group, where we recorded an aspiration of 404.44 ± 211.55 mL (Table 2).

Table 1: Baseline characteristics of the study groups

	Total synovectomy	Partial synovectomy	p-value
Age (years)	70.41±7.25	70.8±7.23	0.71
Gender (M/F)	41/49	44/46	0.77
Preoperative hemoglobin (g/dL)	13.24±1.22	13.22±1.18	0.90

Table 2: Postoperative parameters of the study groups

	Total synovectomy	Partial synovectomy	p-value
Intraoperative bleeding (mL)	367.77±115.71	295.55±106.17	<0.05
Postoperative bleeding (mL)	533.77±281.65	404.44±211.55	<0.05
Postoperative hemoglobin (g/dL)	11.43±1.26	11.88±1.22	<0.05
VAS[1] score (4 weeks)	3.39±1.50	3.79±1.32	0.058
VAS score (12 weeks)	1.3±1.01	1.41±0.89	0.43
KSKS[2] score (4 weeks)	82.28±4.09	82.42±4.46	0.83
KSKS score (12 weeks)	87.9±3.7	88.33±4.11	0.47
Operating Room time (min.)	96.5±7.93	92.2±6.37	<0.05

[1] VAS=Visual Analogue Scale; [2] KSKS=Knee Society Knee Score

The mean preoperative hemoglobin was 13.24 ± 1.22 g/dL for the TS group and 13.22 ± 1.18 g/dL for the PS group ($p = 0.90$). Postoperatively, a statistically significant decrease was recorded in the two groups, the hemoglobin dropping to 11.43 ± 1.26 g/dL and 11.88 ± 1.22 g/dL, respectively, ($p < 0.05$). However, after four weeks, the VAS score was 3.39 ± 1.50 in the TS group and 3.79 ± 1.32 in the PS group, while KSKS was 82.28 ± 4.09 and 82.42 ± 4.46, respectively. Both scores' differences between groups were not significant. At the three months follow-up, both parameters showed improvements for both groups. VAS score was 1.3 ± 1.01 for the TS group, while PS patients had a score of 1.41 ± 0.89. ($p = 0.43$) KSKS score showed no statistically significant differences after three months, the results being 87.9 ± 3.7 and 88.33 ± 4.11, respectively ($p = 0.47$). However, we recorded significant differences regarding the operative room time between groups: 96.5 ± 7.93 minutes for the TS group and 92.2 ± 6.37 minutes for the PS group, respectively ($p < 0.05$).

Regarding complications, we recorded 7 cases of deep vein thrombosis in the total synovectomy group and 5 cases in the partial synovectomy group.

We recorded wound complications in three cases in the first group and two patients in the second group. Also, we noticed hemarthrosis following drain removal in 20 cases that underwent total synovectomy and 5 cases with partial synovectomy**.**

DISCUSSION

The synovial tissue is regarded as a critical component in the locomotor function [20,21]. The synovial tissue of the knee joint plays multiple roles, including the secretion of synovial fluid that lubricates the joint, providing nutrients to the articular cartilage, protection against infection, and removal of waste products from the joint. The aforementioned functions are responsible for the maintenance of the health and proper functioning of the knee joint. On the other hand, progressive joint degeneration, which leads to osteoarthritis, is caused by the involvement of bone, cartilage, and synovial pathological processes [21,22]. For this reason, synovectomy is performed to relieve pain or to attempt the prevention of cartilage and/or bone destruction and to improve knee function [23,24].

During a total knee arthroplasty, total synovectomy may be performed for different reasons. One reason is to remove any inflamed or diseased synovial tissue that may be present in the joint, with the aim of improving joint function and reducing pain and inflammation. Another reason is to prevent the formation of a pseudo capsule, a thick layer of scar tissue that can form around the artificial joint and cause complications. However, total synovectomy may also have drawbacks, such as an increased risk of infection and reduced lubrication and nutrition of the articular cartilage [21,22]. The decision to perform a total synovectomy during a total knee arthroplasty should be taken individually, for each patient, by weighing the potential benefits and risks. Furthermore, proper planning of the procedure is essential to ensure shorter operating times and faster recovery; this includes digital radiological planning, choosing the ideal implant type and model, and controlling the patient risk factors [25-27].

Our study aimed to evaluate the influence of synovectomy on postoperative results and blood loss following total knee replacement. The intraoperative and postoperative blood loss was found to be significantly higher after total synovectomy compared to the partial synovectomy group. We found variable results regarding average blood loss in the literature, which ranged between 300 and 2500 mL [12,28-30]. This variability may be explained by the different surgical techniques, thromboprophylaxis treatments, or implant types that were applied. In our paper, the total synovectomy group had a blood loss from drains of 533.77 ± 281.65 mL for the total synovectomy group compared to the second group, which had 404.44 ± 211.55 mL blood loss. We consider this to be one of the main reasons behind the preference of some surgeons to avoid total synovectomy.

Our study also revealed a significantly lower postoperative hemoglobin level after synovectomy. This result was also recorded in other papers [12,21,31]. However, some studies concluded that even though the bleeding rates are higher, the blood transfusion threshold is not reached when total synovectomy is performed, therefore the two surgical techniques do not yield statistically significant differences in this regard [15,21].

Another significant result of this study was that total synovectomy demonstrated some postoperative improvements but these were not statistically significant compared to the other group and did not show any differences regarding pain relief, despite the considerable blood loss difference. It is well known that the synovium, as well as other articular structures like muscles, joint capsules, or ligaments, is well-innervated and could be a source of pain in osteoarthritis [32,33]. Even though synovectomy reduces knee pain, especially in cases with pathological synovial tissues, the VAS score in our study did not show any significant differences during the follow-ups. Also, the KSKS score showed no significant differences after 4 weeks or after 12 weeks. This result is also reported by other authors who also recorded increased concealed bleeding, higher drainage, and longer operative time in patients that underwent total synovectomy [12]. Moreover, similar studies evaluating the quality of life and complications recorded no significant improvements regarding these parameters [34].

Literature data have indicated that total synovectomy during total knee arthroplasty may be linked to an increased incidence of certain complications such as deep vein thrombosis, hemarthrosis, and wound complications [12,21,33-37]. However, it is essential to note that the available data is currently limited. Therefore, additional investigations with a larger sample size are required to establish a more robust statistical correlation on this subject. Despite this observation, it should be noted that individual patient characteristics and other factors may also contribute to the occurrence of these complications.

One major limitation of our study is that it was conducted in a single center, which may limit the generalization of our results to other study populations. Additionally, we did not evaluate hematocrit levels or blood transfusion requirements, which are important factors that could reveal further associations between total synovectomy and blood loss. Furthermore, we acknowledge that more extended follow-up periods could be beneficial in the thorough evaluation of the functional outcomes and risk of synovial recurrences associated with total synovectomy during total knee arthroplasty. Additionally, it is important to note that our study did not assess the risk of other potential complications that could arise from this procedure. Therefore, while our study provides important insights into the potential risks and benefits of total synovectomy during total knee arthroplasty, additional research with a larger sample size and more extended follow-up periods are needed to fully understand the implications of this procedure for patients undergoing knee arthroplasty.

CONCLUSIONS

Patients with total synovectomy associated with total knee arthroplasty experienced a notably more significant amount of blood loss and lower postoperative hemoglobin levels than the control group. However, no statistically significant differences were observed between the two groups in terms of knee function and pain level. Further multicenter studies with longer follow-up times are required to more comprehensively assess the potential complication rate and risk of synovial recurrence associated with total synovectomy during total knee arthroplasty. Ultimately, more comprehensive research is required to determine the optimal approach to synovectomy and to provide the most beneficial outcomes for patients undergoing total knee arthroplasty.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

M.-A.P. and L.L.P. planned the clinical study and contributed to the concept and design of the study. M.E.G., D.C.C., and A.L. contributed to the data analysis and interpretation as well as to the translation and the critical revision of important intellectual content. C.S. and R.C. contributed to the writing of the manuscript and performed revision, language, and textual editing of the final version. All authors contributed to the original draft preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

Ethics approval and consent to participate

All procedures performed in this paper were by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and according to the national ethical standards. The study received approval from the "Foisor" Orthopaedics Clinical Hospital Ethics Committee of Bucharest, Romania, approval number 6384/2021 (date of approval 10th of December 2021). Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Evaluation of Serum Magnesium Levels in Patients with Hypertension: A Case-control Study

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Abstract: *There are conflicting data on the effects of magnesium on hypertension in the literature. Therefore we aim this study to investigate whether there is a relationship between serum magnesium levels and hypertension in newly diagnosed. This is a case-control study. The patients were selected from the records of patients who applied to the family medicine outpatient clinic between June 2016 and May 2017. A total of 276 patients, 68 patients with newly diagnosed hypertension in the case group and 208 patients without any disease in the control group were included in the study. Binary logistic regression analysis was performed between the case and control groups. The odds ratio for magnesium was 0.1 (0.012-0.810). When the value of the magnesium variable increases by 1mg/dl, the risk of hypertension decreases by 90%. However, in the presence of magnesium as an independent variable, the predictability rate of our model was expected to increase but decreased. The odds ratio for age was 1.14 (1.092-1.186). The odds ratio for exercise was 0.18 (0.03-0.99). The odds ratio for glucose was 1.03 (1.001-1.065). In our study that magnesium deficiency causes an increased risk of hypertension, the predictability rate of our model was expected to increase instead of decrease. These findings show that a cause-effect approach or incidental association cannot explain the relationship between magnesium and hypertension*.

Keywords: *hypertension; magnesium; age; exercise; glucose*

INTRODUCTION

The prevalence of hypertension continues to increase globally [1]. The prevalence of hypertension in the adult population in the world is 31% [2]. For over forty years, the causes of most hypertensive diseases have been unknown and are defined as primary or essential hypertension. Secondary causes are accepted as 5-10% [3].

Recently, risk factors such as age, sex, race, family history, genetic composition, obesity, excessive salt intake, inactivity or lack of exercise, high-fat diet, tobacco use, and alcohol consumption have been proven to lead to the development of hypertension [4].

Magnesium (Mg) can be found in the fourth sequence in the human body and the second after potassium in the intracellular area [5]. Magnesium is an essential cation playing a crucial role in many physiological functions. It is critical in energy-requiring metabolic processes, protein synthesis, membrane integrity, nervous tissue conduction, neuromuscular excitability, muscle contraction, and hormone secretion [6]. Given the role of magnesium in calcium and potassium transport, cell signaling, energy metabolism, genome stability, DNA repair, and replication, it should be expected that hypomagnesemia is associated with many diseases, including neurological disorders, osteoporosis, diabetes, coronary heart disease, and hypertension [7].

Magnesium levels were lower in white hypertensives than in normotensives, while no significant difference was found in black hypertensives compared to normotensives [8]. It has been shown that those with essential hypertension have a significantly higher intra-erythrocytic magnesium level than healthy individuals [9]. A systemic review and meta-analysis study suggested no relationship between serum magnesium and hypertension but that dietary magnesium supplementation reduces blood pressure [10]. Magnesium deficiency was found to be responsible for calcium and magnesium disorders in those with pregnancy-induced hypertension, and magnesium sulfate therapy was effective in these patients [11]. In a meta-analysis of twelve studies, a reduction in diastolic blood pressure was observed, while a decrease in systolic blood pressure was not observed [12]. Again, a meta-analysis of 20 studies found a reduction in systolic blood pressure while no change in diastolic blood pressure [13]. A meta-analysis of 22 studies noted a reduction in both systolic and diastolic blood pressure [14].

The fact that there are conflicting data on the effects of magnesium on hypertension in the literature shows a need for research on this subject. For this purpose, we wanted to evaluate magnesium levels in patients with newly diagnosed hypertension. This study aims to investigate whether there is a relationship between serum magnesium levels and hypertension.

MATERIALS AND METHODS

Study Design

This study is a case-control study. The sample size was determined for logistic regression analysis, considering the number of seven independent variables, such as age, gender, body mass index (BMI), smoking, alcohol consumption, exercise status, and magnesium. For each group, using the power analysis method, the minimum sample size of at least 48 which is needed to detect a significant difference when taken into account at 0.05 type-I error (Alpha), 0.35 effect size, 0,80 power (1-beta).

Population selection

The patients included in the study were selected from those who applied to the family medicine outpatient clinic between June 2016 and May 2017 from the hospital information system and patient records. Sixty-eight people with hypertension constituted the case group. Two hundred-eight people without hypertension formed the control group. The control group was selected considering demographic characteristics such as age and gender.

Inclusion and exclusion criteria

Among the patients tested for magnesium at the time of admission, patients with hypertension newly diagnosed were included in the case group, and those with no chronic disease and no medication were included in the control group. The study did not include those with any illness and drug use before the application, those with a previous hypertension diagnosis and treatment, and those without magnesium checked at the time of application.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (V25). A P-value below 0.05 was considered statistically significant. In the data expression, categorical data, numbers and percentages, and numerical data were expressed with averages and standard deviation. The distribution of demographic data was analyzed by frequency tests, comparison of categorical data by chi-square test, and comparison of numerical data by independent sample t-test. The stepwise enter model was used in the binary logistic regression test to evaluate the effect of magnesium on hypertension. Skewness and kurtosis analyses were used to conform the data to the normal distribution.

Ethical Considerations

Ethical approval was obtained from the İstanbul Training Research Hospital Clinical Research Ethics Committee (Decision no. 14.10.2022, 318).

RESULTS

A total of 276 people, including 68 cases and 208 control groups, were included in the study. There was no found difference between those with hypertension and those without it in terms of sex, smoking, and alcohol consumption. However, the proportion of those without exercise was found to be lower in the hypertension group (Table 1).

Table 1: Comparison of the case and control groups in terms of gender, smoking, alcohol consumption, and exercise status

Characteristics of participants		Hypertension N (%)			
		No	Yes	Total	p
Gender	Women	149 (71.6)	52 (76.5)	201 (72.8)	0.530
	Men	59 (28.4)	16 (23.5)	75 (27.2)	
Smoking	No	167 (80.3)	58 (85.3)	225 (81.5)	0.472
	Yes	41 (19.7)	10 (14.7)	51 (18.5)	
Alcohol	No	204 (98.1)	68 (100.0)	272 (98.6)	0.575
	Yes	4 (1.9)	0 (0.0)	4 (1.4)	
Exercise	No	168 (80.8)	66 (97.1)	234 (84.8)	< 0.001*
	Yes	40 (19.2)	2 (2.9)	42 (15.2)	

Chi-Square Tests: The statistically significant difference was accepted as p < 0.05

The average age and BMI were found to be higher in the group with hypertension. Additionally, glucose, total cholesterol, triglycerides, and LDL- cholesterol (LDL-C) were found to be increased in the case group. Magnesium levels were found to be higher in the control group than in the case group (Table 2).

Table 2: Comparison of case and control groups in terms of age, body mass, and biochemical parameters

	Hypertension			
	No	Yes		
	Mean ± SD		t.	p
Glucose (mg/dl)	95.25 ± 11.04	107.79 ± 15.96	-6.02	< 0.001*
Cholesterol (mg/dl)	206.55 ± 44.32	229.43 ± 35.59	-3.86	< 0.001*
Triglycerides (mg/dl)	111.96 ± 61.58	154.07 ± 60.27	-4.92	< 0.001*
HDL-C (mg/dl)	54.55 ± 12.66	52.68 ± 7.40	1.49	0.137
LDL-C (mg/dl)	129.35 ± 37.07	143.49 ± 29.66	-3.19	0.002*
Calcium (mg/dl)	9.47 ± 0.46	9.54 ± 0.43	-1.11	0.266
Magnesium (mg/dl)	1.99 ± 0.19	1.92 ± 0.15	2.82	0.005*
Sodium (mmol/L)	139.14 ± 1.99	145.24 ± 32.97	-1.52	0.133
TSH (mIU/L)	1.91 ± 1.29	1.90 ± 1.20	0.06	0.950
Vitamin B12 (ng/L)	231.69 ± 127.26	207.32 ± 90.38	1.73	0.085
Vitamin D (ng/ml)	20.08 ± 10.69	19.32 ± 9.60	0.52	0.602
Age (years)	39.69 ± 12.80	59.71 ± 11.65	-11.42	< 0.001*
BMI	25.83 ± 4.48	29.50 ± 6.25	-4.47	< 0.001*

*SD: Standard Deviation; BMI: Body Mass Index; *Independent sample t-test is significant at the 0.05 level (2-tailed)*

Binary logistic regression analysis was performed by considering the different parameters between the case and control groups. In addition to the magnesium, age, exercise, BMI, glucose, triglyceride, and LDL-C variables which were different between the case and control groups, gender and sodium variables were also included in the analysis according to the literature [4]. Total cholesterol was not included in the study because it is affected by LDL-C and triglyceride.

In the analysis in which only magnesium as an independent variable was added, the model is insignificant because the Cox-Snell R2 and Nagelkerke R2 values are smaller than the 0.2 value. However, the model is significant when other independent variables are added. Except for the step in which triglyceride was added, the model's goodness of fit is acceptable since the p-values are bigger than 0.05 in the Hosmer-Lemeshow test. Changes in -2 Log Likelihood values (Chi-square, $p < 0.05$) are significant in magnesium, age, exercise, and glucose steps.

These results show that the logistic regression analysis is generally valid (Table 3).

Table 3: Distribution of data showing the validity of the logistic regression analysis

Metod=Enter: stepwise	-2 Log Likelihood	Chi-square	P	Cox and Snell R Square	Nagelkerke R Square	Hosmer and Lemeshow Test	Predicted percentage
Beginning	308.192						75.4
+ Magnesium	299.999	8.193	0.004*	0.029	0.043	0.150	74.6
+ Age	194.958	105.041	< 0.001*	0.337	0.500	0.493	82.6
+ Exercise	186.608	8.350	0.004*	0.356	0.530	0.642	81.9
+ Gender	186.341	0.267	0.606	0.357	0.531	0.592	81.9
+ BMI	183.822	2.519	0.112	0.363	0.539	0.695	83.3
+ Glucose	179.281	4.541	0.033*	0.373	0.555	0.398	84.1
+ Triglycerides	179.265	0.015	0.901	0.373	0.555	0.044*	83.3
+ LDL-C	178.064	1.201	0.273	0.376	0.559	0.744	82.6
+ Sodium	178.059	0.005	0.944	0.376	0.559	0.746	82.6

**The statistically significant difference was accepted as $p < 0.05$*

The odds ratio for magnesium was found to be 0.1 (0.012-0.810). When the value of the magnesium variable increases by 1mg/dl, the risk of hypertension decreases by 90% (Table 4). However, in the presence of magnesium as an independent variable, the predictability rate of our model was expected to increase but decreased (Table 3). The odds ratio for age was found to be 1.14 (1.092-1.186). When the value of the age variable increases by one unit, the risk of hypertension increases by 1.14 times. The odds ratio for exercise was found to be 0.18 (0.03-0.99). When the reference group is taken as those with non-exercise, the risk of hypertension was found to be 82% less in those with exercise. The odds ratio for glucose was found to be 1.03 (1.001-1.065). When the value of the glucose variable increases by 1 mg/dl, the risk of hypertension increases by 1.03 times (Table 4).between vitamin D and total cholesterol and LDL-C in the young age group. However, serum vitamin D had no significant correlation with serum glucose, triglyceride, HDL-C, calcium, magnesium, sodium, TSH levels, and BMI scores (Table 4).

Table 4: Logistic regression analysis showing the relationship between hypertension and variables of magnesium, age, exercise, gender, BMI, glucose, triglyceride, LDL-C, and sodium

Hypertension[a]	B	SE	Wald	P	Risk (Odds) coefficient	95% C.I.for (Exp B)	
						Lower	Upper
Magnesium (mg/dl)	-2.325	1.079	4.645	0.031*	0.1	0.012	0.810
Age (years)	0.129	0.021	37.532	< 0.001*	1.14	1.092	1.186
Exercise (1: -/+)	-1.723	0.874	3.882	0.049*	0.18	0.032	0.991
Gender (1: Women/Men)	-0.628	0.456	1.898	0.168	0.53	0.219	1.304
BMI	0.031	0.040	0.621	0.431	1.03	0.955	1.115
Glucose (mg/dl)	0.032	0.016	4.140	0.042*	1.03	1.001	1.065
Triglycerides (mg/dl)	0.001	0.003	0.019	0.890	1.00	0.994	1.007
LDL-C (mg/dl)	-0.006	0.006	1.172	0.279	0.99	0.983	1.005
Sodium (mmol/L)	0.002	0.034	1.897	0.947	1.00	0.938	1.071

[a] *Dependent Variable: Hypertension; BMI: Body Mass Index; B: Estimated Coefficients; CI: Confidence Interval; SE: Standard Error; *Logistic regression analysis is significant at the p < 0.05 level (2-tailed)*

DISCUSSION

The fact that the age and BMI values, glucose, total cholesterol, triglycerides, and LDL-C levels are high in those with hypertension and the low rate of exercisers shows that the study groups are compatible with the general population.

In our study, magnesium levels were lower in those with hypertension. When the value of the magnesium variable increases by 1mg/dl, the risk of hypertension decreases by 90%. However, in the presence of magnesium as an independent variable, the predictability rate of our model for hypertensive patients decreased, while it was expected to increase [2]. In two meta-analysis studies involving seven and 11 studies, it has been suggested that magnesium supplementation provides a significant, albeit small, decrease in blood pressure [15,16]. In a meta-analysis in which thirty-four studies were evaluated, it was found that there was a decrease in both systolic and diastolic blood pressure [17]. A recent meta-analysis study claimed that oral magnesium supplementation does not affect blood pressure in normotensives and controlled hypertensives but reduces blood pressure in uncontrolled hypertensives [18]. In addition, there was no difference in magnesium levels between those with hypertensive crisis and the control group [19]. However, in light of these contradictory findings, considering that the number of participants in meta-analysis studies is high, it is a point that should not be overlooked that statistical significance will be high, even if there is no significance in reality.

In our study, it was found that when the value of the age variable increased by one unit, the risk of hypertension increased by 1.14 times. In our working model, the probability of predicting hypertensive patients in the presence of magnesium and age is relatively high. This result indicates that the age variable is an important risk factor. In the literature, age is recognized as a risk factor for hypertension [4]. Studies have been reported showing that magnesium decreases with age [20,21]. It has been suggested that this relationship may be related to decreased absorption with age and slower kidney reabsorption [22,23]. In addition, recent reports indicate that many Americans, especially older people, do not consume the recommended daily amount of magnesium [24]. In countries with high incomes, the rate of hypertension is lower than in poor countries [2]. Again, it is stated that an unhealthy diet causes hypertension [25]. All these findings and literature information suggest that the relationship between magnesium and hypertension may be an incidental association.

Our study found that the risk of hypertension decreased by 82% in those who exercised. In the literature, it is accepted that inactivity or lack of exercise is a risk factor for hypertension [4]. It is stated that regular exercise causes a decrease in blood pressure of 5 mmHg [26]. In addition, emphasis has been placed on the curative effect of both aerobic and moderate-weight exercise on hypertension [27]. Aerobic exercise is assumed to positively affect hypertension by reducing oxidative stress [28]. A recent meta-analysis indicates that aerobic exercise is an effective adjunctive treatment method for lowering ambulatory blood pressure in hypertension patients receiving drug therapy [29].

Our study found no relationship between body mass index and hypertension. However, when the value of the glucose variable increased by one unit, the risk of hypertension increased 1.03 times, albeit very slightly. Obesity is accepted as a risk factor for hypertension in the literature [4,30]. Mechanisms of obesity causing hypertension are explained by the activation of the sympathetic nervous system activated by hormones such as leptin, insulin, melanocortin, and the activation of the renin-angiotensinogen system due to oxidative effects caused by proinflammatory cytokines released as a result of dyslipidemia [31]. Many pathophysiological mechanisms explain the relationship between diabetes and hypertension. These mechanisms are mainly hyperinsulinemia-mediated sympathetic activation, hyperglycemia-induced sodium-fluid retention, the stimulatory effect of hyperglycemia on the renin-angiotensin-aldosterone system, and increased insulin resistance as a result of oxidative stress [32,33].

An increased risk of type-2 diabetes and increased LDL-C levels were found in low magnesium [34]. It has been suggested that the positive effects of magnesium on diabetes occur by inhibiting oxidative stress [35]. It is also stated that the primary pathology in gestational hypertension in which magnesium sulfate is used in the treatment is oxidative stress [36]. The positive effects of regular and aerobic exercise on oxidative stress and its curative effects on diabetes and obesity have been emphasized [37]. Both magnesium deficiency and oxidative stress have been identified as pathogenic factors in aging and age-related diseases. Additionally, severe magnesium deficiency has been shown to increase oxidative stress in experimental animals [38]. In light of these findings, it seems complicated to define the relationship of magnesium with hypertension without fully elucidating the complex effects of oxidative stress on hypertension.

CONCLUSIONS

As a result, it was found in our study that magnesium deficiency causes an increased risk of hypertension. However, in the presence of magnesium as an independent variable, the predictability rate of our model was expected to increase but decreased. These findings show that the relationship between magnesium and hypertension cannot be explained only by a cause-effect connection or incidental association. This may explain the reason for the conflicting results in the literature. More detailed analyzes are needed in this regard.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. This research received no external funding.

Authors' contribution

All authors made substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of data for the work. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Because of the retrospective study, patient consent was not obtained.

Ethics approval and consent to participate

Ethical approval was obtained from the İstanbul Training Research Hospital Clinical Research Ethics Committee (Decision no. 14.10.2022, 318).

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REVIEW

Combat Stress in Military Personnel: Theory, Genesis, Prevention, and Control

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Abstract: *On February 24, 2022, the Russian-Ukrainian war began, in which hundreds of thousands of military personnel are participating. Almost all military personnel experience combat stress. In our opinion, the most fully reflecting the occurrence of stress in humans, including combat stress in military personnel, is the conservation of resource theory proposed by S. Hobfoll. According to this theory, stress occurs when: central or key resources (health, well-being, family, self-esteem, and a sense of purpose and meaning in life) are threatened with loss, are lost, or cannot be retrieved following significant effort. Combat stress in military personnel can manifest itself in the form of negative manifestations of the psychological, physical, psychophysiological, and behavioral register. The most effective system for the prevention and control of combat stress among military personnel was developed in the US Army. Such a program should contain medical and psychological work activities carried out in three stages: preparatory (before performing combat missions), the stage of direct performance of tasks in the combat zone, and the final stage (after completing tasks upon returning to permanent deployment points).*

Keywords: *stress, combat stress, military personnel, fighting, COSC*

INTRODUCTION

There are few areas of contemporary psychology that receive more attention than stress [1,2]. This literature reflects researchers' belief that stress is a major factor affecting people's lives, is intimately tied to mental health, and is very possibly linked with many problems of physical health [1]. The interest in stress has also caught the attention of the popular press, illustrating that stress is of concern to the lay public as well as the academic community [3]. However, despite the breadth of interest in the problem of stress, there is little research to substantiate the relevant theories [2]. But in psychology, as in other sciences, it is difficult to create a sincere body of knowledge without a clear theoretical foundation, because there are no definite limits to the theory that can be denied [4].

An analysis of scientific sources on the subject of combat stress has shown that a lot of applied research has been carried out to solve this rather complex problem. This is because the last fifty years have been characterized by a significant increase in local and regional armed conflicts in the world [5-7]. The localization, time, nature, goals, the pace of hostilities, and military operations varied greatly during this period. But, as a rule, the majority of military personnel in their military career took part only in a series of combat deployments [8].

However, in the 21st century, not a single country, except Ukraine, has faced the armed aggression of the Russian Federation, and a large-scale war has been going on for more than a year. Hundreds of thousands of troops from both sides are taking part in the fighting. Almost all Ukrainian servicemen experience combat stress [9-11]. These circumstances, as well as the duration and complexity of staying in extreme (combat) conditions, put forward high demands on the mental health, army values and motivation of military personnel, as well as on their psychological qualities and the adequacy of behavior for this activity [12-18]. Despite the high motivation to conduct combat operations, the improvement of military professional training and psychological preparation, some military personnel have problems with mental health, manifested by the development of acute stress reactions, post-traumatic stress disorders (PTSD), as well as addictive and delinquent behavior, an increase in suicidal manifestations [19-25].

Similar mental, physical, and behavioral health problems have occurred in US and coalition forces during and after combat deployments in Iraq and Afghanistan [5-7,26-30]. In combatants, these disorders manifested in persistent sleep disturbances, headaches, fatigue, and other symptoms associated with the influence of various stress factors in the combat environment, as well as the occurrence of more complex and severe disorders such as PTSD, traumatic brain injury and depression [31-33].

The conducted studies revealed that the significance of the influence on the psyche of military personnel and the development of the consequences of experiencing combat stress were determined by: factors of the combat situation; individual psychological characteristics of a person; the level of professional and psychological preparation for the conduct of hostilities; the nature and level of motivation of military personnel; the influence of combat stress factors on the further life of military personnel, etc. [9,20,28,34,35].

The objective of this study

The purpose of the article was to systematize scientific research on the problem of stress, combat stress, to study the modern system of mental health care for military personnel of the leading NATO partner countries, and to determine the main directions for improving the Ukrainian system for the prevention and control of combat stress among military personnel.

BASIC STRESS THEORIES

All theories of stress, in particular, combat stress, are based mainly on medical, biological, and psychological studies that reflect the processes of a distinct reaction of the human body to stress. In general, the term “stress” was borrowed by researchers from physics: it was previously believed that people are in some sense similar to physical objects, such as metals, resisting moderate external forces, but losing their stability at some point of higher pressure [1]. Walter Cannon was probably the first researcher to apply the concept of “stress” to humans in these kinds of terms [36]. He concluded that a person can resist initial stressors of moderate strength or lower-level negative factors that cause stress. However, prolonged or more severe stressors will lead to disruption of the body’s biological systems [36].

W. Cannon’s research on stress was continued and developed by Hans Selye. He described stress as a reaction (organized defense) of the body to non-specific harmful agents and called it the “general adaptation syndrome” [37]. According to the author’s definition, the general adaptation syndrome is the body’s response to severe damage by non-specific harmful agents (for example, exposure to cold, surgical shock, excessive physical exertion, or intoxication with sublethal doses of various drugs) and it does not depend on the nature of the damaging agent [37]. Thus, the protective role of the general adaptation syndrome consisted in the formation of nonspecific resistance of the organism to pathogenic agents, regardless of their nature. The researcher believed that there is a response of the body to external factors, which go through three stages in succession: anxiety (anxious response), resistance, and exhaustion [38].

Another direction in the study of the mechanisms of development of stress has already taken into account not only responses to stressors but also directly stressful events, as well as individual differences in their assessment. Spielberger suggested that certain events are stressful if they are thought to be threats to the physical self or the phenomenological self [39]. He proved that although individuals with different personalities responded some-what uniformly to physical threats, people’s responses to ego-threats were related to personality traits. In particular, Spielberger noted that people with high trait anxiety tended to react with state elevations in anxiety to ego-threat, whereas those who were low in trait anxiety tended to be comparatively impervious to ego-threats. Thus, the researcher concluded that it is not the stimulus and not the assessment that is important, but their specific interaction. This three-step approach was a conceptual leap for stress researchers but rarely followed up in further research. Instead, many researchers have reverted to models that only assessed stress, almost completely ignoring actual environmental or personality traits; others reflected only personality characteristics, while others remained tied only to the model’s stimuli.

In modern studies of the problem of stress, the homeostatic and transactional models of stress are most often used. The homeostatic model is presented in detail by McGrath [40] but is based in large part on the work of Lazarus [41]. According to research results, McGrath proposed the definition of stress as “a significant imbalance between the needs of the environment and the ability to respond to the body” [40, p. 17].

In 1966, the concept of cognitive appraisal was proposed by Richard Lazarus [41]. According to this theory, stress is perceived as the imbalance between the demands placed on the individual and the individual's resources to cope [2]. The transactional model of stress and coping contended that a person's capacity to cope and adjust to challenges and problems is a consequence of transactions (or interactions) that occur between a person and their environment. The authors defined stress as "a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being" [2, p. 19].

However, according to Hobfoll, these models and their definitions have a number of shortcomings [1]. First, the author notes, these definitions state that stress is not the product of an imbalance between objective demands and the ability to respond, but is the result of the perception of these factors. Secondly, the consequences of failure should be perceived as very important for a person. Third, McGrath suggested that imbalances might manifest as underutilization rather than overload, i.e. too little need for the ability to cope with stress [1].

In our opinion, one of the leading modern paradigms of the problem of stress, which is used by both scientists and practical psychologists in their work, is the conservation of resource theory proposed by Stephen Hobfoll. According to this theory, stress arises from motivation that encourages people to both maintain their current psychological resources and seek new opportunities [1]. Its main principle is that individuals strive to obtain, retain, foster, and protect those things they centrally value. The main postulates of this theory are understanding that stress occurs when: (a) central or key resources are threatened with loss, (b) central or key resources are lost, or (c) there is a failure to gain central or key resources following significant effort [1].

Essentially, the conservation of resource theory is a motivational theory that explains much of human behavior in terms of the evolutionary need to obtain and maintain resources for survival, which is central to the genesis of human behavior [42]. Resources are defined as those objects, personal characteristics, conditions, or energies that are valued by the individual or that serve as a means for the attainment of these objects, personal characteristics, conditions, or energies. According to the authors, people must acquire and maintain both strong personal characteristics and social ties. Thus, people must use key resources not only to respond to stress but also to create a certain amount of supportive resources in case of future needs. Moreover, the acquisition and maintenance of personal, social, and material resources create in people, families, and organizations the feeling that they are able to cope with stressful situations.

According to Hobfoll, a critical tenet conservation of resource theory is that individual value is secondary to what is most valued and universal to people. Typically, these valuable resources are health, well-being, family, self-esteem, and a sense of purpose and meaning in life [42]. How these evaluations are expressed may vary across cultural backgrounds but always reflects the same basic elements. The conservation of resource theory, in general, explains the mechanisms of development and transformation of the psychological safety of personality in military personnel during activities under different conditions [43].

DEFINITION OF COMBAT STRESS

An analysis of scientific research has shown that there is no generally accepted definition of the "combat stress" construct: depending on the field of its study (medicine, biology, psychology, sociology, other social sciences), there are many definitions of this category. Specialists who study stress in military personnel during combat operations suggest using such constructs as combat mental trauma, combat stress, combat mental pathology, post-traumatic stress disorders, and others definitions.

The official website of the US Department of Defense provides the definition of combat stress, also known as battle fatigue, which is a common response to the mental and emotional strain that can result from dangerous and traumatic experiences. It is a natural reaction to the wear and tear of the body and mind after extended and demanding operations [44]. Combat stress reaction (CSR) is a term used within the military to describe acute behavioral disorganization seen by personnel as a direct result of the trauma of war. CSR is an acute reaction that includes a range of behaviors resulting from the stress of battle that decrease the combatant's fighting efficiency. The most common symptoms are fatigue, slower reaction times, indecision, disconnection from one's surroundings, and the inability to prioritize. The combat stress reaction is generally short-term. The US Army uses the term/acronym COSR (Combat Stress Reaction) in official medical reports. This term can be applied to any stress reaction in the military unit environment. Many reactions look like symptoms of mental illness (such as panic, extreme anxiety, depression, and hallucinations), but they are only transient reactions to the traumatic stress of combat and the cumulative stresses of military operations [45].

Blinov defines combat stress as a reaction of the psyche to a threat to life and health in the form of a process of experiencing mental states at the stages of adaptation using the resource potential [9].

Snedkov makes a distinction between combat stress, combat psychic trauma, and combat psychic pathology [46]. Under combat stress, the author understands “a multilevel process of adaptive activity of the human body in a combat situation, accompanied by a tension in the mechanisms of reactive self-regulation and the consolidation of specific adaptive psychophysiological changes. Combat mental trauma is a pathological condition of the central nervous system, which is formed as a result of combat stress that exceeds the adaptive potential of a particular individual and forms the 'patos' of combat mental pathology. Combat mental pathology is a syndromic and nosologically structured clinical manifestation of combat mental trauma, the occurrence of which is due to the failure of compensation and the generalization of the pathogenetic mechanism” [46, pp. 28-29].

The authors of this article define the term “combat stress” as the process of the influence of combat situation factors on a soldier, accompanied by a decrease in the level of psychological safety of personality and the appearance of non-specific preclinical psychological manifestations, which together are presented by the concept of “combat psychological trauma of personality” or reach specific nosological symptoms of mental disorders, united in the concept of “combat mental trauma”. Combat stress in military personnel can manifest itself in the form of negative manifestations of the psychological, physical, psychophysiological, and behavioral register.

According to American mental health experts, combat and operational stress include all types of psychological and emotional stress that arise as a result of the dangers and requirements of performing tasks in warfare and other military operations [47]. The development of combat stress in military personnel, according to the authors, is directly influenced by: the intensity and duration of a traumatic event or situation; the level of psychological resilience and endurance of the individual; self-efficacy of a person (the ability to take certain actions to achieve a specific goal); collective effectiveness (general belief in the collective power to achieve the desired results); neurobiological factors (heredity, differences in the activity of the sympathetic nervous system, features of cortisol release in response to psychological stress, serotonin metabolism, etc.).

The effects of combat stress are mediated by a complex interaction between changing factors specific to each person and situation, as well as the dynamic interaction between them. Thus, managed moderately mild stressors can have a “hardening” or stress-modifying effect, when the body becomes less reactive to future stressors. Researchers have found that attention and arousal are essential for survival [30,48]. However, when they go beyond the maximum range, this is fraught with detrimental biological consequences: excessive and prolonged nervous excitement after an injury can increase the likelihood of developing PTSD [49].

EFFECTS OF COMBAT STRESS ON MILITARY PERSONNEL

American researchers have proposed a more detailed classification of stressors associated with external and internal factors [47]. They determined that combat-related external stressors included: self-injury; the killing of combatants; monitoring the death of people; death of colleagues; injury resulting in the loss of a limb. External stressors associated with military operations include prolonged exposure to extreme environmental conditions; reduced quality of life and communication resources for a long time; prolonged separation from important support systems (e.g., family); overseeing significant casualties across many missions (for example, overseeing the deaths of several unit comrades during combat operations). External physical stressors include exogenous (heat, cold, moisture, dust; vibration, noise, explosions); harmful odors (smoke, poison, chemical compounds); ionizing radiation; infected environment; physical work; poor visibility (bright light, darkness); difficult terrain; being at height. Physiological stressors that can cause combat stress include sleep deprivation; dehydration; malnutrition; poor hygiene; muscle and hypoxic fatigue; illness or injury; sexual frustration; the use of psychoactive substances (caffeine, alcohol, drugs); obesity; poor physical condition [47].

Mental (internal) stressors include cognitive; requirements or expectations, difficult decisions (combat rules); organizational dynamics and change; difficult choices or lack of it; work that goes beyond their own competence; preliminary failures. Emotional (internal) stressors are new to the unit; isolation, loneliness; fear and threats that cause anxiety (death, injury, failure, or loss); losses that cause grief (mourning); indignation, aggression, and frustrations, causing anger and a feeling of sadness; conflict (ambiguous) motives; inactivity that causes a feeling of sadness; religious confrontation or temptation to lose faith; interpersonal conflicts (in a unit or with comrades); family problems, nostalgia for home; victimization, sexual claims; observation of the battle, dead bodies; murder [47].

During deployment, military personnel is influenced by combat stressors (seeing dead human bodies, being attacked or ambushed, knowing someone who was injured or killed, and handling dead bodies) as well as operational stressors: long deployments, uncertain redeployment date, separation from families and lack of privacy [49,50,51]. Potentially morally compromising war circumstances, as noted by Wortmann et al., could involve prescribed roles (e.g. maiming and killing), proscribed behaviors (e.g. the use of excessive force or cruelty), bearing witness to the aftermath of violence or terror (e.g. handling or uncovering human remains) or being a victim of others' failures to uphold a moral code [52]. As a consequence, many service members suffer moral injury, characterized by guilt, shame, anger, social withdrawal, feelings of worthlessness, self-blame, and spiritual distress [33,52].

Effects of combat stress can also cause psychological trauma in military personnel, which can manifest itself both during the performance of combat missions and in the long-term period after their completion [7,30]. However, according to Born and Zamorski, deployment-related factors and exposure to potentially traumatic deployment experiences have the strongest and most consistent association with post-deployment mental health problems [53]. They may have short-term transient signs of the pre-nosological registry or more complex and severe somatic, psychiatric disorders, which should be well documented [27].

Recovery after exposure to combat stress can be facilitated by: a person's desire to open up and social support; the feeling that the social environment will accept the person's reactions with understanding and will encourage his openness; perception of the veteran as a hero or victim, but not as a victim; a sense of connection with God, a higher power, or a philosophical teaching; trauma-focused treatment to rethink negative reactions, clear intrusive memories, and help distinguish past from present threats [47].

Post-traumatic social support and relatively rare post-traumatic negative events may also serve as protective factors that will aid recovery from trauma [54].

COMBAT AND OPERATIONAL STRESS CONTROL (COSC) PROGRAMS IN MILITARY PERSONNEL

In our opinion, programs for the prevention and control of combat stress among military personnel are more effectively implemented in the Armed Forces of the United States, Great Britain, and Israel; other countries are developing similar activities based on these programs.

Trauma Risk Management (TRiM) is a peer-support program that aims to promote help-seeking military personnel in the aftermath of traumatic events, developed by the UK Royal Navy [55]. This program reflects a peer-led, occupational mental health support process that aims to identify and assist U.K. military personnel with persistent mental ill health related to potentially traumatic events [56]. TRiM seeks to modify attitudes about PTSD, stress, and help-seeking and trains military personnel to identify at-risk individuals and refer them for early intervention.

The use of TRiM may assist in increasing the psychological resilience of military personnel through the facilitation of social support; this may have particular utility during operational deployments [57].

The Israel Defense Forces developed YaHaLOM training to teach service members how to manage ASRs in team members [58] and then adapted into ICOVER by the U.S. Army. YaHaLOM is a novel, rapid, peer-based intervention specifically designed for use amid a high-stress event.

The analysis of scientific sources made it possible to determine that the most effective system for the prevention and control of combat stress among military personnel exists in the US Army. Let's consider this system in more detail. There is a specific terminological apparatus for defining terms related to combat stress that is used by mental health professionals in the US military. They are set out in various legal documents that regulate the professional activities of these specialists: COSC programs are set out in Field Manual 4-02.51 "Combat and Operational Stress Control" and 6-22.5 "Combat and Operational Stress Control Manual for Leaders and Soldiers" [45,59].

Thus, the control of combat and operational stress in the US Army is defined as the developed programs and activities of military leaders aimed at preventing, identifying, and managing dangerous behavior and combat stress reactions that may occur in military personnel [59]. The objectives of the COSC program are optimization of task performance; maintaining combat power; preventing the harmful effects of combat stress reactions on the physical, psychological, intellectual, and social health of military personnel or minimizing these effects; prompt return of soldiers to duty [60].

Also, one of the main tasks of the COSC is to increase unit cohesion and combat capability in a highly stressful military environment and to maximize post-traumatic growth [48]. COSC includes such activities as in-depth medical examination (screening) and psychodiagnostic of recruits; long-term observation of the passage of service, especially before, during, and after combat deployment; ongoing monitoring and consultation of mental health personnel from the US home base to the combat deployment area [61].

In the past, when US military personnel participated in operations in Korea and Vietnam, the consequences of the negative impact of combat stress led to almost half of the psychogenic losses on the battlefield, depending on the complexity of the conditions [47]. Although the level of acute (combat) stress reactions remained high in the wars of the 21st century, losses were significantly reduced. This was due to the introduction of COSC programs into the functioning of military units. According to American mental health professionals, in modern wars, military leaders can expect 95% of military personnel with ASRs should return to combat missions [47]. COSC is effective when it comes not only to military personnel but also to their training and support systems, including meaningful interpersonal relationships, families, and external resources. COSC aims to identify members of the armed forces and their families, as well as US Army civilians who may need assistance due to challenges associated with participating in combat deployments, and to ensure that they are better prepared, stable, and reliable at all stages accomplishment of combat missions. If we consider the personal level of a soldier, then the goal of COSC is to improve the readiness of each soldier and the unit as a whole to perform a combat mission. This can be achieved by: improving adaptive stress responses and preventing unproductive coping strategies; helping military personnel control combat stress responses; helping soldiers with behavioral problems [6].

Overall, the US Army's COSC is one of 10 medical systems active during pre-combat, on the battlefield, and at the end of a mission. Five professional disciplines in mental health and two additional ones are directed to the implementation of COSC. Professional disciplines include social work, clinical psychology, psychiatry, occupational therapy, and psychiatric nursing. Additional disciplines include mental health and vocational rehabilitation.

An analysis of the COSC programs of different armies of NATO partner countries in the field of international security assistance showed that they are similar in terms of organizational principles, main goals, and tasks. However, these programs differ structurally from the US Army COSC and have their own characteristics depending on the conditions of the missions in which the military personnel take part and the performance of combat missions. Such a comparison of COSC programs was made during a mission in Afghanistan among five NATO partner countries: the armed forces of Australia, Canada, the UK, the Netherlands, and the USA [8]. This allowed the authors to identify and compare organizational structures and key practices in providing mental health care and support to military personnel, identify issues, and illustrate new developments in this area.

The main comparative themes of the COSC programs were: education, prevention, early detection, intervention (care), and follow-up (support) in the field of mental health. The main method of organizing support for the mental health of servicemen was training (psycho-prophylaxis) and monitoring their mental state. The educational goal was to enhance the mental stability of individual military personnel while providing a range of mental health services. All mental health officials from these five countries have waived the need for a standard psychological debriefing after critical incidents (military deaths and large numbers of wounded). Instead, these specialists monitored the mental state of military personnel and their commanders during mandatory post-combat checkups and interviews [35].

To organize psychological support for military personnel, specialized mental health units were deployed near the line of combat, the main purpose of which was the early identification, assistance, and early return to normal activities of such military personnel in the units [62]. All countries have deployed mental health support teams made up of a variety of professionals, including psychiatrists, psychologists, social workers, nurses, and priests [63]. There was no consensus on the distribution of mental health disciplines in the war zone.

All countries, except the United States, organized psychological decompression for personnel in temporary deployment elsewhere (outside the combat zone, but in areas of temporary deployment of units) within a few days after the completion of combat deployment. Its main goal was to recognize faces with signs of combat stress reactions, to provide them with assistance, psychological support, and preparation for sending them to permanent deployment points (home). The US Army conducted intra-garrison decompression or reintegration training directly in the US [8]. But psychological decompression had a fairly comparable infrastructure in the field of psychiatric and psychological care.

Common concerns across countries included perceived stigma (unwillingness or fear of military personnel to seek help from mental health professionals), barriers to mental health care, and the need to increase awareness and acceptance of mental health issues among military personnel [64].

In addition to psychological support, modern preventive psychiatric treatment was included in all COSC programs, including a positive approach to strengthening mental stability. There has also been an emphasis on building self-regulatory skills, empowering military personnel, and several other innovations well integrated into the military context [63]. An important innovation in the operational mental health support of military personnel was the recognition of the role of social leadership and the need to increase support from colleagues.

However, not all researchers are positive about the introduction of COSC programs to prevent the development of mental disorders in military personnel that occur during and after combat deployment. Maglione et al. believe that although COSC interventions may play a valuable role in decreasing stress, decreasing absenteeism, and enabling a return to duty, a systematic review of studies that included a control/comparison group found little evidence that COSC is effective in preventing PTSD or decreasing PTSD symptom scores in military personnel [65].

CONCLUSIONS

One of the most important directions in the development of military psychology is the study of the problem of stress. The conservation of resource theory by S. Hobfoll, in our opinion, most fully and reasonably explains the genesis of combat stress that occurs in military personnel during activities in extreme conditions. The main postulate of this theory is that stress occurs when a person's central or key resources (health, well-being, family, self-esteem, and a sense of purpose and meaning in life) are threatened with loss, lost, or there is a failure to gain key resources following significant effort. At its core, the conservation of resource theory is a motivational theory that explains much of human behavior in terms of the evolutionary need to obtain and conserve resources in order to survive.

It was revealed that during the fighting, almost all servicemen experience combat stress. Combat stress is the process of the influence of combat situation factors on a soldier, accompanied by a decrease in the level of psychological safety of personality and the appearance of non-specific preclinical psychological manifestations, which together are presented by the concept of "combat psychological trauma of personality" or reach specific nosological symptoms of mental disorders, united in the concept of "combat mental trauma".

To prevent the occurrence and development of combat stress, it is necessary to develop and implement a system for the prevention and control of combat stress in military personnel. Such a program should contain psychological work activities carried out at three stages: preparatory (before performing combat missions), the stage of direct performance of tasks in the combat zone, and the final stage (after completing tasks upon returning to permanent deployment points).

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Authors' contribution

Conceptualization and project management, I.P.; research, YaM., O.K and S.V.; methodology, V.V.; formal analysis, N.V.; software, L.B.; writing – original draft preparation, V.K. and S.M. All authors read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

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ORIGINAL ARTICLE

A Survey on Mental Health Status in West of Iran: A Spatial Analysis

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Abstract: *Mental disorders clearly are among the major challenges of global health causing many concerns for health systems, health experts, and health policy makers. The present study was conducted to evaluate the mental health program in comprehensive health centers and health houses under the coverage of the Ilam University of Medical Sciences in the West of Iran. The present study was conducted based on the reports obtained from the mental health program in 2019. The data were recorded into registration and evaluation forms of mental disorders with the codes 102-19-03 and 102-19-01 and then entered into the Excel software. The prevalence per 100 and 100,000 people, as well as their 95% confidence intervals, were calculated using Formula 1 ROUND (Number of cases*100000/population) and Formula 2 ROUND (Prevalence ±1.96*SQRT (Prevalence *(1-Prevalence)/population). The results showed that the prevalence of depression was 19,100 [95% CI: 18999-19201] 19100), and the prevalence of anxiety was 304,66 [95% CI: 20348-30584] per 100,000 population. The highest prevalence among mental disorders was related to depression and anxiety. Considering the high average score of mental health status in people over 65 years old, it is necessary to implement measures and interventions to improve these individuals' mental conditions and upgrade their mental health.*

Keywords: *Spatial analysis; Ilam province; Mental health; Iran*

INTRODUCTION

According to the definition provided by the World Health Organization (WHO), mental health is "a health status that turns one's potential talents into reality, enabling people to cope with routine life tensions, accomplish valuable work, and upgrade their social participation" [1]. Mental health is an important component for evaluating the health status of a population and can be evaluated using various indicators [2]. In other words, the concept of mental health falls under the general concept of health and refers to all the methods and measures used to prevent people from developing mental disorders, as well as for the treatment and rehabilitation of affected individuals. Psychologists believe that mental health can be affected by individual characteristics, mental and environmental burdens, economic, social, and familial status, failure to achieve life goals, and lack of motivation [3]. Industrialization of countries, the growth of urbanization, migration to cities, etc. are phenomena that are associated with numerous troubles, including psychological and social problems. For this reason, mental health and mental disorders are considered top priorities in today's world. The high prevalence of mental disorders and their associated morbidities render them an immediate health priority [4].

Excessive attention to physical health problems (such as infectious and contagious diseases) has caused mental health to receive less priority in many countries around the world, including its social and spiritual aspects, which have been overlooked in most cases [5]. Statistics on the global burden of mental illnesses highlight the need to pay attention to these aspects more than ever. In parallel with the estimation of the WHO, Harvard University and the World Bank declared that mental disorders were responsible for about 13% of the total burden of diseases in 2006, which was predicted to reach 15% by 2020. The rate of morbidities caused by these disorders has been reported beyond 33% [6]. In Iran, mental health disorders account for 14.5% of the total burden of diseases, and after traffic accidents, they share the second rank with cardiovascular diseases. In women, depression claims the first place of disease burden, while in men, addiction is considered the third cause of disease burden[7]. According to studies in Iran on individuals older than 15 years old, the prevalence of mental disorders has been 25.9% in women and 14.9% in men, and it was reported that overall, 21% of people suffered from some sort of mental problems. Some studies have assessed the prevalence of mental disorders in Ilam province{8]. In a population-based study by Visani et al. [9], the prevalence of DSM-IV mental disorders in both genders was investigated in the urban areas of Ilam province. Out of 763 participants aged 15 years and older in the recent study, 25.8% showed symptoms of mental distress, and the prevalence of mental disorders was reported to be 20.9% in men and 29.8% in women. Also, the prevalence of anxiety, major depressive disorder (MDD), and obsessive-compulsive disorder was higher in women than in men (23.6% vs. 18.2%; 22.7% vs. 17.4%; 13.9% vs. 10%, respectively). In women, the prevalence of phobia was reported as 10.4%.

Studying mental health status can provide managers and decision-makers of organizations with valuable information for planning educational, therapeutic, and preventive measures to reduce the incidence of these diseases in society. Such information can be obtained by monitoring the mental health status of people in different societies [5].

Assessment and monitoring of mental disorders in the country and in Ilam province can help better understand the condition of mental health and public health in society, which is an important and influential factor affecting social productivity and wealth creation. Therefore, researchers here aimed to investigate the mental health status of the urban population of Ilam using the data recorded in the registration and assessment forms designed for mental disorders. Furthermore, we evaluated the mental health program performed in comprehensive health centers and health houses covered by the Ilam University of Medical Sciences in the West of Iran.

MATERIALS AND METHODS

The present cross-sectional study was conducted on the basis of the reports derived from the mental health program in Ilam province in 2020. According to the statistics announced in 2021, Ilam province harbors a population of over 630,000 people living in ten cities: Ilam, Ivan, Chardavl, Sirvan, Malekshahi, Mehran, Abdanan, Dehloran, Badreh, and Darrehshahr (Figure 1).

Figure 1: The geographical location of Ilam province

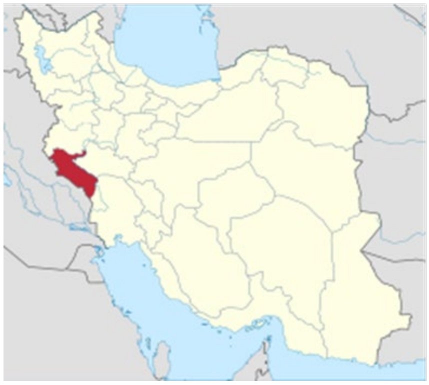

This study was conducted to assess the mental health program implemented in the comprehensive health centers and health houses affiliated with the Ilam University of Medical Sciences in 2019. The study was approved by the institutional ethics committee under the code IR.MEDILAM.REC.1399.266. The data were gathered by reviewing registration and evaluation forms for mental disorders with the codes 102-19-03 and 102-19-01 in all ten cities of Ilam province. Then based on the population of each city in 2021, the prevalence of mental disorders was calculated in 100 and 100,000 people.

Analytic Method

After collection, the data of each city were entered into Microsoft Excel software, and formulas 1 and 2 were used to calculate the prevalence of mental disorders (with 95% CIs) in 100 and 100,000 people.

Formula 1: ROUND (Number of cases*100000/population)

Formula 2: ROUND Prevalence ±1.96*SQRT (Prevalence *(1-Prevalence)/population)

Also, to determine the geographical distribution of mental disorders in the cities of Ilam province, the commands of spatial analysis in the STATA 11 software (Stata Corp. LLC College Station, Texas, USA) were used.

RESULTS

The results showed that in Ilam province, the prevalence of depression was 19100 (95% CI: 18999-19201), and the prevalence of anxiety was 30466 (95% CI: 20348-30584) per 100,000 people. According to the results, the highest prevalence among mental disorders was related to depression and anxiety.

The highest rate of depression belonged to Ilam (20300, 95% CI: 20137-20463) and Malekshahi (21836, 95% I: 21297-22375) cities, while the lowest rate of depression was reported in

Sirvan (16292, 95% CI: 15691-16897) and Badreh (17299, 95% CI: 16706-17892).

Also, the highest rates of anxiety were recorded in the cities of Abdanan (22298, 95% CI: 21922-22674), Ivan (22559, 95% CI: 22228-22970), and Ilam (21200, 95% CI: 210135-21365). On the other hand, the cities of Sirvan (16769, 95% CI: 16184-17404), Chardavl (16499, 95% CI: 16228-16770), and Darrehshahr (19099, 95% CI: 17790-18408) had the lowest rate of anxiety (Table 1).

Table 1: The prevalence of depression and anxiety in the population of Ilam province per 100,000 people in separate cities

Location	Population	Major Depressive Disorder		Anxiety Disorder	
		Prevalence		Prevalence	
		Number of cases	Rate per 100,000 (CI 95%)	Number of cases	Rate per 100,000 (CI 95%)
Ilam	235144	47734	21200(210135-21365)	49850	20300(20137-20463)
Abdanan	46977	9630	22298(21922-22674)	10475	20499(20134-20463)
Ivan	48833	10236	22559(22228-22970)	11036	20961(20600-21322)
Mehran	27506	5281	22402(21909-22895)	6162	19199(18734-19664)
Badreh	15614	2701	17299(16706-17892)	2701	17299(16706-17892)
Dehloran	66339	13135	18900(18602-19198)	12538	19800(19497-20103)
Darrehshahr	59551	10838	18099(17790-18408)	10778	18200(17890-18510)
Shirvan	14404	2347	16796(16184-17404)	2419	1294(15691-16897)
Chardavl	72167	12412	16499(16228-16770)	11907	17199(16924-17474)
Malekshahi	22587	4932	17196(16704-17668)	3884	21836(21297-22375)
Total	580148	110808	30466(20348-30584)	176750	19100(18999-19201)

Regarding age distribution, the results showed that the highest prevalence of depression (29%), anxiety (35.5%), and psychotic disorder (22.6%) was observed in people over 65 years of age. The psychological disorders of mania (6.3%) and obsessive-compulsive disorder (13.3%) were more commonly observed in the age group of 15-24 years compared to other age groups.

Regarding the gender distribution of mental disorders, it was noticed that depression (20.3% vs. 22.7%), mania (2.6% vs. 4.7%), anxiety (23.6% vs. 32.18%), obsessive-compulsive disorder (10% vs. 13.79%), phobia (10.4% vs. 5%), and psychotic disorder (6.17% vs. 2.4%) were more frequent in women than in men (Table 2).

Table 2: The prevalence of mental disorders in Ilam province in various age groups and genders

Age groups/gender	Depression	Mania	Anxiety	OCD*	Phobia	Psychotic
	Prevalence (CI95%)	Prevalence (CI95%)	Prevalence (CI95%)	Prevalence (CI95%)	Prevalence (CI95%)	Prevalence (CI95%)
15-24 years	14.0 (7.08-15.1)	6.3 (5.9-7.3)	14.7 (12.1-16.8)	13.3 (9.04-15.15)	8.4 (6.9-11.1)	0.7 (0.01-5.9)
25-44 years	20.6 (18.2-22.3)	4.0 (2.6-9.1)	21.2 (16.2-18.2)	11.3 (9.13-14.2)	7.5 (6.9-11.1)	4.8 (3.6-6.3)
55-64 years	22.7 (20.2-24.3)	2.3 (1.4-2.9)	23.6 (20.3-25.2)	13.4 (8.1-16.1)	8.3 (7.9-11.3)	3.7 (1.2-5.7)
>65 years	23.6 (20.3-25.2)	0.4 (0.1-1.1)	35.5 (32.3-37.8)	9.7 (7.1-11.2)	9.7 (7.1-12.2)	22.6 (20.2-27.6)
Male	17.4 (15.1-19.6)	2.6 (1.3-8.7)	18.2 (15.2-21.1)	10.0 (8.1-12.4)	5.0 (3.7-8.2)	2.4 (1.4-4.1)

**OCD – Obsessive-compulsive disorder*

The results of the spatial analysis regarding the geographical distribution of mental disorders in Ilam province showed that depression, anxiety, and mania were more commonly observed in the northern compared to southern regions of the province, showing the highest prevalence in Ivan City. Also, panic disorder was more prevalent in southern cities of the province (Abdanan and Dehloran) than in other cities (Figure 2).

Figure 2: The results of spatial analysis. Geographical distribution of the most common mental disorders in Ilam province

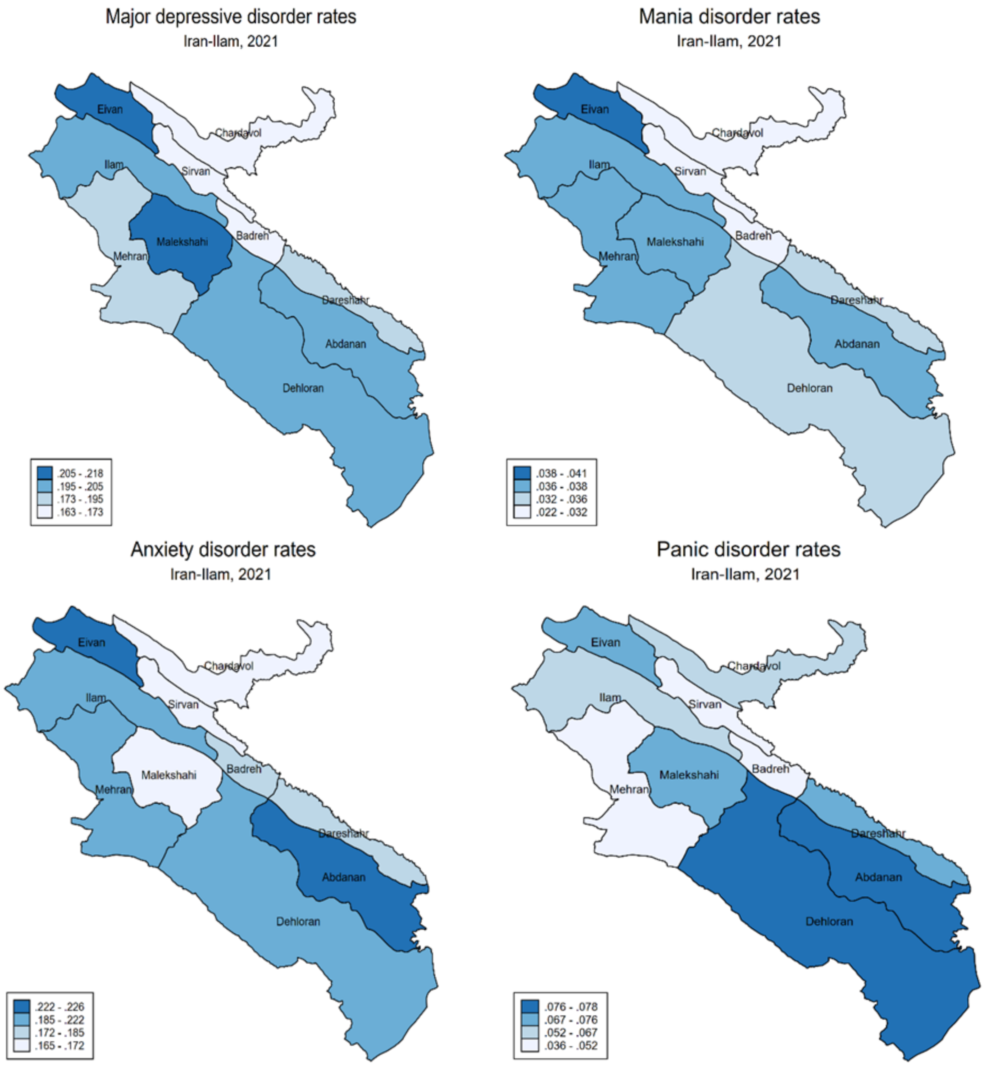
Major depressive disorder rates
Iran-Ilam, 2021
Eivan
Chardavol
Ilam
Sirvan
Badreh
Malekshahi
Mehran
Dareshahr
Abdanan
Dehloran
.205 - .218
.195 - .205
.173 - .195
.163 - .173
Mania disorder rates
Iran-Ilam, 2021
.038 - .041
.036 - .038
.032 - .036
.022 - .032
Anxiety disorder rates
Iran-Ilam, 2021
.222 - .226
.185 - .222
.172 - .185
.165 - .172
Panic disorder rates
Iran-Ilam, 2021
.076 - .078
.067 - .076
.052 - .067
.036 - .052

DISCUSSION

The results of the present study showed that the highest prevalence of depression, anxiety, and psychotic disorders was observed in people over 65 years old. In this study, age showed a direct and significant correlation with having unsatisfactory mental health, indicating the importance of advancing age in the development of mental health problems such as depression, anxiety, and psychotic disorders. In other words, the incidence of mental health disorders increased with age, contradicting the findings of Heidari et al. [10] and Jafari et al. [11]. In recent studies, higher age was reported to be significantly associated with more favorable mental health. Other studies; however, have reported a significant relationship between mental health and advancing age, including a report by Visani et al. [12]. In another study, Noorbala et al. [13] noted that with increasing age, the likelihood of the development of mental disorders increased so the highest prevalence (49.9%) was related to people over the age of 65 years. Among the reasons for the higher prevalence of mental health disorders in older people can be retirement, menopause, loneliness-associated anxiety, and biological alterations. The results of these studies are generally consistent with our findings; nevertheless, some discrepancies can be explained by differences in the assessment tools employed and the age spectra of the studied populations.

The results of this study showed that the highest prevalence of panic disorder and obsessive-compulsive disorder was related to the age group of 15-24 years. Mental disorders in teenagers and young adults exacerbate the predicted future burden of diseases and adversely affect numerous life aspects, including educational performance, employment, income, personal relationships, and social participation, as well as workforce [14]. In a study in Brazil conducted on individuals aged 18 years and older, it was shown that younger people were more vulnerable to mental problems [15]. In another study in Iran, Shirzadi et al. [16] declared a higher prevalence of these disorders in people aged 41 years or older compared to 18-40-year-old individuals.

Also, the results of the present study revealed a lower prevalence of depression and anxiety in women than in men. In a study, Falahzadeh and Momayezi [17], who investigated the prevalence of depression, anxiety, and stress in Yazd city of Iran, noted that depression, anxiety, and stress were more prevalent in women than in men, and this difference was statistically significant for anxiety (P=0.01) and stress (P=0.02), which was not consistent with our observation. We also noticed a higher prevalence of obsessive-compulsive disorders, phobia, and psychotic disorders in women compared to men, which was in agreement with the reports of Pendrez et al. [18], Kesler et al. [19], and Chang et al. [20]. The higher prevalence of mental disorders in women compared to men can be attributed to biological factors, gender-related differences, environmental and occupational stress, as well as limited sources of satisfaction and social participation of females.

Although in the present study, a significant portion of the urban population declared that they had unsatisfactory mental health conditions, this may not exactly match the clinical definition of adverse mental health. Therefore, the results of such subjective studies should not be regarded as a basis for judgment about the true condition of the mental health of respondents. Nevertheless, one should admit that a ratio of people, especially among women, suffer from some degree of mental health disorders. In this regard, the key measures that can be taken may be to initially achieve a correct understanding of the issue and then to implement comprehensive interventions for monitoring populations as large as possible, especially among vulnerable groups. Numerous viable strategies are available to maintain and improve public health. For example, unemployment has been pointed out as an important factor contributing to the development of mental health disorders [21]. So, an important intervention that governments can employ is to create more jobs, especially for youth, and to improve people's livelihoods as much as possible. Also, another effective measure for improving mental health is to remove the sources of distress, psychological pressure, and unnecessary tensions, which can be achieved with the help of mass media by nurturing a comfortable atmosphere. Moreover, the establishment of psychological counseling clinics can be among low-cost and beneficial measures to cover a large population, including young people, middle-aged, and elderly people, so that they can amend and improve their mental health.

CONCLUSIONS

The results of this study affirmed a relatively high prevalence of suspected mental health problems among people over 65 years of age, which is an alarming call,

requiring authorities and researchers to pay more attention to this issue and to identify and modify the factors affecting the mental health of these people. A unique observation in this study was the lower prevalence of anxiety and depression in women than in men. Also, we noticed that panic disorder and obsessive-compulsive disorder were most prevalent in the age group of 15-24 years. A reduction in the prevalence of mental disorders can enhance social liveliness. In this regard, increasing people's awareness of ways to reduce mental disorders, expanding psychological counseling centers across the city, the establishment of counseling phone lines, residing counselors and psychologists in public places such as mosques, expansion of parks and enjoyable environments, equipping parks with sports devices, and finally, encouraging people to use these services can help reduce depression and anxiety in society.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The source of the funding code is the code of IR.MEDILAM.REC.1399.266.

Authors' contribution

Conceptualization, E.N. and Y.V.; methodology, E.N.; formal analysis, H.S; investigation, Y.V.; data curation, F.M. and A.S; writing—original draft preparation, S.A.; and M.Kh.; writing—review and editing, S.A; F.M. and M.Kh. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Investigating the Predictive Power of the Theory of Planned Behavior on the Behavioral Intention of Healthy Eating in Adolescents

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Abstract: *Background: Adolescence is a complex and sensitive period, and learning nutritional concepts during this period is very important. The purpose of the present study is to determine the predictive power of the theory of planned behavior on the behavioral intention of healthy eating in adolescents. Methods: 400 first-grade female students of Bandar Anzali participated in this descriptive-correlational study. Among the first secondary schools of this city, two schools were randomly selected and sampled by census method. The data collection tool was the healthy eating behavior questionnaire based on the theory of planned behavior, which included two sections of demographic information and the constructs of the theory of planned behavior, which were checked and confirmed with the content validity ratio, content validity index, and alpha coefficient. Data were analyzed using SPSS version 22 software. Results: Linear regression analysis showed that a total of 34% of the variance of healthy eating behavior intention is predicted by the constructs of perceived behavioral control and attitude. Conclusion: Considering the high predictive power of the theory of planned behavior in the field of healthy eating behavior, educational interventions based on it and centered on predictive structures are suggested.*

Keywords: *theory of planned behavior, healthy eating, adolescents*

INTRODUCTION

Non-communicable diseases (such as cardiovascular diseases, diabetes, cancer, high blood pressure, obesity, etc.) are the main cause of death worldwide [1]. Due to the increase in the death rate caused by these diseases, especially in developing countries, the World Health Organization (WHO) declared non-communicable diseases among the health priorities in developing countries two decades ago [2]. Iran, as an example of a middle-income country, accounted for 83.5% of all deaths and 78.1% of the total disease burden in 2019 due to non-communicable diseases [3-5].

The role of nutrition as the main cause of these diseases has been proven correctly [6]. In this regard, especially, the unhealthy eating behavior of teenagers is worrying [7]. Rapid growth, and changes in body composition, physiology, and endocrine system accelerate nutritional needs and increase the risk of malnutrition in this group [8]. Adolescents have more decision-making power and independence than children and are heavily influenced by friends, people around them, the environment, and various advertisements, and they turn to foods with an attractive and seductive appearance but lacking in nutritional value, many ready-made foods and snacks are among these materials [9]. Most of them do not consume enough fruits and vegetables, instead, they consume high-fat, energy-rich, and nutrient-poor foods [10].

According to the 2019 GSHS (Global School-based Student Health Survey), almost half of the countries reported that 10-30% of students aged 13-15 did not eat any fruit, a quarter reported that 10-30% of students did not eat any vegetables, and almost 70% of countries reported that at least half of their students eat junk food on a weekly basis and all countries have found that one in five students consumes carbonated drinks at least once a day [11].

Among Iranian teenagers, high consumption of junk foods and unhealthy snacks, not eating breakfast, and low consumption of fruits, vegetables, whole grains, and dairy products have been commonly reported [12] and this will cause them to develop chronic diseases in the future, especially girls who are considered as the future mothers and their eating behavior and learning nutritional concepts affect their own health and that of their children and families [13,14].

Modifying eating habits and behaviors during childhood and adolescence can prevent the occurrence of many non-communicable diseases in adulthood [15]. On the other hand, the future of any country depends on how it takes care of its children and teenagers. If action is taken to meet their health needs, the possibility that the adults of that society will have a healthy and valuable life pattern in the future will increase [16]. On the other hand, the future of any country depends on how it takes care of its children and teenagers. If action is taken to meet their health needs, the possibility that the adults of that society will have a healthy and valuable life pattern in the future will increase [17].

Studies show that the most effective educational programs are based on theory-based approaches that are rooted in behavior change patterns, and the "theory of planned behavior" is one of the well-known ones [18]. According to this theory, attitude, subjective norms, and perceived behavioral control affect the intention to perform a behavior [19]. A person's attitude is a favorable or unfavorable evaluation of behavior formed through past experiences.

Subjective norms refer to the social pressures perceived by the person who agrees or disagrees with the behavior and the media, family, and friends play a role in it. Finally, perceived behavioral control is a person's belief about how easy or difficult it is to perform a certain behavior [20]. According to the explanations provided, the present study was conducted with the aim of determining the predictive power of the theory of planned behavior on the behavioral intention of healthy eating in adolescents.

MATERIALS AND METHODS

This is a descriptive-correlational study, which was conducted in 2022. The research population was 400 first-grade high school girls in Bandar Anzali. From among the first high school girls in this city, two schools were randomly selected and sampled by census method. Inclusion criteria included: students' willingness to participate in research, lack of a special diet and not suffering from chronic diseases, and exclusion criteria were unwillingness to participate and continued cooperation by students.

The study was initiated with the permission of the ethics committee of Iran University of Medical Sciences and the necessary coordination with the education organization of Bandar Anzali city, the principals and officials of the relevant schools, and the informed consent of the students. The data collection tool was the healthy eating behavior questionnaire based on the theory of planned behavior [21].

This questionnaire includes two sections of demographic information and constructs of the theory of planned behavior. The first part of this questionnaire included 8 items related to demographic characteristics, including student age, height, weight, parent’s education, parent's occupation, and household size. The second part or constructs of the theory of planned behavior consists of 32 items, and the third part, or food behavior part includes 7 items, based on the 5-point Likert scale each item, as (strongly disagree, disagree, no idea, agree, strongly agree) with a minimum and maximum score of 1 to 5. The second part of the questionnaire includes attitudes toward behavior with 12 items with an achievable score range of 12 to 60, 13 items for subjective norm with an achievable score range of 13 to 65, perceived behavioral control with 7 items with an achievable score range of 7 to 35, behavioral intention with 6 items with an achievable score range of 6 to 30. In the present study, due to the limitation of answer options for items in the questionnaire, which were 4-point Likert, the questionnaire was re-validated and reliable.

To assess the validity, content validity was used, so that the questionnaire was given to 10 health and nutrition experts, and its validity was confirmed with a content validity ratio (CVR) of 0.81 and a content validity index (CVI) of 0.8. To assess the reliability, Cronbach's alpha test method was used. For this purpose, a questionnaire was given to 20 female high school students. Cronbach's alpha coefficient was 0.85 for the attitude construct, 0.75 for the subjective norm construct, and 0.71 for the perceived behavioral control construct, 0.76 for the behavioral intention construct. Data analysis was done by SPSS software version 24.

RESULTS

In this study, the mean age of students was 14.9 ± 0.78 years. Their mean height and weight were 159.7 ±10.7 and 54.7± 1.4, respectively, and the household size was 4.1 ± 0.8. In terms of employment, the highest frequency was for fathers (45%) as self-employment and 86% of mothers were housewives. In terms of education, 42% of fathers and 45% of mothers were at the level of high school and high school diploma, each of which with the highest percentage of frequency. The mean, standard deviation, and range of obtainable scores for each construct of the theory of planned behavior in the studied students can be seen in Table 1.

Table 1: Mean, standard deviation, and range of obtainable scores for each of the constructs of the theory of planned behavior in the studied students

Variable	Mean ± SD‣	Rang of attainable score
attitude	40 ± 6.21	12-60
subjective norms	38.9 ± 6.44	13-65
perceived behavioral control	24.06 ± 6.44	7-35
behavioral intention	25.47 ± 9.21	6-30

‣ *Standard Deviation*

Table 2 shows the results of Pearson correlation between the constructs of the theory of planned behavior that there is a direct and significant correlation between all constructs (attitude, subjective norm, and perceived behavioral control) with the intention of eating behavior (P<0.001). The construct of perceived behavioral control has the highest correlation with the intention of eating behavior (r=0.302).

The linear regression analysis in Table 3 showed that a total of 34% of the variance of the intention of healthy eating behavior in the studied students is predicted by the constructs of perceived behavioral control and attitude, and perceived behavioral control is the strongest construct related to the intention of eating behavior in this study.

Table 2: Correlation between the constructs of the theory of planned behavior

Variable	Attitude	Subjective norms	Perceived behavioral control	Behavioral intention
attitude	1			
subjective norms	0.191	1		
perceived behavioral control	0.276	0.145	1	
behavioral intention	0.182	0.255	0.302	1

Table 3: Predicting the behavioral intention of healthy eating based on the theory of planned behavior in the studied students

Model		B	Std. Error	Beta	sig
1	(Constant)	5.231	3.424	_	0.127
	attitude	0.157	0.070	0.113	0.024
	subjective norms	0.119	0.085	0.088	0.163
	perceived behavioral control	0.371	0.103	0.230	0.000

a. Dependent Variable: Intention of food behavior; b. R2 = 0.34

DISCUSSION

The present study was conducted with the aim of determining the predictive power of the theory of planned behavior on the behavioral intention of healthy eating in adolescents. The result of the study shows that a total of 34% of the variance of healthy eating behavior intention in the studied students is predicted by the constructs of perceived behavioral control and attitude, and perceived behavioral control is the first and strongest construct related to behavioral intention, which is consistent with studies predicting healthy eating in Danish adolescents [22] and the results of the study by Seo et al in Seoul, which implies a significant relationship between behavioral intention and perceived behavioral control construct [23]. It is also consistent with the findings by Yarmohammadi et al. [24] and Dunn et al. [25] on junk food consumption based on the theory of planned behavior. However, it is not consistent with the findings of Ickes et al. [26] on beverage consumption and the findings of Gholami et al. [27] on fruit and vegetable consumption. Perceived behavioral control may not predict behavior when a person has little information or the resources available to the person change and when new elements such as eating habits are added to the situation [28, 29].

The second construct predicting the intention of healthy eating behavior in this study is the attitude, which is consistent with the results of the study by Mousavi et al. [30] concerning beverage consumption and the study by Gronhoj et al. [22] regarding the prediction of healthy eating behavior and the study by Mamun et al. [31] on the determinants of healthy eating. Attitude shows an individual’s general feeling about the desirability or undesirability of something, and sometimes people's attitude during life as well as sufficient awareness and knowledge, which is a prerequisite for attitude change, leads to the intention of performing the desired behavior in the future [27]. Therefore, the attitude that originates from feelings and internal factors can affect his eating behavior by increasing his information and experiences concerning nutrition.

In this study, the subjective norm is not a predictor of eating behavior intention, which is similar to the finding by Ismawati et al. [32] on the application of the theory of planned behavior in the intention of healthy eating in Malaysia and the study of Brouwer et al. [33] related to healthy eating behavior prediction and finding by Dehdari et al. [34] regarding the consumption of unhealthy foods. However, it is inconsistent with the findings of Khorasani et al. [35] on unhealthy food consumption and White et al. [36] regarding intention to consume low-fat food. The reason for this inconsistency can be the difference in the gender of the participants, in the study of Dehdari et al. [34], as in the present study, the target group is girls, and in the study of Khorasani et al. [35], boys are the target group. On the other hand, diverse food cultures and customs in Iran and other countries of the world can be the reason for the discrepancy of these studies. The diversity and complexities of health behaviors in different groups and in different conditions can lead to the necessity of planning to use the appropriate theories and structures to achieve success in interventions in health education and health promotion.

CONCLUSIONS

Perceived behavioral control and attitude are among the factors predicting on the behavioral intention of healthy eating in the studied students. The research limitation in the present study was the use of self-reporting, and it is necessary to carry out educational interventions based on the "theory of planned behavior" with emphasis on the two predictive constructs mentioned.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research. The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

Authors' contribution

All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

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Ethics approval and consent to participate

This article is taken from the approved research project of Iran University of Medical Sciences with ethics code number: 1401.886 IR.IUMS.REC. Informed consent was obtained from all individual participants included in the study before conducting.

Consent to publish

All participants consented verbally to the publication of the interview data.

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ORIGINAL ARTICLE

A Conceptual Analysis of Post-psychotic Depression

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Abstract: *The conundrum of post-psychotic depression (PPD) is still present in the psychiatric literature, for more than a century since it was first described by Mayer-Gross. After a short existence in the DSM-IV and ICD-10, this nosological construct was excluded from newer versions of the mental disorders classifications. Therefore, an exploratory analysis of the PPD concept was considered useful both from clinical and theoretical perspectives. There is a high prevalence of depressive symptoms in patients with schizophrenia spectrum disorders (SSD) and, although not all of them represent cases of PPD, they are considered risk factors for suicide. The arguments and contra-arguments for/against PPD were reviewed, and the potential pharmacological interventions for this disorder were analyzed. The explored evidence indicates that PPD is worth further investigation, and in order to differentiate it from schizoaffective disorder, negative symptoms of SSD, or depressive manifestations within the acute psychotic episodes, clearly defined criteria should be found. The use of validated scales, like the Calgary Depression Scale for Schizophrenia and the InterSePT Scale for Suicidal Thinking, is recommended for the initial assessment of depression and suicidal risk in patients with SSD, but also for their monitoring during the acute and maintenance phases. Besides the administration of combined, antidepressant and antipsychotic treatment, the use of clozapine, and the recommendation to initiate treatment for SSD with atypical antipsychotics whenever possible, there is a dearth of studies exploring specific interventions for PPD. Future studies are expected to address the validity of the nosological construct of PPD and the most adequate therapeutic and prophylactic interventions in patients with SSD.*

Keywords: *post-psychotic depression, schizophrenia spectrum disorders, atypical antipsychotics, clozapine, typical anti-psychotics, antidepressants*

INTRODUCTION

Depression that may appear in the context of schizophrenia is considered a complex and heterogeneous group of disorders [1,2]. Mayer-Gross was the first author who described in 1920 the reaction to a psychotic experience that manifests itself as a clinical version of depression [3]. He considered "despair" as the main clinical characteristic of this depression that follows a full-blown psychotic episode, and considered this manifestation to be determined by the patient's increased awareness about the severity of his/her illness [3,4]. A few decades later, Semrad also explored the psychodynamic mechanisms for post-psychotic depression (PPD), emphasizing the denial, projection, and distortion of reality after the remission of acute psychotic episodes [5]. PPD was described by several authors as "retarded depression, with strong neurasthenic and schizoid components" emerging after the patient is discharged from the hospital, and often ignored by mental health specialists [6]. Depressive manifestations in patients with SSD have been conceptualized as a psychological reaction to acute psychosis, as previously mentioned, but also as a comorbidity of two distinct pathologies or a proof for the existence of a continuum between schizophrenia and mood disorders [2]. According to psychoanalysts, PPD is a reaction to psychosis, which appears after the remission of the psychotic episode, more specifically, after the Ego regained a significant proportion of its strengths [7,8].

Some authors, however, reported, based on the analysis of the temporal relationship between the onset of depressive and psychotic symptoms in 27 patients, that the first reports on mood manifestations were frequently at the same time same as the onset of psychosis [9]. Therefore, PPD is a controversial concept, as no specific pattern for its onset could be detected, leading to the assumption that depressive manifestations may be only associated with acute psychotic manifestations.

However, a series of studies focused on PPD have tried to delineate the risk factors, clinical features, and prognosis of this nosological entity, as a distinctive pathology from depressive symptoms belonging to acute schizophrenia, mood episodes within a schizoaffective disorder, or adverse events of the antipsychotics or other drugs. For example, a comparison of patients with schizophrenia presenting or not with PPD (N=30 participants in each group) showed that belonging to elementary (or nuclear) families, longer duration of psychotic phase, more frequent hospitalizations, and more sadness and anxiety-somatization during the acute phase of the disease were associated with PPD [10]. A past history of depression was also more frequently reported in the study group vs. controls [10]. Age of onset, sadness during the acute psychosis phase, premorbid adjustment, social support, and life events were significant determinants of severity for PPD, according to the multiple regression analysis [10].

A cohort study with a 10-year follow-up compared the evolution of 29 patients with schizophrenia and PPD vs. only schizophrenia [11]. Patients with PPD presented at an older age with the onset of schizophrenia and more auditory hallucinations; also, they were admitted more often, but their hospitalizations were of shorter duration [11].

A study that explored the main clinical differences between primary depression (N1= 98 patients) and PPD (N2= 71 patients) concluded that depressed mood, reduced energy, and pathological guilt were more frequently detected in patients with primary depression, while sleep disturbance and ideas of reference with a theme of guilt were more consistently associated with PPD [1].

The duration of PPD was estimated by different authors as varying between several days to several months [12], or, more specifically, between 2 and 26 weeks [8].

Depressive symptoms during a psychotic episode may be as frequent as 50%, and they may correlate with the frequency of relapses, defined by re-admission rate and total length of inpatient treatment [13]. By comparison, the prevalence of PPD reported in the literature was around 30-36% [14,15]. Higher rates of PPD were reported in patients with a first episode of psychosis (FEP), up to 50% [3,15]. Older age, a higher familial load of psychoses, a longer duration of untreated psychosis, the presence of an FEP, and a higher number of stressful life events were associated with a higher risk of PPD [14]. Depression was reported by patients with FEP as being linked to the psychotic experience and to the process of recovering from this experience [16]. Feelings of shame, doubt, and embarrassment, but also loss and social isolation, were associated with the presence of the psychotic experience [16]. Also, the onset of PPD has been correlated to the existence of positive symptoms during acute schizophrenia, especially delusions and hallucinations [17]. A study that enrolled 44 patients (aged 10-40 years) with schizophrenia, all of them during their first six months of the illness, explored the risk of developing PPD one year after discharge from the hospital [18]. Four patients were diagnosed with depression in this interval, according to a Hamilton Depression Rating Scale (HAMD) score ≥17 [18]. Only one case recovered from PPD completely during the follow-up [18]. All patients in this trial were undergoing phenothiazine treatment throughout the monitoring period [18].

In a longitudinal study, 17 patients with schizophrenia were monitored for 6 months, using the 18-item Profile for Rating Depressive and Schizophrenic Behavior (PRDSB) [19]. After recovering from acute psychosis, only 2 participants developed major depressive syndrome [19]. However, over 40% of these patients were interviewed only by telephone after their discharge, so a potential bias regarding the sensitivity of the assessment method can not be excluded [19]. Also, the ethnic homogeneity of the study group could be construed as another potential bias that limits the generalizability of the results.

A longitudinal, observational study (N=105 patients with schizophrenia) reported during 12 months of monitoring that depression accompanied acute psychosis in 70% of cases, and remitted together with psychosis, while PPD appeared in 36% of these patients, without a concomitant increase in psychotic symptoms [20].

Focusing the analysis of the PPD concept on the most renowned classifications of mental disorders, this pathology was mentioned for the first time in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), published in 1994 [21]. PPD, according to the cited source, involves the presence of "a Major Depressive Episode that is superimposed on and occurs only during the residual phase of schizophrenia" [21]. The definition of PPD required in the DSM-IV the presence of depressed mood, as a core symptom, and excluded the direct consequences of drug abuse, medication use, or a general medical condition [21]. However, in the fifth edition of the DSM, published in 2013, this nosological entity no longer appears [22].

The tenth edition of the International Classification of Diseases, edited by the World Health Organisation (ICD-10) in 1993, grouped PPD (named "post-schizophrenic depression") together with different types of schizophrenia, in the same chapter, coded F20 [23]. The definition of PPD was "a depressive episode, which may be prolonged, arising in the aftermath of a schizophrenic illness" [29]. It is important, according to the ICD-10, that residual schizophrenic symptoms still be present, although they cannot dominate the clinical status [23]. Newer versions of ICD-10 placed PPD in the category of "other specified depressive episodes" [24]. The latest edition of ICD (ICD-11), the first version published in 2019, did not reference PPD [25].

The convoluted trajectory of the PPD concept from its genesis to the present day is rather difficult to explain, but several important factors contributing to this conundrum represent the main objective of the current review (Figure 1).

Figure 1: Rationale for exploring post-psychotic depression

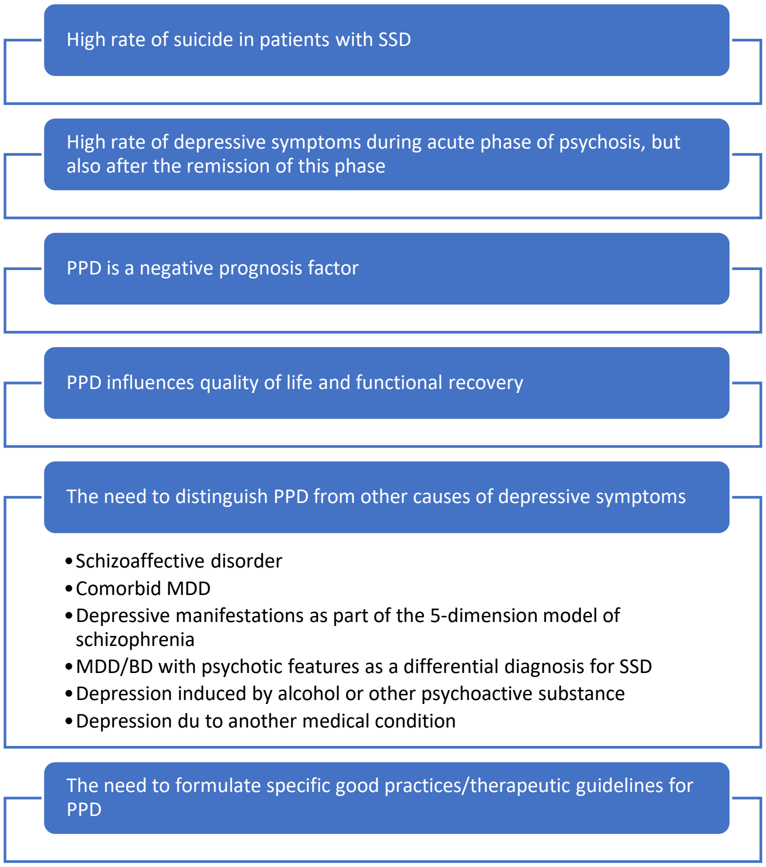

High rate of suicide in patients with SSD

High rate of depressive symptoms during acute phase of psychosis, but also after the remission of this phase

PPD is a negative prognosis factor

PPD influences quality of life and functional recovery

The need to distinguish PPD from other causes of depressive symptoms

- Schizoaffective disorder
- Comorbid MDD
- Depressive manifestations as part of the 5-dimension model of schizophrenia
- MDD/BD with psychotic features as a differential diagnosis for SSD
- Depression induced by alcohol or other psychoactive substance
- Depression du to another medical condition

The need to formulate specific good practices/therapeutic guidelines for PPD

PPD= postpsychotic depression, SSD= schizophrenia spectrum disorders, MDD= major depressive disorder

The rationale for conducting this review derives from several important clinical and theoretical aspects. First, it is essential to obtain adequate control over depressive symptoms in patients with SSD, because of the high rates of suicide reported in this population [26]. PPD seems to be a factor for negative prognosis in the long-term for patients with schizophrenia, especially because of the higher risk for suicide, but also due to its significant impact on the quality of life and general well-being [17]. Second, the need to delineate clinically PPD from other causes of depression in patients with SSD, in order to find the most adequate therapeutic strategies. To support this, the data presented suggest that PPD is a distinct nosological entity from antipsychotic-induced adverse events, negative symptoms of schizophrenia, depression with psychotic symptoms, or schizoaffective disorders [14,17]. This means that good clinical practices in patients with SSD would involve differentiating between PPD and other frequently reported confounding factors.

THE MULTIFACETED CONSTRUCT OF PPD

Although the history of PPD has been over a century, there is a dearth of good-quality studies exploring this pathology's clinical and epidemiological aspects. However, an analysis of the main dimensions of the PPD construct can be done, based on the available evidence (Table 1).

Table 1: Pros and cons in the analysis of the PPD construct

Pros	Cons
PPD was associated with any type of antipsychotic treatment, therefore it is not an adverse event to the first generation antipsychotics	PPD does not exist because it consists of adverse effects of older antipsychotics
There are longitudinal studies that confirm the persistence of depressive symptoms after the remission of acute psychosis and cases where depression followed a period of psychosis remission	The construct should not be treated as a distinct entity, it only accompanies acute schizophrenia
Negative symptoms and depression do not overlap to the point they are indistinguishable	Negative symptoms cover the presence of depression, therefore depression may be observed only after the remission of those symptoms
There are specific scales for measuring depression in patients with SSD, as well as their suicide risk	PPD can not have its own diagnosis criteria and its severity can not be determined, due to the fact that it is secondary to other symptoms

To begin with the presentation of the reasons why PPD is not yet a fully recognized diagnosis, it must be specified that PPD was correlated by different authors with the effects of first-generation antipsychotics, and they called this phenomenon "pharmacological depression" [27-29]. In a multicenter study (N=211 patients with schizophrenia), depression (quantified with HAMD) was detected in 25% of the cases, all of them treated with fluphenazine (either hydrochloride or decanoate) [30]. This study also showed that depression was associated with a more chronic psychiatric history and a higher rate of relapse [30]. Also, older drugs used to treat SSD, such as chlorpromazine or reserpine, have been associated with lethargy, psychomotor retardation, and anergy, which are acknowledged adverse events of these agents [30].

Secondly, the negative symptoms of schizophrenia may cover the depressive symptoms, making them more difficult to detect [14,17]. Anhedonia, defined as a reduced ability to experience pleasant emotions, is considered both a negative symptom in SSD, and a central manifestation in major depressive disorder (MDD) [31-34]. Therefore, it is considered "a transdiagnostic symptom", and its explained by dysfunctions of the reward and aversion pathways in the central nervous system (CNS) [31]. Furthermore, negative symptoms like alogia and avolition may prevent the detection of depressive symptoms, because they interfere with both the interview and the observation of the patients.

The third aspect refers to a contradiction: although current diagnostic classifications do not include mood symptoms in the core criteria for schizophrenia, these are frequently considered part of the clinical picture, as additional manifestations [35]. According to the five-dimension model of schizophrenia, depression is a core clinical component, together with the positive, negative, agitation-excitement, and disorganization symptoms [36]. However, as mentioned in the introductory chapter, there is no reference to PPD in the latest versions of the ICD or DSM [22,25].

The fourth facet of the PPD controversy is represented by the fact that although differentiating between schizophrenia, schizoaffective disorder, and depressive/bipolar disorder with psychotic features is quintessential, in clinical practice it is frequently difficult to make the right diagnosis. Factorial analysis using validated instruments for psychosis and mood symptoms measurement (e.g., Positive and Negative Syndrome Scale – PANSS, Young Mania Rating Scale – YMRS, Montgomery Depression Rating Scale – MADRS, and Calgary Depression Scale – CDSS) confirmed the existence of very complex structure of the clinical manifestations in patients with schizophrenia [37]. Subclinical mood symptoms have been confirmed by this type of analysis, structured within up to 15 factors – e.g., positive, negative, somatic, anxiety, neurocognitive, disorganization and manic, depressive, psychomotor agitation, hostility/violence [37]. These results support the overlapping model of phenotypes between psychotic mood disorders and SSD, and the possibility of misdiagnosing a patient with a mood disorder and associated psychotic features as having an SSD [37].

However, each of these confounding factors may be addressed. The first argument is precarious because even SSD patients treated with newer antipsychotics may develop depressive symptoms and the effect of these pharmacological agents on depressive symptoms is only small to medium [38]. According to a Cochrane review, based on five reports about patients diagnosed with both schizophrenia and depression, there was no significant difference in efficacy (evaluated by PANSS scores) between quetiapine and haloperidol, sulpiride was significantly superior to chlorpromazine (reflected in the severity of depressive symptoms), and clozapine was superior to the association of another antipsychotic and an antidepressant (moclobemide/mianserin) [39]. A significant reduction of depressive symptoms in patients with schizophrenia was reported only if a CDSS score at baseline was ≥5, unlike the positive, negative, and general symptoms (scored with PANSS), in a naturalistic study that enrolled adult patients with ≥2 years of treatment with clozapine (N=23) or other atypical antipsychotics (N=23) [40]. At the follow-up visit, patients who received other antipsychotics led o superior results when compared to clozapine on depressive symptoms, but only when the analysis was limited to those with clinically relevant depression [40]. It can easily be deduced that the results of studies exploring the impact of antipsychotics on depressive symptoms in SSD are quite heterogenous, varying from no difference in outcomes between typicals and atypicals, to superiority of clozapine vs. other antipsychotics combined with antidepressants, and to superiority of other atypicals to clozapine, but only in certain subpopulations.

The second argument is even more difficult to support because authors exploring PPD showed that this syndrome “is usually stable phenomenologically, often lengthy, and may be resistant to all treatment modalities” [6]. Even considering the last part as an exaggeration determined by the lack of adequate therapeutic resources when that statement was formulated, there is still enough ground to back up an independent syndrome, with pharmacological and clinical peculiarities. There are authors that contradict the third argument, stating that depression is “a frequent comorbidity in the course of schizophrenia” [7], and not just a cluster of symptoms intrinsic to SSD.

The fourth argument can also be easily disqualified because there are validated instruments, currently in clinical use, that allow for the monitoring of depressive symptoms in patients with SSD. CDSS is an instrument created by Addington et al., first published in 1990, for the purpose of measuring depression in SSD, starting from selected items within HAMD and Present State Examination (PSE) [41]. This instrument was validated on 50 patients with acute psychosis, and it has 11 items, with a Cronbach’s alpha of 0.84-0.89 [41,42]. The validity of CDSS in evaluating mood symptoms in patients with SSD was confirmed by subsequent studies and reviews, and this scale was significantly correlated with HAMD and MADRS [43,44]. CDSS differentiated depressive symptoms from other clinical manifestations of SSD and also had good predictive validity [44]. Another study comparing CDSS and HAMD (N=119 in-patients with acute schizophrenia) concluded that CDSS had a higher sensitivity than HAMD in assessing mild or severe depression, while the specificity was comparable high for both instruments [45].

The InterSePT Scale for Suicidal Thinking (ISST) has 12 items and was created to assess suicidal ideation in SSD patients [46]. Two validation studies (N=22 and 980 patients with SSD) confirmed that ISST is reliable and valid if used to assess ongoing suicidal ideation in patients with SSD [46]. Both ISST and CDSS can be used to provide information necessary in the case management of patients with SSD and suicidal risk [47].

Based on its properties, CDSS is considered a first-line instrument to determine the severity of depressive symptoms in patients with SSD, and a review that compared 6 different instruments targeting the same symptoms formulated this recommendation [44]. A study that explored the diagnosis of major depressive episodes in 84 patients with schizophrenia found that CDSS was superior to the Beck Depression Inventory (BDI), HAMD, and PANSS-D when evaluating the Receiver Operating Characteristics (ROC) curves [48].

However, the possibility that PPD symptoms may be, in fact, the continuation of depressive symptoms with onset in the acute psychotic phase, can not be completely excluded [7]. On the other hand, if PPD is detected after a period of psychotic symptoms remission, then it may be considered part of a new episode of schizophrenia [7].

PPD may be confounded with negative symptoms of SSD, as previously mentioned. The discussion about primary and secondary negative symptoms is a very complex topic, that clearly exceeds the objective of this paper. As a general definition, primary negative symptoms are intrinsic to schizophrenia, a specific clinical dimension, while secondary negative symptoms derive from the presence of positive symptoms, depression, antipsychotics’ pharmacodynamical properties, substance use disorders (SUDs), and social withdrawal [49-51]. After the remission of psychotic symptoms, the depressive symptoms were still present, although their rate decreased from 60% during the acute psychotic episode, to 40% after 5 years follow-up [52].

In conclusion, a correct diagnosis of PPD should include a clinical and psychometric evaluation of psychotic and depressive symptoms, a thoroughly-conducted screening for comorbidities (organic, toxic, or psychiatric) [53-55], an analysis of the risk factors for the persistence of depressive symptoms [56,57], a clear collection of personal medical history (in order to establish the temporal succession of psychotic and depressive manifestations), and an accurate pharmacological anamnesis.

THERAPEUTIC INTERVENTIONS FOR PPD

Adapting the type and dosage of antipsychotic agents in patients with SSD was signaled by different authors as a method to decrease the risk of PPD [2]. Re-evaluation of the main drugs administered during the acute phase of psychosis or the prescription of adjuvant antidepressants or ECT has also been suggested as a case management strategy in PPD [2]. Psychotherapeutic, social, and institutional support are useful options for patients with PPD [2].

A review (n=20 articles) exploring the results of available pharmacological and psychosocial treatments of PPD after the first episode of psychosis did not find support for one intervention in favor of another [58]. Online psychotherapy and treatment with ω-3 polyunsaturated fatty acids (PUFA) have been associated with some favorable results [58].

Olanzapine (5-15 mg/day) was compared to risperidone (4-8 mg/day) in the treatment of PPD (according to the DSM IV criteria) in patients with schizophrenia (N=76), and a clinically significant decrease in MADRS scores (-4 points vs. baseline) was reported after 8 weeks of treatment with either antipsychotic [59]. This study used a randomized, double-blind, parallel-group design. The comparative analysis of the two interventions did not detect any significant MADRS differences at the end-point [59]. A high response rate (≥20% reduction in MADRS scores) was observed during antipsychotic treatment [59]. In order to control for possible confounders of PPD, patients with akinetic symptoms were excluded from this trial [59]. The same team reported on another randomized, placebo-controlled trial, with 24 patients diagnosed with schizophrenia or schizoaffective disorder and PPD or negative symptoms, who continued their six-month adjunctive imipramine to fluphenazine decanoate/ benztropine mesylate treatment or were tapered to placebo + fluphenazine decanoate/benztropine during one year [60]. More patients receiving a placebo relapsed into PPD, at a statistically significant level; also, patients who received placebo experienced relapses into psychoses [60]. Yet another trial conducted by the same team showed that double-blind, controlled discontinuation of imipramine after stabilization (N=14 participants with schizophrenia undergoing treatment with fluphenazine + benztropine) led to relapse into PPD in all patients with placebo vs. only two out of eight in the antidepressant-receiving group (significant difference, p=0.009) [61].

A controlled trial investigated the effects of imipramine hydrochloride for PPD for six weeks, in a double-blind manner [62]. All patients (N=33) were diagnosed with schizophrenia or schizoaffective disorder and received a stable dosage of fluphenazine decanoate and one week-trial with benztropine mesylate was initiated to rule out akinesia; all participants were either non-psychotic or only residually psychotic at baseline [62]. The PPD diagnosis was based on current major or minor depression for at least four weeks, and a HAMD score ≥12 for at least three consecutive weeks [62]. The depressive symptoms (according to HAMD and SADS, Schedule for Affective Disorders and Schizophrenia scores) were controlled better with imipramine vs. placebo and their global clinical status (according to the CGI scores) also improved more consistently in patients receiving the active drug [62]. The psychotic symptoms and tolerability profile did not change significantly between groups [62]. The use of adjunctive imipramine to fluphenazine was further explored by the same team, in a larger and more heterogenous study group [63]. The positive effects of the combined treatment were still detected in the total sample, but the significance of the results was lower [63]. These differences were explained in a post-hoc analysis by the higher level of psychotic symptoms severity, and by “more refractoriness of depression” due to failed previous trials of antidepressants [63].

In a continuation trial, four out of five patients previously stabilized on imipramine + fluphenazine decanoate + benztropine for PPD relapsed into depression six months after the tapering of the antidepressant [64]. The control group, consisting of four patients who did not discontinue imipramine, did not present depressive relapses [64]. The same research team reported on ten PPD patients with good responses to imipramine who relapsed six months after discontinuation of the antidepressant, that was added to fluphenazine decanoate and benztropine [65].

A 24-week double-blind trial compared haloperidol (mean dose 17.5 mg/day) and thiothixene (mean dose 31.8 mg/day) in 46 outpatients with schizophrenia, from their efficacy and safety properties [66]. One of the outcomes was the incidence of treatment-emergent depression, assessed by the Hamilton Depression Rating Scale (HAMD) and Zung Self-Rating Depression Scale (ZSRS) [66]. Haloperidol was superior to thiothixene on the “cognitive disturbance” HAMD factor [66]. Clinically significant symptoms were reported in five patients treated with haloperidol and three receiving thiothixene [66]. The impact on the psychotic symptoms was also more important for haloperidol, according to the Brief Psychiatric Rating Scale (BPRS) scores [66].

In a trial that compared the antidepressant effects of fluphenazine decanoate (mean dose 32.8 mg fortnightly) and flupenthixol decanoate (mean dose 36.9 fortnightly) administered two-weekly in a group of 48 out- and inpatients with schizophrenia during 15 weeks [67]. No significant differences could be found between groups regarding the number and severity of mood impairments and depression

[67]. Nineteen patients developed depression, and the addition of clomipramine was necessary for five participants, with a mean duration of four weeks, and an average dose of 75 mg/day [67]. The onset of depressive symptoms was reported between days 14 and 70 for outpatients and between days 7 and 77 for inpatients [67]. The onset of depression was unrelated to the antipsychotic dose [67]. The instruments used to assess depression in this trial were HAMD, von Zeersen's self-evaluation scale, and AMP-system [67].

According to a review of 7 studies focused on the use of antidepressants in patients with SSD, tricyclic antidepressants administered in combination with first-generation antipsychotics are a safe choice after the acute psychotic phase is controlled [68]. The risk of exacerbating residual psychotic symptoms is considered minimal by the authors of that review [68]. Still, these antidepressants were ineffective against anergy, considered a core symptom of PPD [68]. This is a controversial conclusion because "anergy" can be easily confounded with several negative signs of schizophrenia (such as avolition, apathy, or even anhedonia) [13,49,50,52,69,70]. The incidence of negative symptoms in patients with SSD is reported to be high, with more than 57% presenting with at least one such symptom, and 41% with at least two negative symptoms [69]. Other authors report a prevalence of negative symptoms that may reach 90% for at least one such manifestation, and up to 60% for at least one depressive symptom in FEP [70]. There is a risk of anticholinergic adverse events because of the pharmacokinetic interactions between the two classes of pharmacological agents [68].

Clozapine is considered a good therapeutic intervention in SSD associating mood disorders [71]. Risperidone was superior to haloperidol in decreasing anxiety and depression scores (at 8 mg/day and 10 mg/day, respectively), and the same atypical antipsychotic was more effective than haloperidol and levomepromazine on the Psychotic Anxiety Scale (at 8.6 mg/day, 9.2 mg/day, and 125 mg/day, respectively) [71]. Another review (n=33 studies focused on treatments for SSD with mood symptoms) concluded that combined treatments are as effective as antipsychotics used in monotherapy for acute psychotic exacerbations with depressive manifestations, although "some evidence" for superiority was detected in the case of antipsychotics [72]. For patients developing a major depressive syndrome after remission of acute psychosis, there has been found evidence to support the administration of antidepressants, as add-ons to antipsychotics [72]. The results regarding therapeutic response to antidepressants in cases of subsyndromal depression were mixed [72].

Figure 2: Potential therapeutic interventions for post-psychotic depression

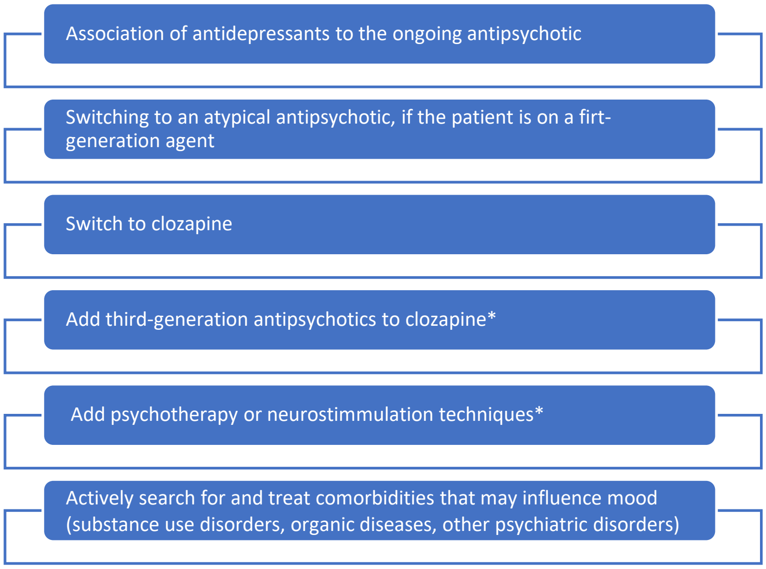
Association of antidepressants to the ongoing antipsychotic
Switching to an atypical antipsychotic, if the patient is on a firt-generation agent
Switch to clozapine
Add third-generation antipsychotics to clozapine*
Add psychotherapy or neurostimmulation techniques*
Actively search for and treat comorbidities that may influence mood (substance use disorders, organic diseases, other psychiatric disorders)

**= extrapolated recommendations from studies with other populations than patients with post-psychotic depression; not yet enough evidence to support their use*

Newer antidepressants have been launched in clinical use, but their efficacy profile in patients with PPD still remains unexplored [73-77]. Also, older antidepressants may be of interest for this topic, if added to atypical antipsychotics, because, as previously reported, the rate of residual depressive symptoms in this population is quite high [52,78]. Other interventions, besides antidepressants and atypical antipsychotics, are waiting to be investigated in clinical trials for depressive symptoms or major depressive episodes associated with SSD [79-81]. Adding third-generation antipsychotics to clozapine, in cases of ultra-resistance to treatment SSD cases, would also have to be considered a viable option for controlling depressive and negative symptoms [82,83]. Exploring organic, toxic, and psychiatric risk factors for treatment resistance is important when analyzing the most adequate treatment plan for patients with depression, PPD included [84-85].

CONCLUSIONS

The controversy surrounding the diagnosis of PPD is still under scrutiny, because (1) SSDs associate a high rate of suicide, even after the introduction of new generation antipsychotics, (2) there is a significant impact of depression on the quality of life and functional recovery in SSD patients, and (3) the case management of PPD may differ from the interventions recommended in patients with schizoaffective disorder, major depression with psychotic features, adverse events of different drugs with CNS depressogenic properties, and other conditions that may present themselves with depressive symptoms. The data about efficient interventions for PPD derives from older studies, therefore new trials exploring new generations of antidepressants are needed for this population. Based on the reviewed data, there are elements referring to risk factors, clinical characteristics, epidemiology, and even treatment for PPD, but they need to be validated by prospective, good-quality trials.

Conflicts of interest and sources of funding

None to declare.

Authors' contribution

OV conceived the idea of the review. OV, AGM, BMP, CAC, CT and CFP designed the study. OV, CFP, AGM, CAC, CFP, CT, DGV, CN and CAS screened the studies during the primary search. OV, AGM, CAC, CFP and CAS drafted the first version of the review. OV, CAC, CFP, DGV, CN and CAS revised the manuscript and elaborated the review's conclusions. All authors had full access to the data contained in this review and decided to submit the manuscript for publication.

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ORIGINAL ARTICLE

Evaluation of Adsorption Therapy as an Extracorporeal Blood Purification Technique in Critically Ill Covid-19 Patients

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Abstract: *We aimed to evaluate the efficacy of adsorption therapy as an extracorporeal blood purification technique in critically ill patients with Coronavirus disease 2019 (COVID-19). A retrospective analysis was performed on 17 adult patients with severe COVID-19, who underwent adsorption therapy using an HA330 cartridge, at Ankara City Hospital. Each adsorption therapy was administered for three consecutive days, and each therapy session lasted three hours. Several parameters, including cytokine levels, blood count, biochemistry panel, clinical status, and mortality rate were assessed before and after each therapy session. The results obtained from this study revealed that adsorption therapy reduced IL-6 levels and improved oxygenation in the short term. However, heart rate, mean arterial pressure and SOFA score did not demonstrate significant changes. Notably, the dose of norepinephrine increased after the third session. Regrettably, only 4 out of 17 patients (23.5%) survived. Consequently, adsorption therapy appears to be effective in reducing IL-6 levels in severe COVID-19 patients. Nonetheless, further investigation is warranted to evaluate its impact on clinical outcomes.*

Keywords: *Adsorption therapy; coronavirus disease 2019; cytokine storm; extracorporeal blood purification*

INTRODUCTION

COVID-19 infects the airway epithelium, leading to widespread damage, severe inflammation, and pneumonia with a cytopathic effect. A cytokine storm, capillary leak syndrome, lung injury, and Acute Respiratory Distress Syndrome (ARDS) are all brought on by the local and circulating cytokines that are produced at this time [1-4]. In patients with severe COVID-19, several inflammatory mediators are involved, and the cytokine storm has a deleterious impact on the disease's progression [5].

Additionally, COVID-19 exhibits immunological dysfunction. Techniques for extracorporeal blood purification have previously been utilized as supportive therapy to manage immunological dysregulation in the treatment of sepsis [6]. Despite the use of a variety of treatments, critically ill COVID-19 patients have consistently significant mortality rates. As a result, the use of cytokine filters as a therapy option for these individuals is now being given further thought.

Patients with COVID-19 have considerably higher levels of proinflammatory cytokines and chemokines like tumor necrosis factor-α, interleukin 1 and 6, granulocyte-colony stimulating factor, monocyte chemoattractant protein-1, interferon gamma-induced protein-10, and macrophage inflammatory proteins 1-α [7]. In individuals with severe COVID-19, these proinflammatory cytokines and chemokines lead to a cytokine storm. The extracorporeal removal of these cytokines from COVID-19 patients' blood circulation using an adsorption therapy is hypothesized to minimize hyperinflammation and calm the cytokine storm. Extracorporeal blood purification systems do not only remove pathogenic toxins and proinflammatory mediators; they also remove regulating cytokines. As a result, their process is non-selective. These regulatory cytokines are also advantageous because they restrain the immune system from overreacting to inflammation [8]. Therefore, while the application of extracorporeal blood purification procedures may have short-term advantages, its possible adverse effects on long-term outcomes have not yet been thoroughly assessed and understood.

An adsorption therapy, which is an extracorporeal blood purification technique can be used as a cytokine filter in severe COVID-19 patients. For this aim, an extracorporeal filter which is a cartridge with a sorbent inside is used for blood purification by combining convection and diffusion for solute removal [9]. Additionally, through ionic attraction, hydrophobic interactions, hydrogen bonds, and van der Waals interactions, the sorbent in the cartridge comes into direct contact with blood to adsorb solutes [10,11]. The sorbent in the cartridge also targets TNF, IL-1, IL-6, IL-10, IL-18, and other cytokines [11]. Thus, cytokines and solutes in the bloodstream bind to the sorbent material. Therefore, adsorption therapy can also be used for patients with autoimmune diseases, chronic uremic symptoms, intoxications, and inflammatory conditions with cytokine storms such as sepsis and pancreatitis [9,12].

For adsorption therapy in COVID-19 patients, the Jafron HA330 cartridge can be used [13]. The neutro-macroporous resin-adsorbing beads in the HA330 cartridge are constructed of the styrene-divinylbenzene copolymer. The resin beads' average diameter ranges from 0.60 to 1.18 mm and is 0.8 mm. The resin's pore sizes range from 500 D to 60 kD, whereas the HA330 cartridge removes molecular weights from 10 to 60 kDa (kiloDalton; Dalton [9,12]. Multiple pore sizes of the sorbent material allow the removal of several molecules with different molecular weights such as free hemoglobin, cytokines, complements, drugs, uremic toxins, and protein-bound or hydrophobic substances. The recommended treatment duration with an HA330 cartridge is 2-2.5 hours, while the sorbent material can be saturated if it is used for a longer period which causes a decrease in clearance efficiency [9,12].

In this study, we wanted to assess the efficacy of adsorption therapy in patients with severe COVID-19. To understand the effects of adsorption therapy using HA330 cartridges in severe COVID-19 patients in the intensive care unit (ICU), we evaluated the changes in the clinical status of patients, cytokine levels, complete blood count, and biochemistry panel before and after the administration of the therapy

MATERIALS AND METHODS

A retrospective analysis was performed on 14 male (82.3%) and 3 female (17.6%) adult severe COVID-19 patients who received adsorption therapy (HA330 cartridge, Jafron) in the ICU at Ankara City Hospital between April 2020 and October 2021 (Table 1). Before and after adsorption therapy with HA330, the patients' laboratory results and clinical outcomes were assessed. Ankara City Hospital (E2-21-983) granted permission for this study with the approval of the local ethics committee.

Table 1: Characteristics of severe COVID-19 patients treated with HA330 adsorption cartridge

Characteristics of COVID-19 patients	
Demographics	
Age (years) (median, IQR)	53.5 (45-62.5)
Gender, male (n, %) female (n, %)	14 (82.3) 3 (17.6%)
Treatment	
Methylprednisolone 40 mg (n, %)	17 (100)
IVIG (n, %)	1 (5.8)
Anakinra (n, %)	9 (52.9)
Tocilizumab (n, %)	1 (5.8)
Outcomes	
Length of ICU stay (days) (median, IQR)	22 (34)
Mechanical ventilator duration (days) (median, IQR)	20 (30)
Survived (n, %)	4 (23.5)
Died (n, %)	13 (76.5)

1 IQR: interquartile range. IVIG: intravenous immunoglobulin. ICU: intensive care unit. Anakinra: An interleukin-1 receptor antagonist. Tocilizumab: A monoclonal antibody against the IL-6 receptor. In this study, 14 male (82.3%) and 3 female (17.6%) adult patients with severe COVID-19 were treated with adsorption therapy (HA330 cartridge, Jafron©) in the ICU. The median age of the patients was 53.5 (IQR: 45-62.5). All 17 patients received a ten-day course of iv methylprednisolone 40 mg once daily. Of the patients, nine were treated with anakinra, one with tocilizumab, and one received IVIG treatment without tocilizumab or anakinra. Four patients died after the first adsorption therapy session, and another four died after the second. Nine patients completed three sessions of adsorption therapy, but five of them died due to sepsis and ARDS-related complications in the ICU. Overall, 4 out of 17 critically ill COVID-19 patients survived after adsorption therapy with an HA330 cartridge (23.5%).

Respiratory distress with peripheral capillary oxygen saturation (SpO_2) 93% in room air, dyspnea with a respiratory rate of at least 30 breaths per minute, a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO_2/FiO_2) less than 300 mmHg, and more than 50% infiltration of the lung fields are all considered to be severe COVID-19 in adult patients [14]. Moreover, cytokine release syndrome was defined as having one or more of C-reactive protein >100 (or >50 mg/L but doubled in the past 48 hours), lymphocyte count <0.6 × 109/L, serum interleukin-6 (IL-6) level higher than 3 times of the upper normal limit, ferritin >300 ug/L with doubling within 24 hours, ferritin >600 ug/L at presentation and LDH >250 U/L, and elevated D-dimer (>1 mcg/mL) [15]. These 17 patients had confirmed COVID-19 diagnoses based on real-time reverse transcription polymerase chain reaction (RT-PCR) test results and ground glass appearance with pneumonia on thorax computed tomography before admission to the ICU. They also had previously defined severe COVID-19 disease and cytokine release syndrome features. The exclusion criteria for this study included being under the age of 18, having severe heart failure with a left ventricular ejection fraction of less than 35%, having coagulation issues, and being pregnant.

All 17 patients received methylprednisolone 40 mg iv once daily for ten days. 9 patients were treated with anakinra, whereas 1 patient was treated with tocilizumab. 1 patient received immunoglobulin (IVIG) treatment without tocilizumab or anakinra.

Adsorption therapy was initially planned for 3 hours daily with an HA330 cartridge for 3 consecutive days. However, adsorption therapy could be applied to 17 patients once, 13 patients twice, and 9 patients three times. Thus, creatinine, glomerular filtration rate, urea, albumin, alanine transaminase (ALT), aspartate transferase (AST), total bilirubin, lactate dehydrogenase (LDH), troponin, fibrinogen, D-dimer, lactate, ferritin, erythrocyte sedimentation rate, C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6), hemoglobin, neutrophil/lymphocyte ratio (NLR), number of leukocytes, neutrophils, lymphocytes, and platelets were noted before and after adsorption therapy sessions. Additionally, the patients' vital signs, requirements for vasopressor medications, partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2), and Sequential Organ Failure Assessment (SOFA) scores were recorded from their medical records before and after adsorption therapy sessions.

Statistical evaluation was performed using the Statistical Packages for the Social Sciences (v17.0, SPSS 24 Inc., Chicago, IL) software. Categorical data were presented as numbers and percentages, while quantitative data were presented as mean standard deviation or median and interquartile range (IQR), as appropriate. The differences between the two groups were evaluated with the Mann-Whitney U test and the Wilcoxon test. The statistical significance level was accepted as p<0.05.

RESULTS

Adsorption therapy with a Jafron HA330 cartridge was applied once to 17 patients, twice to 13 patients, and three times to 9 patients. The median age of the patients was 53.5 (IQR: 45-62.5). The median ICU length of stay was 22 (IQR:34) days. The median mechanical ventilator duration was 20 days (IQR:30). The median APACHE II score was 18 (IQR:10). The median length of stay in the ICU before the initiation of adsorption therapy was 4 days (IQR:2-6) (Table 1).

Table 2: Clinical status of patients before and after 1st, 2nd and 3rd sessions of adsorption therapy with HA330 cartridge

Clinical parameters	Before adsorption therapy (IQR)	After 1st session (IQR)	*P**	After 2nd session (IQR)	*P**	After 3rd session (IQR)	*P**
PaO_2/FiO_2 (mmHg) (n=12)	0.59 (0.46-0.92)	0.69 (0.47-0.95)	*0.12*	0.54 (0.46-0.69)	*0.44*	0.62 (0.51-0.71)	*1.0*
HR (bpm/min) (n=13)	87.0 (66.5-100.0)	80.0 (69.5-102.5)	*0.72*	77.0 (67.5-120.0)	*0.86*	90.0 (72.8-113.8)	*0.74*
MAP (mmHg) (n=14)	80.0 (73.8-102.5)	80.0 (68.8-97.5)	*0.44*	75.0 (65.0-92.5)	*0.23*	71.5 (61.2-96.0)	*0.53*
Dose of NE (µg/kg/min) (n=9)	0.12 (0.04-0.43)	0.15 (0.03-0.67)	*0.46*	0.04 (0.12-0.17)	*0.89*	0.25 (0.07-0.19)	*0.11*

*[2] Numerical data were given as a median and interquartile range (IQR). PaO_2/FiO_2: partial pressure of arterial oxygen/the fraction of inspired oxygen. HR: Heart rate. MAP: Mean arterial pressure. NE: norepinephrine. SOFA: Sequential Organ Failure Assessment. *Wilcoxon test was used.*

The first, second, and third sessions of adsorption therapy using the HA330 cartridge are shown in Table 2 along with the patients' clinical status before and after each session. For each clinical parameter, including PaO_2/FiO_2, heart rate (HR), mean arterial pressure (MAP), norepinephrine dose (NE), and Sequential Organ Failure Assessment (SOFA) score, the median and interquartile range (IQR) are shown in the table. According to these findings, the first adsorption therapy session led to a statistically significant increase in PaO_2/FiO_2 levels (p=0.03), whereas the second and third sessions did not. None of the three sessions of adsorption therapy resulted in statistically significant changes in heart rate, mean arterial pressure, or SOFA score.

The dose of NE showed a statistically significant increase after the third session of adsorption therapy (p<0.11). Overall, these data imply that the use of an HA330 cartridge in adsorption therapy may improve oxygenation in patients with severe COVID-19, but larger sample sizes and additional research are required to confirm these results.

Table 3 shows the variations in cytokine levels, complete blood counts, and biochemistry panels in severely ill COVID-19 patients receiving first, second, and third adsorption therapy sessions using HA330 cartridges. A statistically significant decrease in IL-6 levels was observed after the first, second, and third adsorption therapy sessions (361, 86.5, 28, 20.3, p<0.03, p<0.03, p<0.007, respectively). There was a statistically significant increase in leukocyte count after the first adsorption therapy compared to the leukocyte count before adsorption therapy (12.2 to 14.6, p=0.02). However, the leukocyte count decreased from 14.6 to 12.9 after the 2nd session and increased to 13.5 after the 3rd therapy session, neither of which was statistically significant (p=0.42, p=0.12, respectively). When compared to the neutrophil count prior to the first adsorption therapy (10.9 to 13.6, p=0.03), there was a statistically significant rise in neutrophil count.

However, the neutrophil count climbed to 12.1 after the third therapy session but declined to 11.6 after the second (p=0.46 p=0.19). Neither change was statistically significant (Table 3).

Table 3: Statistical analysis of laboratory parameters of severe COVID-19 patients before and after first, second, and third sessions of adsorption therapy with HA330 cartridge

Laboratory parameters	Before adsorption therapy (n=17) (IQR)	After 1st session (n=17) (IQR)	*P**	After 2nd session (n=13) (IQR)	*P**	After 3rd session (n=9) (IQR)	*P**
Hemoglobin (g/dL)	11.6 (9.1-12.7)	11.0 (9.4-12.9)	0.64	11.0 (10.1-12.4)	0.48	11.0 (9.1-11.6)	0.22
Leukocyte Count x10^{9}/L	12.2 (9.1-14.3)	14.6 (9.7-21.0)	**0.02**	12.9 (8.8-20.2)	0.42	13.5 (8.9-14.6)	0.12
Neutrophil Count x10^{9}/L	10.9 (7.8-13.4)	13.6 (8.2-19.3)	**0.03**	11.6 (7.1-19.2)	0.46	12.1 (7.7-13.0)	0.19
Lymphocyte Count x10^{9}/L	0.64 (0.39-0.77)	0.62 (0.42-0.80)	0.96	0.55 (0.43-0.70)	0.92	0.48 (0.38-0.78)	0.82
Platelet count x10^{9}/L	262.0 (200.0-303.0)	274.0 (162.0-328.0)	0.47	266.0 (195.0-350.0)	0.75	213.0 (175.0-307.0)	0.60
NLR	20.2 (15.9-32.8)	28.8 (17.9-42.1)	**0.03**	24.3 (22.1-49.7)	0.20	23.4 (18.7-29.3)	0.80
Glucose mg/dL	189.5 (114.3-236.8)	211.0 (146.5-224.0)	0.51	153.0 (128.0-205.5)	0.38	151.0 (118.5-210.5)	0.53
Creatinine mg/dL	0.93 (0.56-1.93)	0.86 (0.64-2.0)	0.64	1.0 (0.73-2.40)	0.03	1.09 (0.62-1.30)	0.26
Estimated GFR ml/min/m^{2}	88.0 (41.0-118.0)	94.0 (43.5-112.5)	0.60	78.0 (30.0-99.5)	0.11	83.5 (59.0-108.0)	0.25
Urea mg/dL	62.0 (42.8-98.0)	65.5 (50.3-132.8)	0.06	84.5 (52.3-161.3)	0.07	73.0 (64.0-105.0)	0.24
Albumin g/L	29.0 (26.0-32.0)	28.0 (22.5-31.5)	**0.04**	30.0 (28.3-31.8)	0.20	28.0 (26.3-30.8)	0.49
ALT U/L	46.5 (27.8-126.8)	59.0 (30.0-200.0)	0.55	51.0 (30.0-225.5)	0.64	66.0 (26.0-100.5)	0.99
AST U/L	37.0 (27.3-81.0)	59.0 (32.5-228.0)	0.84	58.0 (22.5-180.5)	0.51	45.0 (21.0-63.0)	0.30
Total bilirubin mg/dL	0.9 (0.5-1.2)	0.8 (0.5-1.2)	0.84	0.6 (0.5-0.8)	0.18	0.75 (0.4-1.3)	0.21
Troponin Ng/L	49.0 (8.8-423.1)	114.4 (24.0-647.0)	0.74	186.0 (22.8-360.7)	0.07	-	-
INR	1.4 (1.2-1.7)	-	-	1.3 (1.2-1.48)	0.60	-	-
Fibrinogen g/L	6.4 (3.7-7.2)	6.5 (3.8-7.3)	0.51	5.7 (3.4-7.8)	0.65	5.0 (1.6-7.0)	0.64
D-dimer mg/L	5.8 (1.9-13.6)	4.9 (2.4-7.3)	0.35	8.6 (1.8-17.7)	0.44	7.90 (4.4-22.0)	0.98
Lactate	2.3 (1.6-3.2)	1.9 (1.3-2.9)	0.07	1.8 (1.4-2.4)	0.21	-	-
LDH U/L	600.5 (516.8-766.5)	659.0 (543.0-963.5)	0.65	671.0 (468.5-889.0)	0.97	578.5 (488.8-674.5)	0.39
Ferritin ml/ng	961.0 (393.5-1540.0)	1500.5 (880.3-5546.0)	0.06	1461.0 (1025.5-3667.0)	0.58	1236.5 (377.5-1611.5)	0.12
ESR mm/h	56.0 (21.8-108.3)	78.0 (15.0-125.0)	0.07	65.0 (22.5-103.0)	0.72	-	-
CRP mg/L	0.16 (0.13-0.218)	0.15 (0.13-0.25)	0.51	0.15 (0.09-0.17)	0.10	0.10 (0.06-0.14)	0.09
Procalcitonin µg/L	1.01 (0.17-11.3)	4.2 (0.28-11.6)	0.44	0.53 (0.22-8.84)	0.35	0.19 (0.11-0.58)	0.06
IL-6 pg/mL	361.0 (68.2-739.8)	86.5 (10.9-167.7)	**0.03**	28.0 (20.8-76.9)	**0.03**	20.3 (7.6-32.4)	**0.007**

*[3] Numerical data are given as a median and interquartile range (IQR). NLR: neutrophil-lymphocyte ratio, GFR: glomerular filtration rate, ALT: Alanine transaminase, AST: Aspartate transferase LDH: lactate dehydrogenase, INR: international normalized ratio, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, IL-6: interleukin-6. (-): data could not be retrieved. There was a statistically significant decrease in IL-6 levels after the first, second, and third adsorption therapy sessions (361, 86.5, 28, 20.3, p <0.03, p<0.03, p<0.007, respectively). *Wilcoxon test was used*

There was a statistically significant increase in the neutrophil-lymphocyte ratio (NLR) after the first adsorption therapy compared to the NLR before adsorption therapy (20.2 to 28.8, p=0.03). Although the NLR dropped from 28.8 to 24.3 after the second session and then further to 23.4 after the third (p=0.20 and p=0.80, respectively), neither of these decreases was statistically significant. When compared to the albumin level before the initial adsorption therapy, there was a statistically significant drop in albumin (29.0 to 28.0, p=0.04). However, albumin increased from 28.0 to 30.0 after the second session and reduced to 28.0 after the third (p=0.20 and p=0.49, respectively), neither of which was statistically significant.

No statistically significant changes were observed in creatinine, glomerular filtration rate, urea, alanine transaminase (ALT), aspartate transferase (AST), total bilirubin, lactate dehydrogenase (LDH), troponin, fibrinogen, D-dimer, lactate, ferritin, erythrocyte sedimentation rate, CRP, procalcitonin, hemoglobin, glucose, lymphocyte, or platelet count after the first, second, and third sessions of adsorption therapy with HA330 cartridges. Four patients died after the first, and another four patients died after the second adsorption therapy session. Nine patients completed three sessions of adsorption therapy. However, five of these nine patients died due to sepsis and ARDS-related complications in the ICU. Only 4 out of 17 critically ill COVID-19 patients survived after adsorption therapy with an HA330 cartridge (23.5%).

DISCUSSION

There are a limited number of publications in the English medical literature related to the use of extracorporeal blood purification techniques with the United States Food and Drug Administration (FDA) approved filters such as oXiris filter (Baxter, Deerfield, IL), CytoSorb filter (CytoSorbents, Monmouth Junction, NJ), Seraph 100 Microbind blood affinity filter (ExThera Medical, Martinez, CA) and the Spectra Optia Apheresis System with the Depuro D2000 Adsorption Cartridge (Terumo BCT, Lakewood, CO) for the treatment of COVID-19 patients [8]. According to Padala et al., using an oXiris filter improved clinical outcomes in two out of three patients and reduced levels of interleukin-6, erythrocyte sedimentation rate, and CRP [16]. According to Zhang et al., CRP, IL-6, IL-8, IL-10, APACHE II, and Sequential Organ Failure Assessment (SOFA) scores all significantly decreased after oXiris filter treatment, while IL-4, IL-8, and procalcitonin showed a nonstatistically significant downward trend. Unfortunately, two of five patients passed away despite treatment [2]. Ugurov et al. reported using oXiris in ten patients with COVID-19. Two of the ten patients passed away related to complications of ARDS. According to Ugurov et al., the number of white blood cells and thrombocytes increased to the normal range whereas IL-6, CRP, and fibrinogen levels decreased [17]. According to Villa et al., treatment with oXiris resulted in a significant drop in IL-6 levels as well as improvements in hemodynamic stability, multiorgan dysfunction, and oxygenation index in 37 patients [18].

Case studies of COVID-19 patients who received treatment with a CytoSorb filter were described by Rizvi et al., Melegari et al., and Berlot et al. [1, 19, 20]. All three patients survived with an improvement in CRP, IL-6, and oxygenation. Rieder et al. treated 4 patients under venovenous extracorporeal membrane oxygenation (vv-ECMO) support with cytokine adsorption with CytoSorb filter, which decreased IL-6 in COVID-19 patients [21]. According to Alharthy et al., continuous renal replacement therapy (CRRT) sessions using a CytoSorb cartridge were administered to 50 patients. Thirty-five patients who survived after CytoSorb therapy had decreased ferritin, lactate dehydrogenase, CRP, IL-6, and D-dimer levels [15].

According to Olson et al., the Seraph-100 filter was utilized in two COVID-19 patients, and as a result, post-treatment vasopressor dose, IL-6, and CRP levels decreased [22]. The use of a Seraph-100 filter on a patient with COVID-19 significantly decreased D-dimer level, according to Pape et al. [23]. Four hemodialysis patients with severe COVID-19 were treated using a Seraph-100 haemoperfusion device, according to Sandoval et al. Despite findings from other research, Sandoval et al. failed to find any appreciable drop in IL-6, D-dimer, or ferritin levels following the treatment [24]. According to Schmidt et al., 78 COVID-19 patients who received treatment with a Seraph-100 microbind affinity blood filter were evaluated. According to Schmidt et al, patients who received Seraph-100 treatment within 60 hours of ICU admission had a reduced mortality rate [25].

Reverse Takotsubo cardiomyopathy associated with COVID-19 was treated with therapeutic plasma exchange utilizing a Spectra Optia apheresis system outfitted with a Depuro D2000 adsorption cartridge, according to Faqihi et al. Following treatment, both lactate levels and left ventricular function returned to normal [26].

Koc et al. published a review of hemadsorption therapy in severe COVID-19 patients [13]. 86 patients with severe COVID-19 were included in 16 trials that were examined by Koc et al. CytoSorb®, oXiris®, Biosky filter, SeaStar® CLR filter, HA280, HA330 Jafron, and resin-directed hemadsorption cartridges were used in hemoperfusion therapy. According to Koc et al., the mortality rate, mean intubation time, and length of stay in the ICU and hospital were, respectively, 29%, 14.93 days, 17.21 days, and 31.7 days. Following hemoperfusion sessions, the mean levels of CRP and interleukin-6 reduced (131.7 to 66.0 and 527.5 to 334.7, respectively). According to Koc et al., hemoperfusion and cytokine adsorption reduced cytokine levels, CPR, and IL-6 relative to pre-therapy, decreased mortality to 29%, and improved clinical conditions. However, these outcomes lacked statistical significance. Hemadsorption therapy was described by Koc et al. as an alternate salvage therapy for COVID-19 patients who were critically unwell. Moradi et. al reported a 73-year-old male patient was treated with hemoperfusion (HA230 cartridge, Jafron) due to a cytokine storm for 4.5 hours [27]. Moradi et al. claimed that the symptoms and need for oxygen support were alleviated during hemoperfusion treatment.

Theoretically, blocking the formation of proinflammatory cytokines can reduce hyperinflammation. The clinical outcome of severely ill COVID-19 patients can be improved by reducing hyperinflammation. Thus, anti-interleukin-6R monoclonal antibodies and corticosteroids were recommended to reduce the inflammatory response. However, IL-6 promotes neutrophil-mediated viral clearance by initiating a preliminary response against viral infections [7]. Moreover, Dienz et al. reported that IL-6 or IL-6 receptor-deficient mice could not resist Influenza A infection, which caused pulmonary damage leading to death [28]. Despite its theoretical benefit, Clark et al. reported that hemoperfusion was an experimental treatment because of its limited evidence of efficacy [29]. However, Kang et al. reported that in patients with severe COVID-19 and non-acute kidney injury, continuous renal replacement therapy using an oXiris filter might not be able to successfully reduce cytokine release syndrome [30].

Hemadsorption therapy has several additional drawbacks. Arrhythmia, coagulation issues, thrombocytopenia, and bleeding were listed as the most typical short-term hemoperfusion consequences in patients with COVID-19 by Darban et al. [31]. However, the majority of the difficulties emerged on the second and third days following hemoperfusion, not during hemoperfusion itself.

A highly biocompatible sorbent covered with a surface that inhibits platelet adherence and clotting activation is necessary for hemoperfusion. The various sorbent types utilized determine how hemoperfusion removes substances, with the effective surface area being a key factor. The sorbent material within the hemoperfusion cartridge can both absorb and lead to the loss of antibiotics and nutrients from the bloodstream [9, 10, 32].

The Jafron HA330 cartridge was the only available single-use hemoperfusion cartridge at Ankara City Hospital. Thus, we performed adsorption therapy on severe COVID-19 patients in the ICU with single-use Jafron HA330 cartridges. We used adsorption therapy as a salvage therapy when the patients did not respond to other treatment options such as antibiotics, corticosteroids, hydroxychloroquine, immunoglobulin, convalescent plasma, tocilizumab or anakinra [33].

There is still no single laboratory parameter with a cut-off value identified to initiate adsorption therapy. Moreover, the duration of therapy and the parameters to monitor the patient’s response to the treatment are still under investigation [6]. Unfortunately, there is no universally accepted standardized adsorption therapy protocol. We preferentially used adsorption therapy to treat severely ill COVID-19 patients with low procalcitonin but high CRP and IL-6 levels.

Except for a decline in IL-6, we were unable to find a statistically significant link between the quantity of adsorption therapy sessions and laboratory values. The majority of the research previously cited showed that adsorption treatment reduced IL-6 levels. However, anakinra and tocilizumab might be responsible for the IL-6 decline in COVID-19 patients [34].

Moreover, our study has certain drawbacks. First of all, the sample size was quite small. Secondly, during the pandemic, we mostly served severe COVID-19 patients in our intensive care unit, which restricts the applicability of our findings to other patient populations. Finally, because a control group was not included in our investigation, additional variables, such as medication modifications and illness progression, might have an impact on the observed changes in laboratory values. Hopefully, our study contributes to the growing body of research on the use of adsorption therapy in COVID-19 patients despite these limitations.

CONCLUSION

After examining this study's data, it can be concluded that in patients with severe COVID-19, the use of an HA330 cartridge during adsorption therapy was connected to a statistically significant drop in IL-6 levels after the first, second, and third therapy sessions. Except for a rise in the leukocyte and neutrophil counts following the first session, there were no statistically significant associations between the quantity of adsorption therapy sessions and other laboratory findings.

To draw a definitive conclusion about the effect of adsorption therapy with HA330 cartridges on COVID-19 patients, further studies with larger and more diverse patient populations are needed. These studies should also consider other potential confounding factors, such as the administration of other treatments and underlying medical conditions. Nevertheless, the findings of this study point to the possibility that adsorption therapy could be a potential supplementary treatment option for individuals with severe COVID-19.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, M.E.Y, N.H.S., C.M.A., S.I.; methodology, M.E.Y., S.I.; software, M.E.Y.; validation, N.H.S., C.M.A., M.E.Y., S.I.; formal analysis, M.E.Y.; investigation, M.E.Y.; resources, N.H.S., C.M.A., M.E.Y., S.I.; data curation, N.H.S, C.M.A, M.E.Y., S.I.; writing-original draft preparation, M.E.Y.; writing-review and editing, N.H.S., C.M.A., M.E.Y., S.I.; visualiza-tion, M.E.Y.; supervision, S.I.; project administration, S.I. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Ankara City Hospital (E2-21-983).

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ORIGINAL ARTICLE

Psychometric Properties of the Method "Evaluation of Negative Mental Reactions and States of Combatants" and Experience of its Application in Short-term Psychological Recovery

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Abstract: *The article shows the development of the method of psychological evaluation called "Evaluation of Negative Mental Reactions and States of Combatants". In the study participated 1300 male servicemen (29.84% from junior lieutenant to colonel and servicemen under contract and demobilized, and 70.16% from private to senior warrant officer). The age of participants varied from 20 to 55 years. The system of evaluation developed consisted of 16 instruments that could help to determine the presence of negative psychological symptoms of servicemen related to their participation in hostilities. The results indicated that the evaluation method developed is a tool that allows determining the presence of negative psychological symptoms related to participation in combat. Likewise, it is a useful and fast method to assess the effectiveness of short-term psychological recovery programs. Unlike existing methods of diagnosing negative mental reactions and states of an individual, which arose after their participation in hostilities, the developed psychodiagnostic toolkit could consider the physical and mental fatigue of the respondents, their deterioration, and other cognitive dysfunctions*.

Keywords: *combat activity, psychological rehabilitation, psychological recovery, post-traumatic stress disorder, evaluation system*

INTRODUCTION

Long participation in hostilities depleted the psychological adaptation resources of servicemen, sharply posing the question concerning the need for psychological recovery [1,2]. This issue was complicated and comprehensive; it related both to the definition of rotation of servicemen and to the determination of the duration and development of effective programs of psychological recovery for all participants of hostilities [3] as well as to the determination of indications for medical and psychological rehabilitation of the servicemen, whose mental health and ability to readaptation was lost [4]. The aim of psychological recovery was to restore the physical and mental resources of servicemen to prolong their ability to perform tasks under the conditions of combat stress factors action [5].

Nowadays, the psychological decompression measures were directed not only to ensuring the adequacy of switching from military service to peaceful life [6], including traditional stress relief and help in civilian life realization, taking into account the acquired military experience, accepting this experience to reduce the probability of post-traumatic stress disorders (PTSD) formation [7,8], but also for the psychological restoration of mental health of servicemen who participated in hostilities to relieve the accumulated stress prolonging the possibility of adequate functioning of servicemen under combat conditions when it was impossible to realize the massive rotations to keep their resistance – psychological stability, ability to resist negative influence from the side of combat stress factors [2]. To the institutions of psychological recovery were sent the servicemen with expressed signs of maladjustment [9], acute stressful reactions [7], significant exhaustion, devastation, and fatigue [10], and servicemen, who had difficulties in adaptation [11] while getting back in line after injuries and concussion and after their stay in hospitals.

Institutions that implemented psychological recovery programs were located in the third echelon of defense in safe places in order to form in the minds of servicemen the idea of the need to return to the performance of combat missions, and military duty, which was a way of posing the direction of recovery – the restoration of combat capacity.

Realization of activities of psychological rehabilitation and implementation of existing psychological programs of decompression on the basis of their personal professional experience and world experience of rehabilitation of servicemen was imposed on the military psychologists of the Armed Forces and the National Guard of Ukraine, as well as on the volunteer psychologists from NGOs who had appropriate training and experience. Usually, the implementation of psychological recovery programs was a combination of procedures of medical treatment orientation with the use of appropriate material and technical bases and the involvement of appropriate specialists.

Even though the system of implementation of the psychological restoration of combat servicemen was well-established and proved its effectiveness in practice, there arose a question on the development of effective psychodiagnostic tools of the mental state control of servicemen before and after a complex of actions by the program of psychological rehabilitation for individual servicemen and correction of the recovery program if required. There were distinguished the following requirements for the psychodiagnostic method:

1) convenient while conducting both individual and group psycho-diagnosis of servicemen, typical for a survey with a large number of people (100-200 people), capable of taking into account the physical and mental fatigue of the respondents, deterioration of their attention, and other cognitive dysfunctions related to stressful experience;

2) suitable for the diagnosis of negative mental reactions and conditions that occur after participation in hostilities;

3) reliable for re-diagnosis due to a minor period of time, excluding the ability to remember accurately their past answers;

4) able to clearly demonstrate changes in psychological state that occurred during their participation in the psychological recovery program;

5) convenient for summarizing data and receiving the psychologist's feedback on the effectiveness of the introduction of new actions into the programs of psychological recovery;

6) useful for obtaining information related to the effectiveness of individual consulting, providing a psychologist with a reflection on his/her professional activity. With existing pressure on psychologists, there occurred a need for supervision and their rotation in institutions that dealt with psychological recovery.

The objective of the study was the development of a psychological evaluation system of the consequences of participation in combat activities for military personnel participating in hostilities. Unlike the existing systems to diagnose the psychological consequences of combat participation, it is intended to develop a set of psychodiagnostic tools that assess the physical and mental fatigue of combatants, their deterioration, and other cognitive dysfunctions. And also, that it is capable of clearly showing the psychological changes achieved by participation in psychological recovery programs.

MATERIALS AND METHODS

Participants and Procedure

The study was conducted in 2022. The study included 1300 servicemen who underwent a psychological recovery program after taking part in a long combat mission. The tested group consisted of officers – 29.84% (from junior lieutenant to the colonel) and servicemen under the contract and mobilized – 70.16% (from private to senior warrant officer). The age of the study participants varied from 20 to 55 years.

While developing the method "Evaluation of negative mental reactions and states of combatants", there were combined the simplest procedures of conditions evaluation which were typical for the method such as "Profile of mood states" (POMS) [12,13], "Self-esteem of psychophysiological state" (Kokun) [14], "Measurement of states" used to express evaluation of emotional states of sportsmen [15]. Also, there was conducted analysis of negative mental reactions and states that often occurred in servicemen while their participation in hostilities, including those who described the ability to perform tasks and the ability to interact with others in the performance of command work [1,16]. Thus, the list of negative mental reactions and states (experience) was quite wide and contained 16 points: irritability; anxiety; aggressiveness;

anger; inattention; self-doubt; devastation; apathy; concern; sense of guilt; sense of powerlessness; lack of concentration; unwillingness to communicate; lack of trust in comrades in the service; lack of trust in commanders; inability to perform the assigned tasks. It was assumed that the use of factor analysis would give the possibility to group these points into the scale.

It was necessary to point out that the simplicity of the instructions and of the testing procedure itself was an important circumstance for effective diagnostic work with servicemen who had been experiencing the influence of combat stress factors for a long time and who had difficulty in the redistribution of attention.

However, a number of points (16) of the evaluation of negative mental states but the evaluation scale itself (from 0 to 10 points) was quite large (exceeding the amount of short-term memory 7±2 elements), which complicated their memorization and accuracy reproduction during re-testing.

The determination of psychometric indicators of the method “Evaluation of negative mental reactions and states of combatants” and its testing was carried out during the participation in a psychological recovery program by servicemen. 1300 servicemen participated at this stage.

The servicemen filled out the form of the method at the beginning of the psychological recovery program and after it. The results obtained in accordance with the method “Evaluation of negative mental reactions and states of combatants” were compared with the results of methods that were conducted with servicemen to control their condition before and after their participation in the psychological recovery program. Before participating in a psychological recovery program, servicemen were surveyed with the following methods: “Maladjustment” (Prykhodko, Matsegora, Kolesnichenko, and Baida) [17], “Mississippi Scale for Combat-Related PTSD” (Keane, Caddell, and Taylor) [18], “Diagnostics of psychological safety of an individual” (Prykhodko, 2012) [19], “Evaluation of traumatism of combat experience of servicemen” (Kolesnichenko) [20]. After participating in the psychological recovery program, servicemen were surveyed in accordance with the following psychodiagnostic methods – “Resistance to psychological traumatism of combat experience” (Kolesnichenko) [21].

All procedures in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants consent for their data to be used in this research.

Instruments and Measures

To determine the constructive validity of the method "Evaluation of negative mental reactions and states of combatants" there were used methods which were standardized on the Ukrainian selection.

Method "Maladjustment" (Prykhodko, Matsegora, Kolesnichenko, and Baida) [17] was an express version of the method "Adaptability" (Maklakov) [22] which had a long tradition of use in the Armed Forces of Ukraine. The "Adaptability" method was concluded by military psychologist A.H. Maklakov [22] to determine the adaptation potential of servicemen based on the individual scales of the method "Minnesota Multiphasic Personality Inventory" (McKinley, Hathaway) [23]. The developer reported satisfaction with the psychometric characteristics of the formed methodology [22]. Since the beginning of hostilities in eastern Ukraine among military psychologists of Ukraine, there was spread a modification of the methodology proposed by Maklakov "Adaptability 200", carried out by Chermianin and Kondratiev [24]. Unlike the previous method, it was supplemented with a number of issues that allowed us to distinguish "military and professional orientation", "tendency to deviant behaviors", and "suicidal risk" and to calculate "the level of resistance to combat stress". This method included 200 questions; thus, there was a need to form a simpler and more reliable toolkit, which was the basis for the development of an express version of the method. The developed method "Maladjustment" (Prykhodko, Matsegora, Kolesnichenko, and Baida) [17] was aimed at the identification of specific violations of adaptation potential which could have a big influence on the ability to adequately perform the tasks. Thus, the method had 4 scales, each with 10 questions – "Violation of behavioral regulation", "Probability of committing suicidal attempts", "Violation of moral normativity" and "Loss of communicative potential". There was highlighted the calculation of the general indicator of "Maladjustment". This calculation was simple and convenient and could be carried out under field conditions without any additional tools. Although working with the method involved the presence of trust in psychologists, the method had a scale of sincerity. The developers provided data about the satisfactory psychometric indicators of the method.

The study used the "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor), which was restored for the Ukrainian selection [18]. Nowadays, it was one of the most common methods used to measure the signs of PTSD. The scale included 35 statements, each of which can be estimated on a five-point scale. The total indicator which was made up in accordance with this method gave the possibility to estimate the degree of influence of the traumatic experience transferred by an individual.

"Diagnosis of Psychological Safety of an Individual" (Prykhodko), which was developed on the basis of the concept of psychological safety of an individual of specialists of extreme activities [19]. According to this concept, psychological safety was not only a lack of danger but also an opportunity for development and self-realization. Psychological safety had a level structure. Each level in the method met a scale with a similar name. The first level was moral and communicative, it meant that knowledge and use of the rules of communication and norms of morality made interaction with the social environment controlled and predictable, formed the ability to escape potentially conflict situations, and involved external resources to overcome problematic situations. The motivational and volitional levels suggested that the developed goal setting, intensity in goal setting, and flexibility in their corrections could ensure the self-realization of an individual in difficult conditions of life. It was assumed that the ability to flexible restructuring of relations with the environment gave the possibility to be leading in these relationships, to control them. The valuable and semantic level was considered as an ability to conscious attitude to one's own life, awareness of one's own influence on the world, and responsibility for its changes. The developer believed that this conscious attitude gave the possibility for an individual to tolerate life difficulties and sufferings that arose in the process of self-realization. Moreover, due to the developer, the hierarchy of the semantic sphere gave the possibility to depreciate the experience in frustration as unimportant and to consolidate efforts to achieve important aims. The level of internal comfort was the factor that provided a change in the direction of activity. Thus, the evaluation of life as safe, and the condition as comfortable contributed to the decision to continue normal regulation. The decisions related to the changes were made in case of non-compliance of the environment with the needs and capabilities of an individual. There was highlighted the calculation of the general indicator of psychological safety of an individual, its height indicated the ability to set new goals of self-realization. When the indicators were reduced the thing was in a supportive reproduction of usual goals of life or in the arrest of development, and further impossibility of self-realization. The developed method had satisfactory psychometric indicators. The National Guard of Ukraine had been using this method for more than 10 years.

The method of "Evaluation of traumatism of combat experience of servicemen" [20] was based on the American method "Combat Exposure Scale" [25]. In the process of its development, there were considered the traumatic events and their intensity which were typical for military actions in Ukraine with the beginning of the Russian invasion in 2014. Unlike the "Combat Exposure Scale" method, the method of "Evaluating of traumatic experience of servicemen" (Kolesnichenko) had been developed; it included a list of phenomena that gave the possibility to reduce the trauma of combat stress factors influence. The method had 5 scales that met 3 groups of combat stress factors and 2 factors that described internal and external resources used to counteract stress factors. In the process of standardizing the method, there was used the procedure of regressive analysis which gave the possibility to determine the contribution of each scale to acute stress disorder which occurred due to the action of combat stress factors. It was reported that the method had satisfactory psychometric characteristics.

The method "Resistance to psychological traumatism of combat experience" (Kolesnichenko) [21] was developed on the basis of the author's concept of combat psychological traumatism of servicemen [1]. The method reflected the given in this concept three levels (stages) of realization of resistance to the action of combat stress factors, which 1) were related to the preparation for activity under the conditions of combat stress factors and under the existing expectations concerning the stress factors' activity and their ability to overcome them, 2) had the direct ability to overcome stressful influence and used adequate situational copings, and 3) had the ability to accept and realize the acquired experience under the stressful conditions. There was provided the calculations of general resistance to combat psychological traumatism. It was reported that this indicator had a reverse correlation with the "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18], r = -0.60, p≤0.01. Moreover, the method of the scale of the second order – "helplessness", "conscious attitude to professional training", "ability to acquire experience", "cognitive dysfunction", and "attitude to military duties". The method had satisfactory psychometric indicators.

Data Analysis

To represent the data, there was used the main descriptive statistics (arithmetic mean M and standard deviation SD). To distinguish the correlational rate between the variables there was used the correlation analysis. To determine the internal coherence of the method "Evaluation of negative mental reactions and states of combatants" there were used calculations based on Cronbach's alpha. To determine the specific negative mental reactions and states which were experienced by servicemen due to their participation in hostilities there was used regression analysis. To determine the structure of the method "Evaluation of negative mental reactions and states of combatants" there was used factor analysis with the help of the method "Principal components". Mathematical data was processed with the help of SPSS 17.0.

RESULTS

We have developed the following form of psychodiagnostic method "Assessment of negative mental reactions and states in combatants" (Table 1). The instructions are as follows:

"Please assess your psychological state at the moment on a scale from 0 - slightly expressed, 10 - strongly expressed". The internal consistency of the method was evaluated using the α-Cronbach indicator, which was 0.944 having the selection of 1300 people for 16 indicators.

Table 1: Form of the methodology "Evaluation of negative mental reactions and states of combatants"

Mental reactions and states	Evaluation of reactions and states										
Irritability	0	1	2	3	4	5	6	7	8	9	10
Anxiety	0	1	2	3	4	5	6	7	8	9	10
Aggressiveness	0	1	2	3	4	5	6	7	8	9	10
Anger	0	1	2	3	4	5	6	7	8	9	10
Inattention	0	1	2	3	4	5	6	7	8	9	10
Self -doubt	0	1	2	3	4	5	6	7	8	9	10
Devastation	0	1	2	3	4	5	6	7	8	9	10
Apathy	0	1	2	3	4	5	6	7	8	9	10
Concern	0	1	2	3	4	5	6	7	8	9	10
Sense of guilt	0	1	2	3	4	5	6	7	8	9	10
Sense of powerlessness	0	1	2	3	4	5	6	7	8	9	10
Lack of concentration	0	1	2	3	4	5	6	7	8	9	10
Unwillingness to communicate	0	1	2	3	4	5	6	7	8	9	10
Lack of trust in comrades in the service	0	1	2	3	4	5	6	7	8	9	10
Lack of trust in commanders	0	1	2	3	4	5	6	7	8	9	10
Inability to perform the assigned tasks	0	1	2	3	4	5	6	7	8	9	10

Despite the fact that the developed method included negative mental reactions and states for evaluation of PTSD, and maladjustment, and testified both negative experiences and inability to manage their behavior, its purposefulness and inability to maintain interaction and consistency in teamwork, factor analysis which was conducted in accordance with "Principal Components" presented the presence of only one factor that included all 16 variables with different factors of factor evaluation: "irritability" (0.743), "anxiety" (0.796), "aggressiveness" (0.692), "anger" (0.722), "inattention" (0.744), "self-doubt" (0.744), "devastation" (0.808), "apathy" (0.733), "concern" (0.808), "sense of guilt" (0,793), "lack of concentration " (0.801), "unwillingness to communicate" (0.714), "lack of trust in comrades in the service" (0.640), "lack of trust in commanders" (0.675), "inability to perform the assigned tasks" (0.746).

Taking into account the received data, the method included the calculations of general indicators. Experience using the method had shown that the most convenient indicator was the calculation of the average arithmetic value for all 16 indicators. Thus, the general indicator had a range from 0 to 10 points, which was convenient to perceive general results by the method and did not require memorization of additional information for manual data processing.

It was necessary to point out that the selection of a single-factor structure of the method was a certain confirmation of less regularity of negative mental reactions and states, a reflection of greater integration of mental processes while operating under stressful conditions.

The determination of the validity of the method was conducted in two stages. In the first stage, to determine the constructive validity, the data of the method "Evaluation of negative mental reactions and states of combatants" was compared with the data of methods which gave the possibility to diagnose the peculiarities of negative mental reactions and states of servicemen, proving that they were related to their participation in hostilities. First of all, the talk was about the method "Maladjustment" which gave the possibility to evaluate the violation of "behavioral regulation", "the probability of committing suicidal attempts", "violation of moral normativity" and "loss of communicative potential" by servicemen [17], and "Mississippi Scale for Combat-Related PPSD" (Keane, Caddell, and Taylor) [18], which gave the possibility to diagnose PTSD symptoms of those who had participated in hostilities and left the battlefield [24]. Moreover, there was used the method of "Evaluation of traumatic experience of servicemen" (Kolesnichenko) [20] and "Resistance to psychological traumatism" (Kolesnichenko) [21], which in this study gave the possibility to connect the experience of negative mental reactions and states with the experience of participation in hostilities, as well as with their peculiarities and preparation for them.

Correlational relations with the method of "Diagnosis of psychological safety" (Prykhodko) [19] indicated the depth of violation of personality structures, which ensured its safety and ability to develop, which was an important factor for making a prognosis for the restoration of an individual in the process of psychological decompression and rehabilitation. The results of correlational analysis, which were obtained at this stage of validity determination were given in Table 2. Considering that the selection of the study at this stage included 1300 people, then all the correlational indicators given reached the level of statistical significance and did not require additional indicators.

Dense correlation relations of the method "Evaluation of negative mental reactions and states of combatants" and methods such as "Maladjustment" (Prykhodko et al.) [17] and "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18] pointed out that according to the data provided by the developed method, it was really possible to consider negative experience, which was connected with adaptation disorders and PTSD symptoms. The data provided by the method "Evaluation of traumatic experience of servicemen" [20] proved that negative experience was mostly related to the force of negative effects than to the existing external support.

However, the formed personality structure (according to the method "Diagnosis of Psychological Safety" [19] including the process of professional and professional-psychological training, due to the method "Evaluation of traumatism of combat experience of servicemen" [20] was able to restrain the development of negative states, providing adequate functioning of an individual under combat conditions. But at this stage, it was impossible to monitor the accumulated influence of traumatic events on the development of negative mental states while exceeding the recommended time for rotation (more than 6 months).

The second stage of determination of the method's validity was developed to determine its ability to be a tool for tracking the dynamics of changes during psychological recovery. The methods which were used at the first stage had satisfactory indicators of reliability, and, therefore, could be insensitive to the changes which occurred during the week (this was a usual term of the program of psychological recovery of servicemen). The method of "evaluation of negative mental reactions and states of combatants" was developed specifically for evaluating the control of negative states' changes before and after participation in the psychological recovery program, including the possibility to evaluate its effectiveness. Considering such purpose, the data obtained by the method "evaluation of negative mental reactions and states of combatants" before and after the participation in the recovery program was compared to the data obtained by Lüsher's color test including the indicator of anxiety. This indicator pointed out the availability of frustrated needs and inadequacy of compensation and, unlike other indicators by this projective method, could be confused with indicators by other methods. The indicator of anxiety in accordance with Lüsher's color test was used as an integrated state evaluation since the main determinants of mental states were the ratio: firstly, conscious and unconscious needs, desires, and aspirations of an individual; secondly, the capabilities of an individual (obvious abilities and hidden potentials); thirdly, environmental conditions (objective influence and subjective perception of the current situation) [25].

40 servicemen participated at this stage of the study. Thus, the general indicator by the method "evaluation of negative mental reactions and states of combatants" correlated with anxiety before the beginning of the decompression program at a moderate level – $r = 0.41$, $p \leq 0.01$; after the decompression program the data was – $r = 0.47$, $p \leq 0.01$. These data proved that the developed method was sensitive to the changes in states and could be used to evaluate the dynamics of states during the programs of psychological recovery which were developed for short-term (up to a week).

It was figured out that the general indicator (see Table 2) had a slightly higher density than individual indicators by the method "evaluation of negative mental reactions and states of combatants" with general indicators of maladjustment, PTSD, psychological safety of an individual, traumatism of combat experience, and resistance to psychological traumatism. It was another piece of evidence that the method would be more reliable if it was used as a single scale.

Then there was used the procedure of regression analysis to determine specific negative mental reactions and states experienced in these psychological categories; thus, there were formed relevant regression equations:

Maladjustment = 3.804 + 0.216*Irritability + 0.307* Aggressiveness + 0.259*Self-doubt + 0.249*Devastation + 0,245*Concern + 0.386*Reluctance to communicate + 0.229* Lack of trust in comrades in the service + 0.350*Lack of trust in commanders +0.264* Inability to perform the assigned tasks + 0,529.

Post-traumatic stress disorder = 54.66 + 1,070*Irritability + 0.719*Aggressiveness + 0.572*Inattention + 0.557* Devastation + 1.230*Apativity + 0.794*Concern + 0.613* Unwillingness to communicate + 0.602*Lack of trust in commanders +0.941* Inability to perform the assigned tasks +0.506.

Table 2: The indicators of correlative relations between the scales of method "Evaluation of negative mental reactions and states of combatants" and the scales of other psychodiagnostics methods which were used to estimate its validity (in points).

Indicators	"Estimation of negative mental reactions and states of combatants"																
	Irritability	Anxiety	Aggressiveness	Anger	Inattention	Self-doubt	Devastation	Apathy	Concern	Sense of guilt	Sense of powerlessness	Lack of concentration	Unwillingness to communicate	Lack of trust in comrades in the service	Lack of trust in commanders	Inability to perform the assigned tasks	General indicators due to the method
"Maladjustment" (Prykhodko, Matsegora, Kolesnichenko, and Baida) [17]																	
Violation of behavioral regulation	0.50	0.56	0.46	0.44	0.49	0.51	0.52	0.51	0.54	0.41	0.54	0.52	0.49	0.42	0.49	0.55	0.68
Probability of committing suicidal attempts	0.41	0.52	0.36	0.39	0.47	0.48	0.54	0.48	0.49	0.41	0.53	0.49	0.48	0.38	0.42	0.51	0.62
Violation of moral normativity	0.34	0.24	0.37	0.33	0.20	0.16	0.25	0.18	0.23	0.14	0.22	0.23	0.29	0.28	0.36	0.19	0.34
Loss of communicative potential	0.36	0.37	0.39	0.38	0.35	0.35	0.38	0.34	0.37	0.27	0.35	0.35	0.43	0.41	0.40	0.36	0.50
General indicators of maladjustment	0.50	0.52	0.49	0.47	0.47	0.47	0.52	0.47	0.50	0.38	0.51	0.49	0.52	0.46	0.52	0.50	0.66
"Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18]																	
PTSD	0.54	0.56	0.51	0.48	0.50	0.50	0.57	0.53	0.54	0.44	0.54	0.53	0.50	0.44	0.48	0.55	0.69
"Diagnostics of psychological safety of an individual" (Prykhodko) [19]																	
Trust	-0.29	-0.34	-0.25	-0.29	-0.29	-0.32	-0.38	-0.30	-0.39	-0.35	-0.39	-0.35	-0.35	-0.33	-0.30	-0.35	-0.44
Respect	-0.25	-0.25	-0.26	-0.29	-0.22	-0.18	-0.28	-0.20	-0.30	-0.24	-0.26	-0.26	-0.32	-0.29	-0.24	-0.21	-0.34
Moral and communicative	-0.30	-0.33	-0.28	-0.32	-0.29	-0.28	-0.37	-0.28	-0.38	-0.33	-0.36	-0.35	-0.37	-0.34	-0.31	-0.32	-0.44
Motivational and volitional	-0.31	-0.38	-0.27	-0.31	-0.34	-0.36	-0.38	-0.33	-0.39	-0.36	-0.40	-0.41	-0.36	-0.28	-0.25	-0.34	-0.46
Valuable and semantic	-0.28	-0.30	-0.27	-0.28	-0.28	-0.28	-0.35	-0.29	-0.32	-0.32	-0.36	-0.33	-0.36	-0.30	-0.25	-0.29	-0.41
Internal comfort	-0.24	-0.30	-0.22	-0.25	-0.30	-0.31	-0.39	-0.33	-0.31	-0.32	-0.37	-0.34	-0.33	-0.27	-0.25	-0.29	-0.40
General indicators of psychological safety of an individual	-0.30	-0.35	-0.28	-0.31	-0.32	-0.32	-0.39	-0.33	-0.37	-0.35	-0.39	-0.38	-0.38	-0.32	-0.28	-0.33	-0.45
"Evaluation of traumatism of combat experience of servicemen" (Kolesnichenko) [20]																	
Unfavorable social conditions of performing service combat tasks	0.47	0.49	0.41	0.37	0.37	0.39	0.42	0.41	0.44	0.33	0.42	0.42	0.34	0.41	0.50	0.45	0.58
Specific traumatic combat conditions	0.27	0.29	0.23	0.22	0.13	0.17	0.14	0.21	0.23	0.21	0.14	0.18	0.11	0.22	0.29	0.15	0.28
Unfavorable social situation of participation under combat conditions	0.21	0.23	0.25	0.26	0.08	0.09	0.09	0.13	0.18	0.06	0.11	0.13	0.15	0.14	0.23	0.09	0.21
Internal resources of resistance	-0.12	-0.14	-0.03	0.00	-0.23	-0.20	-0.24	-0.27	-0.12	-0.10	-0.24	-0.22	-0.10	-0.15	-0.07	-0.27	-0.21
External resources of resistance	-0.11	-0.14	-0.08	-0.10	-0.16	-0.17	-0.20	-0.13	-0.17	-0.12	-0.16	-0.08	-0.07	-0.09	-0.01	-0.14	-0.16
General indicators of traumatism of combat experience	0.32	0.37	0.25	0.24	0.32	0.32	0.38	0.37	0.34	0.23	0.36	0.31	0.17	0.28	0.24	0.36	0.42
"Resistance to psychological traumatism of combat experience" (Kolesnichenko) [21]																	
Expectations of participation in hostilities	-0.32	-0.37	-0.25	-0.15	-0.40	-0.37	-0.39	-0.38	-0.34	-0.31	-0.43	-0.39	-0.26	-0.27	-0.34	-0.46	-0.48
Overcoming a stressful situation	-0.38	-0.38	-0.29	-0.16	-0.40	-0.41	-0.38	-0.31	-0.30	-0.27	-0.40	-0.41	-0.28	-0.27	-0.26	-0.46	-0.46
Implementation of the received experience	-0.36	-0.34	-0.31	-0.19	-0.39	-0.30	-0.44	-0.38	-0.30	-0.25	-0.41	-0.40	-0.30	-0.29	-0.30	-0.42	-0.47
General indicators of resistance to traumatism	-0.40	-0.41	-0.32	-0.18	-0.45	-0.41	-0.45	-0.40	-0.36	-0.31	-0.46	-0.45	-0.32	-0.31	-0.34	-0.50	-0.53
Helplessness	0.28	0.32	0.23	0.16	0.34	0.38	0.30	0.28	0.29	0.26	0.35	0.33	0.27	0.25	0.27	0.39	0.41
Conscious attitude towards professional training	-0.12	-0.07	-0.10	0.01	-0.16	-0.08	-0.17	-0.16	-0.09	-0.07	-0.18	-0.18	-0.07	-0.09	-0.10	-0.17	-0.15
The ability to get experience	-0.35	-0.36	-0.25	-0.17	-0.34	-0.28	-0.33	-0.25	-0.22	-0.23	-0.30	-0.39	-0.19	-0.27	-0.24	-0.34	-0.38
Cognitive dysfunction	0.40	0.38	0.29	0.13	0.37	0.36	0.38	0.32	0.28	0.26	0.39	0.39	0.27	0.23	0.27	0.42	0.45
Conscious attitude towards military duties	-0.21	-0.27	-0.20	-0.09	-0.28	-0.22	-0.34	-0.32	-0.22	-0.18	-0.35	-0.28	-0.18	-0.21	-0.25	-0.38	-0.35

Psychological safety of an individual = 5.325 – 0.072*Irritability – 0.064*Devastation – 0,077*Sense of guilt – 0,095*Sense of powerlessness – 0.071* Reluctance to communicate + 0.775.

Traumatism of combat experience = -17.672 + 0.426*Anxiety + 0.393*Apathy + 0.502*Inability to perform the assigned tasks + 0.789.

Resistance to combat psychological traumatization = 167.165 – 2.514*Anxiety + 2.869*Anger – 1.621*Apathy – 1,510* Lack of trust in comrades in the service – 4.812*Inability to perform the assigned tasks + 0.599.

The procedure of regression analysis had to give the possibility to reduce the dimension of measurements for each state, but as it could be seen, the error of evaluation in the concluded regression equations was quite high, especially in the process of predicting traumatism of combat experience (e = 0.789) and psychological safety of an individual (e = 0.775) that made it inappropriate to use them for calculations. However, the information obtained through regression analysis gave the possibility to better understand the experience of servicemen who had different negative symptoms due to their participation in hostilities.

Moreover, a long-last period of testing and a large selection of the study had shown that the calculations made with the help of regression analysis by individual scales were less accurate than the normalized general indicator by the method (that indicated one more time the importance of using the method as a single scale). It was the regulation for different negative symptoms that prevailed in determining the ways of processing data by the method.

Thus, the obtained data showed that if the general indicator by the method "Evaluation of negative mental reactions and states of combatants" exceeded 3.5 points, then 65.45% of servicemen-participants of the psychological recovery program were likely to be symptoms of maladjustment by the same method. If the average points according to the developed method were 5 or more, then more than 85% of servicemen with this indicator were diagnosed with expressed symptoms of maladjustment.

The comparison of the general indicator by the method "evaluation of negative mental reactions and states of combatants" with the method "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18] showed that while exceeding 3.5 points according to the scale of the developed method more than 90% of servicemen with this indicator had expressed PTSD symptoms. When the average quantity of points was 5.5% – 100% of servicemen who participated in psychological recovery activities had an indicator that exceeded the norm by the "Mississippi Scale for Combat-Related PTSD" (Caddell and Taylor) [18].

The comparison with the data provided by the method "Resistance to psychological traumatism of combat experience" showed that with general points 3 or more in accordance with the method "Evaluation of negative mental reactions and conditions of combatants" 75.59% of servicemen with such quantity of points were diagnosed with an unsatisfactory indicator of resistance (from 0 to 143 points).

If the general indicator was exceeded in accordance with the method "Evaluation of negative mental reactions and states of combatants" the score of 5 points meant that low resistance to combat psychological traumatism was diagnosed in almost 86% of servicemen who participated in psychological recovery activities.

The comparison with the express method "Psychological Safety of an Individual" (Prykhodko) [19] and "Evaluation of traumatism of combat experience of servicemen" (Kolesnichenko) [20] pointed out that if the points were less than 3 by the general scale of the method "Evaluation of negative mental reactions and states of combatants" 89% of servicemen retained satisfactory indicators of psychological safety of an individual, and 85.41% were diagnosed with low trauma of combat experience.

More reliable for these two methods was a forecast concerning psychological well-being than negative symptoms.

Thus, the given data showed that for the general indicator by the method "Evaluation of negative mental reactions and states of combatants" it was normal to have a high probability of negative symptoms' absence (PTSD, maladjustment, instability to combat psychological traumatization, and psychological safety of an individual) and it also showed that high ability to recover was possible when an indicator was less than 3 points.

It was necessary to point out that the results obtained with the help of the method, both individual and group ones, were convenient to present in the form of column histograms. As an example, there was proposed the histogram of the dynamics of negative mental reactions and conditions of servicemen with signs of maladjustment (in particular, violation of behavioral regulation) in the process of one of the decompression courses (Figure 1).

Figure 1: Dynamics of negative mental reactions and conditions while participating in psychological recovery in a group of servicemen with violation of behavioral regulation (in points)

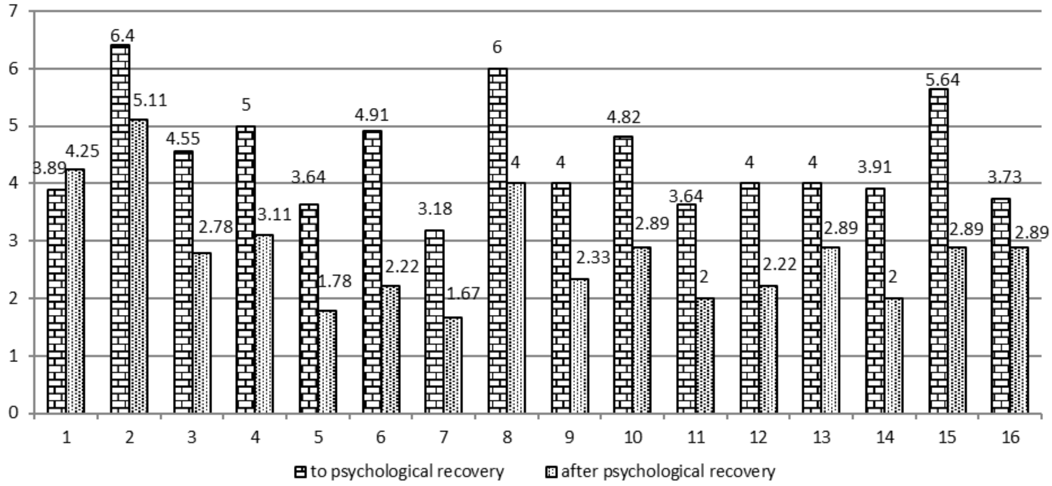

Notes: 1 – inability to perform the assigned tasks; 2 – lack of trust in commanders; 3 – lack of trust in comrades in the service; 4 – unwillingness to communicate; 5 – lack of focus; 6 – sense of impotence; 7 – sense of guilt; 8 – concern; 9 – apathy; 10 – devastation; 11 – self-doubt; 12 – inattention; 13 – anger; 14 – aggressiveness; 15 – anxiety; 16 – irritability

DISCUSSION

According to Ukrainian [14] and foreign specialists [26,27], early diagnosis of negative mental reactions and states [28], post-stress states, and determination of a group of increased psychological attention among personnel gave the possibility to identify servicemen with low personal adaptive potential and high probability of early and severe manifestations of combat psychological traumas [29] and its consequences [30]; also gave the possibility to create individual tactics and methods of prevention and restoration of each surveyed serviceman. To solve the problem of diagnosis of negative mental reactions and states of servicemen, it was suggested to use the following psychodiagnostic tools: "The Hospital Anxiety and Depression Scale (HADS) ", "Brief Scale of Anxiety, Depression, and PTSD", "The Montgomery-Asberd Depression Rating Scale (MADRS)” [31], "Beck Depression Inventory (BDI)" [32], "Primary Screening for Post-Traumatic Stress Disorder (PTSD)", "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18], "Impact of Event Scale–Revised (IES-R)" [33], "Patient Health Questionnaire (PHQ)-9" [34], "Clinical-administered PTSD Scale" [35], "Neurotization and Psychopathization (LNP) questionnaire" [14] and others. As an additional diagnostic tool, at the request of a psychologist, there was used the method "The Suicidal Behaviors Questionnaire-Revised (SBQ-R)" [36] to diagnose the suicidal inclinations of servicemen. However, this psychological study could have been placed under the conditions of medical and psychological rehabilitation which lasted not less than two weeks, but it was quite difficult to realize this tool under the conditions of psychological recovery, which lasted not more than one week.

Traditionally, in clinical practice, there had been used for a long time the projective method such as Lusher's color test, in particular after psychotherapy and training activities for self-regulation and restoration of adaptive capacity, the selection of colors could be compared with the so-called autogenic norm by Wallnöfer [37].

World tendencies in psychological studies showed that the diagnosis of mood, mental reactions, and conditions was relevant not only for the needs of the clinic but also in the workplace. The study of workers’ moods and states was an important component of the psychological well-being (health) of workers. These studies were extremely large-scale and attracted thousands of working people as participants [38]. Such interest in the problem of studying the state of a working person, as an indicator of his/her psychological well-being, was quite clear since the psychological state was a holistic, systematic, integral formation of human psychological activity. The psychological state of an individual could be internally and externally observed, it acted as a regulatory function of adaptation to the external situation and environment [16]. According to the definition by Kirilenko, the psychological state was the formation of psychological activity, which reflected the moment of stability and specificity of psychological processes’ duration, as a form of response, and reflected the attitude of an individual toward his/her own psychological phenomena at a certain point in time under certain conditions [39]. Varii pointed out that the psychological state was a temporary functional level of the psyche, which reflected the interaction of the influence of the internal environment of the body or external factors and determined the orientation of psychological processes' duration at a certain moment and manifestation of psychological properties of an individual [40]. The author defined the following areas in the studies of mental states: 1) mental states were an integral characteristic of the psyche at a particular moment; 2) psychological states were the background on the bases of which operated the psychological activity and orientation of the psychological activity of an individual; 3) it was a systemic reaction of the human psyche to changes of the conditions.

Volzhentseva highlighted that the leading functions of mental states were the regulation and integration into functional units, which were formed in the hierarchy of a single, holistic set of psychological processes and properties [25]. According to the author, the adaptive function of the psychological state was to establish the correspondence between the current needs and capabilities of an individual, considering the external conditions, features of activity, and behavior.

It was likely that the integration and integrity of psychological states became the basis for reducing the dimensions of the methods which were intended for their research. Thus, in foreign scientific sources, it was reported that to determine the moods and conditions, which were important indicators of short-term effects of intervention, was also used in clinical practice, "The Profile of Mood States (POMS)" [12,41]. This scale was developed by American experts, it had the form of a self-report and included 65 elements that related to 7 different scales: depression, anxiety, fatigue, energy, irritability, tension, and confusion.

The answer scale was divided into 5 categories from "not at all" to "completely yes". However, German psychologists in the re-standardization of POMS [12,13] created a shortened version of the method that included only 35 points which belonged to the scale "Depression/anxiety", "Fatigue", "Energy" and "Irritability" [38]. Another study was intended to reduce the dimension of POMS [41]. For this purpose, the POMS scale [12,13] with the help of regression analysis correlated with "The Eight State Questionnaire" (8SQ) [42], which was formed to measure anxiety, stress, depression, regression, fatigue, guilt, extraversion, and excitement. Researchers found that the excessiveness of measurement between these two methods mainly referred to negative emotional states.

CONCLUSIONS

Thus, the developed method "evaluation of negative mental reactions and states of combatants" was a tool that gave the possibility to determine the presence of negative psychological symptoms of servicemen related to their participation in hostilities and was a convenient tool for estimation of the dynamics of the state under the conditions of short-term programs of psychological recovery without the use of complex evaluation procedures and calculation of data.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, O.K. and YaM.; managed the project, I.P.; conducted the research, V.K. and V.P.; contributed to the final draft of the manuscript, A.P., S.S. and N.P.; substantiated the methodology, S.L. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Informed consent was obtained from all subjects for inclusion in the study while maintaining their confidentiality. Written informed consent has been obtained from all subjects to publish this paper.

Ethics approval and consent to participate

All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

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ORIGINAL ARTICLE

Associations Between Medical Students' Opinions on Usage of Internet Services and Digital Teaching Tools

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Abstract: *Currently, the educational process must integrate new tools, to keep pace with technological progress. Our paper aims to investigate the extent to which the preferences of medical students for the autonomous use of multimedia resources in the learning process are correlated with their opinions towards the Internet and the intensity of using it in their daily activities. Methods: We investigated 395 medical students from 4 medical universities in Romania, 75.9% females, and 88.8% in the 1st and 2nd years of study. Students were asked to express on Likert-scales their agreement with a list of statements about Internet services, as well as whether they like learning using multimedia tools and how intensively they use Internet services. Results: The students agree to a medium to large extent with the favorable statements about Internet services but are also aware of the negative influences that indiscriminate use of such technology can have. The intensity of Internet usage is high, although not exaggerated. With small exceptions, the students who enjoy using multimedia resources during the learning process have also favorable opinions about Internet services, and use them constantly, mainly for information and instant messaging on social networks*.

Keywords: *multimedia-based learning; blended learning; Internet services; social networks; medical students*

INTRODUCTION

Currently, digital technology has become increasingly used in everyday activities, and its intensive use in most fields of activity has attracted changes in the structure of society, and even in the psychology of individuals, who gradually change their conceptions about life, work, and education, about the ideal way of spending leisure time and about the features of an optimal work climate. In this general context, it is obvious that the educational process must integrate new tools, in an attempt to keep pace with technological progress. The protagonists of this process, students as well as teachers, are people with a fundamentally different lifestyle than 20 years ago, with different expectations and conceptions.

The most obvious changes are noticed in the case of the current generation of students. This is the so-called "Generation Z" (children born after 1997), also known as iGen, post-millennial, or digitally native. These are children who have been exposed to technology from the beginning of their lives, so using computers and gadgets has become second nature to them, as have the activities online and on social networks. Consequently, these children prefer to study independently, at their own pace, using visual or kinaesthetic didactic tools, which give them immediate feedback. It is important for them to respect the right to privacy and personal space; they are pragmatic, entrepreneurial, and generally reluctant to take risks (because they faced periods of economic recession in childhood); although they generally enjoy interaction with the teacher, Generation Z children have fewer social skills associated with face-to-face communication and the art of conversation, compensated instead by intense online activity [1].

There are currently relatively few studies in the literature concerning Generation Z issues, which usually focus on describing its characteristics [2,3], effective strategies for recruitment in the public sector [4], and individuals' attitudes towards work [5], but without focusing on the specific problems of medical students of this generation, with their own needs, preferences and learning styles.

Obviously, the learning style of Generation Z students has features that differentiate it from the learning styles preferred by other generations of students. The most preferred method is active learning (through discussion and involvement in practical projects), based on sensory stimuli (visual - images, graphics, auditory and kinetic). Knowledge is achieved through observation, practice, and quick and targeted access to the necessary information through the use of technology and online resources: e.g. YouTube for video tutorials, Facebook (for chatting with classmates and sharing projects and teaching materials), or Google (for documenting and searching for scientific references). Students prefer self-study, at their own pace, with a flexible schedule and teaching materials that intensively use multimedia resources: explanatory or demonstrative video sequences, PowerPoint presentations with audio support, simulations, collaborative projects, discussion forums, online questionnaires, and case studies in the classical environment (seminar room or practical works). In this context, the portrait of the ideal teacher has also changed significantly: firstly, he must be a good communicator, close and friendly, transparent, enthusiastic, passionate and competent in his field, able to propose realistic goals and to provide immediate and constructive feedback on their achievement, willing to provide professional and emotional support to his students, both academically and individually, to prepare them for their future careers.

In this context, the concept of blended education, based on the intensive integration of digital resources into the teaching process, is becoming increasingly popular among teaching tools.

Such education programs are usually developed in mixed regimes, online and onsite, combining as much as possible in an optimal manner the advantages of both methods. Blended courses are a current trend that provides a new and effective instructional experience to the students they address, and have several major advantages:

- Flexibility in the teaching schedule, which simplifies students' access to the course, regardless of their physical location and allows them to organize their time and pace of learning according to their own needs and availability;

- More effective teaching: Most scientific studies show that blended teaching is more effective than traditional courses, but also than full online courses. A 2009 report from the United States examined 51 studies comparing online and traditional methods and concluded that students enrolled in blended learning programs score on average better on exams than those enrolled in full-onsite programs [6]; on the other hand, blended teaching programs also perform better than fully online ones as well [7], as they still give to the students the opportunity to interact effectively with the teacher when needed.

- Clearer organization of content: It is the natural result of a well-designed blended course, which must provide students with clear and detailed indications of using the proposed resources, activities, and tests, helping them to concentrate better and proposing an optimal pace of learning, in the same way that the teacher guides students onsite in going through the exposed materials;

- Stimulation of active learning, which emphasizes the student's autonomy in effective learning communities;

- Lower costs: Reducing the number of onsite meetings frees up the teaching spaces of the institution and reduces some utility expenses. The teachers and the students will gain lower transport costs and time savings, although logistics expenses can be slightly increased (it is necessary to purchase adequate personal computers and involves additional costs for electricity consumption and telephone subscription).

Our paper aims to investigate the extent to which the preferences of students at medical faculties for the autonomous use of multimedia resources in the learning process are correlated with their opinions towards the online environment and the Internet and the intensity with which they use such resources in their daily activities, not necessarily related to the learning process.

MATERIALS AND METHODS

We conducted a case study that enrolled 395 medical students from 4 medical universities in Romania. The general features of the sample are shown in Table 1. Students are 3 quarters females (75.9%); most students are in the 1st and 2nd years of study (88.8%), aged between 18-20 years (74.4%), with an average age of 21.78 ± 3,736 years and a range of variation between 18-48 years.

Also, most students come from faculties of Dental Medicine (34.4%) or General Medicine (47.6%), mainly from the "Grigore T. Popa" University of Medicine and Pharmacy in Iasi, Romania (50.9%); 27.1% of the participating students are from University of Medicine and Pharmacy in Craiova, and 20.3% from University of Medicine and Pharmacy "Victor Babeș" in Timisoara.

Students were invited to fill in an online questionnaire in order to express their agreement degree with a list of statements about Internet services (favorable – 7 items and unfavorable – 3 items). In addition, they were asked whether they like learning using multimedia tools and resources and how intensively they use different Internet services (7 items).

The agreement degree with the proposed statements was quantified using a 5-step Likert scale, ranging from 1 – very small agreement to 5 – very large agreement.

Table 1: General features of the study sample

		n	%
Gender	masculine	95	24.1
	feminine	300	75.9
Age group	18-20 years	294	74.4
	21-24 years	78	19.7
	over 25 years	23	5.8
Year of study	1	228	57.7
	2	123	31.1
	3	44	11.1
Specialization	Dentistry	136	34.4
	General medicine	188	47.6
	Dental technique	70	17.7
	Prophylaxis assistance	1	0.3
University	UMPh Iasi	201	50.9
	UMPh Craiova	107	27.1
	UMPh Timișoara	80	20.3
	UMPh Cluj Napoca	7	1.8
Total		395	100.0

The questionnaire was presented and explained individually to each study participant.

Participation in the study was voluntary. Participants were informed about the study and the overall objectives of the research and, at the beginning of the questionnaire, were invited to give their informed consent. The questionnaires were completed anonymously.

The study was approved by the Research Ethics Commission of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (decision no. 21/16.11.2020).

Statistical analysis

Data collected from students were recorded and processed in SPSS 29.0 (SPSS Inc., Chicago, IL) for Windows.

The responses to each item were characterized by frequency distributions and contingency tables. The numerical variables were characterized by descriptive statistics (mean, standard deviation, standard error, range of variation, and median). Comparisons between samples were made using the Kruskal-Wallis test for quantitative data, after having previously verified that they comply with the normal distribution law, using the Shapiro-Wilks normality tests. We considered the p-value ≤ 0.05 to be statistically significant (*) and the p-value ≤ 0.01 to be statistically highly significant (**).

RESULTS

The 7 favorable statements about the Internet were as follows:
- Item 1: The main advantage of the Internet is that it provides information fast;
- Item 2: The main advantage of the Internet is that it provides correct information;
- Item 3: Freedom of expression should not be restricted on the Internet;
- Item 4: Most people would not be able to cope if they no longer had access to the Internet;
- Item 5: I can't imagine my life without Internet access;
- Item 6: People who socialize on the Internet are happier than others;
- Item 7: If I didn't socialize on the Internet I would be sadder.

88.6% of students evaluate the Internet as a fast source of information (item 1) but are more reserved about its correctness (only 30.6% of them think that information on the Internet is much or very much correct – item 2).

68.9% of students believe that most people would not be able to cope if they no longer had access to the Internet (item 4) and 60.7% believe that they themselves would not be able to cope if there is no Internet (item 5). 53.9% of students believe that freedom of expression should not be limited to the Internet (item 3).

On the other hand, only a small percentage of students believe that socializing in the virtual environment ensures a comfortable psychological status, making them happier (item 6 – 10.9%, respectively item 7 – 21.5%) – Table 2.

The 3 unfavorable statements about the Internet were as follows:
- Item 8: Internet is addictive;
- Item 9: Parents should limit their children's access to Internet to at most one hour;
- Item 10: People's privacy is at risk due to social media.

A large part of students believe that the Internet can be addictive (item 8 – 76.2%). Although they generally do not consider it necessary for parents to limit their children's access to the Internet (item 9 – only 41.0% agree to a large or very large extent), they still believe that people's privacy is at risk due to social networks (item 10 – 59.5%) – Table 3.

Table 2: Favourable opinions about Internet services – students' responses

Favorable opinions about Internet services	Agreement:									
	1 – to a very small extent		2 – to a small extent		3 – in average measure		4 – to a large extent		5 – to a very large extent	
	n	%	n	%	n	%	n	%	n	%
Item 1	1	0.3	4	1.0	40	10.1	125	31.6	225	57.0
Item 2	8	2.0	49	12.4	217	54.9	93	23.5	28	7.1
Item 3	9	2.3	16	4.1	157	39.7	107	27.1	106	26.8
Item 4	7	1.8	25	6.3	91	23.0	143	36.2	129	32.7
Item 5	21	5.3	35	8.9	99	25.1	115	29.1	125	31.6
Item 6	81	20.5	121	30.6	150	38.0	34	8.6	9	2.3
Item 7	75	19.0	118	29.9	117	29.6	46	11.6	39	9.9

Table 3: Unfavourable opinions about Internet services – students' responses

Unfavorable opinions about Internet services	Agreement:									
	1 – to a very small extent		2 – to a small extent		3 – in average measure		4 – to a large extent		5 – to a very large extent	
	n	%	n	%	n	%	n	%	n	%
Item 8	3	0.8	17	4.3	74	18.7	139	35.2	162	41.0
Item 9	43	10.9	56	14.2	134	33.9	94	23.8	68	17.2
Item 10	10	2.5	44	11.1	106	26.8	118	29.9	117	29.6

The consumer of Internet services profile was outlined through another 7 questions, regarding the way, intensity, and main purposes for which students use Internet services. The recorded responses are shown in Table 4.

Most students (97.5%) say they use the Internet daily, and 70.9% of them use the Internet at least 3 hours a day. Almost 1/3 of students (29.4%) believe that they could not give up the Internet without problems even for a day - which indirectly means acknowledging an addiction to this practice. On the other hand, half of students (47.6%) indicate the one-week period as the maximum limit for opting out of the Internet, which is also a limited time frame. Most students access the Internet using their mobile phones (84.6%).

Although they use online services quite intensively, the main objective of students is not written communication – thus, almost 3 quarters of them (71.6%) say they receive/send between 1 - 10 emails per day, and 9.4% say they do not use e-mail at all. Half of students (52.4%) are active on social networks, having over 100 friends; however, there is also a significant percentage of students (20.8%) who have fewer than 10 friends online. In contrast, 74.2% of students post on social media only a few times a year or a few times a month. Therefore, students use social networks in a relatively moderate regime, mainly for instant communication and less for permanent posts.

Finally, the students were asked whether they liked learning, even autonomously, using multimedia tools and resources (Item 18). The answers to this question are generally favorable: 41.5% of students say they like much or very much to learn using multimedia tools, 38.0% of students agree on average with this statement, and only 20.5% of students show a total or partial disagreement (Table 5).

In order to express more precisely the obtained results, we quantified them as numerical scores, calculated as arithmetic averages of the responses. We calculated 2 scores, one for favorable opinions about Internet services (INTF) and one for unfavorable opinions (INTNF), with a range of variation between 1 – 5, which we interpreted as follows:

- Values between 1.00 – 1.49 = agreement to a very small extent;
- Values between 1.50 – 2.49 = agreement to a small extent;
- Values between 2.50 – 3.49 = agreement in average measure;
- Values between 3.50 – 4.49 = agreement to a large extent;
- Values between 4.50 – 5.00 = agreement to a very large extent.

Using the same procedure, we calculated also a third score, to characterize the intensity in using Internet services (INTS) – with a range of variation between 1 – 4 and interpreted accordingly:

- Values between 1.00 – 1.49 = very low intensity;
- Values between 1.50 – 2.49 = low intensity;
- Values between 2.50 – 3.49 = high intensity;
- Values between 3.50 – 4.00 = very high intensity.

The analysis of these scores reveals that students agree to a medium to large extent with the favorable statements about Internet services (INTF = 3.44 ± 0.590), but also with the unfavorable statements about these services (INTNF = 3.69 ±

0.817), being therefore aware of the negative influences that indiscriminate use of such technology can have. The intensity of Internet usage is high, although not exaggerated (INTS = 2.85 ± 0.350) – Table 6.

Table 4: Intensity and main purposes in using Internet services – students' responses

		n	%
Item 11. On average, I access the Internet:	several times a month	3	0.8
	several times a week	7	1.8
	daily	385	97.5
Item 12. On average, the amount of time I spend on the Internet during a day is:	less than 1 hour	7	1.8
	1 - 3 hours	108	27.3
	3 - 6 hours	145	36.7
	over 6 hours	135	34.2
Item 13. I could quit the Internet without problems for a time frame of:	1 year	25	6.3
	1 month	66	16.7
	1 week	188	47.6
	not even a day	116	29.4
Item 14. Generally, I access the Internet using:	a desktop	13	3.3
	a laptop	47	11.9
	a tablet	1	0.3
	a mobile phone	334	84.6
Item 15. On average, the number of emails I receive/send daily is:	none	37	9.4
	1 to 10	283	71.6
	11 to 30	58	14.7
	31 to 50	12	3.0
	over 50	5	1.3
Item 16. The number of friends (contacts) I have on the most frequently used social network is:	under 10	82	20.8
	between 11 and 50	68	17.2
	between 51 and 100	38	9.6
	between 101 and 500	109	27.6
	over 500	98	24.8
Item 17. On average, the frequency with which I post new information on my social media profile is:	none	35	8.9
	several times a year	152	38.5
	several times a month	141	35.7
	several times a week	55	13.9
	several times a day	12	3.0

Table 5: General opinion about the use of multimedia resources during the learning process – students' responses

General opinion	**Agreement:**									
	1 – to a very small extent		**2 – to a small extent**		**3 – in average measure**		**4 – to a large extent**		**5 – to a very large extent**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Item 18: I like to learn using multimedia tools and resources	19	4.8	62	15.7	150	38.0	81	20.5	83	21.0

Table 6: Internet services usage scores - descriptive statistics parameters

	n	**Mean (M)**	**Standard deviation (SD)**	**Standard error of the mean (SEM)**	**Min**	**Max**	**Median**
Favourable opinions about Internet usage (INTF)	395	3.44	0.590	0.030	1.57	5.00	3.43
Unfavourable opinions about Internet usage (INTNF)	395	3.69	0.817	0.041	1.00	5.00	3.67
Internet usage intensity (INTS)	395	2.85	0.350	0.018	1.71	3.71	2.86

We compared these scores according to the students' degree of agreement with the usage of multimedia resources during the learning process, to identify the possible links between these two elements. We started with the hypothesis that the students who enjoy using digital technologies for entertainment in leisure time should also enjoy using them professionally, respectively during the learning process.

The obtained results are presented in Table 7 and actually validate this research hypothesis.

Table 7: Internet services usage scores – compared according to the degree of agreement with the use of multimedia resources during the learning process

	Agreement:					**Kruskal-Wallis test**
	1 – to a very small extent	**2 – to a small extent**	**3 – in average measure**	**4 – to a large extent**	**5 – to a very large extent**	
	M ± SD	**M ± SD**	**M ± SD**	**M ± SD**	**M ± SD**	
Favorable opinions about Internet usage (INTF)	3.02 ± 0.705	3.43 ± 0.612	3.32 ± 0.545	3.53 ± 0.522	3.67 ± 0.593	H = 23.634 p < 0.001**
Unfavorable opinions about Internet usage (INTNF)	4.02 ± 0.842	3.68 ± 0.692	3.74 ± 0.807	3.58 ± 0.733	3.63 ± 0.973	H = 5.414 p = 0.247
Internet usage intensity (INTS)	2.75 ± 0.415	2.97 ± 0.325	2.81 ± 0.350	2.88 ± 0.324	2.80 ± 0.358	H = 12.310 p = 0.015*

There are statistically significant differences between the scores of favorable opinions about Internet usage, determined by the degree of agreement with the use of multimedia resources during the learning process. The lowest scores are found in students who agree to a very small extent with the use of multimedia resources (3.02 ± 0.705), and the highest in students who agree to a very large extent with the use of multimedia resources (3.67 ± 0.593), so these 2 elements are in clear correlation.

The situation is different for the scores of unfavorable opinions: the highest score of unfavorable opinions about Internet usage is found in students who strongly disagree with the use of multimedia resources in the learning process (4.02 ± 0.842). As the degree of agreement with the use of multimedia resources increases, the score of unfavorable opinions decreases, but with random fluctuations, so the observed differences are not statistically significant.

The lowest score of Internet usage intensity is also found in students who strongly disagree with the use of multimedia resources in the learning process (2.75 ± 0.415) – therefore they are fundamentally against digital technologies. On the other hand, the highest score of Internet usage intensity (2.97 ± 0.325) is observed in students with little agreement for the use of multimedia resources in the learning process – so they are obviously not against digital technologies but probably prefer to use them exclusively for leisure. Students who agree to a medium or large extent with the use of multimedia resources in the learning process have scores of Internet usage intensity between the 2 categories mentioned above, so they enjoy using digital technologies both professionally and for leisure. The differences between the investigated categories of students are statistically significant.

DISCUSSION

The data from the literature prove that multimedia resources are an effective tool for learning because they are able to stimulate the students' interest [8] and improve their cognitive skills [9], also contributing to the development of supplementary skills – like empathy [10,11] or the academic writing [12]. Such tools are preferred by medical students because they tend to be kinaesthetic learners, who use multiple ways to present a topic for a better understanding [13,14,15] – observations also confirmed by our study.

Medical students are advanced users of smartphones and laptops and easily use such devices during their daily activities and learning [15,16] – a fact also confirmed by our results. The students investigated by us are generally interested in digital technologies and their applicability during the learning process [15].

It is thus natural that our students have generally positive opinions about Internet services, without thinking that they are addicted to them. Moreover, they are even aware of the presence of such a risk, which proves realistic and mature thinking. Most students use the Internet for information, although they are also aware of the risk of not obtaining accurate data – a fact confirmed by other literature data as well [17]. We must admit that the media and entertainment tools familiar to previous generations (TV, radio, written press) have now been joined by new, equally important, or even more important partners – the smart devices, which seem to have become the most convenient ways to access virtual environments.

Almost all students use the Internet daily, from their mobile phones, and most of them for at least 3 hours – which shows a certain degree of dependence on this technology, although it is not recognized as such. The most frequently used Internet services are, according to some authors, WhatsApp (as a fast communication solution) [18,19,20], YouTube, and Blackboard [21].

It is also interesting to note that socializing in cyberspace is not very important for our students and does not replace real socialization. Only half of the students have many friends on social media, but even they don't post very frequently (only a few times a year or a few times a month, which is a moderate pace). Moreover, almost 60% of students believe that people's privacy is at risk due to social networks – a result that is questionable, because not the social networks pose a danger, but the people using them inappropriately – an aspect that seems to have been omitted by students. Literature data also confirms that the students, particularly the medical students, are not very well informed about the ethical issues regarding the use of social media resources [21].

There is an obvious and positive association between the degree of agreement expressed by students towards the use of multimedia resources during the learning process and their favorable opinions towards the use of Internet services. This means that the general openness to modern technologies is reflected by integrating them into personal life, but also professionally. On the other hand, unfavorable opinions towards the use of Internet services are associated sometimes with the general avoidance of modern technologies. We also identified a separate category of students who, although they do not like to use multimedia resources in the learning process, are instead very active on the Internet; they are especially interested in activities on social networks, without being very concerned about the other advantages provided by digital technologies. Anyway, the students who enjoy using multimedia resources during the learning process are also constant users of Internet services, which they use mainly for information, but also for activities on social networks (mainly instant messaging) – a fact also confirmed by previous studies in the literature [21,22,23].

CONCLUSIONS

Technology is only a tool that can improve the teaching process, but not only by itself. Those who are responsible for change are the teachers, who can improve the students' outcomes by using technology to convey knowledge, streamlining their methods, and perfecting themselves [24]. The concept of blended education facilitates such approaches and can trigger positive changes, satisfying for students, who will benefit from flexibility and comfort, and, consequently, also for teachers, who will have the satisfaction that their work achieves its purpose and that the information they transmit is properly heard and acquired.

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Authors' contribution

Conceptualization, C.G.D. and V.L.P.; methodology, C.G.D.; software, C.G.D. and R.S.C.; validation, R.S.C.; formal analysis, C.G.D.; investigation, M.E.A. and R.S.C.; resources, V.L.P.; data curation, C.G.D.; writing—original draft preparation, C.G.D. and M.E.A.; writing—review and editing, C.G.D. and V.L.P.; supervision, V.L.P.; project administration, C.G.D. and M.E.A. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethical Committee of "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Romania (decision no. 21/16.11.2020).

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ORIGINAL ARTICLE

The Impact of Physical Therapy on Psychomotor Functions in a Patient with Systemic Lupus Erythematosus and Diabetes Mellitus – Case Illustrated Literature Review

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Abstract: *The scientific and medical world is making permanent efforts to study, understand, and cure the complicated pathology of Systemic Lupus Erythematosus (SLE) – the disease with 100 faces, due to the diversity and complexity of the symptoms. SLE has a devastating impact on the patient's life, causing multiple metabolic imbalances produced by both the disease itself and the necessary medication. This case report addresses the therapeutic aspects of SLE and diabetes mellitus, on a 47-year-old male with a sensitive multi-pathology and aggressive evolution, medication, and approaching psychomotor rehabilitation therapy, up to now, when SLE treatment has ended. Also, this case study report highlights the importance of flexibility in the psychomotor rehabilitation strategy, in the context of a large range of factors that influence its definition.*

Keywords: *SLE - systemic lupus erythematosus, diabetes mellitus, medication, recovery therapy, psychomotor*

INTRODUCTION

The scientific and medical staff are making permanent efforts to study, understand, and cure the complicated pathology of Systemic Lupus erythematosus (SLE) [1-3]. SLE, an inflammatory autoimmune disease, also named the disease with 100 faces due to the diversity and complexity of the symptoms, has a devastating impact on the patient's life due to the multiple metabolic imbalances produced by the disease itself and the necessary medication [4].

The etiology of SLE is unknown, as are the possible disorders (but not necessarily) – hematological, nephrological, neurological, skin, and ocular, because of cellular and tissue damage [5-7]. There is no chronological sequence of SLE manifestations, even in the same patient. It is problematic to diagnose, hard to treat (due to the various symptomatology from minimum to severe), and difficult to continuously adapt the strategy of preserving psychomotor capacity, sometimes necessary to change in an abrupt way.

Diabetes Mellitus in the case of SLE is a particular situation with the need for continuous monitoring because it can be influenced by this long-term disease and its complex medication treatment [8,9]. Administration of insulin units and/or gliclazide must be dosed by the treating physician according to the particularities of the case [9,10]. It is well known that diabetes can be controlled with careful diet and weight monitoring, and physical exercise as a supplement to medical treatment. That is why we consider the good collaboration between family, patient, doctor, and physical therapist, alongside the regular medical checkouts for blood variables of vital importance. The medical approach therapy is individualized and customized to fit each patient's need depending on the type of disease and each symptom.

Reflection of the theme in specialized literature and conceptual boundaries

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with multisystem involvement. It is triggered by deregulation of the immune system which, due to the autoantibodies produced, attacks the body's healthy cells and tissues. The clinical picture is very rich, it can affect several organs, it has an undulating evolution with periods of exacerbation and remission, and the forms of presentation are from clinical manifestations without life risk (affects the skin, and joints) to potentially severe forms (affects the patient at neurological, renal, cardiovascular level).

There are 4 types of lupus:

- Systemic lupus erythematous – is the most common form of lupus. The manifestation of the disease differs depending on the affected structures.

- Cutaneous lupus – manifests itself in the skin.

- Lupus induced by drugs – iatrogenic lupus occurs as a reaction to the administration of certain drugs.

- Neonatal lupus – is a particular form of the condition and occurs due to the transplacental transmission of anti-Ro antibodies from mother to fetus during pregnancy and can have skin, cardiac (the most serious being atrioventricular block), hematological, and neurological manifestations.

Symptoms can range from mild to very severe, and although they can be chronic, they usually come and go, with the disease having an undulating course.

The most common complaints are fatigue, altered general condition, fever, lymphadenopathy (inflammation of the lymph nodes), and joint and muscle pain. Almost any organ can be affected.

Skin-mucosal damage

It is present in approximately 70% of patients. The classic manifestation of acute lesions is the heliotrope rash, erythema in "butterfly wings" present at the level of the nasal pyramid and the cheeks (redness that extends from the nose to the cheeks, below the eye area), without affecting the nasolabial folds. Erythema can also spread to other areas exposed to UV radiation – forehead, chin, décolleté. Photosensitivity is another characteristic of lupus patients. Subacute lupus may initially present with erythematous lesions that, over time, become papulosquamous, mimicking psoriasis or lichen planus. Discoid lupus erythematosus can appear on the face, scalp, ears, arms, hands, and back, the erythema being followed by hyperkeratosis or atrophy. Discoid lupus can be a strictly dermatological lesion, without being accompanied by systemic manifestations [29].

There may also be alopecia, skin purpura, erythema, small red dots, urticarial eruptions, livedo reticularis, Raynaud's syndrome, and ulcerations of the fingers pulp. Mucous membrane lesions consist of frequently painless petechial and ulceration of the nasal, oral, and, less commonly, genital mucosa.

Musculoskeletal damage

About 90% of patients diagnosed with lupus will have joint pain, arthritis, or muscle pain at some point. The joints of the hands, fists, and then the large joints (knees, ankles, shoulders, elbows) are most frequently affected. They can be painful, swollen, or red, the damage is usually symmetrical. Unlike rheumatoid arthritis, lupus arthritis rarely causes severe joint deformities and is not erosive.

Hematological impairment

Anemia, thrombocytopenia, leukopenia with neutropenia or lymphopenia may occur within the disease or secondary to pharmacological treatment.

Cardiovascular damage

Pericarditis, myocarditis, and endocarditis can occur in this disease.

Lupus endocarditis, called Libman-Sacks endocarditis, has no infectious cause. Atherosclerosis occurs more frequently and develops more rapidly than in the general population.

Lupus patients can also associate antiphospholipid syndrome (SAFL), in which antiphospholipid antibodies are present (anti-beta 2 glycoprotein 1 antibodies, anti-cardiolipin antibodies, lupus anticoagulant).

The presence of SAFL causes thrombosis), and spontaneous abortions.

Lung damage

It can take the following forms: pleurisy, inflammation of the pleura, pleurisy, pneumonitis, interstitial lung disease, pulmonary hypertension, and pulmonary thromboembolism. These can cause coughing, dyspnea (choking hazard), chest pain, and hemoptysis.

Kidney damage (lupus nephritis)

Renal damage determines the severity of the evolution of lupus patients. One-third of patients have kidney damage, suggested by proteinuria or hematuria, serum creatinine is above the upper limit of the laboratory, and creatinine clearance is low.

Neuropsychological impairment

It is the least understood manifestation of systemic lupus erythematosus and one of the most severe. Among the neurological manifestations, we mention convulsions, severe headaches, peripheral neuropathies, cranial nerve damage, cerebral vascular accidents, acute meningitis, and chorea. Psychiatric manifestations include psychoses, cognitive dysfunction, anxiety syndrome, and mood disorders.

Gastrointestinal symptoms

The most common gastrointestinal symptoms are anorexia, nausea, vomiting, and abdominal pain, and may be manifestations of mesenteric vasculitis, peritonitis, pancreatitis, or secondary to the administered medication.

Liver damage is found in up to 60% of patients – hepatomegaly, increased liver enzymes, and hepatic steatosis. Up to 8% of patients develop retinal artery inflammation with thrombosis at this level. The conjunctiva and cornea can be affected by secondary Sjogren's syndrome [30].

Specific immunological abnormalities

The presence of autoantibodies that target their own structures. Antinuclear antibodies (ANA) occur in approximately 95% of patients but have low specificity and may be associated with other conditions (rheumatoid polyarthritis, autoimmune thyroiditis, autoimmune liver diseases, infections, neoplasms, or in healthy individuals). The most frequent antibodies encountered in SLE are the antibodies: anti-double-chain DNA (SLE specific, associated with disease activity), anti-Sm (SLE specific), anti-U1 RNP, anti-Ro, anti-La, anti-ribosomal protein P (SLE specific, appear in neurological, hepatic damage), anti-histones (occur in classical and drug-induced lupus), anti-phospholipids (characteristics of SAFL), rheumatoid factor (non-specific, occurs in many other autoimmune conditions, infections). Decreased serum complement fractions (C3, C4, CH50) are associated with disease activity.

As is the case with many chronic conditions, systemic lupus erythematosus (SLE) negatively affects the quality of life, due to factors such as depression, pain, cognitive dysfunction, and sleep disorders.

Despite a little-understood etiology, fatigue is associated with SLE in approximately 80% of cases, which leads to a decrease in the level of physical activity necessary to carry out usual daily activities and thus dramatically decreases the quality of life.

Physical medicine and recovery in Systemic Lupus Erythematous may involve physical therapy, occupational therapy, speech therapy, recreational therapy, or combinations thereof. The specialist doctor should coordinate the patient's care and be attentive to subtle changes in symptoms that may indicate periods of disease activity [32].

Ultrasound is a frequently used procedure to provide heat in the depth of the affected joint, but it should not be used in the presence of inflammation; locally applied ice is the preferred method for inflamed joints. When ultrasound is used to improve joint mobility, the application of ultrasound and joint movement should be simultaneous.

Hydrokinetic therapy is recommended for the mobilization of inflamed joints, due to the buoyancy that ensures the unloading of the joint and the soothing action of the warm water.

The cardiovascular and pulmonary systems can also be affected by lupus. We try to improve aerobic capacity through physical exercises and specific breathing exercises [31]. These exercises will also contribute to maintaining a strong bone system, as well as strengthening ligaments and joints. Regular practice of sports activities is particularly recommended for patients with lupus.

Occupational therapy has the role of helping the patient regain as much of his functional independence as possible, despite the problems caused by the disease. Activities of daily living are encouraged and may require training with special equipment, techniques, and procedures. These activities include feeding, dressing, bathing, grooming, grooming, and cooking in the home.

A safety assessment at the patient's home may be indicated. Occupational therapy can provide recommendations for equipment (for example bathtub bench, elevated toilet seat, grab bar) to increase the patient's independence and safety at home.

Patients who have neuropsychiatric complications due to lupus may have cognitive dysfunctions, with deficits in attention, language, and memory. The speech therapist can be helpful when a patient with systemic lupus erythematous has slurred speech, difficulty understanding speech, or difficulty speaking properly.

Because systemic lupus erythematosus is a condition that manifests itself episodically, through bouts of activity alternating with episodes of remission, it is important that the patient does nothing without consulting the rheumatologist who deals with his disease – thus, it will reduce the risk of triggering a set of activities.

Unfortunately, there is still no treatment that can cure lupus, but there are treatments that can improve the symptoms while prolonging the episodes of remission of the disease. Adopting a healthy lifestyle also contributes to increasing the quality of life of the lupus patient.

Other things that the lupus patient can do to improve their condition:

- To give up smoking, but also the consumption of caffeine or alcohol.
- To adopt a healthy, balanced diet, based on as many natural foods as possible.
- To rest enough.
- Follow exactly the treatment recommended by the doctor, even during periods of remission of the disease.
- To avoid exposure to the sun because ultraviolet radiation

increases the risk of triggering a flare-up of systemic lupus erythematous.

It is recommended to reduce exposure to UV rays (using creams with a minimum sun protection factor of 50, avoiding exposure to the sun), a diet adapted to the patient undergoing corticosteroid therapy (to prevent its adverse effects – diabetes, hypertension, dyslipidemia), quitting smoking.

To prescribe a drug treatment, it is essential to identify the type of organ damage and its severity.

Non-life-threatening damage (skin-mucous, musculoskeletal, serositis) is indicated for the administration of non-steroidal anti-inflammatory drugs, low-dose systemic cortico-therapy, and synthetic antimalarials.

Potentially vital damage (cardio-pulmonary, hepatic, renal, hematological, central nervous system) requires cortico-therapy in moderate or high doses, associated depending on the affected organ, with immunosuppressants, anticoagulants, anticonvulsants, or even biological therapy.

Systemic lupus erythematosus is a chronic condition with multisystem involvement that presents severe evolutionary potential. Currently, thanks to advances in the knowledge of mechanisms, diagnosis, and treatment, the 10-year survival rate of lupus patients is almost 90% [30].

MATERIALS AND METHODS

This review started with a case study and addresses the therapeutic aspects of SLE and Diabetes Mellitus of a 47-year-old male with a delicate multi-pathology and aggressive evolution, caused by both the diagnostic and the difficult approach of the psychomotor rehabilitation therapy when SLE treatment has ended. In this case study report, we approach the importance of the management of the disease combining not only the multiple therapeutic actions but also the proper medication and nutrition and the psychomotor rehabilitation [11]. The study includes stages of life-threatening biological depreciation, and treatment, until the present when the therapy is positively affecting the patient's quality of life.

We present the short version of the patient's clinical history:

- At 27 years old, he underwent surgery for Non-Hodgkin's Lymphoma, associated with polychemotherapy and radiotherapy, with good response, in remission and without any specific medication from 2003.

- No forms of SLE manifestation until 2017.

- The aggressive form of SLE debuted in September 2017, caused by an unstable lifestyle associated with many detrimental factors accumulated in the last 15 years: unbalanced alimentation (almost exclusively processed meat products/minced meats and semi-prepared products, and carbonated beverages), smoking, stress, lack of sleep, improper self-medication for diabetes mellitus, sustained effort.

- The SLE treatment itself begins in February 2018, the patient’s previous treatment included only medication for his symptoms - edema, hypertension, cytopenia, etc.

- In his delicate condition, with the complex ongoing treatment, the patient beneficiated from the support of his family, nutritionist, priest, and doctors which improved his mental state of being.

- The period 2018-2019 was an equilibrated one when the patient was under observation, good nutritional plan, and 3 weekly sessions of physical therapy.

- In February 2022 the patient gets infected with SARS-CoV-2 Covid 19 Omicron Type which lead to a 10 days hospitalization in the nephrology unit medical care.

- The clinical picture of the subject presented above starts from the aggressive debut in the autumn of 2017 until 2021. We mention that multi-pathology and all associated diagnoses are not included in this presentation.

We also mention that during this period of multiple hospitalizations, there were diagnosed multiple associated pathology such as Diabetes mellitus type 2 with established Diabetic Nephropathy, Hypertensive Nephropathy with Renal Impairment, Hyperuricemia without signs of Inflammatory Arthritis or Tophaceous Disease, Vulgar Ichthyosis, Glomerular disorders in systemic connective tissue disorders, other water and electrolyte disturbances not elsewhere classified, Anemia in other chronic diseases classified elsewhere, Non-inflammatory Pericardial infusion, Medication-induced Specific Polyneuropathies, Secondary hypertension of other kidney disorders, Ascites, Hypo-osmolality and hyponatremia, Chronic nephritis syndrome, etc.

Also, here are some additional landmarks: the dietary regimen consisted of predominant vegetarian and hypoglycemic food, water management with 500 ml more than the urinary removal, medical plant tea indication, measurement of food in carbs no more than 150 per day (50/50/50 for each meal) associated with the prescribed medication; glycemic test each day before meals with calculated insulin administration (rapid and long-acting insulin) and/or gliclazide.

The multidisciplinary team of doctors (diabetologists, nephrologists, cardiologists, and Physiotherapists) [12-17] followed the patient during all the periods.

Treatment of SLE with the administration of combined diuretic treatment: high-ceiling diuretics (furosemide), Vasopressin antagonists (tolvaptan), Diuretic thiazide and potassium-sparing (amiloride hydrochloride and hydrochlorothiazide), thiazide diuretics (hydrochlorothiazide) and immune-suppressive combination (cyclophosphamide, mycophenolate mofetil), cyclosporine and corticosteroids (prednisolone).

Evaluation methods

To carry out this research from the Physiotherapists point of view, we chose the following measurements and evaluation tests:

1. to assess the level of the peripheral inflammatory stage at the level of the lower limbs as well as that of the muscle mass of the subject, we performed the anthropometric-perimetry method for the ankle and calf.

2. to evaluate the degree of mobility of the lower limb necessary for functionality, we used the goniometric method for the ankle and hip joints.

3. to assess static balance, we used the simple bipedal balance test with eyes open with several penalties for imbalance for 5 minutes.

4. to assess dynamic balance we asked the patient to walk on a drawled line of 5 meters distance, eyes open, without imbalance, dizziness, or lateral support.

5. to evaluate the subject's effort capacity, we used the ergometric bike without resistance with heart rate measurement.

Description of the used methods

1. The patient is in the supine position, and the measuring tape is applied at the level of the ankle joint including the most inflamed area, measuring an average of the right/left ankles before the physical therapy program.

2. The patient lying on his back, and the goniometer is applied according to the goniometric measurement and evaluation standards of the ankle and hip joint for the flexion movement, before the physical therapy program.

3. The patient in the standing position with bare feet on the floor for better plantar kinesthesia, eyes open, is timed for how many minutes/seconds he can stand without imbalances, dizziness, or lateral support. The evaluation was carried out at the beginning of the meeting for static balance.

4. For dynamic balance, a 5m line was marked on the floor, and the patient was asked to follow the line walking, eyes open, marking any sign of imbalance, dizziness, or lateral support, evaluated in how many meters the patient succeed to go on, at the end of the session.

5. The patient on the ergometric bicycle without lumbar support, with a distance between the hip and the pedals so that the knee is slightly flexed, without load, he pedals freely at a slow pace, following the heart rate so that it does not exceed 120 beats/min, it is marked how many minutes he can pedal up to the indicated maximum cardiac effort, or any other sign of imbalance, dizziness, numbness or pain. The test was held at the end of the physical therapy session.

Evaluation Instruments

The used instruments were a tape measure, goniometer, stopwatch, liner, ergometric bicycle, and pulse tester.

Research methods used

The research methods used in the paper were:

- the theoretical documentation method.

- pedagogical observation.

- the experimental method.

- the method of tests and evaluation samples.

- the investigation method.

- the method of recording and statistical-mathematical data processing.

- the graphic method.

Applied intervention

To streamline and synthesize the process of describing exercises applied, they are presented below (Tables 1 and 2).

The tables show the means we worked with, the exercise program proposed for every period, and their dosage.

Teaching materials used to fulfill the improvement program of motor functional skills and of balance and effort capacity were the stick, a small hand weight 1 kg, a terra band, the balance board, the inflatable balance disc, the gym ball, the small spikes ball for prehension, the bicycle, marker.

The physical therapy program no. 1 consisted of this period in: specific exercises that do not involve risks factors for strokes, bruises, or bleeding; exercises that do not present risk factors for a patient with high blood pressure; exercises and techniques for lymphatic drainage; specific techniques for tone muscle and equilibrium; specifically adapted methods for connective flexibility and joints mobility; programs for developing effort capacity; outdoor adapted program; exercises that consider the electrolyte imbalance (already existing due to the disease); breathing exercises.

Table 1: The process of describing initial exercises applied – the first program

Type of method	Exercise	Series	Reps or seconds/minutes	Pause (sec)
Mobilization	From the lying position - slow passive mobilization of the ankle in all directions	3	30 reps	30 sec
	From the lying position - slow passive mobilization of the knee for flexion and extension	3	15 reps	30 sec
	From lying position - Slow passive mobilization of the hip in all ranges of motions from simple to complex	3	15 reps	60 sec
	From lying position and sitting - Active mobilization of the upper limbs with a stick in all ranges of motions	3	15 reps	60 sec
	From lying position - Free active mobilization of the upper limb in all ranges of motions	3	15 reps	30 sec
	From lying position - Free active mobilization of lower limbs in all ranges of motion (bicycle, shears, bypasses)	3	15 reps	60 sec
	Quadrupedal position, cat forward and backward	3	10	120 sec
Tone muscle	Lying position - all range motion mobility with 1-kg hand weights	3	15	60 sec
	Sitting position - all range motion mobility with 1-kg hand weights	3	15	60 sec
	Sitting position - terra band extension between arms in abduction and extension	3	10	120 sec
	Sitting position - leg extension with a terra band resistance	3	10	120 sec
	Lying position - knee flexion and extension with terra band resistance	3	10 reps	120 sec
Static balance	Sitting position - imbalance from the shoulders in all directions with the requirement to maintain the posture from the abdomen without the help of the hands	3	10 reps	60 sec
	Standing position - Keep abduction of the hands	3	10-60 seconds	120 sec
	Standing position - maintain the balance without lateral support or movements	3	10-60 seconds	120 sec
Dynamic balance	Standing position - Raise 1 leg on a support ball	3	10-60 seconds	60 sec
	Standing position - walking with a plastic cup with water in the hand	3	10 m	60 sec
	Standing position - walking on a drawled line on the floor without lateral support or lateral deviations	3	10 m	120 sec
	Standing position - with lateral or face hand support - on a balance platform or ball - back straight, crunches	3	10 reps	120 sec
	Standing position - lateral support - kick a ball	3	10 reps	60 sec
Effort capacity	Bicycle ride - level 0	1	5 min	-
	Bicycle ride - level 0	1	15 min	-
	Claiming stairs from 1 to 3 floors	1	15-30 min	5 min
Outdoor training	Walking outdoors on a parc with support (frame, cane or crutch, or human lateral support)	1	15-30 min	-
Breathing techniques	Lying position - costal and abdominal breathing techniques, slowly and following the dizziness or pain	1	5- 10 min	-
	Sitting position - gently inhale with the extension of the trunk and arms, and exhale slowly and long with the return and withdrawal of the navel towards the spine.	1	5-10 min	-
Manual techniques	Manual lymphatic drainage and pump exercises	1	15 – 30 min	-
	Manual therapy for connective tissues	1	15-30 min	-

All exercises and techniques were adapted to the daily dynamic state of functionality of the patient from very simple basic to the maximum possible in his case. The programs were carried out on an outpatient basis at the patient's home, with a frequency of 3 sessions per week (Monday, Wednesday, Friday), the duration of the session being between 30 and 120 minutes depending on the daily state of the patient and the applied methods.

Table 2: The process of describing initial exercises applied – the second program

Type of method	Exercise	Series	Reps or seconds/minutes	Pause (sec)
Mobilization	From the lying position - slow active mobilization of the ankle in all directions	3	30 reps	30 sec
	From the lying position - slow active mobilization of the knee for flexion and extension	3	15 reps	30 sec
	From lying position - Slow active mobilization of the hip in all ranges of motions from simple to complex	3	15 reps	60 sec
	From lying position and sitting - Active mobilization of the upper limbs with a stick in all ranges of motions	3	15 reps	60 sec
	From lying position - Free active mobilization of the upper limb in all range of motions with 1 kg hand weights	3	15 reps	30 sec
	From lying position - Free active mobilization of lower limbs in all ranges of motion with 1 kg leg weights (bicycle, shears, bypasses)	3	15 reps	60 sec
	Quadrupedal position, cat forward and backward	3	10	120 sec
Tone muscle	Face lying position - all range motion mobility with terra band	3	15	60 sec
	Sitting position - all range motion mobility with 1 kg hand weights	3	15	60 sec
	Sitting position - terra band extension between arms in abduction and extension	3	10	120 sec
	Sitting position - leg extension with a terra band resistance or 1 kg leg weights	3	10	120 sec
	Lying position - knee flexion and extension with terra band resistance or 1 kg leg weights	3	10 reps	120 sec
Static balance	Sitting position - imbalance from the shoulders in all directions with the requirement to maintain the posture from the abdomen without the help of the hands or with a ball or a weight of 1 kg in the hands	3	10 reps	60 sec
	Standing position - Keep abduction from 90° - 180° of the hands	3	10-60 seconds	120 sec
	Standing position - maintain the balance without lateral support or movements and keep a 1 kg weight on the hands	3	10-60 seconds	120 sec
Dynamic balance	Standing position - Raise 1 leg on a support ball with 1 kg leg weight	3	10-60 seconds	60 sec
	Standing position - walking with a 1 kg weight on the hand	3	10 m	60 sec
	Standing position - walking on a drawled line on the floor without lateral support or lateral deviations with a cup of plastic water in the hand or on the head	3	10 m	120 sec
	Standing position - with lateral or face hand support- on a balance platform or ball - back straight, squats or lunges	3	10 reps	120 sec
	Standing position - lateral support - kick a ball or roll a 1 kg leg weight	3	10 reps	60 sec
Effort capacity	Bicycle ride - level 1	1	5 min	-
	Bicycle ride - level 1	1	15 min	-
	Claiming stairs from 1 to 6 floors	1	15-30 min	5 min
Outdoor training	Walking outdoors on a parc with or without support (frame, cane or crutch or human lateral support)	1	15-30 min	-
Breathing techniques	Standing position - costal and abdominal breathing techniques, slowly and following the dizziness or pain	1	5- 10 min	-
	Standing or quadrupedal position - gently inhale with the extension of the trunk, leg, and arm, and exhale slowly and long with the return and withdrawal of the navel towards the spine	1	5-10 min	-
Manual techniques	Manual lymphatic drainage and pump exercises	1	15 – 30 min	-
	Manual therapy for connective tissues	1	15-30 min	-

The program contains breathing exercises from decubitus and sitting, easy mobilization exercises for upper limbs with the stick and weights no more than 1 kg, slow exercises for lower limbs at the bedside level, lymphatic drainage, progressive passive mobilizations, progressive active spine mobilizations, static and dynamic equilibrium methods, core and leg muscle tone exercises, home bicycle program from 0 weight, outdoor walking sessions. Monday and Wednesday were addressed to the indoor program and Friday evening (due to the ocular and skin problems in SLE) if the weather was proper and the state of the patient was a good one, was addressed to the outdoor program.

Objectives

The 1 physical therapy program followed the objectives of each period of the evolution of the patient's state.

The initial objectives of the treatment were those of:
- preserving life
- following developments in functionality and evasion of complications.

The main objectives were:
- Improve the quality of life
- Decrease in inflammation and lymph retention
- Preserve and gain flexibility
- Develop tone muscle
- Develop a functional effort capacity
- Obtain a good static and dynamic stability
- Maintain a good psychological state of mind and optimism with the patient and family.

The functional state of the patient translates into functional disability to service and personal care, unable to have an orthostatic position, seated posture, and displacement.

The physical therapy program no. 2 consisted of the same exercises as in the first period but improved and adapted to the new daily possibilities of the patient. Such as upper and lower limb mobilization in a full range motion and with resistance, static and dynamic balance improved techniques for outdoor walking without the support and on uneven terrain, effort capacity development on the bicycle with resistance, maneuverability, and coordination exercises.

The program contains breathing exercises from sitting and standing positions, mobilization, and tonus exercises for upper limbs with the stick and 1 kg weight, exercises for lower limbs at the bedside level and from standing position with terra band, abdominal and diaphragmatic exercises, equilibrium exercises with or without lateral support, progressive active mobilizations, prehension exercises, lymphatic drainage, and manual therapy, bicycle training, outdoor walking sessions.

All exercises and techniques were adapted to the daily dynamic state of functionality of the patient following the rule of "primum non nocere" [26].

Objectives

The 2 physical therapy programs followed the objectives of each period of the evolution of the patient's state.

The initial objectives of the treatment were those of:
- preserving the quality of life and existent functionality
- following developments in functionality and evasion of complications.

The main objectives were:
- Improve the quality of life
- Preserve and gain flexibility and connective tissue elasticity
- Develop tone muscle and regain muscle mass
- Gain a functional effort capacity for outdoor functionality
- Obtain good static and dynamic stability together with coordination skills
- Maintain an optimistic continuous psychological state of mind and optimism with the patient and family.

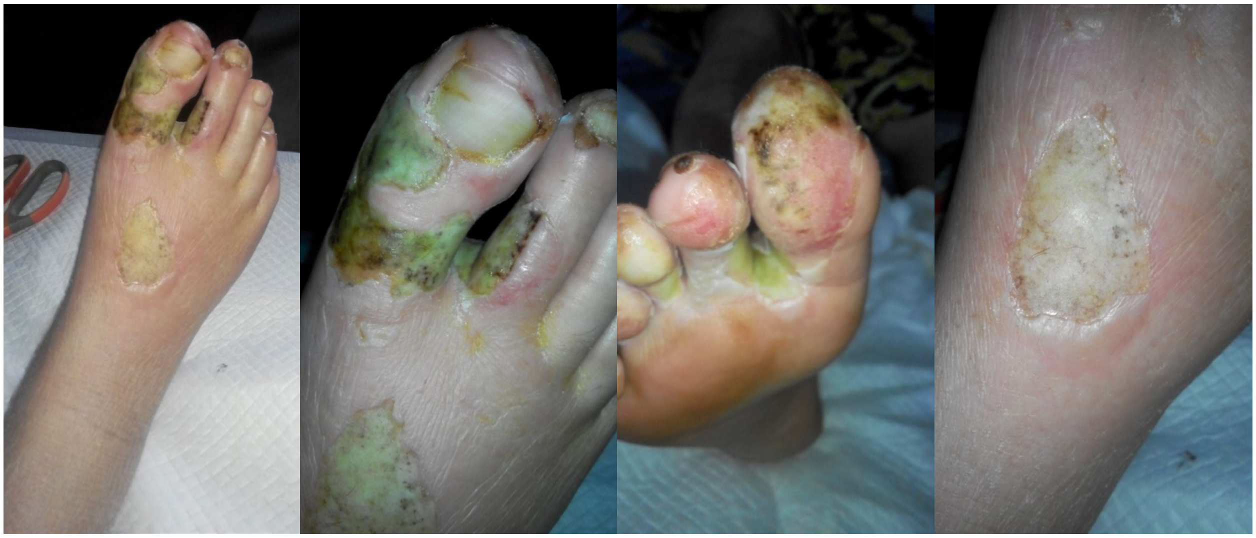

Figure 1: Representative images of the foot edema and neuropathy skin lesions

The recovery protocol consisted of tracking the functionality of each step according to the patient's evolution, following the food and drug indications and the degree of muscle and joint recovery, and also considering the equilibrium function that has been greatly affected.

During these months of complex treatment, the patient had positive or stagnant recovery moments, being affected by atmospheric pressure temperature, and climate change.

The purpose was to perform standing posture and walking with a moderate balance and state of equilibrium.

The functional state of the patient in March 2021 was that of functional and personal care integrity, the ability to perform standing posture, and walking with a moderate balance and state of equilibrium (Figure 3).

The patient continued the prescribed programs on his own at home during the pandemic period and the functional state of the patient in June 2022 was that of functional and personal care integrity, the ability to perform standing posture and walking with a moderate balance, and a state of equilibrium. (Figure 4).

RESULTS

The results concerning our patient were very promising (Table 3).

The perimetric measurements showed a constant decrease between the initial, intermediate, and final evaluations of the dimensions by 6 centimeters for the ankle joint and by 8 centimeters for the calf, relevant to a good drainage process both through the function of the kidney and through the lymphatic system, due to both effective medication and the sustained kinetic program.

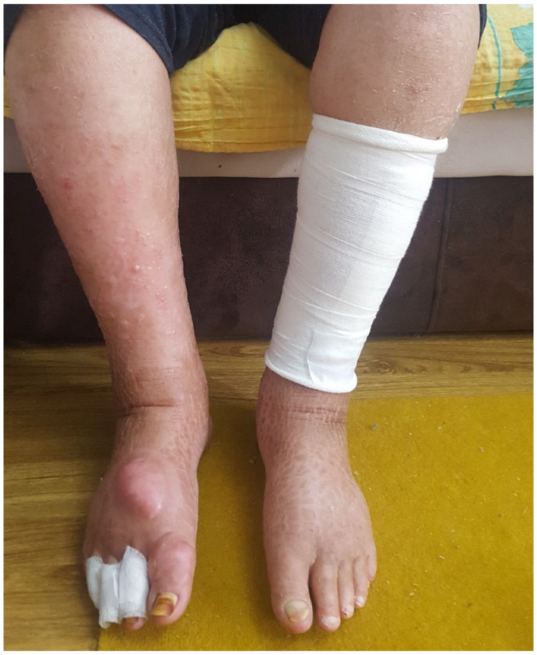

Figure 2: Representative images of the foot edema and neuropathy skin lesions

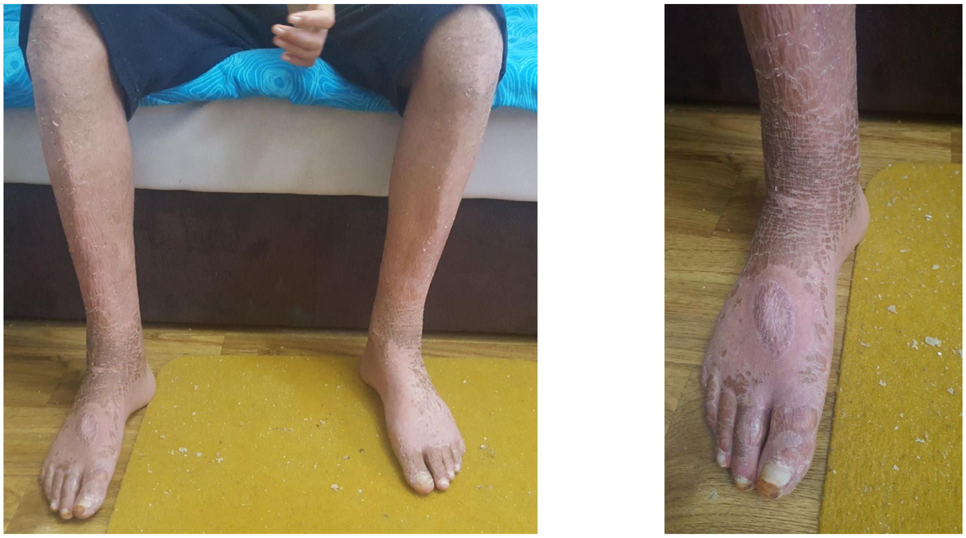

Figure 3: Representative images of the foot from March 2021.

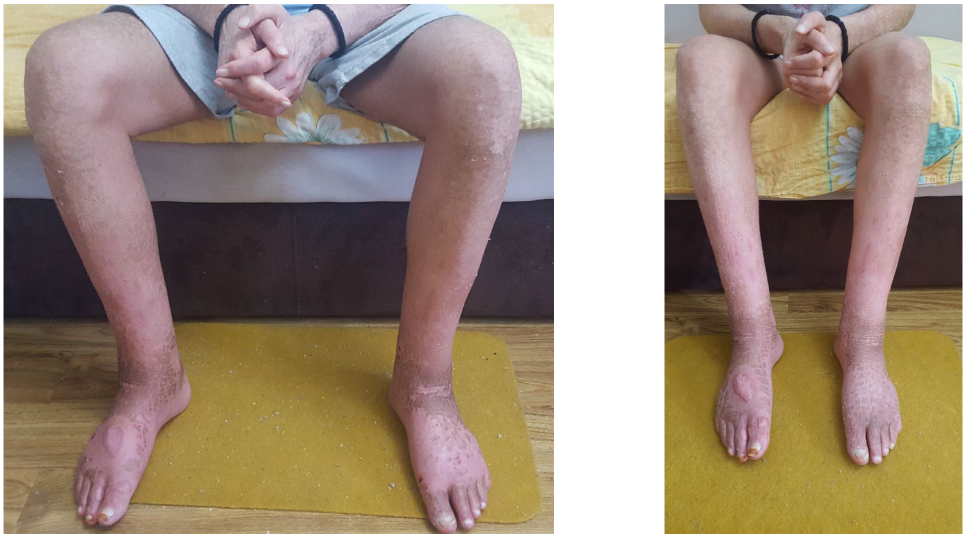

Figure 4: Representative images of the foot from June 2022.

The goniometric measurements for ankle and hip flexion had values of increased joint mobility, thus the ankle gained 10 degrees and the hip 55 degrees in a range of motion, which allowed the patient better mobility and dynamic functionality.

Table 3: Results of evaluation tests

Measurements	Initial testing Feb 2017	Intermediary testing Feb 2018	Final testing March 2019	Observations
Ankle perimeter	30 cm	27 cm	24 cm	Decreased by 6 cm
Calf perimeter	45 cm	43 cm	37 cm	Decreased by 8 cm
Ankle flexion mobility	5°	10°	15°	Improved with 10°
Hip flexion mobility	35°	85°	90°	Improved with 55°
Static stability	0	5 min	10 min	Improved 10 min
Dynamic stability	0	10 meters	30 meters	Improved 30 meters
Effort capacity	0	10 min	15 min	Improved 15 min

The evaluation of static stability recorded beneficial increases for the patient's life from the value of 0 minutes to 10 minutes without imbalance. The dynamic stability tests increased from 0 meters to 30 meters of walking without imbalance and support. Thus, the static stability gained 10 minutes without imbalances, and the dynamic one from value 0 reached a displacement of 30m without imbalances and support, which allowed the patient to go outside the house.

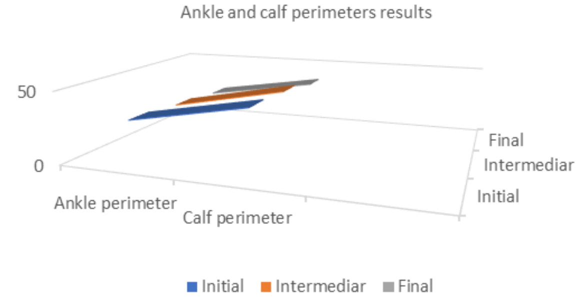

Figure 5: Ankle and calf perimeters measurements results

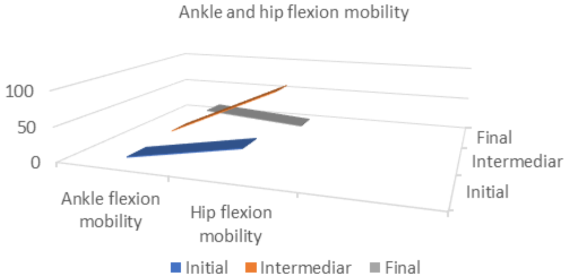

Figure 6: Ankle and hip flexion mobility results

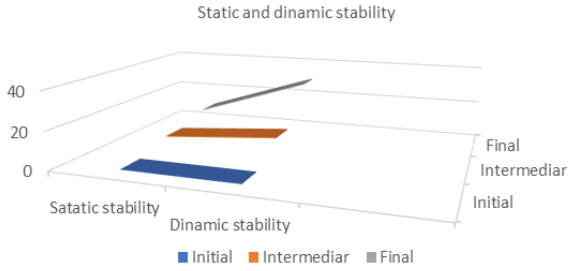

Figure 7: Static and dynamic stability evaluation results

The evaluation of the effort capacity from 0 initial value has reached 10 minutes without effort disorders so that later on the final evaluation on the best day of the patient will be 15 minutes without effort disorders. We mention that every day the patient's dynamics undergo changes from one hour to the next and the time of day matters a lot.

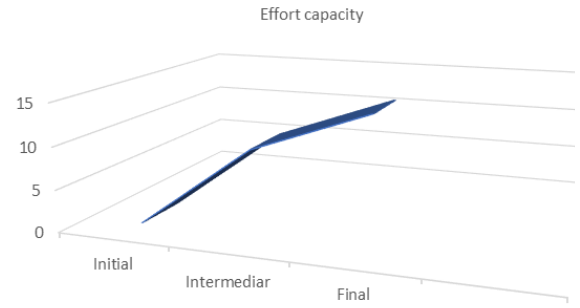

Figure 8: Effort capacity results.

DISCUSSION

The recommended strategy for addressing such a complex SLE case, for recovery of motor function, requires monitoring of at least three lines of observation:

1 Always be aware of disease progression (more in the context of risk of relapse of disease) and medication applied:

- Consulting physician recommendations, the results of medical tests, imaging investigations, etc.

- Check the treatment schedule, especially in the case of a disease involving a long-term treatment scheme with the adjustment of the substances and doses used (side effects of drugs can often have an impact on the patient's condition).

2 Use in an efficient manner the pluses of a positive psychic state (a calm, orderly mind with clear targets) and cultivate the will of the patient.

3 Permanent assessment of patient evolution, in points where difficulties were found: posture, coordination, and motricity.

Following the lines of this strategy, the physical therapist will be able:

- To work together with the patient, in a flexible system, the recovery exercises - maximum 10-30 minutes.

- To recommend to the patient a set of exercises to perform at home (immunosuppressive regimens do not recommend the presence of the SLE patient in the community over long periods of time, even more in the presence of other patients with transmissible diseases).

- And additionally, where appropriate, to apply therapeutic manual therapy and drainage sessions, as well as breathing techniques.

The recommended strategy for addressing the rehabilitation of motor function in such a complex case requires monitoring of at least these five levels of observation:

- Always be aware of the disease progression (even more in the context of risk of relapse) and medication administration, constantly consult physician recommendations, the results of medical tests, imagistic investigations, and so on. In this case, multiple impairments of the organ systems have led to chronic or acute manifestations of imbalances in the body [21-25,33, 34]. Permanently check the treatment schedule, especially in the case of a long-term treatment plan disease with the adjustment of the substances and doses used (side effects of drugs can often have an impact on the patient's condition).

- Permanent assessment of the patient’s evolution, in points where difficulties were found posture, coordination, and motricity, cause many of the associated diseases, both in the kidneys and other organs, make it difficult to choose the correct and balanced diet and medication.

- An important path leading to complete rehabilitation in this case, as in many others, was the combination of sustained social - psychological - spiritual support through faith and management with attention focused on the patient. The psychological support received from the medical staff and family, largely based on the patient’s trust in them, was even more necessary as the treatment with corticosteroids can cause changes in behavior (periods of extreme energy and activity, combined with periods of insomnia and irritability). We identified at least three main engines of the patient’s desire to recover – confidence in the attending physician and successful completion of the treatment, the desire to return to a normal life and sacred therapy.

The multi-pathology, as well as the delicate situation of the patient, have greatly raised the stakes of the entire medical staff in choosing the most suitable treatment management, which is still in progress daily.

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ORIGINAL ARTICLE

The Psychiatric, Psychological, and Psychotherapeutic Approach to Erectile Dysfunction – Between Good Practices and Clinical Challenges

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Abstract: *Erectile dysfunction (ED) has an intricate pathogenesis, with organic and psychosocial factors contributing to the shaping of its clinical manifestations and functional impairment. ED disrupts not only an individual's sexual life but may also contribute to impairments of self-esteem, social functioning, quality of life, overall well-being, mood, etc. The assessment process and therapeutic interventions should be adequate to the specific profile of each patient, therefore an interdisciplinary approach is usually recommended, in order to find the vulnerability factors, recent triggers, psychiatric and organic comorbidities or causes, and elements that contribute to maintaining the sexual dysfunction, such as lifestyle factors, interpersonal conflicts, or inadequate sexual education. This narrative review explores the evidence-based approaches to structured psychiatric, psychological, and therapeutical assessment in patients with ED, and the most validated psychosocial treatments available. The results support the use of recognized diagnostic criteria within DSM-5TR and ICD-11, together with structured instruments (scales, questionnaires, and inventories), in a multidisciplinary approach. The cognitive model and cognitive-emotional model of ED support the initiation of cognitive-behavioral therapy in these patients. Other interventions, like the PLISSIT model, sexual therapy, couple therapy, and sexual-focused psychoeducation are also supported by evidence. In conclusion, ED requires a combined, psychiatric, psychologic, psychotherapeutic, and organic-oriented assessment, with the purpose of tailoring the treatment to the patient's particularities.*

Keywords: *major depressive disorder, erectile dysfunction, antidepressants, antipsychotics, schizophrenia spectrum disorders, psychotherapy, psychoeducation*

INTRODUCTION

Erectile dysfunction (ED) is one of the most frequently reported sexual pathologies, with an estimated global prevalence varying from 3% to 76.5% [1]. The large variations in the reviewed reports regarding the prevalence of ED are explained by the method chosen during studies to define and quantify the manifestations of this disorder. When structured, validated clinical scales, questionnaires, or inventories were used, the prevalence of ED in young men was high [1]. Non-validated instruments have lower sensitivity, therefore the prevalence obtained using such tools is expected to be less accurate [1,2].

It is also important to highlight that ED was positively correlated with cardiovascular diseases, a high risk of all-cause mortality, mortality due to cardiovascular pathology, and a higher risk of developing dementia [1]. Further exploring this topic, ED is considered a "sentinel marker" for cardiovascular disease and could indicate a chronic and undetected coronary artery disease [2]. This correlation was supposed to be more significant in men with diabetes mellitus [2]. ED demonstrated significant value as an indicator of general health in men, irrespective of the cardiovascular risk factors [3].

Therefore, the importance of exploring the most adequate ways to diagnose and treat ED derives not only from the relevance of this disorder on the quality of life and well-being of the patients but also from the potential comorbid conditions this diagnosis may reveal during the multidisciplinary assessment.

OBJECTIVES AND METHODOLOGY

The main objective of this review was to find the most evidence-based assessment methods for patients with ED from the perspective of mental health professionals. Secondary objectives were founding data on (a) the available psychosocial approaches to ED; (b) ways to integrate psychological, psychiatric, and psychotherapeutic evaluations for patients with ED; (c) finding the key aspects that can help the training of mental health specialists in the evaluation and treatment of patients with ED.

The identification of relevant articles involved searching three electronic databases (PubMed, Cochrane, and Clarivate/ Web of Science) as well as their list of references. There was no inferior time limit for the search, while the superior limit was June 2023.

All primary and secondary reports were allowed into the analysis. No language restrictions were applied.

The paradigm used for retrieving sources was “erectile dysfunction” OR “male sexual dysfunctions” AND “assessment” OR “psychotherapy” OR “counseling” OR “diagnosis” OR “cultural aspects” OR “skills training” OR “mental health specialists”.

RESULTS

The results of the literature search were distributed in three sections, according to their relevance for each category of mental health specialists involved in the assistance of patients with ED. The accent is placed on the need to conduct a multidisciplinary exploration of the ED (Figures 1 and 2) and to formulate a therapeutic plan that takes into account the main vulnerability factors identified during the initial assessment of the patient.

The psychiatric approach

The diagnosis of ED implies, according to the 5th edition (revised) of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5TR), significant difficulty in reaching or maintaining an erection, or a marked decrease in the penile rigidity during erection, that appears on almost all occasions of sexual activity (75-100%) [4]. There is also the requirement of a minimal duration of 6 months for these symptoms, and they should cause clinical distress, in order to complete the diagnosis criteria [4]. Like in the case of all sexual dysfunctions, ED should be distinguished from organic, toxic, stress-induced pathology, or other mental disorders [4]. ED may be lifelong (if the patient did not have any period of normal sexual functioning) or acquired (if the dysfunctions started after a period of normal sexual functioning) [4]. ED can also be mild, moderate, or severe (depending on the level of symptoms-induced distress), or it may be generalized or situational (depending on the types of stimulation, situations, or partners that may or may not trigger the symptoms) [4].

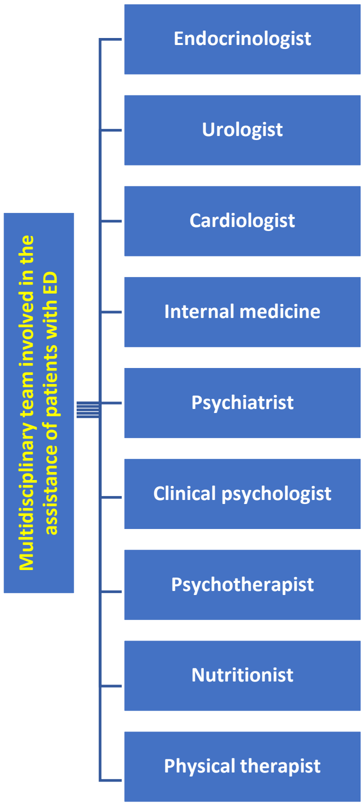

Figure 1: Multidisciplinary assessment of patients with ED

The diagnostic criteria in the DSM-5 and DSM-5TR changed from the previous versions (DSM-IV and DSM-IV TR) [4-7]. In these early editions, only two clinical criteria were mentioned, without any reference to the decrease of penile rigidity, as an independent criterion; also, no frequency of failed sexual acts was mentioned; no severity specifiers existed, but ED could have been classified as exclusively psychogenic or with mixed causation [6,7]. Further back, the third edition (revised) of DSM (DSM-III(R)) included the “male erectile disorder”, defined as a partial or complete, persistent and recurrent, incapacity to obtain and maintain the erection until the end of a sexual act, or recurrent/persistent lack of sexual excitation and pleasure during the sexual activity [8]. ED is included in the category of “male sexual dysfunctions”, together with delayed ejaculation, premature ejaculation, and male hypo-sexual desire disorder (Figure 3) [4-7].

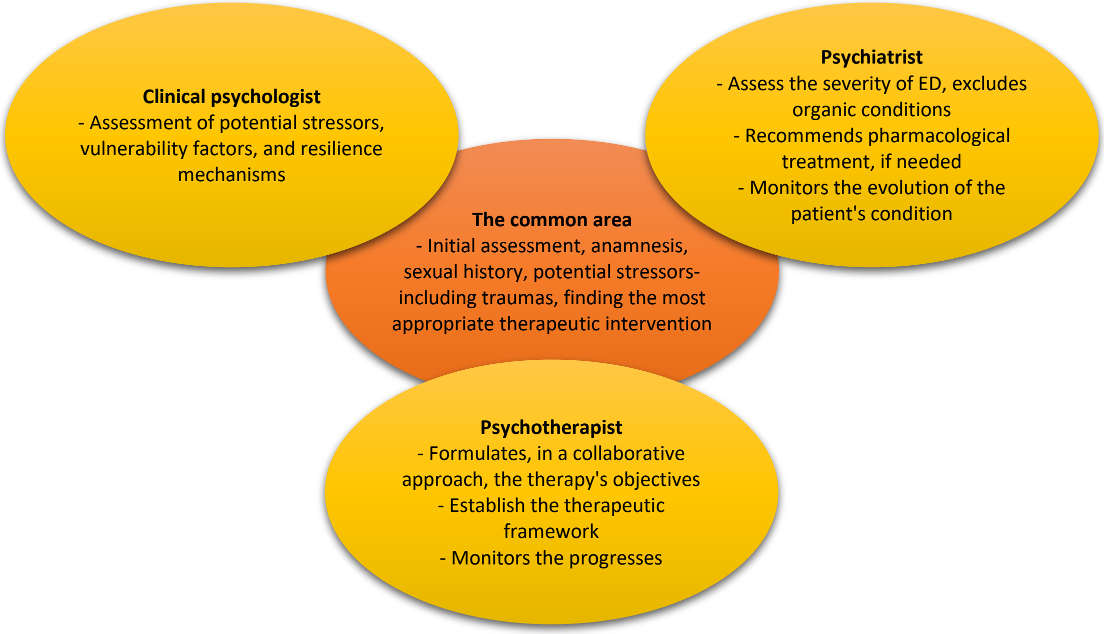
Clinical psychologist
- Assessment of potential stressors, vulnerability factors, and resilience mechanisms
Psychiatrist
- Assess the severity of ED, excludes organic conditions
- Recommends pharmacological treatment, if needed
- Monitors the evolution of the patient's condition
The common area
- Initial assessment, anamnesis, sexual history, potential stressors- including traumas, finding the most appropriate therapeutic intervention
Psychotherapist
- Formulates, in a collaborative approach, the therapy's objectives
- Establish the therapeutic framework
- Monitors the progresses

Figure 2: The roles of the clinical psychologist, psychiatrist, and psychotherapist in the assistance of patients with ED

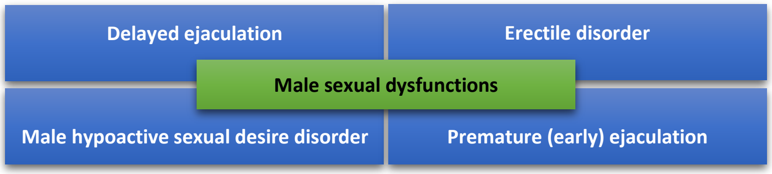
Delayed ejaculation
Erectile disorder
Male sexual dysfunctions
Male hypoactive sexual desire disorder
Premature (early) ejaculation

Figure 3: Male sexual dysfunctions [4-7]

The World Health Organisation’s Classification of Diseases (ICD) mentioned in its latest edition (ICD-11), that ED is the “inability or marked reduction in the ability in men to attain or sustain a penile erection of sufficient duration or rigidity to allow for sexual activity” [9]. Also, ICD-11 specifies that ED appears although the libido and sexual stimulation exist, and its manifestations may be episodic or persistent (several months), and associated with significant distress at the clinical level [9]. In the previous edition, ICD-10 (the version for clinical research), ED had as its main clinical characteristics (1) the impossibility of participating in sexual activity according to the desire of the individual, (2) the dysfunction appears frequently, but may not be observed on every occasion, (3) the manifestations have a history of at least 6 months [10]. The ED may manifest itself as: a complete erection that may be obtained in the initial stage of sexual activity, but then disappears before ejaculation (if this appears); erection may be detected, but only in situations where the sexual activity is unprepared; partial erection, insufficient for sexual activity; or complete absence of erection [10].

The psychiatric assessment of patients with ED is recommended to be carried out from both the clinician's perspective (focusing on signs and symptoms) and the patient's (focusing on the consequences of dysfunction). There may be significant differences between the two perspectives, depending on the criteria for defining ED, for example, the duration of the manifestations, the frequency of their occurrence, and the functional impact. Sometimes the patient can admit the presence of erectile dysfunction and the associated discomfort, but at other times he avoids mentioning any aspect related to sexual functioning, presenting only general symptoms - "weakness", "permanent fatigue", "lack of initiative", etc.

Sexual dysfunction can be an isolated symptom or it can be integrated into a complex context, as it may appear together with multiple psychiatric symptoms (such as anxiety, insomnia, depression, psychotic manifestations, and substance addiction) or organic manifestations (like urinary hesitancy, high blood pressure, pelvic pain, etc.).

Among the main differential diagnoses in ED, major depressive disorder (MDD) is important to be considered, because a decreased libido has been detected in these patients during the acute episodes, and some of the adverse events of antidepressants involve sexual functioning [11-17]. According to a systematic review and meta-analysis (n=49 reports), there is a high comorbidity rate of ED in patients with MDD (OR=1.39) and vice versa (OR=2.92), but a causal relationship can not be determined [11]. The incidence of sexual dysfunctions in patients undergoing treatment for MDD is difficult to be determined, but decreased sexual desire, decreased sexual excitement, reduced ability to reach orgasm, delayed orgasm, ED, delayed ejaculation, priapism, painful ejaculation, penile anesthesia were all reported in this population [12]. Therefore, sexual dysfunction may be a common MDD symptom, but also a frequent adverse effect of antidepressant treatment [14]. Due to this lastly-mentioned phenomenon, premature drug discontinuation in patients with MDD is not an uncommon event [14,18]. Nefazodone and bupropion have been associated with a lower incidence of sexual adverse events, due to their pharmacodynamic properties, unlike the selective serotonin reuptake inhibitors (SSRIs), which are among the most frequent drugs associated with such negative events [18]. At any rate, monitoring sexual adverse events during antidepressant treatment, as well as monitoring sexual functioning in these patients as part of the MDD clinical picture, are very important for preserving therapeutic adherence and quality of life [18-20].

Psychosocial stressors can trigger both major depression and erectile dysfunction [11]. Depression itself can reduce sexual functioning through multiple mechanisms (decreased initiative, fatigue, decreased self-esteem, ideas of uselessness and inability, etc.) [11-13]. Persistent erectile dysfunction can lead to reduced self-esteem, rumination about personal incapacity, and depression. In conclusion, patients with ED should be evaluated for signs of major depression and vice versa [11].

Psychotic disorders involve the presence of delusional ideas (some of which may have a sexual theme), hallucinations (some may be proprio- or interoceptive), thought and language disorganization, behavioral disorganization, negative symptoms (avolition, apathy, flat affect, anhedonia, alogia, etc.). Patients with psychotic disorders frequently associate sexual dysfunctions as a result of negative symptoms, antipsychotic medication, associated depression, etc [21-29]. In patients with psychotic disorders who had never received psychotropic treatment, 17% still accused the presence of sexual dysfunction [22]. Therefore, it is clear that not all sexual dysfunctions reported in this population are secondary to pharmacologic aspects, although a false causal relationship may be construed by patients, relatives, or even treating physicians. It must be mentioned that most mental health professionals do not ask their patients with psychosis about sexual dysfunctions, in clinical settings [23]. The sexual functioning in outpatients with schizophrenia spectrum disorders (SSD) has been associated with the level of subjective quality of life [24]. Also, a correlation between sexual dysfunctions in patients with SSD and their therapeutic adherence has been demonstrated, even when the dose, number, and duration of antipsychotics were not associated with the presence of sexual dysfunctions [25].

In an interesting study, patients with schizophrenia who were drug-naïve (N=20) were compared with patients who received long-acting first-generation antipsychotics (N=51) and with healthy controls (N=51), from the perspective of their sexual functioning [28]. Untreated patients with schizophrenia reported decreased sexual desire, while those who were receiving antipsychotics reported a restoration of the libido, although presented ED, orgasmic and sexual satisfaction problems [28]. Therefore, the structured assessment of sexual function is recommended to be conducted by mental health specialists in all patients diagnosed with SSD.

Anxiety disorders are included in the differential diagnosis of ED, but they may also be comorbid conditions with, or predisposing factors to sexual dysfunctions. While fear is the response to real or perceived imminent danger, anxiety is conceptualized as anticipating an unknown threat or an internal stressor [30]. Both anxiety and fear are associated with an activation of the sympathetic nervous system when anticipating an imminent danger, which prepares the individual for a fight or flight response [30-32].

One of the topics of interest for the current review is the presence of some specific phobias, like genophobia or erotophobia (fear of intimacy and sex), heterophobia (fear of the opposite sex), nosophobia (fear of microbes), coitophobia (fear of sexual intercourse), and haptophobia (fear of being touched by another person) [33-34]. These disorders may negatively affect the quality of sexual intercourse and even lead to avoidant behaviors.

Social anxiety disorder is characterized by fear of being evaluated or criticized by others when an individual is facing social situations, with severe negative consequences over all areas of functioning [35]. Unlike shyness, social anxiety disorder (or social phobia) has a severe impact on the quality of life in affected individuals [35]. According to a study (N=40 participants), men with social phobia report more frequent sexual dysfunctions and are more inclined to pay for sex [36]. The same study found moderate impairment in arousal, orgasm, sexual enjoyment, and subjective satisfaction with sexual activity in men with social phobia [36].

Panic disorder is defined by recurrent and spontaneous panic attacks, with significant negative functional impact [4-10]. Patients with panic disorder have a higher tendency to develop sexual dysfunctions vs. the general population [37]. According to a review (n=6 reports), panic disorder was associated with a high prevalence of sexual aversion and sexual infrequency, while untreated patients with panic disorder may have a higher risk of ED vs. controls [37]. Patients with panic disorder can perceive the similarity between the neurovegetative activation during sexual intercourse and the one with onset during a panic attack [38]. Therefore, this symptomatic overlap maintains a negative conditioning that leads to the continuation of ED [38].

Agoraphobia is characterized by the fear of enclosed spaces, although the focus of this disorder is not limited to such triggers (i.e., newer classifications also include the fear of open spaces, being alone outside the home or being in a crowd, and using the public transportation) [4-10]. The exposure to feared situations can trigger discomfort, anxiety, or panic attacks [4-10]. The exploration of this disorder during the psychiatric evaluation of patients with ED is important because of the significant impact this type of phobia has on the overall functioning and quality of social relationships.

Generalized anxiety disorder has as its core criterion excessive anxiety or worries related to various daily activities, occurring for at least 6 months, and this disorder has been frequently associated with sexual dysfunctions in men [4-10,39]. In a study that evaluated 64 men with ED or premature ejaculation, 23.4% presented with anxiety disorders, and generalized anxiety disorder was the most frequently detected [39]. Men with preexisting anxiety disorders had a higher probability of presenting performance anxiety related to sex and of being diagnosed with premature ejaculation [39].

The prevalence of anxiety disorders in patients with ED ranges from 2.5% to 37%, followed closely by depressive disorders (18-35%) [40,41]. This association was most frequently reported in patients aged between 45 and 54 years [42]. Anxiety, as a non-specific symptom, can impair both sexual functioning and interpersonal behaviors. But the relationship between ED and anxiety is bidirectional because sexual dysfunction can induce anxiety, and consequently lead to a decrease in self-esteem, performance anxiety, and social avoidance.

Obsessive-compulsive disorders (OCDs) are currently conceived as a spectrum, with core symptoms represented by obsessions, compulsions, or both, that occur over a significant period of time or cause discomfort or significant dysfunctions to the patient [4-10]. Individuals with OCDs often present problems with sexual functioning, and 6% of them report the presence of ED, while 39% are dissatisfied with their sexual functioning [43,44]. The specific treatment for OCDs may also play an important role in the onset of ED, starting from tricyclics to SSRIs, especially because this disorder may require higher doses of drugs than MDD or anxiety disorders [44,45].

Body dysmorphic disorder (BDD) is considered the result of a preoccupation with self-perceived defects in physical appearance, which are not observable by others, or are considered minor when evaluated objectively [4-10]. ED was more commonly reported in men with BDD vs. the control group, although libido, frequency of intercourse, and masturbation were similar in the two groups [46]. Patients with BDD had more concerns about their penis size and tried to use different methods to increase it [46].

Post-traumatic stress disorder (PTSD) occurs following exposure (direct or indirect) to life-threatening or severe physical trauma or sexual violence, which translates into intrusion symptoms, avoidance of trauma-related stimuli, negative alterations in cognitions and mood, and alterations in arousal and affectivity [4-10]. The risk of erectile dysfunction in PTSD patients was significantly higher than in the general population [47]. PTSD was associated with overall sexual functioning, sexual desire and satisfaction, and sexual distress [47]. Avoidance and negative alterations in cognition/mood were associated with a higher risk for sexual dysfunctions in this population [47]. Psychotropic medication for patients with PTSD was not significantly associated with the risk of erectile dysfunction [48].

The presence of a defined sexual dysfunction does not exclude the existence of comorbid disorders from the same category. This observation is supported by the results of a study in which 86% of the respondents to an online questionnaire who had a sexual dysfunction also reported the presence of another sexual condition [49]. Men with one severe sexual dysfunction were significantly more likely to have such comorbidities [49]. ED and premature ejaculation were comorbid in 23-29% of cases explored in this study [49].

Substance use disorders may frequently cause the onset of sexual dysfunctions. Alcohol, opioid derivates, cannabis, and ecstasy were associated with higher scores on tests of sexual dysfunction vs. controls in a clinical study (N=101 vs. 43 participants) [50]. The association was not significant for alcohol and cannabis ($p>0.05$), but opioid and ecstasy users had significantly higher scores on the International Index of Erectile Function (IIEF) vs. controls [50]. Patients with alcohol dependence have a 72% rate of being diagnosed with one or more sexual dysfunctions, the most common being ED, reduced libido, and premature ejaculation, according to another study that enrolled 100 male subjects admitted to a detox center [51]. Additionally, illicit substances are sometimes used as aphrodisiacs to enhance sexual performance or pleasure. However, existing data show that most drugs have negative effects on erection, libido, and ejaculation latency [50,51]. The data on cannabis use and its relationship with sexual functioning is controversial, because both men and women perceived this compound may increase their sexual functioning and satisfaction, especially desire and orgasm intensity [52]. Cannabinoids also have been associated with positive effects in a large variety of neurological disorders that may alter sexual functioning [53].

Pornography addiction or excessive use of online pornographic material represents a type of behavioral addiction with a potential negative impact on sexual functioning. There are several studies that have shown a high association between erectile dysfunction and excessive use of pornography, while other studies do not support this association [54-56]. Almost 60% of the patients with sexual dysfunction manifested when having intercourse with their partner, did not report this problem when using pornography [54]. However, a causal relationship between pornography and sexual dysfunctions does not exist, due to the lack of controlled studies and difficulties in designing such studies: sample biases, lack of clear diagnostic criteria, lack of validated instruments, the possibility that pornography abuse may be only a dimension of the more complex entity of sex addiction [54].

Cybersex is an excessive and uncontrolled use of online sexual activities leading to negative functional consequences [57]. Difficulties in forming intimate relationships have been associated with cybersex and pornography craving [58]. Men had higher scores for frequency of pornography online use than women and higher scores for craving the same materials [58].

Chronic organic conditions, such as diabetes mellitus (DM), blood hypertension, dyslipidemias, renal or hepatic failure, and endocrine pathologies (e.g., hypogonadism, hyperprolactinemia, hypothyroidism/hyperthyroidism) may all have an impact on sexual functioning [59]. Also, lifestyle factors such as smoking, chronic alcohol consumption, and excessive use of caffeine may be considered potential nocive stimuli on sexual functioning [59]. Neurological causes of ED that should be considered in the differential diagnosis are multiple sclerosis, spinal cord injuries, Parkinson's diseases, cerebrovascular accidents, etc., and the most common urological causes of ED are priapism, Peyronie's disease, and anatomical abnormalities [59]. The use of drugs like thiazides, spironolactone, clonidine, alpha-blockers, beta-blockers, antipsychotics, antidepressants, digoxin, ketoconazole, phenobarbital, etc. should be included among the potential triggers of ED [59]. Pelvic surgery and radiation therapy in the same area are other possible ED causes [59].

The pathogenetic factors of ED are traditionally split between psychological and organic, but this separation is artificial and, usually, there is mixed pathogenesis of this disorder in clinical practice (Figure 4). According to the results of a study focused on this distinction, in men ≤40 years old, approximately 85% had psychological causes for their ED and only 15% organic factors, while in men >40 years old, the rapport was 60/40 in favor of the organic causes [60]. Anxiety, depression, and relational conflicts were both causes and results of ED [60].

Predominantly organic ED

- Gradual onset
- Progressive evolution
- Constantly diminished response to sexual stimuli
- Better quality of erection while standing
- Elderly patients

Predominantly psychogenic ED

- Abrupt onset
- Intermitent evolution
- Loss of the ability to maintain erection
- Nocturnal penile tumescence is preserved
- Very good response to PDE5 inhibitors

Figure 4: Indicators of psychogenic nature vs. organic causes of erectile dysfunction

Still, the dichotomous model of organic vs. psychological causes in ED is outdated, and the currently suggested multidimensional model, which includes vulnerability factors and precipitating factors, is preferred [61]. This model involves the participation of (1) immediate factors, such as performance anxiety, (2) personal history variables, like recent traumatic events/stressors, and (3) vulnerability factors in childhood and adolescence [61]. Additionally, there are some important elements that should be integrated into the initial evaluation of patients with ED, related to vulnerability: rigid pattern of education in childhood, dysfunctional intrafamilial relationships, uncertainty about gender roles during early phases of development, lack of sexual education, personality disorders [62-64]. Between the most commonly reported factors that contribute to the onset of ED, in combination with vulnerability factors, are a large variety of interpersonal stressors (e.g., dysfunctional marital relationships, extra-marital relationships), psychological reactions to organic diseases, recent physical or mental traumas, occasional failure of erection [61-63]. There are also a variety of elements that maintain sexual dysfunctions, such as performance anxiety, guilt, habituation, fear of intimacy, an untreated sexual dysfunction in the partner, low self-esteem, etc. [61-63].

Sensitivity to sociocultural aspects is needed when patients are evaluated for any sexual dysfunction. Perception of sexuality varies from one population to another, and the impact of social or professional stressors may vary across different cultures [64,65]. Also, attitudes toward sex and the role of sexual activity are variables dependent on cultural context [65]. Gender roles are influenced by social and cultural factors, and they must be taken into account by practitioners when assessing patients with ED who are coming from another culture [64,65].

Studies exploring differences in sexual function and rates of sexual dysfunctions in homosexual and heterosexual populations are extremely few and their results are inconclusive [66]. ED in homosexual men was reported 1.5 times more frequently than in heterosexual individuals, according to a meta-analysis (n=4 studies) [66]. Homosexual men presented, in another study, a 45% higher risk of difficulties in obtaining an erection vs. heterosexual men [67]. Of these, 71% had little difficulty, 22% mild-moderate, 6% moderate, and 2% severe (according to IIEF-5 scores) [67]. Independent predictors for the presence of erectile dysfunction were age, a stable relationship, frequency of sexual activity with the partner, versatile sexual role, libido problems, ejaculation problems, and anodyspareunia [67]. Administration of phosphodiesterase-5 (PDE5) inhibitors was reported in 10% of homosexual men with ED, and 83% of them were satisfied with the effects of the medication [67]. Also, 43% used "legal drugs" to improve erectile dysfunction [67].

To conclude, the psychiatric evaluation of patients with ED involves the following steps: (1) formulating the diagnosis according to DSM-5 or ICD-11 criteria, based on an accurate collection of data on current mental and physical health status, personal medical history, and an assessment of vulnerability factors, triggers, and factors that maintain the sexual dysfunction; (2) verifying if there are concomitant sexual dysfunctions, besides ED; (3) exclusion of organic diseases, by recommending the adequate interdisciplinary and laboratory assessments; (4) formulating a therapeutic strategy, depending on the specific clinical aspects of the ED: psychotherapy, pharmacotherapy, or combined therapy. If non-psychogenic causes of ED or organic comorbidities have been identified, a multidisciplinary approach is recommended.

The psychological perspective

When evaluating a patient with possible ED, it is important to invite his partner to the initial interview, when possible, and to observe the dynamics of the couple's relationship [68]. The following aspects should be investigated during initial visits: fear of rejection, lack of satisfaction in the relationship, inappropriate sexual techniques, and sexual dysfunctions in the partner [68]. If one or more of the problems listed above are detected, the partner is invited to participate in psychotherapy [68].

However, there are also advantages to interviewing the patient alone: this way, it is possible to obtain a higher level of confidentiality, thus self-disclosure would be favored; various fantasies associated with guilt regarding his partner may be verbalized; aggressive sexual drives or traumatic personal experiences can be more easily revealed in the absence of the partner [68].

An example of structure for the first interview is the following: the couple can be interviewed for 10 minutes, to collect relevant historical data and establish the objectives of the assessment sessions; then each partner is interviewed separately, for 20 minutes; then the couple is brought together to formulate the conclusions [68]. Another assessment model considers the couple as the patient, and consequently, all assessments are made in the presence of both partners; the quality of the couple's relationship is a determining factor in the occurrence of erectile dysfunction and its resolution, therefore interviewing the couple is of paramount importance [68].

Forming an alliance with the patient is essential to a successful psychological interview, and to attain this result, the atmosphere must be safe, open, and non-judgmental; a strict agenda is not advisable, and flexibility is preferable [68]. The psychological assessment framework requires unconditional respect toward patients and an empathic, calm, and semantically adequate way to communicate with them [68,69]. The patient’s perspective on his own sexual dysfunction should be assessed and de-structured if based on myths or wrongfully understood scientific information [68,69]. The patient's personality needs to be assessed, as well as his core values, and attitudes towards life and others, his major existential goals, and the importance of relationships for the patient [68,69].

More specifically, the psychologist needs to assess the educational and cultural background of the patients, in order to formulate the questions and conclusions in an adequate manner; the main mechanisms of defense should be assessed, as well as his main coping strategies; gender identity disorders, sexual preferences, paraphilias, other sexual dysfunctions, childhood trauma (i.e., incest, rape, sexual or physical abuse) are essential elements to be evaluated; current level of functioning in all major areas is important to determine- actual relationships, professional performances, social support, relationships with nuclear family, hobbies; stressors in his current environment- bullying, mobbing, sexual harassment, discrimination, conflicts with peers; detailed analysis of the current sexual relationship; which are the main reasons for lack of satisfaction or failure in any functional domain?; dysfunctions in the main stages of the sexual cycle; factors that are associated with the onset and maintenance of the ED; how does it work a typical sexual intercourse for the evaluated individual?; which are the possible factors that negatively interfere with the stages of sexual cycle?; personal sexual history and possible relationship with the current problems [69].

The structured assessment of ED symptoms and functional consequences is done using validated psychological instruments. Arizona Scale for Sexual Experiences (ASEX) was developed by the Department of Psychiatry and Psychology at the University of Arizona and the Department of Psychiatry and Behavioral Sciences at Stanford University [70]. This instrument is self-administered, but it can also be completed during an interview, by a mental health specialist [70]. It measures the following dimensions: sexual desire, sexual arousal, penile erection/vaginal lubrication, ability to achieve orgasm, and orgasmic satisfaction [70]. There are two versions, one for women and one for men [70]. Each item is scored on a six-point Likert scale, with higher scores indicating greater severity of the respective sexual dysfunctions [70].

The Brief Sexual Function Inventory (BSFI) is intended for the evaluation of sexual dysfunction for men only [71]. This inventory has 11 items scored on a Likert scale, which assesses 4 dimensions - sexual desire, the ability to achieve or maintain an erection, ejaculatory function, and satisfaction with one's own sexual activity [71]. Low scores on this scale indicate high severity of sexual dysfunction [71]. For erectile dysfunction there are 3 items, scored from 0 (no dysfunction) to 5 (severe/ persistent dysfunction): frequency of partial/complete erections in the presence of sexual stimulation in the last 30 days; the presence of erections consistent enough to allow sexual intercourse in the last month; difficulty getting an erection in the last 30 days [71]. The scale is self-administered, although it was originally created to be administered by the clinician [71-73].

The UKU scale (Udvalg for kliníske Undersogelser) is an instrument for the assessment of adverse effects of psychotropic medication, sexual adverse events included [74]. It has 48 items scored from 0 to 4 on a Likert scale included in its first section; the second section comprises the assessment of the functional impact of the reported adverse effects; the third part refers to the management of the respective adverse effects [74]. The items represent symptoms and clinical signs distributed by categories: mental (difficulties concentrating, drowsiness, depression, etc.), neurological (dystonia, stiffness, tremors, etc.), neurovegetative (diarrhea, constipation, palpitations, etc.), and other (pruritus, increased/weight loss, photosensitivity, etc.) [74].

The scale also includes 6 items dedicated to sexual functioning: increase/decrease in sexual desire, erectile dysfunction, ejaculatory dysfunction, orgasmic dysfunction, and lack of vaginal lubrication [74]. The scale enables global assessment of the functional impact these symptoms may have [74]. The causal relationship between the medication and the adverse effect is also evaluated as being improbable, possible, or probable [74]. Low scores on this scale mean reduced severity of the respective manifestation [74]. It is administered by a clinician, but there is also a simpler self-administered form (UKU-SERS-Pat) [74,75]. All items can be applied or only relevant ones can be selected; the full version is recommended for clinical trials [75].

International Index for Erectile Dysfunction (IIEF) is a multidimensional tool for assessing sexual dysfunction and comprises 5 factors - erectile function, orgasmic function, sexual desire, sexual satisfaction, and overall satisfaction [76]. These factors result from 15 items formulated as questions, referring to erection frequency, erection firmness, penetration capacity, erection maintenance frequency, sexual satisfaction, etc. [76]. Items are scored from 0-5 and refer to the manifestations detected in the last few weeks; the instrument is self-administered [76]. The scores were used as indicators of the possibility of initiating sildenafil administration (scores below 14 out of a maximum of 30 in the "erectile dysfunction" domain) or psychosexual counseling (scores between 14 and 25 in the same domain) [76]. IIEF version 1 is only administrable to heterosexual men, but IIEF version 2 is applicable to both heterosexual and homosexual men [76,77]. A shortened 5-item version was also in use (IIEF-5), with a total score between 0 and 30; a high severity of dysfunction is present at scores 0–10; scores below 26 indicate the presence of some degree of erectile dysfunction [78].

Erectile Firmness Scale (EHS) contains only one item and is a self-administered instrument [79]. It rates the firmness of the erection from (0) (absent) to 5 (complete) and it is used in clinical trials to determine response to medication [79].

Still, the most important tool for the psychological assessment of patients with sexual dysfunctions remains a detailed history focusing on the history of sexual development. Aspects related to the partner's perception of sex life are essential for building an accurate picture of sexual dysfunction. All the scales previously mentioned are in no way meant to be a substitute for an accurate collection of personal history and psychological examination.

However, these instruments help the psychologist in detecting ED symptoms when patients are embarrassed to talk about the subject, and they are also useful for the monitoring process (i.e., effectiveness or tolerability of a drug or the efficacy of psychotherapy). They may also be needed for screening purposes in vulnerable populations. The main limitations of the scales are the lack of cross-cultural validation and possible differences in symptom reporting based on patient age [80].

The psychotherapeutic approach

According to the results of a review [60], the most frequently therapeutic intervention for ED is sexual therapy (50%), followed by cognitive-behavioral sex therapy (33%), while other types of psychosocial approaches have an equal representation (16%) – cognitive-behavioral therapy, group therapy, and couple therapy.

The psychotherapies dedicated to ED are derived from various interpretative models of this disorder. For example, the cognitive model of ED states that explicit/implicit requests for sexual performance lead to an expectation of performance (e.g., messages received from the partner create the expectation of an erection) [81]. In normal conditions, the attention of the individuals is focused on erotic stimuli, which increases the vegetative excitation, and favors the onset of erection (positive feedback); on the contrary, negative feedback involves catastrophic expectations, lack of perceived control over own body, focus of attention on partner's opinion about a potential failure or on other, non-sexual, stimuli, which increases the vegetative activation and focus on negative consequences, thus leading to ED [81].

The cognitive-emotional model of ED is based on a presumed occasional failure to obtain an adequate erection (no triggers are identified); following this random event, the individual activates cognitive schemata focused on personal incompetence and the misconceptions about the "macho" role; ruminations about future failures and an internal pressure to perform better next time is the following step; possibly as a defense mechanism that tries to protect the individual against a future failure, thoughts and images related to sexual activity disappear, therefore the stimulation decreases; the final stage is the "natural" consequence of the previous steps, and consists in ED's onset [82].

The main questions that help the therapist to formulate a treatment plan are: "What kind of phantasies and related emotions are associated with the current partner?", "Who has the initiative or how does the engagement in sexual activity appear?", "Is achieving sexual arousal within the current relationship and in general an easy process or a difficult one?", "How does the patient consider he can satisfy his partner and how does the partner perceive the satisfaction she receives?" [83]. Also, the therapist should explore the ability of an individual to achieve orgasm and under what conditions is it achieved; to verify what happens when one of the partners does not reach orgasm, and if this becomes a source of relational conflict; which is the definition of satisfactory sexual activity the same for both partners; to determine the level of satisfaction with sex life and with life in general in both partners [83].

According to other therapists specialized in sex-marital therapy, the specialist must keep detailed reports on the patient's status at each visit and of the suggested techniques; the therapist offers operationalized definitions of the problems he/she wants to elaborate on; the therapist evaluates the global functioning of the patient, not only of his sexual function; the therapist schedules monitoring visits at pre-determined intervals [84].

The PLISSIT model comprises "permission", i.e., the patients want to be considered "normal people", not "perverted", "deviant", "sick" or "abnormal"; often they are not disturbed by a certain behavior, but by the thought that they might do something bad or wrong; therefore, reassurance of normality is important for fantasies, thoughts, and dreams (e.g., those related to other people, or homosexual phantasies), for anxiety that can be associated with sexual stimulation (e.g., the appearance of spontaneous erections in inappropriate conditions); there are, of course, limits to validation, like fantasies with extreme sadism, pedophilic drives, etc. [85]. "Limited Information" is another core concept of this model that refers to the fact that clinicians provide limited information on relevant aspects of sexual concerns presented by the patient; the information is adjusted to the particularities of the patient, avoiding unnecessary scientific terminology and over-generalization; it is considered helpful in combating sexual myths [85]. "Specific Suggestions" refer to the fact that the therapist should formulate her/his suggestions according to the specific information obtained from the patient; these are direct attempts to change problematic behavior; these suggestions take place within the theoretical framework of short therapy [85]. "Intensive Therapy" includes specific techniques of sexual therapy [85]. During psychotherapy, the patient is encouraged to elaborate on the topic of his sex life [85]. The psychotherapist's attitude is one of empathetic listening and guiding the interview toward areas of interest in a discreet, non-confrontational manner [85]. The therapist is non-critical, normalizes, and helps validate the patient's emotions [85].

A frequently used technique in sex therapy is sensation focus [86]. These exercises progress from non-genital to genital tactile stimulation, with the instruction for the patient to refrain from vaginal penetration; thus, the patient's anxiety related to the sexual act will be reduced (desensitization), and the partner can be stimulated even in the absence of focusing on the genital area; after the patient has regained the confidence in himself, by managing to focus his attention on bodily sensations, penetration can be re-introduced into the therapy plan, gradually; it is recommended to formulate as objectives sensory focus sessions, 2 times a week, 10 minutes each; by changing roles between partners, each of them being active and passive, the patient gains experience in giving and receiving sexual stimulation [86].

Cognitive therapy and sexual fantasy training involve masturbation using guided imagery to regain control over erection, coupled with cognitive restructuring; homework includes scoring the satisfaction obtained by the patient and his partner [86].

Psychoeducation targets the increase of patients' level of information on the phases of the sexual cycle and encourages open discussions about sexuality [87]. This psychoeducational approach may have as an objective the modification of sexual scripts, which largely depend on social myths and misperceptions about sexual functioning [87].

CONCLUSIONS

The etiology of ED is usually mixed and, as a result, a multidisciplinary approach to both its diagnosis and treatment is recommended. Therefore, this review supports the conclusions of currently available guidelines for sexual dysfunctions, that a multidisciplinary, pharmacological, and psychosocial treatment is superior to monotherapy [86-88]. It is important for mental health specialists to approach the subject of ED in an empathic, non-conflictual, and non-judgmental manner, in order to obtain the information needed not only for the diagnosis but also for the formulation of the therapeutic objectives.

The main factors to be approached during the initial interview are the circumstances of the ED onset, vulnerability factors and triggers, sexual orientation of the patients, gender-related problems, and personal history of trauma [86]. Questionnaires and structured interviews are useful both initially and during the therapeutic process, as a way to document the evolution of ED symptoms. These assessment methods are selected according to the specifics of the case, but a combination of unstructured, structured interviews and scoring techniques using a validated clinical tool is preferred.

There are multiple psychometric methods for evaluating the severity of erectile dysfunction, quality of life, general functionality, etc., and the mental health specialist is the one who selects the most appropriate tools for each individual patient based on objective (severity of symptoms, functional impact), but also on subjective factors (personal experience). The stages of evaluating a patient with ED are flexible but generally involve a stage of constructing the psychiatrist/ psychologist/psychotherapist-patient relationship, describing the objectives and methods used for the evaluation, then a stage of investigating the sexual history, and a phase summarizing the collected data, which paves the way to a therapeutic solution. The desired outcomes of the therapy should be framed around satisfaction, pleasure, and quality, rather than quantity [86]. Directive and non-directive techniques can be combined depending on the particularities of the case. For an experienced mental health specialist, cultural, social, or psychological "barriers" can not represent major obstacles in the relationship with the patient. These aspects are discussed and re-interpreted through the lens of the objectives established by mutual agreement with the patient. The clinician is attentive to cultural, social, and educational details and integrates the patient's values into his/ her discourse. Active listening is essential to obtain "sensitive" information and to create an appropriate therapeutic framework.

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Authors' contribution

OV conceived the idea of the review. OV, AGM, BMP, CAC, CFP and DU designed the study. OV, CFP, AGM, CAC, CFP, DU, MD, DGV, CN and CAS screened the studies during the primary search. OV, AGM, BPM and CAS drafted the first version of the review. OV, CAC, CFP, DU, MD, DGV, CN and CAS revised the manuscript and elaborated the conclusions of the review. All authors had full access to all data contained in the review and decided to submit the manuscript for publication.

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ORIGINAL ARTICLE

Management of Urinary Dysfunction in Patients with Multiple Sclerosis

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Abstract: *Besides the motor and sensory dysfunction that characterizes multiple sclerosis, during the disease evolution and with the accumulation of new demyelinating lesions in specific areas of the central nervous system, autonomic dysfunction be-comes more prevalent among patients suffering from multiple sclerosis. Although not usually addressed, the complex spectrum of autonomic dysfunction manifestations has the potential of a negative impact on the daily activities of multiple sclerosis patients, needing specific investigations and therapeutic measures destined to alleviate symptoms and increase the quality of life. Since urinary dysfunction is evident from the early stages of multiple sclerosis on urodynamic studies even in patients without urinary complaints, it is indicated to thoroughly screen for urinary tract dysfunction as well as other autonomic dysfunctions in all the multiple sclerosis patients.*

Keywords: *autonomic dysfunction, urinary dysfunction, multiple sclerosis*

INTRODUCTION

Multiple sclerosis (MS) is one of the most common causes of disability in young patients. Clinically it does manifest uniquely for every patient, but depending on the site of the demyelinating lesions, some symptoms have a higher frequency in different combinations, particularly sensory symptoms, motor weakness, and bladder dysfunction. Most commonly, patients will present with numbness, tingling, diplopia, Lhermitte sign, and motor weakness, that last for at least 24 hours and may or may not resolve completely. Many patients may have residual symptoms, heat sensitivity with worsening of symptoms in case of increased body temperature, known as Uhthoff's symptom [1–3].

AUTONOMIC DYSFUNCTION IN MULTIPLE SCLEROSIS

As the disease follows its course over the years, inevitably patients will also experience damage to the autonomic nervous system with the involvement of both sympathetic and parasympathetic parts in different proportions. The promoters of the autonomic dysfunction appear to be lesions located in the nuclei of the periventricular regions of the fourth ventricle, medulla, but it was also seen in patients who presented cervical spinal cord atrophy with or without demyelinating lesions, suggesting that autonomic dysfunction may also result from axonal depletion [4].

Although severely overlooked, autonomic dysfunction has a great impact on the quality of life of patients suffering from multiple sclerosis, the most commonly addressed issues being bowel and bladder disturbances, which tend to be more bothersome in the case of longer disease, greater disability, or recent relapses [5].

Approximately 50% of patients experience orthostatic intolerance manifested by dizziness, loss of postural tonus, and tachycardia while transitioning from a supine to an upright position or in case of prolonged standing. The impaired sympathetic vasoconstrictor reflex responsible for maintaining normal blood pressure leads to an increased frequency of orthostatic hypotension among those patients[4].

Sleep disturbances in patients with multiple sclerosis are not very well studied, the most common symptom being difficulty initiating and maintaining sleep. There are several factors like muscle spasms, trigeminal neuralgia, restless legs syndrome, anxiety, depression, and side effects of medication used to treat fatigue, that contribute to insomnia, which can be searched for and addressed [2,4].

Another common but less addressed pathological aspect of multiple sclerosis is the sexual dysfunction that can be a consequence of multiple lesions including spinal cord lesions of the motor and sensory pathways and can be aggravated by the urinary incontinence and the spasticity accompanying upper motor lesions. About 50% of MS patients tend to become sexually inactive during the disease, with consequences like decreased libido, impotence, and infertility [2,5,6].

Bowel dysfunction is present in up to 50% of MS patients, about 43 % of them will complain of constipation partially due to disease exacerbations, and partially to decreased ambulation and certain medications that may alter bowel movement. A great proportion of patients with bowel dysfunction will present fecal incontinence as a consequence of impaired external anal sphincter function [2,4,7].

UROLOGICAL DYSFUNCTIONS

The most frequent autonomic dysfunction present in patients suffering from multiple sclerosis is bladder dysfunction, the prevalence of neurologic lower urinary tract dysfunction varies between 65 to 92% [8]. Approximately 75% of patients report bladder dysfunction secondary to a storage phase dysfunction leading to incontinence, or the voiding phase dysfunction leading to urinary retention and incomplete bladder emptying [2,4]. Bladder control requires complex coordinated functions of the cerebral cortex, pontine and sacral micturition centers, and the integrity of the peripheral nervous system [6]. The demyelinating lesions localized at the spinal cord level disrupt the fiber connections from the pontine micturition center to the parasympathetic sacral micturition center causing the silent C fibers, usually insensitive to bladder filling in normal conditions, to be hypersensitive leading to detrusor hyperactivity [9,10]. Spinal cord centers activate storage reflexes in the filling phase of the bladder, while the brain centers of micturition are responsible for the act of urine output [4,11]. Detrusor overactivity, which translates in clinical terms to symptoms like urgency to void that occurs at a lower bladder volume than normal, frequency or urge and incontinence are the leading complaints. On the opposite side lies detrusor sphincter dyssynergia described as detrusor contraction without urethral sphincter relaxation, which is responsible for the impossibility of bladder emptying causing symptoms like hesitancy, or interrupted stream. While the former urologic abnormality is caused by demyelinating lesions of the frontal cortex, the second one is a consequence of lesions of the pontine centers of micturition or lesions above the sacral parasympathetic centers [2,6,12]. Unlike spinal cord lesions due to multiple sclerosis, spinal cord injuries, even in patients with relatively preserved neurological function, are characterized by universal bladder dysfunction, meaning that there is an impairment of both sensation of bladder fullness as well as the motor control of bladder and sphincter [6,11].

For many patients, urological symptoms arise only in the later stages of disease progression with a proportion of over 50% of patients with severe MS presenting severe bladder symptoms, whereas only 5-10% of MS patients have mild bladder abnormalities at the beginning. Nevertheless, a retrospective study conducted in Ankara, Turkey demonstrated that 52% of their multiple sclerosis patients without urological complaints had altered urodynamic results which could be explained by a subclinical impairment [13,14].

Patients with overactive bladder syndrome usually complain of urinary urgency, increased daytime frequency or nocturia, and sometimes incontinence in the absence of urinary tract infections or other associated pathology that could explain the symptoms. Obstructive symptoms are also an important point of complaint especially when leading to urinary tract infections. These manifestations can be associated and can have a huge impact on patients' quality of life in terms of decreased quality of sleep, decreased work productivity, and depressive symptoms [15,16].

Urine retention is a consequence either of neurogenic underactive bladder or of detrusor sphincter dyssynergia causing bladder obstruction of urine flow, leading to frequent lower urinary tract infections that can be another cause of disease relapse through the autoimmune process which it sets off [9,17,18]. Regarding the complications of the upper urinary tract there have also been mentioned vesica-urethral reflux, hydronephrosis, and end-stage kidney disease have as consequences of the morphological changes in the bladder wall caused by high intravesical pressures [16,17,19–21].

EVALUATION OF LOWER URINARY TRACT SYMPTOMS

Urological evaluation should be specifically made for every patient considering their disease type and duration, the level of disability on the EDSS scale, comorbidities, and current medications especially opiates, antipsychotics, and antidepressants, starting with a complete history taking. The clinical dialogue should be directed towards describing urinary symptoms like frequency, urgency, hesitancy, retention, incontinence, and nocturia and the impact these symptoms have on the quality of the patient's life [16,22]. A bladder diary can provide additional information about the patient's habits of fluid ingestion and the exact frequency of bladder emptying [9].

Regarding physical examination, besides local inspection and examination of pelvic and genital organs including sacral cord mediated reflexes (bulbocavernosus, anal reflex), the neurologist should look for pontine, cerebellar as well as pyramidal signs which are indicative of lower urinary tract symptom severity [16,22]. For an initial evaluation of patients with multiple sclerosis, a bladder and renal ultrasound and a questionnaire for assessing overactive bladder and voiding dysfunction are in order, while for patients with red flags (recurring urinary tract infections, immunosuppressive treatment, EDSS >6, male patients, age >55) should be referred to a neuro-urology clinic or specialist [23].

Furthermore, some investigations can help us better understand the nature of the urological dysfunction. Firstly, all patients complaining of urinary symptoms should undergo a urinalysis and urine culture to assess the presence of a possible urinary tract infection as well as serum creatinine. Ultrasonography can allow us to measure the post-void residual volume, and it can be done at different times a day for better quantification of the bladder emptying pattern. Patients with a known high risk of upper urinary tract disease should benefit from periodical ultrasonography examinations for early detection of upper urinary tract dilation. Since a high post-void residual volume is not enough to distinguish between detrusor contractility problems and obstruction, urodynamic studies are required. Urodynamic studies represent a combination of cystometry and pressure flow study, useful in evaluating the outlet function and compliance of the detrusor and bladder. It can be used as a prognostic tool as well as for guiding the best treatment approach. In the case of hematuria, a urethrocystoscopy evaluation is required to rule out bladder tumors, bladder stones, and urethral stricture [16,20].

TREATMENT APPROACHES IN MULTIPLE SCLEROSIS-ASSOCIATED URINARY DYSFUNCTION

Physical therapy

One of the areas worth addressing is physical therapy, which in this case refers to physical exercises like pelvic floor exercises alone or combined with intravaginal electrical stimulation. Cognitive behavioral therapies may be helpful in some patients in trying to develop a bladder voiding schedule according to each patient's natural voiding pattern which combined with restricted water intake before bedtime can improve sleep quality and reduce complaints like fatigue [16].

Medical treatment

Regarding the drugs efficient for urinary dysfunction, there are several options depending on the main cause of dysfunction, that can be assessed by urodynamic studies. The first treatment line for neurogenic bladder is antimuscarinic drugs (oxybutin, tolterodine). Anticholinergics can alleviate complaints of neurogenic bladder as well as phosphodiesterase inhibitors and desmopressin which can be used as an alternative to antimuscarinics or as an adjuvant [6,20]. In an observational prospective study on the bladder and urodynamic alterations in patients with multiple sclerosis, the degree of urinary symptom severity decreased following symptomatic urological medication by lowering the bladder pressure, improving muscle spasms, and reducing pain caused by urine storage [24].

In case of bladder outlet resistance, autonomic dysreflexia, and postvoid residual volume, alpha-blockers (prazosin, terazosin) can help sphincter relaxation by lowering bladder pressure during contraction, but administered with caution because they can aggravate hypotension [6,20,25].

In patients with hypotonic bladders, cholinergic medications (bethanechol) may help empty the bladder, while in patients with pathologic relaxation of the vesical sphincter, alpha-adrenergic medication may increase bladder storage [6,17].

Minimally invasive treatment

Catheterization, self-catheterization, or third-party catheterization is the preferred method for patients who cannot otherwise empty their bladder. Patient medical education in managing a sterile intermittent catheterization can help reduce complications like urinary tract infections or bacteriuria. The habit of keeping a bladder diary can also help choose the frequency of catheterization so that the volume of the bladder at catheterization does not exceed 400-500 ml. As short-term methods, patients can dispose of condom catheters and adult diapers.

Botulinum toxin injections in the detrusor cause reversible chemical denervation with effects lasting for approximately 9 months, but also with known side effects like urinary tract infections, elevated postvoid residual volume, and increasing need for intermittent catheterization. In the case of bladder neck and urethral obstruction, to reduce the outlet resistance and to protect the upper urinary tract, patients may benefit from chemical denervation of the detrusor sphincter using botulinum toxin [6,17,20].

Surgical treatment

Sphincterotomy for reducing the bladder outlet resistance without completely losing the closure of the urethra comports the risk of secondary narrowing of the bladder neck and needs to be repeated at regular intervals. Bladder augmentation is indicated in patients with overactive or low compliance bladder when conservative methods have not been beneficial [20]. Urethral stents, sphincterotomy, and electrical implantation devices can be taken into account in selected cases, but with limited evidence of efficacy [6,26].

CONCLUSIONS

Multiple sclerosis is a central nervous system inflammatory demyelinating disease with multiple clinical manifestations, including autonomic dysfunctions. The most common and the most addressed is bladder dysfunction, but it is only a small part of the many bothersome effects of multiple sclerosis lesions, hence the need to evaluate and treat accordingly to ensure a higher quality of life for patients.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, C.G.L., C.A.S. and D.M.; methodology, V.I.V., R.A.A., F.A.A. and A.M.R..; resources, D.M., C.A.S., and F.A.A..; data curation, C.G.L., V.I.V., and A.M.R.; writing—original draft preparation, C.G.L., C.A.S., F.A.A., and A.M.R..; writing—review and editing, V.I.V., and R.A.A. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Chronic Pulmonary Sequels on CT Scans of Patients with COVID-19

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Abstract: *Background: Lung tissue changes following COVID-19 may persist for a long time after discharge and recovery, but not enough information is available about these chronic pulmonary sequels. We addressed to evaluation of chronic pulmonary sequels on CT scans of patients with COVID-19. Methods: This cross-sectional study was conducted on 94 patients with COVID-19 who were evaluated by CT scan at the time of discharge and also underwent continuous evaluation by CT scan of the lungs for at least three months. Through reviewing the hospital archive files, patients' backgrounds and three-month follow-up information were collected. Results: Chronic pulmonary involvement during the follow-up of patients was found in 83%. Peripheral and diffuse involvements were revealed in 79.8% and 3.2% respectively. Also, single-lobar and multi-lobar involvements were found in 69.1%% and 13.8% respectively. In terms of abnormal lung findings on CT scan, ground-glass opacity was found in 20.2%, Crazy-paving pattern in 2.1%, reticulation in 3.2%, parenchymal band in 12.8%, consolidation in 1.1% and bronchiectasis in 3.2%. The mean CT score was also 4.21 ± 3.45. The appearance of findings in CT patients was strongly influenced by the patients 'age, body mass index, underlying comorbidities, and also, the severity of initial COVID-19. Conclusion: Within at least three months of disease recovery in hospitalized patients with COVID-19, evidence of pulmonary involvement in 83% in the form of chronic pulmonary sequels can be expected. The most common findings related to pulmonary involvement included GOO in 20.2% and parenchymal band in 12.8% of cases.*

Keywords: *Chronic pulmonary sequels, CT scan, Covid-19*

INTRODUCTION

Although several effective vaccines with high immunity have been developed to prevent the spread of COVID-19, preventing its transmission is now believed to be the only way to combat the disease. In recent months, much effort has been published to explain the epidemiology, clinical manifestations, and clinical features and imaging of COVID-19 [1-4]. In this regard, the occurrence of fever and dry cough are the first and most common symptoms of the disease [5]. However, these symptoms are similar to those of other infections, such as the flu A and B, making it difficult to diagnose. Also, laboratory evaluation in patients is often time-consuming and its diagnostic kit may not be available for homogeneity. On the other hand, laboratory tests are sometimes associated with high levels of false negatives. In this regard, imaging plays a key role in the diagnosis and management of COVID-19-induced pneumonia. CT scan is the first diagnostic modality in patients with suspected disease and also a desirable protocol in following the effect and treatment process in these patients [6]. Especially in patients with a negative RT-PCR result, CT scan evaluation can be very useful [7,8]. Various studies have evaluated and described radiographic changes in the acute phase of COVID-19 disease and have correlated these changes with the severity of clinical manifestations [9-11]. Recent studies have also shown that about 94% of hospitalized patients still experience pulmonary parenchymal changes at discharge [12, 13]. In addition, some have shown that pulmonary opacities persisted in 53% of patients with mild COVID-19 without developing negative and complicating scaffolds for up to three weeks after discharge and clinical recovery [14]. Therefore, it seems that lung tissue changes, even the occurrence of pulmonary fibrosis, may persist for a long time after discharge and recovery of patients [15-17]. But not enough information is available about these chronic pulmonary sequels. What we addressed in the present study was the evaluation of chronic pulmonary sequels on CT scan of patients with Covid-19.

MATERIALS AND METHODS

This cross-sectional study was conducted on patients with COVID-19 who were admitted to our medical center during 2020 and 2021 and were evaluated by CT scan at the time of discharge and also underwent continuous evaluation by CT scan of the lungs for at least three months after treatment and recovery. Initially, through reviewing the hospital archive files, patients' background information including sex, age, history of underlying diseases, body mass index (BMI), and clinical characteristics of patients were collected. Also, in the initial CT scan of patients (during hospitalization) and also at the time of follow-up of patients (at least three months after discharge) and by an experienced radiologist, a complete interpretation of pulmonary involvement patterns was provided in terms of form and extent of involvement and CT score and classified according to the underlying characteristics of the patients. The severity of COVID-19 was stratified into four types mild, moderate, severe, and critical on the basis of patients' symptoms, laboratory results, and imaging findings at admission as previously described [18]. Pulmonary involvement was also assessed based on the type of lesion (peripheral or diffuse), the pattern of lobar involvement (single-lobar or multi-lobar), and the type of pulmonary lesions. To determine the CT score, each of the five lung lobes was visually scored on a scale of 0 to 5, with 0 indicating no involvement and 5 indicating more than 75% involvement. The total CT score was determined as the sum of lung involvement, ranging from 0 (no involvement) to 25 (maximum involvement) [19].

For statistical analysis, results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using the t-test or Mann-Whitney test whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. The categorical variables were compared using the Chi-Square test or Fisher's exact test if required. P values of ≤ 0.05 were considered statistically significant. For the statistical analysis, the statistical software SPSS version 23.0 for Windows (IBM, Armonk, New York) was used. Interventional studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

RESULTS

A total of 94 patients were evaluated and followed up for at least three months after discharge. The average age of patients was 53.02±4.86 years ranged 40 to 62 years and 59.6% were male. The mean BMI was also 25.41±1.91 kg/m^2 and 5.3% were obese. Regarding comorbidity, 22.3% were hypertensive, 13.8% were diabetics, and 6.4% had a history of ischemic heart disease. With respect to disease severity, 4.3% were classified as mild, 86.2% as moderate, 6.4% as severe, and 3.2% as critical.

First, there was no evidence of pulmonary involvement in CT scans in 17.0% and therefore the frequency of chronic pulmonary involvement during the follow-up of patients was 83%. In terms of how the lesions were spread, 79.8% of the involvement was peripheral, and only 3.2% was diffuse. Also, single-lobar and multi-lobar involvements were revealed in 69.1%% and 13.8% respectively. In terms of abnormal lung findings on CT scan, GOO was found in 20.2%, Crazy-paving pattern in 2.1%, reticulation in 3.2%, parenchymal band in 12.8%, consolidation in 1.1% and bronchiectasis in 3.2%. The mean CT score was also 4.21 ± 3.45.

As shown in Table 1, the patterns of chronic pulmonary sequels in patients suffering from COVID-19 were independent of patients' gender. However, the pattern and distribution of pulmonary involvements were different in the younger and the elderly, and the diffuse and multi-lobar patterns were more found in older adults. Also, abnormal lung lesions were more prevalent in older patients. Totally, the mean CT score was significantly higher in older adults than in the younger. Similar patterns were revealed in obese as compared to non-obese patients and thus obesity was found to be another factor predicting more severity of chronic sequels on CT. As shown in Table 1, the presence of comorbidities was in line with more severe pulmonary expansion of abnormal lesions. Also, as indicated in Table 2, we showed diffuse and multi-lobar lesions as well as a higher rate of pulmonary lesions in those with more severe initial COVID-19 disease. There was also a significant association between CT score and disease severity (Figure 1).

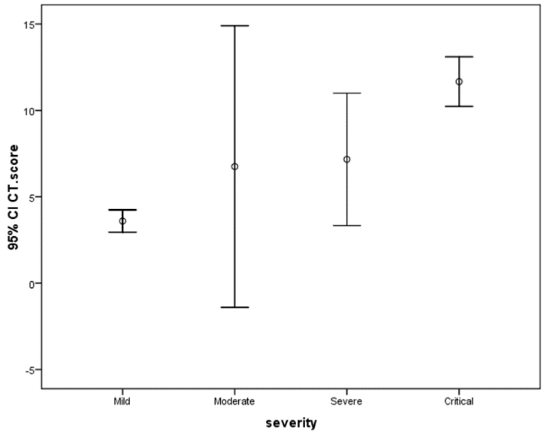

Figure 1: The correlation between CT score and severity of primary Covid-19

Table 1: Status and pattern of pulmonary involvement according to underlying factors

Item	Gender		Age		Obesity		Morbidity	
	Male	**Female**	**≤50**	**>50**	**Obese**	**Non-obese**	**Present**	**Absent**
Distribution								
Peripheral	76.8	84.2	63.4	92.5*	50.0	81.1*	78.3	80.3*
Diffuse	3.6	2.6	0.0	5.7	50.0	1.1	13.0	0.0
Pattern								
Single-lobar	64.3	16.1	53.7	81.1*	50.0	70.0*	52.2	74.6*
Multi-lobar	76.3	10.5	9.8	17.0	50.0	12.2	39.1	5.6
GOO	17.9	23.7	17.1	22.6	50.0	18.9*	43.5	12.7*
Crazy-paving	3.6	0.0	0.0	3.8	25.0	1.1*	8.7	0.0*
Reticulation	3.6	2.6	0.0	5.7	50.0	1.1*	13.0	0.0*
Parenchymal band	14.3	10.5	9.8	15.1	0.0	13.3	26.1	8.5*
Consolidation	1.8	0.0	0.0	1.9	0.0	1.1	4.3	0.0
Bronchiectasis	3.6	2.6	0.0	5.7	0.0	3.3	13.0	0.0*
Mean CT score	4.62±3.49	3.61±3.33	3.37±3.47	4.78±3.32*	7.25±4.92	4.08±3.34*	5.83±3.97	3.69±3.11*

Table 2: Status and pattern of pulmonary involvement according to disease severity

Item	Mild	Moderate	Severe	Critical	P value
Distribution					0.001
Peripheral	100	80.2	100	0	
Diffuse	0	0	0	100	
Pattern					0.001
Single-lobar	77.8	25.0	16.7	0	0.001
Multi-lobar	2.5	75.0	83.3	100	0.001
GOO	9.9	75.0	83.3	100	0.001
Crazy-paving	0	0	0	66.7	0.001
Reticulation	0	0	0	100	0.001
Parenchymal band	50.0	7.4	50.0	33.3	0.201
Consolidation	0	0	0	33.3	0.001
Bronchiectasis	0	1.2	16.7	33.3	0.004
Mean CT score	3.59±2.92	6.75±5.12	7.17±3.65	11.67±0.57	0.001

DISCUSSION

In the acute phase of Covid-19, different patterns of pulmonary involvement are examined in depth and the severity and extent of the disease are classified accordingly. But the question remains, how long will these involvements last after recovery from the disease, and in other words, will there still be evidence of lung involvement due to the disease months after the symptoms have improved? Obviously, the presence of these abnormal findings in the form of chronic scoliosis in CT scans of patients, weeks and months after discharge from the hospital can indicate the continuation of clinical manifestation of the disease and sometimes even exacerbation of these symptoms. What we did in the present study was the evaluation of chronic pulmonary edema on CT scans of patients with COVID-19 and related underlying factors. First, the study showed that for at least three months after treatment and recovery from COVID-19 disease, evidence of pulmonary involvement would still be expected in 83% of patients even after improvement in clinical symptoms. The most common findings of CT as chronic disease sequels included GOO patterns in 20.2% and parenchymal band in 12.8%. Other findings included reticulation in 3.2%, bronchiectasis in 3.2%, Crazy-paving pattern in 2.1%, and consolidation in 1.1% of patients. Therefore, even after about three months of the disease and the relative recovery, the occurrence of some evidence in CT patients with COVID-19, especially GOO, is still predictable. Additionally, in terms of the pattern of involvements, the lesions are mainly in the form of peripheral, and in most cases, they will be single-lobar involvements rather than multi-lobar or diffuse ones. As a second finding, we found that the appearance of these findings in CT patients during the months following recovery from COVID-19 was strongly influenced by the patients 'age, BMI, underlying comorbidities (such as hypertension, diabetes, and history of ischemic heart disease) and also, the severity of initial Covid-19. In other words, the incidence of chronic pulmonary sequels in CT is more predictable in the months following recovery from COVID-19 at older ages, in obese individuals with underlying comorbidities, and in individuals with severe underlying COVID-19.

Various studies have reported the persistence of chronic pulmonary lesions in the weeks and months after discharge from the hospital, although due to differences in patients' underlying characteristics, different follow-up periods, and different intensities of underlying COVID-19 disease, CT scans were quite different during patient follow-up. In a study by Caruso et al and at 6-month follow-up, 72% of patients showed fibrosis-like changes and 42% of patients showed evidence of GOO [20]. In a study by Han et al., fibrotic-like changes were observed in 35% of cases, while 65% of cases either had completely clean lungs or had grand glass opacity or increased interstitial thickness. Age over 50, heart rate above 100 at admission, length of hospital stay over 17 days, respiratory distress syndrome, non-invasive mechanical ventilation, and CT scan score above 18 as factors associated with fibrotic-like changes over six months after discharge [21]. In another study by Dehan Liu et al during the first to third weeks, the GOO opacity and pulmonary fibrosis remained constant [22]. In a study by Safont et al., during a 180-day follow-up with a CT scan, 66% had chronic pulmonary lesions. Among this evidence, 68.3% had GOO and 38.4% had parenchymal bands [23]. In the study by Blanco et al., 19 patients with COVID-19 were evaluated by CT scan during a six-month follow-up period, in which only 48% had a normal CT scan [24]. Also, in the study of Hossein et al., 42.3% had pulmonary sequels in CT scan follow-up. The most common residual patterns in CT scans included GOO at 54.5% followed by grand glass mix at 31.8% and parenchymal bands at 13.7% [25]. Therefore, a wide range of findings related to COVID-19 can be seen in the weeks and months after recovery, sometimes without symptoms and sometimes with signs and symptoms associated with COVID-19. It seems that follow-up of these patients and simultaneous and continuous evaluation of lung CT scan findings along with clinical manifestations in patients will be necessary even after partial recovery and hospital discharge.

CONCLUSIONS

As a final conclusion, within at least three months of disease recovery in hospitalized patients with COVID-19, evidence of pulmonary involvement in 83% in the form of chronic pulmonary sequels can be expected. The most common findings related to pulmonary involvement included GOO in 20.2% and parenchymal band in 12.8% of cases. The presence of chronic pulmonary lesions in COVID-19 patients is strongly influenced by old age, obesity, the presence of underlying comorbidities as well and the severity of early COVID-19 disease.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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None.

Authors' contribution
Design and concepts: TFL, SK, MH, KO; Data gathering and analysis: TFL, SK, MH, KO; Preparing the manuscript: TFL, SK, MH, KO.

Ethics approval and consent to participate
The ethical committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran confirmed the proposal of this study with IR.SBMU.MSP.REC.1400.253 code.

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Investigation of Mutations in H19, IGF2, CDKN1C, KCNQ1, and NSD1 Genes in Iranian Children Suspected of Silver-Russell Syndrome (SRS) and Beckwith-Wiedemann Syndrome (BWS) with MS-MLPA

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Abstract: *Background: Silver-Russell Syndrome (SRS) and Beckwith-Wiedemann Syndrome (BWS) are two syndromes that are poorly diagnosed in many affected people due to mild and subtle symptoms, genetic complexity, and lack of familiarity with the hallmarks. Objective: The present study was conducted with the aim of determining mutations in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes in Iranian children suspected of SRS and BWS by the MS-MLPA method. Methods: In this case series study, which was conducted in 2022 in Pars Genome Laboratory, Karaj, Iran, 10 children suspected of SRS or BWS syndrome were included. These 10 Iranian children were referred by pediatric endocrinologists. 5 ml of peripheral blood was taken per patient for DNA extraction. MS-MLPA method was undertaken for possible mutations (methylation and deletion) in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes. Results: The interpretation of MS-MLPA results showed that out of 10 children (4 boys and 6 girls) suspected of having SRS or BWS syndrome (based on the pediatric endocrinologist's diagnosis), only 3 children were definitively diagnosed with SRS or BWS syndrome. Based on this, methylation changes in the promoter of ICR1 and ICR2, which are related to the genes H19, IGF2, CDKN1C, KCNQ1, and NSD1, lead to the development of SRS or BWS syndrome. Conclusion: The present findings showed that methylation changes in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes are associated with the occurrence of SRS or BWS syndrome. In this study, we show that MS-MLPA can serve as a rapid, inexpensive, and reliable method for the molecular diagnosis of these syndromes.*

Keywords: *Silver-Russell Syndrome (SRS), Beckwith-Wiedemann Syndrome (BWS), Methylation, MS-MLPA*

INTRODUCTION

Silver-Russell Syndrome (SRS) is a rare disorder characterized by intrauterine growth restriction, poor postnatal growth, macrocephaly, a triangular facial appearance, prominent forehead, body asymmetry, and significant feeding difficulties [1]. Patients can have a wide range of clinical findings. The majority of people with SRS have normal intelligence but have motor or speech delays. SRS is genetically heterogeneous, which means that different genetic abnormalities cause this disorder [2]. In around 60% of people with SRS, the disease is related to mutations in chromosome 7 or 11. Genetic defects that may be responsible for this syndrome include uniparental disomy of maternal chromosome 11P15.5, deletion of chromosome 7, or duplication of chromosome 7 or mutation of the KCNQ1, H19 (located on 11p15) and NSD1 (located on 5p35). However, in about 40% of patients with SRS, the cause is unknown. In general, the diagnosis of SRS is based on clinical findings. Since many symptoms are subtle and nonspecific, SRS remains difficult to diagnose [3]. Consensus guidelines for the evaluation and diagnosis of SRS have recently been published based on the Netchine-Harbison clinical scoring system for SRS [4]. Testing for known genetic causes of SRS (chromosomes 7 and 11) can confirm the clinical diagnosis in up to 60% of individuals. Knowing the main genetic cause can also help to select the appropriate treatment approach [5]. Treatment of this syndrome focuses on managing the symptoms [6,7]. SRS is present at birth and many symptoms improve as the patient grows [8].

Beckwith-Wiedemann Syndrome (BWS) is an overgrowth syndrome that affects certain chromosomes of the fetus. People with this syndrome may experience mild to severe signs and symptoms. One out of every 11,000 infants is affected by BWS, which is equally common in both boys and girls. This syndrome causes excessive growth of some body parts. BWS affects many areas of the body and makes the child appear significantly larger than their peers. Patients show different signs and symptoms depending on the affected organs [9,10], including macrosomia, hemihyperplasia, macroglossia, hypoglycemia in infancy, and sometimes long-term hypoglycemia (due to high insulin). The management of this syndrome focuses on its common symptoms and complications.

Physicians monitor and check affected children for low blood sugar and increase the frequency of feeding to obtain optimal blood glucose levels. There are several genetic causes of BWS, usually resulting in changes in one or more genes in a region of chromosome 11 known as 11p15 [11,12]. In most cases, there is genetic mosaicism. Other genetic changes in this syndrome include methylation defects in the region of 11p15, uniparental disomy (UPD) in 11p15, or mutation of the CDKN1C and IGF2. The diagnosis of BWS is clinical and it is often confirmed by genetic testing. To assay these abnormalities, a blood sample or other tissue samples should be taken [13,14].

In addition to organ growth defects, BWS patients are susceptible to other diseases, including cancer (such as Wilms' kidney tumor, hepatoblastoma, and neuroblastoma), heart disease (pericarditis, cardiomyopathy, etc.), hypoglycemia, hemihyperplasia, and digestive disorders [15]. Conducting investigations of H19, IGF2, CDKN1C, KCNQ1 (located 11p15), and NSD1 (located 5p35) genes in these syndromes and increasing information in this field can lead to a more accurate and precise diagnosis; preventing secondary diseases including cancer; and also be effective in preventing the transfer of these genes to the fetus before and during pregnancy [3,13,14]. Considering that no study in this field has been done in Iran, the present research aims to investigate mutations in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes in children suspected of SRS and BWS with Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA) method.

MATERIALS AND METHODS

Study procedures

In this case series study that was conducted in 2022 at Pars Genome Laboratory in Karaj, Iran, 10 children suspected of SRS or BWS were included. These 10 Iranian children were referred to us by a pediatric endocrinologist for final confirmation of the diagnosis. 5 ml of peripheral blood was taken for DNA extraction from these patients. MS-MLPA method was undertaken to identify mutations (methylation and deletion) in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes [16].

Inclusion criteria

The pediatric endocrinologists referred children under 12 years of age and clinically suspicious for the diagnosis of SRS or BWS. Written informed consent from parents and guardians of the children was documented.

Exclusion criteria

Children with multiple comorbidities

DNA extraction and purification

5 ml of peripheral blood was taken from each patient. DNA extraction from peripheral blood leukocytes was performed for all children using a DNA extraction kit (DENA Zist Asia, Cot.No:S-1031-1, made in Iran). Then, the quantity of extracted DNA was determined and confirmed with the nanodrop device, and the quality of the DNA samples was confirmed by using 1% agarose gel.

MS-MLPA

MS-MLPA probe mixture (ME030BWS/SRS) was purchased from the manufacturer (MRC-Holland, Amsterdam/NL).

This mix contains 42 probes, 26 of which are specific for the BWS/SRS region in 11p15. Ten of the 11p15 probes are methylation-specific since they contain a HhaI recognition site. Two probes for NSD1 and 13 reference probes (without HhaI recognition site) and one digestion control probe.

We used a total of 200 ng of genomic DNA extracted from peripheral blood by the salting-out procedure. 10 Mmol/l Tris-EDTA (pH 8.0) was added to DNA if dilution was necessary. After 16 h of hybridization at 60C, samples were equally split into two aliquots. The first aliquot underwent ligation only, whereas the second one underwent ligation followed by enzymatic digestion with HhaI [a restriction enzyme recognizing only unmethylated DNA] and PCR amplification using universal primers.

In the latter case, amplification products were obtained and detected by capillary electrophoresis only if the CpG included in the HhaI site was methylated. Ligation, enzymatic digestion, and PCR amplification were performed according to the manufacturer’s instructions.

All samples were run in duplicate and at least two normal samples were analyzed in each MS-MLPA experiment, as controls. PCR products (1 ml) were mixed with 0.4 ml of internal size standard (500LIZ Genescan; Applied Biosystems) and 9 ml of deionized formamide and injected into an ABI-3130 genetic analyzer (Applied Biosystems) equipped with a 36 cm, four capillaries array using POP7 as polymer. The samples were injected for 20 s at 1.5 kV and run for a total time of 1200 s at 15 kV. Genemarker v1.95 software is used for analysis, which performs all steps of data normalization and sloping data correction.

Ethical considerations

This study was approved by the ethics committee of Azad University of Tehran Medical Sciences (code: IR.IAU.PS.REC.1401.062). Throughout the study, researchers adhered to the Declaration of Helsinki. Written informed consent was obtained from the patients in this research. All information related to children was kept confidential. This study did not interfere with the process of providing medical services and care by physicians in the clinic. This research did not impose any additional costs on the patient.

RESULTS

The interpretation of MS-MLPA findings demonstrated that out of 10 children (4 boys and 6 girls) suspected of SRS or BWS (based on the diagnosis of a pediatric endocrinologist), only 3 were definitively diagnosed with these syndromes. None of the children were the result of a consanguine marriage. No deletion or addition changes were observed in any of the children, especially in the NSD1 gene.

The first patient

A newborn baby girl was diagnosed by an endocrinologist with suspicion of BWS. DNA was extracted from the blood sample and molecular analysis was done with MS MLPA genetic test. Copy number analysis showed that no deletions or duplications occurred in the chromosomal segments and therefore deemed normal (Figure 1A). Methylation analysis showed that hypomethylation of IC2 occurred in the 11p15 section [Figure 1B]. Therefore, this infant indeed had BWS.

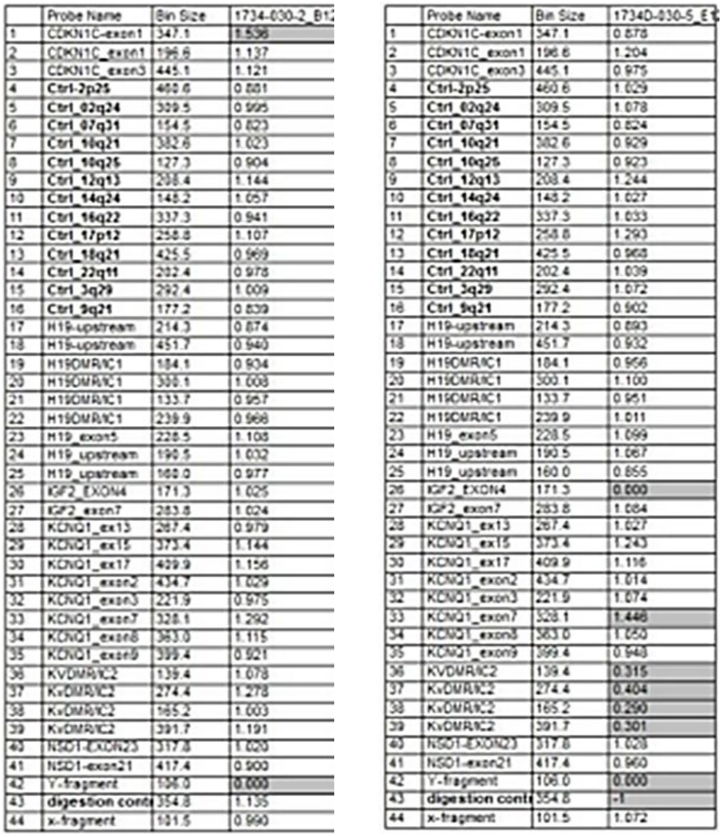

	Probe Name	Bin Size	1734-030-2_B1
1	CDKN1C-exon1	347.1	1.536
2	CDKN1C_exon1	196.6	1.137
3	CDKN1C_exon3	445.1	1.121
4	**Ctrl-2p25**	460.6	0.881
5	**Ctrl_02q24**	309.5	0.995
6	**Ctrl_07q31**	154.5	0.823
7	**Ctrl_10q21**	382.6	1.023
8	**Ctrl_10q25**	127.3	0.904
9	**Ctrl_12q13**	208.4	1.144
10	**Ctrl_14q24**	148.2	1.057
11	**Ctrl_16q22**	337.3	0.941
12	**Ctrl_17p12**	258.8	1.107
13	**Ctrl_18q21**	425.5	0.969
14	**Ctrl_22q11**	202.4	0.978
15	**Ctrl_3q29**	292.4	1.009
16	**Ctrl_9q21**	177.2	0.839
17	H19-upstream	214.3	0.874
18	H19-upstream	451.7	0.940
19	H19DMR/IC1	184.1	0.904
20	H19DMR/IC1	300.1	1.008
21	H19DMR/IC1	133.7	0.957
22	H19DMR/IC1	239.9	0.966
23	H19_exon5	228.5	1.108
24	H19_upstream	190.5	1.032
25	H19_upstream	160.0	0.977
26	IGF2_EXON4	171.3	1.025
27	IGF2_exon7	283.8	1.024
28	KCNQ1_ex13	267.4	0.979
29	KCNQ1_ex15	373.4	1.144
30	KCNQ1_ex17	409.9	1.156
31	KCNQ1_exon2	434.7	1.029
32	KCNQ1_exon3	221.9	0.975
33	KCNQ1_exon7	328.1	1.292
34	KCNQ1_exon8	363.0	1.115
35	KCNQ1_exon9	399.4	0.921
36	KVDMR/IC2	139.4	1.078
37	KvDMR/IC2	274.4	1.278
38	KvDMR/IC2	165.2	1.003
39	KvDMR/IC2	391.7	1.191
40	NSD1-EXON23	317.8	1.020
41	NSD1-exon21	417.4	0.900
42	Y-fragment	106.0	0.000
43	**digestion cont**	354.8	1.135
44	x-fragment	101.5	0.990

A. Copy number analysis

	Probe Name	Bin Size	1734D-030-5_E
1	CDKN1C-exon1	347.1	0.878
2	CDKN1C_exon1	196.6	1.204
3	CDKN1C_exon3	445.1	0.975
4	**Ctrl-2p25**	460.6	1.029
5	**Ctrl_02q24**	309.5	1.078
6	**Ctrl_07q31**	154.5	0.824
7	**Ctrl_10q21**	382.6	0.929
8	**Ctrl_10q25**	127.3	0.923
9	**Ctrl_12q13**	208.4	1.244
10	**Ctrl_14q24**	148.2	1.027
11	**Ctrl_16q22**	337.3	1.033
12	**Ctrl_17p12**	258.8	1.293
13	**Ctrl_18q21**	425.5	0.968
14	**Ctrl_22q11**	202.4	1.039
15	**Ctrl_3q29**	292.4	1.072
16	**Ctrl_9q21**	177.2	0.902
17	H19-upstream	214.3	0.893
18	H19-upstream	451.7	0.932
19	H19DMR/IC1	184.1	0.956
20	H19DMR/IC1	300.1	1.100
21	H19DMR/IC1	133.7	0.951
22	H19DMR/IC1	239.9	1.011
23	H19_exon5	228.5	1.099
24	H19_upstream	190.5	1.067
25	H19_upstream	160.0	0.855
26	IGF2_EXON4	171.3	0.000
27	IGF2_exon7	283.8	1.084
28	KCNQ1_ex13	267.4	1.027
29	KCNQ1_ex15	373.4	1.243
30	KCNQ1_ex17	409.9	1.116
31	KCNQ1_exon2	434.7	1.014
32	KCNQ1_exon3	221.9	1.074
33	KCNQ1_exon7	328.1	1.446
34	KCNQ1_exon8	363.0	1.050
35	KCNQ1_exon9	399.4	0.948
36	KVDMR/IC2	139.4	0.315
37	KvDMR/IC2	274.4	0.404
38	KvDMR/IC2	165.2	0.290
39	KvDMR/IC2	391.7	0.301
40	NSD1-EXON23	317.8	1.028
41	NSD1-exon21	417.4	0.960
42	Y-fragment	106.0	0.000
43	**digestion cont**	354.8	-1
44	x-fragment	101.5	1.072

B. Methylation analysis

Figure 1: MLPA genetic test findings related to the first patient

The second patient

A 21-month-old girl, with the examination of an endocrinologist, was diagnosed with suspected SRS. DNA was extracted from the blood sample and molecular analysis was done with MS-MLPA genetic test. Copy number analysis showed that no deletions or duplications occurred in the chromosomal segments, hence a normal finding (Figure 2A). Methylation analysis showed that hypomethylation of IC1 occurred in the 11p15 segment. This hypomethylation occurred in probes 19 to 22 (Figure 2B). Therefore, this baby definitely had SRS.

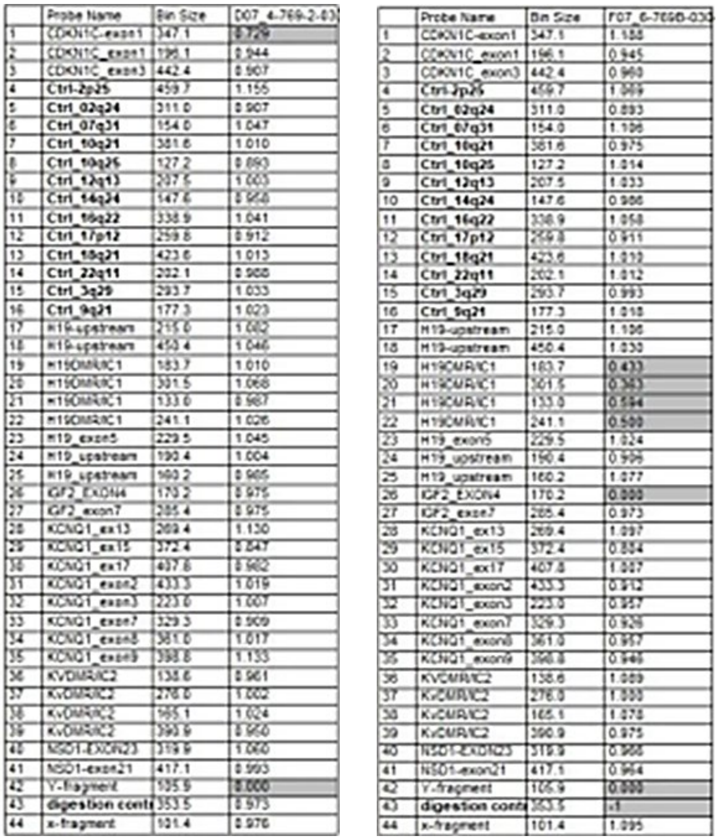

	Probe Name	Bin Size	D07_4-769-2-03
1	CDKN1C-exon1	347.1	0.729
2	CDKN1C_exon1	196.1	0.944
3	CDKN1C_exon3	442.4	0.907
4	**Ctrl-2p25**	459.7	1.155
5	**Ctrl_02q24**	311.0	0.907
6	**Ctrl_07q31**	154.0	1.047
7	**Ctrl_10q21**	381.6	1.010
8	**Ctrl_10q25**	127.2	0.893
9	**Ctrl_12q13**	207.5	1.003
10	**Ctrl_14q24**	147.6	0.958
11	**Ctrl_16q22**	338.9	1.041
12	**Ctrl_17p12**	259.8	0.912
13	**Ctrl_18q21**	423.6	1.013
14	**Ctrl_22q11**	202.1	0.988
15	**Ctrl_3q29**	293.7	1.033
16	**Ctrl_9q21**	177.3	1.023
17	H19-upstream	215.0	1.082
18	H19-upstream	450.4	1.046
19	H19DMR/IC1	183.7	1.010
20	H19DMR/IC1	301.5	1.068
21	H19DMR/IC1	133.0	0.987
22	H19DMR/IC1	241.1	1.026
23	H19_exon5	229.5	1.045
24	H19_upstream	190.4	1.004
25	H19_upstream	160.2	0.985
26	IGF2_EXON4	170.2	0.975
27	IGF2_exon7	285.4	0.975
28	KCNQ1_ex13	269.4	1.130
29	KCNQ1_ex15	372.4	0.847
30	KCNQ1_ex17	407.8	0.982
31	KCNQ1_exon2	433.3	1.019
32	KCNQ1_exon3	223.0	1.007
33	KCNQ1_exon7	329.3	0.909
34	KCNQ1_exon8	381.0	1.017
35	KCNQ1_exon9	398.8	1.133
36	KVDMR/IC2	138.6	0.961
37	KvDMR/IC2	276.0	1.002
38	KvDMR/IC2	165.1	1.024
39	KvDMR/IC2	390.9	0.960
40	NSD1-EXON23	319.9	1.060
41	NSD1-exon21	417.1	0.993
42	Y-fragment	105.9	0.000
43	**digestion contr**	353.5	0.973
44	x-fragment	101.4	0.976

A. Copy number analysis

	Probe Name	Bin Size	F07_6-769B-03
1	CDKN1C-exon1	347.1	1.188
2	CDKN1C_exon1	196.1	0.945
3	CDKN1C_exon3	442.4	0.960
4	**Ctrl-2p25**	459.7	1.069
5	**Ctrl_02q24**	311.0	0.893
6	**Ctrl_07q31**	154.0	1.106
7	**Ctrl_10q21**	381.6	0.975
8	**Ctrl_10q25**	127.2	1.014
9	**Ctrl_12q13**	207.5	1.033
10	**Ctrl_14q24**	147.6	0.986
11	**Ctrl_16q22**	338.9	1.058
12	**Ctrl_17p12**	259.8	0.911
13	**Ctrl_18q21**	423.6	1.019
14	**Ctrl_22q11**	202.1	1.012
15	**Ctrl_3q29**	293.7	0.993
16	**Ctrl_9q21**	177.3	1.018
17	H19-upstream	215.0	1.106
18	H19-upstream	450.4	1.030
19	H19DMR/IC1	183.7	0.433
20	H19DMR/IC1	301.5	0.383
21	H19DMR/IC1	133.0	0.594
22	H19DMR/IC1	241.1	0.500
23	H19_exon5	229.5	1.024
24	H19_upstream	190.4	0.996
25	H19_upstream	160.2	1.077
26	IGF2_EXON4	170.2	0.000
27	IGF2_exon7	285.4	0.973
28	KCNQ1_ex13	269.4	1.097
29	KCNQ1_ex15	372.4	0.884
30	KCNQ1_ex17	407.8	1.087
31	KCNQ1_exon2	433.3	0.912
32	KCNQ1_exon3	223.0	0.957
33	KCNQ1_exon7	329.3	0.926
34	KCNQ1_exon8	361.0	0.957
35	KCNQ1_exon9	398.8	0.946
36	KVDMR/IC2	138.6	1.089
37	KvDMR/IC2	276.0	1.000
38	KvDMR/IC2	165.1	1.078
39	KvDMR/IC2	390.9	0.975
40	NSD1-EXON23	319.9	0.988
41	NSD1-exon21	417.1	0.964
42	Y-fragment	105.9	0.000
43	**digestion contr**	353.5	<1
44	x-fragment	101.4	1.095

B. Methylation analysis

Figure 2: MLPA genetic test findings related to the second patient

The third patient

A 2-year-old boy was suspected of having BWS according to the examination of an endocrinologist. DNA was extracted from the blood sample and molecular analysis was done with MS-MLPA genetic test. Copy number analysis showed that no deletions or duplications occurred in the chromosomal segments and therefore, it was deemed normal [Figure 3A]. Methylation analysis showed that hypermethylation of IC1 occurred in the 11p15 segment. This hypomethylation occurred in probes 36, 38, and 39. IC2 hypomethylation was detected in the 11p15 segment. This hypomethylation occurred in probes 19 to 22 (Figure 3B). Therefore, this boy definitely had BWS. Uniparental disomy occurred in this patient and he inherited both chromosomes from his father.

The fourth patient

A 9-year-old boy was suspected of SRS according to the examination of an endocrinologist. DNA was extracted from the blood sample and molecular analysis was done with MS-MLPA genetic test. Copy number analysis showed that no deletions or duplications occurred in the chromosomal segments and therefore, it was deemed normal (Figure 4A). Methylation analysis showed a normal pattern in the IC1 and IC2 (Figure 4B). Therefore, this child did not suffer from either SRS or BWS.

Fifth to tenth patients

The fifth to seventh patients were a 5-year-old girl, an 8-year-old girl, and a 12-year-old girl, respectively, suspected of SRS. The eighth patient was a 4-year-old boy, the ninth patient was a 6-year-old girl, and the tenth patient was a 7-year-old boy who was suspected of having BWS; according to MS-MLPA, it was found that none had either SRS or BWS.

DISCUSSION

The main reason for investigating mutations and methylation changes in H19, IGF2, CDKN1C, and KCNQ1 (located on 11p15) and NSD1 (located on 5q35.3) genes lies in their function. Alterations in these genes affect function and can give rise to the phenotypes associated with SRS or BWS. The gene CDKN1C (Cyclin Dependent Kinase Inhibitor 1C) is a tumor suppressor and a strong inhibitor and a negative regulator of cell proliferation.

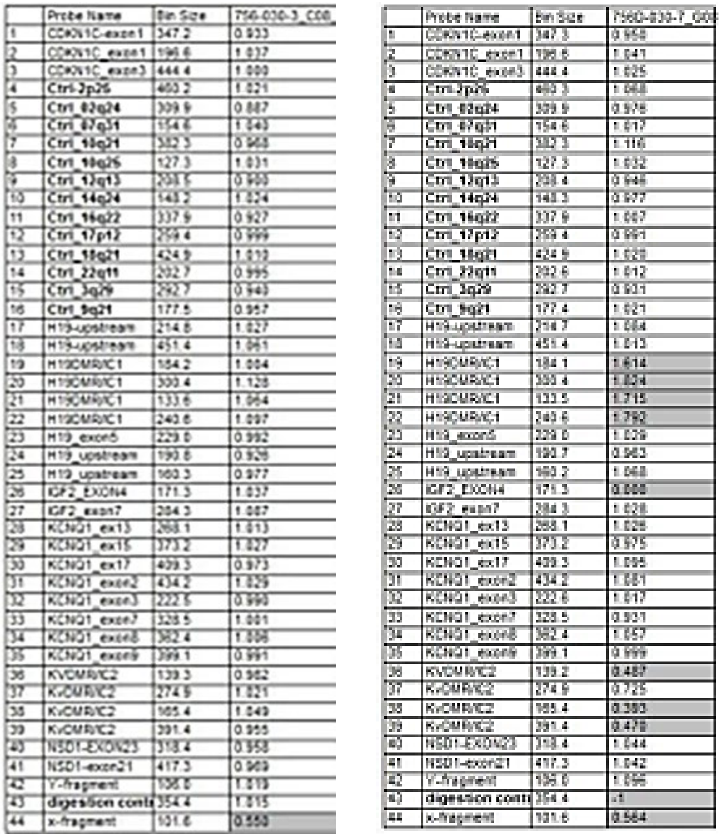

	Probe Name	Bin Size	756-030-3_C08
1	CDKN1C-exon1	347.2	0.933
2	CDKN1C_exon1	196.6	1.037
3	CDKN1C_exon3	444.4	1.000
4	Ctrl-2p25	460.2	1.021
5	Ctrl_02q24	309.9	0.887
6	Ctrl_07q31	154.6	1.043
7	Ctrl_10q21	382.3	0.968
8	Ctrl_10q26	127.3	1.031
9	Ctrl_12q13	208.5	0.900
10	Ctrl_14q24	143.2	1.024
11	Ctrl_16q22	337.9	0.927
12	Ctrl_17p12	259.4	0.999
13	Ctrl_18q21	424.9	1.012
14	Ctrl_22q11	202.7	0.995
15	Ctrl_3q29	292.7	0.943
16	Ctrl_9q21	177.5	0.957
17	H19-upstream	214.8	1.027
18	H19-upstream	451.4	1.061
19	H19DMR/IC1	184.2	1.004
20	H19DMR/IC1	300.4	1.128
21	H19DMR/IC1	133.6	1.064
22	H19DMR/IC1	240.6	1.097
23	H19_exon5	229.0	0.992
24	H19_upstream	190.8	0.926
25	H19_upstream	160.3	0.977
26	IGF2_EXON4	171.3	1.037
27	IGF2_exon7	284.3	1.087
28	KCNQ1_ex13	268.1	1.013
29	KCNQ1_ex15	373.2	1.027
30	KCNQ1_ex17	409.3	0.973
31	KCNQ1_exon2	434.2	1.029
32	KCNQ1_exon3	222.5	0.990
33	KCNQ1_exon7	328.5	1.001
34	KCNQ1_exon8	382.4	1.098
35	KCNQ1_exon9	399.1	0.991
36	KVDMR/IC2	139.3	0.982
37	KvDMR/IC2	274.9	1.021
38	KvDMR/IC2	165.4	1.049
39	KvDMR/IC2	391.4	0.955
40	NSD1-EXON23	318.4	0.958
41	NSD1-exon21	417.3	0.969
42	Y-fragment	108.8	1.019
43	digestion contr	354.4	1.015
44	x-fragment	101.6	0.553

A. Copy number analysis

	Probe Name	Bin Size	7560-030-7_G08
1	CDKN1C-exon1	347.3	0.958
2	CDKN1C_exon1	196.6	1.041
3	CDKN1C_exon3	444.4	1.025
4	Ctrl-2p25	460.3	1.068
5	Ctrl_02q24	309.9	0.976
6	Ctrl_07q31	154.6	1.017
7	Ctrl_10q21	382.3	1.116
8	Ctrl_10q26	127.3	1.032
9	Ctrl_12q13	208.4	0.946
10	Ctrl_14q24	143.3	0.977
11	Ctrl_16q22	337.9	1.067
12	Ctrl_17p12	259.4	0.991
13	Ctrl_18q21	424.9	1.028
14	Ctrl_22q11	202.6	1.012
15	Ctrl_3q29	292.7	0.931
16	Ctrl_9q21	177.4	1.021
17	H19-upstream	214.7	1.084
18	H19-upstream	451.4	1.013
19	H19DMR/IC1	184.1	1.614
20	H19DMR/IC1	300.4	1.824
21	H19DMR/IC1	133.5	1.715
22	H19DMR/IC1	240.6	1.792
23	H19_exon5	229.0	1.029
24	H19_upstream	190.7	0.963
25	H19_upstream	160.2	1.068
26	IGF2_EXON4	171.3	0.888
27	IGF2_exon7	284.3	1.028
28	KCNQ1_ex13	268.1	1.028
29	KCNQ1_ex15	373.2	0.975
30	KCNQ1_ex17	409.3	1.095
31	KCNQ1_exon2	434.2	1.081
32	KCNQ1_exon3	222.6	1.017
33	KCNQ1_exon7	328.5	0.931
34	KCNQ1_exon8	382.4	1.057
35	KCNQ1_exon9	399.1	0.999
36	KVDMR/IC2	139.2	0.487
37	KvDMR/IC2	274.9	0.725
38	KvDMR/IC2	165.4	0.383
39	KvDMR/IC2	391.4	0.479
40	NSD1-EXON23	318.4	1.044
41	NSD1-exon21	417.3	1.042
42	Y-fragment	108.0	1.096
43	digestion contr	354.4	<1
44	x-fragment	101.6	0.564

B. Methylation analysis

Figure 3: MLPA genetic test findings related to the third patient

Mutation in this gene can cause abnormal height and abnormal bladder structure and function. It also provides instructions for making proteins that help growth regulation [17]. NSD1 (Nuclear Receptor Binding SET Domain Protein 1) acts as a basic transcription factor and a regulator in transcription. Mutation in this gene can cause childhood acute myeloid leukemia and abnormal body height [18]. KCNQ1 [potassium voltage-gated channel subfamily Q member 1] gene belongs to a large family of genes responsible for making potassium channels [19]. The IGF2 gene (insulin-like growth factor 2) plays an essential role in the growth and development of the fetus before birth, and studies also showed that IGF2 causes the growth and division (multiplication) of cells in many different tissues [20]. The H19 gene is located near the IGF2 gene and provides instructions for making a non-coding RNA. Despite many genes, the H19 gene does not contain instructions for making a protein. The function of this non-coding RNA produced from this gene is unknown, but researchers believe it may act as a tumor suppressor. The H19 gene is highly active before birth in various tissues and appears to play an important role in early development [21].

The molecular defects of BWS and SRS are very heterogeneous. Many of these abnormalities involve epigenetic or copy number changes within the 11p15.5 gene cluster. Identification of these defects usually requires the use of multiple assays. Molecular diagnosis of SRS and BWS is currently possible only in one-third of patients [22] The aim of this study was to evaluate the methylation status and copy number of genes H19, IGF2, CDKN1C, KCNQ1, and NSD1 in blood samples of 10 Iranian children suspected of SRS or BWS.

Analysis of methylation, deletion, and addition in the mentioned genes in 10 children suspected of SRS or BWS, led to the diagnosis of SRS in one child and BWS in 2 children. In line with the present study, Gaston et al. showed that analysis of KvDMR1 methylation in leukocyte DNA alone identified BWS in 60% of all patients (64% of patients with severe form and 53% of patients with incomplete form of BWS). The analysis of the H19 gene methylation identified 13 other BWS patients with H19 hypermethylation, which confirms the effect of methylation in the occurrence of BWS in the two mentioned genes in line with our study [23].

Therefore, KCNQ1OT and H19 gene analysis is useful in routine evaluation and leads to the diagnosis of BWS or SRS in children suspected of these syndromes [24].

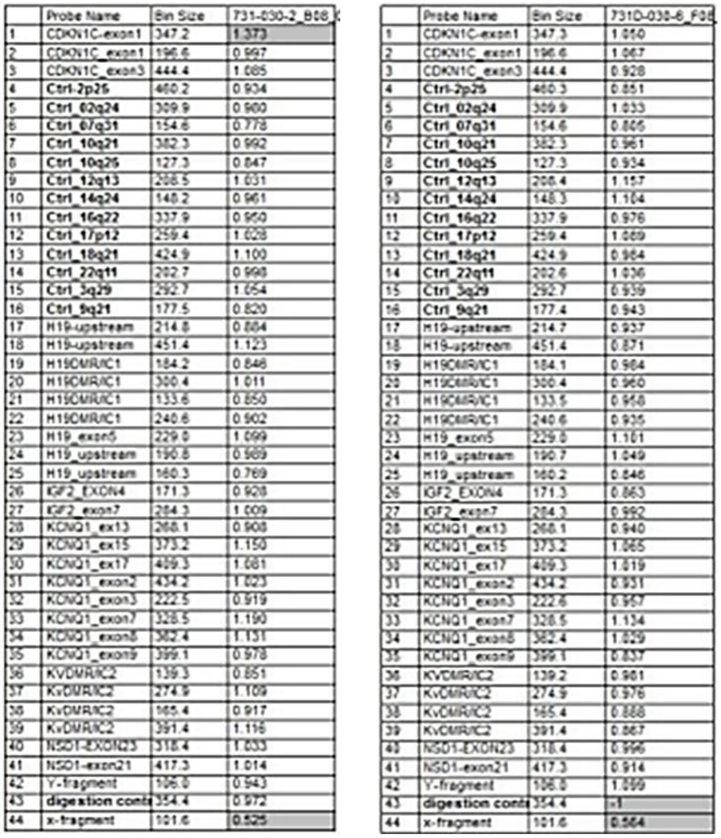

	Probe Name	Bin Size	731-030-2_B08
1	CDKN1C-exon1	347.2	1.373
2	CDKN1C_exon1	196.6	0.997
3	CDKN1C_exon3	444.4	1.085
4	**Ctrl-2p25**	460.2	0.934
5	**Ctrl_02q24**	309.9	0.900
6	**Ctrl_07q31**	154.6	0.778
7	**Ctrl_10q21**	382.3	0.992
8	**Ctrl_10q26**	127.3	0.847
9	**Ctrl_12q13**	208.5	1.031
10	**Ctrl_14q24**	148.2	0.961
11	**Ctrl_16q22**	337.9	0.950
12	**Ctrl_17p12**	259.4	1.028
13	**Ctrl_18q21**	424.9	1.100
14	**Ctrl_22q11**	202.7	0.998
15	**Ctrl_3q29**	292.7	1.054
16	**Ctrl_9q21**	177.5	0.820
17	H19-upstream	214.8	0.884
18	H19-upstream	451.4	1.123
19	H19DMR/IC1	184.2	0.846
20	H19DMR/IC1	300.4	1.011
21	H19DMR/IC1	133.6	0.850
22	H19DMR/IC1	240.6	0.902
23	H19_exon5	229.0	1.099
24	H19_upstream	190.8	0.989
25	H19_upstream	160.3	0.769
26	IGF2_EXON4	171.3	0.928
27	IGF2_exon7	284.3	1.009
28	KCNQ1_ex13	268.1	0.908
29	KCNQ1_ex15	373.2	1.150
30	KCNQ1_ex17	409.3	1.081
31	KCNQ1_exon2	434.2	1.023
32	KCNQ1_exon3	222.5	0.919
33	KCNQ1_exon7	328.5	1.190
34	KCNQ1_exon8	362.4	1.131
35	KCNQ1_exon9	399.1	0.978
36	KVDMR/IC2	139.3	0.851
37	KvDMR/IC2	274.9	1.109
38	KvDMR/IC2	165.4	0.917
39	KvDMR/IC2	391.4	1.116
40	NSD1-EXON23	318.4	1.033
41	NSD1-exon21	417.3	1.014
42	Y-fragment	106.0	0.943
43	**digestion contr**	354.4	0.972
44	x-fragment	101.6	0.525

A. Copy number analysis

	Probe Name	Bin Size	731D-030-6_F0
1	CDKN1C-exon1	347.3	1.050
2	CDKN1C_exon1	196.6	1.067
3	CDKN1C_exon3	444.4	0.928
4	**Ctrl-2p25**	460.3	0.851
5	**Ctrl_02q24**	309.9	1.033
6	**Ctrl_07q31**	154.6	0.806
7	**Ctrl_10q21**	382.3	0.961
8	**Ctrl_10q25**	127.3	0.934
9	**Ctrl_12q13**	208.4	1.157
10	**Ctrl_14q24**	148.3	1.104
11	**Ctrl_16q22**	337.9	0.976
12	**Ctrl_17p12**	259.4	1.089
13	**Ctrl_18q21**	424.9	0.984
14	**Ctrl_22q11**	202.6	1.036
15	**Ctrl_3q29**	292.7	0.939
16	**Ctrl_9q21**	177.4	0.943
17	H19-upstream	214.7	0.937
18	H19-upstream	451.4	0.871
19	H19DMR/IC1	184.1	0.984
20	H19DMR/IC1	300.4	0.960
21	H19DMR/IC1	133.5	0.958
22	H19DMR/IC1	240.6	0.935
23	H19_exon5	229.0	1.101
24	H19_upstream	190.7	1.049
25	H19_upstream	160.2	0.846
26	IGF2_EXON4	171.3	0.863
27	IGF2_exon7	284.3	0.992
28	KCNQ1_ex13	268.1	0.940
29	KCNQ1_ex15	373.2	1.065
30	KCNQ1_ex17	409.3	1.019
31	KCNQ1_exon2	434.2	0.931
32	KCNQ1_exon3	222.6	0.957
33	KCNQ1_exon7	328.5	1.134
34	KCNQ1_exon8	362.4	1.029
35	KCNQ1_exon9	399.1	0.837
36	KVDMR/IC2	139.2	0.981
37	KvDMR/IC2	274.9	0.976
38	KvDMR/IC2	165.4	0.888
39	KvDMR/IC2	391.4	0.867
40	NSD1-EXON23	318.4	0.996
41	NSD1-exon21	417.3	0.914
42	Y-fragment	106.0	1.099
43	**digestion contr**	354.4	>1
44	x-fragment	101.6	0.564

B. Methylation analysis

Figure 4: MLPA genetic test findings related to the fourth patient

Gaston et al. showed complete or nearly complete demethylation (KvDMR1 MI >90%) in 70% of BWS patients, but about one-third showed partial demethylation, suggesting that this abnormality can occur with mosaicism. These findings were supported by the analysis of other pathological tissues that show different demethylation patterns from blood cells. For example, KvDMR1 is incompletely demethylated in the tongue tissue samples of patients while it is fully demethylated in blood cells, indicating that the analysis of blood samples was sufficient to diagnose BWS [23]. Gaston et al., observed that demethylation of the KCNQ1OT gene was never associated with abnormal methylation of the H19 gene [23] except in patients with UPD 11p15, indicating at least two imprinting centers at 11p15.5. These findings were consistent with a recent study by Engel et al [12].

In the present study, the analysis of H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes was normal in 7 out of 10 children. In line with these findings, Gaston et al reported that the analysis of KCNQ1OT and H19 genes was normal for 26 patients (26.8%). CDKN1C gene sequencing was performed in 10 of these patients [5 sporadic forms and 5 patients from families]. No abnormalities were found in sporadic cases. CDKN1C mutations were found in the families of 2 patients, and both patients had exomphalos [23]. Other unknown 11p15 abnormalities may account for other cases. Various mechanisms may cause over-growth syndrome by inducing the activity of growth factors such as IGF2. Several transgenic mouse models have provided evidence for this by showing increased levels of bioactive IGF2 induced by inhibition of the IGF type 2 receptor [IGF2R] or glypican 3, two agents known to bind and downregulate IGF2 expression, making it less accessible to the IGF1R [25]. Further studies on 11p15 genes and IGF2-related regulators in overgrowth syndromes should be conducted to determine the association between genotype and phenotype and to elucidate functional mechanisms.

In a study by Jurkiewicz et al., the MS-MLPA screening test identified the triplication of the IGF2/H19 domain for all probes of these genes in the ME030 kit, although this result was contrary to the results of our study because we did not find any duplication or deletion in individuals. They also showed that the H19 (ICR1) region had increased methylation and also showed normal methylation patterns for the IGF2R KCNQ1OT1 genes, which are in line with the results of our study [26].

In the present study, MS-MLPA was used for the molecular diagnosis of BWS and SRS. The results showed that MS-MLPA is a sensitive and reliable tool for further identification of molecular defects of BWS and SRS. These findings are in line with the study of Priolo et al., which was conducted in Italy [27]. Most epigenetic defects of BWS and SRS occur sporadically and exist in the mosaic form in individuals [28,29]. Therefore, it is important to use a sensitive diagnostic method to identify all mosaic cases. MS-MLPA method for the detection of 11p15.5 defects is very accurate and sensitive [27].

The great advantage of MS-MLPA is that this method simultaneously determines the copy number and methylation level of the loci. This is clearly shown in the third patient who had both hypomethylation and hypermethylation. While the ligation-only reaction revealed an increase in copy number, the ligation-digestion reaction provided information about the parental origin of the amplified region. Another study also confirmed that MS-MLPA can be considered a robust assay for the analysis of chromosome 11p5.5 defects [30]. In conclusion, our experience with these 10 suspected children showed that MS-MLPA is an easy, low-cost, and reliable method for the molecular diagnosis of BWS/SRS cases with im-printed and repetitive defects on chromosome 11p15. However, due to the small sample size as one limitation in this study, these results need to be confirmed in a larger series of patients. Also, for 7 children who were not confirmed to be suffering from BWS and RSS syndromes in this study, we suggest performing a WES test for them because the doctor may have made a mistake in diagnosing their symptoms and they may be suffering from another disease.

CONCLUSIONS

This was the first study to document these two syndromes in Iran. The present findings showed that methylation changes in the control regions of ICR1 and ICR2, which are related to H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes, lead to the development of SRS or BWS. A diagnosis of the two syndromes is not accurate solely based solely on clinical symptoms and without the use of cellular and molecular genetic testing. MS-MLPA is suggested as a low-cost, reliable, and rapid method for the molecular diagnosis of BWS/SRS with imprinting and duplication defects on chromosome 11P15.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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Authors’ contribution

MAE, ME, OM, NS, and SS were responsible for the study concept and design. MAE, ME, OM, NS, and SS led data collection. MAE, ME, OM, NS, and SS were responsible for the analysis and interpretation of data. MAE, ME, OM, NS, and SS wrote the draft.

Ethics approval and consent to participate

This study was approved by the ethics committee of Azaz University of Medical Sciences [code: IR.IAU.PS.REC.1401.062]. Throughout the study, researchers adhered to the Declaration of Helsinki. Written informed consent was obtained from the children's parents in this research.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Body Composition Differences in Army Paratroopers: Effect of Speciality, Age, and Gender

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Abstract: *Body composition has been associated with better performance in military populations. However, there is no consensus about the most suitable body composition values for army paratroopers. To analyze body composition differences among the type of specialty, age group, and gender of army paratroopers, for a better improvement of their operability. 104 paratroopers of the Spanish Army were analyzed. Anthropometric and morphological data were analysed throw an In-Body720. There was a significant effect of age on intracellular water. Older soldiers had lower values of intracellular water, protein, and skeletal muscle mass than middle-aged soldiers. No significant differences (P ≤ 0.05) for the effect of the kind of work and age together or taken separately. Men had significantly higher values than women for height, weight, extracellular water, protein, fat-free mass, and skeletal muscle mass, and significantly lower values for the percentage of body fat, while no differences were found for body fat mass and body mass index. We conclude that paratroopers' body composition depends on age in skeletal muscle mass, intracellular water, and protein. For better unit readiness, soldiers must be monitored to analyze the effect of training and nutrition habits on their body composition.*

Keywords: *military; body fat mass; lean mass; body water*

INTRODUCTION

Physical training is a key factor for soldiers, who are expected to perform efficiently in physical tasks and maneuvers, i.e. lifting and carrying weights, deployments, long displacements, or fighting [26]. Traditionally, Body Composition (BC) has been associated with better performance in military populations [13], like in load-carriage jobs, in which lean mass (together with aerobic power) has been identified as more important than speed of movement [21]. Despite these facts, there is still a tendency towards considering perceived assumptions, like appearance, rather than empirical data to evaluate the capabilities of each soldier, and weight plus Body Max Index (BMI) by themselves are not enough to assess military training since many other factors, like lean mass gain, are positive aspects of training that need to be measured [22,24].

Although some standards have been used and considered in the Army to encourage soldiers to keep fit and with good nutritional habits, there is no consensus about the most suitable BC for all the healthy ranges of weight, soft lean mass, or body fat percentage. This attempt becomes more complicated if we consider the wide range of duties required by the different units of the Army and the different soldiers´ ages [14,28]. The incorporation of women in the Army further complicates this issue, because there is a need to define gender-appropriate standards to maximize female soldiers' health and performance [14]. Taking sports, as a reference, different specializations would require different standards of body composition [2,37], like different jobs would require different BC in the Army, as it was reported for the Army [16,17,38] and Air Forces [4]. Moreover, BC is a variable that could be easily managed by any motivated soldier both with good fitness and nutrition habits, with the aim, not to predict the performance, but to obtain health benefits, resistance to disease or injuries [18,27], and better general capabilities, both mental and physical due to a balanced lifestyle [14,25].

Paratroopers are considered an elite unit of the Army since their deployments are highly operational and involve a parachute jump, which has been reported to increase heart rate and decrease fine motor skills [10,34]. Apart from the physical readiness required for a quick setup, prior to entering the battlefield, they face additional hazards related to high altitudes, like hypoxia, disorientation, or night flights [3,5,6]. The principal tactical jumps are automatic para-chute jumps, in which the soldier has no control of the parachute opening, and manual parachute jumps, in which the subject has control of the parachute in all the maneuvers. Previous research found that both jumps produced a large stress response increasing cortisol, blood lactate, and sympathetic activation [8,10,11]. The health and performance of these kinds of units, which are under extreme psychological and physiological environments [7], can be undermined and there is a need to monitor their body composition [36], especially during their deployment in crisis management operations [29].

MATERIALS AND METHODS

Experimental approach to the problem

To implement the body composition assessment, we followed previous procedures [4,9,30] in which the participants stood upright on foot electrodes on the instrument platform, with arms not touching the torso. They were barefooted and without excess clothing. Four-foot electrodes were used, two of which were oval-shaped and two heel-shaped, and prior to testing the skin was cleaned and dried. Participants were asked to grip the palm and thumb electrodes. The system was calibrated before the testing session and the contacting surface of the electrodes was cleaned with alcohol before each measurement.

Participants abstained from alcohol consumption and vigorous exercise for 24h before the measurement and were measured in the morning after overnight fasting to control hydration status, as in previous research with bioelectrical impedance.

Subjects

A cross-sectional experimental design was conducted to analyze body composition differences of paratroopers, depending on their specialty, group age, and gender. 7 female paratroopers (specialty: automatic parachute; age: M = 32.1, SD = 8.79), and 97 male paratroopers were analyzed and classified into groups of specialty (43 automatic, age: M = 33.3, SD = 9.75; 54 manual, age: M = 34.8, SD = 7.58) and age (21 younger, under 29 years; 48 middle-aged, between 29 and 38 years; and 28 older, above 38 years) of the Spanish Army with a qualification of "fit" according to the periodic medical examination as recorded in the ministerial order 23/2011. Besides, during the research, they were carrying out the periodic aeromedical training included in the ministerial order 23/2011 and the STANAG 3114 "Aeromedical Training of Flight Personnel" (NATO regulations). Before starting the research, the experimental procedures were explained to all the participants, who gave their voluntary written informed consent under the Declaration of Helsinki. The procedures conducted in the present research were designed and approved by the Medical Service of the Aerospace Medicine Instruction Centre of Spanish Air Forces and the University Ethic Committee (CIPI/18/093).

Instruments

A bioimpedance analyzer (InBody 720, Biospace Co. Ltd., Seoul, South Korea) was used for body mass measurement (to the nearest 0.1 kg) and body height (to the nearest 0.1 cm). InBody 720 is a multifrequency impedance body composition analyzer, which uses an eight-point tactile electrode method to take readings from the body. It measures the resistance at five specific frequencies (1 kHz, 50 kHz, 250 kHz, 500 kHz, and 1 MHz) and reactance at three specific frequencies (5 kHz, 50 kHz, and 250 kHz) on each of five segments (right arm, left arm, trunk, right leg, and left leg). Bioelectrical-impedance analysis (BIA) is one of the methods available for measuring body composition in healthy populations. Its simplicity, portability, cost, and subject acceptance make it a very desirable technique. The reliability of BIA compared to the gold standard body composition measurement (DXA) has been successfully demonstrated for a wide range of normal and overweight populations [15,35] and military samples [1].

Data were electronically imported to Excel using Lookin'Body 3.0 software. The following parameters were analyzed: i. Body mass (kg), ii. Height (cm), iii. Body Mass Index (BMI) (kg/m^2), iv. Total Body Water (TBW) (kg) (including left and right arms, trunk, and left and right legs), v. Intracellular Water (ICW) (l) (including left and right arms, trunk, and left and right legs), vi. Extracellular Water (ECW) (l) (including left and right arms, trunk, and left and right legs), vii. Proteins (kg), viii. Minerals (kg), ix. Body Fat Mass (BFM) (kg) (including left and right arms, trunk, and left and right legs, in kg and %), x. Percentage of Body Fat (PBF) (%), xi. Soft Lean Mass (SLM) (kg), xii. Fat-Free Mass (FFM) (kg) (including left and right arms, trunk, and left and right legs, in kg and %), xiii. Skeletal Muscle Mass (kg).

Statistical analysis

The SPSS statistical package (version 21.0; SPSS, Inc. Chicago, Illinois) was used to analyze the data. Normality assumptions were checked with a Shapiro-Wilk test for all the subgroups and a Kolmogorov-Smirnov test for the whole group. Gender differences were analyzed for the same specialty (automatic parachute) with a T-Student test for parametric samples and a Mann-Whitney test for no parametric samples. Homoscedasticity was tested with the Levene test. A factorial ANOVA test was used to compare the type of work, the group of age, and the interaction between the type of work and the group of age on each parameter together with a Bonferroni post hoc test to analyze pairwise comparisons. The level of significance for all the comparisons was set at P ≤ 0.05.

RESULTS

The results are reported with their mean and standard deviation. Table 1 shows the results of body composition. No significant differences were found for the effect of type of work and age together, but there was a significant effect of age [F(2, 91)=3.172, p = 0.047, η2 = 0.06], in ICW. Older soldiers (M = 28.3, SD = 2.8) had lower values (p = 0.041) of ICW than middle-aged (M = 30.3, SD = 3.7). There was a significant effect of age [F(2, 91)=3.108, p = 0.049, η2 = 0.06], in protein. Older soldiers (M = 12.2, SD = 1.2) had lower values (p = 0.043) of protein than middle-aged (M = 13.0, SD = 1.6). There was a significant effect of age [F(2, 91)=3.154, p = 0.047, η2 = 0.07], in SMM. Older soldiers (M = 34.9, SD = 3.6) had lower values (p = 0.041) of SMM than middle-aged (M = 37.5, SD = 4.9).

Table 1: Results of body composition variables by specialty and age groups

	Age < 28				28 < Age < 38				Age > 38				Age and specialty effect		
	Automatic		Manual		Automatic		Manual		Automatic		Manual				
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	F	p	η^2
Height (cm)	178.8	7.5	177.5	7.0	177.3	6.2	175.2	5.9	174.3	5.8	175.0	5.7	0.47	0.66	0.01
Body Mass (kg)	75.3	7.1	79.4	14.1	83.4	15.4	78.9	10.6	73.6	9.4	76.5	9.2	1.40	0.25	0.03
BMI (kg/m^2)	23.6	2.6	25.1	3.6	26.3	3.5	25.6	2.8	24.2	3.0	24.9	2.6	1.03	0.35	0.02
TBW (l)	46.4	3.1	47.6	5.5	49.0	7.8	47.5	4.8	45.5	4.5	45.3	4.4	0.47	0.62	0.01
ICW (l)	29.1	1.9	29.9	3.6	30.7	4.9	30.0	3.0	28.4	2.9	28.3	2.8	0.36	0.69	0.01
ECW (l)	17.2	1.2	17.7	2.0	18.3	2.9	17.5	1.8	17.0	1.5	17.0	1.6	0.72	0.48	0.02
Proteins (kg)	12.6	0.8	12.9	1.5	13.2	2.1	12.9	1.3	12.2	1.2	12.2	1.2	0.33	0.71	0.00
Minerals (kg)	4.2	0.4	4.5	0.7	4.7	0.8	4.4	0.5	4.3	0.4	4.2	0.5	1.17	0.31	0.03
BFM (kg)	11.9	4.1	14.3	10.1	16.3	7.4	13.9	6.4	11.5	6.5	14.6	5.1	1.86	0.16	0.04
PBF (%)	15.6	4.4	16.9	9.1	19.0	6.3	17.1	5.7	15.1	6.8	18.8	5.3	1.83	0.17	0.04
SLM (kg)	59.8	4.0	61.3	7.2	63.2	10.0	61.3	6.2	58.5	5.8	58.3	5.6	0.45	0.64	0.01
FFM (kg)	63.3	4.3	65.1	7.7	67.0	10.8	64.9	6.6	62.1	6.1	61.8	6.0	0.48	0.61	0.01
SMM (kg)	36.0	2.5	37.1	4.7	38.1	6.4	37.1	3.9	35.0	3.8	34.9	3.6	0.38	0.68	0.01

Note. M = Mean; SD = Standard Deviation; F = Fisher-Snedecor test; η2 = Partial eta squared; BMI = Body Mass Index; TBW = Total Body Water; ICW = Intracellular Water; ECW = Extracellular Water; BFM = Body Fat Mass; PBF = Percentage of Body Fat; SLM = Soft Lean Mass; FFM = Fat Free Mass; SMM = Skeletal Muscle Mass

Table 2 shows the results of FFM and BFM by body segments. No significant differences were found for the effect of kind of work and age together or taken separately.

Table 2: Results of Fat-Free Mass and Body Fat Mass by body segments

	Age < 28				28 < Age < 38				Age > 38				Age and specialty effect		
	Automatic		Manual		Automatic		Manual		Automatic		Manual				
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	F	p	η2
FFM Right Arm (kg)	3.65	0.31	3.76	0.52	3.93	0.76	3.79	0.49	3.56	0.55	3.58	0.47	0.39	0.67	0.01
FFM Left Arm (kg)	3.61	0.30	3.72	0.54	3.89	0.78	3.78	0.51	3.50	0.59	3.78	0.51	0.34	0.71	0.01
FFM Trunk (kg)	28.1	1.79	28.7	3.20	29.6	4.44	28.9	2.93	27.2	3.43	27.6	2.88	0.39	0.67	0.01
FFM Right Leg (kg)	10.0	1.01	9.94	1.09	10.1	1.49	9.71	1.02	9.32	0.91	9.37	1.00	0.42	0.65	0.01
FFM Left Leg (kg)	9.92	1.02	9.89	1.13	10.0	1.52	9.64	1.02	9.32	0.80	9.32	0.97	0.37	0.69	0.01
BFM Right Arm (kg)	0.52	0.26	0.75	0.86	0.86	0.56	0.68	0.56	0.55	0.51	0.75	0.35	1.67	0.19	0.04
BFM Left Arm (kg)	0.55	0.28	0.78	0.90	0.90	0.58	0.67	0.56	0.55	0.49	0.76	0.36	1.97	0.14	0.04
BFM Trunk (kg)	6.16	2.59	7.45	5.70	8.80	4.34	7.36	3.74	5.83	3.81	7.78	3.16	1.93	0.15	0.04
BFM Right Leg (kg)	1.82	0.47	2.07	1.22	2.30	0.90	1.98	0.77	1.74	0.82	2.10	0.57	1.87	0.16	0.04
BFM Left Leg (kg)	1.79	0.46	2.02	1.25	2.27	0.89	1.98	0.76	1.74	0.82	2.08	0.59	1.60	0.20	0.03

Note. M = Mean; SD = Standard Deviation; F = Fisher-Snedecor test; η2 = Partial eta squared; FFM = Fat-Free Mass; BFM = Body Fat Mass

Table 3 shows the results of body water (total, intracellular, and extracellular) by body segments. No significant differences were found for the effect of type of work and age together, but there was a significant effect of age [F(2, 91)=3.314, p = 0.041, η2 = 0.06], in the ICW of the trunk. Older soldiers (M = 13.3, SD = 1.51) had lower values (p = 0.035) in the ICW of the trunk than middle-aged (M = 14.2, SD = 1.71). Men had significant higher values than women for height (p=0.000; M = 176.9, SD = 6.6 vs M = 164.1, SD = 5.9), weight (p=0.000; M = 78.0, SD = 12.2 vs M = 60.0, SD = 6.8), TBW (M = 47.2, SD = 5.8 vs M = 34.0, SD = 2.1), ECW (p=0.000; M = 17.6, SD = 2.1 vs M = 12.8, SD = 0.73), ICW (p=0.000; M = 29.5, SD = 3.6 vs M = 21.2, SD = 1.3), Protein (p=0.000; M = 12.7, SD = 1.5 vs M = 9.2, SD = 0.6), Minerals (p=0.000; M = 4.4, SD = 0.62 vs M = 3.27, SD = 0.27), FFM (p=0.000; M = 64.4, SD = 8.0 vs M = 46.5, SD = 2.96), SLM (p=0.000; M = 60.7, SD = 7.5 vs M = 43.8, SD = 2.76), SMM (p=0.000; M = 36.5, SD = 4.8 vs M = 25.7, SD = 1.83), and significant lower values for PBF (p=0.034; M = 16.8, SD = 6.0 vs M = 22.0, SD = 4.3). No significant differences were found for the effect of gender between men and women in BFM (p=0.955; M = 13.5, SD = 6.5 vs M = 13.4, SD = 4.3) and BMI (p=0.065; M = 24.8, SD = 3.29 vs M = 22.34, SD = 2.9).

Table 3: Results of Body Water by Body Segments

	Age < 28				28 < Age < 38				Age > 38				Age and specialty effect		
	Automatic		Manual		Automatic		Manual		Automatic		Manual				
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	F	p	η²
TBW Right Arm (l)	2.84	0.23	2.92	0.40	3.05	0.59	2.94	0.38	2.77	0.42	2.78	0.37	0.39	0.67	0.01
TBW Left Arm (l)	2.80	0.23	2.89	0.42	3.02	0.61	2.93	0.40	2.72	0.46	2.77	0.38	0.37	0.69	0.01
TBW Trunk (l)	21.8	1.42	22.3	2.46	23.0	3.45	22.4	2.28	21.1	2.66	21.4	2.20	0.44	0.64	0.01
TBW Right Leg (l)	7.78	0.80	7.71	0.85	7.86	1.15	7.52	0.79	7.23	0.70	7.28	0.77	0.47	0.62	0.01
TBW Left Leg (l)	7.71	0.80	7.68	0.89	7.81	1.18	7.48	0.80	7.24	0.62	7.25	0.76	0.39	0.67	0.01
ICW Right Arm (l)	1.77	0.14	1.83	0.25	1.91	0.36	1.84	0.23	1.73	0.27	1.73	0.23	0.37	0.68	0.01
ICW Left Arm (l)	1.75	0.14	1.81	0.26	1.89	0.37	1.84	0.24	1.70	0.29	1.73	0.24	0.31	0.72	0.01
ICW Trunk (l)	13.7	0.85	14.0	1.60	14.4	2.17	14.1	1.43	13.2	1.71	13.3	1.40	0.32	0.72	0.01
ICW Right Leg (l)	4.90	0.47	4.88	0.53	4.95	0.73	4.77	0.49	4.55	0.46	4.55	0.49	0.29	0.74	0.01
ICW Left Leg (l)	4.83	0.48	4.82	0.54	4.90	0.74	4.72	0.49	4.53	0.39	4.52	0.47	0.27	0.76	0.01
ECW Right Arm (l)	1.06	0.09	1.08	0.14	1.14	0.22	1.09	1.14	1.04	0.15	1.04	0.14	0.43	0.64	0.01
ECW Left Arm (l)	1.05	0.09	1.08	0.15	1.13	0.23	1.09	1.15	1.02	0.16	1.04	0.14	0.47	0.62	0.01
ECW Trunk (l)	8.15	0.59	8.28	0.88	8.61	1.29	8.27	0.85	7.95	0.95	8.07	0.81	0.68	0.50	0.02
ECW Right Leg (l)	2.88	0.32	2.83	0.33	2.91	0.43	2.74	0.30	2.68	0.24	2.72	0.28	0.86	0.42	0.02
ECW Left Leg (l)	2.87	0.33	2.85	0.35	2.91	0.45	2.75	0.31	2.17	0.22	2.72	0.29	0.63	0.53	0.01

Note. M = Mean; SD = Standard Deviation; F = Fisher-Snedecor test; η2 = Partial eta squared; TBW = Total Body Water; ICW = Intracellular Water; ECW = Extracellular Water

DISCUSSION

This study aimed to analyze body composition differences between the type of specialty, age group, and gender of paratroopers.

The first hypothesis was fulfilled since there were no differences in body composition between specialties. The second hypothesis was partially fulfilled since there were differences in intracellular water, protein, and skeletal muscle mass between middle-aged and older soldiers. The third hypothesis was not fulfilled since there were no significant differences in body composition between male and female paratroopers.

The main parachute jumps conducted by paratroopers' units are automatic or manual. The characteristics of both jumps are different since in automatic parachute jumps there is a lack of parachute control during the flight and especially during the landing (with high impacts that directly affect the muscle and bone structures of jumpers).

On the contrary, in manual jumps the flight and landing phases allow for greater control by the jumpers, leading to a less traumatic landing. But independently of these differences, we did not find differences between jump specialties, a fact probably due to the similar training, instruction, and nutritional regimen of both groups. The only difference was the tactical maneuvers after both jumps, but the rest of the preparation and military context were similar in both groups.

Differences between ages were found in intracellular water, protein, and skeletal muscle mass. The middle-aged group had a better mean than the older group in all those variables. Since the skeletal muscle is mainly built upon protein and water, these results show a tendency towards skeletal muscle loss with age.

The differences found could be related to the fact that middle-aged soldiers have complete growth, they are in their most operational phase in the unit, where they perform a higher number of jumps and the training is more intense, facts that could explain the higher intracellular water, protein, and skeletal muscle mass. This fact would join, with the investigations that support that resistance training promotes an increase in body water, principally by intracellular content [32]. On the other hand, older and younger participants perform a lower number of jumps: older paratroopers begin with the natural muscle mass loss process associated with age [33] and the younger paratroopers have not yet reached their growth zenith and their training in the unit is not yet maximum.

It is important to note that body fat mass, percentage of body fat, and soft lean mass, which have been usually reported as crucial for the performance of the units [13,21] were not different between groups, supporting the idea of the adequate habits of these troops during their service. Also, data analysis for body segments, reinforces the idea of the correct BC maintenance of these units, throughout their military service, despite their age, since no significant differences were found between groups of work and age.

Besides, if we compare the values of body mass index (24.12 kg/m^2 vs 25.88 kg/m^2 for age <28, 25.89 kg/m^2 vs 26.94 kg/m^2 for age between 28 and 38, and 24.64 kg/m^2 vs 26.86 kg/m^2 for age > 38); and body fat percentage (16.0% vs 16.6% for age < 28, 17.8% vs 18.8% for age between 28 and 38, and 17.2% vs 19.7% for age > 38) with some US Army standards, they are better for all the age groups [20]. Nevertheless, we should bear in mind that these groups are considered “elite” inside the Spanish army, and we are comparing them with a combination of soldiers from the combat support, combat arms, and combat service support units. However, results of body mass index and body fat percentage were higher than those reported for soldiers after 6 months of deployment in a crisis management operation (25.89 kg/m^2 vs 24.50 kg/m^2 and 17.8% vs 13.7%), a fact that highlights the need to monitor body composition during field activities, and especially on those with higher length, intensity or more stressful type [36]. Not only fat mass but fat-free mass and protein could be undermined during operational training or deployment [23,24].

If we compare these results with other Army units, body mass index and percentage of body fat are quite similar to airplane and helicopter pilots, but lower to other aircrew support members (25.49 kg/m^2 and 23.79% respectively) [4]. If we take body segments, there is a tendency towards having more fat-free mass in the right arm, which is consistent with previous research in the Air Forces [4], light forces [12], and even in females [39], which could be due to the prominence of right-handedness.

Regarding our third hypothesis about differences in BC according to sex, most of the BC male parameters in absolute terms were higher, while BFM and BMI did not show significant differences. Further investigation is recommended on this issue since the female sample size does not allow the values to be extrapolated and generalized.

Finally, regarding body water composition, there are no significant differences between age groups and manual or automatic parachute jumps in any of the variables analyzed, total, intracellular, and extracellular water. These values suggest an optimal balance of hydration among the healthy population and are correlated with a lower and healthier BMI [19], consequent with the normal-weight distribution among age and type of jump. Indeed, similar values of intracellular and extracellular values have been found in professional athletes depending on their composition whether it is an athlete or not [31].

The main limitation of this study was the small sample size of women. Secondly, we did not have permission to gather other demographic data (like nutrition, economic, education, and physical activity levels in leisure time) that could have helped us to better understand the differences between groups. These background variables could be studied further to design longitudinal research to understand the mechanisms of BC changes.

CONCLUSIONS

Paratroopers' Body Composition is not affected by their type of parachute specialty (automatic or manual) but depends on age for skeletal muscle mass, intracellular water, and protein. Older soldiers had lower values of ICW than middle-aged. Older soldiers had lower values of protein than middle-aged. Older soldiers had lower values of SMM than middle-aged. This data suggests good nutrition and training habits among the paratroopers. When comparing these data with previous research, it suggests that the body fat percentage of paratroopers is similar to airplane pilots, lower than aircrew personnel and combat and service soldiers, but higher than soldiers deployed. Body composition should be monitored to analyze the effect of training and nutrition habits during service, especially during stressful deployments. These facts must be considered to better prepare each unit.

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Authors' contribution

Conceptualization, V.J.C.S. and A.B.S.; methodology, V.J.C.S. and A.B.S.; formal analysis, V.J.C.S. and A.B.S.; investigation, V.J.C.S. and A.B.S..; resources, V.J.C.S. and A.B.S.; data curation, A.B.S.; writing—original draft preparation, A.B.S.; writing—review and editing, V.J.C.S. and A.B.S..; supervision, V.J.C.S.; project administration, V.J.C.S.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of UNIVERSIDAD EUROPEA DE MADRID (protocol code CIPI/18/093).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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ORIGINAL ARTICLE

Functional and Dysfunctional Coping Strategies in Patients Diagnosed with Cancer – From Initial Assessment to Therapeutic Interventions

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Abstract: *The assessment of coping mechanisms in patients diagnosed with oncological diseases is essential for mental health specialists, who have to design the most appropriate case management strategy for comorbid mood disorders, anxiety disorders, adjustment disorders, and other psychiatric conditions that may be detected in this vulnerable population. The adequate treatment of these disorders is important for the preservation of mental health status, quality of life, and overall functionality in patients diagnosed with cancer. Coping mechanisms modulate the vulnerability toward psychiatric disorders, but they also have an impact on treatment adherence, which is an important factor correlating with prognosis. Appraisal-focused, problem-focused, emotion-focused, and occupation-focused coping represent the most well-defined strategies patients use when confronted with a stressful life situation, like a diagnosis with potentially vital consequences. Maladaptive coping strategies may also be identified in these patients, e.g., withdrawal from reality, including complete or partial denial of the disease, substance abuse, behavioral addictions, refusal of the recommended treatment, etc. The psychotherapeutic approach in patients with oncological diseases should include an initial evaluation of the coping strategies used either currently or in the past stressful conditions, an assessment of all the psychosocial resources the patients have (e.,g., support group, professional insertion, hobbies), and screening for mood and anxiety disorders that may have been triggered by the cancer diagnosis. Consequently, within the psychotherapeutic framework, a trained specialist can enhance the role of adaptive coping strategies and highlight the disadvantages of dysfunctional ones. This process may possess a favorable impact on treatment adherence, mental health status, and quality of life in patients with cancer.*

Keywords: *coping mechanisms, treatment adherence, quality of life, cognitive behavioral therapy, executive functions*

INTRODUCTION

Coping mechanisms are defined as thoughts and behaviors activated when finding a solution to either internal or external stressful situations is required [1]. These triggers may vary from different feared situations, a perceived lack of control over own impulses, or difficult life decisions (when internal events are taken into consideration), to physically traumatic stimuli, imposed changes in lifestyle, or interpersonal stressors (when external events are the focus of attention). Combined, internal and external triggers, may exist, as in severe physical conditions, such as oncological diseases, when the diagnosis, the treatment, the stigma, and the overall functionality (personal, familial, social, and professional) are all affected.

A traditional difference exists between the „coping mechanisms", which are conscious and voluntary, and the „defense mechanisms", which are psychodynamically-based constructs, and by their definition unconscious. Coping mechanisms represent sets of structured, stable responses that have a well-defined adaptive function [1]. Defense mechanisms, on the other hand, have as their purpose to disguise internal impulses, in order to protect the Ego; therefore they operate outside the sphere of the conscious mind [2-5]. However, not all authors agree with the assumption that all defense mechanisms are unconscious, because of examples like suppression or humor, which involves, at least partially, a participation of the Ego [6-8]. At any rate, it is considered that defense mechanisms act as „conscious suppression”, and this may be observed in interpersonal relationships [2].

There are many classifications of coping mechanisms because these psychological processes are numerous and quite diverse, therefore different authors group them according to more or less arbitrary criteria [9-15]. One example of such a classification has as its core criterion the targeted stressor(s), and includes (1) reactive coping – the individual responds to a certain stress factor; people who activate this type of coping have presumably better adaptive skills in an unstable environment with many hard-to-predict stressors; (2) proactive coping – the individual activates behaviors/thoughts aimed at neutralizing the effects of anticipated stressors; people who activate this coping perform better in a stable environment with routine tasks [1,16].

Another classification of coping mechanisms includes (a) problem-focused strategies – address the origin of the discomfort, and these may be manifested as active coping, planning, restrictive coping (not acting prematurely, holding back until a good opportunity to act appears), and inhibition of concurrent activities; (b) emotion-focused strategies – targeting the decrease of negative emotions correlated with the ongoing stressor, e.g., positive reframing, acceptance, recourse to religion, and humor; (c) meaning-based strategies, i.e., extraction and management of the meaning the stressful situation has for an individual in a certain moment; (d) social or support-based strategies, which refer to the ability of stress reduction by seeking emotional or behavioral support from the group to which that person belongs [1,17].

The coping mechanisms mentioned above are „adaptive” processes, but there is also a category of „maladaptive” coping strategies [18]. It is supposed that problematic coping may develop in the early stages of conscious life when individuals have to face overwhelming stressors like maltreatment, abuse, or neglect [18]. For example, the excessive activation of primitive coping strategies, lack of modeling for healthy coping alternatives, and repeated exposure to intense stressors in the immediate environment are potential factors that may increase the individual's vulnerability to the development of maladaptive coping strategies [18]. These coping mechanisms are associated with a negative evolution of mental health problems and more severe levels of psychopathological manifestations [1]. Examples of maladaptive or dysfunctional coping are represented by disengagement (defined by efforts to move away from the stress triggers), emotional suppression (i.e., inhibition of emotional reactions), cognitive avoidance (manifested by distraction, worry, and thought suppression), and denial (i.e., the unwillingness to accept reality) [19-21].

The role of maladaptive coping mechanisms in psychopathology has been explored intensively, and conditions like posttraumatic stress disorder, anxiety disorders, depressive disorders, psychosomatic disorders, and somatoform disorders have all been correlated with avoidance-based coping or other maladaptive strategies [1]. The situation of treatment or diagnostic refusal in patients with psychiatric disorders, but also in those presenting with different organic illnesses, including cancer, has been associated with maladaptive coping (i.e., denial) [21].

The rationale for exploring coping mechanisms in patients with oncological and other severe diseases is the need to understand better the psychological strategies activated by patients in stressful situations and to work with these strategies during psychotherapy. One aspect of this process is to allow for the formation of a more solid therapeutic relationship, by exploring the adaptive strategies. Other objectives that may involve coping mechanisms are the training of functional coping skills and helping patients to deconstruct the dysfunctional ones.

There is an additional aspect for exploring these mechanisms in patients with cancer, and it refers to the risk of psychiatric comorbidities. As can be anticipated, this diagnosis, as well as the associated treatment and prognosis, may lead to the onset of adjustment disorders, depressive disorders, stress-related pathology, etc. Many authors are highlighting the need for identifying and treating depression in this specific population because this would have a favorable impact on the quality of life and mortality [22-24]. Cases of major depressive disorders (MDD) have frequently been detected in patients with cancer, reaching prevalences of 2-43.5% [22-24]. The importance of coping mechanisms derives from their impact on the evolution of clinical manifestations and therapeutic responses in patients with MDD [25]. A study exploring this impact concluded that maladaptive mechanisms, mainly ruminating, catastrophizing, and self-blaming were the most frequently used by patients with depression [25]. Accepting the current situation, positive refocusing and re-evaluation are functional coping mechanisms, with a protective role in these patients, and these strategies can be used during the psychotherapeutic process [25].

In another study, the importance of coping strategies for resilience was evaluated in patients with panic disorder [26]. The severity of clinical manifestations and maladaptive coping skills decreased during cognitive-behavioral therapy (CBT) administered in a group format [26]. CBT also had beneficial effects on increasing resilience and quality of life in patients with panic disorder, thus highlighting the role of psychotherapy in modulating the influence of coping mechanisms on daily functioning, besides its positive clinical impact [26].

Adjustment disorders illustrate the failure of coping mechanisms' functionality in preserving a safe distance toward stressful stimuli’ negative impact [27]. Also, acute stress reactions and posttraumatic stress disorder are consequences of coping strategies failure, but in these cases, the stressors represent vital threats [27]. Psychotherapy in these cases targets enhancing coping abilities with the specific stressor and increasing the adaptive responses [28]. This is usually done by identifying negative thoughts and replacing them with more adaptive thoughts (i.e., cognitive restructuring), monitoring the experiences aimed at increasing self-efficacy, and behavioral activation [28-30].

FROM CONCEPTUAL ANALYSIS TO CLINICAL SITUATIONS – AN ANALYSIS OF HOW THE COPING MECHANISMS FUNCTION IN SPECIFIC SITUATIONS

As mentioned in the introductory chapter, coping mechanisms are usually activated in stressful situations, and configuring the individual profile of these mechanisms allows the therapist to represent the specific vulnerability features and the resilience factors to stressful situations, like a vital threat raised by a cancer diagnosis. The presentation of several theoretical situations is considered helpful for a better understanding of how the coping mechanisms may work in clinical settings. For example, when a patient finds out that she has been diagnosed with early-stage breast cancer, she may engage in seeking information about this disease and/or making an appointment for further investigations (which implies active, problem-focused coping); also, she may decide to join a self-help group for patients with the same diagnosis (i.e., a coping based on social support), or she may look for spiritual support from a priest (defining a coping based on recourse to religion). However, continuously looking for information that may invalidate the oncological diagnosis, by exploring different online resources, or by repeated medical consultations in the hope of finding a doctor willing to offer another diagnosis, is a maladaptive coping strategy based on denial. In the case of a patient with prostate cancer who has already been investigated by collecting all the necessary laboratory analyses (prostate biopsy included) and by undergoing all imagistic investigations required, the delay of treatment initiation is caused by his lack of trust in the results of these investigations. Due to this maladaptive coping strategy, the treatment initiation is delayed, and the prognosis may worsen.

Another example is represented by a woman with breast cancer who has undergone a total mastectomy and developed a depressive disorder because she refuses to accept her new body image. As a manifestation of her depression, she does not want to consult a mental health specialist, thus activating a coping strategy based on denial and avoidance.

Yet another typical situation is when a person who goes through a divorce caused by health and financial problems ends up consuming alcohol excessively, to suppress his/her negative emotions. This way of facing the specific life situation is characterized by disengagement and avoiding confrontation with own emotions, i.e., two types of maladaptive coping.

On the other end of the coping mechanisms spectrum, a person who has to retire prematurely due to an oncological disease (now in remission, but with several negative functional consequences) may go initially through an adjustment disorder caused by the recent change of the daily activity regime, but then she/he resignifies the situation and considers that she/he has more free time to spend with own family. This is an example of active coping, planning, and coping based on significance because the individual perceives her/himself as a survivor of the disease and focuses on future prospects.

Of particular importance for understanding how coping mechanisms work, is the exploration of the strategies activated and the solutions each patient has found when confronted previously with difficult situations, such as interpersonal conflicts, social isolation, work conflicts, economic difficulties, and severe illnesses [31].

NEUROBIOLOGICAL FOUNDATION OF COPING MECHANISMS AND THE STRUCTURED APPROACH TO THEIR CLINICAL ASSESSMENT

The prefrontal cortex, responsible for executive functioning (i.e., planning, analysis and synthesis, abstracting, problem-solving, verbal fluency, etc.), and the nucleus accumbens, which is part of the reward system or the mesocorticolimbic circuit, have been associated with the functioning of the coping mechanisms [32,33]. These central nervous system (CNS) structures are sensitive to chronic stress, and disruptions in the functioning of the prefrontal cortex have negative consequences on the ability to perform complex cognitive tasks [32,33]. Executive functions, including cognitive flexibility, planning, inhibitory control, error detection, working memory, and verbal fluency, are largely dependent on the morphological integrity and functionality of the prefrontal cortex [34-39]. These functions are essential for constructing coping mechanisms, their preservation or inhibition, and mediating the relationship between coping strategies and different psychiatric disorders, such as MDD [34,35]. It was even demonstrated that interventions targeting the enhancement of executive functions and/or coping mechanisms are useful in decreasing the risk of MDD recurrence [34]. Executive functions have a role in increasing the risk of suicidality by mediating the relationship between high levels of stress and avoidant coping, therefore enhancing executive functioning through computerized training and cognitive rehabilitation may reduce the impact of stressors on individuals [35]. Also, executive dysfunction and mnestic impairments were associated with lower use of active coping mechanisms in patients with schizophrenia, highlighting the important correlations existing between these variables [39].

The activity of the hypothalamic-pituitary-adrenocortical axis (HPAA) and the plasma level of cortisol have no confirmed direct effect on coping mechanisms, but they may influence intermediate variables with impact on, or associated with, these mechanisms [33,40,41]. Such mediators are depressive mood, anxiety, insomnia, psychomotor restlessness, and attentional and memory deficits [33,40,41]. For example, in a study (N=30 participants), the presence of MDD was correlated with a higher level of morning serum cortisol vs. healthy individuals, and an inverse correlation between these levels and the frequency of activated prosocial coping mechanisms was observed [42].

Serotonergic and dopaminergic pathways in the CNS are responsible for modulating mood, strengthening/decreasing certain behaviors, and influencing memory and attention [43-48]. The prefrontal cortex, hippocampus, and corpus striatum, which are involved in cognition, are densely populated by dopaminergic and serotonergic neurons originating within the raphe complex, mesocortical, and nigrostriatal systems [43,49]. Processes like learning and memory depend on the modulation of serotonergic and dopaminergic activity and imply short- and long-lasting synaptic modifications [43]. Modulation of serotonergic and dopaminergic pathways using pharmacological agents, as is the case with MDD and schizophrenia spectrum disorders, can influence not only the core clinical symptoms of these disorders but, indirectly, may modulate the coping mechanisms [50-53].

The impact of vasopressin and oxytocin on psychological mechanisms that are important for the modulation of coping strategies was also explored [54,55]. These pituitary neuropeptides are responsible for shaping social behavior, aggression, and social memory, according to preclinical studies [54,55].

Data about the importance of vasopressin and oxytocin in humans also emerged recently, and functional neuroimaging studies showed that impaired operation of these neuropeptides appears in mental disorders that imply an early attachment disruption or difficulties in forming social relationships, such as autism spectrum disorders (ASD), social anxiety disorder, borderline personality disorder, and schizophrenia spectrum disorders (SSD) [55].

In conclusion, the biological foundation of cognition is extremely complex, and the adequate functioning of the cognitive processes (as a prerequisite for the operational coping mechanisms) depends heavily on the integrity of multiple neurotransmission and neurohormonal pathways.

The assessment of coping mechanisms is conducted by mental health specialists trained in using validated instruments. Besides the general evaluation, based on a psychological interview targeting current mental status and personal history, the specialists may use various scales, inventories, and questionnaires constructed and validated for this purpose.

Brief-COPE is a 28-item, self-administered instrument that quantifies functional and dysfunctional coping methods in stressful situations [56]. It is often used in clinical settings to assess how patients cope with stressors such as cancer diagnosis, heart failure, trauma, and mental and behavioral disorders [56]. Based on interpreting this questionnaire's results, psychological counseling strategies may be formulated [56]. Brief-COPE evaluates three types of coping: (1) problem-focused – includes the following dimensions: active coping, planning, suppression of competing activities, restraint coping, and seeking instrumental social support; a high score indicates coping strategies aimed at changing stressful situations, psychological stability, a pragmatic approach and is a positive prognostic factor; (2) focused on emotions – includes seeking social support, positive reinterpretation, acceptance, denial, and appeal to religion; high scores indicate strategies aimed at regulating stress-related emotions; (3) avoidant – includees emotional venting, behavioral disengagement, mental disengagement; a high score suggests physical or cognitive disengagement efforts, low scores suggest adaptive coping [56-61]. The original COPE inventory included 15 dimensions, each with 4 items [57,62]. However, this form was criticized because the number of factors differed across studies, varying from 3 to 5, it was considered too long, and the distinction between its dimensions was not supported by further analyses [57].

The most frequently used tools for evaluating coping mechanisms, besides the COPE and Brief-COPE, according to a meta-analysis, are the Ways of Coping Questionnaire, Coping Strategies Questionnaire, Coping Response Inventory, Coping Strategies Inventory, Adolescent Coping Scale, Pain Coping Questionnaire, Religious COPE, and Utrecht Coping List [63]. The same source mentioned that coping scales are often used for the structured assessment of patients with various health problems, such as illnesses, pain, and medical diagnoses [63].

COPING MECHANISMS IN PATIENTS WITH ONCOLOGIC DISEASES

Coping mechanisms influence the decision-making process regarding participation in treatment and implicitly shape the evolution and prognosis of the condition. The same mechanisms impact the patient's desire to be informed about their own illness, their perception of self-efficacy, their relationship with others during the illness, etc. The main coping mechanisms identified in a study (N=603 patients with cancer) were (1) seeking or using social support, (2) focusing on the positive aspects of life, (3) distancing, (4) cognitive avoidance/cognitive abandonment, and (5) behavioral avoidance/behavioral abandonment [64]. This study concluded that the type of oncological disease, the time elapsed since the diagnosis of the disease, and the presence of treatment correlated non-significantly with the type of coping [64]. The stress perception associated with the oncological condition correlated significantly with the activation of coping based on social support, but also with cognitive and behavioral disengagement [64]. Coping based on social support, focusing on the positive, and distancing were all associated with less emotional distress, while cognitive/behavioral withdrawal was associated with more emotional distress [64].

The relationship between coping strategies, quality of life, and mood in patients with incurable oncological conditions was investigated in a randomized trial of palliative care, that enrolled patients with advanced stages of lung or gastrointestinal cancer (N=350), who were in the first 8 weeks after the diagnosis was confirmed [65]. All main outcomes were assessed in a structured way, and the most frequently detected coping mechanism was emotional support (77%), followed by acceptance (49%), self-blame (38%), and denial (28%) [65]. Coping based on emotional support was significantly associated with better quality of life and less severe depressed mood [65]. In the same study, acceptance correlated significantly with better quality of life and inversely with depression and anxiety, denial and self-blame correlated significantly inversely with quality of life and non-significantly with depression and anxiety [65]. The study's authors concluded that interventions aimed at improving the quality of life and mood of these patients should include increasing the use of adaptive coping strategies [65]. It also may be stated that patients with incurable forms employ different coping mechanisms than those with oncological conditions presenting a favorable prognosis [65]. The explanation is that the emotional burden and severity of physical symptoms create a different setting for activating coping mechanisms in the two population subgroups [65].

Types of coping activated by patients with breast cancer predict long-term psychological adjustment, according to the results of a study that explored the strategies for facing difficulties related to this diagnosis in a group of 55 patients [66]. Coping mechanisms, decisional control, expression of frustration, and psychological adaptation in the first 6 months after finding out the diagnosis were assessed initially and after 3 years [66]. Patients who were initially depressed and who responded to finding out the diagnosis with cognitive avoidance (acceptance/resignation) showed significantly less chance of psychological adjustment 3 years later [66]. The poor psychological adjustment was significantly associated with cognitive avoidance and minimal activation of proximity/ contact-based coping [66].

The relationship between quality of life and coping mechanisms was evaluated in women extensively treated for advanced-stage genital cancer (N=64) [67]. A control group included women with genital cancer in the early stages, who consequently received less aggressive treatment [67]. The main stressors in the study group were the adverse effects of chemotherapy, poor physical condition, and poor prognosis [67]. Using the COPE inventory to assess coping mechanisms, it was observed that avoidance and engagement were the dominant strategies in patients with severe forms of cancer [67]. Also, these patients presented more frequently both types of coping (commitment/avoidance) vs. patients in the control group [67]. Avoidant coping strategies correlated with more negative mood and poorer overall well-being, while avoidance and seeking instrumental support were associated with worse prognosis in patients with severe forms of cancer, but not in those with early forms [67]. Thus, coping mechanisms have a stronger impact on prognosis in patients with severe forms of genital cancer [67]. Avoidant coping strategies in particular may adversely affect the quality of life and mood in this population [67].

The predictive value of coping mechanisms in patients who were diagnosed with stage I or II breast cancer no more than 12 months ago was the objective of a longitudinal study that included 70 patients [68]. This study explored the abilities of these patients to activate situational-specific coping mechanisms and hope (considered a more stable factor), as predictors of adaptation after finding out their diagnosis [68]. Active acceptance-based coping at the moment of diagnosis predicted better adjustment over time in this population, while avoidance-based coping correlated with greater fear of cancer recurrence, independent of the patient's initial medical condition and age [68]. Religion-based coping proved more effective for women who had a lower initial level of hope [68]. The conclusion of the study was that protective and risk factors for adaptation in first-year breast cancer survivors can be identified even before surgery [68]. Mood characteristics, such as hope, as well as situation-specific coping strategies, must be considered by the case manager [68].

Optimism was explored as a variable mediated by coping strategies in patients with breast cancer in the early stages, in a study with 59 participants [69]. The level of optimism was determined one day before surgery, 10 days after it, and 3-, 6-, and 12 months post-surgery [69]. Acceptance-based coping, positive reframing, and religion were the most frequently activated strategies, and optimism correlated inversely with the level of distress at each of the study visits [69]. Denial and behavioral disengagement were the least frequently activated coping strategies in this population [69]. In conclusion, optimism and pessimism are mediated by the activation of different coping mechanisms in the adaptive process and they have an important role in favoring or hindering this process [69].

The correlation between coping strategies and psychological distress was investigated in patients with advanced stages of cancer [70]. In such a study, that enrolled 132 patients diagnosed with breast, ovarian, lung, colorectal, and other types of cancer, a correlation between distancing, cognitive avoidance, and behavioral abandonment, on the one hand, and psychological distress, on the other, was demonstrated [70]. Distancing coping correlated negatively with psychological distress, while cognitive and behavioral avoidance correlated positively with the same distress [70]. Overall, coping methods explained 36% of the variation in the level of psychological distress [70]. The most important coping mechanism contributing to psychological distress was behavioral avoidance, followed by cognitive avoidance [70].

Emotional expressive coping has predictive value for physical and mental adjustment to breast cancer diagnosis, according to a prospective, 3-month study (N=92 participants) [71]. Active processing of emotions is a coping mechanism based on promoting emotional contact, that favors adaptation and health status [71]. Women who reported emotional expression-type of coping had fewer visits to the doctor for cancer-related symptoms, had better physical health and energy levels, and had lower levels of distress over the next 3 months compared to patients who had less emotional expression [71]. Quality of life was also better in patients with emotionally expressive coping [71].

MDD, personality disorders, and coping strategies were studied as moderators of the quality of life in patients with non-metastatic breast cancer [72]. A cross-sectional study included 120 patients diagnosed with breast cancer 7 months ago, and the main outcomes were quality of life, coping strategies, biological and physiological variables, presence of MDD and personality disorders, and pain severity [72]. Poorer physical quality of life was associated with MDD, younger age, more severe initial tumor stage, and behavioral disengagement coping [72]. A lower emotional quality of life was associated with MDD, the presence of a personality disorder, more severe pain, a high level of self-blame, and a lower use of acceptance-based coping [72]. Also, lower global quality of life was associated with MDD, the presence of a personality disorder, more intense pain, high levels of self-blame, less use of reframing coping strategies, and the absence of hormone therapy [72]. In conclusion, lower quality of life was associated with premorbid patient characteristics (personality disorder) and coping variables (depression, personality, and specific strategies), less so with cancer-related variables (type of treatment, disease severity) [72].

TRAINING OF THE COPING MECHANISMS IN ONCOLOGICAL PATIENTS

Psychotherapy and psychological counseling in patients diagnosed with severe organic illnesses have, between their objectives, the enhancement of functional coping mechanisms and the reduction of maladaptive ones, in order to enhance resilience to stressful situations (e.g., pain, invalidity, changes in lifestyle, adverse events of drugs). Coping skills training programs have been associated with improving some biological and psychosocial parameters in patients with chronic somatic conditions [73]. For example, a decrease in HbA1c levels and an increase in self-efficacy have been observed in patients with diabetes after applying a coping skills training program [73]. The same programs led to increased self-care capacity in diabetic patients, decreased the severity of depressive symptoms, improved the prognosis of chronic obstructive pulmonary disease (COPD), and ameliorated the quality of life in individuals with various somatic conditions [73]. The training of these skills involves lectures, Q&A sessions, group discussions, role-plays, etc., under the direction of a clinical psychologist/psychotherapist; nurses trained for this purpose can also lead such support groups [73]. The topics addressed are the principles of effective self-care, stress management, communication skills training, cognitive-behavioral restructuring techniques, and problem-solving techniques [73].

A brief coping skills training program for oncology patients was designed to increase the patient’s feelings of self-efficacy, personal control, and resilience to stress [74]. Applied in the perioperative framework, this program was created for nurses, and they were trained on how to enhance the patient’s trust in their own abilities to manage dysfunctional cognitions and behaviors [74]. This training was used to prepare the patients for solving situations or events that may occur before or after surgery [74]. Providing information, education, emotional support, and specific interventions – adapted to the objectives of each individual case, were part of this brief training [74]. Relaxation based on breathing, cognitive restructuring, mindfulness, goal setting, and motivational interviewing may be part of more comprehensive training [74].

Encouraging caregivers of oncology patients to participate in the training of coping mechanisms is considered an important aspect of case management. A study that included 233 patients with lung cancer and their relatives compared two interventions – 14 telephone sessions of coping skills training assisted by a relative vs. education/support with the help of a relative [75]. Patients in both groups showed improvements in anxiety and self-efficacy during the 4-month follow-up [75]. However, family-assisted coping skills training has been associated with better outcomes in patients with stage II or III cancer [75]. Caregiver-assisted education/psychological support was more effective in patients with stage I lung cancer [75].

The effects of coping skills training on caregivers of oncological patients were also explored. Such a study enrolled 70 participants and looked at the effects of coping skills training on the quality of life in daughters of patients with breast cancer [76]. The intervention consisted of an educational program, with workshop-type lectures, followed by group discussions [76]. Relatives' quality of life was considerably improved by this intervention, according to Pediatric Quality of Life Inventory scores [76]. Physical, and emotional functioning and participation in school activities were improved vs. the control group [76].

Particular aspects of adaptive strategies training depend on the specific elements of oncological conditions. The approach in a psychotherapeutic setting to patients with cancer must include the training of coping skills, depending on the particularities of each case. It is essential for the therapist to work with the patient in identifying the stress factors that activate coping mechanisms: self-image changing (e.g. mastectomy, limb amputation), reduced mobility, adverse effects of chemotherapy and radiotherapy (e.g. alopecia, persistent vomiting, swallowing disorders, sexual dysfunctions), lifestyle changes (eg, colostomy, indwelling urinary catheter), physical pain, psychiatric symptoms/ disorders – depression, anxiety, restlessness, insomnia, apathy, anhedonia, etc.

Therapeutic interventions may be focused on the activation of functional coping mechanisms, based on action and social support, e.g. looking for a support group for patients who have undergone similar surgical interventions; planning activities based on the spared skills; analyzing the pros and cons of correcting deficits (prosthetics, plastic surgery); re-signification of the situation, e.g. post-operative scars are an indicator of the battle with the disease and of the fact that the person has overcome it, assuming the state of "survivor" or "victor"; creating an optimal framework for expressing emotions, concerns, fears related to the new situation in which the person finds him/herself, dysfunctions of the social image, self-image, and relationships with those close to the patient, decreased professional functioning, etc.; encouraging acceptance of the present situation and redirecting resources towards functional, healthy aspects of own life; acceptance does not mean resignation; adding non-pharmacological methods to drug pain therapy – mindfulness, progressive muscle relaxation, diaphragmatic breathing, guided imagery, planning hobby activities; specific techniques for augmenting the effect of medication in depressive, anxiety, adaptation disorders, insomnia, etc., e.g., cognitive restructuring in depression and anxiety, aversive therapy or relaxation techniques in insomnia, guided imagery and activity planning for isolated affective/anxiety symptoms, etc.

CONCLUSIONS

Coping mechanisms in patients diagnosed with oncological diseases have an important role in preserving mental health, quality of life, and general functionality. Coping mechanisms modulate the vulnerability towards mood and anxiety disorders in patients diagnosed with cancer, but these mechanisms also have an impact on therapeutic adherence, thus representing important factors that correlate with prognosis. The best-defined functional coping strategies are those centered on realistic assessment, problem-solving, emotion-expression, and occupational activation, therefore it is recommended that the therapist train these healthy mechanisms in patients with severe organic diseases.

Coping resources activated in stressful life circumstances include the participation of family or friends, recourse to religious rituals, at least partial maintenance of professional roles whenever possible, and self-help therapy (online sessions, organized groups, etc.).

In oncology patients, maladaptive coping strategies can also be identified, for example, withdrawal from reality, including complete or partial denial of the disease, substance abuse, behavioral addictions, treatment refusal, requesting the administration of insufficiently validated treatments not recommended by a specialist, etc.

The psychotherapeutic approach to patients with cancer should include an initial assessment of coping strategies used both now and in the past under stressful conditions, an evaluation of the psychosocial resources that patients have previously activated in life-threatening situations, and a screening for mood, stress-related, and anxiety disorders that may have been triggered since the onset of the current illness (Figure 1).

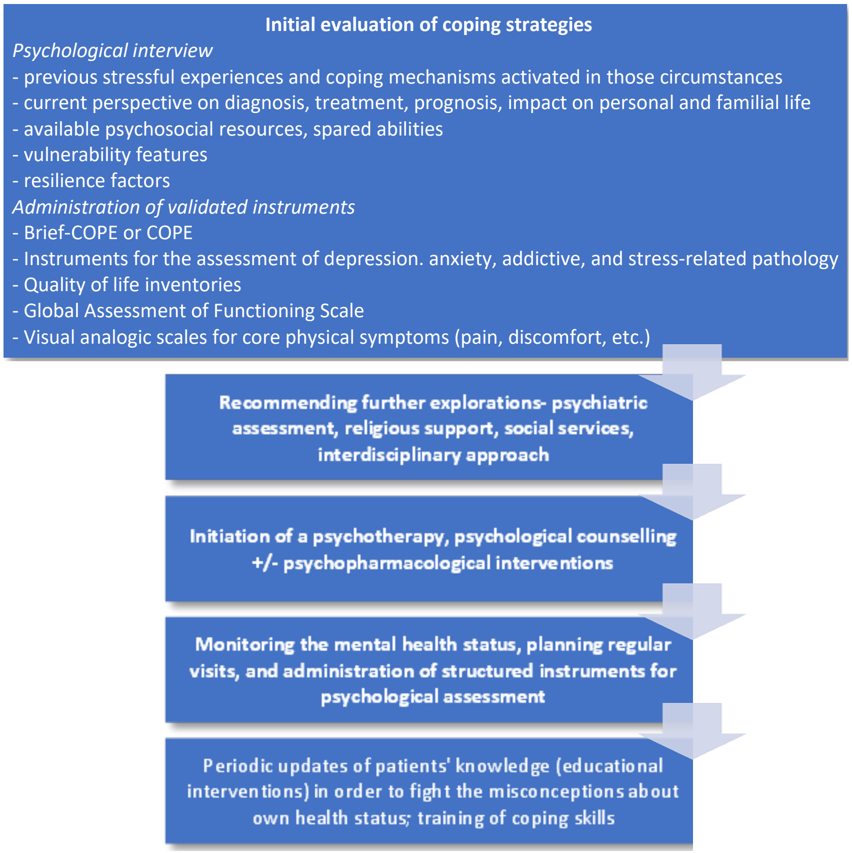
Initial evaluation of coping strategies
Psychological interview
- previous stressful experiences and coping mechanisms activated in those circumstances
- current perspective on diagnosis, treatment, prognosis, impact on personal and familial life
- available psychosocial resources, spared abilities
- vulnerability features
- resilience factors
Administration of validated instruments
- Brief-COPE or COPE
- Instruments for the assessment of depression. anxiety, addictive, and stress-related pathology
- Quality of life inventories
- Global Assessment of Functioning Scale
- Visual analogic scales for core physical symptoms (pain, discomfort, etc.)
Recommending further explorations- psychiatric assessment, religious support, social services, interdisciplinary approach
Initiation of a psychotherapy, psychological counselling +/- psychopharmacological interventions
Monitoring the mental health status, planning regular visits, and administration of structured instruments for psychological assessment
Periodic updates of patients' knowledge (educational interventions) in order to fight the misconceptions about own health status; training of coping skills

Figure 1: The algorithm for assessing and training coping mechanisms in patients with cancer

After this stage, a psychiatric consultation may be recommended, if necessary (acute psychiatric pathology, the need for pharmacological treatment). The initiation of psychological counseling, psychotherapy, and, in specific cases, psychopharmacological treatment may be prescribed, depending on the results of the initial evaluation. Monitoring the mental health status by using standardized tools, and planning follow-up visits to update patients' knowledge and coping skills learned is necessary. The psychotherapeutic intervention should be focused on increasing adaptive coping through systematic training and highlighting the disadvantages of dysfunctional coping mechanisms. This therapeutic process can have a significant favorable impact on treatment adherence, mental health status, and quality of life in patients with cancer

Further research on this topic should investigate the impact of structured coping skills training programs on more homogenous populations, such as patients with a certain type of oncological disease and in a specified stage of the disease's evolution [77-79]. Of major interest would be the exploration of combined therapeutic interventions in this population, and also the assessment of the impact different types of surgical interventions may have on mental health and quality of life [77-83]. As a limitation of this review, it should be mentioned that it was not designed as a systematic approach to this topic, its immediate purpose is to enhance the awareness of mental health specialists about the importance of assessing and addressing during therapy the important aspect of coping strategies.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

OV conceived the idea of the review. OV, AGM, BMP, CAC, CFP and REBB designed the study. OV, CFP, REBB, AFF, MCP, CAS and CN screened the studies and abstracted the primary sources. OV, AGM, BPM and CAS drafted the first version of the review. OV, CAC, CFP, AFA, MCP and CAS revised the manuscript and elaborated the conclusions of the review. All authors had full access to all data contained in the review and decided to submit the manuscript for publication.

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ORIGINAL ARTICLE

Confidentiality in the Pharmaceutical Managed Entry Agreements in Romania

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Abstract: *Background and Aim: Health technology assessment for drugs is a necessary step in developing health policies that are focused on patients and getting the best value for scarce resources. One important feature of health technology assessment is transparency. In many countries, health technology assessment is followed by negotiations between pharmaceutical companies and health authorities to determine whether a drug will be publicly funded. These negotiations often result in Managed Entry Agreements, which typically include confidentiality clauses covering the final price of the drug. Methods: We reviewed Romanian legislation starting in 2014 to assess the level of confidentiality and transparency in drug pricing and reimbursement. Results: We found that for drugs with Managed Entry Agreements, the level of discounts is confidential, the public does not know how much the government is paying for each of these drugs, the volumes (units) of drugs are not transparently published by the payer, the outcomes, in terms of patients treated by therapeutic area, therapeutic success, and resource utilization, are confidential. Conclusions: We consider that too much confidentiality can prevent the public from knowing if the government is getting good value for money, but too little confidentiality can lead to higher drug prices or a lack of drugs from the market.*

Keywords: *managed entry agreements, health technology, confidentiality, pricing, reimbursement*

INTRODUCTION

Every year, the pharmaceutical industry introduces new drugs on the market, constantly developing new solutions for known illnesses. The licensing mechanisms of new drugs are already fully regulated, and the beneficial effects and potential risks have been proven through numerous preclinical and clinical studies [1].

One of the main concerns of each country in establishing its policy on medicines is the permanent increase in the price of new medicines, which generates a growing pressure on the budgets allocated to health [2]. Once they enter the market, the big challenge for a new drug is to be accepted for financing from public funds (or through the health care mechanisms specific to each country). For this, in most of the EU countries, each drug is passed through the very fine sieve of the Health Technology Assessment (HTA) process, the ultimate goal of which is to provide health decision-makers with sufficient relevant information based on which to establish priorities in the field of care, such as the reasonable cost that they afford, and the level of financing, their efficiency, respect for legal and ethical values, and equity.

To develop patient-focused health policies that seek to achieve the best value for scarce resources, one characteristic of the HTA is the transparency of the process [3]. Together or after the HTA process, in numerous countries, there is a practice of negotiations between the Marketing Authorization Holder (MAH) and health authorities to get access to a drug to public financing using Managed Entry Agreements (MEA). In 1993, first-time conventions, lately called MEAs, were introduced between producers and third-party payers, targeting discounts and rebates for innovative drugs [4,5].

The need for these contracts was born from the producers' desire to make price reductions only in the conditions in which they obtain guarantees regarding the avoidance of parallel import and the downward spiral of the price as a result of the fact that the visible price decrease in one country attracts a decrease in all other countries and finally even in the respective country as a result of the use of reference prices. From this perspective, MEAs have a common denominator, respectively, the confidentiality covering the final price of the drug generally as a means to avoid price decreases in other countries.

On the other hand, the third-party payer is interested in keeping costs within a predictable and bearable margin, obtaining superior outcomes for patients, and spending the available resources as efficiently as possible [4]. MEAs can be divided into financial agreements that directly affect the budget (confidential discount or rebate, price-volume agreements, volume or expenditure cap, free initial treatment, etc.) and performance-based agreements that evaluate the treatment's performance (coverage with evidence development, payment by result, conditional treatment continuation, etc.) [4,6,7].

In Romania, a scorecard model called HTA was introduced in 2014. This is based primarily on the results of the assessments or reimbursement status for the respective drugs in EU countries. The health authorities decided in 2015 to increase the financial sustainability of the new drugs through the transfer of financial risk to MAHs via Managed Entry Agreements (known locally as Cost-Volume contracts, which are financial-based agreements, and Cost-Volume-Results contracts, which are performance-based agreements) [8]. The introduction of MEAs allowed a thawing of the introduction of innovative products on the reimbursement list for medicine (the List), after many years of impasse.

To conclude a functional MEA for a drug, there are several mandatory steps in Romania, some of which are confidential and some of which are transparent. These steps are (1) publishing of the List prices in the Official Gazette; (2) submitting the HTA dossier, obtaining the HTA body's approval and publishing the HTA Report; (3) negotiating the MEA, if applicable; and (4) adding the drug on the List.

METHODS

We performed a critical review of the Romanian legislation and rules, from the last 10 years, looking for the confidentiality and transparency aspects covering the pricing and reimbursement of drugs with MEA.

In our research, we worked to identify the elements of the lack of transparency in the price negotiation process for medicines or related to it that could lead to a discrepancy between the benefits offered to patients and the actual price paid.

RESULTS

The Romanian regulations do not stipulate the confidentiality of the final purchase prices. The joint order from the Minister of Health and the President of the National Health Insurance House (NHIH) requires that any confidential information obtained during the execution of the MEA contract (Cost-Volume contract) be used only to fulfill the MEA's purpose and not be disclosed or utilized for other reasons, including the final price of the medication in question [9].

Our analysis revealed that drug reimbursement in Romania through MEAs involves a blend of public and confidential data.

In Romania, there are two List Price catalogs: (1) one used for public reimbursement (Canamed) [10] and (2) one used only by pharmacies or health care providers not having a public reimbursement (Public Catalogue) [11]. Both catalogs are publicly available in the Official Gazette and on the Ministry of Health website [12], while Canamed can be seen also with the reimbursement price on the local payer website [13] (last update on July 1st, 2021). For the International Reference Pricing process done by other countries, the Ministry of Health recommends the use of the Public Catalogue (with higher List prices than Canamed).

The submission of the HTA dossier is a highly confidential process, with most of the steps not transparent: the content of the HTA dossier, the members of the evaluation team from the HTA body, the deadline for the evaluation, and the involvement or not of the Specialty Commissions from the Ministry of Health. Every month, the HTA body (National Agency for Drugs and Medical Devices – ANMDM) publishes a list of the drugs received for HTA evaluation. Next, the HTA report for each drug and the decision and the meeting minutes of the Appeal Committee (only for the drugs where the MAH was not satisfied with the results of the HTA report) are also published.

The HTA methodology allows the MAH to offer (part of the HTA dossier) a "confidential discount" to the payer (NHIH), one key aspect of confidentiality, resulting in a lower price compared to its competitor. This "confidential discount" should be part of a Protocol between MAH and the payer to include the obligation of the MAH to lower the net price (not visible) once the HTA report is positive and then the drug is included in the Reimbursement List 2020 [14].

The negotiation of a MEA is mainly a confidential process. The decision to apply for an MEA relies on the MAH level and also the proposal to be addressed to the Negotiation Committee. The negotiation is contingent upon the number of patients under contract and the eligible population. Ministry of Health (MoH) Specialty Commissions establish the eligible population and publish it on the payer website. Often, these Commissions are not transparent about the data they use to define and establish the eligible population. The number of contracted patients is strictly confidential because the ratio of contracted patients to eligible patients determines the discounts to be paid by MAH as part of MEA (between 25-75%). The table with these discounts is publicly available and published in the Official Gazette (Table 1) [9,15]. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Table 1: Discounts for MEA depending on the market share covered

Percent (%) applied to quarterly consumption (single drug)	Percent (%) applied to quarterly consumption (multiple winners for substitutable drugs)	Percent (%) no. of contracted patients from the no. of total eligible patients
25%	30%	<5%
30%	35%	5-15%
35%	40%	15-25%
40%	45%	25-35%
45%	50%	35-45%
50%	55%	45-55%
55%	60%	55-65%
60%	65%	65-75%
70%	75%	>=75%

The term for these MEAs is one year and is publicly available, their starting point is the first month after the inclusion of the drug in the List.

The renegotiation process involves a simpler approach, with a publication of the moment for negotiation and a clear request for the Specialty Commissions to provide arguments regarding the change in the eligible population.

Other optional aspects of the negotiation, like the coverage by the MAH of some testing or other services, are usually available to the public on the websites of the local affiliates of the payer, due to the need to inform the providers of these aspects [9].

After the conclusion and the execution of the MEA, the MAH will pay the final discount to the payer according to the effective patient share, irrespective of the contracted patient share and discount. The respective data is provided by the payer only to the MAH and nobody else could access this information.

The Parliament approves the payer budget for MEA negotiation and contracting through the State Budget Law. The process of determining this MEA budget is not transparent to the public [16]. Meanwhile, the payer discloses the remaining budget after every round of MEA negotiations. The payer typically reserves a certain amount of its drug budget for drugs that require a new yearly MEA contract to ensure the continued operation of existing MEAs. If the contracting budget is over, no new MEA is signed and for the existing MEAs, there is no renegotiation; only the included patients can continue their treatment and there are no new inclusions of patients.

The budget for drugs with MEA is not available to the public on a monthly or quarterly basis, but at the end of each year, the authorities issue a note detailing the total budget used. MEA's budget information is also included in the Payer Yearly Report, published in the second half of the following year.

The decision to include the drug on the List is highly political, as it requires budget allocation for its introduction. The process for the calculation of budget needs for new drugs is not transparent. The information is only publicly available once the draft of the List is published for public comment.

DISCUSSION

Some authors have pointed out that the classically invoked reasons, namely that the prices of new drugs are very high because most of the income is reinvested in research, are no longer in line with the reality of the time. Drug manufacturers often carry out a series of additional purely financial operations, such as mergers and acquisitions and stock transactions, and play an active role in the capital market, etc. [9,17]. In recent years, concern has grown regarding the confidentiality of the final purchase prices of medicines; in 2019 WHO recommended to member states that they take measures in this regard [18].

The MEA process's opacity creates advantages and disadvantages for the MAH and NHIH regarding the confidentiality of final prices paid by the public payers. The opaque process of setting new budgets extends also for the case when MEAs and the random addition of expenses every time a new product is conditionally approved creates confusion among drug beneficiaries and competing MAHs. Other beneficiaries of the health-care system who require additional funds (except for new drugs) frequently raise concerns.

The legislation for the MEA budget grants access to new drugs on a first-come, first-served basis. Priority criteria are also considered and include the unmet need, the drugs with EU emergency approval, and the public health reasons [15].

The payer does not transparently publish the volume (units) of drugs, including those with MEAs. The only publicly available data on the payer's website is at the ATC3 level and the INN level for the top 10 drugs in terms of value reimbursed in open pharmacies for each category (A, B, C, D, and E) of the reimbursement List [19]. Contract monitoring is challenging because currently there is no way to access treatment outcome data or resource utilization for patients receiving MEA treatments; the payer does not conduct cost-effectiveness analyses or publish data; and manufacturers do not have access to confidential patient outcomes.

The confidentiality stipulated in the MEA prevents not only price comparisons but also the comparisons of the treatment outcomes, making it impossible for any researcher to develop any pharmacoeconomic analysis or to monitor the physician's performance.

There are no transparent rules on the term to publish the updated List (and include the newly evaluated drugs), as the legislation only specifies an update once a year. The practice has shown that shorter terms are needed.

The payer agrees to keep the final prices confidential to avoid the risk of the producer choosing not to sell in Romania than to affect the prices in other countries and decrease the access to new technologies for the population, which would be discriminatory.

The confidentiality of the final price could also be viewed as means to respond to limited resources available in the healthcare sector and is linked with delayed access to innovative technology which could address also the quality of care and patient safety, but even with more resources these aspects will be removed only through a better understanding from the health care professionals [20].

Before 2017, payers and manufacturers would sign collaboration protocols, which included confidential discounts. Protocols aimed to decrease prices in Romania's public healthcare system without affecting the visible drug price or international reference price. Due to unclear legislation, these protocols were abolished and replaced with a new type of protocol where the MAH could support some of the financing burden of the payer [21].

The Payer determines the reimbursement prices (reference price) based on a specific methodology, but its application is not transparent [22]. Anyone can find prices on NHIH's website, and pharmacies will charge co-payments if drug prices are higher than the reimbursement price.

As other authors have already pointed out, the various confidentiality elements of the MAH process prevent other possible cost-containment policies from being used. Meanwhile, in Romania, some of these policies are also used (biosimilars/generic stimulation for prescription, reference pricing, protocols and guidelines for prescriptions, some open tenders for exclusivity) while others are waiting to be implemented (price ceilings, value-based pricing, pay for performance, etc.) [23]

Looking at the future development of MEA in Romania, there are plans to expand from simple cost-volume contracts to other types of MEA, including the payment for performance mechanisms. For this aspect, there will be a need for merging patient-level data with drug-patient data, yet not generally available in Romania at the health system level, but only in some of the hospitals, and the definition and the confidentiality of these patient outcomes will be a new issue during the implementation [24,25].

The future of MEA in Romania will be linked with the new EU Directive on HTA, which will become effective in 2025, and some local projects funded by the MOH in regarding pricing, reimbursement, and HTA. The utilization of a classic HTA model with cost-effectiveness in place could replace the actual scorecard HTA model. This will create the case for the confidentiality of prices used in the cost-effectiveness analysis but will benefit from the public availability of outcomes measures like the local values for Quality Adjusted Life Years (QALY) and will catalyze the efforts to determine the cost of QALY in Romania [26,27,28].

CONCLUSIONS

The negotiation process for the MEA in Romania results in a confidential final price, making it difficult to compare with other EU countries.

Confidential price rises question the legitimacy of some decisions and, in particular, the impossibility of checking over time the cost-effectiveness of drugs in the absence of the knowledge of the cost effectively paid. The solution is finding proper ways to provide more transparency in the process, especially regarding budget allocation. This can be obtained using guidelines as a result of cooperation between all interested actors.

From this perspective, the entire process of delivering drugs to the public domain is a new paradigm that requires new means of increasing the equity of financial allocations within the healthcare system while ensuring profitability for the MAH and accessibility for the patients. The key solution is better collaboration and communication between stakeholders keeping in mind to meet patients' needs and expectations.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, M.S.P. and C.P.R. methodology, M.S.P. and C.P.R., validation, M.S.P. and C.P.R., formal analysis, M.S.P., C.P.R., and B.C.P. resources, M.S.P., C.P.R., B.C.P., data curation, M.S.P., writing—original draft preparation, M.S.P., writing—review and editing, C.P.R., and B.C.P. visualization, B.C.P.; supervision, M.S.P. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable

Patient consent for publication

Not applicable.

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ORIGINAL ARTICLE

Sentinel Node Biopsy Positivity: Is Axillary Dissection Always Necessary? – A Retrospective Analysis

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Abstract: *Breast cancer remains a significant health concern, with predictions indicating a rise in global incidence. While the primary focus is on oncological radicality, the aesthetic and psychological impacts of surgical interventions, such as radical mastectomy, cannot be ignored. This study, conducted over 8 years, aimed to identify factors that can prevent unnecessary lymphadenectomy. The research analyzed various parameters, including age, BMI, tumor size, and immunohistochemistry, to determine their correlation with axillary lymph node invasion. The results highlighted the importance of tumor size and estrogen receptor status in predicting axillary lymph node metastasis. The study underscores the value of sentinel lymph node biopsy in early breast cancer treatment, emphasizing its benefits over complete axillary lymph node dissection.*

Keywords: *breast cancer, lymphadenectomy, sentinel node biopsy*

INTRODUCTION

According to the American Cancer Society, one out of eight women in the United States will develop breast cancer during their lifetime [1]. Predictions regarding the global incidence of breast cancer in women outline the idea that the incidence will reach approximately 3.2 million new cases per year by 2050.

These numbers reflect the magnitude of breast cancer incidence, its impact on women worldwide, and the ongoing and urgent need to take measures towards prevention and proper therapeutic management [2].

The goal of oncological radicality remains essential and obligatory, but breast neoplasm surgery must also consider the aesthetic component due to the psychological impact that a major surgical intervention, such as radical mastectomy, has on patients. In the early stages, breast cancer can be treated without such a procedure being necessary.

Therefore, in today's times, the gold standard of breast cancer surgery is represented by conservative treatment. It involves the resection of the primary tumor with oncological safe margins and sentinel lymph node biopsy, followed by adjuvant radiotherapy [2,3].

The surgical management of the axilla has always been a controversial topic. For a very long time, it was believed that achieving good loco-regional control involves a complete axillary lymph node dissection, with this method being the gold standard of that era.

In recent years, due to the observed increase in morbidity and decrease in the quality of life for these patients, the method has been abandoned and replaced by sentinel lymph node biopsy.

The technique of identifying the sentinel lymph node (SLN) is based on the theory of the lymphatic spread of cancers, with the neoplastic invasion occurring in a lymph node that drains lymph from the site of the tumor [4,5] .

The prime objective of this study is the identification of factors that can prevent unnecessary lymphadenectomy.

MATERIALS AND METHODS

Based on the purpose and objectives of the research, the research design and methodology have been defined, with its main characteristics being: retrospective - spanning over an 8-year interval - January 1, 2014 - December 31, 2021, single-center, descriptive and correlational, non-interventional.

The eligibility criteria were as follows:
- Diagnosis of breast cancer
- The presence of axillary management after a positive sentinel node biopsy
- Female sex
- Stage IIA-IIIB:
    - o IIA (T2N0M0, T1N1M0)
    - o IIB (T2N1M0, T3N0M0)
    - o IIIA (T3N1M0)
    - o IIIB (T4N1M0)
- Single tumor, excluding multifocal and bilateral cases
- The presence of all required data
- Age > 18 years
- M0
- Any Histopathological type

RESULTS

The working hypothesis considers patients who underwent the sentinel lymph node technique, and the result was positive. In this situation, studies have shown that up to 1/3 of cases present a negative result of the axillary lymphadenectomy. To assess the risk factors for a positive axillar biopsy, we made a comparison between group E – a positive result of axillary lymphadenectomy (34 cases), and group D – a negative result of axillary lymphadenectomy (15 cases) (Table 1).

Table 1: Comparative data of groups (E vs. D)

Study lot	D	E
SNB	✓	✓
SNB results	+	+
ALND	✓	✓
ALND results	-	+
Case numbers	15	34

Age

There was no statistical correlation between the age of the patients and the presence of axillary invasion in patients with a positive sentinel lymph node, even though there was a mean difference of 5.85 years - p=0.175 (Table 2 and Figure 1).

Table 2: Comparative statistical data regarding age (E vs. D)

	Age	
	E	D
Valid	34	15
Missing	0	0
Mean	63.324	57.467
Std. Deviation	14.367	12.118
Minimum	28.000	29.000
Maximum	89.000	78.000

	t	df	p	Mean difference	SE difference
Age	1.376	47	0.175	5.857	4.258

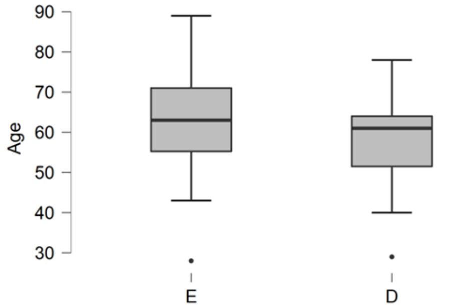

Figure 1: Comparative statistical data regarding age (E vs. D)

BMI

The Body Mass Index (BMI) value calculated for the two groups showed a higher average in the case of patients with axillary invasion, with a difference of 3.58 and moderate statistical significance - p=0.05 (Table 3 and Figure 2).

Table 3: Comparative statistical data regarding BMI (E vs. D)

	BMI	
	E	D
Valid	34	15
Missing	0	0
Mean	28.696	25.107
Median	28.100	24.900
Std. Deviation	6.187	4.608
Minimum	18.970	19.760
Maximum	45.650	33.650

	t	df	p	Mean difference	SE difference
BMI	2.009	47	0.050	3.589	1.786

Bio-clinical analysis

Comparative assessment of routine laboratory test values collected upon admission highlights relatively small differences between the groups discussed in this hypothesis.

The only parameter that demonstrated moderate statistical significance was the white blood cell count - with a mean difference of 1.44 and p=0.065 (Table 4 and Figure 3). Tumor markers did not show statistically significant differences.

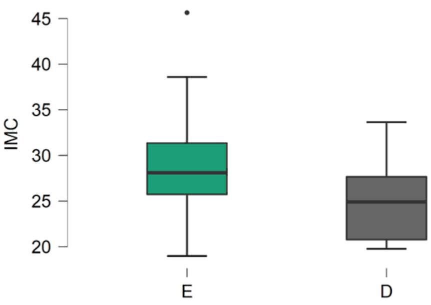

Figure 2: Comparative statistical data regarding BMI (E vs. D)

Table 4: Comparative statistical data regarding bio-clinical analysis (E vs. D)

	Hb		Ht		WBC		ESR		INR		Fibrinogen	
	E	D	E	D	E	D	E	D	E	D	E	D
Valid	34	15	34	15	34	15	34	15	34	15	34	15
Mean	12.515	12.647	40.529	40.067	8.726	7.280	31.971	29.800	1.189	1.130	434.029	378.600
Std. Deviation	1.259	1.148	3.518	4.559	2.550	2.253	18.909	16.618	0.313	0.215	186.974	174.482
Minimum	8.50	9.90	35.00	31.00	5.00	3.90	8.00	3.00	0.830	0.840	201.00	205.00
Maximum	15.30	13.80	48.00	47.00	14.70	10.90	101.00	57.00	1.940	1.60	835.00	823.00

Quadrant

The location of the tumor did not correlate with the extent of the axillary invasion, even though there were differences in the distribution of cases concerning this parameter, the statistical significance was not relevant (Tables 5 and 6).

Table 4: Comparative statistical data regarding bio-clinical analysis (E vs. D) – cont.

	t	df	p	Mean difference	SE difference
Hb	-0.347	47	0.730	-0.132	0.380
Ht	0.387	47	0.701	0.463	1.196
WBC	1.893	47	0.065	1.446	0.764
ESR	0.384	47	0.703	2.171	5.659
INR	0.657	47	0.514	0.059	0.089
Fibrinogen	0.975	47	0.334	55.429	56.830

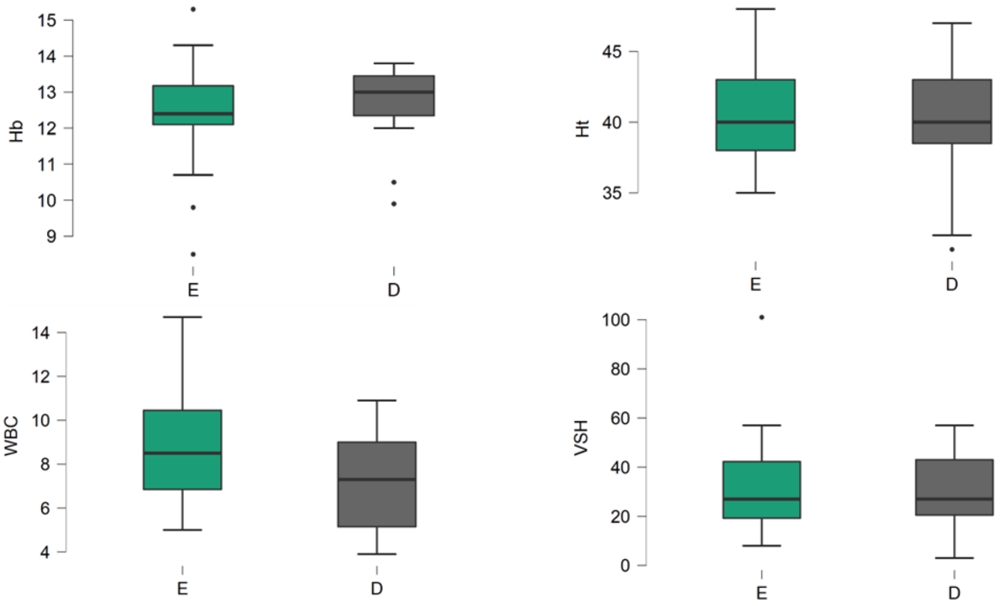

Figure 3: Comparative statistical data regarding bio-clinical analysis (E vs. D)

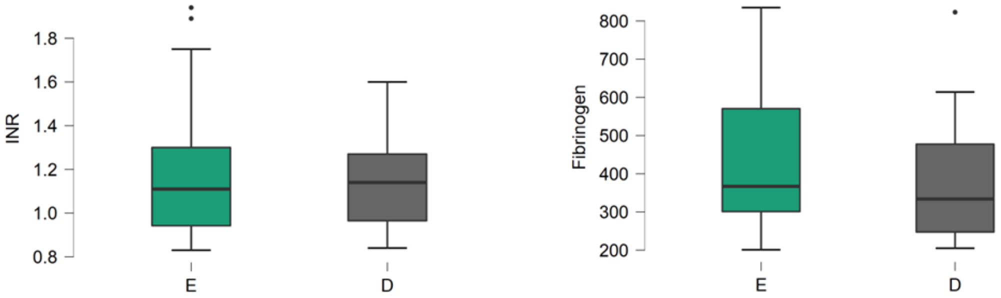

Figure 3: Comparative statistical data regarding bio-clinical analysis (E vs. D) – cont.

Table 5: Comparative statistical data regarding the tumor location (E vs. D)

Frequencies for Quadrant					
Hypothesis 2	**Quadrant**	**Frequency**	**Percent**	**Valid Percent**	**Cumulative Percent**
E	C	3	8.824	8.824	8.824
	SE	20	58.824	58.824	67.647
	SI	3	8.824	8.824	76.471
	IE	7	20.588	20.588	97.059
	II	1	2.941	2.941	100.000
	Missing	0	0.000		
	Total	34	100.000		
D	C	2	13.333	13.333	13.333
	SE	12	80.000	80.000	93.333
	SI	0	0.000	0.000	93.333
	IE	1	6.667	6.667	100.000
	II	0	0.000	0.000	100.000
	Missing	0	0.000		
	Total	15	100.000		

Chi-Squared Tests			
	Value	**df**	**p**
X^2	3.922	4	0.417
N	49		

Tumor size

The distribution of tumor sizes highlights higher values within group E - with axillary lymph node invasion, with a mean difference of 8.7 mm and a statistical significance of p=0.04 (Table 7 and Figures 4 and 5).

Immunohistochemistry

From the perspective of evaluating the presence of hormone receptors in the immunohistochemical assessment of breast resection specimens, only estrogen receptors have been identified as a risk factor for axillary invasion beyond the sentinel lymph node. The calculated risk is 7.33 times with a statistical significance of p=0.005 (Tables 8 - 10).

Subtype

From the perspective of molecular subtypes, the division into Luminal A, Luminal B, and Triple Negative did not show a statistically significant correlation with the presence of axillary lymph node invasion in patients with a positive sentinel lymph node. In the case of the HER2 sub-type, the risk of axillary lymph node invasion was nearly 10 times higher - p=0.084 (Tables 11-14).

Table 6: Distribution of cases concerning the localization in the interior of the exterior quadrant of the tumor (E vs D)

Ext 1 vs Int 0 quadrant		Hypothesis 2		
		0	**1**	**Total**
0	Count	7.000	2.000	9.000
	% within row	77.778 %	22.222 %	100.000 %
	% within column	20.588 %	13.333 %	18.367 %
1	Count	27.000	13.000	40.000
	% within row	67.500 %	32.500 %	100.000 %
	% within column	79.412 %	86.667 %	81.633 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

Table 6: Distribution of cases concerning the localization in the interior of the exterior quadrant of the tumor (E vs D) – cont.

	Log Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	0.522	-1.183	2.227	
Fisher's exact test	0.512	-1.331	2.926	0.702

Table 7: Comparative statistical data regarding the tumor size (E vs. D)

	Size	
	E	D
Valid	34	15
Mean	21.235	12.533
Std. Deviation	14.078	11.154
Minimum	4.000	1.000
Maximum	58.000	47.000

	t	df	p	Mean Difference	SE Difference
Size	2.115	47	0.040	8.702	4.115

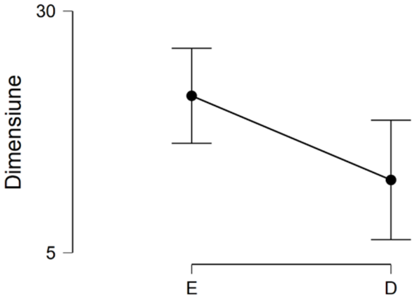

Figure 4: Comparative statistical data regarding tumor size (E vs. D)

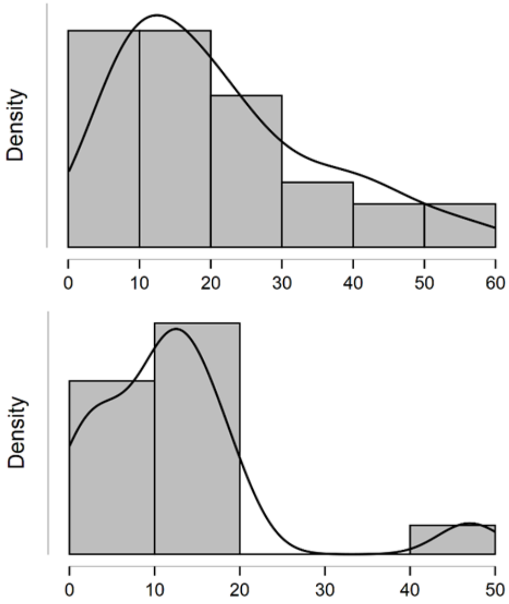

Figure 5: Distribution of cases concerning the tumor size (E left vs. D right)

Table 8: Distribution of cases concerning the presence of ER receptors (E vs D)

ER+		Hypothesis 2		Total
		0	1	
0	Count	22.000	3.000	25.000
	% within row	88.000 %	12.000 %	100.000 %
	% within column	64.706 %	20.000 %	51.020 %
1	Count	12.000	12.000	24.000
	% within row	50.000 %	50.000 %	100.000 %
	% within column	35.294 %	80.000 %	48.980 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	7.333	1.724	31.186	
Fisher's exact test	7.019	1.498	46.390	0.005

Table 9: Distribution of cases with the presence of PR receptors (E vs D)

PR+		Hypothesis 2		Total
		0	1	
0	% within row	72.000 %	28.000 %	100.000 %
	% within column	52.941 %	46.667 %	51.020 %
1	Count	16.000	8.000	24.000
	% within row	66.667 %	33.333 %	100.000 %
	% within column	47.059 %	53.333 %	48.980 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	1.286	0.381	4.344	
Fisher's exact test	1.279	0.321	5.206	0.762

Table 10: Distribution of cases with the presence of PR receptors (E vs D)

HER2+		Hypothesis 2		Total
		0	1	
0	Count	22.000	10.000	32.000
	% within row	68.750 %	31.250 %	100.000 %
	% within column	64.706 %	66.667 %	65.306 %
1	Count	12.000	5.000	17.000
	% within row	70.588 %	29.412 %	100.000 %
	% within column	35.294 %	33.333 %	34.694 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	0.917	0.254	3.308	
Fisher's exact test	0.918	0.198	3.851	1.000

Table 11: The distribution of cases concerning the Luminal A subtype (E vs D)

Luminal A		Hypothesis 2		Total
		0	1	
0	Count	21.000	7.000	28.000
	% within row	75.000 %	25.000 %	100.000 %
	% within column	61.765 %	46.667 %	57.143 %
1	Count	13.000	8.000	21.000
	% within row	61.905 %	38.095 %	100.000 %
	% within column	38.235 %	53.333 %	42.857 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	1.846	0.541	6.302	
Fisher's exact test	1.823	0.455	7.538	0.363

DISCUSSION

The literature describes four parameters as independent factors of lymph node metastasis, namely lymphovascular invasion, primary tumor size, multiple foci, and tumor localization.

An independent prognostic factor for disease-free survival (DFS) that has been recently analyzed in literature for patients with breast carcinoma is the degree of lymphovascular invasion [6].

Additionally, lymphovascular invasion is associated with a higher frequency of axillary lymph node metastases [7]. To use this data as a tool in determining the indication for sentinel lymph node biopsy (SLNB) or axillary lymphadenectomy, more extensive studies are needed to specifically determine the degree of invasion and the cutoff value that necessitates radical intervention.

Table 12: The distribution of cases concerning the Luminal B subtype (E vs D)

Luminal B		Hypothesis 2		Total
		0	1	
0	Count	26.000	10.000	36.000
	% within row	72.222 %	27.778 %	100.000 %
	% within column	76.471 %	66.667 %	73.469 %
1	Count	8.000	5.000	13.000
	% within row	61.538 %	38.462 %	100.000 %
	% within column	23.529 %	33.333 %	26.531 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	1.625	0.428	6.171	
Fisher's exact test	1.608	0.331	7.329	0.500

Table 13: The distribution of cases concerning the Luminal B subtype (E vs D)

Her2		Hypothesis 2		Total
		0	1	
0	Count	27.000	15.000	42.000
	% within row	64.286 %	35.714 %	100.000 %
	% within column	79.412 %	100.000 %	85.714 %
1	Count	7.000	0.000	7.000
	% within row	100.000 %	0.000 %	100.000 %
	% within column	20.588 %	0.000 %	14.286 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	0.118	0.006	2.214	
Fisher's exact test	0.000	0.000	1.466	0.084

A series of studies have demonstrated that younger age is a risk factor for positive axillary lymph nodes (ALN) [8–11]. This has been attributed to more biologically aggressive tumors in this younger age group. The average age of menopause is around 50 years, so most literature establishes 50 years as a cutoff point in exploring the relationship between age and clinicopathological factors in invasive breast cancer [8,12]. The current study did not show correlations between the risk of axillary lymph node spread and age, BMI, and the biological status of breast cancer patients, with results similar to those described in the studies.

Table 14: The distribution of cases concerning the Triple-negative subtype (E vs D)

Triple negative		Hypothesis 2		Total
		0	1	
0	Count	28.000	13.000	41.000
	% within row	68.293 %	31.707 %	100.000 %
	% within column	82.353 %	86.667 %	83.673 %
1	Count	6.000	2.000	8.000
	% within row	75.000 %	25.000 %	100.000 %
	% within column	17.647 %	13.333 %	16.327 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	0.718	0.127	4.051	
Fisher's exact test	0.723	0.063	4.803	1.000

Histologic grade and tumor size (often over 5 centimeters in size) are also important predictors of lymph node metastasis. The probability is high if a patient has a large tumor and a high histologic grade. Our presented data show that patients with larger tumor sizes and a high histologic grade are more likely to have axillary invasion, a finding that aligns with those from previous studies [1,6,13–15]. Patients with small invasive breast cancer might benefit from SLNB.

The inclusion of extranodal extension in the evaluation protocol is necessary to achieve precise oncologic staging. Although attempts have been made to establish cut-off values for the circumferential and perpendicular diameters of invasion extension, these have not yielded significant predictive values. Therefore, more extensive studies are needed to specifically determine the dimensions of invasion from which axillary lymph node metastatic burden can be predicted, subsequently guiding the appropriate therapeutic approach [16,17].

Estrogen receptor status was another factor that showed a significant correlation with axillary lymph node involvement, with a calculated risk of axillary invasion beyond the sentinel lymph node being 7.33 times higher in HER+ patients. Various studies have evaluated the role of hormonal receptor status in predicting axillary lymph node metastasis (ALNM) with contradictory results. Capdet et al. [18,19], in their study of 1416 patients with early breast cancer, found no association between hormonal status and lymph node involvement. In contrast, Lee et al. [20] reported a high incidence of ALNM regardless of tumor size in the Korean population with T1-2 breast carcinoma. Finally, in a recent study conducted by Holm-Rasmussen et al. [21], patients with ER-negative tumors had a reduced risk of ALN involvement at the time of diagnosis compared to other patients.

Large integrative analyses are necessary to explain why ER-negative tumors have a low rate of axillary metastases and a high rate of distant metastases. Conversely, HER2-positive tumors have an axillary metastasis rate closely linked to tumor size and a high rate of distant metastases [22,23].

Among the limitations of the study, the retrospective nature of its design, the small number of tracked patients, and the lack of information regarding the use of chemotherapy, radiotherapy, or hormone therapy are identified.

Systemic therapies, including hormonal therapy, cytotoxic chemotherapy, and/or radiation therapy, remain critically important, especially in the treatment of breast cancer with positive lymph nodes [24–28]. Therefore, the results of this study will contribute to decision-making regarding the addition of axillary lymph node dissection (ALND), systemic therapy, and/or radiotherapy in the case of SLN metastases efforts to determine the cost of QALY in Romania [26,27,28].

CONCLUSIONS

Regional lymph node staging is necessary for tumor staging and surgical planning. Previous studies have identified several important factors in predicting axillary lymph node metastasis (ALNM), but the predictive power has not been satisfactory.

With advancements in surgical techniques, sentinel lymph node biopsy (SLNB) is now widely used in early breast cancer and has become a standard procedure in clinically node-negative patients. The benefits of SLNB include fewer surgical complications than complete axillary lymph node dissection (ALND) and effectiveness and accuracy in long-term follow-up.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, R.A-M. and D.N.P.; methodology, O.A.; software, S.N.; validation, A.B., F.S. and M.C.D.; formal analysis, D.I.; investigation, R.A-M.; resources, D.N.P.; data curation, O.A.; writing—original draft preparation, S.N.; writing—review and editing, A.B.; visualization, F.S.; supervision, M.C.D.; project administration, D.I.; funding acquisition, R.A-M. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted following the Declaration of Helsinki, and approved by the Institutional Review of University Emergency Hospital of Bucharest.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Hepatic Steatosis is the Most Common Finding in Abdominal Ultrasonography Performed in Patients with Psoriasis before Biological Agent Treatment

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Abstract: *Psoriasis has been associated with metabolic syndrome and nonalcoholic fatty liver disease. We wanted to investigate the abdominal ultrasonography results of psoriasis patients before biological agent treatment. Between April 2019 and February 2022, abdominal ultrasonography results of psoriasis patients which were performed before biological agent treatment were reviewed retrospectively. This study included 90 patients, 45 females, and 45 males with a mean age of 48.91±13.43 years. The most common ultrasonography finding was increased echogenicity of the liver parenchyma due to steatosis (58.9%) which was followed by an increase in liver size (25.6%), kidney cysts (17.8%), gallstones (8.9%) and increase in spleen size (7.8%). Hepatic steatosis was more frequent in older patients ($p<0.001$), patients with diabetes ($p=0.007$), and patients who were previously treated with biological agents ($p=0.007$). 70.4% of the patients with moderate or severe hepatic steatosis were female. Herein, hepatic steatosis was detected in 53 (58.9%) patients. Nonalcoholic fatty liver disease which presents as hepatic steatosis in early stages has an asymptomatic course and poor prognosis in psoriasis patients. We suggest that abdominal ultrasonography should be performed in psoriasis patients before biological agent treatment, especially in females, patients with advanced age, patients who were previously treated with biological agents, and patients with diabetes.*

Keywords: *abdomen; biological agents; hepatic steatosis; psoriasis; ultrasonography*

INTRODUCTION

Psoriasis is a systemic inflammatory disorder that primarily affects the skin and may present with various comorbidities such as psoriatic arthritis, nonalcoholic fatty liver disease, diabetes, metabolic syndrome, and inflammatory bowel disease [1]. Tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17), and IL-23 are the cytokines mainly responsible for the inflammation in psoriasis [2]. Chronic inflammation has been implicated in the development of accompanying comorbidities. On the other hand, non-alcoholic fatty liver disease which is an important comorbidity of psoriasis due to its risk of progressing to cirrhosis has been associated with the development of severe psoriasis since it may lead to inflammatory cytokine production from the liver [3]. Biological agents affect specific pathways of the immune system that are implicated in the etiopathogenesis of psoriasis. Several biological agents have been developed and successfully used for the treatment of psoriasis. It has been suggested that biological agents were more effective and safer than conventional systemic treatments of psoriasis which lead to a broad immunosuppressive activity [4]. However, some safety concerns have been raised due to the widespread and long-term use of biological agents. Hepatotoxicity, reactivation of hepatitis B and latent tuberculosis infection, exacerbation of inflammatory bowel disease, and invasive fungal infections have been reported in psoriasis patients treated with biological agents. Side effects vary on each biological agent received [5]. For instance, administration of TNF-α inhibitors for long periods may lead to weight gain which has a negative impact on nonalcoholic fatty liver disease in patients with psoriasis [6].

Furthermore, it is controversial whether biological agent treatment increases the risk of malignancy in patients with psoriasis or not. Fiorentino et al. reported that the risk of malignancy might be increased in patients with psoriasis treated with TNF-α inhibitors for longer than a year [7]. On the other hand, Mastorino et al. stated that the use of biological agents affecting TNF-α, IL-17, IL-12, and IL-23 for the treatment of psoriasis was safe even in patients with a past medical history of malignancy [8]. It has been suggested that abdominal ultrasonography might be performed in patients with psoriasis before the initiation of treatment with biological agents to exclude intraabdominal malignancy such as lymphoma and it might be repeated during the treatment [9]. However, abdominal ultrasonography is not included as a pretreatment test to be performed in psoriasis patients before the initiation of biological agents in major guidelines such as Joint American Academy of Dermatology – National Psoriasis Foundation (AAD-NPF) guidelines of care for the management and treatment of psoriasis with biologics and EuroGuiDerm guideline on the systemic treatment of psoriasis vulgaris [10, 11].

Within this study, we aimed to evaluate abdominal ultrasonography results of patients with psoriasis which were performed before the initiation of biological agent treatment**.**

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained before this study (approval number: 2022-264). Between April 2019 and February 2022, medical records of patients with psoriasis who underwent abdominal ultrasonography before biological agent treatment were evaluated retrospectively. Psoriasis type, disease duration, psoriasis treatment, accompanying psoriatic arthritis, and systemic diseases were reviewed. All patients with psoriasis aged 18 years and over with complete data have been included in the study.

Statistical analysis was performed using SPSS version 20.0. Quantitative variables were represented as mean ± standard deviation or median and interquartile range, categorical variables were represented as counts and percentages. Kolmogorov-Smirnov test was used to determine whether variables were normally distributed or not. Differences in variables between the two groups were evaluated by chi-square test, independent sample t-test, and Mann-Whitney U test. P values less than 0.05 were considered statistically significant.

RESULTS

This study included 90 patients, 45 females and 45 males. The mean age of the patients was 48.91±13.43 years (range: 19-75 years). 79 (87.8%) patients had psoriasis vulgaris, 8 (8.9%) patients had generalized pustular psoriasis, and 3 (3.3%) patients had palmoplantar psoriasis. The mean disease duration was 19.35±11.93 years (range: 2-53 years). The median Psoriasis Area and Severity Index (PASI) was 7.2 (11.4) [12]. Psoriatic arthritis was detected in 34 (37.8%) patients. 34 (37.8%) patients were treated with ustekinumab, 29 (32.2%) patients were treated with IL-17 inhibitors such as secukinumab and ixekizumab, 23 (25.6%) patients were treated with TNF-α inhibitors such as adalimumab, infliximab and certolizumab pegol, 4 (4.4%) patients were treated with IL-23 inhibitors such as risankizumab and guselkumab. All patients previously received conventional treatments such as acitretin, cyclosporine, and methotrexate. 15 (16.7%) patients were previously treated with phototherapy. 37 (41.1%) patients were previously treated with biological agents whereas 53 (58.9%) patients were biologic-naive. None of the patients stated excessive alcohol consumption. Past medical history was unremarkable in 48 (53.3%) patients. However, 23 (25.6%) patients had hypertension, 17 (18.9%) patients had diabetes, 4 (4.4%) patients had chronic obstructive pulmonary disease, 4 (4.4%) patients had asthma, 4 (4.4%) patients had hypothyroidism, 4 (4.4%) patients had chronic hepatitis B virus infection, 3 (3.3%) patients had chronic kidney disease, 3 (3.3%) patients had coronary artery disease, 1 (1.1%) patient had nephrolithiasis, 1 (1.1%) patient had villous adenoma of the colon. In addition, 1 (1.1%) patient had a history of testicular seminoma, 1 (1.1%) patient had cervical cancer, and 1 (1.1%) patient had urothelial carcinoma.

Abdominal ultrasonography revealed an increase in liver size in 23 (25.6%) patients (the mean craniocaudal length in the midclavicular line: 16.69±0.96 mm, range: 15.5-20 mm). Among the patients with increased liver size, 17 (73.9%) were female and 6 (26.1%) were male (p=0.013). Increased liver size was observed in 14 (60.9%) patients with psoriatic arthritis and in 9 (39.1%) patients without arthritis (p=0.008) (Table 1). Increased liver size was more common in patients with hypertension (10, 43.5%) compared to patients without hypertension (13, 19.4%) (p=0.023). Increased liver size was observed in 6 (35.3%) patients with diabetes and 17 (23.3%) patients without diabetes (p=0.309).

The mean psoriasis duration was 22.15±12.47 years in patients with increased liver size and 18.39±11.68 years in patients with normal liver size (p=0.185). Among the patients with increased liver size, the mean craniocaudal length was 17.03±1.10 mm in those who were biologic-naive and 16.25±0.48 mm in those who received biological agents previously (p=0.037). All patients with increased liver size showed increased echogenicity of the liver parenchyma due to steatosis. An increase in echogenicity of the liver parenchyma due to steatosis was reported in 53 (58.9%) patients [mild: 26 (28.9%), moderate: 14 (15.6%), severe: 13 (14.4%)]. The mean age of the patients with and without increased echogenicity of the liver parenchyma due to steatosis was 53.62±11.71 years and 42.16±12.99 years, respectively (p<0.001).

Table 1: Patients with increased liver size and increased echogenicity of the liver parenchyma

	Increased liver size		**P value**
Patient characteristics (n, %)	Female (17, 73.9)	Male (6, 26.1)	0.013
	With psoriatic arthritis (14, 60.9)	Without psoriatic arthritis (9, 39.1)	0.008
	With hypertension (10, 43.5)	Without hypertension (13, 19.4)	0.023
Craniocaudal length (mm, mean±SD)	Biologic-naïve (17.03±1.10)	Previous biologic (16.25±0.48)	0.037
	Increased echogenicity of the liver parenchyma due to steatosis		
Patient characteristics (n, %)	Previous biologic (28, 75.7)	Biologic-naïve (25, 47.2)	0.007
	With diabetes (15, 88.2)	Without diabetes (38, 52.1)	0.007
Moderate/severe (n, %)	Female (19, 70.4)	Male (8, 29.6)	0.021

SD: Standard deviation. Increased liver size was more common in patients with psoriatic arthritis and hypertension. Moderate or severe increase in the echogenicity of the liver parenchyma due to steatosis was more common in female patients.

Among the patients with increased echogenicity of the liver parenchyma due to steatosis, 28 (75.7%) received biological agents previously and 25 (47.2%) were biologic-naive (p=0.007). Increased echogenicity of the liver parenchyma due to steatosis was detected in 15 (88.2%) patients with diabetes and in 38 (52.1%) patients without diabetes (p=0.007). Among the patients with a moderate or severe increase in the echogenicity of the liver parenchyma due to steatosis, 19 (70.4%) were female and 8 (29.6%) were male (p=0.021). In addition, heterogeneous liver parenchyma was reported in 5 (5.6%) patients, hypoechoic areas in the liver which might be compatible with focal fatty sparing in 4 (4.4%) patients, liver cyst in 3 (3.3%) patients, hyperechoic liver lesions which might be compatible with hemangioma in 3 (3.3%) patients and Chilaiditi syndrome was reported in 1 (1.1%) patient.

Moreover, gallstones were detected in 8 (8.9%) patients (the mean diameter: 15±7.05 mm, range: 3-20 mm) and gallbladder polyps in 3 (3.3%) patients (the mean diameter: 4.5±0.5 mm, range: 4-5 mm). Among the patients with gallstones and gallbladder polyps, 7 (63.6%) were female and 4 (36.4%) were male (p=0.141). The mean age was 53.73±13.32 years in patients with gallstones and gallbladder polyps and 48.24±13.40 years in those who had neither (p=0.094). Gallbladder wall thickening (4 mm) and echogenicities in the gallbladder wall (cholesterolosis) were detected in 1 (1.1%) patient. An increase in the transverse diameter of the gallbladder (42 mm) and accompanying gallbladder wall thickening (6 mm) were detected in 1 (1.1%) patient. Furthermore, kidney cysts were detected in 16 (17.8%) patients (the mean diameter: 26.5±15.8 mm, range: 8-50 mm). Increased echogenicity of kidney parenchyma was detected in 5 (5.5%) patients. The mean ages of the patients with and without kidney cysts were 56.81±7.54 years and 47.20±13.85 years, respectively (p=0.008). Kidney stones were detected in 4 (4.4%) patients (the mean diameter: 7.5±5.8 mm, range: 3-16 mm). Lobulated kidney contour was detected in 4 (4.4%) patients. Dilated renal collecting system was detected in 3 (3.3%) patients, focal caliectasis was detected in 1 (1.1%) patient, increase in kidney size was detected in 1 (1.1%) patient, decrease in kidney size was detected in 1 (1.1%) patient and a renal angiomyolipoma was detected in 1 (1.1%) patient. Abdominal ultrasonography also revealed increase in spleen size in 7 (7.8%) patients (the mean craniocaudal length: 13.87±1.05 mm, range: 13-16 mm), accessory spleen in 2 (2.2%) patients, splenic cyst with a diameter of 7 mm in 1 (1.1%) patient, situs inversus totalis in 1 (1.1%) patient, calcifications in prostatic parenchyma in 1 (1.1%) patient, heterogeneous prostatic parenchyma in 1 (1.1%) patient, an ovarian cyst measured 33x27 mm in size in 1 (1.1%) patient, uterine myoma with a diameter of 11 mm in 1 (1.1%) patient, increased bladder wall thickness (6 mm) in 1 (1.1%) patient, echogenic debris within the bladder which might be compatible with urinary tract infection in 1 (1.1%) patient (Table 2).

Based on ultrasonography findings, abdominal computed tomography (CT) was performed in 3 (3.3%) patients for further evaluation of kidney cysts and abdominal magnetic resonance imaging (MRI) in 2 (2.2%) patients for hypoechoic liver lesions and liver cysts. Renal scintigraphy was performed in 1 (1.1%) patient for the dilation of the renal collecting system.

DISCUSSION

Within this study, the most common abdominal ultrasonography finding in patients with psoriasis was increased echogenicity of the liver parenchyma due to steatosis (58.9%). Hepatic steatosis was more frequent in older patients, patients with diabetes, and patients who were previously treated with biological agents (p<0.001, p=0.007, p=0.007, respectively). These results may be related to the fact that patients previously treated with biologic agents were older than biologic-naive ones (p=0.021). In addition, increased echogenicity of the liver parenchyma due to steatosis was moderate or severe in 50.9% of the patients and most of these patients (70.4%) were female. An increase in liver size (25.6%) was the other common abdominal ultrasonography finding followed by kidney cysts (17.8%), gallstones (8.9%), and an increase in spleen size (7.8%). The increased liver size was statistically significantly more prevalent in female patients, and patients with psoriatic arthritis and hypertension (p=0.013, p=0.008, p=0.023, respectively).

Table 2: Abdominal ultrasonography findings in patients with psoriasis before biological agent treatment

	Patients n (%)	Mean size±SD (mm)	Range (mm)
Increased liver parenchyma echogenicity	53 (58.9)		
Increased liver size	23 (25.6)	16.69±0.96	15.5-20
Kidney cysts	16 (17.8)	26.5±15.8	8-50
Gallstones	8 (8.9)	15±7.05	3-20
Increased spleen size	7 (7.8)	13.87±1.05	13-16
Increased kidney parenchyma echogenicity	5 (5.6)		
Heterogeneous liver parenchyma	5 (5.6)		
Kidney stones	4 (4.4)	7.5±5.8	3-16
Lobulated kidney contour	4 (4.4)		
Hypoechoic areas in the liver	4 (4.4)		
Liver cyst	3 (3.3)		
Liver hemangioma	3 (3.3)		
Gallbladder polyps	3 (3.3)	4.5±0.5	4-5
Dilated renal collecting system	3 (3.3)		
Accessory spleen	2 (2.2)		
Gallbladder wall thickening	2 (2.2)		
Echogenicities in the gallbladder wall	1 (1.1)		
Focal caliectasis	1 (1.1)		
Echogenic debris within the bladder	1 (1.1)		
Increased kidney size	1 (1.1)		
Decreased kidney size	1 (1.1)		
Renal angiomyolipoma	1 (1.1)		
Increased bladder wall thickness	1 (1.1)		
Splenic cyst	1 (1.1)		
Uterine myoma	1 (1.1)		
Ovarian cyst	1 (1.1)		
Heterogeneous prostatic parenchyma	1 (1.1)		
Calcifications in the prostatic parenchyma	1 (1.1)		
Chilaiditi syndrome	1 (1.1)		
Situs inversus totalis	1 (1.1)		

SD: Standard deviation. An increase in echogenicity of the liver parenchyma due to steatosis was the most common abdominal ultrasonography finding in patients with psoriasis.

Data on abdominal ultrasonography findings in patients with psoriasis treated with biological agents are scarce. Raudonis et al. reported that hepatic and pancreatic steatosis, hepatomegaly, kidney cyst, splenomegaly, gallbladder polyp, and pancreatic stiffness were commonly detected findings in abdominal ultrasonography which were annually performed in patients with psoriasis treated with infliximab, etanercept, and ustekinumab. Among them, hepatic steatosis (12.7%) and pancreatic steatosis (12.7%) were the most common [13].

Nonalcoholic fatty liver disease is a metabolic disorder that may present with steatosis, and nonalcoholic steatohepatitis and may result in cirrhosis or hepatocellular carcinoma [14-16]. Advanced age and diabetes are the risk factors for the development of nonalcoholic fatty liver disease, whereas the effect of gender on disease remains controversial [17]. In addition, nonalcoholic fatty liver disease is more common in psoriasis patients with a prevalence of up to 65% compared to the general population [15]. Furthermore, poor prognosis has been reported in patients with nonalcoholic fatty liver disease accompanied by psoriasis [14,15]. Dermatologists are recommended to perform abdominal ultrasonography for screening fatty liver in patients with psoriasis, particularly in those who have metabolic syndrome even without elevated liver enzyme levels [6]. Dermatologists must determine accompanying nonalcoholic fatty liver disease in patients with psoriasis to choose appropriate treatment options. Dermatologists should also encourage patients for lifestyle changes or refer them to a gastroenterologist if needed [16]. For instance, Campanati et al. suggested that weight gain during anti-TNF-α treatment might increase the risk of hepatic fibrosis in psoriasis patients with nonalcoholic fatty liver disease [18]. Medications that may contribute to steatosis should be omitted in these patients. However, no specific treatment has been reported or primarily recommended for the treatment of psoriasis patients with nonalcoholic fatty liver disease [16]. On the other hand, TNF-α inhibitors such as adalimumab, etanercept, and infliximab may lead to hepatotoxicity. Among them, infliximab was the most commonly implicated one in the development of adverse effects on the liver such as jaundice, hepatitis, acute liver failure, cholestasis, and autoimmune hepatitis [19]. Even mild and rare, hepatotoxicity has been detected in patients who received ustekinumab [20].

Abdominal ultrasonography may be useful in patients with psoriasis since psoriasis has been associated with nonalcoholic fatty liver disease or intraabdominal malignancies such as hepatocellular carcinoma, gastric cancer, bladder cancer, lymphoma of the stomach and colon adenocarcinoma [21,22]. Intraabdominal malignancy and infections have also been reported in patients who received biological agents for the treatment of psoriasis. For instance, Nosotti et al. reported a 59-year-old male patient with psoriasis treated with adalimumab and diagnosed with B cell lymphoma with gastric, splenic, hepatic, abdominal lymph node, and vertebral involvement. Nosotti et al. suggested that the risk for the development of abdominal lymphoma should be kept in mind in patients with psoriasis under biological agent treatment. Nosotti et al. recommended performing abdominal ultrasonography before and during biological agent treatment for psoriasis [9]. Fortaleza et al. reported a 43-year-old male patient with psoriasis who developed splenic tuberculosis under infliximab treatment. Fortaleza et al. stated that close follow-up of patients treated with TNF-α inhibitors on the risk for extrapulmonary tuberculosis and disseminated tuberculosis was crucial [23]. Bud et al. reported peritoneal tuberculosis in a 32-year-old patient who received adalimumab for the treatment of psoriasis vulgaris. The patient had multiple splenic lesions on computed tomography of the abdomen and underwent surgery due to an initial diagnosis of a possible malignancy. Bud et al. suggested that Quantiferon test, abdominal ultrasonography, and computed tomography might be performed to exclude intestinal tuberculosis in patients with gastrointestinal symptoms treated with TNF-α inhibitors [24].

CONCLUSIONS

We suggest that patients with psoriasis should undergo abdominal ultrasonography before biological agent treatment not only to exclude intraabdominal malignancy and infections but also to determine hepatic steatosis. It should be kept in mind that nonalcoholic fatty liver disease has an asymptomatic course and poor prognosis in patients with psoriasis. Since lifestyle modification and weight loss can reverse early stages of nonalcoholic fatty liver disease such as steatosis [25], early detection of hepatic steatosis is crucial in the treatment of patients with psoriasis. Therefore, we suggest that abdominal ultrasonography should be performed in patients with psoriasis before biological agent treatment, especially in females, patients with advanced age, patients who were previously treated with biological agents, and patients with comorbidities such as diabetes. In addition, considering the high frequency of nonalcoholic fatty liver disease in patients with psoriasis, the most appropriate biological agent treatment should be determined in this population and stated in psoriasis treatment guidelines.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors’ contribution

Conceptualization, F.T., O.A., M.P., E.A.; methodology, F.T.; software, F.T.; validation, F.T., O.A., M.P., E.A.; formal analysis, F.T.; investigation, F.T., O.A.; resources, F.T., O.A., M.P., E.A.; data curation, F.T., O.A., M.P., E.A.; writing-original draft preparation, F.T.; writing-review and editing, M.P., E.A.; visualization, F.T.; supervision, M.P., E.A.; project administration, F.T., O.A., M.P., E.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2022-264).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Essential Oils and their Components in Combating Fungal Pathogens of Human Skin-lipophilic Yeast Malassezia furfur

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Abstract: *The most common problem of modern medicine is the development of increasing antifungal drug resistance nowadays including fungal infections of the skin and its appendages caused by dermatomycetes, yeast-like fungi, and molds so, there will be a need for alternative antifungal treatment. Natural substances with high antifungal properties like essential oils and their components show potential in this regard. Citrus lemon and Citrus sinensis essential oils were screened against Malassezia furfur by disc diffusion method and Minimum Inhibitory Concentration was determined by Modified Microdilution method. Citrus lemon (lemon) and Citrus sinensis (orange) show excellent inhibitory effects on the lipophilic, yeast-like fungus Malassezia furfur which causes Pityriasis versicolor, a chronic superficial fungal disease of the skin. The diameter of the inhibition zone was found to be 70 mm and 60 mm respectively. Malassezia furfur was found to be resistant against Clotrimazole & Ketoconazole. The minimum inhibitory concentration (MIC) of lemon and orange oil against Malassezia furfur was found to be 0.6 µl/ml and 0.8 µl/ml. These findings concluded that the use of Citrus lemon (lemon) and Citrus sinensis (orange) oil showed remarkable and excellent antifungal activity, and can be used as a traditional herbal medicine for the control of Pityriasis versicolor infection of the skin after undergoing clinical trials.*

Keywords: *Pityriasis versicolor, skin, Malassezia furfur, Citrus lemon and Citrus sinensis oils*

INTRODUCTION

Pityriasis versicolor (PV) is a superficial mycosis, that affects the superficial layer of stratum corneum [1]. The causative organism is Malassezia furfur, a yeast-like lipophilic fungus. Previously the mycelial form was called either Pityrosporum ovale or Pityrosporum orbiculare [2]. In 1951, Gordon isolated the organism M.furfur and renamed it P.orbiculare. It was recognized that M.furfur is the correct name and that P.orbiculare and P.ovale are synonyms [3]. The disease is most prevalent in early adulthood and small children are rarely affected [4-5]. PV is common in the post-pubertal age where sebaceous glands are active and in individuals who sweat more [6]. The prevalence in colder climates is less than 1% [7]. M. furfur is a component of normal skin flora in more than 90% of adults living in tropical areas [8]. Infectious diseases account for a high proportion of health problems in developing countries including India. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created [9]. The resistance of the organisms increased due to the indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases. This situation forced the researchers to search for new antimicrobial substances from various sources including medicinal plants [10-11]. A survey of the literature reveals that many essential oils possess antifungal activity. Therefore, we need to search for plant-derived antifungal drugs which are safe and without side effects. Herbal medicines may be in the form of powders, liquids, or mixtures, which may be raw or boiled, ointments, liniments, and incisions [12]. Essential oils have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [13]. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties

[14-15]. Numerous essential oils have been tested for both in vitro as well as in vivo antifungal activity and some pose much potential as antifungal agents. Studies on the antimicrobial, especially antibacterial and antifungal activity of lemon grass oil and its components were reported [16-18]. Orange oil was composed principally of d-limonene, at 94%, and myrcene at about 3%. The essential oils of lemon and orange exhibited antifungal activity against common molds Aspergillus niger, A.flavus, Penicillium chrysogenum, and P.verrucosum. In the present study, the antifungal activities of lemon and orange oils were investigated to discover the medicinal potential of these oils for future application as an antifungal agent for Pityriasis versicolor disease.

MATERIALS AND METHODS

Extraction and Preparation of Oil

In the summer season, extraction of oil from the peels of lemon (Citrus lemon) and orange (Citrus sinensis) was carried out by standard hydrodistillation method, Clevenger's apparatus, and all operations were carried out at room temperature. The fresh peels of lemon and orange were washed to remove soil and sliced. Sliced pieces of fresh peels of lemon and orange (250 g) were placed in a separate flask, together with distilled water (1 L). After 5-6 hours, oil was collected from the apparatus, anhydrous with sodium sulfate for removal of water traces, then this 100% pure essential oil was dispensed into dark bottles and stored at 4°C until used. Essential oil was ready to use for disc diffusion and determination of MIC.

Screening of essential oil using Disc Diffusion method

Oil was screened for its antifungal activity against M.furfur by disc diffusion method [19]. Standard size Whatman No.1 filter paper discs, 6.0 mm in diameter, sterilized by dry heat at 140°C in an oven for one hour were used to determine antifungal activity. SDA medium for the disc diffusion test was prepared. After sterilization, it was poured into sterilized Petri plates and allowed to solidify. Then one day old, fresh culture of yeast will be used for inoculum preparation. A suspension that was just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending yeast in 0.9% NaCl solution and the homogeneous suspension was used for inoculation. Using a sterile cotton swab, yeast cultures were swabbed on the surface of sterile Sabourauds dextrose agar plates. Sterilized filter paper discs were soaked in neat, undiluted (100%) concentration of oils. Using ethanol-dipped and flamed forceps, oil-saturated discs of 100% concentration were aseptically placed over Sabourauds dextrose agar plates seeded with the respective test microorganism. The antibiotic discs of Clotrimazole and Ketoconazole (10 mcg/disc) were also aseptically placed over the seeded Sabouraud's dextrose agar plates as a standard drug for comparison of antifungal activity of lemon and orange oils. The plates were incubated at 37ºC for 24 hours. The diameter of the inhibition zones was measured in mm. Three replicates were kept in each case and average values were calculated. The activity of oils was measured by the following formula:

AI (Activity index) = Inhibition zone of sample/Inhibition zone of standard

Determination of Minimum inhibitory concentration using Microdilution method

The modified microdilution method by Provine and Hadley (2000) was followed to determine MIC [20]. Media used for MIC was semisolid agar media (Brain Heart Infusion Agar) aliquots of semisolid agar media (Bacto Agar; Difco Laboratories) at a pH of approximately 7.4 were poured into a 16-by 125-mm glass tube and autoclaved. A suspension that was just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending the selected fungi in 0.9% NaCl solution, vortexing, and homogeneous suspension was used for inoculation. Different concentrations of lemon and orange oils were added in media containing test tubes, afterwards a standard platinum loopful (~0.001 ml, Himedia, Flexiloop) of the inoculum suspension was inserted deep into each tube of medium containing a different concentration of oils, as well as an oil-free control, by a centered down-up motion to form a two-dimensional inoculum. The tubes were then incubated at 30OC for 48 hours to determine the MIC. MIC was read to be the lowest concentration at which there was no visible growth of the organism. Then, by visual inspection, good growth of the respective fungi in an oil-free medium as a control was detected (48 hrs for yeasts). Afterward, the growth in all tubes at different concentrations of lemon and orange oil was compared with that of the oil-free control to determine inhibition.

RESULTS

In our present work, M.furfur was found main etiological agent of Pityriasis versicolor disease hence antimycotic studies were carried out on M.furfur (Figure 1). The conventional treatment of fungal disease is limited, and part of the reason is due to the limited spectrum of the current antifungal drugs, and the expensive treatment, particularly due to the need for prolonged therapy. Thus, new drugs and alternative therapies are necessary, including natural products. We report here the antimycotic study of lemon and orange oil against M.furfur in vitro (Figure 2). The results of the present work on the antifungal activity of lemon and orange oil against M. furfur studied by two methods i.e Disc diffusion and Microdilution method are presented in Tables 1, 2, and 3.

Table 1: Antifungal activity of lemon and orange oil against Malassezia furfur

Essential Oil	Test Strain	IZ of Essential Oil
C. limon	*Molassezia furfur*	70 mm
C. sinensis	*Molassezia furfur*	60 mm

Table 2: MIC of C. limon oil against Malassezia furfur

Test Strain	Different concentrations of lemon Oil used in µl/ml	Growth visually inspected in different concentrations of oil
Malassezia furfur	0.1	+4
	0.2	+3
	0.3	+3
	0.4	+2
	0.5	+1
	0.6	0
	0.7	0
	0.8	0
	0.9	0
	1	0
	Control without oil	100% growth

Growth was scored in the following manner: *4+, growth comparable to that of the oil free control; 3+, growth approximately 75% that of the control; 2+, growth approximately 50% that of the control; 1+, growth 25% or less that of the control; and 0, no visible growth*

Table 3: MIC of C. sinensis oil against Malassezia furfur

Test Strain	Different concentrations of lemon Oil used in µl/ml	Growth visually inspected in different concentrations of oil
Malassezia furfur	0.1	+4
	0.2	+3
	0.3	+2
	0.4	+2
	0.5	+1
	0.6	+1
	0.7	+1
	0.8	0
	0.9	0
	1	0
	Control without oil	100% growth

Growth was scored in the following manner: *4+, growth comparable to that of the oil free control; 3+, growth approximately 75% that of the control; 2+, growth approximately 50% that of the control; 1+, growth 25% or less that of the control; and 0, no visible growth*

In our study, lemon and orange oils presented higher diameters of inhibition zones. The diameter of the inhibition zone obtained against lemon and orange oil at 100% concentration of pure oil was 70 mm and 60 mm respectively by disc diffusion method (Figure 3). According to our study by comparing with the reference drugs, lemon and orange oil was found to be more effective in inhibiting the growth of M. furfur. Clotrimazole and ketoconazole were found to be resistant to Malassezia furfur (Figure 4). Results of MIC of lemon and orange oils against M. furfur. are summarized in Tables 2 and 3. The results show that the lemon and orange oil exhibited inhibitory action at 0.6 µl/ml and 0.8 µl/ml concentrations against M. furfur. Both oils can be used for the development of natural antifungal agents against Pityriasis versicolor infections.

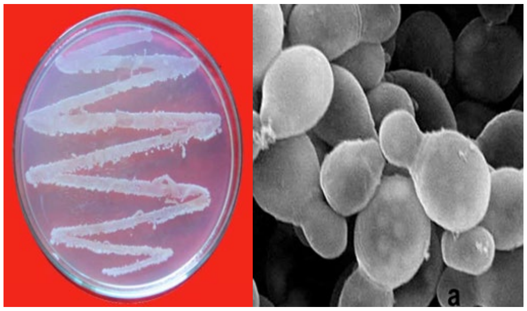

Figure 1: M. furfur on SDA media with olive oil & Bottle shape yeast cells under microscopic examination

Figure 2: Fruit of Citrus lemon (lemon) & Citrus sinensis (sweet orange)

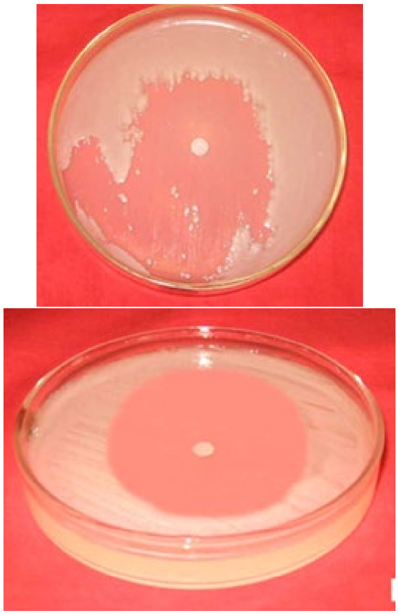

Figure 3: Antifungal activity of C. lemon & C. sinesis against Malassezia furfur

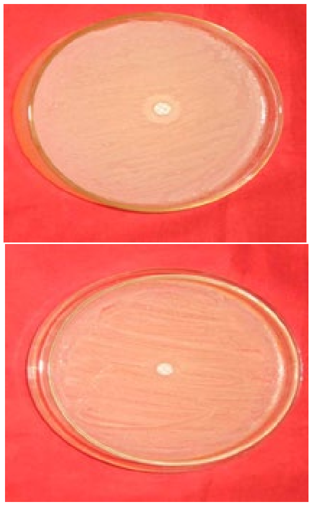

Figure 4: Resistance of Clotrimazole & Ketoconazole against Malassezia furfur

Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage and cell death; blocking the membrane synthesis; and inhibition the spore germination, fungal proliferation, and cellular respiration [22]. Because of the high volatility and lipophilicity of the essential oils, they are readily attached to penetrate the cell membrane to exert their biological effect [23]. The results showed the efficacy of lemon oil on animal skin infections caused by Trichophyton rubrum and Microsporum gypseum. In our study, lemon oil exhibited strong antimycotic activity against M. furfur. In the screening of lemon oil, the diameter of the inhibition zone by the disc-diffusion method was found to be 50 mm. MIC of oil obtained by microdilution method was 0.8µl/ml. Our work is in agreement with the observations of Silvia et al in which screening of lemon grass oil against Candida species was studied and it was found as an effective antifungal agent [24]. Our work also coincides with the previous findings of Ezzat et al which revealed that the essential oil of Citrus lemon at 200 µl concentration showed a diameter of inhibition zone 37 mm against Candida albicans 10261 [25]. According to Rusenova and Parvano Citrus lemon does not show antimicrobial activity by disc-diffusion method against both yeasts, Candida albicans and Malassezia pachydermatis [26]. These results are not in agreement with our studies which showed that antifungal activity by disc-diffusion and minimum inhibitory concentration by microdilution method, lemon oil was found to be effective in inhibiting the growth of M. furfur at a low concentration of oil. Lemon oil is also preferentially more active against Gram-positive bacteria as compared to Gram-negative bacteria. The antimicrobial activity of lemon oil is due to the presence of terpenoids-a-pinene, camphene, b-pinene, sabinene, myrcene, terpinene, b-bisabolene, limonene, trans-a-bergamotene, nerol and neral [27]. Essential oils from citrus offer the potential for all-natural antimicrobials for use in improving the safety of organic or all-natural foods [28]. Shahi et al in 2003 found that the MIC of oil of Citrus sinensis were in the range of 0.7-1 µl/ml against Candida albicans, Aspergillus flavus, A. fumigatus, A. niger, A. ustus, Epidermophyton floccosum, Microporum audouinii, M. canis, M. gypseum, M. nanum, Rhizopus nigricans, Trichophyton tonsurans, T. rubrum, T. mentagrophytes and T. violaceum [29]. The present investigation suggests that essential oils exhibit a narrow or wide range of antifungal activity, which may prove useful in the development of effective antifungal substances. Souza et al (2005) reported MIC of lemon oil at 0.5% concentration against mold strains i.e. Fusarium spp, Penicillium spp, Aspergillus niger, A. flavus, and Rhizopus spp [30]. In our findings, MIC of lemon oil against M. furfur was 0.8 µl/ml i.e. very close to that concentration. The small differences may be due to local environmental and climatic conditions and variations in the choice of microorganism tested.

Our results of orange (Citrus sinensis) and lemon (Citrus lemon) are also similar to the work of Viuda-Martos et al which suggests that both oils were effective in inhibiting the growth of Aspergillus niger, A.flavus, Penicillium chrysogenum and P. verrucosum [18]. The hydrodistilled oil of lemon and orange was also found to be more sensitive against Penicillium digitatum [31]. Antimicrobial activity of Rutaceae family species has been observed on dermatophytes [32] and on some molds [33]. Essential oils of some plants have recently been proven to be successful eco-friendly, bio-control agents [34]. Many authors have reported antimicrobial, antifungal, antioxidant, and radical-scavenging properties of essential oils [35-36].

DISCUSSION

The present results suggest that lemon and orange oils exhibit strong antifungal activity. This is in agreement with the findings of Valero and Frances who reported narrow and wide ranges of antifungal activites. Essential oils derived from many plants are known to possess antifungal activities [21].

CONCLUSIONS

The present study suggests that the oils extracted from the waste product (peels) of C. sinensis and C. lemon hold good promise as an antifungal agent, which could be used in therapeutic remedy against human pathogenic fungi on account of its various antifungal properties, viz. strong fungicidal action, long-shelf life, withstand heavy inoculum density, thermostable, broad range of antifungal activity and absence of any adverse effects. The waste product of C.

sinensis and C. lemon can be used for the development of a potential source of effective and economically viable herbal antifungal against Pityriasis versicolor (fungal skin infection) after undergoing successful clinical trials.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

RS contributed to design of experiments, review, discussion, and editing of the manuscript. GS contributed to the review and writing of the manuscript. RS contributed to all editing of the manuscript. ER contributed to the review of the manuscript. MS contributed to the editing and review of the manuscript.

Ethics approval and consent to participate

This article does not contain any studies with human and animal participants performed by any of the authors,

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REVIEW

From Controversy to Consensus: Neurogenesis and Neuroplasticity

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Abstract: *More and more research in recent years has focused on neurogenesis and neuroplasticity. The hippocampus is a key location of adult neurogenesis. Because this part of the brain is regulated by environmental variables, it must be mentioned the therapeutic potential for neurodegenerative illnesses and brain injuries. Furthermore, this information challenges the historical concept of a static brain and tries to demonstrate its flexibility, adaptability, and possibility for regeneration. Those mechanisms which are found in a variety of mammalian species, including humans, are important when it comes to adaptive learning and memory, thus providing insights into cognitive well-being and mental health. The discovery of the brain's dynamic nature represented by new neurons, marks a shift in the field of neurology with far-reaching potentials. This paper highlights the significant potential of adult neurogenesis when it comes to clinical applications such as Alz-heimer's disease.*

Keywords: *adult neurogenesis; neurodegeneration; neuroplasticity; therapeutical approaches; Alzheimer's disease; hippocampus; ethanol exposure*

INTRODUCTION

From a historical perspective, the brain was frequently defined as a static organ post-development. That means that most of its structures and also its functions are established during the early years of life [1]. This belief was held due to the absence of knowledge regarding neurogenesis, defined as the development of new neurons. The way we understand brain function, its plasticity, and the potential for recovery after an injury has been impacted by this perspective [1].

During the 1960s, remarkable contributions by Altman and Das provided convincing support for the occurrence of neurogenesis in certain regions of the adult rat brain, particularly in the hippocampus [2]. Although these statements led to various debates in the field of neurology, they also initiated a significant change in the way neurosciences is understood.

Based on the latest research, the hippocampus is deeply involved in not only the process of memory formation but also in spatial awareness processes [3-5]. The study of this process at the level of the dentate gyrus has yielded even more insights regarding the brain's innate possibility of self-repair. Neurogenesis also influences cognitive processes such as learning, memory, and mood regulation [6-9].

Advanced techniques ranging from neural imaging to molecular biology were used. The primary objective of these investigations was to ascertain the specific circumstances that facilitate neurogenesis. It is also essential to comprehend the role played by the newly formed neurons and explore the external factors that impact neurogenesis. Researchers have analyzed the effect of stimulating environments and physical activity on this process.

The more robust the evidence supporting adult neurogenesis was discovered, the more researchers became aware of its potential consequences for human health. The inquiry arises as to whether neurogenesis can be utilized for therapeutic purposes, i.e., "What are the possible effects on conditions characterized by neuronal degeneration, such as Alzheimer's disease or traumatic brain injuries?" The scientific community is enthusiastic and motivated about the potential use of adult neurogenesis for treating diverse neurological and psychological disorders [10].

MATERIALS AND METHODS

A rigorous methodology was employed in this narrative review to gather, analyze, and synthesize scholarly literature on adult neurogenesis. Due to the abundance of available research, we employed a systematic approach to thoroughly examine the most relevant and up-to-date studies.

Our analysis involved articles that explored the concept of neurogenesis in adult mammals, their intricacies, implications, and potential therapeutic applications. We methodically sourced diverse academic articles, primarily from PubMed, Scopus, and Google Scholar, ensuring a comprehensive exploration. We searched for various aspects of neurogenesis using both broad and specific search terms, and we prioritized recent research while recognizing foundational studies.

FROM NEUROGENESIS TO NEUROPLASTICITY

Adult neurogenesis is a process involving the formation of new neurons. The process takes place in the mature brain. It has been a topic of intense examination and debate in neuroscience [4,11]. While it is accepted that mammals, particularly rodents, exhibit this phenomenon in their hippocampus, the occurrence of such a process in humans has been controversial [1,12].

Historically, the mature mammalian Central Nervous System (CNS) was believed to possess limited regenerative potential. However, emerging evidence challenges this perspective, proposing that the mature CNS, including that of humans, might harbor a latent regenerative ability influenced by environmental factors [13,14]. Moreover, the adult hippocampal neurogenesis (AHN) observed in rodents is not merely an isolated phenomenon. It manifests neural plasticity prevalent across many mammalian species, including humans. Based on all this information, it is worth mentioning the fact that plasticity which is essential for both memory processes and also adaptive learning, is now starting to shift our understanding of the capabilities of the human brain [12,15-18].

Due to that, further researchers in the field of adult neurogenesis are perceived as being essential. Some of the potential consequences that are highlighted in the literature include an enhancement of cognitive well-being together with the potential of using it as a form of treatment strategy. As this topic becomes more and more prevalent in scientific literature, the current aim is to recognize neurogenesis complexities across different species and try to apply these notions to human brain health and the potential pathologies that might arise at this level.

Based on the results of the current literature review, the most relevant themes referring to neurogenesis and neuroplasticity were clustered into 12 categories (Figure 1).

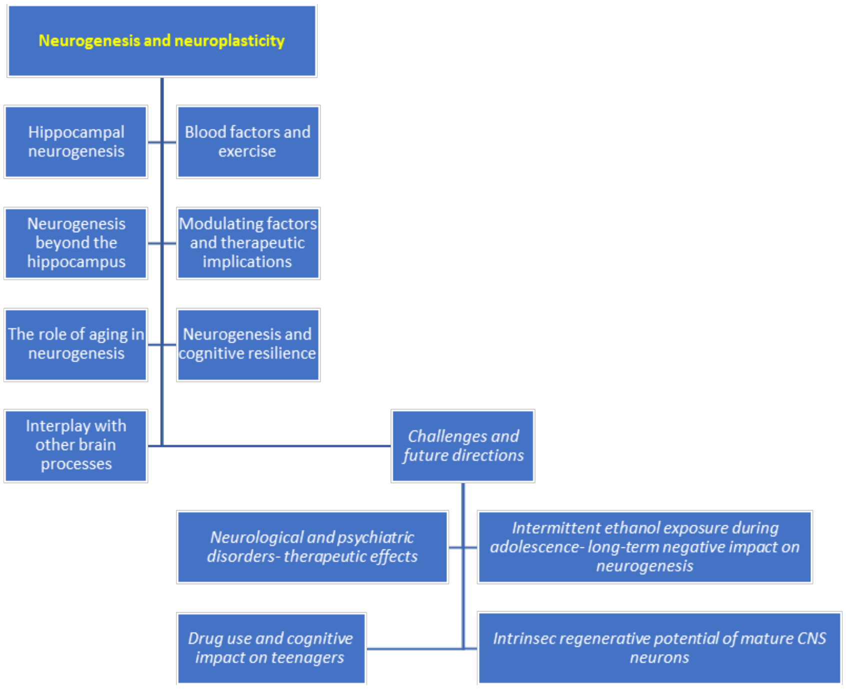
Neurogenesis and neuroplasticity
Hippocampal neurogenesis
Blood factors and exercise
Neurogenesis beyond the hippocampus
Modulating factors and therapeutic implications
The role of aging in neurogenesis
Neurogenesis and cognitive resilience
Interplay with other brain processes
Challenges and future directions
Neurological and psychiatric disorders- therapeutic effects
Intermittent ethanol exposure during adolescence- long-term negative impact on neurogenesis
Drug use and cognitive impact on teenagers
Intrinsec regenerative potential of mature CNS neurons

Figure 1: Neurogenesis and neuroplasticity – paradigm shifters in understanding the brain's functionality

Hippocampal Neurogenesis

Regarding the concept of neurogenesis, the primary focus was on the function of the hippocampus, particularly the area known as the dentate gyrus [7,8,19-22]. This is because, from a historical perspective, this region was frequently mentioned as being associated with various important cognitive functions. It is essential in memory formation, spatial awareness, and emotional control. Discovering neurogenesis in this region has reevaluated our understanding of its importance [2,7].

The incorporation of new neurons into existing hippocampal networks boost plasticity. This could also lead to enhanced cognitive functions. For instance, studies on rodents have shown a positive link between increased neurogenesis and better performance in spatial memory tasks [3,4,5,20,23-26]. Additionally, evidence points to a relationship between disruptions in neurogenesis and mood disorders. This claim suggests a potential link between creating new neurons and emotional regulation [9].

Blood Factors and Exercise

The influence of the environment on neurogenesis is becoming a significant research focus [27]. Evidence suggests that elements present in the bloodstream can affect the brain's ability to regenerate [28,29]. Notably, a study by Horowitz et al. [23] found that plasma from trained animals could transfer the beneficial effects of physical activity on neurogenesis to older individuals. This indicates that a systemic environment shaped by exercise can strengthen the brain's regenerative potential [23, 27].

Such discoveries are of great significance, particularly for the elderly. As concerns about cognitive decline with age intensify, it becomes crucial to grasp how systemic elements can enhance brain health [29]. These blood-derived factors might stimulate neurogenesis in those who can't partake in physical exercise [23].

Neurogenesis Beyond the Hippocampus

While the hippocampus remains a focal point, recent research has expanded the exploration of neurogenesis to other brain areas. Current scientific research has presented persuasive evidence for neurogenesis in various brain regions: hypothalamus, striatum, substantia nigra, cortex, olfactory bulb, and amygdala [28,30-32].

The potential functional impacts of neurogenesis in these areas are still under study. For instance, the striatum, involved in motor functions and reward-driven behaviors, might witness improved plasticity by new neurons. Similarly, neurogenesis in the amygdala, a key center for emotion processing, could shape how emotional memories and knowledge are acquired and stored [3-5,9,20,30].

Modulating Factors and Therapeutic Implications

Numerous intrinsic and extrinsic elements have been identified as influencers of neurogenesis. Neurotrophic factors, signaling molecules that aid neuron growth and differentiation, are known to boost neurogenesis [10,27,33-36]. For example, the Brain-derived neurotrophic factor (BDNF) plays a fundamental role in supporting the survival of new neurons by activating the tropomyosin receptor kinase B (TrKB) receptor, which then triggers pathways related to cell survival and movement [31,36].

Environmental elements, like being in stimulating surroundings or participating in cognitive activities, are associated with heightened neurogenesis [30,37]. Additionally, the development of new neurons in specific brain parts, remarkably in the context of brain injury (such as stroke) and neurodegenerative conditions, can be shaped by particular processes [38]. The importance of understanding neurogenesis is crucial for considering therapeutic approaches [10].

Furthermore, pharmacological interventions targeting specific signaling pathways involved in neuron differentiation offer promising possibilities. As research progresses, more targeted drug interventions might be elaborated to fine-tune the neurogenesis process, providing benefits in conditions where neural regeneration is needed [10,39].

Emerging research also implies that stem cell therapies could be employed to promote neurogenesis. Researchers aim to enhance the brain's regenerative capability, particularly in conditions marked by substantial neuron reduction. This could be achieved by introducing pluripotent stem cells that can differentiate into neurons. The potential of stem cell therapies lies in their ability to replace lost neurons and provide a supportive environment that promotes the growth of new neurons [14,40].

The Role of Aging in Neurogenesis

The implications for aging are one of the most compelling aspects of research on adult neurogenesis [23,24,26,29,41-44]. As individuals age, an inherent decline is observed in multiple cognitive domains. This is accompanied by a reduced brain ability to recuperate from injuries or diseases [45]. Identifying adult neurogenesis, particularly in older mammals, prompts inquiry into the potential harnessing or augmentation of regenerative capabilities in the aging brain [14].

Research has indicated a multifaceted association between the aging process and the generation of new neurons. Although there is an inherent decrease in the rate of neurogenesis with age, it is essential to note that this process does not come to a complete cessation [28]. The population of dormant stem cells in the hippocampal dentate gyri diminishes with advancing age. This happens while the number of actively dividing progenitor cells and immature neurons remains consistent over aging [7,8,28]. This observation implies that the brain maintains its regenerative capacity even during advanced stages of life. Furthermore, while neuroplasticity decreases in older individuals, the volume of granule cells (GCs), glia, and dentate gyrus remains unaltered [28,45].

Significantly, interventions such as exercise have demonstrated the capacity to counteract the process of brain aging [27]. Previous studies have observed that specific blood components present in the plasma of mice subjected to exercise can transmit the advantageous impacts of exercise on adult neurogenesis and cognitive function to mice that had a sedentary lifestyle [33]. The hepatic enzyme glycosylphosphatidylinositol (GPI)-specific phospholipase D1 (Gpld1) exhibited elevated levels following physical exertion. This molecule was associated with enhanced cognitive performance in elderly mice. In a promising manner, high levels of Gpld1 were likewise detected in the bloodstream of physically active and robust older individuals. According to Horowitz et al. [23], when administered systemically in aged mice, it can alleviate age-related impairments in regeneration and cognition.

Neurogenesis and Cognitive Resilience

An emerging area of interest is the role of neurogenesis in cognitive resilience. Cognitive resilience refers to the brain's ability to resist or recover from potential cognitive decline, often attributed to aging, injury, or disease [7,38,46-48]. Generating new neurons and their successful integration into existing neural networks could strengthen the brain's resilience. This adaptation allows the brain to maintain or enhance cognitive functions in the face of adversities [7].

This has implications for age-related disorders and conditions like traumatic brain injuries, stroke, or even chronic stress [38]. If neurogenesis can be stimulated and directed appropriately, it might offer a therapeutic strategy to counteract the cognitive deficits.

Interplay with Other Brain Processes

Neurogenesis is not isolated; it's intricately linked with other brain processes [37,49,50]. Synaptic plasticity, the capability of synapses to toughen or deteriorate over time, is believed to play a role in learning and memory [4]. Introducing new neurons into neural circuits could enhance synaptic plasticity, facilitating better learning and memory retention. Moreover, processes like long-term potentiation, critical for memory formation, might be influenced by new neurons, further emphasizing neurogenesis's role in cognitive functions [49].

Challenges and Future Directions

While the findings on adult neurogenesis are promising, challenges remain (Figures 1, and 2). The exact mechanisms regulating neurogenesis, the factors determining the survival and integration of new neurons, and the functional implications of these neurons are areas of active research [26,29]. Additionally, translating findings from animal models to humans presents its own set of challenges [52]. However, with continued research and technological advancements, the potential to harness neurogenesis for therapeutic benefits appears promising [10].

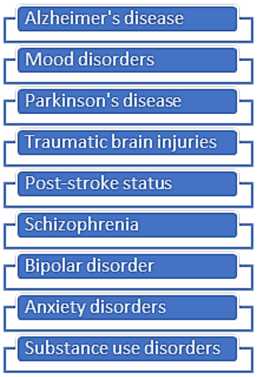

Figure 2: Potential targets of the neurogenic therapies

Implications for Neurological and Psychiatric Disorders

Identifying adult neurogenesis carries significant implications for neurological and psychiatric disorders [38,47,53-61]. Neurodegenerative disorders, such as Alzheimer's disease, are characterized by the loss of neurons. These may benefit from a deeper comprehension of the processes underlying neurogenesis [41,47]. Similarly, mood disorders like depression have been linked to decreased hippocampal volume and disrupted neurogenesis. These could also benefit from interventions aimed at promoting the generation of new neurons [4].

Furthermore, conditions such as Parkinson's disease and Huntington's disease, characterized by specific neuronal cell loss, could be targeted with neurogenic therapies. The interventions should replace the lost neurons or protect the remaining ones. Understanding the factors influencing intrinsic or extrinsic neurogenesis can pave the way for novel therapeutic strategies [57,62-66].

Traumatic brain injuries and strokes resulting in acute neuronal damage might also benefit from enhanced neurogenesis [44]. Strengthening the brain's inherent ability to generate new neurons could speed up recovery, improve cognitive function, and potentially restore lost skills [1].

Moreover, the implications of neurogenesis aren't limited to just the diseases that involve neuronal loss. The balance and health of neurogenesis might influence conditions like schizophrenia, bipolar disorder, and anxiety disorders—these disorders have an unclear etiology that is still under investigation. Disruptions in the process could contribute to the pathophysiology of these disorders [54].

Understanding how lifestyle choices, physical activity, the way we manage stress, and the approach dietary habits might impact the process of neurogenesis are also perceived as being essential to enhancing our understanding of this topic [51].

The impact of intermittent ethanol exposure during adolescence on the process of neurogenesis in the hippocampus

The adolescence period is a key point in neurology due to the high rate of both neuroplasticity and neurogenesis which characterizes it [67-73]. Despite this, during this maturational phase, many young people tend to engage in various risky behaviors such as consuming large amounts of alcohol and binge drinking [29,36,72]. Such behaviors, while transient for many, can have persistent consequences for brain health and function [36].

Recent studies that approached preclinical rodent models have shed light on the possible effects of adolescent intermittent ethanol (AIE) exposure [67]. These models have revealed an alarming pattern, especially because AIE was linked to a significant decrease in the formation of new neurons at the level of the hippocampus [33,36,67-73]. Notably, this decline doesn't merely correspond with the period of ethanol exposure. Instead, the detrimental effects persist into adulthood, even when ethanol exposure has ceased. This indicates a long-lasting impact on the brain's ability to produce new neurons [33].

Recent discoveries have pointed towards neuroimmune and epigenetic pathways. Increased neuroimmune signaling post-AIE exposure suggests an inflammatory response. This might inhibit the normal process of neuron generation [33]. Simultaneously, there's evidence of de-creased neurotrophic support. This implies that the usual signals promoting neuron growth and differentiation are compromised. Epigenetic mechanisms, characterized by modifications in gene expression that do not involve modifications to the underlying DNA sequence, have a crucial role in facilitating these effects. These mechanisms could alter the gene expression for neurogenesis, thereby perpetuating the decline in neuron generation [33,36].

Drug Use and Cognitive Impact on Teenagers

The brain, in particular, undergoes profound developmental processes that give rise to intricate behavioral and mental faculties. However, the trajectory of this development is not solely determined by biological factors; it is also susceptible to environmental influences. Among these, drug exposure is a particularly concerning factor that can derail neurobiological maturation [74-77].

Historically, the health implications of drug use during adolescence have been a subject of considerable concern [75,76]. The predominant focus has been understanding how adolescent drug use might predicate or correlate with drug-using behaviors in adulthood. However, recent scientific inquiries have expanded this focus, revealing that the ramifications of teenage drug use are not confined to future drug-related behaviors. Other behavioral domains, notably learning and memory, have been scrutinized [52].

Emerging evidence suggests that learning and memory are particularly vulnerable to the effects of drugs during adolescence [75]. More alarmingly, deficits in these cognitive domains, once established due to drug exposure, appear to persist into adulthood. These findings indicate a deep-seated interaction between drug exposure and the natural developmental processes of the adolescent brain. This interaction yields consequences that endure well beyond the cessation of drug use, emphasizing the gravity of the issue [74-76].

In light of these revelations, it becomes imperative to understand the exact nature and extent of the cognitive impairments caused by adolescent drug exposure. While the research landscape is vast, some patterns are discernible. Adolescents, due to societal norms and legal frameworks, are more inclined towards drugs that are legally accessible to adults. As such, alcohol, nicotine, and marijuana emerge as substances of primary concern [77,78].

Intrinsic Regenerative Potential of Mature CNS Neurons

For years, the broader field of neuroscience has operated under a prevailing assumption: that the neurons in the adult mammalian CNS possess limited if any, capacity for regeneration following injury. This perspective contrasts our understanding of peripheral nerves, which have consistently demonstrated a robust ability to regenerate after various forms of trauma [14].

Delving into the reasons behind this perceived inability of CNS neurons to regenerate, researchers have pinpointed a few key factors. Paramount among these is the presence of specific extracellular signaling factors in the CNS environment [28]. These factors inhibit axonal growth, blocking the natural regenerative processes that might otherwise occur. Furthermore, there's the notion that mature central neurons, as they age, may lack the adaptive responsiveness required to counteract and recover from damage. This idea aligns with a broader dogma in neuroscience, which suggests that as central neurons mature, they inherently lose their structural plasticity [14].

However, recent discoveries have started to challenge this traditional viewpoint. Emerging evidence suggests that the mature CNS might not be as rigid and unresponsive. More exactly, some of the neurons might be defined by a latent regenerative ability. This capacity might be masked by various environmental factors, but they continue to exist. If this capability can be better understood, it could reform the methodology of treating CNS injuries and neurodegenerative conditions [14].

CONCLUSIONS

The discovery of adult neurogenesis has enhanced our understanding of the concepts of brain plasticity and regeneration. Although we previously believed that the fully developed mammalian brain remained unchanged, this static paradigm was proven wrong based on new discoveries in the field of neuroplasticity and neuroregeneration.

However, the latest academic research in the field of neurosciences highlights that this is incorrect, as our brain is still able to produce new neurons and also can adjust itself based on various internal and external variables. This significant advancement has had a profound impact on various medical fields, as is the case of neurosciences and therapeutic practices regarding the brain.

The phenomenon of adult neurogenesis provides information that contradicts traditional beliefs as it highlights the ever-changing nature of the brain. The process is not limited to only the synaptic plasticity, as completely new neurons can develop. This implies a remarkable durability that provides optimism when it comes to treating patients with neurotrauma or neurodegenerative pathologies.

Therapies that promote neurogenesis might potentially be beneficial for diseases such as Alzheimer's and depression, both of them being conditions that are frequently associated with a significant loss of viable neurons. The understanding of the novel neuron generation process and how this could be modified and incorporated holds significant therapeutical possibilities.

It is also important to mention that the process of neurogenesis can be influenced by external factors such as physical activity. Based on this observation, structured physical exercises must also be considered when trying to facilitate neurogenesis in the case of the elderly population who are diagnosed with dementia and a cognitive decline [79-81]

The neural development of our brains may be significantly influenced not only by our previous experiences but also by the environment in which we live and the decisions we make, often to a greater extent than we are currently aware of. The entire concept of adult neurogenesis research still encounters persistent controversies. This is mostly due to the lack of understanding of this process which is frequently believed to be too complex at this stage or inadequately studied.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, CAS, IGI, OV, MM and CAP; methodology, CAS, MM and CAP; validation, CAS, IGI, MM, and CAP; resources, IGI, OV and CAP; data curation, CAS, OV, and MM; writing—original draft preparation, CAS, IGI, MM and CAP; writing—review and editing, OV and CAP; visualization, CAS, IGI, OV, MM, and CAP; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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REVIEW

Eggshell Calcifications at Thyroid Ultrasound: A Sample-focused Analysis of Cytological Findings and Post-thyroidectomy Pathological Correlates

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Abstract: *Eggshell calcifications at thyroid ultrasound are connected to the challenging field of thyroid nodules and/or malignancy from a multidisciplinary perspective. Calcifications may be found in malignant and benign tumors; typically, micro-calcifications are identified in the papillary type of cancer, being equivalent to psammoma bodies in the pathological report. The risk of macro-calcifications, however, is more nuanced; recent data suggest a potential connection with a differentiated malignancy as well. Eggshell calcifications, a distinct type of macro-calcification, are generally considered benign findings, especially uninterrupted forms. Yet, the real incidence is not clear, which is why the topic remains open. In this narrative review, we analyzed eggshell calcifications in relationship with cytological diagnosis upon fine needle aspiration and post-thyroidectomy histological report. A PubMed-based search of the English-language papers using several keywords identified 171 articles. We only included original studies (no timeline restriction). After removing duplicates and applying the inclusion and exclusion criteria, 14 original studies were analyzed. The number of subjects per study varied (N=61 to 2678) so did the number of analyzed nodules per study (a maximum of 2902). Some studies found connections with benign features; others revealed a higher risk of malignancy at fine needle aspiration. Data remain heterogeneous, and oftentimes, studies investigated it only as a secondary endpoint. A high TI-RADS score is important in malignancy prediction, while eggshell calcification might add to the positive predictive value, yet, this trait is difficult to classify as a standalone element. Nevertheless, an interrupted pattern does not exclude thyroid cancer.*

Keywords: *thyroid, ultrasound, calcification, thyroidectomy, surgery, thyroid nodule, thyroid*

INTRODUCTION

Eggshell calcifications amid thyroid ultrasound are connected to the challenging field of thyroid nodules and/or thyroid malignancy. Recently, an important rise in thyroid nodule detection was brought about by the widespread use of ultrasonography, with detection rates reaching 60% of the general population (noting that the general prevalence of a thyroid nodule is 5%) [1,2]. As a consequence, there was also an apparent increase in over-diagnosis of differentiated thyroid cancer [3]. The increased costs, risks associated with surgical cures such as hypoparathyroidism and dysphonia, and the fact that most thyroid carcinomas are indolent, led current guidelines to lean towards a more conservative and individualized approach to thyroid nodules [4-7]. However, even though the mortality rates of thyroid carcinoma remained stable despite an increasing incidence, this is in opposition to decreasing rates of other solid malignancies [8]. The need for adequate risk assessment of thyroid nodules, therefore, remains clear. The importance of approaching this type of ultrasound lesion comes from providing additional clues and even risk factors for suspected malignancies to assess these patients particularly or to start meticulous screening protocols. Moreover, nowadays, thyroid ultrasound is largely available to a multitude of specialists coming from different backgrounds thus the importance of identifying the risk (if any) behind such lesions [4-8].

Currently, there are several risk stratification systems for thyroid nodules, including the American College of Radiology

Thyroid Imaging Reporting and Data System (ACR TI-RADS) [9], the American Thyroid Association (ATA) guidelines [10], and the European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults EUTIRADS [11]. All of them use similar sonographic characteristics to assess the risk of a thyroid nodule being benign or malignant.

Overall, calcifications may be found both in malignant and benign tumors. Findings such as spongiform or cystic nodules, with regular borders suggest benign nodules, while echogenic foci or micro-calcifications plead for malignancy [12,13]. Typically, micro-calcifications are found in malignant tumors and are the ultrasonographic equivalent of psammoma bodies (that are described in pathological reports) [14,15]. The risk of macro-calcifications, however, is more nuanced, but recent data suggest a possible connection with papillary carcinoma [16,17].

One particular type of peripheral macro-calcification, rim calcifications, which surround at least 120º of the nodule were found to be associated with malignancy [18,19]. Another type of macro-calcification is so-called “eggshell calcification”, a peripheral thin rim-like calcification surrounding the nodule. Typically, they are considered benign findings, especially when they are uninterrupted [20]. They may, however, be found in papillary carcinoma as well, but the real incidence is not yet clear, which is why the topic represents a challenging multidisciplinary approach in the modern era [21].

We aim to identify what type of lesions may present with eggshell calcifications on thyroid ultrasound, what risk of malignancy nodules with eggshell calcification have, and what other sonographic features help in elucidating diagnosis.

MATERIALS AND METHODS

This was a narrative review. A PubMed-based analysis of the English-language medical literature was conducted by using the following keywords: “eggshell”, “peripheral calcification”, “annular calcification”, and “curvilinear calcification” in association with “thyroid”.

The initial search resulted in 171 articles. We only included original studies (no timeline restriction).

Articles were included if they provided data regarding nodules with eggshell calcifications and were selected based on clinical relevance. Case reports and studies exclusively focusing on different types of peripheral calcifications or rim calcifications were excluded.

After removing duplicates and applying the inclusion and exclusion criteria, 14 original studies were analyzed.

RESULTS

Sample-focused analysis

Data regarding eggshell calcifications was heterogeneous, and, oftentimes, studies investigated the relationship between this distinct form of calcification as a secondary objective. The number of subjects varied largely, with the smallest study including 61 subjects and 64 nodules [22]; the largest had 2678 subjects and a total of 2902 nodules [23]. While some studies found connections with benign character [24,25], (others found a higher risk for suspicion of malignancy at fine needle aspiration (FNA) [26]. Some studies, however, did not find statistical significance [22,27-29].

Prevalence of eggshell calcifications amid thyroid ultrasound followed by thyroidectomy

The largest study was performed by Kobayashi et al. [23] and included 2678 subjects who underwent thyroid surgery and a total of 2902 nodules. 26% of nodules had calcifications. This study provided the prevalence of eggshell calcifications in different types of thyroid malignancies. The highest prevalence of eggshell calcifications, 43.90% (N=18/41), was found in undifferentiated carcinomas. In the papillary type of carcinoma, the prevalence was 4.78% (N=45/941), while in follicular carcinoma it reached 11.86% (N=40/337). In some malignancies, such as cyst-forming variants of papillary carcinoma, medullary carcinoma, and primary thyroid lymphoma, no eggshell calcifications were found [23]. The second largest study was conducted by Zayadeen et al. [30] and included 1466 patients and, respectively, 2002 nodules; eggshell calcifications were found in 45 benign nodules and in 11 malignant ones [30].

Eggshell calcifications and thyroid malignancy risk

Overall, no statistically significant association between eggshell calcifications and malignancy was found. However, a link between calcification subtype, namely discontinuous eggshell calcifications, and malignant tumors was reported [31]. Yin et al. [31] conducted a retrospective study on 543 subjects with single nodules who underwent thyroid surgery. Pathology results revealed that 61.32% (N=333) of them were malignant. The relationship between the presence of malignancy and various calcification types, including eggshell calcification, was analyzed. In terms of eggshell sub-type, eggshell in a row (continuous) forms were more frequent in benign nodules (1.7% versus 0.6%, p<0.01), while discontinuous eggshell calcifications were more frequent in malignant nodules (2.2% versus 0.6%, p<0.01). The distribution of calcification types among nodules prompts further studies investigating possible relationships between calcification types and different types of benign and malignant lesions. Of note, in benign nodules, all eggshell calcifications were found in adenomas, while in malignant nodules, all in-a-row calcifications were in medullary carcinoma (N=3/3), while discontinuous calcifications were found in papillary carcinoma (N=12, 3.84% of papillary carcinomas) [31]. Ha et al. [25] also found that nodules with eggshell calcifications are more frequently benign (66.7% benign versus 33.3% malignant, p=0.002) [25].

Another study investigating the connection between eggshell calcifications and malignancy risk was conducted by Arpaci et al. [26]. The study included 907 nodules of 269 subjects. Most were investigated via FNA (N=907). Even though benign nodules were less frequent in cases with eggshell calcifications compared with those without macro-calcifications, and suspicion for malignancy rates were higher in nodules with eggshell calcifications, no statistical significance was found for malignancy or diagnostic rates, based on cytology. Lack of statistical significance in terms of eggshell calcification presence in benign versus malignant nodules was also shown in patients who underwent surgery and had pathologic diagnoses [26]. Another study that failed to find statistical significance between the presence of eggshell calcifications and benign or malignant nodules was that of Lee et al. [22], which included 103 nodules in 92 patients. The diagnosis was made based on FNA and follow-up [22]. Kim et al. [27] did not find a statistically significant association either. However, the study described a prevalence of eggshell calcifications of 2.4% in malignant nodules and 5% in benign nodules, in a population of 1078 subjects with 1432 nodules who underwent thyroidectomy for nodular disease [27].

Yoon et al. [29] did not find a statistically significant difference between benign and malignant groups in terms of the presence and type of peripheral calcifications in a retrospective study on 65 subjects, most of whom were diagnosed by FNA [29]. Regarding differentiated papillary carcinoma in particular, Wang et al. [24] found that eggshell calcifications decreased the risk of papillary carcinoma (OR=0.208, CI=0.049-0.886, p=0.034). Moreover, they were not an independent risk factor for aggressive behavior [24].

Hashimoto's chronic autoimmune thyroiditis

Sonographic features of nodules in patients with Hashimoto's thyroiditis were analyzed by Anderson et al. [32] in 61 subjects with 64 nodules. Overall, eggshell calcifications represented 23% of all calcifications, appeared in 4.7% (N=3) of nodules, and were more frequent in subjects with normal parenchyma [32]. Another study by Anderson et al. [28] extended the search to 82 nodules of 78 subjects with Hashimoto's thyroiditis who underwent FNA or surgery. 84% (N=69) of nodules were benign, while 16% (N=13) were malignant, most of which were papillary carcinoma (N=12). One primary thyroid lymphoma was also found. Even though eggshell calcifications were present in 15% of malignant nodules and 1% of benign nodules, the difference was not statistically significant [28].

Neuroendocrine neoplasia

While eggshells in a row were present in medullary carcinoma in Yin et al.'s study [31], Kobayashi et al. [23] did not find any eggshell calcifications in the medullary type of malignancy [23]. The largest study analyzing eggshell calcification in patients with a form of thyroid cancer was performed by Cai et al. [33] on 27 cases and 50 controls. Even though macro-calcifications, in general, were more frequent in patients with medullary thyroid carcinoma, none of the 27 cases had eggshell calcifications. In controls, however, eggshell calcifications were found in 4 subjects [33].

Potential pitfalls of assessment: eggshell calcifications and non-diagnostic FNA

Anderson et al. [34] investigated ultrasound features in nodules with non-diagnostic FNA results in 393 subjects with single nodules. In terms of eggshell calcifications, they appeared in 9 lesions, but no association with malignancy was found [34].

Factors influencing malignancy risk assessment concerning eggshell calcifications

The retrospective study of Kim et al. [35] focused on eggshell calcifications and included 92 subjects with 93 nodules with eggshell calcifications who underwent thyroid surgery. Sonographic features that had a high sensitivity for malignancy in these patients were the presence of the halo (sensitivity of 62.7%) and disrupted calcification rim (sensitivity of 76.3%) [35]. Regarding malignancy risk detection, Ha et al. [25] found that the positive predictive value of peripheral or eggshell calcification was increased by being combined with high suspicion TIRADS from 33.3% to 66.7-80% [25].

DISCUSSION

Eggshell calcification in a thyroid nodule was described as early as 1976 in a patient with mixed papillary and follicular carcinoma [36], while the first case of a thyroid nodule with eggshell calcification on ultrasonography was reported in 1978 by Gooding [37]. Since then, thyroid ultrasound progressed, but data regarding eggshell calcifications remains scarce. The number of studies providing information regarding eggshell calcifications of thyroid nodules is relatively small when compared to the large volume of data that is published in the specific fields of thyroid ultrasound, FNA, and post-operatory pathological reports. Concerning the specific histological profile amid thyroid malignancies, we discuss the following highlights.

Table 1: Main findings regarding thyroid nodules with eggshell calcifications according to our methods of research, including the PICO analysis [22-35]

First Author/ Publication year/ Reference number **Study design Studied population *P (patients)***	**Positive diagnosis method *I (intervention)***	**Nodules *C (comparison)***	**Main findings *O (outcome)***
Wang/ 2022/[24] Retrospective analysis N=950 subjects (208 males and 742 females) Mean age: 47.0±12 y N=527 patients with papillary carcinoma	surgery	N=1113 Mean size=13.8±9.9 mm Benign: N=486 Malignant: N=627, out of which 527 had aggressive behavior	Eggshell calcifications were a protective factor for papillary carcinoma (OR=0.208, CI=0.049–0.886, p=0.034) Eggshell calcifications were not independent risk factors for aggressive behavior in papillary carcinoma
Yin/2020/[31] Retrospective N=543 Mean age: 49.3±13.2 y	surgery	N=543 Benign: N1=210 (38.67%), out of which 177 adenomas Malignant: N2=333 (61.32%) Macro-calcifications in 204 (37.6%)	Eggshell-in-a-row calcifications were more frequent in benign nodules (1.7% vs. 0.6%, p<0.01) Eggshell discontinuous calcifications were more frequent in malignant nodules (2.2% vs. 0.6%, p<0.01) In benign nodules (N=210): eggshell in a row calcifications N=9 (only adenomas - 7.69%) eggshell discontinuous calcifications N=3 (only adenomas - 2.56%) In malignant nodules: eggshell in a row calcifications, in medullary carcinoma N=3/3 eggshell discontinuous calcifications, in papillary carcinoma N=12/312 (3.84%)
Ha/2019/[25] Retrospective N=954 Mean age: 50.8 y (range: 13–86 y)	surgery CNB FNA	N=1112 nodules (≥ 5 mm) Nodules with echogenic foci N=390 Benign: N=136 Malignant: N=254 Nodules with eggshell calcifications N=21	Nodules with eggshell calcifications (N=21) were more frequently benign (66.7% benign vs. 33.3% malignant, p=0.002 In malignant nodules: surgery (N=413) or CNB (N=4) In benign nodules: surgery (N=65), repeated FNA or CNB (N=41), FNA or CNB with no change in size >12 months (N=589)
Kobayashi/2018/[23] Retrospective N=2678 subjects	surgery	N=2902 Nodules with calcifications=747 (26%) Among patients who underwent surgery: papillary carcinoma N=941 diffuse sclerosing variant of papillary carcinoma N=18 cyst-forming type of papillary carcinoma N=32 metastatic lymph nodes from papillary carcinoma 161 nodes follicular carcinoma N=337 undifferentiated carcinoma N=41 medullary carcinoma N=137 primary thyroid lymphoma N=173	Eggshell calcifications were found in: papillary carcinoma N=45/941 (4.78%) metastatic lymph nodes from papillary carcinoma N=2/161 (1.24%) follicular carcinoma N=40/337 (11.86%) undifferentiated carcinoma N=18/41 (43.90%) No eggshell calcifications were found in diffuse sclerosing and cyst-forming variants of papillary carcinoma, medullary carcinoma and primary thyroid lymphoma.

Zayadeen/2016/[30] Retrospective N=1466 patients (1201 female and 265 male, female-to-male ratio=4.75:1 Mean age: 56 y (range: 11–96 y)	FNA (N=1991) and/or surgery (N=376)	N=2002 nodules 348 nodules in male patients (17%) and 1654 nodules (83%) in female patients Malignant: N=148 (7.4%)	Eggshell calcifications in 45 benign lesions (from 1854 confirmed benign nodules = 2.42%) and 11 malignant lesions (from 148 malignant nodules = 7.43%)
Anderson/ 2014/[34] Retrospective N=393 (328 females, 65 males) subjects with single nodules with non-diagnostic FNA Median age: 57 y (range: 12-94 y)	Following non-diagnostic FNA: Repeated FNA and/or surgery and/or US follow-up	N=393 Nodules with eggshell calcifications N=9/393 (2.3%, 95% CI: 1.1%, 4.3%)	Nodules with eggshell calcifications did not associate with malignancy Increases in volume, anteroposterior diameter, mean or minimum diameter were associated with increases in odds of malignancy
Arpaci/2014/[26] Retrospective N=269 subjects (79.9% female) Mean age: 56.9±13.1 y	FNA (N=907) Surgery (N=43)	N=907 Mean diameters: with macro-calcification: 23.92 ± 14.15 mm without macro-calcification: 15.72 ± 7.53 mm Nodules with macro-calcifications were larger ($p < 0.001$)	Benign nodules rates were higher in nodules without eggshell calcifications compared with nodules with eggshell calcifications (80.3% vs. 67.9%, $p=0.036$) Suspicion for malignancy rates was higher in nodules with eggshell calcifications (7.5% vs. 1.9%, $p=0.010$) In nodules with vs. without eggshell calcifications, no statistical significance was found for: malignant and non-diagnostic rates, pathology results in patients who underwent surgery
Lee/2013/[22] Retrospective N=92 (63 females, 29 males)	FNA (N=103)	N=103 After FNA: Benign N=31 Malignant N=69 Non-diagnostic N=3 After histological confirmation/follow-up: Benign N=34 Malignant N=69	No statistically significant association between eggshell calcification and malignancy
Kim/2013/[27] Retrospective N=1078 subjects (171 males and 907 females) who underwent thyroidectomy for nodular disease Mean age: 47.2 ± 11.1 y (range: 16-82 y)	surgery	N=1432 Benign: N=126 Malignant: N=1305	There was no statistically significant association between annular calcifications and malignant or benign nodules Prevalence of annular calcifications in malignant nodules=31/1305 (2.4%) Prevalence of annular calcifications in benign nodules=5/126 (5%)
Cai/2010/[33] Case-control N=27 cases with medullary carcinoma (13 males and 14 females) Mean age: 45.7±12.8 y N=50 controls (5 males and 45 females) Mean age: 47.2±11.6 y	MTC - surgery controls - NA	N=35 Sizes: 2.39±1.22 cm in the medullary cancer group and 2.42±1.00 in controls - a difference not statistically significant	Eggshell calcifications were found only in controls (N=4) Macro-calcifications were more frequent in patients with medullary carcinoma
Anderson/2010/[28] Retrospective N=61 subjects with nodular Hashimoto's thyroiditis (54 females and 7 males) Median age: 49 y (range: 28–87 y)	FNA	N=64 average mean diameter=15.3 ± 7.33 mm average maximum diameter=19.5 ± 10.39 mm average minimum diameter=11.5 ± 5.63 mm	20% (13/64) nodules had calcifications 4.7% (3/64) nodules had eggshell calcifications 23% (3/13) calcifications were eggshell Eggshell calcifications - more frequent in subjects with normal parenchyma

Anderson/2010/[32] Retrospective N=78 subjects with Hashimoto thyroiditis and nodules Subjects with benign nodules: 85% females Median age: 50 y (range: 30–89 y) Subjects with malignant nodules: 92% females Median age: 54 y (range: 35–89 y)	FNA or surgery	N=82 Benign: N1=69 (84%) Malignant: N2=13 (16%) (12 papillary carcinoma and 1 lymphoma)	Eggshell calcifications were present in: 15% of malignant nodules and 1% of benign nodules - the difference was not statistically significant
Kim/2008/[35] Retrospective N=92	surgery	N=93 nodules and eggshell calcification who underwent surgery Benign: N=34 Malignant: N=59 (papillary carcinoma) Sizes from 4 to 50 mm (mean=16 mm), with no statistically significant difference between groups	In nodules with eggshell calcification, the highest sensitivity for malignancy was found in halo (62.7%) and disrupted calcification rim (76.3%)
Yoon/2007/[29] Retrospective N=65 (57 females) no statistically significant difference in age between benign (54.3 ± 12.7 y) and malignant (54.3 ± 12.7 y) groups	FNA (N=44) surgery (N=3) both (N=18)	mean sizes ± SD: benign nodules (N=53): 13 ± 8 mm (range=3–37 mm) malignant nodules (N=9): 14.4 ± 6.8 mm (range=5–28 mm)	No statistically significant difference between benign and malignant groups in terms of the presence and type of peripheral calcifications

Abbreviations: CI=confidence interval; CNB=core needle biopsy; FNA=fine needle aspiration; N=number of patients; OR=odds ratio; vs.=versus; US=ultrasound; y=years

Papillary thyroid carcinoma

Papillary carcinoma accounts for the majority of thyroid cancer cases and has increased in prevalence in recent years, mainly through the increased use of diagnosis techniques, including ultrasonography and FNA. Even though its prognostic is usually good, properly identifying cases remains an important link in the management of these patients [38-40]. While the presence of micro-calcifications is a highly suggestive feature and may even predict cervical lymph node metastasis [41], the implications of macro-calcifications such as eggshell calcifications are not so clear until the present time thus their importance for daily practitioners in the field of ultrasound of any type, endocrinology or endocrine surgery as well as primary health care. None of the studies linked eggshell calcifications to a higher risk of malignancy. However, many of them did not reach statistical significance for tested hypotheses [22,26,27,29].

One of the most important findings was provided by Wang et al. [24], who found that eggshell calcifications decreased the risk of papillary carcinoma and did not promote aggressive tumor behavior [24]. Even though data does not link eggshell calcifications with malignancy, they have been reported in patients with papillary carcinoma [23,28]. When assessing a nodule with eggshell calcifications, other characteristics should also be taken into consideration, and the risk of papillary carcinoma, even though small, should not be completely overlooked [42].

Medullary thyroid carcinoma

Medullary type of carcinoma involves ultrasound as the most important preoperative imaging technique, as stated by the ATA 2015 guidelines [43], aiding not only in identifying the solitary nodule but also in investigating lymph node metastases [44]. Usually, nodules appear hypoechoic, with a patchy structure, vascularized, and may have micro- or macro-calcifications [24,45-47].

When analyzing data regarding eggshell calcifications and medullary carcinoma, evidence was scarce, and three studies were included [23,31,33]. Even though the largest one did not find any eggshell calcifications in patients with medullary carcinoma, the study of Yin et al. [31] found these calcifications in all three patients with medullary carcinoma. One important difference between these studies is the fact that Yin et al. [31] included only patients with calcifications, while Cai et al. [33] focused on ultrasound features of medullary carcinoma in general. Even though the study of Cai et al. [33] included more patients with medullary carcinoma (N=27), overall, the study of Yin et al. [31] identified the three medullary carcinoma patients from a larger pool of patients with thyroid nodules and calcifications (N=543) [31,33]. Despite the rarity, it is important to keep in mind that there have been cases of eggshell calcifications in patients with medullary carcinoma so that the diagnosis is not overlooked solely based on this characteristic. Further research is needed to understand the significance of egg-shell calcification in relation to the medullary type of carcinoma.

Follicular thyroid carcinoma

Kobayashi et al. [23] found eggshell calcifications in 11.86% of patients with follicular carcinoma and nodules with calcification [23]. Apart from this large study, eggshell calcifications have also been mentioned in case reports of patients with follicular carcinoma. Cai et al. [48] reported the case of a 67-year-old female who presented with a solitary kidney tumor diagnosed by pathology as follicular thyroid carcinoma. Thyroid ultrasound identified two different foci in the same lobe, and one of the nodules presented eggshell calcification with an incomplete halo [48].

Three more cases of follicular thyroid carcinoma were reported by Lee et al. [49]. Two of the three cases involved 75-year-old females who were diagnosed with bone metastases caused by a follicular type of thyroid cancer. Both patients had single nodules with interrupted eggshell calcifications on ultrasonography. A third case involved a 54-year-old female with hyperthyroidism and a thyroid nodule with interrupted eggshell calcifications classified as benign by FNA two years prior. A second FNA classified the nodule as indeterminate, and following thyroidectomy, the patient was diagnosed with follicular carcinoma [49].

It is important to emphasize that interrupted eggshell calcifications may be a sign of malignancy, as stated by the ETA 2017 guidelines [50]. Moreover, a high TI-RADS score remains important in malignancy detection in patients with eggshell calcifications as well, increasing positive predictive value [25].

Thyroid autoimmune disease

Hashimoto's chronic thyroiditis is the most common autoimmune thyroid disease and the main cause of hypothyroidism in the modern era [51]. It may lead to the development of nodules [52] and it is another thyroid condition where eggshell calcifications can be found with a low prevalence of less than 5% [32]. It is unclear why these calcifications are more frequent in nodular thyroiditis with otherwise normal gland parenchyma.

Another aspect worth mentioning is the association of Hashimoto's thyroiditis with thyroid cancers, including primary thyroid lymphoma and papillary carcinoma [53]. The connection with papillary thyroid cancer, however, is still debated, with some studies finding an increased risk while other studies finding thyroiditis as a protective factor [54-56]. This raises the question of how the presence of calcifications in general and eggshell calcifications in particular should be interpreted in patients confirmed with Hashimoto's thyroiditis based on serum thy-roid antibodies and/or cytological reports. The lack of statistically significant data regarding the prevalence of eggshell calcifications in benign versus malignant nodules requires larger studies to confirm or infirm the hypothesis that in patients with Hashimoto's thyroiditis, eggshell calcifications are more frequent in malignant nodules [28,32].

Further researches

For some thyroid pathologies, evidence regarding eggshell calcifications remains at the case report level. Malignancies previously found in patients with nodules with eggshell calcifications include oncocytic thyroid cancer [57], and primary squamous cell carcinoma [58], a rare tumor with similar behavior to anaplastic carcinoma [59]. Other benign conditions reported include follicular thyroid adenoma [60] and Hürthle cell adenoma [61]. Hürthle cell adenomas and carcinomas are classified as subtypes of follicular neoplasms, despite their distinct molecular pathogenesis [62]. Recently, Hürthle cells have been renamed to oncocytic cells [63]. (Figure 1).

Of note, apart from thyroid disease, when faced with eggshell calcifications, clinicians should also be mindful of diseases in surrounding organs. One such example is the case reported by Ahn et al. of a 36-year-old female who underwent a thyroid ultrasound due to a complaint of a cervical mass [64]. The ultrasound revealed a thyroid mass with eggshell calcification. However, surgery revealed that the mass was an oesophageal tumor, and pathology diagnosed it as a schwannoma [64].

Overall, the identification of calcifications at thyroid ultrasound should be assimilated to the larger frame of malignancy criteria according to the current guidelines in the thyroid field [65-67].

On a larger scale, we mention the fact that calcium deposits represent incidental findings in many medical areas outside the specific thyroid exploration, including other malignancies (at the neck, thorax, and mediastinum), infectious diseases, inflammatory, and autoimmune conditions [68-72].

Figure 1: This is a prior unreported unusual case of eggshell calcifications on an 80-year-old male admitted for enlargement of a prior goiter (that he refused to investigate for more than one decade); due to compressive neck symptoms, total thyroidectomy was done with a good post-surgery outcome; the pathological report showed a follicular adenoma with necrosis and hemorrhage

1A. Pre-operatory interrupted eggshell calcification at thyroid ultrasound

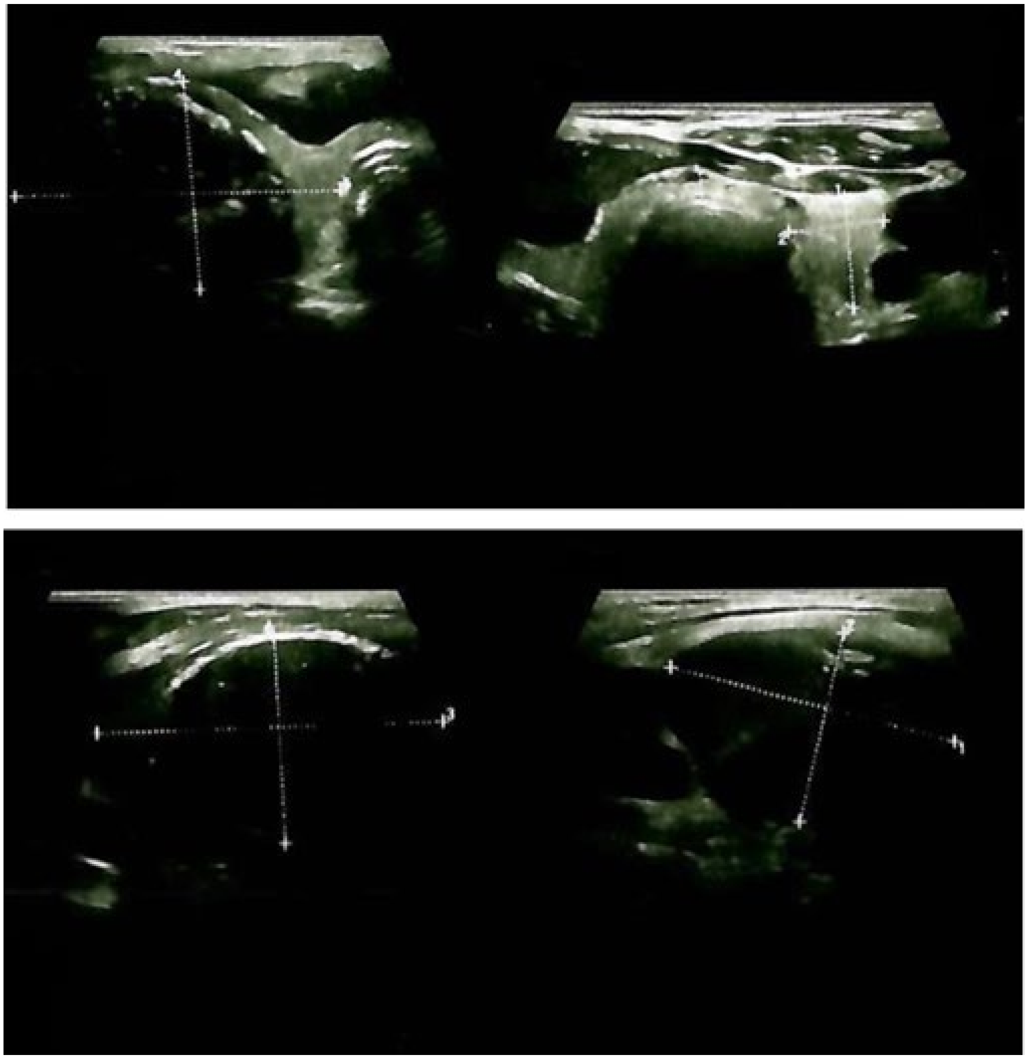

1B. Contrast computed tomography (blue signs show eggshell calcifications)

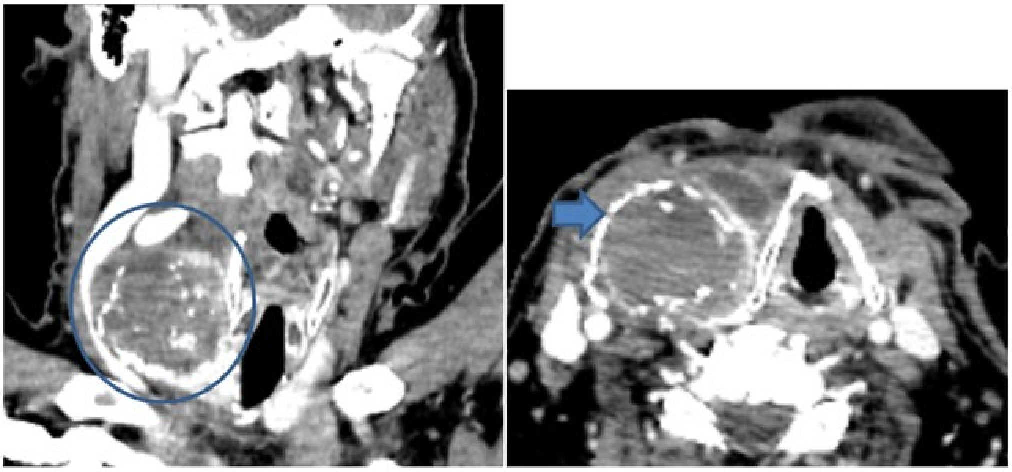

1C. Intra-operatory aspects: right recurrent laryngeal nerve (yellow arrow); trachea (blue arrow); right external jugular vein (green arrow); right common carotid artery (pink arrow); right superior thyroid pedicle (white arrow); right thyroid lobe (black arrow)

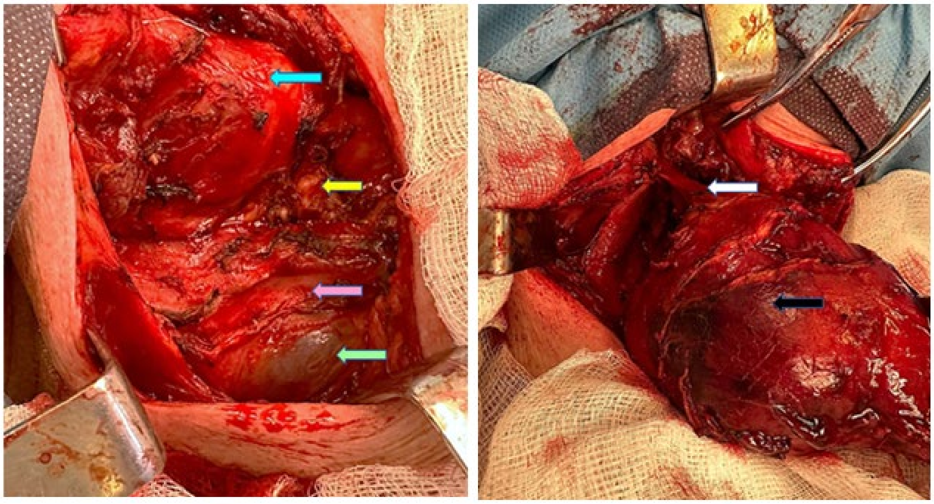

1D. Post-operatory macroscopic specimen (yellow arrow pointing at left lobe)

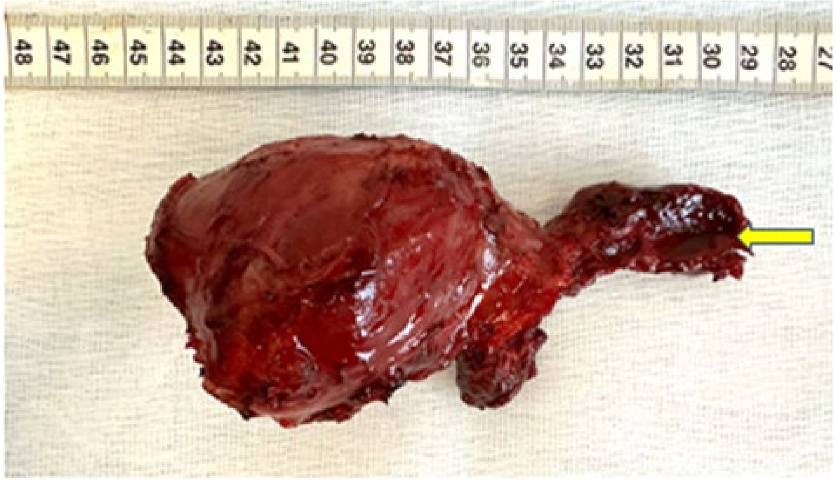

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1E. 1-month post-operatory neck ultrasound check-up shows no thyroid remnants

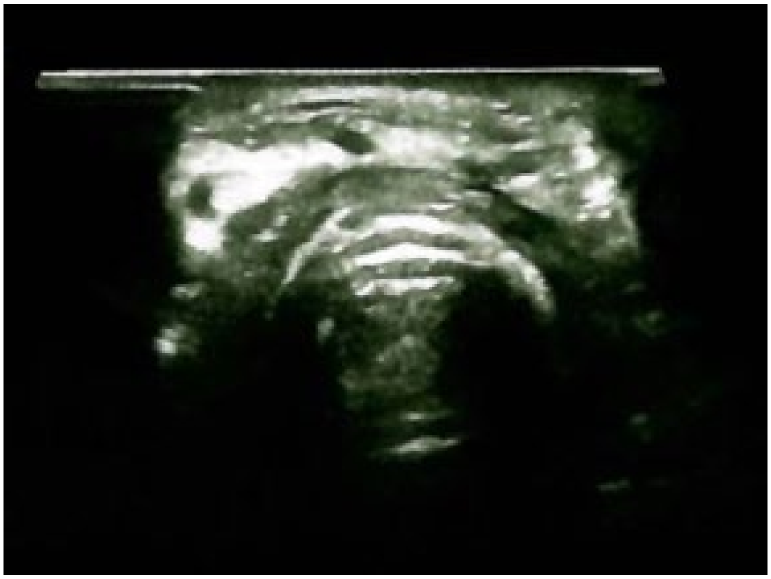

The most common clinical entities associated with eggshell calcification in these mentioned anatomic areas may involve lymphomas, amyloidosis, sarcoidosis, tuberculosis, scleroderma, etc. [73,74]. Another pathogenic trait of calcifications includes calcium and phosphorus anomalies of signal transduction pathways as seen in primary hyperparathyroidism, but, mostly, in chronic renal disease-related tertiary (or secondary) hyperparathyroidism (especially in patients undergoing different types of dialysis) and hypoparathyroidism underlying dysfunction of parathormone and even 25-hydroxyvitamin D regulation [75-77]. Arterial (and generally endothelial) calcifications may be a sign of increased cardiovascular disease in addition to the mentioned anomalies. This might add to a globally increased disease burden leading to a poor outcome [78-82].

CONCLUSIONS

A high TI-RADS score remains important in malignancy prediction while eggshell calcifications might add to the positive predictive value, yet, as a single input this trait is difficult to be classified as a standalone contributor element. Based on these findings, it is safe to say that when faced with eggshell calcifications on thyroid ultrasound, especially if they appear interrupted, clinicians should not rule out the possibility of thyroid carcinoma, as pointed out by our research (Table 1).

Despite the clinical and epidemiological significance, the accurate diagnosis, monitoring of the diagnosed patients, and available licensed treatments remain the challenges of NASH.

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Authors' contribution
Conceptualization, F.I.R., A.-M.G., O.-C.S., M.C., and C.N.; methodology, F.I.R., A.-M.G., M.C. and C.N.; software, F.I.R., A.-M.G., M.C., and C.N.; validation, F.I.R., A.-M.G., O.-C.S., and C.N.; formal analysis, F.I.R., A.-M.G., O.-C.S., M.C., and C.N.; investigation, F.I.R., A.-M.G., O.-C.S., M.C., and C.N.; resources, F.I.R., M.C., and C.N.; data curation, F.I.R., A.-M.G., O.-C.S., M.C. and C.N.; writing—original draft preparation, A.-M.G.; writing—review and editing, F.I.R., A.-M.G., M.C. and C.N.; visualization, F.I.R., A.-M.G., O.-C.S., M.C. and C.N.; supervision, F.I.R., M.C. and C.N.; project administration, F.I.R; funding acquisition, F.I.R. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The Local Committee of Ethics of Dr. Carol Davila Central Military Emergency University Hospital, Bucharest, Romania approved the release of these data (number 608/2023).

Patient consent for publication

The patient signed informed consent during hospitalization and gave us permission to use his medical and surgical records.

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SYSTEMATIC REVIEW

A Systematic Review of the Influence of Aquatic Exercises on the Physical and Psychological Health of Children with Autism Spectrum Disorder

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Abstract: *Background: Children with Autism Spectrum Disorder (ASD) show significant motor deficits and behavioral dysfunctions, as part of their clinical manifestations. Also, these children are facing important difficulties in social learning, which negatively impacts their adjustment to society's requirements. Apart from other modes of exercise, aquatic exercises may be more attractive to children. Aquatic exercises have been shown to have a beneficial effect on gross motor skills and executive functions in children with ASD. Aim: To study the influence of aquatic exercises on the physical and psychological health of children with ASD. Methods: Researchers used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting systematic reviews and meta-analyses. Researchers looked for articles on PubMed, Google Scholar, and by manual search. Searched articles were screened for relevancy. Potential articles were selected using inclusion and exclusion criteria. Results: Out of the 231 articles, four studies were included in the current systematic review. The age of participants ranged from 3−15 years. Overall, included studies found improvements in various aspects of gross motor and executive functions by aquatic exercise in children with ASD. Conclusion: Aquatic exercises can provide health benefits to children with ASD. Aquatic exercises can be useful as a part of therapy in children with ASD. There is wide scope for further studies to evaluate the beneficial effects of aquatic exercises.*

Keywords: *aquatic exercise, autism spectrum disorder, motor skills, cognitive functions*

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by repetitive behaviors, and deficiencies in social and communication skills [1,2,3]. Because of motor deficits, persons with ASD are not involved in social learning experiences to acquire sensory information the same way as others [3,4,5]. Children with ASD often show problems of behavior that negatively impact involvement in the community [6]. Significantly more aggression, inattention, and withdrawal behaviors are shown in children with ASD [6,7]. Self-injurious and stereotypical behaviors are also seen in children with ASD [6].

As movement disorders are seen in ASD [3,4], the goal of physical activity is to control the neural mechanisms that support the acquisition and retention of new skills [3,8]. Exercise programs are useful for improving motor and cognitive skills in children with ASD [3,9,10]. Apart from the other modes of physical exercise, the use of aquatic exercise in children with disabilities is appealing as they are usually not interested in regular physical exercises that may not be attractive to them [3,11,12,13,14,15].

This systematic review evaluates the influence of aquatic exercises on the physical and psychological health of children with ASD. Aquatic exercise's valuable response may provide good hope for therapies in children with ASD.

MATERIALS AND METHODS

Researchers used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting systematic reviews and meta-analyses (Figure 1) [17].

Search Strategy

Relevant studies were identified by a literature search of the papers published only in English, in electronic databases of Google Scholar and PubMed Central. Words used in the search strategy were “therapeutic effect”, “autism spectrum disorder”, “aquatic exercise”, “motor skill”, “cognitive functions”, “social activities”, and “psychological health”. The search string used for PubMed search is (autism spectrum disorder) AND (aquatic exercises OR aquatic therapy) AND (cognitive functions) AND (motor functions) AND (social activities) AND (psychological health). For the search interval, the start date restriction was not applied, and the end date was kept till the end of 2023. A literature search was also done directly online through Google search. A reference list of filtered studies was also searched to get relevant articles, while the grey literature was not searched.

Figure 1: Flow chart article search and study selection

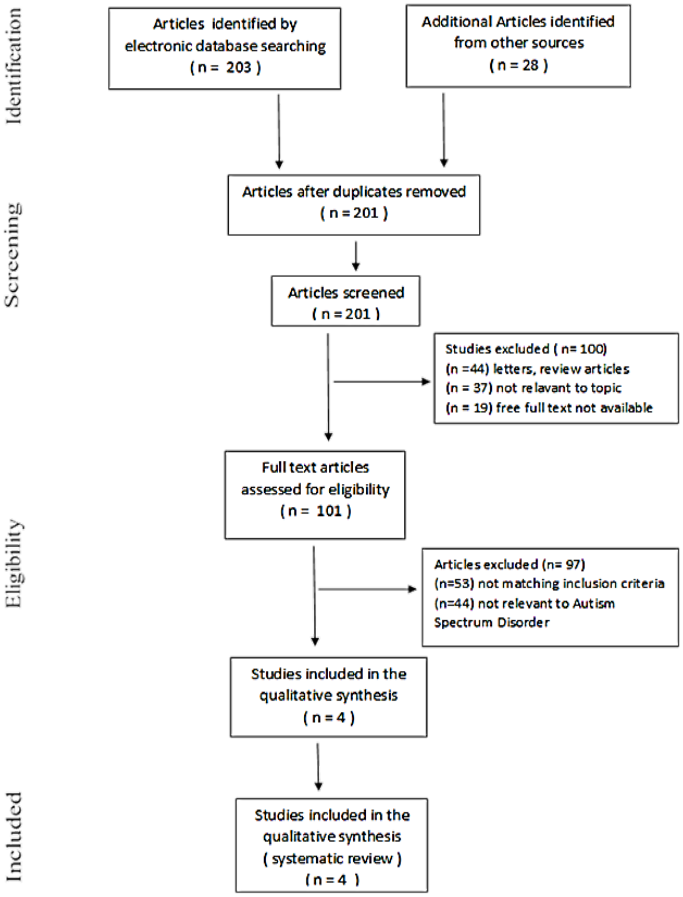
Identification
Articles identified by electronic database searching (n = 203)
Additional Articles identified from other sources (n = 28)
Screening
Articles after duplicates removed (n = 201)
Articles screened (n = 201)
Studies excluded (n= 100)
(n =44) letters, review articles
(n = 37) not relavant to topic
(n = 19) free full text not available
Eligibility
Full text articles assessed for eligibility (n = 101)
Articles excluded (n= 97)
(n=53) not matching inclusion criteria
(n=44) not relevant to Autism Spectrum Disorder
Studies included in the qualitative synthesis (n = 4)
Included
Studies included in the qualitative synthesis (systematic review) (n = 4)

Studies Selection Criteria

Studies filtered in the primary search were assessed for the following inclusion and exclusion criteria. PICO (an acronym for Population, Intervention, Comparison, Outcome) criteria [18,19,20,21], as described in Table 1, was used to retrieve relevant studies.

Table 1: Search strategy (PICO criteria)

	Definition
Population	Children with ASD
Intervention	Aquatic exercises
Comparison	N/A
Outcome	Assessing physical and psychological health through questionnaires or measurement

Abbreviations: ASD - autism spectrum disorder; N/A- not available; PICO - Population, Intervention, Comparison and Outcome

Inclusion criteria:

- Studies including aquatic exercise
- Studies having participants with ASD
- Studies include the evaluation of the aquatic exercise effects

on participants

Exclusion criteria:
- Articles not relevant to ASD
- Free full-text articles not available of studies
- Type of articles as letters and review articles

Study Quality

Reviewers assessed studies independently for inclusion and exclusion criteria mentioned in the study selection criteria. During the detailed reading of studies, queries regarding inclusion and exclusion were sorted out and finally decided by discussion between the authors.

Data Extraction

Reviewers analyzed selected studies under the following headings: name of the first author with initials, year of publication, brief detail of study population, sample size, age, gender, brief detail of aquatic exercise, and brief detail of outcome measure. Reviewers verified the data, and any discrepancies were addressed by discussion and resolved by consensus between the authors.

Data analysis

We extracted aquatic exercise activities and outcome measures in individual articles. Aquatic exercise activities and outcomes in each individual study were analyzed, and collected data was compared.

RESULTS

Literature search

We found 231 eligible articles by combined electronic database search and manual search. After the removal of 30 duplicated studies, the remaining 201 studies were screened by titles and abstracts. In the screening process, a total of 100 studies were excluded for reasons such as 37 studies were not relevant to the topic, 44 studies were letters or review articles, and a free full article was not available for 19 studies. Full-text articles were assessed for eligibility in the remaining 101 studies after the screening process. In this assessment, a total of 97 studies were excluded because 53 studies did not match the inclusion criteria, and 44 studies were not relevant to ASD. Finally, four articles were identified and included in the qualitative analysis. The flow diagram for the study selection process and the reason for exclusion is displayed in Figure 1.

Description of included studies

A brief description of each eligible four studies [3,6,12,22] is detailed in Table 2. The sample size of eligible 4 studies ranged from 7 to 40 participants. All eligible studies were published between 2016 and 2023. Out of the four eligible included studies, one study [3] has not provided information on participants' gender. All four eligible studies [3,6,12,22] included participants with ASD. The ages of participants ranged from 3 to 15 years. Out of four eligible included studies, one study [22] has not provided information on the inclusion and exclusion criteria of participants.

Table 2: Summary of Characteristics of the included studies

First Author, Year	Brief detail of the study population	Sample Size	Age group (Year)	Gender	Brief detail of aquatic exercise	Brief detail of outcome measure
Alaniz LM [12] (2017)	Participants with ASD were recruited. A swim lesson history was gathered. Inclusion and exclusion criteria are described in the study.	7	3 to 7	Both	The intervention was implemented over 3 phases, with eight 1-hour sessions in each phase. Participants attended 8, 16, or 24 hours of group intervention provided in 1-hour increments, once a week. Aquatic occupational therapy intervention is described in the study.	Single-group pre-test and post-test designs were used. Social skills were calculated using a standardized questionnaire, as described in the study, completed by the caregiver before session 1 and again after session 8, session 16, and session 24.
Adin E [22] (2023)	15 individuals with autism, 8 of which were in the experimental group and 7 in the control group, participated in the study	15	10 to12	Both	The experimental group was subjected to swimming exercise for 1 hour, 3 days a week, for 6 weeks. The control group was not included in this exercise.	The respiratory functions and respiratory muscle strength measurements of the subjects were taken 1 day before and 1 day after the 6-week training program. The same pretest–post-test period was applied to the control group.
Faraji S [3] (2023)	40 participants with ASD were recruited and then randomly assigned to a response-oriented aquatic exercise group	40	7 to 10	-----	The response-oriented aquatic group participated in an aquatic program for 8 weeks, three times weekly, for 45 minutes each session at an indoor pool. The control group participants were requested	The pre-and post-test designs were used. Motor skills and executive function were assessed in both groups as described in the

	(n=20) or a control group (n=20). Inclusion and exclusion criteria are described in the study.				not to alter their physical activity patterns for the duration of the study. The control group maintained their daily activities and did not change their physical activities during the intervention.	study, before and after the intervention.
Kanupka JW [6] (2016)	Children with ASD were selected. Inclusion and exclusion criteria are described in the study.	13	3 to 15	Both	The aquatic exercise program was conducted for 1 hour, 1 day per week for 6 weeks. The exercise program consisted of a 10-minute warm-up, a 10-minute strengthening program, 15 minutes of games, a 15-minute free swim, and a 10-minute cool-down, which were all completed in the pool.	Outcome measures as described in the study assessed before and after completion of the aquatic exercise program.

ASD – Autism Spectrum Disorder

In the study by Alaniz LM [12], “social skills” were calculated using the Social Skills Improvement System (SSIS) [23], a 79-item standardized questionnaire completed by the caregiver before session 1 and again after session 8, 16, and 24. The SSIS [23] assesses social skills like cooperation, communication, assertion, empathy, responsibility, engagement, and self-control and problem behaviors like bullying, externalizing, internalizing, hyperactivity/ inattention, and autism spectrum symptoms [12].

In the study by Adin E [22], forced vital capacity measurement, Vital capacity measurement, Breath inspiratory flow, and peak expiratory flow calculation were used.

In the study by Faraji S [3], The Bruininks-Oseretsky Test of Motor Proficiency – Second Edition (BOTMP-2) Short Form [24] was used. This instrument uses 8 subscales, including 14 separate parts, to comprehensively measure motor skills. Four subtests measure fine motor competence: 1) fine motor precision (FMP), 2) fine motor integration (FMI), 3) manual dexterity (MD), and 4) bilateral coordination (BC), and four subtests assess gross motor competence: 1) balance, 2) running speed and agility (RSA), 3) upper-limb coordination (ULC), and 4) strength [3,10,24]. In the study by Faraji S [3], to assess executive function, the computer version of the Wisconsin Card Sorting Test (WCST) was used [25]. This comprises 4 stimulus cards and 128 response cards [3,25].

In the study by Kanupka JW [6], outcome measures included The Pervasive Developmental Disorder Behaviour Inventory (PDDBI) [26] and the Intervention Rating Profile (IRP-15) [27,28,29,30]. The PDDBI is a rating scale designed to assess responsiveness to intervention in children diagnosed with one of the Pervasive Developmental Disorders (PDD) [6,26]. PDDBI subdomains are Sensory/Perceptual Approach Behaviours, Ritualism/Resistance to Change, Social Pragmatic Problems, Semantic/Pragmatic Problems, Arousal Regulation Problems, Specific Fears, Aggressiveness, Social Approach Behaviours, Expressive Language, Learning, Memory, and Receptive language [6,26]. The IRP-15 [6,27,28,29,30] was used to assess the social validity of aquatic exercise as an intervention to improve behavior in children with ASD [6]. One parent of each of the 13 participants reported on the social validity of aquatic exercise as an intervention for their children using a modified version of the IRP – 15 [6,27,28,29,30]. Parents in the study by Kanupka JW [6] rated their opinions of the effectiveness of the aquatic exercise program on a 15-question 6-point scale ranging from 1 (strongly disagree) to 6 (strongly agree), with total scores ranging from 15 to 90 and higher scores indicating greater acceptability [27,28,29,30]. A total score on the IRP - 15 greater than 52.5 indicates that the intervention is acceptable [6,27,28,29,30].

Outcome

All included studies used pre and post-test designs. Alaniz LM [12] observed significant improvement in the overall swim skills score. When examining individual swim skills, the participants advanced most consistently with breath control [12]. At all three time points, the participants, on average, significantly increased their breath control score compared to that of the pre-evaluation [12]. The changing position skill demonstrated significant improvement at 16 and 24 hours [12]. Propulsion skills improved after 24 hours of intervention only [12]. Navigation, exiting the pool, and back float skills did not improve significantly as measured on the Aquatic Skills Checklist (ASC). [12]. Alaniz LM [12] reported analysis of the Social Skills Improvement System (SSIS) [23] scores from baseline to 8 hours of intervention revealed no significant difference in social skills. There was a high rate of noncompliance for completion of the SSIS [23] after 16 and 24 hours of intervention, which prevented statistical analysis of the data [12].

Adin E [22] observed a significant difference between the pre- and post-test in the forced vital capacity (FVC) and forced expiratory volume (FEV1) values of the experimental group. There was no significant difference between the pre-test and post-test in the FVC and FEV1 values of the control group.

The results of the study by Faraji S [3] demonstrated improvements in gross motor skills and executive function following 8 weeks of response-oriented aquatic exercise. Faraji S [3] showed significant differences between re-sponse-oriented aquatic exercise and control groups in receiving, throwing, static balance, dynamic balance, correct responses, conceptual responses, and perseverative errors. A significant difference was observed in the study [3] between response-oriented aquatic exercise groups and control groups from pre- to post-test in bilateral coordination (BC).

In the study by Kanupka JW [6], there was a statistically significant difference in the scores for pre-arousal and post-arousal while there were no other statistically significant differences for pre and post-behavioral subdomains of the PDDBI [6,26]. In the study by Kanupka JW [6], the ratings for all 15 questions on the IRP - 15 ranged from 62 – 90, these scores were all above 52.5, indicating that aquatic exercise is an acceptable intervention for children with ASD [27,28,29,30]. In the study by Kanupka JW,[6], all of the parents agreed the aquatic exercise intervention had a positive effect on their child's behavior (92.3% strongly agree/agree, 7.7% slightly agree), and parents also all indicated that they would be willing to use the intervention again in the future (100% strongly agree) [27,28,29,30].

DISCUSSION

Studies included in the systematic review investigated the effect of aquatic exercises on the physical and psychological health of children with ASD. Overall, included studies found improvements in various aspects of gross motor and executive functions by aquatic exercise in children with ASD.

Studies [3,31,32,33] in the past have shown an improvement in gross motor skills and executive functions with aquatic exercise programs in individuals with ASD. Aquatic exercise may improve arousal levels in children with ASD. [6]. Improved arousal may help children with ASD to learn more efficiently and interact in the aquatic environment [6,16]. A substantial increase in paying attention, tolerating touch, and maintaining eye contact is observed by therapists who have worked with children with ASD in aquatic environments [6,16].

Aquatic therapy may cause complex adaptations of the neuromuscular system and cause improvement in the transmission of neural impulses [3,34]. Aquatic therapy may increase muscle strength by a resistive medium without much pressure on the weight-bearing joints [3,35]. These improvements in executive function in children with ASD could be because of the improvement in activation of the neuron tract and transfer of messages from the brain to the body to perform movement in children with ASD [3,36]. The aquatic environment provides individuals with three-dimensional movements for continuity of movement, which gives more strength to coordination, temporal-spatial knowledge, kinaesthetic perception, and balance [37].

Limitations

In this review, the effect of aquatic exercise on ASD is the primary focus. However, the schedule and duration of the aquatic exercise program module were different among the selected studies. Parameters to measure outcomes were also different among selected studies. As a similar module of the aquatic exercise program is not used in the different studies explored and a similar outcome parameter is not used, it is difficult to generalize the interpretation of the individual studies. Other health problems were not screened for in the study selection criteria. The sample size was also comparatively small in selected studies. No paid tool or utility was used in the assessment of the quality of studies at any stage, which may increase the risk of bias. Another limitation of the search strategy was the English language, and articles that were not freely available also decreased the number of reviewed articles. The selection of articles was done by using only a pre-defined set of electronic databases. Therefore, studies of other electronic databases, which were not included in the search strategy, may contain relevant articles for the topic of this review.

Recommendations for future research

For a more specific evaluation of the positive effects, future research should focus on specific health benefits of aquatic exercise in individuals with ASD. Research should be done using a comparatively large sample size, proper methodology, and standardized outcome measures.

CONCLUSIONS

The present review focused on the effect of aquatic exercises on the health of individuals with ASD. Overall, included studies found beneficial effects of aquatic exercise in individuals with ASD. Further research is required to explore the long-term effects of aquatic exercise and to explore effects with optimal frequency and duration of sessions. However, the role of aquatic exercise seems promising in ASD, but before utilizing it in practice and treatment, properly designed studies are required to examine these effects in more detail, with adequate specificity and accuracy.

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Authors' contribution
Dholakiya JR contributed to the manuscript preparation and assembly of data. Ghuntla TP contributed to the interpretation of results and literature review. All

authors agree with the content of the manuscript.

Ethics approval and consent to participate
Not applicable.

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ORIGINAL ARTICLE

Extracting Data from Medical Records for Monitoring Diseases and Generating Medical Alerts

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Abstract: *Background: Automated data processing is creating and implementing technology that automatically processes data. This computer tool is recommended for doctors because it supports their everyday work, assists in medical diagnosis, and enhances patient care. The aim of this paper is to propose an informatic tool that can extract the values of some parameters of interest from blood test sheets in order to get medical alerts and to monitor the chronic disease. Methods: An application, written in Python, was developed Results: The computer tool extracted automatically the values of glucose, triglycerides, HDL-cholesterol, total cholesterol, and LDL-cholesterol from medical sheets (text-based file or graphic file, respectively), and saved them in a database, accessed and represented in a graphic form the most recent values of these parameters; alerts according to metabolic syndrome criteria and Framingham risk score were generated. Conclusions: This tool contributes to the management of the medical process, saving precious time and helping the doctor in detecting current or future health problems.*

Keywords: *process automation in medicine; Python; medical alerts; metabolic syndrome; Framingham risk score*

INTRODUCTION

Process automation is defined as using software and technologies to automate business processes and functions to accomplish well-defined organizational goals. Automated data processing is creating and implementing technology that automatically processes data [1].

Automated systems, meant to emulate human cognitive capabilities, deploy supervised applications to perform repetitive tasks more accurately and efficiently, saving time and effort for high-volume workloads. In medicine, artificial intelligence has become a valuable tool for improving patient outcomes, particularly in diagnostics, by extracting information from image and text-based files [2].

Chronic patients are followed for years and the disease is monitored according to the clinical status and certain biological parameters. Therefore the medical data about them, saved and quickly accessed, can be of real use in the medical act.

Physicians consult many patients daily, being busy, apart from the actual medical act, with other tasks (electronic filling in of various data, issuing prescriptions, administrative work). Due to the relatively limited time allocated to a consultation, doctors can benefit, using this IT tool, from real support in their work.

Although the computer tool we propose was originally intended for family doctors, specialist doctors can successfully use it (by activating medical alerts, depending on the specialty), especially for chronic patients who return for regular consultations.

The aim of our application is the automation of the medical act, as follows:

- extracting (reading) automatically the values of some parameters of interest from blood test sheets (text-based pdf file or graphic file format);

- saving the blood values and anthropometric parameters for a patient in a database;

- activating medical alerts;

- monitoring the disease by displaying, in a graphic format (chart), the most recent values of the interest parameters**.**

MATERIALS AND METHODS

In this version, the application generates alerts according to metabolic syndrome and Framingham risk score.

Nowadays, the well-established term ‘metabolic syndrome’ (MetS) remains the most useful and widely accepted description of this cluster of metabolically related cardiovascular risk factors, which also predict a high risk of developing diabetes [3].

Many expert groups have attempted to develop a unifying definition for the MetS. We focus, in this paper, on the NCEP ATPIII and IDF criteria.

The NCEP ATPIII (National Cholesterol Education Program - Adult Treatment Panel III) criterion establishes that meta-bolic syndrome is present if three or more of the following five risk factors [3,4]:

• raised triglycerides level: ≥ 1.7 mmol/l (150 mg/ dl);

• reduced HDL-cholesterol: < 1.03 mmol/l (40 mg/dl) in males and < 1.29 mmol/l (50 mg/dl) in females (or specific treatment for these lipid abnormalities);

• raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg) (or treatment of previously diagnosed hypertension);

• raised fasting plasma glucose ≥ 5.6 mmol/l (100 mg/dl);

• raised waist circumference >102 cm for men and >88 cm for women.

The new definition given by the International Diabetes Federation (IDF) says that for a person to be defined as having the MetS, he/she must have central obesity plus any two of four additional factors [3]:

• raised triglycerides level: ≥ 1.7 mmol/l (150 mg/ dl);

• reduced HDL-cholesterol: < 1.03 mmol/l (40 mg/dl) in males and < 1.29 mmol/l (50 mg/dl) in females (or specific treatment for these lipid abnormalities);

• raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg) (or treatment of previously diagnosed hypertension);

• raised fasting plasma glucose ≥ 5.6 mmol/l (100 mg/dl)] (or previously diagnosed type 2 diabetes).

Figure 1: Use-case diagram made in UML

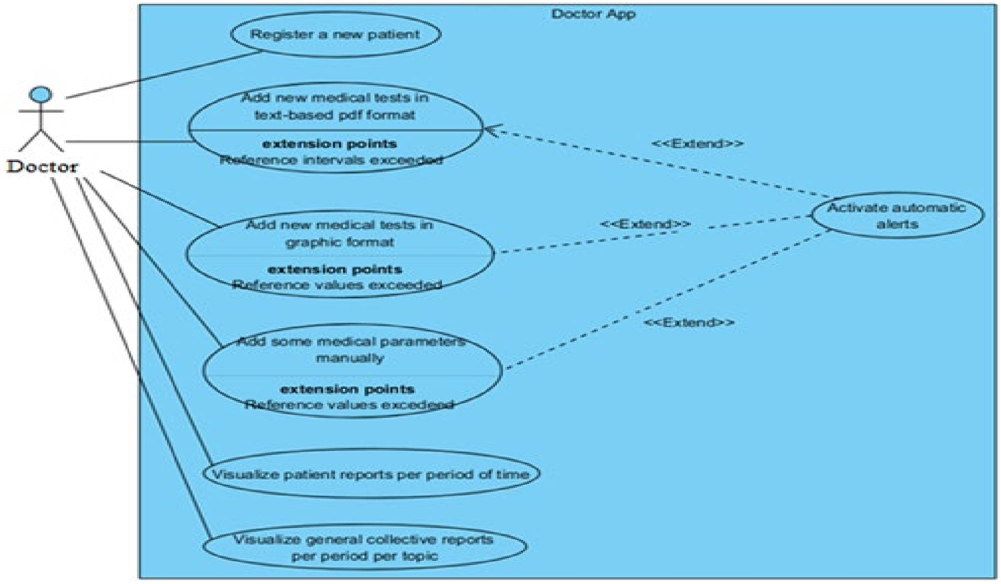
Doctor App
Doctor
Register a new patient
Add new medical tests in text-based pdf format
extension points
Reference intervals exceeded
<<Extend>>
Activate automatic alerts
Add new medical tests in graphic format
extension points
Reference values exceeded
<<Extend>>
Add some medical parameters manually
extension points
Reference values exceded
<<Extend>>
Visualize patient reports per period of time
Visualize general collective reports per period per topic

Central obesity is most easily measured by waist circumference with cut-points that are gender and ethnic-group specific. For example, an abnormal waist circumference for European males is ≥ 94 cm and for European females is ≥ 80 cm. The cut-points for central obesity adopted in the USA by the National Institutes of Health clinical guidelines for obesity were 102 cm for men and 88 cm for women. An abnormal waist circumference for South Asians and Chinese males is ≥ 94 cm and for South Asians and Chinese females is ≥ 80 cm [3,4].

Framingham Risk Score (FRS) and estimation of 10-year Cardiovascular Disease (CVD) Risk is an algorithm adapted from Canadian Cardiovascular Society/Canadian guidelines [5-7].

The PREDATORR (PREvalence of DiAbeTes mellitus, prediabetes, overweight, Obesity, dyslipidemia, hyperuricemia, and chronic kidney disease in Romania) study shows that the prevalence of diabetes mellitus was 11.6% and of prediabetes was 16.5% in the Romanian population aged 20-79 years [8].

Figure 2: Activity diagram made in UML

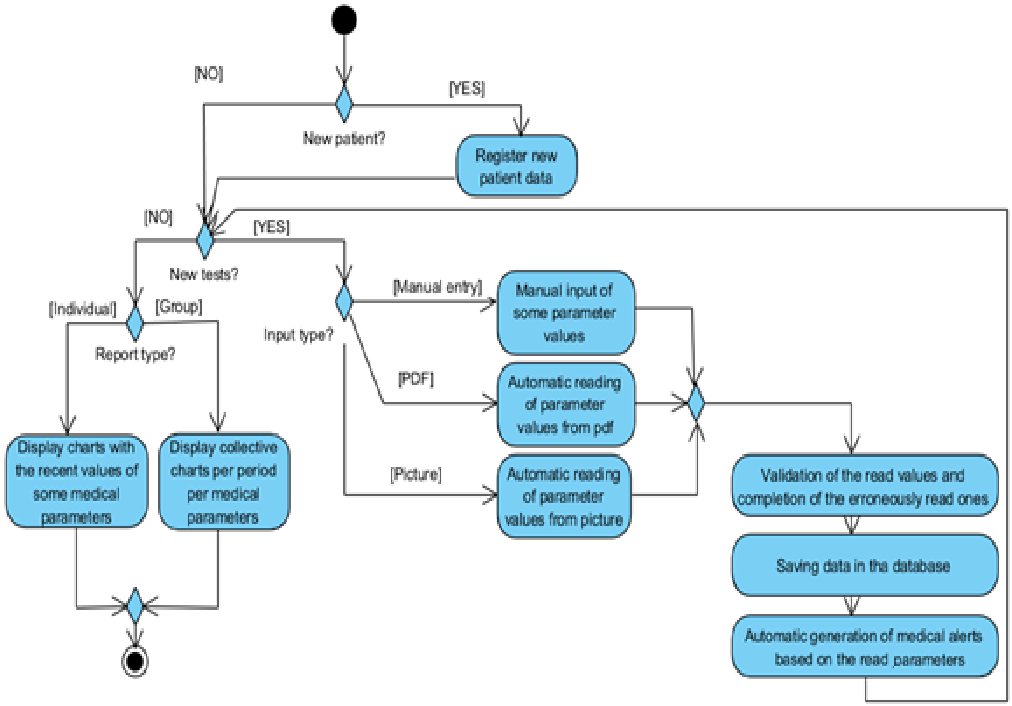
[NO]
[YES]
New patient?
Register new patient data
[NO]
[YES]
New tests?
[Individual]
[Group]
Report type?
Input type?
[Manual entry]
Manual input of some parameter values
[PDF]
Automatic reading of parameter values from pdf
[Picture]
Automatic reading of parameter values from picture
Display charts with the recent values of some medical parameters
Display collective charts per period per medical parameters
Validation of the read values and completion of the erroneously read ones
Saving data in tha database
Automatic generation of medical alerts based on the read parameters

The increased risk for cardiovascular disease in prediabetes is multifactorial, with etiologies including insulin resistance, hyperglycemia, dyslipidemia, hypertension, systemic inflammation, and oxidative stress. The preferred treatment is intensive lifestyle management and aggressive pharmacologic therapies directed toward individual coronary heart disease risk factors [9].

The early diagnosis and screening of prediabetes are essential steps toward the prevention of diabetes and associated complications. The American Diabetes Association (ADA) recommends screening adults over 45 years old without known diabetes every 3 years [10].

The Unified Modeling Language (UML) is a general-purpose visual modeling language that is used to specify, visualize, construct, and document the artifacts of a software system. It captures decisions and understanding about systems that must be constructed [11].

Because of its high level of functionality, Python language is used for web development, data science and data analysis, machine learning, startups, and the finance industry, among others [12].

Our application, written in Python, can be schematically described in Unified Modeling Language, using a use-case diagram (Figure 1), which explains the system's static structure, and an activity diagram (Figure 2), which captures information about the system's dynamic behavior.

As one can see in the above diagrams, the application allows, in a successful scenario:

- automatic reading (extracting) (details are given in Result section) from a text-based pdf file, the values of the parameters required for the metabolic syndrome algorithms (ATP III and IDF), respectively, the Framingham score calculation algorithm;
- automatic reading, from a graphic file (tif) (details are given in the “Results” section), of the values of the parameters required for the metabolic syndrome algorithms (ATP III and IDF), respectively, the Framingham score calculation algorithm;
- generating medical alerts;
- saving the values of parameters in the database;
- displaying charts showing the evolution (the last 3 or less, if the patient hasn’t 3 medical tests yet) of the parameters, each graph showing one parameter.

Suppose the patient does not present himself/herself with any analysis sheet. In that case, the application does not read any parameters and displays the chart showing the evolution of the medical parameters from the previous blood tests. The application also highlights the parameters from the most recent test, which were not within normal limits.

In the current version of the application, the database, created in SQLite 3, contains a single table, called Patient, which has the following structure:

- ID (INT, PRIMARY KEY, NOT NULL) – as the primary key;
- CNP (CHAR(13), NOT NULL) – as personal identification number;
- NAME (TEXT, NOT NULL);
- DT (TIMESTAMP) – as the date and time of analysis collection
- G (REAL) – as fasting glucose (or glycemia), expressed in mg/dl
- TG (REAL) as triglycerides, expressed in mg/dl
- HDL (REAL) – as HDL-Cholesterol, expressed in mg/dl
- WC (INT) - as waist circumference, expressed in cm
- COL (REAL) – as total cholesterol, expressed in mg/dl
- LDL (REAL) – as LDL-Cholesterol, expressed in mg/dl;
- SBP (INT) – as Systolic Blood Pressure, expressed in mm Hg;
- DBP(INT) – as Diastolic Blood Pressure, expressed in mm Hg.

There are situations where the scenario is unsuccessful due to automatic data reading errors (or the absence of some parameters from the analysis sheet), but a message will be displayed in this case. Also, the application uses mg/dl, not mmol/l, as the default measurement unit for triglycerides, plasma glucose, HDL-cholesterol, LDL-cholesterol, and total cholesterol, a shortcoming that will be fixed in future versions of the application.

RESULTS

Launching the application

The doctor can launch the application by double-clicking on the link when consulting a patient. According to the structure of the application presented in the UML diagrams, there can be several use cases of the application, discussed below.

The patient has no new medical tests

The identification of the patient using the personal numerical code is performed in all cases. The medic is asked to complete this information. The personal numeric code is programmatically validated (text should contain 13 digits and no other character):

Figure 3: Identify the patient

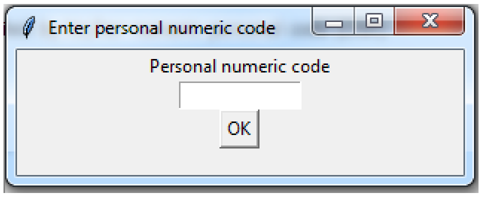

The program will display a chart with the three most recent values for each medical parameter involved in the metabolic syndrome (for example, HDL is displayed in Figure 4), so the doctor can remember the patient's condition. The application automatically retrieves and displays the parameters and their values for the last test made by the patient and programmatically highlights the abnormal values (Figure 5).

Figure 4: Evolution of HDL (the three most recent values) for a patient. The red line indicates the threshold value of the parameter (in this case, all values below 40, being a man, are abnormal)

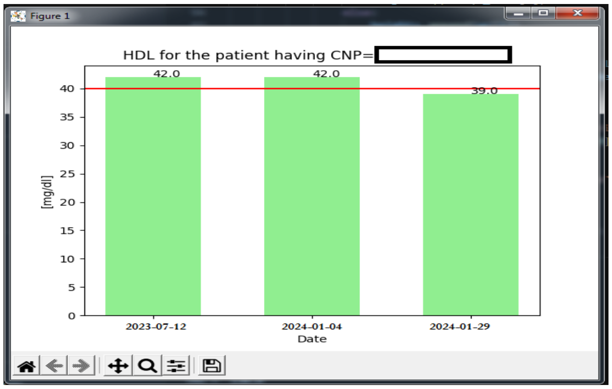

Figure 5: The values from the most recent test

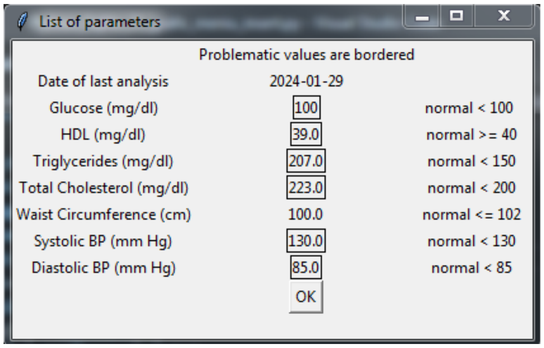

Problematic values are bordered

Date of last analysis	2024-01-29	
Glucose (mg/dl)	100	normal < 100
HDL (mg/dl)	39.0	normal >= 40
Triglycerides (mg/dl)	207.0	normal < 150
Total Cholesterol (mg/dl)	223.0	normal < 200
Waist Circumference (cm)	100.0	normal <= 102
Systolic BP (mm Hg)	130.0	normal < 130
Diastolic BP (mm Hg)	85.0	normal < 85

The patient has a new pdf text-based medical test

The program automatically reads (extracts) values of glucose level, triglycerides, HDL, total cholesterol, and LDL cholesterol from a medical test containing more than 20 parameters. We imported the <PyPDF2> module [13], which allows dividing the document into pages. The requested parameters and their corresponding values were searched on each page, using regular expression facilities (after importing the <re> module [14]).

The doctor will validate the automatically read values (Glucose level, Triglycerides, HDL, Total cholesterol, LDL cholesterol) and fill in other values (Systolic blood pressure, blood pressure treatment, Waist circumference, Smoker status, Diabetes history, and presence of Arterial fibrillation) which appear in the window with default values (shown in Figure 6).

Figure 6: The window for validating the read values and for introducing the other requested values. The first six top parameters have default values, the bottom five parameters have the values programmatically extract from the docu-ment

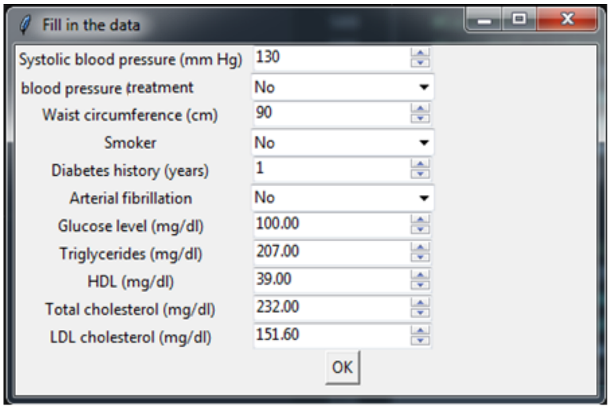

Systolic blood pressure (mm Hg)	130
blood pressure treatment	No
Waist circumference (cm)	90
Smoker	No
Diabetes history (years)	1
Arterial fibrillation	No
Glucose level (mg/dl)	100.00
Triglycerides (mg/dl)	207.00
HDL (mg/dl)	39.00
Total cholesterol (mg/dl)	232.00
LDL cholesterol (mg/dl)	151.60

After validating and completing the data, the values corresponding to the columns in the database are saved as a new record in the database, considering the analysis date as the current date, obtained from the system.

An alert regarding the presence or absence of prediabetes is displayed, as in Figure 7 (Prediabetes according to NCEP-ATP III criterion) and Figure 8 (Prediabetes according to IDF criterion). We observe the differences between the two criteria.

The Framingham score is displayed in Figure 9.

In the final step, charts are displayed with the most three recent values of each parameter, including the one from the control day. For example, in Figure 10, the evolution of glucose level is shown (the red line being the threshold value).

Figure 7: Prediabetes alert according to NCEP ATP III criterion

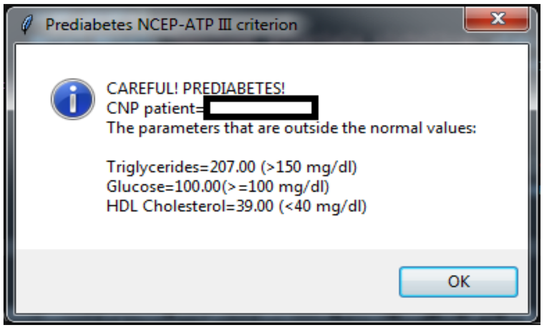

```
CAREFUL! PREDIABETES!
CNP patient=[redacted]
The parameters that are outside the normal values:

Triglycerides=207.00 (>150 mg/dl)
Glucose=100.00(>=100 mg/dl)
HDL Cholesterol=39.00 (<40 mg/dl)
```

Figure 8: Prediabetes alert according to IDF criterion

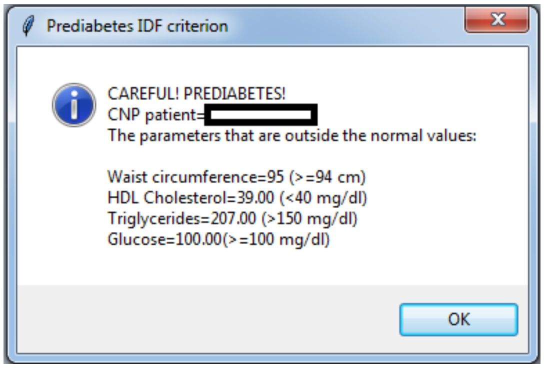

```
CAREFUL! PREDIABETES!
CNP patient=[redacted]
The parameters that are outside the normal values:

Waist circumference=95 (>=94 cm)
HDL Cholesterol=39.00 (<40 mg/dl)
Triglycerides=207.00 (>150 mg/dl)
Glucose=100.00(>=100 mg/dl)
```

Figure 9: Cardiovascular disease risk alert according to Framingham score

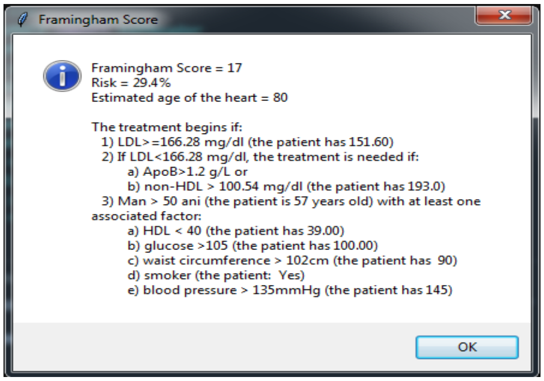

```
Framingham Score = 17
Risk = 29.4%
Estimated age of the heart = 80

The treatment begins if:
  1) LDL>=166.28 mg/dl (the patient has 151.60)
  2) If LDL<166.28 mg/dl, the treatment is needed if:
        a) ApoB>1.2 g/L or
        b) non-HDL > 100.54 mg/dl (the patient has 193.0)
  3) Man > 50 ani (the patient is 57 years old) with at least one
associated factor:
        a) HDL < 40 (the patient has 39.00)
        b) glucose >105 (the patient has 100.00)
        c) waist circumference > 102cm (the patient has  90)
        d) smoker (the patient:  Yes)
        e) blood pressure > 135mmHg (the patient has 145)
```

The patient has a new medical test in graphic format

The program automatically reads values of plasma glucose, triglycerides, HDL-cholesterol, total cholesterol and LDL-cholesterol. We use pytesseract [15], PIL [16] and cv2 [17,18, 19] modules for corrections and extracting requested values. As shown in the activity diagram, the following steps, in this case, coincide with those from the previous subsection, therefore, everything presented above and illustrated by Figures 7, 8, 9, 10 is also valid here.

Situations in which the application does not offer a successful scenario

If a parameter is missing or an error occurs during the automatic reading, a window indicating the missing parameter is displayed (Figure 11), and the medical alert cannot be generated correctly (for example, for the lack of HDL, the prediabetes alert cannot be generated – Figure 12).

Figure 10: Glucose values for the three most recent blood tests

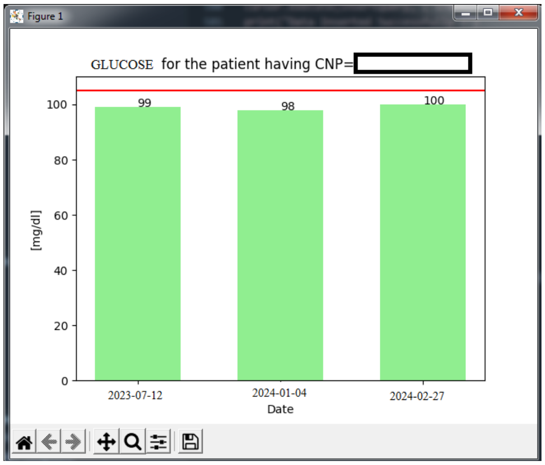

Figure 11: The window indicating a missing parameter

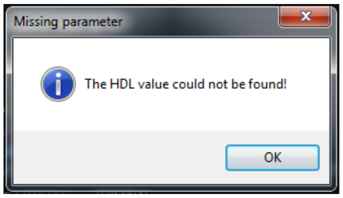

The HDL value could not be found!

Figure 12: The window indicating that the alert could not be generated

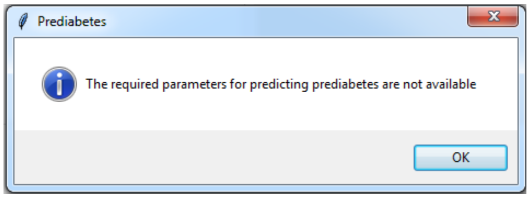

The required parameters for predicting prediabetes are not available

DISCUSSION

In this version, the application automatically generates medical alerts according to metabolic syndrome criteria and the Framingham risk score.

Because risk scores, such as the Framingham Risk Score, indicate the patient’s cardiovascular risk for the next ten years, they are very useful. To apply an algorithm classically, the doctor manually uploads the patient's data in the fields related to the algorithm and this procedure takes time. Also, the dynamic verification of the biological values requires a search of the old documents and this is another task that consumes time. This application resolves these medical objectives at once. Therefore, an advantage of the application is that it saves precious time by automatically reading the values of some blood parameters (Figure 6). The values that are not correctly read by the application will be filled in by the doctor.

In Figure 4 and Figure 10, the charts for monitoring the serum HDLc and glucose values are shown. This IT tool can be adapted (extended) depending on the medical specialty. For example, in rheumatology, monitoring can be done by inflammatory markers (C reactive protein, fibrinogen; in endocrinology, the thyroid function can be monitored by TSH values, and in diabetes, the monitoring can be based on HbA1c values. The examples can go on. Therefore, the application is versatile because it can extract any parameter of interest, depending on the medical specialty.

Medical alerts are generated (Figures 7, 8, 9), indicating, in this case, current (metabolic syndrome) or future problems (Framingham risk score).

If the application, in future versions, will access only blood parameters in order to generate medical alerts, the doctor will not have to fill in the window (Figure 6) any other values (such as, in the present case, systolic blood pressure, waist circumference, etc.) than those automatically extracted, thus saving more time.

The limits of the application refer to errors in the automatic process of reading the values, and to the fact that the application uses as measurement unit mg/dl, not mmol/l (for plasma glucose, triglycerides, HDL-cholesterol, LDL-cholesterol, and total cholesterol). The application also presumes that the patients are European (when taking into account waist circumference). These shortcomings will be fixed in a future version of the application, in which the measurement units will be automatically read from the sheets and the application will take into account the patient's race (including a field in the database).

The current version of the application uses classical algorithms, based on medical guides, but it can also use machine learning and deep learning algorithms for prediction.

CONCLUSIONS

This application, despite some problems that could appear when reading the data automatically, can improve the medical activity of a doctor, making his/her work more efficient and saving valuable time through the automation processes it proposes.

The application offers quick access to the parameters involved in a disease, displaying them in charts that illustrate the evolution of the patient's state of health; in this way, the doctor does not rely on information about what the patient remembers from previous visits or on the papers that the doctor has to look for, but on data stored in the database.

The application automatically activates alerts that can help the doctor in diagnosis. Disease prevention measures can also be recommended by the doctor, based on these alerts.

The application is original and can be easily installed. It is scalable because new facilities can be added. However, the number of medical alerts raised by the application must not be excessively high because instead of helping the doctor's work, it can make it more difficult.

This application is intended to be a support tool and not a substitute for the doctor's work. The collaboration between doctors and computer scientists opens new ways of approaching the medical act.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, O.V., A.R.B. and R.S.C.; methodology, A.R.B., A.C.C. and B.V.; software, O.V and A.R.B.; validation, R.S.C and B.V.; formal analysis, B.V.; investigation, O.V. and A.C.C.; resources, R.S.C. and B.V.; data curation, O.V.; writing—original draft preparation, O.V.; writ-ing—review and editing, O.V. and A.R.B.; visualization, O.V.; supervision, B.V., R.S.C. and A.R.B.; project administration, O.V. and A.C.C.; funding acquisition, R.S.C. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Body Composition Analysis in the Air Force: A Comparative Study of Medical Personnel and Mechanics

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Abstract: *Given the rigorous demands of aerial operations on the human body, this study aims to investigate the body composition differences among aircrew specialties, specifically between medical personnel and mechanics within the Air Force. By examining key body composition metrics such as fat mass, lean mass, and body water, the study seeks to uncover how these variables correlate with aircrew members' distinct occupational duties and overall health. Initial findings indicate subtle but significant body composition variations between groups, suggesting occupational roles may influence physical attributes. There were small differences in body composition between mechanics and medical personnel, primarily based on a greater body mass index, weight, body water, skeletal muscle mass, soft lean mass, and fat-free mass of the mechanics. Body composition analysis by segments suggest a better muscle activation during their duties, but worse nutrition habits in mechanics when compared to medical personnel. This underscores the importance of designing occupation-specific training and nutrition programs to optimize the health and operational performance of aircrew members across specialties.*

Keywords: *aircrew; body composition; nutrition; training; health; performance*

INTRODUCTION

Accurate body composition assessment in military settings is paramount for operational readiness, physical performance, and health outcomes. It emphasized the need for reliable measurement methods within military populations, particularly among Air Force Reserve Officers' Training Corps (ROTC) cadets, highlighting a significant aspect of military fitness assessments, underscoring the necessity of employing accurate and reliable body composition measurement techniques to serve the needs of military personnel better [24]. Other researchers point out the potential adverse effects of unhealthy weight control behaviors, such as weight cycling and disordered eating, prevalent among military personnel [8]. In this line, it was highlighted how body composition is basic to reach operative cardiorespiratory fitness in infantry maneuvers [14]. Additionally, a systematic review and meta-analysis conducted to assess changes in physical performance, body composition, and physical training during military operations revealed significant findings. This study highlighted that deployments could lead to a decrease in endurance performance, among other changes, suggesting the critical role of continuous monitoring and adjustment of training and nutritional strategies to mitigate negative impacts on soldiers' physical conditioning and body composition during deployments [28].

For flight crew members, managing optimal body composition (BC) is crucial due to the unique and stressful nature of their work environment, which includes long durations at high altitudes and irregular working hours that can disrupt sleep patterns and dietary habits [7,13,37]. It is of vital importance to address fatigue among flight crews, especially under extra augmented crew (EAC) schedules implemented during the COVID-19 pandemic, which increases fatigue levels, underscoring the necessity of continuous monitoring and management of crew fatigue to ensure safety and health, suggesting that BC plays a role in maintaining operational efficiency and reducing the risk of fatigue-related errors [19]. It was also found that optimal body composition is critical for pilots, impacting their ability to handle high G-forces and hypoxia at altitude [5,6]. In this line previous authors presented that air force cadets with higher muscle mass and lower fat performed better in G-tests, suggesting that muscle mass improves G-force resistance and cognitive function during flight by enhancing blood flow to the brain [36]. Another important aspect is dysmetria, fact that could negatively impact flight maneuvers, potentially leading to injuries among pilots, showing the necessity to develop specialized training programs aimed at correcting this issue is advisable [10].

Flight crew roles, including pilots, medical staff, and mechanics, are diverse yet integral to ensuring flight safety and efficiency. Pilots helm the aircraft, navigating through various weather conditions and regulatory environments to guarantee a secure journey from start to finish. They communicate with air traffic control and handle emergencies with precision. Medical personnel aboard, such as flight nurses and paramedics, are pivotal in aeromedical transport missions, offering crucial care during emergencies, patient transfers, and providing specialty care mid-flight. Their expertise is indispensable for medical repatriation and in managing healthcare in the unique and stressful conditions of flight. Mechanics and aviation engineers ensure that aircraft are safe and airworthy. Their work involves regular maintenance, pre-flight checks, and addressing any mechanical issues that arise. They are essential for the aircraft's reliability, working behind the scenes to prevent technical failures and ensure that all systems function correctly throughout the flight [20,21].

Understanding body composition standards serves as a foundational step towards enhancing nutritional habits and tailoring training systems to fit the specific realities of individuals, especially those in physically demanding roles such as military personnel. Previous studies emphasize the multifaceted relationship between body composition and military performance, highlighting the diverse implications of BC across various aspects of physical readiness and performance [16]. By recognizing BC benchmarks, individuals and organizations can better design nutritional and training programs that are not only effective but also aligned with the unique demands and realities faced by the individuals. Tailored approaches that consider the specific BC requirements can significantly contribute to improved overall health, fitness, and performance outcomes. This understanding underscores the necessity of integrating comprehensive BC knowledge into the development of personalized nutrition and exercise regimens [7,11]. Therefore, this study aimed to analyze body composition differences between the types of specialty of aircrews, to provide data of their standards, and to improve their preparation for the extra risks they need to face when they are transported in an aircraft. We hypothesized that: i) there would be differences in body composition between specialties, ii) mechanics would have higher values of lean mass and lower values of fat mass than the medical personnel, iii) mechanics would have higher values of fat-free mass in their arms than the medical personnel**.**

MATERIALS AND METHODS

1. Experimental approach to the problem

To implement the body composition assessment, we followed previous procedures [4,9,29] in which the participants stood upright on foot electrodes on the instrument platform, with arms not touching the torso. They were barefooted and without excess clothing. Four-foot electrodes were used, two of which were oval-shaped and two heel-shaped, and prior to testing the skin was cleaned and dried. Participants were asked to grip the palm and thumb electrodes. The system was calibrated before the testing session and the contacting surface of the electrodes was cleaned with alcohol before each measurement. Participants abstained from alcohol consumption and vigorous exercise for 24 hours before the measurement and were measured in the morning after overnight fasting to control hydration status, as in previous research with bioelectrical impedance.

2. Subjects

A randomized cross-sectional experimental study was conducted to analyze body composition differences of aircrews, depending on their specialty. 54 male aircrews were analyzed and classified according to their specialty (mechanics, N = 27, age: M = 43.6, SD = 7.1; medical personnel, N = 27, age: M = 42.2, SD = 11.8) of the Spanish Army with a qualification of “fit” according to the periodic medical examination as recorded in the ministerial order 23/2011. Besides, during the research, they were carrying out the periodic aeromedical training included in the ministerial order 23/2011 and the STANAG 3114 “Aeromedical Training of Flight Personnel” (NATO regulations). Before starting the research, the experimental procedures were explained to all the participants, who gave their voluntary written informed consent under the Declaration of Helsinki. The procedures conducted in the present research were designed and approved by the Medical Service of the Aerospace Medicine Instruction Centre of Spanish Air Forces and the University Ethic Committee (CIPI/18/093).

3. Instruments

A bioimpedance analyzer (InBody 720, Biospace Co. Ltd., Seoul, South Korea) was used for body mass measurement (to the nearest 0.1 kg) and body height (to the nearest 0.1 cm). InBody 720 is a multifrequency impedance body composition analyzer, which uses an eight-point tactile electrode method to take readings from the body. It measures the resistance at five specific frequencies (1 kHz, 50 kHz, 250 kHz, 500 kHz, and 1 MHz) and reactance at three specific frequencies (5 kHz, 50 kHz, and 250 kHz) on each of five segments (right arm, left arm, trunk, right leg, and left leg). Bioelectrical-impedance analysis (BIA) is one of the methods available for measuring body composition in healthy populations. Its simplicity, portability, cost, and subject acceptance make it a very desirable technique. The reliability of BIA compared to the gold standard body composition measurement (Dual-energy X-ray absorptiometry, DXA) has been successfully demonstrated for a wide range of normal and overweight populations [12,33] and military samples [1].

Data were electronically imported to Excel using Lookin'Body 3.0 software. The following parameters were analyzed: i. Body mass (kg), ii. Height (cm), iii. Body Mass Index (BMI) (kg/m2), iv. Total Body Water (TBW) (kg) (including left and right arms, trunk, and left and right legs), v. Intracellular Water (ICW) (l) (including left and right arms, trunk, and left and right legs), vi. Extracellular Water (ECW) (l) (including left and right arms, trunk, and left and right legs), vii. Proteins (kg), viii. Minerals (kg), ix. Body Fat Mass (BFM) (kg) (including left and right arms, trunk, and left and right legs, in kg and %), x. Percentage of Body Fat (PBF) (%), xi. Soft Lean Mass (SLM) (kg), xii. Fat-Free Mass (FFM) (kg) (including left and right arms, trunk, and left and right legs, in kg and %), xiii. Skeletal Muscle Mass (kg).

4. Statistical analysis

The SPSS statistical package (version 21.0; SPSS, Inc. Chicago, Illinois) was used to analyze the data. Normality assumptions were checked with a Shapiro-Wilk test for all the subgroups. Specialty differences were analyzed with a T-Student test for parametric samples and a Mann-Whitney test for no parametric samples. Homoscedasticity was tested with the Levene test. The level of significance for all the comparisons was set at $P \leq 0.05$.

RESULTS

The results are reported with their mean and standard deviation. Table 1 shows the results of body composition. No significant differences were found for the effect of type of work. There was a small effect of the specialty (mechanics had higher values than the medical personnel) in body mass (84.3 kg vs 81.1 kg), BMI (27.1 kg/m^2 vs 25.8 kg/m^2), TBW (46.9 l vs 45.7 l), ICW (29.4 l vs 28.6 l), proteins (12.7 kg vs 12.3 kg), SLM (60.4 kg vs 58.8 kg), SMM (36.4 kg vs 35.2 kg), FFM (64.1 kg vs 62.5 kg).

Table 1: Results of body composition variables by specialty groups

	Medical Personnel		**Mechanics**		**Specialty effect**		
	M	**SD**	**M**	**SD**	**t**	**p**	**Cohen's d**
Height (cm)	177.3	6.54	176.1	6.46	0.703	0.485	0.191
Body Mass (kg)	81.1	14.7	84.3	84.3	-0.848	0.400	-0.231
BMI (kg/m^2)	25.8	4.68	27.1	3.49	-1.175	0.245	-0.320
TBW (l)	45.7	5.41	46.9	5.51	-0.797	0.429	-0.217
ICW (l)	28.6	3.42	29.4	3.39	-0.906	0.369	-0.247
ECW (l)	17.1	2.01	17.5	2.14	-0.608	0.546	-0.166
Proteins (kg)	12.3	1.48	12.7	1.45	-0.860	0.394	-0.234
Minerals (kg)	4.36	0.49	4.44	0.55	-0.609	0.545	-0.166
BFM (kg)	18.6	11.0	20.1	7.74	-0.612	0.543	-0.167
PBF (%)	21.7	9.27	23.3	6.28	-0.701	0.486	-0.191
SLM (kg)	58.8	6.97	60.4	7.06	-0.810	0.421	-0.221
FFM (kg)	62.5	7.37	64.1	7.50	-0.803	0.426	-0.219
SMM (kg)	35.2	4.46	36.4	4.44	-0.920	0.362	-0.250

Note. M = Mean; SD = Standard Deviation; t = t-test; BMI = Body Mass Index; TBW = Total Body Water; ICW = Intracellular Water; ECW = Extracellular Water; BFM = Body Fat Mass; PBF = Percentage of Body Fat; SLM = Soft Lean Mass; FFM = Fat Free Mass; SMM = Skeletal Muscle Mass

Table 2 shows the results of FFM and BFM by body segments. No significant differences were found for the effect of specialty. There was a small effect of the specialty (mechanics had higher values than the medical personnel) in the fat-free mass of the right arm (3.75 kg vs 3.58 kg), the fat-free mass of the left arm (3.69 kg vs 3.53 kg), the fat-free mass of the trunk (28.6 kg vs 27.7 kg), and the body fat mass of the trunk (10.9 kg vs 9.75 kg).

Table 3 shows the results of body water (total, intracellular and extracellular) by body segments. No significant differences were found for the effect of type of work. There was a small effect of the specialty (mechanics had higher values than the medical personnel) in the total body water of the right arm (2.92 l vs 2.78 l), the left arm (2.87 l vs 2.84 l), and the trunk (22.2 l vs 21.5 l). There was a small effect of the specialty (mechanics had higher values than the medical personnel) in the intracellular water of the right arm (1.82 l vs 1.74 l), the left arm (1.79 l vs 1.71 l), and the trunk (13.9 l vs 13.4 l). There was a small effect of the specialty (mechanics had higher values than the medical personnel) in the extra-cellular water of the right arm (1.09 l vs 1.04 l), the left arm (1.08 l vs 1.03 l), and the trunk (8.31 l vs 8.09 l).

Table 2: Results of body composition variables by specialty groups

	Medical personnel		Mechanics		Specialty effect		
	M	SD	M	SD	t	p	Cohen's d
FFM Right Arm (kg)	3.58	0.61	3.75	0.57	-1.052	0.298	-0.286
FFM Left Arm (kg)	3.53	0.61	3.69	0.59	-0.985	0.329	-0.268
FFM Trunk (kg)	27.7	3.62	28.6	3.43	-0.953	0.345	-0.259
FFM Right Leg (kg)	9.64	1.12	9.64	1.21	-0.065	0.948	-0.018
FFM Left Leg (kg)	9.61	1.14	9.61	1.23	0.057	0.489	0.016
BFM Right Arm (kg)	1.20	1.24	1.22	0.71	-0.067	0.947	-0.018
BFM Left Arm (kg)	1.23	1.24	1.25	0.68	-0.081	0.936	-0.022
BFM Trunk (kg)	9.75	5.90	10.9	4.46	-0.834	0.408	-0.227
BFM Right Leg (kg)	2.59	1.26	2.73	0.86	-0.477	0.635	-0.130
BFM Left Leg (kg)	2.56	1.24	2.71	0.86	-0.495	0.623	-0.135

Note. M = Mean; SD = Standard Deviation; t = t-test; FFM = Fat-Free Mass; BFM = Body Fat Mass

Table 3: Results of Body Water by Body Segments

	Medical personnel		Mechanics		Specialty effect		
	M	SD	M	SD	t	p	Cohen's d
TBW Right Arm (l)	2.78	0.48	2.92	0.44	-1.054	0.297	-0.287
TBW Left Arm (l)	2.74	0.47	2.87	0.45	-0.994	0.325	-0.270
TBW Trunk (l)	21.5	2.80	22.2	2.66	-0.931	0.356	-0.253
TBW Right Leg (l)	7.49	0.87	7.49	0.95	-0.031	0.975	-0.009
TBW Left Leg (l)	7.47	0.89	7.45	0.96	0.094	0.926	0.025
ICW Right Arm (l)	1.74	0.30	1.82	0.27	-1.060	0.294	-0.289
ICW Left Arm (l)	1.71	0.29	1.79	0.28	-1.016	0.314	-0.276
ICW Trunk (l)	13.4	1.77	13.9	1.64	-1.026	0.309	-0.279
ICW Right Leg (l)	4.69	0.54	4.72	0.58	-0.183	0.856	-0.050
ICW Left Leg (l)	4.66	0.55	4.67	0.59	-0.067	0.947	-0.018
ECW Right Arm (l)	1.04	0.18	1.09	0.17	-1.038	0.304	-0.283
ECW Left Arm (l)	1.03	0.18	1.08	0.17	-0.952	0.346	-0.259
ECW Trunk (l)	8.09	1.04	8.31	1.02	-0.762	0.450	-0.207
ECW Right Leg (l)	2.79	0.33	2.77	0.37	0.213	0.832	0.058
ECW Left Leg (l)	2.81	0.34	2.77	0.38	0.344	0.732	0.094

Note. M = Mean; SD = Standard Deviation; t = t- test; TBW = Total Body Water; ICW = Intracellular Water; ECW = Extracellular Water

DISCUSSION

This study aimed to analyze body composition differences between mechanics and medical personnel of the Air Force. The first hypothesis was partially fulfilled since there were small differences in body composition between specialties. The second hypothesis was partially fulfilled since the effect of the specialty in the results was small. The third hypothesis was partially fulfilled since there were small differences in the fat-free mass between specialties.

Height, body mass and body mass index measures have been traditionally used as a first basic approach to study body composition [2,11]. Although the differences found were small, it is important to note a greater weight and body mass index in the mechanics group. The morphological distribution of the body mass can be more easily identified through the soft lean mass, the skeletal muscle mass, and the fat-free mass [17], which were greater in mechanics. These differences suggest predominant values of lean mass, rather than body fat, in the body mass values of mechanics. Although mechanics are not as responsible as pilots in the control of aircraft, their performance is crucial to control and maintain the correct functioning of aircraft devices [23]. Mechanics need to use tools in flight time, and to cope with the vibrations, accelerations, and changes of directions of the aircraft, while they maintain their stance [3,15,25]. These aspects could be crucial in the development of muscle mass groups, especially segmental stabilizers. Muscles are made of water and proteins, and the small differences that were found between specialties (greater in mechanics than in medical personnel) are coherent with the previous results and previous research [4].

The analysis of body composition by segments can provide better insights of which are anatomical regions better involved in the morphological differences between specialties. The fat-free mass was higher in the upper extremities for the mechanics' group, and this aspect reinforces the previous assumptions and descriptions about the different jobs of each specialty. Mechanics usually work with tools that need to be controlled by their hands, involving the muscles of their arms, applying different kind of forces and torques to adjust the devices. Many muscle groups in the trunk are responsible of the stability of the core, that is crucial to maintain the stance in flight time [15,18,38]. It could be perceived that the most part of the aircrew are sitting down in the cockpit, as happens with the pilots. However, several factors that happens in an aircraft, like vibrations, accelerations, and 3-axes movements, keep the muscles active to react and stabilize against forces [31], while muscle contractions are even harder when the air-crew stand up to perform their task [3]. Despite the mechanics having higher values for the fat-free mass of the trunk, they also had greater fat mass in this anatomical region when compared to medical personnel. Fat exceedances are normally stored in the abdominal region of the trunk, especially in men [26,32]. If we analyze these two factors together, the results suggest a better activation of the core muscles in the mechanics, but worse nutrition habits than the medical personnel. Healthcare professionals probably have a deeper knowledge of good nutrition habits that could help them to maintain healthier levels of fat, and that could balance their least muscle activation in flight [34]. In any case, it is important to notice that both the aerobic and anaerobic metabolism are activated in an aircraft, as was reported in real flight research [3], and it would be beneficial for these populations to maintain physical training that could help them to perform well in their job environments.

Body water results reinforced the previous assumptions about a greater muscle mass in mechanics than in the medical personnel. Muscle mass is mainly built of water, and there is a coherent relationship between the fat-free mass of the upper extremities and the trunk segments, and higher values of body water [30]. Both body water and fat-free mass, were higher in the right arm than in the left arm for each specialty, which suggests a predominance of the right hand (usually the dominant hand) in the tasks that both job groups must accomplish. If we compare their values with previous research, both mechanics and medical personnel had worse body composition values than pilots [4], and other transported units that usually required to perform high levels of physical activity. Even though there are similarities between transport aircrews, there are still subtle differences related to their occupation needs, and there is a need to monitor and provide body composition standards that gather these gaps [11,27]. Good nutrition habits [35] and training preparation [22] would be beneficial in these units to help them face the hazards that military aircrews need to cope with, both in flight time and when they are deployed.

Practical application

The practical application of this study emphasizes the necessity for air forces to implement specialized training and nutrition programs tailored to the unique demands of different aircrew specialties. Recognizing the specific body composition needs of medical personnel and mechanics can lead to more effective health and performance outcomes, contributing to enhanced operational readiness and safety in aerial missions. This approach supports the development of targeted interventions that promote optimal physical conditioning and well-being among aircrew members, ultimately improving their capacity to handle the physical challenges of flight duties.

Limitations of the study and future research lines

The study's limitations include its narrow focus on specific aircrew specialties within a single air force, potentially limiting the generalizability of the findings to broader populations or different military contexts. Additionally, the cross-sectional design precludes causal inferences between body composition and occupational roles. Lastly, we did not register the years of work experience in this sample, which may help to develop longitudinal studies to assess the evolution of body composition with and without dietary and training interventions. Future research should expand to longitudinal studies across diverse military settings to explore the dynamic changes in body composition over time and their impact on aircrew performance. Investigating the effectiveness of tailored training and nutrition interventions in enhancing operational efficiency and reducing injury risks in varied aircrew roles will also be valuable.

CONCLUSIONS

There are small differences in body composition between aircrew specialties (mechanics and medical personnel) primarily based on a greater body mass index, weight, body water, skeletal muscle mass, soft lean mass, and fat-free mass of the mechanics. Body composition analysis by segments, suggests better muscle activation during their duties, but worse nutrition habits in mechanics when compared to medical personnel. The performance requirements of these units are different from those that are principally responsible for the aircraft (pilots) or those who are transported to be deployed in a conflict area. Perceived assumptions about the tasks that each unit must accomplish need to be clarified by an adequate body composition assessment.

This information could help to understand better each unit requirements and to develop training and nutritional habits that are crucial to performing well in the stressful job contexts of the aircrews.

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Authors' contribution
Conceptualization, V.J.C.S. and A.B.S.; methodology, V.J.C.S. and A.B.S.; formal analysis, V.J.C.S. and A.B.S.; investigation, V.J.C.S. and A.B.S..; resources, V.J.C.S. and A.B.S.; data curation, A.B.S.; writing—original draft preparation, A.B.S.; writing—review and editing, V.J.C.S. and A.B.S..; supervision, V.J.C.S.; project administration, V.J.C.S.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of UNIVERSIDAD EUROPEA DE MADRID (protocol code CIPI/18/093).

Patient consent for publication
Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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LITERATURE REVIEW

Sculpting Hope: Advanced MEN 2 – Associated Tumors. A Narrative Review Starting from a Case Report

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Abstract: *Multiple endocrine neoplasias (MEN) represent a rare and intricate group of disorders with diverse endocrine and extra-endocrine manifestations. Among the five identified types of MEN syndromes, MEN 2 stands out due to its complexity, featuring medullary thyroid carcinoma (MTC), pheochromocytoma, and primary hyperparathyroidism (PHPT). MEN 2 is inherited in an autosomal dominant manner, with nearly a 100% lifetime risk of developing MTC, which can be fatal if not detected early. Comprehensive diagnostic protocols and timely interventions significantly mitigate morbidity. To guide surgical decisions, management strategies include regular imaging and biomarker monitoring (calcitonin, CEA, metanephrines/normetanephrines, and parathyroid hormone). Genetic screening of at-risk families and prophylactic thyroidectomy remain cornerstone preventive measures. Although tyrosine kinase inhibitors are generally reserved for specific cases of MTC and are not first-line therapies for MEN2-associated tumors, they hold the potential for mitigating tumor progression and recurrence despite the risk of endocrine dysfunction. This paper presents a narrative review focusing on multiple endocrine neoplasia type 2 (MEN2) syndrome, encompassing its genetic underpinnings, clinical presentations, diagnostic modalities, and therapeutic approaches. A total of 48 articles were included in this review.*

Keywords: *multiple endocrine neoplasia, medullary thyroid carcinoma, pheochromocytoma, primary hyperparathyroidism*

INTRODUCTION

Multiple endocrine neoplasias (MEN) are rare hereditary syndromes characterized by the co-occurrence of multiple endocrine tumors in the same individual or related individuals of the same family; these tumors usually secrete hormones that give rise to distinct clinical syndromes [1,2]. Currently, five distinct types of MEN have been identified: MEN1, MEN2 (previously known as MEN2A), MEN3 (formerly known as MEN2B) and the recently recognized MEN4 and MEN5 (Table 1) [3–11].

MEN 2 is a complex autosomal dominant inherited syndrome characterized by medullary thyroid carcinoma (MTC), pheochromocytoma, and primary hyperparathyroidism (PHPT)[12,13].

The estimated prevalence of MEN2 is approximately 1 in 35,000 [3]. More than 95% of MEN2 patients develop MTC, and about 50% have pheochromocytomas. HPT occurs in 20–30% of MEN2 patients, generally without evident symptoms, and is found by biochemical and imaging assessments [14].

The majority of pathogenic variants associated with Multiple Endocrine Neoplasia type 2 (MEN2) follow an autosomal dominant inheritance pattern and are situated within the extracellular cysteine-rich domain of the RET proto-oncogene's exon 10 (specifically at codons 609, 611, 618, or 620) or exon 11 (at codon 634). Pathogenic variants at codon 634 are responsible for at least 50% of diagnosed cases [13-20]. Approximately 25% of these cases are hereditary due to activating mutations in the REarranged during Transfection (RET) proto-oncogene. The course of MTC is indolent, and survival rates depend on the tumor stage at diagnosis. Initial management involves total thyroidectomy with bilateral dissection of the central node compartment [22]. Serum calcitonin and carcinoembryonic antigen should be performed pre-operatively. These can be followed over time and used as markers of disease recurrence as their doubling times have been shown to correlate with disease progression and survival [8]. Neck metastases appear early during MTC. Distant metastases are detected in 7-17% of all cases at the time of diagnosis and frequently appear in multiple sites. The most frequent sites of distant metastasis are the liver (49%), bones (45%), and lungs (35%) [23].

Table 1: Update in the classification of MEN syndromes [8–11]

	MEN1 (Wermer Syndrome)	**MEN2 (Sipple syndrome)**	**MEN 3 (Gorlin-Vickers syndrome)**	**MEN4 (MEN X syndrome)**	**MEN5**
Gene alteration	Loss-of-function of the tumor suppressor gene MEN1 (menin)	Germline variants in the *RE*arranged during *T*ransfection proto-oncogene (specially codon 634)	Germline variants in the *RE*arranged during *T*ransfection proto-oncogene (specially codon 918)	Inactivating germline mutation of tumor suppressor gene CDKN1B	Heterozygous missense variant in MAX (only 2 families described in the literature)
Inheritance	Autosomal-dominant	Autosomal-dominant	Autosomal-dominant	Autosomal-dominant	Autosomal-dominant
Dominant endocrine pathology	Primary hyperparathyroidism 85% - usually four-gland hyperplasia	Medullary thyroid carcinoma 95-100%	Medullary thyroid carcinoma 95-100%	Primary hyperparathyroidism 75%	Pheochromocytoma
Associated endocrine disorders	Enteropancreatic tumor: gastrinoma, insulinoma, non-functioning, glucagonoma, VIPoma Pituitary neuroendocrine tumors Bronchopulmonary neuroendocrine tumors, Thymic neuroendocrine tumors Adrenal cortical tumors (usually non-functioning)	Pheochromocytoma (50%) Primary hyperparathyroidism (20-30%): four-gland hyperplasia or single adenoma	Pheochromocytoma (50%)	pNET: non-functional, gastrinoma	Pituitary neuroendocrine tumors Parathyroid adenomas
Associated non-endocrine tumors or disorders	Breast cancer lipomas, angiofibromas, collagenomas, meningiomas	Cutaneous lichen amyloidosis (or) Hirschsprung's disease	Ganglioneuroma of the lips, tongue, or gastrointestinal tract + Marfanoid habitus (95%)		Ganglioneuromas, Neuroblastomas, Chondrosarcoma, Lung adenocarcinoma

The American Thyroid Association (ATA) stratified patients with MEN2 from moderate to the highest-risk groups based on the identified pathogenic variant in RET proto-oncogene and the severity of the clinical course of MTC [24]. According to the 2015 ATA guidelines, the highest-risk pathogenic variant includes MEN3 patients with RET mutations in codon p.Met918Thr [8,25]. High-risk MEN2 pathogenic variants are RET codon p.Cys634 and p.Ala883Phe pathogenic variants. Moderate-risk pathogenic variants include identified RET codon pathogenic variants other than those noted to be at "high" or "highest risk"[26,27].

In metastatic MTC, the focus is on local and systemic treatment modalities. Surgery remains the first recommendation for recurrent cases, dependent on factors like size, extent, and concurrent distant metastases. External beam radiation therapy (EBRT) may be considered when surgical resection is not feasible. Focal treatments such as surgery, embolization, radiofrequency ablation, and EBRT aim at relieving local symptoms and reducing the risk of complications. Embolization or chemoembolization for liver metastases may reduce tumor masses and alleviate symptoms. Multikinase and selective kinase inhibitors represent the systemic treatment for MTC with identified RET mutation [28]. Multikinase inhibitors with anti-RET activity, such as Vandetanib and Cabozantinib, are approved for MTC patients ineligible for surgery with symptomatic or progressive disease [29]. Despite benefits, these inhibitors have limitations, including toxicity impacting quality of life, unclear overall survival benefits, and the potential for tumor cell resistance [28].

Two new highly selective RET inhibitors — Selpercatinib and Pralsetinib — have shown promising results in MTC patients with a RET mutation, regardless of mutation or the number or type of prior systemic therapies received. Selpercatinib exhibited marked antitumor activity in RET-mutant MTC patients, even in those previously treated with Vandetanib or Cabozantinib [30]. Pralsetinib demonstrated increased RET potency and selectivity, with a positive response in RET-mutant MTC patients [31].

Pheochromocytomas are the first manifestation of MEN2 in 13-27% of cases [32]. The clinical picture is similar to that of the sporadic type (hypertension, headache, increased sweating, and tachycardia). However, MEN2-related pheochromocytomas are more likely to be bilateral when compared with sporadic ones. Primary hyperparathyroidism (PHPT) is typically mild and manifests concurrently or after MTC [1].

The objective of this paper is to provide a comprehensive review of multiple endocrine neoplasia type 2 syndrome, focusing on its genetic basis, clinical manifestations, diagnostic approach, and management strategies.

MATERIALS AND METHODS

Search Strategy

A comprehensive literature search was conducted to identify relevant studies on MEN2 syndrome, focusing on treatment strategies and outcomes. The search strategy encompassed electronic databases and academic repositories from January 1995 to February 2024. The following databases were utilized: PubMed/MEDLINE, Scopus, Web of Science, and Embase.

The search strategy combined Medical Subject Headings (MeSH) terms and free-text keywords, including but not limited to: "Multiple Endocrine Neoplasia Type 2", "MEN2 syndrome", "Medullary Thyroid Carcinoma", "Pheochromocytoma", "Hyperparathyroidism", "RET proto-oncogene", "Genetic testing", "Tyrosine kinase inhibitors", "Thyroidectomy", "Treatment outcomes", "Prophylactic surgery", "Patient outcomes", "Targeted therapy", "Clinical trials".

The search paradigm included combinations of Boolean operators (AND, OR) to refine the search results. Example search strings used were: "Multiple Endocrine Neoplasia Type 2" OR "MEN2 syndrome" AND "treatment" OR "selective RET inhibitor", "Medullary Thyroid Carcinoma" OR "MTC" AND "genetic testing" OR "prophylactic thyroidectomy".

Inclusion criteria encompassed peer-reviewed articles, clinical trials, reviews, and observational studies published in English. Exclusion criteria included non-English articles, case reports, letters, and studies not directly related to MEN2 syndrome treatment or outcomes.

Two independent reviewers screened titles and abstracts for relevance. Full-text articles meeting inclusion criteria underwent detailed review. No third reviewer was involved. Data extraction included study design, participant characteristics, interventions, outcomes, and key findings.

A Journey from Personal Medical Cases to Broader Horizons

We presented the clinical case of a 37-year-old patient diagnosed with MEN2 syndrome, characterized by bilateral benign pheochromocytomas, medullary thyroid carcinoma with recurrent disease, and liver metastasis (Figure 1). In her 20s (in 2009), following a severe hypertensive episode and palpitations, abdominal ultrasonography raised suspicion of an adrenal gland tumor.

Figure 1: The evolution of a patient with MEN2 syndrome over 25 years

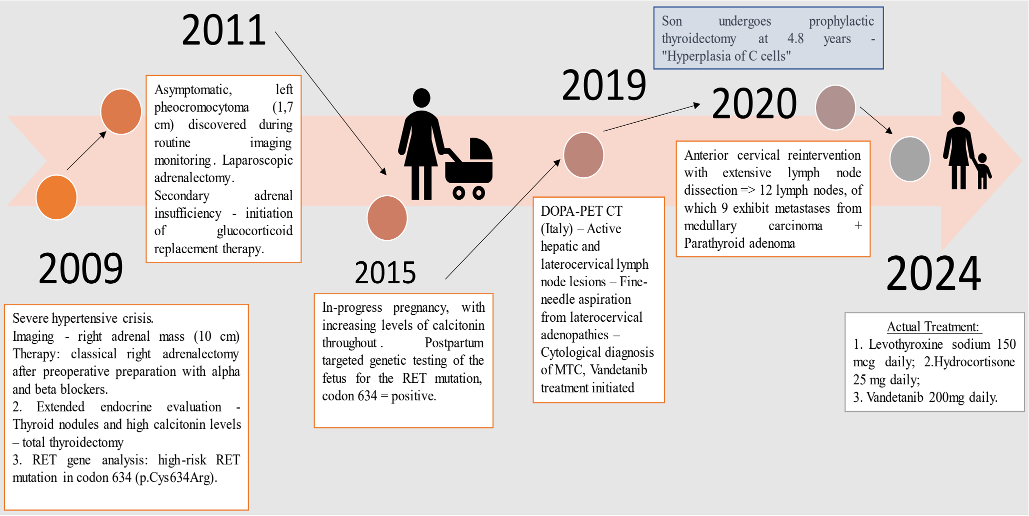

Asymptomatic, left pheocromocytoma (1,7 cm) discovered during routine imaging monitoring. Laparoscopic adrenalectomy. Secondary adrenal insufficiency - initiation of glucocorticoid replacement therapy.

Severe hypertensive crisis.
Imaging - right adrenal mass (10 cm) Therapy: classical right adrenalectomy after preoperative preparation with alpha and beta blockers.
2. Extended endocrine evaluation - Thyroid nodules and high calcitonin levels – total thyroidectomy
3. RET gene analysis: high-risk RET mutation in codon 634 (p.Cys634Arg).

In-progress pregnancy, with increasing levels of calcitonin throughout. Postpartum targeted genetic testing of the fetus for the RET mutation, codon 634 = positive.

DOPA-PET CT (Italy) – Active hepatic and laterocervical lymph node lesions – Fine-needle aspiration from laterocervical adenopathies – Cytological diagnosis of MTC, Vandetanib treatment initiated

Son undergoes prophylactic thyroidectomy at 4.8 years - "Hyperplasia of C cells"

Anterior cervical reintervention with extensive lymph node dissection => 12 lymph nodes, of which 9 exhibit metastases from medullary carcinoma + Parathyroid adenoma

Actual Treatment:
1. Levothyroxine sodium 150 mcg daily; 2.Hydrocortisone 25 mg daily;
3. Vandetanib 200mg daily.

The diagnosis was confirmed by biochemical tests (increased plasma and urinary metanephrines and normetanephrines levels) and MRI, which visualized an ovoid tumor with a diameter of about 10 cm. Therapy with alpha and beta blockers was initiated for preoperative preparation, and later, a classical right adrenalectomy was performed. Examination of the surgically removed adrenal gland revealed multicentric, predominantly alveolar pheochromocytoma (Figure 2).

Figure 2: Macroscopic appearance of the resected adrenal gland including the pheochromocytoma tumor

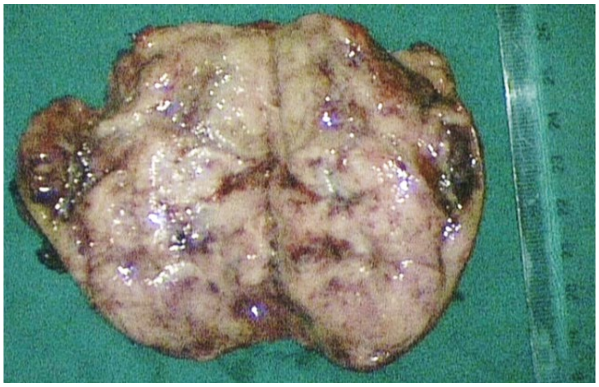

After the discovery of pheochromocytoma, an extensive endocrine evaluation was conducted, revealing high levels of calcitonin (>2000 pg/ml) and multiple heterogeneous thyroid nodules. A neck ultrasound revealed bilateral thyroid nodules. One month following her right adrenalectomy, the patient underwent total thyroidectomy with bilateral dissection of central and lateral lymph nodes of the neck. The thyroid lobes were submitted separately and were enlarged. Well-circumscribed nodules were seen with nests of round cells among fibrous stroma. The histopathological result of the thyroid indicated the presence of thyroid medullary carcinoma.

Given the young age of disease onset and pathological confirmation of the diagnosis of 2 neuroendocrine tumors, suspicion of MEN2 syndrome was raised, and genetic testing to identify RET gene mutations was performed. When the high-risk RET mutation on codon 634 was found, and considering the patient's young age, the potential risks of the condition being passed on to children were discussed with her. While awaiting genetic screening results, biochemical, hormonal, and imaging tests were initiated for all family members. Calcitonin, parathyroid hormone, calcemia, normetanephrine and metanephrine values in plasma and urine were normal, and no thyroid nodules were detected by USG screening among her first-degree relatives. During the years 2009–2011, following the surgical interventions, the patient's condition improved. In 2011, with no characteristic signs, during biannual monitoring, a CT of the abdomen detected a tumor (1.7 cm) on the left adrenal. Laparoscopic left adrenalectomy was performed, and glucocorticoid substitution treatment was initiated.

Between 2012 and 2014, the patient stopped attending endocrinological consultations, and in 2015 she returned for follow-up when she declared she was pregnant and an increase in calcitonin levels was observed during pregnancy. After childbirth, although the calcitonin level did not normalize, it significantly decreased compared to the values at the end of pregnancy. Following birth, the child underwent targeted testing for a mutation in the RET proto-oncogene at codon 634, yielding a positive result (Figure 3). Prophylactic thyroidectomy was performed at the age of 4.8 years. The histopathological result revealed the presence of C-cell hyperplasia.

Figure 3: Targeted testing for RET proto-oncogene indicated the mutation on the level of codon 634-p.Cys634Arg

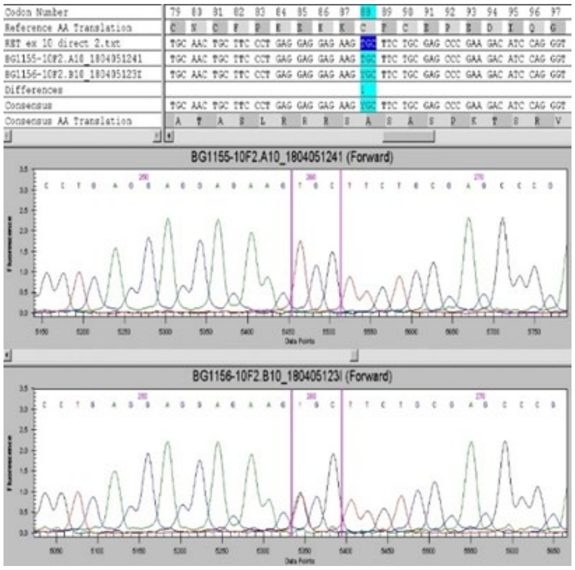

Codon Number	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97
Reference AA Translation	C	N	C	F	P	E	E	E	K	C	F	C	E	P	E	D	I	Q	G
RET ex 10 direct 2.txt	TGC	AAC	TGC	TTC	CCT	GAG	GAG	GAG	AAG	TGC	TTC	TGC	GAG	CCC	GAA	GAC	ATC	CAG	GGT
BG1155-10F2.A10_1804051241	TGC	AAC	TGC	TTC	CCT	GAG	GAG	GAG	AAG	TGC	TTC	TGC	GAG	CCC	GAA	GAC	ATC	CAG	GGT
BG1156-10F2.B10_1804051231	TGC	AAC	TGC	TTC	CCT	GAG	GAG	GAG	AAG	YGC	TTC	TGC	GAG	CCC	GAA	GAC	ATC	CAG	GGT
Differences																			
Consensus	TGC	AAC	TGC	TTC	CCT	GAG	GAG	GAG	AAG	YGC	TTC	TGC	GAG	CCC	GAA	GAC	ATC	CAG	GGT
Consensus AA Translation	A	T	A	S	L	R	R	R	S	A	S	A	S	P	K	T	S	R	V

BG1155-10F2.A10_1804051241 (Forward)
BG1156-10F2.B10_1804051231 (Forward)
Data Points
Fluorescence

Annual reassessments of our patient revealed persistently elevated calcitonin levels. In 2019, due to increasing calcitonin levels, an 18F-DOPA-PET CT scan was conducted in Italy. The results described in the cervical and hepatic regions were suspicious of neoplastic pathology (recurrence) with high absorption of the dopamine tracer. Upon returning to Romania, after performing a fine-needle aspiration from the pointed nodule, the diagnosis of recurrent disease was established.

Recurrent central, bilateral tracheal, and left laterocervical lymphadenectomy was performed in January 2020. Although only one parathyroid gland was removed, the findings suggested parathyroid adenoma. The histopathological diagnosis confirmed the presence of twelve lymph nodes, nine of which exhibited metastases of medullary carcinoma with amyloid stroma.

Following surgical intervention, due to consistently increasing levels of calcitonin and concurrent secondary hepatic determinations, Vandetanib treatment was initiated in October 2020. In subsequent biannual follow-ups, calcitonin levels did not normalize, but there was a significant decrease compared to the values before the initiation of tyrosine kinase inhibitor therapy. Figure 4 presents the evolution of calcitonin levels from 2009 to 2023.

Figure 4: The evolution of the calcitonin level and the main events in the evolution of the patient

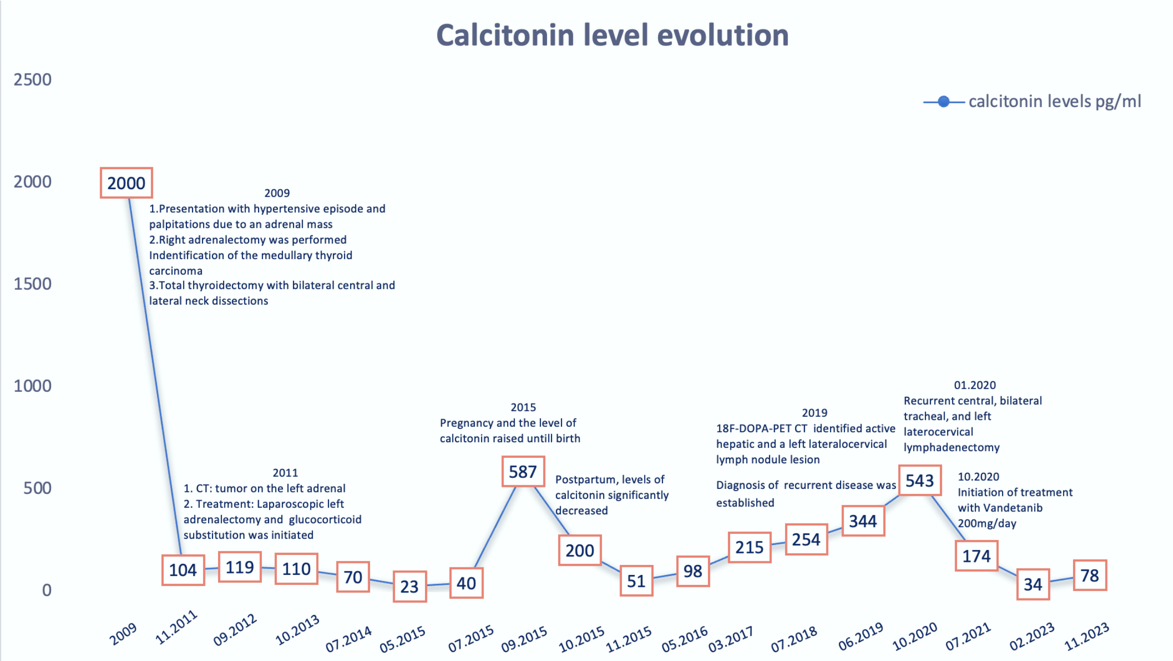

Levels of plasma and urinary metanephrines/ normetanephrines, serum calcium, and parathyroid hormone (PTH) remained stable and within normal ranges throughout the monitoring period after the second adrenal surgical intervention.

The serum PTH level was also normal, including the assessment preceding the surgical re-intervention for the local recurrence of medullary thyroid carcinoma (CMT), during which a parathyroid adenoma was also detected.

Recent laboratory tests have shown stable values, indicating a favorable therapeutic response to the tyrosine kinase inhibitor.

At present, the patient is receiving thyroid and glucocorticoid substitution: 25 mg of cortisone daily and L-thyroxine 150 mcg daily. Additionally, she has been undergoing Vandetanib 200 mg daily treatment since October 2020, for approximately 3.5 years. Calcitonin and CEA values remained stable under this therapy. However, consideration is being given to switching to Selpercatinib, a highly selective RET inhibitor, in the future.

Results of the Narrative Review

In our review, we examined 34 papers to assess their relevance to MEN2 syndrome, and 26 of these papers met our inclusion criteria. Here, we present a summary of the findings from these studies.

The MEN2 syndrome encompasses several neuroendocrine tumors, including medullary thyroid carcinoma (MTC), pheochromocytoma (PCC), and primary hyperparathyroidism (PHPT) [33]. MTC, being the most prevalent and potentially life-threatening component, typically manifests first within the syndrome [31].

The development and severity of tumors in MEN2 syndrome are significantly influenced by specific mutations in the RET gene [34,35]. Prophylactic thyroidectomy is strongly recommended for individuals harboring these mutations, ideally before the onset of MTC [34,35].

Clinical presentations vary, with MTC often presenting as thyroid nodules or symptoms associated with elevated calcitonin levels, while PCC may manifest with symptoms such as palpitations, headache, and diaphoresis. Hyperparathyroidism in MEN2 is frequently asymptomatic and is commonly diagnosed alongside MTC [8].

Surgical removal of MTC and any associated metastases is essential for achieving a favorable outcome [36]. Prognosis in MEN2-related MTC largely hinges on the disease stage at diagnosis [14,37].

Compared to sporadic cases, hereditary MTC typically appears at a younger age and displays distinct pathological features [38–41].

Post-surgery, patients with persistently elevated calcitonin levels require vigilant monitoring using biochemical markers to detect recurrence and assess disease progression effectively [27,42,43].

This narrative review underscores the complex nature of MEN2 syndrome, highlighting its genetic basis, clinical manifestations, therapeutic strategies, and the critical role of ongoing surveillance in managing patient outcomes effectively.

DISCUSSION

MEN2 syndrome consists of several neuroendocrine origin abnormalities: MTC, PCC, and PHPT. Diagnosis of any of these tumors implies correlative analyses and undertaking further investigations for the detection of the other possible associated neoplasia [33]. The case emphasizes the importance of the radical approach to MEN 2 syndrome from both a therapeutic and surgical point of view. The key to the MEN2 phenotype is that the frequency and severity of specific tumors depend on the specific causative mutation (genotype). MEN2 patients with RET mutations require a prophylactic thyroidectomy, that is, ideally, before MTC has developed [34,35]. The timing of this is based on the risk stratification of the RET mutation described by the American Thyroid Association [26].

MTC may present with a thyroid lump or symptoms of calcitonin excess, like diarrhea and flushing. Pheochromocytomas may present with the classic triad of palpitations, headache, and diaphoresis, but can be asymptomatic upon diagnosis. They often exhibit bilateral involvement, and unilateral presentations frequently lead to the development of a contralateral pheochromocytoma within 10 years. Pheochromocytomas in MEN2 syndrome are seldom malignant and the prognosis in MEN2 is primarily influenced by MTC, the most life-threatening component. Hyperparathyroidism in MEN2 is generally without symptoms and is diagnosed concurrently with MTC [8].

One dermatological manifestation associated with MEN2 syndrome is cutaneous lichen amyloidosis (CLA), frequently co-existing with pheochromocytomas. Additionally, reported cutaneous conditions linked to MEN2 comprise dermal hyperplasia and multiple sclerosing fibromas [14].

MTC develops in almost all MEN 2 cases and is usually the first manifestation of the syndrome. Before MTC develops, it is preceded by thyroid C-cell hyperplasia. MEN 2 MTC is typically multifocal and often bilateral [31]. Only a few MEN 2 patients are symptomatic at MTC diagnosis. A recent nationwide population-based study reported that only 15% of the MEN 2 cases with MTC had clinically suggestive characteristics at diagnosis, while the remaining 85% were diagnosed by screening [44]. MTC can result in fatal outcomes within the spectrum of MEN2 when managed ineffectively or remains unrecognized. Because MTC frequently involves the cervical lymph nodes, lymph node dissection, in addition to total thyroidectomy is required [36]. Complete surgical resection of the thyroid tumor and any locoregional metastases is the only curative option for locoregional MTC. Therefore, early diagnosis and detection of MTC are very important for prognosis [38].

There are several key differences in the presentation of hereditary compared to sporadic MTC [39]. Hereditary MTC presents at a significantly younger age, even after excluding patients identified via genetic screening. It is usually diagnosed in the 2nd - 3rd decade [45]. Depending on the specific RET variant, the mean age at diagnosis of normal thyroid/C cell hyperplasia ranges from 5 to 11 years, while the mean age at diagnosis of node-negative MTC ranges from 19 to 33 years [46][40]. In a large cohort of patients with MTC, Saltiki et al. found that compared to sporadic MTC, hereditary MTC patients had significantly more microscopic disease, multifocality, C-cell hyperplasia, and smaller tumor size [41]. Sporadic MTC patients had significantly more capsular invasion, soft tissue invasion, and distant metastases than hereditary MTC patients [42]. According to research, there is a tendency towards a more unfavorable progression in the case of sporadic MTC, and the presented case supports this notion, highlighting the hepatic metastases 10 years after the initial diagnosis of MTC.

In patients with persistently high levels of calcitonin after surgery, biochemical markers with calculated doubling times have been used to monitor MTC recurrence, progression, and survival. Barbet et al. (2005) found in multivariate analysis that calcitonin and carcinoembryonic antigen (CEA) doubling times are independent risk factors for survival. When calcitonin doubling time was 2 years, 100% of patients were alive at 10 years [43]. Some limitations of calcitonin include inter-assay variability, a concentration-dependent half-life, and rapid degradation at room temperature or refrigeration. With the development of increasingly reliable assays, investigation into other biomarkers continues [42].

Survival for patients with MEN2-related MTC treated surgically is dependent upon the disease stage at diagnosis [47]. Post-resection, all patients with MEN2 should be followed with surveillance for recurrent disease. In addition, any patients with MTC, or a constellation of two MEN2 tumors, should undergo RET genetic testing. Children of MEN2 patients should have genetic testing performed within the first 6 months of life [48,49]. Calcitonin and CEA are markers used to assess initial disease burden and followed post-resection to assess disease progression in patients with metastatic MTC [27].

Distance metastasis of MTC depends on tumor stage and calcitonin level. Liver metastases are observed in 13% of MTC with a serum Calcitonin level of more than 400 pg/ml. Liver metastases are generally hypervascular and exhibit variable atypical imaging appearances, therefore, the differentiation between them and other vascular hepatic lesions, especially hemangioma, is challenging. State-of-the-art 68GaDOTA-somatostatin analogue-PET/CT improves specificity in detecting the metastases of NET, especially in liver and lymph nodes; nevertheless, this imaging technique is not yet a routine protocol [50,51].

Before thyroidectomy, the presence of pheochromocytoma should be ruled out by appropriate biochemical analysis in all MEN 2 and MEN 3 patients [52]. If a pheochromocytoma is detected, adrenalectomy should be performed before thyroidectomy or other surgery to avoid severe intraoperative complications. Laparoscopic adrenalectomy is the gold standard in the treatment of pheochromocytoma. Limitations only arise because of technical difficulties or tumor size. Pheochromocytoma is an infrequent initial presentation of MEN2 and is typically recognized during screening evaluations in MEN2 patients [53]. It typically becomes apparent approximately a decade following the onset of MTC or C cell hyperplasia [37]. In our case, the patient presented initially with unilateral, benign left pheochromocytoma, whose symptoms manifested before the diagnosis of medullary thyroid carcinoma. Due to the large dimensions of the adrenal adenoma (10 cm), surgery was performed through the classical approach. When contralateral adrenal involvement was detected 2 years later, the tumor dimensions (1.7 cm) allowed for a laparoscopic approach.

According to the literature, pheochromocytoma has a variable penetrance in MEN2A patients, depending on the mutation of RET [54]. The highest frequency is found in patients with a mutation of codon 634 [55]. It is frequently bilateral, multicentric, and not always synchronous. Patients require alpha blockade, with or without beta-blockade, before and during surgery.

Finally, in MEN2 patients with parathyroid adenomas, open neck exploration is recommended with the removal of enlarged parathyroid glands only, rather than the more aggressive approach adopted for parathyroid tumors in MEN1 patients [56]. Recurrence is uncommon [8]. The clinical manifestations of MEN2-related PHPT are similar to sporadic cases, including nephrolithiasis, osteopenia or osteoporosis. Although parathyroid hyperplasia is more common in MEN syndromes, in the presented case, the discovery of a single parathyroid adenoma was made incidentally, intraoperatively, in the context of the surgical intervention for the removal of metastatic lateral cervical lymph nodes.

What draws our attention and raises questions in this case, are the markedly elevated levels of calcitonin in the last trimester of pregnancy, with a sudden decrease in the last 2 weeks of pregnancy and postpartum. Studies in the literature regarding changes in calcitonin levels throughout pregnancy among patients with a personal history of MEN2 syndrome are limited.

Considering the rare cases described in the literature of an association between MEN2 and pregnancy, we can take this as a limitation of the current study. We can also add the lack of specific guidelines to help manage such a case where elevated calcitonin values are recorded during pregnancy.

As directions for future research, we will consider the continued development of generations of MTTs, which have minimal toxicity and greater specificity for mutated RET and whose purpose is to either cure patients with advanced MTC or change their progressive illness into a chronic, long-lasting.

That is why, in the future, we will be committed to forming and monitoring further studies with larger sample sizes and long-term follow-up to draw definitive conclusions.

CONCLUSIONS

MEN2 is a rare hereditary disorder distinguished by MTC, either unilateral or bilateral pheochromocytoma and primary parathyroid hyperplasia. The risk of developing MTC over one's lifetime is almost 100%, with varying expressions of other symptoms. Neglected or undetected, MTC within the context of MEN2 can lead to fatal outcomes.

Yet, timely identification and thorough diagnostic protocols enable prompt interventions, thereby reducing the associated morbidity and mortality linked to MEN2.

Imaging check-ups in combination with annual monitoring of calcitonin, CEA, metanephrines/normetanephrines, and parathyroid hormone in a patient with MEN 2 syndrome are practical ways to supervise the case, make timely decisions about surgical intervention, and prevent complications.

Genetic testing of MEN2 families and prophylactic thyroidectomy are mandatory. In cases where there is a family history of the RET mutation gene, a proactive approach to performing thyroidectomy significantly reduces the risk of complications and death, as evidenced by the positive outcome observed in our patient's son.

Even in the face of repeated endocrine challenges and recurring illnesses, the potential for utilizing tyrosine kinase inhibitor therapy stands as a robust choice in managing tumors associated with MEN2 syndrome.

By intertwining our clinical observations with established literature, we navigate the complexities of MEN 2 syndrome management. This blend of personalized care and evidence-based medicine sheds light on the nuanced treatment of MEN 2 syndrome. Over 25 years of patient follow-up, we witness sustained health and improved quality of life. As we evolve alongside advancements in targeted systemic therapies, our approach remains grounded in comprehensive understanding and patient-centered care.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

This research received no external funding. All the images are original, not previously published and not collected from other sources.

Authors' contribution

Conceptualization, D.T., and D.P.; methodology, D.T., and D.P.; software, D.T., and A.M.; validation, A.M., and I.S; formal analysis, D.P.; investigation, D.T.; resources, A.A.; data curation, S.P.; writing—original draft preparation, D.T.; writing—review and editing, D.T.; visualization, A.M..; supervision, M.G.; project administration, I.S..

Patient consent for publication

The patient has given written informed consent to publish this paper, including permission for the details/images to be available on the Internet and viewable by the general public.

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NARRATIVE REVIEW

Structural and Functional Imaging in Epilepsy – A Narrative Review

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Abstract: *The primary objective of this narrative review is to evaluate the most frequently recommended structural and functional imaging methods in managing epilepsy cases as effectively as possible from a neurologist's perspective. This review examines the background of current practice, from the beginnings of neuroimaging by computer tomography to the newly introduced methods and their combination, which helps diagnose and monitor these patients. The neuroimaging investigations required for the presurgical evaluation of patients with epilepsy are presented, and recommendations for specific MRI protocols are provided. The clinical use of functional imaging methods is also considered, and correlations between clinical aspects and specific imaging methods are explored, based on the retrieved data in the literature. In conclusion, this review emphasizes using functional and structural imaging methods, guided by clinical evidence, for better therapeutic management in patients with epilepsy.*

Keywords: *Positron Emission Tomography, Single-photon-emission computed tomography, medically refractory epilepsy, HARNESS protocol, MRI*

INTRODUCTION

Epilepsy is one of the most common neurological pathologies globally, with an incidence of 1-2% of the population [1]. According to the 2017 International League Against Epilepsy (ILAE) classification, seizures are stratified into focal, generalized, or unknown onset [2].

Differentiating focal onset from generalized onset seizures is sometimes very difficult because patients may present with bilateral tonic-clonic seizures without a clear description of the initial symptomatology and with inconclusive electro-encephalography (EEG) data. Neuroimaging is crucial because many etiologies can be diagnosed with its help. An epileptogenic area discovered by Magnetic Resonance Imaging (MRI) can support the focal onset of seizures and help for better therapeutic management, either surgical or pharmacological (Figure 1). Unfortunately, about one-third of epilepsy patients become drug-resistant [3]. In this situation, it is necessary to delimitate the epileptogenic area and surgically remove it [4]. The epileptogenic zone is defined by Lüders et al. as "the minimum amount of cortex that must be resected (inactivated or completely disconnected) to produce seizure freedom" [5].

Figure 1: Some non-invasive methods

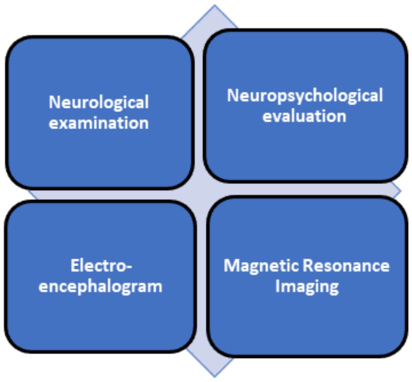

A presurgical evaluation is necessary to decide which candidates are suitable. All patients with pharmacoresistant epilepsy should be evaluated for epilepsy surgery. This may cause or increase the risk of sudden unexpected death in epilepsy (SUDEP), which is why patients should be encouraged to have better therapeutic compliance [6]. This is also helped by good communication between doctor and patient; the better informed the patient is, the more chances there are to provide appropriate therapeutic management [7].

Regrettably, quite a significant number of patients are not considered or cannot access such a program. This happens mainly in underdeveloped or developing countries due to the need for more specialized medical personnel in this field [8].

In many situations, more than these are needed, and more advanced investigations are required (Figure 2) [9].

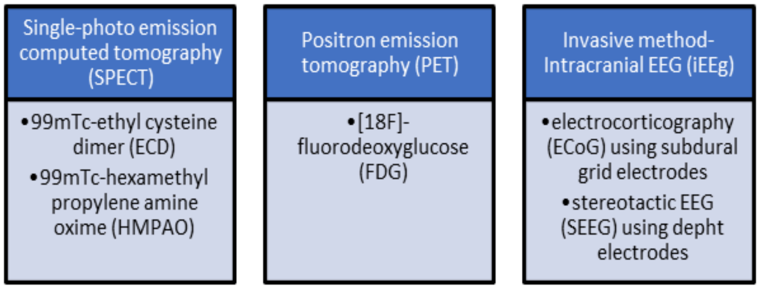
Figure 2: Advanced investigations

Single-photo emission computed tomography (SPECT)
•99mTc-ethyl cysteine dimer (ECD)
•99mTc-hexamethyl propylene amine oxime (HMPAO)
Positron emission tomography (PET)
•[18F]-fluorodeoxyglucose (FDG)
Invasive method-Intracranial EEG (iEEg)
•electrocorticography (ECoG) using subdural grid electrodes
•stereotactic EEG (SEEG) using depht electrodes

These investigations aim to determine the patient's chances of remaining seizure-free postoperatively and, of course, to minimize the post-surgery risks and functional dysfunction. After these steps have been taken, the next one would be to establish a few essential things: the symptomatic area, the irritative area, the ictal area, the epileptogenic area, and the eloquent area [10].

OBJECTIVE

This article aims to summarize the role of structural and functional imaging of the brain by use of CT, MRI, SPECT, FDG PET, and non-FDG PET radio-tracers in epilepsy from a multidisciplinary perspective. Our search strategy on PubMed included terms related to epilepsy, neuroimaging in epilepsy including PET and SPECT, and HARNESS protocol MRI, in studies published from 1995 until January 2024. Only papers published in English were selected.

RESULTS

A number of 44 primary and secondary reports have been selected to be included in the review. These sources explored both structural brain imaging techniques and functional neuroimaging investigations focused on diagnosing and monitoring epilepsy.

STRUCTURAL BRAIN IMAGING

Magnetic Resonance Imaging (MRI)

According to ILAE publications, a brain MRI should be performed after the first seizure to determine therapeutic attitude (Figure 3). It has a high specificity for detecting possible cortical malformations, including cortical dysplasia, mesiotemporal sclerosis, and other structural changes that have an increased epileptogenic effect and may also increase the risk of treatment resistance. As for epileptic syndromes, there is little evidence to recommend advanced imaging, but if abnormal neurological development, cognitive decline, treatment resistance, or focal interictal spikes occur, it should be considered and performed [11].

The ILAE Neuroimaging Task Force has proposed a set of acquisition sequences known as the optimal protocol for epilepsy, the "HARNESS"-MRI protocol (Bernasconi et al., 2019 – Figure 4) [11]. The optional sequences are Gadolinium-enhanced MR and Susceptibility-weighted imaging (SWI) [12].

The role of structural MRI in detecting subtle structural abnormalities

Focal cortical dysplasia (FCD) is one of the most frequent structural changes requiring advanced imaging evaluation and the third most common etiology of medically intractable seizures in adults [13]. There are three types; the first type is associated with a late onset, in adulthood, with changes in the temporal lobe. Clinical symptoms are more severe in type 2, especially in children with predominantly frontal lobe changes. FCD type 2 correlates with other tumors like hippocampal sclerosis, tumor, vascular malformation, or attained pathology during early life [13]. FCD type 3 refers to malformation connected with different cortical lamination abnormalities with a main lesion within the same area/lobe. In a significant number of cases, especially FCD type 1, it may remain undetected.

Additional curvilinear reformatting and morphometric analysis of T1 and T2-FLAIR sequences may be helpful in this situation. For detecting these abnormalities, a satisfactory specificity of 90%, accompanied by a sensitivity of 50%, was observed only by FLAIR sequencing [14-17]. To avoid these limitations, an excellent therapeutic approach is to combine MRI with functional imaging, PET, or SPECT [18].

Another standard structural change (Figure 5) is temporal lobe epilepsy, particularly mesiotemporal sclerosis (MTLE). It refers to damage to the mesial or inner portion of the temporal lobe, with a focus on the hippocampus. This can result from various reasons, including infection, head trauma, or other causes [19]. Symptomatology is polymorphic but may include altered consciousness, memory disturbances, and emotional changes [20]. Patients usually have an increased risk of developing drug resistance, so it is important to refer them to a specialized center. Brain MRI is a central and essential method of diagnosis and assessment. It can show a certain level of atrophy in this area, increased T2 signal, and loss of hippocampal architecture [21,22].

Figure 3: Indications for magnetic resonance imaging (MRI) in patients with epilepsy [22]

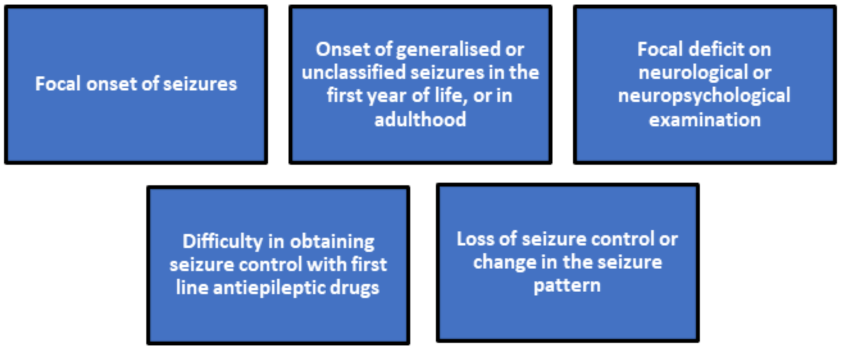
Focal onset of seizures
Onset of generalised or unclassified seizures in the first year of life, or in adulthood
Focal deficit on neurological or neuropsychological examination
Difficulty in obtaining seizure control with first line antiepileptic drugs
Loss of seizure control or change in the seizure pattern

Figure 4: Required sequences of the HARNESS-MRI protocol [11]

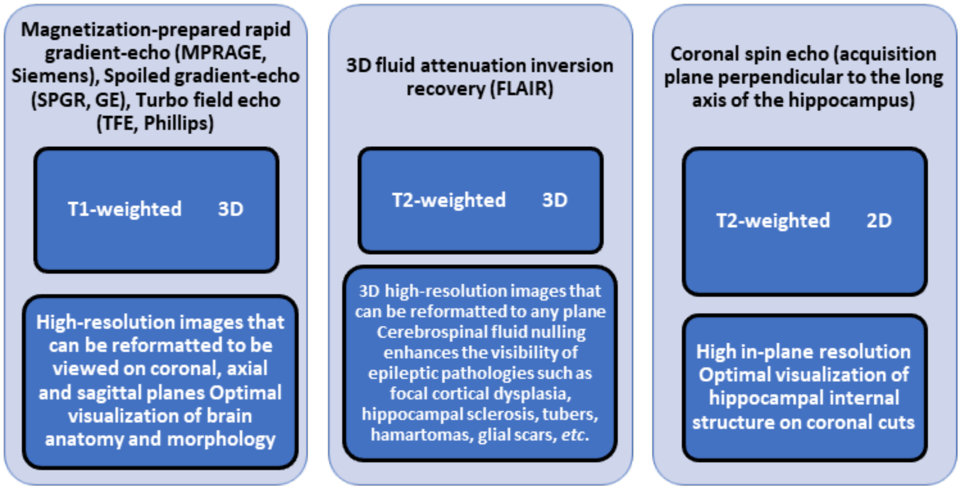
Magnetization-prepared rapid gradient-echo (MPRAGE, Siemens), Spoiled gradient-echo (SPGR, GE), Turbo field echo (TFE, Phillips)
T1-weighted 3D
High-resolution images that can be reformatted to be viewed on coronal, axial and sagittal planes Optimal visualization of brain anatomy and morphology
3D fluid attenuation inversion recovery (FLAIR)
T2-weighted 3D
3D high-resolution images that can be reformatted to any plane Cerebrospinal fluid nulling enhances the visibility of epileptic pathologies such as focal cortical dysplasia, hippocampal sclerosis, tubers, hamartomas, glial scars, etc.
Coronal spin echo (acquisition plane perpendicular to the long axis of the hippocampus)
T2-weighted 2D
High in-plane resolution Optimal visualization of hippocampal internal structure on coronal cuts

Figure 5: Other common epileptic pathologies

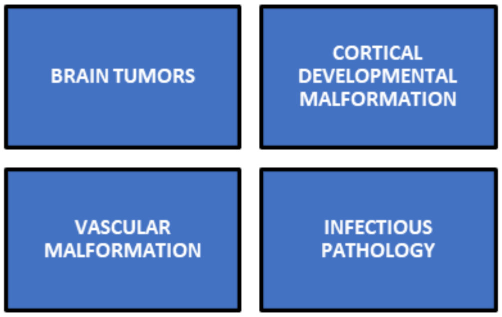
BRAIN TUMORS
CORTICAL DEVELOPMENTAL MALFORMATION
VASCULAR MALFORMATION
INFECTIOUS PATHOLOGY

Computed tomography (CT)

CT can play an essential role in the assessment of patients with epilepsy in certain situations. However, in general, CT is not used as the first option for diagnosing epilepsy but rather to rule out other causes of seizures or to identify possible brain damage. According to the American Academy of Neurology (A.A.N.), this investigation should be performed and recommended to all patients presenting with a first epileptic seizure, especially if they have an abnormal neurological examination or focal onset of the seizure [23]. The role of CT in epilepsy may refer to:

- Exclusion of other causes: CT may be performed to exclude brain lesions or abnormalities that could cause seizures. This may include tumors, vascular malformations, or traumatic injuries.

- Post-traumatic assessment: if the patient has suffered a severe head injury, CT may be used to assess any brain injury or bleeding that could be involved in triggering seizures.

- Preoperative guidance: in cases where epilepsy is resistant to treatment and surgery is considered an option, CT can guide the surgeon in pinpointing the exact brain area responsible for the seizures.

However, for a more detailed evaluation of the brain in epilepsy cases, other imaging techniques, such as MRIs, are usually preferred [24].

FUNCTIONAL IMAGING

Functional imaging (Figure 6) can help us when there are situations where structural imaging is not sufficient or there are negative results (negative MRI) to identify and demarcate the epileptogenic focus, or when multiple epileptogenic foci are detected [25].

A large proportion of patients with drug-resistant epilepsy, almost 50%, have no evident lesion found on brain MRI. In this situation, we can speak of a negative MRI. However, there are cases in which a second interpretation of the same investigation, accompanied by additional information, revealed the lesion [26]. In this case, it should be checked if the HARNESS protocol was used or if there are artifacts that require the imaging to be repeated.

Functional imaging is remarkable in that epileptogenic brain tissue has a different metabolism and electrophysiology than normal tissue. These regions of difference are then visually expressed and projected into existing structural images, usually MRI [27].

Figure 6: The most widely used functional imaging in epilepsy

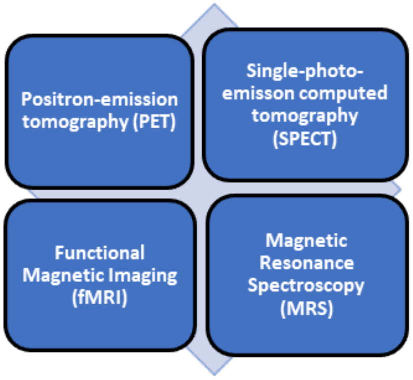
Positron-emission tomography (PET)
Single-photo-emisson computed tomography (SPECT)
Functional Magnetic Imaging (fMRI)
Magnetic Resonance Spectroscopy (MRS)

Functional MRIs can be helpful when the patient is asked to do consecutive, usually simple, tasks. During this time, there may be changes in T2 sequences. This is why this investigation is recommended in some presurgical evaluations to detect hemispheric language dominance, especially when a resection of the dominant temporal lobe is planned [12]. Other indications include motor, somatosensory, or visual cortex mapping. It is beneficial in situations where there is the proximity of tumors or FCD to eloquent areas [28].

In general, areas with changes in contrast-dependent blood oxygenation levels are secondary changes in the ratio of deoxy- to oxyhemoglobin that occur during the performance of cognitive, motor, or other tasks, which permits us to map the neural pathways implicated in the delivery of these tasks. One of the most critical applications of this investigation is the localization and lateralization of cognitive function. A crucial step before surgery is to reduce the risk of causing a significant impairment. This is why a cooperative patient who can smoothly execute the indications and tasks is needed. This investigation is not recommended for patients who are claustrophobic, who have metal devices, or who are not able to perform tasks. For preoperative fMRI, it is advisable to choose appropriate procedures depending on the area of the brain to be resected [29].

In recent years, there has been a growing association between simultaneous electroencephalography monitoring and fMRI [30]. This method makes use of EEG changes, in particular, interictal spikes and discharges that may be associated with hemodynamic changes. This can have a significant impact on the therapeutic decision and case management [31].

Positron Emission Tomography

PET examination of the brain is used to observe a variety of images and biological phenomena such as blood flow, metabolism, protein and DNA synthesis, and more. This happens after injecting a radiolabelled compound.

Radiotracers are imprinted with a positron-emitting isotope manufactured by a cyclotron. After administration, they spread to different areas of the body, even to the brain, crossing the blood-brain barrier and gathering in the tissues to reveal the biological impact of the radiotracer. The mechanism of positron detection is via the coincidence system, which allows PET to have spatial resolution in the mm range. This also differentiates it from SPECT, which has a weaker resolution. Even though there are several PET Radiotracers, the most commonly used for epilepsy is FDG (18F-fluorodeoxyglucose) [32].

FDG-PET imaging

As mentioned above, when MRI and video EEG monitoring are not relevant, we can use PET, and the most common tracer in epilepsy is FDG-PET. This measures glucose metabolism linked to the synaptic and neuronal activity of the cerebral tissue. Even if there is not enough evidence for a precise time window, the indicated postictal period differs depending on the type of seizure. For focal aware seizures, interictal FDG-PET should be performed at least after 24h and 48h after focal impaired awareness and focal to bilateral tonic-clonic seizures. This is important because it can complicate the outcome of investigations. This can be misinterpreted, leading to erroneous lateralization because it should be interictal, and the basis of the interpretation is finding the ratio of hypometabolism to the contralateral side [33]. Another factor that can have an impact by lowering the rate of glucose metabolism is the use of some drugs, for example, barbiturates, valproic acid, phenytoin, and carbamazepine [34].

Some patients need sedation, which can be done 10 minutes before the examination. Several studies have shown that propofol sedation can reduce the rate of glucose metabolism and the rate of cerebral blood flow, which need to be taken into account when these procedures are required [35].

Usually, in interictal periods, FDG-PET shows hypometabolism in epileptogenic areas. This is directly related to seizure duration, frequency, and severity. An increased frequency also leads to increased hypometabolism area and vice versa [36]. There are also some particularities, such as in some cases of focal epilepsy, frontal or temporal, hypometabolism may be present in the ipsilateral thalamus, or contralateral cerebellar hypometabolism may be found especially in frontal lobe epilepsy [36].

In order to avoid false results that could mislead us by hypo or hypermetabolism, a proper strategy is to register the EEG during the 30-40-minute period of FDG capture. If this is not feasible, an epileptologist can be called in to follow the patient. EEG monitoring can be stopped once the patient enters the scanner.

In the case of temporal lobe epilepsies, the specificity and sensitivity are over 80%, but a unique contribution is made in situations where there are dual-pathology cases (coexistence of medial temporal and neocortical seizure foci) [37]. The localization of epileptogenic areas is diminished when dealing with frontal lobe epilepsy especially in some cases of occipital epilepsy [38].

Another essential feature is that FDG-PET can help assess the functionality of the remaining unresected brain tissue when an epileptogenic lesion has been taken into account for surgical resection. The post-surgical neurocognitive status is essential and also depends on the integrity and functionality of the remaining brain tissue.

Also, this type of patient can be associated with other comorbidities, and among the most common are cognitive disorders and depression that contribute to a decrease in quality of life. It has been shown that those with depression and epilepsy have a predisposition to ipsilateral or bifrontal hypometabolism [39]. PET studies in patients with temporal lobe epilepsy using a serotonin transporter ligand as a receptor have shown a decrease in its activity. This supports the hypothesis of using medication with selective serotonin reuptake blockers in patients with epilepsy and depression [40,41]. Memory deficits are among the frequent complaints of these patients. In those with occipital lobe seizures, deficits in verbal memory and executive function have been correlated with left temporal lobe hypometabolism. Patients with postictal amnesia were associated with disturbances in the left mid-temporal metabolism [42]. Under specific circumstances, other radio transmitters can be used, including [^{11}C] flumazenil, which has been shown to have a higher sensitivity to temporal lobe epilepsy, particularly hippocampal sclerosis [43].

SPECT

This type of imaging is used to measure cerebral blood flow in vivo. Although brain perfusion and metabolism are not the same type of process, they are connected in most situations [44,45]. The difference between FDG-PET and SPECT is the lower spatial but higher temporal resolution, leading to the detection of the seizure onset zone. Cerebral blood flow is strongly correlated with neuronal activity, so during the ictal phase, they increase concomitantly. Therefore, for an accurate interpretation of the results, it is necessary to precisely measure the dissimilarity between ictal and interictal SPECT results (which are interrelated with CT or MRI) [46]. The areas of hyperperfusion in the ictal phase are considered to have the most significant potential for delimiting the epileptogenic zone.

In general, interictal SPECT has a lower sensitivity compared to ictal SPECT. In the interictal period, hypoperfusion or normal perfusion may be observed in epileptogenic areas. Even when hypoperfusion is present, it can be of a relatively low intensity, making it quite hard to distinguish from normal tissue. Its primary role is to support the ictal assessment using SISCOM (subtraction ictal SPECT connected to MR imaging; that is, the interictal SPECT images are retrieved from the ictal photos, and the results are displayed on cosigned MR images), by furnishing a baseline blood flow [47].

However, logistics is not an advantage. The patient must be under constant medical supervision, with EEG monitoring and possibly tapering of antiepileptic medication to increase the possibility of a new seizure recurrence. The radiotracer should be placed at the patient's bedside for faster administration. So that immediately at the onset of a seizure, either announced by the patient or by clinical or EEG changes, the radiotracer can be injected. This is important because focal seizures, as we know, bilateralize quite quickly, and a late injection may miss the initial focus. Nevertheless, SPECT remains a crucial investigation in MRI-negative patients [48].

Magnetic resonance spectroscopy (MRS)

This investigation can measure different metabolites. Among those that are detectable are N-acetyl aspartate (NAA), choline, creatine, lactate, glutamate, and gamma-aminobutyric acid (GABA) [49].

Numerous studies examining various brain pathologies have found that NAA, which is predominantly found in neuronal mitochondria, is the most significant informative measure of neuronal function. On the other hand, creatine is found as well in neurons as in astrocytes, but the highest concentration is in the astrocytes [50]. Therefore, when considering the integrity of neuronal function, the NAA/creatine ratio is used. In epileptic patients with cortical anomalies, it has been shown that NAA values are specific to the epileptogenic area and have average values in patients with therapeutically controlled seizures. Even in those patients who have recurrent seizures but with non-epileptogenic structural abnormalities, there's a high likelihood of changing NAA levels [50].

Unlike other investigations, proton MRS does not typically include the whole brain in measurements, and sometimes, a few significant voxels are sampled with proton MRS. A relatively low signal-to-noise ratio is calculated, and this, together with the extended time taken to obtain the spectra, makes the technique of somewhat limited clinical use.

MRS may play a significant role in temporal lobe epilepsy because it is better defined clinically and anatomically. However, the same cannot be said for neocortical or extratemporal epilepsies. In this case, we have a much larger brain volume with the potential to trigger seizures with different propagation paths [51]. Other trials targeting MTLE groups have shown that bilateral metabolic alterations in these patients have predictive value for postoperative outcomes. So, those with significant bilateral or contralateral lesion metabolic changes had a much worse postoperative outcome through poor seizure control [52,53].

Results were compared with EEG localization and surgical results, showing that reduced NAA signal intensity can lateralize the epileptogenic area and those with focal epilepsy, especially MTLE. Nevertheless, these changes are often bilateral in patients with MTLE. After successful surgery, the NAA concentration can normalize [54].

CONCLUSIONS

Neuroimaging plays a vital role in the assessment and management of patients with epileptic seizures. Magnetic resonance imaging can lead to the detection of the etiology of focal seizures and the demonstration of anatomical changes associated with epileptic seizures. Even if its increased efficiency has been demonstrated in some situations, such as in the case of negative MRI or pharmacoresistant epilepsies, other investigations are helpful. One of them is PET, a functional interictal technique that can help us identify the onset of these seizures due to metabolic changes. SPECT is another functional but ictal investigation with preoperative guidance.

SPECT and PET imaging modalities play a vital role in the presurgical evaluation of patients with epilepsy, especially those with negative MRIs or multiple foci on EEG monitoring. Another essential feature is the assessment of the integrity of the rest of the brain tissue, which provides information about the possible pathogenesis of neurocognitive or behavioral disorders. It is important to note and underline that these are not diagnostic tools and should not be used to support a diagnosis of epilepsy.

The aim of these new imaging techniques, together with a multidisciplinary perspective on the presurgical assessment of patients with epilepsy, is to have increasingly better outcomes, with reduced post-surgical neurological deficits and improved quality of life.

The combination of structural and functional methods has been used in multimodal learning models to discover the seizure onset zone and assimilate the more accurate mechanisms involved in structural and functional abnormalities in a very often encountered pathology, i.e., epilepsy.

Authors' contribution
Conceptualization, A.M.M., C.A.S., and R.G.; methodology, O.V., R.I.D., M.M., and O.M.S.; validation, A.M.M., C.A.S., M.M., and R.G.; resources, O.V., R.I.D., and O.M.S.; data curation, A.M.M., C.A.S., M.M., and R.G.; writing—original draft preparation, A.M.M., C.A.S., and R.G.; writ-ing—review and editing, O.V., R.I.D., M.M., and O.M.S.; visualization, O.V., R.I.D., and O.M.S.; supervision, A.M.M., C.A.S., and R.G.; project administration, C.A.S. All authors have read and agreed to the published version of the manuscript reported.

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REVIEW

Comprehensive Review and Update on Multiple Organ Dysfunction Syndrome in Burn Patients: Pathophysiology, Risk Factors, and Management Strategies

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Abstract: *Burn injuries are among the most severe forms of trauma, often leading to multiple organ dysfunction syndrome (MODS), a major contributor to morbidity and mortality in burn patients. This review examines the pathophysiological mechanisms, clinical manifestations, and management strategies associated with MODS in burn patients. Following a severe burn injury, the systemic inflammatory response and subsequent release of pro-inflammatory cytokines precipitate dysfunction across various organ systems. Early recognition and aggressive management of MODS, including fluid resuscitation, infection control, and organ support, are crucial for improving survival outcomes. A comprehensive understanding of the multifactorial etiology and interrelated nature of organ dysfunction in burn patients is essential for developing targeted therapies and optimizing patient care. This review provides a current overview of the topic. In the coming years, new insights into the pathophysiological mechanisms are expected to enhance our comprehension of the subject further.*

Keywords: *burn injury, systemic inflammatory response, multiple organ dysfunction syndrome, cardiac dysfunction, respiratory dysfunction, acute renal failure, hepatic dysfunction, encephalopathy, coagulopathy, sepsis*

INTRODUCTION

Burn injuries are a major threat in military environments, historically present for over 5,000 years. Modern weapons have heightened the risk, causing significant casualties among soldiers and civilians. Advanced weapons, such as napalm, white phosphorus, anti-armor weapons, and fuel-air explosives, pose a high risk of causing severe burns. While protective gear like Kevlar vests has increased survival rates by protecting vital areas, exposed body parts still suffer from serious burns and shrapnel injuries. Advances in battlefield medical care have reduced the fatality rate of combat injuries, but severe burns remain complicated and require long-term treatment and rehabilitation. [1]

In the best circumstances, optimal treatment of war-related burn injuries can salvage about 50% of patients with burns covering 60-70% of their total body surface area (TBSA). Limited resources necessitate prioritizing care for those who will benefit the most, focusing on patients with burns covering 20-70% TBSA. In battlefield triage with resource constraints, patients with burns over 80% TBSA may receive palliative care, while those with burns of 20% or less and who are stable may have care delayed. Triage adjustments should consider coexisting inhalation or mechanical injuries, which reduce the treatment threshold by 10%. Burns on the hands, face, feet, and perineum may require increased care despite a lower TBSA burn percentage. [2]

Multiple organ dysfunction syndrome (MODS) is a severe complication in burn patients, particularly those with extensive burns. Severe burns trigger a systemic inflammatory response syndrome (SIRS), leading to widespread inflammation and potential organ failure. Extensive fluid loss from burn wounds can cause hypovolemic shock, impairing organ perfusion and function. Burn wounds are highly susceptible to infection, which can lead to sepsis and subsequent organ dysfunction. MODS significantly increases morbidity and mortality in burn patients, necessitating a multidisciplinary approach to manage and support affected organ systems effectively. [3-5]

In the early 1970s, Baue characterized the syndrome known as progressive or sequential multiple organ failure [6]. The term "multiple organ dysfunction syndrome" was introduced in 1991 during the Consensus Conference of the American College of Chest Physicians and the Society of Critical Care Medicine and it was defined as the presence of compromised organ function necessitating therapeutic interventions for maintaining homeostasis. The conference also addressed the systemic inflammatory response syndrome (SIRS) to delineate the clinical and paraclinical manifestations of systemic inflammation [7]. Later on, several scoring systems were devised to assess multiple organ dysfunction, including the MOD (Multiple Organ Dysfunction) score [8], the SOFA (Sequential Organ Failure Assessment) score [9], and the LODS (Logistic Organ Dysfunction) score [10], all based on the assessment of six parameters: respiratory, cardiovascular, hepatic, renal, neurological, and coagulation systems.

As the quality of medical care has improved, multiple organ dysfunction has become the leading cause of mortality associated with trauma in patients who survive the initial injury and are admitted to intensive care. The presence of multiple organ dysfunction is associated with increased morbidity and mortality in patients who have survived the first few hours after trauma [11].

PATHOPHYSIOLOGY OF MODS

The pathophysiology of MODS is multifactorial, complex, and still not fully understood. In the 1990s, multiple organ dysfunction was described as a bimodal phenomenon: the one-hit model and the two-hit model [12].

Currently, three models have been proposed to explain the occurrence of MODS:

- One-hit/one-event model: A massive trauma induces an intense SIRS associated with the onset of organ dysfunction.
- Two-hit/two-event model: Describes an initial injury followed by a second, seemingly minor injury that promotes inflammation and immunologic dysfunction. Thus, in the presence of moderate SIRS, a secondary activating event (such as surgical interventions, infections, embolism, transfusions) occurring during the compensatory anti-inflammatory response syndrome (CARS) will lead to late MODS
- Sustained injury model: Describes a continuous injury that acts both as the initial injury and maintains dysfunction [12-13].

Modern theories propose a system in which aggression simultaneously triggers two opposing responses: a pro-inflammatory response (SIRS) and an anti-inflammatory response (SARS, previously called compensatory, CARS), whose intensity and predominance correlate with the occurrence of MODS [14-15]. Early-onset multiple organ dysfunction is induced by the pro-inflammatory status with the activation of the immune system within SIRS, while late-onset multiple organ dysfunction is due to the exacerbation of anti-inflammatory responses within SARS, leading to immunosuppression, delayed healing, and increased susceptibility to infections [16].

There are several mechanisms implicated in the development of MODS, including mitochondrial dysfunction, alterations in immune system regulation, tissue or cellular hypoxia, activation of cellular apoptosis, and bacterial translocation from the gastrointestinal tract [17]. The factors involved in the development of MODS include:

- Persistent thrombocytopenia: It has been demonstrated that the interaction between platelets and neutrophils is involved in the development of acute lung injury (ALI), and antiplatelet therapies have been associated with a reduced risk of ALI, MODS, and decreased mortality in patients with adult respiratory distress syndrome (ARDS) [18].
- Polymorphonuclear leukocytes (PMNs): They act through local cytotoxic effects produced by PMN degranulation and the release of reactive oxygen species, nitric oxide, and pro-inflammatory mediators such as IL-6, IL-8, and TNF-α [19].
- Tissue macrophages and circulating monocytes [19].
- Pro-inflammatory cytokines (TNF-α, IL-1, IL-2, IL-6, IL-8, IL-17, MIP, GM-CSF, IFN-γ) and anti-inflammatory cytokines (IL-4, IL-10, IL-13) [20].
- The complement system: It is activated with the production of pro-inflammatory molecules C3a, C3b, and C5a, and the formation of the C5b-C9 complex, which leads to the lysis of the target cell. The activation of the complement system results in the production of reactive oxygen species, arachidonic acid metabolites, and cytokines [21-22].

In burn patients, it is considered that multiple organ dysfunction syndrome (MODS) is triggered by an inflammatory status, often associated, but not always, with sepsis. The onset of MODS can be caused either by extensive infection or by multiple infections with a smaller systemic impact, but which stimulate inflammation [23].

Other possible causes for MODS include:

- Gastrointestinal barrier impairment: involves bacterial translocation into the portal and lymphatic circulation, promoting the inflammatory response, or causing sepsis [24].
- Presence of uncovered burn wounds: leads to persistent inflammation and elevated levels of IL-6, IL-8, and TNF, contributing to a hypermetabolic and inflammatory status [25].
- Tissue hypoxia associated with hypoperfusion: causes cellular dysfunction through inadequate oxidative mechanisms and produces oxygen radicals [4].

In severely burned patients, MODS is the leading cause of death, with reported mortality rates in studies ranging from 29% to 86% [26-29]. The severity of MODS and mortality in burned patients correlate with the burned body surface area, the presence of deep burns, and the patient's age. The onset of MODS leads to prolonged hospitalization in intensive care, increased duration of ventilatory support, and longer hospital stays [26].

Burns over 30% of the body surface area induce a systemic inflammatory response associated with fluid loss through burn wounds and fluid extravasation into the interstitial space. This results in hypovolemia with hemodynamic changes similar to those seen in hemorrhagic shock, including decreased plasma volume, cardiac output, and urine output, as well as increased peripheral vascular resistance with decreased peripheral blood flow. These manifestations characterize post-burn shock, a particularly severe type of shock in severely burned patients, occurring primarily within the first 24 hours, peaking at 6-8 hours post-burn [31-32].

Inflammatory mediators, both local and systemic, involved in the pathogenesis of post-burn shock include histamine, TNF, interleukins, products of the arachidonic acid cascade (prostaglandins and thromboxanes), serotonin, bradykinin, nitric oxide, and oxygen radicals. Additionally, levels of certain hormones and cardiovascular function mediators are frequently elevated: epinephrine, norepinephrine, vasopressin, angiotensin II, and neuropeptide Y [33].

Similar to the treatment of other forms of hypovolemic shock, the main therapeutic objective is the prompt restoration of intravascular volume to preserve tissue perfusion. However, volume resuscitation in burns is complicated by the presence of edema not only in burned tissues but also in unburned tissues.

Consequently, large volumes of fluids are necessary to maintain intravascular volume in the initial hours following extensive burns [32]. Studies have shown that 50% of the edema fluid volume is found in unburned tissues [34].

RESPIRATORY DYSFUNCTION

Post-burn respiratory dysfunction is multifactorial, with the most common causes being the presence of inhalation injuries and the development of acute respiratory distress syndrome (ARDS) [35]. However, any pulmonary injury can be exacerbated by damage to the small airways (<2mm) and pulmonary alveoli induced by mechanical ventilation [36]. The evolution of respiratory dysfunction is also contributed to by the onset of pneumonia, which has an increased incidence, especially in patients with inhalation burns [37]. The presence of inhalation burns is a negative prognostic factor for morbidity and mortality, as established in the literature [38-39]. The severity of inhalation burns correlates with an increased need for tracheostomy, the number of days of mechanical ventilation, and a longer duration of hospitalization in intensive care [40]. In association with cutaneous burns, the presence of inhalation injuries increases the volume requirements for fluid resuscitation of burn patients and leads to a higher incidence of pulmonary complications [41].

From the perspective of affected structures and the etiopathogenic mechanism, inhalation injuries are divided into three categories:

1. Thermal injury to the upper airways: This results directly from elevated temperatures and consists of edema in the supraglottic structures and obstruction of the upper airways within the first few hours post-burn.

2. Chemical irritation of the lower airways: This is caused by the presence of toxic chemical compounds (such as sulfur dioxide, nitrogen dioxide, ammonia, cyanides, and aldehydes) in smoke, that cause epithelial and capillary endothelial damage to the respiratory tract, with histological features similar to tracheobronchitis. Physiopathological changes include increased local blood flow in the bronchi, local exudate and bronchiolar edema, necrotizing bronchitis, impaired mucociliary clearance with decreased bacterial removal, alveolar collapse, and atelectasis due to surfactant loss, activation of local macrophages with inflammatory response, bronchospasm, and increased capillary permeability leading to enhanced pulmonary edema. After smoke exposure, respiratory failure may occur due to airway obstruction from epithelial detritus, fibrin clots, and mucus within a variable timeframe, from 12 hours to 2 days post-burn.

3. Systemic injury: This occurs through the inhalation of carbon monoxide (CO) or cyanides, which causes both pulmonary effects, such as hypoxia and hypercapnia, and systemic effects mediated by pro-inflammatory cytokines. [41-44].

Acute respiratory distress syndrome (ARDS) is characterized by severe hypoxemia in the context of pulmonary alveolar damage, progressing to acute pulmonary edema and acute respiratory failure [45]. In burn patients, ARDS can occur either in association with airway burns or in their absence due to the systemic inflammatory response associated with burns during the post-burn shock period or septic complications of burns following the post-burn shock period. Inhalation injuries are an independent risk factor for the development of ARDS, with earlier onset and greater severity compared to ARDS induced by cutaneous burns [46]. The severe form of ARDS with refractory respiratory failure is a significant cause of death in severely burned patients [47].

CARDIAC DYSFUNCTION

Cardiac dysfunction leads to organ hypoperfusion, peripheral microcirculation damage, burn wound expansion, and decreased resistance of burned wounds to bacterial infection [48-49]. Cardiac failure is one of the main causes of mortality in severely burned patients within the first two days post-burn [50]

In the first 24-48 hours after a burn, corresponding to the ebb phase, there is a decrease in myocardial contraction and cardiac output [51]. The reduction in cardiac contractility has been associated with cardiomyocyte apoptosis within the first 48 hours post-burn, particularly affecting cells in the subendocardial tissue of the left ventricle [52]. Increased capillary permeability leads to a reduction in circulating plasma volume and an increase in peripheral vascular resistance. Some authors have considered hypovolemia as the primary factor responsible for reduced cardiac output in the early post-burn period [53]. Others have suggested that elevated levels of serum catecholamines, vasopressin, angiotensin II, and neuropeptide Y are responsible for inducing cardiovascular dysfunction, even in the absence of hypovolemia [49].

Data from the literature showed that hypovolemia can persist despite aggressive fluid resuscitation and in the absence of routinely monitored signs of hypoperfusion, such as decreased urine output and decreased mean arterial pressure [51]. Studies have demonstrated that serum lactate levels or the base deficit (the sum of lactate and other acidic metabolites released in the presence of tissue hypoxia) correlate better with the severity of hypoperfusion and remain elevated during fluid resuscitation, even when urine output and blood pressure are adequate [49,54]

From the third day post-burn, corresponding to the flow phase, myocardial contractility improves, and tachycardia and increased metabolic demands with increased catabolism appear [55]. The association of inhalation injuries can further cause right ventricular dysfunction through increased resistance in the pulmonary arteries and pulmonary hypertension, increasing the afterload of the right ventricle [56]. Consequently, left ventricular dysfunction can occur either as global heart failure or due to the displacement of the interventricular septum towards the left ventricle due to increased right ventricular filling [49,57].

Cardiac dysfunction is associated with increased mortality during hospitalization and persistent elevated serum catecholamine levels, tachycardia, and increased oxygen consumption continuing up to two years post-burn [49,58]. Studies in the literature have concluded that sepsis contributes to already present cardiac dysfunction in burn cases [59].

RENAL DYSFUNCTION

Another significant complication in the progression of burn patients is acute kidney injury (AKI), which may manifest either early after the burn injury or later, concurrent with the development of sepsis and in conjunction with other organ dysfunctions [60]. AKI is characterized by a sudden reduction in glomerular filtration with the kidneys' inability to maintain fluid and electrolyte balance, with the first clinical and paraclinical signs being decreased urine output and a subsequent increase in serum creatinine and urea [61]. The incidence of acute renal failure in burn patients has decreased following advancements in the treatment of cardiovascular dysfunction [60].

The causes of early acute renal failure are multiple:

- Myocardial depression: causes decreased cardiac output and renal hypoperfusion [63]

- Hypovolemia and renal hypoperfusion due to underestimation of fluid requirements during fluid resuscitation or delayed fluid resuscitation [62]. Renal hypoperfusion is followed by renal tubular necrosis [63]

- Hemolysis: free hemoglobin can precipitate in renal tubules or cause damage to the tubular epithelium [64]

- Rhabdomyolysis: increased serum levels of myoglobin in extensive deep burns, electrocutions, and compartment syndromes lead to myoglobinuria with myoglobin precipitation in renal tubules, vasoconstriction of afferent arterioles, and production of oxygen free radicals [65]

- Renal hypoperfusion: through increased levels of catecholamines, angiotensin II, aldosterone, and vasopressin, and reduced atrial natriuretic peptide [66]

- Inflammatory mediators such as TNF, IL1, prostaglandins, leukotrienes, thromboxane, and platelet-activating factor: released early post-burn, causing renal tissue damage and microthrombi in renal glomerular capillaries [66]

- Use of nephrotoxic medications, such as aminoglycoside antibiotics, nonsteroidal anti-inflammatory drugs, barbiturates, and hydroxocobalamin [67]

- Respiratory dysfunction: through reduced oxygen supply to the kidneys [68]

Early acute renal failure is usually reversible [252]. Late renal injury typically occurs within the syndrome of multiple organ dysfunction, often associated with severe sepsis. The etiology of acute renal failure in sepsis is also multifactorial, caused by renal arteriole vasoconstriction (prerenal cause), a procoagulant status that induces disseminated intravascular coagulation (DIC), and micro-thrombosis in renal glomeruli and the endothelial damage [67]

Prevention of renal failure is based on monitoring renal function, correcting hypovolemia, hypoxia, and acidosis, limiting nephrotoxic medications, administering thromboprophylaxis in the context of procoagulant status, and early excision-grafting of post-burn lesions to prevent wound infection and sepsis [69].

HEPATIC DYSFUNCTION

Liver injury following burns occurs via multiple mechanisms such as decreased hepatic blood flow, portal hypertension due to the action of endotoxins, release of hepatotoxic products from burn wounds, intrahepatic cholestasis, programmed cell death of hepatocytes through ischemia-reperfusion mechanisms, decreased hepatic blood flow, and the action of proinflammatory cytokines IL1 and TNF-α and hepatic dysfunction due to sepsis from pathogenic microorganisms, toxins, and inflammatory mediators [70-72].

Major burns lead to transient increases in levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, in up to 50% of cases, which typically resolve spontaneously without significant clinical impact [73]. Less frequently, there may be an increase in serum bilirubin, which, however, implies more severe consequences. Severe and persistent increases in liver enzymes, especially bilirubin, often indicate burn-associated complications such as sepsis and are associated with a poor prognosis [74].

During the acute phase response in severely burned patients, there is an increase in the production of acute phase proteins (α1-acid glycoprotein, C-reactive protein, haptoglobin, and α1-antitrypsin) along with a decrease in the levels of constitutive hepatic proteins (prealbumin, transferrin, retinol-binding protein). [75] In the context of meeting the nutritional needs of patients, the decrease in constitutive hepatic proteins is due more to decreased hepatic synthesis and expression. Proinflammatory cytokines such as IL-1β, IL-6, TNF-α, IL-8, and IL-10 act as mediators of the acute phase response and increase immediately post-burn, with their levels remaining elevated for a long time after injury. The uncontrolled and prolonged action of proinflammatory cytokines and acute phase proteins can lead to hypermetabolism and catabolism, which result in multiple organ failure, significantly increasing both morbidity and mortality rates [75-77].

Liver damage has been associated with the development of hepatic edema, with the liver significantly increasing in weight within 2 to 7 days post-burn and reaching a maximum size at 2 weeks post-injury. At 12 months, liver weight can still be increased by 40 to 50% compared to predicted weight [75,78].

Manifestations of liver dysfunction in burns include:

- Hypoalbuminemia due to decreased albumin synthesis; hypoalbuminemia leads to worsening edema and delayed wound healing

- Coagulopathy: due to impaired synthesis of coagulation factors in the liver, increasing the risk of bleeding

- Hyperbilirubinemia

- Glucose metabolism disorders due to impaired hepatic gluconeogenesis and glycogen storage; hyperglycemia is often observed due to deficient glycogenesis and insulin resistance, while hypoglycemia can occur in severe liver failure cases where gluconeogenesis is profoundly affected

- Hepatic steatosis due to altered lipid metabolism

- Sepsis: impaired liver function influences the immune response, making burn patients more susceptible to infections and sepsis [73,75,79].

NEUROLOGIC DYSFUNCTION

Post-burn encephalopathy is defined as acute neurological dysfunction following burns, independent of iatrogenic factors. Patients with post-burn encephalopathy exhibit seizures, agitation, hallucinations, delirium, and other neurological disturbances [80].

Morphopathological studies have demonstrated significant pathological changes in the brain, including massive edema leading to herniation, diffuse intraparenchymal hemorrhage, cerebral infarcts, metabolic encephalopathies, pontine demyelination, infectious complications such as meningitis, cerebral microabscesses, and septic emboli [81-82].

Cerebral ischemia, often underestimated, is a frequent cause of post-burn encephalopathy and is primarily due to hypovolemia associated with severe burns. Hemorrhagic cerebral complications are less common than ischemic ones and present as multiple subarachnoid hemorrhages in both the brain and spinal cord. In patients without hypoxia and hemodynamic disturbances, hemorrhages are more frequently associated with inhalation injuries than with cutaneous burns [83-84].

Proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, along with reactive nitrogen species and oxygen-free radicals released from burned tissues, can alter the integrity of the blood-brain barrier. These molecules, along with neutrophils, can reach the brain, causing microglial cell activation and neuronal apoptosis. The activation of microglial cells in the brain and spinal cord leads to a local inflammatory response and neuronal apoptosis. Activated neutrophils, once they reach the central nervous system, release proteases, oxygen free radicals, reactive nitrogen species, and cytokines, further amplifying the inflammatory process [85].

HEMATOLOGIC DYSFUNCTION

Causes of burn-associated coagulopathy include a systemic inflammatory response with massive cytokine release that interferes with normal coagulation, tissue damage causing direct blood vessel injury and tissue factor release, activating the extrinsic coagulation pathway, impaired normal platelet function, hypoperfusion leading to hypoxia and acidosis, consumption of coagulation factors through clot formation, increased fibrinolytic activity in response to clots and iatrogenic factors (fluid resuscitation and associated hemodilution, as well as blood losses from surgical excision and grafting) [86-87]. Disseminated intravascular coagulation (DIC) is also part of the spectrum of coagulopathy in burn patients. DIC is a clinical-biological syndrome characterized by uncontrolled systemic activation of coagulation, with risks of micro- and macrovascular thromboses and progressive consumptive coagulopathy, increasing the risk of bleeding. [88].

In burn patients, sepsis-induced coagulopathy may also occur, ranging from subclinical coagulopathy to fulminant DIC. [89] Septic patients exhibit a prothrombotic status due to activation of the extrinsic coagulation pathway, amplification of the cytokine-induced coagulation cascade, reduced fibrinolysis, and suppression of natural anticoagulant pathways [90].

Hypercoagulability in burn patients is associated with an increased risk of thromboembolic complications (deep vein thrombosis and pulmonary embolism), with incidence rates in studies ranging from 0.2% to 25% [91]. Contributing factors include patient-specific predispositions (genetic thrombophilia or comorbidities such as cancer history, atherosclerosis, heart failure, chronic kidney disease, previous thromboembolic episodes), iatrogenic factors (presence of central or peripheral venous catheters, repeated venipunctures, certain medications), and the lack of mobilization, serial surgical interventions, and long operative times [92]

Preventive and management measures for coagulopathy in burn patients include general supportive measures such as avoiding dilutional coagulopathy and preventing hypothermia, as well as targeted therapeutic measures like blood product transfusion or antithrombin administration [93].

INFECTIONS IN BURN PATIENTS

The destruction of the skin barrier, as well as the decrease in local cellular and humoral immune responses, predisposes patients with severe burns to an increased risk of infectious complications. Infections can progress to sepsis, septic shock, and multiple organ dysfunction syndrome, and are one of the main causes of morbidity and mortality, accounting for 42-65% of deaths in severely burned patients. [94-95]

The burned wound in deep partial-thickness burns and full-thickness burns is a protein-rich and avascular environment, representing an ideal area for the colonization and proliferation of germs. The lack of vascularization of the post-burn eschar opposes the migration of immune cells to this area and decreases the local availability of systemically administered antimicrobial therapy, while the toxic substances released from the eschar decrease the local immune response. [96] The burned wound is susceptible to colonization with exogenous or endogenous opportunistic microorganisms. Depending on factors such as the patient's age, the burned surface area and burn depth, the type and number of microorganisms, and the production of enzymes and toxins, invasive infections of the burned wound may occur. [97]

Burn patients can be colonized with multiple germs, often resistant to antibiotics, without showing perilesional erythema or cellulitis. In contrast, invasive infections are characterized by the presence of cellulitis in the surrounding tissues, deepening of the burn wounds, debridement of the eschar, or the appearance of necrotic areas. Patients may become febrile or exhibit elevated inflammatory markers, but these clinical and paraclinical signs are difficult to differentiate from those associated with the inflammatory response in severe burns. [94]

According to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. For practical application, organ dysfunction is represented by an increase of 2 or more points in the SOFA score, which is associated with in-hospital mortality greater than 10%. Septic shock is defined as a subset of sepsis in which underlying circulatory, metabolic, and cellular abnormalities are profound enough to substantially increase mortality compared to sepsis without shock. The diagnosis of septic shock corresponds to the need for vasopressor support to maintain a mean arterial pressure above 65 mmHg and an increase in serum lactate levels above 2 mmol/L, in the absence of hypovolemia. [98]

Burn patients exhibit the clinical and paraclinical signs of SIRS even in the absence of sepsis, which has led to the necessity of identifying other diagnostic methods for sepsis. At a consensus conference of the American Burn Association, an attempt was made to establish specific criteria for the diagnosis of sepsis in burn patients. [99] Sepsis should be suspected in the presence of a documented infection and clinical and paraclinical criteria, which are described in Table I.

Table 1: American Burn Association sepsis in burns criteria

At least 3 of the following criteria:	Documented infection:
Temperature >39°C or <36.5°C	Positive culture results
Heart rate >110 bpm	Identification of the pathogen through tissue biopsy
Respiratory rate >25/min in spontaneously breathing adults or Minute respiratory volume >12 L/min in mechanically ventilated adults	Clinical response to antimicrobial treatment
Thrombocytopenia <100,000/µl (considered after more than 3 days from the onset of initial resuscitation)	
Hyperglycemia >200 mg/dl in the absence of pre-existing diabetes mellitus or Insulin resistance is defined as an insulin requirement of more than 7 IU/hour continuous infusion or an increase of more than 25% in insulin requirement over 24 hours	
Inability to administer enteral nutrition for more than 24 hours due to: - Intolerance to enteral nutrition (gastric residual volume twice the volume of administered nutrition) - Uncontrolled diarrhea (more than 2500 ml/day in adults) - Abdominal distension	

In a meeting of experts, Dupuy and colleagues described the characteristics of the ideal biomarker, which should fulfill multiple roles: identifying high-risk patients, stratifying patients based on individual risk factors, and guiding therapeutic management. Thus, an ideal biomarker should be inexpensive, rapid, non-invasive, easily accepted by the patient, routinely available, and should combine diagnostic characteristics (including differentiating between non-infectious SIRS and sepsis, and between bacterial and viral infections) with prognostic characteristics (including mortality prediction) and the ability to evaluate treatment efficacy. [100]

This ideal biomarker has not been established, but inflammatory markers analyzed in studies for identifying sepsis in burn patients include C-reactive protein, procalcitonin, erythrocyte sedimentation rate, presepsin, serum TNFα, interleukins 6, 8, and 10, serum leukocyte levels, serum platelet levels, and serum lactate levels. [101-102]

With the development of burn centers, advances in the fluid resuscitation of patients with severe burns, and the promotion of early excision and grafting of burn wounds, the past trend of early death due to post-burn shock has now shifted to mortality due to infections and sepsis following prolonged hospitalizations. Preventing sepsis is a primary objective in severely burned patients. Several measures have been proposed to reduce the risk of infectious complications. Early excision of burn wounds and coverage with skin grafts reduce bacterial colonization of burn wounds, decrease the release of inflammatory mediators and the incidence of SIRS, and reduce the risk of sepsis and multiple organ dysfunction. [103-104]

In severely burned patients, some succumb early due to the severity of their burns, while others die after prolonged hospitalization complicated by the development of numerous complications. Multiple organ dysfunction syndrome (MODS) associated with sepsis is the leading cause of death in patients who survive early post-burn shock. [27-28]

The risk factors for the development of sepsis in severely burned patients include:

- Total body surface area (TBSA) burned
- Extreme ages
- Pre-existing conditions accompanied by immune-suppression: diabetes, renal or hepatic pathologies, AIDS, malignancies
- Presence of uncovered burn wounds

- Use of urinary catheters, venous and arterial catheters, or mechanical ventilation [105-106]

A particularly concerning group of bacteria in the context of major burns due to their notorious ability to evade standard antibiotic treatments and develop resistance to last-line antibiotics are the ESKAPE pathogens, comprising Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. [107]

In patients suspected of having sepsis, a thorough search for a probable source should be conducted; clinical symptoms and signs, appropriate microbiological cultures, and relevant imaging techniques should be used to try to determine the infectious source. Antimicrobial medication should be administered early and not delayed until culture data are obtained. Broad-spectrum antibiotic therapy, preferably a combination of antimicrobials, should be administered to cover all suspected germs based on the probable source of infection, local microbiological flora, and specific intensive care flora. After obtaining culture results, antibiotic therapy should be reassessed and de-escalated, narrowing the spectrum to optimize treatment efficacy, limit toxicity, prevent the emergence of multidrug-resistant germs, and reduce treatment costs. [108-109]

CONCLUSIONS

Understanding these mechanisms and pathological findings is crucial for managing and preventing complications in burn patients. Early intervention and vigilant monitoring are essential for mitigating the risks associated with severe burns and reducing the incidence of multiple organ dysfunction syndrome. By recognizing the systemic inflammatory responses, fluid loss, and potential for infection that accompany severe burn injuries, healthcare providers can implement targeted strategies to support organ function, prevent sepsis, and optimize patient outcomes. Continuous advancements in burn care and precise triage and treatment approaches are vital for improving survival rates and ensuring better long-term recovery for burn patients. This is a current overview of the subject. In the coming years, new information on the pathophysiological mechanisms will likely emerge to complete the level of understanding on the subject.

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ARTICLE

Investigation of Factors Influencing Soldiers' Disabilities in China: A Cross-Sectional Study

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Abstract: *Due to the frequent occurrence of international military conflicts, there has been an increasing number of soldiers who have suffered injuries and disabilities. The soldiers' disability is a crucial factor influencing combat effectiveness. Objective: This study aims to investigate the current situation of military disability and its influencing factors to provide a basis for reducing the rate of military disability. A total of 430 Chinese military disability assessment materials from 2021 were collected retrospectively between April 2023 and September 2023. The data on injuries, illnesses, and disabilities of soldiers were subjected to statistical analysis with informed consent, and personal information was anonymized to ensure privacy. The occurrence of disability was analyzed based on gender, rank, and different disability systems using unifactor analysis and multiple linear regression analysis. Statistical differences (P<0.05) were found in the degree of disability among soldiers of different ages, status categories, nature of disabilities, disability systems, and causes of disabilities. Age, status category, nature of disabilities, disability systems, and causes were identified as the main factors affecting the degree of soldiers' disabilities, with a total explanation rate of 58.3%. Injuries to the motor system during training were identified as the primary cause leading to soldier disabilities. In conclusion, this study shows that it is essential to prioritize protection against training injuries to minimize soldier disabilities.*

Keywords: *soldier; disabilities; influencing factors; investigation*

INTRODUCTION

Soldiers are the primary component of the national defense force, responsible for safeguarding national security, protecting and defending national borders, maintaining social stability, and other related duties. The physical and mental well-being of soldiers is directly linked to their combat effectiveness, with disability being a major factor affecting this during peacetime operations [1]. The effects of military activities on soldiers include not only physical aspects but also psychological aspects [2].

Previous research has indicated that the impact of military operations on soldiers is irreversible, with some individuals experiencing lasting effects such as physical disabilities [3,4,5,6], pain [7], hearing loss [8], blindness [9], post-traumatic stress disorder (PTSD) [10], traumatic brain injury (TBI) [11], and fractures [12,13]. Data from the US Army, Navy, and Air Force disability agencies [14] show that 10 to 30 out of every 1000 soldiers are disabled each year. However, only 30% of these cases are due to pre-existing conditions; the majority result from injuries sustained during military operations.

The American Veterans Benefits Administration offers disability benefits to individuals and service members who previously served full-time active military duty and were discharged generally or honorably [15]. These benefits can be categorized into three types based on the causes of disabilities: (1) Service-connected disability, which refers to a disability resulting from direct injury or disease while on active duty; (2) Nonservice-connected disability, which is attributed to an injury not acquired during active duty; (3) Presumptive service connection, which encompasses various chronic conditions that appear within 1 year after discharge from active duty.

In China, military training is a significant contributing factor to physical disabilities among soldiers [16]. Research has indicated that the incidence of military training injuries in the new recruit population in China is 21.04%, suggesting that one out of every five individuals will experience a military training injury [17]. Therefore, this study conducts a cross-sectional investigation to analyze the occurrence and influencing factors of disability among Chinese military personnel, to enhance the effectiveness of prevention and treatment of military training injuries.

MATERIALS AND METHODS

Research objects

A total of 430 Chinese military disability assessment materials from 2021 were collected retrospectively between April 2023 and September 2023. The data on injuries, illnesses, and disabilities of soldiers underwent statistical analysis with consent, and personal information was anonymized to ensure privacy. The disability assessment materials included 400 males and 30 females, with an average age of 30.75 ± 7.38 years.

Research methods

According to the "Military Disability Rating Standards"[17], injuries, illnesses, and disabilities of officers and soldiers were graded on a scale from one to ten. A lower rating indicated a more severe injury or disability.

The classification of the causes of disability includes two aspects: (1) Disability due to service, which refers to disabilities directly caused by participating in work or military activities during active duty; (2) Non-service related disability, which refers to disabilities caused by illness during active duty due to physical reasons. The data from disability assessment materials were entered into the computer and checked by two individuals to ensure accuracy.

Inclusion criteria: the disability condition meets the Chinese Military Disability Assessment Standards and is eligible for disability assessment [17], disability assessment standards are mainly based on the site of injury, severity and sequelae to determine the level of disability. Other conditions were excluded from this study, such as cases where recovery after injury was good, and there were no obvious functional abnormalities.

Classifying and analyzing the levels of disability in different groups based on the disability assessment materials. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of The Strategic Support Force Military Center (LS202203, Approved:2022-10-27).

Statistical Methods

Statistical analysis of the data was conducted using SPSS 22.0. Descriptive statistics were used to represent continuous variables as mean and standard deviation and categorical variables as rates or proportions. The data was categorized based on patient medical records, and chi-square tests and multiple linear regression analysis were performed with a significance level set at P<0.05.

RESULTS

The basic situation of disability assessment of military personnel in 2021

In 2021, a total of 430 disability assessment materials submitted to a certain Ministry met the military standards for disability assessment.There were 2 cases (0.5%) of Level 1, 1 case (0.2%) of Level 2, 2 cases (0.5%) of Level 3, 2 cases (0.5%) of Level 5, 31 cases (7.2%) of Level 6, 4 cases (0.9%) of Level 7, 109 cases (25.3%) of Level 8, 175 cases (40.7%) of Level 9, and 104 cases (24.2%) of Level 10.

Statistical differences were observed among different grades of disability in different age groups, status categories, and the nature of disability, as shown in Table 1.

Table 1: Comparison of disability among soldiers with different demographic variables

Divisions	**Disability Grade**									**χ2**	**P**
	Level 1	**Level 2**	**Level 3**	**Level 5**	**Level 6**	**Level 7**	**Level 8**	**Level 9**	**Level 10**		
Gender											
Male	2 (100%)	1 (100%)	2 (100%)	2 (100%)	30 (96.8%)	4 (100%)	104 (95.4%)	161 (92%)	94 (90.4%)	3.854	0.870
Female	0	0	0	0	1 (3.2%)	0	5 (4.6%)	14 (8%)	10 (9.6%)		
Age group (years)											
≦29	2 (100%)	1 (100%)	1 (50%)	1 (50%)	29 (93.5%)	2 (50%)	46 (42.2%)	85 (48.6%)	50 (48.1%)	37.593	0.002
30~≦39	0	0	1 (50%)	0	2 (13.4%)	1 (25%)	54 (49.5%)	68 (38.9%)	44 (42.3%)		
≧40	0	0	0	1 (50%)	0	1 (25%)	9 (8.3%)	22 (12.6%)	10 (9.6%)		
Identity category											
Conscripts	1 (50%)	0	0	0	14 (45.2%)	1 (25%)	9 (8.3%)	28 (16%)	21 (20.2%)	38.999	0.001
NCO	1 (50%)	1 (100%)	2 (100%)	1 (50%)	17 (54.8%)	1 (25%)	56 (51.4%)	91 (52%)	51 (49.0%)		
Officers	0	0	0	1 (50%)	0	2 (50%)	44 (40.4%)	56 (32%)	32 (30.8%)		
Disabling Character											

Non-service	2 (100%)	1 (100%)	2 (100%)	1 (50%)	31 (100%)	0	0	0	0	423.64	0.001
Service	0	0	0	1 (50%)	0	4 (100%)	109 (100%)	175 (100%)	104 (100%)		
Total	2 (0.5%)	1 (0.2%)	2 (0.5%)	2 (0.5%)	31 (7.2%)	4 (0.9%)	109 (25.3%)	175 (40.7%)	104 (24.2%)		

χ2 for chi-square tests.
NCO – Non-commissioned Officers

Grade distribution of disability in different disability systems

In 2021, the majority of cases (90.9%) were related to motor system disability, followed by 4.9% of neurological disability cases and 1.4% of urinary disability cases, and were statistically differentiated by examination (P<0.01). See Table 2 for more details.

Table 2: Comparison of disability among soldiers with different physiological systems

Disability systems	Disability Grade									Total
	Level 1	Level 2	Level 3	Level 5	Level 6	Level 7	Level 8	Level 9	Level 10	
Respiratory System	0	0	0	0	0	0	0	0	1 (0.2%)	1 (0.2%)
Urinary System	0	0	0	0	3 (0.7%)	0	2 (0.5%)	0	1 (0.2%)	6 (1.4%)
Endocrine System	0	0	1 (0.2%)	0	4 (0.9%)	0	0	0	0	5 (1.2%)
Nervous System	2 (0.5%)	1 (0.2%)	0	1 (0.2%)	7 (1.6%)	0	4 (0.9%)	5 (1.2%)	1 (0.2%)	21 (4.9%)
Digestive System	0	0	0	0	1 (0.2%)	0	0	0	0	1 (0.2%)
Circulatory System	0	0	1 (0.2%)	1 (0.2%)	2 (0.5%)	0	0	0	0	4 (0.9%)
Motor System	0	0	0	0	13 (3%)	4 (0.9%)	103 (24%)	170 (39.5%)	101 (23.5%)	391 (90.9%)
Blood System	0	0	0	0	1 (0.2%)	0	0	0	0	1 (0.2%)
Total	2 (0.5%)	1 (0.2%)	2 (0.5%)	2 (0.5%)	31 (7.2%)	4 (0.9%)	109 (25.3%)	175 (40.7%)	104 (24.2%)	430 (100%)

χ2 =351.838, P<0.01, χ2 for chi-square tests

Table 3: Comparison of disability among soldiers with different reasons

Causes of Disability	Disability Grade									Total
	Level 1	Level 2	Level 3	Level 5	Level 6	Level 7	Level 8	Level 9	Level 10	
Illness	1 (0.2%)	1 (0.2%)	1 (0.2%)	1 (0.2%)	29 (6.7%)	0	0	1 (0.2%)	0	34 (7.9%)
Traffic Accident	0	0	0	0	0	0	1 (0.2%)	4 (0.9%)	1 (0.2%)	6 (1.4%)
Physical Activity	0	0	0	0	0	0	1 (0.2%)	1 (0.2%)	2 (0.5%)	4 (0.9%)
Daily Training	1 (0.2%)	0	0	1 (0.2%)	1 (0.2%)	3 (0.7%)	89 (20.7%)	136 (31.6%)	78 (18.1%)	309 (71.9%)
Exercise and Training Tasks	0	0	0	0	1 (0.2%)	1 (0.2%)	9 (2.1%)	28 (6.5%)	13 (3%)	52 (12.1%)
Other	0	0	1 (0.2%)	0	0	0	9 (2.1%)	5 (1.2%)	10 (2.3%)	25 (5.8%)
Total	2 (0.5%)	1 (0.2%)	2 (0.5%)	2 (0.5%)	31 (7.2%)	4 (0.9%)	109 (25.3%)	175 (40.7%)	104 (24.2%)	430 (100%)

χ2=391.947, P<0.01, χ2 for chi-square tests.

Grade distribution of disability caused by different causes

In 2021, the majority of injuries (71.9%) occurred during daily training, followed by 12.1% during exercise and training, and 7.9% due to illness, and were statistically differentiated by examination (P<0.01). See Table 3 for more details.

Multi-factor analysis of the influencing factors of a soldier's assessment of disability

Multiple linear regression analysis was conducted to examine the relationship between disability rating and various independent variables that showed statistical significance in univariate analysis (see Table 4 for assigned values). The findings revealed that the nature of disability, identity category, age, disability system, and cause of disability were the primary factors influencing the degree of disability (F=121.185, P<0.01). The overall explanatory power of these variables was 58.3%, as presented in Table 5.

Table 4: Argument assignment method

Variables	Assignment method
Gender	Male = 1, Female = 2.
Age	Original value input.
Identity category	Conscript = 1, Noncommissioned Officer = 2, Officer = 3.
Disabling Character	Non-service = 1, Service = 2.
Disabling System	Respiratory System = 1, Urinary System = 2, Endocrine System = 3, Nervous System = 4, Digestive System = 5, Circulatory System = 6, Motor System = 7, Blood System = 8.
Causes of Disability	Illness = 1, Traffic Accident = 2, Physical Activity = 3, Daily Training = 4, Exercise and Training Tasks= 5, Other = 6.

Table 5: Multivariate analysis of influencing factors of disability among soldiers

Variables	Beta	SE	Beta '	t	P
Constant	1.210	0.379		3.191	0.002
Age	0.015	0.007	0.086	2.070	0.039
Identity Category	-0.245	0.081	-0.127	3.031	0.003
Disabling Character	3.640	0.211	0.780	17.257	0.001
Disabling System	0.097	0.047	0.074	2.044	0.042
Cause of Disability	-0.129	0.064	-0.081	-1.988	0.047

[Note] R2=0.588, Adjust R2=0.583, F=121.185, P<0.01.

DISCUSSION

In 2021, the majority of soldiers' disabilities were concentrated in grades 8 to 10 (90.2%). This finding is consistent with the research results of Wang [19] and Li [20], indicating that most soldiers injured during normal work experience moderate to low levels of disability, with fewer cases of severe disability. This may be attributed to a greater emphasis on safety measures during peacetime work, more effective preventive measures against major safety accidents, and successful implementation of the safety form analysis system [21,22,23].

In the analysis of the disability system, it is evident that soldiers' disabilities are primarily related to sports activities, with a concentration of disabilities falling within the 8 to 10 level. The causes of these disabilities can be attributed to daily training, and factors such as the nature of the disability, identity category, and age have an impact on the severity of the disability. This pattern is similar to that observed in Chinese Air Force soldiers [24]. In 2020, certain departments under the Central Military Commission made adjustments to the "Military Physical Training Outline", resulting in an increase in training-related injuries due to heightened difficulty levels [25]. Furthermore, young soldiers' competitive spirit often leads them to neglect their physical well-being during training sessions, making them susceptible to injuries or fatigue. Disabilities resulting from training missions are typically linked to officers and soldiers having lax attitudes towards prevention measures and non-standard equipment operation due to psychological tension. Pang [26] also argues that military training missions' high physical load and tension are primary contributors leading to bodily harm, physiological fatigue, and potentially severe consequences.

Methods to Improve the Condition of Injured Soldiers

1. Comprehensive Preventive Measures

In recent years, training organizations at all levels have increasingly prioritized the prevention of various types of training injuries. Despite these efforts, training injuries continue to be the leading cause of military disability. Therefore, it is crucial to strengthen protection in the following areas.

1.1. Pre-training preparation

Proper pre-training preparation is crucial in reducing the occurrence of training injuries. Engaging in warm-up activities can enhance the body's flexibility, adaptability, and malleability, thereby improving its ability to withstand high-intensity training. Furthermore, it can increase blood flow redistribution and velocity in the body, thus reducing the risk of injury to the musculoskeletal system. Additionally, providing comprehensive instruction in operational or action skills is essential for skilled athletes to prevent individual injury.

1.2. Moderation in training is essential

Moderate training plays a key role in preventing physical injuries. It is important for individuals to recognize their own limitations and for training organizations at all levels to consider each individual's body capacity when designing training programs. Gradually increasing the intensity of training can also help avoid injuries caused by overexertion during cross-training activities.

1.3. Post-training recovery

The post-training recovery stage is a critical factor in individual energy and power storage. Following high-intensity training, soldiers often experience exhaustion. Effective recovery can facilitate the body's recuperation and growth, ultimately enhancing soldiers' resilience to future training sessions.

2. Enhance organizational structure and management

Scientific training and program-based organization are fundamental principles for conducting military training. Training and exercise departments at all levels should enhance the planning of various types of training, organize tasks rationally and scientifically, and continuously improve soldiers' physical fitness while enhancing combat effectiveness. It is essential to maintain existing combat effectiveness standards while improving overall combat readiness. When implementing operational actions, organizers should consider operational processes, standards, and proficiency skills to avoid inexperienced individuals operating alone, which could lead to security risks.

Rigorously enforce the annual physical examination system

A well-organized arrangement of officers and soldiers participating in the annual military physical examination task can lead to early detection, diagnosis, and treatment of asymptomatic diseases. This proactive approach helps prevent permanent functional loss such as dysfunction, significantly reducing the disability rate among soldiers.

Limitations and Generalizability

This study is based on a sample of 430 disability assessment materials, which were the result of collaborative efforts from all our researchers. However, it should be noted that these materials may not fully represent all disability assessment outcomes in 2021, thus limiting the generalizability of our results. Additionally, there was a skewed gender ratio in our study with only 30 females compared to 400 males, even though the number of males in the military far exceeds that of females. This imbalance may have impacted the accuracy of our findings regarding gender differences.

Nevertheless, it is important to highlight that this study was able to capture some aspects of disability assessment among Chinese soldiers in 2021. By analyzing the causes of disability among 430 soldiers, we are still able to provide valuable recommendations for preventing disabilities within this population.

CONCLUSIONS

This study adopts the method of cross-sectional investigation to investigate the factors affecting the disabled status of Chinese soldiers. We found that age, rank category, nature of disability, involvement system and cause of disability were the main factors affecting the degree of disability in military personnel. Injury to the musculoskeletal system during training is the main cause of military disability. The prevention and treatment of training injuries is a very important research topic that can provide better health protection for soldiers. Therefore, coaches and managers at all levels need to pay attention to injury prevention in the training process, which can minimize the occurrence of military disabilities.

Conflicts of interest and sources of funding

The authors declare no conflict of interest, and this research has received no external funding.

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Authors' contribution

The initial draft was prepared and revised by LS, while FR and SX contributed by proposing hypotheses and preparing the dataset. QS further refined the analysis and provided crucial support for the manuscript. DNL, XG, SX, and YW conducted data collection in various units and offered theoretical support. LS and QS supervised all aspects of the study, including hypothesis formulation, design of data analysis plan, oversight of data analysis process, verification of results, and development of the manuscript based on the initial draft. All authors participated in reviewing and finalizing the manuscript which has been read and approved by everyone involved.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Strategic Support Force Military Center (LS202203,2022.01.01).

Patient consent for publication

Written informed consent has been obtained from the patient (s) to publish this paper

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REVIEW

Gestational Diabetes: From Mechanism to Therapy – A Short Review

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Abstract: *Gestational diabetes (GDM) is characterized by the initial onset of elevated glucose levels while pregnant throughout the 24th week up until the 28th week of gestation affecting around 21.1 million live births in 2021. GDM is related to significant prolonged health risks for the mother and the unborn baby. A comprehensive research of the PubMed database was conducted between April and June 2024. As a response to IR, maternal beta cells increase in number and ramp up insulin production and release to sustain glucose balance. Physiological IR during pregnancy is driven by hormonal and inflammatory changes. Multiple risk factors contribute to the occurrence of GDM. Glycemic targets during pregnancy are more stringent than those for nonpregnant women. GDM can lead to several short-term or long-term complications during pregnancy for both the mother and the unborn baby. The first step in managing GDM involves lifestyle modifications. The next step in managing GDM involves the use of medication and insulin is the preferred pharmacological treatment. However, there are still many steps to be taken towards establishing an international prevention strategy and accurately determining the mechanisms involved in the development of GDM.*

Keywords: *gestational diabetes; pregnancy; placental hormones; gestational treatment*

INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized as the initial onset of elevated glucose levels while pregnant, typically throughout the 24th week up until the 28th week of gestation. GDM has appeared among many prevalent pregnancy complications, with the rate of occurrence increasing in recent years [1]. The International Diabetes Federation (IDF) specifies that GDM affected around 21.1 million live births in 2021, with a higher prevalence noticed in low- and middle-income countries, where hyperglycemia during pregnancy is more common [2]. More recently, the term “early” gestational diabetes (early GD) has been used to define elevated glucose levels occurring before twenty weeks of pregnancy, and its prevalence is expected to be between 0.7% and 36.8% [3]. The term “late” gestational diabetes (late GD) is defined as elevated glucose levels between 24 to 28 weeks of gestation [4].

The history of diabetes in pregnancy dates back to 1824 when it was first described by Bennewitz [5]. The initial diagnostic criteria for this condition were proposed by Williams in 1909 [6]. Over time international organizations, including the World Health Organization (WHO), the International Association of Diabetes and Pregnancy Study Group (IADPSG), the Canadian Diabetes Association (CDA), and the American Diabetes Association (ADA) have developed guidelines outlining the diagnosis and screening for GDM [7].

GDM is related to significant prolonged health risks for the mother and the unborn baby. Pregnant women diagnosed with GDM face about 8-fold high risk regarding the possibility of developing type 2 diabetes (T2D), especially in the first 5-10 years postpartum [8]. Additionally, GDM can result in a variety of pregnancy complications, including fetal anomalies, spontaneous abortion, fetal demise, preeclampsia, macrosomia, neonatal hyperbilirubinemia, and hypoglycemia [9].

METHODS

A comprehensive research of the PubMed database was conducted between April and June 2024. Keywords used in the search included "gestational diabetes", "prevalence", "hyperglycemia", "risk factors", “diagnosis”, "placental hormones", "mechanism", and “treatment” as well as various combinations of these terms. The research was restricted to studies published in English. Exclusion criteria included studies that identified GDM diagnosis solely by codes, studies focusing on specific populations (e.g., by age, species, or race), and publications in the form of abstracts, letters, or case reports.

DISCUSSIONS

Etiopathogenesis

Insulin is secreted by pancreatic beta cells and plays a vital role in promoting glucose utilization by peripheral cells, decreasing glucose synthesis within the liver, and regulating lipid release from fat stores. When regular levels of insulin fail to obtain the desired response from insulin receptors, insulin resistance (IR) can develop. To compensate, the pancreatic beta cells must release more insulin to ensure glucose levels remain normal, leading to IR [10]. This process is a natural aspect of a healthy pregnancy, induced through placental hormones to guarantee that the unborn baby obtains sufficient essential nutrients for optimal development. As a response to IR, maternal beta cells increase in number and ramp up insulin production and release to sustain glucose balance [11].

GDM’s underlying mechanisms remain unclear, but it is believed that it results from the maternal pancreas's inability to adapt to the increase of insulin demand during pregnancy, leading to hyperglycemia [12,13]. The placenta seems to play a crucial role in GDM formation, as several studies have explored the physiological mechanisms involved.

Physiological IR during pregnancy is driven by hormonal and inflammatory changes [14]. Metabolic changes also occur, with IR typically emerging during the first half of pregnancy and then decreasing. This phenomenon is influenced by raised levels of prolactin (PRL), human placental lactogen (hPL), progesterone, and estrogen, which contribute to the hyperplasia and hypertrophy of maternal pancreatic beta cells. These beta cell modifications are part of the body's physiological adaptation to hPL, a polypeptide hormone secreted by the placenta that peaks around 30 weeks of gestation [15]. This timing may explain why blood glucose levels tend to stabilize after 30-32 weeks of pregnancy. hPL decreases activation of insulin receptor substrate 1 (IRS1) through phosphorylation, thereby inducing IR [16]. PRL, another polypeptide hormone produced by the anterior pituitary gland, also affects insulin sensitivity and lipid metabolism. PRL can induce IR both during and outside the pregnancy period, working alongside hPL to contribute to IR [17]. Prolactin receptors, which are expressed in pancreatic beta cells, play a fundamental role in pregnancy metabolism as well as IR. These receptors regulate pro-proliferative and anti-apoptotic pathways through the mitogen-activated protein kinase (MAPK), protein kinase B (AKT), phosphatidylinositol 3-kinase (PI3K), the Janus kinase 2-signal transducer and activator of transcription 5 (JAK2/STAT5) pathway. After childbirth, beta-cell mass is reduced through apoptosis, cell size reduction, or other mechanisms [18]. Estradiol induces IR by activating the JNK pathway through membrane estrogen receptor (ER)-mediated actions and serine phosphorylation of IRS-1. Progesterone contributes to IR by inhibiting the PI3-kinase pathway and reducing IRS1 expression [19]. Placental growth hormone (PGH) is found in the syncytiotrophoblast and extravillous cytotrophoblast layers of the human placenta [20]. It promotes gluconeogenesis, lipolysis, and anabolism in maternal tissues. PGH plays an important role in maternal adaptation to pregnancy, ensuring adequate fetal growth and development by inducing physiological IR [21,22]. An overproduction of PGH has been observed in GDM, but this phenomenon is not fully understood and deserves further studies [18].

Recent research has emphasized the impact of inflammatory markers in the development of GDM. One mechanism that could induce physiological IR in pregnancy is the reduction in adiponectin synthesis, a protein hormone with more properties such as insulin-sensitizing and anti-inflammatory. Adiponectin inhibits the insulin intracellular signaling pathway, contributing to IR [18]. In contrast, leptin produced by adipocytes, the ovaries, and the placenta inhibits insulin action [23]. GDM is characterized by elevated oxidative stress exceeding levels that are typically seen in normal pregnancies along with an inflammatory state. Elevated oxidative stress leads to increased production of inflammatory cytokines and stimulates the appearance of free radicals. In GDM, reactive oxygen species (ROS) levels are elevated while total antioxidant capacity (TAC) is reduced. Pancreatic beta cells are particularly sensitive to ROS, and oxidative stress can induce beta cell dysfunction through apoptotic events. Additionally, oxidative stress reduces cellular glucose uptake and contributes to IR [24]. The pro-inflammatory environment in GDM may be explained by an increase in platelet-based growth factor and the subsequent formation of other pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and chemokines (CXCL1, CXCL5, CXCL8, CCL2). During pregnancy, there is an elevated secretion of pro-inflammatory cytokines, which reduce insulin release from beta cells, impair insulin signaling, and contribute to IR [25,26].

It is crucial to detect hyperglycemia early in pregnancy because it can have harmful impacts on both the mother and the fetus, over both short and long periods**.**

Risk factors of GDM

Multiple factors contribute to the occurrence of GDM, including financial status, diet, lifestyle, physical activity, and history of diabetes in pregnant women's family or personal history of GDM [27]. The advanced age of the mother represents a significant risk factor. Women over 45 years old have a higher risk for hyperglycemia during pregnancy and associated complications [28]. It appears that women with a history of GDM may develop T2D within 5 to 10 years after childbirth, though not every woman with a history of GDM will progress to diabetes mellitus [2,29]. Excess weight and obesity, are also major contributing factors for GDM. Pregnant women with a higher body mass index (BMI≥ 29 kg/m²) are at heightened chance of developing GDM and experiencing pregnancy-related complications [30]. Excessive body weight has an important role in the deterioration of pancreatic beta cells. After a diagnosis of GDM, the most effective strategy for preventing T2DM is through lifestyle modifications or medications that improve biological outcomes and reduce IR [31]. Diet is another important factor: intake of fat-rich foods, sugary products, as well as red meat increases the likelihood of GDM, while a fiber-rich diet, micronutrients, and polyunsaturated fatty acids can reduce the risk. Additionally, a history of PCOS and multiparity significantly increases the likelihood of GDM and related metabolic complications [32]. Having a family history of diabetes or GDM during a previous pregnancy also raises the chance of developing GDM [2].

Pregnant women with at least one risk factor for diabetes should be early tested (<20 weeks) to establish or not the diagnosis of early GD. Once the diagnosis is established, the treatment must be initiated quickly [33].

Screening and diagnosis of GDM

Over time, the methods for diagnosing GDM have evolved from targeted screening solely on risk factors to the utilization of the oral glucose tolerance test (OGTT) for universal screening [34]. Advanced maternal age (over 30 years) is a major risk factor associated with GDM [28]. Women who have previously given birth to a baby weighing over 4 kilograms or had GDM in a prior pregnancy or are overweight or with a family medical previous occurrence of T2D are considered at higher likelihood for developing GDM. Additionally, women with polycystic ovary syndrome (PCOS) and those with pluriparity are at greater risk [35].

Glycemic targets during pregnancy are more stringent than those for nonpregnant women. According to ADA the screening for GDM is recommended between 24 and 28 weeks of gestational period among pregnant women who have not been before diagnosed with diabetes. Pregnant women with diabetes must consume consistent carbohydrates to establish their insulin doses and avoid both low and high glucose levels, as these conditions can adversely affect the fetus [36]. In 2010, the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study introduced other targets for OGTT, prompting a reassessment of the diagnostic criteria for GDM identified between 24 and 28 weeks of pregnancy. These thresholds have been endorsed by the WHO and Croatian Society for Gynecology-Obstetrics [37].

1. The One-Step Strategy (derived from the IADPSG criteria) involves a 75-gram OGTT. It is performed in the morning, after at least 8 hours of fasting. Blood glucose levels are evaluated at fasting, 1 hour, and 2 hours in individuals who have not been formally diagnosed with diabetes (Table 1). A positive assessment is established if glucose levels are achieved or excelled [36].

Table 1: The One-Step Strategy (adapted from [36])

Fasting	≥ 92 mg/dL (5.1 mmol/L)
1 hour	≥ 180 mg/dL (10.0 mmol/L)
2 hours	≥ 153 mg/dL (8.5 mmol/L)

2. The Two-Step Strategy involves an initial 50-gram OGTT (non-fasting) and followed by a 100-gram OGTT if the first test is positive according to Carpenter-Coustan criteria. The American College of Obstetricians and Gynecologists (ACOG) and the National Institutes of Health (NIH) recommend this strategy for diagnosing GDM [36].

Step 1: A 50-gram OGTT (non-fasting) is administered, and glucose level is measured an hour later. If the plasma glucose value is ≥ 130 mg/dL (7.2 mmol/L), 135mg/dL (7.5 mmol/L), or 140 mg/dL (7.8 mmol/L), the patient proceeds to Step 2.

Step 2: A 100-gram OGTT (fasting) is performed, with glucose levels assessed at fasting, 1 hour, 2 hours, and 3 hours. If two or more of the following glucose thresholds are reached, GDM is confirmed (Table 2).

Table 2: The Two-Step Strategy (adapted from [36])

Fasting	≥ 95 mg/dL (5.3 mmol/L)
1 hour	≥ 180 mg/dL (10.0 mmol/L)
2 hours	≥ 155 mg/dL (8.6 mmol/L)
3 hours	≥ 140 mg/dL (7.8 mmol/L)

The assessment of GDM is based on these standards adapted from [36], [38], [39].

Postpartum evaluation for women experienced GDM is recommended from 4 to 12 weeks after childbirth. This screening should use a 75-gram OGTT, and the diagnosis should be based on non-pregnancy criteria [36].

Complications while pregnant: for mother as well as newborn

Effects on the mother

Short-term complications. GDM can lead to several short-term complications during pregnancy. These include high blood pressure (hypertension), pre-eclampsia, spontaneous abortion, the need for a cesarean section, and difficulties during childbirth [9]. If the fetus is macrosomic, vaginal delivery becomes riskier. Instrumental delivery, using forceps or a vacuum, may be necessary, or an unexpected or emergency cesarean section might be required. Large babies increase the likelihood of perineal tears, vaginal lacerations, and other birth injuries [40]. Additionally, macrosomic births carry risks such as uterine atony, heavy bleeding, postpartum hemorrhage, and genital tract injuries. These risks are further heightened if the mother has previously had a cesarean section [13].

Long-term complications. In chronic complications, mothers experiencing GDM have high risk of recurrence in future pregnancies and a greater likelihood of onset of T2D in the future [2]. The HAPO (Hyperglycemia and Adverse Pregnancy Outcomes) research found that maternal elevated blood sugar during pregnancy was associated with a higher risk of early childbirth, cesarean delivery, macrosomia, congenital malformations, neonatal intensive care unit admission, hyperbilirubinemia, and neonatal hypoglycemia [41]. Generally, GDM resolves after childbirth.

Effects on the newborn

Fetal complications such as premature birth, hypoglycemia at birth, shoulder dystocia, Erb's palsy, congenital anomalies, and fetal macrosomia are common in pregnancy with GDM. Neonatal respiratory distress syndrome and low Apgar scores are also potential issues. This respiratory distress can be linked to alterations in the integrity and composition of fetal pulmonary surfactant, as GDM can delay the secretion of essential surfactant components, such as phosphatidylglycerol [42]. Infants born to mothers with GDM have a higher likelihood of evolving overweight and T2D later in life [14].

Fetal macrosomia is the most common difficulty associated with GDM. It is characterized by a birth weight of 4 kilograms or more. This condition is marked by an increase in the abdominal diameter and scapular area of the fetus [43]. Excess glucose, which crosses the placenta, serves as the primary energy substrate for fetal growth. Elevated maternal glucose levels lead to increased glycogen storage in the fetus, resulting in fetal hyperinsulinemia and an increase in adipose tissue [44,45]. Macrosomic newborns often exhibit asymmetrical growth, characterized by central deposition of subcutaneous fat around the abdomen and back, increased shoulder circumference, and more skin folds [46].

Treatment of late GD brings benefits to both the mother and unborn baby, but more recent studies highlight that the treatment started even earlier (in early GD) brings even more benefits, both in the long term and in the short term [47]. The Treatment of Booking Gestational Diabetes Mellitus (TOBOGM) clinical trial was the first study to evaluate the effectiveness of immediate initiation of treatment in patients diagnosed with early GD in terms of pregnancy complications. There was a significant reduction in the risk of neonatal adverse events in the group in which the treatment was initiated immediately [33].

Treatment (blood glucose monitoring, lifestyle changes, pharmacotherapy)

The main goal of treating GDM is to reach and sustain the optimal blood glucose range. Mothers must understand the condition and aim to manage their blood sugar levels effectively [9,13].

Blood glucose monitoring in pregnancy

In managing GDM daily monitoring of blood sugar at home is very important. Expectant mothers with diabetes should regularly check their glucose levels, including fasting and postprandial (after meal) readings. For those using basal-bolus therapy or insulin pumps, preprandial (before meal) testing is also recommended. ADA sets target values for blood sugar levels as follows: fasting - must be below 95 mg/dL (<5.3 mmol/L), 1-hour postprandial glucose should be below 140 mg/dL (<7.8 mmol/L), and 2 hours postprandial glucose should be below 120 mg/dL (<6.7 mmol/L). For managing hypoglycemia, thresholds are set at less than 70 mg/dL (<3.9 mmol/L) for blood sugar and less than 63 mg/dL (<3.5 mmol/L) for sensor glucose. Glycated hemoglobin (A1C) is used as an additional measure of glycemic control rather than for diagnosing GDM, as A1C levels can be influenced by changes in red blood cell exchange during pregnancy. Consequently, A1C may need to be monitored more frequently, such as monthly [9]. Factors like increased vitamin C intake accelerated red blood cell renewal, and higher iron demands during pregnancy can lower A1C levels [48,49]. Continuous glucose monitoring (CGM) is another valuable tool, providing real-time tracking of glucose levels throughout the day and night. This method helps in monitoring glucose fluctuations and offers data that can improve both patient and clinician management of GDM, enhancing patient compliance and reducing the discomfort associated with frequent finger pricks

[49].

Lifestyle changes

The first step in managing GDM involves lifestyle modifications, including nutritional support therapy, regular active lifestyle, and body mass control based on weight before pregnancy [9,13]. For many pregnant women, these changes may be sufficient to control GDM.

Nutrition. The dietary plan should ensure sufficient calorie intake to aid both the mother and the unborn baby, although the ideal calorie amount during pregnancy for women with GDM has not been established. The nutrition plan should be aligned with the recommendations of the National Academy of Medicine. It is suggested that the dietary reference intake for all expecting women includes a starting amount of 175 grams of carbohydrates (thirty-five percent of 2,000 calories per day), at least 71 grams of protein, and 28 grams of fiber [9, 50]. Emphasizing superior quality, carbohydrates rich in nutrients help to maintain regulated pre-meal and post-meal glucose levels, whereas simple carbohydrates can lead to higher post-meal glucose excursions [9]. A diet rich in whole foods, such as fresh produce, veggies, fruits, beans, whole cereals, and healthy fats (including omega-3 fatty acids from nut products, seeds, as well as fish) is highly recommended. Processed foods, sweetened foods, beverages, and consumption of fatty red meats should be reduced. An expert panel on nutrition advises a balanced intake of primary nutrients and cautions against very restrictive diets, trans fats, ketogenic diets lacking carbohydrates, dairy-restricted diets, and those high in saturated fats [9,51].

Physical activity can improve blood glucose control and decrease the need for insulin. Recommended exercises include aerobic activities, resistance training, or a combination of both. The duration should last between 20 to 50 minutes daily, 2 to 7 days a week, at medium force [9,52].

Pharmacotherapy

The next step in managing GDM involves the use of medication. Up to 30 percent of women with GDM will require pharmacotherapy [53]. Insulin is the preferred pharmacological treatment for GDM when lifestyle modifications (diet and exercise) fail to achieve blood glucose targets. Due to its large molecular size, insulin does not cross the placenta, being safe for the fetus [9,54]. The insulin doses must be calculated according to glycemic values and the amount of carbohydrates consumed. Long-acting insulin (detemir or glargine) may be used for basal glucose control. Rapid (lispro or aspart) or short (regular) insulin is used to control elevated post-prandial blood sugars [55,56,57]. In addition, short-acting insulin analogs have a lower risk of hypoglycemia and offer greater flexibility in administration compared to human insulin [58]. For pregnant women with both higher fasting and postprandial blood sugar basal-bolus is used: 50% of the total daily dose of long-acting insulin and 50% of the total daily dose of short or rapid-acting insulin. In the first trimester of pregnancy, insulin is initiated with 0.7 U/Kg/day and can reach up to 1U/kg/day until birth. In some cases of pregnancy, intermediate-acting insulin (neutral protamine Hagedorn (NPH)) can also be used: 2/3 of total daily insulin before breakfast and 1/3 of TDD before dinner [55,56, 57]. Recently ultra rapid-acting insulin aspart (Fiasp) and lispro (Lyumjev) have received approval for use in pregnancy [58]. Emerging yet limited evidence suggests that insulin degludec may be suitable for use in pregnancy [59]. Also, the data regarding the effectiveness and risks of glulisine and glargine U-300 insulin administration during pregnancy is still limited [4]. Insulin pumps are not yet recommended for women with GDM because the clinical data is still limited [58].

Oral medications that have been researched include metformin and glyburide. These oral antidiabetic drugs do pass through the placental membrane and should not be used as first-line treatments. Sulfonylureas, such as glyburide, have been linked with elevated risks of neonatal hypoglycemia, macrosomia, and a larger abdominal circumference [9,54]. Fetuses exposed to metformin have shown smaller head and chest circumferences, reduced birth size, and decreased lean mass [60]. These oral antidiabetic drugs can impact fetal development and growth.

CONCLUSIONS

GDM is an increasingly common complication with negative outcomes for both the mother and the unborn child. It is described by the body's lack to compensate for the physiological IR that occurs during pregnancy. Although many factors of risk contribute to the development of GDM. IR looks to be the primary factor which in turn triggers other mechanisms. The placenta also plays an important role in the onset of GDM. Inflammatory markers, along with placental hormones, are involved in this process which is not yet fully understood. These changes impact fetal development and growth, making early diagnosis and prompt treatment of GDM crucial to reducing the risk of complications.

Additionally, early postnatal preventive approaches should be considered in reducing the development from GDM to T2D, thereby increasing awareness of this condition during pregnancy. However, there are still many steps to be taken towards establishing an international prevention strategy and accurately determining the mechanisms involved in the development of GDM.

Conflicts of interest and sources of funding

The authors declare no conflict of interest, and this research has received no external funding.

Ethics approval and consent to participate

Not applicable.

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Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

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The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Academic Editor. The Editor-in-Chief reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

ETHICAL CONSIDERATIONS

Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed following the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at: http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent before their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects from being recognized (or an eye bar should be used).

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

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Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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ORIGINAL ARTICLE

The Impact of Ketone Salt Supplementation on Blood Pressure and Health Parameters in Adults Diagnosed with PTSD – A Pilot Study

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Abstract: *Post-traumatic stress disorder (PTSD) is a condition associated with high levels of stress often linked to elevated resting blood pressure (BP). Beta-hydroxybutyrate, a fuel source and signaling molecule, has been shown to improve metabolic and neurological conditions, as well as decrease systolic BP in healthy populations. The purpose of this study was to determine if long-term beta-hydroxybutyrate administration via ketone salt (KS) supplementation alters BP, complete blood count (CBC), or comprehensive metabolic panel (CMP) results in adults diagnosed with PTSD. Sixteen adults (ages 21-65; 12 women/4 men) with PTSD completed this randomized, double-blinded, and placebo-controlled study by taking KS or a placebo supplement for 6 weeks. Blood was collected (for CMP and CBC assessment) and BP measurements were taken before and after the intervention period. None of the variables changed significantly based on the condition (p > .05 for all interaction effects). The reduction in BP measures following the KS supplementation, though non-significant, may be clinically meaningful in PTSD populations and others subject to increased cardiovascular mortality risk. Chronic KS supplementation did not negatively impact BP, CBC, or CMP measures in adults with PTSD. Further investigation of KS as a hypertension treatment approach is warranted in this population.*

Keywords: *PTSD, ketone salts, blood pressure, cardiovascular, comprehensive metabolic panel, complete blood count, beta-hydroxybutyrate, hypertension*

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a highly prevalent and impairing mental condition brought on by the occurrence of a traumatic life event(s) [1,2]. According to the U.S. Department of Veterans Affairs, an estimated 6 in 100 people will suffer from PTSD at some point during their lifetime; in 2020 approximately 13 million American adults were estimated to have PTSD [3]. Only a minority of those with PTSD obtain treatment [1]. Individuals who suffer from this may experience flashbacks and invasive thoughts concerning the event, as well as nightmares, emotional numbness, and loss of interest in daily activities [4-6]. In addition to psychological ramifications, PTSD symptoms manifest in the physical health of these individuals [7].

Hyperarousal is characteristic of PTSD which may result in elevated resting blood pressure and/or heart rate commonly found in this population [8,9]. Previous findings, though controversial, demonstrate an association between PTSD and elevated levels of proinflammatory blood markers [10]. Compared to the general population, those diagnosed with PTSD are at a higher risk of developing cardiovascular disease, autoimmune disorders, metabolic syndrome, obesity, diabetes, and atherosclerosis [11-14]. Negative health behaviors previously associated with PTSD, such as smoking, increased weight, and physical inactivity, may attribute to these increased risks [15,16]. Since a minority seeks professional treatment options, holistic, at-home remedies need to be identified and examined for efficacy.

Elevating circulating ketone bodies, specifically beta-hydroxybutyrate, has been shown to significantly improve a range of metabolic and neurological disorders [17-19]. For example, beta-hydroxybutyrate has been shown to inhibit the NLRP3 inflammasome, a multiprotein complex that induces neuroinflammation in the hippocampus, with antidepressant and anti-inflammatory effects on rats exposed to acute and chronic stress [20]. Through several mechanistic effects, increased circulating beta-hydroxybutyrate may positively affect psychiatric disorders by enhancing brain energy metabolism, promoting repolarization of the neuronal membrane, decreasing hyperexcitability and neuronal firing, modulating neurotransmitter release and balance, decreasing inflammatory processes, and providing neuroprotective effects [21-26]. Strategies to increase circulating beta-hydroxybutyrate levels include maintaining a low-carbohydrate, high-fat ketogenic diet, and/or supplementing with exogenous ketones; both approaches demonstrate therapeutic benefits [22,27,28]. Rigorous adherence to a ketogenic diet is required to increase ketone levels in the blood but is difficult to achieve, especially in the psychiatric population [19]. Exogenous ketone supplements increase circulating ketone levels and require no dietary changes other than consuming the ketones, usually in drink form [27,29]. Similar to dietary-induced endogenous ketones, exogenous ketone supplements provide a fuel source and evoke signaling-based physiological modifications [30,31]. Therefore, elevating circulating ketone levels via long-term administration of exogenous ketone supplements may allow for a more accurate examination of the effects of ketones in the PTSD-diagnosed population.

Previously, we have demonstrated that supplementing with ketone salts for 6 weeks seems safe in terms of blood health parameters and blood pressure in a healthy population, even showing a significantly reduced systolic blood pressure [29]. Prior to examining the therapeutic effects of ketone salts, it is important to determine if they alter basic health variables and seem safe. Studies demonstrating the effects of increased circulating ketone levels on psychiatric disorders in humans are limited and need to be explored due to their therapeutic properties [21]. Therefore, the aim of this 6-week pilot study was to determine if daily consumption of exogenous ketone salts alters blood pressure and/or blood health parameters in individuals diagnosed with PTSD.

MATERIALS AND METHODS

Experimental Design

This randomized, double-blinded, placebo-controlled, six-week clinical trial consented individuals previously diagnosed with PTSD. Participants were randomized to either a placebo (PLA) or ketone salt (KS) group. Both the PLA and KS groups were asked to maintain a normal diet, consume their provided supplement twice daily, and maintain their normal medication schedule throughout the 6-week period. The PLA and KS supplements were sent to the experimental laboratory by the manufacturer in white packages coded “A” or “B”, and participant numbers 1-24 were randomly assigned to supplement ‘A’ or ‘B’ prior to recruiting or consenting participants. The study was approved by Augusta University’s Institutional Review Board, and all procedures were in compliance with the institutional guidelines. All participants signed an informed consent with a study-approved investigator prior to participating. The study is registered with ClinicalTrials.gov with the ID# NCT04083352.

Participants

Inclusion criteria to participate in this study were: 1) a previous diagnosis of PTSD and 2) men and non-pregnant women between 18-65 years old. Participants were recruited through email solicitation via the University’s Veteran Success Department, the Augusta Warrior Project, as well as through the local news station. Twenty-one participants consented to the study and 14 completed it, including 3 males and 11 females. Participant demographics are provided in Table 1.

Table 1: Participant demographics and p-value of t-test comparing baseline characteristics between groups (ketone salts, KS; placebo, PLA)

Measure	KS (*n*=7; 5 women)	PLA (*n*=7; 6 women)	*p*
Age (years)	42.85 (9.01)	49.14 (11.05)	.266
Height (cm)	171.78 (9.31)	166.71 (8.57)	.310
Weight (kg)	93.37 (23.08)	86.16 (18.26)	.529
BMI (kg/m^2)	31.21 (4.76)	31.07 (6.61)	.964

Procedures

Participants visited the laboratory twice for data collection, once at the beginning of the study for baseline data and again 6 weeks later after supplementing with their assigned supplement.

Prior to the two laboratory visits, participants were asked to fast and refrain from exercise for at least 8 hours, refrain from caffeine and nicotine for at least 12 hours, and refrain from consuming alcohol for at least 24 hours. During the two laboratory visits, participants filled out a health history questionnaire, took a pregnancy test if female, had height, weight, resting heart rate, and blood pressure measured, and then had a venous blood draw to use for comprehensive metabolic panel (CMP) and complete blood count (CBC) analyses.

Measures

Height was measured using a stadiometer (Stadi-o-meter; Novel Products, Inc., Rockton, IL, USA), and weight was measured using a beam balance scale (Healthometer; Continental Scale Corporation, Bridgeview, IL). Resting blood pressure and heart rate were recorded 5 minutes after resting with an automated blood pressure cuff (10 Series Upper Arm Blood Pressure Monitor; Omron Healthcare, Inc, Kyoto, Japan).

Venous blood samples were collected into an ethylenediaminetetraacetic acid (EDTA) anticoagulant-sealed vacutainer and two lithium heparin-sealed vacutainers. The samples were then transported directly to the Clinical Pathology Lab at Augusta University Hospital for analysis of CMP and CBC. The CMP included analysis of plasma sodium, potassium chloride, carbon dioxide, blood urea nitrogen (BUN), creatinine, calcium, glucose, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate amino-transferase (AST) and alanine transaminase (ALT) levels. The CMP was performed on an automated chemistry analyzer (ADVIA 1800 Chemistry System; Siemens Healthineers, Erlangen, Germany). The CBC included analysis of whole blood levels of white blood cells (WBC), red blood cells (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width percent (RDW), platelets (PLT) and mean platelet volume (MPV). The CBC was analyzed with a cellular analysis system (UniCel DxH 800 Hematology Analyzer Workstation; Beckman Coulter, Brea, CA).

Supplement

Participants were randomized to consume two servings per day of either KS or PLA supplements for 6 weeks (84 total servings) after the first data collection visit. Participants were encouraged to consume one serving in the morning and one in the late afternoon while maintaining their typical diet. Participants were asked to fill out daily supplement logs to confirm adherence to consuming the assigned supplement.

The KS and PLA supplements were flavor- and calorie-matched (fruit punch and 41 calories, respectively), and were provided in a powder form that was to be mixed with water. The KS supplement consisted of 7 grams of beta-hydroxybutyrate combined with erythritol, L-Taurine, fermented L-Leucine, citric acid, natural flavor, vegetable juice color, stevia, xanthan gum, beta carotene and approximately 920 mg of sodium (KETO//OS® MAX, Pruvit Ventures, Inc., Melissa, TX). The PLA supplement included similar color, sodium, and flavoring as the KS supplement but with maltodextrin in place of the beta-hydroxybutyrate.

Statistical Analysis

All analyses were performed with SPSS version 29 by a statistician who was blinded from any information pertaining to the group assignment of all participants. A predetermined alpha level of .05 was used in all cases unless otherwise specified. Any missing data was excluded from analysis via pairwise deletion, leading to slightly different sample sizes for some analyses. Demographic variables (age, height, weight, and BMI) at baseline were compared between the KS and PLA groups using paired-sample t-tests to verify similar participant characteristics in each group. Body weight was compared before and after the intervention period in each group using paired samples t-tests to assess weight change during the intervention period.

A series of 2 (group: control or ketones) x 2 (time: PRE and POST) mixed factorial ANOVAs was performed to assess the changes in each dependent variable in each group over time for BP, CMP, and CBC measures. Data were screened for outliers (> 3.0 SD units from the mean) prior to analysis. The assumptions of homogeneity of variance and covariance were assessed with Levene's test and Box's test, respectively. Violations of these assumptions are reported with relevant results. In the case of a significant interaction effect, Bonferroni-adjusted post hoc tests were performed. Effect size is reported as partial eta squared (η^2) and is interpreted according to the guidelines described by Cohen (2013) as small (η^2=0.01), medium (η^2=0.06), and large (η^2=0.14) [32].

RESULTS

Cardiovascular and body weight measures

There was no interaction effect, or main effects of condition or time, respectively, for SBP (p=.120 and η^2=.175; p=.154 and η^2=.150; p=.849 and η^2=.003), DBP (p=.174 and η^2=.137; p=.442 and η^2=.046; p=.701 and η^2=.012), or MAP (p=.124 and η^2=.172; p=.266 and η^2=.094; p=.743 and η^2=.009). Results for SBP, DBP, and MAP are presented graphically in Figure 1.

Similarly, there were no significant effects for RHR (p=.349 and η^2=.080; p=.832 and η^2=.004; p=.286 and η^2=.103) which increased from PRE to POST in the KS condition (73.86±15.61 bpm vs. 81.14±15.91 bpm) and the PLA condition (75.67±13.26 bpm vs. 76.17±12.58 bpm). Among participants with complete data, there was no significant change in body weight from PRE to POST for KS (103.32±23.82 kg vs. 103.3±25.23 kg; p=.973) or PLA (87.1±17.11 kg vs. 87.95±17.12 kg).

Figure 1: Cardiovascular measures before (PRE) and after (POST) 6-week intervention period (ketone salts, KS; or placebo, PLA). SBP (systolic blood pressure), DBP (diastolic blood pressure), and MAP (mean arterial pressure) values are presented. Bars represent mean values and whiskers represent standard deviations

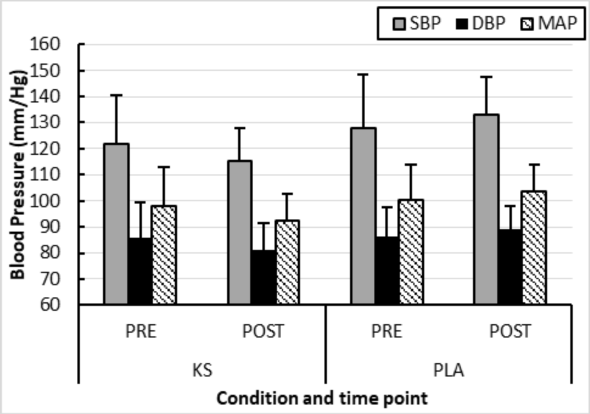

Comprehensive metabolic panel

ALT did not meet the assumption of homogeneity of variance for either time point. No interaction effect or main effect of time was observed for ALT (p=.870 and η^2=.006; p=.941 and η^2=.001, respectively). However, there was a main effect of condition for ALT (p=.009 and η^2=.775). ALT was higher in KS (39.13±7.08 U/L) than in PLA (16.67±7.08 U/L).

There was no interaction effect, or main effects of condition or time, respectively, for ketones (p=.349 and η^2=.080; p=.832 and η^2=.004; p=.286 and η^2=.103), sodium (p=.918 and η^2=.002; p=.160 and η^2=.300; p=.798 and η^2=.012), potassium (p=.551 and η^2=.062; p=.350 and η^2=.146; p=.480 and η^2=.086), chloride (p=.941 and η^2=.001; p=1.00 and η^2=.000; p=.768 and η^2=.016), CO2 (p=.852 and η^2=.006; p=.287 and η^2=.185; p=.666 and η^2=.033), BUN (p=.114 and η^2=.363; p=.762 and η^2=.017; p=.197 and η^2=.260), creatinine (p=.902 and η^2=.003 ; p=.670 and η^2=.032; p=.206 and η^2=.251), glucose (p=.691 and η^2=.028; p=.554 and η^2=.061; p=.898 and η^2=.003), calcium (p=.117 and η^2=.358; p=.897 and η^2=.003; p=.291 and η^2=.183), protein (p=.690 and η^2=.028; p=.707 and η^2=.025; p=.909 and η^2=.002), albumin (p=.212 and η^2=.245; p=.906 and η^2=.003; p=.828 and η^2=.009), AST (p=.706 and η^2=.025; p=.075 and η^2=.435; p=.510 and η^2=.076); alkaline phosphate (p=.770 and η^2=.015; p=.388 and η^2=.126; p=.363 and η^2=.139), or bilirubin (p=.775 and η^2=.015; p=.463 and η^2=.093; p=.496 and η^2=.081). Descriptive statistics for all comprehensive metabolic panel variables are presented in Table 2.

Complete blood count

No interaction effect or main effect of time was observed for MCV (p=.520 and η2=.061; p=.203 and η2=.220, respectively). However, a main effect of the condition for MCV (p=.018 and η2=.573). MCV was higher in KS (90.72±2.84) than PLA (84.55±2.84).

There was no interaction effect, or main effects of condition or time, respectively, for WBC (p=.565 and η^2=.050; p=.227 and η^2=.200; p=.664 and η^2=.028), RBC (p=.597 and η^2=.042; p=.597 and η^2=.042; p=.943 and η^2=.001), Hgb (p=.527 and η^2=.060; p=.393 and η^2=.106; p=.831 and η^2=.007), Hct (p=.749 and η^2=.016; p=.434 and η^2=.089; p=.831 and η^2=.007), MCH (p=.649 and η^2=.031; p=.129 and η^2=.297; p=.304 and η^2=.149), MCHC (p=.456 and η^2=.082; p=.904 and η^2=.002; p=.823 and η^2=.008), RDW (p=.727 and η^2=.019; p=.129 and η^2=.298; p=.312 and η^2=.145), PLT (p=.333 and η^2=.134; p=.228 and η^2=.200; p=.786 and η^2=.011), or MPV, which did not meet the assumption of homogeneity of covariance (p=.815 and η^2=.008; p=.299 and η^2=.152; p=.132 and η^2=.293). Descriptive statistics for all complete blood count variables are presented in Table 3.

DISCUSSION

The findings from this pilot study demonstrate that chronic ketone salt supplementation is safe for individuals diagnosed with PTSD. After supplementing with ketone salts twice daily for 6 weeks, blood pressure and blood health parameters including CMP and CBC remained unaltered. There was a trend toward a reduction in blood pressure after consuming ketone salts and may have clinical significance as it changed the average from hypertensive to normal levels.

Individuals diagnosed with PTSD suffer from health issues including, but not limited to, high blood pressure, depression, and anxiety with increased risk for cardiovascular disease, diabetes, and autoimmune disease [11-13]. Because of the compounding negative health effects related to PTSD, and the large population of individuals affected by this condition, it is important to find therapeutic approaches that may reduce PTSD symptoms and risk factors [1,2]. Ketone salt supplementation has been shown to improve many of these health issues in animal and/or human models [17,18].

Table 2: Descriptive statistics for the comprehensive metabolic panel before (PRE) and after (POST) 6-week intervention period (ketone salts, KS; placebo, PLA). Mean values are reported with standard deviations in parentheses, i.e. "M (SD)". Measure units are specified next to each variable name

Measure	KS		PLA	
	PRE	POST	PRE	POST
Ketones (mg/dL)	0.13 (0.10)	0.13 (0.08)	0.17 (0.12)	0.10 (0.06)
Sodium (mEq/L)	136.40 (1.52)	137.80 (1.30)	136.67 (1.53)	137.67 (0.58)
Potassium (mEq/L)	4.54 (0.74)	4.16 (0.26)	4.13 (0.12)	4.10 (0.46)
Chloride (mEq/L)	103.40 (2.79)	103.60 (1.67)	103.33 (1.53)	103.67 (1.53)
CO_2 (mEq/L)	26.40 (2.97)	26.00 (3.54)	25.00 (2.65)	24.00 (1.00)
BUN (mg/dL)	12.80 (2.77)	13.20 (3.19)	14.00 (1.73)	10.67 (4.73)
Creatinine (mg/dL)	0.84 (0.04)	0.81 (0.07)	0.88 (0.16)	0.85 (0.19)
Glucose (mg/dL)	115.60 (43.18)	114.40 (37.21)	98.67 (12.89)	101.00 (11.14)
Calcium (mg/dL)	9.26 (0.29)	9.32 (0.27)	9.40 (0.17)	9.13 (0.29)
Protein (g/dL)	7.36 (0.50)	7.42 (0.65)	7.57 (0.58)	0.75 (0.57)
Albumin (g/dL)	4.42 (0.31)	4.54 (0.50)	4.53 (0.21)	4.37 (0.21)
AST (U/L)	27.20 (7.98)	24.80 (4.66)	18.67 (5.03)	18.00 (1.00)
ALT (U/L)	39.25 (12.99)	39.00 (5.48)	16.33 (2.52)	17.00 (2.65)
Alkaline Phosphate (U/L)	71.40 (19.76)	75.20 (25.99)	58.33 (13.43)	60.33 (17.79)
Bilirubin (mg/dL)	0.60 (0.24)	0.68 (0.31)	0.50 (0.10)	0.53 (0.15)

Table 3: Descriptive statistics for the comprehensive metabolic panel before (PRE) and after (POST) 6-week intervention period (ketone salts, KS; placebo, PLA). Mean values are reported with standard deviations in parentheses, i.e. "M (SD)". Measure units are specified next to each variable name

Measures	KS		PLA	
	PRE	POST	PRE	POST
WBC (x10^9L)	8.18 (2.40)	8.88 (3.31)	6.37 (1.52)	6.27 (1.14)
RBC (x10^{12}L)	4.68 (0.41)	4.72 (0.35)	4.88 (0.47)	4.82 (0.42)
Hgb (g/dL)	14.40 (1.10)	14.50 (1.23)	13.90 (0.36)	13.70 (0.95)
Hct (%)	42.58 (3.89)	42.68 (3.36)	41.10 (1.82)	40.60 (2.60)
MCV (fL)	91.05 (1.61)	90.38 (1.11)	84.67 (4.58)	84.43 (5.00)
MCH (pg)	30.77 (0.99)	30.70 (0.89)	28.70 (2.90)	28.53 (2.91)
MCHC (g/dL)	33.80 (1.37)	33.98 (0.91)	33.83 (1.50)	33.73 (1.43)
RDW (%)	13.45 (0.74)	13.25 (0.45)	14.00 (0.10)	13.90 (0.30)
PLT (x10^9L)	247.17 (59.17)	267.50 (41.19)	300.67 (14.01)	289.00 (34.83)
MPV (fL)	9.05 (0.96)	8.80 (0.86)	8.17 (1.89)	7.83 (1.47)

Ketones are a preferred fuel source, especially for the brain as they can cross the blood-brain barrier to enter the brain cells and ultimately provide energy for the central nervous system [32]. Animal studies have demonstrated therapeutic benefits for similar psychiatric symptoms including anti-anxiety and antidepressant effects possibly due to a reduction in neuroinflammation [20,22].

We previously demonstrated that chronic ketone salt supplementation reduced systolic blood pressure in a healthy population; the PTSD study described herein demonstrated a trend toward reduced blood pressure. Kimura et al. (2011) demonstrated that beta-hydroxybutyrate inhibits sympathetic activity by interacting with G protein-coupled receptor 41, a receptor involved in the regulation of the sympathetic nervous system [34]. The inhibition of the sympathetic nervous system from beta-hydroxybutyrate is a possible explanation for reduced blood pressure after chronic consumption of ketone salts [35]. A common concern of ketone salt supplements is the potential negative health effects that may result from the high sodium content of the supplement. One serving of the ketone salt supplement used in this study contained 914 mg of sodium which is almost 40% of the daily recommended sodium content. However, our study in healthy individuals as well as in this PTSD population found unaltered blood sodium levels and blood pressure were not negatively affected after consuming two servings of ketone salts for 6 weeks [29].

The effect of KS on BP measures over time, although not statistically significant, was substantial as noted by the large effect sizes reported and corresponding reductions in SBP, DBP, and MAP (6.5, 4.8, and 5.3 mmHg, respectively). Hypertension is typically defined as resting values of SBP>120 or DBP>80, though there is a clearly documented positive linear relationship between BP and mortality from cardiovascular causes at any level above 115/75 mmHg, wherein a higher BP is associated with increased risk [36,37]. To add perspective, Ettehad et al. (2016) estimated that for every 10 mmHg reduction in SBP, people with hypertension experience >20% reductions in CVD events, stroke, and heart failure, as a population [38]. If a reduction in blood pressure of any magnitude (at or above healthy levels) is considered clinically meaningful based on these findings from large-scale clinical trials, then supplementing one's diet with KS could be seen as an attractive alternative to traditional hypertension medications which may be contraindicated or have adverse effects [39]. It is also noteworthy that the reductions in SBP, DBP, and MAP observed here were in participants who were for the most part very close to healthy BP levels. If a similar intervention was completed in participants with hypertension, a more substantial, and possibly significant, antihypertensive effect may result.

Studies examining the efficacy of exogenous ketone supplementation on PTSD or other psychiatric disorders in humans are limited, and therefore this needs further investigation. Future studies exploring the effects of exogenous ketone supplementation on cardiovascular parameters in people with PTSD should include a group without PTSD for comparison and larger sample size, as these were two primary limitations of this study.

CONCLUSION

Chronic ketone salt supplementation did not alter CMP or CBC, blood health parameters indicative of liver and kidney function as well as infection and disease, in individuals diagnosed with PTSD. Blood pressure and heart rate were unaltered, although blood pressure trended toward a reduction from hypertensive to normal levels, which may be clinically meaningful in terms of cardiovascular mortality risk. This small pilot study suggests that ketone salt supplementation does not negatively impact cardiovascular or blood health parameters and should be further explored as a potential therapeutic approach for PTSD and related cardiovascular comorbidities.

Conflicts of interest and sources of funding

The authors declare no conflicts of interest. Angelia Holland-Winkler received funding for this project from Augusta University's College of Education and Human Development and Prüvit Ventures, Inc. Ketone and placebo supplements were provided by Prüvit Ventures, Inc.

Authors' contribution

Conceptualization, A.H-W; methodology, A.H-W; formal analysis, A.M.; investigation, A.H-W; writing—original draft preparation, J.L., A.M., A.H-W; writing—review and editing, J.L., A.M., A.H-W; supervision, A.H-W; funding acquisition, A.H-W. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

This study was approved by Augusta University's Institutional Review Board with approval number 907405. All subjects signed an approved and stamped informed consent document prior to participating.

Patient consent for publication

Informed consent was obtained from all subjects involved in this study.

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ORIGINAL ARTICLE

The Effect of an Empowerment Program on the Spiritual Care Competence of Nursing Students: A Randomized Clinical Trial Study

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Abstract: *Spiritual care is an integrated section of nursing. Proper implementation of it to patients requires that care providers have the necessary competencies in this regard. Therefore, this study was performed to investigate the effect of an empowering program on spiritual care competence (SCC) in nursing students. This study was a randomized controlled trial that was performed on 60 nursing students at Kashan University of Medical Sciences, Iran. Participants were randomly assigned to two groups. The intervention group was under the spiritual empowering plan in seven sessions. The control group was traditionally trained. Data were collected by a spiritual care competence scale and analyzed using Fisher, independent and paired t-test in SPSS 16v. Baseline characteristics were similar in the two groups. The mean score of SCC was significantly increased post-empowerment for the intervention group. The differences between the two groups were statistically significant in the SCC scores (p<0.037). A significant difference was observed between the mean of the SCC score of the intervention group before and after the empowering (p < 0.0001). While, no significant changes were observed in the control group, before and after the study (p= 0.44). Empowering spiritual care skills in nursing students develops the quality of care in patients, which requires more attention in the educational curriculum.*

Keywords: *holistic care; spiritual care; competence; empowerment program; nursing students*

INTRODUCTION

A holistic attitude is a dynamic way of educating an individual with a holistic view, and the nursing profession is always focused on caring for a person as a whole and covering the physical, mental, social, and spiritual dimensions of a person [1]. Along with the physical and mental dimensions, the spiritual dimension is considered an important factor [2] and it is the basis that all principles, ideas, thoughts, beliefs, choices, actions, and final goals of a person are focused on it [3]. Considering this important human dimension and providing comprehensive care will promote the recovery and well-being of patients [4]. Hence, spiritual care is an integrated section of the nursing process [5]. Spiritual care contains religious and non-religious skills [6]. The religious skills contain such things as patient accompaniment in worship and provision of the necessary requisites for religious ritual, talking with him about God, clarifying the patient's relationship with God, applying religious books, and referring to religious institutions. The non-religious skills also contain relationship skills, empathy, respect, counseling, emotional support of people, and encouraging the client to express feelings and avoid negative thoughts [7]. The correct implementation of spiritual care requires having the necessary skills in this field [8]. This competence is very important for nurses and nursing students because the patients' spiritual needs become apparent when disease or crisis occurs [9]. Spiritual competence empowers nurses and increases their knowledge and skills to nurture hope, meaning, and purpose in patients [2]. During the course of the disease, nursing students are admitted to the private domain of patients, along with health workers, so they should be able to deal with their spiritual issues [10]. Students have different cultural and spiritual views, beliefs, and values than current nurses [11], and institutionalization of ethical principles among students and preparing them for future professional life is an important part of university responsibility. The task of universities is to organize the educational environment in accordance with the culture and religion of the country and the needs of the community [12]. Studies show that the spiritual competence of nurses and nursing students is not in good condition [11, 13-15]. A study showed that only 42% of nurses in the clinic pay attention to the patient’s spiritual needs [13]. Also, Ross et al. (2014) reported that only 25% of nursing students are qualified to perform spiritual care [16], and most nursing students in clinical practice ignore spiritual care [17]. Although nurses are motivated to become qualified in spiritual care [18] and nursing students value it [11], they do not implement spiritual care, and lack of attention to spirituality in undergraduate programs is stated as the predominant obstacle to the lack of competence in implementation spiritual care [19-21]. The results of the studies show that the nursing curriculum needs to integrate spirituality and spiritual care more than ever [22, 23] so that nurses become more aware of the patients’ spiritual needs [2]. Culatto and Summerton (2015) reported that spirituality is an important part of the curriculum before and after a clinical period, and 64.2% of educational institutions considered spirituality a valuable and significant part of education, and only 14.3% considered this part worthless [24]. Nursing literature also recognizes the need for nurses' empowerment in the field of spiritual care [25-27]. However, so far this issue has not been formally and explicitly included in nursing education programs [16]. Spiritual empowerment can improve students' attitudes and awareness about the spirituality of patients, assess spiritual needs, respond differently to their needs, the ability to refer patients, and their communication skills [28,29]. It was also found in another qualitative study that spiritual care education had a positive effect on their personal, academic, and professional lives [30]. In spite of the benefits of spirituality education, a review study by Cooper et al. (2013) noted its negative effects, such as the concern and the sense of coercion in changing personal opinions about spirituality [31], and in another study, little effect of spiritual care education was found on students' attitude towards spirituality and communication skills [32]. Few studies have been done on the form and manner of empowering spirituality [33]. Therefore, it is necessary to conduct numerous studies to evaluate the benefits of empowering methods in the field of spirituality [34]. The purpose of this study was to investigate the effect of an empowering plan on the competence of implementation of spiritual care in nursing students.

MATERIALS AND METHODS

The present study is a randomized control clinical trial that was performed on senior nursing students of the Faculty of Nursing and Midwifery of Kashan University of Medical Sciences.

Participants and data sampling

The sample size was estimated to be 30 in each group based on the mean and standard deviation of the previous study [28, 29] at a 95% confidence level and 80% test capacity ($\alpha = 0.95$, $\beta = 0.2$, $s1 = 10$ and $s2 = 10$). Sampling in the first stage was simply random so the samples were selected from the list of senior nursing students of the faculty using the random number table. Then, students were randomly assigned to two intervention (30) and control (30 people) groups by block randomization (four blocks). Inclusion criteria included students who were studying in the seventh and eighth semesters, were not guests and free from academic probation or transfer, had no known physical and psychological problems, and were satisfied with the research. Students who were reluctant to continue to cooperate at each stage of the research, had severe crises (like the death of a close family member, divorce, separation, and quarrel with the spouse), and had two sessions or more absences in the classes, were excluded from the study.

Procedure

The researchers gave information on the objectives, type, advantages, and confidentiality of the study to the students. If they were willing to collaborate in the study, written informed consent was completed and the study instrument was self-reported by them. The samples were then assigned to intervention and control groups. The intervention group was under the empowering plan. The intervention method was such that the students participated in a group and in a single month in seven 60-minute sessions. The control group was trained under the former and routine curriculum of the university. Educational pamphlets about care were also provided. In the final of the study (one month after starting the study), the study tool was completed by the samples again.

Intervention

The empowerment content of the present study is taken from past studies [29,33,35-37] and consists of spiritual subjects such as the description of spirituality and spiritual care, assessment and implementation of spiritual care, communication skills, respect for human dignity, individual support, counseling, hope therapy, and betterment the quality of life, which was explained and taught in the form of lectures and small group discussions (Box 1). This content was confirmed by 10 experts and faculty members who had the awareness and experience in this background.

Data Collection

The study instrument consists of two sections of individual social information inventory including 9 items containing age, gender, marital status, semester, accommodation status, interest in the subject of spirituality, passing the course of spiritual care, participation in the ethics seminar or workshop, and the degree of personal religiosity and the scale spiritual competence.

Box 1: Subjects of the training plan

Session 1:
- The description of spiritual health and its dimensions
- Acceptance of life and relationship with God, self, society, and the environment
- The expression of the integrity of the biological, psychological, and social components
- Essential characteristics of spirituality
- Differences and similarities between spirituality and religion
- Explain spirituality and spiritual care
- Religious and non-religious care

Session 2:
- Types of spiritual care (personal, procedural, cultural, and evangelical)
- Essential skills for performing its
- Spiritual distress, the influence of disease on individual life
- Detect meaning and goal of life
- The role of hope in patient spirituality
- Facilitate coping and adaptation strategies used by patients during illness

Session 3:
- Dimension of professionalism and betterment of the quality of life
- Obstacles to the implementation of its
- Strategies to improve its

Session 4:
- Assessment and implementation of its
- The patients’ spiritual needs during illness and how to assess them
- How to provide spiritual care by nurses
- The nursing process in spirituality
- Communication skills

Session 5:
- Communication techniques, trust building, and patient acceptance
- Active listening
- How to express feelings

Session 6:
- Importance of human dignity
- Efficacy of observance of privacy in patient’s spirituality
- Paying attention to spiritual care in the charter of patient rights
- Personal support
- Counseling and referral

Session 7:
- Attention and support of religious rituals
- Religious and spiritual referral
- Protective presence

The Spiritual Care Competence Scale (SCCS) was designed by Van Leeuwen & Cusveller (2009). This scale has 6 dimensions, the first dimension is "assessment and implementation of spiritual care" (Questions 1-6), the second dimension "professionalism and betterment of the quality of spiritual care" (Questions 7-12), the third dimension "personal support and patient counseling" (Questions 13-18), the fourth dimension "referral" (Questions 19-21), the fifth dimension "attitude towards patients' spirituality" (Questions 22-25) and finally the sixth dimension is "communication" (Questions 26-27) that the responses were ranked according to the 5-point Likert scale (fully agree, to fully disagree). The total score of this scale is between 27 and 135 and it has three grades. A score of 27 to 62 indicates a low level, a score of 63 to 98 indicates an average level and a score of more than 98 indicates a high level of spiritual competence. The validity and reliability of this instrument were investigated by Van Leeuwen et al. In 2009, the Cronbach's alpha coefficient for the sub-scales was between 0.56 to 0.82 and the mean inter-item correlation ((MIIC ≥ 0.25) was reported as an acceptable level of internal consistency or reliability [38]. The validity and reliability of this scale have been confirmed in Iran. In the study of Nasehi et al. (2013), the Cronbach's alpha coefficient was 0.78 and in the other study, the Cronbach's alpha coefficient for the whole instrument was 0.77, and for the subcategories have been reported between 0.65 and 0.85 [8,10].

Data Analysis

Data analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine the normal distribution of the data. Descriptive statistics (i.e., mean, standard deviation, frequency, and percentage) were calculated. Fisher and t-tests were used to compare demographic variables in the two groups. The independent-sample t-test was also used to examine the differences between the competence of spiritual care mean scores, and the subscale mean scores within the two groups at the beginning and the final of the study. In addition, a paired-sample t-test was used to compare the changes in the competence of spiritual care mean scores of the individual groups at the beginning and the final of the study. The significance level was considered less than 0.05 in all tests.

RESULTS

Out of 60 participants in this study, eight persons in the intervention group and five persons in the control group were excluded from the analysis due to irregular attendance at training sessions and incomplete answers in the post-test instrument, respectively. Finally, the data of 47 samples were analyzed (Figure 1).

The mean age of participants was 22.43 ± 0.85. About 25.5% of the participants in the intervention group and 36.2% in the control group were female.

Figure 1: CONSORT flow diagram

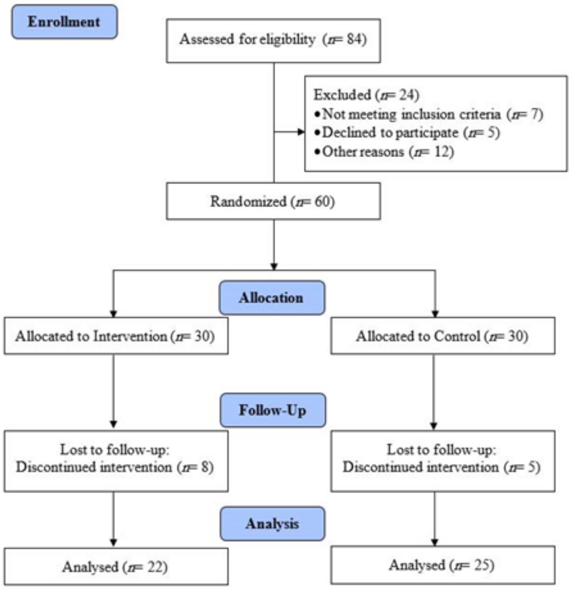
Enrollment
Assessed for eligibility (n= 84)
Excluded (n= 24)
•Not meeting inclusion criteria (n= 7)
•Declined to participate (n= 5)
•Other reasons (n= 12)
Randomized (n= 60)
Allocation
Allocated to Intervention (n= 30)
Allocated to Control (n= 30)
Follow-Up
Lost to follow-up: Discontinued intervention (n= 8)
Lost to follow-up: Discontinued intervention (n= 5)
Analysis
Analysed (n= 22)
Analysed (n= 25)

The results showed that there was no significant difference between the two groups' demographic variables, including age, semester, sex, marital status, accommodation status, interest in the subject of spirituality, and attending spiritual care of patient course ($p > 0.05$) (Table 1). The results obtained from conducting independent sample t-tests revealed that there was no significant difference between the mean scores of spiritual care competence and its subscales for the two groups at the beginning of the study ($p > 0.05$) (Table 2). However, the mean score of spiritual care competence (104.9 ± 9.7 for the intervention group, and 97.4 ± 14 for the control group), was significantly increased post-intervention for the intervention group. The differences between the two groups were statistically significant in both the overall spiritual care competence scores ($p < 0.037$) and across two of the subscales, including "assessment and implementation of spiritual care" and "professionalism and betterment of the quality of spiritual care" ($p < 0.05$) (Table 3). Using paired t-tests, we found that spiritual care competence and its subscales, including "assessment and implementation of spiritual care", "professionalism and betterment of the quality of spiritual care", "personal support and patient counseling" and "referral" in the intervention group were significantly different before and after the spiritual education ($p < 0.0001$). There was no significant difference between the mean scores of the spiritual care competence and its subscales in the control group at the beginning and the final of the study ($p > 0.05$) (Table 4).

Table 1: Demographic information of samples

Variables	Intervention group *(n= 22)*	Control group *(n= 25)*	*p*-value
Age, year†	22.27 ± 0.76	22.56 ± 0.91	0.25*
Semester, number†	7.55 ± 51	7.52 ± 51	0.86*
Gender††			0.38**
Male	10 (21.3)	8 (17)	
Female	12 (25.5)	17 (36.2)	
Marital status††			0.17**
Single	19 (40.4)	17 (36.2)	
Married	3 (6.4)	8 (17)	
Accommodation status††			0.21**
Personal house	17 (77.3)	14 (56)	
Dormitory	5 (22.7)	11 (44)	
Interest in the subject of spirituality††			0.21**
Low	17 (77.3)	14 (56)	
High	5 (22.7)	11 (44)	
Passing the course of spiritual care††			0.4**
Yes	3 (13.6)	6 (24)	
No	19 (86.4)	19 (76)	

† Mean ± Standard Deviation
†† No. (%)
** Obtained from independent t-test*
*** Obtained from Fisher test*

Table 2: Comparison of spiritual care competence at the beginning of the study between the intervention and control group

Variables	Intervention group† (*n*= 22)	Control group† (*n*= 25)	*p*-value*
Assessment and implementation of spiritual care	18.9 ± 3.8	20.08 ± 3.17	0.26
Professionalism and betterment of the quality of spiritual care	17.81 ± 3.6	19.28 ± 3.28	0.15
Personal support and patient counseling	20 ± 3.3	21.08 ± 3.87	0.31
Referral	9.77 ± 1.6	10.2 ± 2.04	0.43
Attitude toward patients' spirituality	15.68 ± 2.57	16.56 ± 2.32	0.22
Communication	7.45 ± 1.53	8.2 ± 1.63	0.11
Spiritual care competence	89.63 ± 10.57	95.33 ± 10.82	0.07

† Mean ± Standard Deviation

** Obtained from independent t-test*

Table 3: Comparison of spiritual care competence at the final of the study between the intervention and control group

Variables	Intervention group† (*n*= 22)	Control group† (*n*= 25)	*p*-value*
Assessment and implementation of spiritual care	23.54 ± 2.08	21.28 ± 3.72	0.01
Professionalism and betterment of the quality of spiritual care	22.5 ± 3.01	20.36 ± 3.86	0.03
Personal support and patient counseling	23.13 ± 2.73	21.8 ± 3.3	0.14
Referral	11.5 ± 1.76	10.76 ± 2	0.18
Attitude toward patients' spirituality	16.54 ± 1.81	15.44 ± 2.61	0.1
Communication	7.68 ± 1.61	7.76 ± 1.76	0.8
Spiritual care competence	104.9 ± 9.7	97.4 ± 14	0.03

† Mean ± Standard Deviation

** Obtained from independent t-test*

Table 4: Comparison of spiritual care competence pretest-posttest between the intervention and control group

Variables	Intervention group (*n*= 22)			Control group (*n*= 25)		
	Baseline†	After intervention†	*p*-value*	Baseline†	Final of study†	*p*-value*
Assessment and implementation of spiritual care	18.9 ± 3.8	23.54 ± 2.08	0.00	20.08 ± 3.17	21.28 ± 3.72	1.37
Professionalism and betterment of the quality of spiritual care	17.81 ± 3.6	22.5 ± 3.01	0.00	19.28 ± 3.28	20.36 ± 3.86	0.26
Personal support and patient counseling	20 ± 3.3	23.13 ± 2.73	0.00	21.08 ± 3.87	21.8 ± 3.3	0.53
Referral	9.77 ± 1.6	11.5 ± 1.76	0.00	10.2 ± 2.04	10.76 ± 2	0.22
Attitude toward patients' spirituality	15.68 ± 2.57	16.54 ± 1.81	0.19	16.56 ± 2.32	15.44 ± 2.61	0.05
Communication	7.45 ± 1.53	7.68 ± 1.61	0.59	8.2 ± 1.63	7.76 ± 1.76	0.19
Spiritual care competence	89.63 ± 10.57	104.9 ± 9.7	0.00	95.33 ± 10.82	97.4 ± 14	0.44

† Mean ± Standard Deviation

** Obtained from independent t-test*

DISCUSSION

The purpose of this study was to investigate the efficacy of empowerment programs on the competence of implementation of spiritual care in nursing students it showed that spiritual empowerment generally increases the competence of implementation of nursing students' spiritual care, but its impact on the dimensions of "assessment and implementation of spiritual care", "professionalism and betterment of the quality of spiritual care", "personal support and patient counseling" and "referral" are more than the dimensions of "attitude toward patients' spirituality" and "communication skills". The findings of this study are consistent with the findings of studies with the aim of determining the efficacy of the empowering plan in betterment of the competence of providing spiritual care by nursing students [32], the effectiveness of an educational intervention in the ability to perform spiritual care and spiritual well-being of undergraduate and postgraduate students [39], the effectiveness of simulated-spiritualized training in implementation of spiritual care for nursing students [28], and the changes in the spiritual experiences of medical and nursing students before and after a period of clinical and spiritual care [40]. Also, in a qualitative study, the survey of experiences of nursing students after the course of spiritual care showed that spirituality had a positive effect on their personal, academic, and professional lives, and led to increased self-awareness, individual development, knowledge about spirituality and spiritual care, sensitivity to the patient's spiritual needs, familiarity with adaptation methods, and providing holistic care [33]. In fact, students who are trained in spirituality, realize the concept of spirituality and give more notice to the patient's spiritual needs [41]. Increasing their moral sensitivity and ethical decision-making ability in clinical settings [29], in addition, they do it in their daily routines. Concerning the cause of the effect of the empowerment plan, the following may be mentioned: since spiritual awareness is prior to the spiritual assessment of patients [41], spirituality empower-ing by increasing awareness of students about patients' spiritual needs and improving their attitude toward spirituality, provides the required competencies in assessing spiritual needs, responding differently to needs, referring patients and communication skills [28]. Empowering also leads to an increase in understanding of the value of spirituality [40] and the ability to provide spiritual care to nursing students in the future.

The method of spiritual empowerment has also been effective in the efficacy of education. According to Taylor, the best method of spiritual care education is to induce a positive attitude and compassionate skills in order to create a sensitivity to spiritual care, which is not achieved except through student discussion [42]. In this study, students used the ideas of each other and recognized their and other's religious views to understand and develop spiritual self-awareness. In addition, inspirational stories were also assisted.

The reason for the low impact of the plan on some aspects of the competence of providing spiritual care, including the attitude toward spirituality and communication, is probably lack of work experience because spirituality is not only rooted in one's beliefs, but also rooted in his experience [4] and factors such as individual spirituality, degree of beliefs, experience, and age of individuals are associated with their attitude to spiritual care [43]. Of course, there is another point to consider that communication is more effective in responding to this topic and is also a kind of research limitation, and it is a self-report of students. Hence, the use of other instruments in assessing these skills is suggested. Considering the influence of the group discussion method, it is suggested that other active educational methods be evaluated and compared in subsequent studies to better spiritual care education and to choose the best em-powering method for spirituality and spiritual care.

CONCLUSION

Based on the results, the empowerment plan is effective and useful in the field of spiritual care competence of nursing students. Therefore, authorities should pay more attention to the importance of empowering spiritual care to nursing students, because the more nursing students have knowledge of spiritual care is greater, the higher the quality of care provided to the patient. Nursing students need to be trained in a wider range of spiritual care skills and it is best to pay more notice to this topic in the nursing educational curriculum. Valuing spiritual care and proper nurse-patient spiritual communication enriches the nursing profession increases nurses' job satisfaction and provides opportunities for better performance.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization and supervision: KS and MA; Data collection: ZS, MH; Data analysis: MA; Writing-original draft and Writing-review & editing: ZS and MA; Final approval: All authors.

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Ethics approval and consent to participate

This study was confirmed by the institutional review board and the ethics committee of the University (IR.KAUMS.NUHEPM.REC.1396.41). The objectives of the study were stated for all nursing students and informed written consent was obtained from them. All student's participation in this study was voluntary and they had the right to withdraw from the study at any time and stage of the study. Also, they were reassured that their personal information would remain confidential. The current research was registered with registration code IRCT20130721014086N11.

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ORIGINAL ARTICLE

The Effect of COVID-19 and Vaccination against COVID-19 on Patients with Psoriasis

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Abstract: *COVID-19 has not been adequately investigated in patients with psoriasis. We wanted to evaluate the effect of COVID-19 and COVID-19 vaccines on disease course in patients with psoriasis. We retrospectively reviewed the medical records of patients who were admitted for the treatment of psoriasis between March 2020 and May 2022. Afterward, patients were contacted by telephone call to collect data on COVID-19. This study included 257 patients, 118 females, and 139 males. 238 (92.6%) patients were vaccinated against COVID-19. The mean age of the vaccinated and unvaccinated patients was 49.23±14.60 and 40.37±11.66 years, respectively (p=0.010). Exacerbation of psoriasis after COVID-19 vaccination was detected in 32 (13.4%) patients. 114 (44.4%) patients had been diagnosed with COVID-19 and 18.4% of them stated exacerbation of psoriasis after COVID-19. No association was detected between COVID-19 and having an accompanying disease and psoriasis treatment (p=0.843, p=0.845, respectively). Hospitalization was required in 12 (10.5%) patients. Unvaccinated patients with psoriasis were significantly younger than those who were vaccinated. Exacerbation of psoriasis was reported in 13.4% of vaccinated patients and in 18.4% of patients after COVID-19. Nevertheless, accompanying disease and conventional or biological agent treatment were associated neither with COVID-19 nor COVID-19-related hospitalization.*

Keywords: *COVID-19; psoriasis; vaccine*

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease that affects 2-3% of the general population [1]. Psoriasis may be triggered by infections and various vaccines such as influenza and tetanus-diphtheria vaccines [2]. Recently, exacerbation and onset of psoriasis have been reported after Coronavirus disease 2019 (COVID-19) and vaccination against COVID-19 [3,4]. Common inflammatory cytokine production has been reported both in patients with psoriasis and in patients with COVID-19 or vaccinated against COVID-19 [5,6]. However, the exact mechanism of exacerbation of psoriasis after vaccination against COVID-19 has not been clearly understood yet. Increased Th17 response in psoriasis has also been detected in patients with severe COVID-19. Therefore, mRNA COVID-19 vaccines may lead to exacerbation of psoriasis by inducing Th17 cells [7].

In addition, the course of COVID-19 in patients with psoriasis remains controversial. It has been suggested that psoriasis might be a risk factor for severe COVID-19 since psoriasis comorbidities such as hypertension, diabetes, and cardiovascular disease have been associated with poor prognosis of COVID-19 [8]. Increased serum level of angiotensin-converting enzyme (ACE) has been reported in psoriasis patients with comorbidities such as cardiovascular disease and atherosclerosis. Therefore, hyperactivity of ACE in COVID-19 may lead to susceptibility to cardiovascular events in psoriasis patients with COVID-19 [9].

Moreover, data on the association between immune-suppressive treatment and increased risk for COVID-19 are inadequate and it is a matter of debate that cessation of immunosuppressive treatment and biological agents in psoriasis patients with COVID-19 is required or not [6,8]. On the other hand, vaccination against COVID-19 is of utmost importance in patients with psoriasis, especially in those under immunosuppressive treatment. Nevertheless, vaccination against COVID-19 in patients with psoriasis has not been adequately investigated [10]. Within this study, COVID-19 vaccination status and the effect of COVID-19 and Pfizer-BioNTech and Sinovac-CoronaVac COVID-19 vaccines on disease course were evaluated in patients with psoriasis.

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained before this study (approval number: 2022-700). We retrospectively reviewed the medical records of patients who were admitted for the treatment of psoriasis between March 2020 and May 2022. Patients aged 18 years and over with complete data were included in the study. The exclusion criteria were pregnancy, lactation, and receiving any vaccines other than COVID-19 such as the flu shot. Data on age, gender, psoriasis type, psoriasis duration, psoriatic arthritis, past medical history, and psoriasis treatment were evaluated. Afterward, patients were contacted by telephone call to collect the data on COVID-19 vaccination status, vaccine type, vaccine dose, effect of COVID-19 vaccines on psoriasis, time to initiation of exacerbation of psoriasis after vaccination, other cutaneous side effects of vaccines, diagnosis of COVID-19, effect of COVID-19 on psoriasis, time to initiation of exacerbation of psoriasis after COVID-19, other cutaneous effects of COVID-19 and requirement of hospitalization. Exacerbation of psoriasis was regarded as an increase in the size and number of the psoriasis lesions.

Statistical analysis was performed using SPSS version 20.0. Data were presented as mean ± standard deviation or median (interquartile range) for quantitative variables, counts, and percentages for categorical variables. Differences between the groups were evaluated by using the chi-square test and Mann-Whitney U test. P values less than 0.05 were considered statistically significant.

RESULTS

This study included 257 patients, 118 (45.9%) females and 139 (54.1%) males. The mean age of the patients was 48.57±14.57 years (range: 18-84 years). 233 (90.7%) patients had psoriasis vulgaris, 14 (5.4%) had palmoplantar pustular psoriasis, 7 (2.7%) had generalized pustular psoriasis, 1 (0.4%) had inverse psoriasis, 1 (0.4%) had nail psoriasis and 1 (0.4%) had guttate psoriasis. The mean psoriasis duration was 17.56±12.36 years (range: 1-54 years). Psoriatic arthritis was observed in 70 (27.2%) patients. The past medical history was unremarkable in 140 (54.5%) patients. However, hypertension was detected in 60 (23.3%) patients, diabetes in 36 (14%), chronic hepatitis B virus infection in 20 (7.8%), coronary artery disease in 16 (6.2%), hypothyroidism in 11 (4.3%), hyperlipidemia in 11 (4.3%), chronic obstructive pulmonary disease in 5 (1.9%), chronic renal disease in 5 (1.9%) and asthma in 5 (1.9%) patients. Malignancies such as thyroid, bladder, non-small cell lung, and breast cancers were detected in 5 (1.9%) patients, rheumatic diseases such as rheumatoid arthritis, ankylosing spondylitis, gout, and Sjögren's syndrome in 4 (1.6%) patients, benign prostatic hyperplasia in 4 (1.6%), chronic venous insufficiency in 2 (0.8%) and migraine in 2 (0.8%) patients. 129 (50.2%) patients were treated with biological agents. 52 (20.2%) patients received ustekinumab, 35 (13.6%) received interleukin-17 (IL-17) inhibitors such as secukinumab and ixekizumab, 34 (13.2%) received tumor necrosis factor-α (TNF-α) inhibitors such as adalimumab, etanercept, infliximab and certolizumab pegol, 8 (3.1%) patients received IL-23 inhibitors such as guselkumab and risankizumab. In addition, 93 (36.2%) patients were treated with methotrexate, 27 (10.5%) with acitretin, 5 (1.9%) with cyclosporine, and 3 (1.2%) with narrowband ultraviolet B therapy.

238 (92.6%) patients were vaccinated against COVID-19 whereas, 19 (7.4%) patients were unvaccinated (Table 1). The mean age of the vaccinated and unvaccinated patients was 49.23±14.60 and 40.37±11.66 years, respectively (p=0.010). 114 (44.4%) patients received Pfizer-BioNTech, 77 (30%) patients received both Pfizer-BioNTech and Sinovac-CoronaVac, 47 (18.3%) patients received Sinovac-CoronaVac COVID-19 vaccines. The mean age of the patients vaccinated with only Pfizer-BioNTech and only Sinovac-CoronaVac COVID-19 vaccine was 44.12±12.72 and 49.49±15.19 years, respectively (p=0.023). The mean psoriasis duration of the patients vaccinated with only Pfizer-BioNTech and only Sinovac-CoronaVac COVID-19 vaccine was 14.75±11.29 and 19.72±13.23 years, respectively (p=0.017). 29 (61.7%) patients vaccinated with only Sinovac-CoronaVac had at least one accompanying disease whereas, 39 (34.2%) patients vaccinated with only Pfizer-BioNTech had at least one accompanying disease (p=0.002). 8 (17%) patients vaccinated with only Sinovac-CoronaVac had diabetes whereas, 5 (4.4%) patients vaccinated with only Pfizer-BioNTech had diabetes (p=0.007). Among 191 (74.3%) patients who received Pfizer-BioNTech COVID-19 vaccine, 93 (36.2%) received 2 doses, 68 (26.5%) received 3 doses, 29 (11.3%) received 1 dose and 1 (0.4%) received 4 doses of vaccine. Among 124 (48.2%) patients who received Sinovac-CoronaVac COVID-19 vaccine, 88 (34.2%) received 2 doses, 16 (6.2%) received 4 doses, 14 (5.4%) received 3 doses, 4 (1.6%) received 1 dose and 2 (0.8%) received 5 doses of vaccine. No accompanying disease has been detected in 22 (73.3%) patients who were unvaccinated or received only a single dose of vaccine and in 118 (52%) patients who received two or more doses of vaccine (p=0.027). 206 (86.6%) patients reported no effect of COVID-19 vaccines on psoriasis, however, 32 (13.4%) patients reported exacerbation of psoriasis after COVID-19 vaccines. Among them, 24 (75%) received Pfizer-BioNTech, 7 (21.9%) received Sinovac-CoronaVac and 1 (3.1%) received both Pfizer-

BioNTech and Sinovac-CoronaVac COVID-19 vaccines. Exacerbation of psoriasis lesions was reported in 28 (87.5%) patients, generalized psoriasis lesions in 3 (9.4%) patients, and exacerbation of nail involvement in 1 (3.1%) patient (Table 2). The median time to initiation of symptoms after COVID-19 vaccination was 15 (25.25) days. Moreover, 1 (0.4%) patient had urticaria, 1 (0.4%) had papules and pustules on soles and 1 (0.4%) had COVID arm following Pfizer-BioNTech COVID-19 vaccination.

Table 1: Vaccination status of patients with psoriasis against COVID-19

	Vaccinated	**Unvaccinated**	**P value**
Patients, n (%)	238 (92.6%)	19 (7.4%)	
Mean age ± SD (years)	49.23±14.60	40.37±11.66	0.01
Administered vaccine, n (%)	Pfizer-BioNTech, 114 (44.4%) Pfizer-BioNTech and Sinovac-CoronaVac, 77 (30%) Sinovac-CoronaVac, 47 (18.3%)		
	Pfizer-BioNTech	**Sinovac-CoronaVac**	
Mean age ± SD (year)	44.12±12.72	49.49±15.19	0.023
Mean psoriasis duration ± SD (year)	14.75±11.29	19.72±13.23	0.017
Accompanying disease, n (%)	39 (34.2%)	29 (61.7%)	0.002
Diabetes, n (%)	5 (4.4%)	8 (17%)	0.007

COVID-19: Coronavirus disease 2019, SD: Standard deviation. Vaccination with Pfizer-BioNTech was more common in younger patients and patients with shorter psoriasis duration compared to vaccination with Sinovac-CoronaVac COVID-19 vaccine (p=0.023, p=0.017, respectively)

Table 2: The effect of COVID-19 and Pfizer-BioNTech and Sinovac-CoronaVac COVID-19 vaccines on patients with psoriasis

Exacerbation of psoriasis after vaccines, n (%)	32 (13.4%)
Vaccine type, n (%)	Pfizer-BioNTech, 24 (75%) Sinovac-CoronaVac, 7 (21.9%) Pfizer-BioNTech and Sinovac-CoronaVac, 1 (3.1%)
Effect of vaccines on psoriasis, n (%)	Exacerbation of the lesions, 28 (87.5%) Generalized lesions, 3 (9.4%) Exacerbation of nail involvement, 1 (3.1%)
Initiation of the symptoms after vaccination, median time (IQR)	15 (25.25) days
Cutaneous effects of Pfizer-BioNTech vaccine, n (%)	Urticaria, 1 (0.4%) Papules and pustules on soles, 1 (0.4%) COVID arm, 1 (0.4%)
Exacerbation of psoriasis after COVID-19, n (%)	21 (18.4%)
Time to onset of COVID-19 and exacerbation of psoriasis, (mean ± SD)	11.83±9.59 days
COVID-19 related hospitalization, n (%)	12 (10.5%)

COVID-19: Coronavirus disease 2019, IQR: Interquartile range, SD: Standard deviation. Exacerbation of psoriasis after vaccines was reported in 32 (13.4%) patients. Among them, 24 (75%) patients received the Pfizer-BioNTech COVID-19 vaccine

114 (44.4%) patients had been diagnosed with COVID-19 whereas, 143 (55.6%) patients never had the infection. No statistically significant difference was observed between patients who had been diagnosed with COVID-19 and those who never had COVID-19 in age, gender, psoriasis duration, having an accompanying disease, psoriatic arthritis, hypertension, and diabetes (p=0.660, p=0.154, p=0.254, p=0.843, p=0.093, p=0.194, p=0.726, respectively). 21 (18.4%) patients stated exacerbation of psoriasis after COVID-19, however, 93 (81.6%) patients stated no effect of COVID-19 on psoriasis. The mean time between the onset of COVID-19 and exacerbation of psoriasis was 11.83±9.59 days (range: 1-30 days). Exacerbation of psoriasis after COVID-19 was detected in 6 (37.5%) patients who reported exacerbation of psoriasis after COVID-19 vaccines and in 11 (12.5%) patients who reported no effect of vaccines on psoriasis (p=0.013). Besides psoriasis, none of the patients developed any skin lesions which might be associated with COVID-19. No statistically significant difference was observed between patients who had been diagnosed with COVID-19 and those who never had COVID-19 in psoriasis treatment such as biological agents and conventional medication, in biological agents received such as anti-TNF-α, anti-IL-17, anti-IL-12/23 and anti-IL-23 agents and in vaccination status or vaccine type (p=0.845, p=0.274, p=0.452, p=0.694, respectively). 12 (10.5%) patients were hospitalized for COVID-19. None of the patients required

treatment in an intensive care unit. No statistically significant difference was observed between patients who were hospitalized and did not require hospitalization for COVID-19 in age, gender, psoriasis duration, having an accompanying disease, hypertension, diabetes, psoriatic arthritis, treatment with conventional and biological agents, and vaccination status (p=0.462, p=0.587, p=0.972, p=0.309, p=0.615, p=0.705, p=0.473, p=0.249, p=0.309, respectively).

DISCUSSION

Vaccination of patients with psoriasis is crucial in the fight against COVID-19 since immunosuppressive treatment leads to predisposition to infections and psoriasis patients with comorbidities such as metabolic syndrome may be more vulnerable to severe COVID-19 [11]. Therefore, vaccination against COVID-19 is recommended for all psoriasis patients without vaccine allergies, especially for those who receive immunosuppressive treatment [12]. However, it has been suggested that safety concerns might lead to decreased vaccination rates in patients with psoriasis [13,14]. In the United States, Gondo et al. reported that 35.9% of patients with psoriasis were unlikely to get the COVID-19 vaccine. Gondo et al. reported no association between age, gender, having comorbid disease, and being likely to get the COVID-19 vaccine [15]. Sotiriou et al. stated that younger patients with psoriasis, females, patients under biological agent treatment, patients with diabetes, chronic obstructive pulmonary disease, and malignancy had positive beliefs towards COVID-19 vaccines. Concerns about the safety and effectiveness of vaccines and the risk of the exacerbation of psoriasis led to unwillingness to vaccination among patients with psoriasis [16]. Wang et al. reported that 68.9% of patients with psoriasis were vaccinated against COVID-19 in China. Young age, anti-IL-17 treatment, psoriatic arthritis, pustular psoriasis, erythrodermic psoriasis, anxiety, and depression were related to unvaccinated patients [13]. Within this study, 92.6% of the patients with psoriasis were vaccinated. Unvaccinated patients were younger than those who were vaccinated (p=0.010). In addition, most (73.3%) of the psoriasis patients who were unvaccinated or received only a single dose of vaccine had no accompanying systemic disease. Vaccination with Pfizer-BioNTech was more common in younger patients and patients with shorter psoriasis duration compared to vaccination with Sinovac-CoronaVac COVID-19 vaccine (p=0.023, p=0.017, respectively). However, vaccination with Sinovac-CoronaVac was more common in patients with diabetes or at least one accompanying disease compared to vaccination with the Pfizer-BioNTech COVID-19 vaccine (p=0.007, p=0.002, respectively).

Despite the importance of vaccination, data on the safety of COVID-19 vaccines in patients with psoriasis are scarce [17]. New onset and exacerbation of psoriasis have been reported following the administration of COVID-19 vaccines [18]. COVID-19 vaccines have been associated with plaque, guttate, pustular, nail, and erythrodermic psoriasis [5,18-21]. However, the effect of COVID-19 vaccines on psoriasis has not been clearly identified [11]. Wang et al. reported deterioration of psoriasis in 21% of patients and rash and itching in 4% of patients after COVID-19 vaccination [13]. El-Qushayri et al. reviewed the published data on patients who experienced flare-ups of psoriasis due to COVID-19 vaccines. The flare-up of psoriasis was reported in 51 patients, 55% of whom were female after Pfizer-BioNTech, Oxford-AstraZeneca, Moderna, Sinovac-CoronaVac and Covaxin COVID-19 vaccines [17]. Wu et al. reviewed the data on 279 patients with flare-ups of psoriasis following COVID-19 vaccines. Wu et al. reported slight female predominance and plaque psoriasis as the most commonly observed psoriasis type. The time to onset of the flare was 1 to 90 days and the Pfizer-BioNTech COVID-19 vaccine was the most commonly implicated one [22]. Within this study, 32 (13.4%) patients reported exacerbation of psoriasis after COVID-19 vaccines. Exacerbation of skin lesions was the most common finding (87.5%) followed by generalized skin lesions (9.4%) and exacerbation of nail involvement (3.1%). Pfizer-BioNTech COVID-19 vaccine was implicated in most of the cases and the median time to onset of symptoms after COVID-19 vaccination was 15 (25.25) days. Urticaria, papules, and pustules on soles and COVID arm were the other cutaneous side effects detected after the administration of the Pfizer-BioNTech COVID-19 vaccine.

Moreover, COVID-19 has been implicated in the exacerbation and onset of psoriasis [23]. It has been suggested that the synthesis of cytokines such as TNF-α, IL-17, and IL-23 due to activation of toll-like receptor 3 in patients with COVID-19 might trigger psoriasis [23]. For instance, Rouai et al. reported a patient with guttate psoriasis which was started 5 days after recovery from COVID-19 [23]. Shah et al. reported exacerbation of psoriasis following COVID-19 in 3 patients who were well-controlled under treatment with ustekinumab, apremilast, and risankizumab [4]. Erythroderma has also been reported in patients with psoriasis due to COVID-19 [24-26]. Furthermore, concerns have been raised on whether immunosuppressive treatment in patients with psoriasis might negatively affect the course of COVID-19, especially in those with comorbidities [27]. Kridin et al. reported no difference in the risk for COVID-19 and hospitalization and mortality due to COVID-19 in patients with psoriasis treated with IL-17 inhibitors compared to patients who received methotrexate or non-systemic treatments [27]. Yan et al. reported COVID-19 in 33.5% of 173 patients with psoriasis and psoriatic arthritis. Requirement of hospitalization and intensive care stay was detected in 19% and 10% of patients, respectively. Yan et al. suggested that hypertension was associated with severe COVID-19 whereas, treatment with IL-12/23 and IL-23 inhibitors led predisposition to COVID-19. However, methotrexate, apremilast, TNF-α, and IL-17 inhibitor treatment did not affect COVID-19 outcomes [28]. Nevertheless, a meticulous approach is recommended in psoriasis patients treated with biological agents [29]. Within this study, 114 (44.4%) patients had been diagnosed with COVID-19. No association was detected between COVID-19 and age, gender, having an accompanying disease, psoriasis treatment such as biological agents and conventional medications, COVID-19 vaccination status, and vaccine type ($p=0.660$, $p=0.154$, $p=0.843$, $p=0.845$, $p=0.452$, $p=0.694$, respectively). 21 (18.4%) patients stated that COVID-19 exacerbated their psoriasis and the mean time to onset of symptoms was 11.83±9.59 days. Exacerbation of psoriasis after COVID-19 was more frequent in patients who reported exacerbation after COVID-19 vaccines. This result may indicate a common mechanism of action of both COVID-19 and COVID-19 vaccines on psoriasis flare. Hospitalization for COVID-19 was required in 12 (10.5%) patients. No association was detected between the requirement of hospitalization and age, having an accompanying disease, treatment with conventional or biological agents, and vaccination status ($p=0.462$, $p=0.309$, $p=0.249$, $p=0.309$, respectively).

CONCLUSION

The rate of vaccination against COVID-19 in patients with psoriasis was 92.6%. Besides the high vaccination rate, unvaccinated patients with psoriasis were at a younger age. Most (44.4%) of the patients were vaccinated with the Pfizer-BioNTech COVID-19 vaccine. However, age, psoriasis duration, having an accompanying disease and diabetes affected the preferred vaccine type. 13.4% of the patients stated exacerbation of psoriasis after vaccination which more commonly occurred after the administration of the Pfizer-BioNTech COVID-19 vaccine. COVID-19 exacerbated psoriasis in 18.4% of the patients. Nevertheless, having an accompanying disease and treatment with conventional medications or biological agents were associated neither with COVID-19 nor hospitalization due to COVID-19. Our study is noteworthy in that it included psoriasis patients vaccinated with both Pfizer-BioNTech and Sinovac-CoronaVac COVID-19 vaccines. The results will hopefully contribute to the literature on the effect of COVID-19 and COVID-19 vaccines on patients with psoriasis, which has not been clearly elucidated yet.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.
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Authors' contribution

Conceptualization, F.T., Y.A., A.B.A.; methodology, F.T.; software, F.T.; validation, F.T., Y.A., A.B.A.; formal analysis, F.T.; investigation, F.T., Y.A.; resources, F.T., Y.A., A.B.A.; data curation, F.T., Y.A., A.B.A.; writing—original draft preparation, F.T.; writing—review and editing, F.T., Y.A., A.B.A.; visualization, F.T.; supervision, A.B.A.; project administration, F.T., Y.A., A.B.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2022-700).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Effectiveness of Pre-procedural Psycho-education on Pain Perception and Anxiety in Women Undergoing Colposcopy

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Abstract: *Cervical cancer is a major cause of morbidity and mortality among women. It is the fourth most commonly diagnosed cancer in females. Screening strategies such as colposcopy can cause anxiety, discomfort, and pain in patients during this procedure and poor quality of life. The aim of this study was to evaluate the effect of psychoeducation on pain perception and anxiety in women undergoing colposcopy. This randomized clinical trial included 60 women, candidates for colposcopy referred to the surgical clinic of Kashan, Iran. The patients were randomly assigned into two groups: the experimental group, which received 60 minutes of psychoeducation, and the control group. Data were collected with the Spielberger anxiety inventory and visual analog score of pain and were analyzed using independent and dependent t-tests, Chi-square, and co-variance performed with SPSS version 16. The means of anxiety scores were significantly decreased post-psychoeducation in the intervention group, and the differences between the two groups were statistically significant in the anxiety scores ($P < 0.0001$). A significant difference was observed between the mean anxiety scores of the intervention group prior to and after the psychoeducational program (PEP) intervention (99.50 ± 5.28 vs. 84.89 ± 8.80) ($P < 0.0001$). No significant changes were observed in the control group pre- and post-psychoeducation ($P > 0.05$). There was no statistically significant difference between the mean pain score between the study and the control group at the end of the study ($P = 0.17$). Psychoeducation resulted in reduced anxiety in women undergoing colposcopy. Therefore, it is recommended to consider this intervention as a suitable and effective complementary, non-invasive, low-cost method to reduce anxiety in these patients.*

Keywords: *anxiety; colposcopy; pain; psychoeducation, woman*

INTRODUCTION

Cervical cancer is a major cause of morbidity and mortality among women. It is the fourth most commonly diagnosed cancer in females [1]. According to global estimates from 2008, there are approximately 530,000 new cases of cervical cancer and it is responsible for 275,000 deaths annually [2]. Screening strategies such as colposcopy have successfully decreased cervical cancer incidence and mortality [3]. Colposcopy is the visual inspection of the cervix under magnification and is one of the first steps in the diagnostic workup of women with abnormal cervical cytology [4]. Like any other medical intervention, colposcopy can cause anxiety, discomfort, and pain in patients during this procedure. High anxiety during colposcopy can lead to a more difficult procedure for the doctor and the patient, as well as a lack of follow-up for the next steps [5].

Studies have reported long-term anxiety in the majority of women after colposcopy [6]. Although in these patients increased anxiety can result in impairment of health-related quality of life [5].

Anxiety is defined as a non-specific, unpleasant feeling of fear and discomfort, associated with a perceived danger, feelings of powerlessness, and prolonged tension in preparation for the expected danger/event [7]. General anxiety caused by the possibility of having cancer can be troublesome for some patients and high levels of anxiety before colposcopy can have adverse consequences, including pain and discomfort during the procedure and a high rate of loss to follow-up various interventions such as group educational counseling, and information leaflets. Listening to music during colposcopy and video colposcopy have been used to reduce anxiety during colposcopy examinations [7-11].

A majority of the studies on PEPs have been conducted on patients with psychiatric disorders while being limitedly implemented in patients with medical disorders [12]. This study aimed to examine the effects of a PEP intervention on anxiety and pain in women undergoing colposcopy.

MATERIALS AND METHODS

This randomized controlled trial was conducted on 60 women candidates for colposcopy who were referred to the surgical clinic of Kashan, Iran, in 2019. According to previous studies, the sample size was determined to be 30 patients in each group. The patients were recruited into the study consecutively. The subjects who met the inclusion criteria were randomly allocated into experimental and control groups through a randomized block sampling method. Blocking is used to ensure that comparison groups will be generated according to a predetermined ratio, 1:1. Blocking be used to ensure a close balance of the numbers in each group for every block of eight participants, for example, four would be allocated to each arm of the trial

The inclusion criteria were the age of 20- 50 years, anxiety scores higher than 30, no history of colposcopy, absence of psychological disorders, and recent use of anti-anxiety drugs. The exclusion criteria were the occurrence of any acute or urgent medical or psychological upheaval, and the patient's decision to leave the study. A written informed consent was obtained from each participant.

Procedure

In addition to routine medical care, the experimental group received PEP intervention, which included discussions and training materials on skills for coping with anxiety and pain, with an emphasis on muscular relaxation techniques, as presented in Box 1. The STAI inventory was assessed before and after the PEP session in the intervention group.

Patients in the control group only received routine medical care, plus a training pamphlet from the patient care and health info (colposcopy) containing general information about the colposcopy. These patients answered the STAI inventory at the beginning of the recruitment and again after studying the pamphlet.

Both the intervention and control group's pain was assessed using the VAS scale after colposcopy.

Box 1: Content of the Psychoeducation session

Timing
5 to 10 minutes
Introducing the participants to each other, presenting the objectives of the procedure, and listening to the participants' feelings and problems.
10 minutes
Providing general information about the pap-smear, colposcopy, and its importance in cervical cancer prevention
5 minutes
Introducing the medical staff and providing information about the way the procedure is done by using educational photos, slides, and videos.
30 to 40 minutes
emotional support, through empathy, congruence, and unconditional positive acceptance and trying to encourage the patients in times of stress and improve their tendency to accept the importance of the procedure to decrease anxiety, Relaxation training and pain reduction techniques
10 minutes
informing the patients about possible side effects of the procedure

Instruments

A three-part instrument was used in this study. The first part contained questions about socio-demographic characteristics (the patient's age, marital status, family history of cancer, and delivery history). The second part of the instrument included a section on sociodemographics (such as educational level, employment status, parity, and smoking), State-Trait Anxiety Inventory (STAI), and VAS.

State-Trait Anxiety Inventory (STAI)

The State-Trait Anxiety Inventory (STAI) measures how a person feels in a certain situation at a certain period. It consists of 40 statements, and subjects are asked to indicate how they felt "at the moment", using a 4-point scale ranging from "not at all" to "very much so". The test is split into the State Anxiety Scale which is considered a subjective and temporary strain and the Trait Anxiety Scale (T-Anxiety) which evaluates relatively stable aspects of anxiety, including general states of calmness, confidence, and security. Total scores for each score obtained from the STAI range from 20 to 80. In this study, we classified the score into three categories: less than 40, low anxiety; between 40 and 60, average anxiety; and greater than 60, high anxiety class [13]. In the study, the reliability of the Persian version of STAI was determined from Cronbach's alpha which was 0.94 for the overall score [14]. The third part of the instrument was the VAS scale.

The VAS is a scale that measures the intensity of acute pain. It is a trustable, valid, and useable asset of measurement of pain in repeated measurements. The VAS is a scale of about 10 cm in length, and it can be utilized in a horizontal or vertical fashion. It starts with the "no pain" and ends with the "unendurable pain" definitions. The patient is asked to point out the choice of his/her perception of the intensity of his/her pain between two endpoints. The distance between the marked point and the outset of the line (0: no pain) is measured in centimeters and the result indicates the intensity of pain in a numeric fashion [15].

Ethical Considerations

Permission for this study was obtained from the ethics committee of the University of Medical Sciences. Ethical issues in this study involved the assurance of confidentiality and the anonymity of the participants. All participants were informed of the purpose and design of the study and understood that their participation was voluntary. Participants signed a written informed consent form before their participation.

Data Analysis

Data analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The normal distribution of the study data was tested with Shapiro-Wilk. The Student's t-test was performed for the analysis of the normally distributed permanent variants. The mean values were expressed using central tendency indicators: mean, median, standard deviation, 95% confidence interval of the mean, and range. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 60 patients participated in the study. Eighty-nine percent of the women in the intervention group and 93% of the women in control group were married. The mean participant age was 39.19 ± 6.28. The majority of patients had a history of childbirth and had children and had a normal vaginal delivery (67.9% in the intervention group and 53.6% in the control group). The mean age in the intervention group was 39.07 years, the majority of whom had a diploma and university education (42.9%), while the mean age in the control group was 39.31 years, and the majority (44.8%) had a secondary and high school education. Twenty-five percent of the intervention group and 34.5 % of the control group reported a history of cancer in family members and friends. There were no significant differences between the intervention and control groups in age, marital status, education, family history, or parity.

The findings of the present study showed that PEP reduces anxiety levels in women undergoing colposcopy. Of the total participants, two in the intervention and one in the control group were excluded from the analysis due to incomplete answers on the post-test questionnaire. In total, the data from 57 patients were analyzed (Figure 1).

Figure 1: Study's data

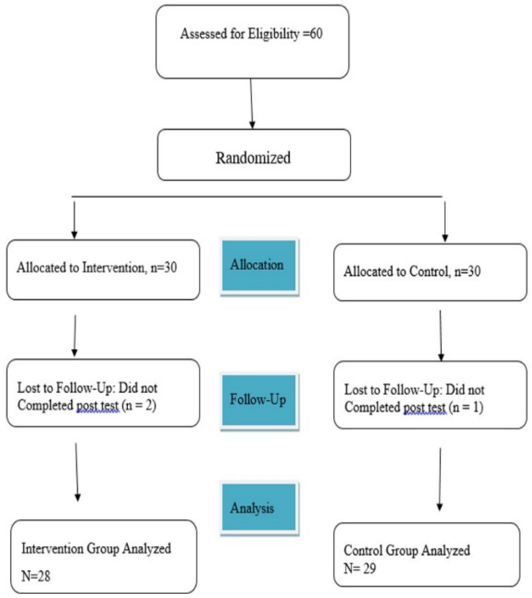
Assessed for Eligibility =60
Randomized
Allocated to Intervention, n=30
Allocation
Allocated to Control, n=30
Lost to Follow-Up: Did not Completed post test (n = 2)
Follow-Up
Lost to Follow-Up: Did not Completed post test (n = 1)
Analysis
Intervention Group Analyzed N=28
Control Group Analyzed N= 29

Table 1: Demographic composition of the two randomly assigned groups

	Control	Intervention
Age*	39.31±6.27	39.07±6.51
Marital status		
Married	27 (93.1)**	25 (89.3)
Divorced/widow	2 (6.9)	3 (10.7)
Education		
Primary	7 (24.1)	8 (26.8)
Secondary	13 (44.8)	8 (26.8)
Graduated	9 (31)	12 (42.9)
Family history		
Yes	10 (34.5)	7 (25)
No	19 (65.5)	21 (75)
Children		
Yes	28 (96.6)	28 (100)
No	1 (3.4)	0 (0)
Delivery		
Yes	28 (96.6)	28 (100)
No	1 (3.4)	0 (0)
Type of delivery		
Vaginal	15 (53.6)	19 (67.9)
Cesarean section	8 (28.6)	5 (17.9)
Both	5 (17.9)	4 (14.3)

** Mean ± Standard Deviation ** No. (%)*

No significant differences were found between the two groups in terms of socio-demographic variables (Table 1).

Analysis of covariance showed the means of overall anxiety and its attributes (state, trait) in the intervention group was significantly less than the control group at the end of the study (P<0.0001). Moreover, the t-dependent test results showed a significant decrease in overall anxiety score post-intervention (P<0.0001) while no significant decrease was seen in anxiety score in the control group at the end of the study (P>0.05). Using the independent t-test no significant differences were observed between the intervention and control groups in terms of the means of VAS score at the end of the study (Tables 2, 3).

Table 2: Comparison of anxiety scores between and within two groups

Anxiety		Intervention group	Control group	p-value*
State	Before	50.86 ± 3.99	49/79 ± 3.56	0.0001
	After	43.78 ± 3.94	48.24 ± 3.58	
	p-value**	0.0001	0.0001	
Trait	Before	48.64 ± 3.58	49.79 ± 2.66	0.0001
	After	41.11 ± 4.05	48.93 ± 3.68	
	p-value**	0.0001	0.3	
Total	Before	99.5 ± 5.28	99.57 ± 5.38	0.0001
	After	84.89 ± 6.37	97.17 ± 5.56	
	p-value**	0.0001	0.11	

Table 3: Comparison of pain score at the end of the study in two groups

	Control group		Intervention group			
	mean	SD	mean	SD	t	P value
Pain	3.60	2.05	2.75	2.36	-1.45	0.15

DISCUSSION

This randomized trial showed that psychoeducation based on providing information and emotional support in a psychotherapeutic atmosphere and relaxation techniques before a colposcopy reduced anxiety among the participants. This finding is consistent with the results of Lungulescu, who studied the effects of PEP intervention on anxiety in women undergoing their initial breast cancer screening mammography [13]. Azogh et al. studied the effect of Psychoeducation on anxiety in subsequent pregnancy following stillbirth [16]. Ozturk et al. studied the effect of psychoeducation on anxiety and pain in patients with mastalgia [15]. Sancak et al have also reported that providing verbal and written information reduces anxiety in patients during third molar extraction [17]. PEPs combine methods of relaxation with psychological training, such as trying to convince patients to accept the importance of the procedure and muscle relaxation and pain relief techniques. Several studies have used single methods, such as anger management or relaxation, and reported positive effects. For instance, a systematic review study conducted confirmed the positive effect of relaxation techniques on reducing anxiety and depression among older adults [18]. Some studies [19, 20] used relaxation and distraction in patients with cardiac disorders and post-cardiac surgery and reported that these methods were effective in reducing patients' anxiety. However, some studies that used PEPs did not find any positive significant effects. This could be attributed to differences in the content and techniques used or to the methods. For example, Howells et al. provided information leaflets before colposcopy and Hartford et al. implemented psychological training methods through telephone contact [10,21]. It appears that PEP is more effective if it is applied in a face-to-face approach.

In general, it seems that PEP can reduce patient's anxiety by providing general information about the screening program, teaching relaxation techniques, emotional support with empathy and unconditional positive acceptance, and trying to encourage the patients in times of stress and improve their tendency to accept the importance of the procedure. It was shown with the results of this study that psychoeducation is not effective in reducing the patient's pain during the colposcopy.

Various studies have been performed on the effect of PEP on pain caused by therapeutic interventions, especially after surgery. For example, Horn et al. studied preemptive and preventive pain psychoeducation and its potential application as a multimodal perioperative pain control option [22]. Ozturk et al. confirmed the positive effects of psychoeducation on anxiety and pain in patients complaining of mastalgia [15]. Jochum et al. studied the effect of psychoeducation and physiotherapy on the reduction of chronic myofascial pain [23]. These studies showed the positive effect of psychological training on pain reduction, which contradicted the results of our study.

It seems that PEP can reduce pain and the need for analgesics after surgery by raising awareness, reducing anxiety, changing attitudes toward pain, and providing information about expected pain and how to deal with it [22,24,25].

The inconsistency of the results of the present study with the previous research may be due to differences in the nature, and severity of pain, the acuity and chronicity, and the nature of disease. Since women who are candidates for colposcopy do not experience pain before colposcopy and the procedure itself does not cause severe pain, this pain is not clinically notable.

CONCLUSION

Based on the results, it seems that PEP is a useful, effective, and economic method that can reduce anxiety. Also, considering the psychological needs of women and the positive effect of this intervention on women's psychological condition, it is recommended that PEP interventions be added to medical and nursing education curricula, and it is also recommended to study the effect of this intervention on other aspects of women's mental health as well as other medical interventions.

Conflicts of interest and sources of funding

The authors do not declare a conflict of interest. This study was extracted from the general medical thesis of Kashan University of Medical Sciences. This article was funded and supported by the Deputy of Research, Kashan University of Medical Sciences, Grant No: 2806.

Authors' contribution

Conceptualization and supervision: Z.V and M.A; Data collection, M.J; Data analysis: F.S.A; Writing-original draft and Writing-review & editing: M.A and F.S. A; Final approval: All authors.

Ethics approval and consent to participate

This study was registered in the Iranian Registry for Clinical Trials (IRCT) with registration code 20130721014086N12.

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REVIEW

Ethical Challenges for Healthcare Providers in War, an Integrative Review

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Abstract: *Background: Healthcare providers should care for everyone without discrimination based on gender, race, religion, ethnic background, and social and economic status. Therefore, considering the importance of ethical challenges in war, this study was conducted with the aim of investigating the ethical challenges of healthcare providers in war. Method: This integrative review study was conducted in the field of ethical challenges for healthcare providers in war. Using the keywords of ethics, challenge, war, and healthcare providers that were selected based on the words of the medical subject heading (Mesh); a targeted search was conducted under the title and abstract in Google Scholar, ProQuest, SID, Web of Science, PubMed, Elsevier, and Scopus from 2000 to November 2022 and in Persian and English languages. Findings: twelve articles of 1160 were reviewed. Findings showed that healthcare providers face ethical challenges in war, including discrimination in care, unprofessional work, cultural and gender care, ethics versus security, and ethical decisions. Conclusion: Recognizing ethical challenges and providing solutions helps to provide better care and save people's lives in war. Developing programs for the preparation of health service providers, using existing experiences, emphasizing religious and humanitarian teachings, and holding periodical missions, can prepare health service providers to face these ethical challenges.*

Keywords: *challenge, ethics, morality, war, healthcare providers*

INTRODUCTION

Patient care is based on ethics [1]. In addition, compliance with the principles of professional ethics is the right of recipients of health care [2]. Crises and natural disasters are exceptional situations that make healthcare providers face many ethical challenges along the way in providing care. The professional competence of health care providers depends on adherence to the principles of professional ethics. The issue of professional ethics becomes more important, especially in difficult situations and natural disasters. On the other hand, healthcare providers, who play the main role in disaster-based relief, when they are in critical situations, face many ethical challenges [3].

Ethical performance is one of the main components in providing comprehensive and high-quality care to patients, especially in critical situations [4]. It is necessary to pay attention to the issues of professional ethics, especially in times of crisis and natural disasters, because the provision of health care in a normal situation is different from a crisis, and it causes more ethical responsibilities [5].

Wartime care is an undeniable reality in current clinical practice. Being at war situation can happen to anyone, including care providers [6]. The review and comparison of various studies regarding the experiences of healthcare workers during the war show that most of the results of these studies indicate the continuous and self-sacrificing efforts of this group in providing medical services to the wounded and injured despite the enormous pain and suffering they endure [7]. Health service providers are obliged to take care of everyone without discrimination based on gender, sexual orientation, race, religion, ethnic background, and social and economic status. This standard is true in all the countries of the world and is expressed in rituals such as the code of ethics for nurses of the International Council of Nurses [8].

According to the declaration of the World Medical Association (WMA) published in 2012, medical ethics in wartime is the same as medical ethics in peacetime, and adherence to medical ethics standards in wartime and peacetime is essential [9]. Since healthcare personnel are also present in the war zone along with military personnel, and they work in different environments such as battlefield field hospitals and air transport and evacuation, they face very important ethical challenges [10]. Studies show that healthcare providers suffer many problems and hardships during the war, including moral challenges, (for example: discrimination in care, care of enemies, and safety) which have many positive and negative effects on them [11]. During the Iran-Iraq war, healthcare providers faced ethical dilemmas caused by challenges such as insufficient personnel and facilities to deal with critically ill patients when referring large numbers of wounded soldiers and insufficient information on the care of victims of chemical attacks [12]. The experience of moral challenges in the war zones of Iraq made health service providers face psychological distress [13].

The Syrian civil war has created an environment of violence and instability in which humanitarian health workers have repeatedly faced ethical challenges that have resulted in ongoing threats to personal safety and lasting psychological trauma. Challenges include disruption of services during an attack, relocation or reconstruction of health facilities, limited access to resources, equipment, or medical supplies, lack of qualified personnel, dealing with armed groups, working with local communities, gaining organizational support for decisions difficult, deciding to stay despite the risks, facing discrimination based on gender and experiencing distress and internal conflict [14]. In the Lebanon war in 2006, ethical challenges were experienced including the protection of employees, the continuation of normal treatment of patients, and the protection of patients by health care providers [15]. In the war in Bosnia and Serbia, the health care providers of The Netherlands, who were stationed in the conflict centers, faced ethical challenges in providing care to the wounded, such as discrimination in care and conflict with military duties [16].

There are certainly cases of terrible or tragic behavior during war that many ordinary citizens, and most military members, do not remember with pride or approval. The My Lai massacre during the US war in Vietnam, prisoner abuse at the Abu Ghraib prison in Iraq, “saturation” or “terror bombing” of German cities by Allied forces toward the end of World War II, the use of nuclear weapons against civilian targets in Japan, the killing or severe mistreatment of prisoners of war by all sides in the Atlantic and Pacific theaters during World War II. The list is not a short one. The preparation of healthcare providers to face moral challenges in unusual conditions and to use appropriate strategies during war is very important [10].

Therefore, due to the importance of ethical challenges in war zones for healthcare providers and Less attention is paid to this issue, in this study, we decided to investigate the ethical challenges of healthcare providers in war.

It should be noted that today we are facing wars in different parts of the world and healthcare providers in the context of these wars are facing many ethical challenges and our information about them is very limited. Since there is an information gap in the healthcare provider's ethical challenges in war, this study was conducted with the aim of investigating the ethical challenges of healthcare providers in war. The question of this study: What are the ethical challenges that healthcare providers face in war?

MATERIALS AND METHODS

Because the methodology of the reviewed studies was different and unsuitable for statistical analysis, an integrated review method was used. This integrated study was carried out with the help of the guidelines provided in Hanna Snyder's article [17]. An integrative review is a method that collects and synthesizes the knowledge generated through the analysis of results extracted from selected studies. This study was carried out in the following steps: Setting the research question. Sampling or selection of studies for review; Introduction of reviewed studies; Analysis of selected studies; Interpretation of results and final report [18].

The research question was: "What ethical challenges do health service providers face in war?" In this regard, the researchers searched native and international databases such as SID.ir, Iranmedex, PubMed, Web of Science, Scopus, and Google Scholar, with the keywords War, Healthcare Providers, Challenge, morality, and ethics in the years 2000 to 2022. For Iranian databases, keywords were searched in Farsi and English. In this way, related articles in this field with quantitative and qualitative approaches are extracted. Gray sources, such as unpublished results, dissertations, and articles published in sources with low credibility, were excluded due to their insufficient credibility (Table 1).

The criteria for entering the study were: relevance and continuity in the subject of study, publication of the article in Persian and English language, and the availability of the full text of the article; The criterion for selecting the article after the search was the relevance to the article context for the question from the study. At first, 1160 articles were extracted, and after evaluation by the research group based on the inclusion criteria, 12 articles were studied and analyzed by removing irrelevant and duplicate articles. Figure 1 describes the PRISMA method in extracting the target articles.

Table 1: Example of the search strategy in the PUBMED database

Query	Sort By	Filters	Search Details	Results
((((health care provider) AND (war)) AND (morality)) AND (ethics)) AND (challenge)	Publication Date	from 2000 - 2022	(("health personnel"[MeSH Terms] OR ("health"[All Fields] AND "personnel"[All Fields]) OR "health personnel"[All Fields] OR ("health"[All Fields] AND "care"[All Fields] AND "provider"[All Fields]) OR "health care provider"[All Fields]) AND ("armed conflicts"[MeSH Terms] OR ("armed"[All Fields] AND "conflicts"[All Fields]) OR "armed conflicts"[All Fields] OR "war"[All Fields]) AND ("morale"[MeSH Terms] OR "morale"[All Fields] OR "morales"[All Fields] OR "moralism"[All Fields] OR "moralities"[All Fields] OR "moralization"[All Fields] OR "moralize"[All Fields] OR "moralized"[All Fields] OR "moralizing"[All Fields] OR "morally"[All Fields] OR "morals"[MeSH Terms] OR "morals"[All Fields] OR "moral"[All Fields] "morality"[All Fields]) AND ("ethic s"[All Fields] OR "ethicality"[All Fields] OR "ethically"[All Fields] OR "ethics"[MeSH Terms] OR "ethics"[All Fields] OR "ethic"[All Fields] OR "ethics"[MeSH Subheading] OR "morals"[MeSH Terms] OR "morals"[All Fields] OR "ethical"[All Fields]) AND ("challenge"[All Fields] OR "challenged"[All Fields] OR "challenges"[All Fields] OR "challenging"[All Fields])) AND (2000:2022[pdat])	34
(((health care provider) AND (war)) AND (ethics)) AND (morality)	Publication Date	from 2000 - 2022	(("health personnel"[MeSH Terms] OR ("health"[All Fields] AND "personnel"[All Fields]) OR "health personnel"[All Fields] OR ("health"[All Fields] AND "care"[All Fields] AND "provider"[All Fields]) OR "health care provider"[All Fields]) AND ("armed conflicts"[MeSH Terms] OR ("armed"[All Fields] AND "conflicts"[All Fields]) OR "armed conflicts"[All Fields] OR "war"[All Fields]) AND ("ethic s"[All Fields] OR "ethicality"[All Fields] OR "ethically"[All Fields] OR "ethics"[MeSH Terms] OR "ethics"[All Fields] OR "ethic"[All Fields] OR "ethics"[MeSH Subheading] OR "morals"[MeSH Terms] OR "morals"[All Fields] OR "ethical"[All Fields]) AND ("morale"[MeSH Terms] OR "morale"[All Fields] OR "morales"[All Fields] OR "moralism"[All Fields] OR "moralities"[All Fields] OR "moralization"[All Fields] OR "moralize"[All Fields] OR "moralized"[All Fields] OR "moralizing"[All Fields] OR "morally"[All Fields] OR "morals"[MeSH Terms] OR "morals"[All Fields] OR "moral"[All Fields] OR "morality"[All Fields])) AND (2000:2022[pdat])	232

Figure 1: PRISMA diagram in extracting articles

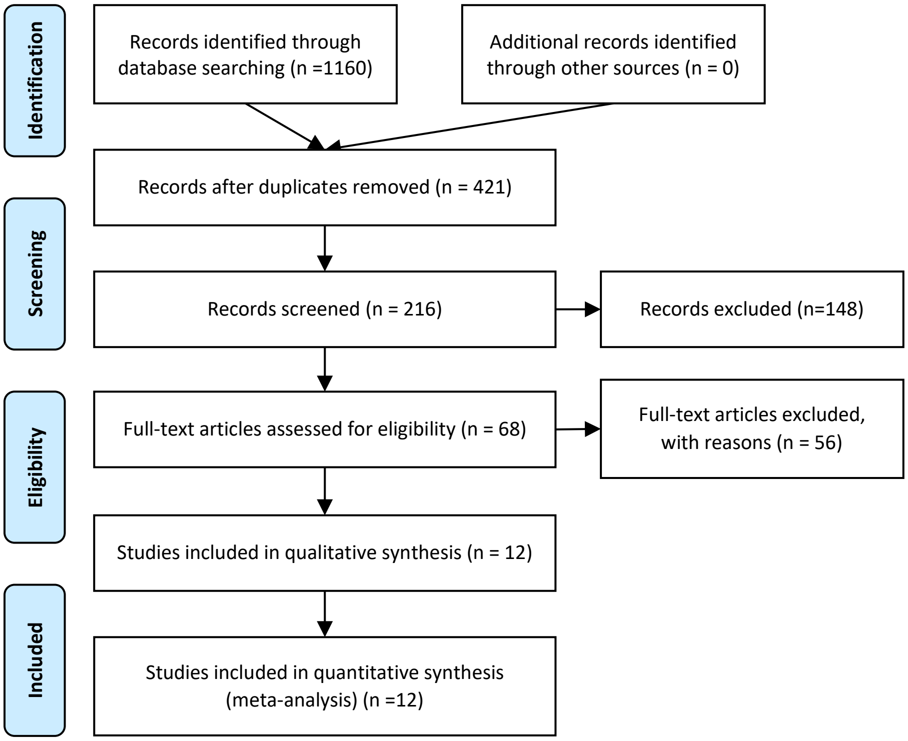
Identification
Records identified through database searching (n =1160)
Additional records identified through other sources (n = 0)
Records after duplicates removed (n = 421)
Screening
Records screened (n = 216)
Records excluded (n=148)
Eligibility
Full-text articles assessed for eligibility (n = 68)
Full-text articles excluded, with reasons (n = 56)
Studies included in qualitative synthesis (n = 12)
Included
Studies included in quantitative synthesis (meta-analysis) (n =12)

RESULTS

From 1160 articles that obtained in the first stage by searching the key words mentioned, after removing the articles not related to the subject of the study and complying with the inclusion and exclusion criteria, finally 12 articles that related to the purpose of the study examined. The findings of this study showed the ethical challenges of health service providers in war zones in terms of: Discrimination in care, Unprofessional work, Cultural and Gender care, Ethics versus Security and Ethical Decisions. A summary and complete description of the reviewed studies showed in the Table 2. This review summary includes authors, year of publication, methodology and results (Table 2).

Table 2: Final articles used in the study

Title	Authors	Journal	Year	Method	Ethical Challenges
Army nursing practice challenges in humanitarian and wartime missions [19]	Janice Agazio	International Journal of Nursing Practice	2010	Descriptive exploratory design	Use and/or disposal of medications while caring for local nationals The dilemma of taking care of coalition forces or local wounded
Making the hard decisions: Ethical care decisions in wartime nursing practice [8]	Janice Agazio, Petra Goodman	Nursing outlook	2017	Secondary analysis	Management of ethical situations in war Use of recognized ethical codes Ethical decision making
Military Nursing Care of Iraqi Patients [13]	COL Petra Goodman, et al	Military Medicine	2013	Focus groups interview	Bias in providing care to Iraqi patients. lack of trust in translators, Discomfort from not being able to guarantee
The challenges and psychological impact of delivering nursing care within a war zone [20]	Alan Finnegan, William Lauder, Hugh McKenna	Nursing outlook	2016	Constructivist grounded theory	The challenge of caring for prisoners of war or the wounded in the army
Lived experiences of military nurses from war - a mini-review [10]	Fateme Teymoury, Zahra Farsi	Journal of Military Medicine	2019	Mini-review	Discrimination in providing care for the injured. Ethical challenges in facing the native culture and cultural differences
Military physicians' ethical experience and professional identity: a Canadian perspective [21]	Christiane Rochon	JRAMC	2018	Miles and Huberman's mixed approach	Ethical challenge in caring for indigenous people in war-torn areas The challenge of using limited facilities in dealing with the injured Safety of injured or employees
Experiences of military nurses in Iraq and Afghanistan [22]	Louisa Jane Kenward. Gary Kenward	Nursing standard	2015	Review	The challenge of choosing the injured due to limited facilities. The challenge of caring for a wounded enemy
Women's Health and Hygiene Experiences During Deployment to the Iraq and Afghanistan Wars, 2003 through 2010 [23]	Mary Ellen Doherty, Elizabeth Scannell-Desch,	Journal of Midwifery & Women's Health	2011	Phenomeno-logical	Providing cultural and gender care Provision of support for female employees and privacy protection Moral protection of employees
Ethical Issues Encountered by Military Nurses During Wartime [24]	Janice Agazio, et al	Annual review of nursing research	2016	Review	Feelings of confusion, uncertainty, frustration, anger, discouragement and anxiety in caring for captives Ethical conflicts in care
Nursing Ethics and the 21st-Century Armed Conflict: The Example of Ciudad Juárez [25]	Kathleen O'Connor	Journal of Transcultural Nursing	2015	Review	Personal safety and time to evacuate a situation Caring for both sides without bias, Witnessing suffering and intervening in torture. Support and defend the rights of others. Fixing conditions that harm others, Helping the disabled and saving people in danger.

'We will never give up': a qualitative study of ethical challenges Syrian health workers face in situations of extreme violence [14]	Namrita S. Singh et al	Disasters	2002	Qualitative	Service disruption during attack. Moving or renovating health centers. Limited access to medical resources, equipment, or supplies. Lack of qualified personnel, dealing with armed groups. Cooperation with local communities. Obtain organizational support for difficult decisions. Decided to stay despite the risks. Facing discrimination based on gender, and experiencing distress and internal conflict.
Legal framework versus moral framework: military physicians and nurses coping with practical and ethical dilemmas [16]	Francesca Baukje Hooft	J R Army Med Corps	2019	Personal view	Discrimination in providing care when facilities are lacking Care in unsafe conditions Taking care of the wounded enemy Facing anger, rage and shame.

DISCUSSION

The purpose of this study was to consolidate the results of studies related to the ethical challenges of healthcare providers in war. Ethical challenges of healthcare providers in war mentioned in the areas of discrimination in care, unprofessional work, cultural and gender care, ethics versus security and ethical decisions.

Discrimination in care

Discrimination, in the non-moralized sense, is ubiquitous. Discrimination is a surprisingly slippery concept, and much of the attention it has received in recent years has focused on constructing a precise definition of the term. At least in part, this may be because the concept appears to cover such a wide variety of disparate cases crossing traditional boundaries in applied ethics and political theory, from distributive justice, medical ethics, and criminal justice to multiculturalism, liberal rights, and feminism [26].

Everyone is entitled to human rights without distinction of any kind, such as nationality, race, religion, age, gender, sexual orientation, economic background, or disability. Even if humanitarian workers are trying to do things well, they usually cannot do everything that is needed. In most situations, humanitarian organizations are faced with rationing problems difficult choices about exactly who they can help and what they can do. With limited resources and typically widespread needs, organizations have to focus. To focus responsibly means finding a need that one's agency is good at meeting—this may be health, food, or protection—and maximizing their programs in some way so that as many people benefit as possible [27].

When supplies are short, who is first in line? Health service providers at war environment always faced by a lack of medical facilities due to the special conditions prevailing in war. Using these limited facilities to save the lives of many people who injured in the war environment is one of the ethical challenges of health service providers. These challenges will be more difficult when caring for enemies wounded, prisoners of war and indigenous people. In addition, the decision to save a person in a critical situation, which may be accompanied by neglecting to save sicker or dying people, is a challenging situation for health service providers [13,19, 20,22].

The evidence indicates that the health service providers faced by a series of moral dilemmas, including discrimination in providing care to patients, and due to political beliefs and culture, they tried to have less contact with the enemy wounded. In this regard, the results of Desch-Scannell and Doherty's study showed that nurses did not want to provide care to the enemy wounded. In addition, the results of Lal and Spence's study showed that due to the limitations of medical equipment, human and technical resources, nurses had to perform differently than the injured and make difficult decisions about providing care to people. Limitation of resources in providing care knew as one of the ethical challenges of health service providers in war [10,14,16,21]. The decision to save the seriously injured for whom there is no hope for a healthy life or the wounded who are at risk of death and their care in war conditions lead to a waste of limited resources is one of the ethical challenges of health service providers [8,14,21].

Discrimination is sometimes the product of what the discriminator takes to be a fundamental distinction between classes of people. For example, placing a lesser value in war on the life of an enemy than on a country's own citizens is typically premised on a belief in a basic distinction between friend and foe, or between citizens and non-citizens. When prisoners captured they should receive treatment as prisoners of war, unless they are known to have committed criminal acts on the battlefield in contravention of the laws of war. They have a right to self-defense against other combatants, unlike terrorists, and their use of deadly force against their enemy is not considered murder [28]. International laws, say they should not, for example, be tortured during questioning. Nor can they be beaten, starved or somehow medically experimented on. They cannot be used as shields between oneself and the opposing side; the understanding is that captured enemy soldiers are to be incarcerated far away from the front lines. Moreover, very basic medical and hygienic treatment is supposed to be offered: things like aspirin, soap, water, and toothbrushes [29].

Thus, caring of prisoners under torture and cooperation of healthcare providers with investigators causes many ethical challenges. Here, hard ethical problems arise when doctors, nurses, and medics are called upon to assist in interrogation or force-feed hunger-striking inmates [30]. The use of medical staff to determine the amount of torture that a person endures, the use of psychological methods to obtain information, keeping captives alive to receive information, and the use of therapeutic tools for torture are among these challenges. In these situations, emotional responses such as anger, fear, resentment, hate and prejudice are in conflict with moral requirements [24,31,32].

Unprofessional work

The quality of humanitarian action is a constant concern in humanitarian ethics. The great majority of humanitarian workers recognize that it is not enough simply to try to help people. One must help them professionally and well. As a result, there has always been a considerable effort put into standard-setting, professional guidelines, training, and competence [27]. One of the ethical challenges of health service providers in the war is the employment of people in non-specialist positions in the conditions of shortage of specialist personnel. Although in many cases this issue has caused more growth and training in health service providers, it faces people with ethical challenges in providing care that they do not have enough skills. For example, health service providers were faced with the challenge of caring for children injured in war that they may harm them during care [19,20, 24]. Furthermore, healthcare providers realized that they have not fully trained to treat a wide range of injured patients, including severe traumas, extensive bleeding and critical care, health problems, care of infants, women, children, and the elderly [13]. Because of staff shortages, participants emphasized the difficulty of taking on new roles for which they not trained. They feel forced to play multiple roles or provide care beyond their skill level. In addition, volunteers who came to hospitals without educational qualifications will cause ethical challenges [14].

Cultural considerations

Today, official declarations abound avowing the indispensability of gender inclusiveness and diversity for military effectiveness in the twenty-first-century operating environment. Today's "militaries rely more and more on women and members of visible minority and Aboriginal groups to fill their ranks, rendering the recruitment, retention, and optimum employment of these members important to the success of the organization from the perspectives of both operations and public accountability. In some units and services, women and members of nonwhite ethnic, non-Judeo-Christian religious, or non-heterosexual minorities have been integrated effectively. However, equal opportunity and diversity policies have not been uniformly successful. Oftentimes, our inability to understand other's culture is a direct cause of needless strife and deprivation. The past decade of armed conflict speaks emphatically to the increased pain and suffering that follow from our violating the cultural norms of countries that have the misfortune of playing host to militaries [30].

Taking care of an injured patient of the opposite gender

The presence of women in military teams as well as their use as health service providers in the war environment is facing issues such as moral challenges. Providing security, meeting the needs of women, maintaining privacy, preventing sexual assaults, and providing health care to injured women can be among the ethical challenges of health service providers in war. Furthermore, not paying attention to local culture in the place of war, especially in relation to women, can cause health service providers to face ethical challenges [23].

Difference in values

Another ethical dilemma in the field of cultural difference between nurses and injured people was the difference in values, especially regarding gender. Some patients believed that women's bodies should not be exposed to men, even if that man is a health service provider [10,13]. Cultural differences identified between health care providers and patients lead to ethical dilemmas. Findings indicated that health care providers had difficulty confronting cultural norms that conflicted with care delivery. Gender issues can lead to differences in care and expectations regarding female patients. Based on the male dominant role, men were the main decision-makers and could dictate the provision or care of female members or children of the family. Cultural customs in terms of social, political and economic factors are effective on ethical challenges when providing care [8,13,24]. Furthermore, women's health service providers face ethical dilemmas while taking care of men who avoid contacts with women [14].

Ethics versus security

One area of humanitarian ethics that is often uppermost in the minds of humanitarian workers in armed conflict is the level of risk they are prepared to accept for themselves and their teams as they try to help others at risk. Humanitarian workers like soldiers, policemen, and firefighters may genuinely expect to suffer and die in pursuit of their duty. Humanitarian workers have often died while doing their jobs—most usually from infectious diseases or traffic accidents. Armed actors of various kinds also deliberately kill them as humanitarian workers. The ethics of personal risk in humanitarian action is not discussed publicly very much in order to maintain the moral and legal taboo on killing humanitarian workers. However, in reality, many humanitarian workers decide to put their lives at risk every day. To do so responsibly means balancing risks against results how important is it to deliver this convoy today? It also means working with a strong duty of care towards one's staff and with their full and informed consent when it comes to engaging in risky operations [27].

In addition, one of the ethical dilemmas of healthcare providers at war environment is maintaining the safety of employees and recognizing when to leave the war environment. Sometimes health care providers have to make a difficult choice between saving someone's life and their own safety and health, because laws regarding the immunity of health care providers in war not always respected. When the order to leave an environment announced, healthcare providers may face with a lack of facilities to evacuate the injured. In this situation, they should act between staying and protecting the injured or leaving the situation according to the order [14,25].

Sometimes leaving a wounded person alone for a few minutes, for example, during the warning of an imminent attack, may endanger his life. In such a situation, the decision to go in to the shelter or stay with the injured will create a significant challenge [15,16]. Facing organizational needs in opposition to the needs of the injured, such as applying pressure from the commanders to provide service to an injured person, or releasing some of the injured, or transferring the injured who are not ready to be transferred, puts healthcare providers in a situation of ethical challenges [21].

The intervention or pressure of armed groups to leave the triage process and accepted clinical practice was one of the ethical challenges of the treatment staff. This included verbal threats, harassment, humiliation, acts of violence, and requests for preferential treatment for some patients, such as members of armed groups or their family members. Obligation to comply with laws contrary to medical requirements, requests for participation in decision-making, and intervention in medical affairs, under threat from the military, are among the ethical challenges of health service providers in war [14].

Ethical decisions

“Working to enhance national security,” writes the BMA, “may not always be compatible with the fundamental tenets of medical ethics.” This is one of the central dilemmas of military medical ethics: To whom do medical practitioners—doctors, nurses, and medics—owe their loyalty? Are they caregivers or soldiers first? Here is a dual-loyalty dilemma in the most quotidian terms: the patient’s interests or those of the military? The mission of the Army Medical Department is to conserve the fighting strength. Combat health support maximizes the system’s ability to maintain presence with the supported soldier, to return injured, sick, and wounded soldiers to duty, and to clear the battlefield of soldiers who cannot return to duty [30].

The most controversial issue was that military medical ethics based more on the expediency of returning soldiers to battle than treating the wounded, meaning that the priority was returning soldiers to duty rather than providing care to the seriously wounded. The moral challenge for many was about returning to war, including soldiers who wanted to return to combating but were judged unsuitable and those who did not want to return but were deemed fit. Health service providers considered this as a great moral responsibility both towards the patients and towards the mission [21,24].

The most troubling ethical issues cited related to conflict with physicians in treatment decisions, working with incompetent or unprofessional colleagues, and staffing patterns. Among the resources that helped nurses in dealing with ethical issues, role models, staff meetings, and physician, supervisor, and peer consultations more used. However, nurses also used professional sources (journal articles and books) and personal sources (church) [24].

CONCLUSION

The findings from this study showed that health service providers face many ethical challenges in providing health services while in war zones. Understanding these challenges in order to provide solutions to face them helps to provide better health services and save people's lives in the battle scene. One of the applications of this study for health service providers is that it informs them about the ethical challenges in the battlefield so that they can make the right decision in the event of war. Decision-making training in critical situations seems essential for healthcare providers. Healthcare providers must have adequate military training to protect themselves and the injured. Developing programs for the preparation of health service providers, using existing experiences, emphasizing religious and humanitarian teachings, and holding training maneuvers can be effective in preparing them to face these ethical challenges. Holding classes to be acquainted with the local culture of the war zone and the values governing the society before deployment is effective in reducing moral problems. Managers should consider providing mental health services to people who provided care in war zones and faced moral challenges.

Conflicts of interest and sources of funding

The authors declare that there is no conflict of interest in the present study.

Authors' contribution

GDM conceived of the presented idea. KhM, VZ contributed to documentation on the topic of interest, work conception and revised the manuscript. GDM, KhM, VZ performed the final revision of the manuscript. All authors discussed the results and contributed to the final manuscript. KhM and VZ gave final approval. All authors have read and agreed to the published version of the manuscript. PH, HS, GDM had equal contributions.

Ethical considerations

In the present study, all ethical considerations, including the condition of trust and honesty, have been taken into consideration. The present study was written in line with the results of the doctoral dissertation in nursing approved by Baqiyatullah University of Medical Sciences with the ethics code IR.MBSU.REC.13990442.

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REVIEW

Current Trends, Challenges for Research, and Therapeutic Perspectives in Nutritional Psychiatry

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Abstract: *Nutritional interventions in the field of psychiatry have been explored more intensively in the last two decades, but definitive conclusions based on the retrieved data in the literature about the efficacy of this therapeutic approach are difficult to formulate. This observation has multiple explanations, e.g., these therapeutic interventions are very heterogenous, cohort studies usually enroll different psychiatric populations or healthy subjects with minor psychiatric symptoms, nutritional supplements or special diets are commonly recommended as add-ons to the pharmacological interventions (thus making it difficult to differentiate the impact of each therapy on the outcomes), placebo-controlled randomized studies are still very few, etc. Therefore, a narrative review focused on the main challenges reported during the research in the field of nutritional psychiatry (NP) may be useful for clinicians interested in strategies to augment the efficacy of pharmacological treatments or to find new solutions with low risk of adverse events in patients with organic comorbidities or multiple concomitant medications. Data about the efficacy and tolerability of nutritional interventions explored for psychiatric disorders were searched in four electronic databases (PubMed, Cochrane, Clarivate/Web of Science, and EMBASE). Omega-3 polyunsaturated fatty acids supplementation, healthy diet interventions, folate and vitamin B12, S-adenosyl-L-methionine, N-acetyl cysteine, vitamin B1 and B6, vitamin D, minerals, psychobiotics, inositol, and herbal products were the main interventions identified, and their demonstrated efficacy was quite heterogenous, while their tolerability and safety were good. Therefore, NP is a domain of research that may benefit from more good-quality and longer-duration trials, in order to verify the effectiveness of such augmentation strategies to the already validated treatments.*

Keywords: *prebiotics, probiotics, synbiotics, vitamin D, folate, B vitamins, S-adenosyl-L-methionine, N-acetyl-cysteine, inositol, micronutrients*

INTRODUCTION

Nutritional psychiatry (NP) explores the impact of nutrient-based interventions on clinical symptoms and behavioral models of mental disorders. The number of published research in this field has continually increased during the past decades [1]. Dietary recommendations and the use of nutraceutical supplements sparked interest for psychiatrists in the management of psychiatric disorders or in the prophylactic strategies targeting the same type of disorders [1]. Nutrition has been considered able to modulate different pathophysiological mechanisms of psychiatric disorders, especially inflammation, oxidative stress, the structure of the gut microbiome, epigenetic influences, and neuroplasticity [2]. Associations between variables related to diet quality and mental health have been supported by epidemiological research, and they do not seem influenced significantly by lifestyle characteristics, demographic factors, or reverse causality [2]. There is accumulating evidence supporting the relationship between diet quality and/or feeding imbalances, on one side, and mental health, on the other [3]. Such evidence already exists for other medical specialties, such as endocrinology, cardiology, and gastroenterology [3]. Nutrient-based supplements and augmenting strategies could be of major interest to psychiatry, if proven efficient and well tolerated, because of their lack of significant pharmacokinetic and pharmacodynamic interactions with the drugs, and due to their more socially-accepted, non-stigmatizing, image as therapeutic interventions in patients with mental disorders.

The founding in the year 2013 of the International Society for Nutritional Psychiatry Research (ISNPR) reflects the importance gained by this domain in the last decade [4]. According to a position statement of this institution, diet is part of an integrative psychiatric model, and evidence-based nutraceuticals should be considered elements of case management in psychiatry [4]. The education of both clinicians and patients about evidence-based nutrition or nutraceuticals is also a key element of the ISNPR position statement [4].

Another domain of intensive exploration for nutritional psychiatry is represented by the connection between gut microbiota (GM) and the pathogenesis of mental disorders. This aspect was explored through the lens of the bidirectional signaling that was observed between the intestinal flora and the brain functioning, i.e., the microbiome-gut-axis [5]. The possibility of changing the composition of GM through probiotics, prebiotics, synbiotics, or fecal material transplantation (FMT) was considered important as an approach to psychiatric disorders [6,7]. Also, the impact of early-life nutrition and maternal diet has been investigated in connection with the onset of psychiatric disorders in offspring [1].

The results of studies investigating the efficacy of nutraceuticals in the treatment of psychiatric disorders are still far from being convincing [1]. Heterogeneity, residual confounding, measurement errors, and difficulties in quantifying and ensuring adherence to a certain diet have been considered challenges for experimental research in NP [8]. A high rate of placebo effect could be one of the factors that mitigate the difference between study groups and hinder the development of nutraceutical-oriented therapies in psychiatry [1]. Therefore, methodological aspects should be considered when interpreting the results of such trials, and the use of biomarkers, e.g., specific nutrient deficiencies, allelic variants, and metabolomic variables, should be encouraged [1]. Also, taking into consideration individual dietary patterns and nutrient necessities is important when constructing a therapeutic strategy for patients with mental disorders from the perspective of NP [1]. However, the effects of nutraceuticals are also actively investigated in different categories of psychiatric disorders and advances have been recorded in the field of mood-modifying and cognitive-improving agents in clinical populations and healthy individuals [9]. Systemic and central vascular function, inflammation, metabolism, and neuroprotection may explain the positive impact of nutraceuticals [9]. A synthesis of the methodological difficulties in finding answers to the main questions in NP is represented in Figure 1.

Figure 1: Challenges identified in the research of nutritional interventions in patients with psychiatric disorders *(SSD= schizophrenia spectrum disorders, MDD= major depressive disorder, GM= gut microbiota)*

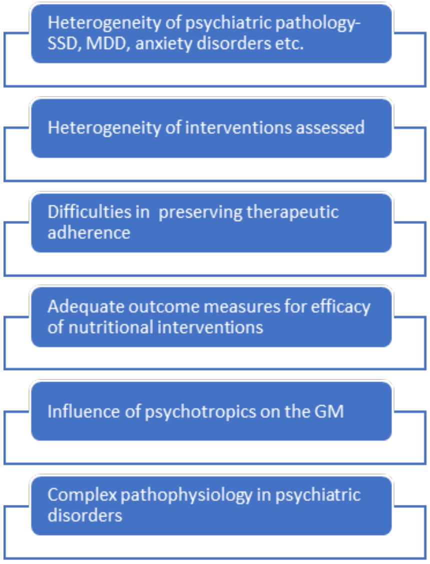
Heterogeneity of psychiatric pathology- SSD, MDD, anxiety disorders etc.
Heterogeneity of interventions assessed
Difficulties in preserving therapeutic adherence
Adequate outcome measures for efficacy of nutritional interventions
Influence of psychotropics on the GM
Complex pathophysiology in psychiatric disorders

Psychotropics may exert different effects on the GM, thus influencing the gut-brain axis [21]. Antipsychotics have been associated with anti-commensal activity, which is difficult to explain since these agents are targeting monoaminergic neurotransmission in the central nervous system (CNS) [21]. According to a complex study that observed the influence of various non-antibiotic drugs on the growth of 40 representative bacterial isolates, which can be found in the human GM, almost all antipsychotic categories present anti-commensal activity [21]. There are suppositions that direct bacterial inhibition of antipsychotics might not be an adverse event, but a potential mechanism of action attributed to these agents [21]. However, the negative effects of this antimicrobial activity have been reported, although the significance of this effect was not yet clearly demonstrated. For example, an alteration of GM by antipsychotics may result in body weight gain and metabolic dysfunctions [22].

Antidepressants, like monoamine oxidase inhibitors (MAOIs), serotonin selective reuptake inhibitors (SSRIs) and tricyclic antidepressants also have antimicrobial properties by different mechanisms, e.g., inhibition of DNA replication, blocking of the bacterial efflux pumps, etc. [23,24]. Dysbiosis induced by this type of pharmacological agents has been considered a possible contributor to the effectiveness but also to the treatment resistance in patients with major depressive disorder (MDD) [23].

OBJECTIVE AND METHODOLOGY

Because the methodology of the reviewed studies was different and unsuitable for statistical analysis, an integrated review method was used. This integrated study was carried out with the help of the guidelines provided in Hanna Snyder's article [17]. An integrative review is a method that collects and synthesizes the knowledge generated through the analysis of results extracted from selected studies. This study was carried out in the following steps: Setting the research question. Sampling or selection of studies for review; Introduction of reviewed studies; Analysis of selected studies; Interpretation of results and final report [18].

SPECIFIC INTERVENTIONS IN NUTRITIONAL PSYCHIATRY

Data supporting the influence of nutrition, as a category of interventions, on mental health are derived from meta-analyses and systematic reviews, based on randomized controlled trials and cohort studies [25]. The main nutritional interventions found during the literature review are represented in Figure 2.

Figure 2: Main nutritional interventions explored for the management of psychiatric disorders *(PUFA= polyunsaturated fatty acids, SAME= S-adenosyl L-methionine)*

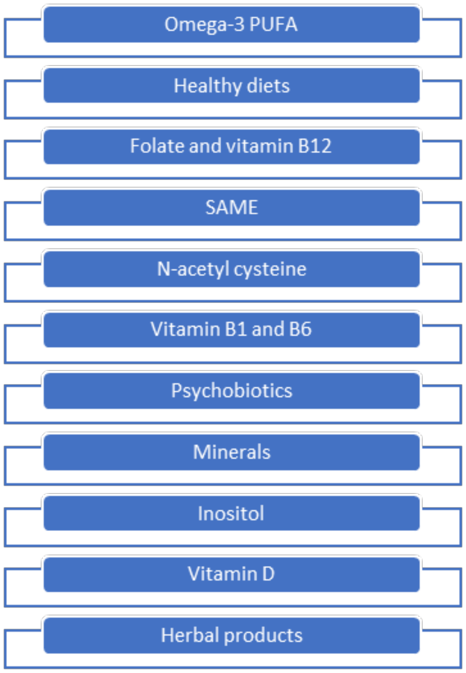

For example, a meta-review of the meta-analyses focused on nutritional interventions in the field of psychiatry (N=10951 individuals) concluded that the strongest evidence supports the use of PUFAs, especially eicosapentaenoic acid (EPA), as an adjunctive treatment for MDD, and the therapeutic potential of these interventions for ADHD; folate supplements, and especially high-dose methyl folate regimens, were associated with positive results in MDD; emergent evidence for N-acetylcysteine as an adjunctive for mood disorders and schizophrenia were reported [26]. All nutritional interventions assessed had good safety profiles, without evidence of serious adverse effects or contraindications in psychiatric disorders [26]. Another review (n=69 trials) reported the statistically significant or clinically important changes observed in the majority of the research conducted (n=48 trials) when nutritional interventions were used as supplementation for psychiatric disorders; however, the heterogeneity of the psychiatric diagnoses, clinical outcomes, and treatments assessed preclude the extraction of definitive conclusions [27].

Omega-3 polyunsaturated fatty acids supplementation

EPA and docosahexaenoic acid (DHA) are the two ω-3 PUFAs explored in the treatment of psychiatric disorders based on their anti-inflammatory role, involvement in preserving neurons' stability, and possible importance for the proper functioning of neurotransmission [28]. The American Psychiatric Association practice guideline for depression recommends ω-3 PUFAs as adjunctive therapy in patients diagnosed with MDD [29].

In a placebo-controlled study, 2.4 g/day ω-3 PUFAs were added to venlafaxine in a group of 72 patients presenting their first episode of depression [30]. After 12 weeks, both groups (active and placebo) had improvements in their clinical status vs. baseline, but the rate of responders for anxiety symptoms was significantly higher in patients who received PUFA at week 4 [30]. These results support a possible faster response in patients who received venlafaxine + PUFA regarding their associated anxiety symptoms, although the final scores did not differentiate between the two groups [30]. Also, childhood trauma and lack of social support have been associated with poor prognosis in patients with first episodes of depression in this study [30].

A double-blind, placebo-controlled trial evaluated the effects of ω-3 PUFAs (3.4 g/day) as monotherapy vs. placebo in pregnant women (N=36) with MDD during eight weeks [31]. Patients in the active intervention group had significantly lower HAMD (Hamilton Depression Rating Scale) scores at week 6 and at the endpoint, higher response and remission rates [31]. Also, the changes in the secondary outcome measures (EPDS- Edinburgh Postnatal Depression Scale and BDI- Beck Depression Inventory) supported the significant efficacy of PUFAs vs. placebo [31]. The tolerability of PUFAs was good, without adverse effects reported by either mothers or newborns [31].

A meta-analysis (n=19 trials, N=2240 participants) supported the existence of a beneficial effect of ω-3 PUFAs vs. control interventions over anxiety symptoms [32]. The subgroup analyses showed that patients with well-defined psychiatric conditions responded better to adding ω-3 PUFAs than individuals without clinical diagnoses [32]. The anxiolytic effect of ω-3 PUFAs was superior to controls in subgroups with a higher dosage (≥2 g/day) vs. a lower-dose regimen [32].

Epidemiological studies reported a correlation between reduced levels of ω-3 PUFAs or low intake of fish products and the increased risk for age-related cognitive decline or AD [33]. Also, increased dietary consumption or blood levels of DHA are protective against AD and other dementia in epidemiological studies, with a possible negative interference from the apoE4 genotype [33].

Possible mechanisms explaining the protective effects of DHA against cognitive decline and AD are the following: some of its metabolites have neuroprotective effects, there is a decrease in the production of arachidonic acid metabolites, the transduction for trophic factors is increased, and the inhibition of signal transduction pathways induced by β-amyloid is more intense [33].

The addition of ω-3 PUFAs in patients with ADHD has been explored starting from the potential benefits of these agents on oxidative stress (which can be increased in this population), inflammation (by competitive inhibition of the ω-6 PUFAs, which are pro-inflammatory), and increasing levels of dopamine and serotonin neurotransmission [34]. Meta-analyses report small to insignificant effect sizes on ADHD behavioral symptoms of ω-3 PUFAs when added to ongoing medication [35-38]. The overall tolerability of ω-3 PUFAs was good, and no severe adverse were reported in these meta-analyses [35-38]. Dyspepsia and transient nosebleeds have been the most frequently reported adverse events [34-38]. In patients with ADHD and developmental disorders or other comorbidities, the add-on of ω-3 PUFAs may lead to superior results when compared to other groups [35].

Schizophrenia has been associated with alterations in lipid metabolism and antipsychotic treatment supplementation with ω-3 PUFAs has been explored as a potential way to improve these patients' prognosis [39]. A recent systematic review (n=26 clinical studies) found that the results of ω-3 PUFAs supplementation may significantly improve positive and negative symptoms during the prodromal phase, ameliorates the negative symptoms during the first episode of psychosis (FEP), and has a favorable, although partial, effect in patients with chronic schizophrenia [39]. The effects of ω-3 PUFAs in this population are modulated by demographic variables (e.g., age), clinical variables (e.g., duration of untreated psychosis), and the status of antioxidant processes in individuals with psychosis [39].

Healthy diet interventions

According to a review dedicated to dietary interventions for the prevention of depression, five recommendations are supported by evidence: "traditional" diets (e.g., Mediterranean, Norwegian, or Japanese diet); increased consumption of fruits, vegetables, legumes, wholegrain cereals, nuts, and seeds; high use of foods rich in ω-3 PUFAs; replacement of unhealthy foods with wholesome, nutritious foods; reduction of processed foods, " fast-foods", sweets, etc. [40]. Low-carbohydrate high-fat diets may be useful in improving the outcome of anxiety disorders and ADHD [28,41]. This type of ketogenic diet has anti-inflammatory effects due to the lower production of reactive oxygen species and free radicals [28].

A multicenter, randomized study focused on preventing cardiovascular disease evaluated the effects of the Mediterranean diet supplemented with nuts in community-dwelling men (age 55-80 years) and women (age 60-80 years) at high risk of cardiovascular disease vs. the control group (low-fat diet) [42]. A number of 224 new cases of depression were identified at follow-up (after three years), and an inverse association with depression was reported for patients with the Mediterranean diet vs. the control group, but this was not significant [42]. When only patients with type 2 diabetes mellitus were included in the analysis, the magnitude of the effect determined by the dietary intervention did reach statistical significance [42].

Dietary interventions as adjuvants to the treatment of moderate to severe MDD were evaluated by comparison with social support group, in a single-blind, randomized controlled trial for 12 weeks, with 67 participants [43]. The nutritional intervention consisted of personalized dietary advice and counseling conducted by a clinical dietician who respected the "ModiMedDiet" program [43]. Whole grains, vegetables, fruit, legumes, low-fat and unsweetened dairy foods, raw and unsalted nuts, fish, lean red meats, chicken, eggs, and olive oil were the core ingredients of this dietary intervention (18% protein, 40% fat 37% carbohydrates, 2% alcohol, and fiber/other 3% of the total energy) [43]. After 12 weeks, the results of MADRS supported the superiority of the active intervention vs. social support (control) at a significant level [43]. Remission (MADRS score <10) was reported in 32.2% of the active intervention group vs. only 8% of the control group, a statistically significant difference [43].

In a prospective, randomized trial, adults (N=152) who had depressive symptoms received MedDiet cooking workshops and food hampers for 12 weeks, plus fish oil supplements for six months vs. participation in social groups for three months [44]. The MedDiet group used more vegetables, fruit, nuts, legumes, whole grains, and vegetable diversity, less unhealthy snacks and red meat/chicken [44]. At the endpoint, the MedDiet group presented a lower severity of depression and improved quality of life scores; at six months improved diet and mental health were sustained in this group [44]. Reduced depression correlated with increased MedDiet score, use of nuts and vegetable diversity [44].

The relationship between adherence to the Mediterranean dietary pattern and the prevalence of psychological disorders in adults was investigated in a cross-sectional study (N=3172 participants), using the Hospital Anxiety and Depression Scale (HADS) and General Health Questionnaire (GHQ) as outcome measures for mental health [45]. A high intake of fruits and vegetables was associated with a lower risk of depression, anxiety, and psychological stress [45]. On the contrary, a high intake of grains was more frequently detected in individuals who presented depression, anxiety, and psychological distress [45].

A cross-sectional study (N=5371 individuals from the general population) reported a significant correlation between a healthy diet (vegetables, salads, fruits, rice, pasta, cereals, fish, wine, non-processed meats) quality score (based on Food Frequency Questionnaire, FFQ) and a lower risk for depression and anxiety in women and a similarly lower risk for depression in men (evaluated in HADS) [46]. A traditional Norwegian diet (fish, shellfish, potatoes, fruits, vegetables, butter, margarine, milk and yogurt, bread, pasta, rice, meat spreads, legumes, and eggs) was associated with a reduced risk of depression in women and anxiety in men, while a western-type diet (meat, liver, processed meats, pizza, salty snacks, chocolates, sugar and sweets, soft drinks, margarine, mayonnaise, French fries, beer, coffee, cake, and ice cream) correlated with higher anxiety in men and women [46].

Also, the “healthy Japanese” diet (high intake of vegetables, mushrooms, seaweed, soybean products, potatoes, fish, shellfish and fruits) was inversely correlated with depressive symptoms severity during an exposure-response association study 47]. It is hypothesized that this type of diet exerts its beneficial effect (as opposed to “sweets-fat” and “ seafood-alcohol” patterns) through an anti-inflammatory diathesis, which might act as a factor for decreasing the risk for mood and anxiety disorders [47].

Folate and vitamin B12

Preclinic evidence to support the direct influence of folate and cobalamin on CNS functioning has been gathered, and the relationship between the deficits of these substances and the onset or worsening of different psychiatric disorders has also been explored with convincing results [48]. Folate deficiency has been associated with neurocognitive disorders and MDD, while the cobalamin deficit can be connected to psychosis [48]. Both folate and vitamin B12 are essential for the process of homocysteine methylation and for the synthesis of S-adenosyl-L-methionine (SAMe) [49]. A defect in the methylation process has been hypothesized as the key to the pathophysiological basis of many neuropsychiatric disorders [49]. The toxic effects of homocysteine can also be part of the negative effects of folate and vitamin B12 deficiency [49]. The causal relationship is, however, still unclear, and multiple variables are modulating the effects of vitamin B12 deficiency [48]. Also, it is important to note that normal hematological indices do not automatically exclude vitamin deficiencies [48].

According to a meta-analysis (n=43 studies) that explored the relationship between levels of folate and depression, a small but statistically significant effect size was found (low levels of folate correlated with a higher risk of depression) [50]. This meta-analysis had a high heterogeneity (over 84%), which could not be explained by the folate measurement methods or study population [50]. Regarding the relationship between cognition and vitamin B12 deficiency, the context of its investigation is more complex. According to a meta-analysis (n=21 observational studies, adults ≥45 years old), higher levels of vitamin B12 and folate were associated with better cognitive functioning in cross-sectional research, but not in prospective studies [51]. Yet, another meta-analysis (n=95 studies, N=46175 participants) concluded that B vitamins have a positive impact on cognitive functioning (assessed by Mini-Mental State Examination, MMSE) [52]. B vitamins can slow cognitive decline in prospective studies, especially on long-term administration (over 12 months) [52]. In individuals without dementia, B vitamin supplementation slowed cognitive decline vs. placebo, but this effect was not observed in patients with neurocognitive disorders [52]. Lower folate levels, but not lower B12 or B6 vitamins level, and higher homocysteine levels were significantly correlated with a higher risk of dementia [52]. Also, a higher intake of dietary folate, but not of B12 or B6, was associated with a lower risk for dementia in the old age population [52].

Adjunctive folate for psychiatric disorders can be an efficient intervention, but the results of good-quality clinical trials differ in terms of results. According to a systematic review (n=14 studies), adjunctive folate did not improve the total psychopathology, positive, negative, or general symptoms more than placebo in patients with schizophrenia, but it was superior in patients with bipolar or unipolar depression, in terms of decreasing the severity of the depressive symptoms [53]. Also, in patients with bipolar disorder, adding folate to the conventional treatment of acute mania had a favorable impact in terms of efficacy [53]. The overall tolerability of folate was good, in terms of discontinuation due to adverse events [53].

Another review (n=23 studies) concluded that oral levomefolic acid or 5-methyl folate was the most frequent nutritional factor associated with improvements in clinical status in patients with psychiatric disorders, mainly MDD, including postpartum and post-menopausal depression, schizophrenia, autism spectrum disorders (ASD), ADHD, and bipolar disorder, with a variable magnitude of effect [54]. The tolerability of folate supplements was reported as good [54].

Vitamin B1 and B6

Thiamine (vitamin B1) is a cofactor for many enzymes involved in energy metabolism, biosynthesis of nucleic acids, and anti-oxidative stress protective mechanisms [55]. Because of the importance this vitamin has in ATP mitochondrial production, thiamine deficiency can be associated with neurologic, cardiovascular, and psychiatric dysfunctions manifested as confusion, memory deficits, sleep problems, encephalopathy, ataxia, or congestive heart failure, but it can also lead to death, in severe cases [55]. Wernicke encephalopathy is the result of an acute thiamine deficiency and usually appears as a consequence of alcohol abuse [55]. This disease has as its main clinical features ataxia, loss of coordination, memory deficits, confusion, and ophthalmoplegia, and it could result in death in 17% of the cases, in the absence of appropriate treatment [55]. Korsakoff's syndrome may result from a thiamine deficit and has a chronic evolution, with short-term memory deficiencies and hallucinations [55]. Also, depression in adults has been associated with low levels of thiamine, and more severe mood symptoms have been correlated with lower levels of thiamine in the erythrocytes [56].

Pyridoxine (vitamin B6) is part of the synthesis processes of certain neurotransmitters and it has been invoked as a potential contributor to the pathogenesis of AD, ASD, ADHD, anxiety disorders, depression, and psychosis [57]. Patients with mood disorders had low pyridoxine and riboflavin levels in a study with 172 participants [58].

A cross-sectional study evaluated the correlation between dietary intake of vitamin B6 and the risk of depression and anxiety in 3362 adults [59]. The supplementation of the ongoing antipsychotic treatment with vitamin B6 (up to 400 mg/day) in a 9-week, double-blind, placebo-controlled, cross-over study in 15 patients with SSD did not lead to differences between active and placebo groups, according to the Positive and Negative Syndrome Scale (PANSS) [57]. However, a subgroup of patients with schizophrenia and comorbid minor depression may benefit from pyridoxine adjuvant treatment, as reported during an open-label study (N=9 patients) [60].

Vitamin D

A favorable impact of vitamin D was suggested for the preservation of mental health because this substance may interfere with complex homeostatic, trophic, and immunomodulatory mechanisms that can modulate different processes in the CNS [61]. A systematic review of the studies (n=14) which included various doses of vitamin D supplementation explored the effects of this intervention on mental status in healthy adults [62]. The majority of studies do not support a favorable impact of vitamin D supplementation, but data on depression outcomes were conflicting [62]. Vitamin D combined with physical activity could be more efficient than vitamin D in these patients, and there is evidence to recommend vitamin D from food sources instead of vitamin supplementation [62].

Another review (n=61 articles) concluded that a relationship between vitamin D and depression is possible, but its causality is uncertain [61]. A more important effect of this supplementation was observed in patients with MDD and vitamin D deficiency [61]. Serum vitamin D levels inversely correlate with the clinical severity of depression, but the results of clinical trials are not consistent enough to recommend a regular supplementation of the diet with this vitamin in patients with MDD [61]. A meta-analysis demonstrated that vitamin D supplements administered in patients with psychiatric disorders (N=9 trials) can reduce the BDI scores, improves the quality of sleep on Pittsburgh Sleep Quality Index (PSQI), and increase the glutathione and total antioxidant capacity while decreasing the level of C-reactive protein [63].

Neonatal vitamin D deficiency is correlated with an increased risk of schizophrenia, and preclinical studies support the alteration of brain development by vitamin D deficiency leading to subtle neurochemical and cognitive changes that may represent vulnerability factors for schizophrenia [64]. Also, patients with schizophrenia or even first-episode of psychosis may have an increased risk of vitamin D deficiency vs. healthy controls [64].

A cohort study (N=152 participants with various psychiatric diagnostics) assessed the correlation between the Brief Psychiatric Rating Scale (BPRS) and the plasma levels of 25-HO-vitamin D and parathyroid hormone (PTH), as well as the calcium serum levels [65].

The study concluded that a negative relationship may exist between serum levels of 25-OH-vitamin D and BPRS total and items scores, and inpatients had lower PTH and 25-OH-vitamin D levels [65]. This trend toward vitamin D deficiency in hospitalized patients was not dependent on the specific diagnosis or diagnosis category [65].

S-adenosyl-L-methionine

SAMe is a compound with essential roles in multiple biochemical reactions, which impact the production of molecules involved in the regulation of monoaminergic transmission [66]. Therefore, SAMe can modulate the pathophysiological mechanisms of MDD, and there are studies suggesting the antidepressant efficacy of this compound [66]. SAMe in monotherapy has been proven superior to placebo and tricyclic antidepressants, while other studies demonstrated its efficacy in patients with SSRIs- and serotonin and norepinephrine reuptake inhibitors (SNRIs)-non-responsive MDD [66]. These data are derived from open-label trials and placebo-controlled randomized trials, which investigated the effects of oral and parenteral SAMe, in doses varying from 200 mg/day (i.v.) to 1600 mg/day (p.o), and durations of treatment between 2 and 21 weeks [66-69].

According to a meta-analysis (n=13 uncontrolled trials and 25 controlled studies), the response to SAMe was superior to the placebo, and the global effect size ranged between 17% and 38% [70]. The antidepressant effect was comparable to that of tricyclic antidepressants in this meta-analysis [70].

The results of a Cochrane systematic review (n=8 studies comparing SAMe with placebo, imipramine, desipramine, or escitalopram, N=934 adults with MDD) showed that there are no differences in the depressive symptoms severity scores change between endpoint and baseline in patients treated with SAMe or placebo as monotherapy [71]. Low evidence quality supported a favorable effect of SAMe similar to imipramine when administered as monotherapy [71]. There was low-quality evidence that SAMe was superior to placebo as an add-on to SSRIs in the change of depressive symptoms severity from baseline to endpoint [71].

N-acetyl cysteine

This compound influences CNS activity through multiple mechanisms, i.e., it contributes to the production of glutathione and oxidative stress, and dysfunctions at this level have been frequently reported in many psychiatric disorders; also, it attenuates dopamine and glutamate dysfunctions in vitro, and it may be a useful adjuvant in the modulation of inflammatory pathways [72-74]. N-acetyl cysteine (NAC) is the acetylated form of cysteine and it is able to cross the blood-brain barrier [72]. The preclinical evidence in animal models of schizophrenia, bipolar disorder, MDD, anxiety disorders, and obsessive-compulsive disorders (OCD) spectrum disorders support the potential benefits of NAC administration [75].

Clinical evidence supports the use of NAC as an add-on treatment for patients with schizophrenia, with a favorable effect being reported on total and negative symptoms of psychosis [72]. A positive impact of NAC was observed in decreasing the craving and risk of relapse in patients with substance use disorders (SUDs), but proof of a clear effect of NAC on OCD spectrum disorders, ASD, and mood disorders is still lacking [72]. Orally administered NAC is considered safe and no severe tolerability problems have been detected in the clinical trials when recommended dosage (2000-2400 mg/day) was used [72].

In 24 patients with cannabis use disorders (CUD), NAC 1200 mg BID was administered for four weeks in an open-label design [76]. The treatment was well tolerated and had a favorable impact on the addictive pathology, decreasing the self-reported measures of marijuana use and craving [76]. The results of a mixed-models study (preclinical and clinical) indicated that NAC reduced the number of cigarettes smoked in patients with nicotine use disorder (NUD) [77]. In a group of treatment-refractory OCD patients (N=48), NAC (up to 2400 mg/day) was compared to a placebo for 12 weeks, and the results supported the efficacy of the active intervention, according to the Yale-Brown Obsessive Compulsive Scale (Y-BOCS); 52.6% of the NAC-receiving patients were full responders at the endpoint vs. only 15% in the placebo group [78]. NAC (1200-2400 mg/day) was also evaluated in a group of 50 patients with trichotillomania during a placebo-controlled randomized trial with a 12 weeks duration; NAC reduced significantly more than placebo the Massachusetts General Hospital Hair Pulling Scale (MGHPS) and the Psychiatric Institute Trichotillomania Scale (PITS) scores; 56% of the patients on NAC were “much” or “very much improved” vs. 16% on placebo [79]. A randomized, double-blind trial enrolled 66 participants diagnosed with excoriation (skin-picking) disorder who received NAC (1200-3000 mg/day) or placebo for 12 weeks; NAC was associated with significant improvements on the modified Y-BOCS vs. placebo at the endpoint; the overall tolerability of NAC was good [80].

Most studies evaluating NAC were underpowered and of short duration, which are important methodological limitations [75]. Therefore, although NAC demonstrated positive effects on animal models of psychiatric disorders, the clinical efficacy of this compound has not been fully explored [81].

Psychobiotics

Prebiotics, probiotics, and synbiotics have been explored in the treatment of psychiatric disorders based on their presumed properties of gut microbiota (GM) modulation. Potential mechanisms underlying their therapeutic effect in psychiatric disorders are very complex and include the participation of the immune system, vagus nerve, various neurotransmitters, and metabolic or endocrine factors [82].

Probiotics are defined as living organisms without pathogenic properties that can produce beneficial effects in hosts, and in this category are included a large variety of lactobacilli, bifidobacteria, strains of Escherichia coli, certain enterococci, Saccharomyces boulardii, etc. [83]. The probiotic products available on the market are usually containing a combination of strains, which makes quite challenging the interpretation of results in clinical trials exploring this intervention (i.e., high heterogeneity of the active product) [83]. Prebiotics are mainly fibers, non-digestible ingredients of the diet, that may have a positive impact on the host's health through different mechanisms, e.g., selective enhancement of the activity and/or growth of some GM genera [83]. Prebiotics are, for example, inulin and pectin, which have been associated with increased bioavailability and uptake of minerals, lower risk of cardiovascular disease, and promoted satiety and weight loss [83]. Synbiotics represent mixtures of pre and probiotics, which presumably improve the survival and implantation of live microbial dietary supplements in the gastrointestinal tract [83].

A meta-analysis (n=34 trials) concluded that prebiotics did not differ from placebo for depression or anxiety, while probiotics had small but significant effects for depression and anxiety [84]. Larger effects were reported for probiotics in clinical samples of depressed patients, than in community samples, but the pooled effects were low, due to a paucity of trials focused on mental health status-related outcomes [84]. According to another systematic review (n=54 randomized placebo-controlled trials) and meta-analysis (n=30 trials), the impact of most probiotics on mood, anxiety, depression, and psychiatric distress was not relevant vs. placebo at a qualitative level, but only at a quantitative level [85]. The same source concluded that depression severity and psychiatric distress registered but minor improvements in the probiotic group [85].

A systematic review (n=11 randomized controlled trials) showed that probiotics can be beneficial in MDD and AD, with potentially favorable effects in mania, by reducing the rehospitalization rate in these patients [86]. In the case of ASD, the results of the trials were mixed, but early-in-life administration of probiotics may have a preventive role [86]. The tolerability was good in most of the reviewed studies [86].

A randomized, double-blind, placebo-controlled trial enrolled 48 healthy adults with a body mass index (BMI) > 25 kg/m2 and investigated the impact of 21 g oligofructose/day or placebo (maltodextrin) for 12 weeks on food intake and related variables [87]. Body weight decreased by 0.45+/-0.31 kg during three months, and the self-reported caloric intake was correlated with a lower area under the curve (AUC) for ghrelin and a higher AUC for peptide YY in the oligofructose-receiving group [87]. Insulin concentrations mirrored the evolution of glucose, which decreased in the oligofructose group and increased in the control group at the endpoint vs. baseline [87]. The overall tolerability of oligofructose was good [87].

Another double-blind, randomized clinical trial explored the effect of supplementation with synbiotics and probiotics on mental health, quality of life, and anemia in patients undergoing hemodialysis for 12 weeks, and the results showed a significant decrease in BDI and Beck Anxiety Inventory (BAI) scores vs. placebo [88]. Health-related quality of life was not significantly improved by probiotics/synbiotics vs. placebo [88].

Minerals: Zinc, magnesium, copper, selenium, and iron

Micronutrients have been linked with the status of mental health and potential neuroprotective effects [89]. Selenium is important for the construction of selenoproteins, which are involved in the protection against oxidative stress in the CNS, and low selenium levels have been associated with cognitive loss and neurotransmitter turnover rate changes [89]. Zinc is involved in numerous cellular processes, potentially significant for the pathophysiology of depression and neurocognitive disorders; probable antidepressant effects of zinc may be mediated by its glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonistic properties [89]. Copper homeostasis is important, and an excessive accumulation of this micronutrient leads to Wilson disease, which includes depressive and anxiety manifestations; copper could be involved in the neuroplasticity mediated by BDNF (brain-derived neurotrophic factor) and NGF (nerve growth factor); immune function could be affected by copper imbalances, and may influence the pathogenesis of depression [89]. Magnesium participates in the enhancement of DNA replication, transcription and translation, and it is a coenzyme or activator for many enzymatic systems; also, it is an NMDA glutamate receptor antagonist and is involved in memory functioning [89].

Micronutrient supplementation in healthy individuals has a limited favorable impact on mood and anxiety manifestations, but minor general improvements may still be reported [90]. In adults with physical or psychiatric conditions, the effect can be more favorable, although this consideration is limited by small samples and nutrient heterogeneity in the administered products [90]. Broad-spectrum nutrient products are preferable to a few preselected micronutrients [90].

A study exploring the long-term safety of micronutrients (N=17, average length of monitoring= 2.66 years) reported good preliminary tolerability and long-term safety [91]. The overall evaluation concluded that substantial psychiatric benefits outweigh the minimal risks reported in these patients

[91]. However, screening for potential medical diseases is recommended before the initiation of treatment with micronutrients, and long-term monitoring is also considered useful [91].

There are preliminary data about the potential benefits of micronutrients in autism, depressed mood, stress-related conditions, and elderly populations, but less significant benefits in individuals presenting with bipolar disorder, ADHD, and SUDs [92].

Inositol

This is a simple precursor included in the phosphatidylinositol cycle, a secondary messenger system important for CNS activity [93,94]. In patients with MDD, a low level of inositol in the cerebrospinal fluid (CSF) has been reported [93]. This compound was investigated in patients diagnosed with MDD, panic disorder, and OCD with positive results, while in cases of SSD, AD, ADHD, and ASD the results were negative [93]. In preclinical models, chronic administration of inositol reduced anxiety-like and depression-like behaviors in rats but did not lead to significant effects on amphetamine-induced hyperactivity, apomorphine-induced stereotypy, or on memory functioning in monkeys [94].

A meta-analysis of the trials focused on the effects of inositol in patients with depression and anxiety disorders (n=11 and 4 reports, respectively, reaching a total of 242 and 70 participants) did not detect statistically significant results vs. placebo on depressive, anxiety, and obsessive-compulsive symptoms [95]. The discontinuation rate reported in these trials was not significantly influenced by inositol, either [95]. The percentage of responders was marginally higher in the patients with depression treated with inositol vs. placebo, and patients with premenstrual dysphoric disorder had a trend towards a better evolution if they received inositol [95]. Gastrointestinal discomfort was reported marginally more frequently in patients treated with inositol compared to placebo [95].

Herbal products

St. John's wort, kava, Ginkgo biloba extract, and valerian are the most explored herbal supplements in patients with psychiatric disorders [96], but their efficacy is controversial due to many potential confounders: lack of adequate, good quality trials; the complexity of the standardized products investigated; difficulties in differentiating the effects of a herbal add-on from the effect of the concomitantly administered psychotropics, etc.

According to a review (n=9 controlled trials), while several studies reported herbs' superiority vs. placebo, others did not find differences between herbs and antidepressants in terms of effectiveness [96]. Kava has been associated with anxiolytic properties in studies with short duration; clinically significant improvements were detected during Ginkgo biloba administration in domains like memory impairments, concentration, fatigue, anxiety, and depressed mood; valerian may decrease sleep latency and nocturnal awakenings, but the effect was not consistently reported across studies as superior to placebo; St. John's wort was associated with positive results when administered for patients with depressive symptoms, but there are several negative studies, also [96].

Another review of studies exploring 27 herbal medicines and formulas in the treatment of psychiatric disorders reported that kava and St. John's wort benefit from good quality evidence in the treatment of anxiety and depression, respectively; other phytotherapies (saffron, passionflower, etc.) are not, at least for now, supported by enough data derived from well-designed trials [97].

Continuous research on the efficacy and safety of herbal products is necessary [98]. Although this type of therapeutic intervention has been increasingly explored and is an attractive alternative due to its potential superior tolerability and acceptability for patients with psychiatric disorders, clinicians must be aware of the limitations associated with the generally low-quality evidence on which such products are used [98].

DISCUSSION

The interest of clinicians and researchers in NP is increasing, and a continuously growing body of literature is published regarding the potential benefits of different nutritional interventions in patients with psychiatric disorders. This may be a consequence of the inherent limits of the pharmacological and psychotherapeutic interventions in this population, reflected in the high rate of non-response or partial response to conventional approaches. For example, more than one-third of all patients diagnosed with MDD have treatment resistance [99-101], and almost a quarter of individuals with FEP will become treatment-resistant in their early stages [102,103].

Omega-3 PUFAs were explored in clinical and epidemiological studies, meta-analyses, and systematic reviews, and the available evidence supports a potential benefit of this intervention in patients with MDD, ADHD, FEP, anxiety disorders, and AD [30,31,33-39]. The main positive effect involved in the PUFAs' favorable consequences, when used as an add-on to current psychiatric treatment, was supposed to be their influence on oxidative stress and lipid metabolism [34,39]. However, the number of reviewed sources was small, and the duration of the studies was too short to allow for definitive conclusions.

Healthy diets, especially Mediterranean, Norwegian, and Japanese, were explored in prospective clinical trials and literature reviews, and the main mechanism of action supposed for these interventions was an antiinflammatory activity [8,40-47]. The results of these interventions were positive in lowering the severity of MDD, ADHD, anxiety symptoms, depressive symptoms, and psychological stress, but also in increasing the quality of life in patients with depression [40-47]. The methodology of these sources was of moderate and high quality, but the high degree of heterogeneity of these diets prevents the extraction of clearly-cut recommendations.

Vitamins added to the diet as a therapeutic strategy (vitamins B1, B6, B12, D, and folate) were also explored in clinical and preclinical studies, reviews, and meta-analyses, with mixed results [48-53,55-64]. Except for some well-established recommendations, e.g., vitamin B1 for Wernicke encephalopathy and Korsakoff syndrome, other psychiatric disorders, like MDD, bipolar disorder, ASD, anxiety disorders, and SSD, are still explored as potential targets for vitamin therapy. The potential beneficial effect of B vitamins in slowing cognitive decline is also a subject of interest for researchers [52]. The main mechanism of action of vitamin therapy is patients with psychiatric disorders is supposed to be this intervention's positive effects on multiple metabolic processes within the CNS.

Preclinical studies, clinical trials, reviews, and meta-analyses regarding the effects of SAMe and NAC were analyzed [66-81]. While SAMe benefits from good-quality evidence to be recommended in patients with MDD, due to its efficacy comparable to that of certain antidepressants, the main recommendation for NAC regards the patients with SUD (especially NUD and CUD) [66,70,71,76-78]. The importance of NAC and SAMe on the normal function of neurotransmission has been supported by preclinical models [66,81], but the positive results obtained on preclinical models for a diversity of psychiatric disorders were not yet confirmed in clinical trials [75].

Psychobiotics interfere with the microbiome-gut-brain axis and data about prebiotics and synbiotics are encouraging in MDD, ASD, and anxiety disorders, but not so much for AD, according to the primary and secondary sources reviewed [84-88]. Micronutrients lead to minor general improvements in mood and anxiety symptoms, and preliminary data about their usefulness in ASD, stress-related conditions, and elderly populations also exist [90,92]. The number of reviewed sources for this intervention was low; therefore it could be expected that further research may change its level of recommendation. Inositol was associated with good results in MDD, panic disorder, and OCD, and negative results in SSD, AD, ADHD, and ASD, according to limited sources [93-95]. Herbal products are largely used nowadays, but the evidence to support their evidence is scarce; according to the reviews analyzed here, kava may be useful for the treatment of anxiety symptoms (on short duration) and St.John's wort for depressive symptoms [96,97]. It is important to note that NP interventions have been associated with good tolerability, and no significant adverse events were reported in the reviewed reports.

These conclusions about mixed results of NP interventions support the results of other available secondary reports. For example, a meta-analysis concluded that the response to treatment in the case of adding ω-3 PUFAs and zinc in patients with MDD and antidepressant treatment was similar [103]. Folic acid was not supported by studies in terms of efficacy; the preliminary data for inositol was negative, and the evidence for vitamin D was considered scarce [104]. Mixed results also were reported for vitamin B12 and SAMe [104]. Another review of 356 sources concluded that the highest therapeutic potential in the prevention or treatment of psychiatric disorders can be attributed to the rational diet, physical activity, use of psychobiotics, and consumption of antioxidants [11].

There are a large number of ongoing studies that approach the subject of psychobiotics augmentation in patients with psychiatric disorders. For example, the augmentation of antipsychotic treatment with probiotics (bifidobacteria, lactobacillus, and maltodextrin) or prebiotics (inulin + maltodextrin) in patients with schizophrenia is intended to be explored in a 26-week prospective, randomized, controlled multicenter trial [108]. Dietary supplements (microbiotherapy, GynMDD) are explored as add-ons to venlafaxine in patients with MDD in a 12-week randomized trial, after failure of a first-line antidepressant [109]. The exploration of a potentially favorable impact of prebiotics (Akkermanisa muciniphila, through enteric-coated orally-administered acetate) on the most problematic side effects of psychotropics, i.e., weight gain and metabolic syndrome, is the objective of a 5-month trial, which is expected to enroll 16-25-year old patients undergoing psychiatric treatment [110]. In children, the effects of probiotics, more specifically a combination of BB-12 and LGG (Lacticaseibacillus rhamnosus GG and Bifidobacterium animalis subsp. lactis) are explored in a randomized, placebo-controlled trial with an expected target of enrollment of 70 patients (4-16-year old) with confirmed ASD, who will be monitored for 84 days [111].

It is expected that further research in the field of nutritional interventions for psychiatric disorders would include long-term, double-blind, controlled design, in order to obtain high-quality data. Also, decreasing the heterogeneity of the study population would be beneficial for detecting a signal in efficacy studies. This objective can be attained by different means, such as using structured interviews, excluding different confounders (like concomitant use of antibiotics for studies with psychobiotics), and applying cut-off values at baseline for certain psychological instruments used to quantify the severity of core psychiatric symptoms.

Limitations of this review refer to its narrative design, due to which certain important sources may be missed. However, based on the reviewed material, a systematic review of the nutritional interventions, and even an umbrella review, may be granted. Also, due to its design, there was no structured assessment of the quality of evidence; therefore reports of different design and methodological quality have been treated equally. There are some data about the utility of fecal material transplantation in patients with psychiatric disorders, and about the potential benefits of cannabinoids [6,105-107], but these aspects were not approached because it was appreciated that they would increase too much the heterogeneity of the explored interventions.

As strengths of this review, it is important to mention its aim, i.e., to explore the available evidence for the nutritional interventions both at clinical and preclinical levels. Also, the inclusion of a large variety of psychiatric disorders undergoing intense exploration from the perspective of NP may be considered another strength. Last but not least, the awareness of available nutritional interventions is still precarious in mental health specialists, therefore, a presentation of the current trends, challenges, and perspectives in the field of NP was considered beneficial for the case managers responsible for treating patients with psychiatric disorders

CONCLUSION

NP is still a young science and the systematic exploration of its core interventions requires intensive investigation in good-quality, large-scale trials, before they can be included in the therapeutical guidelines. Based on this review of the literature, preliminary data to support the use of omega-3 PUFAs, healthy diets, vitamin therapy, SAMe, NAC, psychobiotics, micronutrients, inositol, and herbal products exist, and most of the evidence refers to potential benefits of these augmentative strategies in patients with anxiety and depressive symptoms. The translation of data from preclinical models to clinical populations is difficult, and a difference between the results in animal studies and those in human trials has been reported. The main advantages of NP interventions refer to their good tolerability, their superior acceptability (due to lower stigma), and their flexibility, which allows to use them as add-ons to other treatments.

Conflicts of interest and sources of funding
None to declare.

Authors' contribution
OV conceived the idea of the review. OV, AGM, BMP and CFP designed the study. OV, CFP, AGM, CFP, DU, MM, CF, AID, CN and CAS screened the studies during the primary search. OV, AGM and CAS drafted the first version of the review. OV, CFP, DU, MM, CF, CN and CAS revised the manuscript and elaborated the conclusions of the review. All authors had full access to the data contained in this review and decided to submit the manuscript for publication.

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ORIGINAL ARTICLE

A Survey on Mental Health Status in West of Iran: A Spatial Analysis

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Abstract: *Mental disorders clearly are among the major challenges of global health causing many concerns for health systems, health experts, and health policy makers. The present study was conducted to evaluate the mental health program in comprehensive health centers and health houses under the coverage of Ilam University of Medical Sciences in the West of Iran. The present study was conducted based on the reports obtained from the mental health program in 2019. The data were recorded into registration and evaluation forms of mental disorders with the codes 102-19-03 and 102-19-01 and then entered into the Excel software. The prevalence per 100 and 100,000 people, as well as their 95% confidence intervals were calculated using Formula 1 ROUND (Number of cases*100000/population) and Formula 2 ROUND (Prevalence ±1.96*SQRT (Prevalence *(1-Prevalence)/population). The results showed that the prevalence of depression was 19,100 [95% CI: 18999-19201] 19100), and the prevalence of anxiety was 304,66 [95% CI: 20348-30584] per 100,000 population. The highest prevalence among mental disorders was related to depression and anxiety. Considering the high average score of mental health status in people over 65 years old, it is necessary to implement measures and interventions to improve these individuals' mental condition and upgrade their mental health.*

Keywords: *Spatial analysis; Ilam province; Mental health; Iran*

INTRODUCTION

According to the definition provided by the World Health Organization (WHO), mental health is "a health status that turns one's potential talents into reality, enabling people to cope with routine life tensions, accomplish valuable work, and upgrade their social participation" [1]. Mental health is an important component for evaluating the health status of a population and can be evaluated using various indicators [2]. In other words, the concept of mental health falls under the general concept of health and refers to all the methods and measures used to prevent people from developing mental disorders, as well as for the treatment and rehabilitation of affected individuals. Psychologists believe that mental health can be affected by individual characteristics, mental and environmental burdens, economic, social, and familial status, failure to achieve life goals, and lack of motivation [3]. Industrialization of countries, the growth of urbanization, migration to cities, etc. are phenomena that are associated with numerous troubles, including psychological and social problems. For this reason, mental health and mental disorders are considered top priorities in today's world. The high prevalence of mental disorders and their associated morbidities render them an immediate health priority [4].

Excessive attention to physical health problems (such as infectious and contagious diseases) has caused mental health to receive less priority in many countries around the world, including its social and spiritual aspects, which have been overlooked in most cases [5]. Statistics on the global burden of mental illnesses highlight the need to pay attention to these aspects more than ever. In parallel with the estimation of the WHO, Harvard University and the World Bank declared that mental disorders were responsible for about 13% of the total burden of diseases in 2006, which was predicted to reach 15% by 2020. The rate of morbidities caused by these disorders has been reported beyond 33% [6]. In Iran, mental health disorders account for 14.5% of the total burden of diseases, and after traffic accidents, they share the second rank with cardiovascular diseases. In women, depression claims the first place of disease burden, while in men, addiction is considered the third cause of disease burden [7]. According to studies in Iran on individuals older than 15 years old, the prevalence of mental disorders has been 25.9% in women and 14.9% in men, and it was reported that overall, 21% of people suffered from some sort of mental problems. Some studies have assessed the prevalence of mental disorders in Ilam province{8]. In a population-based study by Visani et al. [9], the prevalence of DSM-IV mental disorders in both genders was investigated in the urban areas of Ilam province. Out of 763 participants aged 15 years and older in the recent study, 25.8% showed symptoms of mental distress, and the prevalence of mental disorders was reported to be 20.9% in men and 29.8% in women. Also, the prevalence of anxiety, major depressive disorder (MDD), and obsessive-compulsive disorder was higher in women than in men (23.6% vs. 18.2%; 22.7% vs. 17.4%; 13.9% vs. 10%, respectively). In women, the prevalence of phobia was reported as 10.4%.

Studying mental health status can provide managers and decision-makers of organizations with valuable information for planning educational, therapeutic, and preventive measures to reduce the incidence of these diseases in society. Such information can be obtained by monitoring the mental health status of people in different societies [5].

Assessment and monitoring of mental disorders in the country and in Ilam province can help better understand the condition of mental health and public health in society, which is an important and influential factor affecting social productivity and wealth creation. Therefore, researchers here aimed to investigate the mental health status of the urban population of Ilam using the data recorded in the registration and assessment forms designed for mental disorders. Furthermore, we evaluated the mental health program performed in comprehensive health centers and health houses covered by the Ilam University of Medical Sciences in the West of Iran.

MATERIALS AND METHODS

The present cross-sectional study was conducted on the basis of the reports derived from the mental health program in Ilam province in 2020. According to the statistics announced in 2021, Ilam province harbors a population of over 630,000 people living in ten cities: Ilam, Ivan, Chardavl, Sirvan, Malekshahi, Mehran, Abdanan, Dehloran, Badreh, and Darrehshahr (Figure 1).

Figure 1: The geographical location of Ilam province

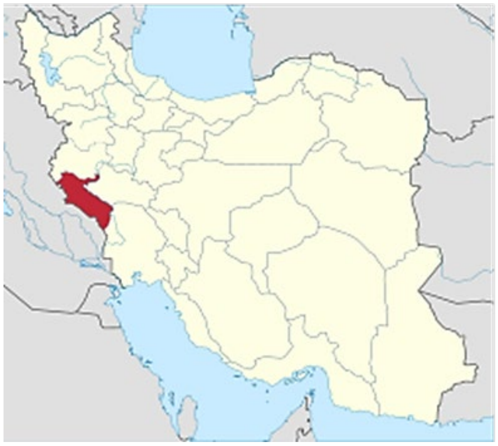

This study was conducted to assess the mental health program implemented in the comprehensive health centers and health houses affiliated with the Ilam University of Medical Sciences in 2019. The study was approved by the institutional ethics committee under the code IR.MEDILAM.REC.1399.266. The data were gathered by reviewing registration and evaluation forms for mental disorders with the codes 102-19-03 and 102-19-01 in all ten cities of Ilam province. Then based on the population of each city in 2021, the prevalence of mental disorders was calculated in 100 and 100,000 people.

Analytic Method

After collection, the data of each city were entered into Microsoft Excel software, and formulas 1 and 2 were used to calculate the prevalence of mental disorders (with 95% CIs) in 100 and 100,000 people.

Formula 1: ROUND (Number of cases*100000/population)

Formula 2: ROUND Prevalence ±1.96*SQRT (Prevalence *(1-Prevalence)/population)

Also, to determine the geographical distribution of mental disorders in the cities of Ilam province, the commands of spatial analysis in the STATA 11 software (Stata Corp. LLC College Station, Texas, USA) were used.

RESULTS

The results showed that in Ilam province, the prevalence of depression was 19100 (95% CI: 18999-19201), and the prevalence of anxiety was 30466 (95% CI: 20348-30584) per 100,000 people. According to the results, the highest prevalence among mental disorders was related to depression and anxiety. The highest rate of depression belonged to Ilam (20300, 95% CI: 20137-20463) and Malekshahi (21836, 95% I: 21297-22375) cities, while the lowest rate of depression was reported in Sirvan (16292, 95% CI: 15691-16897) and Badreh

(17299, 95% CI: 16706-17892). Also, the highest rates of anxiety were recorded in the cities of Abdanan (22298, 95% CI: 21922-22674), Ivan (22559, 95% CI: 22228-22970), and Ilam (21200, 95% CI: 210135-21365). On the other hand, the cities of Sirvan (16769, 95% CI: 16184-17404), Chardavl (16499, 95% CI: 16228-16770), and Darrehshahr (19099, 95% CI: 17790-18408) had the lowest rate of anxiety (Table 1).

Table 1: The prevalence of depression and anxiety in the population of Ilam province per 100,000 people in separate cities

Location	Population	Major Depressive Disorder Prevalence		Anxiety Disorder Prevalence	
		Number of cases	Rate per 100,000 (CI95%)	Number of cases	Rate per 100,000 (CI95%)
Ilam	235144	47734	20300 (20137-20463)	49850	21200 (210135-21365)
Abdanan	46977	9630	20499 (20134-20463)	10475	22298 (21922-22674)
Ivan	48833	10236	20961 (20600-21322)	11036	22559 (22228-22970)
Mehran	27506	5281	19199 (18734-19664)	6162	22402 (21909-22895)
Badreh	15614	2701	17299 (16706-17892)	2701	17299 (16706-17892)
Dehloran	66339	13135	19800 (19497-20103)	12538	18900 (18602-19198)
Darrehshahr	59551	10838	18200 (17890-18510)	10778	18099 (17790-18408)
Shirvan	14404	2347	1294 (15691-16897)	2419	16796 (16184-17404)
Chardavl	72167	12412	17199 (16924-17474)	11907	16499 (16228-16770)
Malekshahi	22587	4932	21836 (21297-22375)	3884	17196 (16704-17668)
Total	580148	110808	19100 (18999-19201)	176750	30466 (20348-30584)

Regarding age distribution, the results showed that the highest prevalence of depression (29%), anxiety (35.5%), and psychotic disorder (22.6%) was observed in people over 65 years of age. The psychological disorders of mania (6.3%) and obsessive-compulsive disorder (13.3%) were more commonly observed in the age group of 15-24 years compared to other age groups. Regarding the gender distribution of mental disorders, it was noticed that depression (20.3% vs. 22.7%), mania (2.6% vs. 4.7%), anxiety (23.6% vs. 32.18%), obsessive-compulsive disorder (10% vs. 13.79%), phobia (10.4% vs. 5%), and psychotic disorder (6.17% vs. 2.4%) were more frequent in women than in men (Table 2).

Table 2: The prevalence of mental disorders in Ilam province in various age groups and genders

Age groups/ gender	Depression	Mania	Anxiety	Obsessive-compulsive disorder	Phobia	Psychotic
	Prevalence (CI 95%)	Prevalence (CI 95%)	Prevalence (CI 95%)	Prevalence (CI 95%)	Prevalence (CI 95%)	Prevalence (CI 95%)
15-24 years	14.0 (7.08-15.1)	6.3 (5.9-7.3)	14.7 (12.1-16.8)	13.3 (9.04-15.15)	8.4 (6.9-11.1)	0.7 (0.01-5.9)
25-44 years	20.6 (18.2-22.3)	4.0 (2.6-9.1)	21.2 (16.2-18.2)	11.3 (9.13-14.2)	7.5 (6.9-11.1)	4.8 (3.6-6.3)
55-64 years	22.7 (20.2-24.3)	2.3 (1.4-2.9)	23.6 (20.3-25.2)	13.4 (8.1-16.1)	8.3 (7.9-11.3)	3.7 (1.2-5.7)
> 65 years	23.6 (20.3-25.2)	0.4 (0.1-1.1)	35.5 (32.3-37.8)	9.7 (7.1-11.2)	9.7 (7.1-12.2)	22.6 (20.2-27.6)
Male	17.4 (15.1-19.6)	2.6 (1.3-8.7)	18.2 (15.2-21.1)	10.0 (8.1-12.4)	5.0 (3.7-8.2)	2.4 (1.4-4.1)

The results of spatial analysis regarding the geographical distribution of mental disorders in Ilam province showed that depression, anxiety, and mania were more commonly observed in the northern compared to southern regions of the province, showing the highest prevalence in Ivan City. Also, panic disorder was more prevalent in southern cities of the province (Abdanan and Dehloran) than in other cities (Figure 2).

DISCUSSION

Sciences. The results of the present study showed that the highest prevalence of depression, anxiety, and psychotic disorders was observed in people over 65 years old. In this study, age showed a direct and significant correlation with having unsatisfactory mental health, indicating the importance of advancing age in the development of mental health problems such as depression, anxiety, and psychotic disorders. In other words, the incidence of mental health disorders increased with age, contradicting the findings of Heidari et al. [10] and Jafari et al. [11]. In recent studies, a higher age was reported to be significantly associated with more favorable mental health. Other studies; however, have reported a significant relationship between mental health and advancing age, including a report by Visani et al. [12]. In another study, Noorbala et al. [13] noted that with increasing age, the likelihood of the development of mental disorders increased so the highest prevalence (49.9%) was related to people over the age of 65 years. Among the reasons for the higher prevalence of mental health disorders in older people are retirement, menopause, loneliness-associated anxiety, and biological alterations. The results of these studies are generally consistent with our findings; nevertheless, some discrepancies can be explained by differences in the assessment tools employed and the age spectra of the studied populations.

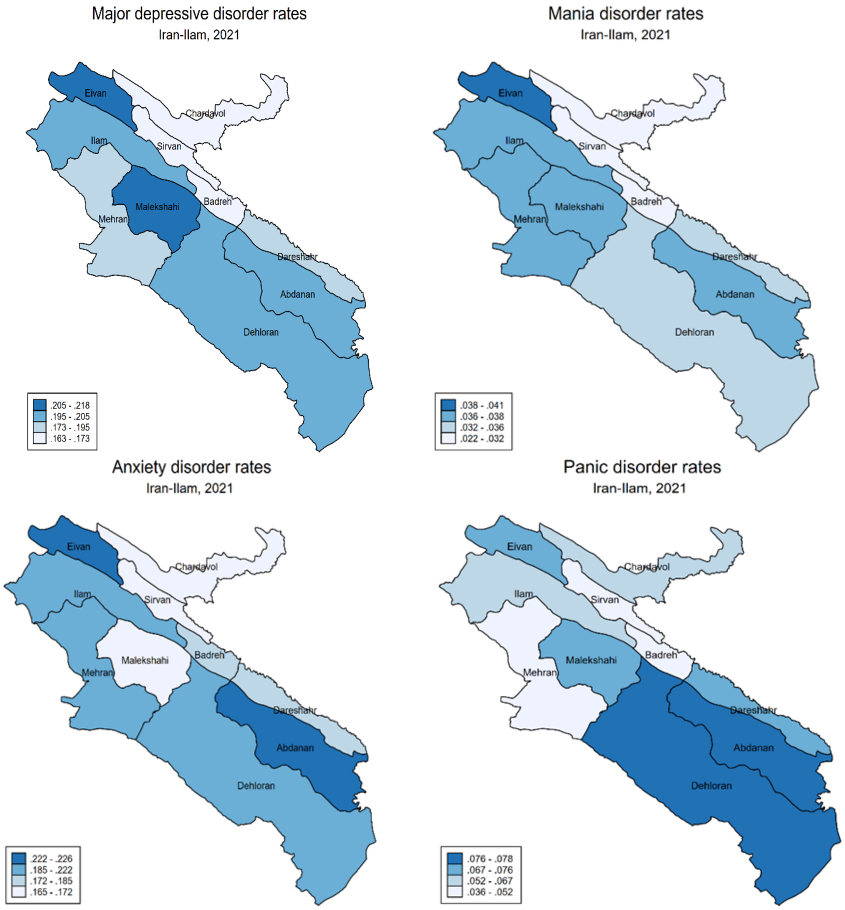
Major depressive disorder rates
Iran-Ilam, 2021
Eivan
Chardavol
Ilam
Sirvan
Malekshahi
Badreh
Mehran
Dareshahr
Abdanan
Dehloran
.205 - .218
.195 - .205
.173 - .195
.163 - .173
Mania disorder rates
Iran-Ilam, 2021
Eivan
Chardavol
Ilam
Sirvan
Malekshahi
Badreh
Mehran
Dareshahr
Abdanan
Dehloran
.038 - .041
.036 - .038
.032 - .036
.022 - .032
Anxiety disorder rates
Iran-Ilam, 2021
Eivan
Chardavol
Ilam
Sirvan
Malekshahi
Badreh
Mehran
Dareshahr
Abdanan
Dehloran
.222 - .226
.185 - .222
.172 - .185
.165 - .172
Panic disorder rates
Iran-Ilam, 2021
Eivan
Chardavol
Ilam
Sirvan
Malekshahi
Badreh
Mehran
Dareshahr
Abdanan
Dehloran
.076 - .078
.067 - .076
.052 - .067
.036 - .052

Figure 2: The results of spatial analysis. Geographical distribution of the most common mental disorders in Ilam province

The results of this study showed that the highest prevalence of panic disorder and obsessive-compulsive disorder was related to the age group of 15-24 years. Mental disorders in teenagers and young adults exacerbate the predicted future burden of diseases and adversely affect numerous life aspects, including educational performance, employment, income, personal relationships, and social participation, as well as the workforce [14]. In a study in Brazil conducted on individuals aged 18 years and older, it was shown that younger people were more vulnerable to mental problems [15]. In another study in Iran, Shirzadi et al. [16] declared a higher prevalence of these disorders in people aged 41 years or older compared to 18-40-year-old individuals.

Also, the results of the present study revealed a lower prevalence of depression and anxiety in women than in men. In a study, Falahzadeh and Momayezi [17], who investigated the prevalence of depression, anxiety, and stress in Yazd City, Iran, noted that depression, anxiety, and stress were more prevalent in women than in men, and this difference was statistically significant for anxiety (P=0.01) and stress (P=0.02), which was not consistent with our observation.

We also noticed a higher prevalence of obsessive-compulsive disorders, phobia, and psychotic disorders in women compared to men, which was in agreement with the reports of Pendrez et al. [18], Kesler et al. [19], and Chang et al. [20]. The higher prevalence of mental disorders in women compared to men can be attributed to biological factors, gender-related differences, environmental and occupational stress, as well as limited sources of satisfaction and social participation of females.

Although in the present study, a significant portion of the urban population declared that they had unsatisfactory mental health conditions, this may not exactly match the clinical definition of adverse mental health. Therefore, the results of such subjective studies should not be regarded as a basis for judgment about the true condition of the mental health of respondents. Nevertheless, one should admit that a ratio of people, especially among women, suffer from some degree of mental health disorders. In this regard, the key measures that can be taken may be to initially achieve a correct understanding of the issue and then to implement comprehensive interventions for monitoring populations as large as possible, especially among vulnerable groups.

Numerous viable strategies are available to maintain and improve public health. For example, unemployment has been pointed out as an important factor contributing to the development of mental health disorders [21]. So, an important intervention that governments can employ is to create more jobs, especially for youth, and to improve people’s livelihoods as much as possible.

Also, another effective measure for improving mental health is to remove the sources of distress, psychological pressure, and unnecessary tensions, which can be achieved with the help of mass media by nurturing a comfortable atmosphere. Moreover, the establishment of psychological counseling clinics can be among low-cost and beneficial measures to cover a large population, including young people, middle-aged, and elderly people, so that they can amend and improve their mental health**.**

CONCLUSION

The results of this study affirmed a relatively high prevalence of suspected mental health problems among people over 65 years of age, which is an alarming call, requiring authorities and researchers to pay more attention to this issue and to identify and modify the factors affecting the mental health of these people. A unique observation in this study was the lower prevalence of anxiety and depression in women than in men. Also, we noticed that panic disorder and obsessive-compulsive disorder were most prevalent in the age group of 15-24 years. A reduction in the prevalence of mental disorders can enhance social liveliness. In this regard, increasing people’s awareness of ways to reduce mental disorders, expanding psychological counseling centers across the city, establishing counseling phone lines, residing counselors and psychologists in public places such as mosques, expansion of parks and enjoyable environments, equipping parks with sports devices, and finally, encouraging people to use these services can help reduce depression and anxiety in society.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The source of the funding code is the code of IR.MEDILAM.REC.1399.266.

Authors’ contribution

Conceptualization, E.N., and Y.V.; methodology, E.N.; formal analysis, H.S; investigation, Y.V.; data curation, F.M., and A.S; writing—original draft preparation, S.A.; and M.Kh.; writing—review and editing, S.A; F.M. and M.Kh. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Dermatologists' Views on the Use of Teledermatology

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Abstract: *Boosted by the COVID-19 pandemic, the adoption of technology has shifted people's lifestyles, impacting social interactions and leading to an increase in the use of telemedicine, including teledermatology (TD). Social media platforms facilitate communication between patients and physicians, between primary care physicians and specialists, and between dermatologists. The study aimed to assess the attitudes, views, and usage of dermatologists regarding TD consultations. A cross-sectional study was performed with the use of an anonymous, web-based questionnaire intended to be completed by dermatologists. A total number of 73 physicians, both resident and board-certified dermatologists completed the survey. Almost everyone (98.6%) used some form of teledermatology, the most frequently used platforms being WhatsApp, followed by classical telephone calls/messages, email, and other forms of TD. TD is considered to be especially useful in communicating with peer colleagues (94.4%), with physicians of other specialties (90.0%), with family doctors (72.9%) and with patients (69.9%). Since TD can facilitate the treatment-related follow-up of adverse effects, while also being useful in the triage of patients, its use will likely increase in the future. The main concerns were related to the accuracy of the diagnosis through TD, a less developed doctor-patient relationship, a less informative consultation, an increased workload as well and a diminished financial reimbursement. Dermatologists currently use TD in addition to the classical consultations of patients, as well as to communicate with family physicians and peer colleagues to better manage complex cases. Furthermore, TD proves to be an important tool in the education of young dermatologists.*

Keywords: *teledermatology, consultations, education*

INTRODUCTION

Skin diseases are frequent pathologies encountered in the general population that require standard or specialized care, depending on disease rarity or difficulty. The Global Burden Disease study that was performed over 10 years in 187 countries revealed that skin conditions ranged from the 2nd to the 11th leading cause of years lived with disability, and, at a global level, cutaneous conditions were the 4th cause of nonfatal disease burden [1]. Such a high prevalence of skin disorders is met with a significant effort on the part of the medical personnel and the medical system, requiring new solutions to optimize medical care. Teledermatology (TD) developed in recent years as a response to this need. Its use, boosted by the COVID-19 pandemic [2], is expected to increase soon. Technology facilitates professional communication between patients and healthcare providers, as well as between healthcare professionals of identical or different specialties.

Several social media platforms facilitate communication between patients and healthcare providers, allowing individuals to contact specialized personnel more quickly. During the past years, departments already offering asynchronous TD created new systems to support the direct interaction between specialists and patients [3].

As expected, the use of TD increased along with other branches of Telemedicine, such as Tele-echography[4], women's health telemedicine, family medicine telemedicine, telecardiology, and others. Taking into consideration these changes in technology and social interactions, we aimed to evaluate the attitudes, views, and usage patterns of dermatologists.

MATERIALS AND METHODS

A cross-sectional study was performed between February and July 2022 among specialist and resident dermatologists using a web-based survey. Contact details were retrieved from the database of the Dermatology Department at the University of Medicine and Pharmacy "I. Hatieganu" Cluj Napoca, Romania. Both email and phone messages were used to distribute the questionnaire to dermatologists, explaining that the completion of the survey was anonymous and voluntary.

The survey was developed by the authors based on previously published data and was sent via Google Forms (Google LLC, Mountain View, CA, USA) to the participants. The questionnaire was divided into several sections: Initially, the participants were invited to provide certain anonymous personal data such as age, gender, area of dwelling, workplace, and amount of time spent using appliances such as telephone, tablet, etc. This introductory part was followed by a second section assessing the current use of TD, a section focusing on the accuracy of medical consultation via TD, the advantages and disadvantages of TD, and future perspectives.

Statistical analysis

Data was collected and further analyzed using SPSS, version 12, SPSS Inc., Chicago, IL, USA. Spearman correlation was used between ordinal variables and between ordinal and numeric variables. The biserial correlation coefficient was used between ordinal and nominal variables. A p-value under 0.05 was considered statistically significant.

RESULTS

A total of 73 participants completed the online questionnaire, of which 25 (34.2%) were during residency and 48 (65.8%) were board-certified dermatologists (Table 1). The female gender predominated with 65 answers (89%), with the following age distribution: 20 participants (27.4%) in the 20–30-year age group, 27 (37%) in the 30–40-year age group, 16 (21.9%) in the 40-50 year age group and 10 (13.6%) above 50 years. Most responders worked under contract with the national health care system, while 12 (16.4%) participants only worked in private settings. 72 (98.6%) out of 73 responders use some form of TD, the most frequently utilized platform being WhatsApp with 69 users (95.8%), followed by classical telephone call/message in 52 participants (76.5%) and email-based TD in 35 participants (57.4%) (Figure 1). TD is being used for the diagnosis and treatment of dermatological disorders daily by 23 (31.5%) participants and every few days by 30 (41.1%) participants.

Table 1: Participant characteristics

Total no of responders	**73**	
	No	**%**
Gender		
Female	65	89%
Male	8	11%
Area of dwelling		
Urban	73	100%
Years in practice		
0-10 years	24	32.9%
11-20	11	15.1%
21-30	5	6.8%
31+	8	11%
Current resident	25	34.2%
Setting		
Out-patient clinic public	11	15.1%
Out-patient clinic private	10	13.7%
Inpatient public	23	31.5%
Both public and private settings	27	37%
Inpatient private	2	2.7%

TD is being used as a tool to professionally discuss cases between peer colleagues daily by 21 (29.2%), every few days by 27 (37.5%), once a week by 10 (13.9%), and less than once a week by 14 (19.4%) of participants.

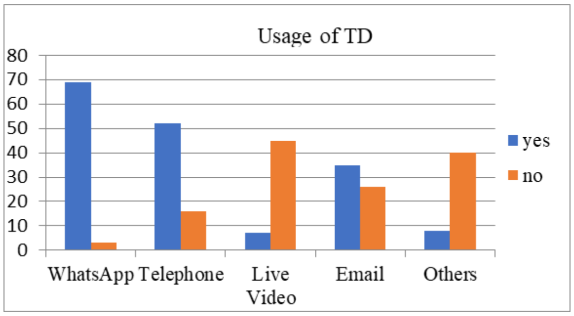

Figure 1: Usage of TD among dermatologists

TD is considered to be most useful as tertiary TD, in communicating with peer dermatologists, with 68 (94.4%) affirmative responses; it may be useful to some extent to communicate with physicians of other specialties 63 (90.0%), with family doctors 51 (72.9%) and with patients 51 (69.9%). Only 4 responders (5.5%) consider that TD did not increase workload, while 19 physicians (26.0%) consider that the workload mostly increased during their free time and 48 (65.8%) realized that TD-related tasks surged both during working hours and leisure period (Figure 2).

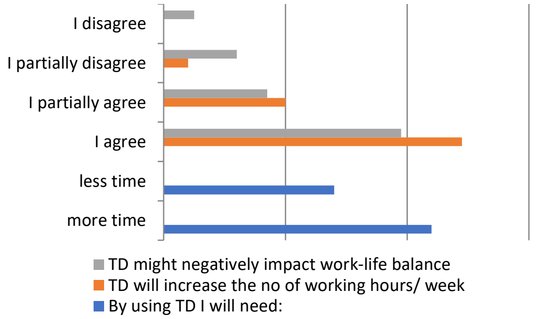

Figure 2: TD and work-life Balance

58 (79.5%) participants agreed to some extent that the relationship between a patient and the physician may be less developed in a TD setting and 67 (91.8%) responders contend that it may be less informative compared to classical consultations. Most replies, 55 (75.3%), were not reimbursed for the service of TD consultations; for 7 (9.6%) it was expected from the work environment, while another 7 (9.65) were partially or fully reimbursed 4 (5.5%).

41 (56.2%) dermatologists agree that consultations performed via TD might have a lower accuracy compared to classical consultations, while 29 (39.7%) partially agreed with this statement; clear images, complete anamnesis, and communication with the patient were considered key factors that might enhance diagnostic accuracy. Both forms of TD, synchronous and asynchronous TD, were considered as having advantages and disadvantages. Participants were asked the most common pathology they were contacted for during the last week through TD and the most frequent skin disorders were allergic reactions with 25 (34.7%) answers, acne with 10 answers (13.9%), bacterial infections with 7 (9.7%), followed by viral infections and malignant lesions with 6 (8.3%) answers each. Most dermatologists 61 (83.6%) felt uncomfortable offering medical information regarding malignant cutaneous pathology through TD. Physicians partially agree 38 (52.1%) and agree 22 (30.1%) that TD may facilitate the follow-up of treatment related to adverse effects. Regarding the statement that TD will increase the number of working hours/week, 49 (67.1%) agreed and 20 (27.4%) partially agreed. A large number of responders, 45 (61.6%) agreed and 24 (32.9%) partially agreed that TD might negatively impact the work-life balance. Participants agreed in 53.4% (39 answers) and partially agreed in 23.3% (17 answers) that by using TD many patients trivialize the dermatological consultation. Also, dermatologists feel that TD should be financially sustained in 76.4% (55) of answers or partially agree with the statement in 20.8% (15). In our cohort, 51 (69.8%) physicians believe that they will use more TD in the future. 41 (56.2%) agree and 28 (38.4%) partially agree that this rise will be due to patients request. Most health care professionals agree 29 (39.7%) and partially agree 31 (42.5%) that TD could be employed as a useful method in the triage of patients.

Upon statistical analysis, age was inversely correlated with the custom of discussing certain cases with colleagues ($p=0.009$), with the utility of TD with the family doctor ($p=0.018$) and peer dermatologists ($p=0.02$), with the future usage of TD ($p=0.015$) and the utility of TD in the triage of patients ($p=0.015$). The usefulness of TD in communicating with the patient and with general practitioners was correlated with the usefulness of TD as a tool in the triage of patients ($p<0.1$ and $p=0.01$). Future use of TD was inversely correlated with a less developed doctor-patient relationship ($p<0.01$), with a lower diagnostic accuracy ($p<0.01$), with the trivialization of consultation ($p<0.01$) and longer working hours ($p<0.01$) and positively related to better monitoring of adverse effects ($p=0.0$). Lack of information through TD was related to more time invested by the physician ($p<0.01$) and inversely correlated with monitoring of adverse effects ($p=0.02$). The lack of accuracy of TD was also related to more time invested by the physician ($p<0.01$) and longer working hours ($p<0.01$). Disturbance of work-life balance was related to more time required by TD ($p=0.05$) and consult trivialization ($p<0.01$). Finding a financial solution for TD was related to better monitoring of adverse effects through TD ($p<0.01$).

DISCUSSION

A relatively high usage of TD was noticed in our cohort, with 53 (72.6%) participants employing it a few times a week. Taking into account that 60 (82.2%) participants consider that TD could be a useful method for selecting patients in the future, we believe that some of these consultations are aimed at prioritizing patients according to pathology. Indeed, TD might be useful in the triage of patients, its' cost- effectiveness increasing when patients need to travel farther distances to benefit from the dermatological service [5].

Tertiary dermatology, or a "second opinion" among specialists, is a valuable tool for reinforcing or modulating the physician's perspective on certain pathology but also as a learning tool for residents/young specialists. In our study, TD was used for the means of tertiary dermatology by 48 (66.7%) participants daily or every few days and once or less than once a week by 24 (23.3%) participants. The vast majority of our responders, 68 (94.4%), considered TD as being useful in communicating with peer colleagues while younger age was also correlated with the use of TD in communicating with peer colleagues, family, or specialist physicians. A recent systematic review that focused on TD education both in medical students and postgraduate trainees indicated high satisfaction rates and improvement in educational outcomes among medical students, residents, and other trainees, some residents mentioning that TD provided a low-stress learning environment and some students considering that eConsult-based training enhanced their education [6,7].

Secondary to tertiary-care teleconsultation is applied less frequently, however, a study performed in the Netherlands that included 39 dermatologists showed that it had important advantages such as fast response time, formalized records, and data and privacy security(6). However, physicians also prefer to use means of communication they are accustomed to because of ease of use and direct personal network[8,9]. A study evaluating practice models in TD in Spain indicated that the main advantages of TD were prioritization in cancer screening, rapid emergency care, training of and communication with primary care physicians, and screening for trivial conditions while also reducing in-person visits [10]. A TD service between primary healthcare centers and the National Skin Centre in Singapore using "store and forward TD" indicated satisfaction for the TD service (by both primary and specialist doctors), more convenience for the patients but also an educational value for family physicians, while considering challenging the quality of the images, lack of personal assessment of the skin pathology and of the patient as a whole [11,12].

In our study, 56 (76.7%) agreed (fully or to a certain extent) that patients trivialize dermatological consultations when performed as TD. This usually implies a lack of complete information regarding the skin problem but also carelessness regarding compensation, leading to incomplete medical advice and frustration.

Financial aspects will directly influence the usage of TD. A cross-sectional study performed in the US involving 591 dermatologists documented low reimbursement (69.8%) as one of the most common barriers to the implementation of TD while the majority of respondents (85.2%) felt reimbursement for store-and-forward TD was too low [13]. A global survey developed by WHO in 2010 indicated that TD is more acknowledged in high-income countries, difficulties in implementing were legal issues concerning the privacy of patients, limited reimbursement for practitioners as well and competing health system priorities. High costs are also an issue in developing countries, alongside resource, infrastructure, and technical limitations [14,15]. Therefore, we may observe that financial issues do influence in different manners the implementation and use of TD. In certain areas, specialists do not receive extra compensation for addressing professional questions from primary care physicians but time slots in the weekly schedule were allocated for such tasks, integrating TD alongside other tasks [16].

In our cohort, the use of TD has increased workload both during working hours and in free time, physicians expect a further increase in the future while 69 (94.5%) dermatologists partially/completely agree to the fact that TD might negatively impact the work-life balance. A cross-sectional study evaluating usage patterns on WhatsApp TD (WhatsApp being the most frequently used platform in our study as well) underlined the fact that the added workload both during and after work hours was the biggest disadvantage of WhatsApp, potentially leading to burnout. Both primary health care physicians, as well as specialists, reported being consulted during working hours but also after 20:00, during weekends, holidays, or when abroad [16].

The most common pathologies dermatologists were contacted for were allergic reactions, acne, bacterial and viral infections as well as malignant lesions. A study performed in pediatric patients described acne, atopic dermatitis, benign melanocytic nevi, and verruca as being the most common diseases treated by TD in the adolescent age group. In contrast, atopic dermatitis, verruca, and molluscum were the most frequent diseases in the 3-12 year age group [17]. Most dermatologists who participated in the survey considered that TD might have a lower accuracy compared to classical consultations. This concern has previously been reported by US-based pediatric dermatologists who, even though positive about TD's present and prospects, considered TD as being inferior to in-person care regarding accuracy and management plans [9,18]. This may pose the specialist in an uncomfortable position, having to give a diagnosis and offer a treatment plan while not being fully confident of the diagnosis. However, this situation is avoided when there is a face-to-face meeting for the initial diagnosis and the monitoring is performed by TD. A randomized clinical trial including patients with atopic dermatitis, where the initial visit was in-person while the follow-up was through TD versus personal consultation for a year, showed similar improvement in the disease severity of patients [19]. A similarly structured- study demonstrated that the quality of life of patients with atopic dermatitis improved both when followed up through TD or in person [20]. Very good results for monitoring were also obtained for store-and-forward TD in case of pathologies like acne and psoriasis [21].

When asked about which pathologies they felt uncomfortable offering consultation through TD, 59 (80.8%) answered malignant tumoral lesions. Indeed, the accuracy of TD in skin neoplasms was inconsistent between studies, with a moderate accuracy reported by Giavina-Bianchi et al [22] and a high accuracy reported by Moreno-Ramirez et al [23] and Kroemer et al [24]. One should take into consideration that diagnostic precision increases when both clinical and dermoscopic images are available, but, also the cautiousness of dermatologists of not missing a melanoma and, while a severe dysplastic nevus would still be in a "benign" category, the excision of such a lesion would be in the benefit of the patient. Another aspect to take into consideration is that dermatological consultation of patients referred through TD detected "unobserved" malignant lesions both on exposed and covered skin [25], highlighting the usefulness of intertwining both methods of consultation.

The future perspectives of TD look promising. Most of our participants 51 (69.8%) agreed or partially agreed to the statement that they will be using TD in the future and 69 (94.6%) to the statement that it will requested by patients in the future. A cross-sectional study performed in the US aiming at dermatologists also showed that 70.9% of the physicians foresee that TD could become an ongoing component of their practice, provided that reimbursement for video visits remains similar to that of in-person visits [9].

Among the limits our study has, we would like to mention that participants were selected from the database of the clinic, therefore, we believe most answers were from the Transylvanian part of the country. Number of participants can virtually always be increased to obtain more accurate results. Still, we believe the data we obtained is relevant, especially because the official implementation of TD differs more between employers and the type of work setting compared to a certain region in the country. Rapid development of social media platforms and artificial intelligence quickly alters the work environment for dermatologists, leading to changes in perception and usage that only be captured by repetitive assessments performed at different time-frames**.**

CONCLUSION

In conclusion, dermatologists currently use TD in addition to classical consultations for patients, to manage cases from family physicians but also to discuss and better manage complex cases. TD proves to be an important tool in the education of young dermatologists. The swift evolution of technology brought us this tool that is under continuous development but that is here to stay.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, CE, MNM, AB; methodology, CE, MNM, SD.; software, PAI; validation, all authors; investigation, CE, MNM, F.S.; data curation, SD, PAI., A.N.; writing—CE, X.X., F.S., M.C.D.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted following the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Medicine and Pharmacy "I. Hatieganu" Cluj Napoca number 172.

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Supplementary material – Dermatologists' views on the use of teledermatology

Within the first section, anonymous personal data was collected through several questions. Participants had the option of selecting gender, area of the dwelling, and age (by selecting between age groups, namely "20-30","30-40","40-50", "50-60", "60-70" and ">70"), the setting they provide dermatological health care ("public ambulatory", "private ambulatory", "public hospital", "private hospital" and "public & private ambulatory/hospital") and the years of clinical experience ("Resident", "Specialist for 0-10 years", "Specialist for 10-20 years", "Specialist for 20-30 years", "Specialist for 30-40 years" and finally "Specialist for >40 years"). A question assessing the time of current use of computer/tablet/phone/television was included in the survey but due to its lack of statistical significance, it was not reported in the manuscript. The second section of the questionnaire focused on the current use of Teledermatology. Participants were first asked to express the method they currently employ for TD by choosing from the following possible answers: "WhatsApp messenger", "Telephone", "Email", "Live Video conferencing" and "other devices". To assess the frequency of TD in skin disease diagnosis/treatment and the frequency of using TD in the management of cases, four possible answers were offered to the two questions: "never", "daily", "every couple of days", "once per week", "less than once per week". To evaluate the workload, the statement "The use of TD increased my workload during:" was followed by four possible answers "working hours", "free time", "both working hours and free time" and "it did not increase my workload". The following question referred specifically to the impact of COVID-19 on TD, asking "Usage of TD increased:" with the following answers: "during the COVID-19 pandemic", "after the COVID-19 pandemic" and "COVID-19 did not influence the usage". Regarding compensation, participants were asked whether they received any benefits for TD consultations and also whether they believed they were supposed to; responders had the option of selecting the following: "Yes", "No", "Partially" and "not applicable (part of the contract)".

To answer the questions of whether through TD the doctor-patient relationship is less developed and the TD-consultation is less informative, four choices were given: "I don't agree, I don't agree, I partially agree, I agree". Participants were given the option of selecting "yes, no, partially agree" to whether TD is more useful in communicating with patients, family physicians, dermatologists, and other specialists.

The next section of the questionnaire was directed towards how physicians feel about their diagnostic accuracy through TD. To the question of whether TD- followed diagnosis accuracy could be lower compared to in-person consultations and whether accuracy increases when collaborating with family physicians, the answer options were: I don't agree", "I rather not agree", "I agree partially" and "I agree". To improve the diagnostic accuracy in TD, the following options were given: "Clear images with optimal resolution", "Complete information from anamnesis", "Prior appointment for the TD consultation", "the presence of a dialogue with the patient". In the following question, dermatologists could select which type of TD is more useful, what was the cutaneous pathology they were most frequently contacted for during the last week and what is the pathology for which they don't feel comfortable offering TD-consultations.

To evaluate the workload, participants were asked whether they would be more or less time-efficient by using TD and whether the use of TD would increase the number of working hours/week. The agreement of participants regarding the trivialization of consultation, regarding ease in the follow-up of treatment-related adverse effects was also assessed.

Future perspectives and usage trends of TD were assessed by asking physicians whether they believe they will use more TD in the future, whether they believe patients will request more TD in the future, whether TD could be an option for triage, whether TD would affect their work-life balance and whether the future of TD would depend on finding a financial reimbursement, with the following scale of agreement: "I don't agree", "I rather not agree", "I agree partially" and "I agree".

Nevertheless, throughout the study, all participants had the chance to add personal ideas after each section, if they wanted to express answers that were not displayed.

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REVIEW

Monkeypox: Pathogenesis, Prevention, Threats, Challenges and Immune Response Against It

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Abstract: *A rare zoonotic disease, monkeypox is brought on by the Monkeypox virus, which is a member of the Orthopoxvirus genus, Chordopoxvirinae subfamily, and Poxviridae family. Smallpox-like symptoms are brought on by the monkeypox disease, which is closely linked to the variola virus (smallpox virus). In the past, it has been demonstrated that smallpox vaccination with the vaccinia virus (another orthopoxvirus) is 85% protective against monkeypox. The two most likely forms of monkeypox transmission are animal-human transmission and human-human transmission. Transmission between people has been connected to respiratory droplets, coming into contact with bodily fluids, contaminated patient things or surroundings, and skin lesions on infected people. Numerous strategies or tactics have been developed by monkeypox to subvert or exacerbate the host's immune reaction to infection. A crucial element of innate immunity, natural killer [NK] cells employ cytokines to kill virus-infected cells and interfere with the operation of other cell types like T cells and dendritic cells. Studies have shown that the smallpox vaccine provides cross-protection against other Orthopoxvirus species, including monkeypox. This study was out to provide a thorough overview of the pathogenesis, prevention, risks, immune response problems, and analysis of possible concerns associated with the monkeypox virus.*

Keywords: *Monkeypox, pathogenesis, prevention, threats, challenges, immune response*

INTRODUCTION

Numerous levels of worry, terror, mistrust, and rumors have been circulated among the general public as a result of the quick spread of new diseases. Rapid scientific knowledge dissemination is a useful strategy for reducing the fear and worry that emerge with new outbreaks. This won't be possible unless epidemiologists and statistical modelers offer in-the-moment studies to forecast future conditions and potential treatment effects [1]. In order to solve the health issue as soon as is practical, it should be underlined that real-time analysis is the analysis of fresh available data utilizing logic and mathematics as soon as the data is created. In Wuhan, China, instances of pneumonia with no known cause were first reported in late December 2019. A unique form of coronavirus was later found to be the source of those pneumonia cases in January 2020 [2]. The World Health Organization (WHO) officially named the ailment COVID-19 (short for coronavirus illness 2019), and the virus was given the moniker SARSCOV-2 (severe acute respiratory syndrome coronavirus 2). Iran and other nations across the world made major steps in this direction despite the COVID-19 epidemic being alarming and it being crucial to design and implement appropriate regulations [3]. In Wuhan, China, instances of pneumonia with no known cause were first reported in late December 2019. A unique form of coronavirus was later found to be the source of those pneumonia cases in January 2020. The World Health Organization (WHO) officially named the ailment COVID-19 (short for coronavirus illness 2019), and the virus was given the moniker SARSCOV-2 (severe acute respiratory syndrome coronavirus 2). Iran and other nations across the world made major steps in this direction despite the COVID-19 epidemic being alarming and it being crucial to design and implement appropriate regulations [4]. Since the late 1970s, there have been no confirmed cases of smallpox. As a result of their disease, up to 30% of people passed away. Toximemia and systemic shock, a condition that affects the entire body, were the causes of death (toxins in the blood). This study set out to provide a thorough overview of the pathogenesis of the monkeypox virus, its hazards, obstacles to the immune system's response, and an analysis of potential threats.

SMALLPOX

In a second outbreak, up to 80% of household contacts could contract the highly contagious disease known as smallpox. Those who survived the illness typically experienced long-term effects like blindness and significant scarring [5]. In order to continue studying vaccines and treatments, researchers saved some samples of the smallpox-causing variola virus. Only two locations on Earth contain these virus samples. They are secure at the State Research Center of Virology and Biotechnology in Russia and the Centers for Disease Control and Prevention (CDC) in Atlanta (VECTOR Institute). Public health officials are only concerned about smallpox spreading as a result of biological warfare because it is no longer present in nature [6]. There hasn't been any recent evidence of terrorists using smallpox as a weapon. On the other side, scientists are prepared to react if smallpox is employed as a weapon. The CDC has enough smallpox vaccine to protect everyone living in the United States in the event that the virus reappears. The variola virus is what causes smallpox. In the past, prolonged, direct face-to-face contact with people was the main method of spreading smallpox. When they coughed or sneezed, respiratory particles would travel through the air. If other people inhaled these large drops, they would catch the infection [7]. A less frequent way for people to contract an infection is by direct contact with the rash or crust material from a swab. People can pass the virus to one another by sharing linens, towels, and clothing. The condition is most contagious when throat and mouth ulcers initially appear. On the other hand, smallpox remains contagious for a number of weeks after the initial lesions manifest. Since smallpox no longer occurs naturally, smallpox diagnoses are extremely uncommon today. Any symptoms you have are almost always a sign of another illness or condition [8]. Prior to its elimination, smallpox was easy to distinguish from a few other common illnesses, such as severe chickenpox. If other people breathed in these large drops, they would catch the infection. A less frequent way for people to contract an infection is by direct contact with the rash or crust material from a swab. People can pass the virus to one another by sharing linens, towels, and clothing. The condition is most contagious when throat and mouth ulcers initially appear. Contrarily, smallpox is infectious for several weeks following the onset of the first lesions [9]. Since smallpox no longer occurs naturally, smallpox diagnoses are extremely uncommon today. Any symptoms you experience are almost certainly a sign of another illness. Prior to its elimination, smallpox was easy to distinguish from a few other common illnesses, such as severe chickenpox. Human monkeypox was discovered in 1970 after regional smallpox elimination revealed isolated cases of a comparable illness in remote areas of the DRC. Since then, extensive research has been done on the epidemiological and clinical features of the condition, but no specific medical countermeasures have been created [10]. It could take anywhere between 5 and 21 days from the moment of exposure to the onset of symptoms. On average, symptoms endure between two and four weeks. Although it is unknown to what extent it can happen without symptoms, there may be very few indicators.

MONKEYPOX

There is currently no known animal reservoir host for the zoonosis monkeypox (Figure 1), making preventative strategies challenging. In addition to the DRC (CAR), cases have recently been reported from Nigeria, the Republic of the Congo (ROC), Sierra Leone, Liberia, Cameroon, and the Central African Republic [11].

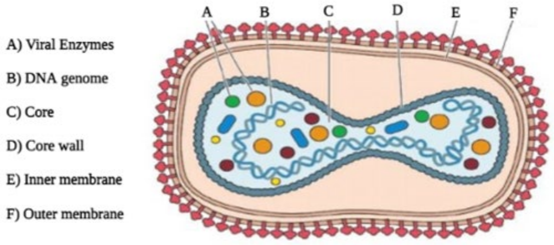

Figure 1: Structure of Monkeypox

In several of these nations, there had been no reports of human monkeypox for decades. The cessation of routine smallpox immunization, which led to a decline in orthopoxvirus immunity, has been blamed for the rise in cases of monkeypox. Since May 13, 2022, cases of monkeypox have been reported to WHO from 12 Member States that are not endemic for the virus and span three WHO regions. Epidemiological research is ongoing, but to date, there have been no established links to travel to endemic regions [12]. According to the information that is currently available, occurrences have mainly — but not exclusively — been found among men who have sex with men (MSM) who visit general care and sexual health clinics for treatment. This Disease Outbreak News aims to raise awareness, offer technical support for urgently advised measures, and provide information to support readiness and response operations. The situation is evolving, and according to WHO, additional cases of monkeypox will be discovered as surveillance in non-endemic countries increases [13]. In order to stop the disease from spreading further, the first step will be to accurately notify those who are most at risk of contracting monkeypox. The most vulnerable individuals, according to the available studies, are those who have had close personal contact with a monkeypox patient. The WHO is also creating policies to safeguard frontline healthcare providers and other vulnerable healthcare employees, such as housekeepers. The WHO will offer more technical recommendations over the coming days [14].

Monkeypox signs and symptoms

Early signs of monkeypox frequently include headaches, muscle pains, fever, and fatigue, thus they may initially resemble the flu (Figure 2). Swollen glands are a symptom of the illness, which might mimic chickenpox, measles, or smallpox. These generally show up behind the ear, below the jaw, in the neck, or in the groin before the rash does. After a few days of the fever, lesions often start on the face before spreading centrifugally to the palms of the hands and soles of the feet [15].

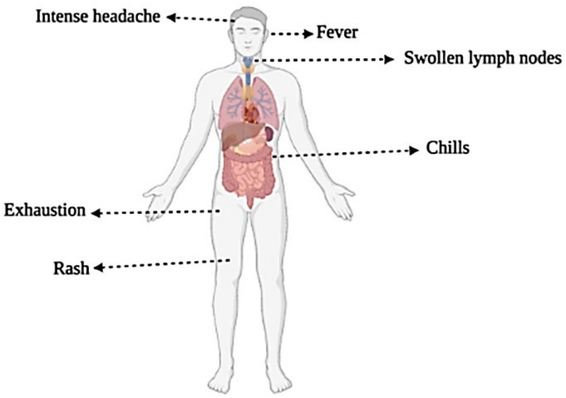

Figure 2: Symptoms of monkeypox disease (illness lasts for 2-3 weeks)

Patients with HIV may have a number of symptoms. Several people in the 2022 monkeypox outbreak also experienced fever, swollen lymph nodes, and difficulties swallowing in addition to vaginal and perianal lesions. One in five infected people have lesions in their eyes, more than two-thirds have lesions in their mouths, more than two-thirds have lesions in their genital area, and more than two-thirds have lesions on their palms and soles. They begin as little flat patches, then transform into tiny lumps, filling with clear, then yellow fluid, finally bursting and scarring [16]. There may be a few or many thousand lesions, which occasionally combine to form larger lesions. Every area of the body that is afflicted by lesions develops at the same stage. It resembles a smallpox rash in appearance. The rash often lasts for ten days or less. Affected people may feel under the weather for two to four weeks. After the lesions have healed, they may leave behind pale marks before leaving behind black scars. Only a small amount of infection from person to person has been observed in Africa's disease-endemic regions [17].

Pathogenesis of monkeypox

With the exception of the fact that viral entry from a wildlife source most frequently occurs through small lesions on the skin or oral mucous membranes, the pathogenesis of human monkeypox is substantially the same as that of smallpox. Viral entry can also happen through the respiratory tract in rare instances of person-to-person transmission. Although the monkeypox virus replicates in lymphoid tissue similarly to the smallpox virus, it results in greater lymphadenopathy [18]. The virus initially manifests in mononuclear phagocytic cells, moves through the bloodstream, and eventually resurfaces in skin cells. The virus swiftly infects nearby lymph nodes after an infection. On the third or fourth day following infection, the virus enters the bloodstream and moves to the bone marrow, sleep, and other lymph nodes. Once the virus has been in the blood for 8 to 12 days, fever and toxemia set in [19]. The virus has now been localized in the oral mucosa and dermal blood vessels, resulting in the initial enanthem and exanthem. By day 14, the host is contagious, and high viral titers are found in the kidneys, liver, spleen, lymph nodes, and bone marrow. Skin lesions develop as a result of endothelial cell vessel walls and dermal capillaries dilating [20]. When these lesions reach the epidermis, swelling cells eventually grow so enormous that their membranes rupture. Pus forms after vesicular lesions as a result of the concentration of polymorph nuclear cells inside the vesicle. The legions scramble when the pus progressively vanishes. The most frequent cause of mortality is overwhelming toxemia, which is most likely brought on by circulating immune complexes. The long-term repercussions of secondary infections include encephalitis, blindness, and arthritis [21].

Epidemiology of the disease

While clinically less severe than smallpox, monkeypox is a viral zoonosis (a virus that spreads from animals to people) with symptoms that are strikingly similar to those experienced by smallpox patients in the past. It is brought on by the orthopoxvirus genus monkey poxvirus, which is a member of the Poxviridae family. The West African and Congo Basin (Central African) clades of the monkeypox virus are separated. Monkeypox is the name given to the virus because it was originally identified in primates in a Danish laboratory in 1958. [22]. The first human case was found in a child in the Democratic Republic of the Congo in 1970. The monkeypox virus can be transferred from one person to another by close contact with lesions, bodily fluids, respiratory droplets, and infected surfaces like beds. Monkeypox can incubate for anywhere from 5 and 21 days, but it typically takes 6 to 13 days. Numerous animal species have been identified to be susceptible to the monkeypox virus's contagiousness [23]. Further investigation is required to identify the precise reservoir(s) and how virus circulation is maintained in nature because the natural history of the monkeypox virus is still unknown. Eating raw meat and other animal products from diseased animals may pose a risk [24]. Although it can be severe in some people, such as children, expectant mothers, or those whose immune systems have been weakened by other illnesses, monkeypox is typically self-limiting. With a case fatality rate of 3.6% compared to 10.6% for the Congo Basin clade, the West African clade appears to induce less severe disease in humans [25].

Geographical distribution and high-risk areas

With 106 persons confirmed to have the disease as of May 26, 2022, the UK has the highest number of cases in a non-endemic country, according to the WHO, followed by 49 people in Portugal and 26 in Canada (Figure 3).

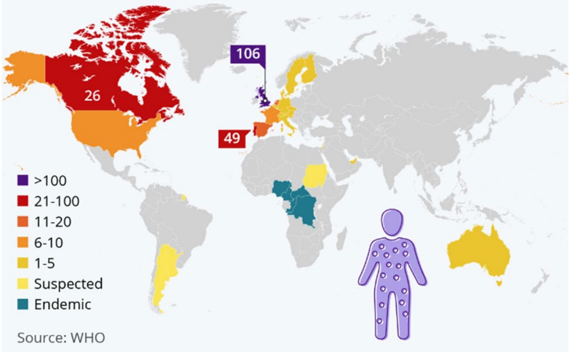

Source: WHO

Figure 3: Number of recorded monkeypox cases per country (May 13-26, 2022) and where the disease is endemic (Source WHO).

No fatalities have been connected to the incident as of yet. In the instances that have been recorded so far, there have been no confirmed travel connections to an endemic region [26]. According to the information that is currently available, occurrences have mainly — but not exclusively — been found among men who have sex with men (MSM) who visit general care and sexual health clinics for treatment. All cases whose samples had the West African lineage of the virus confirmed by PCR to date have been identified. A genomic sequencing from a swab sample from a confirmed case in Portugal showed a close match to the monkeypox virus driving the current outbreak to cases shipped from Nigeria to the United Kingdom, Israel, and Singapore in 2018 and 2019. [27]. Finding confirmed and probable cases of monkeypox without any known travel history to an endemic region is unusual. Until recently, surveillance in non-endemic areas was minimal, but it is currently being increased. According to the WHO, more instances are anticipated to be reported in non-endemic regions. The research suggests that those who come into contact with symptomatic instances directly on a bodily level are where human-to-human transmission happens [28]. Aside from this new epidemic, WHO continues to receive data through established surveillance techniques on the status of ongoing cases of monkeypox in endemic countries (Integrated Disease Surveillance and Response). Monkeypox is endemic in Benin, Cameroon, Central African Republic, Gabon, Ghana (only in animals), Ivory Coast, Liberia, Nigeria, Republic of the Congo, Sierra Leone, South Sudan, and the Central African Republic [29].

WHO risk assessment

Only West and Central Africa often experience the endemic monkeypox disease. There is an urgent need to increase awareness of monkeypox and carry out thorough case finding and isolation [along with supportive care], contact tracing, and supportive care to prevent further onward transmission because it is unusual to find confirmed and suspected cases of monkeypox in multiple countries without a history of travel to an endemic area [30]. Cross-protective immunity from smallpox immunization will be restricted to the elderly since populations under the age of 40 or 50 no longer benefit from the protection offered by prior smallpox vaccination programs. Younger individuals have only a limited level of immunity to monkeypox because the virus has not been seen in non-endemic regions. In the past, it has been discovered that the smallpox vaccine can prevent monkeypox [31]. Despite the licensing of one vaccine (MVA-BN) and one specific treatment (Tecovirimat) for monkeypox in 2019 and 2022, respectively, these preventative interventions are still not generally accessible. According to the information that is currently available, occurrences have mainly — but not exclusively — been found among men who have sex with men (MSM) who visit general care and sexual health clinics for treatment. No fatalities have been noted to date [32]. The extent of local transmission is currently uncertain, though, because of the scant observation. There is a good likelihood that other cases, including in different population subgroups, with unidentified transmission networks, will be found. Cases of monkeypox have been recorded in several countries spanning several WHO regions, and it is very likely that cases will be found in more nations [33]. The situation is evolving, and according to WHO, additional cases of monkeypox will be discovered as surveillance in non-endemic nations grows. So far, there have been no fatalities connected to this outbreak. The first efforts should concentrate on informing those most at risk of monkeypox infection accurately, stopping the disease's spread, and safeguarding first responders [34].

Host Immune Responses to Monkeypox

Poxviruses have developed a variety of methods or tactics to circumvent or worsen the host's immune reaction to infection. Although much study has been done on the pathology and pathogenesis of monkeypox, the innate and adaptive immune responses to monkeypox infection are still poorly understood due to a paucity of data. A crucial element of innate immunity, natural killer (NK) cells employ cytokines to kill virus-infected cells and interfere with the function of other cell types like T cells and dendritic cells [35]. Activating or inhibitory NK cell receptors engage with their ligands, such as major histocompatibility complex 1 (MHC-1) molecules, to cause activation or inhibition of NK cells. Granule secretion, which includes perforin and granzyme, and cell-cell contacts are the mechanisms by which NK cells carry out their killing activity. IFN- and TNF-, which are generated by NK cells in the early stages of infection, regulate dendritic cells to polarize T-helper type 1 (Th1) cells and mediate inflammatory responses in inflamed tissues [36]. Infections with human monkeypox have not been associated with T cell or antibody responses in Nigeria. Additionally, there are no data or reports on the immunological reactions that occur after experimental infections in animal models. An immunologically naive population that is very susceptible to monkeypox infection has been caused by the lack of cross-protection produced by the smallpox vaccination, as well as the probable weakening of herd immunity and immunosuppression as a result of HIV/ AIDS. This may be the cause of the current global resurgence of monkeypox (Figure 4) [37].

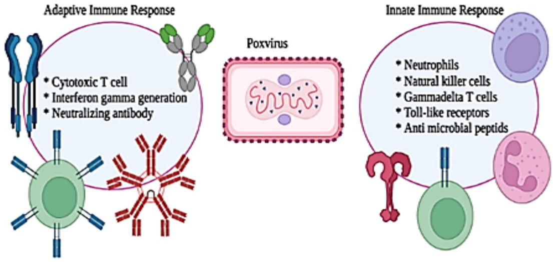

Figure 4: Host Immune Responses to Monkeypox

Prevention and Vaccination

It is expected that smallpox vaccination will protect against human monkeypox infection because the two viruses are closely related and the vaccine shields animals from lethal monkeypox challenges in experiments. This has not been properly demonstrated in individuals because systematic smallpox immunization was stopped when smallpox was eradicated. The smallpox vaccine has been demonstrated to reduce the risk of monkeypox in previously immunized individuals in Africa. A reduction in poxvirus immunity in communities that have been exposed affects the occurrence of monkeypox [38]. It is attributed to a rising proportion of unvaccinated people as well as a decline in cross-protective immunity among those who received an immunization before the cessation of mass smallpox vaccinations in 1980. The US Centers for Disease Control and Prevention (CDC) advises being vaccinated against smallpox in order to protect themselves from the disease if they are going to be handling sick people or animals or looking into outbreaks of monkeypox. Anyone who has had close or direct contact with people or animals that have monkeypox infection is also advised to get vaccinated [39]. The CDC advises against pre-exposure immunization for veterinary professionals, veterinary assistants, and animal control officers unless they are taking part in field investigations. The CDC advises healthcare professionals to put on a full set of personal protective equipment before treating an infected person (PPE). Included are a gown, mask, pair of goggles, and a disposable filtering respirator (such as an N95). An infected person should be isolated in a negative air pressure chamber or, at the very least, a private exam room to prevent others from coming into touch with them [40]. In the United States and the European Union, Tecovirimat has a license to treat a variety of poxviruses, including monkeypox. BMJ Best Practice recommends tecovirimat or the smallpox drug Brincidofovir, together with supportive care, if antiviral treatment is required [including antipyretic, fluid balance, and oxygenation]. Aciclovir or empirical antibiotic therapy may be used if a subsequent bacterial or varicella zoster infection is suspected [41].

AN ANALYSIS OF POTENTIAL THREATS

While the COVID-19 epidemic is still going strong, other viral diseases are starting to show warning symptoms in various nations around the world. Since the disease's initial identification in 1958, small, recurring outbreaks of monkeypox have been noted. However, with 100 confirmed cases and almost 300 suspected cases recorded in more than 16 countries, we are now witnessing a larger spread of the virus [42]. 13 solitary cases of monkeypox have been documented in recent years, the majority in a single nation. The virus can be found in Central and West Africa, where it is believed to be present. Three cases of monkeypox were reported in Britain in May and June 2021; each case was linked to travel to West Africa. The World Health Organization claims that the current outbreak is different and that there are far more cases, more cases of human-to-human transmission, and more cases of infection outside of Africa [43]. Although there is limited human-to-human transmission, monkeypox is typically spread from diseased animals to humans in Central and West Africa. Before skin irritation manifests as blisters on the skin, mouth, genitals, eyes, or soles of the feet, smallpox first causes fever, headache, exhaustion, and enlarged lymph nodes. It's probable that the short-term increase in instances of monkeypox transmission has resulted from the resumed travel and big social gatherings after the coronary limitations were loosened [44]. Although current statistics indicate that this strain of the virus is quite similar to the West African variant that has previously caused modest outbreaks, it is also likely that the virus has been changed or modified to allow for wider transmission. However, because of prior coronary infections, this outbreak could develop differently [45]. Our immune systems can be affected by infections from the COVID-19 virus, and current research from Australian experts reveals that this condition may last for weeks to months after the initial infection. According to scientists, it's also conceivable that this immunological dysfunction may modify clinical illness, accelerate viral transmission, or raise vulnerability to infection [46]. Despite the peculiarity of the present monkeypox epidemic, the US Centers for Disease Control and Prevention predict that there will certainly be more in the following weeks. It takes some time to determine the entire scope of an epidemic since symptoms can appear up to three weeks after exposure and the virus can spread for up to a month. Given that the primary way that monkeypox is transmitted is via intimate contact with someone who has a rash or blister, it is unlikely that the illness will provide a fresh hazard at this time [47].

CONCLUSION

Considering what has been said so far, healthcare executives ought to provide staff members with the proper training in four areas: potential threats, prevention, detection, and care, as well as reporting.

Potential threats: Monkeypox is most commonly transferred by intimate contact with someone who has a rash or blister, thus under the current circumstances it is unlikely that the illness would pose a new threat.

Prevention: Direct contact with an infected person, especially sexual contact, puts one at risk for contracting monkeypox. Self-protection measures include avoiding skin-to-skin or face-to-face contact with people who are exhibiting symptoms, engaging in safer sex, washing your hands often with soap and water or an alcohol-based hand rub, and following respiratory etiquette.

Detection and care: People should visit their doctor and get tested for monkeypox if they get a rash that is accompanied by a fever, a feeling of discomfort or sickness, or both. If you have monkeypox, you should stay home until the scabs come off and avoid intercourse, especially oral sex. Patients can get supportive care to ease their monkeypox symptoms throughout this period. Anyone providing care for someone who has monkeypox should take the necessary measures, such as donning a mask and wiping down all objects and surfaces that have been touched.

Reporting: As soon as possible after returning home, anybody with a rash-like illness should consult a doctor and provide information about all recent travel, sexual history, and smallpox vaccinations. Avoid interacting with ill animals including rats, marsupials, and nonhuman primates (alive or dead), as well as eating or handling wild game, if you reside in or visit a country where monkeypox is prevalent (bush meat).

Conflicts of interest and sources of funding

The authors declare that there were no confict of interest regarding this paper.

Authors' contribution

Conceptualization, H.E.G.G. and M.F.; methodology, S.Y.S.; validation, T.B., and M.J.; formal analysis, M.M.; investigation, A.R.; resources, M.M.; data curation H.E.G.G.; writing original draft, A.R.; writing, review and editing, A.R.; visualization, H.E.G.G.; supervision, A.R. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

PET-CT with 18F-FDG in Gynecological Malignancies. Prognostic Significance of the Standardized Uptake Value

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Abstract: *Gynecologic cancers are among the most common cancers in women and are a significant cause of mortality in women worldwide. FDG-PET images play a critical role in several areas of oncology and are an essential tool for the diagnosis and staging of gynecologic cancer, providing information about tumor metabolism. Aim: The aim of this study was to investigate the relationship between tumor differentiation, type of malignancy, and glucose metabolism in patients with confirmed gynecologic cancer. Materials and Methods We performed a retrospective review of 60 consecutive patients (ages 31-76) with histopathologically confirmed gynecologic cancer who underwent PET-CT in our department between January 2021 and March 2022. Quantitative data on glucose metabolism were collected using the maximum standardized uptake value normalized by lean body mass (SULmax). We reviewed a total of 60 patients with a mean age of 60±9 years, and a median of 62. Results: 14 patients were diagnosed with cervical cancer, 20 with endometrial cancer, and 26 with ovarian cancer; 13 patients had well-differentiated cancer, 11 had moderately differentiated cancer, and 36 had poorly differentiated cancer. A positive correlation was observed between the measured SULmax and the degree of tumor differentiation, indicating higher glucose uptake in poorly differentiated cancer. When examining the relationship between the type of cancer and SULmax, we found that endometrial cancer exhibited the highest mean SULmax (8.36±6.75), followed by ovarian cancer and cervical cancer. Additionally, we noted that glucose uptake was higher in patients aged over 62 years. Regardless of the tumor differentiation, the findings in endometrial cancer showed the highest mean SUVmax values in all grades of tumor differentiation. Conclusion: Our study showed that glucose consumption correlates not only with the degree of differentiation but also with age and type of tumor, with the highest values in patients older than 62 years with endometrial cancer. Given the correlation between glucose consumption and tumor aggressiveness, this finding may be important for prognostic evaluation of patients with gynecologic malignancies.*

Keywords: *18F-FDG, PET-CT, Gynecologic cancer, glucose consumption, cancer differentiation*

BACKGROUND

Gynecologic cancers are among the most common cancers in women and are a major cause of mortality in women worldwide. There are five main types of gynecologic cancers: cervical cancer, ovarian cancer, uterine cancer, vaginal and vulvar cancer.

Despite advances in diagnosis, gynecologic cancers remain a major health problem worldwide, with a large number of cases already in a locally advanced stage of the disease at presentation.

Of all gynecologic cancers, only cervical cancer has screening tests that can detect it at an early stage when treatment is most effective. However, despite existing screening programs, cervical cancer is the third most common cancer in women worldwide [1]. With the exception of cervical cancer, there is no simple and reliable method for early detection of gynecologic cancers, making it especially important to recognize the warning signs.

Based on histologic criteria, gynecologic cancers are classified into three grades. Grading is a measure of cell differentiation in the tumor, based on the similarity of the tumor to the tissue of origin. The grade describes the appearance of the cancer cells as follows: Grade 1 (G1; well differentiated) and Grade 2 (G2; moderately differentiated) are the most common categories, while Grade 3 (G3; poorly differentiated) indicates a very aggressive tumor [2].

Positron emission tomography (PET) with fluorine-18-fluorodeoxyglucose (FDG) is an important tool for the diagnosis and staging of gynecologic cancer, providing information about tumor metabolism [3].

FDG-PET images play a critical role in several areas of oncology, primarily because of its ability to detect metabolic changes that may occur earlier and provide more meaningful insights into the effects of therapy compared with morphologic changes alone [4]. As detected by FDG-PET, tumor metabolic activity can provide a faster and more accurate assessment of treatment response. This allows physicians to assess the effectiveness of therapies earlier and adjust treatment plans in a timely manner if needed. By providing valuable metabolic information, FDG-PET contributes to more precise and personalized cancer management that can support treatment decision-making and improve patient outcomes [5].

PET is used in conjunction with computed tomography (CT) to precisely localize regions of increased focal uptake and to compensate for the attenuation effect of incident photons (attenuation correction). With the introduction of integrated PET/CT scanners, the limitations associated with inadequate anatomical definition of functional examinations have been overcome and image quality has been significantly improved [6,7].

In routine clinical practice, PET lesions are analyzed using the standardized uptake value (SUV), a semiquantitative parameter that allows assessment of metabolic activity but is also useful for quantifying the post-therapeutic metabolic response of the tumor. Since 1999, the European Organization for Research and Treatment of Cancer (EORTC) has introduced metabolic criteria that use the standardized uptake value (SUV) in positron emission tomography (PET) to evaluate the response to therapy. These criteria were developed to assess tumor metabolic activity and monitor changes in SUV levels before and after treatment. By analyzing SUV values, these criteria are expected to provide valuable information about the efficacy of cancer treatments and support clinical decision-making [8].

Response to therapy can also be assessed using CT imaging according to the Response Evaluation Criteria in Solid Tumors, but these criteria do not reflect metabolic changes. Tumors detected by morphologic imaging may contain nonviable necrotic tissue. Therefore, measurements from PET may provide more accurate information than a purely anatomic evaluation such as CT [9,10].

The aim of this retrospective study was to investigate the relationship between glucose metabolism, characterized by the uptake of 18F-FDG, measured by the maximum standardized uptake value normalized to lean body mass (SULmax), tumor differentiation, and the type of gynecologic cancer. The study was performed on the 3 major types of gynecologic malignancies – cervical, endometrial, and ovarian cancer.

MATERIALS AND METHODS

Patients

We retrospectively reviewed 60 patients with a type of gynecologic cancer aged 31 to 76 years who underwent FDG-PET/CT between January 2021 and March 2022. PET/CT images were evaluated for primary tumors, locoregional spread, and distant metastases. Quantitative data were collected using the maximum standardized uptake value normalized to lean body mass (SULmax) calculated in the pathologic regions of interest.

PET-CT imaging

PET/CT examinations were performed at the Central Emergency Military University Hospital in Bucharest, Romania, using a dedicated scanner. The weight and blood glucose level of each patient were measured before the examination. The actual injected and residual radioactive doses were also measured.

Before the scan, patients fasted for 6 hours, and only patients with a serum glucose level of < 200mg/dl were injected intravenously with 18F-FDG. Image acquisition started 50-60 minutes after injection. Patients were positioned with their arms raised above their heads. All examinations were performed according to a standard protocol, scanning from tentorium to mid-thigh.

PET/CT data analysis

Maximum SUV normalized to lean body mass (SULmax), was determined from FDG-PET studies and was measured in primary tumors (when possible) and in secondary lesions.

Statistical data analysis

The mean SUV was calculated for each type of cancer and for each of the three grades of differentiation.

RESULTS

From January 2021 to March 2022, a total of 60 patients with a mean age of 60±9 years, and a median of 62, were enrolled in the study. Of these, 14 patients were diagnosed with cervical cancer, 20 with endometrial cancer, and 26 with ovarian cancer. After analyzing the grade of tumor differentiation, we found that 13 patients were diagnosed with well-differentiated cancer, 11 patients with moderately differentiated cancer, and 36 patients with poorly differentiated cancer (Figure 1).

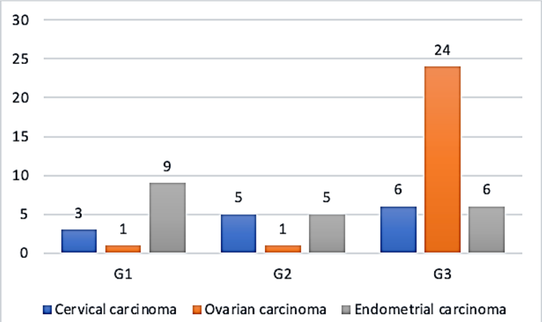

Figure 1: The relationship between gynecologic cancer type and tumor differentiation

The patients' and tumors' characteristics are listed in Table 1.

Table 1: Patient and tumor characteristics

	Age, year
Mean	60+/-9
Range	31-76
Type of cancer, nr.	
Cervical cancer	14
Ovarian cancer	26
Endometrial cancer	20
Tumor differentiation, nr.	
Well-differentiated cancer	13
Moderate differentiated cancer	11
Poorly differentiated cancer	36

The mean SULmax value for the entire group of patients was calculated as 7.83±5.73, with a mean SULmax value of 4.20±3.42 in the group of patients with well-differentiated cancer; 7.96±3.74 in the group with moderately differentiated cancer, and 8.40±6.28 in poorly differentiated cancer. There was a positive correlation between the measured SULmax, tumor differentiation, with higher glucose uptake in poorly differentiated cancer (Figure 2).

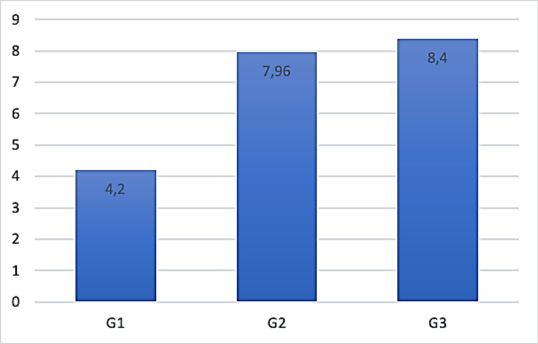

Figure 2: The relationship between glucose metabolism and tumor differentiation

When we analyzed the relationship between cancer type and SULmax, we found that endometrial cancer had the highest mean SULmax (8.36±6.75), followed by ovarian and cervical cancer.

Regardless of differentiation grade, endometrial cancer had the highest mean SULmax values in all differentiation grades. Among moderately differentiated tumors, cervical carcinoma showed higher values than ovarian carcinoma and lower values than endometrial carcinoma, and among poorly differentiated tumors, ovarian carcinoma showed higher values than cervical carcinoma and lower values than endometrial carcinoma (Figure 3).

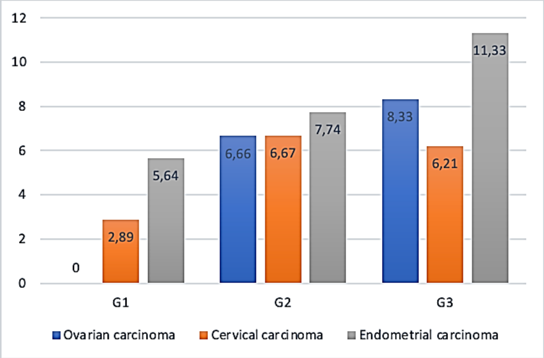

Figure 3: The relationship between glucose metabolism, type of malignancy, and tumor differentiation

The degree of tumor differentiation positively correlated with age, with patients diagnosed with G1 tumors having a mean age of 57±10 years, while patients with G3 tumors have a mean age of 62.7 ±9 years. We also found that glucose uptake was higher in patients older than 62 years.

DISCUSSION

In this study, we found that FDG uptake in primary tumor and secondary lesions varied according to cancer type and degree of differentiation. The SULmax value correlated with the degree of tumor differentiation in all gynecologic cancers.

Endometrial cancer had a significantly higher SULmax value than ovarian and cervical cancer for all tumor differentiation grades. Cervical cancer had higher values than ovarian cancer for moderately differentiated tumors, and for poorly differentiated tumors, the SULmax value was higher for ovarian cancer than for cervical cancer.

The current study also found that poorly differentiated tumors had a higher SULmax value than well- or moderately-differentiated tumors, with a significant difference in SULmax value for well- and poorly-differentiated tumors.

Similar results were obtained by Takagi et al. on a group of patients with endometrial carcinoma. They discovered that the level of glucose uptake significantly differs in patients with different grades of differentiation, being higher in those with G2 and G3. They also found that the degree of glucose uptake correlates with tumor size [11].

Another study that refers to the prognostic value of glucose uptake and its relationship with the degree of differentiation was conducted by Walentowicz-Sadlecka et al. The results demonstrated a progressive increase in glucose uptake as the degree of tumor differentiation advanced. Specifically, patients categorized as G1 exhibited lower uptake, while those in G2 and G3 displayed higher values. However, in their study, statistically significant differences between the three grades of differentiation were observed only in the case of patients classified in the G1 group compared to those in the G2 and G3 groups [12].

Kidd et al. show that tumor differentiation affects 18F-FDG uptake in cervical cancer. They found the same results regarding the correlation between FDG uptake and tumor differentiation as we did in our study. They also found that higher glucose uptake was correlated with an increased risk of lymph node metastasis in patients with cervical cancer [13,14].

More recently in patients with recurrent ovarian cancer, Jiang et al demonstrated that the level of glucose uptake in peritoneal lesions was a significant independent prognostic element [15].

On the other hand, in the study of patients with cervical cancer conducted by Mocciaro V et al, no correlation was found between the SULmax value and the degree of tumor differentiation [16].

The presence of conflicting results indicates that this is a complex area of research that requires prospective studies with a larger number of patients to evaluate the correlation between the parameters of PET and the histopathological features of gynecologic tumors.

Glucose uptake has also been associated with tumor aggressiveness in other cancers. Sachs S et al. reached similar results in a study of patients with lung cancer, showing that higher glucose uptake in the primary lesion was predictive of lymph node involvement and more aggressive tumor behavior [17]. Some authors also suggested that the correlation between FDG uptake in non-small cell lung cancer (NSCLC) and tumor proliferation may allow FDG-PET to serve as a non-invasive method for tumor grading. This is the case in the work published by Vesselle et al. in which tumor differentiation in NSCLC was found to correlate exactly with glucose uptake, with well-differentiated tumors having lower FDG uptake. [18].

As for the relationship between the age of patients at diagnosis and the aggressiveness of the cancer, expressed by the degree of tumor differentiation and SUVmax values, the results of our study suggest a positive correlation. These findings are also supported by studies conducted by Koual et al. and Gonzales-Rodilla et al. who described similar results when studying patients with endometrial cancer [19,20].

The current study provides some new insights into the relationship between gynecologic cancer, tumor differentiation, and glucose metabolism. We believe that the relationship between glucose uptake and the degree of tumor differentiation in gynecologic cancer could serve as a valuable prognostic tool and add to the existing clinical knowledge in this field.

Our study has some limitations – the relatively small number of patients and also the histologic type of malignancies, which included only carcinomas. As mentioned earlier, larger prospective studies are needed to validate glucose uptake, expressed as SUL values, as a prognostic marker in gynecologic malignancies.

CONCLUSIONS

In conclusion, the current study provides valuable insight into gynecologic cancer by correlating glucose uptake and the degree of tumor differentiation. The study also shows that glucose metabolism is highest in endometrial cancer, followed by ovarian and cervical cancer. In addition, the results show that glucose metabolism tends to increase with age. These findings contribute to our understanding of gynecologic cancer and highlight the importance of considering SULmax values and glucose metabolism as potential factors in disease evaluation and treatment.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

R.M, D.C and P.M.S. planned the clinical study and contributed to the concept and design of the study. C.S, C.M., D.C., M. J and F.I. contributed to the data analysis and interpretation as well as to the translation and the critical revision of important intellectual content. T.M. and L.M. contributed to the writing of the manuscript and performed revision, language, and textual editing of the final version.

All authors contributed to the original draft preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

Ethics approval and consent to participate

All procedures performed in this paper were by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and according to the national ethical standards.

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ORIGINAL ARTICLE

Influence of Combat Experience on Psychophysiological and Fine Motor Skill Responses in Air Force Warfighters under Acute Stress

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Abstract: *This study investigates the influence of experience on stress responses and fine motor skills among professional male military parachutists. We analyzed 132 paratroopers, divided into a non-experienced group (NEG; ≤6 parachute jumps) and an experienced group (EG; >50 jumps). Baseline samples were collected 2.5 hours before participants performed a 500 m jump, with post-jump evaluations conducted immediately after landing. Results indicated that experience significantly influences fine motor skills and stress responses. Non-experienced jumpers showed greater degradation in fine motor skills and higher cortical arousal pre-jump levels, suggesting an increased anticipatory state. In contrast, experienced parachutists maintained more consistent fine motor skills and exhibited a ceiling effect in stress response. Leg strength was higher in non-experienced jumpers, reflecting youth and greater muscle mass, with no significant difference in hand strength. These findings highlight the role of experience in managing stress and maintaining motor skills under pressure. The study suggests further exploration into psychological aspects like motivation and resilience, along with the long-term effects of stress exposure. The importance of experience in high-stress military operations emphasizes the need for targeted training interventions to enhance stress-coping mechanisms in less experienced individuals.*

Keywords: *military parachutists; experience; stress response; fine motor skills; psychophysiological analysis*

INTRODUCTION

Fine motor skills are critical for various professionals, including athletes, and are notably influenced by an array of stressors, both psychological and physical [1]. Specifically, acute fatigue resulting from physical exertion can significantly impair these skills [1]. The impact of fatigue is modulated by several factors such as exhaustion, intensity, duration, and type of exercise, as well as the individual's experience level [2]. Traditionally, physiological explanations for the decline in fine motor skills due to fatigue have been focused on biochemical changes, such as the accumulation of hydrogen ions, inorganic phosphate, or ammonia [3]. These alterations adversely affect muscle contractility, hindering ATP-producing enzymes, disrupting the reuptake of calcium ions (Ca^{2+}), and leading to increased sarcoplasmic calcium concentrations [4]. Consequently, these physiological shifts can detrimentally affect the quality of neural connections and electrical activity, ultimately impairing fine motor coordination [3].

Supporting this concept, prior research has revealed notable impacts of physical exertion on motor and cognitive functions [5]. For example, soccer players exhibited a decrease in passing accuracy following a session of intense lower-body resistance training [3]. Similarly, cognitive functions like attention and reaction time were significantly impaired in individuals cycling at 70-100% of their maximum heart rate on an ergometer [6]. These findings underscore the complex interplay between physical activity, physiological stress, and fine motor skill performance, further elucidating the importance of understanding how different variables like exercise type, intensity, and individual training background influence these outcomes [7].

Further on, stress is a pivotal factor that can significantly affect fine motor skills, particularly in high-pressure environments such as the military [8]. The intense demands of military tasks often lead to brain overstimulation as soldiers must allocate extensive attentional resources to their surroundings and activities [9]. This overstimulation can result in concentration difficulties and distractions, shifting focus away from primary tasks and disrupting automatic motor execution [10]. Additionally, anxiety, a common response to the high-stress situations often encountered in military settings, can lead to reduced blood flow to the prefrontal cortex [2]. This reduction means less oxygen and nutrients reach the brain, thereby diminishing the effectiveness of executive functions [11]. Such impairments can negatively influence various brain regions, including the hippocampus, leading to issues with concentration, memory, decision-making, working memory, and ultimately, fine motor skills [11].

Moreover, in a military context, where decisions and actions often have critical consequences, the impact of stress and anxiety on fine motor skills becomes even more significant. Therefore, authors have noted that extreme social pressure, such as pre-competitive anxiety in military exercises or operations, exacerbates these effects [2,12-17]. On this line, both somatic and cognitive anxiety, often heightened in combat or high-intensity training scenarios, can impair motor skills. Furthermore, environmental factors typical in military settings, like exposure to loud noises exceeding 89 dB, can also contribute to the decline in fine motor skills [18]. These factors, combined with the need for precise and coordinated movements in various military operations, underline the importance of understanding and mitigating the effects of stress and anxiety on motor performance in military personnel [18]. Addressing these challenges through training, mental health support, and adaptive strategies can enhance the overall effectiveness and safety of soldiers in demanding operational environments [19].

In addition, the psychophysiological stress response and its modulation by experience is a critical area of study, particularly relevant in high-stakes environments such as medicine, the military, and sports. It has been demonstrated that experience plays a pivotal role in this modulation [20]. For example, studies show that the stress response of novice paratroopers after a low-altitude jump is significantly more pronounced compared to their experienced counterparts [21]. This difference highlights how experience directly influences the psychophysiological reaction to stress, an essential consideration for learning and performing under pressure [21-23]. The presence of stress hormones like cortisol, glucocorticoids, and corticotropin can impair the central nervous system and higher cognitive processes, affecting overall performance [22,23]. Skilled and experienced individuals, therefore, might exhibit optimal motor performance due to a more selectively tuned stress response, coupled with potentially enhanced cognitive automation and executive function efficiency under stress [21,24,25].

However, the intricate relationship between experience in stressful contexts and fine motor skills remains not fully elucidated. This uncertainty underscores the objective of our current research, which is to analyze the impact of experience on the psychophysiological response and fine motor skills under intense stress. We hypothesize that: i) non-experienced participants will show a more substantial negative effect on fine motor skills compared to their experienced counterparts, and ii) higher cortical arousal states and self-confidence, along with lower levels of cognitive and somatic anxiety, will be positively correlated with enhanced fine motor skills. This investigation aims to provide a deeper insight into how experience shapes our physiological and psychological responses in stress-filled scenarios, offering critical implications for training and performance optimization in various high-pressure fields.

MATERIALS AND METHODS

Participants

In this study, we conducted a comprehensive analysis of 132 male professional paratroopers, meticulously categorized into two distinct cohorts: i) the Non-Experienced Group (NEG), comprising younger members (mean age: 23.2 ± 2.3 years, ≤ 6 parachute jumps) primarily recruited from the first-year cohort of the air academy; and ii) the Experienced Group (EG), consisting of significantly seasoned practitioners (mean age: 34.8 ± 4.0 years, > 50 parachute jumps), each boasting over five years of dedicated service within the unit. We applied stringent exclusion criteria to both groups, disqualifying individuals presenting any medical or physical conditions potentially hindering participation or firearms usage, such as cardiovascular diseases, as well as those currently using dietary supplements, stimulants, or any other ergogenic aids.

Participants from both groups were uniformly outfitted in standard-issue military attire, which included boots, helmets, tactical fighting load carriers, body armor, and an HK G-36 rifle. Additionally, each individual was equipped with regulatory emergency parachutes for the execution of the jump. The total gear weight amounted to approximately 39.3 kilograms. Before their involvement, detailed explanations of the experimental procedures were provided to all participants, from whom voluntary written informed consent was subsequently obtained.

It is crucial to note that the entire study adhered rigorously to the ethical standards outlined in the Helsinki Declaration (as revised in Brazil, 2013). The research protocol received the necessary approval from the University's ethical committee, ensuring the highest standards of research integrity and participant welfare.

Procedure

To comprehensively address the objectives of our study, we conducted a detailed investigation into the physiological and psychological responses of both non-experienced and experienced paratroopers before and after performing a parachute jump.

Baseline data were meticulously collected 2 hours and 30 minutes before the embarkation, ensuring a robust understanding of the participants' initial state.

The participants boarded a Hercules T-21 aircraft for the jump, which was executed from an altitude of 500 meters. In this structured activity, the parachute deployment was automatic, triggered immediately upon exiting the aircraft. This process ensured a standardized jumping experience for all participants, crucial for the reliability of our data.

Following the descent and immediate landing, participants were swiftly escorted to a specially equipped field laboratory situated near the landing site. This arrangement was critical to capture the immediate post-jump physiological and psychological responses with minimal delay or external influence, thereby preserving the integrity and immediacy of the data.

In the field laboratory, a comprehensive post-jump evaluation was conducted, encompassing a series of assessments designed to measure the effects of the jump on various fine motor skills, cognitive functions, and stress responses. This timely assessment was vital to understanding the acute impact of parachuting on these key variables in both inexperienced and seasoned military personnel.

Materials

Before and after the automatic parachute jump the following parameters were analyzed according to previous authors [21]:

Psychophysiological parameters

Lower body muscular strength manifestation using a vertical jump test. We used the Sensorize FreePowerJump system (SANRO Electromedicina, Madrid. Spain) which recorded flight time (s) and jump height (cm) to evaluate the Squat Jump (SJ). Three attempts were recorded, and only the best attempt was considered.

Isometric hand-grip strength

It was measured using a TKK 5402 dynamometer (Takei Scientific Instruments CO. LTD)

Each participant's grip strength was measured on the shooting hand while standing with 0 degrees of shoulder flexion, 90 degrees of elbow flexion, and the forearm in neutral. Idem, was done pre-post and among series.

Cortical arousal by the critical flicker fusion threshold (CFFT)

An increase in CFFT suggests an increase in cortical arousal and information process, by contrast, when the values fall below the baseline, it suggests a reduction in the efficiency of processing information and fatigue of the central nervous system [26]. Subjects were seated in front of a viewing chamber (Lafayette Instrument Flicker Fusion Control Unit Model 12021), which was constructed to control extraneous factors that might distort CFFT values. Two light-emitting diodes (58 cd/m^2) were presented simultaneously in the viewing chamber, one for the left eye and one for the right eye. The stimuli were separated by 2.75 cm (center to center) with a stimulus-to-eye distance of 15 cm and a viewing angle of 1.9°. The inside of the viewing chamber is painted flat black to minimize reflection. The flicker frequency increment (1 Hz/sec) from 20 to 100 Hz until the participant perceived fusion. After a fovea binocular fixation, participants were required to respond by pressing a button upon identifying the visual fusion thresholds. Before the experiment, they performed as many practice trials as needed to become familiarized with the exigencies of the CFF test. Then, five trials were performed with an interval of 5 seconds. In each one of the tests, time was quantified as the amount of time that a subject took to detect the changes in the lights from the beginning of the test until the moment of pressing a button. The average of these times was calculated to determine the CFFT [15,27,28]

Cognitive Anxiety (CA), Self Confidence (SC), and Somatic Anxiety (SA)

Those parameters were evaluated using the CSAI-2R [29]. In this questionnaire, 17 items are evaluated: 5 items for CA, 7 items for SA, and 5 items for SC, respectively. The response scale evaluated the intensity of each symptom on a scale from 1 (not at all) to 4 (very much). Higher scores on cognitive and somatic anxiety indicated a higher level of anxiety, as well as higher scores on the self-confidence scale, indicated a higher level of self-confidence.

Fine motor skills were evaluated through the time of re-loading a 15 bullet of 9 mm caliber into a Beretta pistol magazine since previous authors used the same protocol to analyze specific fine motor skills for the population used in the present research [30–32].

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Ill., USA). Means and SDs were calculated using traditional statistical techniques. Normality was tested with the Kolmogorov-Smirnov test. A one-factor ANOVA for intergroup comparisons (i.e. pre and post-test differences; change score (Δ) differences) and a Student's T-test for intragroup comparisons were conducted. The Effect Size was tested by Cohen's D. Pearson correlation coefficient was calculated, and separate multiple regression analysis using the Forward method was performed to determine the presence of predictor variables on the cortical arousal and the fine motor performance. In all these analyses, the significance level was set at $p \leq 0.05$.

RESULTS

The analysis of psychophysiological and fine motor skills in non-experienced and experienced paratroopers before and after a jump revealed some key insights (Table 1). Both groups demonstrated significant improvements in Squat Jump performance, indicating enhanced lower-body muscular strength, likely influenced by the acute stress or adrenaline rush associated with the jump. Notably, this improvement was more pronounced in non-experienced jumpers. In terms of Isometric Hand Strength, there was a stable performance in the non-experienced group, while the experienced group showed a slight but significant decrease post-jump. This outcome might suggest a more nuanced impact of experience on the regulation of grip strength under stress. Critical Flicker Fusion Threshold results indicated an increase in both groups, with the experienced group showing a significant improvement. This finding implies an enhanced capacity for cortical arousal and information processing, especially among the experienced paratroopers.

Regarding fine motor skills, measured through Pistol Magazine Reload Time, there was a significant improvement in the non-experienced group but no change in the experienced group. This difference highlights the possible influence of prior experience on maintaining consistent performance under stress.

Cognitive and Somatic Anxiety levels decreased in both groups, with a notable reduction in physical symptoms of anxiety post-jump across both groups. Interestingly, while Self-Confidence remained unchanged in non-experienced paratroopers, it significantly increased among the experienced ones after the jump, suggesting a boost in self-assurance likely stemming from their familiarity and proficiency in such high-stress scenarios.

Overall, these results underscore the significant effects of experience in shaping the psychophysiological and fine motor skills responses of paratroopers to stress. While certain responses like improvements in muscular strength and reductions in anxiety seem universal to both groups, aspects like grip strength regulation, cortical arousal, fine motor skills, and self-confidence exhibit distinct patterns, emphasizing the nuanced impact of experience in high-stress military operations.

Table 1: Modifications of study variables in non-experienced and experienced participants

Variable	Non-experienced					Experienced				
	Pre	Post	t	p	Effect Size	Pre	Post	t	p	Effect Size
SJ (m)	35.7±1.5 (0.070)	37.7±2.9 (0.021)	-5.686	0.000	0.67	33.4±4.1	35.6±4.6	-7.976	0.000	0.54
IHS (N)	48.2±7.7	48.0±7.3	0.255	0.800	0.03	51.2±7.3 (0.027)	49.8±8.1 (0.039)	2.310	0.024	0.19
CFFT (Hz)	39.3±3.3 (0.000)	39.8±2.0 (0.030)	-1.316	0.194	0.15	37.1±3.2	38.5±3.6	-4.544	0.000	0.44
PMTR (s)	75.1±17.8 (0.000)	59.5±14.9 (0.050)	6.562	0.000	0.56	41.1±11.5	40.7±12.1	0.515	0.608	0.03
CA	8.9±4.2 (0.003)	7.8±4.7 (0.009)	1.947	0.057	0.26	2.8±3.2	2.4±2.9	1.547	0.126	0.13
SA	12.2±5.9 (0.000)	6.0±4.9 (0.002)	5.991	0.000	1.05	5.8±3.8	1.7±1.7	9.097	0.000	1.08
SC	15.2±2.6	15.6±5.1	-0.697	0.489	0.15	18.6±1.7 (0.037)	19.3±1.2 (0.020)	-4.155	0.000	0.41

Between parenthesis p values lower than 0.05 between novel and veterans' paratroopers. Squat Jump (SJ), Isometric Hand Strength (IHS), Critical Flicker Fusion Threshold (CFFT), Pistol Magazine Reload Time (PMRT), Cognitive Anxiety (CA), Somatic Anxiety (SA) and Self-Confidence (SC).

In Table 2, the results of a one-factor ANOVA analysis on various psychophysiological and performance variables are presented, shedding light on the impact of an unstated factor (likely experience or a specific training intervention) on these variables. Data suggests that whatever factor was being analyzed (experience, training, or another intervention) had a pronounced and often significant effect on physical abilities (like SJ and PMTR), cognitive function (as in CFFT), and psychological states (CA, SA, and SC). The lack of significant changes in some deltas might indicate that while the factor made a significant initial impact, its effect on the rate of change over time was not always strong, possibly due to a ceiling effect, adaptation over time, or other intervening variables (Table 3 and Table 4). The strong influence of age also suggests that demographic factors can be crucial in how individuals respond to training, experience, or other interventions in these domains.

Table 2: Results of the variables analyzed in the one-factor ANOVA

Variable	F	P	Variable	F	P
Age	373.128	0.000	PMTR Post	65.687	0.000
SJ Pre	9.185	0.003	Δ PMTR	26.330	0.000
SJ Post	8.831	0.004	CA Pre	88.429	0.000
Δ SJ	0.003	0.960	CA Post	66.286	0.000
IHS Pre	4.992	0.027	Δ CA	3.643	0.058
IHS Post	1.650	0.201	SA Pre	57.671	0.000
Δ IHS	2.536	0.114	SA Post	50.266	0.000
CFFT Pre	13.882	0.000	Δ SA	0.083	0.774
CFFT Post	5.404	0.022	SC Pre	79.531	0.000
Δ CFFT	2.986	0.086	SC Post	38.843	0.000
PMTR Pre	93.494	0.000	Δ SC	0.512	0.476

Squat Jump, Isometric Hand Strength (IHS), Critical Flicker Fusion Threshold (CFFT), Pistol Magazine Reload Time (PMRT), Cognitive Anxiety (CA), Somatic Anxiety (SA) and Self-Confidence (SC)

Table 3: Correlations between the Δ cortical arousal

		Cortical arousal pre-test	**Cortical arousal post-test**	**Vertical jump post-test**	**Vertical jump (Δ)**	**Fine motor performance pre-test**	**Fine motor performance post-test**	**Fine motor performance (Δ)**	**Self-confidence pre-test**	**Self-confidence (Δ)**
Cortical arousal (Δ)	Pearson correlation coefficient	-0.527**	0.303**	-0.222*	-0.174*	0.127	0.080	-0.149	0.251**	-0.378**
	Sig. (bilateral)	0.000	0.000	0.011	0.046	0.146	0.364	0.088	0.004	0.000
	N	132	132	132	132	132	132	132	132	132

**p<0,05; **p<0,01*

Table 4: Correlations between the fine motor performance (Δ) and psychophysiological parameters

		Age	**Records of jumps**	**Vertical jump pre-test**	**Fine motor performance pre-test**	**Fine motor performance post-test**	**Cognitive anxiety pre-test**	**Self-confidence pre-test**	**Cognitive anxiety post-test**	**Somatic anxiety post-test**	**Self-confidence post-test**
Fine motor performance (Δ)	Pearson correlation coefficient	0.355**	0.410**	-0.213*	-0.598**	-0.172*	-0.246**	0.284**	-0.341**	-0.340**	0.202*
	Sig. (bilateral)	0.000	0.000	0.014	0.000	0.048	0.005	0.001	0.000	0.000	0.020
	N	132	132	132	132	132	132	132	132	132	132

**p<0,05; **p<0,01*

Table 4 shows the correlations between changes in fine motor performance and various psychophysiological parameters. Significant findings include positive correlations of changes in fine motor performance with age and records of jumps, suggesting that improvements in fine motor skills are associated with older age and more jumping records. A negative correlation is observed with pre-test vertical jump performance, indicating that better initial physical performance might relate to lesser improvements in motor skills. Furthermore, there is a strong negative correlation with pre-test fine motor performance, suggesting that higher initial skill levels might see smaller subsequent improvements. Furthermore, negative correlations of changes in fine motor performance with cognitive and somatic anxiety post-test, and a positive correlation with self-confidence pre-test were also seen.

DISCUSSION

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and the working hypotheses. The findings and their implications should be discussed in the broadest context possible.

Future research directions may also be highlighted. The present research aimed to analyze the effect of experience on the psychophysiological response and fine motor skills in a highly stressful situation. Hypothesis i. was partially supported, as non-experienced participants demonstrated a more pronounced decline in fine motor skills compared to their experienced counterparts. This outcome underscores the impact of experience in managing the physiological and psychological stressors affecting motor skill performance. For hypothesis ii., the findings revealed a nuanced relationship between psychophysiological responses and fine motor skills. While greater cortical arousal and self-confidence, as well as lower levels of cognitive and somatic anxiety, generally correlated with enhanced fine motor skills, these associations were more complex than initially hypothesized. Experienced participants displayed better stress management and resilience, leading to superior fine motor skill execution under pressure. However, this relationship was not strictly linear and suggests the influence of additional moderating factors, such as individual stress tolerance, coping mechanisms, and task-specific training. These results highlight the multifaceted nature of psychophysiological responses in high-stress scenarios and their differential impact on fine motor skill performance based on an individual's experience level.

On this line, the data analysis revealed an intriguing pattern: experienced participants exhibited an increase in Critical Flicker Fusion Threshold (CFFT) following their jumps, suggesting heightened cortical demands imposed by this complex and perilous task. Notably, before the jump, non-experienced participants displayed significantly higher CFFT compared to their experienced counterparts. This unexpected finding could be attributed to an increased level of cortical hyperactivation. While previous studies have identified sympathoadrenal hyperactivation in similar contexts [2,20,33], our results indicate a concurrent elevation in cortical arousal. This heightened state in non-experienced participants may be a preparatory response to the novel and challenging situation, potentially facilitating mental anticipation of potential hazards and the formulation of strategies to address them. This phenomenon also includes a thorough mental review of the jump procedures. Similar patterns of elevated cortical arousal in novice participants have been reported in military personnel engaged in ground operations [2,34], lending support to our observations. Conversely, the lower cortical arousal observed in experienced paratroopers may indicate a tendency to rely on ingrained standard operating procedures, honed through extensive training and requiring lesser cognitive exertion. This strategy, as suggested by prior research, likely optimizes performance by conserving cognitive resources, allowing for a more efficient and focused execution of well-practiced tasks.

Following the parachute jump, participants exhibited an increase in leg strength, a phenomenon previously linked to enhanced sympathetic modulation triggered by the body's fight-or-flight response system [30,35]. However, in this instance, the significant muscular demand on the leg muscles, due to the impact of landing, may also explain this strength augmentation. Conversely, no significant changes were observed in isometric hand strength post-jump, possibly reflecting the less intense muscular engagement required by these muscles during an automatic parachute jump. Yet, notable differences emerged between experienced and non-experienced participants: non-experienced paratroopers showed higher leg strength, but lower hand strength compared to their experienced counterparts. This variation in major muscle groups like the legs might be attributable to the generally greater muscle mass in the younger, non-experienced group [36], though this factor appears less relevant in smaller muscle groups such as the hands.

From a psychological standpoint, experienced paratroopers reported lower levels of anxiety compared to their non-experienced peers, aligning with findings from prior studies involving similar demographic groups [37]. Interestingly, we noted a reduction in somatic anxiety among non-experienced participants post-jump, a shift potentially linked to the alleviation of immediate stress following the completion of the parachute jump. This differential response highlights the nuanced interplay between physical exertion, psychological stress, and experience in high-stress environments like military parachute jumping [31].

Furthermore, the established negative impact of anxiety on fine motor skills has been well documented in previous research [38]. In this context, it was anticipated that the anxiety reduction observed in non-experienced jumpers, despite their significant anticipatory anxiety response, would enhance their performance in fine motor skills tasks. Correspondingly, improvements were seen in Pistol Maga-zine

Reload Time (PMTR) among non-experienced jumpers, which might also be linked to the sustained physiological arousal following the jump [32]. In contrast, experienced paratroopers exhibited no notable differences in PMTR before and after the jump, a phenomenon potentially explained by a performance ceiling effect. In such a highly skilled group, PMTR appears to be minimally affected by the stress and excitement inherent in a parachute jump. This outcome suggests an optimal level of coping in challenging scenarios, a trait similarly observed in seasoned military parachute jumpers [39]. Indeed, a strong correlation has been established between Isometric Hand Strength (IHS) and better performance in both PMTR and shooting tasks [40]. This finding is consistent with the observations that experienced paratroopers displayed superior IHS and PMTR at both pre-jump and post-jump measurements. Their advanced skills and training may facilitate a more consistent and effective engagement in tasks requiring fine motor control, underscoring the significant advantage conferred by experience and expertise in military operations.

Finally, regarding the correlational analysis, data suggest that the interplay between psychophysiological states and fine motor skills is complex, particularly under high-stress conditions. Significant correlations were found between fine motor skills and various psychophysiological parameters, including arousal and anxiety, suggesting a relationship that is more intricate than the traditional perspectives on stress and performance. Indeed, there seems to be a nuanced connection between the level of initial cortical arousal, its changes over time, and motor skill performance. In this vein, studies have supported this assertion, indicating that higher levels of initial cortical arousal correlated positively with enhanced performance in precision tasks under pressure. For instance, in archery, an optimal level of initial arousal was found conducive to increased focus and steadier hand movements, thereby improving accuracy [41]. Similarly, studies on musicians revealed that those with lower initial levels of stress and anxiety, and who exhibited smaller physiological arousal changes during performances, tended to maintain better motor control, thus delivering superior performance. These findings corroborate our data and underscore the idea that managing arousal levels can be crucial in fine motor skill activities where precision and control are paramount [42]. Nonetheless, it has been proposed that the straightforward interpretation of the arousal-performance relationship must also consider factors such as task complexity, individual stress tolerance, and prior training [43].

Furthermore, research indicates that variables like age and self-confidence significantly influence fine motor performance under stress. These insights are in line with studies demonstrating that older individuals often excel in high-pressure medical scenarios, likely benefiting from their life experiences and heightened self-confidence [44]. Similarly, police officers with greater self-confidence have been observed to exhibit superior performance in stress-induced precision tasks [45].

Therefore, acknowledging these multifaceted influences underscores the necessity for bespoke training and stress management strategies. Such recognition heralds a pivotal shift towards more personalized approaches in performance training and preparation for high-stress environments. This paradigm shift not only reflects an evolving understanding of stress and performance dynamics but also emphasizes the importance of individual differences, advocating for more nuanced and effective training methods that cater to these variances.

Limitations of the study

One limitation of this study is its focus solely on male professional parachutists, restricting the generalizability of findings to other populations, including females and amateur or non-military parachutists. The specific stressor — military parachuting — may elicit unique psychophysiological responses, different from those induced by other stressors in various professional or athletic settings. Additionally, the measurement of fine motor skills and stress responses was confined to specific methods and tools, which might not capture all nuances of these complex phenomena. Future studies should consider a broader, more diverse participant base and a wider range of stressors and assessment techniques.

Practical applications

Our research on the effects of experience on psychophysiological responses and fine motor skills, with a focus on military parachutists, reveals critical insights with broad practical applications, particularly for high-stress professions. We discern that targeted interventions could markedly improve stress management and fine motor skills in less experienced individuals. In this line, simulation-based training stands out as an influential tool, offering a realistic, yet controlled, platform for developing crucial skills and adapting to stress. The use of VR and AR in simulations could provide immersive, risk-free environments for practitioners to build experience and confidence. This technique not only enhances skill acquisition but also allows individuals to mentally prepare for the high-stress scenarios they will face. Moreover, mindfulness and cognitive training programs, targeting attention control, emotional regulation, and mental resilience, present another viable strategy. These programs could help mitigate stress effects, enhance decision-making, and maintain fine motor control during high-pressure situations. Particularly, mindfulness exercises can reduce the psychophysiological impacts of stress, leading to improved performance.

Furthermore, physical conditioning specific to the professional demands also plays a key role. Appropriate fitness training can alleviate physiological stress responses, indirectly benefiting cognitive functions and fine motor skills. Regular psychological assessments can identify individual stress and anxiety responses, enabling more personalized training approaches. Building resilience, managing anxiety, and learning effective stress coping mechanisms are crucial in these trainings. In addition, neurofeedback and biofeedback techniques offer innovative methods for monitoring and controlling physiological stress responses. For example, controlling heart rate variability might be an effective way to manage anxiety and bolster focus in tense environments. Also, training that emphasizes team dynamics and communication is particularly valuable where teamwork is crucial under stress. Effective team interactions can significantly lessen individual stress levels and elevate collective performance.

Therefore, implementing these strategies not only aims to boost individual performance in stressful settings but also contributes to long-term well-being, professional durability, and the efficiency of teams in such environments. For less experienced individuals, these interventions can be crucial in bridging the experience gap, enhancing their capacity to manage stress, and improving their fine motor skills, ultimately leading to better overall performance and adaptability in their respective high-stress professions.

Future research lines

Considering the intricate connections between psycho-physiological responses and fine motor skills as observed in military parachutists, future research avenues appear both extensive and varied. Investigating these dynamics across different high-stress professions and sports could offer a broader understanding of how distinct stress levels influence fine motor skills in contexts such as surgery, law enforcement, or competitive disciplines like archery. Longitudinal studies focusing on the long-term impact of chronic stress exposure could unveil critical insights into the effects on mental health, well-being, and the maintenance of fine motor skills, highlighting the need for proactive intervention strategies. Further exploration into psychological factors beyond anxiety, including resilience, motivation, and risk perception, would enrich our comprehension of their roles in performance under stress. There's also a pressing need to develop and evaluate intervention strategies and training programs, including mindfulness, cognitive-behavioral approaches, and simulated environments, aimed particularly at enhancing the skills and stress management capabilities of the less experienced. Assessing the efficacy of simulator training in preparing individuals for high-stress scenarios, understanding the influence of physical fitness on stress response, and integrating neuroimaging and physiological monitoring to decrypt the brain-body interactions during stress, could provide transformative insights. Furthermore, exploring genetic and biochemical markers like cortisol levels might lead to more tailored training and development protocols. Lastly, cross-cultural studies would offer a valuable perspective on how cultural variations impact stress perception and coping mechanisms, potentially guiding global strategies in stress management and skill enhancement. This wide-ranging research agenda underscores the complexity of stress and performance, especially in high-stakes, high-stress environments, promising not only to deepen our understanding but also to significantly improve training and support for professionals in such challenging roles.

CONCLUSIONS

This study's exploration into the effects of experience on psychophysiological responses and fine motor skills in military parachutists underscores the intricate relationship between stress, experience, and motor performance. Notably, experienced parachutists displayed superior motor skills and lower stress responses, likely attributed to their habitual exposure to high-stress environments and refined coping mechanisms. Contrarily, non-experienced individuals showed heightened stress markers and impacted motor skills, although some improvement in performance post-exposure suggests potential adaptability. These findings illuminate the importance of experience in moderating stress and enhancing motor proficiency. They also highlight the need for tailored training programs, especially for novices, focusing on stress management, skill acquisition, and psychological resilience to bridge the experience gap. This research contributes valuable insights into stress-induced motor skill impairment, suggesting that experience plays a crucial role in optimally managing high-stress situations essential in military and other high-risk professions.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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Authors' contribution
All authors contributed equally to the development of this manuscript. All

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Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Investigating EGFR, BRAF, and RAS Mutations in Oral and Cutaneous Squamous Cell Neoplasms: A Preliminary Report on Romanian Patients

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Abstract: *Background: Head and neck cancers, and particularly, oral cancers have a complex pathogenesis that includes genetic mutations and epigenetic alterations which interfere with cellular signaling and can trigger tumor development. The purpose of this study was to reveal whether low-frequency hotspot mutations may be detected in a study lot with histopathological evidence of squamous cell carcinoma (SCC) of the oral mucosa and skin of the head and neck. Methods: Tumor biopsies from treatment-naïve patients were tested for BRAF V600, NRAS G12/G13, NRAS Q61, KRAS Q61 mutations, and EGFR exon 19 deletions (Ex19Del) using droplet digital PCR (ddPCR). The tumors were also analyzed for EGFR T790M mutations by RT-PCR, using a CE-IVD validated kit, with a limit of detection of 0.05%. Results: None of the examined cases exhibited NRAS G12/G13, NRAS Q61, KRAS Q61, BRAF V600, or EGFR T790M mutations, indicating that these alterations are rare events in SCC pathogenesis. Interestingly, among the 12 specimens tested by ddPCR for EGFR Ex19Del, an HPV-negative cSCC tumor occurring in the parotid region tested positive for this drug-sensitizing mutation, offering unexplored therapeutic perspectives to the patient from whom it was collected. Conclusions: Our study highlights the important clinical implications of detecting low-frequency hotspot mutations in tumor biopsies by ddPCR. We believe that the ddPCR-assisted analysis of these mutations in larger SCC cohorts may provide us with mechanistic insights regarding their role in SCC pathogenesis and guide the development of novel therapeutic strategies for this problematic disease.*

Keywords: *head and neck squamous cell carcinoma; droplet digital PCR (ddPCR); targeted therapy, immunotherapy*

INTRODUCTION

Squamous cell carcinomas (SCCs) are one of the most unpredictable and widespread neoplasms globally, presenting a significant socio-economic burden on healthcare systems worldwide [1]. SCC development has been linked to the dysregulation of several molecules involved in different stages of squamous lineage commitment and keratinocyte terminal differentiation [2]. However, the exact identity and the precise order of the genetic and epigenetic alterations facilitating keratinocyte malignant transformation remain elusive. Several research groups postulated that epidermal clones with mutated p53 may be at the origin of SCC, as they lead to squamous dysplasia and in situ SCC, which subsequently progress to metastatic SCC [3]. At the same time, emerging evidence suggests that the disruption of the mitogen-activated protein kinase (MAPK) signaling pathway, activated by epidermal growth factor receptor (EGFR), facilitates the development and progression of various malignancies, including head and neck squamous cell carcinoma (HNSCC) and cutaneous SCC (cSCC) [3,4]. The MAPK pathway may be depicted as a complex regulatory network that links the tumor microenvironment with the cell nucleus, converting the extracellular signals into intracellular responses such as cell proliferation, differentiation, or apoptosis [4,5]. RAFs (A-/B-/C-RAF), RASs (N-/H-/K-RAS), MEKs, and MAPKs kinases are the most notorious constituents of the MAPK signaling pathway, behaving as powerful oncogenes in various human cancers. Thus, any alteration within those signaling components may lead to apoptosis resistance and uncontrolled cellular growth [4]. Beyond the critical role of EGFR/MAPK signaling in HNSCC development, the various nodal points within the signaling pathway can be converted into therapeutic vulnerabilities in these neoplasms. For instance, BRAF V600E-mutant ameloblastomas may be targeted with BRAF inhibitors such as vemurafenib and dabrafenib, whereas HRAS-mutant HNSCC can be approached with farnesyltransferase inhibitors (FTis) like tipifarnib [4,6]. Despite these recent discoveries, the main therapeutic approach for HNSCC and cSCC combines surgery with radiation therapy and systemic chemotherapy, with ancillary reconstructions depending on case complexity and structures involved [7–9]. Surgical excision of the tumor mass remains the cornerstone of treatment for resectable SCC, followed by adjuvant radiotherapy, with or without chemotherapy [10,11]. The tumoral resection may carry additional risks when considering local infiltration, anatomical variants, or surgical approach [12–14].

The stratified epithelium from which SCC originates can be found distributed on the surface of the outer integument or covering organs and cavities such as the oral cavity, sinonasal cavity, oropharynx, larynx, esophagus, lung, and anogenital region [15]. SCCs are currently classified and examined taking into account the anatomical divisions of the human body; therefore, the SCCs of the head and neck are treated differently from those of the lung [2]. Here, we will focus exclusively on SCCs of the skin and of the mucosal epithelium of the oral cavity and lips (OSCC). Although both tumor types arise from the disruption of the same differentiation programs, cSCC and OSCC are considered distinct entities with different risk factors [16,17]. The main etiological factors for OSCC include tobacco and alcohol usage, and human papillomavirus (HPV) infection [15,18,19]. For cSCC and lower lip SCC, the most prominent risk factor remains increased exposure to ultraviolet radiation (UVR), followed by a personal history of skin cancer and HPV infection [19,20].

cSCC is the second most common cancer worldwide, and its incidence is steadily increasing in the Caucasian population. As cSCC is often invasive, inaesthetic, and locally destructive, such diagnosis usually involves complex skin surgeries and facial reconstructions [21]. While the majority of cSCC patients present with a very good prognosis, 1.5-5.2% of cases may develop life-threatening metastases [22,23]. Moreover, owing to the high incidence rates, in the United States, the mortality rate associated with cSCC is comparable to that reported for more aggressive tumors such as leukemia and melanoma [22]. The number of cSCC-associated deaths is hence expected to surpass that of melanoma in the next 5 years, mainly due to the rise in HPV infections [24]. HNSCC, the sixth most common type of cancer globally, represented mainly by OSCC, also exhibits a poor overall survival (OS) rate. Despite advancements in diagnostic and treatment methods, the HNSCC 5-year OS rate remains approximately 50% [25], suggesting that more intensive scientific efforts should be devoted to understanding the complex biology of HNSCC tumors. Molecular studies to identify the key factors driving cSCC and OSCC development are ongoing, aiming to identify the most promising diagnostic biomarkers and therapeutic targets for these problematic diseases [5,26,27].

The latest advancements in omics technologies facilitated the identification of several hotspot mutations in the EGFR/MAPK signaling pathway which have been harnessed for targeted pharmacological interventions in cancer patients. For instance, the observation that 80% of HNSCCs exhibit EGFR overexpression led to the development and subsequent approval by the Food and Drug Administration (FDA) of Cetuximab, an anti-EGFR monoclonal antibody, to be used in combination with radiotherapy in locally advanced HNSCC [28]. Yet, the dissection of the mutational spectrum of the EGFR gene served as a foundation for the development and implementation of novel EGFR-targeted therapies in clinical settings. For instance, genetic alterations such as exon 19 deletions (Ex19Del) and exon 21 substitutions (e.g., L858R), representing the most common EGFR mutations in non-small cell lung cancer (NSCLC), confer sensitivity to first (gefitinib and erlotinib) and second-generation (nilotinib, dasatinib) tyrosine kinase inhibitors (TKIs) [29]. In addition, exon 20 substitutions which include EGFR T790M mutations and are generally associated with NSCLC resistance to first-generation TKIs, may be also matched with targeted therapy in cancer patients, particularly with third-generation TKIs such as osimertinib and alflutinib [29]. In HNSCC/cSCC clinical cohorts, single-target TKIs, such as gefitinib and erlotinib were shown to improve modestly patient survival rates when given as monotherapy [30]. However, promising results have been reported when small TKIs were combined with other agents such as pembrolizumab, cisplatin, or docetaxel, in recurrent and metastatic settings [31]. This suggests that EGFR mutations may be exploited for targeted therapeutic interventions in the future, once the clinicians validate the most beneficial drug combinations. Alternative strategies for tailored therapy in HNSCC include a) FTIs, such as tipifarnib, for HRAS-mutant HNSCC (recently FDA-approved); b) cetuximab for SCC presenting with KRAS germline variants; c) EGFR inhibitors for HNSCC tumors harboring MAPK1 mutations; d) BRAFi and

MEKi for BRAF V600E-mutant ameloblastomas, combined or not with immunotherapies [4].

As we described earlier, the genetic assessment of BRAF, a serine/threonine protein kinase mutated in 60% of cutaneous melanomas and more than 15% of human cancers, may also expand the anti-cancer armamentarium in cSCC/HNSCC [32]. Despite the low prevalence of BRAF mutations in SCC of the head and neck (2%), HNSCC patients carrying these alterations present with improved clinical outcomes after BRAF and MEK inhibition [33]. RAS is another key oncogene in the pathogenesis of human cancers. KRAS mutations were identified in 2.89% of HNSCC cases, most frequently in tumors with sinonasal localization, whereas NRAS alterations were reported in 2.2% of cases, especially in tumors of the nasopharynx [34]. A recent meta-analysis highlights that oncogenic KRAS may be associated with significantly enhanced immune checkpoint molecule (ICM) expression and immune evasion programs within the tumor microenvironment, making KRAS-mutant tumors promising candidates for immune checkpoint inhibition (ICI) in the clinical setting [35]. However, several recent reports highlight that the MAPK-mutant status may serve as an indicator of a favorable response to immunotherapy in HNSCC, mainly due to the increased CD8+ T cell and dendritic cell infiltrations reported within these tumors, representative of an active cytolytic CD8+T-inflamed status [36].

The present study aims to explore the role and prevalence of KRAS, NRAS, BRAF, and EGFR mutations in a pilot group of patients diagnosed with SCC of the oral mucosa and skin.

MATERIALS AND METHODS

Patients and samples

Twelve patients diagnosed with squamous cell neoplasms of the head and neck between February 2017 and October 2019 were included in the present study. Three patients presented with cSCC, whereas nine had OSCC. Archived formalin-fixed paraffin-embedded (FFPE) tissues from these 12 subjects were obtained from the Department of Oral and Maxillofacial Surgery of the Carol Davila Military Emergency Hospital in Bucharest, Romania. All participants provided written informed consent to take part in this study. Ethical approval for this retrospective analysis was obtained from the Ethics Committee of the Carol Davila Central Military Emergency Hospital. The study including all procedures and handling of patients' samples was carried out in accordance with the guidelines of the Declaration of Helsinki and national as well as institutional guidelines for research ethics. The clinical and histopathological characteristics of the SCC tumors were analyzed following the latest version of the international standard American Joint Committee on Cancer (AJCC) tumor/ node/metastasis (TNM) system for the classification and staging of tumors. Presence of the EGFR T790M mutation was analyzed with the gb ONCO EGFR (T790M) Real-Time PCR assay (PN: 3245-048, Generi Biotech, Czech Republic); additional mutations in the EGFR-RAS-RAF mutations were tested using the droplet digital PCR (ddPCR) Screening assays for BRAF V600 (PN: 12001037), NRAS G12/G13 (PN: 12001627), NRAS Q61 (PN: 12001006), KRAS Q61 (PN: 12001626) mutations and EGFR exon 19 deletions (PN: 12002392) (all BioRad, Hercules, USA); details of these techniques are outlined below.

DNA extraction

The total genomic DNA was extracted from FFPE tissues (3 x 15-μM-thick sections) using the ReliaPrep™ FFPE gDNA Miniprep kit (PN: A2352, Promega). The protocol includes a proprietary deparaffinization method that does not rely on the use of xylene or any other hazardous solvents, ensuring at the same time the extraction of high-purity nucleic acids from FFPE samples. Following the deparaffinization of the FFPE tissue sections, the extraction protocol involves the lysis of the tissues. Nucleic acids released from the cells are further bound on the surface of a spin filter membrane and eluted in 50 μL of elution buffer after several rounds of washing. The purified genomic DNA was kept on ice and quantified using a Nanodrop spectrophotometer.

Droplet digital PCR

Before carrying out the ddPCR reaction, the genomic DNA samples were subjected to uracil-DNA-glycosylase treatment (UDG; PN: 1B1634, VWR) following the manufacturer's instructions. This step aimed to eliminate potential C>T transitions that could arise from tissue fixation, therefore reducing the risk of false-positive ddPCR reactions. All gDNA samples were analyzed in triplicate.

The ddPCR mixture requires the addition of 10 μL of 2x ddPCR Supermix for Probes (no dUTP) per reaction. The 2x ddPCR Supermix is a proprietary reagent containing an optimized reaction mix for primers and probes. Additionally, 1 μL of 20x BRAF (NRAS/KRAS/EGFR) primer and 3 μL of UDG-treated gDNA (7 ng/μL) were included in each ddPCR reaction, and the final reaction volume was adjusted to 20 μL by adding an appropriate volume of RNase/DNase-free water. Each analytical run included negative (wild-type control, WT) and positive controls at the same concentration as the analyzed gDNA samples. The positive controls were provided by Horizon Discovery (PN: HD701) and contained recurrent mutations in BRAF, KRAS, NRAS, cKIT, EGFR, and PIK3CA genes, at allelic frequencies ranging from 1% to 24.5%. The negative controls were WT genomic DNA samples acquired from Promega

Corporation (PN: G1471).

In the droplet generation step, individual reaction mixtures were loaded into disposable DG8 cartridges (Bio-Rad) along with 70 µL of droplet generation oil and passed through the microfluidic channels of a droplet generator. The resulting droplets from each well were transferred into a 96-well PCR plate, heat-sealed, and subjected to the following PCR conditions: 95°C for 10 min, followed by 40 cycles of 94°C for 30 s, 60°C for 1 min, and 98°C for 10 min. Post-PCR, the droplets from each well were analyzed using a QX200 droplet reader (Bio-Rad) and quantified based on target DNA fluorescence. The obtained data were analyzed using the QuantaSoftTM Analysis Pro software, version 1.0.596 (Bio-Rad). Additional details were reviewed recently [37].

Real-Time PCR

The EGFR T790M mutational status was evaluated using the CE-IVD validated gb ONCO EGFR (T790M) Real-Time (RT-) PCR kit (PN: 3245-048, Generi Biotech, Czech Republic). All reagents included in the kit were prepared for use according to the manufacturer's instructions. For each sample, we set two separate RT-PCR reactions. One reaction contained the Assay qPCR EGFR Control T790M and the other, the Assay qPCR EGFR MUT T790M, both in a total volume of 20 µL. 5 µL of the same gDNA sample (diluted to a final concentration of 50 ng/µL) was added to each of the two reactions. Thermal cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 50 cycles with denaturation at 95°C for 10 s, and annealing/elongation at 60°C for 20 s. Fluorescence acquisition was set on the FAM/SYBR channel. All gDNA samples were analyzed in triplicate for the detection of EGFR T790M mutation. Each run contained two types of positive controls according to manufacturer instructions. We included one EGFR wild-type (WT) control and one EGFR T790M positive control, with 5 µL of the provided kit standards pipetted to each. Finally, to check the purity and quality of the PCR reagents, we introduced a third assay control, the so-called 'negative control'. In this case, RNase/DNase-free water was used to replace the corresponding volume of gDNA. The qRT-PCR assays were run on the Rotor-Gene Q 5plex HRM equipment (Qiagen, Germany) and the results were analyzed using the Rotor-Gene Q Series Software (version 2.3.1) (Qiagen, Germany). For each sample, the assessment of the mutational status was based on calculating fold change (FC) values from the Ct values obtained in the two separate reactions, as per the manufacturer's specifications.

Table 1: Demographic and clinicopathological data of SCC patients

Patient #	Gender	Age (years)	Area	Tobacco usage	Alcohol use	The primary site of the tumor	HPV infection	TNM	T stage	Lymph node metastases	Outcome
1	M	80	rural	no	no	lower lip	no	I	1	0	alive
2	M	67	urban	yes	no	tongue	no	IV A	3	2	dead due to disease progression
3	M	50	urban	yes	yes	tongue	no	IV A	2	3	alive
4	M	65	rural	no	yes	lower lip	no	I	1	0	alive
5	M	45	urban	yes	no	lower lip	no	III	3	1	alive
6	F	54	urban	yes	no	tongue	no	II	2	0	alive
7	M	60	rural	no	no	retromolar trigone	no	IV A	4	5	dead due to disease progression
8	M	69	urban	yes	yes	buccal mucosa	no	IV A	3	1	alive
9	F	85	rural	no	no	lower lip	no	II	2	0	alive
10	M	88	urban	no	no	cSCC of the parotid region	no	III	3	1	alive
11	M	51	rural	yes	yes	cSCC of the nasal region	no	IV A	4	2	dead due to disease progression
12	F	86	urban	no	no	cSCC of the genian region	no	I	1	0	alive

The limit of detection (LoD) declared for this assay is 5 copies per 25 µL reaction. This corresponds to a minimum detectable mutant allelic fraction of 0.05% in a background of 100,000 WT copies.

Statistical analysis

Data analysis was performed using The GraphPad Prism software (Boston, Massachusetts USA, www.graphpad.com) version 10.0.0. Patients' demographic and clinicopathological data were analyzed by descriptive statistics.

RESULTS

Patients' general characteristics

Table 1 describes the demographic and clinicopathological characteristics of the SCC patients. The clinical cohort consisted of 12 patients diagnosed with SCC of the head and neck region, encompassing 9 men (75%) and 3 women (25%). Among them, nine patients presented with OSCC (Patients #1-#9), while three subjects had cSCC (Patients #10-#12). The median age of the patients was 66 years, ranging from 45 to 88 years.

Fifty-nine percent (n=7) of the analyzed patients are from an urban environment, and 50% (n=6) of them are active smokers. Additionally, 33% (n=4) of the patients declared that they frequently consume alcohol, and 58% (n=7) presented with pathologically confirmed lymph node metastasis. Five patients were diagnosed with TNM stage IVA disease, three with stage I, and two patients each with stages II and III. All patients tested negative for the presence of high-risk HPV strains.

DNA extraction

The average concentration of DNA extracted from the archived FFPE tissues was 255.7 ng/µL (ranging from 70.2 to 484.3 ng/µL), and the average A260/A280 value was 1.83.

The A260/A280 values obtained for gDNA from FFPE tissues are within the optimal range (~1.8), indicating that the extracted gDNA is free from protein contamination. In addition, all A260/A230 values fall within the recommended range (2.0-2.2), indicating that gDNA has minimal contamination by polysaccharides. These observations confirm the obtained gDNA's suitability for subsequent molecular manipulations.

ddPCR results

The ddPCR analysis demonstrated that BRAF V600, KRAS Q61, NRAS G12/G13, and NRAS Q61 mutations were not present in the examined samples, emphasizing the rarity of these genetic alterations in the pathogenesis of squamous cell cancers. Figure 1 illustrates the absolute quantification of the NRAS Q61 mutation in FFPE samples using the ddPCR system. In addition, by exploiting the ultrahigh sensitivity of ddPCR technology, we successfully identified the presence of the EGFR Ex19Del mutation in a cSCC tumor developing in an 88-year-old male diagnosed with stage III disease (Figure 2). None of the remaining 11 SCC patients within the clinical cohort exhibited this particular mutation.

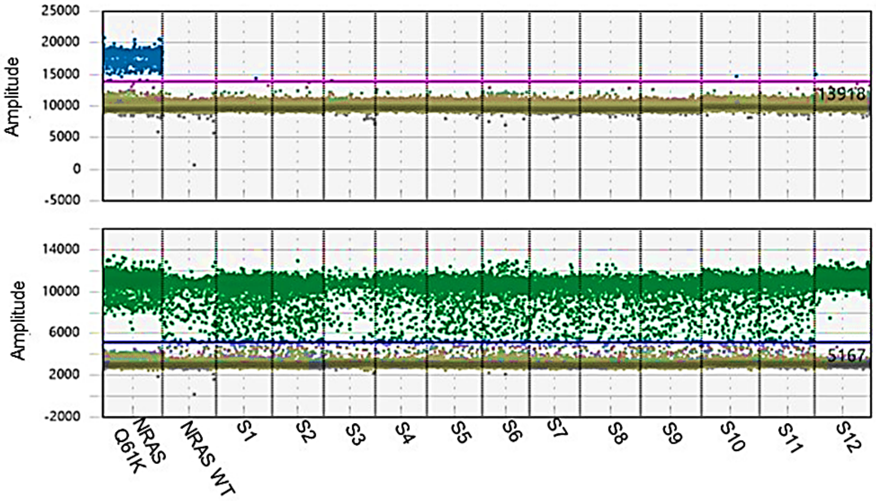

Figure 1: One-dimensional (1D) droplet fluorescence plot for NRAS Q61 mutation analysis. Blue dots represent the FAM-labeled droplets containing the NRAS Q61 -mutant allele, while green dots depict the HEX-labeled fluorescence probes associated with the NRAS WT allele. The pink line represents the threshold between positive and negative signals of the droplets. This determination enables precise NRAS Q61 mutation quantification in FFPE samples (S1-S12- SCC samples; NRAS Q61K- positive control; NRAS WT- negative control).

Real-Time PCR results

The 12 SCC samples were additionally subjected to qRT-PCR analysis to detect the EGFR T790M mutation. All SCC samples included in the study tested negative for the presence of the EGFR T790M mutation.

According to the manufacturer's recommendations, each analytical run included a positive control (containing the investigated mutation at an allelic frequency of 1%), a WT control (where only the WT allele was amplified), and a negative control (without gDNA), to investigate the purity and the quality of the reagents used. Figure 3 illustrates the positive control of the EGFR T790M RT-PCR assay, the negative control along with a sample negative for this mutation (EGFR wild-type; in blue).

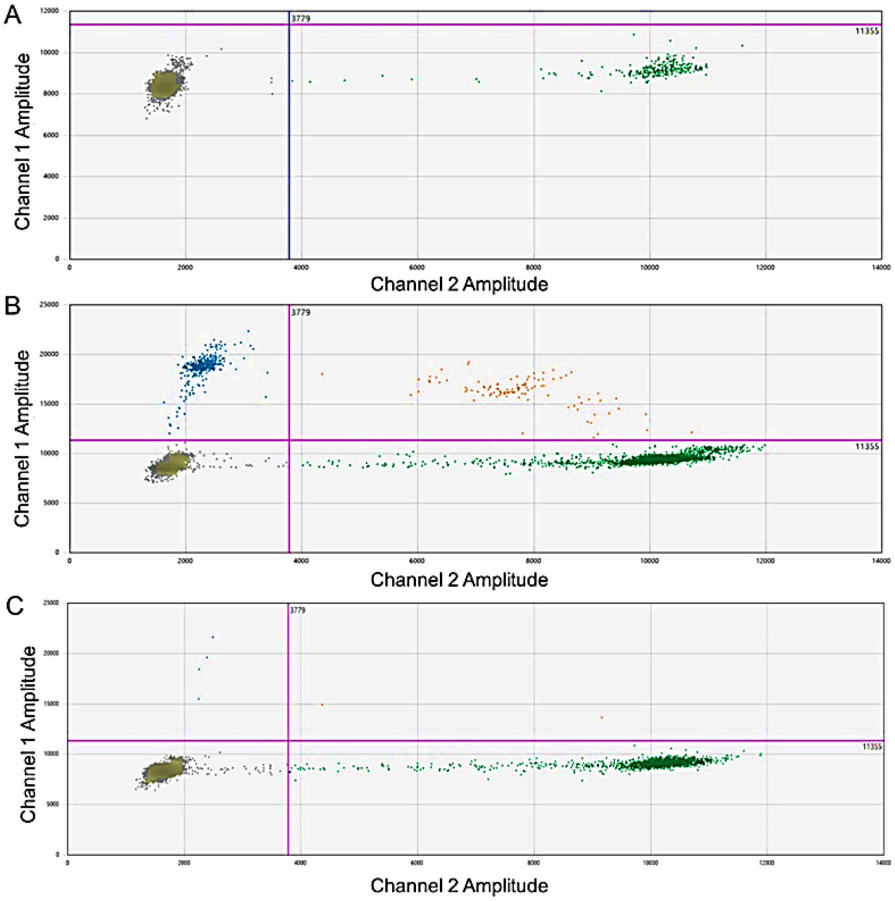

Figure 2: Two-dimensional (2D) ddPCR plots of EGFR Ex19Del mutation detection in FFPE samples. (A) ddPCR results for an EGFR wild-type DNA sample (negative control). The 2D plot illustrates that no droplets are positive for EGFR Ex19Del mutant alleles on channel 1 (FAM labeled, in blue) while EGFR Ex19 wild-type alleles are abundantly detected on channel 2 (HEX labeled, in green). Empty droplets (in grey) do not contain the EGFR Ex19Del target amplicon. (B) ddPCR results for the positive control (presenting the EGFR Ex19Del mutation). Mutant alleles are detectable on channel 1 (FAM labeled, blue). Double-positive droplets (orange) contain both mutant and wild-type EGFR Ex19 alleles. (C) ddPCR results for the cSCC tumor tested positive for EGFR Ex19Del.

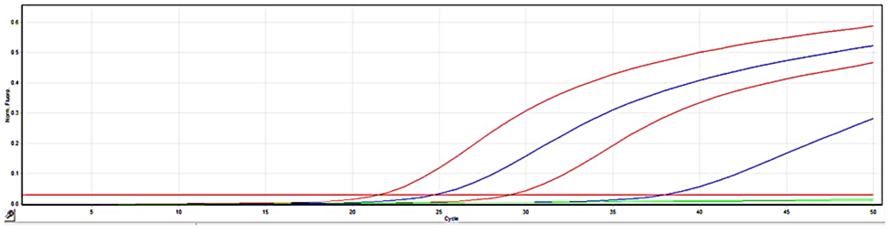

Figure 3: Determination of the EGFR T790M mutational status by RT-PCR. The positive control of the qRT-PCR analysis is marked in red, whereas the negative control is in green. The amplification curve of the EGFR WT SCC sample is depicted in blue. The threshold for the entire run was set at 0.03. A sample can be considered mutated when its FC value is lower than the cut-off FC value and the difference between the CtM of a sample and the CtC of the WT standard is lower than 13. If the latter condition is not met, or the FC value of a sample is higher than the cut-off FC, the sample is evaluated as WT.

DISCUSSION

SCC neoplasms of the head and neck are one of the most heterogeneous and aggressive tumors ever described [10]. Despite recent advances in SCC diagnosis and therapy, the prognosis of advanced patients remains poor, highlighting the need for a more comprehensive evaluation of these tumors [38–40]. From the beginning of modern medicine until now, SCCs have always been approached separately, depending on the tissue from which they originate (e.g., cutaneous SCC, oral SCC) or the anatomical region where they occur. However, in the present study, we wanted to deliver a unifying approach regarding cSCCs and HNSCCs, by analyzing the shared molecular mechanisms involved in their pathogenesis. Our point of view is also supported by the fact that the cSCC and HNSCC pathogenesis converge toward the same risk factors and genetic alterations [2,41]. Additionally, we believe this approach may facilitate a comparative examination of intrinsic tumor cell heterogeneity, stromal composition, and therapeutic efficacy across SCCs occurring in different anatomical sites, an idea that is attracting considerable research interest from the international community as well [2].

Here, we embarked on a preliminary analysis of the frequency and role of hotspot mutations in the EGFR-RAS-RAF signaling components within a clinical cohort encompassing 12 Romanian patients diagnosed with SCCs (3 cSCCs and 9 OSCC cases). We employed the third-generation PCR technology, also known as ddPCR, to screen for mutations such as BRAF V600, NRAS G12/G13, NRAS Q61K, KRAS Q61, and EGFR Ex19Del, while the mutational status of EGFR T790M was assessed using RT-PCR. None of the examined tumor specimens exhibited mutations in the BRAF, KRAS, and NRAS genes. Our findings are consistent with previous observations in the field, supporting the assertion that these genes are rarely mutated in cSCCs and HNSCCs [34,42].

The analysis of the demographic data of the subjects enrolled in the study revealed that 75% of them are men (n=9) and half (n=6) are smokers. We also observed that 58% of the subjects (n-=7) have metastases with lymph node involvement and all SCC tumors are HPV-negative (n=12).

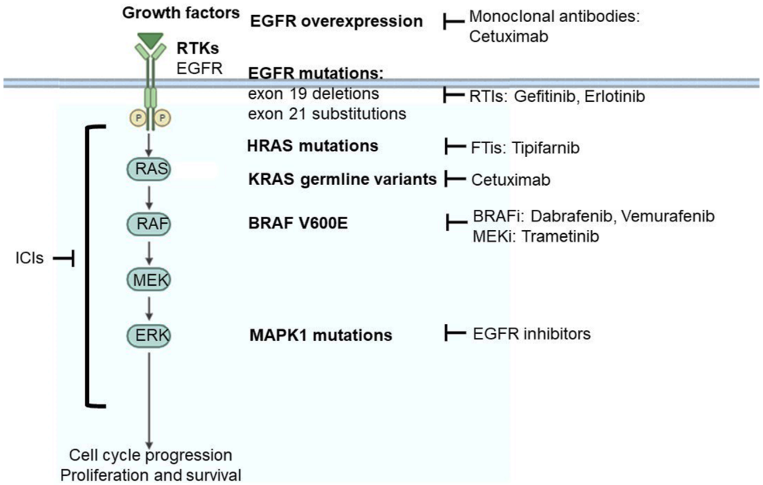

Figure 4: Precision treatment strategies for EGFR/MAPK pathway-mutated head and neck squamous cell neoplasms. ICIs- Immune checkpoint inhibitors; RTKs- Receptor tyrosine kinases; RTis- Receptor tyrosine kinase inhibitors; FTis- Farnesyl transferase inhibitors; BRAFi- BRAF inhibitors; MEKi- MEK inhibitors

However, among the 12 FFPE samples analyzed for EGFR Ex19Del, one HPV-negative cSCC tumor identified in the parotid region of a male tested positive for this mutation. Interestingly, the patient presenting with this tumor is an 88-year-old man from an urban environment who neither smokes nor drinks alcohol. Nevertheless, current literature emphasizes the role of HPV infection status as a valuable prognostic indicator for HNSCC/cSCC patients. Usually, HPV-negative SCCs display alterations in the tumor suppressors such as p53 and cell cycle regulators and present with an unfavorable prognosis compared to HPV-positive tumors, which show less genomic complexity [43]. Several research groups have examined the prevalence of EGFR Ex19Del mutations in head and neck SCCs, reporting a 1% occurrence in the Caucasian population [44] and 7.3% in the Asian population [45]. Despite providing tumor cells with different adaptive advantages via downstream hyperactivated pathways (ERK/MAPK, PI3K/Akt, and JAK/STAT), these mutations also confer sensitivity to TKIs [3]. Several ongoing clinical trials are investigating TKIs' clinical safety and efficacy in cSCC/HNSCC. Single-target TKIs gefitinib and erlotinib given as monotherapy have not demonstrated clinical efficacy in

HNSCC and cSCCs [46,47]. However, remarkable benefits have been reported when small-molecule TKIs were administered in conjunction with other therapies such as cisplatin, docetaxel, or immunotherapy [31,48]. Therefore, these mutations might be exploited for targeted pharmacological interventions in the future, once the most effective drug combinations are identified.

The evaluation of the mutational status of the MAPK signaling pathway is another hot topic in the field of SCC bio-medical research. Several recent studies potentiated that mutations in different components of the MAPK signaling pathway, such as BRAF and RAS, may serve not only as important therapeutic targets but also as valuable indicators of prognosis in SCC, indicating those patients who are most likely to respond to immunotherapy (Figure 4) [36].

This is why we focused our research on investigating these mutations in a small group of patients diagnosed with SCC of the skin and oral mucosa, the present study being also the first one of this type conducted in Romania. Recent studies have shown that even in p53-mutated HNSCC tumors, notorious for their unpredictable evolution, MAPK pathway mutations may help identify those patients with superior outcomes [36]. It is also worth mentioning that the positive prognostic value of these mutations in HNSCC has been reported independently of HPV status [36].

Bioinformatic analysis of HNSCC patient data retrieved from The Cancer Genome Atlas (TCGA) revealed that MAPK-mutant HNSCC tumors present with an increased infiltration of CD8+ T cells and dendritic cells and immune-active cytolytic and interferon-gamma signatures in situ, data that were also reported by our group [36,38,49].

Those features are not observable in HNSCC tumors with mutations in the PI3K, WNT, NOTCH, JAK/STAT, NF-kB, or TGF-beta signaling pathways [36]. Moreover, independent of tumor mutational burden (TMB), MAPK pathway mutations alone predicted those HNSCC patients who benefited the most from clinical anti-PD1/PD-L1 immunotherapy [36].

Similar prognostic roles of MAPK pathway mutations were reported in pan-cancer immunotherapy studies, suggesting that the mutational analysis of this signaling pathway might be essential to improve SCC patient risk stratification.

CONCLUSIONS

Oral and cutaneous squamous cell neoplasms (SCCs) remain a public health challenge and a topic of current scientific interest. Genetic analysis is essential to understand the biology and the clinical behavior of SCCs, unraveling potential biomarkers and therapeutic targets with practical applications in this challenging disease. Therefore, to align with the precision medicine initiatives in the field, we initiated a clinical study to explore the prevalence and significance of hotspot mutations in the EGFR-RAS-RAF signaling pathway within a small clinical cohort of Romanian patients diagnosed with squamous cell neoplasms of the head and neck. Taking into account the shortcomings reported with traditional chemotherapies, administered on a 'one size fits all' basis and resulting in poor efficacy, limited selectivity, and increased toxicity in HNSCCs/cSCCs, we are actively supporting the transition towards the adoption of more individualized anticancer agents in SCC clinical practice.

The present retrospective study emphasizes the absence of mutations in the NRAS, BRAF, and KRAS genes within a small clinical cohort of cSCC and HNSCC Romanian patients, marking the first study of this type conducted in Romania. In addition, our research underlines the remarkable sensitivity of the third-generation PCR technology, the so-called 'droplet digital PCR' (ddPCR), which may be instrumental for detecting low-frequency actionable mutations in cSCC/OSCC tissue biopsies.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research was funded by grants from the Ministry of Research, Innovation and Digitization, CCCDI-UEFISCDI, project number [PN-III-P4-PCE-2021-0549] and [PN-III-P2-2.1-PED-2021-2243], and within PNCDI III by the Core Program, implemented with the support of NASR, project [PN 23.16.01.03].

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Authors' contribution

Conceptualization, M.N., and EG.D.; methodology, EG.D.; software, A.C, and C.Constantin.; validation, A.N.M., C.Constantin., M.S. and C.S.; formal analysis, C.Caruntu.; investigation, A.C.; resources, M.N. and C.Carruntu.; data curation, EG.D.; writing—original draft preparation, EG.D., A.C., A.N.M., M.S., C.Constantin, C.Caruntu, C.S., M.N.; writing—review and editing, M.N. and C.Caruntu.; funding acquisition, M.N., and C.Caruntu. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee from Carol Davila Central Military Emergency Hospital, Bucharest (No. 333/28.03.2019).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Should Serum Levels of Creatine kinase be Evaluated in Patients with Acne Vulgaris Treated with Systemic Isotretinoin?

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Abstract: *The laboratory tests that should be performed and their frequency during isotretinoin treatment are controversial. We wanted to investigate muscle and joint pain and laboratory tests in acne patients who received isotretinoin. Between April 2019 and February 2023, serum CK, AST, ALT, and GGT levels were retrospectively evaluated in acne patients before and three months after systemic isotretinoin treatment. This study included 410 patients. The median serum AST, ALT, and GGT levels significantly increased, whereas serum CK levels were similar in all patients three months after treatment. 23 (5.6%) patients revealed muscle or joint pain after treatment. No significant difference was detected in patients with or without pain in the median serum CK, AST, ALT, and GGT levels after treatment. Isotretinoin treatment at a dose of 10 mg/day was more common in patients with pain than in those without pain, whereas none of the patients who received isotretinoin 40 mg/day developed muscle or joint pain. We suggest that routine evaluation of serum CK levels is unnecessary in acne patients treated with isotretinoin. However, muscle and joint pain may develop even at doses as low as 10 mg/day. Furthermore, monitoring AST, ALT, and GGT levels may be beneficial in acne patients treated with isotretinoin.*

Keywords: *acne vulgaris; creatine kinase; isotretinoin; joint pain; muscle pain; treatment*

INTRODUCTION

Acne vulgaris is a common and chronic inflammatory skin disease that usually occurs during adolescence. Acne vulgaris may also affect adults or even initiate in adulthood. The etiopathogenesis of acne includes seborrhea, follicular hyperkeratinization, Cutibacterium acnes, and inflammation. Lesions mainly appear on the face and may lead to scarring and pigmentation changes. Therefore, acne has been associated with psychological complications such as depression and anxiety. Prompt and appropriate treatment is crucial both to improve lesions and to prevent the development of hyperpigmentation and scars [1].

Oral isotretinoin is a systemic retinoid used to treat patients with moderate to severe acne. Oral isotretinoin inhibits the function of sebaceous glands and shows anti-inflammatory effects [2]. Cheilitis is the most frequent dose-dependent side effect of isotretinoin, observed in approximately 90% of patients [3]. However, various side effects such as xerosis, pruritus, hair loss and photosensitivity have been reported after systemic isotretinoin treatment. In addition, musculoskeletal system disorders such as tendinitis and muscle and joint pain can also develop [2,3].

It has been suggested that about 50% of patients under isotretinoin treatment might develop increased serum creatine kinase (CK) levels, which indicate muscle damage [4]. Slight elevations in creatine kinase are usually reversible, while significant elevations may show rhabdomyolysis. Half of the patients with rhabdomyolysis are asymptomatic, and a minority of patients may develop fatigue and myalgia. Therefore, it has been proposed that it might be useful to evaluate serum CK levels before the initiation of isotretinoin treatment and regularly or when necessary during treatment [5].

In addition, patients with acne are usually monitored monthly, performing laboratory tests for potential side effects such as deterioration of the lipid profile and liver enzymes or complete blood count abnormalities such as leukopenia [6]. However, no consensus exists on the tests that should be performed in patients treated with isotretinoin. For instance, routine evaluation of complete blood count has not been recommended by Hansen et al. [6,7]. Moreover, it is controversial whether frequent laboratory testing is necessary during systemic isotretinoin treatment. It has been reported that laboratory tests might be checked before and two months after treatment, and in case the results are within normal limits, there is no need for further evaluation [6,8].

Herein, we investigated muscle and joint pain and serum levels of CK, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) in patients with acne vulgaris treated with systemic isotretinoin.

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained before this study (approval number: 2023-210). Between April 2019 and February 2023, medical records of patients with acne vulgaris treated with systemic isotretinoin were reviewed retrospectively. Age, sex, past medical history, chronic medication use, duration of acne, the age of onset of acne, and the severity of acne were evaluated. The severity of acne was classified as papulopustular and nodular acne. Isotretinoin dose, muscle or joint pain developed after isotretinoin treatment, localization of the pain, and time to onset of pain after treatment were also noted. In addition, serum levels of CK, AST, ALT, and GGT before and three months after the initiation of systemic isotretinoin treatment were evaluated by the biochemistry panel. The reference range was (46-171 U/L) for CK, (<34 U/L) for AST, (<49 U/L) for ALT and (<73 U/L) for GGT. Exclusion criteria were having liver and kidney diseases, using medications that may affect liver or muscle enzymes and intense exercise.

Statistical analysis was performed using SPSS version 25.0. Quantitative variables were stated as mean ± standard deviation or median (interquartile range). Categorical variables were stated as counts and percentages. The normality of the distribution of the data was evaluated by Kolmogorov-Smirnov test. Wilcoxon signed ranks test, chi-square test, and Mann-Whitney U test were used to determine the differences between the groups. P value less than 0.05 was considered statistically significant.

RESULTS

This study included 410 patients, 324 (79%) females and 86 (21%) males. The median age of the patients was 21 (6) years (range: 14-34 years). The past medical history was unremarkable in 391 (95.4%) patients. The characteristics of the patients are stated in Table 1. None of the patients reported regular use of a medication or excessive alcohol consumption. The patients were treated with oral isotretinoin at a dose of 10 to 40 mg daily. Among females, 220 (67.9%) had papulopustular, 104 (32.1%) had nodular acne, whereas among males, 44 (51.2%) had papulopustular and 42 (48.8%) had nodular acne (p=0.005). The median acne duration was 5 (5) years, (range: 1-20 years) in patients with papulopustular acne and 5 (4) years, (range: 1-20 years) in nodular acne (p=0.174). The median age of onset of disease was 15 (4) years, in patients with both papulopustular and nodular acne (p=0.693).

Table 1: Characteristics of patients with acne vulgaris

	Patients with acne vulgaris (n = 410)
Sex, n (%)	Females: 324 (79) Males: 86 (21)
Age Median (IQR), years Range, years	 21 (6) 14-34
Medical history, n (%)	Unremarkable, 391 (95.4) Seborrheic dermatitis, 3 (0.7) Asthma, 3 (0.7) Depression, 2 (0.5) Hashimoto's thyroiditis, 2 (0.5) Insulin resistance, 2 (0.5) Thalassaemia carrier, 2 (0.5) Allergic rhinitis, 1 (0.2) Urticaria, 1 (0.2) Familial Mediterranean fever, 1 (0.2) ITP, 1 (0.2) Mitral valve insufficiency, 1 (0.2)
Acne duration Median (IQR), years Range, years	 5 (5) 1-20
Age of onset of acne Median (IQR), years Range, years	 15 (4) 10-22
Severity of acne, n (%)	Papulopustular, 264 (64.4) Nodular, 146 (35.6)

IQR: Interquartile range. ITP: Idiopathic thrombocytopenic purpura. This study included 324 females and 86 males with acne between the ages of 14 and 34 years

The median serum levels of AST (p<0.001), ALT (p=0.024), and GGT (p<0.001) statistically significantly increased in all patients three months after systemic isotretinoin treatment. However, no statistically significant difference was ob-served in serum CK levels in all patients three months after systemic isotretinoin treatment (p=0.991) (Table 2). On the other hand, high serum GGT levels were significantly more frequent after isotretinoin treatment than those before treatment (p=0.012) (Table 3).

23 (5.6%) patients revealed muscle or joint pain during systemic isotretinoin treatment, while 387 (94.4%) patients did not have any musculoskeletal complaints. Low back pain was the most common and detected in 16 (3.9%) patients. In

addition, widespread pain was observed in 5 (1.2%) patients and leg pain in 2 (0.5%) patients (Table 4).

Table 2: Laboratory test results of patients with acne vulgaris before and three months after isotretinoin treatment

	Before treatment	After treatment	P value
CK, median (IQR) U/L Range: U/L	74 (43) 30-756	75 (40) 32-717	0.991
AST, median (IQR) U/L Range: U/L	18 (5) 9-215	21 (6) 8-853	<0.001
ALT, median (IQR) U/L Range: U/L	13 (9) 3-95	14 (9) 4-250	0.024
GGT, median (IQR) U/L Range: U/L	13 (6) 5-60	16 (7) 6-110	<0.001

ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CK: Creatine kinase. GGT: Gamma-glutamyl transferase. IQR: Interquartile range. A statistically significant increase was observed in serum levels of AST, ALT, and GGT, whereas no significant difference was observed in serum CK levels three months after systemic isotretinoin treatment.

Table 3: The frequency of high enzyme levels in patients with acne vulgaris before and three months after isotretinoin treatment

	Before treatment n (%)	After treatment n (%)	P value
CK	Normal: 379 (92.4) High: 31 (7.6)	Normal: 377 (92) High: 33 (8)	0.739
AST	Normal: 403 (98.3) High: 7 (1.7)	Normal: 398 (97.1) High: 12 (2.9)	0.251
ALT	Normal: 398 (97.1) High: 12 (2.9)	Normal: 395 (96.3) High: 15 (3.7)	0.532
GGT	Normal: 404 (98.5) High: 6 (1.5)	Normal: 393 (95.9) High: 17 (4.1)	0.012

ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CK: Creatine kinase. GGT: Gamma-glutamyl transferase. High serum GGT levels were significantly more frequent after isotretinoin treatment

Table 4: Patients with acne vulgaris who revealed muscle or joint pain after systemic isotretinoin treatment

	Patients n (%)
Muscle or joint pain	Yes: 23 (5.6) No: 387 (94.4)
Localization of the pain	Low back: 16 (3.9) Widespread: 5 (1.2) Leg: 2 (0.5)
Time to onset of pain after treatment	Within the first month: 5 (1.2) Within the second month: 9 (2.2) Within the third month: 9 (2.2)
Isotretinoin dose	10 mg/day: 6 (26.1) 20 mg/day: 10 (43.5) 30 mg/day: 7 (30.4)

Muscle or joint pain was detected in 23 (5.6%) patients with acne vulgaris after systemic isotretinoin treatment. Among them, low back pain was the most common

When patients were evaluated according to whether muscle and joint pain developed or not, no difference was observed in age (p=0.640), gender (p=0.436), duration of acne (p=0.919), the age of onset of acne (p=0.898) and the severity of acne (p=0.660). Nevertheless, among patients who developed muscle or joint pain, 6 (26.1%) received oral isotretinoin 10 mg/day, 10 (43.5%) received 20 mg/day, and 7 (30.4%) received 30 mg/day, whereas, among patients without pain, 13 (3.4%) received oral isotretinoin 10 mg/day, 177 (45.7%) received 20 mg/day, 174 (45%) received 30 mg/day and 23 (5.9%) received 40 mg/day (p=0.001). Furthermore, no statistically significant difference was detected in patients with or without pain in the median serum levels of CK, AST, ALT and GGT before and after systemic isotretinoin treatment (Table 5).

DISCUSSION

Musculoskeletal symptoms have been reported in about 25% to 87% of patients under isotretinoin treatment [9,10]. Various adverse effects have been described in patients with acne following systemic isotretinoin treatment such as myalgia, arthralgia, gout, spondyloarthritis, low back pain, muscle weakness, muscle stiffness, and myopathy [9,11,12]. Among them, temporary arthralgia or myalgia is the most common [13]. Musculoskeletal symptoms have been described even with low-dose regimens [9]. Drozd et al. revealed that low back pain was the most frequent musculoskeletal complaint in patients with skin diseases who received isotretinoin and that symptoms initiated within the first month of the treatment in 32.9% of patients, whereas between the second and the sixth month in 64.7% of patients. A dose reduction or temporary discontinuation of isotretinoin was recommended in patients with muscle pain or high CK levels [10].

On the contrary, De Francesco et al. stated that arthritis did not have a dose-dependent effect that it was unnecessary to discontinue the drug or reduce the dose, and that isotretinoin treatment could be continued at the same amount without complications [13]. Furthermore, Feily reported patients with acne aged 18 to 29 years who developed musculoskeletal symptoms such as back and leg pain within two months after the initiation of oral isotretinoin treatment and responded to folic acid and vitamin B12 supply [14].

Table 5: Characteristics and laboratory test results of patients with and without muscle or joint pain after systemic isotretinoin treatment

	Patients with pain	Patients without pain	P value
Sex, n (%)	Female: 20 (87) Male: 3 (13)	Female: 304 (78.6) Male: 83 (21.4)	0.436
Age, median (IQR) years Range, years	21 (4) 16-31	21 (6) 14-34	0.640
Acne duration, median (IQR) years Range, years	5 (2) 1-14	5 (5) 1-20	0.919
Age of onset of acne, median (IQR) years Range, years	15 (3) 10-22	15 (4) 10-22	0.898
Acne severity, n (%)	Papulopustular: 16 (69.6) Nodular: 7 (30.4)	Papulopustular: 248 (64.1) Nodular: 139 (35.9)	0.660
Patients received different isotretinoin doses, n (%)	10 mg/day: 6 (26.1) 20 mg/day: 10 (43.5) 30 mg/day: 7 (30.4)	10 mg/day: 13 (3.4) 20 mg/day: 177 (45.7) 30 mg/day: 174 (45) 40 mg/day: 23 (5.9)	0.001
Before treatment CK, median (IQR) U/L n (%)	70 (29) Normal: 22 (95.7) High: 1 (4.3)	75 (45) Normal: 357 (92.2) High: 30 (7.8)	0.494 0.549
AST, median (IQR) U/L n (%)	17 (3) Normal: 22 (95.7) High: 1 (4.3)	18 (5) Normal: 381 (98.4) High: 6 (1.6)	0.389 0.334
ALT, median (IQR) U/L n (%)	14 (7) Normal: 23 (100) High: 0	13 (9) Normal: 375 (96.9) High: 12 (3.1)	0.620 0.392
GGT, median (IQR) U/L n (%)	13 (5) Normal: 23 (100) High: 0	13 (6) Normal: 381 (98.4) High: 6 (1.6)	0.299 0.548
After treatment CK, median (IQR) U/L n (%)	69 (39) Normal: 22 (95.7) High: 1 (4.3)	75 (41) Normal: 355 (91.7) High: 32 (8.3)	0.147 0.502
AST, median (IQR) U/L n (%)	21 (8) Normal: 23 (100) High: 0	21 (5) Normal: 375 (96.9) High: 12 (3.1)	0.649 0.392
ALT, median (IQR) U/L n (%)	15 (14) Normal: 21 (91.3) High: 2 (8.7)	14 (9) Normal: 374 (96.6) High: 13 (3.4)	0.832 0.203
GGT, median (IQR) U/L n (%)	16 (9) Normal: 23 (100) High: 0	16 (7) Normal: 370 (95.6) High: 17 (4.4)	0.285 0.613

Muscle damage due to isotretinoin treatment is generally mild and presents with well-tolerated pain or tenderness of muscles and increased serum CK levels [15,16]. However, isotretinoin can lead to severe muscle damage such as rhabdomyolysis which may result in renal insufficiency and even mortality [5,16]. Evaluation of CK has been recommended in patients treated with oral isotretinoin, especially in those who exercise regularly [5,15]. Furthermore, during follow-up, muscle and joint pain should be questioned [16]. Besides CK, laboratory tests performed to evaluate possible side effects in patients treated with systemic isotretinoin include serum levels of AST, ALT, lipid panel, and complete blood count [17]. The American Academy of Dermatology guidelines of care for the management of acne vulgaris recommend monitoring liver function tests and serum lipids until treatment response is achieved [18]. Zane et al. stated that among patients who used isotretinoin, 11% developed increased serum levels of AST or ALT which was usually mild and reversible, and that they principally evaluated ALT levels since ALT was more specifically indicated liver damage than AST [19]. On the other hand, Webster et al. stated that although AST and ALT were used to detect liver damage, they were not specific to the liver, they were also found in muscles, whereas GGT was a liver-specific and CK was a muscle-specific enzyme. In addition, Webster et al. suggested that monitoring serum GGT level was more beneficial than AST and ALT to detect liver damage in patients treated with isotretinoin for acne and that the increase in AST originated from the muscle rather than the liver [17].

Within this study, the median serum levels of CK, AST, ALT, and GGT were increased three months after oral isotretinoin treatment, however, a statistically significant increase was detected in serum AST (p<0.001), ALT (p=0.024) and GGT (p<0.001) levels. The number of patients with high serum CK, AST, ALT, and GGT levels increased after treatment. Nevertheless, a statistically significant increase was observed only in the number of patients with high GGT levels (p=0.012).

Muscle or joint pain occurred in 23 (5.6%) patients, among them, low back pain was the most common. There was no significant difference in age, gender, acne severity, acne duration, age of onset of acne, and serum CK, AST, ALT, and GGT levels between patients who developed and did not develop pain after isotretinoin treatment. On the other hand, isotretinoin treatment dose differed significantly in patients with and without pain (p=0.001). Treatment with isotretinoin at a dose of 10 mg/day was more common in patients with muscle or joint pain than in those without pain, whereas none of the patients who received isotretinoin 40 mg/day developed muscle or joint pain.

CONCLUSIONS

In conclusion, since no significant change was detected in serum CK levels with isotretinoin treatment, as well as no association was observed between serum CK levels and muscle or joint pain, we suggest that routine evaluation of serum CK levels is not necessary in patients with acne vulgar-is treated with isotretinoin. Although muscle or joint pain was observed in 5.6% of patients with acne vulgaris used isotretinoin, it should be kept in mind that muscle and joint pain may develop regardless of the isotretinoin dose, even at doses as low as 10 mg daily. Our results revealed a significant increase in serum AST, ALT, and GGT levels due to isotretinoin which indicated that monitoring serum AST, ALT, and GGT levels might be beneficial in patients with acne vulgaris treated with systemic isotretinoin.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, F.T., L.F., E.A.; methodology, F.T., L.F.; software, F.T.; validation, F.T., L.F., E.A.; formal analysis, F.T.; investigation, F.T., L.F.; resources, F.T., L.F., E.A.; data curation, F.T., L.F., E.A.; writing-original draft preparation, F.T.; writing-review and editing, E.A.; visualization, F.T.; supervision, E.A.; project administration, F.T., L.F., E.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2023-210).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

A Comprehensive Review of the Factors Affecting the Redefinition of Therapeutic Use in Metropolitan Cities during the Emerging and Re-emerging Pandemics

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Abstract: *Resilience refers to the means and ability to deal with adverse conditions. Resilience is defined as the ability of the system to recover easily after disturbances and changes in the system and the ability of the organization to respond quickly to them. The resilient system can withstand environmental pressures so that it can provide optimal performance in critical situations. Resilience in the use of healthcare services is related to reducing vulnerability to shocks caused by natural disasters and increasing adaptive capacity due to improved measures and opportunities. Considering the importance of evaluation, monitoring, and planning to improve user resilience against accidents, spatial evaluation and redefinition of the use of health and medical services to prepare a comprehensive model for measuring the resilience of hospitals against accidents and calamities Naturally, at the top of which is the coronavirus epidemic, it is necessary. The aim of the current study is a comprehensive review of the factors influencing the redefinition of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a pandemic disease with a resilience approach. It is expected that the managers and decision-makers of the country's health field can use the results of the present research for better planning.*

Keywords: *Resilience, Economic factors, Metropolis, Healthcare services, Coronavirus*

INTRODUCTION

The accelerated and unbalanced development of physical space in some countries in the last few decades has brought adverse social, economic, and physical effects and consequences [1].

The exorbitant costs of urban transportation and services, energy waste, wasting material and social capital in the city, intensifying social segregation, environmental destruction, lack of beauty and cohesion of the city environment, social identitylessness, and instability are among the most important problems of cities in the discussion [2]. Undesirable development of physical space and low density of cities are considered. We are now living in the era of urbanization and urban development, where the ecology and morphology of cities have been increasingly affected by human and natural activities, with the development of urbanization and the desire of people to live in cities, as well as the occurrence of such diseases [3]. In recent years, the existing healthcare spaces in big cities have been seriously challenged. So during the peak of the epidemic, it reaches its peak time and the lack of these uses (hospitals and other medical centers) is felt in urban communities. For this reason, in recent years, many cities have implemented measures to create such spaces (sanitary-therapeutic) during the crisis of pandemic diseases [4].

In Iran, especially since the 1950s, the need to compile different levels of the health network cycle of the country has been felt and based on specific studies of culture, geographical environment, the population of each region, and various types of treatment facilities, from health centers to hospitals. This important thing has been specified and defined. Of course, the country's goal of these divisions is to create the possibility of greater efficiency of financial, equipment, construction, human resources, etc. [5]. In the shortest possible time and with the highest quality of treatment and health, especially when pandemic diseases (coronavirus) occur. But the format of city managers looks for other things beyond these necessities [6]. Improving access to medical services as an indicator of quality of life and social welfare plays an important role. Equal access to medical services for different sections of society in a fair and balanced distribution of these services is one of the important foundations of social welfare [7]. Treatment spaces can be designed in 2 formats: 1) Health spaces and 2) Health-treatment spaces The basis of work in the health management system of countries is the discussion of the care and survival system of health centers and the chain of health spaces. Along with and parallel to the completion of this chain, the treatment system is proposed and then the treatment spaces are defined [8]. Although in most countries, the health care system is necessarily more complete than the treatment chain, but the possibility of full adaptation of these two chains to provide health services in cities is one of the ideals of every country. Today, the growth and development of urbanization have led to the creation of many facilities, but it should be kept in mind that with the growth of urbanization, the factors that because crises have also increased [9]. The issue of access to urban services is one of the important issues that affect the city from various dimensions. In some cases, the lack of proper access to some uses puts people's health at risk. And suitable for it is necessary for all members of the society to access services from two dimensions: the access of the target space is not the space that can be examined [10]. Access is one of the key components affecting the important quality of the urban environment, among the important ideals in this field is homogeneous access with the highest degree related to urban facilities [11]. The sustainability of cities and even megacities when pandemic diseases start, depends on the function and ability and more optimal use of health and treatment spaces, especially urban hospitals, therefore, based on the announced policies of the urban planning and treatment system of each country, it is effective for the life of that city [12]. It will be useful, however, when such crises (pandemic disease) occur, in such a way that it shows the best reaction and response in the shortest possible time during the accident. This will require the review, definition, and re-review of the uses of health and treatment spaces during pandemic crises (after the start of the coronavirus) [13]. Natural and man-made disasters inflict various types of damages, injuries, and death on the affected communities. Such disasters can disrupt various infrastructures and facilities, including hospitals, schools, transportation systems, and emergency services [14]. Non-attendance or lack of preparation in the continuity of services in emergencies caused by accidents has destructive and unfavorable effects on the responsiveness of hospitals. Recent disasters around the world have resulted in irreparable damage to hospitals and disruption of medical services [15]. One of the calamities that has spread all over the world today and has affected all the countries of the world is the coronavirus. In December 2019, the first case of coronavirus was reported in Wuhan, China, and due to the high death rate caused by it, it caused global panic. The rapid transmission of this virus has led to the diagnosis of more than a few million patients over the past two years [16]. A significant increase in the number of infected patients has made the work of medical and health centers difficult in dealing with this disease. Disruption and inconsistency in the provision of medical and health services can lead to irreparable losses and damages [17]. Therefore, it is very important to encourage researchers and decision-makers in order to prepare strategies for improving resilience in health and treatment centers [18]. The aim of the current study is a comprehensive review of the factors influencing the redefinition of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a pandemic disease with a resilience approach.

METHODS

This research is a scoping review that has been conducted by searching for systematic studies to identify the factors influencing the redefining of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a new and re-emerging pandemic disease with a resilience approach using the Cochrane protocol in 1402. accepted. The study inclusion criteria were defined as follows:

Publication date: Due to the comprehensiveness and breadth of both keywords of the research, preparedness against emerging and re-emerging pandemic diseases and the spatial pattern of therapeutic uses of megacities were searched with a resilience approach. In terms of time, only researches that were published in the last decade, from 2010 to 2023 AD and from 1390 to 1402 AD, were examined.

Language: Due to time, financial, and manpower limitations, only the research published in English or Farsi was examined. On the other hand, to evaluate the quality of the studies and use them in the review, only the researchers whose texts are accessible to the researcher have been used.

Place of publication: Relevant sources published in reputable scientific journals, conferences, and theses were used.

Type of studies: Quantitative, qualitative, mixed, or review studies were examined.

The extracted sources were examined by two researchers independently. In this way, the mentioned keywords were first searched in reliable Persian and English scientific databases, including Direct Science, Scopus, PubMed, Google Scholar, and SID in the period from 2010 to 2023. The reason for choosing these databases was their validity and comprehensiveness including a large number of authoritative English-language publications in the field of health and environmental studies, as well as the availability of access to most of the articles and books of these databases for the researcher. In the next step, articles whose full text could not be accessed were excluded from the study. Then, the articles that appeared more than once in the search results were removed using Endnote software, and according to the title of the article, articles not related to the topic were also removed. In the next step, the abstracts of these articles were studied more deeply, and according to the proposed limitations, at this stage, the articles that did not meet the mentioned criteria were removed. Finally, to evaluate the quality of the articles, the standard evaluation checklist (Critical Appraisal Skills Program: CASP) of qualitative studies was used, and the studies that received a score of 25 or higher were included in the study. Due to the inconsistency of included studies and the impossibility of meta-analysis, the meta-synthesis method was used to analyze the data. Out of 2583 reviewed articles, after considering the entry and exit criteria, 37 articles were included in the final review.

RESULTS AND DISCUSSION

The topic of social justice was first introduced to the science of geography by David Harvey in the book Social Justice and the City in 1976. In this book, Harvey has examined social justice with a radical Marxist perspective. In general, many articles and theses have been presented in the discussion of social justice in Iran, the most important of which are mentioned in the following. English et al. (2021) in the research they conducted in the city of Seoul, South Korea, have concluded that in a city, despite having enough services per capita (such as a park), due to improper distribution, they may be available to everyone.

Therefore, the residents have evaluated the distribution of parks concerning the population and other uses of the city and the proposed density of the areas. The results of their findings show that some of the residential areas in Seoul are deprived of access to city parks [19]. Esser et al. (2019) conducted research titled Capacity in Education: A Step Towards Social Justice, which was carried out from a 3-year collaborative activity of 8 primary schools in Darussalam and the coastal region of Powani in Tanzania, and concluded that there are countless obstacles to capacity (Education) In the world, communities and school levels exist, and in Tanzania some progress has been made in terms of (education) capacity, but equity and quality of education still have a long way to go [20].

In their article, Yasobant et al. (2019) express the idea of locating hospital centers in cities, in general, there is no long history in the field of locating medical and health service centers, and it goes back to the 1970s, since 1979, the department Social security health in England paid attention to the strategic development of medical and health service centers in cities, and it was followed in Austria in 1980 and 1982 [21].

Manenti et al. (2020) in an article on urban health addressed the evidence, challenges and guidelines. As a result, we note that although in this review we highlight challenges, this is due to the potential power of urban health research and the importance of studying health. Although the study of urban health encompasses considerable complexity, research with specific research questions and appropriate study designs can improve our understanding of the relationship between specific characteristics of urban nature and health in both specific cities and as generalizable to National cities and recent methodological advances, especially the widespread adoption of multilevel methods in public health research, have made it possible to test hypotheses about urban characteristics and their association with specific health outcomes. Finally, newer methods may contribute to a better understanding of competing influences on the health of urban populations over time. Such research can inform local interventions and policies in urban areas. We hope that efforts such as this review aim to build Our thinking about cities and health will help stimulate both empirical and theoretical developments that can lead to improved health in cities around the world [22].

Schäfer et al. (2021) in research have examined the spatial access of primary care, including methods, challenges, and concepts, and finally concluded that almost all healthcare centers to date, whether based on simple or complex measures, are limited to social inequality in access to health services and to investigate how much primary care centers affect people's health [23]. Sevá et al. (2017) have conducted a research regarding the access and use of health services in Ghana, in which the aim is to investigating the impact of access and socioeconomic factors on the use of health services in developing countries (specifically Ghana). Utilization of health services in Ghana distribution shows a significant difference between rural and urban areas in all variables, except health status and age. Even though the metropolis uses relatively more health services than the villages, the difference in the health status (need) of the two districts is not significant. The coefficient between the two districts is insignificant. Therefore, there is a difference between the need and use between the districts. Success is part of a local understanding of the dimensions and determinants of access to health services, as well as concrete efforts to improve services for the poor. Part of the innovations in health care financing and regulation of care promises to improve access for the poor in any case.

The challenge remains to find ways to ensure that vulnerable populations have a say in how strategies are developed, implemented, and formulated, and to ensure that information and incentives are channeled in ways that can lead to improvements in access for the poor. It is expressed whether or not it can be given [24].

Morris et al. (2021) researched regarding the regulation of access and use of health services. The results showed, first, descriptively, how the countries are using different doctors to refer to a specialist. Second, a multilevel analysis shows how regulatory indicators affect each quantity of care consumed in a country, and finally, they examine how institutional structure affects differences in service utilization among groups with different incomes, education levels, and Health statuses [25].

Lingam et al. (2021) studied the determinants and pattern of health service consumption among graduate students and the result of this research showed that the healthcare system in Pakistan includes public and private health centers. In Pakistan, the majority of doctors are in the regions. They live in the city and attract the educated classes than the private sector. The use of public healthcare facilities is not the main priority of the educated class of the country, and government officials should pay more attention to making it more common for the people [26].

Gatto et al. (2022) studied poverty and access to health care in developing countries. The result of this research showed that despite the progress in access to health care in developing countries, a significant number of their population has limited access. Poor countries have a higher burden of disease but usually have less access to health care. Whether based on geographical access, accessibility, physiological access, acceptance or quality of care. However, recent studies show that this exodus is inevitable [27].

Peng (2020) discussed the optimal location for the construction of a hospital. In this study, the AHP method was used as one of the methods of multi-criteria analysis and GIS. The result of the study showed the use of the hierarchical analysis process in location. The hospital has been very efficient in the study area [28].

Lewis et al. (2021) investigated the improvement of access to health services in urban and regional areas for indigenous people. The results of this literature study provide information about barriers to access to health and mental health services for local people living in urban and regional communities. These barriers can be classified as barriers of accessibility (physical accessibility), affordability, appropriateness, and cultural acceptance [29].

Sousa et al. (2021) analyzed the equity and use of health services in Uganda in a research that focused on several factors (supply and demand) that affect the access and use of health services in Uganda, such as Education, age, household expenses, use of the outpatient department, hospital capacity and the distance from medical services, this study has analyzed a number of measures that are used in the implementation of policies, especially in the main fields. Key policies such as malaria, tuberculosis, and HIV/AIDS have been created. The analysis shows, however, that despite efforts to improve health by improving health outcomes for people, mountainous challenges still remain. For example, living near essential health services, although they work as an incentive to use care, does not necessarily ensure that health services are actually used. Secondly, the elimination of the usage fees of all government centers will increase the utilization of new healthcare facilities, especially fixed and fee-based service providers with catastrophic costs [30].

Joshi et al. (2022) in a study investigated the access and use of health care among rural households in Nigeria. The results showed that the average age of the respondents was 46 years and the average household was 8 people. The majority of the respondents, 43.5%, do not have formal education and the main occupations are agricultural officials. 58% of the respondents have access to medical services, while only 42.5% use these services. Most of the respondents, 40.5%, are 5-9 kilometers away from accessing medical centers. Accessibility indicators show unequal access to modern health facilities in the study area, so this study recommends that rural development policies should create an organizational environment to increase participation and access to justice in the provision of modern health care throughout rural areas. strengthen in the country [31].

McKenzie 2017, in an article on the access and use of health services in Massachusetts, the United States, conducted a qualitative study of the views and experiences of immigrant women in Brazil. The findings of the research show that illegal immigrant women in Brazil are more likely to access health services in countries that have health services. They are satisfied with the global level. Health care is critical to quality of life, but many immigrants and ethnic minorities often lack adequate access to health care, increasing health risks. This study adds to the current literature on healthcare access and use by immigrants living in the United States and also provides new information on Brazilian immigrant women's perspectives and experiences of healthcare access and use in Massachusetts, USA to give While identifying the opportunities to improve the services and health of the entire immigrant population [32].

Allam et al. (2022) examined the relationship between transportation networks and healthcare services and their impact on urban sprawl. The findings of this study show that there are more dynamic relationships between the content of these urban components and access to health care [33].

Das et al. (2021) investigated the design of decision support systems for access to space services. The results of the research showed that improving access to health care can help reduce health costs, increase healthcare equity, and improve health outcomes. Through our research, we have designed a new application-based framework for measuring access to health care and a practical example of SDSS that is rooted in the theoretical concepts of BM and existing FCA methods. Our unique method to obtain the weight of the population characteristics is provided by the use of predictive analysis using real data to evaluate the need to integrate non-spatial factors into existing algorithms in the creation of SPAI. The findings should encourage future research to further examine how demographic characteristics may shape the demand for health care gives the hardness by considering the actual manufacturer's needs [34].

Cotta et al., 2020, reviewed the approaches to measuring potential spatial access to urban health services in a research where they examined the types of distances and their related issues [35].

Berendes et al. (2018) examined the urban status and health competence of critical issues and exercises, and it was concluded that if the global community is serious about the sustainable development goals of SDGs, more attention should be paid to understanding and taking action to improve urban conditions to have a life and social and economic conditions that can promote health competence. The result of the research showed that healthy and fair urban places are very important to achieve health for all the population, however, strict emphasis is placed on built environments or risk factors. A single person does not address the numerous and often cumulative social and environmental obstacles that exist in cities today and does not help the inequalities that burden poor urban communities. To collaborate or help the urban poor to analyze and solve problems. We realize that this will not be easy and the evidence base for how to do this effectively is still emerging. Our challenge is great, but in an urban planet public health Environment is urban health and health justice should be our goal [36].

Pak et al. (2018) investigated network and spatial analysis to evaluate and guide decision-making about equal access and health services in the general patient care system in Ederdamora (Spain). The simultaneous use of access and coverage maps to show the relative distribution of resources in a population, while showing the coverage levels according to the time of access to physical and professional facilities with and without administrative restrictions, can help decision-makers to improve the optimal level of stocks in a region. Large and low-density geography is very valuable [37].

Coccia et al. 2020 studied the issue of access to health care by urban refugees and the host population around Uganda. The results of this research showed that health services were available every five kilometers for both refugees and the host population. Refugees are generally unable to pay for access to health services, including transportation, research, essential drugs, and other informal costs. Refugees' perception of public health services is weaker compared to general citizens. Primarily due to long-term expectations of a small number of inadequate essential drug services, perceived discrimination and the existence of unofficial fees [38].

Paris et al. (2020) in a study to measure the resilience of traditional societies against natural disasters have concluded that traditional societies face natural disasters using four dimensions: nature of risk, level of resilience, cultural structure and policies. Managers can show resilience in the face of disasters [39].

Finn (2022) conducted a research as a place-based model to understand the resilience of local communities against natural disasters. This study presents a new framework of the place of urban resilience in order to improve the method of assessing resilience against disasters at the local and regional level, and a set of variables have been considered in this study as the first step in the target research. The final output of this research is presented in the form of environmental, social, economic, institutional, infrastructure, and competence of society [40].

Park et al (2022) in an article titled "Towards resilient regions: regional development policy" use a descriptive-analytical method and emphasizes the economic dimension of resilience and analyzing indicators of employment rates, large-scale economic centers, income and production gross and knowledge mobility introduced the process of making the regions resilient [41].

Plastina et al (2019) worked on an article entitled Regional planning and the future of resilience in the Ning ladder coral coastal region of Australia in 2019, which was the indicator of resilience and the amount of tourist attraction, which finally reached the correlation between resilience and tourist attraction [42].

Teng et al (2022) in an article titled "Tissue-based identification of old urban neighborhoods in Hyderabad, India using remote sensing data with a renovation approach, concluded that the location map of worn-out tissue is an efficient tool in identifying areas." The densely populated city can be used as a reliable source for vulnerability and resilience assessment in the next step. The presented method allows for quick analysis and comparison of multi-temporal data and can be used in many densely developing cities around the world [43].

Acharya et al. (2017) collected the articles and opinions of different people in one collection and investigated the reconstruction of built environments after disasters in order to increase resilience and found that resilience should be considered among the necessities of reconstruction [44].

Hasson et al. (2022) categorized urban resilience indicators from a landscape ecologist perspective and the result of this article introduces five urban design and planning indicators to create urban resilience, which include redundancy, multi-functional (biodiversity) Being an urban system, urban ecological networks and connection with connections, adaptive design or adapting to changes, he still emphasizes the combination of ecological principles and urban planning to achieve urban resilience [45].

Ferdowsi and Arkhodi (2016) in an article entitled "Evaluation of health and medical resilience of Semnan province cities against natural disasters" stated that due to the high importance of health and medical resources in order to improve the resilience of settlements against natural disasters Naturally, managers and planners should have a special approach to providing healthcare facilities and infrastructure. The purpose of this research is to evaluate the cities of Semnan province based on health-medical indicators effective in resilience. In this research, the desired indicators have been evaluated using the Vikor model. According to the results, the cities of Shahrood, Damghan, Garmsar, Aradan and Sorkheh have a more efficient performance in providing services during the occurrence of natural disasters by having proper medical facilities and infrastructures, and in other words, they have favorable health-medical resilience in the face of natural disasters. They will have accidents. On the other hand, the cities of Mahdishahr, Miami and Semnan, due to the lack of appropriate proportion between the population and medical facilities and infrastructure, will have low resilience when faced with accidents compared to other parts of the province. In general, by evaluating and analyzing this process in Semnan province and resilience strategies, managers and planners can lead the cities of the province to strengthen resilience against natural hazards, considering the existing capacities and competitive advantages [46].

Saffari Darberazi et al. (2019) in an article entitled "Designing a Comprehensive Hospital Resilience Model in the Face of Corona Disease" stated that healthcare centers should be able to quickly adapt to catastrophic events such as natural and human disasters. One of the ways to deal with various disasters in healthcare centers is to increase resilience.

The current research aimed to design a comprehensive hospital resilience model by identifying the factors affecting hospital resilience, and the way these factors are related in the face of the corona disease. At first, by using the background study of the research, the effective factors on hospital resilience were identified, and then by using the interpretive structural modeling technique, the communication model between the identified factors was obtained. The conceptual model obtained from interpretive structural modeling was fitted using Smart PLS software version three. For this purpose, the questionnaire containing 33 questions was provided to 80 managers, experts, and employees of Yazd teaching hospitals. The research results indicate the identification of eight general factors affecting hospital resilience. The eight factors identified in the present study were structured in four general levels by interpretive structural modeling. The starting level of the model is the factors of "sustainability" and "information technology and communication system". Also, the model-fitting results confirm the relationships formed by interpretive structural modeling. Based on the results of the present research, the country's health and treatment managers can use to make the country's hospitals resilient in the face of natural disasters and unforeseen events [47].

Ebrahimzadeh et al (2009) in an article entitled "Spatial-spatial planning and organization of health and medical services using GIS case: Zanjan city" found that in most cities there is a lack of appropriate allocation of space and optimal placement of service and physical elements. The city, especially the medical centers (hospitals) and the number of effective factors in the location of these centers, has followed and continues to increase the problems of the city and its citizens. Therefore, this has revealed the necessity of using effective methods and computer systems in the optimal location of these centers. The current location of most of the medical centers and hospitals in Zanjan city does not match the scientific criteria of the needs of this land use, at the same time, the findings of this analysis show that the current need of medical centers in Zanjan city is at least 7 hospitals according to the city's per capita, which the city officials They can use 11 points extracted from gis to solve this deficiency [48].

Rahnama and Amirfakhrian (2010) analyzed spatial access to healthcare services in the city of Mashhad. They stated that access planning, as a method of integrated planning of users and transportation networks, has two spatial and non-spatial dimensions. The availability and availability of services is one of the dimensions of spatial access, and this study examines medical services in Mashhad. The study method is of descriptive-analytical type, based on which, after forming a spatial data bank of therapeutic spaces in ARC-GIS software (including 6 major uses with 4528 frequency items) and using accessibility models. two-stage floating basin", the condition of Mashhad's districts' access index to each of the treatment spaces was determined. The advantage of using this method, compared to other methods, is the simultaneous use of indicators such as distance, functional radius, and number of facilities. The result of this analysis shows the mismatch between the spatial distribution of the population and the distribution of medical services. In other words, the central areas of the city have the best access and the peripheral areas have the weakest access to medical services. In the following, while using SPSS software and the "two-stage cluster analysis" model, 1266 population zones of Mashhad city were classified in terms of access to health care services. In this section, the results of the findings showed that the city of Mashhad can be classified into two clusters, including the first cluster with an average accessibility index of 3.24, including 2.3% of urban areas (areas with excellent access conditions) and the cluster The second one with an average accessibility index of 0.061 includes 97.7% of the domains, which does not show a good situation compared to the first cluster. The graphs prepared from the evaluation of this model showed that the most important indicator regarding the difference between the first cluster and the second cluster is the index of access to pharmacies. On the other hand, the index of access to emergency bases shows the least role in this classification [49].

Shojaiyan and Rahimpour (2017) in an article entitled "Evaluation of land suitability of health-treatment centers using CF analysis (case study: regions one and eight of Ahvaz city)" stated that the assessment of urban land suitability is the basis of urban planning. Especially in the field of proper use of land. This important issue directs the existing uses in the city in such a way as to prevent incorrect and incompatible adjacencies and ultimately lead to the establishment of land suitability. The present research was carried out with a descriptive-analytical approach in order to evaluate the land suitability of health-therapeutic centers in areas one and eight of Ahvaz city. ArcGIS software and CF relationship were used to achieve the mentioned goal and analyze the obtained information. Finally, the spatial distribution of land and the suitability percentage of each of the selected uses were determined with health-therapeutic use. The results of the research showed that among the uses studied, industrial use with 16.17%, residential use with 8.17%, and then fire stations with 7.53% had the highest incompatibility, and main roads with 0.71% had the lowest incompatibility. Is. In the end, 41.09% of the total area of the studied area is occupied by incompatible areas, 18.66% by neutral areas, and 13.02% by compatible areas. According to the conducted studies, the suitability of the land and the proximity of the selected uses in relation to the health-therapeutic use in the studied area have not been proportionately observed [50].

Motamedi (2019) in a research article entitled "Investigation and locating the access of the citizens of Shirvan city" to therapeutic uses. In this article, one of the basic and important tasks of urban and regional planners is to allocate land to various urban uses while paying attention to the role and function of the city, the economy of the city, and also the effect of mutual factors of uses on each other. The results showed that in terms of location, the studied clinics are concentrated in the eastern side of the city, and the central and western parts of the city are limited in terms of access to the clinics, and only 28,136 people out of the city's population of 83,000 have access to the standard radius range of the clinics; In other words, it can be said that about 75% of the city's population is outside the standard radius of the clinics, and the access radius of the clinics is not suitable for the citizens of the city, and there is a need for a well-equipped hospital in the west of Shirvan [51].

Hataminejad et al (2017) in a research entitled "Analysis of access to urban services in Esfrain" which was conducted using a questionnaire and unbalanced Morris index, concluded that the upper socio-economic classes in the city of Esfrain, in terms of having better financial conditions, they have more favorable uses, and the distribution pattern of service uses is in favor of more prosperous groups so that it is not consistent with the concepts of social justice [52].

Rostami (2010) in the research "Investigation and analysis of urban public service distribution from the perspective of spatial justice, case study: Yasouj city" the degree of access and need of residents to public services using the access network analysis model, hierarchical analysis model and analysis model local spatial autocorrelation has been investigated and analyzed. He also used the Gini coefficient to analyze the inequality of access to urban services and the Moran coefficient to analyze the pattern of distribution of injustices among urban blocks and finally concluded that urban public services are based on accessibility, public benefit, efficiency Population distribution and residents' needs for public services have not been fair and facilities are concentrated in certain areas [53].

Rahnama and Zabihi (2011) analyzed the distribution of public facilities in line with spatial justice with the integrated model of access in Mashhad in 2013 and in this research they concluded that the integrated access analysis of services with the help of geographic information system and spatial autocorrelation technique This indicates that the spatial distribution of public facilities based on Moran's index is clustered and based on the general index, it is a cluster with low concentration. The final result is that the spatial distribution of public facilities is not fair based on the integrated model [54].

CONCLUSIONS

The range of topics and topics of strategic urban research is very wide. For example, studying sports, recreational, therapeutic, educational spaces, police stations, fire stations, etc. All of them are topics that the researcher can investigate. To scientifically and accurately examine urban issues, it is necessary to carefully examine the components and influencing factors according to their type and degree of importance. In addition, due to the rapid growth and expansion of cities, the existence of a multitude of variables affecting them, as well as the incredible amount of information, the use of traditional methods in the analysis of problems is not very reasonable and expressive, and it is misleading. Factors such as population growth, migration and its consequences, and the fair and optimal distribution of services in the city environment and especially the metropolises are associated with obstacles and problems, therefore, adopting efficient and appropriate policies in relation to the provision of services and optimal allocation of space for the population living in different sectors of the city (outskirts, center, etc.) is one of the important goals of urban planning for quick and low-cost access to citizens. In this chapter, theories and models such as spatial allocation models, access models, attraction models, therapeutic approaches, sustainable development theory, and spatial theories were discussed.

Therefore, considering the problems stated above and the large spatial scale and analysis of a large amount of mechano-descriptive and statistical information, the researcher was motivated to solve various problems by having analytical functions (especially network analysis facilities, spatial allocation models and modeling in general) including analysis of accesses, determination of sphere of influence, location, and allocation of resources, sustainable development, development of social justice and so on.

With the help of these tools and statistical analysis, reach scientific results. Biological accidents and threats, re-emerging, and emerging diseases are increasing, and in these diseases, accidents, and threats, the first place to get involved is the Ministry of Health, especially hospitals. Hospitals must have the necessary preparation to face these incidents and threats so that in case of such incidents, they have the least vulnerability reduce casualties, and can provide the best and safest care to clients. One of the ways to create readiness is to conduct exercises and maneuvers, therefore, for this purpose, hospitals must write and practice several scenarios regarding various types of accidents and biological threats.

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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REVIEW

Immunomodulatory Effects of Statins in the Treatment and Prophylaxis of Influenza Viruses

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Abstract: *Background and aim: Influenza, commonly referred to as "the flu," is an infectious illness caused by influenza viruses (IV). Despite the availability of efficient antiviral drugs, the IV still significantly increases mortality. Antiviral drugs swiftly remove these viruses from pulmonary secretions, suggesting that a prolonged inflammatory response may be to blame for the poor result. Therefore, using immunomodulatory medications looks advantageous. Numerous anti-inflammatory effects are produced by statins. Numerous retrospective studies recommended that statins be considered for IV therapy. This study set out to comprehensively examine the immunomodulatory potential of statins in IV prophylaxis and therapy. Methods: From the beginning until 25 March 2022, a number of electronic databases (Scopus, EMBASE, PubMed, Web of Science, ProQuest, OVID, EBSCO, and CINAHL) were searched for cohort studies and randomized trials assessing the association between outcomes or risk of infections and statin therapy. Data were gathered on the investigated characteristics, measuring statin usage, quality evaluation, and results (set for potential confounders). Results: Finding additional therapeutic agents for influenza prevention and management is crucial since there are uncertain public health issues regarding the clinical efficacy of conventional medications. Pro-inflammatory cytokines are released as a result of influenza. According to the results, by using anti-inflammatory medications, severe influenza infections can be prevented along with pleiotropic effects. As a result, statins, which are cardioprotective medications with immunomodulatory and anti-inflammatory properties, may be beneficial for individuals with IV. Conclusion: Based on the positive immunomodulatory effects of statin administration, our systematic review's findings suggested that IVs may be prevented and treated.*

Keywords: *immunomodulation, antiviral, anti-inflammation, statin, prophylaxis, Influenza viruses*

INTRODUCTION

Despite the Influenza virus (IV) being recognized as a serious pathogen during the past century, influenza continues to cause a sizable number of ailments. According to estimates, only in the US in 2017–2018. (Table 1) [1].

Universally, The worldwide WHO estimates that each year, influenza epidemics cause 3,000,000–5,000,000 serious illnesses, with 290,000–650,000 recorded fatalities due to the severity of IV [2]. Although yearly influenza vaccinations are advised and antiviral drugs are available, both have a number of limitations. Following influenza infection, immunological dysregulation, which is determined to release the pro-inflammatory cytokines generating major risk factors for mortality and morbidity, is the cause of the severity of the disease and clinical presentations [3]. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are the most significant implicated cytokines, generating innate immune responses and inflammation [4]. The antigenic changes over time (requiring a precise strain match annually), vaccine strain egg-adapted changes, slow manufacturing processing, the absence of cross-reactivity, brief duration of protection, and poor immunogenicity in certain populations (for example, the elderly) all play a significant role in the effectiveness of the seasonal influenza vaccine [5,6]. If given at the right moment during the illness, antiviral medications such as neuraminidase inhibitors might have a profound effect; otherwise, they have only a subtle impact on clinical symptoms [7]. Prophylaxis is defined as a preventative measure against disease.

A preventative measure has immediate effects against response-triggering and can reduce the systemic reaction to physiological stress or illness. This development might greatly reduce the detrimental disease-related circumstances [8]. The most often given medications in the world are statins, which decrease cholesterol. Statins block the enzyme 3-hydroxy-3-methyl glutaryl coenzyme-A reductase, which catalyzes the rate-restrictive step of cholesterol production. Statins have been shown to have positive cardiovascular benefits (Figure 2). [9].

Table 1: It was extremely well-established how viral respiratory tract illnesses compare epidemiologically

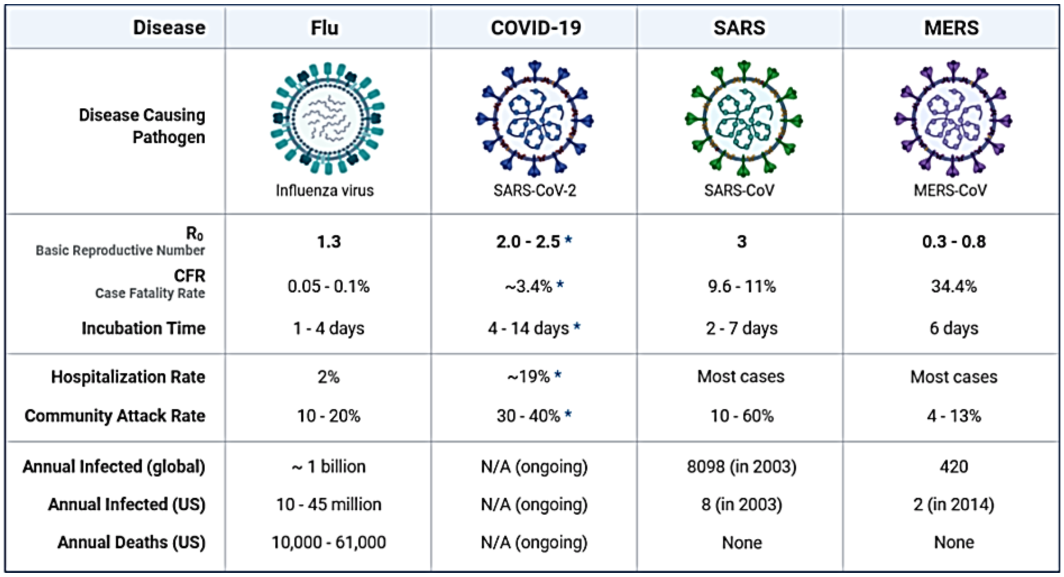

Disease	Flu	COVID-19	SARS	MERS
Disease Causing Pathogen	Influenza virus	SARS-CoV-2	SARS-CoV	MERS-CoV
R_0 Basic Reproductive Number	1.3	2.0 - 2.5 *	3	0.3 - 0.8
CFR Case Fatality Rate	0.05 - 0.1%	~3.4% *	9.6 - 11%	34.4%
Incubation Time	1 - 4 days	4 - 14 days *	2 - 7 days	6 days
Hospitalization Rate	2%	~19% *	Most cases	Most cases
Community Attack Rate	10 - 20%	30 - 40% *	10 - 60%	4 - 13%
Annual Infected (global)	~ 1 billion	N/A (ongoing)	8098 (in 2003)	420
Annual Infected (US)	10 - 45 million	N/A (ongoing)	8 (in 2003)	2 (in 2014)
Annual Deaths (US)	10,000 - 61,000	N/A (ongoing)	None	None

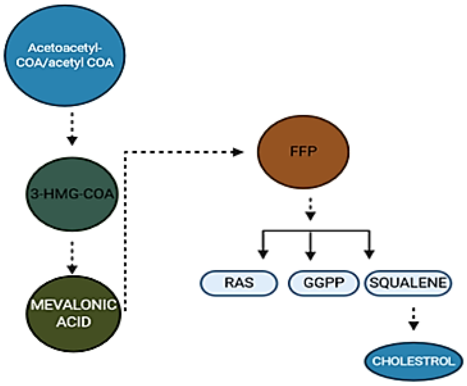

Figure 1: Statins' advantageous cardiovascular effects

Regardless of its effects on cholesterol levels, statins have been shown in clinical and experimental studies to decrease inflammation, improve endothelial function, and stabilize atherosclerotic plaques through a direct mechanism [10]. Statins can also alter cell shape, which is necessary for viral propagation [11]. Despite conflicting scientific findings, several retrospective observational analyses identified a link between statin use and decreased IV morbidity effects in people. However, these evaluations are constrained by the significant variation in medication delivery timing and duration across contributors or the participation of pneumonia morbidity in the absence of laboratory-approved IV infection [12]. A systematic review of statins' possible immunomodulatory properties in the treatment and prevention of IVs was the goal of this assessment.

METHODS

From the beginning until 25 March 2020, a number of electronic databases (Scopus, PubMed, EMBASE, Web of Science, ProQuest, OVID, EBSCO, and CINAHL) were searched for cohort studies and randomized trials exploring the connection between statin prescription and the result or risk of infections. Data on trial characteristics, statin consumption measurement, quality analysis, and outcomes were retrieved (set for potential confounders).

Influenza and the immune system

IVs are single-stranded RNAs that belong to the Orthomyxoviridae family and are divided into 4 categories based on their negative sense, segmented, and ssRNA genomes [12]. (classes of A, B, C, and D). In the meantime, many animal species might contract the influenza A virus (IAV) [13]. The IAV genome is almost 13,500 bases long and has 8 ribonucleoproteins (RNP) units, which together may encode at least 17 different proteins, including the recently identified

NS3, M42, PA-N155, and PA-N182 [14].

The genetic characteristics and molecular makeup of the neuraminidase (NA) and hemagglutinin (HA) proteins allow for further classification of IAVs. However, 9 NA and 16 HA subtypes were shown to be present in both human and animal bodies [15]. There have been reports linking many important IAV-encoded proteins to the host immune response and virus pathogenesis to the viral infection. According to research, changes in viral proteins' amino acid composition are linked to greater immune evasion and illness severity in humans and birds caused by IAVs [16]. In order for B cells to start producing neutralizing antibodies, HA contains epitopes. Therefore, the primary factors influencing recombination processes and viral mutation are the HA epitopes [17]. An innate immune response is the initial line of defense for the body against viral infection. IAV replicates itself by concentrating on respiratory tract epithelial cells. Understanding the innate immune systems defending these cells against IAV infection is crucial. Pathogen-associated molecular patterns (PAMPs), which are present in airway epithelial cells, are identified by receptors such Toll-like receptor (TLR) 3, melanoma differentiation-associated protein 5 (MDA5), and retinoic acid-inducible gene I (RIGI) [18]. Different autocrine signaling pathways can trigger the production of class 1 and 3 interferons (IFNs) and other cytokines instead of locating PAMP [19]. IFN lambda receptor 1 (IFNLR1-IL10R2) and IFN alpha/beta receptor (IFNAR1-IFNAR2), which are the receptors for class 1 and class 3 IFNs, respectively, can result in the paracrine response, which in turn activates a number of ISGs with antiviral activity and, as a result, induces the defensive activity of neighboring cells against viral infection [20]. The majority of adaptive immunity against IAV infection comes from B and T cells. T lymphocytes are mostly recognized as CD8+ and CD4+. In order to create cytokines and other powerful chemicals that can stop viral replication and kill virus-infected cells, CD8+ T cells are developed into cytotoxic T lymphocytes (CTLs). T cells are therefore essential to limit viral infection [21]. The naive CD8+ T cells are stimulated by the DCs to go from the lungs to the draining lymph nodes' T-cell zone during IAV infection, where they differentiate and multiply into CTLs [22]. Additionally, IL-12, IL-2, class 1 IFN-, and IFNs help to differentiate CD8+ T cells into CTLs [23]. It has also been demonstrated that IFN- increases T-cell proliferation during IV [24]. The workings of CTL have been extensively studied. The CTLs create cytotoxic granules that contain chemicals like granzymes and perforin while the virus targets the cells [25]. Additionally, CTLs can activate death receptors in IAV-infected cells by secreting cytokines including TNF, TRAIL, and FASL [26]. Following infection, lymphoid organs, blood, and the infection site are circulated with virus-specific DCs and CTLs [27]. The differentiation and activation process during the first infection increases the memory CTL cells' effectiveness and proficiency in secondary infection, and the memory CTL cells respond swiftly to subsequent IAV infection [28]. Although neutralizing antibodies are protected from subsequent infection with the same IAV serotype, CTLs are epitope-specific in conserved IAV proteins including NP, PA, and M1. As a result, the CTL response is heterosubtypic [29]. CD4+ T cells are a crucial subset of immune cells that are involved in adaptive immunity against IAV infection. In mouse models, CD4+ T lymphocytes may promote MHC type 2 expression in epithelial cells and target IAV-infected epithelial cells [20]. The CD40 ligand (CD40L) is notable among the several co-stimulatory ligands produced by CD4+ T cells that are important in antibody production and B cell activation [21]. TNF, IL-2, and IFN- are examples of antiviral cytokines generated by Th1 effector CD4+ T cells that activate alveolar macrophages [22]. IFN- and IL-2 released by Th1 cells control CD8+ T-cell differentiation to clear viral infection [23]. Additionally, CD4+ T cells can develop into killer, Th17, Th2, follicular helper, regulatory, and rarely Th2 cells [24]. Antigen-offering cells attach to MHC type II-related peptides produced from viruses, and Th2 cells produce IL-13 and IL-4 primarily to encourage B cell responses [25]. It was discovered that the control of cellular immunity against IAV infection includes the Treg and Th17 cells.

Immunopathogenesis of Influenza

The primary targets of IV for infection are respiratory epithelial cells, which are also amplified cytokines by the choreographers [26]. Following first exposure, the offspring viruses that have multiplied inside of these cells can spread to other cell types, including alveolar macrophages. The inflammatory reactions start when infected cells die through necrosis or apoptosis [27]; acute inflammation is thought to be the body's main defense mechanism against damaging stimuli. In order for leukocytes and plasma to reach the site of extra-vascular injury, blood flow must be increased. Acute inflammation is identified by an increase in local temperatures and discomfort [3]. Acute inflammation is indicated by the activation of pro-inflammatory chemokines or cytokines [28]. Inflammatory cell recruitment may happen as a result of these pro-inflammatory chemokines or cytokines. Then, there is extensive tissue damage, immune cell infiltration, and a gradual expression of antiviral, inflammatory, and apoptotic genes [29]. Both the repair of the damage and the regeneration processes begin at the same time. Most of the time, this repair technique can fully restore the function [30].

The formation of hyaline membranes, diffuse alveolar damage, fibrotic healing, and fibrin exudate (Figure 3), which are thought to be signs of severe capillary damage, persistent organ dysfunction, and immunopathologic injury, are among the more pathologically severe alterations found for severe inflammation related to cytokine storm. Additionally, the release of strong inflammatory chemokines and cytokines into the bloodstream can result in systemic cytokine storms and multiple organ failure [32].

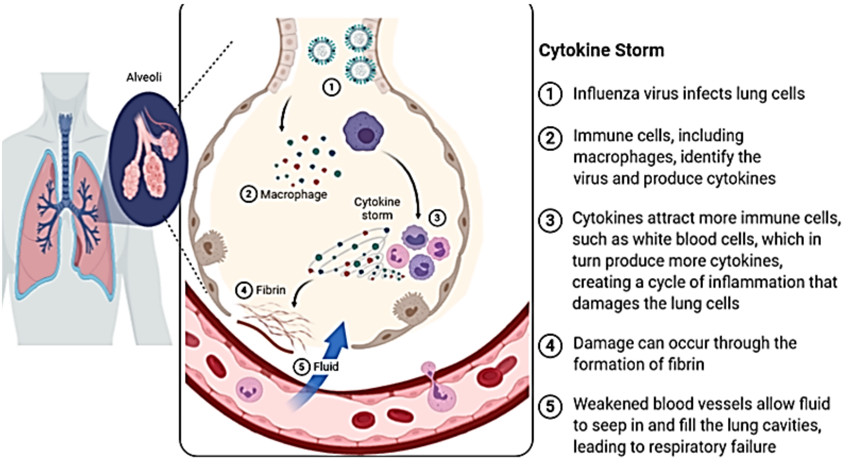
Alveoli
①
② Macrophage
Cytokine storm
③
④ Fibrin
⑤ Fluid
Cytokine Storm
① Influenza virus infects lung cells
② Immune cells, including macrophages, identify the virus and produce cytokines
③ Cytokines attract more immune cells, such as white blood cells, which in turn produce more cytokines, creating a cycle of inflammation that damages the lung cells
④ Damage can occur through the formation of fibrin
⑤ Weakened blood vessels allow fluid to seep in and fill the lung cavities, leading to respiratory failure

Figure 2: Severe inflammation related to cytokine storm

Because viral virulence and host resistance both have a role in the severity of influenza outcomes, it is imperative to use immunomodulatory treatment in addition to traditional antiviral medication. Indeed, several studies have shown that administering immunomodulatory medicines, with or without antiviral medications, can reduce organ damage and death from severe influenza. Additionally, the majority of these medications are not costly and are made simply, making them potentially useful in large-scale influenza pandemics. Here, the characteristics of statins and some instances of these were discussed.

Statins as immunomodulatory agents

The enzyme hydroxyl methylglutaryl-coenzyme A (HMG-CoA) reductase is inhibited by statins. By catalyzing the reduction of HMG-CoA for mevalonate during cholesterol synthesis, this enzyme can gauge the pace of the early step of the cholesterol biosynthesis pathway [33]. In addition to their ability to lower cholesterol levels, statins are pleiotropic cardioprotective medications that are gradually becoming recognized as direct cellular effect mediators. Statins inhibit downstream molecules, which are essential for viral contagiousness [34]. They reduce or prevent the production of downstream isoprenoid intermediates such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGPP) (FPP). These isoprenoid intermediates interact with membranes when post-translational proteins are modified. Statins regulate GDP and GTP-bound states by controlling their isoprenylation and preventing them from switching from the cytosol to the membrane when they are inactive [35].

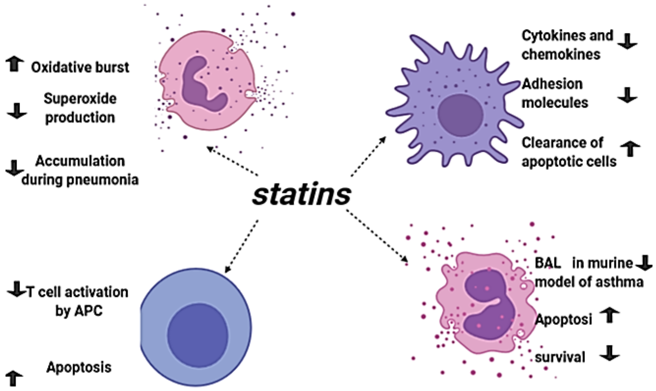
Oxidative burst ↑
Superoxide production ↓
Accumulation during pneumonia ↓
statins
Cytokines and chemokines ↓
Adhesion molecules ↓
Clearance of apoptotic cells ↑
T cell activation by APC ↓
Apoptosis ↑
BAL in murine model of asthma ↓
Apoptosi ↑
survival ↓

Figure 3: Some functions of statins

As a result, statins stop the activity and membrane translocation of isoprenoid intermediates and reduce their production. It is thought that lowering the isoprenylation of several proteins mediates the pleiotropic effects of statins.

This is best shown by the Rho GTPase family, which includes RhoA, Cdc42, and Rac, and which performs distinct roles dependent on the shape, secretion, motility, control, and proliferation of gene expression in cells [36]. To apply their activities and membrane interactions, members of the Ras family must add a farnesyl group to processes such as platelet activation, cell division, differentiation, intracellular vesicle transport, and the production of oxygen radicals [37]. These effects of statins include immunomodulatory actions such as the reduction of T-cell activation, the inhibition of inflammatory responses in neutrophils and macrophages, and the inhibition of a number of pro-inflammatory cytokines like TNF, IL6, and IL8. Furthermore, it has been demonstrated that blocking MAPK pathways such p38 MAPK, JNK, and ERK1/2 prevented the expression of RANKL, the production of cytokines and MMPs, and the degeneration of cartilage in different cells from RA patients (Figure 3). [38-42]

Use of immunomodulatory features of statins on IV treatment

According to three categories, including in vitro, human, and animal investigations, the effects of statin-related immune-modulation were examined to treat influenza in a number of studies. The following sections were used to review particular research from each category.

In vitro study

In the US Vaccine Effectiveness Network trial across 6 influenza courses (2011-2012 to 2016-2017), Havers et al. evaluated the influenza VE in statin users and nonusers with age higher than 45. All individuals with acute respiratory illnesses were screened for influenza at outpatient clinics. According to the data, out of 11692 eligible contributors, 3359 (30%) used statins, and 2806 (24%) tested positive for IV illness. Meanwhile, only 60% of nonusers and 78% of statin users obtained influenza vaccination. Setting for possible confounders came in at 39 percent (95 percent CI, 32 percent -45 percent) among nonusers and 22 percent (95 percent confidence interval [CI], 22 percent -47 percent) among statin users, with influenza VE coming in at 36 percent. They discovered no appreciable VE alteration as a result of statin use. Between nonusers and statin users, VE against influenza A(H3N2), A(H1N1) pdm09, and B viruses were comparable. When they analyzed the data by type and subtype, they discovered that the use of statin administration did not change the effect of vaccination on influenza [43].

According to research by Gower et al., lovastatin can stop virally induced cell-to-cell fusion and RSV replication. The experimental research showed that isoprenylation inhibition is a potential new antiviral strategy for treating or preventing severe RSV infection [44]. Statins, a widely used and affordable pharmacological medication, may be effective in preventing current and future influenza epidemics, particularly in less developed and underdeveloped nations, according to Mehrbod et al. By lowering cytokine over-expression and modulating strong inflammatory responses, statin therapy may protect the body from inflammatory reactions in the pulmonary system and other inflammatory reactions connected to severe influenza sickness. The availability of statins, which might be used as preventative or supplemental drugs to lessen the severity of the disease, and possible preventive medical therapy for unanticipated pandemics, may help to diminish the inflammatory repercussions of influenza that are connected with it [45]. Simvastatin and H1N1 elevated membrane localization and LC3 protein lipidation, according to Mehrbod et al. Simvastatin, however, has a direct influence on LC3 lipidation through a reduction in cholesterol, while H1N1's contribution to autophagy is more convoluted due to its effects on the activation of several autophagy-related genes to boost the autophagic flux. Their results were in line with those of an earlier study on the primary human lung mesenchymal cells, highlighting the relationship between the relative amount of LC3-II and the dynamic turnover of LC3-II through lysosome activity [46].

Animal study

Despite the ambiguous results, several animal studies showed that alternative statin medications may be protective against acute lung damage. In C57BL/6 mice infected by IAV, Haidari et al. observed that atorvastatin (40 mg/kg/day) prescription decreased the lung viral titers and mortality. Statins work directly against influenza by inhibiting the Rho/Rho-kinase pathway. The statins' protection against influenza might be viewed as a component of their cardiovascular event prevention benefits [47]. According to Lee et al., the treatment of simvastatin plus the neutralizing RANTES antibody effectively inhibited the growth of neutrophils caused by poly I:C in the lungs. Additionally, they said that simvastatin inhibits neutrophilia in poly I:C-induced pneumonia by inhibiting STAT3-mediated RANTES production. Understanding the etiology of viral pneumonia is crucial in order to develop effective anti-inflammatory or antiviral medications for certain inflammatory conditions. Simvastatin should be considered as a potential therapeutic option for viral pneumonia, particularly in neutrophilic inflammation due to a viral component, they further said. Simvastatin can reduce airway inflammation, but its exact mechanism of action has to be understood [48]. Simvastatin's efficacy against influenza A/PR/8/34 virus infection in the mouse model was evaluated by Glück et al. The first trial used oral administration of simvastatin. In a subsequent investigation, intraperitoneal administration was used to achieve high plasma levels. Bodyweight loss, survival rate, histologic lung damage, and viral titers in the lungs and trachea were evaluated. Surprisingly, simvastatin treatment caused the mice to lose more body mass and have lower survival rates than the virus-infected control animals. Simvastatin also did not affect histological lesions, tracheas, or lung viral loads. In general, they demonstrated that simvastatin did not shield the mice from IV infection [49]. According to Radigan et al., rosuvastatin treatment did not successfully treat influenza A illness. Inflammation of the lungs, lung damage, and the degree of weight loss were comparable in rosuvastatin- and control-treated mice. Rosuvastatin treatment had no impact on mortality in influenza A-infected mice (A/WSN/33) Statins did not affect influenza A replication in vitro or its removal from the lung in vivo. Statins did not affect influenza A-related mortality and did not lessen the severity of the lung damage caused by the virus. According to them, there is no connection between HMG CoA reductase inhibitors and improved outcomes for patients with sepsis and pneumonia related to their effects on influenza A infection [50].

Human study

In critically ill patients with severe sepsis, Kruger et al. investigated the clinical and biochemical effects of atorvastatin treatment. Before using statins, the site stratified a phase II, prospective, multicenter, double-blind, randomized, and placebo-controlled experiment. A matching placebo or atorvastatin was given to 250 individuals in a cohort trial who had severe sepsis and acute infection (127 received placebos, 123 received statins) (20 mg daily). The results showed no interactions between treatment groups, showing that there were distinct behaviors across the groups throughout time (P = 0.26), and no variations in IL-6 concentrations (initial endpoint) between the placebo and atorvastatin groups (P = 0.76). The baseline IL-6 plasma titer was decreased (129 [87-191] vs. 244 [187-317] pg/ml; P = 0.01) in those who had previously taken statins. There was no difference between the two groups in terms of the length of hospital stays, hospital discharges at 28 or 90 days (15% vs. 19%), changes in Sequential Organ Failure Assessment scores, death at intensive care unit release, or negative effects. Patients on atorvastatin had reduced cholesterol (2.6 [0.06] vs. 2.4 [0.07] mmol/L; P = 0.006). Greater than 28-day mortality was detected for those randomly assigned to the placebo (28 percent vs. 5 percent; P = 0.01) than for those getting atorvastatin in the selected group of 77 past statin users. At 90 days, there were no statistically significant differences (11 percent vs. 28 percent; P = 0.06). A therapy with atorvastatin for severe sepsis did not appear to have an impact on IL-6 levels. A lower baseline IL-6 concentration in this population was associated with improved survival, while prior statin usage was associated with the continuation of atorvastatin [51].

According to Papazian et al., simvastatin (60 mg) prescriptions for supplementary VAP therapy did not lower day-28 mortality. Simvastatin did not substantially worsen any side effects. Although the superiority of a placebo to simvastatin was not tested in our investigation, it is important to note that day-28 mortality in the simvastatin group increased by approximately 6% absolute and by almost 8% absolute in the statin-naive category. It would be pertinent to look at the potential supremacy of a placebo over a statin as normal practice does not involve de novo statin administration in ICU patients with infection. They thus investigated the claim that simvastatin is preferable to a placebo. The results did not support the use of supplementary statin medication in ICU patients with VAP. Therefore, this result may be extended to ICU patients who have any type of nosocomial infection [52].

With the aid of connected administrative databases, Kwong et al. conducted a population-based cohort analysis across 10 influenza seasons (1996–2006) in Ontario, Canada. Before the start of the influenza season, they identified all persons over the age of 65 who had had an influenza vaccination, and they also separated those who had received prescription statins (23 percent) from those who had not (77 percent). A final cohort of 2,240,638 patients—exactly half of those taking statins—was obtained by matching based on propensity, which took into consideration each patient's likelihood of receiving a statin. Statins had poor protective effects against pneumonia hospitalization (odds ratio [OR] 0.92; 95 percent confidence interval [CI] 0.89-0.95), all-cause mortality (0.87; 95 percent CI 0.84-0.89), and pneumonia mortality within 30 days (0.84; 95 percent CI 0.77-0.91). The significantly decreased with time, varied between risk groups and propensity score quintiles, and remained unaltered across post-influenza season periods after multivariate regulation and while omitting multiple clarifications for each individual. They suggested that statin use could have a statistically significant, but little, protective effect on influenza morbidity, which was merely attributable to public health authorities and residual confounding. Additionally, doctors must focus on minimizing mortality and morbidity from the ensuing influenza epidemic [53].

CONCLUSIONS

The immunomodulatory effects of statins are mediated by a number of mechanisms, including direct activation of heme oxygenase and inhibition of major histocompatibility type II complexes, which interfere with the interaction between leukocytes and endothelium. The most crucial mechanism is intracellular inflammatory signaling, however. GTP-binding proteins serve as molecular on/off switches for various protein kinases, which makes them essential for intracellular inflammatory signaling. Both the cycle's inactive and active stages require isoprenylation for the route to be activated. By blocking 3-hydroxy-3-methylglutaryl-CoA reductase, statins lessen but do not completely abolish protein isoprenylation. The response strength of the impacted signaling pathway is decreased by this drop in prenylated protein concentration. As a result, it could balance out the "cytokine storm" associated with IVs. Based on the aforementioned effects, statins were found to have direct antiviral effects in some cases when used at physiologically appropriate concentrations. This suggests that they may prove to be an effective adjuvant treatment in the fight against pandemic and seasonal influenza, particularly in health systems that cannot afford expensive vaccines or antiviral medications. Despite conflicting results from several research, statins are affordable and easily available medicines in underdeveloped nations. By controlling immune system cytokine overexpression and modulating severe inflammatory reactions, they may be used as preventative medication or therapy for influenza sickness.

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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ORIGINAL ARTICLE

Pyloric-preserving Pancreaticoduodenectomy with Duct to Mucosa Pancreatico-gastrostomy Reconstruction: Long-term Follow-up in a Tertiary Center

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Abstract: *Background: Duct to mucosa Pancreatico-gastrostomy (DMPG) is a method for reconstruction of pancreatic mass following pancreaticoduodenectomy (PD). There are still controversies about the benefits of this surgical technique compared to the old ones. Objectives: This study aimed to evaluate the long-term outcomes of DMPG reconstruction following PD operation. Method: Through a prospective observational setting, 164 patients, undergoing PD surgery, with diagnosed pancreatic head or peri-ampullary cancer, were enrolled. A pylorus-preserving PD and DMPG were done for all patients to reconstruct the pancreatic mass. Patients were followed up to a mean of 21.06±14.12 months. Demographic data, risk factors, tumoral staging, and postoperative follow-up data were collected for the analytic study. Results: The mean survival of the patients was 21.06±14.23 months after surgery. DGE (delayed gastric emptying) (34.75%), pancreatic fistula (21.34%), bile duct fistula (7.31%), and hemorrhage (5.5%) were recorded as the most important complication in patients. Some post-operative complications were significantly observed in patients with diabetes mellitus (pancreatic fistula, increased amylase, and DGE), Smokers (bile duct fistula, hemorrhage, and increased Amylase), and the elders (bile duct fistula) (p-value <0.05). Conclusions: Although our results indicate that DMPG reconstruction is a safe technique, our findings did not confirm the priority of this method over the older techniques.*

Keywords: *pancreatic neoplasms, gastrostomy, jejunostomy, pancreaticoduodenectomy*

INTRODUCTION

Pancreaticoduodenectomy (PD) is the main surgical treatment option for ampullary and peri-ampullary cancers, and some modifications have been described for this surgical operation. Therefore, these surgical modifications, complications, and outcomes need to be evaluated.

Despite the surgical techniques and management improvements, there are still many concerns about its high incidence of post-operative complications and morbidity rate (30-50%) [1,2]. Pancreatic fistula is considered the most common postoperative complication followed by bile duct fistula, thromboembolism, intra-abdominal hemorrhage, intra-abdominal abscess, wound infection, and gastro-intestinal bleeding as other important complications [2].

Reconstruction with pancreaticojejunostomy (PJ) is the preferred anastomosis technique following PD surgery. While this method has been commonly used for several decades, its high prevalence of complications (40-50%) prompts surgeons to come up with new approaches [3-5].

Duct to mucosa pancreaticogastrostomy (DMPG) is a method of reconstruction following PD surgery. There are still controversies about its benefits compared to previous ones. Few studies are available evaluating DMPG's outcomes, and complications [6-8]. This study aimed to evaluate the long-term outcomes of DMPG reconstruction following PD operation.

MATERIALS AND METHODS

Patients

In this prospective observational study, patients with the diagnosis of pancreatic head or peri-ampullary tumors who underwent PD surgery at Baqiyatallah Hospital from January 2007 to February 2016 were enrolled. Informed consent was taken from patients. All operations were performed by the same surgeon. Patients were excluded from the study if failed to follow or their data were incomplete or inaccessible. Altogether 191 patients were included. During the study, 27 patients were excluded, (12, due to changes in their address, and 15, because of incomplete data and/or failure to follow). The study was approved by the ethics committee of Baqiyatallah University of Medical Sciences (national approval number: IR.BMSU.REC.1393.87).

Surgical Procedure

In all operations, a right subcostal incision was made and a pylorus-preserving PD and DMPG (using 4-0 polydioxanone sutures) were performed for the reconstruction. The Head of the pancreas was transected with at least a 2 cm macroscopic surgical margin from the mass, usually at the margin of the head and neck of the pancreas. The proximal duodenum was transected about 1.5-2 cm distal to the pylorus.

During the DMPG reconstruction, a small piece of the feeding tube was inserted into the DMPG anastomosis, like a stent, to secure the patency of the anastomosis within the first few days. The tube was not fixed with sutures. An end-to-side duodeno-jejunostomy hand-sewn anastomosis was made using 3-0 vicryl sutures. The coledoco-jejunostomy anastomosis was created about 50 cm distal to duodeno-jejunostomy using 3-0 Vicryl sutures. Tumor Lymphadenectomy was performed in all patients with palpable regional lymph nodes. A Penrose drain was inserted in the bed of the surgical site, and a closed-suction drain was placed in the Morison pouch.

Data Collection

Demographic information including age, sex, risk factors for the disease and surgery, history and laboratory, data essential to calculate the American Society of Anesthesiology scoring (ASA) class, and clinical staging of the tumor were collected and recorded into a checklist before surgery.

Duration of the operation, length of stay (LOS) in the hospital, post-operative complications, short- and long-term outcomes, pathologic result of the sample, morbidity, and mortality were recorded into the checklist during close follow-up. The patients were followed up to a maximum of 11 years or up to the date of death. The post-operative complications include pancreatic fistula, bile fistula, thromboembolic events, intra-abdominal fluid collection such as hemorrhage and abscess, wound infection, gastrointestinal bleeding, and gastroparesis. International Study Group on Pancreatic Fistulas (ISGPF) criteria was used to estimate pancreatic fistula [9]. Delayed gastric emptying (DGE) was graded according to the validated criteria of the ISGPF classification [10]. Pre-operative ASA classification was also assessed according to the ASA classification of physical status [11].

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows (version 21, IBM Corp, NY). Averages were expressed as mean ± SD. Kolmogorov-Smirnov test was used to assess the normal distribution of data. Possible statistical relationships between the risk factors and complications after the surgery were calculated using student t-test, ANOVA, and Chi-Square tests. The p-values equal to or less than 0.05 were defined as statistical significance levels.

RESULTS

In this study, a total of 164 patients with a mean age of 62.21± 9.34 years (including 93 males and 71 females) were included. The demographic information, risk factors, and also the number of cases in each ASA class are demonstrated in Table 1. Most of the patients were in class II of ASA classification (45.73%). According to the exclusion criteria, the patients with class V of ASA classification were excluded from the study.

Table 1: Demographic information, risk factors, and ASA class of patients

Variable (N=164)		
Age, (Mean± SD)	62.21 ± 9.34	
Sex, n (%)	Male	93 (56.70)
	Female	71 (43.29)
Smoking, n (%)	58 (35.36)	
DM, n (%)	48 (29.24)	
IHD, n (%)	42 (25.60)	
ASA Class n, (%)	I	15 (9.14)
	II	75 (45.73)
	III	64 (39.02)
	IV	10 (6.09)
	V	0 (0.00)

DM: Diabetes Mellitus, IHD: Ischemic Heart Disease, ASA: American Society of Anesthesiology scoring

Adenocarcinoma was the most prevalent tumoral type in this study (41.46%). Less common pathologies were cholangiocarcinoma, ampullary carcinoma, high-grade dysplasia, neuroendocrine carcinoma, and intraductal papillary mucinous neoplasm (IPMN). The mean pancreatic duct size was 4±1 millimeters. According to the TNM tumoral staging, 55 (33.5%), 70 (42.7%), and 39 (23.8%) of tumors were classified as T1, T2, and T3, respectively.

Post-operative data such as complications, mortality rate, and their relationship with the risk factors are illustrated in Table 2. In brief, DGE (delayed gastric emptying) (34.75%), pancreatic fistula (21.34%), bile duct fistula (7.31%), and hemorrhage (5.5%) were recorded as the most important post-operative complications in patients. Some post-operative complications were significantly observed in patients with diabetes mellitus [pancreatic fistula (p-value 0.038), increased amylase (p-value 0.010) and DGE (p-value 0.024)], smokers [bile duct fistula (p-value 0.027), hemorrhage (p-value 0.026) and increased amylase (p-value 0.045)] and the elders [bile duct fistula (p-value 0.006)]. No patient reported a history of alcohol consumption as a risk factor for pancreatic cancers.

Table 2: Post-operative data; and their relationship with the risk factors

Complications		Descriptive, n(%)	Age (P)	Sex (P)	Smoking (P)	DM (P)	IHD (P)	HTN (P)
Pancreatic fistula	0	129 (78.65)	0.690	0.607	0.293	0.038*	0.777	0.919
	A	6 (3.65)						
	B	17 (10.36)						
	C	12 (7.31)						
Bile duct fistula		12 (7.31)	0.006*	0.626	0.027*	0.99	0.506	0.134
PGIB		9 (5.48)	0.075	1.00	0.722	0.722	1.00	0.733
IAAC		23 (14.02)	0.174	0.176	0.062	0.272	0.9701	0.120
TE		18 (10.97)	0.503	0.266	0.740	0.134	1.00	0.375
SSC		23 (14.02)	0.117	0.664	0.950	0.392	0.955	0.493
Amylase[a]		34 (20.7)	0.943	0.619	0.045*	0.010*	0.755	0.170
DGE		57 (34.75)	0.611	0.791	0.117	0.024*	0.418	0.949
Hemorrhage		9 (5.5)	0.409	0.459	0.026*	0.199	0.838	0.896
Reoperation		18 (10.97)	0.332	0.366	0.169	0.213	0.567	0.908
R-resection[b]		30 (18.29)	0.503	0.223	0.869	0.429	0.883	0.909
Survival (month)		21.06±14.23	0.282	0.915	0.665	0.924	0.616	0.291
LOS (day)		13.12±7.15	0.115	0.880	0.800	0.580	0.700	0.241

PGIB=Post-operative-Gastrointestinal Bleeding, IAAC= Intra-abdominal abscess collection, TE= Thromboembolism, SSC= Surgical Site Complication, DGE= Delayed gastric emptying, LOS= Long of stay, P= p-value

[a]: *Significant increasing amylase = triple time level for more than 3 days*

[b]: *Surgical resection margins were not restricted to malignant tumors, and the surgeon had to conduct more resection to reach a free margin*

*The character * in the above P values indicates that there is a significant statistical relationship between the two methods of surgery*

The mean follow-up time of the patients was about 21.06±14.12 months. The mean survival of the patients was 21.06±14.23 months after surgery. There were 5 early post-operative mortality; one case due to massive lung embolism, three because of post-operative intra-abdominal hemorrhage, and one patient due to myocardial infarction. The survival rate of patients who were diagnosed with adenocarcinoma was 19.93±15.02 months. Survival rates for other groups are shown in Table 3. Also, the mean survival years in each pathologic type of tumor are shown in Figure 1. The longest survival is seen in non-malignant masses and the shortest survival is seen in PMN tumors.

DISCUSSION

Pancreaticoduodenectomy is an accepted surgical treatment for pancreatic head and peri-ampullary cancers. However, its complications have made surgeons find a better surgical technique in recent years [12].

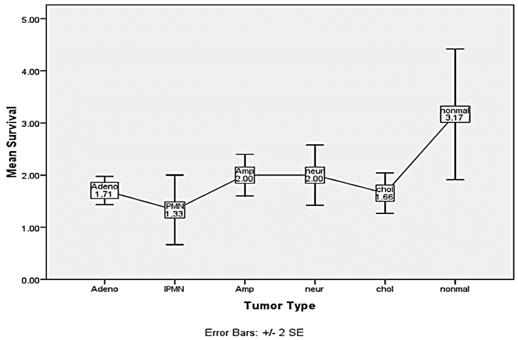

Figure 1: Mean survival year of the patients for each tumor type (*Adeno: Adenocarcinoma, IPMN: Intraductal papillary mucinous neoplasm, Amp: Ampullary Carcinomacarcinoma, Neur: Neuroendocrine tumor, Chol: Cholangiocarcinoma*)

PJ reconstruction is one of the accepted and common methods of anastomosis after the excision of the pancreatic head and duodenum. However, the current method has many challenges such as a high rate of postoperative pancreatic fistula (POPF) and abdominal collections that are reported to be decreased in PG reconstruction in comparison to PJ reconstructions in different studies [13].

Table 3: The patients' survival rate (±SE); according to the pathologic type of tumor

Tumor Survival	Ade (%)	IPMN (%)	Amp-Car (%)	Neur (%)	Chol (%)	Non-mal (%)	Total (%)
1-year	94±3	83±17	97±4	100	90±6	100	93.9±1.8
2-year	51±5	50±22	65±10	67±17	48±10	75±13	54.8±3.8
3-year	15±4	0	67±17	33±17	24±8	50±15	23.1±3.3
4-year	4±2	0	33±17	0	3±3	33±14	4.8±1.6
5-year	1±1	0	0	0	0	25±13	2.4±1.2
6-year	1±1	0	0	0	0	17±11	1.8±1.0
7-year	1±1	0	0	0	0	17±11	1.8±1.0
8-year	1±1	0	0	0	0	0	0.6±0.6
9-year	1±1	0	0	0	0	0	0.6±0.6
10-year	1±1	0	0	0	0	0	0.6±0.6

Ade: Adenocarcinoma, IPMN: Intra-ductal papillary mucinous neoplasm, Amp-Car: Ampullary Carcinomacarcinoma, Neur: Neuroendocrine tumor, Chol: Cholangiocarcinoma

Table 4: The Difference of Overall POPF Complication Rate in Recent Studies between PG and PJ Anastomosis after PD Surgery

Study	Year of Publication	PG		PJ		P value*
		N (%)	Total	N (%)	Total	
Wang et al. (DMPG) [7]	2014	2 (2.7)	75	-	-	-
Figueras et al. [4]	2013	10 (15.38)	65	20 (34.48)	58	0.006*
Topal et al. [14]	2013	13 (8.02)	162	33 (19.76)	167	0.002*
Wellner et al. (grade B/C) [20]	2012	6 (10.16)	59	7 (12.28)	57	0.775
Current study (DMPG)	2018	35 (21.32)	164	-	-	-

PG= Pancreatico-gastrostomy, PJ= Pancreatico-jejunostomy, DMPG= Duct to mucosa Pancreatico-gastrostomy
*The character of * in the above P values indicates that there is a significant statistical relationship between the two methods of surgery*

Table 5: Comparison of the various complication rates after PG Anastomosis in the previous studies

Complications/Study (Year, N)	Wang et al. (2014, 75) [7]	Figueras et al. (2013, 65) [19]	Topal et al. (2012, 162) [14]	Wellner et al. (2012, 59) [20]	Current Study (2018, 164)
Bile duct Fistula, n (%)	1 (1.3)	1 (2)	-	-	12 (7.31)
IAAC, n (%)	3 (4.0)	5 (8)	9 (6)	7 (12)	23 (14.02)
SSC, n (%)	3 (4.0)	-	65 (40)	_	23 (14.02)
DGE, N (%)	13 (17.3)	19 (29)	25 (15)	14 (27)	57 (34.75)
Hemorrhage, n (%)	2 (2.7)	13 (20)	21 (13)	6 (10)	9 (5.5)
Reoperation, n (%)	1 (1.3)	4 (6)	14 (9)	7 (12)	18 (10.97)
R-resection[a], n (%)	-	-	22 (10)	-	30 (18.29)
LOS (day, min-max)	19 (12-39)	12 (1-52)	19 (14-25)	15 (7-135)	14 (7-40)

PGIB=Post-GI Bleeding, IAAC= Intra-abdominal abscess collection, SSC= Surgical Site Complication, DGE= Delayed gastric emptying, LOS= Long of stay
a: Surgical resection margins were not restricted to malignant tumors, and the surgeon had to conduct more resection to reach a free margin

Duct to mucosa pancreatico-gastrostomy anastomosis is a method of anastomosis following PD surgery. There are few studies to show the distribution of complication rates after the DMPG surgical technique [7].

There are many accepted risk factors for POPF. Many risk factors are related to the pancreas itself and are different among patients; these include the size of the duct, histologic features, and the blood supply of the tail. However, many risk factors are related to the techniques of surgery such as anastomosis type after PD, stenting of the anastomosis, and blood loss during surgery [5,7,14].

In this study, there was also a significant relationship between the history of diabetes mellitus and the development of POPF (P=0.038). In a previous study, the risk of POPF was significantly higher in patients with diabetes mellitus (P=0.03). There was no significant relationship between age and POPF (P=0.69). Our finding in this regard is different from other studies [15]. Amylase levels and delayed gastric emptying (DGE) in diabetic patients were higher than in other patients.

In most studies, the male gender has a strong risk factor for pancreatic cancers. In our study, 56.7% of patients were male. However, gender was not an important risk factor for complications after surgery, the rate of bile duct fistula was higher in the elders significantly (P=0.006).

Smoking is another major risk factor for pancreatic cancer. The actual mechanism of this process is unknown. However, cigarettes contain thousands of carcinogenic compounds that can cause cancers [16]. Smoking is also an important risk factor for complications after surgery as seen in our study smokers developed higher rates of bile duct fistula and hemorrhage after the surgery. In these patients, the increase in amylase level was also higher than in other patients (Table 2).

In the current study, the prevalence of POPF was higher than the similar study on DMPG (21.32% vs. 2.7%), and also higher compared with PG (pancreatico-gastrostomy) reconstruction [17]. Although have no justification for that, it seems that DMPG doesn't have any priority in this regard. There are few reports on the comparison between POPF rates following DMPG and PJ anastomosis (21.32% vs. 12.28 and 34.48%) [18, 19]. In other studies, some complication rates such as hemorrhage, bile duct fistula, DGE, and also LOS are higher in PG in comparison to PJ anastomosis [17,19].

In this study, the prevalence of DGE was 34.75% which is higher compared to another study [5]. It might be because of the pylorus-preserving surgery done for the patient; however, this finding is not in favor of the priority of DMPG (Table 4). The prevalence of hemorrhage as a life-threatening post-operation complication was lower in this study (5.5%). On the other hand, the prevalence of bile duct fistula after PG was higher than in other studies. (7.31%, Table 5).

We calculated the DMPG survival rate for the first time according to a recommendation for future researchers by Topal et al [17]. The Survival rate after a mean follow-up of 21.06±12 months was 21.06±14.23 months in our study [17]. Survival rates for all pathologic types were similar and ranged from 1.33-2 years except for non-malignant tumors which resulted in a higher mean survival of 3.17 years (Figure 1).

Although our results indicate that DMPG reconstruction is a safe technique following PD, the claimed lower rate of post-operative complications and lower hospital stay is still in question and needs further investigation. Compared to other studies and the prevalence of this condition, the number of patients in this study is acceptable, however, it had some limitations. We did not perform subgroup analysis based on duct diameter. Also, pancreatic remnant consistency was not documented after our operations. Results might also be influenced by the surgical technique used for PG here. Also, we did not compare outcomes after DMPG with PJ anastomosis as a control group.

CONCLUSIONS

This study with a rather large number of patients and long-term follow-up after DMPG showed a lower rate of many post-operative life-threatening complications such as hemorrhage. On the other hand, the prevalence of bile duct fistula after PG was higher than in other studies. Though our results indicate that

Duct-to-mucosa pancreatico-gastrostomy reconstruction is a safe technique following PD however, does not confirm the priority of this method over older techniques. However, this contradiction calls for more studies including clinical trials and metaanalysis to clear all obscure dimensions.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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None

Authors' contribution

SNS, JAM, HG, ARK, MH, MR, SM were responsible for the study concept and design. SNS, JAM, HG, ARK, MH, MR, SM led data collection. SNS, JAM, HG, ARK, MH, MR, SM were responsible for the analysis and interpretation of data. SNS, JAM, HG, ARK, MH, MR, SM wrote the draft.

Ethics approval and consent to participate

This study was approved by the ethics committee of Baqiyatallah University of Medical Sciences. Throughout the study, researchers adhered to the Declaration of Helsinki. Written informed consent was obtained from the participants in this research.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Primary Headaches and Their Most Important Imagistic Features in Clinical Practice

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Abstract: *Background. Primary headaches are comprised of three big categories: migraines (with or without aura), the third most common and the 6th most disabling disease worldwide, tension-headaches, and trigeminal autonomic cephalalgias, each with different, complex, and not completely understood pathophysiological, clinical, and neuroimagistic characteristics. Structures like the brain stem, hippocampus, or hypothalamus have been depicted as being the main triggers of these types of headaches, which have a significant impact on the quality of life. Methods. We performed a broad literature review on primary headaches from January 2016 to June 2023, analyzing the latest scientific updates and emphasizing the pathophysiological and neuroimagistic characteristics. Results. We provide a concise review of the main neuroimaging studies that help in differential diagnosis between several types of primary headaches. Conclusions. It is difficult to approach the pathophysiology of these three primary headache categories. Keeping in mind that neuroimagistic methods are rapidly developing (especially in the MRI sphere), the most important endpoint of future studies will be to establish a reliable distinction system for all these types of cephalalgias, thus updating the protocols and guidelines. Currently, imaging studies are indicated only in certain circumstances.*

Keywords: *migraine; tension-type headache; pathophysiology; trigeminal autonomic cephalalgias; imaging studies; cluster headache; SUNCT; MRI; fMRI; SUNA*

INTRODUCTION

Headache is a recurrent and upsetting symptom in the neurological sphere, also being one of the most frequent ones, patients often present to the physician for it. Most of the studies suggested that more than 90% of the population has suffered from headaches at least once in their lives [1]. The diagnostic standard for headaches of any cause has been offered over the past two decades by ICHD since 2004 and today reaches the updated ICHD-3 [2]. This accounts for approximately 3% of emergency department admissions, but some authors have identified percentages as high as 8% [3,4]. It is important to emphasize that the repeated presentation of a patient to ED for the same symptoms significantly increases the costs associated with medical care. These recurrent presentations occur in approximately 10% of headache patients [3]. When a severe headache begins, patients present to the ED frightened by the thought of a serious condition. However, one study showed that of 3026 imaging studies of patients presenting to the emergency department, pathology was objectified by imaging in less than 3% of them [5].

Given the high prevalence of this type of neurologic symptomatology, clinicians must carefully consider the indication for imaging studies because they are expensive and usually do not provide the desired information to guide subsequent treatment. In addition, headaches can lead to absenteeism from school or work, increasing the indirect costs associated with pathology. In a recent study, workers with headaches were observed to have low productivity or, more rarely, to be absent from work for up to 7.5 days/month [6].

Headaches of a primary cause are set apart by the presence of headache without any underlying pathology. These are far more common than secondary headaches. For example, the prevalence of TTH is as high as 70% during a person's lifetime [7]. In the past, categorization of these pathologies has been difficult because symptoms vary from person to person, but also in the same individual over time [8]. Standardization of primary cause headaches has been achieved by the ICHD-3 classification, which divides primary headaches into migraine, both with aura or without it, tension-type headache (TTH), and cephalalgias which belong to the trigeminal autonomic sphere [2,9]. The mechanism of action of these pathologies is not yet known, but research is ongoing. In this paper, we propose to present the situations in which imaging studies are indicated.

MATERIALS AND METHODS

We reviewed the literature related to primary cause headaches by searching the Google Scholar and Pubmed English databases between 2000 and 2023 with keywords such as "headache", "cephalalgia", "imaging", "MRI", "primary", "migraine", "tension-type", "cluster", "trigeminal", "autonomic", or any logical combination of these terms. In the first stage, the retrieved studies were filtered by a title check to exclude duplicates and assess their relevance. After this first step, the outstanding studies were distributed evenly and randomly to the authors of this study, in the first phase by reviewing the abstract to determine relevance and later by studying the entire text. We have extracted relevant, basic information from these articles, but also important news that may change the paradigm of primary headaches.

RESULTS

Migraine

Migraine is a disabling disease, being the most prevalent type of primary headache, with 12-15% of the general population suffering from it [10,11]. Prevalence is different between males and females, and also it varies by the age group: interestingly, in children, puberty, this pathology is more common in boys. In young adults, it differs, being more usual in females, probably due to the hormonal changes they undergo [12]. Throughout life, the prevalence is 12-17% for women and 4-6% for men [13]. It manifests as moderate to severe headache episodes, usually unilateral, with mild to moderate cognitive deficit and cortical hyper-excitability manifestations such as phonophobia or photophobia, nausea, and vomiting. Two phases of migraine are described: the ictal phase, which occurs during a migraine attack, and the interictal phase, which occurs between two migraine attacks.

Up to 30% of patients with headaches accuse migraine attacks, and their frequency also influences cognitive deficits [14,15]. The ratio of men to women is 1:3. According to the ICHD-3, the main types of migraine are those without aura, those with aura, chronic-type migraine, complications of migraine, probable migraine, and episodic syndromes associated with migraine [16]. Migraine appears as a result of electrical abnormalities occurring in the brainstem, and vascular abnormalities in the cerebrum, and when we talk about migraine with aura, the result of cortical spreading depolarization [17].

The aura manifests most often as a visual aura (80-90% of cases), characterized by flashes of light in the simplest situations to scotomas or complex hallucinations [18].

Four phases of migraine have been described: the prodromal phase, in which the first warning symptoms appear (depression, fatigue, hunger, food cravings), which are most likely associated with dopaminergic activation and precede the headache by 2-48 hours [19]; the aura (which occurs in one-third of migraine patients) represents reversible neurological symptomatology that usually lasts about one hour and it usually manifests as sensory, verbal or motor symptoms. However, the visual ones are the most common. These changes come before the start of the cephalalgia [16,20]; headache is commonly on only one side of the head, having a progressively aggravating pattern at the beginning, being linked with autonomic symptoms like photophobia or phonophobia, or with symptoms like nausea, sickness-like condition or vomiting [20]; the postdromal phase, which is associated with a localized headache that is aggravated by movement, fatigue, or dizziness.

Migraine can be clinically assessed by structural MR imaging. For example, we now know that both aura and headache are mainly associated with vasoconstrictive changes in cerebral arteries and veins. Hypersensitivity in the region innervated primarily by cranial nerve V, but also in extracranial regions, causes allodynia when a normally painless stimulus becomes painful, such as wearing glasses or a light touch to the face.

MRI examinations are not routinely recommended in migraine patients without aura when preventive treatment is not necessary, and some authors recommend imaging studies when three lines of preventive treatment fail [21]. Another recent study emphasizes that imaging examinations are not needed in migraine patients without red flags [22]. In migraine patients with aura, no examinations are recommended in typical cases, and in the remaining ones, MRI or MRA of the brain is recommended to rule out vascular anomalies [21]. Cerebral MRI is also recommended for vascular comorbidities or headache onset after 50 years of age. MRI is not recommended for chronic migraine, especially if the history shows progressive worsening of headache with typical symptoms [21]. In patients with persistent aura, hospitalization, and diffusion-type imaging are mandatory to rule out cerebral ischemia.

Migraineurs are sensitive to olfactory stimuli. Up to 40% of them complain of headache exacerbation when exposed to strong, irritating, or sensitizing odors, even in the interictal phase, with studies demonstrating abnormal activation of the amygdala and insular cortex in the case of certain crises caused by odor stimuli [23,24]. On the other hand, other studies determining thermal stimulation showed unequal activation of certain areas between migraine patients and the control population, such as in the lentiform nucleus, parahippocampal gyrus, subthalamic nucleus, prefrontal cortex, or temporal lobe [25,26]. 40% of migraineurs report that their migraines are activated by strong light stimuli in the interictal phase, and 90% of them report that they suffer from photophobia in the ictal phase. Some studies demonstrate visual-nociceptive integration at the brainstem level [27].

Usually, imaging studies are not required in patients with a normal clinical examination who present with typical migraine symptoms according to the ICHD-3 definition [20,28]. To distinguish between patients who do not require additional imaging studies and patients with warning signs who do require imaging studies, the SNOOP algorithm is used successfully, as follows: S for systemic disease (e.g., infections, HIV), N for neurologic assessment (confusion, seizures, focal neurologic signs), O for onset (sudden onset, first episode after age of 50), O from other associated features (medications, toxins, trauma, association of headache with Valsalva maneuver), and P from previous episodes (or headache progression). The American College of Emergency Physicians 2019 (ACEP 2019) and American Academy of Neurology (AAN) guidelines recommend imaging studies only for warning signs that are not indicative of a common headache [29,30]. The American College of Radiology (ACR) emphasizes that clinical physicians should not perform repeated imaging studies when the headache is not complicated [31].

However, in migraine, deep white matter lesions (Figure 1A, T2, and FLAIR hyperintensities) are seen in 20-30% of MRI examinations, regardless of age or periventricular white matter lesions. Their exact pathophysiology is not known, but two theories have been mainly discussed: the theory of ischemia due to circulatory disturbances in the small perforating vessels together with inflammatory changes with consequent hypoperfusion, evidenced in MRI imaging by the appearance of small focal ischemic spots, mainly at the level of the frontal lobe (Figure 1B), but also in the corpus callosum, and the theory of neuroinflammation [32,33]. The latter emerged after the CAMERA study, which highlighted in females that deep WML takes place without a connection with ischemic lesions [32]. It is noteworthy that numerous subsequent studies have highlighted the fact that in migraine, the deep supratentorial white matter lesions are important but these do not correlate with the development of the disease, whereas the infratentorial lesions do not affect migraine attacks [30]. In addition, migraine patients appear to have a higher risk for cerebrovascular disease, but also doubling the risk of ischemic stroke [34,35].

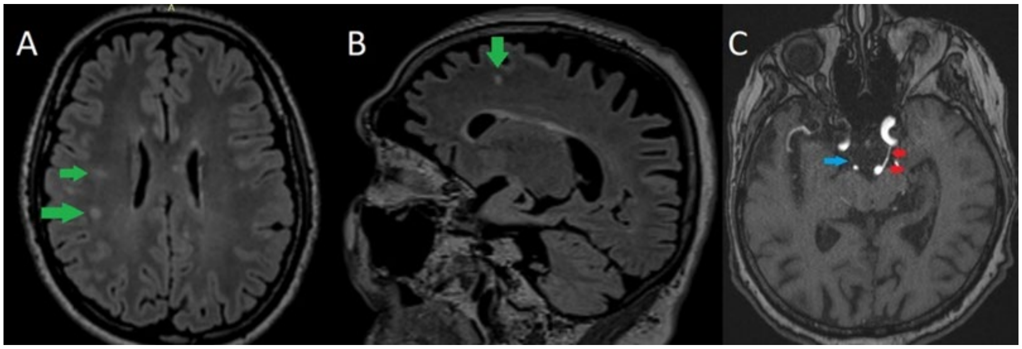

Figure 1: A: T2-weighted and FLAIR hyperintense lesions, in the centrum semiovale on the right side and deep white matter in a patient accusing repeated migraine attacks (green arrows). **B**: T2-weighted and FLAIR hyperintense lesion in the superior frontal gyrus (green arrow), the usual location for demyelinating lesions in migraine patients. **C**: asymmetry of the circle of Willis, showing a filiform right posterior communicating artery (blue arrow) compared to the contralateral one that shows significant blood flow (red arrows).

In migraine with aura, there may be transient restricted diffusion in the affected areas during the aura, in many studies taking place, especially in the corpus callosum [36].

Following some recent studies using MRI angiography (MRA), a new theory arises, advocating that there are asymmetries pointed out in the circle of Willis in a bigger population of migraine patients, particularly with aura (Figure 1C) [37,38].

In summary, in migraine patients without aura, imaging studies are not recommended for usual symptomatology that does not require treatment. For treatment-emergent migraine episodes, some authors recommend cerebral MRI or MRA when three lines of therapy have failed. In migraine patients with aura, an MRI of the brain is almost always recommended, or MRA if posterior fossa symptoms are present. In chronic migraine patients, additional imaging studies are not recommended as long as the symptomatology is not progressing, as long as the symptoms do not progress, but fundoscopy should be an integral part of the clinical examination in all patients with headaches [2,21].

Tension-type headache (TTH)

Tension-type headache represents a mild to moderate cephalalgia with a pressure-like character and usually occurs bilaterally, sometimes with anterior or posterior topography and sometimes it is longitudinal, lasting from minutes to days or even weeks. In contrast to migraine, it does not go along with autonomic manifestations or nausea and vomiting, and the presence of the above features precludes the diagnosis of tension-type headache. ICHD-3 classified TTH into four broad categories: infrequent epi-sodic TTH lasting < 1 day/month, frequent episodic TTH lasting 1-14 days/month throughout> 3 months, chronic TTH with a duration of > 14 days over > 3 months, and probable TTH [16]. The best diagnostic tool for tension-type headaches is the creation of a diary in which the patient records the frequency of painful episodes, their intensity, and their characteristics [39]. Considering the lower pain intensity, a lower proportion of patients with TTH consult a physician compared with migraine patients. One study has shown that the number of physician visits for TTH is 3 times lower than for migraine patients [40].

TTH is the second leading cause of chronic disease worldwide and is a socioeconomic burden, with 820 days missed from work or school per 1000 patients [41,42]. The most common differential diagnosis is migraine, as migraine patients often overlap with tension-type headaches.

The reported prevalence is 70% of the population, although 59% of patients report only episodic TTH [7,43]. Prevalence peaks in the 30-to-39-year-old age group and then decreases slightly [44]. Several studies have observed that sleep deprivation in combination with a disorganized lifestyle contributes to the occurrence of TTH [42,45].

Central factors are particularly important in tension-type headaches. While the pain threshold is normal in patients with eTTH, this threshold is somehow lower in tension-type headache patients with a chronic form [46,47]. Negative states, like depression or anxiety, and also chronic stress in TTH patients can increase the risk of headaches by lowering the nociceptive threshold [48,49].

In a study from Taiwan, it was suggested that the risk factors for TTH were more than 7 days of headache and female gender [50].

To date, although we use more and more advanced imaging techniques, the TTH pathophysiology is still not completely elucidated. Myofascial factors are incriminated to have a crucial role in episodic TTH along with pericranial muscle contractures, whereas sensitization of nociceptive sensory pathways by repetitive headaches leads to chronic TTH [51-53].

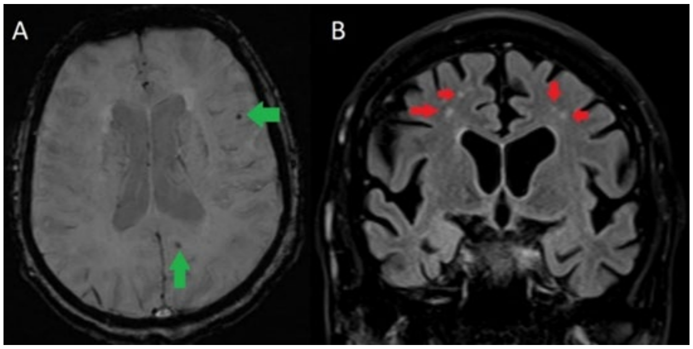

Figure 2: A. Magnetic susceptibility sequence in the axial plane. Changes in the magnetic susceptibility sequence with a suggestive appearance for microhemorrhages in the deep white matter and cortico-subcortical junction. **B**. Lesions in T2 and FLAIR hypersig-nal at the level of the bilateral corona radiata, in TTH patients without being specifically associated with some deep localizations such as in migraineurs.

As we already found out in the migraines chapter, imaging studies should not be carried out with typical-ly-described symptoms. More specifically, in patients with infrequent tension-type headaches, imaging is not recommended. In patients with frequent or chronic TTH, similar to migraine, some authors recommend an imaging examination when three lines of preventive treatment have failed [21]. Patients with chronic tension-type headaches should undergo one single imaging examination, either MR or CT, at the initial admission in the ED to exclude other differential diagnosis pathologies. The same applies to the TTH patients. The latter ones will generally have small foci of hemorrhage defined as hypodensities on susceptibility-weighted sequences deep in the white matter (Figure 2A) and deep WML, having the same physiopathological substrate as the migraineurs, increasing the risk of dementia or stroke later in life [54-56]. TTH and migraine patients generally have a similar number and amplitude of deep WML, but contrary to migraineurs, the TTH-associated WML does not show a preferred topography (Figure 2B). However, studies have shown that imaging examinations are not recommended because they do not improve the diagnosis and treatment, but they can cause and increase anxiety related to health status [57,58]. Acetaminophen and aspirin are thought to be efficient in cutting down acute symptomatology of hypertensive headaches. Opioids or triptans should be avoided. Ibuprofen and ketoprofen are the most effective NSAIDs for pain relief. Most patients benefit from lifestyle changes that promote a healthy lifestyle by quitting smoking and eating foods rich in antioxidants, and nonpharmacologic and behavioral therapies such as relaxation, biofeedback, or acupuncture can be helpful in most cases.

Trigeminal autonomic cephalalgias (TAC)

Cluster headache

Cluster headache is not a common headache, but is the most prevalent one in the group of TAC cephalalgias, having a prevalence of about 0,1% in the general population, affecting males and females differently, in a 6:1 manner. Many of the patients affected are older than 20 years of age [14,59]. Its most characteristics are severe pain with spontaneous remission, recurrent (usually circadian), unilateral, lasting 15-180 minutes, associated with autonomic ipsilateral cranial clinical signs such as lacrimation, conjunctival injection, or nasal congestion, the latter often leading to the misdiagnosis of sinus pain, with a completely different treatment from cluster headache [16,60]. In rare cases, cluster headaches may occur without autonomic symptoms [61].

80-90% of patients have episodic cluster headaches that occur apart from circadian development related to season (especially spring and autumn), number of daily light hours, and sleep stage, being more frequent during REM sleep [62]. Moreover, the relationship between sleep and cluster headache is very close. Many studies have shown that approximately 80% of patients who have manifestations of cluster headache will present cephalalgia attacks in the middle of the night, disturbing their sleep, but only 30% of them will show cephalalgias when they are napping during the day [63]. However, most of the patients complain of disturbed sleep quality, whether it occurs during or outside the headache period, and as a compensatory measure, some patients forget nightly sleep to avoid developing cephalalgic episodes [64]. An association between cluster headaches and sleep apnea has also been noted [63]. 10-20% of patients suffer from chronic cluster headaches, which have a very short period of remission, generally of the order of days.

The physiopathologic mechanism of this pain is not fully known. Both neurovascular causes and central mechanisms are suspected [65,66]. Given the predominant development with circadian recurrences, it has been suggested that the hypothalamus is of central importance for the development of pain, and also the trigeminovascular system and the parasympathetic systems being incriminated for starting and maintaining the symptomatology, those being involved in the autonomic trigeminal reflex [67]. Even though the V1 division of the 5th cranial nerve plays an important role in the development of algic symptoms, the autonomic ones are developed by the parasympathetic fibers of the facial nerve [68]. Since the 1990s, but also in more recent research, a genetic substrate is suspected, as there is a familial predisposition [69-71].

An association between cluster headaches and smoking has been confirmed. A recent study showed that 88% of the participating patients were active smokers or had smoked in the past [72]. When partial Horner syndrome occurs, cerebral MRI scans and targeted imaging studies at the level of the lung apex must be performed to identify lesions of the recurrent laryngeal nerve. Polysomnography may also be performed in cluster headache patients, as it is often associated with restless legs syndrome and helps to identify and treat this pathology [21].

In all types of TAC, cerebral MRI with high-resolution sequences of the cranial nerves is required at baseline to evaluate the parasellar structures. Similar to migraine, MRA is recommended if the first three lines of preventive treatment fail [21]. Changes in some brain structures were observed on imaging, particularly at the level of the thalamus, nucleus cinguli, prefrontal, parietal, and insular cortex [73,74]. Some older studies showed structural changes in the hypothalamus by MRI scans when headache was active, whereas other studies did not find these changes [75-77]. Some authors suggested that a decrease in grey matter volume in the visual cortex can occur, but it is non-specific [78,79].

Treatment of cluster headaches is divided into acute episode treatment and prophylactic treatment [80]. It includes drug treatment as well as patient education and lifestyle modification. As lifestyle changes, we can mention the avoidance of alcohol consumption, daytime sleep, or excessive physical activity. Treatment of the acute episode is initially by administering 100% oxygen by mask, up to 10 L/minute, for 15-20 minutes, which can relieve the cephalalgic episode in up to 15 minutes. Other therapeutic options include sumatriptan, a serotonin receptor agonist known to be the most effective therapeutic option and effective within 15 minutes when administered intravenously; dihydroergotamine, effective within 10 minutes when administered intravenously; zolmitriptan, effective within 30 minutes when administered orally; and local anesthetics [81]. Prophylactic treatment consists mainly of prednisone, dexamethasone, and ergotamine derivatives.

SUNCT and SUNA

SUNCT and SUNA are the other two types of TAC. Together, they are referred to as SUNHA [82]. The etiology and pathophysiology are not fully known. The association between these pathologies and posterior fossa lesions is well known, and recently the association between pituitary adenomas and these headaches has been discussed [83]. The pain generally occurs in the trigeminal area, more specifically in the V1 division and there is a high difficulty in differentiating between SUNHA and trigeminal neuralgia. These headaches have a sharp or burning character, last for a minimum of 5 seconds to a maximum of 10 minutes, and have a "sawtooth" development. A minimum of 20 pain attacks correlating with autonomic symptoms is required for clinical diagnosis, criteria defined by ICHD-3 [16,84]. The incidence is 0.1 per 10,000 population and the prevalence is 0.6 per 10,000 population [85].

The physiopathologic mechanism incriminated is represented by the activation of the trigeminal reflex and, similar to cluster headache, by triggering the hypothalamus [86]. Some recent studies have shown that trigeminal neurovascular conflict is important in the etiology of SUNCT and probably SUNA, with neuroimaging highlighting the similarity of changes in both pathologies, even assuming that the distinction between these two entities can be discarded [87,88]. High-resolution MRI imaging revealed neurovascular conflicts in more than 85% of patients with SUNCT or SUNA [87]. In addition, a recent study demonstrated ischemic changes in the left ventricular septal defect in approximately 15% of patients, leukoaraiosis in 5% of patients, and brainstem infarction in 5%. 88% of all patients had a vasculonervous conflict between the trigeminal nerve and certain vascular branches [85].

Therapeutically, the administration of many drugs used in other primary headaches has been tried, but with rather poor results. In this respect, SUNCT and SUNA differ from the other primary headaches. They respond mainly to the treatments used to treat neuropathic pain. Lamotrigine is effective in a large percentage of patients with the first pathology and less effective in the latter and is administrated at a dose of 100 to 300 mg daily. Topiramate isn't as effective as lamotrigine, and a smaller percentage of patients report symptomatology improvement. Carbamazepine is extensively studied and given in the treatment of these two pathologies, although with some poor results for the improvement of symptomatology in the SUNA group of patients. It is generally administrated in the standard dose of 200-1600 mg. Lidocaine is administered in an intravenous infusion of 1-4 mg/kg/hour to relieve severe headache attacks. Attempts may also be made to damage the trigeminal nerve or Gasser's ganglion by stereotactic surgery, radiofrequency stimulation, glycerol gangliolysis, or mechanical compression [89]. It is important to note that SUNCT and SUNA may also occur secondary to lesions at the level of the posterior fossa: damage to the cerebellum or brainstem by ischemic, tumoral, or vascular mechanisms. It is important to note that high-resolution MRI imaging studies can provide details about neurovascular compression, but these are not essential [2,21].

Hemicrania continua (HC)

Hemicrania continua is another primary headache that presents as a unilateral headache of moderate intensity with development over several months and belongs to the group of TACs according to ICHD-3 [2]. As the name implies, the pain is continuous and constant, but sometimes exacerbations associated with autonomic symptoms may occur. The response to indomethacin is pathognomonic [90]. It is estimated that the incidence of pathology is 1.0% and that continuous hemicrania accounts for approximately 1.5% of all primary headache cases [90]. Cases of continuous bilateral hemicrania were rarely presented [91]. According to ICHD-3, a clinical diagnosis requires a unilateral headache with pain duration of more than 3 months, ipsilateral autonomic symptoms, restlessness associated with the symptoms, and response to indomethacin treatment. Exacerbations may occur as a result of stress, alcohol consumption, or, in women, menstruation [90]. If HC is clinically suspected, cerebral MRI is recommended in all patients, and MRA or CT angiography of the head and neck should be indicated if an aneurysm or vascular pathology is suspected. Response to treatment with indomethacin is pathognomonic. Start with a dose of 25 mg three times daily, and if the patient does not respond to this treatment within 2-3 days, the dose is gradually increased up to a maximum of 300 mg per day [92].

Other primary headaches

Primary cough headache (PCH) represents a rare disorder in which bilateral cephalgia starts suddenly during coughing and may last up to two hours. According to ICHD3, the clinical diagnosis of primary-type headache is made by at least two episodes of pain that occur only after coughing or the Valsalva maneuver and last between one second and two hours, with no other associated intracranial pathology. However, up to 50% of patients hospitalized for this type of pain may have underlying lesions, such as Chiari malformation type 1, obstructive hydrocephalus, or tumors. Therefore, it is strongly recommended that imaging studies of the brain be performed, such as MRI or MRA [2,21,93]. Indomethacin is the treatment of choice for this type of headache [2].

Primary exercise headache (PEH) is a rare pathology similar to PCH, characterized by a bilateral pulsating headache that occurs only in the context of prolonged physical activity, lasting 45 minutes or less [2]. According to ICHD-3, when the patient first presents to the hospital with symptoms, it is mandatory to perform imaging studies to rule out "subarachnoid hemorrhage, arterial dissection, or vasoconstriction syndrome." Indomethacin is the treatment of choice for this pathology, in a dose of 25-150 mg before starting physical activity [94].

Primary headache associated with sexual activity is a diffuse bilateral headache that begins during sexual activity and lasts from 1 minute to 24 hours, accompanied by nausea or vomiting but without autonomic symptoms. These may present as progressive or sudden headaches, most often beginning at the moment of climax. Although it is generally not a serious medical problem, brain imaging is recommended to rule out other pathologies, such as subarachnoid hemorrhage or aneurysm [95]. It is difficult to quantify the epidemiology of this pathology because patients are reluctant to go to the doctor to be examined due to shame or ethical reasons. Some authors recommend prophylaxis with beta-blockers and symptomatic treatment with indomethacin [96,101].

Because of the rarity of these pathologies, there are no large, homogeneous studies quantifying the imagistic changes specific to each of these three pathologies, but rather small studies and case reports. For this reason, it is important to undergo a CT scan, especially in cases of sudden onset headache, to rule out secondary causes such as rupture of an aneurysm or subarachnoid hemorrhage, and subsequent vascular studies such as CT angiography or MRA are recommended in specific cases.

DISCUSSION

Despite the use of increasingly advanced imaging examination methods that have improved the understanding of the pathophysiology and mechanisms of development of primary headaches, the medical community still cannot fully understand and fully, optimally treat primary headaches. Structural MR imaging has demonstrated the presence of T2 and FLAIR hypersignal lesions that can be correlated with different pathologies that mimic the primary types of headache, although those appear especially with aura migraine. MR Perfusion imaging has shown several abnormalities in blood circulation and neuronal activity during cephalalgia exacerbations in every type of primary headache, demonstrating a lowering of pain threshold and, compared with the general population, deficient activation of many different areas in the brain responsible for interpreting pain, and in the case of migraineurs in particular, there is both a supernumerary activation of the areas as well as a more important or intense activation of them. Many authors have demonstrated that the autonomic nervous system takes part in the mechanism of headache development, due to age-related development and the decrease in headache amplitude in the case of noninvasive stimulation of the vagal endings for cluster headache prevention. These suspicions, especially in migraine, are not confirmed by imaging studies, either structural or functional. The use of imaging studies, regardless of their type, to diagnose migraine or tension-type headaches is not recommended because of the lack of specificity of the studies. Because these are debilitating diseases that affect both individual lives and society, sufferers search for answers and go from one physician to another in search of them. Repeated imaging studies in these cases represent a significant waste of resources, and each pseudonormal imaging result leads to even greater concern and anxiety about health status. The correlation of the new, recently discovered data with the clinical evolution of the patient can improve the selection and treatment of patients at risk for primary headache development, sometimes even from childhood. Today we know that primary headaches begin around the age of 5 years and the highest prevalence is found in the age group between 20 and 40 years, decreasing with age. The phenotypes and treatments of each pathology are different, even if they belong to the same class of pathologies. Currently, we can summarize that there are ischemic lesions, circulatory disturbances, vascular conflicts, 5th cranial nerve activation, and a lower pain threshold, all having their role in the development of these pathologies. Current studies include far too small and inhomogeneous groups of patients to be able to present convincing results regarding structural changes in the brain parenchyma or the various neuronal circuits.

CONCLUSIONS

In recent years, significant progress has been made in understanding the pathophysiologic mechanisms, both central and peripheral, of the primary cause of headaches, by using highly advanced technologies like structural high-resolution

MR, both of these imaging studies of cerebral structures allow the detection of cerebral parenchyma lesions with greater precision.

Pharmacologic interventions may help alleviate headaches, and recently several minimally invasive interventions have been tried, mainly targeting the physiopathologic mechanism that occurs via the cranial V nerve, obtaining promising results. However, there are still physiopathologic and imaging ambiguities related to these primary pathologies.

There is still an unmet need for studies in all fields, in large groups of homogeneous patients, to answer these questions.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, RID, FAA, AMR, and CAS; methodology, AFN, FAA, AMS, and CAP; software, RID, AFN, OV, and AMR; validation, AMR, OV, CAS, and CAP; formal analysis, RID, AFN, AMR and FAA; investigation, RID, AMR, OV, AMS, and CAS; resources, AFN, FAA, and OV; data curation, RID, AMR, AMS, CAS, and CAP; writing—original draft preparation, RID, AMR and CAP; writing—review and editing, AFN and AMS; visualization, RID; FAA, AMR, and CAS; project administration, FAA. RID and AMR contributed equally to this paper. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

E-cigarettes and Associated Medical Burden: For Better and For Worse

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Abstract: *The harmful effects of smoking cigarettes on human health are well documented; thus various smoking cessation methods have been assessed and new options are currently under development to provide a "better' alternative to the "worse" one, namely smoking. However, no ideal method of ending or replacing conventional smoking has been developed so far, and actually, none of them has been proven entirely effective or safe. The laxity, or rather the absence of regulations in the first decade of existence of electronic nicotine delivery systems (ENDS) allowed their use with prohibited substances instead of nicotine or other substances with detrimental effect, with the emergence of a severe syndrome - e-cigarette and vaping-associated lung injury (EVALI), whose treatment required even lung transplantation in young people without any prior underlying lung disease. This narrative review aims to provide a brief overview of concerns about medical issues associated with e-cigarette use, particularly cardiovascular and respiratory panels. We organized the data in several micro-sections varying from practical aspects of understanding ENDS to clinical issues. To combat tobacco addiction, electronic cigarettes are increasingly widely accepted. A global regulatory framework is required to prevent the emergence of an illicit e-cigarette business with detrimental impacts on health.*

Keywords: *e-cigarettes, EVALI, smoking, lung, respiratory, cardiovascular, pulmonary, electronic cigarette, ENDS*

INTRODUCTION

The harmful effects of smoking cigarettes on human health are well documented – from a simple addiction to a significant risk factor for lung cancer. Recent data places smoking as the leading risk factor for lung cancer, the most common type of cancer in the world, which is responsible for more deaths annually. The detrimental effects on cardiovascular, pulmonary, and reproductive systems are also considerable. Therefore, various smoking cessation methods have been assessed – behavioral, pharmacological, and combined methods and new options are currently under development to provide a "better' alternative to the "worse" one, namely smoking. However, no ideal method of ending or replacing conventional smoking has been developed so far, and actually, none of them has been proven entirely effective or safe [1-4].

Therefore, that's why there has been a continuous struggle to find smoking methods that do not involve burning nicotine, with heat-not-burn devices being the best, safest alternative, and an efficient smoking cessation tool. However, in the last decade, considerable evidence has been presented showing an induction of addiction in adolescents and smoking initiation in those who have never smoked both adolescents and adults. On the other hand, the laxity, or rather the absence of regulations in the first decade of existence of electronic nicotine delivery systems (ENDS) allowed their use with prohibited substances instead of nicotine or other substances with detrimental effects, with the emergence of a severe syndrome – e-cigarette and vaping-associated lung injury (EVALI), whose treatment required even lung transplantation in young people without any underlying lung disease. After the emergence of EVALI, which unfortunately has been overshadowed by the coronavirus disease-19 (COVID-19) pandemic, an increasing number of countries around the world have imposed regulations on the use of ENDS, which are now considered medical devices in some countries and are banned in others. Since it has been determined that the numerous flavors available to users have led to an increase in their use by adolescents, current legislation in most countries that have regulations limits the number and type of flavors available [5-8].

Aim

We aim to provide a brief overview of concern for medical issues associated with e-cigarette use.

METHODS

This is a narrative review of literature with no timeline restriction of research. We organized the data in several micro-sections varying from practical aspects of understanding ENDS to clinical issues.

ENDS – electronic nicotine delivery systems

ENDS include various devices, such as electronic cigarettes, e-hookahs, advanced personal vaporizers, and cig-a-likes. Currently, there are two main types of heat-not-burn products – tobacco heating devices and electronic cigarettes (four generations). From its first release in China in 2003 until today, the e-cigarette has undergone numerous improvements, with the current generation allowing a complex control of the device with significantly improved user safety. Electronic cigarettes are composed of an energy source (a rechargeable battery available from the second generation), a heating device - an atomizer, responsible for the generation of aerosols, a flow generator, a refillable (available from second generation) cartridge for e-liquid (water, propylene glycol, nicotine, flavors, and glycerol) and a mouthpiece. The third generation of e-cigarettes allows resistance and power adjustment while the fourth generation offers the possibility of temperature control (generally temperatures range from 40-65 Celsius), allowing for the modulation of the oropharyngeal experience [9-13].

It has been observed that by increasing the heating temperature, the aerosol generation rate also increases, and the aerosol particle size is modified as well (staying in the 100-600 nm range), with a potential impact on aerosol toxicity. Tobacco heating devices consist of a holder, an accumulator, and a nicotine heating unit (blade or induction heat technology) with aerosol generation. The differences between tobacco heat devices and e-cigarettes consist of user experience, the first one having a different operating time (6 minutes and a maximum of 14 puffs) and the temperature at which it operates (conventional smoking requires 200-600 Celsius). Nicotine is present in the tobacco that is used in the device. Also, not all e-cigarette devices use e-liquid with nicotine [14-19].

ENDS chemical compounds

E-liquid compounds include solvents (humectants or stabilizing agents), nicotine, cannabinoids, metals (in both the nicotine extract and the device constituents – nickel, manganese, copper, iron, arsenic, and lead), and flavors. The harmful effects of smoking are due to the inhalation of toxic products in cigarette smoke, generated by the combustion and pyrolysis of nicotine. Smoke from regular cigarettes contains more than 7000 different substances, including at least 70 carcinogens [20-24].

Unfiltered cigarette smoke is an aerosol with more than 400-500 gaseous components (nitrogen, oxygen, carbon monoxide and carbon dioxide, water, hydrogen, ammonia, argon, nitric oxide, hydrogen cyanide, hydrogen sulfide, methane, volatile alkenes, volatile alkenes, aromatic hydrocarbons - benzene, toluene, styrene, acids - formic acid, acetic acid, propionic acid, aldehyde - formaldehyde, acetaldehyde, acrolein, acetone, methanol, furan, pyridine, methylamine, etc.) as well as over 3500 other particulate constituents (nicotine, nornicotine, anatabine, anabazine, non-volatile hydrocarbons, carcinogenic polynuclear aromatic hydrocarbons, phenols, phthalates, polyphenols, acids – palmitic, stearic, oleic, linoleic, linolenic, lactic, indoles, quinolones, aromatic amines, nitrosamines and glycerol). In addition to these, various flavorings, pesticides, and metals are also present. Cigarette smoke contains 72 carcinogens and at least six compounds that harm reproduction [25-29].

Because they inhale both mainstream and sidestream smoke, passive smokers are more exposed to these dangerous substances than regular smokers. The amount of nicotine delivered by the e-cig is the result of several factors – smoking pattern (experienced versus inexperienced), aerosol characteristics (pH, particle size), pharmacokinetic and pharmacodynamics features, the site of absorption (oral or pulmonary), and of course the nicotine level in the e-liquid. The new generations of e-cigarettes deliver more aerosols and consequently higher amounts of nicotine, as demonstrated by their serum levels. The amount of nicotine in traditional cigarettes ranges between 10-15 mg nicotine/cigarette, of which 1-2 mg nicotine/cigarette is systemically absorbed, compared to 0-36 mg/mL liquid in electronic cigarettes [30-35].

E-liquid includes propylene glycol, vegetable glycerine, nicotine, and flavors. The e-cig structure includes absorbent materials in the atomizer (silicon, organic cotton, rayon fibers, stainless steel mesh, bamboo yar), as well as heating coils manufactured from different iron, chrome, aluminum, or nickel alloys. The usual heating temperature in early generation ENDS ranged from 45 to 60 degrees, but in new generations that allow the user to adjust the temperature, it can reach over 200 Celsius, resulting in the release of potentially harmful carbonyl products, such as acrolein in increased quantities at voltages above 3.3 V [36-39].

The most commonly encountered flavorings in e-cigarettes are diacetyl, acetoin (buttery flavor), benzaldehyde (almond/ cherry aroma), cinnamaldehyde (cinnamon aroma), cresol (leather aroma), eugenol (clove aroma) or isoamyl acetate (banana flavor). The products resulting from the conversion of liquid into aerosol can vary, the temperature obtained by heating in e-cigs not being uniform and depending on several factors – the volatilization rate, the effectiveness of aerosol absorption, product design, and battery voltage. Thus, by converting solvents, formaldehyde, acetaldehyde, acrolein, methyl glyoxal, and glyoxal are obtained, and from vitamin E, ketene, benzene, and alkene are generated [33-39].

Cardiovascular effects

As a result of nicotine absorption, there is a release of catecholamines with a secondary increase in heart rate, blood pressure, and heart contractility, concomitant with reduced coronary vascular flow and increased musculoskeletal flow. The decreased risk of cardiovascular disease has been demonstrated in both in vitro and in vivo studies. It has been shown a reduction in monocyte cell adhesion to endothelial cells, a reduction in platelet activation markers (thromboxane B2), oxidative stress markers (prostaglandin F2 alpha), and C-reactive protein and cholesterol [37-40].

The currently available studies have assessed both short-term and medium to long-term effects (years). Heart rate and blood pressure abnormalities have been shown to vary according to the smoking status of e-cigarette or former conventional tobacco smokers. Thus, in short-term use of e-cigarettes with or without nicotine, an increase in heart rate and blood pressure was observed, whereas in chronic smokers the data are not conclusive. In e-cig smokers, an increase in arterial pressure was found only for short-term users. In contrast to conventional cigarette smokers converted to e-cigarettes, the switch to ENDS reportedly promotes a reduction in cardiovascular risk and BP [41-45].

Respiratory system effects

Studies have consistently identified a significant association between using electronic cigarettes and respiratory disorders. At the pulmonary level nicotine acts by activating nicotinic receptors for acetylcholine resulting in bronchoconstriction by action on bronchial smooth muscle. Epigenetic changes resulting in reduced PPAR-gamma (mesenchymal peroxisome proliferator-activated receptor gamma) receptor expression results in an asthmatic phenotype. Lung nicotine levels are more than 200 times the systemic peak in both conventional smokers and vapers [46-48].

Propylene glycol and vegetable glycerine are used as humectants, but repeated inhalation is not associated with significant changes in lung function if the exposure is limited. On the other hand, the new generations of devices, which can reach temperatures above 300 Celsius, can generate toxic carbonyl volatile compounds – formaldehyde and acetaldehyde (at temperatures above 215 Celsius) and acrolein at temperatures above 270 Celsius, two compounds that are associated with exacerbation of bronchial asthma. Diacetyl (2,3 butanedione) a butter flavoring is known to induce irreversible acute bronchiolitis obliterans (long popcorn). Other flavorings such as cinnamaldehyde are known irritants for asthma and benzaldehyde is an irritant for the mucous membranes of the respiratory tract. Yet, benzaldehyde higher levels provided by newer generation ENDS were proven to suppress allergic inflammation in a murine model by decreasing the number of inflammatory cells in lung and nasal tissues [49-53].

Destructive effects on respiratory epithelial cells have been demonstrated in several in vitro studies, as well as on nasal epithelial cells [49-53]. In vivo research has shown reduced lung cancer risk in ENDS users [54-56]. Increased oxidative stress and mitochondrial dysfunction have also been demonstrated in in vivo studies [57-60].

Shortly before the COVID-19 pandemic, a new outbreak occurred in ENDS users- EVALI, with more than 2500 cases reported in the US over 5 months [61-72]. The predominant symptomatology was respiratory – dyspnea, cough, and chest pain, associated also digestive symptoms and fever. Most cases were caused by exposure to tetrahydrocannabinol while the remainder by exposure to vitamin E acetate which was used as a thickening agent. The latter is thought to alter the structure of the surfactant and by heating may generate keten, a toxic compound [73-77]. The introduction of regulations on ENDS status has led to a dramatic decrease in EVALI cases.

DISCUSSION

Apart from the mentioned inflammatory issues and the cardiovascular/respiratory associated entities related to the use of e-cigarettes, other medical consequences have been identified, yet, with various levels of statistical significance and some require further exploration to adequately assess their clinical impact.

For instance, while most studies have focused on pulmonary and cardiovascular effects, smoking is a well-known risk factor for oral cancer.

Current data shows a similar effect of e-cigarettes when compared to traditional tobacco smoking in terms of oxidative stress, wound healing arrest, promotion of a pro-inflammatory environment by increased pro-inflammatory cytokines, and incidence of periodontal disease, caries, and edentations.

However, the few available data on the carcinogenic effect of e-cigarette use have not led to any relevant practical conclusions, as they did not have a well-defined duration of exposure that enabled an objective risk assessment [78-84]. In addition to the direct effects on the user's health, environmental effects are described, which are attributed to the combination of compounds in mainstream and sidestream smoke.

Finally, as mentioned the most dramatic consequence in relationship with e-cigarette use is EVALI which particularly affects young individuals. While severe COVID-19 forms of infection might mimic an episode of EVALI, during pandemic years a growing number of hospitalizations for this syndrome had been registered.

The medical community increased the level of awareness concerning EVALI and recently various public health resources and scientific papers have been released to address this particular aspect [85-88].

CONCLUSIONS

Although ENDS are promoted as a safe alternative to smoking tobacco, it has been shown that adolescents who use them are more likely to start smoking tobacco again or start smoking it for the first time. Smoking is a significant global public health issue. The methods for quitting smoking have changed over time, but none have achieved perfect success. To combat tobacco addiction, electronic cigarettes are increasingly widely accepted. A global regulatory framework is required to prevent the emergence of an illicit e-cigarette business with detrimental impacts on health.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; methodology, C.N., A.-P.C., A.C., A.-I.T, A.-M.G., and M.C.; software, C.N., A.-P.C., O.-C.S., A.-M.G., and M.C.; validation, C.N., A.-P.C., O.-C.S., A.C., A.-M.G., and M.C.; formal analysis, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; investigation, C.N., A.-P.C., A.-M.G., and M.C.; resources, C.N., A.-P.C., O.-C.S., A.C., and M.C.; data curation, C.N., A.-P.C., and M.C.; writing—original draft preparation, C.N., and A.-P.C.; writing—review and editing, C.N., A.-P.C., and M.C.; visualization, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; supervision, C.N., A.-P.C., and M.C.; project administration, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; funding acquisition, C.N, and M.C.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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REVIEW

The Spectrum of Neurological Complications after Bariatric Surgery – Review of the Literature

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Abstract: *Bariatric surgery represents one of the most efficient treatments addressing obesity and its associated complications with an increasing trend. Even if the medical benefits for patients are huge, due to weight loss and despite technical improvements in bariatric procedures, it is still associated with diverse types of complications. Most of the complications appear due to nutrient deficiency, as absorption is compromised after surgical anatomy modification. Neurological complications after bariatric surgery are very diverse with an occurrence within days to years after surgery, most frequently in patients with gastrointestinal or surgical complications. Any segment of the nervous system can be affected with distinct types of manifestations ranging from peripheral axonal neuropathy, nerve palsy secondary to compartment syndrome, encephalopathy, and myelopathy, to psychiatric and demyelinating central nervous system diseases. Identifying patients at risk and systematic screening for nutrient deficiency with proper supplementation is essential for preventing neurological complications and lifelong sequelae. In this paper, we present the spectrum of neurological complications reported after bariatric surgery.*

Keywords: *bariatric surgery; nutrient deficiency; neurological complications*

INTRODUCTION

Bariatric surgery still represents one of the most efficient treatments addressing obesity and because the incidence of severe obesity is rising worldwide, more and more bariatric procedures are done annually in specialized medical centers. Even if the medical benefits for the patients are huge due to weight loss and despite the technical improvements for bariatric procedures, it is still associated with different types of complications.

Most of the complications are due to the modified anatomy which leads to impaired absorption of the nutrient, besides the inflammatory state and mechanical stress induced by surgical procedure. The most common complications are gastrointestinal and surgical complications and include but are not limited to reflux disease, vomiting, dumping syndrome, leaks with high mortality getting up to 15%, stenosis, twists and kinks causing dysphagia, bleeding, enteric perforations, internal hernias, bowel obstruction, ulcerations, and gallstones disease. Other complications seen following bariatric surgery are weight regain, pulmonary thromboembolism, metabolic and hematological disorders, such as anemia, leucopenia, and neuropsychiatric complications. [1]

MATERIALS AND METHODS

The purpose of our paper was to review the existing literature data regarding the neurological complications that occurred after bariatric surgery and the presence of nutrient impairment.

We performed a scoping review of the published literature, and we included original studies, reviews, and a series of cases by searching the Medline, PubMed, and Google Scholar databases. We included articles written in the English language.

We searched for the terms: “bariatric surgery”, “neurological complications and bariatric surgery”, “nutrient deficit and bariatric surgery”, “vitamin deficit and bariatric surgery”, “gastric sleeve”, “gastric banding”, “gastric bypass”, “vertical gastroplasty”, “obesity surgical treatment”.

We excluded papers with respect to bariatric surgery-related complications and pre-operative nutritional deficiencies.

NEUROLOGICAL COMPLICATIONS

Neurological complications after bariatric surgery were reported in 3-16% of cases and usually affected more than one component of the nervous system [2,3].

Neurological disorders are various and affect mainly the peripheral nervous system and less common central nervous system, but both are associated with a poorer quality of life and substantial morbidity. Neurological complications of these patients might occur days to years after surgery and include peripheral neuropathy, compartment syndrome, plexopathy, mononeuropathy, encephalopathy, myelopathy, ataxia, myopathy, myotonia, behavioral and psychiatric disorders [2,3,4].

The physiopathology of neurological impairment comprises nutritional deficiency, rapid weight loss, and compartment syndrome. The vitamin B12 shortage, thiamine deficit, and decreased levels of copper are the most commonly deficient nutrients responsible for neurological conditions development after bariatric surgery. Deficiency of riboflavin, pyridoxine, folate, vitamin D, E, phosphorus, calcium, selenium, zinc, and iodine can also contribute to neurologic dysfunction [3,5].

Impact Of Vitamin and Micronutrient Deficiency After Bariatric Surgery and Neurological Complications

Nutritional impairment after bariatric surgery is quite common and manifests a large spectrum of vitamin and micro-nutrient deficiencies affecting both, the water-soluble vitamins (thiamine, riboflavin, pyridoxine, folate, cyanocobalamin, vitamin C), and the liposoluble ones (A, D, E, K), alongside the fat malabsorption, loss of proteins, and minerals deficiencies (iron, zinc, copper, selenium). All these associate different clinical manifestations within a variable time frame of appearance and usually are overlooked by clinicians. Some bariatric procedures, such as Roux-en-Y gastric bypass and post-biliopancreatic diversion/duodenal switch put the patient at higher risk of developing multiple nutritional deficiencies since the absorptive surface is reduced or bypassed [5,6].

The most common deficiencies reported after bariatric surgery are iron deficiency (which was found in more than 30% of patients) [5], cobalamin and folate deficiency in up to 35%, respectively 39% after five years [6], and thiamine deficiency in 49% of patients [7].

Reports from a French cohort study showed a high rate of overall vitamin B deficiency (74%), with thiamine deficit in more than 47% of the cases [8]. A significant number of patients, up to 30%, already had micronutrient deficiency before bariatric surgery which increased the risk of new complications [9]. Hypovitaminosis D after bariatric surgery was reported in 25-70% of the patients, with calcium deficiency in 10%, but it should be considered that obesity is associated with suboptimal 25-hydroxy-vitamin D and 30% of obese patients could have vitamin D deficiency [10,11]. Vitamin E deficiency was reported up to 65.7% in a recent systematic review, and vitamin A in 5-69% in 2-4 years following surgery. Clinical manifestations of vitamin E deficiency are usually seen after Roux-en-Y gastric bypass, being mainly neurological (ataxia, peripheral neuropathy), but also hemolytic anemia, impaired immune response, ophthalmological disorders, infertility, and nystagmus can occur [12]. Although rarely clinically manifested, deficiency of vitamin C and vitamin K was reported in almost half of the patients [6,12].

Low zinc level after bariatric surgery was associated with high inflammatory status. There are reports of necrolytic migratory erythema with severe bacteremia and optic neuropathy due to zinc depletion [13,14]. The zinc repletion therapy should be done cautiously because it can induce copper deficiency. The copper deficiency occurs mostly after gastric bypass as its absorption occurs in the stomach and duodenum. Many cases of copper deficiency had been reported up till 9 years after bariatric surgery with capricious clinical expression, the majority mimicking vitamin B12 deficiency, consisting of myelopathy, ataxia, peripheral neuropathy, optic neuropathy, blindness, muscle weakness, anemia, and neutropenia [15,16, 17,18].

The diagnosis of copper deficiency usually requires the measurement of serum levels associated with the ceruloplasmin level under 20 mg/dl and C reactive protein because ceruloplasmin is an acute phase protein and levels could be misinterpreted in inflammatory states. MRI study with T2 hyperintensity in the posterior dorsal column of the spinal cord can suggest the diagnosis [19,20].

Most of the neurological complications are often seen in patients with surgical complications, longer hospitalization time, or those with gastrointestinal symptoms, especially protracted vomiting. Moreover, these patients underwent a surgical reintervention in 8-32% of the cases [8,21].

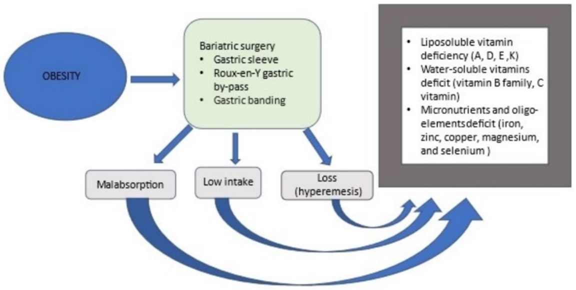
OBESITY
Bariatric surgery
• Gastric sleeve
• Roux-en-Y gastric by-pass
• Gastric banding
Malabsorption
Low intake
Loss (hyperemesis)
• Liposoluble vitamin deficiency (A, D, E ,K)
• Water-soluble vitamins deficit (vitamin B family, C vitamin)
• Micronutrients and oligo-elementsdeficit (iron, zinc, copper, magnesium, and selenium)

Figure 1: Pathophysiology mechanisms of neurological complications after bariatric surgery and types of nutrient deficiencies

Peripheral nervous system complications

Neuropathy is the most frequent neurological complication described after bariatric surgery and has been reported in 16% of patients after this type of procedure [22]. Its occurrence is induced by vitamin deficiency, especially thiamine, and cobalamin, which are involved in myelin synthesis, but also riboflavin, pyridoxine, and copper deficiency [21].

Another mechanism of occurrence is attributed to entrapment and mechanical compression. Rapid weight loss with subcutaneous protective fat tissue disappearance and inaccurate leg postures can cause compression neuropathy, frequently unilateral. Involvement is usually sensory predominant with late motor progression, chronic and symmetrical, mostly affecting distal limbs, but also small fiber neuropathies and bilateral neuropathies were several times described, contrary to plexopathy which was rarely seen [23,24].

The median nerve compression, the peroneal nerve palsy, and the ulnar nerve palsy were the commonest nerves affected after bariatric surgery. Peroneal nerve palsy is usually unilateral, but cases with bilateral affection were described.

Rapid weight loss with a mean of 45-50 kg reduction in several months is the main risk factor identified for peroneal nerve injury as the nerve's superficial anatomic localization makes it susceptible to impingement [24]. One case of meralgia paresthetica after bariatric surgery was described in an Asiatic report [25]. Rare cases of dry beriberi disease characterized initially by nausea, vomiting, constipation, severe weakness and then progressing to feet tingling and burning were described in the literature with late diagnosis, which was only suggested after performing electromyography [26].

A few cases of acute axonal polyneuropathy resembling Guillain Barre syndrome, rapidly progressive were described in the medical literature after bariatric surgery with good response to vitamin B1 and B12 high dose replacement [27]. There are also recently reported cases of mixed polyneuropathy after bariatric surgery both axonal and demyelinating after five to 24 months post-surgery and some case reports of Guillain Barre syndrome, raising the suspicion that bariatric surgery could induce immunological changes which predispose to autoimmunity [14,28]. Cases of plexopathies affecting the lumbar plexus were reported, manifesting with unilateral severe pain with sensory loss in the hip and thigh region, muscle wasting, patellar reflex loss, and extensive denervation on electromyography study [25].

Several other risk factors for predicting neuropathy development, despite rapid weight loss, were previously reported, such as low albumin and transferrin levels, gastrointestinal complications, surgical complications with long hospitalization, jejunoileal bypass, and lack of nutritional assessment after bariatric surgery [22]. Symptoms of neuropathies include pain, paresthesia, burning feet in small fiber neuropathy, usually progressive weakness, and rarely acute, foot drop, slapping gait, loss of reflection, and objective sensory loss. The diagnosis is confirmed by electromyography studies indicating distal axonal neuropathies. Biopsies of the affected nerves show axonal degeneration and peri-vascular inflammation [24,29].

Treatment includes a high dose of vitamin B12 dose of 1000 ug intramuscular once daily for a week and then weekly, vitamin B1 500 mg daily, intravenous for three days, and afterward 100 mg oral route and acid folic supplementation 5 mg per day, with good response and resolution of symptoms. Vitamin supplementation should be done for long periods and started as soon as possible when diagnosing neuropathy in post-bariatric surgery patients without waiting for confirmation of vitamin deficiency tests since vitamin levels can be normal. Nerve decompression was cited as the most efficient therapy for peroneal nerve entrapment after bariatric surgery [23]. Intravenous immunoglobulin is the treatment of choice for Guillain Barre syndrome, with a good response. Physical therapy can be used as a complementary treatment to vitamin replacement when treating neuropathy [24,29].

Functional impairment is usually mild, but it can be debilitating when the neuropathy becomes chronic or when the patient experiences weakness and foot drop. Also, small fiber neuropathy can evolve into distal neuropathic chronic pain syndrome in all extremities, deteriorating life quality [24,29].

Central nervous system complications (CNC)

The most reported CNS complications after bariatric surgery are encephalopathy, myelopathy, neuropsychiatric disorders, and optic neuropathy [30].

Encephalopathy

The most common type of encephalopathy seen after bariatric surgery is Wernicke encephalopathy secondary to thiamin deficiency, but also different types of encephalopathy are being described, such as hyperammonemia encephalopathy due to high plasmatic levels of glutamine and ammonia, zinc deficiency and urea cycle disorders. Episodic encephalopathy associated with D lactic acidosis is another type of encephalopathy encountered after bariatric surgery, usually precipitated by high carbohydrate intake, and hardly diagnosed by clinicians [31,32].

Clinical manifestation of Wernicke encephalopathy consists of acute confusion, ataxia, nystagmus, sometimes associated with cranial nerve palsies (third and sixth), seizures, dysarthria, myoclonus, asterixis, papilledema, vestibular symptoms, blurred vision, and polyneuropathy. Almost 80% of patients surviving encephalopathy develop Korsakov syndrome defined by amnesia (retrograde and anterograde), confabulation, and loss of insight, being more common in alcohol consumers. Most cases of Wernicke encephalopathy occur between 14 days to 1-2 years, mostly in women, and are usually precipitated by several factors. Vomit-ing is the most common gastrointestinal symptom precipitating Wernicke encephalopathy, usually preceding thiamine (Gloy VL) deficiency manifestations [8,12,33]. Other factors include rapid weight loss, food avoidance, poor appetite, and lack of vitamin supplementation, as thiamine reserves are depleted in less than 3 weeks without supplementation. In the absence of rapid diagnosis and appropriate treatment, the disease is fatal. Diagnosis of Wernicke encephalopathy is usually clinical, supported by laboratory and IRM studies. Tissue thiamine levels are accurately determined by red blood assays of specific enzymes (erythrocyte transketolase activity coefficient since blood and urine levels are not reliable [34].

IRM changes in Wernicke encephalopathy include symmetrical increased signal on T2/FLAIR phase in dorsomedial thalami, mamillary bodies, tectum plate, periaqueductal area, and around the third ventricle. Treatment of Wernicke encephalopathy consists of intravenous/intramuscular of thiamine 100 mg for 3-5 days minimum, followed by 30 mg twice orally for several months and correction of hypo-magnesemia since magnesium is a cofactor in thiamine-dependent metabolism. Prevention is mandatory and patients at risk should get thiamine supplementation of 100 mg intramuscularly followed by 50 orally once daily. Recovery in Wernicke encephalopathy induced by bariatric surgery after optimum thiamine supplementation is seen in more than half of the cases, the others remaining with cognitive deficiencies, gait disturbances, nystagmus, psychosis, and rarely death [15,30,33].

Hyperammonemia encephalopathy represents a particular type of encephalopathy recognized in the past years in patients who have undergone Roux-en-Y gastric bypass with a high mortality rate, characterized by a high level of ammonia in the absence of liver disease [31]. In a review from Andrew Z. Fenves et all in 2015 a mortality rate of 50% was reported in these patients with a predominance of women as the conditions were most often seen in this category of patients [35].

Laboratory findings include mild to moderate increased levels of ammonia, high levels of plasma glutamine, low albumin and zinc levels, essential amino acids deficiency, and reactive hypoglycemia [36].

The precise mechanism of onset is not yet understood, but several factors were until now described in the hyperammonemia state after bariatric surgery. Most of the lethal cases of hyperammonemia encephalopathy showed genetic predisposition caused by urea cycle abnormalities with a partial ornithine transcarbamylase deficiency or a mild carbamyl phosphate synthetase (CPS) deficiency [32].

One of the predisposing factors for hyperammonemia is the presence of huge nitrogenous residues in the liver. Intake reduction triggers a high catabolic state with protein breakdown. Ammonia level is also increased by altered gut microbiota that produces more urea lithic strains because of small bowel pouches created by bariatric surgery [31,36]. Case reports from patients with this neurological complication after bariatric surgery reported lower citrulline levels in the intestine affecting ureagenic capacity. Moreover, other predisposing conditions as the presence of a splenorenal shunt should be suspected and ruled out in patients developing hyperammonemia [32,35].

Therapeutic options include protein intake reduction, zinc and amino acid repletion, parenteral glucose, lipid supplementation, and oral lactulose. In refractory cases, hemodialysis and setting back of surgical procedures could be an option [37].

D-lactic acidosis encephalopathy is a rare condition reported mostly in short bowel syndrome, but also described years after bariatric surgery, especially after jejunum-ileostomy, and should be taken into consideration in a patient with anatomic changes of the digestive tract who develops confusion syndrome [38]. Confusion episode is associated with behavioral changes, dysarthria, ataxia, lethargy, and weakness, and is usually triggered by high carbohydrate intake. D lactate serum elevation is a result of carbohydrate fermentation in the small bowel or colon and is hardly diagnosed as serum and urine measurement of D lactate is not commonly done, L lactate being the only form that is most frequently checked when assessing lactic acidosis. A level over 3 mmol/l is diagnostic for this pathology and is often associated with a high serum anion gap [38]. Treatment involves metabolic acidosis correction with intravenous bicarbonate, fasting reducing carbohydrate levels, and intravenous amino acids, but also modulating intestinal microbiota with antibiotics which improve neurological symptomatology [39].

Myelopathy is usually seen years after bariatric surgery being one of the most disabling complications. It commonly affects posterior columns with the dorsal ganglia root and can be also associated with neuropathy (myeloneuropathy) or optic neuropathy. Manifestations consist of vibration and proprioception loss, gait ataxia, hyperreflexia, and Babinsky sign, sometimes accompanied by sphincter dysfunction and limb weakness [40].

When neuropathy occurs sensory changes in glove and stocking patterns are also seen in these patients after bariatric surgery. This complication is caused primarily by cobalamin deficiency but is also attributed to other deficient nutrients such as copper, vitamin E, and pyridoxine. Correction of these micronutrients initially intravenously and then long-term orally or intramuscularly for vitamin B12 improves neurologic symptomatology but without full recovery in some patients. For optimum management copper levels should be regularly checked and supplemented as quickly as needed initially intravenous and then oral administration [41].

Optic neuropathy affecting one or both side nerves is associated with bariatric procedures because of nutritional deficiencies in vitamin B12 and vitamin A, copper, and rarely zinc, causing malfunction of nerve fibers. The onset can vary during a large period and can be acute or progressive causing blindness. As optic neuropathy pathogenesis is linked to demyelination, permanent blindness may not be overcome [22,42].

Central nervous system demyelination presenting as multiple sclerosis which fulfilled the McDonald criteria for diagnosis was reported in a series of cases after various types of bariatric surgery with relapsing-remitting forms [4,43,44], without understanding the mechanism of appearance or the exact relationship between the events, but it might be attributed to rapid and substantial weight loss. Other previous case reports also described multiple sclerosis onset and myasthenia gravis after rapid weight loss induced by bariatric surgery or highly restrictive diets [45]. Two cases of monophasic central nervous system demyelination were also reported by Falah et al in a series of patients with neurological complications after bariatric surgery, and in a Brazilian study [44].

NEUROPSYCHIATRIC DISORDERS

Neuropsychiatric disorders are seen after bariatric surgery, usually consisting of eating disorders (anorexia and bulimia nervosa, binge eating, avoidance eating (post-surgical avoidance eating disorder), reports of acute mania [46], and higher risk of suicide. These disorders frequently appear in preexisting psychiatric conditions, making psychiatric evaluation before surgery mandatory to find patients at risk who need to be followed. Several cases of cognitive complaints were described following bariatric surgery in Roux-en-Y Gastric Bypass induced by focal brain atrophy in the bilateral posterior thalamus [47] but need to be further studied.

Other conditions described after bariatric surgery with higher incidence are stroke, seizures [48,49], spontaneous intracerebral hypotension linked to vitamin A, and D deficiency, and spontaneous leakage of cerebrospinal fluid, but the exact relationship needs to be established in future studies [30].

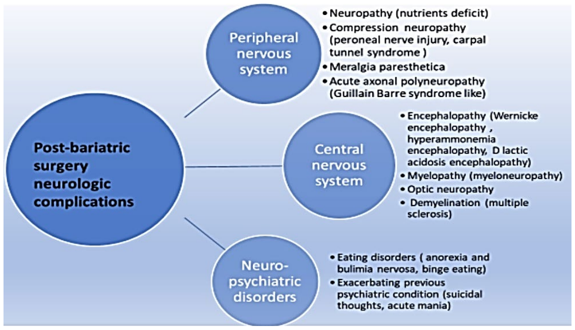
Post-bariatric surgery neurologic complications
Peripheral nervous system
• Neuropathy (nutrients deficit)
• Compression neuropathy (peroneal nerve injury, carpal tunnel syndrome)
• Meralgia paresthetica
• Acute axonal polyneuropathy (Guillain Barre syndrome like)
Central nervous system
• Encephalopathy (Wernicke encephalopathy , hyperammonemia encephalopathy, D lactic acidosis encephalopathy)
• Myelopathy (myeloneuropathy)
• Optic neuropathy
• Demyelination (multiple sclerosis)
Neuro-psychiatric disorders
• Eating disorders (anorexia and bulimia nervosa, binge eating)
• Exacerbating previous psychiatric condition (suicidal thoughts, acute mania)

Figure 2: The spectrum of neurological complications described after bariatric surgery. From peripheral to central nervous complications, to neuropsychiatric disorders.

CONCLUSIONS

Complications after bariatric surgery have a broad spectrum of manifestations involving both the peripheral and central nervous system and should be suspected and diagnosed as soon as possible to prevent long-term sequelae. The risk of developing central nervous system demyelination or stroke events after bariatric surgery should be further evaluated in future studies. Patients with rapid weight loss and those who develop gastrointestinal symptoms and surgical complications are considered at high risk for developing neurological complications, which is why nutritional deficiency assessment and supplementation should be done as early as possible.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization: FIR; Methodology: MJ, RSC, PN; Writing - original draft preparation: MC; writing-review and editing SB, MC; Supervising: FIR. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

Roadmap of Photoplethysmography Technology in Advanced Cardiovascular Assessment

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Abstract: *This review explores the trajectory of photoplethysmography (PPG) technology from its inception in 1934 to its integration into smart devices in 2013. While PPG has proven effective in estimating left ventricular ejection time (LVET) and distinguishing between hypertensive and normotensive patients, challenges persist. The need for a robust mathematical model to explain physiological behaviors, address calibration protocols, and handle waveform variability is emphasized. Despite limitations, PPG is on the cusp of achieving clinical-grade confidence, particularly in estimating blood pressure and tracking vasomotor states. Integration into wearable devices is a prominent trend, with major companies exploring applications for improved health monitoring. Open-source databases and the availability of physiological data aim to enhance understanding, paving the way for universally accepted protocols. As PPG advances, it holds promise for personalized healthcare, revolutionizing treatment evaluations and preventive measures. However, potential disagreements over protocols may impede progress. Nevertheless, the technology's potential to remotely monitor cardiovascular markers could reduce physicians' workload for routine tasks. PPG stands as a beacon for the future of noninvasive cardiovascular assessment.*

Keywords: *photoplethysmography roadmap; cardiovascular assessment; cardiovascular disorders; left ventricular ejection time; blood pressure estimation*

INTRODUCTION

Cardiovascular diseases are, according to WHO, the most common cause of death globally. There are made continuous efforts to reduce the incidence, morbidity, and mortality of these conditions. All over the world, the physicians involved in preventing, detecting, and treating cardiovascular diseases are insufficient and subjected to an overwhelming workload: theoretically, after a certain age, virtually all individuals have a certain risk of developing cardiovascular conditions and these patients, after diagnosis, need periodical evaluation for monitoring, assessment of treatment efficacy and, also, management of complications. Therefore, in the context of nowadays technology capabilities, it has raised the question if there could be developed some methods to monitor these patients over a distance, using suitable medical technologies to non-invasively measure some biomarkers.

At present, it is available to remotely measure, using various wearable devices, pulse, SpO_2 and to record an ECG. However, in detecting or monitoring the risk of adverse events, it would be useful to be able to remotely monitor arterial blood pressure, hypertension being the most prevalent cardiovascular risk factor, or, more advanced, to monitor various parameters of heart performance.

Photoplethysmography (PPG) technology has already demonstrated promising efficiency in distinguishing between normotensive and hypertensive patients or in estimating left ventricular ejection time. In our opinion, with more research in this field and with better mathematical models that can explain all physiological behaviors, PPG could help healthcare professionals offer personalized care regarding the prevention, treatment, and follow-up of cardiovascular diseases.

PHOTOPLETHYSMOGRAPHY APPLICATIONS

Short History

The founding concept of the PPG technique is older than a century when German physicist M.R. Bonnsman invented a photocell device in 1934 intended for peripheral blood volume evaluation [1]. This novel method was taken over by Alrick Hertzman [2,3] for further investigations in the physiological domain. Based on numerous experimental investigations, but limited by the technology capabilities at that time, he proposed blood volume variation as the origin of the PPG signal. No new progress in this technique has been made in the next years since the associated sensor was more of a tool in circulation research.

A valuable clinical application emerged in the form of blood oxygen saturation ratio (SpO_2) [4], pioneered by Takuo Aoyagi in the 1970s [5]. The device, now known as a pulse oximeter, was introduced into U.S. clinical monitoring a few years later, confirming its utility. Following this significant advancement, there was no immediate surge in research within the PPG technology domain; instead, efforts were concentrated on enhancing the accuracy of SpO_2 measurements. The pulse oximeter device became commercially accessible in the 2000s, owing to technological advancements and its availability in the open market [6].

Technology nowadays

The subsequent period that drew attention to PPG was marked by the integration of this technology into the first smartwatches in 2013 [7]. This significant development raised awareness among both academic and industry researchers regarding the potential for cardiovascular monitoring through wearable devices. While the well-established SpO_2 measurement and heart rate determination are valuable for assessing heart performance during resting periods or intense activity [8,9], they do not constitute a comprehensive tool for physicians to remotely monitor a patient in terms of cardiovascular disease (CVD) prevention or tracking.

Therefore, intense research has commenced in the PPG domain over the last decade, exploring various capabilities as depicted in Figure 1. One sought-after application is noninvasive arterial blood pressure (ABP) monitoring, considered a crucial hemodynamic marker in the evaluation of cardiovascular diseases (CVD) [10,11]. By incorporating this targeted assessment into smart devices such as watches, bracelets, and rings, personalized healthcare becomes accessible for end-users. The confidence in this feature has prompted major companies like Samsung [12] to integrate it into their devices. Start-ups such as Valencell [13] and Aktiia [14] assert their ability to provide this measurement using Machine Learning (ML) techniques. Additionally, numerous individual research entities report significant progress in this direction, leveraging artificial intelligence (AI) networks [15].

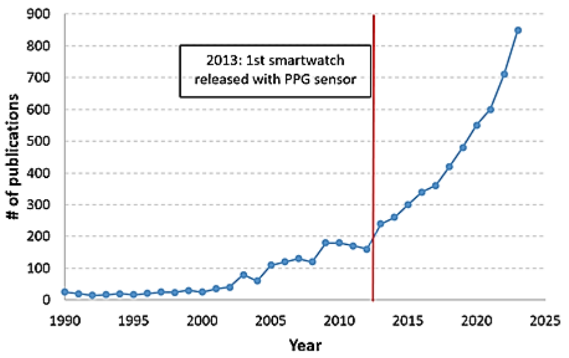

Figure 1: A timeline of PPG-related publications every year from 1990 to 2023. Data obtained from Google Academic, last accessed on 15th December 2023

The key factors contributing to the popularity of PPG encompass the mitigation of motion artifacts, the precision of SpO_2 readings and heart rate measurements, initiatives for extracting arterial compliance, and the estimation of blood pressure. Furthermore, a novel approach, remote photoplethysmography (rPPG) [16], has emerged in recent years, focusing on extracting hemodynamic information through facial recognition. The advancement of information technology, notably the internet and easy access to information, has played a significant role in augmenting the popularity of PPG.

However, as depicted in Figure 1, the upward trend in the number of publications does not align with the maturity of the technology. Regrettably, numerous topics are repetitive, merely contributing to the validation of specific assessments rather than yielding outstanding discoveries or breakthrough research. As will be presented in the next section, a strategy focused solely on PPG signal interpretation is not sustainable. Reflective sensors are commonly employed for wearable applications, as illustrated in Figure 2, in contrast to the transmissive ones more prevalent in clinical settings.

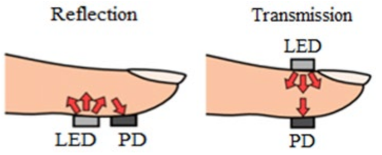

Figure 2: Two types of PPG modes: reflective and transmissive. In wearable devices, the first mode is prevalent, while in clinical settings the second one is present.

Eligible models must be developed to comprehensively explain all observed physiological behavior, and the correlation or causation with arterial blood pressure needs to be elucidated. Without such a focused direction, the repetition of topics will persist without yielding new and significant discoveries.

PPG as a standalone biomarker

As a standalone biomarker, information extracted from PPG waveform contour analysis becomes crucial. Following this approach, significant features such as the tidal wave (TDW) [17] and the dicrotic wave (DCW) [18] can be associated with the vasomotor state. The latter waveform component proves to have a strong correlation in estimating vasomotor tone, as observed in Figure 3, where the dicrotic notch sharpens proportionately with the level of vasodilation [19]. When combined with other waveform markers, such as the ratio between systolic upstroke and downstroke, and aided by Machine Learning (ML) models, the hypertensive state can be reliably detected [20].

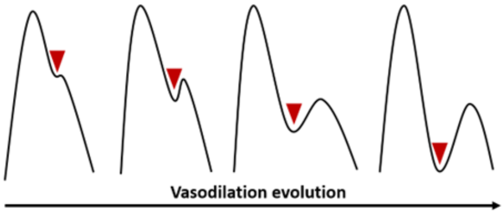

Figure 3: Evolution of PPG waveform during vasodilation progress, by highlighting incisura or DCW feature. Reconstructed after the findings on [19]

Another application involves the myocardial performance index (MPI or TEI) [21], as the PPG waveform encompasses information about isovolumetric contraction time (IVCT) [22], isovolumetric relaxation time (IVRT), and left ventricular ejection time (LVET) [23]. Despite the potential for overestimation and underestimation of measurements due to the hemodynamic state, these values closely align with the golden standard method of Doppler echography.

PPG in sensor fusion

One popular technology in this category is represented by the pulse pressure transit time (PTT) method [24], developed for arterial compliance and blood pressure assessment. The current literature protocol [25] guides the measurement of this marker from the electrocardiogram (ECG) R peak to the PPG systolic point, as depicted in Figure 4.

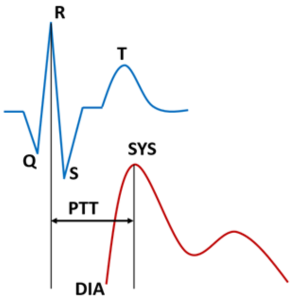

Figure 4: PTT protocol based on guidelines [25]

Based on the physical equation developed by Moens–Korteweg [26], which describes the traveling behavior of mechanical pulse pressure, blood pressure estimation can be extracted. Thus, a stiffer system will promote rapid traveling of the pulse wave, resulting in a shorter PPT time. Conversely, an elastic system, due to its ability to absorb mechanical energy, will slow down the traveling mode, leading to a longer measured time.

Another application that combines PPG capabilities involves fusion with a medical cuff. During cuff inflation, PPG acts as a monitor of blood supply at the targeted site. After a certain point, the externally induced pressure will trigger blood vessel occlusion, which is reflected by a missing PPG waveform. This measurement approach was embedded in the Omron smartwatch [27] to enhance the accuracy of blood pressure assessment.

A recent application involves remote photoplethysmography [16], which targets face recognition in combination with pulsatile detection of facial skin. By selecting two separate regions of interest (ROI), such as the chin and forehead, equivalent approaches to the PPT technique are employed to compute and estimate blood pressure, as depicted in Figure 5.

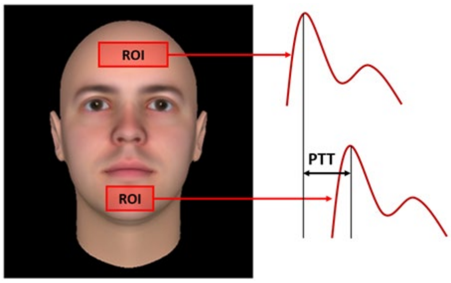

Figure 5: Principle of rPPG. Two locations of skin pulsation generate PPG waveform but with a time delay in between. This delay is exploited by a similar to the PPT method to extract blood pressure estimation

PHOTOPLETHYSMOGRAPHY LIMITATIONS

Physiological model

Despite the important and useful applications of the PPG technique, either standalone or in conjunction with other methods, the technology has demonstrated some limitations.

The first significant limitation pertains to the proper understanding of PPG origin, particularly in relation to pulse formation. While Alrick Hertzman proposed blood volume variation as a working model in the 1930s, recent investigations have revealed some drawbacks. One common limitation arises during reduced SpO_2 readings [28], which actually represent the ratio between oxygenated and deoxygenated blood volume. Additionally, the hand position concerning the heart level [29] modulates the PPG waveform amplitude according to specific rules, contrary to the initially proposed volume variation model. Therefore, significant progress in the hemodynamic domain cannot be achieved without a suitable PPG model that can explain and predict different physiological states. At this juncture, two mathematical models are required: one to elucidate the optical nature of the recorded signal and another to describe the mechanical nature that provides a specific waveform morphology. Training Machine Learning (ML) or Artificial Intelligence (AI) systems with a large set of hemodynamic data will not unveil the true relationship between these two physical quantities. Instead, it serves as an interim step to understand which waveform components are predominant in blood pressure assessment.

A recently developed model proposes the red blood cell deformation effect as the origin of PPG formation [30]. The mere presence of erythrocytes in the measurement area modulates the direct component or baseline of the recorded waveform. Interestingly, as cells move under a defined velocity field, they deform in the capillary regime. Thus, targeting red blood cells with light results in the absorption of radiant flux proportional to the deformation level. Given that many publications attempt to correlate arterial blood pressure (ABP) waveform with PPG, it is observable that the physiological stimuli contributing to PPG formation are the blood flow velocity at the capillary level, ranging from 50 µm/s to 2000 µm/s [31]. As a result, as depicted in Figure 6, the PPG waveform is exclusively modulated by the velocity values through the capillary network, reflected in the final waveform by red blood cell deformation.

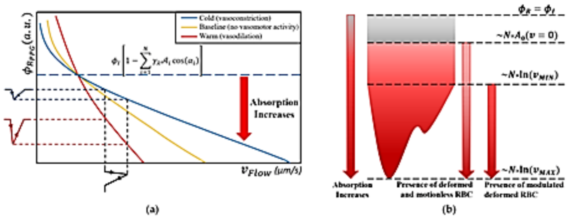
Cold (vasoconstriction)
Baseline (no vasomotor activity)
Warm (vasodilation)
$\phi_{R_{PPG}}(a.u.)$
$\phi_I\left[1-\sum_{i=1}^{N}\gamma_{Ri}A_i\cos(\alpha_i)\right]$
Absorption Increases
v_{Flow} (µm/s)
(a)
$\phi_R = \phi_I$
$\sim N\cdot A_0(v=0)$
$\sim N\cdot \ln(v_{MIN})$
$\sim N\cdot \ln(v_{MAX})$
Absorption Increases
Presence of deformed and motionless RBC
Presence of modulated deformed RBC
(b)

Figure 6: RBCM MODEL. Reused from [30]

It is important to note that both the mechanical blood pressure pulse and red blood cell deformation—precursors for PPG—are driven by blood flow velocity. Therefore, this physical quantity serves as the same input stimulus, the causal factor, for both associated waveforms. In this context, a correlation rather than direct causation exists between arterial blood pressure (ABP) and PPG information. From this standpoint, estimations of hemodynamic markers can be extracted with the aid of wearable technologies. However, due to the logarithmic nature of PPG origin, as depicted in Figure 6, the hemodynamic information will undergo modifications that could lead to wrongly interpreted results.

Finally, without a proper optical model, the improvement of pulse oximeter sensors becomes challenging. The most critical design considerations are related to mechanical features, such as the frequency of the LED used, initial light flux power, and the arrangement of the photodiode. In terms of signal processing, important design inputs include signal amplification, sampling frequency, and filtering stages.

Measurement protocols

All the aforementioned PPG measurement capabilities require a universally agreed-upon protocol to ensure that different devices provide consistent results for the same user or patient. This requirement is crucial for accurate measurements to be eligible for clinical applications. Moreover, regardless of the device's manufacturer, doctors need to rely on the measured data to provide correct treatment or formulate an observational plan.

While the PPT method has demonstrated high-confidence results in estimating blood pressure with the defined protocol, there is still an applicability problem on the user end. Measuring PPT involves two sensor readings simultaneously: EKG and PPG, as discussed earlier. Even though the latter doesn't require user intervention, the former necessitates the user to close the electrical circuit of their body to perform an EKG. Consequently, the measurement cannot be performed frequently without user intervention, for example, during sleep or physical exercise. In essence, it shares the same measurement limitations as a traditional oscillometric blood pressure device [32].

For assessments that involve standalone PPG sensors, a major limitation is the calibration protocol process. Given the optical nature of the recorded quantity, the readings need to be calibrated with a mechanical reference, especially for applications intended for blood pressure estimation. In the assessment of vasomotor state, where absolute values aren't critical, a protocol should outline which PPG waveform components, besides DCW, need to be evaluated for high-confidence results. This is necessary due to the various waveform morphologies that appear in the real world, apart from the idealized perfect shape.

The same approach is necessary for measurements involving time values, such as left ventricular ejection time (LVET) determination. While the association with the TDW feature yields good results, a common agreement on the onset and ending points is essential. Given that the TDW component comprises a finite disturbance event in combination with different morphologies, a protocol is also required for consistency in interpretation.

Finally, from a numerical standpoint, all the measured hemodynamic markers should be evaluated by a specialist within the proper context. The obtained data must be assessed to determine if the measurements were intended for prevention, treatment evaluation, or cardiovascular disease monitoring.

PHOTOPLETHYSMOGRAPHY FUTURE

An important stride in pulse oximeter technology has already been taken by integrating it into wearable devices. The increased computational power per integrated circuit in these devices holds the potential to enhance the signal processing part of the technology.

The availability of open-source databases containing hemodynamic and physiological data intended for researchers [33,34] will contribute to a proper understanding of PPG technology. In the final analysis, having such resources will help reduce, mitigate, or avoid the discussed drawbacks with a supportive tool.

The existing technological capabilities are poised to elevate PPG technology to clinical grade, enabling it to provide high-confidence measurements. Consequently, cardiovascular markers such as LVET and MPI can be remotely monitored, reducing the need for unnecessary physician office visits. This advancement holds potential benefits for both patients and doctors.

The capability to monitor patients' markers with a higher frequency rate would enhance the evaluation of the efficiency of the treatment plan. Additionally, cardiovascular diseases (CVDs) could be prevented or tracked more effectively, enabling better control and management.

An important assumption pertains to future protocols. The competition to embed hemodynamic markers into wearable devices will likely lead the first entity with proven and credible tracking capabilities to establish standalone protocols. Consequently, the community researching in the respective domain will likely follow the defined rules. However, a potential drawback is that this could trigger disagreements over a common, acceptable protocol, which may delay the accessibility of personalized healthcare [35].

CONCLUSIONS

The age-old noninvasive method, photoplethysmography, holds immense potential for enhancing cardiovascular assessment. However, realizing this potential requires the development of a proper mathematical model that can link and explain all physiological behaviors.

While the interest in PPG surged with the advent of the first smart devices in 2013, no disruptive progress has been achieved since then. Instead, some recurrent topics have been validated rather than proposing novel waveform markers for association with clinical applications. Blood pressure estimation and vasomotor state tracking are currently receiving the most attention, with popular companies piloting projects to integrate them into wearable devices.

Despite current limitations, PPG has shown validity, especially in estimating LVET and in binary detection between hypertensive and normotensive patients. Given the current context of technological advancement, there is optimism that this technology will achieve clinical-grade confidence. Consequently, it is anticipated to become an integral part of human lives by providing easy accessibility to personalized healthcare. This, in turn, will unlock improved health monitoring and contribute to a reduced workload for physicians in handling less critical tasks.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, L.E. and L.D; methodology, L.E.; software, L.E.; validation, L.E., A.F and L.D.; formal analysis, L.E; investigation, L.E.; re-sources, L.D.; data curation, A.F.; writing—original draft preparation, L.E.; writing—review and editing, A.F; visualization, L.E.; supervision, A.F.; project administration, L.D.; All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Ethics approval and consent to participate

Not applicable.

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REVIEW

Non-alcoholic Steatohepatitis Diagnosis and Treatment – Current Concepts

Ana M. Ijacu[1,2], Laura G. Gagiu[1,2], Iulia M. Staicu[1,2], Corina Zugravu[3], Ciprian Constantin[1,2,4]

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Abstract: NAFLD (Non-alcoholic fatty liver disease) is a condition of high importance due to its increased prevalence, estimated at 24% of the global population, and its potential progression to advanced liver disease, cirrhosis, and hepatocellular carcinoma. One-quarter of NAFLD patients have NASH (non-alcoholic steatohepatitis), which is histologically described as liver steatosis, inflammation, fibrosis, hepatocyte injury, and death. Metabolic disruptions, such as obesity, diabetes, and dyslipidemia, are considered to have a great impact on the development of NASH. Patients may have NAFLD for a long time before progressing into NASH, which highlights the necessity of periodic imaging and biochemical evaluation of the liver, and also presents an optimal window of therapeutic intervention. The challenge in developing treatment for NASH stems from the slow progression of the disease and the reliance on sequential biopsies as the diagnostic and staging tools. Several non-invasive tests have been developed as potential future alternatives to the current invasive gold standard. This review aims to assess the current available methods of diagnosis and treatment targeting inflammation.

Keywords: NAFLD; NASH; MASH; liver steatosis; liver fibrosis; metabolic syndrome; lanifibranor; obeticholic acid; semaglutide

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is an advanced form of non-alcoholic fatty liver disease (NAFLD), characterized by liver inflammation and hepatocyte injury, that is progressively evolving due to the accumulation of fat in the liver [1]. NAFLD results from an unhealthy diet and sedentary lifestyle, without high alcohol consumption, and is usually a silent disease before the advanced stages, including cirrhosis, liver failure, and hepatocellular carcinoma [2].

The highest rates of NAFLD were reported in the Middle East (32%) and South America (31%), followed by Asia (27%), USA (24%) and Europe (23%), whereas the lowest rates were in Africa (14%) [3-5]. It is estimated that 25% of the patients with NAFLD are thought to have NASH and a quarter of them are diagnosed with cirrhosis [3-5].

Recent studies show the association between metabolic disruptions and NASH, which is why a new medical terminology has emerged introducing the term MASH as metabolic-associated steatohepatitis. A bidirectional correlation exists between NAFLD and metabolic syndrome (obesity, insulin resistance, and dyslipidemia). Also, patients with type 2 diabetes mellitus (T2DM) have a greater risk of developing NAFLD, with a prevalence estimated at 57.80% (95%CI: 53.88% - 61.62%) [6]. NASH has been reported in 65.26% (95% CI: 51.73%-76.71%) of the patients with type 2 diabetes mellitus, who underwent liver biopsy [6]. In a meta-analysis of more than 8.5 million people from 22 countries, the results show that 80% of the patients with NASH are overweight or obese, 72% have dyslipidemia, and 44% are diagnosed with type 2 diabetes mellitus [3].

The evolution of NASH is non-linear. Some patients progress to cirrhosis, and most of them develop cardiovascular disease, but some patients, even those with fibrosis, may spontaneously regress. Cardiovascular mortality is the most common cause of death in patients with NASH. In contrast, in the U.S., due to decompensated liver disease, NASH is among the top three indications for liver transplantation [6,7].

In a study on patients with liver cirrhosis, secondary to HCV infection or NASH, over 4 years, it was estimated that the annual cumulative risk of HCC (hepatocellular carcinoma) was 2.6% per year in patients with NASH cirrhosis compared with 4.0%

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per year in those with HCV cirrhosis [8]. These figures suggest that NASH carries a risk of developing HCC that rivals the risk in patients with HCV-cirrhosis. As such, patients with NASH cirrhosis should be closely monitored for HCC. The decrease in HCV-related transplantation due to the significant effect of the HCV therapies placed NASH and ALD (alcohol-related liver disease) as the most common etiologies of liver disease among liver transplant waiting list registrants without HCC, and NASH is becoming a leading indication in patients with HCC [9].

MATERIALS AND METHODS

This review aims to assess the currently available methods of diagnosis and treatment targeting liver inflammation and fibrosis, named NAFLD, NASH, or similar.

We have selected over 200 English, French, and Romanian studies highlighting information on diagnosing and treating these hepatic diagnoses. For the development of this review, we further selected the articles based on our inclusion criteria, consisting of diagnosis and treatment-focused articles and recently published articles to ensure the current advancements in the field.

Databases such as PubMed, Up-to-date, Medline, and NCBI were used to perform the search using keywords: NAFLD, NASH, liver steatosis, liver fibrosis, and metabolic syndrome. Exclusion criteria were imposed to select the papers, such as the article publication date not exceeding 20 years old, and the publications to be in English, French, and Romanian, addressing the diagnosis and treatment concepts for NASH.

RESULTS

Diagnosis

Identifying a person with NASH can be difficult because neither the diagnosis nor the severity of the liver fibrosis can be predicted based on the risk factors (obesity, insulin resistance, or hepatic steatosis) alone. Accurate fibrosis staging, through biopsy series, is important for diagnosis and mortality assessment. All-cause and liver-specific mortality appear to increase once the F2 fibrosis stage has developed [10]. The risk of liver disease death rises by a factor of 50 to 80 for patients with NASH who have F3/F4 fibrosis, as compared with those who have little or no fibrosis [11,12]. People at risk for NASH, such as overweight or obese patients with metabolic syndrome, elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and negative noninvasive results for other causes of liver disease, should undergo further testing. Also, patients with type 2 diabetes mellitus over the age of 45 years are likely to have advanced fibrosis and bad liver outcomes [5].

Before advanced testing for NASH, patients should be thoroughly examined to exclude other causes of steatosis and liver damage and to determine the presence of commonly associated comorbidities (obesity, dyslipidemia, insulin resistance, diabetes, sleep apnea, hypothyroidism, and polycystic ovary syndrome). Excessive alcohol consumption, steatogenic medications, high serum ferritin with increased iron saturation, and viral or autoimmune hepatitis should be excluded [7].

The liver biopsy is an invasive procedure, currently considered the gold standard for diagnosing and staging NASH. Numerous non-invasive methods of diagnosis based on serum markers and imaging tests have been investigated, but their performance is not comparable with the biopsy (Table 1). The need for non-invasive tests has been enhanced to develop new treatments for NASH. Various emerging diagnostic tools are presented in Table 1 [7,13-25].

MRI-PDFF [7,13] (Proton density fat fraction MRI) is the most accurate method of detecting steatosis, whereas MRE [7,14] (Magnetic resonance elastography) is the most accurate tool of staging liver disease, by the "stiffness'' indirectly related to fibrosis. Although availability and costs are a major challenge for clinicians, combining these tests can provide a risk stratification in patients with NAFLD. Non-invasive serum markers include alanine aminotransferase levels, and circulating keratin18 fragment levels and there are also tests such as Fibrometer, microRNA panels, and lipidomic panels. In patients with ALT>70U/L, NASH ALT prediction testing was found to have a 50% sensitivity and 61% specificity [7,15]. Considering the prediction ability of the serum ALT, it should not be used for definitive diagnosis, staging, or evolution of the disease, but only to guide further testing. The NASH Test combines demographic characteristics (age, gender, and BMI), serum markers (ALT and lipids), and alpha-2 macroglobulin, ApoA1, and haptoglobin, with a sensitivity of 33% and a specificity of 94%, indicating that it has a good negative predictive value (NPV) [7,16]. The Fibrometer NAFLD (based on age, weight, fasting glucose, AST, ALT, ferritin, and platelet count) achieves reasonably good results for mild to moderate fibrosis. The positive predictive value (PPV) for correctly identifying F=1 was 84.9%, the NPV was 66.7% and the diagnostic accuracy was 80%. The PPV for F=3 was 74.5%, the NPV was 86.2% and the diagnostic accuracy was 82.1% [17-20]. Fibromax, a diagnostic tool composed of 4 non-invasive biomarker panels, can be used for the diagnosis of steatosis (estimated by the SteatoTest), necrosis and inflammation (evaluated by ActiTest and NashTest-2), and fibrosis (FibroTest). Although it is a well-validated and relatively frequently used test, it may not be accurate for patients with T2DM [21]. OWLiver (Overweight Liver) [16,18] is an alternative for overweight patients and PROC3 (Pro Collagen III) can be used as a differentiating test between steatosis and NASH [24]. NASH Index Score 4 (NIS4) [23] and Enhanced liver fibrosis (ELF) [25] are tools that exclude AST and ALT as parameters in the diagnosis process.

There are two main scoring systems for the biopsy results in NASH. The NAS (NAFLD Activity Score) sums the score of steatosis, lobular inflammation, and ballooning of the liver cells. The score (0-8) is not diagnostic. It was designed to evaluate the histological changes in seriate biopsies. It is usually interpreted by adding the fibrosis score (0-4) (Table 2) [7,26-27]. Another available score is SAF

(Steatosis, Activity, and Fibrosis). By evaluating 1000 biopsies, there was an excellent concordance between the two scores in determining the definite NASH, whereas 84% of the NAS borderline biopsies (3-4), were identified by SAF score as NASH [7,26-27].

Table 1: Non-invasive methods of diagnosis [7,13-25]

Test	Parameter measured	Observations
MRI-PDFF [7,13] (Proton density fat fraction magnetic resonance imaging)	steatosis	most accurate method of detecting steatosis
MRE [7,14] (Magnetic resonance elastography)	liver stiffness	most accurate modality of staging liver disease, by the ''stiffness'' indirectly related to fibrosis.
NASH Test [7,16]	age, sex, height, weight, serum triglycerides, cholesterol, a-macroglobulin, apolipoprotein A1, haptoglobin, gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin	sensitivity of 33% specificity of 94%
FibroMeter VCTE [17-20] (Vibration Controlled Transient Elastography)	Fibrosis, liver stiffness Combines TE (transient elastography) with biochemical markers (age, sex, AST, urea, platelets, prothrombin time, GGT, and alpha2-macroglobulin)	shows good results for mild to moderate fibrosis By combining biochemical markers with imagistic results it increases the accuracy
Fibromax [21]	Combines 4 non-invasive biomarker panels SteatoTest, ActiTest, NashTest-2, and FibroTest	affordable and simple score, but it may not be accurate for patients with T2DM
FNI [22] (The Fibrotic NASH Index)	fibrosis AST, high-density lipoprotein cholesterol, and hemoglobin A1c	accurate, affordable, and simple score for patients with fibrotic NASH
NIS4 [16,18,23] (NASH Index Score 4)	miR-34a-5p, alpha-2 macroglobulin, YKL-40, and glycated haemoglobin A1c	Identifies at-risk patients, defined as [NAS] ≥4 and fibrosis stage ≥2 on biopsy
OWLiver [16,18] (Overweight Liver)	extensive analyses of triglycerides panel by high-performance liquid chromatography and mass spectrometry	Used for individuals with BMI>25 kg/m^2
PROC3 [18,24] (Pro Collagen III)	Pro collagen III	Differentiates steatosis from NASH
ELF [18,25] (Enhanced liver fibrosis)	fibrosis hyaluronic acid, procollagen III, and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1)	stages liver fibrosis independently of AST, ALT elevations

Treatment

The main pathogenic processes identified in NASH are metabolic stress, inflammation, and fibrosis. Pharmacologic agents that target these key mechanisms are being investigated in clinical trials [2].

Due to the lack of pharmacological therapies for NASH, lifestyle modifications remain the main intervention for mitigating histological impairment. Weight loss appears to bring the highest benefit.

Usually, the recommendations include 5-10% weight reduction for obese and overweight patients, physical activity, and a Mediterranean diet. Ultra-processed foods and drinks, fructose-enriched beverages, and alcohol should be avoided. In addition, associated comorbidities (T2DM, dyslipidemia, POS) should be treated [1,26].

Vitamin E, pioglitazone, and statins are available options for patients with NASH. Studies have indicated the role of vitamin E in the reduction of steatosis and fibrosis [28,29]. Pioglitazone, a treatment used for type 2 diabetes mellitus, has been reported to significantly reduce fibrosis in NASH patients. In addition, statins have been tested on NAFLD/NASH patients, with substantial results in improving steatosis and fibrosis [30].

The PIVENS trial focused on NASH patients without diabetes, who were randomly assigned to one of three groups (pioglitazone 30 mg daily, vitamin E 800 IU daily, or placebo). The vitamin E group had a significantly higher rate of histology improvement than the placebo group 43% vs. 19%, but pioglitazone did not meet the primary outcome [31]. Based on several studies, there is a variability in treatment response for vitamin E supplementation. Genetic variations in haptoglobin and FADS1/FADS2 (fatty acid desaturase 1 and 2) have been suggested to interfere with the treatment response, determining whether a patient will show histological improvement or not [32].

Many new potential therapies for NASH are currently studied in phase III trials. The pan-PPAR agonist (Peroxisome proliferator-activated receptors), lanifibranor, has completed the phase IIb trial with 247 participants, the results show a reduction in the SAF activity score (> 2 points), with no increase in fibrosis, achieved by 49% of patients compared with 27% on placebo. The results were significantly higher among those who received 1200 mg of lanifibranor vs. the group with 800 mg dose. In the lanifibranor groups, there was an improvement in liver enzyme levels and also in the majority of lipid, inflammatory, and fibrosis biomarkers [33]. There is an ongoing phase 3 study conducted to evaluate the safety and efficacy of lanifibranor compared to placebo on 1000 participants

with NASH and liver fibrosis histological stage F2 or F3 [34].

The GLP-1 receptor agonist, semaglutide, has also completed a 72-week, phase 2 trial (NN9931-4296) with 320 participants, showing resolution of NASH and no worsening of fibrosis for 59% of patients, in the 0.4mg group, and 40% of patients, in the 0.1mg group, compared with 17% on placebo. The percentage of participants with an improvement in NAS score was 82.9% in the 0.4mg group compared with 43.8% on placebo [35]. A phase 3 study, ESSENCE (The Effect of Semaglutide in Subjects With Non-cirrhotic Non-alcoholic Steatohepatitis), was initiated in 2021 and the interim results are expected in 2024 [36].

Table 2: NAS score and fibrosis staging (adapted from [7,27])

Characteristic	Score
Steatosis	<5%=0 5-33%=1 (mild) 33%-66%=2 (moderate) >66%=3 (severe)
Lobular inflammation	No foci = 0 <2 foci per 200 field =1 2-4 foci per 200 field =2 >4 foci per 200 field = 3
Ballooning	None = 0 Few = 1 Many cells = 2
NAS Score	0-8
Fibrosis	None = 0 Mild = perisinusoidal or periportal (stage 1) Moderate = perisinusoidal and portal/periportal (stage 2) Severe = bridging fibrosis (stage 3) Cirrhosis = stage 4

For liraglutide, also a GLP-1 analog, a multicenter, double-blinded, randomized, placebo-controlled phase 2 trial (the LEAN trial) has demonstrated histological resolution of non-alcoholic steatohepatitis and a significant effect on liver fibrosis after 48 weeks of treatment. In addition, the comparison between pioglitazone and liraglutide showed better results for the latter [37-39].

Obeticholic acid, a selective FXR (farnesoid X receptor) agonist, approved by the FDA in 2016 for primary biliary cholangitis, previously completed a phase III trial (REGENERATE) on 1968 NASH participants with fibrosis F2–F3. The results show that the drug improved the fibrosis score by at least one stage, but did not meet the endpoint of NASH resolution [40]. The FDA rejected the approval of obeticholic acid for NASH in May 2023, based on the modest benefits that did not outweigh the risks.

A phase IIb randomized study of the efficacy and safety of Namodenoson, an A3 adenosine receptor (A3AR) agonist, in the treatment of NASH is ongoing. Previous trials on Namodenoson showed normalization in ALT and AST levels after 12 weeks of treatment [41].

Pegozafermin, a long-acting glycopegylated fibroblast growth factor 21 (FGF21) analog, showed improvements in fibrosis in a phase 2b trial (ENLIVEN). The fibrosis improvements occurred in 7% of the placebo group and between 22-27% in the pegozafermin groups (22% with 15 mg – pegozafermin, 26% on 30 mg dosage, and 27% on 44 mg dosage). The NASH resolution was 2% in the placebo group, 37% in the 15mg pegozafermin group, 23% in the 30 mg pegozafermin group, and 26% in the 44 mg pegozafermin group. The study completion date is estimated around September 2024, demonstrating favorable prospects for advancing to phase 3 [42,43].

Oxidative stress and the formation of reactive oxygen species (ROS) are often associated with the progression of NAFLD to NASH. Excessive ROS formation impairs insulin sensitivity and inflammatory responses. The release of pro-inflammatory molecules and uncontrolled ROS production in many cells is partially the result of TAGE (toxic advanced glycation end-products) and receptor for AGEs (RAGE) interactions [44,45]. Hyperglycemia contributes to the production of advanced glycation end-products (AGEs), especially AGEs formed from glyceraldehyde (GA), which are highly cytotoxic, damaging the hepatocytes. AGEs and TAGE were measured in a study of 66 patients with histologically confirmed NASH without cirrhosis, 10 with NAFL, and 30 control subjects [46]. The TAGE levels of NASH patients were significantly higher than those with NAFL. Ultra-processed foods with a high content of sugar (fructose-enriched or sugar-sweetened drinks) and dietary AGEs have been suggested to contribute to the development of obesity, metabolic syndrome, and the progression of NAFLD/NASH when consumed excessively [47].

Antioxidants such as pentoxifylline (PTX), silybin, resveratrol, and vitamins A, C, and E are used in clinical trials against NAFLD and NASH [47].

PTX treatment with three divided doses of 400 mg for one year improved hepatic histological activity. NAS ≥ 2 improved by (75%) in the PTX group and 30% in the placebo group, but there was no impact on fibrosis [48]. Also, another study on PTX (1200 mg/day) and fenofibrate showed a beneficial effect on indirect biochemical markers of hepatic fibrosis. After 24 weeks, the association of fenofibrate and PTX resulted in reduced liver fibrosis and stiffness, insulin resistance, and inflammation [49]. The combined administration of PTX (1200 mg/daily) and metformin (1500 mg/ daily) for six months reduced AST, and ALT levels and improved

insulin resistance in patients with NASH [50]. A meta-analysis based on 5 RCT on patients with NAFLD/NASH reported that PTX treatment could reduce glucose serum levels, liver transaminases, body weight, and TNF-α levels and also improve NAS and fibrosis scores [51]. Another study used combinational therapy (PTX+Vitamin E) and the results showed regression of fibrosis compared to vitamin E alone [52]. In a phase 2 study, pentoxifylline (PTX) alone significantly improved steatosis, lobular inflammation, and liver fibrosis and was well tolerated [53]. Therefore, PTX can be used along with other drugs as an adjuvant for metabolic diseases.

A phase 4 study conducted on 102 NASH patients divided into 3 intervention groups (PTX, vitamin E, and ursodeoxycholic acid) measured the levels of several serum markers (AST, ALT, IL6, and CCL2/ MPC-1, albumin, total bilirubin, direct bilirubin, total cholesterol, triglyceride, LDL, HDL) for 3 months [54].

In 2017 a study [55] based on an experimental rat model of NASH demonstrated for the first time that the combination of SAMe (S-Adenosyl-L-Methionine) and DLPC (Dilinoleoylphos-phatidylcholine), two physiologic antioxidants, significantly opposed liver injury generated by fat excess.

Numerous studies on hepatoprotective drugs, probiotics, and antioxidants, such as Siliphos—Selenium—Methionine—Alpha Lipoic Acid, omega-3 fatty acids, and vitamins A, C, and E, are currently ongoing [47,52,56-58].

CONCLUSION

The treatment for non-alcoholic steatohepatitis is, unfortunately, an unmet need. At present, there are no FDA-approved therapies for this condition, and the same holds for the EU. Considering the increasing prevalence and the risk for progression toward advanced liver disease, cirrhosis, and hepatocellular carcinoma, there is a major demand for reliable non-invasive biomarkers and specific therapy. In addition to healthy diet and exercise, many new pharmacological agents, such as lanifibranor, semaglutide, and obeticholic acid are being studied. Antioxidants as well are used in clinical trials against NAFLD and NASH.

Despite the clinical and epidemiological significance, the accurate diagnosis, monitoring of the diagnosed patients, and available licensed treatments remain the challenges of NASH.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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Authors' contribution

Conceptualization, CC and AI; Methodology, Search of materials: CC and AI; Writing—original draft preparation, CC and AI; writing—review and editing: AI, LG, CC; visualization, IS, CZ; supervision, CZ, CC. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

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Patient consent for publication

Not applicable.

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REVIEW

Multinodular and Vacuolating Neuronal Tumor – Challenges and Perspectives

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Abstract: A multinodular and vacuolating neuronal tumor (MVNT) is a rare non-malign central nervous system tumor. The cerebral cortex of young to middle-aged adults is affected and seizures and focal neurological deficits could reveal it. Despite being considered a low-grade tumor, MVNT can be challenging to diagnose and manage due to its histopathological similarities to other neuronal tumors. The limited number of reported cases makes it difficult to establish definitive guidelines for treatment and follow-up. Given the relatively recent discovery of MVNT, there is a need for further research to better understand its pathogenesis, optimal treatment and long-term strategies. Furthermore, studying MVNT can contribute to the broader understanding of brain tumor biology and the intricacies of tumor heterogeneity. This paper explores the challenges and perspectives surrounding MVNT to improve clinical decision-making and patient outcomes. Based on the present narrative review, advancements in diagnostic technologies have shown promise in addressing the challenges associated with diagnosing MVNTs. Liquid biopsy is an innovative and non-invasive diagnostic tool that analyzes biomarkers to provide information about tumors, and radiomics is a method that extracts quantitative data from medical images to provide detailed insights into tumor characteristics. Such innovative diagnostic technologies are important because they have the potential to significantly improve the current diagnostic landscape for MVNTs, allowing for earlier detection, accurate classification, and personalized treatment strategies.

Keywords: multinodular and vacuolating neuronal tumor; radiomics; liquid biopsy; diagnostic methods; histological examination; FDG-PET/CT; immunohistochemistry

INTRODUCTION

First described in 2013, multinodular and vacuolating neuronal tumor (MVNT) was classified as a benign ganglion cell grade I tumor, potentially associated with seizures, with a specific molecular marker, as outlined in the WHO 2016 and 2021 classification [1-3]. The discovery of MVNT is often accidental, and it is frequently asymptomatic [1,2]. Studying MVNT is of utmost significance in neuro-oncology. Understanding the molecular mechanisms underlying the development and progression of MVNT can provide insights into potential therapeutic targets and treatment strategies.

Typically identified in adults, MVNTs seem to appear during neurogenesis and present as subcortical clusters of nodular lesions within the cortex and superficial subcortical white matter [4]. Structure abnormalities in the cortex may be present with it [4,5]. Initially classified as either neoplastic or malformation, recent research on MVNTs emphasizes their stable nature over time, suggesting that they are “leave me alone” lesions with minimal changes in volume, thus underscoring the potential for less frequent imaging surveillance [6,7]. Whether the nodules of vacuolar neurons are a definite neoplastic transformation or aberrant development is still up for debate [6]. Overall, these insights contribute to a nuanced understanding of MVNTs, highlighting the complexities and diagnostic considerations surrounding these distinct neuronal tumors. Moreover, the purpose of this research is to shed light on the challenges and perspectives surrounding MVNT.

MATERIALS AND METHODS

A narrative review was conducted through five electronic databases (PubMed, CINAHL, Google Scholar, Cochrane, Web of Science/Clarivate), using as keywords “multinodular and vacuolating neuronal tumor” AND “diagnosis” OR “pathogen*” OR “imaging” OR “treatment”. All primary and secondary reports found from the inception of the respective databases up to July 2024 were explored for relevant data. Only papers that addressed explicitly the pathogenesis, diagnosis methods,

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and treatment strategies of MVNT were included in the secondary analysis**.**

RESULTS

Based on the literature search, 34 primary and secondary reports were reviewed in detail. The results were classified according to the three areas of interest, i.e., (1) pathogenesis and classification of MVNT, (2) imaging characteristics, and (3) diagnosis and treatment.

Classification and pathogenic characteristics of MVNT

Recent studies have highlighted the challenge of defining and classifying MVNT. Due to its rarity and unique histopathological features, there is ongoing debate within the scientific community regarding its precise definition and classification. Some researchers argue that MVNT should be considered a separate entity from other neuronal tumors due to its distinct morphological characteristics and clinical behavior. On the other hand, some propose classifying it under the umbrella of neuronal and mixed neuronal-glial tumors based on its neurocytic differentiation and immunohistochemical profile.

The purely neuronal tumors in the 2021 WHO classification include multinodular and vacuolating neuronal tumors, gangliocytoma, extraventricular neurocytoma, central neurocytoma, dysplastic cerebellar gangliocytoma, and cerebellar liponeurocytoma.

The mixed glioneuronal tumors include dysembryoplastic neuroepithelial tumor, diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters, ganglioglioma, desmoplastic infantile ganglioglioma/astrocytoma, papillary glioneuronal tumor, rosette-forming glioneuronal tumor, myxoid glioneuronal tumor, and diffuse leptomeningeal glioneuronal tumor [13].

There is a consensus on the need for standardized diagnostic criteria to identify and classify MVNT cases accurately. This would facilitate better communication among pathologists and clinicians, improving management strategies and outcomes for patients with MVNT.

The WHO 2016 classification defines MVNT as a benign ganglion cell tumor with specific pathologic hallmarks [2]. These nodular lesions are typically located in specific brain regions and can present differently across age groups, emphasizing the importance of histopathological examination for accurate diagnosis [1,14,15].

Histopathological analysis of MVNT reveals several distinctive features that aid in their diagnosis and differentiation from other neuronal tumors. One of the hallmark features of MVNT is the presence of numerous vacuoles within the tumor cells. This vacuolation can create a “floating” appearance of the cells and is a critical diagnostic criterion. MVNT typically exhibits a multinodular architecture. The tumor forms distinct nodules, which may vary in size, often surrounded by a gliotic background. Such detailed histopathological analyses are essential for elucidating the distinct features of MVNT and guiding effective clinical management strategies. Nevertheless, no histopathological evidence is available to confirm the diagnosis since only a small percentage of patients with suspected MVNT undergo surgery [10].

Immunohistochemical markers play a crucial role in the diagnosis and classification of MVNT. Various markers have been studied, differentiating MVNT from other tumors with similar histological features, such as dysembryoplastic neuroepithelial tumor (DNT) or ganglioglioma [16,17]. So, immunohistochemistry (IHC) is a crucial tool in the diagnosis and characterization of MVNT, establishing the neuronal nature of MVNT, as seen with positive staining for neuronal markers [18]. MVNT typically shows positivity for several neuronal differentiation markers: NeuN, a neuronal nuclear antigen typically expressed in mature neurons, β-III tubulin, which is highly expressed in neuronal cells and can help confirm the tumor’s neuronal lineage. Synaptophysin, a protein associated with synaptic vesicles, indicates neuroendocrine differentiation and is often used to demonstrate the presence of neurons. Although MVNT is primarily neuronal, some glial markers may also be expressed, reflecting maybe the mixed composition of the tumor. GFAP (Glial Fibrillary Acidic Protein) positivity can indicate a glial component in the tumor. Phenotypic analyses may also include checking for markers that indicate low proliferation rates, which can help distinguish MVNT from more aggressive tumors. Markers like Ki-67 are occasionally assessed but typically show low proliferation in MVNT. The absence of expression for markers associated with high-grade tumors, such as p53 or other markers linked to aggressive histology, reinforces its benign nature [18,19].

Understanding the molecular pathogenesis of Multinodular Vacuolating Neuronal Tumors (MVNT) presents a significant challenge due to the tumor’s unique histological features and clinical behavior. MVNTs are characterized by vacuolated tumor nodules with glial and neuronal differentiation, primarily affecting the cerebrum in adults. The pathognomonic radiological features, such as the soap-bubble morphology and lack of enhancement post-gadolinium injections, provide important clues for differential diagnosis and management strategies [4,11,20,21]. The molecular mechanisms driving its formation and growth remain elusive. Further research is warranted to elucidate the genetic alterations and signaling pathways involved in the development of MVNT. The MAP (Mitogen-Activated Protein) kinase signaling pathway is a critical cellular signaling pathway that regulates various cellular processes, such as growth, proliferation, differentiation, survival, and apoptosis. This pathway is activated by various extracellular signals, including growth factors, cytokines, and environmental stresses. MAP kinase signaling pathway possibly sheds light on targeted therapeutic approaches [22].

Research has identified specific genetic mutations that may contribute to the development of MVNTs. For instance, missense mutations in the BRAF gene or HRAS gene have been linked to the pathogenesis of tumors [22,23]. Vacuolization and neuronal differentiation in MVNTs are characterized by vacuolated neurons. This cellular morphology suggests that disruptions in normal

neuronal differentiation and cellular homeostasis may be due to genetic and epigenetic factors. Investigating the presence of other oncogenes and tumor suppressor genes may provide further insights. In exploring the molecular underpinnings of MVNT, a deeper understanding of its pathogenesis may pave the way for personalized treatment strategies and improved patient outcomes in the future. Like many tumors, MVNTs may exhibit altered vascularization patterns [24]. The tumor microenvironment, including the interaction between tumor cells and surrounding glial cells, immune cells, and extracellular matrix components, plays a crucial role in tumorigenesis [25,26]. Studying these interactions can show how the tumor ecosystem supports MVNT growth and survival.

Table 1: Multinodular and vacuolating neuronal tumor (MVNT) characteristics

Characteristics	Descriptors
Definition [2,3]	Low-grade tumors (grade I) comprising monomorphous neuronal elements in discrete and coalescent nodules, with vacuolar changes both in tumor cells and the neuropil, with a molecular pattern impaling MAPK pathway
Localization [4,9,10]	Parietal lobe, frontal lobe, temporal lobe, occipital lobe, spinal, rare cases of multiple localization, mainly subcortical white matter rare cases with cortical extension
Imaging features [4,11,12]	**Brain CT**- is usually normal or shows a non-enhancing/non-calcifying cortical/subcortical white-matter hypodense lesion, absence of mass effect and edema; **Brain MRI** – intra-axial multiple clustered nodules hyperintense on T2WI and FLAIR; T1WI: small hypointense, homogeneous, and well-defined nodules, some clustered tightly or with a small interface between them, located at the cortical-subcortical junctional side; Abnormal white matter signal surrounding the bubble-like or bunch of grape appearances lesions; No contrast enhancement; Restricted diffusion could be present with high ADC values rare **FDG-PET/CT** brain imaging showed tumor hypometabolism
Histological examination [4,8,12]	No evidence of mitosis, necrosis, or microvessel proliferation; Abnormal clusters and numerous vacuoles within the tumor cells, with different sizes, often surrounded by gliotic tissue; Glioneuronal hamartomas; Hypomyelinated plaque-like nodules extending into the deep cortex;
Immunohistochemical stains [4,8]	Many neuronal cells show weak immunoreactivity for synaptophysin and negative immunoreactivity for neuronal nuclear and neurofilament proteins; Variable background of positive dendritic staining for CD34; Negative for IDH1-R132H mutation antibody; Staining was normal for ATRX and p53; The Ki67 proliferation refraction was very low; GFAP highlighted the reactive astrocytes within the nodules; The vacuolated cells were immunoreactive for synaptophysin, while the fibrillary tumor matrix showed decreased synaptophysin expression compared to the adjacent cortex
Natural evolution [4]	Stable on time, „leave me alone” tumor

MAPK- Mitogen-Activated Protein Kinase; CT – computer tomography; MRI- magnetic resonance imaging; T2 FLAIR – space inversion recovery with fluid attenuation; FDG-PET/CT- fluorodeoxyglucose positron emission tomography/computed tomography; CD34 - transmembrane phosphoglycoprotein encoded by the CD34 gene; IDH1-isocitrate dehydrogenase; ATRX- chromatin remodeling · nucleosome assembly; p53- tumor suppressor gene; Ki67 – a nuclear nonhistone protein present in all active phases of cell cycle; GFAP – Glial fibrillary acidic protein responsible for astroglial cytoskeleton and is not found outside the CNS

Imaging Characteristics of MVNT

Imaging characteristics of MVNT reveal distinctive features that aid in their diagnosis and differentiation from other brain tumors. Described as low-grade lesions primarily found in the cerebrum, MVNT presents as clustered multinodular structures within the cortex and subcortical regions (Table 1). The brain MRI revealed multinodular T2-hyperintense lesions in both the deep cortex and superficial white matter of the frontal lobes or temporal lobes, exhibit a unique soap-bubble morphology, hyperintensity on T2 FLAIR sequences, and minimal or absent contrast enhancement [10,16,27]. Based on the signals from T1, T2, and T2 FLAIR, the nodules have nonhomogeneous signal intensity due to low T1, high T2, and low FLAIR vacuole aspects. Confluent T2/FLAIR hyperintensity overlying cortex was present in some cases [4]. The larger nodules have an inner core that is hypointense, a rim that is hyperintense, and areas with a high signal intensity that connect the nodules. The smaller nodules exhibit a signal intensity that is not uniform, with a central dot of low-intensity [11]. The nodules' periphery shows high signal intensity on FLAIR because of high protein content and neuronal demyelination [11].

Sometimes MR spectroscopy indicates an increase in the choline peak and a decrease in the N-acetyl aspartate peak, and tractography shows a partial disruption of the typical structure caused by the absence of lesional cortical layers [11,28]. No increase in perfusion was observed by perfusion-weighted imaging in another study, and abnormal peaks were not detected by MR spectroscopy [10]. Despite their benign nature, the pathologic hallmark of MVNT, questions their neoplastic or malformative origin and potential association with seizures [29]. Cases of a spinal cord lesion resembling cerebral MVNT of unknown significance were named MVNT-like spinal cord lesions. With intramedullary cystic ovoid nodules within the white matter, some immediately contiguous to the grey matter, this lesion was stable in a follow-up [9,30]. The presence of well-delineated intramedullary cystic ovoid nodules within the spinal cord raises questions about the underlying pathophysiology of MVNT [9]. A comprehensive MR protocol has been suggested to

increase the diagnostic confidence of MVNT [10].

Diagnostic and treatment of MVNT

Despite advancements in imaging and molecular techniques, MVNT can present with various clinical manifestations, posing diagnostic challenges. Usually, MVNT is revealed as an incidentaloma [31]. The clinical presentation is nonspecific, with symptoms such as seizures, headaches, or focal neurological deficits, often mimicking other more common brain tumors or epilepsy syndromes [1,14]. Additionally, the radiological features of MVNT on MRI can be subtle and easily overlooked, leading to misdiagnosis. The lack of specific diagnostic criteria for MVNT further complicates accurate identification, underscoring the need for histopathological examination for definitive diagnosis [16,32]. Biopsy remains the gold standard for confirming MVNT, highlighting the importance of integrating clinical, radiological, and pathological findings to navigate the diagnostic challenges posed by this rare entity in neuro-oncology. Additionally, the classification of MVNT as a benign neuronal tumor, possibly associated with seizures, further complicates the understanding of its neurological manifestations, especially in young patients [33].

Table 2: Immunohistochemical analysis [8,19]

Neuronal markers		Glial markers	
NeuN (Neuronal Nuclei)	A marker for neurons, specifically in post-mitotic neurons. It is widely used to identify neuronal populations in various brain regions.	GFAP (Glial Fibrillary Acidic Protein)	A specific marker for astrocytes. Increased expression is often seen in reactive astrocytes following injury
MAP2 (Microtubule-Associated Protein 2)	A neuron-specific protein that stabilizes microtubules and is important for dendritic structure.	Olig2	A transcription factor and marker for oligodendrocyte precursor cells and mature oligodendrocytes, essential for myelination in the central nervous system
βIII-Tubulin (Tuj1)	A marker commonly used to identify neurons, particularly in developmental studies.	S100	A protein found in astrocytes, used for identifying glial cells
Synaptophysin	A presynaptic vesicle protein, often used to identify synaptic structures and neuronal activity	CD68	A marker for microglia, reflecting macrophage activity in the central nervous system and often used in studies of neuroinflammation.

Distinguishing MVNT from entities such as dysembryoplastic neuroepithelial tumor (DNET), ganglioglioma, extraventricular neurocytoma, and low-grade glioma is sometimes challenging [34]. The bright diffusion sign and absence of cortical involvement could differentiate MVNT from DNET [35]. Moreover, incorporating genetic and molecular analyses can further aid in narrowing down the list of differential diagnoses and confirming the diagnosis of MVNT. Immunohistochemical analysis for neural and glial markers are also helpful (Table 2). Antibodies against neuronal protein HuC/HuD (HuC/HuD), pan-neurofilament (pan-NF), neurofilament 200 KD (NF200 KD), chromogranin, and antibodies against glial fibrillary acidic protein (GFAP), nestin, CD3, CD45, CD68, CD34, human leukocyte antigen-DR (HLA-DR), isocitrate dehydrogenase1 (IDH1), calbindin, parvalbumin, calretinin, Ankyrin-G, p62 and Ki67, BRAF(V600E) enhance diagnostic accuracy [8,19]. Immunopositivity for HuC/HuD and Olig2 proves that MVNT appears during neuronal development [4].

The latest WHO tumor brain classification has classified MVNTs as grade I, and the mitogen-activated protein kinase (MAPK) is considered a molecular marker [3,8]. The MAPK is a key signaling pathway that transmits signals from the cell surface to the nucleus and regulates various cellular processes, including growth, proliferation, differentiation, and survival. Further research in this area, particularly investigating specific diagnostic markers and biomarkers, is warranted to refine the differential diagnosis process for MVNT.

Factors such as tumor size, location, and the presence of associated neurologic deficits can influence treatment strategies and prognosis.

One of the foremost challenges in the preoperative diagnosis of MVNT is the difficulty in distinguishing them from other neoplastic or malformative lesions to avoid unnecessary surgical interventions [36]. The characteristic imaging features of MVNT, such as the subcortical nodular lesions with unique signal properties on MRI, can often be mistaken for more aggressive tumors due to their atypical presentation in younger patients. The rarity of MVNT in young individuals poses a diagnostic dilemma, questioning the

classification of these lesions as true neoplasms or malformative anomalies [22,31,21].

Imaging modalities and treatment strategies play pivotal roles in managing epileptic patients, especially those with drug-resistant epilepsy considered for neurosurgery. Utilizing advanced imaging techniques such as the Harmonized Neuroimaging of Epilepsy Structural Sequences (HARNESS-MRI) protocol and 7-Tesla MRI can aid in identifying previously occult epileptogenic lesions and improving surgical outcomes [37,38]. Additionally, the development of MRI postprocessing techniques and functional imaging modalities like EEG-functional MRI contribute to enhanced sensitivity in lesion detection and focus localization [39].

Surgery is often considered the primary treatment option for multinodular vacuolating neuronal tumors (MVNTs) under several circumstances. If the MVNT is causing neurological symptoms (such as seizures, headaches, or focal neurological deficits), surgical intervention may be necessary to alleviate these symptoms, when the tumor is accessible and can be safely resected without causing significant damage to surrounding brain tissue. Histological confirmation by surgical resection can provide a definitive diagnosis through the histopathological exam, which is crucial for confirming the nature of the tumor and ruling out other possible lesions. However, the precise location of the tumor within the brain can sometimes pose challenges in obtaining a biopsy sample. In such cases, advanced imaging techniques and neurosurgical expertise are indispensable in ensuring accurate sampling for an accurate diagnosis and subsequent management of MVNT. It is essential that surgical decisions are made in consultation with a team of specialists, including neurosurgeons and oncologists, who can evaluate the risks and benefits specific to each patient's condition and tumor characteristics. Functional MRI is as accurate as WADA testing, for determining language dominance [40].

The management of asymptomatic MVNT often involves careful observation rather than immediate intervention. However, a strategy of active surveillance can be appropriate for asymptomatic or minimally symptomatic MVNT. This involves regular follow-up through serial neurological examinations and imaging studies (usually MRI) every 6-12 months**.**

DISCUSSION

Advancements in diagnostic technologies have shown promise in addressing the challenges associated with diagnosing MVNTs. One emerging technology that holds potential is liquid biopsy, an innovative and non-invasive diagnostic tool that analyzes biomarkers (circulating tumor cells and genetic material) from bodily fluids (such as blood, urine, or cerebrospinal fluid) to provide information about tumors [41-46]. Circulating Tumor DNA (ctDNA) from the blood allows for the detection of genetic mutations and alterations associated with MVNT. Liquid biopsy is less invasive than traditional tissue biopsy, making it a more feasible option for patients, especially in complex cases where surgical options may be limited. It allows for repeated sampling without the risks associated with multiple surgical procedures, enabling ongoing monitoring.

Additionally, the development of radiomics, a method that extracts quantitative data from medical images to provide detailed insights into tumor characteristics, could aid in distinguishing MVNTs from other brain tumors with similar radiographic features [44-46]. These innovative diagnostic technologies have the potential to revolutionize the current diagnostic landscape for MVNTs, allowing for earlier detection, accurate classification, and personalized treatment strategies. Furthermore, integrating these technologies into clinical practice could improve patient outcomes and enhance our understanding of MVNT biology.

More studies are needed to elucidate the genetic mutations and molecular pathways involved in the development of MVNT. This would not only provide valuable insights into the underlying mechanisms of this rare brain tumor but also potentially identify novel therapeutic targets. Additionally, clinical research focusing on the efficacy of different monitoring modalities is crucial for optimizing patient outcomes**.**

CONCLUSION

Based on the results of this narrative review, emerging technologies allow for a re-conceptualization of the MVNT diagnosis and treatment. Although data regarding the validity of the new imaging and molecular techniques are still gathering, the fact that WHO includes MAPK as a marker for MVNT indicates the need for further refinement of the differential diagnosis based on molecular explorations in this pathology. The references in the literature related to genetic mutations and alterations associated with MVNT suggest new research directions congruent with the paradigm of personalized medicine.

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Authors' contribution

Conceptualization, M.M., O.V., and O.M.S.; methodology, O.V., A.P., and O.M.S.; software, M.M., A.P., and O.M.S.; validation, M.M., O.V., and A.P.; formal analysis, O.V.; investigation, M.M.; resources, A.P., and O.M.S.; data curation, O.V.; writing—original draft preparation, M.M., O.V., and O.M.S.; writing—review and editing, M.M., O.V., and O.M.S. All authors have read and agreed to the published version of the manuscript.

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ARTICLE

Functional Evaluation and Neuro-Muscle-Skeletal Recovery in Lyme Disease: A Study on Motor Control Theories and Rehabilitation

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Abstract: Background: Patients with Lyme disease often suffer from neuro-motor sequelae following Borrelia infection, leading to impairments in balance, posture, and spinal musculoskeletal functionality. This study aims to evaluate the effectiveness of physical therapy in addressing these impairments. Methods: Initial assessments were conducted using the GPS Posture-test System to evaluate static bipodal balance and posture, and BACKFIX Technology for unipedal balance and spinal functionality. Disturbances in balance, postural asymmetries, and a forward-shifted center of gravity were observed. The patient then underwent two 10 sessions (each during two weeks) of computer-assisted physical therapy focused on neuro-muscular movement and balance control. Results: Post-therapy evaluations showed significant improvements in both bipodal and unipedal static balance, segmental and overall body posture, and spinal musculoskeletal functionality. The therapy effectively addressed the postural asymmetries and shifted the center of gravity closer to the optimal vertical posture point. Conclusion: Computer-assisted physical therapy with visual feedback significantly improved posture, balance, and spinal functionality in patients with Lyme disease-related neuro-motor sequelae. This approach facilitated the restoration of muscle coordination and enhanced motor control, aligning with findings from the existing literature.

Keywords: Lyme Disease; Global Postural System; unipedal static balance; BACKFIX; global posture; spine; physical therapy

INTRODUCTION

The specialized literature lacks scientific studies on the functional evaluation and neuromusculoskeletal recovery in Lyme Disease (LD). Approximately 80 articles from the past decade are available in databases, primarily focusing on early detection and antibiotic treatment. No studies provide information on potential methods for functional evaluation or physical therapy-based recovery for musculoskeletal dysfunctions caused by inflammation secondary to Borrelia infection.

LD is caused by the bite of a tick infected with Borrelia. The disease progresses through three stages and can result in multisystem complications. When the nervous system is affected, it is referred to as neuroborreliosis, which may manifest as meningitis, mononeuritis multiplex, or cranial neuritis, including inflammation of the vestibulocochlear nerve [1]. Exclusive involvement of the vestibular system can lead to dizziness and balance instability [2]. Musculoskeletal symptoms can appear at any stage, including early localized infection, often resulting in patients being misclassified into the incorrect stage [3].

We describe motor control and the potential effects of systemic inflammation caused by borreliosis, followed by the stages of assessment and recovery, and the conclusions drawn from these steps.

Although the exact mechanism by which LD causes small fiber neuropathy is unknown, several potential mechanisms of neuronal damage are considered. These include the direct neurotoxic effect of the spirochete, neuroinflammation, and increased autoimmunity affecting the peripheral nervous system (PNS) with subsequent impacts on muscles, joints, and specific post-infection pains. However, a clear causal link between neurological symptoms and Borrelia infection cannot be

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established for patients with polyneuropathy/polyneuritis who test positive for Borrelia [4].

Among the genospecies of Borrelia burgdorferi sensu lato: Borrelia burgdorferi sensu stricto (s.s.), Borrelia garinii and Borrelia afzelii, Borrelia burgdorferi s.s. has the strongest potential to damage joints [5]. It is the most common cause of LD in the United States [3, 6], while infections with this pathogen are much less common in Europe [7-9]. The pathogenesis of LD is primarily driven by a strong inflammatory reaction [5], resulting in a perivascular collection of lymphocytes, macrophages, and scattered mast cells in the interstitial tissue, as typically observed in histopathological examinations of severe LD cases [9].

Previous studies have shown that late manifestations affecting the nervous system or joints can appear months or years after the initial infection, presenting symptoms such as fatigue, difficulty walking, posture disorders, and muscle pain. These symptoms are also present in the subject of our study, secondary to the diagnosis of borreliosis [10].

The examination and treatment of patients with motor control disorders are based on theories of motor control that guide the understanding of movement mechanisms [11-14]. In our study, we utilize systems theory as a foundation for clinical applications. Systems theory posits that movement arises from the interaction of various processes, including perceptual, cognitive, and motor processes, as well as the interactions between the individual, the activity, and the environment.

The severity of symptoms observed in all the patients with LD that we have evaluated has led us to pay more attention to this condition. As a result, our study focuses on evaluating and recovering a patient with LD using methods and applications informed by previous scientific studies. We analyzed a patient with sequelae following LD, including postural, walking, and static and dynamic balance disorders, muscle fatigue, changes in balancing synergies, walking base, and the projection of the General Center of Gravity (GCG). We conducted initial and final tests after a series of 10 and 20 sessions of specific physical therapy exercises.

Pioneers in the study of motor control and movement, such as Bernstein and Latash [15-17], along with Charles Sherrington (1857 - 1952), Thomas Graham Brown (1882-1965), and Walter Hess (1881-1973), have significantly contributed to our understanding of joint freedom, redundancy, and muscle-articular synergies. Charles Sherrington contributed to the understanding of inhibition, spinal-muscle connectivity, and the sensory-motor cortex. Thomas Graham Brown researched central movement programming and pattern generation mechanisms. Walter Hess studied the central coordination of the autonomic system and anticipatory motor behavior. Bernstein, recognized posthumously by Western colleagues, demonstrated a central hierarchy of postural control mechanisms and distributed, parallel processing of movement.

Bernstein also participated in developing theories of motor programs, recognizing that understanding neural control of movement requires knowledge of the system's characteristics in response to external and internal stimuli [16]. His distributed model of motor control showed that central commands could produce different motions due to external forces and initial conditions. His student, Mark Latash, furthered this work by demonstrating that synergies evolve to ensure flexible and stable performance in motor activities [17].

Postural control for stability and body orientation in space requires a complex interaction between the musculoskeletal and neural systems. This includes joint amplitudes, intersegmental biomechanical relationships, muscle physiology, spinal flexibility, neuromotor processing, synergies, perceptual and sensory processes, and their central integration.

Research has concluded that postural control is not managed by a single system but depends on various intrinsic and extrinsic factors [18]. Initial studies highlighted the importance of maintaining the center of mass (COM) within certain limits for static posture. However, subsequent studies demonstrated that stability limits are not fixed and vary with individual characteristics or actions undertaken. When recovering balance, individuals tend to restore COM disturbances rather than segmental alignment disturbances, suggesting that COM is a key variable controlled by the central nervous system during postural control.

Further studies have shown that analyzing the relationship between the center of gravity (COG) and the center of pressure (COP) provides a better picture of stability [19-24]. Their interaction has been considered and used to measure and estimate posture control effectiveness in our study.

As actions have both stability and orientation components, and certain actions prioritize orientation over stability, research by Shumway-Cook & McCollum [25] and Horak & Macpherson [26] has demonstrated that stability and orientation vary with each action. The body can behave like an inverted pendulum, employing various strategies (ankle, hip) for both static and dynamic balance [21].

Bernstein's assertion that "Movement coordination is the process of mastering the redundant degrees of freedom of a mechanism in motion" [16] encapsulates the essence of motor control theories used in our study.

Equilibrium

While bipedal standing is characterized by minor postural imbalances, dynamic balance involves the integration of sensory information from the visual, vestibular, and proprioceptive systems in conditions of postural instability.

There are several theories about how sensory organization adapts to a changing environment, with two primary ones being: the intermodal theory of sensory organization by Stoffregen and Riccio [27], which posits that all sensory systems equally and continuously contribute to postural orientation; and the sensory weighting model, which suggests that the central nervous system adjusts the importance of each sensory system based on their accuracy for orientation and postural stabilization.

Torres and Oviedo [28] examined the structure and organization of muscle synergies for balance control, highlighting that a muscle can contribute to multiple synergies, each with a unique role. They demonstrated that muscle synergies can be continuously modulated by feedback signals to create postural adjustments, indicating that the central nervous system uses both pre-programmed synergies and continuous feedback control to regain stability.

Nashner et al. [29] investigated the selection of sensory information by the CNS for postural control by designing a dynamic platform with a moving visual wall to study sensory interactions in maintaining posture and balance. This approach allowed for the measurement and recording of posture and balance under various conditions.

Several studies on normative values using static platforms have examined the performance of normal subjects under varying sensory information conditions [29-33]. These studies have shown that adults and children over seven years old (the age of vestibular maturation) maintain balance well under all conditions of sensory disturbance.

The inability to maintain a stable static posture has significant functional consequences in neurological pathologies. It necessitates using the arms and hands for balance, detracting from daily activities [34-38]. Moreover, postural instability and the inability to stand on one leg for more than six seconds increase the risk of falls threefold.

Springer's 2007 study [39] showed that balance disorders are prevalent in patients with specific diseases or traumas, particularly head injuries, peripheral neuropathies, or acquired neurological conditions. Neurological pathologies can disrupt one of the postural control strategies (e.g., loss of pre-programmed synergies) while leaving the other (continuous feedback control) intact, necessitating complementary studies to distinguish between these aspects and propose specific solutions.

For the main types of static/dynamic balance (postural control/anticipatory and reactive postural control), numerous clinical tests measure performance, including the unipedal stance and computerized posturography. Our study used such tests to assess the patient's condition at the beginning and end of the recovery program.

Unipedal standing is a highly challenging situation for humans and allows researchers and clinicians to assess the effects of aging, disease, trauma, and the influence of multiple sensory systems. The single-leg stand test, which requires minimal equipment, measures balance aspects and shows abnormal values in conditions such as peripheral neuropathies and intermittent claudication. [35,36]

Various studies demonstrated the importance of maintaining unipedal posture and balance by examining corticomuscular coherence between the ankle joint and the sensorimotor cortex. This study showed that the sensorimotor cortex is directly involved in postural control during unipedal standing, compensating for high postural demands due to the low support base. Normative values in the literature indicate a significant decrease in unipedal stance time with age and pathology [40, 41].

Alain Berthoz et al. [20] demonstrated that during complex movements, the head is intermittently stabilized under gaze and fixation control, allowing it to act as an inertial guidance platform for controlling multisegmental movements. The otolithic organs, the saccule, and utricle, detect the linear accelerations of the head and provide a gravitational reference used not only to correct posture but also for perceptual actions requiring spatial orientation, including mental map rotation based on sensory information from all senses.

Merton's Servo-Control Hypothesis

The interaction between motor and sensory processes is bidirectional, and the importance of sensory information for motor control was established many years ago. The traditional feedforward and feedback control mechanisms could not fully explain the coactivation of the alpha-gamma muscle loops, necessitating alternative theories such as the equilibrium point hypothesis and the hierarchy of synergies to explain how correct posture is maintained during external destabilizations [42].

Merton proposed a model that adjusts the equilibrium point, where the CNS controls both speed and position during rapid segmental movements. Degenerative, infectious, and metabolic diseases have disrupted the transmission of sensory information from the periphery to the CNS, affecting the parameters and components of posture and movement control. Disorders of nerve conduction from proprioceptors to the brain, as seen in neurological or diabetic patients, result in significant balance issues, making it difficult for patients to stand upright with their eyes closed on a static plane [42].

Posture, a term introduced by André Bregegère in 1955 [43], is defined as a momentary attitude or body position [44]. It involves the synergistic and coordinated action of the musculoskeletal system and the central and peripheral nervous systems to maintain body stability, balance, and constant relations between body segments and the environment [45-47]. Correct posture involves the alignment of all joints, and any misalignment can lead to muscle-joint imbalances [48].

Maintaining postural stability requires the co-participation and complementarity of actions from all support components, followed by motor actions (balancing, distribution of motor control tasks), which can be synchronous or asynchronous, simultaneous, or alternating depending on the demands [44,49]. Optimal body alignment in an upright position involves:

Head: Maintained by neck muscles. Insufficiency or habitual forward tilt increases muscle demand.

Trunk: Tends to fall forward due to its weight and the head's weight, counteracted by vertebral muscles.

Shoulders: The scapular belt and upper limbs' weight cause the shoulders to fall and scapulae to separate from the spine, counteracted by shoulder girdle muscles.

Pelvis: Key to maintaining correct posture. Anteversion is balanced by gluteal and posterior thigh muscles, whose hypotonia causes anterior trunk tilt. Retroversion is balanced by rectus abdominis, obliques, and iliopsoas muscles, whose hypotonia causes posterior tilt.

Lower limbs: Stability is ensured by plantar flexor muscles and triceps surae, especially the soleus [45].

Postural defects can lead to fatigue, muscle strain, and pain in later stages, potentially affecting vital organs, particularly in the abdominal region. Our study integrates current scientific conclusions and hypotheses to address parameters affecting motor control and static and dynamic postural control in our subject, considering the literature review.

Motor control theories are inherently unfinished, always allowing room for new information from recent studies on normal and pathological brain and PNS functioning. Neuroscientists identify the scientific basis for movement disorders, while clinicians develop applications of this research, as theories are considered more or less useful based on their problem-solving efficacy for patients with movement disorders.

In this context, we present a study where a patient with Lyme disease sequelae is compared to literature findings regarding posture, balance, and neuromuscular control of movement. Previous works by Schor D, Schor V et al.[50-52] introduced the BACKFIX Method, used for spine functional impairments in preventive care. This study evaluates its application to a complex disease like LD, characterized by neurological symptoms affecting balance, walking patterns, general movement control, and muscle strength imbalance.

We demonstrate the patient's condition evolution using physical therapy with visual feedback on the rectangular force-amplitude model. Improvements in agonist-antagonist cooperation lead to enhanced muscle strength, functional muscle-joint balance of the spine, posture, and walking pattern. The patient exhibited muscle cramps in the calf and foot, pronounced thigh flexor fatigue during walking, insecurity in unipedal support, jerky walking with a wide support base, and reduced standing time in unipedal support.

MATERIALS AND METHODS

We performed the following measurements:

- Computerized somatoscopic examination
- Forces, amplitudes, and mechanical work on the supporting muscles of the trunk, enabling a computerized muscle-articular evaluation of the trunk
- Bipodal static balance - stabilometry
- Unipedal static balance

We utilized the GPS System (Global Postural System) for computerized somatoscopy and computerized static bipodal balance tests, provided by UNEFS Bucharest (University of Physical Education and Sport from Bucharest), and the Ergocontrol System for musculo-articular functionality tests of the spine using the Backfix Method, technology provided by Medisport Company.

An initial evaluation and two intermediate evaluations were conducted. For the static one-legged balance test, we measured the hold time while standing on one leg. An initial evaluation and two intermediate evaluations were conducted. For the static one-legged balance test, we measured the hold time while standing on one leg

RESULTS

Initial Assessment Results

Computerized Somatoscopy Test

Front:

- Right shoulder slightly lowered and rotated anteriorly
- Left shoulder slightly raised
- Right hip slightly lowered
- Left hip slightly raised

Profile:

- Abdominal hypotonia with increased lumbar curvature
- Projection of the GCG (general center of gravity) 11 cm anterior to the normal position

Back:

- Left shoulder blade normally positioned
- Right shoulder slightly lowered and rotated anteriorly
- Left shoulder slightly raised
- Right hip slightly lowered
- Left hip slightly raised

Evaluation of the Muscle-Articular Functionality of the Spine

The results of the computerized musculo-articular measurements regarding the muscular support of the spine are presented in Table 1. The movements were performed with difficulty and were jerky, which is characteristic of the neurological sequelae of the condition.

Table 1: Initial muscle-articular evaluation (3.02.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	5.5	26	14.47
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	9.31	49	45.45
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	8.47	39	32.85

Resources and methods used in recovery

The recovery protocol involved 20 sessions of exercises utilizing visual feedback on force-amplitude curves, performed on the Ergocontrol System. This protocol aimed to restore agonist-antagonist cooperation within muscle groups and chains responsible for global body posture while also focusing on strength enhancement through movement control exercises targeting deficient muscle groups and chains.

Each session included walking exercises designed to integrate the improvements from the neuro-muscular movement control exercises, to enhance the walking pattern.

Results After 20 Recovery Sessions

There was a noticeable improvement in overall body posture and a tendency towards optimizing the indices of musculo-articular functionality of the spine. The results from the intermediate evaluations demonstrate progressive improvements in all measured parameters, as detailed in Tables 2 and 3.

Table 2: Intermediate muscle-articular evaluation (27.02.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	15.6	41	64.93
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	181	61	110.29
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	179	58	104.37

Table 3: Intermediate muscle-articular evaluation (26.03.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	20.8	49	103.5
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	21.5	66	140.7
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	213	56	118.2

The evolution of the somatic functionality of the trunk

Spine Pathology and Accident Risk Scale:
- < 0.50 = High Risk
- 0.50-0.75 = Medium Risk
- 0.75-0.95 = Low Risk

Initial Personal Levels:
- Lumbar: High
- Antero-posterior Thoracic: Medium

After Two Series of 10 Physical Therapy Sessions:
- Lumbar: Medium
- Antero-posterior Thoracic: Low

Chart number 1 illustrates the progress in muscle-articular functionality indices of the spine, specifically Thoracic Antero-posterior and Thoraco-lumbar, as assessed by Test 2 and Test 3. These improvements are also evident in the overall body posture, with notable

enhancements in the static balance and the projection of the GCG (see Figure 1).

Table 4: Segmental and intersegmental muscle-articular functionality ratios of the spine

Segmental and inter-segmental ratio	Optimal values	Personal values 03.02.2023	Personal values 27.02.2023	Personal values 26.03.2023
Thoracic anterior - posterior	0.85 - 0.95	0.73	0.94	0.84
Thoraco-lumbar	0.85 – 0.95	0.31	0.59	0.73

Chart number 1: Indices of muscle-articular functionality of the spine - Thoracic antero-posterior and Thoraco-lumbar

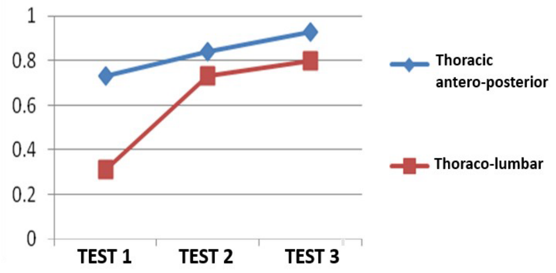

Figure 1: The improvements of the static ballance and the projection of the General Center of Gravity (CGG)

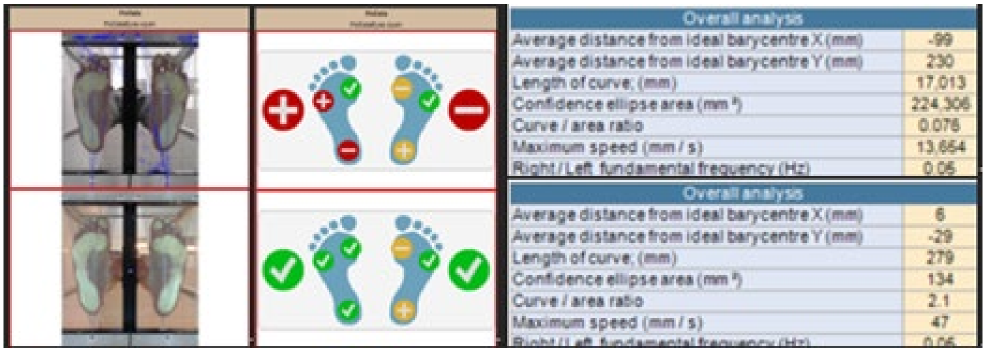

Overall analysis

Average distance from ideal barycentre X (mm)	-99
Average distance from ideal barycentre Y (mm)	230
Length of curve; (mm)	17,013
Confidence ellipse area (mm ²)	224,306
Curve / area ratio	0.076
Maximum speed (mm / s)	13,664
Right / Left fundamental frequency (Hz)	0.06

Overall analysis

Average distance from ideal barycentre X (mm)	6
Average distance from ideal barycentre Y (mm)	-29
Length of curve; (mm)	279
Confidence ellipse area (mm ²)	134
Curve / area ratio	2.1
Maximum speed (mm / s)	47
Right / Left fundamental frequency (Hz)	0.06

Unipedal Balance:
•Initially, unipedal support was impossible.
•After ten sessions, balance time increased to 21 seconds.
•After 20 sessions, the balance time reached 30 seconds.

General Center of Gravity (GCG)

Projection: Figures 2, 3, 4, and 5 depict the improvement in the migration of the General Center of Gravity in four positions: standing with eyes open, standing with eyes closed, facing left, and facing right.

Figure 2: The improvement of the migration of GCG in the position of stand-up face to the right side

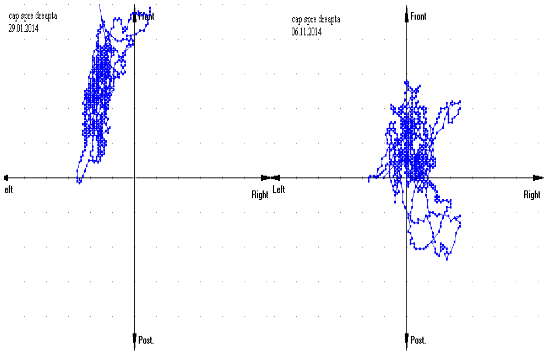

Figure 3: The improvement of the migration of GCG in the position of stand-up face to the left side

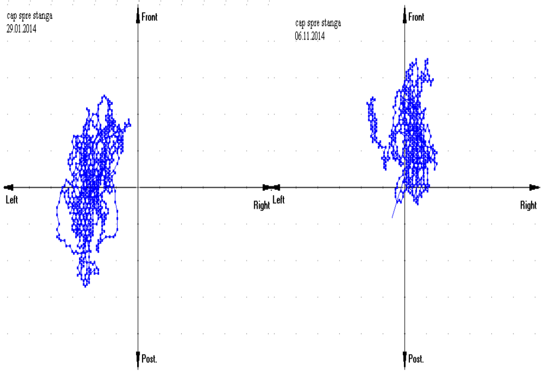

Figure 4: The improvement of the migration of GCG in the position of stand-up eyes closed

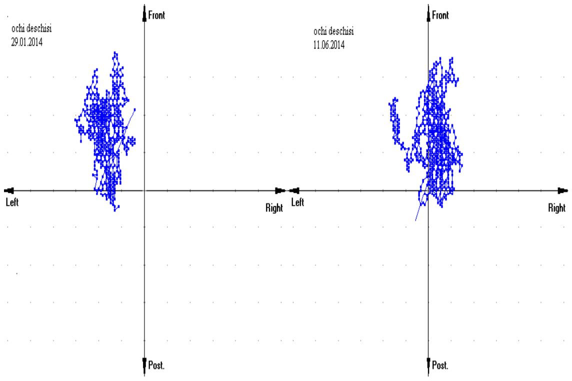

Figure 5: The migration of GCG improved in the position of standing up and eyes open

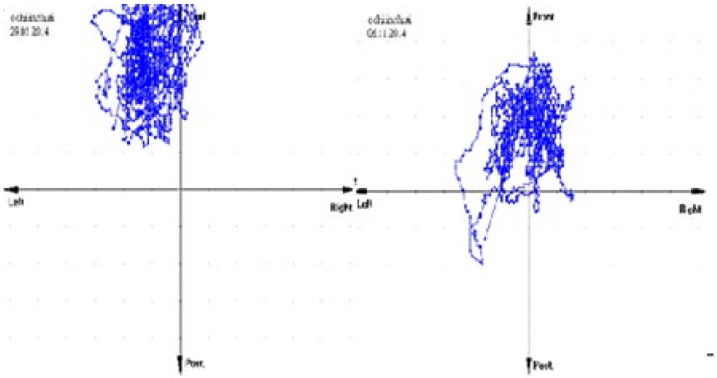

DISCUSSION

The key outcomes and benefits observed after completing two series of 10 physical therapy sessions in the study are presented in the following.

After Two Series of 10 Physical Therapy Sessions:
- Increased muscle strength and joint amplitude at the shoulder girdle.
- Continued need for abdominal muscle strength improvement to balance spinal muscle support.
- Enhanced muscle-articular functionality indices for the Thoracic Antero-Posterior and Thoraco-Lumbar regions, showing improvement from 0.73 to 0.84 and from 0.31 to 0.73, respectively.
- Balance time in the unipedal position with eyes open increased from 0 to 30 seconds.
- Improved gait from a jerky, insecure pattern to a smooth, balanced walk.
- During computerized somatoscopic evaluation, overall body posture improved, with the anterior projection of the CGG reducing from 11 cm to 5 cm from the normal projection.

The BACKFIX Method:
- Led to improved posture by optimizing muscle-articular functionality indices of the spine.

Visual Feedback on Force-Amplitude Patterns:
- Restored agonist-antagonist cooperation, positively affecting gait and balance motor control.

Optimized Functional Musculo-Articular Balance:
- Resulted in visible improvements in posture and walking pattern.

Risk Reduction:
- The risk of spinal pathology and accidents improved, moving from medium to low risk, with enhanced muscle chain synergies supporting proprioceptive and sensory system integration in unipedal stance.

The observed benefits from targeted physical exercises confirm previous studies on the uniqueness of bipodal and unipedal stance control, inter-segmental coordination, and the antigravity function of the leg extensors. Most cases of LD resolve after one course of therapy, although some patients experience gradual symptom relief even after treatment ends; therefore, follow-up assessments are recommended 3 months post-therapy. In some cases, symptoms persist despite antibiotic treatment, leading to the term "antibiotic-refractory LD", defined as persistent synovitis lasting more than 1 month after two four-week oral antibiotic courses or more than 2 months after intravenous ceftriaxone treatment [53].

Untreated arthritis may eventually resolve on its own, but it can persist for years, with some cases of LD lasting 7-8 years [54,55]. Potential treatments for patients with post-arthritis LD include non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), biologic agents, intra-articular steroids, and arthroscopic synovectomy [55,56].

The strengths of this study lie in its focus on the functional evaluation and rehabilitation of patients with Lyme disease-related neuro-muscular issues, an area with limited prior research. The use of computer-assisted physical therapy, employing technologies like the GPS Posture-test System and BACKFIX Technology, allowed for a precise and objective assessment of balance and posture, leading to significant improvements in the patients. These technologies facilitated a more thorough understanding of musculoskeletal impairments and offered targeted interventions that improved both static and dynamic balance, posture, and muscle coordination.

However, some limitations must be noted. The rehabilitation was conducted on one patient, and this limits the generalizability of the results. Additionally, the long-term sustainability of the therapy outcomes remains uncertain, and further follow-up studies are needed to verify the persistence of improvements beyond the immediate post-treatment period. Another limitation is the lack of control, which would have strengthened the validation of the study's findings.

Future research in this domain should focus on expanding the sample size and incorporating control groups for comparative analysis. Long-term studies are also crucial for understanding the chronic impact of rehabilitation techniques. Finally, integrating more advanced technologies, such as virtual reality or machine learning algorithms for personalized therapy adjustments, could further enhance patient outcomes in neuro-muscular recovery for Lyme disease.

CONCLUSION

This study demonstrates the effectiveness of computer-assisted physical therapy using technologies such as the GPS Posture-test System and BACKFIX Method in addressing the neuro-muscular and postural impairments associated with Lyme disease sequelae. Patients exhibited significant improvements in static and dynamic balance, posture, and musculo-articular functionality after completing two series of physical therapy sessions. The visual feedback provided during therapy sessions played a crucial role in restoring agonist-antagonist cooperation and enhancing motor control.

In conclusion, targeted physical therapy combined with advanced postural assessment technologies offers an effective means of improving functional recovery in Lyme disease sequelae patients, supporting their overall motor control and quality of life.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Acknowledgment

The current manuscript does not contain previously published materials or self-generated AI text.

Authors' contribution

Conceptualization D.S. and V.S.; methodology D.S.; software V.S.; validation D. S., L.G.E. and A.G.; formal analysis R.P.; investigation P.S.F.; resources S.S. and M.B; data curation R.R.G.; writing—original draft preparation D.S.; writing—review and editing L.G.E and A.G.; visualization R.R.G.; supervision L.G.E.; project administration D.S.; funding acquisition V.S. All authors have read and agreed to the published version of the manuscript elaboration.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of MEDISPORT Physical Therapy Clinic (11/30.03.2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

Navigating Cancer: Mental Adjustment as Predictor of Somatic Symptoms in Romanian Patients-A Cross-Sectional Study

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Abstract: Objective: This study aimed to examine the relationship between mental adjustment mechanisms and somatic symptoms in cancer survivors, a topic with limited research in Psycho-oncology. Methods: In this exploratory cross-sectional study, 88 cancer patients were surveyed using the Somatic Symptoms Scale (SSS-8), the Somatic Symptom Disorder Scale (SSD-12), and the Mini-Mental Adjustment to Cancer Scale (MINI-MAC). Additional questions were asked regarding cancer stage, treatment, and illness relapses. Data collection occurred over one month at a local oncology unit. Results: Several significant correlations were observed between the scales and subscales of the instruments applied during this study. The most important results showed that maladaptive adjustment mechanisms were significantly associated with somatic symptoms, while adaptive mechanisms were not. However, "fighting spirit," an adaptive mechanism, acted as a moderator between somatic symptoms and their psychological impact (effect size $t = 0.157$, $p < 0.05$). Lower "fighting spirit" scores correlated with higher SSS-8 and SSD-12 scores, indicating its potential protective role. Conclusion: A more specific assessment of somatic symptoms and their link to mental adaptation to cancer is needed. Psycho-oncological interventions should address both adaptive and maladaptive adjustment mechanisms due to their significant influence on patient outcomes.

Keywords: cancer, mental adjustment, somatic symptoms, adaptive mechanisms, maladaptive mechanisms, Psycho-oncology

INTRODUCTION

The prevalence of cancer is increasing worldwide, with almost 18.1 million cases in 2020 and a rise of 28 million cases in 2040 [1]. Romania has a lower incidence of cancer related to EU mean value, but the mortality of this disease is higher, which reflects the overall weakness in the cancer approach in our country, Romania having among the highest oncological mortality rates in Europe [2,3]. Approximately 100,000 people are diagnosed with cancer in Romania every year, a number that could be even higher. In response, Romania approved a National Plan for Beating Cancer in 2022 to be implemented during 2023-2026. Addressing the cancer burden will require sustained efforts to improve cancer prevention, early diagnosis, treatment access, and cancer surveillance systems [4,5,6]. Key measures included in the country`s National Plan for Beating Cancer are integrated and present a multidisciplinary approach, health innovation fund, cancer registry, and data collection, clinical guidelines and protocols, and oncological experts' boards. Among these last ones, a special place will be highlighted by the Psycho-oncological approach. Psycho-oncology is facing significant challenges, including major gaps in services and a critical shortage of specialized professionals, resulting in a nationwide deficit in Psycho-oncology care. However, the concepts of mental adjustment, psychological distress, and quality of life are very scarcely mentioned in local literature. Broader searches for Eastern Europe yielded limited similar results, indicating a general lack of research [7].

Patients with cancer may present more frequent anxiety, depressive symptoms, somatic symptoms, and overall psychological imbalance due to the perceived poor outcome and recurrence of the disease. Recent research has reinforced the link between psychological distress and an increased likelihood of mortality and worse survival outcomes [8]. Psychological distress in patients with cancer is defined as the psychological damage that impairs an individual's ability to operate. This illness comprises a range of emotional and behavioral features arising from the diagnosis and prognosis uncertainty [9]. Prior studies have emphasized the importance of depressive disorders and extreme emotional response patterns as crucial elements in diminishing the quality of life of these patients. It is important to acknowledge the

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interconnection between anxiety, sadness, and somatic symptoms in the context of cancer experience [10].

The cancer adjustment frame was developed by Watson and includes five adjustment mechanisms: fighting spirit, avoidance–denial, fatalism–stoic acceptance, helplessness–hopelessness, and anxiety [11]. Mental adjustment to cancer refers to the process of coping with emotional distress and restoring mental balance after a cancer diagnosis. It involves specific attitudes and strategies that patients use to adapt to the challenges of cancer and its treatment. Patients with a fighting spirit tend to have better quality of life and lower anxiety/depression compared to those with a helpless/hopeless attitude [12,13]. Maladaptive coping styles like helplessness/hopelessness are associated with a higher risk of mortality in some studies, although the evidence is not yet conclusive [12]. Factors like age, performance status, social support, and satisfaction with support can influence mental adjustment. Mental adjustment, economic status, perceived disease severity, and symptom severity directly affect the comprehensive quality of life outcome (CoQoLO) in terminally ill patients [12,13].

Somatic symptoms constitute about 50% of all general medical consultations, remaining prone to further investigations and bringing frustration to both patients and doctors in approximately one-third to one-half of cases. Both physiological and psychological variables seem to contribute to the reporting of somatic symptoms, especially in individuals with long-term medical conditions [14]. These symptoms are linked to significant limitations in functioning, disability, and utilization of healthcare services, even when considering other medical and mental conditions [14,15,16]. Research on the frequency of somatic symptoms in cancer has frequently concentrated on individuals with advanced stages of the disease or specific forms of cancer observed at specialized medical facilities [12,13,17,18]. Research revealed that a minimum of 30% of patients with cancer experience the following symptoms: fatigue and low energy, unspecified pain, loss of appetite, constipation, sleep disorders, and dizziness. The connection between psychological distress and reporting of somatic symptoms, also known as somatization, has only been examined in a limited number of small-scale research on cancer [15, 16,19,20]. This gap is significant since several studies in non-cancer medical populations have shown a high occurrence of somatic symptoms, even among symptoms that are regarded to be more specific to a particular disease. Moreover, the somatization concept refers to the expression of psychological distress through physical symptoms. Somatic symptoms, the term we chose to use throughout this paper, conversely denotes the way emotional stress can materialize as physical symptoms. A thorough comprehension of this matter is crucial to effectively address the psychological requirements of individuals with cancer, thereby enhancing their quality of life and their capacity to manage the illness [13,16,21]. Despite the recognized importance of mental adjustment on QoL and prognosis in cancer care [12], there is limited research focusing on the interplay between mental adjustment and somatic symptoms specifically.

This study aims to fill this gap by exploring the relationship between mental adjustment to cancer and somatic symptoms in patients with cancer. The findings could provide valuable insights for Psycho-oncology professionals enhancing supportive care strategies, ultimately improving patient outcomes and quality of life. The primary objectives of this study are to assess the levels of mental adjustment to cancer among Romanian patients, to measure the prevalence and severity of somatic symptoms, and to investigate their relationship with mental adjustment as being predicted by certain types of adaptive and maladaptive mechanisms. We hypothesize that maladaptive coping styles will predict somatic symptoms and their burden, while adaptive coping styles may represent protective factors in the onset and maintenance of somatic symptoms. This cross-sectional study involved a sample of Romanian patients, implementing validated psychological assessment tools to evaluate mental adjustment and somatic symptoms. Statistical analyses were conducted to explore correlations and identify significant predictors.

MATERIALS AND METHODS

Study design

This study employed a cross-sectional design to investigate the relationship between mental adjustment to cancer and somatic symptoms and their burden in Romanian cancer survivor outpatients.

Participants

A total of 88 patients with cancer were recruited from the cancer department of Elias University and Emergency Hospital in Bucharest, Romania, for one month, between May and June 2023. Inclusion criteria were: a confirmed diagnosis of cancer in the last three years, age 18 years or older, out-patients regularly monitored during their cancer treatment follow-up, and ability to understand and provide informed consent. Exclusion criteria included: severe cognitive impairment, DSM-5-TR diagnosis of a severe psychiatric disorder such as schizophrenia, depression or bipolar disorder currently or in the past based on information obtained from behavioral scales, a clinical psychiatric evaluation, and the Structured Clinical Interview for DSM Disorders [22]; diagnosis of neurological conditions (e.g., Parkinson's disease, multiple sclerosis) that could affect cognitive or emotional processing, other genetic diseases or syndromes that might affect mental adjustment mechanisms or cognitive function.

Ethical approval

The study was approved by the Ethics Committee of the Elias University and Emergency Hospital of Bucharest, Romania (nr. 9286/2023). All participants provided written informed consent before participation.

Data Collection

Data were collected through structured interviews and self-administered questionnaires. The following tools were used:

Socio-demographic data- this section included information such as age, gender, education level, employment status, marital status, residence, income level, number of children, cancer stage, cancer location, chemotherapy, hormonal, surgery, and radiotherapy history.

Mini-Mental Adjustment to Cancer (Mini-MAC) – The questionnaire, designed by Watson[11], used the Romanian-translated version [23]. The mini-MAC questionnaire consists of 29 items, assessing different ways of coping with cancer: Anxiety- refers to fear concerning the disease without prior control which is anxiety-causing; Fighting spirit- refers to framing cancer as a challenge that may prone the patient to take action and fight it; Helplessness–hopelessness- refers to feelings of passivity and fatalism towards cancer leading to submission; Positive revaluation- refers to framing the life already lived in the light of satisfaction. Coping with cancer can be made within a constructive framing including fighting spirit and positive revaluation while anxiety and helplessness may represent a destructive pattern of coping. The items are rated on a scale of 1 to 4 representing variations of responses from „definitely not" to „definitely yes" which may offer results for each of the coping strategies from 7 to 28 points. The higher the score the more frequent and intense each mental adjustment mechanism is represented.

Somatic Symptoms Scale-8 (SSS-8)- The 8-item was developed after a version of the Patient Health Questionnaire-15 (PHQ-15) [24] and validated in the general population and Romanian population [25] as well as inpatient and outpatient settings, with SSS-8 not yet validated in Romanian language [26] to detect somatic symptoms burden. The level of the experienced somatic symptoms is scored from 0 meaning „not at all" until 4 which represents „very strongly". The results are grouped into different categories with minimum, low, medium, and high groups containing symptoms such as pain in the arms, legs, or joints, gastric problems, insomnia, unspecified thoracal pain or trouble breathing, fatigue, or different ranges of headaches.

Somatic Symptom Disorder-12 (SSD-12)- this tool was first developed by Toussaint et al. [27] and focused on criterion B of the Somatic Symptom Disorder (SSD) diagnosis including subscales on cognition, affect, and behavior. 12 items are scored from 0 meaning „never" to 4 meaning „very often". The scale was already validated in outpatient settings [28], and the Romanian version used, was previously translated and validated [19,29]. 18 score was chosen to screen for potential cases by the creators of the scale with studies still being concerned with the validity of cut-off points [30].

Procedures

Participants were approached during their routine medical visits and invited to participate in the study. Those who consented were given the questionnaires to complete on-site, with instructions for returning them. Structured interviews were conducted on-site by trained researchers and clinical psychologists to ensure consistency and accuracy in data collection and that the data collection processes did not interfere with the regular cancer follow-up procedure.

Statistical Analysis

Data were analyzed using JASP software [31]. In line with the explorative nature of our study, we first described the demographics of the study participants. The internal consistency of psychometric scales was assessed using Cronbach's alpha coefficient, and all instruments had coefficients above 0.7, which is the minimum acceptable value for proper reliability. Descriptive statistics (means, standard deviations, frequencies) were used to summarize the demographic and clinical characteristics of the sample. Pearson correlation coefficients were calculated to examine the relationship between mental adjustment and somatization scores. Multiple regression analyses were conducted to identify significant predictors of somatic symptoms, controlling for potential confounders and considering standard p-value < 0.05

RESULTS

Psychometric scales reliability

All the psychometric tools used proved to be reliable. As confirmed in our analysis, a tool needs to have a Cronbach Alpha of at least 0.7 to be used. Moreover, SSS-8 presented values of 0.815, SSD-12 of 0.910, and Mini-MAC of 0.879, with all subscales also confirmed to be reliable.

Descriptive analysis

Socio-demographic data

Table 1 presents the socio-demographic characteristics of the participants. The most frequent age interval of the participants was 41-50 years with over 70% being in the age interval of 41-60 years. The sample included 86 females (97.7%). 69,3% were married, over 68% graduated from at least one university, and 75% declared a good financial status. Concerning cancer, 82,5% had local cancer, and 71% presented breast cancer with various periods since surgery, from less than one year (20,4%) to over three years (36.6%). 79% declared chemotherapy intervention, while 64% declared radiotherapy, with over 90% presenting surgery history.

Psychometric scales scores

The scores for SSS-8 indicated moderate somatic symptoms burden (mean= 10.214, SD=5.903), while SSD-12 presented a mean of 16.753, close to the cut-off point of 18, considered for SSD screening [30]. Also, the behavioral aspect of SSD-12 presented a mean of

6.110 (SD=3.256). Concerning Mini-MAC subscales, we identified anxious preoccupation (mean=17.352; SD=4.857) and helplessness (mean=14.082; SD=4.198) among the highest-scoring mechanisms checked.

Table 1: Socio-demographic characteristics

Variable	Category	Frequency	Percent	Valid Percent	Cumulative Percent
Age	20-30	3	3.409	3.409	3.409
	31-40	6	6.818	6.818	10.227
	41-50	36	40.909	40.909	51.136
	51-60	27	30.682	30.682	81.818
	>60	16	18.182	18.182	100.000
Gender	Feminine	86	97.727	97.727	97.727
	Masculine	2	2.273	2.273	100.000
Marital Status	Married	61	69.318	69.318	69.318
	Single	5	5.682	5.682	75.000
	Divorced	17	19.318	19.318	94.318
	Widowed	5	5.682	5.682	100.000
Education	Elementary	1	1.136	1.136	1.136
	Medium	25	28.409	28.409	29.545
	University	33	37.500	37.500	67.045
	Master, PhD	29	32.955	32.955	100.000
Financial Situation	Bad	16	18.182	18.182	18.182
	Good	66	75.000	75.000	93.182
	Very good	6	6.818	6.818	100.000
Cancer Stage	Local	73	82.955	83.908	83.908
	Advanced	14	15.909	16.092	100.000
Cancer Location	Breast	71	80.682	80.682	80.682
	Digestive	1	1.136	1.136	81.818
	Genital	15	17.045	17.045	98.864
	Respiratory	1	1.136	1.136	100.000
Time Since Surgery	<1 year	18	20.455	22.785	22.785
	< 2 years	14	15.909	17.722	40.506
	2-3 years	15	17.045	18.987	59.494
	>3 years	32	36.364	40.506	100.000
Chemotherapy	No	16	18.182	18.605	18.605
	Yes	70	79.545	81.395	100.000
Radiotherapy	No	30	34.091	34.483	34.483
	Yes	57	64.773	65.517	100.000
Hormone Therapy	No	35	39.773	39.773	39.773
	Yes	53	60.227	60.227	100.000
Surgical History	No	8	9.091	9.091	9.091
	Yes	80	90.909	90.909	100.000

Table 2: Psychometric scales scores characteristics

Variable	Valid	Missing	Mean	Std. Deviation	Minimum	Maximum
SSS-8	84	4	10.214	5.903	1.000	32.000
SSD-12	73	15	16.753	8.052	1.000	39.000
SSD--12_Cognitive	88	0	4.170	2.649	0.000	14.000
SSD12_Afective	88	0	5.784	3.758	0.000	16.000
SSD-12_Behavioral	73	15	6.110	3.256	1.000	15.000
MAC-Helplessness	85	3	14.082	4.198	8.000	28.000
MAC-Anxious preoccupation	85	3	17.353	4.857	8.000	29.000
MAC-Fighting Spirit	85	3	12.388	2.111	5.000	16.000
MAC-Cognitive avoidance	85	3	11.153	2.432	4.000	16.000
MAC-Fatalism	85	3	14.847	2.780	7.000	20.000

The missing data pertains specifically to the behavioral aspects subscale of the SSD-12, with approximately 17% of participants not completing this part of the scale. This indicates that the missing data is not spread across the entire SSD-12 scale but is confined to a particular subset. To address this, analyses related to the behavioral component were conducted on available data. This approach

minimizes data loss but may introduce bias if the missing data is systematic (e.g., patients with more severe symptoms avoiding completion). No imputation was used for the missing data, and as such, the findings should be interpreted with caution. Sensitivity analyses showed that the inclusion or exclusion of these cases did not substantially alter the overall findings of the study.

Correlation between all psychometric scales analysis

We found significant correlations between SSS-8 scores and overall SSD-12 score including affective and behavioral aspects subscales (r=0,63; r=0,57; p<.001), anxious preoccupation (r=0.328; p<.01), helplessness (r=0.354, p<.01) and significant negative correlations with fighting spirit (r=-0.299; p<.001). SSD-12 scores were significantly correlated with helplessness and anxious preoccupation (r=0.558, p<.001; r=0.578, p<.001) as seen in Table 3.

Table 3: Pearson correlation coefficients between different variables

Variable	SSS8	SSD12	SSD12_ Cog	SSD12_ Affect	SSD12_ Beh	Help-lessness	Anxious preoccupations	Fighting spirit	Cognitive avoidance	Fatalism
SSS8	—									
Pearson's r	—	0.537***	0.420***	0.630***	0.570***	0.354**	0.328**	-0.299**	-0.023	-0.171
p-value	—	1.615×10^-6	7.014×10^-5	1.318×10^-10	2.592×10^-7	0.001	0.003	0.007	0.837	0.126
SSD12	0.537***	—	0.796***	0.950***	0.919***	0.558***	0.578***	-0.195	0.257*	-0.004
p-value	1.615×10^-6	—	3.648×10^-17	1.515×10^-37	1.814×10^-30	3.581×10^-7	1.084×10^-7	0.101	0.030	0.971
SSD12_ Cog	0.420***	0.796***	—	0.695***	0.562***	0.407***	0.469***	-0.111	0.104	0.110
p-value	7.014×10^-5	3.648×10^-17	—	5.610×10^-14	2.360×10^-7	1.124×10^-4	5.838×10^-6	0.312	0.344	0.315
SSD12_Afectv	0.630***	0.950***	0.695***	—	0.844***	0.520***	0.614***	-0.199	0.198	-0.029
p-value	1.318×10^-10	1.515×10^-37	5.610×10^-14	—	6.586×10^-21	3.423×10^-7	4.054×10^-10	0.068	0.069	0.790
SSD12_Beh	0.570***	0.919***	0.562***	0.844***	—	0.565***	0.544***	-0.165	0.241*	-0.013
p-value	2.592×10^-7	1.814×10^-30	2.360×10^-7	6.586×10^-21	—	2.278×10^-7	7.828×10^-7	0.167	0.043	0.914
Helplessness	0.354**	0.558***	0.407***	0.520***	0.565***	—	0.693***	-0.414***	0.057	-0.275*
p-value	0.001	3.581×10^-7	1.124×10^-4	3.423×10^-7	2.278×10^-7	—	2.733×10^-13	9.071×10^-5	0.606	0.012
Anxious preoccupations	0.328**	0.578***	0.469***	0.614***	0.544***	0.693***	—	-0.239*	0.152	-0.132
p-value	0.003	1.084×10^-7	5.838×10^-6	4.054×10^-10	7.828×10^-7	2.733×10^-13	—	0.028	0.170	0.233
Fighting spirit	-0.299**	-0.195	-0.111	-0.199	-0.165	-0.414***	-0.239*	—	0.311**	0.734***
p-value	0.007	0.101	0.312	0.068	0.167	9.071×10^-5	0.028	—	0.004	2.878×10^-15
Cognitive avoidance	-0.023	0.257*	0.104	0.198	0.241*	0.057	0.152	0.311**	—	0.425***
p-value	0.837	0.030	0.344	0.069	0.043	0.606	0.170	0.004	—	6.071×10^-5
Fatalism	-0.171	-0.004	0.110	-0.029	-0.013	-0.275*	-0.132	0.734***	0.425***	—
p-value	0.126	0.971	0.315	0.790	0.914	0.012	0.233	2.878×10^-15	6.071×10^-5	—

*p < 0.05, **p < 0.01, ***p < 0.001

Tables 3 and 4 show the contribution and significance of each predictor of MAC mechanisms to somatization as follows. Helplessness and anxious avoidance negatively predict somatization (t=2.520, p<.01; t=2.462, p<.01) while fighting spirit is moderator of the relationship between somatization (SSS-8) and its burden (SSD-12) (t= 0.157, p<.01) with low and medium values having a positive correlation which disappears at highest levels.

Table 4: Multiple regression analysis

Model	Unstandardized Coefficient (B)	Standard Error	Standardized Coefficient (Beta)	t	p-value
H_0 (Intercept)	17.060	1.002	—	17.026	0.013
H_1 (Intercept)	-22.708	9.161	—	-2.479	0.016
Helplessness	0.744	0.295	0.385	2.520	0.014
Anxious preoccupations	0.583	0.237	0.342	2.462	0.017
Cognitive avoidance	0.701	0.377	0.191	1.858	0.068
Fatalism	0.601	0.479	0.174	1.254	0.215
Fighting spirit	0.108	0.685	0.025	0.157	0.876

DISCUSSION

Our study showed that mental adjustment to cancer and somatic symptoms are significantly interrelated in patients with cancer. Specifically, only negative mechanisms such as anxious preoccupation and helplessness predicted somatic symptoms burden and not the positive ones, while fighting spirit moderated the relationship between somatic symptoms burden and their overall impact on a cognitive, affective and behavioral level, suggesting a potential protective factor in the relationship between SSS-8 and SSD-12.

Previous research has proved that psychological distress can exacerbate somatic symptoms in patients with cancer [32]. Also, somatic symptoms were highlighted to have mediating effects between psychological distress and quality of life in patients with cancer, while screened-positive SSD patients represented a significant predictor of lower quality of life (QOL) ($B = -0.476$, $p < 0.00$) [33]. Also, previous studies reported somatic symptoms as being risk factors for cancer patients' prognosis using the same psychometric tool of SSD-12 but without an emphasis on mental adjustment mechanisms [34]. Moreover, research reported greater psychological adaptation and maturity in coping mechanisms are associated with lower levels of somatic symptoms and depression in patients with cancer [35]. Also, maladaptive emotional responses to cancer, characterized by strong associations between emotional and somatic symptoms, can lead to higher levels of somatization and poorer quality of life in patients with cancer [36]. To our knowledge, until now, no other study explored mental adjustment to cancer mechanisms in relationship with somatic symptoms and their burden.

Concerning specific mental adjustment to cancer mechanisms, previous research linked positive and negative affect to fatalism and fighting spirit with fighting spirit being negatively correlated to fatalism and negative affect [12,37], while another study highlighted anxious preoccupation as a predictor for significant decreases in all the domains of quality of life, with helplessness-hopelessness predictor for significant decreases in emotional and functional wellbeing domains [38].

We showed the strong association between helplessness and somatic symptoms suggesting that patients who feel more overwhelmed and powerless by their illness are more likely to report physical symptoms. Similarly, anxious preoccupation, characterized by excessive worry about the disease, may lead to heightened awareness and reporting of somatic symptoms. An interesting finding of our study relies on fighting spirit as an adaptive mechanism that moderates the relationship between somatic symptoms and overall impact on patients with low and medium values, having a positive correlation that disappears at the highest levels. This may explain fighting spirit as a protective factor in the relationship, an aspect worth considering in modern psycho-oncological approaches and in accordance with previous research on its link to positive affect [39]. The absence of significant effects for adaptive mechanisms in our sample may be attributed to the homogeneity of the sample, particularly regarding cancer type and stage, as well as potential measurement limitations of the Mini-MAC scale in capturing nuanced coping responses. These findings align with literature suggesting that adaptive mechanisms may exert more influence during acute or early-stage illness phases and less during chronic or long-term survivorship. However, our results contrast with studies reporting sustained benefits of fighting spirit over time. This discrepancy highlights the need for future research to explore these mechanisms in more diverse and heterogeneous patient populations and consider longitudinal designs to capture temporal changes in coping efficacy. Tailored interventions that account for individual patient contexts and preferences may be more beneficial than a one-size-fits-all approach focusing solely on adaptive mechanisms like fighting spirit [40].

Concerning somatic symptoms assessment, although the SSD-12 and SSS-8 have good reliability and validity, they cannot replace the diagnostic accuracy of a psychiatric interview. Our descriptive results obtained from this study for both SSD-8 and SSD-12 need to be interpreted appropriately. For many diagnoses, the DSM-5 requires that symptoms from SSD should not be the results of an underlying organic cause [41]. For patients with a medical condition such as cancer, this criterion is not realistic as symptoms may indeed be associated with both the underlying medical condition [42].

Somatic symptom identification in patients with cancer needs more attention to be paid to the psychological care of patients with physical symptoms. However, the observed relationship between mental adjustment mechanisms, such as fighting spirit, and somatic symptoms could be influenced by several confounding variables not fully controlled in our study. For instance, time since diagnosis may alter the effectiveness of coping strategies, with recently diagnosed individuals potentially exhibiting more distress and somatic symptomatology compared to long-term survivors who have developed adaptive coping mechanisms. Additionally, the type and intensity of treatment—particularly chemotherapy and radiotherapy—can exacerbate physical symptoms like fatigue and cognitive impairment complicating the assessment of psychological coping. Furthermore, the disease stage significantly impacts psychological well-being and symptom burden, with advanced stages often associated with higher levels of distress and lower fighting spirit. These

confounders could attenuate or amplify the observed relationships between mental adjustment and somatic symptoms, suggesting the need for future studies to incorporate these variables more comprehensively into their analytical models in order to disentangle their effects.

Therefore, it is necessary to conduct a comprehensive assessment of the patient`s psychological dimensions of the clinical presentation. Hence, we hope to present the phenomenon of somatic symptom prevalence as a consequence of poor mental adjustment mechanisms in patients with cancer, thus highlighting the need to consider this issue by both oncologists and psychiatrists [43,44].

Clinical Implications

Somatic symptoms can be as easily confusing as physical symptoms in this specific type of population, which results in both overmedication and insufficient psychosocial care. In our study, we demonstrated a significant correlation between somatic symptoms and maladaptive mental adjustment mechanisms, a finding that should warrant greater attention to this unique issue among patients with cancer. Identifying patients with high levels of helplessness and anxious preoccupation can help Psycho-oncologists target interventions to reduce it. Psycho-oncology programs focusing on enhancing coping skills and reducing anxiety may be beneficial in this regard for Romania and other countries as costs for invasive and time and financially resources consuming investigations may overwhelm an already limited and insufficiently developed multidisciplinary approach, which may lead to confusion and medical shopping among patients [3].

Limitations

Several limitations should be acknowledged. This was a cross-sectional study, which was unable to determine the causal relationship between mental adjustment mechanisms and somatic symptoms. We selected a single cancer department, including only one hospital from one area in the country, which may limit the generalizability of the findings considering the relatively small sample size. The patient population from this hospital may not be representative of the broader population of patients with cancer in other regions or countries considering factors such as regional healthcare practices, socioeconomic status, higher in Bucharest region, and cultural differences [2,45,46]. Also, the gender imbalance significantly limits the generalizability of the findings, especially for male cancer patients. Studies incorporating larger and more diverse cancer samples, as well as longitudinal studies, are recommended to further understand the relationship between previously described variables. In this study, a formal power analysis was not conducted prior to data collection. Given the complexity of the moderation analyses, particularly in examining the role of 'fighting spirit,' the current sample size of 88 participants may not provide sufficient power to detect small to medium interaction effects reliably. This limitation should be considered when interpreting the findings, as true moderating effects may not have been detected due to insufficient statistical power. Future research should aim to recruit larger samples and conduct a priori power analyses to ensure adequate power for detecting moderation effects. Many patients have more than one concomitant disease, especially advanced patients with cancer and those who have received or are undergoing conventional anticancer treatments. However, we collected information on all somatic symptoms but did not distinguish between symptoms derived from cancer, those derived from treatments and those that resulted from other illnesses. We would like to consider this issue in our future studies on somatic symptoms in cancer populations.

Future directions

Research on patients with cancer focusing on somatic symptoms is still scarce. Most of the studies remain in the stage of presenting descriptive outcomes. More research on the mechanism of somatic symptoms in cancer, whether it has a profound impact on quality of life or survivorship, how to identify it properly and in a timely manner, and which kind of psychosocial interventions would be more effective are necessary in the future. This is the first study to explore how mental adjustment to cancer may predict and moderate somatic symptoms and its burden in Romania and beyond. Future research should consider longitudinal designs to establish causal relationships between the described variables. Studies involving diverse cultural groups are also needed to explore the generalizability of these findings, considering the cultural aspect of somatic symptoms an important point to consider. Furthermore, investigating the effectiveness of psychological interventions in reducing maladaptive adjustment mechanisms would be a valuable contribution to the field of Psycho-Oncology**.**

CONCLUSION

In conclusion, this study highlights the significant relationship between mental adjustment mechanisms as predictors of somatic symptoms in patients with cancer. Considering the intricate nature of somatic symptoms and targeting through Psycho-oncologic interventions, helplessness, anxious avoidance, and fatalism while also exploring fighting spirit may help mitigate somatic symptoms and improve overall well-being in this population, leading to high-quality care for patients.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, M.L., C.G.I. and L.S.; methodology, L.S.; software, D.M.P.; validation, M.L.., O.V., and A.C.; formal analysis, D.M.P.; investigation, L.S.; resources, M.L.; data curation, C.G.I.; writing—original draft preparation, C.G.I.; writing—review and editing, M.L. and A.C.; visualization, O.V.; supervision, M.L.; project administration, L.S.; funding acquisition, M.L. All authors have read and agreed to the published version of the manuscript.

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Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Elias Emergency and University Hospital, Bucharest, Romania (nr. 9286/2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

The Neurocardiogenic Impact of Ischemic Stroke: Intricacies of Cardiac Enzymes and the Vegetative System

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Abstract: There is a potential lateralization of vegetative influences at the cerebral level. We sought to understand the impact of these influences in the setting of ischemic stroke. We conducted a prospective study on 110 who presented with acute ischemic stroke (symptom onset maximum 24 hours before admission). We investigated correlations between stroke subtype, insular location or lateralization, stroke severity, hospital mortality, and the dynamic of enzymes (Tn, TnT, CK, CK-MB). We demonstrated that a higher cardiac enzyme value is associated either with stroke severity or with a higher risk of death in the short term, this growth being a marker for a more severe prognosis of a large stroke rather than an independent cause of mortality. Cardiac monitoring in the acute phase of ischemic stroke can prevent cardiac morbidity and mortality, which is why it is important to identify patients at high risk of heart complications after stroke.

Keywords: stroke; vegetative system; cardiac enzymes

INTRODUCTION

The brain structures involved in cardiac autonomic activity regarding the modulation of the force of contraction, heart rate, and stress response are the anterior cingulate cortex, the parabrachial nucleus, the hypothalamus (the paraventricular nucleus, the dorsomedial nucleus, the lateral hypothalamic area), insula (especially its anterior portion), periaqueductal gray matter, and some parts of the medulla (ventrolateral rostral medulla, nucleus ambiguus, nucleus of the solitary tract) [1].

Right insular lesions lead to increased parasympathetic activity to the detriment of sympathetic activity, and the opposite occurs in left insular lesions [2]. Thus, it can be stated that there is a lateralization of vegetative influences at the cerebral level. This lateralization hypothesis applies to the intrinsic control of the cardiac vegetative system [3].

In a study of approximately 865,000 patients, it was observed that the incidence of acute myocardial infarction (MI) in patients with acute stroke reaches 1.6% (approximately 80% being non-STEMI and 20% STEMI). Advanced age is the strongest predictor of MI in stroke patients, along with ischemic heart disease, chronic kidney disease (CKD), and atrial fibrillation (AF) [4,5]. In the trial RANTTAS [6], it has been observed that in 6% of acute ischemic strokes, cardiac complications may occur, such as angina pectoris, acute MI, and cardiac ischemia. Cardiac monitoring is crucial after a stroke. Prolonged cardiac monitoring is a weak link in stroke care in Romania overall – evaluation past the first 24-48 hours is a rarity [7]. Acute MI after stroke may have the following etiopathogenic mechanisms: concomitant coronary artery occlusion or exacerbation of a pre-existing coronary artery disease secondary to excessive and abnormal catecholamine discharge in the acute phase of stroke [4,8,9]. Sympathetic hyperactivity in the acute phase of stroke leads to excessive stimulation of beta receptors, with the opening of Ca^{2+} channels with the intracellular flow of Ca^{2+}, contractile myocardial dysfunction, and ATP depletion, leading to mitochondrial dysfunction, and finally, cell death, translated into the paraclinical image of ECG changes and increased troponin (Tn) values [10].

In patients with acute ischemic stroke, myofibrillar degeneration, myocytolysis or the appearance of contraction band necrosis may occur, the death of myocardial

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cells occurring in a state of exaggerated contraction, followed by the rapid appearance of calcifications and infiltration of mononuclear cells. In contrast, in acute MI, the important occurrence of necrosis contraction bands is not identified, and the process of necrosis of myocardial cells takes hours - days, occurs in a state of relaxation, and calcifications are delayed. Thus, neurogenic myocytolysis involves the subendocardium with a high arrhythmogenic potential, different from the coagulative necrosis encountered in acute MI [11]. Scheiz et al. [12] proposed a method of classifying Tn growth in patients with acute stroke into two categories: patients with an acute Tn increase and patients with a chronic Tn increase. In those with an acute troponin (Tn) increase, after excluding acute MI, it was observed that, most likely, this increase is the consequence of neurogenic lesions.

MATERIALS AND METHODS

Patients selection

We conducted a prospective study on 110 patients hospitalized in the Neurovascular Emergency Unit who presented with acute ischemic stroke (symptom onset taking place a maximum of 24 hours before admission). We excluded patients with:

- Ischemic stroke with onset >24 hours before admission
- Hemorrhagic stroke
- The presence of simultaneous acute pulmonary edema
- Clinical and laboratory signs of simultaneous acute myocardial infarction
- History of MI in the last year
- Recent cardiac surgery (<1 year)
- History of severe CKD (terminal stage)
- History of lung disease with severe acute respiratory failure
- History of clinically relevant arrhythmias (especially atrial fibrillation)
- Advanced cardiac insufficiency (Stage III/IV NYHA)
- History of active malignancy

Objective

The study's objective was to highlight the correlation between enzymatic changes (their dynamic during the first five days after stroke onset) and insular involvement/insular lateralization, stroke etiology, severity of acute ischemic stroke (using the NIHSS score at admission and at seven days), and in-hospital mortality.

Data collection

Before inclusion in the study, patients were informed about the methodology and the clinical, paraclinical, imaging, and other explorations performed. Each patient (or caregiver if the patient has severe language disorder, impaired understanding, cognitive impairment, or impaired alertness) has signed informed consent, respecting the conditions of medical ethics. The approval of the Scientific Research Ethics Board of the University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania, was obtained for this study.

Etiological diagnosis of stroke

The etiological diagnosis of stroke was performed using the TOAST classification [13] after paraclinical tests (brain CT / MRI, Doppler ultrasound, standard ECG, 24-hour Holter monitoring, 5-day continuous ECG monitoring, and transthoracic echocardiogram).

Stroke severity

The clinical severity of ischemic stroke was calculated using the NIHSS (National Institutes of Health Stroke Scale) by a neurologist certified to use the NIHSS scale. According to the NIHSS scale, stroke patients were divided into 3 categories:

- mild stroke (NIHSS ≤8 points)
- moderate stroke (NIHSS = 9-15 points)
- severe stroke (NIHSS ≥16 points).

Statistical methods and statistical calculation tools

For both Tn and creatine-kinase MB (CK-MB) values, the Kolmogorov-Smirnov normality test (which determines to what extent the data have a normal/Gaussian distribution) was applied, which invalidated normality, which is why the Student t-test could not be applied. We resorted to a non-parametric test, Mann-Whitney (MW), which demonstrated the absence of a significant difference between troponin/CK-MB values on any 2 days (of the 5 days studied). The Mann-Whitney test was used to analyze the correlation

between stroke subtype, insular location/insular lateralization, stroke severity, hospital mortality, and enzymatic elevations (Tn, TnT, CK, CK-MB). The chi-squared test was applied to investigate the possible correlation between increased values of myocardial lesion indicators (choosing those with a higher cardiac specificity: TnT and CK-MB) and acute ischemic stroke.

RESULTS

Etiology, risk factors and demographic factors of patients

The mean age of the patients was 67.2+/-12.93 years, and the male-to-female ratio was 64.55%:35.55%. The prevalence of vascular risk factors and associated pathologies was the following – high blood pressure (HBP) 73.64%, obesity 31.82%, diabetes 23.64%, dyslipidemia 46.36%, active smoking 50%. Chronic alcohol usage was recorded in 27.27% of patients, stroke history in 21.82%, transient ischemic attack (TIA) in 1.82%, heart failure (HF) NYHA class 1 or 2)= in 5.45%, past MI in 8.18%, dementia in 10%, peripheral arteriopathy in 3.64%, chronic obstructive pulmonary disease in 1.82%, CKD (stage 1 or 2) in 0.91% of patients. Death during admission occurred in 14 of 110 patients (12.72%).

The etiological classification of stroke was performed based on the TOAST system criteria (Trial of Org 10172 in Acute Stroke Treatment) [13] in cardioembolic (29.09%), atherosclerosis of large vessels (50.90%) [including a small proportion atheroembolic (6.36%)], lacunar (3.64%), other causes (internal carotid artery dissection 1.81%) and cryptogenic (14.54%).

Insular localization of ischemic stroke and insular lateralization

Regarding the topographic location of stroke, out of the total of 110 patients included in the study, 63 patients (42.73%) had a stroke located in the insula. Of the 63 patients with insular localization, 23 patients (36.5%) had right insular localization and 40 patients (63.5%) had left insular localization.

The growth dynamic of cardiac enzymes was analyzed. There was no sustained increase in cardiac enzymes during the 5 days of follow-up. In all calculations concerning cardiac enzymes, the 3 patients who were found to have had acute MI after stroke onset were excluded.

Patients were divided into 2 categories according to Tn T levels: patients with normal values (≤0.02ng/ml) and patients with values above the upper limit (>0.02ng/ml). A similar division of patients was performed according to the CK-MB value: patients with normal values (≤25IU/L) and patients with superior values (>25IU/L). Following this analysis, it was observed that out of the total 110 patients, 60.86% had at least one elevated TnT value (in at least one of the 5 days of follow-up) (Figure 1), and 36.52% had at least one increased CK-MB value (in at least one of the 5 days of follow-up) (Figure 2).

For each patient, we took into consideration the highest value obtained in the 5 days of follow-up. The relatively high percentage of patients with elevated TnT is within the limits demonstrated in the literature, in a review [14] on increased Tn in patients with acute stroke (ischemic and hemorrhagic), this percentage difference is pending on various factors, such as the chosen day of reference, the number of patients and the arbitrary baseline. When applying the chi-squared test to analyze the correlation of TnT value above the normal limit of every 5 days with insular localization, on day 4 and 5, the TnT value correlation with insular involvement was statistically significant (Table 1). No statistically significant correlation with insular lateralization was observed. The increase in Tn (less specific for cardiac distress than TnT) did not correlate with insular involvement nor with left-right insular lateralization.

Figure 1: The distribution of TnT values in the studied group

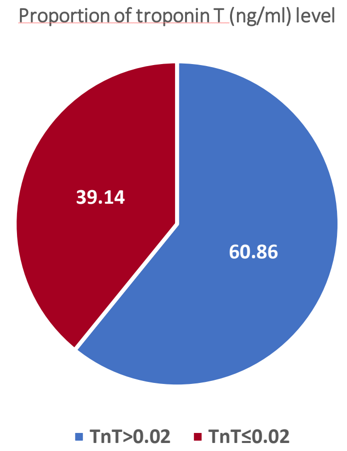

Figure 2: The distribution of CK-MB values in the studied group

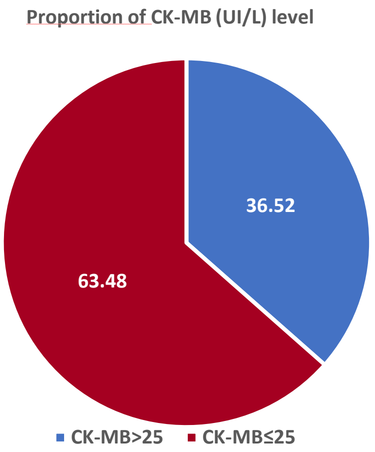

Table 1: TnT value and stroke location

Parameter	Insular	Non-insular	P-value
TnT day 4, [median (Q1-Q3)]	0.029(0.01-0.029)	0.01(0.01-0.029)	0,007 (Mann-Whitney)
TnT day 5 [median (Q1-Q3)]	0.029(0.01-0.029)	0.01(0.01-0.029)	0,02 (Mann-Whitney)

On average, CK-MB was significantly higher in patients with insular localization on each follow-up day (day 1 to day 5) (Table 2), but this was not statistically significant depending on insular lateralization. There was also no statistically significant correlation between insular location or insular lateralization and the CK value.

Table 2: CK-MB value related to stroke location

Parameters	Insular	Non-insular	P-value
CK-MB day 1, [median (Q1-Q3)]	17 (14-23)	14 (12-16)	0.006 (Mann-Whitney)
CK-MB day 2, [median (Q1-Q3)]	17 (13,21.5)	13 (11-15.25)	0,0004 (Mann-Whitney)
CK-MB day 3, [median (Q1-Q3)]	16.25 (13.25,20.75)	14 (11.25-17.75)	0,03 (Mann-Whitney)
CK-MB day 4, [median (Q1-Q3)]	19 (13.75-26.25)	13 (12-16)	0.0002 (Mann-Whitney)
CK-MB day 5, [median (Q1-Q3)]	16 (13-18.75)	13 (10.25-15)	0,003 (Mann-Whitney)

Stroke subtypes and cardiac enzymes

Regarding stroke etiology, a statistical correlation between stroke subtype and an increase in CK-MB or TnT during any of the 5 days of follow-up has not been demonstrated (neither by the Mann-Whitney test nor by the Chi-Square test).

Stroke severity and cardiac enzymes

When analyzing the correlation between stroke severity and cardiac enzyme elevations, the mean CK-MB on days 2, 3, 4, and 5 was statistically significantly higher in patients with severe stroke (NIHSS score ≥16) than in patients with moderate and mild stroke (NIHSS<16). CK (day 3 and 4) and Tn (day 4) were, on average, statistically significantly higher in patients with severe stroke than in patients with moderate to mild stroke (Table 3). TnT, much more sensitive for myocardial injury, did not record statistically significantly higher values in patients with severe stroke.

Table 3: Correlation between cardiac enzymes and stroke severity

Parameters	NIHSS<16 (moderate stroke)	NIHSS≥16 (severe stroke)	P-value
Tn day 4, [median (Q1-Q3)]	0.2 (0.2-0.2)	0.2 (0.2-0.26)	0.003 (Mann-Whitney)
CK day 3, [median (Q1-Q3)]	91 (51.9-139)	132 (82-254)	0.001 (Mann-Whitney)
CK day 4 (median (Q1-Q3))	100 (59-147)	115.5 (71.75-296)	0.004 (Mann-Whitney)
CK-MB day 2, [median (Q1-Q3)]	13 (12-16)	17 (13-20.75)	0.002 (Mann-Whitney)
CK-MB day 3, [median (Q1-Q3)]	14 (12-18)	17 (13-21)	0.002 (Mann-Whitney)
CK-MB day 4, [median (Q1-Q3)]	15 (12-19.5)	18.5 (13.75-26.25)	0.002 (Mann-Whitney)
CK-MB day 5, [median (Q1-Q3)]	13 (11-16)	15.5 (13-18.25)	0.002 (Mann-Whitney)

Cardiac enzymes and mortality

The correlation between enzyme increases and in-hospital mortality was analyzed. For Tn, a statistically significant correlation was observed between its increase on days 3, 4, 5 and hospital mortality. TnT on day 4 was, on average, significantly higher in patients who died during hospitalization than in those who survived. The increase in CK and CK-MB on days 4 and 5 correlated significantly with death during hospitalization (Table 4).

Table 4: Correlation between enzyme values and in-hospital death

Parameters	Death	No death	P-value
Tn day 3 [median (Q1-Q3)]	0.378 (0.2-1.129)	0.2 (0.2-0.2)	0,01 (MW)
Tn day 4 [median (Q1-Q3)]	0.506 (0.31-1.33)	0.2 (0.2-0.2)	0.002 (MW)
Tn day 5 [median (Q1-Q3)]	0.426 (0.26-0.81)	0.2 (0.2-0.2)	0,009 (MW)
TnT day 4 [median (Q1-Q3)]	0.049 (0.02-0.2)	0.01 (0.01-0.03)	0,008 (MW)
CK day 4, median (Q1, Q3)	326 (180.75-1621)	101 (61-177)	0,02 (MW)
CK day 5 [median (Q1-Q3)]	15 (188-4468)	288 (55.5-160)	0,01 (MW)
CK-MB day 4 [median (Q1-Q3)]	38 (21.5-55.75)	16 (12-20)	0.006 (MW)
CK-MB day 5 [median] (Q1-Q3)]	29 (19-63)	14 (12-17)	0,01 (MW)

MW=Mann-Whitney

DISCUSSION

With regard to the growth dynamics of cardiac enzymes, a time-based increase was not observed as in other studies [15]. No statistical correlation has been demonstrated (either by the Mann-Whitney test or by the chi-square test) between the stroke subtype and CK-MB or TnT elevation on any of the 5 follow-up days.

The increase in myocardial injury indicators has been interpreted differently in existing studies, with some authors considering it a marker of stroke severity and not an independent factor of poor prognosis [16] and others see it as an independent predictor of disability or mortality [17,18]. Apart from the current study, the literature has revealed only one study [19] that previously targeted the timed growth (days 1, 2, 3, 4, 5) of cardiac enzymes in stroke patients; other studies chose a variety of values of cardiac enzymes.

The possible pathophysiological mechanisms of increasing TnT and CK-MB in patients with acute stroke are multiple, but none of the studies existing so far in the literature could establish the exact cause. There is evidence of neurocardiogenic influences on myocytolysis in the acute phase of stroke, but there is great controversy regarding the cardiogenic increase in CK-MB. This increase may be possible in the case of a stroke location with a high potential for heart damage. Supporting the theory of a cardiac CK-MB increase secondary to neurogenic influences, in our study, 36.52% of patients had values above the normal limit of CK-MB, and the value of CK-MB (and TnT) correlated with insular involvement.

The current study offers different results than other literature references [20] - which concluded that TnT, unlike CK-MB, does not increase in patients with acute ischemic stroke. Also, the current study demonstrates contradicting conclusions to Barber et al. [21], which showed that in patients with acute ischemic stroke and elevated TnI, this increase did not correlate with insular location, one explanation being that in the cited study, only one TnI value was measured upon admission, whereas in the current study, statistically significant results were obtained on day 4 and 5 from stroke onset. Other examples from the literature [22] demonstrated a correlation between increased TnI and right hemisphere ischemia, but no insular involvement was specified, and no differences in insular lateralization were observed. One explanation could be that this increase is not related to other brain locations except insular ones, although further studies on other brain locations will need to be conducted to prove this claim. An argument against the theory mentioned above would be that the results obtained are controversial when it comes to the correlation of TnT alone with cardiac function, which makes it possible that CK-MB increase is not of cardiac origin and demonstrates that TnT is a much more reliable parameter to highlight the cardiac impact of chronic ischemia.

The statistical significance of elevated TnT and CK-MB values at days 4 and 5 with insular location may actually be due to a large ischemic stroke, and the increased TnT may reflect the systemic severity of stroke that may worsen the neurological status. A possible pathophysiological mechanism would be that in the case of large ischemic strokes, the growth curve of cerebral edema is maximum on days 4-5-6 from the onset of cerebral ischemia. Cerebral edema during these days may cause an exacerbation of sympathetic activity; this explanation is supported in a previous study [15], which revealed that CK-MB values are higher on day 5 of stroke onset. Obviously, this hypothesis will require further research in the future, by measuring catecholamines and enzymes simultaneously. Increased CK-MB value may originate elsewhere (muscle damage due to venous punctures, fluid restriction, and negative caloric

intake) [20]. In support of this argument comes the association of growth and CK.

In fact, it is well known that TnT is much more sensitive for assessing cardiac ischemia, either acute or chronic. The authors are of a similar opinion to the study conducted by Jensen et al. [18] that the increase in TnT is rather the consequence of HF. Another plausible explanation for the increase in TnT could be the existence of unstable atheroma plaques in the coronary arteries that could cause recurrent myocardial ischemia. It is probably necessary to dose other types of Tn (hsTnI) to correctly evaluate cardiac myocytolysis in patients with acute ischemic stroke and to redefine the concept of neurogenic cardiac ischemia.

Although this study cannot accurately state the pathophysiology of the increase in cardiac enzymes, we demonstrated that a higher value is associated either with stroke severity or with a higher risk of death in the short term, this growth being a marker for a more severe prognosis of a large stroke rather than an independent cause of mortality.

Looking at cardiac enzymes, the value of TnT and CK-MB was related to stroke severity, which indicates that in well-selected cases cardiac enzyme dosing may be useful for stratifying the risk of cardiac complications. It cannot be stated that the dosing of TnT and CK-MB should be performed routinely in all patients with acute stroke, being an expensive method and not providing additional net benefit**.**

CONCLUSION

Cardiac complications are common in patients with acute ischemic stroke, some of which may be potentiated by autonomic hyperactivity. Careful monitoring of patients at high risk of heart complications may change the short-term prognosis.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization and design, MMM, DD, ST; data analysis, MMM, DD; preparing the manuscript, MMM, IIE, ICS, MIG, OV, ST, DD, CV, CAS; writing, review and editing, MMM, DD.; visualization, MMM, DD; supervision, MMM. All authors have read and agreed to the published version of the manuscript.

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Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of "CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY (protocol code PO-35-F-03 no. 83 and date of approval: 29.02.2016).

Patient consent for publication

Written informed consent has been obtained from the patient(s) to publish this paper.

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REVIEW

Radiotherapy and Reconstructive Surgery: Challenges, Implications and Optimized Therapeutic Approaches

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Abstract: Radiotherapy plays an indispensable role in cancer treatment, with its ability to target malignant cells and improve patient survival. However, its impact on healthy tissues poses significant challenges for reconstructive surgery. The altered tissue environment resulting from radiation, including fibrosis, vascular damage, and compromised wound healing, complicates reconstructive efforts and increases the risk of surgical complications. As cancer therapies advance, the approaches for managing radiation-induced complications must also adapt accordingly.

This article explores the complex interaction between radiotherapy and reconstructive surgery, particularly in the treatment of head and neck cancers, breast reconstruction following mastectomy, and sarcoma management. Special attention is given to the customization of surgical approaches based on cancer type, with the goal of optimizing both functional and aesthetic outcomes. A multidisciplinary approach, integrating advanced imaging techniques, modern radiation delivery methods, and innovative surgical techniques is essential for improving patient outcomes.

Keywords: radiotherapy, reconstructive surgery, head and neck cancer, breast reconstruction, sarcoma

INTRODUCTION

Radiotherapy is an essential part of therapeutic management, utilized in approximately 50-60% of all cancer patients during their treatment. It plays an important role in controlling tumor growth, reducing recurrence rates, and improving overall survival. However, despite its therapeutic efficacy, radiotherapy can have serious adverse effects on surrounding healthy tissues, leading to complications such as fibrosis, vascular damage, and impaired wound healing. These radiation-induced changes significantly complicate subsequent reconstructive surgery, particularly in areas where structural architecture and functional restoration are critical. [1-3]

The association between radiotherapy and reconstructive surgery presents unique challenges, especially in cases of head and neck cancers, breast cancer treatment, and sarcomas. This article aims to explore the impact of radiotherapy on reconstructive surgery, focusing on these three specific cancer types, the final goal being to optimize both functional and aesthetic outcomes for cancer survivors.

OVERVIEW OF PARTICULAR SITUATIONS REGARDING RADIOTHERAPY AND RECONSTRUCTIVE SURGERY

Head and neck cancer treatment

Head and neck cancers are frequently treated with radiotherapy, either as a primary modality or adjuvant therapy following surgery. While radiotherapy effectively targets malignant cells, it also damages surrounding healthy tissues, leading to fibrosis, vascular compromise, and wound healing complications. [4] Novel strategies for improving radiotherapy protocols are currently being explored. In the case of radioresistant tumors, for head and neck oncological cases, proton and carbon ion therapies are emerging as superior options. Proton therapy facilitates the delivery of high-dose radiation accurately, concurrently preserving adjacent healthy tissue, thereby rendering it appropriate for the eradication of microscopic disease.

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Carbon ion therapy, with its higher linear energy transfer, is more effective for larger tumors with significant burdens, offering enhanced biological effectiveness in tumor cell damage. [5]

For locoregional recurrences or second primary malignancies, reirradiation (RERT) with advanced techniques like intensity-modulated radiation therapy (IMRT) and stereotactic body radiotherapy (SBRT) is a viable option. These methods improve survival rates and reduce toxicity by precisely targeting the tumor while sparing normal tissues. [6]

Intraoperative Radiation Therapy (IORT) has been described to be efficient when utilized alongside surgical procedures. IORT provides a targeted radiation boost during surgery, potentially reducing the need for high doses of external beam radiation and minimizing exposure to normal tissues, particularly beneficial in recurrent cases where additional radiation is required. [7]

Radiotherapy in the head and neck region presents several unique challenges. One of the primary concerns is fibrosis and soft tissue contracture; radiation induces progressive fibrosis, which can result in trismus, dysphagia, and restricted neck mobility. Soft tissue contracture can further complicate flap elevation and wound closure. Compromised vascularity is another challenge, as radiotherapy damages the microvasculature, leading to poor perfusion and an increased risk of flap necrosis. Additionally, the complex anatomy of the head and neck region, which includes multiple vital structures such as the facial nerve and carotid artery, makes surgical navigation particularly challenging. [7-11]

To address these challenges, several surgical strategies are currently employed. Reconstructive surgery after head and neck cancer aims to restore function, appearance, and quality of life. Free tissue transfer, utilizing free flaps such as the anterolateral thigh (ALT) flap or radial forearm flap, remains a mainstay due to their robust vascularity and versatility. Preoperative imaging modalities like CT angiography or MR angiography are crucial for assessing recipient vessel patency and planning the anastomosis. In cases where free flap transfer is not feasible, pedicled flaps, such as the pectoralis major myocutaneous flap, provide reliable coverage. Additionally, functional reconstruction, including dynamic reconstruction of facial nerve function using gracilis muscle transfer and nerve grafts, is performed to restore facial symmetry and expression. [11-14]

Emerging techniques in this field show promise for improving outcomes. For mandibular reconstruction, the fibula flap remains the gold standard, followed by scapular flaps in selected cases; however, recent advancements in 3D printing technology have enabled the creation of custom-made implants, which can enhance functional outcomes. Despite their effectiveness, free flaps carry a higher risk of complications, such as pedicle thrombosis, especially when using irradiated vessels. [13,15-17]

Breast Reconstruction after breast cancer

Breast cancer is the most common malignancy among women and the leading cause of cancer-related mortality in females. [18,19] Surgical options for breast cancer treatment generally include breast-conserving therapy associated with radiotherapy or mastectomy, both of which offer comparable survival rates for patients with early-stage disease. [18,20] With the advent of magnetic resonance imaging (MRI), BRCA-1 and BRCA-2 genetic screening, and advancements in surgical techniques, mastectomy followed by reconstruction has become an increasingly preferred treatment option. [18] For patients undergoing mastectomy with reconstruction, a decision must be made between autologous tissue reconstruction and implant-based reconstruction. Both approaches can be performed either immediately following the mastectomy or at a later stage. Radiotherapy is recommended for high-risk cases, significantly increasing the complication rate associated with any type of reconstruction. [18,21-23]

Breast reconstruction after radiation therapy is a challenging process that aims to restore the breast's appearance while managing a series of adverse effects introduced by radiation. [24]

Deciding on the type of breast reconstruction involves a thorough discussion between the patient and her reconstructive surgeon, focusing on the expected outcomes and potential complications. Autologous reconstruction, which uses the patient’s own well-vascularized soft tissue, closely resembles natural breast tissue, can be easily shaped, and generally yields more natural-looking results. Moreover, autologous reconstruction is known to be more resilient to the effects of post-mastectomy radiation therapy (PMRT). [25,26]

A significant challenge in breast reconstruction, particularly for patients who have received or will receive radiation therapy, is determining the optimal timing for reconstruction. Radiation therapy, while crucial for reducing the risk of cancer recurrence, can complicate reconstruction efforts. Post-mastectomy radiotherapy is a common treatment that can reduce local recurrence rates and improve survival, especially in patients with lymph node-positive breast cancer. However, PMRT can negatively impact the outcome of flap reconstructions, leading to complications such as flap loss, wound contracture, and fat necrosis. [27-30]

Breast reconstruction using autologous tissue is favored because it results in a more natural-looking breast with a soft texture, consistent temperature, and a certain degree of ptosis that closely mimics the natural breast. Furthermore, autologous tissue is more resilient to the effects of radiation therapy, making it a suitable option for patients who have undergone or will undergo radiation. Autologous reconstruction is considered the gold standard by many plastic surgeons due to its superior aesthetic outcomes and the fact that it allows the reconstructed breast to age naturally alongside the other breast. It is also more customizable, enabling surgeons to tailor the reconstruction to the individual patient's needs. [31-34]

Multiple flap options are available for breast reconstruction, each tailored to meet specific patient anatomical considerations and reconstructive goals. The latissimus dorsi myocutaneous (LD) flap remains a versatile and reliable choice, particularly in cases where moderate tissue volume is required. Abdominally-based flaps, such as the transverse rectus abdominis myocutaneous (TRAM) flap,

deep inferior epigastric perforator (DIEP) flap, and superficial inferior epigastric artery (SIEA) flap, are considered the gold standard for autologous breast reconstruction due to their abundant soft tissue, robust vascularity, and the potential to achieve highly natural aesthetic outcomes. Thigh-based flaps, including the transverse upper gracilis (TUG), transverse myocutaneous gracilis (TMG), profunda artery perforator (PAP), and lateral thigh perforator (LTP) flaps, provide valuable alternatives for patients who are unsuitable candidates for abdominal donor sites, offering well-vascularized tissue with satisfactory volume and contour. Gluteal flaps, such as the superior gluteal artery perforator (SGAP) and inferior gluteal artery perforator (IGAP) flaps, are particularly beneficial in cases where other donor sites are not viable or in patients requiring a larger volume of tissue. The thoracodorsal artery perforator (TDAP) flap and lumbar artery perforator (LAP) flap represent additional options for autologous breast reconstruction, particularly in cases where more conventional donor sites are unsuitable. [35-46]

Two main strategies exist for combining flap reconstruction with radiotherapy: immediate reconstruction, where the reconstruction is performed immediately after the tumor is removed and before radiation therapy, and delayed reconstruction, where radiation is completed first, and reconstruction is performed 6-12 months later. Immediate reconstruction has the advantage of preserving the breast envelope, making the surgery easier and potentially resulting in better aesthetic outcomes. However, it also comes with significant risks. Radiation therapy can cause fibrosis, shrinkage, and fat necrosis in the reconstructed breast, leading to higher rates of complications compared to unirradiated reconstructions. Additionally, complications from immediate reconstruction can delay the start of radiotherapy, which could negatively affect the patient's cancer prognosis. [26,46,47]

Delayed reconstruction, on the other hand, allows the skin and tissues to recover from the effects of radiation before undergoing reconstruction. This approach may reduce the risk of complications but often results in less favorable cosmetic outcomes. The skin and tissues may become fibrotic and scarred, limiting the options for reconstruction and making it harder to achieve a natural breast shape. [48,49]

To minimize radiation injury to the flap while preserving the breast mound, a two-stage approach called delayed immediate autologous reconstruction (DIAR) can be considered. This technique, described by Kronowitz et al., allows for the preservation of the native breast skin and supports the patient’s psychological well-being. DIAR offers the advantages of skin-sparing mastectomy while reducing radiation-induced complications. However, the use of a tissue expander in this method is not without risks. It is debated whether deflating the tissue expander before radiotherapy is necessary. Historically, deflation was thought to give the radiotherapist better control, similar to treating a flat chest. However, more recent studies suggest that deflation is not essential, and keeping the expander inflated may actually reduce radiation-induced complications such as seroma formation and capsular contracture. [50-56]

While autologous reconstruction is often preferred for its aesthetic appeal and lower overall complication rates, prosthetic reconstruction is a practical alternative for some patients. Despite the well-documented increased risks of complications, infections, capsular contracture, the need for revision surgery, and overall reconstruction failure associated with prosthetic reconstruction in the setting of PMRT, it remains an option for a select group of patients. [57,58]

Prosthetic reconstruction, like autologous reconstruction, can be performed in either a single stage or two stages. Single-stage reconstruction, commonly known as direct-to-implant (DTI) reconstruction, involves placing the final implant during the initial surgery. The two-stage approach involves first placing a tissue expander, which is later replaced with a permanent implant. A key debate in prosthetic reconstruction is whether radiotherapy should be administered to the implant or the tissue expander. Recent systematic reviews indicate a significantly higher failure rate when PMRT is delivered to a tissue expander compared to an implant. Although the complication rates are generally lower when using implants rather than tissue expanders, these reviews often overlook the long-term results. Capsular contracture, which can negatively impact patient quality of life and satisfaction with the cosmetic outcome, often does not become apparent until years after the reconstruction. Patients who have previously undergone radiation therapy have the highest rates of reconstruction failure across all groups. Due to the significant morbidity associated with tissue expansion in irradiated skin, pure prosthetic delayed reconstruction is relatively contraindicated. [54,59-63]

Autologous fat grafting, or fat transfer, is frequently used in breast reconstruction, either as a complementary technique or a primary modality. This procedure implies the harvesting of adipose tissue from donor sites such as the abdomen, thighs, or flanks through liposuction, followed by purification and injection into the breast to augment volume, contour, and symmetry. Fat grafting improves the aesthetic results of both flap-based and implant-based reconstructions, addressing contour deformities and enhancing the overall cosmetic outcome. Moreover, fat grafting has demonstrated the capacity to improve the quality of irradiated skin by promoting neovascularization and decreasing radiation-induced fibrosis, providing not only simple volume restoration but also further therapeutic advantages. The grafted fat contains adipocyte-derived stem cells, whose regenerative capacity has been well-documented in various clinical contexts, contributing to improved tissue repair and functional recovery. Although long-term outcomes of fat grafting are still under investigation, current evidence supports its role as a versatile, minimally invasive technique that can significantly improve both the functional and aesthetic results of breast reconstruction. [64-69]

Reverse expansion is a technique used in breast reconstruction after nipple-sparing mastectomy, where a tissue expander is initially inflated to maintain the skin envelope of the breast and to provide adequate coverage for reconstruction. The expander is then gradually deflated, allowing the tissue to contract naturally, resulting in a more contoured and natural breast shape for the final reconstruction. In selected, reverse expansion can be combined with lipofilling to achieve very good outcomes in breast reconstruction, a technique described also as “intratissular expansion with serial deflation–lipofilling sessions”. While reverse expansion helps prepare the tissue and create the necessary space for reconstruction, lipofilling in multiple sessions is used to provide

volume, enhance the aesthetic result, smooth out any irregularities, and improve the quality of irradiated or compromised skin. [70-72]

Radiotherapy in the treatment of breast cancer also increases the risk of secondary lymphedema. A key area of focus is the prevention of lymphedema and the development of advanced therapeutic strategies. These approaches encompass not only conservative management but also cutting-edge super-microsurgical techniques, including the use of robotic microsurgery, to enhance treatment outcomes. [73-76]

These advanced reconstructive options enable the customization of surgical approaches to optimize both functional and aesthetic outcomes in breast reconstruction following mastectomy.

Sarcoma treatment

Sarcomas are highly aggressive tumors that pose a significant risk to patient survival, necessitating prompt and effective treatment. Comprehensive management strategies, including a combination of surgery, radiation therapy and chemotherapy in specific cases, are essential to optimize outcomes and mitigate the high potential for local recurrence and metastasis. Early diagnosis and a multidisciplinary approach are crucial to improving prognosis and reducing the substantial morbidity and mortality associated with these tumors. Radiation therapy plays a crucial role in the management of sarcomas, particularly in cases where surgical resection alone may not achieve optimal outcomes. Given the diverse histological subtypes and varying radiosensitivity of sarcomas, radiation therapy can be tailored to enhance local control, minimize recurrence, and improve overall survival rates. Preoperative radiation is often utilized to reduce tumor size, making surgical resection more feasible while preserving surrounding tissues and critical structures. Conversely, postoperative radiation may be employed to eradicate microscopic residual disease in cases where negative surgical margins are difficult to achieve. [77-79]

Concerns about negative microsurgical outcomes in patients receiving preoperative radiation therapy are justified. Irradiated wounds are described as challenging surgical environments, marked by dense fibrosis, tissue edema, and fragile recipient vessels that are prone to rupture. At the cellular level, in vitro studies have shown that radiation therapy induces cytokine upregulation in the endothelium and accumulation of inflammatory cells. [80,81]

Some studies suggested that a key strategy to successful free tissue transfer is selecting recipient vessels outside of the irradiated field. This is a more practical approach in head and neck reconstruction where there are a plethora of available recipient vessels. However, in limb reconstruction, the irradiated field often spans the entire limb circumference and getting out of the irradiated field may require the use of vein interposition grafts, which themselves can increase free flap failure rate. [82]

Surgical resection with wide margins, combined with perioperative radiation therapy, is the standard treatment for extremity soft tissue sarcomas. [83]

The timing of radiotherapy in the treatment of soft tissue sarcoma remains a topic of debate. Radiation protocols typically include either pre-operative or post-operative external beam radiotherapy, or brachytherapy. Pre-operative irradiation offers several benefits, including the delivery of a lower radiation dose to a smaller field, the potential for tumor downsizing before resection, and improved long-term functional outcomes. However, these advantages are offset by a higher incidence of wound healing complications compared to post-operative radiotherapy. [84]

Intraoperative radiotherapy (IORT) can be employed to enhance margin control by delivering a concentrated dose of radiation (typically 10-12 Gy) to the tumor bed during surgery. This approach is particularly useful when tumors are near vital neurovascular structures or when the resection margin is within several millimeters of the sarcoma. [85]

Regarding microsurgical outcomes, there are additional concerns about the effects of radiotherapy on the quality of recipient vessels, the recipient bed, and ultimately, the survival of the flap. When planning reconstruction for a soft tissue defect, key considerations include the size, location, and depth of the defect. Small-sized defects with a limited zone of injury can be effectively covered using local or perforator-based flaps. For medium-sized defects, axial pattern pedicled flaps are suitable, as they offer a greater reach while still utilizing local tissue.

Large defects, which cannot be adequately covered with local tissue, require distant or free flaps, as these are not constrained by local tissue availability. Locoregional flaps are often effective for reconstructing defects of moderate size. Among these, local fasciocutaneous flaps and pedicled muscle or myocutaneous flaps are viable options. The pedicled rectus abdominis flap, which can be oriented vertically (vertical rectus abdominis musculocutaneous, VRAM), obliquely, or transversely (TRAM), is versatile for reconstructing various defects.

Commonly used muscle flaps include the gracilis, rectus femoris, sartorius, and vastus lateralis. The anterolateral thigh (ALT) flap is the primary fasciocutaneous option, and keystone and freestyle propeller flaps can also be designed for a variety of thigh or truncal defects. For larger defects, the latissimus dorsi (LD) and rectus abdominis are commonly used free muscle flaps, both of which can be harvested with a skin paddle. Additionally, the subscapular and lateral femoral circumflex arterial systems (LFCA) allow for the harvesting of large chimeric flaps for composite tissue replacement when necessary. For reconstructing large defects, the ALT, TRAM, and latissimus dorsi free flaps are excellent choices. For smaller defects, options include the free gracilis, radial forearm, parascapular, lateral arm, and superficial circumflex iliac artery perforator (SCIP) flaps. [86-90]**.**

CONCLUSION

Radiotherapy, while essential for cancer treatment, introduces significant challenges to reconstructive surgery due to the damage it causes to healthy tissues. Advances in radiotherapy techniques, such as proton and carbon ion therapies, offer promising solutions for minimizing damage to surrounding tissues, thereby enhancing reconstructive outcomes. Surgical innovations, including the use of autologous flaps, 3D printing, and intraoperative radiotherapy, have significantly improved the ability to restore function and aesthetics in patients undergoing reconstruction after radiotherapy. However, the complexity of irradiated tissues requires a specific, multidisciplinary approach that takes into consideration the particular needs of each patient and cancer subtype. As reconstructive techniques continue to evolve, further research and collaboration between oncology, radiology, and surgery will be of utmost importance in the improvement of cancer survivors' outcomes.

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ORIGINAL ARTICLE

Multidrug-resistant ESKAPE Pathogens in a Romanian Secondary-Care Hospital during the COVID-19 Pandemic

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Abstract: The study aims to assess the frequency of ESKAPE pathogens and their antimicrobial resistance profile during the COVID-19 pandemic in a multidisciplinary hospital. Based on microbiological reports, the present study retrospectively evaluated the frequency and antimicrobial resistance (AMR) profile of ESKAPE microorganisms during 2020-2022 in a multidisciplinary hospital in Romania. There were reported 1117 bacterial isolates during the study and ESKAPE pathogens account for 33.5% of the strains. The specific AMR of ESKAPE pathogens cumulates 30.9%, while antimicrobial multidrug resistance characterizes 21% of all ESKAPE strains. The AMR profile of K. Pneumoniae, followed by S. aureus, and P. aeruginosa are alarming on ESKAPE surveillance. The most frequent bacterial isolates and antibiotic resistance reports were identified in the urology department. Based on the local AMR data we suggest the first-line antibiotic recommendations when ESKAPE pathogens are suspected. The directions of the near future antibiotic stewardship program focus on revising the clinical protocols of diagnosis and treatment in each department, increasing the bacteriological samples collection, improving the microbiological diagnostic techniques for AMR identification, and intensifying the epidemiological actions of surveillance, control, and education.

Keywords: ESKAPE; COVID-19; antimicrobial resistance; multidrug resistant organisms; antibiotic stewardship

INTRODUCTION

The World Health Organization (WHO) considers antimicrobial resistance (AMR) among the top 10 priorities for global public health. This phenomenon has developed and spread due to the selection pressure resulting from the excessive or incorrect use of antibiotics [1]. The number of deaths caused by antimicrobial resistance (AMR) could exceed 10 million annually by the year 2050 without active measures promoting the prudent use of antibiotics, improving hand hygiene in hospitals and environmental healthcare hygiene, as well as enhancing food biosecurity [2].

The emergence of multidrug-resistant (MDR) bacteria poses a concerning issue for public health due to the increased risk of healthcare-associated infections and limited treatment options. "ESKAPE" is an acronym for six bacteria of major interest in AMR surveillance systems, namely: (E) Enterococcus faecium, (S) Staphylococcus aureus, (K) Klebsiella pneumoniae, (A) Acinetobacter baumannii, (P) Pseudomonas aeruginosa, and (E) Enterobacter species [3, 4].

The "ESKAPE" pathogens are frequently implicated in severe infections, ranking high among the causes of morbidity and mortality worldwide. However, the WHO has developed a list of priorities within this group. Gram-negative bacilli are considered "critical": carbapenem-resistant CBR Acinetobacter baumannii (A. baumannii), CBR Pseudomonas aeruginosa (P. aeruginosa), third-generation cephalosporin-resistant

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(C3R) Klebsiella pneumoniae (K. pneumoniae), and C3R Enterobacter spp. In the second line of priority are Gram-positive cocci, namely vancomycin-resistant (VAN-R) Enterococcus faecium (E. faecium) and Methicillin-Resistant Staphylococcus aureus (MRSA) [5,6].

Antibiotics are frequently used to treat COVID-19-related bacterial infections. The excessive use of antibiotics during the COVID-19 pandemic can accelerate the emergence and spread of antibiotic-resistant bacteria [7].

In the first year of the COVID-19 pandemic, although many E.U. countries experienced a general decrease in antibiotic consumption both in the community and in healthcare facilities, certain antibiotics, such as azithromycin and ceftriaxone, saw an increase in usage. The reduction in prescriptions was induced by isolation, decreased mobility, and a decline in elective surgical interventions, but the impact of reduced antibiotic use on AMR, especially concerning the ESKAPE group, is insufficiently understood. In a survey conducted in 26 E.U. countries, monitoring antibiotic prescribing behaviors in healthcare facilities was halted in 9 countries, and antibiotic prescriber education programs were interrupted in 11 countries [8,9]. Understanding the link between COVID-19 and antibiotic use can contribute to the development of strategies to limit the impact of infections on vulnerable populations, thereby aiding the global effort to combat infectious diseases.

The study aims to assess the frequency of ESKAPE pathogens and their antimicrobial resistance profile during the COVID-19 pandemic (2020-2022) in a multidisciplinary Romanian hospital.

MATERIALS AND METHODS

A retrospective analysis of the isolated bacterial strains was conducted in the Microbiology Laboratory of the Emergency Military Hospital " Dr. Aristide Serfioti" Galati, derived from various biological samples obtained from hospitalized patients. The hospital is found in the Southeast region of Romania, on the eastern border of the European Union, and includes surgical departments (75 beds), medical departments (100 beds), and an intensive care unit (15 beds).

Escherichia coli and microorganisms in the ESKAPE group had their antibiotic susceptibility evaluated. Escherichia coli was included in the analysis due to its frequent association with horizontal gene transfer of resistance genes from other Enterobacterales, potentially reflecting the resistance profile of Klebsiella pneumoniae or Enterobacter spp, both of which are part of the ESKAPE pathogens [10].

The study was conducted from January 1, 2020, to December 31, 2022. This timeframe coincides with the hospital's focus on COVID-19 support, meaning that the hospital provided healthcare to COVID-19 patients, along with healthcare assistance to the general population, mostly in emergencies.

Microbiological Bacterial Identification Procedures

According to the current work procedures, bacterial isolation was performed by inoculating biological samples either "in sector" or "by exhaustion" on solid media, including Columbia blood agar, Chapman agar, and MacConkey agar (GRASO Biotech, Oxoid - Thermo Scientific, USA), followed by incubation for 24-48 hours at a temperature of 37°C. Biochemical identification of isolated bacterial strains was done through multi-testing and the automatic Vitek 2 Compact method (bioMérieux-l'Etoile).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using either the classic disk diffusion method or the minimum inhibitory concentration (MIC) method with the Vitek 2 Compact automated system, adhering to the current CLSI standards of the working period. The choice of the MIC testing method was contingent upon laboratory availability, the clinical severity of the patient, and the need for an urgent result, particularly in cases of multidrug-resistant bacteria or discrepancies found in the disk diffusion testing.

Depending on the isolated bacteria and the type of sample, specific sets of antibiotics were used for the antibiotic susceptibility testing, including Penicillin's (P – Penicillin - 10 U, A – Ampicillin - 10µg), β-lactam combination agents (AMC – Amoxicillin-clavulanate - 20/10, TZP – Piperacillin-tazobactam - 100/10 µg), cephems (CXM – Cefuroxime - 30 µg; FOX – Cefoxitin - 30 µg, CTX – Cefotaxime - 30 µg, CAZ – Ceftazidime, FEP – Cefepime - 30 µg), monobactams (AZM – Aztreonam - 30 µg), carbapenems (IMP – Imipenem - 10 µg, MEM – Meropenem - 10 µg, ERT – Ertapenem - 10 µg), fluoroquinolones (CIP – Ciprofloxacin - 5µg), macrolides (E – Erythromycin - 15 µg), TE – Tetracycline - 30 µg, lincosamides (DA – Clindamicyn); ansamycins (RIF – Rifampicine - 5 µg), sulfamides (SXT – Trimethoprim/suphamethoxazole - 25 µg), nitrofurans (NF – Nitrofurantoin - 300µg), aminoglycoside (CN – Gentamicin - 10 µg), glycopeptides (VAN – Vancomycin - 30µg, TEI – Teicoplanine - 30µg) and oxazolidinones (LNZ – Linezolid - 30 µg). The disk diffusion testing method used Oxoid Antimicrobial Susceptibility Disks from Thermo Scientific, USA, and Mast Group Ltd from the U.K. BioMérieux-l'Etoile products were employed for minimum inhibitory concentration (MIC) testing, defined as the lowest antibiotic concentration (µg/mL) that inhibits the bacterial growth. Only acquired antimicrobial resistance was taken into consideration, excluding intrinsic resistance. Results of susceptibility for each antibiotic were expressed as resistant, intermediate, or sensitive, and interpretation was made by the Clinical and Laboratory Standards Institute (CLSI) for the respective testing year [11,12,13]. Quality control procedures incorporated the use of reference strains for both identification and antibiotic resistance testing, including Staphylococcus aureus ATCC 29213, ATCC 25923; E. coli ATCC 25922; Pseudomonas aeruginosa ATCC 27853, Enterococcus casseliflavus ATCC 700327, Enterobacter hormaechei ATCC 700323, and Stenotrophomonas maltophilia ATCC 176666.

Data Collection, Interpretation, and Statistical Analysis

After removing duplicates, the antibiotic susceptibility test results were input into the electronic database of the laboratory. Analysis

of the antibiotic susceptibility profile facilitated the identification of significant resistance issues and risks posed by healthcare-associated infections in the hospital. Microorganisms that have minimum inhibitory concentrations (MIC) above the standard cut-off levels are resistant to antibiotics. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), a microorganism classified as sensitive (S) indicates therapeutic success with standard antibiotic doses, intermediate (I) responds to therapy after exposure to higher antibiotic concentrations, and resistant (R) exhibits therapeutic failure even after elevated antibiotic concentrations [14].

Multidrug resistance (MDR) was considered if non-susceptibility was found to be at least one antibiotic from two or more classes while maintaining susceptibility to at least one antibiotic from three or more categories15. Other patterns of AMR were also notified: extended-spectrum beta-lactamases (ESBL) in Enterobacterales, carbapenem resistance (CBR) in P. aeruginosa and A. baumannii, methicillin resistance in S. aureus (MRSA) and vancomycin resistance of enterococci (Van-R) [16,17,18].

Statistical analysis of the data used version 19 of the Statistical Package for Social Sciences (SPSS). Descriptive statistics tests were applied to determine frequency, incidence, and prevalence. The χ2 test was used for comparing categorical variables, considering a significance level of p<0.01.

RESULTS

Microbial Profile of the Isolates from Hospital

The microbiology laboratory obtained 1117 bacterial isolates, during the pandemic 2020-2022. The lowest annual frequency of positive cultures was recorded in 2020 (266 strains), when the specific activity of the hospital's departments was most restricted. The average length of hospitalization per year varied over the three years, ranging between 4.32 and 4.96 days.

Table 1: Distribution of the Bacterial Isolates in the Hospital's Departments

Hospital Department		Other surgery	ERT	General Surgery	Urology	ICU	Internal Medicine	Neurology	Pneumo	Other Medical	Total
GPC	*Enterococcus faecium*	-	-	-	1	3	1	1	-	-	6
	Non-faecium	1	1	5	65	12	6	7	3	1	102
	Staphylococcus aureus	1	39	18	3	20	2	2	3	3	91
	Co-Negative	-	-	2	13	13	3	5	1	-	37
	Partial sum	***1***	***40***	***25***	***82***	***48***	***12***	***15***	***7***	***2***	***236***
NF-GNR	*Pseudomonas aeruginosa*	1	18	2	20	18	1	-	7	4	71
	putida	-	-	-	1	-	-	-	-	-	1
	Acinetobacter baumanii	-	-	-	1	1	-	-	2	-	4
	Partial Sum	***2***	***18***	***2***	***22***	***19***	***1***		***9***	***4***	***76***
GNR	*Klebsiella Pneumoniae*	-	8	2	71	27	13	13	13	8	155
	Others	-	6	1	8	1	4	2	4	1	27
	Enterobacter spp	1	6	3	19	5	5	-	4	5	48
	E. coli	1	5	22	179	28	99	82	8	55	479
	Proteus spp	1	10	-	36	4	4	-	-	3	60
	Morganella spp	-	-	1	8	-	1	1	-	-	11
	Citrobacter spp	-	1	2	3	2	2	1	1	1	12
	Serratia marscenscens	-	1	1	4	1	-	1	-	-	8
	Providencia stuartii	-	-	1	1		-	-	1	-	3
	Hafnia alvei	-	-	-	-	1	-	-	-	-	1
	Salmonella spp	-	-	-	-		1	-	-	-	1
	Partial sum	***3***	***37***	***34***	***329***	***69***	***129***	***100***	***31***	***73***	***805***
Total		***6***	***95***	***61***	***433***	***136***	***142***	***115***	***47***	***79***	***1117***

The isolated bacteria were grouped into Gram-positive cocci (GPC), Gram-negative bacilli from the Enterobacterales order, and Gram-negative non-fermentative rods (NF-GNR). The highest frequency of GPC was recorded during the first pandemic year, 25.5% compared to 17% in 2021 and 21.1% in 2022, while NF-GNR was identified more frequently in the second pandemic year, representing 9.1% compared to 6% in 2020 and 5.6% in 2022.

Urine cultures were the most requested microbiological investigations, constituting 80% of bacterial cultures. Much fewer analyses were performed on other biological samples such as 5% purulent secretions, 5% respiratory secretions, 3% ear secretions, and 1% nasal and pharyngeal exudates. Blood cultures represented only 0.45% of the tested biological samples.

The urology department reported the highest number of isolates, representing 38.76% of the total bacterial strains. The Internal

Medicine department accounts for 12.71%, the Intensive Care Unit for 12.17%, and Neurology for 10.29%. Bacterial strains were obtained from between 4.9% and 2.68% of the identified isolates from the ENT, Pneumology, Rheumatology, Surgery, and Cardiology departments. Each other hospital's departments were associated with <1% of bacterial isolates (Table 1).

E. coli was isolated with the highest frequency, of 42.8% of species, potentially influencing antibiotic resistance in other Enterobacterales. At the same time, ESKAPE pathogens account for 33.5% of the bacterial strains.

Antimicrobial Resistance Profile of the ESKAPE Pathogens

Enterococcus spp.

Enterococci were identified in 108 isolates with the following distribution: 50.9% E. faecalis, 6% E. faecium, and 43% unspecified Enterococcus spp. E. faecium strains were notified in 6 cases, obtained from the urine cultures (3/6), blood cultures (2/6), and sputum (1/6), mostly on men (4/6), from the ICU (3/6). Each department of pulmonology, internal medicine, and urology reported a single strain. Among the six strains of E. faecium, one-third (2/6) were resistant to Vancomycin and Teicoplanin, and half of the strains (3/6) were multidrug-resistant (MDR). None of the strains were sensitive to Ciprofloxacin (Figure 1a).

Figure 1: Antibiotic susceptibility Profile of the ESKAPE group: a - E. faecium (n=6); b - S. aureus (n=128); c - K. Pneumoniae (n=155); d - A. baumannii (n=4); e - P. aeruginosa (n=71); f - Enterobacter spp. (n=48).

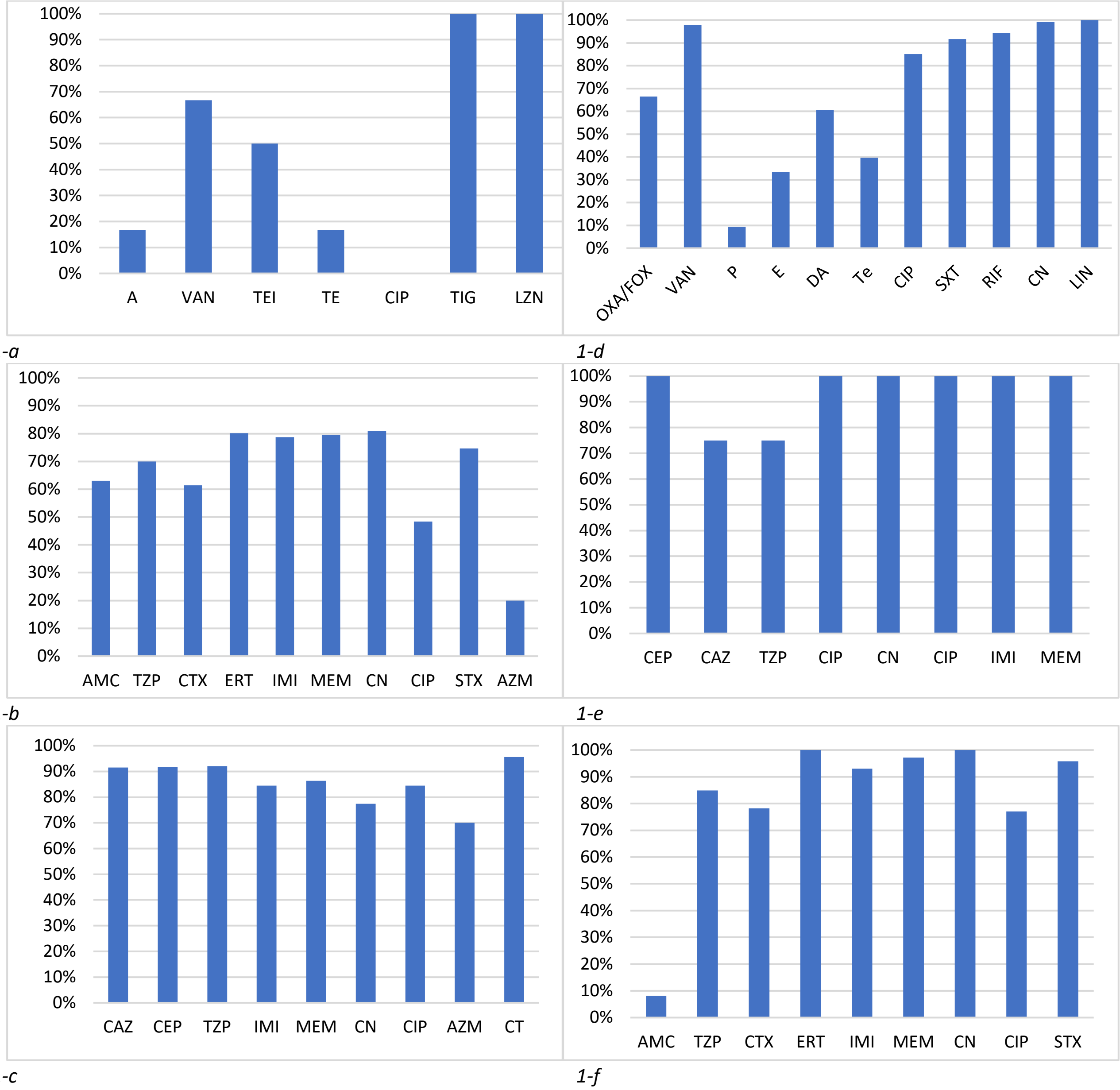

1-a *1-d*

1-b *1-e*

1-c *1-f*

Legend: P–penicillin; A–ampicillin; AMC-amoxicillin-clavulanate; TZP–piperacillin-tazobactam; CXM – cefuroxime; FOX – cefoxitin; CTX- cefotaxime; CAZ-ceftazidime; CEP-cefepime; CT: colistin; AZM-aztreonam; IMP-imipenem; MEM-meropenem; ERT-ertapenem; CIP- ciprofloxacin; E-erythromycin; TE-tetracycline; TIG- tigecycline; DA-clindamicyn; RIF-rifampicine; SXT– trimethoprim/suphamethoxazole; CN-gentamicin; VAN- vancomycin; TEI- teicoplanin; LNZ-linezolid.

Staphylococcus aureus

A total of 128 staphylococcal strains were isolated and identified as follows: 71% S. aureus and 29% Coagulase-Negative Staphylococcus (Co-NS), represented by S. epidermidis (23), S. warneri (4), S. haemolyticus (3), S. hominis (2), S. lugdunesis (1), S. saprophyticus (1), and unspecified Co-NS (3). Most S. aureus strains were identified in nasal or pharyngeal exudates (29.7%), purulent secretions (18.7%), and ear secretions (17.6%). Most samples were taken from patients in the Otorhinolaryngology (ENT) department, Intensive Care Unit, and Surgery departments (85.7%). The frequency of MRSA strains was 33.6%. The MDR rate for S. aureus was 44.5%. The lowest susceptibility of S. aureus was recorded for Penicillin at 9.4%, Erythromycin at 30%, Daptomycin at 60.1%, and Tetracycline at 39.6%. Susceptibility remained at 85% for CIP, 91.66% for SXT, 94.2% for RIF, and 99% for CN (Figure 1b).

Klebsiella pneumoniae

A total of 182 Klebsiella spp strains were isolated, including 85.16% K. pneumoniae, 5.5% K. oxytoca, and 9.3% unspecified species. Most of the cases (64.55%) were found in male patients, and most of the strains came from urine cultures (69%), sputum (9%), and purulent secretions (6%), mainly from the urology department (43.4%), intensive care (15.38%), pulmonology (9.3%), and internal medicine (9.3%). The antibiotic susceptibility profile highlighted AMC 69.8%, CTX 64.1%, TZP 75%, CN 80.9%, CIP 48.3%, STX 76.1%. Resistance to Aztreonam was found in 80% of antibiograms, noting that only 25 strains were tested. The resistance rate to carbapenems in K. pneumoniae strains was 21%, while MDR was found in 18.7% of the isolates (Figure 1c). There is no available data on the susceptibility of reserve antibiotics, as colistin, Ceftazidime + Avibactam. Meropenem + Vaborbactam.

Pseudomonas aeruginosa

Pseudomonas strains were identified in samples taken from 72 patients, of which 71 were P. aeruginosa and one was P. putida. 60.56% of the patients were males. The main products from which P. aeruginosa was isolated included urine (30.55%), ear secretion (23.6%), purulent secretions (15.27%), and sputum (6.9%), mainly from urology, ENT department, and intensive care units. The CBR rate is 15.5%, and the MDR rate is 6.8%. Among the 50 strains tested, resistance to Aztreonam (AZM) was 30%. Resistance issues were also identified for CN 22.5%, CIP 15.5%, but susceptibility was maintained for CAZ 91.55%, CEP 91.66%, TZP 92.1%, and CT 95.56% (Figure 1 e).

Enterobacter spp

Forty-eight strains of Enterobacter spp were isolated (identified) and distributed as follows: 16.66% E. aerogenes, 50% E. cloacae, and 33.33% unspecified strains. Most strains were isolated from male patients (34/48), in urine cultures (25/48), sputum (7/48), wound swabs (6/48), and pus (5/48). They originated predominantly from the urology department (39.85%), ENT department (12.5%), ICU (10.41%), internal medicine (10.41%), pneumology (8.33%), surgery (3/48), and cardiology (2/48). Isolates were also found in the orthopedics, rheumatology, and diabetology departments. ESBL frequency was found to be 14.58% among isolates, and CBR was identified in 2.85% of strains and MDR in 4.16%. The most concerning antibiotic resistance issues were observed with beta-lactams, influencing the following susceptibility profiles: AMC 8.1%, CTX 78.2%, TZP 84.85%, CIP 77%, SMX 95.75%, CN 100% (Figure 1f).

Antimicrobial Resistance Profile of E. coli

A total of 479 strains of E. coli were isolated, with 61.8% from female patients and 89.5% originating from urine cultures. E. coli strains were obtained from the following departments: urology 37.3%, internal medicine 20.6%, neurology 17.1%, ICU 5.8%, surgery 4.5%, and 14.7% from other departments. Identified antimicrobial resistance issues had frequencies of 15% MDR, 2.6% CBR, and 7.5% ESBL. The lowest susceptibility was found for AMP (37.5%), AZM (62%), CIP (64%), and SXT (72.4%). Better susceptibility was reported for AMC (86.7%), CTX (85.4%), TZP (95.5%), and CN (91.4%). Fosfomycin and Nitrofurantoin testing was limited to the E. coli strains isolated from urine culture, which proved to be susceptible in 100% and 98.25%, respectively (Figure 2).

Figure 2: Antibiotic Susceptibility Profile of E. coli (N=479)

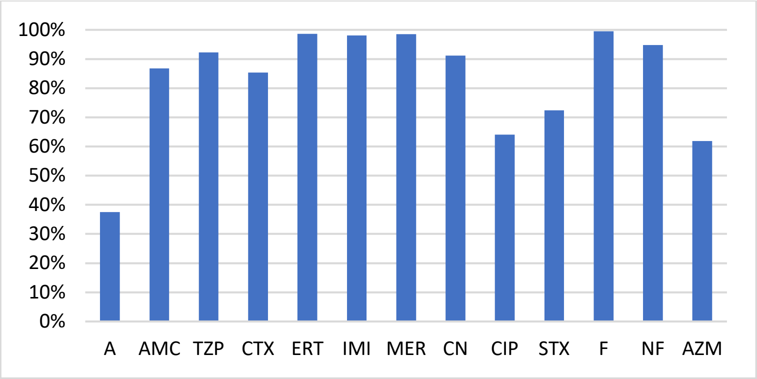

Legend: A – Ampicillin; AMC – amoxicillin-clavulanate; TZP – piperacillin-tazobactam; CXM – cefuroxime; CTX – cefotaxime; AZM – aztreonam; IMP – Imipenem; MEM – meropenem; ERT – ertapenem; CIP – ciprofloxacin; F – fosfomycine; SXT – trimethoprim/suphamethoxazole; CN – gentamicin.
Resistance of E. coli strains to AMC and CTX correlated with the resistance profile of K. pneumoniae (χ2 test: p<0.001) and Enterobacter spp (χ2 test: p<0.001).

Spread of ESKAPE Pathogen Resistance in Hospital Wards

Antibiotic resistance issues among ESKAPE pathogens have variable frequency and impact, both regarding the microorganisms surveyed and within each word, influenced by the characteristics of specific diseases to each specialty.

Acinetobacter baumannii is one of the most concerning public health problems worldwide but CBR has not been identified in any of the hospital's departments. All strains highlighted maintained sensitivity to carbapenems and other commonly tested antibiotics, but a single case evidenced an intermediary pattern, signaling decreasing susceptibility that requires vigilant epidemiological surveillance.

Pseudomonas aeruginosa - Carbapenem-resistant cases were found in isolated instances in the ICU, urology, and surgery departments without being considered a source for healthcare-associated infections. The urology department was the primary source for these isolates, but a sporadic case was also reported from the surgery department.

Enterobacter spp - Third-generation cephalosporin-resistant (C3R) was found occasionally, only in the urology department, having the slightest impact among "critical" Gram-negative bacteria on antibiotic stewardship.

The "critical" pathogen Klebsiella pneumoniae C3R is causing the most problems because it was most identified and is spreading the most in hospital wards. It mainly affects the urology department, then the intensive care unit, pneumology, internal medicine, and neurology departments. Epidemiological unrelated cases have also been reported rarely from the ENT, rheumatology, and psychiatry departments (Figure 3 a).

Figure 3: Distribution of ESKAPE Germs in Hospital Departments: a – by Specific AMR; b – by MDR

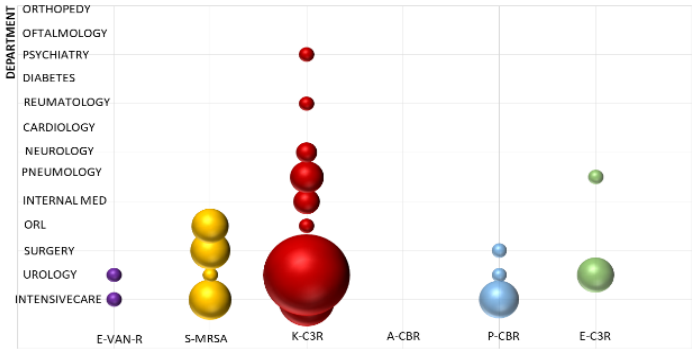

a: Specific AMR

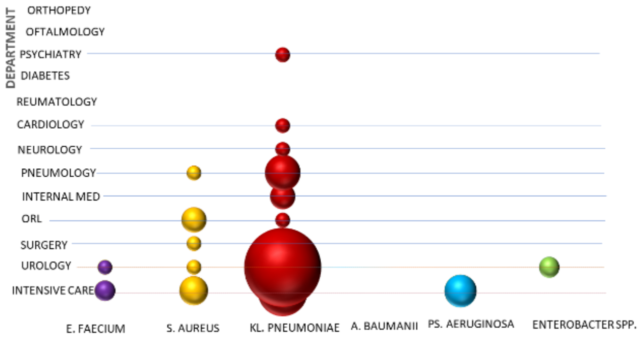

b. MDR

Among ESKAPE Gram-positive cocci, MRSA draws attention in the intensive care, surgery, and ENT departments, while only a single case was identified in urology. Vancomycin resistance in Enterococcus faecium is reported in isolated cases in the intensive care and urology departments (Figure 3 a).

Total ESKAPE pathogens cumulate specific AMRs (E. faecium Van-R, MRSA, P. aeruginosa CBR, A. baumannii CBR, K. pneumoniae, and Enterobacter spp- C3R) of 30.9%. In addition, MDR is superposing to 21% of these strains. The multidrug resistance of S. aureus, K. pneumoniae, and P. aeruginosa was associated with respect to Methicillin Resistance ($\chi 2$ test: $p < 0.001$), C3R ($\chi 2$ test: $p<0.001$) and CBR ($\chi 2$ test: $p<0.001$). Concerning E. faecium and Enterobacter spp, we found a small number of isolated and MDR very few strains, that are not considered epidemiologically significant.

DISCUSSION

The reported bacterial burden is low, with a yearly average of 1.95 bacterial strains per hospital bed. This result could be explained by the hospital's multidisciplinary profile, mainly in short hospitalization, for patients from the community. The reorganization of COVID-19 support wards has disturbed the specific activities of specialized departments. Additionally, in COVID-19 wards there were

few recommendations for microbiological investigations and properly sampling bacteriological specimens was frequently difficult.

The major challenges of the COVID-19 pandemic for Antibiotic Stewardship, Infections Prevention, and Control were the increase in the number of critical cases, diminution of vigilance in infection control, short supply of specialized staff resources, and absence of guidelines for COVID-19 diagnostic and treatment at the beginning of the pandemic [1,19,20,21]. Moreover, redistribution of lab resources was required during the pandemic, by prioritizing virologic tests and reducing the bacteriological investigations1,19,20. Apart from these general respects, Romania is considered “in the red zone” of European antimicrobial resistance, since is the country with the second highest consumption of antibacterials in Europe and 11% of the population declared antibiotic prescription from the pharmacy, without medical consultation. Therefore, we could assume that patients hospitalized in most wards used antibiotics before hospital admission, so the results of this report should be taken with caution [22,23].

WHO report on antimicrobial resistance (AMR) surveillance in the European Region highlighted a decrease in E. coli isolates in 2020 compared to previous years, attributed to reduced non-COVID-19 care activities and a decline in AMR surveillance vigilance [20,24]. Unlike other countries in the European Region that reported an increase in the frequency of commonly associated healthcare pathogens, such as Acinetobacter baumannii and Enterococcus faecium, these issues were not observed in our study. However, monitoring VAN-R enterococci remains a priority for AMR surveillance in our hospital, given the elevated regional prevalence of Clostridioides difficile infections and the intensive use of oral vancomycin, exerting selective pressure on enteric bacteria [25,26].

Like other European countries, resistance to carbapenems in E. coli and Enterobacter spp. remains low, below 3%, while the rates of C3R in our study exceed 14%, consistent with the European report [1]. The complete susceptibility to Fosfomycin of urinal E. coli isolates justifies the local guideline recommendation of this antibiotic in the initial antibiotic therapy for uncomplicated infections, preserving the β-lactam class [27].

Our study's carbapenem-resistant Pseudomonas aeruginosa (CBR) rate was consistent with the European report (15.5% vs. 17.8%). However, it showed a higher resistance to aminoglycosides (22.5% vs. 9.4%) [1]. Surprisingly, susceptibility to cephalosporins of P. aerugionosa in our setting is above 90%, recommending piperacillin/tazobactam as a first-line therapeutic option for this presuming etiology of infections.

Klebsiella pneumoniae demonstrated the highest frequency among "critical" pathogens but also exhibited the most concerning antibiotic resistance. Resistance rates exceeded 20% for all antibiotic classes, including carbapenems, reaching over 50% for quinolones. It is necessary to systematically the antibiotic testing of K. pneumoniae strains for reserve antibiotics considering colimicin and combinations of ceftolozane–tazobactam, imipenem–cilastatin/relebactam, meropenem–vaborbactam or ceftazidime–avibactam [16]. The recommendation for the first-line treatment of suspected carbapenem-resistant strains should consider these reserve antibiotics. Other additional potent drugs for carbapenem-resistant K. pneumoniae such as plazomicin, eravacycline, cefiderocol or temocillin could become available by governmental regulations [28]. Furthermore, the emergence of K. pneumoniae hypervirulent strains, especially in association with CBR, is linked to antibiotic treatment failure and an elevated mortality rate. Monitoring these variants necessitates using advanced molecular techniques to identify virulence and resistance factors [29].

We have tested the susceptibility on Aztreonam, according to the standardized procedures, although Aztreonam has not been available in Romania for therapeutic use. We found over 30% resistance between the tested Gram-Negative rods, predicting low benefit as a first-choice antibiotic in clinical practice.

Staphylococcus aureus ranks second in the frequency of pathogens with antibiotic resistance risk. The MRSA rate is among the highest in Europe, meaning over 33% of strains. The notably high rate of multidrug-resistant (MDR) strains is mainly attributed to intensive community usage of penicillin, macrolides, and tetracyclines. Vancomycin and Linezolid are valuable options for first-line antibiotic treatment in severe invasive infections, while sulfamethoxazole could be used in non-severe infections. Although not within the study's scope, we observed a higher MDR rate in Coagulase-Negative Staphylococci (Co-N), which colonizes the skin, mucous membranes, and environmental surfaces in the community and the hospital. This may contribute to the transmission of antibiotic resistance to Staphylococcus aureus [30].

ESKAPE pathogens are found in the community, but more frequent in healthcare settings. The mechanisms of antibiotic resistance emergence and persistence of the ESKAPE pathogens are heterogeneous, but they share the ability to build biofilms related to biotic and abiotic surfaces [14]. Regarding the characteristics of our health care services, the source of the ESKAPE pathogens is more probable community than acquired in the hospital but could reflect the regional antimicrobial practices of the practitioners in family medicine and other specialists.

Monitoring and analyzing antimicrobial resistance in the hospital setting is essential for identifying the risks associated with the emergence of "critical" strains and healthcare-associated transmission. Specific resistances within the ESKAPE group, as well as MDR within this group, were more frequently identified in males compared to E. coli, which predominantly originated from females. Both Gram-negative ESKAPE pathogens and E. coli were predominantly associated with urinary tract infections in most cases. This observation could be considered for the risk evaluation in the first-line antibiotic treatment decision, in addition to the predictive clinical scores, such as CarbaSCORE, Tumbarello score, Duke score, or machine learning models [30,31,32,33].

This is the first surveillance report of AMR in the Emergency Military Hospital "Dr. Aristide Serfioti" from Galati, developed to understand the local impact of COVID-19 on bacterial charge, diversity, frequency, and antibiotic resistance, for updating the regional antibiotic stewardship strategy. Revising the clinical protocols for diagnosis and treatment in each department, intensifying the

bacteriological samples collection, and improving the microbiological diagnostic techniques for AMR identification, including high technologies of molecular investigations and point of care antimicrobial resistance tests are highly interests for the near future stewardship. Systematic epidemiological monitoring and control of the hospital environment, hand hygiene procedures stimulation, strict assurance of safe medical procedures, and continuing education are ongoing necessary components of the antibiotic stewardship program.

Limits of the Study

This is a retrospective study, based only on microbiological records. Consequently, the patient history and clinical data were not available. Furthermore, the community or healthcare-acquired source of infection was not analyzed. Hemocultures accounted for only 1% of the total positive samples and 3.18% of them are categorized in ESKAPE group. Therefore, comparing the antimicrobial resistance profile resulting from our study with reference data reported by representative laboratories that monitor antibiotic resistance in epidemiological surveillance networks for invasive bacterial strains, has limited significance [24]. The molecular investigation of antimicrobial resistance mechanisms was not affordable. The antibiotic consumption was not included in the present study but should be correlated with the antimicrobial resistance pattern of the hospital.

CONCLUSION

The COVID-19 pandemic impaired the hospital practice for infections and bacterial diagnostics and weakened the vigilance of antibiotic stewardship. ESKAPE pathogens are almost one-third of the total bacterial isolates, with 21% associated MDR.

The “red flag” for antibiotic stewardship between ESKAPE pathogens is Klebsiella pneumoniae. The directions of the near future antibiotic stewardship program focus on revising the clinical protocols of diagnostic and treatment in each department, to intensify the bacteriological samples collection, to improve the microbiological diagnostic techniques for AMR identification, and to intensify the epidemiological actions of surveillance, control, and education.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

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The current manuscript does not contain previously published materials or self-generated AI text.

Authors’ contribution

Conceptualization, M.A., C-M.V.; methodology, A-V.I, , M.A., C-N.D.; software, I.D., C-S.S; validation, M.A., C-M. V. and A-V-I.; formal analysis, I.D., C-N.D., A.N.; investigation, C-M.V., I.D.; resources, C-M.V. and C-S.S.; data curation, A-V.I.; C-N.D.; writing—original draft preparation, C-M.V., C-S.S, A-V.I., I.D., C-N.D., A.N.; writing—review and editing, M.A.; visualization, C-S.S., A. N.; supervision, M.A.; project administration, C-M.V., A.N. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board Institutional Board of Emergency Military Hospital "Dr. Aristide Serfioti" from Galati (RCM 212/M1-544/2023) for studies involving humans.

Patient consent for publication

Not applicable. Our retrospective study used laboratory management which regularly collected data from the databases. The consent for the use of personal data was given by each patient as a routine procedure of the hospital health care provision.

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ORIGINAL ARTICLE

Impact of Physical Performance on Hemodynamic and Cognitive Status in Romanian Older Adults

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Abstract: Background: Frailty is a major challenge for the aging population, increasing the risk of falls, disability, hospitalization, and death. It's common with age, and often linked to reduced physical activity and poor diet, especially in those prone to sarcopenia. Gait speed tests are frequently used to assess mobility in older adults. Materials and methods: This six-month study at "Sfântul Luca" Chronic Disease Hospital involved 143 patients over 65 (mean age 72.85 ± 10.07), 113 of whom were women. The Tensiomed Arteriograph measured heart rate, pulse wave velocity, and central aortic blood pressure to assess acute vascular stiffness. Results: As patients aged, their physical circumstances changed: in the age group 75-84 years ("Very elderly") only 39% of the subjects achieved medium- and good-physical performance, while 56% of them were characterized by low-physical activity (Chi-square test, $p < 0.001$). The mean Mini-Mental State Examination (MMSE) values of individuals with inadequate physical activity were significantly lower (21.02 ± 4.68 points) than those with adequate physical activity (Student t-test, $p < 0.001$). Conclusion: Regular physical activity has been shown to improve hemodynamic factors and potentially slow down arterial aging, thus promoting healthy aging. It is imperative for healthcare professionals to promptly evaluate whether older, frail patients can benefit from rehabilitation, enabling swift recovery through multidisciplinary care.

Keywords: physical performance; hemodynamic status; older adults; cognitive status; personalized rehabilitation program

INTRODUCTION

The clinical state of frailty is one of the most difficult issues facing the senior citizen population. Frailty is linked to a higher risk of falls, disability, hospitalization, and mortality [1] and is thought to be common as people age. The onset of frailty in the elderly is often characterized by muscular weakness, reduced mobility, and constraints in performing routine physical tasks. [2]. Sarcopenia is characterized by the loss of muscle mass and can stem from chronic malnutrition, musculoskeletal changes related to aging, chronic illnesses, micro-inflammation, and related comorbidities. For elderly individuals, being admitted to the hospital is one of the most distressing experiences. The gait speed test is a widely used tool for assessing the mobility of elderly individuals [3]. The level of physical activity and adequate nutrition intake considerably declined among older adults at risk of developing sarcopenia [4]. Muscle weakness and reduced movement speed leading to disabilities characterize sarcopenia, a health condition associated with the aging process among the geriatric population. [5]. Physical activity and gait speed are protective factors for the occurrence of sarcopenia in older adults. The primary physical activity for seniors is walking. Clinical research and clinical objectives are increasingly relying on the evaluation of walking ability [6]. Evaluation of gait speed and its effects on hemodynamic parameters and cognitive status in older persons was the aim of this study.

MATERIALS AND METHODS

From the 160 patients hospitalized in the months of January-March who met the eligibility criteria: 8 patients refused to participate, 4 patients presented a new diagnosis of oncological disease in the middle of the hospitalization and 5 patients withdrew.

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Our study involved 143 patients, 113 of whom were women, and had a mean age of 72.85 ± 10.07 years. This six-month observational, clinical trial was conducted at the Geriatrics Clinic "Carol Davila" in the Chronic Disease Hospital "Sf. Luca." Enrolment was contingent upon the participants fulfilling the subsequent eligibility requirements: being 65 years of age or older; having no self-reported health conditions that would preclude physical examinations; being able to perform basic mobility tasks (such as walking without extreme pain, aching, or stiffness); and not being undergoing cancer treatment, knee or hip replacement, heart or spinal surgery, myocardial infarction within the previous six months, or experiencing chest pain while walking within the previous 30 days. A MMSE score < 20 points resulted in the exclusion of participants. Before participating, each participant gave written, informed consent. The Clock Drawing Test (CDT) is a simple tool used to check for early signs of dementia, scoring up to 10 points. We used the Short Physical Performance Battery (SPPB) to measure physical activity, which includes the tandem standing, 4-meter walk test, and Up-and-Go [7]. For functional capacity, we used the standardized scales Activity Daily Living (ADL) with ranges from 0 to 6 points and Instrumental Activity Daily Living (IADL) with ranges from 0 to 8 points, where a high score indicates high function [8, 9]. We measured hand grip strength using a hydraulic dynamometer and used the Tensiomed Arteriograph to evaluate arterial vascular stiffness: pulse wave velocity (PWVao), heart rate (HR), central aortic systolic blood pressure (SBPao), and central aortic pulse pressure (PPao) [10].

To illustrate the degree of comorbidity, we categorized the patients into three groups based on a comorbidity index: low comorbidity index, which represented patients with up to two chronic pathologies; moderate comorbidity index, which represented patients with three to five chronic pathologies; and high comorbidity index, which represented patients with more than five chronic pathologies.

The inclusion of patients with metabolic syndrome was achieved by evaluating those with 3 criteria (MS3) or with the presence of 4 of them (MS4) and all 5 criteria (MS5). In addition, we conducted a search through reference lists of pertinent publications, Google Scholar, EBSCOhost, and Medline in order to find published hand grip strength norms.

All determinations are performed according to the protocol and the results are analyzed according to standardized scales. This study was approved by the Ethics Commission (9/09.12.2023).

Statistical Analysis

All the data from this study were analyzed using IBM SPSS Statistics 25 and illustrated using Microsoft Office Excel/Word 2021 Stundent's t-test was used for parametric distributions. Qualitative variables were written as absolute frequencies with percentages and were tested between groups using Fisher's Exact Test.

RESULTS

The age caused changes in the physical status of patients. Thus, 56% of the subjects with low physical activity were in the age group 75-84 years, and only 39% of very elderly subjects were characterized by medium and good physical activity (Chi-square test, $p < 0.001$). Also, in the "very, very elderly" age group (over 85 years), we noticed the same statistically significant difference between the two groups (see Figure 1).

Figure 1. Distribution of physical activity by age

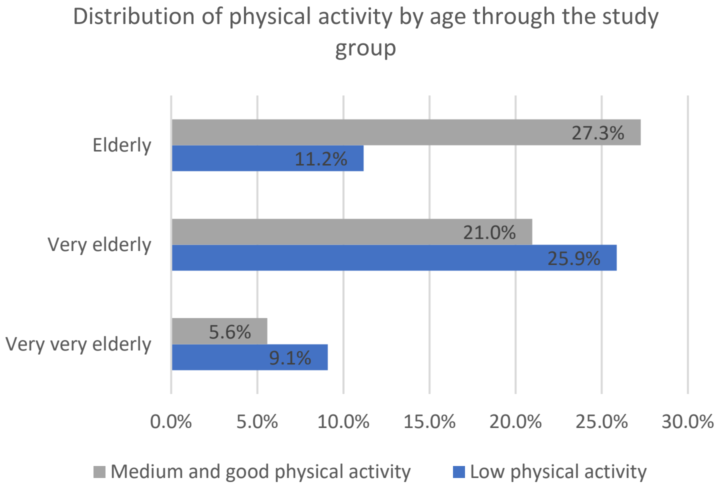

Within the group, there is no statistically significant difference in terms of physical performance and the number of associated diseases (expressed by the comorbidity index, student T-test) (see Table 1).

The functional capacity of the elderly in the study group was relatively normal, with no more than two activities affected, especially in the instrumental activities of living: the mean IADL is 6.66 ± 1.71 (out of a maximum of 8 points), and the mean ADL is 5.74 ± 0.92 (out of a maximum of 6 points). Quantitative independent variables were tested between groups using Mann–Whitney U tests (see

Table 2).

Table 1: Physical activity and comorbidity index

Characteristic	Low physical activity (N = 66)	Medium and good physical activity (N= 77)	p
Comorbidity index Low Moderate High	1 (2%) 52 (79%) 13 (19%)	2 (2%) 66 (86%) 9 (12%)	0.389
Metabolic syndrome criteria Without MS[1] MS3 MS4 and MS5	18 (27%) 23 (35%) 25 (38%)	14 (18%) 34 (44%) 29 (38%)	0.353
Diabetes Yes No	29 (44%) 37 (56%)	40 (52%) 37 (48%)	0.339

Table 2: Physical performance and functional capacity in the studied group

Characteristic	Low physical activity (N = 66)	Medium and good physical activity (N = 77)	p
ADL	5.50 ± 0.93	5.95 ± 0.87	0.004
IADL	6.06 ± 1.81	7.17 ± 1.43	< 0.001
Subjective fatigue Rare Sometimes Often	8 (12%) 9 (14%) 49 (74%)	17 (22%) 37 (48%) 23 (30%)	< 0.001
Hand grip strength	11.74 ± 2.9	17.08 ± 4.94	< 0.001
Walking speed	0.34 ± 0.1	0.69 ± 0.13	< 0.001
Statin treatment Yes Not	29 (44%) 37 (56%)	39 (51%) 38 (49%)	0.423

Physical performance and impact on daily activity scores show a direct relationship between the functionality and the SPPB values. A statistically significant difference was found in IADL scores within the group with low physical activity (6.06 ± 1.81 compared to 7.17 ± 1.43, Student's t-test, p < 0.001). Additionally, basic individual needs, such as eating and washing, are impacted in the group with reduced functionality, as measured by the ADL, like those with lower physical performance (Student's t-test, p = 0.004) (see Table 2).

Hand grip strength measured by a dynamometer, adjusted for age, gender, and height, revealed a significant difference in the group with low physical activity, with an average strength of just 11.74 ± 2.9 kg (Student's t-test, p < 0.001) (see Table 2).

In the context of long-term treatment of elderly patients with chronic cardiovascular disease, statins are often associated with the development of sarcopenia, chronic fatigue, and reduced physical activity. However, our study findings revealed no statistically significant correlation between the use of statins and the decline in hand grip strength or physical performance among the elderly patients in our study group. Remarkably, more than half (51%) of the elderly patients undergoing statin treatment maintained satisfactory levels of physical activity (see Table 2).

In our analysis of walking speed, we calculated patients' movement rates and correlated them with some hemodynamic parameters (HR, PWVao, PP) and functional capacity, through multiple regression analyses. We divided the patients into three groups: the first group (home movement) consisted of elderly individuals at heightened risk of disability who have limited mobility confined to their residence, with a walking speed of up to 0.4 m/s; the second group (limited community movement) included elderly people engage in movement within the community and are at moderate risk of losing their autonomy, with a walking speed between 0.4 - 0.8 m/s; the third group (full mobilization) comprised individuals who are not exposed to the risk of disability and are fully mobile with a walking speed exceeding 0.8 m/s (see Table 3).

The results showed a significant statistical difference on the pulse pressure (PP) (p = 0.001). This indicator could potentially serve as a valuable predictor for gauging cardiovascular risk and assessing disability risk, particularly in relation to walking speed.

Regarding the cognitive level there is a clear difference between the two groups. The mean MMSE values, which assess the cognitive status of the subjects, are significantly lower (21.02 ± 4.68 points) among those with low activity compared to those with good physical activity (Student t-test, p < 0.001) (see Table 4).

Table 3: Distribution of patients according to walking speed

	Group 1 = home movement (N = 48)	Group 2 = limited community movement (N = 71)	Group 3 = full mobilization (N = 24)	p
ADL	5.46 ± 0.84	5.80 ± 0.95	6.13 ± 0.85	0.011
IADL	6.19 ± 1.8	6.75 ± 1.71	7.33 ± 1.09	0.021
PWVao	10.37 ± 1.74	9.94 ± 1.93	9.95 ± 1.63	0.419
PP	**66.71 ± 14.23**	**65.17 ± 15.31**	**53.42 ± 12.75**	**0.001**
HR	68.81 ± 12.7	66.61 ± 11.36	65.33 ± 7.02	0.404

Table 4: Correlation of hemodynamic and cognitive parameters with walking speed

Characteristic correlation	Coefficient	p
PWVao and SPPB	- 0.141	0.092
HR and SPPB	- 0.166	0.047
Walking speed and SBPao	- 0.149	0.076
Walking speed and Body Mass Index (BMI)	0.125	0.138
Walking speed and MMSE	**0.339**	**< 0.001**
Right-Hand grip strength and SPPB	**0.422**	**< 0.001**
Left-Hand grip strength and SPPB	**0.440**	**< 0.001**
Fatigue and statin treatment	0.064	0.450

The presence of this difference comes as an important finding that supports the potential role of physical exercise in preventing cognitive impairments in the elderly. Given the limited effectiveness of current drug treatments for dementia, geriatric clinicians should place greater emphasis on incorporating physiotherapy programs for these elderly patients.

Another important finding is the difference between the two groups in the case of the Clock Drawing Test (CDT), which is easy to use, quick to administer, and can be implemented as a screening tool in primary care settings [11].

The group of those with low physical activity had an average CDT score of 6.63 ± 1.33 points (out of a max. 10 p), compared to an average score of 7.96 ± 1.72 points among those with good physical performance (Student t-test, $p < 0.001$). Our findings once again emphasize the importance of the age-old adage, "Mens sana in corpore sano".

DISCUSSION

Regular physical activity is not only crucial for maintaining autonomy but also for preserving cognitive function [12]. It is widely recognized that consistent aerobic exercise enhances cardiorespiratory function, reduces arterial stiffness [13], diminishes cardiovascular risk factors, and lowers cardiovascular mortality. Conversely, anaerobic exercises promote skeletal muscle strength and are instrumental in preventing age-related muscle atrophy [14]. Elderly patients can benefit from the high level of education of nurses, which influences the degree of clinical autonomy. The nurse needs to be aware of any barriers to learning, like loss of hearing or decline of vision and should tailor his or her education for that specific patient [15].

Physical activity is an effective strategy for protecting against sarcopenia and promoting healthy aging. Most studies on exercise in elderly people show that participants achieve positive results, although maintaining muscle strength appears to depend on the continued implementation of certain types of physical activities. One of the conclusions from a meta-analysis of 10 studies was that there was no consistency in the measurement of sarcopenia, indicating a need for further investigations [16].

For elderly people, regular exercise (at least one hour, at least twice a week) through personalized exercise programs can have beneficial cardiovascular effects, such as lowering blood pressure, improving skeletal muscle perfusion, and enhancing glucose utilization in myocytes. Additionally, regular exercise helps maintain muscle fiber quantity and supports skeletal muscle strength [17]. Numerous studies have demonstrated the significant impact of this factor on recovery rates in neurological system disorders and cardiovascular diseases, both of which are primary contributors to disability and diminished quality of life (QoL) [18-20].

The QoL significantly influences patients' adherence to treatment and their overall recovery, making it a pivotal endpoint in clinical research. Aspects such as pain management, emotional well-being, social interactions, and independence profoundly impact patient outcomes. Consequently, enhancing QoL becomes a primary objective from the point of diagnosis. While both patients and physicians acknowledge the importance of prolonging survival, maintaining an optimal quality of life throughout the treatment process is equally imperative [21]. We consider that physical activity plays a major role in enhancing QoL. Due to the Coronavirus (COVID-19) pandemic, many elderly individuals—already a high-risk group for severe illness and adverse SARS-CoV-2 outcomes—opted to remain at home out of fear of contracting the virus, regardless of their associated comorbidities. Consequently, it is hypothesized that a significant

number of older adults are now facing the repercussions of that period, notably sarcopenia, which is linked to a lack of physical activity. As a result, these individuals are now tasked with recovering from that phase, underscoring the need to increase awareness about the importance of physical exercise in this population [22]

In our study, the measurement of muscle strength through the dynamometer revealed a significant difference in the group of elderly patients with low physical activity (Student t-test, $p < 0.001$). Traumatic injuries accounted for the largest percentage of rehabilitation cases in Romania (73.17%), with musculoskeletal illnesses coming in second (70.06%). Reduced recovery was observed in cases of neurological system disorders (50.56%) and cardiovascular diseases (44.23%) [18].

Although other studies have debated the potential link between statin administration in the elderly and the development of sarcopenia, fatigue, and decreased physical activity [23], our group did not find a positive correlation supporting this relationship. Hence, it endorses the recommendations for the continued utilization of statins, even in advanced age, as they have exhibited protective advantages in averting cardiovascular incidents.

Analyzing and interpreting walking speed presents a challenge for clinicians, but recent specialist studies have provided new insights into survival and mortality predictors. Normal walking speed requires a relatively low level of energy consumption [24, 25]. Our study found statistically significant results related to pulse pressure (PP), which is considered a predictor for evaluating disability risk in the elderly population [26].

Cognitive status can be improved by exercise. Several prospective studies have demonstrated that maintenance of higher levels of physical fitness helps protect against cognitive deterioration, even at an advanced age [12,27,28]. Conversely, poor physical performance among older adults may accelerate cognitive decline and lead to early onset of dementia [29, 30]. Additionally, muscle strength has been linked to cognitive performance [31,32]. Promising trials utilizing AI and technology, which show significant improvements in autonomy and reduced reliance on human resources point to a bright future for the medical care and recovery of elderly patients with cognitive impairments [33,34,35].

Several limitations of the present study should be acknowledged. First, the study sample was predominantly female, which could potentially influence the results. Second, the study group was relatively small, and there was a lack of homogeneity, which may affect the comparability of the findings with the broader Romanian population.

CONCLUSION

Maintaining physical performance, especially in the elderly with associated chronic diseases, has an important role in maintaining autonomy and quality of life, especially in the very old and very, very old age groups that become frailer. Assessing hand grip strength can provide valuable insights into physical performance and guide lifestyle optimization and treatment recommendations for elderly patients. Exercise training has been shown to improve hemodynamic status (including SBP, PP, SBPao, PPao) and may help delay arterial aging, playing an important role in healthy aging. Muscle strength, balance, flexibility, endurance, and mobility are all aspects of whole-body functioning that are included in physical performance. When older adults perform poorly physically, they experience a decline in their quality of life, a loss of cognitive function, an increase in their risk of falling, poorer clinical outcomes, and a reduction in their independence when it comes to doing activities of daily living (ADL).

Early detection of rehabilitation potential in frail elderly patients is essential for initiating timely recovery interventions through specialized medical services and multidisciplinary teams.

From a practical and clinical perspective, developing a consistent program of adapted, personalized physical activities is necessary. Such programs can positively affect functional parameters and significantly enhance the quality of life for elderly individuals. In order to corroborate these findings and draw further conclusions, it is essential to conduct studies encompassing a more diverse and extensive demographic.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, J.A., S.M.A., A.Z. and C.O.; methodology, R.M., A.P..; software, S.M.G, C.D.G., A.G.P .; validation, J.A., S.M.A., A.Z. and C.O; formal analysis, J.A., A.Z., V.M.A.M., M.N; investigation, V.M.A.M., C.D.G., S.M.G.; resources, A.G.P., S.M.A., M.N., C.O.; data curation, S.M.G, C.D.G, A.G.P., M.N.; writing—original draft preparation, J.A, S.M.A, A.Z., C.O.; writing—review and editing, J.A., S.M.A., C.O.; visualization, A.Z., R.M., S.M.A.; supervision, S.M.A, J.A, C.O.; project administration, A.Z., J.A.; funding acquisition. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Commission of the HOSPITAL OF CHRONIC DISEASES "SF. LUCA" (protocol code 9, date of approval 09.12.2023).

Patient consent for publication

Written informed consent to participate in the research was obtained from each patient/subject..

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REVIEW

Case Management of Delirium in Patients with Major Neurocognitive Disorders

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Abstract: Delirium is associated with lower quality of life, increased healthcare costs, longer hospitalizations, and worse prognosis when detected in patients who are already confronted with a severe and invalidating disease, like the major neurocognitive disorder (MND). In order to identify the most evidence-based interventions that could be included in case management for patients with both delirium and MND, this narrative review explored three electronic databases (Pubmed, Cochrane, and Web of Science/Clarivate) for primary and secondary sources dedicated to this topic. All papers published in English between the inception of each database and September 2024 were screened for relevant data on pharmacological and non-pharmacological therapies targeting delirium. The results supported the use of a multicomponent approach as the first line of treatment, mitigating the precipitating factors, and only as a second line, the use of pharmacological instruments. There is a lack of validated therapies targeting specifically the delirium superimposed on MND, most of the recommendations being extrapolated from studies with delirium in the general population or in elderly patients with different organic diseases. Further studies are required to investigate the specifics of delirium in patients with MND and potential strategies focused on neurobiological mechanisms instead of just clinical manifestations.

Keywords: delirium; acute confusional states; dementia; major neurocognitive disorders; cognitive impairment; delirium assessment tools; antipsychotics

INTRODUCTION

Establishing adequate case management strategies for patients diagnosed with delirium is among the most challenging aspects of liaison psychiatry due to the complexity of the interactions between psychiatric phenomenology and organic pathology. Psychiatrists working in general hospitals are frequently called for consultation by colleagues with different medical specialties to „calm down” patients who suddenly become confused and agitated or, on the contrary, to „wake up” patients who abruptly become non-responsive to external stimuli. This psychiatric disorder is, however, far from being possible to approach by a single therapeutic method and requires an individualized case conceptualization and treatment. While in some cases, identification and correction of the causal factor(s) are simple and may be conducted promptly, such as in the case of severe dyselectrolytemia or hypoglycemia, in most cases, extensive investigations and multiple interventions are needed before the symptoms of delirium may be adequately managed. In this context, it would be necessary to mention that delirium is frequently deceptive because it can be the final manifestation of multiple organic comorbid conditions and a superficial investigation and treatment of the patient may make his or her prognosis worse. Therefore, psychiatrists should be aware of the intricate etiopathogenesis of delirium and of the necessity to investigate and treat such patients within a multidisciplinary framework. These considerations become more obvious when patients with severe chronic cognitive deterioration are evaluated since their anamnesis is reputedly difficult and the psychiatric exam also is frequently challenging.

Due to the changes in the names and definitions of neurocognitive disorders along the main classifications of psychiatric disorders, edited by the World Health Organization (WHO) and American Psychiatric Association (APA) [1-5], a trans-

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nosographic analysis is considered necessary to configure the framework of case management in patients affected by these conditions. Although there is a clear tendency to increase the concordance between the APA's Diagnostic and Statistical Manual of Mental Disorders (DSM) and the WHO's International Classification of Diseases (ICD) criteria for the diagnosis of psychiatric disorders, there are still important nosographic differences that need to be acknowledged by clinicians due to practical reasons.

Major neurocognitive disorders (MND) are defined, according to the latest editions of the DSM (DSM-5 and 5 TR, published in 2013 and 2022, respectively), by significant cognitive decline compared to the individual's previous level of performance in at least one cognitive domain; this cognitive impairment has to be supported by concerns reported by the patient, significant caregiver or clinician; also, a substantial deficit in cognitive performance, documented by a quantitative clinical assessment is required [1,2]. Also, significant interference of these cognitive impairments with the individual's ability to perform independent daily tasks is needed for the diagnosis, and the exclusion of other mental disorders is required as a differential diagnosis in these cases [2]. It is important not to consider the diagnosis of MND in patients who present cognitive impairment exclusively during the periods of delirium [2]. Once the diagnosis of MND is made, the presumed type of disease must be formulated, and the specifiers „with/without behavioral disturbance" and „severity" (mild/moderate/severe) are added [2].

The previous edition, DSM IV-TR, not only used different terminology for MNDs, which were called „dementias", but also the diagnosis criteria included neurological signs, i.e., aphasia, apraxia, agnosia, and the existence of disturbances in executive functioning, besides the memory impairment [3]. Regarding the ICD-10 and ICD-11, their perspective on the terminology is quite similar, and the term „dementia" is preserved in both editions, but in the latest version of ICD, there is an increased focus on the behavioral or psychological disturbances in dementia (BPDD), which received their own set of codes [5].

According to DSM-5 and 5 TR, to formulate a diagnosis of delirium, the clinician should confirm the existence of a disturbance in attention and awareness, accompanied by disturbances in cognition, i.e., memory, orientation, language, visuospatial abilities, or perception [1,2]. Additionally, this diagnosis requires an abrupt onset (e.g., hours to a few days), a change from the baseline level of attention/awareness, and a tendency towards fluctuation in severity during a single day [1,2]. The clinician must exclude the presence of a preexisting or evolving neurocognitive disorder and the presence of a coma or related conditions [1,2]. DSM-5 and 5 TR also state, as an essential criterion, the evidence supporting the direct causal-ship between a general medical condition, substance withdrawal/intoxication, exposure to a toxin/medication, or multiple etiologies and the symptomatology of delirium [1,2]. There are several specifiers for delirium, i.e., those related to the etiology, specifiers of severity (acute/persistent), and level of behavioral symptoms (hyperactive/hypoactive/mixed level of activity) [1,2].

When comparing the diagnostic criteria for delirium in the DSM-IV TR, DSM-5, and DSM-5 TR, several differences emerge [1-3]. The latest editions emphasize the need for disturbances in attention and awareness to represent a change from baseline, exclude coma from the category of delirium, and recognize the potential for multiple cognitive domains to be impaired by this disorder. Furthermore, both DSM-5 and DSM-5 TR provides a more comprehensive list of etiological types of delirium than does the DSM-IV TR [1-3].

The WHO's ICD-10 (the 2019 version) specifies that delirium is „an etiologically non-specific organic cerebral syndrome" that is characterized by disturbances of consciousness and attention, perception, thinking processes, psychomotor behavior, emotions, and the sleep-wake rhythm [4]. ICD-10 mentions that the duration of delirium is variable and the degree of severity may be different, from mild to severe [4]. Regarding the possible etiological considerations, ICD-10 includes different codes for delirium not induced by psychoactive substances, in cases where it is not superimposed on dementia, in cases of delirium superimposed on dementia, and contains codes for other types of delirium (e.g., mixed delirium, or post-surgery), and delirium-unspecified; delirium is also a complication of various withdrawal/intoxication states or somatic diseases (e.g., thyroid storm, kidney failure) [4].

The latest edition of the WHO Classification of Diseases (ICD-11) expands the definition of delirium (but preserves the core dimensions-phenomenological description, changes in functionality) and specifies the main differential diagnoses and potential etiologies, i.e., direct physiological effects of a medical condition, direct physiological effects of a substance or medication (including withdrawal), and unknown factors [5]. Residual categories, such as multiple etiological factors and other specific causes, are also coded [5].

The phenomenon of delirium in patients diagnosed with MND is very complex since the relationship between the two pathologies is far from being easy to define in each case. Individuals with MND have an increased risk of delirium onset when compared to the general population, but delirium in itself represents an independent risk factor for developing MND [6]. Also, delirium may be more difficult to diagnose in patients with MND because disentangling cognitive changes and sensorium clouding in a patient with an already deteriorated cognitive status, based on clinical evaluation, is challenging. Even more, confusional episodes appearing in patients with MND can worsen the cognitive decline and the overall functioning level [6]. It must also be mentioned that MNDs associate multiple well-defined psychiatric disorders (e.g., mood, anxiety, psychotic) as well as BPDDs, which creates supplementary difficulties in detecting new-onset cognitive decline due to delirium [7]. These convoluted interactions between delirium and MND are represented in Fig.1., but caution is recommended when interpreting these relationships due to the intricacy of organic and psychological factors (e.g., infectious diseases may trigger delirium, and delirium may, in patients with MND, worsen the psychological long-term evolution, or, a psychological trigger may be responsible for the clouding of sensorium in a patient with MND, which also may worsen the long-term biological evolution of the patient, if the trigger is not properly identified and corrected; also, psychosocial factors may inadvertently worsen a somatic disease that, in turn, may aggravate the preexisting MND).

Figure 1: The interaction between delirium and major neurocognitive disorders

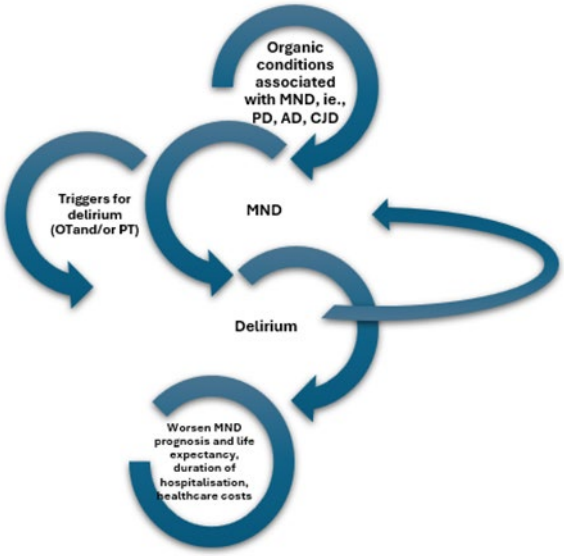
Organic conditions associated with MND, ie., PD, AD, CJD
Triggers for delirium (OTand/or PT)
MND
Delirium
Worsen MND prognosis and life expectancy, duration of hospitalisation, healthcare costs

AD= Alzheimer's disease; CJD= Creutzfeldt-Jakob disease; MND= major neurocognitive disorder; OT= organic triggers; PT= psychological triggers; PD= Parkinson's disease

Although the phenomenology of delirium encompasses prodromal and subsyndromal stages [8], no consensus about corresponding specific symptoms exists, making it difficult for clinicians to identify such forms of delirium in daily practice. Due to the complexity of the phenomenological presentation of delirium, the differential diagnosis is another important challenge for clinicians [8]. More exactly, delirium consists of core manifestations, which are expected to be present in all cases (e.g., awareness alterations), frequently met symptoms (e.g., motor activity dysfunctions), and associated, variable symptoms (e.g., hallucinations), which may reflect the etiological medical or toxic factors [8]. The evolution of delirium is difficult to predict because it is dependent on etiological factors, but it was reported that almost 57% of hospitalized patients may still have delirium after discharge from the hospital [9].

The reported prevalence in hospitalized patients varies across studies, depending on the criteria used, the type of department reporting the cases, the instruments applied for quantifying the severity of the symptoms, etc. The prevalence of delirium was reported between 1 and 31%, and the incidence between 3 and 25% [10-12]. For example, 39% of the patients admitted to intensive care units (ICUs) were diagnosed with delirium, according to a cross-sectional multicenter study (N=256 participants) that used a screening checklist as the core methodology of assessment [13]. In hospitalized patients aged ≥65, delirium was reported in the case of 55.4% of the 327 participants using the 3-Minute Diagnostic Confusion Assessment Method (3D-CAM) [9]. A surprisingly high rate of patients with delirium (35.4%) were not identified by the treating team, indicating the need for increased awareness about the existence of this neurocognitive disorder [9]. Also, it is important to explore the possibility of multiple etiologies in patients with delirium, including organic acute diseases, chronic illnesses that are acutely decompensated, new drugs recently introduced for the treatment of a physical or mental disorder, different environmental stressors, etc. [14-16].

The presence of delirium was independently associated with worse clinical prediction of survival, lower quality of life, and increased healthcare costs through longer hospitalizations have been reported in patients with delirium [17-19]. More specifically, an economic analysis of healthcare for older hospitalized patients with or without Alzheimer's disease and related dementias associated with delirium showed that in the first group, the adjusted mean difference in costs related to delirium was almost 35000 USD, and these costs increased progressively over one year vs. non-MND patients [20]. The same study revealed that patients with Alzheimer's disease-related MND were more likely to develop delirium (51% vs. 24%) and die within one year (38% vs. 21%), all values being statistically significant [20]. Because delirium is considered a modifiable factor for prognosis in patients with MND and, frequently, a preventable one, the necessity for a correct diagnosis and early treatment, whenever delirium is identified, could have both short-term and long-term substantial benefits for the MND population. According to a systematic review, the prevalence of delirium superimposed on dementia varies between 22% and 89% in hospitalized patients aged over 65 [21]. Such high prevalence indicates, once again, that there is an acute need to detect and treat delirium actively in vulnerable populations.

This narrative review aims to identify the most valid interventions that could be included in case management for patients with delirium associated with MND. Because it was anticipated that the sources for this specific population might be scarce, the secondary objective included finding case management recommendations for patients with delirium in clinical populations, regardless of the organic or psychiatric background; these supplementary data were explored to see if they allow for being extrapolated to MND + delirium cases.

MATERIALS AND METHODS

This review explored three electronic databases (Pubmed, Cochrane, and Web of Science/Clarivate) for primary and secondary sources dedicated to this topic, using as keywords „delirium", OR „confusional episodes" OR „confusion superimposed on dementia"

AND „major neurocognitive disorder" OR „dementia" AND „pharmacologic*" OR „drug" OR „non-pharmacologic*" OR „psychosocial". All papers published in English between the inception of each database and September 2024 were screened for relevant data on pharmacological and non-pharmacological therapies targeting delirium. The results were grouped into two classes: (1) guidelines for patients with delirium in clinical populations and (2) case management for patients with delirium and neurocognitive disorders.

RESULTS

Based on the 36 sources reviewed in the first section, dedicated to delirium case management in clinical populations, and 15 sources (partially overlapping) focused on the evaluation and treatment of delirium superimposed on MND, the following results are presented.

Recommendations for case management in patients with delirium in the hospital settings

The papers reviewed and grouped in this section were distributed according to their main clinical use, i.e., (1) validated tools for screening and monitoring delirium and (2) therapeutic recommendations, since these two components were considered essential for clinicians to construct a case management plan (Fig.2).

Instruments for detection of delirium and its severity quantification in clinical population

There are many instruments for the diagnosis and monitoring of the presence of delirium, some of them being more widely used and largely validated than others. CAM was developed by an expert panel through a consensus process and has the clear advantage of its brevity and easiness of administration [22]. CAM may be applied in less than five minutes and consists of nine criteria derived from DSM III R, the main symptoms of delirium being acute onset and fluctuating course, lack of attention, disorganized thinking, and altered sensorium [22]. Different versions of this instrument emerged, such as CAM-ICU, which is dedicated to patients admitted to intensive care units and can be administered by non-specialists [23].

4-AT is another tool conceived for the screening of delirium symptoms in elderly patients and demonstrated good sensitivity and specificity for hospitalized subjects [24]. This instrument has four items, i.e., alertness, two items for cognitive function, and one for fluctuation in mental status [24].

The NEECHAM (Neelon and Champagne) Confusion Scale is also used for rapid assessment of delirium and has been proven to have good internal consistency and interrater reliability, and it also correlated well with the Mini-Mental State Examination [25]. This tool has nine scale items, is easy to use, and can be scored by nurses with minimal training. Also, NEECHAM may be administered initially and periodically to monitor mental status [25].

The Observational Scale of Level of Arousal (OSLA) can be used for the identification of abnormal levels of arousal associated with delirium and may be administered for screening and monitoring purposes [26]. The items refer to eye-opening, eye contact, posture, and movement, and the total score is between 0 and 15 [26].

Therapeutic and prophylactic recommendations in patients with delirium

Regarding the therapeutic approaches to delirium, after the diagnosis of an acute confusional state was formulated, the first step is to analyze the potential contributors to the onset of this disorder. Predisposing and precipitating factors must be evaluated, and modifiable etiological factors should be addressed [27]. The case management of patients with delirium has as the main objective the correction of the biological disequilibrium that fueled the confusional state, but the prevention of relapse is also of major interest since several causes may be chronic or recurrent. According to an expert consensus on delirium management, „early identification and intervention of subclinical delirium can reduce the risk of clinical delirium" [28]. Also, daily assessment of at-risk patients is important for early detection of delirium and adequate therapeutic interventions [28].

The most common precipitating factors that should be approached during the evaluation of a patient with delirium are infections, electrolyte imbalances, drugs (medications, drugs of abuse-intoxication/withdrawal, toxic substances), pain, organ failures, traumas, metabolic and nutritional imbalances, central nervous system (CNS) pathologies, environmental factors (e.g., sensorial deficits for patients with neurocognitive disorders, immobilization, sleep-wake cycle dysfunctions), but also psychiatric factors (e.g., anxiety, insomnia or depression in elderly patients with severe organic disease).

The ABCDE/ABCDEF protocol was suggested for managing delirium [29,30]. This acronym stands for (a) assessing, preventing, and managing pain, (b) both Spontaneous Awakening Trials and Spontaneous Breathing Trials (SAT and SBT, respectively), (c) choice of analgesia and sedation, (d) delirium - assessment, prevention, and management, (e) early mobility and exercise, and (f) family engagement and empowerment [31].

Another integrated approach algorithm for critically ill patients targeted the prevention of early deep sedation in the ICU as a risk factor for poor long-term outcomes, including mortality and neurocognitive disorders [32]. Flexible multimodal analgesia, with the minimal use of opioids, is the main concept of this algorithm, together with the use of the lowest doses of sedatives that are still efficient [32]. Promoting sleep, early mobilization, and improved communication of patients with medical personnel and caregivers are also included in the eCASH (early Comfort using Analgesia, minimal Sedatives, and maximal Human care) model as methods to avoid isolation, delirium, and a decreased quality of life [32].

Figure 2: Case management in patients with delirium in clinical populations

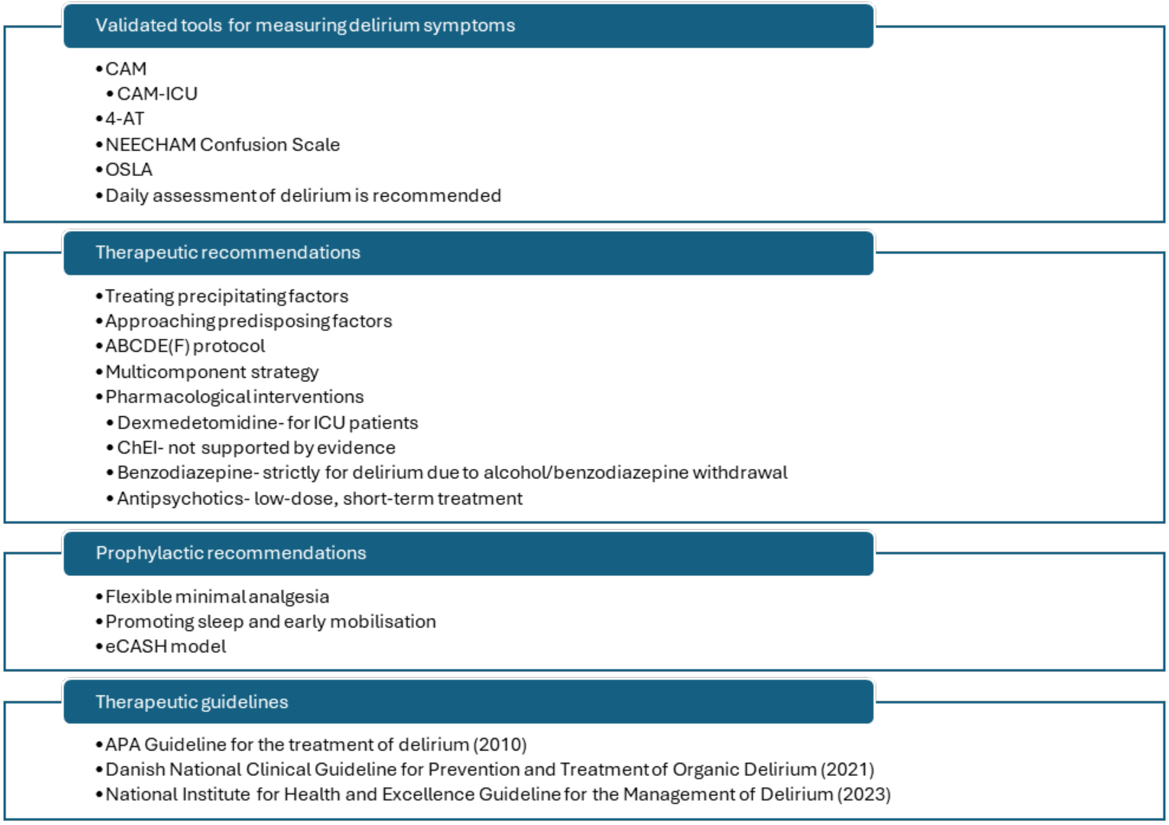

Validated tools for measuring delirium symptoms

- CAM
  - CAM-ICU
- 4-AT
- NEECHAM Confusion Scale
- OSLA
- Daily assessment of delirium is recommended

Therapeutic recommendations

- Treating precipitating factors
- Approaching predisposing factors
- ABCDE(F) protocol
- Multicomponent strategy
- Pharmacological interventions
  - Dexmedetomidine- for ICU patients
  - ChEI- not supported by evidence
  - Benzodiazepine- strictly for delirium due to alcohol/benzodiazepine withdrawal
  - Antipsychotics- low-dose, short-term treatment

Prophylactic recommendations

- Flexible minimal analgesia
- Promoting sleep and early mobilisation
- eCASH model

Therapeutic guidelines

- APA Guideline for the treatment of delirium (2010)
- Danish National Clinical Guideline for Prevention and Treatment of Organic Delirium (2021)
- National Institute for Health and Excellence Guideline for the Management of Delirium (2023)

APA= American Psychiatric Association, CAM= Confusion Assessment Method, ChEI= cholinesterase inhibitors, eCASH= early Comfort using Analgesia, minimal Sedatives and maximal Human care, ICU= Intensive Care Unit, NEECHAM= Neelon and Champagne, OSLA= Observational Scale of Level of Arousal

The multi-component strategy for delirium in the elderly includes six intervention directions, i.e., cognitive deficits, sleep deprivation, immobility, visual deficits, hearing impairments, and dehydration [33,34]. Early psychomotor and cognitive action may reduce the duration of delirium in critically ill patients [35].

Methods may be specific to the identified causal factors, such as the treatment of alcohol withdrawal, Wernicke encephalopathy, acute kidney injury, hepatic encephalopathy, diabetic ketoacidosis, etc. [36-40]. Exploring neurological disorders that may represent vulnerability factors for the onset of delirium is an important step in the prophylaxis of these patients [14,41,43]. In patients with neurocognitive disorders due to Alzheimer's disease, Parkinson's disease, or vascular diseases, the initiation of acetylcholinesterase inhibitors (AChEI) may help in reducing the risk of delirium onset/recurrence or its severity [44,45].

Dexmedetomidine was associated with an improved diagnosis for patients with delirium and mechanical ventilation admitted to ICUs [29]. Dexmedetomidine combined with analgesics may reduce the duration of delirium in patients with agitated forms of confusional states [29]. Dexmedetonimide is usually administered in the ICU for adult patients who require a level of sedation that allows for awakening as a response to verbal stimulation, meaning a Richmond Agitation and Sedation Scale (RASS) score between 0 and -3 [46]. Dexmedetomidine is administered by infusion and requires continuous monitoring of the blood pressure, cardiac rhythm, and respiratory functioning [46].

Impairment of cholinergic neurotransmission by high levels of circulating proinflammatory cytokines is considered a critical contributor to the onset of delirium; therefore, the administration of pharmacological agents that increase acetylcholine may be an adequate method to prevent or treat confusional episodes [47,48]. Following this line of reasoning, cholinesterase inhibitors (ChEI) were explored as symptomatic treatments for delirium, independent of the presumed pathophysiological substrate. However, a systematic review found only one study matching the inclusion/exclusion criteria (N=15 participants, out of which 7 have comorbid dementia) did not find a significant difference between rivastigmine and placebo regarding the duration of delirium, adverse events, use of rescue medication, mortality, and study discontinuation [49].

Benzodiazepines are used in cases of delirium due to withdrawal of substances in patients with substance use disorders, and where antipsychotics are contraindicated [29,50]. SAT, which is one of the core elements of the ABCDEF bundle previously mentioned, refers to the need to use a daily discontinuation strategy of sedatives or a continuous titration of these drugs in order to preserve the minimum level of sedation [31].

Antipsychotics are associated with variable results in this population. Based on the results of a meta-analysis and systematic review (n=5 randomized controlled trials, N=1750 patients), the use of antipsychotics in critically ill adults with delirium did not increase the

number of days without delirium or coma vs. placebo and did not show a significant effect on mortality, duration of mechanical ventilation, ICU stay or hospital length [51]. Another meta-analysis (n=19 studies) focused on the prevention and treatment of delirium by using antipsychotics showed these agents had no significant effect on delirium incidence, duration, severity, or ICU length of stay [52]. Yet another systematic review (n=14 sources) showed that uncontrolled studies reflect limited support for the use of low-dose, short-term treatment of delirium with several antipsychotics [53].

APA Guidelines for the Treatment of Delirium (2010) recommends (1) a close collaboration between the psychiatrist and other physicians to ensure appropriate care for these patients; (2) identify the precipitants; (3) initiate interventions for acute conditions; (4) provide disorder-specific treatment for co-occurring diseases; (5) monitor and ensure the safety of the patient; (6) assessment and monitoring of the psychiatric status; (7) assessment of individual and family psychosocial and social characteristics; (8) maintain the therapeutic alliance with the patient and his/her family; (9) education of the patient and family regarding the illness; (10) provide post-delirium management [54]. Environmental interventions, structuring and ensuring support for the patient, and support and education for the family are the most important non-pharmacological interventions, while antipsychotics (orally or intramuscularly, low doses, and for the shortest time needed), benzodiazepines (but there are only limited data, except for the treatment of delirium related to alcohol or benzodiazepine withdrawal), pro-cholinergic drugs (but there is a risk for cholinergic adverse events), vitamins (in specific cases, such as patients with hemodialysis, or alcohol use disorder), morphine or other opiates (in palliative care, and there is a risk of exacerbating the delirium) are considered the most important pharmacological strategies for the management of delirium [54].

The Danish National Clinical Guideline for Prevention and Treatment of Organic Delirium (2021) recommends non-pharmacological therapy to be initiated in all patients with a risk of delirium and in those with delirium (i.e., sensory optimization, orientation and cognitive stimulation, mobilization, nutrition, rehydration, supporting the circadian rhythm) [55]. Placing the delirious patient in a protected environment, actively involving relatives in the treatment of patients with delirium, and constantly reviewing medication for all these patients (i.e., adjusting, including stopping non-essential drugs), and avoiding routinely using antipsychotics for all delirious patients are also recommended by this guideline [55]. Benzodiazepines should not be used in this population except for palliative patients with agitation and terminal delirium or for patients in ICU where respiratory support and monitoring are accessible [55]. Melatonin is not considered by the Danish guidelines a useful intervention, but electroconvulsive therapy may be recommended in refractory cases where other therapeutic options have failed [55].

The National Institute for Health and Excellence (NICE) guideline for the management of delirium (updated January 2023) recommends, for the prevention of delirium, a multi-component approach conducted by a multidisciplinary team, addressing the cognitive impairment and/or disorientation by environmental measures, and address all potential triggers for confusion [56]. Treating delirium involves management of the underlying causes, ensuring communication and reorientation, de-escalation of the situation using verbal and non-verbal techniques, followed, if necessary, by short-term haloperidol, and, if there is still little or no improvement, re-evaluation of the potential precipitants and vulnerability factors, dementia included [56].

Case management for patients with delirium superimposed on a major neurocognitive disorder

The distribution of the sources retrieved during the literature search for the case management of patients with delirium overimposed on MND was similar to that mentioned in the previous section for delirium in clinical populations, and the results are synthetically represented in Figure 3.

Diagnostic aspects of delirium in patients with MND

Regarding the diagnosis aspects, an analysis of the potential biomarkers for delirium superimposed on MND showed that genetic, epigenetic, and pharmacogenetic variables might be used in the near future to construct personalized case management for these patients [57].

Gene-gene, protein-protein, gene-drug, and gene-miRNA interactions have been detected as potentially significant in this specific population, with several essential genes, like BDNF (brain-derived neurotrophic factor), APP (amyloid beta precursor protein), DNMT1 (DNA methyltransferase 1), APOE (apolipoprotein E), and TNF (tumor necrosis factor) presenting gene x drugs interactions also relevant for this pathology [57]. However, there is no single independent, validated biomarker for delirium, which leaves, for now, at least, the diagnosis to the clinicians [58].

A review dedicated to the best tools for identifying delirium in patients with MND concluded there is no concordance between validated diagnostic criteria, psychometric instruments, and therapeutic guidelines, which makes it difficult for clinicians to identify and treat this pathology appropriately [59]. Taking into account such limitations, CAM and CAM-ICU are the most recommended instruments for the diagnosis of delirium in dementia, based on the review of clinical studies [21,59]. Other instruments, such as 4-AT or 4-DSD (Delirium superimposed on dementia), have also been studied in this population, but there is less data to support their clinical use [59]. The 4-AT may be beneficial for the correct and timely detection of delirium in this population because it can help in the exclusion of cognitive impairments in emergency rooms and hospital admissions [33]. Therefore, as a screening tool, 4-AT is a helpful instrument that requires little time for administration.

The 4-DSD has four items and is derived from 4-AT, but the scoring algorithm is different, and several items have a changed content compared to the initial tool [59]. This instrument assesses the level of alertness, altered brain function, attention integrity, and acute change and fluctuation in mental status [59].

The use of the OSLA in combination with another very simple attention test (that requires the patient to identify a certain letter in a sequence) correctly identified more than 90% of the cases of delirium superimposed on dementia [33,60].

Figure 3: Case management of patients with delirium superimposed on major neurocognitive disorder

Diagnosis and monitoring

- genetic, epigenetic, and pharmacogenetic variablesfor delirium + MND are still in early phase of validation
- CAM, CAM-ICU
- 4-AT
- 4-DSD
- OSLA

Prophylactic interventions

- Identification and treatment of precipitating factors, including BPDD
- Multi-component approach
- Involving the family members in the prevention of delirium associated to MND
- Multiprofessional evaluation on a regular basis in high-risk patients with MND
- Efficient collaboration between medical team members
- Melatonin, ramelteon

Therapeutic interventions

- Pharmacological interventios are second-line
  - Melatonin
  - Antipsychotics (but NOT in LBD!)
- First line is represented by non-pharmacological interventions
  - Cognitive training
  - Occupational therapy
  - Nursing interventions

BPDD= behavioral and psychological disturbances in dementia, CAM= Confusion Assessment Method, DSD= Delirium Superimposed on Dementia, ICU= Intensive Care Unit, LBD+ Lewy body dementia, MND= major neurocognitive disorder, OSLA= Observational Scale of Level of Arousal

Preventative and treatment-focused recommendations in patients with delirium and MND

Mentioning that there is no „gold standard" for the diagnosis and treatment of delirium in patients with MND, a systematic review showed that a multi-professional evaluation regularly of high-risk patients with MND is recommended as a step toward the prevention of delirium [61]. An efficient collaboration between multiple members is also needed within the team, which is involved in case management [61]. Eliminating the risk factors for delirium in this vulnerable population is essential [61]. Screening for all the major causes of delirium in patients with MND is strongly recommended and could have significant benefits [33]. Constipation, medications with central nervous system adverse events, infections, vascular factors, electrolytic imbalance, low mobility, malnutrition, pain, incorrect sleep hygiene, and urinary retention should be screened for in patients with MND and sudden clouding sensorium [33].

A non-pharmacological approach is initially indicated in all individuals presenting delirium and MND, based on decreasing the number of medications used, maximization of family support, orienting techniques, adequate hydration, and correction of visual and hearing deficits, whenever possible [21]. However, the controlled studies did not show a consistent effect of environmental strategies in preventing delirium in patients with MND [21].

Pharmacological therapies are considered second-line interventions and applied only when non-pharmacological strategies have failed or are insufficient [61]. The use of melatonin and antipsychotics to treat delirium in MND is not supported enough by evidence [62].

As for the prevention of delirium, the identification of the precipitants and their treatment, initiation of multicomponent interventions, prevention of complications, and monitoring the resolution of the core symptoms are the main directions of case management in patients with delirium and MND [58,62]. The participation of the family members is strongly encouraged by the case managers because the evolution of the patients is often unpredictable even after hospital discharge, and caregivers may play an important role in re-orienting a confused individual by offering him or her a point of reference [58]. Re-assessment of precipitating and vulnerability factors is the first step, and only after that can non-pharmacological and pharmacological treatment be considered [62].

Special care should be given to patients with MND and BPDD, a sub-population that is more vulnerable to the onset of delirium than patients with dementia but without BPDD [63]. The recovery of delirium is slower in patients with both dementia and BPDD; therefore,

identifying early signs of delirium, like sleep impairments, mood lability, and psychomotor agitation, should trigger increased attention and more intensive monitoring [63].

Cognitive training was explored as a possible intervention for MND, to facilitate neuroplasticity in these patients [64]. Based on several similar cognitive features between dementia and MND, the use of recreational activities to enhance attentional abilities and offer cognitive stimulation was suggested to reduce delirium severity and duration, improving quality of life and reducing healthcare costs [64]. A pilot study dedicated to this intervention (N=16 participants) demonstrated a greater decrease in physical function and mental status during the 30 days of monitoring vs. the control group [65]. Delirium, but also the severity of delirium and attention had a trend toward significance in the study vs. control group [65].

Occupational therapy, based on preliminary evidence, can be another option for patients with delirium and MND [66]. A case series (N=6 patients with dual disorders) reported positive results of occupational therapy conducted twice daily for a total of 40 minutes until the delirium was remitted [66].

Nursing interventions for delirium over-imposed on MND were associated with good results [67]. A 10-step intervention was reported to help in the dissolution of delirium in a patient with Alzheimer's dementia: interdisciplinary communication with care providers, reduction of medications administered, communicating calmly and slowly with the patient, avoidance of physical restraints, re-hydration, regulation of the sleep-wake cycles, assess and prompt treatment of pain, education of the family and encouraging family members to participate in care, mobilize the patient as soon as possible, and regulation of the excretory function [67].

Melatonin and ramelteon may be recommended in older patients for the prevention of delirium when admitted to an acute geriatric facility [68,69]. According to the results of a multicenter, randomized study (N=67 patients admitted in ICUs), ramelteon administered nightly was associated with a lower risk of delirium, even after controlling the risk factors, vs. placebo [69].

Antipsychotics are frequently used in clinical practice for this population, but a trial with these medications should be recommended only for a short time, in low doses, and under careful supervision [33]. Special care should be taken when administering antipsychotics in patients with MND due to Parkinson's disease or Lewy Body Dementia [33].

DISCUSSION

The existing evidence reviewed in the previous chapter supports the use of validated instruments for (1) screening in vulnerable populations, patients with MND included; (2) supporting the clinical diagnosis of delirium in the absence of validated biomarkers; (3) monitoring the evolution of delirium during treatment, until the complete resolution of its symptoms [22-26, 33,61]. Also, preventative measures could be applied in vulnerable populations, which may lead to a reduction in the risk of delirium onset, i.e., treating risk factors, close assessment of the clinical status, multi-component interventions, and even limited data supporting pharmacological interventions for short periods of time [58,62,63]. Therapeutic measures that can be initiated in cases of delirium superimposed on MND are mainly non-pharmacological since, for the pharmacological agents, there is only limited data to support their use [29,33,46-50,53-56].

Limitations of this review are related to its narrative nature, and, as a result, not all significant sources may have been found and reviewed. Also, due to the narrative character of the review, no quality of data was assessed, and all sources were treated equally. Finally, there is a paucity of data derived from good-quality trials focused on delirium overimposed on MND, which limits the validity of the review's results, since most data are extrapolated from studies with patients presenting delirium in the clinical population, regardless of the causal factors.

The strengths of the present review are related to the enlarged focus on both pharmacological and non-pharmacological components of case management and the integration of structured assessment instruments into the clinical practice in vulnerable populations.

Future directions of research should include better-quality trials with homogenous populations, e.g., delirium with onset in patients with MND due to Alzheimer's disease, or the prevention of delirium in patients with MND due to Parkinson's disease, but also active comparators and placebo for the pharmacological trials, and a large number of participants with long-period of follow-up, in order to assess the risk of recurrence.

CONCLUSION

When formulating the case management plan for patients with delirium superimposed on MND, the clinician should take into account (1) the need for a multidisciplinary team since the precipitating and vulnerability factors may be complex and have intricate relationships; (2) regularly and frequently assess the patient using instruments like CAM, 4-AT, OSLA, or 4-DSD; (3) include the family in the monitoring of the patients, whenever possible; (4) non-pharmacological interventions, such as cognitive training and occupational therapy, nursing techniques, multi-component approaches, are first-line strategies; (5) pharmacological interventions are considered second-line, and they are administered only for short time and low doses; (6) prophylaxis of delirium in vulnerable populations, MND patients included, remains the single most important way to reduce the complications associated with this pathology.

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ORIGINAL ARTICLE

Gender-Dependent Associations Between Digit Ratio and Genetic Polymorphisms, BMI, and Reproductive Factors

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Abstract: The digit ratio (2D:4D) has been associated with prenatal hormonal influences and various traits and pathologies. This article explores the relationship between 2D:4D and a series of common polymorphisms and Torque Teno Viruses. In this study, 120 healthy participants were included. The IGF2 Apa I, ACE I/D, INS -23 Hph I, VDR Fok I, VDR Apa I, VDR Taq I, AT1R A1166C polymorphism were genotyped by PCR-RFLP technique, and the IL-6 -174 G/C polymorphism by tetra-primer ARMS-PCR. The presence of TTV was identified by a hemi-nested PCR technique. Haplotype analyses were performed using the SHEsis software. The average 2D:4D values were similar for men and women. Overweight men presented higher 2D4D ratios than normal-weight women ($p<0.05$). Lower 2D:4D values were recorded in women with pregnancy loss or one child or none ($p<0.001$). Men with lower 2D4D ratios reported a higher number of children ($p<0.001$). The IGF2 GG and ACE DD were associated with a higher digit ratio in all subjects and in the women's subset. A significant association was found in men between 2D:4D and the INS-23 Hph I – IGF2 Apa I T-G haplotype ($p<0.01$). The data obtained in this study indicate a sexual dimorphism for the digit ratio. The associations between 2D:4D and the genetic polymorphisms studied could be influenced by gender.

Keywords: 2D:4D, Insulin-IGF2 haplotype, ACE I/D, parity, BMI

INTRODUCTION

The ratio between the length of second and fourth digits (2D:4D) is determined prenatally, and remains stable throughout life, serving as a marker of early maternal hormonal influences [1,2]. This length is influenced by the ratio between testosterone and estrogen during the fetal period. A smaller digit ratio has been associated with a higher concentration of fetal testosterone and a lower concentration of fetal estrogen [3]. Additionally, the 2D4D ratio can be influenced by the density of receptors for testosterone and estrogen, which is greater for 4D than 2D [2], and also by the maternal environment [4]. Nevertheless, testosterone production during the first years of life could influence secondary sexual differences developing before puberty as 2D4D ratio [4]. Furthermore, the digit ratio was correlated with specific genetic particularities of the child such as a polymorphism in the androgen receptor gene [5]. Therefore, lower digit ratios are common in males whereas higher values are identified in females [6].

The study of the digit ratio offers a compelling gateway into understanding the intricate interplay of genetic, hormonal, and environmental factors from the intrauterine environment. These interactions also may shape an individual's predisposition for different physiological (e.g. height, weight, BMI, athletic performance, reproductive success) [7,8,9,10] or psychological traits (e.g. academic performance) [11]. In addition, the 2D:4D has been associated with various categories of pathologies, ranging from autoimmune diseases (e.g. vitiligo [12], thyroid disease [13]), metabolic disorders [14], and malignant tumors [15] to mental disorders such as autism, ADHD, and depression [16]. A series of polymorphisms such as Insulin, IGF2, angiotensin I-converting enzyme (ACE), angiotensin II type 1

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receptor (AT1R), vitamin D receptor (VDR), and IL-6 genes may imply fetal growth and development and have been associated with some of these pathologies [17,18,19].

Insulin-like growth factor two (IGF2) is a key protein regulating growth, secreted, mostly via the placenta, and after birth by hepatocytes [20]. INS-23Hhp influences IGF2 transcription in the human placenta and it may be implicated in insulin-regulated intrauterine growth and insulin secretion in adults [21].

ACE, a zinc metallopeptidase, plays a pivotal role in the renin-angiotensin-aldosterone system (RAAS), responsible for homeostasis, blood pressure regulation, and water balance in the body. ACE I/D polymorphism has been associated with different pathologies, including pregnancy complications and infertility [22]. Angiotensin II type 1 receptor (AT1R) is a well-reported biomarker for hypertension and AT1R A1166C was strongly associated with the risk of developing pregnancy-induced hypertension [23].

Vitamin D (VD) is a molecule involved in regulating various processes, including phosphate and calcium metabolism, bone mineral density, and immune response [24], but also plays an important role in fetal development. Low maternal VD levels during pregnancy are associated with various adverse obstetric outcomes, such as gestational diabetes mellitus [25]. Vitamin D, as cortisol and sex hormones are all derived from cholesterol, creating an intricate network of interactions. Cortisol and testosterone concentrations might be influenced by vitamin D [26] through the activity of the vitamin D receptor, which is present in Leydig cells [27] (that plays a role in testosterone synthesis) and regulates the activity of the adrenal gland [28] (to produce cortisol). Therefore, three of the most studied VDR polymorphisms may be of interest: VDR Fok I (C/T), VDR ApaI (A/C), and VDR Taq I (C/T). Additionally, a low cortisol reactivity was associated with a low digit ratio [29]. Nevertheless, no significant association between 2D:4D and vitamin D has been reported in the literature so far.

Having pro-inflammatory and anti-inflammatory properties, interleukin 6 (IL-6) plays a major contribution in modulating the immune response, being reported as a biomarker of inflammation and intraamniotic infection [30]. Moreover, IL-6 was associated with right 2D:4D in girls, suggesting that the gender of the subjects and the hand's position in the sagittal plane influence the correlation between IL-6 and digit ratio [31]. The synthesis of pro-inflammatory cytokines, including IL-6, may be influenced by Torque Teno viruses (TTV) [32]. TTV are non-enveloped viruses that belong to the Anelloviridae family that are widespread globally [33]. They have multiple transmission routes, including transplacental transmission, which may have implications in prenatal development, although studies are not consistent on this matter [34, 35]. TTV may represent a potential biomarker of immune function and for some diseases [36].

This article aims to investigate the associations between digit ratio, gender, certain characteristics (such as height, weight, BMI, and reproductive success), and a series of common polymorphisms.

MATERIALS AND METHODS

Selection of subjects.

This study included 120 healthy, unrelated, volunteers (61 women, 59 men), aged between 42 and 60 years (52.73 ± 3.6 years). All participants were informed about the purpose of the study. Selection criteria for the participants included alcohol intake and smoking. Alcohol intake consisted of no more than two standard drinks a day for men and one standard drink a day for women. A beverage is considered alcoholic if it contains up to 10 g of ethanol, according to the World Health Organization [37]. As for smoking, the threshold was 20 cigarettes per day, based on the participants' answers.

Methods and Protocols.

The length between the second digit and the fourth digit was measured by the caliper three times, therefore the value obtained is the average of these three consecutive measurements. It was measured on the ventral surface of the left hand, from a midpoint on the metacarpophalangeal flexion crease to the tip of the digit. The measurements were considered valid if the difference between the maximum and minimum values measured for a finger was less than 5 mm. If the difference was greater, the measurement process was repeated for that participant. The analysis was restricted to subjects with a digit ratio range of 0.96-1.005, with more extreme values excluded.

A peripheral blood sample (300 mL) was collected from each subject in an EDTA tube and genomic DNA was extracted using the Promega Wizard® Genomic DNA Purification Kit. The presence of TTV was identified using a hemi-nested PCR protocol [38]. The ACE I/D polymorphism (rs1799752) was identified by PCR with a primer set for insertion [39], while the PCR-RFLP technique was used for genotyping the polymorphisms IGF2 Apa I (820 G/A, rs680) [40], INS -23 Hph I (-23 A/T, rs689) [41], AT1R A1166C (rs5186) [42], VDR Fok I (C/T, rs2228570), VDR Apa I (A/C, rs7975232), VDR Taq I (C/T rs731236) [43, 44]. IL-6 -174 G/C (rs1800795) was genotyped by the tetra-primer ARMS-PCR technique [45]. Restriction products were resolved by electrophoresis.

Statistical Analysis.

The distribution of qualitative data in subgroups of subjects was analyzed with Chi-square or Fisher tests. The outlier values for 2D:4D were considered values that exceed the mean value ±3 standard deviations. The quantitative values were analyzed using ANOVA, one-way ANOVA with post-hoc Tukey HSD test. Simple linear regression analyses were used to assess the variation in anthropometric parameters with variation in 2D:4D. The statistical analyses were performed using the R Studio software, version 2023.12.1.+402. Haplotype analyses were performed using the SHEsis software [46]. Differences were considered statistically significant if $p<0.05$.

RESULTS

The average age (52.73 ± 3.6; 42-60), weight (71.71±5.06; 63-86), height (1.70±0.02; 1.65-1.75) and BMI (24.96±1.78; 21.89-29.41) and 2D/4D (0.9858 ±0.01; 0.97-1.01) of subjects were estimated. The characteristics of the investigated subjects are presented in Table 1.

We have identified no outliers for 2D:4D in the case of men or women investigated in this study. Men have a higher digit ratio than women, but this difference was not statistically significant (0.9871 vs 0.9845, p >0.05).

Table 1: Characteristics of subjects

Characteristics	Values
Male / Female	59 /61
Age: men vs women	52.89 vs 52.57
Weight: men vs women	74.10 vs 69.39^{b}
Height: men vs women	1.70 vs 1.69
BMI: men vs women	25.72 vs 24.21^{b}
Smokers: men vs women	24 vs 17
Alcohol drinkers: men vs women	12 vs 3^{a}
2D/4D: men vs women	0.9871±0.007 (0.97-0.998) vs 0.9845 ±0.009 (0.97-1.005)
TTV positive samples: men vs women	45 vs 39
INS -23 Hph I: AA/AT/TT	49/66/5
IGF2 Apa: GG/GA/AA	55/57/8
VDR Fok I: CC/CT/TT	53/50/17
VDR Apa I: AA/AC/CC	41/53/26
VDR Taq I: TT/TC/CC	25/52/43
ACE I/D: II/ID/DD	35/52/33
AT1R A1166C: AA/AC/CC	57/48/15
IL-6 -174G/C: GG/GC/CC	75/38/7

^{a} p<0.05; ^{b} p<0.0001

The IGF2 GG and ACE DD were associated with a higher digit ratio in all subjects and also in women (Table 2). No other genotype shows a significant association with the digit ratio.

Table 2: Characteristics associated with 2D:4D

Subgroup compared	2D: 4D average (interval)
All: IGF2 GG vs. (GA+AA)	0.9877±0.0074 (0.97-1.002) vs 0.9841±0.008 (0.97-1.005)^{b}
Women: IGF2 GG vs (GA+AA)	0.9873±0.007 (0.97-1.002) vs 0.9823±0.009 (0.97-1.005)^{a}
Men: IGF2 GG vs (GA+AA)	0.9881±0.006 (0.973-0.998) vs 0.9861±0.006 (0.97-0.997)
All: ACE DD vs. II	0.9893±0.007 (0.97-1.002) vs 0.9843±0.009 (0.97-1.005)^{b}
Women: ACE DD vs. II	0.9899±0,008 (0.97-1,002) vs 0.9811±0.009 (0.97-1.005)^{c}
Men: ACE DD vs. II	0.9885±0.005 (0.98-0.996) vs 0.9878±0.007 (0.97- 0.998)
All: ACE (DD+ID) vs II	0.9844±0.008 (0.97-1.005) 0.9843±0.009 (0.97-1.005)
Women: ACE (DD+ID) vs I	0.9823±0.008 (0.97-1.005) vs 0.9811±0.009 (0.97-1.005)
Men: ACE (DD+ID) vs ACE II	0.9866±0.007 (0.97-0.998) vs 0.9877±0.007 (0.973-0.998)

^{a} p<0.05; ^{b} p<0.01; ^{c} p =0.006

The INS and IGF2 (11p15.5) polymorphisms or VDR (12q13.11) polymorphisms were mapped in close proximity. As a consequence, we evaluated the effects of Ins -23 Hph - IGF2 Apa I and VDR Fok I – Apa I – Taq I haplotypes on 2D:4D. The INS-23 Hph I – IGF2 Apa I T-G haplotype was found to be significantly associated with 2D:4D in men (p<0.01), but not in women (p>0.05). No statistical associations were identified between the digit ratio and VDR Fok I – Apa I – Taq I haplotypes (Table 3).

Twenty-four (39.34%) women reported at least a pregnancy loss. The 2D:4D value of these women was lower compared to those who did not report such an event (0.9799 vs 0.9876, p<0.001). The height in these subsets of women was similar (1.691 vs 1.697, p<0.05) (Figure 1). The average age at which they had their first child was similar between those who had one child and those who had multiple

children (22.93 vs 22.2, p>0.05).

The 2D:4D values were lower in women who had no children compared to those who had children (p <0.001), who had at least two children (p <0.001), or one child (0.9809 vs 0.9865, p < 0.3). In the case of men, the differences identified were significant between men who had at least two children and those who had one child (p<0.001) or who did not have children (p<0.02). In the subset of subjects who did not have children, the lowest average 2D:4D values were recorded in men compared to women (0.9883 vs 0.9809, p< 0.001). The height of men and women stratified by the number of children did not show any statistically significant differences (Figure 2).

Table 3: Association of INS-IGF2 and of VDR haplotypes with 2D:4D

Polymorphisms	Haplotypes	The total count in women's sublot	The total count in men's sublot
VDR Fok I – Apa I – Taq I	CAC	34	31
	TCT	20	7
	CCT	18	24
	TAC	15	22
	CAT	15	7
	CCC	13	14
	TCC	5	4
	TAT	-	9
INS -23Hph I – IGF2 Apa	AG	51	49
	TG	34	33[a]
	AA	35	29
	TA	-	7

(Haplotypes with frequency <0.03 were not taken into consideration; [a] p <0.01)

Figure 1: Analysis of 2D:4D ratio and number of children by gender

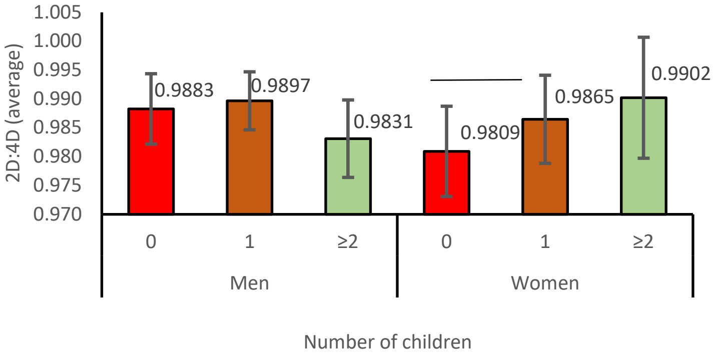

Figure 2: Analysis of height and number of children by gender

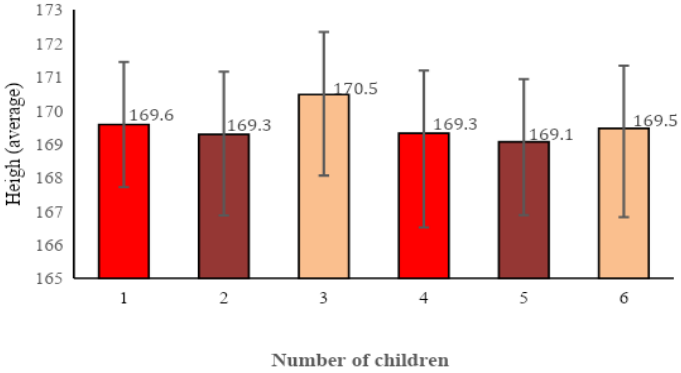

The 2D:4D values were higher in overweight subjects than in normal-weight participants (0.984 vs 0.988, p<0.008). The weight (74.1 vs 69.39 kg, p<0.0001) and BMI (25.72 vs 24.21 kg/m^2, p<0.0001), but not height (170 vs 169 cm, p>0.05), were significantly higher in men compared to women. Each gender was subsequently stratified according to BMI into normal-weight and overweight subgroups. There was only a significant difference between the 2D:4D values of overweight men and normal-weight women (0.9884 vs 0.9837,

p<0.05). No significant results were found when the male and female subsets were stratified according to height.

There appears to be a positive correlation between 2D:4D and BMI, regardless of gender (F=0.02) (Figure 3), and an inverse correlation between the 2D:4D and height of men (F = 0.03) (Figure 4). However, the proportion of 2D:4D variance that may be predicted based on BMI (R2=0.047) or height (R2 =0.08) appears to be weak.

The tested genetic polymorphisms were not associated with the height or BMI of men and women (p>0.05). These variables were similar in women stratified by parity.

Figure 3: Linear regression for 2D:4D and BMI (both genders)

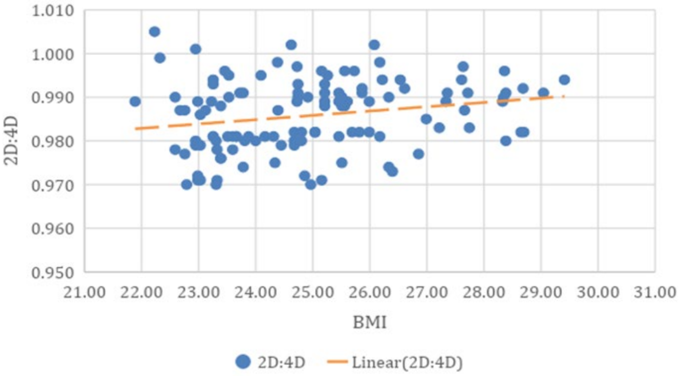

Figure 4: Linear regression for 2D:4D and height of men and women

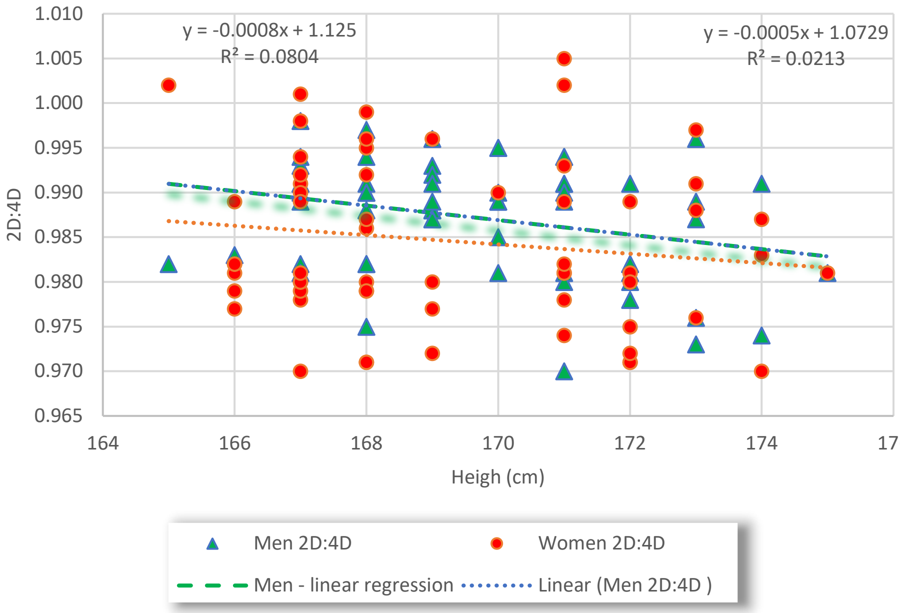

DISCUSSION

The digit ratio is determined during the prenatal period and in general remains stable throughout life [1]. A study that analyzed the 2D:4D ratio in 327 deceased fetuses (14 to 42 weeks old) concluded that the values were lower than those reported for children and adults in both sexes, suggesting 2D:4D could increase after birth [48]. In addition, a study has shown that this ratio can change, to a significantly reduced extent, during childhood, between the ages of one and five years [47]. Therefore, age may influence to some extent the length between the index and ring fingers. In the present study, the 2D:4D values were not correlated with age, neither at the level of the entire group of adult participants nor at the subgroup level by gender.

The difference between genders appears to be moderated by the type of finger length measurement and by the hand that was measured. Some studies suggest that there are greater differences between genders when measurements are taken on the right hand, which would better reflect prenatal hormone exposure [49,50]. In our sample, measurements were taken on the left hand, for which we obtained higher average values in men than in women, but the differences were not significant (0.9871 vs 0.9845, p >0.05). This result is contrary to the results reported by other studies [3,4]. In a meta-analysis that studied the representation of women in politics and was based on data collected in an online survey, the reported data for Romania indicated higher values in women than in

men for both the left hand (1 vs. 0.985) and the right hand (0.997 vs. 0.986) [50].

In contrast to the 2D:4D, height, weight, and BMI are characteristics that change postnatally, influenced by both genetic and environmental factors. Weak correlations between 2D:4D and these variables were identified in this study. Studies are not consistent on this matter. For example, 2D:4D was positively related to BMI in a very large sample of adults [51]. On the contrary, other studies have reported no significant correlations between these variables and 2D:4D [52,53].

The analysis of anthropometric data we performed showed that the values for weight and BMI were higher in men than in women. Moreover, we identified a significant difference between the 2D:4D values of overweight men and normal-weight women (0.9884 vs 0.9837, $p<0.05$). In the literature, significant positive correlations have been reported between higher (feminized) digit ratios and postnatal parameters (body weight, BMI, and waist and hip circumference) [54,55]. There were no statistically significant differences between height of men and women ($p>0.05$). The linear regression showed a negative correlation between 2D:4D and the height for males ($F = 0.03$), but not for females or both genders. One study reported that body height was negatively correlated with right- and left-hand digit ratios and the correlations were significant for the left-hand digit ratio in men and for the right-hand digit ratio in women [7].

Our data suggested a significant association between 2D:4D and reproductive success which is influenced by gender. Thus, lower average 2D:4D values were recorded in women who reported a pregnancy loss or who had no or one child ($p <0.001$). In the case of men lower 2D:4D values were associated with a higher number of children. Our results are in agreement with some results reported previously in the literature [9,10].

The minor allele frequencies for IGF2 Apa I (MAF A: 0.3 vs. 0.24) [56, 57] and ACE I/D (D: 0.49 vs 0.51) [58] in our lot are consistent with those previously reported in the Romanian population. Previous studies have reported that the IGF2 G allele may contribute to weight gain and eating disorders, and BMI [59]. Another study, which investigated multiple single nucleotide polymorphisms in the IGF2 gene region, concluded that IGF2 genetic variation is a significant determinant of body weight in middle-aged males [60]. The data we have obtained suggest that nearby genes, IGF2 or Insulin, could influence the digit ratio. The IGF2 GG genotype was associated with a higher digit ratio, especially in women ($p<0.05$) whereas the Insulin - IGF2 T-G haplotype was significantly associated with 2D:4D, but only in men. The closely linked IGF2 and Insulin genes have been previously associated with various conditions such as obesity, diabetes, metabolic syndrome, and coronary heart disease [61, 62]. Genetic imprinting influences IGF2 expression [63] and the association between the parent-of-origin allele and various phenotypes. The design of our study did not permit the investigation of IGF2 parent-of-origin.

ACE converts angiotensin I into angiotensin II and most of its functions are mediated by AT1R. ACE D was associated with a higher enzymatic activity of ACE [64]. Carriers of ACE DD have higher values of 2D:4D than ACE II in our lot of subjects ($p<0.01$) and especially in women ($p<0.01$). In a previous study, it was also reported that ACE DD individuals have higher 2D:4D than carriers of ACE II [65]. ACE I/D was also associated with various diseases, such as cardiovascular diseases and hypertension [66] or physical endurance or competitiveness [67]. The association may bias the results we have detected in our healthy subjects. Given the fact that ACE I/D polymorphism and 2D:4D ratio might play a role in cardiovascular pathology, our subjects (42-60 years old), although clinically healthy, may still have a predisposition to develop these diseases. This predisposition might influence the results obtained in this study, potentially causing a bias. The results could also be affected by the small number of subjects included in the study ($n=120$), as well as by measurement errors related to the length between the index and ring fingers.

Although no significant association was identified between the digit ratio and Torque Teno viruses, the high prevalence of TTV in the population was confirmed, being present in 70% of the subjects included in the study, in line with previously obtained results [32,68].

CONCLUSION

In our dataset, the digit ratio was similar in men and women. The data obtained in this study indicated that INS - IGF2 T-G haplotype, ACE I/D, pregnancy loss, number of children, and BMI are associated with digit ratio. These relations could be also influenced by the gender of the subject. Additional studies are needed to bring new data regarding the relationships between 2D:4D and the genetic polymorphisms of anthropometric characteristics to see if the results we obtained in this study could be replicated. This data could contribute to a broader understanding of how genetics can shape developmental outcomes through prenatal hormones.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, N.S., R.S., R.M., and D.C.; methodology, O.C.; software, R.M. and M.T.; validation, M.I.N., M.L.C., and C.I.; formal analysis, M.T and M.I.N.; investigation, N.S., D.C., L.B., O.A.; resources, L.B., D.C.; data curation, S.R. and O.A..; writing—original draft preparation, N.S., E.S., R.M, O.C.; writing—review and editing, D.C., M.T, I.R., M.L.C.; visualization, M.T.; supervision, D.C. and O.C.; project administration, L.B, and D.C.; funding acquisition, L.B. and S.R.. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the National Research and Development Institute for Food Bioresources – IBA Bucharest (protocol code 285/20.03.2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Non-alcoholic Steatohepatitis Diagnosis and Treatment – Current Concepts

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Abstract: NAFLD (Non-alcoholic fatty liver disease) is a condition of high importance due to its increased prevalence, estimated at 24% of the global population, and its potential progression to advanced liver disease, cirrhosis, and hepatocellular carcinoma. One-quarter of NAFLD patients have NASH (non-alcoholic steatohepatitis), which is histologically described as liver steatosis, inflammation, fibrosis, hepatocyte injury, and death. Metabolic disruptions, such as obesity, diabetes, and dyslipidemia, are considered to have a great impact on the development of NASH. Patients may have NAFLD for a long time before progressing into NASH, which highlights the necessity of periodic imaging and biochemical evaluation of the liver, and also presents an optimal window of therapeutic intervention. The challenge in developing treatment for NASH stems from the slow progression of the disease and the reliance on sequential biopsies as the diagnostic and staging tools. Several non-invasive tests have been developed as potential future alternatives to the current invasive gold standard. This review aims to assess the current available methods of diagnosis and treatment targeting inflammation.

Keywords: NAFLD; NASH; MASH; liver steatosis; liver fibrosis; metabolic syndrome; lanifibranor; obeticholic acid; semaglutide

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is an advanced form of non-alcoholic fatty liver disease (NAFLD), characterized by liver inflammation and hepatocyte injury, that is progressively evolving due to the accumulation of fat in the liver [1]. NAFLD results from an unhealthy diet and sedentary lifestyle, without high alcohol consumption, and is usually a silent disease before the advanced stages, including cirrhosis, liver failure, and hepatocellular carcinoma [2].

The highest rates of NAFLD were reported in the Middle East (32%) and South America (31%), followed by Asia (27%), USA (24%) and Europe (23%), whereas the lowest rates were in Africa (14%) [3-5]. It is estimated that 25% of the patients with NAFLD are thought to have NASH and a quarter of them are diagnosed with cirrhosis [3-5].

Recent studies show the association between metabolic disruptions and NASH, which is why a new medical terminology has emerged introducing the term MASH as metabolic-associated steatohepatitis. A bidirectional correlation exists between NAFLD and metabolic syndrome (obesity, insulin resistance, and dyslipidemia). Also, patients with type 2 diabetes mellitus (T2DM) have a greater risk of developing NAFLD, with a prevalence estimated at 57.80% (95%CI: 53.88% - 61.62%) [6]. NASH has been reported in 65.26% (95% CI: 51.73%-76.71%) of the patients with type 2 diabetes mellitus, who underwent liver biopsy [6]. In a meta-analysis of more than 8.5 million people from 22 countries, the results show that 80% of the patients with NASH are overweight or obese, 72% have dyslipidemia, and 44% are diagnosed with type 2 diabetes mellitus [3].

The evolution of NASH is non-linear. Some patients progress to cirrhosis, and most of them develop cardiovascular disease, but some patients, even those with fibrosis, may spontaneously regress. Cardiovascular mortality is the most common cause of death in patients with NASH. In contrast, in the U.S., due to decompensated liver disease, NASH is among the top three indications for liver transplantation [6,7].

In a study on patients with liver cirrhosis, secondary to HCV infection or NASH, over 4 years, it was estimated that the annual cumulative risk of HCC (hepatocellular carcinoma) was 2.6% per year in patients with NASH cirrhosis compared with 4.0%

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per year in those with HCV cirrhosis [8]. These figures suggest that NASH carries a risk of developing HCC that rivals the risk in patients with HCV-cirrhosis. As such, patients with NASH cirrhosis should be closely monitored for HCC. The decrease in HCV-related transplantation due to the significant effect of the HCV therapies placed NASH and ALD (alcohol-related liver disease) as the most common etiologies of liver disease among liver transplant waiting list registrants without HCC, and NASH is becoming a leading indication in patients with HCC [9].

MATERIALS AND METHODS

This review aims to assess the currently available methods of diagnosis and treatment targeting liver inflammation and fibrosis, named NAFLD, NASH, or similar.

We have selected over 200 English, French, and Romanian studies highlighting information on diagnosing and treating these hepatic diagnoses. For the development of this review, we further selected the articles based on our inclusion criteria, consisting of diagnosis and treatment-focused articles and recently published articles to ensure the current advancements in the field.

Databases such as PubMed, Up-to-date, Medline, and NCBI were used to perform the search using keywords: NAFLD, NASH, liver steatosis, liver fibrosis, and metabolic syndrome. Exclusion criteria were imposed to select the papers, such as the article publication date not exceeding 20 years old, and the publications to be in English, French, and Romanian, addressing the diagnosis and treatment concepts for NASH.

RESULTS

Diagnosis

Identifying a person with NASH can be difficult because neither the diagnosis nor the severity of the liver fibrosis can be predicted based on the risk factors (obesity, insulin resistance, or hepatic steatosis) alone. Accurate fibrosis staging, through biopsy series, is important for diagnosis and mortality assessment. All-cause and liver-specific mortality appear to increase once the F2 fibrosis stage has developed [10]. The risk of liver disease death rises by a factor of 50 to 80 for patients with NASH who have F3/F4 fibrosis, as compared with those who have little or no fibrosis [11,12]. People at risk for NASH, such as overweight or obese patients with metabolic syndrome, elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and negative noninvasive results for other causes of liver disease, should undergo further testing. Also, patients with type 2 diabetes mellitus over the age of 45 years are likely to have advanced fibrosis and bad liver outcomes [5].

Before advanced testing for NASH, patients should be thoroughly examined to exclude other causes of steatosis and liver damage and to determine the presence of commonly associated comorbidities (obesity, dyslipidemia, insulin resistance, diabetes, sleep apnea, hypothyroidism, and polycystic ovary syndrome). Excessive alcohol consumption, steatogenic medications, high serum ferritin with increased iron saturation, and viral or autoimmune hepatitis should be excluded [7].

The liver biopsy is an invasive procedure, currently considered the gold standard for diagnosing and staging NASH. Numerous non-invasive methods of diagnosis based on serum markers and imaging tests have been investigated, but their performance is not comparable with the biopsy (Table 1). The need for non-invasive tests has been enhanced to develop new treatments for NASH. Various emerging diagnostic tools are presented in Table 1 [7,13-25].

MRI-PDFF [7,13] (Proton density fat fraction MRI) is the most accurate method of detecting steatosis, whereas MRE [7,14] (Magnetic resonance elastography) is the most accurate tool of staging liver disease, by the “stiffness’’ indirectly related to fibrosis. Although availability and costs are a major challenge for clinicians, combining these tests can provide a risk stratification in patients with NAFLD. Non-invasive serum markers include alanine aminotransferase levels, and circulating keratin18 fragment levels and there are also tests such as Fibrometer, microRNA panels, and lipidomic panels. In patients with ALT>70U/L, NASH ALT prediction testing was found to have a 50% sensitivity and 61% specificity [7,15]. Considering the prediction ability of the serum ALT, it should not be used for definitive diagnosis, staging, or evolution of the disease, but only to guide further testing. The NASH Test combines demographic characteristics (age, gender, and BMI), serum markers (ALT and lipids), and alpha-2 macroglobulin, ApoA1, and haptoglobin, with a sensitivity of 33% and a specificity of 94%, indicating that it has a good negative predictive value (NPV) [7,16]. The Fibrometer NAFLD (based on age, weight, fasting glucose, AST, ALT, ferritin, and platelet count) achieves reasonably good results for mild to moderate fibrosis. The positive predictive value (PPV) for correctly identifying F=1 was 84.9%, the NPV was 66.7% and the diagnostic accuracy was 80%. The PPV for F=3 was 74.5%, the NPV was 86.2% and the diagnostic accuracy was 82.1% [17-20]. Fibromax, a diagnostic tool composed of 4 non-invasive biomarker panels, can be used for the diagnosis of steatosis (estimated by the SteatoTest), necrosis and inflammation (evaluated by ActiTest and NashTest-2), and fibrosis (FibroTest). Although it is a well-validated and relatively frequently used test, it may not be accurate for patients with T2DM [21]. OWLiver (Overweight Liver) [16,18] is an alternative for overweight patients and PROC3 (Pro Collagen III) can be used as a differentiating test between steatosis and NASH [24]. NASH Index Score 4 (NIS4) [23] and Enhanced liver fibrosis (ELF) [25] are tools that exclude AST and ALT as parameters in the diagnosis process.

There are two main scoring systems for the biopsy results in NASH. The NAS (NAFLD Activity Score) sums the score of steatosis, lobular inflammation, and ballooning of the liver cells. The score (0-8) is not diagnostic. It was designed to evaluate the histological changes in seriate biopsies. It is usually interpreted by adding the fibrosis score (0-4) (Table 2) [7,26-27]. Another available score is SAF

(Steatosis, Activity, and Fibrosis). By evaluating 1000 biopsies, there was an excellent concordance between the two scores in determining the definite NASH, whereas 84% of the NAS borderline biopsies (3-4), were identified by SAF score as NASH [7,26-27].

Table 1: Non-invasive methods of diagnosis [7,13-25]

Test	Parameter measured	Observations
MRI-PDFF [7,13] (Proton density fat fraction magnetic resonance imaging)	steatosis	most accurate method of detecting steatosis
MRE [7,14] (Magnetic resonance elastography)	liver stiffness	most accurate modality of staging liver disease, by the ''stiffness'' indirectly related to fibrosis.
NASH Test [7,16]	age, sex, height, weight, serum triglycerides, cholesterol, a-macroglobulin, apolipoprotein A1, haptoglobin, gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin	sensitivity of 33% specificity of 94%
FibroMeter VCTE [17-20] (Vibration Controlled Transient Elastography)	Fibrosis, liver stiffness Combines TE (transient elastography) with biochemical markers (age, sex, AST, urea, platelets, prothrombin time, GGT, and alpha2-macroglobulin)	shows good results for mild to moderate fibrosis By combining biochemical markers with imagistic results it increases the accuracy
Fibromax [21]	Combines 4 non-invasive biomarker panels SteatoTest, ActiTest, NashTest-2, and FibroTest	affordable and simple score, but it may not be accurate for patients with T2DM
FNI [22] (The Fibrotic NASH Index)	fibrosis AST, high-density lipoprotein cholesterol, and hemoglobin A1c	accurate, affordable, and simple score for patients with fibrotic NASH
NIS4 [16,18,23] (NASH Index Score 4)	miR-34a-5p, alpha-2 macroglobulin, YKL-40, and glycated haemoglobin A1c	Identifies at-risk patients, defined as [NAS] ≥4 and fibrosis stage ≥2 on biopsy
OWLiver [16,18] (Overweight Liver)	extensive analyses of triglycerides panel by high-performance liquid chromatography and mass spectrometry	Used for individuals with BMI>25 kg/m^2
PROC3 [18,24] (Pro Collagen III)	Pro collagen III	Differentiates steatosis from NASH
ELF [18,25] (Enhanced liver fibrosis)	fibrosis hyaluronic acid, procollagen III, and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1)	stages liver fibrosis independently of AST, ALT elevations

Treatment

The main pathogenic processes identified in NASH are metabolic stress, inflammation, and fibrosis. Pharmacologic agents that target these key mechanisms are being investigated in clinical trials [2].

Due to the lack of pharmacological therapies for NASH, lifestyle modifications remain the main intervention for mitigating histological impairment. Weight loss appears to bring the highest benefit.

Usually, the recommendations include 5-10% weight reduction for obese and overweight patients, physical activity, and a Mediterranean diet. Ultra-processed foods and drinks, fructose-enriched beverages, and alcohol should be avoided. In addition, associated comorbidities (T2DM, dyslipidemia, POS) should be treated [1,26].

Vitamin E, pioglitazone, and statins are available options for patients with NASH. Studies have indicated the role of vitamin E in the reduction of steatosis and fibrosis [28,29]. Pioglitazone, a treatment used for type 2 diabetes mellitus, has been reported to significantly reduce fibrosis in NASH patients. In addition, statins have been tested on NAFLD/NASH patients, with substantial results in improving steatosis and fibrosis [30].

The PIVENS trial focused on NASH patients without diabetes, who were randomly assigned to one of three groups (pioglitazone 30 mg daily, vitamin E 800 IU daily, or placebo). The vitamin E group had a significantly higher rate of histology improvement than the placebo group 43% vs. 19%, but pioglitazone did not meet the primary outcome [31]. Based on several studies, there is a variability in treatment response for vitamin E supplementation. Genetic variations in haptoglobin and FADS1/FADS2 (fatty acid desaturase 1 and 2) have been suggested to interfere with the treatment response, determining whether a patient will show histological improvement or not [32].

Many new potential therapies for NASH are currently studied in phase III trials. The pan-PPAR agonist (Peroxisome proliferator-activated receptors), lanifibranor, has completed the phase IIb trial with 247 participants, the results show a reduction in the SAF activity score (> 2 points), with no increase in fibrosis, achieved by 49% of patients compared with 27% on placebo. The results were significantly higher among those who received 1200 mg of lanifibranor vs. the group with 800 mg dose. In the lanifibranor groups, there was an improvement in liver enzyme levels and also in the majority of lipid, inflammatory, and fibrosis biomarkers [33]. There is an ongoing phase 3 study conducted to evaluate the safety and efficacy of lanifibranor compared to placebo on 1000 participants

with NASH and liver fibrosis histological stage F2 or F3 [34].

The GLP-1 receptor agonist, semaglutide, has also completed a 72-week, phase 2 trial (NN9931-4296) with 320 participants, showing resolution of NASH and no worsening of fibrosis for 59% of patients, in the 0.4mg group, and 40% of patients, in the 0.1mg group, compared with 17% on placebo. The percentage of participants with an improvement in NAS score was 82.9% in the 0.4mg group compared with 43.8% on placebo [35]. A phase 3 study, ESSENCE (The Effect of Semaglutide in Subjects With Non-cirrhotic Non-alcoholic Steatohepatitis), was initiated in 2021 and the interim results are expected in 2024 [36].

Table 2: NAS score and fibrosis staging (adapted from [7,27])

Characteristic	Score
Steatosis	<5%=0 5-33%=1 (mild) 33%-66%=2 (moderate) >66%=3 (severe)
Lobular inflammation	No foci = 0 <2 foci per 200 field =1 2-4 foci per 200 field =2 >4 foci per 200 field = 3
Ballooning	None = 0 Few = 1 Many cells = 2
NAS Score	0-8
Fibrosis	None = 0 Mild = perisinusoidal or periportal (stage 1) Moderate = perisinusoidal and portal/periportal (stage 2) Severe = bridging fibrosis (stage 3) Cirrhosis = stage 4

For liraglutide, also a GLP-1 analog, a multicenter, double-blinded, randomized, placebo-controlled phase 2 trial (the LEAN trial) has demonstrated histological resolution of non-alcoholic steatohepatitis and a significant effect on liver fibrosis after 48 weeks of treatment. In addition, the comparison between pioglitazone and liraglutide showed better results for the latter [37-39].

Obeticholic acid, a selective FXR (farnesoid X receptor) agonist, approved by the FDA in 2016 for primary biliary cholangitis, previously completed a phase III trial (REGENERATE) on 1968 NASH participants with fibrosis F2–F3. The results show that the drug improved the fibrosis score by at least one stage, but did not meet the endpoint of NASH resolution [40]. The FDA rejected the approval of obeticholic acid for NASH in May 2023, based on the modest benefits that did not outweigh the risks.

A phase IIb randomized study of the efficacy and safety of Namodenoson, an A3 adenosine receptor (A3AR) agonist, in the treatment of NASH is ongoing. Previous trials on Namodenoson showed normalization in ALT and AST levels after 12 weeks of treatment [41].

Pegozafermin, a long-acting glycopegylated fibroblast growth factor 21 (FGF21) analog, showed improvements in fibrosis in a phase 2b trial (ENLIVEN). The fibrosis improvements occurred in 7% of the placebo group and between 22-27% in the pegozafermin groups (22% with 15 mg – pegozafermin, 26% on 30 mg dosage, and 27% on 44 mg dosage). The NASH resolution was 2% in the placebo group, 37% in the 15mg pegozafermin group, 23% in the 30 mg pegozafermin group, and 26% in the 44 mg pegozafermin group. The study completion date is estimated around September 2024, demonstrating favorable prospects for advancing to phase 3 [42,43].

Oxidative stress and the formation of reactive oxygen species (ROS) are often associated with the progression of NAFLD to NASH. Excessive ROS formation impairs insulin sensitivity and inflammatory responses. The release of pro-inflammatory molecules and uncontrolled ROS production in many cells is partially the result of TAGE (toxic advanced glycation end-products) and receptor for AGEs (RAGE) interactions [44,45]. Hyperglycemia contributes to the production of advanced glycation end-products (AGEs), especially AGEs formed from glyceraldehyde (GA), which are highly cytotoxic, damaging the hepatocytes. AGEs and TAGE were measured in a study of 66 patients with histologically confirmed NASH without cirrhosis, 10 with NAFL, and 30 control subjects [46]. The TAGE levels of NASH patients were significantly higher than those with NAFL. Ultra-processed foods with a high content of sugar (fructose-enriched or sugar-sweetened drinks) and dietary AGEs have been suggested to contribute to the development of obesity, metabolic syndrome, and the progression of NAFLD/NASH when consumed excessively [47].

Antioxidants such as pentoxifylline (PTX), silybin, resveratrol, and vitamins A, C, and E are used in clinical trials against NAFLD and NASH [47].

PTX treatment with three divided doses of 400 mg for one year improved hepatic histological activity. NAS ≥ 2 improved by (75%) in the PTX group and 30% in the placebo group, but there was no impact on fibrosis [48]. Also, another study on PTX (1200 mg/day) and fenofibrate showed a beneficial effect on indirect biochemical markers of hepatic fibrosis. After 24 weeks, the association of fenofibrate and PTX resulted in reduced liver fibrosis and stiffness, insulin resistance, and inflammation [49]. The combined administration of PTX (1200 mg/daily) and metformin (1500 mg/ daily) for six months reduced AST, and ALT levels and improved

insulin resistance in patients with NASH [50]. A meta-analysis based on 5 RCT on patients with NAFLD/NASH reported that PTX treatment could reduce glucose serum levels, liver transaminases, body weight, and TNF-α levels and also improve NAS and fibrosis scores [51]. Another study used combinational therapy (PTX+Vitamin E) and the results showed regression of fibrosis compared to vitamin E alone [52]. In a phase 2 study, pentoxifylline (PTX) alone significantly improved steatosis, lobular inflammation, and liver fibrosis and was well tolerated [53]. Therefore, PTX can be used along with other drugs as an adjuvant for metabolic diseases.

A phase 4 study conducted on 102 NASH patients divided into 3 intervention groups (PTX, vitamin E, and ursodeoxycholic acid) measured the levels of several serum markers (AST, ALT, IL6, and CCL2/ MPC-1, albumin, total bilirubin, direct bilirubin, total cholesterol, triglyceride, LDL, HDL) for 3 months [54].

In 2017 a study [55] based on an experimental rat model of NASH demonstrated for the first time that the combination of SAMe (S-Adenosyl-L-Methionine) and DLPC (Dilinoleoylphos-phatidylcholine), two physiologic antioxidants, significantly opposed liver injury generated by fat excess.

Numerous studies on hepatoprotective drugs, probiotics, and antioxidants, such as Siliphos—Selenium—Methionine—Alpha Lipoic Acid, omega-3 fatty acids, and vitamins A, C, and E, are currently ongoing [47,52,56-58].

CONCLUSION

The treatment for non-alcoholic steatohepatitis is, unfortunately, an unmet need. At present, there are no FDA-approved therapies for this condition, and the same holds for the EU. Considering the increasing prevalence and the risk for progression toward advanced liver disease, cirrhosis, and hepatocellular carcinoma, there is a major demand for reliable non-invasive biomarkers and specific therapy. In addition to healthy diet and exercise, many new pharmacological agents, such as lanifibranor, semaglutide, and obeticholic acid are being studied. Antioxidants as well are used in clinical trials against NAFLD and NASH.

Despite the clinical and epidemiological significance, the accurate diagnosis, monitoring of the diagnosed patients, and available licensed treatments remain the challenges of NASH.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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REVIEW

Multinodular and Vacuolating Neuronal Tumor – Challenges and Perspectives

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Abstract: A multinodular and vacuolating neuronal tumor (MVNT) is a rare non-malign central nervous system tumor. The cerebral cortex of young to middle-aged adults is affected and seizures and focal neurological deficits could reveal it. Despite being considered a low-grade tumor, MVNT can be challenging to diagnose and manage due to its histopathological similarities to other neuronal tumors. The limited number of reported cases makes it difficult to establish definitive guidelines for treatment and follow-up. Given the relatively recent discovery of MVNT, there is a need for further research to better understand its pathogenesis, optimal treatment and long-term strategies. Furthermore, studying MVNT can contribute to the broader understanding of brain tumor biology and the intricacies of tumor heterogeneity. This paper explores the challenges and perspectives surrounding MVNT to improve clinical decision-making and patient outcomes. Based on the present narrative review, advancements in diagnostic technologies have shown promise in addressing the challenges associated with diagnosing MVNTs. Liquid biopsy is an innovative and non-invasive diagnostic tool that analyzes biomarkers to provide information about tumors, and radiomics is a method that extracts quantitative data from medical images to provide detailed insights into tumor characteristics. Such innovative diagnostic technologies are important because they have the potential to significantly improve the current diagnostic landscape for MVNTs, allowing for earlier detection, accurate classification, and personalized treatment strategies.

Keywords: multinodular and vacuolating neuronal tumor; radiomics; liquid biopsy; diagnostic methods; histological examination; FDG-PET/CT; immunohistochemistry

INTRODUCTION

First described in 2013, multinodular and vacuolating neuronal tumor (MVNT) was classified as a benign ganglion cell grade I tumor, potentially associated with seizures, with a specific molecular marker, as outlined in the WHO 2016 and 2021 classification [1-3]. The discovery of MVNT is often accidental, and it is frequently asymptomatic [1,2]. Studying MVNT is of utmost significance in neuro-oncology. Understanding the molecular mechanisms underlying the development and progression of MVNT can provide insights into potential therapeutic targets and treatment strategies.

Typically identified in adults, MVNTs seem to appear during neurogenesis and present as subcortical clusters of nodular lesions within the cortex and superficial subcortical white matter [4]. Structure abnormalities in the cortex may be present with it [4,5]. Initially classified as either neoplastic or malformation, recent research on MVNTs emphasizes their stable nature over time, suggesting that they are "leave me alone" lesions with minimal changes in volume, thus underscoring the potential for less frequent imaging surveillance [6,7]. Whether the nodules of vacuolar neurons are a definite neoplastic transformation or aberrant development is still up for debate [6]. Overall, these insights contribute to a nuanced understanding of MVNTs, highlighting the complexities and diagnostic considerations surrounding these distinct neuronal tumors. Moreover, the purpose of this research is to shed light on the challenges and perspectives surrounding MVNT.

MATERIALS AND METHODS

A narrative review was conducted through five electronic databases (PubMed, CINAHL, Google Scholar, Cochrane, Web of Science/Clarivate), using as keywords "multinodular and vacuolating neuronal tumor" AND "diagnosis" OR "pathogen*" OR "imaging" OR "treatment". All primary and secondary reports found from the inception of the respective databases up to July 2024 were explored for relevant data. Only papers that addressed explicitly the pathogenesis, diagnosis methods,

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and treatment strategies of MVNT were included in the secondary analysis**.**

RESULTS

Based on the literature search, 34 primary and secondary reports were reviewed in detail. The results were classified according to the three areas of interest, i.e., (1) pathogenesis and classification of MVNT, (2) imaging characteristics, and (3) diagnosis and treatment.

Classification and pathogenic characteristics of MVNT

Recent studies have highlighted the challenge of defining and classifying MVNT. Due to its rarity and unique histopathological features, there is ongoing debate within the scientific community regarding its precise definition and classification. Some researchers argue that MVNT should be considered a separate entity from other neuronal tumors due to its distinct morphological characteristics and clinical behavior. On the other hand, some propose classifying it under the umbrella of neuronal and mixed neuronal-glial tumors based on its neurocytic differentiation and immunohistochemical profile.

The purely neuronal tumors in the 2021 WHO classification include multinodular and vacuolating neuronal tumors, gangliocytoma, extraventricular neurocytoma, central neurocytoma, dysplastic cerebellar gangliocytoma, and cerebellar liponeurocytoma.

The mixed glioneuronal tumors include dysembryoplastic neuroepithelial tumor, diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters, ganglioglioma, desmoplastic infantile ganglioglioma/astrocytoma, papillary glioneuronal tumor, rosette-forming glioneuronal tumor, myxoid glioneuronal tumor, and diffuse leptomeningeal glioneuronal tumor [13].

There is a consensus on the need for standardized diagnostic criteria to identify and classify MVNT cases accurately. This would facilitate better communication among pathologists and clinicians, improving management strategies and outcomes for patients with MVNT.

The WHO 2016 classification defines MVNT as a benign ganglion cell tumor with specific pathologic hallmarks [2]. These nodular lesions are typically located in specific brain regions and can present differently across age groups, emphasizing the importance of histopathological examination for accurate diagnosis [1,14,15].

Histopathological analysis of MVNT reveals several distinctive features that aid in their diagnosis and differentiation from other neuronal tumors. One of the hallmark features of MVNT is the presence of numerous vacuoles within the tumor cells. This vacuolation can create a “floating” appearance of the cells and is a critical diagnostic criterion. MVNT typically exhibits a multinodular architecture. The tumor forms distinct nodules, which may vary in size, often surrounded by a gliotic background. Such detailed histopathological analyses are essential for elucidating the distinct features of MVNT and guiding effective clinical management strategies. Nevertheless, no histopathological evidence is available to confirm the diagnosis since only a small percentage of patients with suspected MVNT undergo surgery [10].

Immunohistochemical markers play a crucial role in the diagnosis and classification of MVNT. Various markers have been studied, differentiating MVNT from other tumors with similar histological features, such as dysembryoplastic neuroepithelial tumor (DNT) or ganglioglioma [16,17]. So, immunohistochemistry (IHC) is a crucial tool in the diagnosis and characterization of MVNT, establishing the neuronal nature of MVNT, as seen with positive staining for neuronal markers [18]. MVNT typically shows positivity for several neuronal differentiation markers: NeuN, a neuronal nuclear antigen typically expressed in mature neurons, β-III tubulin, which is highly expressed in neuronal cells and can help confirm the tumor’s neuronal lineage. Synaptophysin, a protein associated with synaptic vesicles, indicates neuroendocrine differentiation and is often used to demonstrate the presence of neurons. Although MVNT is primarily neuronal, some glial markers may also be expressed, reflecting maybe the mixed composition of the tumor. GFAP (Glial Fibrillary Acidic Protein) positivity can indicate a glial component in the tumor. Phenotypic analyses may also include checking for markers that indicate low proliferation rates, which can help distinguish MVNT from more aggressive tumors. Markers like Ki-67 are occasionally assessed but typically show low proliferation in MVNT. The absence of expression for markers associated with high-grade tumors, such as p53 or other markers linked to aggressive histology, reinforces its benign nature [18,19].

Understanding the molecular pathogenesis of Multinodular Vacuolating Neuronal Tumors (MVNT) presents a significant challenge due to the tumor’s unique histological features and clinical behavior. MVNTs are characterized by vacuolated tumor nodules with glial and neuronal differentiation, primarily affecting the cerebrum in adults. The pathognomonic radiological features, such as the soap-bubble morphology and lack of enhancement post-gadolinium injections, provide important clues for differential diagnosis and management strategies [4,11,20,21]. The molecular mechanisms driving its formation and growth remain elusive. Further research is warranted to elucidate the genetic alterations and signaling pathways involved in the development of MVNT. The MAP (Mitogen-Activated Protein) kinase signaling pathway is a critical cellular signaling pathway that regulates various cellular processes, such as growth, proliferation, differentiation, survival, and apoptosis. This pathway is activated by various extracellular signals, including growth factors, cytokines, and environmental stresses. MAP kinase signaling pathway possibly sheds light on targeted therapeutic approaches [22].

Research has identified specific genetic mutations that may contribute to the development of MVNTs. For instance, missense mutations in the BRAF gene or HRAS gene have been linked to the pathogenesis of tumors [22,23]. Vacuolization and neuronal differentiation in MVNTs are characterized by vacuolated neurons. This cellular morphology suggests that disruptions in normal

neuronal differentiation and cellular homeostasis may be due to genetic and epigenetic factors. Investigating the presence of other oncogenes and tumor suppressor genes may provide further insights. In exploring the molecular underpinnings of MVNT, a deeper understanding of its pathogenesis may pave the way for personalized treatment strategies and improved patient outcomes in the future. Like many tumors, MVNTs may exhibit altered vascularization patterns [24]. The tumor microenvironment, including the interaction between tumor cells and surrounding glial cells, immune cells, and extracellular matrix components, plays a crucial role in tumorigenesis [25,26]. Studying these interactions can show how the tumor ecosystem supports MVNT growth and survival.

Table 1: Multinodular and vacuolating neuronal tumor (MVNT) characteristics

Characteristics	Descriptors
Definition [2,3]	Low-grade tumors (grade I) comprising monomorphous neuronal elements in discrete and coalescent nodules, with vacuolar changes both in tumor cells and the neuropil, with a molecular pattern impaling MAPK pathway
Localization [4,9,10]	Parietal lobe, frontal lobe, temporal lobe, occipital lobe, spinal, rare cases of multiple localization, mainly subcortical white matter rare cases with cortical extension
Imaging features [4,11,12]	**Brain CT**- is usually normal or shows a non-enhancing/non-calcifying cortical/subcortical white-matter hypodense lesion, absence of mass effect and edema; **Brain MRI** – intra-axial multiple clustered nodules hyperintense on T2WI and FLAIR; T1WI: small hypointense, homogeneous, and well-defined nodules, some clustered tightly or with a small interface between them, located at the cortical-subcortical junctional side; Abnormal white matter signal surrounding the bubble-like or bunch of grape appearances lesions; No contrast enhancement; Restricted diffusion could be present with high ADC values rare **FDG-PET/CT** brain imaging showed tumor hypometabolism
Histological examination [4,8,12]	No evidence of mitosis, necrosis, or microvessel proliferation; Abnormal clusters and numerous vacuoles within the tumor cells, with different sizes, often surrounded by gliotic tissue; Glioneuronal hamartomas; Hypomyelinated plaque-like nodules extending into the deep cortex;
Immunohistochemical stains [4,8]	Many neuronal cells show weak immunoreactivity for synaptophysin and negative immunoreactivity for neuronal nuclear and neurofilament proteins; Variable background of positive dendritic staining for CD34; Negative for IDH1-R132H mutation antibody; Staining was normal for ATRX and p53; The Ki67 proliferation refraction was very low; GFAP highlighted the reactive astrocytes within the nodules; The vacuolated cells were immunoreactive for synaptophysin, while the fibrillary tumor matrix showed decreased synaptophysin expression compared to the adjacent cortex
Natural evolution [4]	Stable on time, „leave me alone" tumor

MAPK- Mitogen-Activated Protein Kinase; CT – computer tomography; MRI- magnetic resonance imaging; T2 FLAIR – space inversion recovery with fluid attenuation; FDG-PET/CT- fluorodeoxyglucose positron emission tomography/computed tomography; CD34 - transmembrane phosphoglycoprotein encoded by the CD34 gene; IDH1- isocitrate dehydrogenase; ATRX- chromatin remodeling · nucleosome assembly; p53- tumor suppressor gene; Ki67 – a nuclear nonhistone protein present in all active phases of cell cycle; GFAP – Glial fibrillary acidic protein responsible for astroglial cytoskeleton and is not found outside the CNS

Imaging Characteristics of MVNT

Imaging characteristics of MVNT reveal distinctive features that aid in their diagnosis and differentiation from other brain tumors. Described as low-grade lesions primarily found in the cerebrum, MVNT presents as clustered multinodular structures within the cortex and subcortical regions (Table 1). The brain MRI revealed multinodular T2-hyperintense lesions in both the deep cortex and superficial white matter of the frontal lobes or temporal lobes, exhibit a unique soap-bubble morphology, hyperintensity on T2 FLAIR sequences, and minimal or absent contrast enhancement [10,16,27]. Based on the signals from T1, T2, and T2 FLAIR, the nodules have nonhomogeneous signal intensity due to low T1, high T2, and low FLAIR vacuole aspects. Confluent T2/FLAIR hyperintensity overlying cortex was present in some cases [4]. The larger nodules have an inner core that is hypointense, a rim that is hyperintense, and areas with a high signal intensity that connect the nodules. The smaller nodules exhibit a signal intensity that is not uniform, with a central dot of low-intensity [11]. The nodules' periphery shows high signal intensity on FLAIR because of high protein content and neuronal demyelination [11].

Sometimes MR spectroscopy indicates an increase in the choline peak and a decrease in the N-acetyl aspartate peak, and tractography shows a partial disruption of the typical structure caused by the absence of lesional cortical layers [11,28]. No increase in perfusion was observed by perfusion-weighted imaging in another study, and abnormal peaks were not detected by MR spectroscopy [10]. Despite their benign nature, the pathologic hallmark of MVNT, questions their neoplastic or malformative origin and potential association with seizures [29]. Cases of a spinal cord lesion resembling cerebral MVNT of unknown significance were named MVNT-like spinal cord lesions. With intramedullary cystic ovoid nodules within the white matter, some immediately contiguous to the grey matter, this lesion was stable in a follow-up [9,30]. The presence of well-delineated intramedullary cystic ovoid nodules within the spinal cord raises questions about the underlying pathophysiology of MVNT [9]. A comprehensive MR protocol has been suggested to

increase the diagnostic confidence of MVNT [10].

Diagnostic and treatment of MVNT

Despite advancements in imaging and molecular techniques, MVNT can present with various clinical manifestations, posing diagnostic challenges. Usually, MVNT is revealed as an incidentaloma [31]. The clinical presentation is nonspecific, with symptoms such as seizures, headaches, or focal neurological deficits, often mimicking other more common brain tumors or epilepsy syndromes [1,14]. Additionally, the radiological features of MVNT on MRI can be subtle and easily overlooked, leading to misdiagnosis. The lack of specific diagnostic criteria for MVNT further complicates accurate identification, underscoring the need for histopathological examination for definitive diagnosis [16,32]. Biopsy remains the gold standard for confirming MVNT, highlighting the importance of integrating clinical, radiological, and pathological findings to navigate the diagnostic challenges posed by this rare entity in neuro-oncology. Additionally, the classification of MVNT as a benign neuronal tumor, possibly associated with seizures, further complicates the understanding of its neurological manifestations, especially in young patients [33].

Table 2: Immunohistochemical analysis [8,19]

Neuronal markers		Glial markers	
NeuN (Neuronal Nuclei)	A marker for neurons, specifically in post-mitotic neurons. It is widely used to identify neuronal populations in various brain regions.	GFAP (Glial Fibrillary Acidic Protein)	A specific marker for astrocytes. Increased expression is often seen in reactive astrocytes following injury
MAP2 (Microtubule-Associated Protein 2)	A neuron-specific protein that stabilizes microtubules and is important for dendritic structure.	Olig2	A transcription factor and marker for oligodendrocyte precursor cells and mature oligodendrocytes, essential for myelination in the central nervous system
βIII-Tubulin (Tuj1)	A marker commonly used to identify neurons, particularly in developmental studies.	S100	A protein found in astrocytes, used for identifying glial cells
Synaptophysin	A presynaptic vesicle protein, often used to identify synaptic structures and neuronal activity	CD68	A marker for microglia, reflecting macrophage activity in the central nervous system and often used in studies of neuroinflammation.

Distinguishing MVNT from entities such as dysembryoplastic neuroepithelial tumor (DNET), ganglioglioma, extraventricular neurocytoma, and low-grade glioma is sometimes challenging [34]. The bright diffusion sign and absence of cortical involvement could differentiate MVNT from DNET [35]. Moreover, incorporating genetic and molecular analyses can further aid in narrowing down the list of differential diagnoses and confirming the diagnosis of MVNT. Immunohistochemical analysis for neural and glial markers are also helpful (Table 2). Antibodies against neuronal protein HuC/HuD (HuC/HuD), pan-neurofilament (pan-NF), neurofilament 200 KD (NF200 KD), chromogranin, and antibodies against glial fibrillary acidic protein (GFAP), nestin, CD3, CD45, CD68, CD34, human leukocyte antigen-DR (HLA-DR), isocitrate dehydrogenase1 (IDH1), calbindin, parvalbumin, calretinin, Ankyrin-G, p62 and Ki67, BRAF(V600E) enhance diagnostic accuracy [8,19]. Immunopositivity for HuC/HuD and Olig2 proves that MVNT appears during neuronal development [4].

The latest WHO tumor brain classification has classified MVNTs as grade I, and the mitogen-activated protein kinase (MAPK) is considered a molecular marker [3,8]. The MAPK is a key signaling pathway that transmits signals from the cell surface to the nucleus and regulates various cellular processes, including growth, proliferation, differentiation, and survival. Further research in this area, particularly investigating specific diagnostic markers and biomarkers, is warranted to refine the differential diagnosis process for MVNT.

Factors such as tumor size, location, and the presence of associated neurologic deficits can influence treatment strategies and prognosis.

One of the foremost challenges in the preoperative diagnosis of MVNT is the difficulty in distinguishing them from other neoplastic or malformative lesions to avoid unnecessary surgical interventions [36]. The characteristic imaging features of MVNT, such as the subcortical nodular lesions with unique signal properties on MRI, can often be mistaken for more aggressive tumors due to their atypical presentation in younger patients. The rarity of MVNT in young individuals poses a diagnostic dilemma, questioning the

classification of these lesions as true neoplasms or malformative anomalies [22,31,21].

Imaging modalities and treatment strategies play pivotal roles in managing epileptic patients, especially those with drug-resistant epilepsy considered for neurosurgery. Utilizing advanced imaging techniques such as the Harmonized Neuroimaging of Epilepsy Structural Sequences (HARNESS-MRI) protocol and 7-Tesla MRI can aid in identifying previously occult epileptogenic lesions and improving surgical outcomes [37,38]. Additionally, the development of MRI postprocessing techniques and functional imaging modalities like EEG-functional MRI contribute to enhanced sensitivity in lesion detection and focus localization [39].

Surgery is often considered the primary treatment option for multinodular vacuolating neuronal tumors (MVNTs) under several circumstances. If the MVNT is causing neurological symptoms (such as seizures, headaches, or focal neurological deficits), surgical intervention may be necessary to alleviate these symptoms, when the tumor is accessible and can be safely resected without causing significant damage to surrounding brain tissue. Histological confirmation by surgical resection can provide a definitive diagnosis through the histopathological exam, which is crucial for confirming the nature of the tumor and ruling out other possible lesions. However, the precise location of the tumor within the brain can sometimes pose challenges in obtaining a biopsy sample. In such cases, advanced imaging techniques and neurosurgical expertise are indispensable in ensuring accurate sampling for an accurate diagnosis and subsequent management of MVNT. It is essential that surgical decisions are made in consultation with a team of specialists, including neurosurgeons and oncologists, who can evaluate the risks and benefits specific to each patient's condition and tumor characteristics. Functional MRI is as accurate as WADA testing, for determining language dominance [40].

The management of asymptomatic MVNT often involves careful observation rather than immediate intervention. However, a strategy of active surveillance can be appropriate for asymptomatic or minimally symptomatic MVNT. This involves regular follow-up through serial neurological examinations and imaging studies (usually MRI) every 6-12 months**.**

DISCUSSION

Advancements in diagnostic technologies have shown promise in addressing the challenges associated with diagnosing MVNTs. One emerging technology that holds potential is liquid biopsy, an innovative and non-invasive diagnostic tool that analyzes biomarkers (circulating tumor cells and genetic material) from bodily fluids (such as blood, urine, or cerebrospinal fluid) to provide information about tumors [41-46]. Circulating Tumor DNA (ctDNA) from the blood allows for the detection of genetic mutations and alterations associated with MVNT. Liquid biopsy is less invasive than traditional tissue biopsy, making it a more feasible option for patients, especially in complex cases where surgical options may be limited. It allows for repeated sampling without the risks associated with multiple surgical procedures, enabling ongoing monitoring.

Additionally, the development of radiomics, a method that extracts quantitative data from medical images to provide detailed insights into tumor characteristics, could aid in distinguishing MVNTs from other brain tumors with similar radiographic features [44-46]. These innovative diagnostic technologies have the potential to revolutionize the current diagnostic landscape for MVNTs, allowing for earlier detection, accurate classification, and personalized treatment strategies. Furthermore, integrating these technologies into clinical practice could improve patient outcomes and enhance our understanding of MVNT biology.

More studies are needed to elucidate the genetic mutations and molecular pathways involved in the development of MVNT. This would not only provide valuable insights into the underlying mechanisms of this rare brain tumor but also potentially identify novel therapeutic targets. Additionally, clinical research focusing on the efficacy of different monitoring modalities is crucial for optimizing patient outcomes**.**

CONCLUSION

Based on the results of this narrative review, emerging technologies allow for a re-conceptualization of the MVNT diagnosis and treatment. Although data regarding the validity of the new imaging and molecular techniques are still gathering, the fact that WHO includes MAPK as a marker for MVNT indicates the need for further refinement of the differential diagnosis based on molecular explorations in this pathology. The references in the literature related to genetic mutations and alterations associated with MVNT suggest new research directions congruent with the paradigm of personalized medicine.

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Authors' contribution

Conceptualization, M.M., O.V., and O.M.S.; methodology, O.V., A.P., and O.M.S.; software, M.M., A.P., and O.M.S.; validation, M.M., O.V., and A.P.; formal analysis, O.V.; investigation, M.M.; resources, A.P., and O.M.S.; data curation, O.V.; writing—original draft preparation, M.M., O.V., and O.M.S.; writing—review and editing, M.M., O.V., and O.M.S. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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ARTICLE

Functional Evaluation and Neuro-Muscle-Skeletal Recovery in Lyme Disease: A Study on Motor Control Theories and Rehabilitation

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Abstract: Background: Patients with Lyme disease often suffer from neuro-motor sequelae following Borrelia infection, leading to impairments in balance, posture, and spinal musculoskeletal functionality. This study aims to evaluate the effectiveness of physical therapy in addressing these impairments. Methods: Initial assessments were conducted using the GPS Posture-test System to evaluate static bipodal balance and posture, and BACKFIX Technology for unipedal balance and spinal functionality. Disturbances in balance, postural asymmetries, and a forward-shifted center of gravity were observed. The patient then underwent two 10 sessions (each during two weeks) of computer-assisted physical therapy focused on neuro-muscular movement and balance control. Results: Post-therapy evaluations showed significant improvements in both bipodal and unipedal static balance, segmental and overall body posture, and spinal musculoskeletal functionality. The therapy effectively addressed the postural asymmetries and shifted the center of gravity closer to the optimal vertical posture point. Conclusion: Computer-assisted physical therapy with visual feedback significantly improved posture, balance, and spinal functionality in patients with Lyme disease-related neuro-motor sequelae. This approach facilitated the restoration of muscle coordination and enhanced motor control, aligning with findings from the existing literature.

Keywords: Lyme Disease; Global Postural System; unipedal static balance; BACKFIX; global posture; spine; physical therapy

INTRODUCTION

The specialized literature lacks scientific studies on the functional evaluation and neuromusculoskeletal recovery in Lyme Disease (LD). Approximately 80 articles from the past decade are available in databases, primarily focusing on early detection and antibiotic treatment. No studies provide information on potential methods for functional evaluation or physical therapy-based recovery for musculoskeletal dysfunctions caused by inflammation secondary to Borrelia infection.

LD is caused by the bite of a tick infected with Borrelia. The disease progresses through three stages and can result in multisystem complications. When the nervous system is affected, it is referred to as neuroborreliosis, which may manifest as meningitis, mononeuritis multiplex, or cranial neuritis, including inflammation of the vestibulocochlear nerve [1]. Exclusive involvement of the vestibular system can lead to dizziness and balance instability [2]. Musculoskeletal symptoms can appear at any stage, including early localized infection, often resulting in patients being misclassified into the incorrect stage [3].

We describe motor control and the potential effects of systemic inflammation caused by borreliosis, followed by the stages of assessment and recovery, and the conclusions drawn from these steps.

Although the exact mechanism by which LD causes small fiber neuropathy is unknown, several potential mechanisms of neuronal damage are considered. These include the direct neurotoxic effect of the spirochete, neuroinflammation, and increased autoimmunity affecting the peripheral nervous system (PNS) with subsequent impacts on muscles, joints, and specific post-infection pains. However, a clear causal link between neurological symptoms and Borrelia infection cannot be

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established for patients with polyneuropathy/polyneuritis who test positive for Borrelia [4].

Among the genospecies of Borrelia burgdorferi sensu lato: Borrelia burgdorferi sensu stricto (s.s.), Borrelia garinii and Borrelia afzelii, Borrelia burgdorferi s.s. has the strongest potential to damage joints [5]. It is the most common cause of LD in the United States [3, 6], while infections with this pathogen are much less common in Europe [7-9]. The pathogenesis of LD is primarily driven by a strong inflammatory reaction [5], resulting in a perivascular collection of lymphocytes, macrophages, and scattered mast cells in the interstitial tissue, as typically observed in histopathological examinations of severe LD cases [9].

Previous studies have shown that late manifestations affecting the nervous system or joints can appear months or years after the initial infection, presenting symptoms such as fatigue, difficulty walking, posture disorders, and muscle pain. These symptoms are also present in the subject of our study, secondary to the diagnosis of borreliosis [10].

The examination and treatment of patients with motor control disorders are based on theories of motor control that guide the understanding of movement mechanisms [11-14]. In our study, we utilize systems theory as a foundation for clinical applications. Systems theory posits that movement arises from the interaction of various processes, including perceptual, cognitive, and motor processes, as well as the interactions between the individual, the activity, and the environment.

The severity of symptoms observed in all the patients with LD that we have evaluated has led us to pay more attention to this condition. As a result, our study focuses on evaluating and recovering a patient with LD using methods and applications informed by previous scientific studies. We analyzed a patient with sequelae following LD, including postural, walking, and static and dynamic balance disorders, muscle fatigue, changes in balancing synergies, walking base, and the projection of the General Center of Gravity (GCG). We conducted initial and final tests after a series of 10 and 20 sessions of specific physical therapy exercises.

Pioneers in the study of motor control and movement, such as Bernstein and Latash [15-17], along with Charles Sherrington (1857 - 1952), Thomas Graham Brown (1882-1965), and Walter Hess (1881-1973), have significantly contributed to our understanding of joint freedom, redundancy, and muscle-articular synergies. Charles Sherrington contributed to the understanding of inhibition, spinal-muscle connectivity, and the sensory-motor cortex. Thomas Graham Brown researched central movement programming and pattern generation mechanisms. Walter Hess studied the central coordination of the autonomic system and anticipatory motor behavior. Bernstein, recognized posthumously by Western colleagues, demonstrated a central hierarchy of postural control mechanisms and distributed, parallel processing of movement.

Bernstein also participated in developing theories of motor programs, recognizing that understanding neural control of movement requires knowledge of the system's characteristics in response to external and internal stimuli [16]. His distributed model of motor control showed that central commands could produce different motions due to external forces and initial conditions. His student, Mark Latash, furthered this work by demonstrating that synergies evolve to ensure flexible and stable performance in motor activities [17].

Postural control for stability and body orientation in space requires a complex interaction between the musculoskeletal and neural systems. This includes joint amplitudes, intersegmental biomechanical relationships, muscle physiology, spinal flexibility, neuromotor processing, synergies, perceptual and sensory processes, and their central integration.

Research has concluded that postural control is not managed by a single system but depends on various intrinsic and extrinsic factors [18]. Initial studies highlighted the importance of maintaining the center of mass (COM) within certain limits for static posture. However, subsequent studies demonstrated that stability limits are not fixed and vary with individual characteristics or actions undertaken. When recovering balance, individuals tend to restore COM disturbances rather than segmental alignment disturbances, suggesting that COM is a key variable controlled by the central nervous system during postural control.

Further studies have shown that analyzing the relationship between the center of gravity (COG) and the center of pressure (COP) provides a better picture of stability [19-24]. Their interaction has been considered and used to measure and estimate posture control effectiveness in our study.

As actions have both stability and orientation components, and certain actions prioritize orientation over stability, research by Shumway-Cook & McCollum [25] and Horak & Macpherson [26] has demonstrated that stability and orientation vary with each action. The body can behave like an inverted pendulum, employing various strategies (ankle, hip) for both static and dynamic balance [21].

Bernstein's assertion that "Movement coordination is the process of mastering the redundant degrees of freedom of a mechanism in motion" [16] encapsulates the essence of motor control theories used in our study.

Equilibrium

While bipedal standing is characterized by minor postural imbalances, dynamic balance involves the integration of sensory information from the visual, vestibular, and proprioceptive systems in conditions of postural instability.

There are several theories about how sensory organization adapts to a changing environment, with two primary ones being: the intermodal theory of sensory organization by Stoffregen and Riccio [27], which posits that all sensory systems equally and continuously contribute to postural orientation; and the sensory weighting model, which suggests that the central nervous system adjusts the importance of each sensory system based on their accuracy for orientation and postural stabilization.

Torres and Oviedo [28] examined the structure and organization of muscle synergies for balance control, highlighting that a muscle can contribute to multiple synergies, each with a unique role. They demonstrated that muscle synergies can be continuously modulated by feedback signals to create postural adjustments, indicating that the central nervous system uses both pre-programmed synergies and continuous feedback control to regain stability.

Nashner et al. [29] investigated the selection of sensory information by the CNS for postural control by designing a dynamic platform with a moving visual wall to study sensory interactions in maintaining posture and balance. This approach allowed for the measurement and recording of posture and balance under various conditions.

Several studies on normative values using static platforms have examined the performance of normal subjects under varying sensory information conditions [29-33]. These studies have shown that adults and children over seven years old (the age of vestibular maturation) maintain balance well under all conditions of sensory disturbance.

The inability to maintain a stable static posture has significant functional consequences in neurological pathologies. It necessitates using the arms and hands for balance, detracting from daily activities [34-38]. Moreover, postural instability and the inability to stand on one leg for more than six seconds increase the risk of falls threefold.

Springer's 2007 study [39] showed that balance disorders are prevalent in patients with specific diseases or traumas, particularly head injuries, peripheral neuropathies, or acquired neurological conditions. Neurological pathologies can disrupt one of the postural control strategies (e.g., loss of pre-programmed synergies) while leaving the other (continuous feedback control) intact, necessitating complementary studies to distinguish between these aspects and propose specific solutions.

For the main types of static/dynamic balance (postural control/anticipatory and reactive postural control), numerous clinical tests measure performance, including the unipedal stance and computerized posturography. Our study used such tests to assess the patient's condition at the beginning and end of the recovery program.

Unipedal standing is a highly challenging situation for humans and allows researchers and clinicians to assess the effects of aging, disease, trauma, and the influence of multiple sensory systems. The single-leg stand test, which requires minimal equipment, measures balance aspects and shows abnormal values in conditions such as peripheral neuropathies and intermittent claudication. [35,36]

Various studies demonstrated the importance of maintaining unipedal posture and balance by examining corticomuscular coherence between the ankle joint and the sensorimotor cortex. This study showed that the sensorimotor cortex is directly involved in postural control during unipedal standing, compensating for high postural demands due to the low support base. Normative values in the literature indicate a significant decrease in unipedal stance time with age and pathology [40, 41].

Alain Berthoz et al. [20] demonstrated that during complex movements, the head is intermittently stabilized under gaze and fixation control, allowing it to act as an inertial guidance platform for controlling multisegmental movements. The otolithic organs, the saccule, and utricle, detect the linear accelerations of the head and provide a gravitational reference used not only to correct posture but also for perceptual actions requiring spatial orientation, including mental map rotation based on sensory information from all senses.

Merton's Servo-Control Hypothesis

The interaction between motor and sensory processes is bidirectional, and the importance of sensory information for motor control was established many years ago. The traditional feedforward and feedback control mechanisms could not fully explain the coactivation of the alpha-gamma muscle loops, necessitating alternative theories such as the equilibrium point hypothesis and the hierarchy of synergies to explain how correct posture is maintained during external destabilizations [42].

Merton proposed a model that adjusts the equilibrium point, where the CNS controls both speed and position during rapid segmental movements. Degenerative, infectious, and metabolic diseases have disrupted the transmission of sensory information from the periphery to the CNS, affecting the parameters and components of posture and movement control. Disorders of nerve conduction from proprioceptors to the brain, as seen in neurological or diabetic patients, result in significant balance issues, making it difficult for patients to stand upright with their eyes closed on a static plane [42].

Posture, a term introduced by André Bregegère in 1955 [43], is defined as a momentary attitude or body position [44]. It involves the synergistic and coordinated action of the musculoskeletal system and the central and peripheral nervous systems to maintain body stability, balance, and constant relations between body segments and the environment [45-47]. Correct posture involves the alignment of all joints, and any misalignment can lead to muscle-joint imbalances [48].

Maintaining postural stability requires the co-participation and complementarity of actions from all support components, followed by motor actions (balancing, distribution of motor control tasks), which can be synchronous or asynchronous, simultaneous, or alternating depending on the demands [44,49]. Optimal body alignment in an upright position involves:

Head: Maintained by neck muscles. Insufficiency or habitual forward tilt increases muscle demand.

Trunk: Tends to fall forward due to its weight and the head's weight, counteracted by vertebral muscles.

Shoulders: The scapular belt and upper limbs' weight cause the shoulders to fall and scapulae to separate from the spine, counteracted by shoulder girdle muscles.

Pelvis: Key to maintaining correct posture. Anteversion is balanced by gluteal and posterior thigh muscles, whose hypotonia causes anterior trunk tilt. Retroversion is balanced by rectus abdominis, obliques, and iliopsoas muscles, whose hypotonia causes posterior tilt.

Lower limbs: Stability is ensured by plantar flexor muscles and triceps surae, especially the soleus [45].

Postural defects can lead to fatigue, muscle strain, and pain in later stages, potentially affecting vital organs, particularly in the abdominal region. Our study integrates current scientific conclusions and hypotheses to address parameters affecting motor control and static and dynamic postural control in our subject, considering the literature review.

Motor control theories are inherently unfinished, always allowing room for new information from recent studies on normal and pathological brain and PNS functioning. Neuroscientists identify the scientific basis for movement disorders, while clinicians develop applications of this research, as theories are considered more or less useful based on their problem-solving efficacy for patients with movement disorders.

In this context, we present a study where a patient with Lyme disease sequelae is compared to literature findings regarding posture, balance, and neuromuscular control of movement. Previous works by Schor D, Schor V et al.[50-52] introduced the BACKFIX Method, used for spine functional impairments in preventive care. This study evaluates its application to a complex disease like LD, characterized by neurological symptoms affecting balance, walking patterns, general movement control, and muscle strength imbalance.

We demonstrate the patient's condition evolution using physical therapy with visual feedback on the rectangular force-amplitude model. Improvements in agonist-antagonist cooperation lead to enhanced muscle strength, functional muscle-joint balance of the spine, posture, and walking pattern. The patient exhibited muscle cramps in the calf and foot, pronounced thigh flexor fatigue during walking, insecurity in unipedal support, jerky walking with a wide support base, and reduced standing time in unipedal support.

MATERIALS AND METHODS

We performed the following measurements:
- Computerized somatoscopic examination
- Forces, amplitudes, and mechanical work on the supporting muscles of the trunk, enabling a computerized muscle-articular evaluation of the trunk
- Bipodal static balance - stabilometry
- Unipedal static balance

We utilized the GPS System (Global Postural System) for computerized somatoscopy and computerized static bipodal balance tests, provided by UNEFS Bucharest (University of Physical Education and Sport from Bucharest), and the Ergocontrol System for musculo-articular functionality tests of the spine using the Backfix Method, technology provided by Medisport Company.

An initial evaluation and two intermediate evaluations were conducted. For the static one-legged balance test, we measured the hold time while standing on one leg. An initial evaluation and two intermediate evaluations were conducted. For the static one-legged balance test, we measured the hold time while standing on one leg

RESULTS

Initial Assessment Results

Computerized Somatoscopy Test

Front:
- Right shoulder slightly lowered and rotated anteriorly
- Left shoulder slightly raised
- Right hip slightly lowered
- Left hip slightly raised

Profile:
- Abdominal hypotonia with increased lumbar curvature
- Projection of the GCG (general center of gravity) 11 cm anterior to the normal position

Back:
- Left shoulder blade normally positioned
- Right shoulder slightly lowered and rotated anteriorly
- Left shoulder slightly raised
- Right hip slightly lowered
- Left hip slightly raised

Evaluation of the Muscle-Articular Functionality of the Spine

The results of the computerized musculo-articular measurements regarding the muscular support of the spine are presented in Table 1. The movements were performed with difficulty and were jerky, which is characteristic of the neurological sequelae of the condition.

Table 1: Initial muscle-articular evaluation (3.02.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	5.5	26	14.47
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	9.31	49	45.45
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	8.47	39	32.85

Resources and methods used in recovery

The recovery protocol involved 20 sessions of exercises utilizing visual feedback on force-amplitude curves, performed on the Ergocontrol System. This protocol aimed to restore agonist-antagonist cooperation within muscle groups and chains responsible for global body posture while also focusing on strength enhancement through movement control exercises targeting deficient muscle groups and chains.

Each session included walking exercises designed to integrate the improvements from the neuro-muscular movement control exercises, to enhance the walking pattern.

Results After 20 Recovery Sessions

There was a noticeable improvement in overall body posture and a tendency towards optimizing the indices of musculo-articular functionality of the spine. The results from the intermediate evaluations demonstrate progressive improvements in all measured parameters, as detailed in Tables 2 and 3.

Table 2: Intermediate muscle-articular evaluation (27.02.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	15.6	41	64.93
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	181	61	110.29
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	179	58	104.37

Table 3: Intermediate muscle-articular evaluation (26.03.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	20.8	49	103.5
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	21.5	66	140.7
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	213	56	118.2

The evolution of the somatic functionality of the trunk

Spine Pathology and Accident Risk Scale:
- < 0.50 = High Risk
- 0.50-0.75 = Medium Risk
- 0.75-0.95 = Low Risk

Initial Personal Levels:
- Lumbar: High
- Antero-posterior Thoracic: Medium

After Two Series of 10 Physical Therapy Sessions:
- Lumbar: Medium
- Antero-posterior Thoracic: Low

Chart number 1 illustrates the progress in muscle-articular functionality indices of the spine, specifically Thoracic Antero-posterior and Thoraco-lumbar, as assessed by Test 2 and Test 3. These improvements are also evident in the overall body posture, with notable

enhancements in the static balance and the projection of the GCG (see Figure 1).

Table 4: Segmental and intersegmental muscle-articular functionality ratios of the spine

Segmental and inter-segmental ratio	Optimal values	Personal values 03.02.2023	Personal values 27.02.2023	Personal values 26.03.2023
Thoracic anterior - posterior	0.85 - 0.95	0.73	0.94	0.84
Thoraco-lumbar	0.85 – 0.95	0.31	0.59	0.73

Chart number 1: Indices of muscle-articular functionality of the spine - Thoracic antero-posterior and Thoraco-lumbar

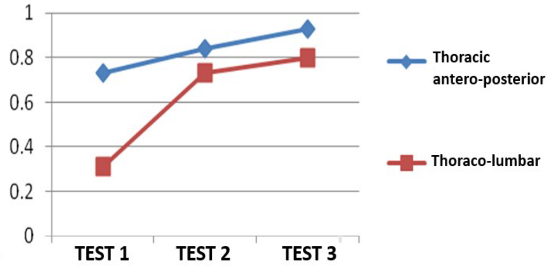

Figure 1: The improvements of the static ballance and the projection of the General Center of Gravity (CGG)

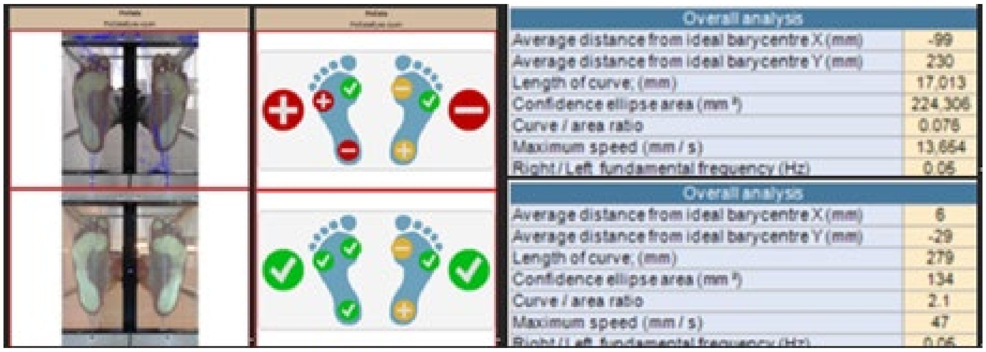

Overall analysis	
Average distance from ideal barycentre X (mm)	-99
Average distance from ideal barycentre Y (mm)	230
Length of curve; (mm)	17,013
Confidence ellipse area (mm^2)	224,306
Curve / area ratio	0.076
Maximum speed (mm / s)	13,664
Right / Left fundamental frequency (Hz)	0.06

Overall analysis	
Average distance from ideal barycentre X (mm)	6
Average distance from ideal barycentre Y (mm)	-29
Length of curve; (mm)	279
Confidence ellipse area (mm^2)	134
Curve / area ratio	2.1
Maximum speed (mm / s)	47
Right / Left fundamental frequency (Hz)	0.06

Unipedal Balance:
•Initially, unipedal support was impossible.
•After ten sessions, balance time increased to 21 seconds.
•After 20 sessions, the balance time reached 30 seconds.

General Center of Gravity (GCG)

Projection: Figures 2, 3, 4, and 5 depict the improvement in the migration of the General Center of Gravity in four positions: standing with eyes open, standing with eyes closed, facing left, and facing right.

Figure 2: The improvement of the migration of GCG in the position of stand-up face to the right side

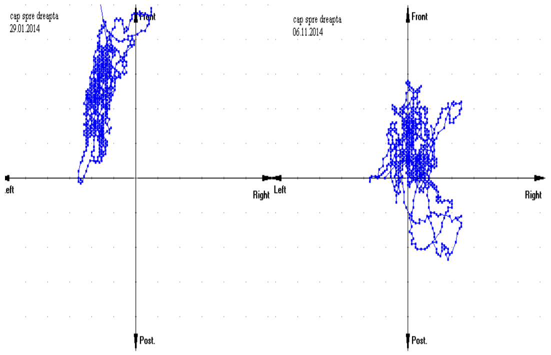

Figure 3: The improvement of the migration of GCG in the position of stand-up face to the left side

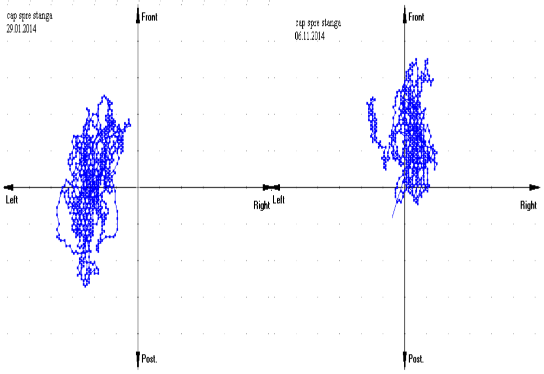

Figure 4: The improvement of the migration of GCG in the position of stand-up eyes closed

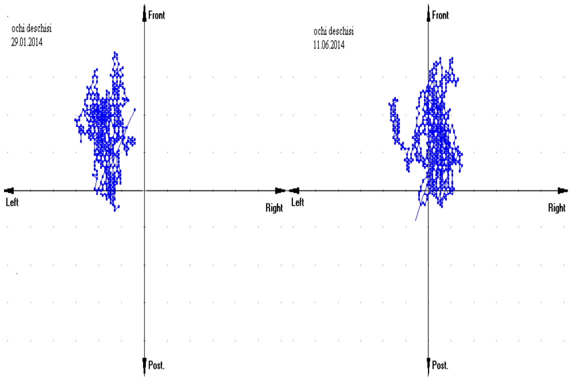

Figure 5: The migration of GCG improved in the position of standing up and eyes open

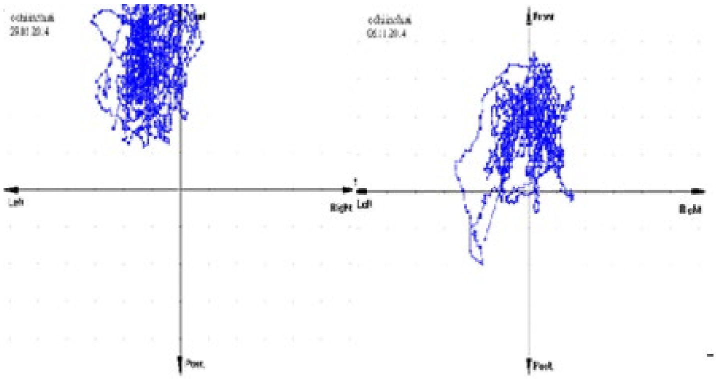

DISCUSSION

The key outcomes and benefits observed after completing two series of 10 physical therapy sessions in the study are presented in the following.

After Two Series of 10 Physical Therapy Sessions:
- Increased muscle strength and joint amplitude at the shoulder girdle.
- Continued need for abdominal muscle strength improvement to balance spinal muscle support.
- Enhanced muscle-articular functionality indices for the Thoracic Antero-Posterior and Thoraco-Lumbar regions, showing improvement from 0.73 to 0.84 and from 0.31 to 0.73, respectively.
- Balance time in the unipedal position with eyes open increased from 0 to 30 seconds.
- Improved gait from a jerky, insecure pattern to a smooth, balanced walk.
- During computerized somatoscopic evaluation, overall body posture improved, with the anterior projection of the CGG reducing from 11 cm to 5 cm from the normal projection.

The BACKFIX Method:
- Led to improved posture by optimizing muscle-articular functionality indices of the spine.

Visual Feedback on Force-Amplitude Patterns:
- Restored agonist-antagonist cooperation, positively affecting gait and balance motor control.

Optimized Functional Musculo-Articular Balance:
- Resulted in visible improvements in posture and walking pattern.

Risk Reduction:
- The risk of spinal pathology and accidents improved, moving from medium to low risk, with enhanced muscle chain synergies supporting proprioceptive and sensory system integration in unipedal stance.

The observed benefits from targeted physical exercises confirm previous studies on the uniqueness of bipodal and unipedal stance control, inter-segmental coordination, and the antigravity function of the leg extensors. Most cases of LD resolve after one course of therapy, although some patients experience gradual symptom relief even after treatment ends; therefore, follow-up assessments are recommended 3 months post-therapy. In some cases, symptoms persist despite antibiotic treatment, leading to the term "antibiotic-refractory LD", defined as persistent synovitis lasting more than 1 month after two four-week oral antibiotic courses or more than 2 months after intravenous ceftriaxone treatment [53].

Untreated arthritis may eventually resolve on its own, but it can persist for years, with some cases of LD lasting 7-8 years [54,55]. Potential treatments for patients with post-arthritis LD include non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), biologic agents, intra-articular steroids, and arthroscopic synovectomy [55,56].

The strengths of this study lie in its focus on the functional evaluation and rehabilitation of patients with Lyme disease-related neuro-muscular issues, an area with limited prior research. The use of computer-assisted physical therapy, employing technologies like the GPS Posture-test System and BACKFIX Technology, allowed for a precise and objective assessment of balance and posture, leading to significant improvements in the patients. These technologies facilitated a more thorough understanding of musculoskeletal impairments and offered targeted interventions that improved both static and dynamic balance, posture, and muscle coordination.

However, some limitations must be noted. The rehabilitation was conducted on one patient, and this limits the generalizability of the results. Additionally, the long-term sustainability of the therapy outcomes remains uncertain, and further follow-up studies are needed to verify the persistence of improvements beyond the immediate post-treatment period. Another limitation is the lack of control, which would have strengthened the validation of the study's findings.

Future research in this domain should focus on expanding the sample size and incorporating control groups for comparative analysis. Long-term studies are also crucial for understanding the chronic impact of rehabilitation techniques. Finally, integrating more advanced technologies, such as virtual reality or machine learning algorithms for personalized therapy adjustments, could further enhance patient outcomes in neuro-muscular recovery for Lyme disease.

CONCLUSION

This study demonstrates the effectiveness of computer-assisted physical therapy using technologies such as the GPS Posture-test System and BACKFIX Method in addressing the neuro-muscular and postural impairments associated with Lyme disease sequelae. Patients exhibited significant improvements in static and dynamic balance, posture, and musculo-articular functionality after completing two series of physical therapy sessions. The visual feedback provided during therapy sessions played a crucial role in restoring agonist-antagonist cooperation and enhancing motor control.

In conclusion, targeted physical therapy combined with advanced postural assessment technologies offers an effective means of improving functional recovery in Lyme disease sequelae patients, supporting their overall motor control and quality of life.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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The current manuscript does not contain previously published materials or self-generated AI text.

Authors' contribution

Conceptualization D.S. and V.S.; methodology D.S.; software V.S.; validation D. S., L.G.E. and A.G.; formal analysis R.P.; investigation P.S.F.; resources S.S. and M.B; data curation R.R.G.; writing—original draft preparation D.S.; writing—review and editing L.G.E and A.G.; visualization R.R.G.; supervision L.G.E.; project administration D.S.; funding acquisition V.S. All authors have read and agreed to the published version of the manuscript elaboration.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of MEDISPORT Physical Therapy Clinic (11/30.03.2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

Navigating Cancer: Mental Adjustment as Predictor of Somatic Symptoms in Romanian Patients- A Cross-Sectional Study

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Abstract: Objective: This study aimed to examine the relationship between mental adjustment mechanisms and somatic symptoms in cancer survivors, a topic with limited research in Psycho-oncology. Methods: In this exploratory cross-sectional study, 88 cancer patients were surveyed using the Somatic Symptoms Scale (SSS-8), the Somatic Symptom Disorder Scale (SSD-12), and the Mini-Mental Adjustment to Cancer Scale (MINI-MAC). Additional questions were asked regarding cancer stage, treatment, and illness relapses. Data collection occurred over one month at a local oncology unit. Results: Several significant correlations were observed between the scales and subscales of the instruments applied during this study. The most important results showed that maladaptive adjustment mechanisms were significantly associated with somatic symptoms, while adaptive mechanisms were not. However, "fighting spirit," an adaptive mechanism, acted as a moderator between somatic symptoms and their psychological impact (effect size $t = 0.157$, $p < 0.05$). Lower "fighting spirit" scores correlated with higher SSS-8 and SSD-12 scores, indicating its potential protective role. Conclusion: A more specific assessment of somatic symptoms and their link to mental adaptation to cancer is needed. Psycho-oncological interventions should address both adaptive and maladaptive adjustment mechanisms due to their significant influence on patient outcomes.

Keywords: cancer, mental adjustment, somatic symptoms, adaptive mechanisms, maladaptive mechanisms, Psycho-oncology

INTRODUCTION

The prevalence of cancer is increasing worldwide, with almost 18.1 million cases in 2020 and a rise of 28 million cases in 2040 [1]. Romania has a lower incidence of cancer related to EU mean value, but the mortality of this disease is higher, which reflects the overall weakness in the cancer approach in our country, Romania having among the highest oncological mortality rates in Europe [2,3]. Approximately 100,000 people are diagnosed with cancer in Romania every year, a number that could be even higher. In response, Romania approved a National Plan for Beating Cancer in 2022 to be implemented during 2023-2026. Addressing the cancer burden will require sustained efforts to improve cancer prevention, early diagnosis, treatment access, and cancer surveillance systems [4,5,6]. Key measures included in the country`s National Plan for Beating Cancer are integrated and present a multidisciplinary approach, health innovation fund, cancer registry, and data collection, clinical guidelines and protocols, and oncological experts' boards. Among these last ones, a special place will be highlighted by the Psycho-oncological approach. Psycho-oncology is facing significant challenges, including major gaps in services and a critical shortage of specialized professionals, resulting in a nationwide deficit in Psycho-oncology care. However, the concepts of mental adjustment, psychological distress, and quality of life are very scarcely mentioned in local literature. Broader searches for Eastern Europe yielded limited similar results, indicating a general lack of research [7].

Patients with cancer may present more frequent anxiety, depressive symptoms, somatic symptoms, and overall psychological imbalance due to the perceived poor outcome and recurrence of the disease. Recent research has reinforced the link between psychological distress and an increased likelihood of mortality and worse survival outcomes [8]. Psychological distress in patients with cancer is defined as the psychological damage that impairs an individual's ability to operate. This illness comprises a range of emotional and behavioral features arising from the diagnosis and prognosis uncertainty [9]. Prior studies have emphasized the importance of depressive disorders and extreme emotional response patterns as crucial elements in diminishing the quality of life of these patients. It is important to acknowledge the

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interconnection between anxiety, sadness, and somatic symptoms in the context of cancer experience [10].

The cancer adjustment frame was developed by Watson and includes five adjustment mechanisms: fighting spirit, avoidance–denial, fatalism–stoic acceptance, helplessness–hopelessness, and anxiety [11]. Mental adjustment to cancer refers to the process of coping with emotional distress and restoring mental balance after a cancer diagnosis. It involves specific attitudes and strategies that patients use to adapt to the challenges of cancer and its treatment. Patients with a fighting spirit tend to have better quality of life and lower anxiety/depression compared to those with a helpless/hopeless attitude [12,13]. Maladaptive coping styles like helplessness/hopelessness are associated with a higher risk of mortality in some studies, although the evidence is not yet conclusive [12]. Factors like age, performance status, social support, and satisfaction with support can influence mental adjustment. Mental adjustment, economic status, perceived disease severity, and symptom severity directly affect the comprehensive quality of life outcome (CoQoLO) in terminally ill patients [12,13].

Somatic symptoms constitute about 50% of all general medical consultations, remaining prone to further investigations and bringing frustration to both patients and doctors in approximately one-third to one-half of cases. Both physiological and psychological variables seem to contribute to the reporting of somatic symptoms, especially in individuals with long-term medical conditions [14]. These symptoms are linked to significant limitations in functioning, disability, and utilization of healthcare services, even when considering other medical and mental conditions [14,15,16]. Research on the frequency of somatic symptoms in cancer has frequently concentrated on individuals with advanced stages of the disease or specific forms of cancer observed at specialized medical facilities [12,13,17,18]. Research revealed that a minimum of 30% of patients with cancer experience the following symptoms: fatigue and low energy, unspecified pain, loss of appetite, constipation, sleep disorders, and dizziness. The connection between psychological distress and reporting of somatic symptoms, also known as somatization, has only been examined in a limited number of small-scale research on cancer [15, 16,19,20]. This gap is significant since several studies in non-cancer medical populations have shown a high occurrence of somatic symptoms, even among symptoms that are regarded to be more specific to a particular disease. Moreover, the somatization concept refers to the expression of psychological distress through physical symptoms. Somatic symptoms, the term we chose to use throughout this paper, conversely denotes the way emotional stress can materialize as physical symptoms. A thorough comprehension of this matter is crucial to effectively address the psychological requirements of individuals with cancer, thereby enhancing their quality of life and their capacity to manage the illness [13,16,21]. Despite the recognized importance of mental adjustment on QoL and prognosis in cancer care [12], there is limited research focusing on the interplay between mental adjustment and somatic symptoms specifically.

This study aims to fill this gap by exploring the relationship between mental adjustment to cancer and somatic symptoms in patients with cancer. The findings could provide valuable insights for Psycho-oncology professionals enhancing supportive care strategies, ultimately improving patient outcomes and quality of life. The primary objectives of this study are to assess the levels of mental adjustment to cancer among Romanian patients, to measure the prevalence and severity of somatic symptoms, and to investigate their relationship with mental adjustment as being predicted by certain types of adaptive and maladaptive mechanisms. We hypothesize that maladaptive coping styles will predict somatic symptoms and their burden, while adaptive coping styles may represent protective factors in the onset and maintenance of somatic symptoms. This cross-sectional study involved a sample of Romanian patients, implementing validated psychological assessment tools to evaluate mental adjustment and somatic symptoms. Statistical analyses were conducted to explore correlations and identify significant predictors.

MATERIALS AND METHODS

Study design

This study employed a cross-sectional design to investigate the relationship between mental adjustment to cancer and somatic symptoms and their burden in Romanian cancer survivor outpatients.

Participants

A total of 88 patients with cancer were recruited from the cancer department of Elias University and Emergency Hospital in Bucharest, Romania, for one month, between May and June 2023. Inclusion criteria were: a confirmed diagnosis of cancer in the last three years, age 18 years or older, out-patients regularly monitored during their cancer treatment follow-up, and ability to understand and provide informed consent. Exclusion criteria included: severe cognitive impairment, DSM-5-TR diagnosis of a severe psychiatric disorder such as schizophrenia, depression or bipolar disorder currently or in the past based on information obtained from behavioral scales, a clinical psychiatric evaluation, and the Structured Clinical Interview for DSM Disorders [22]; diagnosis of neurological conditions (e.g., Parkinson's disease, multiple sclerosis) that could affect cognitive or emotional processing, other genetic diseases or syndromes that might affect mental adjustment mechanisms or cognitive function.

Ethical approval

The study was approved by the Ethics Committee of the Elias University and Emergency Hospital of Bucharest, Romania (nr. 9286/2023). All participants provided written informed consent before participation.

Data Collection

Data were collected through structured interviews and self-administered questionnaires. The following tools were used:

Socio-demographic data- this section included information such as age, gender, education level, employment status, marital status, residence, income level, number of children, cancer stage, cancer location, chemotherapy, hormonal, surgery, and radiotherapy history.

Mini-Mental Adjustment to Cancer (Mini-MAC) – The questionnaire, designed by Watson[11], used the Romanian-translated version [23]. The mini-MAC questionnaire consists of 29 items, assessing different ways of coping with cancer: Anxiety- refers to fear concerning the disease without prior control which is anxiety-causing; Fighting spirit- refers to framing cancer as a challenge that may prone the patient to take action and fight it; Helplessness–hopelessness- refers to feelings of passivity and fatalism towards cancer leading to submission; Positive revaluation- refers to framing the life already lived in the light of satisfaction. Coping with cancer can be made within a constructive framing including fighting spirit and positive revaluation while anxiety and helplessness may represent a destructive pattern of coping. The items are rated on a scale of 1 to 4 representing variations of responses from „definitely not" to „definitely yes" which may offer results for each of the coping strategies from 7 to 28 points. The higher the score the more frequent and intense each mental adjustment mechanism is represented.

Somatic Symptoms Scale-8 (SSS-8)- The 8-item was developed after a version of the Patient Health Questionnaire-15 (PHQ-15) [24] and validated in the general population and Romanian population [25] as well as inpatient and outpatient settings, with SSS-8 not yet validated in Romanian language [26] to detect somatic symptoms burden. The level of the experienced somatic symptoms is scored from 0 meaning „not at all" until 4 which represents „very strongly". The results are grouped into different categories with minimum, low, medium, and high groups containing symptoms such as pain in the arms, legs, or joints, gastric problems, insomnia, unspecified thoracal pain or trouble breathing, fatigue, or different ranges of headaches.

Somatic Symptom Disorder-12 (SSD-12)- this tool was first developed by Toussaint et al. [27] and focused on criterion B of the Somatic Symptom Disorder (SSD) diagnosis including subscales on cognition, affect, and behavior. 12 items are scored from 0 meaning „never" to 4 meaning „very often". The scale was already validated in outpatient settings [28], and the Romanian version used, was previously translated and validated [19,29]. 18 score was chosen to screen for potential cases by the creators of the scale with studies still being concerned with the validity of cut-off points [30].

Procedures

Participants were approached during their routine medical visits and invited to participate in the study. Those who consented were given the questionnaires to complete on-site, with instructions for returning them. Structured interviews were conducted on-site by trained researchers and clinical psychologists to ensure consistency and accuracy in data collection and that the data collection processes did not interfere with the regular cancer follow-up procedure.

Statistical Analysis

Data were analyzed using JASP software [31]. In line with the explorative nature of our study, we first described the demographics of the study participants. The internal consistency of psychometric scales was assessed using Cronbach's alpha coefficient, and all instruments had coefficients above 0.7, which is the minimum acceptable value for proper reliability. Descriptive statistics (means, standard deviations, frequencies) were used to summarize the demographic and clinical characteristics of the sample. Pearson correlation coefficients were calculated to examine the relationship between mental adjustment and somatization scores. Multiple regression analyses were conducted to identify significant predictors of somatic symptoms, controlling for potential confounders and considering standard p-value < 0.05

RESULTS

Psychometric scales reliability

All the psychometric tools used proved to be reliable. As confirmed in our analysis, a tool needs to have a Cronbach Alpha of at least 0.7 to be used. Moreover, SSS-8 presented values of 0.815, SSD-12 of 0.910, and Mini-MAC of 0.879, with all subscales also confirmed to be reliable.

Descriptive analysis

Socio-demographic data

Table 1 presents the socio-demographic characteristics of the participants. The most frequent age interval of the participants was 41-50 years with over 70% being in the age interval of 41-60 years. The sample included 86 females (97.7%). 69,3% were married, over 68% graduated from at least one university, and 75% declared a good financial status. Concerning cancer, 82,5% had local cancer, and 71% presented breast cancer with various periods since surgery, from less than one year (20,4%) to over three years (36.6%). 79% declared chemotherapy intervention, while 64% declared radiotherapy, with over 90% presenting surgery history.

Psychometric scales scores

The scores for SSS-8 indicated moderate somatic symptoms burden (mean= 10.214, SD=5.903), while SSD-12 presented a mean of 16.753, close to the cut-off point of 18, considered for SSD screening [30]. Also, the behavioral aspect of SSD-12 presented a mean of

6.110 (SD=3.256). Concerning Mini-MAC subscales, we identified anxious preoccupation (mean=17.352; SD=4.857) and helplessness (mean=14.082; SD=4.198) among the highest-scoring mechanisms checked.

Table 1: Socio-demographic characteristics

Variable	Category	Frequency	Percent	Valid Percent	Cumulative Percent
Age	20-30	3	3.409	3.409	3.409
	31-40	6	6.818	6.818	10.227
	41-50	36	40.909	40.909	51.136
	51-60	27	30.682	30.682	81.818
	>60	16	18.182	18.182	100.000
Gender	Feminine	86	97.727	97.727	97.727
	Masculine	2	2.273	2.273	100.000
Marital Status	Married	61	69.318	69.318	69.318
	Single	5	5.682	5.682	75.000
	Divorced	17	19.318	19.318	94.318
	Widowed	5	5.682	5.682	100.000
Education	Elementary	1	1.136	1.136	1.136
	Medium	25	28.409	28.409	29.545
	University	33	37.500	37.500	67.045
	Master, PhD	29	32.955	32.955	100.000
Financial Situation	Bad	16	18.182	18.182	18.182
	Good	66	75.000	75.000	93.182
	Very good	6	6.818	6.818	100.000
Cancer Stage	Local	73	82.955	83.908	83.908
	Advanced	14	15.909	16.092	100.000
Cancer Location	Breast	71	80.682	80.682	80.682
	Digestive	1	1.136	1.136	81.818
	Genital	15	17.045	17.045	98.864
	Respiratory	1	1.136	1.136	100.000
Time Since Surgery	<1 year	18	20.455	22.785	22.785
	< 2 years	14	15.909	17.722	40.506
	2-3 years	15	17.045	18.987	59.494
	>3 years	32	36.364	40.506	100.000
Chemotherapy	No	16	18.182	18.605	18.605
	Yes	70	79.545	81.395	100.000
Radiotherapy	No	30	34.091	34.483	34.483
	Yes	57	64.773	65.517	100.000
Hormone Therapy	No	35	39.773	39.773	39.773
	Yes	53	60.227	60.227	100.000
Surgical History	No	8	9.091	9.091	9.091
	Yes	80	90.909	90.909	100.000

Table 2: Psychometric scales scores characteristics

Variable	Valid	Missing	Mean	Std. Deviation	Minimum	Maximum
SSS-8	84	4	10.214	5.903	1.000	32.000
SSD-12	73	15	16.753	8.052	1.000	39.000
SSD--12_Cognitive	88	0	4.170	2.649	0.000	14.000
SSD12_Afective	88	0	5.784	3.758	0.000	16.000
SSD-12_Behavioral	73	15	6.110	3.256	1.000	15.000
MAC-Helplessness	85	3	14.082	4.198	8.000	28.000
MAC-Anxious preoccupation	85	3	17.353	4.857	8.000	29.000
MAC-Fighting Spirit	85	3	12.388	2.111	5.000	16.000
MAC-Cognitive avoidance	85	3	11.153	2.432	4.000	16.000
MAC-Fatalism	85	3	14.847	2.780	7.000	20.000

The missing data pertains specifically to the behavioral aspects subscale of the SSD-12, with approximately 17% of participants not completing this part of the scale. This indicates that the missing data is not spread across the entire SSD-12 scale but is confined to a particular subset. To address this, analyses related to the behavioral component were conducted on available data. This approach

minimizes data loss but may introduce bias if the missing data is systematic (e.g., patients with more severe symptoms avoiding completion). No imputation was used for the missing data, and as such, the findings should be interpreted with caution. Sensitivity analyses showed that the inclusion or exclusion of these cases did not substantially alter the overall findings of the study.

Correlation between all psychometric scales analysis

We found significant correlations between SSS-8 scores and overall SSD-12 score including affective and behavioral aspects subscales (r=0,63; r=0,57; p<.001), anxious preoccupation (r=0.328; p<.01), helplessness (r=0.354, p<.01) and significant negative correlations with fighting spirit (r=-0.299; p<.001). SSD-12 scores were significantly correlated with helplessness and anxious preoccupation (r=0.558, p<.001; r=0.578, p<.001) as seen in Table 3.

Table 3: Pearson correlation coefficients between different variables

Variable	SSS8	SSD12	SSD12_ Cog	SSD12_ Affect	SSD12_ Beh	Help-lessness	Anxious preoccupations	Fighting spirit	Cognitive avoidance	Fatalism
SSS8	—									
Pearson's r	—	0.537***	0.420***	0.630***	0.570***	0.354**	0.328**	-0.299**	-0.023	-0.171
p-value	—	1.615×10^-6	7.014×10^-5	1.318×10^-10	2.592×10^-7	0.001	0.003	0.007	0.837	0.126
SSD12	0.537***	—	0.796***	0.950***	0.919***	0.558***	0.578***	-0.195	0.257*	-0.004
p-value	1.615×10^-6	—	3.648×10^-17	1.515×10^-37	1.814×10^-30	3.581×10^-7	1.084×10^-7	0.101	0.030	0.971
SSD12_ Cog	0.420***	0.796***	—	0.695***	0.562***	0.407***	0.469***	-0.111	0.104	0.110
p-value	7.014×10^-5	3.648×10^-17	—	5.610×10^-14	2.360×10^-7	1.124×10^-4	5.838×10^-6	0.312	0.344	0.315
SSD12_Afectv	0.630***	0.950***	0.695***	—	0.844***	0.520***	0.614***	-0.199	0.198	-0.029
p-value	1.318×10^-10	1.515×10^-37	5.610×10^-14	—	6.586×10^-21	3.423×10^-7	4.054×10^-10	0.068	0.069	0.790
SSD12_Beh	0.570***	0.919***	0.562***	0.844***	—	0.565***	0.544***	-0.165	0.241*	-0.013
p-value	2.592×10^-7	1.814×10^-30	2.360×10^-7	6.586×10^-21	—	2.278×10^-7	7.828×10^-7	0.167	0.043	0.914
Helplessness	0.354**	0.558***	0.407***	0.520***	0.565***	—	0.693***	-0.414***	0.057	-0.275*
p-value	0.001	3.581×10^-7	1.124×10^-4	3.423×10^-7	2.278×10^-7	—	2.733×10^-13	9.071×10^-5	0.606	0.012
Anxious preoccupations	0.328**	0.578***	0.469***	0.614***	0.544***	0.693***	—	-0.239*	0.152	-0.132
p-value	0.003	1.084×10^-7	5.838×10^-6	4.054×10^-10	7.828×10^-7	2.733×10^-13	—	0.028	0.170	0.233
Fighting spirit	-0.299**	-0.195	-0.111	-0.199	-0.165	-0.414***	-0.239*	—	0.311**	0.734***
p-value	0.007	0.101	0.312	0.068	0.167	9.071×10^-5	0.028	—	0.004	2.878×10^-15
Cognitive avoidance	-0.023	0.257*	0.104	0.198	0.241*	0.057	0.152	0.311**	—	0.425***
p-value	0.837	0.030	0.344	0.069	0.043	0.606	0.170	0.004	—	6.071×10^-5
Fatalism	-0.171	-0.004	0.110	-0.029	-0.013	-0.275*	-0.132	0.734***	0.425***	—
p-value	0.126	0.971	0.315	0.790	0.914	0.012	0.233	2.878×10^-15	6.071×10^-5	—

**p < 0.05, **p < 0.01, ***p < 0.001*

Tables 3 and 4 show the contribution and significance of each predictor of MAC mechanisms to somatization as follows. Helplessness and anxious avoidance negatively predict somatization (t=2.520, p<.01; t=2.462, p<.01) while fighting spirit is moderator of the relationship between somatization (SSS-8) and its burden (SSD-12) (t= 0.157, p<.01) with low and medium values having a positive correlation which disappears at highest levels.

Table 4: Multiple regression analysis

Model	Unstandardized Coefficient (B)	Standard Error	Standardized Coefficient (Beta)	t	p-value
H_0 (Intercept)	17.060	1.002	—	17.026	0.013
H_1 (Intercept)	-22.708	9.161	—	-2.479	0.016
Helplessness	0.744	0.295	0.385	2.520	0.014
Anxious preoccupations	0.583	0.237	0.342	2.462	0.017
Cognitive avoidance	0.701	0.377	0.191	1.858	0.068
Fatalism	0.601	0.479	0.174	1.254	0.215
Fighting spirit	0.108	0.685	0.025	0.157	0.876

DISCUSSION

Our study showed that mental adjustment to cancer and somatic symptoms are significantly interrelated in patients with cancer. Specifically, only negative mechanisms such as anxious preoccupation and helplessness predicted somatic symptoms burden and not the positive ones, while fighting spirit moderated the relationship between somatic symptoms burden and their overall impact on a cognitive, affective and behavioral level, suggesting a potential protective factor in the relationship between SSS-8 and SSD-12.

Previous research has proved that psychological distress can exacerbate somatic symptoms in patients with cancer [32]. Also, somatic symptoms were highlighted to have mediating effects between psychological distress and quality of life in patients with cancer, while screened-positive SSD patients represented a significant predictor of lower quality of life (QOL) ($B = -0.476$, $p < 0.00$) [33]. Also, previous studies reported somatic symptoms as being risk factors for cancer patients' prognosis using the same psychometric tool of SSD-12 but without an emphasis on mental adjustment mechanisms [34]. Moreover, research reported greater psychological adaptation and maturity in coping mechanisms are associated with lower levels of somatic symptoms and depression in patients with cancer [35]. Also, maladaptive emotional responses to cancer, characterized by strong associations between emotional and somatic symptoms, can lead to higher levels of somatization and poorer quality of life in patients with cancer [36]. To our knowledge, until now, no other study explored mental adjustment to cancer mechanisms in relationship with somatic symptoms and their burden.

Concerning specific mental adjustment to cancer mechanisms, previous research linked positive and negative affect to fatalism and fighting spirit with fighting spirit being negatively correlated to fatalism and negative affect [12,37], while another study highlighted anxious preoccupation as a predictor for significant decreases in all the domains of quality of life, with helplessness-hopelessness predictor for significant decreases in emotional and functional wellbeing domains [38].

We showed the strong association between helplessness and somatic symptoms suggesting that patients who feel more overwhelmed and powerless by their illness are more likely to report physical symptoms. Similarly, anxious preoccupation, characterized by excessive worry about the disease, may lead to heightened awareness and reporting of somatic symptoms. An interesting finding of our study relies on fighting spirit as an adaptive mechanism that moderates the relationship between somatic symptoms and overall impact on patients with low and medium values, having a positive correlation that disappears at the highest levels. This may explain fighting spirit as a protective factor in the relationship, an aspect worth considering in modern psycho-oncological approaches and in accordance with previous research on its link to positive affect [39]. The absence of significant effects for adaptive mechanisms in our sample may be attributed to the homogeneity of the sample, particularly regarding cancer type and stage, as well as potential measurement limitations of the Mini-MAC scale in capturing nuanced coping responses. These findings align with literature suggesting that adaptive mechanisms may exert more influence during acute or early-stage illness phases and less during chronic or long-term survivorship. However, our results contrast with studies reporting sustained benefits of fighting spirit over time. This discrepancy highlights the need for future research to explore these mechanisms in more diverse and heterogeneous patient populations and consider longitudinal designs to capture temporal changes in coping efficacy. Tailored interventions that account for individual patient contexts and preferences may be more beneficial than a one-size-fits-all approach focusing solely on adaptive mechanisms like fighting spirit [40].

Concerning somatic symptoms assessment, although the SSD-12 and SSS-8 have good reliability and validity, they cannot replace the diagnostic accuracy of a psychiatric interview. Our descriptive results obtained from this study for both SSD-8 and SSD-12 need to be interpreted appropriately. For many diagnoses, the DSM-5 requires that symptoms from SSD should not be the results of an underlying organic cause [41]. For patients with a medical condition such as cancer, this criterion is not realistic as symptoms may indeed be associated with both the underlying medical condition [42].

Somatic symptom identification in patients with cancer needs more attention to be paid to the psychological care of patients with physical symptoms. However, the observed relationship between mental adjustment mechanisms, such as fighting spirit, and somatic symptoms could be influenced by several confounding variables not fully controlled in our study. For instance, time since diagnosis may alter the effectiveness of coping strategies, with recently diagnosed individuals potentially exhibiting more distress and somatic symptomatology compared to long-term survivors who have developed adaptive coping mechanisms. Additionally, the type and intensity of treatment—particularly chemotherapy and radiotherapy—can exacerbate physical symptoms like fatigue and cognitive impairment complicating the assessment of psychological coping. Furthermore, the disease stage significantly impacts psychological well-being and symptom burden, with advanced stages often associated with higher levels of distress and lower fighting spirit. These

confounders could attenuate or amplify the observed relationships between mental adjustment and somatic symptoms, suggesting the need for future studies to incorporate these variables more comprehensively into their analytical models in order to disentangle their effects.

Therefore, it is necessary to conduct a comprehensive assessment of the patient`s psychological dimensions of the clinical presentation. Hence, we hope to present the phenomenon of somatic symptom prevalence as a consequence of poor mental adjustment mechanisms in patients with cancer, thus highlighting the need to consider this issue by both oncologists and psychiatrists [43,44].

Clinical Implications

Somatic symptoms can be as easily confusing as physical symptoms in this specific type of population, which results in both overmedication and insufficient psychosocial care. In our study, we demonstrated a significant correlation between somatic symptoms and maladaptive mental adjustment mechanisms, a finding that should warrant greater attention to this unique issue among patients with cancer. Identifying patients with high levels of helplessness and anxious preoccupation can help Psycho-oncologists target interventions to reduce it. Psycho-oncology programs focusing on enhancing coping skills and reducing anxiety may be beneficial in this regard for Romania and other countries as costs for invasive and time and financially resources consuming investigations may overwhelm an already limited and insufficiently developed multidisciplinary approach, which may lead to confusion and medical shopping among patients [3].

Limitations

Several limitations should be acknowledged. This was a cross-sectional study, which was unable to determine the causal relationship between mental adjustment mechanisms and somatic symptoms. We selected a single cancer department, including only one hospital from one area in the country, which may limit the generalizability of the findings considering the relatively small sample size. The patient population from this hospital may not be representative of the broader population of patients with cancer in other regions or countries considering factors such as regional healthcare practices, socioeconomic status, higher in Bucharest region, and cultural differences [2,45,46]. Also, the gender imbalance significantly limits the generalizability of the findings, especially for male cancer patients. Studies incorporating larger and more diverse cancer samples, as well as longitudinal studies, are recommended to further understand the relationship between previously described variables. In this study, a formal power analysis was not conducted prior to data collection. Given the complexity of the moderation analyses, particularly in examining the role of 'fighting spirit,' the current sample size of 88 participants may not provide sufficient power to detect small to medium interaction effects reliably. This limitation should be considered when interpreting the findings, as true moderating effects may not have been detected due to insufficient statistical power. Future research should aim to recruit larger samples and conduct a priori power analyses to ensure adequate power for detecting moderation effects. Many patients have more than one concomitant disease, especially advanced patients with cancer and those who have received or are undergoing conventional anticancer treatments. However, we collected information on all somatic symptoms but did not distinguish between symptoms derived from cancer, those derived from treatments and those that resulted from other illnesses. We would like to consider this issue in our future studies on somatic symptoms in cancer populations.

Future directions

Research on patients with cancer focusing on somatic symptoms is still scarce. Most of the studies remain in the stage of presenting descriptive outcomes. More research on the mechanism of somatic symptoms in cancer, whether it has a profound impact on quality of life or survivorship, how to identify it properly and in a timely manner, and which kind of psychosocial interventions would be more effective are necessary in the future. This is the first study to explore how mental adjustment to cancer may predict and moderate somatic symptoms and its burden in Romania and beyond. Future research should consider longitudinal designs to establish causal relationships between the described variables. Studies involving diverse cultural groups are also needed to explore the generalizability of these findings, considering the cultural aspect of somatic symptoms an important point to consider. Furthermore, investigating the effectiveness of psychological interventions in reducing maladaptive adjustment mechanisms would be a valuable contribution to the field of Psycho-Oncology**.**

CONCLUSION

In conclusion, this study highlights the significant relationship between mental adjustment mechanisms as predictors of somatic symptoms in patients with cancer. Considering the intricate nature of somatic symptoms and targeting through Psycho-oncologic interventions, helplessness, anxious avoidance, and fatalism while also exploring fighting spirit may help mitigate somatic symptoms and improve overall well-being in this population, leading to high-quality care for patients.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, M.L., C.G.I. and L.S.; methodology, L.S.; software, D.M.P.; validation, M.L.., O.V., and A.C.; formal analysis, D.M.P.; investigation, L.S.; resources, M.L.; data curation, C.G.I.; writing—original draft preparation, C.G.I.; writing—review and editing, M.L. and A.C.; visualization, O.V.; supervision, M.L.; project administration, L.S.; funding acquisition, M.L. All authors have read and agreed to the published version of the manuscript.

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Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Elias Emergency and University Hospital, Bucharest, Romania (nr. 9286/2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

The Neurocardiogenic Impact of Ischemic Stroke: Intricacies of Cardiac Enzymes and the Vegetative System

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Abstract: There is a potential lateralization of vegetative influences at the cerebral level. We sought to understand the impact of these influences in the setting of ischemic stroke. We conducted a prospective study on 110 who presented with acute ischemic stroke (symptom onset maximum 24 hours before admission). We investigated correlations between stroke subtype, insular location or lateralization, stroke severity, hospital mortality, and the dynamic of enzymes (Tn, TnT, CK, CK-MB). We demonstrated that a higher cardiac enzyme value is associated either with stroke severity or with a higher risk of death in the short term, this growth being a marker for a more severe prognosis of a large stroke rather than an independent cause of mortality. Cardiac monitoring in the acute phase of ischemic stroke can prevent cardiac morbidity and mortality, which is why it is important to identify patients at high risk of heart complications after stroke.

Keywords: stroke; vegetative system; cardiac enzymes

INTRODUCTION

The brain structures involved in cardiac autonomic activity regarding the modulation of the force of contraction, heart rate, and stress response are the anterior cingulate cortex, the parabrachial nucleus, the hypothalamus (the paraventricular nucleus, the dorsomedial nucleus, the lateral hypothalamic area), insula (especially its anterior portion), periaqueductal gray matter, and some parts of the medulla (ventrolateral rostral medulla, nucleus ambiguus, nucleus of the solitary tract) [1].

Right insular lesions lead to increased parasympathetic activity to the detriment of sympathetic activity, and the opposite occurs in left insular lesions [2]. Thus, it can be stated that there is a lateralization of vegetative influences at the cerebral level. This lateralization hypothesis applies to the intrinsic control of the cardiac vegetative system [3].

In a study of approximately 865,000 patients, it was observed that the incidence of acute myocardial infarction (MI) in patients with acute stroke reaches 1.6% (approximately 80% being non-STEMI and 20% STEMI). Advanced age is the strongest predictor of MI in stroke patients, along with ischemic heart disease, chronic kidney disease (CKD), and atrial fibrillation (AF) [4,5]. In the trial RANTTAS [6], it has been observed that in 6% of acute ischemic strokes, cardiac complications may occur, such as angina pectoris, acute MI, and cardiac ischemia. Cardiac monitoring is crucial after a stroke. Prolonged cardiac monitoring is a weak link in stroke care in Romania overall – evaluation past the first 24-48 hours is a rarity [7]. Acute MI after stroke may have the following etiopathogenic mechanisms: concomitant coronary artery occlusion or exacerbation of a pre-existing coronary artery disease secondary to excessive and abnormal catecholamine discharge in the acute phase of stroke [4,8,9]. Sympathetic hyperactivity in the acute phase of stroke leads to excessive stimulation of beta receptors, with the opening of Ca^{2+} channels with the intracellular flow of Ca^{2+}, contractile myocardial dysfunction, and ATP depletion, leading to mitochondrial dysfunction, and finally, cell death, translated into the paraclinical image of ECG changes and increased troponin (Tn) values [10].

In patients with acute ischemic stroke, myofibrillar degeneration, myocytolysis or the appearance of contraction band necrosis may occur, the death of myocardial

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cells occurring in a state of exaggerated contraction, followed by the rapid appearance of calcifications and infiltration of mononuclear cells. In contrast, in acute MI, the important occurrence of necrosis contraction bands is not identified, and the process of necrosis of myocardial cells takes hours - days, occurs in a state of relaxation, and calcifications are delayed. Thus, neurogenic myocytolysis involves the subendocardium with a high arrhythmogenic potential, different from the coagulative necrosis encountered in acute MI [11]. Scheiz et al. [12] proposed a method of classifying Tn growth in patients with acute stroke into two categories: patients with an acute Tn increase and patients with a chronic Tn increase. In those with an acute troponin (Tn) increase, after excluding acute MI, it was observed that, most likely, this increase is the consequence of neurogenic lesions.

MATERIALS AND METHODS

Patients selection

We conducted a prospective study on 110 patients hospitalized in the Neurovascular Emergency Unit who presented with acute ischemic stroke (symptom onset taking place a maximum of 24 hours before admission). We excluded patients with:

- Ischemic stroke with onset >24 hours before admission
- Hemorrhagic stroke
- The presence of simultaneous acute pulmonary edema
- Clinical and laboratory signs of simultaneous acute myocardial infarction
- History of MI in the last year
- Recent cardiac surgery (<1 year)
- History of severe CKD (terminal stage)
- History of lung disease with severe acute respiratory failure
- History of clinically relevant arrhythmias (especially atrial fibrillation)
- Advanced cardiac insufficiency (Stage III/IV NYHA)
- History of active malignancy

Objective

The study's objective was to highlight the correlation between enzymatic changes (their dynamic during the first five days after stroke onset) and insular involvement/insular lateralization, stroke etiology, severity of acute ischemic stroke (using the NIHSS score at admission and at seven days), and in-hospital mortality.

Data collection

Before inclusion in the study, patients were informed about the methodology and the clinical, paraclinical, imaging, and other explorations performed. Each patient (or caregiver if the patient has severe language disorder, impaired understanding, cognitive impairment, or impaired alertness) has signed informed consent, respecting the conditions of medical ethics. The approval of the Scientific Research Ethics Board of the University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania, was obtained for this study.

Etiological diagnosis of stroke

The etiological diagnosis of stroke was performed using the TOAST classification [13] after paraclinical tests (brain CT / MRI, Doppler ultrasound, standard ECG, 24-hour Holter monitoring, 5-day continuous ECG monitoring, and transthoracic echocardiogram).

Stroke severity

The clinical severity of ischemic stroke was calculated using the NIHSS (National Institutes of Health Stroke Scale) by a neurologist certified to use the NIHSS scale. According to the NIHSS scale, stroke patients were divided into 3 categories:

- mild stroke (NIHSS ≤8 points)
- moderate stroke (NIHSS = 9-15 points)
- severe stroke (NIHSS ≥16 points).

Statistical methods and statistical calculation tools

For both Tn and creatine-kinase MB (CK-MB) values, the Kolmogorov-Smirnov normality test (which determines to what extent the data have a normal/Gaussian distribution) was applied, which invalidated normality, which is why the Student t-test could not be applied. We resorted to a non-parametric test, Mann-Whitney (MW), which demonstrated the absence of a significant difference between troponin/CK-MB values on any 2 days (of the 5 days studied). The Mann-Whitney test was used to analyze the correlation

between stroke subtype, insular location/insular lateralization, stroke severity, hospital mortality, and enzymatic elevations (Tn, TnT, CK, CK-MB). The chi-squared test was applied to investigate the possible correlation between increased values of myocardial lesion indicators (choosing those with a higher cardiac specificity: TnT and CK-MB) and acute ischemic stroke.

RESULTS

Etiology, risk factors and demographic factors of patients

The mean age of the patients was 67.2+/-12.93 years, and the male-to-female ratio was 64.55%:35.55%. The prevalence of vascular risk factors and associated pathologies was the following – high blood pressure (HBP) 73.64%, obesity 31.82%, diabetes 23.64%, dyslipidemia 46.36%, active smoking 50%. Chronic alcohol usage was recorded in 27.27% of patients, stroke history in 21.82%, transient ischemic attack (TIA) in 1.82%, heart failure (HF) NYHA class 1 or 2)= in 5.45%, past MI in 8.18%, dementia in 10%, peripheral arteriopathy in 3.64%, chronic obstructive pulmonary disease in 1.82%, CKD (stage 1 or 2) in 0.91% of patients. Death during admission occurred in 14 of 110 patients (12.72%).

The etiological classification of stroke was performed based on the TOAST system criteria (Trial of Org 10172 in Acute Stroke Treatment) [13] in cardioembolic (29.09%), atherosclerosis of large vessels (50.90%) [including a small proportion atheroembolic (6.36%)], lacunar (3.64%), other causes (internal carotid artery dissection 1.81%) and cryptogenic (14.54%).

Insular localization of ischemic stroke and insular lateralization

Regarding the topographic location of stroke, out of the total of 110 patients included in the study, 63 patients (42.73%) had a stroke located in the insula. Of the 63 patients with insular localization, 23 patients (36.5%) had right insular localization and 40 patients (63.5%) had left insular localization.

The growth dynamic of cardiac enzymes was analyzed. There was no sustained increase in cardiac enzymes during the 5 days of follow-up. In all calculations concerning cardiac enzymes, the 3 patients who were found to have had acute MI after stroke onset were excluded.

Patients were divided into 2 categories according to Tn T levels: patients with normal values (≤0.02ng/ml) and patients with values above the upper limit (>0.02ng/ml). A similar division of patients was performed according to the CK-MB value: patients with normal values (≤25IU/L) and patients with superior values (>25IU/L). Following this analysis, it was observed that out of the total 110 patients, 60.86% had at least one elevated TnT value (in at least one of the 5 days of follow-up) (Figure 1), and 36.52% had at least one increased CK-MB value (in at least one of the 5 days of follow-up) (Figure 2).

For each patient, we took into consideration the highest value obtained in the 5 days of follow-up. The relatively high percentage of patients with elevated TnT is within the limits demonstrated in the literature, in a review [14] on increased Tn in patients with acute stroke (ischemic and hemorrhagic), this percentage difference is pending on various factors, such as the chosen day of reference, the number of patients and the arbitrary baseline. When applying the chi-squared test to analyze the correlation of TnT value above the normal limit of every 5 days with insular localization, on day 4 and 5, the TnT value correlation with insular involvement was statistically significant (Table 1). No statistically significant correlation with insular lateralization was observed. The increase in Tn (less specific for cardiac distress than TnT) did not correlate with insular involvement nor with left-right insular lateralization.

Figure 1: The distribution of TnT values in the studied group

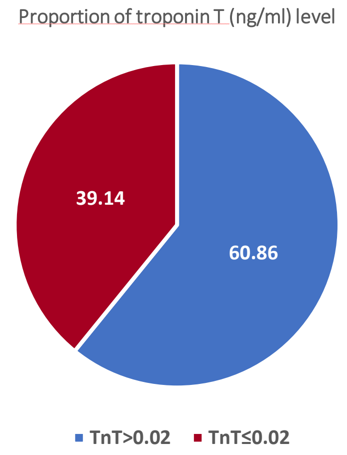

Figure 2: The distribution of CK-MB values in the studied group

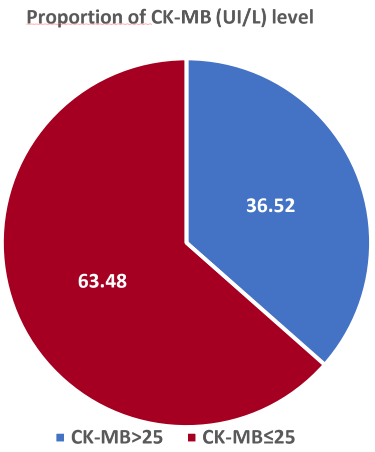

Table 1: TnT value and stroke location

Parameter	Insular	Non-insular	P-value
TnT day 4, [median (Q1-Q3)]	0.029(0.01-0.029)	0.01(0.01-0.029)	0,007 (Mann-Whitney)
TnT day 5 [median (Q1-Q3)]	0.029(0.01-0.029)	0.01(0.01-0.029)	0,02 (Mann-Whitney)

On average, CK-MB was significantly higher in patients with insular localization on each follow-up day (day 1 to day 5) (Table 2), but this was not statistically significant depending on insular lateralization. There was also no statistically significant correlation between insular location or insular lateralization and the CK value.

Table 2: CK-MB value related to stroke location

Parameters	Insular	Non-insular	P-value
CK-MB day 1, [median (Q1-Q3)]	17 (14-23)	14 (12-16)	0.006 (Mann-Whitney)
CK-MB day 2, [median (Q1-Q3)]	17 (13,21.5)	13 (11-15.25)	0,0004 (Mann-Whitney)
CK-MB day 3, [median (Q1-Q3)]	16.25 (13.25,20.75)	14 (11.25-17.75)	0,03 (Mann-Whitney)
CK-MB day 4, [median (Q1-Q3)]	19 (13.75-26.25)	13 (12-16)	0.0002 (Mann-Whitney)
CK-MB day 5, [median (Q1-Q3)]	16 (13-18.75)	13 (10.25-15)	0,003 (Mann-Whitney)

Stroke subtypes and cardiac enzymes

Regarding stroke etiology, a statistical correlation between stroke subtype and an increase in CK-MB or TnT during any of the 5 days of follow-up has not been demonstrated (neither by the Mann-Whitney test nor by the Chi-Square test).

Stroke severity and cardiac enzymes

When analyzing the correlation between stroke severity and cardiac enzyme elevations, the mean CK-MB on days 2, 3, 4, and 5 was statistically significantly higher in patients with severe stroke (NIHSS score ≥16) than in patients with moderate and mild stroke (NIHSS<16). CK (day 3 and 4) and Tn (day 4) were, on average, statistically significantly higher in patients with severe stroke than in patients with moderate to mild stroke (Table 3). TnT, much more sensitive for myocardial injury, did not record statistically significantly higher values in patients with severe stroke.

Table 3: Correlation between cardiac enzymes and stroke severity

Parameters	NIHSS<16 (moderate stroke)	NIHSS≥16 (severe stroke)	P-value
Tn day 4, [median (Q1-Q3)]	0.2 (0.2-0.2)	0.2 (0.2-0.26)	0.003 (Mann-Whitney)
CK day 3, [median (Q1-Q3)]	91 (51.9-139)	132 (82-254)	0.001 (Mann-Whitney)
CK day 4 (median (Q1-Q3))	100 (59-147)	115.5 (71.75-296)	0.004 (Mann-Whitney)
CK-MB day 2, [median (Q1-Q3)]	13 (12-16)	17 (13-20.75)	0.002 (Mann-Whitney)
CK-MB day 3, [median (Q1-Q3)]	14 (12-18)	17 (13-21)	0.002 (Mann-Whitney)
CK-MB day 4, [median (Q1-Q3)]	15 (12-19.5)	18.5 (13.75-26.25)	0.002 (Mann-Whitney)
CK-MB day 5, [median (Q1-Q3)]	13 (11-16)	15.5 (13-18.25)	0.002 (Mann-Whitney)

Cardiac enzymes and mortality

The correlation between enzyme increases and in-hospital mortality was analyzed. For Tn, a statistically significant correlation was observed between its increase on days 3, 4, 5 and hospital mortality. TnT on day 4 was, on average, significantly higher in patients who died during hospitalization than in those who survived. The increase in CK and CK-MB on days 4 and 5 correlated significantly with death during hospitalization (Table 4).

Table 4: Correlation between enzyme values and in-hospital death

Parameters	Death	No death	P-value
Tn day 3 [median (Q1-Q3)]	0.378 (0.2-1.129)	0.2 (0.2-0.2)	0,01 (MW)
Tn day 4 [median (Q1-Q3)]	0.506 (0.31-1.33)	0.2 (0.2-0.2)	0.002 (MW)
Tn day 5 [median (Q1-Q3)]	0.426 (0.26-0.81)	0.2 (0.2-0.2)	0,009 (MW)
TnT day 4 [median (Q1-Q3)]	0.049 (0.02-0.2)	0.01 (0.01-0.03)	0,008 (MW)
CK day 4, median (Q1, Q3)	326 (180.75-1621)	101 (61-177)	0,02 (MW)
CK day 5 [median (Q1-Q3)]	15 (188-4468)	288 (55.5-160)	0,01 (MW)
CK-MB day 4 [median (Q1-Q3)]	38 (21.5-55.75)	16 (12-20)	0.006 (MW)
CK-MB day 5 [median] (Q1-Q3)]	29 (19-63)	14 (12-17)	0,01 (MW)

MW=Mann-Whitney

DISCUSSION

With regard to the growth dynamics of cardiac enzymes, a time-based increase was not observed as in other studies [15]. No statistical correlation has been demonstrated (either by the Mann-Whitney test or by the chi-square test) between the stroke subtype and CK-MB or TnT elevation on any of the 5 follow-up days.

The increase in myocardial injury indicators has been interpreted differently in existing studies, with some authors considering it a marker of stroke severity and not an independent factor of poor prognosis [16] and others see it as an independent predictor of disability or mortality [17,18]. Apart from the current study, the literature has revealed only one study [19] that previously targeted the timed growth (days 1, 2, 3, 4, 5) of cardiac enzymes in stroke patients; other studies chose a variety of values of cardiac enzymes.

The possible pathophysiological mechanisms of increasing TnT and CK-MB in patients with acute stroke are multiple, but none of the studies existing so far in the literature could establish the exact cause. There is evidence of neurocardiogenic influences on myocytolysis in the acute phase of stroke, but there is great controversy regarding the cardiogenic increase in CK-MB. This increase may be possible in the case of a stroke location with a high potential for heart damage. Supporting the theory of a cardiac CK-MB increase secondary to neurogenic influences, in our study, 36.52% of patients had values above the normal limit of CK-MB, and the value of CK-MB (and TnT) correlated with insular involvement.

The current study offers different results than other literature references [20] - which concluded that TnT, unlike CK-MB, does not increase in patients with acute ischemic stroke. Also, the current study demonstrates contradicting conclusions to Barber et al. [21], which showed that in patients with acute ischemic stroke and elevated TnI, this increase did not correlate with insular location, one explanation being that in the cited study, only one TnI value was measured upon admission, whereas in the current study, statistically significant results were obtained on day 4 and 5 from stroke onset. Other examples from the literature [22] demonstrated a correlation between increased TnI and right hemisphere ischemia, but no insular involvement was specified, and no differences in insular lateralization were observed. One explanation could be that this increase is not related to other brain locations except insular ones, although further studies on other brain locations will need to be conducted to prove this claim. An argument against the theory mentioned above would be that the results obtained are controversial when it comes to the correlation of TnT alone with cardiac function, which makes it possible that CK-MB increase is not of cardiac origin and demonstrates that TnT is a much more reliable parameter to highlight the cardiac impact of chronic ischemia.

The statistical significance of elevated TnT and CK-MB values at days 4 and 5 with insular location may actually be due to a large ischemic stroke, and the increased TnT may reflect the systemic severity of stroke that may worsen the neurological status. A possible pathophysiological mechanism would be that in the case of large ischemic strokes, the growth curve of cerebral edema is maximum on days 4-5-6 from the onset of cerebral ischemia. Cerebral edema during these days may cause an exacerbation of sympathetic activity; this explanation is supported in a previous study [15], which revealed that CK-MB values are higher on day 5 of stroke onset. Obviously, this hypothesis will require further research in the future, by measuring catecholamines and enzymes simultaneously. Increased CK-MB value may originate elsewhere (muscle damage due to venous punctures, fluid restriction, and negative caloric

intake) [20]. In support of this argument comes the association of growth and CK.

In fact, it is well known that TnT is much more sensitive for assessing cardiac ischemia, either acute or chronic. The authors are of a similar opinion to the study conducted by Jensen et al. [18] that the increase in TnT is rather the consequence of HF. Another plausible explanation for the increase in TnT could be the existence of unstable atheroma plaques in the coronary arteries that could cause recurrent myocardial ischemia. It is probably necessary to dose other types of Tn (hsTnI) to correctly evaluate cardiac myocytolysis in patients with acute ischemic stroke and to redefine the concept of neurogenic cardiac ischemia.

Although this study cannot accurately state the pathophysiology of the increase in cardiac enzymes, we demonstrated that a higher value is associated either with stroke severity or with a higher risk of death in the short term, this growth being a marker for a more severe prognosis of a large stroke rather than an independent cause of mortality.

Looking at cardiac enzymes, the value of TnT and CK-MB was related to stroke severity, which indicates that in well-selected cases cardiac enzyme dosing may be useful for stratifying the risk of cardiac complications. It cannot be stated that the dosing of TnT and CK-MB should be performed routinely in all patients with acute stroke, being an expensive method and not providing additional net benefit**.**

CONCLUSION

Cardiac complications are common in patients with acute ischemic stroke, some of which may be potentiated by autonomic hyperactivity. Careful monitoring of patients at high risk of heart complications may change the short-term prognosis.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization and design, MMM, DD, ST; data analysis, MMM, DD; preparing the manuscript, MMM, IIE, ICS, MIG, OV, ST, DD, CV, CAS; writing, review and editing, MMM, DD.; visualization, MMM, DD; supervision, MMM. All authors have read and agreed to the published version of the manuscript.

Acknowledgment

The current manuscript does not contain previously published materials or self-generated AI text.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of "CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY (protocol code PO-35-F-03 no. 83 and date of approval: 29.02.2016).

Patient consent for publication

Written informed consent has been obtained from the patient(s) to publish this paper.

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REVIEW

Radiotherapy and Reconstructive Surgery: Challenges, Implications and Optimized Therapeutic Approaches

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Abstract: Radiotherapy plays an indispensable role in cancer treatment, with its ability to target malignant cells and improve patient survival. However, its impact on healthy tissues poses significant challenges for reconstructive surgery. The altered tissue environment resulting from radiation, including fibrosis, vascular damage, and compromised wound healing, complicates reconstructive efforts and increases the risk of surgical complications. As cancer therapies advance, the approaches for managing radiation-induced complications must also adapt accordingly.

This article explores the complex interaction between radiotherapy and reconstructive surgery, particularly in the treatment of head and neck cancers, breast reconstruction following mastectomy, and sarcoma management. Special attention is given to the customization of surgical approaches based on cancer type, with the goal of optimizing both functional and aesthetic outcomes. A multidisciplinary approach, integrating advanced imaging techniques, modern radiation delivery methods, and innovative surgical techniques is essential for improving patient outcomes.

Keywords: radiotherapy, reconstructive surgery, head and neck cancer, breast reconstruction, sarcoma

INTRODUCTION

Radiotherapy is an essential part of therapeutic management, utilized in approximately 50-60% of all cancer patients during their treatment. It plays an important role in controlling tumor growth, reducing recurrence rates, and improving overall survival. However, despite its therapeutic efficacy, radiotherapy can have serious adverse effects on surrounding healthy tissues, leading to complications such as fibrosis, vascular damage, and impaired wound healing. These radiation-induced changes significantly complicate subsequent reconstructive surgery, particularly in areas where structural architecture and functional restoration are critical. [1-3]

The association between radiotherapy and reconstructive surgery presents unique challenges, especially in cases of head and neck cancers, breast cancer treatment, and sarcomas. This article aims to explore the impact of radiotherapy on reconstructive surgery, focusing on these three specific cancer types, the final goal being to optimize both functional and aesthetic outcomes for cancer survivors.

OVERVIEW OF PARTICULAR SITUATIONS REGARDING RADIOTHERAPY AND RECONSTRUCTIVE SURGERY

Head and neck cancer treatment

Head and neck cancers are frequently treated with radiotherapy, either as a primary modality or adjuvant therapy following surgery. While radiotherapy effectively targets malignant cells, it also damages surrounding healthy tissues, leading to fibrosis, vascular compromise, and wound healing complications. [4] Novel strategies for improving radiotherapy protocols are currently being explored. In the case of radioresistant tumors, for head and neck oncological cases, proton and carbon ion therapies are emerging as superior options. Proton therapy facilitates the delivery of high-dose radiation accurately, concurrently preserving adjacent healthy tissue, thereby rendering it appropriate for the eradication of microscopic disease.

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Carbon ion therapy, with its higher linear energy transfer, is more effective for larger tumors with significant burdens, offering enhanced biological effectiveness in tumor cell damage. [5]

For locoregional recurrences or second primary malignancies, reirradiation (RERT) with advanced techniques like intensity-modulated radiation therapy (IMRT) and stereotactic body radiotherapy (SBRT) is a viable option. These methods improve survival rates and reduce toxicity by precisely targeting the tumor while sparing normal tissues. [6]

Intraoperative Radiation Therapy (IORT) has been described to be efficient when utilized alongside surgical procedures. IORT provides a targeted radiation boost during surgery, potentially reducing the need for high doses of external beam radiation and minimizing exposure to normal tissues, particularly beneficial in recurrent cases where additional radiation is required. [7]

Radiotherapy in the head and neck region presents several unique challenges. One of the primary concerns is fibrosis and soft tissue contracture; radiation induces progressive fibrosis, which can result in trismus, dysphagia, and restricted neck mobility. Soft tissue contracture can further complicate flap elevation and wound closure. Compromised vascularity is another challenge, as radiotherapy damages the microvasculature, leading to poor perfusion and an increased risk of flap necrosis. Additionally, the complex anatomy of the head and neck region, which includes multiple vital structures such as the facial nerve and carotid artery, makes surgical navigation particularly challenging. [7-11]

To address these challenges, several surgical strategies are currently employed. Reconstructive surgery after head and neck cancer aims to restore function, appearance, and quality of life. Free tissue transfer, utilizing free flaps such as the anterolateral thigh (ALT) flap or radial forearm flap, remains a mainstay due to their robust vascularity and versatility. Preoperative imaging modalities like CT angiography or MR angiography are crucial for assessing recipient vessel patency and planning the anastomosis. In cases where free flap transfer is not feasible, pedicled flaps, such as the pectoralis major myocutaneous flap, provide reliable coverage. Additionally, functional reconstruction, including dynamic reconstruction of facial nerve function using gracilis muscle transfer and nerve grafts, is performed to restore facial symmetry and expression. [11-14]

Emerging techniques in this field show promise for improving outcomes. For mandibular reconstruction, the fibula flap remains the gold standard, followed by scapular flaps in selected cases; however, recent advancements in 3D printing technology have enabled the creation of custom-made implants, which can enhance functional outcomes. Despite their effectiveness, free flaps carry a higher risk of complications, such as pedicle thrombosis, especially when using irradiated vessels. [13,15-17]

Breast Reconstruction after breast cancer

Breast cancer is the most common malignancy among women and the leading cause of cancer-related mortality in females. [18,19] Surgical options for breast cancer treatment generally include breast-conserving therapy associated with radiotherapy or mastectomy, both of which offer comparable survival rates for patients with early-stage disease. [18,20] With the advent of magnetic resonance imaging (MRI), BRCA-1 and BRCA-2 genetic screening, and advancements in surgical techniques, mastectomy followed by reconstruction has become an increasingly preferred treatment option. [18] For patients undergoing mastectomy with reconstruction, a decision must be made between autologous tissue reconstruction and implant-based reconstruction. Both approaches can be performed either immediately following the mastectomy or at a later stage. Radiotherapy is recommended for high-risk cases, significantly increasing the complication rate associated with any type of reconstruction. [18,21-23]

Breast reconstruction after radiation therapy is a challenging process that aims to restore the breast's appearance while managing a series of adverse effects introduced by radiation. [24]

Deciding on the type of breast reconstruction involves a thorough discussion between the patient and her reconstructive surgeon, focusing on the expected outcomes and potential complications. Autologous reconstruction, which uses the patient’s own well-vascularized soft tissue, closely resembles natural breast tissue, can be easily shaped, and generally yields more natural-looking results. Moreover, autologous reconstruction is known to be more resilient to the effects of post-mastectomy radiation therapy (PMRT). [25,26]

A significant challenge in breast reconstruction, particularly for patients who have received or will receive radiation therapy, is determining the optimal timing for reconstruction. Radiation therapy, while crucial for reducing the risk of cancer recurrence, can complicate reconstruction efforts. Post-mastectomy radiotherapy is a common treatment that can reduce local recurrence rates and improve survival, especially in patients with lymph node-positive breast cancer. However, PMRT can negatively impact the outcome of flap reconstructions, leading to complications such as flap loss, wound contracture, and fat necrosis. [27-30]

Breast reconstruction using autologous tissue is favored because it results in a more natural-looking breast with a soft texture, consistent temperature, and a certain degree of ptosis that closely mimics the natural breast. Furthermore, autologous tissue is more resilient to the effects of radiation therapy, making it a suitable option for patients who have undergone or will undergo radiation. Autologous reconstruction is considered the gold standard by many plastic surgeons due to its superior aesthetic outcomes and the fact that it allows the reconstructed breast to age naturally alongside the other breast. It is also more customizable, enabling surgeons to tailor the reconstruction to the individual patient's needs. [31-34]

Multiple flap options are available for breast reconstruction, each tailored to meet specific patient anatomical considerations and reconstructive goals. The latissimus dorsi myocutaneous (LD) flap remains a versatile and reliable choice, particularly in cases where moderate tissue volume is required. Abdominally-based flaps, such as the transverse rectus abdominis myocutaneous (TRAM) flap,

deep inferior epigastric perforator (DIEP) flap, and superficial inferior epigastric artery (SIEA) flap, are considered the gold standard for autologous breast reconstruction due to their abundant soft tissue, robust vascularity, and the potential to achieve highly natural aesthetic outcomes. Thigh-based flaps, including the transverse upper gracilis (TUG), transverse myocutaneous gracilis (TMG), profunda artery perforator (PAP), and lateral thigh perforator (LTP) flaps, provide valuable alternatives for patients who are unsuitable candidates for abdominal donor sites, offering well-vascularized tissue with satisfactory volume and contour. Gluteal flaps, such as the superior gluteal artery perforator (SGAP) and inferior gluteal artery perforator (IGAP) flaps, are particularly beneficial in cases where other donor sites are not viable or in patients requiring a larger volume of tissue. The thoracodorsal artery perforator (TDAP) flap and lumbar artery perforator (LAP) flap represent additional options for autologous breast reconstruction, particularly in cases where more conventional donor sites are unsuitable. [35-46]

Two main strategies exist for combining flap reconstruction with radiotherapy: immediate reconstruction, where the reconstruction is performed immediately after the tumor is removed and before radiation therapy, and delayed reconstruction, where radiation is completed first, and reconstruction is performed 6-12 months later. Immediate reconstruction has the advantage of preserving the breast envelope, making the surgery easier and potentially resulting in better aesthetic outcomes. However, it also comes with significant risks. Radiation therapy can cause fibrosis, shrinkage, and fat necrosis in the reconstructed breast, leading to higher rates of complications compared to unirradiated reconstructions. Additionally, complications from immediate reconstruction can delay the start of radiotherapy, which could negatively affect the patient's cancer prognosis. [26,46,47]

Delayed reconstruction, on the other hand, allows the skin and tissues to recover from the effects of radiation before undergoing reconstruction. This approach may reduce the risk of complications but often results in less favorable cosmetic outcomes. The skin and tissues may become fibrotic and scarred, limiting the options for reconstruction and making it harder to achieve a natural breast shape. [48,49]

To minimize radiation injury to the flap while preserving the breast mound, a two-stage approach called delayed immediate autologous reconstruction (DIAR) can be considered. This technique, described by Kronowitz et al., allows for the preservation of the native breast skin and supports the patient’s psychological well-being. DIAR offers the advantages of skin-sparing mastectomy while reducing radiation-induced complications. However, the use of a tissue expander in this method is not without risks. It is debated whether deflating the tissue expander before radiotherapy is necessary. Historically, deflation was thought to give the radiotherapist better control, similar to treating a flat chest. However, more recent studies suggest that deflation is not essential, and keeping the expander inflated may actually reduce radiation-induced complications such as seroma formation and capsular contracture. [50-56]

While autologous reconstruction is often preferred for its aesthetic appeal and lower overall complication rates, prosthetic reconstruction is a practical alternative for some patients. Despite the well-documented increased risks of complications, infections, capsular contracture, the need for revision surgery, and overall reconstruction failure associated with prosthetic reconstruction in the setting of PMRT, it remains an option for a select group of patients. [57,58]

Prosthetic reconstruction, like autologous reconstruction, can be performed in either a single stage or two stages. Single-stage reconstruction, commonly known as direct-to-implant (DTI) reconstruction, involves placing the final implant during the initial surgery. The two-stage approach involves first placing a tissue expander, which is later replaced with a permanent implant. A key debate in prosthetic reconstruction is whether radiotherapy should be administered to the implant or the tissue expander. Recent systematic reviews indicate a significantly higher failure rate when PMRT is delivered to a tissue expander compared to an implant. Although the complication rates are generally lower when using implants rather than tissue expanders, these reviews often overlook the long-term results. Capsular contracture, which can negatively impact patient quality of life and satisfaction with the cosmetic outcome, often does not become apparent until years after the reconstruction. Patients who have previously undergone radiation therapy have the highest rates of reconstruction failure across all groups. Due to the significant morbidity associated with tissue expansion in irradiated skin, pure prosthetic delayed reconstruction is relatively contraindicated. [54,59-63]

Autologous fat grafting, or fat transfer, is frequently used in breast reconstruction, either as a complementary technique or a primary modality. This procedure implies the harvesting of adipose tissue from donor sites such as the abdomen, thighs, or flanks through liposuction, followed by purification and injection into the breast to augment volume, contour, and symmetry. Fat grafting improves the aesthetic results of both flap-based and implant-based reconstructions, addressing contour deformities and enhancing the overall cosmetic outcome. Moreover, fat grafting has demonstrated the capacity to improve the quality of irradiated skin by promoting neovascularization and decreasing radiation-induced fibrosis, providing not only simple volume restoration but also further therapeutic advantages. The grafted fat contains adipocyte-derived stem cells, whose regenerative capacity has been well-documented in various clinical contexts, contributing to improved tissue repair and functional recovery. Although long-term outcomes of fat grafting are still under investigation, current evidence supports its role as a versatile, minimally invasive technique that can significantly improve both the functional and aesthetic results of breast reconstruction. [64-69]

Reverse expansion is a technique used in breast reconstruction after nipple-sparing mastectomy, where a tissue expander is initially inflated to maintain the skin envelope of the breast and to provide adequate coverage for reconstruction. The expander is then gradually deflated, allowing the tissue to contract naturally, resulting in a more contoured and natural breast shape for the final reconstruction. In selected, reverse expansion can be combined with lipofilling to achieve very good outcomes in breast reconstruction, a technique described also as “intratissular expansion with serial deflation–lipofilling sessions”. While reverse expansion helps prepare the tissue and create the necessary space for reconstruction, lipofilling in multiple sessions is used to provide

volume, enhance the aesthetic result, smooth out any irregularities, and improve the quality of irradiated or compromised skin. [70-72]

Radiotherapy in the treatment of breast cancer also increases the risk of secondary lymphedema. A key area of focus is the prevention of lymphedema and the development of advanced therapeutic strategies. These approaches encompass not only conservative management but also cutting-edge super-microsurgical techniques, including the use of robotic microsurgery, to enhance treatment outcomes. [73-76]

These advanced reconstructive options enable the customization of surgical approaches to optimize both functional and aesthetic outcomes in breast reconstruction following mastectomy.

Sarcoma treatment

Sarcomas are highly aggressive tumors that pose a significant risk to patient survival, necessitating prompt and effective treatment. Comprehensive management strategies, including a combination of surgery, radiation therapy and chemotherapy in specific cases, are essential to optimize outcomes and mitigate the high potential for local recurrence and metastasis. Early diagnosis and a multidisciplinary approach are crucial to improving prognosis and reducing the substantial morbidity and mortality associated with these tumors. Radiation therapy plays a crucial role in the management of sarcomas, particularly in cases where surgical resection alone may not achieve optimal outcomes. Given the diverse histological subtypes and varying radiosensitivity of sarcomas, radiation therapy can be tailored to enhance local control, minimize recurrence, and improve overall survival rates. Preoperative radiation is often utilized to reduce tumor size, making surgical resection more feasible while preserving surrounding tissues and critical structures. Conversely, postoperative radiation may be employed to eradicate microscopic residual disease in cases where negative surgical margins are difficult to achieve. [77-79]

Concerns about negative microsurgical outcomes in patients receiving preoperative radiation therapy are justified. Irradiated wounds are described as challenging surgical environments, marked by dense fibrosis, tissue edema, and fragile recipient vessels that are prone to rupture. At the cellular level, in vitro studies have shown that radiation therapy induces cytokine upregulation in the endothelium and accumulation of inflammatory cells. [80,81]

Some studies suggested that a key strategy to successful free tissue transfer is selecting recipient vessels outside of the irradiated field. This is a more practical approach in head and neck reconstruction where there are a plethora of available recipient vessels. However, in limb reconstruction, the irradiated field often spans the entire limb circumference and getting out of the irradiated field may require the use of vein interposition grafts, which themselves can increase free flap failure rate. [82]

Surgical resection with wide margins, combined with perioperative radiation therapy, is the standard treatment for extremity soft tissue sarcomas. [83]

The timing of radiotherapy in the treatment of soft tissue sarcoma remains a topic of debate. Radiation protocols typically include either pre-operative or post-operative external beam radiotherapy, or brachytherapy. Pre-operative irradiation offers several benefits, including the delivery of a lower radiation dose to a smaller field, the potential for tumor downsizing before resection, and improved long-term functional outcomes. However, these advantages are offset by a higher incidence of wound healing complications compared to post-operative radiotherapy. [84]

Intraoperative radiotherapy (IORT) can be employed to enhance margin control by delivering a concentrated dose of radiation (typically 10-12 Gy) to the tumor bed during surgery. This approach is particularly useful when tumors are near vital neurovascular structures or when the resection margin is within several millimeters of the sarcoma. [85]

Regarding microsurgical outcomes, there are additional concerns about the effects of radiotherapy on the quality of recipient vessels, the recipient bed, and ultimately, the survival of the flap. When planning reconstruction for a soft tissue defect, key considerations include the size, location, and depth of the defect. Small-sized defects with a limited zone of injury can be effectively covered using local or perforator-based flaps. For medium-sized defects, axial pattern pedicled flaps are suitable, as they offer a greater reach while still utilizing local tissue.

Large defects, which cannot be adequately covered with local tissue, require distant or free flaps, as these are not constrained by local tissue availability. Locoregional flaps are often effective for reconstructing defects of moderate size. Among these, local fasciocutaneous flaps and pedicled muscle or myocutaneous flaps are viable options. The pedicled rectus abdominis flap, which can be oriented vertically (vertical rectus abdominis musculocutaneous, VRAM), obliquely, or transversely (TRAM), is versatile for reconstructing various defects.

Commonly used muscle flaps include the gracilis, rectus femoris, sartorius, and vastus lateralis. The anterolateral thigh (ALT) flap is the primary fasciocutaneous option, and keystone and freestyle propeller flaps can also be designed for a variety of thigh or truncal defects. For larger defects, the latissimus dorsi (LD) and rectus abdominis are commonly used free muscle flaps, both of which can be harvested with a skin paddle. Additionally, the subscapular and lateral femoral circumflex arterial systems (LFCA) allow for the harvesting of large chimeric flaps for composite tissue replacement when necessary. For reconstructing large defects, the ALT, TRAM, and latissimus dorsi free flaps are excellent choices. For smaller defects, options include the free gracilis, radial forearm, parascapular, lateral arm, and superficial circumflex iliac artery perforator (SCIP) flaps. [86-90]**.**

CONCLUSION

Radiotherapy, while essential for cancer treatment, introduces significant challenges to reconstructive surgery due to the damage it causes to healthy tissues. Advances in radiotherapy techniques, such as proton and carbon ion therapies, offer promising solutions for minimizing damage to surrounding tissues, thereby enhancing reconstructive outcomes. Surgical innovations, including the use of autologous flaps, 3D printing, and intraoperative radiotherapy, have significantly improved the ability to restore function and aesthetics in patients undergoing reconstruction after radiotherapy. However, the complexity of irradiated tissues requires a specific, multidisciplinary approach that takes into consideration the particular needs of each patient and cancer subtype. As reconstructive techniques continue to evolve, further research and collaboration between oncology, radiology, and surgery will be of utmost importance in the improvement of cancer survivors' outcomes.

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ORIGINAL ARTICLE

Multidrug-resistant ESKAPE Pathogens in a Romanian Secondary-Care Hospital during the COVID-19 Pandemic

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Abstract: The study aims to assess the frequency of ESKAPE pathogens and their antimicrobial resistance profile during the COVID-19 pandemic in a multidisciplinary hospital. Based on microbiological reports, the present study retrospectively evaluated the frequency and antimicrobial resistance (AMR) profile of ESKAPE microorganisms during 2020-2022 in a multidisciplinary hospital in Romania. There were reported 1117 bacterial isolates during the study and ESKAPE pathogens account for 33.5% of the strains. The specific AMR of ESKAPE pathogens cumulates 30.9%, while antimicrobial multidrug resistance characterizes 21% of all ESKAPE strains. The AMR profile of K. Pneumoniae, followed by S. aureus, and P. aeruginosa are alarming on ESKAPE surveillance. The most frequent bacterial isolates and antibiotic resistance reports were identified in the urology department. Based on the local AMR data we suggest the first-line antibiotic recommendations when ESKAPE pathogens are suspected. The directions of the near future antibiotic stewardship program focus on revising the clinical protocols of diagnosis and treatment in each department, increasing the bacteriological samples collection, improving the microbiological diagnostic techniques for AMR identification, and intensifying the epidemiological actions of surveillance, control, and education.

Keywords: ESKAPE; COVID-19; antimicrobial resistance; multidrug resistant organisms; antibiotic stewardship

INTRODUCTION

The World Health Organization (WHO) considers antimicrobial resistance (AMR) among the top 10 priorities for global public health. This phenomenon has developed and spread due to the selection pressure resulting from the excessive or incorrect use of antibiotics [1]. The number of deaths caused by antimicrobial resistance (AMR) could exceed 10 million annually by the year 2050 without active measures promoting the prudent use of antibiotics, improving hand hygiene in hospitals and environmental healthcare hygiene, as well as enhancing food biosecurity [2].

The emergence of multidrug-resistant (MDR) bacteria poses a concerning issue for public health due to the increased risk of healthcare-associated infections and limited treatment options. "ESKAPE" is an acronym for six bacteria of major interest in AMR surveillance systems, namely: (E) Enterococcus faecium, (S) Staphylococcus aureus, (K) Klebsiella pneumoniae, (A) Acinetobacter baumannii, (P) Pseudomonas aeruginosa, and (E) Enterobacter species [3, 4].

The "ESKAPE" pathogens are frequently implicated in severe infections, ranking high among the causes of morbidity and mortality worldwide. However, the WHO has developed a list of priorities within this group. Gram-negative bacilli are considered "critical": carbapenem-resistant CBR Acinetobacter baumannii (A. baumannii), CBR Pseudomonas aeruginosa (P. aeruginosa), third-generation cephalosporin-resistant

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(C3R) Klebsiella pneumoniae (K. pneumoniae), and C3R Enterobacter spp. In the second line of priority are Gram-positive cocci, namely vancomycin-resistant (VAN-R) Enterococcus faecium (E. faecium) and Methicillin-Resistant Staphylococcus aureus (MRSA) [5,6].

Antibiotics are frequently used to treat COVID-19-related bacterial infections. The excessive use of antibiotics during the COVID-19 pandemic can accelerate the emergence and spread of antibiotic-resistant bacteria [7].

In the first year of the COVID-19 pandemic, although many E.U. countries experienced a general decrease in antibiotic consumption both in the community and in healthcare facilities, certain antibiotics, such as azithromycin and ceftriaxone, saw an increase in usage. The reduction in prescriptions was induced by isolation, decreased mobility, and a decline in elective surgical interventions, but the impact of reduced antibiotic use on AMR, especially concerning the ESKAPE group, is insufficiently understood. In a survey conducted in 26 E.U. countries, monitoring antibiotic prescribing behaviors in healthcare facilities was halted in 9 countries, and antibiotic prescriber education programs were interrupted in 11 countries [8,9]. Understanding the link between COVID-19 and antibiotic use can contribute to the development of strategies to limit the impact of infections on vulnerable populations, thereby aiding the global effort to combat infectious diseases.

The study aims to assess the frequency of ESKAPE pathogens and their antimicrobial resistance profile during the COVID-19 pandemic (2020-2022) in a multidisciplinary Romanian hospital.

MATERIALS AND METHODS

A retrospective analysis of the isolated bacterial strains was conducted in the Microbiology Laboratory of the Emergency Military Hospital " Dr. Aristide Serfioti" Galati, derived from various biological samples obtained from hospitalized patients. The hospital is found in the Southeast region of Romania, on the eastern border of the European Union, and includes surgical departments (75 beds), medical departments (100 beds), and an intensive care unit (15 beds).

Escherichia coli and microorganisms in the ESKAPE group had their antibiotic susceptibility evaluated. Escherichia coli was included in the analysis due to its frequent association with horizontal gene transfer of resistance genes from other Enterobacterales, potentially reflecting the resistance profile of Klebsiella pneumoniae or Enterobacter spp, both of which are part of the ESKAPE pathogens [10].

The study was conducted from January 1, 2020, to December 31, 2022. This timeframe coincides with the hospital's focus on COVID-19 support, meaning that the hospital provided healthcare to COVID-19 patients, along with healthcare assistance to the general population, mostly in emergencies.

Microbiological Bacterial Identification Procedures

According to the current work procedures, bacterial isolation was performed by inoculating biological samples either "in sector" or "by exhaustion" on solid media, including Columbia blood agar, Chapman agar, and MacConkey agar (GRASO Biotech, Oxoid - Thermo Scientific, USA), followed by incubation for 24-48 hours at a temperature of 37°C. Biochemical identification of isolated bacterial strains was done through multi-testing and the automatic Vitek 2 Compact method (bioMérieux-l'Etoile).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using either the classic disk diffusion method or the minimum inhibitory concentration (MIC) method with the Vitek 2 Compact automated system, adhering to the current CLSI standards of the working period. The choice of the MIC testing method was contingent upon laboratory availability, the clinical severity of the patient, and the need for an urgent result, particularly in cases of multidrug-resistant bacteria or discrepancies found in the disk diffusion testing.

Depending on the isolated bacteria and the type of sample, specific sets of antibiotics were used for the antibiotic susceptibility testing, including Penicillin's (P – Penicillin - 10 U, A – Ampicillin - 10µg), β-lactam combination agents (AMC – Amoxicillin-clavulanate - 20/10, TZP – Piperacillin-tazobactam - 100/10 µg), cephems (CXM – Cefuroxime - 30 µg; FOX – Cefoxitin - 30 µg, CTX – Cefotaxime - 30 µg, CAZ – Ceftazidime, FEP – Cefepime - 30 µg), monobactams (AZM – Aztreonam - 30 µg), carbapenems (IMP – Imipenem - 10 µg, MEM – Meropenem - 10 µg, ERT – Ertapenem - 10 µg), fluoroquinolones (CIP – Ciprofloxacin - 5µg), macrolides (E – Erythromycin - 15 µg), TE – Tetracycline - 30 µg, lincosamides (DA – Clindamicyn); ansamycins (RIF – Rifampicine - 5 µg), sulfamides (SXT – Trimethoprim/suphamethoxazole - 25 µg), nitrofurans (NF – Nitrofurantoin - 300µg), aminoglycoside (CN – Gentamicin - 10 µg), glycopeptides (VAN – Vancomycin - 30µg, TEI – Teicoplanine - 30µg) and oxazolidinones (LNZ – Linezolid - 30 µg). The disk diffusion testing method used Oxoid Antimicrobial Susceptibility Disks from Thermo Scientific, USA, and Mast Group Ltd from the U.K. BioMérieux-l'Etoile products were employed for minimum inhibitory concentration (MIC) testing, defined as the lowest antibiotic concentration (µg/mL) that inhibits the bacterial growth. Only acquired antimicrobial resistance was taken into consideration, excluding intrinsic resistance. Results of susceptibility for each antibiotic were expressed as resistant, intermediate, or sensitive, and interpretation was made by the Clinical and Laboratory Standards Institute (CLSI) for the respective testing year [11,12,13]. Quality control procedures incorporated the use of reference strains for both identification and antibiotic resistance testing, including Staphylococcus aureus ATCC 29213, ATCC 25923; E. coli ATCC 25922; Pseudomonas aeruginosa ATCC 27853, Enterococcus casseliflavus ATCC 700327, Enterobacter hormaechei ATCC 700323, and Stenotrophomonas maltophilia ATCC 176666.

Data Collection, Interpretation, and Statistical Analysis

After removing duplicates, the antibiotic susceptibility test results were input into the electronic database of the laboratory. Analysis

of the antibiotic susceptibility profile facilitated the identification of significant resistance issues and risks posed by healthcare-associated infections in the hospital. Microorganisms that have minimum inhibitory concentrations (MIC) above the standard cut-off levels are resistant to antibiotics. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), a microorganism classified as sensitive (S) indicates therapeutic success with standard antibiotic doses, intermediate (I) responds to therapy after exposure to higher antibiotic concentrations, and resistant (R) exhibits therapeutic failure even after elevated antibiotic concentrations [14].

Multidrug resistance (MDR) was considered if non-susceptibility was found to be at least one antibiotic from two or more classes while maintaining susceptibility to at least one antibiotic from three or more categories15. Other patterns of AMR were also notified: extended-spectrum beta-lactamases (ESBL) in Enterobacterales, carbapenem resistance (CBR) in P. aeruginosa and A. baumannii, methicillin resistance in S. aureus (MRSA) and vancomycin resistance of enterococci (Van-R) [16,17,18].

Statistical analysis of the data used version 19 of the Statistical Package for Social Sciences (SPSS). Descriptive statistics tests were applied to determine frequency, incidence, and prevalence. The χ2 test was used for comparing categorical variables, considering a significance level of p<0.01.

RESULTS

Microbial Profile of the Isolates from Hospital

The microbiology laboratory obtained 1117 bacterial isolates, during the pandemic 2020-2022. The lowest annual frequency of positive cultures was recorded in 2020 (266 strains), when the specific activity of the hospital's departments was most restricted. The average length of hospitalization per year varied over the three years, ranging between 4.32 and 4.96 days.

Table 1: Distribution of the Bacterial Isolates in the Hospital's Departments

Hospital Department		Other surgery	ERT	General Surgery	Urology	ICU	Internal Medicine	Neurology	Pneumo	Other Medical	Total
GPC	*Enterococcus faecium*	-	-	-	1	3	1	1	-	-	6
	Non-faecium	1	1	5	65	12	6	7	3	1	102
	Staphylococcus aureus	1	39	18	3	20	2	2	3	3	91
	Co-Negative	-	-	2	13	13	3	5	1	-	37
	Partial sum	***1***	***40***	***25***	***82***	***48***	***12***	***15***	***7***	***2***	***236***
NF-GNR	*Pseudomonas aeruginosa*	1	18	2	20	18	1	-	7	4	71
	putida	-	-	-	1	-	-	-	-	-	1
	Acinetobacter baumanii	-	-	-	1	1	-	-	2	-	4
	Partial Sum	***2***	***18***	***2***	***22***	***19***	***1***		***9***	***4***	***76***
GNR	*Klebsiella Pneumoniae*	-	8	2	71	27	13	13	13	8	155
	Others	-	6	1	8	1	4	2	4	1	27
	Enterobacter spp	1	6	3	19	5	5	-	4	5	48
	E. coli	1	5	22	179	28	99	82	8	55	479
	Proteus spp	1	10	-	36	4	4	-	-	3	60
	Morganella spp	-	-	1	8	-	1	1	-	-	11
	Citrobacter spp	-	1	2	3	2	2	1	1	1	12
	Serratia marscenscens	-	1	1	4	1	-	1	-	-	8
	Providencia stuartii	-	-	1	1		-	-	1	-	3
	Hafnia alvei	-	-	-	-	1	-	-	-	-	1
	Salmonella spp	-	-	-	-		1	-	-	-	1
	Partial sum	***3***	***37***	***34***	***329***	***69***	***129***	***100***	***31***	***73***	***805***
Total		***6***	***95***	***61***	***433***	***136***	***142***	***115***	***47***	***79***	***1117***

The isolated bacteria were grouped into Gram-positive cocci (GPC), Gram-negative bacilli from the Enterobacterales order, and Gram-negative non-fermentative rods (NF-GNR). The highest frequency of GPC was recorded during the first pandemic year, 25.5% compared to 17% in 2021 and 21.1% in 2022, while NF-GNR was identified more frequently in the second pandemic year, representing 9.1% compared to 6% in 2020 and 5.6% in 2022.

Urine cultures were the most requested microbiological investigations, constituting 80% of bacterial cultures. Much fewer analyses were performed on other biological samples such as 5% purulent secretions, 5% respiratory secretions, 3% ear secretions, and 1% nasal and pharyngeal exudates. Blood cultures represented only 0.45% of the tested biological samples.

The urology department reported the highest number of isolates, representing 38.76% of the total bacterial strains. The Internal

Medicine department accounts for 12.71%, the Intensive Care Unit for 12.17%, and Neurology for 10.29%. Bacterial strains were obtained from between 4.9% and 2.68% of the identified isolates from the ENT, Pneumology, Rheumatology, Surgery, and Cardiology departments. Each other hospital's departments were associated with <1% of bacterial isolates (Table 1).

E. coli was isolated with the highest frequency, of 42.8% of species, potentially influencing antibiotic resistance in other Enterobacterales. At the same time, ESKAPE pathogens account for 33.5% of the bacterial strains.

Antimicrobial Resistance Profile of the ESKAPE Pathogens

Enterococcus spp.

Enterococci were identified in 108 isolates with the following distribution: 50.9% E. faecalis, 6% E. faecium, and 43% unspecified Enterococcus spp. E. faecium strains were notified in 6 cases, obtained from the urine cultures (3/6), blood cultures (2/6), and sputum (1/6), mostly on men (4/6), from the ICU (3/6). Each department of pulmonology, internal medicine, and urology reported a single strain. Among the six strains of E. faecium, one-third (2/6) were resistant to Vancomycin and Teicoplanin, and half of the strains (3/6) were multidrug-resistant (MDR). None of the strains were sensitive to Ciprofloxacin (Figure 1a).

Figure 1: Antibiotic susceptibility Profile of the ESKAPE group: a - E. faecium (n=6); b - S. aureus (n=128); c - K. Pneumoniae (n=155); d - A. baumannii (n=4); e - P. aeruginosa (n=71); f - Enterobacter spp. (n=48).

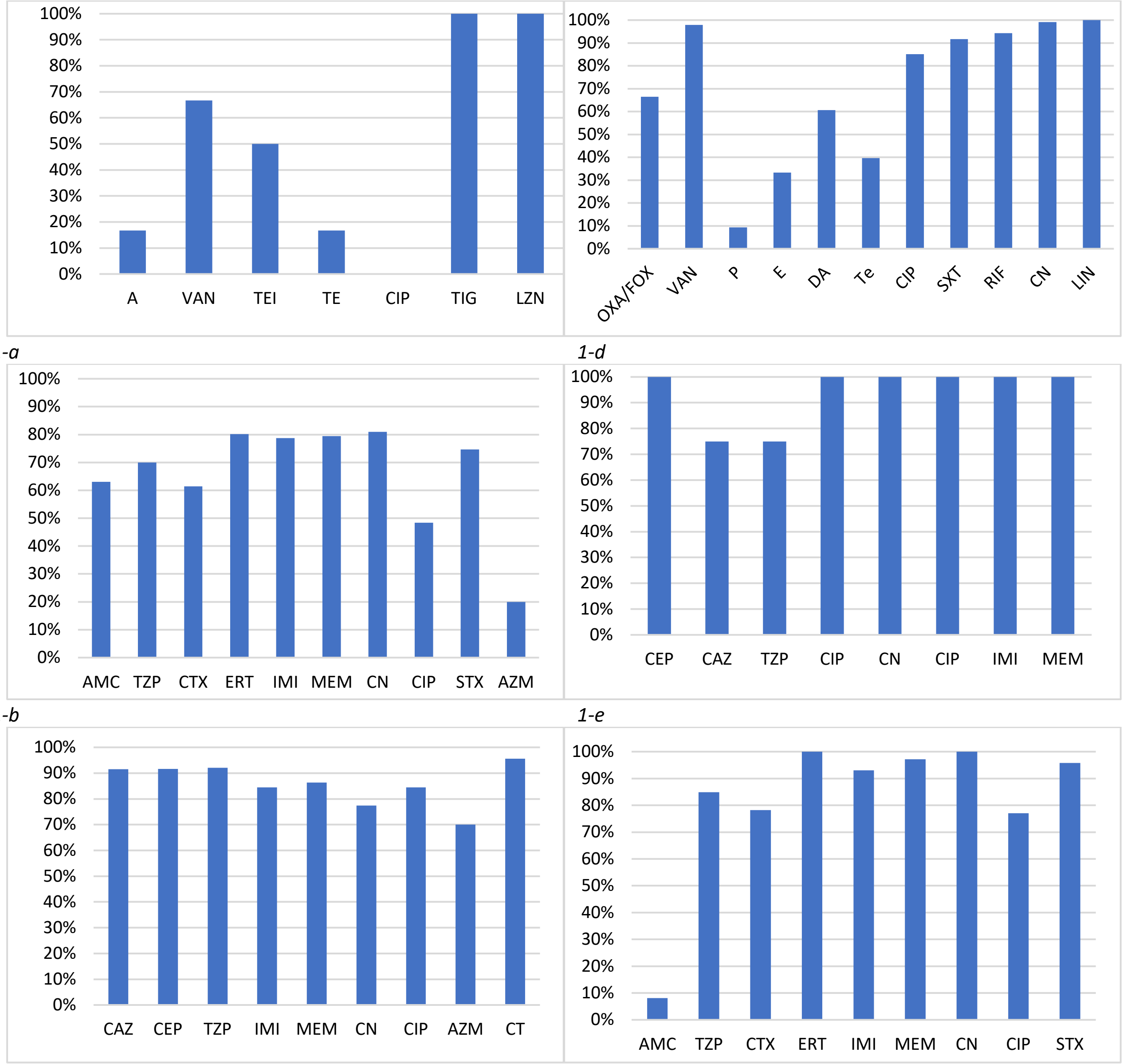

1-a *1-d*

1-b *1-e*

1-c *1-f*

Legend: P–penicillin; A–ampicillin; AMC-amoxicillin-clavulanate; TZP–piperacillin-tazobactam; CXM – cefuroxime; FOX – cefoxitin; CTX- cefotaxime; CAZ-ceftazidime; CEP-cefepime; CT: colistin; AZM-aztreonam; IMP-imipenem; MEM-meropenem; ERT-ertapenem; CIP- ciprofloxacin; E-erythromycin; TE-tetracycline; TIG- tigecycline; DA-clindamicyn; RIF-rifampicine; SXT– trimethoprim/suphamethoxazole; CN-gentamicin; VAN- vancomycin; TEI- teicoplanin; LNZ-linezolid.

Staphylococcus aureus

A total of 128 staphylococcal strains were isolated and identified as follows: 71% S. aureus and 29% Coagulase-Negative Staphylococcus (Co-NS), represented by S. epidermidis (23), S. warneri (4), S. haemolyticus (3), S. hominis (2), S. lugdunesis (1), S. saprophyticus (1), and unspecified Co-NS (3). Most S. aureus strains were identified in nasal or pharyngeal exudates (29.7%), purulent secretions (18.7%), and ear secretions (17.6%). Most samples were taken from patients in the Otorhinolaryngology (ENT) department, Intensive Care Unit, and Surgery departments (85.7%). The frequency of MRSA strains was 33.6%. The MDR rate for S. aureus was 44.5%. The lowest susceptibility of S. aureus was recorded for Penicillin at 9.4%, Erythromycin at 30%, Daptomycin at 60.1%, and Tetracycline at 39.6%. Susceptibility remained at 85% for CIP, 91.66% for SXT, 94.2% for RIF, and 99% for CN (Figure 1b).

Klebsiella pneumoniae

A total of 182 Klebsiella spp strains were isolated, including 85.16% K. pneumoniae, 5.5% K. oxytoca, and 9.3% unspecified species. Most of the cases (64.55%) were found in male patients, and most of the strains came from urine cultures (69%), sputum (9%), and purulent secretions (6%), mainly from the urology department (43.4%), intensive care (15.38%), pulmonology (9.3%), and internal medicine (9.3%). The antibiotic susceptibility profile highlighted AMC 69.8%, CTX 64.1%, TZP 75%, CN 80.9%, CIP 48.3%, STX 76.1%. Resistance to Aztreonam was found in 80% of antibiograms, noting that only 25 strains were tested. The resistance rate to carbapenems in K. pneumoniae strains was 21%, while MDR was found in 18.7% of the isolates (Figure 1c). There is no available data on the susceptibility of reserve antibiotics, as colistin, Ceftazidime + Avibactam. Meropenem + Vaborbactam.

Pseudomonas aeruginosa

Pseudomonas strains were identified in samples taken from 72 patients, of which 71 were P. aeruginosa and one was P. putida. 60.56% of the patients were males. The main products from which P. aeruginosa was isolated included urine (30.55%), ear secretion (23.6%), purulent secretions (15.27%), and sputum (6.9%), mainly from urology, ENT department, and intensive care units. The CBR rate is 15.5%, and the MDR rate is 6.8%. Among the 50 strains tested, resistance to Aztreonam (AZM) was 30%. Resistance issues were also identified for CN 22.5%, CIP 15.5%, but susceptibility was maintained for CAZ 91.55%, CEP 91.66%, TZP 92.1%, and CT 95.56% (Figure 1 e).

Enterobacter spp

Forty-eight strains of Enterobacter spp were isolated (identified) and distributed as follows: 16.66% E. aerogenes, 50% E. cloacae, and 33.33% unspecified strains. Most strains were isolated from male patients (34/48), in urine cultures (25/48), sputum (7/48), wound swabs (6/48), and pus (5/48). They originated predominantly from the urology department (39.85%), ENT department (12.5%), ICU (10.41%), internal medicine (10.41%), pneumology (8.33%), surgery (3/48), and cardiology (2/48). Isolates were also found in the orthopedics, rheumatology, and diabetology departments. ESBL frequency was found to be 14.58% among isolates, and CBR was identified in 2.85% of strains and MDR in 4.16%. The most concerning antibiotic resistance issues were observed with beta-lactams, influencing the following susceptibility profiles: AMC 8.1%, CTX 78.2%, TZP 84.85%, CIP 77%, SMX 95.75%, CN 100% (Figure 1f).

Antimicrobial Resistance Profile of E. coli

A total of 479 strains of E. coli were isolated, with 61.8% from female patients and 89.5% originating from urine cultures. E. coli strains were obtained from the following departments: urology 37.3%, internal medicine 20.6%, neurology 17.1%, ICU 5.8%, surgery 4.5%, and 14.7% from other departments. Identified antimicrobial resistance issues had frequencies of 15% MDR, 2.6% CBR, and 7.5% ESBL. The lowest susceptibility was found for AMP (37.5%), AZM (62%), CIP (64%), and SXT (72.4%). Better susceptibility was reported for AMC (86.7%), CTX (85.4%), TZP (95.5%), and CN (91.4%). Fosfomycin and Nitrofurantoin testing was limited to the E. coli strains isolated from urine culture, which proved to be susceptible in 100% and 98.25%, respectively (Figure 2).

Figure 2: Antibiotic Susceptibility Profile of E. coli (N=479)

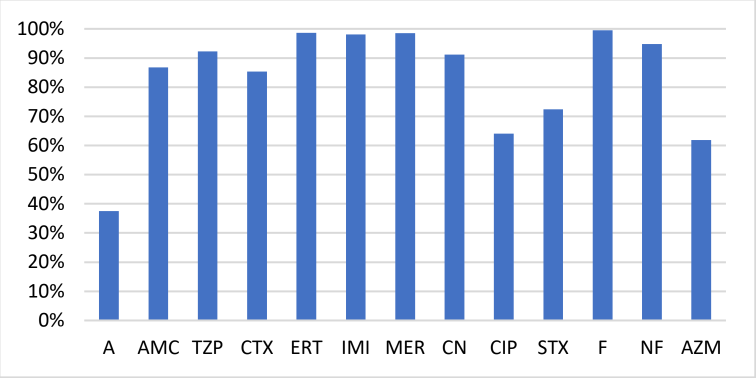

Legend: A – Ampicillin; AMC – amoxicillin-clavulanate; TZP – piperacillin-tazobactam; CXM – cefuroxime; CTX – cefotaxime; AZM – aztreonam; IMP – Imipenem; MEM – meropenem; ERT – ertapenem; CIP – ciprofloxacin; F – fosfomycine; SXT – trimethoprim/suphamethoxazole; CN – gentamicin.
Resistance of E. coli strains to AMC and CTX correlated with the resistance profile of K. pneumoniae (χ2 test: p<0.001) and Enterobacter spp (χ2 test: p<0.001).

Spread of ESKAPE Pathogen Resistance in Hospital Wards

Antibiotic resistance issues among ESKAPE pathogens have variable frequency and impact, both regarding the microorganisms surveyed and within each word, influenced by the characteristics of specific diseases to each specialty.

Acinetobacter baumannii is one of the most concerning public health problems worldwide but CBR has not been identified in any of the hospital's departments. All strains highlighted maintained sensitivity to carbapenems and other commonly tested antibiotics, but a single case evidenced an intermediary pattern, signaling decreasing susceptibility that requires vigilant epidemiological surveillance.

Pseudomonas aeruginosa - Carbapenem-resistant cases were found in isolated instances in the ICU, urology, and surgery departments without being considered a source for healthcare-associated infections. The urology department was the primary source for these isolates, but a sporadic case was also reported from the surgery department.

Enterobacter spp - Third-generation cephalosporin-resistant (C3R) was found occasionally, only in the urology department, having the slightest impact among "critical" Gram-negative bacteria on antibiotic stewardship.

The "critical" pathogen Klebsiella pneumoniae C3R is causing the most problems because it was most identified and is spreading the most in hospital wards. It mainly affects the urology department, then the intensive care unit, pneumology, internal medicine, and neurology departments. Epidemiological unrelated cases have also been reported rarely from the ENT, rheumatology, and psychiatry departments (Figure 3 a).

Figure 3: Distribution of ESKAPE Germs in Hospital Departments: a – by Specific AMR; b – by MDR

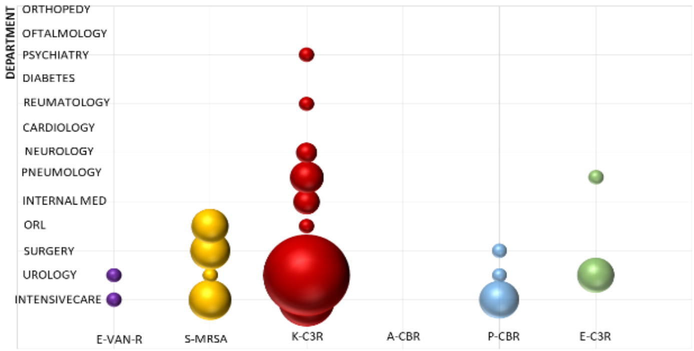

a: Specific AMR

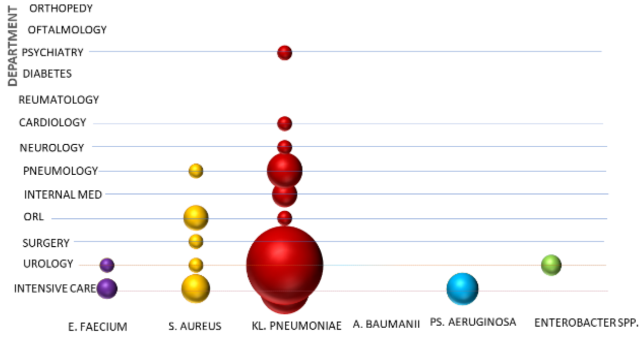

b. MDR

Among ESKAPE Gram-positive cocci, MRSA draws attention in the intensive care, surgery, and ENT departments, while only a single case was identified in urology. Vancomycin resistance in Enterococcus faecium is reported in isolated cases in the intensive care and urology departments (Figure 3 a).

Total ESKAPE pathogens cumulate specific AMRs (E. faecium Van-R, MRSA, P. aeruginosa CBR, A. baumannii CBR, K. pneumoniae, and Enterobacter spp- C3R) of 30.9%. In addition, MDR is superposing to 21% of these strains. The multidrug resistance of S. aureus, K. pneumoniae, and P. aeruginosa was associated with respect to Methicillin Resistance ($\chi 2$ test: $p < 0.001$), C3R ($\chi 2$ test: $p<0.001$) and CBR ($\chi 2$ test: $p<0.001$). Concerning E. faecium and Enterobacter spp, we found a small number of isolated and MDR very few strains, that are not considered epidemiologically significant.

DISCUSSION

The reported bacterial burden is low, with a yearly average of 1.95 bacterial strains per hospital bed. This result could be explained by the hospital's multidisciplinary profile, mainly in short hospitalization, for patients from the community. The reorganization of COVID-19 support wards has disturbed the specific activities of specialized departments. Additionally, in COVID-19 wards there were

few recommendations for microbiological investigations and properly sampling bacteriological specimens was frequently difficult.

The major challenges of the COVID-19 pandemic for Antibiotic Stewardship, Infections Prevention, and Control were the increase in the number of critical cases, diminution of vigilance in infection control, short supply of specialized staff resources, and absence of guidelines for COVID-19 diagnostic and treatment at the beginning of the pandemic [1,19,20,21]. Moreover, redistribution of lab resources was required during the pandemic, by prioritizing virologic tests and reducing the bacteriological investigations1,19,20. Apart from these general respects, Romania is considered “in the red zone” of European antimicrobial resistance, since is the country with the second highest consumption of antibacterials in Europe and 11% of the population declared antibiotic prescription from the pharmacy, without medical consultation. Therefore, we could assume that patients hospitalized in most wards used antibiotics before hospital admission, so the results of this report should be taken with caution [22,23].

WHO report on antimicrobial resistance (AMR) surveillance in the European Region highlighted a decrease in E. coli isolates in 2020 compared to previous years, attributed to reduced non-COVID-19 care activities and a decline in AMR surveillance vigilance [20,24]. Unlike other countries in the European Region that reported an increase in the frequency of commonly associated healthcare pathogens, such as Acinetobacter baumannii and Enterococcus faecium, these issues were not observed in our study. However, monitoring VAN-R enterococci remains a priority for AMR surveillance in our hospital, given the elevated regional prevalence of Clostridioides difficile infections and the intensive use of oral vancomycin, exerting selective pressure on enteric bacteria [25,26].

Like other European countries, resistance to carbapenems in E. coli and Enterobacter spp. remains low, below 3%, while the rates of C3R in our study exceed 14%, consistent with the European report [1]. The complete susceptibility to Fosfomycin of urinal E. coli isolates justifies the local guideline recommendation of this antibiotic in the initial antibiotic therapy for uncomplicated infections, preserving the β-lactam class [27].

Our study's carbapenem-resistant Pseudomonas aeruginosa (CBR) rate was consistent with the European report (15.5% vs. 17.8%). However, it showed a higher resistance to aminoglycosides (22.5% vs. 9.4%) [1]. Surprisingly, susceptibility to cephalosporins of P. aerugionosa in our setting is above 90%, recommending piperacillin/tazobactam as a first-line therapeutic option for this presuming etiology of infections.

Klebsiella pneumoniae demonstrated the highest frequency among "critical" pathogens but also exhibited the most concerning antibiotic resistance. Resistance rates exceeded 20% for all antibiotic classes, including carbapenems, reaching over 50% for quinolones. It is necessary to systematically the antibiotic testing of K. pneumoniae strains for reserve antibiotics considering colimicin and combinations of ceftolozane–tazobactam, imipenem–cilastatin/relebactam, meropenem–vaborbactam or ceftazidime–avibactam [16]. The recommendation for the first-line treatment of suspected carbapenem-resistant strains should consider these reserve antibiotics. Other additional potent drugs for carbapenem-resistant K. pneumoniae such as plazomicin, eravacycline, cefiderocol or temocillin could become available by governmental regulations [28]. Furthermore, the emergence of K. pneumoniae hypervirulent strains, especially in association with CBR, is linked to antibiotic treatment failure and an elevated mortality rate. Monitoring these variants necessitates using advanced molecular techniques to identify virulence and resistance factors [29].

We have tested the susceptibility on Aztreonam, according to the standardized procedures, although Aztreonam has not been available in Romania for therapeutic use. We found over 30% resistance between the tested Gram-Negative rods, predicting low benefit as a first-choice antibiotic in clinical practice.

Staphylococcus aureus ranks second in the frequency of pathogens with antibiotic resistance risk. The MRSA rate is among the highest in Europe, meaning over 33% of strains. The notably high rate of multidrug-resistant (MDR) strains is mainly attributed to intensive community usage of penicillin, macrolides, and tetracyclines. Vancomycin and Linezolid are valuable options for first-line antibiotic treatment in severe invasive infections, while sulfamethoxazole could be used in non-severe infections. Although not within the study's scope, we observed a higher MDR rate in Coagulase-Negative Staphylococci (Co-N), which colonizes the skin, mucous membranes, and environmental surfaces in the community and the hospital. This may contribute to the transmission of antibiotic resistance to Staphylococcus aureus [30].

ESKAPE pathogens are found in the community, but more frequent in healthcare settings. The mechanisms of antibiotic resistance emergence and persistence of the ESKAPE pathogens are heterogeneous, but they share the ability to build biofilms related to biotic and abiotic surfaces [14]. Regarding the characteristics of our health care services, the source of the ESKAPE pathogens is more probable community than acquired in the hospital but could reflect the regional antimicrobial practices of the practitioners in family medicine and other specialists.

Monitoring and analyzing antimicrobial resistance in the hospital setting is essential for identifying the risks associated with the emergence of "critical" strains and healthcare-associated transmission. Specific resistances within the ESKAPE group, as well as MDR within this group, were more frequently identified in males compared to E. coli, which predominantly originated from females. Both Gram-negative ESKAPE pathogens and E. coli were predominantly associated with urinary tract infections in most cases. This observation could be considered for the risk evaluation in the first-line antibiotic treatment decision, in addition to the predictive clinical scores, such as CarbaSCORE, Tumbarello score, Duke score, or machine learning models [30,31,32,33].

This is the first surveillance report of AMR in the Emergency Military Hospital "Dr. Aristide Serfioti" from Galati, developed to understand the local impact of COVID-19 on bacterial charge, diversity, frequency, and antibiotic resistance, for updating the regional antibiotic stewardship strategy. Revising the clinical protocols for diagnosis and treatment in each department, intensifying the

bacteriological samples collection, and improving the microbiological diagnostic techniques for AMR identification, including high technologies of molecular investigations and point of care antimicrobial resistance tests are highly interests for the near future stewardship. Systematic epidemiological monitoring and control of the hospital environment, hand hygiene procedures stimulation, strict assurance of safe medical procedures, and continuing education are ongoing necessary components of the antibiotic stewardship program.

Limits of the Study

This is a retrospective study, based only on microbiological records. Consequently, the patient history and clinical data were not available. Furthermore, the community or healthcare-acquired source of infection was not analyzed. Hemocultures accounted for only 1% of the total positive samples and 3.18% of them are categorized in ESKAPE group. Therefore, comparing the antimicrobial resistance profile resulting from our study with reference data reported by representative laboratories that monitor antibiotic resistance in epidemiological surveillance networks for invasive bacterial strains, has limited significance [24]. The molecular investigation of antimicrobial resistance mechanisms was not affordable. The antibiotic consumption was not included in the present study but should be correlated with the antimicrobial resistance pattern of the hospital.

CONCLUSION

The COVID-19 pandemic impaired the hospital practice for infections and bacterial diagnostics and weakened the vigilance of antibiotic stewardship. ESKAPE pathogens are almost one-third of the total bacterial isolates, with 21% associated MDR.

The "red flag" for antibiotic stewardship between ESKAPE pathogens is Klebsiella pneumoniae. The directions of the near future antibiotic stewardship program focus on revising the clinical protocols of diagnostic and treatment in each department, to intensify the bacteriological samples collection, to improve the microbiological diagnostic techniques for AMR identification, and to intensify the epidemiological actions of surveillance, control, and education.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

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The current manuscript does not contain previously published materials or self-generated AI text.

Authors' contribution

Conceptualization, M.A., C-M.V.; methodology, A-V.I, , M.A., C-N.D.; software, I.D., C-S.S; validation, M.A., C-M. V. and A-V-I.; formal analysis, I.D., C-N.D., A.N.; investigation, C-M.V., I.D.; resources, C-M.V. and C-S.S.; data curation, A-V.I.; C-N.D.; writing—original draft preparation, C-M.V., C-S.S, A-V.I., I.D., C-N.D., A.N.; writing—review and editing, M.A.; visualization, C-S.S., A. N.; supervision, M.A.; project administration, C-M.V., A.N. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board Institutional Board of Emergency Military Hospital "Dr. Aristide Serfioti" from Galati (RCM 212/M1-544/2023) for studies involving humans.

Patient consent for publication

Not applicable. Our retrospective study used laboratory management which regularly collected data from the databases. The consent for the use of personal data was given by each patient as a routine procedure of the hospital health care provision.

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ORIGINAL ARTICLE

Impact of Physical Performance on Hemodynamic and Cognitive Status in Romanian Older Adults

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Abstract: Background: Frailty is a major challenge for the aging population, increasing the risk of falls, disability, hospitalization, and death. It's common with age, and often linked to reduced physical activity and poor diet, especially in those prone to sarcopenia. Gait speed tests are frequently used to assess mobility in older adults. Materials and methods: This six-month study at "Sfântul Luca" Chronic Disease Hospital involved 143 patients over 65 (mean age 72.85 ± 10.07), 113 of whom were women. The Tensiomed Arteriograph measured heart rate, pulse wave velocity, and central aortic blood pressure to assess acute vascular stiffness. Results: As patients aged, their physical circumstances changed: in the age group 75-84 years ("Very elderly") only 39% of the subjects achieved medium- and good-physical performance, while 56% of them were characterized by low-physical activity (Chi-square test, p <0.001). The mean Mini-Mental State Examination (MMSE) values of individuals with inadequate physical activity were significantly lower (21.02 ± 4.68 points) than those with adequate physical activity (Student t-test, p <0.001). Conclusion: Regular physical activity has been shown to improve hemodynamic factors and potentially slow down arterial aging, thus promoting healthy aging. It is imperative for healthcare professionals to promptly evaluate whether older, frail patients can benefit from rehabilitation, enabling swift recovery through multidisciplinary care.

Keywords: physical performance; hemodynamic status; older adults; cognitive status; personalized rehabilitation program

INTRODUCTION

The clinical state of frailty is one of the most difficult issues facing the senior citizen population. Frailty is linked to a higher risk of falls, disability, hospitalization, and mortality [1] and is thought to be common as people age. The onset of frailty in the elderly is often characterized by muscular weakness, reduced mobility, and constraints in performing routine physical tasks. [2]. Sarcopenia is characterized by the loss of muscle mass and can stem from chronic malnutrition, musculoskeletal changes related to aging, chronic illnesses, micro-inflammation, and related comorbidities. For elderly individuals, being admitted to the hospital is one of the most distressing experiences. The gait speed test is a widely used tool for assessing the mobility of elderly individuals [3]. The level of physical activity and adequate nutrition intake considerably declined among older adults at risk of developing sarcopenia [4]. Muscle weakness and reduced movement speed leading to disabilities characterize sarcopenia, a health condition associated with the aging process among the geriatric population. [5]. Physical activity and gait speed are protective factors for the occurrence of sarcopenia in older adults. The primary physical activity for seniors is walking. Clinical research and clinical objectives are increasingly relying on the evaluation of walking ability [6]. Evaluation of gait speed and its effects on hemodynamic parameters and cognitive status in older persons was the aim of this study.

MATERIALS AND METHODS

From the 160 patients hospitalized in the months of January-March who met the eligibility criteria: 8 patients refused to participate, 4 patients presented a new diagnosis of oncological disease in the middle of the hospitalization and 5 patients withdrew.

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Our study involved 143 patients, 113 of whom were women, and had a mean age of 72.85 ± 10.07 years. This six-month observational, clinical trial was conducted at the Geriatrics Clinic "Carol Davila" in the Chronic Disease Hospital "Sf. Luca." Enrolment was contingent upon the participants fulfilling the subsequent eligibility requirements: being 65 years of age or older; having no self-reported health conditions that would preclude physical examinations; being able to perform basic mobility tasks (such as walking without extreme pain, aching, or stiffness); and not being undergoing cancer treatment, knee or hip replacement, heart or spinal surgery, myocardial infarction within the previous six months, or experiencing chest pain while walking within the previous 30 days. A MMSE score < 20 points resulted in the exclusion of participants. Before participating, each participant gave written, informed consent. The Clock Drawing Test (CDT) is a simple tool used to check for early signs of dementia, scoring up to 10 points. We used the Short Physical Performance Battery (SPPB) to measure physical activity, which includes the tandem standing, 4-meter walk test, and Up-and-Go [7]. For functional capacity, we used the standardized scales Activity Daily Living (ADL) with ranges from 0 to 6 points and Instrumental Activity Daily Living (IADL) with ranges from 0 to 8 points, where a high score indicates high function [8, 9]. We measured hand grip strength using a hydraulic dynamometer and used the Tensiomed Arteriograph to evaluate arterial vascular stiffness: pulse wave velocity (PWVao), heart rate (HR), central aortic systolic blood pressure (SBPao), and central aortic pulse pressure (PPao) [10].

To illustrate the degree of comorbidity, we categorized the patients into three groups based on a comorbidity index: low comorbidity index, which represented patients with up to two chronic pathologies; moderate comorbidity index, which represented patients with three to five chronic pathologies; and high comorbidity index, which represented patients with more than five chronic pathologies.

The inclusion of patients with metabolic syndrome was achieved by evaluating those with 3 criteria (MS3) or with the presence of 4 of them (MS4) and all 5 criteria (MS5). In addition, we conducted a search through reference lists of pertinent publications, Google Scholar, EBSCOhost, and Medline in order to find published hand grip strength norms.

All determinations are performed according to the protocol and the results are analyzed according to standardized scales. This study was approved by the Ethics Commission (9/09.12.2023).

Statistical Analysis

All the data from this study were analyzed using IBM SPSS Statistics 25 and illustrated using Microsoft Office Excel/Word 2021 Stundent's t-test was used for parametric distributions. Qualitative variables were written as absolute frequencies with percentages and were tested between groups using Fisher's Exact Test.

RESULTS

The age caused changes in the physical status of patients. Thus, 56% of the subjects with low physical activity were in the age group 75-84 years, and only 39% of very elderly subjects were characterized by medium and good physical activity (Chi-square test, p < 0.001). Also, in the "very, very elderly" age group (over 85 years), we noticed the same statistically significant difference between the two groups (see Figure 1).

Figure 1. Distribution of physical activity by age

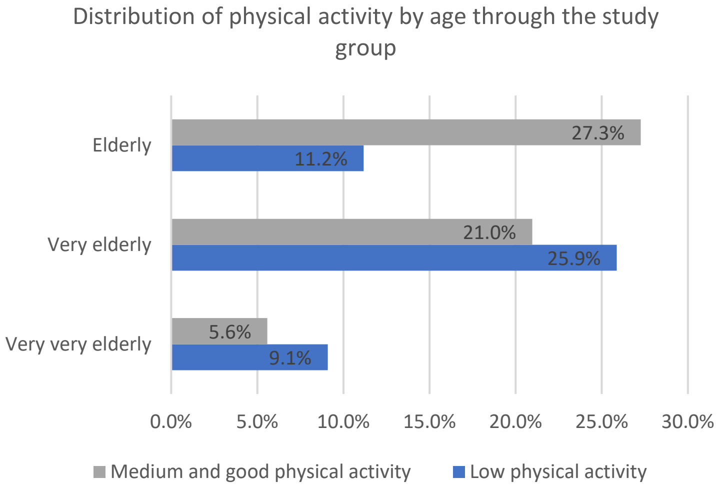

Within the group, there is no statistically significant difference in terms of physical performance and the number of associated diseases (expressed by the comorbidity index, student T-test) (see Table 1).

The functional capacity of the elderly in the study group was relatively normal, with no more than two activities affected, especially in the instrumental activities of living: the mean IADL is 6.66 ± 1.71 (out of a maximum of 8 points), and the mean ADL is 5.74 ± 0.92 (out of a maximum of 6 points). Quantitative independent variables were tested between groups using Mann–Whitney U tests (see

Table 2).

Table 1: Physical activity and comorbidity index

Characteristic	Low physical activity (N = 66)	Medium and good physical activity (N= 77)	p
Comorbidity index			
Low	1 (2%)	2 (2%)	0.389
Moderate	52 (79%)	66 (86%)	
High	13 (19%)	9 (12%)	
Metabolic syndrome criteria			
Without MS[1]	18 (27%)	14 (18%)	0.353
MS3	23 (35%)	34 (44%)	
MS4 and MS5	25 (38%)	29 (38%)	
Diabetes			
Yes	29 (44%)	40 (52%)	0.339
No	37 (56%)	37 (48%)	

Table 2: Physical performance and functional capacity in the studied group

Characteristic	Low physical activity (N = 66)	Medium and good physical activity (N = 77)	p
ADL	5.50 ± 0.93	5.95 ± 0.87	0.004
IADL	6.06 ± 1.81	7.17 ± 1.43	< 0.001
Subjective fatigue			
Rare	8 (12%)	17 (22%)	< 0.001
Sometimes	9 (14%)	37 (48%)	
Often	49 (74%)	23 (30%)	
Hand grip strength	11.74 ± 2.9	17.08 ± 4.94	< 0.001
Walking speed	0.34 ± 0.1	0.69 ± 0.13	< 0.001
Statin treatment			
Yes	29 (44%)	39 (51%)	0.423
Not	37 (56%)	38 (49%)	

Physical performance and impact on daily activity scores show a direct relationship between the functionality and the SPPB values. A statistically significant difference was found in IADL scores within the group with low physical activity (6.06 ± 1.81 compared to 7.17 ± 1.43, Student's t-test, $p < 0.001$). Additionally, basic individual needs, such as eating and washing, are impacted in the group with reduced functionality, as measured by the ADL, like those with lower physical performance (Student's t-test, $p = 0.004$) (see Table 2).

Hand grip strength measured by a dynamometer, adjusted for age, gender, and height, revealed a significant difference in the group with low physical activity, with an average strength of just 11.74 ± 2.9 kg (Student's t-test, $p < 0.001$) (see Table 2).

In the context of long-term treatment of elderly patients with chronic cardiovascular disease, statins are often associated with the development of sarcopenia, chronic fatigue, and reduced physical activity. However, our study findings revealed no statistically significant correlation between the use of statins and the decline in hand grip strength or physical performance among the elderly patients in our study group. Remarkably, more than half (51%) of the elderly patients undergoing statin treatment maintained satisfactory levels of physical activity (see Table 2).

In our analysis of walking speed, we calculated patients' movement rates and correlated them with some hemodynamic parameters (HR, PWVao, PP) and functional capacity, through multiple regression analyses. We divided the patients into three groups: the first group (home movement) consisted of elderly individuals at heightened risk of disability who have limited mobility confined to their residence, with a walking speed of up to 0.4 m/s; the second group (limited community movement) included elderly people engage in movement within the community and are at moderate risk of losing their autonomy, with a walking speed between 0.4 - 0.8 m/s; the third group (full mobilization) comprised individuals who are not exposed to the risk of disability and are fully mobile with a walking speed exceeding 0.8 m/s (see Table 3).

The results showed a significant statistical difference on the pulse pressure (PP) ($p = 0.001$). This indicator could potentially serve as a valuable predictor for gauging cardiovascular risk and assessing disability risk, particularly in relation to walking speed.

Regarding the cognitive level there is a clear difference between the two groups. The mean MMSE values, which assess the cognitive status of the subjects, are significantly lower (21.02 ± 4.68 points) among those with low activity compared to those with good physical activity (Student t-test, $p < 0.001$) (see Table 4).

Table 3: Distribution of patients according to walking speed

	Group 1 = home movement (N = 48)	Group 2 = limited community movement (N = 71)	Group 3 = full mobilization (N = 24)	p
ADL	5.46 ± 0.84	5.80 ± 0.95	6.13 ± 0.85	0.011
IADL	6.19 ± 1.8	6.75 ± 1.71	7.33 ± 1.09	0.021
PWVao	10.37 ± 1.74	9.94 ± 1.93	9.95 ± 1.63	0.419
PP	**66.71 ± 14.23**	**65.17 ± 15.31**	**53.42 ± 12.75**	**0.001**
HR	68.81 ± 12.7	66.61 ± 11.36	65.33 ± 7.02	0.404

Table 4: Correlation of hemodynamic and cognitive parameters with walking speed

Characteristic correlation	Coefficient	p
PWVao and SPPB	- 0.141	0.092
HR and SPPB	- 0.166	0.047
Walking speed and SBPao	- 0.149	0.076
Walking speed and Body Mass Index (BMI)	0.125	0.138
Walking speed and MMSE	**0.339**	**< 0.001**
Right-Hand grip strength and SPPB	**0.422**	**< 0.001**
Left-Hand grip strength and SPPB	**0.440**	**< 0.001**
Fatigue and statin treatment	0.064	0.450

The presence of this difference comes as an important finding that supports the potential role of physical exercise in preventing cognitive impairments in the elderly. Given the limited effectiveness of current drug treatments for dementia, geriatric clinicians should place greater emphasis on incorporating physiotherapy programs for these elderly patients.

Another important finding is the difference between the two groups in the case of the Clock Drawing Test (CDT), which is easy to use, quick to administer, and can be implemented as a screening tool in primary care settings [11].

The group of those with low physical activity had an average CDT score of 6.63 ± 1.33 points (out of a max. 10 p), compared to an average score of 7.96 ± 1.72 points among those with good physical performance (Student t-test, $p < 0.001$). Our findings once again emphasize the importance of the age-old adage, "Mens sana in corpore sano".

DISCUSSION

Regular physical activity is not only crucial for maintaining autonomy but also for preserving cognitive function [12]. It is widely recognized that consistent aerobic exercise enhances cardiorespiratory function, reduces arterial stiffness [13], diminishes cardiovascular risk factors, and lowers cardiovascular mortality. Conversely, anaerobic exercises promote skeletal muscle strength and are instrumental in preventing age-related muscle atrophy [14]. Elderly patients can benefit from the high level of education of nurses, which influences the degree of clinical autonomy. The nurse needs to be aware of any barriers to learning, like loss of hearing or decline of vision and should tailor his or her education for that specific patient [15].

Physical activity is an effective strategy for protecting against sarcopenia and promoting healthy aging. Most studies on exercise in elderly people show that participants achieve positive results, although maintaining muscle strength appears to depend on the continued implementation of certain types of physical activities. One of the conclusions from a meta-analysis of 10 studies was that there was no consistency in the measurement of sarcopenia, indicating a need for further investigations [16].

For elderly people, regular exercise (at least one hour, at least twice a week) through personalized exercise programs can have beneficial cardiovascular effects, such as lowering blood pressure, improving skeletal muscle perfusion, and enhancing glucose utilization in myocytes. Additionally, regular exercise helps maintain muscle fiber quantity and supports skeletal muscle strength [17]. Numerous studies have demonstrated the significant impact of this factor on recovery rates in neurological system disorders and cardiovascular diseases, both of which are primary contributors to disability and diminished quality of life (QoL) [18-20].

The QoL significantly influences patients' adherence to treatment and their overall recovery, making it a pivotal endpoint in clinical research. Aspects such as pain management, emotional well-being, social interactions, and independence profoundly impact patient outcomes. Consequently, enhancing QoL becomes a primary objective from the point of diagnosis. While both patients and physicians acknowledge the importance of prolonging survival, maintaining an optimal quality of life throughout the treatment process is equally imperative [21]. We consider that physical activity plays a major role in enhancing QoL. Due to the Coronavirus (COVID-19) pandemic, many elderly individuals—already a high-risk group for severe illness and adverse SARS-CoV-2 outcomes—opted to remain at home out of fear of contracting the virus, regardless of their associated comorbidities. Consequently, it is hypothesized that a significant

number of older adults are now facing the repercussions of that period, notably sarcopenia, which is linked to a lack of physical activity. As a result, these individuals are now tasked with recovering from that phase, underscoring the need to increase awareness about the importance of physical exercise in this population [22]

In our study, the measurement of muscle strength through the dynamometer revealed a significant difference in the group of elderly patients with low physical activity (Student t-test, $p < 0.001$). Traumatic injuries accounted for the largest percentage of rehabilitation cases in Romania (73.17%), with musculoskeletal illnesses coming in second (70.06%). Reduced recovery was observed in cases of neurological system disorders (50.56%) and cardiovascular diseases (44.23%) [18].

Although other studies have debated the potential link between statin administration in the elderly and the development of sarcopenia, fatigue, and decreased physical activity [23], our group did not find a positive correlation supporting this relationship. Hence, it endorses the recommendations for the continued utilization of statins, even in advanced age, as they have exhibited protective advantages in averting cardiovascular incidents.

Analyzing and interpreting walking speed presents a challenge for clinicians, but recent specialist studies have provided new insights into survival and mortality predictors. Normal walking speed requires a relatively low level of energy consumption [24, 25]. Our study found statistically significant results related to pulse pressure (PP), which is considered a predictor for evaluating disability risk in the elderly population [26].

Cognitive status can be improved by exercise. Several prospective studies have demonstrated that maintenance of higher levels of physical fitness helps protect against cognitive deterioration, even at an advanced age [12,27,28]. Conversely, poor physical performance among older adults may accelerate cognitive decline and lead to early onset of dementia [29, 30]. Additionally, muscle strength has been linked to cognitive performance [31,32]. Promising trials utilizing AI and technology, which show significant improvements in autonomy and reduced reliance on human resources point to a bright future for the medical care and recovery of elderly patients with cognitive impairments [33,34,35].

Several limitations of the present study should be acknowledged. First, the study sample was predominantly female, which could potentially influence the results. Second, the study group was relatively small, and there was a lack of homogeneity, which may affect the comparability of the findings with the broader Romanian population.

CONCLUSION

Maintaining physical performance, especially in the elderly with associated chronic diseases, has an important role in maintaining autonomy and quality of life, especially in the very old and very, very old age groups that become frailer. Assessing hand grip strength can provide valuable insights into physical performance and guide lifestyle optimization and treatment recommendations for elderly patients. Exercise training has been shown to improve hemodynamic status (including SBP, PP, SBPao, PPao) and may help delay arterial aging, playing an important role in healthy aging. Muscle strength, balance, flexibility, endurance, and mobility are all aspects of whole-body functioning that are included in physical performance. When older adults perform poorly physically, they experience a decline in their quality of life, a loss of cognitive function, an increase in their risk of falling, poorer clinical outcomes, and a reduction in their independence when it comes to doing activities of daily living (ADL).

Early detection of rehabilitation potential in frail elderly patients is essential for initiating timely recovery interventions through specialized medical services and multidisciplinary teams.

From a practical and clinical perspective, developing a consistent program of adapted, personalized physical activities is necessary. Such programs can positively affect functional parameters and significantly enhance the quality of life for elderly individuals. In order to corroborate these findings and draw further conclusions, it is essential to conduct studies encompassing a more diverse and extensive demographic.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

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Authors' contribution

Conceptualization, J.A., S.M.A., A.Z. and C.O.; methodology, R.M., A.P..; software, S.M.G, C.D.G., A.G.P .; validation, J.A., S.M.A., A.Z. and C.O; formal analysis, J.A., A.Z., V.M.A.M., M.N; investigation, V.M.A.M., C.D.G., S.M.G.; resources, A.G.P., S.M.A., M.N., C.O.; data curation, S.M.G, C.D.G, A.G.P., M.N.; writing—original draft preparation, J.A, S.M.A, A.Z., C.O.; writing—review and editing, J.A., S.M.A., C.O.; visualization, A.Z., R.M., S.M.A.; supervision, S.M.A, J.A, C.O.; project administration, A.Z., J.A.; funding acquisition. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Commission of the HOSPITAL OF CHRONIC DISEASES "SF. LUCA" (protocol code 9, date of approval 09.12.2023).

Patient consent for publication

Written informed consent to participate in the research was obtained from each patient/subject..

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REVIEW

Case Management of Delirium in Patients with Major Neurocognitive Disorders

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Abstract: Delirium is associated with lower quality of life, increased healthcare costs, longer hospitalizations, and worse prognosis when detected in patients who are already confronted with a severe and invalidating disease, like the major neurocognitive disorder (MND). In order to identify the most evidence-based interventions that could be included in case management for patients with both delirium and MND, this narrative review explored three electronic databases (Pubmed, Cochrane, and Web of Science/Clarivate) for primary and secondary sources dedicated to this topic. All papers published in English between the inception of each database and September 2024 were screened for relevant data on pharmacological and non-pharmacological therapies targeting delirium. The results supported the use of a multicomponent approach as the first line of treatment, mitigating the precipitating factors, and only as a second line, the use of pharmacological instruments. There is a lack of validated therapies targeting specifically the delirium superimposed on MND, most of the recommendations being extrapolated from studies with delirium in the general population or in elderly patients with different organic diseases. Further studies are required to investigate the specifics of delirium in patients with MND and potential strategies focused on neurobiological mechanisms instead of just clinical manifestations.

Keywords: delirium; acute confusional states; dementia; major neurocognitive disorders; cognitive impairment; delirium assessment tools; antipsychotics

INTRODUCTION

Establishing adequate case management strategies for patients diagnosed with delirium is among the most challenging aspects of liaison psychiatry due to the complexity of the interactions between psychiatric phenomenology and organic pathology. Psychiatrists working in general hospitals are frequently called for consultation by colleagues with different medical specialties to „calm down” patients who suddenly become confused and agitated or, on the contrary, to „wake up” patients who abruptly become non-responsive to external stimuli. This psychiatric disorder is, however, far from being possible to approach by a single therapeutic method and requires an individualized case conceptualization and treatment. While in some cases, identification and correction of the causal factor(s) are simple and may be conducted promptly, such as in the case of severe dyselectrolytemia or hypoglycemia, in most cases, extensive investigations and multiple interventions are needed before the symptoms of delirium may be adequately managed. In this context, it would be necessary to mention that delirium is frequently deceptive because it can be the final manifestation of multiple organic comorbid conditions and a superficial investigation and treatment of the patient may make his or her prognosis worse. Therefore, psychiatrists should be aware of the intricate etiopathogenesis of delirium and of the necessity to investigate and treat such patients within a multidisciplinary framework. These considerations become more obvious when patients with severe chronic cognitive deterioration are evaluated since their anamnesis is reputedly difficult and the psychiatric exam also is frequently challenging.

Due to the changes in the names and definitions of neurocognitive disorders along the main classifications of psychiatric disorders, edited by the World Health Organization (WHO) and American Psychiatric Association (APA) [1-5], a trans-

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nosographic analysis is considered necessary to configure the framework of case management in patients affected by these conditions. Although there is a clear tendency to increase the concordance between the APA's Diagnostic and Statistical Manual of Mental Disorders (DSM) and the WHO's International Classification of Diseases (ICD) criteria for the diagnosis of psychiatric disorders, there are still important nosographic differences that need to be acknowledged by clinicians due to practical reasons.

Major neurocognitive disorders (MND) are defined, according to the latest editions of the DSM (DSM-5 and 5 TR, published in 2013 and 2022, respectively), by significant cognitive decline compared to the individual's previous level of performance in at least one cognitive domain; this cognitive impairment has to be supported by concerns reported by the patient, significant caregiver or clinician; also, a substantial deficit in cognitive performance, documented by a quantitative clinical assessment is required [1,2]. Also, significant interference of these cognitive impairments with the individual's ability to perform independent daily tasks is needed for the diagnosis, and the exclusion of other mental disorders is required as a differential diagnosis in these cases [2]. It is important not to consider the diagnosis of MND in patients who present cognitive impairment exclusively during the periods of delirium [2]. Once the diagnosis of MND is made, the presumed type of disease must be formulated, and the specifiers „with/without behavioral disturbance" and „severity" (mild/moderate/severe) are added [2].

The previous edition, DSM IV-TR, not only used different terminology for MNDs, which were called „dementias", but also the diagnosis criteria included neurological signs, i.e., aphasia, apraxia, agnosia, and the existence of disturbances in executive functioning, besides the memory impairment [3]. Regarding the ICD-10 and ICD-11, their perspective on the terminology is quite similar, and the term „dementia" is preserved in both editions, but in the latest version of ICD, there is an increased focus on the behavioral or psychological disturbances in dementia (BPDD), which received their own set of codes [5].

According to DSM-5 and 5 TR, to formulate a diagnosis of delirium, the clinician should confirm the existence of a disturbance in attention and awareness, accompanied by disturbances in cognition, i.e., memory, orientation, language, visuospatial abilities, or perception [1,2]. Additionally, this diagnosis requires an abrupt onset (e.g., hours to a few days), a change from the baseline level of attention/awareness, and a tendency towards fluctuation in severity during a single day [1,2]. The clinician must exclude the presence of a preexisting or evolving neurocognitive disorder and the presence of a coma or related conditions [1,2]. DSM-5 and 5 TR also state, as an essential criterion, the evidence supporting the direct causal-ship between a general medical condition, substance withdrawal/intoxication, exposure to a toxin/medication, or multiple etiologies and the symptomatology of delirium [1,2]. There are several specifiers for delirium, i.e., those related to the etiology, specifiers of severity (acute/persistent), and level of behavioral symptoms (hyperactive/hypoactive/mixed level of activity) [1,2].

When comparing the diagnostic criteria for delirium in the DSM-IV TR, DSM-5, and DSM-5 TR, several differences emerge [1-3]. The latest editions emphasize the need for disturbances in attention and awareness to represent a change from baseline, exclude coma from the category of delirium, and recognize the potential for multiple cognitive domains to be impaired by this disorder. Furthermore, both DSM-5 and DSM-5 TR provides a more comprehensive list of etiological types of delirium than does the DSM-IV TR [1-3].

The WHO's ICD-10 (the 2019 version) specifies that delirium is „an etiologically non-specific organic cerebral syndrome" that is characterized by disturbances of consciousness and attention, perception, thinking processes, psychomotor behavior, emotions, and the sleep-wake rhythm [4]. ICD-10 mentions that the duration of delirium is variable and the degree of severity may be different, from mild to severe [4]. Regarding the possible etiological considerations, ICD-10 includes different codes for delirium not induced by psychoactive substances, in cases where it is not superimposed on dementia, in cases of delirium superimposed on dementia, and contains codes for other types of delirium (e.g., mixed delirium, or post-surgery), and delirium-unspecified; delirium is also a complication of various withdrawal/intoxication states or somatic diseases (e.g., thyroid storm, kidney failure) [4].

The latest edition of the WHO Classification of Diseases (ICD-11) expands the definition of delirium (but preserves the core dimensions-phenomenological description, changes in functionality) and specifies the main differential diagnoses and potential etiologies, i.e., direct physiological effects of a medical condition, direct physiological effects of a substance or medication (including withdrawal), and unknown factors [5]. Residual categories, such as multiple etiological factors and other specific causes, are also coded [5].

The phenomenon of delirium in patients diagnosed with MND is very complex since the relationship between the two pathologies is far from being easy to define in each case. Individuals with MND have an increased risk of delirium onset when compared to the general population, but delirium in itself represents an independent risk factor for developing MND [6]. Also, delirium may be more difficult to diagnose in patients with MND because disentangling cognitive changes and sensorium clouding in a patient with an already deteriorated cognitive status, based on clinical evaluation, is challenging. Even more, confusional episodes appearing in patients with MND can worsen the cognitive decline and the overall functioning level [6]. It must also be mentioned that MNDs associate multiple well-defined psychiatric disorders (e.g., mood, anxiety, psychotic) as well as BPDDs, which creates supplementary difficulties in detecting new-onset cognitive decline due to delirium [7]. These convoluted interactions between delirium and MND are represented in Fig.1., but caution is recommended when interpreting these relationships due to the intricacy of organic and psychological factors (e.g., infectious diseases may trigger delirium, and delirium may, in patients with MND, worsen the psychological long-term evolution, or, a psychological trigger may be responsible for the clouding of sensorium in a patient with MND, which also may worsen the long-term biological evolution of the patient, if the trigger is not properly identified and corrected; also, psychosocial factors may inadvertently worsen a somatic disease that, in turn, may aggravate the preexisting MND).

Figure 1: The interaction between delirium and major neurocognitive disorders

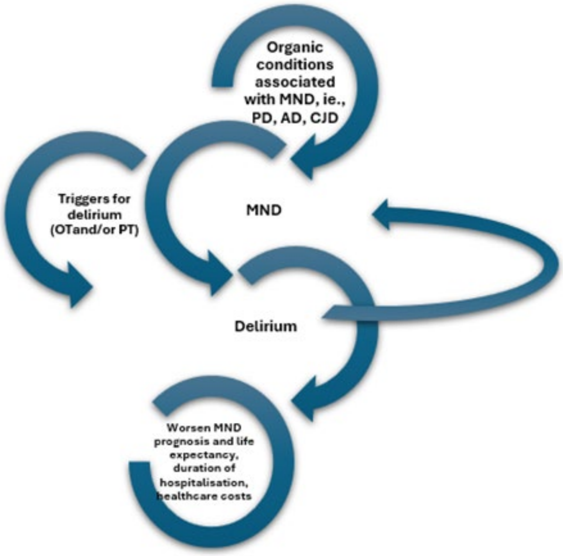
Organic conditions associated with MND, ie., PD, AD, CJD
Triggers for delirium (OTand/or PT)
MND
Delirium
Worsen MND prognosis and life expectancy, duration of hospitalisation, healthcare costs

AD= Alzheimer's disease; CJD= Creutzfeldt-Jakob disease; MND= major neurocognitive disorder; OT= organic triggers; PT= psychological triggers; PD= Parkinson's disease

Although the phenomenology of delirium encompasses prodromal and subsyndromal stages [8], no consensus about corresponding specific symptoms exists, making it difficult for clinicians to identify such forms of delirium in daily practice. Due to the complexity of the phenomenological presentation of delirium, the differential diagnosis is another important challenge for clinicians [8]. More exactly, delirium consists of core manifestations, which are expected to be present in all cases (e.g., awareness alterations), frequently met symptoms (e.g., motor activity dysfunctions), and associated, variable symptoms (e.g., hallucinations), which may reflect the etiological medical or toxic factors [8]. The evolution of delirium is difficult to predict because it is dependent on etiological factors, but it was reported that almost 57% of hospitalized patients may still have delirium after discharge from the hospital [9].

The reported prevalence in hospitalized patients varies across studies, depending on the criteria used, the type of department reporting the cases, the instruments applied for quantifying the severity of the symptoms, etc. The prevalence of delirium was reported between 1 and 31%, and the incidence between 3 and 25% [10-12]. For example, 39% of the patients admitted to intensive care units (ICUs) were diagnosed with delirium, according to a cross-sectional multicenter study (N=256 participants) that used a screening checklist as the core methodology of assessment [13]. In hospitalized patients aged ≥65, delirium was reported in the case of 55.4% of the 327 participants using the 3-Minute Diagnostic Confusion Assessment Method (3D-CAM) [9]. A surprisingly high rate of patients with delirium (35.4%) were not identified by the treating team, indicating the need for increased awareness about the existence of this neurocognitive disorder [9]. Also, it is important to explore the possibility of multiple etiologies in patients with delirium, including organic acute diseases, chronic illnesses that are acutely decompensated, new drugs recently introduced for the treatment of a physical or mental disorder, different environmental stressors, etc. [14-16].

The presence of delirium was independently associated with worse clinical prediction of survival, lower quality of life, and increased healthcare costs through longer hospitalizations have been reported in patients with delirium [17-19]. More specifically, an economic analysis of healthcare for older hospitalized patients with or without Alzheimer's disease and related dementias associated with delirium showed that in the first group, the adjusted mean difference in costs related to delirium was almost 35000 USD, and these costs increased progressively over one year vs. non-MND patients [20]. The same study revealed that patients with Alzheimer's disease-related MND were more likely to develop delirium (51% vs. 24%) and die within one year (38% vs. 21%), all values being statistically significant [20]. Because delirium is considered a modifiable factor for prognosis in patients with MND and, frequently, a preventable one, the necessity for a correct diagnosis and early treatment, whenever delirium is identified, could have both short-term and long-term substantial benefits for the MND population. According to a systematic review, the prevalence of delirium superimposed on dementia varies between 22% and 89% in hospitalized patients aged over 65 [21]. Such high prevalence indicates, once again, that there is an acute need to detect and treat delirium actively in vulnerable populations.

This narrative review aims to identify the most valid interventions that could be included in case management for patients with delirium associated with MND. Because it was anticipated that the sources for this specific population might be scarce, the secondary objective included finding case management recommendations for patients with delirium in clinical populations, regardless of the organic or psychiatric background; these supplementary data were explored to see if they allow for being extrapolated to MND + delirium cases.

MATERIALS AND METHODS

This review explored three electronic databases (Pubmed, Cochrane, and Web of Science/Clarivate) for primary and secondary sources dedicated to this topic, using as keywords „delirium", OR „confusional episodes" OR „confusion superimposed on dementia"

AND „major neurocognitive disorder” OR „dementia” AND „pharmacologic*” OR „drug” OR „non-pharmacologic*” OR „psychosocial”. All papers published in English between the inception of each database and September 2024 were screened for relevant data on pharmacological and non-pharmacological therapies targeting delirium. The results were grouped into two classes: (1) guidelines for patients with delirium in clinical populations and (2) case management for patients with delirium and neurocognitive disorders.

RESULTS

Based on the 36 sources reviewed in the first section, dedicated to delirium case management in clinical populations, and 15 sources (partially overlapping) focused on the evaluation and treatment of delirium superimposed on MND, the following results are presented.

Recommendations for case management in patients with delirium in the hospital settings

The papers reviewed and grouped in this section were distributed according to their main clinical use, i.e., (1) validated tools for screening and monitoring delirium and (2) therapeutic recommendations, since these two components were considered essential for clinicians to construct a case management plan (Fig.2).

Instruments for detection of delirium and its severity quantification in clinical population

There are many instruments for the diagnosis and monitoring of the presence of delirium, some of them being more widely used and largely validated than others. CAM was developed by an expert panel through a consensus process and has the clear advantage of its brevity and easiness of administration [22]. CAM may be applied in less than five minutes and consists of nine criteria derived from DSM III R, the main symptoms of delirium being acute onset and fluctuating course, lack of attention, disorganized thinking, and altered sensorium [22]. Different versions of this instrument emerged, such as CAM-ICU, which is dedicated to patients admitted to intensive care units and can be administered by non-specialists [23].

4-AT is another tool conceived for the screening of delirium symptoms in elderly patients and demonstrated good sensitivity and specificity for hospitalized subjects [24]. This instrument has four items, i.e., alertness, two items for cognitive function, and one for fluctuation in mental status [24].

The NEECHAM (Neelon and Champagne) Confusion Scale is also used for rapid assessment of delirium and has been proven to have good internal consistency and interrater reliability, and it also correlated well with the Mini-Mental State Examination [25]. This tool has nine scale items, is easy to use, and can be scored by nurses with minimal training. Also, NEECHAM may be administered initially and periodically to monitor mental status [25].

The Observational Scale of Level of Arousal (OSLA) can be used for the identification of abnormal levels of arousal associated with delirium and may be administered for screening and monitoring purposes [26]. The items refer to eye-opening, eye contact, posture, and movement, and the total score is between 0 and 15 [26].

Therapeutic and prophylactic recommendations in patients with delirium

Regarding the therapeutic approaches to delirium, after the diagnosis of an acute confusional state was formulated, the first step is to analyze the potential contributors to the onset of this disorder. Predisposing and precipitating factors must be evaluated, and modifiable etiological factors should be addressed [27]. The case management of patients with delirium has as the main objective the correction of the biological disequilibrium that fueled the confusional state, but the prevention of relapse is also of major interest since several causes may be chronic or recurrent. According to an expert consensus on delirium management, „early identification and intervention of subclinical delirium can reduce the risk of clinical delirium” [28]. Also, daily assessment of at-risk patients is important for early detection of delirium and adequate therapeutic interventions [28].

The most common precipitating factors that should be approached during the evaluation of a patient with delirium are infections, electrolyte imbalances, drugs (medications, drugs of abuse-intoxication/withdrawal, toxic substances), pain, organ failures, traumas, metabolic and nutritional imbalances, central nervous system (CNS) pathologies, environmental factors (e.g., sensorial deficits for patients with neurocognitive disorders, immobilization, sleep-wake cycle dysfunctions), but also psychiatric factors (e.g., anxiety, insomnia or depression in elderly patients with severe organic disease).

The ABCDE/ABCDEF protocol was suggested for managing delirium [29,30]. This acronym stands for (a) assessing, preventing, and managing pain, (b) both Spontaneous Awakening Trials and Spontaneous Breathing Trials (SAT and SBT, respectively), (c) choice of analgesia and sedation, (d) delirium - assessment, prevention, and management, (e) early mobility and exercise, and (f) family engagement and empowerment [31].

Another integrated approach algorithm for critically ill patients targeted the prevention of early deep sedation in the ICU as a risk factor for poor long-term outcomes, including mortality and neurocognitive disorders [32]. Flexible multimodal analgesia, with the minimal use of opioids, is the main concept of this algorithm, together with the use of the lowest doses of sedatives that are still efficient [32]. Promoting sleep, early mobilization, and improved communication of patients with medical personnel and caregivers are also included in the eCASH (early Comfort using Analgesia, minimal Sedatives, and maximal Human care) model as methods to avoid isolation, delirium, and a decreased quality of life [32].

Figure 2: Case management in patients with delirium in clinical populations

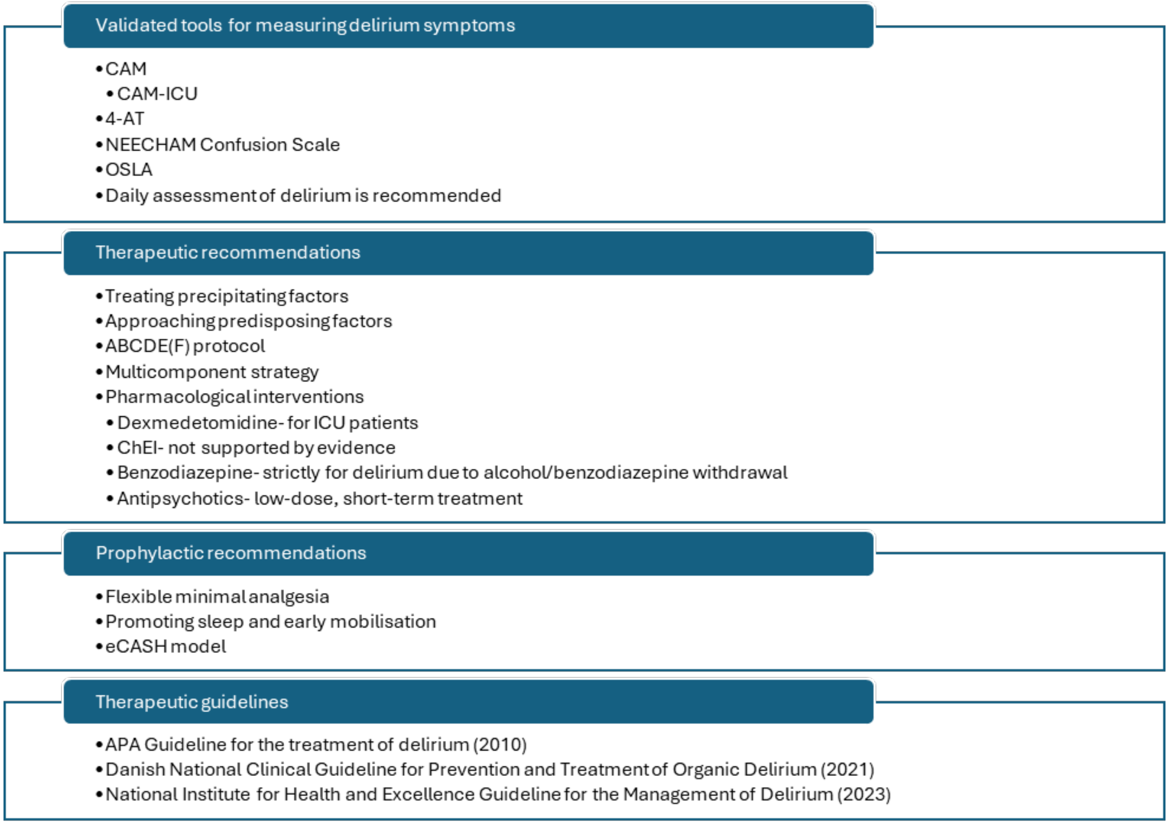

Validated tools for measuring delirium symptoms

- CAM
  - CAM-ICU
- 4-AT
- NEECHAM Confusion Scale
- OSLA
- Daily assessment of delirium is recommended

Therapeutic recommendations

- Treating precipitating factors
- Approaching predisposing factors
- ABCDE(F) protocol
- Multicomponent strategy
- Pharmacological interventions
  - Dexmedetomidine- for ICU patients
  - ChEI- not supported by evidence
  - Benzodiazepine- strictly for delirium due to alcohol/benzodiazepine withdrawal
  - Antipsychotics- low-dose, short-term treatment

Prophylactic recommendations

- Flexible minimal analgesia
- Promoting sleep and early mobilisation
- eCASH model

Therapeutic guidelines

- APA Guideline for the treatment of delirium (2010)
- Danish National Clinical Guideline for Prevention and Treatment of Organic Delirium (2021)
- National Institute for Health and Excellence Guideline for the Management of Delirium (2023)

APA= American Psychiatric Association, CAM= Confusion Assessment Method, ChEI= cholinesterase inhibitors, eCASH= early Comfort using Analgesia, minimal Sedatives and maximal Human care, ICU= Intensive Care Unit, NEECHAM= Neelon and Champagne, OSLA= Observational Scale of Level of Arousal

The multi-component strategy for delirium in the elderly includes six intervention directions, i.e., cognitive deficits, sleep deprivation, immobility, visual deficits, hearing impairments, and dehydration [33,34]. Early psychomotor and cognitive action may reduce the duration of delirium in critically ill patients [35].

Methods may be specific to the identified causal factors, such as the treatment of alcohol withdrawal, Wernicke encephalopathy, acute kidney injury, hepatic encephalopathy, diabetic ketoacidosis, etc. [36-40]. Exploring neurological disorders that may represent vulnerability factors for the onset of delirium is an important step in the prophylaxis of these patients [14,41,43]. In patients with neurocognitive disorders due to Alzheimer's disease, Parkinson's disease, or vascular diseases, the initiation of acetylcholinesterase inhibitors (AChEI) may help in reducing the risk of delirium onset/recurrence or its severity [44,45].

Dexmedetomidine was associated with an improved diagnosis for patients with delirium and mechanical ventilation admitted to ICUs [29]. Dexmedetomidine combined with analgesics may reduce the duration of delirium in patients with agitated forms of confusional states [29]. Dexmedetonimide is usually administered in the ICU for adult patients who require a level of sedation that allows for awakening as a response to verbal stimulation, meaning a Richmond Agitation and Sedation Scale (RASS) score between 0 and -3 [46]. Dexmedetomidine is administered by infusion and requires continuous monitoring of the blood pressure, cardiac rhythm, and respiratory functioning [46].

Impairment of cholinergic neurotransmission by high levels of circulating proinflammatory cytokines is considered a critical contributor to the onset of delirium; therefore, the administration of pharmacological agents that increase acetylcholine may be an adequate method to prevent or treat confusional episodes [47,48]. Following this line of reasoning, cholinesterase inhibitors (ChEI) were explored as symptomatic treatments for delirium, independent of the presumed pathophysiological substrate. However, a systematic review found only one study matching the inclusion/exclusion criteria (N=15 participants, out of which 7 have comorbid dementia) did not find a significant difference between rivastigmine and placebo regarding the duration of delirium, adverse events, use of rescue medication, mortality, and study discontinuation [49].

Benzodiazepines are used in cases of delirium due to withdrawal of substances in patients with substance use disorders, and where antipsychotics are contraindicated [29,50]. SAT, which is one of the core elements of the ABCDEF bundle previously mentioned, refers to the need to use a daily discontinuation strategy of sedatives or a continuous titration of these drugs in order to preserve the minimum level of sedation [31].

Antipsychotics are associated with variable results in this population. Based on the results of a meta-analysis and systematic review (n=5 randomized controlled trials, N=1750 patients), the use of antipsychotics in critically ill adults with delirium did not increase the

number of days without delirium or coma vs. placebo and did not show a significant effect on mortality, duration of mechanical ventilation, ICU stay or hospital length [51]. Another meta-analysis (n=19 studies) focused on the prevention and treatment of delirium by using antipsychotics showed these agents had no significant effect on delirium incidence, duration, severity, or ICU length of stay [52]. Yet another systematic review (n=14 sources) showed that uncontrolled studies reflect limited support for the use of low-dose, short-term treatment of delirium with several antipsychotics [53].

APA Guidelines for the Treatment of Delirium (2010) recommends (1) a close collaboration between the psychiatrist and other physicians to ensure appropriate care for these patients; (2) identify the precipitants; (3) initiate interventions for acute conditions; (4) provide disorder-specific treatment for co-occurring diseases; (5) monitor and ensure the safety of the patient; (6) assessment and monitoring of the psychiatric status; (7) assessment of individual and family psychosocial and social characteristics; (8) maintain the therapeutic alliance with the patient and his/her family; (9) education of the patient and family regarding the illness; (10) provide post-delirium management [54]. Environmental interventions, structuring and ensuring support for the patient, and support and education for the family are the most important non-pharmacological interventions, while antipsychotics (orally or intramuscularly, low doses, and for the shortest time needed), benzodiazepines (but there are only limited data, except for the treatment of delirium related to alcohol or benzodiazepine withdrawal), pro-cholinergic drugs (but there is a risk for cholinergic adverse events), vitamins (in specific cases, such as patients with hemodialysis, or alcohol use disorder), morphine or other opiates (in palliative care, and there is a risk of exacerbating the delirium) are considered the most important pharmacological strategies for the management of delirium [54].

The Danish National Clinical Guideline for Prevention and Treatment of Organic Delirium (2021) recommends non-pharmacological therapy to be initiated in all patients with a risk of delirium and in those with delirium (i.e., sensory optimization, orientation and cognitive stimulation, mobilization, nutrition, rehydration, supporting the circadian rhythm) [55]. Placing the delirious patient in a protected environment, actively involving relatives in the treatment of patients with delirium, and constantly reviewing medication for all these patients (i.e., adjusting, including stopping non-essential drugs), and avoiding routinely using antipsychotics for all delirious patients are also recommended by this guideline [55]. Benzodiazepines should not be used in this population except for palliative patients with agitation and terminal delirium or for patients in ICU where respiratory support and monitoring are accessible [55]. Melatonin is not considered by the Danish guidelines a useful intervention, but electroconvulsive therapy may be recommended in refractory cases where other therapeutic options have failed [55].

The National Institute for Health and Excellence (NICE) guideline for the management of delirium (updated January 2023) recommends, for the prevention of delirium, a multi-component approach conducted by a multidisciplinary team, addressing the cognitive impairment and/or disorientation by environmental measures, and address all potential triggers for confusion [56]. Treating delirium involves management of the underlying causes, ensuring communication and reorientation, de-escalation of the situation using verbal and non-verbal techniques, followed, if necessary, by short-term haloperidol, and, if there is still little or no improvement, re-evaluation of the potential precipitants and vulnerability factors, dementia included [56].

Case management for patients with delirium superimposed on a major neurocognitive disorder

The distribution of the sources retrieved during the literature search for the case management of patients with delirium overimposed on MND was similar to that mentioned in the previous section for delirium in clinical populations, and the results are synthetically represented in Figure 3.

Diagnostic aspects of delirium in patients with MND

Regarding the diagnosis aspects, an analysis of the potential biomarkers for delirium superimposed on MND showed that genetic, epigenetic, and pharmacogenetic variables might be used in the near future to construct personalized case management for these patients [57].

Gene-gene, protein-protein, gene-drug, and gene-miRNA interactions have been detected as potentially significant in this specific population, with several essential genes, like BDNF (brain-derived neurotrophic factor), APP (amyloid beta precursor protein), DNMT1 (DNA methyltransferase 1), APOE (apolipoprotein E), and TNF (tumor necrosis factor) presenting gene x drugs interactions also relevant for this pathology [57]. However, there is no single independent, validated biomarker for delirium, which leaves, for now, at least, the diagnosis to the clinicians [58].

A review dedicated to the best tools for identifying delirium in patients with MND concluded there is no concordance between validated diagnostic criteria, psychometric instruments, and therapeutic guidelines, which makes it difficult for clinicians to identify and treat this pathology appropriately [59]. Taking into account such limitations, CAM and CAM-ICU are the most recommended instruments for the diagnosis of delirium in dementia, based on the review of clinical studies [21,59]. Other instruments, such as 4-AT or 4-DSD (Delirium superimposed on dementia), have also been studied in this population, but there is less data to support their clinical use [59]. The 4-AT may be beneficial for the correct and timely detection of delirium in this population because it can help in the exclusion of cognitive impairments in emergency rooms and hospital admissions [33]. Therefore, as a screening tool, 4-AT is a helpful instrument that requires little time for administration.

The 4-DSD has four items and is derived from 4-AT, but the scoring algorithm is different, and several items have a changed content compared to the initial tool [59]. This instrument assesses the level of alertness, altered brain function, attention integrity, and acute change and fluctuation in mental status [59].

The use of the OSLA in combination with another very simple attention test (that requires the patient to identify a certain letter in a sequence) correctly identified more than 90% of the cases of delirium superimposed on dementia [33,60].

Figure 3: Case management of patients with delirium superimposed on major neurocognitive disorder

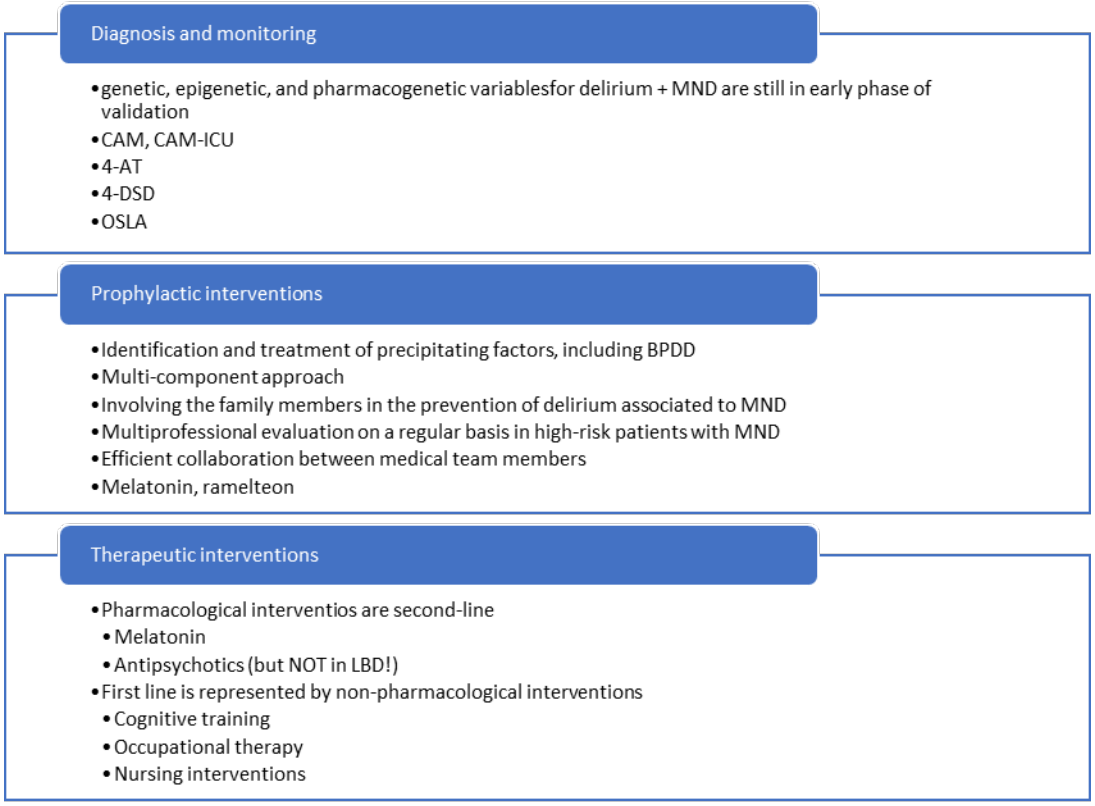

Diagnosis and monitoring

- genetic, epigenetic, and pharmacogenetic variablesfor delirium + MND are still in early phase of validation
- CAM, CAM-ICU
- 4-AT
- 4-DSD
- OSLA

Prophylactic interventions

- Identification and treatment of precipitating factors, including BPDD
- Multi-component approach
- Involving the family members in the prevention of delirium associated to MND
- Multiprofessional evaluation on a regular basis in high-risk patients with MND
- Efficient collaboration between medical team members
- Melatonin, ramelteon

Therapeutic interventions

- Pharmacological interventios are second-line
  - Melatonin
  - Antipsychotics (but NOT in LBD!)
- First line is represented by non-pharmacological interventions
  - Cognitive training
  - Occupational therapy
  - Nursing interventions

BPDD= behavioral and psychological disturbances in dementia, CAM= Confusion Assessment Method, DSD= Delirium Superimposed on Dementia, ICU= Intensive Care Unit, LBD+ Lewy body dementia, MND= major neurocognitive disorder, OSLA= Observational Scale of Level of Arousal

Preventative and treatment-focused recommendations in patients with delirium and MND

Mentioning that there is no „gold standard" for the diagnosis and treatment of delirium in patients with MND, a systematic review showed that a multi-professional evaluation regularly of high-risk patients with MND is recommended as a step toward the prevention of delirium [61]. An efficient collaboration between multiple members is also needed within the team, which is involved in case management [61]. Eliminating the risk factors for delirium in this vulnerable population is essential [61]. Screening for all the major causes of delirium in patients with MND is strongly recommended and could have significant benefits [33]. Constipation, medications with central nervous system adverse events, infections, vascular factors, electrolytic imbalance, low mobility, malnutrition, pain, incorrect sleep hygiene, and urinary retention should be screened for in patients with MND and sudden clouding sensorium [33].

A non-pharmacological approach is initially indicated in all individuals presenting delirium and MND, based on decreasing the number of medications used, maximization of family support, orienting techniques, adequate hydration, and correction of visual and hearing deficits, whenever possible [21]. However, the controlled studies did not show a consistent effect of environmental strategies in preventing delirium in patients with MND [21].

Pharmacological therapies are considered second-line interventions and applied only when non-pharmacological strategies have failed or are insufficient [61]. The use of melatonin and antipsychotics to treat delirium in MND is not supported enough by evidence [62].

As for the prevention of delirium, the identification of the precipitants and their treatment, initiation of multicomponent interventions, prevention of complications, and monitoring the resolution of the core symptoms are the main directions of case management in patients with delirium and MND [58,62]. The participation of the family members is strongly encouraged by the case managers because the evolution of the patients is often unpredictable even after hospital discharge, and caregivers may play an important role in re-orienting a confused individual by offering him or her a point of reference [58]. Re-assessment of precipitating and vulnerability factors is the first step, and only after that can non-pharmacological and pharmacological treatment be considered [62].

Special care should be given to patients with MND and BPDD, a sub-population that is more vulnerable to the onset of delirium than patients with dementia but without BPDD [63]. The recovery of delirium is slower in patients with both dementia and BPDD; therefore,

identifying early signs of delirium, like sleep impairments, mood lability, and psychomotor agitation, should trigger increased attention and more intensive monitoring [63].

Cognitive training was explored as a possible intervention for MND, to facilitate neuroplasticity in these patients [64]. Based on several similar cognitive features between dementia and MND, the use of recreational activities to enhance attentional abilities and offer cognitive stimulation was suggested to reduce delirium severity and duration, improving quality of life and reducing healthcare costs [64]. A pilot study dedicated to this intervention (N=16 participants) demonstrated a greater decrease in physical function and mental status during the 30 days of monitoring vs. the control group [65]. Delirium, but also the severity of delirium and attention had a trend toward significance in the study vs. control group [65].

Occupational therapy, based on preliminary evidence, can be another option for patients with delirium and MND [66]. A case series (N=6 patients with dual disorders) reported positive results of occupational therapy conducted twice daily for a total of 40 minutes until the delirium was remitted [66].

Nursing interventions for delirium over-imposed on MND were associated with good results [67]. A 10-step intervention was reported to help in the dissolution of delirium in a patient with Alzheimer's dementia: interdisciplinary communication with care providers, reduction of medications administered, communicating calmly and slowly with the patient, avoidance of physical restraints, re-hydration, regulation of the sleep-wake cycles, assess and prompt treatment of pain, education of the family and encouraging family members to participate in care, mobilize the patient as soon as possible, and regulation of the excretory function [67].

Melatonin and ramelteon may be recommended in older patients for the prevention of delirium when admitted to an acute geriatric facility [68,69]. According to the results of a multicenter, randomized study (N=67 patients admitted in ICUs), ramelteon administered nightly was associated with a lower risk of delirium, even after controlling the risk factors, vs. placebo [69].

Antipsychotics are frequently used in clinical practice for this population, but a trial with these medications should be recommended only for a short time, in low doses, and under careful supervision [33]. Special care should be taken when administering antipsychotics in patients with MND due to Parkinson's disease or Lewy Body Dementia [33].

DISCUSSION

The existing evidence reviewed in the previous chapter supports the use of validated instruments for (1) screening in vulnerable populations, patients with MND included; (2) supporting the clinical diagnosis of delirium in the absence of validated biomarkers; (3) monitoring the evolution of delirium during treatment, until the complete resolution of its symptoms [22-26, 33,61]. Also, preventative measures could be applied in vulnerable populations, which may lead to a reduction in the risk of delirium onset, i.e., treating risk factors, close assessment of the clinical status, multi-component interventions, and even limited data supporting pharmacological interventions for short periods of time [58,62,63]. Therapeutic measures that can be initiated in cases of delirium superimposed on MND are mainly non-pharmacological since, for the pharmacological agents, there is only limited data to support their use [29,33,46-50,53-56].

Limitations of this review are related to its narrative nature, and, as a result, not all significant sources may have been found and reviewed. Also, due to the narrative character of the review, no quality of data was assessed, and all sources were treated equally. Finally, there is a paucity of data derived from good-quality trials focused on delirium overimposed on MND, which limits the validity of the review's results, since most data are extrapolated from studies with patients presenting delirium in the clinical population, regardless of the causal factors.

The strengths of the present review are related to the enlarged focus on both pharmacological and non-pharmacological components of case management and the integration of structured assessment instruments into the clinical practice in vulnerable populations.

Future directions of research should include better-quality trials with homogenous populations, e.g., delirium with onset in patients with MND due to Alzheimer's disease, or the prevention of delirium in patients with MND due to Parkinson's disease, but also active comparators and placebo for the pharmacological trials, and a large number of participants with long-period of follow-up, in order to assess the risk of recurrence.

CONCLUSION

When formulating the case management plan for patients with delirium superimposed on MND, the clinician should take into account (1) the need for a multidisciplinary team since the precipitating and vulnerability factors may be complex and have intricate relationships; (2) regularly and frequently assess the patient using instruments like CAM, 4-AT, OSLA, or 4-DSD; (3) include the family in the monitoring of the patients, whenever possible; (4) non-pharmacological interventions, such as cognitive training and occupational therapy, nursing techniques, multi-component approaches, are first-line strategies; (5) pharmacological interventions are considered second-line, and they are administered only for short time and low doses; (6) prophylaxis of delirium in vulnerable populations, MND patients included, remains the single most important way to reduce the complications associated with this pathology.

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ORIGINAL ARTICLE

Gender-Dependent Associations Between Digit Ratio and Genetic Polymorphisms, BMI, and Reproductive Factors

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Abstract: The digit ratio (2D:4D) has been associated with prenatal hormonal influences and various traits and pathologies. This article explores the relationship between 2D:4D and a series of common polymorphisms and Torque Teno Viruses. In this study, 120 healthy participants were included. The IGF2 Apa I, ACE I/D, INS -23 Hph I, VDR Fok I, VDR Apa I, VDR Taq I, AT1R A1166C polymorphism were genotyped by PCR-RFLP technique, and the IL-6 -174 G/C polymorphism by tetra-primer ARMS-PCR. The presence of TTV was identified by a hemi-nested PCR technique. Haplotype analyses were performed using the SHEsis software. The average 2D:4D values were similar for men and women. Overweight men presented higher 2D4D ratios than normal-weight women ($p<0.05$). Lower 2D:4D values were recorded in women with pregnancy loss or one child or none ($p<0.001$). Men with lower 2D4D ratios reported a higher number of children ($p<0.001$). The IGF2 GG and ACE DD were associated with a higher digit ratio in all subjects and in the women's subset. A significant association was found in men between 2D:4D and the INS-23 Hph I – IGF2 Apa I T-G haplotype ($p<0.01$). The data obtained in this study indicate a sexual dimorphism for the digit ratio. The associations between 2D:4D and the genetic polymorphisms studied could be influenced by gender.

Keywords: 2D:4D, Insulin-IGF2 haplotype, ACE I/D, parity, BMI

INTRODUCTION

The ratio between the length of second and fourth digits (2D:4D) is determined prenatally, and remains stable throughout life, serving as a marker of early maternal hormonal influences [1,2]. This length is influenced by the ratio between testosterone and estrogen during the fetal period. A smaller digit ratio has been associated with a higher concentration of fetal testosterone and a lower concentration of fetal estrogen [3]. Additionally, the 2D4D ratio can be influenced by the density of receptors for testosterone and estrogen, which is greater for 4D than 2D [2], and also by the maternal environment [4]. Nevertheless, testosterone production during the first years of life could influence secondary sexual differences developing before puberty as 2D4D ratio [4]. Furthermore, the digit ratio was correlated with specific genetic particularities of the child such as a polymorphism in the androgen receptor gene [5]. Therefore, lower digit ratios are common in males whereas higher values are identified in females [6].

The study of the digit ratio offers a compelling gateway into understanding the intricate interplay of genetic, hormonal, and environmental factors from the intrauterine environment. These interactions also may shape an individual's predisposition for different physiological (e.g. height, weight, BMI, athletic performance, reproductive success) [7,8,9,10] or psychological traits (e.g. academic performance) [11]. In addition, the 2D:4D has been associated with various categories of pathologies, ranging from autoimmune diseases (e.g. vitiligo [12], thyroid disease [13]), metabolic disorders [14], and malignant tumors [15] to mental disorders such as autism, ADHD, and depression [16]. A series of polymorphisms such as Insulin, IGF2, angiotensin I-converting enzyme (ACE), angiotensin II type 1

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receptor (AT1R), vitamin D receptor (VDR), and IL-6 genes may imply fetal growth and development and have been associated with some of these pathologies [17,18,19].

Insulin-like growth factor two (IGF2) is a key protein regulating growth, secreted, mostly via the placenta, and after birth by hepatocytes [20]. INS-23Hhp influences IGF2 transcription in the human placenta and it may be implicated in insulin-regulated intrauterine growth and insulin secretion in adults [21].

ACE, a zinc metallopeptidase, plays a pivotal role in the renin-angiotensin-aldosterone system (RAAS), responsible for homeostasis, blood pressure regulation, and water balance in the body. ACE I/D polymorphism has been associated with different pathologies, including pregnancy complications and infertility [22]. Angiotensin II type 1 receptor (AT1R) is a well-reported biomarker for hypertension and AT1R A1166C was strongly associated with the risk of developing pregnancy-induced hypertension [23].

Vitamin D (VD) is a molecule involved in regulating various processes, including phosphate and calcium metabolism, bone mineral density, and immune response [24], but also plays an important role in fetal development. Low maternal VD levels during pregnancy are associated with various adverse obstetric outcomes, such as gestational diabetes mellitus [25]. Vitamin D, as cortisol and sex hormones are all derived from cholesterol, creating an intricate network of interactions. Cortisol and testosterone concentrations might be influenced by vitamin D [26] through the activity of the vitamin D receptor, which is present in Leydig cells [27] (that plays a role in testosterone synthesis) and regulates the activity of the adrenal gland [28] (to produce cortisol). Therefore, three of the most studied VDR polymorphisms may be of interest: VDR Fok I (C/T), VDR ApaI (A/C), and VDR Taq I (C/T). Additionally, a low cortisol reactivity was associated with a low digit ratio [29]. Nevertheless, no significant association between 2D:4D and vitamin D has been reported in the literature so far.

Having pro-inflammatory and anti-inflammatory properties, interleukin 6 (IL-6) plays a major contribution in modulating the immune response, being reported as a biomarker of inflammation and intraamniotic infection [30]. Moreover, IL-6 was associated with right 2D:4D in girls, suggesting that the gender of the subjects and the hand's position in the sagittal plane influence the correlation between IL-6 and digit ratio [31]. The synthesis of pro-inflammatory cytokines, including IL-6, may be influenced by Torque Teno viruses (TTV) [32]. TTV are non-enveloped viruses that belong to the Anelloviridae family that are widespread globally [33]. They have multiple transmission routes, including transplacental transmission, which may have implications in prenatal development, although studies are not consistent on this matter [34, 35]. TTV may represent a potential biomarker of immune function and for some diseases [36].

This article aims to investigate the associations between digit ratio, gender, certain characteristics (such as height, weight, BMI, and reproductive success), and a series of common polymorphisms.

MATERIALS AND METHODS

Selection of subjects.

This study included 120 healthy, unrelated, volunteers (61 women, 59 men), aged between 42 and 60 years (52.73 ± 3.6 years). All participants were informed about the purpose of the study. Selection criteria for the participants included alcohol intake and smoking. Alcohol intake consisted of no more than two standard drinks a day for men and one standard drink a day for women. A beverage is considered alcoholic if it contains up to 10 g of ethanol, according to the World Health Organization [37]. As for smoking, the threshold was 20 cigarettes per day, based on the participants' answers.

Methods and Protocols.

The length between the second digit and the fourth digit was measured by the caliper three times, therefore the value obtained is the average of these three consecutive measurements. It was measured on the ventral surface of the left hand, from a midpoint on the metacarpophalangeal flexion crease to the tip of the digit. The measurements were considered valid if the difference between the maximum and minimum values measured for a finger was less than 5 mm. If the difference was greater, the measurement process was repeated for that participant. The analysis was restricted to subjects with a digit ratio range of 0.96-1.005, with more extreme values excluded.

A peripheral blood sample (300 mL) was collected from each subject in an EDTA tube and genomic DNA was extracted using the Promega Wizard® Genomic DNA Purification Kit. The presence of TTV was identified using a hemi-nested PCR protocol [38]. The ACE I/D polymorphism (rs1799752) was identified by PCR with a primer set for insertion [39], while the PCR-RFLP technique was used for genotyping the polymorphisms IGF2 Apa I (820 G/A, rs680) [40], INS -23 Hph I (-23 A/T, rs689) [41], AT1R A1166C (rs5186) [42], VDR Fok I (C/T, rs2228570), VDR Apa I (A/C, rs7975232), VDR Taq I (C/T rs731236) [43, 44]. IL-6 -174 G/C (rs1800795) was genotyped by the tetra-primer ARMS-PCR technique [45]. Restriction products were resolved by electrophoresis.

Statistical Analysis.

The distribution of qualitative data in subgroups of subjects was analyzed with Chi-square or Fisher tests. The outlier values for 2D:4D were considered values that exceed the mean value ±3 standard deviations. The quantitative values were analyzed using ANOVA, one-way ANOVA with post-hoc Tukey HSD test. Simple linear regression analyses were used to assess the variation in anthropometric parameters with variation in 2D:4D. The statistical analyses were performed using the R Studio software, version 2023.12.1.+402. Haplotype analyses were performed using the SHEsis software [46]. Differences were considered statistically significant if $p<0.05$.

RESULTS

The average age (52.73 ± 3.6; 42-60), weight (71.71±5.06; 63-86), height (1.70±0.02; 1.65-1.75) and BMI (24.96±1.78; 21.89-29.41) and 2D/4D (0.9858 ±0.01; 0.97-1.01) of subjects were estimated. The characteristics of the investigated subjects are presented in Table 1.

We have identified no outliers for 2D:4D in the case of men or women investigated in this study. Men have a higher digit ratio than women, but this difference was not statistically significant (0.9871 vs 0.9845, $p > 0.05$).

Table 1: Characteristics of subjects

Characteristics	Values
Male / Female	59 /61
Age: men vs women	52.89 vs 52.57
Weight: men vs women	74.10 vs 69.39[b]
Height: men vs women	1.70 vs 1.69
BMI: men vs women	25.72 vs 24.21[b]
Smokers: men vs women	24 vs 17
Alcohol drinkers: men vs women	12 vs 3[a]
2D/4D: men vs women	0.9871±0.007 (0.97-0.998) vs 0.9845 ±0.009 (0.97-1.005)
TTV positive samples: men vs women	45 vs 39
INS -23 Hph I: AA/AT/TT	49/66/5
IGF2 Apa: GG/GA/AA	55/57/8
VDR Fok I: CC/CT/TT	53/50/17
VDR Apa I: AA/AC/CC	41/53/26
VDR Taq I: TT/TC/CC	25/52/43
ACE I/D: II/ID/DD	35/52/33
AT1R A1166C: AA/AC/CC	57/48/15
IL-6 -174G/C: GG/GC/CC	75/38/7

[a] $p<0.05$; [b] $p<0.0001$

The IGF2 GG and ACE DD were associated with a higher digit ratio in all subjects and also in women (Table 2). No other genotype shows a significant association with the digit ratio.

Table 2: Characteristics associated with 2D:4D

Subgroup compared	2D: 4D average (interval)
All: IGF2 GG vs. (GA+AA)	0.9877±0.0074 (0.97-1.002) vs 0.9841±0.008 (0.97-1.005)[b]
Women: IGF2 GG vs (GA+AA)	0.9873±0.007 (0.97-1.002) vs 0.9823±0.009 (0.97-1.005)[a]
Men: IGF2 GG vs (GA+AA)	0.9881±0.006 (0.973-0.998) vs 0.9861±0.006 (0.97-0.997)
All: ACE DD vs. II	0.9893±0.007 (0.97-1.002) vs 0.9843±0.009 (0.97-1.005)[b]
Women: ACE DD vs. II	0.9899±0,008 (0.97-1,002) vs 0.9811±0.009 (0.97-1.005)[c]
Men: ACE DD vs. II	0.9885±0.005 (0.98-0.996) vs 0.9878±0.007 (0.97- 0.998)
All: ACE (DD+ID) vs II	0.9844±0.008 (0.97-1.005) 0.9843±0.009 (0.97-1.005)
Women: ACE (DD+ID) vs I	0.9823±0.008 (0.97-1.005) vs 0.9811±0.009 (0.97-1.005)
Men: ACE (DD+ID) vs ACE II	0.9866±0.007 (0.97-0.998) vs 0.9877±0.007 (0.973-0.998)

[a] $p<0.05$; [b] $p<0.01$; [c] $p =0.006$

The INS and IGF2 (11p15.5) polymorphisms or VDR (12q13.11) polymorphisms were mapped in close proximity. As a consequence, we evaluated the effects of Ins -23 Hph - IGF2 Apa I and VDR Fok I – Apa I – Taq I haplotypes on 2D:4D. The INS-23 Hph I – IGF2 Apa I T-G haplotype was found to be significantly associated with 2D:4D in men ($p<0.01$), but not in women ($p>0.05$). No statistical associations were identified between the digit ratio and VDR Fok I – Apa I – Taq I haplotypes (Table 3).

Twenty-four (39.34%) women reported at least a pregnancy loss. The 2D:4D value of these women was lower compared to those who did not report such an event (0.9799 vs 0.9876, $p<0.001$). The height in these subsets of women was similar (1.691 vs 1.697, $p<0.05$) (Figure 1). The average age at which they had their first child was similar between those who had one child and those who had multiple

children (22.93 vs 22.2, p>0.05).

The 2D:4D values were lower in women who had no children compared to those who had children (p <0.001), who had at least two children (p <0.001), or one child (0.9809 vs 0.9865, p < 0.3). In the case of men, the differences identified were significant between men who had at least two children and those who had one child (p<0.001) or who did not have children (p<0.02). In the subset of subjects who did not have children, the lowest average 2D:4D values were recorded in men compared to women (0.9883 vs 0.9809, p< 0.001). The height of men and women stratified by the number of children did not show any statistically significant differences (Figure 2).

Table 3: Association of INS-IGF2 and of VDR haplotypes with 2D:4D

Polymorphisms	Haplotypes	The total count in women's sublot	The total count in men's sublot
VDR Fok I – Apa I – Taq I	CAC	34	31
	TCT	20	7
	CCT	18	24
	TAC	15	22
	CAT	15	7
	CCC	13	14
	TCC	5	4
	TAT	-	9
INS -23Hph I – IGF2 Apa	AG	51	49
	TG	34	33[a]
	AA	35	29
	TA	-	7

(Haplotypes with frequency <0.03 were not taken into consideration; [a] p <0.01)

Figure 1: Analysis of 2D:4D ratio and number of children by gender

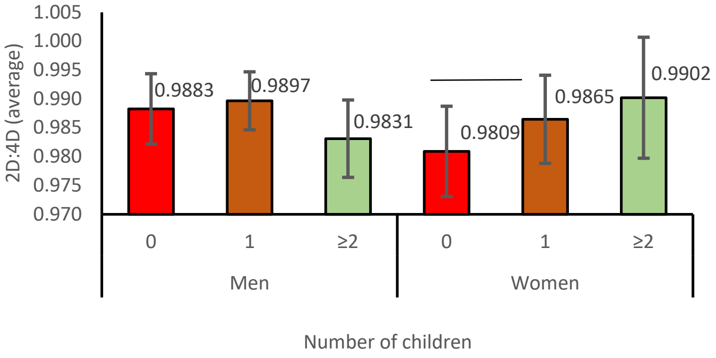

Figure 2: Analysis of height and number of children by gender

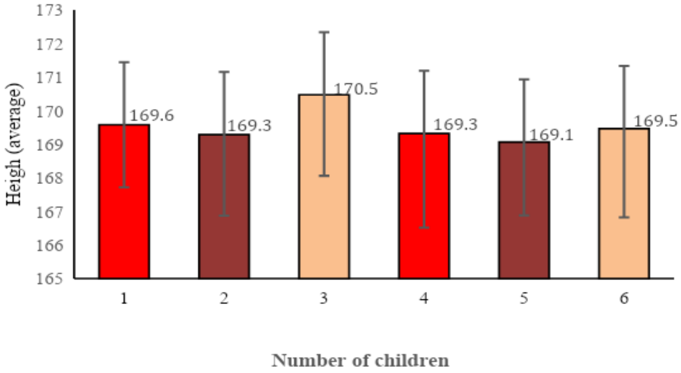

The 2D:4D values were higher in overweight subjects than in normal-weight participants (0.984 vs 0.988, p<0.008). The weight (74.1 vs 69.39 kg, p<0.0001) and BMI (25.72 vs 24.21 kg/m^2, p<0.0001), but not height (170 vs 169 cm, p>0.05), were significantly higher in men compared to women. Each gender was subsequently stratified according to BMI into normal-weight and overweight subgroups. There was only a significant difference between the 2D:4D values of overweight men and normal-weight women (0.9884 vs 0.9837,

p<0.05). No significant results were found when the male and female subsets were stratified according to height.

There appears to be a positive correlation between 2D:4D and BMI, regardless of gender (F=0.02) (Figure 3), and an inverse correlation between the 2D:4D and height of men (F = 0.03) (Figure 4). However, the proportion of 2D:4D variance that may be predicted based on BMI (R2=0.047) or height (R2 =0.08) appears to be weak.

The tested genetic polymorphisms were not associated with the height or BMI of men and women (p>0.05). These variables were similar in women stratified by parity.

Figure 3: Linear regression for 2D:4D and BMI (both genders)

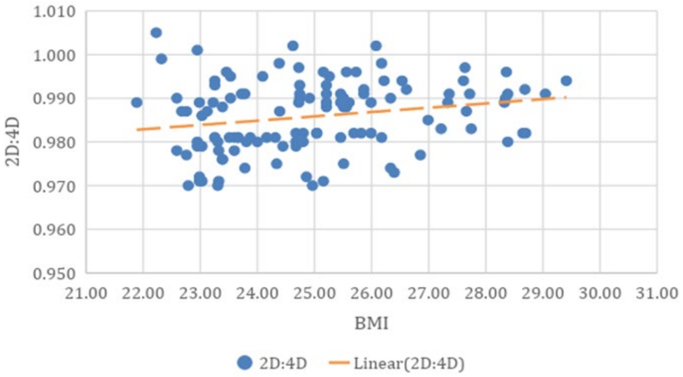

Figure 4: Linear regression for 2D:4D and height of men and women

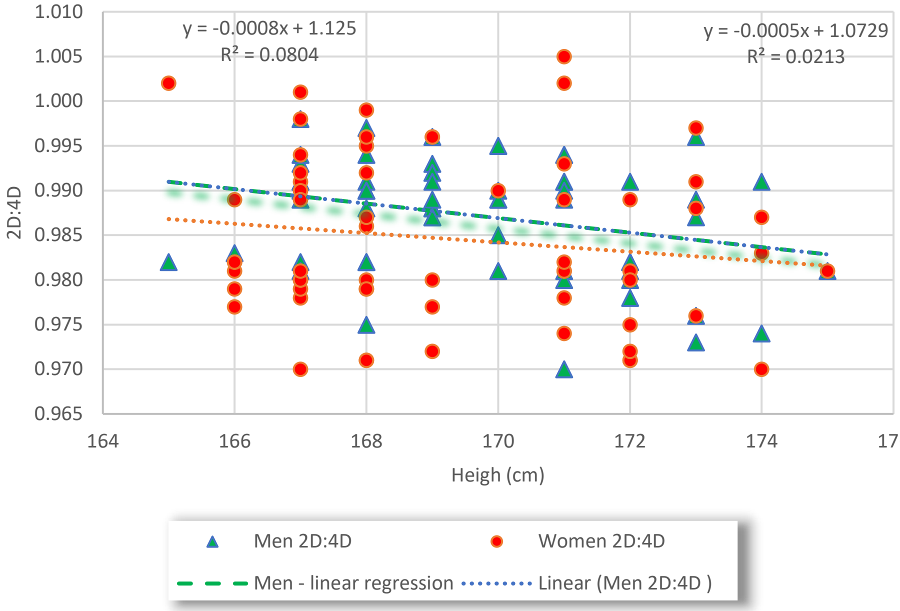

DISCUSSION

The digit ratio is determined during the prenatal period and in general remains stable throughout life [1]. A study that analyzed the 2D:4D ratio in 327 deceased fetuses (14 to 42 weeks old) concluded that the values were lower than those reported for children and adults in both sexes, suggesting 2D:4D could increase after birth [48]. In addition, a study has shown that this ratio can change, to a significantly reduced extent, during childhood, between the ages of one and five years [47]. Therefore, age may influence to some extent the length between the index and ring fingers. In the present study, the 2D:4D values were not correlated with age, neither at the level of the entire group of adult participants nor at the subgroup level by gender.

The difference between genders appears to be moderated by the type of finger length measurement and by the hand that was measured. Some studies suggest that there are greater differences between genders when measurements are taken on the right hand, which would better reflect prenatal hormone exposure [49,50]. In our sample, measurements were taken on the left hand, for which we obtained higher average values in men than in women, but the differences were not significant (0.9871 vs 0.9845, p >0.05). This result is contrary to the results reported by other studies [3,4]. In a meta-analysis that studied the representation of women in politics and was based on data collected in an online survey, the reported data for Romania indicated higher values in women than in

men for both the left hand (1 vs. 0.985) and the right hand (0.997 vs. 0.986) [50].

In contrast to the 2D:4D, height, weight, and BMI are characteristics that change postnatally, influenced by both genetic and environmental factors. Weak correlations between 2D:4D and these variables were identified in this study. Studies are not consistent on this matter. For example, 2D:4D was positively related to BMI in a very large sample of adults [51]. On the contrary, other studies have reported no significant correlations between these variables and 2D:4D [52,53].

The analysis of anthropometric data we performed showed that the values for weight and BMI were higher in men than in women. Moreover, we identified a significant difference between the 2D:4D values of overweight men and normal-weight women (0.9884 vs 0.9837, $p<0.05$). In the literature, significant positive correlations have been reported between higher (feminized) digit ratios and postnatal parameters (body weight, BMI, and waist and hip circumference) [54,55]. There were no statistically significant differences between height of men and women ($p>0.05$). The linear regression showed a negative correlation between 2D:4D and the height for males ($F = 0.03$), but not for females or both genders. One study reported that body height was negatively correlated with right- and left-hand digit ratios and the correlations were significant for the left-hand digit ratio in men and for the right-hand digit ratio in women [7].

Our data suggested a significant association between 2D:4D and reproductive success which is influenced by gender. Thus, lower average 2D:4D values were recorded in women who reported a pregnancy loss or who had no or one child ($p <0.001$). In the case of men lower 2D:4D values were associated with a higher number of children. Our results are in agreement with some results reported previously in the literature [9,10].

The minor allele frequencies for IGF2 Apa I (MAF A: 0.3 vs. 0.24) [56, 57] and ACE I/D (D: 0.49 vs 0.51) [58] in our lot are consistent with those previously reported in the Romanian population. Previous studies have reported that the IGF2 G allele may contribute to weight gain and eating disorders, and BMI [59]. Another study, which investigated multiple single nucleotide polymorphisms in the IGF2 gene region, concluded that IGF2 genetic variation is a significant determinant of body weight in middle-aged males [60]. The data we have obtained suggest that nearby genes, IGF2 or Insulin, could influence the digit ratio. The IGF2 GG genotype was associated with a higher digit ratio, especially in women ($p<0.05$) whereas the Insulin - IGF2 T-G haplotype was significantly associated with 2D:4D, but only in men. The closely linked IGF2 and Insulin genes have been previously associated with various conditions such as obesity, diabetes, metabolic syndrome, and coronary heart disease [61, 62]. Genetic imprinting influences IGF2 expression [63] and the association between the parent-of-origin allele and various phenotypes. The design of our study did not permit the investigation of IGF2 parent-of-origin.

ACE converts angiotensin I into angiotensin II and most of its functions are mediated by AT1R. ACE D was associated with a higher enzymatic activity of ACE [64]. Carriers of ACE DD have higher values of 2D:4D than ACE II in our lot of subjects ($p<0.01$) and especially in women ($p<0.01$). In a previous study, it was also reported that ACE DD individuals have higher 2D:4D than carriers of ACE II [65]. ACE I/D was also associated with various diseases, such as cardiovascular diseases and hypertension [66] or physical endurance or competitiveness [67]. The association may bias the results we have detected in our healthy subjects. Given the fact that ACE I/D polymorphism and 2D:4D ratio might play a role in cardiovascular pathology, our subjects (42-60 years old), although clinically healthy, may still have a predisposition to develop these diseases. This predisposition might influence the results obtained in this study, potentially causing a bias. The results could also be affected by the small number of subjects included in the study ($n=120$), as well as by measurement errors related to the length between the index and ring fingers.

Although no significant association was identified between the digit ratio and Torque Teno viruses, the high prevalence of TTV in the population was confirmed, being present in 70% of the subjects included in the study, in line with previously obtained results [32,68].

CONCLUSION

In our dataset, the digit ratio was similar in men and women. The data obtained in this study indicated that INS - IGF2 T-G haplotype, ACE I/D, pregnancy loss, number of children, and BMI are associated with digit ratio. These relations could be also influenced by the gender of the subject. Additional studies are needed to bring new data regarding the relationships between 2D:4D and the genetic polymorphisms of anthropometric characteristics to see if the results we obtained in this study could be replicated. This data could contribute to a broader understanding of how genetics can shape developmental outcomes through prenatal hormones.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. This study was funded by the Ministry of Research and Education, grant number PN 23 01 03 03.

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Authors' contribution

Conceptualization, N.S., R.S., R.M., and D.C.; methodology, O.C.; software, R.M. and M.T.; validation, M.I.N., M.L.C., and C.I.; formal analysis, M.T and M.I.N.; investigation, N.S., D.C., L.B., O.A.; resources, L.B., D.C.; data curation, S.R. and O.A..; writing—original draft preparation, N.S., E.S., R.M, O.C.; writing—review and editing, D.C., M.T, I.R., M.L.C.; visualization, M.T.; supervision, D.C. and O.C.; project administration, L.B, and D.C.; funding acquisition, L.B. and S.R.. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the National Research and Development Institute for Food Bioresources – IBA Bucharest (protocol code 285/20.03.2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

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ORIGINAL ARTICLE

Off-Label Percutaneous Solution for Primary Degeneration of a Bioprosthetic Valve in the Tricuspid Position: Case Report and Literature Review on Transcatheter Valve-in-Valve Insertion

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Abstract: Transcatheter valve-in-valve implantation in failing bioprosthesis is an emerging field in cardio-vascular surgery and cardiology. If a valve plasty is not possible, a biological valve is implanted with limited durability. Due to valve degeneration repeated valve exchanges are necessary in some patients. To expand the lifetime of a bioprosthesis in the tricuspid position percutaneous transcatheter valve-in-valve implantation was introduced recently. This is a promising new catheter interventional technology. There are no published outcomes of repeat surgical, but overall early mortality after tricuspid valve replacement (TVR) was 37% in patients undergoing TVR after prior tricuspid valve (TV) repair. The off-label use of transcatheter aortic valve prostheses for tri-cuspid valve–in–valve implantation within dysfunctional surgical tricuspid valve bioprosthesis has been described in small reports but has become of interest nowadays.

Keywords: valve-in-valve tricuspid, redo-surgery, endovascular surgery, off-label valve implantation

INTRODUCTION

A severely diseased tricuspid valve (TV) is classically treated by tricuspid valve repair (TVr) or, when the valve is too damaged, by tricuspid valve replacement (TVR) in classical open-heart surgery. In the case of TVR usually, a biological valve is used considering the thrombo-embolic risk, however, the life expectancy of this valve is limited due to degeneration [1]. Tricuspid stenosis is not an infrequent late complication of TVR. The 8-year follow-up of 31 patients showed that 1 out of 3 patients developed degeneration of the prosthesis with an average gradient above 5 mmHg, and 21% of patients needed surgical redo [2]. Redo surgery is associated with very high risk, with reported early mortality after TVR being 37% in patients undergoing TVR after prior TV repair [3,4]. The off-label use of transcatheter aortic valve prostheses for tricuspid valve–in–valve implantation within dysfunctional surgical tricuspid valve bioprosthetics has been described in small reports and has become of interest nowadays [5,6] especially in patients with high surgical risk or contraindications for surgery [7,8].

Case report

We describe the case of a 64-year-old female patient who presented in our department for fatigability and dyspnea with mild exertion. She had a past medical history of surgical excision of a right ventricle myxoma in 2008, followed by TVR with a biological prosthesis for residual severe tricuspid regurgitation in 2011, VVI pacemaker for iatrogenic grade III atrioventricular (AV) block in 2011 followed by lead fracture and DDD pacemaker upgrade with one lead in the right atrium and second lead in the coronary sinus in 2016. Between 2016 and 2021 the patient had several admissions for heart failure symptoms and degeneration of the tricuspid

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valve bioprothesis was diagnosed with moderate-severe regurgitation and moderate stenosis. The patient developed progressive signs of cardiac failure despite the administration of high doses of diuretics.

At the time of admission, the patient had signs of peripheral congestion and hepatomegaly. Lab tests showed hepatic failure, low albumin, thrombocytopenia, prolonged prothrombin time, and a BNP of 144 pg/mL. Repeated echocardiography showed severe stenosis of the bioprosthesis (max/medium gradient of 20/10 mmHg) and moderate-severe regurgitation. After a careful evaluation of the case by our Heart Team (which includes cardiac surgeons, a cardiologist, an interventional cardiologist, and an anesthesiologist), the patient was contraindicated for open heart cardiac surgery due to an evaluated very high surgical risk. An off-label transcatheter valve–in–valve procedure was considered and an Angio-CT was performed to confirm the feasibility. After careful clinical, biological, and anatomical evaluation, the procedure was scheduled, with the patient's consent.

The procedure was performed by transfemoral right vein approach with a percutaneous Proglide suture implanted before sheath insertion. The 16 F system delivery sheath was inserted without events. A long 0.035 regular wire was advanced with the aid of a JR catheter, passing the wire through the stenotic TV, and positioning a Pigtail catheter in the right ventricle (RV). An exchange was performed with the p of a Safari wire in the apex of the RV. A 26 mm balloon valvuloplasty catheter was then advanced and pre-dilatation was performed with good expansion of the balloon. Then the valve system was introduced into the sheath with a 180-degree counterclockwise turn by inserting the valve system with the Edwards E logo facing downward. The passage through the valve proved to be very difficult with prolapse of the guidewire in the superior vena cava (SVC) even with the use of the flex features of the system.

After several maneuvers, a 90-degree clockwise turn allowed us to obtain a loop into the RV as seen in Figure 1 (a), that stabilized the system and proved to be useful to cross with adequate alignment the degenerated valve, 3 mm below the ventricular edge of the surgical valve frame. A very slow inflation during implantation was performed. This gave us the possibility in the last part of the implant, when the valve tended to protrusion into the RV, to control the position and obtain a good final result as observed in Figure 1 (b, c). The procedure was finalized with the closure of the implanted Proglide suture. There was a good postprocedural result with no para-valvular leak and a mean gradient of 2 mmHg as observed in Figure 2. No vascular complications were encountered during and after the procedure.

Figure 1: Angiographic images during intervention: a – Loop of the system with valve; b – Valve implantation crossing the degenerated prosthesis; c – Final position of the valve-in-valve prosthesis

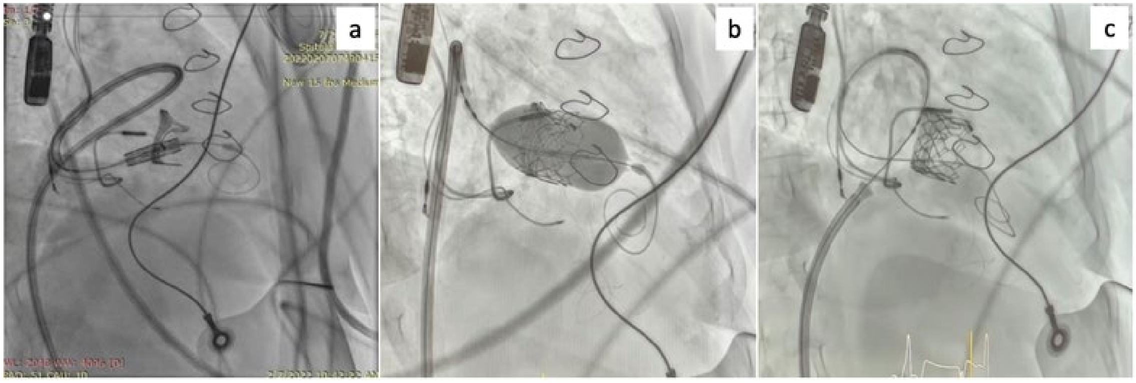

Figure 2: Echocardiographic aspect

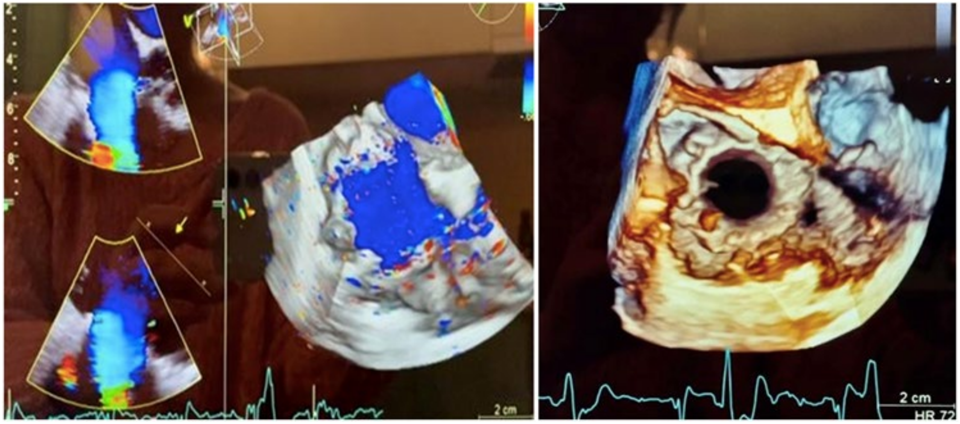

The patient had a favorable postprocedural evolution with symptom improvement. She was discharged on the 4th postprocedural day. At 1 month follow-up the patient had good clinical status (NYHA functional class II). Biologically, there was a significant decrease in natriuretic peptides and normalization of the hepatic markers. On echocardiography, there was no para-valvular leak, with a stationary mean gradient of 2 mmHg.

DISCUSSION

With the technical evolution of percutaneous valvular systems and clinically proven real benefits of transcatheter aortic valve implantation, a new treatment option has become available for patients with inoperable tricuspid valve stenosis [9]. The calcified degenerated native valves have a rough inner surface that acts as an effective anchor for the prosthesis [10]. The irregular inner surface and metal frame of a degenerated bio-prosthetic have similar characteristics and form an ideal docking station for a transcatheter valve. This has allowed valve-in-valve implants, mostly in the aortic position [11-13]. The first case of transcatheter valve-in-valve tricuspid valve replacement was described by Hon et al [14]. Their approach was through a thoracotomy and direct puncture of the right atrium [14]. This has definite advantages regarding the delivery of the device but remains more invasive. The first transcatheter tricuspid valve-in-valve procedure was performed by Leen van Garsse, aided by an extracorporeal circulation machine [15]. Today, with the aid Edwards Sapien valve system, the procedure result is similar and offers real benefits in severe clinical cases.

A successful procedure is highly dependent on the preprocedural evaluation of the patient. A good selection is mandatory in out-of-label cases. Confirmation of the tricuspid stenosis can be made by echocardiography with additional data from the trans-esophageal evaluation. A transvalvular gradient across the tricuspid valve of more than 5-10 mmHg at a heart rate of 70 beats per minute suggests severe stenosis. Gradients are affected by the patient's current hemodynamic status and evaluation needs to be performed with the patient under maximal medical therapy. Additional parameters may be evaluated: a pressure half-time of more than 190 msec, right atrial enlargement, and plethoric inferior vena cava suggest severe TS. Right ventricular performance is exceedingly difficult to assess after surgical tricuspid valvular replacement. Published data suggests that TAPSE and S' wave may be inaccurate parameters when we need to evaluate right ventricular function, at least in the first year after surgery, being highly dependent on the load volume [16]. However free wall longitudinal strain is a valid parameter even after immediate surgery. The mechanism of the prosthesis failure can be analyzed by echo and additional data on the stability of the surgical implanted prosthesis and paravalvular leak are mandatory to evaluate the procedural success. Also, clinical and echocardiographic assessment needs to eliminate the presence of acute endocarditis which is often caused by bacteria entering the bloodstream, adhering to damaged or abnormal heart tissue, and forming colonies [17-19].

The sizing of the valve is made considering three aspects: the dimensions and type of the surgically implanted valve with the aid of the ViV software developed by the Minneapolis Heart Institute Foundation, the Angio-CT sizing, and the echocardiographic findings.

As we demonstrated in this case the presence of hepatic failure appears to regress after the treatment, and this preprocedural condition should not be a contraindication.

The presence of cardiac stimulation leads may need judgment before the procedure, but several case reports suggest that the presence of a transvalvular lead in a valve-in-valve tricuspid procedure is safe and does not affect the functionality of the pacemaker. In our case, however, the ventricular lead was positioned in the coronary sinus, a position that reduced the risk of electric disturbances and paravalvular leak after the implant.

The procedure is done by general anesthesia considering the high dependency of the atrioventricular plane during positioning and implant of the valve with the respiratory activity, general anesthesia allows the control of respiratory movements with apnea if needed during the implant. Conscious sedation and local anesthesia may be considered in selected cases when good anatomy indicates a straightforward procedure.

There are three ways for valve delivery: the transfemoral, transjugular, and direct surgical trans-atrial approach. The decision is made considering the anatomy of the tri-cuspid plane: the valve is vertical, and the transfemoral approach is indicated. Nowadays, with the complex features of the systems that allow us flex movements, the trans-atrial and transjugular approaches are almost abandoned, transfemoral approach allowing good crossing and alignment in the valve [20]. The transjugular approach may remain a good option in case of inferior vena cava thrombosis. The trans-atrial approach is nowadays considered too invasive and is almost abandoned.

The transfemoral approach that we perform is a gradual one, with a first 7F approach, one Proglide suture implanted, and an 8F sheath insertion. As demonstrated in MitraClip studies [21], pre-closure of the femoral vein with Proglide suture is feasible and safe for the 14-16 F access needed for a tricuspid valve-in-valve procedure, considering the pressure and volume vein overload status before the valve treatment.

The insertion of the 16 F large system sheath is performed by gentle movements on a stiff wire, with the positioning of the sheath 5 to 10 cm below the entrance into the right atrium to allow free movement without tension of the system.

The crossing of the valve may be done with the aid of an angulated catheter (JR or vertebral catheter) with a regular J wire, a strait wire, or a hydrophilic wire depending on the degree and anatomy of the valvular complex. After the passage into the right ventricle, two final positions of the wire can be chosen: one in the apex of the RV and another one in the pulmonary artery. There are significant differences between the two positions: the RV position has a higher risk of complications by ventricular rupture and pericardial effusion but allows a better coaxially of the system in the valve with better control during the implant; the pulmonary position may give better support with the minus of poor coaxially and a higher risk of distal pulmonary perforation with pulmonary hemorrhage.

The more vertical the tricuspid plane is, the more the RV position is indicated. The positioning of the stiff wire should be made on a pigtail catheter to diminish the possible trauma on the RV muscle.

If many studies proved the benefit of a direct implant in the aortic position without pre-dilatation, in a tricuspid position during a valve-in-valve procedure we consider that pre-dilatation with a similar-sized balloon is mandatory to anticipate the behavior of the system in the valve, to facilitate the passage through the valve with the system and to anticipate the movement of the system during the gentle inflation.

As we already anticipated for the Edwards systems the insertion should be made opposite to an aortic implant so that the flex movement is possible on the left and not on the right side. This allows typical anatomies to align and cross into the valve without major difficulties. As we presented in our case prolapse of the wire into the right atrium (RA), especially in enlarged anatomies, is possible and in such case, there are two strategies to be tried: one is using a stiffer wire such as Lunderquist Extra-stiff wire by Cook Medical, eventually positioned into the PA- this strategy needs to be prepared in advance because once the system is inserted repositioning of another wire is virtually impossible. If this doesn't allow the positioning of the system in the TV the technique that we used was a gentle two-people synchronized maneuver with the return of the system in an aortic position, developing a loop with the wire in the RA and crossing the valve from the lateral wall of the RA using the loop as a stabilizer to increase pushability (a maneuver similar for the right heart catheterization by femoral approach in severe tricuspid regurgitation (TR) with the loop-technique with the Swan-Ganz catheter).

Once the system is positioned there is no need for rapid pacing during inflation, but several aspects may be considered: the inflation needs to be very slow to allow small adjustments, especially in the last part of the inflation when the rigidity of the full inflated balloon may change the position of the valve in the AV plane. Small movements of the wire and the system in slow inflations can achieve perpendicular lines on the valvular plane with a perfect result. Also, during implantation, apnea may help in maintaining the position.

We consider that transesophageal echocardiography (TEE) during the implant is mandatory to evaluate the result of the procedure, and the presence of the paravalvular leak, and evaluate complications such as pericardial effusion. There is no consensus about an antithrombotic treatment after a valve-in-valve procedure in the tricuspid position. We believe that the low regimen pressure of the right heart indicates us to consider oral anticoagulant treatment (OAC) treatment (without consideration of the presence or absence of sinus rhythm) associated with at least 6 months of clopidogrel. The regimen should be adapted to the patient, evaluating the bleeding risk. This strategy is highly debatable and further studies are needed to define the correct antithrombotic treatment.

CONCLUSION

In conclusion, tricuspid valve-in-valve implantation using the Edwards SAPIEN valve system is a feasible and effective procedure for selected patients with failing bio-prosthesis where redo open heart surgery presents considerable risk. The procedure proved a good clinical improvement for the patients in NYHA III or IV functional class at baseline, with consistent results at 1-year follow-up. Further studies are needed to analyze patient selection, implant technique, results, and antithrombotic treatment following a valve-in-valve procedure in the tricuspid position.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, H.M. and M.R.; methodology, H.M., V.A.I., A.G., E.N., G.G. and M.R.; software, A.G., E.N., A.B., A.Z., M.S.S. and A.B.; validation H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; formal analysis, H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; investigation, H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; resources, H.M., A.B., M.R. and A.B.; data curation, H.M., V.A.I., A.G., E.N., G.G. and M.R.; writing—original draft preparation, H.M., A.G., A.B., A.Z., M.S.S., M.R. and A.B.; writing—review and editing, H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; visualization, H.M., V.A.I., A.G., E.N., A.B., A.Z., M.S.S., G.G., M.R. and A.B.; supervision, H.M., V.A.I. and G.G.; project administration, H.M., G.G. and M.R; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Asymptomatic Bacteriuria – A Permanent Challenge in Clinical Practice: When Should We Treat It?

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Abstract: Asymptomatic bacteriuria (ABU) is a common finding in everyday clinical practice. Many patients, many of them with significant comorbidities, will present, when tested, with bacterial colonization of the urine even if they have no lower urinary tract symptoms at all. Therefore, the clinician is having a dilemma: Should I prescribe antibiotics to sterilize the urine or not? This article aims to update and eventually support physicians in the difficult decision of whether to treat or not to treat patients with ABS and when.

Keywords: asymptomatic bacteriuria, urinary tract infections, antibiotics, pregnant women, elderly patients

INTRODUCTION

Bacterial infections represent a serious health problem worldwide [1]. The growth of bacteria in the urine of a patient who has no symptoms is named as asymptomatic bacteriuria (ABU). ABU is quite a common finding in clinical practice and corresponds to a commensal colonization of the urinary tract [2]. Enterobacterales, B Streptococcus group, and gram-negative bacilli are often met in the urine of ABU patients [3]. Among these species, Escherichia coli, followed by Klebsiella sp, were frequently detected in ABU-urine patients [3-5]. Studies have reported ABU may potentially protect against superinfecting organisms that can determine urinary tract infections (UTIs), therefore the treatment of ABU should be offered only to selected patients. Avoiding unnecessary antibiotic treatment in a significant number of patients with ABU is highly likely to avoid the potential risk of acquired antimicrobial resistance and the risk of removing a potentially effective defense mechanism [4,5]. On the other hand, in some categories of patients, the treatment of ABU proved to be beneficial, avoiding possible serious complications, some of them life-threatening like urosepsis. ABU is quite common in everyday practice. It can be identified in healthy pre-menopausal women(1-5%), with the incidence increasing to 4-19% in postmenopausal women. The ABU varies between 0.7 and 27% in diabetic patients, up to 10% in pregnant patients, and impressive rates varying between 15 and 50% are found in patients living in care homes, and in those who have had spinal cord injuries between 23 and 89% [6]. The bacteria identified in patients with ABU are not different than those found in patients with symptomatic uncomplicated or complicated UTIs.

DISCUSSIONS

ABU diagnosis

By definition, ABU is characterized by a mid-stream urine sample (MSU) culture with a bacterial growth of more than 105 colony-forming units/mL in a patient without any urinary tract symptoms. Two consecutive samples should be positive in female patients and only one in males [7,8].

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No further investigations are required if the history is unremarkable. However, if the urine cultures confirm the presence of urease-producing bacteria, an ultrasound of the kidneys and urinary tract might be performed to rule out the urinary tract stones [8,9]. Digital rectal examination (DRE) needs to be performed in men to exclude the risk of prostate pathology, either infectious or benign/malignant. In selected cases, cystoscopy, either flexible or rigid, can offer a significant diagnostic or sometimes even treatment benefit when bladder wash-out, biopsies, or bladder stone laser disintegration is needed.

ABU management in patients with no identified risk factors

Studies have shown that ABU by itself in patients without any risk factors does not cause any urinary tract infections, or renal disease and does not determine acute or chronic pyelonephritis that can potentially alter the kidney function [10].

One study investigated the value of antibiotic treatment in adult, non-diabetic, non-pregnant women with ABU the incidence of symptomatic UTI was similar in both treated and not treated groups[4]. It was concluded that screening for ABU and the antibiotic treatment of ABU are not routinely recommended in patients with no risk factors and no lower urinary tract symptoms.

A study published in 2012 reviewed the ABU treatment in women with recurrent symptomatic UTIs otherwise healthy and demonstrated an increased risk of symptomatic UTIs in treated patients, compared to non-treated ones. This observation proves that ABU can play a prophylactic role in female patients with recurrent UTIs. Hence the treatment of ABU is not recommended in patients with no risk factors[5].

ABU management in pregnant women

ABU is present in pregnant women in between 2 to 10% of cases. Many studies were dedicated to this category of complex patients when potentially both the mother and the fetus can be affected.

A couple of RCTs [9,10] have been performed on pregnant women trying to identify if ASB treatment is needed or not. Those RCTs compared the groups with ABU treated with antibiotics versus placebo and versus no-treatment patients, also using various antibiotics and regimens. Therefore, these trials reported that the antibiotic treatment has significantly reduced the number of symptomatic UTIs in this group, versus placebo or no treatment ones. Another conclusion of these trials was that the ABU treatment has significantly decreased the rates of low birth weight and the rates of preterm delivery.

Other authors have remarked that the ABU treatment in pregnant women induces decreased incidence of symptomatic UTIs, pyelonephritis, and low birthweight or preterm delivery [11-14]. In conclusion, it seems that it is beneficial for both mother and fetus if the screening for ABU is used in pregnant women and, if bacterial growth is found in the urine, the patients should be subsequently treated appropriately. On the other hand, considering the variety and complexity of pregnant women as patients, it is always advisable to consult national guidelines and recommendations but also to consider each patient on a case-by-case basis.

As it seems that the ABU treatment in pregnant women is generally accepted, another question is which antibiotics and for how long should be used in pregnancy. A significant number of randomized clinical trials have been performed trying to answer these questions. [11-21]. These trials essentially compared different antibiotics, different regimens, or the same antibiotic prescribed in different doses or various durations. Many antibiotics have been used including mainly Nitrofurantoin, Sulphamethopirazine, Pivmecillinam, Amoxicillin, Amoxicillin associated with Clavulanic Acid, Cephalexin, Co-trimoxazole, Fosfomycin [13-15, 17, 18]. Different treatment groups were identified and used for comparison when the treatment length was considered: single dose; short course varying from 2 days to 7 days); long course considered between 8 and 14 days; and long-term course until delivery [17-20]. The data showed that single-dose treatment determined fewer side effects but more babies with low birth weight compared with the short course of treatment.

The value of Fosfomycin single dose was reviewed in a meta-analysis which compared the use of it in pregnant women with uncomplicated UTIs or ABU versus short-term courses of treatment with various antibiotics. The successful treatment of ABU was found to be not significantly different between the patients who received short-term courses of antibiotics, whatever the antibiotic used was. It was also apparent that both standard short-course treatment with various antibiotics and single dose Fosfomycin could be effective options in treating ABU in pregnant patients; however, further studies are needed [21,22].

ABU management in patients with identified risk factors- Diabetes Mellitus

If in 2019, worldwide 463 million people were diagnosed with diabetes mellitus (DM), in 2045 the number can increase to 700 million [23]. Long-term hyperglycemia induces several side effects affecting the eyes, nervous system, kidneys, cardiovascular disorders, [24], and periodontal disease [25]. Therefore, the diabetic patient has an increased risk of developing ABU. Even if diabetes is well controlled with the appropriate medication and diet, a significant proportion of cases with ABU [26]. A randomized control trial was addressed to these patients and found that the ABU treatment, with consecutive negative mid-stream urine, did not reduce the risk of symptomatic UTIs or pyelonephritis. On the other hand, the lack of ABU antibiotic treatment did not correlate with an increased risk of developing diabetic nephropathy [27]. It is now accepted that the ABU screening and treatment in well-controlled DM patients is not routinely recommended.

However, poorly controlled diabetes represents a significant risk factor for various infectious complications. In these patients, the doctors and nurses should make every effort to get the diabetes controlled using both medication and diet. If ABU and infectious complications are present, sounds sensible to not only treat the ABU but also further investigate these patients to rule out urological causes.

ABU management in patients with identified risk factors – Postmenopausal women

Another category of patients who have an increased incidence of ABU is represented by postmenopausal women. Randomized control trials were addressed to these patients and compared the groups with ABU antibiotic treatment vs the placebo or no treatment groups, using different antibiotics and regimens [28]. There were no reported benefits of antibiotic treatment regarding both the incidence rate of symptomatic UTIs and the incidence of bacteriuria resolution [29, 30]. Therefore, as currently there is no demonstrated benefit of ABU antibiotic treatment in post-menopausal women, ASB should be managed the same in both pre-menopausal and post-menopausal women and the screening and treatment of ABU are not recommended.

ABU management in patients with identified risk factors –Elderly/institutionalized patients

Studies have revealed that in elderly institutionalized patients, the rate of ABU is significantly elevated, varying between 15% to 50% [4,5]. Considering that the differential diagnosis between ABU and UTIs is extremely difficult or even impossible in bed-bound, poor mobility patients who also have multiple comorbidities and are potentially mentally deteriorated, it seems that unfortunately unnecessary antibiotic treatments are prescribed in a significant number of patients [30, 31].

An important number of randomized control trials were addressed to the elderly patient population (either institutionalized or not). They were divided into three groups and the group in whom they were treated with antibiotics (different antibiotics, different doses, and regimens were prescribed), was compared with placebo controls or no-treatment groups [30-34]. These RCTs reported that the ABU treatment did not show any significant benefit, compared to placebo or no treatment groups, in reducing the rate of symptomatic UTIs. Also, there was no reported benefit of the antibiotic treatment versus placebo in obtaining a persistently negative MSU culture and sensitivity. One randomized control study compared the incidence of urinary incontinence before and after the resolution of ABU and found no effect in the group of patients who were treated with antibiotics. Even more, other studies reported that the antibiotic treatment of ABU determined significant side effects without any clinical benefit [34]. Therefore, based on the current scientific literature, we can conclude that the screening and treatment of ASB are not recommended in this group.

ABU management in patients with identified risk factors - Renal Transplant Patients

Renal transplant patients represent a very important category, as they are complex patients with associated pathology and sophisticated medication frequently [35-38]. A meta-analysis including randomized control trials and retrospective studies compared the effect of antibiotic treatment to no treatment in renal transplant patients and did not find any benefit in decreasing the risk of symptomatic UTIs between 12 and 22 months after transplant. No benefit was reported in ABU resolution, graft loss, or renal function preservation for up to 24 months [38].

Other publications reported that the incidence of UTIs and pyelonephritis (15% vs. 2.5%) was higher in patients receiving antibiotic treatment for ABU compared to no treatment group [37]. In another RCT, in the first year after transplantation, no difference in the acute graft pyelonephritis was found, but, not surprisingly, the incidence of antimicrobial resistance was higher in the treatment group [36]. Therefore, routine screening and treatment are not routinely recommended in patients who had renal transplantation.

ABU management in patients with identified risk factors – Patients with dysfunctional or reconstructed lower urinary tract

Patients with dysfunctional or reconstructed lower urinary tracts become frequently colonized having ABU [39, 40]. A study reported that the incidence of ABU varies from 25 to 86% for intestinal urinary conduits and from 9.1 to 85% for orthotopic neobladders. It has demonstrated no long-term benefit of ABU treatment in this group [40]. Interestingly, the deliberate colonization with an ABU strain (Escherichia coli 83972) has shown a protective effect against symptomatic UTI recurrences [41]. ABU screening and treatment in these patients is therefore generally not recommended.

ABU management in patients with identified risk factors –Patients with urethral/suprapubic catheters, nephrectomies, or ureteral stents

Patients with urethral or suprapubic catheters and nephrostomy tubes routinely become colonized with bacteria and is generally accepted nowadays that antibiotic treatment is not needed as determines no benefit. No screening for ABU or antibiotic treatment is recommended for patients with indwelling ureteral stents. Hence routine antibiotics for ABU are not recommended, as offer no benefit and determine a significant risk of acquired antibiotic resistance.

In patients who need routine urethral/suprapubic catheter changes, there is no need to be screened for ABU or to have antibiotics. On the other hand, in patients subjected to nephrostomy tube replacement or ureteral stent change, ABU is a risk factor, and therefore prophylactic antibiotics or treatment of ABU are needed [42,43].

ABU management in patients with identified risk factors – Chronic Kidney Disease (CKD)

Worldwide, kidney failure represents another serious complication, especially among diabetic patients [44]. Studies reported an incidence between 9-20% of UTI among female DM patients, while for male DM patients, the incidence is between 3-11% [45].

Patients with CKD are another group in whom ABU can be present. There is no study to investigate if ABU is identified in patients with CKD needs to be treated or not. As it seems that in this group there is an increased risk of antimicrobial resistance, highly likely no routine screening and treatment is needed [46]. This physician should not forget that, if treatment is needed, then it will be necessary to prescribe the antibiotics in renal dose, considering the patients' kidney function test.

ABU Treatment options

If the clinician decides to treat ABU, the same antibiotics, regimens, and duration as in uncomplicated UTIs can be offered, depending on multiple characteristics like gender, co-morbidities, risk factors, allergy status, other medication the patient is taking, potential medication interactions, etc. Treatment should be always tailored on a case-by-case approach based on mid-stream urine culture and sensitivity and should not be empirical.

CONCLUSION

ABU is a clinical entity frequently found in everyday practice. Despite this, there are only a few indications to treat it. Unfortunately, even though the guidelines are clear, inappropriate antibiotic treatment is still offered and determines antimicrobial resistance and selection of extremely aggressive uropathogens. As we presented above, the screening and the antibiotic treatment of ABU are recommended only in pregnant women and in selected individuals who will have endourological procedures that can potentially determine the urothelial mucosa trauma. It remains that future studies could identify alternative methods and treatments, to better manage the patients with asymptomatic bacteriuria.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, F.L.T.M and I.A.V., methodology, A-R.M., D.E.G., M.R.M., and A.E.B-S., software, A.M.S., M.R.M.., I.A.V., and F.L.T., validation, I.A.V., and M.R.M.., formal analysis, F.L.T., A-R.M., D.E.G., A.M.S., and A.E.B-S., investigation, F.L.T., I.A.V., and M.R.M., resources, F.L.T., and I.A.V., data curation, A-R.M., D.E.G., and A.M.S writing—original draft preparation F.L.T.M and I.A.V., writing—review and editing, F.L.T.M, D.M., A.E.B-S., and I.A.V., visualization, F.L.T.M and I.A.V.; supervision, I.A.V., and D.M.; project administration F.L.T.M and I.A.V.; funding acquisition, F.L.T.M. All authors have read and agreed to the published version of the manuscript.

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REVIEW

Conventional and Modern Methods in the Diagnosis of Sepsis and Septic Shock: A Narrative Review

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Abstract: Background/Objectives: Sepsis and septic shock are critical conditions associated with high mortality rates and substantial impacts on healthcare systems. Accurate and rapid diagnosis is essential for the management of these conditions. The objective of this study is to assess the accuracy of contemporary and traditional methods for diagnosing sepsis and to determine whether improvements have been made concerning the integration of novel diagnostic approaches, to facilitate a prompt diagnosis, taking into account the rapid progression of complications associated with this disease. For this purpose, studies published between 2014 and 2024 were examined to highlight the benefits and limitations of each approach. Methods: A systematic literature review was conducted, including randomized clinical trials, observational studies, and retrospective studies assessing both conventional diagnostic methods (blood cultures and clinical scoring systems) and modern methods (rapid molecular tests, specific biomarkers, and machine learning algorithms). The studies included were selected based on strict design and methodology criteria to ensure a rigorous comparative evaluation of the interventions and technologies used in diagnosing and monitoring patients with sepsis. Results: A total of 23,822 patients were reviewed across the studies included in this systematic analysis. Modern methods, such as continuous monitoring through integrated biosensors and the use of molecular panels for pathogen detection, demonstrated high potential for the early and accurate diagnosis of sepsis. The reviewed studies suggest that these methods can significantly reduce diagnostic time and improve the ability to stratify mortality risk compared to conventional methods. Conclusions: Integrating modern diagnostic technologies, such as rapid pathogen identification tests and specific biomarkers, may complement traditional methods and bring significant benefits in the management of sepsis.

Keywords: sepsis; septic shock; diagnostic methods; biomarkers; rapid pathogen detection; machine learning; clinical outcomes; intensive care; advanced monitoring; mortality prediction

INTRODUCTION

Sepsis and septic shock are critical medical conditions that occur as a systemic inflammatory response to severe infections and have a devastating impact on global public health [1,2]. These conditions are considered medical emergencies, as they can quickly lead to multiple organ dysfunction and death, with mortality rates reaching up to 40% in severe cases [1,3].

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Sepsis is triggered by the presence of pathogenic microorganisms, such as bacteria, viruses, or fungi, which activate a complex and often excessive immune response [4]. The initial immune response to infection involves the activation of macrophages and other immune cells, which release cytokines and other inflammatory substances [5]. These proinflammatory molecules play a crucial role in defending the body; however, in sepsis, this response becomes disproportionate, damaging tissues and organs [6]. This excessive activation leads to vasodilation, increased vascular permeability, microvascular dysfunction, and, ultimately, multiple organ failure [7].

In essence, sepsis represents an overreaction of the immune system to an infection which, rather than protecting the body, causes extensive tissue and organ damage [8]. When this reaction escalates, resulting in a severe drop in blood pressure and inadequate perfusion of vital organs, septic shock occurs—an extreme form of sepsis that requires urgent and complex therapeutic interventions to prevent patient death [9].

The challenge of sepsis is compounded by the variability of symptoms, which can differ significantly from one patient to another, and the difficulty in quickly identifying the responsible infection [10]. This clinical heterogeneity makes timely diagnosis of sepsis challenging, contributing to its high mortality rate [11]. The fact that sepsis presents with symptoms common to other conditions—fever [12], tachycardia [13], rapid breathing [14], and low blood pressure [15]—can delay intervention.

In light of the aforementioned evidence, it can be surmised that the specific manifestations of sepsis fall within the differential diagnosis of other pathologies. In such cases, the therapeutic approach to sepsis (volemic repletion) may precipitate a worsening of the patient's condition if the underlying diagnosis is not correctly identified. This could potentially lead to delays in establishing an accurate diagnosis and initiating appropriate treatment. It is therefore imperative to identify more rapid and efficient diagnostic methods, to save lives and reduce the significant costs associated with the care of patients with sepsis and septic shock.

MATERIALS AND METHODS

Given the significant impact of sepsis and septic shock on public health and the limitations of current diagnostic methods, this study aims to identify the efficacy and clinical relevance of conventional diagnostic methods and modern approaches. This comparison will be conducted through a systematic review of the literature from 2014 to 2024, providing insights into the benefits, limitations, and potential of each clinical approach.

The study will synthesize evidence regarding the accuracy of diagnoses obtained through conventional methods, such as blood cultures and modern methods, including advanced molecular tests and specific biomarkers associated with sepsis. Additionally, it will examine the practical aspects of integrating these methods into clinical practice, with a focus on their potential to reduce diagnostic time and improve patient prognosis.

By highlighting how recent innovations can complement or even replace traditional methods, the study will provide a solid evidence base to support informed clinical decision-making. Ultimately, this review aims to contribute to the discussion on the broader integration of modern diagnostic methods within healthcare systems and to support the development of standardized practices that incorporate these advanced technologies in the management of sepsis and septic shock.

The methodology for this systematic review has been designed in accordance with the PRISMA guidelines to ensure transparency and rigor in the search, selection, and analysis process. This approach facilitates a detailed and comparative assessment of conventional and modern methods used in the diagnosis of sepsis and septic shock [16].

Article Identification Strategy

In our search strategy, we explored renowned international databases to obtain a representative and up-to-date selection of relevant scientific articles. The primary databases included MDPI and PubMed, as these provide access to a wide range of clinical studies, research articles, and other academic sources addressing the diagnosis of sepsis and septic shock.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were rigorously established to maintain the quality and relevance of selected articles. Only studies that include specific diagnostic methods for sepsis, whether conventional or modern, were included. Additionally, only articles published in peer-reviewed journals with complete data available were selected for analysis to ensure a robust data foundation and minimize publication bias.

Regarding exclusion criteria, review articles, opinion pieces, case reports, and studies lacking clinical validation were excluded. These types of publications do not provide direct comparative data or are not based on systematic evaluations of diagnostic methods, and their inclusion would have distorted the analysis results.

To ensure transparency and traceability in the selection process, we included a PRISMA flow diagram in the study, detailing the number of studies identified and filtered at each stage. The diagram specifically illustrates the initial number of articles obtained from the search, the number excluded after reviewing titles and abstracts, and finally, the number included in the detailed analysis. The reasons for excluding articles are also specified to clarify why certain studies were not included in the final analysis.

The PRISMA diagram is an essential tool for transparency, as it enables readers to follow the decision-making process behind article selection, ensuring that the criteria established at the beginning of the review are adhered to [16,17].

Keywords and Boolean operators were used in the search process to refine the results and select only articles relevant to the study objectives. The main search terms included phrases such as “sepsis diagnosis” (29,700 results), “septic shock” (21,800 results), “conventional methods sepsis and septic shock” (17,300 results), and “modern methods sepsis and septic shock” (17,200 results). These terms were combined using the AND, OR, and NOT operators to ensure a highly precise selection.

The timeframe set for the included articles was 2010–2024 to reflect recent developments in sepsis diagnosis and ensure the relevance and currency of the data analyzed.

Figure 1: PRISMA Flow Diagram of Articles Related to the Diagnosis of Sepsis and Septic Shock

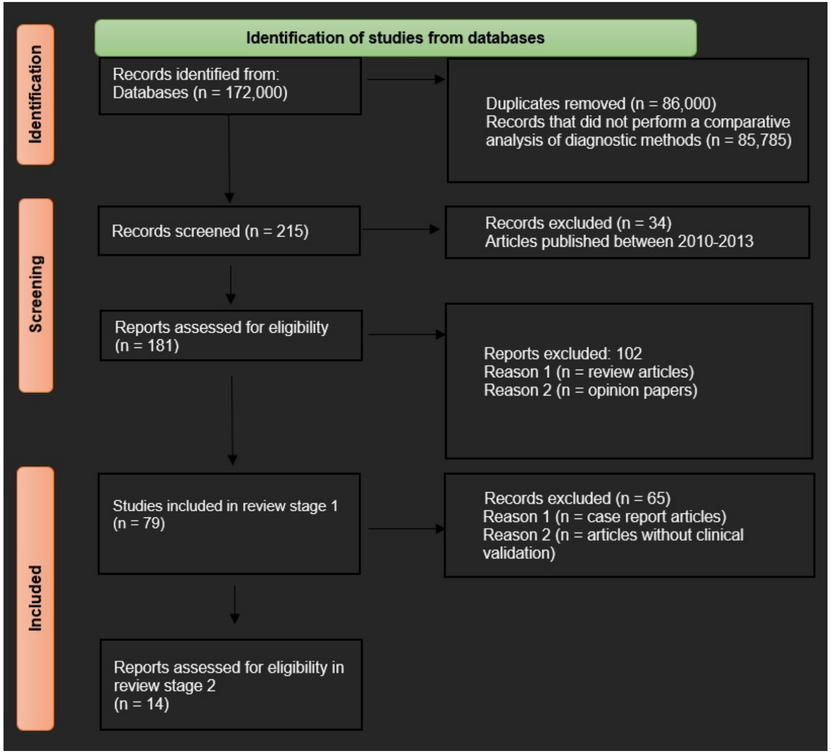
Identification of studies from databases
Identification
Records identified from: Databases (n = 172,000)
Duplicates removed (n = 86,000)
Records that did not perform a comparative analysis of diagnostic methods (n = 85,785)
Screening
Records screened (n = 215)
Records excluded (n = 34)
Articles published between 2010-2013
Reports assessed for eligibility (n = 181)
Reports excluded: 102
Reason 1 (n = review articles)
Reason 2 (n = opinion papers)
Included
Studies included in review stage 1 (n = 79)
Records excluded (n = 65)
Reason 1 (n = case report articles)
Reason 2 (n = articles without clinical validation)
Reports assessed for eligibility in review stage 2 (n = 14)

In Figure 1, we can see that the study identification process from the databases began with the selection of two main databases. In the course of this process, a total of 172,000 studies were identified. Of these, 86,000 records were eliminated as duplicates, as well as a further 85,785 records that did not perform a comparative analysis of diagnostic methods. Following these initial exclusions, 215 records remained for screening.

Of these, 34 were excluded after the selection process. These were articles published before 2014. We chose a period of 10 years rather than 14 because the literature on sepsis is constantly changing and the period 2010-2013 seemed outdated for today's requirements. A total of 181 reports were assessed for eligibility. At this stage, reports were excluded for several reasons, including review articles, and opinion pieces.

In the end, 79 were included in the phase 1 review, with studies without clinical validation and case reports being discarded. 14 studies were selected for inclusion in stage 2 of the review.

RESULTS

Two distinct groups of modern and conventional methods were generated. The conventional methods included: basic immunophenotyping, cardiac function monitoring and histopathology, manual assessment of CRT, serum lactate, MAP, vasopressor use, standard intravenous fluid administration, ICD-9 coding for sepsis, clinical scores (e.g., SOFA, APACHE II, SAPS II/III), standard biomarkers (CRP, PCT, lactate), and blood clutches and antibiogram. Modern methods included: PAR2 receptor inhibition with 1-PPA, proteomic analysis (2D-nanoUPLC-UDMSE), restrictive intravenous fluid administration, SOFA score and albumin combination, NLR, PLR, MLR, PLT/MPV ratio, AR stimulation (PediSepsisAR), remote photoplethysmography (rPPG) and aCRT, T2Bacteria® panel and T2MR®, continuous monitoring with biosensors (PO, NIRS, ST), advanced biomarkers (e. (e.g. IL-6, suPAR, irisin), ML (machine learning) algorithms.

In Table 1, we present a detailed analysis of 14 studies, each examining innovative interventions, biomarkers, and technologies used in the diagnosis and management of sepsis and septic shock. Data were extracted from recently published articles and structured according to study design, type of intervention, methodology, and key results.

Table 1: Comparison of Conventional and Modern Diagnostic Methods for Sepsis and Septic Shock

Author(s) and Year	Study Design	Sample	Intervention	Intervention Duration	Conventional Methods	Modern Methods	Key Results	Conclusions
Meyhoff et al., 2022 [18]	International, randomized, open-label study (CLASSIC trial)	1554 ICU patients with septic shock	Restriction of IV fluids vs. standard fluids	Until ICU discharge (max. 90 days)	Standard IV fluid administration according to guidelines	Restrictive IV fluid administration	Similar mortality between groups: 42.3% (restrictive) vs. 42.1% (standard). Similar incidence of adverse events: 29.4% (restrictive) vs. 30.8% (standard). Similar life support-free days and out-of-hospital days between groups	Fluid restriction did not improve survival rates compared to standard therapy. Fluid restriction did not reduce the incidence of severe adverse events. Restrictive intervention showed no significant benefits over standard intervention in the ICU.
Liberski, Szewczyk, & Krzych, 2020 [19]	Retrospective case-control study	138 ICU patients	Evaluation of NLR, PLR, MLR, PLT/MPV ratios vs. CRP, PCT, and lactate	On ICU admission	CRP, PCT, lactate	NLR, PLR, MLR, PLT/MPV	High NLR has predictive value for septic shock (AUROC = 0.66); combining NLR + CRP improves septic shock prediction (AUROC = 0.88)	NLR can identify patients with septic shock but must be interpreted alongside CRP/PCT; no index predicts ICU mortality
Toto et al., 2021 [20]	Randomized controlled feasibility study	50 paediatric participants	Simulation of septic shock with and without AR (PediSepsisAR)	Short-term simulation (per exercise)	Traditional mannequin simulation	AR simulation (PediSepsisAR)	Fluid administration time and volume were measurable in both groups; no significant differences in patient status recognition or fluid administration.	PediSepsisAR is feasible for simulation; improved perception of perfusion in most participants, but no impact on response times.

Wang et al., 2023 [21]	Retrospective bioinformatics study	98 sepsis patients (GSE26440) and 32 control; external test: 51 sepsis patients, 22 control	Diagnostic model using PCD-associated genes	Retrospective, pre-existing data	SOFA score and traditional inflammatory biomarkers	ML algorithms (RF, SVM, DNN) for gene-based diagnosis	ML models based on 10 genes (e.g., ITGAM, KIF1B) achieved AUC=0.7951 (internal) and AUC=0.9627 (external); analysis of immune cell infiltration in sepsis	Gene-based ML models show high diagnostic capacity for sepsis; potential to improve diagnosis and identify therapeutic targets
Lungu et al., 2024 [22]	Longitudinal observational study	121 neonates (39 late-onset sepsis, 35 early-onset sepsis, 47 control)	Multimodal monitoring (pulse oximetry - PO, near-infrared spectroscopy - NIRS, skin temperature - ST) with biosensor signal integration through ML algorithm	Max 72 hours of continuous monitoring	Standard biomarkers (CRP, PCT), cultures	Continuous monitoring with PO, NIRS, ST, ML algorithm	The model had 87.67% accuracy and detected sepsis 6-48 hours before clinical diagnosis; NIRS and ST had the highest predictive accuracy (0.90 and 0.85)	Non-invasive monitoring methods (NIRS, PO, ST) significantly improve early neonatal sepsis detection, complementing conventional tests and reducing the potential for clinical deterioration; require validation in varied clinical settings
Pipitò et al., 2024 [23]	Retrospective study	15,373 sepsis patients admitted to hospitals in Sicily between 2016-2020	Retrospective data collection from standard discharge forms for all sepsis cases in hospitals	5 years	ICD-9 for coding and identifying sepsis and septic shock	Temporal trend analysis through regression	Overall in-hospital mortality was 36.3%, higher in septic shock cases (66.7%) and ICU patients (74%). Rates increased over the analyzed years.	Sepsis and septic shock significantly contribute to in-hospital mortality; and require a focus on infection prevention, antimicrobial stewardship, and improved diagnostic coding.

Luka et al., 2024 [24]	Prospective observational study	67 sepsis and septic shock patients, ED, Cluj-Napoca, Romania	Evaluation of 6 biomarkers (IL-6, suPAR, AZU1, hsCRP, sTREM-1, PCT) combined with severity	28 days	Severity scores: SOFA, APACHE II, SAPS II/III, NEWS, CCI, GCS, qSOFA, SIRS, MEDS	Advanced biomarkers (IL-6, suPAR, AZU1) combined with clinical scores	Mortality was 49.25%; IL-6 was the most accurate biomarker, and SAPS II/III were the most predictive scores for mortality	IL-6 and SAPS II/III are the best predictors of 28-day mortality in ED sepsis cases; using biomarkers with clinical scores improves prognostic accuracy
Klibus et al., 2024 [25]	Prospective observational study	20 patients with bacterial septic shock or COVID-19-associated sepsis, ICU, Latvia	Evaluation of microcirculation via remote photoplethysmography (rPPG) and automated capillary refill time (aCRT) analysis in infusion	Various stages during the protocol	MAP, manual CRT, serum lactate levels, skin temperature	rPPG, aCRT	rPPG and aCRT detected perfusion changes more sensitively than manual CRT, highlighting significant differences between bacterial and COVID-19-associated sepsis	rPPG and aCRT are promising tools for microcirculation monitoring in intensive care, providing more precise assessments than conventional methods, useful for sepsis patient management
Bonura et al., 2024 [26]	Retrospective observational study	85 patients with septic shock or sepsis-induced hypotension, ICU	Evaluation of T2 Magnetic Resonance (T2MR®) technology for early ESKAPE pathogen detection using T2Bacteria®	First 6 hours after sepsis onset	Blood cultures and antibiogram	T2Bacteria® Panel for rapid bacterial identification	81% concordance rate between T2Bacteria® and blood cultures; T2Bacteria® detects pathogens in 5.15 hours vs. 94.62 hours for traditional cultures	T2Bacteria® Panel provides rapid and concordant ESKAPE pathogen detection, allowing faster decisions on antibiotic escalation or de-escalation in severe sepsis cases

Ayenew Mekuria et al., 2024 [27]	Retrospective cohort study	386 ICU septic shock patients	Septic shock management in ICU	2019-2023	Vasopressors (adrenaline, dopamine)	ICU surveillance, continuous monitoring	Mortality rate of 58.29%; predictive factors: age >60 years, delayed ICU admission, low MAP, comorbidities, unidentified pathogen	ICU mortality for septic shock remains high; need for early recognition and treatment protocols, in line with sepsis survival guidelines
Karampela et al., 2024 [28]	Prospective observational study	102 critical patients with sepsis or septic shock, 102 healthy control	No specific intervention, comparative analysis	28 days	CRP, procalcitonin as standard biomarkers	Serum irisin determination via ELISA	Patients with low circulating irisin had significantly higher 28-day mortality; irisin was negatively associated with sepsis severity and APACHE II and SOFA scores.	Circulating irisin decreases at sepsis onset and could be a promising biomarker for prognosis and diagnosis; needs confirmation in larger studies.
Alves et al., 2024 [29]	Observational proteomic study	5 septic shock patients, 10 healthy control	No specific intervention	N/A (not applicable)	Monocyte immunophenotyping	Proteomic analysis via 2D-nanoUPLC-UDMSE	Identified 67 proteins with altered expression in septic shock patients' monocytes, linked to immune dysfunction, hypotension, vasodilation, and endothelial damage	Identified proteins could serve as biomarkers for diagnosis, prognosis, and treatment of septic shock, offering new insights into this condition's pathophysiology.
Kim et al., 2024 [30]	Retrospective observational study	5805 septic shock patients from 12 EDs in South Korea	No specific intervention	N/A (not applicable)	Initial SOFA assessment	Combination of SOFA with serum albumin level	28-day mortality inversely proportional to albumin level; SOFA with albumin improved mortality prediction (AUC 0.71 vs. 0.68 SOFA alone)	Combining initial SOFA score with albumin level may enhance prognostic accuracy, providing a risk stratification tool in septic shock

Luisetto, Scarpa, Villano, Martini, et al., 2024 [31]	Experimental study, in vitro and in vivo	Mouse model of LPS-induced endotoxemia	Administration of 1-PPA molecule to inhibit PAR2 receptor	1 and 3 hours post-LPS injection (early and delayed administration)	Survival monitoring, cardiac function analysis, histopathological assessments	Real-time PCR, Western blot, cardiac ultrasound, flow cytometry	1-PPA administration reduced mortality and sepsis symptoms, improved cardiac function, and decreased inflammation via PAR2 inhibition	1-PPA demonstrates efficacy in reducing sepsis effects by blocking PAR2, preventing pro-inflammatory pathway activation, and reducing multiorgan dysfunction

*ICU: Intensive Care Unit; CRP: C-Reactive Protein, an inflammatory marker; PCT: Procalcitonin – Procalcitonin, a specific biomarker for bacterial infections; NLR: Neutrophil-to-Lymphocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; MLR: Monocyte-to-Lymphocyte Ratio; PLT/MPV: Platelet to Mean Platelet Volume Ratio; AR: Augmented Reality; PediSepsisAR: AR simulation for paediatric septic shock recognition; PCD: Programmed Cell Death; ML: Machine Learning; RF: Random Forest classification algorithm; SVM: Support Vector Machine classification algorithm; DNN: Deep Neural Network; AUC: Area Under the Curve; PO: Pulse Oximetry; NIRS: Near-Infrared Spectroscopy; ST: Skin Temperature; ICD-9: International Classification of Diseases, 9th Revision; AUROC: Area Under the Receiver Operating Characteristic; IL-6: Interleukin-6, an inflammatory biomarker; suPAR: Soluble Urokinase Plasminogen Activator Receptor; AZU1: Azurocidin 1 – Protein associated with inflammation and immune response; sTREM-1: Soluble Triggering Receptor Expressed on Myeloid Cells-1 – Triggering Receptor on Myeloid Cells-1; MAP: Mean Arterial Pressure – Mean Arterial Pressure; CRT: Capillary Refill Time; rPPG: Remote Photoplethysmography; aCRT: Automated Capillary Refill Time; T2MR®: T2 Magnetic Resonance; CCI: Charlson Comorbidity Index; GCS: Glasgow Coma Scale; NEWS: National Early Warning Score; qSOFA: Quick Sequential Organ Failure Assessment; SIRS: Systemic Inflammatory Response Syndrome; MEDS: Mortality in Emergency Department Sepsis; 2D-nanoUPLC-UDMSE: Two-dimensional Ultra Performance Liquid Chromatography-Ultimate Data-Independent Mass Spectrometry Enhancer – Advanced proteomic technology for monocyte analysis; ELISA: Enzyme-Linked Immunosorbent Assay – Enzyme-Linked Immunosorbent Assay, used for irisin determination; PAR2: Protease-Activated Receptor 2 – Protease-Activated Receptor 2, associated with inflammation.

The analyzed studies included ICU patients who were assessed and monitored for sepsis and septic shock using both conventional and modern methods. These studies comprised randomized, observational, retrospective, and prospective designs, with data covering clinical variables such as mortality, incidence of adverse events, length of hospital stay, and response to various interventions.

The interventions analyzed included techniques for intravenous fluid administration, the use of inflammatory biomarkers (NLR, PLR, MLR, CRP, PCT, lactate), machine learning algorithms for continuous monitoring (e.g., photoplethysmography, capillary refill time analysis), and gene expression-based models for diagnostics (e.g., using a panel of PCD genes in machine learning algorithms). Each study employed specific statistical analysis methods and regression models to evaluate the effect of these interventions on mortality and clinical outcomes of patients.

For biomarkers and continuous monitoring, predictive analysis methods were used, combining clinical scores (SOFA, APACHE II, SAPS II/III, qSOFA) with advanced biomarkers (IL-6, suPAR, sTREM-1). Additionally, rapid molecular technologies for pathogen identification, such as the T2Bacteria® panel using magnetic resonance, were utilized to enable quick identification of infectious agents.

The analysis also included proteomic diagnostic technologies that identified altered proteins in septic shock, used as potential biomarkers for diagnosis and prognosis. Experimental studies also involved animal models to assess the efficacy of interventions such as blocking inflammatory receptors (e.g., PAR2) with specific molecules aimed at reducing sepsis effects by preventing organ dysfunction.

In Table 1, we also observe that recent studies emphasize the importance of personalized approaches and advanced monitoring in sepsis and septic shock, incorporating both conventional clinical interventions and innovative methods. Although fluid restriction and standard biomarkers have shown limitations in predicting and managing sepsis effectively, emerging technologies like monitoring through machine learning algorithms, rapid diagnostics with molecular panels, and the use of specific biomarkers, such as irisin, offer promising perspectives for improving diagnostic and prognostic accuracy. However, further studies are recommended to validate these methods across various clinical settings and to implement them into intensive care protocols.

In terms of conventional methods, the most frequently used are clinical scores (e.g. SOFA, APACHE II, SAPS II/III) and standard biomarkers (CRP, PCT, lactate). These are each mentioned four times across studies, which serves to underscore their central role in traditional clinical practice.

Other conventional methods, such as blood cultures and antibiograms, appear twice, while standard intravenous fluid administration, ICD-9 coding for sepsis, vasopressor use, and cardiac function monitoring are each mentioned only once.

The high frequency of clinical scores (e.g., SOFA, APACHE II, SAPS II/III) and standard biomarkers (CRP, PCT, lactate) in traditional clinical practice is because these methods are well-validated, accessible, and easy to apply across various clinical settings. These tools are an integral part of international guidelines for the diagnosis and monitoring of sepsis and septic shock, with widely recognized utility in assessing patient severity and risk stratification [32,33].

Conversely, less frequently used conventional methods, such as blood cultures and antibiograms, standard intravenous fluid administration, ICD-9 coding for sepsis, vasopressor use, and cardiac function monitoring, are employed less often for various reasons. Blood cultures and antibiograms are useful for identifying pathogens but require a long time to yield results, making them less practical in emergencies. ICD-9 coding is important for epidemiological data collection but has a limited impact on immediate patient care.

Therefore, the more frequent use of clinical score assessment and standard biomarkers is due to their ability to provide rapid and reliable information about the patient's condition, making them suitable for initial assessment and real-time monitoring of patient progress.

On the other hand, among modern methods, the use of advanced biomarkers (e.g., IL-6, suPAR, irisin) has the highest frequency (3 mentions), followed by machine learning algorithms and continuous monitoring with biosensors (e.g., PO, NIRS, ST), each with two mentions. Other modern methods, such as molecular diagnostic panels (T2Bacteria® and T2MR®), remote photoplethysmography, AR simulations, and PAR2 receptor inhibition, were used less frequently, each mentioned only once, suggesting that they are either emerging technologies or applied only in specific contexts.

The high frequency of advanced biomarkers (e.g., IL-6, suPAR, irisin), machine learning algorithms, and continuous monitoring with biosensors (e.g., PO, NIRS, ST) as modern methods in the diagnosis and monitoring of sepsis and septic shock are due to their enhanced capacity for early detection and precise patient assessment. Advanced biomarkers are valued for their increased sensitivity in identifying inflammation and organ dysfunction associated with sepsis, and their inclusion in clinical practice is supported by growing evidence showing their predictive value.

Machine learning algorithms and continuous monitoring with biosensors are also highly attractive to researchers and clinicians, as they can analyze complex data and provide real-time alerts, enabling faster and more personalized interventions. In particular, machine learning algorithms can integrate information from multiple sources (biomarkers, vital signs) to improve risk stratification and allow continuous, proactive monitoring of patients.

On the other hand, modern methods with lower usage frequency, such as molecular diagnostic panels (T2Bacteria® and T2MR®), remote photoplethysmography, AR simulations, and PAR2 receptor inhibition, are relatively new and are typically used only in specific contexts or experimental studies. These technologies are still in the evaluation phase for widespread implementation and require additional studies to validate their effectiveness and cost-efficiency across various clinical settings. Additionally, the higher costs and infrastructure requirements for some of these emerging technologies limit their broad use in everyday clinical practice.

Thus, there is a continued preference for conventional methods in clinical practice, alongside a gradual trend towards the integration of more advanced modern methods, which have the potential to provide faster and more accurate detection of sepsis and septic shock.

DISCUSSIONS

A total of 23,822 patients were reviewed across the studies included in this systematic analysis. These patients come from various observational, randomized, and retrospective studies, each with different designs and intervention methods aimed at evaluating and monitoring sepsis and septic shock.

The results of this systematic analysis demonstrate that the treatment of sepsis and septic shock can be enhanced by combining conventional interventions with modern methods, including advanced biomarkers, digital monitoring, and artificial intelligence (AI) techniques. The studies reviewed cover a wide range of therapeutic approaches, from fluid therapy strategies and inflammatory biomarkers to continuous monitoring technologies and predictive genetic analyses.

A central aspect of the analysis is the impact of conventional and modern interventions. For example, the study by Meyhoff et al. (2022) shows that intravenous fluid restriction does not offer significant advantages over standard therapy in terms of mortality or reduction of severe adverse events [18]. Although fluid restriction may reduce the risk of volume overload, the results of this study indicate that there are no significant differences between restrictive intervention and the conventional approach regarding the survival of patients with septic shock. This suggests that the optimization of fluid volume should be evaluated based on the individual characteristics of each patient.

Similarly, the study by Liberski et al. (2020) highlights the role of biomarkers such as NLR (neutrophil-to-lymphocyte ratio) in assessing the risk of septic shock [19]. NLR has shown predictive value for identifying patients prone to septic shock, but its combination with conventional biomarkers like CRP and PCT significantly increases predictive accuracy. This suggests that advanced biomarkers may contribute to the early identification of high-risk patients, facilitating faster interventions. However, since no single biomarker directly predicted mortality, their optimal use remains as a complement to standard clinical measures, for more effective risk stratification.

Another essential aspect is the inclusion of modern monitoring technologies, such as near-infrared spectroscopy (NIRS) and remote photoplethysmography (rPPG). The study by Lungu et al. (2024) indicates that continuous monitoring using NIRS, PO, and skin temperature can detect sepsis 6–48 hours before clinical diagnosis, providing an important advantage in preventing the rapid progression of the disease [22]. NIRS and PO demonstrated the highest predictive accuracy, highlighting the potential of these non-invasive methods for continuous monitoring and early detection of neonatal sepsis. Microcirculation monitoring via photoplethysmography (rPPG) showed greater sensitivity than conventional methods for assessing perfusion, which could have important implications for managing critically ill patients with septic shock, according to the study by Klibus et al. (2024) [25]. The use of these technologies offers a more sensitive way to monitor subtle changes in tissue perfusion, which could precede overt clinical deterioration.

AI-based methods also bring innovative contributions to the diagnosis and prognosis of sepsis. The study by Wang et al. (2023) highlights the capability of machine learning (ML) algorithms to identify sepsis-associated genes, such as ITGAM and KIF1B, with significant accuracy [21]. These models achieved high-performance scores (AUC), indicating that ML-based approaches can support diagnosis and risk stratification by identifying specific genetic profiles. Using AI to analyze genetic profiles paves the way for personalized treatments and improved identification of therapeutic targets.

Additionally, the administration of the molecule 1-PPA, investigated by Luisetto et al. (2024), demonstrated positive effects by reducing mortality and sepsis symptoms in animal models [31]. This molecule inhibits the PAR2 receptor, reducing systemic inflammation and protecting cardiac function, suggesting that interventions targeting specific molecular pathways could offer new therapeutic solutions for patients with septic shock. Although these results are preliminary and obtained from in vitro and in vivo animal studies, they show promising potential for developing targeted therapies capable of blocking inflammatory cascades at the molecular level.

Overall, the results of this analysis underscore the need for an integrated approach that combines conventional interventions, such as fluid administration and vasopressors, with modern diagnostic and monitoring methods. Continuous monitoring, advanced biomarkers, and AI algorithms have the potential to improve prognosis and reduce mortality in sepsis, although their widespread applicability depends on validation in extensive clinical studies. While some methods, such as fluid restriction, have shown limited results, others, like biomarkers or digital monitoring, have significant potential in risk stratification and early identification of patient deterioration.

Thus, the combination of conventional and modern methods allows for better adaptation of treatment to the individual specificities of each patient, while also offering opportunities to improve survival and quality of care in intensive care units.

CONCLUSION

This comparative study of conventional and modern methods for diagnosing and monitoring sepsis and septic shock highlights the importance of adopting advanced technologies in the care of critically ill patients. While traditional methods, such as blood cultures and standard biomarkers, remain fundamental, the results of the reviewed studies suggest that modern technologies – including rapid molecular tests, continuous monitoring through biosensors, and machine learning algorithms – offer greater accuracy in early diagnosis and superior risk stratification capabilities. These advanced tools enable faster and more informed interventions, thereby contributing to improved prognosis and reduced mortality in sepsis and septic shock.

The comparative analysis between conventional and modern methods of diagnosis and monitoring for sepsis and septic shock reveals several practical implications for clinical practice. Conventional methods, such as the use of standard biomarkers (CRP, PCT, lactate) and clinical scores (SOFA, APACHE II, SAPS II/III), remain essential due to their accessibility and the familiarity healthcare professionals have with them. These methods provide a solid foundation for the initial assessment of patients and risk stratification in intensive clinical settings.

However, integrating modern methods, such as machine learning algorithms, continuous monitoring with biosensors, and the use of molecular diagnostic panels (e.g., T2Bacteria®), has the potential to significantly transform clinical approaches. These advanced technologies can improve diagnostic accuracy, enable early detection of sepsis, and facilitate faster and more informed decision-making. For instance, rapid molecular panels can identify pathogens within hours, thereby reducing the time needed to initiate appropriate antibiotic therapy, which is crucial for improving patient prognosis.

Implementing these modern methods in hospitals requires initial investments in infrastructure and medical staff training, but the potential benefits are substantial, including reduced mortality, shortened hospital stays, and optimized resource utilization in intensive care units. The use of advanced biomarkers and machine learning algorithms could also facilitate continuous monitoring of patients with sepsis and septic shock, providing real-time alerts for patient deterioration and supporting early intervention.

In the long term, these innovations may contribute to the standardization of more effective clinical protocols and the personalization of treatments for sepsis patients, tailoring interventions to the specifics of each patient. Furthermore, the gradual integration of these modern technologies could help raise the quality of medical care and improve the overall efficiency of the healthcare system.

To fully integrate these technologies into clinical practice, further clinical studies are needed to validate their effectiveness across a variety of clinical settings. It is essential to develop standardized protocols that include rapid molecular tests and machine learning algorithms for early detection and personalized monitoring of patients. Additionally, future research should explore the potential of new biomarkers and non-invasive monitoring technologies to support rapid clinical decision-making and facilitate integrated sepsis management in intensive care units.

This systematic analysis included studies with varied designs (randomized, observational, and retrospective studies), which may impact the direct comparability of results. Differences in study design, patient populations, and clinical settings (pediatrics, adults, ICU) make generalizing conclusions challenging, as each type of study may have its methodological limitations and biases. The present study encompasses a diverse range of patient populations, including neonates, children, adults, and the elderly. It is therefore evident that the newborn with sepsis has almost no defense mechanisms, whereas the middle-aged population demonstrates the most effective response to sepsis through early secretion of various mediators, a robust response to systemic inflammation, and a high capacity for regeneration. The elderly are included in the category of individuals who are considered to be frail, and as a result, may demonstrate a reduction in their ability to respond effectively to an encounter with a pathogen. Consequently, sepsis can be identified even in its most advanced stages in these patients. Furthermore, the capacity for regeneration is diminished in this population, resulting in more pronounced manifestations and consequences of sepsis and septic shock. Furthermore, patients with at least one additional health condition (such as autoimmune, cardiovascular, respiratory, or renal disease) are at significantly elevated risk of developing more severe forms of sepsis, including septic shock. Consequently, developing a diagnostic methodology for sepsis in the general population is challenging, necessitating the use of specific diagnostic techniques and combinations of traditional and modern methods adapted to the individual's age, associated diseases, and other factors.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, G.P.G. and A.N.D.; methodology, R.M.S and L.P; software, A.B. and I.S.C.; validation, V.T.G and D.R.T.; formal analysis, D.C, D.O.C. and E.F.L.; investigation, G.P.G, A.N.D and A.B.; data curation, V.T.G. and E.F.L ; writing—original draft preparation, A.B and R.M.S.; writing—review and editing G.P.G, D.O.C. and D.C.; visualization, L.P. and I.S.C.; supervision, G.P.G and D.R.T.; project administration, G.P.G and D.C. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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REVIEW

Assessment of Suicide Risk in the Civilian Population, Active Military Personnel and Veterans Using Psychometric Instruments – A Literature Review

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Abstract: The assessment of suicide risk is a highly debated topic in the literature due to the complex interplay between social, psychological, cultural, and biological factors that contribute to the pathogenesis of self-harm. There is no unanimously accepted predictive model of suicide and no widely recognized evidence-based algorithm for psychiatric and psychological evaluation in clinical and non-clinical populations for early detection of self-harm. Therefore, this narrative review is focused on identifying the validated clinical instruments that may contribute to the construction of a possible screening and monitoring plan for suicide risk in military and civilian populations. Four electronic databases were searched (PubMed, CINAHL, Google Scholar, and Clarivate/Web of Science) for relevant reports on psychometric tools for detecting suicide risk published between the inception of each archive and January 2025. Seventeen instruments dedicated to the measurement of suicide and suicide-related aspects were reviewed, as well as three tools for the evaluation of depression severity that include specific items for assessing self-harm. The advantages and vulnerabilities of each instrument were assessed, and the particular features of using these tools in the military population were also explored. In conclusion, although a large number of validated instruments for the assessment of suicide risk exist, it is not possible to recommend the use of a single tool, either for clinical and nonclinical populations or for military and civilian personnel. The endeavor of finding an algorithm for the assessment of suicide risk is still far from reaching its end, as new psychometric instruments and possibly a new paradigm for the phenomenon of self-harm are sorely needed.

Keywords: suicide risk, self-harm, depression, military personnel, veterans, suicide prevention

INTRODUCTION

Suicide prevention is one of the most challenging phenomena in clinical psychiatry due to the social impact of self-harm, its high prevalence, and the lack of unanimously accepted predictive models. More than 720000 individuals die by suicide each year, and self-aggression is the third leading cause of death among individuals aged 15 to 29 years old [1]. More than one in every 100 deaths worldwide occurs due to suicide, and methodological improvements are needed in order to increase the accuracy of reports regarding complete suicide and suicide attempts [2]. In this context, structured assessment of suicidal ideation and behaviors is an important aspect of the psychiatric clinical practice, and the development of validated instruments (scales, inventories, and questionnaires) for assessment of suicidal risk is essential for both civilian and military populations.

Suicide rates are still high among military personnel, both active and retired [3]. The Army Study To Assess Risk and rEsilience in Servicemembers (Army STARRS) is a large investigation targeting the prediction of suicide in active duty soldiers who were hospitalized (N=53769 participants), in the next year after discharge [4]. The incidence of suicide in this population was 12% of all US Army suicides, equivalent to 263.9 suicides per 100,000 person-years vs. 18.5 suicides per 100,000 person-years in the entire US Army [4]. This data indicates the need for more careful post-discharge monitoring of active duty soldiers, but the specific means to be implemented for this purpose are far from being clear. A potential contributor to the higher risk of suicide among military personnel has been the existence of stigmatizing beliefs since these can act as deterrents for help-seeking [3]. In a cohort study (N=153,736 patients) of US veterans, the risk assessment suggested that only a few variables were associated with subsequent suicide, especially suicidal ideation, firearm access, and preparatory behaviors, which is completely insufficient to develop a risk prediction algorithm [5]. According to the US Department of Veterans Affairs, the risk of suicide in veterans was 1.57 to 1.66 higher than in non-veterans between 2017 and 2020 [6].

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Searching for psychological predictors of suicide is but only one side of the complex process of identifying relevant contributors to the pathogenesis of suicide. Biological factors, such as chronic diseases, and social vulnerability factors, like stigmatization, migration, or bullying, cultural and economic influences, e.g., poverty, acculturation stress, being part of a cultural minority, transgenerational conflicts, etc. [7-12]. However, all these potential pathogenetic factors have intricate relationships, and disentangling them is necessary as part of constructing an evidence-based approach to a predictive model for self-harm behavior.

Based on all these data, it becomes clear that finding adequate tools for detecting the risk of suicide is essential for creating preventative measures and thus decreasing the incidence of completed suicide. To attain this objective, the current review focuses on the detection of the most validated and widely used psychometric instruments for the detection of suicide risk in military personnel, veterans, and civilians. It is expected that such an endeavor could provide the basis of an algorithm for the evaluation of vulnerable populations, as a screening strategy, and for the monitoring of at-risk groups during and after exposure to severe stressors. This review does not target only clinical populations derived from civilian or military environments but also non-clinical populations, which may be exposed to significant risk of suicide ideation and behavior.

MATERIALS AND METHODS

A narrative literature review was conducted by searching four major databases – PubMed, CINAHL, Google Scholar, and Clarivate/Web of Science – for all primary and secondary reports published between the inception of each archive and January 2025, using as keywords "suicide" OR "self-harm" AND "scale" OR "inventory" OR "questionnaire" OR "profile" OR "psychometric instrument" OR "psychometric tool" AND "civilian" OR "military personnel" OR "veterans. Only papers in English were selected. The inclusion criteria were adolescents and adults, clinical and nonclinical groups, civilian and military populations, primary outcomes related to suicide, clinician-rated and self-rated instruments, both prospective and retrospective studies, but also systematic reviews, narrative reviews, and meta-analyses. Exclusion criteria were undefined measurement methods, exclusively non-structured methods of evaluation, poorly defined study design, and children or unspecified population characteristics.

RESULTS

A number of 17 instruments dedicated to the measurement of suicide and suicide-related aspects were found during the literature search, and three tools that include at least one item related to suicide, that were investigated in multiple studies were also included in the analysis.

The **Suicide Attempt Self-Injury Interview** (SASII) was developed by Linehan et al. (2006) to assess the multiple factors associated with suicide attempts and self-aggressive behaviors without a clear suicidal intent [13]. According to the authors of this instrument, its major strength is the use of a standardized definition of suicidal and other self-injurious activities, allowing the practitioners to assess the suicidality/suicide intent regardless of the particular aspects and consequences of the acts themselves [13]. This tool also offers individual ratings for the components of lethality, such as the method used, the consequences of the gesture, and the therapy needed [13]. The scales of SASII are „suicide intent", „lethality", „rescue likelihood", „risk/rescue ratio", „suicide communication", „interpersonal influence", and „emotional relief" [13]. SASII allows for each episode of non-suicidal self-injury behavior to be coded separately and described by its characteristic details. The major variables are assessed by the interview and the frequency of self-injury behaviors, the medical treatment required secondary to the behaviors, and a set of four factors for each episode of non-suicidal behavior - medical risk, suicidal intent, instrumental intent, and impulsivity [13].

A study investigating patients (N=60) with repetitive nonsuicidal self-injury (NSSI) tried to validate subtypes of these behaviors, based on multiple self-report scales, and two structured interviews, SCID-5-CV (Structured Clinical Interview for DSM-5 Clinical Version) and SASII [14]. The results supported a two-subtype model, i.e., (a) substance abuse and suicide attempt subtype- with a higher rate of lifetime suicide plans, number of lifetime suicide attempts, higher severity of self-harm, borderline personality features, anger, posttraumatic manifestations, and difficulties in regulation of own emotions; (b) cutting and scratching subtype [14]. The use of SASII in this study supported the validity of this model and, consequently, the existence of clinical heterogeneity of individuals with NSSI [14].

In another study, SASII was combined with the Beck Scale for Suicide Ideation (BSI), Beck Depression Inventory (BDI), and Beck Hopelessness Scale (BHS), to characterize the self-harm behaviors in a group of Pakistani patients (N=221) [15]. The results showed the possibility of common causal mechanisms and processes underlying self-harm between the investigated population and other groups, with factors such as financial, relationship, or interpersonal difficulties being considered [15]. SASII was administered to collect data on details regarding the time, methods, circumstances, motivations, and treatment for self-harming behaviors [15].

According to a systematic review that explored self-harm measuring tools, seven such validated instruments were identified, but there was a high variation detected in their overall quality [16]. Out of the identified tools, SASII appeared to be the most detailed and psychometrically solid instrument currently available for measuring self-harm behaviors [16]. The other instruments identified were Self-Harm Inventory (SHI), Deliberate Self-Harm Inventory (DSHI), Self-Harm Behavior Questionnaire (SHBQ), Self-Injury Questionnaire (SIQ), Self-Injurious Thoughts and Behaviors Interview (SITBI), and Inventory of Statements About Self-Injury (ISAS) [16]. The reported reliability of SASII was α=0.63-0.93, and the convergent validity with therapist notes (r=0.76-0.86) and the medical records (r=0.75-0.82) were also high [16].

A study investigating the influence of NSSI onset age in adolescents is associated with the frequency of subsequent episodes of self-harm (N=103 participants) showed that a lower age of onset and a longer duration of self-harm were both associated with an increased frequency of other NSSI episodes and a higher risk of a first suicide attempt [17]. Therefore, early identification of NSSI using validated instruments is an essential way to create adequate management strategies for these behaviors [17]. In this important study, SASII was used to collect data on most of the parameters targeted, including time, modality, and frequency of self-harm behavior [17].

A recent cross-sectional study evaluated the presence of factors associated with self-harm behavior during military service in the Israel Defense Forces and used SASII and the Columbia Suicide Severity Rating Scale (C-SSRS) to characterize this population [18]. A number of 1238 episodes of self-harm behavior were identified between 2017 and 2021 and included in the analysis [18]. NSSIs correlated with the presence of adjustment difficulties, and higher rates of previous psychiatric diagnoses were found in individuals with suicide attempts [18]. The risk of dying by suicide during military service was twice as high in soldiers with previous suicide attempts and psychiatric diagnoses [18]. Also, if these soldiers were serving in a combat unit, the risk of suicide was four times higher [18].

Another two-year prospective study explored the predictive value of NSSI for suicide attempts in a clinical sample of US active duty military personnel (N=152, mean age=27.53 years), using SASII and Beck Scale for Suicide Ideation (BSSI-C) [19]. All participants reported current suicidal ideation and/or had a suicide attempt during the last month [19]. The results showed 40% of soldiers with a history of NSSI and 25% of those with a history of suicide attempt made a suicide attempt during the two years of follow-up [19]. Soldiers with a history of NSSI were twice as likely to engage in another suicide attempt, but those with a previous suicide attempt had no increase in suicidal behavior vs. soldiers without a history of suicide attempts [19]. However, 30% of the soldiers with a suicide attempt in their history had engaged in NSSIs, and the total number of NSSIs was significantly associated with an increased risk for future suicide attempts [19].

The **Columbia Suicide Severity Rating Scale** (C-SSRS) is one of the most widely used instruments in Psychiatry to determine suicide risk, but it must be noted that it cannot replace a complete clinical assessment. The C-SSRS has very good convergent and divergent validity with other scales for assessing suicidal ideation and behavior, its subscales are sensitive to changes over time, and the suicidal ideation subscale has a moderately high internal consistency [20]. The C-SSRS is structured in such a way that it allows separate assessments of the severity and intensity of suicidal ideation, autolytic behavior, and lethality of the attempt [20]. The severity of ideation is rated on a five-point scale, the intensity of ideation subscale also on a 5-point ordinal scale (i.e., frequency, duration, controllability, deterrents, and reason for ideation), the behavior subscale is rated on a nominal scale (i.e., aborted, actual, and interrupted attempts, preparatory behavior, and NSSI), while the fourth scale is the lethality subscale for the assessment of actual attempts (there is a 6-point ordinal scale, and if the current lethality is zero, potential lethality of attempts is rated on a 3-point ordinal scale) [20].

The cut-off value of 27 on C-SSRS has very good sensitivity and specificity for classifying suicide attempts in the first three months, according to a prospective multicenter cohort study (N=804 patients with a recent episode of self-harm) [21]. The CSSRS and the Suicide Assessment Scale (SUAS) had a better chance to classify the suicide attempts in the first months but also during a one-year follow-up, although the cut-off value for long-term monitoring could not be established for either instrument [21]. The same study showed that the Suicide Intent Scale (SIS) was the only instrument able to classify suicides adequately at 3 and 12 months, with a cut-off score of at least 21 and at least 17, respectively [21].

C-SSRS had proven a sensitivity of 67% and a specificity of 76% for identifying suicidal behaviors at the follow-up (mean period of 64 days) in a large study (N=3776 patients) [22]. Negative life events were associated with a 2.4% subsequent risk of suicidal behavior, and patients reporting lifetime suicidal ideation with intent/prior suicide attempt had a 4 to 9 times higher risk to report prospectively suicidal behavior [22].

C-SSRS score includes information collected from the patient, but also from other significant sources, and has been validated in emergency settings, as well as in the outpatient population [23]. Also, C-SSRS has been validated in children and adolescents, military veterans, and patients with different psychiatric disorders [23]. The US Food and Drug Administration recommends the use of C-SSRS for clinical trials, and it has been approved by the Centers for Disease Control and Prevention to define and monitor suicidal ideation and behavior [23,24].

The administration of C-SSRS in a large sample of US veterans (N=15373) resulted in confirmation of concurrent validity by comparison with self-reports from the last three months and attempts documented in medical records [25]. Nevertheless, a significant number of discordant responses were reported [25]. The predictive value for future attempts was more than 80% [25]. Another study evaluated the psychometric assessment of C-SSRS and Suicidal Behaviors Questionnaire-Revised (SBQ-R) in 200 active-duty personnel and concluded acceptable reliability for both SBQ-R and C-SSRS ideation severity subscale, while the confirmatory factor analysis supported the SBQ-R, but not the self-reported version of C-SSRS [26]. Yet another study that included US military personnel with lifetime attempted suicide (N=121) used C-SSRS to test the hypothesis that NSSIs elevate the suicide risk; the results showed that a history of NSSI is relevant for increasing the service member's social alienation and acquired tendency for suicide, but did not correlate with the number of lifetime suicide attempts or the controllability of suicide ideation [27].

The **Scale for Suicide Ideation** (SSI) and its self-administered version, Beck Suicidal Ideation Scale (B-SSI), were developed by Beck et al. and are widely used in clinical studies, although their dimensional structure is debated in the literature [28-30]. For SSI, the internal consistency was high, and there were recorded moderately high correlations with clinical ratings of suicidal risk and self-administered measures of self-harm, while the sensitivity to changes in hopelessness and depression over time was also good [28]. For B-SSI, the

Cronbach alpha coefficient was 0.90, indicating high internal consistency for both paper-pencil and computer versions [29]. B-SSI has 21 items, and its items are scored on a 3-point Likert scale, with a total score varying from 0 to 38 [29,31]. The cut-off score of 6 for B-SSI was defined in a prospective study as presenting accuracy for future suicidal behavior, and a reduction to less than eight items with stochastic curtailment was sufficient for this purpose [32]. There is a version for the assessment of current ideation, i.e., the SSI-C, and another for the worst moment in the patient's life, i.e., the SSI-W, both with 19 items [33]. Both SSI-C and SSI-W were positively correlated with a diagnosis of mood disorder, a personality disorder, and measures of depression and hopelessness [33]. The internal consistencies of both scales were high, i.e., alpha 0.84 for SSI-C and 0.89 for SSI-W [33].

A study that involved US military service members showed that summing up five items of the B-SSI (i.e., 1, 2, 4, 6, and 15) is an acceptable strategy to shorten this tool while preserving the predictive value of 74% for a cut-off score ≥1 [34]. A study that included 39 Israeli soldiers (mean age 19 years) who were suicide attempters during their military service included evaluations using B-SSI, C-SSRS, and Suicide Intent Scale (SIS); the results supported seven items of the B-SSI, one of the C-SSRS and two of the SIS were associated with severe suicide attempts [35].

The **Beck Hopelessness Scale** (BHS) explains hopelessness through the concept of a cognitive schema focused on unfavorable expectations, both in terms of the short-term and long-term perspective. This instrument, developed by Beck et al. in 1974, includes 20 items with a dichotomous format, and its primary use is the evaluation of suicide risk in clinical and non-clinical adult populations [36,37]. The original version has three factors, i.e., affective, motivational, and cognitive, but other alternative factorial structures have been suggested [37]. The BHS can be self-administered, as well as administered by the clinician, to hospitalized and non-hospitalized subjects, in specialized outpatient clinics or in emergency hospitals [37]. The predictive validity of BHS has been confirmed by multiple studies in patients with psychiatric disorders [38]. The Cronbach alpha was 0.81 in the general population, according to a study (N=1500 participants) [39]. Shorter versions of BHS have been created, in order to increase its applicability for clinical populations, such as BHS-9 or BHS-4 [38,40,41].

A study focused on improving the detection and prediction of suicidal behavior among military personnel by measuring suicidal beliefs used a set of tools, BHS included, in order to evaluate a new instrument, i.e., the Suicide Cognitions Scale (SCS) [42]. Another study included BHS in the set of instruments dedicated to the assessment of psychological pain in suicidal veterans and concluded that psychological pain accounted for the most shared variance for suicidality, but most relevant correlations were determined for the C-SSRS [43].

The **SAD PERSONS scale** is a mnemonic with each letter representing a risk for suicide, and the total score varies from 0 to 10 [44]. The interpretation is low risk for scores from 0 to 4, medium risk from 5 to 6, and high risk from 7 to 10 [44]. Modified SPS (M-SPS) is characterized by a substitution of new factors and changes in scoring (low risk = 0-5, medium risk = 6-8, and high risk = 9-14) [45]. These are simple and easy-to-administer instruments developed for screening suicide risk in community mental health settings [46].

The SAD PERSONS Scale (SPS) was assessed in a systematic review (N=9 studies) and the data were considered insufficient to support its use in the assessment or prediction of suicidal behavior [47]. In a large study (N=5462 consecutive adults evaluated by psychiatrists in tertiary emergency departments), the suicide rates at six months, 12 months, and five years were determined [48]. Low-risk assessed by SPS at baseline died by suicide at 6 and 12 months, while high-risk scores were significantly associated with death by suicide over the five-year interval by both SPS and M-SPS [48]. Another study (N=4019 consecutive referrals to psychiatric services in emergency departments) used both SPS and M-SPS and showed that high-risk baseline values presented low sensitivity and low positive predictive power [49]. SPS did not predict suicide better than chance in this study, but when only the five original scale items that accounted for the higher proportion of suicide attempt variance were used, the sensitivity was 93.5%, and the predictability was 5-fold higher for suicide attempt presentation [49].

To evaluate the possibility of using SAD PERSONS in veterans, a study (N=271 participants from the Midwest Veterans Affairs Medical Center) concluded that the rate of false-positive and false-negative results is unacceptable [46].

The **Reasons for Living Inventory** (RFLI) was created by M. Linehan in 1983, and it consists of a checklist of motives for choosing to continue living, instead of self-harming with suicidal intent [50]. RFLI has 72 items (long version) or 48 items (short version). The six factors originally describing the structure were replicated by other authors, the internal consistency was proven acceptable (Cronbach alpha = 0.72-0.92), and the test-retest reliability was also demonstrated [51]. A high score on RFLI predicted fewer future suicide attempts in the next two years interval for initially hospitalized women with depression, but not for men, in a study (N=386 participants) [52].

RFLI was administered in a veteran male population (N=421) with various combat exposures and a history of suicide attempts, and the initial results were supportive of this tool being used in the specified population [53]. The reliability and validity of RFLI were confirmed by exploratory factor analysis and bi-factor analysis, correlates between total scores and subscale scores, and estimates of internal consistency reliability on the original instrument subscale scores [53].

The **Reasons for Living Scale-Military Version** (RFL-MV) was created later by Deutsch & Lande, who added 20 items to the items of RFLI-SV, items specifically designed to reflect the military environment characteristics [54]. RFLI-MV was evaluated on 200 patients from the military personnel, and a six-factor structure was defined, with the subscale of "Military Values" reflecting the coherence of the new dimension added to Linehan's version of the tool [54].

The **Reasons for Living versus Reasons for Dying Assessment** is another tool that combines the protective factors and triggers for suicide, to offer a more complete interpretation of the individual's risk [55]. In a study (N=49 suicidal university counseling center patients), 173 reasons for living and 145 reasons for dying were obtained and structured in eight and, respectively, nine categories, and different chi-square results for the two dimensions were obtained, suggesting a different salience of those dimensions for suicidal patients [55]. The evaluation of the reasons for dying vs. reasons for living (RFD-RFL) index in military psychiatric inpatients (N=167) following a suicide event showed that family was the most reported supporting factor (40% of the participants), while the most frequently invoked reasons for dying were general descriptors of self (27%), general statements about escape (19%), and others/relationships (19%) [56]. Higher values of this index were associated with a more significant wish to die vs. wish to live, higher levels of hopelessness, and a history of multiple suicide attempts [56]. When individuals can find more reasons for dying than for living, this could be indicative of a higher suicide risk [56].

Another study evaluated the risks of dying vs. the risks of living in patients admitted after a suicide attempt (N=60) and a monitoring period of 24 months, showing that the number of reasons for dying was the most relevant predictor for increased suicidal ideation at baseline, and reasons for dying, depressive symptoms and baseline suicide ideation predicted suicide reattempt up to 12 months later [57]. In the mediation analysis, reasons for dying mediated the effect of depressive symptoms at baseline and one-year follow-up on suicidal ideation [57]. However, reasons for living did not prove themselves a protective factor against suicide risk [57].

The **Suicide Opinion Questionnaire** (SOQ) was designed to assess the different approaches to the event of suicide in different cultures and demographic groups, being administered in multiple settings and populations, e.g., college students, medical students, and mental health professionals [58,59]. The test-retest reliability coefficient for SOQ was r=0.78 to 0.91, and the intercorrelations between the scales were in the range of r=0.20 [59]. An exploratory factor analysis detected a weak 2-factor structure in one study, and this model failed in the second study, thus raising doubts about the factorial structure of this instrument [60].

In a group of undergraduate volunteers (N=738), the responses to SOQ were compared between attempters, contemplators, and nonattempters, and a factor analysis indicated the existence of seven factors, with two significant functions discriminating between the respondents based on their gender and prior suicide history [61]. In another study (N=91 psychiatric inpatients admitted for suicidality), the SOQ was included in a battery of tests focused on suicidality, and the results showed that 20 items differed between patients with suicide attempts vs. those without at baseline and follow-up [58]. The linear discriminant analysis supported the sensitivity and specificity of the test for post-discharge suicide attempts for the 9-item scale [61]. The potential of SOQ to be useful in identifying prospectively the individuals with a risk of suicide is supported by the available data, but maybe including it in a module of tests would be more beneficial than using it alone, and using it in high-risk patients would increase its predictive power [58].

In a study (N=1758 Marine Non-Commissioned Officers) that evaluated the usefulness of SOQ in military personnel, a 4-factor structure was detected, which accounted for approximately 30% of the total variance, with sex, education, and prior exposure to suicide within the individual's military unit being significantly associated with suicide options [62].

The **Suicide Trigger Scale** (STS) is a self-report instrument to assess the trigger states for suicide, which showed a strong internal consistency, with a Cronbach alpha of 0.94, and good validity related to past suicidal behavior [63,64]. Its third version, STS-3, showed that scores may be predictive for post-discharge suicide attempts [63]. This scale has 42 items and three factors explaining 43% of the variance- Frantic Hopelessness, Ruminative Flooding, and Near-Psychotic Somatization [64]. In a validation study (N=183 adult psychiatric patients with suicidal ideation/attempt in the psychiatric emergency room) with a 1-year follow-up, suicidal subjects with suicide attempt history had a mean of 7 points more than those without such a history, and Frantic Hopelessness was a significant predictor for current suicide attempt, if only attempters requiring at least some medical attention were considered [64].

A subset of six items on the STS-3 improved the prediction of post-discharge suicide attempts, and patients with ultra-high scores differed significantly from those with ultra-low scores on mood intensity, depression, impulsiveness, abuse history, and attachment security, in a validation study (N=161 adult psychiatric patients admitted to hospital for suicidal ideation or attempt) [63].

The **Manchester Self-Harm Rule** (MSHR) was based on an exploration of multiple variables related to self-harm, i.e., those which include the nature of the act, details of self-harm episode, the main reason for current distress/precipitant to the episode of self-harm, social and demographic information, clinical history, and current medical state [65]. The four questions of MSHR refer to the history of self-harm, previous psychiatric treatment, current psychiatric treatment, and benzodiazepine taken as an overdose [65]. The tool has a sensitivity of 97%, a specificity of 26%, and a positive predictive value of 22% [65]. A related tool is the ReACT Self-Harm Rule, derived from a prospective cohort study, which analyzed 29571 self-harm presentations to hospital Emergency Departments [66]. Recent self-harm (in the last year), living alone/homelessness, cutting as a method of self-harm, and treatment for an ongoing psychiatric disorder were factors associated with higher risk [66]. This tool had 95% sensitivity and 21% specificity, with a positive predictive value of 30%, identifying 83 out of the subsequent 92 suicides [66].

The **Suicidal Behaviors Questionnaire** (SBQ) is a 34-item self-assessment instrument for suicidal thoughts and behaviors, developed by Linehan in 1981 [67]. Another, much shorter form of SBQ, containing only four items, and another 14-item tool [67-60]. Other versions of SBQ are available, but this multitude of variants precludes the finding of definite recommendations and psychometric properties for each population (adolescents vs. adults, clinical vs. nonclinical) [70,71]. SBQ 4-item version correlated with SSI scores significantly and it is recommended as a screening tool [71]. A revised version exists, SBQ-R, with four items, which benefits from empirical support as a risk measurement tool that differentiates between suicide-risk and non-suicidal risk individuals at a cut-off value of 7 (nonsuicidal sample) or 8 (clinical samples) [70]. SBQ-R has good internal consistency (Cronbach alpha = 0.97), convergent validity, a one-factor solution (89.5% of the total variance), good sensitivity (82%), and specificity (63%) when differentiating between serious planner/ideator and attempter groups [70]. SBQ-R has been translated and validated in numerous languages due to its brevity, ease of administration, and good psychometric properties [72,73].

The first item ("Have you ever thought about or attempted to kill yourself?") of the SBQ-R and the total scores have been recommended for use in both clinical and non-clinical populations [70]. A Bayesian approach to the SBQ-R supports the usefulness of item 1 for identifying other parameters of suicide, e.g., thoughts and behaviors [74]. The same analysis showed the relevance of each SBQ-R item when exploring the suicide elements and suggests that the individual item evaluation can offer clinically meaningful insights [74].

SBQ-R and the Risk-Taking Questionnaire 18 (RT-18) helped differentiate between individuals with suicide attempts and healthy controls by the variable "risk-taking" [75]. Individuals who reported a recent suicide attempt also had a higher tendency to sensation-seeking and impulsive behavior as correlates to risk-taking behaviors [75]. SBQ-R and other instruments were administered in a study (N=1310 medical graduate students) that suggested a significant risk of suicide (6.7%) among specific populations of students in China [76]. Poor relationships with the supervisor increased the risk of suicidality, suggesting more care is needed when educators are interacting with medical students, especially if the last ones are coming from families with low socio-economic status [76]. The administration of the original SBQ version in clinical populations was also explored in a study with patients diagnosed with bipolar disorder (N=98 participants); the association between anxiety dimensions and SBQ scores was relevant in a similar way to the association with depressive rumination [77]. Depressive rumination was a significant predictor of higher SBQ scores, and emotional processing was a significant predictor for lower SBQ scores for women and the entire sample [77].

In an exploration of the temperament dimensions most associated with the progression from ideation to suicide attempts, an adapted version of SBQ with 17 items was used together with the Affective and Emotional Composite Temperament Scale (AFECTS) [78]. The depressive, cyclothymic, and volatile were the most frequently associated temperaments with suicide attempters, while the cyclothymic, depressive, and euphoric temperaments were associated more with progression from ideation to suicide attempt [78].

The use of SBQ-R in a study that included post-9/11 veterans and service members seeking treatment for psychiatric symptoms (N=261 participants) reflected the high rates of suicidal ideation and behavior in this population [79]. A rate of 40% was obtained in this sample, based on the SBQ-R, indicating a high risk of suicide [79]. Negative posttraumatic thoughts about self, gender, military branch of service, higher levels of severity, anger and anger expression, less impulse control, and lower rank, all these factors were associated with a higher risk of suicidal ideation and behavior [79].

Another retrospective analysis included 3356 cases (age 55+) collected from the US National Health and Resilience in Veterans Study (2019-2020) and used SBQ-R in correlation with various demographical and personal history data [80]. The rate of suicidal ideation during the last year was 6.6%, for lifetime suicide plans was 4.1%, and for future suicide intent 0.9% [80]. A higher level of loneliness and a lower level of purpose in life was most strongly associated with last year's suicide ideation, while a lifetime history of major depressive disorder correlated with suicide plans and suicide attempts [80].

In another cohort study (N=3078 US military veterans), the rates of suicide ideation and suicide attempts did not present higher values during the COVID-19 pandemic vs. pre-pandemic level, but a small percentage of veterans (2.6%) developed new-onset suicidal ideation during the pandemic [81]. SBQ-R items 1 and 2 were used in this study, together with the UCLA Loneliness Scale, Alcohol Use Disorders Identification Test, Life Events Checklist for DSM-5, Adverse Childhood Experience Questionnaire, Medical Outcome Study Social Support Scale-5, and the Coronavirus Health and Impact Survey [81].

The **Military Suicide Attitudes Questionnaire** (MSAQ) is a 32-item instrument with a four-factor structure designed to be used by military service members [82]. The confirmatory analysis included 317 US military personnel, and good levels of test-retest reliability were found; the four factors identified were "individual-based rejection vs. acceptance", "psychache vs. pathological", "unit-based acceptance vs. rejection", and "moral vs. immoral" [82]. The validation study did not control for social desirability, which may be an important limitation [82].

Two different studies of MSAQ validation included 200 and 1116 individuals, respectively, from the military environments, and factorial analyses support the previously reported four-factor structure [3]. The reliabilities ranged from 0.77 to 0.83; men had more negative suicide-related beliefs vs. women, and discomfort and unacceptability beliefs were more positively associated with perceived barriers to care [3].

The **Suicide Probability Scale** was developed in 1982, it contains 36 items and is a self-report tool [83]. This scale was designed to assess suicide risk in adolescents and adults, and it only takes 5 to 10 minutes to complete [83]. The score is obtained by adding the answers using a 4-point scale; there are four factors: Hopelessness, Suicide Ideation, Negative Self-evaluation, and Hostility [83]. The Cronbach alpha for this scale was 0.92 in a sample of hospitalized patients with psychiatric disorders, and the results correlated with the scores on the Beck Depression Inventory-II and Adult Suicidal Ideation Questionnaire [83,84]. The utility of this scale did not confirm its utility in adolescents to assess suicide potential, and the factorial structure was not confirmed [85]. However, other studies support its utility for this population, with good convergent and discriminant validity [86].

The **Sheehan Suicidality Tracking Scale** (SSTS) has a standard version with 16 items that assess the seriousness of suicidality, the frequency of key phenomena, and the overall time spent in suicidality on a Likert scale (0-4) [87]. It is available in a patient and clinician-rated format, but a version that reconciles the eventual discrepancies between the previous two in specific items also exists [87]. There is a clinically meaningful change version of the SSTS and a pediatric version (P-SSTS) [87,88]. Another version of SSTS (v.10) has an 8-item instrument that measures variables related to self-injury, self-harm, suicide ideation and suicide attempt, with each item being scored on a Likert scale, from 0 (absent) to 4 (extremely) [89]. SSTS proved good convergent, divergent, internal consistency, and test-retest stability, according to a cross-sectional study (N=303 undergraduate students) [89]. All SSTS items were loaded on a single factor, suggesting it could be useful as a screening tool [89].

In a clinical study (N=82 subjects with generalized anxiety disorder) that assessed an experimental corticotropin-releasing factor antagonist vs. placebo and an active comparator (escitalopram), SSTS had a good sensitivity, as well as item 3 on the Hamilton Depression Rating Scale for Depression (HAMD) for identification suicidal thoughts or behaviors (100% and 63%, respectively) [90].

In another clinical trial, which investigated the evolution of depressive symptoms during citalopram + lithium vs. citalopram + placebo (N=80 patients), SSTS and Montgomery Asberg Depression Rating Scale (MADRS) were administered as primary measures, and the results showed a larger reduction on SSTS vs. MADRS (43% vs. 25%) [91]. A subgroup of patients who received lithium and citalopram had higher SSTS remission rates (45% vs. 19%) [91].

The **InterSePT scale for suicidal thinking** (ISST) has 12 items and assesses the current suicidal ideation in patients with chronic psychosis [92]. In two studies, with 22 inpatients with schizophrenia and schizoaffective disorders who had a recent suicide attempt or ideation, and 980 patients with the same disorders and a history of suicidal ideation in the past 36 months, respectively, ISST was used as a monitoring tool for two years, together with Clinical Global Impression for Severity of Suicide (CGI-SS), Calgary Depression Scale (CDS), Scale of Functioning (SOF) and Positive and Negative Symptom Scale (PANSS) [92]. The Cronbach alpha was high (0.86-0.89) for individual items, as well as the overall value (0.88) [92].

The ISST total score was highly correlated with the CGI-SS by the blind rater [92]. During the InterSePT trial that evaluated clozapine efficacy, ISST and CDS were administered to predict suicide attempts or hospitalizations [93,94]. The results of the InterSePT trial suggest both tools may be useful for assisting clinical decision-making regarding the suicide risk in patients with chronic psychoses [93].

The **Acquired Capability for Suicide Scale** (ACSS) is a 20-item tool administered based on the Interpersonal Psychology Theory of Suicide and assesses factors that facilitate individuals to acquire the ability to engage in suicidal acts, such as fearlessness about death and habituation to pain (which are considered needed to overcome the self-preservation reflexes) [95]. The items are scored on a Likert scale, from 0 to 4, and the reliability of the scale was calculated in a study to be alpha=0.80 [95]. In a study that included suicide ideators, depressed suicide attempters, and control participants (N=44 participants), based on the ACSS administration, suicide attempters showed the highest level of fearlessness and pain insensitivity and a greater history of painful and provocative life events [96].

A systematic review evaluated the use of ACSS among US military personnel and veteran samples (n=31 studies), and the results, based on a high risk of bias sources, indicated inconsistent findings across studies [97]. The authors of the respective analysis recommended caution in the interpretation of ACSS empirical data [97].

Regarding the instruments for the assessment of depression, which include items specific for suicidality, three tools were analyzed based on their wide use in clinical and epidemiological trials and their validated psychometric profile.

The **Beck Depression Inventory** (BDI) is among the most widely used self-rating scales for depression severity, with a long history of being administered in therapeutic and other various studies, starting from its publication in 1961 [98]. The internal reliability was confirmed in clinical and nonclinical samples and the average Cronbach alpha was 0.75; the factorial validity of this instrument showed a variable number of factors, from 1 to 9, and the convergent and discriminant validity was also proven [98]. Of interest for this review is the suicide item for its predictive value for deaths by suicide, which is scored from 0 ("I do not have any thoughts of killing myself") to 3 ("I would kill myself if I had the chance") [99].

A study that included 5319 patients showed that the single BDI item significantly predicted both deaths by suicide and repeated suicide attempts [100]. Each successive rating of the suicide item on BDI conferred greater risk, suggesting a direct proportionality between the two values, with an optimal cutoff score of ≥1 for suicide and ≥2 for suicide attempts providing the maximum equilibrium between sensitivity and specificity [100]. A value of ≥1 on item 9 on BDI-II was also used as a marker for suicide ideation in a study that concluded type D personality could be a risk factor for suicide ideation in individuals with major depression with (N=318) [101].

The **Hamilton Rating Scale for Depression** (HAMD) represents, in fact, a variety of scales that differ by the number of items and modalities of administration [102]. HAMD is a valid and sensitive clinimetric index and requires informed use, with the specific type of HAMD to be selected for each context [102]. The HAMD includes an item on the assessment of suicide risk (scored from 0 – "absent" to 4- "attempts at suicide") [99]. It correlates significantly with the number of suicide attempts and the age at the first attempt in a study with 281 suicide attempters [103].

The **Montgomery Asberg Depression Rating Scale** (MADRS) is a 10-item tool dedicated to the measurement of depressive severity that includes an item dedicated to „suicidal thoughts" (scored from 0 – „enjoys life or takes it as it comes" to 6 – „explicit plans for suicide when there is an opportunity/active preparations for suicide") [99]. This item of MADRS and the suicide item on HAMD correlated with the first five items on SSI at a significant level (r>0.80) in a study that assessed the efficacy of ketamine on the severity of depression [99].

DISCUSSION

Table 1 presents a synthesis of the instruments explored in this review. All of the reviewed instruments present benefits and shortcomings, making the unanimous recommendation of only one such instrument for evaluating suicide risk highly unlikely. However, based on the characteristics of each population or group that needs to be explored (screened or monitored) for suicide risk, a certain set of tools may be selected from the available resources, depending on their psychometric properties and previous studies' results available.

Table 1: Analysis of the psychometric tools for the assessment of suicide

Psychometric instrument	Studies on active military personnel and /or veterans	Studies on the civilian population	Advantages	Disadvantages	Observations
SASII	Yes [18,19]	Yes [14-17]	Standardized definition of suicidal and other self-injurious activities; individual ratings for the components of lethality, such as the method used, the consequences of the gesture, and the therapy needed.	More studies on military personnel are needed to confirm its psychometric properties in this population and its predictive power. The necessary time to administer is quite long.	Reliability and convergent validity were determined. Studies that compared this instrument with other validated instruments were performed. Evaluates both suicidal and non-suicidal self-injury behaviors.
C-SSRS	Yes [23,25-27]	Yes [21-23]	Separate assessments of the severity and intensity of suicidal ideation, autolytic behavior, and lethality of the attempt.	Data about the self-reported version of C-SSRS in military personnel is insufficient to recommend its use in this population.	Very good convergent and divergent validity with other scales used for the same purposes.
SSI, B-SSI, SSI-C, SSI-W	Yes [34,35]	Yes [28-20,33]	The set of SSI scales offers a more nuanced choice for different settings and populations.	The dimensional structure is debated.	Good internal consistency and sensitivity to changes for several important suicide factors.
BHS	Yes [42,43]	Yes [38]	Evaluation of suicide risk in clinical and non-clinical adult populations. Self-administered or clinician-administered.		Demonstrated predictive validity and reliability. Shorter versions of this tool have been created.
SPS, M-SPS	Yes [46]	Yes [47-49]	Ease of administration; the existence of a shorter version.	The predictive value of suicidal behavior was considered inconsistent.	A further refinement of the items included in this scale may be needed to increase its predictive value.
RFLI, RFL-MV	Yes [53,54]	Yes [51,52]	A self-administered version and a clinician-administered version exist. A military version of this scale also has been published.	The RFLI scales (both long and short versions) are quite long due to the large number of items.	A checklist of motives for living that can represent useful resources to work with in psychotherapy. Acceptable internal consistency and test-retest reliability.
RFD-RFL Index	Yes [56]	Yes [55,57]	It combines two distinct lists of reasons for living and reasons for dying.	It is a complex and long instrument to administer (17 domains).	Reasons for living are not a valid protective factor against suicide risk. However, reasons for dying may be useful as a predictor of suicide risk.
SOQ	Yes [62]	Yes [58,61]	It was constructed to be sensitive to cultural and demographic variability.	Different studies did not support the factorial structure.	Test-retest reliability was demonstrated. It was administered in different groups. It has the potential to help identify the risk of suicide.
STS	None were identified	Yes [63,64]	It has several versions, some of them presenting scores with good predictive values.	More studies are needed to support its utility, especially in military personnel.	Good internal consistency and validity related to past suicidal behavior. A subset of items could be further explored for higher predictive value than the overall scale.

MSHR	None were identified	Yes [65]	Based on multiple variables related to self-harm	Psychometric properties are poor.	Good sensitivity but poor specificity and low predictive value.
ReACT SHR	None were identified	Yes [66]	Based on a large populational study, and includes multiple variables related to suicide.	More research is needed to validate this instrument.	Good sensitivity but poor specificity and low predictive value.
SBQ, SBQ-R	Yes [79-81]	Yes [70,72,73,75-78]	Many versions of this instrument exist. It was explored in multiple studies that enrolled veterans.	Psychometric properties are poorly defined, except for SBQ-R.	It was extensively explored in both military and civilian populations.
MSAQ	Yes [3,82]	Not applicable	It was explored in two studies with US military personnel.	The currently available studies have methodological limitations.	More studies are needed to explore the validity of this instrument.
SUPS	None were identified	Yes [83-86]	It is a short instrument, easy to administer.	It is a self-report tool. The factorial structure is debated.	Good validity and reliability. The utility of this tool in adolescents to predict suicide was not confirmed.
SSTS	None were identified	Yes [89-91]	Patient and clinician-rated format, and a version that reconciles the differences between the two also exists. A shorter version exists.	No study was identified with military populations.	Good convergent, divergent, internal consistency, and test-retest stability. Only one factor was identified: good screening tools.
ISST	None were identified	Yes [92]	A valuable instrument for assessing suicidal ideation in patients with schizophrenia spectrum disorders.	It applies to a limited population, i.e., a clinical population with chronic psychoses.	High construct validity, good correlation with other scales.
ACSS	Yes [97]	Yes [95,96]	Short, easy-to-administer tool.	Caution is recommended in the interpretation of this scale's results in the military population.	Good validity and reliability.
Scales with single items evaluated for suicide predictive power					
BDI - item 9	None were identified	Yes [99-101]	The suicide item predicted deaths by suicide and suicide attempts.	Insufficient data to support scores on single items as independent predictors of suicide.	Good internal reliability in clinical and nonclinical samples for depressive disorders.
HAMD – item 3		Yes [103]	The suicide item correlated with the number of suicide attempts.		Valid and sensitive tool for depression.
MADRS – item 10		Yes [99]	Short and easy-to-administer tool. The suicide item correlated with items on SSI.		A useful tool for the quantification of depressive symptoms across age intervals.

SASII= Suicide Attempt Self-Injury Interview, C-SSRS= Columbia Suicide Severity Rating Scale, SSI= Scale for Suicide Ideation, B-SSI= Beck Suicidal Ideation Scale, BHS= Beck Hopelessness Scale, SPS= SAD PERSONS, M-SPS= Modified SAD PERSONS, RFLI= Reasons for Living Inventory, RFL-RFD= Reasons for Living versus Reasons for Dying Assessment, RFL-MV= Reasons for Living- Military Version, SOQ= Suicide Opinion Questionnaire, STS= Suicide Trigger Scale, MSHR= Manchester Self-Harm Rule, ReACT SHR= ReACT Self-Harm Rule, SBQ= Suicidal Behaviors Questionnaire, MSAQ= Military Suicide Attitudes Questionnaire, SSTS= Suicide Probability Scale Sheehan Suicidality Tracking Scale, ISST= InterSePT scale for suicidal thinking, ACSS= Acquired Capability for Suicide Scale, BDI= Beck Depression Inventory, HAMD= Hamilton Rating Scale for Depression, MADRS= Montgomery Asberg Depression Rating Scale

A prospective comparative analysis of multiple scales for the assessment of suicide risk included MSHR, ReACT Self-Harm Rule, SAD PERSONS, M-SPS, and Barratt Impulsivity Scale (BIS) in patients who would repeat self-harm within six months [104]. By evaluating 483 episodes of self-harm, the recurrence rate was 30%, and the sensitivity of these tools varied from 1% for SAD PERSONS, to 97% for MSHR [104]. The positive predictive value also varied, from 13% for M-SPS to 47% for the clinician assessment of risk [104]. The authors of the respective study concluded that risk scales for self-harm have limited clinical utility, as they are no better than clinician or patient ratings of risk [76]. A secondary analysis of data collected from multicenter prospective cohort studies showed that some individual items outperformed the scale in which they are included, but no items were superior to clinician or patient risk estimations

[105]. Based on the data from the same analysis, the use of suicide risk scales was less cost-effective than the clinician and patient ratings based on QALY (Quality-Adjusted Life Years) exploration [106].

A systematic review that evaluated the level of evidence for instruments assessing the suicide risk (n=21 studies) included 15 such tools and for the outcome suicide attempt, SPS had a sensitivity of 15% with a specificity of 97%, MSHR 97% and 20%, respectively, ReACT a similarly low specificity with MSHR; BHS presented a sensitivity of 89% and a specificity of 42% for the outcome suicide [107].

A direct comparison of the psychometric properties of CSSRS, STS, and SSTS in 199 psychiatric inpatients was conducted in a randomized manner by three trained judges [108]. All three assessments had very good accuracy for suicidal ideation and attempts (k=0.72 to 1.00, and 0.82 to 0.95, respectively), with an interrater agreement about CSSRS categories being more varied (k=0.48 to 1.00) [108]. Cronbach alpha value was less than 0.55 for CSSRS ideation and 0.78-0.92 for STS and SSTS [108]. The administration of any of these three instruments would improve reliability vs. unstructured assessment in the evaluation of suicidal ideation and behavior [108].

A study included 40 subjects with suicidal ideation and behavior who were interviewed using the InterSePT Scale for Suicidal Thinking-Plus, the SSTS, and the CSSRS, administered in a random sequence [109]. An agreement between these scales and the 2012 Food and Drug Administration (FDA) Classification Algorithm of Suicide Agreement categories was sought [109]. SSTS and InterSePT scale had an acceptable agreement with CSRSS in the detection of passive ideation, active ideation with method, intent, and plan, completed suicide, preparatory actions, and self-injurious behaviors [109]. However, these tools did not detect the presence or absence of other active suicidal ideation combination categories and aborted and interrupted attempts [109]. These results show the importance of validity exploration regarding the widely used scales for suicide risk assessment and the concordance with the FDA categories [109].

The use of single items from validated scales for depression as indicators for the assessment of suicidal ideation is a debated approach in clinical practice. A comparison of BDI and HAMD suicidal items and SSI scores was conducted in 281 suicide attempters and a positive correlation between the first two and the specific scale for suicide scores (SSI) [103].

The importance of searching for chronic diseases as predictors for suicide that can interfere with mental health due to negative impact on multiple levels (i.e., low functionality, poor quality of life, pain, low self-esteem) can not be overemphasized [110-113]. Therefore, any predictive model for suicide should take into account not only personality features, psychiatric symptoms, and actual psychological stressors but also organic diseases and their essential effects on mental health. This involves the necessity of a complex evaluation of individuals with a risk of suicide by a team of specialists, not only psychiatrists and clinical psychologists.

Treatment of the pathological background in patients with suicide risk can not be overemphasized [114-117]. Although significant advances in the field of psychopharmacology can be observed, as well as multiple psychotherapies focused on reducing the risk of self-harm, it is essential to note that no single intervention can be appropriate for all cases with self-harm tendencies, due to the unique configuration of vulnerability factors and triggers that exist in each case.

The current review's limitations are related to its narrative design, which explains why important sources may be missed from the analysis and the lack of assessing the quality of each report. The review's strengths are its focus on clinical tools used for military and civilian populations and the inclusion of psychometric properties together with relevant clinical and epidemiological studies for each instrument.

Directions for further research in this field are the need to further investigate the psychometric properties of some scales presented in this review, like MSAQ, which is dedicated to military personnel, and the comparative exploration of such instruments focused on clinical and non-clinical samples, to highlight the strengths and vulnerabilities of each. The final purpose of further research could be the construction of an algorithm able to detect individuals vulnerable to self-harm behavior.

CONCLUSION

Many good-quality, validated instruments allow for assessing the risk of suicide in civilian populations, and some of them have been explored in military personnel, like SBQ, C-SSRS, SASII, SOQ, etc., while others have been constructed especially for military populations, like MSAQ. Unfortunately, each of these tools has shortcomings that are expected to be corrected by further research.

Therefore, any algorithm for the detection of suicide risk based exclusively on the existing scales, interviews, and questionnaires would be vulnerable to a more rigorous analysis since the complex pathogenesis of the suicide phenomenon would impair its predictability.

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The author declares no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

As the only author, I assume the responsibility for data collection, writing, and revision of the manuscript.

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REVIEW

Oxidative Stress and Nutritional Antioxidants in Neurological Diseases

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Abstract: A significant number of neurological diseases are pathogenetically related to oxidative stress, including but not limited to cerebrovascular afflictions such as ischemic and hemorrhagic stroke, Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, and so on. Natural nutrients may help limit the impact of oxidative stress and, therefore, delay or prevent the impairment of these diseases. Although these natural components have not entered routine use, many have been studied in preclinical or even clinical settings with promising results. Therefore, the need to find the stage of the research in the field of validating the properties and clinical usefulness of such nutrients represents the main reason this narrative review was conducted. This analysis explored the PubMed database for papers related to the influence of natural nutrients on the onset and evolution of some of the most severe neurological disorders. The results of the review provide an overview of the pathways oxidative stress may undertake and how the use of nutrients may counteract these pathways. In conclusion, natural nutrients may have beneficial effects that can be impactful on clinical outcomes, but more good quality research in this field is needed before formulating any clear recommendation.

Keywords: oxidative stress, neurodegeneration, antioxidants, nutrients

INTRODUCTION

This narrative review aims to provide readers with a brief, yet pinpointed overview as to the therapeutic potential of nutritional antioxidants. Given that oxidative stress has a key involvement in the pathogenesis of many neurological diseases (neurodegenerative, cerebrovascular, or inflammatory), targeting oxidative stress through such components (known to medicine for hundreds, if not thousands of years) may prove efficient. Moreover, this paper should be viewed as an incentive to inspire future research, preclinical and particularly clinical, in this field, so that the usage of such components may one day become a validated treatment with a probable wide spectrum. A step-by-step approach to nutrient intervention in neurological diseases is provided in Figure 1.

MATERIALS AND METHODS

Comprehensive research has been conducted by consulting the PubMed database for relevant reports published between the inception of the archive and January 2025.

The following keywords have been used by themselves or in various combinations to achieve optimal results: "Parkinson's disease", "amyotrophic lateral sclerosis", ”Alzheimer's disease", ”multiple sclerosis", ”small vessel disease", ”ischemic stroke", ”hemorrhagic stroke", "inflammation", "oxidative stress", "reactive oxygen species", "mitochondrial dysfunction", "apoptosis", "necrosis", "excitotoxicity", "lipid peroxidation", "vitamin C", "vitamin E", "coenzyme Q10", "black tea", "green tea", "taurodeoxycholic acid", "flavonoids", "curcumin", "polyphenol", "antioxidant", "Ginkgo biloba", "mecasin", "citicoline", "Artemisia", "Silybum", "curcumin", "melatonin", "saffron", "vitamin D3", "Gastrodia elata", "lavender oil".

Case reports, preprints, and standalone abstracts have been excluded. Only papers published in English were included in the selection.

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Figure 1: Addressing neurological diseases through nutrient intervention

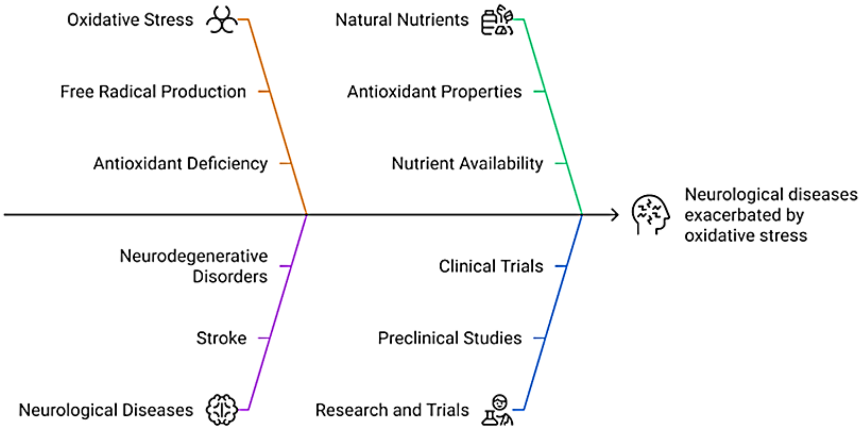

Oxidative stress is a central component in the pathophysiology of a plethora of neurological diseases. Nutrients may be an appealing method to target oxidative stress and, therefore, mitigate or perhaps even prevent these afflictions. However, the impact of nutrients has to be appropriately measured through validated, reproducible clinical trials before they can broadly enter therapeutic practice.
This figure has been generated using Napkin AI.

RESULTS AND DISCUSSIONS

Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative disease with a characteristic loss of the dopaminergic neurons in the pars compacta of the substantia nigra [1]. Literature has shown that environmental factors (herbicides, pesticides (such as paraquat), industrial pollutants, neurotoxins (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or MPTP) and its metabolite (1-methyl-4-phenylpyridinium), as well as genetic factors (mutations of DJ-1, Parkin, PINK1) [2] involved in PD pathogenesis induce oxidative stress (OxSt) in the mitochondria [3], reduce the production of adenosine triphosphate (ATP), facilitate the generation of reactive oxygen species (ROS), and finally lead to the apoptosis of dopaminergic cells [2].

Pathogenesis

1 Oxidative stress and mitochondrial dysfunction

OxSt contributes to the degeneration of dopaminergic cells in PD [4]. Also, OxSt is tightly linked to other pathogenic mechanisms involved in PD (mitochondrial dysfunction, excitotoxicity, and inflammation); therefore, it is difficult to ascertain whether it is a primary factor in PD or a consequence of the aforementioned mechanisms [3].

In PD, lipid peroxidation is upregulated, and antioxidant pathways (superoxide dismutase – SOD, catalase, glutathione peroxidase – GPx, ceruloplasmin, vitamin E, vitamin C, copper, zinc, selenium) may be insufficient to protect the cells from pathological oxidation [4]. Increased OxSt in association with diminished antioxidant protectors leads to the loss of neurons in the pars compacta, hence the development of PD [4]. Studies have proven lower levels of vitamin C and E in PD patients versus control groups, launching the hypothesis of increased consumption of these vitamins in PD due to OxSt [4].

Excessive ROS production, mitochondrial transport changes, and increased iron deposition in the pars compacta of the substantia nigra are key pathways in PD pathogenesis [1]. There is a close link between ROS formation and mitochondrial dysfunction [1]. Cellular death in PD is thought to be connected to excessive lipid peroxidation, occurring as a consequence of increased oxidation and expenditure of compensatory mechanisms [1]. ROS have a direct cytotoxic impact, as well as an indirect one by impacting mitochondrial complex I, with higher lipid peroxidation [1]. The reduction of this complex leads to the production of more ROS [1].

2 Neuroinflammation

In PD, α-synuclein deposition induces an activation of the microglia in the pars compacta, thus activating nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase) [1]. The latter's consequences are, on the one hand, ROS production in the extracellular compartment afflicting nearby neurons, and on the other hand, redox signaling in the microglia, amplifying the pro-inflammatory response with the induction of chronic inflammation [6].

The presence of excessive OxSt in PD is supported by studies that have proven an increase in carbonyl derivatives [7], in indicators of lipid peroxidation [8], and 8-hydroxy-2'-deoxyguanosine (a marker of nucleic acid damage) [9].

Nutrients

Given the involvement of OxSt in the occurrence of PD and the fact that PD treatment (especially L-dopa) may determine, after a long period of usage, the appearance of free radicals, the administration of multiple antioxidant agents in high dosage along with L-dopa may increase the latter's efficiency [10,11].

In experimental studies, vitamin E was proven to protect cells from OxSt-related damage in PD and the chronic administration of high doses of vitamin E may be a therapeutic strategy in PD treatment or prevention [3,12]. In other experimental studies, vitamin E diminished the OxSt induced by midbrain iron deposits in PD patients, suggesting it may have neuroprotective effects in these patients [13].

Other observational studies on human subjects suggested that a combined administration of high doses of vitamins E and C may determine a slowed PD progression through their antioxidant effect (14), although their efficacy was not proven in double-blind randomized clinical trials (RCTs) [15].

Taurodeoxycholic acid is an antioxidant agent, decreasing ROS reduction, an effect that has been proven when in vivo PD lab models were used [16].

Coenzyme Q10 has an important antioxidant role in the mitochondria and the lipid membrane, some of its antioxidant effects being mediated by its interaction with α-tocopherol. A phase 2 clinical trial on the efficiency of the antioxidant effect of the coenzyme on early PD patients (i.e., patients who did not require specific therapy) versus placebo showed a decrease in the treated patients' disability [17].

Black and green tea, due to high polyphenol concentration, exert an antioxidant effect and may have a neuroprotective and neuroregenerative effect in this disease, although clear evidence to support this assertion, derived from clinical research, is scarce [18].

Flavonoids enhance the endogenous activation of antioxidant enzymes, inhibit the peroxidation of lipids, and reduce the production of inflammatory mediators [19]. Due to all these actions, flavonoids exert a favorable effect on dopaminergic neurons and mitigate PD manifestations in laboratory studies [19].

An association between PD and a lack of adherence to the Mediterranean diet suggests a potential neuroprotective effect of this diet, through an undetermined mechanism that may attenuate the OxSt and inflammation, which are both recognized pathogenetic factors in PD [20].

Alzheimer's disease and small vessel disease

Alzheimer's disease (AD) is a neurodegenerative affliction characterized by the dysfunction of memory, attention, and other cognitive processes; it is the most common form of dementia [21]. Small vessel disease (SVD) may affect microvasculature systemically or mainly in the brain, the latter constituting a devastating cause of stroke and dementia worldwide [22,23].

Pathogenesis

AD mainly determines a synapse affliction, with the development of senile plaques mainly containing β-amyloid (Aβ). Aβ derives from the proteolysis of the amyloid precursor protein performed mainly by the enzymatic actions of β- and γ-secretase, with an imbalance between production and clearance causing the Aβ buildup [21]. The lesions produced by OxSt in AD are associated with an accumulation of Aβ [11]. Aβ buildup is also a central feature of the cerebral amyloid angiopathy form of SVD, to which AD is intimately connected; some might argue that the two constitute an inseparable spectrum [22]. The other form of SVD, deep perforator arteriolosclerosis, centrally features a blood-brain barrier (BBB) dysfunction, subsequent morphological and functional changes to the vascular wall, as well as myelin impairment [22,23].

Aβ determines the release of ROS by attacking mitochondrial enzymes (particularly cytochrome c oxidase), leading to mitochondrial dysfunction, increasing superoxide radicals, conversion to hydrogen peroxide (H2O2), the release of cytochrome, affecting the production of ATP and cellular apoptosis [21].

OxSt increases with aging and it is an important causal factor in the appearance of AD and other neurodegenerative age-related diseases, such as SVD. Moreover, OxSt is thought to bridge the connection between SVD and AD [24]. It is not yet fully understood how neurochemical factors influence the appearance of age-related cerebral lesions, but more and more data has shown the involvement of biometals (Cu, Fe, Zn) in Aβ accumulation. Copper is a potent mediator for the hydroxyl radical, and the high-affinity link between copper and Aβ (via histidine and tyrosine), as well as between zinc and the amyloid precursor protein and Aβ may contribute to the increased OxSt in AD [11].

Apolipoprotein E is a lipid transport molecule believed to be related to ROS and directly to lipid peroxidation in AD [25]. Carriers of apolipoprotein E4 more often have cerebral amyloid angiopathy [24].

To conclude, the involvement of OxSt in AD pathogenesis is supported by the high concentrations of Cu, Fe, Al, and Hg in the brain parenchyma, a high level of lipid peroxidation and DNA oxidation, as well as a decrease in polyunsaturated fatty acids, in cytochrome c oxidase activity, and energy metabolism [11]. OxSt could become a promising link between AD and SVD; therefore, research focusing on OxSt may decrease the neurological manifestations of both illnesses [24].

Nutrients

In preclinical research, caffeine has been associated with a tendency to lower the Aβ levels in early (familial) AD, because of its antioxidant properties [26]. Studies on cerebral amyloid angiopathy models are lacking.

Curcumin is an antioxidant and anti-inflammatory agent (inhibiting cyclooxygenase and lipoxygenase) that has been shown to reduce carbonyl derivatives, facilitating Aβ disaggregation, with possible beneficial effects in AD in animal studies [26]. Curcumin may also aid endothelial function, which is frequently impaired in SVD, as it has been shown to diminish the risk of stroke in animal models [27].

Vitamin E is a potent antioxidant that prevents oxidizing lesions induced by Aβ in cell cultures; it also delays memory impairment in animal studies on AD [28]. Some RCTs suggested vitamin E, through its antioxidant effects, may delay functional disability in patients with mild to moderate AD [29]. Vitamin E tocotrienols have demonstrated a positive impact on white matter lesions (associated with SVD) in an RCT [30].

Polyphenols (such as anthocyanins from berries, catechins and flavonoids from tea, curcumin from turmeric, and resveratrol from grapes) have an antioxidant effect with possibly associated neuroprotection, observed in preclinical models of AD [26]. Dizziness improved in SVD patients taking polyphenols [31].

The association between AD and a lack of adherence to the Mediterranean diet indicates that the latter may have a neuroprotective effect through an as-of-yet incompletely understood mechanism, potentially including an influence on OxSt and inflammation, both involved in AD pathogenesis [32]. Compliance with a Mediterranean diet lowered the burden of white matter lesions in SVD patients [33].

Due to their antioxidant properties, alkaloids such as groenlandicine, berberine, and palmatine from Coptis chinensis rhizomes may find their use in AD treatment [26]. Berberine promotes endothelial health and has been proven to support proper arterial elasticity in human subjects [34].

Milk thistle (Silybum marianum) diminishes OxSt and memory decline in laboratory studies of AD [35]. Milk thistle extracts also favored proper endothelial function in mice [36].

Ginkgo biloba, through its capacity to purge ROS, may reduce Aβ aggregation in AD, which is suggested by certain in vitro studies [26], but on the other hand, this theory has been contested in the literature [37]. Currently, there is little proof to support the routine use of Ginkgo biloba in AD patients [26]. Ginkgo extract had a beneficial yet limited effect on cognitively impaired mice models of SVD [38].

Melatonin overcomes apoptosis, prevents Aβ from interfering with mitochondrial DNA, diminishes lipid peroxidation, and, in high concentrations, protects from the OxSt induced by Aβ [39]. However, more studies are needed to prove its efficiency in delaying progression in early AD stages or in AD prevention [39]. Melatonin was shown to be a potential BBB integrity protector in cell cultures [40].

Coenzyme Q10 is an antioxidant factor that maintains mitochondrial membrane potential during OxSt, while also protecting neural cells from excessive Aβ deposition in laboratory studies of AD [26]. It also eliminates peroxyl radicals and may prove efficacious in AD [17].

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease defined by a loss of central and peripheral motor neurons [41].

Pathogenesis

The pathophysiology of ALS onset is quite complex, probably heterogeneous, and not fully understood [42]. OxSt is a common link between various pathogenetic mechanisms of ALS, contributing to: (a) mitochondrial dysfunction: high ROS levels may augment mutations in mitochondrial DNA, and mutations of the Cu-Zn-SOD1 mitochondrial gene are synchronous with increased OxSt;(b) protein aggregation: OxSt is a contributor to the mechanisms of abnormal aggregation of SOD1; (c) axonal transport: certain fragments of axonal neurofilaments are targeted by OxSt [43].

Environmental or modifiable risk factors may be the source of OxSt, incriminated in ALS pathogenesis: (a) smoking induces lipid peroxidation, DNA damage, and cellular death, smokers having a higher risk of ALS (sporadic form); (b) chemicals used in agriculture (pesticides, organophosphates) are linked to ALS and induce mitochondrial dysfunction and ROS production [44].

It is not clear whether OxSt is the main cause of ALS or if it is a consequence of all the other pathogenetic mechanisms involved, but the existence of SOD1 gene mutation among familial cases suggests OxSt is central to the pathophysiology of genetic forms of ALS, at the very least [43].

OxSt could also interfere with ALS pathophysiology by deteriorating the mitochondria that support presynaptic transportation and, hence, affecting presynaptic mechanisms involved in neurotransmission [45]. ROS and reactive nitrogen species (RNS) alter RNA processing, generating mitochondrial dysfunction that eventually leads to neuronal death [46].

In ALS, indicators of OxSt are increased, such as carbonyl derivatives (in sporadic forms), 3-nitrotyrosine (a marker of oxidative lesions, with a higher expression in sporadic forms as well as familial forms associated with SOD1 mutation), 8-hydroxy-2'-deoxyguanosine (a sign of damaged DNA), 4-hydroxynonenal (showing lipid peroxidation) [43].

Nutrients

Laboratory studies have proven that mecasin (also known as KCHO-1, a herbal extract) diminishes OxSt via the gp91phox and MAPK pathways in ALS patients. Mecasin improved motor function and delayed ALS onset in study models [47].

EGb761, a standardized Gingko biloba extract with antioxidant and neuroprotective effects, could prove useful in treating ALS patients [48].

To prevent OxSt from interfering in ALS pathogenesis, various antioxidant agents have been tested, such as vitamin E and vitamin B complex; however, there were no significant proven benefits [43].

Multiple sclerosis

Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system [49,50], defined by perivenous lymphocytic infiltration and macrophage presence in the cerebral parenchyma [51].

Pathogenesis

MS neuropathology is characterized by focal demyelinating lesions and axonal lesions. Neurodegeneration is induced by inflammation and mitochondrial dysfunction, with ROS production as a common element. ROS promotes transendothelial migration of leukocytes, contributing to axon and oligodendrocyte degeneration [52].

ROS occurs mainly by the activation of macrophages, microglia, and astrocytes and are produced through NADPH-oxidase, myeloperoxidase, and inducible NO-synthase. ROS leads to neuron, axon, myelin, and oligodendrocyte destruction and may contribute to mitochondrial dysfunction, which in turn exacerbates ROS production, augmenting MS lesion appearance [52].

Through lipid, protein, and DNA oxidation, OxSt leads to cellular dysfunction and, finally, to necrosis or apoptosis [53]. In the early stages of MS, OxSt is associated with microglial activation and the inflammatory process, whereas in advanced stages, it is linked to mitochondrial dysfunction, pathological iron accumulation in cerebral structures and its release in demyelinating lesions [54]. Myelin and oligodendrocytes are the most damaged by OxSt in this disease, a phenomenon that has been supported by the cytoplasmic accumulation of oxidated lipids and nuclear DNA alterations [54].

Nutrients

Melatonin presents neuroprotective effects and may decrease the OxSt in individuals with secondary progressive MS [55] by ROS neutralization and induction of antioxidant enzymes [49].

Coenzyme Q10 potentially diminishes OxSt and stimulates the activity of antioxidant enzymes in relapsing-remitting MS [56].

Saffron extract attenuated OxSt in experimental studies on MS [57].

Vitamin D3 has antioxidant properties and has been shown to halt lipid peroxidation in MS literature [58].

Ischemic stroke and intracerebral hemorrhage

Ischemic stroke (IS) is the most widespread type of cerebrovascular affliction and a relevant cause of death and disability worldwide. Intracerebral hemorrhage (ICH) is about 30% of all non-traumatic strokes worldwide, with a higher disability rate compared to IS [59]. The major cause of ICH is small vessel disease, a complex microvascular affliction of the brain [23].

Pathogenesis

Normally, the BBB protects against neurotoxins. After ischemia occurs, various mediators of inflammation, as well as other potentially neurotoxic molecules, cross the altered BBB to the brain parenchyma, contributing to neuronal lesions [60]. A breakdown of the BBB also occurs in ICH, as well as an enhanced proinflammatory response with a release of ROS [61,62].

OxSt is an important factor involved in the pathophysiology of IS [63]. Immediately after a cerebral injury is produced in IS as a result of vascular obstruction, the rapid increase of ROS determines the extension of the ischemic area [64]. OxSt also occurs in ICH and may surprisingly have a disseminated impact, such as the induction of glomerular dysfunction [65].

The brain is particularly sensitive to oxidative lesions. The main sources of ROS in the brain are the mitochondrial respiratory chain, NADPH-oxidase (especially isoforms 2,3,4 that are notably expressed in the central nervous system) and xanthine oxidase (XO). Superoxide ($O2-$), physiologically produced by the mitochondria, is converted via SOD to $H2O2$ and acts as a cellular messenger, with a role in neuronal signaling in the central and peripheral nervous system. Oxygen depletion in ischemic neurons favors anaerobic glycolysis, with higher lactate levels and acidosis. The latter contributes to OxSt, providing $H+$ and hence facilitating $O2-$ conversion to $H2O2$ [60].

After cerebrovascular flow is reestablished, the mitochondrial respiratory chain is reestablished with the generation of a new peak of ROS [63], contributing to cerebral reperfusion lesions [60]. $O2-$ is involved in reperfusion lesions and NADPH-oxidase contributes to its production [60]. Mitochondrial malfunction has also been detected in ICH around the hematoma [66].

During cerebral ischemia, ATP is broken down to hypoxanthine, which accumulates in the ischemic cerebral parenchyma, and during the reperfusion phase, XO facilitates hypoxanthine oxidation to xanthine and subsequently to uric acid, a process during which O2− and H2O2 are generated [60].

An increase in excitotoxic amino acids such as glutamate in cerebral lesions as well as in the perihematoma region in ICH also favors the production of ROS [63,67].

Excessive ROS leads to lipid peroxidation and a deterioration of proteins and nucleic acids, hence the lesion area is extended and finally, necrosis and apoptosis occur [63]. Lipid peroxidation is the main pathway through which ROS impacts neurons: phospholipase A2 is activated by ROS and subsequently releases arachidonic acid, further enhancing the production of ROS. By impacting lipids, ROS produces aldehydes, dienals, and alkanes, thus provoking neuronal apoptosis. The process of DNA oxidation has an early onset in ischemia, and, although it is initially reversible, then an irreversible deterioration of DNA and cellular death appear due to all the ROS production pathways and a blockage of antioxidant mechanisms [60].

Nutrients

Vitamin C, playing the role of an oxygen donor, can revert oxidative processes [60]. Vitamin E is a lipid antioxidant capable of inhibiting lipid peroxidation and acting as a ROS scavenger [68]. In an experimental study, vitamin C and E administration diminished lipid peroxidation and IS volume [69], but their therapeutic effects were not proven in human observational studies [68,70,71]. Vitamin C plasma levels are reduced in ICH patients [72] and administering vitamin C and E in combination with ICH rat models had positive effects [73].

Coenzyme Q10 is a component of the mitochondrial transport chain, the latter being involved in ROS production [74]. Coenzyme Q10 builds up in the mitochondria, having a powerful antioxidant effect, proving its efficacy in neuronal protection in IS experimental models [75]. Coenzyme Q10 therapeutic strategies in ICH are also under investigation [76].

Citicoline is a natural component that may reduce the release of fatty acids during lipid peroxidation [74]. Citicoline has demonstrated protective effects in experimental IS studies [77], but these results were not obtained in IS patients [78]. Small double-blind trials on citicoline in ICH have shown an increase in muscular strength, however, this needs to be proven in larger studies [79].

Wormwood (Artemisia absinthium), a plant of the Asteraceae family, increases ROS purging and diminishes lipid peroxidation, managing to reduce IS volume in experimental literature [80]. Eupatilin, an Artemisia-derived flavone with antioxidant properties, had neuroprotective effects by reducing inflammation in the microglia in ICH cellular models [81].

Ginkgo biloba extract reduces lipid peroxidation and protects neurons against OxSt in experimental studies [82]. There is no convincing data to support its routine use in IS patients [83]. A systematic review on ginkgo biloba extract in ICH patients has recently shown promise for this therapy, but further validation is needed [84].

Gastrodia elata is a plant belonging to the Orchidaceae family with antioxidant activity, capable of inhibiting glutamate neurotoxicity [85]. In animal studies, it confers neuroprotection against cerebral lesions caused by ischemia and reperfusion [86].

In animal models, lavender oil proved to have antioxidant properties, reducing ROS levels in ischemic or reperfusion lesions [87].

Cannabinoids, mainly cannabidiol, have been associated with neuroprotective effects and the studies with rat cortical neuron cultures exposed to toxic levels of glutamate support a further investigation of these compounds in different pathologies, including ischemic stroke [88,89]. Also, cannabidiol is increasingly investigated in other disorders, including psychiatric illnesses, due to its anxiolytic, antidepressant, and antipsychotic properties, and the benefit of being administered as an adjuvant to current treatment, thus potentially decreasing the rate of treatment resistance [90,91].

CONCLUSION

To summarize, whereas it may play a variety of roles in the pathogenesis of various neurological diseases, oxidative stress is a worthy target to consider in future therapeutic strategies. Natural nutrients may have beneficial effects that can be impactful, pending more validation in the literature. Vitamin E appears as a versatile option, with potential neuroprotective effects in PD, a possible functional role in AD, and an antioxidant capacity in both ischemic and hemorrhagic stroke, although its clinical role requires further, solid proof. Coenzyme Q10 could also be useful to PD, AD, MS, and stroke patients, with a vast potential awaiting exploration. Ginkgo biloba might also hold promise in AD and stroke, but there is limited evidence to support its routine use, despite clinicians nowadays often adding it to therapeutic regimens. Pending improved technologies to refine and standardize natural extracts, as well as solid and validated methodology usage in RCTs, novel therapies may include nutrients in a plethora of neurological diseases.

GLOSSARY

Aβ = β-amyloid; AD = Alzheimer's disease; ALS = amyotrophic lateral sclerosis; ATP = adenosine triphosphate; BBB = blood-brain barrier; GPx = glutathione peroxidase; ICH = intracerebral hemorrhage; IS = ischemic stroke; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NADPH = nicotinamide adenine dinucleotide phosphate; OxSt = oxidative stress; PD = Parkinson's disease; RNS = reactive nitrogen species; ROS = reactive oxygen species; SOD = superoxide dismutase; SVD = small vessel disease; XO = xanthine oxidase.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization I.I.E., M.M.M., S.M.G., D.D.; methodology M.M.M, D.D, R.N., C.I.S., I.I.E; software M.M.M, I.I.E.; validation M.M.M, S.M.G., D.D,; formal analysis M.M, D.D., I.I.E, S.M.G; investigation M.M.M., I.I.E, resources I.I.E., M.M.M.; data curation M.M.M.; writing—original draft preparation, I.I.E., M.M.M., R.N., C.I.S., D.D.; writing—review and editing I.I.E., M.M.M., R.N., C.I.S, D.D.; visualization M.M.M.; supervision M.M.M, D.D., I.I.E; project administration M.M.M, D.D., S.M.G. All authors have read and agreed to the published version of the manuscript.

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Patient consent for publication

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REVIEW

A Review of the Ethical and Legal Challenges in Clinical Trials

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Abstract: Clinical research is the cornerstone of the progress recorded in psychiatry in the last six decades, or of what is called the psychopharmacological revolution. Regardless of the stage of clinical research, there are sensitive ethical and legal aspects that may appear during this process, and such challenges have to be acknowledged in order to preserve its scientific value, integrity, and centered-on-the-patient-wellbeing core principles. Also, adequate strategies focused on preventing the risk of ethical misconduct and litigation have to be found to avoid a loss of trust in the results of clinical research, to protect vulnerable populations from abuse, and to ensure a legally stable environment for investigators. In order to explore these practical problems, a narrative review was performed through a search in PubMed and Google Scholar databases. The main ethical risks detected in clinical research were related to errors in the methodology of obtaining informed consent, monitoring the participant's safety during the clinical trial, and falsifying collected data. Several famous cases of ethical misconduct were found and analyzed, and methods to decrease the risk of the re-appearance of such problems have been listed. In conclusion, this review is an invitation to explore the complexity of the methodology of clinical research and its ethical and legal risks, as well as to find new ways to mitigate the possibility of such risks related to the research process.

Keywords: clinical trial; volunteers; informed consent; vulnerable population; pharmacological agents; psychopharmacology; ethical misconduct

INTRODUCTION

Clinical research is an essential component of the broader paradigm of evidence-based medicine that is focused on ensuring scientific progress in healthcare. Although the evolution of medicine is an important objective, it must always be kept in mind when discussing clinical trials that the ultimate goal is to increase the quality of services offered to patients and improve their prognosis and chances of regaining a normal life. To refer only to the field of psychiatry, the developments recorded in the armamentarium of psychotropics in the last six decades represent a veritable revolution that allowed a change in the nosological paradigm of psychiatric disorders. Due to this change, which showed there is a biological basis for mental illnesses and that pharmacological agents can mitigate their symptoms, the stigmatization and the tendency to institutionalize patients with such diagnoses decreased and are expected to diminish further in the near future.

The importance of clinical research is illustrated by the high number of trials identified by a search in the International Clinical Trials Registry Platform (ICTRP), a World Health Organization database, that reported the existence of 10606 depression studies in 2024 [1]. According to the US National Library of Medicine database for clinical trials (ClinicalTrials.gov), more than 530000 studies are registered, out of which over 200000 are exploring a drug or a biological product, regardless of the pathology targeted, at the beginning of 2025 [2].

The need to conduct clinical trials in psychiatry is supported by a large number of practical difficulties when confronting the reality of mental health problems. One of the most critical challenges for clinicians is to increase the rate of responsiveness in treatment-resistant psychiatric illnesses, being known that 30-35% of depressive disorders, 30% of schizophrenia spectrum disorders, and up to 25% of bipolar disorders do not respond well enough to the usually administered pharmacological agents [3-7]. Another significant motive to foster clinical research in the field of psychiatry is the continuous quest to find new formulae to enhance therapeutic adherence (e.g., other ways of administration for a particular substance, coupling

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the active drug with a vehicle that allows a slower release of the active substance so that the interval between administration could increase, or to find molecules with superior tolerability). Changes in the pharmacodynamic or pharmacokinetic properties of certain drugs that are already available for clinical use could lead to the discovery of new drugs with potentially superior properties. Covering emerging health needs, including mental health-related problems, such as the long COVID-19 syndrome, new psychoactive substances (NPS) use disorders, etc., is another important reason for conducting clinical trials.

Some of the above-mentioned directions of research have been more productive than others, while high expectations are related to the objectives insufficiently accomplished, like detecting pathogenic rather than symptomatic treatments or improving the tolerability of already existent drugs for treatment-resistant cases. For example, when discussing the improvement of treatment adherence, the discovery of long-acting antipsychotics that allow patients with schizophrenia to receive only two injections each year (i.e., paliperidone palmitate with 6-month release) could be considered significant progress [8-10]. The production of active metabolites as independent drugs, a process starting with tricyclic antidepressants, like nortriptyline from amitriptyline, or desipramine from imipramine, and continuing with new-generation antidepressants and atypical antipsychotics, like desvenlafaxine from venlafaxine, or paliperidone from risperidone, allowed for improving and diversification of pharmacokinetic and pharmacodynamic properties of the parent drugs [11-15]. Another direction of research is improving the pharmacodynamic properties of a racemic mixture by extracting only the most potent enantiomer, as was the case of escitalopram, levomilnacipran, or eszopiclone [16-20]. Even one of the most sensitive topics in psychopharmacology, i.e., the pathogenic vs. symptomatic treatments, acknowledged significant progress in the last years by the Food and Drug Administration (FDA) approval of monoclonal antibodies that target beta-amyloid peptides, like aducanumab, donanemab or lecanemab [20-23].

The responsibility for conducting clinical trials belongs to medical professionals, primarily doctors, but also to other categories of healthcare personnel – registered nurses, clinical psychologists, etc., who follow specific training programs before engaging in any research activity. Completing these training programs provides professionals with a common background of knowledge and skills necessary for participation in pharmacological studies. Specific certifications, such as Good Clinical Practice (GCP)– ICH Guidelines E6 R2, or qualifications for scoring scales and inventories used to measure the severity of clinical symptoms, have the aim to protect participants in clinical studies, reduce the risks of malpractice for investigators, increase the validity of data obtained from the research, and decrease the inter-rater variability of clinical scale results [24-27].

The Declaration of Helsinki regarding human experimentation originated in 1964 in the World Medical Association (WMA) statement and has been constantly revised ever since, with the last revision in 2024 [28-30]. This declaration is an essential step in the history of conducting research with human subjects, as it stipulates the fundamental principles of this domain, for example, the unconditioned respect for individuals and their right to self-determination and making informed decisions regarding their own health, including aspects pertaining to participation in clinical studies [28,30]. The primary duty of the investigators is to the patient or volunteer included in clinical studies, and the participant's well-being takes precedence over the interests of science and society [28-30]. This declaration also stipulates that ethical aspects are primordial and laws and regulations are only second in importance [28-30].

Ethical and legal errors in pharmacological research have the potential to harm not only study participants but also the integrity of the entire research process, thus indirectly affecting a very large number of patients who could benefit from a specific investigational product once it is approved. This narrative review is dedicated to highlighting the most relevant domains of clinical research that are vulnerable to malpractice risk and to investigate possible ways to decrease the probability of its onset.

MATERIALS AND METHODS

Two electronic databases (PubMed and Google Scholar) were searched for the terms „malpractice," OR „ethical issues," OR „methodological errors," OR „legal issues," AND „clinical trials," OR „pharmacological research," OR „drug research," OR „psychopharmacology." All primary and secondary reports published in English were selected if they corresponded to the inclusion criteria and did not meet the exclusion criteria. In order to ensure adequate coverage of this topic, grey literature was also searched for papers published in English. The period chosen for published papers was between the inception of each database and January 2025. Inclusion criteria: all types of research and commentaries, press releases and related sources of information; all stages of clinical trials, if related to the field of psychiatry; pharmacological interventions, either alone or in combination with other interventions. Exclusion criteria: non-pharmacological interventions used alone; reports that did not contain precise data on ethical or legal issues related to clinical trials in psychiatry; undefined outcomes or imprecise source of the referenced studies.

RESULTS

From the clinical research perspective, ethical and legal error problems appear when a doctor participating in clinical trials (hereinafter referred to as "investigator") does not comply with the correct way of obtaining adequate informed consent, mismanages patient safety measures, or falsifies research data. These actions can lead to serious consequences for participants, undermine the validity of clinical trial results, and negatively impact the medical community and society as a whole through the decrease of individuals' chances of getting the optimal cure.

Ethical and legal challenges related to obtaining informed consent

Signing informed consent is the first procedure a participant in a clinical trial has to perform prior to any study-related procedures [31]. This step is mandatory in the era of clinical trials conducted under rigorous ethical principles, according to the principles of the

Declaration of Helsinki [28-30]. The procedure of informed signed consent is mentioned in the GCP guidelines and reflects the principles of absolute respect for patients' autonomy and their right to be informed in every aspect of the trial that is relevant to their own health [24-27]. Also, the Declaration of Helsinki stipulates that whenever the participants are unable to give their own consent, surrogate consent may be obtained from an individual acting in the participant's best interest, but the consent of the participant still needs to be obtained if at all possible [28-30,32]. The cases when this procedure is applicable are also presented in the Declaration of Helsinki [28]. The investigator must ensure that this consent is given freely, without any form of coercion, and with a comprehensive understanding of the implications of the study [28].

Possible errors in this domain, which are compatible with a risk of malpractice, are (a) a failure to disclose potential risks of the trial, (b) providing false or intentionally incomplete information, and (c) coercing patients to participate in the study.

For example, if an investigator conducting a study fails to properly explain to participants the adverse events or health risks of the investigational product, patients could be exposed to a danger to their health that they did not voluntarily assume in the very beginning. A study exploring the proportion of participants in clinical trials (N=135 cohorts) who were aware of the different elements of informed consent showed that only 52-75.8% understood what they were signing [33]. According to this study, 74% of the participants were aware of potential benefits and less than 70% of the study's aim, while 66% understood the rule of confidentiality [33]. Also, the proportion of participants who understood the informed consent did not increase during the 30 years of the studies included in the review [33]. These data indicate the need for better communication between investigators and participants during the signing of the informed consent form.

According to the GCP-ICH, both the informed consent discussion and the written informed consent form and any other written information to be provided to subjects should include explanations of the testing hypothesis process involved by the clinical trial, which is the purpose of the trial, understanding the fact that the treatment is not validated and what is the probability of random assignment to each treatment/placebo [24-27]. Also, the participant must receive adequate information about the study procedures to be followed, including all invasive procedures, which are his/her responsibilities, those aspects of the trial that are experimental, and the reasonable risks that can be anticipated or inconveniences to the subject and, also, the reasonably expected benefits [24-27]. Other elements included in the informed consent, according to the same source, refer to the alternative procedure(s) or treatment(s) that may be available to the subject, and their potential significant benefits and risks, the compensation and/or treatment available to the subject in the event of study-related harm, and the anticipated payment, if any, to the subject for participation in the study [24-27]. The list of all elements that constitute an informed consent form is too extensive to be reproduced here, but this complexity explains the long time necessary for the investigator to explain the totality of the relevant aspects to each participant in order to obtain his/her approval to participate in the trial.

Patient safety and monitoring of adverse events

Investigators are responsible for carefully monitoring the psychobiological parameters of each participant in a trial, ensuring that subjects are not exposed to avoidable health risks [24-27]. This process presumes a regular verification for the existence of adverse reactions, offering adequate interventions if these appear and discontinuing participants' involvement if they develop a serious adverse event. Failure to comply with this principle is a severe form of malpractice and a clear violation of the core principles of the Declaration of Helsinki [28-30].

For example, if an investigator continues to administer an investigational product to a patient despite clear signs suggesting the onset of severe side effects, he or she is engaging in a form of negligence that can have severe legal consequences far beyond the violations of ethical conduct. In this area are placed the cases when participants who remain pregnant during the clinical trials are still receiving the investigational product or when adverse events to the experimental drugs are treated with medications not allowed by the study protocol, thus disregarding the potential of pharmacologically dangerous interactions. According to a study [34] focused on the understanding of potential trial participants regarding the monitoring and communication of serious adverse events during a clinical trial, it was observed that their awareness of safeguards or regulations in this domain was very scarce; also, many of them expressed the desire to be more informed about the potentially serious adverse events and short reporting deadlines. The same study reported on the concerns of participants about potential financial conflicts of interest in monitoring and further reporting serious adverse events during a clinical trial [34].

In the same direction, the 2024 revision of the Declaration of Helsinki notes that unproven interventions focused on alleviating pain or suffering must not bypass ethical safety measures or avoid evaluation by controlled clinical trials [35], highlighting the importance of conducting an accurate assessment of all health parameters even in the most severe patients.

New tools have been suggested for the most accurate monitoring of safety in clinical trials, including those using graphical visualizations that are congruent with the FDA/EMA (European Medicines Agency) guidelines [36]. Such tools are interactive and support regular medical monitoring and oversight [36]. The National Institutes of Health in the US require data and safety monitoring boards for all phase III clinical trials, which are conceived as a supplementary barrier to errors in safety monitoring [37].

Data falsification and breaking the research integrity

The accuracy of data collection in the clinical research process and the integrity of the analysis of information regarding the efficacy and tolerability of the investigational product are essential to provide valid results. Ethical breaks and legal issues can be detected in this field when an investigator manipulates or falsifies clinical data to obtain some desired results [38,39]. Data falsification exerts significant prejudice on the validity of research, endangers the patient's safety, and violates the core ethical principles of medicine.

For example, such an unethical approach can lead to the approval by competent institutions of harmful or ineffective treatments for clinical use, which can significantly endanger future patients [38-41]. Furthermore, new research could be based on a false presumption, and this could exponentially amplify the negative economic and medical consequences of a single false study [38-41].

Several examples of data falsification and research integrity break are: (a) selective reporting, which is a form of reporting only data that supports the drug's efficacy while omitting negative or inconclusive results; (b) data harvesting, a phenomenon that refers to the selective analysis of certain variables that fit a desired outcome, ignoring others; (c) partial monitoring of tolerability data, which may be observed when there is a lack of monitoring or inadequate reporting of adverse effects that occur during the study; (d) lack of adequate monitoring of participants after the study ends to confirm that the investigator ensures that any long-term effects of the drug are documented and managed appropriately; (e) underestimation of risks, which refers to the situation when the investigators minimize or ignore potential risks that expose participants to short, medium, and long-term negative effects [38-43].

Another example of phenomena that negatively interact with research integrity is the participation in clinical trials of investigators with insufficient experience or without enough credentials, and this is a direct violation of the Declaration of Helsinki [28-30]. Thus, allowing individuals without the necessary certifications or knowledge to conduct or supervise critical aspects of a trial is an example of ethical misconduct [28-30]. Failure to provide adequate training to staff on ethical practices, safety protocols, or specific clinical trial procedures is also a breach of research integrity [28-30].

Ethical integrity can also be breached by improper recruitment and enrollment of participants [28-30,38]. For example, the inclusion of ineligible participants might refer to the enrollment of individuals who do not meet the inclusion criteria for the study, such as those with contraindicated health conditions or who are already taking other medications prohibited by the study protocol. On the other hand, but in the same category of ethical breaches, the exclusion of eligible participants refers to discrimination against specific populations, such as vulnerable groups, in order to distort results or save time. Such examples are the inclusion of participants diagnosed with major depression and active suicide ideation, although the study protocol prohibits their enrollment, and, on the other hand, exclusion of patients with severe depression due to the investigator's fear they will be challenging to manage, although the study protocol allows their participation.

Ethical breaches may be derived from protocol deviations and protocol violations [44,45]. The first case refers to divergences between the study activities from the institutional review board (IRB)-approved protocol with minimal impact, while the second refers to divergences that reduce the quality or completeness of the data, inaccuracies in the informed consent form, or endanger the subject's safety, rights, or welfare [45]. For example, if the test procedures are modified or the treatment administration algorithm is changed during the trial without a clear rationale, these may affect the results and induce harmful reactions in the participants. Also, failure to adequately document protocol changes or deviations makes it impossible to assess their potential impact on the trial. Failure to report or address ethical concerns is a significant methodological and ethical or legal error that can be observed, for example, when an ethical review is neglected by failing to obtain approval from an IRB or ethics committee before the trial begins or failing to address ethical concerns raised during the review process [46].

Several ways to decrease the risk of protocol deviations are enhanced training for the investigators, regular monitoring visits, involvement of the Ethics committee in site monitoring, etc. [44]. In a study that explored 80 postgraduate dissertations, representing observational studies (74%) and interventional trials (10%), a significant proportion of protocol deviations (more than 42%) were due to nonreporting and incomplete documentation of the divergencies [47].

Consequences of malpractice in clinical trials

Clinical trial litigation has been rapidly escalating during the last decades, and defendants are not only principal investigators or sponsors but also IRBs, individual IRB members, study coordinators, etc. [48,49]. Beyond the legal repercussions, malpractice in clinical trials can have long-lasting effects on public opinion about medical research. Patients may become hesitant to participate in future clinical trials, fearing that their health may be at risk. As a consequence, the interest of the general public in research could decrease, and thus, the availability of new treatments for various disorders could be delayed, or potentially valuable drugs may even be abandoned.

Furthermore, the reputation of healthcare practitioners can be significantly damaged when cases of malpractice in clinical trials are discovered and exploited by mass media [50,51]. Such phenomena can fuel an environment of skepticism and mistrust, leading to the erosion of the relationship between the patient and the healthcare specialists. The impact is, consequently, negative not only at an individual level but also at a macro-social level, with consequences beyond the level of a research site or a certain clinical trial.

Examples of famous cases when ethical and legal violations of clinical research were detected

One of the first cases of ethical misconduct after the Nuremberg Trials, which brought to the general interest the dangers of experiments without ethical restraints, was related to the MKUltra studies (1955–1967) [52,53]. These involved „mind-control" techniques using LSD-25, and were sponsored by the CIA [52]. Many of the subjects were given LSD and other psychoactive substances without consent, and some were subjected to psychological manipulation; also, not all of the „investigators" were healthcare specialists, but they came from very diverse professional backgrounds, e.g., one of them was a magician, i.e., a student of Harry Houdini, while another was a CIA consultant with a shady history; tragically, this so-called experiment for mental illnesses became medical torture not very unlikely to the Nuremberg Trials [52,53]. Different reports describe the effects of these experiments on mental health, including the Veterans Health Initiative Report in 2003 [53].

The study 329 (1994–2001) investigated the use of paroxetine and imipramine in adolescents, and this research was later found to have serious ethical and scientific flaws, including selective reporting of results [54,55]. An independent reanalysis of the data from Study 329 (the RIAT initiative) showed that acute and long-term paroxetine and imipramine were harmful and ineffective in adolescents with major depression [54]. In the original study, paroxetine proved itself well-tolerated and effective in that population on multiple outcomes, i.e., the Hamilton Depression Rating Scale (HAMD), Schedule for Affective Disorders and Schizophrenia for Adolescents-Lifetime version (K-SADS-L), and Clinical Global Impression (CGI), while the withdrawal rates due to adverse events were 9.7% vs. 6.9% (paroxetine vs. placebo) [55]. The RIAT analysis presented several ways in which the presentation of safety data in clinical trials may influence the apparent tolerability of a drug, e.g., the use of an idiosyncratic coding system, failure to transcribe all adverse events from clinical records to an adverse event database, or filtering data on adverse events through statistical techniques [55]. This case is interesting because it represents an argument to make it available for independent analysis of all primary data available from a clinical trial [56].

The Ranbaxy Case (2006-2008) involved a major Indian pharmaceutical company in a significant controversy related to drug safety, more specifically to the quality of various generic drug products, including gabapentin and the antibiotic ciprofloxacin [57]. Ranbaxy was accused of falsifying data in clinical trials, including trials of generic drugs, related to the purity, strength and quality of these generics, and the case resulted in the company being fined $500 million by the US Department of Justice for selling counterfeit drugs and submitting false data to the FDA [57]. Incomplete testing records and inadequate programs focused on the assessment of the stability characteristics of drugs were detected by an FDA inspection in the Paonta Sahib facility in 2006 [57].

Other doubts about the regulation authorities failing to keep up with ethical issues in clinical research in India have been raised [58]. Although most of the alleged ethical violations were not confirmed, the controversy about the clinical trials in India has decreased dramatically the trust in clinical research originated in India [58]. Lack of informed consent from volunteers, conflict of interests surrounding the members of independent ethics committees, suspicions about the use of vulnerable populations, and lack of insurance or compensation in case of serious adverse events were subjects presented by activists [58]. In 2017, a guideline passed by the Indian Council of Medical Research was launched to improve the quality of clinical research in that country [59]. These guidelines approach sensitive topics, like the procedure to obtain informed consent, the respect for voluntariness, the definition of vulnerable population, the assessment of the benefits vs. risks balance, and the compensation for harm derived from participation in clinical trials [59]. Also, regularization of the Ethical Committee structure and functions are included in these revised guidelines [59].

Prevention of malpractice in clinical trials

The complexity of clinical research has attracted numerous debates over the last decades, and national institutions with responsibilities in the field of drug regulation have initiated their own rules and norms, besides international or regional ones. EMA, WHO (World Health Organisation), and WMA are the leading institutions with expertise in the regularization of clinical research [60-65]. National agencies, like the FDA in the US or the PMDA (Pharmaceuticals and Medical Devices Agency) in Japan, are also regulatory organizations with rich experience and ethical guidelines dedicated to clinical research [66,67].

In order to decrease the risk of ethical misconduct and litigations in this field, such institutions with responsibilities in the domain of monitoring the research activity must provide continuing education and training to physicians involved in clinical trials, ensuring that they understand the ethical, legal, and scientific responsibilities they bear. The activity of pharmacovigilance is also a topic that needs to be regularized because this stage is essential for ensuring post-marketing feedback from the patients. Phase IV studies are not at all less important than other clinical trials due to their importance in maintaining clinical use of only those drugs that prove themselves useful and tolerable, even after they pass the pivotal trials. The real safety profile of a certain drug may be defined only by continuing surveillance, and this requires an intense effort from patients, doctors, and regulatory institutions [68].

There is a need for effective oversight mechanisms, including local ethics committees, which evaluate the ethical, methodological, and safety aspects of clinical trials before and during their implementation. These committees act as a core protection for human research participants due to their independent review of the ethical acceptability or proposal for human research [69]. The importance of these organisms has been mentioned in the previous chapter, as avoidance of the conflict of interest within IRBs/Ethical Committees was a topic raised in the controversy surrounding clinical research in India [59].

Also, the transparency of research data implies compliance with rigorous protocols, with clear documentation of all procedures, decisions and results, which can be audited at any time by competent national and international institutions. This aspect's importance was highlighted by the Study 329 case, where an independent re-analysis of the original data provided different results [54].

DISCUSSION

The progress recorded in the treatment of psychiatric disorders in the last six decades could not be possible without clinical research based on the dedication of the investigators and volunteers who contributed in a significant way to this endeavor. Like any scientific domain, clinical research is not free of ethical and legal challenges, especially because, due to its sensitive nature, it mixes epistemological aspects (i.e., the experimental nature of the investigational process), with axiological (i.e., the liberty of the individual as a core value), and ontological ones (e.g., the biological vs. spiritual causation of our mental life). Thus, hypersensitive topics are related to each dimension; starting from the acknowledgment of the participants that they assume certain health risks in a clinical trial, continuing with the voluntariness vs. coercion dilemma when being recruited for a study, or with the liberty to decide when to withdraw from a trial, all these aspects may be negatively exploited and consequently may trigger ethical misconduct and litigation. Clinical research has received a lot of attention from both the scientific world and the public, not to mention the vehicle between the two, i.e., the media. Avoiding dogmatic thinking and understanding the complexity of clinical research is important for any investigator, as is the ability of scientists to maintain the transparency of the research to society, which is the final beneficiary of their work. The legislation in the field of clinical trials is an evolving organism, and the Declaration of Helsinki is the result of the conjugated effort of society and the scientists trying to prevent the repeating of tragic ethical misconduct as in the Nuremberg Trials, MK Ultra experiments, etc.

Because the number of clinical trials is continuously increasing as a result of the social pressure to find more performant and more tolerable pharmacological agents, not to mention the need to respond to newer threats to human health, there is an ever-higher need to control the potential risk factors for ethical deviations in clinical research. Therefore, the vulnerabilities related to the clinical research methodology explored in this review, i.e., those about the obtaining of informed consent, those connected to patients' safety, and those derived from data falsification, need to be continuously monitored and regulated by authorized institutions. The failure to do so would lead to a decrease of public trust in clinical research and virtually erase the line between scientific, validated-through-trials pharmacological agents and any other substance promoted by non-regularized commercial entities. This is a very important and actual topic because psychedelics (LSD included) or other substances with a risk of addiction are nowadays explored again for the use of various mental disorders, while various therapies with innovative mechanisms (e.g., focused on immune factors or gene modulation) are also emerging as potential solutions for multiple, psychiatric or somatic, illnesses [70-75]. Therefore, a continuous updating of ethical guidelines is needed to protect the participants in clinical trials from any type of abuse and to allow the investigators to work in a legally secure environment.

CONCLUSION

Pharmacological research activity is an essential component of the approach aimed at increasing the quality of medical practice by multiplying the existing therapeutic options for a specific medical condition. Evolution in the field of medicine is impossible to achieve, at least taking into consideration the current level of scientific knowledge, without clinical trials. Through this type of activity, progress in pharmacology is ensured, and the quality of patients' lives is increased while their chances of functional recovery and prognosis are improved.

Ethical and legal risks in clinical research derive mainly (but in no way exclusively) from (1) irregularities in obtaining informed consent of participants in the study, (2) failure of investigators to respect the safety of participants in the clinical study, and (3) incorrect reporting of data obtained from the research.

Controlling these risks involves respecting the stages of professional training of clinical trial investigators, as well as the efficient functioning of institutional control mechanisms in the field of pharmacological research.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, O.V, A.M.C.., and A.G.M.; methodology, B.M.P.; formal analysis, O.V, A.M.C.; investigation, O.V., A.M.C., B.P.M., A.G.M.; resources, O.V.; data curation, A.M.C..; writing—original draft preparation, O.V., A.M.C.; writing—review and editing, O.V., A.M.C., B.M.P.; visualization, A.G.M.; supervision, O.V., A.M.C.; project administration, O.V. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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ORIGINAL ARTICLE

Efficacy and Patient Satisfaction of Laser Labiaplasty for Labia Minora Hypertrophy: A Retrospective Analysis

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Abstract: Background: Labia minora hypertrophy can lead to aesthetic dissatisfaction and functional discomfort among women, affecting their quality of life and sexual self-esteem. Traditional surgical labiaplasty techniques are effective but are associated with higher complication rates and longer recovery times. This study aims to evaluate the efficacy, safety, and patient satisfaction of laser labiaplasty as a minimally invasive alternative for labia minora reduction. Methods: A retrospective analysis was conducted on 60 female patients who underwent laser labiaplasty. Preoperative assessments included medical history, gynecological examination, and patient-reported concerns. The procedure utilized a CO_2 laser for tissue excision without the need for sutures. Postoperative outcomes were evaluated through follow-up visits and telephone interviews, focusing on complications, recovery time, and patient satisfaction measured by the Subjective Global Aesthetic Improvement Scale (sGAIS). Results: The primary motivations for surgery were aesthetic concerns (85%) and functional discomfort (55%). The mean age was 34.7 years. Minor complications included transient postoperative bleeding (10%) and mild discomfort (25%), with no major complications reported. High satisfaction rates were observed, with 92% of patients reporting significant improvement (sGAIS scores of 4 or 5). Conclusions: Laser labiaplasty is an effective, minimally invasive procedure with high patient satisfaction and low complication rates. It offers a viable alternative to traditional surgical methods for women seeking labia minora reduction due to aesthetic or functional concerns.

Keywords: laser labiaplasty; labia minora hypertrophy; patient satisfaction; minimally invasive procedure; genital aesthetics

INTRODUCTION

Labia minora hypertrophy, characterized by the enlargement or elongation of the inner vaginal lips, has been a largely recognized condition [1,2]. Women experiencing this condition often report both aesthetic dissatisfaction and functional issues, such as discomfort during physical activities or sexual intercourse [3,4]. The increased visibility and awareness of genital aesthetics in contemporary society have led to a growing number of women seeking corrective procedures to address these concerns [5,6].

Genital aesthetics significantly impact a woman's sexual self-esteem and overall quality of life [7,8]. Studies have demonstrated that dissatisfaction with genital appearance can lead to psychological distress, reduced sexual function, and decreased confidence [9,10]. The rise of media portrayal of idealized genital images has further influenced women's perceptions, prompting many to pursue medical interventions to achieve their desired appearance [11].

Traditional surgical labiaplasty techniques, including edge resection, wedge resection, Z-plasty, and deepithelialization, have been employed to reduce labial tissue [12,13]. While effective, these methods are associated with risks such as scarring, altered sensation, asymmetry, and extended recovery periods. These potential complications have led both patients and clinicians to explore less invasive alternatives that offer effective results with fewer risks [14].

Laser labiaplasty has emerged as a minimally invasive option that utilizes laser technology to excise excess labial tissue precisely [15,16]. Introduced more than 15 years ago using a neodymium-doped yttrium aluminum garnet (Nd) laser, the technique has evolved with the adoption of carbon dioxide (CO_2) and erbium-doped yttrium aluminum garnet (Er) lasers [17]. These lasers offer advantages such as reduced intraoperative bleeding, minimal thermal damage, and enhanced healing, contributing to improved patient outcomes.

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Despite the growing popularity of laser labiaplasty, there is a paucity of comprehensive studies evaluating its efficacy, safety, and patient satisfaction. Most existing literature comprises small case series or focuses on surgical techniques without detailed analysis of patient-reported outcomes [18,19]. This gap underscores the need for robust clinical data to inform practitioners and patients about the benefits and limitations of this procedure. Therefore, the objective of this study is to retrospectively analyze our center's experience with laser labiaplasty in treating labia minora hypertrophy. By examining preoperative motivations, procedural details, postoperative outcomes, and patient satisfaction, we aim to provide valuable insights into the efficacy and safety of laser labiaplasty as a minimally invasive alternative to conventional surgical methods.

MATERIALS AND METHODS

This retrospective study analyzed data from 60 female patients who underwent laser labiaplasty between June 2020 and September 2024. Inclusion criteria encompassed women aged 18 years or older diagnosed with labia minora hypertrophy, who expressed aesthetic or functional concerns and completed a minimum of six months of follow-up. Exclusion criteria included active genital infections, uncontrolled systemic diseases, previous labiaplasty, or other genital surgeries.

All patients underwent a comprehensive preoperative evaluation. Medical and gynecological histories were obtained, focusing on reproductive history, menstrual cycle details, prior pregnancies, and any gynecological conditions or surgeries. Physical examination included measurement of labia minora dimensions using standardized calipers. Preoperative investigations included a Papanicolaou (PAP) smear, vaginal secretion analysis for infections, and a transvaginal ultrasound to assess pelvic anatomy.

The classification of labial hypertrophy identifies three types of labial hypertrophy, based on Stephane Smarrito's study [20]: Type I, known as the "flag," affects the anterior one-third and was found in 11% of the cases; Type II, or the "oblique," involves the middle third and accounted for 29% of cases; and Type III, termed "complete," includes the posterior third, observed in 60% of the patients. Symptomatically, Type I patients typically reported aesthetic concerns and discomfort from tight clothing without dyspareunia. Type II was noted for a fuller appearance, and Type III frequently presented with dyspareunia more so than the other types. Surgical approaches were tailored accordingly, with Type I cases treated using a superior pedicle flap technique for moderate labial resection, while Types II and III were approached with the lambda laser technique. This classification system allows for more targeted surgical strategies that align closely with patient symptoms and expectations, providing a significant improvement over the traditional method based solely on dimensional measurement.

The procedure was performed under local anesthesia. Initially, a topical anesthetic cream containing lidocaine 2.5% and prilocaine 2.5% was applied to the labia minora for 30 minutes. After the removal of the cream and disinfection with povidone-iodine solution, infiltration anesthesia was administered using 1% lidocaine with epinephrine 1:100,000 via a 30G needle. Preoperative markings were made to delineate the extent of tissue excision (Figure 1).

Figure 1: Preoperative markings

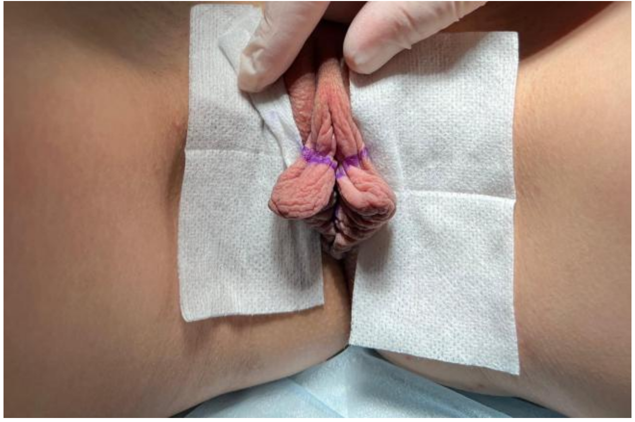

A CO_2 laser (CO_2RE, Syneron Candela) equipped with a surgical handpiece was set to a continuous wave mode at 10 Watts. The laser was used to excise excess labial tissue along the marked lines, ensuring symmetrical reduction and preservation of the natural contour (Figure 2). Hemostasis was achieved through the laser's coagulative properties, eliminating the need for sutures. After the procedure, the area was treated with topical fusidic acid ointment and covered with a sterile dressing.

Patients received detailed postoperative instructions, including applying fusidic acid ointment twice daily for one week; maintaining local hygiene with gentle cleansing using mild soap and water; avoiding sexual activity, strenuous exercise, swimming pools, and saunas for six weeks; and wearing loose-fitting clothing to minimize friction.

Follow-up visits were scheduled at one week, one month, three months, and six months postoperatively. Evaluations focused on wound healing, identification of any complications, and assessment of patient satisfaction using the Subjective Global Aesthetic Improvement Scale (sGAIS), a validated 5-point scale ranging from 1 (no improvement) to 5 (very significant improvement).

After the clamps were removed, the treated area was managed with targeted local applications of anti-inflammatory and antibiotic solutions. Specifically, Fucidin H cream, containing fusidic acid 20 mg/g and hydrocortisone acetate 10mg/g, was used in conjunction with Baneocin powder, which includes zinc bacitracin 250 UI and neomycin sulphate 500 UI/g. To control bleeding and further protect the site, a hemostatic sponge was placed over these topical agents, and the area was subsequently dressed. Pain management was addressed systemically through an intravenous administration of acetaminophen 10mg/ml, amounting to 100 ml, and an oral dose of Ketonal Forte, which contains ketoprofen at a concentration of 100 mg.

Figure 2: Excised excess labial tissue

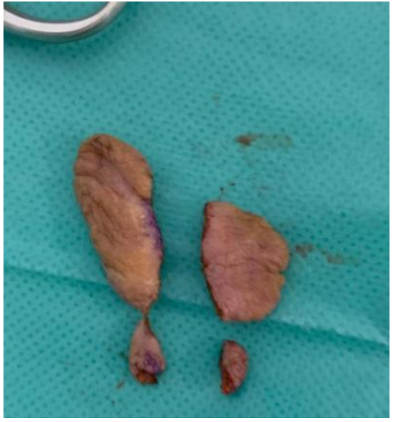

Discharge instructions provided to the patient emphasized crucial care steps to ensure proper healing. The patient was instructed to maintain local compression and restrict themselves to bed rest on the day of the procedure to minimize movement and stress at the site. For the initial three weeks post-procedure, the patient was advised to clean the area using Betadine and apply the previously mentioned Fucidin H cream and Baneocin powder twice daily. In the subsequent three weeks, the regimen shifted to cleaning with water and soap followed by the application of a protective repairing cream to foster skin restoration and barrier function.

Figure 3: Case 1. (a) Before and (b) six weeks after laser labiaplasty

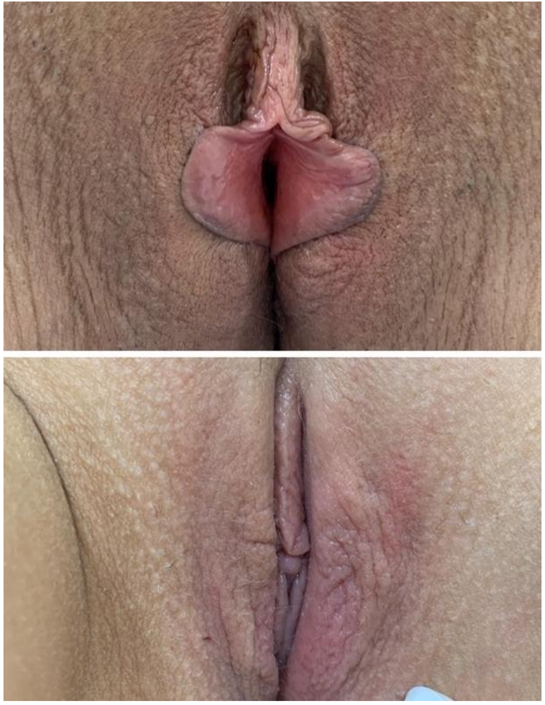

At the six-week post-procedure mark, a follow-up consultation was arranged to evaluate the healing progress and the cosmetic outcome of the laser labiaplasty. The provided figures (Figure 1 and Figure 2), display the condition of the area before the procedure and at the six-week follow-up. These images illustrate a notable reduction in labial size and improved symmetry, indicating a successful aesthetic enhancement. The area appeared to be well-healed without signs of infection or significant inflammation, underscoring the effectiveness of the postoperative care regimen.

Figure 4: Case 2. (a) Before and (b) six weeks after laser labiaplasty

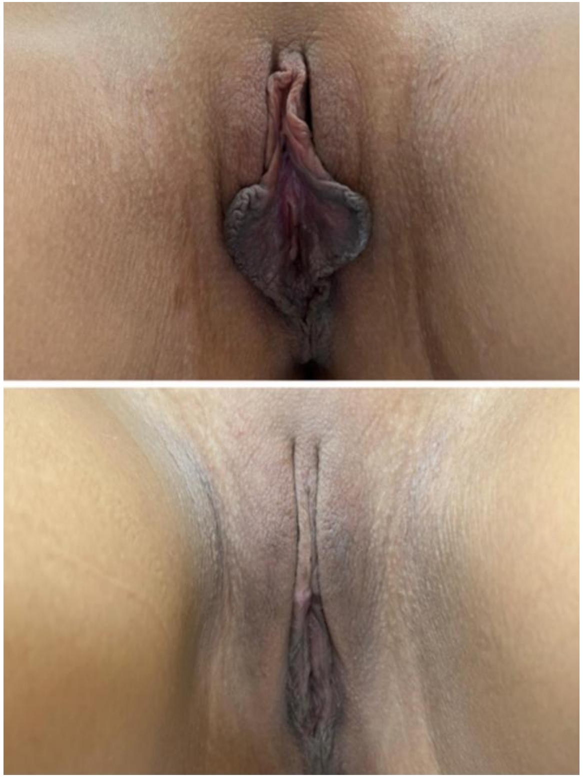

RESULTS

The study included 60 women aged between 19 and 52 years, with a mean age of 34.7 years. The average age at menarche was 13.1 years. Regarding reproductive history, 40% of patients were nulliparous, 35% had one pregnancy, and 25% had two or more pregnancies. Positive gynecological history, such as polycystic ovary syndrome, previous cesarean sections, or uterine fibroid removal, was present in 30% of patients. This demographic distribution indicates a diverse patient population seeking laser labiaplasty (Table 1).

Table 1: Demographic and Clinical Characteristics

Parameter	Mean (SD)	Range
Age (years)	34.7 (7.8)	19 - 52
Age at menarche (years)	13.1 (1.4)	11 - 16
Number of pregnancies	0.9 (1.1)	0 - 4
Positive gynecological history (%)	30%	N/A

SD – Standard Deviation

Aesthetic concerns were the primary motivation for 85% of patients, highlighting the significance of genital appearance in this cohort. Functional discomfort during daily activities, such as exercise or wearing tight clothing, was reported by 55% of patients. Discomfort during sexual intercourse affected 45%, indicating that labia minora hypertrophy can significantly impact sexual function. Recurrent infections or hygiene difficulties were less common but still noteworthy at 15% (Table 2).

Table 2: Preoperative Motivations

Motivation	Number of Patients (%)
Aesthetic concerns	51 (85%)
Functional discomfort during activities	33 (55%)
Discomfort during sexual intercourse	27 (45%)
Recurrent infections/hygiene issues	9 (15%)

Postoperative complications were minimal. Mild bleeding occurred in 10% of patients, resolving within an average of 2.3 days without intervention. Mild to moderate discomfort was experienced by 25% of patients, lasting approximately 4.2 days. Notably, statistical analysis revealed a significant association between previous pregnancies and postoperative discomfort (p=0.03), suggesting that multiparous women may experience more discomfort due to tissue changes from childbirth. No infections or hematomas were reported, indicating a favorable safety profile (Table 3).

Table 3: Postoperative Complications and Recovery

Complication	Number of Patients (%)	Duration (Days)	p-value
Mild bleeding - Total	6 (10%)	2.3 (1.1)	0.12
Mild to moderate discomfort – Nulliparous	6 (10%)	4.0 (1.5)	-
Mild to moderate discomfort – One pregnancy	5 (8.3%)	4.1 (1.6)	-
Mild to moderate discomfort – Multiparous	4 (6.7%)	4.5 (1.8)	0.03*
Infection	0 (0%)	N/A	N/A
Hematoma	0 (0%)	N/A	N/A

* *Student's t-test*

Table 4 provides a comprehensive overview of patient satisfaction following laser labiaplasty, as measured by the standardized Global Aesthetic Improvement Scale (sGAIS). The table categorized patient responses into five distinct levels, highlighting the overwhelmingly positive feedback received. Specifically, a majority of 33 patients, accounting for 55% of the total surveyed, reported a "Very significant improvement" in their condition, with an sGAIS score of 5. This indicated that these patients perceived the results of their surgery as greatly surpassing their expectations in terms of both aesthetic appeal and functional benefits. Additionally, 22 patients, representing 37% of the total, observed a "Significant improvement," scoring a 4 on the sGAIS. This suggests that for a large number of patients, the procedure substantially met their initial goals and resulted in marked enhancements.

On the lower end of the scale, 5 patients, making up 8% of the total, felt they had only "Moderate improvement," denoted by a score of 3 on the sGAIS. This group perceived the changes as noticeable but not as extensive as perhaps hoped for. Importantly, the table showed that no patients reported a lack of change or a worsening of their condition post-procedure, with zero reports in both the "No change" and "Worsened condition" categories (scores of 2 and 1, respectively). This absence of negative feedback underscored the effectiveness and safety of laser labiaplasty in achieving desired outcomes without adverse effects. The data clearly demonstrated a high level of patient satisfaction overall, with 92% of participants rating their improvement as significant or very significant, highlighting the procedure's ability to meet or exceed patient expectations in the vast majority of cases.

Table 4: Patient Satisfaction (sGAIS Scores)

Satisfaction Level	No/Mild Complications n (%)	Moderate Complication n (%)	Total	p-value*
Very Significant Improvement	32 (97%)	1 (3%)	33	
Significant Improvement	16 (73%)	6 (27%)	22	
Moderate Improvement	0 (0%)	8 (100%)	5	
p-value				<0.001

* *Chi-square test*

Among 60 patients, 34 who were satisfied and 17 who were less satisfied cited aesthetic concerns, against expected counts of 37.4 and 13.6, respectively. Functional discomfort during activities was reported by 28 satisfied and only 5 less satisfied patients, compared to expected figures of 24.2 and 8.8. For discomfort during sexual intercourse, 20 satisfied and 7 less satisfied patients were recorded, versus an expected count of 19.8 for satisfied and 7.2 for less satisfied. Lastly, recurrent infections or hygiene issues were less commonly reported, with 6 satisfied and 3 less satisfied patients, aligning closely with expected values of 6.6 and 2.4. The overall chi-square analysis resulted in a p-value of 0.306, indicating no significant statistical association across the groups (Table 5).

Table 5: Analysis of Complaints and Patient Satisfaction Post-Laser Labiaplasty

Complaint Type	Satisfied Patients (n = 40, %)	Less Satisfied Patients (n = 20, %)	Expected (Satisfied)	Expected (Less Satisfied)	p-value
Aesthetic concerns	34	17	37.4	13.6	
Functional discomfort during activities	28	5	24.2	8.8	
Discomfort during sexual intercourse	20	7	19.8	7.2	
Recurrent infections/hygiene issues	6	3	6.6	2.4	
p-value					0.306

Patients with Type III hypertrophy showed a substantially higher improvement in sexual discomfort (p < 0.001), aligning with their higher baseline rates of dyspareunia. Differences in aesthetic and functional improvements were not statistically significant across the three groups (p = 0.43 and p = 0.07, respectively), although Type II and Type III trended toward slightly greater gains than Type I. Reduction in hygiene-related complaints was similar among all types (p = 0.73). Overall satisfaction (sGAIS) remained high across all groups, with no statistically significant variation (p = 0.56), as presented in Table 6.

Table 6: Postoperative Quality of Life Differences by Labial Hypertrophy Type

Variable	Type I	Type II	Type III	p-value
Mean Improvement in Aesthetic Concerns (0-10 scale, higher = greater improvement)	4.7 ± 1.3	4.9 ± 1.1	5.1 ± 1.2	0.43
Mean Improvement in Functional Discomfort (0-10 scale)	5.4 ± 1.3	6.2 ± 1.4	6.3 ± 1.6	0.07
Mean Improvement in Sexual Discomfort (0-10 scale)	3.3 ± 1.0	4.6 ± 1.2	6.6 ± 1.5	<0.001
Patients Reporting Fewer Hygiene Issues (n, %)	2 (28.6%)	4 (23.5%)	10 (27.8%)	<0.001
Mean Postoperative sGAIS Score (1-5 scale*)	4.4 ± 0.5	4.3 ± 0.6	4.5 ± 0.4	0.56

* *Standardized Global Aesthetic Improvement Scale (SGAIs): 1 – Worsened; 2 – No Change; 3 – Moderate Improvement; 4 – Significant Improvement; 5 – Very Significant Improvement.*

DISCUSSION

Literature findings

The study demonstrates that laser labiaplasty is a safe and effective minimally invasive procedure for treating labia minora hypertrophy. The high patient satisfaction rates align with existing literature, indicating that laser techniques offer significant advantages over traditional surgical methods. The predominance of aesthetic concerns as a motivation reflects societal trends emphasizing genital aesthetics and their impact on women's self-esteem and sexual health.

The low incidence of postoperative complications, such as bleeding and discomfort, highlights the safety profile of laser labiaplasty. The precise excision and coagulative properties of the CO_2 laser reduce intraoperative bleeding and eliminate the need for sutures, contributing to faster healing and reduced postoperative pain. The absence of infections and hematomas in our cohort further supports the procedure's safety.

The significant association between previous pregnancies and increased postoperative discomfort may be attributed to changes in tissue elasticity and sensitivity resulting from childbirth. This finding suggests that multiparous women may require additional preoperative counseling and postoperative support to manage expectations and enhance recovery.

In the realm of female genital cosmetic surgery, the studies by Pardo et al. [21] and Bizjak-Ogrinc et al. [22] provide valuable insights into the efficacy and patient satisfaction associated with different techniques of laser labiaplasty of the labia minora. Pardo et al. [21] reported on 55 cases conducted between October 2003 and November 2004, where the primary indications were moderate hypertrophy and labial asymmetry, with 67% of cases due to these reasons. They noted a high satisfaction rate, with 91% of patients reporting being very satisfied and 9% satisfied, despite a 7% incidence of minimal suture dehiscence during the early postoperative period. In a similar manner, the study by Bizjak-Ogrinc et al. [22], which analyzed 80 patients from February 2015 to April 2018, found an equally high level of patient satisfaction with the sutureless laser labiaplasty technique. Their approach, interestingly, reported minimal side effects—7 instances of wound infection, and one case each of bleeding and hematoma formation, yet no patients were partly satisfied or dissatisfied. sutures.

Similarly, Li et al.'s study on 17 Chinese women, with an average age of 36.4 years, demonstrated significant improvements in vulvar texture and pigmentation after three treatment sessions, with a scoring system showing substantial changes ($p < 0.05$) and no adverse reactions [23]. Patients self-reported enhanced satisfaction, indicating both perceived and observed effectiveness. Similarly, Lauterbach et al. [24] assessed vaginal CO_2 laser treatment in a cohort of 25 women with sexual dysfunction and pelvic floor issues. They reported significant increases in vaginal elasticity and tightening post-treatment (54.8 ± 5.2 vs. 41.5 ± 6.3, $P = 0.0027$; 1.97 ± 0.25 vs. 1.32 ± 0.31, $P = 0.0014$), as well as improvements in pelvic muscle contraction strength (25.9 ± 3.5 vs. 16.5 ± 4.2, $P = 0.0011$) and FSFI and VHI scores. This study utilized vaginal tactile imaging (VTI) for objective assessment, adding robust quantification to support the clinical effectiveness of CO2 laser treatment in enhancing vaginal biomechanical properties.

Moreover, Messas et al. [25] reviewed 40 studies involving 3466 participants to assess the efficacy of fractional CO_2 ($FrCO_2$) laser therapy in treating genitourinary syndrome of menopause (GSM) and vulvovaginal atrophy (VVA) [25]. Their findings highlight the therapy's good efficacy and safety, with high patient satisfaction noted across studies. However, they also pointed out the lack of level I evidence and called for more randomized, sham-controlled trials to validate these results further and address clinical questions such as cost-effectiveness and potential synergistic effects with other treatments. Similarly, Géczi et al. [26] conducted a systematic review and meta-analysis of various labiaplasty techniques, analyzing data from 86 studies. They reported a high overall satisfaction rate (94%, CI 93%-95%) with specific techniques like deepithelialization, reaching even higher satisfaction rates (97%, CI 85%-99%). Despite these high satisfaction rates, they noted variations in complication rates across different techniques, with some, like the wedge resection, showing higher incidences of complications such as dehiscence. Both studies underscore the importance of personalized treatment approaches and the need for high-quality research to optimize outcomes in cosmetic genital surgeries.

The study by Smarrito et al. [27] provided a focused examination of secondary labiaplasties, reporting on 44 cases split into two main categories: those involving wound dehiscence primarily from lambda laser procedures and those related to over-resections or amputations. The authors highlighted the use of local flaps and lipofilling techniques to address aesthetic and functional issues resulting from over-resection, emphasizing the importance of surgical precision and anatomical knowledge to prevent such complications. In a similar manner, the systematic review and meta-analysis by Escandón et al. [28] surveyed a much larger cohort of 3804 patients across 46 studies, reporting a high satisfaction rate of 99% for labia minoraplasty. However, they also noted significant complications, such as a 5% incidence of dehiscence from laser-assisted labiaplasty and up to 8% hematoma formation from W-shape resection. Both studies underscore the necessity of thorough training in surgical techniques and patient-specific approaches to optimize outcomes and minimize the need for subsequent corrective procedures. The findings from Escandón et al. [28] further support the observations of Smarrito et al. [27] by quantitatively demonstrating the impact of technique selection on complication rates and patient satisfaction, thereby reinforcing the call for meticulous surgical practice and adequate anatomical understanding in labiaplasty procedures.

Limitations

This study has several limitations. While the study provides valuable insights, limitations include its retrospective design and the reliance on patient-reported outcomes, which may introduce bias. The lack of a control group undergoing traditional surgical labiaplasty limits direct comparison of efficacy and safety between techniques. The retrospective design may introduce selection bias, as only patients who returned for follow-up were included. The sample size, while larger than some previous studies, is relatively small and may not represent the broader population. Reliance on subjective measures like the sGAIS may not capture objective functional improvements. Additionally, the absence of a control group limits the ability to compare laser labiaplasty directly with traditional surgical methods. Future prospective, randomized studies with larger sample sizes are warranted to validate these findings and optimize patient selection and procedural protocols.

CONCLUSION

Laser labiaplasty is an effective and safe, minimally invasive procedure for women seeking labia minora reduction due to aesthetic or functional concerns. The high patient satisfaction rates and low incidence of complications make it a valuable alternative to traditional surgical techniques. Personalized patient counseling and careful consideration of individual medical histories can further enhance outcomes. Future research should focus on long-term results and direct comparisons with other surgical modalities to establish standardized guidelines.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, F.D.; methodology, F.D.; software, F.D.; validation, M-M.C. .; formal analysis, M-M.C.; investigation, M-M.C..; resources, S.A.; data curation, S.A.; writing—original draft preparation, F.D., M.M.C. and S.A.; writing—review and editing, D.O.C. and F.D.; visualization, D.O.C. and FD.; supervision, D.O.C.; project administration, D.O.C. and F.D.; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Elos Clinic from Bucharest, Romania (protocol code NR 29 and date of approval 6 January 2020).

Patient consent for publication

Consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

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REVIEW

Dermatoporosis. What We Know and What to Expect

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Abstract: With the increase of the medium lifespan in developing countries, skin aging rise attention more and more. In 2007, Kaya and Saurat rebranded the extreme expression of skin fragility under the name of ''dermatoporosis'' or chronic cutaneous insufficiency/fragility syndrome. Dermatoporosis is still underused in medical literature and nurses and wound specialists know also about this condition, but they call it "skin tears". There is obvious confusion cause the nursing literature talks about skin tears while dermatologists write about dermatoporosis. Clinical aspects comprise atrophy, purpura, and pseudo-stellate scars. Firstly, the British dermatologist Thomas Bateman described senile purpura in 1818 as purpuric patches arising in elderly people after minimal trauma, especially on the dorsum of the hands. Furthermore, between 1970 and the full documentation of Kaya and Saurat in 2007, we found medical papers about senile purpura, pseudoscars, skin tears, and so on. Starting from this historical data, they bring valuable information according to clinical aspects, histopathology, ultrasonographic aspects, pathophysiology, and therapeutics. This paper aims to analyze published data for improving the diagnosis and management of dermatoporosis.

Keywords: dermatoporosis

HISTORY

With the increase of the medium lifespan in developing countries, a new type of dermatosis was described in 2007 by Kaya and Saurat under the name of 'dermatoporosis' of chronic cutaneous insufficiency/fragility syndrome [1,2,3]. Clinical aspects comprise atrophy, purpura, and pseudo-stellate scars (Figure 1). Firstly, the British dermatologist Thomas Bateman described senile purpura in 1818 as purpuric patches arising in elderly people after minimal trauma, especially on the dorsum of the hands [4]. Later, in 1967 Colomb reported pseudo-stellate scars as fibrous scars with a stellate aspect arising on the forearms of the elderly [5]. One year after this description, Frederik and Zak made the connection between stellate pseudoscars and senile purpura, describing that both arise on the forearms of the elderly after minimal traumas. They also report that the shape of these scars can be round or linear, not only stellate [6]. In 1972, Colomb described the trade consisting of atrophy, Bateman's purpura, and spontaneous pseudo-scars The term pseudoscars seems to be related to the fact that there is no solution of continuity with the exterior, the lesions are produced by a compression trauma without true lesions in the extreme external part of the skin, the epidermis [5,7]. Furthermore, there is a lack of reports between 1970 and the full documentation of Kaya and Saurat in 2007. Starting from this historical data and personal clinical cases, they bring valuable information according to clinical aspects, histopathology, ultrasonographic aspects, pathophysiology, and therapeutics [3]. The prevalence of dermatoporosis is about 30% in patients older than 65 years, but this aspect can vary according to the recruitment group: non-hospitalized patients, inpatients, outdoor patients, and patients for rehabilitation centers. The aim of this paper is to analyze published existing data for improving the diagnosis and management of dermatoporosis.

RISK FACTORS AND COMORBIDITIES

Only the future will decide if dermatoporosis is the expression of the general process of senescence, chronic sun exposure or even both [3]. Firstly, some data indicate that patients could have a genetic predisposition according to the connection with atopic dermatitis [8]. Furthermore, analyzing some aspects of aquaporines and skin aging previous statement is increased [9]. Aging plays a major role, the aging process acts mainly through chronic sun exposure, especially the professional type [3].

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Figure 1: Clinical aspects of dermatoporosis: A) Dermatoporosis located on the forearms; B) Dermatoporosis located on the forearms and anterior thorax; C) Superficial hematoma after a minimal trauma; D) Deep hematoma in a patient with severe and extended dermatoporosis

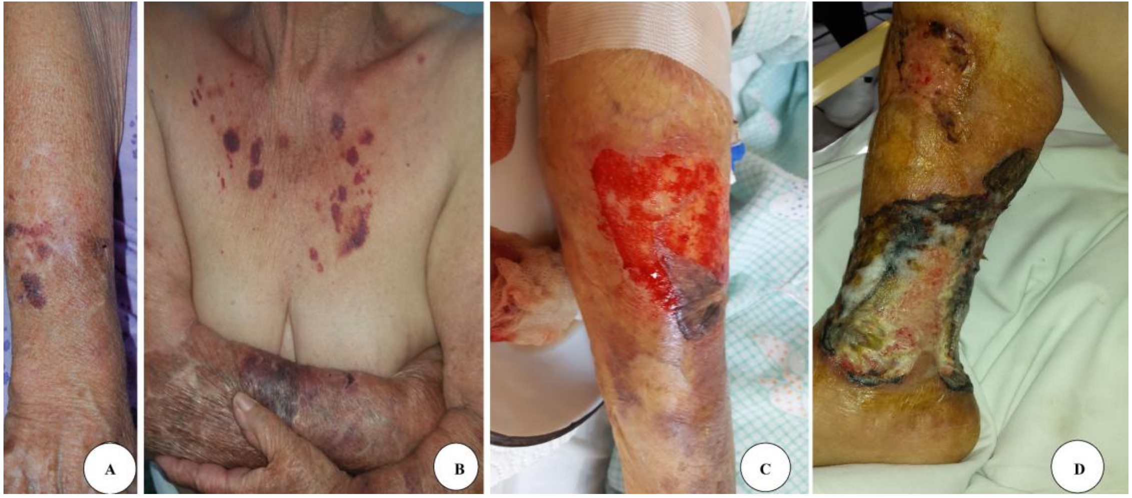

Figure 2: Bleeding after repeated procedures for peripheral vein catheterization in a female patient with primary dermatoporosis

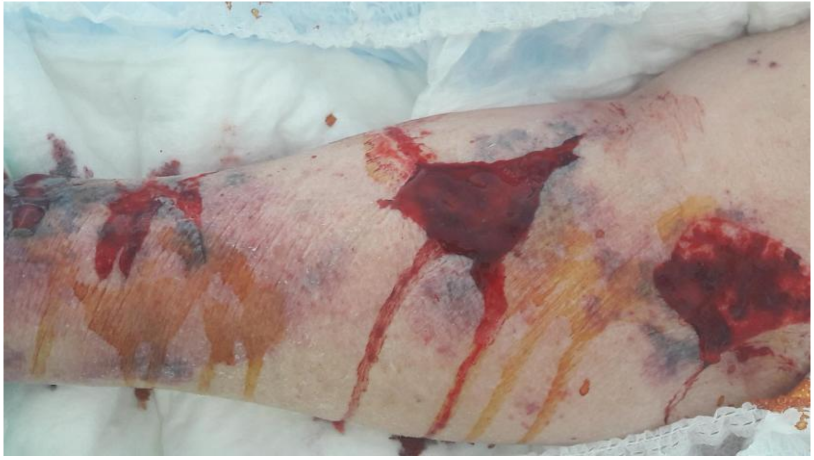

Intrinsic aging or endocrine aging plays a role, the disease is more frequent in postmenopausal women [10]. On the other way, seems that maternal age > 40 years at last pregnancy, breastfeeding for > 7 months per pregnancy and > 18 cumulative months, age < 20 years at first pregnancy, and menopause after 45 years play a protective role against dermatoporosis [11]. Iatrogenic factors like systemic steroid usage especially in patients with blistering diseases can induce dermatoporosis by decreasing the extracellular matrix leading to skin atrophy.

Regarding topical steroids seems that only clobetasol propionate 0.5% can induce dermatoporosis, can it be used for more than six months, this aspect can be visible on the forearms in patients with psoriasis (Figure 3) [12]. The author documented also an interesting case of a 42 female old patient suffering from a secreting cortisol tumor with a Cushing syndrome aspect and typical lesions of dermatoporosis on the forearms (Figure 4). Other medications could be anticoagulants, and epidermal growth factor inhibitors (EGFR) used in the treatment of different carcinomas (Figure 5). Associated comorbidities like osteoporosis, renal failure, and anemia were cited [13].

Moreover, there is a strong connection between the degree of osteoporosis and dermatoporosis [14,15]. It is not clear yet if osteoporosis has a direct influence on the onset of dermatoporosis or if both are the result of the general aging process having hormonal disturbances at the base. Additional information was brought by Kluger in 2016 describing the possible connection of dermatoporosis with the lack of vitamin C levels [16]. Laboratory data about skin expression or serum levels of vitamin C and dermatoporosis are lacking. This correlation is based only on the benefits of topical or systemic administration of vitamin C [16,17].

Recently, vitamin D deficiency was also proposed as a factor by Romano et al based on the connection between osteoporosis and dermatoporosis. Anyway, correlation data between decreased levels of vitamin D and dermatoporosis degree in the elderly are necessary for validation [18]. Finally, most of the patients present an association of these risk factors.

Figure 3: Secondary dermatoporosis: A) Secondary dermatoporosis in a female patient treated with 0.5 mg prednisone per kilo of body weight for 4 months for bullous pemphigoid; B, C) Secondary dermatoporosis in 2 patients treated with 1 mg per kilo of body weight prednisone for 5 months for pemphigus vulgaris.

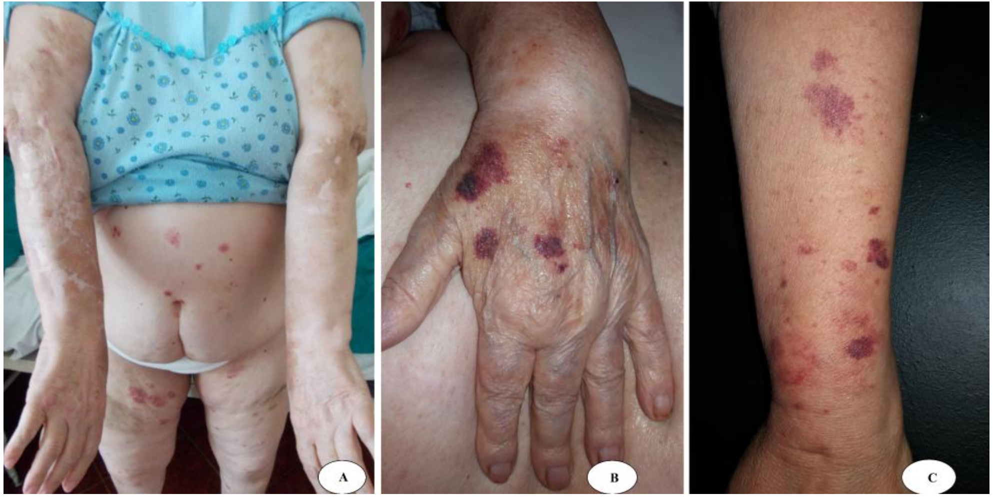

Figure 4: Secondary dermatoporosis in a 42-year-old-female patient diagnosticated with a secreting cortisol tumor: A) Cushing syndrome; B) Facial telangiectasia; C) Dermatoporosis on the forearms; D) Dermatoporosis on the shins.

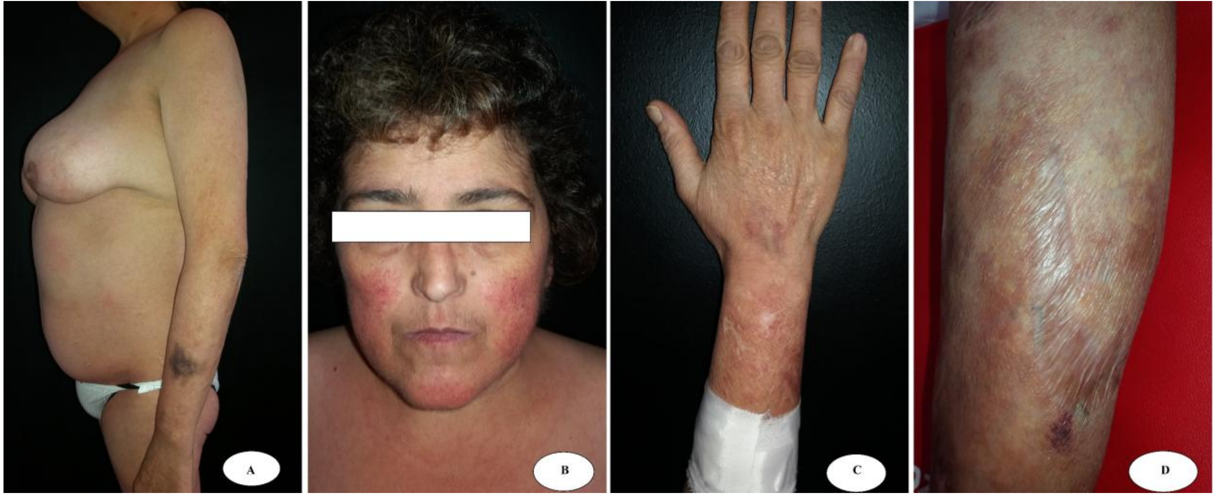

Figure 5: Dermatoporosis in patients undergoing erlotinib in a 67- year-old female patient undergoing erlotinib for lung cancer.

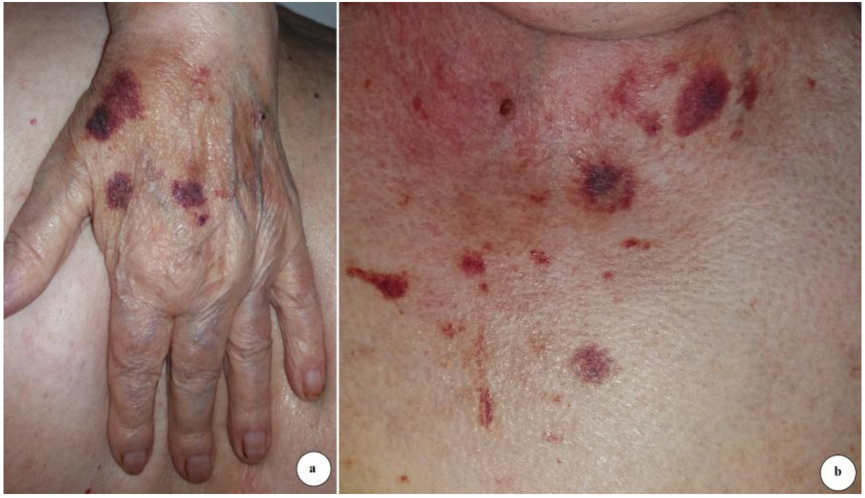

CLINICAL ASPECTS

The clinical changes are skin atrophy, purpura, pseudoscars, hyperpigmentation, and ulcerations. Due to atrophy, there is an increased risk of the appearance of superficial or dissecting hematomas of the skin that form by the accumulation of blood in between the hypodermis and the muscular fascia due to local traumas [3,19]. Most lesions are located at the level of the forearms and shins [3]. Other sites are the chest, arms, and dorsal parts of the hands. It is a tendency for females to develop lesions on the chest and face compared with men. The appearance of lesions at the level of the face and scalp is reported in rare and isolated cases [3,10]. Skin atrophy is the main sign of dermatoporosis. It is essential for the development of the other changes, so that, on the background of exposure to trauma of the underlying structures, purpura and skin ulcers develop. These heal with hyperpigmentation and scars [3]. Senile purpura (Thomas Bateman, 1818) occurs in the elderly, especially on the backs of the hands, and is often found in the context of dermatoporosis [3.4]. It has a complex mechanism, being produced by both exposure of blood vessels due to atrophic skin, and by a decrease in the elastic fibers of the vascular endothelium. An increase in capillary permeability with age is also taken into account [4,20]. Colomb stellate pseudoscars are found on the forearms of the elderly and are produced by defective remodeling of the extracellular matrix due to functional disturbances of enzymes such as metalloproteinases [21]. Appearance on the shins and other sites is also possible. They can be stellate, linear, or plaque-like. The stellate ones are the most common [3,10]. They are formed by the rupture of collagen fibers but without clinical evidence of a break in continuity at the level of the epidermis. Repair is done by excessive proliferation of fibrous tissue, which leads to a clinically hypertrophic scar appearance [5,7]. Pseudoscars are found in approximately 30% of people over the age of 70. Hyperpigmentations appear as residual lesions and as a sign of photodamaged skin [3,10,21]. Cutaneous ulcerations appear based on the cutaneous atrophy after some unnoticeable frequent minimal traumas. They appear at the level of the extremities [3].

Figure 6: Dermatoporosis in a 52-year-old-female patient undergoing erlotinib for breast cancer

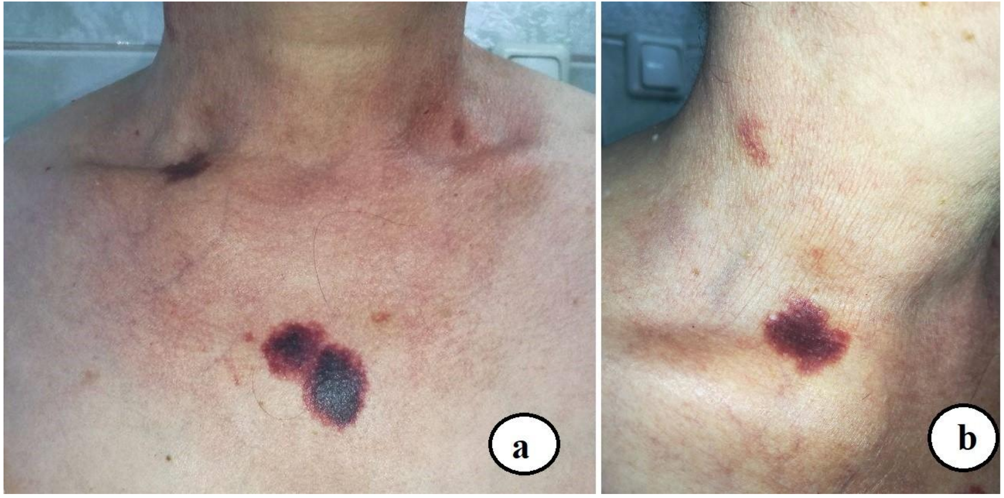

NATURAL COURSE AND COMPLICATIONS

When the cause is obvious like usage of corticosteroids, local or oral, disruption of the treatment can improve greatly and stop the development of further purpura. Superficial hematomas are due to blood accumulation between the dermis and the subcutaneous fat. Clinically appears as a detachment of a skin region from the subjacent adipose tissue. They appear in the advanced stages of dermatoporosis and are found at the level of the shins as well as at the dorsal surface of the forearms [3]. Deep Dissecting hematomas of the skin (DDH) represents the main complication of dermatoporosis. They form as the accumulation of blood between subcutaneous fat and muscular fascia. It represents a surgical emergency due to the risk of block detachment of the cutaneous structures with exposure to infections of major muscular tissue areas. Due to the profound localization of blood collection, the detachment of the skin does not occur immediately. Thus, the lesions mimic cellulitis, presenting as painful erythematous-edematous plaques. People over 80 years with are affected, especially the females and they are located strictly on the calves. The positive diagnosis is done through magnetic resonance imaging (MRI) that shows a hypoechogenic band in the virtual space located between the hypodermis and the muscular fascia. DHH represents a major complication of the disease, the emergency surgical drainage being necessary. The repair of the residual defects is done slowly by the application of topical hyaluronic acid or with skin grafting [3,19] The development of non-melanocytic skin cancer is possible also on dermatoporotic skin, the author saw a case of actinic keratosis and one of cutaneous horn arising in the area of dermatoporosis (Figure 6).

DIAGNOSIS

Clinical aspects are usually enough for the diagnosis. More than that, Saurat developed a self-diagnosis tool (IDA: Index Dermatoporosis Assessment) consisting of a two-module questionnaire that is addressed to individuals aged ≥65 years. The technique was validated in France and Mexico. Skin ultrasonography quantifies the grade of atrophy showing a thickness of skin approx. 0.7-0.8 mm at the level of the regions with dermatoporosis (normal value = 1.3-1.5 mm). The usefulness of reflectance confocal microscopy

(RCM) for the diagnosis of dermatoporosis and quantifying skin thickness was proven by Mezinger, but is expensive and not accessible for routine. Electronic microscopy highlights the disorganization of collagen with parallel exposure of the fibers on the surface of the epidermis (88). Anyway, skin biopsy is an invasive tool, so is recommended to be avoided in such patients and usually is unnecessary when the diagnosis is obvious but might be necessary to rule out other diagnoses in case of atypical location or isolated lesions. For these cases, a 3 mm punch biopsy can be performed on non-erosive/ulcerated lesions without any risk. A general blood count to exclude bleeding disorders, exploration of renal function, and osteoporosis assessment is strongly recommended [3,8]. The fragility of dermatoporotic areas with bleeding and delayed healing results in significantly limited skin biopsies and secondary histopathological and immunohistochemistry studies.

CLASSIFICATION AND STAGING

Depending on the main etiological factors, dermatoporosis can be classified as primary or secondary. Regardless of the form, the lesions are located on the same regions of election such as forearms, shins, anterior part of the thorax, and dorsum of hands [3,10]. Face and scalp location was also cited [10]. Primary dermatoporosis or actinic is due to chronic exposure to the sun, especially in patients over 60 years who are affected. Secondary dermatoporosis lies behind iatrogenic factors, especially the prolonged utilization of corticotherapy. It is characterized by the possibility of the lesions appearing under the age of 60 as well as by partial or complete resolution of the lesions after corticotherapy cessation [3]. The systemic usage as well as topic one can lead to the development of the lesions. Regardless of the way of administration or the cutaneous location in the case of local therapy, the lesions develop in s the same regions/ zones as well as in primary dermatoporosis [3,6]. The disease presents four evolutionary stages. The first stage is characterized by atrophies, purpura as well as pseudo scars, and adding the presence of cutaneous ulcerations leads to the second stage. In the third stage, the ulcerations are increased in size and number. Development of DDH is characteristic of the fourth stage [3,6,19].

PREVENTION AND TREATMENT

The prevention consists firstly in avoidance of the triggers. Regarding this, there is the necessity to use the factors for photoprotection and the avoidance of trauma in patients who are already suffering from primary dermatoporosis. Careful organization at home with the furniture (« coffee table syndrome »), prevention of falling, also proper information of nursing staff the way they handle the patients, no adhesive directly on the skin, avoid excessive application of potent or highly potent corticosteroids. The first topical was proposed by Kaya in 2007, at the same time as the disease was described. The application of hyaluronic acid 1% leads to the improvement of the lesions [3]. In a recent study, topical or oral hydrolyzed collagen does not improve precocious lesions of dermatoporosis – stage I [23]. Topical retinoids are cited to be efficient in the treatment of dermatoporosis since their role is the re-establishment of the differential cellular process. The use of the combination of hyaluronic acid and retinyl-aldehyde is more effective than the separate application of these two [24]. Alpha-hydroxy-acids (AHA) are efficient by diminishing the grade of atrophy [25]. Humbert and colleagues sustain that dermatopororis, especially the Bateman senile purpura, is due to a deficit of vitamin C skin level and it shows an abatement of the lesions after applying topically vitamin C 5% [17]. There is limited data regarding the efficacy of topical application of topical human epidermal growth factor or dehydroepiandrosterone [26,27]. Systemic administration of vitamins C and D could bring some benefits [16,18]. An isolated case of dermatoporosis in the upper limbs treated with polymethylmethacrylate (PMMA) using the BioSculpt®technique was reported.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, MAB; methodology, MAB, ACC; software, ACC; validation, DOC, ALT, NK; formal analysis, NK; investigation, MAB, ACC; resources, DOC, ALT, NK; data curation, ALT, NK; writing—original draft preparation, MAB, NK, ACC; writing—review and editing, DOC, ALT; visualization, ALT, NK; supervision, MAB, NK; project administration, DOC, ALT. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki.

Patient consent for publication

Consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

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ORIGINAL ARTICLE

Physiological Demands of Real and Simulated Combat Flight

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Abstract: Combat aircraft pilots operate in highly demanding environments where their expertise and psychophysiological responses impact performance. This study evaluated the acute psychophysiological effects of simulated and real combat flight maneuvers. Twelve Spanish Air Force fighter pilots (mean age = 33.08 ± 5.21 years) participated, averaging 13.25 ± 5.15 years of military service, including deployments in international missions. Various physiological and metabolic variables—isometric hand strength, lower body strength, pulmonary capacity, blood oxygen saturation, urinary hydration levels, cortisol, and blood lactate concentrations— were measured before and after both simulated and real combat flights. Results showed no statistically significant differences between real and simulated flights for most variables, including blood oxygen saturation, lactate, glucose, cortisol, urine color, and physical fitness indicators. However, glucose levels significantly decreased after simulated flights ($p = 0.021$), and horizontal jump performance improved post-real flights ($p = 0.004$). The similarity in physiological responses suggests that simulators effectively replicate real combat conditions, reinforcing their value as a safe, effective training tool. Simulators enhance pilot preparedness while minimizing exposure to combat risks. These findings contribute to aviation psychophysiology, improving training protocols and operational readiness for both military and civilian applications, ultimately optimizing pilot performance and safety in high-risk environments.

Keywords: combat flight, fighter pilots, psychophysiological response, flight simulation, operational training

INTRODUCTION

The operational limits of military personnel are frequently challenged in a range of extreme scenarios, including parachute operations (both High Altitude High Opening [HAHO] and High Altitude Low Opening [HALO]), engagements in symmetrical and asymmetrical warfare, subterranean missions, and close-quarters combat situations. Among these, combat aviation is widely regarded as one of the most physiologically and psychologically taxing environments. Military pilots are exposed to both acute and chronic stressors, such as heightened autonomic nervous system activity, pronounced cardiovascular and hemodynamic responses, and sustained exposure to gravitational accelerations (G-forces), which can exceed 9G during high-intensity maneuvers. These conditions can substantially compromise functional capacity, manifesting in acute loss of consciousness, grey-out episodes, spatial disorientation, and blackouts, even when utilizing oxygen supplementation systems and G-suits. Furthermore, combat aviators must operate at altitudes exceeding 15 kilometers (~50,000 feet), with cabin pressurization and the administration of supplemental oxygen typically required above 7 kilometers (~20,000 feet) to mitigate the dangers associated with hypoxia and decompression illness during tactical missions [1,2,3,4,5].

Exposure to acute psychophysiological stress elicits pronounced sympathetic nervous system activation, increases in anaerobic metabolism—as evidenced by elevated blood lactate concentrations—and disturbances in several cognitive domains, including cortical arousal, memory retention, temporal perception, and perceived exertion. Repeated exposure to such stressors can induce habituation, which is associated with attenuated physiological responses and improved task performance. However, when stress exposure is prolonged and recovery is inadequate, this may result in deleterious long-term effects on both physiological and psychological health. As such, implementing training strategies that closely

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mimic the stressors encountered in combat within a controlled setting is essential to enhance both operational effectiveness and the overall well-being of military personnel and aviators [5,6,7,8].

In this context, simulation-based training has gained increasing recognition as a method to reproduce the multifaceted stressors characteristic of combat environments. In aviation, flight simulators are commonly employed to replicate conditions such as high-G exposure, complex tactical maneuvers, and the cognitive load inherent to aerial combat. These simulated environments enable pilots to adapt to task-specific challenges without facing the direct risks associated with live combat operations. Prior investigations have demonstrated the utility of such simulators in assessing variables like mental workload, spatial orientation, and perceptual illusions under stress-inducing conditions. Nonetheless, despite their widespread implementation, research remains limited regarding the extent to which simulators replicate the full spectrum of psychophysiological responses observed during actual combat aviation [6,7,8,9].

Existing literature examining the physiological demands of real combat aviation has largely focused on variables such as G-force exposure, hypoxia, and the cumulative stress of extended tactical engagement. These studies have documented substantial physiological strain on the respiratory and muscular systems, as well as on metabolic processes, with measurable alterations in biomarkers such as lactate and cortisol. In contrast, flight simulators are engineered to recreate these stressors predominantly at a cognitive level, often falling short of reproducing the mechanical and gravitational stresses encountered in real flight. Although some findings suggest simulators effectively induce mental stress, there is insufficient empirical evidence to support their capacity to fully emulate the psychophysiological impact of actual combat flying [10,11,12].

A comprehensive understanding of the differential psychophysiological responses elicited by real versus simulated combat flight is imperative for refining pilot training regimens and operational planning. Given the considerable implications for pilot health and mission effectiveness, accurate replication of combat-induced stress in training environments is of paramount importance. This study seeks to address a notable gap in the current literature by directly comparing the physiological and metabolic responses of fighter pilots engaged in both real and simulated combat maneuvers. The outcomes of this research have the potential to inform the development of optimized training protocols, thereby enhancing preparedness for the demands of aerial warfare.

Accordingly, the present study hypothesizes that real combat flight induces significantly greater acute psychophysiological stress in pilots compared to simulated flight conditions. This hypothesis is grounded in the premise that, although simulation technologies are capable of replicating many cognitive stressors, they are inherently limited in their ability to reproduce the physical elements of combat aviation, including the effects of G-forces and mechanical loading. Elucidating these distinctions is essential for the advancement of simulation-based training strategies that adequately reflect the realities of combat flight.

MATERIALS AND METHODS

Participants

To achieve the objective of this research we analyzed the physiological response of fighter pilots before and after a simulated and real attack flight maneuver.

A total of 12 fighter pilots (age=33.08±5.21) from the Spanish Air Force participated in this cross-sectional study. Participants had mean military service experience of 13.25 ± 5.15 years, with experience in international missions in the current conflict in Lebanon, Afghanistan, Bosnia, Kosovo, and Iraq. Before participation, the experimental procedures were explained to all the participants, who gave their voluntary written informed consent in accordance with the Declaration of Helsinki. In addition, All the procedures were approved by the University research ethics committee (approval number: 206/219), and all participants agreed and gave written consent to participate in the study. Participants were equipped with the standard flying suit and boots, combat gear composed of parachute harness, life support jacket, and G-suit with a total weight of 10kgs.

Procedures

Pilots were evaluated before and after a real fight mission and a simulated one. Conditions, protocols, and procedures were exactly the same either in simulated or real combat. F5 combat aircraft was used for the real manoeuvres (Figure 1). For the simulated maneuvers, an operational F-5 M (Indra Company, Madrid, Spain) flight simulator was used (Figure 2). All pilots conducted both protocols, and 48 hours of rest between them were assigned. The tactical combat maneuvers were performed in the Air Fighting School of the Spanish Air Force at Talavera de la Real Air Base (Badajoz, Spain), in the middle of April month, between 08:00 and 15:00, with temperatures between 20 and 24 ºC and clear sky. Order and type of protocol were also randomized, in both cases, the protocol consisted of:

In both cases, pilots conducted both maneuvers at altitudes between 8000 and 18,000 ft, with G force between 0.5 and 5.9, with oxygen mix supply over 8000 ft with a combat duration between 30 and 35 min. The offensive maneuver consisted of a fighter aircraft that started at a 1-mile separation and a speed of 400 knots (unit of speed equal to one nautical mile). Both aircraft maintained a sustained rate of 410 knots and 5.5 Gs for 30 to 40 seconds until the target aircraft (referred to as the 'bandit') was within effective gun range. By this point, the bandit had closed the distance to the offensive aircraft, causing the speed to drop to 350–300 knots and the G-load to decrease to 4–4.5 Gs. After the initial bullet stream, a repositioning maneuver could be performed to solve range, aspect, and closure. Speed will decrease to 300 knots and gravity forces to 3G's to return to a control position of effective gun employment. The exercise started at 16000 ft and it progressively decreased to 10,000 ft. In the defensive maneuver, the lead aircraft turned to one side to keep the pursuing offensive aircraft in sight. The exercise began at an altitude of 17,000 feet and a speed of 350 knots. Defensive aircraft executed a break turn at 4 G's slowing down at the same time, looking over the shoulder at the offensive aircraft to assess his pursuit curve. At 300 knots, the defensive aircraft executed turning maneuvers, gradually reducing G-forces from 3.5 to 2 Gs and speed to 200–250 knots. Depending on the progression of the air combat, the defensive aircraft could maintain an airspeed of 350 knots and a G-load of 3.5–4 Gs while descending. Exercise finished at 10000 ft.

Figure 1: F5 combat aircraft

Figure 2: F5 Indra Company (Madrid, Spain) flight simulator

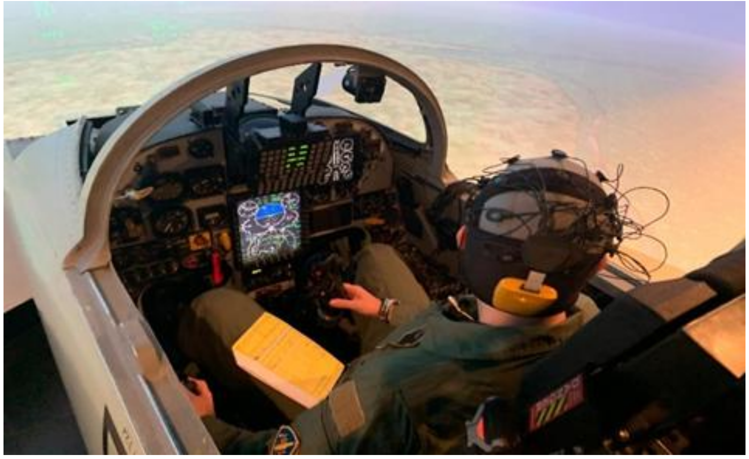

Materials

One hour before and thirty minutes after finishing the air combat maneuvers the following variables were measured:

Lower body muscular strength employing horizontal jump test. Subjects performed a standardized warm-up consisting of 2x10 vertical jumps and then, they performed two maximal horizontal jumps as in the previous report (-), and the best attempt was used for the statistical analysis.

The forced expiratory volume in the first second (FEV1) corresponds to the maximum volume of air exhaled in the first second of the FVC. And mean expiratory flow or FEF25-75, which is between 25% and 75% of the forced expiratory maneuver. Were analyzed using a QM-SP100 (Quirumed, Spain) spirometer in a maximum inhale-exhale cycle.

Urine samples were collected to analyze dehydration levels which were examined by the urine color (UC) chart (color range 1-8; where 1 = very pale-yellow urine, reflected a good level of hydration, and 8 = very dark yellowish-brown, reflected a significant level of dehydration); the number closest to the sample color was recorded.

Isometric hand strength (IHS) by a grip dynamometer (Takei Kiki Koyo, Japan).

Blood oxygen saturation ($SatO_2$) and heart rate (HR) by a pulse oximeter (Pulse Oximeter 30 Beurer Medical).

Lactate and glucose samples were collected by taking a sample of 5 µl of capillary blood from the fingers of the pilots and analyzed by Accutrend Plus (Roche Diagnostics, Switzerland).

Direct Salivary Melatonin ELISA (Bühlmann, Schönenbuch, Switzerland) was used to measure cortisol concentrations.

Statistical analysis

Wilcoxon signed-rank test was used to examine the difference between the pre and post-measures. The baseline was subtracted to post-values to normalize and compare the acute effects of real vs. simulated flight. Effect sizes [r] were calculated for the non-parametric tests which are classified as follows: 0.5 is a large.

RESULTS

The results of the study revealed notable differences between the acute psychophysiological effects of real and simulated combat flights on specific metabolic, respiratory, and physical fitness variables. A significant reduction in glucose levels was observed following simulated combat flights (p = 0.021, effect size = 0.668), while this effect was not present after real flights. This finding suggests that the metabolic demands in simulated conditions may differ from those experienced during real combat maneuvers. Additionally, a significant improvement in horizontal jump performance was found after real flights (p = 0.004, effect size = 0.839), indicating enhanced lower body strength or neuromuscular efficiency induced by real combat flight conditions.

No significant differences were detected in other variables, including blood oxygen saturation, blood lactate levels, cortisol concentrations, urine color, respiratory function (FEV1, FER25, FEF75), or handgrip strength, either between pre- and post-measurements or when comparing real and simulated flight conditions. These results highlight the comparable physiological demands of simulated and real combat flights across most variables, emphasizing the potential of simulation-based training to closely replicate real flight conditions. However, the observed differences in glucose levels and horizontal jump performance underline specific areas where real combat flight may impose unique demands.

These findings suggest that flight simulators are effective tools for replicating many of the psychophysiological demands of combat aviation, while also identifying areas where real combat conditions elicit distinct responses. This information is critical for optimizing training protocols and ensuring pilots are adequately prepared for operational demands.

Table 1: Acute effects of real and simulated combat flight on metabolic, respiratory and physical fitness variables

	Variables	**Baseline Mean (SD)**	**Post Mean (SD)**	**Baseline vs post measure**		**Acute effects of a real vs a simulated mission**	
				p-value	**Effect Size**	**p-value**	**Effect Size**
Real flight	Blood oxygen saturation (%)	98.58 (0.51)	98.00 (0.74)	.100	0.475	.476	0.205
Simulated flight		98.08 (0.67)	98.08 (0.67)	.527	0.182		
Real flight	Lactate (mmol/l)	1.92 (0.78)	2.86 (3.04)	.367	0.261	.790	0.077
Simulated flight		2.62 (1.78)	3.25 (2.97)	.533	0.180		
Real flight	Glucose (mg/dl)	96.42 (9.57)	96.25 (8.61)	.844	0.056	.084	0.498
Simulated flight		102.75 (14.04)	92.50 (4.74)	.021*	0.668		
Real flight	Cortisol (µg/dl)	9.70 (5.78)	7.56 (6.30)	.158	0.408	.445	0.220
Simulated flight		6.30 (4.83)	6.87 (7.01)	.386	0.250		
Real flight	Urine color (a.u.)	3.75 (1.71)	3.17 (0.94)	.176	0.391	.887	0.044
Simulated flight		3.08 (1.08)	2.58 (1.44)	.092	0.486		
Real flight	Fev1 (l)	5.11 (1.19)	4.81 (0.66)	.450	0.218	.142	0.424
Simulated flight		4.83 (0.68)	4.85 (0.65)	.123	0.445		
Real flight	FER25 (l)	3.92 (0.61)	4.08 (0.44)	.308	0.294	.722	0.103
Simulated flight		4.13 (0.64)	4.16 (0.75)	.260	0.324		
Real flight	FEF75 (l)	10.10 (1.89)	9.72 (2.07)	.477	0.205	1.000	<0.001
Simulated flight		9.98 (1.91)	9.27 (1.99)	.678	0.120		
Real flight	Handgrip (N)	52.62 (6.08)	51.92 (7.43)	.759	0.088	.386	0.250
Simulated flight		50.62 (7.23)	51.09 (5.47)	.858	0.051		
Real flight	Horizontal jump (cm)	141.33 (11.10)	147.42 (12.99)	.004*	0.839	.878	0.044
Simulated flight		137.42 (9.10)	144.09 (10.32)	.055	0.553		

**p-value <0.05*

DISCUSSION

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible.

Future research directions may also be highlighted.

This study aimed to analyze the acute psychophysiological effects of simulated and real combat flights in military pilots. The findings emphasize the utility of flight simulators for training purposes, as no significant differences were observed in most physiological and metabolic variables between simulated and real combat flights. These results align with previous research demonstrating that modern simulators effectively replicate many of the stressors encountered in real-world conditions, such as mental workload and physical demands while avoiding the risks associated with live operations [13,14,15]. The high fidelity of modern simulators in recreating operational environments supports their role as indispensable tools in pilot training.

Despite the overall similarity in physiological responses, certain differences were evident, providing nuanced insights into the unique demands of real combat flights. A significant improvement in horizontal jump performance following real flights was observed, suggesting that real combat maneuvers may elicit greater neuromuscular activation compared to simulated conditions. This response could be linked to the heightened cortical arousal and increased recruitment of fast-twitch muscle fibers required to cope with the physical and cognitive demands of real flight maneuvers. Similar findings have been reported in high-intensity military tasks, such as close-quarter combat and parachute jumps, where lower body strength improves due to acute neuromuscular adaptations to high-stress conditions [16,17,18]. These findings reinforce the hypothesis that real-world stressors induce unique physiological responses that are challenging to replicate entirely in simulated environments.

Interestingly, no significant changes were detected in handgrip strength, highlighting the distinct demands placed on upper body fine motor skills in piloting. Unlike lower body strength, which benefits from gross motor activation, piloting requires sustained precision and control over joystick or yoke movements, which rely on fine motor skills rather than maximal force. Previous research has similarly noted that tasks involving fine motor coordination are less affected by acute fatigue compared to those demanding gross motor output [19,20,21]. These results suggest that while simulators effectively mimic many neuromuscular demands, they may not fully capture the precise motor control required during real flight operations.

The absence of significant changes in blood lactate levels following real flights contrasts with findings in other high-stress combat scenarios, where anaerobic metabolism typically increases due to intense physical exertion [22,23]. This discrepancy may be attributed to the effects of G-forces during real flights, which redistribute blood flow, potentially reducing capillary perfusion at peripheral sampling sites. Simulated flights, lacking such mechanical stressors, exhibited lactate levels closer to the anaerobic threshold, further supporting the influence of G-forces on metabolic responses [24,25,26]. Research on the physiological effects of G-forces in combat aviation highlights their role in altering cardiovascular and metabolic regulation, with implications for pilot performance and endurance [27,28].

Respiratory function, as assessed by spirometry and blood oxygen saturation, showed no significant differences between simulated and real flights. The stability of these variables suggests that the participants' high level of operational experience and training enabled them to maintain respiratory efficiency across both conditions. Previous studies have identified respiratory fatigue as more prevalent among less experienced pilots or during extended operations [29,30]. The lack of observed respiratory impairments in this study underscores the resilience of experienced pilots. However, the reliance on spirometry alone may have limited the scope of respiratory assessments. Advanced techniques such as wearable strain gauge sensors or optoelectronic plethysmography could provide a more detailed understanding of respiratory patterns during flight [31,32].

Cortisol levels remained stable across both flight conditions, a finding that may reflect the effects of habituation associated with extensive operational experience. High-stress environments often elicit significant cortisol responses in less experienced individuals, but research has shown that habituation to stress reduces these responses over time in seasoned personnel [33]. This phenomenon is consistent with studies in other military contexts, where experienced individuals exhibit attenuated psychophysiological responses to acute stressors [34]. The stability of cortisol levels in this study further emphasizes the importance of experience in mitigating stress responses during high-pressure scenarios.

Hydration profiles, assessed through urine colorimetry, did not differ significantly between simulated and real flights. However, the hydration levels observed were close to dehydration thresholds, highlighting the need for improved pre-flight hydration strategies. Dehydration has been shown to impair both physical and cognitive performance, particularly in high-stress environments such as combat aviation [35,36,37]. The use of urine color charts for hydration monitoring has been validated in clinical and operational settings, offering a practical tool for maintaining optimal hydration status among pilots [38]. Addressing hydration deficits could enhance both safety and performance during flight operations.

The results of this study contribute to the growing body of literature emphasizing the effectiveness of flight simulators in replicating the majority of psychophysiological demands associated with real combat flights. However, the observed differences in neuromuscular and metabolic responses highlight specific areas where real flight conditions impose distinct challenges that simulators may not fully replicate. For instance, while simulators effectively mimic the mental workload and basic physiological demands of combat aviation, they may not accurately replicate the physical effects of G-forces or the fine motor requirements of real piloting.

These findings have important implications for the design and implementation of simulation-based training programs. By identifying the limitations of current simulators, training protocols can be refined to better prepare pilots for the unique demands of real combat scenarios. For example, incorporating supplementary training exercises that target neuromuscular activation and motor coordination could address the gaps identified in this study. Additionally, integrating advanced physiological monitoring tools, such as portable biosensors and electroencephalography, could enhance the precision and effectiveness of simulation-based training [39,40,41].

Limitations of the study

This study has several limitations that should be considered when interpreting the results. First, the relatively small sample size (n = 12) limits the generalizability of the findings, particularly given the elite status of the participants as experienced military pilots. Access to larger samples in similar operational contexts remains a challenge, but it would enhance the robustness of the conclusions. Second, the study did not control for gender differences, which may introduce variability in psychophysiological responses. Given the increasing presence of female pilots in military aviation, future studies should aim to include a gender-balanced sample.

Another limitation is the reliance on conventional physiological and metabolic measures, such as spirometry and blood lactate levels, which may not fully capture the complexity of pilot responses during flight. The lack of advanced monitoring techniques, such as electroencephalography (EEG) or respiratory inductive plethysmography, restricts the scope of insights into neuromuscular and respiratory dynamics. Additionally, the study did not evaluate psychological measures such as perceived exertion, stress, or workload, which are critical components of the psychophysiological response in high-stress environments.

Finally, while the study focused on acute effects, it did not assess the cumulative impact of repeated exposure to simulated and real flight conditions. Chronic stress and its potential long-term effects on pilot health and performance warrant further exploration.

Practical applications

Our research on the effects of experience on the findings of this study has direct implications for improving pilot training and operational readiness. The demonstrated effectiveness of flight simulators in replicating many psychophysiological demands of real combat flights supports their continued use as a safe, cost-effective, and practical training tool. Simulators allow pilots to acclimate to combat stressors without exposure to the risks associated with live operations, such as physical injury or equipment failure. These insights reinforce the value of simulators in enhancing pilot preparedness, particularly during the early phases of training.

The observed differences in neuromuscular activation and metabolic responses between real and simulated flights highlight areas where simulation-based training can be refined. Incorporating supplementary neuromuscular exercises, such as lower body strength training, may enhance physical preparedness for real combat scenarios. Additionally, addressing hydration deficits through pre-flight hydration protocols could mitigate the risk of dehydration-related performance decrements.

Moreover, the study underscores the importance of tailoring training protocols to individual pilot characteristics, including experience level and physiological baseline. Advanced monitoring technologies, such as wearable biosensors, could be integrated into training programs to provide real-time feedback on physiological and psychological responses, enabling personalized training adjustments.

Future research lines

Future research should build upon the findings and limitations of this study by addressing several key areas. First, future studies should include larger sample sizes and greater diversity, particularly through the inclusion of female pilots. This approach would improve the generalizability of findings and allow for a deeper exploration of potential gender differences in psychophysiological responses. Additionally, investigations into the cumulative effects of repeated exposure to both simulated and real combat flight conditions are essential. These studies could provide valuable insights into the long-term impact of operational stressors on pilot health, performance, and resilience, areas that remain underexplored.

Advanced physiological and psychological monitoring tools should also be integrated into future research. Techniques such as electroencephalography (EEG), respiratory inductive plethysmography, and portable biosensors could offer a more comprehensive understanding of pilot responses. These tools would enable the assessment of critical parameters, including brain activity, detailed breathing dynamics, and stress biomarkers, allowing for a more nuanced analysis of the interaction between physiological and psychological stressors.

Incorporating psychological measures, such as perceived exertion, workload, stress, and fatigue, will also enhance future studies by providing a holistic view of pilot performance under stress. These measures would complement physiological data and help elucidate the interplay between mental and physical demands in combat aviation. Furthermore, comparing psychophysiological responses between novice and experienced pilots would yield valuable insights into how training and habituation influence operational readiness, offering opportunities to tailor training programs to the specific needs of pilots at different stages of their careers.

Another important area for future research involves enhancing the fidelity of flight simulators. Efforts should focus on better replicating the physical effects of G-forces and other unique stressors encountered during real combat flights. Advanced simulation technologies that integrate neuromuscular and metabolic stressors could bridge the gap between simulated and real flight conditions, thereby improving training effectiveness.

Hydration and nutritional strategies represent another critical avenue for investigation. Tailored interventions to optimize hydration status and metabolic recovery could significantly enhance pilot performance during combat scenarios. Research in this area would address a practical operational need and contribute to the development of evidence-based protocols to support pilot health and performance.

Finally, cross-operational comparisons should be conducted to broaden the scope of research. Investigating psychophysiological responses across different aviation contexts, such as helicopter pilots or unmanned aerial vehicle (UAV) operators, would provide a more comprehensive understanding of aviation-specific stressors and their management. These comparisons would also facilitate the identification of universal stressors and strategies applicable across various aviation roles, contributing to the overall advancement of the field.

CONCLUSION

The present study underscores the essential function of flight simulators within pilot training frameworks, particularly in their capacity to replicate a substantial portion of the psychophysiological demands encountered in actual combat aviation scenarios. Nevertheless, the discrepancies identified in neuromuscular activation patterns and metabolic responses indicate that simulators may fall short in fully emulating the intricate physiological challenges of real-world flight operations. Bridging these limitations through the integration of targeted training interventions and sophisticated monitoring methodologies has the potential to enhance pilot preparedness and physiological resilience, thereby contributing to improved performance and mission safety. These results highlight the necessity of ongoing research aimed at refining training protocols to ensure that pilots are effectively equipped to meet the complex requirements of contemporary aerial warfare.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

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Authors' contribution

Conceptualization, V.J.C.-S., S.V. and J.P.F.-G.; methodology, V.J.C.-S., S.V. and J.P.F.-G.; formal analysis, J.F.T.-A., V.J.C.-S., S.V. and J.P.F.-G.; investigation, J.F.T.-A., V.J.C.-S., S.V., M.A.M.-G. and J.P.F.-G.; resources, J.P.F.-G. and V.J.C.-S.; data curation, J.F.T.-A., V.J.C.-S., S.V., M.A.M.-G. and J.P.F.-G.; writing—original draft, J.F.T.-A.; writing—review & editing, J.P.F.-G. and V.J.C.-S; visualization, M.A.M.-G.: supervision, V.J.C.-S. and J J.P.F.-G.; project administration, J.P.F.-G.; funding acquisition, J.P.F.-G. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the University of Extremadura (approval number: 206/219: July 24, 2019).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Therapeutic Particularities of Depression in Relapsing-Remitting Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) is an autoimmune disease in which the myelin sheath is damaged by the body itself. Because of the symptoms and progressive forms, in most cases, patients experience recurrent depressive disorder or anxious depression disorders. Depression treatment for MS patients stands in two categories, one is psychotherapy and the other is pharmacological. This article presents the research results on customized pharmacological treatment schemes applied to a group of relapsing-remitting multiple sclerosis (RRMS) patients with moderate, or severe, depression. All of them are included in the national treatment program for multiple sclerosis and their health state is continuously monitored. The therapeutic scheme based on four different antidepressants had encouraging results, meaning that any of the antidepressants used is efficient and with the patient's good tolerance. There were no side effects that could determine interruption of the treatment. Further research development would focus on improved therapeutic plans for patients with depression disorders in relapsing-remitting multiple sclerosis.

Keywords: depression disorder; therapeutic particularities; multiple sclerosis; HAM-D scale

INTRODUCTION

In 1868, the neurologist Jean-Martin Charcot mentioned for the first time multiple sclerosis (MS) as a neurological affection with unpredictable evolution varying from benign (mild) up to aggressive. This illness especially affects people, aged between 30-50 years, most of them being diagnosed in their mid-thirties. There are also teenagers affected, mostly the ones who smoke and the overweight young women. The official reports [1] mention the ratio of males and females as 1:3.

There is no identified specific factor to trigger MS, so the assumption is that of a series of factors that could cause the disease, like:

- genetic factors - usually, if one parent has a diagnosis of multiple sclerosis, the risk for their children to have MS is about (2 - 3)%, and, more importantly, if both parents have a diagnosis of multiple sclerosis, the risk for their children to have this disease is about (12 - 30)%;

- Environmental factors - are diverse and perhaps complementary, of which the following are considered relevant: prolonged sun exposure during childhood and adolescence - determines high serum vitamin D levels and thus lower risk of MS; smoke from smoking - affects systemic immunity and can damage the central nervous system (CNS); obesity - especially in teenage girls, when hormonal changes that occur secondary to obesity are related to inflammatory processes; sleep disturbance - wake cycle and poor quality of rest; brain trauma - when being a child or adolescent could cause the disease.

Multiple sclerosis is an autoimmune neurodegenerative disease that acts on and destroys the central nervous system [2,3]. It is an inflammatory disease that leads to demyelination and axonal loss, resulting in manifest cognitive, motor, or sensory symptoms such as depression, emotional distress, or anxiety. As mentioned in [4],

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the clinical course of MS is classified as follows: Clinically Isolated Syndrome (CIS); Relapsing-Remitting (RRMS); Primary Progressive (PPMS); Secondary Progressive (SSMS).

Once the evolution of MS is on, the predominant emotional disorder is stated to be [5,6] depression. Patients with depression also have a poor psychosocial quality of life, manifest diminished capacity to work, and manifest decreased therapeutic compliance.

Most of the studies [7,8,9,10,11] mention fatigue, depression, and pain as inherent disorders associated with structural and functional changes in the brain in MS. These changes have been noticed in the basal ganglia, prefrontal cortex, and limbic system [12,13,14].

Comorbid fatigue and/or depression symptoms reported in patients with MS are also linked to impairment of the hypothalamic-pituitary-adrenal (HPA) axis [15]. Because chronic fatigue and depression are low levels of cortisol and dehydroepiandrosterone (DHEA) it is possible to be endocrine contribution. Studies [16,17] have proved that MS patients state to have increased energy after taking corticosteroids as treatment for MS relapse.

As mentioned in [18,19] depressive disorder (MDD), or clinical depression, represents a debilitating disease exhibiting at least one major depressive episode for at least two weeks, with an evident bad mood, low interests and pleasure (anhedonia), poor cognition and neurovegetative symptoms (fatigue, loss of appetite, insomnia). There is a high focus on improving the quality of life for MS patients. Depression treatment usually is individualized and is split between associated pharmacological and non-pharmacological ones.

The pharmacological treatment with Desipramine (tricyclic antidepressant) 200 mg/day [20] and Paroxetine (selective serotonin reuptake inhibitor) 40 mg/day [21] is mentioned in comparison with placebo, for a period of five and, respectively, twelve weeks. As a result, there was noticed a slight improvement of depression symptoms but, not a statistically significant result.

In [22] it is analyzed how a specialized exercise training program with prescriptive guidelines [23, 24] having as background the social cognitive theory sustains the reduction severity of depressive symptoms in MS patients.

This article presents the research results on therapeutic particularities of depression, a survey carried out on a group of relapsing-remitting multiple sclerosis (RRMS) patients with moderate, or severe, depression forms. The therapy plan was developed based on the authors' expertise in the treatment of multiple sclerosis and mainly consists of the administration of one of the following antidepressants: venlafaxine; sertraline; mirtazapine; and trazodone. The diagnosis and the severity of depression were evaluated by the total Hamilton Depression Rating Scale (HAM-D).

Aspects of materials and methods used in research, as well as results, discussion, and conclusion of the obtained results, are evidenced in the next chapters. Further research development focus is also mentioned.

MATERIALS AND METHODS

There are different signs of multiple sclerosis [25,26] caused by the location and severity of the myelin sheath lesions. Troubles in moving arms or legs, disorders in equilibrium, visual disturbances, or, when severe, loss of ability for independent motion - these are the alarm signals for this illness.

For the moment, there is no specific and targeted treatment for curing MS, but there are therapeutic schemes usually focusing on fast and high recovery from attacks, reducing probabilities of relapse, slowing down the disease progression, and adequate managing of symptoms. There are also cases when the symptoms are mild and patients do not need specific treatment.

While MS attacks are on, for reducing inflammation of the nerves are used intravenous corticosteroids. If there is no positive response to corticosteroids and the symptoms are extremely severe, plasma exchange can be used for ease of symptoms (not for curing). There are also treatment schemes for lowering the rate of relapse and reducing the risk of brain atrophy, such as immunomodulatory medication, monoclonal antibodies, or antihistamines. Solutions for MS symptom relief are various and customized on patients, like: kynetotherapy to increase muscle strength; muscle relaxers for reducing pain in muscle rigidity; specific medication against pain, insomnia, and depression; and psychological counseling.

There is a common opinion regarding the risk of immobilization for patients with multiple sclerosis but, the highest risk for them is that of suicide. Psychological manifestations have been noticed since the 19th century [27], once the clinical signs of this illness have been described.

About 50% of patients with multiple sclerosis exhibit at least one depression symptom, and a percentage varying in-between (15 ÷ 30)% of these patients have severe depression disorder.

A comprehensive study [28] indicates that the majority of MS patients present with both anxiety and depression symptoms. However, due to the presence of multiple overlaps in diagnostic criteria, it is challenging to ascertain the exact frequency of each of these conditions. The predominant feature of their syndrome points towards depression, anxiety, or, mixed anxiety-depressive disorder. Even for now, it has not been identified yet a direct relationship between multiple sclerosis and depression, the main mechanisms are assumed to be the ones that follow:

- Depression is determined by the process of damaging specific brain zones, related to the control and manifestation of emotions. There are also immune and endocrine changes induced by MS that could determine bad mood feelings.

- Depression is triggered by the emotional impact of the patient when finding out the medical diagnosis of multiple sclerosis.

- Depression manifests as a side effect of pharmacological treatment, like interferon-based medications or corticosteroids.

The criteria for diagnosis of depression, as mentioned by the Diagnostic and Statistical Manual of Mental Disorders, DSM-5 [27] are evidenced in Table 1.

Table 1: Criteria of depression by DSM-5 [26]

No.	Criteria	Tick √ if YES within two weeks
1	Mood of depression - long time a day, almost every day	
2	Diminished interest/pleasure in most activities - long time a day, almost every day	
3	Significant weight loss/gain without specific dieting	
4	Slow down in decision-making and performing physical activities (relevant to the others)	
5	Fatigue and poor energy	
6	Bad feelings and guilt for most of the things done - almost every day	
7	Difficulty in concentration and thinking, indecisiveness - almost every day	
8.	Repeated thoughts of death, suicidal behavior	

An individual is diagnosed with depression if, at least, five of the above symptoms are exhibited within two weeks and one of them should be depressed mood and/or diminished interest or pleasure. Attention is to be given so that symptoms do not result from any substance abuse.

There are also two additional specifiers for the diagnosis, of depression:

- with mixed features – such as manic symptoms;

- with anxious distress – the presence of anxiety symptoms;

The depression causes are multiple and commonly classified in four main categories (A ÷ D), as follows:

A. Psychosocial stress - that represents the disability in MS because it is generated by: global physical impairment; illness evolution in pus, with no possible estimation in time; motor deficits; diminished social role / inadequate support from the family.

B. Neuro-immune-endocrine triad (see figure 1) - as in MS patients the severity of depression depends directly on the amount of cytokines, IFN⍰. In this research, there was noticed decrease in IFN⍰ amount after antidepressant treatment, once the depression symptoms diminished. The cytokines stimulate the axis: HHA (hypothalamic-hypophyseal-adrenal) ⍰ CRH (Corticotropin-releasing hormone) ⍰ ACTH (Adrenocorticotropic hormone) ⍰ CORTISOL. The cytokines disturb the metabolism of monoamines (serotonin, non-adrenaline, dopamine) by prostaglandins - in cerebral areas highly important for depression: the hippocampus and hypothalamus.

C. Relationship between brain injuries and depression - based on neuroimaging evidence (RMN). Multiple sclerosis illness does affect myelin and nerve conduction so depression could be a direct consequence of the disease. The severity of depression directly depends on the right temporal lesions of the frontal lobe and the area adjacent to the basal ganglia.

D. Pharmaceutical treatment - there is no relevant evidence of increased occurrence of depression symptoms caused by the MS pharmacological treatment. Mainly, if the depression gets worse it is caused by the worsening of disability. Depression treatment for MS patients stands in two categories, one is psychotherapy and the other is pharmacological. Psychotherapy commonly consists of: psychological counseling; support therapy and cognitive-behavioral techniques.

To diminish depression, there are some additional steps [29] that could be done by multiple sclerosis patients, complementary to psychotherapy and pharmacological treatment, such as:

- physical exercises - that determine higher endorphins rate and, as a consequence, reduce fatigue and better mood;

- stress management program - meditation;

- talk about feelings and emotions - to a friend, family member, or, priest;

- belong to a community - to take part in targeted activities (walks, talks, reunions);

- find something that makes you happy and enjoy the day;

- help other people in need - get a sense of worthiness and utility;

- pet adoption - if possible and allowed by doctors;

- excellent sense of humor.

Figure 1: Neuro-immune-endocrine triad scheme

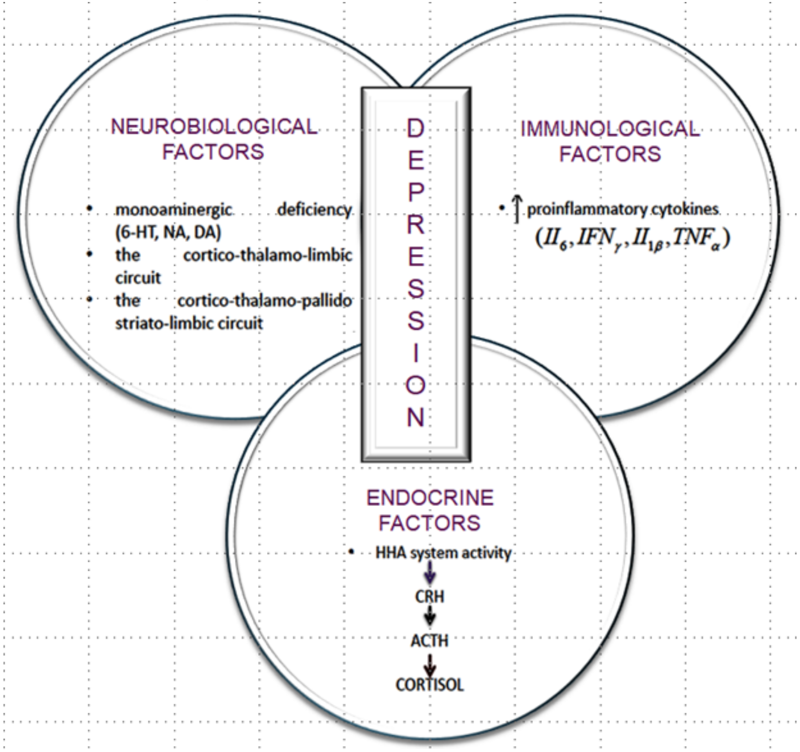
NEUROBIOLOGICAL FACTORS
monoaminergic deficiency (6-HT, NA, DA)
the cortico-thalamo-limbic circuit
the cortico-thalamo-pallido striato-limbic circuit
DEPRESSION
IMMUNOLOGICAL FACTORS
↑ proinflammatory cytokines
$(Il_6, IFN_\gamma, Il_{1\beta}, TNF_\alpha)$
ENDOCRINE FACTORS
HHA system activity
CRH
ACTH
CORTISOL

In this research the focus is not on either psychotherapy, or additional actions, even the patients in the sample group also practiced some of these therapies. This research is focused on the therapeutic particularities of depression, in relapsing-remitting multiple sclerosis (RRMS) patients.

Pharmacological treatment in depression for relapsing-remitting multiple sclerosis patients is evidenced in Table 2. These therapeutic schemes are particularly used in neurology clinics the authors work. All the patients of the survey are included in the national program for the treatment of multiple sclerosis and monitored by: neurological examination; brain imaging; psychological examination; and psychiatric examination.

Table 2: Pharmacological treatment in depression

Class	Representative	Dose [mg/day]	Comments
ADT (antidepressants)	Nortriptyline Desipramine	50 ÷ 200 100 ÷ 300	- limited use because of anticholinergic side effects (dry mouth, constipation, urinary and cardiovascular disorders)
SSRI (selective serotonin reuptake inhibitors)	Sertraline Fluoxetine S-Citalopram	50 ÷ 200 20 ÷ 80 10 ÷ 20	- good tolerance; - efficiency; - side effects (sexual dysfunctions, insomnia)
NaSSA (noradrenergic and specifically serotonergic antidepressants)	Mirtazapine	15 ÷ 45	- efficiency; -minimum effects on sexual function; - side effects (weight gain, drowsiness)
SARI (reuptake inhibitors and serotonin 2A antagonists)	Trazodone	150 ÷ 300	- efficiency; - minimum effects on sexual function; - side effects (drowsiness, orthostatic hypotension)
NDRI (noradrenaline and dopamine reuptake inhibitors)	Bupropion	150 ÷ 400	- efficiency; - minimum effects on sexual function; - side effects (convulsions, psychotic symptoms)
SNRI (serotonin and noradrenaline reuptake inhibitors)	Venalafaxine	75 ÷ 225	- efficiency; - side effects (intestinal disorders, psychotic symptoms, arterial hypertension)

The patients in the research group were diagnosed and subsequently admitted to the hospital when acute attacks occurred. Because of the relapsing attacks, patients develop anxious depressive disorder with panic attacks. Once the severe symptoms were relieved, they were discharged from the hospital and followed up the therapeutic scheme so that to get into the survey.

Initially, it was considered for the study a group of 102 patients, registered within a period of 4 years (2019 - 2023). Because of changes in their health conditions, mental and willingness to take part in the study, not the least family involvement, some of them had to retire. So, the size of the study (sample) group got down to 58, but its size remains fit for a research study (as the sample size is greater than 30).

The survey group is made of 58 relapsing-remitting multiple sclerosis patients with moderate, or severe, depression, out of which 18 are males with ages in between 18 and 30 years. The others are 40 females with ages between 30 and 40 years.

One can notice the higher percentage of females with MS diagnostic (40 out of 58, meaning around 69%) aspect revealed by most of the specific literature references. There has to be mentioned the fact that young people (above 25 years old) usually suffer from anxiety or anxious depression disorder, while mature people suffer from anxious depression disorders or recurrent depressive disorders. The survey period lasted for 16 weeks.

The customized therapeutic scheme applied for diminishing depression, based on the authors' expertise in the diagnosis and treatment of (relapsing-remitting) multiple sclerosis consists of the administration of one of the antidepressants (see Figure 2):

- venlafaxine - for 7 patients (12%), in flexible dosing of 75 ÷ 150 mg/day;

- sertraline - for 8 patients (14%), in flexible dosing of 50 ÷ 100 mg/day;

- mirtazapine - for 19 patients (33%), in a dose of 30 mg/day;

- trazodone - for 24 patients (41%), in a dose of 150 mg/day;

The selected percentages for medication considered the medical history of patients and estimated positive effects of the antidepressant. Also, previous experience of the authors (medical team) in the administration of these types of medicines is important. If there are multiple relapsing attacks, an increase in dosage has to be considered.

The antidepressant medication has to be administrated only in consideration of the following rules (see Figure 3):

- the patients who do not exhibit hypnic jerk were administered sertraline or venlafaxine which do not have sedative effects;

- the patients with hypnic jerk were administered mirtazapine or trazodone, as they also have sedative effects;

- the patients with appetite decrease/weight loss were administered mirtazapine.

Figure 2: Schematic representation of the depression medication for RRMS patients

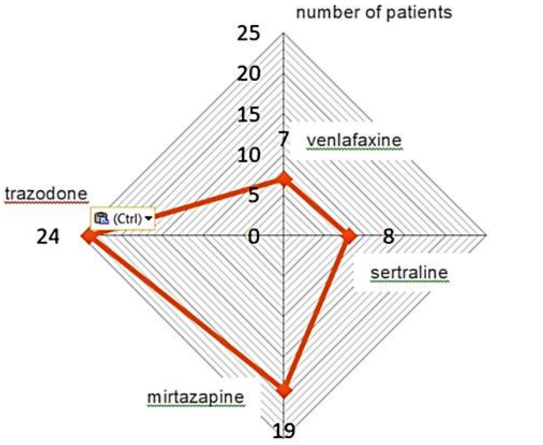

Figure 3: Customized therapeutic plan for RRMS patients

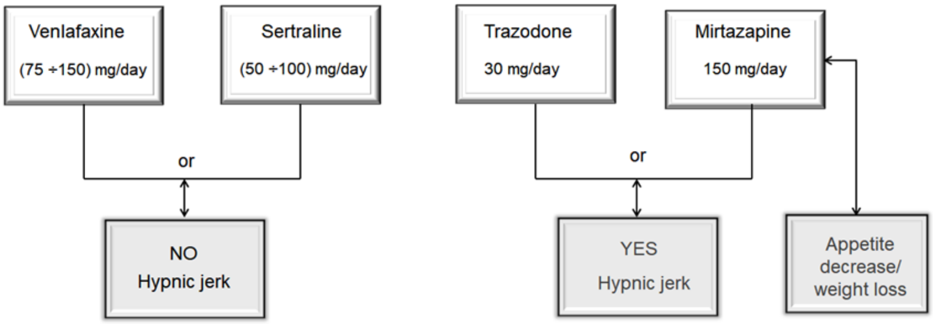

RESULTS

In diagnosing depression among patients with relapsing-remitting multiple sclerosis, it's essential to apply DSM-5 criteria tailored for MS. Recent guidelines from the National Multiple Sclerosis Society (2025) highlight the interplay between neurological symptoms and psychological distress in MS. These updated guidelines advocate for a nuanced diagnostic approach beyond traditional tools like the Hamilton Depression Rating Scale (HAM-D), ensuring diagnoses reflect current clinical insights.

This scale is a multiple-item questionnaire, providing a self-evaluation tool for depressive symptoms. The advantage of using HAM-D is that of high fidelity [30] in terms of clinical symptom assessment due to multiple questions for most of the items. It is compatible with DSM and provides an indicator for the severity of depression reported by an adult, based on a threshold score of clinical value.

Some relevant aspects of HAM-D and its scores are mentioned [31,32] next:

- the scale is scored between 0 and 4;

- scores of 0–7 suggest normal state;

- scores of 8–16 suggest mild depression;

- scores of 17–23 suggest moderate depression;

- scores starting from 24 suggest severe depression.

Before starting the customized depression disorder therapeutic schemes, based on the HAM-D test results, patients were divided into five groups, depending on test scores, as follows:

- 14 patients with HAM-D = 25 (group 1, G1);

- 5 patients with HAM-D = 21 (group 2, G2);

- 22 patients with HAM-D = 19 (group 3, G3);

- 14 patients with HAM-D = 18 (group 4, G4);

- 3 patients with HAM-D = 17 (group 5, G5).

Survey and evaluation after two weeks of treatment proved the improvement of depression symptoms, evidenced by psychiatric examination and HAM-D scores. The same trend was evidenced by further evaluation after six weeks of treatment, as evidenced in Figure 4). One can notice that patients with a HAM-D score of 25 at the beginning of the survey (severe depression, group G1) got to a score of 19 (moderate depression) after 2 weeks of treatment and, further, achieved a score of 11 (mild depression) by the end of the survey (within 6 weeks of therapeutic scheme applied). The same improvements in HAMD-D scores are observed for the patients classified in the other four groups (G2-G5).

Figure 4: HAM-D scores for RRMS patients after the therapeutic plan

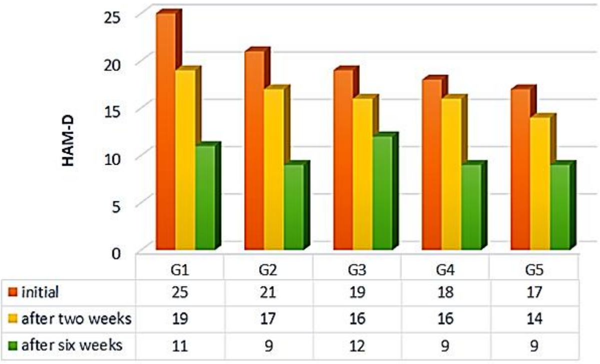

	G1	G2	G3	G4	G5
🟥 initial	25	21	19	18	17
🟨 after two weeks	19	17	16	16	14
🟩 after six weeks	11	9	12	9	9

DISCUSSION

There are different signs of multiple sclerosis caused by the location and severity of the myelin sheath lesions. Some alarm signals for this illness are: disorders in equilibrium, visual disturbances, and loss of ability for independent motion.

There is a common opinion regarding the risk of immobilization for patients with multiple sclerosis but, the highest risk for them is that of suicide. Depression represents a real problem in patients with multiple sclerosis, with about 50% of them exhibiting at least one depression symptom.

This research study is focused on therapeutic particularities of depression, in patients with relapsing-remitting multiple sclerosis (RRMS). Even though the patients in the sample group previously practiced either psychotherapy or additional actions for healing depression, the results did not prove to be fundamentally encouraging.

In the sample group, there are 18 males - from 18 up to 30 years old and 40 females aged between 30 and 40 years. This proves the higher percentage of females having the diagnosis of multiple sclerosis.

Depression disorder diagnostic was given to the 58 sample patients based on psychiatric and psychological examination by the total Hamilton Depression Rating Scale. There were considered five groups, depending on HAM-D test scores, all pointing toward severe to moderate depression disorders. The patients are included in the national program for the treatment of multiple sclerosis.

Analyzing the obtained results, important aspects of therapeutic schemes are evidenced. First, it is obvious that any of the four antidepressants are efficient and with patients good tolerance. Surveys and evaluations after two weeks of treatment proved the improvement of depression symptoms. The same trend was evidenced by further evaluation after six weeks of a customized therapeutic scheme.

Then, it is to be emphasized the lack of side effects that could determine interruption of the treatment. None of the sample group patients showed relevant side effects.

After two weeks of treatment, the HAM-D scores turn from severe depression up to moderate depression (for groups G1, and G2) and from moderate depression to mild depression (groups G3, G4, G5). After 6 weeks of the therapeutic scheme applied, in each of the groups, the result was that of mild depression.

The variation of HAM-D scores, through the survey, for each of the groups, G1-G5, is evidenced in Figure 5. Regression models (dash lines) that fit the survey data (curved lines) are marked along each of the regression lines, just to prove the tendency (by the negative slope) of decreasing the HAM-D results.

Statistical processing of data resulted in mean values of HAM-D scores as follows: 20.28 when starting the survey (initially); 16.69 after two weeks of customized therapeutic scheme and 10.67 at the end of the survey (after 6 weeks), as evidenced in Figure 6. It can be noticed that the trend was that of decreasing HAM-D scores so that the patients' symptoms of depression turned from moderate depression (scores of 17–23) to mild depression (scores of 8–16).

Figure 5: HAM-D models during the survey

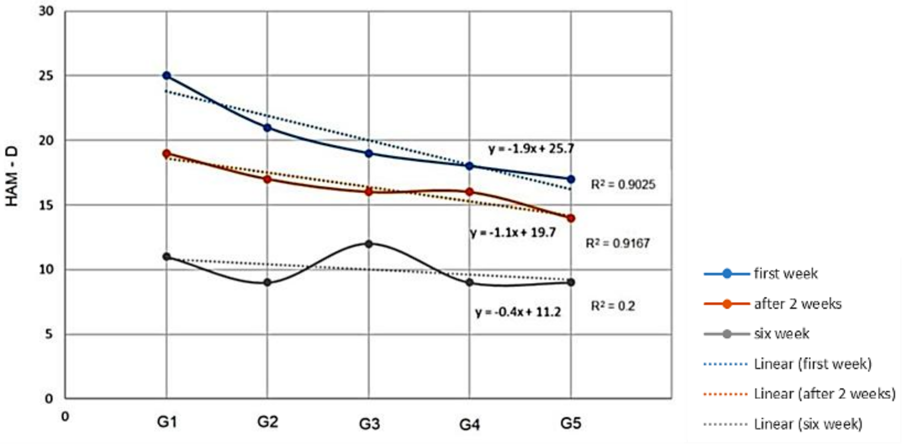

Figure 6: HAM-D mean scores

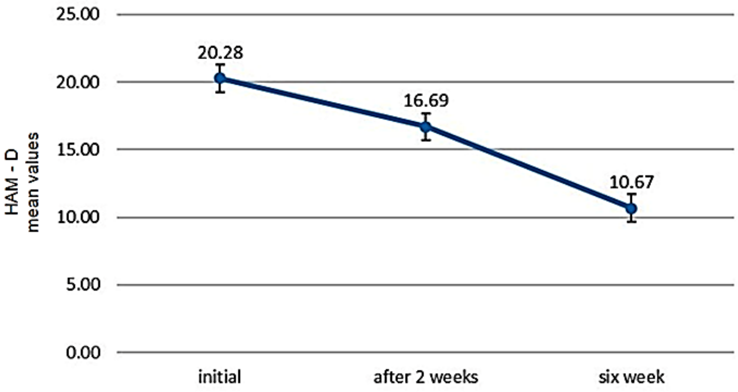

The items of the HAM-D scale that target somatic aspects (psychomotor retardation, agitation, depth of sleep, wake-up insomnia) are less specific when evaluating depression and anxious depression in patients with associated relapsing-remitting multiple sclerosis.

CONCLUSION

Depression is, relatively, common in MS patients, with approximately half of them being diagnosed with it. There is no exact knowledge of its relevance but it is assumed the influence of many factors like: psychosocial, immunological, and brain injuries. It is not sure if some MS patients have depression as a multiple sclerosis reaction or if depression is part of this illness's biology.

Many times, depression is underdiagnosed in MS patients because some of the depression symptoms also belong to the disease (fatigue, concentration difficulties, psychomotor slowness, sleep disorders, sexual disorders).

There is a negative relationship between multiple sclerosis and depression, as depression can be a factor for worsening MS and reverse. Most of the MS patients exhibit both anxiety and depression symptoms, the predominant feature of their syndrome being the one that points towards depression, anxiety, or, mixed anxiety-depressive disorder. The depressive symptoms are in remission with adequate treatment (psychotherapy and/or pharmacological) thus improving the quality of patient life.

Depression treatment for MS patients stands in two categories, one is psychotherapy and the other is pharmacological. Additionally, these, complementary actions could be performed by the patients as, for example: doing things that make them happy, activating a stress management program, belonging to a group of people that share similar problems, etc.

The research results on therapeutic particularities of depression are presented in this article. The sample group used in the study is made of 58 patients with relapsing-remitting multiple sclerosis, out of which 69% are females. They are between 18 to 40 years old.

The customized therapeutic scheme developed is based authors' expertise in the treatment of relapsing-remitting multiple sclerosis and mainly consists of the administration of one of the following antidepressants: venlafaxine; sertraline; mirtazapine; trazodone. The antidepressants are prescribed only considering particularities of the patient's health, like: exhibiting or not exhibiting hypnic jerk; evidence of appetite decrease, or weight loss.

The diagnosis and the severity of depression were evaluated by the total Hamilton Depression Rating Scale (HAM-D). Periodic evaluation of patients' depression symptoms evidenced improvement from severe to moderate to mild depression, after two weeks and, respectively, six weeks of therapeutic scheme.

Further research development would focus on different treatment schemes for depression disorders in MS patients, as well as on psychotherapy and sustained family involvement. It would also be pay attention to the complementary/additional steps in depression customized treatment as: stress management program - meditation; belonging to a community - so that to take part in targeted activities (walks, talks, reunions); find something that makes happy and enjoy the day; help other people in need - gets a sense of worthiness and utility.

Maybe, more adequate screening could be done using the Beck Depression Inventory (BDI) as it does exclude the items on somatic symptoms. It was not used in this research as the clinic where the survey was developed did not commonly apply the inventory for assessing depression severity.

Finally, it could be stated that a customized therapeutic scheme for depression disorders in patients with multiple sclerosis is considered to be the main tool for reducing symptoms and improving, even if possible, eliminating the depressed mood.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

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Authors' contribution

Conceptualization, E.F.L. and S.L.; methodology, D.G.C. and G.P.G.; software, A.B.; validation, G.P.G.; formal analysis, S.L.; investigation, H.M. and D.G.C.; resources, E.F.L. and S.L. data curation, G.P.G. and A.B.; writing—original draft preparation, D.G.C. and E.F.L.; writing—review and editing, E.F.L. and G.P.G.; visualization, S.L. and D.G.C.; supervision, G.P.G. and A.B.; project administration H.M. and S.L. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Clinical Hospital CF2, Bucharest, Romania; (protocol code 1121/12.10.2023).

Data Availability Statement

Data is available on reasonable request.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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ORIGINAL ARTICLE

Assessment of the Sensitivity and Specificity of CT Investigations in Characterizing and Evaluating the Post-treatment Evolution of Ovarian Tumor Lesions

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Abstract: This study analyzes the contribution of Computed Tomography (CT) in the detection and characterization of ovarian tumors, particularly malignant ones, to enhance the accuracy of preoperative diagnosis and postoperative follow-up, thereby optimizing therapeutic planning. This paper presents a retrospective study conducted on a group of 63 patients with ovarian tumors who were operated on at the Department of Gynecology of the Central University Emergency Military Hospital "Dr. Carol Davila" in Bucharest. The patients were investigated preoperatively using Computed Tomography to characterize the ovarian tumors and assess the sensitivity and specificity of the imaging diagnosis. Computed Tomography utilizing new multidetector technologies (MDCT) provides superior characterization of adnexal masses, with a sensitivity of at least 90% and a specificity of 80%. This imaging modality is recommended for the preoperative staging of ovarian cancer.

Keywords: CT, MDCT, ovarian tumor, postoperative follow-up, postoperative evolution, sensitivity, specificity, accuracy

INTRODUCTION

Ovarian cancer is a heterogeneous disease that is the eighth most common cancer in women and the leading cause of gynecologic-related cancer death [1-3].

In relation to the postoperative histopathological/immunohistochemical diagnosis for each operated patient, the contribution of Computer Tomography was evaluated for the preoperative characterization of ovarian tumors and assessment of the post-interventional evolution through monitoring at 6 and 12 months, respectively [4-6].

The Computer Tomography (CT) examination is an imaging method that uses ionizing radiation, but demonstrates good sensitivity and specificity, thanks to advancements in multidetector technology. Computed tomography using new multidetector technologies (MDCT) achieves a better characterization of adnexal masses, with a sensitivity of at least 90% and a specificity of 80%. This type of imaging is recommended for preoperative staging of ovarian cancer [4]. This is especially recommended for the preoperative staging of ovarian tumors [7,8,9]. The CT examination is usually used in addition to an inconclusive abdominopelvic ultrasound for the differential diagnosis of pelvic pain syndrome [8]. Although the accuracy of CT is generally lower than that of magnetic resonance imaging (MRI) or transvaginal ultrasound for assessing ovarian pathology, its multiplanar reconstructions enable effective evaluation of the extent of ovarian lesions and identification of coexisting pathologies [4,10,11].

The ovaries are visualized near the iliac vascular axes as oval structures with low densities due to the presence of peripheral follicles. Ovarian ligaments may appear as dense linear structures, especially when free intraperitoneal fluid outlines them. The corpus luteum is thick-walled and shows peripheral contrast uptake [5,7,10,11].

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This study aimed to evaluate the performance of diagnostic imaging concerning postoperative histopathological results and the features that may be useful for the classification of tumors. The diagnostic imaging introduction should briefly place the study in a broad context and highlight its importance. It should define the purpose of the work and its significance.

MATERIALS AND METHODS

This retrospective study investigated a group of patients with ovarian tumors (n = 63), operated in the Gynecology Department of the Central University Emergency Military Hospital, who underwent preoperative CT examinations. The studied group included patients with benign ovarian tumors, histopathologically diagnosed postoperatively (n=30), and with malignant ovarian tumors (n = 33); 3 patients with malignant ovarian tumors were considered borderline.

The main imaging characteristics that were considered suspicious for malignancy were the following: solid components; endo/exocystic vegetations; wall with irregular thickness and intramural nodules; irregular intratumoral septa with thicknesses over 3mm; contrast uptake of the solid components (intratumoral, parietal or septal) with enhancement curve type II/III appearance (early contrast uptake of lower intensity/equal to the myometrium, persistent in the plateau, respectively intense early contrast uptake, more pronounced than the myometrial and dynamic "wash-out"); dimensions (>3 cm); secondary lesions involving the peritoneal lining, mesenteric or omental fat; ascites; pelvic extension; locoregional nodal disease; distant metastases (extraperitoneal) [5,8].

Fisher's test and Mann Mann-Whitney (Wilcoxon) test were used to determine the significance between groups. The correlation between age at diagnosis and CA125 values was performed using Pearson's correlation coefficient.

The Sensitivity [TPR =TP/(TP+FN)], False negative ratio [FNR=FN/(TP+FN)]; False positive ratio [FPR=FP/(FP+TN)], Specificity [TNR =TN/(FP+TN)], Predictability of positive values [Precision PPV=TP/(TP+FP)]; Omission rate [Omission rate = FN/(FN+TN)] and Accuracy [Accuracy = (TP+TN)/Total] were estimated. For this estimation, the values for True positive (TP); True negative (TN); False positive (FP) and False negative (FN) were used.

RESULTS

This retrospective study analyzed 63 patients with ovarian tumors who were preoperatively CT investigated. Nine patients with malignant tumors and eight with benign tumors had bilateral tumors. Histopathological diagnosis established the presence of epithelial (n = 40), germ cell (n = 17), or stromal and sex cord tumors (n = 6). Epithelial tumors are more common in patients with malignant tumors compared to benign tumors. (31 vs 9, p<0.0001). Multilocular tumors were identified in 29 patients with malignant tumors and 4 patients with benign tumors (p<0.001). Endo or exocytic vegetations were identified in 29 of the patients with malignant or borderline tumors, but were not present in those with benign tumors.

Of the patients analyzed, 23 resided in rural areas (9 with benign tumors) and 36 (21 with benign tumors) in urban areas. In this sample, the distribution of malignant and benign tumors was not associated with place of residence (p>0.05). The age at diagnosis of patients from urban and rural areas was similar (44.58±13.72 vs 50.44±10.50, p>0.05).

The median age of these patients was 46 years (23-72 years old). The age of patients with benign tumors (average: 41.40±12.17 years; 23-67 years old) was significantly lower (p=0.0007) than that of those with malignant tumors (average: 51.88±10.61; 35-72 years old). The age at diagnosis in the subplot of women with tumors over 4 cm was lower in subjects with benign tumors when compared with carriers of malignant tumors (40.12±12.88 vs 51.88±10.61 vs p=0.003). The age of patients with malignant tumors, stratified according to the presence or absence of metastases, was similar (52.44±10.77 vs 51.35±10.76, p>0.05).

CA125 was elevated more frequently found to be elevated in carriers of benign tumors (15 patients with benign tumors, all 3 patients with borderline tumors, in 5 patients with malignant tumors, p<0.05). The values of this parameter were not significantly correlated with the age of the patients in the entire group of subjects (r = 0.25) or the groups of patients with benign tumors (r = 0.16) or malignant tumors (r = -0.06).

The ROMA score had increased values in all patients with malignant tumors and 53.33% of patients with benign tumors. The size of the tumors over 4 cm was recorded in all 33 malignant and borderline tumors and 17 of the benign lesions. Analysis of the intratumoral solid component. A solid intratumoral component was detected in 52 of the studied ovarian tumors (30 malignant tumors and 22 of benign tumors, p<0.05). The presence of irregular intratumoral septa with a thickness of over 3mm was found in 28 malignant and borderline tumors and only in 4 benign tumors (p<0.0001).

Contrast uptake analysis of the solid/parietal/septal tumor components. Of the benign tumors, 80% showed contrast uptake (80% with a progressive loading curve type I), and in the remaining 20%, contrast uptake was absent. Bilateral ovarian serous cystadenocarcinoma, with extensive peritoneal disease (especially at the level of the great omentum - "omental cake") and a small amount of ascites near the liver, spleen and in the pouch of Douglas. Ascites were detected in 22 of 30 malignant tumors, in none of the borderline tumors, and in one case of a benign tumor (p<0.0001).

Analysis of locoregional lymph node disease. Several 32 tumors from the 63 cases presented with enlarged locoregional lymph nodes. Of these, most were malignant and borderline tumors (78% of this group), confirmed histopathologically with positive lymph node disease. Out of the total of benign tumors, only 20 % of benign tumors had locoregional ganglionic hypertrophies, confirmed histopathologically as reactive lymph nodes (inflammatory). These differences between malignant and benign tumors are statistically significant (p<0.0001).

Analysis of cases with macroscopic peritoneal disease. Macroscopic peritoneal disease was highlighted only in malignant tumors, 78% of malignant tumors presented this aspect. The age of women with or without macroscopic peritoneal disease was similar (51.80±10.89 vs 52.13±10.40, p>0.05).

Analysis of pelvic parietal extension/to the neighboring organs. Most of the malignant tumors (70%) and 2 of the borderline ones showed pelvic parietal extension/to the neighboring organs. Among the benign tumors, only one presented this aspect, which represents 3%.

Figure 1: Contrast-enhanced CT examination

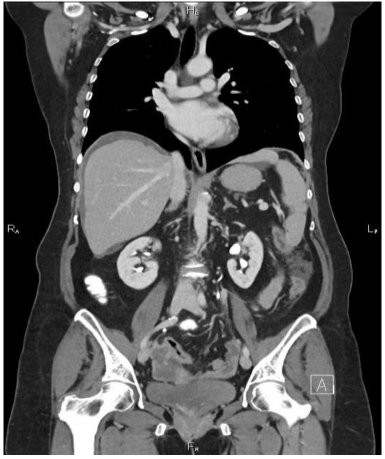

Analysis of cases with distant metastasis. This category included cases in which distant (non-peritoneal) metastases occurred. Among the malignant tumors, 53% showed distant metastases, while benign or borderline tumors did not show such metastases.

Evaluation of the sensitivity and specificity of CT examination. From the sample of 63 cases investigated by CT examination (the groups of patients evaluated by ultrasound and CT or ultrasound, CT, and MRI), the number of cases identified as positive for malignant tumors was 36, in relation to the post-interventional histopathological examination that established the diagnosis of malignancy in 33 of the analyzed cases.

There were 27 cases evaluated by CT with a benign appearance, and the post-interventional histopathological examination established the diagnosis of benignity in 30 of them.

The confusion matrix for assessing the sensitivity and specificity of CT evaluation of malignant ovarian tumors showed a sensitivity of 91%, with a specificity of 80% and an accuracy of 85%.

Table 1: Confusion matrix for sensitivity and specificity of CT examination

Results	Final Positive	Final Negative	
CT positive	TP=30	FP=6	Predictability of positive values, =0.83
CT negative	FN=3	TN=24	Omission rate =0.11
	Sensitivity = 0.91	False positive ratio =0.2	Accuracy=0.85
	False negative ratio=0.09	Specificity =0.80	

CT evaluation was particularly beneficial in cases of large tumors with distant extension, allowing for the assessment of secondary lesions in other organs and the presence of associated comorbidities, in order to optimize therapeutic management.

From the group of 63 cases investigated by CT examination, the number of cases identified as positive for malignant tumors was 36, compared to the post-interventional histopathological examination, which confirmed the diagnosis of malignancy in 33 of the analyzed cases.

The method remains useful in assessing nodal extension and distant metastatic disease and allows a sensitivity of 90%, a specificity of 89%, a positive predictive value of 78%, and a negative predictive value of 95%, with an overall accuracy in diagnosing malignancy of about 89% [2,7,10].

DISCUSSION

This retrospective study analyzed 63 patients with ovarian tumors who were preoperatively CT investigated. Histopathological analysis reveals that epithelial tumors, multilocular tumors, and cystic vegetations were more common in malignant tumors. These differences highlight the heterogeneity and differences between ovarian tumors.

The median age of patients investigated in this study was 46 years (23-72 years old). This result is concordant with data previously published [15], the majority of women diagnosed with ovarian cancer are in the perimenopausal state.

The size of the tumors over 4 cm was recorded in all 33 malignant and borderline tumors and 17 of the benign lesions. The large sizes found in patients diagnosed with malignant tumors may be due to the non-specific symptoms at the onset and the late presentation at the gynecological examination, which determines a long time until diagnosis, ovarian tumors being most frequently discovered incidentally during a routine examination.

A solid intratumoral component was detected in 52 of the studied ovarian tumors (30 malignant tumors and 22 benign tumors, $p<0.05$). The presence of a solid intratumoral component with enhancement curve type II/III is correlated with a higher risk of malignancy, as the Ovarian-Adnexal Reporting and Data System shows [16].

The presence of irregular intratumoral septa with a thickness of over 3mm was significantly more frequent in malignant and borderline tumors than in benign tumors ($p<0.0001$). The irregular septa with a thickness of over 3mm in any ovarian tumor seen on imaging studies should raise suspicion of malignancy, requiring further investigations.

Ascites was detected only in 22 of 30 malignant tumors. In most cases, ascites is present in ovarian cancer because neoplastic cells in the peritoneal lining affect the physiological drainage of peritoneal fluid [17]. Very rarely, ascites can be associated with a benign ovarian tumor, as we found in our patient who had Demos-Meigs syndrome [18].

Most of the malignant tumors (70%), 2 of the borderline ones, and one benign tumor showed pelvic parietal extension/to the neighboring organs. Parietal extension/invasion to the neighboring organs is an imaging feature of malignancy until proven otherwise. Sometimes, in imaging studies, it can be misinterpreted as malignancy in cases of chronic infections or previous surgeries [4].

The ROMA score had increased values in all patients with malignant tumors and 53.33% of patients with benign tumors. In some studies, high ROMA scores were correlated with advanced ovarian cancer before surgery [19,20].

Different imagistic methods can be used to differentiate between physiologic, benign, and malignant ovarian conditions in women [21,22]. Computer Tomography has increased value, especially for the preoperative staging of ovarian cancer, considering the increased spatial resolution of the method, the increased accuracy in the detection and characterization of masses through the new multi-detector technologies (MDCT) implemented, and the reduced radiation doses due to low-dose protocols optimization [6,23].

Computed tomography (CT) is the imaging modality of choice for staging ovarian cancer. Multidetector computed tomography (MDCT) provides clinically relevant information about these tumors (e.g., size of the primary tumor, size and location of peritoneal implants, and lymph nodes). These data can be used to help evaluate surgical options. The overall accuracy of MDCT in diagnosing malignant adnexal masses is as high as 89%. MDCT signs predictive of malignancy include the presence of papillary projections in a cystic lesion, necrosis in a solid mass, and peritoneal metastases. CT is frequently used to detect ovarian carcinoma and to assess response to therapy. CT has limitations in detecting small peritoneal metastases. CT can detect tumors larger than 1 cm with a sensitivity of 85–93% and a specificity of 91–96% [24].

CONCLUSION

Computed Tomography has significant value in the management of ovarian cancer patients, especially in the preoperative staging of ovarian cancer, given its high spatial resolution and improved accuracy in detecting and characterizing ovarian tumor masses and metastatic lesions. This is achieved through newly implemented multi-detector tomography (MDCT) technologies and significantly reduced radiation doses using optimized low-dose protocols. The method remains useful in assessing lymph node extension and distant metastatic lesions, offering high sensitivity and specificity, as demonstrated in this article.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, N.I.A. and N.M.F.; methodology N.N, S.N.M.E.D, A.F.C, A.D.S and D.L.M; software, N.F. and S.N.M.E.D.; validation, N.I.A. ,C.I.M, A.D.S, A.F.C. and N.M.F.; formal analysis, N.M.F.; investigation N.I.A.; resources A.D.S, S.N.M.E.D.; data curation N.F.; writing—original draft preparation, N.I.A.; writing—review and editing, N.I.A, N.M.F, D.L.M; visualization, N.I.A.; supervision, N.M.F; project administration, N.I.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the hospital's ethics committee Nr. 601/ 24.05.2023 and is performed under the ethical standards laid down by the Declaration of Helsinki.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Exploring Gliosarcoma by Light Microscopy, Two-Photon Excitation Microscopy, and Radiological Imaging Techniques

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Abstract: Gliosarcoma is a rare and aggressive variant of glioblastoma, characterized by both a malignant glial component and a mesenchymal sarcomatous component. Gliosarcomas have genetic alterations with glioblastomas, including TP53, PTEN mutations, and EGFR amplification, but may also exhibit additional changes related to epithelial-mesenchymal transition pathways. Management is similar to glioblastoma, involving safe, maximal surgical resection followed by radiotherapy and chemotherapy with temozolomide, but the prognosis remains poor, with a median survival of 6-14 months. Both Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) play crucial roles in the detection, characterization, and treatment planning of gliosarcoma. While MRI is the gold standard, CT remains valuable in certain situations. Light microscopy is essential for definitive diagnosis, allowing pathologists to examine cellular morphology and tissue architecture. It is essential for distinguishing gliosarcoma from other high-grade gliomas and for guiding treatment decisions. In addition, two-photon excited fluorescence (TPEF) microscopy is an advanced optical imaging technique that enables real-time, high-resolution visualization of tumor tissue without the need for staining or contrast agents and enhances visualization of collagen structure and vascularization, key factors in gliosarcoma assessment. The study of radiological and histopathological (light microscopy) features in primary gliosarcomas of the brain is a priority to achieve an early diagnosis that can be translated into better outcomes. Here, we describe the radiological and histopathological features observed in multiple cases of gliosarcoma in current practice.

Keywords: gliosarcoma; histopathology; light microscopy; two-photon excited fluorescence; radiology; magnetic resonance imaging; neurosurgery

INTRODUCTION

Gliosarcoma is a rare and highly malignant primary neoplasm of the central nervous system (CNS), classified as WHO grade 4. It is characterized by a biphasic histopathological pattern, comprising both glial and mesenchymal components. The peak incidence occurs around the sixth decade of life, with a male-to-female ratio of 1.8:1 [1,2,3]. While most cases are primary, secondary gliosarcomas may develop in patients with previously resected glioblastomas or as a consequence of cranial irradiation. Clinically, gliosarcoma often presents with symptoms such as headache, seizures, and hemiparesis. Due to their aggressive nature, tumors are typically large at diagnosis and frequently cause midline shift [4,5], particularly in the temporal lobe, as revealed by computed tomography (CT) and magnetic resonance imaging (MRI).

Brain and other nervous system malignancies constitute a significant proportion of cancer-related deaths, particularly among children and young adults [6]. Gliomas, the most common primary brain tumors in adults, originate from glial cells and are highly invasive, diffusely infiltrating the surrounding brain parenchyma. Glioblastoma multiforme (GBM), the most aggressive glioma subtype, has a particularly poor prognosis, with a median survival of less than one year and a five-

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year survival rate below 5% [7,8]. The dismal survival outcomes are attributed to the tumor's extensive infiltration, limiting the effectiveness of surgical resection, and leading to inevitable recurrence.

Preoperative imaging modalities such as MRI, CT, single-photon emission computed tomography (SPECT), and positron emission tomography (PET) provide critical insights into tumor localization and characteristics [9]. However, intraoperative identification of residual tumor tissue remains a major challenge. The extent of surgical resection is a key determinant of overall survival and progression-free survival in glioma patients. Achieving maximal safe resection necessitates advanced intraoperative imaging techniques [10] to differentiate tumor tissue from normal brain structures with high specificity and sensitivity.

Recent advances in molecular biology have significantly improved the classification and diagnosis of brain tumors. The integration of molecular markers has refined tumor characterization, enabling more precise prognostic assessments [11]. However, the routine implementation of molecular testing is limited by accessibility constraints, particularly in resource-limited settings [12,13,14]. Furthermore, molecular data interpretation can be complex, and in certain cases, tumors are categorized as "not otherwise specified" (NOS) when genetic features are ambiguous or unavailable.

Histopathological evaluation remains a cornerstone in brain tumor diagnosis [15], with light microscopy providing critical insights into cellular morphology, mitotic activity, necrosis, and vascular proliferation. Hematoxylin and eosin (H&E) staining, alongside immunohistochemical markers, enhances tumor characterization. Despite its essential role, conventional light microscopy alone may not always provide sufficient contrast for distinguishing malignant from benign or reactive tissues. Consequently, there is growing interest in complementary optical imaging techniques for improved tumor assessment.

Emerging optical imaging modalities, including confocal microscopy and nonlinear optical (NLO) microscopy [16,17,18], offer promising non-invasive diagnostic capabilities. Among these, two-photon excited fluorescence (TPEF) microscopy [19] has gained attention due to its ability to generate high-resolution images of biological tissues with minimal photodamage [20,21,22]. TPEF operates by simultaneously absorbing two lower-energy photons, exciting endogenous or exogenous fluorophores, and producing a fluorescence signal. This technique enables detailed visualization of cellular and extracellular structures, making it a valuable tool for characterizing brain tumors, including gliosarcomas.

Most studies utilizing TPEF microscopy in brain tumor research have relied on exogenous fluorescent dyes or genetically expressed fluorescent proteins [23] to achieve image contrast for visualizing tumor cells, neurons, and blood vessels [24,25,26]. However, intrinsic fluorescence signals from endogenous biomolecules [27], such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), can also be leveraged for label-free tumor imaging. TPEF microscopy has demonstrated significant potential in various biomedical applications, including neurobiology [28], tissue engineering [29], and in vivo optical biopsies [30], highlighting its versatility in tumor diagnostics.

In this study, we explore the potential of TPEF microscopy as a complementary imaging tool for gliosarcoma diagnosis, alongside conventional modalities such as CT, MRI, and histopathology. By leveraging TPEF's high-resolution and label-free imaging capabilities, we aim to enhance the visualization of gliosarcoma-specific features, ultimately contributing to improved tumor identification and diagnostic precision.

MATERIALS AND METHODS

These cases were retrieved from the files of our department. In the past 9 years (2016-2024), the department's archive contained and diagnosed 10 cases of gliosarcoma. From these cases, we obtained MRI and CT images for illustration, which we compared with images acquired from tissue sections embedded in paraffin.

The tissue was fixed in a 10% buffered formalin and embedded in paraffin, with 4-5 microns-thick paraffin sections. On hematoxylin-eosin (HE) stained sections, the glial component showed malignant astrocytes, with associated microvascular proliferation and geographic necrosis, while the sarcomatous components showed pleomorphic spindle cells. Features identified on CT and MRI scans were partial uptake of contrast agent with a ring-enhancement pattern, peripheral edema, and central calcification. The tumor displayed a specific pattern in TPEF imaging. Selected regions of interest were examined using a Leica TCS SP laser scanning confocal microscope optimized for nonlinear imaging. The excitation source was a Ti: Sapphire laser (Coherent Chameleon Ultra II) tuned to 860 nm, with a pulse width of 140 fs and a repetition rate of 80 MHz. During scanning, the laser power at the objective focus was kept below 10 mW. A 10× objective (NA 0.3) was used to focus the excitation beam onto the samples and collect the TPEF signal in epi-detection. The resulting field of view was 1 × 1 mm^2. Spectrally resolved detection, inherent to the Leica TCS SP system, was used to capture TPEF signals in the 450–700 nm range. Also, the distinction between tumor and peritumoral brain zone was easy in TPEF analysis, allowing a clear visualization of the brain/tumor interface. TPEF imaging is a fast and efficient method allowing analysis of brain tumor tissue.

RESULTS

MRI and CT analysis

The images were obtained in our hospital, and the MRI characteristics were evaluated. Gliosarcomas can be very similar to glioblastomas in appearance. These are usually widely localized peripheral lesions with possible direct dural invasion or only reactive dural thickening (dural tail). On MRI, gliosarcomas typically present as heterogeneously enhancing lesions with irregular margins, often associated with significant peritumoral edema and central necrosis. They may also demonstrate areas of hemorrhage and variable diffusion restriction. The tumor frequently involves the cerebral hemispheres, with a predilection for the temporal and frontal lobes.

Figure 1: MRI images demonstrating a gliosarcoma with typical localization in the left temporal lobe – A) Sagittal section in T1-weighted imaging; B) Axial section in T2-weighted imaging; C) Coronal section in Fluid Attenuated Inversion Recovery (FLAIR) sequence; D) Sagittal section in post-contrast T1-weighted imaging; E-F) Maximum Intensity Projection (MIP) reconstruction; G) Diffusion-weighted imaging in axial plane; H) Apparent Diffusion Coefficient (ADC) in axial plane; I) Axial section in Susceptibility Weighted Imaging (DWI) sequence

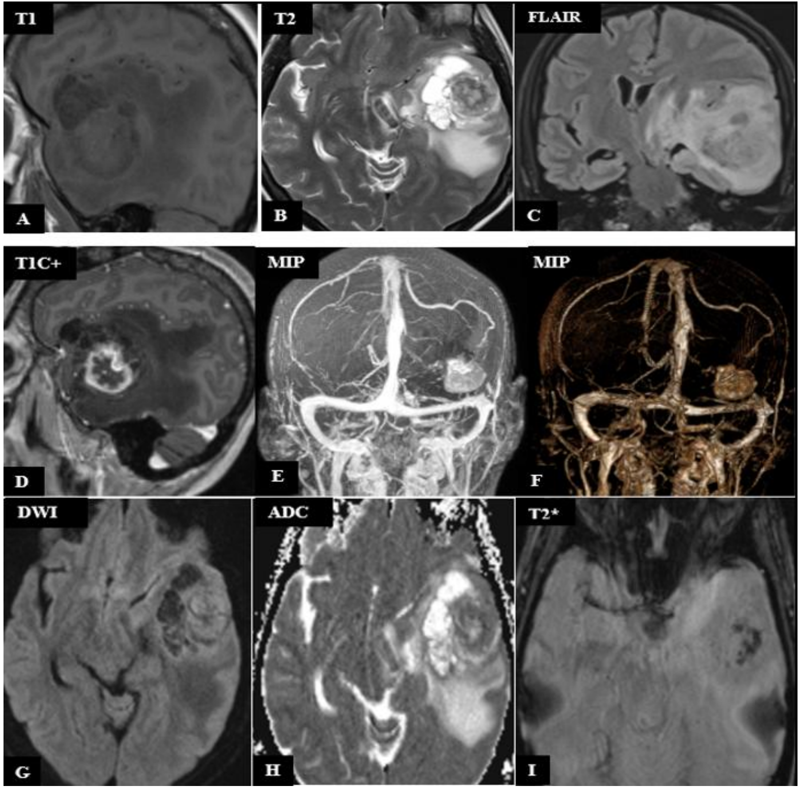

Figure 2: MRI image with postoperative appearance 4 days after surgery - No tumor debris - A) Sagittal section in T1-weighted imaging; B) Axial section in T2-weighted imaging; C) Sagittal section in post-contrast T1-weighted imaging; D) Axial section in subtraction imaging between native T1 and post-contrast T1; E) Diffusion-weighted imaging in axial plane; F) Apparent Diffusion Coefficient (ADC) in axial plane

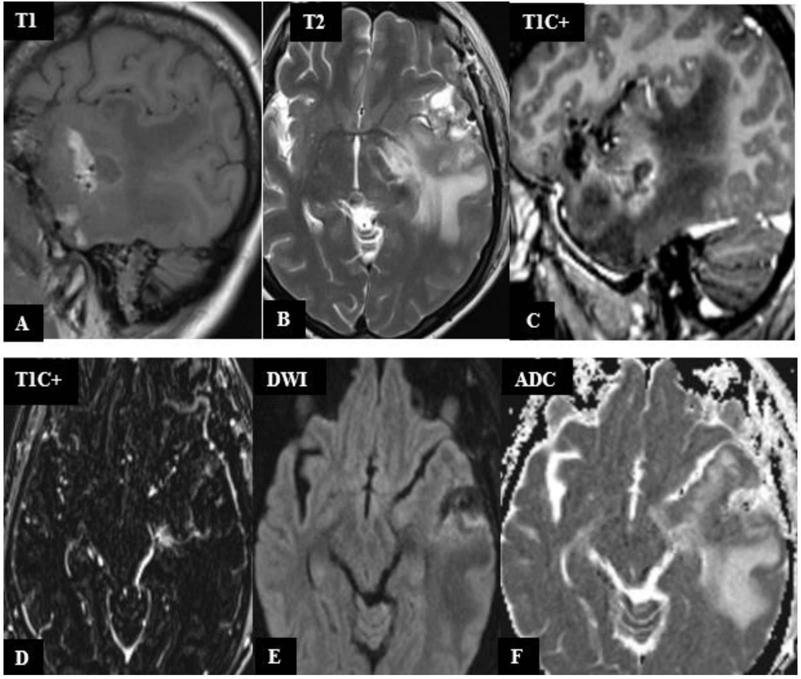

As can be observed in Figure 1, MRI confirms an intra-axial mass in the left temporal lobe. The lesion demonstrates a heterogeneous pattern of central hypersignal and marginal hyposignal on T1-weighted images (Figure 1A) and peripheral hypersignal with central hyposignal on T2-weighted images (Figure 1B), which is consistent with a solid-cystic mass. We can see the ring-enhancement pattern on T1-weighted Gadolinium images (Figure 1D) with central necrosis, as seen in DWI and ADC sequences (Figures 1G-H). A great amount of perilesional edema can be noticed on FLAIR images (Figure 1C). The high vascularity and intratumoral neo-vascularization can be observed on angio-MRI reconstructed images (Figures 1E-F). Small areas of intratumoral hemorrhage can be noted on T2*-weighted images (Figure 1).

In Figure 2, we have MRI images with postoperative appearance 4 days after surgery, with the presence of a marginal contrast-enhanced (Figure 2C, D), left temporal post-interventional porencephalic cavity (Figure 2A, B). No tumor debris present or diffusion restrictions in the surgical area were identified (Figure 2E, F).

MRI images of postoperative appearance 6 days after surgery showed postoperative left temporal changes with a slight decrease in the porencephalic cavity compared to the previous examination (Figure 3). Significant reduction of cerebral edema. On the MIP reconstructions performed from the T1 subtraction sequence with contrast, the disappearance of the tumor seen in Figure 1 is observed (Figure 3F).

Figure 3: MRI images of postoperative appearance 6 days after surgery - No tumor debris present - A) Sagittal section in T1-weighted imaging; B) Axial section in T2-weighted imaging; C) Coronal section in Fluid Attenuated Inversion Recovery (FLAIR) sequence; D) Axial section in post-contrast T1-weighted imaging; E) Coronal section in post-contrast T1-weighted imaging; F) Maximum Intensity Projection (MIP) reconstruction; G) Diffusion-weighted imaging in axial plane; H) Apparent Diffusion Coefficient (ADC) in axial plane; I) Axial section in Susceptibility Weighted Imaging (SWI) sequence

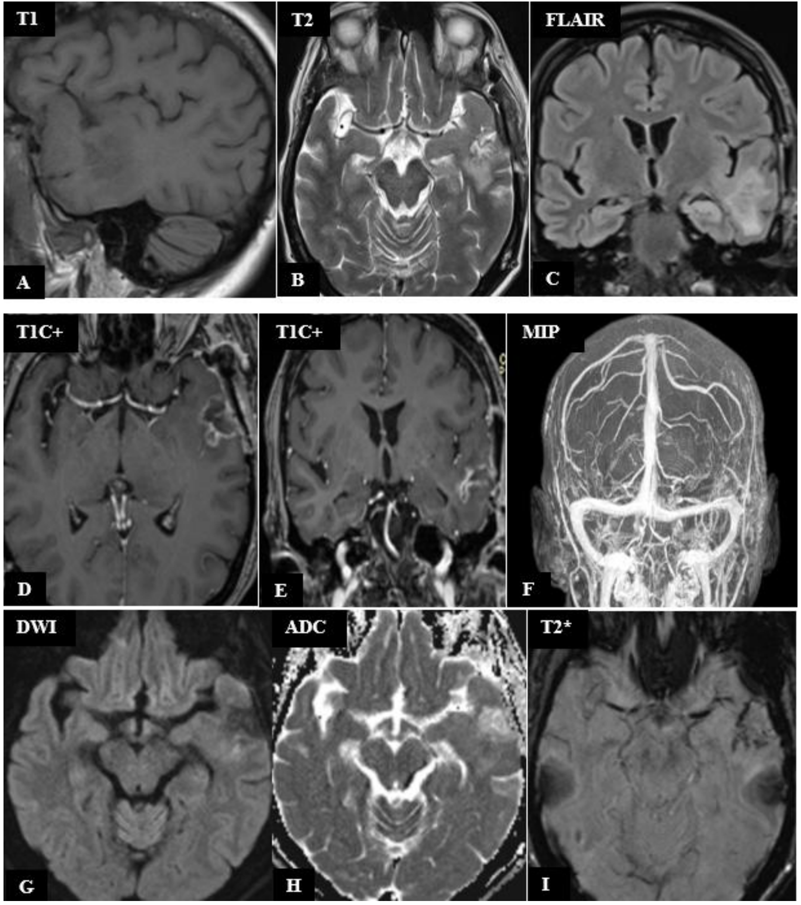

Unfortunately, after 4 months postoperatively, the patient presented again to the emergency department, and he had a head CT scan (Figure 4). The diagnosis was tumor recurrence, which causes mass effect (Figure 4A, C), peritumoral edema (Figure 4A, B, C), displacement of midline shift due to tumor and edema (Figure 4A, C) and herniation of the left contralateral cerebral parenchyma with a subfalcine herniation appearance (Figure 4C).

Gliosarcomas, like glioblastomas, exhibit a high recurrence rate, with most recurrences occurring within the first year following initial treatment. Recurrence often presents with rapid growth and can be more aggressive than the initial tumor, due to the tumor's inherent heterogeneity and resistance to therapy. The prognosis for patients with recurrent gliosarcoma is poor, with limited survival rates. The overall survival after recurrence is typically shorter than for primary tumors.

Figure 4: CT images were obtained 4 months postoperatively - tumor recurrence - A) Axial section (native); B) Sagittal section (native); C) Coronal section (native).

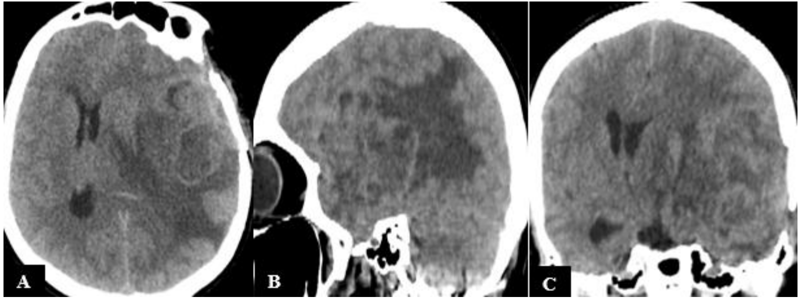

Light microscopy

Images acquired with bright-field microscopy on H&E-stained tissue sections highlight the aspects of a gliosarcoma, which exhibits a biphasic histological pattern, with alternating regions of glial and mesenchymal differentiation. The glial component resembles glioblastoma, characterized by atypical astrocytic cells with pleomorphic nuclei, increased mitotic activity, and areas of necrosis with pseudopalisading (Figure 5A; Figure 6B). The mesenchymal component consists of spindle-shaped cells arranged in fascicles, resembling fibrosarcoma (Figure 5B). Optical microscopy is essential for detecting vascular proliferation, a hallmark of gliosarcoma, including endothelial hyperplasia and abnormal vascular structures. This feature is significant in distinguishing gliosarcoma from other tumors (Figure 6A).

Figure 5: (A) Glial component showing large, pleomorphic, fibrillary astrocytic cells with atypical mitosis in the center (marked with an arrow). (B) Sarcomatous component showing spindle cells arranged in a pattern of densely packed, long bundles

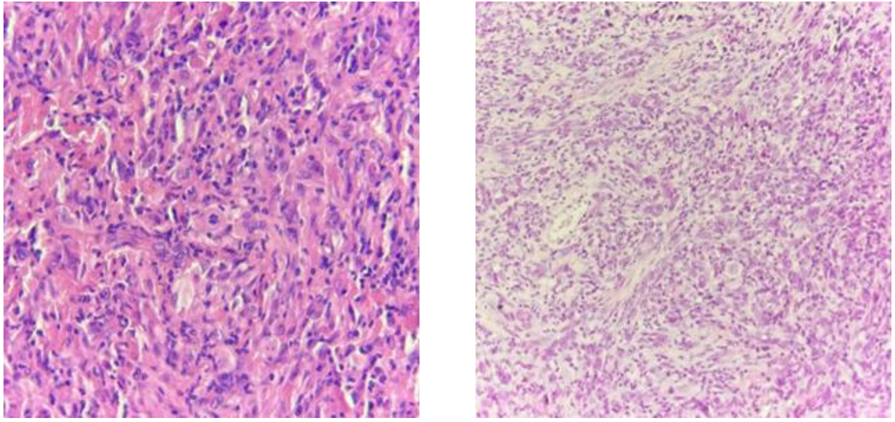

The mitotic index and cellular density observed through light microscopy are useful for grading the tumor. High mitotic activity correlates with a higher grade and a more aggressive tumor. Light microscopy can help assess the peritumoral edema and the extent of surrounding tissue invasion, giving insights into how the tumor interacts with the surrounding brain structures.

Figure 6: (A) Microvascular proliferation is represented by multilayered, small-caliber blood vessels with a glomeruloid appearance (several blood vessels marked with arrows). (B) Geographic necrosis (pseudopalisading neoplastic cells surrounding a central area of tumor necrosis), marked with an arrow

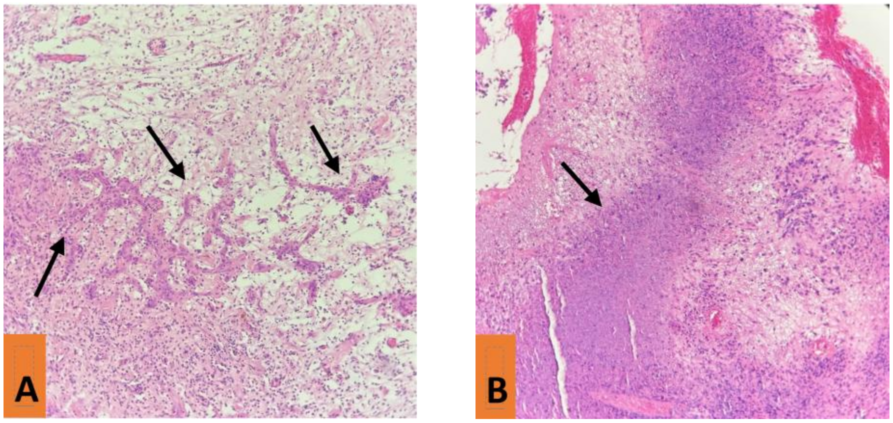

Recurrences often show similar histological features to the original tumor but may have increased cellularity, more prominent necrosis, or increased vascular proliferation.

Understanding the exact histopathological features through light microscopy assists clinicians in treatment planning, as the tumor's aggressiveness, heterogeneity, and vascular properties influence the choice of therapy (e.g., surgical resection, radiotherapy, chemotherapy).

Light microscopy compared to two-photon excited fluorescence (TPEF)

By integrating optical microscopy and TPEF, this study aims to enhance the understanding of gliosarcoma's complex microenvironment, exploring the advantages and limitations of each method in tumor characterization. Our findings provide insights into the potential of TPEF as a complementary diagnostic tool, bridging the gap between traditional histopathology and advanced optical imaging techniques.

Figure 7: (A) Light microscopy image of large, highly pleomorphic glial cells, with bizarre nuclei, mitotic activity in the center (arrow), and microvascular proliferation on the right (asterisk) with corresponding TPEF image – Figure 7 (B).

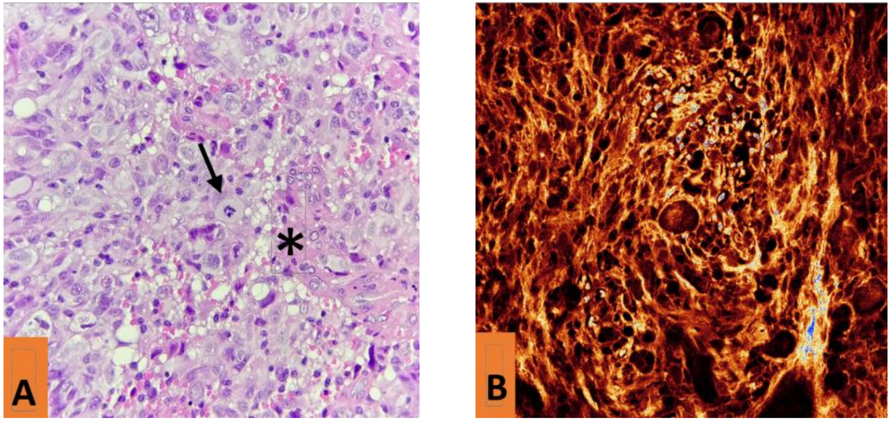

Figure 8: (A) Light microscopy image showing a biphasic pattern, represented by atypical, glial cells intermingled with spindle, sarcomatous cells. (B) Corresponding TPEF microscopy showing the corresponding image

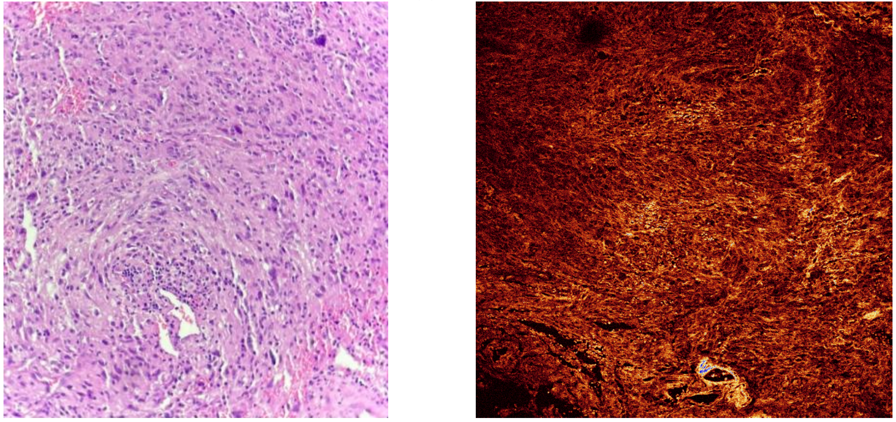

As can be observed in Figures 7 and 8, the pleomorphic nature of cells in gliosarcoma is one of the most striking features observed under light microscopy and TPEF. The glial component of the tumor typically consists of atypical astrocytic cells with varying sizes, shapes, and nuclear characteristics, which include prominent nucleoli, irregular nuclear contours, and increased mitotic figures (Figure 7A). This cellular pleomorphism reflects the aggressive nature of the tumor and contributes to its histopathological diagnosis as a high-grade glioma. In contrast, the mesenchymal component of gliosarcoma exhibits spindle-shaped cells with less defined borders, further adding to the pleomorphic architecture of the tumor (Figure 8A).

In Two-Photon Excitation Fluorescence (TPEF) imaging, the pleomorphic cellular structure is also identifiable, but through label-free imaging that highlights differences in tissue architecture based on endogenous fluorescence. While TPEF cannot provide the same cellular details as optical microscopy, it offers insights into the variations in cellular and extracellular matrix composition that correspond to the pleomorphic nature of gliosarcoma cells. The increased autofluorescence in areas with high cellularity and metabolic activity, as seen in the glial component (Figure 7B), contrasts with the more fibrous and structured mesenchymal regions, providing a complementary visualization of the tumor's heterogeneity (Figure 8B). TPEF's ability to image deep tissue also offers a broader view of how pleomorphic regions interact with surrounding structures, complementing the histopathological observations made under optical microscopy.

Figure 9: (A) Light microscopy image showing a vessel-rich, fibrosarcomatous area of the tumor, composed of spindle cells, with (B) the corresponding TPEF microscopy image

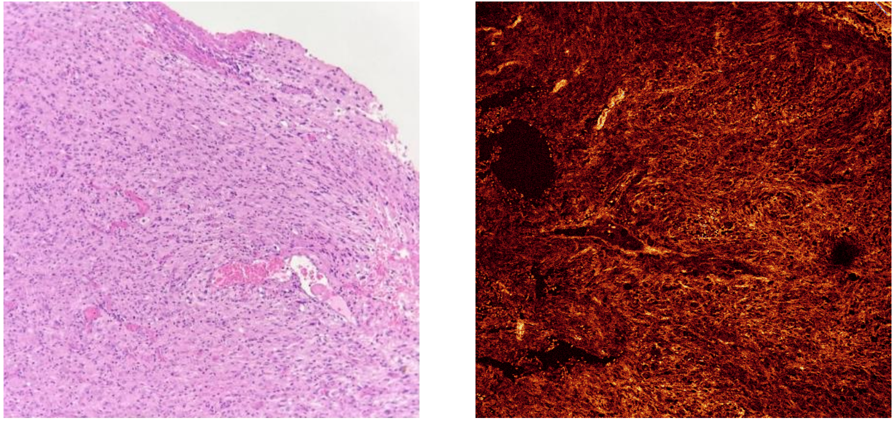

Under light microscopy, these vascular changes are readily observed, particularly the presence of abnormal, hyperplastic blood vessels that often exhibit endothelial cell proliferation and vascular lumen formation. These blood vessels may appear as tortuous and irregular in shape, contributing to the high degree of neovascularization characteristic of high-grade gliomas (Figure 9A and Figure 10A). In TPEF imaging, the vascularization in gliosarcoma is also detectable, albeit in a more indirect manner. While TPEF does not specifically stain blood vessels, the increased autofluorescence in regions of high vascularity can reveal the areas where blood vessels are more concentrated, especially in regions of new blood vessel formation and vascular leakage. TPEF imaging can also visualize the extracellular matrix surrounding the vessels, allowing for the assessment of the tumor's interaction with its vascular network (Figure 9B and Figure 10B). Furthermore, the deep-tissue penetration of TPEF offers a more comprehensive view of the tumor’s vasculature, providing insights into both the microvascular architecture and the surrounding tissue environment that may not be fully captured with optical microscopy alone.

Figure 10: (A) Light microscopy image showing the gliomatous component, with foci of microvascular proliferation. (B) TPEF microscopy showing the corresponding image

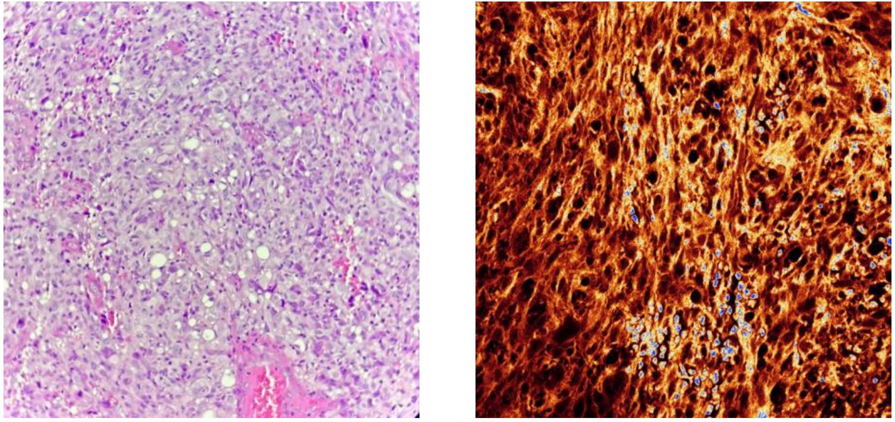

DISCUSSION

Early detection of brain tumors, including gliosarcoma, is crucial for improving treatment outcomes and patient survival. Delayed diagnosis can lead to uncontrolled tumor growth, reducing the effectiveness of therapeutic interventions. Conventional diagnostic approaches, including MRI, CT, and histopathological analysis using light microscopy, remain the gold standard for tumor identification and classification. However, these methods have inherent limitations, such as variability in manual interpretation and challenges in distinguishing tumor margins with high precision.

Light microscopy provided valuable histopathological insights into gliosarcoma, enabling the identification of distinct tumor cell characteristics and assessment of tissue architecture. However, conventional light microscopy is often complemented by immunohistochemical staining, which can introduce variability and require additional processing time. In contrast, TPEF microscopy offers a label-free, rapid, and high-resolution imaging approach for characterizing brain tumors. Our study demonstrates that TPEF microscopy effectively differentiates individual tumor cells and delineates their distribution in gliosarcoma samples.

Recent studies have explored the application of multiphoton imaging techniques in oncology. For example, TPEF microscopy has been successfully employed for brain tumor visualization, leveraging the endogenous fluorescence of metabolic coenzymes such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) to assess cellular metabolism [31,32]. Several studies have demonstrated the utility of metabolic imaging for distinguishing between normal and cancerous tissues [33,34]. These findings align with our results, where TPEF microscopy provided high-contrast visualization of gliosarcoma cells based on fluorescence.

Furthermore, recent advances in label-free optical imaging techniques, such as second-harmonic generation (SHG), have also been explored for brain tumor detection [35]. Compared to SHG, which primarily targets fibrillar collagen, TPEF provides direct cellular imaging, making it particularly useful for distinguishing tumor cells from surrounding neural tissue. Additionally, while confocal microscopy has been employed for intraoperative brain tumor imaging, it lacks the penetration depth and reduced photodamage advantages offered by TPEF microscopy [36].

This study introduces a novel application of TPEF microscopy for the analysis of gliosarcoma, showcasing several key advantages. Firstly, TPEF provides high-resolution with potential label-free imaging, eliminating the need for exogenous stains and thus reducing sample preparation time. The potential for intraoperative use is another strength, as real-time, high-contrast imaging of tumor margins could assist neurosurgeons in achieving maximal safe resection.

Despite these strengths, the study does have certain limitations. The analysis was performed on a limited number of gliosarcoma samples, and further validation on a larger dataset is necessary to establish robustness. Additionally, the ex vivo imaging of paraffin-embedded tissue sections, rather than fresh, in vivo samples, limits the immediate application of TPEF in clinical settings. Future studies should focus on exploring real-time, intraoperative applications of TPEF.

Looking ahead, several avenues for future research are proposed to expand the clinical applicability of TPEF microscopy. These include the integration with multimodal imaging, such as combining TPEF with SHG or Raman spectroscopy for more comprehensive tumor characterization. Further development of in vivo applications is also critical, including the creation of fiber-optic or handheld TPEF imaging systems for intraoperative brain tumor resection. Additionally, the incorporation of artificial intelligence-assisted analysis could help automate the classification of TPEF images, improving diagnostic accuracy. Finally, validation on diverse brain tumors, including glioblastoma and metastases, is essential to assess the broader diagnostic potential of TPEF microscopy.

CONCLUSION

Our study highlights the complementary roles of various imaging modalities in the comprehensive analysis of gliosarcomas. Light microscopy provides detailed insights into tissue morphology and cellular architecture, essential for initial tumor identification and classification. Two-photon excitation microscopy offers deeper tissue imaging with reduced photodamage, enabling visualization of tumor structures in their native environment. Radiological imaging techniques, such as MRI and CT scans, facilitate non-invasive assessment of tumor location, size, and progression within the brain. Integrating these methodologies enhances diagnostic accuracy and informs effective therapeutic strategies for gliosarcoma patients. When used as an additional technique with practical application in clinical settings, it may help to prevent under or overdiagnosis of certain lesions, whose accurate diagnosis is necessary for sufficient and effective treatment. Additionally, we will prevent the abuse of other currently available, somewhat expensive supplemental diagnostic methods (molecular biology techniques, genetic tests, IHC tests, electron microscopy, etc.).

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors' contribution

Conceptualization A.E. and L.G.E.; methodology A.E., L.G.E and R.R.G..; software R.H.; validation L.G.E., R.H., O.M.S, M.S. and G.A.S.; formal analysis M.B. and O.C.V; investigation A.E.; resources L.G.E. and O.C.V.; data curation R.R.G.; writing—original draft preparation A.E.; writing—review and editing L.G.E, R.H., O.M.S. and G.A.S.; visualization R.R.G, M.B. and O.C.V.; supervision O.M.S., M.S. and G.A.S.; project administration A.E..; funding acquisition L.G.E, and R.H. All authors have read and agreed to the published version of the manuscript elaboration

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The approval of the Local Ethical Committee was obtained under the number 392/9G /2025. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols. Before the study, the committee reviewed and approved the methodology, including data collection and analysis procedures, to guarantee ethical standards were met. Additionally, all patient data used in the study were anonymized to maintain confidentiality and adhere to ethical principles.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Traditional Ethnomedical and Ethnobotanical Applications and Uses of Piper Nigrum

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Abstract: Ethnomedicine and ethnobotany have been at the forefront of current scientific focus. The focus of this review is Piper nigrum, for which there exists a wide host of traditional ethnomedical and ethnobotanical applications, recorded mostly from India and the surrounding regions where black pepper is native. These applications cover a wide range of pathologies, including cardiovascular, pulmonary, gastrointestinal, gynaecological, endocrine, integumentary and neurological, and viral diseases and colds. While some of these applications are confirmed by modern research, the majority have not been tested using modern scientific methods, and perhaps many more applications remain to be documented. Finally, it must be noted that some interesting non-medical applications comprise the application of black pepper, as an insecticide/larvicide, are worth further consideration, especially given the newer policies at a European and international level.

Keywords: *Piper nigrum*; black pepper; ethnomedicine, traditional uses

INTRODUCTION

Herbs and spices have been used for medicinal, preservative, and culinary purposes since antiquity in numerous traditional medicine systems [1-4]. In recent years, there has been an increase in research efforts, dealing both with recording ethnomedical and ethnobotanical practices across different regions, and verifying the reported uses by employing modern analytical and experimental methods [5-8]. The focus of this review is black pepper (*P. nigrum*), which has a long history of culinary history and use.

In Indian culinary tradition, black pepper has been an integral part since about the 2nd millennium BC, and numerous traditional herbal recipes are mentioned by Venkatachalapathi et al. [9]. Many ethnozoological uses of *P. nigrum*, combined with other components, also exist in India [10]. Given that *Piper nigrum* was originally cultivated in India [11], it makes sense that the record of relevant ethnomedicinal and ethnobotanical applications will be rich in this country and in the surrounding regions. Traditional uses of *P. nigrum* are reported from nearby regions as well.

TRADITIONAL ETHNOBOTANICAL AND ETHNOMEDICINAL APPLICATIONS OF BLACK PEPPER

Applications in Cardiovascular System Pathologies

Regarding cardiovascular pathologies, the fruit of *P. nigrum* is mixed with other ingredients in a powder in the area of Odisha, India, to combat hypertension [17]. Based on in vivo experiments in rats, it was proven that piperine could normalise

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hypertension and aortic ring reactivity [62]; therefore, there is at least experimental confirmation of the previous usage. An additional use for cardiovascular pathologies is recorded in the area of Tiruvallur where *P. nigrum* is used in different formulations, along with other plants and herbs, in these uses [16].

Table 1: Traditional ethnomedical and ethnobotanical applications of P. nigrum.

Part of the Plant Used	Country/Region	Type of Ailment	Year	Reference
Cardiovascular Pathologies				
Seeds (used to make a decoction with *Z. officinale* leaves)	Maharashtra, India	Generalised oedema	2006	[12]
Seeds (used to make a paste with leaves of *C. ternatea* L.)	Tamil Nadu, India	Leg swelling	2012	[13]
Seeds (made into paste with other plant parts, and used to make tablets)	Odisha, India	Haemorrhoids	2012	[14]
Fruits	Tamil Nadu, India	Good circulation promotion	2015	[15]
Different parts in various formulations	Tamil Nadu, India	Cardiovascular pathologies	2016	[16]
Fruits (powdered with other ingredients)	Odisha, India	Hypertension	2018	[17]
Respiratory Pathologies				
Seeds (powder for internal administration)	Tamil Nadu, India	Cough	2007	[18]
		Bronchial disorders		
Seeds, fruits	Orissa, India	Cough	2010	[19]
Different parts of the plant (combined with *E. cardamomum*, *A. galanga*, *Z. officinale*, and sugar)	Western Ghats, India	Asthma	2011	[20]
Seeds (powdered added in *V. negundo L.* leaf decoction with honey)	Odisha, India	Cough	2012	[14]
Raw fruits	Kerala, India	Sinusitis	2015	[21]
Seed (powder combined with dried leaf powder of *G. bosvallia*)	Madhya Pradesh, India	Whooping cough	2018	[22]
Gastrointestinal Pathologies				
Leaves (leaf juice is mixed with leaf and root bark powder of *K. grandiflora*, and leaf powder of *C. halicacabum*)	Tamil Nadu, India	General gastric problems	2005	[23]
Seeds (dried and swallowed)	Tamil Nadu, India	Stomach ache	2006	[24]
Seeds (powdered, mixed with root of *P. margaritiferum* Scott)	Odissa, India	Dysentery	2010	[25]
Seeds (powdered, with bark decoction of *P. xylocarpum*)		Infantile diarrhoea		
Fruits (powdered, mixed with *T. dioica* leaf decoction)		Diarrhoea		
Different parts of the plant (combined with different plants)	Western Ghats, India	General gastric problems	2011	[20]
Different parts of the plant	Tamil Nadu, India	General gastric problems	2012	[26]
Fruits (combined with numerous different plants and made into a decoction)	Karnataka, India	Intestinal ulcers	2012	[27]
		Aphthae		
Seeds [combined with *T. asthmatica* (L. f.) Wight &Arn roots, garlic and fruits of *S. cumini*, made into paste]	Karnataka, India	Jaundice	2012	[28]
Fruits (in powder form – oral adm.)	Assam, India			
Seeds (mixed with seeds of *B. roxburghii* and jaggery, made into tablets)	Maharashtra, India			
Fruits (combined with *C. dactylon* and *P. amarus* leaves – subsequently an extract is produced)	Tamil Nadu, India			
Seeds	Assam, India	Indigestion	2013	[29]
Leaves (used to make an infusion)	Misiones, Argentina	Digestive complaints	2014	[30]
Fruits (macerated with other plants to make pills)	Jhenaidah, Bangladesh	Ulcer	2014	[31]
Fruits (powder for oral administration)	Kerala, India	Stomach ache,	2014	[32]
		Indigestion		
Raw fruit	Kerala, India	Vomiting	2015	[21]
Seeds (crushed and mixed with different ingredients)	Phnom Penh, Cambodia	Liver pathologies	2017	[33]

Different parts (combined with *C. diococcum*, *C. aromatica*, *P. nigrum*, and *P. betle* and salt)	South Western Ghats, India	Stomachache	2018	[9]
Fever, General Anti-Inflammatory Actions, Musculoskeletal Complaints and Stomatological Pathologies				
Fruit (powdered with *A. vasica* Nees)	Karnataka, India	Fever	2005	[34]
Seeds (mixed with powder of *H. isora* L., *A. sativum* rhizome, and gingelly oil, and boiled)	Tamil Nadu, India	Earache	2007	[18]
Different parts of the plant (combined with *A. vasica* and honey to form a paste)	Western Ghats, India	Headache, earache	2011	[20]
Different parts of the plant (combined with *E. cardamomum*, *A. galanga*, *Z. officinale*, and sugar)		Headache		
Fruits (mixed and macerated with other plant parts and then made into a paste)	Jessore & Narail, Bangladesh	Bone fractures	2011	[35]
Seeds (paste with *Caesalpinia bonduc* L. and honey)	Odisha, India	Fever	2012	[14]
Seeds (powdered and applied with salt)	Tamil Nadu, India	Toothache	2012	[26]
Fruits (combined with different plant parts, ground and boiled in water)	Karnataka, India	Gingival wounds	2012	[27]
Seeds [with macerated leaves of *E. indica* (L.) Gaertn]	Rajshahi & Nawabganj, Bangladesh	Spinal cord pain	2013	[36]
Seeds [powdered with macerated roots of *L. aspera* (Willd.) Link]		Headache due to fever or dog bite		
Seeds (powdered with crushed bark of *D. peregrina* L.)		Chest pain (various causes)		
Seeds (powdered with macerated whole *S. cordata* to create a paste)		Abscess		
Fruit (decoction or infusion)	Batan, Philippines	Toothache	2013	[37]
Seeds	Assam, India	Pain (of various causes)	2013	[29]
Fruit (powdered in *C. serratum* decoction/root extract)	Bengal & Andhra Pradesh, India	Fever	2014	[38]
Fruits	Tamil Nadu, India	Throat pain	2015	[15]
Seeds (paste)	Kerala, India	Fever, cuts, wounds	2015	[21]
Seeds/fruits (as part of a multi-plant mixture)	High Atlas, Morocco	Musculoskeletal complaints	2016	[39]
Seeds/fruit (powdered)	Khushab, Pakistan	Malarial fever and body pain	2017	[40]
Seeds (combined with animal and plant parts)	Chota Nagpur Plateau, India	Headache	2017	[41]
Fruit (powdered in *O. sanctum* L. leaf decoction with palmgur)	India (different regions)	Fever	2018	[42]
Different parts (combined with *C. caudata*, *O. sanctum*, *C. monophyla*, and *P. nigrum*, combined with milk and honey)	South Western Ghats	Fever	2018	[9]
Gynaecological and Reproductive System Pathologies				
Fruits (mixed with *C. pareira* L.)	Assam, India	Post-partum birth control	1982	[43]
Fruits	Assam, India	Post-partum pain	2008	[44]
		Lactation difficulties		
Seeds [combined with a paste of *T. cordifolia* (Willd.)]	Eastern Rajasthan, India	Leucorrhoea	2010	[45]
Fruit (powdered mixed with the root juice of *A. aspera*)	Narail & Jessore, Bangladesh	Menstrual pain	2011	[35]
Fruits (powdered and added to a macerated mixture of different components to make pills)		Prolapse of uterus		
Seeds (macerated with roots of *P. daemia* Forsk., and made into pills)	Rajshahi district, Bangladesh	Menstrual irregularities	2012	[46]
Seeds	Assam, India	Post-labour ailments	2013	[29]
Fruit (powdered, mixed with *P. hydropiper* leaf paste and made into pills)	Jhenaidah, Bangladesh	Menstrual pain	2014	[31]
Seeds (ground with newly-sprouted *N. arbor-tristis* leaves to make pills)	Jammu, India	Menorrhagia	2014	[47]

Seeds (powdered with leaves of *P. granatum* and mixed with water; a filtrate is then produced)		Leucorrhoea		
Seeds (powdered with eight spoons of *S. indicum* seeds and jaggery and boiled in water)		Oligomenorrhea		
Seeds (powdered with two spoons of *S. indicum* seeds and jaggery and boiled in water)		Hypomenorrhoea		
Fruit (macerated)	Andaman & Nicobar islands, India	Lactation difficulties	2015	[48]
Seeds/fruits (as part of a multi-plant mixture)	High Atlas, Morocco	General gynaecological complaints	2016	[39]
Different parts (combined with *A. excelsa*, *C. aromatica*, *P. nigrum* and *P. betle,* and coconut oil)	South Western Ghats, India	Menstrual irregularities	2018	[9]
Neurological Pathologies				
Seeds [powdered and added into the flower extract of *Calotropis procera* (Aiton)]	Uttarakhand, India	Epilepsy	2013	[49]
Leave extract	Thailand	Insomnia	2014	[50]
Seeds (made into pills combined with *L. interrupta* roots)	Chota Nagpur Plateau, India	Epilepsy	2017	[41]
Seeds (made into pills combined with other plants)				
Seeds (mixed with honey)		Insomnia		
Endocrine Pathologies				
Fruits (crashed with fruits of *C. nymphaeifolia* Vent.)	Jessore, Bangladesh	Goiter	2011	[51]
Seeds (added to a juice of three leaves of *M. charantia* L.)	Mauritius	Type 2 diabetes mellitus	2014	[52]
Fruits (used to make a decoction)	Songkhla, Thailand	Diabetic neuropathy	2015	[53]
Different parts (with *A. marmelos*, *C. aromatica*, *P. betle*, coconut oil and honey to make a herbal preparation)	South Western Ghats, India	Diabetes mellitus	2018	[9]
Ailments of Viral Cause and Common Cold				
Different parts of the plant (combined with other plants into a paste)	Shimoga, India	Common cold	2010	[54]
Seeds, fruits	Orissa, India	Common cold	2010	[19]
Different parts of the plant (combined with *A. vasica* and honey to form a paste)	Western Ghats, India	Common cold	2011	[20]
Seeds (powdered, with dried ginger and palm sugar made into a coffee-like preparation)	Tamil Nadu, India	Common cold	2012	[26]
Seeds (powdered, added with salt in an omelette)				
Fruits (mixed with multiple plant components in a complex preparation)	Rajshahi, Bangladesh	Influenza	2012	[55]
Seed (powder with roots of *S. virginianum* L.)	Rajshahi & Nawabganj, Bangladesh	Chickenpox	2013	[36]
Leaves (mixed with ginger and honey)	Kodagu, India	Common cold	2013	[56]
Fruits (powdered for oral administration)	Tamil Nadu, India	Viral hepatitis	2014	[57]
Seeds (used to make a decoction)	South Western Ghats, India	Common cold	2018	[9]
Different parts (combined with *E. variegata*, *O. tenuiflorum*, *P. nigrum* and *P. betle*, and honey)				
Treatment of Parasitic Infections				
Fruits (crushed and mixed with ghee)	Assam, India	Scabies	2006	[58]
Seeds (boiled in milk and water)	Eastern Rajasthan, India	Malaria	2010	[45]
Leaves (crushed, applied either alone or in combination with other components)		Ringworm		
Fruits	Karnataka, India	Scabies	2014	[59]
Treatment of Bites				
Seeds (powder for internal administration)	Tamil Nadu, India	Snake bite	2007	[18]
Seeds (powdered and mixed with butter)	Eastern Rajasthan, India	Snake bite	2010	[45]
Fruits (macerated with leaves of *A. indica*)	Narail & Jessore, Bangladesh	Snake bite	2011	[35]

Fruits (made into a paste with lime juice and the roots of *L. indica*, and *C. medica*)	Karnataka, India	Snake bite	2012	[27]
Fruits (ground with root of *D. metel*)	Jhenaidah, Bangladesh	Dog bite	2014	[31]
Flowers (mixed with ghee)	Various regions, India	Snake bite	2017	[60]
Different parts (combined with *C. diococcum*, *C. aromatica*, *P. nigrum*, and *P. betle* and salt)	South Western Ghats, India	Dog bite	2018	[9]
Different parts (combined with *T. indica*, C. *aromatica*, *P. betle* and coconut oil)		Scorpion bite		
Treatment of Skin Diseases				
Seeds (powdered and added to crushed, soaked and filtered *P. murex* L., mixed with sugar candy)	Eastern Rajasthan, India	Various skin diseases	2010	[45]
Seeds (macerated with part plants and honey to make pills)	Jessore, Bangladesh	Various skin diseases	2011	[51]
Fruits (ground with minerals, camphor and other plant parts)	Karnataka, India	Foot xeroderma	2012	[27]
Fruits (made into a paste with other plant parts and coconut oil, and boiled)		Gangrene		
Seeds (powder mixed with betel leaf paste)	Hyderabad, India	Eczema	2013	[61]
Fruits	Karnataka, India	Dandruff	2014	[59]
		Pruritus		
		Eczema		
		Bed sores		
		Boils		

Another application regarding the cardiovascular system concerns the use of seeds of *P. nigrum*, combined with roots of *Rawolfia serpentina*, dried rhizome of *Zingiber officinale*, and seeds of *Piper longum*, all in paste form, which are used to make tablets; these are then administered orally for 10 to 15 days to treat haemorrhoids [14]. In Tamil Nadu, India, the fruit of *P. nigrum* is used to promote a state of overall health and good circulation [15].

Many ethnomedical and ethnobotanical applications are concerned with swelling and the presence of oedema, either localised or generalised. In many cases, a pathology of cardiovascular nature is the cause of oedema formation [63]. In the district of Amravati, India, a decoction made from the leaves of *Zingiber officinale* and P. *nigrum* seeds is taken once daily for 5 days to treat generalised swelling [12]. A paste made from leaves of *Clitoria ternatea* L. and *P. nigrum* seeds is applied on swollen legs in Sivagangai district, India [13].

Applications in Respiratory System Pathologies

In the region of Western Ghats, the Kani healers combine *Elettaria cardamomum*, *Alpinia galanga*, and *Zingiber officinale*, combined with sugar, to treat headache and asthma [20]. In the region of Kerala, the raw fruit of black pepper is used to treat sinusitis [21], while the seed and fruit are used to treat cough [19], in Orissa, India. In Odisha, a decoction of black pepper seeds and *Vitex negundo* L. leaves mixed with honey is taken orally to relieve cough [14].

In Madhya Pradesh, India, a preparation consisting of the dried leaf powder of *Glossocardia bosvallia* and the powder of *P. nigrum* seeds is administered to patients with whooping cough [22]. This pathology is caused by *Bordetella pertussis*, a bacterial pathogen whose presence has been recorded in human populations for centuries. Its importance is currently considered to be of endemic proportions despite the existence of an appropriate vaccine [64].

Applications in Gastrointestinal System Pathologies

For general gastric problems, in the region of Western Ghats, India, *P. nigrum* is combined with *Tephrosia purpurea*, *Carmona retusa*, and *Piper hymenophyllum* and used by the Kani healers [20]. In the Nadar community of the Atoor village, in Tamil Nadu, black pepper is used to treat gastric complaints of various causes [26]. In another area of Tamil Nadu, gastric complaints are treated with a mixture of *P. nigrum* leaf juice, leaf powder, and root bark powder of *Kleinia grandiflora*, and powder from *Cardiospermum halicacabum* leaves [23].

The powder of *Piper nigrum* fruits is administered in the Silent Valley of Tamil Nadu, to treat stomach ache and indigestion [32]. In other parts of India, the dried seeds are taken orally [24]. An infusion made from *P. nigrum* leaves is used to treat digestive problems by Polish migrants in Misiones, Argentina [30].

In Assam, the seeds of the plant are given to treat indigestion [29]. The raw fruit of *P. nigrum* is given to treat vomiting in regions of Kerala [21]. In the case of a stomach ache, the same preparation as for dog bites is used in some regions of the SW Ghats, India [9]. In Bargarh, the powder of seeds and fruits is combined with different parts of different parts, in various formulations, to treat infantile diarrhoea, in infants and adults, and dysentery [65].

Quite a number of formulation exist regarding the treatment of jaundice; for example, in the region of Karnataka, *P. nigrum* seeds are mixed with roots of *Tylophora asthmatica* L., garlic and fruits of *Syzygium cumini*; in the region of Assam, the fruits of black pepper are used in powder form; in the region of Maharashtra, its seeds are mixed with jaggery and *Balanotis roxburghii* seeds; in the region of and Tamil Nadu, its fruits are is combined with ground leaves of Cynodon dactylon and Phyllanthus amarus and an extract is produced [28]. This last use is also mentioned by [66], based on the earlier research of [67]. Regarding liver pathologies, a paste containing crushed black pepper seeds and four other ingredients is used by the Khmer traditional healers in Cambodia [33].

For the treatment of ulcers, in the Jhenaidah district of Bangladesh, leaves of *Polygonum hydropiper*, nutmeg, a fruit of *Terminalia chebula*, *Carum carvi*, and young leaves of *Cynodon dactylon* are mixed with *Piper nigrum*, and macerated to prepare a pill mixture [31]. Another therapy, this time specifically for intestinal ulcers, comes from the Uttara Kannada district of India, where the fruits of *P. nigrum* are used in combination with about 10 different plant parts and made into a water decoction with jaggery to be orally administered; the same preparation is used in the case of aphthae.

Antipyretic and General Anti-Inflammatory Applications and Musculoskeletal Complaints

In the village of the Shimoga district, Karnataka, India, black pepper powder is mixed into the leaf paste of *Adhatoda vasica* Nees; this formulation is then made into pills for oral administration [34]. In other parts of India, black pepper powder is added to *Clerodendrum serratum* decoctions or aqueous root extracts, which are then administered once per day orally, for three days [38]. The Kani healers of Western Ghats have a preparation containing *P. nigrum*, honey, and parts of *Alpinia vasica*, which they use to cure headaches and earaches; the same tribe uses another formulation to treat headaches as well as asthma [20]. In the region of Assam, seeds of black pepper are used to treat pains in different parts of the body, from different causes [29]. The fruits of the plant are used to treat throat pain in the area of Tamil Nadu [15].

In different regions of India, *P. nigrum* and palmgur are added to the leaf decoction of *Ocimum sanctum* L. [42]. A similar preparation is used in the SW Ghats [9]. In the region of Kerala, the paste of the seeds is known to have an anti-inflammatory effect in cases of fever, cuts, and wounds [21]. In the district of Odisha, *Caesalpinia bonduc* L. is made into a paste along with seeds of *P. nigrum*, and taken with honey to cure fever [14]. A complex mixture of plant and animal parts, containing black pepper seeds, is made into pills and administered orally once per day, for 10 days, to treat headache in the Chota Nagpur Plateau [41].

For the treatment of earache, in the area of Tamil Nadu, the fruit powder of *Helicteres isora* L. is boiled with *P. nigrum* seeds, *A. sativum* rhizome, and gingelly oil; the resulting fluid is applied locally in the affected area [18].

In Pakistan, black pepper seeds are also known for their antipyretic properties, and also for their action against fever-induced body pain, when the fever is of malarial origin [40].

In Bangladesh, there are many different anti-inflammatory uses of black pepper reported, such as a paste made from black pepper seeds and macerated leaves of *Eleusine indica* (L.) Gaertn applied once daily for 21 days, in cases of pain in the spinal cord. In the case of severe headache due to fever or dog bites, the macerated roots of non-flowering *Leucas aspera* (Willd.) Link is mixed with 10-15 powdered seeds of *Piper nigrum* L., and prepared for oral administration. As a form of abscess treatment, a paste made from macerated *Sida cordata* (Burm.f.) Borss.Waalk. is mixed with black pepper seed powder and applied around the abscess. Finally, in the case of chest pain of various causes, seed powder is mixed with crushed bark of *Diospyros peregrina* L., and applied locally, particularly in the cases where the chest pain is caused by gout [36].

In other regions of Bangladesh, in Jessore and Narail, some of the leaves of *Morus indica* are mixed with rhizomes of *Zingiber officinale*, 20 fruits of *Piper nigrum*, and 25-30 drops of oil prepared from seeds of *Brassica campestris*; this mixture is then macerated thoroughly and applied as a thick paste over the affected area. A bandage is then placed over the applied paste and changed, along with the mixture, every 7 days [35]. Another preparation, containing *P. nigrum* as part of a multi-plant mixture called Msahan, is used in the High Atlas region of Morocco [39], to treat general musculoskeletal complaints.

In the Philippines, a decoction or infusion of black pepper is used to cure a toothache [37]. In India, again, to treat toothache, in the Atoor village, powdered pepper and salt are applied in dental cavities [26]. Still on the subject of oral-related pathologies, a complex infusion is made, containing many different plant parts along with *P. nigrum fruits*, which is then used, when lukewarm, to gargle, when there are gingival complaints [27].

Applications in Gynaecological and Reproductive System Pathologies

One ethnomedicinal preparation containing *P. nigrum*, as part of a multi-plant mix, comes from the High Atlas region of Morocco, and is named Msahan; it is used generally to treat various types of gynaecological complaints [39]. For post-labour ailments, the seeds of *P. nigrum* are used in Assam, India [29]; specifically for post-partum pain, the fruit of *P. nigrum* is administered in Tinsukia district, India [44]. In the same regions, there also exist some ethnomedical recipes for post-partum birth control [68]; for example, in some cases, the fruits of *P. nigrum* are used, along with pieces of *Cissampelos pareira* L., and the duration of administration depends on the desired length of contraception [43].

Menorrhagia, i.e., heavy menstrual bleeding, is defined as a bleeding of over 80 mL per menstrual cycle, and usually occurs as a result of uterine abnormalities, endometrial abnormalities, ovulation, or coagulation disorders; the treatments and their effectiveness vary depending on the primary cause [69]. In Udhampur, India, the newly sprouted leaves of *Nyctanthes arbor-tristis* are ground along with Piper nigrum seeds to make 2 g tablets, which are administered twice per day for two weeks [47].

Oligomenorrhoea is another type of menstrual cycle disorder, where the menstrual cycle lasts more than 35 days and less than 90 days [70], or the total menstrual cycles per year are between 5 and 7 [71]. It is a problem of increasing importance in recent years [72]. The usual causes are associated with endocrine disorders [73]. In Udhampur, India, eight spoons of *Sesamum indicum* seeds and the powder of *Piper nigrum* seeds and jaggery are boiled in water until the volume is reduced by half; the produced liquid is taken twice a day, for 15 days, before the due date of each successive period [47]. A similar treatment is used in cases of hypomenorrhea [47], which is defined as a menstrual bleeding of 1-2 days or when the amount of blood is too low, even in the context of a normal duration of menstruation [74].

Leucorrhoea, or fluor albus, may be physiological in women, during menstruation, or pathological; in this latter case, a foul smell, colour changes, and other symptoms such as itching and a burning sensation are present. Usually, pathological leucorrhoea is associated with a urogenital infection [75]. There are two recorded ethnobotanical treatments for leucorrhoea containing *P. nigrum* in India. The first one is from the region where a paste of *Tinospora cordifolia* (Willd.) is combined with five seeds of black pepper and administered once in the morning [45]. In the district of Udhampur, India, the leaves of *Punica granatum* are crushed, the powder from *P. nigrum* seeds is added, and then both are mixed with water; a filtrate is then produced, which is consumed twice a day [47].

Another common gynaecological problem is menstrual pain, i.e., primary dysmenorrhoea, which affects both young and adult females, especially from a quality-of-life perspective; treatment remains mostly symptomatologic [76]. The Kavirajes tribe in Bangladesh mixes one fruit of *P. nigrum* with the juice of one root of *Achyranthes aspera*, and gives it to affected females on an empty stomach once only [35]. In the same country, in the district of Jhenaidah, a paste obtained from the leaves of *Polygonum hydropiper* is mixed with 1 powdered fruit of *Piper nigrum*, to make pills that are taken thrice per day for 2-3 days [31].

Menstrual cycle irregularities are a relatively common problem in many populations, affecting between 5% and over 30% of females of reproductive age [77]. There exists several modifiable and non-modifiable risk factors associated with irregular menstruation [78,79]; regardless, irregular menstruation is associated with some pathologies and a decrease in quality of life (80). In the SW Ghats, India, *Ailanthus excelsa*, *Curcuma aromatica*, *Piper nigrum*, and *Piper betle* are combined with coconut oil and made into a herbal remedy for the cure of menstrual problems [9]. In the Rajshahi district, Bangladesh, the roots of *Pergularia daemia* Forsk. are macerated with 2-3 seeds of *Piper nigrum L.*, and then pills are made, which are taken thrice daily [46].

Uterine prolapse, one of the manifestations of pelvic organ prolapse in women, affects over 5% of most female population worldwide [81-83]. In Bangladesh, in the Jessore and Narail districts, the roots of *Abutilon indicum*, *Glycosmis pentaphylla*, *Eleusine indica*, and *Amaranthus spinosus*, are mixed together and macerated, and then sugar and 21 powdered black pepper fruits are added and 42 pills are prepared from this mixture; 1 pill is taken daily for 14 days [35].

In the case of lactation difficulties, the macerated fruit of *P. nigrum* is orally administered by the healers of the tribes of Andaman and Nicobar islands, India [48]. In Tinsukia district, the fruit of *Piper nigrum* is used to increase breast milk production [44].

Applications in Neurological Problems

Of particular note are the mentions of *P. nigrum* in the context of traditional Indian medicinal approaches to epilepsy. The seed of the plant, when combined with either *Laportea interrupta* roots or the stem of *Cissus repanda*, and the roots of *Leea macrophylla*, and *Mucuna pruriens*, is used to treat epilepsy in the Chota Nagpur Plateau, in India. Also in the same area, for the treatment of insomnia, *P. nigrum* is mixed with fresh honey from the Indian honey bee (*Apis cerana indica*), and applied inside the nose [41]. In the region of Uttarakhand, powdered seeds of *P. nigrum* are mixed with the flower extract of *Calotropis procera* (Aiton) [49]. Meanwhile, in Thailand, the leaf extract from *P. nigrum*, which has a demonstrably high melatonin content, is traditionally used to treat insomnia [50].

Applications in Endocrine Pathologies

In Jessore, Bangladesh, the crushed fruits of P. nigrum are combined with crushed fruits of *Colocasia nymphaeifolia* Vent. and taken twice daily for 7 days, to treat goiter [51]. Goiter can be attributed to a number of causes, the most common of them being iodine deficiency; the worldwide prevalence of goiter is quite high [84].

For the treatment of diabetes mellitus, in the Walayar valley, India, different parts of *Aegle marmelos*, *Curcuma aromatica*, *Piper nigrum*, and *Piper betle* are combined with coconut oil and honey to make the relevant herbal medicine [9]. Specifically for type 2 diabetes mellitus, a juice from three leaves of *Momordica charantia* L. and *Piper nigrum*, to be consumed once a week, is administered [52]. For the treatment of one of the complications of diabetes, diabetic neuropathy-associated numbness, a decoction made from the fruits of black pepper in *Songkhla province*, Thailand, its administration has a proven hypoglycaemic effect in rats [85].

Applications in Ailments of Viral Cause and Colds

In Bangladesh, the roots of *Solanum virginianum* L. are powdered and mixed with black pepper seed powder to cure chickenpox [36], an extremely contagious viral disease caused by the Varicella-Zoster virus [86]. In Tamil Nadu, India, the powder from *Piper nigrum* fruits is administered orally in cases of viral hepatitis [57]; viral hepatitis is attributable in most cases to the five different hepatitis viruses [87].

In the district of Shimoga, India, to treat the common cold, *P. nigrum* Linn. and *Allium sativum* Linn. are combined with plants of the Caesalpiniaceae spp. and made into a paste, which is taken orally for 3-4 days [54]. In Tirunelveli hills of Western Ghats, India, *P.*

nigrum is combined with *Alpinia vasica* and honey, and the resulting paste is used by Kani traditional healers against the common cold, but also against headache, and earache [20]. In the Karnataka region of India, the leaves of *P. nigrum* are pounded and mixed with ginger and honey; they are taken for 3-5 days orally to treat the common cold and associated cough [56]. The fruits and seeds of *Piper nigrum* are used to treat the common cold in Orissa, India [19]. Seed powder is swallowed to treat cough and bronchial disorders in Coimbatore district, India [18].

In the Kanyakumari district, Tamil Nadu, India, black pepper is used in a number of formulations for different ailments. Regarding the treatment of the common cold, black pepper, dried ginger, and palm sugar are mixed along with water to prepare a coffee-like drink, which is then administered to patients; omelettes are also made with powdered pepper and salt and given to such patients [26].

In the Walayar valley of India, a decoction made from seeds of *P. nigrum* is used as a treatment for cough and cold; another more complex herbal preparation containing black pepper, from the same area, also exists [9].

For the treatment of influenza, caused by the viruses of the Orthomyxoviridae family [88] in Rajshahi district, Bangladesh, a complex preparation comprising 12 fruits of black pepper, along with a number of different plant parts, is used [55].

Applications in the Treatment of Parasitic Infections

There are reports that if black pepper seeds are boiled in milk and water and administered orally, once in the morning for a period of 4 to 5 days, the multiplication of malarial parasites is decreased and splenomegaly regresses [45]. In the case of ringworm infection, the crushed leaves of the plant are rubbed directly onto the parasite; in other cases, the leaves are crushed and mixed with cow milk or mustard oil or crushed with bulblets of garlic and mixed with lemon juice is applied on ringworm to affect a complete cure [45]. Both of these antiparasitic applications are common in the region of Eastern Rajasthan, India.

Scabies is increasingly being diagnosed, at least in certain countries [89]; it is caused by the host-specific human scabies mite (Sarcoptes scabiei varietas hominis). While the infection is not life-threatening on its own, bacterial superinfections are common [90]. In the Central Western Ghats region, India, the fruits of black pepper are used to treat scabies [59]. In Assam, the crushed fruits of *P. nigrum* are mixed with ghee and the resulting product is orally administered [58].

Applications in the Treatment of Bites

In Eastern Rajasthan, seed powder mixed with butter is given orally against snake bite [45]. A similar preparation exists for snake-bitten animals in Uttaranchal, India [91]. The same recipe is also known in other areas of India, while the paste of *P. nigrum* flowers with ghee is administered orally for the same purpose [60]. In the region of Karnataka, the fruits of *P. nigrum* along with the root of *Citrus medica* and *Leea indica*, are used to make a paste, mixed with lime juice, which can be both applied externally and swallowed, in cases of snake bite [27]. The powder of black pepper seeds is administered internally to counteract the effects of snake poison, in the *Coimbatore district*, India [18]. In Bangladesh, several leaves of *Aristolochia indica* are mixed with 2-3 fruits of *Piper nigrum*, and are then macerated; finally, a juice is obtained which is administered immediately [35].

Finally, for the treatment of dog bites, in the district of Jhenaidah, fruits of *P. nigrum* are mixed with roots of Datura metel and ground. The resulting ground mass is administered orally as soon as possible [31]. In the SW Ghats, India, *Canthium diacocum* is combined with *Curcuma aromatica*, *Piper nigrum*, and *Piper betle*, and salt, to make a preparation used in the case of dog bites [9]. In the same region, *P. nigrum* is combined with *Tamarindus indica*, *Curcuma aromatica*, *Piper betle*, and coconut oil to treat scorpion bites [9].

Applications in the Treatment of Skin Diseases

Crushed *Pedalium murex* L. is soaked in water overnight, filtered the next morning, mixed with sugar candy and seed powder of black pepper, and then administered orally as a treatment for various skin diseases in Eastern Rajasthan, India [45]. Again, for skin diseases generically, seeds of *P. nigrum* are macerated with roots of *Rauwolfia serpentina* (L.) Benth. ex Kurz and rhizomes of *Zingiber officinale* Roscoe, with honey added afterwards; in this way, pills are made which are administered daily for 3 weeks, for prophylaxis, in Jessore, Bangladesh [51]. In Central Western Ghats, India, the fruits of *P. nigrum* are used to treat dandruff, pruritus, eczema, bed sores, and boils [59]. Regarding eczema, a different traditional cure, comprising the powder of black pepper seeds and betel leaf paste, applied daily, is used in Hyderabad, India [61].

For foot xeroderma, the fruit of *P. nigrum* along with the seed and bark of *Semecarpus anacardium*, the fruit of *Vernonia anthelmintica*, the flower bud of *Syzygium aromaticum*, and the rhizome of *Curcuma longa* are ground with copper sulphate, camphor tablets, and boiled in cow's ghee, grease, and paraffin; they are then applied externally [27]. A rather more complex preparation with more than 10 plant parts, comprising also the fruits of *P. nigrum*, is made into a paste with coconut oil and boiled; it is applied externally after it has cooled [27].

DISCUSSION

Research in phytochemistry has revealed the remarkable therapeutic properties of black pepper. While its antimicrobial, antiparasitic, and antiviral effects are well-known, many other beneficial properties of black pepper extracts have been researched thoroughly, including its anti-inflammatory, anticonvulsant, and analgesic properties [92-94]. It seems that it may be possible to use it, in an adjuvant role, in a mode similar to that of capsaicin [95,96]; perhaps its integration in 3D-printed biomaterials [97-99] as a potent antimicrobial would represent another promising application. Related to inflammation, based on a review of available data, concluded that the terpenes present in black pepper, in combination with taurine, can have an anti-atherogenic effect, with parallel antimicrobial and metabolic benefits [100]. Given the extensive number of ethnomedical and ethnobotanical data, as presented in this text, and also discussed by various authors [11,101,102], it can be observed that a number of them are corroborated by recent experimental data, while the effectiveness of others is still to be determined. For example, the addition of piperine in the treatment of jaundice or of various gynecological problems warrants further research. Cutaneous applications may show effectiveness due to the potential of piperine to modulate cellular and humoral components in the skin, as well as interact with inflammatory pathways, in a similar manner as other phytochemicals [103-106]. Other traditional applications of piperine in neurological problems, namely epilepsy, may be of importance, given the prevalence and severity of certain epileptic syndromes [107-109]. In addition to testing the effectiveness and applicability of reported uses, a sustained effort should be made to complete the recording of such uses across different cultures, where black pepper has been used extensively for centuries.

Combination of Black Pepper with Acupuncture

Given that capsaicin has been proven an effective anti-bacterial [110] anti-inflammatory agent when applied at acupuncture points [95], a promising research direction seems to be the application of black pepper cream in similar points, alone or combined with capsaicin and other phytochemicals. Currently, there exist protocols for acupuncture in diabetic patients, with the acupuncture points Zusanli (ST36), Shenshu (BL23), Sanyinjiao (SP6), Yishu (EX-B3), and Zhongwan (CV12) being the most studied; protocols for diabetic neuropathy, one of the main complications, also exist and are presented in detail by Ma et al. [111]. The available evidence indicates that acupuncture is useful as an adjuvant therapy in diabetic patients [112], although the design and completion of more long-term trials is considered necessary.

Continuing on the potential combinations of acupuncture with the traditional applications of black pepper, it is noted that based on the research from the area of Bangladesh, black pepper is applied as a paste in case of gout-related chest pain [36]. Regarding gout and gouty arthritis, there are currently available acupuncture protocols [113-115], while based on a recent case study, it is possible to use acupuncture and moxibustion in the context of renal insufficiency [116]. Notably, recent research has demonstrated the renoprotective potential of piperine in the case of hyperuricemic nephropathy [117].

Black Pepper in Metabolic Diseases

Another interesting research direction concerns the effect of black pepper on glucose and lipid regulation. Regarding glycaemic control, in rats, black pepper extract was found capable of reducing blood glucose [85,118]. Inhibition of aldose reductase was noted by [119]; aldose reductase is the enzyme most associated with the complications of diabetes [120,121]. More recent research results in vitro are also in favour of the hypoglycaemic activity of black pepper [122,123].

These findings are important in the context of increasing incidence and prevalence and complications of diabetes mellitus [124-132], the associated mortality [133-135], the adverse effects of antidiabetic drugs [136-138], and the burden of disease [139-141]. Moreover, intestinal dysbiosis, specifically with Enterobacteriaceae and Candida species, was found to be a risk factor for diabetes mellitus type 1 [131]; piperine is effective against such pathogens and thus it could, hopefully, be used in a dual antiglycemic-antimicrobial role.

In rats, the addition of black pepper to their diet had a hypolipidemic effect [142,143]. A more recent successful experiment, performed by administering the methanolic leaf extract of *Piper guineense*, a species of pepper native to West Africa, in female diabetic rats, indicates that it can be used in the management of diabetic patients, with no adverse biochemical effects [144]. A leaf extract from the same plant species has demonstrated properties beneficial for glycaemic control in vitro [145]. In rats, immunohistochemical and histological data support the antidiabetic potential of black pepper in combination with other compounds [146], although further research is required on the matter. Such experiments are in accordance with a recent trend in evaluating the potential beneficial effects of spices in diabetic patients [147].

Hyperlipidaemia is associated with diabetes mellitus [148,149] and is, in all contexts, a risk factor for morbidity and mortality [150-152]. Therefore, the administration of black pepper extracts in general and piperine in particular, in high enough – but tolerable – concentrations, will benefit glycaemic and lipidaemic control both in diabetic and non-diabetic patients. Based on evidence on the interplay between obesity and inflammation [153], and taking into account the evidence in favour of piperine application in cases of obesity and inflammation, it is reasonable to consider the potential of a two-pronged effect of piperine administration in such cases.

Anticarcinogenic Effect of Piperine

It appears that there is a significant corpus of data in favour of anticarcinogenic effects of piperine [11,154-157]. When taking into account the different pathways, like molecular and metabolic, implicated in carcinogenesis (e.g., [158-169]), it is clear that there are numerous potential pathways upon which piperine may act to exert its anticarcinogenic potential. We would like to highlight the potential effectiveness of piperine in the treatment of hepatocellular carcinoma [170], the most prevalent form of liver cancer [171], and other phytochemicals were already shown to be good candidates [172,173]; this raises the possibility of combining piperine and the already used somatostatin analogues [174,175], to increase their effectiveness, in this and other forms of cancers. Piperine could be combined with a number of other natural compounds, which have a known anticarcinogenic potential [168,176] to maximize their efficiency; furthermore, taking into account the potential associations of certain pathogens with cancer [177,178], it could function both in an anticarcinogenic and anti-inflammatory role. The association of diabetes and colorectal cancer [179] also opens new avenues for piperine applications with a dual effect.

Non-medical properties of P. nigrum

Black pepper also has some notable ethnomedical veterinary uses, with the fruit being included in pastes or creams or infusions, to treat blotting [180], constipation, skin infections [181], various ailments [55], and helminthic infections [182] in animals. Other uses are also reported in the literature [183-187].

Perhaps more important are the applications of *P. nigrum* as a component of insecticides and larvicides. Early evidence of the insecticidal and larvicidal potential of black pepper, piperine, and its derivatives was provided by a number of experiments [188-190]. A later experiment by [191], documented the toxicity of black pepper extract on the rice weevil, *Sitophilus oryzae* (L.). It was previously observed that when exposed to white pepper, all the *Anopheles larvae* died [192], and it was then that the potential of this plant as an anti-malarial agent was first realised. A mix of ground black pepper and piperine was found to be very effective against a number of larvae of the same species [193]. Crucially, the mode of action of black pepper extracts is different from that of pyrethroids, meaning that potential resistance to the latter will not affect the action of the former; they both exhibit a marked potential for synergistic action between them [194]. Even more importantly, black pepper extracts and piperine are not toxic to other organisms and do not persist in the environment [195,196]. Piperine itself has been shown to be effective against the larvae of *Aedes aegypti*, the vector of the yellow fever and Dengue fever viruses [197,198]. Regarding a less dangerous species of mosquito, *Culex pipiens*, the common house mosquito, the study of [199] demonstrated that piperine-based dienehydrazide derivatives were more effective than piperine itself or Deltamethrin. Another study investigated the potency of *P. nigrum* against ant species, along with that of other plant extracts; the results were promising, revealing a viable alternative [200].

Given the adverse impacts of larvicides and insecticides in both the environment and human health [201-203], and existing threats to the biodiversity of different environments [204-207], efforts to incorporate natural compounds into insecticides and larvicides should intensify. The increased effectiveness of such compounds is important both in the context of agriculture [208-210] and, perhaps more importantly, in the context of the insect-borne diseases [211-213].

In response to the aforementioned health and safety concerns, both the European Union (EU) and the United Nations (UN) (https://sdgs.un.org/goals) have implemented measures to regulate and reduce reliance on hazardous chemicals, while exploring natural alternatives. The EU's regulatory framework (e.g., Regulation (EC) No 1107/2009 [214], Regulation (EU) 2022/1438 [215], Directive 2009/128/EC [216]), multilateral treaties like the Rotterdam Convention [217,218] and the Stockholm Convention [219,220], and research and development (R&D) programs strongly emphasize promoting directly or indirectly innovation in plant-based substances. The EU also provides funding opportunities through Horizon Europe [221], the largest research and innovation program supporting projects in sustainable agriculture, biodiversity, and green technologies.

Additionally, global initiatives such as the International Plant Protection Convention (IPPC) [222], the intergovernmental treaty that aims to protect the world's plants, agricultural products and natural resources from plant pests and the FAO (Food and Agriculture Organization of the United Nations) Guidelines on Pesticide Management [223,224] provide the necessary foundation for the establishment of national legislation and policies for pest and pesticides management, with the potential inclusion of more favourable conditions for biopesticides. Other organizations, such as OECD (https://www.oecd.org/), have dealt with the topic of biopesticides while there are countries worldwide with dedicated frameworks on the matter, like the US EPA (www.epa.gov/pesticides/biopesticides) and China’s Plan for Green and Sustainable Agricultural Development [225], encouraging biological control methods to reduce synthetic pesticide use.

CONCLUSION

Piperine and black pepper extracts exhibit ethnomedical and ethnobotanical applications, which should be explored further, with the aim of developing novel therapeutic solutions for a number of different pathologies. Future research should concentrate on elucidating the effectiveness of such applications in in vivo settings, using modern methods, and exploring the potential of increasing the active concentration via the application of novel delivery methods.

Finally, the non-medical applications of piperine and black pepper in general are promising in the field of insecticides/larvicide development, in accordance with the new directives of the EU and of other nations.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere. This research received no external funding.

Authors’ contribution

Conceptualization AP, ATP, AFG, DOC and CC; methodology AP, AFG, DOC and CC; validation AP, MP, DOC, and CC; formal analysis AP, LT, KP, ATP, GMA, AFG and CC; investigation AP, LT, KP, ATP, GMA, SPG, AFG and DLC; resources AFG and DLC; data curation DOC; writing—original draft preparation AP, ATP, SPG, AFG; writing—review and editing AP, ATP, DOC, and CC; supervision AP, MP, DOC and CC; project administration AP, AFG, and DLC. All authors have read and agreed to the published version of the manuscript elaboration

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ORIGINAL ARTICLE

Assessing the Effectiveness of an Ethical Communication Course for Romanian Medical Students: A Pretest-Posttest Study

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Abstract: Effective communication is essential in healthcare, prompting the inclusion of communication courses in medical education. However, the effectiveness of such courses for Romanian medical students has not been evaluated. This pilot study investigates the impact of an ethical communication course on Romanian medical students' communication self-efficacy, communication skills, and the perceived importance of communication. Students attended a 3-month course on ethical communication, covering active listening, assertive communication, conflict management, non-verbal communication, patient-centered care, and ethical considerations in various medical fields. The course was developed based on the European Psychiatric Association guide regarding ethical aspects of communication with patients and their families. The data were analyzed on a sample of 24 students who completed the questionnaires at pretest and posttest. Students reported higher levels of medical communication self-efficacy and perceived importance of medical communication after the course. However, no statistically significant differences were found in health professionals' communication skills. Future courses should include more patient interactions and varied teaching methods. Further research with larger samples and control groups is needed.

Keywords: communication self-efficacy; healthcare communication skills; ethical communication course; healthcare students

INTRODUCTION

Ethical issues are a daily reality in healthcare, affecting both patient care and professionals' well-being. Studies indicate that nurses and physicians frequently encounter dilemmas related to patient autonomy, informed consent, resource allocation, and end-of-life care [1,2,3]. Ethical conflicts often arise when professional values clash with institutional constraints, leading to moral distress and demoralization [4]. The World Medical Association (WMA) International Code of Medical Ethics (2022) and the International Council of Nurses (ICN) Code of Ethics [5] emphasize that healthcare professionals must uphold patient rights, confidentiality, and informed decision-making, yet the ability to navigate these obligations depends on effective communication skills. Nurses, in particular, report high levels of stress and burnout due to ethical conflicts, with staffing shortages and inadequate support being major contributors [3]. Younger and less experienced professionals struggle more with ethical dilemmas, highlighting the need for early training in ethical reasoning and communication during medical education [2].

Without structured guidance, healthcare professionals may feel powerless or unprepared to address ethical concerns, negatively impacting patient trust and care quality. The increasing ethical complexity of medical practice demands that future healthcare professionals receive formal training in ethical communication. Research demonstrates that ethical challenges are frequent and stressful, particularly for younger and less experienced clinicians [2,3]. Demoralization, moral distress, and institutional constraints further complicate decision-making, highlighting the need for early intervention through ethics education [4]. A structured ethical communication course for medical students would provide the necessary skills to navigate ethical dilemmas, improve patient relationships, and support professional integrity in healthcare practice.

Effective communication is a critical tool for resolving ethical dilemmas, reducing stress, and fostering patient-centered care [3,4]. The professionalization of healthcare ethics has gained momentum, emphasizing the need for structured training in ethical decision-making [6]. Ethical communication courses can serve as a preventive strategy against demoralization, equipping students with tools to

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navigate ethical tensions proactively. Current medical education often provides limited exposure to ethics beyond theoretical frameworks, leaving students unprepared for real-world ethical dilemmas [4]. Communication skills are considered essential in the healthcare professions, prompting the inclusion of courses aimed at developing these competencies within many medical education programs [7,8]. These courses generally aim to improve students' attitudes towards patient communication (i.e., their perception of the importance of communication skills), to increase students' self-efficacy regarding communication with patients (i.e., their confidence in their ability to communicate effectively), and to improve their actual communication skills [8]. Furthermore, the majority of medical education programs addressing communication skills encompass an ethical dimension related to patient communication [9]. Communication with patients is also a topic of interest for the Romanian academic medical community, with experts in the Romanian healthcare system highlighting the necessity of cultivating students' communication skills [10]. However, the efficacy of such courses for Romanian medical students has yet to be evaluated. To cover this gap, the purpose of the current study is to investigate the effectiveness of a course on ethical considerations in communication with patients for Romanian medical students. Specifically, we examined the course's impact on increasing students' medical communication self-efficacy, health professionals' communication skills, and perceived importance of medical communication.

Previous studies support the effectiveness of courses designed to enhance communication skills with patients. In one study [11], nursing students were trained in communication skills using complex care scenarios, with professional actors portraying patients. The training effectively enhanced self-efficacy and communication skills, with sustained benefits observed 6 months post-intervention. In another study [12], a communication skills training program that included experiential learning methods (e.g., role-playing) and a medical psychology course significantly improved medical students' positive attitudes toward communication skills. A recent research [13] evaluated the acceptability and efficacy of a communication skills training program tailored for nursing students. The program focused on building empathy and discussing complex situations such as end-of-life care. Results indicated that the training significantly improved nursing students' confidence and usage of essential communication skills in complex clinical scenarios. One study found that three experiential teaching methods (practicing patient interviews with peers, followed by debriefing and feedback; conducting interviews with trained actors simulating patients, followed by feedback sessions; watching actors playing a theatrical performance that included communication challenges between doctor and patient) are equally effective in teaching interpersonal communication skills to medical students [14].

In addition to effective communication, recent medical literature emphasizes the importance of ethical competence in protecting patients and promoting high-quality care [15]. It requires a combination of personal virtues, professional ethics, reflective practice, and a commitment to confidentiality and honesty [15,16]. Ethical competence enhances the quality of care and supports healthcare professionals in making ethical decisions [16]. In a systematic review that explored the literature on ethics education aimed at fostering ethical competence among healthcare professionals and students [17], based on the thematic analysis of 40 articles, the review highlights that ethics education is crucial for developing the ability to recognize and manage ethical issues in healthcare settings. Key themes identified include the importance of creating supportive conditions for ethical learning, designing effective educational strategies, and fostering interaction among learners. The studies reviewed indicate that ethics education can help healthcare professionals and students become more aware of ethical problems, enhance their ethical reasoning skills, and ultimately improve their ability to act in the best interests of patients. Indeed, another literature review based on 23 empirical studies [18] revealed that ethics interventions enhanced ethical knowledge, moral sensitivity, and the ability to identify ethical problems among healthcare workers and students. Interventions also improved ethical empowerment, including moral courage and the ability to cope with moral distress.

These findings underscore the importance of integrating comprehensive ethical communication skills training into medical students' education programs. Such training can significantly enhance medical students' confidence and skills, particularly in handling complex and emotionally charged interactions with patients and families, the ability to recognize and manage ethical issues, and ethical reasoning skills. Following this general trend of integrating communication and ethics courses in medical educational programs, the purpose of the current study is to evaluate the effectiveness of an ethical communication course for Romanian medical students in increasing students' medical communication self-efficacy, health professionals' communication skills, and perceived importance of medical communication.

MATERIALS AND METHODS

Participants

No a priori power analysis was conducted, as the pilot study relied on a small convenience sample of available students. As such, the findings should be interpreted with caution, as the limited sample size reduces statistical power and increases the likelihood of both Type I and Type II errors. Fifty-eight healthcare students completed the questionnaire. Five responses were excluded because the participants answered an attention check question incorrectly ("Do you think stones float on water?", Agree/Disagree). One case was excluded due to missing values. The final sample at the pre-test consisted of 42 women (80.8%) and 10 men (19.2%). Of these, 32 (61.5%) previously participated in other communication courses, and 45 (86.5%) did medical practice or volunteering in a hospital or clinic. The pilot study participants had an average age of 20.21 years with a standard deviation of .72. The posttest questionnaire was completed by 24 students. Among them, 16 (66.7%) were women, 8 (33.3%) were men, 21 (87.5%) had previously done medical practice or volunteering in a hospital or clinic, and 11 (45.8%) had participated in a communication course in the past. The students had an average age of 20.25 years with a standard deviation of .67.

To assess potential differences between the students who completed only the pretest and those who completed both the pretest and posttest, we conducted non-parametric and categorical comparisons. A Mann–Whitney U test showed no significant difference in age between the two groups (U = 334.00, Z = –.04, p = .97). However, Chi-square tests indicated significant differences in gender distribution ($\chi^2(1) = 5.71$, p = .02), with a higher proportion of male students among those who completed both assessments. There was also a significant association between attrition and prior participation in communication courses ($\chi^2(1) = 4.65$, p = .03), suggesting that students without previous communication training were more likely to remain in the study. No significant difference was found regarding prior clinical or volunteering experience ($\chi^2(1) = 1.01$, p = .32).

Procedure

The students participated in a 3-month course called Ethical Considerations in Communication with Patients. The first session, called Ethical Communication with Patients and Relatives, included topics such as active listening, assertive communication, conflict management, non-verbal communication, and patient-centered care. The second session, Ethical Communication in Oncology and Palliative Care, included the presentation of the WHO principles of patient care, case studies, and ethical dilemmas. The third session was about ethical considerations in organ transplantation. The fourth session addressed the ethical considerations in communication within the field of psychiatry and mental health. Finally, the last session was about social media, mass media, and public health issues.

The sessions were developed based on the European Psychiatric Association guide regarding ethical aspects of communication with patients and their families [19]. The basic principles of communication that were presented were: respecting the patients' autonomy, dignity, opinions, values, privacy, and confidentiality; sincere and clear communication; encouraging the expression of emotions; using a language adapted to the patient's age and culture; avoiding time constraints [19]. Regarding the communication about the diagnosis, four principles were proposed: informing patients about their diagnosis after analyzing what they know and what they want to know about the disease; the step-by-step and flexible approach to presenting the diagnosis, avoiding the communication of unreliable information; prior discussion with patients to clarify what can be communicated to relatives [19]. Finally, the recommendations regarding communication about treatments were followed: presentation of all relevant information about treatments (risks and benefits, duration, possible adverse events); presenting the advantages and disadvantages of refusing treatment; helping the patient to make an informed decision; informing the relatives about the treatment [19].

Participation in the ethical communication course was optional. Since the present pilot study included only students who voluntarily attended the course, there was no inter-group comparison performed, and variability between groups was not controlled for. The course sessions were delivered by faculty members from the medical university, specifically two psychologists and one psychiatrist with clinical and teaching experience. All lecturers were course coordinators with expertise in communication skills training and medical ethics. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the University (Approval No. 8371, dated March 29, 2024).

Measurements

Medical communication self-efficacy was evaluated with the scale developed by Parle, Maguire, and Heaven (1997) that measures self-efficacy regarding nine components of communication with the patient (e.g., helping a patient deal with the uncertainty of his/her situation, breaking bad news to a patient) [20]. Students reported on a scale from 0 (not at all confident) to 100 (totally confident) how confident they were that they could successfully manage each situation.

Health professionals' communication skills were measured with the Health Professionals Communication Skills Scale (HP-CSS) [21], which contains 18 items (e.g., When I give information to patients, I do so in understandable terms.) on a scale from 1 (seldom) to 6 (many times) that evaluates four communication skills dimensions (empathy, informative communication, respect, and social ability/assertiveness).

Perceived importance of medical communication was measured with a modified form of the medical communication self-efficacy scale [20], in which the students reported, on a scale from 1 (not important at all) to 5 (very important), how important they consider the behaviors in the respective scale (e.g., concluding a patient interview with an agreed problem list and a plan of action) in the physician-patient relationship.

The instruments were translated into Romanian by two psychologists with expertise in health psychology using the back-translation method. Internal consistency was evaluated using Cronbach's alpha coefficients for each scale at both pretest and posttest, indicating good reliability (values presented in Table 1).

RESULTS

Descriptive Statistics

We performed the descriptive analysis for the study variables at the pretest and posttest for the students who completed the questionnaires at both time points. Results are presented in Table 1.

The Differences between Pretest and Posttest

We analyzed differences between the pretest and posttest using the Wilcoxon signed-rank test. Students reported significantly higher levels of medical communication self-efficacy after the course, Z = –3.29, p = .001, with a large effect size (r = .67). Similarly, the perceived importance of medical communication significantly increased from the pretest to the posttest, Z = –2.26, p = .02, with a medium effect size (r = .46) (Table 2). No significant change was observed in health professionals' communication skills, Z = –1.72, p = .09, although the effect size was in the medium range (r = .35).

Table 1: Descriptive Statistics for Pretest and Posttest (N = 24)

Variable	Condition	Mean	Standard Deviation	Cronbach's alpha
Medical communication self-efficacy	Pretest	55.99	13.76	.82
	Posttest	68.86	15.83	.89
Health professionals' communication skills	Pretest	4.70	.61	.86
	Posttest	4.92	.58	.86
Perceived importance of medical communication	Pretest	4.23	.58	.87
	Posttest	4.43	.61	.91

Table 2: The Differences between Pretest and Posttest (N = 24)

Variable	Z	p	Effect size (r)	Interpretation
Medical communication self-efficacy	-3.29	.01	.67	Large effect
Health professionals' communication skills	-1.72	.09	.35	Medium effect (non-significant)
Perceived importance of medical communication	-2.26	.02	.46	Medium effect

DISCUSSION

This pilot study aimed to assess the effectiveness of an ethical communication course for Romanian medical students in enhancing students' self-efficacy in medical communication, improving health professionals' communication skills, and increasing the perceived importance of medical communication. The course followed the European Psychiatric Association's guide on ethical communication [19]. Key principles included respecting patients' autonomy, dignity, privacy, and confidentiality; clear communication; encouraging emotional expression; using appropriate language; and avoiding time constraints. For diagnosis, principles included informing patients, using a step-by-step approach, avoiding unreliable information, and clarifying what can be shared with relatives. For treatment, recommendations included presenting all relevant information, discussing treatment refusal consequences, aiding informed decisions, and informing relatives.

Ethical communication is increasingly recognized as an essential competency in healthcare, yet many medical students receive inadequate formal training in this area [1,2,3]. Research shows that healthcare professionals frequently encounter ethical dilemmas that require effective communication skills, particularly in cases of informed consent or end-of-life decision-making [4]. The ICN Code of Ethics (2021) and the WMA International Code of Medical Ethics (2022) stress the importance of ensuring clear, transparent, and compassionate communication in patient care, yet studies indicate that many healthcare professionals struggle to implement these principles effectively [3,22]. Ethical stress and moral distress are also common among nurses and physicians who feel unprepared to navigate these complex interactions, leading to demoralization and job dissatisfaction [4]. Given these challenges, structured ethical communication training is essential for enhancing professionalism, patient trust, and ethical decision-making in clinical practice [6].

Students reported higher levels of medical communication self-efficacy and perceived importance of medical communication after completing the course. These results are in line with previous studies in which different types of communication courses had a positive effect on healthcare students' communication self-efficacy and attitudes toward communication skills [11,12,13]. Our results are also in accordance with the studies highlighting that ethical interventions enhance ethical knowledge [17,18]. Research further suggests that integrating ethics training into medical curricula can reduce moral distress and improve ethical resilience, helping future healthcare professionals navigate value conflicts and ethical dilemmas with greater confidence [2,3].

No statistically significant differences were found in terms of health professionals' communication skills. This result is in contradiction with other studies in the field [7,13,14,17]. There are two possible explanations for this result. Firstly, without ongoing reinforcement and opportunities to practice these skills in clinical settings, the impact of the course might diminish. It is possible that the students did not have enough opportunities to interact with patients and practice the new skills. Future courses could offer students more practical contexts in which to interact with patients. Secondly, the lack of effect may be because the course did not include didactic methods used in the training that had positive results in increasing communication skills (e.g., care scenarios with professional actors playing the role of patients, practicing patient interviews with peers, watching actors perform a theatrical piece depicting communication challenges between doctor and patient) [11,14]. Future courses could include a greater variety of teaching methods.

The present pilot study has several limitations. First of all, the sample is very small. Second, the study was based on a convenience sample. Thirdly, the sample lacked diversity, as most students shared similar educational backgrounds, age ranges, and academic experiences. This limited representativeness may affect the generalizability of the results to broader and more heterogeneous student populations. Fourth, some of the students did not complete the questionnaire at both time points. Additionally, the voluntary nature of participation may have introduced self-selection bias, as more motivated or engaged students were likely overrepresented in the post-test sample, potentially inflating the observed effects. These limitations regarding the participants make the conclusions of the study inconclusive. Future studies could be based on larger statistically representative samples. Fifthly, there was no control group of students to compare their scores with those of the students who attended the course. Therefore, it was not possible to test whether the increases in self-efficacy and the perception of the importance of communication were due to the participation in the communication course or due to other factors (e.g., the participation in other courses, and the experience with patients in hospitals). Future studies could also include a control group to test the effectiveness of the course. Another limitation of this study is the exclusive use of self-report questionnaires, which are subject to personal biases and may not accurately reflect actual improvements in communication skills. Without objective assessments, such as knowledge tests, role-playing evaluations, or clinical observations, it is difficult to determine whether students have effectively internalized and applied the skills learned. Future research should combine subjective and objective measures to better evaluate training outcomes. In addition, the statistical power of the study was limited due to the small sample size and lack of a priori power analysis. Future studies should conduct formal power calculations to ensure adequate sensitivity for detecting meaningful changes. Finally, although the students participated in the course, they did not have extensive practical experience with patients in hospitals. Therefore, the study participants could not evaluate whether they improved their communication skills in real contexts with patients. Future studies could test the effectiveness of such courses after students have more practical experience with patients. Future studies could also explore the integration of structured pedagogical frameworks (such as experiential or transformative learning models) into course design, to better support behavioral change and deepen students' engagement with ethical communication principles.

CONCLUSION

This pilot study explored the effectiveness of an ethical communication course for Romanian medical students, focusing on its impact on communication self-efficacy, perceived importance of communication, and communication skills. The results indicate that theoretical training can improve students' confidence in communicating with patients and raise their awareness of the ethical significance of medical communication. However, the course did not produce measurable changes in actual communication skills. These findings highlight the need to complement theoretical instruction with practical components such as role-playing, simulations, or supervised patient interactions to support skill development. Given the small, non-representative sample and lack of a control group, the results should be interpreted with caution. Future research should test similar interventions using larger, randomized samples and objective outcome measures. Additionally, future courses could consider incorporating structured pedagogical frameworks to enhance student engagement and better support the internalization and application of ethical communication principles.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, M.L., C.G.I., A.C.C. and A.C.; methodology, B.O.; software, D.M.P.; validation, M.L.., B.O., and A.C.; formal analysis, D.M.P.; investigation, S.G.; resources, M.L. and A.C.C.; data curation, C.G.I.; writing—original draft preparation, B.O..; writing—review and editing, M.L. and A.C.; visualization, B.O..; supervision, M.L.; project administration, L.S.; funding acquisition, M.L. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the University (Approval No. 8371, dated March 29, 2024).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Correlational Analysis of Performance Outcomes during the Education of Combat Medics at the Tactical Medical Training Cell of the Belgian Defense

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Abstract: Combat medics must deploy multiple cognitive, manual, and social skills to perform efficient life-saving interventions and ensure the safe transportation of casualties. In 2021, the Tactical Medical Training Cell of the Belgian Army introduced an immersive and integrative educational program for Advanced Emergency Medicine Technicians (EMTa) certification. This study aims to provide a correlational analysis of performance outcomes during the certification period. While conducting their assessment, candidates were evaluated in three different but complementary roles: Medical Leader (ML), Medical Practitioner (MP), and Tactical Leader (TL). The individual outcomes in theoretical and practical examinations of one hundred thirty-seven candidates were collected during 2021 and 2022. The mean success scores for the theoretical and total practical examinations were 90.6% and 80.1%, respectively. Candidates scored significantly better as TL. Theoretical results were significantly correlated with the ML function, and performances in all three positions were correlated with each other. This study showed that within the combat medic team, individual performances, even if initially well-defined, were associated with the success rates of the other positions. This finding should be considered in the further consolidation of combat medic education.

Keywords: military medicine; education; first aid; combat medic; tactical leader; emergency medicine

INTRODUCTION

In today's operational theaters, combat medics must deploy multiple cognitive, manual, and social skills to perform efficient life-saving interventions, provide basic medical and nursing care, and ensure the safe transportation of casualties off the battlefield. In a contemporary warfare environment, first-line healthcare professionals face disruptive tactical challenges and novel technological threats [1]. Due to these particularly complex working conditions and this broad spectrum of responsibilities, educational and training programs must emphasize a strong knowledge base and intense practice of basic and advanced damage control resuscitation and surgical procedures. Furthermore, when combined, this knowledge and these skills should stimulate agile physiological thinking, appropriate clinical prioritization and time management, and the conservation of material and human resources, while considering cultural differences and ethical issues where necessary [2].

The integration of adult education principles can actively contribute to enhancing these learning performances. Adult education is defined as a teaching model that incorporates strategies of student self-direction toward goals of practical relevance for the learner [3]. According to Illeris, learning has individual cognitive, emotional, and external environmental dimensions [4]. An integrative and cross-fertilization process – the start of any learning process – takes advantage of five types of stimuli: (a) perception of the raw material; (b) transmission by another person (e.g., in lectures and instruction or through questions); (c) experience; (d) imitation; and (e) participation [5]. Consequently, accommodative and transformative learning that enlarges stimulation modes can contrast with restricted cumulative and assimilative learning. More specifically, theories of adult education aim to value the prior learning and experience of adults [6]. In a systematic review of faculty development initiatives designed to improve teaching effectiveness in medical education, the use of experiential learning, feedback, and effective peer relationships was found to be paramount to the success of these programs [7].

However, for a long time, the superiority of adult education principles in the specific domain of healthcare professionals' education was a matter of debate [8-10]. Interactive learning methods can be used in new contexts that do not resemble the original learning situation [8]. Scientific evidence supports the view that multimodal

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and interactive learning principles can lead to higher performance during medical education and training [11-13]. Additionally, problem- or case-based learning appears to promote greater critical thinking, better integration of facts, and improved self-evaluation compared to traditional teaching models with teacher-directed learning and non-interactive modes of teaching [14].

Although these principles of adult learning have been well-described for over 50 years, comparatively little is known regarding their integration into combat medic education or training programs.

In 2021, the Belgian Defense, Tactical Medical Training Cell (TacMed) introduced an integrative and immersive model of an educational program in the Advanced Emergency Medicine Technicians (EMT) certification. All Combat Medics candidates for the advanced course are systematically exposed to this learning model. The principles of the course shape theoretical and practical skills as support for individual professional development and, by extension, leadership maturing. The essential components for individual and collective medical training were identified following the NATO SOF doctrine [15]. Different training principles, such as Life Tissue training (LT) and simulation with role players (SIM), are combined. The last serves as a core education pivot because of the abundance of peer-reviewed evidence from military studies that clearly demonstrates that training methods using human patient simulators for teaching trauma care skills were superior and more cost-effective than many other learning techniques [16]. Finally, specific efforts are put into hyperrealistic and immersive simulation training scenarios as stimulators of team performance [17]. Like other authors [18,19], the course organizers conceived team performance as a multilevel process that includes the interrelation between individual-level and team-level taskwork and teamwork processes. The coordinated activities of a team of well-educated and trained individuals were considered the main objective [20,21].

The primary objective of the study was to establish, within an integrative hyperrealistic education model, the associative principles between theoretical and practical domains in order to explore global performance outcomes. As a secondary objective, success rates for specific achievements were inventoried, and a complementary analysis of their association profiles was performed.

MATERIALS AND METHODS

Study design

Advanced Combat Medic candidates presenting at the Medical Component Competence Centre, Belgian Defense, were included in this single-center retrospective cohort study.

Study setting and data collection

This study was carried out from January 2021 to December 2022. Instructors, evaluators, and Exercise Controllers systematically collected and processed all theoretical and practical examination results.

Population

A yearly refresher Tactical Combat Casualty Care (TCCC) educational program must be attended by all Belgian soldiers. The operational units of the Belgian Medical Component and combat units, including Special Operation Forces, detached their Combat Medic candidates. In their respective units, they applied voluntarily for a specific position as first-line responders. All candidates were consecutively included. To exclusively explore the course benefits, only students who failed the entrance examination were excluded from this study.

Intervention

A three-week theoretical module covering TCCC, Pre-Hospital Trauma Life Support (PHTLS), and Advanced Medical Life Support (AMLS) principles is preceded by an initial entrance exam on general anatomy and physiology. At the end of this sequence, and after succeeding in the theoretical exam in these domains, a three-week practical module is launched. This module includes dead tissue training, trauma patient simulation (using role players or manikins), clinical scenarios (individual or team-based), and a broad spectrum of clinical practicals. These are focused on amphibious medical incidents, mass casualty situations, complex casualty extrication, and CASEVAC (Casualty Evacuation). During a 72-hour final synthesizing on-field examination, all candidates were evaluated based on three independent secondary domains: their capability to act as Medical Practitioner, Medical Leader, and Tactical Leader.

Continuous monitoring by experienced healthcare professionals and psychologists during the on-field certification period ensured adherence to ethical guidelines and psychological safeguards.

Outcomes

Two main domains (total theoretical and practical scores at the final examination) and three practical secondary domains were considered as performance outcomes. The three practical secondary domains were designed to cover cognitive, procedural, and environmental dimensions. These three practical domains were linked to the following positions:

a. The Medical Leader (ML) was responsible for overall clinical assessment and management. The ML's responsibilities included: preliminary medical assessment, medical decision-making, guidance of medical activities, securing medical evacuation, and leadership of medical providers. Evaluation methods for this function assessed the quality of the medical approach (both diagnostic and therapeutic), the effectiveness of control over hard skills by team members, the quality of general clinical guidance, adequate and effective communication with the superior clinical echelon, control of safety rules, and final clinical responsibility.

b. The mission of the Medical Practitioner (MP) was to execute technical medical skills under the leadership and supervision of the ML. Particular attention was paid to the quality of material preparation, completeness, and safety in execution. The specific soft skills evaluated were: empathy towards the casualty, reactivity to ML demands, quality of reporting to the ML, and interaction and communication with the ML and other MPs.

c. Finally, the Tactical Leader (TL) was responsible for team security, controlling situational awareness, and coordinating the medical team's tactical actions. This function was evaluated through quality indicators of situational awareness, safety rules, planning of team actions, and consolidation of the medical evacuation plan proposed by the ML.

Evaluation tools and variables

During the theoretical evaluation, the candidates were evaluated on the percentage of correct responses at the end of the theoretical module. At least two independent evaluators from TacMed scored the respective ML, MP, and TL performances during the practical examination. Concerning the scenarios with role players, three main objectives were preliminarily defined and specifically evaluated through a scoring system reportable on a percentage scale. The first evaluation objective was always dealing with the hard skills, the second with the individual soft skills, and the third with the individual contribution to the collective dynamics.

The evaluators had received the same education and training for this purpose. The education of evaluators respects the NATO standards and includes additional skills from the civilian evaluation systems developed for health care. They regularly train for this function and are obliged to participate in all evaluation exercises organized by TacMed. At least one of the observers is educated and certified in an Observer/Trainer function following the NATO standards (International Military Medical Observer Trainer Course (IMMOT) course, NATO MED-MS-36887, NATO Centre of Excellence for Military Medicine). During the preparation of the scenario, the defined objectives and evaluation tools per objectives are revised together within the evaluator’s team. All evaluators use the same evaluation scale.

Evaluators are blinded to theoretical scores and scores from the other scenarios run by the candidate.

The evaluation indicators were independently evaluated by each evaluator and compiled into a final score. The final score for each function was obtained by simple cumulation of the individual scores of all of the examinators. Each candidate went through the different positions at least two times.

The final global practical score corresponded to the sum of all scenarios’ scores of the candidate.

In support of this scoring system materials, additional videos or pictures were captured.

At the end of each scenario, during the debriefing, individual and collective learning points were pointed out by each candidate as well as by the entire team.

Statistical analysis

An overall description of all populations/outcomes was statistically explored. Categorical variables were summarized using frequencies and percentages. Continuous variables were summarized using medians and standard deviations (SD). After checking for normality using the Kolmogorov-Smirnov test, the data were found to be not normally distributed (statistic = 0.127; df = 107, $p < 0.001$). The Wilcoxon Rank Sum test was used to compare two groups, paired or unpaired. The Mann-Whitney U and Kruskal-Wallis tests were used to compare a continuous variable with a categorical variable with two or more categories, respectively. Correlations between theoretical/practical results and individual results from the secondary domains were calculated using Spearman's rank correlation, two-tailed. The overall practical results (%) were defined as the dependent variable for multivariate linear regression, with multiple independent variables being demographic or individual results. A p-value < 0.05 was considered statistically significant. Biostatistical analyses were supported by IBM SPSS Statistics Version 29.0.1.0 (171) (License Ghent University, Belgium).

RESULTS

One hundred thirty-seven combat medic candidates for EMTa certification were recruited, and their individual scores were collected. Their participation was well-distributed during the two-year study period: from January 2021 to December 2022 (Table 1). With a mean seniority in the army of 8.99 years (SD = 5.13 years, range = 1-22 years), the candidates had a mean age of 30.30 years (SD = 5.89 years, range = 21-49 years). The vast majority of candidates were male (97.8%), with only 2.2% being female. In the total group, 71.5% of candidates succeeded in the final test, and 9.5% failed. Nineteen percent of the total group did not complete the entire course or final certification examination, mainly due to changed work schedules. Although not statistically significant, older candidates were found to be more resilient regarding non-completion of the course.

Theoretical and practical domains

A mean success score of 90.6% (SD = 6.5%) was found for the theoretical examination. On the other hand, the mean of the total practical examinations was 80.1% (SD = 4.8%) at the end of the 72-hour certification field exercise. All age and seniority groups presented this drop between theoretical and practical results, with no significant difference observed between younger and older candidates.

Secondary domains during practicals

The mean results displayed the following respective scores: for the ML function, the success rate was 79.1% (SD = 6.49%); for the MP, 80.8% (SD = 6.28%); and for the TL function, 81.1% (SD = 5.68%). Candidates scored significantly better as TL (p = 0.014) in comparison with their function as ML. When observing the secondary domain success scores, no significant difference was observed between the age or seniority categories.

Table 1: Population characteristics

Characteristic	N	Mean	StdDev	Range
Age (years)	137	30.20	5.89	21-49
Seniority (years)	137	8.99	5.13	1-22
		Count	**Percentage**	
Gender				
Male		134	97.8%	
Female		3	2.2%	
Year of completion				
2021		75	55.5%	
2022		61	44.5%	

Figure 1: Descriptive map of observed associations

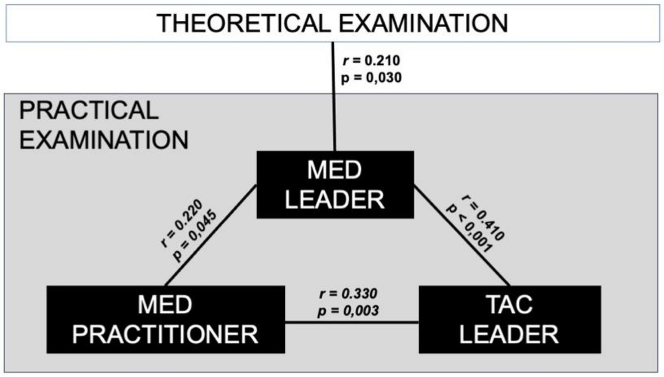

Correlations between main and secondary domains

Interestingly, the theoretical results were found to be weakly but significantly correlated not with the total practical score, but only with the ML function (r = 0.210, p = 0.030) (Figure 1). As expected, the total practical scores were significantly correlated with their secondary domains: ML (r = 0.210, p < 0.001), MP (r = 0.565, p < 0.001), and even more strongly with TL (r = 0.793, p < 0.001) functions. Furthermore, the secondary domains were found to be statistically correlated with each other: weakly or fairly but significantly correlated between ML and MP (r = 0.220, p = 0.045), and between MP and TL (r = 0.330, p = 0.003), while the correlation between ML and TL was found to be statistically stronger (r = 0.410, p < 0.001).

Multivariate linear regression

With the total results at the practicals as the dependent variable, the multivariate linear regression identified all practical secondary domains as independent predictors of the total practical results (p < 0.001), with the theoretical results as a weaker but significant independent predictor (p = 0.04) (Table 2). Candidate age and seniority were not found to be predictors of higher performance scores at the final practical examination.

DISCUSSION

In this study, we explored performance scores during military educational and training programs for combat medics. An integrative and immersive combat medic-specific training was proposed in a structured and stratified manner. During the practical examination, three different positions were tested, and this analysis shows how they were associated with each other. During the educational phase of the program, candidates were stimulated in their leadership (medical and tactical) positions through an integrative and cross-fertilization process, taking advantage of five types of stimuli identified by Jensen [5]. On the other hand, the role of the MP was not restricted to the adequate and safe execution of medical hard skills. Although well-structured assessment testing of medical hard skills is beneficial [22], additional soft skills such as communication, reporting, or mutual support were also added to the MP's educational program.

Table 2: Results of regression analyses performed to determine the effect of indicators in predicting performance scores at the practical examination

Coefficients*				
	Coefficient Std Error	**Standardized coefficients β**	**t**	**Sig**
Age	0.005	-0.005	-0.771	0.443
Seniority	0.006	0.002	0.254	0.800
Candidate category	0.041	0.003	0.876	0.384
Scores theoretical results	0.003	-0.008	-2.095	0.040
Medical leader	0.003	0.553	128.107	<0.001
Medical provider	0.003	0.353	83.922	<0.001
Tactical leader	0.003	0.412	103.293	<0.001

$R^2 = 0.999$
F Change = 10945.960
Sig F. Change <0.001
StdError of the estimate = 0.15587
N=137

** Dependent variable: Total scores at practicals*

Objectives of the Course

Our education and training principles were largely inspired by the eight learning fundamentals of Haraldseid-Driftland et al. (2023) – categorizing collaborative, practical, and content elements – to help develop learning principles and tools to translate resilience into healthcare practice [23]. We complemented these with some of the six guiding recommendations of Sousa et al., including simulation, milestones as course learning objectives, and a multi-dimensional, competency-based assessment system [24]. The ultimate goals of this approach were to optimize crew resource management on one hand, and the individual resilience of first-line responders on the other. Especially in healthcare, communication, teamwork, and leadership top the list of nontechnical skills pivotal for clinical success [25,26]. As an illustration, deficiencies in nontechnical skills are among the leading causes of adverse events in the operating room [27,28]. In the early 1990s, Crew Resource Management principles initially developed to reduce pilot errors were applied to the medical field by anesthesia instructors, who developed the concept of Anesthesia Crisis Resource Management [29,30], with later extension to other actors in the operating room [31]. Across levels and contexts, this approach was found to stimulate healthcare professionals to collaborate, adapt to change, maintain processes and functions, and improve quality and safety through interactions such as exchanging information, coordinating, negotiating, aligning needs, and developing buffers [32]. Increased resilience has been shown to positively impact nursing practice by Thomas and Asselin [33]. In this context, resilience appears to be an important asset [34], and resilience-enhancing interventions to prepare healthcare students for their future professional practice were found to be beneficial [35].

Conceiving a Balanced Educational Program

Observing performance results, this study identified statistical associations between different positions within a combat medic team. For instance, in our study, the TL position – even if primarily more oriented towards situational awareness – was associated not only with global performance scores but also with the quality of more specific medical functions. Over the past century, educational psychologists and researchers have posited many theories to explain how individuals acquire, organize, and deploy skills and knowledge [36,37]. The three different processes of learning identified by Illeris – cognitive, emotional, and social – can occur simultaneously, forming a holistic learning process [4]. Additional dimensions of learning, such as physical or spiritual, could probably be added to this scheme.

At a cognitive level, our study has shown that the execution of medical techniques is not simply linked to previous knowledge acquisition. MP performance was correlated with the presence of two leading functions. Based on our methodological limitation of using a retrospective observational protocol, we cannot establish any intrinsic causal strength. Cognitive learning theories support the view that learning is a targeted internal process focusing on thinking, understanding, organizing, and consciousness. Even if successful in many learning aspects, such as information processing, curiosity stimulation, and motivation, they were found not to be strong enough to transfer the required knowledge to all learners. Additionally, they require considerable time and a trained workforce [37]. This evidence justified our choice not to exclusively concentrate on hyperrealism or single structural fidelity. We hypothesized that it may be more productive from an educational standpoint to consider the fidelity of the simulation scenario relative to clinical task demands along with physical resemblance [38-41], referring to evidence from the literature in this field [39-44]. This advantage of using multimodal simulation was supported by recent literature [42,43,45,46].

The emotional dimension of learning was not specifically explored in our study. However, our educational approach took advantage of an additional social dimension by encouraging and persuading previously learned behaviors, drawing the attention of learners, and creating strong responses [39-44]. Thanks to selective attention and memory, humans learn more from their experiences and organize these experiences into internal representations that facilitate future behavior [40-45]. Passive formats, lack of relevance, and disconnection from students' needs are some arguments supporting the apparent lack of efficacy of formal lectures [38, 46]. It appeared that the candidate's age or seniority in the army was not correlated with performance scores. Seniority and its consequent experience in the army, initially considered as a possible confounding factor or indirect advantage in this integrated learning model, cannot exclusively support the entire learning process. This finding stressed that medical and tactical leadership functions within a Combat Medic team must be learned and trained in addition to acquiring the necessary medical hard skills.

Finally, to emphasize the social dimension of this learning process, we chose event-based assessment as an essential method to deal with the complexity of authentic clinical interactions [47]. It potentially overcomes the shortcomings of not being able to observe tacit behaviors and cognitions and allows team members to assess the unobservable elements of teamwork [48,49].

Holistic Approach for the Education of Combat Medics

Our results only describe performance patterns, and our study was not designed to measure inferences within intrinsic cognitive mechanisms. Further analyses should be conducted to describe causality between indicators, identify all influencers, and measure their respective effect sizes on performance. The reported interactions could be considered the basis for a virtuous circle between these three functional abilities: medical guidance, execution, and external situational vigilance.

First, this study confirmed existing scientific evidence stating that a strong integration of various learning methods or tools leads to solid theoretical and practical results, even when considering specific practical tasks.

Secondly, a strict tasking of ML, MP, and TL functions within a combat medic team facilitated the organization and the detection of medical or tactical issues. These multiple but complementary views on the same medical situation positively influenced the final performance of each individual in the group. This task was initially designed to avoid interference and improve the quality of healthcare. All these tasks appear not to act separately but can reinforce each other. However, this approach imposes complementary education and training in soft skills, such as communication and reporting.

Finally, these considerations are not restricted to the specific environment of the Belgian Army. The program developed by TacMed at Belgian Defense was based on existing national or international protocols and studies, whether developed by or for armed forces or not. It took into account the military culture in Belgium and existing programs in other professional communities. The combination of educational tools was tested and adapted within the military, but external civilian experts adjusted program content or assessment methods. For this reason, some of these findings can be generalized beyond the specific Belgian military context.

Limitations

This study has limitations. The education program benefited from knowledge accumulated from the trainers' expertise, general trends in adult education, and guiding principles in EMT practice. Although efforts were made to minimize their effects, bias and unmeasured factors due to this retrospective design are still an issue. Because of their retrospective and observational nature, correlations could not establish causation between parameters. The conclusions of this study are based on the 71.5% of candidates who successfully completed the certification process. This high attrition rate could introduce a bias towards overestimating competency and positive correlations. Furthermore, the study is subject to all the limitations of a monocentric study design. Finally, while this is a preliminary qualitative investigation of integrative practice and leadership views in a military prehospital context, the perspectives represented may not reflect the entire profession, given the small overall sample size. Despite these limitations, these results provide valuable insight into some performance indicators in a complex work environment.

CONCLUSION

Inspired by recent findings in adult education, our integrative and immersive education program for advanced combat medic certification has demonstrated high success rates at both theoretical and practical levels. Furthermore, the performance levels of the different functions within the Combat Medic team, even if initially well-delimited, were found to be correlated with each other. These reported interindividual interactions open new perspectives for the consolidation of learning outcomes.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, F.W., J.J. and J.B.W.; methodology, J.B.W.; validation, F.W., J.J. and N.D.; formal analysis, J.B.W.; investigation, F.W. and J.B.W.; resources, J.B.W.; data curation, J.B.W.; writing—original draft preparation, J.B.W.; writing—review and editing, F.W., J.J., N.D. and J.B.W.; visualization, J.B.W.; supervision, J.B.W.; project administration, F.W. All authors have read and agreed to the published version of the manuscript.

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The authors did not use a generative artificial intelligence (AI) tool or service to assist with preparation, data analysis, or interpretation of results. AI was utilized to refine the language of the main body text after initial editing and review. The authors take full responsibility for the content of this publication.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki, and approved by the institutional office for data protection and the Ethics Committee (EC) of Ghent University and Ghent University Hospital (UZ Gent) and registered under number ONZ-2023-0600.

Patient consent for publication

Not applicable. Our retrospective study used examination results which regularly collected data from the databases. The consent for the use of personal data was given by each candidate as a routine procedure of the Centre of Medical Expertise, Belgian Defense provision.

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REVIEW

The Effect of Biological Therapies on Psoriasis and Its Associated Comorbidities – New Facts and Future Options

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Abstract: Psoriasis is a chronic and complex disease that consists of characteristic cutaneous lesions and is frequently accompanied by systemic comorbidities caused by the chronic inflammatory state. The most common such comorbidities are hypertension, diabetes mellitus, dyslipidemia, inflammatory bowel diseases, depression, anxiety, neoplasia, and non-alcoholic fatty liver disease. Therefore, it is of utmost importance to simultaneously treat both psoriasis and the associated conditions with the best therapies available to improve patients' life quality. Therefore, this review aims to evaluate the effects of biological therapies on both psoriasis and its comorbidities and also to highlight other future options.

Keywords: psoriasis, chronic inflammation, biological therapies

INTRODUCTION

Psoriasis is a chronic, immune-mediated inflammatory disease that affects approximately 125 million people, or around 2% of the world population [1-3]. It has a bimodal incidence, with peaks between 16-20 years and between 57-60 years, with almost 70% of the cases before the age of 40 years [4]. On the other hand, due to an increase in life expectancy, the number of patients with psoriasis who are over 65 years is increasing, and that represents a therapeutic challenge, as the treatment has to be modulated in order to prevent drug interactions and not interfere with other comorbidities [4].

Psoriasis is mainly characterized by specific cutaneous lesions, but also by systemic involvement, the most frequent associated comorbidities being hypertension, diabetes mellitus, dyslipidemia, depression, anxiety, neoplasia, and non-alcoholic fatty liver disease [1,5]. The most common form of psoriasis is psoriasis vulgaris, which consists of well-defined erythematous plaques covered with silvery scales, most often found on knees, elbows, and scalp [6,7]. Other possible forms are guttate psoriasis, which affects most frequently children and young people, usually after streptococcal infections, and consists of small, teardrop lesions on trunk and extremities, pustular psoriasis, a rare form, which is characterized by sterile pustules, and inverse psoriasis, which affects flexion surfaces [7,8]. In severe cases, psoriasis can evolve toward an erythrodermic form affecting over 90% of the body surface, a condition that requires urgent medical intervention [9]. The pathophysiologic mechanisms are represented by an abnormal activation of the immune system, especially of T helper lymphocytes – Th1 and Th17, the next step being the production of proinflammatory cytokines like tumor necrosis factor alpha (TNF-α), interleukin 17 and 23 [10]. The result is the proliferation of keratinocytes and local inflammation, with the formation of the characteristic psoriasis plaques [10].

The diagnosis is mainly clinical, but there are also a few methods that can help in confirming the diagnosis, like reflectance confocal microscopy, which can show an increase in the size and number of dermal papillae, along with a dilation of capillary vessels, and dermoscopy [11,12].

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In this review, we aim to evaluate the psoriasis-associated comorbidities and to identify the best therapeutic approach in order to treat the patient as a whole.

PSORIASIS-ASSOCIATED COMORBIDITIES

The main link between psoriasis and all the comorbidities involved is the systemic inflammation, mediated by pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), and interleukin (IL) 17 and IL-23 [2]. This inflammation process involves mainly the vessels, aggravating atherosclerosis and making the cardiovascular diseases the most important comorbidities in patients with psoriasis, leading to a decrease in life expectancy of approximately 4-5 years [13-15]. Also, the risk of cardiovascular diseases is increased by over twofold in patients with psoriasis, independently of other risk factors, especially in severe forms and in patients under the age of 50 years, the mechanism involved being vascular inflammation that affects mainly coronary arteries and promotes early atherosclerosis [3,13,16]. Another very important aspect is that dyslipidemia, characterized by high levels of triglycerides and low-density lipoprotein cholesterol (LDL) and low levels of high-density lipoprotein cholesterol (HDL), has an essential role in the relationship between psoriasis and cardiovascular diseases [17]. Therefore, a correct management of the lipid levels is very important in order to lower the cardiovascular risk [17].

Obesity is another important comorbidity with a greater prevalence in patients with psoriasis, the severity of the last one being proportional to the body mass index [18]. The relationship between obesity and psoriasis is bidirectional. Psoriasis, due to chronic inflammation, leads to insulin resistance, promotes visceral fat deposition, and, in severe forms, limits mobilization, all of which increase the risk of becoming overweight. On the other hand, obesity is associated with a great number of pro-inflammatory adipokines, like leptin, and a low number of anti-inflammatory ones, an imbalance that aggravates local and systemic inflammation and also increases the risk of psoriasis by over one and a half times [18-21]. Another fact worth mentioning is that obesity can reduce the treatment efficiency due to a larger distribution volume and, therefore, indirectly aggravate the severity of psoriasis [19]. Moreover, almost 50% of the patients with psoriasis have metabolic syndrome, defined as the presence of at least three of the following: abdominal obesity (waist at least 102 cm in men and at least 88 cm in women), insulin resistance or increased level of blood glucose (at least 100 mg/dL), dyslipidemia (tryglicerides at least 150 mg/dL and/or HDL under 40 mg/dL in men and under 50 mg/dL in women), arterial hypertension (over 130/85 mmHg) [22]. The presence of metabolic syndrome is very important, as it is linked with a greater prevalence of cardiovascular diseases, fact that creates a vicious circle [20]. In addition, osteoporosis is more frequently encountered in patients with psoriasis due to the activation of the osteoclasts by the pro-inflammatory cytokines, and it also aggravates the prognosis [23].

Diabetes mellitus is one of the most frequent diseases worldwide and also a common comorbidity associated with psoriasis, both by chance and by similar pathophysiological mechanisms, the first link between these two diseases being noted as early as 1897 [14,24]. As it was already mentioned, psoriasis is characterized by systemic inflammation that can lead to insulin resistance and promote visceral fat deposition, mechanisms that can increase the risk for developing diabetes by up to two times compared with the general population [22]. Moreover, diabetes may be induced in certain patients with psoriasis by the use of corticosteroids in an attempt to control the severity of the disease, especially if other therapies are lacking [23].

Regarding mental ailments, patients with psoriasis have a greater risk than the general population, especially for depression, anxiety, and suicide [2]. The explanation is that the presence of cutaneous lesions leads to shame, low self-esteem, and isolation from other people, all of which result in a reduction of the quality of life [2]. In addition, a low level of melatonin is very frequent in this category of patients and may contribute to depression by inducing changes in the sleep pattern [25].

Table 1: Common hepatic adverse reactions of classical psoriasis treatment

Treatment	Effects on the liver	Sources
Methotrexate	• significant toxicity • can induce hepatic fibrosis and cirrhosis if used long time, especially in NAFLD	[32,33]
Cyclosporine A	• can increase lipid levels and favor hepatic steatosis • treatment should be carefully monitored in NAFLD	[34]
Acitretin	• can aggravate dyslipidemia and NAFLD in patients with metabolic syndrome	[35]
Dimethyl fumarate	• has a better safety profile and can reduce hepatic inflammation and oxidative stress • however, the liver function should be monitored carefully	[36]

NAFLD – non-alcoholic fatty liver disease

Last, but not least, one of the most common comorbidities recently associated with psoriasis is non-alcoholic fatty liver disease (NAFLD) [26]. It consists of excessive deposition of lipids in the liver that is not caused by alcohol consumption and that can progress to non-alcoholic steatohepatitis (NASH) and cirrhosis [27]. Epidemiological studies indicate that the prevalence of NAFLD is three times higher in this category of patients and also that the severity of psoriasis is proportional to that of NAFLD [28-30]. Moreover, an interesting study has revealed that almost 47% of the patients with psoriasis had NAFLD, and 22% even had biopsy-confirmed NASH

[29]. The diagnosis of NAFLD is established through imagistic methods like abdominal echography and elastography and laboratory tests for liver function, and fully confirmed by liver biopsy [31]. It is worth mentioning that NAFLD has a significant impact not only on the liver but also on other systems, including the heart, kidneys, and thyroid gland, which have to be carefully evaluated [29]. Thus, the combination of psoriasis and NAFLD leads to a greater cardiovascular risk and mortality, emphasizing once again the importance of treating the patient as a whole [28]. However, some of the therapies available for psoriasis can have a direct impact on the liver and be dangerous, especially if patients already have NAFLD [31]. Table I highlights the most common adverse hepatic reactions.

The dermatologist has an essential role in the early identification of psoriasis-associated comorbidities. The general management consists of carefully evaluation of the liver function and of cardiovascular risk using physical examination – body mass index calculation, arterial pressure, signs of dyslipidemia like the presence of xanthomas, imagistic methods – abdominal echography, liver elastography, coronarography in selected cases and laboratory tests, a wise choice of medication, especially in the presence of NAFLD, where biological therapies are the first recommended, and inclusion of non-pharmacological treatment options like a healthy diet with anti-inflammatory products and physical activity [13,17,18,27].

However, an integrated approach is mandatory for a correct and complete treatment, the most commonly involved medical fields being cardiology, gastroenterology, and psychology [37].

TREATMENT OF PSORIASIS AND ITS ASSOCIATED COMORBIDITIES

The treatment of psoriasis has significantly evolved in the last century, from empirical therapies to modern biological medications based on a more profound understanding of the pathophysiological mechanisms [19]. Initially, between 1920 and 1950, the treatment of psoriasis was focused on the clinical aspect, and the most common medications included arsenic, coal tar, salicylic acid, and dithranol [19]. In the 1950s, significant progress had been made by the development of topical corticosteroids, a very efficient form of medication, that rapidly became the gold standard of the treatment due to anti-inflammatory properties, but which lacked specific targets [38]. Moreover, if used extensively, adverse reactions occurred in the form of skin atrophy or tachyphylaxis [39]. A more important step toward better control of psoriasis was made when methotrexate and cyclosporine were discovered, both of them used in severe forms of psoriasis, but are also limited by the risk of significant hepatic and kidney adverse reactions or toxicity [32].

Table 2: Treatment of psoriasis: benefits on comorbidities, risks, and side effects

Medication	Benefits on comorbidities	Risks	Side effects	Sources
Methothrexate	↓systemic inflammation ↓cardiovascular risk	can aggravate NAFLD	hepatotoxicity	[32,33]
Cyclosporine	rapid control of severe psoriasis ↓ inflammation	arterial hypertension dyslipidemia	nephrotoxicity	[34]
TNF-α inhibitors (Infliximab, Adalimumab, Etanercept, Certolizumab)	↓ inflammation ↓cardiovascular risk ↓ risk of hepatic fibrosis ↓ insulin resistance first line in psoriatic arthritis	↓ efficiency in overweight patients modest weight gain	infections, especially TB autoimmune diseases: SLE or arthritis hepatotoxicity congestive HF rare: lymphoma and skin cancer	[49]
IL-17 inhibitors (Secukinumab, Ixekizumab, Brodalumab)	↓ inflammation improve dyslipidemia of choice in overweight patients	may ↑ IBD	fungal infections, especially local/systemic candidosis and respiratory infections pain, erythema, edema at the site of the injection rare: autoimmune diseases – SLE-like, anterior uveitis	[44,50]
IL-23 inhibitors (Guselkumab, Risankizumab, Tildrakizumab)	↓ inflammation ↓ insulin resistance improve hepatic function very good in the long term, with no negative impact on comorbidities	none	respiratory tract infections, especially viral pain, erythema, and edema at the site of the injection rare – autoimmune diseases – less frequent than with TNF-α or IL-17 inhibitors	[50]
Dual IL-12/23 inhibitors (Ustekinumab)	↓ vascular inflammation improve endothelial dysfunction	none		[51,52]
Small molecules (Apremilast)	↓ systemic inflammation improve glycemic metabolism modest weight loss, which can be of benefit	diarrhea		[53,54]

HF – heart failure; IBD – inflammatory bowel disease; IL – interleukin; NAFLD – non-alcoholic fatty liver disease; SLE – systemic lupus erythematosus; TB – tuberculosis; TNF-α – tumor necrosis factor alpha; ↓ - lower; ↑ - increase/aggravate

The true revolution of psoriasis treatment, especially of the severe forms, was provided by biological medication, which targeted specific inflammatory cytokines and modulated the pathophysiological mechanisms involved [40]. However, significant side effects are also common, therefore, a good collaboration with other subspecialities is mandatory [40]. The main representatives of this therapeutic class are: TNF-α inhibitors, IL-17 inhibitors, IL-23 inhibitors, and dual IL-12/23 inhibitors [41,42]. Of particular importance regarding comorbidities are the effects of these therapies on atherosclerosis, as a main cardiovascular risk factor. It is evaluated using the thickness of the intima and media of the vessels as measured by echography, the pulse-wave velocity, the aspect of the atherosclerotic plaques by CT angiography, and the level of C-reactive protein [43]. For example, it was revealed that IL-17 inhibitors like secukinumab significantly reduce the thickness of the intima and media of the vessels and also the arterial rigidity, much more than classical treatments like methotrexate and cyclosporine [44]. On the other hand, they seem to not affect the vascular inflammation evaluated by PET/CT or on metabolic markers like insulin or adiponectin [43]. Further studies are necessary to better explore these effects, especially in the long term, in order to simultaneously reduce both the severity of psoriasis and of cardiovascular risk, eventually by using therapies that target multiple cytokines at the same time [45].

As was mentioned before, another very frequent psoriasis-associated comorbidity is obesity, with almost 50% of the patients with psoriasis having metabolic syndrome [18,21,22]. Hence, a wise choice of medication is mandatory, especially because not all the therapeutic classes are effective in this context [13,37,46,47]. For example, TNF-α inhibitors have low efficiency in overweight patients and can even lead to an increase in weight [37,46,47]. IL-17 inhibitors, IL-23 inhibitors, and also small molecules (Apremilast) are of choice in this situation, due to their improvement in dyslipidemia, insulin resistance, and weight loss [47].

Last, but not least, NAFLD is a very important comorbidity that has to be taken into consideration when choosing a therapeutic approach, especially in patients with severe psoriasis, in whom this comorbidity also has a high prevalence [28,29]. Methotrexate or cyclosporine should be avoided if NAFLD is suspected, because they can aggravate it [28,47]. In this context, a much better solution nowadays is represented by the use of biological therapies, especially of TNF-α inhibitors, which can decrease the risk of hepatic fibrosis and decrease inflammation and dyslipidemia [1,48].

For an easier understanding, the most common therapies used in psoriasis are presented in Table 2, along with their benefits on psoriasis-associated comorbidities, risks involved, and the most important side effects.

NON-PHARMACOLOGICAL MANAGEMENT OF PSORIASIS AND ITS ASSOCIATED COMORBIDITIES

As was mentioned before, in this category of patients, it is of utmost importance to adopt an integrative approach, from modification of the lifestyle to a careful selection of the best medications in order to have as many benefits as possible with the minimum of side effects. Interventions on the lifestyle are mainly represented by aerobic exercises for at least 150 minutes per week, which reduce the risk of obesity and also improve the lean mass and the glucidic metabolism, management of the stress and of anxiety, and by adopting a healthy diet [46,55]. This last aspect is very important, as the Mediterranean diet is well documented to reduce inflammation and to prevent metabolic comorbidities [46]. It consists of healthy fats like olive oil, good quality proteins from fish, chicken, or eggs, fibers, vitamins, and minerals from fresh fruits and vegetables, and also of cereals and fermented dairy products [56]. In psoriasis, the Mediterranean diet has many important benefits: it reduces systemic inflammation, insulin resistance, and dyslipidemia and, consequently, reduces the risk of atherosclerosis, obesity, and NAFLD [46,55]. Regarding alcohol consumption, in modest quantities, the red wine has antioxidative properties due to resveratrol, but the frequent use has to be discouraged because it has a negative impact both on the severity of the disease, increasing the levels of pro-inflammatory cytokines like TNF-α and IL-17 that can aggravate the skin lesions and NAFLD, and on the treatment adherence and efficiency [46,57].

Table 3: Plants most commonly used in psoriasis

Plant	Effects	Sources
Aloe vera (Aloe barbadensis miller)	↓ local inflammation ↑ skin hydration prevents exfoliation	[58,64]
Turmeric (Curcuma longa)	– TNF-α – IL-6 ↓ local inflammation	[58,59]
Fish oil (Omega-3)	↓ systemic inflammation ↑ skin hydration ↑ PASI score	[56,58]
Chamomile (Matricaria chamomilla)	soothing skin effects ↓ local inflammation ↓ pruritus	[58,60]
Capsicum (Capsicum annuum)	↓ pain and pruritus ↓ local inflammation sometimes can irritate the skin	[58,65]
Fenugreek (Trigonella foenum-graecum)	↓ local inflammation ↑ skin barrier	[58,66]

IL – interleukin; TNF-α – tumor necrosis factor alpha; ↓ - decrease; ↑ - increase; – - inhibits

Medicinal plants are used in psoriasis as associated therapies due to their anti-inflammatory, antioxidative, and immune modulation properties. Even though the efficiency of these therapies is not well studied, some plants significantly reduced the symptoms of the disease when taken orally or applied topically [58]. Some of these plants are Aloe vera (Aloe barbadensis miller), Curcuma longa, omega-3 rich fish oil, Matricaria chamomilla, Capsicum annuum, and Trigonella foenum-graecum [58]. Aloe vera hydrates the skin and reduces local inflammation, while curcumin inhibits pro-inflammatory cytokines like TNF-α și IL-6 [58,59]. Omega-3 fatty acids from fish oil modulate the immune response and can decrease the severity of cutaneous lesions [56,58]. In addition, chamomile has soothing effects and is frequently topically utilized [60]. Last, but not least, capsaicine, which is a chili pepper extract, has analgesic and anti-inflammatory properties, decreasing pruritus and redness when used topically, while fenugreek can also be used orally, with similar anti-inflammatory and anti-oxidative characteristics [61–63]. On the other hand, future studies are needed to better explore their properties, and, because of this, patients must visit a specialist before taking any of these plants to prevent side effects or drug interactions. These plants and their properties in treating psoriasis are presented in Table 3.

CONCLUSION

Psoriasis is a complex disease, with a multitude of comorbidities that significantly affect the quality of patients` lives. Thus, the concomitant treatment is essential in reducing the risks, and a careful selection of the medication is mandatory, as not to aggravate the pre-existing or newly appeared conditions. The most common psoriasis-associated conditions are cardiovascular diseases, especially due to systemic inflammation that promotes early atherosclerosis, obesity, metabolic syndrome, dyslipidemia, NAFLD, diabetes, mental ailments like anxiety and depression, neoplasia, and inflammatory bowel disease. The greatest mortality is caused by cardiovascular diseases, the prevalence of them being over two times higher in patients with psoriasis. Consequently, pro-active management is essential, using both pharmacological and non-pharmacological measures, ideally in a personalized manner.

The novel, modern therapeutic options, which include biologic therapies, are very promising. They reduce systemic inflammation by targeting specific cytokines involved in the pathogenesis of psoriasis, like TNF-α, IL-17, IL-23, and, therefore, can improve both the cosmetic appearance and other conditions, especially when concurrently used with non-pharmacological interventions. Of them, IL-17 inhibitors seem to be of real benefit in reducing atherosclerosis and ameliorating cardiovascular diseases, the most dangerous risk in psoriasis patients. Also, IL-23 inhibitors and the small molecules (Apremilast) are of choice in overweight patients, decreasing dyslipidemia, insulin resistance, and obesity. Moreover, TNF-α inhibitors reduce the risk of NAFLD.

It can be concluded that nowadays there are many therapeutic options for treating psoriasis, and novel therapies targeting specific pathophysiological mechanisms that can modulate both psoriasis and its associated conditions. However, further studies are necessary to better identify the long-term effects of these modern, biological therapies.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization AFG, RSC, DOC and CC; methodology AFG, RSC, DOC and CC; validation MP, RSC, DOC, and CC; formal analysis AFG and CC; investigation AFG and DLC; resources AFG and DLC; data curation DOC; writing—original draft preparation AFG; writing—review and editing DOC, and CC; supervision MP, DOC and CC; project administration AFG, and DLC. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Avascular Bone Necrosis in Systemic Lupus Erythematosus: An European Review Uncovering Hidden Risks, Early Diagnostic Gaps and Clinical Case Insight

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Abstract: Aseptic bone necrosis (ABN), also known as avascular necrosis (AVN), is a serious and often overlooked complication of systemic lupus erythematosus (SLE), a chronic autoimmune disease with diverse symptoms. AVN in SLE is caused by multiple factors, including blood supply disruption due to clotting disorders, chronic inflammation, and especially prolonged use of moderate to high-dose corticosteroids. This review analyzes 17 out of 62 peer-reviewed European studies (2015–2024) from the PubMed database, focusing on osteonecrosis in lupus. ABN affects between 0.8% and 33% of SLE patients, with a mean symptomatic rate of 9%, while up to 29% may be asymptomatic. The femoral head is most commonly involved. Besides corticosteroid use, other risk factors include antiphospholipid antibodies (especially anticardiolipin IgM), endogenous hypercortisolemia, hypercoagulability, dyslipidemia, and active disease. In Romania, where no national registry exists, a representative case of a 27-year-old woman with undiagnosed SLE and bilateral femoral head necrosis without steroid exposure highlights the role of autoimmune coagulopathy. Misdiagnosis of her facial rash as rosacea delayed proper identification, emphasizing the importance of clinical awareness in atypical cases.

Keywords: osteonecrosis, autoimmune disease, systemic erythematosus lupus disease

INTRODUCTION

Aseptic bone necrosis, also known as avascular necrosis (AVN), is a condition defined by the disruption of the bone's blood supply, leading to bone tissue death in the absence of infection. It is among the primary causes of disability in individuals diagnosed with systemic lupus erythematosus (SLE) [1,2].

Systemic lupus erythematosus is a prototypical connective tissue disorder of presumed autoimmune origin, exhibiting a broad spectrum of clinical manifestations, ranging from mucocutaneous involvement to life-threatening organ dysfunction [2].

Rheumatic diseases, in general, present either diagnostic challenges or management issues, and SLE is no exception. The complexity of SLE arises from two primary concerns: the diagnostic challenge posed by its diverse clinical presentations and the management difficulties associated with its multisystem involvement, compounded by a long list of complications related to both the disease itself and its treatment [3,4]. Due to the disease's aggressive nature and heterogeneous course, therapeutic interventions remain intensive, particularly in cases involving organ systems.

The complications of SLE stem not only from the disease pathophysiology but also from the treatment regimens. AVN represents a frequent complication in SLE, occurring in approximately 10–30% of patients, with the incidence depending on the clinical presentation [2,5]. AVN is a significant contributor to morbidity in lupus patients. The development of AVN in SLE is associated with various factors, including the use of corticosteroids, thromboembolic events, and potential vasculitis.

This manuscript is a review article focused on synthesizing current medical knowledge regarding aseptic bone necrosis in patients with systemic lupus erythematosus. The literature search was restricted to peer-reviewed publications.

The underlying hypothesis guiding this peer-reviewed literature analysis is that AVN in patients with SLE is not attributable to corticosteroid exposure, but rather results

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from a multifactorial pathophysiological process involving autoimmune-mediated endothelial dysfunction, prothrombotic states, and systemic inflammatory activity. Furthermore, it is hypothesized that a significant proportion of AVN cases may present before or concurrently with the diagnosis of SLE, thereby positioning osteonecrosis as a potential sentinel manifestation of an underlying undiagnosed autoimmune process. This review aims to elucidate these associations and patterns based on contemporary European evidence.

MATERIALS AND METHODS

This review was conducted through a structured search of the PubMed biomedical database, targeting peer-reviewed literature published between January 2015 and March 2024. The search strategy included combinations of the following MeSH (Medical Subject Headings) terms and keywords: “systemic lupus erythematosus,” “avascular necrosis,” “aseptic bone necrosis,” “osteonecrosis,” and “Europe.” Only studies conducted on human subjects within European populations were included to maintain epidemiological and healthcare system comparability.

Inclusion criteria were:

- Peer-reviewed original research articles or systematic reviews;
- Studies involving patients diagnosed with SLE who developed avascular or aseptic bone necrosis;
- Publications between 2015–2024;
- Geographic focus limited to European cohorts.

Out of a total of 62 publications identified on the topic of osteonecrosis in the context of lupus, 17 studies were selected for in-depth analysis based on specific predefined variables, as described below.

Data were extracted and organized into a Microsoft Excel database. The following variables were evaluated: patient age and sex; timing of SLE diagnosis relative to AVN onset; osteonecrosis staging (where available); identified risk factors (corticosteroid use, antiphospholipid antibodies, hypercoagulability, dyslipidemia, hypercortisolemia); and diagnostic modalities used (MRI vs. conventional radiography). The aim was to synthesize consistent clinical patterns and identify the multifactorial contributors to AVN in the SLE population within the European context.

Statistical analyses were performed using both descriptive and inferential techniques.

RESULTS AND DISCUSSION

This section presents the synthesized findings obtained from the systematic review. The results aim to elucidate clinical patterns, identify significant associations, and integrate the findings into the broader framework of rheumatological, orthopedic, and radiological practice across Europe. These insights are subsequently integrated into a critical discussion evaluating the implications for early detection, risk stratification, and clinical management of AVN in SLE patients.

SLE is a chronic autoimmune multisystem disease with variable incidence and prevalence across Europe (Figure 1), which may reflect differences in genetic susceptibility, environmental exposures, healthcare accessibility, and diagnostic practices [6,7].

In Romania, epidemiological data remain limited.

Figure 1: Prevalence of ABN in Europe

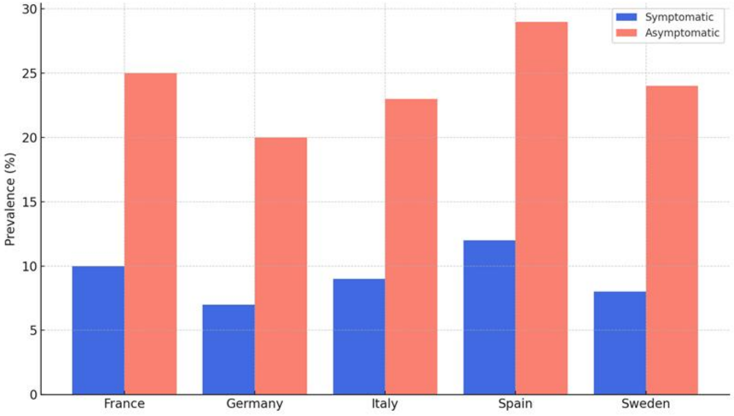

The above chart illustrates the prevalence of both symptomatic and asymptomatic aseptic bone necrosis among patients with systemic lupus erythematosus across five European countries. Notable inter-country variations are observed, with the highest prevalence reported in Spain (97/100,000 persons) and the lowest in Germany (36.7/100,000 persons) [7,8,9,11].

Based on the data from the reviewed literature, avascular necrosis in systemic lupus erythematosus exhibits a clear sex-related disparity. Approximately 85% of the reported cases occurred in female patients, reflecting the known female predominance of SLE itself. However, male patients with SLE appear to have a disproportionately higher risk of developing AVN relative to their representation in the general SLE population, suggesting a potential sex-related susceptibility to osteonecrotic complications [12,13,14,15,16,17].

The association between sex and the presence of AVN in SLE patients was assessed using the Chi-square test, appropriate for examining relationships between categorical variables. The analysis revealed a statistically significant association, indicating that although SLE predominantly affects women (~90%), male patients exhibit a disproportionately elevated risk of developing AVN relative to their population size within the SLE cohort (Figure 2).

Figure 2: Sex Distribution of AVN in SLE patients

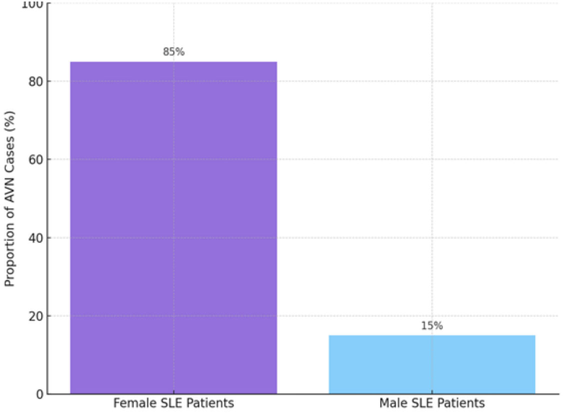

The age-related prevalence of AVN among SLE patients is shown above (Figure 3). The majority of AVN cases (52%) occurred in patients aged 20–40 years, reflecting the typical age of SLE onset and highest disease activity. This age group often coincides with early exposure to corticosteroids and aggressive immunosuppressive therapy, which may contribute to AVN risk.

Figure 3: Age distribution of AVN in SLE patients

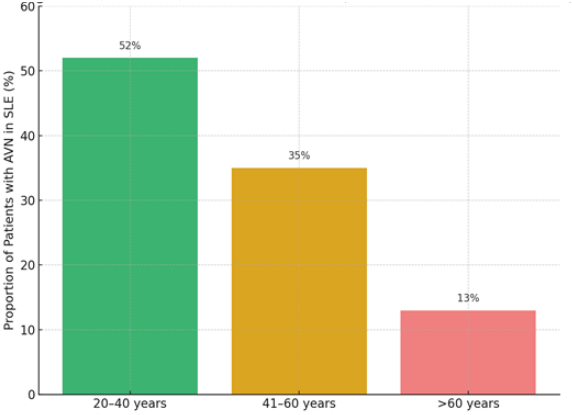

A further 35% of cases were reported in the 41–60 years group, often associated with chronicity of disease, cumulative glucocorticoid burden, and vascular comorbidities. Only 13% of AVN cases were identified in patients over 60 years, likely reflecting both survivor bias and reduced use of high-dose steroids in elderly populations [11,12,13,14,16].

Based on the findings from the reviewed articles, the four most frequently reported anatomical sites of AVN in patients with SLE were as follows: Femoral head – 68% of cases (the most commonly affected site, due to its high susceptibility to ischemic injury and limited collateral blood supply), Knee (distal femur/proximal tibia) – 14% of cases (often associated with weight-bearing stress and microvascular compromise), Humeral head (shoulder) – 10% of cases (involvement tends to occur bilaterally and may be underdiagnosed due to subclinical presentation), Talus (ankle) – 5% of cases and the remaining 3% included other locations such as the jaw (mandible), vertebral bodies, and small bones of the wrist [7,11,12,13,14,16].

These findings emphasize the need for focused imaging of high-risk joints, particularly in symptomatic or corticosteroid-exposed SLE patients.

According to synthesized data from the reviewed literature:

- 78% of patients had a confirmed diagnosis of SLE before the development of AVN, indicating that osteonecrosis is predominantly a complication of established disease, often related to corticosteroid exposure or disease-related coagulopathy.

- 22% of patients were diagnosed with SLE concurrently with AVN, suggesting that in some cases, osteonecrosis may serve as a sentinel clinical event, prompting further investigation that leads to the identification of underlying autoimmune pathology.

This emphasizes the importance of considering systemic autoimmune disorders such as SLE in the differential diagnosis of atraumatic osteonecrosis, particularly in young patients or those presenting with systemic signs [11,12,13,14,15,16,17].

Based on pooled data from the reviewed articles, the majority of systemic lupus erythematosus (SLE) patients diagnosed with AVN were identified in advanced radiologic stages (Figure 4):
- Stage I: 10% (early, MRI-specific findings)
- Stage II: 20% (mild structural changes)
- Stage III: 35% (subchondral collapse)
- Stage IV: 35% (joint space narrowing, secondary osteoarthritis)

Figure 4: Imaging stage distribution of patients

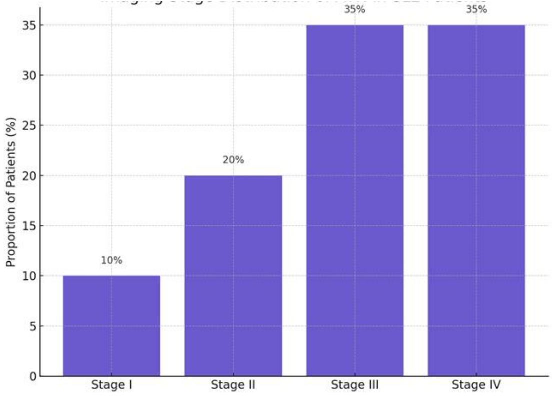

This distribution underscores the delayed detection of AVN in clinical practice, with over 70% diagnosed at stages III or IV—often beyond the window for joint-preserving interventions [11,17].

Regarding the imaging modalities employed for diagnostic purposes, the following distribution was observed among the reviewed cases: 45% of patients were diagnosed using MRI alone, underscoring its superior sensitivity in detecting early-stage AVN prior to radiographic changes, 25% of cases relied exclusively on plain radiography (X-ray), despite its limited sensitivity in early-stage AVN and potential for false-negative results in the absence of structural alterations and 30% of patients underwent both MRI and X-ray, highlighting clinical awareness of radiographic limitations and the recognized diagnostic value of MRI as a complementary tool.

These findings (Figure 5) emphasize the critical role of MRI in the early identification and accurate staging of AVN.

Figure 5: Imaging modalities used in AVN diagnosis among SLE patients

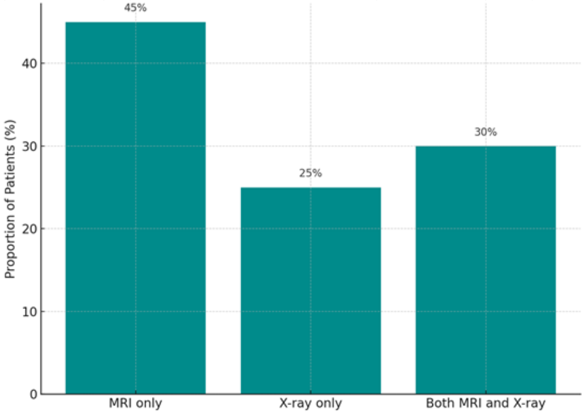

The distribution of key risk factors identified among patients with SLE who developed ABN, based on data synthesized is shown above (Figure 6).

The compiled data revealed the following distribution of risk factors:
- 76% of cases had a history of corticosteroid use, affirming its central role as the predominant modifiable risk factor for AVN.
- 48% demonstrated positivity for antiphospholipid antibodies (aPL), particularly anticardiolipin IgM, supporting the contribution of autoimmune-mediated vascular injury.

- 42% showed laboratory evidence of hypercoagulability, including elevated D-dimer levels, indicative of a prothrombotic state.
- 37% were reported with dyslipidemia, reflecting the vascular and metabolic burden in chronic autoimmune disease.
- 33% exhibited hypercortisolemia, either iatrogenic or endogenous.

These findings emphasize the multifactorial nature of AVN pathogenesis in SLE, warranting comprehensive risk stratification and early intervention strategies [7,10,11,12,13,15,17].

Figure 6: Distribution of risks factors in SLE patients with ABN

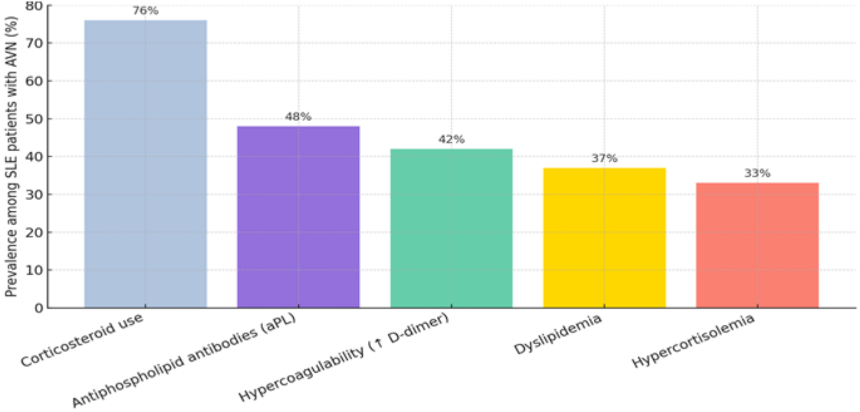

To explore the relationships between clinical variables associated with aseptic bone necrosis (ABN) in systemic lupus erythematosus (SLE), a Pearson correlation analysis was conducted (Figure 7). The following continuous variables were selected based on their pathophysiological relevance and availability in the reviewed literature:

- Corticosteroid dose (mg/day)
- D-dimer level (µg/mL) as a marker of hypercoagulability
- Imaging-based ABN stage (1–4)

Figure 7: Pearson Correlation matrix between risks factors and ABN severity

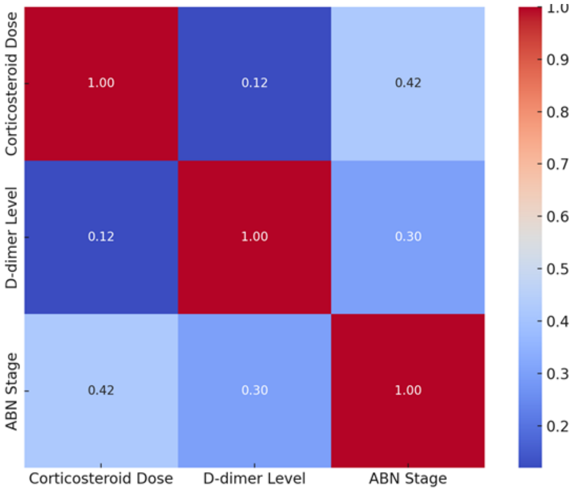

A moderate positive correlation (r ≈ 0.42) was observed between corticosteroid dose and ABN stage, indicating that higher glucocorticoid exposure is associated with more advanced osteonecrotic changes.

A weak-to-moderate correlation (r ≈ 0.30) was noted between D-dimer levels and ABN stage, suggesting a potential link between thrombotic activity and disease severity.

Minimal correlation was found between corticosteroid dose and D-dimer levels (r ≈ 0.12), reflecting the multifactorial and potentially independent contributions of these variables.

These findings support the multifactorial pathogenesis of AVN in SLE and highlight the importance of integrating both inflammatory and thrombotic parameters in clinical risk assessment.

To assess whether a correlation exists between corticosteroid dosage and the imaging-defined stages of ABN, a One-Way ANOVA test was employed.

The boxplot presented (Figure 8) demonstrates the distribution of corticosteroid doses (mg/day) among three ABN stages in patients with SLE: Stage I, Stage II, and Stage III–IV.

Figure 8: Corticosteroid dose across ABN stages (one Way ANOVA test)

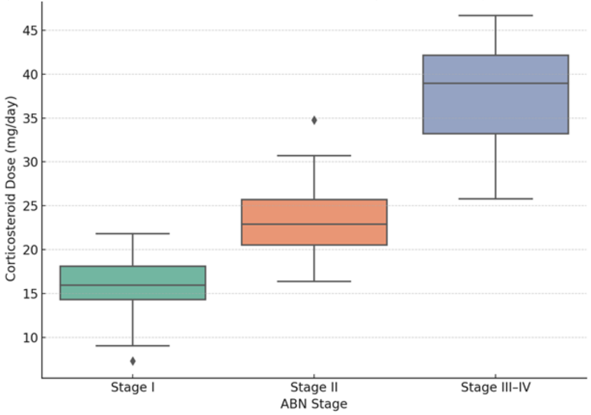

The ANOVA test produced a highly significant p-value (< 0.0001), confirming that the differences in corticosteroid exposure across the three groups are statistically significant.

These findings strongly support the hypothesis that greater cumulative corticosteroid use is associated with more advanced stages of osteonecrosis in patients with SLE.

In conclusion, the development of ABN in SLE patients reflects a multifactorial pathological process that extends beyond corticosteroid exposure. These findings underscore the necessity for a multidisciplinary approach — involving rheumatologists, radiologists, and orthopedic surgeons — to facilitate early detection, individualized risk stratification, and timely therapeutic intervention.

Following the comprehensive review of the European medical literature on ABN in SLE, we present a Romanian clinical case in order to identify and explore potential parallels between the data reported in the literature and the findings observed in a real-life patient scenario.

ROMANIAN CASE REPORT

The case is about a 27-year-old male patient who initially presented with osteonecrosis of the bilateral femoral head, in the context of previously undiagnosed systemic lupus erythematosus (SLE). The patient sought consultation due to persistent bilateral hip joint pain, which had been progressively worsening over the past month.

Medical History

Approximately one year ago, he had been diagnosed with rosacea after presenting with erythema on the nasal pyramid and cheeks. The erythema was identified as a typical rash associated with rosacea, and he was treated with topical medications. However, the erythema did not resolve with the prescribed treatment, prompting further investigation. The patient had no significant past medical history beyond these episodes.

The patient denied alcohol consumption and was a non-smoker. He had not been on corticosteroids or any other medications prior to this event and had not taken any traditional or alternative medicine. Additionally, there was no significant family history of connective tissue diseases or other autoimmune disorders.

Orthopedic Consultation

Upon presentation to the orthopedic department of our hospital, the patient described the bilateral hip pain as deep and localized primarily to the groin and gluteal regions. Initially intermittent, the pain had become progressively persistent, worsening with ambulation, prolonged standing, and stair climbing, raising suspicion of significant intra-articular pathology. He reported marked restriction in hip mobility, particularly during internal rotation and abduction, along with morning stiffness lasting more than 30 minutes, indicative of advanced joint involvement.

Physical examination revealed significant pain on passive hip mobilization, especially during flexion and internal rotation. Orthopedic provocative tests (FABER, log roll test) were positive bilaterally, further supporting the suspicion of intra-articular pathology.

The patient also reported occasional swelling in other joints, particularly in the wrists and knees.

The suspected diagnosis at presentation was: Osteonecrosis of the bilateral femoral heads, under etiological observation, as well as facial erythema, which was also under etiological observation.

Paraclinical Investigations

Laboratory Tests

Routine laboratory tests were performed, which revealed mildly elevated inflammatory markers (moderately elevated erythrocyte sedimentation rate (ESR) of 78 mm in the first hour), hemoglobin level was 11.1 g/dL, and both her white blood cell count along with C-reactive protein were within normal limits. She presented with thrombocytopenia, with a platelet count of less than 100,000/mm, Low C3 and C4 levels (below 60 for C3 and below 15 for C4). The routine coagulation tests, including APTT, INR, and prothrombin time (Quick), were within normal limits and showed no abnormalities.

Radiological Investigations

The antero-posterior X-ray of the pelvis reveals bilateral femoral head involvement consistent with stage III (FICAT-Arlet) osteonecrosis, with loss of sphericity of both femoral heads, absence of femoral head collapse into the acetabulum, and no significant joint space narrowing (Figure 9).

Figure 9: Hip X-ray

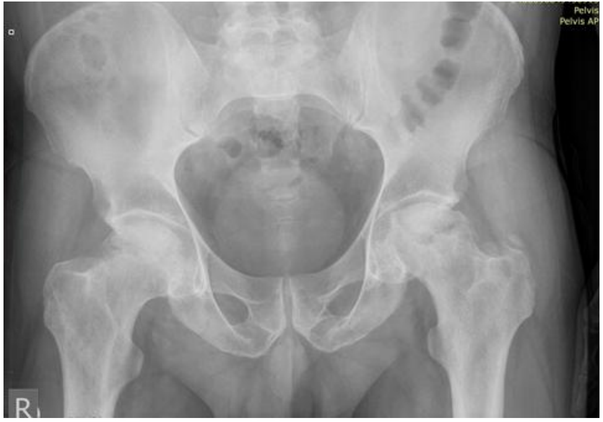

To further evaluate the extent of the lesion, an MRI of the hip was performed (Figures 10a,10b, 11), which revealed characteristic findings of osteonecrosis of both femoral heads, including areas of bone marrow edema and subchondral collapse.

Figure 10: a – MRI of the right hip; b – MRI of the left hip

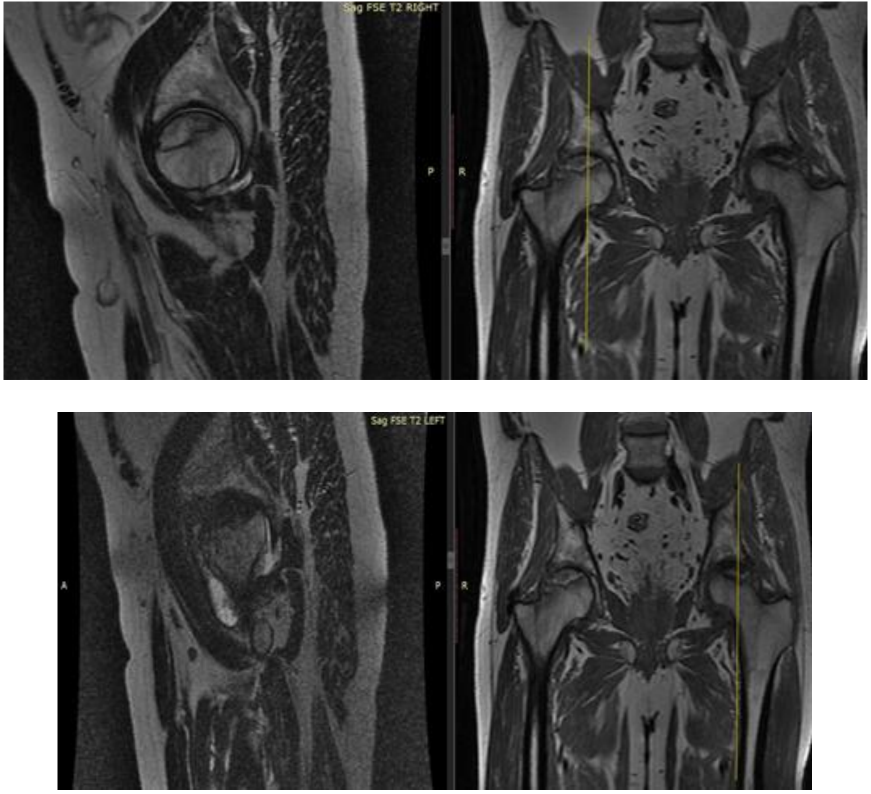

Figure 11: Hip X-ray

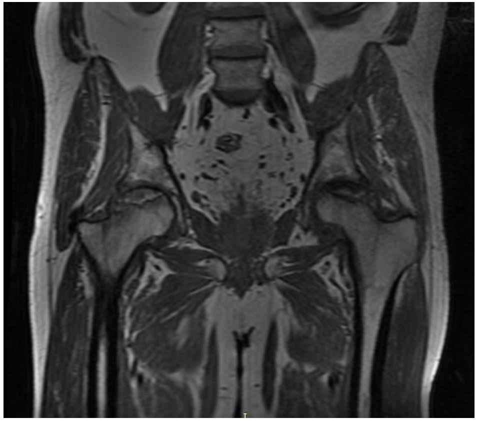

A CT scan of the hips was available from previous investigations performed by the patient, as shown above (Figure 12).

Pelvic CT reveals bilateral femoral head osteonecrosis consistent with ARCO stage IIIA, characterized by collapse of the subchondral bone, loss of femoral head sphericity, and crescent sign of the hip joints.

Figure 12: CT scan of the hip

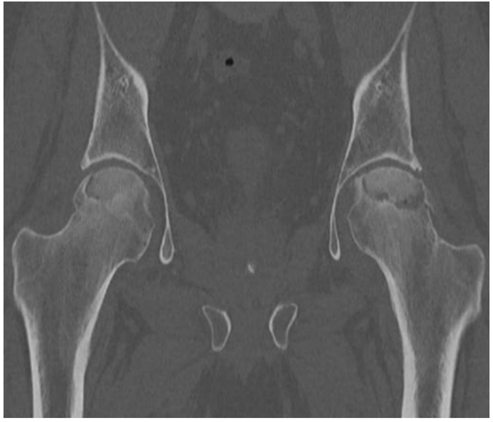

To exclude other potential diagnoses and perform a differential diagnosis, several conditions were considered, including:

1. Infectious etiologies such as tuberculosis and chronic osteomyelitis.
2. Sickle cell disease due to its potential association with avascular necrosis.
3. Hypercoagulable states such as antiphospholipid syndrome, which may predispose to AVN.
4. Systemic vasculitis that could cause both joint involvement and AVN.
5. Rheumatoid arthritis with associated joint damage and systemic manifestations.
6. Other connective tissue diseases, such as mixed connective tissue disease (MCTD) or dermatomyositis, can present with musculoskeletal involvement.

As part of the diagnostic workup and given the elevated ESR and the presence of osteonecrosis lesions, both dermatologic and rheumatologic consultations were sought, and additional tests were performed to further evaluate these differential diagnoses.

Dermatological Consultation

Given the patient's positive history of erythema and the unusual persistence of the rash, a dermatology consult was requested. The dermatologic examination revealed a butterfly-shaped erythema over the cheeks and the bridge of the nose, a classic sign of systemic lupus erythematosus. In addition to the skin findings, the patient reported arthralgia with an inflammatory character, particularly in the wrists, knees, and shoulders. Based on these findings, the dermatologist suggested a referral to a rheumatologist for further evaluation of a possible autoimmune etiology.

Rheumatologic Evaluation

Considering the clinical findings, including the butterfly-shaped erythema, a history of pregnancy loss, and joint involvement, the rheumatologist ordered further tests to assess for autoimmune disease. Additionally, the patient's coagulation profile showed a potential underlying thrombotic risk.

The following tests were conducted, and the results were as follows:

- Antinuclear antibody (ANA): Positive at a high titer of 1:1280, indicative of an autoimmune process.
- Anti-dsDNA (double-stranded DNA) antibodies: Positive at a titer of 120 IU/mL, confirming the diagnosis of systemic lupus erythematosus (SLE).
- Complement levels (C3, C4): Both C3 and C4 were significantly reduced, with C3 at 50 mg/dL (normal range: 90-180 mg/dL) and C4 at 10 mg/dL (normal range: 20-50 mg/dL), which is typical of active SLE.
- Anti-Sm (anti-Smith) antibodies: Positive at 25 U/mL, further supporting the diagnosis of lupus.
- Antiphospholipid antibody panel: Positive for both anticardiolipin antibodies (IgG) at 40 GPL U/mL (normal range: <10 GPL U/mL) and lupus anticoagulant, consistent with antiphospholipid syndrome (APS), which is frequently associated with SLE.

These results helped confirm the diagnosis of systemic lupus erythematosus with associated antiphospholipid syndrome.

Diagnosis

The clinical presentation, laboratory findings, and positive autoimmune tests confirmed the diagnosis of Systemic lupus erythematosus with associated antiphospholipid syndrome (APS). The diagnosis of osteonecrosis of the bilateral femoral head was also established as a complication of the underlying SLE, potentially exacerbated by the presence of antiphospholipid antibodies.

Treatment and Management

The patient was started on hydroxychloroquine at a standard dose of 200 mg twice daily to manage the underlying lupus. Additionally, prednisone was initiated at a low dose of 10 mg per day to control systemic inflammation and prevent further disease progression.

Given the presence of antiphospholipid antibodies and the history of pregnancy loss, an antiplatelet drug was prescribed at a dose of 75 mg daily to reduce the risk of thrombosis. As the patient tested positive for lupus anticoagulant, coumarin anticoagulant (Trombostop) was considered for anticoagulation therapy, with an initial dose of 4 mg daily, adjusted according to the patient's INR to maintain a therapeutic range of 2.0–3.0.

The patient was referred to orthopedic surgery for definitive management of bilateral femoral head osteonecrosis and underwent staged total hip arthroplasty procedures (Figure 13).

Figure 13: Intraoperative imaging of femoral head

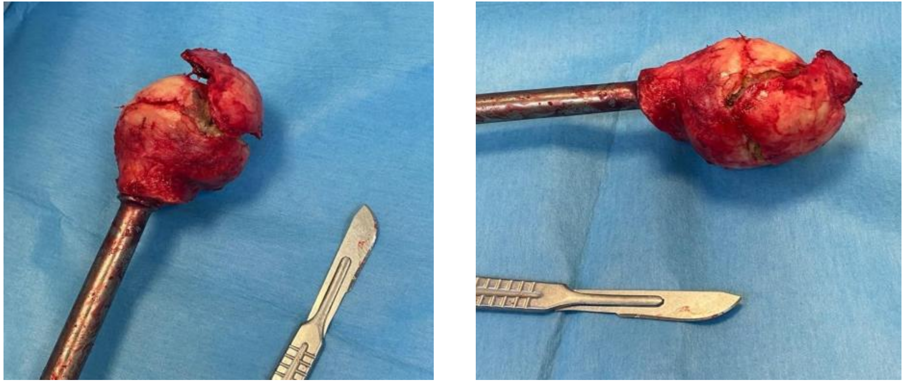

Postoperatively, pelvic x-ray demonstrates satisfactory positioning and alignment of bilateral total hip prostheses, with no immediate complications identified (Figure 14).

The patient was also was referred to a structured physiotherapy program for postoperative rehabilitation.

Figure 14: Postoperative X-ray of the hip

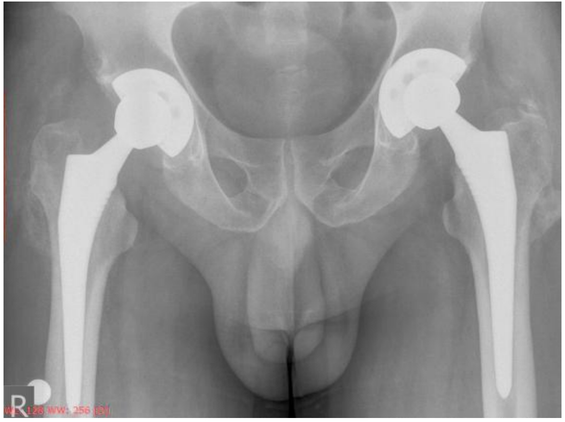

Follow-up and Outcome

The patient is currently being followed up regularly in both the rheumatology and orthopedic clinics. Her lupus symptoms are under control with the current treatment regimen, and she reports significant improvement in her joint pain.

At the six-month postoperative follow-up, the patient demonstrated significant functional recovery, with restoration of full range of motion in both hip joints and only minimal residual pain during high-demand activities.

The patient is also being monitored for any potential long-term complications related to the use of corticosteroids, such as bone density loss.

CONCLUSION

This case highlights the importance of considering systemic lupus erythematosus in the differential diagnosis of young patients presenting with unexplained osteonecrosis, particularly when accompanied by characteristic dermatologic signs and autoimmune findings. Early diagnosis and appropriate management of SLE and associated complications, such as aseptic necrosis and antiphospholipid syndrome, are crucial to improving patient outcomes and preventing further morbidity.

The uniqueness of this case lies in the fact that the patient's presentation began with bilateral femoral head osteonecrosis, which was the first manifestation of the disease. The diagnosis of SLE was made later, after the development of osteonecrosis. The delay in diagnosis, compounded by an incorrect dermatologic diagnosis, led to the inability to initiate early treatment, which is crucial for a better prognosis in autoimmune diseases like SLE.

This case also underscores the necessity of close collaboration between dermatologists, rheumatologists, and orthopedic specialists.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, L.I.S. and C.A.; methodology, L.I.S. and C.A.; software, L.I.S.; validation, A.C. and D.G.M.; investigation, L.I.S.; resources, C.C. and L.I.S..; data curation A.C. and L.I.S.; writing—original draft preparation, L.I.S.; writing—review and editing, L.I.S. and A.C.; visualization, C.C. and D.G.M.; supervision, C.C. and D.G.M.; project administration, L.I.S. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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REVIEW

The Importance of Screening and Early Diagnosis for a Good Outcome in Patients with Colorectal Cancer

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Abstract: Colorectal cancer (CRC) ranks third worldwide in terms of morbidity and second in mortality. In Romania, CRC represents the second leading cause of malignancy, accounting for 13.3% of all diagnosed cancers and associated with a five-year survival rate close to 50%. Despite the availability of effective CRC screening programs proven to reduce incidence and mortality, low participation rates contribute to a high occurrence of advanced disease complications. As a result, bowel obstruction develops in approximately 25% of colorectal cancer cases, significantly worsening patient outcomes. This review aims to highlight the role of screening in reducing the incidence of such complications and to assess the outcomes associated with surgical management of malignant bowel obstruction. A comprehensive review of current literature was conducted, focusing on the incidence, clinical presentation, and management of intestinal obstructions in CRC patients. Emphasis was placed on studies evaluating screening programs, risk factors, and surgical interventions. Data indicate a continuous rise in emergency presentations due to obstructive CRC, correlating with low screening uptake. Early detection through organized screening significantly lowers the risk of obstruction and improves outcomes. Enhanced screening programs and early identification of high-risk individuals are crucial in preventing advanced CRC complications. Timely diagnosis not only reduces emergency surgical interventions but also improves prognosis and overall survival rates.

Keywords: colorectal cancer; screening programs; bowel obstruction; surgical management; early detection

INTRODUCTION

Colorectal adenocarcinoma is a significant global health concern, affecting approximately one million individuals annually and causing nearly 500,000 deaths each year [1-2]. Although rare before the age of 40, its incidence sharply increases after age 45, with most cases diagnosed beyond the age of 50. In Romania, colorectal cancer ranks second among all cancers, accounting for about 13% of cases and a five-year survival rate close to 50% [3-5]. Despite a declining population, Romania saw an increase in cancer incidence from 2011 to 2018, reflecting challenges in healthcare access and prevention [6].

Colorectal cancer is frequently diagnosed at advanced stages due to insufficient screening programs and common risk factors such as smoking, poor diet, and lack of physical activity [7-8]. Additionally, Romania's healthcare system faces significant challenges, including financial and logistical barriers, which hinder early detection and treatment [9-12]. Some studies indicate that approximately 40% of cases occur in the right colon, 31% in the left colon, and 29% in the rectum [13].

The rising incidence of early-onset colorectal cancer has led to changes in screening guidelines, with organizations such as the American Cancer Society lowering the recommended starting age from 50 to 45 years. This shift aims to enhance early detection and improve patient outcomes, though its overall impact remains under evaluation. The optimal screening approach, whether through non-invasive tests followed by confirmatory colonoscopy or direct colonoscopic screening, continues to be debated [14-15].

Effective screening methods such as colonoscopy, flexible sigmoidoscopy, CT colonography, capsule endoscopy, and stool-based detection tests play a crucial role in identifying and removing precancerous lesions, ultimately reducing cancer-related mortality. This paper asserts the importance of increasing awareness and implementing systematic screening programs to curb colorectal cancer incidence and mortality, potentially preventing severe complications such as bowel obstructions [16-19].

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MATERIALS AND METHODS

This narrative review systematically examines the existing literature concerning colorectal cancer screening, diagnosis, and the management of acute complications. A comprehensive search was conducted across four prominent electronic databases, namely PubMed, NCBI, Google Scholar, and MDPI. Relevant articles were identified using targeted keywords, such as "colorectal cancer", "screening", "early diagnosis", and "treatment." To ensure the inclusion of contemporary advancements, priority was given to recent publications, primarily from the past five years. Only studies specifically addressing methods for colorectal cancer screening, diagnostic processes, and therapeutic interventions for advanced stages were selected for detailed evaluation in this review.

RESULTS

Screening Methods

Colorectal cancer (CRC) screening is a critical tool in the early detection and prevention of the disease, significantly lowering both incidence and mortality rates. Several screening strategies are available, each with its own benefits and limitations (Figure 1). As technology advances, newer approaches are being incorporated to enhance detection accuracy, patient compliance, and accessibility. The current landscape of CRC screening includes invasive procedures such as colonoscopy, non-invasive stool-based tests, imaging modalities, molecular biomarker assessments, and emerging techniques such as artificial intelligence (AI) and liquid biopsies [20].

Figure 1: Screening guidelines

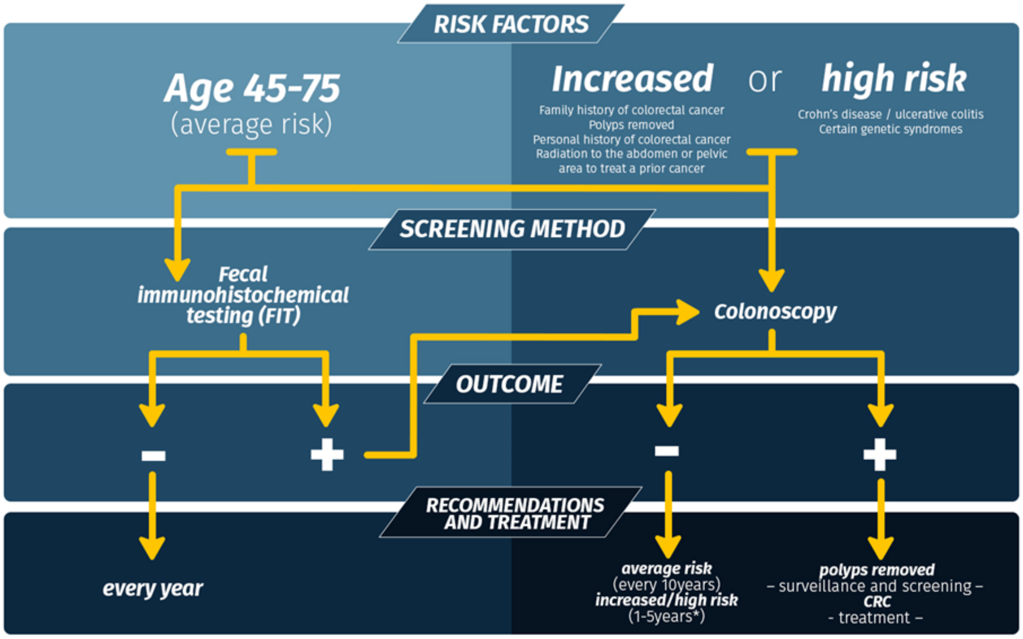
RISK FACTORS
Age 45-75
(average risk)
Increased
Family history of colorectal cancer
Polyps removed
Personal history of colorectal cancer
Radiation to the abdomen or pelvic area to treat a prior cancer
or
high risk
Crohn's disease / ulcerative colitis
Certain genetic syndromes
SCREENING METHOD
Fecal immunohistochemical testing (FIT)
Colonoscopy
OUTCOME
-
+
-
+
RECOMMENDATIONS AND TREATMENT
every year
average risk
(every 10years)
increased/high risk
(1-5years*)
polyps removed
– surveillance and screening –
CRC
- treatment –

**family history of colorectal cancer – screening starting before age 45*
polyps removed – 3 years
personal history of colorectal cancer - 1 year after surgery
radiation to the abdomen or pelvic area to treat a prior cancer – after age 35 or between 3 to 10 years
Crohn's disease or ulcerative colitis – 1 to 3 years
certain genetic syndromes - begin at a young age, more frequently depending on genetic syndrome

Colonoscopy remains the most effective and widely used screening method due to its ability to directly examine the entire colon and rectum. This procedure enables the detection and removal of precancerous polyps, thereby preventing progression to malignancy. With a sensitivity exceeding 95% for advanced neoplasia, colonoscopy is a highly reliable diagnostic and preventive tool. However, its drawbacks include the need for extensive bowel preparation, sedation, and the expertise of trained specialists. Additionally, although rare, complications such as perforation and post-procedural bleeding can occur. Given these factors, adherence to colonoscopy screening remains suboptimal, particularly in average-risk individuals. Nevertheless, it is strongly recommended for high-risk populations, such as those with a personal or family history of CRC or genetic syndromes like Lynch syndrome and familial adenomatous polyposis [21-22].

Flexible sigmoidoscopy is another visualization-based screening tool that examines only the rectum and the lower part of the colon. While it is less invasive and does not require sedation, it is limited in its ability to detect right-sided lesions in the proximal colon. Despite this limitation, studies have shown that sigmoidoscopy reduces CRC incidence and mortality, particularly for left-sided tumors. It is often used in conjunction with stool-based tests to improve overall detection rates [23].

Capsule endoscopy is another innovation being explored for CRC screening. This technique involves swallowing a small, camera-equipped capsule that captures images of the gastrointestinal tract as it moves through the digestive system. Unlike traditional colonoscopy, capsule endoscopy does not require sedation and is non-invasive. However, current challenges include incomplete visualization of the colon, difficulty detecting small lesions, and limitations in image interpretation. Research is focused on enhancing camera resolution, battery life, and image processing technology to improve its effectiveness as a screening tool [24-25].

Computed tomography (CT) colonography, also referred to as virtual colonoscopy, is a radiological screening technique that generates three-dimensional images of the colon and rectum using low-dose CT scans. This non-invasive method eliminates the need for sedation and is generally more tolerable for patients. CT colonography has demonstrated high sensitivity for detecting polyps larger than 10 mm but is less effective for smaller lesions. Unlike conventional colonoscopy, it does not permit biopsy or polyp removal, requiring follow-up colonoscopy for confirmation and intervention when abnormalities are detected. Radiation exposure is another factor that must be considered, particularly for younger individuals undergoing repeated screenings [26].

Blood-based testing has emerged as a promising direction in colorectal cancer (CRC) screening, providing a minimally invasive alternative to traditional methods. These assays aim to detect tumor-associated molecular alterations in circulation, offering improved patient compliance, especially among those hesitant to undergo invasive procedures such as colonoscopy. Among the most studied blood-based tools are circulating tumor DNA (ctDNA) tests, which identify somatic mutations, copy number alterations, and epigenetic changes shed by tumor cells into the bloodstream. ctDNA-based screening has demonstrated high specificity for CRC detection and holds potential for both early diagnosis and post-treatment surveillance. Despite these advances, the sensitivity of ctDNA for detecting early-stage CRC and precancerous adenomas remains limited [27]. This poses a challenge for its application as a standalone screening modality, particularly for detecting lesions before malignant transformation. Additional liquid biopsy strategies, including the analysis of circulating tumor cells (CTCs), exosomal RNA, and methylated DNA, are under active investigation. These approaches aim to enhance detection sensitivity by capturing a broader spectrum of tumor-derived signals, though most remain in the experimental phase and require extensive validation in large-scale prospective studies before being adopted into routine practice. Parallel to blood-based diagnostics, biomarker-driven innovations in stool and urine-based screening have gained traction. Advances in genomics and proteomics have led to the identification of novel molecular signatures associated with CRC, including aberrantly methylated DNA sequences, microRNAs, and tumor-specific proteins. These biomarkers have contributed to the development of next-generation assays that improve the performance of non-invasive tests. The multitarget stool DNA test (sDNA-FIT), which combines fecal immunochemical testing with molecular markers for DNA mutations and methylation, exemplifies this approach and has already been integrated into clinical practice. Nevertheless, ongoing research seeks to refine these assays further, expand biomarker panels, and improve sensitivity and specificity—particularly for early-stage lesions [27-28].

Stool-based screening tests offer an alternative for individuals who prefer non-invasive methods. The fecal immunochemical test (FIT) has largely replaced the traditional guaiac-based fecal occult blood test (gFOBT) due to its improved specificity and sensitivity. FIT detects human hemoglobin in stool samples, reducing the risk of false positives from dietary or upper gastrointestinal sources. This test is typically performed annually and has been associated with a reduction in CRC-related mortality. However, its lower sensitivity for detecting advanced adenomas necessitates frequent retesting. A more advanced stool-based approach, the multitarget stool DNA test (sDNA-FIT), combines FIT with molecular markers to detect genetic mutations and epigenetic changes linked to CRC. This method enhances sensitivity beyond FIT alone and improves the detection of precancerous lesions. However, it has a higher false-positive rate and requires a confirmatory colonoscopy for abnormal findings [29-31].

Artificial intelligence (AI) is increasingly being applied to CRC screening, particularly in colonoscopy and imaging-based methods. AI-enhanced colonoscopy utilizes deep learning algorithms to analyze real-time endoscopic images and improve the detection of adenomas. Studies have demonstrated that AI-assisted colonoscopy increases the identification of subtle mucosal abnormalities, reducing the risk of missed lesions. AI is also being integrated into stool and blood-based screening tests, where machine learning models optimize the interpretation of molecular signatures to improve accuracy. Additionally, computational models incorporating genetic, environmental, and clinical data are being developed to personalize CRC risk assessment and screening recommendations [32-33].

Despite the variety of available screening methods, adherence to CRC screening guidelines remains a significant concern, particularly in populations with limited healthcare access. Socioeconomic barriers, healthcare infrastructure, and patient awareness all play critical roles in screening participation. Efforts to improve accessibility include at-home testing kits, such as FIT and stool DNA tests, which allow individuals to collect and submit samples without visiting a healthcare facility (Figure 2). Increasing public awareness, healthcare provider engagement, and offering personalized screening options based on individual risk factors are essential strategies for enhancing participation in screening programs [34].

Diagnosis Methods

Colorectal cancer (CRC) diagnosis relies on a combination of clinical evaluation, laboratory testing, endoscopic procedures, and imaging techniques to confirm malignancy and determine disease stage. Despite advancements in screening and early detection, a significant proportion of patients are still diagnosed at advanced stages, often presenting with complications such as bowel obstruction. Colonoscopy remains the gold standard for CRC diagnosis, allowing for direct visualization, biopsy, and removal of suspicious lesions during a single procedure. Late-stage detection is associated with poorer outcomes, underscoring the need for timely and effective diagnostic strategies [35].

Imaging modalities, including contrast-enhanced CT scans, magnetic resonance imaging (MRI), and positron emission tomography (PET) scans, play a critical role in staging CRC and evaluating tumor spread. These techniques help determine the extent of local invasion, lymph node involvement, and distant metastases. Accurate staging is essential in guiding treatment decisions, including surgical resection, chemotherapy, radiation therapy, or a combination of modalities for advanced cases. Imaging is particularly important for assessing resectability in metastatic disease, as well as monitoring treatment response in patients undergoing systemic therapy [36].

Figure 2: Screening methods

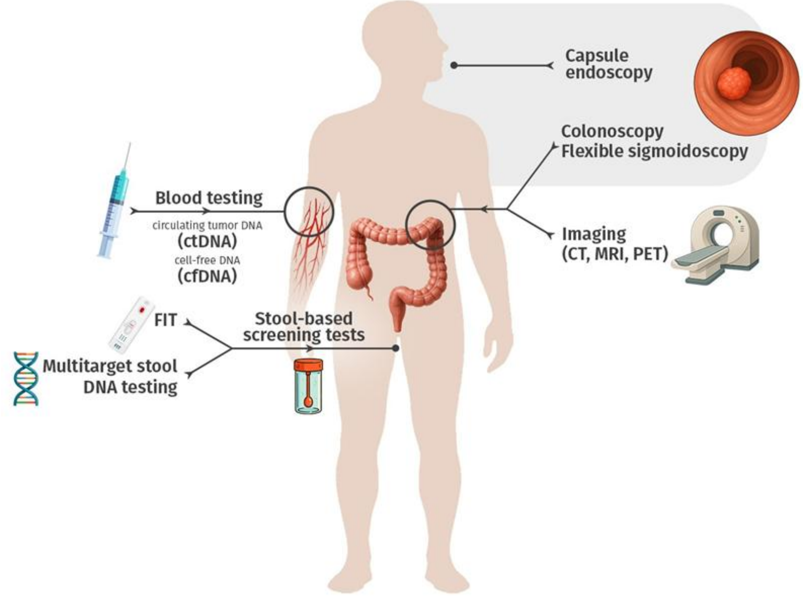

Despite these diagnostic advancements, many CRC cases are still identified at later stages when symptoms become severe. One of the most common emergency presentations of advanced CRC is bowel obstruction, occurring in approximately 20% of cases. This complication results from tumor-induced narrowing of the intestinal lumen, leading to symptoms such as severe abdominal pain, bloating, nausea, vomiting, and the inability to pass stool or gas. Bowel obstruction often necessitates urgent medical intervention, with many patients requiring emergency surgery to relieve the blockage and resect the affected segment of the bowel. Compared to elective surgery for early-stage CRC, emergency surgical interventions are associated with increased postoperative complications, prolonged hospital stays, and higher mortality rates. The occurrence of bowel obstruction as an initial manifestation of CRC highlights the ongoing challenges in early detection, particularly among individuals who do not undergo regular screening. Patients presenting with obstruction often have advanced tumors that limit treatment options, negatively impacting prognosis. Research has shown that individuals diagnosed with obstructive CRC have lower survival rates compared to those diagnosed through routine screening, emphasizing the need for widespread and consistent preventive measures [37-38].

Treatment

For patients diagnosed with early-stage CRC, particularly stage I and stage II, surgical resection is the primary treatment. In stage I, where the tumor is confined to the bowel wall without lymph node involvement, removal of the affected segment of the colon with regional lymphadenectomy is typically curative. The preferred surgical approach may be open or laparoscopic colectomy, with the latter offering benefits such as reduced postoperative recovery time and lower complication rates. In stage II disease, the cancer has penetrated the muscular layer of the colon but has not spread to regional lymph nodes. Surgery remains the standard of care, but adjuvant chemotherapy may be considered in cases where high-risk pathological features are present, including T4 tumors, lymphovascular invasion, perineural invasion, poor differentiation, or inadequate lymph node sampling. The decision to administer chemotherapy is individualized, considering both the potential survival benefit and the risk of treatment-related toxicity [39-41].

Management of stage III CRC, where the cancer has spread to regional lymph nodes, involves a multimodal approach integrating surgery and systemic chemotherapy. The primary surgical treatment is colectomy with complete mesocolic excision and lymphadenectomy to achieve optimal oncologic outcomes. Adjuvant chemotherapy is recommended in nearly all cases to eliminate micrometastatic disease and reduce recurrence risk. Standard chemotherapy regimens include FOLFOX (5-fluorouracil, leucovorin, and oxaliplatin) and CAPOX (capecitabine and oxaliplatin), with treatment duration typically spanning six months. In patients who cannot tolerate oxaliplatin, fluoropyrimidine monotherapy remains an option, though with slightly lower efficacy [39-40].

Stage IV CRC is characterized by distant metastases, most commonly involving the liver, lungs, or peritoneum. In these cases, systemic therapy is the cornerstone of treatment, intending to prolong survival and maintain quality of life. Chemotherapy regimens such as FOLFOX and FOLFIRI (5-fluorouracil, leucovorin, and irinotecan) are frequently utilized, often in combination with targeted agents. Bevacizumab, an anti-VEGF monoclonal antibody, is commonly added to chemotherapy to inhibit tumor angiogenesis, particularly in patients with RAS-mutant tumors. In contrast, EGFR inhibitors such as cetuximab and panitumumab are used in RAS-wild type tumors, demonstrating improved response rates when combined with chemotherapy. Molecular profiling, including assessment of KRAS, NRAS, and BRAF mutations, is crucial in guiding treatment selection, as tumors harboring certain mutations exhibit resistance to EGFR-targeted therapies. Additionally, immunotherapy has emerged as a novel treatment option for patients with microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR) tumors, with checkpoint inhibitors such as pembrolizumab and nivolumab demonstrating promising efficacy in this subset of patients [39-40, 42-43].

A significant proportion of CRC cases, particularly those diagnosed at advanced stages, present with bowel obstruction, a life-threatening complication resulting from tumor-induced luminal narrowing. This condition manifests as severe abdominal pain, nausea, vomiting, and bowel distension, often necessitating urgent medical intervention. The management of bowel obstruction is guided by the patient’s overall prognosis, performance status, and treatment goals. In patients with potentially resectable disease and good functional status, surgical intervention, such as resection of the obstructing tumor or creation of a diverting ostomy, may be performed to restore bowel function and facilitate further oncologic treatment. However, in patients with widespread metastases or poor surgical candidacy, non-surgical approaches such as endoscopic stenting are preferred. Self-expanding metal stents (SEMS) can be placed to relieve obstruction and maintain luminal patency, serving as either a bridge to surgery in operable patients or a palliative measure in those with unresectable disease. While SEMS placement offers symptom relief with a lower risk of perioperative morbidity, potential complications include stent migration, re-obstruction, and perforation, particularly in patients receiving anti-angiogenic therapy [44-46].

Medical management plays an essential role in symptom control for patients with bowel obstruction. Corticosteroids may be administered to reduce tumor-associated edema and improve bowel patency. Antiemetics, including dopamine antagonists and serotonin receptor antagonists, are used to alleviate nausea and vomiting, while opioid and non-opioid analgesics help manage pain. In cases where bowel function cannot be restored, a venting gastrostomy tube may be placed to decompress the stomach and relieve symptoms of persistent vomiting. Total parenteral nutrition (TPN) may also be considered for select patients to prevent malnutrition when enteral feeding is not feasible. Given the complex nature of malignant bowel obstruction, a multidisciplinary team approach, including oncology, gastroenterology, surgery, and palliative care specialists, is necessary to ensure comprehensive management tailored to the patient's individual needs [47].

DISCUSSION

Colorectal cancer (CRC) remains a major health challenge despite advances in screening, diagnosis, and treatment. A key issue is the persistent late-stage diagnosis, which is often associated with complications such as malignant bowel obstruction. The need for improved screening adherence and the integration of novel technologies to enhance early detection is evident, as early intervention significantly improves survival outcomes. However, real-world implementation of screening recommendations remains suboptimal due to accessibility issues, patient reluctance, and limitations of existing tests.

The effectiveness of CRC screening depends on balancing sensitivity, specificity, patient compliance and healthcare system feasibility. While colonoscopy is the most definitive method, its invasiveness and preparation requirements often deter patients. Non-invasive alternatives like fecal immunochemical tests (FIT) and stool DNA testing provide practical options but require frequent testing and have limitations in detecting precancerous lesions. CT colonography offers another alternative but lacks the ability to remove polyps during the procedure. The future of CRC screening may lie in risk-adapted strategies that personalize screening intervals and methods based on genetic predisposition, lifestyle factors, and emerging biomarkers.

A significant portion of CRC cases are detected at advanced stages, often when symptoms such as bowel obstruction arise. These cases require urgent medical intervention, typically involving surgery or endoscopic stenting. While self-expanding metal stents (SEMS) are increasingly used for temporary relief, their risks, including perforation and migration, necessitate careful patient selection.

Addressing late-stage CRC diagnosis requires increased awareness of early warning signs, improved access to screening programs, and adherence to recommended screening guidelines. Healthcare providers play a crucial role in recognizing potential symptoms and encouraging high-risk individuals to undergo regular screening. Strategies to improve early detection include expanding non-invasive screening options, utilizing risk-based stratification models to identify individuals at greater risk, and integrating emerging technologies such as liquid biopsy and artificial intelligence-assisted diagnostics. By strengthening early detection efforts, the burden of late-stage CRC and its associated complications, including bowel obstruction, can be significantly reduced, leading to better patient outcomes and improved survival rates.

Disparities in CRC outcomes highlight the need for broader access to screening and timely treatment. Socioeconomic barriers, healthcare infrastructure disparities, and patient education gaps contribute to late-stage diagnoses. Efforts to integrate artificial intelligence (AI) in colonoscopy and risk prediction models could enhance detection accuracy and optimize screening protocols. AI-assisted imaging analysis has demonstrated the ability to improve adenoma detection rates, reducing missed lesions and potentially lowering CRC incidence.

The treatment of CRC is continually evolving, with research focusing on optimizing multimodal therapeutic strategies to improve survival and quality of life. While surgery remains the foundation for early-stage disease, systemic therapy plays a critical role in advanced CRC, particularly with the integration of targeted agents and immunotherapy. The management of complications such as malignant bowel obstruction requires a patient-centered approach, balancing aggressive interventions with supportive care to maintain comfort and dignity. Ongoing clinical trials continue to explore novel therapeutic approaches, including next-generation targeted therapies and personalized medicine strategies, which aim to refine treatment paradigms and improve outcomes for patients diagnosed with colorectal cancer.

CONCLUSION

While significant progress has been made in CRC screening, diagnosis and treatment, challenges remain in optimizing early detection, improving treatment outcomes for advanced-stage disease and addressing disparities in care. A multidisciplinary approach integrating surgery, systemic therapy and supportive care remains crucial in managing CRC across different disease stages. Ongoing research into AI, molecular profiling and liquid biopsies holds promise for further refining CRC management strategies and improving patient outcomes. Continued efforts to enhance screening adherence, personalize treatment approaches and expand access to novel therapies will be key in reducing the global burden of colorectal cancer.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization- M.T., J.C. and B.I.; methodology - M.T.; investigation - M.T., J.C. and B.I.; writing of the manuscript- M.T. and J.C.; review, editing - M.T., J.C. B.I. and H.S; data curation - J.C. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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REVIEW

Multiple Intestinal Neuroendocrine Tumors: A Literature Review and Case Report

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Abstract: Neuroendocrine tumors (NETs) are a group of rare malignancies characterized by heterogeneous clinical and biological presentations. Their estimated annual incidence ranges from 3 to 6 cases per million inhabitants, with a notable rise in prevalence due to improved diagnostic capabilities and medical awareness. Gastrointestinal NETs predominantly affect the small intestine (~45%), followed by the rectum, appendix, colon, and stomach. Despite advancements in imaging modalities such as CT, MRI, and somatostatin receptor-based PET-CT scans, the diagnosis of small intestinal NETs remains challenging due to nonspecific symptoms, frequently leading to delayed recognition until advanced disease stages. Surgical resection with comprehensive lymphadenectomy remains the cornerstone of curative management for localized disease, offering improved survival outcomes when R0 resection is achieved. Nevertheless, recurrence rates remain high, underscoring the critical importance of postoperative surveillance. This review provides a comprehensive synthesis of recent literature regarding the epidemiology, clinical manifestations, diagnostic methods—including imaging and immunohistochemical markers—and therapeutic approaches, particularly emphasizing surgical management strategies. A clinical case example is integrated into the review to illustrate the real-world complexity and challenges in the diagnosis, treatment, and follow-up of patients with small intestinal NETs.

Keywords: multiple gastrointestinal neuroendocrine tumors, small intestine, nonspecific abdominal pain

INTRODUCTION

Gastrointestinal neuroendocrine tumors (NETs) are rare, slow-growing tumors characterized by specific histological, biological, and clinical features. These tumors have shown a significant increase in incidence and prevalence over recent decades, reaching an increase of over 100% in the past 40 years, due to advancements in diagnostic methods and increased medical awareness [1-6]. Gastrointestinal NETs represent an important subtype of neuroendocrine tumors, affecting various segments of the gastrointestinal tract, as follows: the small intestine (45%), rectum (20%), appendix (16%), colon (11%), and stomach (7%) [7].

One of the most notable characteristics of these tumors is their predominant setting in the small intestine, particularly in the terminal ileum, approximately 60 cm from the Bauhin valve. This makes them the most frequent malignancy of the small intestine [8]. Small intestinal neuroendocrine tumors are often diagnosed in advanced stages because clinical symptoms are frequently nonspecific and can include abdominal pain, intestinal obstruction, and symptoms caused by carcinoid syndrome [2]. Additionally, these tumors develop distant metastases more frequently than neuroendocrine tumors from other locations, further complicating their clinical management. Mesenteric lymph node metastases are found in over 80% of patients at the time of diagnosis, regardless of the primary tumor's size [9-14]. Imaging techniques such as contrast-enhanced CT, MRI, and somatostatin receptor-based PET scans have improved detection, while immunohistochemical markers such as chromogranin A and synaptophysin aid in confirming the diagnosis.

Histologically, gastrointestinal neuroendocrine tumors are characterized by the presence of neuroendocrine cells that secrete various peptides and biogenic amines. These substances can cause diverse clinical manifestations, including diarrhea, flushing, and wheezing, within the context of carcinoid syndrome [15].

Biologically, the tumors exhibit variable expression of neuroendocrine markers such as chromogranin A and synaptophysin, which are used in the diagnosis and monitoring of the disease [7, 16-18].

Recent studies have highlighted the genetic and molecular diversity of gastrointestinal neuroendocrine tumors. Mutations in the MEN1, DAXX, and ATRX genes are frequently associated with these tumors. The identification of these mutations has allowed for a better understanding of the tumor pathogenesis and opened new perspectives for targeted therapies [8, 19-21].

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The clinical management of patients with gastrointestinal neuroendocrine tumors involves a multidisciplinary approach, including advanced imaging, endoscopic techniques, surgery, and systemic therapies. Surgical intervention remains the treatment of choice for resectable tumors [22], while systemic therapies, including somatostatin analogs, mTOR inhibitors, and radionuclide therapies, are used for advanced or metastatic disease [23-29].

EPIDEMIOLOGY AND DEMOGRAPHICS

Gastrointestinal neuroendocrine tumors (NETs) represent a relatively rare group of malignancies, yet recent literature indicates a significant rise in their incidence worldwide. Small intestinal NETs account for approximately 45% of all gastrointestinal NETs, establishing these tumors as the most prevalent malignancy of the small bowel [1,7]. The estimated global annual incidence ranges between 3 to 6 cases per million inhabitants, with a clear upward trend observed over the last four decades, primarily attributed to improved diagnostic methods and increased physician awareness [2,4].

A comprehensive analysis of epidemiological data from the Surveillance, Epidemiology, and End Results (SEER) database (1977–2016) by Liu et al. (2023) demonstrated that gastrointestinal NETs have exhibited a dramatic increase of over 100% in diagnosed cases during recent decades [2]. This significant upward trend reflects advances in diagnostic imaging modalities and improved endoscopic techniques, facilitating earlier and more accurate diagnosis [2,7].

In line with findings reported by Taal and Visser (2004), small intestinal NETs show notable geographical variation, yet consistently demonstrate an annual incidence ranging from 3 to 6 cases per million inhabitants globally [4]. This consistency suggests a common biological behavior and potentially shared risk factors influencing the occurrence of these tumors [4-5].

Epidemiological patterns also reveal that the diagnosis of small bowel NETs most commonly occurs between the ages of 50 and 70 years, highlighting the importance of clinical suspicion within this age group when nonspecific gastrointestinal symptoms are present. The age distribution emphasizes the need for targeted diagnostic vigilance in middle-aged and older patients presenting with chronic abdominal complaints without an obvious etiology [6-7,12].

In addition, several retrospective studies have documented the high likelihood of delayed diagnosis due to nonspecific symptomatology, often resulting in advanced disease at initial presentation, with a high frequency of mesenteric lymph node involvement and distant metastasis at the time of diagnosis [6-7].

Therefore, awareness of these epidemiological trends, along with improved understanding of the disease's clinical behavior, is crucial for clinicians to achieve earlier diagnosis and intervention, ultimately enhancing patient outcomes.

CLINICAL PRESENTATION AND DIAGNOSIS

The clinical presentation of gastrointestinal neuroendocrine tumors (NETs), especially those of the small intestine, is highly variable and nonspecific, frequently contributing to significant delays in diagnosis. Commonly reported symptoms include chronic abdominal pain, often intermittent and vague, unexplained weight loss, changes in bowel habits such as chronic diarrhea or intermittent constipation, and episodes of intestinal obstruction due to either tumor mass or mesenteric fibrosis [7,25,27].

In certain instances, especially when tumors exhibit secretory activity (functional NETs), clinical presentation may include symptoms associated with the classic carcinoid syndrome. This syndrome occurs predominantly when liver metastases are present, allowing serotonin and other vasoactive substances to enter systemic circulation. Characteristic features include episodic flushing, chronic diarrhea, bronchospasm, and occasionally cardiac valvular lesions [15,29].

The nonspecific clinical picture typically leads to prolonged diagnostic latency, with literature reporting that many cases are incidentally identified during surgeries performed for unrelated reasons, such as intestinal obstructions or abdominal hernias, rather than direct suspicion of NETs [25,27].

Imaging plays a crucial role in diagnosing NETs and determining disease extent. Computed tomography (CT), Magnetic Resonance Imaging (MRI), and somatostatin receptor imaging (68Ga-DOTATATE PET/CT) are the mainstay diagnostic tools. Of these, somatostatin receptor PET-CT (68Ga-DOTATATE PET-CT) has demonstrated superior sensitivity and specificity, especially for detecting small primary tumors and distant metastases compared to conventional imaging methods [3,8].

Histopathological confirmation is indispensable for accurate diagnosis and characterization of NETs. Immunohistochemical markers such as chromogranin A, synaptophysin, and the transcription factor CDX2 are routinely employed and demonstrate high sensitivity and specificity for neuroendocrine differentiation [3,6].

Additionally, the proliferation index assessed by the Ki67 index is crucial in grading these tumors and assessing their malignant potential. The World Health Organization (WHO) classification categorizes NETs as G1 (Ki67 <3%), G2 (Ki67 3-20%), or G3 (Ki67 >20%), directly impacting the choice of therapeutic strategies and prognostic evaluation [6,12,22].

Given the nonspecific nature of the symptoms, the combination of clinical suspicion, advanced imaging modalities, and specific immunohistochemical analyses is paramount to timely diagnosis and improved outcomes for patients with small intestinal NETs.

Surgical management of small intestinal neuroendocrine tumors

Surgical intervention remains the cornerstone of management for localized small intestinal neuroendocrine tumors (SI-NETs). The primary goals of surgical treatment include complete tumor resection (R0 resection), adequate lymph node dissection, and alleviation of symptoms associated with tumor burden or obstruction.

Given that SI-NETs frequently present with multifocal lesions (25-44% of patients), a thorough exploration and palpation of the entire small intestine from the ligament of Treitz to the ileocecal valve is critical to achieve adequate surgical clearance. Consequently, open laparotomy remains widely accepted as the gold standard, as it allows complete assessment and palpation to detect subcentimeter tumors that are often missed by minimally invasive techniques [9,14,23-27,28].

Despite this, several studies have explored the efficacy of minimally invasive approaches such as laparoscopy. Figueiredo et al. (2014) described laparoscopic resection of small-bowel NETs in a cohort of 12 patients, demonstrating comparable outcomes in selected cases. Similarly, Reissman et al. (2013) successfully employed laparoscopic resections in 20 patients with primary midgut carcinoid tumors, emphasizing the feasibility and safety of this method in carefully selected cases. However, both studies highlighted critical limitations, notably the difficulty in detecting sub-centimetric lesions, which may necessitate additional techniques such as hand-assisted laparoscopy or open exploration to achieve complete resection [25-27].

Comprehensive lymphadenectomy is also a fundamental aspect of surgical treatment, as metastases in mesenteric lymph nodes are present in more than 80% of patients at initial diagnosis. Optimal lymph node dissection has demonstrated improved long-term outcomes and decreased recurrence rates. Motz et al. (2018), in a comprehensive retrospective analysis from the National Cancer Database (NCDB), emphasized that thorough lymphadenectomy significantly correlates with improved survival outcomes, particularly when combined with R0 resections [10-11,28].

Following curative surgery, long-term follow-up is essential due to the notably high recurrence rate, estimated around 50% even after achieving an R0 resection. Le Roux et al. (2011), in a large multicenter French retrospective analysis, highlighted that factors such as tumor size, presence of lymph node metastases, and microscopic residual disease significantly increase the risk of recurrence. Regular postoperative surveillance involving biochemical markers, such as chromogranin A, and imaging modalities, including CT and somatostatin receptor PET-CT, is therefore mandatory to ensure early detection of recurrence and prompt therapeutic intervention [29-33].

In summary, optimal surgical management of small bowel NETs requires careful evaluation and meticulous surgical techniques, aiming at complete resection (R0) and adequate lymph node clearance. Given the frequent recurrence even after successful surgery, ongoing surveillance and follow-up are critical components in the comprehensive management of patients with SI-NETs.

Case example: multifocal small intestinal net

A 68-year-old male, with history of right inguinal hernia surgery in 2005 and no significant family medical history, who has been exposed to toxic environment, presents to our department complaining of diffuse abdominal pain amplified in the left lower quadrant, particularly postprandial, anorexia, the presence of a left inguinal pseudotumoral mass, and 15 kg weight loss in the last 3 months.

The symptoms began approximately 2 months before admission, with severe abdominal pain, predominantly in the left lower quadrant, unresponsive to usual analgesics, anorexia, intestinal motility disorders – loose stools in the last 3 weeks, weight loss – 15 kg in 3 months, and 2 episodes of minor upper gastrointestinal bleeding.

The patient was evaluated in another medical center through upper gastrointestinal endoscopy, lower gastrointestinal endoscopy, and no significant findings. Abdominal ultrasound showed a left lower abdominal quadrant mass.

The patient presented to the Surgery Department of “Dr. Carol Davila” University Central Emergency Military Hospital, in an emergency setting, with a left lower quadrant mass, painful, enlarged (7/8 cm), irreducible, with normal overlying skin and a diffusely painful abdomen.

Subsequent investigations were performed. Thoracic-abdominal-pelvic CT scan revealed a left inguinal hernia with a 4.5 cm abdominal wall defect, narrow neck, and larger herniating sac containing omentum and a large bowel loop. There were proximal intestinal obstructive changes and colonic distension with stasis content and air-fluid levels. Additionally, a tumor mass was identified in the intramesenteric mesogastric region, antero-left para-aortic, cranial to the iliac bifurcation, measuring approximately 5/4/3 cm. The mass had a heterogeneous structure with hypodense areas included and no surgical cleavage plane to the loco-regional digestive loop.

The patient underwent emergency surgery, consisting of exploratory laparotomy. Intraoperatively, an intense adhesive process (Figure 1), multiple whitish jejunal tumor masses up to 3 cm in size, with two mesenteric tumoral masses measuring 3 cm and 5 cm in diameter with adherence to neighboring loops and incarcerated left inguinal hernia (Figure 2) were found, without ascites and no peritoneal or liver nodule. Extensive enterectomy with side-to-side entero-enteric anastomosis and non-mesh hernia repair were performed (Figures 3, 4). There were no signs of ischemia in the bowel loop contained within the herniated sac, hence, there was no need for resection. The non-mesh surgical technique was preferred in order to prevent the risk of infection associated with the mesh.

During hospitalization, the patient had a favorable evolution and was discharged on the 7th postoperative day.

Figure 1: Abdominal adhesions

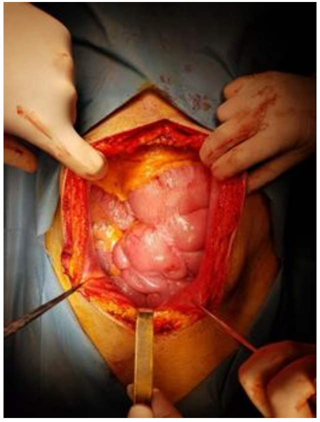

Figure 2: Mesenteric tumoral mass

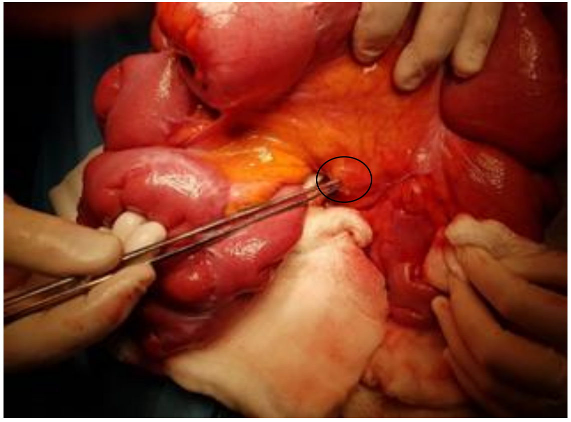

Figure 3: Side-to-side anastomosis

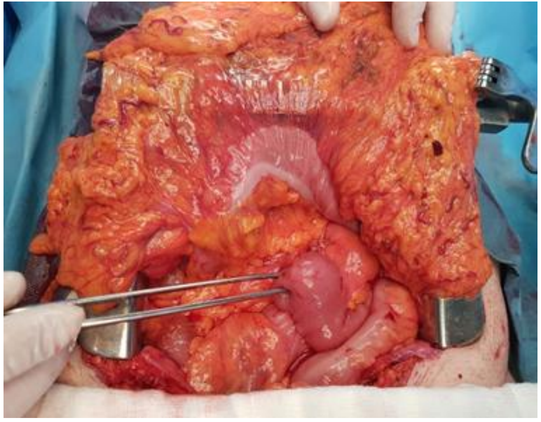

Figure 4: Resected specimen

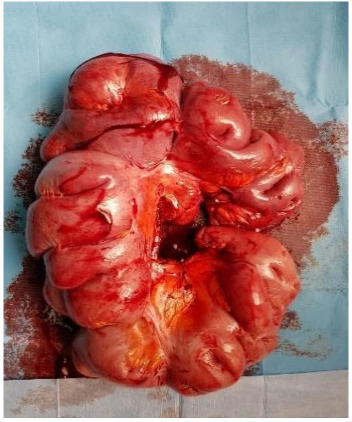

Histopathologic result confirmed the diagnosis of neuroendocrine tumor involving multiple levels of the small intestine with lymph node metastases (3/10) (TNM: pT4 N2 Mx).

The immunohistochemical examination revealed that CDX2 (EPR2764Y) exhibited a uniform positive reaction with both intensity and distribution in the tumor cells, indicating consistent expression throughout the sample. Similarly, CHROMO (LK2H10) showed a uniform positive reaction with intensity, while Synaptophysin (SP11) demonstrated a uniform positive reaction with diffuse intensity in the tumor cells, suggesting widespread expression. Furthermore, Ki67 (30-9) displayed an intense nuclear reaction in 1% of the tumor cells, indicating a low proliferative index within the examined sample. These findings collectively support the diagnosis and provide insights into the tumor's characteristics.

Table 1: Immunohistochemical results

CDX2 (EPR2764Y): Uniform positive reaction with intensity and distribution in tumor cells
CHROMO (LK2H10): Uniform positive reaction with intensity in tumor cells
Synaptophysin (SP11): Uniform positive reaction with intensity, diffuse in tumor cells
Ki67 (30-9): Intense nuclear reaction in 1% of tumor cells

Immunophenotypic data correlated with the morphological cells in the examined fragment are compatible with a neuroendocrine tumor of reduced histological grade (G1).

The patient did not receive chemotherapy, somatostatin analogues, or radiotherapy. He underwent routine follow-ups and regular imaging investigations. At the five-year evaluation, no tumor recurrences or new pathological findings were detected.

DISCUSSION

Primary tumors in the jejunum or ileum are frequently very small, requiring meticulous palpation of the small intestine from the ligament of Treitz to the ileocecal valve. In 25% to 44% of patients, there are multifocal primary tumors, many of which are subcentimeter in size and can only be detected through careful digital palpation [24]. The use of laparoscopy in these cases is controversial, as it may not allow the detection of such small tumors and does not enable the comprehensive palpation of the entire small intestine that is essential for identifying multifocal disease. Therefore, the limitations of laparoscopy must be taken into account when determining the surgical strategy for these patients. The literature contains limited studies on the laparoscopic resection of small bowel neuroendocrine tumors (SBNETs). Figueiredo et al. [25] reported successful laparoscopic resections in 12 patients, while Reissman et al. [26] documented similar success in 20 patients. Wang et al. [27] described successful laparoscopic or minimally invasive resections of ileal neuroendocrine tumors (NETs) in six patients who presented with NETs of unknown primary origin. In their study, the authors highlighted the critical role of palpation during minimally invasive surgery to identify the small, often multifocal primary tumors. To facilitate this, they employed a hand-assisted laparoscopic device or a soft tissue wound retractor to exteriorize the jejunum and ileum. This technique allows for comprehensive palpation, resection of the primary tumors, dissection of mesenteric lymph nodes and associated fibrosis, and subsequent intestinal anastomosis.

Current literature regarding the optimal management of neuroendocrine tumors recommends achieving an R0 resection to increase survival rates. However, an R0 resection is feasible in only 20% of cases due to the typically advanced stage at diagnosis [28]. It is important to highlight that the patient presented as an emergency case with symptoms caused by intestinal obstruction due to an incarcerated inguinal hernia, and not from the intestinal tumors, which were discovered incidentally. Therefore, it cannot be claimed that there was an intent for radical treatment, even though an R0 resection was achieved.

Adjuvant treatment options for neuroendocrine tumors may include chemotherapy, somatostatin analogues, and radiotherapy. However, following curative surgery for small intestinal neuroendocrine tumors, there is no established benefit for adjuvant systemic therapy. Somatostatin analogues are recommended for their antiproliferative properties as a first-line treatment for slowly progressive, well-differentiated metastatic small intestinal neuroendocrine tumors. Additionally, they are used for antisecretory purposes to manage persistent secreting syndromes or refractory diarrhea by inhibiting gastrointestinal secretions [29-31].

The overall survival rate appears favorable when compared to other malignancies. However, achieving reliable survival data is challenging due to variations in terminology, inconsistencies in coding practices, conflicting results from smaller cohort studies, and recent advancements in treatment modalities [32].

Several studies have indicated that the long-term recurrence rate for small intestinal neuroendocrine tumors (SI-NETs) following curative R0 resection, in the absence of distant metastases, is approximately 50%. This high recurrence rate underscores the need for vigilant postoperative surveillance and ongoing management strategies to monitor for disease recurrence. Factors contributing to this recurrence include residual microscopic disease and the inherent biological behavior of SI-NETs. Consequently, patients require comprehensive follow-up protocols, including regular imaging and biochemical assessments, to promptly identify and address any recurrence or progression of the disease [33].

CONCLUSION

The case highlights the importance of considering gastrointestinal neuroendocrine tumors in the differential diagnosis of nonspecific abdominal pain.

Advancements in diagnostic and treatment methods have significantly improved the outlook for many patients with NET, but challenges remain, especially for advanced or poorly differentiated cases.

Although neuroendocrine tumors are rare, their increasing incidence and clinical complexity underline the need for a multidisciplinary approach for effective diagnosis and treatment. Progress in the genetic and molecular understanding of these tumors has opened new avenues for targeted therapies, offering improved hopes for patients.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

MT, BI, JC, HS performed the surgical procedure. MT contributed to the manuscript's conceptualization, methodology, investigation, discussion, review, editing, and supervision. BI contributed to the conceptualization, investigation, data curation, review, and writing of the manuscript. JC contributed to the manuscript's conceptualization, investigation, review, and editing. HS contributed to the editing and review of the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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REVIEW

From Genetic Links to Personalized Therapies: Understanding Melanoma and Urological Cancer Overlap

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Abstract: Malignant melanoma and urological cancers originate from different tissues and organs, yet several studies highlight connections between these malignancies, including common risk factors, genetic predispositions, and immunological pathways. Evidence from recent studies suggests that a prior diagnosis of melanoma may increase the likelihood of subsequently developing renal cell carcinoma (RCC), and, conversely, patients with RCC appear to face a heightened risk of being diagnosed with melanoma. Shared factors such as a personal or family history of cancer, UV radiation exposure, smoking, and obesity have all been linked to an increased incidence of various cancer types. A major link between malignant melanoma and urological cancers is the presence of shared genetic mutations and familial cancer syndromes. Key mutations, including germline mutations in BRCA1, MITF, CDKN2A, TP53, and alterations in the PI3K/AKT pathway, significantly contribute to the risk of both types of malignancies. Personalized medicine, which tailors prevention and treatment strategies to an individual's genetic, environmental, and lifestyle factors, has significantly improved cancer care. The primary aim is to select the most effective treatment for each patient, maximizing therapeutic outcomes, reducing side effects, and minimizing the risk of drug resistance. Advances in genomics and immunology are driving the development of personalized therapies that target specific molecular pathways and immune responses common to both melanoma and urological cancers. Angiogenesis inhibitors and checkpoint inhibitors have demonstrated notable success in treating these cancers, with tumor mutational burden serving as a valuable biomarker for predicting the efficacy of immune checkpoint inhibitors.

Keywords: malignant melanoma; urological cancers; risk factors; shared genetic mutations; lifestyle changes; personalized treatment; tumor mutational burden; angiogenesis inhibitors; checkpoint inhibitors

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INTRODUCTION

Cancer currently ranks as the leading cause of mortality worldwide. The growing number of cases and related deaths is primarily driven by shifts in demographic patterns, such as population aging and growth, along with greater exposure to environmental and lifestyle-related carcinogenic factors [1].

Although malignant melanoma and urological cancers arise from different anatomical sites and tissues, multiple studies have identified links between them. These connections include overlapping risk factors, common genetic susceptibilities, and similarities in immune-related pathways involved in their development.

Gaining insight into the association between malignant melanoma and urological malignancies may contribute to the improvement of approaches for early diagnosis, risk reduction, and therapeutic management.

Although melanoma accounts for less than 2% of cancer cases globally, it remains one of the most frequently diagnosed malignancies across Europe (5th place). Survival outcomes have improved considerably in recent years, with the overall 5-year survival now approaching 93.3%. However, patients diagnosed with stage IV disease continue to face a poor prognosis, as the survival rate in advanced stages drops significantly, reaching only 29.8% [2].

Urological cancers (UC), which include kidney, bladder, prostate, testicular, penile, and urethral cancers, differ significantly in incidence, risk factors, and prognosis.

Renal cell carcinoma (RCC) represents roughly 3% of all malignant tumors diagnosed globally. The occurrence of this malignancy tends to be more frequent in industrialized nations, especially in Western societies, where lifestyle habits, environmental risk factors, and advancements in medical imaging likely play a role in higher diagnosis rates. Renal cell carcinoma represents a major public health issue due to its frequently silent evolution in early stages and the elevated risk of distant spread if not recognized in time [3]. Globally, it is estimated that more than 430,000 new cases are identified annually, underlining the increasing impact of this disease on healthcare systems. Of these cases, approximately 138,000 are reported in Europe alone, underscoring the significant prevalence of RCC across the continent. Such statistics emphasize the importance of ongoing efforts in early detection, prevention, and the development of effective treatment strategies. [4].

Urothelial carcinoma is the second most common urological malignancy in developed countries [5]. Bladder cancer (BC) accounts for 90–95% of the UC [6]. It ranks as the seventh most frequently diagnosed cancer among men globally [7].

Primary urethral carcinoma is considered a rare cancer, accounting for < 1% of all genitourinary malignancies [8].

Approximately 1.5 million new cases are reported worldwide each year. This high incidence places it as the second most commonly identified malignancy in the male population [9].

Testicular cancer (TC) accounts for 1% of adult neoplasms and 5% of urological tumors. Its incidence has risen in recent decades, particularly in industrialized countries, and continues to increase [10].

Penile cancer is a rare condition in industrialized countries, with an incidence of approximately 1 per 100,000 men in Europe and 0.5 per 100,000 in the USA [11].

The aim of this article is to evaluate the potential connections between malignant melanoma and the most common urological cancers—renal cancer, bladder cancer, and prostate cancer—from the perspectives of epidemiological factors, shared genetic mutations and molecular pathways, clinical implications, and personalized medicine.

EPIDEMIOLOGICAL LINKS

Environmental and Lifestyle Risk Factors for Malignant Melanoma and Urological Cancers

Advancing age, genetic predisposition, prior cancer diagnoses, radiation exposure (such as ultraviolet rays), contact with certain hazardous substances, tobacco use, alcohol intake, and excess body weight are all factors that have been linked to a higher likelihood of developing different forms of cancer.

Ultraviolet (UV) exposure and vitamin D

The role of ultraviolet (UV) sunlight radiation in the development of skin cancers, particularly malignant melanoma, is well-established. UVA (320–400 nm) penetrates deeper into the skin, leading to indirect DNA damage through reactive oxygen species (ROS), while UVB (280– 320 nm) directly damages DNA by inducing cyclobutane pyrimidine dimers [12]. UVB radiation can also induces mutations in genes such as TP53 and BRAF, contributing to melanomagenesis [13].

However, the association between UV exposure and RCC remains unclear, recent studies have attempted to clarify the relationship but have produced mixed results. Grasso et al. [14] found no statistically significant differences between sun exposure in patients affected by kidney cancer and controls, both during childhood and adult life. Contrary to this, Karami et al. [15] suggest that among males there is an inverse association between occupational UV exposure and renal cancer risk. Replication studies are warranted to confirm these results.

Similar to the RCC, the potential involvement of vitamin D in the development and progression of bladder cancer and upper urinary tract urothelial tumors remains underexplored. Recent epidemiological studies offer conflicting results regarding the potential link between blood levels of vitamin D and the risk of bladder cancer onset. However, several investigations suggest that individuals with a higher intake of vitamin D through diet, particularly when not accompanied by elevated calcium consumption, may have a reduced risk of developing bladder malignancies [16]. In a meta-analysis, Zhang et al. [17] concluded that Vitamin D deficiency has been linked to a heightened risk of bladder cancer. According to Gislefoss et al. [18] educed serum concentrations of 25-hydroxy vitamin D, along with obesity, may be linked to a higher risk of developing bladder cancer. The 2024 guidelines of the European Association of Urology (EAU) highlight that both insufficient and excessive levels of vitamin D have been linked to a higher likelihood of developing prostate cancer, with a particularly stronger connection observed in cases involving high-grade tumors [19,20]. Additionally, Murphy et al. demonstrated that Vitamin D deficiency was linked to higher Gleason scores and advanced tumor stages at diagnosis [21].

Smoking

The effect of smoking on melanoma outcome still remains an enigma [22]. In a recent study, Arafa et al. [23] found that individuals who currently smoke or have a heavy smoking history tend to exhibit a greater susceptibility to developing squamous cell carcinoma (SCC), while simultaneously displaying a lower probability of malignant melanoma. Interestingly, no significant relationship was observed between past smoking and the overall risk of skin cancer. In contrast to established risk patterns, a case-control study by Sondermeijer et al. [24] reported an inverse association between cigarette smoking and melanoma incidence in men. However, smoking remains a well-documented risk factor for various urologic malignancies.

Smokers face a significantly elevated likelihood—estimated at around 1.5 times higher—of being diagnosed with renal cell carcinoma (RCC) when compared to non-smokers [25]. Encouragingly, long-term smoking cessation has been shown to gradually diminish this elevated risk [26]. Multiple carcinogens (N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), polycyclic aromatic hydrocarbons (PAHs), Cadmium and lead (Pb), 2-naphthylamine) are absorbed through the lungs into the bloodstream and then lipophilic carcinogens may accumulate in renal tissue [27].

Cigarette smoking represents the leading modifiable contributor to bladder cancer development and is estimated to be responsible for nearly half of all diagnosed cases [28]. Low-tar cigarettes are not associated with a lower risk of developing BC. The risk associated with electronic cigarettes has not been adequately assessed. Research by Bjurlin et al. [29] revealed the presence of carcinogen-related biomarkers in the urine of individuals who use e-cigarettes, several of which have a well-established association with bladder cancer. Kispert et al. [30] recently presented a study showing that smoking is associated with increased accumulation of platelet-activating factor (PAF) and elevated expression of the PAF receptor (PAF-R), which may contribute to tumor progression and metastasis in smokers.

Current cigarette smoking has been linked to an increased risk of prostate cancer (PCa) mortality, as well as more aggressive tumor characteristics and a poorer prognosis, even after smoking cessation [31].

Obesity

Obesity promotes chronic inflammation, a recognized risk factor for cancer development. Inflammatory cytokines can stimulate the growth of both melanoma and renal cell carcinoma (RCC), potentially linking these two malignancies in obese patients [32].

Obesity appears to be associated with malignant melanoma, particularly in patients with a BMI >30 [2].

Several retrospective studies have shown that obesity appears to be a factor that positively influences the prognosis of patients with RCC, called the "obesity paradox" [33]. Recently, Graff and colleagues [34] provided a comprehensive review with the main purpose of disentangling the "obesity paradox" in renal cancer. The results support obesity with a BMI >35 as a risk factor for total and fatal RCC. They undermine the obesity paradox by suggesting that weight loss around diagnosis, and not low BMI itself, is associated with worse prognosis.

In the REDUCE study [35], obesity was associated with a lower risk of low-grade PCa and a higher risk of high-grade PCa.

Occupational Exposure

Occupational exposure to carcinogens can increase the risk of various cancers. One common risk factor for both malignant melanoma and urological cancers is exposure to polycyclic aromatic hydrocarbons.

In a 45-year follow-up study conducted across five Nordic countries, Pukkala et al. [36] found an elevated incidence of prostate cancer, malignant melanoma, non-melanoma skin cancer, and lung adenocarcinoma among firefighters, with skin absorption identified as a primary route of exposure. Similarly, individuals working in the petroleum industry are exposed to comparable risks [37].

According to the 2024 EAU Guidelines [38], occupational exposure to polycyclic aromatic hydrocarbons and other aromatic amines or hydrocarbons, mainly in the paint, dye, or petroleum industries, is the second most important risk factor for bladder cancer.

While certain studies have indicated a potential association between occupational exposure to polycyclic aromatic hydrocarbons and an increased risk of renal cancer [39,40], there is no high-quality evidence to definitively confirm this link [41].

SHARED GENETIC MUTATIONS AND MOLECULAR PATHWAYS

Integrating molecular profiling into clinical oncology practice, particularly through mutation-focused approaches, has become essential in the evolution of precision medicine. By identifying specific genetic alterations within tumors, clinicians can better tailor immunotherapeutic interventions that modulate immune checkpoints. Therapeutic approaches that focus on modulating immune checkpoints have identified key molecular pathways, such as those involving PD-1 and its ligand, as well as CTLA-4, as critical elements in regulating immune responses against tumors. Targeting these mechanisms has opened promising prospects for the treatment of a wide range of cancers [42]. These immunotherapeutic agents have significantly reshaped treatment approaches across several types of cancers with overlapping characteristics.. Such technology was entirely unavailable before the completion of the Human Genome Project in 2001 [43]. However, current advances now enable the routine identification of relevant therapeutic mutations in many cancers, particularly in malignant melanoma and also in UC.

Genetic Mutations in Malignant Melanoma

Melanoma exhibits one of the highest mutation frequencies among cancers [44]. The most frequent mutations are BRAF, NRAS, NF1, the TERT promoter, and CDKN2A, while KIT and TP53 mutations occur at a much lower frequency. [45].

- The BRAF mutation is the most common in malignant melanoma, occurring in 2/3 of these patients. [46]. This BRAF mutation (especially the BRAF V600 mutation) promotes cellular growth and inhibits apoptosis and is more commonly found in patients with intermittent sun exposure compared to those with chronically sun-exposed skin or unexposed skin [47].

- NRAS mutations. Among the three main proto-oncogenes of the RAS family (NRAS, KRAS, and HRAS), NRAS mutations play a key role in continuously activating pathways that lead to cell growth, proliferation, and cell cycle dysfunction. They are found in approximately 1/4 of patients with malignant melanoma, particularly in those with non-exposed skin, and are associated with aggressive clinical forms and poor prognosis [48,49].

- The neurofibromatosis type 1 (NF1) mutation leads to a loss of function in certain tumor suppressors. NF1 mutations appear to be correlated with high UV exposure, resulting in a high mutational burden [50,51].

- The TERT (telomerase reverse transcriptase) promoter mutation is a UV-induced mutation associated with poor clinical prognosis. This mutation typically occurs in the promoter region, resulting in increased expression of the gene [52].

- CDKN2A Mutation. The CDKN2A gene contributes to cellular homeostasis by limiting uncontrolled cell division through mechanisms that halt the progression of the cell cycle. Its function is essential in preventing the unchecked proliferation characteristic of tumor development. When mutations occur in this gene, normal growth regulation is disrupted, promoting tumor development. Individuals who carry CDKN2A mutations face a markedly increased risk of developing multiple primary melanomas, often at an earlier age. [53,54].

- KIT is a proto-oncogene receptor tyrosine kinase and binds to stem cell factor. By activating the KIT protein, cell growth, proliferation, survival, and migration can be affected. KIT mutations occur more commonly in areas of chronic sun damage [55].

- BAP1 Tumor Predisposition Syndrome (BAP1-TPDS) is an inherited cancer syndrome marked by germline mutations in the BRCA1-associated protein 1 (BAP1) gene [56]. The consequences include genomic instability, uncontrolled cell proliferation, and resistance to apoptosis. In the case of cutaneous melanoma, patients with germline mutations in BAP1 develop multiple atypical melanocytic lesions (dome-shaped, skin-colored to reddish papules), which are histologically distinct from conventional melanomas [57,58].

- TP53 Mutation. In human biology, the TP53 gene plays a major role in preserving genomic integrity and is often described as the "guardian of the genome." This designation stems from its crucial function in cellular defense mechanisms, particularly in detecting DNA damage and initiating appropriate responses such as cell cycle arrest, DNA repair, or apoptosis. By preventing the propagation of cells with genetic errors, TP53 serves as a key tumor suppressor and a vital component in maintaining cellular homeostasis and protecting against malignant transformation [10]. The TP53 mutation has been found in association with BRAF, NRAS, and NF1 mutations and seems to appear at a later stage in tumor development. Even though TP53 is rarely mutated in melanoma, reduced levels of p53 contribute to aggressiveness and resistance to therapy for patients with malignant melanoma [59,60].

Genetic Mutations in Urological Cancers

There is increasing evidence that genetic susceptibility factors and familial associations may influence the incidence of UC, although their role is not as well defined as in the case of malignant melanoma.

- BAP1 Tumor Predisposition Syndrome (BAP1-TPDS) can predispose individuals not only to cutaneous and uveal melanoma but also to renal cell carcinoma (RCC) [57].

In case of cutaneous melanoma, patients with Germline mutations in BAP1 develop multiple atypical melanocytic lesions (dome-shaped, skin-colored to reddish papules), histologically distinct from conventional melanomas [58].

Kapur et al [61] in a retrospective analysis on 145 patients with primary clear cell renal cell carcinoma (ccRCC) concluded that in RCC, the clear cell subtype (ccRCC) germline mutations occur in approximately 10–15% of ccRCC. This study concluded that these patients are associated with poorer overall survival compared to those without the mutation.

Studies on the expression and prognostic role of BAP1 in prostate cancer are currently lacking. However, Steurer et al. immunohistochemically analyzed more than 17,000 prostate cancer cases, assembled on a tissue microarray over 10 10-year period, and concluded that their study shows BAP1 upregulation is associated with prostate cancer progression and aggressiveness [62].

- CDKN2A Mutation. The role of CDKN2A mutations in renal cell carcinoma is less clear. Recently, in a study, Kiatprungvech and colleagues [63] demonstrated that CDKN2A mutations may be associated with higher tumor grade and poorer prognosis in RCC, development, and sarcomatoid changes.

Deletions of CDKN2A are a molecular risk factor for tumour progression in non-muscle-invasive bladder cancers, an indicator of increased aggressiveness and worse prognosis in muscle-invasive bladder cancers [64].

In a meta-analysis investigating the involvement of CDKN2A methylation in prostate cancer, Cao et al. [65] found no strong evidence supporting a significant association between this epigenetic alteration and the onset or advancement of the disease. Nevertheless, lower levels of CDKN2A expression were correlated with poorer disease-free survival outcomes, suggesting potential prognostic relevance.

- MITF Mutation. The Microphthalmia-associated Transcription Factor (MITF) regulates genes involved in melanogenesis, melanocyte survival and proliferation, and DNA repair. The MITF-E318K variant is a heterozygous missense mutation characterized by the replacement of glutamic acid with lysine at the 318th amino acid position in the protein sequence.. Carriers have a higher risk of developing early-onset melanoma [66].

This mutation was also identified in patients with RCC, suggesting a shared genetic risk factor with melanoma. In their study, Lang et al. [67] observed that the presence of the pathogenic MITF variant in a family with bilateral and multifocal type 1 papillary renal cell carcinoma reinforces its potential role as a genetic risk factor for RCC. These findings also highlight the need to consider MITF variant screening regardless of the tumor's histological subtype.

- TP53 mutation. The role of the p53 pathway in renal cancer is underestimated. In RCC, there is a low rate of p53 mutations, which leads to poor response to conventional therapies or even therapy resistance [68].

According to findings reported by Wu et al. [69], alterations in the TP53 gene were identified in half of the individuals diagnosed with bladder cancer. It is worth noting that these genetic alterations appeared with greater frequency in tumors that had penetrated the bladder's muscular layer, as opposed to those confined to the superficial, non-muscle-invasive stages. Patients with TP53 mutations were associated with lower TP53 mRNA expression levels, more advanced tumor stages, and higher histologic grades.

- Loss of function in the VHL gene, often caused by inherited mutations, is associated with the development of von Hippel-Lindau hereditary cancer syndrome. This rare genetic condition can predispose affected individuals to various tumor types, including, in some cases, clear-cell renal cell carcinoma [70].

- Alterations in the BRCA1 and BRCA2 gene pathways have been linked to more aggressive disease phenotypes and less favorable prognoses in individuals affected by metastatic castration-resistant prostate cancer [71]. Specifically, inherited BRCA2 mutations are frequently observed in early-onset prostate cancer and are recognized as independent predictors of metastatic progression and reduced prostate cancer-specific survival [72,73]. Nyberg and colleagues [74] in a prospective cohort study confirmed the BRCA2 association with aggressive PCa. This mutation does not occur in other urological cancers or malignant melanoma.

CLINICAL IMPLICATIONS AND PERSONALIZED MEDICINE

Prevention is the main principle in reducing morbidity and mortality, as well as in lowering healthcare system costs.

Personalized medicine, which customizes prevention and treatment strategies based on an individual's genetic, environmental, and lifestyle factors, has greatly advanced cancer care [75].

Understanding the link between malignant melanoma and urological cancers is a key advancement toward personalized medicine.

Identifying genetic mutations and syndromes that link melanoma to urological cancers highlights the importance of genomic profiling in cancer diagnosis and treatment. The development of next-generation sequencing (NGS) technologies has made it possible to identify particular genetic alterations that may increase a person's susceptibility to various types of cancer or impact how they respond to specific therapeutic interventions [76].

Tailoring Prevention Strategies (Table 1).

Reduce Ultraviolet Exposure and the Role of Vitamin D

Excessive UV exposure is widely recognized as the leading environmental risk factor for melanoma. In people with an increased hereditary predisposition to skin cancer [77], tailored prevention approaches may involve regularly applying sunscreens that offer protection against both UVA and UVB rays, opting for clothing that minimizes sun exposure, and refraining from the use of artificial tanning devices.

In a systematic review and meta-analysis, Song et al. [78] concluded that a moderate dietary vitamin D supplement to prevent serum 25(OH)D deficiency may be beneficial for the long-term survival of melanoma patients.

A prospective study conducted by Lin et al. [58] suggested that exposure to ultraviolet radiation may be associated with a reduced incidence of prostate cancer. Additionally, research has shown that both low and excessively high levels of vitamin D may contribute to a higher likelihood of developing prostate cancer, particularly in cases involving more aggressive tumor forms [19]. Therefore, maintaining normal vitamin D levels is essential.

Table 1: Recommendations for lifestyle changes

	Malignant melanoma	**RCC**	**Bladder cancer**	**Prostate cancer**
Smoking cessation	uncertain	yes	yes	yes
Reduce ultraviolet exposure	yes	-	-	no/increase
Vitamin D	moderate increase dietary	normal vitamin D levels		
Obesity	uncertain	yes	yes	yes
Dietary antioxidants	yes	yes	yes	yes

Smoking Cessation in Malign Melanoma and Urological Cancers Prevention

While the relationship between smoking and malignant melanoma remains inconclusive, tobacco use is widely recognized as a major contributor to the development of several urologic malignancies, especially bladder cancer and renal cell carcinoma. Smoking cessation programs, aided by pharmacological treatments such as nicotine replacement therapy (NRT), have proven effective in reducing the incidence or recurrence of progression of RCC [26] and bladder cancer [80]. In a network meta-analysis of 20 randomized controlled trials, Gou et al. [81] concluded that Varenicline and Bupropion increased the odds of smoking abstinence.

Obesity and Dietary Interventions in Malignant Melanoma and Urological Cancers Prevention

Studies that focus specifically on the link between obesity and melanoma prognosis are limited [61]. Obesity may be a risk factor for bladder cancer recurrence [83] and a higher risk of death from prostate cancer [84].

The use of dietary antioxidants has shown promising results in studies, supporting the potential role of antioxidants in the prevention of malignant melanoma related to UV exposure.

Obesity and dietary habits are well-established risk factors for prostate cancer and RCC.

Personalized weight management programs guided by genetic and metabolic profiles, and dietary recommendations, such as reducing red and processed meats and increasing intake of plant-based foods rich in antioxidants (e.g., lycopene), regular physical activity, are essential for individuals at high risk for prostate cancer and RCC [64,65].

Screening and Surveillance

Patients with a history of melanoma or urological cancer need careful monitoring for the potential development of additional malignancies. Routine screenings, such as skin examinations and urological assessments, can aid in early detection and improve patient outcomes. Screening for malignant melanoma is recommended for individuals with certain risk factors, including family or personal history, lifestyle or occupational predispositions, and being an older white male.

Patients with germline mutations in BAP1, CDKN2A, MITF, and other cancer-related genes may benefit from more extensive cancer screening protocols. These could include regular skin examinations for melanoma, imaging studies to detect urological cancers, and continuous monitoring for other associated malignancies.

Skin cancer screening remains a topic of debate.

There is an ongoing debate regarding the value of routine screening for skin cancer.

According to current assessments by major public health authorities, existing evidence is insufficient to clearly confirm or reject the benefits of regularly screening asymptomatic adults in the general population [87]. In contrast, screening is recommended for high-risk individuals by both the Australian Cancer Network [88] and the Canadian Cancer Society [89].

The group of patients at higher risk for developing skin cancer includes individuals with a personal history of skin cancer, those with light-colored skin, eyes, and hair, a high number of moles or freckles, and a history of multiple blistering sunburns during childhood [87]. In individuals at elevated risk, initial screening typically involves assessment of skin lesions using the "ABCDE" criteria, focusing on asymmetry, irregular borders, variations in color, and lesions exceeding 6 mm in diameter. Another widely used approach is the visual examination of the skin to detect pigmented lesions that appear noticeably different from the patient's other moles, a method commonly referred to as the "ugly duckling" sign [90].

The American Academy of Dermatology advises that patients diagnosed with cutaneous melanoma undergo cancer risk counseling if they present specific risk factors. These include having a family history involving three or more relatives on the same side affected by invasive melanoma or pancreatic cancer; the presence of three or more primary invasive melanomas, particularly if at least one was diagnosed before the age of 45; the occurrence of one or more melanocytic BAP1-mutated atypical intradermal tumors (MBAITs) accompanied by a family history of cancers such as mesothelioma, meningioma, or uveal melanoma; the presence of two or more MBAITs; or a broader family history that includes other forms of cancer. [91]. According to the National Comprehensive Cancer Network (NCCN), genetic testing for p16/CDKN2A mutations is advised in cases where an individual or family history includes three or more invasive cutaneous melanomas, or a combination of invasive melanoma, pancreatic cancer, and/or astrocytoma. Furthermore, individuals diagnosed with invasive cutaneous melanoma who also have a first-degree relative affected by pancreatic cancer are considered appropriate candidates for genetic evaluation. [92].

Renal cancer screening.

There is currently no evidence to support primary screening in the general population. The most pragmatic approach is to screen high-risk individuals or integrate this screening with other routine screening programs. This strategy improves the cost-effectiveness ratio, particularly for patients at risk of RCC [93]. Genetic assessment is advised in individuals under the age of 47, as well as in those presenting with tumors affecting both kidneys or multiple sites within the same kidney. It is also warranted for patients who have a direct or extended family history of renal cell carcinoma or relatives carrying a confirmed pathogenic genetic alteration. Additionally, testing is appropriate when histopathological characteristics raise suspicion of an inherited subtype of renal cancer [94].

Bladder cancer screening.

Current evaluations by major public health experts indicate a lack of sufficient high-quality evidence to clearly assess whether bladder cancer screening in asymptomatic adults provides more benefits than harms [95]. Similar recommendations are also found in the EAU Guidelines 2024 [96].

Prostate cancer screening.

Screening for PCa is still controversial. [97]. Prostate screening is associated with an increased diagnosis of prostate cancer (PCa), the detection of more localized disease, and fewer cases of advanced PCa. However, no PCa-specific survival benefit has been observed, and there has been no overall survival (OS) benefit [98]. Consequently, population-based screening has not been widely adopted.

According to the 2024 EAU Guidelines, PSA testing for prostate cancer screening is recommended for well-informed men at higher risk of PCa. It is generally recommended for men aged 50 and above. Earlier screening may be appropriate for those over 45 who have close relatives previously diagnosed with the disease or who belong to high-risk populations, such as individuals of African ancestry. Furthermore, men with confirmed BRCA2 mutations are advised to initiate screening starting at age 40 [99].

Hereditary genetic testing is recommended for men who have several relatives affected by prostate cancer at a relatively early age—specifically before turning 60, or in cases where a family member has died as a result of the disease. Genetic testing may also be appropriate for men who have a familial pattern of high-risk inherited mutations or a history of multiple cancer diagnoses occurring on the same side of the family [99].

Personalized Treatment

The goal of personalized medicine for cancer patients is to select the most effective treatment tailored to each individual, aiming for maximum efficacy, reduced side effects, a lower risk of drug resistance, and ultimately, improved overall survival. With advancements in technology, genetic testing has become increasingly accessible and is now part of routine clinical practice. [100]. Advancements in genomics and immunology are driving the development of personalized treatments that focus on specific molecular pathways and immune mechanisms common to both melanoma and urological cancers. The convergence of these genetic pathways offers promising potential for the creation of targeted therapeutic approaches.

In most cancer cases, treatment follows well-established standards that are outlined in specialized guidelines. It includes surgical therapy (nephrectomy, prostatectomy, radical cystectomy, melanoma surgery, etc.), radiotherapy, and/or hormone therapy.

In the final stages, palliative therapies are offered to patients with metastatic cancer, and these treatments can help enhance quality of life while potentially prolonging disease-free survival.

Angiogenesis inhibitors and immunotherapies have shown notable effectiveness in treating malignant melanoma and urological cancers, particularly kidney and bladder cancers. Most prostate cancers exhibit resistance to immunotherapies, particularly immune checkpoint inhibitors [101].

Angiogenesis inhibitors.

VEGF inhibitors function by blocking the interaction between VEGF and its receptors (VEGFRs) on endothelial cells, effectively stopping the signaling processes responsible for initiating new blood vessel formation [102]. VEGF inhibitors are primarily categorized into two groups: monoclonal antibodies, which bind directly to VEGF ligands to prevent receptor interaction, and tyrosine kinase inhibitors (TKIs), which target VEGFRs along with associated kinases to block angiogenesis signaling pathways [103,104]. These inhibitors have shown notable effectiveness in adjuvant treatment for renal cell carcinoma (RCC) and malignant melanoma, though their use is more restricted in other types of urological cancers.

Monoclonal VEGF antibodies, particularly Bevacizumab, have demonstrated effectiveness in the treatment of both renal cell carcinoma (RCC) and malignant melanoma [105,106].

Tyrosine kinase inhibitors are commonly prescribed as initial or subsequent targeted treatments for patients with kidney cancer that has progressed locally or spread to distant sites [107,108]. Initial treatment with sunitinib alone has demonstrated significant benefits by delaying disease progression and enhancing the overall tumor response. Some tyrosine kinase inhibitors, such as pazopanib, have shown similar effectiveness to sunitinib when used in the initial treatment of patients with metastatic kidney cancer [109]. Certain agents, including cabozantinib, have shown clinical benefit in patients with advanced clear-cell renal cell carcinoma that no longer responds to therapies targeting the VEGF receptor pathway. This option is particularly relevant for individuals who have not yet received immune checkpoint inhibitors and who have previously undergone one or two systemic treatment regimens [110].

For patients with malignant melanoma, sunitinib may show efficacy, particularly in those with KIT mutations [111].

Checkpoint inhibitors

Immunotherapeutic strategies—especially those directed at modulating checkpoint pathways—have shown significant efficacy in the treatment of various cancers. A prominent category within these therapies includes drugs designed to block the binding between PD-L1 and its associated receptor, PD-1, both of which are crucial in helping tumors evade immune surveillance. Interrupting this interaction enables the reactivation of T cells, thereby strengthening the immune system’s capacity to detect and destroy malignant cells.

Tumor mutational burden (TMB) serves as an essential biomarker for predicting immunotherapy outcomes, representing the total count of somatic mutations within a tumor's DNA. Although tumor mutational burden (TMB) does not appear to impact the success of treatments aimed at inhibiting angiogenesis, it is considered an important biomarker in forecasting patient responsiveness to immunotherapeutic strategies. These treatments work by altering specific immune checkpoints involved in immune regulation, including pathways mediated by proteins like PD-1, PD-L1, and CTLA-4 [112]. Tumors with a high mutational burden typically generate a higher quantity of neoantigens (abnormal proteins created by genetic mutations). This abundance of neoantigens improves the chances of immune system recognition and response, thereby boosting the effectiveness of immunotherapy [113]. Cancers such as melanoma, lung cancer, and urothelial cancers often have a high tumor mutational burden (TMB), while prostate and breast cancers typically show lower TMB [69]. Tumors with high TMB tend to express a wider array of neoantigens, making them more prone to immune system recognition and response, particularly when treated with checkpoint inhibitors. As a result, immunotherapy is generally more effective in cancers with elevated TMB [114,115].

Both malignant melanoma and RCC are highly immunogenic tumors, meaning they can provoke an immune response. The strong immunogenic nature of both cancer types has contributed to their responsiveness to immunotherapeutic approaches, especially those involving checkpoint blockade with agents targeting PD-1 and CTLA-4. However, the application of PD-L1 inhibitors in the treatment of renal cell carcinoma remains relatively restricted [116].

After the introduction of ipilimumab in 2011 for the systemic treatment of melanoma, immunotherapy has made significant strides in treating both resectable and unresectable melanoma [117]. The CheckMate 037 [118,119]and CheckMate 066 trials established nivolumab's efficacy in patients with metastatic melanoma. Additionally, the KEYNOTE-006 trial, which compared pembrolizumab and ipilimumab for advanced melanoma, showed pembrolizumab's superiority in terms of the 2-year overall survival rate [120].

In the 2023 ASCO Guideline Update on Systemic Therapy for Melanoma, checkpoint inhibitors are positioned as the primary treatment choice for malignant melanoma. They are recommended for neoadjuvant therapy (in resected stage III B-IV melanoma), as adjuvant therapy (for resected stage II B or higher), and for unresectable melanoma. For patients with unresectable or metastatic cutaneous melanoma, or resected stage IV melanoma, the combination of ipilimumab and nivolumab serves as one of the primary treatment options [121].

In selected cases of metastatic kidney cancer, monotherapy with an immune checkpoint inhibitor, such as pembrolizumab, can represent a suitable therapeutic choice, particularly when combination regimens are not indicated or tolerated. However, in metastatic patients whose disease is categorized as intermediate or poor risk and who are suitable candidates for immunotherapy, current clinical guidelines tend to favor starting with a dual-agent immunotherapeutic regimen as the initial line of systemic treatment [122]. Currently, five immunotherapy-based combination treatments have received approval for clinical use. These include the pairing of ipilimumab with nivolumab, pembrolizumab with axitinib, as well as other therapeutic duos such as nivolumab combined with cabozantinib, avelumab administered alongside axitinib, and pembrolizumab used in conjunction with Lenvatinib [123]. Among these, the ipilimumab and nivolumab combination is specifically approved for intermediate- and poor-risk mRCC patients [124].

Despite their efficacy, these agents also cause immune-related adverse effects that may be life-threatening if not detected and controlled appropriately [125].

Targeting the PD-1/PD-L1 immune checkpoint pathway has demonstrated significant therapeutic benefits in patients with advanced or inoperable bladder cancer, including those with metastatic disease. Nivolumab has received FDA approval as adjuvant therapy in individuals with urothelial carcinoma who face an elevated risk of disease recurrence after undergoing surgery. Additionally, the PD-1 inhibitor pembrolizumab was authorized for use in patients whose condition has worsened despite prior treatment with platinum-based chemotherapy, a decision supported by findings from the phase III clinical study known as KEYNOTE-045 [126].

For prostate cancer, the use of angiogenesis and checkpoint inhibitors in therapeutic strategies is limited. Currently, pembrolizumab is the only FDA-approved treatment option, although it has not been approved by the EMA. [127].

Beyond the therapies previously mentioned, each of these pathologies benefits from specific treatments targeting different types of genetic mutations and molecular pathways.

For malignant melanoma with BRAF gene mutations (V600E/K), treatment combinations such as dabrafenib with trametinib (or encorafenib with binimetinib) are indicated [128,129].

In prostate cancer, besides androgen receptor pathway inhibitors, non-hormonal, non-cytotoxic treatments like poly-ADP ribose polymerase inhibitors (PARPi) have emerged, specifically designed to target cancer cells harboring BRCA mutations [130]. While no AKT inhibitors are currently approved, the investigational drug ipatasertib has demonstrated significant activity in clinical trials when combined with abiraterone acetate in patients with loss of the tumor suppressor protein PTEN, as confirmed by immunohistochemistry [131].

CONCLUSION

The link between malignant melanoma and urological cancers is underpinned by common epidemiological factors and genetic mutations, establishing a foundation for progress in personalized medicine. As understanding of the shared molecular pathways in these cancers expands, opportunities for tailored prevention strategies, screening practices, and individualized treatments become increasingly apparent. Identifying genetic predispositions—such as BAP1 and CDKN2A mutations—early on, along with employing targeted therapies and immunotherapies, is expected to meaningfully enhance patient care in the near future.

Personalized medicine holds great promise for patients with elevated risks of both melanoma and urological cancers. By leveraging genomic profiling alongside advancements in immunotherapy and targeted therapies, more tailored and effective treatment options become possible, enhancing patient outcomes. However, ongoing research and clinical trials are essential to fully understand the effects of shared genetic mutations and molecular pathways and to establish comprehensive, individualized treatment protocols. Further refinement in treatment sequencing approaches will also be necessary to optimize care for these patients.

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The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

DPH contributed to the manuscript's conceptualization, methodology, discussion, supervision, and writing. MAB contributed to the manuscript's editing, data curation, and writing. NM contributed to the editing and data curation of the manuscript. OSC contributed to the conceptualization, discussion, and supervision of the manuscript. MDV contributed to the manuscript's conceptualization, editing, and data curation. CT contributed to the discussion, data curation, and writing of the manuscript. IA contributed to the methodology, review, and editing of the manuscript. BOF contributed to the conceptualization, discussion, review, and supervision of the manuscript. VMG contributed to the editing, data curation, and writing of the manuscript. OKIM contributed to the conceptualization, discussion, and supervision of the manuscript. CTM contributed to the conceptualization, methodology, investigation, discussion, review, editing, supervision, data curation, and writing of the manuscript.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

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ORIGINAL ARTICLE

Relationship Between Psychological Resilience and Attitudes Towards A.I. in Emergency Workers

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Abstract: The impact of artificial intelligence (AI) on our lives can no longer be neglected, and thus, understanding this phenomenon and how humans interact with it is becoming more important than ever. The study emphasizes one of the psychological characteristics that helps us in accepting AI and using it to its fullest, namely the psychological resilience that each of us, and in particular emergency service workers, possesses in different proportions. The analysis of the relationship between the two dimensions (psychological resilience and AI) highlights, among other things, the importance of how organizations can use this interaction to achieve a framework for developing the psychological resources of their employees so that they are increasingly receptive to the use of AI in their work, pursuing the benefits it brings. The study is conducted on 89 emergency service workers and focuses on the positive and highly statistically significant relationship between psychological resilience and attitudes towards AI. The study also particularly emphasizes the relationship between tenacity and self-efficacy, self-confidence, ability to learn from life experiences, rapid recovery from negative events, social and family resources as dimensions of psychological resilience in relation to attitude towards IA of emergency workers.

Keywords: mental resilience, artificial intelligence (A.I.), attitude, emergency workers

INTRODUCTION

People everywhere have suffered from various life events, but these have also provided an open research background from which we can affirm that some psychological characteristics, including psychological resilience, can support a sense of well-being, the ability to quickly restore happiness, post-traumatic growth, and some positive coping strategies after experiencing these events. One of the occupations where increased resilience is needed is that of emergency workers (firefighters, paramedics, police, psychologists, search and rescue teams).

Intervening in emergencies places maximum demands on the cognitive and neurological resources of each person working in such situations. It is thus imperative to understand how the brain processes information, manages stress, and makes quick decisions, because this understanding will lead to optimizing performance and maintaining the long-term mental health of these workers [1, 2, 3, 4]. Also, if we take into account that repeated exposure to traumatic events and high levels of stress can negatively affect the hippocampus, leading to difficulties in concentration, altered prosexic function, memory errors, or even freezes, all this makes it even more important to study the effects of stress on these workers and how they build resilience. One answer to all this may be A.I. through the various ways it offers to stimulate understanding of situations, acceptance of differences in reaction, stimulate coping mechanisms, highlight stress levels (including its various health effects through wearable devices), etc. A.I. can be used in different simulations to understand how they learn, or the different reactions in interventional cases, or afterwards; monitoring with wearable devices of different data and then analyzing them with A.I. (early signs of deterioration of health or well-being can be identified and proactive interventions can be proposed), identification of training needs, alternative learning routes customized on different worker characteristics, personalized training scenarios etc.

AI can be used to create a resilient infrastructure, a resilient organization, and resilient ways of working, and thus contribute to increasing the resilience of the participants in the process. Health is an important component for all people and the systems in which they function [5, 6, 7, 8, 9, 10].

A.I. is very helpful to emergency workers by:

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- rapid data collection and analysis (huge volumes of information can be processed in a short time to provide a quick and accurate overview of the situation);
- predictability and anticipation (A.I. can predict the evolution of an emergency by analyzing existing data, movement patterns of fire, typhoon, etc.);
- optimization of resource allocation (A.I. can help to optimize the distribution of crews, vehicles, and equipment, taking into account factors such as distance, traffic, type of emergency, and availability of resources);
- decision support (through rapid data analysis, scenario generation, AI can provide emergency workers with critical information for decision-making in situations of stress, and uncertainty - e.g., rapid patient resuscitation, prioritizing serious cases, etc.);
- robotics, and automation (robots, drones can penetrate areas that are dangerous for humans, and collect data to streamline intervention);
- effective, and improved communication (A.I. systems can help with real-time translation, answering emergency calls, and relaying information efficiently to teams and the public).

All these provide emergency workers with increased safety, increased efficiency in situations they would not have coped with on their own (e.g. dangerous areas they could not enter), better decisions through access to more complete information or a large volume of information processed quickly, reduced cognitive load (A.I. can take over repetitive tasks or data analysis, allowing staff to focus on human, and operational aspects).

Over the years, researchers have defined resilience in various ways. For example, in some studies [11], psychological resilience is considered to be the ability of individuals or outcomes to adapt successfully to challenging or threatening circumstances. Resilience also reflects a person's ability to maintain stability and equilibrium in the face of difficulties caused by particular life situations, including the epidemics that we are increasingly facing, and people with resilience might also experience positive emotions. It also states that resilience is characterized by positive behaviour, and attitudes in the face of adversity, courage, and perseverance in coping with life's difficulties, possessing a positive attitude even in the face of adversity, enjoying intimate relationships, and expanding social circles.

Other studies [12,13] highlight some key components of resilience, including self-improvement, and particularly positive attitudes maintained during adverse situations, learning from negative and/or positive experiences, novelty seeking, emotional regulation, positive future orientation, and the pursuit of meaningful life goals. Research on mental resilience considers it to be a person's ability to adapt positively to adverse circumstances or to maintain adequate functioning even in the midst of disastrous situations.

Experts are more and more concerned about the effects of life events on the psychological apparatus, especially long-term psychological problems, such as a decrease in subjective well-being, a decrease in adaptability, the onset of psychological trauma, etc., and how to combat them [14]. When the adversity experienced in PTG (Posttraumatic Growth) is high, it can stimulate the person's ability to withstand hostile and traumatic environments, therefore also increases the ability to enhance or accelerate his or her experience of posttraumatic growth [15]. However, research over the last two decades indicates that not all people who face adversity will suffer from mental or emotional health problems. It is known that some people experience positive emotions and psychological growth/development, and this is known as positive mental health, and changes include post-traumatic growth and resilience [16,17]. Scientists' concerns for resilience are all the greater as the world is constantly changing, natural disasters have multiplied, and people are often in stressful situations due to these changes. All the more, emergency workers have more challenges in the current context of climate change and the presence of many natural disasters. Certainly, A.I. facilitates knowledge of data that predicts the occurrence and evolution of traumatic events, and through this, can be useful for building resilient cities, resilient communities, and resilient people in general [18].

Although A.I. can be of real help to emergency workers in building their resilience, there are also some challenges they face in relation to A.I.:

- acceptance of A.I. – the level of acceptance of A.I. is related to the age of people (digital immigrants or digital natives, for example), the degree to which they find it useful, and easy to use;

- dependence on technology – over-reliance on A.I. can lead to problems in the event of technical failures or cyber-attacks;

- data quality – the proper functioning of the A.I. depends on the quality and accuracy of the input data;

- privacy, and ethics – the use of A.I. raises questions about the privacy of personal data, and the ethical aspects of decisions made by algorithms, and this is not yet sufficiently regulated;

- training, and adaptation – emergency workers need to be trained to understand, and use A.I. systems effectively (and this depends on how they are implemented in each institution or system in which they work);

- cost – Implementing and maintaining advanced AI systems can be expensive.

AI is a powerful tool that can revolutionize emergency management by providing essential support to emergency workers and can actively contribute to building their resilience. Resilience has a positive impact on both mental health, and wellbeing, it can help maintain subjective wellbeing, it maintains people's optimism, and belief that things will get better when faced with various challenges, it includes positive adaptive patterns that are developed over time in response to adversity, and people with high resilience can recover effectively from the impact of everyday stressors, so it becomes all the more important that we can optimize it.

Based on some research in the international literature that has highlighted that positive interaction with technology provides a framework for the development of psychological resources [19, 20], and that artificial intelligence (AI) promotes more effective coping mechanisms by increasing resilience, especially in a professional setting, and during emergencies [21], the objective of the present research was to analyze the relationship between psychological resilience – as a resource that ensures effective adaptation to various situations (new or difficult) –, and the attitude towards AI of Romanian employees.

MATERIALS AND METHODS

The general objective of the research was investigated by means of a questionnaire-based online survey, through a Typeform link containing information about the subjects' informed consent, their socio-demographic data, and the items of the two questionnaires - one analyzing psychological resilience, and the other analyzing attitudes towards AI.

Research hypotheses

The following hypotheses have been formulated to fulfill the general research objective:

Hypothesis 1 - We assume that there are interdependent relationships between psychological resilience and some of its dimensions (tenacity, self-efficacy, self-confidence, ability to learn from life experiences, rapid recovery from negative events, social, and family resources), and the attitude towards IA of emergency workers.

Hypothesis no. 2 - We anticipate that the attitude towards IA can be predicted and explained in terms of the psychological resilience of emergency workers, and its dimensions.

Subjects

The study participants were 89 people, employed in emergency services, from the South, aged between 19 and 43 years (M=29.56, SD=14.26), 51 male, and 38 female. The research sample was a convenience sample, utilizing available subjects, and the only selection criterion was that they were employed. Subjects participated in the research voluntarily, being informed about the research topic, that the results will be confidential, anonymized, and used for research purposes only, and also that they had to fill in two questionnaires (and a set of socio-demographic data) sent via a Typeform link.

Instruments

Psychological resilience was investigated by applying the adapted ARES-i25 scale [22], which contains 25 items distributed on the following scales:

1. tenacity, and self-efficacy – the competence to evaluate the life situation, and the necessary steps to solve the various problematic contexts, and personal resources/limits, as well as the ability to organize resources to solve problems;

2. self-confidence – reflects a positive self-image obtained in objective relation to intuition, and capabilities;

3. ability to learn from life experiences (personal, and/or other people's);

4. rapid recovery from negative life events - tolerance of negative affect, and uncertainty, recovery from failure to adopt resolving means, identification of sources of support, focus on goal achievement, and resistance to disruptive factors;

5. social, and family resources – are the factors external to the person that play a supportive role in coping with various life problems, and in manifesting resilience.

Participants' responses are scored in five steps (1 = strongly disagree, 5 = strongly agree), and the results can be reported both overall and by dimension. ARES-i25 was applied and validated on 423 participants in Bucharest, aged between 19 and 42 years, 260 female, 163 male. Following the factorial analysis, it was found that the scale, at the general level, explains 72.1% of psychological resilience, with coefficients showing that it has a very good internal consistency (Cronbach's $\alpha = 0.83$) at the general level, but also by dimensions ($0.71< \alpha > 0.89$). ARES-i25 also has content/construct validity, which was also verified by the Delphi method (10 experts). For the present study, the internal consistency of the scale items that were applied to the research subjects (Cronbach's α coefficient = 0.78) demonstrated the fidelity of the ARES-i25 scale in the new context in order to draw valid conclusions.

Attitudes towards artificial intelligence were investigated using the scale constructed by [23], abbreviated ATTARI-12, the authors building on other studies [24, 25]. The 12 items of the scale represent the three facets of attitude (cognitive, affective, and behavioral), with the scale containing an equal number of items for each of these, and reflecting equally positive or negative attitudes towards AI. Participants' responses are scored in five steps (1 = strongly disagree, 5 = strongly agree), and results can be reported both overall and by dimension. The ATTARI-12 was administered and validated on 488 U.S. participants, aged 19-72 years, 211 female, 273 male, and 4 subjects who did not indicate gender. The factor analysis found that the scale, overall, explains 79% of attitudes towards AI, with coefficients showing that it is a unidimensional scale with very good internal consistency (Cronbach's $\alpha = 0.93$), and that it also has convergent validity, with a statistically significant positive correlation with the scale of attitudes towards specific AI applications (r = 0.93), and that it has convergent validity, with a statistically significant positive correlation with the scale of attitudes towards specific AI applications (r = 0. 60), which proves the psychometric characteristics of the ATTARI-12 scale [26]. For the present study, the scale was translated, and the internal consistency of the items (indicated by Cronbach's α = 0.76) demonstrated the fidelity of the ATTARI-12 scale to draw valid conclusions.

RESULTS

THE IBM SPSS Statistics v23 program was used to analyze and process the data from this research.

For analyzing the first hypothesis, which assumes that between psychological resilience, and some of its dimensions (tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources -, and employees' attitude towards AI there are interdependent relationships), and the employees' attitude towards AI, the Pearson correlation statistical procedure was used. The obtained results are presented in Tables 1 and 2.

Table 1. Descriptive statistics for the variables psychological resilience, its dimensions, and attitude towards IA

Research variables	Mean	Standard Deviation
Psychological resilience	64.19	22.35
Tenacity, and self-efficacy	13.22	5.39
Self-confidence	12.97	4.24
The ability to learn from life experiences	16.45	5.13
Quick recovery from negative life events	13.89	3.94
Social, and family resources	17.38	5.36
Employee attitudes towards AI	32.44	11.57

Table 2. Correlations between psychological resilience, its dimensions, and employee attitudes towards AI

Research variables	Employee attitudes towards AI
Psychological resilience	0.91**
Tenacity, and self-efficacy	0.79**
Self-confidence	0.74**
The ability to learn from life experiences	0.83**
Quick recovery from negative life events	0.82**
Social, and family resources	0.75**

*** Correlation is significant at the 0.01 level (2-tailed)*

These results demonstrate a positive and highly statistically significant correlation between psychological resilience and employees' attitudes towards artificial intelligence (AI). Specifically, the more psychologically resilient a person is, the more positive their attitudes towards AI. This strong relationship (r = 0.87) is also supported by the analysis of individual dimensions of resilience. All the dimensions of psychological resilience - including tenacity, self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative events, and social and family resources - show strong positive correlations with employees' attitudes towards IA (with correlation coefficient values ranging from 0.74 to 0.91). This suggests that each aspect of resilience contributes to a favorable perception of IA. In conclusion, psychological resilience is a key factor in shaping employee attitudes towards AI. These findings may have important implications for organizations in the context of the ever-accelerating adoption of AI technologies, suggesting that developing employees' psychological resilience could facilitate a smoother and more positive integration of AI in the workplace. There are also statistically significant correlations ($p < 0.05$) between psychological resilience and its dimensions, and between all dimensions of resilience, but these are not within the focus of the research and have not been analyzed or presented in this paper. The effect size of each statistically significant correlation between different variables was calculated using the r2 coefficient; the values of this coefficient ranged between 0.54 and 0.82, thus indicating a high level of association between variables [27], which, beyond the statistical level, adequately reflects reality.

The results of the second hypothesis, predicting that attitudes towards AI can be predicted and explained by the psychological resilience of employees and its dimensions, were obtained by multiple regression. The standard analysis method (Enter) was used, considering 6 predictor variables (psychological resilience, tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources) for employees' attitude towards AI (criterion variable).

Examination of the multiple correlation coefficient (with R-value = 0.81) indicates a high correlation between the predictor variables, and the criterion variable,, and its R2 value (0.65) shows that 65% of the variance in employees' attitudes towards AI is determined by the predictor variables (psychological resilience, tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources).

The analysis of variance tested the significance of R, and the value of F (68.22 at p = 0.01) denotes that the predictor variables jointly predict the variance of the criterion variable.

The significance of the individual regression coefficients was assessed using the t-test, and their values (significant at $p < 0.05$) reveal that all predictor variables are important for the estimation of the criterion variable (attitude towards AI). The results are summarized in Table 3.

Table 3. Degree of contribution of predictor variable values to the regression line, and significance of multiple regression

	Unstandardized coefficients		**Standardized coefficients**	**t**	**p**	**R**	**R^2**	**F**	**p**
Model	B	Std. error	Beta						
(Constant)	87.13	11.16		38.45	0.01	0.81	0.65	68.22	0.01
Psychological resilience	42.15	12.43	2.31	10.57					
Tenacity, and self-efficacy	28.52	10.24	1.98	9.38					
Self-confidence	22.14	8.28	0.88	10.22					
The ability to learn from life experiences	21.76	8.33	0.71	11.54					
Quick recovery from negative life events	18.33	5.66	0.56	10.87					
Social, and family resources	14.99	8.47	0.42	11.07					

Based on these data the regression equation can be written: ***Employees' attitude towards*** AI = 87.13 + (42.15) * *psychological resilience* + (28.52) * *tenacity, and self-efficacy* + (22.14) * *self-confidence* + (21.76) * *ability to learn from life experiences* + (18.33) * *quick recovery from negative life events* + (14.99) * *social, and family resources*.

The reliability of the regression equation prediction was verified by calculating the bivariate correlation between the employees' attitude towards AI and its estimation (pre_1), obtaining r = 0.81 (value identical to the multiple correlation coefficient).

The statistical data obtained in the present research indicate that there are 6 predictors of employees' attitude towards artificial intelligence, which both individually and jointly estimate this attitude.

The results show that psychological resilience is the strongest predictor of employees' attitude towards AI, with a regression coefficient of 42.15. This suggests that, independent of its specific dimensions, an overall high level of resilience contributes significantly to a positive attitude towards AI and is a fundamental factor shaping employees' perceptions. The equation shows that attitude towards AI is not only influenced by overall resilience but also by the independent contribution of each dimension. Even though there is a conceptual overlap between overall resilience and its dimensions, the regression equation allows us to see the added impact of each component.

At the same time, these results suggest that organizations wishing to improve employee attitudes towards AI should focus on developing psychological resilience through specific programs, which would lead to easier acceptance of AI and multiple possibilities of its use.

DISCUSSION

The present research started from a number of studies in the literature that have emphasized that positive interaction with technology provides a framework for the development of psychological resources, and that artificial intelligence promotes more effective coping mechanisms by increasing resilience, especially in a professional setting, and during emergencies.

The objective of this research was to analyze the relationship between psychological resilience – as a resource that ensures effective adaptation to various new or difficult situations –, and the attitude of Romanian employees towards artificial intelligence.

The research results (through the significant bivariate correlations identified) proved that people with a high level of psychological resilience have an open attitude towards AI, but also that this attitude is directly proportionally associated with psychological resilience, and its dimensions (tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events,, and employees' social, and family resources).

Deeper statistical analyses have shown that these variables associated with employees' attitudes towards AI are also predictors of AI.

The novelty of the research is represented by the association between the level of psychological resilience - seen as a resource that ensures adaptation (to uncertainty, difficulties in life) of people from a psychological perspective -, and the employees' reporting to artificial intelligence.

Implementing AI in the workplace often brings with it significant changes: new tasks, the need to learn new skills, redefinition of roles, and sometimes even the fear of replacement. In such a context, psychological resilience acts as a protective shield and an adaptive engine. Resilient employees are, by definition, better able to manage stress and uncertainty, to adapt quickly, to see opportunities rather than threats in AI. In terms of the contribution of specific dimensions of resilience in attitudes towards AI:

- tenacity, and self-efficacy - people who believe in their ability to complete tasks, and overcome obstacles (self-efficacy), and who persist in the face of difficulties (tenacity) will approach AI integration with a proactive mindset, seeing it as a challenge that can be overcome;

- self-confidence - a strong self-esteem helps employees not to feel threatened by AI, but to perceive it as a tool that can enhance their own capabilities, not diminish them;

- the ability to learn from life experiences - this dimension emphasizes the importance of cognitive flexibility and openness to new things. Employees who have demonstrated this ability in the past will be more likely to embrace the new knowledge needed to work with AI.

- rapid recovery from negative events - in the context of AI, this translates to the ability to bounce back quickly from initial frustrations or learning difficulties. They do not give up easily but find ways to move on;

- social, and family resources - a strong support system provides a sense of security and stability, reducing anxiety about change, and allowing employees to better focus on adapting and learning.

This deep underst, anding of the link between resilience, and attitudes towards AI has major practical implications for organizations, namely investing in resilience development programmes, transparent, and empathetic communication (an organizational culture that promotes open dialogue, and underst, anding of employees' concerns can strengthen collective, and individual resilience, facilitating acceptance of AI), .creating a supportive work environment (where difficulties can be discussed, and employees find support in resolving them). In essence, the success of integrating AI into organizational structures depends not only on the technology itself but also, to a large extent, on the psychological preparation of the workforce. By cultivating resilience, organizations not only improve attitudes towards AI but also build a more adaptable, motivated, and prepared workforce for future challenges.

These results cannot be considered as exhaustive conclusions, but in relation to other particularities that could be mediating factors - for example, certain personality traits, openness to novelty, resistance to change, adoption of certain coping strategies, etc. [28, 29, 30].

The results of the present research are in line with the existing literature, with the concept of the importance of socio-technical adaptation of employees,, and the experience, and type of organization play a crucial role in facilitating adaptation to AI; conversely, social, and psychological adaptation of employees can be facilitated with the help of AI-based gadgets, and can reflect on the level of well-being (Giudici, Bento & Falk, 2024). In other words, the differences between organizations in their digital readiness are reflected in the individual's ability to adapt, which supports the socio-technical adaptation framework. Resilience as a central coping mechanism enables individuals to maintain their psychological equilibrium in diverse contexts, including those involving adaptation to new technologies [31, 32, 33, 34, 35, 36, 37].

From the literature review and the data obtained in the present research, it can be considered that psychological resilience as a personal resource helps to adapt to job demands, including those related to AI, helps to understand how AI can be used to increase performance, and well-being at work.

To clarify the influential relationship of psychological resilience on attitudes towards AI research, several directions should be pursued:

- exp, anding the number of subjects (starting from the main limit of this study, consisting of N = 89 subjects);

- to equalize the sample by gender to increase the validity of the conclusions drawn;

- analyzing the role of personality, and other particularities (such as openness to new, resistance to change, stress management, etc.) to establish a psycho-socio-demographic profile of people who would have an open but rational, and cautious attitude towards AI (maintaining a balance between addiction, and fear), respecting ethical principles in the use of AI products;

- exploring the involvement of the organization in developing the psychological resilience of employees to adapt more easily to new technologies, and to ensure an open but rational attitude towards AI.

CONCLUSION

In conclusion, starting from the fact that the research sample contains only 89 subjects, the research has the characteristics of a pilot study,, and the conclusions cannot be extrapolated to the general population of employed people, this being the main limitation of the study. The results of this research are important, however, in that they help to identify trends in the relationship of the influence of psychological resilience on open, rational attitudes towards AI, and to draw directions for further studies to complete the picture of the analysis of this relationship.

Conflicts of interest, and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript,, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, R.M., A.P., and M.L; methodology, R.M; software, A.C.; validation, R.M., A.P., and M.L; formal analysis, R.M; investigation, M.L, and A.C.C.; resources, A.C.; data curation, A.P., and M.L.; writing—original draft preparation, R.M.; writing—review, and editing, R.M, and A.P.; visualization, M.L..; supervision, R.M; project administration, R.M.; funding acquisition, M.L., and A..C. All authors have read, and agreed to the published version of the manuscript.

Ethics approval, and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines, and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Positive Experience and Patient Satisfaction – Determinants of Paradigm Shifts in Healthcare

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Abstract: Today's recognition of health as a fundamental value of humanity and the spectacular digital evolution of the environment dominated by permanent and dynamic changes has brought healthcare marketing to the forefront. The trends continuously affecting medical services have generated the need for increased involvement of marketing, with all current developments in healthcare having an indispensable marketing dimension. The successful implementation of a health marketing strategy aimed at enhancing patient satisfaction and fostering a positive care recipient experience, coupled with the cost-effectiveness of the health services offered, has necessitated the development of an adaptive marketing function that harmonizes the set objectives, addresses capability gaps, accommodates cultural realities, and considers individual mentalities and situational circumstances. The direct interaction with patients has made it possible to understand the need for a new paradigm of healthcare services, the demand to comprehend patient satisfaction and positive experience, the call for creating innovative models to address the patient experience, and, above all, the requirement to communicate with the patient continuously and effectively throughout the continuum of healthcare. The challenge for the management of future health systems and organizations is encapsulated in the words of Professor Drucker, who stated that the best way to predict the future is to create it. An exploratory research on the state-of-the-art of the literature was conducted with key references to the most important aspects captured by specialists on positive patient experience and satisfaction, which contributes by highlighting the determining factors that have led to the paradigm shift in healthcare.

Keywords: patient satisfaction, positive experience, new healthcare paradigm, adaptive healthcare marketing, management of healthcare, digital evolution

INTRODUCTION

Given the crucial role of a positive patient experience and satisfaction in enabling individuals who require medical care to make informed treatment choices and prioritize their own needs, the demand for innovative models to address this aspect is evident. These models must reflect the patient-centeredness of healthcare, rather than focusing on the doctor, procedure, or healthcare institution. Also, the models have to consider the medical condition which needs to be seen over the full cycle of care, reflecting the empathy and respect given to patients, the way communication with staff is carried out, the methods by which the information-communication binomial works during the hospitalization period and, of course, confirm the moral values of the hospital unit.

Experience is a core strategy in modern marketing, defined as "the perceptions, emotions and thoughts that consumers have when they come into contact with market services and engage in consumption activities" [1,2]; therefore, the experience is deeply personal and subjective in nature, requires learning and is based on human senses and is persistent over time [3].

Experience is used to influence consumers and is directly related to their level of satisfaction [4]. Four possible strategies are used for its planning:

- designing compelling experiences that stimulate the consumer's interest, attention, and desire to retain information about the service;
- engagement, which is achieved when the consumer feels that they resonate with the service, which they find relevant and necessary;
- emotion induction, which stimulates attention and learning,

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- creating temporary memories, which over time become permanent memories and define new experiences.

The ultimate strategy is to interconnect all these experiences in a holistic, intensive approach, designed to improve the life of the healthcare consumer significantly.

One innovative model for approaching the patient experience is the seven-step model, which includes the following processes: define, research, conceptualize, prototype, choose, implement, and learn. At Stanford University, students took a two-day course offered by the Hasso Plattner Institute of Design, seeking ways to enhance the patient experience in the Stanford Hospital Emergency Department. For the exercise, they role-played patients and their family members to get a sense of what it's actually like to be in the often chaotic atmosphere of an emergency department [5]. The following, among others, were highlighted in this context:

- empathy is a key element of design thinking, a step-by-step approach to problem solving that involves observing and interviewing patients as they go through an experience and then using this information to realize both a prototype and reliable ways to improve the product or process.

- patients wanted a regular flow of information to help them better understand what was happening and how their care providers communicated with each other;

- coordinated and clear communication, in the patients' view, is most helpful in calming anxiety and natural feelings of fear [5].

As is well known, the patient experience, one of the three activities that drive outcomes, along with diagnosis and safe, error-free execution, can be improved by coordinating the patient journey, reducing handoff time, focusing on the audiences of interest, the Disney management approach (every team member is trained to be an effective communicator), and building on traditional health systems (such as the holistic Ayurveda, which emphasizes the unbreakable connections between body, mind and spirit), progressing touchpoints and reforming interactions.

The HealthLeaders Media Intelligence Unit is a division of HealthLeaders Media and has for many years been considered a premier source for health care management research, providing analysis and forecasts through digital platforms, customized reports, customized reports, printed books, conferences, roundtables, peer-to-peer networking technology opportunities (where each computer has equal rights and responsibilities, with network devices sharing resources and communicating directly with each other), and various presentations for senior management [6].

The measurement of patient satisfaction is the application in health care of the concept of customer satisfaction, a concept that comes from both quality management and marketing [6].

As specialists argue, patient satisfaction has an impact on: retaining existing patients and gaining potential ones; the perceived quality of healthcare services by patients as a result of both the interaction with medical staff and the communication through which care recipients are informed about solutions to the medical problems they face, solutions offered by the healthcare facility in comparison to its competitors; compliance with doctors' recommendations; patients seeking a second medical opinion; health outcomes achieved by the health facility in question; satisfaction of the health facility's medical staff; patients not resorting to malpractice lawsuits; patients becoming promoters of the health facility's brand [7].

Several different types of satisfaction questionnaires exist in the medical literature, an established benchmark being, for example, the PSQ-III Patient Satisfaction Questionnaire (a more recent version of the PSQ), a survey of global satisfaction with health care, as well as satisfaction with six aspects of care: technical quality, interpersonal manner, communication, financial aspects of care, time spent with the doctor and accessibility of care [8].

On the other hand, as a short-form version of a hospital patient satisfaction questionnaire, we can consider a combination of a myriad of variants, including the one used by St. Joseph's General Hospital, Elliot Lake [9].

The constant concern about the satisfaction of patients receiving health care services in one of the health care organizations allows resorting to a questionnaire that would allow to objectively assess the degree of patient satisfaction (a significant indicator of health care) treated in a health care facility, and based on the interpretation of the results, to recommend to practitioners solutions to improve this experience, with the effect of optimizing health outcomes and increasing brand awareness in the relevant market.

OBJECTIVES AND METHODS

The main objectives of this study are twofold. First, it aims to examine the key determinants of the emerging patient-centered paradigm in healthcare, with particular emphasis on how positive patient experiences and high levels of patient satisfaction catalyze this paradigm shift. Second, the study evaluates the role of adaptive and innovative marketing strategies in healthcare, including experiential marketing approaches to enhance patient satisfaction and align healthcare delivery with patient needs and expectations. Taken together, these objectives underscore that patient satisfaction and positive patient experiences are central pillars of contemporary healthcare marketing, highlighting the necessity of adopting patient-centered strategies and illustrating how such approaches can drive the transformation of healthcare systems toward more empathetic, responsive, and digitally integrated services.

To accomplish these objectives, an exploratory review of relevant literature was conducted, complemented by a theoretical analysis of key sources. This bibliographic research focused on existing studies addressing the patient-provider service paradigm, experiential marketing in healthcare, and established tools for measuring patient satisfaction, thereby grounding the discussion in well-founded concepts and evidence. Through the synthesis of these sources, the study provides a comprehensive theoretical perspective on the factors driving the patient-centric shift and on the evolution of marketing practices tailored to patient expectations.

RESULTS AND DISCUSSIONS

Paradigm shift in healthcare: patient as healthcare provider

It is well known that paradigms are fundamental frameworks around which disciplines and schools of thought are generated, and most of the paradigms associated with service management have, according to numerous studies, originated from the marketing discipline and perspective. Inevitably, given the generous perspectives that marketing offers, many paradigms associated with services have emerged, with a particular focus on the management sphere.

Given that certain paradigms within service marketing may diverge from traditional operations management principles, leading scholars in both domains have advocated for the development of a renewed and integrative service paradigm.

The review of paradigms in the literature led to the proposal of a customer-supplier service paradigm superior to alternative service paradigms. Thus, the client-provider paradigm (patient-provider of health care services, in the case of health care services) is considered a useful and justified basis for the present and future study of (health care, in our case) services.

The service operations paradigm considered to be prominent is the customer contact model, introduced in the late eighties by R.B. Chase, whose fundamental argument is that the potential efficiency of service operations is limited by the amount of customer contact with the service provider's employees [9].

Furthermore, Chase recommended the famous equation below [10]:

$$\text{Operational potential efficiency} = {}^{\int}(1-)\frac{\mathit{Client\ time\ contact}}{\mathit{Service\ time\ creation}}$$

To understand the customer-supplier paradigm, it is necessary to realize that the customer-supplier paradigm does not focus on what customers do but on what they provide as inputs to service processes. This paradigm shift is that customer-provided inputs are the defining characteristic of services, as long as the customer is identified and the process being analyzed is specified. Also, processes rarely occur in isolation; rather, they exist within supply (value) chains, where one process feeds into another process. Thus, the customer-supplier service paradigm clarifies service supply chains, showing how they involve customers both as providers of inputs and as consumers of outputs [10].

For a correct understanding of this paradigm in the sphere of health services, two essential aspects must be taken into account, according to the rich literature [10]:

- Supply Chain Management: the patient-provider paradigm of health care services identifies the complexity of the supply of health care services and shows how to involve patients in their dual roles, those of input providers and output consumers.

- The universal input-output model: the patient-provider paradigm focuses on the patient's needs and wants, which is the defining characteristic of health services.

The primary paradigm in health services has been naturally generated by the interaction between the patient and the healthcare provider and has proven superior to alternative service paradigms, thus providing a viable basis for studying health services.

Examples of adaptive marketing in healthcare

The exponential developments in healthcare necessitate a continuous search for innovative solutions to meet the needs of existing patients, retain them, and attract new ones, while providing high-quality services that take into account the diverse perceptions of all patients. This requires dynamic and creative management strategies, as well as specific patient-centered marketing actions.

The service revolution continues, always presenting new challenges for marketing management, new rules of competition, new methods of organization, new commitments regarding the communication process and interpersonal relationships, as well as new approaches to economy and intelligence [11]. Dynamism, innovation, and technology are now considered essential tools in a scientific approach [11].

Professor Richard K. Thomas argued that "health professionals, especially physicians, fall into a special category, and the fact that they make most of the decisions about patients creates a unique dynamic to health care." [12]

Kotler recommended the development of stronger marketing (i.e., making it more important than production), replacing old marketing with new marketing, the latter of which should be holistic, strategic, technologically oriented, and financially focused [13].

The strong development and affirmation of marketing in the health sector are due to the rapid and diverse evolution of marketing tools, the marketing mix, and especially the various types of marketing that have adapted quickly and effectively in the health sector. One of these is experience marketing, also known as experiential marketing, a specific strategy through which organizations target different categories of consumers with varying cultural values, satisfying their wants and needs by offering emotionally triggering experiences that stimulate their senses and facilitate the formation of personalized bonds [14].

Experiential marketing can include five types of experiences [4]:

1. *Sensory experiences*: occur when human senses are stimulated:

- sight, through attractive colors and shapes, which are part of the brand identity and intended to be memorized by consumers;
- hearing, through music and sounds used as positioning elements;
- sense of touch, to which men are more sensitive than women;
- the sense of smell, which can trigger certain emotions.

Marketing strategies aim to differentiate an organization and its services from other similar organizations and to add value to services through their sensory appeal.

2. *Affective experiences*: These are determined by the inner feelings of the consumer, to whom the use of the service triggers certain emotions, feelings and moods. Marketing strategies are geared towards increasing the intensity of emotions and developing a sense of anticipation of the pleasant feelings that will follow after using the service.

3. *Experiences through thinking*: stimulates the consumer's creativity and challenges them to solve problems, actually encouraging them to get involved in evaluating the service received and the organization. The marketing strategy works in several directions, designed to surprise the consumer, arouse their curiosity, and create controversy by breaking conventional boundaries - actions that address both convergent, rigorous, and systematic problem analysis thinking and divergent, free thinking.

4. *Experiences through action*: involves the consumer in direct interaction with the health care service, towards which he or she takes an attitude and changes some of his or her lifestyle habits. Strategic directions refer to either physical reactions (such as reflex actions or body signals) or changes in mindset and lifestyle.

5. *Relational experiences*: They provide the consumer with the opportunity to learn about entities as a result of the buying and consuming process.

Marketing strategies also have multiple directions:

- public awareness of a particular social category and its advantages;
- identification of the consumer with an established reference group, so that he/she is convinced that he/she has the same needs as the members of the reference group;
- highlighting, whereby membership of the reference group is perceived as beneficial.

The evolution of holistic organizational ecosystems in the context of the broad and ongoing process of digital transformation, as well as the importance of aligning external trends with internal strategic priorities in addressing the new digital transformation model - digital marketing and digital health- are evident [15].

The potential of digital technologies and data to enrich understanding and marketing as a cultural architect is huge. Digital transformation plays a crucial role in advancing healthcare ecosystems, particularly through the strategic use of digital marketing [16]. This has underscored the importance of adopting a strong human-centered approach, effectively managing digital marketing efforts, and integrating artificial intelligence (AI) with the Internet of Things (IoT). Additionally, leveraging AI-driven insights for diagnostics and other applications is becoming increasingly essential.

Nowadays digital technology has become more and more used in everyday activities, and its intensive use and in most areas of activity has brought changes in the structure of society, and even in the psychology of individuals, who are gradually changing their conceptions about life, work, health and education, the ideal way of spending leisure time and the attributes of an optimal work climate. In this general context, it is clear that conceptions of the healthcare process are also changing, and, naturally, they should keep pace with technological progress and incorporate new tools, new treatments, new procedures, and new medicines, in an attempt to simplify and streamline it. Some of the recent spectacular developments in digital marketing worldwide:

- Digital transformation is enabling a closer connection with customers, responding to the new demands of supply chain management (few business functions are as profoundly disrupted by digitization as marketing).
- In the context of an organization's greater interactivity with more empowered customers, amidst facing particular challenges, marketing is now obliged to offer something for each and every customer [17].

- We live, today, in a much more complex world of digitally enabled smart customers, digital products, digital business operations, and digital competitors [18].
- It is important to identify the most useful insights by understanding what is happening at the confluence of people and technology, taking into account the ability of marketing technologies to bridge different technologies [19].
- Marketers need to enhance the customer experience by getting a complete picture of their customers and delivering real-time experiences [20].
- Marketers now have a critical role in the process of continuously identifying digital opportunities along the entire value chain.
- Marketers must first, and best, understand the difference between change and transformation.
- Marketers must integrate people, technology, and best practices, given the accelerated social interaction of social media.
- There are already new digital tools and new ways to drive change [9].

CONCLUSION

The beneficiaries of healthcare services, the patients, are people with fundamentally different lifestyles, varying ages, diverse social backgrounds, differing levels of education, diverse health conditions, and, of course, different expectations and conceptions.

To resonate with these characteristics, the portrait of the ideal doctor has also changed significantly: first and foremost, he must be an excellent professional, a good communicator, approachable and friendly, transparent, enthusiastic, passionate, able to draw realistic conclusions and objectives and provide immediate and effective feedback on health issues, willing to offer professional and emotional support to his patients.

In the midst of continuously identifying digital opportunities along the entire value chain, marketers need to understand the difference between change and transformation better, integrating people, technology and best practices, taking into account the accelerated social interaction of social media, enhancing the patient experience by improving knowledge of patients' needs, wants and attitudes, gaining a complete picture of them and delivering real-time experiences [20].

The transformation of economies into service economies - i.e., economies in which the production and consumption of services predominate - fundamentally changes the structure of society. This economic transformation is being significantly driven by rapid advances in technology, particularly in information and digital technology [21].

This new, highly topical paradigm in healthcare requires not only innovative models of approaching the patient experience and especially patient satisfaction, but also the obligation of permanent, accurate, and clear communication with the patient.

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Authors' contribution

Conceptualization MCMS, ITS, ADS, DLDM, AGS, and VLP; methodology MCMS and ITS; software, MCMS and ITS; validation MCMS, ITS, ADS, DLDM, and VLP; formal analysis, MCMS; investigation, MCMS, and ITS; resources, ADS, DLDM, and MCMS; data curation, MCMS, and ITS; writing-original draft preparation, MCMS; writing-review and editing, MCMS; visualization, MCMS; supervision, ADS and DLDM; project administration, MCMS, and ITS. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Not applicable.

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REVIEW

Psychotherapeutic Interventions for Combat Soldiers in Complex Clinical Settings

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Abstract: This narrative review examines psychotherapeutic interventions for combat soldiers who deal with post-traumatic distress and face a complex therapeutic setting where the relationships between clients and therapy providers are often conflictual. Despite available emotional aid, gaps between traumatized soldiers' emotional needs and institutional solutions are inevitable and are challenging for clients and therapists alike. These issues are discussed through Israeli soldiers' experiences, a population at high risk for trauma-related distress, due to this country's ongoing security threats. Following a narrative literature review, four key themes emerged: mutual difficulties in admitting mental injury, intergenerational trauma, preserving the soldier identity upon discharge, and choosing among therapeutic opportunities. The study highlights the need and rationale for diverse biopsychosocial interventions for veterans and proposes considerations for enhancing treatment success. Results emphasize the importance of tailored approaches that address both individual and systemic factors affecting veterans' mental health. Guidance for psychosocial interventions is provided, considering the multifaceted nature of combat-related trauma.

Keywords: combat, Israeli soldiers, military, psychotherapy, soldier identity, posttraumatic stress disorder, psychological trauma, veterans

INTRODUCTION

Military service and exposure to war zones affect millions worldwide, putting soldiers and veterans at high risk of physical, emotional, and psychological responses, from resilience to stress-related psychopathologies [1,2]. Posttraumatic stress disorder (PTSD) rates are higher among veterans than in the general population, estimated at 15% of combat veterans [3]. In Israel, military service is mandatory for Jewish and Druze men and Jewish women; many other citizens voluntarily enlist to promote social integration [4].

Active service begins at age 18 for 2–3 years, followed by reserve duty until age 40 [5]. Despite widespread exposure to potentially traumatic combat events, PTSD rates among Israeli Defense Forces (IDF) personnel remain unclear. Recent studies reported a trauma exposure rate of 96% among combat veterans [6], with PTSD estimates ranging from 2% to 16.5% [7–9]. However, the current ongoing conflict has likely increased these rates [10].

Official recognition of mental injuries in Israel, similar to that in other countries, is more complex than physical harm. Providing psychological aid to traumatized soldiers involves dilemmas about the nature, timing, and recipients of interventions, as well as the personal and social consequences [11,12].

The current narrative review [13,14] examines the psychological treatment of military personnel in conflict settings, a perspective that is rarely discussed [15]. It considers the diverse landscape of mental health opportunities offered for Israeli veterans, including official services, the private sector, and non-profit organizations. It aims to contribute to the international discourse on mental health care for military personnel in complex, conflict-prone environments by illuminating considerations for clinicians and policymakers dealing with emotionally injured soldiers or veterans.

This review explores two main research questions:

- What obstacles and dilemmas do professionals face when providing veterans with therapeutic solutions?
- What lessons can be learned from the Israeli experience to help clinicians worldwide better plan therapeutic interventions for military populations?

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MATERIALS AND METHODS

This paper presents a narrative review [13,14] of the literature on psychological interventions for Israeli combat veterans, supplemented by clinical case descriptions. This approach was chosen to provide a comprehensive overview of the topic while allowing for the integration of diverse perspectives and critique, and deepening the understanding of clinical insights through the inclusion of case materials.

A thorough literature search was conducted using PsycINFO and Medline (PubMed), supplemented with manual searches on Google Scholar. The search was restricted to peer-reviewed articles published in English and Hebrew between 1990 and May 2025. Additionally, clinical papers and book chapters that present the treatment of combat soldiers and veterans were also included. Non–peer–reviewed sources were intentionally sought to describe the study's socio-political context, and they included major local and international press. Papers and unofficial media or press publications were excluded if they were not initially published in Hebrew or English. No private or social media forums were included in the current narrative review. Search terms included psychological intervention-related keywords such as Psychotherapy, Group Therapy, Public health system, Private Sector; trauma-related keywords such as Trauma, Post-traumatic stress symptoms, PTSD, Suicidality; and military-related keywords such as Veterans, Soldiers, and Israeli Defense Forces.

The narrative synthesis of the literature focused on identifying key themes and issues relevant to the psychotherapeutic treatment of Israeli combat veterans, in light of global psychotherapeutic trends. These themes were organized into four major categories:

1. Admitting the Injury: Psychological Help for Mentally Injured Israeli Soldiers

2. Intergenerational Traumatic Consequences

3. Preservation of the "Soldier Identity"

4. Making Sense among Therapeutic Opportunities

To illustrate the clinical implications of these themes, case examples were drawn from the author's professional experience as a therapist and clinical supervisor. These case descriptions reflect patterns seen frequently in clinical practice and exemplify complex clinical situations. These composites offer a bridge between research findings and clinical practice, providing concrete examples of how theoretical concepts manifest in real-world therapeutic settings. All presented cases are composites of multiple real cases, with names and identifying details altered to protect client confidentiality.

The integration of literature review findings with clinical case descriptions allows for a rich, nuanced exploration of the psychological and psychosocial interventions that Israeli soldiers receive after developing traumatic distress during exposure to military-related trauma [14]. This approach aligns with the narrative review format, enabling a comprehensive discussion of complex clinical scenarios that may not be fully captured by more structured review methodologies. By combining a broad overview of the literature with detailed clinical examples, this study provides valuable insights for researchers and practitioners working with combat veterans, highlighting the unique challenges and opportunities in this field of psychotherapy. Since the majority of IDF combat soldiers are men, the examples presented describe treatment cases involving male soldiers only.

However, the ideas they illustrate are equally applicable to the experiences of female soldiers. Still, further research is needed to examine the unique challenges faced by female combat soldiers in coping with the emotional post-service impact on their lives, as well as how they utilize therapeutic services.

RESULTS

Admitting the Injury: Psychological Help for Mentally Injured Soldiers

Due to mandatory IDF service, Israel provides various treatment options for military-related distress [16]. IDF mental health services involve about 260 professionals, mostly psychotherapists and psychiatrists [17]. According to the press, these numbers have tripled since October 2023 due to increased demand [18]. Mental health professionals can be accessed across military units, and soldiers can seek help during or after service through their unit's psychologist or the Unit for the Treatment of Combat-Related PTSD. Professionals offer evidence-based psychotherapies in various settings. However, many veterans complain of insufficient or inadequate treatment, according to media reports [19].

The case of Sergeant Y.S., a 26-year-old Israeli veteran who self-immolated in 2021 to protest inadequate mental health support, highlights the challenges that IDF veterans face in seeking help. Unofficial reports indicate that this incident led to increased public awareness and the implementation of the "Nefesh Achat" (One Soul) reform to improve care for traumatized veterans [20]. However, the media keeps raising the voice of many veterans who still report insufficient mental health care [19,21]. Such reports highlight the risk of suicidality and suicide attempts, which have increased among Israeli soldiers in recent years [22], raising questions about the reform's effectiveness. According to these sources, many soldiers and veterans avoid seeking help due to stigma, fear of self-exposure, bureaucracy, or underestimation of their condition [23,24].

These barriers persist despite efforts to improve mental health services.

Intergenerational Traumatic Consequences

In Israel, most soldiers are second- or third-generation veterans, creating a unique context in which combat experience is viewed as a "civilian duty." The country's small size means combat often occurs near where soldiers live. Negative world views among parents can increase trauma-related stress in children through behavioral imitation and cognitive interpretation [25]. Indeed, mothers can play a crucial role in transmitting or buffering intergenerational trauma. These conditions make the effects of military service tangible for other family members and can lead to intergenerational and bidirectional traumatic stress, frequent shared traumatic reality, and secondary stress reactions in families of veterans [26]. Hence, therapists should consider the broader context of trauma, including its connections to a veteran's personal and interpersonal world.

Exemplifying this condition is the case of Josh, 27, who sought therapy 6 years after he finished his mandatory service. Growing up in a village known for its military sacrifices, he served in the armored corps, like his father did 30 years before him. During his service, Josh experienced traumatic events that triggered anxiety, doubt, and PTSD symptoms. Despite this, he described initially feeling undeserving of support and believing physical injuries were necessary to qualify for help. Only years later, when he experienced the horrific sights and fear of the October 7th, 2023, surprising attack by Hamas in the south of the country, he realized how his life followed a reliving of his father's traumatic experiences. As he joined his combat comrades on that day, he understood his emotional reaction was a transgenerational family burden, one he did not choose for himself. Encouraged by his girlfriend, Josh sought treatment for his military-related distress. Although emotionally clear to him, proving the connection between his current condition and past events was complicated, requiring careful navigation of bureaucracy and emotional sensitivities by both Josh and the professionals involved.

Preservation of the "Soldier Identity"

After service, soldiers face stress while readjusting to civilian life due to the stark contrast between military and civilian cultures [5]. Military service emphasizes power, aggression, obedience, and dichotomous thinking, whereas civilian life requires autonomous thinking, contextual understanding, and restraint. Transitioning between these worlds involves a challenging identity shift [27], affecting veterans in terms of personal, interpersonal, familial, and professional aspects of life. Due to their long-term reserve commitments, Israeli combat soldiers must maintain these conflicting identities for years and often never fully relinquish their military ways [28]. Combat experiences can lead to moral injury that exacerbates emotional burdens [29]. Facing longitudinal emotional stress and feeling trapped between ingrained military responses and unfamiliar, emotionally regulated approaches to problem-solving, veterans may resort to substance use [30].

David, aged 34, exemplifies these challenges. As a former infantry soldier, he struggled to adapt his combat instincts to civilian life. His aggressive responses, once valued in the military, became problematic in everyday situations. A turning point occurred when he reacted violently to his 5-year-old son's playful behavior that triggered memories of past military operations. In therapy, David realized how automatic responses to stress that he developed during service were now maladaptive. He grappled with guilt over his current actions as a father and past experiences as a soldier. This led to anger toward the Ministry of Defense, and David questioned the lack of support for veterans transitioning to civilian life and described feeling "used" and "disposable." This example illustrates the complex and long-term impact of military service on personal and family life.

Trauma exposure can interact with established traits among combat veterans, potentially leading to violent behavior [31]. Israeli therapists who are usually veterans may face challenges in helping other veterans process these complex situations, as they often struggle with their own identification or attempts to restore emotional balance in the face of military-related stress. This aspect often becomes a central transferential issue in treatment [32]. Helping veterans process such complicated situations and acknowledging their various emotional origins, as well as their current implications, is a challenging therapeutic task. Clinicians who deal with such conditions often find themselves either identifying with their patients or trying to help them reach a more balanced state, in which they can once again find order in their inner world. These reactions by clinicians are reflected in their hardships related to bearing witness to these violent acts [33]. Press and media reports indicate this situation is especially complicated for minority soldiers, who may face racism during service [33–35]. These individuals can experience both peer criticism and the stigma of seeking help [36]. Providing therapy in such contexts with multiple conflicts requires significant effort to build trust and emotional safety, along with a mature clinical attitude [37].

Choosing among Therapeutic Opportunities

Israeli veterans often struggle with the emotional toll of their service and rely on functional coping methods that can generate emotional avoidance and loneliness [5,38]. Like in other countries, many veterans hope time will heal their wounds or perceive their PTSD symptoms as minor, leading to underutilization of official support systems [40,41]. A recent study found 21% of Israeli combat veterans dealt with subthreshold PTSD, while 14% had clinical levels [39]. To address this increasing need, nongovernmental organizations have developed group intervention programs focusing on raising awareness, adjusting to civilian life, and enhancing emotional resilience [42,43]. These programs combine being in nature and outdoor activities, stress reduction techniques, and group processing of service-related events. They aim to enhance veterans' emotional coping, reduce guilt, and promote social recognition in the army service's psychological impact, helping veterans resolve unfinished business from their service in a supportive environment.

An example of these group interventions is the case of Daniel, 32, who grew up in an orthodox, traditional, low-income family. He described army service as a chance to achieve social mobility and volunteered for an elite commando unit, where he served as a sniper. After his service, he experienced emotional and physical stress, depression, and life dissatisfaction. He didn't connect these issues to his military service until participating in a group intervention program with his unit. There, he realized his depression stemmed from his role in killing others while in the military. The program helped him process a particularly conflictual incident during which he shot a young attacker. This shared experience with comrades helped him feel legit for his distress, relieve the big parts of it which were related to social demands and expectations, and seek further future therapy.

As described, army service often provides the first opportunity for minority youth to integrate into broader Israeli society [44]. However, minority soldiers often cope with both inner stress and social criticism when facing traumatic events and their consequences. Their race and socioeconomic background can impair resilience, increasing emotional vulnerability [37]. In such conditions, acknowledging combat-related emotions in therapy requires confronting difficult feelings, leading many young soldiers and veterans to avoid emotional processing and dwell in emotional numbing [45].

Sharon's case, a 23-year-old soldier who participated in a "friendly fire" incident where an Israeli soldier was killed, demonstrates this state of affairs. Right after this deadly incident, a group session with the unit's psychologist was provided, but all the squad's members remained silent throughout this whole session. In a later individual session, Sharon gave mostly rational answers to the therapist's attempts to reach his emotions, detaching from any emotional connection. Recognizing this as a defense mechanism, the therapist acknowledged the normalcy of stress and emotional detachment in such situations. He validated the ongoing challenges of combat service and encouraged Sharon to seek support, including after service completion. This message of partnership and hope proved crucial for Sharon's present coping and future development.

DISCUSSION

Balancing the Opposite Ends

Israeli society, like many others, often overlooks veterans' needs, focusing primarily on major psychopathologies while missing cases of long-term subthreshold PTSD [40,42,43]. Awareness has grown in recent years that combat soldiers need time to integrate into civilian life after service, with volunteer and "third-sector" organizations supporting this transition [5]. Providing therapy in this context is challenging due to unresolved questions about optimal timing and format [46]. Even though early and tailored interventions that leverage established social cohesion are considered beneficial [47], the effectiveness of psychological aid for young, cynical soldiers or veterans remains uncertain. The multicultural nature of Israeli society makes this tough clinical situation even more complex. Although army units are cultural melting pots, veterans often return to their original communities after service [44]. Balancing soldier and civilian identities is complicated, especially for people from traditional, patriarchal backgrounds in which admitting emotional hardships and seeking help through individual therapy can be intimidating [28,48]. Social workers must recognize both general and culture-specific aspects of these situations to support the integration of veterans into civilian life effectively [38,49]. This requires addressing controversies and the unique cultural needs of diverse populations when providing beneficial psychological aid. According to the press, these challenges have become even more difficult during the past 2 years, because Israeli reservists have been required to undertake numerous extended service rotations amid an atmosphere of extreme social division in Israeli society [49].

Resilience and social support are critical resources that can help combat veterans cope with PTSD and other stress-related symptoms. Although resilience levels among veterans are generally high, their impact often depends on individual coping skills [6]. Prior research and clinical experience have suggested that interventions should encourage authentic emotional expression, strengthen social relationships, and provide stress-management strategies, thereby reducing distress following military service without compromising resilience. Social support can play a particularly important role in mitigating symptoms of depression, like social withdrawal and emotional restriction [50]. Concurrently, identity integration remains a central challenge for many veterans during the transition from military to civilian life, particularly as they reconcile traumatic experiences and navigate societal expectations [27]. These processes differ based on cultural, social, and political contexts, highlighting the need for culturally competent practice. Group-based processing with peers can facilitate the integration of traumatic experiences into veterans' personal and social identities. Prior research [51] and clinical practice provide evidence of veterans who benefit from such interventions across a wide range of ages, depending on their emotional preparedness to utilize psychological help. Hence, practitioners must remain attentive to local cultural and familial attitudes toward military service and norms around emotional expression, which shape how veterans manage post-service adjustment and distress. These issues may be even more complex when dealing with female veterans' post-service emotional distress, which requires further study.

This study has limitations related to its narrative review methodology, which may have introduced author bias in the selection and interpretation of materials. Unlike systematic reviews, this approach does not reflect objectivity, reproducibility, or an exhaustive evidence search. However, it allows for holistic analysis, interpretation, and critique of the focal topic. This review synthesized current knowledge on treatment for Israeli veterans and highlighted knowledge gaps. It emphasizes the need for carefully considered solutions to treating traumatized veterans and encourages therapists to address their service-related emotional wounds, which may surface during therapy. Future research should explore the complex professional and relational aspects of this clinical area.

CONCLUSION

Both professionals and lay people who deal with PTSD symptoms often lack knowledge about the complexity of this condition and its effective treatments. Social workers and psychotherapists should provide traumatized veterans with comprehensive bio-psycho-social solutions. Professional coordination across sectors is crucial for optimal therapeutic outcomes, and public education is needed to help veterans recognize when and where to seek care. Although support for PTSD among veterans is a global need, recent developments in Israel and other countries demonstrate that progress in this field is achievable and should be prioritized by professionals and policymakers.

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Authors' contribution

As the only author, the author is responsible for the design, data collection, reviewing, interpreting the results, and revision of the manuscript.

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REVIEW

The Impact of miRNAs in Diabetes Mellitus

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Abstract: Diabetes mellitus refers to metabolic disorders whose main characteristic is chronic hyperglycaemia. The cause is either disturbed insulin secretion, insulin resistance, or usually both. MicroRNAs represent a subclass of non-coding RNA molecules that are short in length, about 17-26 nucleotides. Since they are circulant and can be tissue-specific, their use as diagnostic biomarkers or screening for different diseases is currently undergoing deeper studies. It was found that 22 miRNAs were associated with the pathophysiology of T1DM, 34 with T2DM, and 16 miRNAs were identified to be common amongst T1DM and T2DM. All of them were reconfirmed in at least two separate studies.

Keywords: microRNA; diabetes mellitus; insulin resistance; pancreatic β cells; gene silencing

INTRODUCTION

Diabetes mellitus (DM) encompasses a group of heterogeneous diseases characterized by hyperglycemia. The most prevalent forms of disease are Type 1 DM (T1DM) and Type 2 DM (T2DM). IDF has also just confirmed type 5 diabetes. It is reportedly characterized by severe insulin deficiency, mainly caused by undernutrition during childhood or adolescent years [1]. T1DM affects approximately 8.75 million individuals globally, whereas T2DM currently impacts about 6.28% of the world's population, accounting for 80% of all diabetes cases. DM represents a significant health issue both in terms of prevalence and the impact of its chronic complications, which can lead to organ failure (e.g., kidneys, retina, heart) [2-4]. Projections indicate that these values are expected to increase significantly in the coming years [5].

T1DM and T2DM are multifactorial diseases. T1DM is typically diagnosed following extensive destruction of insulin-secreting pancreatic β cells (70-80%) by T lymphocytes, resulting in the presence of self-reactive autoantibodies (T1A-DM). Non-immune mechanisms account for 10-30% of T1DM cases (type 1B - T1B-DM subclass), which are classified as idiopathic T1DM [6-8]. Idiopathic T1DM does not have an autoimmune background. Although it can present with insulin deficiency, this subclass of DM does not have a clear trigger. Fulminant T1DM represents another subclass that is also non-immune-mediated. It manifests by fast β-cell death, which may be caused by environmental and genetic backgrounds [9]. Altered insulin secretion, often paired with insulin resistance, represents the main mechanism involved in T2DM [10].

The predisposition for DM remains only partially understood. With some exceptions, a complex network of genetic and epigenetic factors can predispose individuals to T1DM and T2DM [11-15]. Since 2004-2005, exosomal microRNAs (miRNAs) have been included in the list of factors associated with DM and its chronic complications [16-17].

miRNA biosynthesis

Small RNA molecules (e.g., microRNAs -miRNAs; small interfering RNAs -siRNAs) are synthesized in the nucleus and subsequently transported to the cytoplasm. The origin of the miRNA molecules is a primary transcript that contains hairpin-shaped

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structures [18]. The RNase Drosha recognizes and cuts these hairpin structures in RNA, resulting in a miRNA precursor (pre-miRNA) of about 70 nucleotides in length. These molecules exit the nucleus (by means of exportin-5 complex) and in the cytoplasm are recognized and cut at the loop part by the enzyme Dicer [19]. The double-stranded miRNA is then loaded into the Argonaute (Ago) protein complex, and a complete silencing complex is formed, and the miRNA is used as a template for target recognition [18].

These double-stranded RNA molecules are utilized by the RNA-Induced Silencing Complex (RISC) to regulate gene expression [19]. It has been reported that the structural similarity of these miRNA hairpins and anti-Tn-C aptamers indicates miRNA functionality beyond RISC, making them even more sophisticated regulators [20]. The RISC complex acts as a translational repressor or as an mRNA breaker, depending on the number of base pairs that form between base pairs. One miRNA can target multiple genes, and they often return to silence the primary transcript from which they were synthesized.

The silencing process has critical roles in the physiological processes (e.g., cell differentiation, organism development) [21]. The concentration of miRNA was estimated between 1000 copies/ 1000 mm3 cells and around 200 copies/cell (or 166pM). It has been reported in previous studies that the median half-life of miRNA is approximately 5 days, with some exceptions (e.g., miR-155 was reported to have lower stability) [20]. The temporal and spatial deregulation of miRNA expression and the silencing process can result in pathological consequences (e.g., by modulating the expression of pro-inflammatory and pro-apoptotic effectors), which impact disease onset, progression, and severity [18,22]. The possibility of repeated sampling has increased scientists' interest in assessing the potential of miRNAs as biomarkers for malignant (e.g., lung adenocarcinoma, gastric or breast cancers) and non-malignant diseases (e.g., including DM) [23-27]. It should be taken into consideration that the multi-role of these molecules when comprising a panel for clinical usage. Thus, miR-21, miR-326 (e.g., glioblastoma), miR-34a, miR-2a (e.g., hepatocellular carcinoma), miR-155 (e.g., pancreatic ductal adenocarcinoma), miR-23b (e.g., melanoma, prostate cancer), miR-98 (e.g., squamous cell carcinoma; head and neck carcinoma), miR-186 (e.g., Hodgkin's lymphoma), miR-223 (e.g., chronic myeloid leukemia) were associated with different malignant diseases. But some of these miRNAs were also associated with different nonmalignant diseases (e.g., miR-21- cardiomyocyte hypertrophy; immune response in sepsis; miR-326 and miR-186 - multiple sclerosis; miR-590- extrinsic cardiomyopathy, autoimmune myocarditis; miR-223 - hereditary neutrophilia) [28].

STUDY OBJECTIVES

The study aims to perform a bibliometric analysis to evaluate research trends in the field and to identify and characterize miRNAs implicated in DM using curated bioinformatic resources.

MATERIALS AND METHODS

This study was conducted in two main phases: an analysis of the scientific literature and the identification of miRNAs altered in DM based on records in specialized databases.

Bibliometric Analysis. A comprehensive literature search was performed using the Scopus database [29]. The search strategy targeted the Title and Abstract fields with the following keywords: ("hsa-microRNA" OR "hsa-miRNA" OR "microRNA" OR "miRNA") AND ("type 1 diabetes" OR "type 2 diabetes" OR "T1D" OR "T2D") AND ("human" OR "humans" OR "Homo sapiens") NOT ("mouse" OR "mice" OR "rat" OR "rats" OR "murine" OR "animal model" OR "in vivo" OR "non-human" OR "rodent"). The initial search yielded 1730 articles. After excluding irrelevant subject areas (e.g., Engineering, Mathematics, Materials Science, Arts and Humanities, Social Sciences, Physics and Astronomy), 1,707 articles remained. Further filtering by document type (research articles only) reduced the number to 1085. Applying language (English) and publication stage filters resulted in a final dataset of 1051 articles. The literature analysis was conducted using the Bibliometrix R-package (version 4.4.0) and Biblioshiny (version 5.0), which enabled descriptive statistics, thematic mapping, and trend analysis [30]. All analyses were performed in R (version 2025.05.0, Build 496).

MiRBase [31] and TargetScanHuman 8.0 [32] were consulted to check for predicted target transcripts and conservation status of each miRNA. Diseases associated with miRNA were extracted from the Human ncRNA Gene Database [28].

RESULTS

Literature data analysis

The timespan of the analyzed publications was 2008 to 2025. The dataset comprised 1051 documents published across 440 sources, involving a total of 6409 authors and citing 49120 references. The annual growth rate of scientific output was 18.34%. The average age of the documents was 5.34 years, and the mean number of citations per document was 28.12.

Interest in the publication of this data began between 2008 and 2012 (Figure 1). A high increase was noticed from then on until 2015, with a spike from 2016 to 2022.

As for the average number of citations per year, it aligned with the wave of scientific production (Figure 2). The first spike was observed in 2009, followed by a second spike in 2012. A slight rise was noticed again, from 2018 to 2020.

Figure 1: Annual scientific production of data regarding miRNA and DM.

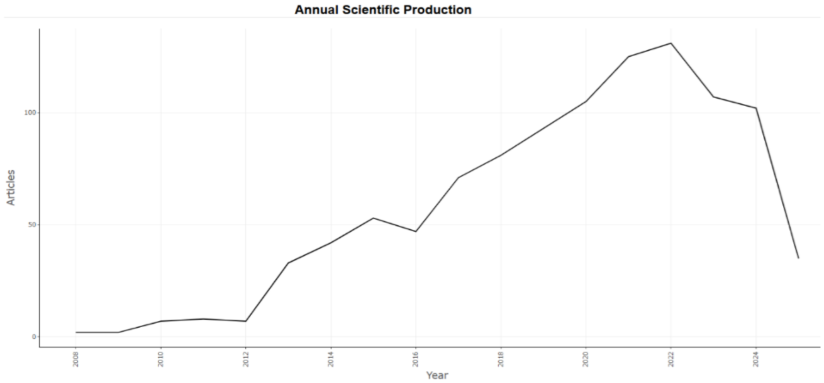

Figure 2: Annual scientific production of data regarding miRNA and DM.

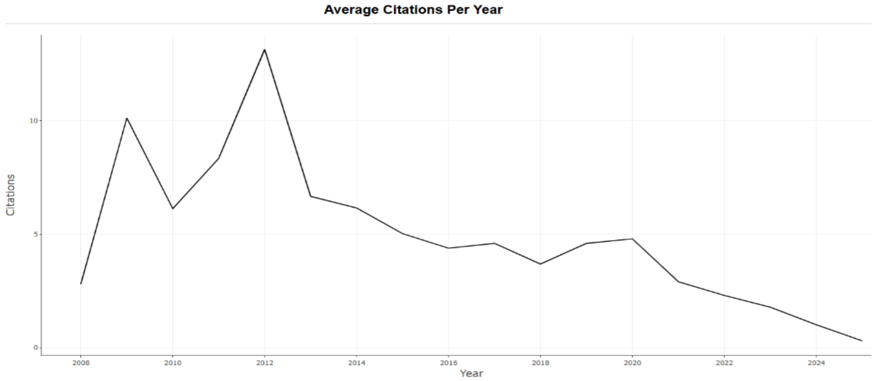

The 1051 articles were published in various sources. The majority of them can be found in PLOS One, Frontiers in Endocrinology, International Journal of Molecular Sciences, and Scientific Reports. Other sources included: Acta Diabetologica, Journal of Clinical Endocrinology and Metabolism, and Molecular Medicine Reports (Figure 3).

Figure 3: Most Relevant Sources containing the included articles.

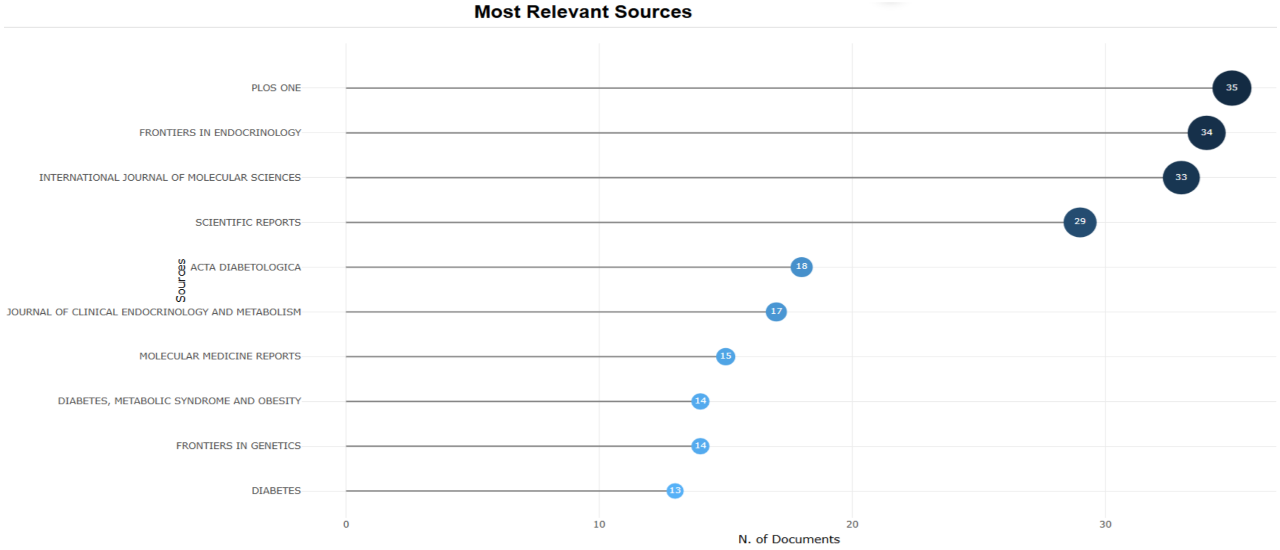

Figure 4: Most relevant authors of articles included in this study.

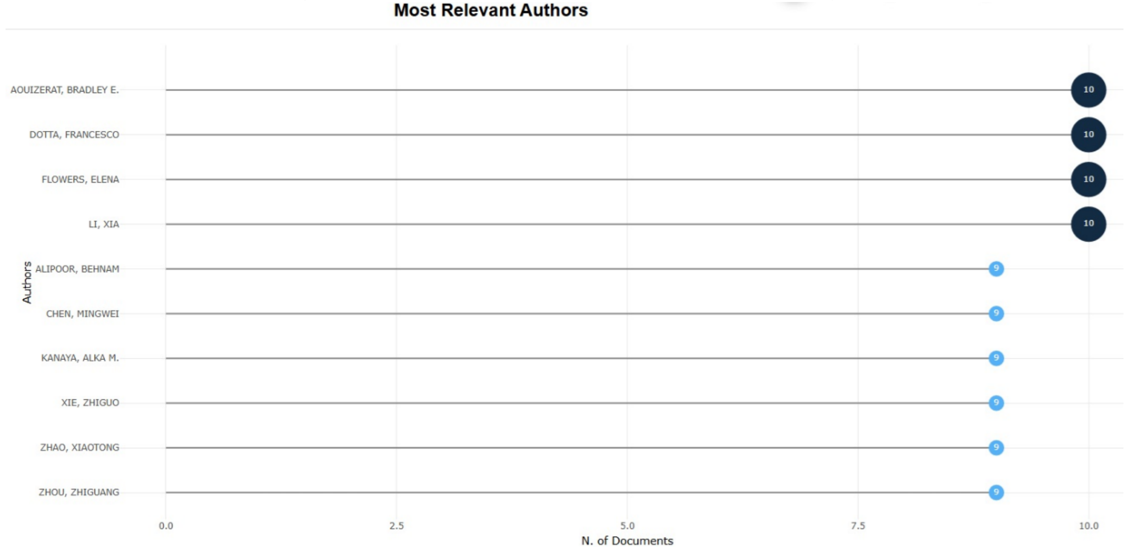

Figure 5: Most relevant affiliations of authors whose papers were cited in this article.

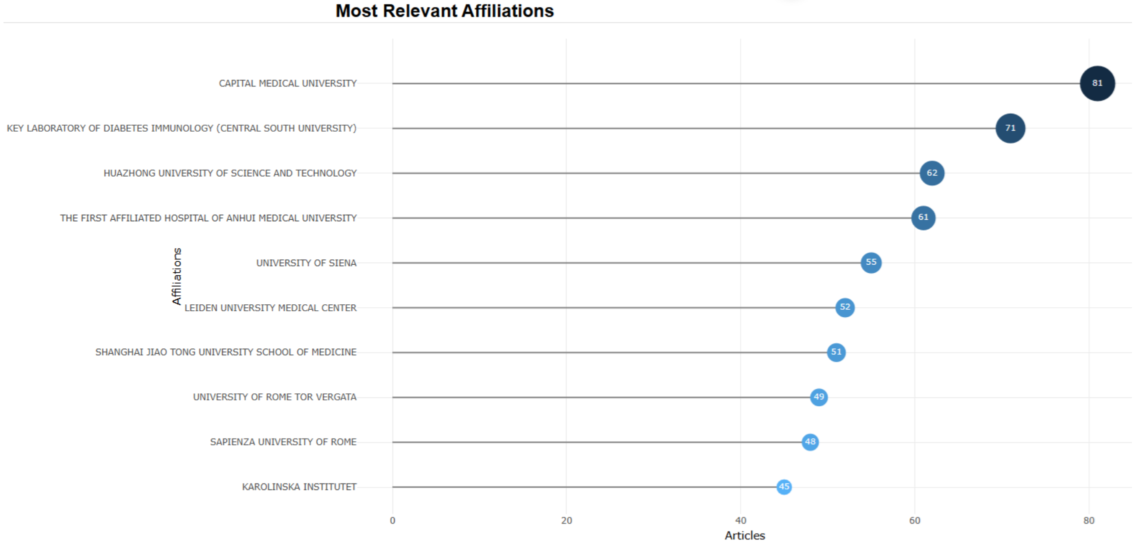

From the 6049 authors who participated in the research included in this paper, some of the most relevant are Dotta F., Flowers E., Li X., Chen M., and Zhao X. (Figure 4). Their affiliations include: Capital Medical University, Key Laboratory of Diabetes Immunology, University of Siena, and Huazhong University of Science and Technology (Figure 5).

Speaking at a global level, the papers with the most citations included in this study were [2,33-36]. The sources in which the works of the aforementioned authors were published include, in order: Oxidative Medicine and Cellular Longevity, Acta Diabetologica, The FASEB Journal, Journal of the American College of Cardiology, and The Journal of Clinical Endocrinology & Metabolism (Figure 6).

The trending topics identified in the reviewed articles highlight the evolving focus areas and emerging themes within the research field over time (Figure 7). Authors' keywords pinpointed in articles that constitute the database change over the years. First time span consisted of keywords such as: “cytokines”, “miR-375”, “single nucleotide polymorphism”. In recent years, keywords such as “diagnosis”, “molecular mechanisms”, “biomarker”, and “type 2 diabetes mellitus” were employed.

The co-occurrence network of authors’ keywords and the microRNA species miR-21, miR-126, and miR-146a highlights the relationship and thematic connections within the literature contained in the database of this paper (Figure 8).

The distribution of corresponding authors by country mirrors the ranking of the most cited countries (Figure 9; Figure 10). Leading the list are countries such as China, the United States, and Italy, which also represent the most active contributors to DM research. In terms of citation impact, Italy is followed by the United Kingdom, Iran, Spain, and Japan.

Figure 6: Most Globally Cited Documents cited in this paper.

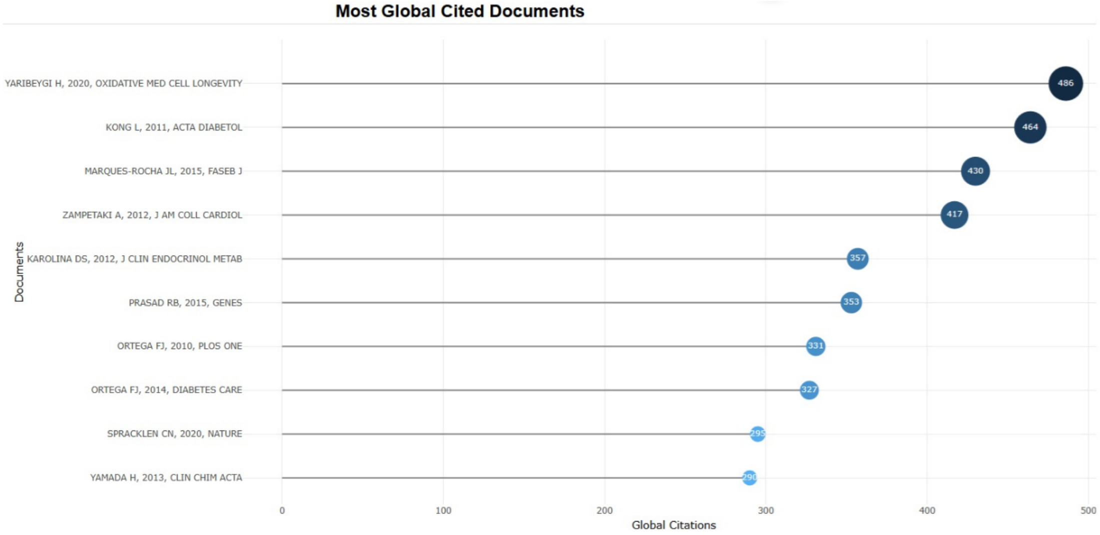

Figure 7: Trend topics identified in articles used in this systematic study.

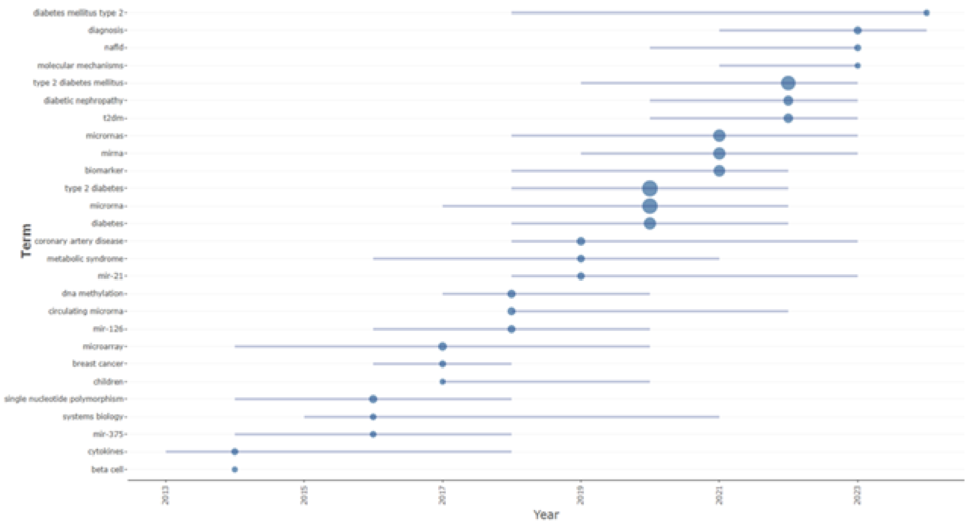

Figure 8: Co-occurrence network of authors' keywords (miRNAs: miR-21, miR-126, and miR-146a are represented in a green triangle).

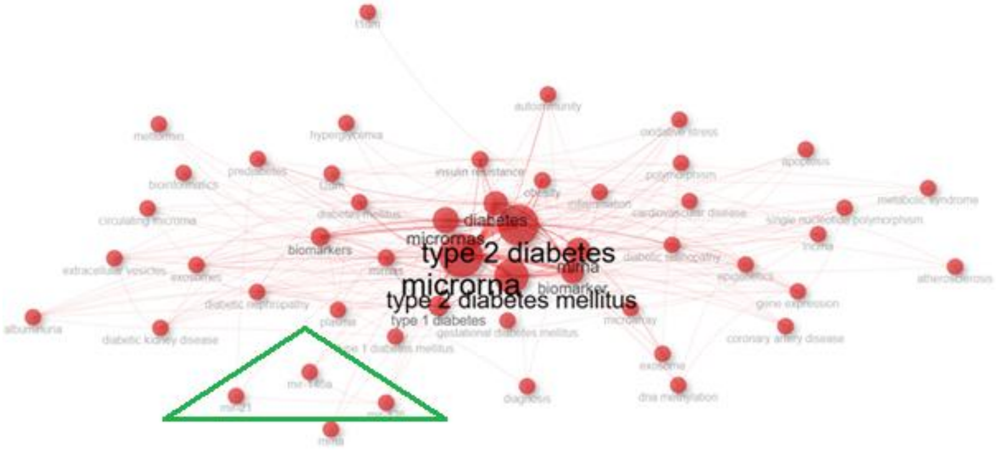
t1dm
autoimmunity
metformin
hyperglycemia
oxidative stress
apoptosis
prediabetes
insulin resistance
polymorphism
bioinformatics
t2dm
obesity
metabolic syndrome
inflammation
cardiovascular disease
circulating microrna
diabetes mellitus
diabetes
single nucleotide polymorphism
micrornas
lncrna
biomarkers
type 2 diabetes
diabetic retinopathy
extracellular vesicles
exosomes
mirnas
mirna
epigenetics
atherosclerosis
microrna
biomarker
diabetic nephropathy
type 2 diabetes mellitus
gene expression
albuminuria
plasma
type 1 diabetes
microarray
diabetic kidney disease
gestational diabetes mellitus
coronary artery disease
type 1 diabetes mellitus
exosome
mir-146a
mir-21
mir-126
diagnosis
dna methylation
mirna

Figure 9: Authors' corresponding countries that were included in this study.

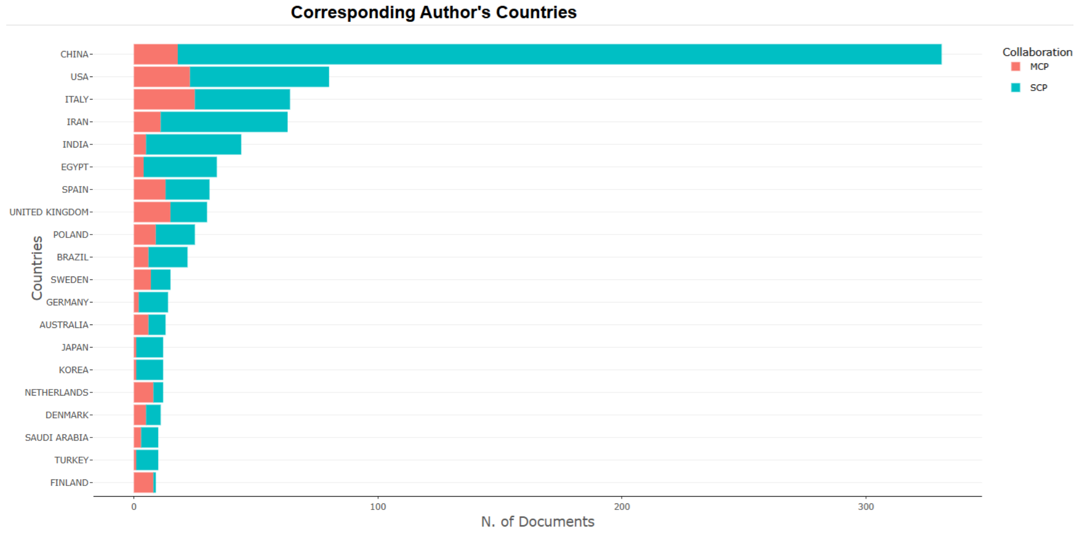

Figure 10: Most cited countries on subjects referring to miRNA and DM.

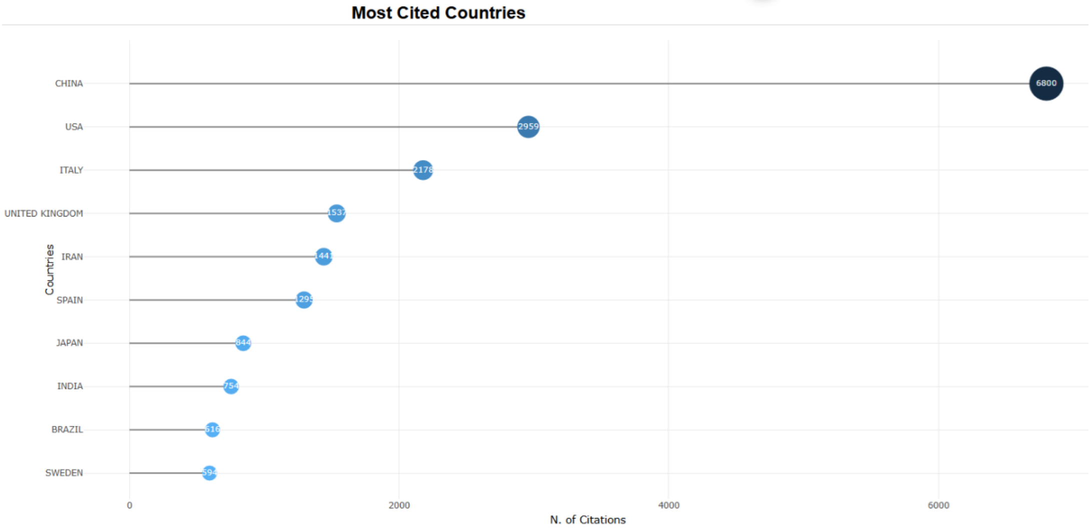

Figure 11: World map of countries collaborating on research on miRNA and DM

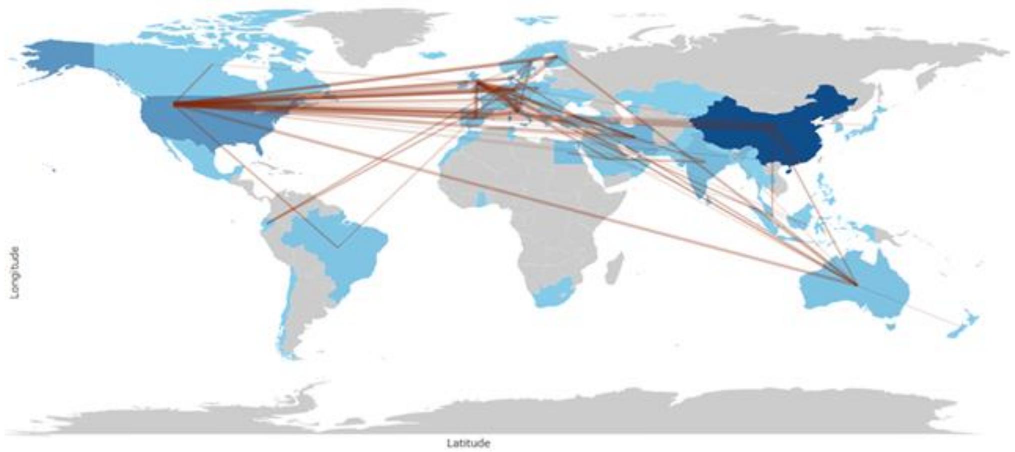

Visualization of international research collaborations by country is seen in Figure 11. Highlighting the global networks formed through co-authorship and cross-border scientific partnerships, its scope encompasses every continent.

miRNAs expression and DM

Bioinformatic analysis can be conducted in order to predict possible miRNA targets. Examples of databases constructed for this purpose are miRBase [31], TargetScan [32], and miRecords [37]. For this research, miRbase [31] and TargetScanHuman v8.0 [32] were used for consulting target transcripts and conservation status for each miRNA. Target gene IDs can be found in Tables 1, 2, and 3. MiR ID was normalized according to at least two articles and two miR databases, according to that miR's sequence.

Table 1: microRNAs downregulated (↓) or upregulated (↑) in T1DM analyzed in at least two studies.

miR in T1DM	Target Gene/Mechanism in which they are involved	Referenes
miR-326 ↑	TNFSF14, 15; CEP85	Zheng et. al., 2017
miR-23 ↑; miR-98 ↑; miR-590-5p ↑	Trail, Faslg	
miR-186 ↑	CXCL13; IRF8; STAT4	[38]
miR-223 ↑	HSP90B1; TP53; MCL1	
miR-142-5p ↑	HERPUD1	[39]
miR-155 ↑ / ↓	CASP3	[39]; Katsarou et. al., 2017
miR-100-5p ↓	RAVER2; AP1AR	Ferraz et. al., 2022
miR-181a-5p ↑	ZNF; TNFSRF11B	Liu et. al., 2019
miR-1275 ↑	KCNC3; KIR2DL4	[22]
miR-30b-3p ↑	CCL28, 22; TAF8; NCR3	Nizam et. al., 2024
miR-25-3p ↑	HIPK3; SNAPC1; RBM47	Liu et. al., 2019
miR-10a-5p ↑	CRLF2; HOXA3	Santos et. al., 2022
miR-98 ↑	IGDCC3; IGF2BP2; CCL3	Khan et. al., 2020
miR-22-3p ↓	H3F3C; GADD45A	Kaur et. al., 2015
miR-16-5p ↑	TNFSF13B; BTLA	Gao et. al., 2020
miR-574-3p ↓	NKG7; IL6; ATPIF1; IFNLR1; SRF	Garcia-Contreras et. al., 2017
miR-103a-3p ↑	SPI1 1; AGO4	Assmann et. al., 2018
miR-454-3p ↑	IRF1; IGF	Erener et. al., 2017
miR-450a-2-3p ↑	IFG2; IL6ST; NOX5; IL17A	Takahashi et. al., 2014
miR-100-5p ↓	INSM1; AGO2	Hezova et. al., 2010

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

Some factors involved in the pathophysiology of T1DM (e.g., ILs, tumor necrosis factor) can interfere with the expression of these miRNAs and thereby modulate the efficiency of silencing processes. These observations complicated the relationship between T1DM and miRNAs.

ABERRANT MICRORNA EXPRESSION IN TYPE II DIABETES

Database analysis

MiRNAs can interfere with T2DM physiopathological mechanisms [40] (Table 3). Some of these molecules were associated with both T1DM and T2DM (Table 2).

Although there is significant interest in developing new biomarkers for DM, only a few miRNAs have been consistently associated with T2DM in at least two studies (Table 3).

Table 2: microRNAs downregulated (↓) or upregulated in (↑) T1DM & T2DM analyzed in at least two studies.

miR in T1DM & T2DM	Target Gene/Mechanism in which they are involved	Referenes
miR-21 ↑	IL12A; FASLG; CCL1	[41]
miR-34a ↑	CDIP1; MSR1; PDCD6	
miR-146a ↓	INSR; ZNF; GRIN2B	
miR-29 ↑	NFIA; ATAD2B	Massart et. al., 2017
miR-142-3p ↑	CASP3	[39]; Zhu & Leung, 2015
miR-24-3p ↑	FASLG; BCL2L14	Blanco et. al., 2023; Garavelli et. al., 2020
miR-148a-3p ↑	ATP6AP2; GADD45A	Grieco et. al., 2018; Ghoreishi et. al., 2022

miR-150-5p ↓	MYB; TADA1; AIFM2	Kim et. al., 2019; Qiu et. al., 2021
miR-210-5p ↑	ERP29; GDF11	Patra et. al., 2023
miR-342-3p ↓	OSER1; SPI	Jiang et. al., 2020
miR-375 ↑	INS; HOXA5; ATP1B1	Higuchi et. al., 2015; Marchand et. al., 2016
miR-101 ↑	UBE2D1; APP	Santos et. al., 2019; Higuchi et. al., 2015
miR-199a ↑	PAWR; ACOX1	Wang, et. al., 2018; Yang et. al., 2020
miR-335-5p ↓	CASP; IL17RD	Li & Zhang, 2021; Hezova et. al., 2010
miR-20b-5p ↓	PDCD; IRF9	Katayama et. al., 2019; Hezova et. al., 2010
miR-15b ↑	CYP7A1; CASP	[42]
miR-186	CRP	
miR-155 ↑ / ↓	BCL2	Corral-Fernández et. al., 2013; Katsarou et. al., 2017

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

Table 3: microRNAs downregulated (↓) or upregulated (↑) in T1DM analyzed in at least two studies.

miR in T2DM	Target Gene/Mechanism in which they are involved	Authors & Year
miR-143	IGFBP; FADS6; ITM2B	[43]
miR-142-3p	BCL2	Zhu & Leung, 2015
miR-126	INSR; SCL7A5	Zhu & Leung, 2015
miR-375	INS; VEGFA; HOXA5; ATP1B1	Poy et. al., 2009; Chakraborty et. al., 2014
miR-26a-5p	POLR3G; INS; BAX	Xu et. al., 2020;
miR-146a-5p	IGSF1; IRAK1; PSMA4	Li et. al., 2012
miR-1 ↓	CYP7A1; XCR1; PRPF39	Chakraborty et. al., 2014
miR-16 ↓	RRM2B; MRPS22; C8B; PDX1	Bork-Jensen et. al., 2015
miR-30e ↓	GABRB1; IL1R1APL2; SOCS1	Dieter et. al., 2019
miR-9 ↑	DSCC1; GLT1D1	Al-Muhtaresh et. al., 2018
miR-33a ↑	TEX38; TLR4; PTPLAD1	Saeidi et. al., 2023
miR-320a ↑	ETFA; TRIAP1; RGS9BP	Du et. al., 2021
miR-148b ↑	GADD45A; MMD; TGFA	Liang et. al., 2020
miR-21 ↓	IL12A; FASLG; GPR64	[44]
miR-29a ↑	MANEA; HOXA10; RTP3	Massart et. al., 2017
miR-34a ↑	MSR1; PDCD6; GORAB	Shen et. al., 2017
miR-103	RNF38; TRIAP1; AGO4	Rafiee et. al., 2024
miR-15a ↓	POLR3K; SLC22A9; RAVER2;	Al-Kafaji et. al., 2015
miR-155 ↓	H3F3A; ZNF652; FGF7	Katsarou et. al., 2017
miR-221 ↑	GABRA1; KCNQ3; SOCS5	Liu et. al., 2018
miR-145 ↓	ATP6V0B; ITGB8; CDC37L1	He et. al., 2020
miR-24 ↓	SNN; BCL2L11; KCNK2	Xiang, 2015
miR-182 ↑	TMEM50B; CHIC1; VLDLR	Krause et. al., 2024
miR-192 ↑	insulin-like growth factor 2; SLC44A1	Jaeger et. al., 2018
miR-194 ↑	PRKAR1A; SLC10A7; BNIP2	Jaeger et. al., 2018
miR-223 ↑	FBXW7; IL6ST; HSP90B1	Sánchez-Ceinos et. al., 2021
miR-130a ↑	IGF1; TGFBR2; HIVEP2	Ofori et. al., 2017
miR-19a ↑	QKI;	Yan et. al., 2020
miR-26b ↑	ID2; IGFBP3; SOCS1	Xu et al., 2015
miR-27a	FOXO1, FOXA2, STAT3	[45]
miR-27b ↓	GOLM1; GPAM	Ghoreishi et. al., 2022
miR-28 ↑ / ↓	TRAF3	[45;46]

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

DISCUSSION

Literature data analysis

The utility of miRNAs as biomarkers for screening or diagnosis of T1DM is of high interest. There have been some attempts to propose a panel of miRNAs for elaborating these tests, although normalization of miRNA levels is still necessary [47].

A systematic review published in 2017 mentioned 48 miRNAs that were investigate in tissues relevant for T1DM pathogenesis (e.g. serum, plasma, pancreatic tissue, blood cells); for 11 of them the expression level differ significantly in T1DM patients and control subjects (e.g. miR-21-5p, miR-24-3p, miR-100-5p, miR-146a-5p, miR-148a-3p, miR-150-5p, miR-181a-5p, miR-210-5p, miR-342-3p, miR-375 and miR-1275) [22]. There are certain miRNAs (e.g., miR-375) that directly influence specific pathways leading to diabetogenesis. These molecules target genes that modulate immune T cell activity. Human and mouse T lymphocytes have been shown to release exosomal miRNAs (e.g., miR-142-3p, miR-142-5p, and miR-155), which can predispose or support the apoptosis of pancreatic β cells [39]. Additionally, overexpression of some miRNAs can reduce insulin synthesis, insulin secretion, or their biological effects [48].

Cytotoxic attack in T1DM is mediated by proinflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α). These cytokines have been found to induce the synthesis of miR-21, miR-34a, and miR-146a in vitro, in human and mouse pancreatic cells [41].

miR-21 is predicted to target multiple transcripts (e.g., IL12- natural killer cell stimulatory factor and cytotoxic lymphocyte maturation factor, CXCL1- chemokine ligand 1). Increased levels of IL-12 could be predictors of T1DM. IL-12 plays a part in determining the levels of CD4+T cells and increasing the CD8+ T cells' toxicity in DM [49]. It also has a role in activating the apoptotic pathway of the β cells. Existing literature proved the implication of the CXCR1/2 axis, CCL4-CCR5 axis, and CXCL10-CXCR3 axis in the inflammatory background of DM. These pathways were proven to be engaging more aggressive immune T-cells. This furthermore accentuates the β cell death [50].

A decrease in miR-155 expression has been shown to impair the function of regulatory T cells (Tregs), which exhibit an upregulated expression of suppressor of cytokine signaling 1 (SOCS1) upon maturation. Treg cells have a primary role in alleviating the aggressivity of CD4+ and CD8+ T cells. Therefore, their underdevelopment has been linked with one of the causes of T1DM, implicating the STAT3 mutations. The signal transducer was reported to recognize and enhance the miR-155 promoter. The development of Treg cells is also threatened by mutations in the FoxP3 gene, and its altered transcriptional activity has been linked with the presence of miR-155 [51].

The apoptotic genes (Trail, Faslg) are predicted to be targeted by miR-23, miR-98, and miR-590-5p. These miRNAs are overexpressed in CD8+ T cells of T1DM patients [52]. MiR-326 targets different genes (e.g., the gene coding the vitamin D receptor), which modulate the function of the immune system [44]. Expression levels of miR-326 were found to be increased in lymphocytes of T1DM patients who tested positive for the islet-specific autoantibodies IA-2A and GADA [53].

MiR-34a is known to inhibit macrophage polarization, thus impairing tissue homeostasis and repair. This miRNA is involved in the regulation of insulin signaling and glucose metabolism through the targeting of specific mRNAs, including those of sirtuin 1 (SIRT1) and vesicle-associated membrane protein 2 (VAMP2) [54]. Dysregulated miR-34a in cancer patients has been proven to lead to low survival chances. It targets multiple oncogenes and plays a part in apoptosis mediated by the p53 complex, the tumor suppressor gene [55].

In a cross-sectional study, Januszewski and his coworkers demonstrated increased levels of miR-186 and miR-223 in T1DM patients. MiR-186 is known for its role in apoptosis and chemokine and insulin signaling pathways [42]. MiR-186 is also known for being a tumor suppressor in prostate cancer. There was an increase in granulocytes in model mice with miR-223 deficiency, and its expression was highly decreased in patients with active nephritis. This miR-223, along with others, has been associated with multiple cellular processes: p53 pathway regulation, cell cycle regulation, tumor metabolism, and apoptosis [38].

The cumulative roles of miRNAs in T1DM are various, implicating many cellular and molecular pathways and mechanisms. Extensive research is needed to further validate the expression levels in vivo model organisms [47].

miRNAs expression and DM

Certain miRNAs found in scientific publications or databases are associated with the pathogenesis of diabetes or with particular features of diabetic patients. Subjects with impaired glucose tolerance presented abnormal levels of miR-18b [45]. miR-143 has a role in precursor adipocyte differentiation and in predisposition for T2DM (e.g., by targeting the insulin-AKT pathway) [43]. MiR-21 targets mainly FOXO1, FOXA2, and STAT3 transcripts. Its levels were considered informative for gluconeogenesis [56], insulin resistance [57], and altered glucose tolerance [45]. Markers of low-grade inflammation in T2DM (e.g., IL6, TNFα) are also associated with changes in miRNAs. TNFa enhances the intracellular accumulation of miR-223, which targets genes involved in lipid and glucose metabolism, inflammation, and T2DM (e.g., IGF-IR, Hsp90, FOXO1) [58;59].

A randomized community-based study analyzed a sample of 1000 individuals. It was identified several downregulated miRNAs (e.g. miR-126, miR-15a, miR-29b, miR-223, miR-20b, miR-197) in the plasma samples from T2DM patients [46]. Upregulated (e.g., miR-140, miR-142, miR-222) or downregulated (e.g., miR-125b, miR-126, miR-130, miR-192, miR-195, miR-423-5p, miR-532-5p) miRNAs in T2DM patients were also identified in a cross-sectional, double-blinded study. It was observed that the expression levels of four miRNAs (e.g., miR-126, miR-140-5p, miR-195, miR-423-5p) demonstrated a strong ability to distinguish subjects with T2DM from those with normal fasting glucose levels [60]. MiR-126 has been reported by a considerable number of research groups to be a suitable biomarker for the assessment of T2DM progression [44, 46, 61]. MiR-186 expression levels were found to be statistically significantly associated with HbA1C levels in T2DM patients and with the risk of diabetic neuropathy [62].

There are numerous factors to consider when evaluating the potential of miRNAs as biomarkers for DM. For instance, the dynamics of miRNA concentration may be correlated with causes of dysregulated expression (e.g., number of cells releasing these small non-coding molecules or with miRNA overexpression in individual cells), miRNA half-life, and factors influencing their stability. Other factors can also significantly impact the significance of results. For example, an increase in adipose tissue volume can increase the levels of some miRNAs; these associations may influence the results, particularly in studies involving obese patients with T2DM [45]. The timing of miRNA testing poses a challenge, especially in presymptomatic stages of diabetes [63]. It has been hypothesized that dysregulated miRNAs may be present only in specific cells or tissues. Comprehensive research involving paired analyses of body fluids and biopsy tissues, along with validation studies, could be beneficial for future investigations [64]. There also remains a critical need for the standardization of miRNA analysis across different research platforms, tissues, and conditions to facilitate the development of clinically useful biomarkers [65].

The size of the sample pool influences the study's power and the reliability of results. Studies with small sample sizes (e.g., fewer than 10 patients and 10 control subjects) may yield biased outcomes [66]. Consequently, findings from such studies may not be corroborated by research involving larger sample sizes. Therefore, the statistical significance and clinical relevance of some miRNA studies may be limited. Reliable statistics are needed against large sample pools and of different categories.

CONCLUSION

MiRNAs can target a large number of transcripts, and their levels in specific tissues change during the natural history of the disease. But these factors also increase the difficulty of identifying the relevant genes for diabetogenesis and validating the clinical use of specific miRNAs. Standardization in profiling techniques and validation of these biomarkers across large, diverse populations remain essential steps toward their clinical application.

This review underscores the emerging role of miRNA as one of the key molecular players in the pathogenesis, diagnosis, and potential treatment of DM. Numerous studies have identified distinct expression patterns of specific miRNAs (e.g., miR-21, miR-126, miR-143, miR-155, miR-223, and miR-375) that correlate with immune dysregulation, β-cell apoptosis, insulin resistance, and metabolic imbalance.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, X.I.P. and DC.; methodology, X.I.P., RN and DC; software, D.C. and X.P; validation, X.I.P., O.A.A.T, I.R, L.B., I.A.E. and S.N.; formal analysis, D.C.; investigation, D.C, X.I.P. and R.N; resources, D.C.; data curation, X.I.P., O.A.A.T, I.A.E, S.N, and R.N.; writing- X.I.P. and D.C; writing-review and editing, O.A.A.T, I.R, L.B., I.A.E, S.N. and R.N.; visualization, D.C.; supervision, D.C.; project administration, L.B.; funding acquisition, L.B. All authors have read and agreed to the published version of the manuscript.

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REVIEW

The Novelty of Contrast-enhanced Ultrasound in the Pathology of Liver Tumors. A Narrative Review of the Literature

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Abstract: Liver pathology represents, nowadays, a major cause of mortality and morbidity worldwide. Since MRI helps in limited cases in the differential diagnosis of various pathologies concerning liver tumors, other modern diagnostic strategies should be prioritized. Besides various tumoral biomarkers, contrast-enhanced ultrasound (CEUS) plays a substantial role in differentiating various liver pathologies due to its availability and specificity. Various contrast-enhanced substances used for CEUS assist mainly in detecting focal liver lesions and differentiating their malignant character. This review focuses on the main indications and limits of this novel investigation, considering that patients presenting with various liver pathologies are more challenging. We highlighted in our review the similarities with the CT/MRI imaging methods in reference to the specificity and sensitivity of the method, in its potential ability to detect various liver tumors. Moreover, the contrast agents used by CEUS are eliminated throughout the lungs, showing no evidence of hepatotoxic, nephrotoxic, or cardiotoxic events. In conclusion, the accessibility and safety of the procedure and the absence of the use of ionizing radiation point out that CEUS could be a stand-alone technique in the diagnosis and evaluation of complex liver pathologies.

Keywords: contrast-enhanced ultrasound; hepatocellular carcinoma; viral hepatitis; liver cirrhosis

INTRODUCTION

The diagnosis of various liver tumors remains, nowadays, challenging even if new diagnostic and prognostic tools are continuously developed [1–5]. In this context, contrast-enhanced ultrasound (CEUS) has been a widely used imaging method in Europe and Asia for over 10 years. CEUS consists of an ultrasound that employs contrast agents for increasing the accuracy of the investigation, typically injected intravenously. It can be compared with other contrast-enhanced imaging methods, such as CT or MRI, and it possesses the advantage of a real-time assessment of different focal liver lesions, improving their detection and characterization [1,2]. CEUS has become more popular in recent years than CT or MRI due to the fact that it has almost no adverse reactions, does not make use of any type of ionizing radiation, and can be employed successfully in patients with impaired renal function [1].

CEUS uses a special type of contrast agent that consists of a microsphere with an albumin, phospholipid, or surfactant-based shell and a gas filling, which subsequently forms a microbubble [2]. These microbubbles do not usually surpass 7 µm in size and have a mean diameter of 2.5 µm. These small sizes allow the microbubbles to freely circulate in the capillaries and impede them from passing through the vascular endothelium into the interstitial space, thus rendering them exclusively intravascular [2,6,7].

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The microbubbles are excreted in two different ways depending on their components – the shell is metabolized in the liver, while the gas filling is eliminated through the lungs in about 10 to 15 minutes, even in patients with chronic obstructive pulmonary disease. This characteristic allows the utilization of these contrast agents in patients with renal failure, thus posing no risk of nephrotoxicity, when other contrast-enhanced methods such as CT or MRI are not indicated [2,6,8,9].

There are many contrast agents available around the world, but the most conventional are the sulfur hexafluoride microbubbles (SonoVue, Bracco), the perflutren lipid microspheres (Definity, Luminity), and the perfluorobutane microbubbles (Sonazoid).

SonoVue is the contrast agent most often used in Western countries and comprises a phospholipid shell that surrounds the sulfur hexafluoride microbubbles, resulting in a diameter of 2.5 µm. The agent is usually injected as a bolus of 2.4 mL in a peripheral vein (the antecubital vein), together with a 5 mL to 10 mL saline solution, using a large enough needle (more than 20-gauge) to ensure that the bubbles do not rupture during the procedure [1,2,6].

Sonazoid is mainly employed in Japan, Norway, and South Korea, and uses perfluorobutane microbubbles. Although it is similar to the other contrast agents used in CEUS, unlike those, Sonazoid can be phagocytized by the Kupffer cells and allows an additional evaluation of the liver in the Kupffer phase or the post-vascular phase. The agent can persist up to several hours in the liver and spleen after its vascular phase [1,6].

INDICATIONS - GENERAL CONTEXT

Even though the most common clinical application of CEUS is the characterization of focal liver lesions and differentiating between malignant and benign masses, there are studies and guidelines that show there are numerous other uses for this technique. As an intravascular contrast-agent imaging method, CEUS can provide information about different vascular diseases that may affect the carotid arteries or the aorta and other abdominal blood vessels, or it can help assess the thrombosis of the portal vein [10,11].

CEUS plays an important role in the imaging of the extravascular structures, mainly cavities, either physiological, such as the peritoneal, pleural cavities, biliary and gastrointestinal tracts, or non-physiological, including cysts, abscesses, or diverticula [12,13]. Thus, CEUS is used in gynecology to evaluate the uterus and the fallopian tubes, in nephrology and urology to help diagnose ureteral reflux and to discern between bladder or renal tumors and cysts or testicular masses. Excluding the liver evaluation, CEUS also has other applications in the assessment of the gastrointestinal tract.

It is successfully used in patients with inflammatory bowel diseases to estimate disease activity or with fistulae and abscesses, and at the same time, it can be administered orally in order to examine the bowel lumen [12–14].

CEUS can be successfully used for assessing different focal liver lesions seen on standard ultrasound and is indicated for the following: hepatic nodules larger than 10 mm (for nodules smaller than 10 mm CEUS can be attempted by an expert examiner); LI-RADS-graded liver lesions as LR-3, LR-4 or LR-M on CT or MRI: monitoring enhancement pattern changes over time for LI_RADS-graded lesions as LR-3 or LR-4; detecting arterial phase hyperenhancement (APHE) when it is absent on CT or MRI, but still suspected; guiding liver biopsies or treatments for difficult to visualize lesions, aiding in choosing the right lesion to be biopsied or reevaluating prior biopsied lesions with inconclusive histopathology; assessing treatment response; making the differential diagnosis between a tumor inside a vein or a simple thrombus [15].

One of the most important indications for CEUS is liver mass characterization and not just detection, with some studies showing an equivalence or even a superiority compared to CT or MRI scans. This ultrasound can also help in differentiating benign from malignant nodules [16].

The most common benign lesions that can be diagnosed using CEUS are hemangiomata, focal nodular hyperplasias (FNH) and hepatic adenomata. Malignant masses which are evaluated by CEUS are hepatocellular carcinomata (HCC) and non-hepatocellular tumors, that include metastases, intrahepatic cholangiocarcinomata (ICC) and lymphomata [16–18].

Liver lesions in CEUS can be described using two features that appear in different phases of the ultrasound: arterial phase hyperenhancement (APHE) and washout [1,16].

If there is no washout present and the enhancement in the arterial phase is sustained, there is a high probability that the mass is benign. In comparison, malignant masses show a decline in the enhancement (washout) in the portal or late phase. Furthermore, the intensity and the timing of the washout can be employed to differentiate between types of liver malignancies.

HCC nodules are most likely to display a late (in over 1 minute) and weak washout, while non-hepatocellular malignancies exhibit early (in less than 1 minute) and intense washout. In order to detect liver metastasis, a sweep of the entire liver in the portal venous phase is essential [1,2,16,17,19]. The benign lesions are usually described by specific patterns of enhancement in the arterial phase. Some examples of these patterns are peripheral discontinuous nodular enhancement (hemangiomata), stellate vessels with centrifugal filling (FNH) and centripetal filling (some adenomata) [16,20].

TECHNICAL EXECUTION

To correctly perform an ultrasound using contrast agents, firstly, it is necessary to have a properly trained examiner who should be able to recognize focal liver lesions and vascular disorders, to assess the effect of a treatment over a lesion and to compare different findings with other imaging methods such as CT, MRI or PET-CT. Secondly, the ultrasound equipment needs to have appropriate hardware, for example, scanners that can cancel soft-tissue enhancement, and software that can display both the standard ultrasound image and the CEUS image simultaneously [15,16]. The patient should be in a supine position with the abduction of the right arm, and before injecting the agent, the examiner should be aware of the medical history of the patient, of the clinical aspects and also of any existing cysts or calcifications that may be wrongly interpreted as malignant [1,12,15,16,21].

For liver imaging, the recommended dose of SonoVue, the most frequently used contrast agent, is 2.4 mL. This value can be decreased by 20-25% if the patient has a low BMI in order to avoid the excessive enhancement of the liver or it can be increased if the lesion is very superficial [15]. The substance is then injected through a large IV line (20-gauge minimum) as a bolus in the span of 2-3 seconds, immediately followed by a saline flush of 5-10 mL [2,7,15].

Interpretation of CEUS is made by evaluating the three vascular phases that follow the injection of the contrast agent. These phases appear as a result of the double vascularization of the liver, namely the hepatic artery and the portal vein [21].

The arterial phase begins at 10 to 20 seconds after injection and ends at 30-45 seconds and appraises the arterial vascular supply of the liver lesion by degree and pattern [1,21]. The portal venous phase begins immediately after the arterial phase at 30-45 seconds and can last up to 120 seconds (2 minutes). This phase marks the entry of the contrast agent in the portal vein and is characterized by diffuse and maximal enhancement of the liver parenchyma [1,21]. The late phase is represented by the amount of time passed from the end of the previous phase until the clearance of the contrast agent from circulation and it can vary from 2 to 8 minutes (120–480 seconds), depending on the type and dose of the agent [1,21]. The post-vascular phase only appears if Sonazoid is injected and it is the result of the contrast loading of the Kupffer or other phagocytic cells. This particular phase usually starts after 8 minutes (480 seconds) following the initial injection and can be seen even after several minutes or hours [1,21] (Table 1).

Table 1: Vascular phases and their duration in contrast-enhanced ultrasound (CEUS)

	START	END
Arterial	10-20 s	30-45 s
Portal venous	30-45 s	120 s
Late	>120 s	Approximately 4-8 min (bubble disappearance)
Post-vascular	>480 s	Approximately 30 min

In the following images, an HCC nodule can be seen during a contrast-enhanced ultrasound. The hyperenhancement in the arterial phase and the late and mild washout in the later phases should be noted (Figure 1).

Figure 1: HCC nodule as seen during CEUS. (a) After 0 seconds (moment of contrast injection), (b) After 30 seconds (arterial phase); (c) After 90 seconds (portal venous phase); (d) After 140 seconds (late phase). Image credits: Teodora Isac.

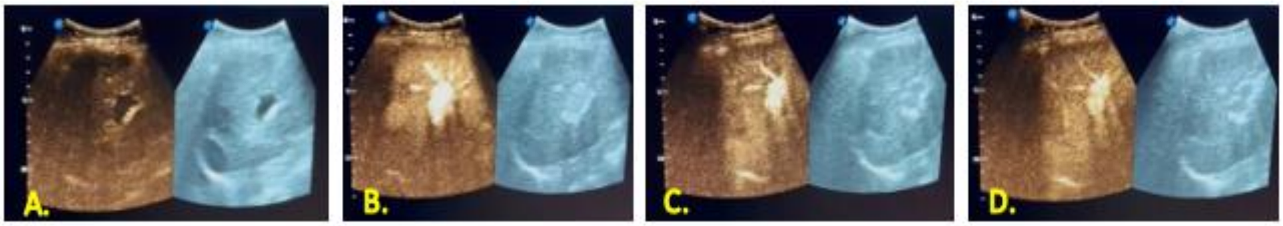

BENEFITS

The contrast agents used for CEUS have a few major advantages in comparison to those used for CT or MRI. The most important characteristic is the increased safety of administration, with almost no side effects reported in the literature. There is no evidence of either cardiotoxic, nephrotoxic or hepatotoxic events, nor any interference with the thyroid function as they do not contain iodine and no need for any specific laboratory testing or fasting before the procedure [2,15,21]. Therefore, these contrast agents can be harmlessly administered to patients with renal failure and CEUS can be considered the first-line imaging method for these particular cases [2,21]. Some studies show that when adverse reactions do appear, they are: headaches, chest pain or chest discomfort and nausea, all other symptoms being extremely rare [21,23,24].

Compared to CT contrast agents, CEUS agents have a significantly lower incidence of hypersensitivity events and the probability of an anaphylactoid-type reaction is extremely low, with a rate of 0.001% and a rate of 0.0006% of fatalities after SonoVue administration [2,21]. Some studies show that CEUS is also safe for use in pediatric patients or pregnant women [21]. Following these observations in the population, the 30-minute period of monitoring previously recommended after injection of contrast agents was eliminated [15].

CEUS has the advantage of being a real-time investigation with a higher sensitivity for detecting APHE in focal liver lesions than CT or MRI and is one of the most important imaging methods for identifying HCC nodules [6,21]. CEUS can categorize a rapidly enhancing hemangioma as LR-1, often wrongly diagnosed as LR-3 or LR-4 by CT or MRI, and can also differentiate an arterioportal shunt from a true HCC nodule, which can confuse the previously mentioned types of scanning [6].

CEUS was successfully employed for guiding treatment procedures or for evaluating treatment response. When performing a US-guided thermal ablation of a liver nodule, often an HCC one, CEUS showed a rate of 95.2% of patients with complete tumor ablation after a single procedure compared to 32% of patients after using a standard US. The rate of tumor recurrence was reduced to 16% as compared to 48% and the patients that benefited from CEUS guidance showed better progression-free survival rates [16,25,26]. The sensitivity of CEUS for post-treatment assessment is more than 80% and the specificity nears 100%, in some studies exceeding other imaging modalities, and, if used immediately after the ablation, is able to evaluate hemorrhages or hepatic infarctions [26].

Another treatment procedure after which CEUS can be used to determine residual tumor is transarterial chemoembolization (TACE). When employed after 4 weeks, it can successfully differentiate between a real active tumor and post-procedural inflammation. Some studies show that CEUS can visualize residual HCC nodules after only 1 day following the procedure, with higher rates of detection than CE-CT after 1 month [25]. It also has the advantage of detecting smaller areas of viable tumor that cannot be correctly appreciated by CT or MRI [26].

Though limited, there are studies that attest to the use of CEUS for evaluating patients one week after transarterial radioembolization (TARE) based on its ability to detect changes in tumoral perfusion [26].

Some studies evaluate CEUS as a monitoring tool for systemic therapies and their effects on tumor angiogenesis [25].

LIMITS

Even if CEUS is one of the most efficient imaging methods for liver nodules, there are some limits that need to be taken into consideration.

First of all, being a real-time evaluation, it is not possible to examine more than one lesion at the same time or to visualize the entire liver in the arterial phase; for this instance, a second bolus of contrast agents is needed for the same patient [2,15]. For patients with multiple liver nodules, contrast-enhanced CT or MRI is recommended [22].

Secondly, areas that are difficult to examine on a standard ultrasound are most likely the same when using CEUS and it can be troublesome when the lesion is smaller than 10 mm or is located subdiaphragmatic or very deep [15,22]. Small, simple cysts can be misinterpreted as metastatic lesions because both of them appear hypoechoic on CEUS [2].

For nodules that require complete imaging staging or monitoring of changes in size over time, CT or MRI are more likely to be of use, because CEUS alone is either not sufficient or it is very difficult to capture exactly the same image as the first time on serial US exams [6,22,27].

Even though CEUS is better than standard US, it is still examiner-dependent and the quality of the images may differ with movement artifacts or other interferences [2]. Some limits may also appear in the technique itself, for example, artifacts related to the dosing of the contrast agent, destruction of the microbubbles by the US beam, or inadequate tissue suppression as seen in a fatty liver [16].

CEUS LI-RADS and HCC

In order to create standardized reports for liver nodules, mainly HCC, seen on CEUS examinations, a scoring system has been designed called LI-RADS (Liver Reporting and Data System) [1,28,29]. It helps improve communication between different examiners and reduce confusion and diagnostic errors in patients with high risk for HCC [28]. The nodules observed with CEUS are then classified as: LR-1 (definitely benign), LR-2 (probably benign), LR-3 (intermediate malignancy probability), LR-4 (probably HCC) and LR-5 (definitely HCC) [1,28] (Table 2). Because CEUS is also able to evaluate other types of liver masses, additional categories have been added: LR-M (malignant, but not specific for HCC), LR-TIV (tumor present in the vein) and LR-NC (not categorized due to omission or image degradation) [1,15,30,31]. The sensitivity of CEUS for detecting HCC lesions is dependent on the mass size. For nodules of less than 2 cm, the sensitivity can vary in different studies from 53.6% to 83.3% and for nodules bigger than 2 cm, it ranges from 91.3% to 94.5%, with a mean of approximately 78.3% and a specificity of around 93.1% [32].

If a nodule shows rim-like APHE, which means the enhancement is more intense in the periphery, forming a halo around it, the category according to CEUS LI-RADS should be LR-M and they are usually liver metastasis or other malignant lesions, but not HCC. If the nodule has a peripheral discontinuous globular pattern, it should be included in the LR-1 category as a benign hemangioma [1,33].

Table 2: CEUS LI-RADS diagnosis (modified after [6])

APHE (arterial phase hyperenhancement)	**NO**		**YES** (not rim and not peripheral discontinuous globular)	
Rim APHE or early washout (<60 s) or marked washout – LR-M Peripheral discontinuous globular – LR-1 (hemangioma)				
Nodule size	Nodule size	≥20 mm	<10 mm	≥10 mm
No washout	LR-3	LR-3	LR-3	LR-4
Late and mild washout	LR-3	LR-4	LR-4	LR-5

In a study that took place between January 2010 and December 2018, 382 patients (with a total of 464 liver lesions) with chronic liver disease at high-risk for hepatocellular carcinoma were included [28]. The exclusion criteria were: transarterial chemoembolization, radiofrequency ablation, and systemic treatment for HCC [28]. The 464 liver lesions were divided into: 68 non-HCC non-malignant lesions (hemangiomata, simple cysts, fatty infiltration, regenerative nodules), 359 HCCs and 37 non-HCC malignant lesions (liver metastases, other types of malignancies). By using CEUS LI-RADS, all of the 464 nodules were classified and the percentage of HCC was established for each category. From the total number of lesions examined, 22 of them were part of the LR-1 category and the other 8 lesions were classified as LR-2. Among those, the HCC rate was 0%. 26 lesions were included in the LR-3 class, but 11 (42.3%) of them were HCC. From the 106 nodules that were included in the LR-4 category, 85 (80.2%) were also confirmed to be HCC. 264 nodules from the total of 464 were categorized as LR-5 by means of CEUS and the HCC rate in this category was 258 (97.7%). For the remaining lesions, 11 were classified as LR-TIV and all of them (100%) were confirmed as HCC, and 38 were classified as LR-M; only 5 (13.2%) of those actually were HCC [28].

The sensitivity of the LR-5 category for the diagnosis of hepatocellular carcinoma was 71.9%, with a specificity of 94.3%, a positive predictive value of 97.7%, a negative predictive value of 49.5% and an overall diagnostic accuracy of 76.9% [28].

By combining the LR-4 and LR-5 categories, they managed to improve the diagnostic accuracy to 90.7%. In this case, the sensitivity was 95.5%, the specificity 74.3%, the positive predictive value 92.7% and the negative predictive value 82.9% [28].

This study has once more shown that CEUS is comparable to CE-MRI and CE-CT in the diagnosis of hepatocellular carcinoma and can be used as a first step to describe liver lesions in high-risk patients during the HCC surveillance. Moreover, using CEUS instead of CT/MRI limits the use of unnecessary imaging techniques in the case of non-malignant tumors [28].

Differentiating an intrahepatic cholangiocarcinoma from a hepatocellular carcinoma in patients with liver cirrhosis can be difficult. The CEUS pattern of the intrahepatic cholangiocarcinoma in this study was a heterogeneous and rim-like enhancement in the arterial phase and a portal venous and late vascular phase washout. The key feature of the HCC is represented by the arterial hyperenhancement of the nodule, followed by a late (after one minute) or absent washout [28]. CEUS has shown a good performance in the diagnosis of HCC, with good sensitivity and specificity using the LI-RADS algorithm. However, an HCC nodule lower than 2 cm has shown a lower sensitivity (56.3%) as compared to 78.9%, representing the sensitivity of HCC nodules higher than 2 cm.

In spite of all the limitations of CEUS presented in this study, the CEUS LI-RADS algorithm for HCC diagnosis remains a useful non-invasive clinical practice [28].

In conclusion, for a nodule to be diagnosed as HCC, it needs to show an APHE, not rim-like and not peripheral discontinuous globular patterns and late and mild washout, as compared to other malignant lesions of the liver categorized as LR-M, that reveal a rim APHE and early, marked washout [30,31,33].

Specific HCC-related conditions and CEUS contribution

CEUS could help differentiate AFP-negative hepatocellular carcinoma from other liver malignancies. A total of 99 patients with liver disease and AFP-negative primary liver malignant tumors were included in a study that took place between January 2018 and February 2021. While the inclusion criteria were the following: serum AFP negative for all patients, the CEUS LI-RADS criteria applied to all patients and CEUS performed within 2 weeks before the liver surgery or biopsy, the exclusion criteria were the following: tumor lesion below 1 cm, thrombosis of the vein, multiple tumors, other treatment modalities in advance of the CEUS examination [28]. They performed CEUS using Siemens ACUSON Sequoia and Philips IU22/EPIQ7 and SonoVue as the contrast agent [33].

According to the results of the biopsy, the 99 intrahepatic lesions were divided into 38 cases of other malignancies (3 cases of HCC combined with ICC, 31 cases of ICC, 3 cases of lymphoma, 1 case of spindle cell sarcoma and 1 case of angiosarcoma) and 61 cases of HCC [33]. The study showed that there were no patients in the LR-1, LR-2 or LR-3 categories in both the HCC group and the OM group. In the HCC group, there were 13 patients in the LR-4 category, 38 patients in the LR-5 category and 10 cases in the LR-M category, while in the OM group, there were 3 patients in the LR-5 category and 35 cases in the LR-M category [33].

The table below shows the sensitivity, specificity and both positive and negative predictive values for the diagnosis of hepatocellular carcinoma or other liver malignancies using the CEUS features [33] (Table 3).

Table 3: CEUS characteristics and their efficacy in the diagnosis of both HCC and OM (modified after [33])

HCC characteristics	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic compliance rate
APHE (but non-rim)	95.1%	63.2%	80.6%	88.9%	82.8%
Mild washout	88.5%	65.8%	80.6%	78.1%	79.8%
Late washout	75.4%	84.2%	88.5%	68.1%	78.8%
LR-4	21.3%	97.4%	100.0%	44.2%	51.5%
LR-5	62.3%	92.1%	92.7%	60.4%	73.7%

Other primary malignancies (OM)	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic compliance rate
Rim APHE	57.9%	96.7%	91.7%	78.7%	81.8%
Marked washout	65.8%	95.1%	89.3%	81.7%	83.8%
Early washout	84.2%	82.0%	74.4%	89.3%	82.8%
LR-M	92.1%	83.6%	77.8%	94.4%	86.9%

Although AFP is a serological marker of great importance in the early screening of HCC, there is a important number of patients who are AFP-negative and present intrahepatic nodules. Therefore, CEUS is a safe, convenient, cost-effective and radiation-free tool that can reveal microvascular vascularization of the tumor. However, it may be difficult to differentiate HCC nodules from ICC nodules in cirrhosis due to the overlap between the enhancement patterns [33].

This study shows that the LR-5 category has a specificity of 92.1% for HCC nodules that are AFP-negative and a specificity of 62.3%. The LR-M category represents an optimal way to differentiate other liver malignant lesions from HCC nodules. The LR-M category in this study revealed a sensitivity of 92.1% and a specificity of 83.6%. [33] Moreover, CEUS LI-RADS has a great application value for the diagnosis of HCC versus OM in patients at risk of HCC. CEUS LI-RADS can be used to determine the presence of focal intrahepatic lesions, even at an early stage when AFP is negative. Nevertheless, CEUS LI-RADS needs continuous improvement in order to diagnose and differentiate other liver malignancies efficiently [33].

Furthermore, CEUS proved to be helpful in the evaluation of the post-treatment response in patients who underwent transarterial chemoembolization (TACE) for HCC nodules. TACE is a therapeutic option widely used for unresectable hepatocellular carcinomas, given the fact that the majority of patients with HCC are usually diagnosed when the disease is at an advanced stage [34]. Patients who underwent TACE as treatment for HCC need to be accurately evaluated to establish the subsequent plan of therapy. CEUS can depict tumor necrosis after TACE, as early as 1-2 months, while the shrinkage of the tumor can take longer to occur [34]. This study wanted to describe the patterns of residual HCC on CEUS and compare the accuracy of CEUS with that of CT in the assessment of HCC response to TACE [34].

This study took place between February 2010 and June 2015 and included patients with HCC who had undergone TACE. They were divided into two groups: those who were assessed for the very first time after the procedure for tumor response and those who were suspected of tumor recurrence due to raised serum AFP.

The inclusion criteria in the study were the following: single mass or multinodular HCC less than 50% of the liver, entire nodule seen on standard US, nodule smaller than 15cm, while the exclusion criteria were represented by the presence of comorbidities (renal and heart failure), severe cirrhosis, HCC involving more than 50% of the liver and breastfeeding women. After applying the exclusion criteria, 50 patients with 70 liver lesions were evaluated by CEUS, but only 57 masses by CEUS, CT and MRI [34].

MRI detected residual disease in 36 masses out of 57; meanwhile, CEUS successfully identified 34 out of 36, resulting in a sensitivity of 94%. The specificity of CEUS compared to MRI was 100% regarding the 21 masses with no recurrent disease. The positive and negative predictive values of CEUS were 100% and 91% [34].

Compared to the multiphase CT, the sensitivity of CEUS was 94% (vs. 50%), while the specificity was 100% for both imaging tools. These results may be explained by the Lipiodol substance used for TACE, which may appear similar to a residual viable tumor. Therefore, the CT scan may be confusing, while CEUS, by not being affected by Lipiodol, can detect even tiny nodules as early as one week after TACE. After 4 weeks post-TACE, CEUS achieved superior sensitivity rates than CT and MRI (100% vs. 50%) [34].

CEUS could be considered an effective alternative technique for identifying early tumor response of HCC after TACE (using Lipiodol substance). However, it is recommended that, in the long term, CEUS be used together with MRI/CT for a better evaluation of the tumor response [34].

CONCLUSION

CEUS is a real-time imaging method used in many areas of interest, including the study of liver masses. It is shown in many studies that the sensitivity and the specificity of CEUS compared to CT/MRI are similar, or even superior, in the diagnosis and surveillance of hepatocellular carcinoma and other malignant liver nodules. The contrast agents used by CEUS are eliminated throughout the lungs, showing no evidence of hepatotoxic, nephrotoxic, or cardiotoxic events. In order to systematize the assessment of the HCC nodules, the CEUS LI-RADS classification was introduced.

Ultimately, although CEUS has its limitations, there are great perspectives in the appraisal of liver nodules using this imaging technique. The accessibility and safety of the procedure and the absence of the use of ionizing radiation reinforce the idea of using CEUS in the near future as a stand-alone technique in the diagnosis and evaluation of the response to TACE for the hepatocellular carcinoma nodules.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research work received no external funding. No AI tools were used to draft or edit the manuscript.

Authors' contribution

All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Not applicable.

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REVIEW

The Transformative Role of Artificial Intelligence in the Future of Radiology

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Abstract: Recent advances in technology and artificial intelligence have transformed radiology. Artificial intelligence, which uses machine learning, deep learning algorithms, and cellular neural networks, is now applicable to image analysis, enabling the analysis of large volumes of data and the early identification of lesion features, such as tumors. Integrating artificial intelligence into clinical workflows increases the efficiency of medical report generation and enables efficient prioritization of complex or urgent cases. Predictive analytics based on artificial intelligence uses extensive patient data, including demographics, medical history, tests, and imaging, to build more accurate predictive models. Among the areas where artificial intelligence is successfully implemented in radiology is the automated detection of lung nodules. In the field of neurology, artificial intelligence helps track progressive lesions through volumetric analysis of the brain and surveillance of demyelinating diseases. In oncologic radiology, artificial intelligence is used for automated image segmentation, lesion review, and standardized report generation. Therefore, in this ever-evolving landscape, radiologists must embrace emerging technological advances in order to occupy a unique position at the intersection of different diagnostic fields.

Keywords: artificial intelligence; radiology; magnetic resonance imaging; computed tomography

INTRODUCTION

The Current State of Radiology and Its Importance in Modern Medicine

Radiology has been closely linked to the degree of technological evolution and has experienced revolutionary developments in recent years. This review examines the implementation of Artificial Intelligence (AI) in radiology, examining its practical applications, associated challenges, and potential paths for progress.

Radiology is a medical field that, through several imaging technologies, including Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Positron Emission Tomography (PET), ultrasound, and conventional radiology, contributes to the formation of medical diagnoses [1].

Challenges facing radiology today include a high workload that is not adapted to the existing workforce, corporatization, streamlining of workflow, and burnout. All of these contribute to the “perfect storm,” creating pressure on radiologists and the institutions in which they work [2].

In this context, AI can contribute to the evolution of radiology, from improving image acquisition to image interpretation with the help of advanced image analysis [1].

Understanding Terminology: AI, ML, and DL

Artificial intelligence has a contribution in radiology through machine learning (ML) and deep learning (DL) algorithms that play a role in image analysis.

Artificial intelligence is the replication of human intelligence in machines programmed to simulate human thought and action, with the aim of creating intelligent systems that operate autonomously and have the ability to solve problems that would normally require human intelligence.

Machine learning algorithms have emerged in recent years and are based on the introduction of decision trees, support vector machines, and neural networks, with these algorithms forming the basis of image analysis.

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Deep learning is a subset of ML that uses multilayer artificial neural networks, which allow DL algorithms to understand complex data, which is successful in visual recognition. The layers are learned from the data, with architecture inspired by the human brain [1, 3].

HOW ARTIFICIAL INTELLIGENCE IS CHANGING THE FUTURE OF RADIOLOGY

AI is crucial to radiology's future in many aspects:

Intelligent image analysis algorithms

Through DL, AI can analyze large volumes of unstructured data, such as images, and learn patterns to automatically recognize features from the raw data. Through these technologies, and particularly through Convolutional Neural Networks (CNNs), AI has revolutionized the early identification of lesion patterns in CT scans [3].

Increased workflow speed

Integrating AI into clinical workflows gives radiologists quicker access to advanced imaging, speeding up results and helping prioritize complex or urgent cases [1]. There are areas where the use of artificial intelligence tools has reduced the workflow of radiologists by about 34%, such as in the case of breast cancer screening [4]. One of the main problems that arises when implementing AI in radiology is the lack of standardization between platforms and the fact that there are multiple AI tools with different user interfaces, which ultimately leads to an inefficient workflow, as radiologists must access different systems to reach the information provided by AI. The existence of a centralized AI platform, which integrates multiple diagnoses, with a single interface, can facilitate the workflow [5].

Predictions based on image analysis

The ability to make an accurate prediction of a patient's prognosis is essential for timely interventions. The emergence of predictive analytics through artificial intelligence allows the analysis of a large volume of patient data, including demographic data, medical history, and previous tests, which, together with imaging, can be used to establish predictive models with greater accuracy [6].

The role of AI in education

The current approach to education emphasizes a series of theoretical notions and a practical case-based experience. However, the method has its limitations, exposing learners to a limited number of cases compared to real-world work situations, as well as limited access to expert radiologists in certain fields. This is an area where AI can have a transformative impact through its ability to analyze large volumes of data and provide real-time feedback to complement the knowledge acquired through traditional education. Also, the existence of work platforms with simulations of real cases, connected to large databases, can increase the quality of training for students and residents [7].

Another transformative element is the inclusion of interdisciplinary education; radiologists must go beyond the traditional focus and have experience in fields such as genomics, pathology, laboratory medicine, oncology, molecular medicine, and immunology. Thus, the radiologist can understand the complex data that modern AI systems introduce into analysis to generate multimodal results. Radiologists occupy a unique position at the intersection of diverse diagnostic fields and play a central role in the diagnosis and care of patients, which is why their expertise in placing imaging data in an integrative clinical context is essential. Interdisciplinary collaboration is the way to ensure that radiologists are active users of artificial intelligence. Another area that can be improved is communication skills, an area in which the existence of structured reports also helps interdisciplinary communication and the transmission of clear conclusions [8].

Radiology training programs should prepare radiologists to understand artificial intelligence and to be able to make decisions under uncertainty without being influenced by cognitive biases, a situation that training in risk assessment and probabilistic reasoning helps to improve. This shift involves more than simply adapting the curriculum; it involves an integrative mindset focused on continuing education and an education that emphasizes interdisciplinary collaboration. These are the values that can help radiologists adapt to the complexity of the modern data they face [8].

AREAS WHERE AI IS SUCCESSFULLY INTEGRATED IN RADIOLOGY

Oncology

Correct interpretation of images, considering tumor heterogeneity and imaging characteristics, is a key factor in cancer diagnosis. By integrating AI into the oncology imaging workflow, multidimensional information about tumors can be extracted, which together can aid in tumor staging and stratification, molecular diagnosis, metastasis prediction and treatment response [9]. The main directions of focus for the inclusion of AI in oncology imaging include automatic image segmentation, lesion review, and the generation of standardized responses based on automated analysis. A significant aspect in oncology is the development of radiomics, but also the contribution of AI in radiotherapy.

Among the areas where artificial intelligence is successfully integrated into radiology is oncology, where techniques for automatic image segmentation, lesion review, generation of standardized responses based on automated analyses, and the development of radiomics have been successfully implemented.

Automated Image Segmentation

DL significantly contributes to image segmentation and classification and therefore increases the speed and accuracy of diagnosis. CNNs contribute significantly to the ability of artificial AI to learn complex patterns, which are found in fields with visual components, such as radiology. This is possible due to the complex, layered architecture of these networks, which have demonstrated increased ability in object detection and segmentation. CNNs have shown a key role in segmenting brain tumors in MRI examinations. Other applications where CNN segmentation has been successfully applied in radiology are the individualization of lung nodules, also distinguishing between benign and malignant tumors in mammography [1, 10, 11].

Review of lesions

Radiology exploits the most useful AI algorithms to automatically identify, locate, and recognize abnormalities found on medical images, whether they are tumors, fractures, or other types of injuries. Among these, DL and reinforcement learning help AI systems increase diagnostic accuracy, helping to halve the radiologist's workload and medical decision-making time. DL models can identify small lesions more easily through resolution enhancement techniques such as interpolation and super-resolution. To increase the performance of AI analysis algorithms in medical object detection, image pre-processing procedures are applied. Among the image preprocessing techniques used in artificial intelligence for object detection in radiology, there are some important ones, such as spatial resampling, intensity normalization, image registration, data augmentation, noise reduction, and segmentation. Data augmentation techniques include rotating, scaling, and flipping images to create larger data sets for use in AI models. Radiological applications of AI object detection apply to both tumors and other types of lesions in a wide range of examinations, including X-ray and CT scans, but one of the most innovative applications is CNN-based segmentation in MRI imaging [12].

Development of standardized reports based on automated analysis

The existence of structured reports in radiology improves workflow efficiency by faster generation of medical reports, especially when there are variable templates for various clinical aspects. The European Society of Radiology (ESR) emphasizes the need to use structured medical reports to streamline workflow and reduce the potential for errors. The use of structured reports that include a standardized list of issues reduces the risk of error by guiding the radiologist through a system of reviewing key points. At the same time, a structured approach ensures better communication between specialties and ultimately contributes to better patient care. With multiple AI tools, there is a risk that radiologists will be overwhelmed, but AI algorithms for rapid image analysis and standardized reporting can create consistency and make these disparate AI tools work together more effectively [5].

Radiomics

Among AI techniques, ML and radiomics involve extracting predefined features from radiological images through a data characterization algorithm. These features encompass various aspects of tumors, such as intensity-based measurements, shape characteristics, volume, heterogeneity, vascularity, and effect on surrounding structures. The entire process aims to increase diagnostic and predictive capabilities [1, 9].

Radiotherapy

Technological progress in diagnostic imaging has improved the efficiency of radiotherapy by introducing artificial intelligence software that optimizes the segmentation of tumors and organs at risk, prognostic assessment, treatment planning and optimization, thus saving considerable time for the radiation oncologist [13].

Lung nodule detection and classification

An advantage and a significant improvement of the workflow in radiology services appeared with the programs for automatic analysis and segmentation of pulmonary nodules [14]. These programs have become routine and are permanently attached to PACS systems, identifying, measuring dimensions, analyzing the structure, density and location of pulmonary nodules with dimensions between 3-30 mm [14].

There are also limitations in the automated analysis of pulmonary nodules, among which the most significant are the false identification of vascular trajectories as nodular hyperdensities, and other limits in identifying lesions in areas with ventilation disorders, with motion artifacts or with fibronodular sequelae. However, automated analysis programs are continuously improving and shortening the times for preparing medical reports, increasing work efficiency, and becoming working tools for the redistribution of highly qualified human resources in other directions where they are still irreplaceable [15].

Another useful contribution is the automated comparative analysis of the evolution of pulmonary nodule sizes, in accordance with the intervals specified by specialist guidelines, such as that of the Fleischner Society, an aspect that contributes to establishing the most appropriate therapeutic and follow-up conduct [16]. Automated analysis generates medical reports that enter patients' electronic records in parallel with the generation of a set of images with automatically marked lesions [15]. A concrete example in practice is that provided by CAD-type analyses in the identification of undetected tuberculous lesions.

Trauma with applications in military medicine

In imaging departments serving emergency rooms in civilian or military field hospitals, cerebral hemorrhagic lesions, or hemorrhages in any part of the body, represent the most common cause of post-traumatic death. Rapid recognition of hemorrhages and the most appropriate therapeutic interventions are of paramount importance. The role of AI systems using ML is to optimize diagnosis in these situations through algorithms that recognize hemorrhagic lesions and assess their risk potential through CT and FAST ultrasound methods [17]. These programs use semiological notions of lesion recognition, such as Language Models (LM) for automatic 3D labelling to recognize hemorrhage subtypes based on brain segmentation, midline deviation, mass effect, cerebral edema, and hyperdensity values. Diagnostic models in neurotraumatology utilize various emergency diagnoses, including hemorrhage, ischemia, cerebral infarction, space-occupying lesions, mass effect, hydrocephalus, and midline deviation. Neurotraumatology lesion recognition models based on imaging data obtained through radiography, computed tomography, and MRI can also be used for the rest of the body, through 3D anatomical segmentation, 2D CNNs software, and Recurrent Neural Network (RNN) [18].

Neurology

By using the Picture Archiving and Communication System (PACS) system, lesions with complex neurological substrates can be effectively detected and characterized through automatic algorithms that have entered routine analysis in some types of cerebral pathologies.

Automatic Cerebral Volumetry consists of an efficient automated analysis based on segmentation using the 3D T1 MPRAGE sequence. For reference, the images and measurement values are compared with those of a comparative database of patients from the same age groups for whom the brain volume is reported. Thus, this program provides cerebral volumetric assessment in case of suspected dementia, by evaluating the frontal, parietal and temporal lobes, and particularly the hippocampus. Brain volume assessment can also be extremely useful in multiple sclerosis and other degenerative diseases, such as Multiple Systemic Atrophy (MSA), Progressive Supranuclear Palsy (PSP), and Cortico-basal Degenerative Disease (CBD).

The program can also analyze the volume of specific brain areas such as the caudate nucleus, putamen, globus pallidus, cerebellar cortex, and midbrain [19].

The *Evaluation of Arterial Intracranial Vascular Lumen* uses a Time-of-flight (TOF) acquisition for analysis before being processed with the Maximum Intensity Projection (MIP) program. The analysis program is attached to the PACS system and generates a medical report that includes measurements and imaging of the identified lesions. In the case of intracranial aneurysms, it is estimated that 10-15% of them are underdiagnosed, and these automated analysis programs can improve the anomaly detection rate with an accuracy of almost 100% [20].

The *Characterization of a Demyelinating Lesion* is based on the analysis of 3D Fluid-Attenuated Inversion Recovery (FLAIR) sequences that highlight the location of the lesions. The analysis includes classification of the lesions using various reference systems, such as the McDonald and Fazekas criteria.

The program can generate a medical report that includes data on the location and size of the lesions, the presence of new lesions compared to previous examinations, and key illustrative images that are included in the report. Thus, this type of analysis can be extremely useful in all demyelinating diseases, including multiple sclerosis [20].

Brain tumor analysis allows for the automated assessment of tissue structures associated with tumor presence in entities with different anatomopathological substrates. Automated tumor lesion analysis is performed after intravenous administration of a contrast agent, which evaluates the behavior and uptake of the contrast. Automated lesion analysis is applied to highlight and differentiate various lesions and tissue components, which can be especially useful in the characterization of glioblastoma-type lesions, metastases and other tumor types, such as meningiomas. Also, complex data analysis allows for the possibility of integration into radiotherapy and neurosurgery programs [21].

Prostate

AI has become a commonly used tool for the diagnosis of prostate cancer, useful for both radiologists and urologists in multiparametric MRI examinations. The speed and efficiency of prostate cancer diagnosis and staging are among the greatest achievements for AI in prostate MRI evaluation [22].

Prostate cancer staging is performed using the PIRADS classification system and is integrated into the PACS system. The AI-generated medical report provides PIRADS classification, prostate volume, and detailed multiparametric analysis of prostate lesions, including nodule identification and categorization. In addition, when available, previous examinations can be evaluated comparatively. Medical reports also include appropriate graphical representations and dimensional assessments, as well as mapping of prostate lesions [22, 23].

When the patient's PSA analysis is also available, this aspect can be represented in tables and associated with the presentation of prostatic density in the workflow report. This medical report can also be effectively used for image-guided biopsy, depending on the representation and arrangement of the most representative lesions [22, 23].

CLINICAL IMPLEMENTATION OF ARTIFICIAL INTELLIGENCE, CASE STUDIES AND APPLICATIONS

Case 1. Detection and analysis of intracerebral aneurysms using artificial intelligence

By analyzing brain MRI, TOF sequences, AI software can analyze the intracerebral vascular lumen and thus identify the presence of intracerebral arterial aneurysms and estimate their location and size (Figure 1).

Figure 1: Brain MRI examination. (A) Axial TOF images. (B) Automated detection of an aneurysm in the anterior communicating artery.

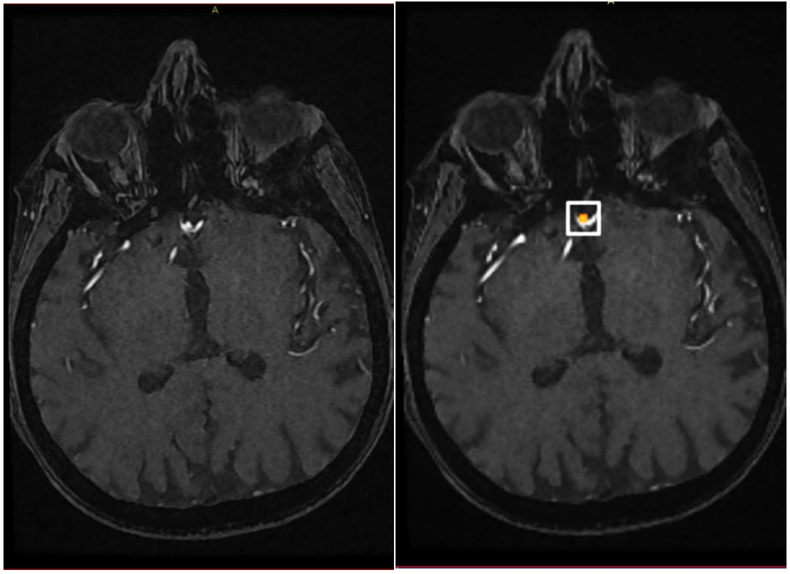

(A) **(B)**

Case 2. Automatic identification and dimensional monitoring of pulmonary nodular lesions

With the help of automated AI programs, in the case of thoracic CT examinations, the lung parenchyma can be analyzed for the presence of lesions, thus nodular lesions are identified, counted and measured automatically, and if there are previous examinations, the dimensional progression of the lesions can also be automatically assessed. Thus, with the help of AI tools, such as Rayscape, clinical impact improvement and shortening of diagnostic times are observed [24] (Figure 2).

Figure 2. CT examination of axial sections, lung parenchyma window. (A) Automatic identification of pulmonary nodular lesions and dimensional assessment. (B) Automatic comparative analysis of pulmonary lesions with dimensional progression assessment.

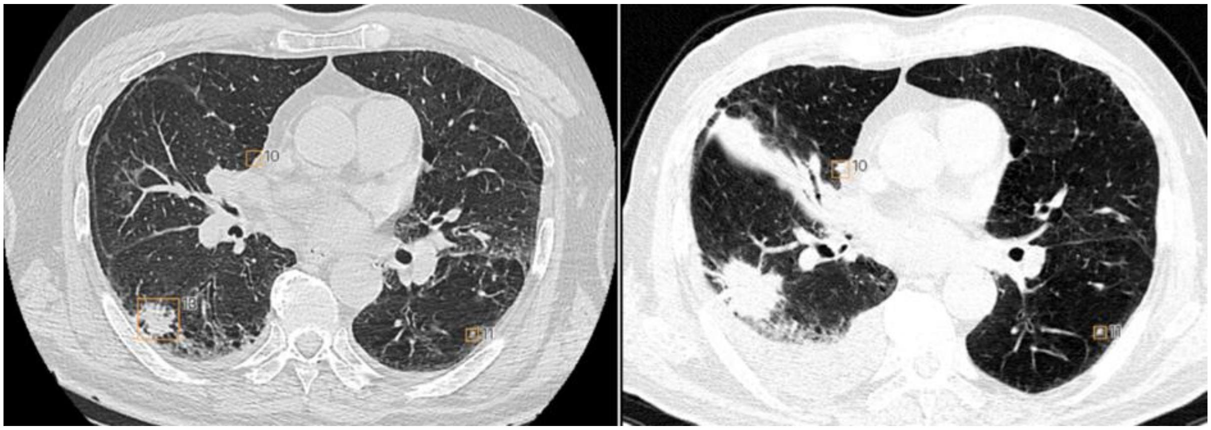

Case 3. Automated prostate volume analysis and lesion characterization

Automated prostate analysis on MRI examinations is an area where AI has demonstrated progress and efficiency, so that prostate volume is assessed, lesions are identified, measured and classified according to PI-RADS classification, and a mapping of the most significant lesions is made in the report (Figure 3).

Figure 3. MRI examination, coronal T2 images of the prostate with automated prostate volume analysis and characterization of nodular lesions.

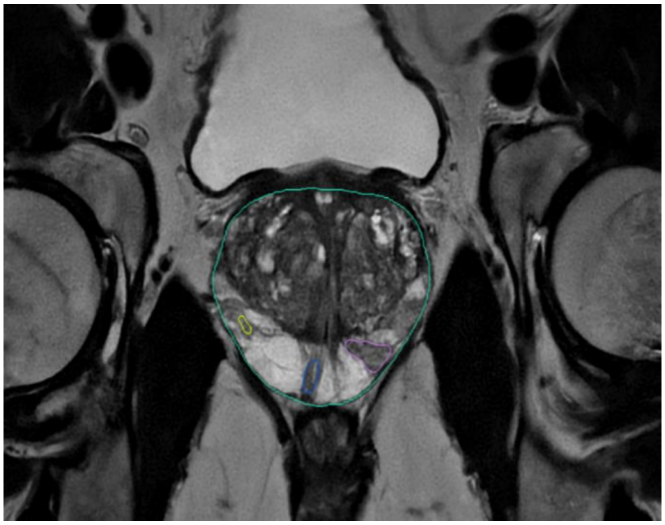

METHODS AND INDICATORS FOR EVALUATING THE EFFECTIVENESS OF AI PROGRAMS

The most useful evaluation method is the one that uses the Area Under the Curve (AUC) indicator in binary detection. The method is derived from the Receiver Operating Characteristic (ROC) curve, which uses sensitivity and specificity as parameters to state whether a lesion is present or not [17]. In the ideal mode, the optimal value of AUC is 1. Between 1 and 0.5 AUC has the appearance of a random model and below 0.5, it cannot be brought into discussion. At the current time, the interpretation in an efficient model has AUC values close to 0.9. The evaluation of the AUC indicator has, in turn, references and standards related to diagnosis established at the level of junior and senior radiologists with expertise. This level of over 0.9 is more easily achieved in lesions defined by few parameters and with clear values, such as hemorrhages, fractures, pneumothorax, lesions from mammographic examinations, while multiparametric defined lesions, such as prostatic or hepatic ones, require a senior radiologist-type standard of expertise. In the radiological evaluation of polytrauma in emergency services, high AUC values, above 0.9, and short processing time are required [25].

CONSIDERATIONS AND CHALLENGES

- Legal and ethical implications

The legal and social implications of artificial intelligence technologies in radiology need to be seriously assessed. One concern is the lack of transparency behind the decision-making process through artificial intelligence. Another key aspect in the implementation of artificial intelligence is the lack of a legislative system that regulates the level of involvement of artificial intelligence. Medical liability in case of malpractice is attributed to the radiologist. AI tools must meet rigorous validation and approval standards to establish their safety. Once approved, they must be continuously monitored to ensure that the results they provide are dependable. The implementation of AI systems requires adherence to established standards, appropriate regulatory approval, and awareness among radiologists and medical practitioners regarding their limitations, ensuring these factors are carefully considered [1, 26].

- Bias and generalization

Despite the development of artificial intelligence, when implemented in real life, its performance varies for certain subgroups. The question arises whether the goal should be fairness to the individual or fairness to the group. It has been observed that artificial intelligence takes shortcuts in the learning process, which can lead to biased results towards certain subgroups, for example, when multiple factors, including age, gender, and race, are taken into account in the diagnosis [27].

- Familiarization, incorporating AI into existing workflow systems

The integration of artificial intelligence into clinical radiology faces several challenges. One of these is the existence of robust hardware capable of managing the substantial amounts of data generated by medical imaging systems, which implies investment in such infrastructure. On the other hand, this new hardware and software must be able to integrate with existing radiology systems. There is also a need for training of radiologists and for them to collaborate with artificial intelligence developers [1].

A LOOK INTO THE FUTURE OF RADIOLOGY

The future transformation of radiology includes the integration of Augmented/Virtual Reality (AR/VR) and AI. AR/VR technologies are expected to play a significant role in radiological practice and radiological education by improving the visualization of radiological images, thereby contributing to diagnosis and treatment planning. Also, through machine learning, AI improves its image analysis capabilities, thereby contributing to reducing diagnostic errors. A tool based on AI is Computer-Aided-Diagnosis (CAD), which can contribute to the development of an integrative diagnosis by incorporating radiological, anatomopathological and genomic information into the AI-assisted workflow [1].

CONCLUSION

Artificial intelligence is developing rapidly, and understanding its use in medical practice is mandatory. Radiology is transforming, and specialists must keep up with it, adopting these innovative technologies and being integrated into multidisciplinary clinical teams, with collaboration between healthcare providers being paramount to fully capitalize on the benefits of this scientific knowledge.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, D.C., and A.S.C.; methodology, D.C., and A.S.C.; software, D.C., and A.S.C..; validation, D.C., and A.S.C.; formal analysis, D.C., and A.S.C.; investigation, D.C., and A.S.C.; resources, D.C., and A.S.C.; data curation, D.C; writing—original draft preparation, D.C., and A.S.C.; writing—review and editing, D.C., and A.S.C.; visualization, D.C.; supervision, D.C. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Not applicable.

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ARTICLE

Salivary Investigation of the Complex Relationship between NLRP3 Inflammasome, Leptin and Total Antioxidant Capacity in the Context of Periodontal Disease

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Abstract: Periodontal disease is a chronic pathology, resulting from the action of microbial communities that become dysbiotic, accompanied by immune response impairment in periodontal tissues, leading to an inflammatory process that promotes progressive tissue destruction. Abnormal activation of nucleotide-binding oligomerization domain (NOD-), leucine-rich repeat (LRR-), and pyrin domain (PYD)-containing protein 3 (NLRP3) promotes chronic inflammation. Research shows that leptin and oxidative stress (OS) are involved in the activation of the inflammatory pathways, including the activation of the NLRP3 inflammasome. In this context, the main objective of our study was to determine the levels of the NLRP3, leptin, and total antioxidant capacity (TAC) in the saliva of patients with periodontal disease. The present cross-sectional study included 44 patients with periodontitis, while the control group was represented by 19 clinically and biologically healthy adults. Saliva samples were collected from all participants in the study, and salivary levels of NLRP3, leptin, and TAC were determined using the Enzyme-linked immunosorbent assay (ELISA) and commercial kits. The results show that NLRP3 had statistically significantly increased levels in the study group versus the control group ($p < 0.00001$). Although TAC was also increased in the patients compared to the healthy subjects ($p=0.491$), no statistically significant differences were found. On the other hand, leptin levels were significantly lower in periodontitis patients compared to the control group ($p < 0.00001$). The results obtained in the present study are promising, but further studies are needed to obtain a more comprehensive understanding of the complex molecular mechanisms underlying periodontal disease.

Keywords: periodontal disease; NLRP3 inflammasome; leptin; TAC; saliva

INTRODUCTION

Around 700 species of oral bacteria form the oral microbiome [1], a term used by molecular biologist and Nobel Prize laureate Joshua Lederberg [2, 3] to define the mixture of symbiotic, commensal, and pathogenic microorganisms present in our organism [4]. Periodontal disease is a chronic inflammatory disease [5] with origins in the dental plaque [6], being a result of microbial communities that become dysbiotic, corroborated with immune response impairment in periodontal tissues, leading to an inflammatory process that promotes progressive tissue destruction [7, 8].

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In the beginning, the inflammation induced by anaerobic bacteria [6] leads to gingival bleeding, pain, and swelling, which are clinical traits of gingivitis, which affects almost 90% of the population and is considered to be the mildest form of periodontal disease [9, 10]. The progression of the disease, in the lack of appropriate treatment, leads to irreversible attachment loss of the periodontium and alveolar bone destruction, thus causing periodontitis [9], which, if left untreated, results in tooth loss [11, 12].

Periodontal disease affects not only the quality of life of the patients because of the associated symptoms and dysfunctions, but also has a considerable effect on society, even from an economic point of view [13], becoming a global health burden [14]. Moreover, by favoring the transfer of periodontal pathogens and pro-inflammatory agents to other tissues, periodontal disease has been linked to systemic diseases [15], including diabetes and heart disease [9], respiratory infections [16], Alzheimer's disease [17], neurodegenerative diseases[18], autoimmune diseases, and even malignancy [19].

Recent research has shown that the development of periodontal disease is not promoted only by bacterial, but also by viruses, environmental factors (e.g., smoking) and lifestyle of the host, and it involves genetic mechanisms and immune response [5, 7], new studies focusing on the development of alternative therapies such as vaccines or antioxidant agents to improve the prevention of the disease [20].

The activation of inflammasomes represents one of the core pathways linked to inflammatory diseases [21]. The nucleotide-binding oligomerization domain (NOD-), leucine-rich repeat (LRR-) and pyrin domain (PYD)-containing protein 3 (NLRP3) inflammasome [22], found in the cytosol, is a supramolecular complex that comprises receptor protein NLRP3, adapter protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and caspase-1 [23]. These three components of the inflammasome have different functions. While receptor protein NLRP3 can detect endogenous danger signals and enlist downstream targets [23, 24], ASC links NLRP3 to caspase-1, which is involved in cytokine release and pyroptosis (inflammatory cell death) [22, 24, 25] via gasdermin D [24]. NLRP3 can be activated by cellular alterations [23], microbial infections, or environmental stimuli [26]. The activation of NLRP3 leads to protease caspase-1 activation, which in turn promotes the production of cytokines IL-1β and IL-18, thus upholding inflammation [26]. Furthermore, abnormal activation of NLRP3 promotes chronic inflammation, hence being involved in the development of numerous diseases [25], such as cardiovascular diseases [27], metabolic diseases [25], autoimmune diseases [28], cancer [25], periodontal disease [29], and, as it was recently discovered, COVID-19 [30].

So, the NLRP3 inflammasome is responsible for the transformation of IL-1β, one of the most important pro-inflammatory cytokines linked to periodontal disease, into its biologically active form [31]. This mediator of inflammation can induce osteoclast differentiation, thus playing a crucial role in alveolar bone resorption, specific to periodontitis. Moreover, NLRP3 activation mediates osteoblasts' apoptosis via IL-1β and IL-18, affects the fibroblasts in the periodontal ligament via IL-1β and IL-6, and is involved in leukocytes' regulation in inflamed periodontal tissue [32].

Research shows that leptin and reactive oxygen species (ROS) can activate inflammatory pathways, including the NLRP3 inflammasome [33]. Leptin, a peptide hormone produced primarily by adipocytes [34], is involved in the regulation of food intake, additionally having an important role in proinflammatory immune responses [35]. It is also produced by the salivary glands, which are responsible for leptin storage and secretion as well [36]. In periodontal disease, leptin has been shown to be able to regulate the ratio between osteoprotegerin and receptor activator of nuclear factor kappa beta (RANKL) in gingival fibroblasts [37], hence influencing bone remodeling [38]. In a recent study conducted on mice, Han et al revealed that leptin promotes macrophage polarization in periodontitis via NLRP3 inflammasome [39]. Furthermore, leptin was found to promote oxidative stress (OS) [40].

OS has already been proven to be one of the key players in periodontal disease development and progression, contributing to tissue destruction and tooth loss [41]. OS is characterized by the loss of balance between oxidants and antioxidants, promoting the formation of ROS and free radicals, which can play an important role in inflammation, despite their reduced lifespan [42]. In periodontal disease, the activation of the innate immune system produces a storm of proinflammatory cytokines and overproduction of ROS [41]. On the other hand, in chronic inflammation, such as the one present in periodontitis, overproduction of ROS, mainly by neutrophils [43], can not only induce OS, and activate proteolytic enzymes but can also activate different signaling pathways [44].

In order to investigate the complex mechanisms involved in the pathogenesis of this condition, the main objective of our study was to determine the levels of the NLRP3, leptin, and TAC (total antioxidant capacity) in the saliva of periodontitis patients.

MATERIALS AND METHODS

Study groups

The present cross-sectional study included 44 patients with periodontitis, diagnosed according to the 2018 AAP/EFP Classification of Periodontal & Peri-implant Diseases [45, 46].

Inclusion criteria for the study group:

- Presence of at least 14 teeth on both dental arches combined;

- Interdental clinical attachment loss (CAL) is detectable in ≥2 non-adjacent teeth or buccal/ oral CAL ≥3 mm with periodontal pocket depth >3 mm is detectable in ≥2 teeth;

- CAL cannot be attributed to non-periodontal causes such as:

1) gingival recession of traumatic origin;

2) dental caries extending into the cervical area of the tooth;

3) presence of CAL on the distal aspect of a second molar and associated with malposition or extraction of a third molar;

4) a combined endo-periodontal lesion;

5) occurrence of a vertical root fracture [46].

Exclusion criteria for the study group:

- chronic alcohol consumption;
- administration of contraceptive medication;
- pregnancy or breastfeeding;
- periodontal treatment less than 6 months before sampling.

Among the 46 patients initially recruited from the Periodontology Department of the Faculty of Dentistry, “Carol Davila" University of Medicine and Pharmacy, and two private practices, 2 were excluded because they had active autoimmune diseases, which could have altered the results, given the fact that inflammation is also at the center of these pathologies.

The control group was represented by 19 clinically and biologically healthy adults with no periodontal disease, other oral disorders, and any known systemic pathology.

All the participants in this study were informed about the aim of the study and their voluntary participation. It was also explained to them that by agreeing to take part in the research, they would have no additional risks to their periodontal treatment, nor any costs or benefits of any kind. Moreover, the subjects included in the study were assured of their anonymity in the processing and publication of the data obtained. Furthermore, they were informed of the right to freely withdraw from this study at any time without any type of repercussions or penalties. After they completely understood the nature and purpose of this research, they signed the informed consent. This study was approved by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy (22976/17.08.2022).

Sample collection and biomarker determination

All subjects included in our study received written information regarding the collection procedure before sampling. Saliva was collected during their next appointment, in the morning, between 8 and 10. They were recommended to avoid food and drink intake, as well as any oral hygiene method, for 12 hours before the collection of the sample. If this was not possible, they had to refrain from eating and drinking for at least 60 minutes before sampling.

Additionally, smoking and chewing gum were also restricted in the last hour before the sample collection, while physical effort was forbidden 30 minutes before the collection. Another requirement was to avoid using lipstick or similar products that morning. In order to remove food scraps and exfoliated cells, each participant in the study rinsed their mouth with distilled water before the collection of the samples.

During the procedure, they were advised to relax, not swallow, and not communicate with others. Three mL of unstimulated total saliva was collected from each participant in the study. Once retrieved in sterile tubes, the saliva was transported to the laboratory on ice in a special transport box for biological samples. Samples were then centrifuged at 3000 rpm for 15 minutes and aliquoted. Until further analysis, they were kept in the freezer, at -80°C.

The three biomarkers investigated in this study were determined using the Enzyme-linked immunosorbent assay (ELISA) and commercial kits (DRG Instruments GmbH, Germany – TAC and leptin; Abbexa LTD, Cambridge, UK for NLRP3). The determination of all parameters was performed according to the instructions of the manufacturer.

Statistical analysis

All the data from the study were analyzed using IBM SPSS Statistics 26 (IBM, USA). Data were expressed as means ± std. Deviation, as well as medians, depend on the distribution's normality. The Shapiro-Wilk test was used to evaluate data distribution. The non-parametric Mann-Whitney U test was then applied to compare quantitative independent variables between the groups. Correlations were assessed using Spearman's rho correlation coefficient. A p-value lower than 0.05 was considered statistically significant.

RESULTS

The patient group included 44 subjects, among whom 21 were males (47%). The mean age was 51.8 ± 9.9 years, with a median of 51.5 years. The average number of remaining teeth in the patient group was 21.8 ± 4.6, with a median of 23. Eleven patients (25%) suffered from hypertension, diabetes mellitus, hyperthyroidism, or osteoporosis. The control group was represented by 19 healthy subjects, among whom 5 were males (26%). Their mean age was 28.5 ± 13.8 years, with a median of 19 years.

The results obtained show that when all patients with periodontal disease were compared with the control group, NLRP3 had statistically significantly increased levels in the study group (Table 1, Figure 1). Although TAC was also increased in the patients compared to the healthy subjects, no statistically significant differences were found (Figure 2). At the same time, leptin was significantly decreased in patients with periodontal disease compared to the control group (Figure 3).

Table 1: NLRP3, TAC and leptin salivary levels in periodontal patients and the control group

Biomarker	Group	Median	Mean ± Std. Deviation	p-value*
NLRP3	Patient	1.1372 ng/mL	1.117 ± 0.332 ng/mL	**< 0.00001**
	Control	0.7382 ng/mL	0.776 ± 0.208 ng/mL	
TAC	Patient	76.741 µmol/L	114.127 ± 119.398 µmol/L	0.491
	Control	70.838 µmol/L	94.553 ± 101.928 µmol/L	
Leptin	Patient	5.894 ng/mL	6.124 ± 2.064 ng/mL	**< 0.00001**
	Control	8.827 ng/mL	8.618 ± 1.478 ng/mL	

**Mann-Whitney U Test*

Figure 1: Salivary levels of NLRP3 in the patient group *versus* the control group

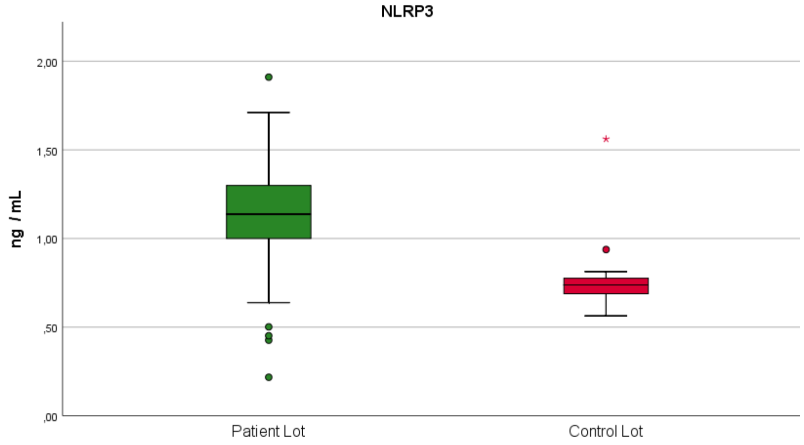

Figure 2: Salivary levels of TAC in the patient group *versus* the control group

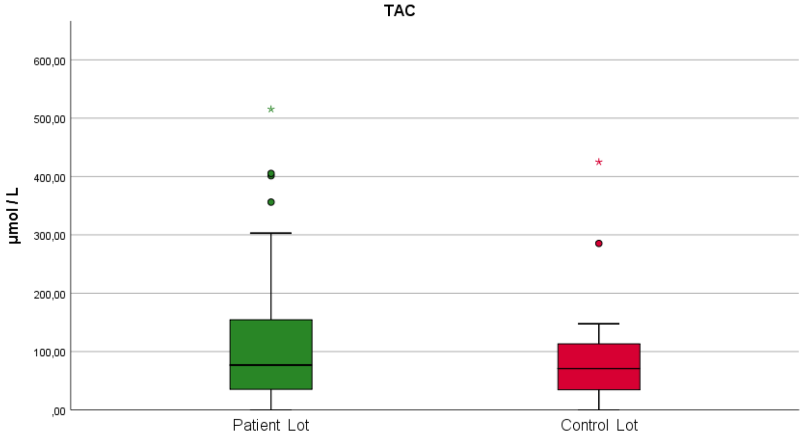

Figure 3: Salivary levels of leptin in the patient group *versus* the control group

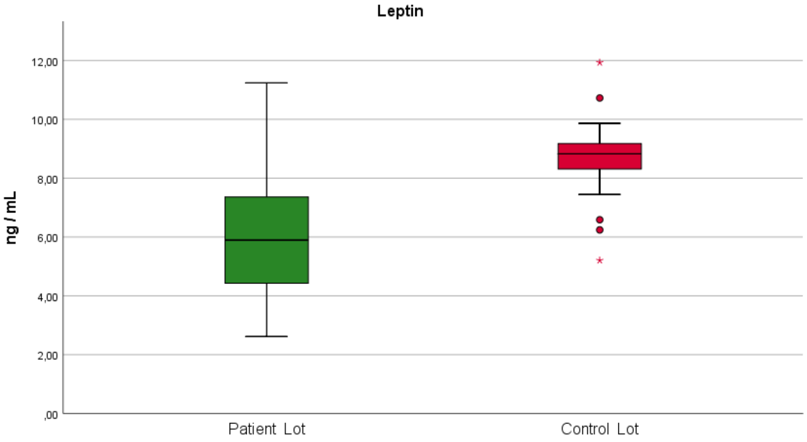

None of the three determined parameters displayed significant differences when comparing patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions (Table 2, Figures 4, 5, 6).

Spearman's rank correlation coefficient was used to test intragroup possible associations between salivary parameters. No significant correlation was found between salivary levels of leptin and NLRP3 when including all patients or when analyzing just patients with periodontal disease without associated systemic conditions. Also, no significant correlation was found between salivary levels of NLRP3 and TAC or TAC and leptin when including all patients or when analyzing just patients with periodontal disease without associated systemic diseases. No statistically significant correlations were found between the concentrations of the analyzed biomarkers and the number of remaining teeth when taking into consideration all periodontal disease patients, nor when including only the patients without associated systemic diseases (Table 3).

Table 2: Comparison of the biomarkers' salivary levels in periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

Biomarker	Group	Mean ± Std. Deviation	p-value*
NLRP3	No Associated Diseases	1.13 ± 0.369 ng/mL	0.356
	Associated Diseases	1.077 ± 0.189 ng/mL	
TAC	No Associated Diseases	112.041 ± 112.434 µmol/L	0.828
	Associated Diseases	120.387 ± 115.215 µmol/L	
Leptin	No Associated Diseases	6.346 ± 2.011 ng/mL	0.115
	Associated Diseases	5.457 ± 2.174 ng/mL	

**Mann-Whitney U Test*

Figure 4: Salivary levels of NLRP3 in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

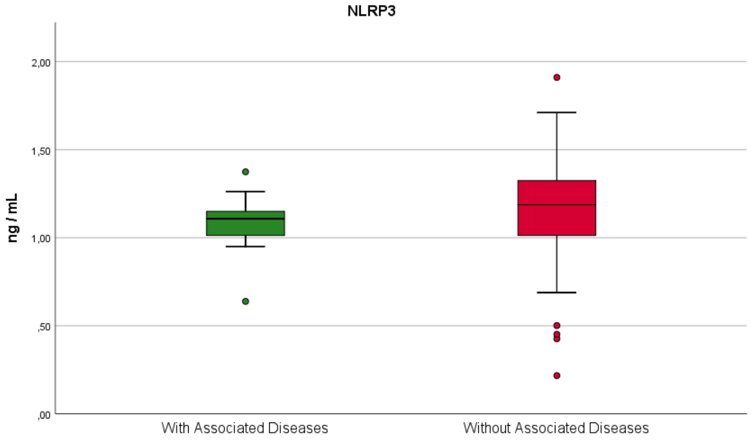

Figure 5: Salivary levels of TAC in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

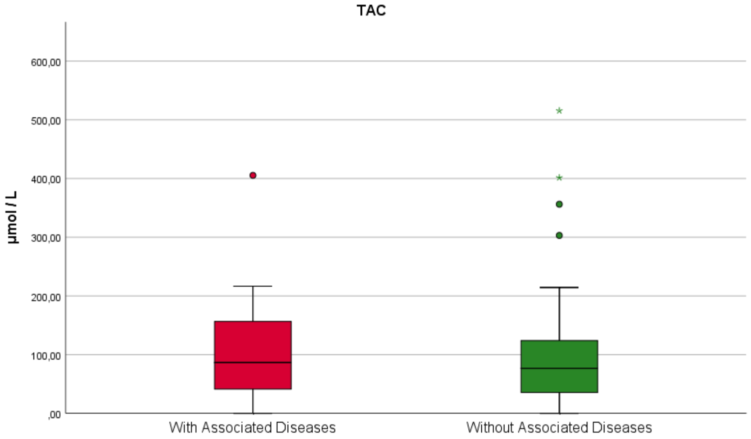

Figure 6: Salivary levels of leptin in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

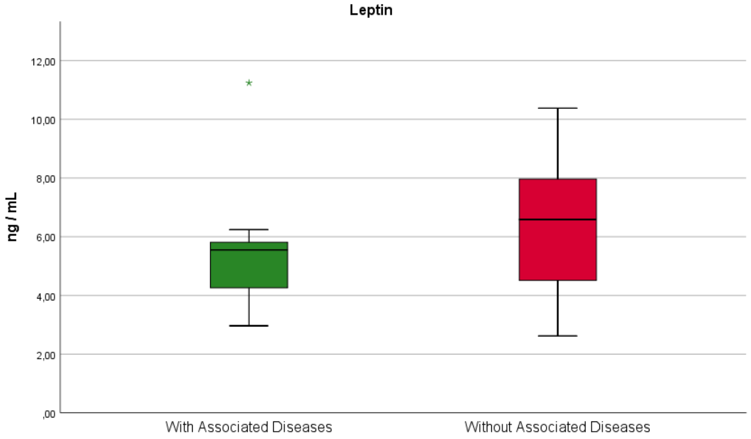

Table 3: Correlations between the determined biomarkers

Correlation	Spearman Rho	p – value
Between the number of remaining teeth and the NLRP3 levels for all patients	0.175	0.255
Between the number of remaining teeth and Leptin levels for all patients	0.095	0.541
Between the number of remaining teeth and the TAC levels for all patients	-0.158	0.305
Between the number of remaining teeth and the NLRP3 levels for patients without associated disease	0.223	0.213
Between the number of remaining teeth and leptin levels without associated disease	-0.063	0.729
Between the number of remaining teeth and leptin levels for patients with associated diseases	0.378	0.252
Between the number of remaining teeth and the TAC levels for patients without associated disease	-0.088	0.626
Between leptin and NLRP3 in all patients	0.147	0.340
Between leptin and NLRP3 in patients with no associated diseases	0.234	0.189
Between TAC and NLRP3 in all patients	-0.171	0.267
Between TAC and NLRP3 in patients with no associated diseases	-0.131	0.467
Between TAC and leptin in all patients	-0.168	0.276
Between TAC and leptin in patients with no associated diseases	-0.059	0.746

DISCUSSION

Saliva has already proven to be a reliable diagnostic, prognostic, and monitoring fluid for both oral and systemic diseases, being very rich in molecules and macromolecules that can be used as biomarkers [48]. Furthermore, the collection procedure is easy, it is more efficient in terms of costs, has a lower risk of cross-infection compared to the blood, and most importantly, the sampling is non-invasive [48, 49]. However, when determining the salivary levels of these biomarkers, we have to keep in mind that their concentrations are frequently decreased compared to the blood. Additionally, there are several factors that can influence their levels, including the moment of the day or the level of stress [49].

In recent years, inflammasomes have represented a major research interest due to their potential to determine an inflammatory response by integrating signals from different pathogens and altered cells [50, 51]. The most studied of the inflammasomes in systemic diseases, NLRP3, has also gained attention regarding its connection with periodontal disease [52]. The dysbiosis and inflammation associated with periodontal disease lead to the activation of the components of the NLRP3 inflammasome. Apart from promoting pyroptosis and neutrophil recruitment, once activated, NLRP3 promotes the overproduction of cytokines and antibodies, as well as the release of prostaglandin E2. In the end, all these events result in the destruction of the periodontium [53].

The results of previous studies performed on animal models were not conclusive. Yamaguchi et al induced periodontal disease in mice by orally injecting them with Porphyromonas gingivalis, showing that this microbial species, involved in the inflammatory process characteristic of chronic periodontitis, activated the NLRP3 inflammasome, leading to a response from the cells of the innate immune system and involvement of the IL-1 family of cytokines [54]. Nevertheless, a recent study by Rocha et al. performed on mice with induced periodontal disease revealed different results, showing that the inflammation associated with periodontitis and the consequent bone resorption was not determined by NLRP3, which played a minor role in the process, but rather by caspase-1 [55]. The results of our study showed statistically significant increased salivary levels of NLRP3 in patients with periodontitis compared to the control group ($p< 0.00001$, Table 1, Figure 1), suggesting a possible association of NLRP3 with the pathogenesis of this oral condition. Furthermore, the data we obtained did not show any correlation between NLRP3 salivary levels and the number of remaining teeth on the dental arches (Table 3).

When comparing the salivary levels of NLRP3 in patients with periodontitis and without systemic diseases to those who have associated systemic diseases, we were unable to find any statistically significant difference (Table 2). These results indicate that NLRP3 levels are mainly due to the presence of periodontal inflammation and not to the coexistence of other pathologies. Isola et al reported that patients with periodontitis and patients with periodontitis and type 2 diabetes had increased NLRP3 levels, both in the serum and saliva, not only compared to healthy controls, but also versus patients who had type 2 diabetes, but did not have periodontal disease [53]. Using samples from the gingival crevicular fluid and gingival biopsies, Garcia-Hernandez et al. showed that patients with periodontitis and uncontrolled type 2 diabetes displayed upregulated genes and proteins associated with NLRP3 activation compared to patients who had only periodontitis [56].

A systematic analysis from 2020 concluded that the levels of leptin both in plasma and saliva reported in the analyzed studies were too different, and although a decrease in salivary levels of leptin was observed in periodontal patients compared to healthy subjects, that data was not sufficient to establish the relationship between the disease and levels of leptin [57].

Similar to other studies, our results showed that leptin had significantly decreased levels in the saliva of patients with periodontal disease compared to those of the control group ($p< 0.00001$, Table 1, Figure 2) [58]. Leptin has already been shown to activate NLRP3 inflammasome in other inflammatory diseases, including systemic lupus erythematosus, and autoimmune disease characterized by the presence of the inflammatory process [59]. Despite both NLRP3 and leptin showing increased levels in periodontitis, we could not find a correlation between the two biomarkers.

TAC was defined by Miller as the "measure of the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound” [60]. Since ROS are naturally counteracted by antioxidants, determining the total antioxidant capacity has become one of the common and important ways to monitor the effect of oxidative stress in periodontal disease. However, the results regarding TAC levels in periodontal disease are controversial, with research showing both significant increases in periodontitis patients compared to healthy controls, as well as no significant differences [61].

On the other hand, Toczewska et al reported lower levels of TAC in both the saliva (which was a mixture of non-stimulated and stimulated saliva) and the gingival crevicular fluid of periodontal patients [62]. A decrease in the antioxidant capacity of patients with periodontal disease was also reported by Diab-Ladki et al [63]. Moreover, similar results were obtained by Brock et al, although their data did not show a statistically significant difference for salivary levels [64]. In our study, the determination of TAC displayed higher salivary levels in periodontitis patients compared to the control group ($p=0.491$, Table 1, Figure 3), but no statistical significance was found. This difference could be explained by the fact that the control group consisted of healthy subjects who had a mean age lower than that of the periodontitis patients. Limberaki et al previously reported that, contrary to expectations, younger people have lower TAC levels in the serum than older people [65].

Previous research revealed that while mitochondrial dysfunction and ROS overproduction can promote NLRP3 activation, their existence is not imperative for this to happen. Additionally, DNA damage caused by ROS can activate the inflammasome, but its release can happen downstream of the activation of the inflammasome [66]. This could explain why our results showed no statistically significant correlation between the salivary levels of NLRP3 and those of TAC.

Our study has several limitations, including the study groups being relatively small. Additionally, there was a significant mean age difference between the study group and the control group. Moreover, although saliva was collected following the same recommendations for all participants in the study, the salivary flow of each participant could have played a role in the level of the biomarkers [67]. Nevertheless, they reveal that the data obtained by our research group suggests the involvement of NLRP, leptin, and oxidative stress in periodontitis.

CONCLUSION

The results obtained in the present study are promising, but they incite further research that should be more focused on the relationship between these biomarkers and other factors that could influence the outcome, for example, the different types of bacteria involved in periodontal disease. Moreover, analyzing not just the saliva of the patients, but their gingival crevicular fluid could provide better insights. Future perspectives could be offered by determining these biomarkers in dynamic, before and after periodontal treatment, as well as correlating them to clinical parameters. All these represent objectives for our future research, which would offer a more complete picture of the mechanisms involved in periodontal disease development and progression and thus, show how NLRP3 activation can promote periodontal tissue destruction and bone loss.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, I.-I.S.-S., A.C.D; methodology, A.-S.D, D.M., B.-F.M; software, R.I., S.C.B; validation, T.-C.S., A.M.C..; formal analysis, D.M., S.C.B; investigation, T.-C.S., B.-F.M; resources, T.-C.S., A.M.C.; data curation, D.M., B.-F.M; writing-original draft preparation, I.-I.S.-S., T.-C.S., R.I.; writing-review and editing, I.-I.S.-S., D.M., A.-S.D, A.C.D; visualization, R.I., A.M.C.; supervision, A.-S.D, A.C.D; project administration, I.-I.S.-S., A.C.D.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy (22976/17.08.2022).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Evaluating Arbitrary and Histological Margins in High-Risk Nonmelanoma Skin Cancers: A One-Center Experience

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Abstract: Background: Nonmelanoma skin cancers (NMSCs), including basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC), often occur in high-risk areas like the face, ears, and scalp. Surgical excision is standard, but achieving tumor-free margins while preserving function and appearance is challenging. This study evaluates the adequacy of arbitrary surgical margins versus histopathological clearance. Methods: A retrospective analysis was conducted on 147 patients with BCC or cSCC in high-risk areas treated at Elias Emergency University Hospital (2020–2024). Data included demographics, tumor type, size, location, excision margins, and histological clearance. Margins were grouped from 0 mm to >10 mm. Results: Most patients were male (59.2%) with a mean age of 70.6 years. BCC accounted for 70.7% of cases. Arbitrary margins averaged 5.4 mm; mean histological margins were ~2.5 mm. Complete excision was achieved in 91.2%; positive margins were most common on the nose and scalp. Discussion: Arbitrary margins in high-risk areas are often narrower than guidelines recommend, especially where tissue is limited, increasing the risk of incomplete excision. Conclusion: While aesthetic and functional considerations often necessitate smaller margins, improved intraoperative margin assessment could enhance oncological outcomes in high-risk NMSC.

Keywords: Nonmelanoma skin cancer (NMSC), Basal cell carcinoma (BCC), Cutaneous squamous cell carcinoma (cSCC), Surgical margins, Histopathological clearance, High-risk anatomical areas, Mohs micrographic surgery, Arbitrary excision margins, Incomplete excision, Oncologic safety

INTRODUCTION

Basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC) represent the two most common forms of nonmelanoma skin cancer (NMSC), with BCC being the most prevalent malignancy globally. While BCC is characterized by slow growth and minimal metastatic risk, its potential for local destruction and disfigurement, especially in anatomically sensitive areas, underscores the importance of timely and adequate treatment. cSCC, although less frequent, poses a relatively higher risk of local invasion and lymphatic metastasis, particularly in immunosuppressed patients [1].

The cornerstone of NMSC treatment remains surgical excision, favored for its efficacy, cost-effectiveness, and accessibility across healthcare systems. Surgical excision allows for histopathological evaluation of the margins and is generally sufficient for low-risk lesions. However, for high-risk tumors located in functionally and cosmetically sensitive areas (defined as Area H: central face, ears, eyelids, nose, genitals, and hands), standard excision can be technically challenging. In these zones, even small tumors may have subclinical extension, increasing the risk of incomplete excision and recurrence [2].

A surgical margin refers to the outer edge of the tissue removed during surgery. The tumoral involvement of the resected margins is a crucial prognostic element. Evaluating the tumoral invasion of the margins is essential for determining whether the excision is sufficient or incomplete. In instances of cancer, follow-up management choices depend on whether the margins are devoid of tumors. Consequently, precise and timely margin evaluation is essential. Attaining clear surgical margins guarantees the complete removal of cancerous tissues, minimizing the chances of local recurrence [3].

While standard excision remains widely used, it has notable limitations in high-risk areas. One of the primary drawbacks is the inability to assess 100% of the peripheral

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and deep margins during surgery. Mohs micrographic surgery (MMS) is considered the gold standard for treating high-risk NMSCs, particularly recurrent or infiltrative lesions. MMS provides complete circumferential and deep margin control (CCPDMA), resulting in superior cure rates—over 98% for primary BCCs and above 95% for recurrent cases. Moreover, MMS allows for maximal tissue conservation, which is crucial in cosmetically sensitive areas. However, its disadvantages include longer operative times, higher costs, and limited availability, which restrict its universal application [4].

This study aims to demonstrate that standard surgical excision can yield acceptable oncological outcomes in the management of high-risk nonmelanoma skin cancers, particularly in under-resourced hospitals where Mohs micrographic surgery is not available.

MATERIALS AND METHODS

This study included 147 patients who underwent surgical excision of non-melanoma skin cancer at the Plastic Surgery Department in „Elias Emergency University Hospital", Bucharest, Romania, between 2020-2024.

Inclusion criteria – all patients admitted to our plastic surgery department with skin lesions with high suspicion of non-melanocytic skin cancer/histopathological confirmation, in a high-risk area, who accepted the surgical treatment. The high-risk areas were defined using NCCN guidelines depending on anatomical localization and size: head, scalp, genital area, trunk, and extremities >2cm.

Exclusion criteria – patients with BCC/cSCC in low-risk areas, premalignancies, melanoma, psychological disorders, and refusal of future treatments.

Arbitrary surgical margins were defined as preoperative markings based on local tissue availability, skin elasticity, and the surgeon's experience. Data regarding sex, age, comorbidities, type of anesthesia used, type of reconstruction, surgical dissection plane, arbitrary surgical margins, peripheral and deep clean margins, tumor diameter, and ulceration were collected and analysed. Surgical standard excision was performed using a no.15 blade in a full-thickness fashion. The deep dissection plane was chosen depending on the case. Excisions were performed under local, regional, or general anesthesia. For wound closure, depending on defect size and location, we used primary closure, skin grafts, and/or flaps. Specimens were oriented using sutures as markings for histopathological evaluation. Excised specimens were sent for histopathological examination to assess tumor clearance.

The histological clearance margins of the lesions were measured peripherally and in depth, recorded in millimeters to one decimal place. They were categorized into five groups:

- zero clearance (indicating margin involvement)
- clearance under 1 mm (0.1–0.9 mm)
- clearance between 1 and 4.9 mm
- clearance between 5 and 9.9 mm
- clearance greater than 10 mm.

Those with zero clearance were advised to have a re-excision following their histopathological results. Patients with clearance margins under 1 mm underwent dermatoscopy surveillance every 3 months; in case of recurrence, the tumors were re-excised. Patients were monitored for wound healing and recurrence.

RESULTS

Demographic

In the 4 years, there were 147 patients, predominantly men with 87 cases (59.2%), with an average age of 70.6 years (see Table 1). BCCs were the predominant cancer, with 104 excisions (70.7%), while cSCCs had 43 excisions (29.3%).

Regarding comorbidities, cardiovascular diseases were predominant in 68 cases (46,2%), followed by diabetes mellitus in 11 cases (7.4%) and other malignancies in 10 cases (6.8%). The anesthesia of choice was local in 143 cases (97.3%), regional and general anesthesias were less prevalent at 2 and 3 cases, respectively. The preferred method of reconstruction was the local flap, with 54 cases (36.7%), followed by full-thickness skin grafts with 38 cases (25.9%), and direct sutures with 36 cases (24.5%). On the other hand, reconstruction techniques such as regional flaps and split-thickness skin grafts were rarely used.

The surgical plane of choice was subcutaneous with 62 cases (42.2%), suprafascial with 42 cases (28.6%), while deeper planes such as supracondylar (14.3%) and supraperiostal (8.2%) have been rarely used.

Incomplete excisions

There were 13 cases of incomplete excisions (see Table 2). The areas with incomplete excisions were located on the nose, with 5 out of 13 cases, followed by the scalp, 4 out of 13 cases. The cheek and peri-orbital regions had a lower rate of incomplete excisions, accounting for 2 cases. Notably, no incomplete excisions were recorded for lesions located on the lips, limbs, or trunk.

Table 1: Demographics and Clinical Characteristics

Sex	
- female	60 (40.8%)
- male	87 (59.2%)
Age	70.6 years (29 – 94 years)
Type	
- BCC	104(70.7%)
- SCC	43 (29.3%)
Comorbidities	
- CV	68(46.2%)
- Diabetes Mellitus	11(7.4%)
- other malignancies	10 (6.8%)
Type of anesthesia	
- local	143 (97.3%)
- regional	2 (1.4%)
- general	3 (2%)
Type of reconstruction	
- direct suture	36 (24.5%)
- skin graft	
-STSG	6 (4.1%)
-FTSG	38 (25.9%)
- local flap	54 (36.7%)
- regional flap	3 (2%)
Surgical dissection plane	
- subcutaneous	62 (42.2%)
- suprafascial	42 (28.6%)
- supracondral	21 (14.3%)
- supraperiostal	12 (8.2%)
Arbitrary surgical margins (mean, mm)	5.4 mm (2-15mm)
Largest diameter (mean, mm)	21.6 mm (5 – 184mm)
Peripheral clearance margins (mean, mm)	2.52 mm (0-9.2mm)
Deep clearance margins (mean, mm)	2.65mm (0-20mm)

Table 2: Incomplete excisions expressed as % of all incomplete excisions depending on anatomic site

Anatomic site	Total Lesions	Incomplete Excisions	% of All Incomplete Excisions	Mean Diameter (mm)	Mean Arbitrary Margin (mm)	Presence of Ulceration (n)
Cheek	17	1	7.7%	17.11	4.3	16
Forehead	15	2	15.4%	18.6	4.4	9
Nose	33	5	38.5%	14.1	4.3	24
Peri-orbital	24	1	7.7%	15.45	5.2	15
Lips	8	0	0%	13.3	6.2	8
Scalp	26	4	30.8%	22.6	5.0	19
Limbs	14	0	0%	35.2	9.2	11
Trunk	10	0	0%	58.6	7.6	6
	147	**13**				

The majority of cancers (64.6%) had peripheral histological clearance margins between 1.0–4.9 mm, as we can see in Table 3. Margins with clearance under 1 mm were present in 15.6% of cases. Complete margin involvement (0 mm clearance) was identified in 4.8% of tumors, comparable to the 8.8% incomplete excision rate. No cases exceeded peripheral margins larger than 10 mm, balancing oncological safety with tissue preservation.

The most common deep margin range was 1.0–4.9 mm, present in 57.1% of cases. Deep margins between 0.1–0.9 mm were observed in 19% of cases. Complete deep margin involvement (0 mm clearance) was identified in 8.8% of cases, reinforcing the necessity of achieving adequate excision depth to minimize recurrence risk. Only 4.1% of lesions had deep clearance margins exceeding 10 mm.

Table 3: Margin Clearance Distribution Among Excised Tumors

Margins (mm)	Peripheral total	% total lesions	Deep total	% total lesions
0	7	4.8%	13	8.8%
0.1-0.9	23	15.6%	28	19.0%
1-4.9	95	64.6%	84	57.1%
5.0-9.9	13	8.8%	16	10.9%
>10	0	0%	6	4.1%

Peripheral Margins >1 mm

The highest histological clearance rates, with peripheral margins over 1 mm, were noted in lesions located on the limbs (100%) and scalp (73%), suggesting that these regions allow a wider excision. In contrast, the lowest rates of peripheral clearance beyond 1 mm were documented in lesions on the forehead (6.1%) and lips (4.8%), revealing the technical difficulties of obtaining wider margins in these areas.

Table 4: Distribution of Peripheral and Deep Histological Clearance Margins depending on anatomic site

Anatomic site	Total lesions	Peripheral histological clearance margins > 1mm	%	Deep histological clearance margins (0.5-4.9 mm)	%
Cheek	17	13	8.8%	17	11.6%
Forehead	15	9	6.1%	11	7.5%
Nose	33	21	14.3%	27	18.4%
Peri-orbital	24	16	10.9%	22	15.0%
Lips	8	7	4.8%	8	5.4%
Scalp	26	19	12.9%	21	14.3%
Limbs	14	14	9.5%	13	8.8%
Trunk	10	8	5.4%	10	6.8%
	147	**107**	**72.8%**	**129**	**87.8%**

Deep Margins (0.5–4.9 mm)

The nasal region had the greatest number of cases with deep margins in the 0.5-4.9 mm range, with 27 cases (18.4%), as seen in Table 4, explaining its elevated rate of incomplete excisions. Areas such as the peri-orbital region, presented 22 cases (15.0%), the scalp with 21 cases (14.3%), reveal the difficulties of achieving sufficient deep margins in said locations.

DISCUSSION

Basal cell carcinoma is the most prevalent cancer, presenting with a slow growth rate and a very low metastatic risk, located predominantly on the head and neck, but also on the limbs and trunk [5]. BCCs tend to be locally destructive and may cause deformity or recurrences if not fully excised [6]. The most important risk factor is long-term/chronic sun exposure, followed closely by a history of either BCC or SCC, with patients being more likely to develop another BCC compared to patients without a history of NMSCs. The rate of developing BCC increases with age, with an average age of 68 years; men have higher rates of BCC than women [7]. The treatment of choice is removing the tumor through different methods, such as Mohs micrographic surgery, peripheral and deep en face margin assessment, cryosurgery, electrodesiccation, and surgical excision. Surgical excision remains the staple method of removing the tumor [8,9].

Cutaneous squamous cell carcinoma, while not as prevalent as BCC, is still a frequent malignancy with similar risk factors as the BCC, such as sun exposure, Fitzpatrick skin types I-II, male gender, and other etiologies such as immunosuppression and chronic wounds (e.g., Marjolin Ulcer). Regarding the recurrence rate following surgical excision, cSCCs located in areas prone to prolonged sun exposure yielded a higher rate of recurrence compared to areas with less sun exposure. The primary risk of recurrence is invasion of the dermis. On the other hand, there was no noticeable difference between the 2 areas regarding the rate of metastases [10,11]. SCCs pose a low metastatic risk, the preferred metastatic site being the lymph node [12,13]. They can be more aggressive than BCCs, with a higher likelihood of local invasion and potential for metastasis, especially in high-risk cases. Understanding the local invasiveness of NMSCs is crucial for determining appropriate treatment strategies and improving patient outcomes [14].

Surgical excision of NMSCs aims to completely remove the tumoral tissue while sacrificing as little normal tissue as possible. This can be achieved through simple elliptical excision. However, Mohs micrographic surgery is the gold standard for treating high-risk tumors. Its primary benefit lies in conserving healthy tissue, preserving anatomical structures, and ensuring tumor-free margins. A recent randomized clinical trial found that Mohs surgery yielded superior outcomes for recurrent BCC compared to direct excision, although no significant differences were observed for primary BCC treatment [15].

Area H refers to high-risk regions, including the central face, ears, genitals, and hands, which require balancing oncologic control with cosmetic and functional outcomes [16]. Mohs surgery is an excellent option for preserving function and enhancing cosmetic outcomes. From a reconstructive perspective, imaging techniques help to identify optimal blood supply for flaps, minimizing complications and improving success rates. As a result, the focus of skin cancer surgery has evolved from simple excision and flap coverage to superior functional and aesthetic outcomes [17].

Peripheral and deep en face margin assessment (PDEMA), also known as complete circumferential peripheral and deep margin assessment (CCPDMA), is associated with the highest cure rates for NMSCs, due to complete peripheral and deep margin visualization, and re-excision of residual tumor [18]. Mohs is the most common method of PDEMA; however, there are also other techniques, such as the Tubingen method, which differs from Mohs by having a histopathological examination in a 1-5 day interval over Mohs's rapid frozen section and interpretation [19].

The mean age of patients with NMSCs in our study was 70.6, with a tendency to be more represented in males. Ciążyńska et al. observed no significant differences between genders. However, the mean age of men in their group was 70.9 +/- 11,4 years, and for women 71.4 +/- 11.9 years [20].

According to NCCN Guidelines Version 2.2025, high-risk criteria for both BCCs and cSCCs are: tumor diameters >= 2 cm in the trunk and extremities, any size pertaining to the head, neck, feet, pretibial and anogenital area, recurrence, immunosuppression, and poorly defined borders [21,22].

The issue with arbitrary surgical margins is that they can lead to either removing more normal tissue than necessary or less than required, and thus leading to recurrence. A study from the Hospital Universitari Vall d'Hebron in Barcelona reviewed histopathological reports from all BCCs treated by different specialties, aiming to determine the influence of a surgical specialty on assessing and excising a tumor with proper safety margins. It revealed that dermatologists had the lowest rate of positive margins out of other specialties, such as plastic surgery, general surgery, etc. attributed to their broader knowledge and expertise in evaluating tumor margins macroscopically. Therefore, proper identification of macroscopic tumoral margins for excision must be emphasized [23].

Australian Journal of Plastic Surgery conducted a study in which they monitored subjects with different levels of experience, as they were drawing a 3 mm, 5 mm, and 10 mm margin around different lesions without using a measurement device, with acceptable margins being considered to be approximately within 0.5 mm of the recommended surgical margin. Margins were underestimated, no matter the level of experience, being smaller in size for each bracket. Margins of 3 mm have been the most accurately estimated out of the 3, increasing the margin size leads to an increase in inaccuracy. This serves to enforce the importance of proper safety margins and the risk of re-excisions caused by improper measurements [24,25].

The majority of excised tumors (64.6%) displayed peripheral histological clearance margins ranging between 1.0–4.9 mm. Narrow peripheral margins (0.1–0.9 mm) have been observed in 15.6% of cases. Complete margin involvement (0 mm clearance) was identified in 4.8% of tumors. No cases exceeded peripheral margins larger than 10 mm, balancing oncological safety with tissue preservation. The most common deep margin range was 1.0–4.9 mm, present in 57.1% of cases. Deep margins between 0.1–0.9 mm were observed in 19% of cases. Complete deep margin involvement (0 mm clearance) was identified in 8.8% of cases, reinforcing the necessity of achieving adequate excision depth to minimize recurrence risk. Only 4.1% of lesions had deep clearance margins exceeding 10 mm. Khalid-Raja M. suggested identifying the peripheral margin of excision by using the wet blotting technique and applying a 4-mm margin of excision to all NMSCs. Their results showed a 95% successful excision rate and a peripheral clearance margin of 0.1-14 mm, a mean peripheral margin of 3.73 in the case of BCCs, and a 96% successful excision rate with peripheral margins varying from 0.5 to 19.0 mm, a mean peripheral margin of 4.86. Comparatively, our study showed a 92% successful excision rate and a mean peripheral margin of 2.52 mm, with the mean average surgical margin being 5.4 mm. The highest number of incomplete excisions was located at the nose and scalp with arbitrary surgical margins of 4.3 and 5.0 mm, respectively. Lesions over 2 cm lead to a 91.2% complete excision with a 4 mm margin while using the wet blotting technique. This coincides with our findings [26,27].

J Kiely et al. recommend the excision of poorly differentiated cSCCs with a minimum peripheral margin of 6 mm, well-differentiated cSCCs were excised with an average peripheral margin of 4.72 mm, and the poorly differentiated ones with an average margin of 6.42 mm. This led to an involvement of peripheral margins in 3% of well-differentiated lesions and 13.2% of poorly differentiated ones. Deep margins were also close or involved in 50% of poorly differentiated cSCCs, demonstrating that poorly differentiated cSCCs are not properly excised in both peripheral and deep planes, in spite of the guidelines [28].

Australian Journal of General Practice recommends for high-risk lesions over 5 mm margins for BCCs and a 6 mm margin for cSCCs, claiming selection of surgical margin should aim to balance between incomplete excision of the tumor and normal tissue sacrifice, affecting cosmetic and functional outcomes [29].

Eva Van Loo et al. found the recommended excision margins for BCC too defensive, evaluating the Dutch Guideline, which states that the margin for low-risk BCC should be 3-4 mm, and the high-risk BCC up to 20 mm, incorporating the H-zone as a high-risk BCC. Currently, the Dutch and European consensus is that high-risk BCC should have a minimum of 5 mm, or 5-15 mm (European guideline). The findings indicate that adherence to the guideline in high-risk BCC was not adequate, yet it had a limited effect on the incomplete excision rate. A margin of 3 mm was sufficient for 99.6% of low-risk BCCs, and a 5 mm margin for high-risk BCCs up to 94% [30].

High incomplete excision rates (8.8%) suggest that in certain areas, such as the nose and scalp, wider margins might be needed. The lowest incomplete excision rates were found in the peri-orbital region and the cheek, while no incomplete excisions were documented on the lips, trunk, and limbs. Ceder H found higher incomplete excision rates located on the face and scalp, the highest ones being on the nose, ear, and peri-orbital area; also noteworthy, circular excisions were also more often incomplete compared to elliptical excisions [31].

Despite its high cure rates and tissue-sparing advantages, Mohs micrographic surgery (MMS) presents several notable disadvantages that limit its universal application. One of the primary concerns is the limited availability of specialized centers and trained personnel, making access difficult in under-resourced or rural healthcare settings. MMS is also more time-consuming than standard excision, as it involves intraoperative histological analysis of multiple tissue layers, requiring patients to remain in the facility for extended periods. Additionally, the procedure is costlier due to the need for specialized equipment and trained histopathologists, potentially placing a financial burden on healthcare systems and patients alike. From a logistical standpoint, not all lesions are suitable for MMS—tumors with extensive subclinical spread, poorly defined borders, or aggressive histologic subtypes may require complementary or alternative approaches. Lastly, in settings where rapid frozen-section pathology is not feasible, delays in diagnosis and treatment can occur, reducing the practicality of MMS outside high-volume dermatologic surgery centers [32]. While Mohs surgery offers excellent cure rates, its use in elderly patients requires careful consideration. The prolonged procedure time, need for patient immobility, and potential for postoperative care challenges may be poorly tolerated by those with comorbidities or cognitive impairment. Therefore, treatment should be individualized based on the patient's overall health, functional status, and life expectancy [33].

CONCLUSION

This study highlights the challenges of achieving adequate surgical margins in high-risk anatomical areas affected by nonmelanoma skin cancers. Although arbitrary margins based on surgeon experience averaged 5.4 mm, histopathological evaluation revealed that a significant number of excisions had suboptimal peripheral or deep clearance, particularly in constrained areas like the nose and scalp. Despite a 91.2% overall rate of complete excision, the 8.8% rate of positive margins underscores the limitations of visual and tactile margin estimation alone. The findings support existing guidelines recommending wider margins ≥5 mm for BCC and ≥6 mm for cSCC, while also emphasizing the need to balance oncologic safety with aesthetic and functional preservation. In high-risk regions, individualized surgical planning, improved intraoperative margin assessment, and consideration of techniques like Mohs micrographic surgery in selected cases may optimize outcomes and minimize recurrence. Ultimately, refining margin estimation and tailoring surgical strategy to both tumor characteristics and anatomical context are essential for improving the quality of care in patients with high-risk NMSCs.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, S.M.R and K.A.J, methodology, A.L.P.; formal analysis, A.L.P.; data curation, P.A.C; writing—original draft preparation, K.A.J, A.L.P; writing—review and editing, S.M.R..; visualization, R.D.S..; supervision, R.D.S.; project administration, A.L.P. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Elias University Emergency Hospital, Bucharest, Romania (protocol code 3606 and 19.06.2025).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Targeting Heart Disease in Cardiovascular–Kidney–Metabolic Syndrome: A Review of Novel Therapeutic Approaches with Prognostic and Quality-of-Life Impact

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Abstract: Cardiovascular-kidney-metabolic (CKM) syndrome describes the complex, bidirectional interplay among cardiovascular, renal, and metabolic dysfunctions, where impairment in one system accelerates decline in the others. This interconnected pathophysiology significantly elevates the risk and progression of heart disease, particularly heart failure, through mechanisms involving insulin resistance, systemic inflammation, neurohormonal activation, and endothelial dysfunction. In this context, therapeutic strategies targeting heart disease must address the multifaceted drivers of CKM. Recent advances highlight the need for an integrated, patient-centered approach that combines lifestyle interventions with pharmacological treatments tailored to individual cardiovascular risk. While foundational therapies such as RAAS inhibitors and statins remain essential, novel agents now offer additional prognostic and quality-of-life benefits. SGLT2 inhibitors have emerged as a cornerstone therapy, improving outcomes in heart failure with both preserved and reduced ejection fraction, independent of glycemic control. GLP-1 receptor agonists and dual GLP-1/GIP agonists like tirzepatide demonstrate cardiometabolic and renal protection, while finerenone shows promise in diabetic kidney disease and several heart failure phenotypes. These therapies not only target glycemic control but also reduce cardiovascular mortality, hospitalizations, and renal decline. Optimizing cardiovascular outcomes in CKM syndrome requires early, multifactorial therapeutic intervention, informed by evolving evidence and a deeper understanding of the heart–kidney–metabolism axis.

Keywords: heart failure, diabetes mellitus, obesity, chronic kidney disease, GLP-1 receptor agonists, SGLT2 inhibitors, mineralocorticoid-receptor antagonists, finerenone, cardiovascular-kidney-metabolic syndrome

INTRODUCTION

Heart disease is frequently observed in patients with CKM syndrome. There is a pathophysiological interplay between the cardiovascular, renal, and metabolic systems, where dysfunction in one organ can exacerbate impairment in others, which substantially increases the risk of developing heart failure. Patients with CKM syndrome usually present with overlapping conditions as type 2 diabetes, chronic kidney disease, and atherosclerotic cardiovascular disease, all of which contribute to functional and structural cardiac dysfunction.

Heart disease and cardiovascular mortality represent the main drivers of prognosis in all CKM syndrome comorbidities. Hence, special consideration should be directed toward understanding pathophysiological mechanisms through which all these metabolic risk factors converge toward cardiovascular impairment. Additionally, emphasis should be placed on the early and appropriate initiation of the right medication for the right patient, tailored to the estimated individual risk, particularly concerning the novel drug classes that have demonstrated beneficial effects across all components of the CKM syndrome: GLP1 receptor agonists, SGLT2 inhibitors, and the non-steroidal mineralocorticoid receptor antagonist finerenone.

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This review emphasises the role of the heart in the CKM spectrum, with particular interest in the therapeutic implications.

GLP-1 RECEPTOR AGONISTS

GLP-1 (glucagon-like peptide-1) is a natural hormone mainly released by intestinal L enteroendocrine cells residing in the distal jejunum, ileum, and colon, as a result to oral glucose intake. GLP-1 is classified as an incretin hormone because it stimulates insulin release from beta pancreatic cells, inhibits the release of glucagon and, along with that, hepatic glucose output, and reduces the rate of gastric emptying and promotes satiety by central appetite suppression. GLP-1 has a very short half-life of approximately 2 minutes, being quickly cleaved by dipeptidyl peptidase IV (DPP-4), a widely expressed enzyme, thereby limiting the strong insulin secretagogue effect of this hormone [1–3].

Owing to the very short half-life, the molecule of GLP-1 is not suitable for an adequate therapeutic agent. Nevertheless, researchers developed molecules capable of being adequate therapeutic agents: inhibitors of DPP-4, which are capable of prolonging the effects of natural GLP-1, and molecules that represent long-acting GLP-1 receptor agonists [1].

Apart from pancreas, GLP-1 receptors are expressed on various types of cells throughout the body and the finding of this widespread localization of receptors led to studies that demonstrated multiple effects beyond the well-known incretin actions, the so-called “pleiotropic effects”: in central nervous system, being involved in the regulation of appetite, in the cardiovascular system, being expressed both in cardiomyocytes and vascular cells, in the musculoskeletal system, gastrointestinal tract, regulating the motility or secretion, interfering in the gut-brain axis, in adipose tissue influencing fat metabolism through interaction with leptin, in kidneys at multiple levels (arterial and arteriolar smooth cells, glomerulus, juxtaglomerular cells, proximal tubule), modulating renal function both directly by altering glomerular hemodynamics and indirectly through less known direct interactions between gastrointestinal system and kidneys known as gut-renal axis [3–7]. Even in beta pancreatic cells, besides altering insulin or glucagon levels, the stimulation of GLP-1R could enhance beta-cell proliferation, inhibit apoptotic pathways, and induce expansion of islet mass [4].

Considering that diabetes mellitus and obesity serve as risk factors in the context of cardiovascular diseases, the effects of GLP-1 receptor agonists on the cardiovascular system are, to a considerable extent, mediated through modulation of these metabolic conditions.

Regarding their role as antidiabetic medications, all GLP-1RAs proved effective in glycemic control by reliably reducing fasting plasma glucose and HbA1c (up to a reduction of 56.2 mg/dl FPG levels and 2.1% for HbA1c levels, respectively) [8].

Obesity is strongly associated with the development of T2DM as excessive adipose tissue, particularly visceral fat, contributes to insulin resistance [9]. The central downregulation of appetite, accompanied by slowing the gastric evacuation, which induces a feeling of fullness, facilitates the reduction of overall calorie intake and the maintenance of a favorable long-term energy balance [3]. Large meta-analyses of the available RCTs proved that GLP-1 RA are effective in inducing weight loss and a reduction in the body mass index and in the waist circumference [8,10].

Conversely, an increasing body of evidence suggests that GLP-1RAs exert direct beneficial effects on various components of the cardiovascular system, independently of their impact on glycemic regulation and weight control.

As far as cardiomyocytes are concerned, the presence and the exact extent of GLP-1R expression are unclear, corroborated by limited evidence regarding the cellular localization of GLP-1R protein [11]. However, in cellular and animal models, besides metabolic effects, which could indirectly improve cardiac function, there is evidence that GLP-1 could restore crucial signaling pathways that could be altered by external stressors [7]. For example, GLP-1 could ameliorate the extent of ischemia-reperfusion injury by activating specific signaling pathways and inhibiting cardiomyocyte apoptosis [3]. Moreover, GLP-1 signaling is involved in reducing hyperglycemia-induced apoptosis of the cardiomyocytes by optimizing the signaling pathways from the sarcoplasmic reticulum and in lowering the extracellular matrix remodeling [12].

There is also important evidence that GLP-1 receptor stimulation influences various pathogenic mechanisms that are involved in atherogenesis. First of all, GLP-1 can mitigate endothelial dysfunction by inducing NOS-mediated vasodilation and by limiting the formation of advanced glycosylation end-products [3,13]. Additionally, GLP-1 is involved in improving lipid metabolism at several levels: with respect to gene expression, it downregulates genes of lipogenic enzymes and upregulates genes of lipolytic enzymes, reduces lipotoxicity by lowering lipid peroxidation, stimulates fatty acid oxygenation, inhibits HMG-CoA reductase and lowers cholesterol levels, ameliorates hepatic steatosis, lowers serum triglyceride levels and even reduces intestinal lipid absorption [3,14]. There are also described effects of enhanced cholesterol efflux from foam cells or suppression of foam cell formation [13]. Even an anti-inflammatory pathway is proposed with the downregulation of genes involved in inflammation and oxidative stress. Moreover, GLP-1 promotes an atherosclerotic plaque remodeling towards stabilization by reduction of matrix metalloproteinases (MMP), proteolytic enzymes involved in weakening of plaque fibrous caps and in increasing the risk of rupture [3,13].

GLP-1RA proved effects of blood pressure reduction both in preclinical and clinical studies, by as much as 6 mmHg (as it was found in DURATION-1 trial) [11,15]. There are several mechanisms possibly involved in this effect: the capacity of GLP-1RA to counteract the sympathetic nervous system hyperactivation, both at the central and peripheral nervous system level, the improvement in the endothelial function and therefore in vasodilation, and the possible effect on renal tubular mechanisms by increased natriuresis, which was observed in experimental models of hypertension [16,17]. The effect of lowering blood pressure was consistent throughout the entire spectrum of renal function, from normal to severely reduced [18].

Diabetes affected individuals are at high risk of chronic kidney disease (CKD) (as many as 40% diabetic patients develop chronic kidney disease), diabetic nephropathy representing the leading cause of chronic kidney disease worldwide. However, in the CKD population, cardiovascular events constitute the primary contributor to mortality, due to enhanced atherosclerosis, arterial and valvular calcification, and amplification of myocardial fibrosis [19]. There is a clear rationale that reducing both the risk and progression of chronic kidney disease could play a pivotal role in decreasing the burden of cardiovascular complications in diabetic patients.

Apart from indirect beneficial effects of glycemic control, weight loss and blood pressure reduction upon renal function, there are described direct effects: increased natriuresis and diuresis, at least partially explained by inhibiting sodium-hydrogen exchanger 3 in proximal tubular cells, anti-inflammatory effects by inhibition of cytokine production and chemotactic recruitment of inflammatory cells, free-radical scavenging effects and direct glomerular hemodynamic effects [5,17,19].

Another pathophysiological mechanism seemingly targeted by this therapeutic class is the modulation of the renin–angiotensin–aldosterone system, as there was been proven that GLP-1RA could induce a decrease in plasma levels of renin and angiotensin II. The effect of lowering the activity of this system could contribute to the effect of lowering blood pressure, the enhanced natriuresis, and could influence glomerular hemodynamics by vasodilation of the efferent arteriole and reduction of glomerular capillary pressure [18].

Beyond the pathophysiological effects demonstrated at the experimental level, the clinically relevant renoprotective effects include the reduction of albuminuria and, as evidenced by several studies, a reduction in eGFR decline [18,20].

Transitioning from experimental models and pathophysiological mechanisms to clinical evidence, GLP-1RA use in practice is supported by results from representative large-scale cardiovascular outcome trials. These studies have positioned GLP-1 receptor agonists as a distinct class among antidiabetic medications, with benefits that go beyond mere glycemic regulation.

There are 8 cardiovascular outcomes randomized controlled trials (CVOTs) for 7 different GLP-1RA (lixisenatide, liraglutide, semaglutide s.c., oral semaglutide, exenatide, albiglutide, dulaglutide, efpeglenatide) which included type 2 diabetic patients with high cardiovascular risk or previous cardiovascular disease. Four different GLP-1RAs, namely liraglutide, semaglutide s.c., dulaglutide, and efpeglenatide, proved efficient in reducing a composite end-point of major adverse cardiac events MACE (comprising cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke)[21]. However, analyzing the hazard ratio for individual components of MACE, there is a clear heterogeneity between these molecules. A meta-analysis which included all 8 RCTs, comprising a total of 60.080 patients (72.4% having established cardiovascular disease), concluded that GLP-1RA class reduce MACE by 14% (HR = 0.86, 95% CI 0.79-0.94, P = 0.006), with significant effects on the risk of cardiovascular death by 13% (P = 0.016) and nonfatal stroke by 16% (P = 0.007) [22]. Another meta-analysis, which included the same RCTs but ELIXA (the RCT examining lixisenatide), because lixisenatide is a short-acting agent that raised concerns regarding the lack of sustained GLP-1 inhibition in patients, GLP-1 RA proved effective in reducing MACE by 14% (HR 0.86 [95% CI 0.80–0.93]; p<0.0001), cardiovascular death by 13% (HR 0.87 [0.80–0.94]; p=0.0010), stroke by 17% (0.83 [0.76–0.92; p=0.0002), and, in addition to the other meta-analysis, myocardial infarction by 10% (0.90 [0.83–0.98]; p=0.020) [23]. Additionally, both meta-analyses proved a significant 12% reduction in all-cause mortality [22,23].

Table 1: Major CVOTs of GLP-1RA [21] (Although promising results, albiglutide was withdrawn from the market by the manufacturer due to commercial reasons)

TRIAL	ELIXA	LEADER	SUSTAIN-6	EXSCEL	Harmony Outcomes	REWIND	PIONEER-6	AMPLITUDE-O	SELECT
DRUG	***Lixisenatide***	***Liraglutide***	***Semaglutide s.c.***	***Exenatide***	***Albiglutide***	***Dulaglutide***	***Semaglutide oral***	***Efpeglenatide***	***Semaglutide s.c.***
POPULATION	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	BMI>27 kg/m^2
MACE HR (95% CI)	1.02** (0.89–1.17)	0.87 (0.78–0.97)	0.74 (0.58–0.95)	0.91 (0.83–1.00)	0.78 (0.68–0.90)	0.88 (0.79–0.99)	0.79 (0.57–1.11)	0.73 (0.58–0.92)	0.80 (0.72–0.90)
CV DEATH HR (95% CI)	0.98 (0.78–1.22)	0.78 (0.66–0.93)	0.98 (0.65–1.48)	0.88 (0.76–1.02)	0.93 (0.73–1.19)	0.91 (0.78–1.06)	0.49 (0.27–0.92)	0.72 (0.50–1.03)	0.85 (0.71–1.01)
MI HR (95% CI)	1.03 (0.87–1.22)	0.86 (0.73–1.00)	0.74 (0.51–1.08)	0.97 (0.85–1.10)	0.75 (0.61–0.90)	0.96 (0.79–1.15)	1.18 (0.73–1.90)	0.78 (0.55–1.10)	0.72 (0.61–0.85)
STROKE HR (95% CI)	1.12 (0.79–1.58)	0.86 (0.71–1.06)	0.61 (0.38–0.99)	0.85 (0.70–1.03)	0.86 (0.66–1.14)	0.76 (0.61–0.95)	0.74 (0.35–1.57)	0.80 (0.48–1.31)	0.93 (0.74–1.15)

Taking into account the various pleiotropic effects of GLP-1, there has emerged the hypothetical idea of using GLP-1RA in individuals without diabetes. In 2023, the results of the SELECT trial were published, a randomized controlled trial that included overweight patients (BMI>27 kg/m^2) with a history of cardiovascular disease but without diabetes. Subcutaneous semaglutide proved efficient, compared to placebo, in reducing MACE by 20% (HR 0.80 [0.72 to 0.90]; p<0.001), mainly driven by a statistically significant reduction of myocardial infarction risk by 28% [24].

These solid results, from large, reputable RCTs, have been translated into guideline recommendations. According to the 2024 ESC Guidelines for the management of chronic coronary syndromes and the 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes, GLP-1RAs have a class I (level of evidence A) recommendation in patients with diabetes mellitus type 2 and history of atherosclerotic cardiovascular disease to reduce incident MACE [25,26]. This recommendation stands regardless of the baseline or target HbA1c or any other antidiabetic medications the patient may be taking. Additionally, consistent with the SELECT trial, the 2024 ESC Guidelines for the management of chronic coronary syndromes (CCS) included a IIa (level of evidence B) recommendation for the utilization of subcutaneous semaglutide in overweight (BMI > 27 kg/m^2) CCS patients to mitigate the risk of cardiovascular mortality and myocardial infarction [24,25].

As far as kidney outcomes are concerned, there is an RCT specifically designed to assess renal endpoints. The FLOW trial included 3533 patients with type 2 diabetes mellitus and CKD (defined as eGFR 50-75 ml/min/1.73m^2 and macroalbuminuria UACR >300 mg/g or eGFR 25-50 ml/min/1.73m^2 and albuminuria UACR >100 mg/g) and compared subcutaneous semaglutide with placebo. The results showed that subcutaneous semaglutide reduced the risk of a primary outcome (defined as a composite of major renal adverse events comprising new onset of kidney failure, a reduction of eGFR of at least 50% from baseline, or death from kidney or cardiovascular-related causes) with 24% (HR 0.76; [0.66 to 0.88]; p=0.0003) [27]. There is also some evidence toward improving kidney outcomes emerging from the pivotal CVOTs of GLP-1RA and from the meta-analyses which assessed them, but with a lower degree of statistical power as the conclusions are derived from secondary outcomes or exploratory assessments, and the renal benefits were demonstrated through the reduction in the progression of albuminuria, with fewer effects observed on hard end-points [21].

With regard to heart failure, the aforementioned meta-analyses reported a 10% risk reduction of heart failure hospitalizations, but this result was driven only by the reduction reported in the AMPLITUDE-O trial, which investigated efpeglenatide [21–23]. As a result, this outcome could not be generalized as a class effect.

2 clinical trials specifically investigated GLP-1RA in HFrEF patients: FIGHT and LIVE. Neither of the two trials demonstrated the beneficial effects of GLP-1 receptor agonists in patients with HFrEF. Notably, the LIVE trial even raised concerns regarding a potential trend toward an increased risk of mortality, ventricular arrhythmias, and atrial fibrillation [21,28]. In terms of HFpEF, two complementary RCTs were published, which investigated subcutaneous semaglutide in obese patients (BMI>30 kg/m^2) without DM (STEP-HFpEF) and with DM (STEP-HFpEF DM). In both trials, there are reported similar results: significant reductions in primary end-points such as a reduction of 7.8 points in Kansas City Cardiomyopathy Questionnaire (KCCQ) (95% CI [4.8 to 10.9]; P<0.001) in STEP-HFpEF and a reduction of 7.3 points in KCCQ in STEP-HFpEF DM (95% [CI] [4.1 to 10.4]; P<0.001) and an average of 10.7% reduction in body weight (95% CI [−11.9 to −9.4]; P<0.001) in STEP-HFpEF and 6.4% reduction in body weight (95% CI [−7.6 to −5.2]; P<0.001) in STEP-HFpEF DM. There are also reported significant results concerning secondary end-points, such as an average improvement of 20.3 m at 6-minute walk distance (95% CI [8.6 to 32.1]; P<0.001) in STEP-HFpEF and 14.3 m (95% CI [3.7 to 24.9]; P=0.008] in STEP-HFpEF DM, respectively. Essentially, these two trials proved that semaglutide improves symptoms and exercise capacity in obese HFpEF patients with or without type 2 DM, but neither study was powered enough to evaluate the influence on hard clinical events, such as heart failure-related hospitalizations or urgent hospital visits [29,30]. It has been suggested that the clear weight reduction effect had a major contribution to the improvement of symptoms and exercise capacity in these patients [21].

Tirzepatide has recently emerged as a new star in the GLP-1 modulation field, being the latest discovered molecule in this class. More specifically, tirzepatide is a dual agonist that acts both on the receptors of GLP-1 and the receptors of GIP (glucose-dependent insulinotropic polypeptide). GIP represents another incretin hormone, naturally released by K-cells of the upper small intestine. Tirzepatide was validated as an effective antidiabetic medication (up to 2.4% reduction in HbA1c), as proved in the series of trials SURPASS 1-5, when tirzepatide was subsequently compared to placebo, or to active comparators, such as semaglutide, to which it was shown to be non-inferior or insulin degludec, to which it was shown to be superior in terms of glycemic control [31]. In terms of weight management, in SURMOUNT 1-4 clinical trials, tirzepatide proved a potent effect, with a mean weight loss of up to 26% [32]. In a recent network meta-analysis, compared to GLP-1 RA, tirzepatide proved higher efficacy in terms of glycemic control and weight control, probably owing to the dual incretin action [8]

Besides direct effects in the metabolic pathway, the SUMMIT trial yielded noteworthy findings in both the cardiovascular and, to some extent, the renal pathways of CKM syndrome. It included 731 obese patients (BMI over 30 kg/m^2) with HFpEF (LVEF of at least 50%), regardless of their diabetes status, who were randomized to receive either tirzepatide or placebo. The trial yielded positive results with regard to the both primary end-points: first one representing a composite end-point formed by death from cardiovascular causes or a worsening heart failure event (hazard ratio, 0.62; 95% confidence interval [CI], 0.41 to 0.95; P=0.026), a result mainly driven by a reduction in worsening heart-failure events (hazard ratio, 0.54; 95% CI, 0.34 to 0.85), and the second one a reduction in KCCQ score, with a between-group difference at the end of the study of 6.9 (95% CI [3.3 to 10.6]; P<0.001). There was no difference in cardiovascular death [33]. Therefore, for the first time, the SUMMIT trial offered evidence derived from an RCT that influencing metabolic pathways with tirzepatide, regardless of diabetes diagnosis, we could influence hard cardiovascular end-points.

An interesting, recently published subanalysis of the SUMMIT trial found that the beneficial effects of tirzepatide upon worsening heart failure events or various metrics pertaining to functional capacity or quality of life are not influenced by the presence or absence of CKD, namely, eGFR does not seem to influence the effects on major adverse heart failure outcomes. In a head-to-head comparison pertaining to the primary composite end-point of death from cardiovascular causes or a worsening heart failure event, tirzepatide showed a larger absolute risk reduction in the CKD group (preventing 3.6 primary endpoint events for 100 patients treated for 1 year) compared to the group without CKD (preventing 1.6 primary endpoint events for 100 patients treated for 1 year). This may probably be an effect at least partially explained by the larger global risk of heart failure events in CKD patients. Moreover, tirzepatide seems to significantly ameliorate glomerular function at 52 weeks (+2.9 [95% CI: 0.9-4.9]; P = 0.004), an effect which proved constant throughout the broad spectrum of baseline eGFR (between 15 ml/min/1.73 m^2 and 70 ml/min/1.73m^2), as well as UACR at 24 weeks (−25%; 95% CI: −35.5% to −12.7%; P < 0.001) [34].

Although we have a very probable class effect of GLP-1RA in reducing MACE and unique evidence for tirzepatide in influencing hard end-points in HFpEF, currently, there is no direct evidence from RCTs that the dual GLP-1R/GIPR agonist reduces MACE. There exists an ongoing trial (SURPASS-CVOT) which currently evaluates the effect of tirzepatide in comparison to dulaglutide on MACE in patients with type 2 diabetes mellitus.

In general, GLP-1RAs are considered relatively well-tolerated medications, and evidence derived from clinical trials supports their effectiveness. However, there are described side effects such as hypersensitivity reactions (including, in rare cases, anaphylactic shock), a hypothetical enhanced risk of C-cell thyroid neoplasm (based on animal studies and theoretical risk), gastrointestinal adverse effects (such as nausea or vomiting, especially during initiation or dose escalation, increased ileus risk), an increased risk of cholelithiasis, cholecystitis and pancreatitis, and concerns of increased pancreatic cancer risk emerging from observational studies. Generally, gastrointestinal adverse effects are the most common and the most frequent cause of drug discontinuation [35].

SGLT2 INHIBITORS

SGLT2 inhibitors were created to target the SGLT2 transporter, a protein mainly located in the first segment of the proximal convoluted tubule, which is responsible for the absorption of 90-97% of the filtered glucose. The reabsorption of one molecule of glucose is coupled with one sodium ion, the latter being transported along the transmembrane concentration gradient created by the Na/K ATP-ase pump [36]. The residual glucose that escapes reabsorption is transported via SGLT1, which is located in the more distal parts of the proximal convoluted tubule. In case of SGLT2 blockage, SGLT1 could partially compensate for its effects, with only 50-60% of the filtered glucose excreted in the urine [37]. Although SGLT2 is mainly located in the kidney, it is also found in the brain, liver, thyroid, and striated muscle [36,37].

The development of SGLT2 inhibitors was inspired by phlorizin, a naturally occurring compound known to be a SGLT2 inhibitor, with the therapeutic objective of promoting natriuresis and glycosuria in order to obtain glycemic control [37]. In the context of efforts to preserve the biologically active properties of phlorizin, while also improving its oral bioavailability and stability, a series of novel molecules were developed: canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin. Moreover, a dual inhibitor that blocks both SGLT1/SGLT2, namely sotagliflozin, was approved, which adds the effect of delaying intestinal glucose absorption by inhibiting SGLT1 at this level [37,38]. All molecules share very attractive pharmacologic properties: optimal oral absorption, once-daily administration as a result of the long half-life, metabolism occurring primarily in the liver, reduced renal clearance, and absence of clinically significant drug interactions [37].

Throughout studies, SGLT2 inhibitors have shown only modest effectiveness with regard to glycemic control, with reductions in HbA1c levels within the range of 0.5-1% [36,39]. Despite continuous glycosuria, inhibition of SGLT2 reduces insulin release and increases glucagon levels, which stimulates gluconeogenesis besides activation of lipolysis and lipid oxidation [40]. Similar to GLP-1 receptor agonists, and constituting an important observation for the largely overweight type 2 diabetes population, it was observed that SGLT2 inhibitors also exert a weight-reducing effect. This effect was observed to be approximately 2 to 4 kg, a consistent and durable reduction documented across all studies and for all molecules. To refer to specific figures, the inhibition of glucose reabsorption at the nephron level causes a loss of 75 g of glucose per day, which means nearly 300 kcal per day. It would be expected that a yearly weight loss of 10 kg, which is much more than was noted in clinical studies [40]. There are proposed mechanisms for this discrepancy between theory and practice, and the most prominent one is compensatory hyperphagia induced by a reduction in leptin, a hormone released by adipose tissue which inhibits appetite at a central level [40,41].

After the finding that rosiglitazone, a thiazolidinedione, is associated with an increased risk of adverse cardiovascular events, the scientific community requested that the new SGLT2i need to undergo cardiovascular outcome trials in order to prove their safety, similar to the aforementioned GLP-1RA.

The EMPA-REG OUTCOME trial tested empagliflozin in over 7000 patients with established cardiovascular disease and proved that this SLGT2i is associated with a significant 14% (HR 0.86; 95% confidence interval [CI], 0.74–0.99; p=0.04) reduction in the risk of major adverse cardiovascular events, a 32% reduction of all-cause mortality (HR, 0.68; 95% CI, 0.57–0.82]; p<0.001), and a 35% reduction of HF hospitalization risk (HR, 0.65; 95% CI, 0.50–0.85; p=0.002) [42,43]. Similarly, the DECLARE TIMI trial, for dapagliflozin, showed non-inferiority to placebo with respect to MACE (p<0.001) and a reduction with 17% of the risk for cardiovascular death or hospitalization for heart failure (HR, 0.83; 95% CI, 0.73-0.95; p=0.005) [42,44]. The CANVAS trial, for canagliflozin, showed a reduction with 14% of the combined risk of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke (HR, 0.86; 95% CI, 0.75 to 0.97; P<0.001 for noninferiority; P=0.02 for superiority) [42,45]. Ultimately, the VERTIS trial, which studied ertugliflozin, found a significant reduction in the risk of heart failure hospitalizations [42]. Based on these preliminary studies, the ESC guidelines for the management of heart failure mention a class I indication for the utilization of SGLT2i in diabetic patients at high risk for CV events to reduce the risk of hospitalization for heart failure events, MACE, end-stage renal dysfunction, or cardiovascular death [46].

These initial trials prompted the conduction of future dedicated trials in order to establish a potential role of SGLT2i in the management of heart failure patients.

The further DAPA-HF trial proved to be a pivotal study, which paved the way for the actual large indications of SGLT2i in heart failure with reduced ejection fraction (HFrEF). This trial included 4744 patients with II-IV HYHA class heart failure, with an ejection fraction of less than 40% and proved that dapagliflozin (10 mg single dose) is associated with the reduction of a primary composite outcome of cardiovascular death, HF hospitalizations, and worsening HF (HR, 0.74; 95% CI, 0.65 to 0.85; P<0.001), with all components of this composite outcome being positively and significantly influenced. This trial also found that the effect is not influenced by the diabetic status of the patient [42,47].

For empagliflozin, the EMPEROR-Reduced trial showed similar results: a reduced composite outcome of cardiovascular death and HF hospitalizations (HR, 0.75; 95% CI, 0.65–0.86; p<0.001), similarly independent of the diabetic status of the patient. In the case of empagliflozin, the effect on the primary outcome was mainly driven by the reduction of the rate of HF hospitalizations, as the reduction of cardiovascular death proved to be non-significant [42,48]. A meta-analysis of these 2 landmark trials found significant reductions in all-cause death (pooled HR 0.87, 95% CI 0.77-0.98; p=0.018) and in cardiovascular death (pooled HR 0.86, 0.76-0.98; p=0.027) [49].

These findings have been incorporated into guideline recommendations, as both the ESC guidelines and the ACC/AHA guidelines on heart failure mention dapagliflozin and empagliflozin as indicated in all HFrEF patients, irrespective of their diabetic status, to reduce the risk of heart failure-related death or hospitalization (class I level of recommendation A) [46,50].

Encouraged by the positive results in the HFrEF field and taking into account the lack of available medications with prognostic effects in HFpEF, the idea of investigating the efficacy of these medications in this prevalent HF phenotype has emerged. And initial evidence emerged from the SOLOIST-WHF trial, which evaluated the effects of sotagliflozin, the dual SGLT1/SGLT2 inhibitor, initiated soon after an episode of decompensation of heart failure; sotagliflozin proved a lower risk of a primary composite end-point formed by cardiovascular death or hospitalizations and urgent visits for heart failure (HR, 0.67; 95% confidence interval [CI], 0.52 to 0.85; P<0.001). The result, suggesting a potential effect of SGLT2i in HFpEF, was that the risk reduction of the primary end-point was consistent across the entire spectrum of ejection fraction [51].

Empagliflozin gained evidence for HFpEF and HFmrEF attributable to the results of the EMPEROR-Preserved trial (which included patients with an ejection fraction above 40%). Empagliflozin 10 mg once daily reduced a composite end-point of worsening heart failure or cardiovascular death (hazard ratio, 0.79; 95% confidence interval [CI], 0.69 to 0.90; P<0.001), a result driven by a significant reduction in the risk of worsening heart failure (with no difference in death from cardiovascular causes) [52]. Likewise, in the DELIVER trial (heart failure patients with an ejection fraction above 40%), dapagliflozin 10 mg od reduced the events of a similar primary composite end-point (hazard ratio, 0.82; 95% confidence interval [CI], 0.73 to 0.92; P<0.001). Once again, this effect is attributable to the reduction of the worsening heart failure events, without significant effects on cardiovascular mortality [53]. As a result, clinical guidelines have been harmonized and currently recommend empagliflozin and dapagliflozin (unique dose of 10 mg per day) in HFpEF and HFmrEF for the reduction of the risk of HF hospitalization or cardiovascular death (class I recommendation in European guidelines, class IIa recommendation in American guidelines) [50,54].

Important evidence for using SGLT2i in heart failure derives from the EMPULSE trial, in which empagliflozin was initiated in hospitalized acute heart failure after stabilization, regardless of ejection fraction or diabetes status. The primary analysis was a win ratio, being a composite of death, heart failure events, time to first heart failure event, and modifications in KCCQ. Empagliflozin proved a better win ratio, with a statistically significant result (win ratio 1.36, 95% CI 1.09-1.68, p=0.0054), with consistent results across the prespecified subgroups [55]. Besides this combination of prognostic and symptomatic benefits, empagliflozin also proved significant results on various indices of decongestion, suggesting that early initiation of empagliflozin in hospitalized acute heart failure patients also has the potential to contribute to an early and sustained decongestion [56].

Regarding the benefits of SGLT2 inhibitors in another acute setting—this time early after myocardial infarction—a recently published meta-analysis concluded that early administration of SGLT2i post-myocardial infarction may be beneficial by reducing the risk of recurrent major adverse cardiovascular events (MACE) or hospitalization for heart failure. However, this meta-analysis did not demonstrate a significant reduction in mortality among these patients [57].

Similar to GLP-1RA, SGLT2i is also an effective medication in chronic kidney disease. Based on findings from early studies on cardiovascular safety, specific trials have been designed to directly assess the potential beneficial effects of SGLT2i in CKD. 4 trials, CREDENCE (for canagliflozin), DAPA-CKD (for dapagliflozin), SCORED (for sotagliflozin), and EMPA-KIDNEY (for empagliflozin) proved that SGLT2i slow the progression of CKD. Whereas for canagliflozin and sotagliflozin, the effects were proven only in T2DM populations, in contrast, for empagliflozin and dapagliflozin, the effects were irrespective of the diabetic status, similar to HF trials. It is important to mention that the recommendation is to initiate dapagliflozin at an eGFR of at least 25 ml/min/1.73m^2 and empagliflozin at an eGFR of at least 20 ml/min/1.73m^2, but the administration may continue even if the renal function further deteriorates (this indication derives from the design of the studies and the characteristics of the included patients) [58].

Table 2: Major CVOTs of SGLT2i [42]

	Type 2 diabetes mellitus	**Heart failure**	**Chronic kidney disease**
Proved effects	In high-risk cardiovascular patients, it reduces the risk of major adverse cardiac events or cardiovascular death. (Besides improved glycemic control and weight loss)	Reduce the risk of heart failure-related death or hospitalization (across the entire ejection fraction spectrum) Improves decongestion in stabilized acute heart failure (empagliflozin)	Mitigate the progressive reduction of eGFR and lower the risk of renal or cardiovascular-related death
Drugs approved	Canagliflozin, Dapagliflozin, Empagliflozin, Ertugliflozin	Dapagliflozin, empagliflozin	Canagliflozin, Dapagliflozin, Empagliflozin
Guideline recommendations	Recommended as first-line add-on therapy to metformin, especially in cardiovascular high-risk, heart failure, or CKD patients	Class I recommendation in HFrEF Class I recommendation in ESC guidelines, class IIa recommendation in ACC/AHA guidelines for HFpEF	T2DM and CKD with eGFR>20 ml/min/1.73m^2 ACR>200 mg/g (and eGFR>20 ml/min/1.73m^2) ACR<200 mg/g with eGFR 20-45 ml/min/1.73m^2 CKD + heart failure

MINERALOCORTICOID RECEPTOR ANTAGONISTS (MRAs)

It is known that overactivation of the renin-angiotensin-aldosterone system and aldosterone receptors (mineralocorticoid receptors, MR) in particular, has effects beyond salt and water retention and hypertension. MR overactivation is capable of inducing inflammation and fibrosis in various organs, being involved in CKD and CV disease progression, situations in which RAAS is overly activated and aldosterone is in excess [73]. As far as metabolic derangement is involved, diabetes mellitus also induces a state of overactivation of MRs, and in obese patients there was observed a significant aldosterone production from adipose tissue was observed [74,75].

The deleterious effects of excess aldosterone are facilitated by the widespread expression of mineralocorticoid receptors throughout the body, as they are present not only in the kidneys but also in the heart, blood vessels, gastrointestinal tract, adipose tissue, and central nervous system [73].

The first therapeutic options to block MRs were the steroidal agents spironolactone and eplerenone, respectively.

The first results for these agents came from RCTs, which tested them in HFrEF, a common entity in advanced stages of cardio-renal-metabolic syndrome. In RALES trial, conducted in 1999, spironolactone reduced all-cause mortality by 30% (RR, 0.70; 95% CI, 0.60 to 0.82; P<0.001) and the risk of heart failure hospitalization by 35% (RR 0.65; 95% CI, 0.54 to 0.77; P<0.001), besides observed benefits on improvement regarding the symptoms of heart failure [76]. Eplerenone gained evidence from the EMPHASIS-HF trial, which proved a significant reduction of the primary composite endpoint of CV death or heart failure hospitalization (HR, 0.63; 95% CI, 0.54 to 0.74; P<0.001) [77]. Further trials provided evidence for steroidal MRAs, namely eplerenone, early after myocardial infarction. EPHESUS and REMINDER trials found that eplerenone, early initiated after MI, in patients with clinical signs and symptoms of heart failure and an ejection fraction of less than 40% could reduce all-cause mortality, length of hospitalization due to heart failure, ventricular arrhythmias, or the risk for deterioration of ejection fraction [78,79]. The beneficial effects of MR blockade in HFrEF and after myocardial infarction emphasize the important effects of aldosterone in cardiac adverse remodelling.

Regarding the HFmrEF and HFpEF, the available results are not as solid as in HFrEF. In the TOPCAT trial, 3,445 patients with symptomatic heart failure and a left ventricular ejection fraction of ≥45% were randomized to receive either spironolactone or placebo. Although the study narrowly failed to meet its primary composite endpoint (including cardiovascular death, aborted cardiac arrest, and hospitalization for heart failure), its results generated considerable debate [80]. The difference between a significant HR in American cohort (HR, 0.82; 95% CI, 0.69-0.98; p = 0.026) and a non-significant HR in Russian and Georgian cohort (HR, 1.10; 95% CI, 0.79-1.51; interaction p = 0.12) has led to speculation that the apparent neutral results of the TOPCAT trial may be due to issues with enrollment and drug adherence in the Eastern European cohort [81]. Based on the significant results on American cohort, there is a 2b recommendation in ACC/AHA guidelines on heart failure for steroidal MRA use in HFpEF and HFmrEF [50].

Hypertension in general and resistant hypertension in particular are common entities in chronic kidney patients or in obese, insulin-intolerant, or diabetic patients. Resistant hypertension is associated with a poor prognosis, causing target organ damage. The PATHWAY-2 RCT established spironolactone as a first-line agent in resistant hypertension, on top of maximum tolerated doses of ACEI/ARB, calcium-channel blocker, and thiazide diuretic [82].

The main limitation of steroidal MRAs' use is the risk of hyperkalemia, a risk observed since the early trials and considered relatively significant, particularly in a population with chronic kidney disease. A meta-analysis that included the landmark RCTs of steroidal MRAs proved that the risk of hyperkalemia and the benefits are inversely proportional to the eGFR (with unclear benefits compared to high risk of hyperkalemia in patients with an eGFR<30 ml/min/1.73m^2) [83].

Gynecomastia and breast pain are other well-known and relatively frequent adverse effects of spironolactone (with a 10% to 1% ratio observed in the RALES trial) [73].

There is a limitation in the steroidal MRAs' potential for broader cardiorenal protection, given the fact that their widespread use is limited by hormonal adverse effects or, in advanced CKD, by a significant risk of hyperkalemia. Moreover, data regarding the direct effects of steroidal MRAs on slowing CKD are unconvincing. There is a meta-analysis that proposes some benefits on intermediate endpoints such as albuminuria or eGFR slope, albeit at the cost of significant risk of hyperkalemia, worsening renal function, or gynecomastia [84].

The novel class of non-steroidal mineralocorticoid receptor antagonists (nsMRAs) has emerged in an effort to overcome these limitations while preserving the beneficial effects of the steroidal MRAs.

Finerenone is the most prominent agent of this pharmacological class, with several advantages over its steroidal counterparts: a comparable potency of MR inhibition but with a much higher selectivity, virtually no sexual side-effects, a more balanced tissue distribution between kidneys and the heart (in comparison with sMRA which are more concentrated at the renal level) and a shorter plasma half-time, which might contribute to a lower risk of hyperkalemia [85,86].

The first RCTs investigating finerenone were FIDELIO-DKD and FIGARO-DKD. FIDELIO-DKD primarily enrolled diabetic patients suffering from advanced CKD with prominent albuminuria, and there was a prespecified primary composite kidney endpoint. FIGARO-DKD enrolled diabetic patients with moderate albuminuria associated with a wider spectrum of CKD and stated a primary composite cardiovascular endpoint [85]. The results from these 2 RCTs and from their pooled analysis (FIDELITY) form the basis for the KDIGO guideline recommendation of finerenone in CKD: in patients with diabetic nephropathy, with an eGFR>25 ml/min/1.73 m^2 and with an albuminuria >30 mg/g, despite a maximum dose of ACEI/ARB [72]. Apart from reducing the kidney disease progression, finerenone reduced cardiovascular risk (HR 0.86, 95% CI: 0.78-0.95) and the risk of heart failure hospitalization (HR 0.78, 95% CI 0.66-0.92) in diabetic CKD patients [86].

The latter FINEARTS-HF trial provides the first quality results for MRAs (particularly nsMRA, finerenone) in HF with EF above 40%. In this RCT, finerenone reduced the risk of a primary composite endpoint formed by worsening heart failure events and cardiovascular death with 16% (rate ratio, 0.84; 95% confidence interval [CI], 0.74 to 0.95; P=0.007), driven by a significant reduction in worsening heart failure events, similar to SGLT2i results in the same patient population [87]. Compared to the previous ones, FINEHEARTS was a revolutionary trial also by the population included, with approximately 60% not having CKD or diabetes, suggesting that there may be important direct effects on the heart [88].

The recent FINE-HEART meta-analysis allows for the formulation of global conclusions regarding finerenone as far as cardiovascular and renal outcomes are concerned. This meta-analysis included the three RCTs, FIDELIO-DKD, FIGARO-DKD, and FINEARTS-HF, encompassing a total of 18991 patients. 12.1% of the included patients concurrently had HF, CKD, and diabetes, the main conditions encompassed in the CKM syndrome. The results indicate that finerenone reduces the risk of heart failure hospitalization (HR: 0.83; 95% CI: 0.75-0.92; $P < 0.001$) and of a composite kidney outcome, thus delaying CKD progression (HR: 0.80; 95% CI: 0.72-0.90; $P < 0.001$). Although not succeeding in proving risk reductions in cardiovascular death (HR 0.89; 95% CI: 0.78-1.01; P = 0.076), finerenone seems to reduce the risk of all-cause death (HR: 0.91; 95% CI: 0.84-0.99; P = 0.027) [88].

Currently, the sole recommendation for finerenone within cardiology guidelines is the class IA recommendation in the 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes, in patients with type 2 DM and CKD (UACR>300 mg/g or eGFR 25-60 ml/min/1.73m^2 and UACR>30 mg/g), in addition to ACE-I or ARB, to reduce the risk of cardiovascular events or kidney failure [26].

This recommendation, similar to those related to SGLT2i or GLP-1RA, empowers all medical doctors involved in the management of CKM syndrome to take proactive measures aimed at improving the prognosis of these patients. It remains to be seen what future heart failure guidelines will unveil regarding nsMRA indications, probably related to future trials that may investigate the effects of finerenone in this population.

CONCLUSION

Management of cardiovascular disease within the cardiorenal metabolic (CKM) syndrome requires a comprehensive, individualized approach, guided by cardiovascular risk stratification. While lifestyle interventions remain foundational, pharmacologic therapy plays a pivotal role in improving prognosis and quality of life.

Standard treatments such as RAAS blockade—with ACE inhibitors, ARBs, and steroidal MRAs—form the therapeutic backbone. However, recent advances support the early initiation of novel agents with proven benefits across all three CKM components.

GLP-1 receptor agonists and dual GLP-1/GIP agonists (e.g., tirzepatide) not only promote glycemic control and weight reduction but also lower the risk of major adverse cardiovascular events and may preserve renal function. SGLT2 inhibitors have demonstrated robust prognostic benefits in heart failure—regardless of ejection fraction—and chronic kidney disease, independent of glycemic status. Finerenone, a non-steroidal mineralocorticoid receptor antagonist, is recommended in diabetic kidney disease with albuminuria and shows emerging evidence of efficacy in HFpEF and HFmrEF, although formal guideline recommendations are still pending.

Optimizing therapeutic strategies with both established and novel agents is essential for altering the course of heart disease in CKM syndrome and addressing its complex, multisystem pathophysiology.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, A.G.F., S.H. and S.M.S., methodology, M.J..; formal analysis, M.F.; data curation, E.R.; writing—original draft preparation, A.G.F., S.A.P.; writing—review and editing, E.C., R.D.G., M.F., E.R.; visualization, R.D.G.; supervision, S.M.S.; project administration, S.H. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

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ORIGINAL ARTICLE

Investigating Digital Skills of Healthcare Professionals in Greek Military and Civil Hospitals: A Cross-Sectional Study

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Abstract: Background and Aim: Digital skills of healthcare professionals are recognized as critical for the successful digital transformation of healthcare. The present study aims to examine the relationship between the digital skills of healthcare professionals and the organizational structures of hospitals. Methods: In this cross-sectional study, using the convenience sampling method, 857 healthcare professionals, working in Greek military and civilian hospitals, voluntarily participated. Data were collected between July and November 2021 through a structured questionnaire, based on the Digital Competence framework. Digital skills were categorized into five subscales: information, communication, content creation, problem solving, and safety skills. Statistical analysis was performed with SPSS 26.0, with p-values <0.05 considered statistically significant. Results: Cronbach's alpha values for all subscales were (α)>0.8, indicating high reliability. Younger participants showed higher scores (p<0.001). The mean scores for information, communication, content creation, problem solving and safety skills in military hospitals were calculated as 52.1 (SD=32.3), 50.6 (SD=31.8), 48.4 (SD=33.3), 49.8 (SD =30.8) and 52.8 (SD=34.1) respectively, while in civilian hospitals they were calculated as 45.1 (SD=32.2), 44.5 (SD=29.5), 41.8 (SD=32.4), 41.5 (SD=30.8) and 47.1 (SD=34.5) respectively. Conclusions: Healthcare professionals in military hospitals exhibit higher digital competence compared to their counterparts in civilian hospitals. Identifying the organizational factors that promote greater digital competence among healthcare professionals provides essential information to policymakers and healthcare managers for implementing targeted organizational improvements.

Keywords: Digital skills, Healthcare professionals, Military hospitals, Digital Competence framework

INTRODUCTION

The management of competencies and skills in the healthcare sector has drawn significant attention from international organizations, recognizing that a well-trained workforce is essential for ensuring patient safety and delivering high-quality care [1,2]. In alignment with the continuous advancements in medical technology, the necessity for healthcare professionals to develop new skills has become increasingly urgent [3,4]. This need was starkly highlighted during the recent COVID-19 pandemic, where the heightened digital demands of healthcare services underscored the importance of sufficient digital capabilities [5,6].

In particular, digital skills are deemed critical not only for the effective management of healthcare organizations but also for medical diagnosis, treatment monitoring, patient care, and decision-making processes [7,8]. Within the healthcare domain, digital competence encompasses knowledge of digital literacy and technology as essential tools for the patient-centered provision of quality healthcare services. It reflects the dynamic interaction between healthcare professionals, patients, and colleagues, as well as the drive to acquire professional expertise in digital technology [9,10].

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It is widely recognized that digital competencies are essential for the effective integration of health technologies into routine clinical practice. Numerous processes are now carried out digitally, such as patient monitoring, where electronic health records and vital sign monitoring systems enable doctors and nurses to track patient status in real time. Material procurement is also facilitated through electronic systems, enhancing both accuracy and efficiency. Furthermore, telemedicine plays a pivotal role, particularly in military hospitals, by enabling the delivery of healthcare services to remote areas and military bases. Digital tools further support decision-making by allowing healthcare professionals to analyze data and make evidence-based decisions more efficiently [3,11,12].

In Greece, military hospitals offer more structured training programs for digital skills, supported by military frameworks that emphasize continuous professional development of their personnel. These programs encompass modules on the management of digital tools, telemedicine, and electronic patient record systems, reflecting the military's long-standing tradition of structured training and lifelong education. Additionally, military healthcare professionals are often encouraged to engage in e-learning programs to further enhance their expertise in healthcare technologies.

Conversely, in public hospitals, training programs for digital skills vary depending on the institution and the extent of technological integration. Significant advancements have been observed in large hospitals that have adopted electronic medical records; however, training opportunities remain limited due to resource constraints and time pressures. Healthcare professionals, including nurses and doctors, typically receive training through Ministry of Health initiatives or European-funded programs such as eHealth and Horizon 2020 [9,10].

Despite extensive research on digital skills, a literature review from the past decade indicates a scarcity of studies that examine the digital competencies of healthcare professionals within a specific and well-defined conceptual framework. One such framework is the European Digital Competence (DigComp) Framework, created by the European Union as a common umbrella of digital competences for its citizens, which defines digital competence domains, indicators for each domain, competence levels, examples of knowledge, skills, and attitudes, as well as examples of application [13]. Moreover, limited research has explored the associations between digital skills and the organizational work environment. To the best of our knowledge, no prior studies have investigated differences in this domain between military and civilian healthcare professionals.

Therefore, the objective of this paper is to address this research gap by examining the relationship between digital skills of healthcare professionals and the organizational structures of their work environments. We aim to contribute evidence on the organizational factors influencing healthcare professionals' digital competencies, which can inform policymakers and healthcare managers' efforts to implement targeted organizational improvements.

MATERIALS AND METHODS

Study Design

A cross-sectional survey utilizing a descriptive study design was conducted based on prior research, which demonstrated that the Digital Competence Indicator tool is a reliable and valid measurement scale applicable to Greek doctors and nurses [14]. The study addressed two primary research questions: (1) How are healthcare professionals' digital skills assessed? and (2) Are there differences in the digital skills of healthcare professionals between military and civilian hospitals?

The present study was conducted as part of the research activities of the Department of Nursing at the Hellenic Mediterranean University and received ethical approval from the Review Committee (Protocol No.: 4634/29-03-2021). Additionally, the necessary permissions were obtained from the Scientific Committees of all participating hospitals (Protocol No.: 5584/1973/26-06-2021). Data were collected using a self-assessment questionnaire, with the principal investigator maintaining sole access to the collected data. Participation was entirely voluntary, and all participants were informed both verbally and via an information/consent form that completing and returning the questionnaire constituted their consent to participate in the study. The information/consent form was approved by the above Scientific Committees. The research adhered strictly to the European Union's General Data Protection Regulation, ensuring full compliance with personal data protection standards.

Participants and settings

A convenience sample of doctors and registered nurses was selected from seven tertiary care hospitals in Greece. This particular sampling method was preferred because it offers time and cost savings, ease of access, and an abundance of qualitative data. However, it carries the risk of bias and systematic errors, and therefore, research findings cannot be accurately generalized beyond the study sample. The selection of hospitals was based on the diversity of organizational structures between military and civilian institutions, as well as the aim of achieving a large sample size. The study population size was calculated using a 95% confidence interval and a 5% margin of error, ensuring that the final sample accurately represented healthcare professionals in Greek hospitals. The target sample size was N>800. A total of 1207 questionnaires were distributed, and 876 were collected, of which 19 were invalid due to incomplete responses. Thus, the study population is N=857.

The inclusion criteria for participation were employment in either a military or civilian hospital, professional status as a doctor or nurse, and, for nurses, registration as per the European Qualifications Framework (EQF). The distribution of questionnaires was proportional to the size of each hospital, based on the number of medical and nursing staff within the institutions. Data collection occurred during staff meetings, where participants from various work positions within the hospitals were approached, provided with explanations of the study objectives, and given clarifications on completing the questionnaire. Data collection was conducted between July and November 2021.

Research tool

Data were collected using a validated 37-item paper-based questionnaire. It is built on the European DigComp Framework, which comprehensively defines the elements of digital competence as a combination of skills, abilities and attitudes essential for incorporating data processing into the professional careers of healthcare providers [13,15].

The questionnaire consisted of two parts. The first part captured demographic characteristics, including gender, age, educational level, profession, specialty, hospital type, professional experience, administrative responsibilities, and possession of digital skills certification. The second part assessed 26 digital skills, organized into five competency domains (subscales): information, communication, content creation, problem-solving, and safety skills (Table 1).

Table 1: Competence area and digital skills of the questionnaire. Adapted from the European Commission's DigComp framework [13]

Competence Area	Digital Skills
Information Skills	1. Finding information about goods and services
	2. Obtaining information from public authority websites
	3. Reading or downloading online news/newspapers/news magazines
	4. Copying or moving a file or folder
	5. Seeking health-related information
Communication Skills	6. Sending/receiving emails
	7. Telephoning over the internet/video calls (via webcam) over the internet
	8. Participating in social networks
	9. Posting messages to chat sites
	10. Uploading self-created content to any website to be shared
Content Creation Skills	11. Creating websites or blogs
	12. Writing a computer programme using a specialized programming language
	13. Using copy and paste tools to duplicate or move information within a document
	14. Creating electronic presentations with presentation software (e.g., slides), including e.g., images, sound, video, or charts
	15. Using basic arithmetic formulae to add, subtract, multiply, or divide figures in a spreadsheet
Problem-Solving Skills	16. Connecting and installing new devices
	17. Installing a new or replacing an old operating system
	18. Modifying or verifying the configuration parameters of software applications
	19. Doing an online course
	20. Buying or ordering goods or services for private use (last 12 months) over the internet
	21. Selling online
	22. Job search or sending an application
	23. Internet banking
	24. Making an appointment with a practitioner via a website
Safety Skills	25. Using any kind of IT security software or tool (anti-virus, anti-spam, firewall, etc.) in order to protect private computers and data
	26. Updating one or more security products at least occasionally

Subscale values were calculated on a scale ranging from 0 to 100, with scores approaching 0 reflecting a more negative evaluation of the variable and scores approaching 100 reflecting a more positive assessment.

The questionnaire provided a concise yet compelling assessment of the Information Technology (IT) skills of hospital doctors and nurses. Designed for ease of use and brevity, it was particularly suitable for hospital settings often characterized by high workloads and limited time for activities outside core medical and nursing responsibilities. Participants rated the extent of their computer usage for each activity on a five-point Likert scale, ranging from 1 ("not at all") to 5 ("very much").

Data Analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) version 26.0. Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables were presented as absolute numbers (n) and percentages (n%). The normality of variables was assessed using the Shapiro-Wilks test, alongside graphical methods such as the Normal Q-Q plot, Detrended Normal Q-Q plot, and Box Plot. For all statistical tests, differences were considered significant at $p < 0.05$. Effect sizes weren't reported. The independent t-test was employed to compare the means of two groups, while one-way analysis of variance (ANOVA) was used for comparisons involving more than two group means.

RESULTS

Participant's characteristics

The study population comprised 857 Greek healthcare professionals employed across seven tertiary care hospitals. The results indicate that 538 participants (62.8%) were female, while 319 (37.2%) were male, with a mean age of 39.7 years (SD = 10.0). Of the total, 657 participants worked in military hospitals, while 200 worked in civilian hospitals. Indicatively, it is reported that the number of military doctors and nurses in hospitals, at the present time, amounts to approximately 1,700 people.

Specifically, among the military hospitals, 384 participants were from the 251 Hellenic Air Force General Hospital, 110 from the 401 General Military Hospital of Athens, 66 from the Athens Naval Hospital, and 97 from the General Military Training Hospital. Among civilian hospitals, 82 participants were from the Sismanoglio General Hospital of Attika, 51 from the Asklepieio Voulas General Hospital, and 67 from the Henry Dunant Hospital Center.

The majority of participants were registered nurses (70.2%), with doctors comprising 29.8% of the sample. Considering the nature of the study population, where nurses outnumber doctors by more than twice, the preponderance of the female gender can be explained by the fact that the nursing profession in Greece has traditionally been considered female-dominated for many years. The average professional experience was estimated at 15.3 years. Regarding educational levels, 528 participants (61.6%) held a Bachelor of Science (BSc) degree, 275 (32.1%) had also completed a Master's degree, and 54 (6.3%) held a PhD degree. Examining the level of education by profession, doctors have a higher proportion of PhD holders (5.4%) than nurses (0.9%). Conversely, nurses have a higher percentage of master's degree holders (22.8%) than doctors (9.3%). The demographic characteristics of the study population are summarized in Table 2.

Table 2: Demographic characteristics of participants (N = 857).

		Hospital				**Total**		
		Civilian hospital		***Military hospital***				
		N	N %	N	N %	N	N %	p-value
Age (years)*		40.6 (9.9)		39.4 (10)		39.7 (10)		0.143
Gender	Female	134	67.0%	404	61.5%	538	62.8%	0.158
	Male	66	33.0%	253	38.5%	319	37.2%	
Educational level	BSc	136	68.0%	392	59.7%	528	61.6%	0.092
	MSc	52	26.0%	223	33.9%	275	32.1%	
	PhD	12	6.0%	42	6.4%	54	6.3%	
Profession	Medical Doctor	53	26.5%	202	30.7%	255	29.8%	0.250
	Registered Nurse	147	73.5%	455	69.3%	602	70.2%	

**Note. Age expressed as mean (standard deviation)*

Digital Skills

Regarding digital skills, the results are overall. The mean scores of all digital skills subscales indicate an average level and are as follows: information skills, 52.1 (SD = 32.3); communication skills, 50.6 (SD = 31.8); content creation skills, 48.4 (SD = 33.3); problem-solving skills, 49.8 (SD = 30.8); and safety skills, 52.8 (SD = 34.1). Median scores, interquartile ranges (IQR), and p-values of the above subscales are presented in Table 3.

The reliability of the subscales was confirmed, with Cronbach's alpha values indicating high internal consistency: information skills (5 items; $\alpha = 0.95$), communication skills (5 items; $\alpha = 0.95$), content creation skills (5 items; $\alpha = 0.95$), problem-solving skills (9 items; $\alpha = 0.97$) and safety skills (2 items; $\alpha = 0.92$). Cronbach's alpha was calculated by group. It is worth noting that the high Cronbach's alpha values (>0.9) observed in the study results are likely due to the narrowness of the aspect structure of the measurements' tool subscales. However, the measurement scale was taken as an existing structure from the European DigComp Framework and was not developed by the researchers. Future survey to explore the factor structure by removing some potentially redundant items or adding new ones is a challenge for the present investigators.

Table 3: Median scores, IQR, and p-values of subscales (information skills, communication skills, content creation skills, problem-solving skills, safety skills) per type of hospital

		Hospital		
		Civilian hospital	**Military hospital**	**Total**
Information skills	Median	45.00	60.00	55.00
	IQR	63.75	60.00	60.00
	p-value	<0.001		
Communication skills	Median	40.00	55.00	50.00
	IQR	50.00	65.00	60.00
	p-value	0.002		
Content creation skills	Median	40.00	50.00	50.00
	IQR	60.00	67.50	65.00
	p-value	0.001		
Problem-solving skills	Median	30.60	52.80	50.00
	IQR	58.30	52.80	55.60
	p-value	<0.001		
Safety skills	Median	37.50	62.50	62.50
	IQR	71.88	62.50	62.50
	p-value	0.007		

The mean scores across all five subscales of the measurement tool were found to differ significantly between military and civilian hospitals. Specifically, the mean score for the "Information skills" subscale in military hospitals was M = 54.2 (SD = 32.1), compared to M = 45.1 (SD = 32.2) in civilian hospitals, with t(855) = -3.522, $p < 0.001$. For the "Communication skills" subscale, the mean score in military hospitals was M = 52.4 (SD = 32.3), while in civilian hospitals it was M = 44.5 (SD = 29.5), with t(855) = -3.088, p = 0.002.

Similarly, the "Content creation skills" subscale showed a mean score of M = 50.4 (SD = 33.3) in military hospitals versus M = 41.8 (SD = 32.4) in civilian hospitals, with t(855) = -3.236, p = 0.001. For "Problem-solving skills," the mean score in military hospitals was M = 52.4 (SD = 30.4), compared to M = 41.5 (SD = 30.8) in civilian hospitals, with t(855) = -4.395, $p < 0.001$. Lastly, the "Safety skills" subscale yielded a mean score of M = 54.6 (SD = 33.8) in military hospitals and M = 47.1 (SD = 34.5) in civilian hospitals, with t(855) = -2.740, p = 0.006.

The mean values (SD) of digital skills (information, communication, content creation, problem-solving, and safety skills) by hospital type are illustrated in Figure 1.

DISCUSSION

Based on the theoretical framework outlined, the findings of this study align with existing research emphasizing the growing importance of digital skills for healthcare professionals. Digital competence is essential not only for the effective delivery of healthcare services but also for ensuring patient safety and enhancing the overall efficiency of healthcare systems [16,17]. These competencies enable healthcare professionals to manage patient data effectively, navigate electronic health records [18,19], process large volumes of information[20], and utilize telemedicine tools [21,22]—capabilities that are no longer optional, but essential and fundamental to healthcare professionals' expertise [10,23,24].

In the present study, digital skills of the Greek study population can be characterized as good, as they are proven to be above the median level, with safety and information skills gathering the highest averages, communication and problem-solving skills being at the median level, and content creation skills being the weakest, with a score just below the median level. It is noteworthy that content creation skills require particular IT knowledge and technical skills, such as developing or maintaining computer systems, hardware, or software, and writing a computer program using a specialized programming language. Thus, they appear as the digital skills category with the lowest proficiency rates in the majority of occupations in Europe [25].

The above levels of digital skills are in line with a similar Greek study, where the digital competence of healthcare professionals is moderate to good [26]. Research conducted on healthcare professionals in European countries shows that the majority have medium levels of digital competence. A study carried out in Moldova found advanced levels of digital skills in the areas of information and communication and moderate levels in the areas of content creation, safety, and problem solving[6]. In Finland, the levels of digital competence are relatively high[27], while in some regions in Finland and Sweden, digital skills are reported as adequate but with several shortcomings[16] and in Catalonia, they appear at a basic and intermediate level [28]. According to the European Centre for the Development of Vocational Training (Cedefop), in terms of digital intensity measurements in the EU for 2021, the level of digital skills for health professionals appears as basic to intermediate, and only in a percentage of 13.4% as advanced [29].

Figure 1: Clustered Bar Mean (95% CI) of Information skills, Mean of Communication skills, Mean of Content creation software skills, Mean of Problem-solving skills, Mean of Safety skills by Hospital (Error Bars: 95% CI).

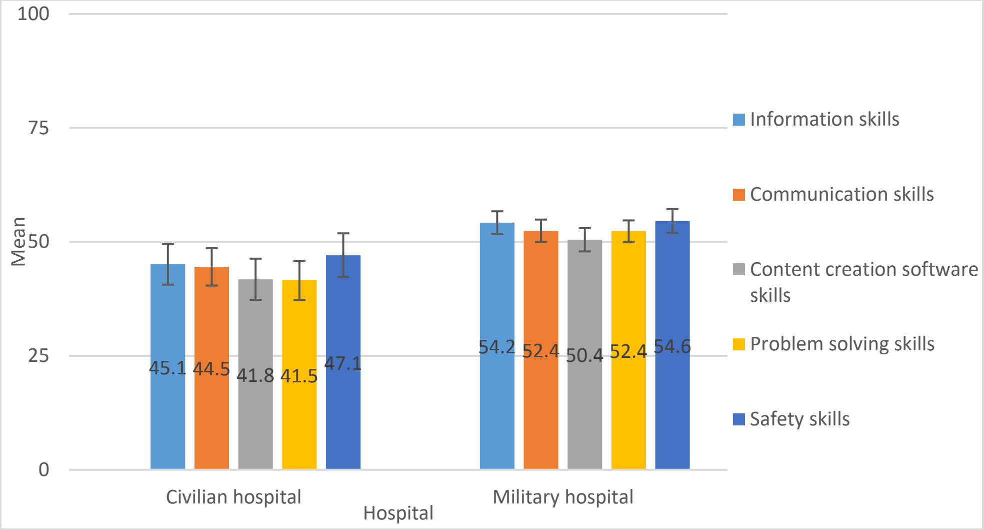

Consistent with prior research, this study also revealed that younger healthcare professionals demonstrated higher levels of digital competence, particularly in domains such as communication and content creation. Literature suggests that younger professionals tend to exhibit greater eHealth literacy and are more adept at using digital tools [30,31]. This generational disparity in digital skills underscores the need for targeted interventions that provide older healthcare professionals with the necessary training and support to enhance their digital proficiency. Hospital management can organize continuing education programs that are easily accessible and tailored explicitly to older professionals, such as online courses, interactive sessions, or specially designed workplace courses.

It is worth noting that, in the measurement tool, all possible demographic confounding factors were included to avoid biased conclusions. According to the literature review, the demographic factors found to be associated with digital skills levels were age, type of occupation, gender, professional experience, and educational level, which were studied in our research [30,32,33]. However, the results of this study found no significant differences in digital skills based on gender, professional experience, specialty, or educational level.

In addition, research underscores the critical role of the working environment in shaping digital literacy. For instance, De Veer and Francke (2010) observed that attitudes toward electronic health records varied significantly depending on the type of healthcare organization [11]. Similarly, Jarva et al. (2023) as well as Rippen et al. (2013), emphasized that strong organizational support—through timely interventions and the provision of adequate resources—is essential for the successful adoption of digital technologies by healthcare professionals [9,34]. Similarly, the findings of the present survey revealed significant differences in digital skills between military and civilian hospitals, with the predominance of health professionals in military hospitals.

This superiority can be attributed to several factors, including the structured nature of military institutions, where digital training and protocols are more systematically integrated into daily operations [35,36]. Military hospitals often place a stronger emphasis on technical proficiency and operational readiness [37,38], which likely fosters the development of higher digital literacy among their staff. Moreover, they prioritize digital training for their personnel as part of their operational strategies and compliance with applicable protocols. In contrast, civilian hospitals may face a broader array of challenges, including inconsistent administrative policies and slower technology adoption due to resource constraints [35,39].

In Greece, in recent years, the Ministry of Digital Governance, in coordination with the Ministry of Health and other bodies, has been working to strengthen the digital competence of doctors and nurses through the implementation of a national e-health strategy. Among the objectives are to improve the continuous education of health professionals and to ensure access to reliable digital information and tools [40]. However, training programs for civilian health professionals are usually their personal responsibility, as organizational procedures are inadequate. In contrast, in military environments, such programs are supported by the respective military education sectors, financed with their own resources and means, and organized on a regular and periodic basis for the training of all their personnel.

The operation of military hospitals, with adherence to hierarchical procedures, is a significant factor in their ability to implement and train staff in digital technologies efficiently. These institutions often adopt new systems at a faster pace, which may explain the consistently higher levels of digital proficiency observed among healthcare personnel in military settings. Such an organizational advantage aligns with the findings of Ludwick and Doucette (2009), who emphasized that organizational readiness and robust support systems are critical to the successful implementation of electronic health records [41].

Furthermore, the constant exposure to technology [36,42] and digital tools [43,44] observed in military settings provides healthcare professionals with more opportunities to enhance their digital skills. In contrast, civilian hospitals face a variety of challenges that may hinder the development of such competencies. In Greece, these challenges include diverse administrative structures, slower decision-making processes, and limited access to resources for digital training [45,46]. The inherent flexibility within civilian hospital operations may further contribute to inconsistencies in the application and development of digital skills among healthcare professionals. This discrepancy could reflect differences in organizational priorities and the availability of technological resources within these institutions.

A noteworthy area where military healthcare professionals excel is in safety skills, likely due to the stringent data protection and cybersecurity protocols mandated within military environments [47,48]. This focus on safeguarding sensitive patient and operational data, cultivates a more advanced understanding of cybersecurity measures among military hospital staff. Although civilian hospitals also prioritize data security, they often encounter greater challenges in implementing uniform protocols due to variations in organizational structure and resource constraints.

Another significant disparity lies in problem-solving skills. Military healthcare professionals appear to be better equipped to handle technical challenges, potentially due to the emphasis on problem-solving as an integral part of military training and operational effectiveness [49]. In contrast, the less regimented procedures commonly found in civilian hospital settings may provide healthcare professionals with fewer structured opportunities to develop and refine these skills.

We consider that the Ministry of Health or the EU frameworks could adopt military-style training modules emphasizing techniques such as extensive practical training, simulation exercises, the development of specialized centers of expertise, field training techniques, the use of existing training frameworks, needs- and context-based design, continuous harmonization with modern standards, and synergies between civilian and military professionals. One such model is the Common Security and Defence Policy (CSDP) Training Programme, an EU policy to provide senior civil and military officers with the necessary skills and knowledge in crisis management situations [50].

Future Implications

We believe that civil-military cooperation, by embracing strengths and avoiding weaknesses, could improve the overall digital capability of health professionals. As military hospitals provide a more conducive environment for developing digital skills, civilian hospitals could benefit from adopting some of the structured approaches used in military hospitals. With the continuous evolution of healthcare technology, it is essential that all healthcare professionals, irrespective of their work environment, are supported in acquiring and maintaining high levels of digital competence.

Digital skills among healthcare professionals represent a burgeoning field of research that has experienced rapid growth over the past decade. The findings of this study highlight the need to explore further the role of institutional support in fostering digital skills, as well as the factors contributing to digital competence gaps among healthcare professionals across different organizational settings. These results hold international relevance, given the global significance of digital transformation in the healthcare sector. With additional research conducted in similar settings across various countries, these findings could be generalized to further advance digital literacy and transformation in healthcare systems worldwide.

Limitations

This study is subject to three key limitations that may affect the generalizability of the findings. First, the participating nurses were all registered nurses (European Qualifications Framework - EQF 6) and not practicing nurses (EQF 5), and therefore, the measurement tool is validated and reliable only for registered nurses. Second, a self-assessment questionnaire was used as a measurement tool, which entails the risk of subjectivity due to overestimation or underestimation of the actual skills of the participants. Finally, the convenience sampling approach carries the risk of bias and systematic errors.

CONCLUSION

This study underscores the critical importance of digital skills among healthcare professionals, drawing attention to notable differences between staff in military and civilian hospitals. The findings indicate that military healthcare professionals generally demonstrate higher levels of digital competence, likely attributable to the structured and resource-rich environments characteristic of military hospitals. These organizational factors, which foster a more digitally skilled workforce, serve as a foundation for identifying and implementing necessary organizational improvements that healthcare managers can leverage.

However, it is important to note that both military and civilian healthcare professionals exhibited moderate levels of digital skills overall, signifying substantial room for improvement across both settings. Strengthening digital competence across the healthcare sector remains essential for meeting the demands of modern healthcare systems.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

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No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere.

Authors' contribution

Conceptualization, A.K. and C.M.; methodology A.K. and C.M.; software, A.K. and C.C.; validation, A.K. and C.M.; formal analysis, C.C. and V.A.K.; investigation, A.K., D.K., V.A.K., M.M., V.T. and C.M.; resources, A.K. and C.M.; data curation, A.K., D.K., C.C. and C.M.; writing—original draft preparation, A.K.; writing—review and editing, A.K., D.K., C.C., V.A.K., M.M., V.T. and C.M.; visualization, A.K.; supervision, C.M., D.K. and C.C.; project administration, A.K., C.C. and C.M. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Hellenic Mediterranean University Ethics Committee (Nursing Department's Executive Board 4634/29-03-2021).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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EDITORIAL AND CONTENT CONSIDERATIONS

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

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The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Academic Editor. The Editor-in-Chief reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials), are exempt.

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Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background and description of the disorder of interest, together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication, as presented at http://www.ICMJE.org/.

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Abbreviations should be used sparingly and only where they ease the reader's task by reducing the repetition of long technical terms. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter, use the abbreviation.

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Acknowledgments

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ORIGINAL ARTICLE

A brief overview of the Epstein Barr virus and its association with Burkitt's lymphoma

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Abstract: *Epstein Barr virus (EBV) is known as an oncovirus and associates with several human malignancies such as Burkitt's lymphoma, other non-Hodgkin lymphomas, nasopharyngeal carcinoma, Hodgkin's disease, gastric adenocarcinoma, etc. in Burkitt's lymphoma, and the key event is the translocation of MYC gene, that increase of cell survival and aberrant expression of MYC gene. The biology of EBV and its function in the development of Burkitt's lymphoma are discussed in this review.*

Keywords: *Epstein Barr virus, oncovirus, Burkitt's lymphoma, MYC gene*

INTRODUCTION

Epstein–Barr virus (EBV), discovered in 1964 by Epstein, Achong, and Barr from cultured cells of Burkitt's lymphoma by electron microscopy [1]. The EBV is recognized as the first human tumor virus. It is associated with lymphomas, epithelial cell cancers, nasopharyngeal carcinoma, and gastric cancer [2, 3]. EBV infection is mainly transmitted by saliva. The prevalence of EBV in the worldwide population is more than 90%. Most of the EBV carrier individuals do not have any symptoms. However, it is estimated that EBV has a role in the development of approximately 200,000 new cancer cases worldwide each year. Also, it is responsible for almost 2% of all human cancers, and according to current evidence, EBV may be a co-factor in the development of other malignancies, such as breast and cervical cancer [4].

EBV virion structure

EBV (also knowns as human gammaherpesvirus 4) is a member of the Herpesviridae family and a common virus among humans. EBV has a linear, double-stranded DNA genome with a length of 184 kb in a nucleocapsid with 162 capsomeres, which is encapsulated in an envelope with external glycoprotein spikes. The virion diameter is roughly 120-180 nm [5, 6].

Replication cycle

EBV infection targets epithelial and B-cells. Eight glycoproteins are involved in virus entry depends on the type of cell (Table 1) [7]. One of the most abundant glycoproteins of EBV called GP350\220 is responsible for the binding to B-cells, in which it binds to complement receptor type 2 (CR2) on B-cells with high affinity [8]. Although this is a key step for virus attachment to the target cells, other factors also are required for infecting target cells. Major histocompatibility complex class II (MHC-II) on B-cell surface act as a co-factor that interacts with the three viral glycoprotein gp42, gL, and gH to bind the virus to B-cell [9-11].

The fusion of the viral envelope with the cell membrane begins with the interplay of gp42 and HLA class II that eventually leads to endocytosis of the virus into the cell [11]. Complement receptor 1 (also known as CD35) (CR1) is an additional adhesion factor that binds to gp350\220 and can provide a pathway for EBV entry into CD21 negative cells such as immature B-cell. Furthermore, CD35 is sufficient for EBV binding, MHC-II expression is also needed for cell entry and infection. The gp350 mediates the attachment of EBV to epithelial cells, however, it can be fulfilled by gHgL complex on CR2 negative cells [20]. The gHgL complex binds specifically to epithelial cells, not B-cells, and the gH-null virus loses the ability to link to a CR2-negative epithelial cell but still can use gp350 to bind to CR2-positive cells [21-23]. The ability of gHgL to bind to CR2 negative epithelial cells can be blocked by the presence of gp42 [21]. It also prevents the virus's ability to infect CR2-positive cells [24]. In epithelial

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cells lacking CD21, the BMRF-2 protein of the virus interacts with cellular β1 integrin, and gHgL proteins interact with αvβ6\8 integrin to initiate virus fusion [25]. Endocytosis of the EBV into the vesicle and fusion of the virus with the vesicle membrane results in the release of the nucleocapsid into the cytoplasm. As the nucleocapsid dissolves, the genome is transferred to the nucleus [26].

Table 1: Roles of envelope glycoproteins in entry to epithelial and B-cells

Gene	Glycoprotein	Role in virus entry	REF
BLLF1	Gp350	Binding to CR2\CD21 receptor in B cell	[12]
BXLF2	gH	Attachment of virus to epithelial cells, also essential for penetration to B-cells	[13]
BKRF2	gL	Heterodimer gH\gL is critical for entry into the host cell	[14]
BZLF2	gp42	Acting as co-receptor for fusion and entry of EBV into the B-cell	[15]
BALF4	gB	Required for fusion with both epithelial cell and B-cell	[16]
BLRF1	gN	It may play a role in post-fusion events	[17]
BBRF3	gM	Co-expressed with gN	[18]
BMRF2	BMRF2	Binding to host beta 1 integrin family and facilitates virus attachment to the epithelial cell	[19]

Lytic replication

Lytic replication leads to the production of infectious viruses. Lytic replication can occur in both B-cell and epithelial cells. The frequent replication cycle in B-cell occurs after reactivation from latency, while the replication cycle in epithelial cells initiates after entry of the virus [27]. For lytic replication of the viral life cycle, its genome must be linear [28]. In the latent phase, the genome is circular, so it should be linearized in the process of lytic reactivation [28]. During lytic replication, the viral DNA polymerase is responsible for the replication of the viral genome, but in the latent phase, it uses the host cell DNA polymerase to replicate its genome [26]. Three consecutive steps are involved in the production of lytic genes: intermediate early (IE), early (E), and late [29]. In the IE step, two essential genes BZLF1 and BRLF1, are expressed.

These genes act as trans-activators in activating the virus and initiating the cascade of viral genes expression [26]. Expression of the IE BZLF1 gene can induce a switch from latent to lytic infection. BZLF1 binds to the promoters of E genes and encodes proteins that are crucial for viral DNA replication. It recruits RNA polymerase II on their promoters and inducing the lytic cascade mechanism [29].

Expression of one of the BZLF1 or BRLF1 genes is enough to induce lytic replication in both epithelial cells and B-cells. The expression of one IE protein causes the expression of other proteins [30]. Early lytic gene products include a wide range of activities such as replication, metabolism, and blockade of antigen processing [31]. BNLF2a is one of the EBV viral genes expressed in the early lytic stage that plays an important role in escaping the virus from the immune system by expression of a 60 amino acid protein that interferes with antigen presentation to CD8+ cells [32-34]. Late lytic gene products often have a structural role, such as viral capsid antigen (VCA), which is involved in the production of the viral capsid. Several late lytic gene products such as BCRF1 are involved in escaping the virus from the immune system [35].

Latency

Unlike the lytic phase, progeny virions are not produced in the chronic phase, and it is a persistent viral infection. EBV persistent infection is mainly seen in memory B-cells but is also found in epithelial cells [36]. Actually, it is thought that one in the millions of B-cells can carry the virus genome as an episome or integrated DNA after recovering from an acute infection [6, 37].

During the latent phase, episome replication occurs by the host DNA polymerase. In the latency phase, only a limited portion of the genes are expressed [38]. These genes contain five EBV-encoded nuclear antigens (EBNA), two latent membrane proteins (LMP), EBV-encoded small RNA (EBER), and BART RNA (Table 2) [39, 40]. Three patterns of expression of latency genes exist that which of them have different expression patterns and are known as latency programs. Latency programs include latency I, latency II and latency III that each of which can lead to the expression of specific viral genes [41, 42]. Three latency program patterns have been determined in different Burkitt lymphoma and lymphoblastoid cell lines (LCL) and are reported in Table 3 [36].

Table 2: expression of Epstein-Barr virus latent genes

Latent gene	Roles	REF
EBNA-1	Responsible for DNA replication, mitotic segregation, EBV transcriptional activation, autoregulation	[36]
EBNA-2	Main viral trans-activator, critical for B-cell activation	[37]
EBNA-LP	Co-operate with EBNA-2 in viral and cellular gene transcription	[38]
EBNA-3A	With EBNA-3C block EBNA-2 by interaction with RBPG proteins, decrease expression of MYC	[39]
EBNA-3B	It is essential for B-cell transformation, tumor suppressor	[40]
EBNA-3C	Essential for efficient B-cell transformation, also like EBNA-2, LP and EBNA-3C regulates viral and cellular gene expression	[41]
LMP-1	B-cell and epithelial cell transformation, critical role as the primary EBV-encoded oncogene, and active NF-KB and P38 pathway, immune regulation	[42]
LMP-2A	Play a key role in improving cell adhesion, cell motility, and inhibition of epithelial cells also, can activation signaling pathways such as the PI3K\AKT, NF-KB, B-Catenin, STAT, and syk tyrosine kinase and immune modulation	[43]
LMP-2B	-	[43]
EBER	Play a key role in modulating innate immunity response, cell resistance to PKR-mediated apoptosis	[44]
BART	Immune regulation, cancer progression	[45]

Table 3: Epstein-Barr virus latency pattern

Gene expressed	EBNA-1	EBNA-2	EBNA-3A	EBNA-3B	EBNA-3C	EBNA-LP	LMP1	LMP-2A	LMP-2B	EBER	BART
Latency I	+	-	-	-	-	-	-	+	+	+	+
Latency II	+	-	-	-	-	+	+	+	+	+	+
Latency III	+	+	+	+	+	+	+	+	+	+	+

Epstein-Barr Virus microRNAs

The first human viral-encoded miRNAs were discovered in EBV in 2004 by Pfeffer et al. [43]. microRNAs (miRNAs) are small non-coding RNA containing about 20-22 nucleotides that play a crucial role in regulating gene expression [44]. For cell-to-cell transmission, miRNAs can be packaged into exosomes and released into the extracellular space [45]. various cell types can secreted exosomes into body fluids, including blood, urine, saliva, breast milk, cerebrospinal fluid, ascites, etc [46] miRNA plays an important role in the pathogenesis of EBV-associated cancers.

EBV encodes over 40 miRNAs, which are derived from approximately 25 viral-miRNA precursors [47]. Forty of them are located to BamHI-A rightward transcript (BART), and four are located to the BamHI fragment H rightward open reading frame 1 (BHRF1) [48]. These miRNAs have distinct levels of expression at different phases of latency. EBV miRNAs can modulate infection and pathogenesis by regulating the expression of viral genes [49]. The miRNA can be effective in escaping the EBV from the immune system by suppressing the release of pro-inflammatory cytokines, such as IL-12, and repress the differentiation of CD4+ T cells. miRNA directly targets the transported TAP2 and decreases levels of TAP1 and MHC class 1 molecule, which can cause the EBV-infected cells to evade CD8+ T cells [50].

Epidemiology

In developing countries, the infection occurs in children (in the first years of life), whereas in developed countries, it is usually delayed and seen in adolescence [51]. In some developed countries, however, the rate of infection is bimodal, with peaks in children under five years and again after ten years [52]. The cause of this bimodality is the transmission of the virus through oral excretion between parents and infants and from partners in adults [53]. The EBV antibody titer in seropositive individuals shows a U-shaped pattern, and the highest antibody titer is seen in infants and the elderly [54]. The high antibody titer in children is usually a reflection of primary infection and, in the elderly, is due to a reduced immune response [55, 56]. EBV has spread worldwide but with remarkable geographical variety in the distribution of EBV genotypes [57]. EBV contains two major types, I and II, that differ in the genes expressed in latently infected cells [58]. Both types are detectable all over the world, but type I is more common.

However, in some areas such as Africa, Papua New Guinea, and Alaska the second type is the most common [59, 60]. The prevalence of the virus is not significantly different between men and women, but overall, antibody titers are higher in women than men [53]. EBV is also associated with socioeconomic conditions. Poor socioeconomic status is related to primary EBV infections, whereas late primary EBV infection occurs more frequently in people with higher socioeconomic status [61].

Transmission

EBV usually is transmitted through oropharyngeal secretions. In adults and adolescents, the primary cause of virus transmission is kissing, whereas saliva on stuff such as toys or fingers is the primary cause of transmission among children. EBV is detectable in cervical secretions of 8-28% of young girls and adult women and also in the seminal fluid of men. In rare circumstances, the virus can be transmitted through breast milk. It can also be transmitted through blood transfusion and transplanted allogeneic hematopoietic cells or solid donor organs [53, 62].

Vaccine for EBV

Vaccination is the most effective way to avoid contracting EBV. More than 90% of the world's human population is infected by EBV, therefore, the vaccine is greatly important. The evidence shows that the EBV vaccine can prevent more than 200,000 new malignancies per year worldwide. gp350 viral antigen is the most abundant glycoproteins on the surface of EBV that known as the first candidate for the vaccine. gp350 has an important role in the efficient infection, although the gp350 deficient virions can still infect B cell, gp350 antibodies predominantly neutralize B cell infection. The amino-acid sequence of gp350 is extremely conserved among diverse strains, especially in the amino-terminal region, and 97% amino-acid similarity between EBV type1 and type 2 [63]. Among all EBV glycoproteins, gp350 is a promising vaccine target to block b cell infection and is also a target for T cell-mediated immunity (64-66). gp350 in combination with the AS04 adjuvant demonstrated can prevent the development of infectious mononucleosis (IM) after EBV infection [67]. Monoclonal antibodies against gp42 can inhibit EBV infectivity of B cells, while infection of epithelial cells inhibited by a monoclonal antibody against gH\gL or BMFR2 antigens [68, 69]. EBV neutralizing antibody titers correlate more with gp350 antibody compared with antibody titers to gp42 [70]. Furthermore, the latent antigen EBNA3A with tetanus toxoid formulated in water-in-oil adjuvant acts as a vaccine that is designated to induce EBV-specific T cell response. In almost 90% of vaccines, CD8 T-cell responses are obvious but can not prevent EBV infection. Although it seems that vaccination can reduce the incidence of IM [71].

Diagnosis

There is a spectrum of laboratory approaches for the diagnosis of EBV infection. The strategy of diagnosis is different between immunocompromised and immunocompetent individuals because therapeutic interventions in these groups are different. The diagnosis of primary infection is based on antibody assays [72]. Three serological patterns such as immunoglobulin (Ig) G and M antibodies to viral capsid antigens (VCA) and IgG antibodies to EBNA1 are a minimal requirement for detection of primary EBV infection. Immunofluorescence assay (IFA) is a gold standard for the diagnosis of EBV infection [73]. enzyme immunoassay (EIA) and chemiluminescence immunoassay (CLIA) are often performed as screening tests, while western blot analysis is used for confirmation [74]. For detecting and quantifying the virus in body fluids, polymerase chain reaction (PCR) analysis is the best approach, and tissue samples as well as, it can be used for measuring the viral load [75]. Additionally, the detection of EBV-encoded RNA transcripts (EBERs) by in situ hybridization is the gold standard for finding EBV in tissue [76].

Treatment

For the treatment of EBV infection, there is no approved antiviral medication. More physicians prescribe acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) to alleviate fever and pain [77]. Fluids and nutrition are highly recommended, especially for febrile cases. Since most patients have anorexia in the first and seconds weeks, nutrition is very important [78]. Several antiviral drugs are used for EBV infection, although non specifically against EBV, and most of these drugs are nucleoside-analogue [79]. The only class of drugs that assess for EBV infection in clinical trials is nucleoside-like agents. The nucleoside analogue in the drug disrupts the synthesis of viral DNA by inhibiting viral DNA polymerase. Acyclovir is a nucleoside analogue that can reduce EBV shedding but without evidence of clinical benefit [80]. Studies have also reported the use of other agents, such as interleukin 2 [81], interferon-alpha [82], and Intravenous immunoglobulins (IVIg) [83] in the management of EBV related diseases.

EBV AND BURKITT LYMPHOMA

For the first time, Burkitt lymphoma (BL) was described 60 years ago by Denis Burkitt as a disorder with jaw tumors in African children, which he called “African lymphoma” [84, 85]. BL is an aggressive B-cell malignancy among non-Hodgkin lymphoma (NHL) types [86, 87]. It is divided into three classes, which vary in geographic distribution and relation with EBV: endemic (eBL), sporadic (sBL), and HIV-associated BL [85, 88].

- **Endemic BL** (eBL) is one of the most common childhood cancers in Equatorial Africa and is accounts for 75% of malignant disorders in children aged 4-7 years [89]. Evidence suggested a supportive relationship between EBV and eBL, which almost all eBL cases are associated with EBV [90]. Plasmodium falciparum contributes to the etiology of eBL first by disrupting immune response against EBV, thereby increase virus replication, second by stimulating B-cell activation, which leads to EBV lytic reactivation and abnormal expression of activation-induced cytidine deaminase (AID) that elevate the probability of MYC translocation triggering oncogenesis [91]. Jaw and kidney involvement are frequent, as well as the central nervous system (CNS), ileal, cecal, gonads, facial bone, thyroid, and breast can be affected [92].

- **Sporadic BL** (sBL) is almost occurring worldwide, without an association between geographic and climatic conditions. sBL is responsible for 1-2% of adult lymphoma, up to 40% of children’s lymphoma in western Europe and the United States. The abdomen, particularly the ileocecal region and lymph node, are the most common site of involvement. sBL is associated with EBV infection, ranging from 10-30% positivity in various regions [93].

- **HIV-associated BL** mainly occurs in human immune-deficiency virus (HIV) infection. HIV expansion in developed and developing countries has increased EBV-dependent BL in adult populations and is an early marker for AIDS progression in HIV-positive individuals [94]. EBV is observed in 30-40% of HIV-associated BL cases (Table 4) [95].

Table 4: Characteristic features of the various Burkitt lymphoma subtypes

	Endemic BL	**Sporadic BL**	**HIV-associated BL**
Geographical distribution	Equatorial Africa And Papua New Guinea	Worldwide	Worldwide
Incidence	$5\text{-}15\backslash 10^{5}$	$1\text{-}2\backslash 10^{6}$	Variable
Age	4-7 years	All ages	All ages
EBV-association	>90%	10-30%	30-40%
Co-factor	EBV, malaria	Unknown	HIV
Location of MYC breakpoint	More than 1 Kb upstream of first exon	Exon1 the MYC gene	Exon1 the MYC gene
Location of Ig breakpoint	Joining (j) region, Sμ switch region of Ig	Sμ, sα and j region	Sμ
Tumor site	Most common in the jaw, it can also occur in the abdomen, kidney, and ovaries	Most common in the abdomen, it can also occur in the kidney	Lymph node, bone marrow, abdomen, and CNS

The hallmark feature of all BL is the translocation of the MYC gene, which results in the upregulation of MYC protein. Most frequent translocation (approximately 80% of cases) between the MYC gene with a heavy chain of immunoglobulin (14q32). The two other translocations can be found in BL, translocation of c-MYC with the light chain of immunoglobulin at 2p12 and 22q11 [96, 97]. The c-MYC has essential roles in different cellular processes, such as regulates the transcription of various genes, cell growth, signal transduction, regulation of RNA processing, immune function, and apoptosis. MYC overexpression does not alone adequate to initiate lymphomagenesis. Dysregulation of microRNAs (miRNA) may lead to increase expression of MYC independent of chromosomal translocation or gene amplification [98].

Furthermore, in sporadic Burkitt's lymphoma, additional mutations related to the MYC gene have been discovered. Mutations in TCF3 or ID3, which is a protein that blocks TCF3 activity, are observed in about 70% of cases. In addition, almost one-third of sBL cases have a mutation in CCND3, which encodes cyclin D3 [99, 100]. sBL mostly causes abdominal tumors, while eBL is often present in the jaw and kidney but can also be present in the abdomen, ovaries, facial bone, and other extranodal sites [101, 102].

Burkitt's tumors have historically been monomorphic medium-sized cells with rounded nuclei, many nucleoli, and copious basophilic cytoplasm. Due to the presence of high numbers of macrophages, Tumours show a ‘‘starry sky’’ pattern [103]. The proliferation of BL cells is rapid, and their doubling time is 24-48 hours [104]. Their rate of apoptosis is also high. BL cells usually express B-cell markers including CD19, CD20, CD22, and IgM and germinal center markers such as CD10, BCL6, and human germinal center associated lymphoma (HGAL) protein [105]. The cell surface phenotype of BL cells reflects the origin of the germinal center, whereas jaw and ovaries are the primary sites of tumor growth, do not usually contain germinal center [106]. It seems that translocation of the MYC gene to immunoglobulin chains in eBL occurs during the VDJ recombination [107, 108]. This process occurs by a VDJ recombinase activating an enzyme called RAG-1/2 expressed in both pre-B and germinal center B cells [109]. However, in sBL and HIV associated, this chromosomal breakage happens mainly in the class switch region [110]. The GC involvement is an essential factor in the pathogenesis of this disease, whether in MYC translocation or association with EBV, malaria, and HIV infection as a co-factor [111]. Malaria and HIV infection, for example, has been determined to activate B cells [112]. Evidence from clonal EBV genomes in Burkitt's lymphoma tissues showing that the tumor is originated from a single infected cell. EBV gene products can directly aid cell survival in the BL cells. Both EBNA-1 and the EBERs, for example, have a function in BL cell survival and apoptosis prevention.

CONCLUSION

Burkitt's lymphoma is known as a highly aggressive non-hodgkin lymphoma with a diverse pattern of clinical presentation. It is a multi-factorial disease, although MYC translocation accounts for one of the main causes of its development. Other risk factors contribute significantly to MYC translocation. Finding the detailed role of each risk factor in the occurrence and progression of Burkitt's lymphoma is a good area in medical research. A better understanding of the detailed mechanism of Burkitt's lymphoma pathogenesis, as well as the precise role of each factor, can lead to the introduction of more effective prevention and therapy strategies.

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EDITORIAL

About malpraxis, with love… for your information

Dan Mischianu

The title of this article for the Romanian Journal of Military Medicine, is inspired from the title of an American movie from 1967, ''To Sir with love'', starring Sidney Poitier in the lead role. Maybe some of you still remember it!... It is said that once, when school did not oscillate between "variable concepts" and "palpable realities", between protocols and procedures, a hydrogeology professor, in a practical work with his students, asked them if they knew the significance of the walnuts planted near the houses of the Romanian peasants. All the students were amazed by the question and kept silence. Nobody made any gesture. The professor knew that this would be the reaction of the students - because this was the answer to the following question: "Why does the world need teachers?" The professor answered the questions and taught the students, as much as a professor can teach, that ''a walnut needs plenty of water to grow and the roots of the walnut are very deep, and that is why houses that have walnut trees next to them will not have problems with dampness...''

This parable, as understood by our mind, aims on one hand to draw the reader’s attention to the fact that "malpractice is defined as an improper or negligent treatment applied by a doctor to a patient, which causes the latter any kind of harm, in relation to the degree of damage of his physical and mental capacity", according to DEX, and on the other hand to state that the "WALNUT" – that is, the Teacher, in the way he teaches his students will keep the "house" (i.e. the workplace) safe against possible future malpractice charges.

And after all, malpractice can even be compared to dampness... for any professional organization. A superb allegory! I consider it a proper allegory and that is why I wanted to introduce it to you.

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I do not pretend to be an expert in the field of "medical malpractice" but being involved and exposed to it in my daily activities, I welcome some considerations on this topic, which I have found out from books, from the media, and also from journalistic practice.

This article also wishes to draw attention of the medical community and to make it more aware of the responsibilities that we have when we dare to practice this noble profession. All of us – physicians, nurses, stretcher-bearers, technical staff – have civil liability (and here is the context of a possible allegation of medical malpractice), disciplinary liability (in the relationships we have as full members of our professional organizations) and criminal liability (most often as defendants, sometimes as investigated parties or as witnesses).

I will present you several cases that have been the subject of disciplinary, civil and criminal investigations, all of them in the “urological filed”.

Case 1

A 55-year-old patient is admitted in the Surgery Department for urgent mumps and blurred urine. Diagnosis at admission: ''Urinary infection. Prostate adenoma. Hematuria.” Diagnosis after three days since admission: ''Medium periurethral adenoma with urethral fistula microabs. Piohematuria. Urinary Infection." Under antibiotic treatment, the symptomatology improves and an open surgery is performed – with the patient's consent – practicing transvesical adenomectomy with favorable post-surgery progress.

14 days after surgery, the urinary incontinence at effort is detected, which subsequently worsens with the occurrence of episodes of urinary tract infection recorded on a posterior urethral stricture. Urethrotomy and TUR-P for remaining adenomatous tissue are performed within urological university department. The histopathological result revealed prosthetic adenocarcinoma microfocus and chronic urethritis. Unfortunately, the functional diagnosis is probative: High overall deficiency, Invalidity degree II.

The patient's complaint was lodged against the surgery after which the patient was diagnosed with urinary incontinence that can only be cured by means of an artificial sphincter costing about 5,000 euros and retirement due to illness.

The Superior Disciplinary Commission of the Romanian College of Physicians considered that the moment when surgery was performed and the surgical approach were inappropriate, therefore it decided to sanction the physician. [2]

Case 2

The son of a patient lodges a complaint against his family physician for the unauthorized delay of the diagnosis of his father's renal carcinoma. The physician treated him for a year and six months for macroscopic hematuria and a so-called renal colic. After one year and 6 months, the patient is diagnosed with renal neoplasm, he goes through surgery and dies one year later.

The Superior Discipline Commission analyzes the facts and notes the following: Hematuria is a diagnostic urgency and therefore its etiology needs to be confirmed as soon as possible by an urologist, the diagnosis of renal carcinoma is not the responsibility of the family doctor, therefore the applied sanction has remained in force.

Case 3

This is a case distinct from the others due to a capital error. It involves a 74-year-old patient admitted in a Urology department and diagnosed with: prostate adenoma, complete urine retention, prostate litiation. Twenty-four hours after admission, the urologist intervenes and performs transvesic adenomyctomy.

Immediate post-surgery progression is favorable with the subsequent occurrence of a third degree urinary incontinence. Two years after the surgery, the patient lodges a complaint against the doctor.

The Territorial Discipline Committee ordered the action to be discontinued, but the patient filed an appeal with the Supreme Discipline Commission. The latter did not find any irregularities neither in terms of the technique used nor of the complication that has arisen, not even regarding the postsurgery treatment administered by the urologist, that is, Driptane. The lack of the informed consent from the patient's file was the only and actually the capital irregularity found, for which the physician was sanctioned.

The medical deontology code in Romania is very clear: "for any diagnostic or therapeutic medical intervention, the informed consent of the patient is necessary". Moreover, the consent is given only after informing the patient about his/her diagnosis, prognosis, therapeutic alternatives, along with their risks and benefits. [2]

Case 4

In the same spirit, there is also the case of a 39-year-old patient admitted in emergency with the diagnosis: right kidney colic. Two days after the admittance in hospital, surgery is performed: right pielolithotomy with internal drainage for calculus included in the right skin-ureteral junction and secondary uropionefrosis. The immediate post-operative progression was favorable up to the occurence of a cardio-respiratory stop during the first post-surgery night which was found to be irreversible. The cause of death was a massive pulmonary thromboembolism with probable causes either the lithotomy position, or more likely the presence of urinary sepsis.

The Superior Discipline Commission decided to sanction the physician for the lack of informed consent, lack of anticoagulation therapy and poor post-surgery follow-up [2, 3]

Case 5

The following is the case of a 54-year-old patient with a transient ischemic stroke, recently treated in a hospital from the country, where a Foley autostatic bladder ureter was mounted. From the patient’s personal history, we mention: type II diabetes, hypertension and ischemic heart disease. Approximately 8 (eight) days after urethro-bladder probe was mounted (according to the patient's report), physicians found the presence of an irreducible paraphimosis. Upon admission to the urology clinic, glandular necrosis, lack of local sensitivity, denuded penile body, penile scar tissue and necrosis, present at the base of the penis, free cutaneous mucous suture, skin remnants, cavernous body having an indurated appearance at palpation are found. The consultation of plastic and reparative surgery is entirely consistent with the urological clinical examination performed.

Figure 1: Preoperative aspect; the devitalization of the gland and the partially necrotic areas of the tegument can be seen

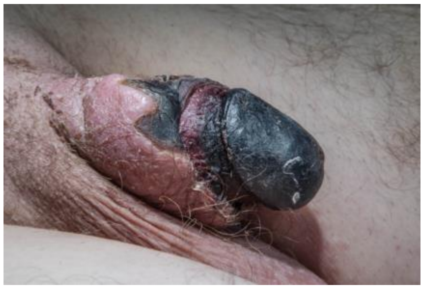

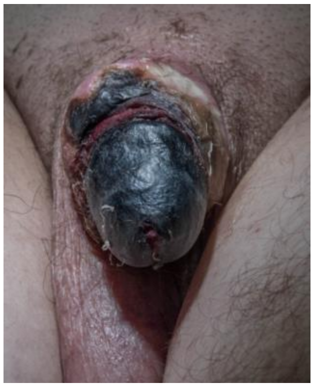

Figure 2: Postoperative final aspect; ureteral bladder and definitive perineal urethrostoma

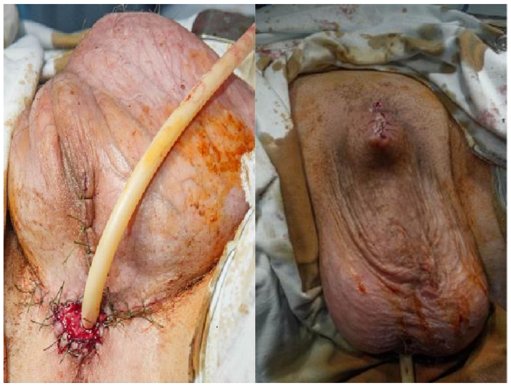

A surgical intervention is performed, after the patient gave his written consent, consisting of the following: noncrectomy, lavage, double drainage both by the uretro - bladder probe and by suprapubic cistostomy.

After limiting the infectious process, a new surgical intervention is performed consisting of definitive perineal urethrostomy and closure of the penile arch. Post-operative evolution is surgically favorable [4]

The patient contacted the media, the criminal investigation organs, the territorial discipline commission, and is in charge of the Superior Discipline Commission.

CONCLUSIONS

We wanted to point out, against all inconsistencies, errors, mistakes and malpractice, which perhaps appear at first sight incriminating up to the contrary proof, that the presence during the professional training of a ''Teacher'' of a “Walnut'' absorbing all ''professional dampness'' is defining for the next years of professional maturity.

In relation to all the exposed cases, some of them not so relevant anymore, others still ''incendiary'' on which the public opinion and the indigenous media keep a close eye, I believe this journalistic warning should give us all something to think about.

The old Romanian saying teaches us: "The good deed speaks for itself", but here, in Romania, there is a new saying which has been quoted for about thirty years, in my opinion without any substantial background, which says: "No good deed remains unpunished!"

However, I have the absolute conviction that although you have performed 10,000 perfect surgeries, if you make a mistake on your last day of surgical practice, that mistake will not be forgiven!

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EDITORIAL

Academician Eugeniu Gh. Proca, founder of modern urology school in Romania. A memory

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Motto: *"You only live once – but if you work it right, once is enough."* Joe E. Lewis

The history of Romanian Surgery will record, I am absolutely convinced, the name of Academician Eugeniu Gh. Proca (January 12, 1927 Godeni Muscel - 07.03.2004 Bucharest). As he testified in a televised interview, he wanted to become a general surgeon, he did not like urology at first, but in the end, as it happens in life, "you love who you live with." [1]

Image 1: Academician Eugen Proca at the age of great responsabilities and professional fulfilments

He didn't regret at all the fact that he became a Romanian urologist, transforming the Romanian urology to which he added new development opportunities; therefore the title of this article is perfectly true. Moreover, after many years, I can see that his first love – general surgery –seems to disappear as a stand-alone specialty, slowly but surely…

Urological surgery is a noble, beautiful, difficult and extremely consuming profession. As one of my colleagues, a surgeon from Constanta – Professor Vasile Sârbu – wrote: "The Romanian surgery amazed through two miracles. One of them happened at the end of the nineteenth century when, in two to three decades, starting from almost nothing, it came to the level of the brightest surgical schools in the world. The second miracle took place in the twentieth century and it refers to the ability to improvise, to do great surgery in all areas, under dramatic conditions, with instrumental, material and technology shortcomings. "[2] Perhaps the chance of Romanian surgeons was the fact that in the twentieth century the three Ministers of Health, who were surgeons, proved an exceptional organizational spirit in their mandates, (Acad. N. Hortolomei 1939-1940, Prof. Dr. Voinea Marinescu 1954 -1966 and Acad. Eugen Proca 1978-1985). I do not mention the name of Prof. Constantin I. Angelescu, a surgeon and a professor at Brâncovenesc Hospital and then at Filantropia Hospital because his ministerial mandate – excelent in substance! – took place at the Ministry of Public Works 1914-1916 and at the Public

Instruction.

Image 2. At the Hospital Prof. Dr. Th. Burghele (previously called Panduri) near his colleagues: primary urologists and secondary urologists

Having all the available means of information from the present, in which we all become when we want and especially when it is not the case, curious "researchers" in the biographies and in the personal identification numbers of everyone from everywhere, I do not want to present the commonly known things regarding the life and work of an overwhelming man, such as Acad. Eugen Proca, I only want to reveal some rather special information.

The professor was born on January 12, 1927, in Godeni, from the former county Muscel. He was a "trueborn inhabitant of Muscel", the only son of Gheorghe C. Proca and Maria Proca. The people from Muscel, who lived close to the mountains, are known to be fiery and combative spirits (see "partisans from Muscel"). Brilliant graduate of "Dinicu Golescu" High School in Câmpulung Muscel, he becomes a student of the Faculty of Medicine in Bucharest in 1945 and at the same time, a student of the Medical-Military Institute. This educational ambivalence has almost always been a solution for many skilled children coming from families with modest material conditions.

Image 3. Standing beside Acad. L. Gherasim and Gl. Prof. Dr. Doc. Iuliu Şuteu

I will tell you something that I saw with my eyes and I heard with my ears a long time ago. A very long time ago...

I was a student of the Medical-Pharmaceutical Institute (currently University of Medicine and Pharmacy Carol Davila, Bucharest) and a Corporal student at the Medical-Military Institute. By the kindness of the Party Secretary of the Medical-Military Institute, as I was a student with honorable academic achievements, in the summer session 1974-1975, I was allowed to prepare the exams to come, late at night, in a room which was more protected against the ubiquitous noise in the area of the North Railway Station. It was then that I discovered the catalogues of former graduates, in the room called "IMM Museum". There were large, thick-tatted books in a dusty cabinet. This is how I browsed, with curiosity, the catalogues of promotions of future military doctors, in which the high school photo was attached to each student. The promotions of 1940 and 1942 showed me the young and confident faces of the future generals and surgeon professors – Iuliu Suteu and Traian Oancea, once emblematic people for the Central Military Hospital. Looking at the pages which turned yellow over time, I was contradicted to find out at some point (it was midnight), the name Eugene Gh. Proca, Godeni, Muscel 1927 January, who entered the Medical-Military Institute in 1945 and below, across the whole sheet of paper, it was written with a red, thick pencil, I have such a vivid recollection of the word: "EXPELLED!".

Image 4. Minister Eugen Proca beside some of his collaborators

Years passed incredibly fast for all of us and while I was a secondary physician, studying my second specialty – urology, during a short break between two surgeries, I told the Professor what I had discovered 20 years before. We were living in difficult times, quite similar to the present ones, but I saw his unblinking blue-grey eyes shining and he even told me the story which was also mentioned by Dr. M. Mihailide in the footnotes of one of his books [3]. He said he had been expelled from the institute because he had lit the fire in a stove using the newspaper published by the party – “Scanteia” newspaper. He also mentioned the way in which, on a Sunday from April 1961, being on duty at the hospital, he took care with full empathy and unaltered human kindness of his former faculty colleague, who needed dialysis. He was uremic and only dialysis prolonged his life.

His faculty colleague was the one who had informed the State authorities about the fire lit in the stove!

Academician Eugen Proca was a Christian orthodox who believed in life after death and who, I am sure, is looking at us with empathy from up there. Academician Eugen Proca is not only the creator of the modern urology school from our country. He is and will always be an emblematic figure for Romanian surgery.

For as long as I was around him, in my perception he was a true "Imperator", intelligent to extreme, self-exigent, caustic with cheaters, tenacious, with a huge culture, always eager to self-improve and aware, at all times, that a surgeon's job does not have a program.

Anyone using a computer can easily find out about his scientific and clinical achievements: performing the first hemodialysis – April 7, 1959, World Health Day – at the hospital raised by the Professor or Academician Th. Burghele, performing the first kidney transplant in Romania in February 1980 (the operation for which he had been mentally and spiritually prepared for a very long time) and about his paideic model. What almost nobody knows is that when he was the second Minister of Health as term of office (7 years), he used to work 24/7. At six o'clock in the morning he was examining patients. Then he went in the surgery room. At 11.00 a.m. – 12.00 p.m., he went to his office from the Ministry where he tried to solve many problems of that time: the birth issue (which is now of almost no interest to anyone), the introduction of medical promotion contests and many more, and in the evening, at 8.00 p.m. – 9.00 p.m., he returned at the hospital to see his patients...

Apparently, Ceausescu’s wife was very proud of Minister Prof. Dr.Proca saying: “He is our only Minister who actually works.”

The fact that the name of Academician Professor Dr. Eugeniu Proca, 15 years after passing into a better and fairer world, is not found in the memory of some of our institutions, is an additional proof that the Latin parable "Nemo propheta in patria sua " (Luke's Book, Chapter IV, verse 24) or in English translation: "No one is a prophet in his country" remains forever and, unfortunately, perfectly true!...

It seems that Oscar Wilde also enlightened our understanding with the following aphorism: “the truth is rarely pure and never simple…”

I dared to reveal something from my own truth about the work and life of this Man.

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REVIEW

A comprehensive review of the factors affecting the work-life balance of nurses

Alireza Rahmani[1], Moloud Radfar[1], Afsaneh Azarabadi[1]

Abstract: *The field of nursing has a lot of responsibilities and workload and is considered as a hard job. If a person does not have the spirit of being responsible for work or interest in service, he cannot survive in the difficult conditions that exist in the field of nursing, such as fatigue, work shifts, stress. Occupational concerns such as insufficient staff, high workload, long working hours and work pressure make nurses unable to play their role in front of their families. On the other hand, nurses are usually unable to perform their duties in the workplace due to family problems. In this study, related articles from the years 2000-2020 were reviewed. The results of studies showed that due to the importance of work-life balance in nurses, it is recommended that managers create the conditions for creating effective methods and techniques for job flexibility for nurses and help nurses to better perform these approaches with the necessary training. Taking care of nurses living conditions, question and answer sessions and interviews with nurses, establishing kindergartens with long working hours can also be effective in creating balance*.

Keywords: *work-life balance, nurse, management, satisfaction*

INTRODUCTION

Nurses are important members of the health care team that human society needs and they exert their influence in the health system by playing different roles [1]. For nurses, like other sections of the working community, the profession is considered as the most important factor in shaping social identity, the source of providing the family and shaping social relationships [2]. But despite all the positive effects that work and the resulting income have on the personal life of every human being, the concept of work has changed imperceptibly over time, exposing human social and family relationships to wide-ranging changes and causing problems for employed people [3].

One of the biggest problems that almost every working person complains about is the lack of balance between work and family activities, so much so that it can be said that the lack of balance between work and life has become a new companion for today's families [4]. Today, the nursing community around the world faces many problems such as insufficient staff, high workload, long working hours, irregular work schedule and work pressure. In studies on job stress over the past 32 years, the nursing profession has been considered as the first source of stress due to insufficient number of nurses and as a result of high work pressure on nurses [5]. Stress is a well-known component of modern nursing, which in the long run leads to chronic diseases such as hypertension, cardiovascular disease and asthma. In today's competitive world, the issue of work-life balance has attracted the attention of many researchers and academics around the world [6]. Today, the challenge of balancing work and family roles is one of the main concerns for individuals. They have the most and strongest connection with the individual and society, so that creating a balance between them is of great value and importance, and if the relationship between work and life is not managed, the conflict between them will cause irreparable damage to the individual, organization and society [7].

A study by Boston Consulting Group on HR managers across Europe found that balancing work and life is one of the three major challenges facing HR managers. Work-life balance is a self-identifying term that is achieved by a person who is able to manage responsibilities in the field of work and family, and this balance is maintained by physical and mental health by preventing grief and other negative effects on life [8].

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Researcher shows that failure to achieve the right balance between effort and rest leads to a feeling of lack of control over workload and lack of energy to achieve individual goals and commitment, resulting in imbalance leading to fatigue, poor performance and reduced quality of life [9]. There are several definitions of work-life balance, with some studies defining work-life balance as follows: The extent to which an individual's effectiveness and satisfaction in work and family roles are consistent with his or her life priorities. Balancing work and life is one of the most important concerns of employees in organizations today. In other words, the most successful employees are those who can balance work and life [10]. One of the tools to balance work-family life is to create flexible programs in organizations. Hospitals are one of the organizations that have more justification for implementing flexible programs among their employees (nurses) [11]. The purpose of this study is a comprehensive review of the factors affecting the balance between work and life of nurses.

LITERATURE REVIEW

The Table 1 lists the most important studies conducted in the field of work-life balance.

Table 1: Literature review

Study specifications	Conclusion
Clark AJ. Nasica and fides. The Classical Quarterly. 2007 May;57(1):125-31.	Defines work-life balance as good and satisfying performance at work and at home, regardless of role conflicts (12)
Raisinghani M, Goswami R. Model of Work Life Balance Explaining Relationship Constructs. Int J Appl Innov Eng Manage 2014;3(2):46-59.	Work-life balance is defined as the satisfactory and appropriate performance of work at work and at home with minimal role conflict (13).
Littig B. Work Life Balance-catchword or catalyst for sustainable work? Vienna: Institute for Advanced Studies 2008.	On the other hand, work-life balance is mainly related to issues related to employee productivity, performance and job satisfaction (14).
Fapohunda TM. An exploration of the effects of work life balance on productivity. J Hum Resour Manage Labor Stud 2014;2(2):71-89	Various studies show that when employees are happy at home, workplaces are free of conflict and become enjoyable places that ultimately lead to increased employee productivity (15).
Engel CC, Bray RM, Jaycox LH, Freed MC, Zatzick D, Lane ME, et al. Implementing collaborative primary care for depression and posttraumatic stress disorder: design and sample for a randomized trial in the U.S. military health system. Contemp Clin Trials. 2014;39(2):310-9	Excessive workload and poor work environments severely affect the psychological aspects of employees and make it difficult for them to strike the right balance between work and private life (16).
Grzywacz JG, Frone MR, Brewer CS, Kovner CT. Quantifying work-family conflict among registered nurses. Res Nurs Health. 2006;29(5):414-26.	Hospital staff are more exposed to work-life conflict due to occupational conditions such as low organizational support, number of shifts, hard work, low work flexibility and long working hours (17).
Buerhaus PI, DesRoches CM, Dittus R, Donelan K. Practice characteristics of primary care nurse practitioners and physicians. Nurs Outlook. 2015;63(2):144-53	Long working hours in medical settings for nurses can cause severe depression and hormonal imbalances. As a result, nurses often tend to eat more, use drugs to relieve fatigue, stress, and drowsiness in their lives (18).
Kunaviktikul W, Wichaikhum O, Nantsupawat A, Nantsupawat R, Chontawan R, Klunklin A, et al. Nurses' extended work hours: Patient, nurse and organizational outcomes. Int Nurs Rev. 2015;62(3):386-93	Various studies show that nurses' mistakes at work, such as mistakes in injections and medication errors, cause more problems that may cause patients dissatisfaction (19).
Poulose S, Sudarsan N. Assessing the influence of work-life balance dimensions among nurses in the healthcare sector. J Manage Dev 2017;36(3):427-37.	Nurses also have responsibilities in relation to their family and social life, so that an imbalance between nurses' personal and professional activities can lead to reduced organizational commitment, burnout, increased work stress, severe job dissatisfaction and disruption in personal, family and life (20).
Konrad AM, Mangel R. The impact of work-life programs on firm productivity. Strategic Manage J. 2000;21(12):1225-37.	Balance programs in three types of organizations have significant profit margins, which are: organizations that have a high percentage of skilled labor, have made large investments in connection with the specific skills of their organization, and a high percentage of their workforce are women (21).

Study specifications	Conclusion
Wong SC-k, Ko A. Exploratory study of understanding hotel employees' perception on work– life balance issues. Int J Hosp Manage 2009;28(2):195-203	In their research, the factors affecting work-life balance are important, including having free time, work support in case of family problems, work commitment, job flexibility, attention to life issues and voluntary reduction of working hours in order to solve family problems (22).
Hasanqulipour T, Seyed Javadin S, Safiri K, Roshannezhad M. [Presentation and explanation of a model for causes and factors affecting the balance of work life]. J Public Adm. 2012;5(2):37-54.	In their research, they identified job flexibility, work and family support, and individual skills as factors affecting work-life balance (23).
Hasanqulipour T, Seyed Javadin S, Safiri K, Roshannezhad M. [Presentation and explanation of a model for causes and factors affecting the balance of work life]. J Public Adm. 2012;5(2):37-54.	Free time, work support, work commitment, job flexibility, attention to life issues and reduction of working hours voluntarily (24).
Rahimaghaee F, Dehghan Naiery N, Mohammadi E. [Iranian nurses perception of their professional growth and development]. Online J Issues Nurs. 2010;9(10).	In their research, they concluded that many nurses believe that in no other profession are conditions such as the nursing profession possible for them to help others, this attitude is largely rooted in the nature of Iranian society. Iranian society is associated with spirituality and religion, so that some nurses participating in the study of their presence in the nursing profession considered it a divine destiny that caused them to become closer to spirituality, so the commitment to the nursing profession regardless of material needs (25).
Hinami K, Whelan CT, Wolosin RJ, Miller JA, Wetterneck TB. Worklife and satisfaction of hospitalists: toward flourishing careers. J Gen Intern Med. 2012;27(1):28-36.	In their study, they showed that 1.63% of the nurses studied are weak in balancing work and life, which will lead to high job transfer rates, poor job satisfaction and ultimately low quality of life for nurses (26).
Hebrany P, Behdani F, Mobtaker M. [Exploring of stressors in nurses]. J Ment Health 2010;10(3):231-7.	The study identified job stressful factors for nurses including job insecurity, financial problems, communication conflicts, time constraints, irregular working hours, high work pressure, problems with psychological contracts, unequal job opportunities, inadequate vocational training and hospital policies. And introduced one of the ways to reduce these problems to support nurses (27).
Almalki MJ, Fitzgerald G, Clark M. Quality of work life among primary health care nurses in the Jazan region, Saudi Arabia: a cross-sectional study. Hum Resour Health. 2012;10(1):30.	In their study, they concluded that 91% of the nurses studied in the hospital environment need times or places away from patients to be free from any stress for hours (28).
Bahrami M. [Quality of life of nurses working in internal-surgical wards of Oncology center of medical university Isfahan]. J Isfahan Univ Med Sci. 2016;3(7):36-46.	In her research, she showed that the quality of life of nurses is far from the desired situation (29).
Baljani E, Rahimi J, Safari S. [Health Promotion Needs, Stress Resources, and Nurses' Workplace Problems]. J Hamedan Nurs Midwifery Fac. 2013;21(1):49-56	Showed that more than half of the nurses participating in the study needed help and promotion in the areas of exercise and physical activity, stress management, improving nutrition and avoiding continuous work (30).
Alhani F, Seyyed Nematollah Roshan F. [Explaining the Problems of Improving Nursing Healthy Lifestyle and Providing Solutions]. Razi Med J. 2014;21(125):1-8.	The biggest problem of nurses in promoting a healthy lifestyle related to the managerial-executive field was the high working hours of nurses with a score of 1.72 (31).

CONCLUSION

Considering the importance of nurses 'health as the main member of the country's health system, which is responsible for maintaining the health of themselves, family and society, and since nurses' health is directly related to the quality of their performance in caring for patients and playing family roles The importance of conducting this research in identifying the factors affecting the work-life balance of nurses is becoming increasingly clear. According to various studies in the present study, the factors affecting work-life balance, respectively, the importance of the effect, including job flexibility, commitment, work support, free time, attention to life issues and reduction of working hours were voluntarily extracted. Therefore, if hospital managers provide conditions for nurses to have some control over their working hours. In order to reduce the problems of nurses in this dimension, it is recommended that managers provide less working conditions for nurses in exchange for lower salaries and maintaining job security. This approach can be useful in solving many of the problems mentioned by nurses. The findings of this study emphasize the importance of creating a balance in the family and professional life of nurses, so that by informing the officials of the results of this study, an important step can be taken to reduce the problems of nurses and increase the quality of services provided to them.

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VARIA

Acute kidney injury incidence and models for mortality prediction in acute coronary syndromes

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Abstract: *Background and aim. The presence of acute kidney injury (AKI) in subjects diagnosed with acute coronary syndromes (ACS) represents a major predictor of a negative outcome.The purpose of this study is to reveal the incidence of AKI in ACS subjects and to illustrate the prognosis of these subjects, using special models for mortality prediction, based on the creatinine values and the presence of acute heart failure (AHF).*

Methods. Using multiple linear regression, several models were designed to predict the mortality including: comorbidities of the subjects, initial and highest value of the serum creatinine (sCr) during hospitalization, presence of theAHF and Killip class compared with sCr values, the last model illustrating the influence of all the previous variables.

Results. AKI had a significant incidence in the chronic kidney disease (CKD) group (53.11%). The mortality rate and the hospital readmission were higher in the CKD subjects (41.14%) versus the patients with normal kidney function (21.23%, p<0.01). The association of AKI and AHF, known as cardio-renal syndrome, was related to a negative outcome in the CKD group. The models for prediction of mortality emphasize that the age, dyslipidaemia, worst sCr, Killip class and AHF presence can be used to assess the mortality risk of patients with AKI and ACS. Previous pathological conditions such as diabetes, peripheral artery disease, coronary artery disease (CAD) and obesity, failed to achieve statistically significance for prediction of death.

Conclusions. AKI represents a serious pathological condition with a high prevalence in subjects with CKD; the association with AHF increases the risk of hospital readmission and fatal outcome.

Keywords: *acute coronary syndromes, acute kidney injury,acute heart failure, serum creatinine, predictors, mortality*

INTRODUCTION

AKI represents a pathological condition that resumes a complex interaction between various factors like subsequent renal failure, utilization of iodinated contrast substance during percutaneous coronary intervention (PCI), renal hypoperfusion caused by decreasing of the cardiac output during an ischemic event [1]. Manifestations of AKI may consist in a simple increase in sCr or severe renal failure with anuria requiring kidney replacement therapy [1]. There is a strong relationship between the renal function impairment and the CAD development, regardless the other common risk factor involvement. The prospective study realized by Jurkovitz et al. including 13,329 participants,

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without previous CAD, reflected that increased values of baseline sCr (defined as ≥1.2 mg/dL in women and ≥1.5 mg/dL in men) were associated with a high incidence of acute myocardial infarction (AMI) and death due to ischemic events, compared to normal values of the sCr [2]. The renal impairment has an important influence on the patient's outcome during cardiovascular events and a small decrease in glomerular filtration rate worsens the prognosis of these patients [3].

The complexity of the atherosclerosis process in CKD subjects is a result of the interaction between common and uncommon risk factors. Diabetes mellitus, hypertension, dyslipidaemia and smoking represent essential factors in atherosclerosis development by promoting chronic inflammation, oxidation of the low-density lipoproteins and reactive oxygen species formation [4]. Even secondary hypertension in young persons has an important role in atherosclerosis occurrence and the level of plasma renin in these subjects represents a clear indication [5, 6]. Systolic and diastolic values of the blood pressure are strongly related to alimentation in young persons and there are several actions to change the unhealthy eating habits, beginning with education regarding healthy diet from early age [7].

Various terms were attributed to AKI and that is the reason why the comparison of studies is difficult. There are more than 30 definitions of AKI and the most used are: the Risk, Injury, Failure, Loss and End-stage renal disease (RIFLE), criteria revised by the Acute Kidney Injury Network (AKIN) and the Kidney Disease: Improving Global Outcomes (KDIGO) AKI Work Group classification.

AKI definition (according to KDIGO) expresses an increase in sCr by 0.3 mg/dL (≥ 26.5 µmol/L) within 48 hours or increase in sCr 1.5 to 1.9 times baseline value, with known or presumed abrupt onset within the prior 7 days. There are 3 stages according to the sCr values and the urine output. The first stage is consistent with sCr variations mentioned in the definition and a urine output of less than 0.5 mL/kg/h for 6-12 h, whereas the second stage involves increase in sCr 2.0 to 2.9 times baseline and a urine output of 0.5 mL/kg/h for 12 h. The last stage represents an association between increase in sCr to ≥ 4.0 mg/dL (≥ 353.6 µmol/L) or ≥ 3.0 times baseline or usage of renal replacement therapy and a urine output of less than 0.3 mL/kg/h for ≥ 24 h or anuria for ≥12 hours [8, 9, 10].

Usually, AKI remains undetected due to the fact that the onset is earlier in ACS patients and the sCr from admission represents the reference value, without actually knowing the evolution of the creatinine before hospitalization. If the sCr does not have a remarkable increase after admission, there may be an error of AKI diagnosis. New biomarkers able to early detect the AKI are required in order to initiate the preventive treatment and minimalize the renal insult [11]. The beginning of AKI is usually in the early phase of the ACS, fact that determines the late detection of this condition.

ACS represents a challenging diagnosis in CKD subjects because most of them remain asymptomatic. The explanation is suggested by the fact that uremic milieu and associated diabetic neuropathy influence the perception of pain in these patients [12, 13].Inflammation and oxidative stress represent common elements for both CKD and ACS, and the association of these diseases increases the risk to develop AKI. High levels of inflammatory markers (such as C reactive protein (CRP), fibrinogen and procalcitonin (PCT)) were found in subjects who developed AKI after PCI. Kurtul et al. revealed that PCT represents an independent factor for the development of AKI in ACS patients undergoing PCI [14]. Subjects without AKI emphasize PCT values less than 0.1 µg/L15. Gao et al. analysed 7000 PCI and illustrated that CRP levels were higher in subjects with AKI16. Gu et al. illustrated that determination of both markers, CRP and PCT, has higher predictive value in identifying subjects at high risk of AKI development. Also, the AMI is an independent risk factor involved in AKI occurrence [17].The role of antioxidants in the cardiovascular field is well known and vegetables/fruits consumption has demonstrated its effect for people at risk of cardiovascular diseases, on immunologic, clinical and antioxidant markers [18].

The main purpose of the present research is to highlight the incidence of AKI in ACS patients, and to present prediction for these patients based on particular models for mortality prognosis taking into account the presence of AHF and creatinine values.

METHOD

This study designed a retrospective single-centre analysis, which enrolled 435 patients admitted with ACS diagnosis in the Cardiology department of the Clinical Emergency County Hospital of Oradea, January 2016 - January 2018. The study lot involved 209 patients with ACS and CKD; the control lot comprised 226 patients with ACS and normal kidney function. The exclusion criteria included allergy to contrast media, exposure to the contrast media 2 days before the coronary angiography procedure, malignant conditions.The AKI patients were detected using the KDIGO classification because it contains lower values of sCr than the AKIN criteria, so the underdiagnosis of the condition was avoided.

ACS was revealed according to the Fourth Definition of the

Myocardial Infarction using the association of thoracic pain, elevated cardiac necrosis enzymes, electrocardiographic changes and imaging evidence of new regional wall motion disturbances.ST-segment elevation myocardial infarction (STEMI) was defined due to the presence of persistent ST-segment elevation more than 1 mm in two contiguous leads with the exception of precordial leads that require an elevation of 1.5 mm in women, more than 2.5 mm in men under 40 years-old and less than 2 mm in men older than 40 years. Myocardial infarction in subjects with previous left bundle branch was revealed due to Sgarbossa’s criteria. Non-ST-segment elevation myocardial infarction (N-STEMI) illustrates either a ST-segment depression ≥0.5 mm in two contiguous leads or deep inverted T wave more than 1 mm, along with elevated myocardial necrosis enzymes. Unstable angina (UA) expresses cardiac necrosis enzymes in normal limits associated with T negative wave, ST-segment depression or transitory ST-segment elevation [19]. Left ventricle ejection fraction (LVEF) was assessed using the Simpson’s method. The normal value was considered between 50-72% in men and 52-74% in women [20].

Cardiac necrosis enzymes, cTnI (cardiac troponin I) and hscTnI (high-sensitive cardiac troponin I), were tested and abnormal values were defined as elevated when the result showed at least one value above the upper limit of the 99th percentile. Normal ranges were considered whenthe values were lower than 0.04 ng/mL for cTnI and 29 ng/L for hscTnI. Serial measurements were required to exclude thediagnosis of myocardial injury [21].

Killip Kimball classification was assessed in each patient with STEMI and the four classes. The first class illustrated subjects without signs of cardiac decompensation, the second class included patients with rales, third heart sound and increased jugular venous pressure. The third class was consistent with acute pulmonary oedema and the last stage revealed subjects with cardiogenic shock [22].

The statistical analysis was performed using Biostat software. Chi-square test was used when comparing prevalence in the two-groups, while Anova test was used to compare the mean values of certain parameters in the two groups. Univariate and multivariate regressions were done to assess the factors that correlate with the risk of death. A value of p<0.0 was considered statistically significant.

This research was performed respecting the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects), and it was approved by the Ethics Committee of the Clinical County Emergency Hospital of Oradea, Bihor County, Romania (decision no. 30088/December 10th, 2019); each patient included in the study signed the informed consent form.

RESULTS

The prevalence of the common risk factors and associated comorbidities was higher in the CKD lot. Conditions like hypertension, diabetes mellitus, dyslipidaemia and peripheral artery disease were present in an increased proportion in CKD subjects in comparison with patients without CKD (84.21% vs 63.27%, p<0.01; 58.85% vs 33.62%,p<0.01; 60.09% vs 49.95%, p=0.02; 20% vs 4.9%, p<0.01).The distribution of the ACS types was different between the two lots. STEMI was characteristic in subjects without CKD whereas the CKD lot revealed mostly N-STEMI cases, UA had a similar incidence in both CKD and non-CKD lots (Figure 1).

Figure 1: Categories of coronary syndrome according to the presence/absence of CKD

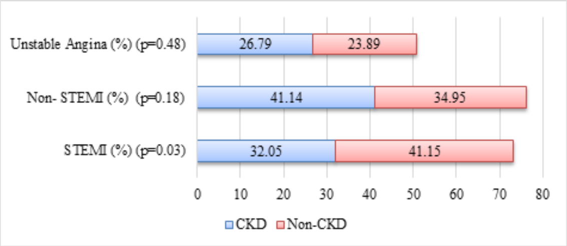

Myocardial interventional revascularization through PCI and stent implantation represented the therapeutic option for 53.59% of CKD subjects and 63.72% non-CKD patients (p=0.03). Coronary artery bypass surgery (CABS) was performed in 15.79% of CKD subjects, whereas the non-CKD lot had a proportion of surgical myocardial revascu1arization of 16.81% (p=0.77). The use of conservative treatment instead of PCI, known as renal nihilism, was objectified mostly in the CKD lot (p<0.01) (Figure 2).

Figure 2: Treatment of ACS according to the presence/absence of CKD

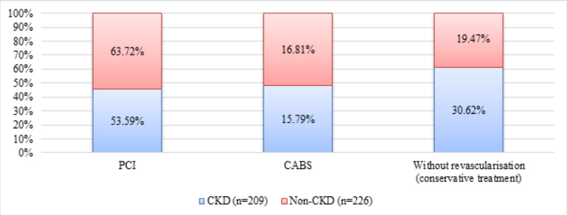

AKI has occurred in the CKD lot, revealing statistically significance (p<0.01). The association of two pathological conditions, such as AKI and AHF, had a major impact in subjects' outcome and was revealed in the CKD group (p<0.01). The uncomplicated evolution of the ACS cases was noticed in patients with normal kidney function (p<0.01) (Figure 3).

Figure 3: Presence of AKI or AHF according to the presence/ absence of CKD

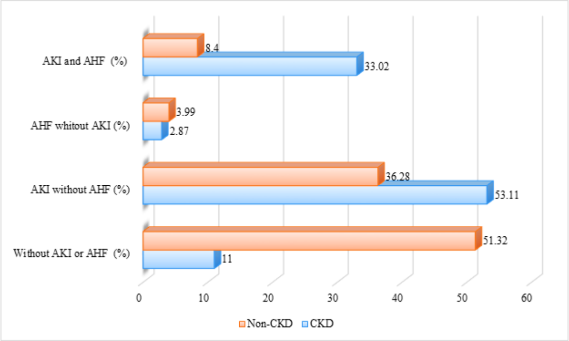

Deteriorated LVEF was noticed in a higher proportion in CKD lot, compared to patients without CKD (23.5% vs 20.17%, p=0.4). The average value of sCr was higher in the CKD lot (1.66 mg/dL vs 0.8, p<0.01), the standard deviation (Q1=1.01, Q2=0.20). Another important parameter was the worst value of the sCr which revealed a high level in the CKD lot with statistical significance (2.71 mg/dL vs 1.1 mg/dL, p<0.01), standard deviation (Q1=2.11, Q2=0.36).The analysis of the average sCr variation illustrated an increased value in the CKD group (1.03 mg/dL vs 0.3 mg/dL, p<0.01).The mortality rate was statistically significant in CKD subjects compared to patients with normal kidney function (13.39% vs 6.1%, p<0.05, SD CKD 8.39-17.61, SD non-CKD 4.39-7.69). Killip class assessment revealed that most of the subjects without CKD had first Killip class at admission (p<0.01), whereas the CKD patients were included in the third and fourth Killip classes (p<0.01).

The initial value of sCr illustrated importance in univariate analysis and predicted the development of AHF and AKI (p<0.01) (Figure 4, Figure 5, Table 1).

Figure 4: Correlation between worst value of sCr (mg/dL) and AKI development

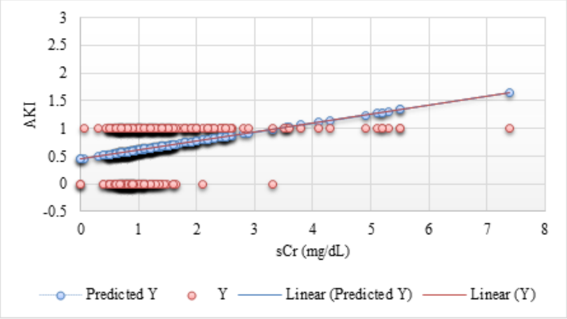

Models for mortality prediction were designed using multivariate analysis and including different factors to demonstrate the impact of AKI and other comorbidities or biomarkers regarding the outcome of patients with ACS (Table 2). The first model, based on the medical history and the associated cardiovascular risk factors of the subjects, illustrated that only the age and dyslipidaemia reached statistical importance to predict the mortality risk in ACS subjects with and without CKD (p=0.0404, p=0.0332).

Figure 5: Correlation between worst value of sCr (mg/dL) levels and AHF development

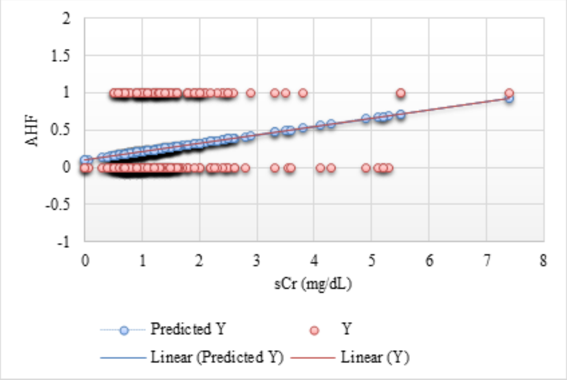

Table 1: Parameters of linear regression

	Coefficient	Standard error	LCL	UCL	t Stat	p value
Between worst value of sCr (mg/dL) and AKI						
Intercept	0.4489	0.0389	0.3724	0.5253	11.5427	*0.0000
Worst value of sCr (mg/dL)	0.1619	0.0263	0.1102	0.2136	6.1518	1.7451E-9
Between sCr (mg/dL) and AHF						
Intercept	0.1005	0.0352	0.0314	0.1696	2.8576	*0.0045
Worst value of sCr (mg/dL)	0.1120	0.0238	0.0652	0.1587	4.7053	3.4162E-6

**p<0.05, respectively p<0.001; LCL –low confidence limit; UCL–upper confidence limit; sCr – serum creatinine*

Diabetes, hypertension, smoking condition, history of peripheral artery disease, stroke did not predict the risk of death in the presented model. The second model included an analysis regarding the mortality risk of the study groups based on the presence of complications and biomarkers values. The presence of AHF and Killip class were statistically significant in anticipating the fatal outcome (p=0.0002, p<0.01). Other parameters like sCr value, age, troponin, LVEF failed to demonstrate their role in predicting the mortality. The third model evaluated the difference between baseline sCr and the worst value of sCr in identifying the patient's outcome. The worst value of sCr was the most important factor in mortality prediction in this model (p=0.0117). The fourth model represented an evaluation of all the variables in detecting the mortality risk. The Killip class and the AHF demonstrated statistical importance (p=0.0132, p= 0.0000).

Table 2: Prediction of mortality risk using multivariate analysis, based on 3 models

Parameter	Coefficient	Standard error	p value
Model 1. Associated cardiovascular risk factors and comorbidities			
Intercept	-0.0037	0.1521	0.9805
Age	0.0027	0.0013	*0.0404
Gender	-0.0004	0.0342	0.9918
BMI (kg/m^2)	-0.0030	0.0034	0.3700
Smoking	0.0137	0.0187	0.4656
Hypertension	-0.0342	0.0348	0.3270
Diabetes mellitus	-0.0111	0.0162	0.4945
CKD	0.0498	0.0327	0.1288
Anaemia	0.0496	0.0328	0.1318
Peripheral artery disease	0.0866	0.0469	0.0661
Chronic obstructive pulmonary disease	0.0131	0.0583	0.8229
Stroke	-0.0728	0.0491	0.1389
CAD	0.0020	0.0117	0.8651
Model 2. Complications and paraclinical results			
Intercept	-0.2572	0.0972	*0.0085
Age	0.0009	0.0010	0.3625
Initial value of sCr (mg/dL)	-0.0066	0.0328	0.8410
Worst value of sCr (mg/dL)	0.0144	0.0176	0.4138
AKI	-0.0175	0.0271	0.5200
AHF	-0.2284	0.0598	*0.0002
cTnI (ng/mL)	0.0057	0.0286	0.8426
Killip class	0.2300	0.0303	*<0.001
Decreased LVEF	0.0431	0.0322	0.1818
Model 3. Initial sCr and worst value of sCr			
Intercept	0.0176	0.0249	0.4791
Initial sCr (mg/dL)	-0.0027	0.0350	0.9375
Worst value of sCr (mg/dL)	0.0439	0.0173	*0.0117

**p<0.05, respectively p<0.001; BMI – body mass index; CKD – chronic kidney disease; CAD – coronary artery disease; AKI – acute kidney injury; AHF – acute heart failure; ctnI –cardiac troponin I; LVEF – left ventricular ejection fraction; sCr – serum creatinine.*

DISCUSSIONS

The present study outlines two significant ideas: (1) The serial determination of sCr is essential in identifying subjects with AKI and special attention should be paid to subjects with CKD undergoing the coronary procedure; (2) The presence of AKI in patients with ACS and CKD can be a fatal prognosis, especially when associated with AHF. Despite following accurate indications regarding coronary imaging, like the use of a low-osmolarity contrast agent (600-800 mOsm/kg) and the corresponding post-procedural hydration associated with diuresis stimulation, the incidence of AKI varies from 5% to 55% [1, 23, 24]. The variable incidence resides in variations of the nomenclature of this term [25].

As there is no safe and highly efficient prevention method for AKI, it is considered necessary to develop prediction scores to identify high-risk patients [26]. A first element that should be included is the Killip class, evaluated upon admission to the hospital, along with the presence of AHF. These elements should be identified particularly for CKD subjects, as multivariate analysis demonstrates the role of independent risk factors for the risk of death in these patients. The reduction of the number of functional nephrons, evidenced by the initial sCr analysis and the worst value of the sCr, together with an increased rate of reduced

LVEF are major predictors of AKI and AHF occurrence, an association known as cardio-renal syndrome [27].

Common cardiovascular risk factors have failed to outline the risk of mortality in multivariate analysis, except for dyslipidaemia, since in the case of CKD there are also factors specific to the uremic environment that significantly influence the prognosis of these patients. Early myocardial revascularization reduces the risk of AHF. A recent study confirms the importance of this therapeutic method, especially in subjects with N-STEMI and CKD, that associate increased values of cardiac biomarkers, such as the N-terminal fragment of the brain natriuretic peptide (N-terminal pro b-type natriuretic peptide, NT-proBNP, BNPT) or hscTnI, or a Killip class three or four [28]. For patients with ACS undergoing PCI, an independent predictor of developing AKI is the level of NT-proBNP at admission, being connected with high costs, extended hospitalization, high rates of terminal-stage renal failure, repeat revascularization, myocardial infarction and early and late mortality [29]. LVEF shows low values in subjects with ACS and CKD due to the increased incidence of multivariate CAD and especially to the impairment of left coronary artery trunk. Choosing the method of myocardial, interventional or surgical revascularization should consider the possibility of resolving all significant coronary lesions, the complexity of CAD and the surgical risk for the subjects [30].

The increased mortality evidenced in the group of subjects with CKD is explained by the increased incidence of AKI in these subjects. The mean hospitalization period was longer for patients with ACS and CKD who developed AKI, highlighting the severity of this complication (15.71 days vs. 13.15 days, $p < 0.01$). For patients with STEMI, accentuated oxidative stress, sympathetic activation and pronounced inflammatory response were found to synergistically accelerate AKI [31]. The most powerful independent predictor of mortality within hospital in case of dangerous settings like for STEMI associated with cardiogenic shock was identified to be AKI [32].

A recent and further argument of the way AKI influences the long-term effect for patients suffering from STEMI subjected to primary PCI is presented by Narula et al. The 3-year follow-up revealed that the mortality rate for patients suffering from AKI was 16%, while for those without AKI it was 4%. As for the association of severe ischemic cardiac side effects and serious bleeding, the intensification of the side effects was more accentuated after three years, being of 40% for subjects with AKI and about 25% for patients without AKI. Corroborated, these data show that AKI evolution influences the complete prognosis of ACS patients [33]. Throughout primary PCI for STEMI, increased contrast volume is connected with increased levels of AKI and mortality, the contrast ratio and contrast volume determining AKI evolution [34]. A general complication in the case of patients suffering from shock and myocardial infarction is nephropathy, as recently revealed by a CULPRIT-SHOCK study that showed a high percentage of subjects (11.6-16.4%) who needed renal replacement therapy [35].

The decrease of renal mass is thought to be the main pathophysiology that is associated with CKD, AKI and the end stage renal disease. Considering these interesting findings and the fact that the common therapies do not influence the short-term prediction in AKI, supplementary preventive actions meant to ameliorate the health status for AKI survivors are extremely important. There is general evidence that most of the patients suffering from AKI are not regularly surveyed by nephrologists and the current clinical practice does rarely include follow up evaluation for AKI, dealing with this issue representing a considerable option for important change and better caring for this category of population [36]. Even patients with mild and moderate AKI, which in most cases is short lived and clinically reversible renal failure, had an elevated risk of death after hospitalization that did not dissipate over time. These conclusions arenovel and do not support the conventional teaching that AKI in its mild-form is inconsequential [37].

CONCLUSIONS

AKI negatively influences the short- and long-term prognosis of subjects diagnosed with ACS. CKD and AHF are other negative predictors of ACS evolution and their association with AKI has shown the highest mortality rate. Although it is not an accurate marker, the initial sCr and the worst value of sCr can be used in AKI detection to establish appropriate therapeutic measures. New predictive scores able to identify AKI in a timely manner are necessary, considering the fatal prognosis for patients with ACS and CKD.Early and complete myocardial revascularization in ACS is an optimal method, which decreases the risk of developing AHF and AKI, known as cardio-renal syndrome.

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ORIGINAL ARTICLES

Addressability to the Emergency Department of patients with acute post-COVID 19 clinical symptoms

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Abstract: *The long-distance post-SARS-CoV2 infection effects were partially known. The study aims to follow the evolution of the patients with acute post-COVID 19 clinical symptoms presented in the Emergency Department. Methods: We analyzed 262 presentations more than 14 days after the initial COVID-19 diagnosis and with at least one acute COVID-19-related symptom. This study included presented between 01.01 and 31.05.2021 in the Emergency Department of Bucharsti-Emergency University Hospital. Data were obtained in real-time, at the presentation, from the patients' medical charts, and afterward from SUUB's database. Results. Out of the total of 262 presentations, 40.84% required patient hospitalization. The highest number of presentations with (18.70%) or without (22.90%) hospitalization was recorded in the first month after the COVID-19 diagnosis. Patients with less than 30 days between presentations had significantly fewer hospitalization days, compared to those with more than 30 days between presentations (8.3 vs 14.14 days, p=0.009). We found a high amount of hospitalization days (1093 days), with 29.88% deaths. Conclusions: COVID-19 complications require prolonged hospitalization, with implications on the patients' quality of life, the overcrowding of intensive care facilities, high-cost treatment, and unfavorable evolution for a great number of patients.*

Keywords: *long COVID, ongoing COVID, COVID-19 complications, hospitalization*

INTRODUCTION

Worldwide, the Emergency Departments were at the front line of the fight against the SARS-CoV2 pandemic, which began on March 11, 2020 [1]. Presently, there are published many COVID-19 studies, which bring information regarding the physiopathological mechanisms of action, the long-term effects, and complications of the disease, treatment, and the potential leverage of prevention, as well as the psychologic and socio-economic implications of COVID-19 [2]. Males seem to be more frequently affected by COVID-19 whereas the risk for poor prognosis is increased by age and chronic illnesses [3]. These factors seem to contribute to the extreme variability of COVID-19, from asymptomatic to severe illness and death, as well as to its unpredictable response to treatment.

In the Czech Republic, a study over 2 ½ months, that enrolled 7,455 patients with positive RT-PCR SARS-CoV2 tests shows that 15.9% required hospitalization and 6.2% developed severe complications [4].

The term "ongoing symptomatic COVID-19" is used when the symptoms persist between 4 and 12 weeks whereas the frequently used terms "post-COVID syndrome" or "long COVID" implies the persistence of symptoms over 12 weeks, in the absence of other causes [5,6]. The Office for National Statistics (UK) estimates that 1 of 10 persons with positive COVID-19 tests has symptoms for a period of more than 12 weeks, and 1 of 5 persons for a period of more than 5 weeks. In England, an ample review of 508,707 COVID-19 patients identified several risk factors for the persistence of symptoms for more than 12 weeks (e.g. age, female gender, obesity, and hospitalization for COVID-19) [7].

National Institute for Health Research mentions a study

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conducted on patients discharged from a hospital in Rome with "ongoing COVID-19 symptoms". Of them, 87% had at least one symptom present 60 days after the debut of SARS-CoV2 infection, 55% had 3 or more symptoms, and 40% had a reduced quality of life [8].

NHS England estimated that out of 95,000 patients admitted to hospitals until June 5, 2020, 45% had "ongoing support" and up to 50% required rehabilitation services [9].

Long COVID affects multiple and different body systems, influences daily routine activity [10], impacts family life, and decreases workplace productivity, with psychological and socio-economic repercussions. A study that followed 3,762 patients from 56 countries, which had symptoms for more than 28 days, showed that 90% of them had symptoms for up to 60 days and 91% needed more than 35 weeks to recover; the most frequently reported symptoms after 6 months were fatigue, decreased resistance to exertion, and cognitive disfunction [11].

Persistent clinical charges for up to 6 months after COVID-19 were reported mostly for inpatients, but also for those who did not require hospitalization [12]. A cohort of patients, discharged in the period 07.01-29.05.2020, mentioned fatigue, weakness, sleeping disorders, anxiety, and depression, 6 months after acute infection [13]. The persistence of symptoms (e.g. fatigue, shortness of breath, memory loss, hair loss) was correlated with the number of days of hospitalization [14]. A New York City study that evaluated 929 survivors out of 1,190 hospitalized COVID-19 patients at 3 months and 6 months after discharge, found the persistence of cardiopulmonary symptoms (35.7%, respectively 28%), especially shortness of breath (22.1%, respectively 15%). After 6 months from discharge, general (26.4%) and neurological (24.2%) symptoms persist. There were also 33 deaths during the 6 months [15]. A study conducted in Rome, on 143 patients, shows that a high number of patients still have more than 2 symptoms at discharge. The patients entered a recovery program after discharge and, after 60 days from the debut 12.6% had no symptoms, 32% had 1-2 symptoms, and 55% had more than 3 symptoms [16].

A review of 24 articles published until 01.01.2021 concludes that the presence of more than 5 symptoms in the first week of acute infection is associated with the development of long COVID, regardless of age or gender. Increased risk of symptoms' persistence is associated with advanced age, hospitalization at the debut, shortness of breath at debut, chest pain, and comorbidities [17].

Hypertension, considered independent or in association with diabetes mellitus, predisposes to longer persistence of symptoms [18] and the increased mortality after COVID-19 [19]. COVID-19 also is a risk factor for other diseases, like stroke, myocardial infarction, and thrombosis [20].

Taking all this info into account, we followed the patients with previous COVID-19 diagnoses presented, in the Emergency Department of the University Emergency Hospital in Bucharest (SUUB).

METHOD

We analyzed 262 emergency presentations, unique or multiple, in the period January-May 2021, with at least one acute COVID-19 related symptom, that necessitated or did not hospitalization. Clinical data were obtained in real-time, at the moment of the presentation and afterward, from SUUB's database.

Inclusion criteria were: at least 14 days after prior COVID-19 diagnosis, confirmed by RT-PCR test, age over 18 years, and the presence of at least one persistent, acute, or newly appeared symptom associated with SARS-CoV2 infection. Presentations in Emergency Department that are not related to the previous COVID-19 diagnosis were not included in this study.

Statistical analysis was performed using the StatsDirect software.

RESULTS

The total number of presentations with acute post-SARS-CoV2 infection symptoms in the Emergency Department of SUUB was 262 (Figure 1); out of these, 155 (59.16%) were discharged and 107 (40.84%) were hospitalized.

Figure 1: Total number of presentations (262) in the period 01.01.-31.05.2021

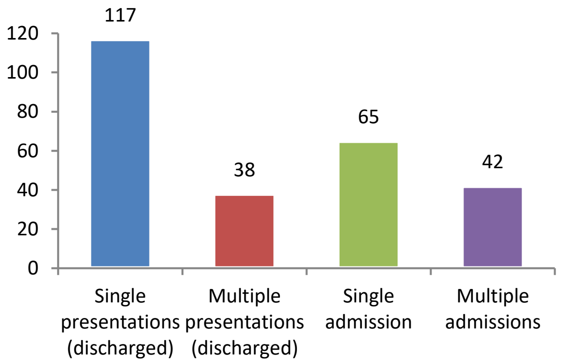

117 patients didn't need hospitalization, and 2 of them had 2 presentations. 87 patients were hospitalized; out of these, 65 patients had a single presentation with admission and the other 22 patients had multiple presentations (in total 78

presentations) out of which 42 presentations were followed by admission, and 36 by home discharge (Figure 1).

There is a high variability regarding the 22 patients with multiple presentations, as follows: 12 patients with 2 presentations, 1 patient with 3 presentations, 7 patients with 4 presentations, 1 patient with 8 presentations, and another with 15 presentations, all of them with at least 1 admission.

Most presentations are in the first month after the initial diagnosis, regardless of severity: 60 presentations with discharge (22.90%), and 49 presentations with hospitalization (18.70%).

The decreasing trend is maintained at 4 months distance for both types of patients and continues after 4 months for the patients that needed hospitalization.

There is an increase in presentations that do not require hospitalization, with less severe symptoms, like fatigue, decreased resistance to exertion, sleeping disorders, physical and mental asthenia, influencing daily activity, after 4 months from the COVID-19 diagnosis (Figure 2).

Figure 2: Interval between COVID-19 diagnosis and ED presentation

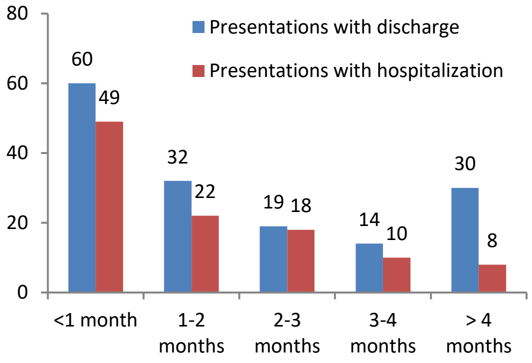

Concerning the time of hospitalization, there is high variability, from 1 to 36 days. The 65 patients with single presentation and admission cumulate a total of 643 days of hospitalization.

In the case of the 22 patients with multiple presentations, they have a variable number of hospitalizations and hospitalization days, as follows: 7 patients with 1 hospitalization (5-36 days), 11 patients with 2 hospitalization periods (6-46 days), 3 patients with 3 hospitalizations (15-31 days), and 1 patient with 4 hospitalizations (30 days). The total number of hospitalization days for all the patients in the study was 1093 days (Figure 3).

Figure 3: Number of presentations with admission and hospitalization days

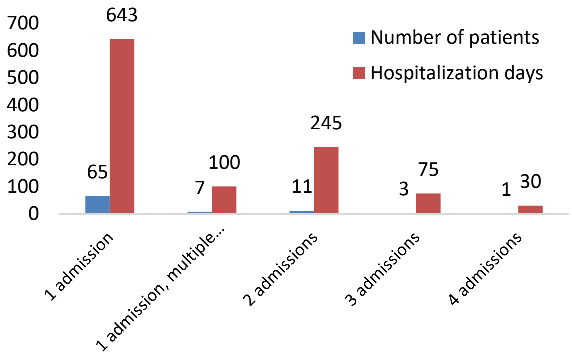

The analysis of the sublot of patients with multiple presentations and admissions shows that the average value of hospitalization time for presentations in the first 30 days from COVID-19 diagnosis is 8.3 (±4.5) days, respectively 14.14 (±4.98) days for presentations after the first 30 days (0.01). Out of the 87 hospitalized patients, 61 (70.12%) were discharged, and the rest 26 (29.88%) died.

The 117 patients that didn't require admission were 63 (53.85%) males and 54 (46.15%) females. The highest age group was 40-59 years, respectively 49 patients. There were no patients aged less than 20 years. 5 patients had between 80-89 years (Figure 4). The most frequent symptoms for the discharged patients were: respiratory (36.77%), cardiac (19.35%), digestive (18.06%), neurological (18.06%), ENT (4.52%), anxiety (3.23%).

Figure 4: Age distribution for discharged/admitted patients

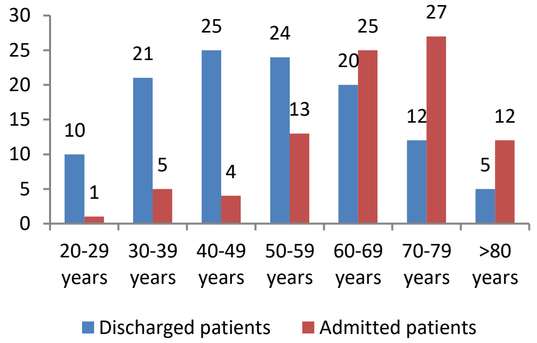

Out of the 87 admitted patients, 16 (18.39%) males and 10 (11.49%) females died. Most deaths occurred in men (43.75%) aged 60-69 years and in women in the age group 70-79 years (50% of female deaths) (Figure 5).

Out of 107 admissions in the period covered by the study, 46 patients (43%) had a positive RT-PCR test at the time of admission. During hospitalization, 15 patients (32.6%) from this lot; 11 patients (18.03%) of the 61 patients with negative

RT-PCR at the admission died (p>0.05).

Figure 5: Age distribution for deaths

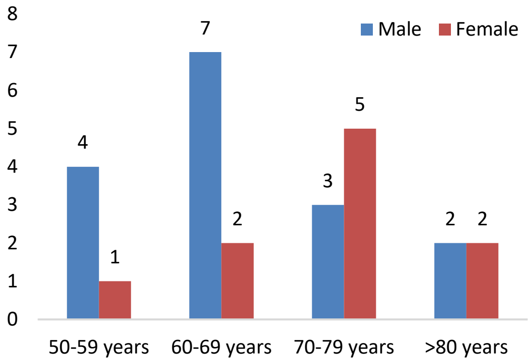

Out of the 22 patients with multiple admissions, only 8 had positive RT-PCR at first admission (5 of them had 2 admissions and 3 patients had 3 admissions). The other 14 patients had negative RT-PCR tests. Eighty-seven patients (81.30%) had comorbidities versus 20 patients (18.69%) without comorbidities (Figure 6). The most frequent conditions diagnosed at the admission of patients needing hospitalizations were an acute respiratory failure (25.68%), stroke (22.47%), acute decompensated heart failure (6.42%), Clostridioides difficile infection (6.42%), acute coronary syndrome (5.35%), pulmonary thromboembolism (4.28%), and deep venous thrombosis (4.28%). Out of the deceased patients, 5 (25%) had no medical history before the SARS-CoV2 infection, 2 males (53 years and 68 years old), and 3 females (68, 70, and 81 years old).

Figure 6: Comorbidities in hospitalized patients

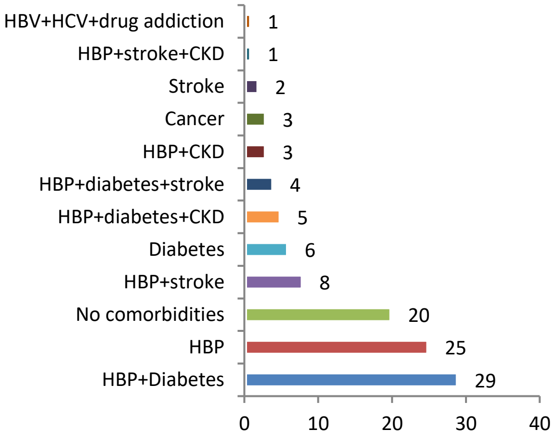

Neutrophilia was found in 66.5% of patients with a single presentation. History of stroke, neutrophilia, heart rhythm disorders, and neurological diagnoses at admission presented significant differences between patients stratified according to the number of presentations (Table 1). The most deaths were in patients with a single which suggests the severity of the post-COVID 19 pathologies.

Table 1: Differences identified between subjects who required hospitalization (n=87)

Characteristics	Number of hospitalizations		Statistical significance (p)
	Single	Multiple	
Neutrophilia (normal/yes)	22/43	14/8	<0.05
History of stroke (no/yes)	46/19	20/2	<0.05
Neurological diagnoses at admission (no/yes)	44/21	20/2	<0.05
Heart rhythm disorders	18/47	0/22	0.002
Discharge/Death	43/22	21/1	0.004

DISCUSSION

In the third wave of the pandemic, at the same time with newly infected COVID-19 patients, there were 262 post-COVID presentations in the Emergency Department, in the first 5 months of the year 2021.

Out of these, 59.16% of presentations did not require hospitalizations, and 40.84% were hospitalized. For both categories of patients, most came to the hospital with acute or persistent symptoms in the first month after the initial COVID-19 episode. 22 patients came repeatedly to the ED, out of which 7 patients had 4 presentations, 1 patient had 8 presentations and 1 patient had 15 presentations.

The patients presented in the first 30 days after SARS-CoV2 infection had significantly fewer hospitalization days, versus those presented after 30 days or more (8.3 vs 14.14 days, p=0.01).

The number of hospitalization days is variable, from 1 day to 36 days, for a single admission. 15 patients (17.24%) required multiple hospitalizations (2-4). Four of these patients cumulated over 30 days of hospitalization (maximum 46 days for 1 patient). The total number of hospitalization days for an small number of patients (87) was 1093.

Out of the total number of admissions, those with negative RT-PCR tests at the time of admission were more (57%) than those with positive RT-PCR tests. 18.03% of patients with negative RT-PCR died, vs 32.60% of those with positive RT-PCR. The results show that a negative RT-PCR test after the initial COVID-19 episode isn't equivalent to favorable evolution or total recovery.

Our study, like other studies, argues that associated comorbidities increase the risk of COVID-19 symptoms' persistence, the risk of complications, and is a factor in poor prognosis.

Hypertension is the most common comorbidity (28.73%), with the highest risk of unfavorable evolution, followed by diabetes (6.89%). In hospitalized patients, the most frequent comorbidities are hypertension and diabetes (33.33%), hypertension and stroke (9.19%), or hypertension, diabetes mellitus, and chronic kidney disease (5.75%) [21].

Acute stroke was diagnosed in 22.47% of admitted patients [22].

Treatment included interventional procedures, like mechanical thrombectomy for 2 patients with acute stroke, or coronary angioplasty, for 1 patient with STEMI.

The high percentage of deaths among patients who presented with post-COVID 19 symptoms and required hospitalization (29.88%) shows the severity of the complications of COVID-19.

The hospitalized patients without prior medical history (18.69% - 20 patients) are apparently few, but 25% of them (5 patients) died. It is therefore important to monitor the evolution of post-COVID-19 patients without prior comorbidities.

The limitations of the study are the small number of presentations, the study being conducted in a single emergency department, and a lack of data related to the vaccination status of patients (vaccination began in Romania at the end of December 2020).

CONCLUSIONS

The overcrowding of emergency departments and hospitals caused by post-COVID-19 complications becomes a real logistic problem in the context of the currently ongoing SARS-CoV2 pandemic. It appears from the study that late presented patients have unfavorable evolution, with significant mortality and prolonged hospitalization. Some patients died after just 1-3 days of hospitalization.

The importance of the study is great, data being collected from the biggest emergency department in Romania, with the highest number of presentations. The high number of hospitalization days has multiple implications, both for the patients and the medical personnel. The results of the study point out the frequency and severity of post-COVID-19 pathology.

Prompt intervention, targeting cases investigated clinically and radiologically in an outpatient setting, could go the relief of emergency departments overcrowding and a decrease in the rate of hospitalization, and implicitly a decrease in morbidity and mortality, with positive effects on each patient's health, and also in their familial and socio-economic circumstances.

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ORIGINAL ARTICLES

Alteration of levels of thyroid hormones in acute ischemic stroke and its correlation with severity and functional outcome

Brinder M.S. Lamba[1], Shalini Sharma[1], Pulin Gupta[1]

Abstract: *Introduction – With increasing life expectancy and changing trends in diseases, lifestyle diseases come forward and become a major concern. Ischemic stroke is an emerging public health issue leading to significant morbidity and mortality. To improvise positive outcome in acute ischemic stroke (AIS) and optimizing healthcare resources, accurate and timed prognostication of functional outcome is important. Various studies implied derangement of Hypothalamo-pituitary-thyroid (HPT) axis in AIS, thereby advocates neuroprotective properties to these hormones. This is, still, an open field for active research. Thyroid hormones can, therefore, be used as a surrogate tool for outcome predilection in AIS. This prospective observational cohort study aimed to investigate the alteration of levels of thyroid hormones in AIS and simultaneously correlate their levels in assessment of clinical severity and predicting the functional outcome.*

Materials and methods – We had collected data from 60 patients of, radiologically confirmed new cases of AIS who sought medical help within 72 hours of initiation of stroke symptom and had no previous history of thyroid disease.

Blood samples for thyroid function test [TSH, FreeT3 (FT3), and FreeT4 (FT4)] were collected within 24 hours after admission and subsequently at 7th and 30th day of discharge .On admission neurological clinical severity was assessed by using National Institute of Health Stroke Scale (NIHSS). Functional outcome was assessed by Modified Rankin Scale (mRS) at 7th and 30th day of discharge.

Result – Out of 60 AIS patients, 40 were having Non thyroidal illness Syndrome (NTIS). Low T3 was most common NTIS observed. Negative correlation was observed between FT3 levels and on admission NIHSS score and mRS score on follow up.

Conclusion – This study thereby concludes that low T3 levels were associated with clinical severity and poor functional outcome in AIS.

Keywords: *stroke, thyroid profile, correlation, NIHSS, mRS, NTIS*

INTRODUCTION

Stroke, described by the WHO, as the clinical syndrome of rapid onset of focal (or global, in SAH) neurological deficit, persisting more than 24 hrs or leading to death with no apparent cause other than a vascular one. [1]

It is an emerging health problem all over the world. Worldwide every year, 15 million people suffer a stroke. It is the fourth most common cause of death behind the disease of heart, cancer and chronic lower respiratory disease [2]. Globally, stroke is the second leading cause of death above the age of 60 years [3].

After dementia, CVA is the second chief cause of disability. Disability comprise of deprivation of speech and /or vision, confusion and paralysis [3].

Burden of stroke is increasing, which can be, due to

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increasing longevity, smoking, changing habits &lifestyles and type A personality. Beside these, other known risk factors include hypertension, obesity, diabetes mellitus and metabolic syndrome.

In comparison to developed countries, where the incidence of stroke has been decreased or reached a plateau, burden of stroke has been increasing in developing countries [3, 4, 5]. India, like other developing countries, is in the midst of epidemic of stroke.

In rural areas, the estimated adjusted stroke prevalence rate range 84-262/ 100,000 and 334-424/100,000 in urban areas. The incidence rate is 119-145/100,000 based on recent population based studies. [6] In India, nearly one fifth of patients with first ever stroke admitted to hospital is estimated to be of age 40 years or less [7]. Globally, a third of all strokes occur in 20-64 year age group [8].

Stroke can be classified into ischemic and hemorrhagic type. The Indian Collaborative Acute Stroke Study (ICASS) reported that up to 80% stroke were of the ischemic type [9]. The ICMR has advocated tobacco use, diabetes mellitus, hypertension and low concentration of hemoglobin as main risk factors for ischemic CVA [10].

Review of the literature reveals a significant derangement in thyroid hormones in patients with critical illness, specifically during the acute phase [11]. Euthyroid Sick Syndrome or Non Thyroidal Illness Syndrome (NTIS) is a condition in which derangement of thyroid hormones occurs in association with severity of disease and stress like surgeries [12] The most common pattern found under NTIS is low T3 level with normal level of T4 and TSH [13,14].

In acute ischemic stroke (AIS), HPT axis are significantly deranged. Various studies advocate low T3 levels as an indicator of poor prognosis in AIS [15, 16]. A reduction of serum T3 without elevation of TSH is associated with severity and worse clinical outcome in stroke [15, 17, 18]. Whether the prognosis is direr in AIS patients than those with the same disease but without the low T3 syndrome remain unknown. In AIS, implication of thyroid hormones is hence, controversial.

Low T4 syndrome, a condition found in severe illness, in which T3 and T4 levels are reduced while TSH level does not show expected pituitary thyroid axis reactivity [13, 14]

After acute stroke, better survivals were reported even when the cut-off point used was the median T3 levels [15], showing that lower T3, even it is in the normal range, may be associated with poorer prognosis. However, the association was not confirmed by other studies [19-21]. Therefore, it is not clear if, in ischemic CVA, lower T3 levels, especially within the normal range, on hospital admission provide worthy prognosticative information. A better understanding of this issue can be used for risk stratification in AIS and hence better healthcare resource utilization.

The basic aim of our study was to find out whether there occurs any alteration in thyroid hormone levels in AIS and also to correlate their levels in assessment of stroke severity and functional outcome as evaluated by using NIHSS and modified Rankin scale (mRS) respectively.

MATERIALS AND METHODS

This study was performed at the Department of Medicine at PGIMER (Post Graduate Institute of Medical Education and Research), Dr RML Hospital, New Delhi from November 2016 to March 2018. It was a prospective observational study in which a cohort of 60 patients of AIS was admitted and followed up at 7th and 30th day after discharge.

Inclusion criteria

Any patient of age 18 years or older & with no previous history of thyroid hormone abnormality, with first ever radiologically confirmed ischemic CVA presented within 72 hours of onset of stroke symptoms.

Exclusion criteria

1. Patients with known thyroid disease or biochemically defined overt thyroid disease,
2. Patients with transient ischemic attack, or past history of stroke,
3. Those using medications that can affect thyroid functions,
4. Those with cancer, severe renal or liver failure, inflammatory or infectious diseases and hematological diseases.

The enrolment of patients was done after informed consent. All the issues, including ethical issues of the study had been reviewed by the Institutional Review Board and approved.

The patient was first evaluated clinically which include a detailed history and examination.

Baseline characteristics, include demographic data such as age, gender, and concurrent diseases, thyroid disorder related medication use, risk factors of stroke, such as a history of diabetes mellitus, coronary artery disease hypertension, smoking and TIA.

Acute ischemic CVA was confirmed by using radiological modality NCCT head /CECT head/MRI brain was used.

On admission, stroke severity was assessed by using NIHS scale and was divided into 3 categories:-

- Mild NIHSS score <8
- Moderate NIHSS score 8-14
- Severe NIHSS score ≥ 14

Blood samples for assessment of the thyroid profile was drawn within the first 24hrs of presentation and then subsequently during follow up at 7th day and 30th day after discharge.

FT3, FT4 and TSH was measured by using chemiluminescent micro particle immunoassay.

Normal range in our laboratory is

- TSH 0.5-5.0 U/ml
- Free T3 (FT3) 2.0-4.4 pg/dl
- Free T4 (FT4) 0.7-2.0 ng/dl

On the basis of thyroid profile, the patients were divided into five categories:

1. NTIS was diagnosed as a low FT3, low or normal FT4, and low or normal TSH. Low T3 syndrome was defined as low FT3 levels with normal TSH and FT4 levels. [19, 22, 23, 28]

2. Euthyroid was diagnosed as normal FT3, FT4, and TSH. [19, 22, 23]

3. Subclinical hypothyroid was diagnosed as normal FT3, normal FT4, and high TSH. [24, 25, 26, 27, 29]

4. Hyperthyroid was diagnosed as low TSH with or without high FT4 and FT3 [22, 30, 31]

5. Hypothyroid was diagnosed as high TSH with low FT4 and normal FT3. [23, 30, 31]

Functional outcome was evaluated by using the Modified Rankin Scale (mRS) at the first follow up clinic visit at 7th day and subsequently 30th day after discharge. The mRS [32, 33, 34] is a scale to determine the disability after stroke. It has a score of 0-6, with 0 referring to no limitations or symptoms, 5 being severe disability requiring constant care and 6 being dead

On the basis of this scale, patients were divided into 2 outcome groups:

- Poor functional outcome (mRS > 2)
- Good functional outcome (mRS ≤ 2)

Data was analysed by using SPSS version 21.0 Qualitative & categorical or nominal variables were expressed as numbers (percent) or proportion. Quantitative & continuous or ordinal variables were expressed as medians or mean ± standard deviation (SD) Association and comparison of two categorical variables was done by using Chi square. The association between thyroid hormone levels and stroke severity and functional outcome was evaluated by Pearson correlation. The chi square and independent sample t test were used to compare respective categorical and continuous variables between different outcome groups. Variables with significant difference between different outcome groups were included in multiple logistic regression analysis. The criterion of statistical significance was set at p value < 0.05.

RESULTS

The mean age of the study population was 63.75 ±11.755 SD years with maximum age reported was 87 years and the minimum age was 38 years (Figure 1). Out of 60 study subjects 14 were female and 46 were male (Figure 2), suggesting ischemic stroke was more frequent in male (76.7%) as compared to female (23.3%) and male to female ratio was 3.28:1.

Figure 1.

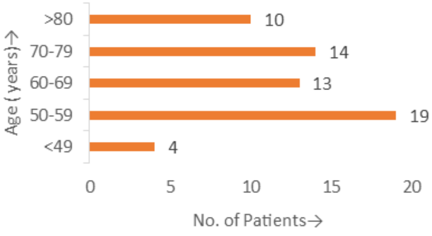

Figure 2.

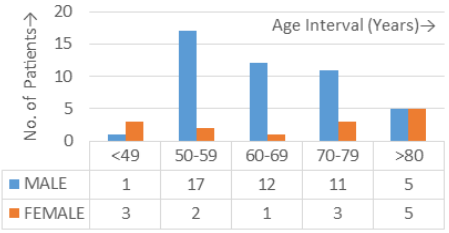

	<49	50-59	60-69	70-79	>80
■ MALE	1	17	12	11	5
■ FEMALE	3	2	1	3	5

The incidence of stroke increases with presence of risk factors. Common vascular risk factors, as shown in Table 1, included in the study were hypertension (n=40, 66.7%), diabetes (n=23, 38.3%), smoking (n=38, 63.3%) and dyslipidaemia (n=17, 28.3%).

Figure 3.

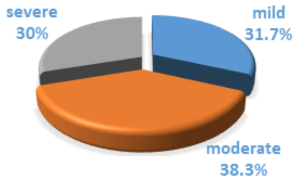

At admission, 19 patients (31.7%) had mild, 23 (38.3%) had moderate, whereas 18 (30%) had a severe stroke on NIHS scale (Figure 3). There was no statistical significant difference found between NIHSS score and age, gender, vascular risk factors for stroke studied (p value >0.05).

Table 1: Distribution of risk factors according to age group

Age (year)		<49	50-59	60-69	70-79	>80	Total	p-Value
Hypertension (HTN)	HTN	3	11	10	8	8	40	>0.05
	non HTN	1	8	3	6	3	20	
Diabetes (DM)	Diabetes	1	9	5	4	4	23	>0.05
	non DM	3	10	8	10	6	37	
Smoker	Smoker	1	12	8	11	6	38	>0.05
	non	3	7	5	3	4	22	
Dyslipidemia	Dyslipidemia	1	2	5	5	4	17	>0.05
	non	3	17	8	9	6	43	

Figure 4: Comparison between thyroid patterns observed

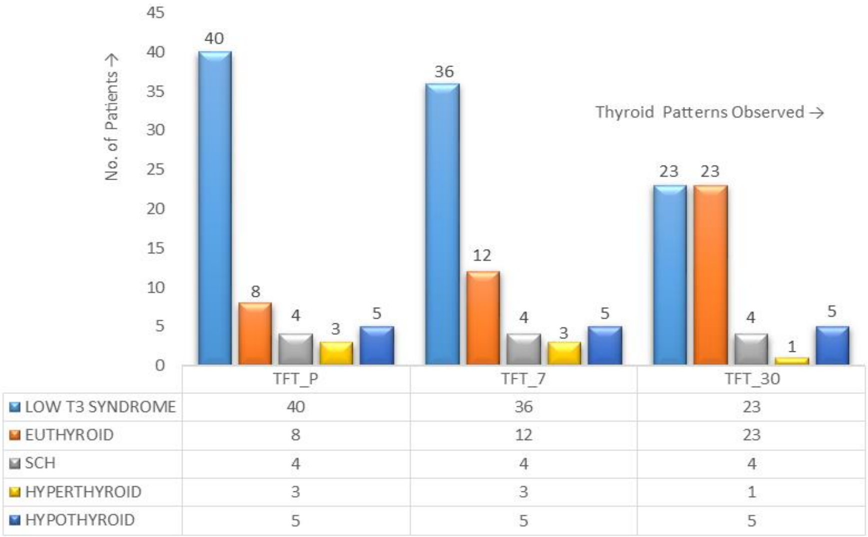

	TFT_P	TFT_7	TFT_30
LOW T3 SYNDROME	40	36	23
EUTHYROID	8	12	23
SCH	4	4	4
HYPERTHYROID	3	3	1
HYPOTHYROID	5	5	5

Figure 5: NIHSS score and thyroid dysfunction on admission

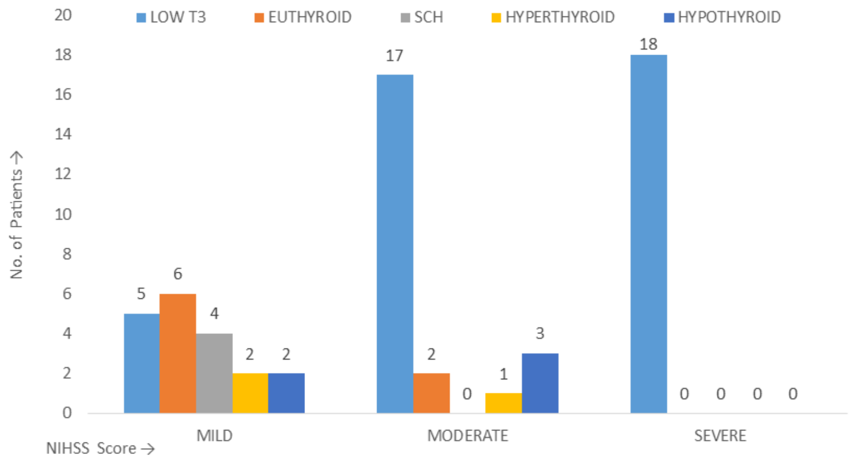

Table 2: Characteristics of AIS with low T3 & normal T3 values

	low T3 group (n=40)	normal T3 group (n=20)	p value
Clinical variables			
Age (years)	65.75	65.50	0.819
Sex (% male)	31 (77.5%)	15 (75%)	0.829
Hypertension	27 (67.5%)	13 (65%)	0.846
Diabetes	16 (40%)	7 (35%)	0.707
Smoking	25 (62.5%)	13 (65%)	0.850
Dyslipidemia	8 (20%)	9 (45%)	0.064
NIHSS (median)	11	6	0.000
Baseline NIHSS score	12.35±4.5	7.15±2.3	0.000
Distribution of NIHSS scores			
Mild	5 (12.5%)	14 (70%)	0.000
Moderate	17 (42.5%)	6 (30%)	0.000
Severe	18 (45%)	0	0.000
Thyroid function test (on admission)			
TSH	2.40±1.12	3.81±2.87	0.05
FT3	1.28±0.50	2.37±1.0	0.05
FT4	1.60±0.64	1.57±0.77	0.05
mRS_7 (median)	3	2	0.000
Pt with mRS_7 <2	8 (20%)	16 (80%)	0.000
mRS_30 (median)	3	2	0.000
Pt with mRS_ 30 <2	13	18	0.000

Thyroid pattern observed in this study (Figure 4) was low T3 syndrome (n=40, 66.7%), euthyroid (n=8, 13.3%), subclinical hypothyroid (SCH, n=4, 6.7%), hyperthyroid (n=3, 5%) & hypothyroid (n=5, 8.3%) during admission.

A statistically significant (r=0.436, p value <0.05, Figure 5) direct relationship found between NIHSS score and the number of patients with the low T3 syndrome.

Figure 6. Scatter plot graph between FT3 values and NIHSS scores on admission. Each circle represents a patient

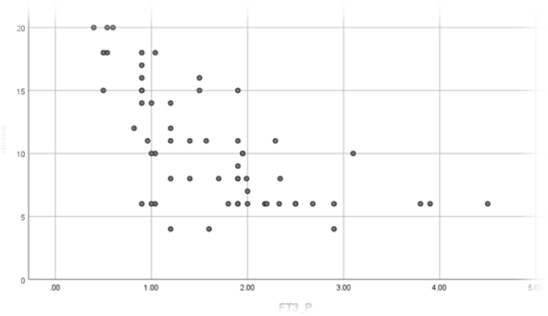

Table 3. Correlation between FT3 & NIHSS scores on admission

		NIHSS	FT3_P
NIHSS	Pearson Correlation	1	-0.712**
	Sig. (2-tailed)		0.000
	N	60	60
FT3_P	Pearson Correlation	-0.712**	1
	Sig. (2-tailed)	0.000	
	N	60	60

*** Correlation is significant at the 0.01 level (2-tailed).*
FT3_P: free T3 levels on presentation /admission

In order to study the impact of T3 levels of severity & outcome of stroke in our study, we divided cohort of 60 patients into two groups – low T3 and normal T3 group and also analyze the distribution of risk factors among them (Table 2). No significant difference observed between age, gender & vascular risk factors between these two groups.

There were significant negative correlations between free T3 (FT3) levels and NIHSS scores on admission (n=60, r= -0.712, r2=0.507, p=0.000, 99%CI, Figure 6, Table 3) among all patients. This implies, after acute stroke, that the lower FT3 values are, the worse neurological impairment will be.

NIHSS Score was negatively correlated with TSH on admission as well as 7th and 30th day of discharge (Table 4 – correlation is significant at the 0.01 level 2-tailed). NIHSS Scores were shown a negative correlation with FT4 values only on follow up. Although the strength of the correlation was not as stronger as it was with FT3 levels.

Table 4: Associations between thyroid hormones and NIHSS score on admission and on 7th and 30th day of discharge

	NIHSS	Pearson Correlation (r)	r^2	Sig (2 –tailed)
TSH_P	-0.227	-0.227	0.051	0.041
FT3_P	-0.712	-0.712	0.507	0.000
FT4_P	-0.043	-0.043	0.002	0.747
TFT_P	-0.402	-0.402	0.162	0.001
TSH_7	-0.259	-0.259	0.101	0.044
FT3_7	-0.753	-0.753	0.567	0.000
FT4_7	-0.264	-0.264	0.070	0.042
TFT_7	-0.455	-0.455	0.207	0.000
TSH_30	-0.343	-0.343	0.117	0.007
FT3_30	-0.787	-0.787	0.619	0.000
FT4_30	-0.365	-0.365	0.133	0.004
TFT-30	-0.479	-0.479	0.229	0.000

ASSESSMENT OF FUNCTIONAL OUTCOME

Final outcomes were evaluated at 7th and 30th day of discharge and the mRS score so obtained was compared with on admission NIHSS Score as well as thyroid hormone levels measured concurrently.

Out of 60 patients, 36 patients (60%) had a poor functional outcome (mRS>2) at 7th day of discharge in comparison to 29 patients (48.3%) who still had a poor functional outcome at 30th day of discharge.

No mortality either in hospital or hospice was observed on first following up clinic visit. However, on second follow up clinic visit, 4 patients, among those with poor functional outcome had mRS Score 6 (dead) constituting 13.7 % of patients with mRS score>2. Retrospective analysis of these patients revealed that they had a severe stroke on NIHS scale. Analysis of thyroid hormone pattern on admission and at 7th day of discharge were suggesting that they belong to the low T3 syndrome. They had very low levels of free T3. TSH and free T4 levels were also low. These mortalities thereby emphasize severe derangement of hypothalamo-pituitary-thyroid axis.

Univariate analysis showed that mRS scores at first and second follow up visit, were not associated with age, gender and vascular risk factors like hypertension, smoking, diabetes & dyslipidaemia (p>0.05, Table 5).

Table 5: Demographic and vascular risk factors according to outcome at 7th & 30th day of discharge

Modified Rankin Scale (mRS)		Age (years)	Sex (male %)	Smoking (n, %)	HTN (n, %)	Diabetes (n, %)	Dyslipidemia (n, %)
TSH_P FT3_P FT4_P	mRS≤2 (n=24)	64±11.4	19 (79.2%)	20 (83.3%)	13 (54.1%)	07 (29.2%)	09 (37.5%)
	mRS>2 (n=36)	63.6±12.1	27 (75.0%)	18 (50.0%)	27 (75.0%)	16 (44.4%)	08 (22.2%)
	p value	> 0.05 (NS)					
TFT_P TSH_7 TFT-30	mRS≤2 (n=31)	61.4±12.6	23 (74.2%)	22 (70.9%)	19 (61.2%)	09 (29.0%)	10 (32.3%)
	mRS>2 (n=29)	66.2±10.3	23 (79.3%)	16 (55.2%)	21 (72.4%)	14 (48.3%)	07 (24.1%)
	p value	> 0.05 (NS)					

Table 6: Admission clinical and lab findings according to outcome at 7th & 30th day of discharge

	Modified Rankin scale at 7th day		Modified Rankin scale at 30th day		P value
	mRS_7≤2 (n=24)	mRS_7>2 (n=36)	mRS_30≤2 (n=31)	mRS_30>2 (n=29)	
Baseline NIHSS (median)	6	13	6	15	<0.05
Baseline NIHSS (mean±SD)	6.83±2.26	13.14±3.92	7.23±2.14	14.24±3.56	<0.05
Distribution of NIHSS score					
Mild (n)	17	2	19	0	<0.05
Moderate (n)	6	17	12	11	<0.05
Severe (n)	1	17	0	18	<0.05
Thyroid hormone levels at admission					
TSH	3.15±2.23	2.69±1.81	3.27±2.29	2.45±1.28	<0.05
FT3	2.16±0.83	1.30±0.72	2.08±0.87	1.18±0.59	<0.05
FT4	1.60±0.61	1.50±0.73	1.57±0.73	1.62±0.64	<0.05
Thyroid hormone levels at 7th day of discharge					
TSH	3.09±2.21	2.62±1.59	3.19±2.29	2.40±1.16	<0.05
FT3	2.20±0.84	1.33±.73	2.13±0.86	1.20±0.61	<0.05
FT4	1.56±0.65	1.47±0.64	1.60±0.72	1.42±0.45	<0.05
Thyroid hormone levels at 30th day of discharge					
TSH	3.17±2.05	2.66±1.47	2.42±1.36	2.40±1.23	<0.05
FT3	2.30±0.81	1.42±0.82	3.31±2.01	1.24±0.71	<0.05
FT4	1.58±0.61	1.35±0.54	2.30±0.81	1.24±0.43	<0.05

Patients with poor functional outcome were having high baseline & median NIHSS Score. The majority of patients with poor outcome had moderate to severe score on NIHS Scale (p value <0.05, Table 6). Free T3 levels were significantly lower in them during first as well second follow up visit (p value <0.05, Table 6).

Figure 7. Scatter plot between FT3 values and mRS_7 scores at 7th day of discharge

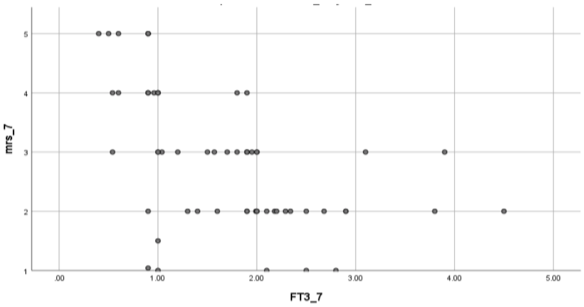

These findings, consistent with the findings reported by Xu and his colleagues [21], which is due to the fact that in patients with poor functional outcome there might be decreased peripheral conversion of FT4 to FT3 which lead to low levels of FT3.

Table 7. Correlation between FT3_7 values and mRS_7 scores at 7th day of discharge

		mRS_7	FT3_7
mRS_7	Pearson Correlation	1	-0.692**
	Sig. (2-tailed)		0.000
	N	60	60
FT3_7	Pearson Correlation	-0.692**	1
	Sig. (2-tailed)	0.000	
	N	60	60

***Correlation is significant at the 0.01 level (2-tailed).*
mRS_7: modified Rankin scale at 7th day of discharge
FT3_7: free T3 levels at 7th day of discharge

On the 7th day of discharge, there was a significant negative correlation between mRS scores and free T3 levels [correlation between mRS & FT3 on admission (n=60, r= -0.599, r^2=0.301, p<0.05, 99%CI, Table 8) and at 7th day of discharge (n=60, r= -0.692, r^2=0.479, $p<0.05$, 99% CI, Figure 7, Table 7)]

Table 8: Associations between thyroid hormones on admission & at 7th day of discharge and mRS scores at 7th day

	mRS _7 scores	Pearson Correlation (r)	r^2	Sig (2 –tailed)
TSH_P	-0.215*	-0.215*	0.046	0.009
FT3_P	-0.599**	-0.599**	0.301	0.000
FT4_P	-0.021*	-0.021*	0.0004	0.874
TFT_P	-0.384**	-0.384**	0.147	0.002
TSH_7	-0.242*	-0.242*	0.058	0.006
FT3_7	-0.692**	-0.692**	0.479	0.000
FT4_7	-0.150*	-0.150*	0.023	0.254
TFT_7	-0.445**	-0.445**	0.198	0.000

Table 9: Associations between mRS_30 scores and thyroid hormones on admission and on 7th and 30th day of discharge

	mRS_30 scores	Pearson Correlation (r)	r^2	Sig (2 –tailed)
TSH_P	-0.250*	-0.250*	0.063	0.054
FT3_P	-0.600**	-0.600**	0.360	0.000
FT4_P	-0.095*	-0.095*	0.009	0.570
TFT_P	-0.376**	-0.376**	0.141	0.003
TSH_7	-0.281*	-0.281*	0.466	0.029
FT3_7	-0.683**	-0.683**	0.028	0.000
FT4_7	-0.166*	-0.166*	0.042	0.204
TFT_7	-0.432**	-0.432**	0.186	0.001
TSH_30	-0.376*	-0.376*	0.141	0.003
FT3_30	-0.724**	-0.724**	0.524	0.000
FT4_30	-0.387*	-0.387*	0.149	0.002
TFT-30	-0.477**	-0.477**	0.228	0.000

***Significance of Correlation at the 0.01 level (2-tailed).*

** Significance of Correlation at the 0.05 level (2-tailed).*

mRS_30: mRS score at 30th day of discharge

On 2nd follow up clinic visit, similar trends of negative correlation was observed between mRS score and free T3 levels (Figure 8, Table 9).

This implies that AIS patients with the low T3 syndrome tend to have poor outcome at follow up. From Table 8, it was inferred that at first follow up visit, no statistically significant correlation found between mRS score and FT4 levels (neither an admission nor at 7th day of discharge). There were a statistically significant negative correlation between TSH levels (on admission, at 7th and 30th day of discharge) and mRS score at 30th day of discharge (p value <0.05, Table 9). No relation was seen between FT4 levels (on admission, & at 7th day of discharge) and mRS score at 2nd follow up visit (p value >0.05, Table 9). But a negative relationship seen between the two at 30th day of discharge (r = -0.387, CI 95%, p value <0.05, Table 9).

Figure 8. Scatter plot graph between FT3_30 values and mRS_30 scores at 30th day of discharge.

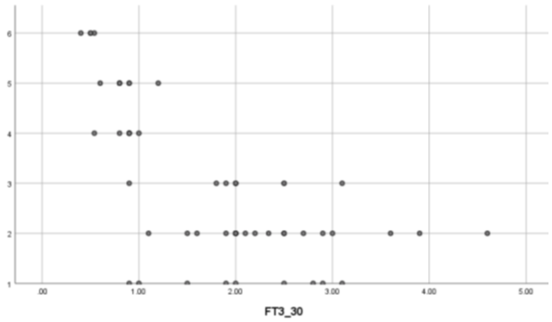

This findings can be explained by the fact that even with the severity of stroke, levels of T3 decrease immediately, but it requires some time for T4 levels to show changes in their levels.

On multiple logistic regression analysis (Table 10), lower FT3 concentration (odds ratio OR=1.166, 95%CI, p value <0.05) remained independently related to poor outcomes.

Table 10: Multiple logistic regression analysis

	NIHSS* OR (95% CI), p	TSH* OR (95% CI), p	FT3* OR (95% CI), p	FT4* OR (95% CI), p
7th day outcome mRS ≤2 vs mRS >2 or death	0.641 (0.457-0.845) P<0.05	0.936 0.661-1.326 P<0.05	1.166 (0.425-3.200) P<0.05	0.803 (0.289-2.229) P>0.05
30th day outcome mRS ≤2 vs mRS >2 or death	0.418 (0.241-0.725) p<0.05	1.125 (0.744-1.701) p<0.05	1.296 (0.276-6.087) p<0.05	0.255 (0.052-1.258) p>0.05

** values at the time of admission*

Table 11: ΔmRS score vs Δthyroid pattern during follow up

		Δthyroid pattern		Total	
		no change	euthyroid		
ΔmRS	improvement	10	4	14	Pearson chi square= 3.08 p value 0.215
	same statuts	27	11	38	
	detoriation	8	0	8	
Total		45	15	60	

ΔmRS = change in functional outcome (mRS_30 score - mRS_7 score)
Δthyroid pattern = change in thyroid pattern

Figure 9. Thyroid hormones pattern observed at various time interval in this study

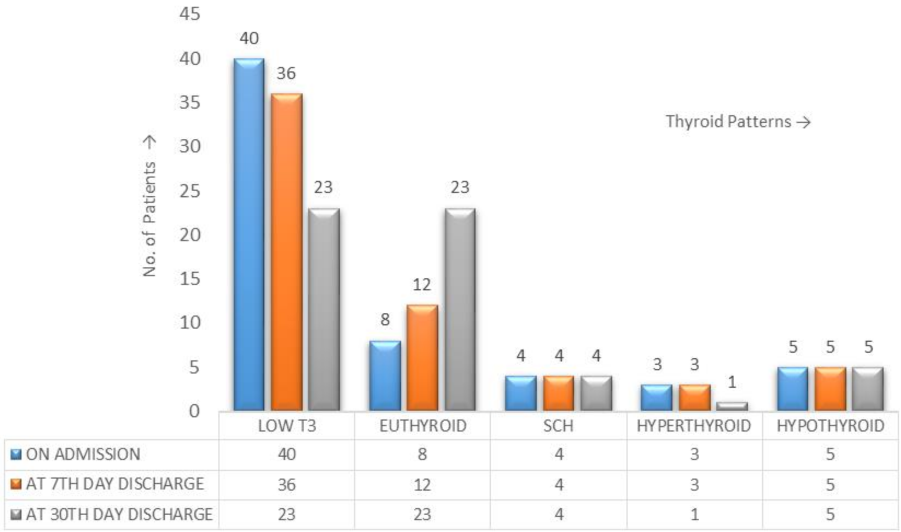

	LOW T3	EUTHYROID	SCH	HYPERTHYROID	HYPOTHYROID
■ ON ADMISSION	40	8	4	3	5
■ AT 7TH DAY DISCHARGE	36	12	4	3	5
■ AT 30TH DAY DISCHARGE	23	23	4	1	5

In addition to the FT3, the only other variables independently associated with poor functional outcome were NIHSS score (OR=0.641, 95% CI, p value <0.05) and TSH levels (OR=0.936, 95%CI, p value <0.05).

Of 40 patients with the low T3 syndrome, 13 patients became euthyroid on follow up (Figures 4 & 9, Table 11). This can be explained by the fact that during disease recovery, thyroid hormones and TSH concentrations return to normal [35]. This emphasize the need to follow up of such patients to confirm suspected cases of sick euthyroid. Similarly, on follow up, a very small percentage had shown improvement in their functional outcome. However, this finding was not statistically significant (p>0.05).

DISCUSSIONS

Conduction of this study was done with an objective to decipher the relationship between thyroid hormone levels and functional outcome in AIS. Observations recorded were similar to studies conducted by Alvezaki et al [17] and Zhang et al [19]. In hospitalized patients, especially in ICUs, the most common type of NTIS seen is low T3 [36, 37]. In ICH from either trauma or hemorrhagic CVA, low T3 has been reported [38, 39]. The present study showed thyroid hormones derangement in AIS and most common type of it was NTIS/low T3 syndrome.

An inverse relationship between T3 levels and NIHSS score was observed in our study. To follow up, patients with poor functional outcomes were found to have low free T3 levels. Thus, an inverse relationship observed between FT3 value and NIHSS score as well as mRS score, thereby strengthening its predictive role in estimating the severity and functional outcome in AIS.

The degree of decrease in T3 levels is related to worse neurological impairment. Thus, monitoring levels of FT3 could potentially be an easy, quick and surrogate marker in the future if other studies confirmed it further. The observations and findings delineated in the present study were similar to studies done by Alvezaki et al [17] which stated that low T3 is a possible independent predictor of stroke outcome.

In severe illness, T3 levels would decline to spare energy, considering it as an adaptive response to stressful conditions. [40-44].

Similar to other systemic severe diseases, a decline in serum T3 with no increment in TSH levels seem to be related to stroke severity and poor clinical outcome [17, 20, 45-47].

Possible mechanisms include the following:

(1) Change in the peripheral metabolism of thyroid hormones due to alterations in enzyme activity involved in peripheral T4 to T3 conversion, [42, 48]

(2) Involvement of pro-inflammatory cytokine action, [49]

(3) A disturbed shift in thyroid hormone distribution or change in binding proteins, [41, 49, 50]

(4) In severe illness, excessive glucocorticoids release, which lead to inhibition of hypothalamic-pituitary- thyroid axis activities and T4 to T3 conversion. [51]

Similar to various studies conducted in ICUs, AIS patients with low T3 or combination with low T3 and T4 found to have a worse prognosis. [15, 43, 48, 52-54]

Interestingly, studies showed that derangement in T3 levels is not related with regional distribution of stroke (anterior vs posterior circulation). This change in thyroid hormone levels can, therefore, be due to disturbance in their metabolism instead of any structural defect induced due to abnormality in blood supply to hypothalamic-pituitary-thyroid axis. [19, 23]

A significant negative correlation observed between NIHSS score and TSH (p value < 0.05) on admission and on follow up. On admission, no correlation was observed between NIHSS score and free T4, however, negative correlations observed between them only to follow up. Unlike, NIHSS score and FT3 levels, strength of association between NIHSS score and TSH & T4 levels were not strong.

After CVA onset, within hours, the decline in serum T3 levels and degree of alteration in its levels is associated with the severity of stroke.[48 ,55-57] In severely ill patients, levels of T4 also decrease [55, 57]. A drop in FT4 levels represents a continuum of the NTIS spectrum in which as illness progresses in its severity, levels of FT4 would decrease. Therefore, low FT4 levels also correlated with bad prognosis.

However, no relationship of NIHSS score with FT4 levels on admission can be explained by the fact that with the severity of stroke there occurs decrease in the peripheral conversion of T4 to T3 as a result of which T3 levels would decrease but T4 levels would either normal or increases. Similar findings were reported by Xu and his colleagues in their study [21] that T4 levels were found to be increased in patients with higher scores on NIHS scale and hence those with poorer prognosis can have higher or normal T4 levels.

On univariate analysis, during follow up, no relationship was observed between mRS score and baseline parameters, i.e. age, gender and vascular risk factors. It was observed that AIS patients with high moors scores had low levels of free T3 and also had a high NIHSS score, both baseline and median. These findings, consistent with the findings reported by Xu and his colleagues [21], which is due to the fact that in patients with poor functional outcome there might be decreased peripheral conversion of FT4 to FT3 which lead to low levels of FT3.

At first follow up clinic visit, only other thyroid parameters with which mRS score can be correlated was TSH. Similarly, on 2nd follow up visit, mRS score was negatively correlated with TSH levels. A negative correlation was observed between free T4 and 2nd follow up visit mRS score only at 30th day of discharge. To sum up, apart from free T3, TSH levels would be next important parameter to determine functional outcome in AIS. However, free T4 would not show any significant change in its level immediately, but ultimately its levels also decrease in those with severe stroke and poor outcome which would be observed only on follow up.

In the present study, no significant difference observed between age, gender and vascular risk factors among AIS patients with low T3 and those with normal T3 levels. Zhang et al [19] study, however, showed that low T3 group patients were slightly but not significantly older. Alvezaki et al [17] study showed that patients under low T3 group were significantly older. In elderly, baseline thyroid function is usually at the lower side and when stressful events like stroke occurs, they tended not to compensate fully. This was the explanation offered by his study.

Except low T3 subgroup, none of the other subgroups had shown any mortality. In this subgroup on follow up, 4 patients (10%) were expired. Retrospective analysis of these patients showed that they had a severe stroke on NIHS Scale and had very low levels of FT3.TSH and free T4 levels were also at the lower range. These mortalities emphasize a severe derangement in HPT axis. These findings were consistent with studies which advocate that long term survival is poor in patients with the low T3 syndrome and emphasized that ischemic stroke patients with the low T3 syndrome were at higher risk of poor outcome on follow-up that was arranged from 2 to 4 weeks after ischemic CVA and 1-year survival was remarkably worse in CVA patients with the low T3 syndrome. The outcomes were supported by other studies. [17, 19]

We, in the end, conclude that there is alteration in levels of thyroid hormones in AIS and low T3 levels are predictive of severity and functional outcome in these patients. However, the understanding of pituitary-thyroid axis, factors controlling action of thyroid hormone at the cellular level and explanation of different arrays of thyroid function tests may be considered cardinal in the management of ischemic CVA.

CONCLUSIONS

In acute ischemic stroke, there is the major impact of thyroid dysfunction. In such patients non thyroidal illness (NTIS) is not uncommon and reflect the increase in physical stress associated with the insult. The present study showed that severity of initial (functional) impairment measured by an NIH Stroke scale on admission, and free T3 & TSH levels had an independent predictive role in determining the functional outcome.

LIMITATIONS OF THE STUDY

Although the research was prepared carefully, there were some inevitable limitations such as small size of the sample, shortfall of long term follow up, selection biases, etc. Due to time constraint, this study was conducted on a small sample size, but it is advised to conduct it on large population so that better results and better comprehension of subjects can be made.

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A marketing perspective on the efficient strategies in Romanian family medicine

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Abstract: *Family Medicine has always been regarded as an interdisciplinary specialty, but lately, in most Eastern-European countries, such as Romania, it has been supported by many laws and regulations. The outcome of this prolific background has brought both advantages and disadvantages. Some advantages relate to a sustainable structure, and, at the same time, Family Medicine has been recognized as being the gate-keeper in almost any health care system. Further, due to the privatization of most family practice offices, in Romania, appeared a modern organizational approach, in the shape of a consortium of family physician practices. Some family physicians consider providing their services in a private office as a benefit, but for others the freedom and autonomy to become responsible of their own practice is considered to be a drawback because, apart from the clinical activity, many family physicians have to deal with managerial and marketing issues. In Romania, a consortium of family practice offices has to overcome a very competitive environment and some Marketing specific trends. The aim of this paper is to investigate the usefulness of marketing strategies in the Romanian family physician practice.*

Keywords: *family medicine, family physician, primary care, marketing, word-of-mouth, WOM*

INTRODUCTION

From its beginnings, the Family Medicine specialty has raised considerable discussion and debates regarding its scope and borders from an interdisciplinary perspective [1-3]. Since the Alma Ata conference in 1978 [4] and the World Health Organization's program, called "Health for All" [5], the importance of efficient primary care has been recognized as being the foundation of health systems in many parts of the world [6]. However, today Family medicine continues to face an unclear identity, as the scope of the practice is not well-defined and its consequences may trigger issues that relate to medical malpractice, healthcare coverage and insurance reimbursement [7].

In Central and Eastern European countries, health care systems are very much specialist and hospital oriented but the importance of family care still remains in the background, and it is not fully supported by focused strategies [8]. In the pandemic context, most of these countries have undergone rapid political changes and challenges that led to the necessity of developing and supporting the activities of the Family Medicine.

Since the collapse of the communist regime, the Eastern-European countries have made a radical transformation in the family care practice [9]. Once, under the umbrella of the impersonal polyclinic services and the purview of the law, the family practice offices have been privatized and are often managed by the family physicians themselves. Moreover, this privatization triggered an emergent decentralized primary care sector, which lacks the team-based care approach.

Although in most of this region many countries still struggle for the on-going acceptance and recognition, in Romania, there has been an upsurge of laws supporting the family practice activity and discipline-specific associations that recognize its fundamental position in the health care system. For instance, solo family practices have mostly disappeared in the Romanian urban areas, being replaced by a modern perspective, namely, building consortium of family physician practices [10]. In 2019, in Romania, there were 11,682 family physicians, who provided around 30,720,485 receipts [11].

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Further, the organizations that promote and support the modern Family Medicine are: The Romanian Society of Family Medicine/General Medicine, The Family Medicine Commission of the Medical College of Physicians and the National Centre for Studies in Family Medicine. The most important organization is the Romanian Society of Family Medicine/General Medicine because it is the biggest association of family physicians and has a long tradition in supporting the development of Family Medicine, as a distinct specialty in Romania [12].

The self-employed status of a family physician has brought with it a wide range of other responsibilities besides the clinical activity. The family physician has to take responsibility for the equipment and supporting staff as well as for the management of the practice. In fact, family physicians had to learn about management and marketing strategies in order to develop and implement a modern concept of their practice and offer a competitive advantage.

The aim of this paper is to investigate the usefulness of marketing strategies in the Romanian family physician practice

THE PARTICULARITIES OF FAMILY MEDICINE IN ROMANIA

Family physicians are the core stakeholders in delivering any health policy by providing primary care services, and the first contact of patients with the health system [13]. Although, the World Health Organization has defined their role in the primary health practice and their importance is widely accepted, there may be registered some differences between countries [14]. Specifically for the Family Medicine, the scientific literature revealed that the most often encountered differences relate to the incentives, practice information capacity, access, the use of quality indicators, staff and patient safety and the number of allowed patients in a portfolio of a family physician [15].

In Romania, the family physician is a person responsible with the provision of comprehensive and continuing care to every individual in need, regardless of age, gender and illness, but who is under his supervision [16]. Broadly, family physicians integrate in their professional practice activities that focus on the health and prevention measures as well as activities that concentrate on the provision of cure, care or palliation. A family physician provides these services either directly or through the services of other specialists, according to the health needs and the resources available within a community.

The particularities of Family Medicine in Romania are [16]:

- The family practice office is normally the point of first medical contact within the health care system, which provides open and unlimited access to its users, dealing with all health problems regardless of the age, sex, or any other characteristic of an individual;

- The family physician makes efficient use of health care resources through coordinated care, working with other professionals in the primary care setting, having an advocacy role for the patient in need;

- The family physician has a person-centred approach, orientated towards both the individual, his/her family, and their community;

- The family physician has a unique consultation process, which should establish a long-term relationship with the patients, through effective communication strategies;

- The family physician is responsible for the provision of longitudinal continuity of care as determined by the needs of the patient;

- The family physician has a specific decision making process determined by the prevalence and incidence of illness in the community;

- The family physician manages simultaneously both acute and chronic health problems of patients;

- The family physician manages illnesses at early stages of evolution;

- The family physician promotes health and well-being ideas for individuals through appropriate and effective interventions.

- The family physician deals with health problems in their physical, psychological, social, cultural and existential dimensions.

According to the Primary Health Care Activity Monitor for Europe [17], Romania has one of the strongest primary care structures, along with Denmark, Finland, Italy, Portugal and United Kingdom, but the critical issues in a family physician activity resumes to buying modern equipment, competence involvement and using appropriate appointment systems [18].

Despite having an interdisciplinary approach, family physicians have identified the following challenges in providing their specialty [18]:

- Less attractive – Due to the economical and managerial freedom, most family physicians no longer find this medical specialty attractive. The former enjoyment of working on a private basis has been replaced by disappointment because the workload has not been reduced, especially in the pandemic context.

- Constant need to buy modern equipment – In order to deliver quality health care services, family physicians need to keep track of the latest equipment and, as the patient becomes more empowered with information, they need to understand that the patients' needs have been raised as well. In Romania, some family physician offices have been equipped with computers and printers, electrocardiographs, spirometers, CT scanners and even office-based laboratory tests.

- Raise the continuing professional development credits – The family physician's activity is influenced by the quality improvement initiatives of their practice in the shape of a constant participation to conferences and other specific events or publish certain articles, books and other materials that would help them build their knowledge and learn about the latest medical information. Yearly, getting the credits imposed by the laws, leads to a re-certification of the family physician.

- Impairment in accessing the academic field – Although the academic positions opened for family practitioners have improved significantly, there are a few issues that need to be addressed, as for instance the salience of Family Medicine as a discipline and its connections with other medical specialties [19].

Moreover, the indicators specific to Family Medicine relate to health care quality, and the elements that need to be addressed are linked to continuity, coordination, comprehensiveness of care and community orientation. [20] Continuity is described by informational, relational and management activities, while the coordination is reflected in the organization and the monitoring of the services delivery. In Romania, the family physician's continuity and coordination are connected to the medical records and electronic medical records and with the collaboration with other specialists. The comprehensiveness is reflected in the scope of the practice and in the range of services provided, whereas the community orientation is important to be ensured for the sustainability of the health care system. In Romania, both the comprehensiveness and the community orientation are extremely low because the family physician provides the basic services or ancillary services, with a specific interest, for ultrasound or gynaecology related services, and the community orientation is non-existent because family physicians are too busy with their clinical activities in order to have other voluntary activities.

All four elements reduce to the doctor-patient relationship, this being the core characteristic of the Family Medicine [21]. If the practice gets influenced in a negative direction by the doctor-patient relationship, than there would be significant implications at the image level and the patients' desirability to continue going to the family physician. In addition, since 1999, when the family physician's form of payment switched from a salary paid by the system to a yearly contract based model, the family physicians became the gate keepers of the health system but was also introduced the freedom of patient choice of a family physician. Therefore, family physicians have to catch the attention of patients through different strategies and build their portfolios as well as offer a competitive advantage. In fact, family physicians had to implement efficient marketing strategies in order to survive on a market.

CURRENT CHALLENGES OF FAMILY MEDICINE IN ROMANIA

Health care is a field in a permanent and constant evolution, being filled with a wide range of opportunities that should stimulate creativity and enthusiasm of the specialists in the field. Still, health care services are not like other services because they are unwanted, and this may be considered highly troublesome by patients and, at the same time, critically important for their well-being [22]. Actually, health care services have always been archetypal in nature because consumers lack the technical knowledge to understand it, and have to rely on credence qualities [23]. Consequently, entropy of information is formed and consumers will assess the quality of the provided health care services based on their perceptions and not on their real experiences. If we take into consideration the pressure which consumers face in making health care decisions, we may conclude that patients confront with a challenging lack of accessible and medically verified information that would, eventually, help them in rationally think about the benefits and drawbacks of the medical procedures [24]. Moreover, frequently, the desires and the needs of health care consumers come in conflict, resulting in their reluctance to play the role of co-producers [25], but physicians motivate them to assume their part in the health care process by using ethics, compassion, empathy, and solidarity [26].

Doyle [27] mentioned that patient experience consists of two aspects: rational and functional. The rational aspect focuses on the 'interpersonal aspects of care', such as the physicians' ability to treat patients with respect and compassion, to empower patients with information in order to prevent other illnesses, as well as, to highly engage patients and their family members in the decision-making process, whereas the functional aspect relates to the patient's basic expectations about the health care service, which includes elements such as the effectiveness and efficiency of health care delivery and the cleanliness and safety of the health care background.

Due to the emergence of technology, patient emancipation or patient empowerment became the most substantial and visible trend [28], prior to sickness in the shape of prevention methods or upon its primary form, in the shape of diagnosis, treatment, and during the treatment period, shaped by the degree of compliance. This trend has fundamentally changed not only the doctor–patient relationships, but also the relationships established between doctor–society– patient, as a whole. As such, the passive patient has changed and became involved and proactive in designing the process of care and taking decisions. Also, this trend triggered several changes in the providers' perspectives that emphasized the necessity for greater authority and involvement in care from the regulatory and funding bodies as well as from the stakeholders, who had to change their behaviors, through financial, structural, and organizational mechanisms. These strategies had to significantly rely on patient expectations, and basically, to improve patient support by using personalized communication about preventive advice and reorientation based on consumption analyses, and, implement Marketing instruments and principles [29]. Marketing plays an important role in helping health care professionals to create, communicate, and provide value to their target market- the health care consumers- by building a sustainable relationship [30].

Although many experts have considered the emergence of Health Marketing as a distinct field, few specialists have provided clear and integrative definitions [31].

Over the past decade, some market specific trends contributed to integrating Marketing in the health care field. These trends are the following [32]:

- From a mass-marketing approach to a more specific approach;
- From "one measure for all" to personalization;
- From the emphasis on a health episode to a long-lasting relationship;
- From low-technology market to high-technology market.

From a Marketing perspective, the process of providing health care services must be conducted according to the patient requirements, because these activities are designed to satisfy their needs and wants. The main argument in adopting a Marketing perspective in health care services steams from the objectives of the health care systems around the world, namely, assessing a balance of financial-territorial resource allocation and the control of costs, reducing the inadequate technology employment and introducing modern managerial methods [33]. Further, a high performing health system is characterized by the following features: accessibility, pertinence toward the needs, equity, chance of selection, efficacy and efficiency, and large scale social accessibility [30]. So, everyone has the right to non- discriminant medical attendance and health care organizations should satisfy the needs of the health care consumers in a timely manner. In addition, along with these challenges, competition in the health care market has increased in certain medical specialties, as in the case of Family Medicine.

In Family Medicine, Marketing strategies should be tailored differently for patients and families, communities, other physicians with whom they work with and other categories of medical personnel, in the shape of promotion strategies. As such, promotion strategies include a carefully designed set of activities intended to induce change with the help of a message.

Frequently, promotion strategies concentrate on various elements such as specific communication objectives for each target audience, guidelines for designing attention-catching and effective messages, and designing appropriate communication channels [34]. However, promotion is considered discreditable among the medical profession because there is a strong social norm against advertising in the health care field [35]. Although, consumers typically receive most of the information about health care services from Mass-Media, in fact, the most important information comes from recommendations from family or peers, which take the form of word-of-mouth.

WOM is the most powerful form of Marketing that can be easily used by physicians [36], and that may influence consumer expectations [37], pre-usage attitudes [38] and post-usage perceptions of a service [39]. In Family Medicine, WOM may be useful for consumer decision-making because it is quick, convenient, cost-free and it saves time and effort. Actually, the two categories of sources have complementary functions in Family Medicine, namely, Mass-Media informs and builds awareness, whereas the personal sources raise the potential evaluation process in looking for information. In the pandemic context, many family physicians made use of the WOM in the online environment by keeping up-to-date informative blogs and articles published on Social Media. Hence, the main benefits of implementing promotion strategies in a Family Medicine are the following:

- to improve the competitive advantage;
- to increase visibility;
- to create a positive reputation among patients;

- to monitor the expectations of consumers;
- to build a brand.

eWOM generated by patients is based on their referrals, and these recommendations, in their turn, are the outcomes of physician-patient interactions or experiences [40]. For instance, negative eWOM in health care services is expressed in the shape of online messages, in which are embedded emotions, such as anger [40]. In order to avoid the spread of negative eWOM, family physicians may elaborate or contribute to the design of a patient journey.

Patient journey maps [41] are used to measure patient experience after an interaction with a physician from the patient's perspective. The aim of a patient journey map is to capture the key elements of a journey, consisting of patient persona, medical timeline, medical pathway, relationships between elements and rules of getting from the starting point to the ending point. Going further with an in-depth analysis, the patient persona offers details about the user group and their emotional, physical and technological journey, and each interaction with the physician should be divided into tasks.

CONCLUSION

Health Care Marketing strategies have proved to be useful in Family Medicine. Marketing strategies may focus on the referral or recommendation solutions generated by patients, known as word-of-mouth. The outcome of WOM is to build a positive reputation and to attract more patients for a portfolio of a family physician. In order to avoid negative WOM, family physicians should design a patient journey map.

Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Analysis of COVID-19-related psychiatric disorders – clinical manifestations and therapeutic considerations

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Abstract: *During the COVID-19 pandemic, neuropsychiatric disorders have been diagnosed both in the acute phase, and in the aftermath of this disease. Inpatients, as well as outpatients recently diagnosed with mild, moderate or severe forms of Coronavirus infection have reported neuropsychiatric manifestations with variable duration, symptoms that could be directly or indirectly related to the viral pathogenic agent. Patients who are recovering from the acute phase of COVID-19 also may exhibit various psychopathological manifestations, during the so-called "post-COVID-19 syndrome" or "long COVID-19 syndrome". Explanations regarding the onset of this type of clinical manifestations and the SARS-CoV-2 infection are extremely diverse, ranging from biological factors (e.g., direct central nervous system viral activity, cerebral hypo-oxygenation, high level of inflammatory response) to psycho-social stressors (e.g., isolation, fear of death, anxiety related to possible somatic complications or sequelae). Objectives: The primary objective of this article was to analyze psychiatric manifestations in patients with acute COVID-19 disease and in patients during post-COVID-19 phase. The secondary objective was to propose a conceptual framework for the evaluation and treatment of these patients. Methods: Patients included in this analysis were screened positive for COVID-19 infection in the last 6 months before their first psychiatric examination. These patients were further evaluated to detect any personal history of psychiatric disorders, somatic comorbidities, or significant concomitant pharmacological treatments. Specific scales for the measurement of symptoms severity and functional impairment were administered in all patients. Results: Five patients were included in this analysis, one during the acute phase of COVID-19 infection, and four patients were evaluated after the complete remission of this disease. Hyperactive delirium, mild neurocognitive disorder, major depressive episode, panic disorder with agoraphobia, or acute psychotic disorder were the main diagnoses in these patients. All patients received adequate treatment and they were monitored using psychological scales until symptoms remission or stabilisation. Conclusions: Psychosocial stressors, neurobiological changes, systemic inflammatory reaction, and individual vulnerability factors may contribute to a diathesis-stress model for psychiatric disorders onset within the acute phase or after the remission of acute manifestations in COVID-19-diagnosed patients.*

Keywords: *COVID-19, long COVID-19 syndrome, psychotic disorders, major depression, neurocognitive disorder, delirium, panic disorder*

INTRODUCTION

Although the primary target cells for SARS-CoV-2 are epithelial cells of the respiratory and gastrointestinal tract, it is believed that the penetration of the virus in the human body are far from being limited to these organs [1,2]. The presence of neurological manifestations, such as headache, anosmia/ageusia, dizziness, seizures, and strokes suggests also the invasion of the central nervous system (CNS) during COVID-19 infection [2,3]. Data derived from both patient and experimental models of β-coronaviruses infection support this hypothesis of CNS involvement, due to their evident neurotropism (SARS- CoV-2, MERS-CoV, HCoV-HKU1, HCoV-NL63, HCoV-OC43, HCoV-229E) [2]. This invasion of the CNS is presumed to occur directly, through peripheral nerves or via systemic blood circulation, but aberrant immune responses and respiratory insufficiency associated with COVID-19 are indirect mechanisms that could also explain neurologic manifestations [3]. However, only few studies have reported the presence of SARS-CoV-2 in cerebrospinal fluid or brain parenchyma [3]. It is speculated that a potential trigger of multi-organ autoimmunity in COVID-19

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infection could be the molecular mimicry between SARS-CoV-2 proteins and different human cell antigens, CNS cells included [3,4].

The importance of psychosocial factors in the onset of COVID-19-related psychiatric disorder is significant, starting from social isolation, to negative impact over the financial status or professional performances, lower quality of life, changes in day-to-day functioning etc. [5,6]. All these factors might translate into different psychiatric disorders, including post-traumatic stress disorder, depression, sleep disorders, or isolated neuropsychiatric symptoms [6]. A frequently reported finding is that the pandemic had a significant negative effect over the mental health status in people compared to their average mental health levels during previous years, although this difference is mediated by age, gender, ethnicity and other factors [6]. This observation is supported by an increased prevalence of depression, post-traumatic stress, and sleep disorders in the last years [6,7]. According to a review, symptoms of anxiety and depression (16-28%) and self-reported stress (8%) are common psychological reactions to the COVID-19 pandemic, and they may be associated with disturbed sleep [7]. Both clinical and sub-syndromal mental health problems should be actively investigated, especially in vulnerable populations, as possible reactions to the COVID-19 pandemic. If left untreated, these problems may worsen and lead to long-term complications, with negative, invalidating consequences.

Anxiety and depression spectrum symptoms and disorders in COVID-19 survivors were frequently identified at one month after discharge from hospital in a study (N=402 adults), with rates reaching 28% for post-traumatic stress disorder, 31% for depression, 42% for anxiety, 20% for obsessive-compulsive symptoms, and 40% for insomnia [8]. More than half of the individuals questioned in this study about the existence of psychiatric manifestations or disorders scored in the pathological range in at least one clinical dimension [8]. Patients with psychiatric history showed increased scores on most psychopathological measures, even in the presence of similar baseline level of inflammation [8]. Anxiety and depression scores at follow-up were associated with baseline systemic immune-inflammation index, which evaluates the immune response and systemic inflammation [8].

A study evaluating 841 patients hospitalized with COVID-19 (mean age 66.4 years) showed that 57.4% developed at least one type of neuropsychiatric symptoms, since early stages of infection [9]. Disorders of consciousness occurred commonly (almost 20%), mostly in older patients and in severe advanced COVID-19 stages, but also headache (14%), dizziness (6%), seizures (0.7%) etc. were reported [9]. Anxiety (8%), depression (5.2%), insomnia (13%), and psychosis (1.3%) were also detected during this study [9]. All CNS analyses performed were negative for the viral RNA in patients who presented neurologic manifestations, therefore the inflammatory response with blood-brain-barrier breakdown seems more probable as a pathogenic mechanism [9].

Based on the literature analysis, a comprehensive evaluation of patients diagnosed with COVID-19 should integrate both somatic and psychological aspects, on short- and long-term. A case series to illustrate this necessity is included in the next chapter of this article, with focus on the methodological and therapeutic dimensions.

METHODS

The primary objective of this article was to analyze psychiatric manifestations in patients with acute COVID-19 infection and in patients during post-COVID-19 phase. All these patients were evaluated by multidisciplinary teams, either during hospitalisation or as out-patients, depending on the severity of their clinical manifestations. The secondary objective was to propose a conceptual framework for the evaluation and treatment of these patients.

In order to attain these objectives, patients presented here were screened positive for COVID-19 infection in the last 6 months prior to their initial psychiatric evaluation. All of these patients were further evaluated to detect any personal history of psychiatric disorders, somatic comorbidities, or significant concomitant pharmacological treatments.

CASE SERIES

The first patient was a male, 75 years old, diagnosed positive for SARS-CoV-2 three days prior to the initial psychiatric evaluation. He had no significant history of somatic diseases or psychiatric disorders, and presented an acute onset of spatial and temporal disorientation, together with disturbance in attention and short-term memory, visual and auditory hallucinations, irritability, sleep-wake cycle disturbance, restlessness and agitation, while being admitted to an ICU department for a severe form of COVID-19. Regarding the blood tests, inflammatory markers (procalcitonin, C-reactive protein, fibrinogen) were increased, and CT scan showed bilateral pneumonia. This patient had a slowly favorable evolution of his respiratory infection, and the severity of his confusional state also gradually declined. Treatment with haloperidol 0.5 mg b.i.d. was initiated and maintained for 5 consecutive days, in order to control his psychomotor agitation and hallucinatory-induced behavior. The patient gradually recovered his attentional and mnestic abilities, as well as his sleep-wake cycle at day 5, while hallucinatory manifestations disappeared. Therefore, psychotropic treatment was discontinued starting with day 6. Follow-up visits took place at day 10 after the first psychiatric examination (when the patient was discharged from ICU), and at day 17 (hospital discharge). Clinical Global Impression – Severity (CGI-S) decreased from 6 to 3 and 1, respectively, and Delirium Rating Scale (DRS, 10-item version) [10] total score decreased from 26 to 5, and 2, respectively. Another visit was planned at day 60 after discharge, and the patient was in good physical and mental health, without blood tests abnormalities, chest-X-ray opacities or psychometric cognitive impairment on Mini-Mental State Examination (MMSE).

Discussion

The "cytokine storm" and the neurotropism of coronoviruses were invoked as potential explanations for neuroinflammation and delirium in COVID-19 patients [11]. Prolonged hospitalization, multiple pharmacological concomitant treatments, corticotherapy, higher age, and social isolation may also be contributing factors to the delirium onset [11]. Regardless of causation, either there is a direct effect of coronavirus or a multifactorial ethiology, delirium should be interpreted as a negative prognosis factor in older patients [12]. In an Italian cohort study with 80 COVID-19 inpatients (mean age 74.7+/-14.5 years), 68.8% had delirium with agitation [11]. A larger study (N=516 inpatients, mean age 78 years, SARS-CoV-2 positive) showed that 14.1% of these patients presented delirium on admission, and the most significantly factors associated with delirium were dementia (OR=4.66), the number of chronic diseases (OR=1.20), and chest-X-ray or CT opacity (OR=3.29-3.35 for multiple/bilateral opacities and single opacity vs. no opacity, respectively) [13]. The rate of in-hospital deaths was higher in patients with delirium (58.9% vs. 33.4%), and a multivariable analysis showed an almost twofold increased hazard ratio for delirium-positive patients vs. non-delirium patients for in-hospital mortality [13]. Another study (N=91 participants) supported an even higher difference of in-patient death in cases with delirium vs. non-delirium (72% vs. 31.8%, OR=3.98) [12].

Based on the data in the literature, special attention should be given in elderly developing delirium while being hospitalised for COVID-19. In our presented case, the evolution was favorable, although the patient was admitted in an ICU unit, and he had bilateral pneumonia. According to a longitudinal study, delirium during the hospitalisation in elderly patients with respiratory failure or shock (N=821) was associated with cognitive impairments on long-term in a significant proportion of the participants, and a longer duration of delirium was independently associated with significant worse global and worse executive function cognition at 3 and 12 months [14]. Therefore, follow-up visits are considered necessary in old age patients with delirium during hospitalisation for COVID-19, with screening for possible long-term psychiatric consequences, especially for neurocognitive impairments.

The second patient was a female, 37 years old, diagnosed with acute psychotic disorder, after two months since her discharge from hospital for COVID-19, with an acute onset of delusions of contamination and incurability ("I am still positive for SARS-CoV-2", "No one can really be ever cured from this infection", "I feel like I am condemned to being sick forever"), somatic hallucinations ("My lungs are pressured and they are getting smaller and smaller", "All my body is aching because the virus is spreading in me"), delusional-induced behavior (continuously disinfecting her environment and her body, repeatedly consulting physicians in spite of the fact that all her COVID-19 tests were negative), and lack of insight into her pathology. These symptoms were correlated by the patient with the re-occurrence of several respiratory symptoms (dyspnea, cough) which were attributed by her GP to a non-coronavirus respiratory infection, without pulmonary negative impact, according to the chest X-ray results. Atypical antipsychotics should be preferred in young and adult population, based on their superior tolerability profile [15,16]. Olanzapine 5 mg q.d. was initiated, and the dose was increased up to 15 mg q.d. during the next 7 days. After two weeks, the patient presented an improvement of her delusions and hallucinations, and of her psychosis-induced behavior. Also, a better insight was detected, as she admitted she may need a psychotropic treatment for her condition, and that there could be a possible psychiatric origin of her symptoms. Follow-up visits were performed at 4, 8, and 24 weeks. The clinical evolution reflected in the Positive and Negative Syndrome Scale (PANSS) score was favorable, with gradual decline in psychotic positive symptoms, from 98 (initial visit) to 80, 67, and 56, respectively. CGI-S score evolution reflected this improvement, with decline from 5 (initial visit) to 2 (at week 24). Global Assessment of Functioning (GAF) scores increased from 40 (initial visit) to 60, 75, and 80, respectively.

Discussion

Evidence from previous pandemics, especially with viral respiratory pathogens, support the correlation between schizophrenia-like disorders and the status of infected patient [17]. In a review of 42 cases of psychosis as acute or post-infectious manifestation of COVID-19 could not find evidence for a specific mechanism of psychosis onset in this population, 4 hypotheses being considered: direct neuronal viral infection, post-infectious neuronal autoimmunity, vasculopathies from impaired coagulation, and systemic effects of a pervasive severe pathogen and/or critical illness [17]. In our case, no biological causal relationship could be established between the COVID-19 disease and the acute psychotic disorder, but a psychological relationship is easy to infer from the available data.

There are several similar reports in the literature about new onset of psychotic disorders in the context of COVID-19 pandemic, e.g. a 38-year-old woman, with no psychiatric history, developed psychotic symptoms caused by fear of SARS-CoV-2 contamination [18]. She started to feel that “a demonic force” visit her at night and tries to possess her soul, while presenting insomnia, auditory hallucinations which became imperative, visual hallucinations, and induced aggressive behavior [18]. She did not have positive SARS-CoV-2 tests or IgM/IgG antibodies against this virus, and after being diagnosed with acute psychotic disorder, treatment with quetiapine 500 mg/day and clonazepam 1 mg/day was initiated [18]. Psychotic symptoms, anxiety and insomnia improved rapidly during the first week of hospitalisation and the patient was discharged [18].

Another case series included 6 patients (3 male and 3 female) with first-episode psychosis, admitted in an Italian hospital during the COVID-19 pandemic lockdown [19]. Blood tests, toxicological screening, SARS-CoV-2 antigen tests, and brain imaging were unremarkable, except for a mild cortical atrophy in a 73-year old male [19]. All patients had a negative psychiatric history, despite their advanced age [19]. Rapid discharge from hospital (less than 14 days) and fast remission of symptoms were observed in this case series after relatively low doses of antipsychotic treatment [19]. The follow-up visits confirmed the remission of psychosis in all these cases, suggesting the correlation between intense psychosocial stress induced by COVID-19 lockdown measures and the abrupt onset of acute psychosis [19].

Regarding the presence of psychotic symptoms in recently-diagnosed COVID-19 patients, a case series (N=3) showed a relatively rapid remission of these clinical manifestations under treatment (9 days, 10 days, and also 10 days, respectively) with antipsychotics (ziprasidone 40 mg/day, ziprasidone 20 mg/day, or haloperidol 15 mg/day, respectively) [20]. A direct causal relationship between SARS-CoV-2 infection and the onset of psychosis was not established in these cases [20].

Therefore, onset of acute, brief psychotic disoders is not uncommon in individuals without psychiatric history and non-positive for SARS-CoV-2 infection, possible triggered by prolonged social isolation during lockdown or quarantine. In patients with COVID-19 infection, psychotic episodes were also reported, without assessing a possible direct causality. In our presented case, the patient recovered from COVID-19, and the most likely trigger for her psychosis was an acute and mild, viral respiratory, non-SARS-CoV-2 infection.

The third patient was a female, 24 years old, diagnosed with panic disorder with agoraphobia, who presented the onset of her anxiety disorder 4 months after the discharge for COVID-19 pneumonia. This patient reported feelings of choking, shortness of breath, nausea, palpitations, dizziness, paresthesias, derealization, and intense fear of dying with abrupt onset and limited duration (5-15 minutes): “I cannot find myself, it’s like I’m in a trance for several minutes”, “I feel like I’m choking… I can’t breathe, and I need to sit down because everything is spinning around”, “I feel my heart is beating erratic, and my legs are numb… I feel nauseated and my belly hurts”, “I think that I will certainly die when this happens”. The frequency of these panic attacks was 2-3 times/week and the patient actively avoided going out in open spaces for fear of getting new panic attacks. When asked about the first time she had these symptoms, she said “panic attacks are like the choking sensations I had when I was hospitalised for COVID-19... I actually believed I have this disease again, so I repeatedly tested myself for COVID infection”. Clinical, chest-X-ray and spirometry could not objectify any respiratory dysfunction, and the RT-PCR (reverse transcriptase-polymerase chain reaction) assay from nasal and pharyngeal swabs for SARS-CoV-2 infection was negative. No other comorbid conditions, either psychiatric, or organic, were detected. Initial GAF score was 50 and Hamilton Anxiety Rating Scale (HAMA) score was 32. Treatment with paroxetine 20 mg q.d. was initiated, and it was increased up to 20 mg b.i.d in the next 7 days, and gabapentin 100 mg t.i.d [21] was also added from day 1. After 14 days the patient was re-evaluated, and GAF score increased up to 65, while HAMA score decreased to 24. The follow-up visits at day 30 and 90 showed continuous improvement on both scales, GAF scores were 70 and 80, while HAMA scores reached values of 16, and 10, respectively. A cognitive-behavioral therapy (CBT) was suggested to the patient since the initial evaluation, but she declined.

Discussion

Anxiety symptoms are not rarely reported in patients diagnosed with COVID-19, even if the respiratory or general status was not severely affected [7]. In a case report, a 38-

year-old man developed recurrent palpitation, worries, shortness of breath, chest discomfort, fear of death, feeling of impending doom which lasted for about 10 minutes; these symptoms first appeared within two days after he was tested positive for COVID-19 [22]. Although he had a mild form of COVID-19, his symptoms of panic persisted after his test became negative [22]. The patient had no psychiatric history, and he was started on sertraline 50 mg/day and clonazepam 0.75 mg/day [22]. Regarding the possible mechanisms of panic disorder onset in COVID-19 patients, various hypotheses have been formulated: respiratory symptoms, like coughs and dyspnea, are very frequently associated with the SARS-CoV-2 infection, so fear conditioning to abnormal breathing patterns, and hypervigilence towards breathing abnormalities may increase the risk of developing a panic disorder in COVID-19 survivors; other somatic factors associated with COVID-19 or its treatment may fuel the worries and anxiety related to personal health [23].

A systematic review and meta-analysis focused on anxiety in the general population during COVID-19 pandemic (n=43 studies) found an estimated prevalence of 25% (vs. 7.3% normally), varying across the different tools used to measure anxiety [24]. Initial or peak phase of the COVID-19 outbreak, female gender, younger age, marriage, social isolation, unemployment, student status, financial hardship, low educational level, insufficient knowledge about COVID-19, epidemiological or clinical risk of disease and lifestyle and personality variables were the most common risk factors for anxiety [24].

In our presented case, the patient re-activated her memories related to the COVID-19 four months later, in the absence of any current respiratory infection. The main focus of her attention were respiratory symptoms, associated with fear of death, triggered by the same feeling she had when she found out she was diagnosed with COVID-19. The evolution of anxiety symptoms under treatment was favorable, even if no CBT was initiated, based on the patient's preference.

The fourth patient was a female, 72 years old, diagnosed with major depressive episode, severe, and she had the onset of her mood symptoms after she was discharged from hospital with a diagnosis of "remitted COVID-19 disease". This patient was already diagnosed with recurrent major depressive disorder for 8 years, but she was in remission for more than 2 years prior to the COVID-19 diagnosis. She invoked the COVID-19-related stress as the only major stressor in her recent history, and her family confirmed the absence of any other stresors in her immediate environment. She said that she felt threatened by this infection and she was afraid she might die: "When I heard I was positive, I immediately though I will die, and I thought I have only a few hours to live... I was almost certain I could not survive this". Although she had a moderate form of COVID-19, which did not require ICU admission, she necessitated psychiatric evaluation after her discharge from hospital, due to specific depressive symptoms, i.e. anhedonia, depressed mood, insomnia, loss of appetite for food, ideas of incurability, and recurrent thoughts of death. New generation antidepressants, with superior tolerability profile, should be recommended in this population [25,26]. Sertraline 50 mg q.d. was initiated, and gradually increased up to 50 mg t.i.d in the next 14 days. Zolpidem 5 mg q.d. for insomnia was also recommended in this case, for 2 weeks. Geriatric Depression Scale (GDS-30) initial score was 23, and decreased to 16 after 14 days, and 8 after two months. GAF scores increased from 40 (baseline value) to 65, and 82, respectively.

Discussion

A cross-sectional, observational study included hospitalised, COVID-19 infected patients (N=93) and their immediate family members (N=54), and its objective was to assess depression, anxiety, and stress symptoms in the given population [27]. Almost one-third of this sample showed high scores on depression and anxiety subscales of DASS21 (Depression Anxiety Stress Scale-21), with 30-45% of the family members presenting higher scores than the patients [27]. Another observational study that used Patient Health Questionnaire-9 (PHQ-9) in adults during the COVID-19 pandemic vs. 2015-2019 period in Canada showed a substantially increase of depression prevalence in the first time interval [28].

There is a debate in the literature regarding the specifics of the post-COVID depression, i.e. if there are neurobiological differences between the commonly diagnosed major depression and the one detected in COVID-19 survivors. Analysis of data from USA (N=91791 respondents to a questionnaire) collected during COVID-19 pandemic showed that age, gender, race, education, and income exhibited an interaction with prior COVID-19 in risk for moderate to severe depressive symptoms [29]. Among people with such mood symptoms, motoric symptoms and suicidality were significantly greater among those with prior COVID-19, and the depression risk increased with greater interval following acute infection [29]. Little evidence are available until now to suggest different pathogenetic substrates for the two types of depression.

In patients with severe mental illnesses (affective disorders and schizophrenia), a systematic review (n=36 studies) tried to detect special needs during the COVID-19 pandemic [30]. A general impairment of the mental health in individuals with severe mental illnesses could be detected, especially in patients with mood disorders, as compared to those with schizophrenia [30]. Symptoms of depression, anxiety, and stress were more pronounced in patients with history of severe mental disorders, and resilience, age, and socioeconomic factors impacted significantly their health status [30].

In the here-presented case, the patient was already known with history of major depression, but she was in remission prior to the onset of COVID-19 symptoms. She clearly admitted that her worries were related to perceived threats to her personal safety and fear of dying, and she considered these worries as the origin of her currently diagnosed depressive episode. Based on her psychiatric history, the need for prolonged treatment with antidepressants is obvious, as it is the need to closely monitor suicidal ideation and severity of depression on long-term. In patients with psychiatric history, COVID-19 infection may be a significant stressor that could re-activate mood disoders, or may aggravate their mental health. Therefore, supplying patients with psychiatric history of depression with adequate information about the COVID-19 disease and increasing their stress resilience is important [30].

The fifth patient was 69 years old, and presented neurocognitive deterioration possible related to COVID-19, after 5 months since the remission of the infectious disease. She was admitted in a hospital due to COVID-19 pneumonia and had a severe evolution, which required an ICU admission for 3 days. After 5 months since COVID-19 remission, her family reported the gradual onset of low attention and memory functioning, social isolation, speech difficulties, spatial disorientation and low involvement in activities of daily living. Neuroimagistic evaluation (CT scan) detected a temporal and parietal cerebral atrophy, and leukoaraiosis at 5 months after discharge from hospital. Unfortunately, no comparison of this result is possible, as no prior cerebral CT or MRI imagery was available. During the initial psychiatric exam, no other comorbidities were detected. Baseline MMSE score was 22, and GAF score was 45. Treatment was initiated with donepezil 5 mg q.d., and the dose increased during the next month to 10 mg q.d., in association with pramiracetam 600 mg b.i.d. The follow-up visits at 3 and 6 months detected no change in the MMSE score, and the GAF slowly increased to 50.

Discussion

Multiple mechanisms for the onset of neurocognitive disorders in COVID-19 survivors have been suggested, e.g. hypoxic brain injury secondary to severe pneumonia; centyral nervous system invasion by coronaviruses via blood circulation or neuronal pathway; immune-mediated injury [31]. Under-detection of delirium in patients with COVID-19 may be a silent contributor to the development of neurocognitive disorders, especially if there are cases of hypoactive delirium [31,32]. Pre-existing delirium and small vessel cerebrovascular disease often detected in elderly may result in greater vulnerability to accelerated cognitive deficits in COVID-19 survivors [33]. In an observational study, the cognitive function was evaluated among individuals with persistent cognitive complaints about 5.5 months after COVID-19 infection [34]. Mild cognitive deficits were detected on tests involving attention, processing speed, and executive function, and 70% of these patients were diagnosed with a mood disorder prior to COVID-19 infection [34]. The pattern of cognitive deficits consists in mild impairments, and the contribution of psychological factors related to COVID-19 infection, as well as residual symptoms may increase the severity of subjective cognitive complaints in this population [34].

In the case we presented, no delirium was diagnosed during the COVID-19 infection, but the patient was admitted in ICU. Lack of any previous neuroimagistic evaluation or cognitive assessment makes it difficult to establish the impact of COVID-19 infection over cognitive function in this case. Increased attention should be given to the existence of delirium (either hypoactive or hyperactive) in elderly patients with COVID-19 infection, and especially if they had severe forms of this disease. Cognitive evaluation in elderly with delirium during hospitalisation, or prolonged hospitalisation related to COVID-19 infection should be included in their monitoring plan.

CONCLUSIONS

Patients diagnosed with COVID-19 may have a high rate of neuropsychiatric symptoms or disorders [35,36], due to the direct, biological impact of the SARS-CoV-2 virus, or due to the psychological stress related to this disease (table 1). The direct effects of COVID-19 over the mental health status is difficult to verify in clinical practice [37,38], and data in the literature does not support such a clear, causal relationship. However, evaluation of the COVID-19-diagnosed patients from the perspective of psycho-social stress consequences could help physicians in the detection of vulnerability factors, which may indicate the necessity for early pharmacological and/or psychotherapeutic interventions.

Patients with prior psychiatric diagnoses, prolonged ICU/hospital admissions, multiple stressors (psychological, social, economic, health-related), or personality vulnerabilities (e.g., reduced coping mechanisms, low tolerability to stressors, anxiety features) may require a psychological screening after acute respiratory symptoms remission. When early signs of psychiatric disorders or isolated symptoms are identified, structured psychiatric evaluation, pharmacologic and/or psychological support should be recommended. Mood symptoms, especially depressive, neurocognitive impairment, anxiety symptoms/ disorders, or psychosis have been reported in COVID-19 survivors, even after several months since the remission of respiratory symptoms. In the acute phase of the COVID-19, delirium and psychosis, but also anxiety or mood symptoms should be actively monitored and adequately treated. Because mood disorders are associated with severe complications, like suicide or addictive disorders [39,40], and neurocognitive disorders have a dramatic impact over patients' and caregivers' quality of life [41], early detection and treatment of these conditions are needed, under constant medical monitoring.

Table 1: Synthetic presentation of clinical and therapeutic characteristics in COVID-19-related psychiatric disorders

Case number	Diagnosis	Scales administered	Treatment	Evolution	Observations
1	Delirium, hyperactive	DRS, CGI-S, MMSE	Haloperidol 0.5 mg b.i.d	Favorable, symptoms remited in 5 days	Hyperactive delirium is easy to detect, but hypoactive type should also be searched for in vulnerable populations
2	Acute psychotic disorder	PANSS, CGI-S, GAF	Olanzapine 5 mg q.d., increased to 15 mg q.d. during 7 days	Favorable, symptoms declined significantly after 2 weeks	Psychological stressors related to COVID-19 may be important for the onset of acute psychosis in patients without psychiatric history
3	Panic disorder with agoraphobia	HAMA, GAF	Paroxetine 20 mg q.d., increased up to 20 mg b.i.d in the next 7 days + gabapentin 100 mg t.i.d.	Favorable, improvements on both scales up to 60 days of follow-up	Respiratory distress during COVID-19 may represent a fixation point for anxiety, and it may be subsequently activated
4	Major depressive episode, severe, without psychotic features	GDS-30, GAF	Sertraline 50 mg q.d., gradually increased to 50 mg t.i.d; zolpidem 5 mg q.d. for insomnia was also recommended	Favorable under treatment, up to 60 days of follow-up	Patients with MDD in personal history may be susceptible to new episodes due to COVID-19 distress
5	Mild neurocognitive disorder	MMSE, GAF	Donepezil 5 mg q.d., increased during the next month to 10 mg q.d.; pramiracetam 600 mg b.i.d	Stable, up to 6 months of follow-up	Cognitive function should be monitored in vulnerable populations after COVID-19 infection.

DRS= Delirium Rating Scale, CGI-S= Clinical Global Impression- Severity, MMSE= Mini-Mental State Examination, Positive and Negative Syndrome Scale, HAMA= Hamilton Anxiety Rating Scale, GDS= Geriatric Depression Scale- 30-item version, MDD= major depressive disorder

In conclusion, psychosocial stressors related to the COVID-19 pandemic (e.g., vital threat, isolation, hospitalisation), direct neurobiological influence of SARS-CoV-2 infection, systemic inflammatory reaction, and individual vulnerability factors (e.g., personal psychiatric history, dysfunctional coping mechanisms) may contribute to a diathesis-stress model for psychiatric disorders onset in patients during the acute phase or after the remission of COVID-19 symptoms.

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SYSTEMATIC REVIEW

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Analysis of risk factors for antipsychotic-resistant schizophrenia in young patients – A retrospective analysis

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Abstract: *Treatment resistant schizophrenia (TRS) is a severely disabling disorder, which decreases dramatically the quality of life and overall functionality, while it increases the rate of hospital admissions and overall healthcare costs. The main objective of this research was to evaluate the risk factors for TRS in a group of patients based on a retrospective analysis. The secondary objective was to design an algorithm for initial evaluation in patients with schizophrenia, in order to detect the candidates at risk for developing TRS. Medical charts and consultation records of all patients aged between 18 and 30, diagnosed with TRS, evaluated during 1-year in our department, were selected for analysis. The most significant risk factors for TRS found in univariate model were younger age at schizophrenia onset, male gender, living in rural areas, co-morbid drug dependence, lower therapeutic adherence, and premorbid personality disorder. Marginally significant were higher Positive and Negative Syndrome Scale (PANSS) scores at previous admissions, higher scores on PANSS negative symptoms sub-scale, and lower educational background. In the multivariate model, TRS was still significantly predicted ($p<0.05$) by younger age at the disease onset, addictive co-morbidity, and lower therapeutic adherence. An algorithm based on these risk factors is suggested, based on (a) structured PANSS evaluation using SCI-PANSS and Informant Questionnaire for PANSS, (b) a scale for the detection of co-morbid drug dependence (i.e. Inventory of Drug Taking Situations, IDTS), (c) an interview for detecting premorbid personality disorders (i.e. Structured Clinical Interview for DSM IV – Axis II Disorders, SCID-II), and (d) Treatment Satisfaction Questionnaire for Medication (TSQM) for therapeutic adherence monitoring. Also, the inclusion of several pharmacogenetic parameters (at least CYP450 2D6 panel for detection of poor/ultrarapid metabolizers) could be useful when establishing an adequate therapeutic management, and may help in decreasing the rate of non-response due to variations in antipsychotics plasma levels.*

Keywords: *antipsychotics, treatment-resistant schizophrenia, pharmacogenetics, therapeutic adherence, co-morbidity*

TREATMENT-RESISTANT SCHIZOPHRENIA IN YOUNG PATIENTS – CURRENT STAGE OF RESEARCH

Young age is an important negative prognosis factor in patients diagnosed with schizophrenia, and individuals aged 26 or less at their first psychotic episode have a higher risk to be treatment-resistant [1]. This phenomenon has significant impact over patients and their caregivers functionality, but also involves higher costs for society, and especially for the healthcare system.

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Treatment resistant schizophrenia (TRS) is defined by the lack of significant clinical improvement despite therapy with two different antipsychotics from at least two different chemical classes (at least one is an atypical agent) in the previous 5 years at recommended doses for at least 2-8 weeks each drug [2]. Other definitions are more inclusive, stating that resistance is observed when the patient has inadequate response to at least two antipsychotic drugs at the maximally tolerated dose within the recommended therapeutic range in trials of at least 6 weeks [3].

Pseudo-resistance may be attributed to a large spectrum of factors, i.e. lack of therapeutic adherence, co-morbid psychiatric and/or somatic disorders, pharmacokinetic interactions, and pharmacodynamic negative impact of concomitant medication. Also, from a pragmatic perspective, cases of partial response or lack of response may be related to changes of generic antipsychotics, i.e. in patients who take different products (although with the same non-proprietary names) during successive months, due to economic or administrative issues. This observation is supported by at least a literature review which confirms clinical deterioration, adverse effects, and changes in pharmacokinetics associated with generic substitution of several psychotropics, antipsychotics included, while generics do not always lead to anticipated monetary savings and raise compliance issues [4].

To make things more complicated, certain pharmacological strategies could produce ambiguous results, i.e. combining two or more antipsychotic agents is a practice observed in more than 40% of patients with schizophrenia [5]. It's very difficult in these cases to differentiate the effect of each drug, and to monitor the clinical evolution of such patients.

A re-evaluation of the diagnosis is granted in all cases of non-response, and various psychological factors that could worsen the patient's overall status should be addressed by the case manager (i.e. housing instability or homelessness, lack of integration in the medical health system, familial conflicts). Integration of these factors in the therapeutic management of schizophrenia as possible factors for decreasing the response rate should be considered good practice.

Currently it is assumed that one third of the patients who has schizophrenia don't respond to an adequate treatment [6]. Even if these patients are switched on clozapine, after two failed trials with different antipsychotics, up to 40% of them will experience only partial remission, and full functional recovery is impossible for more than half of these individuals [7].

Various predictors for poor treatment response were reported, i.e. severity of negative symptoms, or low level of premorbid functioning [8]. Treatment-resistant patients present glutamatergic abnormalities, a lack of dopaminergic abnormalities, higher familial loading, and significant decrease in grey matter compared to responsive patients, which raises the question if a distinct type of schizophrenia could be detected from the very beggining [8]. An analysis of adult patients with TRS (N=8624) included in the Danish National Registry showed that higher rates of treatment-resistance were observed in younger individuals, living in rural or provincial areas, with lower education level, hospitalised over 30 days in the year before first schizophrenia diagnosis, who were in-patients at first schizophrenia diagnosis, with paranoid subtype, comorbid personality disorder, and/or previous suicide attempt, who used multiple psychotropic drugs (antipsychotics, antidepressants, or benzodiazepines) [9].

Pharmacogenetic studies detected associations between genes polymorphisms in pharmacokinetic (i.e. cytochrome P450 1A2 and 2D6 isoenzymes) and pharmacodynamic (i.e. D2, D4, and 5HT2A receptors) factors, and response to antipsychotics [10]. These genes are involved in various stages of processing and action of antipsychotics, from the hepatic metabolism to generation of various adverse events. If all these factors could be defined on large-scale trials, they could improve the efficacy and tolerability of antipsychotic treatment, in a future personalised medicine. Until then, several studies and case reports linked functionally 5HT3 receptors gene single-nucleotide polymorphisms (SNP) to the clozapine responsivity, and CYP1A2*1F genetic polymorphism to the clozapine metabolism [11,12]. Both these observations could explain several cases of TRS, but larger trials are needed to confirm these reports.

A predictive model for therapeutic response in schizophrenia, based on logistic regression analyses, was suggested, and it showed that 76% of the patients carrying combination of four SNPs will have a lower response to atypical antipsychotic monotherapy [13].

Another interesting suggestion is classifying schizophrenia in different subtypes, based on biological markers, clinical staging (illness severity, prognosis, and therapeutic options), and treatment response [14]. Antipsychotic responsive schizophrenia, clozapine responsive schizophrenia, and clozapine resistant schizophrenia are the subtypes suggested by this model [14]. This classification may have important prognostic value and could help clinicians in formulating an adequate therapeutic management. Unfortunately, no good quality data exist for the next step in the treatment of clozapine-resistant patients, although co-prescribing a second atypical antipsychotic, a selective serotonin reuptake inhibitor, lithium or divalproex, glycine or D-cycloserine, and memantine has been investigated with low to moderate success [15].

Table 1. Demographic and disorder-related variables at the evaluation visit

Variables	Values	Observations
Current age	26.1 (range 18-30)	Only young patients were included in the analysis
Age at onset of the disorder	22.9 (range 18-29)	
Gender		
Female	8	53.3%
Male	7	46.7%
Environment		
Rural	7	46.7%
Urban	8	53.3%
Marital status		
Single (including unmarried and divorced)	11	73.3%
Married	4	26.7%
Social status/income		
High	1	6.7%
Medium	4	26.7%
Low	10	66.6%
Independent living	14	This proportion could be due to the addressability of our department
Institutionalized	1	
Number of previous psychotic episodes	0.6 (range 0-3)	Most of the patients were at their second psychotic episode
Substance related disorders present (nicotine dependence included)	11	9%
Personality disorders present	8	53.3%
PANSS overall score	91.8 (range 86-122)	
PANSS-negative Scale	30.5 (range 15-40)	
PANSS-positive Scale	32.6 (range 20-42)	
Length of the current episode (weeks)	12.5 (range 6-30)	

OBJECTIVE AND METHODS

The main objective of this research was to evaluate the risk factors for TRS in a group of patients based on a retrospective analysis.

The secondary objective was to design an algorithm for initial evaluation in patients with schizophrenia, in order to detect the candidates at risk for developing TRS.

Medical charts and consultation records of all patients aged between 18 and 30, diagnosed with TRS, evaluated during 1-year in our department, were selected for analysis. A number of 15 TRS cases were detected from a total of 68 patients diagnosed with schizophrenia. The diagnosis of TRS was based on DSM IV TR criteria and the World Federation of Societies of Biological Psychiatry Guidelines for Schizophrenia criteria [2].

A logistic regression analysis using SPSS 22.0 was performed on 25 demographic, clinical, and therapeutic variables, in a univariate, followed by a multivariate model. No pharmacogenetic variables could be identified in the selected medical documents.

RESULTS

The most significant risk factors for TRS in univariate model were younger onset age ($p<0.01$), male gender ($p<0.01$), living in rural areas ($p<0.05$), co-morbid drug dependence ($p<0.05$), lower therapeutic adherence ($p<0.05$), and premorbid personality disorder ($p<0.05$).

Marginally significant in the same univariate model were higher Positive and Negative Syndrome Scale (PANSS) score at previous admissions ($p=0.056$), higher score on PANSS negative symptoms scale ($p=0.058$), and lower educational background ($p=0.061$).

In the multivariate model, TRS was still significantly predicted ($p<0.05$) by younger age at onset ($p=0.022$), addictive co-morbidity ($p=0.042$), and lower therapeutic adherence ($p=0.048$).

Table 2. Risk factors for TRS in young adults

Factors	Values	
	Spearman's ρ	**Linear regression p**
Younger age at the current episode onset	0.467	<0.01
Gender – male	0.592	<0.01
Substance-related disorder comorbidity	0.320	<0.05
Living in rural areas	0.367	<0.05
Lower therapeutic adherence	0.322	<0.05
Premorbid personality disorder	0.298	<0.05
PANSS overall score at previous admission	0.155	0.056
PANSS-Negative Scale current score	0.152	0.058
Lower educational background	0.142	0.061

An algorithm based on these risk factors is suggested, based on:

(a) Structured PANSS evaluation based on SCI-PANSS and Informant Questionnaire for PANSS, because the previous PANSS overall scores and current Negative Scale score are correlated with the patients risk for developing TRS;

(b) A scale for the detection of co-morbid drug dependence (i.e. Inventory of Drug Taking Situations, IDTS), because current drug-related disorders should be integrated in the therapeutic management, and it has prognostic value;

(c) An interview for detecting premorbid personality disorders (i.e. Structured Clinical Interview for DSM IV – Axis II Disorders, SCID-II), because of the correlation between the risk for TRS and several premorbid disorders (especially cluster A disorders);

(d) Treatment Satisfaction Questionnaire for Medication (TSQM) for the monitorisation of therapeutic adherence, because a low therapeutic adherence is a major risk factor for developing treatment-resistance

Also, the inclusion of several pharmacogenetic parameters (at least CYP450 2D6 panel for detection of poor/ultrafast metabolizers) could be useful when establishing an adequate therapeutic management, and may help in decreasing the rate of non-response due to variations in antipsychotics plasma levels. This could have important pharmacoeconomic impact, since many antipsychotics undergo hepatic metabolism, not to mention the risk of potential pharmacokinetic interactions between antipsychotics, or between antipsychotics and other co-administered drugs.

CONCLUSIONS

Analysis of risk factors for TRS should be included in an evaluation algorithm for all patients diagnosed with schizophrenia. While some risk factors could not be influenced therapeutically, still others can be addressed, with targeted intervention for co-morbid addictive disorders, personality disorders, negative symptoms, or with techniques for increasing therapeutic adherence.

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VARIA

Anatomical study of the anterolateral ligament in Romanian population

Radu Paraschiv[1], George Dinache[2,3], Mark E. Pogarasteanu[2,3], Sorin Lazarescu[1]

Abstract: *The purpose of this paper was to evaluate the existence of the anterolateral ligament of the knee in the Romanian population. Multiple studies have investigated the anterolateral structures of the knee, but there is some inconsistency regarding the existence of the anterolateral ligament.*

Materials and methods: A cadaver dissection study was performed on 10 knees (5 left and 5 right), 3 males and 2 females

Results: The anterolateral ligament was observed in all 10 knees. The mean length was 32±6mm, the width was 5.1±2 mm, and the thickness at the articular line was 1.1±5 mm.

Conclusions: A 100% presence was found, with a slight difference from the length, width, and thickness in other countries, race not being a decisional factor in differences.

Keywords: *anterolateral ligament, anatomy, lateral collateral ligament, dissection*

INTRODUCTION

In the year 1879, the French surgeon Paul Segond [1] described the existence of a fibrous band laying on the anterolateral facet of the knee. Until this moment in the specialty literature, we find ambiguous naming of this band, like lateral capsular ligament [2], the osseous-capsular layer of the iliotibial band [3, 4], or anterolateral ligament [5]. In this study made on 10 knees from the cadavers from The "Carol Davila" University of Medicine and Pharmacy Bucharest, there had been studied the femoral origin and tibial insertion, the trajectory and anatomical rapport with neighboring structures. So the anterolateral ligament was found like being a distinct ligamentous structure on the lateral facet of the knee, anteriorly its origin is on the lateral femoral epicondyle anterior of the collateral ligament and insertion on the anterolateral facet of the tibia at the half distance between the tibial tubercle and Gerdy's tubercle, being distinct of the iliotibial tract, with an oblique trajectory from its origin towards the insertion.

The knee joint is the largest joint in the body, and the following structures that are contributing to its stability are anterior and posterior cruciate ligament, medial and lateral collateral ligaments, quadriceps tendon, patellar tendon, popliteus tendon, and the anterolateral ligament [6].

MATERIALS AND METHODS

In this study were used 5 cadavers (10 knees), 3 males and 2 females. The mean height was 170 cm, the mean weight was

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62 kg, and the mean age was 75.1. For the dissection, three incisions were made: two circumferential in the medial 1/3 of the thigh and the medial 1/3 of the calf and 1 sagittal incision on the anterior aspect of the knee. The skin and subcutaneous tissue were removed using these incisions. The lateral vastus and iliotibial tract were detached. Once the lateral collateral ligament was shown, the tibia was internally rotated and the knee flexed to highlight the anterolateral ligament. Once shown, the knee was extended, and the origin and insertion of the ALL were marked, its length, width, and thickness were measured.

Figure 1: Superficial layer of the knee

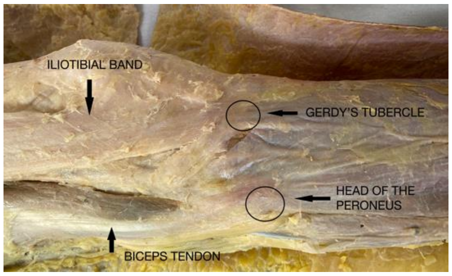
ILIOTIBIAL BAND
GERDY'S TUBERCLE
HEAD OF THE PERONEUS
BICEPS TENDON

Figure 2: Dissection of the superficial layer

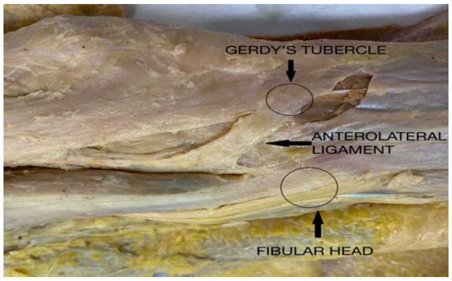
GERDY'S TUBERCLE
ANTEROLATERAL LIGAMENT
FIBULAR HEAD

RESULTS

The anterolateral ligament was observed on the anterolateral aspect of the intraarticular capsule of the knee after the detachment of the iliotibial tract. The ALL appeared as a white thickening that could be seen without a microscope and can be palpated. ALL was shown in all the 10 knees (5 right knees and 5 left knees).

Table 1: Prevalence of anterolateral ligament in the Romanian population

Total	Right	Left	Female	Male
100 % (5/5)	100 % (5/5)	100 % (5/5)	100 % (2/2)	100 % (3/3)

The origin of the ALL is at the lateral femoral epicondyle, posteriorly from the popliteus muscle-tendon, and has an oblique trajectory towards its insertion on the tibial plateau, and the half distance between de fibula head and Gerdy Tubercle. Fibers from the ALL insert on the lateral meniscus. ALL insertion is at 16±4mm anterior of the fibula head. Once the knee is extended the length was 32±6 mm, the width was 5.1±2 mm, and the thickness at the articular line was 1.1±5 mm.

Figure 3: Dissection of the deep layer

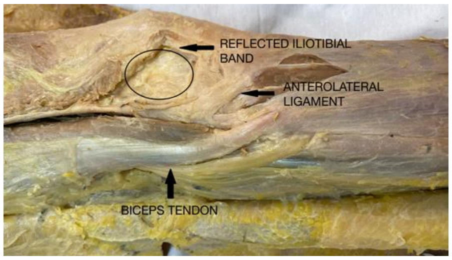
REFLECTED ILIOTIBIAL BAND
ANTEROLATERAL LIGAMENT
BICEPS TENDON

Figure 4: Expose anterolateral ligament

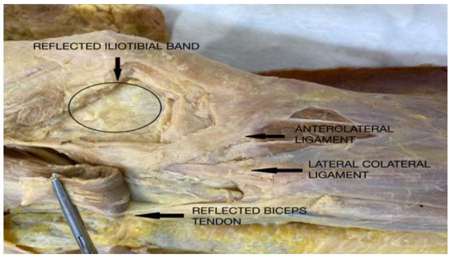
REFLECTED ILIOTIBIAL BAND
ANTEROLATERAL LIGAMENT
LATERAL COLATERAL LIGAMENT
REFLECTED BICEPS TENDON

Table 2: Prevalence of anterolateral ligament in the Romanian population

	Female	Male	Total	P-value
Length (knee extended)	33 ± 5.9 mm	31.6 ± 5.8 mm	32 ± 6mm	0.56
Width (articular line)	5.2 ± 2.2 mm	5 ± 1.8 mm	5.1 ± 2mm	0.78
Thickness (articular line)	1.2 ± 5.3 mm	1 ± 4.8 mm	1.1 ± 0.5 mm	0.16

There were multiple researches conducted regarding the presence and anatomical features of the anterolateral ligament in various populations. We choose a few studies that we considered more relevant for our ethnicity and we compared them with our study. Our results were similar with some of them (Kennedy et al (2015), Vincent et al (2012)), but others described the presence of the ALL in a minority of cases – Potu et al (2016), Roessler et al (2016)) – as it is seen in Table 3.

Table 3: Anterolateral ligament prevalence in the world

Autor (year)	Subject (nr)	Population (mean age)	Prevalence (%)	Lenght (mm)	Width (mm)	Thickness (mm)
This study	Cadaver (5)	Romania (75.1)	100	32 ± 6	5.1 ± 2	1.1 ± 0.5
Dodds et al (2014)	Cadaver (40)	UK (75)	83	59 ± 4	6 ± 1	
Kennedy et al (2015)	Cadaver (15)	USA (58.2)	100	36.8		
Vincent et al (2012)	Cadaver (10)	France (85.3)	100	34.1 ± 3.4	8.2 ± 1.5	2-3
Runer et al (2016)	Cadaver (44)	Austria (78.1)	45.5	42.2 ± 6.2	5.6 ± 1.3	1.2 ± 0.3
Claes et al (2013)	Cadaver (41)	Belgium (79)	97	38.5 ± 6.1	6.7 ± 3.0	1.6 ± 0.6
Helito et al (2013)	Cadaver (20)	Brazil (61.5)	100	37.3 ± 4.0	7.4 ± 1.7	2.7 ± 0.6
Caterine et al (2015)	Cadaver (19)	Canada (70)	100	40.3 ± 6.2	5.1 ± 1.8	1.4 ± 0.6
Potu et al (2016)	Cadaver (24)	Caucasian	4.16	34.23	4.04	1.78
Roessler et al (2016)	Cadaver (20)	Germany (79.4)	60	39.63 ± 0.78	5.28 ± 0.33	1.52 ± 0.31

DISCUSSION

Although the number of cadavers selected in this study was low (five cadavers), one of the most important observations was that the anterolateral ligament was highlighted on all 10 knees. In the specialty literature until now there was shown inconsistency in discovering the ALL [7-9]. After Claes et al. [5] conducted the study, the anterolateral ligament began to get high importance and from this more studies had been made, but had uncertain results. While some authors redact that they have a 100% prevalence rate for ALL [10-13], others describe its presence in a minority of cases [7-9]. Another important detail is that the anterolateral ligament is a capsular-ligamentous structure in the lateral aspect of the knee, and although many studies were made, some of them discovered the absence of this structure [14-16]. Seebascher described that the lateral facet of the knee has three layers [17], and Getwood said that the ALL is situated in the Seebacher layer [18]. Through the dissection of the cadavers, I had concluded to agree with Getwood and that the anterolateral ligament is situated proximal attached to the lateral epicondyle and distally to the lateral plateau, having fibers that unite with the lateral meniscus. Regarding other studies, in our dissection, we found the presence of the anterolateral ligament in all the 10 knees (5 right and 5 left knees). The study also demonstrated a shorter, thinner, and narrow ligament comparative with others. The mean length was 32±6 mm, 33±5.9 mm in females, 31.6±5.8 mm in males. The mean width was 5.1±2 mm, 5.2±2.2 mm in females, 5±1.8 mm in males. The mean thickness was 1.1±5 mm, 1.2±5.3 mm in females, 1±5.3 mm in males. It couldn't be demonstrated a significant difference between sexes.

CONCLUSIONS

The study was conducted to confirm the presence of the ALL in the anterolateral region of the knee and to analyze its anatomical characteristics using Romanian cadavers. A 100% presence was found, with a slight difference from the length, width, and thickness in other countries, race not being a decisional factor in differences. Therefore, the capsular thickening thas has been controversial with its naming can be called the anterolateral ligament.

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ORIGINAL ARTICLE

Anatomic-surgical study on the interparietoperitoneal space from a laparo-endoscopic perspective

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Abstract: *The interparietoperitoneal (IPP) space, by a broad definition, is the space between the musculo-fascial walls of the abdomen and the parietal peritoneum. A review of the literature on this subject has been performed through a search in the databases according to the following keywords: Bogros space, Retzius space, preperitoneal approach, and urogenital fascia. We have also analyzed the video recordings of the dissections of the inguinopreperitoneal region during TAPP, conducted by a single team, evaluating the dissection planes in the two compartments (medial and lateral) of the IPPS. Based on the latest data from the literature, as well as on our own experience in laparo-endoscopic herniation surgery, we aim to provide answers to several questions. IPPS from the inguinal region is an area as complex as it is narrow, but of great current surgical interest (approaches of hernias, vessels, prostate). The fascial distribution has its origin in the embryological development of the urogenital apparatus. The basic fascial structure in understanding IPPS compartmentalization is the UGF, along with its extensions.*

Keywords: *interparietoperitoneal space, Bogros space, Retzius space, preperitoneal approach, urogenital fascia*

INTRODUCTION

The interparietoperitoneal (IPP) space, by a broad definition, is the space between the musculo-fascial walls of the abdomen and the parietal peritoneum.

In classic anatomy descriptions, it is depicted as a virtual space, filled with adipose/fibroareolar tissue [1]. It is Bogros' merit to have broadened the perspective on a portion of the IPPS in 1823, one of only 13.5/15.5 mm, through which continuity between the anterior and posterior IPP space is obtained [2].

Bogros' initial description was in relation to the inferior epigastric artery and went along the lines of: “a loose layer of cellular tissue separating the inferior epigastric artery from the transversalis fascia anteriorly. Posterior, a thicker layer of the same tissue separates it from the peritoneum” [3].

We could therefore call this space located immediately below the FT (Fascia transversalis), where Bogros recommends the approach of the epigastric artery, as "original sp. of Bogros". The term Bogros space was given to it by Poirier as late as 1912 [4]. In 1858 the Swiss Anders Adolph Retzius described the so-called prevesical space as a compartment of the extraperitoneal space (a division of the at-large extraperitoneal space), as it turns out, without the knowledge of Bogros' work [5]. Since then, the two compartments of the IPPS have long been debated in anatomical literature.

A “historical review” of the authors who endeavored with the anatomical clarification of the IPPS, in terms of

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delimitations, content, and surgical applications, was made by R.C. Read [6]. The laparo-endoscopic approach of interparietoperitoneal space has brought many clarifications concerning regional anatomy, through in vivo exploration, obtained by magnification using specific optical instrumentation.

Based on the latest data from the literature, as well as on our own experience in laparo-endoscopic herniation surgery, we aim to provide answers to several questions:

- Which is the structure described by Bogros as a "loose layer of cellular tissue" in the first part of the quote?

- Which is structure described as a “thicker layer”?

- Is there an anteroposterior division of the Bogros space?

- Is the Retzius space a continuation within the same plane of the Bogros space?

- Which are the landmarks in terms of the fasciae for optimal dissection during the transabdominal preperitoneal laparoscopic (TAPP)/totally extraperitoneal laparoscopic (TEP) approach?

MATERIALS AND METHODS

A review of the literature on this subject has been performed through a search in the databases according to the following keywords: Bogros space, Retzius space, preperitoneal approach, urogenital fascia.

We have also analyzed the video recordings of the dissections of the inguinopreperitoneal region during TAPP, conducted by a single team, evaluating the dissection planes in the two compartments (medial and lateral) of the IPPS.

RESULTS

Ever since 1965, R Stoppa has approached the inguinal pro-peritoneal space by median sub umbilical incision, to carry out the prosthetic procedure called giant prosthetic reinforcement of the visceral sac (GPRVS). Dissecting the sperm cord elements in the IPPS, he described the internal spermatic fascia as an extension of the urogenital fascia [7].

This latter structure is the key to understanding the layout of the anatomical elements within Bogros and Retzius spaces. Embryology data show that the two layers of the UGF are a direct continuation of the renal fasciae, the anterior one (Gerota) and the posterior one (Zukerkandl), in-between themselves containing the urogenital elements developed from the mesonephros (kidneys, adrenal glands, gonads, ureters, ductus deferens), as well as the vessels and nerves that serve them, being compared to a border of these organs [8]. In the 5-week-old embryo, the mesonephros is attached to the posterior abdominal wall through a "broad urogenital mesentery" [9]. One must mention here that the urinary bladder and the urachus have another embryonic origin, which is the cloaca and allantois respectively, so they are located behind the UGF (Figure 1).

Figure 1: IPPS from the “new perspective” of TAPP

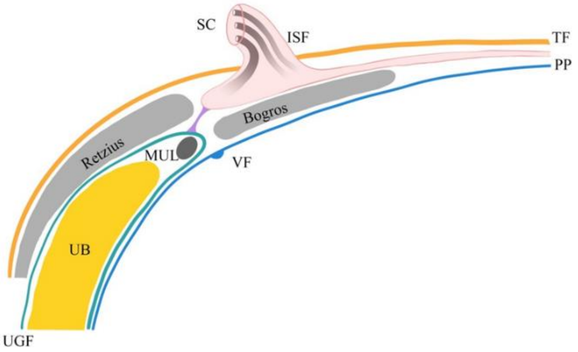

UGF – Urogenital fascia; MUL – Medial umbilical ligament; UB –Urinary bladder; SC –Spermatic cord; ISF – Internal spermatic fascia; TF – Fascia transversalis; PP – Parietal peritoneum; VF – Vesicular fold

Therefore, the presence of the UGF in the IPPS sub-divides the two compartments – medial and lateral into another two – superficial and deep, as shown in the following diagram (Table 1):

1 – the superficial medial compartment is the actual Retzius space, previously delimited by the FT (superior)/pubic (inferior) and posterior to it by the UGF.

2 – the deep medial compartment is limited by the UGF anterior to it and peritoneum posterior to it, containing the urinary bladder/urachus and the medial umbilical ligament (MUL), and also known as the perivesical space.

3 – the superficial lateral compartment between the FT (bilaminated) in the anterior plane and the UGF contains the initial portion of VEI, femoral vessels, and genito-femoral nerves. It is the compartment in which Bogros suggested the approach of the inferior epigastric artery be performed.

4 – the deep lateral compartment, delimited by the UGF in the anterior plane and the parietal peritoneum in the posterior plane, i.e., "the new Bogros space".

Table 1: IPPS – Interparietoperitoneal Space Compartments

	Medial	Lateral
Superficial	Retzius prevesical space	Old Bogros space
Deep	Prevesical space	New Bogros space

From this perspective, the structure described by Bogros as “a loose layer separates the inferior epigastric artery from the transversalis fascia" is now understood to be the FT's posterior layer, as described by Cooper. The structure described as "a thicker layer" is identified today as the UGF, having as well a bilaminated structure [10].

Anatomical descriptions of the IPP are sometimes confusing because the posterior layer of FT is often described as fused with or identical to the anterior layer of the UGF [11].

The two extensions of the UGF – the spermatic fascia and the umbilical-prevesical fascia have a connection/continuity area at the level of the umbilical artery origin point in the internal iliac artery located in front of the sacroiliac joint. This could correspond to the peritoneal fold visible during TAPP exploration and is described as a "transverse vesicular fold" [12].

From the laparo-endoscopic perspective of inguinal hernia, the Retzius space was located anterior to the UGF, and the new Bogros space was posterior to it (Figure 2).

Figure 2: IPPS Diagram

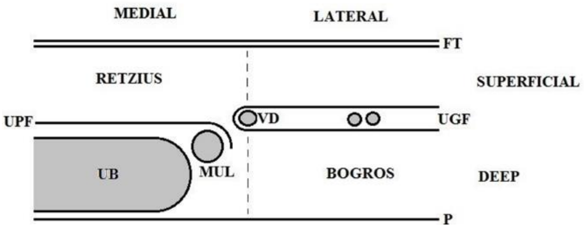

In the TAPP approach of indirect inguinal hernia, medial dissection is therefore conducted anterior to the UGF, the mark being the MUL located posterior to it. In TEP, one arrives from the retromuscular space in the avascular Retzius space penetrating the posterior rectus sheath [13].

Wandering medial to the posterior space of the UGF leads to bleeding from perivesical venous plexus or even to opening the bladder!

Regarding the creation of the new Bogros space during the laparo-endoscopic approach, our own experience has shown that the most suitable place to initiate the lateral compartment dissection is lateral to the deep inguinal opening, advancing towards the iliac spine in strict contact with the parietal peritoneum. If the dissection is started from the iliac spine, it most often determines the sectioning of the entire peritoneo-fascial cover, revealing the muscular fibers of the transversus abdominis and a variable degree of bleeding from the muscular branches. As one descends towards the ilio-pubic tract, the risk of bleeding from "the Bendavid venous circle" is added [14]. Therefore, lateral compartment dissection should aim strictly for the space between the peritoneum and UGF.

There are authors [15] with descriptions of the dissection plane in the two compartments (medial and lateral) similar to ours, but who use the term "preperitoneal tissue" or "areolar tissue" to denote what we consider to be the UGF or its prolongations.

In a sagittal section, the two spaces, Retzius and Bogros, are located anterior and posterior to the UGF, hence the confusion of some authors who speak, in general, of IPPS and properitoneal space without specifying the differences between the two compartments, the medial and the lateral [16].

Laparoscopic anatomy data of the IPP are confirmed imagistically in various pathological entities involving this region [17-22]. Osteoporosis of different etiology, especially in obese people has multiple anatomical implications for this region through fractures and defective post-healing remodeling, so the surgical approach to this region must be correlated with the condition of the patient [23]. An interesting area of future research is the possibility that the miscarriages occurring in the context of a uterine septum may be explained by the poor vascularization of fibromuscular tissue composing the septum and the approach of the IPPS from the inguinal region can treat the problem with less invasive surgical procedures [24]. Therefore, the prosthesis placed via TAPP or TEP in the Retzius and Bogros spaces is located anterior and posterior to the UGF [25].

DISCUSSION

- The IPPS from the inguinal region is an area as complex as it is narrow, but of great current surgical interest (approaches of hernias, vessels, prostate).
- The fascial distribution has its origin in the embryological development of the urogenital apparatus.
- The basic fascial structure in understanding IPPS compartmentalization is the UGF, along with its extensions.
- Starting from the requirement of reuniting the anatomical terms in this matter we suggest the following equivalents:

- preperitoneal/properitoneal space = interparietoperitoneal space (IPPS)
- old Retzius space= is the space between the UB and FT/pubic branch
- new Retzius space = the space between the UGF and FT/ pubic branch
- old Bogros space = the space between FT and the UGF
- new Bogros space = the space between the UGF and the parietal peritoneum
- UGF = preperitoneal fascia = retroperitoneal fascia.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors' contributions
DA drafted the manuscript and critically revised the final form, AB researched the literature, RDSB researched the literature and revised the initial form, OA is the corresponding author, AMO drafted the manuscript, AP critically revised the data from the literature, MCD researched the literature, DNP approved the final form. All authors have contributed equally to writing, reading, and approving the final manuscript.

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that they have no competing interests

Abbreviations
FT: Transversalis Fascia; UGF: Urogenital fascia; P: Peritoneum; UPF: umbilical prevesical fascia; MUL: Medial umbilical ligament; VD: Vas Deferens; UB: Urinary bladder

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ORIGINAL ARTICLES

An organizational chart of an Emergency Health Operation Center

Athanasios Zafeirakis[1], Panagiotis Efstathiou[2]

Abstract: *Nowadays, a wide range of natural and man-made disasters, disease outbreaks and pandemics give rise to health emergencies at a global level. Thus, a health security strategy is urgently required through central planning. At the national level, that vision can be implemented with a properly organized and fully functional Emergency Health Operation Center (EHOC). The main executive sections of an EHOC are Command, Planning, Operations, Logistics, Administration, Communications/Intelligence, and Reporting/Briefing. Each of the above sections has several functions and sub-functions, all operating according to a concrete legal basis and detailed operating procedures.*

Keywords: *emergency planning, public health, operation center, health crisis*

INTRODUCTION

In our epoch, due to the extreme complexity of the modern age, natural and man-made threats the management of health crises should imply an effective and integrated action plan, qualified with characteristics that define the effectiveness and success of such a system. An EHOC is such a system that can lead and coordinate the overall national medical response in every emergency incident, especially when involving mass casualties that either exceeds the management capabilities of the nation's health mechanism or disorganize the existing procedures planned for routine conditions. A successful EHOC should be based on two pillars: Legal authority and organizational structure; both will be herein briefly discussed.

DISCUSSION

Legal authority

The legal authority of an EHOC refers to the development of a concrete legislation directive for the public health authorities as regards roles, responsibilities, and coordination with national disaster management resources for legal health operations. That legislation should establish policy guidance, officials of the government and involved organizations, and other executive officers and professionals tasked to provide leadership at a strategic level. The legal authority of an EHOC varies among countries, while the key governmental agencies usually involved are unfortunately often scattered in various Ministries. The long list of those national agencies includes, but is not limited to the following: Fire Brigade; Police; Ministry of National Defence; Coast Guard; Ministry of Transport and Communications; Civil Airlines; Medical School Laboratories; National Public Health Organization; National Center for Emergency Assistance (NCEA); Hospital units (public and private); National Atomic Energy Committee; Forensic Services; Ministry of Foreign Affairs; Non-governmental healthcare and humanitarian responders [1]. The concomitant spread of leadership centers combined with defective multi-ministerial coordination has been proved devastatingly ineffective in the last decades in many environmental or man-made disasters throughout the world. Hence, an EHOC

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must be set under a unified command, a sort of an all-hazard ministry or deputy ministry "of Interior Threats", or "of Civil Protection"; that Ministry can substitute roles, authorities, personnel, and infrastructure of other Ministries and also replace at the highest level both the GSCP and all sorts of emergency committees, mechanisms and operation centers of other Ministries.

Organizational structure

An EHOC should operate within the framework of a thoroughly planned national incident management system, with dedicated personnel and a consolidated operational terminology. Organizational prerequisites for such a system are described in detail in relative handbooks [2-4] and involve at minimum the following executive sections:

1. Command
2. Planning
3. Operations
4. Logistics
5. Administration
6. Communications/Intelligence
7. Reporting/Briefing

The function of an EHOC should be based on competent personnel and equipment installed in a capacious building, consisting of at least an operation-command room, a communication room, and a multipurpose meeting room. The operational doctrine of an EHOC is based on the C4-I principle, i.e. Command – Control – Communication – Coordination – Intelligence. These procedures are prioritized for the planning and response to potential public health risks, albeit they do not replace the daily operational control of resources and services. The goal of C4-I is operational cross-disciplinary coordination at the regional, national and international level and is an integral part of the Concept of Health Operations [5]. Each of the above sections has several functions and sub-functions, (data illustrated in Figure 1), operating according to relevant response actions, called Standard Operating Procedures (SOPs), as described below:

1. Command Section: The EHOC director exerts ex officio the vertical, unified command. He/she is a senior medical officer, with documented expertise in the field of Health Crises and with vast experience in managing complex health operations. The EHOC director will be subordinated to the GSCP and will be supported at his command by a deputy director. Two branches are straight subordinated to the EHOC director:

a) A *Legal Operations Unit* (LOU), staffed by dedicated legal advisers, supports the EHOC leader in taking complicated decisions without unacceptably high legal risks (mainly in emergencies, when some regulations of normal life might need to be temporarily revised or suspended for EHOC operations to be carried out).

b) The *Ancillary Services Unit* (ASU), a supportive bundle of services. Apart from full secretarial support, that unit covers personnel's health, safety and security, cleaning, and janitorial affairs of buildings.

Apart from managing, coordinating, and collaborating skills, the EHOC director must be able to set and follow priorities and optimize results based on available resources. A thorough presentation of the required qualifications of an EHOC director is described by Efstathiou et al [6]. The EHOC director is responsible for the following specific functions:

- The overall operational function and legal responsibility of the EHOC.

- The application of effective command systems, which clearly define roles and responsibilities for all EHOC staff.

- The final responsibility for verification of any possible health event, mobilization of the national mechanism, and information of government. That highly demanding decision is accomplished through the systematic gathering of relevant information from various sources, such as operational contacts, expert networks, printed reports, and media information.

- The coordination (called "interoperability") among EHOC's sections and also between EHOC as a concrete system and any participant organization, including local and private agencies, when a major health incident/operation takes place. As major participants, apart from the national agencies are considered the following international stakeholders:

- Center for Disease Control and Prevention (CDC), with worldwide action; a European-CDC, as well as national CDCs also exist (Planning Section, bellow).
- Monitoring and Information Center (MIC): The operational heart of the United Nations Health Security Committee; mobilizes international aid for mass disaster incidents.
- World Health Organization (WHO): Provides the ultimate health assistance worldwide.
- North Atlantic Treaty Organization (NATO): An intergovernmental military alliance, also providing some specific health standardization agreements (STANAGs).

2. Planning Section: It emphasizes coordination, interoperability, and cooperative team working through relevant SOPs, with the following branches and sub-functions:

Figure 1: Organizational structure of an EHOC

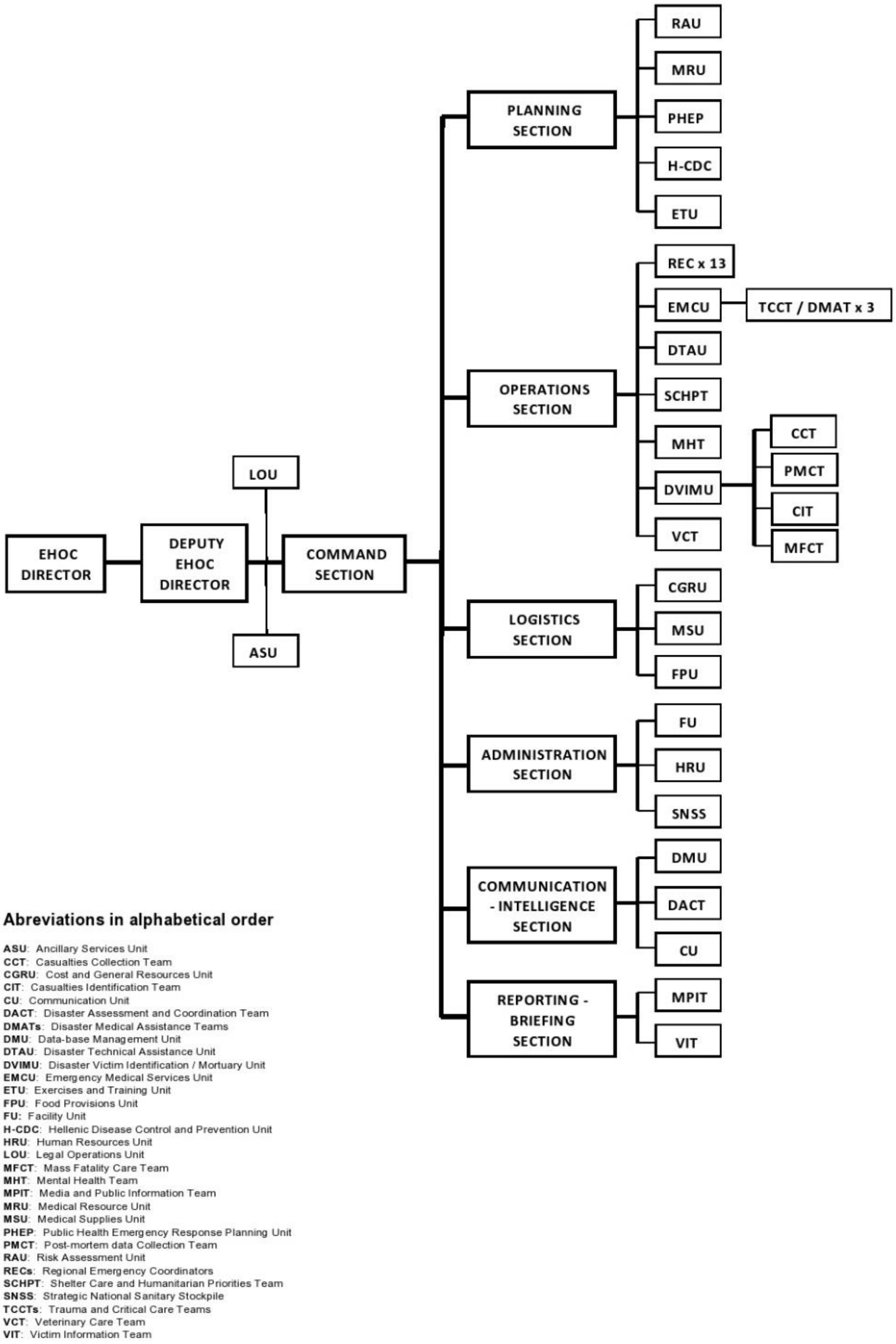
EHOC DIRECTOR
DEPUTY EHOC DIRECTOR
LOU
ASU
COMMAND SECTION
PLANNING SECTION
RAU
MRU
PHEP
H-CDC
ETU
OPERATIONS SECTION
REC x 13
EMCU
TCCT / DMAT x 3
DTAU
SCHPT
MHT
DVIMU
CCT
PMCT
CIT
MFCT
VCT
LOGISTICS SECTION
CGRU
MSU
FPU
ADMINISTRATION SECTION
FU
HRU
SNSS
COMMUNICATION - INTELLIGENCE SECTION
DMU
DACT
CU
REPORTING - BRIEFING SECTION
MPIT
VIT

Abreviations in alphabetical order

ASU: Ancillary Services Unit
CCT: Casualties Collection Team
CGRU: Cost and General Resources Unit
CIT: Casualties Identification Team
CU: Communication Unit
DACT: Disaster Assessment and Coordination Team
DMATs: Disaster Medical Assistance Teams
DMU: Data-base Management Unit
DTAU: Disaster Technical Assistance Unit
DVIMU: Disaster Victim Identification / Mortuary Unit
EMCU: Emergency Medical Services Unit
ETU: Exercises and Training Unit
FPU: Food Provisions Unit
FU: Facility Unit
H-CDC: Hellenic Disease Control and Prevention Unit
HRU: Human Resources Unit
LOU: Legal Operations Unit
MFCT: Mass Fatality Care Team
MHT: Mental Health Team
MPIT: Media and Public Information Team
MRU: Medical Resource Unit
MSU: Medical Supplies Unit
PHEP: Public Health Emergency Response Planning Unit
PMCT: Post-mortem data Collection Team
RAU: Risk Assessment Unit
RECs: Regional Emergency Coordinators
SCHPT: Shelter Care and Humanitarian Priorities Team
SNSS: Strategic National Sanitary Stockpile
TCCTs: Trauma and Critical Care Teams
VCT: Veterinary Care Team
VIT: Victim Information Team

a) *Risk Assessment Unit* (RAU); as a component of a comprehensive risk management program, RAU conducts hazard-specific vulnerability analyses according to the following flow-chart: Information gathering – data analysis – assessment – prioritization of actions – follow up of resultant outcome.

b) *Medical Resource Unit* (MRU); it predicts the local needs in medical resources, supervises hospital operation in emergency status, and registers medical devices, pharmaceutical stock, beds and bedding, laboratory capabilities, supplies and reserves, personal safety equipment, bio-safety facilities, etc.

c) *Public Health Emergency Planning Unit* (PHEP); it devises hazard generated procedures, priorities and risks, preparedness programs, and all relevant SOPs, both hazard- and response-specific, in a form of an operation manual or handbook. Expected activities are described in flow-chart diagrams and regard all actions to take place from the first hour up to several weeks after the incident. Such SOPs exist for all levels of response and describe, among others, personnel, equipment, supplies and also define operational time points. In their standard form, SOPs include a basic plan describing current legislation, operational phases, and appendices describing incident functions, response specific and deactivation plans, checklists, templates, job cards, resources, and relevant literature [7].

d) *Disease Control and Prevention Unit* (CDC); the national link to the European-CDC.

e) *Exercises and Training Unit* (ETU) for continuous and efficient training of the EHOC staff according to international standards [8]; ETU also conducts preparatory planning for future emergency response scenarios, reviews existing national and international regulations, frameworks, and planning guidelines, and organizes periodically full-scale exercises on a realistic base. A final goal for ETU is an overall improvement of awareness, competence, operational readiness, and continuous updating of plans and procedures.

3. Operations Section: The cardinal EHOC branch comprises preparedness and response activities provided by trained personnel capable of responding within two hours after identification of a major health incident. Specific SOPs are:

- Gathering and analyzing all relevant pieces of information.
- Stepwise analysis and evaluation of all factors affecting the mission; in these are included not only medical factors but also general ones (environment, weather conditions, available time, humanitarian implications, cooperation reluctance or conflict of interest among various public and private participants, staff security, etc).
- Consideration of all alternative case-based actions; that is called mitigation, i.e. threat/hazard/disease-specific, on occasion, actions to minimize health-related impact after hazard occurrence.
- Activation of preparedness levels, from Level 3 (lowest) up to Level 1 (highest), according to the existing situation after interaction with bio-surveillance laboratories network and with the national response framework given by GSCP. The preparedness SOPs include a detailed and continuously updated catalog of involved personnel, methods of fast recall on a 24h/7d basis, key-persons, and all other necessary details of an early warning protocol.
- Flexibility, surge capacity, i.e. ability to respond in multiple emergency health incidents, surge capability, i.e. ability to cope with unusual/special medical needs, and adequate durability, i.e. long-lasting response [9].
- Close collaboration and engagement with scientific entities in strategic preparedness and tactical operations, such as associates from the academic world, seismologists, meteorologists, building engineers, forensic scientists, laboratory experts, anthropologists, epidemiologists, etc.
- Linkage and integration of healthcare volunteers, non-governmental organizations, and major humanitarian responders, e.g. Red Cross.
- Recovery actions at multiple levels, which restore the stricken environment to as close to former “normal” status after a major health incident.
- Promotion of environmental health, social engagement, mobilization, and solidarity of the community.

Hence, according to the above functions the Health Operations Section acts in the following response stages: Incident recognition – Notification – Activation – Mobilization – Operations – Demobilization - Transition to recovery. The Operations Section is divided into several branches [9], as follows:

a) *Regional Emergency Coordinators* (RECs), acting as operational liaisons between central EHOC and the corresponding regional departments of Civil Protection for each of the administrative districts of the nation’s territory; for each REC there should be established a mobile (in a vehicle) operation center with satellite intercommunication with the regional Civil Protection liaison.

b) An *Emergency Medical Services Unit* (EMCU); which is a field-level rapid deployment medical force subdivided into three (3) Trauma and Critical Care Teams (TCCTs). Depending on the incident, these teams can be reorganized in equipment and staffing to Disaster Medical Assistance Teams (DMATs) [10]. DMATs provide primary and acute care, triage of mass casualties, initial resuscitation and stabilization, advanced life support, and preparation of sick or injured for evacuation. All victims are managed by use of the internationally standardized procedure of colored cards which illustrate a brief medical history and mainly the medical priority for each victim (by the respective color). After prompt evacuation from the disaster area (when it is such), a rapid pre-hospital triage, followed by emergency initial stabilization and then by urgent transport of casualties constitutes the operational "holy trinity" for these teams. At the disaster point the TCCT/DMAT leader should be the first official responder who will undertake the following SOPs:

- Rapid estimation of hazard situation on the operation field along with an estimation of a probable number of casualties.
- Prompt notification to his/her superiors about urgent requirements in staff and equipment (ambulances, bedding, paramedics, body bags for fatalities, cold storage facilities, drinking water supply, etc.).
- Together with the (non-medical) on-site delegate of Civil Protection, they immediately delineate operational disaster zones around the disaster point.
- Outside the "hot zone" the above two operational delegates minister to the needs of less injured victims; the TCCT/DMAT leader, in particular, activates the operational teams of sub-paragraphs (c-g) of the EHOC Operation Section (described below).
- Remains in the "hot zone" and coordinates the rescue teams until he/she receives a replacement order.

c) A *Disaster Technical Assistance Unit* (DTAU), consisting of non-medical technical specialists who will provide, support, and repair mechanical and electrical equipment of operation.

d) A *Shelter Care and Humanitarian Priorities Team* (SCHPT); it coordinates humanitarian affairs, especially of the more vulnerable population groups, and prioritizes on-site non-medical, but still life-threatening needs that can heavily affect a disaster area, such as shelter, covers, safety, nutrition, drinkable water, etc. This unit cooperates with the Construction/Engineering Branch of the Civil Protection for shelters and sanitation systems construction.

e) A *Mental Health Team* (MHT), consisting of psychiatrists, psychologists, and social workers. They assess early post-disaster stress within the affected population and responders and support their mental health status by interventional and/or pharmaceutical practices and by cooperation with corresponding church/religion representatives.

f) A *Disaster Victim Identification/Mortuary Unit* (DVIMU); provides expertise, technical assistance, and support for casualties and human remains documentation, in collaboration with the Reporting/Briefing Section of the EHOC (described below). The unit further develops four operational branches; the Casualties Collection Team (employs laborers), the Post-mortem data Collection Team (collects and evaluates fingerprints, DNA/dental samples, etc, for forensic medicine purposes), the Casualties Identification Team, and the Mass Fatality Care Team (offers disaster mortuary services).

g) A *Veterinary Care Team* (VCT); offers care to animal casualties and to operating animals (e.g. rescue dogs), using veterinary medical support, equipment, supplies, and pharmaceuticals.

4. Logistics Section: It determines and supervises the provision of emergency resource needs for the unceasing operational function of the EHOC on a 24h/7d basis. It is divided into the following branches:

a) *Cost and General Resources Unit* (CGRU); it monitors cash flow and expenses and coordinates all sources of funding. Additionally, it can provide direct cash from a special account for immediate access in urgent needs of high priority, thus avoiding time-consuming bureaucracy.

b) *Medical Supplies Unit* (MSU); it monitors needs and provides required quantities of medical and pharmaceutical supplies as well as personal protective equipment for frontline health care workers.

c) *Food Provisions Unit* (FPU); it provides all services, resources, and supplies regarding nutrition to operation needs and also ensures that operating EHOC personnel have sufficient supplies of food and drinkable water.

5. Administration Section: It provides facility systems and supports the overall physical infrastructure (premises, furniture, equipment, etc.) of the EHOC; The section is divided into the following branches:

a) *Facility Unit* (FU); it organizes building and sectional layout of EHOC units in dedicated, purpose-built facilities, with adequate space for its staff, physically and environmentally secure, accessible, and survivable in the event of a threat or disaster.

b) *Human Resources Unit* (HRU); it formulates job descriptions and classifies staff requirements based on the knowledge, skills, and abilities required for the role and function of each person in the EHOC.

c) *Strategic National Sanitary Stockpile* (SNSS); it develops and deals with the national repository of basic life-support medications (antibiotics, antidotes, etc) and surgical supplies for any nation-wide event of a mass health emergency.

6. Communications/Intelligence Section: The equipment of that sector includes high technology infrastructure (computers, servers, internet stations, software, etc.) and all tools available to support an effective, all-weather-resistant continuous communication and data management according to high-level privacy and security standards. Of utmost importance is the timely, efficient, and accurate information flow on a 24 hour/7 day basis, between the Command section and each of the corresponding field-units from the Operations sector. For that to be accomplished a sophisticated web-based digital tool allowing fast, real-time information sharing to multiple recipients is constantly in function. Some specific sub-functions of that section are:

- A continuous surveillance system, for gathering and exchanging information about sensitive civil national territories (e.g. cities and forest areas), by use of a broad camera-surveillance network and by electronic processing of the data-flow from an advanced, interactive mapping application. The relevant SOPs address all kinds of environmental and man-made threats, monitoring procedures, and actions according to certain hazard thresholds.
- An internet monitoring and medical intelligence/analysis system that verifies information to prevent duplication/ contradictory data collection.
- A rapid early warning/alert system triggering the coordinated rapid recall of staff back to their positions.
- A classified and secure web-based priority system for high-level communication and providing of information among national and transnational decision-makers.

The Communications/Intelligence sector is divided into the following branches:

a) *Data-base Management Unit* (DMU); it controls and centralizes information distribution to appropriate stakeholders for coordinated resource mobilization and correct decision-making to a wide range of emergencies.

b) *Disaster Assessment and Coordination Team* (DACT); it links the abovementioned data with relevant colleagues and experts from both country-level contacts and from the European Monitoring and Information Centre (MIC), as well as from UN/WHO.

c) *Communication Unit* (CU), equipped with telecommunication infrastructure and emergency communication services for sharing urgent relevant information among operational delegates from national and European governmental and private health agencies.

7. Reporting/Briefing Section: Chief is a senior Public Information Officer with pronounced communication skills to prevent inaccurate information spreading and panic diffusion, thus public composure and confidence to be preserved. The chief of the section applies uniform medical reporting SOPs according to the basic requirements: Who – when – where - what and how to report. The Reporting / Briefing section is divided into two branches:

a) *Media and Public Information Team* (MPIT), for regular management of the media and consequently of public expectations for the incident, by the timely transmission of valid information, carefully filtrated for any confidential or higher classified evidence.

b) *Victim Information Team* (VIT); it acts as the liaison between the afflicted population (victims or their families) and state authorities to serve their needs, to manage the list of missing persons, and to provide discreet information, in cooperation with DVIMU and MFCT of the Operations Section. The VIT also formulates antemortem medical records for the deceased and provides forensic support by coordination with local and national law enforcement agencies.

CONCLUSIONS

Albeit the proper function of an EHOC depends on numerous factors, qualifications, and principles, only two ones can be regarded as primary; first, the political will to radically reform the national and trans-national administrative “status quo” at the highest level, so that various authorities to be centralized under the same command through an appropriate legislative framework and second, a properly structured and fully functional organizational plan of an exemplary EHOC.

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REVIEW

An overview of the complications of acute and chronic mercury exposures. Past, present, and future

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Abstract: *Mercury is a chemical element that has been known since prehistoric times and has been used over time as a component of paints, in the treatment of various diseases, and later in various industrial compounds. Mercury enters the body transcutaneously, by inhaling or by ingesting food contaminated with mercury. The most common cause of mercury poisoning is eating seafood, but people can get mercury poisoning from industrial processing, thermometers, dental work, and old paints. The most vulnerable are people at occupational risk due to chronic exposure, as well as fetuses in the intrauterine period. Mercury poisoning produces extremely serious effects on the nervous, renal, and gastrointestinal systems. The nature and extent of mercury poisoning depend on factors such as the route of exposure, the rate of exposure, the distribution and biotransformation of mercury in the body, the chemical form of mercury or the mercury compound. Both mercury in the blood and the urine should not exceed 10 µg/L. The critical values of mercury in the blood are >150 µg/L and the lethal ones exceed 800 µg/L. New diagnostic methods allow the rapid and accurate identification of the concentration of mercury in biological products. Treatment is based on the use of chelating agents. However, the prognosis of mercury poisoning is extremely variable, due to the potentially irreversible nature of the lesions.*

Keywords: *mercury exposure, neurotoxicity, gastrointestinal toxicity, diagnosis, chelation therapy*

INTRODUCTION

Mercury or hydrargyrum (from the Greek language), is one of the 6 liquid elements at a temperature and pressure close to those of the room [1, 2]. It is found in nature in the form of sulfide, also known as cinnabar. Under the name of vermillion, it was used by people to paint the walls of caves in Spain and France in prehistoric times. Archaeological discoveries have shown that mercury has been known since antiquity by the Romans, Greeks, Chinese, and Hindus [3].

Beginning in the 16th century, new information regarding mercury emerged. Agricola detailed the use of mercury and analyzed the effects of inhalation of its vapors on human health [4]. Thanks to the writings of Paracelsus [5], mercury had become, along with antimony [6], an element used in the treatment of venereal diseases [7]. Its anti-inflammatory and antiseptic effects have also determined its use in the treatment of syphilis. It was used in the form of dimethylmercury, but its action on the central nervous system led to the cessation of these treatments [8]. Around

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1913, diuretics based on organic mercury compounds were used, which were released on the market for more than 30 years [8]. Mercury is also used in dental work (especially fillings), in some antiseptics, or vaccine preservatives. Mercurochrome was used to treat wounds, later being given up due to not being recognized as safe and effective.

SOURCES OF MERCURY

Mercury is a pollutant of global concern owing to its great toxicity even at very low concentrations [9]. Most of it is deposited in oceanic sediments and soil. The global mercury cycle involves its release from the earth's crust, the air transport of mercury vapor, and its storage in soil and water. Aquatic ecosystems are an essential component of the global biogeochemical cycle of mercury, as inorganic mercury can be converted to toxic methylmercury in these environments [10]. Through burning organic matter and industrial processes, volatile forms of mercury can be released into the atmosphere. The origin of mercury is determined by the type of fuel that was used, such as coal, oil, or municipal waste. Mercury also enters the environment due to the incineration of materials such as car components, batteries, fluorescent bulbs, medical products, thermometers, and thermostats [11]. Leather manufacturing companies or tanneries are highly polluting, generating enormous amounts of solid and liquid waste, including mercury compounds [12].

The most common cause of mercury poisoning is from eating seafood, but people can get mercury poisoning from industrial processing, thermometers, and blood pressure machines, dental work, and old paints [13]. Methylmercury can be absorbed from the water by all sea creatures, but it also continues through the food chain.

Small sea creatures, such as shrimps, often ingest methylmercury and are then eaten by other fish. These fish will now have more methylmercury in them than the original shrimp. This process continues up the food chain, so that a large fish may contain much more mercury than the fish it has eaten.

ROUTES OF PENETRATION

Mercury enters the body transcutaneously, by inhalation, or by ingesting food contaminated with mercury [14]. Transcutaneous absorption of mercury is low. The physical properties of elemental mercury limit its absorption through intact skin. Inhalation of toxic mercury vapors leads to their absorption into the circulatory system, then being distributed throughout the body [15]. The absorbed mercury circulates through the bloodstream, and about half of it is bound to plasma albumin (in association with sulfhydryl groups), while the other half enters the red blood cells and then is rapidly distributed to the tissues; in a few hours, the highest concentration is found in the kidneys. Mercury can build up in the bones, bone marrow, and liver for a short time. One week after exposure, 85-95% of the body's mercury is stored in the kidneys [16].

MERCURY TOXICITY

The primary mechanism of mercury toxicity involves its irreversible inhibition of selenoenzymes, such as thioredoxin reductase (IC50 = 9 nM) [17]. Thioredoxin reductase restores vitamins C and E, as well as several other important antioxidant molecules, back into their reduced forms, enabling them to counteract oxidative damage [18].

Mercury in its various forms is particularly harmful to fetuses as an environmental toxin in pregnancy, as well as to infants. Women who have been exposed to mercury in substantial excess of dietary selenium intakes during pregnancy are at risk of giving birth to children with serious birth defects [19].

The destruction of the proteins that form the glomerular basement membrane leads to the alteration of the renal filtration capacity, with the appearance of severe proteinuria. At the digestive level, mercury destroys the gastric mucosa, a process that favors its absorption in the body. Gastrointestinal symptoms are extremely polymorphic, including digestive hemorrhages, nausea and vomiting, diffuse abdominal pain, and transit disorders, such as constipation or diarrhea [20, 21]. The differential diagnosis should consider other causes of the acute surgical abdomen, such as acute cholecystitis, gastroenteritis, or intestinal occlusion [22-24]. Even less frequent causes such as Amyand hernia or pheochromocytoma may be taken into account [24-26]. Lately, during the COVID-19 pandemic, the SARS-Cov 2 infection has been another cause to be excluded, due to the tropism of the virus for angiotensin convertase (ACE) receptors at the level of the intestinal epithelium [27-29]. A carefully obtained history is necessary to evaluate the risk of mercury poisoning and to avoid unnecessary surgeries in suspected cases. Implementing the principle of safe surgery in the emergency ward is important for a comprehensive evaluation of the patient during admission and to minimize the anesthetic-surgical risk [30].

Mercury poisoning causes some neuropsychiatric, sensory, visual, cognitive, and motor disorders. Previous clinical trials have reported symptoms of tremor, emotional lability, insomnia [31, 32] memory disorders, muscle weakness, muscle atrophy and spasms, headaches, polyneuropathy (paresthesia, hyperactive tendon reflexes, decreased nerve conduction and motor speed), and cognitive impairment [32, 33].

Recent studies found a good correlation between exposure to mercury and other heavy metals and the risk of type 2 diabetes in young adults [34-36]. One possible mechanism is the direct toxic effect of mercury on the beta-pancreatic cells [37].

Toxicity of mercury compounds

There are three different forms of mercury that cause health problems. They are:

- Elemental mercury, also known as liquid mercury or quicksilver;
- Inorganic mercury salts;
- Organic mercury.

The nature and extent of mercury poisoning depend on factors such as the route of exposure, the rate of exposure, the distribution and biotransformation of mercury in the body, the chemical form of mercury or the mercury compound. Organic mercury compounds often produce severe and irreversible toxicity to the nervous system in overdoses, while inorganic compounds produce primary peripheral effects, such as gastroenteritis and renal failure [38-40] (Table 1).

Table 1: Manifestations due to the route of exposure, the rate of exposure, the distribution, and the biotransformation of mercury in the body [34].

	Elemental mercury	Inorganic mercury	Organic mercury
Primary route of exposure	Inhalation	Oral	Oral
Primary tissue distribution	Central Nervous System, Kidney	Kidney	Central Nervous System, Kidney, Liver
Clearance	Renal clearance GI clearance	Renal clearance GI clearance	Methyl: GI Aryl: GI, Renal
Clinical Effects			
Central Nervous System	Tremor	Tremor, Heresy	Paresthesias, ataxias, tremor, dysarthria, tunnel vision
Pulmonary	+++	-	-
GI	+	+++ (caustic)	+
Renal	+	+++ (ATN)	+
Acrodinia	+	++	-
Therapy			
	BAL, succimer	BAL, succimer	Succimer (Early)

GI - gastrointestinal

Quicksilver (liquid metallic mercury) is poorly absorbed by ingestion (Figure 1). Metallic mercury, due to its high volatility and low absorption, has no systemic toxicity. Mercury compounds tend to be much more toxic than the elemental form, and the organic forms are extremely toxic, being involved in kidney and brain destruction. Mercury cyanide can enter the body both by inhalation and by transcutaneous absorption and ingestion. Ingestion of mercury cyanide can cause clinical phenomena similar to both mercury ingestion and cyanide ingestion, leading to extremely severe clinical signs. The degree to which cyanide poisoning occurs is thought to be correlated with the cyanide ions released in the stomach, which depend on the amount ingested, gastric acidity, and volume of gastric contents [41].

Figure 1: Liquid metallic mercury in the large bowel. Source - ICU Toxicology Prof. Macovei collection

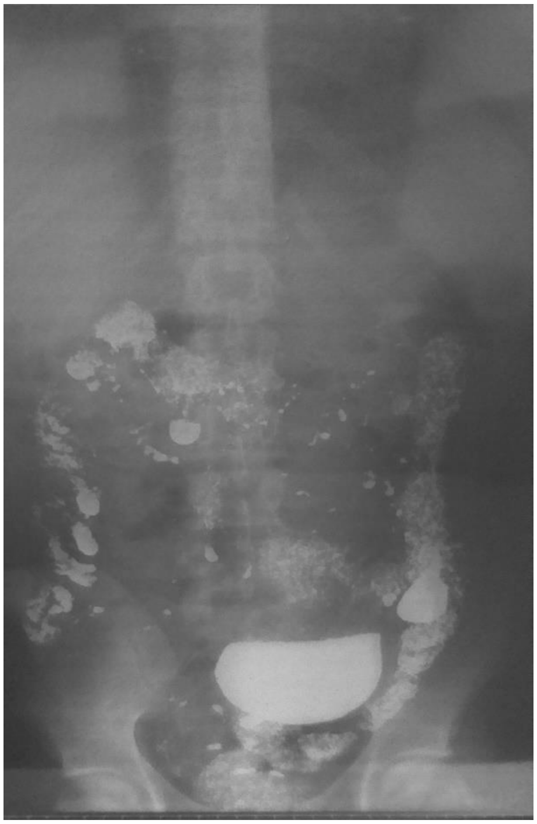

Mercury occurs as salts such as mercuric chloride ($HgCl_2$) and mercurous chloride (Hg_2Cl_2), the latter also known as calomel. Because they are more soluble in water, mercuric salts are usually more acutely toxic than mercurous salts. Their higher solubility allows them to be more readily absorbed from the gastrointestinal tract. Mercury salts primarily affect the gastrointestinal tract and the kidneys, and can cause severe kidney damage [20]; however, as they cannot cross the blood-brain barrier easily, these salts inflict little neurological damage without continuous or heavy exposure. Low-grade chronic inorganic mercury poisoning is the basis of acrodynia (pink disease) in children, who typically developed irritability after exposure to mercury-containing teething powder, ointments, and drugs [41-43].

In 1960, JA Freeman of Orange, New Jersey, described the Mad Hatter syndrome, characterized by Danbury-type tremor, found in workers in hat factories [44]. Mercury was inhaled by workers during the textile dyeing process with mercury nitrate. The characteristic symptoms are tooth loss, salivation, neurological deficits described by Ramazzine, the father of occupational medicine, in 1970. One of the most toxic mercury compounds is dimethylmercury. The toxicity is so high that, at doses of a few microliters in contact with the skin, they can cause death.

Methylmercury (MeHg) is the main source of organic mercury. Due to bioaccumulation, it achieves high concentrations found in some species, such as tuna or swordfish [45]. Clinical phenomena occur after a latency varying from a few weeks to a few months after a single exposure [46]. Paresthesias appear as the first manifestation [47], followed later by much more severe manifestations, such as coma. High-level exposure to methylmercury is known as Minamata disease [48, 49].

Another organic mercury compound responsible for human intoxications is ethylmercury, derived from the metabolism of thimerosal (o-carboxyphenyl-thio-ethyl-sodium salt), which is the most widely used form of organic mercury, still being used as a preservative in some human and animal vaccines [50]. The biologic profile is different from methylmercury, with a shorter blood half-life and increased renal elimination [50, 51]. The nervous system affected by intoxication with this substance has the same symptoms as intoxication with methylmercury, adding signs of kidney tissue damage, such as proteinuria, digestive disorders such as nausea, vomiting, and abdominal and dermatological pain [8, 52].

Under the 2013 Minamata Convention on Mercury, organized by the United Nations Environment Program (UNEP), 140 countries have committed themselves to end mercury emissions [53].

LABORATORY DIAGNOSTIC OF MERCURY INTOXICATION

Diagnosis of elemental or inorganic mercury poisoning involves determining the history of exposure, physical findings, and an elevated body burden of mercury. Although whole-blood mercury concentrations are typically less than 6 μg/L, diets rich in fish can result in blood mercury concentrations higher than 200 μg/L [54, 55]; it is not very useful to measure these levels for suspected cases of elemental or inorganic poisoning because of mercury's short half-life in the blood. Free mercury is not well absorbed through the gastrointestinal tract and is excreted almost completely in the feces after ingestion; however, when it persists, mercury volatilizes at room temperature into vapors that are well absorbed by the lungs.

The diagnosis of organic mercury poisoning differs in that whole-blood or hair analyses are more reliable than urinary mercury levels [56].

Both mercury in the blood and the urine should not exceed 10 μg/L. The critical values of mercury in the blood are more than 150 μg/L and the lethal ones exceed 800 μg/L.

Mercury levels in the blood and urine are normally very low. The result of the test, which does not show mercury, indicates that it is likely that the tested person was not exposed to excessive levels of mercury, at least not during the test. Elevated levels of mercury in the blood suggest a relatively recent exposure to mercury. Mercury levels in the blood or urine will not indicate the form or amount of mercury a person has been exposed to.

Blood and urine mercury concentrations are commonly used as biomarkers of mercury exposure. Hair has been used as a biomarker of exposure to methylmercury [57]. Occupational studies show that recent mercury exposure is reflected in blood and urine [54, 58]. A 24-hour urine sample is the most useful in determining the degree of exposure.

The preanalytical phase in determining the species of mercury or total mercury involves a succession of procedures such as sample collection, conservation, and storage, extraction, purification, preconcentration, and separation of species of interest (if applicable).

Exhaustive handling of samples but also the properties of mercury (especially mobility and high volatility) can lead to erroneous results due to either the loss of analyte or contamination of the sample. In addition, interconversion of the species can occur by manipulating the sample. Quantification is usually performed by atomic spectrometry techniques [59].

Some detection techniques that have been used for total Hg analysis are cold vapor atomic fluorescence spectrophotometry (CV-AFS), cold vapor atomic absorption spectroscopy (CV-AAS), graphite furnace atomic absorption spectrophotometry (GF-AAS), cold vapor inductively coupled plasma atomic emission spectrophotometry (CV-ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) [60]. Mercury determination through flame atomic absorption spectroscopy (FAAS) gives rise to poor sensitivity [61].

Of all these available methods, cold atomic absorption spectrometry is rapid, sensitive, and accurate, but through this method, the forms of mercury cannot be distinguished. Thin layer and technical gas chromatography are used in the determination of mercury species because with the help of these techniques, the forms of mercury can be distinguished.

Analytical parameters of reviewed research papers about the speciation and determination of mercury by spectrometric instruments (CV-AAS, AAS, AFS, GC-AFS, HPLC-CV-ICPMS, ICP-MS) are presented in Table 2 [60-69].

Table 2: Analytical parameters of reviewed research papers about the speciation and determination of mercury by spectrometric instruments (after Suvarapu LN et al)

No.	Analyte	Detection	Method	Limit of detection	Linearity range	Samples	Ref.
1	Total Hg	CV-AAS	Digestion	3.98×10^{-10} M	2.5–10.0 μg L^{-1}	Biological samples	[61]
2	Total Hg	AAS	Amalgamation	0.2 ng/g for hair 0.02 ng/g for blood	—	Hair Blood	[62]
3	Hg(II)	AFS	Fluorescence optical sensor	9.57×10^{-12} M	2.27×10^{-11} 1.13×10^{-3} M	Hair Urine	[63]
4	MeHg	GC-AFS	SPE	12 ng /g	> 1.5 ng/ mL	Biological samples	[64]
5	MeHg, Hg(II), EtHg	HPLC-CV-ICPMS	Extraction and separation	5.98×0^{-11} (Hg(II)) 2.17×10^{-11} (EtHg) 1.8×10^{-8} (MeHg) M	—	Plasma/serum	[65]
6	Total Hg	ICP-MS	Isotope dilution and UV-photochemical vapor generation	0.5 pg/ g	—	Biological tissues	[66]
7	Hg Total	ETAAS	Extraction of mercury species in toluene	2 mcg/dm^3	-	Blood	[67]
8	Total Hg	GC/MPD	Cleavage with HCl, toluene extraction, addition of Grignard reagent	0.4 mcg/L	-	Blood	[68]

The most powerful tool for the determination of trace elements in a variety of matrices is chemical vapor generation in conjunction with atomic absorption spectroscopy techniques. An absorption technique such as cold vapor atomic absorption spectroscopy (CV-AAS) is one of the most sensitive methods for mercury determination. A reducing agent, stannous chloride ($SnCl_2$), is used to convert ions of Hg_2^+ (aq) in solution to Hg0 (g) which produces a higher analytical absorbance signal. Mercury, having a high vapor pressure, is liberated and entrained in an inert gas of argon after passing through the liquid-gas separator. The Hg vapor is transferred into the optical path of the atomic absorption spectrophotometer where it absorbs light of a specific wavelength at room temperature [60].

Another method of determining mercury is atomic absorption spectrometry (AAS) with graphite furnace atomization, using palladium and magnesium nitrate as modifiers; their use makes it easier to separate the analyzed element from the matrix.

Due to its simplicity and low operating costs, AAS is widely applied in the single-elemental analysis [70, 71]. The samples are exposed to concentrated acids and extended heating, which increase analyte loss. Atomic absorption spectrometry is an analytical method for determining the number of metals or metalloids in a sample, based on the amount of UV or VIS electromagnetic radiation absorbed by the atoms brought into the atomizing state. To establish the atomization temperatures, it is necessary to highlight the interferences that can occur through reaction mechanisms in the graphite furnace. In the case of volatiles, the use of chemical modifiers aims to:

- Shift the value of the atomization temperature outside the non-atomic absorbance that may occur due to the complexity of the matrix, which is especially characteristic of biological samples.
- Obtaining a much higher calcination temperature due to the reduced volatility of the analyte, which allows the elimination of compounds that could give a non-atomic absorbance.
- Increasing the sensitivity of the method by simultaneously atomizing the different forms in which the analyte is present in the matrix.

When obtaining optimal operating parameters of the GF-

AAS system, it is desirable to reduce non-atomic absorbance by optimizing drying, calcination, and atomization temperatures and by using the deuterium lamp.

For example, a method for mercury analysis was established by the authors of this paper using a Graphite Furnace Atomic Absorption Spectrophotometer (GF- AAS) Varian SpectraAA-880, with a hollow cathode lamp (Agilent Technologies) and a deuterium lamp (Agilent Technologies) for background correction, coupled to a GTA-100 atomizer and a programmable sample dispenser (Varian, Australia).

The calibration curve was obtained by measuring 5 concentration levels of the standard mercury solution. The results are shown in Figure 2.

Figure 2: The calibration curve used for the determination of mercury

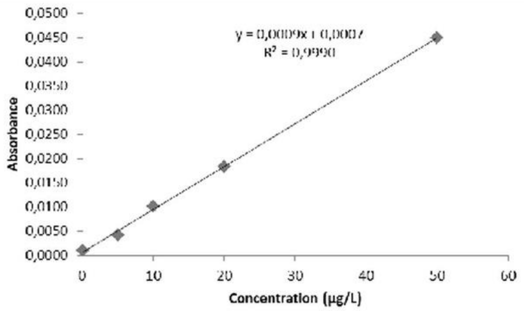

A linear relationship was found between the absorbance at 253.7 nm and the concentration of mercury in the range of 5.0 to 50 µg/L. The representative linear equation was $y = 0.0009\,x + 0.0007$ where: y is the absorbance and x is the mercury concentration (µg/L) calculated by the least-squares method.

The coefficient of determination for the calibration curve was 0.9990 (Figure 2) indicating a good correlation between the absorbance read using the equipment and the concentration of mercury in the standard calibration solution.

The method was applied to the urinary determinations of mercury involved in acute intoxications and to monitor urinary mercury levels during chelation therapy.

However, according to several authors, mercury levels in urine less than 10 mcg/L and bloodless than 20 mcg/L are considered normal. Mercury concentrations in the blood and urine poorly support the neurological clinical findings on electrophysiological measurements. Organic forms of mercury, including phenylmercury, are metabolically transformed into inorganic mercury, compared to methyl- and ethylmercury, which do not undergo metabolic transformations but are eliminated in the bile and feces, so alkylmercury compounds cannot be detected in urine [72].

Urinary elemental mercury levels are useful in assessing acute or chronic mercury vapor poisoning. The concentration of mercury in urine samples in approximately 98% of all evaluated subjects without known mercury exposure is less than 5 µg/L [24].

So, in methyl mercury poisonings, for example, urinary mercury levels do not provide suggestive information, but because methylmercury is concentrated in red blood cells, the whole-blood level may reflect acute methylmercury exposure but does not correlate with the severity of the poisoning/exposure.

THERAPEUTIC OPTIONS

Acute ingestion of mercuric salts can be treated by inducing vomiting or gastric lavage. Resins with polythiol can be administered orally to bind mercury in the gastrointestinal tract. The most effective chelating agents are dimercaprol, succimer, and penicillamine, which have active mono- and dithiol groups.

Acute inorganic mercury poisoning can be treated with dimercaprol in doses not exceeding 24 mg/kg daily, administered intramuscularly in several doses [73]. The treatment is divided into 5-day courses, separated by a few days off. The N-acetylated form of penicillamine is also useful at doses of 30 mg/kg daily in divided doses. Peritoneal dialysis, hemodialysis, and regional extracorporeal hemodialysis with succimer complexation have been used with some success in the treatment of patients with renal impairment [73, 74].

Chelation therapy for acute inorganic mercury poisoning is based on DMSA, 2,3-dimercapto-1-propanesulfonicacid (DMPS), D-penicillamine (DPCN), or dimercaprol (BAL).

Children who ingest mercury can be treated with DMSA. DMSA is the most frequently used agent for severe methylmercury poisoning [75]. When administered orally, it is superior to BAL, DPCN, and DMPS. α-Lipoic acid (ALA) requires correct dosing, as inappropriate dosages increase toxicity. N-acetylcysteine (NAC) as a glutathione precursor is recommended by physicians but has been shown to increase mercury concentrations in the kidneys and the brain.

CONCLUSIONS

Generally, two population groups are more sensitive to the effects of mercury. One of these is fetuses who are most susceptible to developmental effects due to mercury. The second group consists of people who are regularly exposed (chronic exposure) to high levels of mercury (such as populations that rely on subsistence fishing or people who are occupationally exposed). Once the source of mercury poisoning has been identified, the exposure must be discontinued. Novel laboratory methods, such as GF-AAS, allow quick identification of mercury in biological products. However, the prognosis of mercury poisoning is highly variable, depending on the irreversible neurological and organ damage that is present at the moment of diagnosis.

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ORIGINAL ARTICLES

Antibiotherapy: latest current affairs – state of the art 2019

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Abstract: *Given the continuous growth of the bacterial resistance phenomenon, the appropriate choice of antibiotherapy represents an important step in the treatment and prophylaxis of infectious diseases. Depending on the symptomatology, the dose required for the effective treatment of an infection may vary. Inadequate dosing not only reduces the effectiveness of the antibiotic, but also promotes the emergence of resistant bacterial strains. Also, too high of a dose increases the toxic side effects of antibiotics. Therefore, a personalised antibiotherapy according to: the patient's clinical status, body mass, associated chronic diseases, etc. represents a promising approach in the treatment of infectious diseases.*

The purpose of this study is to present the most important current affairs regarding the emergence of new antibiotics, the maximum doses recommended by the latest version of the "European Committee on Antimicrobial Susceptibility Testing" (EUCAST - version 9/2019) and providing an indication on whether these antibiotics are used in Romania.

Keywords: *antibiotics, antibiotherapy, maximum recommended dose, antimicrobial chemotherapics*

INTRODUCTION

Antibacterial chemotherapics (antibiotics) have become the emblematic drugs of the 20th century starting with the first antibacterial chemotherapic, Salvarsan (invented by Paul Ehrlich, of German origin, in 1909) and the beta-lactamase Penicillin (discovered by the American Alexander Fleming, in 1929). Currently, there are hundreds of antibacterial antibiotics (AB), of which 269 have been approved for antibiotherapy (ABT). It was believed that bacterial diseases could be eradicated, but the practice has shown since the middle of the last century that the phenomenon of bacterial resistance to antibiotics emerges, that much higher doses are now needed, with modified conditioning and dosage, targeted use, in phases and combined or associated, even the use of new antibiotics, as far as they can be produced [1-7].

ANTIBIOTHERAPY

Over time, the efficacy of ABT has been reduced, despite advances in medicine in general and pharmacology in particular. Resistant, multi-resistant and all-resistant bacteria create the most serious problems in medical practice and more and more patients die from infections or over-infections that do not respond to treatment. This worrying phenomenon has been thoroughly investigated, starting with the application manner of ABT. Thus, the website www.longitudeprize.org published in 2014 an international medical statistic [6] having as results the inappropriate use and prescription of ABs:

- 90% of the physicians prescribed AB under the pressure of the patient who wanted a certain AB (namely, "Customers is King?!");
- 70% of the physicians prescribed AB without being sure

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that the etiology of the disease is bacterial or viral;

• 49% of the physicians prescribed AB for a week or more, without a prolonged treatment being needed;

•44% of the physicians prescribed AB only preventively, after discharge from surgery.

Following the highlight of the multiple factors that lead to the irrational use of ABs, medical and especially non-medical, in 2017 UN/WHO launched a new conception on prophylaxis and treatment – One health – and officially formulated recommendations for the Member States, with the goal of maintaining the efficiency of ABT worldwide. Based on these recommendations, each country sets its own research directions for medical and pharmaceutical scientific research. In this respect, a prize was established worldwide, Longitude Prize (2014). It has a prize pool of £ 10m to reward a competitor who will develop a test targeted on the rapid diagnosis of the diseases that require ABT. Through the rational use of ABs, the test would lead to the conservation of efficiency and shall revolutionise healthcare worldwide. The test should be accurate, fast, inexpensive, easy-to-use and accessible to anyone, anywhere in the world. By this test, shall be identified which ABs are needed and which shall be used. But these requirements seem utopian in the current stage of development of science and technology, it being unlikely to discover something that corresponds at least partially. Therefore, the prize has not been awarded so far.

Inventing new ABs is costly and difficult. These must meet divergent conditions: high toxicity for microorganisms (thus, antimicrobial efficacy), but little or no toxicity for the treated macroorganism; also, it must not have significant side effects; it must be produced industrially with reasonable costs; it must be stored and managed conveniently etc.

For the in vitro evaluation of the antibacterial effect of the AB, the Minimum Inhibitory Concentration (MIC) testing is performed on the respective bacterium (Breakpoint by determination in microdilutions in liquid culture medium) and the Kirby-Bauer diffusimetric antibiogram (ABG) with the measurement of the diameter of the Inhibition Zone (IZ) on the solid culture medium [2, 7]. One can choose from theoretically efficient ABs [1, 3, 4, 5] those that inhibit isolated and identified bacteria from the patient, by interpreting in the SIR system (Susceptible, Intermediate and Resistant) the diameter measured in millimeters, compared to EUCAST tables [2]. Because often the result obtained in vitro was between susceptible and resistant, this is noted as intermediate and for clinicians it was equivalent to resistant, because it would not have the desired therapeutic effect. In the current conditions of spread of the bacterial resistance phenomenon towards antibiotics, it is recommended to increase the dose of ABs, where the pharmacological properties allow, so that the bacteria pass from the intermediate to the susceptible area. By this approach of bacterial susceptibility, the intermediate bacteria pass from the resistant group to the susceptible group, and the SIR system gains another significance. The paraclinical findings become a practical indicator for the guidance of the clinician:
S = susceptible to standard dosing regimen (usual dosage)
I = intermediate, susceptible to high exposure (high doses, where possible)
R = resistant (therefore, useless for use).

As a result, the most handy method of combating bacterial resistance is the justified increase of the consumption of ABs, by increasing the single dose, daily or per cure, the frequency of administration or the duration of treatment, where the pharmacological characteristics allow (for example, in order not reach the toxic dose). The maximum recommended doses can be per dose, per day or per cure, and the cure can be short (1-5 days), medium (5-10 days), long (10-15 days) or very long (15-60 days). The doses are approximated depending on the conditioning of the drug (single antibiotic package or fixed combination), as well as the body mass of the patient. Any drug is theoretically dosed for an average patient, with a net body weight of 70 kg; so for a patient over 140 kg it should be double dose and for one below 35 kg it should be half dose. Some drugs, including ABs, are given by calculating in mg/kg body weight, especially in paediatrics, where children may have very different weights. When choosing ABs and in determining the dose, one must take into account, in addition to the pharmacological criteria imposed by antibiotherapy (which are well known), also: the general and particular contraindications, the age, the general patient status, the associated diseases, possible allergies or intolerances, the need for combinations with anti-beta lactamases or combinations with ABs or other anti-infective or adjuvant drugs. Consideration should also be given to possible antagonisms, inactivation with drugs and food, unwanted adverse or secondary phenomena as well as possible Herxheimer-type reactions. All this leads to a bi-unequivocal personalized ABT: depending on the needs and characteristics of the patient as well as the susceptibility of the pathogenic bacterium [8-13].

THE CLASSIC RECOMMENDATIONS

Professor Balş, the professional owner of the hospital that bears his name formulated the basic principles of antibiotherapy (also called antibiotic therapy) regarding: indications, contraindications, errors, associations of ABs, which are still valid today. These principles have been applied in Romanian medical practice by Prof. Angelescu and his students, with undeniable successes. But at present, although all these principles have remained valid, their effectiveness in ABT practice is progressively diminished, due to the the spread of bacterial resistance to ABs, worldwide. As a result, we must complement these principles with new observations and discoveries in order to maintain the effectiveness of ABT. The most important news for ABT are not the new ABs, but the increased doses of those already known and their grouping by type of bacterial resistance.

MAXIMUM DOSES CURRENTLY RECOMMENDED

The new maximum doses are recommended by EUCAST 2019, p.96-99, systematized on AB groups, International Common Names (DCI) and with mentions for different clinical situations (2), and in the following we shall provide a summary, with the mention, „*" If used in Romania:

Penicillins: Benzylpenicillin* 1.2 g (2MU) x 4-6 iv, or 2.4 (4 MU) x 6 iv in meningitis and pneumonia; Ampicillin* 2 g x 4 iv, or 2 g x 6 iv in meningitis; Ampicillin+Sulbactam* 3 g x 4 iv; Amoxicllin* iv 2 g x 6 iv; Amoxicllin - oral 1 g x 3; Amoxicllin+clavulanic acid* iv 2.2 g x 3 iv; Amoxicllin+ clavulanic acid - oral 1 g x 3; Piperacillin* 4 g x 4 iv; Piperacillin+tazobactam* 4.5 x 4 iv; Ticarcillin* 3 g x 6 iv; Ticarcillin+clavulanic acid* 3.1 g x 6 iv; Phenoxymethyl-penicillin 2 g x 4 oral; Oxacillin* 1 g x 6 iv; Cloxacillin 1 g x 4 oral or 2 g x 6 iv; Dicloxacillin 2 g x 4 oral or 2 g x 6 iv; Flucloxacillin* 1 g x 4 oral or 2 g x 6 iv; Mecillinam 0.4 g x 3 oral.

Cephalosporins: Cefaclor* 0.25-1 g tidpo; Cefadroxil* 0.5-1 g bidpo; Cephalexin* 0.25-1 g bid-tid po; Cefazolin* 1 g tid-qid (or 2 g tid) iv; Cefepime* 2 g tid iv; Cefixime* 0.2- 0.4 g bid po, or 0.4 g po in gonorrhea; Cefotaxime* 2 g tid iv, or 2 g qid in meningitis; Cefpodoxime* 0.1-0.2 g bid po; Ceftaroline* 0.6 g tid iv (slow infusion – 2 hrs); Ceftazidime* 2 g tid iv or 1 g x 6 iv; Ceftazidime+avibactam* 2.5 g tidpev (2 hr infusion); Ceftibuten 0.4 g po; Ceftobiprole 0.5 g tid iv (slow infusion – 2 hrs); Ceftolozane+tazobactam* 1.5 g tid iv (slow infusion – 1 hr); Ceftriaxone* 2 g bid iv, or 4 g iv in meningitis; Cefuroxime* 1.5 g tid iv; Cefuroxime 0.5 g bid po.

Carbapenems: Ertapenem* 1 g x 1 iv; Imipenem* 1 g qid iv (30 mins perfusion); Meropenem* 2 g tid iv (3 hrs perfusion), or in meningitis 2 g tid iv; Meropenem+vaborbactam 4 g tid iv.

Monobactams: Aztreonam 2 g qid iv.

Fluoroquinolones: Ciprofloxacin* 0.75 g bid po, or 0.4 g tid iv; Levofloxacin* 0.5g bid po, or 0.5g bid iv; Moxifloxacin* 0.4g x 1 po, or 0.4g x 1 iv; Norfloxacin* 0.4g bid po; Ofloxacin* 0.4 g bid po, or 0.4 g bid iv.

Aminoglycosides: Amikacin* 30 mg/kg x 1 iv; Gentamicin* 7 mg/kg x 1 iv; Netilmycin* 7 mg/kg x 1 iv; Tobramycin* 7 mg/kg x 1 iv.

Glycopeptides and glycolipopeptides: Dalbavancin* 1 g x 1 iv; Oritavancin 1.2 g x 1 iv; Teicoplanin* 0.8 g x1 iv (single dose in slow infusion – 3 hrs); Telavancin 10 mg/kg x 1 (slow infusion – 1 hr); Vancomycin* 0.5 g qid iv, or 1 g bid iv, or 2 g x 1 in perfusion.

Macrolides, lincosamides and streptogramins: Azithromycin* 0.5 g x 1 po, or 0.5 g x 1 iv, or 2 g po in single dose; Clarithromycin* 0.5 g bid po; Erythromycin* 1 g qid, or 1 g qid iv; Roxithromycin 0.15 g bid po, Telithromycin 0.8 g x 1 oral; Clindamycin* 0.3 g qid po, or 0.9 g tid iv; Quinupristin+dalfopristin 7.5 mg/kg tid iv.

Tetracyclines: Doxycycline* 0.2 g x 1 po; Eravacycline 1 mg/kg bid iv; Minocycline* 0.1 g bid po; Tetracycline* 0.5 g qid po; Tigecycline*0.1 g followed by 50 mg bid iv.

Oxazolidinone: Linezolid* 0.6 g bid po, or 0.6 g bid iv; Tedizolid* 0.2 g x 1 po.

Other antibiotics: Chloramphenicol* 2 g qid po, or 2 g qid iv; Colistin* 4.5 MU bid iv; Daptomycin* 6 mg/kg x 1 iv; Fosfomycin* 8 g tid iv or 3 g x 1 po in a single dose; Fusidic acid* 0.5 g tid iv; Metronidazole* 0.5 g tid po, or 0.5 g tid iv; Nitrofurantoin* 50-100 mg tid-qid po; Nitroxoline* 0.25 g tid po; Rifampicin* 0.6 g bid po, or 0.6 g bid iv; Spectinomycin 2 g x 1 im; Trimethoprim 0.16 g bid po; Trimethoprim+ sulfamethoxazole* 1.44 g bid po, or 1.44 g bid iv.

CLINICAL GROUPING OF ABs

Changing the posology and increasing the doses of ABs, where necessary and possible, contributes to maintaining a reasonable susceptibility of pathogenic bacteria, maintaining the efficiency of ABT. Clinicians should rationally use ABs and shall apply, where appropriate, the new recommendations, which move the category of bacteria with intermediate sensitivity, from resistant to susceptible [2]. Consideration should also be given to the recent clinical classification of ABs in the three groups: for infections with common bacteria (Access group antibiotics), with resistant bacteria (Watch group antibiotics) and the reserve (Reserve group antibiotics), as well as special antibiotics (eg anti-tuberculosis, anti-leprosy), according to the WHO Model List Essential Medicines, no 20/2017 and 21/2019 [1]. The practical advantage for medicine is immense, but if in medical practice it is found that many clinicians still do not follow the rules of good practice of ABT, action will have to be taken. One of them could be, in extremis, that according to the recommendations of WHO 2017 and 2019, ABs from the therapeutic groups should be prescribed according to the competence of the doctor:

ABs group I, usual, by any doctor with the right to free practice,

ABs group II, for resistant or multidrug-resistant bacteria, by specialized clinicians,

ABs group III, reserved, by primary care clinicians,

Special ABs, by the clinicians, according to their specialty.

Obviously, "off-label prescriptions" could represent the exception, in justified cases; As an example, it has been found that the antiparasitic Metronidazole is also the most effective antianaerobic AB.

There are also some ABs, which, although authorized, are so little used in national medical practice that they could be considered as new ABs. It is interesting to see how many new ABs will be authorized in the coming years. It is very important to discover new anti-infective drugs, both with new molecules, and by highlighting antibacterial and/or antibiofilm properties in pharmaceuticals known for other pharmacological effects. This type of applied research involves smaller investments, and the results can be faster, going up to the patenting of new innovative anti-infective drugs, in the near future.

CONCLUSIONS

Antibiotherapy is constantly dynamic, in order to keep up with the adaptation of pathogenic bacteria to the selection of pressure exerted by ABs, We must be aware of these trends, apply new acquisitions in the field of drugs, dosage conditioning and dosage, to help maintain an effective ABT, and abandon, where appropriate, the old methods and habits. Unfortunately, it is possible that some healthcare workers may continue to prescribe ABs, not for treating the patient, but for their own peace of mind and as well as their patients' or for any other reasons.

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ORIGINAL ARTICLES

A practical approach of the pediatric patient with obstetrical brachial plexus palsy starting from 28 cases – Single-center experience over 6 years

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Abstract: *Neonatal brachial plexus palsy is a complication that occurs during difficult vaginal delivery or due to unexplained causes, probably prenatal in which some or all brachial plexus branches are affected (avulsion, rupture, stretching). A cohort of 28 cases evaluated in our clinic in 6 years and 6 months (Jan 2007-July 2013) from the clinical and paraclinical point of view, treatment, and the outcome were analyzed. 17 (60.7%) of the patients were boys and 11 (39.2%) were girls. 14 (50%) had right brachial plexus palsy, 12 (42.8%) had left brachial plexus palsy and 2 (7.1%) had bilateral involvement. 5 (17.8%) of them had reconstructive surgery done. 11 (39.2%) had good to complete recovery. The purpose of this paper is to underline the importance of early presentation for neurological evaluation and the beginning of physical therapy.*

Keywords: *brachial plexus palsy, physical therapy, reconstructive surgery of brachial plexus*

INTRODUCTION

Neonatal brachial plexus palsy (NBPP) – is a functional disturbance due to brachial plexus injury, characterized by deficits in the areas served by the brachial plexus branches (C5 to T1). The following NBPP types have been described according to the lesion's level: total (C5 to T1) or partial – superior (C5 to C6), middle (C5 to C7), inferior (C8 to T1). The name of this dysfunction is controversial, some authors calling it obstetrical, because the trauma during a difficult delivery is the most common cause, while others name it congenital including obstetrical and unexplained cases due to a prenatal cause. In these circumstances, an umbrella term will be NBPP [2, 3, 6, 9]. Some or all the fibers of the brachial plexus branches suffer stretching, rupture or avulsion. The incidence is 0.1 - 0.4% of live births and has remained relatively constant over time [3, 6, 7, 9]. A multidisciplinary approach and consensus are needed for a better outcome.

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OBJECTIVES

This paper aims to analyze the cohort of patients with NBPP followed in the Pediatric Neurology Department, to highlight the variability of NBPP symptoms at presentation, to identify and correlate specific items related to clinical signs, investigations, and outcome, and to establish a protocol of diagnosis and follow-up for children with NBPP, according to the international recommendations adapted to the local needs. We propose a treatment algorithm, allowing optimum outcomes for these children.

MATERIALS & METHOD

The archive of the Pediatric Neurology Clinic was retrospectively analyzed over 6.5 years (January 2007 – July 2013), selecting the patients with NBPP. Approval from the Local Ethics Committee was obtained. Narakas's classification was used at the first admission to diagnose the NBPP type: superior, middle, inferior, and total [7]. The clinical status was registered at different times: at presentation, at 3, 6, 12 months follow-up. Active motor scale [1, 2, 4, 8, 9], Toronto score [1, 2, 4, 8, 9], and protocol for obstetrical brachial plexus palsy (Hospital for Sick Children Seattle) [8] were retrospectively applied, using registered neurological examinations, reports of physical therapy specialist, from files. Laboratory findings, EMG and nerve conduction velocities of the upper limb and brachial plexus MRI (performed if needed), treatment choices, and their results were also evaluated [10]. We designed a separate questionnaire for present status evaluation, including data about active movements, strength, trophic changes summarized in a score. The prognosis was quantified as follows: score 0-12 – poor recovery, score 13-24 – moderate recovery, 25-36 – good recovery, or complete remission. Statistical analysis used the SPSS17 program.

RESULTS

28 patients with the diagnosis of NBPP were identified between January 2007 – July 2013.

Evaluation schedule. All patients came at 3 months for neurological evaluation; 23 children (82.1%) returned for reevaluation at 6 months, and only 10 (35.7%) at 12 months.

History and clinical data. Among them, 17 were boys (60.7%) and 11 girls (39.3%), 4 days to 13 years old at admission. 14 (50%) cases had the right side, 12 (42,8%) had left side, and 2 (7.1%) had bilateral brachial plexus involvement. 6 (21.4%) of the cases have total NBPP and 22 (78.6%) – partial – superior (C5-C6) or extended superior (C5-C7) NBPP. All the children were born spontaneously, 13 (46.4%) of them had > 4000 g, another 8 (28.5%) had birth weight > 3900 g and eight (28.5%) patients had 2800 – 3900 g, but with difficult labor and delivery. Four patients (14.2%) had Claude Bernard Horner syndrome (CBH) and 3 (10.7%) had further associated pathology – global developmental delay, diplegia, microcephaly in evolution. 13 patients (46.4%) were first evaluated in our clinic in the first month of life, 12 (42.8%) between 1 and 3 months, 2 (7.1%) between and 1 (3.5%) after 6 months. 2 (7.1%) mothers had prenatal hypertension and hypertension during pregnancy, but that didn't affect the children.

Investigations. 6 patients (21.4%) had electrophysiological studies (EMG and nerve conduction velocities) performed in the first 2 months of life, in 3 cases (10.7%) repeated at 3 months and in 5 (17.8%) after 6 months. 6 patients were evaluated with MRI of the cervicobrachial region. 3 (10,7%) of them had avulsion (preganglionic lesions, usually associate pseudomeningoceles), 1(3.5%) had rupture (usually are postganglionic lesions, could associate pseudomeningoceles) and 2 (7.1%) had stretching/ elongation (postganglionic lesions, neuroma or hematoma or diffuse lesions). 4 (14.2%) of the children who had brachial plexus surgery had pre- and postganglionic lesions and 1 (3.5%) had postganglionic lesions.

Treatment. All patients received physical therapy, Vojta method (in our clinic); among them, two patients (7.1%) had already started physiotherapy through the Bobath method (in another Clinic). 12 patients (42.8%) were included in an intensive physical therapy program and 16 (57.1%) received inconstant or for a short period physical therapy. Good recovery was registered in 6 patients (21.4%) who performed intensive physical therapy and 5 patients (17.8%) among those with inconsistent therapy. Five patients (17.8%) had surgical reconstruction of the brachial plexus, 3 (10.7%) of them had total brachial plexus palsy and two (7.1%) had extended superior brachial plexus palsy.

Follow-up. Evolution at 3 month after physical therapy, evaluated with Toronto score (T score) showed score > 3.5 in five patients (17.8%) and values < 3.5 in 23 patients (82.1%). From the 23 patients (82.1%) with T score < 3.5, 6 (21.4%) were evaluated for surgical procedure and five (17.8%) were operated, in one (3.5%) case it was not necessary. The rest continued physical therapy. 18 patients (64.2%) with a T score <3.5 were not evaluated for surgery because they did not come for their scheduled evaluation/ follow-up.

Outcome. Eight (28.5%) patients had a poor recovery, 9 (32.1%) patients had a moderate recovery, 11 children (39.2%) had good recovery, among them 6 (21.4%) with complete remission. All of the children with good recovery to complete remission had superior or extended superior

NBPP.

DISCUSSIONS

Most of the children (18 – 64.2%) did not comply with the follow-up schedule. That is because they recovered with physiotherapy or spontaneously or because they were lost to follow-up. The last evaluation, consisting of a questionnaire applied on the phone showed that the majority had a good outcome, most probably because most of the cases were mild, superior type, and a good response to therapy (even those who have made physical therapy at home with their parents). Besides, we could not compare the types of reconstructive surgery, because the number of children undergoing surgery was too small (5). Among them, 4 children had reconstruction of the brachial plexus, and for one patient neurotomy was performed (the fibrous sleeve around the brachial plexus was removed).

Figure 1: Algorithm for diagnosis and treatment of NBPP

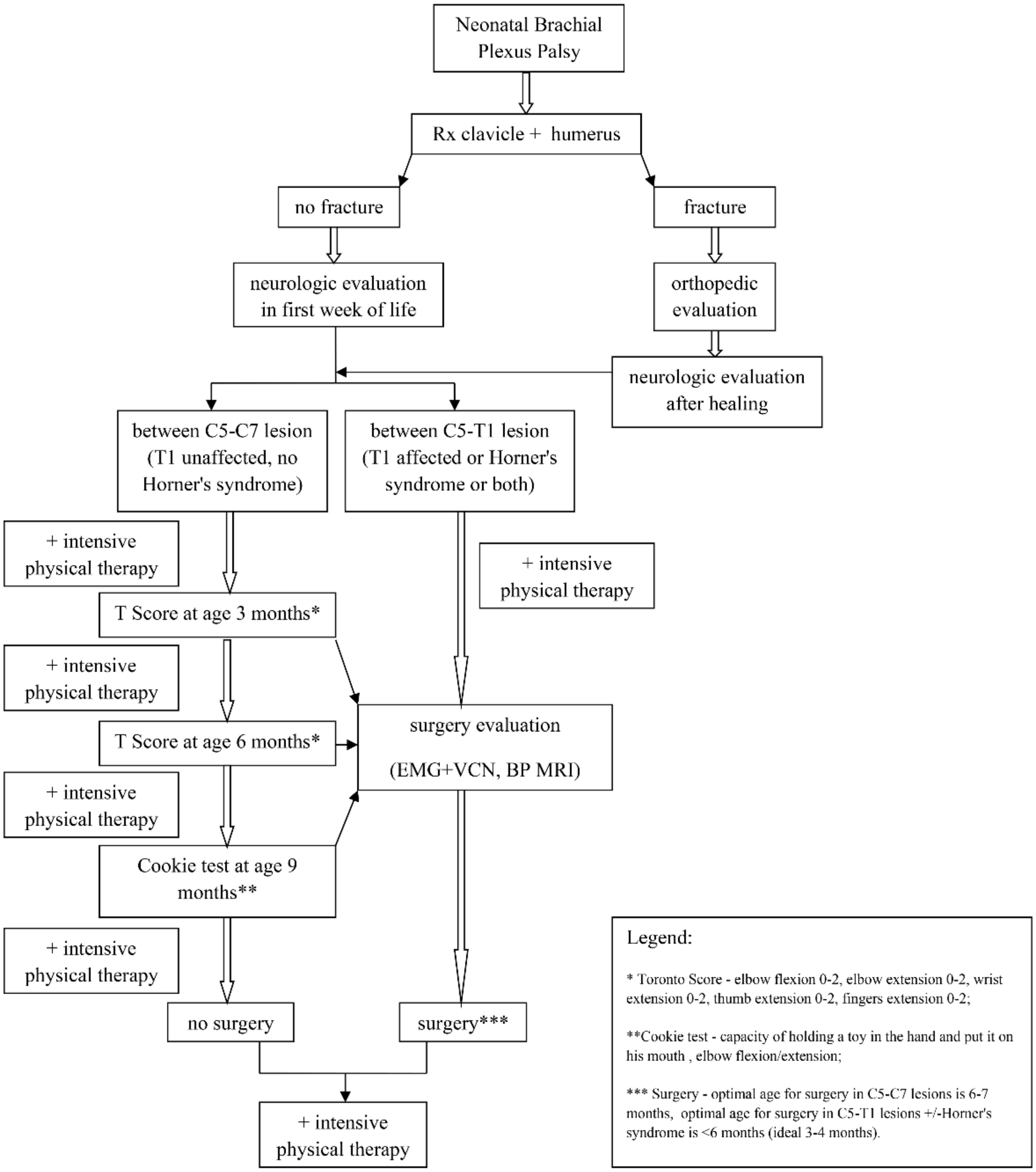
Neonatal Brachial Plexus Palsy
Rx clavicle + humerus
no fracture
fracture
neurologic evaluation in first week of life
orthopedic evaluation
neurologic evaluation after healing
between C5-C7 lesion (T1 unaffected, no Horner's syndrome)
between C5-T1 lesion (T1 affected or Horner's syndrome or both)
+ intensive physical therapy
T Score at age 3 months*
+ intensive physical therapy
+ intensive physical therapy
surgery evaluation (EMG+VCN, BP MRI)
T Score at age 6 months*
+ intensive physical therapy
Cookie test at age 9 months**
+ intensive physical therapy
no surgery
surgery***
+ intensive physical therapy

Legend:

* Toronto Score - elbow flexion 0-2, elbow extension 0-2, wrist extension 0-2, thumb extension 0-2, fingers extension 0-2;

**Cookie test - capacity of holding a toy in the hand and put it on his mouth , elbow flexion/extension;

*** Surgery - optimal age for surgery in C5-C7 lesions is 6-7 months, optimal age for surgery in C5-T1 lesions +/-Horner's syndrome is <6 months (ideal 3-4 months).

Age at first presentation in NBPP is of paramount importance for the subsequent treatment choice. It is important that the children with BPP to be early referred – even from birth for evaluation and treatment, in this way – T score at age 3 months will be the most valuable tool for an indication of early surgery if needed.

Cervico-brachial MRI was performed only in some of the patients with a T score <3.5. The importance of MRI is high for the treatment choice – in case of the complete avulsion, surgery is indicated.

Although some studies report 80-90% of the good recovery in unilateral, superior brachial plexus palsy, in our cohort we had only 11 (39.2%) patients with good recovery, probably due to inconsistent physical therapy and non-compliance to scheduled evaluations.

The results cannot be applied in the general population due to the small number of patients analyzed. A larger, prospective study is needed in the future to fill in the gaps. To be noted the good clinical practice of neonatology specialists, pediatricians, and general practitioners who evaluate the children in the first days of life and immediately take action, sending them immediately to pediatric neurology for evaluation and treatment.

– the age of first neurological evaluation and initiation of physical therapy decreased and the number of evaluated children with NBPP increased in the last few years in Romania although this is purely based on anecdotal data as no national registry exists for NBPP. That is why we strongly agree that the first protocol of diagnosis and treatment was needed in our country (Figure 1). Of course, that the experience of the next few years and the discoveries in the field will help us to permanently improve the protocol for better practice, according to the international rules.

CONCLUSIONS

The use of protocols for diagnosis and follow-up makes us „speak the same language”, have the same criteria for diagnosis, evaluation, and treatment, with some particular situations due to the local needs.

Early diagnosis and treatment are important because the outcome is better – than raises the quality of life of these patients and the addressability for physical therapy. Vojta therapy has good results in NBPP. Better information of the patients concerning the steps of evaluation of NBPP and the importance of compliance with the protocol is critical for a better outcome and for avoiding a permanent deficit.

Reconstructive surgery must be done in selected cases for a better recovery.

This is a pilot study, with a proposal of protocol for diagnosis and treatment, according to the international protocols in force, but larger, prospective studies are mandatory to complete and refresh the existing data (Figure 1).

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ORIGINAL ARTICLES

Aptamer as a proper alternative instead of monoclonal antibody in diagnosis and neutralization of menacing biological agents

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Abstract: *Of the major threats to contemporary mankind, is the use of very dangerous and lethal biological agents as the biological weapons. The first step in confronting with this serious threat after prevention, is the accurate and rapid detection of this agents and neutralization of them. In this article, the role of molecules known as aptamer, has been studied in biological defense against these menacing biological agents. Traditional methods for detection of these agents are based primarily on immuno-affinity assays and the use of antibody molecules. While the modern methods, based on aptamer-affinity assays, are being replaced with traditional methods, due to the abundant advantages of them. The selection and preparation method of specific aptamer with high binding affinity to these biological agents is known as SELEX and the use of magnetic nanoparticles to perform this procedure (Mag-SELEX) is very common. The isolated aptamers with high specificity can also be used in neutralization and inhibition of menacing agents function, in addition to, quick and accurate diagnosis of these agents, utilizing them in nano-biosensors, based on aptamers (as the nano-aptasensors).*

Keywords: *Aptamer, SELEX, nano aptasensor, diagnosis and neutralization, menacing biological agents*

INTRODUCTION

Nowadays virulent and dangerous biological agents which can be used as biological weapons are becoming a considerable threat to us [1]. Some of very important agents used in bioterrorism are namely botulinum neurotoxins which are some of the strongest toxins known to men and produced by the anaerobic bacteria called clostridium botulinum [2]. Botulinum neurotoxins, especially three types of A, B and E, have been known as dangerous biologic weapons. Among these three serotypes, serotype A is more potentially virulent [3]. The first action against these very dangerous biological agents, after prevention is to identify and detect the agent accurately and rapidly and then to neutralize it [4]. Modern methods of identification and neutralization is based on molecules called aptamer [5]. In this article we will first introduce aptamer and chemical modifications possible to make on this molecule to approach certain purposes, and compare them to antibody molecules as a conventional tool in diagnoses and neutralization of the biological and viral agents or their exclusive products (such as toxins and etc.) and we will interpret concisely the works done around the world.

APTAMER, AS A NEW MOLECULAR DEVICE

Aptamer is generated from the Latin word of "Aptus" which means "to fit". In fact, aptamers are oligopeptides or single stranded oligonucleotides (DNA or RNA) with high affinity for binding to target molecule (Figure 1). They create a bond with high specificity through their third structure to a target molecule [6]. In this article we discuss about oligonucleotide aptamers, especially DNA aptamers. By development of

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molecular sciences and setting up the SELEX technique, the specific DNA/RNA aptamers have been separated for binding to many molecules. These molecules are consisted of nucleic acids, amino acids, sugars and their polymers, and also mineral compounds, enzymes, growth factors, antibodies, the molecules binding to cells, chitins and even viral components [7].

Figure 1. A schematic representation of the binding of aptamer molecule to the target protein

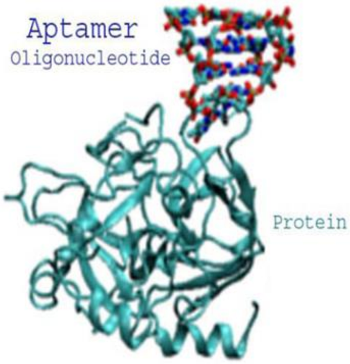

Natural aptamers in living systems

Naturally, aptamer is found in bacteria, archaea bacteria, fungi and plants, and acts as a part of riboswitch structures and often plays a role in regulation of expression of the genes involved in production of vitamins (co-enzymes) and in some cases is efficient in regulation of amino acids' production [8].

Evolution of aptamers in non-living systems

In 1990, Gold and Tuerk attained RNA fragments which were able to bind to T4 bacteriophage DNA polymerase. These oligonucleotide fragments were selected by a new combinatory technique called SELEX (systematic evolution of ligands by exponential enrichment). The meaning of this expression is that among a collection of aptamers with various sequences, it is possible to attain the specific sequence with high binding affinity to target molecule through some screening steps [9].

Aptamer library (AptLib)

The initial library for performing the SELEX process includes single stranded DNA (or RNA) oligonucleotides with a central core (approximately 40 bases) containing random and variable sequences (more than 1,015 different fragments), in which every oligonucleotide is consisted of certain flanking sites in both ends that are fixed positions for primer attachment [10]. In the middle of these constant regions (i.e. the central core), sequences of 15 to 75 (e.g. 40) nucleotides are randomly situated and every four base (A, G, C, T) may be situated in that position with the same probability [11]. These fragments are prepared by frequent synthetizations, with mixture of four types of nucleotides, coincidentally and by synthetization machine (will extendedly be discussed in results and discussion section) [12].

Aptamer library screening by SELEX cycles

In this stage, fragments with the highest affinity and specificity for target protein is selected from the aptamer library, through several SELEX cycles (usually 13-15 cycles). Repetition of these cycles is required for attaining the suitable affinity [13].

Using magnetic nano-particles for screening by SELEX cycles (Mag-SELEX)

Some of Mag-SELEX benefits include:
1) No requirement for special facilities or equipment,
2) Easiness of gathering and separation of aptamers by SELEX process only using a magnet or a simple centrifugation,
3) Possibility of using nano particles for two purposes: stabilization of target protein and also making aptamers single stranded in each cycle,
4) Possibility of performing the methods such as flow cytometry for evaluation of affinity,
5) Needing to little amount of protein in this technique (around 100 micrograms),
6) Possibility of coating nano particles with streptavidin for very strong interaction with biotin and thus prevention of wastage of stabilized protein during the washing process in which the conditions becomes tougher by rising cycle number of SELEX,
7) Expansion of protein contact surface with existent aptamers in the solution and rise in SELEX efficiency and abate of errors,
8) Easiness of stabilizing protein on these Nano particles.

It is worth mentioning that the main procedure of SELEX in different methods such as cell SELEX, CE SELEX, microfluidic SELEX (M-SELEX) and etc. are the same [14-16].

Corrections and chemical rearrangements of aptamer molecules

For increasing the stability and resistance of these molecules in clinical use, labeling or increasing the performance of these molecules, some modifications are made on these aptamer oligonucleotides.

The most important modifications include:

1) Corrections on carbon 2' of sugar including: a) Adding fluorine and amino group to pyrimidine bases. b) Adding hydroxyl group to purine bases. c) Adding o-methyl group to purine and pyrimidine bases. In clinical applications, 2'-O-

methyl oligomers are in priority, for they are less toxic than other corrections on carbon 2' (these structures exist naturally in ribosomal RNAs) [17].

2) Capping of 3'-terminus of oligonucleotide including: a) Attachment of inverted thymidine (3'-idT) to 3'-end of oligonucleotide and creation of 3'-3' connection. b) Attachment of biotin to the 3'-end [18]. 3) Capping of 5'-terminus of oligonucleotide including: a) Adding alkyl-amine group to 5'-end phosphate. b) Attachment of 5'-end of oligonucleotide to polyethylene glycol or on surface of liposome. c) Adding cholesterol, fatty acids and protein [19]. 4) Correction and chemical rearrangement of phosphate group which means replacement of sulfur group instead of the oxygen that doesn't attend in phosphodiester bond (thio-aptamer). 5) Adding signal peptides to aptamers such as penetrating peptides including Tat and antennapedia and fusogenic peptides [20]. 6) Labeling aptamers with fluorescent molecules (for example derivatives of fluorescein such as FITC, FAM and etc.) for flow cytometry or with biotin molecules for stabilizing aptamer on solid surface coated with streptavidin (for performing ELISA or in biosensors design) [21].

It's rarely seen that the corrections and chemical rearrangements made for resistance improvement are effective on the main features, such as binding affinity and third structure. That's while in performance most of these modifications are possible in primary library before SELEX [22].

Comparison of aptamers with antibody molecules

1) Antibodies' half-life is limited, while aptamers are very stable despite of the chemical rearrangements of their nucleotides. 2) Antibodies unlike aptamers are sensitive to environmental conditions such as temperature, pH and etc. 3) Antibodies have irreversible denaturation, that's while aptamers have denaturation and renaturation capacity because of their nucleic acid structure. 4) Antibodies unlike aptamers are immunogenic and this causes their rapid elimination in the body in clinical applications. 5) Size of antibodies unlike aptamers is massive (around 150 KDa) which causes long-term pause of them in blood and slow penetration in tissues and as a result, causes bone marrow toxicity in radioactive form [23]. 6) The procedure of antibody production is very difficult and for aptamers it is only difficult in the first time, during the panning steps of SELEX and then by sequencing of the aptamer selected from library, it is possible to synthesize the sequence attained easily. 7) Possibility of using aptamers in the chips that are based on DNA, so that in protein staining process aptamers do not get color. 8) Impossibility of antibody production for little non-immunogenic molecules; and possibility of aptamer production regarding to these molecules. 9) Factors like pH variation, salt concentration, heat, and chelating agents may help the process of aptamer reactivation and creation of functional structures in them. But for antibodies these agents would cause denaturation of them. 10) Stabilization of aptamers and labeling them are easier than antibodies.

These prominences have caused them to be widely used as molecular detectors in biosensors based on aptamer molecules (apta-sensors) and nanobiosensors based on aptamer molecules (nano-apta-sensors) [24].

AN OVERVIEW OF THE RESEARCH WORKS DONE UNTIL NOW, IN RELATION TO APTAMERS AND THEIR APPLICATIONS

Selection of aptamers for binding to antibodies, proteins and growth factors

- In 1996, Wiegand et al. succeeded in isolating aptamers) single stranded DNA and also RNA with 2'-amino chemical arrangement) against human IgE to inhibit the interaction of this molecule with "FcεRI" receptor on surface of basophilic leukemia cells of rat (as a new drug for the treatment of allergic diseases) [25].

- In 1995, Doudna et al. succeeded in selecting a RNA aptamer to detect the main epitope of insulin receptor antigen on surface of human cells [26].

- In 1995, Geling et al. selected RNA aptamers (with 2'-amino chemical arrangement) to inhibit the activity of fibroblast growth factor [27].

- In 2002, Lupold et al. isolated the RNA aptamer detecting prostate specific membrane antigen (PSMA) on surface of prostate cancer cells and labeled that aptamer by fluorescent molecules to observe its ligand-binding function in condition of "in vivo" [28].

Disruption of translation pathway signs of lymphocytes by Aptamar

- In 1997, Kubic et al. separated the binding and inhibiting RNA aptamer against the interferon gamma (INF-γ) which caused the inhibition of MHCI and ICAMI expression induction by INF-γ [29].

Selection of anti-viral aptamers

Some of these aptamers resulted in the elimination of virus cell cycle by interaction with the proteins necessary for virus proliferation and some of them were able to block surface binding proteins and consequently resulted in the prevention of attachment and entry of viruses into cells [30].

- In 1995, Pan et al. separated the detecting and neutralizing RNA aptamer against Rose Sarcoma virus to inhibit the binding of the virus [31].

Selection of specific aptamers for binding and inhibiting the function of enzymes

- In 1998, Brindonau et al. succeeded in isolating 2'-amino RNA aptamer by SELEX (as a new drug) for inhibition of phospholipase A2 enzymatic activity in patients [32].

- In 2011, Tzu-Wang Chang et al. succeeded in isolating the RNA aptamer specified for inhibition of enzymatic activity of botulinum neurotoxin type A [33].

Selection of aptamers for detection of small molecules

- In 1998 and some years later, one DNA aptamer for Sulforhodamine B, two RNA aptamers for sulforhodamine B and fluorescein and one RNA aptamer for malachite green were separated by Wilson and Szostak [34].

Aptamers for cell diagnosis

- In 1999, Bruno et al. detected Bacillus anthracis spore by a single stranded DNA (ssDNA) aptamer using magnetic beads [35].

- In 2005, Qin Pan et al. isolated the RNA aptamer detecting the strains of Solmonella enterica serovar Typhi containing type IVb pili [36].

- In 2007, Fan Chen et al. isolated the ssDNA aptamer detecting a virulent strain of Mycobacterium tuberclosis called H37Rv [37].

- In 2006, Vivekananda et al. isolated 25 sequences of the ssDNA aptamer identifying surface bacterial antigens of Francisella tularensis japonica [38].

- In 2007, Ikanovic et al. designed an optical biosensor based on aptamer for detection of Bacillus thuringiensis bacteria [39].

Aptamers as tools for drug delivery

- In 2006, Farrokhzad et al. prepared nano-capsules of poly lactic acid (PLA) containing drug and linked to them the special aptamer related to prostate cancer cells through the poly-ethylene glycol linkage (PEG) [40].

Using aptamers for designing biosensors (apta-sensors) and nano-biosensors (nano-aptasensors)

- In 2011, Lakshmi et al. designed a chemical-resistant nano-aptasensor, for detection of anthrax PA toxin based on single wall carbon nanotubes (SWNT) [41].

- In 2012, Jeffery Degrasse et al. isolated a selective DNA aptamer for detection of Staphylococcus aureus enterotoxin B (SEB) for designing nano-aptasensor on the basis of surface plasmon resonance (SPR) (Figure 2 – as seen in the figure, biotinylated apatamers have been stabilized on streptavidin-coated gold nanoparticles, and the above nanoparticles are also fixed onto gold discs and by use of corresponding device, signals from surface plasmon resonance (SPR) changes are measured after detecting the target ligand by aptamer.) [42].

Figure 2. A schematic representation of aptamer molecule application in the design of nano-aptasensor.

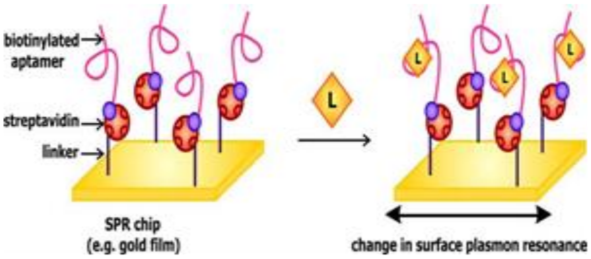
biotinylated aptamer
streptavidin
linker
SPR chip (e.g. gold film)
L
change in surface plasmon resonance

APTAMER AS A PROPER TOOL IN MODERN METHODS OF DIAGNOSIS

Nowadays traditional methods based on immune-affinity assays, in which antibody molecules are used, while transposing with new methods based on aptamers-affinity assays [43].

Functions and features of modern determination methods based on immune-affinity assays:

1. Aptamers are produced in vitro conditions and they do not require any living organisms, to which they allow us to use any molecules regardless of it being toxic for the organism and separate exclusive aptamers for determination.

2. After separation and selection of exclusive aptamers by SELEX, we could easily reproduce and mass-produce it in high purity of specified aptamers.

3. Mass production expenses are very low and they have proved to be cost effective.

4. Nucleic acids (including DNA-aptamers) by having some functional groups can more easily be adapted to their target exclusive ligand. Without any malfunctions or lessening in tendency for binding with exclusive ligand in the specified domain.

5. Aptamers are originally stable in a broader domain of conditions.

6. Aptamers have very high tendency to synthesize and dedicate compared to antibodies.

7. Aptamers with high affinity can be used for determination and detection of different sorts of ligands such as toxins, allergens, sugars (that are weak immunogens), haptens, nucleic acids, proteins and different kinds of little biological and non-biological molecules like morphine and etc. can be separated and used. It is while in previous methods there were many limitations regarding to this matter [44].

USING APTAMERS MOLECULES IN DETECTION AND NEUTRALIZATION (INHIBITION) OF BOTULINUM NEUROTOXINS

As latterly mentioned, until today there have been many researches around the globe on these oligonucleotides with detection and neutralization purposes specifically on botulinum neurotoxins (such as type A).

Reports have indicated that aptamers molecules separated from library are suitable and proper for this matter [45].

Diagnostic purposes

In diagnosis of botulism agent as the one of biologic threat factors, we can separate some aptamers with high binding tendency with SELEX method. Aptamers separated to interact with botulinum toxin and also with different neurotoxin regions including catalytic domain can be very effective in diagnosis purposes. But catalytic domains are more efficient and useful in diagnosis purposes for neurotoxin types specially type E [46].

Inhibitory goals

In inhibiting and neutralizing this extremely lethal factor we can get our hands on molecules with inhibiting and neutralizing effects on operational sites, by investigating inhibiting effects of separated aptamers for exclusive connections with high tendency to catalytic site [47].

These aptamers might hinder the activation of this operational site by connecting to de-activated catalytic site of the toxin’s body or by binding themselves to the freed catalytic site and cause the enzyme activity to stop [48]. In fact, inhibiting this position’s enzymatic function leads to restraining the toxin’s function. But antidotal aptamers can do the neutralizing process by connecting to other sites like connector sites and actually prevent the toxin from connecting to the cell and entering it [49].

Separating the aptamer against the catalytic domain of botulinum neurotoxin for recognizing and counteracting this toxin

Choosing the catalytic site from other operational sites of botulinum neurotoxin is important according to reasons mentioned below:

1. Results gained from conducted studies around the world show that this operational site is very suitable for getting the recognizing and counteracting goals.

- In 2011, Tzuu-Wang Chang et al. separated RNA-aptamer exclusively for inhibiting botulinum neurotoxin type A enzymatic activity [50].

- In 2009, Fang Wei et al. used separated aptamer for recognizing botulinum neurotoxin type A in designing electrochemical sensors [51].

- In 2009, Xinhul Lou et al. separated the special conjugative aptamers to catalytic domain of recombinant botulinum neurotoxin type A for recognizing this neurotoxin [52].

2. The main and operational part of this neurotoxin is the catalytic part and the quality of this neurotoxin is basically related to this part, in a way that said toxin will have no effect or special toxic function without this part [53]. So in conclusion the diagnosis and neutralizing value of this location will be much higher than other ones. And recognizing this location will lead to the certain recognition of infection to this type of neurotoxin, whereas recognizing other locations won’t necessarily mean that and it’s possible that other locations be used as recombinant with other factors [54].

3. The unique structure of botulinum neurotoxin type E and the importance of epitopes situated on this location and symmetric bond to the connector site, in a more general sense epitope makes the selection of this site for the toxin for this serotype exclusively more suitable (Figure 3 – the red represents the catalytic region, the green color indicates the translocating region and the yellow color indicates the binding region of the neurotoxin. As can be seen, both the catalytic and binding regions are located on one side of the translocating region, not on two sides as seen in the other types.) [55].

RESULTS AND DISCUSSION

Studies have shown that aptamers with central core of 75-100 base length are not suitable aptamers and on the other hand aptamers with the central core of lesser than 15-20 base also cannot be suitable aptamers [56]. If the length of aptamers fragments is longer than the above:

Figure 3. A schematic representation of second structures of neurotoxin botulinum type E and a symmetric relationship between catalytic and binding domains of toxin.

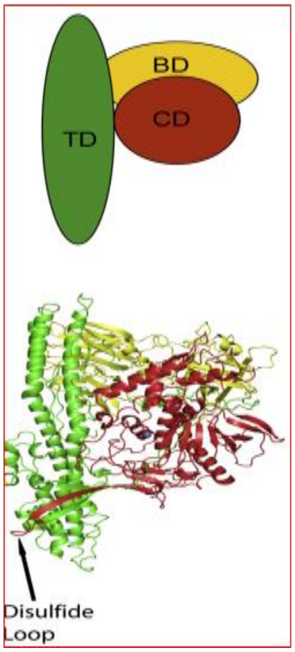

1. The possibility of creating similar sequences with primer in the central core is increased and with binding primer to this central parts and amplification, no particular fragments with shorter length will be the outcome.

2. On the other hand the possibility of connecting flanking sites of 2 sides (primer binding sites) with central sequences of various fragments, is increased and will cause an unhindered length increase and generation of fragments with larger sizes and PCR product of these two processes will be seen as smear.

3. Also the larger of these fragments be their particular operationally will also be decreased. Because after the formation of the 3rd structure there will be more binding sites to bind to target protein that each of which will have different affinity and can bind to our protein from different sites and positions and one of these position might have a high infinity to our target protein but no other position. So in conclusion the average affinity will drop and this means the decrement of specificity (affinity and specificity are two completely separate parameters) [57-59].

If the length of aptamers fragments is shorter than the above:

1. We'll witness a significant decrease in genetically variety of aptamer fragments and this will significantly limit our screening stages.

2. On the other hand the 3rd structures and conformation of aptamer which is the basis of aptamers binding to biomolecules will not form properly or won't form at all and this means the inefficiency of aptamer.

3. Another problem is the drawback in performing PCR and evaluating the target binding which distinguishing it from dimer primer is not easily achievable.

4. On the other side we'll face up to a huge problem in the purification of a production with such dimensions. Studies have shown that lengths about 40 base will be adequate and as we advance to larger or smaller numbers we will encounter the problems mentioned above more frequently although we are free to choose between 15-75 (20-100 according to some sources) (Figure 4) [60-62].

Figure 4. A schematic form of the single-stranded oligonucleotide structure of aptamer with a central core of 40 nucleotides. Scientifically and statistically, there is the probability of having a variety of 440 aptamer fragments (which is 1024 fragments) with a central core of 40 nucleotides, but optimistically virtually this variation is about 1015 aptamer fragments.

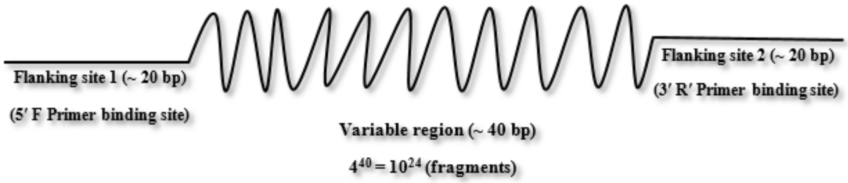
Flanking site 1 (~ 20 bp)
(5′ F Primer binding site)
Variable region (~ 40 bp)
$4^{40} = 10^{24}$ (fragments)
Flanking site 2 (~ 20 bp)
(3′ R′ Primer binding site)

In Figure 5 the schematic form of SELEX cycle on RNA aptamer library is shown. The primary library made of DNA is firstly turned into a RNA library during a transcription process and after being added to nano particles covered with target proteins and performing the stages of washing and reunifying by performing the process of "asymmetric RT-PCR", is transformed to c-DNA and after single threading and removing the non-original strand the next cycle is again continued by transacting on the product mentioned above and creation of a RNA aptamer library [63-65]. It is necessary to perform a stage of negative selection in the presence of similar proteins.

Figure 6 shows the increment of aptamers' affinity as with the progress of SELEX stages, which is evaluable using the

ELASA method (aptamer-based ELISA). Then we can perform a software based test on aptameric sequences using M-fold software.

Figure 5. A schematic representation of SELEX cycle on RNA aptamer library

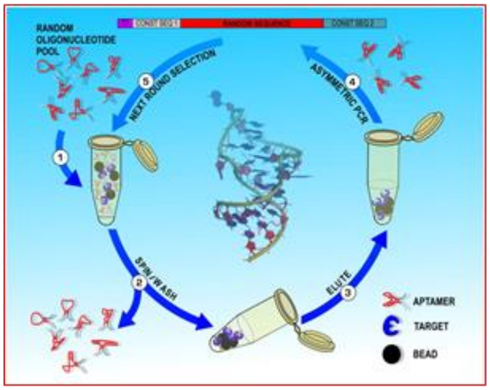
RANDOM OLIGONUCLEOTIDE POOL
CONST SEQ 1
RANDOM SEQUENCE
CONST SEQ 2
5
NEXT ROUND SELECTION
4
ASYMMETRIC PCR
1
2
SPIN/WASH
3
ELUTE
APTAMER
TARGET
BEAD

This investigation will give us the chance to understand how nucleotide changes in the final aptameric sequences causes a change in operational structure [66]. On the other hand, the software itself will predict different kinds of possible operational structures for that aptamer molecule and it will also suggest the best possible form. Sometimes the result of such investigations will show us the existence of a necessary operational site in the central core, which will have a key role in binding to the target protein [67].

Figure 6. Binding affinity of aptamers increases with the progress of the SELEX steps

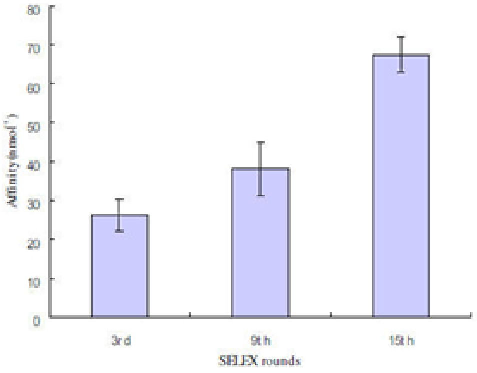

In a more accurate investigation for getting aptamers with higher affinities and higher specificity for binding to target protein we can put this specified operational site in the central core of a new aptamer library and perform the SELEX stages on that library again (Figure 7a and 7b) [68].

Figure 7. A schematic picture of two types of aptamers. 7a: An aptamer library with a central core containing a fixed region that is obtained by software evaluating by the M-Fold software. 7b: A simple aptamer library with a completely variable core

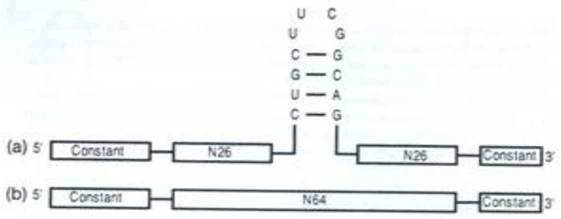
U C
U G
C — G
G — C
U — A
C — G
(a) 5'
Constant
N26
N26
Constant
3'
(b) 5'
Constant
N64
Constant
3'

In Figure 8 the pattern of aptamer nucleotides labeled with digoxigenin (DIG) recognizing the target molecule is shown, so in part A the flow cytometry is used for identifying the interaction between aptamer and target molecule and in part B the chemiluminescence is used. Part C shows the chemical formula of digoxigenin [69].

Figure 8. A) Digoxigenin labeled aptamer identifying the target molecule and measuring the binding by flow cytometry B) Digoxigenin labeled aptamer identifying the target molecule and measuring the binding with the ELISA method C) The chemical formula of digoxigenin.

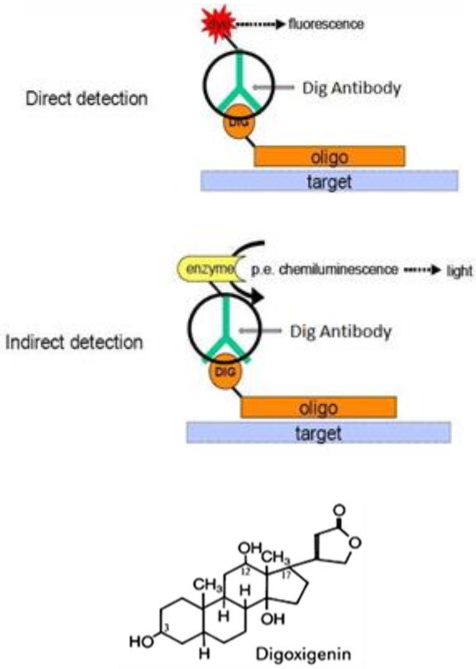
fluorescence
Direct detection
Dig Antibody
DIG
oligo
target
enzyme
p.e. chemiluminescence
light
Indirect detection
Dig Antibody
DIG
oligo
target
OH
CH3
CH3
H
H
OH
HO
H
12
17
3
Digoxigenin

In Figure 9 an image of the function of flow cytometer machine is shown. In a way that the sample containing the aptamers marked with fluorescent (marked aptamers bound to nanoparticles) is passed through a fluorescent sensor and after radiating laser light on that the number of marked nanoparticles are counted [70].

Figure 9. Flow cytometer device operation

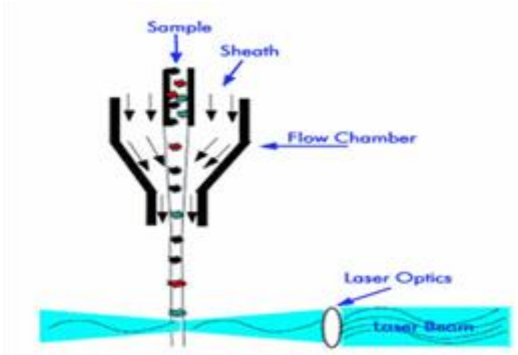

CONCLUSION

The most recent discoveries related to aptamer molecule shows the existence of particular properties and unique attributes in this biomolecule for the means of getting to the considered goal and the beneficial role of this molecule as a powerful tool for recognizing and neutralizing (inhibiting) the threatening factors shows great promise. These days the traditional methods for recognizing the biological factors based on immuno-affinity assays in which antibody molecules are used are being replaced with the new methods based on aptamer-affinity assays. The selected aptamers during the different stages of SELEX, with the ability of creating exclusive bonds and high tendency to interact with operational sites of biologic factors, in addition to having precise and fast recognition of this factors e.g. utilizing them in biological nano-sensors based on aptamer (nano-aptasensor), they could also be used in neutralizing and inhibiting the function of these threatening biological agents.

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VARIA

Are there only records of success in urology, or are there errors, mistakes, and risks as well?

Viorel Tode

The history of urology dates back to the antiquity, before Christ. The panels decorated in low relief of Saqqara, located at the Institute for the History of Medicine in London, depict the oldest surgical procedure in the world – the circumcision – dating from 2200 BC.

Hippocrates knew about genito-urinary disorders and treated them with medication, leaving bladder stones to be treated by lithotomists.

Cystolithectomy is also known to be dating back to ancient times, having been performed in Egypt, the Middle East, and Ancient Greece.

Urology has always been and still is, an exceptional profession, requiring both intelligence and effort in scheduling and executing the necessary techniques, in assuming risks, and in accomplishing spectacular results.

I will only mention here several of the events that marked the exceptional evolution of urology: defeating pain using anesthesia, progresses of pathological anatomy, the development of the cystoscope by Nitze and the beginnings of endoscopic explorations, the use of Roentgen rays in medicine, Pasteur's contribution to the study of microbes and Lister's in asepsis and antisepsis, the renal transplantation – using which, the urologist gives another lease of life to the patient – the tremendous achievement of Mathias, Jaboulay and Alexis Carrel, percutaneous, laparoscopic and robotic urology as well as other exceptional accomplishments.

The title of this article is addressed as a question: are there only records of success in urology, or are there errors mistakes, and risks to take as well?

However, the word urology can be replaced by any other specialty: surgery, gynecology, ENT, ophthalmology, and why not even internal medicine, neurology, psychiatry, endocrinology, family medicine, and so on.

Hippocrates said: "In medicine, the one who has to be praised is the one who makes the fewer mistakes".

Reflecting upon my professional life, recalling now at senescence, I concluded that I remember less about my many successes in healing patients, but have more vivid memories of some intra or postoperative complications that I have lived intensely.

One can hardly forget, for instance, bleedings that were hard to contain, the slip of a ligature on a vascular pedicle, a vena cava or iliac tear, a large posttraumatic retroperitoneal hematoma, a complex kidney rupture, a pelvic fracture that affects the urethra, urinary bladder or rectum, a postoperative urinary fistula and so on.

In 1971, in medical literature, the second edition of a valuable work by Academy Professor Th. Burghele was published in French – "Erreurs, fautes et risqué en Urologie", from which I will cite only the title of the first chapter – The complexity of urology, factors of error and faults (La complexite de l'urologie, factor d'erreurs et de fautes) and the title of the last chapter – The ontological aspects of faults and errors in urological practice (Les aspects deontologiques des fautes et des erreurs de la practique urologique).

Specialty medical journals are rich in articles presenting, and rightfully so, the successes accomplished by urologists, however, very few publish the occasional failures: errors, mistakes, and risks met or faced during surgery. Professor Th. Burghele once introduced a chapter in the journal "Surgery" – deaths on the surgical table – but after a few case studies, no one published anything related anymore.

It would be difficult for me to believe that in medical practice, one could fully believe one never made a mistake. Medical mistakes in general, and surgical-urological ones in particular, given the human element on which the work is being done, can sometimes generate severe consequences and are thus not admitted.

Momentous errors punishable by law notwithstanding, some mistakes often look insignificant, that occasionally pass undetected by some and that only a urologist in the know would notice. Those are the kinds of mistakes that sometimes would genuinely shake the morality of the urologist tougher than any legal sanction.

Technical progress, modern means of investigation, and new therapeutic options have made urology more benign, with lesser risks and fewer possibilities for errors. The extension of urology to new areas, some not attempted before, exposed other possibilities for complications and postoperative incidents. However, with all the developments of urology and medicine in general, what we still only partially fathom are the reactions of the organism.

Despite technical or analytical errors, it is sometimes possible for the organism, utilizing its reactions to avert or at least limit important consequences that might follow. Other times, through a lack of response, paradoxical or unanticipated reactions, complications and mistakes might be aggravated, thus requiring re-intervention to ensure positive results.

No one in the world, in any profession, can be above mistake: the urologist has the moral duty to do whatever he can to minimize errors.

All active people, and especially those who try new things in the name of growth made mistakes. Progress in all fields, in medicine and particularly in surgery, meant repeated attempts with a mistake, repetition, effort, and sacrifice, and people often paid dearly for surgical advance. But often, from mistake came more knowledge than from success.

To admit errors and to look for their source is the essential condition for a better tomorrow.

Marshal Foch, the highest commander of allied armed forces, victorious in World War I said: “of course I have made many mistakes, but if I hadn’t made them, I wouldn’t have accomplished everything I did in 1918”.

But to misstep as little as possible is no simple or easy task. For the urologist, few mistakes mean having thorough professional training that never ceases to perfect.

For the urologist, making few mistakes means having the chance to stay young at heart, to have the will to constantly improve, and at the same time to keep an unshaken belief in the promise of a better future. It also means understanding and cherishing this ingrate but unimaginably beautiful profession which is urology. And to assimilate it well, the urologist is bound to forfeit his youth, sacrifice his freedom, and surrender all thoughts.

In my personal opinion, a resident in urology should only train in clinics that alongside highly qualified faculty, have the means of instructing residents in all areas of the specialty: open surgery, low and high endoscopic urology, ESWL, abundant imaging possibilities, laparoscopy, robotic surgery and so on.

Once a specialist, the urologist has to constantly oversee his professional progress and keep up with all modern attainments in our specialty.

To obtain the title of the primary physician, there should be a return to a competitive exam form factor, with a limited number of positions, exams not attestations, held before a commission with valued members of the faculty from the country. The primary physician title is a higher degree within the specialty, one that should be aspired to, and only received by the valuable urologists.

Competitive exams should be set to obtain the position of head of the unit, on a period of no more than 4 or 5 years, same as the presidency of the country. The head of the unit should be the best trained professional, highly competent, and capable of solving the most complex cases, known through his profession and case studies held at conferences, symposiums, or published in specialty publications.

There was once even an institute for specializing and perfecting doctors in Romania, housed within Fundeni Hospital. Bygone…

The urologist, like any other person, can slip, and without a doubt will make errors the more he works and the more intricate, tough problems he attempts. But what portrays a proper urologist is not that he is never mistaken, but the fact that he recognizes the mistake and has the conscience and power to fix it.

Celsius, the great anatomist and surgeon of the middle ages said from the beginnings of surgery:” recognizing mistakes is only fit for the intelligent man”.

Diagnosing and identifying complications that necessitate re-intervention is far from being a simple, easy feat.

The numerous paraclinical investigations available have particular importance in appreciating postoperative evolution and occurrences, with imaging being one of the most important means of investigating in diagnosing and indicating re-intervention.

However, all those, without minimizing their importance, cannot replace the clinical assessment, repeated examinations, careful and continuous observation of the patient, and especially the evaluation of the sometimes subtle signs of aggravation.

The character Sherlock Holmes, by Sir Arthur Conan Doyle, said: ”You know my method. It is founded upon the observation of trifles”.

Joseph Okinczy wrote the famous book that will forever remain in the history of surgery ”The small rules of perfect surgery”.

Due to the rapid progress of the last years in the area of biomedical sciences, and in cellular and molecular biology, genetics, and medical imaging, in particular, some clinicians developed the tendency to put less of an emphasis on the anamnesis and clinical examination, relying solely on "adequate" paraclinical examinations, which often prove to be inadequate. This leads frequently to not only unnecessary spending but to diagnosing faults which reflect on the patient's well-being.

The problem of re-intervening reflects upon the character of the urologist and his professional condition in the most profound dimensions of the specialty and subtlest forms of knowledge.

Whenever complications occur, the experience, expertise, and professional value, as well as the temper of the urologist are often put to the test.

Urology was not always as we see it currently. To achieve perfection and the shine we see today, every civilization contributed in spirit, every century laid its rocks, every people brought their input. In its constant evolution, it was only natural that the passive attitude towards incidents and complications had to change.

Currently, such accidents can undergo surgery, a large number of patients can be healed. A detailed study has shown how to best avoid those incidents.

Sure enough, several re-interventions have nothing to do with the primary procedure, nor any urological error. But when in doubt and more importantly when errors exist and the following complications require surgical remedy, re-intervention, no matter how severe, is the only act which allows the urologist to fix, often successfully, that which was wrong.

Within our need and tendency to better ourselves, we must not only see the mistakes of others. It would be despicable to comment in bad faith any errors of our colleagues while justifying our own.

The constant growth of urology offers indisputable potential for great success, but also occasions to fail.

As far as I am concerned, whenever I made a mistake, I prayed to the providence that it be the last one.

But I never forgot that the urologist, as long as he is working, cannot say that he has made his final mistake.

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REVIEW

A review of biovitroceramic PAW1 – Romanian bioactive implant material for ossiculoplasty

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Abstract: *A successful ossiculoplasty (reconstruction of the ossicular chain of the middle ear), even now, almost a hundred years after the first attempts and countless tested materials, remains a challenge through the complexity of the surgical act and through the repeated attempts to find the most adequate prosthesis to re-establish, in the long term, the sound conductive mechanism.*

In this article, we present a review of the main implanted materials, including the biotechnological aspects of ossicular chain reconstruction with an emphasis on the Romanian contribution to this field of study.

Keywords: *prostheses, implants, ossiculoplasty, ceramics, ossicular chain, biotechnology, biocompatibility, biovitroceramic PAW1*

INTRODUCTION

Ossiculoplasty is a surgical procedure used to restore hearing loss by reconstructing the ossicular chain mechanism between the tympanic membrane and the oval window, in the presence of a discontinuity or fixation, with or without a concomitant disease. The medium-term and especially the long-term success of this intervention depends on several important factors: the skill of the surgeon, the material used in the reconstruction of the ossicular chain, and, most importantly, a preserved, quality middle ear.

Chronic suppurative otitis media (COM), with or without cholesteatoma [1], is the most common pathology that influences the outcome of surgery and the long-term functional and anatomic results. The functional results become even more important if you consider that hearing loss of variable etiology represents one of the most challenging public health issues confronting the world's population [2]. "The integrity of the auditory system is one of the prerequisites for the acquisition and the proper development of oral language and a person suffering from hypoacusis is more likely to have poorer professional results than his colleagues, and will be less competitive in the labor market, and will have smaller chances to complete higher education" [3,4].

The next most important factor influencing results is the type and the quality of the material. Over time many types of materials have been tested (biological and synthetic), some entering into medical practice a shorter period, others remaining in current use, but the surgeon's choice for the ossicular chain reconstructions depends on their own experience in the field, the ossicular deficiency or the patient's immunological status [5]. The purpose of all these attempts is to implant a type of material with excellent biocompatibility, and stability, to provide natural sound transmission with the minimum risk of potential bacterial colonization or graft rejection [6].

THEORY AND CONCEPT

Ossicular substitution or reconstruction has been attempted with an array of materials but the only conclusion that has been drawn is that "the ideal prosthesis for ossicular reconstruction should be made of a material that maintains

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its shape, rigidity, and acoustic properties, as well as being cost-effective. This material should also be biocompatible, safe, and easily inserted and modified" [7].

From the acoustical point of view, a prosthesis is well designed, if its shape and material reach a high stiffness combined with a low mass but is also small, relatively cheap, and easy to modify [8,9,10,11].

From this point of view, titanium has advantages compared with gold, ceramics, or plastics because of its low density and high mechanical strength. These properties of titanium significantly reduce possible loss in sound transfer due to internal resonances. The higher mass of gold and ceramic prostheses decreases the motion of the entire system above the first resonance.

This inertia influence can also be seen by a decrease in the first resonance frequency of the (reconstructed) middle ear system. "By contrast, the low mass of the light titanium prostheses contributes to a better sound transfer performance, especially in the high-frequency range." [12]

A further significant parameter is the quality of prosthesis coupling to both tympanic membrane/malleus and stapes. The dynamics of the reconstructed middle ear are very sensitive to where and how the prosthesis is coupled to the tympanic membrane and ossicles, to the spatial direction in which the prosthesis is oriented, and to the prosthesis attachment (near rigid or rather loose attachment). In a patient, the prosthesis attachment may be influenced by the resulting tension that is achieved both by the surgeon and by postoperative wound healing.

Prosthesis placement is paramount for obtaining good functional results, but this represents no guarantee that postoperative migration relative to the malleus handle, TM, or stapes will not occur [13,14]. This is highly dependent on the skill of the surgeon; the type of prosthesis and we should never forget that medical research can sometimes require a high degree of abstraction and theorization [15].

"Ossicular reconstruction materials are categorized as autografts, homografts, and alloplastic prosthetics. Each of these materials has advantages and disadvantages for use in the middle ear" [7].

BIOLOGICAL MATERIALS

Hall and Rytzner (1957) performed the first chain reconstruction using autogenous incus [16]. Several otologists, including Farrion, Sheehy, and Guilford, began reporting on the success of using autografts for ossicular reconstruction, especially for the body of the incus [17]. However, the patient's incus is not always available for repositioning and Farrion first advocated the use of cortical bone as an ossicular substitute [17,18].

As a biological alternative to ossicles, cartilage has been extensively used in tympanoplasty and ossiculoplasty [18]. Autologous tragal and/or conchal cartilage is, however, not always suitable for ossicular chain reconstruction, especially in cases where the stapes arch is missing since they do not have the required mass, stiffness, and thickness [19]. Zini et al. (1984) introduced ossicular prostheses made from costal cartilage and reported no extrusion at 10 years of follow-up with stable functional results over the years [19].

Autologous materials presented a high degree of tolerance within the middle ear and provided reliable hearing results. Their disadvantages were the laborious and time-consuming sculpting into the required shape and the scarceness of material in chronically diseased ears [4] loss of rigidity (especially with cartilage), and possible fixation to the soft tissues of the middle ear [17].

Homografts (as an option for unreliable autografts) were first used for middle ear reconstruction in 1966 by House and colleagues and later led to the use of "irradiated ossicles, cartilage, and even homograft tympanic membranes with en bloc ossicles" [20,21]. The hearing results and biocompatibility of homografts and autografts proved similar but the risk of disease transmission of HIV and prions (i.e., Creutzfeldt-Jakob disease) brought their decline in use – see Table 1.

Donor (D) – Receiver (R) Relationship:
1. Autologous: D ≡ R
2. Isologous: D·R Homozygotic Twins
3. Homologous (Alloplastic): D·R Same species
4. Heterologous (Xenoplastic): D·R Different species

Donor Situs (D.S.) to Receiving (R.S.) situs Relationship:
1. Orthotopic (same situs): D.S. ≡ R.S.
2. Heterotopic (different situs): D.S. ≠ R.S.

Although many surgeons preferred biological prostheses due to increased compatibility with the middle ear and low rate of rejection, the lack of materials and prolonged intraoperative time led the researchers to look for the ideal material in the reconstruction of sound conducting mechanisms.

SYNTHETIC MATERIALS

Other than the natural or biological materials (autologous and homologous), polymers, ceramics, and metals are currently used for ossicular chain reconstruction, each with its advantages and disadvantages. These synthetic materials are defined as biomaterials.

In 1958, Shea used for the first-time polyethylene prostheses (Polytef – Teflon and silicone elastomer – Silastic) for middle ear reconstruction [17] with excellent short-term hearing results. The high extrusion rate, significant middle ear reactivity towards synthetic materials, and risk of penetration into the inner ear [17] caused this material to gradually drop out of use.

Table 1: Types of natural biomaterials used in ossiculoplasty

Natural Biomaterials (Grafts, Transplants)				
Type	**Status**	**Donor–Receiver Relationship**	**Species**	**Author, Year**
Autologous	Fresh	Orthotopic	Remodeled malleus	Wullstein, 1953
		Heterotopic	Cartilage	Williams, 1958
			Tragal cartilage	Utech, 1960
			Septal cartilage	Jansen, 1962
			Rib cartilage	
			Mastoid cortical bone	
Homologous (Allo)	Preserved	Orthotopic	Isolated ossicles	Portmann, 1959
			Ossicular chain	Marquet, 1966
		Heterotopic	Mastoid cortical bone	Wullstein, 1952
			Septal cartilage	Jansen, 1960
			Rib cartilage	
			Meniscus cartilage	
			Dentine	Zini, 1967
Heterologous (Xeno)	Preserved	Orthotopic	Veal incus	Sultan, 1983
		Heterotopic	Veal rib cartilage	Zini, 1959
	Normal	Normal	Coral	Robier, 1987
				Schmorll, 1990

After almost 20 years of research, in 1976, Shea reported on the use of a high-density polyethylene sponge (Plastipore), a porous, nonreactive material that could allow the in-growth of tissue [22]. "A similar thermal-fused porous polyethylene (Polycell) was developed later and allowed the prosthesis to be coupled to other materials, such as stainless steel thus enabling various prosthesis designs" [17]. The most-reported problem of such porous materials was the high extrusion rate when in direct contact with the TM. However, a disc of cartilage placed between the head of the prosthesis and the tympanic membrane gives excellent long-term results even today [22,23].

In 1979 the era of ceramics in ossicular reconstruction begins by introducing a few categories of alloplastic materials, either bioinert or bioactive. Implantable materials that do not release detectable trace substances are termed bioinert. The prototype bioinert material is dense aluminum oxide ceramic (Al_2O_3), popular in Germany and Japan in the 1970s. The implant can be fitted to the under-surface of the tympanic membrane without cartilage coverage [24].

By studying the way, the body reacts to the implanted materials, there can be three major recipients defined (Tables 2, 3, 4):

● "bioinert" – the body does not react to the implant material.

● "bio-tolerated" – the local tissue regards the implant as a foreign body but does not react, the material is not excluded.

● "bioactive" – the local tissue reacts and tends to create a stable bound to integrate it [6].

"Bioactive implants, such as glass-ceramic (Ceravital), were biocompatible and reacted with surrounding soft tissue and adjacent bone allowing a coupling between the implant and the ossicle" [19,25] and were used, with various results, around the world, even in the former communist bloc, including Romania [26].

Hydroxyapatite, found in the mineral matrix of living bone, was introduced for tympanoplasty surgery in 1984 by Grote (calcium phosphate ceramic) [27]. It is a bioactive material that can be easily integrated into surrounding tissues (bone). The first results by Wehrs et al. reported functional success and a low extrusion rate 4 years after implanting hydroxyapatite incus or incus/stapes prosthesis. Since that time, this material has been adapted to various uses and prosthesis designs [17,20].

Titanium prostheses were developed and first used in Germany in 1993 and have been growing in popularity ever since. The material is rigid and biocompatible just like hydroxyapatite, but much lighter. It is also nonmagnetic and allows it to be manufactured in many precise shapes. “Most of the titanium prostheses possess an open head, allowing better visualization during placement” [17].

Table 2: Types of synthetic bioinert materials used in ossiculoplasty.

Name	Composition, Chemical structure, Family, Order	Author, Year
ALUMINA FRIALIT CORINDON	Al_2O_3 Oxide Ceramics	Plester & Jahnke, 1979
BIOCERAM	Al_2O_3 Y_2O_3	Yammamoto & Ywanaga, 1983
MACOR CERAMIC	Fluor phlogopite mica-Borosilicate glass $SiO_2 \cdot MgO \cdot Al_2O_3 \cdot K_2O \cdot B_2O_3 \cdot F$	Pauler & Jahnke, 1984
VITROCARBON	Glass - Carbone	Blemke et al. 1987

Table 3: Types of synthetic bioactive materials used in ossiculoplasty.

Name	Composition, Chemical structure, Family, Order	Author, Year
BIOGLASS 45-S-5	Silicon-Phosphorus Bio-glass $SiO_2 \cdot CaO \cdot Na_2O \cdot P_2O_5$	Hench et al., 1970
CERAVITAL	Silicon-Phosphorus Bio-vitro-ceramic (Hydroxyapatite – Glass) $Li_2O \cdot ZnO \cdot SiO_2$	Brömer et al., 1973
CERABONE A-W	Hydroxyapatite – Glass- β-Wollastonite ($CaO \cdot SiO_2$)	Kokubo et al., 1982
IMPLANT L-I	Hydroxyapatite – Glass- β-Wollastonite ($CaO \cdot SiO_2$)	
BIOVERIT I	Mica – Apatite – Glass $SiO_2 \cdot (Al_2O_3) \cdot MgO \cdot Na_2O \cdot K_2O \cdot F \cdot KO \cdot P_2O_5$	Holland &Vogel, 1987
BIOVERIT II	Mica – Glass – Corderite	Holland & Vogel, 1993
BIOVITROCERAMICS PAW-1	Fluro-hydroxyapatite – Wollastonite – Glass $B_2O_3Fl_2$	Rădulescu & Negreanu, 1993

Table 4: Types of synthetic bio-tolerated materials used in ossiculoplasty.

Name	Composition, Chemical structure, Family, Order	Author, Year
PALAVIT	Vinyl-acrylic resin	Wullstein, 1952
SUPRAMID	Polyamide (Nylon)	Kley, 1955
POLYETHYLENE	Non-porous, low-density polyethylene	House, 1960
TEFLON	Polytetrafluoroethylene (C2F4)n (PTFE)	Shea, 1962
NON-OXIDATIVE STEEL TANTALUM SILVER ZIRCONIUM TITANIUM	Non-oxidative metals	Schucknecht, 1971
PROPLAST	Polytetrafluoroethylene (TEFLON) – Carbon fibre composite	Shea, 1974
PLASTIPORE	High-density polyethylene sponge (HDSP)	Shea, 1974
POLYCEL	Thermal fused HDSP	Fisch, 1981

Classification of Synthetic Biomaterials:

1. Destination:
   - 1.1. Tissue
   - 1.2. Organ
   - 1.3. Function

2. Composition:
   - 2.1. Monocomponent
   - 2.2. Policomponent

3. Structure – Mass - Form:
   - 3.1. Monostructure
   - 3.2. Polistructure

4. Relationship to Host (Recipient):
   - 4.1. Biotolerated
   - 4.2. Bioinert
   - 4.3. Bioreactive (Active, Bio-reactive, Bioactive)
   - 4.4. Composite (e.g., Tolerate + Active)

BIOVITROCERAMIC PAW-1 AND THE ROMANIAN CONTRIBUTION

Synthesized in 1990 by Dr. Ing. Popescu-Negreanu, PAW-1 was tested physically, chemically, and biologically (in vitro and in vivo) between 1991 and 1992 and homologated on March 31st, 1993.

Bio-vitro-ceramic PAW 1 is a solid, bio-reactive material, made up of fluoro-hydroxyapatite and wollastonite microcrystals encompassed into a vitreous mass (glass). Hydroxyapatite, which is the mineral matrix of living bone, is a well-tolerated osteoinductive material that does not produce necrotic phenomena in adjacent tissues and does not give foreign body reactions [26] – see Table 5.

Table 5: Taxonomy of PAW1.

General Taxonomy of PAW1
Class: Solids with biphasic structure (crystalline and non-crystalline)
Order: Ceramics
Family: Bio-vitro-ceramics
Genus: Silicon – Phosphorus
Species: Controlled porosity
Variety: α - closed porosity (dense) β– opened porosity (porous)
Special Taxonomy of PAW1
Synthetic
Destination: Organ
Composition: Poli-component
Structure – Mass – Form: Monostructure
Relationship to Host (Recipient): Bioactive

Medical use:

1. Dentistry: Central Military Hospital, Bucharest since June 1993
- Parodontopathy
- Granules

2. Oral and maxillofacial surgery/Esthetics: Prof. Burlibașa, Prof. Gănuță since 1993
- Granules
- Shaped implants

3. Orthopedics: Central Military Hospital, Bucharest and Foișor Orthopedics Hospital, Bucharest, Prof. Popescu Mihai since 1993
- Granules
- Shaped implants

4. Neurosurgery: “Bagdasar-Arseni” Neurosurgical Hospital, Bucharest, Prof. Dănăilă since 1994
- Shaped implants

5. Otorhinolaryngology (Otology): IFACF-ORL Prof. Dorin Hociota, Dr. Marian Rădulescu since June 1993
- Ossicular chain implants (PORP, TORP)

Chemical composition:
1. Fluorohydroxyapatite: $[Ca_{10}(PO_4)_6(OH)xFl_2\text{-}x]$
2. β-Wollastonite ($CaO \cdot SiO_2$)
3. Boron trioxide: B_2O_3
4. Fluoride: Fl_2

The material is obtained by thermally controlled crystallization (ceramization) of glass from the Silicium-Calcium-Magnesium-Phosphorus system ($SiO_2 \cdot CaO \cdot MgO \cdot P_2O_5$) with minute additions of Borum trioxide (B_2O_3) and molecular Fluoride.

Biological properties:

1. Biocompatibility:
- Predictive: Good, due to the identity of the crystalline phase of the material to the mineral phase of the mature bone.
- Experimental: tested through the low rejection rate of middle-ear implants.

2. Biodegradation:
- Predictive: Null without inflammation/infection. Osteogenesis on the implant-tissue interface.

Identical to the bone with inflammation/infection.
- Experimental: Clinical – the study of rejected implants degradation

Paraclinical – Rx for integrated implants/HP for rejected.

3. Bioactivity:
- Predictive: Good, based on the physical and chemical structure.

Porosity – cell colonization and mechanical anchorage.

Chemical structure: Chemical anchorage (direct) = covalent ties at the implant-tissue interface. Osteogenesis on the interface.
- Experimental: tested through the rate of histological integration and rate of tympanic membrane perforation.

4. Bio-functionality:
- Predictive: Good, based on mechanical and vibro-mechanical properties.
- Experimental: tested through therapeutic efficiency (audiological gain – dB): Air-bone Gap (ABG) pre-operatively vs. post-operatively.

CONCLUSION

Although numerous techniques and materials have been used for ossicular reconstruction, the quest for the ideal prosthesis and the best-suited materials is ongoing. This research took place all over the world during the past century and even developing countries such as Romania played a role.

The autochthonous bio-vitro-ceramic PAW1 proved to be a reliable, resilient material with a high degree of biocompatibility and good functional results.

The best combination of biocompatibility, toughness, ease of shaping, and inserting are still to be found.

Today, autogenous, and alloplastic prostheses are equally used with good outcomes, but the final decision remains to the surgeon, and it would be wise that he chooses the one that is most comfortable for him and provides consistent and favorable results.

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Authors' contribution
Conceptualization: RM, AIM. Data curation: AIM, RM. Formal analysis: AIM, RM. Methodology: RM. Project administration: AIM. Visualization: AIM. Writing - original draft: AIM, RM. Writing - review & editing: RM, AIM. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present work represents a review of the literature and therefore requires no agreement from the Research Ethics Committee.

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Not applicable.

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A review of the need to provide hyperbaric medical research infrastructure for the treatment of combat-related injuries

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Abstract: *HBOT has been used quite extensively in the last couple of decades. But there are not many centers that offer this treatment, even in civilian situations. This review has described the rationale and clinical evidence for equipping medical centers with HBO systems, especially in military areas.*

A narrative review of all the literature available on HBOT in combat-related injuries, studies were retrieved from systematic searches on Science Direct, PubMed, SCOPUS, ISC, Google Scholar, and by cross-referencing until 2020.

First, the etiological mechanisms of action of HBOT were discussed. Furthermore, we have provided an overview of 117 studies that clinically investigated the effect of HBOT in the last couple of decades. Therefore, based on the number of studies showing a positive effect, to speed up the treatment process, especially in war-related injuries, it is better to provide its infrastructure and equip hospitals and centers with HBO.

Keywords: *hyperbaric, hyperbaric therapy, trauma, war trauma*

INTRODUCTION

The trauma of firearms has caused many casualties in human societies since world wars military facility up to date with the advancement of military facilities, and a large part of the medical staff has always been dedicated to treating and improving the disability of injuries caused by war trauma [1]. Many studies have been done in this regard, and the beneficial effect of hyperbaric treatment has been proven [2].

Hyperbaric Oxygen therapy is the treatment of patients with oxygen respiration in a high-pressure treatment room. Increasing oxygen and delivering it to tissues has beneficial effects for various diseases. Some of these injuries are related to combat-related injuries [3]. Although the number of hyperbaric oxygen therapy sessions varies depending on the type of disease, and there is no absolute definition for the duration or pressure of oxygen in each treatment session, when hyperbaric oxygen therapy timely and properly is done, the evolution and the final result will be improved [4]. However, due to the technical limitations of treatment such as the need for a high-pressure treatment room, adequate oxygen, and compressed air supply, medical and paramedical staffs, the centers that provide this treatment are not numerous, even in civilian situations [5]. Hyperbaric Therapy Working Group investigates the possible military applications of hyperbaric oxygen therapy and defines the terms of its use. The amount of damage caused by war traumas inside or outside the city varies depending

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on the geographical location, which necessitates a comprehensive study to provide a suitable solution to prevent trauma-related injuries [1]. Although it is not possible to set up hyperbaric treatment centers near the operation site, it seems possible to organize medical evacuation routes. It appears that this collection of information in the form of a review paper will benefit to help shorten the overall treatment time of combat-related injuries and optimize the final recovery by building the right infrastructure [6, 7]. It appears that this collection of information in the form of a review paper will benefit to help shorten the overall treatment time of combat-related injuries and optimize the final recovery by building the right

METHOD

To find available data and information in the literature on the role of Hyperbaric oxygen therapy (HBOT) induced combat-related injuries via vasoconstriction and Increase oxygen supply after trauma and we performed a search in multiples data banks such as Science Direct, PubMed, SCOPUS, ISC, Google Scholar, and the paper published Until 2020 were reviewed.

It should be mentioned that the search terms and keywords for evaluation of all published papers in this manner were "Hyperbaric Oxygen Therapy", "Refractory Osteomyelitis ", "Intracranial Abscess", "Radiation Injuries", "Burns", "Wound", "Traumatic Wound", "Traumatic Brain injury", "crush injury", "military Medicine" and "inflammation", "Acoustic trauma "Also, we used MESH term "OR" operator between synonyms keywords and "AND" operator between independent keywords for finding articles and papers as well as articles with the most consistency with our goal. For a review of all papers, we had inclusion criteria, and in 203 published papers which in them the hyperbaric oxygen therapy in combat-related injuries, 117 papers were selected for review and citation of all published papers.

DISCUSSIONS

The effects of hypoxia vary depending on the cause, whether the condition is acute or chronic, as well as the overall health of each individual. Cellular hypoxia may occur in multiple organ failure syndromes due to increased tissue oxygen demand or due to the ability of oxygen to break down at the cellular level; to repair, oxygen transfer and metabolic support are important components of treatment.

Definition and mechanism of action of HBOT

Since the 1960's HBO has been used for the treatment of various diseases and illnesses [8]. HBO involves the respiration of pure oxygen under high air pressure [8]. HBOT often use in complex trauma. Lamm [9] and Appaix [10] are credited for their pioneering work on the role of HBOT in these two conditions

According to the laws of physics and physiology, breathing oxygen at high pressure increases alveolar oxygen pressure and pharmacological effects with increased arterial O_2 content leading to improvement of ischemic state due to lack of oxygen such as chronic unhealed wounds, causes vasoconstriction and special antibacterial effect against anaerobic bacteria on the body [11].

In this procedure, the patient is completely enclosed in a special chamber, and by breathing 100% oxygen, the pressure inside the chamber gradually increases to more than one atmosphere [12]. So HBOT is a relatively time-consuming therapy for patients because patients must be physically present in the oxygen chamber [13]. HBOT is considered a safe therapy; however, side effects do occur in some studies. Contraindications to hyperbaric oxygen therapy are low, including untreated pneumothorax and some chemotherapy drugs such as bleomycin, which can lead to pulmonary fibrosis [14].

The effect of HBOT on the treatment of war traumas

Acoustic trauma

Acoustic trauma is damage to the auditory structures in the inner ear due to loud noise. Hearing loss and tinnitus are two of the most common disabilities caused by military veterans. Reports indicate a worrying increase in these complications in the military population [13]. Recent war scenes have seen soldiers face numerous dangers that lead to these effects, such as explosions, ototoxic chemicals, and loud noises [15]. Damage to the auditory mechanism can be caused by an explosion near the ear, shooting, or prolonged exposure to loud noises. Hearing loss, the fourth leading cause of medical visits to soldiers [16]. Of the more than 5,000 troops stationed in Iraq and Afghanistan who underwent audiometry, 1550 people have been reported to have been exposed to acute trauma of a sound explosion; of these, 72% have lost their hearing. Of all deployed personnel who underwent audiometry, 28% had a degree of hearing loss. More than two-thirds of English soldiers who have returned from Afghanistan, from injuries suffered severe and permanent hearing loss [5]. Animal experiments showed that 60 hours after acoustic trauma injury, the number of morphologically damaged inner ear sensory cells was lower in animals treated with HBO than in those treated without HBO [17, 18]. The best therapeutic effect on noise-induced hearing loss was obtained with a combination of HBO and prednisolone [19]. Animal studies in rats [20], pigs [18], and chickens [21] all showed that the HBO-treated group had a significantly reduced signal-to-noise ratio. Also in human studies, victims of acoustic trauma in military operations were treated with HBO [22]. There was a significant improvement in hearing loss. Therapeutic results confirmed that 65% of patients who were not treated with various methods had improved hearing [23]. Based on studies performed at different times and conditions of hearing loss compared to other treatments, it can be concluded that HBO is recommended within 3 months after the onset of the disorder, and treatment with it is guaranteed [24].

Soft tissue infection

Soft-tissue infections, occur after trauma and around the foreign body, and even spontaneously [25]. A common factor, tissue hypoxia that suppresses the immune defense mechanisms [26]. In animal models (mice, rats, rabbits), mortality was significantly lower during treatment with antibiotics and HBO. But (depending on the specific type of bacteria) seen a series of adverse effects was treated with HBO, the streptococcal infection worsened [27]. The level of evidence for soft tissue infections and HBO is low. There are only case reports and retrospective studies, and no RCTs are available. Almost all patients studied were endangered hosts [28-30].

In the study of war-related organ injuries including type 3 combat fractures and the description of the effect of HBOT on wound healing and complications of infection, it was shown that the reduction of infectious complications in patients who have used HBOT in the treatment process (31). Recent studies aimed at determining the effect of HBOT in the treatment of acute surgical wounds and trauma have shown a lack of quality clinical trial studies and pointed to the need for further studies [32-34].

Crush Injury

Crushing causes severe tissue damage and calls into question tissue survival. These injuries lead to local hypoxia. Ischemia, on the other hand, causes edema due to direct vascular trauma, fluid secretion, or compartment syndrome. Edema is either vasogenic or cytogenic and increases the distance of oxygen delivery to the cells. Edema also leads to capillary collapse, thereby increasing the severity of hypoxia [35]. The main purpose of using hyperbaric oxygen in crushing injuries is to cause excessive tissue oxidation.

Animal models (rodents, dogs) Crush injury studies have shown significant effects of HBO treatment [36]. HBO also regulates inflammatory gene expression in endothelial cells and can reduce the expression of apoptosis.

In human studies, most data are obtained from case reports or series of up to 35 patients. And there is only one RCT that compares crush injuries with and without HBO treatment [37], which improved treatment and significantly reduced surgery. Patients over the age of 40, in particular, benefited greatly from HBO treatment. From the available data, experts conclude that the adjuvant use of HBO in crushing injuries may lead to a favorable outcome for selected patients [34, 38].

Burns

Burns are followed by hypoxia and tissue ischemia [39], which increase edema with loss of capillary wall integrity (endothelial cell contraction). This fluid loss, by changing the oncotic pressure gradient in the capillary wall, further reduces the intra-capillary fluid pressure and thus increases stasis [40]. As a result, areas of second-degree superficial burnt tissue (minor burns), develop in the first 24 hours after the burn, it progresses to a deep second or third-degree burn, which requires early surgery and a transplant to heal [41].

In all of these pathophysiological mechanisms, hypoxia plays a pivotal role. Oxygenation of ischemic tissues should be performed rapidly and in large volumes, to reduce tissue damage with ischemic-reperfusion. Hyperbaric oxygenation is the only treatment that can ensure that the treatment is successful [42, 43].

Several reports show the help of HBOT in the treatment of severe burns [44]. The use of HBOT in severe burns in a war scene may reduce the need for surgery and length of hospital stay [45], and possibly reduce mortality [46].

When soft tissues (such as skin and muscle) are burned, blood flow to the affected area is severely reduced. This causes the area to swell, and the swelling spreads from the affected area in all directions. The swelling may reach deep into the muscles and surrounding skin, causing more damage than the original injury. HBO should be used very soon to limit initial debridement and gradual tissue destruction [47].

The theoretical beneficial effect of HBOT on burn wounds is in the antibacterial effects of possible infections, in reducing edema and systemic inflammatory response, in limiting the size of burned tissue, and in increasing collagen formation. It seems that the best results from starting hyperbaric treatment in the first hours of injury. However, studies indicate a low and unclear role of HBOT in the management of severe burns. A randomized study supports the use of HBOT to evaluate the potential positive effects of HBOT on experimental burn wound healing [48]. In this study, the authors demonstrated a beneficial effect on HBOT wound healing and hypothesized that such an effect would be determined by reducing edema and maintaining microcirculation. Although the quantity and quality of evidence-based studies on the potential benefits of HBOT in patients with burns are poor, its potential use in medicine, war is worthy of further investigation. To achieve the best result, careful selection and screening of patients are recommended [47].

Special attention has been paid to the use of HBO in respiratory injuries. There is currently concern that HBO may worsen lung damage, especially in patients with high levels of inhaled O_2 [49]. Studies have not reported any evidence of oxidative stress in HBO-treated burn patients who used lipid peroxidation exhalation products as a symptom [50].

However, a series of compelling prospective and retrospective studies have been published [47, 51] that show less need for surgery, reduced mortality, shorter hospital stays, and reduced treatment costs when using HBO concomitantly with conventional therapy. But so far no prospective placebo-controlled trials have been published that explicitly prove the effectiveness of HBO treatment [52, 53]. Therefore, acceptance of HBO as a valuable adjunctive therapy is limited to those burn centers that can provide early treatment of HBO without adding additional risks (infectious, hemodynamic) to the patient's condition [54, 55].

Frostbite

Frostbite-like thermal burns a profound impact on local microcirculation that it can be cold or an injury or an acute and prolonged exposure to cold be defined. It has been shown that after heating, a long-term damage phase occurs, which is very similar to the ischemia-reperfusion injury observed in thermal burns. At least, in theory, hyperbaric oxygen therapy could have the following benefits in frostbite injury.

Animal studies (especially rabbit studies) have been conducted sporadically over different years [56, 57], with each study showing different results [58-60]. These different results suggest that deep and rapid freezing damage can indicate several important differences in pathogenesis and tissue damage from slow to moderate freezing.

The experiments carried out on animals, the early application of HBO during or immediately after the heat re-use. Some sporadic case reports suggest that HBO may be helpful, even long after warming up. Because these are uncontrolled and isolated reports, the evidence supporting these claims is not very strong. In 2001, he reported a case in which HBO was used after only 4 days for a young boy who had both hands frozen, and was able to save all his fingers [61]. Similarly, cases have been reported, 12 and 22 days after frost damage and the use of HBO led to the rescue of the individual fingers [62, 63].

Although the experience is relatively limited because HBO is generally not recognized as a complementary treatment standard for frostbite; clinically, HBO appears to provide early, good and rapid separation of growing tissue from non-growing tissue. MR angiography, one week after the onset of the injury, looks promising in estimating the likelihood of resuscitation of the fingers [64].

Spinal cord injury

Many studies have examined the mechanism of action of HBOT in SCI [65, 66]. Some studies have shown the protective effect of HBOT in the treatment of SCI [67, 68] and others recommended a review of the care process [66]. The number of treatment sessions for patients with spinal cord injuries is determined by the patient's condition and response to treatment, but according to studies, at least 30 sessions of standard HBO treatment 5 sessions per week were recommended to begin treatment [69].

Carbon monoxide poisoning

Because lipid peroxidation is an important aspect of CO-induced neuronal damage, inhibition of neutrophil O_2 integrins at the cellular level by HBO therapy has been identified as a significant advantage of that treatment. HBO relieves cerebral edema through vasoconstriction [70]. HBO therapy is unique in increasing oxygen delivery while causing blood vessels to constrict.

Experimental studies have shown that HBO treatment restores mitochondrial oxidative metabolism in addition to eliminating tissue hypoxia [71] and prevent lipid peroxidation [72] and prevents endothelial leukocyte adhesion [73]. Although many studies have talked about the healing process and preventing the disease from getting worse [74-76], In some studies, the authors failed to demonstrate a significant advantage of an HBO treatment session over NBO treatment in patients with loss of consciousness. Similarly, treatment with two sessions of HBO showed no advantage over one session of HBO in patients who were in a coma [77].

Decompression sickness (DCS)

Decompression sickness (DCS) occurs when atmospheric pressure decreases too rapidly. During this syndrome, bubbles of inert gas dissolved in blood or tissues lead to blood supply blockage, direct mechanical damage, or biochemical actions. DCS can be expected after scuba diving, ascent with flying, or hypobaric or hyperbaric exposure [78, 79].

The endothelium of vessels can be injured by the bubbles causing DCS. This damage induces platelet aggregation, lipoproteins denaturation, and leukocytes activation. These events eventually lead to capillary leaks and proinflammatory events [80, 81].

Hyperbaric oxygen therapy (HBOT) by using an oxygen gradient and also through pressure is ale to reduce the size of the bubbles. According to Boyle's law, pressure increases can decrease the volume of the bubble. The bubble causing DCS is thought to be composed of nitrogen.

In the process of DCS, when a tissue ascends to a decreased atmospheric pressure, bubbles derived from nitrogen seep out of blood, tissue, or both. By breathing 100% oxygen in HBOT, the blood becomes rich in oxygen and poor in nitrogen that leads to a gradient of nitrogen between the blood and the bubble. So nitrogen goes from the bubble into the bloodstream which makes the bubble smaller [79].

Recompression is the choice of treatment. The greatest success from recompression, as the definitive treatment, occurs when treatment is done as soon as possible but no exclusionary time from symptom onset has been established [79, 81]. Assessment patient's symptom response to therapy determines the time a patient is kept at 60 or 30 fsw [82].

Recovery therapy

Military response is now accepted around the world to assist civil society in natural disasters. Most of the time, military support is provided only in large-scale disasters. In contrast, local resource capacity can be used in small-scale disasters.

On the other hand, due to the proposed vascular etiology, many other treatment modalities were proposed and experimentally tested. Examples of treatments are blood flow-promoting agents, dextran, vasodilators, and pentoxifylline. However, many of these studies have did not show effectiveness within the clinical setting [83-87].

According to some authors, contraction of the prostheses caused by HBOT treatment reduces tissue edema and secretion of acute post-traumatic inflammatory factors, which can have an analgesic effect on acute pain [88, 89]. So we conclude that, at least from a pathophysiological point of view, HBO should be started as soon as possible, so HBO delayed effects can be considered reasonable, even so, it is impossible to define a period in which HBO can be considered anything more than a tool [90].

Recommendations

For future studies, we recommend starting therapy as early as possible, preferably within 48 h and to use combination therapy. Furthermore, we recommend investigating the relationship between the effects of HBO and age, sex, and underlying disease. In future studies, randomization and having a control group also seem necessary.

CONCLUSIONS

HBOT has been used quite extensively in the last couple of decades. Even though most studies were not randomized controlled trials, we think that based on the number of studies showing a positive effect, HBOT should be discussed with patients (shared decision making) as optional therapy.

This narrative review has described the rationale and clinical evidence for equipping medical centers with HBO systems, especially in military areas. Equipping military hospitals with this treatment system can perform the first stage of treatment for the individual before the transfer and speed up the treatment process, especially in combination therapies.

Therefore, centers can be classified based on belonging to different categories of hospitals, and for each pathology, a suitable hospital category can be defined. Given that so far no formal coordination has taken place between the military hyperbaric centers of different countries, interoperability facilitated the organization of cooperation in the field of multinational operations.

We emphasize international cooperation on the applications of hyperbaric medicine in military medicine. At the International Congress of Military Medicine (ICCM) held in Iran in 2018, a panel on hyperbaric medicine was held with the participation of hyperbaric medical professors from the participating countries. In this research panel, Dr. Shahram Oliaei, hyperbaric medical researcher, emphasized research and educational cooperation to further coordinate the hyperbaric medical centers of the member countries.

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REVIEW ARTICLE

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Towards an integrated medical system for radiological medical imaging investigations

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Abstract: *The continuously increasing number of medical investigations using radiological methods imposes the strong necessity of informing patients about benefits and risks regarding radiation absorbed doses. Tracking the radiation doses absorbed by patients must be a future challenge of any medical system. The effective doses received by patients in many types of medical investigations must be calculated, transformed, recorded and cumulated. Doctors and patients must be very responsible in prescribing or demanding new radiological medical investigations. Radiological standards, legislation, guidelines, programmers and practice supervised by international commissions on radiology protection must include new specific measures for patients' cumulated doses. A pilot Romanian project had tried to accomplish some of these tasks.*

INTRODUCTION

Radiation is commonly associated with nuclear accidents or modern irradiative artificial sources such as: mobile phones, great antennas, satellites, food irradiation, and radioactive building materials.

Generally, radiation is associated with all kind of human activities.

As a huge surprise, in fact, radiation is quite a natural phenomenon in our lives. There are many radioactive substances in air, water and soil that are called natural background radiation. During a long airplane flight one can also receive an important radiation dose. Typically doses from natural sources are measured in mSv. The Sievert (Sv) is the International System of Units (SI) derived unit for radiation dose.

There are many other different units for absorbed radiation and transformations can be made in order to compare them.

A recent United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) publication [1] estimates worldwide distribution of radiation exposure:

- 13% from cosmic sources
- 16% from soil
- 9% from food
- 20% from medical investigations
- 42% from radon sources

Radon-222 is a radionuclide in the form of a gas that normally emanates from the soil. Radon is a colorless,

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odorless, radioactive gas. Most exposure to radon comes from being indoors in buildings. A strong interaction between smoking and radon has been proven, so radon is considered as a primary cause of lung cancer, smokers being more vulnerable [2]. There are no widely available medical tests to measure radon exposure. So radon belongs to natural sources of radiation.

The increased doses of radiation in our climate and habitat is surveyed by national or international organizations, there are standards, policies and strategies for general radiation protection.

International commissions on radiology protection are worldwide active for radiological standards, legislation, guidelines, programmers, and practice.

The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) highlights the levels and effects of exposure to ionizing radiation [1].

The International Commission on Radiological Protection (ICRP) has published a lot of reports on generally human protection and the specific protection of patients exposed to ionizing radiation [3].

In Romania, CNCAN is the national competent authority in nuclear field “having responsibilities of regulation, authorization and control stipulated in this Law is the National Commission for Nuclear Activities Control, public institution of national interest, legal entity, with the head office in Bucharest, being headed by a President having the rank of State Secretary, coordinated by the Prime Minister [4].

RADIOLOGICAL MEDICAL INVESTIGATIONS

Medical imaging is a complex process of visualization of the interior of a human body. A lot of techniques and engineering tools are used in order to obtain a visual representation:

- Radiology using X-rays;
- Computed Tomography (CT scan) using also uses special X-ray equipment;
- Nuclear medicine, using internal radioactive substances including scintigraphy that uses radio-isotopes travelling to organs, positron emission tomography (PET) or Single-photon emission computed tomography (SPECT) ;
- Mammography as a specific type of breast imaging that uses low-dose x-rays;
- Magnetic resonance imaging using the property of hydrogen atoms from human body to absorb or emit radiofrequency energy when placed in an external magnetic field;
- Medical ultrasonography or ultrasound, a diagnostic imaging technique based on the application of ultrasound;
- Endoscopy using an instrument inserted in the human organ;
- Thermography, using thermographic cameras and infrared imaging techniques;
- Elastography;
- Tactile imaging or mechanical imaging.

Only the first four investigations imply radiological methods in medical investigations. The last ones do not imply radiological risks, even there are scientific debates on SAR (specific absorption rate) as a dosimetric quantity for Electromagnetic Fields bio effects [5].

Patient exposure to ionizing radiation can be made externally, internally nor both. CTs doses are usually externally absorbed, while in angiography, fluoroscopy or scintigraphy investigations, a radioactive substance is ingested. Contrast media are also used on radiography and CT to visualize vessels or tissues. Iodine is a modern intravenous contrast agent, but barium is also used. Usually iodine contrast media is used intravenously, it can also be used intra arterially. Barium sulfate is usually swallowed. Magnetic Resonance Imaging or CTs also use contrast agents.

Differences between exposure and contamination can also be debated [6]. Exposure to a radiation source does not imply that this person becomes a new radioactive source. Contamination incorporates the radioactive agent in the body, such as in thyroid gland.

Exposure to X- rays increases the future incidence of cancer, but quantitative models predicting the level of risk are still not worldwide accepted. Different methods for radiation risk calculus are available. The

linear no-threshold (LNT) model assumes the proportionality between cancer risks versus absorbed dose. The relationship between dose and DNA damage is considered linear. The International Commission on Radiological Protection (ICRP) recommends the use of the LNT model [7].

Significant uncertainties are related to dose assessment, low dose and low dose-rate extrapolation approaches, transfer of risks from a population for which health effects data are available to one for which such data are not available [8]. Adaptive responses of human cells have also been observed at low doses [9].

Classical radiographies, computed tomographies (CTs) and scintigraphies are worldwide used medical radiological investigations that expose the patients to significant doses of radiation.

The actual volume of medical prescription, the contrast substances used in order to increase their accuracy, the overlapped investigations are general causes for a strong increasing of the cumulative radiation dose absorbed by patients. Patients and doctors usually excess in demanding, using and prescribing medical investigations in spite of harmful effects associated with radiation delivery.

The prescribed medical investigations total volume, starting from dental radiographies and ending at multiple regions CT scans prove a gap in monitoring and tracking of the cumulative radiation doses in many health services all over the world. The received radiation doses by patients are quit impossible to be cumulated in spite of the fact that in our modern world, 20 % of absorbed radiation dose is due to medical investigation.

The patients travel from one hospital to another, all over the country or all over the world.

An integrated system must be implemented. In order to cumulate all the absorbed doses by a patient in different types of radiological investigations, the doses measurement units must be the same and the recorded doses must be kept together in a single patient monitoring unit.

RADIATION DOSE RISK AND DIAGNOSTIC BENEFIT [10]

The treating physician and the radiologist can prescribe and carry out radiological imaging procedures. Several objectives and subjective elements are implied in a prescription:

- Medical request
- Imaging investigation algorithm
- The personal experience of the examiner
- Patient history
- The results of any other previous investigations

All the time a correct judgment concerning the benefit/risk ratio must be performed. Medicine and the radiation risks knowledge must be well handled. The aim of managing radiation exposure is to minimize the irradiative risk without sacrificing, or unduly limiting, the obvious benefits in the prevention, diagnosis and also in effective cure of diseases (optimization).

When too little radiation is used for diagnosis or therapy there is an increase in risk. Too low an amount of radiation in diagnosis will result in an image that does not have enough information to make a correct diagnosis. There are several ways that will minimize the risk without sacrificing the valuable information that can be obtained for patients' benefit. A possible measure is to carefully justify the examination before sending a patient to the radiologist or nuclear medicine physician. Repetition should be avoided in recently investigations performed into another clinic or hospital. An investigation may be seen as a useful one if its outcome – positive or negative – influences management of the patient. Another factor, which potentially adds to usefulness of the investigation, is strengthening confidence in the diagnosis. Most common examples of unjustified examinations include: routine chest radiography at admission to a hospital or before surgery in absence of symptoms indicating cardiac or pulmonary involvement (or insufficiency); skull radiography in asymptomatic subjects of accidents; lower sacrolumbal radiography in stable degenerative condition of the spine in the 5th or later decade of life, but there are of course many others [11]. From medical experience, the diagnosis

benefit is a priority. For a lung Rx examination any suspected image is repeated or supplementary investigated in a Computed Tomography.

Many times, the personal experience of the radiologist is the dominant factor in high radiation doses investigations:

- urgent exam such as trauma and polytrauma, fractured skull or spine where CT exams are a real benefit for the patient and an economic benefit for the hospital. They can shorten the patient’s hospital stay.
- scintigraphic investigation in thyroid diseases
- repeated mammography
- bone disease with seemingly benign tumors requiring an examination for differential diagnosis.

RADIATION MEASUREMENT UNITS

„The International Commission on Radiological Protection (ICRP) recommends that the public limit of artificial irradiation should not exceed an average of 1 mSv effective dose per year, not including medical and occupational exposures. ICRP limits for occupational workers are 20 mSv per year, averaged over defined periods of five years, with the further provision that the dose should not exceed 50 mSv in any single year“ [12].

The Sievert (Sv) is a derived unit in the International System of Units (SI) used for equivalent absorbed radiation dose measurement. This must be the central unit of a tracking medical system.

There are many different units for absorbed radiation used on a large scale. Becquerel (Bq) and Curie (Ci) as SI units are used for released radioactivity, while Coulomb/Kilogram (C/kg) and Roentgen (R) are used for the dose travelling through the air, Gray (Gy) and Rad, used with quantities of absorbed dose. A lot of Internet sites provide convertors between them.

The biological effects of the absorbed amount of radiation can be described with Roentgen equivalent man (rem) and Sievert (Sv). They are specific measurement units.

The Sievert can better describe the effective equivalent dose absorbed by biological tissues while the Gray can describe the absorbed dose of any material.

Table 1: Radiation Measurement Units

Type	Measurement unit		
Released radioactivity	Curie (Ci)	Non-SI	**1 curie = 3.7×10^{10} radioactive decays per second**
	Becquerels (Bq)	SI unit Derived	One becquerel represents the activity of a quantity of radioactive material in which one nucleus decays per second – **$1\ Bq = 1\ s^{-1}$**
Dose travelling through air (Exposure)	Coulomb/Kilogram (C/kg)	SI unit	**1 C/kg = 3876 R**
	Roentgen (R)	Non-SI	**1 C/kg = 3876 R**
Absorbed Radiation dose	Gray (Gy)	SI unit Derived	It is defined as the absorption of one joule of radiation energy per kilogram of matter **$1Gy=1J/Kg=1m^2/s^2$**
	Rad	Deprecated, replaced by the Gray	**1 rad = 0.01 Gy = 0.01 J/kg**
Dose equivalent (the biological effects of the absorbed amount of radiation)	Röntgen equivalent man (rem)	Non-SI	**1 rem=0,01Sv** **1 mrem/h = 8766 mrem/yr**
	Sievert (Sv)	SI unit Derived	represents the equivalent biological effect of the deposit of a joule of radiation energy in a kilogram of human tissue

Modern radiological apparatus for computerized tomographies or scintigraphies can provide the radiation doses during a particular investigation, but the recorded doses’ types and the radiation

measurement units in different types of investigations are not the same.

In the classic radiological investigation an important topic for data management is the measurement and the calculation of radiation dose expressed in Dose area product (DAP), expressed in (Gy*cm2). The dose length product (DLP) was chosen as input data for the system in CTs investigations and it is expressed in mGy*cm. The DLP must be converted to mSv in order to unify the results.

REQUIREMENTS OF A DESIGNED TRACKING SYSTEM

A tracking proposed system must include computers, smartcards for storing data and suitable software applications for recording, retrieving and alert. The effective doses received by patients must be unified and cumulated.

The system provides the replication of the information stored in central databases, local databases and patient cards to cover all the following possible situations:

- The patient goes to the doctor without the patient card. In this case, the system provides the data corresponding to the patient based on the local database, if the patient has been investigated in that hospital unit; if the patient is new, the system must provide the data from the central database to the local database, so as to take the optimal decision in recommending the type of investigation. After the investigation, the system must store the new local database accumulated radiation dose to the patient, the information arriving in the central database. Later, when the patient goes to the doctor for further investigations with the card, the system must ensure synchronization between the information stored on the card and information stored in the local and central database.
- The patient goes to the doctor with no card and the hospital unit’s information system does not have access to the central database (ex: for mobile laboratories). If the local database contains the information corresponding to the patient, the doctor must be able to use them for recommending a particular type of investigation. After the investigation, the system must store in the local database the new radiation doses accumulated by the patient. Later, when the local system can access the central system, the information corresponding to the patient must be synchronized between the two databases.
- The patient presents the card to the doctor but the doctor does not have access to any database (local or central). In this case the doctor, using a computer with a card reader can access the history of investigations and the doses received by the patient, as the current cumulative dose calculated and can recommend the most appropriate investigation. After investigation, the appropriate dose must be recorded on the patient’s card and next time when the patient goes to the doctor with the card, this information must be stored in both in the local and the central database.

Additionally the system provides applications for:

- Viewing in real time the history of investigations, of the doses delivered to the patient and of the current cumulative calculated dose expressed in mSv.
- Aiding the medical staff in taking the adequate decisions regarding the indication of investigations according to the current calculated cumulative doses and the maximum doses allowed for the risk and age groups.
- Performing of various periodic reports in order to take different types of decisions related with the existing radioprotection rules.

The proposed system includes the following modules:
a) Applications running on smart cards, called on card applications.
b) Off-card applications that are running on medical stations. They include smart card readers and writers in order to record and retrieve the information about the type of investigations and the specific emitted doses
c) A data base records all the necessary information in order to replicate a lost or destroyed card but also this database must provide the possibility of collecting data about the patients on several criteria, it must provide the possibility of standard or customized reports’ creation.
d) The security solutions such as public key infrastructure PKI in order to achieve a high level of

security of recording and retrieving data. A public-key infrastructure (PKI) is a set of hardware, software, people, policies, and procedures needed to create, manage, distribute, use, store, and revoke digital certificates. A PKI establishes and maintains a trustworthy networking environment by providing key and certificate management services that enable encryption and digital signature capabilities across applications — all in a manner that is transparent and easy to use. Digital certificates are electronic credentials that are used to assert the online identities of individuals, computers and other entities on a network. They are issued by certification authorities (CAs) that must validate the identity of the certificate-holder both before the certificate is issued and when the certificate is used.

The entire system must be designed around the two types of cards: the patients' cards and the medical cards and it ensure many requirements ensuring secured communications.

Two different data flows must be implemented:

- Electronic cards flow management that includes: smart cards issuance, their renewal in case of loss, damage or theft.
- Operational cards flow management including: patients and doctors authentication using unique PINs.

Figure 1: Designed system

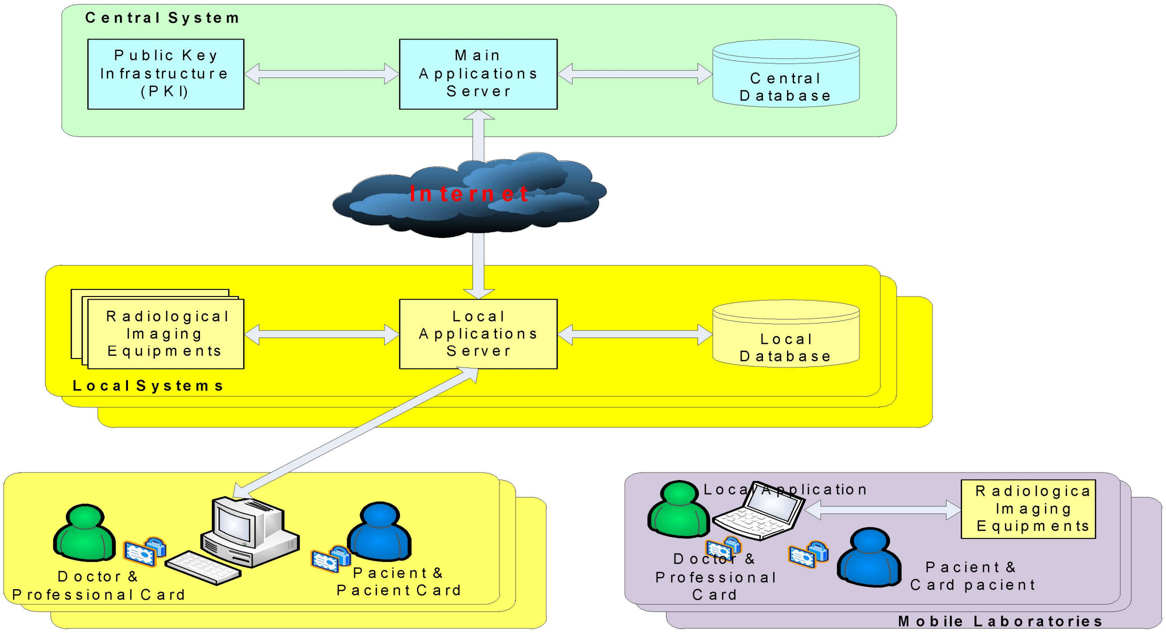
Central System
Public Key Infrastructure (PKI)
Main Applications Server
Central Database
Internet
Radiological Imaging Equipments
Local Applications Server
Local Database
Local Systems
Doctor & Professional Card
Pacient & Pacient Card
Local Application
Radiologica Imaging Equipments
Doctor & Professional Card
Pacient & Card pacient
Mobile Laboratories

DESIGNED SYSTEM

A Romanian pilot study has been implemented the International System of Units uses the Sievert (Sv) as a derived unit in order to estimate the effective dose.

The new Romanian system is based on smart cards and Public Key Infrastructure and its structure is represented in figure 1. The system was implemented in Dr. Carol Davila Central Military Universitary Emergency Hospital, Bucharest, Romania.

The whole designed system and software application was the main result of the 187/2012 SRSPIRIM project in a Romanian National Partnerships Program, Collaborative Applied Research Projects – PCCA 2.

The system includes the following components:

- smart cards dedicated to patients: Citizen Radiation Safety Card (CRSC) and
- professional Radiation Safety Card (PRSC), designed for medical and investigation laboratories personnel. Among the last ones, one or more Administrator Radiation Cards are also designed and used in order to enroll patients in the application.

A common patient card and a doctor card are shown

in Figure 2.

Figure 2: Types of cards

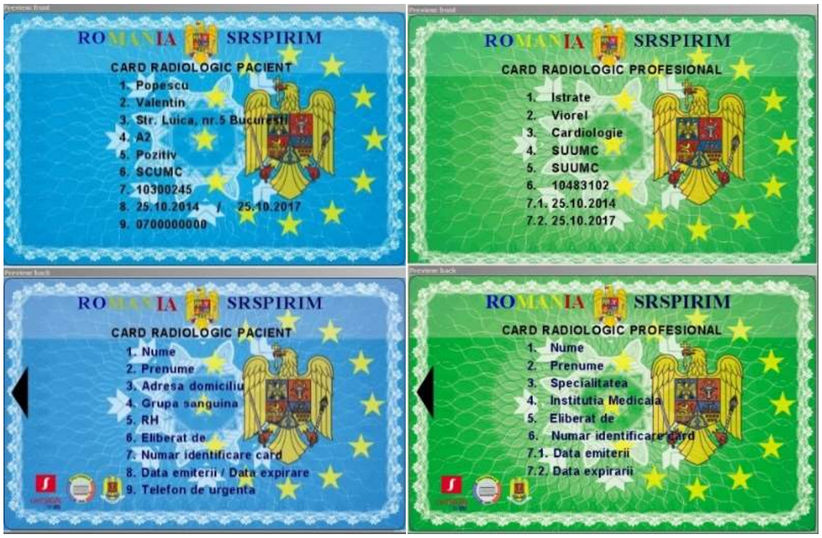

ROMANIA SRSPIRIM
CARD RADIOLOGIC PACIENT
1. Popescu
2. Valentin
3. Str. Luica, nr.5 Bucuresti
4. A2
5. Pozitiv
6. SCUMC
7. 10300245
8. 25.10.2014 / 25.10.2017
9. 0700000000

ROMANIA SRSPIRIM
CARD RADIOLOGIC PROFESIONAL
1. Istrate
2. Viorel
3. Cardiologie
4. SUUMC
5. SUUMC
6. 10483102
7.1. 25.10.2014
7.2. 25.10.2017

ROMANIA SRSPIRIM
CARD RADIOLOGIC PACIENT
1. Nume
2. Prenume
3. Adresa domiciliu
4. Grupa sanguina
5. RH
6. Eliberat de
7. Numar identificare card
8. Data emiterii / Data expirare
9. Telefon de urgenta

ROMANIA SRSPIRIM
CARD RADIOLOGIC PROFESIONAL
1. Nume
2. Prenume
3. Specialitatea
4. Institutia Medicala
5. Eliberat de
6. Numar identificare card
7.1. Data emiterii
7.2. Data expirarii

The hardware part of the system consists in computers, smart cards and card readers in order to record and retrieve the information about the type of investigations and the specific emitted doses.

The software includes a data base that records the commonly information on doctors, patients, types of medical procedures but also designed applications in order to replicate a lost or destroyed card.

Figure 3: Central database

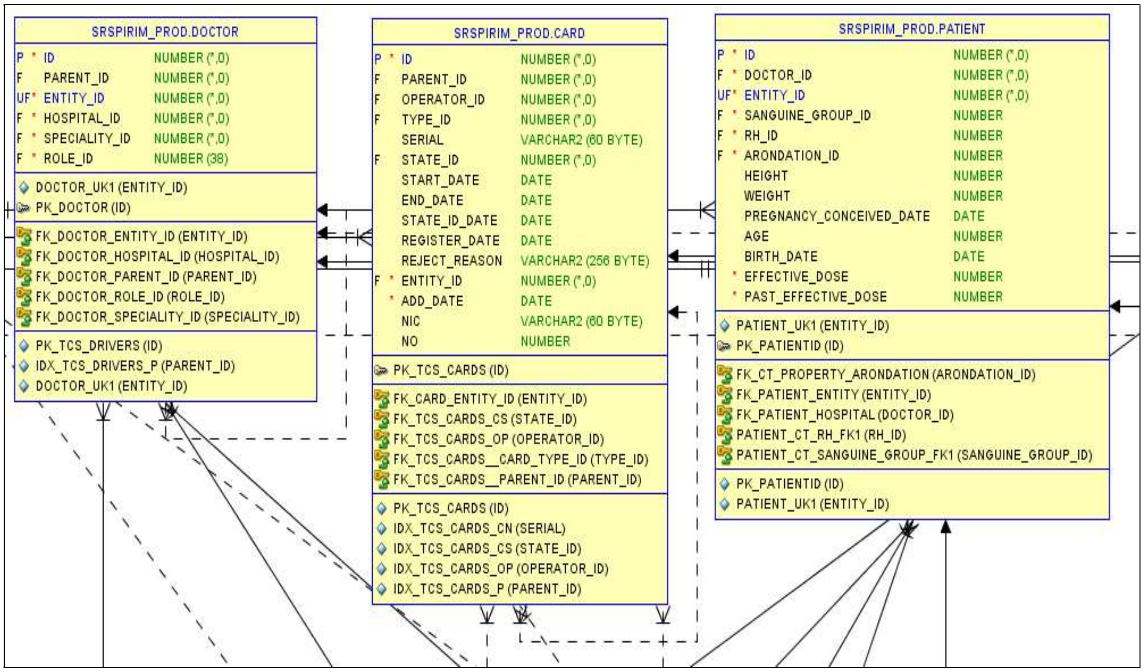

SRSPIRIM_PROD.DOCTOR

P *	ID	NUMBER (*,0)
F	PARENT_ID	NUMBER (*,0)
UF*	ENTITY_ID	NUMBER (*,0)
F *	HOSPITAL_ID	NUMBER (*,0)
F *	SPECIALITY_ID	NUMBER (*,0)
F *	ROLE_ID	NUMBER (38)

DOCTOR_UK1 (ENTITY_ID)
PK_DOCTOR (ID)

FK_DOCTOR_ENTITY_ID (ENTITY_ID)
FK_DOCTOR_HOSPITAL_ID (HOSPITAL_ID)
FK_DOCTOR_PARENT_ID (PARENT_ID)
FK_DOCTOR_ROLE_ID (ROLE_ID)
FK_DOCTOR_SPECIALITY_ID (SPECIALITY_ID)

PK_TCS_DRIVERS (ID)
IDX_TCS_DRIVERS_P (PARENT_ID)
DOCTOR_UK1 (ENTITY_ID)

SRSPIRIM_PROD.CARD

P *	ID	NUMBER (*,0)
F	PARENT_ID	NUMBER (*,0)
F	OPERATOR_ID	NUMBER (*,0)
F	TYPE_ID	NUMBER (*,0)
	SERIAL	VARCHAR2 (60 BYTE)
F	STATE_ID	NUMBER (*,0)
	START_DATE	DATE
	END_DATE	DATE
	STATE_ID_DATE	DATE
	REGISTER_DATE	DATE
	REJECT_REASON	VARCHAR2 (256 BYTE)
F *	ENTITY_ID	NUMBER (*,0)
*	ADD_DATE	DATE
	NIC	VARCHAR2 (60 BYTE)
	NO	NUMBER

PK_TCS_CARDS (ID)

FK_CARD_ENTITY_ID (ENTITY_ID)
FK_TCS_CARDS_CS (STATE_ID)
FK_TCS_CARDS_OP (OPERATOR_ID)
FK_TCS_CARDS__CARD_TYPE_ID (TYPE_ID)
FK_TCS_CARDS__PARENT_ID (PARENT_ID)

PK_TCS_CARDS (ID)
IDX_TCS_CARDS_CN (SERIAL)
IDX_TCS_CARDS_CS (STATE_ID)
IDX_TCS_CARDS_OP (OPERATOR_ID)
IDX_TCS_CARDS_P (PARENT_ID)

SRSPIRIM_PROD.PATIENT

P *	ID	NUMBER (*,0)
F *	DOCTOR_ID	NUMBER (*,0)
UF*	ENTITY_ID	NUMBER (*,0)
F *	SANGUINE_GROUP_ID	NUMBER
F *	RH_ID	NUMBER
F *	ARONDATION_ID	NUMBER
	HEIGHT	NUMBER
	WEIGHT	NUMBER
	PREGNANCY_CONCEIVED_DATE	DATE
	AGE	NUMBER
	BIRTH_DATE	DATE
*	EFFECTIVE_DOSE	NUMBER
*	PAST_EFFECTIVE_DOSE	NUMBER

PATIENT_UK1 (ENTITY_ID)
PK_PATIENTID (ID)

FK_CT_PROPERTY_ARONDATION (ARONDATION_ID)
FK_PATIENT_ENTITY (ENTITY_ID)
FK_PATIENT_HOSPITAL (DOCTOR_ID)
PATIENT_CT_RH_FK1 (RH_ID)
PATIENT_CT_SANGUINE_GROUP_FK1 (SANGUINE_GROUP_ID)

PK_PATIENTID (ID)
PATIENT_UK1 (ENTITY_ID)

This database provides the possibility of collecting data about the patients on designed criteria and future designed reports. Central database diagram, containing the recorded medical information are stored in many tables. A part of the design of the complex relationships between these tables is presented in Figure 3.

The security solutions include a Public Key

Infrastructure base (PKI), in order to achieve a high level of security of recording and retrieving data. A public-key infrastructure (PKI) is a set of hardware, software, people, policies, and procedures needed to create, manage, distribute, use, store, and revoke digital certificates. A PKI establishes and maintains a trustworthy networking environment by providing key and certificate management services that enable encryption and digital signature capabilities across applications — all in a manner that is transparent and easy to use. The application ensures a secured authentication of doctors and patients using digital certificates and cryptographic hardware. The whole logic application is stored in the central server. The clients use the minimum functional software. The applications users have access through a web browser. The system uses cryptographic libraries FIPS 140-2 or CC EAL4. The keys are RSA 1024 bits minimum length. The cryptographic hardware must support PKCS#11, PKCS#15 and Microsoft CryptoAPI. The cryptographic hardware must ensure the recovery of the cryptographic context. The certification system ensures roll–over facilities. The PKI system has an own relational database storing all the emitted certificates.

Figure 4: Effective dose calculus in radiological investigations

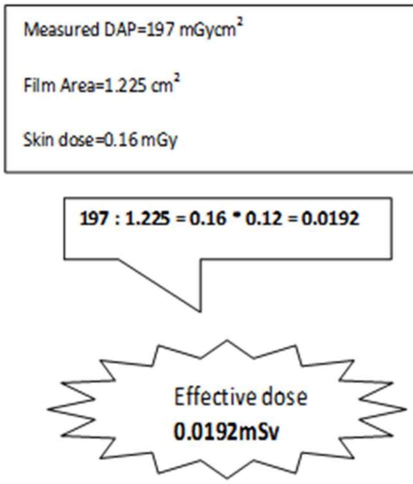

EXPERIMENTAL IMPLEMENTING

In the classic radiological investigation the calculus for radiation dose uses Dose Area Product (DAP), expressed in (Gy*cm^2) as an indicator of the total risk of inducing cancer. It also has the advantages of being easily measured, with a DAP meter on the X-ray set. In order to obtain the effective dose measured in Sv, the DAP is divided by the film area and then it is multiplied by the tissue factor [13] as shown in Figure 4. A sensible situation was determined by CT-s registered dose. More related CT radiation doses are available on CT consoles. Dose length Product DLP was chosen as input data for the system in CTs investigations but the required reported data is the effective dose. Conversion factors were used. The conversion factors can slightly vary from different manufacturers. Conversion factors for normalized effective dose per DLP depend only on the region of the body being scanned. All CT manufacturers provide tables with such conversion factors.

Conversion factors are also available for children of various ages, but this project does not include children [14]. Conversion problems were detected for multiple CT scans in one single examination, because more DLP are measured in the same examination and recording them becomes a long procedure.

EXPERIMENTAL RESULTS

Different studies were performed.
During a starting one month study in the hospital, the maximum cumulative dose has been overpass The CT exams are the most concerning prescribed imaging investigations that can lead to high cumulative doses.

The initially Figure 5 reveals some aspects from the initial study at the beginning of the project. The initial proposed threshold radiation dose of 20 mSv was frequently over passed and maybe a higher dose may be accepted for medical investigations in the future.

During a three years study in the hospital, different studies were performed.

The database contains 1125 records with 333 patients. From these records 42 patients were rejected in the final analysis because incomplete data.

These records contain all the investigations performed and all patients.

A lot of patients were examined in more than one investigation, so the system enrolled only 291 unique patients.

Figure 5: Cumulative radiation doses received by patients in the initial study

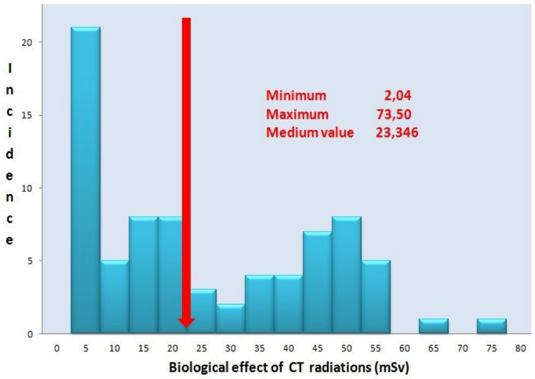

The recorded cumulated effective doses are shown in figure 6 and their distribution on decades is shown in figure 7.

Figure 6: Recorded doses in the project

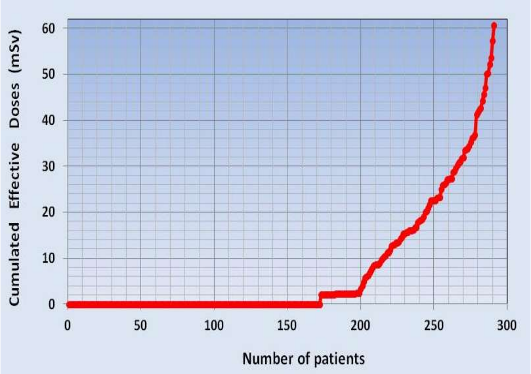

Figure 7: Recorded doses on decades

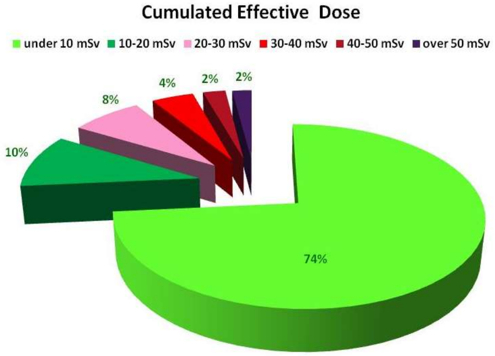

From these 291 patients, shown in figure 8 by gender, many of them have performed multiple investigations as in figure 9 and from them, in 47 cases, the received cumulated dose exceed the 20 mSv threshold dose, meaning 16,5% of cases.

Figure 8: Patients recorded in the project

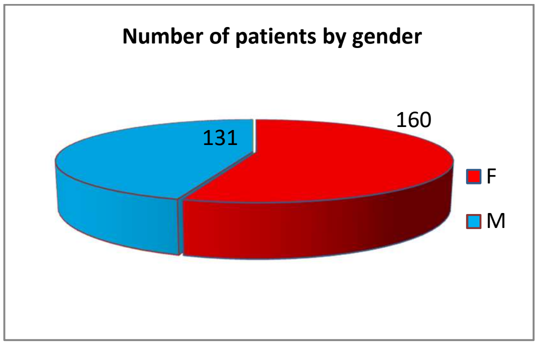

Figure 9: Patients recorded in the project

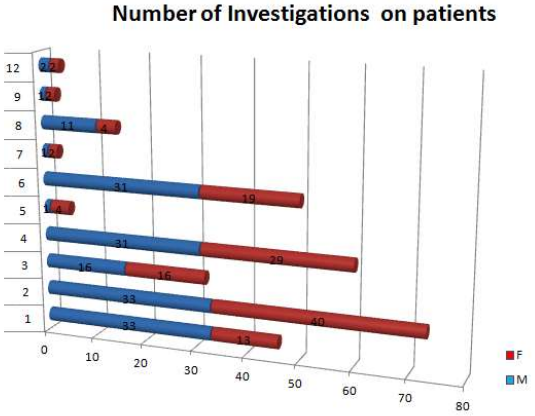

ACKNOWLEDGMENT

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REVIEW ARTICLE

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General considerations regarding the infections with the Escherichia coli pathogen

Marius Necşulescu[1], Bogdan-Ioan Coculescu[1,2], Simona Bicheru[1], Diana Popescu[1], Lucia Ionescu[1], Gabriela Dumitrescu[1], Viorel Ordeanu[1,2]

Abstract: *Escherichia coli is the species of the genus Escherichia with the greatest epidemiological impact. Escherichia coli infections are found mainly in places with poor hygiene; the infants with ages between 1 and 3 years old are included in the category with the highest risk. It is a "fecal-oral" transmission mechanism as a result of consumption of contaminated food or water, or by "dirty hands". The foods most commonly implicated in the transmission of the infection are unpasteurized milk and milk products, beef, especially the one insufficiently cooked, unpasteurized fruit juice, lettuce and insufficiently washed vegetables. The disease has been reported worldwide, being described numerous episodes of infection with Escherichia coli that caused multiple illnesses and deaths. Escherichia coli has three types of antigens: antigen "O" (somatic), antigen "H" (flagella) and antigen "K" (capsular). Clinical manifestations are present in the form of non-specific diarrhea, a dysentery form of enteritis, choleriform enteritis, hemorrhagic colitis and hemolytic uremic syndrome (HUS). The Escherichia coli infection diagnosis is made by identifying the etiologic agent and/or by highlighting the VTI toxin in the feces. The treatment consists in precautionary antibiotherapy, hydrodynamics and electrolyte rebalancing, blood transfusions and dialysis, if in the case of renal failure. The prevention of infections with Escherichia coli is achieved by personal hygiene, food hygiene and work hygiene.*

Keywords: *Escherichia coli, infection, toxin, treatment, hygiene*

INTRODUCTION

Escherichia coli is the species of Enterobacteriaceae, of the genus Escherichia, with the greatest epidemiological impact. Escherichia coli infections are found mainly in places with poor hygiene; infants are included in the category with the highest risk. Also, adults can develop severe forms of infections, especially in regions where underdevelopment, poverty and lack of hygiene are the dominant picture.

The disease can occur in particular through the "fecal-oral" transmission mechanism as a result of consumption of contaminated food or water, or by "dirty hands". The foods most commonly implicated in the transmission of the infection are unpasteurized milk and milk products, beef, especially the one insufficiently cooked, unpasteurized fruit juice, lettuce

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[2] Titu Maiorescu University, Bucharest

and insufficiently washed vegetables. Processing raw food can contaminate kitchen utensils, especially if these are not separately used for each food category and are not cleaned between uses. Contamination can occur through contact with carrier animals, if the basic rules of personal hygiene are not complied with, such as washing hands with soap and water.

In recent years, in nearly all parts of the world have been reported numerous episodes of infection with Escherichia coli, resulting in numerous illnesses and even deaths. We recall the tragedy of Walkerton (Ontario/Canada), when approximately 5,000 people from the community showed symptoms specific to the digestive infection, the etiological agent being Escherichia coli [1]. In 2011, in Germany broke one of the largest outbreaks of acute gastroenteritis with hemolytic uremic syndrome (HUS) caused by the E. coli O104: H4 strain (German hemolytic uremic syndrome/ enterohemorrhagic E. coli - GHUSEC). The outbreak has had an unusual epidemiological origin only partially resolved. Investigations conducted by authorities showed that the illnesses occurred amid the consumption of contaminated water by agricultural runoff into a water well and subsequently there was the groundwater contamination [2].

The latest outbreak of the infection with Escherichia coli is evolving in Romania with this disease being contracted by 12 children (so far), aged between 1 and 3 years, all coming from localities in Argeș County.

The particularly severe infection resulted in the death of 3 children and even though the competent bodies through their health authorities have sought to find the source of the outbreak, they have not been able until now to find the source of the infection.

After the latest information, this seems to have been about a false "outbreak of diarrheal disease with uremic syndrome"; in reality the infectious etiology is varied, as confirmed by the Cantacuzino Institute.

In conclusion, due to the lack of a common source for the contagion, there cannot be a question of an epidemic or outbreak as the cases are isolated; however, due to the media pressure the incident could be termed as a "media outbreak".

ETIOLOGY

The genus Escherichia is of the Enterobacteriaceae family which comprises of 44 genera, 25 of which are found in human pathology. Most of these genres include commensal bacteria very abundant in nature (soil, water, etc.) [3], their natural habitat being the gastrointestinal tract of humans and warm-blooded animals, their feces containing more than 1010 enterobacteria/gram [4]. The constant presence of Escherichia coli strains in human and animal intestines and implicitly in the feces, determined that this bacterium represents the indicator of fecal pollution of the environment, especially of the water. Within the genus, are known 6 species: Escherichia coli, Escherichia fergusonii, Escherichia hermannii, Escherichia vulneris, Escherichia albertii and Escherichia blattae, the latter being isolated due to the fact that it only comes from specific species of insects.

Figure 1: Escherichia coli
(http://www.telegraph.co.uk 620 × 387)

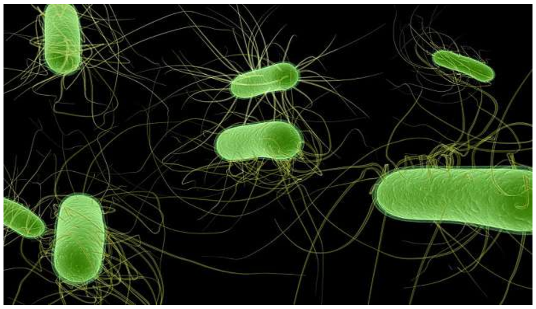

The most important representative of the genus is Escherichia coli, bacterium discovered by Austrian pediatrician Theodor Escherich. In 1886, after a hard clinical and laboratory activity he has presented at the Faculty of Medicine in Munich and subsequently published in Stuttgart, a monograph on the link between intestinal bacteria and the physiology of digestion in children entitled “Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung” [5]. In 1919, Castellani and Chalmers proposed that the name of the isolated bacillus from the colon (Bacterium coli commune), by the renowned Austrian pediatrician Theodor Escherich be changed in Escherichia coli, in his honor, a name that was officially approved in 1958 [6]. Bacteria are present in the form of straight or slightly curved bacilli with rounded ends,

the presence of flagella and therefore the mobility being constant in most species (Figure 1). It has a size between 2-5 µm/0.5-1 µm and from a tinctorial point of view is Gram-negative.

It grows on simple culture media (simple glucose broth, nutrient agar and blood agar) developing specific Type S colonies, round, domed, shiny with a smooth surface (Figure 2) and regular edges or with dry R colonies with irregular edges.

Figure 2: Escherichia coli culture on blood agar (http://www.wzzm13.com 534 × 401)

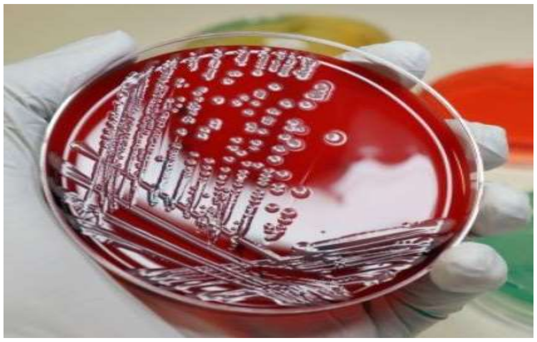

Biochemically, is fermenting the glucose with the production of gas, it produces indol, β-galactosidase and ferments lactose, characteristic that is allowing the differentiation from other enterobacteriaceae. Escherichia coli has an aerobic or optionally anaerobic metabolism and develops at an optimum temperature of 370C and pH between 7.2-7.8 [7], but differs from other coliform bacteria by increasing to 44^{0}C.

THE ANTIGENIC STRUCTURE

Escherichia coli has three types of antigens that can be highlighted by serologic antigen-antibody reactions:

- The O antigen (somatic) represented by a lipopolysaccharide surface antigen, thermostable, common to all S forms of the Enterobacteriaceae family;
- The H antigen (flagella) is a proteinaceous antigen (flagellin), heat-labile present in bacteria flagella;
- The K antigen (capsular) which may be of a polysaccharide or protein (fimbriae) nature. The O and H antigens are transmitted via plasmids and can be found together with the K antigens that are chromosomally determined.

Up until now, have been identified more than 170 types of the O antigen, more than 50 types of H antigen, 90 types of K antigen and several types of fimbriae antigens (F antigens). Combining all these antigenic variants, results in more than 1,000 antigenic types of Escherichia coli.

CHARACTERISTICS OF PATHOGENICITY

Colonic microflora in healthy persons (normal intestinal microbiota) is dominated by strains of Escherichia coli that live in a symbiotic relationship with the host. These infections may occur only if the host is immunocompromised or when it crosses the intestinal barrier and reaches areas which would normally be sterile. There have also been described pathogenic strains of Escherichia coli which have an exacerbated virulence with the possibility of being able to multiply, triggering infectious processes in immunocompetent individuals. Currently, have been described 6 intestinal Escherichia coli pathotypes involved in the EPEC diarrheal syndrome (intestinal pathotypes) and a pathotype involved in extraintestinal infections (urinary tract infections, meningitis, septicemia), ExPEC [8]. It is the most common etiologic agent in urinary infections, being incriminated in over 80% of urinary tract infections (UTI).

The health of the host (diabetes, immunodeficiency, colon malformations, obstructive uropathies, etc) is predisposing factors for infection with Escherichia coli.

Intestinal pathotypes are: enteropathogenic Escherichia coli (EPEC), enterotoxigenic Escherichia coli (ETEC), enteroinvasive Escherichia coli (EIEC), verotoxin producing Escherichia coli (VTEC), enteroaggregative Escherichia coli (EAEC) and diffuse adhesion Escherichia coli (DAEC). Within the VTEC pathotype stands out, mainly to highlight the association with severe clinical manifestations (haemorrhagic colitis), the subset of strains of the enterohaemorrhagic Escherichia coli (EHEC), whose prototype is the O157: H7 serotype. The pathogenic Escherichia coli strains produce virulence factors that contribute and affect the processes undertaken at cell level in the host (the protein synthesis the cytoskeleton’s function, the cell division, ion secretion,

the mitochondrial function, apoptosis). Adhesins are adhesion structures located on the fimbriae/pili (P fimbriae from the uropathogenic strains of Escherichia coli, the CFA colonization factors from the ETEC strains) or on the surface of the outer bacteria membrane (Afa afimbriae adhesins from DAEC strains). They are important virulence factors that allow bacteria to colonize areas of the human body (the small intestine, the urinary tract, the vagina), in which it does not normally reside. Toxins are another category of virulence factors. Endotoxins are part of the structure of the outer membrane (the A lipid from the LPZ complex) and are present in all Gram-negative bacteria. Released upon the elimination of the bacterial cell wall, they manifest their toxic effects being involved in the occurrence of the fever and the toxic shock from septicemia. Exotoxins are synthesized in the processes of the bacterial metabolism, being specific to the various pathotypes of Escherichia coli. Currently, are known the structure and the functions of many toxins responsible for the pathogenicity of strains of Escherichia coli (e.g., heat-labile enterotoxin produced by ETEC strains, verotoxins 1 and 2 synthesized by the VTEC strains, enteroaggregative Escherichia coli heat-stable enterotoxin 1 of EAEC strains etc.). Another important component of the pathogenicity of the species is the capsule. The main role of the capsule is to inhibit the action of the serum complement and the phagocytosis, thus imparting bacterial resistance. The K1 capsular polysaccharide antigen is present in 80% of Escherichia coli strains, isolated from the cases of neonatal meningitis.

THE DISEASE

Most of the Escherichia coli outbreaks were associated with the presence of the O15: H7 (VTEC) serotype. The incubation period of the disease varies between 1 and 9 days, averaging 3-5 days. Clinical manifestations are extremely varied ranging from non-specific diarrhea, dysentery form of enteritis (with an invasion of the colon), choleriform enteritis (with the invasion of the jejunum-ileum mucosa) to hemorrhagic colitis and consecutively the hemolytic uremic syndrome (HUS). The hemolytic uremic syndrome is characterized by acute renal failure, thrombocytopenia and angiopathic hemolytic anemia. The most common form of HUS is associated with diarrhea and has the highest incidence in children, being the major cause of acute renal failure in children [9, 10]. The factor responsible in HUS manifestations occurred after the VTEC infection and the Shiga dysentery is a protein enterotoxin called the Shiga toxin or VTI. Initially, the hemolytic uremic syndrome mortality rate reached to 50%, but the evolution of medicine and modern treatment techniques have reduced the mortality rate to 3-5%, indicating that approximately 30% of the healed patients remain with long-term sequelae, such as acute kidney failure [8]. Escherichia coli is the cause in 80% (ExPEC pathotype) of ITU (urethral cystitis, cystitis, nephritis, pyelonephritis, etc.). Contamination may be downward (blood-borne) or upward, this route being the most common. Infection is favored by the existence of obstructive congenital lesions, post gonococcal infection strictures, urinary stasis via decubitus or pregnancy. Once the bladder mucosa is invaded, subsequently occurs the upward progression to the kidney and the infection stabilizes at this level as a result of slow mobilization of the phagocytes [11].

Lab diagnosis

The diagnosis of the Escherichia coli infection is made by addressing two strategies namely, identifying and highlighting the etiological agent of the VTI toxin. The usual method used by most diagnostic laboratories is the selective isolation of the etiologic agent (Escherichia coli O157H7; O157H), on the MacConkey with sorbitol culture media from stool [12]. The method's principle is that the strains of Escherichia coli O157: H7 do not ferment sorbitol in 24 hours and the colonies appear colored on the culture, characteristic that differentiates them from the other strains of Escherichia coli. If the isolated strains are suspected as belonging to the serotype O157, but ferment the sorbitol in 24 hours, then will be used the specific antisera for titration and the agglutination tests [13]. The detection of the VTI toxin can be achieved through the cytotoxicity method, cultivation of isolated strains from feces on Vero or HeLa cell lines. The genetic methods (PCR) are based on the detection of specific sequences of polynucleotides that encode the synthesis of VTI. A new and very precise method is MALDI TOF, namely the proteomics analysis through

mass spectrometry of the specific proteins extracted from the ribosomes [14]. The principle of the method consists in measuring the mass of the weights of the polypeptides from the sample to be examined, generating and comparing its spectrum with the spectrum from the data base of the mass spectrometer (figure 3).

Figure 3: Maldi TOF operating flow (after Bruker Daltonix)

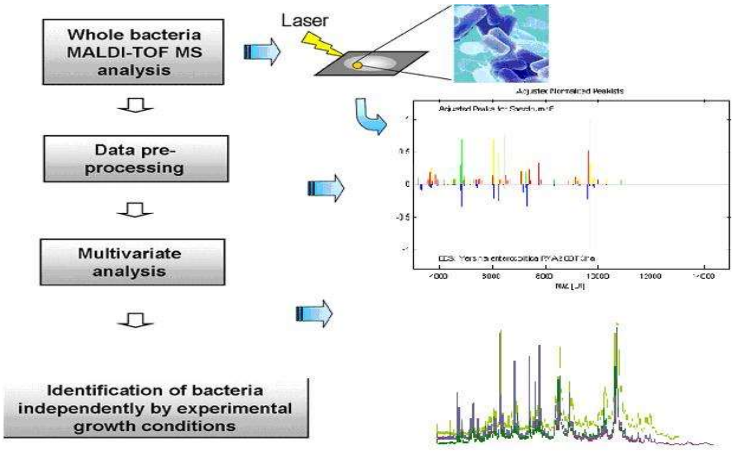
Whole bacteria MALDI-TOF MS analysis
Laser
Data pre-processing
Multivariate analysis
Identification of bacteria independently by experimental growth conditions

Other lab diagnostic techniques that are being used are the ELISA, for the detection of the O 157 antigen from feces and the serological methods which, however, are of limited value because Escherichia coli does not always develop serum antibodies against the VTI toxin. Also, the LPS antigen used may develop cross-reactions with other biological agents, such as Brucella abortus.

Treatment and Prevention

The treatment of the infection with Escherichia coli O157:H7 is often symptomatic and consists in the treatment of the complications arising due to massive fluid loss, and consequently of the acute diarrhea condition. As a result of dehydration and permeabilization of the blood vessels, pathological changes take place in the kidney function with renal impairment and severe anemia, that may become complicated with hemolytic uremic syndrome (HUS), as apparently is the case in the current outbreak in Romania, since winter-spring 2016. The therapeutic management must take into account the avoidance of the abuse of antibiotics that can foster the development of strains of Escherichia coli, avoiding the administration of antispasmodic drugs with effect in slowing bowel, thus maintaining more toxins in the digestive tract and amplifying its harmful effects. It recommended avoiding administering products based on salicylates and ibuprofen that can increase digestive bleeding. The symptomatic treatment must include hydrodynamics and electrolyte rebalancing, blood transfusions and, if in severe forms of renal failure, dialysis. Prevention of disease is achieved by non-specific measures such as: washing hands with soap and water before and after eating; processing animal products and by-products; washing fruits and vegetables before consumption or industrial processing; avoid the consumption of raw animal products (milk) or semi-raw (hamburger); avoid the consumption of water from bacteriologically unverified sources; washing utensils that came in contact with meat or raw meat products; avoiding the use of unpasteurized juices, in particular for children, etc. In serious cases, potentially fatal, especially in young children, may be needed the treatment from the Intensive Care with perfusions, resuscitation and even peritoneal dialysis.

CONCLUSIONS

- Escherichia coli is from the Enterobacteriaceae species present in the colonic microflora of a healthy human, which has the largest epidemiological impact.
- The transmission mechanism of the infection is

"fecal-oral" as a result of the consumption of contaminated food or water, or by "dirty hands".

• The disease has been reported worldwide, being described numerous episodes of infection with Escherichia coli that caused multiple illnesses and sometimes even deaths (Walkerton Ontario/Canada, GHUSEC - Germany).

• Escherichia coli has three types of antigens: antigen "O" (somatic) antigen "H" (flagella) and antigen "K" (capsular) that can be highlighted by serologic antigen-antibody reactions. Combining all these antigenic variants theoretically result in 1,000 antigenic types of Escherichia coli.

• There are six known pathotypes of Escherichia coli involved in the EPEC diarrheal syndrome (intestinal pathotypes) and one pathotype involved in extraintestinal infections (urinary tract infections, meningitis, septicemia), ExPEC.

• Toxins are the most important category of virulence factors in Escherichia coli (heat-labile and heat-stable enterotoxin produced by ETEC strains, verotoxins 1 and 2 synthesized by VTEC strains, enteroaggregative heat-stable enetrotoxin of EAEC strains etc.).

• Clinical manifestations are extremely varied ranging from non-specific diarrhea, dysentery form of enteritis (with an invasion of the colon), choleriform enteritis (with the invasion of the jejunum-ileum mucosa) to hemorrhagic colitis and consecutively the hemolytic uremic syndrome (HUS).

• The Escherichia coli infection diagnosis is made by identifying the etiologic agent (biochemical tests, agglutination reactions, PCR, mass spectrometry) and by highlighting the VTI toxin (cell culture cytotoxicity assay, immuno-chromatographic methods, ELISA).

• The treatment is symptomatic and includes hydrodynamics and electrolyte rebalancing, blood transfusions and, if in severe forms of renal failure, dialysis.

• The prevention of infections with Escherichia coli is achieved by non-specific measures such as: washing hands with soap and water before and after eating; processing animal products and by-products; washing fruits and vegetables before consumption or industrial processing; avoid the consumption of raw animal products (milk) or semi-raw (hamburger); avoid the consumption of water from bacteriologically unverified sources.

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REVIEW ARTICLE

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Intellectual mobility in medical higher education system

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Abstract: *Intellectual mobility brings change, there is the primary factor in the way of progress and optimal premise of human being development from theoretic and practice regards. Medical Higher Education, worldwide, is generally similar in structure and consistency, but different in typology of presentation, teaching, learning and assessment. In fact, general medicine, as a subject refers to the same biological body, but presented differently depending on culture, space and under various methods of teaching and learning.*

The idiom of intellectual mobility is not new, but according to globalization, which we live at the present times, brought the mobility in the main plan of Europeanization, a new plan, with continues sustainable development and maybe of success. By institutional mobility, both for students and for academic staff, an exchange means a period of one academic year or a semester, for students and, for two days to several months for academic staff, into a foreign university. These stages of study, practice, and teaching take place most frequently within the Erasmus + framework, have been of 30 years in Europe and 20 years in Romania. Also, there are other programs that can perform intellectual mobility, but the most well-known is Erasmus program, where European Commission has allocated the biggest legal and financial budget framework. Overall activity program features has a variety of tools to be deployed and an inter-institutional framework with qualified staff to manage it.

Keywords: *medical education, recognition, higher education, mobility, Erasmus program*

INTRODUCTION

General Medicine as an important branch of study has a special status in the conduct of mobility as importance of the field and also under emotional aspect.

Intellectual mobility takes a certain mental patterns generated by education and a native predisposition, regardless of the field. Along with this added dynamic appeal cases handled by the abstract representations. In the field of medicine is worked with a high degree of abstraction. Or spatial relocation and mobility means mental derived including concrete way of their operationalizing in the two phases of the medical profession: correct diagnosis and treatment of the patient, not the disease. Appeal to intellectual mobility, is done often in the holistic approach to the patient. The doctor represents, on the one hand a mechanic and on the other hand a sociologist. This would be the condition of being a successful doctor. There is also the assumption that both treatment and technique coordinate the doctor to a successful performing.

Casuistry with high risk requires determination,

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curiosity and professional beliefs. However, it is based on a dominant personality characteristic correlated with a predominantly emotional intelligence, and then on IQ. But there is a less explored area of the mind of a physician or a future doctor, a student, how to manage frustration generated by failure in high-risk cases, but not only. Failure, in any other domain generates lessons learned that are grouped in a simple taxonomy:

1. Lessons indicating approaches “know how";
2. Lessons to refute or confirm hypotheses absolutely absurd;
3. Lessons to optimize cases generally valid but wrong managed.

Thus, intellectual mobility in general, depends of subjective perception of reality itself the objective of playfulness and cognitive operators with which man is accustomed routinely to operational abstractions. Medical education has the same topology and provides almost the same bibliographic regarding symptoms of the disease; unfortunately get to treat the disease using methods quite invasive.

Such patterns of study are known in medical education through student mobility from one education system to another. Exchange of experience, for a period from one university to another can bring new knowledge, new methods of learning, but also a personal self, psycho-emotional development.

Erasmus mobility can bring competitive doctors on the labor market and ensure a quality structure, but the real problem is the distribution on the labor market.

As it is well known Romanian doctors prefer to work in European hospitals or beyond Europe, which is not bad, on the one hand, but on the other hand it led to a destabilization of the health system in Romania. So Erasmus mobility tended by a medical mass migration.

Naturally, in this context a basis is economically and socially disadvantaged in Romanian hospitals and moral degradation of the system.

MEDICAL HIGHER EDUCATION IN DRESDEN, ROME AND BUCHAREST

A brief history

A brief history of medicine proves that it was practiced of ancient times from trained professional. History and times prove how the society have changed and it is also in a continuous changes in the approach to sickness and disorder from ancient beginnings

It is well known in the world that medical services were provided for the poor people in monastic hospitals. The care was rudimentary way and rather palliative. As we can observe also medical services and school education, in any domains started from the monastic area, in churches. As just a thin remembering it can be named that culture and civilization started around the human necessity of norms and rules issued by the spirituality.

In the 9th century there were some medical schools in Italy. The influence from other nations as: Greek, Latin, Arabic and Hebrew gave an international dimension. Students learn three years as preliminary courses and five years of medical schools. Nowadays they study five, six or seven years in Europe and in SUA more than ten years.

Italy is the place where medical universities were founded, after came France and England which developed medical schools. So we can see from the beginning the health science started in an internationally manner and mobility and migration are quit ancient and were very important in the development of it.

Today according to the progress of technology, techniques and information system mobility is very used and normal in the society.

Recognition of studies

In order to define studies in Erasmus Program we have to analyze its framework. The main problem is the recognition in making Erasmus.

Although there is a desired of full recognition of Erasmus studies, according to the Erasmus Charter, it is not possible, discussing the case by case,

depending on the curriculum and structure. Of course, that is an ideal situation that a degree program can be accepted fully recognized, but there are features that can be dealt with individually. Although Erasmus Charter directs the full recognition and, in general, universities are trying to respect this principle, even though at the end of the program, there are people involved in Agencies of Recognition and Accreditation of Studies from all over Europe, not easily accept that Erasmus has a special pattern, easily convertible by ECTS and also has all instruments and forms provided. We will see a simple case which presents two distinct situations of curricula on a few subjects of study. We'll see how a curriculum in three different states differ and how to work through the transformation to studies from a curriculum to another, by grades, ECTS obtained both from practical or clinical training and courses. Quantification of studies must be easy and in interest of students. Of course it is of great important qualitative component, especially in the field of medicine. But always should take into account the socio-cultural characteristics, adjusting the student in a new cultural space and psycholinguistics barrier.

In the context of Europeanization and for exemplifying the above situation exposes three major universities with medical schools, in Europe.

Universitatsklinikum "Carl Gustav Carus" Dresden Technische Universität of Germany, especially the ENT discipline; Universita Degli Studi di Sapienza, Rome, Italy, with examples on general surgery and internal medicine and Titu Maiorescu University in Bucharest, Romania, as a university of origin/home university, which makes recognition and equivalence studies.

Germany is a country of art, technique and study continuously, so the team from ENT created a standardized teaching maneuvers examination by a small guide, so that students can observe organized clinical examination of Otolaryngology. Consideration is made all the standardized by checking maneuvers in a form, for a period of 6 minutes of examining a patient. This calculates a score of the exam, and the form can be filled by two examiners for the compared results.

Such an experience has brought by an academic staff that has benefited of teaching stage in Dresden. Today, the ENT method was implemented in Bucharest for teaching and assessment the subject. One such example is enlightening to harmonize the methods of teaching and assessment, job shadowing lead to the development of new skills.

Regarding the Recognition of Studies facts are the discussions become slightly rigid and austere although the program is provided with all the necessary tools.

A student who chose as subjects of study maxillofacial surgeon, a course of a single module named Head has 1 ECTS, but the workload is the same as that of a course of otolaryngology at Bucharest, which has 4 ECTS. Also, the grading system is different. In Germany the scale grades are from 1 to 5, and in Italy from 18 to 30; 1 ECTS has 25 hours of workload. In Romania, at faculty of medicine 1 ECTS has approximately 14 hours of workload. Depending on the workload is denoted by ECTS, 30 for a semester and 60 per academic year, which should be equivalent between higher education systems, but they are not. Module Thorax includes the following disciplines: Cardiology, Angiology, Pulmonology, Vascular Surgery, Thoracic and Cardiac has a volume of 10 ECTS, if studied together, if it divides, then the number of ECTS is divided too, in Romania they are separate disciplines. In Italy, Internal Medicine and General Surgery is a module that measures 12 ECTS together, and in Romania internal Medicine has 6 ECTS for the first semester and for the second semester it has 5 ECTS, in the end there are 11 ECTS, just Internal Medicine. General Surgery is another subject and it has 4 ECTS for the first semester and 4 ECTS for the second semester; they are totally different from one system to other. Such recognition is based on workload and always is made in the favour of the student, or should be.

In Germany the focus is on clinical stages, more than in Italy. In Romania, specialized practice/training is done since of the first year of study, according to the students' testimony.

Although there are differences in the three systems

of teaching and learning, Erasmus has provided the tools; study contract/Learning Agreement, and students can choose their subjects to be studied and disciplines which will be equate to return. Studies are, or should be recognized, integrum, full recognition under the Erasmus Charter. If the student does not fulfill the learning agreement he/she will support additional exams from local education until completion of ECTS number needed to pass the academic year. These are predetermined patterns of procedure and related methods for classification and institutionalization of each institution. What becomes interesting is the prospect of personal development of each individual differs from person to another depending on operators and cognitive education.

There are three factors that can prevent full recognition, as following:

1. Changing subjects of study during mobility, Erasmus Learning Agreement provides that rule can be changed, only in the first 14 days of mobility. Thus, changing the curriculum content, can prevented full recognition procedure because of the time period from the moment of making the new choice of new disciplines and to the approval by the academic tutor from home institution.

2. A second factor that keeps the procedure and otherwise representing a procedural error is soliciting approvals for the recognition and equivalence studies to the professors who are tutor of the disciplines. Thus, the holder of course, may be not sufficiently informed and decide in the detriment of the student. Erasmus Rule requires the application of Charter based on acceptance of Erasmus in function. So is forbidden that a tutor can decide regarding his/her discipline.

3. Finally a third factor, which prevents full recognition, is negative influence of the party who decide subjective and would not assumes the recognition of Learning Agreement. These are isolated cases, and in recent years almost no longer exist. The procedure for recognition and equivalence is the essential characteristic of students in the decision to go in Erasmus mobility.

According to the magazine Prime 2010, only 19% of students surveyed are convinced that it will not benefit from an exchange. The rest of the students who responded to the questionnaire in the same magazine argued that regardless of the recognition of studies, mobility itself and experience are more important than the recognition of studies, thus they assuming full academic exchange activities. Of course that always the activity must be tried separately according to each case. If the student wishes on its own initiative to have examinations in the subjects of home university, it is not prevented, or if the student did not follow important disciplines for future examinations of competence, then it will have them at the return from the mobility without charge of any fee.

There is also a risk that the student take courses that are done in the near future/years of study in the home university and through full recognition, the student would be forced to repeat subject mobility in the coming years. As such, the choice of subjects, from a curriculum structured around six years can be challenging even for academic tutor. This happens because the curricula are not similar, nor how to be similar. Bologna process does not seek to standardize the Higher Education, but seek to a better harmonization of curricula, a socio-cultural and economic uniformity.

If the student is studying disciplines in the curriculum of the home university is doing in a upper year, the University, study case of this research, recognizes full program of study at the partner university and mention the time spent abroad in the Diploma Supplement, and recognizes discipline by discipline in the years that match the local program, and to promote appropriate student take exams in the subjects of study sessions legal up, and in special cases can be organized special sessions for Erasmus students. Whatever, the situation of recognition and equivalence of studies is made, only in students' favor without affect the merit place at home university.

Despite these shortcomings of procedures for recognition of studies, students wishing to repeat the experience to the extent permitted by the Erasmus program.

Most often students after followed a study program they would like to follow a clinical internship. From the experience of the university concerned, they apply after completing their studies in residency programs in the world. University receives per few times a year, forms requesting certification of studies by agencies of recognition of medical studies in the USA and Canada.

Table 1: Example of a model of recognition studies, in Germany
(it can be observed that the students pass more than 5 ECTS at home university)

Name of the subject	ECTS	Grade in Germany	Grade in Romania
Maxillofacial Surgery	1	1,5	10
Cardiology, Angiology, Pulmonology, Vascular Surgery, Thoracic and Cardiac	10	2	9
Dermatology & Venereology	3	4	5
Rheumatology	2	attended	
Pathophysiology & Medical Biochemistry and Laboratory Diagnostics	9	4	5

It is mention were equated the subjects studied in Germany in the V th year, semester 1 of the study, under the Erasmus Charter, after the student has pass the remaining number of 5 ECTS, as follows:

Name of the subject	ECTS	Grade in Romania
Pneumology	4	7
Occupational diseases	5	8
Radiology	5	9
Gerontology		10
TOTAL	14	

Analyzing the situation of a student who was Erasmus, in the fifth year of study at the University of Dresden, after she came back home observed willingness to continue the study and mobility training, such as she obtained a period of 2 months in a hospital in Germany, combining theoretical and practical work with a clinical internship. Besides the recognition and equivalence of procedural, student mobility has made a qualitative leap in medical education, as well as psycholinguistic improving linguistic competence and went beyond customary in the sphere of national education.

CONCLUSION

Mobility in medical education can make steady progress in what is called globalization, harmonization of curricula and skills in the labor market. The main objective of globalization and mobility function is preventing and overcome poverty and habits that can hinder knowledge and human progress. The internationalization of education is a process of development and a better preparation of students for a globalized society, based on knowledge and skills. Higher education institutions have the main role to prepare graduates for the labor market in the line with international policies of globalization. Mobility brings extra value to Europe and is a sine qua non of human development in relation to the labor market, the practical problem that remains in the knowledge era is the economic and political, social and moral worldwide crisis, otherwise unmanageable. Now it is felt the effects of the global turmoil, but concrete results are likely to be known around 2050.

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REVIEW ARTICLE

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The influence of homocysteine on osteoporosis

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Abstract: *Osteoporosis is a major health problem, and the economic costs are expected to rise due to an increase in life expectancy throughout the world. Its major consequence is fractures, and especially hip fractures are associated with institutionalization and increased mortality. Homocysteine is an amino acid intermediate formed during the metabolism of methionine. Homocysteinuria is a rare autosomal recessive biochemical abnormality which causes elevated plasma concentrations of homocysteine and severe occlusive vascular disease. In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. The high prevalence of osteoporosis among patients with homocysteinuria suggests that high levels of plasmatic homocysteine may also increase the risk of fractures. Nutritional factors such as vitamins B12, B6, and folate are cofactors in homocysteine metabolism, and vitamin intakes may inversely affect plasma homocysteine levels.*

Keywords: *osteoporosis, homocysteine, hip fracture*

INTRODUCTION

Osteoporosis is a major health problem, and the economic burden is expected to rise due to an increase in life expectancy throughout the world. Its major consequence is fractures, and especially hip fractures are associated with institutionalization and increased mortality. The prevalence of osteoporosis increases with age due to an imbalance in the rate at which bone is removed and replaced during the bone remodeling cycle, which is an important physiological process that is essential for maintenance of a healthy skeleton.

Pharmacological interventions may prevent 30-60% of fractures in patients with osteoporosis. Common sites for osteoporotic fracture are the spine, hip, distal forearm and proximal humerus. The remaining lifetime probability in women at the menopause of a fracture at any one of these sites exceeds that of breast cancer (approximately 12%), and the likelihood of a fracture at any of these sites is 40% or more in developed countries [1].

The level of bone mass can be assessed with adequate precision by measuring bone mineral density using dual X-ray absorptiometry. It has

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been suggested that bone strength may be reflected, independently of bone mineral density level, by ultrasonic measurements of bone and by measuring bone turnover using specific serum and urinary markers of bone formation and resorption.

Physical activity as a way to prevent osteoporosis is based on evidence that it can regulate bone maintenance and stimulate bone formation including the accumulation of mineral, in addition to strengthening muscles, improving balance, and thus reducing the overall risk of falls and fractures. It is well known the important influence of hormones as well as dietary and specific nutrient abundance on bone, growth and health is emphasized and premature bone loss associated with dietary restriction and estradiol withdrawal in exercise-induced amenorrhoea [2].

OSTEOPOROSIS

It is becoming increasingly clear that there is a relationship between growth and development in early childhood and bone health in old age. In fact, suboptimal bone development leads to a reduction in peak bone mass, and a higher risk of osteoporotic fracture later in life. Osteoporosis is a skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility. Preventative strategies against osteoporosis can be aimed at either optimizing the peak bone mass obtained, or reducing the rate of bone loss.

One of the largest risk factors for fractures is a reduction in bone mineral density. Risk factors for fracture can be purely skeletal-related affecting bone mass, bone geometry, bone micro-architecture and bone turnover, or solely fall-related such as neuromuscular dysfunction, poor balance, cognitive impairment, cardiovascular instability, reduced visual acuity and sedative medications. Others risks are both skeletal and fall related such as age, genotype, and family history of fracture, weight, weight change and mobility [3]. Falls history is a further independent risk factor for fracture, in particular in men [4]. About 50% of white women and 20% of men will have an osteoporosis-related fracture in their lifetimes. Fractures of the hip and spine may be disabling and are associated with mortality rates that are about 20% greater than that of an age-matched population. The goal of any treatment for osteoporosis is to improve bone strength, thereby decreasing fracture risk.

Bone remodeling is the result of two opposite activities, the production of new bone matrix by osteoblasts and the destruction of old bone by osteoclasts. The rates of bone production and destruction can be evaluated either by measuring predominantly osteoblastic or osteoclastic enzyme activities or by assaying bone matrix components released in the bloodstream and excreted in the urine [5].

Factors which influence negatively the osteogenetic potential are age, nutrition diseases and endocrinopathy, ionized radiations treatments and different toxic factors. It has been scientifically proved the fact that elder patients have decreased general biological potential. Regarding the bone system, there occur structural changes which weaken the resistance, to which we can add a diminishing of the response potential towards harmful factors. These lacks are considered to belong to multiple causes: a diminishing of the vitamin D action and of calcitonin, hyperactivity of the parathyroid hormones with osteoblast inhibition, etc. [6] As a result, osteolysis-osteo-synthesis dynamics is reversed, resorbing processes become dominant: osteodystrophy, osteoporosis, senile osteopenia, metabolic and endocrine osteopathy, which, affecting the very bone structure, are the main causes of diminish in the osteogenic potential in elderly patients. Nutrition diseases and endocrinopathies seem to have the most harmful effects on osteogenesis and the repairing processes of the bone tissue.

The evaluation of biochemical markers of bone turnover has been useful in clinical research. However, the predictive factor of these

measurements is not defined clearly, and these findings should not be used as a replacement for bone density testing [7]. There is a high prevalence of calcium, protein and vitamin D insufficiency in the elderly. Calcium and vitamin D supplements decrease secondary hyperparathyroi-dism and reduce the risk of proximal femur fracture, particularly in the elderly living in nursing homes. Sufficient protein intakes are necessary to maintain the function of the musculoskeletal system, but they also decrease the complications that occur after an osteoporotic fracture.

HOMOCYSTEINE

Sulfur is the seventh most abundant element measurable in the human body and is supplied mainly by the intake of methionine, an indispensable amino acid found in plant and animal proteins. Inhibition of cystathionine-β-synthase activity causes the upstream sequestration of homocysteine and the downstream drop in cysteine and glutathione [8]. Homocysteine is an aminoacid which contains a thiol group formed by methionine intracellular demethylation (alpha amino gama methylthio butyric). In plasma, homocysteine is found free in oxidized or disulphidic form, linked to proteins. Homocysteine has two ways to be metabolised, a metabolic path is represented by transsulfuration to cysteine through cystatin synthetasis, enzymes which need vitamin B6 as co-factor (proving the need of vitamin B6 administration during the treatment). Synthesis of cysteine as a product of the transsulfuration pathway can be viewed as part of methionine or homocysteine degradation, with cysteine being the vehicle for sulfur conversion to end products (sulfate, taurine) that can be excreted in the urine. Transsulfuration of homocysteine to cysteine is catalyzed by two pyridoxal 5′-phosphate-dependent enzymes, cystathionine β-synthase and cystathionine γ-lyase. The transsulfuration pathway is responsible for catabolism of the carbon chain of methionine, release of the amino nitrogen in a form that can be funneled into pathways of nitrogen excretion, and transfer of Met sulfur to serine to synthesize cysteine [9].

Another metabolic path is remethylation to methionine in the presence of methylentetra-hydrofolate reductase (MTHFR) and methionine synthesis in the presence of folic acid as an under layer for vitamin B12 as co-enzyme (proving the need of folic acid and vitamin B12 administration during the treatment). There are studies which proved that the level of homocysteine in blood is inversely proportional with folate levels, vitamin B12, vitamin B6 and oxygen intake induced by these vitamins [10].

Homocysteinuria is a rare autosomal recessive biochemical abnormality which causes elevated plasma concentrations of homocysteine and severe occlusive vascular disease. In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. Blood levels of total homocysteine increase throughout life in men and women. Prior to puberty, both sexes enjoy optimally healthy levels (6 µmol/L). During puberty, levels rise, more in males than women, reaching, on average, almost 10 µmol/L in men and more than 8 µmol/L in women. As we age, mean values of homocysteine continue to rise and the concentrations usually remain lower in women than in men. Adults without homocysteinuria who have high homocysteine levels are also at risk for fractures. Thus, elevated plasma homocysteine concentrations (>14 µmol/L) are associated with osteoporosis and may increase the risk of hip fracture, in both men and women. These can lead to substantial disability, high medical costs, and death [11]. Elevated plasma homocysteine concentrations are associated with reduced physical performance and muscle strength in older women [12].

Hyperhomocysteinemia may contribute to the development of osteoporosis. High hyper-homocysteine and low vitamin B12 concentrations were significantly associated with low bone turnover markers, high markers of bone turnover, and increased fracture risk [13]. Folate and vitamins B12 and B6 are major determinants of homocysteine concentrations in older persons [14]. The B vitamins folate, B12, and B6 are important cofactors in homocysteine metabolism, and low status of these

nutrients is the primary determinant of elevated plasma homocysteine concentrations in elders. Patients with pernicious anemia have decreased bone mineral density at the lumbar spine, and in comparison with the general population they have almost double the risk of hip fracture. Gene polymorphisms related to homocysteine metabolism also may result in high homocysteine levels. Thus, nutritional factors such as B12/folate deficiency and genetic factors may affect homocysteine levels and contribute to fracture risk.

Hyperhomocysteinemia is regarded as a risk factor for ischemic stroke and for hip fractures in Parkinson's disease patients receiving levodopa [15]. The high prevalence of osteoporosis among patients with homocysteinuria suggests that hyperhomocysteine may also increase the risk of fractures [16]. Higher plasma levels of total homocysteine and folate were independent predictors of coronary heart disease [17]. In the Framingham study authors had shown that plasma total homocysteine concentration is inversely related to the intake and plasma levels of folate and vitamin B6 as well as vitamin B12 plasma levels. Almost two-thirds of the prevalence of high homocysteine is attributable to low vitamin status or intake. Elevated homocysteine concentrations in plasma are a risk factor for prevalence of extracranial carotid artery stenosis of at least 25% in both men and women [18]. Some researchers want to determine if there is a possibility that plasma total homocysteine may serve as an indicator of the status and perhaps the intake of a number of vitamins, including folic acid, vitamin B12, and vitamin B6. This possibility derived from the large number of studies that implied that methionine metabolism is tightly regulated and from other studies that showed that deficiencies in the above vitamins are often associated with hyper-homocysteinemia.

It was shown that premenopausal women have lower homocysteine levels than men and postmenopausal women. Higher plasma levels of total homocysteine concentrations in men than in women may be explained by differences in muscle mass, hormone and vitamin status [19]. Factors that influence total homocysteine plasma levels in the general population include diet, in particular folate intake, blood levels of folate, vitamin B12, and betaine, renal function, and the MTHFR 677C>T polymorphism [20]. There are studies to establish that increasing evidence that plasma total homocysteine is inversely associated with bone health. It has been speculated that moderately elevated total homocysteine levels could contribute to osteoporotic changes, based on the fact that osteoporosis is a common phenomenon in homocysteinuria. High total homocysteine and low vitamin B12 concentrations are significantly associated with high levels of markers of bone turnover, and relations have been reported between total homocysteine and markers of bone resorption [13].

Elevated plasma total homocysteine, deficiencies of folate and vitamin B12 are associated with risk of osteoporosis and fracture. In some studies, there were examined whether plasma levels of elevated plasma total homocysteine, folate, and vitamin B12 predicted hip fracture [21]. They found that elevated plasma total homocysteine is a predictor for hip fracture among elderly men and women.

CONCLUSION

In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. The high prevalence of osteoporosis among patients with homocysteinuria suggests that hyperhomocysteine may also increase the risk of fractures. Nutritional factors such as vitamins B12, B6, and folate are cofactors in homocysteine metabolism, and vitamin intakes may inversely affect plasma homocysteine levels.

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REVIEW ARTICLE

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Clinical features of oral angiolipoma – A review

Shishir Ram Shetty[1], Sura Ali Ahmed Foud Al- Bayati[1], Shakeel Santerbennur Khazi[1], Sesha Reddy Manchala[1]

Abstract: *The aim of this paper was to analyze the data obtained from case reports of oral angiolipoma in terms of age and gender distribution, site of occurrence, nature (intraosseous/ extraosseus) and infiltration. an internet search using Google scholar and Pubmed engine was carried out using search terms 'angiolipoma' and 'oral'/'mouth'. English literature full text articles and abstracts of oral angiolipoma obtained from 1976-2016 were analyzed for clinical data and presented in this article.*

Keywords: *angiolipoma, oral, mouth*

INTRODUCTION

Angiolipoma (AL) is rare variant of lipoma which consists of both fatty and vascular elements. [1] ALs were first describe by Bowen in the year 1912. [2] The first published report of an oral angiolipoma was by Davis et al in 1976. [3] Angiolipomas account for 5 to 17% of all the lipomas occurring in the oral cavity, lipomas themselves account for 1-5% of all oral benign tumors [1]. Since oral angiolipoma is rare benign tumor, published reports are mainly restricted to case reports. [4,5]

CLASSIFICATION OF ANGIOLIPOMAS

Based on their studies Gonzalez-Crussi et al. classified angiolipomas into infiltrating and non-infiltrating. angiolipomas.[6] The non-infiltrating angiolipomas are encapsulated, whereas infiltrating angiolipomas lack this capsule.[6] Among the two, infiltrating angiolipoma (IAL) is a rare lesion typically characterized by infiltration of the surrounding structures, especially skeletal muscle.[7] Based on whether the angiolipoma occur in bone or soft tissues they can be classified as intra-osseous or extra-osseous types.[8] The intra-osseous variety is extremely rare in comparison to the extraosseus variety. [8,9]

METHODOLOGY FOR ANALYSIS OF THE CASE REPORTS

An analysis of the data obtained from case reports of oral angiolipoma in terms of age and gender distribution, site of occurrence, nature (intraosseous/ extraosseus) and infiltration. An internet search using Google scholar and pubmed engine was carried out using search terms 'angiolipoma' and 'oral'/'mouth'. Full text and abstracts of oral angiolipoma cases obtained from 1976-2016 were analyzed. (Table 1)

GENDER PREDICTION OF ORAL ANGIOLIPOMA

Fregnani ER et al (2003) stated that male to female

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ratio from these intraoral angiolipomas was 1.4:1, in contrast to cutaneous angiolipomas and intraoral lipomas as a group, which either shows no sex predilection or a slight female predilection.[10]

Table 1: Clinical data obtained from full texts and abstract of oral angiolipoma case reports published from the year 1976-2016

Researcher	Year of publication	Type infiltrating/ non-infiltrating	Gender	Age group	Site	Extraosseous/ intraosseous
Davis et al [3]	1976	-	-	-	hard palate	
Polte HW et al [30]	1976	-	-	-	mandible	intraosseous
Weitzner et al [35]	1978	-	male	4-year-old	cheek	
Campos et al [34]	1980	-	-	-	cheek	
Lewis DM et al [31]	1980	-	-	-	mandible	intraosseous
Brahney CP et al [21]	1981	-	-	-	tongue	
Flaggert et al [19]	1986	-	female	8-year-old	palate	
Reilly et al [15]	1988	-	female	infant	parotid	
Lin SC et al [17]	1989	-	male	49-year-old	tongue	
Manganaro AM et al [32]	1994	-	-	-	mandible	intraosseus
Ali MH et al [36]	1996	-	-	-	cheek	
Sugiura J et al [16]	1999	infiltrating	-	-	muco-buccal fold	
Ida-Yonemochi H et al [12]	2005		male	69-year-old	buccal mucosa	
Altug HA et al [11]	2009	non-infiltrating	male	22-year-old	cheek	
Gerard N et al [20]	2009	-	-	-	upper lip	
Thakur B eta al [37]	2010	non-infiltrating	female		cheek	
Palaia G et al [38]	2011	non-infiltrating	female	66-year-old	cheek	
Yanase S et al [13]	2011	non infiltrating	male	76-year-old	cheek	
Sah K et al [4]	2012	non infiltrating	female	9-year-old	upper lip	
Hemavathy S et al [9]	2012	-	female	21-year-old	mandible	intraosseus
Silva-Junior GO et al [5] 2 cases	2013	non-infiltrating	male	57-year-old	cheek	
	2013	non-infiltrating	female	29-year-old	masseteric region	
Shahi AK et al[14]	2014	infiltrating	female	9-month-old	cheek	
Mohiuddin SA et al [39]	2014	non-infiltrating	male	46 year old	mandibular buccal vestibule	
Ohnishi Y et al [7]	2015	infiltrating	female	74 year old	lower lip	
Chandrasekaran D et al [1]	2016	non infiltrating	female	55-year-old	palate	

However most of the other researchers have stated that no gender predilection. [1,7,11,12] According to the data obtained from the analysis of the reports, seven cases were in males, ten cases occurred in females. However we could not obtain enough data regarding gender in 9 case reports.

AGE PREDILECTION OF ORAL ANGIOLIPOMA

Fregnani ER et al (2003) stated that the mean age of the affected patients was 37 years (range, 4 to 81 years),which was in contrast to other variants of lipomas occurring in the oral cavity that occur in the 50-60 year age group.[10] On analysis of the available case reports we found that occurrence of oral angiolipoma ranged from a 4-month-old to 76-year-old individual.[13,14,15] Infiltrating angiolipomas are usually diagnosed in older individuals.[7, 16, 17, 18]

SITE PREDILECTION OF ORAL ANGIOLIPOMA

Angiolipoma have a prediction of occurrence in the

buccal mucosa, followed by lip.[5] Occurrence in palate has been reported in few cases.[1,3,19]. Upper lip involvement is more common than lower lip.[4, 20] Few cases have also been reported in tongue.[17, 21] However many of the cases of angilipoma of tongue have the muscle component along with fat and vasculature and thus are termed as angiomyolipoma.[22,23, 24, 25]. The occurrence of angiolipoma in the parotid has also been reported.[15] The benign angilipomas occurring the salivary gland have glandular component apart from adipose and vascular components and are called as sialoangiolipoma.[26] Such cases have been reported to have been observed in parotid and submandibular gland.[27, 28] A rare case of a lipoma with vascular and salivary gland components has been reported in palate also.[29]

INTRAOSSEOUS ANGIOLIPOMA

The first case of intraosseous angiolipoma was by Polte in the year 1976.[30] Mandible is the most common site of occurrence of intraosseous angiolipoma.[9,31,32] Intraosseous lipoma may present a range of clinical manifestations such as swelling, or presence of neurologic signs (hypesthesia or paresthesia) or can be totally asymptomatic.[9] On radiographic examination the lesion usually presents as well-circumscribed radiolucency.[9] Extraction related trauma is believed to be cause for intraosseous angiolipoma of the jaws. Fatty metamorphosis of a central hemangioma and possible variation osteoporotic bone marrow defect are other suspected etiologies. [33]

INFILTRATING ANGIOLIPOMA

Infiltrating angiolipomas lack encapsulation and are typically characterized by infiltration of the surrounding structures.[6,7,14,16] In contrast to the non-infiltrating angiolipomas are well encapsulated. [34,35,36,37,38]. Infiltrating angiolipoma usually occur in older individuals but a report of 9-month-old diagnosed with infiltrating angiolipoma has recently been published.[14] Infiltrating angiolipomas are also associated with symptoms muscular pain and neural deficits.[39,40]

CONCLUSION

In this review we have made an attempt to analyze the clinical features of oral angiolipoma in terms of its gender predilection, site predilection, infiltrative nature, intra/extraosseus types. The information obtained from this review could aid the clinicians in diagnosis this extremely rare benign lesion.

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SYSTEMATIC REVIEW

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Hepatocellular carcinoma – A review

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Hepatocellular carcinoma is a major public health issue, occupying the fifth place of the most common cancers and the third leading cause of cancer in the world. The incidence of HCC has increased in the past decade thanks to higher rates of HCV infection and improvements in the management of cirrhotic patients. The regions with the highest incidence of HCC are, in descending order: Eastern Asia, sub-Saharan Africa, South-East Asia and southern Europe. It has been observed that etiological factors which lead to HCC have a heterogeneous distribution; thereby, in Asia and Africa the most common risk factor is HBV infection, increased by ingestion of aflatoxin B1-contaminated food that is associated with a specific mutation in the p53 tumor suppressor gene. HCC may develop earlier in these regions, most common in a noncirrhotic liver. In developed countries, the principal risk factors are HCV infection and alcohol-related cirrhosis [1].

RISK FACTORS

The main risk factors involved in the development of HCC are chronic viral infections, hemochromatosis and alcoholic cirrhosis. Other risk factors which may lead to HCC are cirrhosis secondary to autoimmune hepatitis and Wilson disease. In the last years, diabetes was suggested as HCC risk factor in numerous studies. However, it is difficult to study the possible association between the two concepts, because diabetes is a risk factor for nonalcoholic liver disease and nonalcoholic steatohepatitis , which may lead to the appearance of liver fibrosis, of cirrhosis, and finally to HCC, being known the fact that the principal risk factor is the presence of cirrhosis, no matter of the cause. On the other hand, end stage liver disease may herself determine glucose intolerance and diabetes. Studies show that >90% of patients with cirrhosis have glucose intolerance and 30% have diabetes [2].

Cirrhosis is a premalignant statement independent to the etiology. Liver cell dysplasia is probably the intermediate step. Patients with cirrhosis and high rates of cellular proliferation are at high risk to develop hepatocellular carcinoma.

HBV and HVC. It has been shown that there is an interval of 15-40 years between the development of chronic hepatitis and the appearance of hepatocellular carcinoma and the distance is shorter for the virus C infection. For hepatitis B, is important to know the age at which the infection occurred- patients infected in the prenatal period or in childhood are at high risk to develop chronic hepatitis or to become chronic carriers and at the same time to develop cirrhosis and hepatocellular carcinoma.

HBV is responsible for almost 80% of hepatocellular

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carcinoma. Vaccination against HBV and antiviral treatment are the most efficient ways for prevention. HBV seems to be directly and indirectly carcinogenic. There is a connection between the level of replication of virus B (viral load) and the risk of hepatocellular carcinoma. HBV integrates into the genome host and the viral load precedes almost always the development of hepatocellular carcinoma, leading to mutations at the place of integration and high levels of TNF-α. HBx protein inhibit the p53 tumor suppressor gene and activate genes implied in the control of cellular growth. Patients with HB antigen positive infected with delta virus have a reduced incidence of hepatocellular carcinoma, probably because delta virus suppress HBV.

HCV, despite HBV, is an ARN virus which does not have reverse transcriptase and can't integrate into the genome host. The manner of carcinogenesis production is uncertain, but probably is generated by chronic hepatitis and cirrhosis. Persistent hepatitis with high levels of alanine aminotransferase is a semnificative predictor of hepatocellular carcinoma development. Accelerated cellular turnover is implied in the pathogenesis of cirrhosis; at the same time, it has been demonstrated that patients with persistent hepatic injury develops a sort of HCV species that metamorphoses in time, meanwhile patients with normal aminotransferase levels have a stabile infection with a single species of HCV. It seems that during this process are produced species more ,,oncogenic” of HCV core protein. HCV reacts with cellular genes that regulates the cellular growth and differentiation. P53 activity is suppressed and tumor suppressor functions are disabled. HCV core proteins are probably oncogenic [3].

Alcohol intake increases four time the risk of hepatocellular carcinoma development, especially in older patients; cirrhosis is always present. The risk of hepatocellular carcinoma is highest in patients with chronic hepatitis and persistent hepatocellular injury. Patients who stop the alcohol intake reduce considerably the risk of hepatocellular carcinoma development. The enzyme induction mediated by alcohol increases the conversion of co-carcinogens in carcinogens.

Aflatoxin B1, produced by Aspergillus flavus and Aspergillus parasiticus is a pro-carcinogen which is converted in the liver into a metabolite by the cytochrome P450 system at the 8,9-vinyl bond with the formation of an unstable reactive intermediate, aflatoxin B1-8,9-epoxide, which can bind covalently to DNA. The result is the formation of Aflatoxin B1-guanine adducts and protein- aflatoxin B1-albumin and other protein adducts. In addition, aflatoxin B1 acts as a carcinogen by mechanisms that include the formation of reactive oxygen species leading to increased hepatic damage [4].

Sex and genetic predisposition. Globally, hepatocellular carcinoma is 3 times more frequent in males than females. In regions with increased incidence, familial history of hepatocellular carcinoma is an important factor. It is possible to exist a genetic predisposition for hepatocellular carcinoma or a familial predisposition for an extended phase of HBV replication.

Other factors. Hepatocellular carcinoma is a rare complication of autoimmune hepatitis and primary biliary cirrhosis; it may occur frequently in hemochromatosis (in almost 45% of cases, due to excessive levels of free iron submitted in tissues, carcinogenic by the formation of reactive oxygen species). α1-antitrypsin deficiency, tyrosinosis, porphyria cutanea tarda, Clonorchis sinensis infection may also increase the risk of hepatocellular carcinoma development.

There have been identified certain factors which may decrease the risk of HCC in patients with cirrhosis : high serum retinol levels, high coffee consumption, alcohol cessation, clearance of HCV virus with treatment, iron depletion of hemochromatosis patients [5].

PATHOGENESIS

The pathogenesis of HCC is not fully understood, but most probably HCC results from activation of proto-oncogenes, deactivation of tumor suppressor genes (p53, pRb), changes in growth factors signaling processes (insulin-like growth factor-IGF, transforming growth factor-TGF), changes in telomeric length and

activity or microsatellite instability. Genetic injury may result from a variety of sources, such as HBV and HCV viruses. HBV is associated with a relatively high frequency of mutations as it replicates through RNA-mediated reverse transcription and the HBV reverse transcriptase (HBV-RT) does not have proofreading function. Unlike HBV, HCV is a single-stranded non-retroviral RNA virus which does not integrate into the host genome.

Although there have been identified multiple genetic abnormalities in the development of HCC, it is probably that the process is complex and genetic studies are continuing [1].

DIAGNOSIS

Hepatocellular carcinoma should be suspected in patients with previously compensated cirrhosis who develop decompensation, such as encephalopathy, ascites, jaundice or variceal bleeding associated with the extension of the tumor into the hepatic or portal veins or arteriovenous shunt induced by the tumor. Other uncommon presentations include: para-neoplastic syndromes – hypercalcemia (by PTH-like synthesis), erythrocytosis (by EPO-like synthesis), carcinoid syndrome, porphyria cutanea tarda, vascular angioma.

Diarrhea may be the reason for presentation to hospital, caused by cholestasis or the production of prostaglandins by the tumor.

Cutaneous manifestations include pemphigus foliaceus, dermatomyositis, Leser-Trelat sign (suddenly installed seborrheic keratosis, accompanied by freckles, very itchy).

Acute presentation is determined by spontaneous rupture of the tumor into the peritoneum or invasion of the liver capsule. In this case, the preferred treatment is the chemoembolization of the bleeding artery. Capsule invasion is causing severe pain, similar to biliary colic, lasting less than 24 hours.

In end stages, patients may present consumptive syndrome with impaired general condition, anorexia, weight loss and fatigue.

Physical examination can detect an abdominal mass and a bruit related to arterioportal shunting may be heard. A supraclavicular lymphadenopathy (Virchow-Troisier), Sister Mary Joseph or axillar lymph-adenopathies are rarely present [6].

Laboratory studies can show high levels of aminotransferase, but can be also normal. Alkaline phosphatase may be elevated in infiltrative tumor or if there is biliary obstruction due to malignancy. α-fetoprotein, the tumor marker most frequently associated with hepatocellular carcinoma (a glycosylated protein expressed in proliferating hepatocytes) has a sensitivity of 41-65% and specificity of 80-94% when the cut-off value is 20 ng/ml; levels higher than 400 ng/ ml are likely to be caused by hepatocellular carcinoma. AFP>1000 ng/ml associated with a liver mass is diagnostic for hepatocellular carcinoma. However, AFP level is elevated in only 60-70% of patients and false-positive results may occur during pregnancy, in active liver disease, certain gastrointestinal tumors and embryonic tumor. Additional studies of AFP have revealed that AFP has three glycoforms (AFP-L1, AFP-L2, AFP-L3) according to their binding ability to the lectin lents agglutinin (LCA). It has been shown that AFP-L3 exists only in the serum of patients with hepatocellular carcinoma at a cut-off value of 15%, with a sensitivity and specificity of 96.9% and 92%, respectively, in detecting hepatocellular carcinoma [7].

Des-gamma carboxyprothrombin (DCP-prothrombin,a protein induced by vitamin K-absence, PIVK-II) has been described as a protein produced by malignant hepatocytes. Different studies have shown than a combined analysis of AFP and DCP can lead to a better prediction in early stages of hepatocellular carcinoma [8]. Glypican-3, a member of the glypican famlily, has been revealed as a diagnosis marker, having the capacity to differentiate hepatocellular carcinoma from cirrhotic liver or benign lesion. It has been demonstrated that Glypican-3 and AFP together increase sensitivity and specificity for the diagnosis of small and unicentric HCC, respectively [9].

Heat shock protein 70 (HSP) – a protein produced by cells as a response to exposure to stressful conditions, may also be used as a marker for diagnosis in hepatocellular carcinoma [10].

The International Consensus Group of Hepatocellular Neoplasia recommends to define a pathological diagnosis of hepatocellular carcinoma if at least 2 markers are positive.

Imaging diagnosis

A minimum of 2 imaging exploration is required for staging and to confirm/exclude vascular invasion (ultrasound, CT with contrast, MRI); additionally is performed chest CT and bone scintigraphy if taken into account liver transplantation. In case of portal vein thrombosis associated with a small hepatic lesion, Doppler ultrasound is performed and thrombus punction ultrasound guided; the presence of thrombus exclude the possibility of radical treatment. Arterial signal into the thrombus indicate the tumoral etiology.

Abdominal ultrasonography has a 50% sensitivity in hepatocellular carcinoma detection for liver with advanced cirrhosis. Lesions under 2 cm can usually be detected.

Ultrasound appearance is variable. Lesions are usually hypoechoic. In large hepatocellular carcinoma, hyperechoic or heterogenic appearance is due to necrosis, hemorrhage or fat infiltration. Small hepatocellular carcinomas can be homogeneously hyperechoic and can be similar to hemangioma (due to a large portion of fat being present in the tumor) or can appear hypoechoic. Doppler ultrasonography can be used in the detection of portal vein, hepatic artery or inferior vena cava invasion. Portal venous invasion is more common in hepatocellular carcinoma, but hepatic vein invasion is more specific.

Small hyperechoic masses seen on ultrasound require further examination, because they can represent hemangioma (most frequently), metastatic disease or, less likely, hepatocellular carcinoma. Further imaging with CT scanning or MRI during dynamic contrast enhancement shows the typical peripheral, nodular contrast enhancement pattern of hemangioma. MRI or CT scanning can further characterize many nonspecific hepatic masses seen on ultrasonography.

CT scan – dynamic CT, spiral CT is the investigation of election for hepatocellular carcinoma diagnosis. The CT appearance of hepatocellular carcinoma is variable, depending on image phase and tumor size. The most common attenuation pattern is iso-hyper-iso-attenuation on prephase, arterial phase and venous phase, respectively; however, this pattern is common to other hepatocellular nodules, including regenerative and dysplastic nodules.

Unenhanced CT typically shows an iso-hypodense mass; for large masses central areas of necrosis may be seen.

In the hepatic arterial phase, nodules appear hyperdense (relative to hepatic parenchyma), as a result of hepatic arterial supply. Larger tumors may have necrotic central regions that are usually hypodense during this imaging phase. Neovascularity indicate the presence of inconspicuous lesions.

In the portal venous phase, small nodules may appear isodense or hypodense and larger nodules with necrotic regions remain hypodense.

In the delayed-postcontrast phase, small nodules may be inconspicuous on late phases.

Spiral-CT evidentiate 17% of lesions <1 cm, 29% of lesions between 1 and 2 cm and 63% of lesions higher than 2 cm.

MRI has higher sensitivity for focal hepatic lesions than CT.

Precontrast and postcontrast MRI has a 70-85% chance of detecting a solitary mass of HCC.

MRI can differentiate cirrhotic nodules from HCC: if the mass is bright on T2-weighted images, it is HCC until proven otherwise; if the mass is dark on T1- and T2-weighted images, it is a siderotic regenerative nodule or siderotic dysplastic nodule; if the mass is bright on T1-weighted images and dark or isointense on T2-weighted images, it is a dysplastic nodule or low-grade HCC [11].

Hepatic angiography.

Hepatocellular carcinoma is vascularized by hepatic artery and selective arteriography of superior mesenteric artery or celiac artery may identify the lesion. This is an invasive procedure used for hepatic chemoembolisation.

Liver biopsy.

For small lesions detected on ultrasound or CT, histological confirmation is very important. Biopsy is performed with ultrasound or CT guidance. There is a possibility of sowing on the needle path.

In present, the diagnosis of hepatocellular carcinoma is established by clinical examination associated with typical imaging aspect, with/without increased α-fetoprotein, histological confirmation not being absolutely necessary.

EASL 2016 recommend to perform liver biopsy for lesions with atypical imaging aspect and in non-cirrhotic patients [12].

DIFFERENTIAL DIAGNOSIS OF HEPATOCELLULAR CARCINOMA IN THE PRESENCE OF CIRRHOSIS

The major problem consist in the differentiation between hepatocellular carcinoma and a dysplastic nodule. Hepatocellular carcinoma appear hyper-vascularised compared to surrounding tissues.

For lesions higher than 2 cm identified in 2 different imaging studies, in the presence of cirrhosis, the diagnosis of hepatocellular carcinoma can be established. Also, if the lesion is identified in 2 different imaging studies and α-fetoprotein level is higher than 400 ng/ml, the diagnosis is very probable and histological confirmation is not really necessary [1].

SCREENING

Hepatocellular carcinoma screening is recommended in patients at high risk, such as male patients, HBV, HCV viruses, age > 40 years and chronic liver disease, especially cirrhosis and high grade dysplasia.

EASL 2016 clinical practice guidelines on the management of hepatocellular carcinoma recommend screening for patients with chronic HBV infection, even without cirrhosis, Asian males >40 years, females >50 years, patients with family history of hepatocellular carcinoma, African Americans >20 years, primary biliary cirrhosis stage 4, hemochromatosis, α1-antitrypsin deficiency.

Recommended screening methods are abdominal ultrasound combined with α-fetoprotein every 6 months. In cirrhotic patients, a nodule <1 cm detected must be supervised every 4 months for 1 year, than every 6 months.

For nodules 1-2 cm in diameter, the diagnosis is established by non-invasive criteria or histological examination.

Nodules higher than 2 cm may be diagnostic for hepatocellular carcinoma in case of typical aspect; for atypical aspect, histological examination is required [12].

TREATMENT OPTIONS

The treatment is established using the Barcelona-Clinic Liver Cancer (BCLC) staging system which defines very early stage cancer (single nodule,<2 cm, Child-Pugh A) as stage 0, early stage cancer (1-3 nodules, <3 cm each, Child-Pugh A-B) as stage A, intermediate stage for multinodular HCC (stage B), advanced stage which involves vascular invasion or extrahepatic spread (stage C) and terminal stage at patients with Child-Pugh C cirrhosis. Resection, ablation and transplantation is recommended for stage 0 and A, transcatheter arterial chemoembolization for stage B, Sorafenib – stage C and best supportive care for terminal stage.

Surgical treatment

Resection is curative, but limited in patients without cirrhosis or in the presence of cirrhosis and without portal hypertension. It is accepted as treatment for localized hepatocellular carcinomas. Intrahepatic recurrence is common, because the potential of neoplastic liver remain unchanged. The rate of recurrence at 5 years is almost 52 %. Partial hepatectomy is indicated in the absence of cirrhosis or in Child-Pugh stage A with normal portal pressure and normal bilirubin. The best results are obtained in patients with unicentrical disease, without vascular invasion, under 5 cm and with liver disease relatively inactive (5% of hepatocellular carcinomas). 5-year survival rate for curative treatment (resection, transplantation and local ablation therapy) is almost 80%.

Liver transplantation – the technique is successful in

selected patients, but it requires immunosuppressive treatment for life. It is indicated in patients with advanced cirrhosis (Child-Pugh B and C) who cannot survive on surgical resection. Liver transplantation can be applied independently on the liver stage disease and treat both hepatocellular carcinoma and neoplastic potential of cirrhotic liver.

Liver transplantation is indicated for unique tumors under 5 cm or <3 nodules (Milano criteria), each less than 3 cm.

It is difficult to elect between resection and transplantation for a tumor <5 cm. Altered liver function is in favor of transplantation.

Nonsurgical treatment

Chemotherapy

Sorafenib, a Raf-kinase and tyrosine kinase inhibitor, is indicated in patients with normal liver function (Child-Pugh A) and portal vein thrombosis, extrahepatic tumors and failure of other therapies. In patients with altered liver function, only best supportive care is indicated.

Local ablation-is potentially curative in patients with small tumors, <3-5 cm that are not candidates for resection or transplantation.

TACE (Transarterial chemoembolisation) – hepatic artery catheterization via the femoral artery or celiac axis allows the nutrient vessel embolization of tumor, and chemotherapeutic agents can be delivered in high concentrations. (Cisplatinum, Doxorubicim, foam gel, Epirubicin). Chemoembolization is indicated in unresecable tumors, patients with Child-Pugh A-B. The result is partial/total tumoral necrosis. The most frequent side effects are fever, nausea, encephalopathy, ascites and high levels of aminotransferase.

Percutaneous injection of ethanol (PEI) is efficient in 2-3 cm tumors (<5 cm), usually less than 3 nodules, even in the presence of advanced cirrhosis. The technique suppose the ethanol injection under ultrasound/CT guidance; outpatient treatment may be carried out 2 times per week, 3-15 sessions, using 2-16 ml of absolute alcohol. 3-year survival rate is 71% in patients with Child-Pugh A and 41% in Child-Pugh B.

Radiofrequency ablation (RFA) is a treatment that uses imaging guidance to place a needle electrode through the skin into a liver tumor. High-frequency electrical currents are passed through the electrode, creating heat that destroys the cancer cells. RFA is more effective than PEI in larger tumors (3-5 cm) and it requires fewer sessions. Recurrence rates are higher than in resection, but survival rate is similar to resection.

Trans-catheter arterial chemoembolization uses angiography and allows the nutrient vessel embolization of tumor. TACE is recommended in palliative treatment for unresecable tumors and in patients on transplantation list; the effectiveness of the method depends on the tumor dimension. TACE it is not recommended in the treatment of large and unresecable tumors.

CONCLUSION

In conclusion, hepatocellular carcinoma remain a fatal disease. All treatments used increases survival. Some studies have shown that there were no big differences between the results given by resection, transplantation and arterial embolization [13].

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Lipid profile and comorbidities in patients with psoriasis vulgaris

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Abstract: *Background and Aim: Psoriasis is an inflammatory skin disorder with important influence on the lipid profile, insulin resistance and cardiovascular disease. The aim of this study is to compare the lipid profile of classical therapy versus anti-TNFs patients and asses the associated diseases.*
Methods: We conducted a prospective study. Patients' data were collected from two Romanian Dermatology Clinics, during the period January 2015 – June 2016. The patients were divided into two groups: study group (on TNF inhibitors) and control group (on classical treatment).
Results: The subjects in the study group had more associated diseases and much more patients suffered from dyslipidemia. There were not statistically significant differences in the lipid profile between the two groups. But eliminating patients treated with Acitretin and Methotrexate, a p-value of 0.0259 (significant statistically) was observed when comparing the values of triglycerides in the two groups.
Conclusions: We did not observe a beneficial effect of the TNF inhibitors therapy in psoriatic patients on the lipid profile. Subjects on biological treatment suffer from a more severe form of psoriasis vulgaris and associate much more comorbidities.

Keywords: *lipid profile, comorbidities, anti-TNF agents*.

INTRODUCTION

Psoriasis is a chronic, inflammatory skin disorder [1]. The inflammation predisposes to a pro-atherogenic lipoprotein profile, large vessel inflammation, and adipokine dysregulation [1] thus leading to insulin resistance and cardiovascular diseases [2]. There is a strong link between psoriasis and risk factors for metabolic syndrome (central obesity, insulin resistance [3], abnormal lipid metabolism [4], hypertension [5]. Patients with psoriasis have a more increased risk of developing atherosclerosis due to inflammation and dyslipidemia [4]. Moreover, lipid abnormalities may play an important role in the development of psoriasis [4].

The inflammation mediated changes at lipids and lipoproteins levels have more likely a role in protecting the host. The treatment of psoriasis was shown to lead to a normalization of the lipid levels [6]. TNF induces a reduction in cholesterols (especially HDL) by interfering with LDL receptors, Apolipoprotein A and B, and 7A1 and 7B1 cytochromes with no changes in HMG-Co reductase action [7].

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In Romania, however, the inclusion criteria for biological therapies require previous two systemic non-biological therapies (retinoids, systemic glucocorticoids, methotrexate, PUVA, UVB narrow band).

Moreover, most of these therapies are known as lipid modifiers [4].

The aim of this study was to examine two groups of patients (biological versus non-biological therapy psoriatic patients) in terms of comorbidities and lipid profile.

MATERIAL AND METHODS

Study design

We carried out a prospective study. Patients' data were collected from two Romanian Dermatology Clinics: Dermatology Department, Carol Davila Emergency Military Hospital, Bucharest, (January – June 2016) and Dermatology Department, Mureş County Clinical Hospital, (January – December 2015).

All subjects had a physical examination. Body max index (BMI) was calculated as weight (kg)/height (cm²) (normal weight: 18.5–24, 9; overweight: 25–29, 9; class I obesity: 30–34, 9; class II obesity: 35–39, 9; class III obesity: ≥ 40). Each patient completed a DLQI form and a dermatologist filled the PASI form.

The data collected included (i) patients' demographic characteristics: date of birth, gender, weight, height, BMI; (ii) psoriasis-related data: type of psoriasis, current therapies (topical treatment, Methotrexate/ MTX, Acitretin, PUVA, UVB TL01, anti-TNFs: Infliximab original and biosimilar, Adalimumab, Etanercept); (iii) comorbidities, with an accent on metabolic syndrome (hypertension, diabetes mellitus, obesity); (iv) cholesterol/cho and triglycerides/TG levels.

Patients were divided into two groups: study group (biological therapy) and control group (topical treatment, Methotrexate, Acitretin, PUVA, UVB TL01).

All participants gave written informed consent.

Statistical analysis

Data were analyzed using 2017 GraphPad Software.

RESULTS

At the end of the follow-up period, 102 subjects were included for the analysis: study group – 53 patients: 39 males (73.58%) and 14 females (26.42%) and control group – 49 patients: 25 females (51.02%) and 24 males (48.98%).

The biological therapy patients were distributed as follows: Infliximab (9, 16.98%), Infliximab biosimilars (4, 7.54%), Entanercept (17, 32.07%), Adalimumab (23, 43.39%). Four subjects had a switch of anti-TNF therapy, two changed Infliximab for Etanercept, the other two switched Infliximab for Adalimumab. Only 16 (15.68%) patients out of the 102 achieved both PASI and DLQI equal to zero during the study.

Biological patients were screened for associated diseases. Table 1 includes the number/(%) and comorbidities.

Table 1: Comorbidities of biological patients

Associated diseases	Number of patients /(%)
HTA	10/(18.86%)
Diabetes mellitus type 2	3/(5.66%)
Obesity	40/(77.35%)
Latent tuberculosis	5/(9.43%)
Hepatitis VHB	1/(1.88%)
Herpes zoster	1/(1.88%)
BPOC	1/(1.88%)
Chronic bronchitis	2/(3.77%)
Hepatic steatosis	1/(1.88%)
Glaucoma	1/(1.88%)
Arthropathy	15/(28.3%)
Synovitis right knee	1/(1.88%)
Disc hernia	1/(1.88%)
Anemia	1/(1.88%)
Allergy to penicillin and erythromycin	1/(1.88%)
Renal litiasis	1/(1.88%)
Hidradenitis suppurativa	3/(5.66%)
Bipolar disorder	1/(1.88%)
Amputation of right police	1/(1.88%)
Thyroid nodule operation	1/(1.88%)
Cholecystectomy	3/(5.66%)
Appendectomy	2/(3.77%)

The subjects with criteria of metabolic syndrome were

41 (77.35%) with obesity, 3 (5.66%) had diabetes mellitus type 2, 10 (18.86%) with hypertension. Other comorbidities were previous surgery (cholecystectomy, thyroid nodule operation, appendectomy), infection (latent tuberculosis, hepatitis VHB, herpes zoster), accidents (amputation of right police), pulmonary disease (BPOC, chronic bronchitis), inflammatory disease (hidradenitis suppurativa), hepatic disease (hepatic steatosis), renal disease (renal litiasis), ocular disorder (glaucoma), skeletal problems (arthropathy, synovitis right knee, disc hernia), hematological disease (anemia), psychiatric disorder (bipolar disease), allergy to drugs (allergy to penicillin and erythromycin).

The classical therapy patients associated obesity – 20 (40.81%), diabetes mellitus type 2 – 3 (6.12%), hypertension – 4 (8.16%). Other associated diseases were: hematological (anemia, thalassemia), skin cancer, arthropathy, osteoporosis, Cushing disease, venous disease, operated pituitary gland (table 2).

Table 2: Associated diseases of control group

Associated diseases	Number of patients
HTA	4
Diabetes mellitus type 2	3
Obesity	20
Anemia	1
Thalassemia	1
Skin cancer	2
Arthropathy	9
Osteoporosis	1
Cushing disease	1
Operated pituitary gland tumor	1
Chronic venous insufficiency	1

Patients with psoriasis have an important weight problem. The normal weight patients in the study group represented 12 (22.64%) versus 16 (32.65%) control group patients. Increased BMI was found in 74 (72.54%) psoriatic patients. (Table 3)

Some patients on anti-TNFs required additional drugs for disease control. 10 (18.86%) biological therapy patients used only topical treatment versus the majority of control group – 45 (91.83%).

Table 3: BMI for the two groups

BMI	Study group	Control group
Normal weight	12	16
Overweight	20	20
Obesity class I	15	13
Obesity class II	5	-
Obesity class III	1	-

Methotrexate was prescribed to 6 (11.3%) patients in the study group and to 4 (8.16%) patients on classical therapy.

Retinoid (Acitretin) was used only in 1 (1.88%) biological patient versus 2 (4.08%) patients in the control group. (Tables 4, 5)

Table 4: Additional therapy of anti-TNFs patients

Patient	Additional therapy
1	Acitretin
2	Methotrexate
3	Methotrexate
4	Methotrexate
5	Calcipotriol and betamethasone gel
6	Clobetasol propionate 0.0525% ointment
7	Calcipotriol and Betamethasone gel
8	Calcipotriol and Betamethasone gel
9	Methotrexate
10	Calcipotriol and Betamethasone gel
11	Calcipotriol and Betamethasone gel
12	Calcipotriol and Betamethasone gel
13	Keratolytic creams, Dermatocorticoids
14	Calcipotriol and Betamethasone gel
15	Calcipotriol and Betamethasone gel
16	Dermatocorticoids, Methotrexate
17	Methotrexate
18	Methotrexate

Table 5: Therapies of patients in the control group

Patient	Therapy
1	calcipotriol and betamethasone gel, 15% urea cream, UVB TL01
2	0.1% mometasone furoate cream, vitamin A, emollient, cream with salicylic acid, UVB TL01
3	topical corticosteroid, calcipotriol and betame-thasone gel, keratolytics, emollient cream, Methotrexate, UVB TL01
4	clobetasol propionate 0.0525% cream, Methotrexate, PUVA
5	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, PUVA
6	0.1% mometasone furoate cream, UVB TL01
7	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, UVB TL01
8	0.1% mometasone furoate cream, Methotrexate
9	betamethasone dipropionate and salicylic acid cream, clobetasol propionate 0.0525% cream, vitamin A, vitamin E,UVB TL01
10	15% urea cream
11	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, 15% urea, vitamin A, vitamin E, PUVA
12	0.1% mometasone furoate cream, emollient cream, keratolytic cream, UVB TL01
13	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, Vitamin E, PUVA
14	topical corticosteroid, UVB TL01
15	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, Vitamin E, PUVA
16	clobetasol propionate 0.0525% cream, calcipotriol and betamethasone gel, Vitamin E, Vitamin A, salicylic acid cream, PUVA
17	clobetasol propionate 0.0525% cream, Vitamin E, Vitamin A, UVB TL01
18	0.1% mometasone furoate cream, emollient cream, betamethasone dipropionate and salicylic acid cream, UVB TL01
19	0.1% mometasone furoate cream, emollient cream
20	clobetasol propionate 0.0525% cream, Vitamin E, Vitamin A, UVB TL01
21	calcipotriol and betamethasone gel, Vitamin E, Vitamin A
22	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, emollient cream, Vitamin E, Vitamin A, PUVA
23	vitamin E, 30% urea cream
24	clobetasol propionate 0.0525% cream, emollient cream, UVB TL01
25	calcipotriol and betamethasone gel, 15% urea cream, PUVA
26	calcipotriene hydrate and betamethasone solution, 0.1% mometasone furoate cream, Vitamin A, Vitamin E, Methotrexate, PUVA
27	0.1% mometasone furoate cream, Desloratadine cp, UVB TL01
28	0.1% mometasone furoate cream, clobetasol propionate 0.0525% cream, Desloratadine cp, PUVA
29	clobetasol propionate 0.0525% cream, emollient cream, vitamin A, vitamin E, PUVA
30	0.1% mometasone furoate cream, emollient cream, Methotrexate
31	0.1% mometasone furoate cream, emollient cream, salicylic acid cream, Clarithromycin
32	0.1% mometasone furoate cream, emollient cream, UVBTL01
33	clobetasol propionate 0.0525% cream, Ciprofloxacin cp, UVB TL01
34	clobetasol propionate 0.0525% cream, salicylic acid cream, UVB TL01

35	calcipotriol and betamethasone gel, vitamin E, Vitamin A, UVB TL01
36	0.1% mometasone furoate cream, UVB TL01
37	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, vitamin A, vitamin E, PUVA
38	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, vitamin A, vitamin E, UVB TL01
39	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, silver nitrate solution, vitamin A, vitamin E, UVB TL01
40	UVB TL01
41	Methotrexate
42	0.1% mometasone furoate cream, calcipotriol and betamethasone gel
43	0.1% mometasone furoate cream, Desloratadine cp, UVB TL01
44	clobetasol propionate 0.0525% cream, UVB TL01
45	0.1% mometasone furoate cream, UVB TL01
46	calcipotriol and betamethasone gel
47	0.1% mometasone furoate cream, 15% urea cream, emollient cream, Methotrexate
48	0.1% mometasone furoate cream, 15% urea cream, emollient cream, Methotrexate
49	calcipotriol and betamethasone gel, 15% urea cream, vitamin A, vitamin E, Methotrexate, PUVA

The lipid profile was screened in both groups of patients. But there was no difference between the two groups of patients. The p-value comparing the two groups of cholesterol (study vs. control) was 0.8674, and for the triglycerides groups (study vs. control) was 0.218. On the other hand, a significant statistically p-value of 0.0259 was observed when comparing the values of triglycerides in the 2 groups, but after eliminating the patients on Acitretin and Methotrexate.

We compared the cholesterol and triglycerides values from each group for patients treated only Methotrexate or Acitretin and the ones on anti-TNFs and Methotrexate or Acitretin, but no significant p-values were found.

We observed high triglycerides values in patients treated with Infliximab – 7 (58.33%) followed by Etanercept – 5 (35.71%) and Adalimumab – 4 (22.22%). The highest levels of cholesterol were observed in the Etanercept and Adalimumab subgroup 11 (78.57%). The others subgroups contained only small samples of patients. (Table 6)

The study group was divided into subgroups of years on biological therapy and the levels of cholesterol and triglycerides were compared.

Table 6: Patients with high lipid values divided into systemic therapies

Therapy	High cholesterol No/(%)	High triglycerides No/(%)
Infliximab (original + biosimilar)	2 (16%)	7 (58.33%)
Etanercept	11 (78.57%)	5 (35.71%)
Adalimumab	11 (61.11%)	4 (22.22%)
Adalimumab + Methotrexate	3 (75%)	3 (75%)
Etanercept + Methotrexate	2 (100%)	0 (0%)
Adalimumab + Acitretin	1 (100%)	0 (0%)
Methotrexate	2 (50%)	3 (75%)
Acitretin	0 (0%)	0 (0%)

The subgroup 4-6 years had the highest level of dyslipidemia as it can be observed in Table 7.

DISCUSSION

Analyzing the two groups, the patients on biological therapy seemed to have more associated comorbidities than the control group. Cardiovascular events appear more often in patients with severe (8) and therapy resistant psoriasis like the ones treated

with anti-TNFs.

The two groups showed a difference in the frequency of hypertension (study group – 18 (86%) vs. control group – 8 (16%)). Even though, in normotensive and rheumatoid arthritis individuals, Infliximab reduced blood pressure levels, we observed in our study that the anti-TNFs do not improve alone the blood pressure values in psoriatic hypertensive patients [9].

Table 7: Levels of cholesterol and triglycerides divided into subgroup of years of anti-TNF therapy

Years of biological therapy	High cholesterol	High triglycerides	Cholesterol %	Triglycerides %
≤ 3.5 years	7	4	13.20	7.54
4-6 years	22	15	41.50	28.30
7-8 years	2	2	3.70	3.70

Many studies observed that high blood pressure is more often noted in patients with psoriasis compared to the non-psoriatic population [10], especially psoriatic patients who were male, middle-aged and elderly people [11] as our study group (39 males (73.58%) versus 14 females (26.42%)). Men are more predispose to severe forms of psoriasis [12].

High blood pressure is independent associated with psoriasis [8].

Apparently, in psoriasis the pathophysiologic mechanisms for high blood pressure are related to the underlying autonomic nervous system dysfunction [13], elevated plasma renin activity, elevated angiotensin converting enzyme activity, and elevated endothelin-1, TNFG308A polymorphism [14].

Psoriasis is thought to promote insulin resistance by overproduction of Th1 cytokines [10]. An association can be observed between high levels of TNFα, IL6, IL17, leptin, and C-reactive protein, and increases in BMI, which contribute to alterations in insulin biochemical pathways, leading to insulin resistance, type II diabetes [15]. Our patients are overweight, dyslipidemic and topical corticosteroid users for long periods of time, more over they have an inflammatory disease which is pro-atherogenic. Although only 6 patients out of 103 were diagnosed with diabetes mellitus type 2, the screening is continuous, because psoriasis is an independent risk factor for type 2 DM, the risk being greater in severe disease. (Marius Irimie, 2015).

Most of our patients with psoriasis vulgaris are above normal weight (72.54%). It has been reported that psoriasis favors weight increase and obesity [15]. In obesity there is an unbalanced production of pro- and anti-inflammatory adipokines in obesity, leading to a chronic low-grade inflammation state, which seems to favor worsening of psoriasis lesion and a poorer response to treatment [16].

Although we did not evaluate the weight gain before and after anti-TNF treatment, several studies have reported an increase in weight and body mass index (BMI) with the use of anti-TNF agents (adalimumab, etanercept and infliximab) [9, 17]. Most of our patients in the study group are overweight (77.35%).

On the other hand, weight loss (greater than or equal to 5% of the initial weight) is associated with a higher success rate in achieving control of disease activity in patients with overweight or obesity with psoriatic arthritis treated with anti-TNF-α [9]. A study with patients on low-energy diet lost weight which led to significant reductions of several cardiovascular risk-associated factors, moreover the weight loss improved HbA1c levels by 0.7%, equivalent to reductions achieved by treatment with an anti-diabetic drug [18]. Moreover, the psoriatic patients with the lowest monounsaturated fatty acid (MUFA) intake presented the highest values of PASI score and inflammation marker [19].

The mean age of psoriasis patients in our study was 52.07 years similar to international studies, thus confirming the average age of type 2 psoriasis patients worldwide [20]. Moreover the biological patients are the ones that do not respond to classical therapy

suggesting the severe form. The equal distribution in the classical therapy group can be due only to a mild form of disease.

Between the two groups there were not statistically significant differences in the lipid profile. Our results are similar with those of [21], their psoriatic patients treated with etanercept, adalimumab, or infliximab (each patient serving as their own control), did not present a significant change in total cholesterol, LDL and triglycerides. On the other hand, a p-value of 0.0259 (significant statistically) was observed when comparing the values of triglycerides in the two groups (eliminating the patients on Acitretin and Methotrexate).

We discovered that patients with 4-6 years of anti-TNF therapy were the majority group with dyslipidemia. However, the study of Ehsani et al. demonstrated an insignificant 5% increase in LDL and triglycerides after 24 weeks of treatment with anti – TNF agents [7], but all the changes in lipid profile by biological therapy are temporary, and the negative feedback will balance these changes over time [7]. Others have proven that patients with long term psoriasis have an increase in the serum total cholesterol levels and triglyceride levels [22]. Several authors found increased cholesterol levels (more than 5 years), but the lipid profile changes from the early stage to the late chronic stage of the disease. In contrast, Jorge Santos-Juanes et al. did not find a relationship between lipid levels and disease duration [23]. Maybe lipid profile abnormalities might be genetically determined rather than acquired [8].

Comparing only patients on MTX or Acitretin versus patients on anti-TNFs and MTX or Acitretin we did not observe a significant statistically important difference. But in similar studies there was a significant increase in triglycerides in patients treated with TNF inhibitors + MTX compared with patients treated with MTX alone. In our study, the highest triglycerides levels had patients on Infliximab – 7 (58.33%), followed by Etanercept – 5 (35.71%) and Adalimumab – 4 (22.22%). Concerning cholesterol levels were higher in Adalimumab and Etanercept – 11 subjects (61.11%; 78.57%). The patients on systemic classical treatment (Methotrexate or Acitretin) or on anti-TNF + MTX/ Acitretin were too few to have statistical significance [21].

Our control group (excluding MTX and Acitretin) showed increased cholesterol (26/53.06%) versus triglycerides levels (6/12.24%). Other studies showed that TG levels were significantly higher in psoriatic subjects than control groups [24].

We did not exclude patients on Methotrexate and Acitretin.

These may cause or influence the dyslipidemia. Recent studies proved that retinoid acid treatment could produce unsaturated fatty acids and induce triglyceride breakdown, bile acid secretion, lipolysis, and retinoids elimination. But, retinoid x receptor α deficiency might induce the synthesis of saturated fatty acids, triglyceride, cholesterol, bile acids, and retinoids [25]. Retinoids, especially Acitretin increase triglycerides levels [26], but also a mild and transient reduction in HDL levels and insulin sensitivity [27].

Regarding MTX, there are conflicting studies on the effect of Methotrexate on the lipid metabolism. The HDL profile function is favorable influenced by Methotrexate [28], Methotrexate has an anti-atherogenic potential [29] with cardioprotective properties [30]: tendency to decrease the risk of heart failure [9], a lower incidence of vascular disease (cardiovascular, cerebrovascular and arteriosclerosis) [17]. But it has been proven that cholesterol, LDL cholesterol and HDL cholesterol are increased short-termed after the initiation of Methotrexate therapy [31].

CONCLUSION

Our study did not show a positive effect of anti-TNF therapy in psoriatic patients on the lipid profile. Most likely, the abnormalities of the lipids are due to genetically inherited mechanisms. Our patients on biological therapy have a more severe form of psoriasis vulgaris, with many comorbidities.

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ORIGINAL ARTICLES

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Management of burning mouth syndrome (BMS) in patients with diabetes mellitus

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Abstract: *Unlike the therapy of primary BMS, the treatment of secondary BMS benefits, in addition to symptomatic therapy, from the possibility of using means and methods to correct biological disturbances and/or morphological, psycho-emotional changes respectively. The use of these therapeutic means in secondary BMS is recommended to be established as a first step of therapeutic conduct. Their success is reflected in homeostasis restoring, correcting morphological and functional changes of the field, which frequently leads to no longer use prescription of symptomatic treatment.*

DEFINITION

The International Association for the Study of Pain (IASP) defines burning mouth syndrome (BMS) as a "distinct nosological entity" characterized by "oral sensation of burning or pain, persistent and non-remitting in time in the absence of clinical objective changes of the mouth mucosa" [1]. A relatively similar vision is supported by Grushka (2014). BMS defining a sensory sensation, the author admits that this is manifested mainly by: a) subjective characteristics: changes in taste perception; bilateral burning sensation associated with altered taste mouth; sensation of "dry mouth", although salivary flow is not reduced; perceived foreign body in the oropharynx and b) objective criterion: the oral tissues with clinically normal aspect. The author specifically mentions that the 4 subjective lists, encountered in BMS come forth from the absence of results after an objective clinical exam, a defining element for this condition [2].

EPIDEMIOLOGY

Setting the epidemiology of BMS is difficult and imprecise because there is no universally accepted definition. The various epidemiological studies often refer to different clinical entities in which oral mucosal lesions lack [3]. This disease has a high rate that varies according to the studies from 0.7% to 14.8 - 15% [3,4,5]. The prevalence of painful syndrome in the Service of Oral Pathology of the Faculty of Dental Medicine - "Carol Davila" University of Medicine and Pharmacy, Bucharest (Romania) is 16.23% [6].

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ETIOLOGY. PREDISPOSING FACTORS

Pathogenic, sex, age, menopause can be considered contributing factors. Therefore:
1. Although it was observed in any age group between 27-87, BMS has a clear predisposition related to age. The average age of reporting the disease is 61 [2]. BMS rarely occurs before the age of 30 [7].
2. Sex is also placed between contributory factors. The women are 2.5 to 7 times more frequently affected than men.
3. The body functioning as a whole, at menopause, besides circulating electrolyte changes, it suffers from other disturbances such as:
a) Lipid disorders with consequences in the development of atherosclerosis;
b) exacerbated production of ROS/RNS, including liquid in the gingival sulcus (where ROS = reactive oxygen species, and RNS = reactive nitrogen species);
c) changes in the hormonal constellation at menopauses the quantity of estrogen become responsible for the development of female cardiovascular pathologies same as men.

Data from the literature considers that up to 90% of the female patients with BMS are around menopause, the onset of symptoms characteristic of peri-menopausal period taking place three years before and 12 years after climax [8].

The explanation for BMS distribution predominantly in women during perimenopause, according to Woda et al. (2009) is that BMS is the result of hormonal changes (hypo-estrogenism) in women during menopause, which often associates with metabolic disorders and neuropsychological chronic anxiety or stress. Mental disorder in menopausal women explains anxiety and depression from BMS [9].

4. Another pathogenic explanation takes into consideration the interrelationship taste-saliva-pain. Laboratory tests showed that female patients with disorders in climax present a growth of salivary phosphate concentrations, proteins, Na, K+, Ca2+ and Mg2+. The referred electrolyte disturbances trigger the disruption of the membrane potential, including those of the cells involved in collecting taste excitations or cells responsible for saliva secretion [10].

Certain hormonal changes (hypoestrogenemia, diabetes mellitus, hypothyroidism, thyroid dysfunction type) and functional morphological and immunological disorders affecting the endocrine glands were also among factors described as possible cause of this disorder [11]. Regarding the relationship between diabetes and BMS various assumptions were formulated, including the existence of metabolic changes in the mouth due to peripheral neuropathy, which can generate among other disturbances the hypo-function of the salivary glands, thus reducing the secondary salivary flow [3,12,13].

The mode of action of psycho-neuro-endocrine factor is even more difficult to explain since it has been shown that patients presenting BMS have a greater tendency to somatization and to develop psychiatric symptoms [11]. For this reason the theory was accepted that burning mouth syndrome is a form of neuropathic pain [14]. The neuropathy mechanism in the pathogenesis of BMS is still controversial, the expert literature admitting the possibility of dysfunction via peripheral or central reflex arc and cortical excitation [8,10].

BMS SYMPTOMS

Subjectively, patients describe the syndrome BMS as the presence of oral mucosa burning sensation, the most common manifestation present in the disease. Other features of the pain in this syndrome are itching sensation, numbness, impaired taste (taste “spicy”), dry mouth etc. As topography, the symptom is located almost constantly close to the lingual mucosa (especially in the front third) and the lip. Recently, Demarosi (2013) stresses however that it is not possible that all the oral mucosa could be involved without being identified any precise anatomic distribution [3].

Even since 2003, Scala et al. underlined the semiotic nature of BMS as being more complex, consisting of main and associated symptoms:
a) The main symptom is a triad, consisting of:
1. Pain in the oral mucosa: burning, stinging, tingling, swelling;

2. Dysgeusia (altered taste): the persistence of a certain flavor/taste perception alteration;
3. Xerostomia, with dry mouth.
b) Other associated symptoms: thirst, headache, pain in the temporomandibular joint (TMJ), tenderness/ pain during mastication and/or in some regions of the neck suprahyoid or shoulder level muscles [15].

The amount of positive clinical examination in the diagnosis and any associated distress, which act as causative factors in BMS syndrome

BMS diagnosis is essentially one of exclusion [16]. It is based on a historic and very thorough clinical examination. An accurate diagnosis requires careful investigation of BMS medical history of each patient (such as a preexisting condition at the oral mucosa level, malnutrition, cerebral dysfunctions or drug intoxication) [17,18].

1. The contribution of general clinical examination in the diagnosis of BMS

At their first visit at the dentist's, patients frequently report present disorders and diseases already diagnosed under therapy. When there is a suspicion of disease present and undiagnosed, the patient is advised to consult a physician in the respective domain [19].

2. The role of local clinical examination in the diagnosis of BMS

Local clinical examination often does not reveal any changes. Sometimes clinical examination can detect minor changes or variations of normality: fissured tongue, exfoliative glossitis, depapillations of various origins, geographic tongue or coated tongue [16].

If physical examination reveals the absence of clinically obvious lesions on the oral mucosa, it is reasonable to suspect that intraoral burning is a possible indicator of a systemic disorder [3].

Identifying the possibility of the existence of systemic diseases associated with or responsible for installing the BMS syndrome is by clinical examination and by performing general laboratory or laboratory tests [16].

THE MAIN THERAPIES EMPLOYED IN THE BMS

Based on a comprehensive assessment of the patient's disease state that integrates general clinical examination results, psychiatric/psychological balance and the local dental examination, a therapy in BMS is set. The characteristic of glossodynia treatment is its individualization and adapting to each individual case [19].

Primary stage in establishing BMS therapy requires the differentiation of the primary from the secondary. This is because if the balance sheet allowed the identification of predisposing and etiologic factors, therapy will target their harmful action through appropriate specific methods.

Clinical attitude to be adopted to establish the diagnosis and clinical form of BMS is summarized in figure 1. Schematic representation is useful because it allows to facilitate the understanding of therapeutic medical conduct to follow. According to Demarosi (2013), clinical, biochemical and hematological test results represent two phases of special importance in the etiological diagnosis of the BMS [3].

The therapy goals of the BMS syndrome are administered to target a triple purpose:
1) Improvement of symptoms;
2) Correction of biological and/or morphological disturbances (if there are), commonly referred to as correction therapy;
3) Changes in psycho-emotional therapy [3].

Symptomatic treatment

Symptomatic treatment is similar in both cases of BMS (idiopathic or secondary). While pain is a symptom of many chronic diseases, persistent pain should not be regarded simply as a symptom. Pain itself can become a disease where pain signals origins fail to be controlled due to deterioration of the pain alarm system, leaving the patient with persistent pain. Whatever the cause, chronic pain can transcend ordinary physical pain. Persistent pain interferes with daily life, other important relationships and puts a mark on the mind, body and spirit of the BMS patient. The pain and related problems (e.g., medication side effects, depression and anxiety) can be best managed

using a complex program of integrative treatments tailored to each patient. The name of integrative treatment derives from the means/method by which it is achieved, the approach of a "multi-modal" one.

Figure 1: The current clinical attitude in the presence of a case with BMS [3]

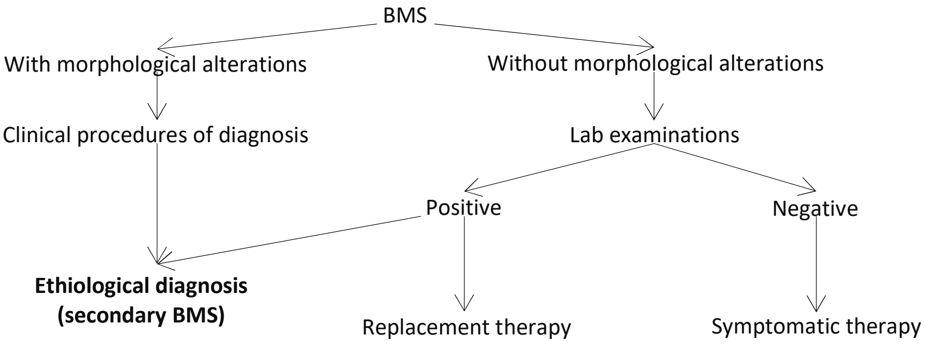
BMS
With morphological alterations
Without morphological alterations
Clinical procedures of diagnosis
Lab examinations
Positive
Negative
Ethiological diagnosis (secondary BMS)
Replacement therapy
Symptomatic therapy

The method involves combining treatments from conventional medicine with those of complementary and alternative therapies. Scientifically, for the efficiency of the latter, evidence have been provided for both the quality and safety of the therapy. In this category, the treatment of patients with BMS can use homeopathic products, cognitive-behavioral methods, aromatherapy, herbal extracts, vitamins, dietary supplements, some combination of minerals etc. [21,22]. Also, acupuncture as an adjunctive therapy method is referred to in the expert literature as being beneficial for the relief of symptoms in patients with BMS [14].

Generally speaking, it is acceptable that the treatment of idiopathic BMS is essentially a symptomatic treatment. In the context of such therapy, the dentist has available a number of symptomatic therapies that can be administered by the general or local means with topical use. Some of these are summarized in the table shown above. The purpose of a symptomatic therapy aims at either improving the intensity of symptoms or halting the clinical manifestations. The results are uncertain, including failure.

Therapeutic strategies include benzodiazepines (clonazepam), tricyclic antidepressants (amitriptyline), opioids (gabapentin), selective inhibitors of serotonin receptors (paroxetine and sertraline), capsaicin topical/systemic, alpha-lipoic acid (neurological antioxidant) hydrochloride, benzydamine 0.15%, hormone replacement therapy, vitamin supplementation and/or zinc, iron and psycho-cognitive therapy [3].

Tricyclic antidepressants (amitriptyline/doxepin/ nortriptyline) are extremely useful for patients with chronic pain. Although developed for the treatment of depression, they have a spectrum of biological activities depending on the dose, including inducing analgesia in a variety of clinical conditions. Although the mechanism is not known, the analgesic effect of antidepressants have a more rapid onset and occurs at a dose lower than that typically necessary to treat depression. The maximum effect is achieved by tricyclic antidepressants in patients with chronic pain who are not depressed, reduced pain being a certainty. Anticonvulsants (carbamazepine) and tranquilizers (clonazepam) are particularly useful for patients with neuropathic pain [23].

Capsaicin, a substance extracted from red peppers, is used for its revulsive and analgesic action. Capsaicin is specifically detected by the "transient receptor potential vanilloid receptor" (TRPV1) distributed in the oral mucosa. The explanation is that the capsaicin molecule is a selective agonist vanilloid receptors TRPV1. Binding means stimulating the receptor, which is found in the nociceptors (pain receptors) in the oral cavity. Overstimulation of TRPV1 receptors make them become "desensitized", no longer able to respond to the stimuli that normally cause pain in patients with peripheral neuropathic pain [24].

Topical application of capsaicin has been shown to sometimes be useful in some pain syndromes, in particular for relieving the symptoms of herpes neuralgia or diabetic polyneuropathy hyperalgesia [25].

Similar conclusions on the effectiveness of topical application of capsaicin (0.02%/0.002%) in BMS are made by Silvestre et al. (2012) and Spanemberg et al. (2011) [26,27].

Other authors speak of the efficacy of capsaicin. Spadari et al. (2014) reported a success rate of over 70% for each control visits after 1, 3, 6 and 12 months of topical therapy (as by rinsing the mouth with a solution of capsaicin 3 times/day) [28].

Among the most widely used symptomatic therapies for BMS are salivary substitutes, from the most simple and economical specific physiological solution to gel. Their use is motivated by the fact that xerostomia is a frequent syndrome in patients with BMS [3].

Grushka (2014) suggests that treatment involves a combination of several BMS drugs that act together, increasing each other's effect (they are given together as dose so that a low-dose should enhance the action). From such an attitude, “low dose” can achieve favorable results on major symptoms present in patients. Achieving success involves on the one hand an individualized (personalized) therapy, on the other successive explorations, to identify the effective drug combination. Based on the results of his studies, Grushka (2014) admits that ⅔ of the patients respond to the treatment aimed at suppressing the effects of benzodiazepine receptor agonists group GABA-A and inhibition of pain. The combination drug likely to complete such a success is a triad of medications: clonazepam 0.5 mg before bed + 10 mg amitriptyline + gabapentin 100-300 mg 3-4 times +/or 25-75 mg Pregabalin 3-4 times daily. Again Grushka (2014) states that zinc therapy is effective in taste changes, prescribing zinc gluconate 50 mg 1-3 times a day for a period of 3-4 months [2].

In conclusion, according to the expert literature, the current level of knowledge about the disease does not include any certainly effective treatment for primary BMS.

Treatment of secondary BMS

According to Ţovaru et al. (2015), for the treatment of secondary BMS the major objectives are:

• detection and treatment of general factors involved in the emergence of glossodynia (anemia, vitamin B deficiency, diabetes, autoimmune disorders of the digestive tract, disorders during menopause, and esophageal reflux caused by gastric hyperacidity);

• detection and removal of the local causes of BMS (incorrect prostheses, changed vertical dimension of the lower floor of the face, improper dental alloys, chronic mucosal infections – for example, Candida – contact allergies, dry mouth, migratory glossitis;

• detection and treatment of coexisting psychiatric disorders (anxiety neurosis or depression, with stress, chronic states of conflict, emotional pressure, cancer-phobia) [19].

Achieving this objectives requires expert advice and patient's hospitalization for the neuropsychological disorder, often lasting for years.

Etiological treatment

A priori, BMS secondary treatment is etiologic, addressed to fight against the general and local factors that maintain the syndrome. The success of etiologic therapy is dependent on the precision with which the causative factor was identified [8,19]. Thus, in the case of the presence of allergic contact reactions, simply removing the suspected allergen (e.g., the material / dental alloy) determines remission of the symptoms of BMS.

If the necessary examinations revealed the presence of anemia, therapy should be prescribed to correct hemoglobin assets and deposits of iron and vitamin B12. Completion of these deposits is mandatory because suppressing the substitute anti-etiological treatment after restoration of erythrocyte hemoglobin level, allows a recurrence of anemia after a certain period of time.

Etiological treatment is required in other cases of BMS secondary diseases such as diabetes mellitus, gastric ulcer, gastro-esophageal reflux disease, mycosis, and the burning mouth is one of the manifestations of

these disorders [3].

Psychological and psychiatric treatment

The second place in the treatment of secondary BMS is psycho-pharmaceutical correction. Frequently, symptomatic treatment [3] is no longer resorted to.

Psychological and psychiatric treatment in the syndrome of secondary BMS is applied not only to the patients with psychiatric disorders or behavioral disorders diagnosed during the first medical checkup, but also to patients who do not respond to dental treatment. For this purpose, patients are directed to consult a specialist who would in fact perform a reassessment if the psychotherapeutic treatment is not appropriate to be set [3,29].

Specialized treatment is achieved by two means:

a) psychotherapy (psychological exam);

b) with drugs, therapy monitored by a psychiatrist;

Psychological treatment aims at controlling and alleviating the mental disorders that occurred. Typically, psychological treatment complements the psychiatric one, but it is the first to start with, especially in patients who have problems with mental BMS, such as those with dental treatment failure. Results of consequent studies have concluded that pharmacological products such as benzidamine-HCl solution, sucralfate, antihistamines, lidocaine, capsaicin have not shown significant improvement statistically [2,3,16].

Frequently followed conduct is the psychoanalytic association with administration of specific psychotropic medication (anxiolytic, antidepressant). The usefulness of psychotherapy is to reduce symptoms, if there is a psychological component to glossodynia [19].

Counselling treatment

BMS syndrome treatment conduct includes counselling, preferably applied by a physician demonstrating empathy for the patient. The purpose of counselling is to provide the patient information and explanations about the sickness, benignity of the lesion, its correlation with notions of its correlation with field (age and sex). These patients should always know that their disorders are often related to stress and that if they make it go away, at least in part their suffering can diminish [3].

The doctor should always have the status of patients with BMS, the suffering and disease, so they have to devote the necessary time to communicate and discuss the patients' problems. This is because one of the major concerns of patients with BMS is to undergo a treatment to alleviate the symptoms [3].

During counselling sessions the BMS patient will be informed about the need to respect therapeutic indications, even if obvious improvements do not occur. Also, we will clarify that for the relief of symptoms, duration of treatment is for months/years.

Starting from his own medical experience, Demarosi (2013) states that patients rarely comply with prescribed BMS treatment, the recommended therapy is often performed incorrectly and doctor visits are random. Simultaneously, the patient will be clearly advised that the outcome of the treatment consisting in improvement or even the disappearance of the disease is often only temporarily effective [3].

Since the treatment of BMS is generally unsatisfactory even if it is appropriate, and the pain is chronic, it is necessary that patients be counselled with correct information on expectations that are to be understood realistically. Otherwise, the patient loses confidence in his regular doctor and becomes psychotic, going from doctor to doctor.

EVOLUTION AND PROGNOSIS IN BMS SYNDROME

Sardella et al. (2006) in a retrospective study of 18 months showed that 28.3% of the BMS (basic form) cases registered moderate improvement, 49% not any significant change, 18.9% of the cases presented a worsening of symptoms in patients who have not received any treatment. Spontaneous complete remission is rare and has been reported by the same team in 3% of cases investigated over a period of 5 years [30,31].

Cure rates measured in glossodynia of type I and II were almost comparable (61% versus 59%). 70% of all

cases that have not responded to treatment present associated psychiatric disorders [19].

Idiopathic nonspecific painful syndromes are most rebellious to treatment. Some cases even exclude any possibility of allopathic treatment, in which case additional treatment, such as acupuncture is resorted to (14). Frequently, the results of acupuncture are not always stable, and in some cases a patient can get addicted to this kind of treatment [19].

In other words, where allopathic treatment + psychological + counseling fail, acupuncture may be used with relative efficacy and limited duration, which requires to repeat the complementary procedure.

In another study, a group of 91 patients with BMS symptoms were analyzed and it was concluded that in a small number of patients symptoms may resolve spontaneously within five years from the onset. Moreover, a partial improvement was observed in 38.4% of these cases under various treatments (for example, clonazepam in local administration). This rate of improvement is greater (up 61.7%) when patients were administered clonazepam locally associated with psychotherapy [32].

Fenelon et al. (2014), evaluating the results of short-term therapy with clonazepam versus amitriptyline in the pain relief of BMS highlighted that after 6 weeks of treatment there was an improvement in the symptoms of pain in both situations, noting that the effect of amitriptyline was lower than that of clonazepam. To explain such particularity of the therapy, the authors imply a slow onset of action of the first drug. After 3 months, the results were similar in the two groups of patients evaluated [33].

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New synthesized oximes active in nerve agents' hazards

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Abstract: *Object: The aim of the study is to select the most active new imidazolium-quinuclidinum-oxime, from some similar chemical compounds synthesized in our chemistry department, with sufficient efficacy to decrease the acute toxicity of neurotoxic organophosphates known as nerve agents. Method: The experimental study consist in vivo testing the antidotal efficacy of obidoxime and of selected imidazolium oximes synthesized in our chemistry department. Each oxime was included, by equimolar replacing the obidoxime, in an antidotal formula, which also contains atropine. The above mentioned formula containing atropine and obidoxime was used as reference. The protective ratio, defined as the ratio between the lethal median dose of the poisoned and treated study group and the median lethal dose (LD50) of the poisoned and untreated study groups was one of the used parameters in order to select a new active chemical structure in counteracting the neurotoxic organophosphorus compounds acute toxicity. Another studied parameter was the erythrocyte acetylcholinesterase value measured in whole blood 24 hours after exposure. Results: The protective ratio against an organophosphorus compound were the follow: obidoxime chloride: 2; 1,3-dimethyl-2-hydroxyethyl-imidazolyliodide: 1,75;3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl]quinuclidin-dichl-oride: 2,5; 1-methyl-quinuclidin-3-iodide: 1,5. The erythrocyte acetycholinesterase main values were the following: the unpoisoned and untreated study group:3,45 ±0,13mmol/dl; the poisoned and untreated study group: 0,89 ±0,09 mmol/dl; the poisoned and 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl]quinuclidindichloride treated study group:2,89 ±0,11 mmol/dl; the poisoned and obidoxime treated study group: 2,53±0,15 mmol/dl. Conclusions: 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl] quinuclidindichloride synthesized in our chemistry department, has shown a better protective ratio and a more prolonged surviving time than the reference (obidoxime). It has shown the best AChE reactivation of all the synthetized compounds. This compound can be a cheap and good option for replacing obidoxime in the antidotal formula active in nerve agent exposure.*

Keywords: *obidoxime chloride; 1,3-dimethyl-2-hydroxyethyl-imidazolyl-iodide;3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl] quinuclidin dichloride; 1-methyl-quinuclidin-3-on-iodide; organophosphorus compounds; nerve agents*

INTRODUCTION

Strategic expert analyzes have shown that in the next period, there may be international conflicts, amplifying the risk of attempts to use highly toxic chemicals as weapons of mass destruction.[1]

Romania through its geostrategic position, being the member of NATO, can provide a better potential target of such actions.

Neurotoxic organophosphorus compounds (sarin, soman, tabun, most insecticides) are chemical compounds, phosphonic acid esters with central and

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peripheral properties, acetyl-cholinesterase and butyryl-cholinesterase irreversible inhibitors with toxic effects on smooth and striated muscles and central nervous system.[2-4]

They were registered on the list of substances to be destroyed in accordance with international regulations (Convention on the Prohibition of Development, Production and Stockpiling Chimice-Geneva).

Although banned by international conventions, these toxic substances have been used in terrorist attacks against civilians (Tokyo 1995 Halabja in 1987, Anfal-1988). In this context geostrategic and due to soaring use of organophosphorous insecticides, development of medical measures to protect against the lethal toxicity is one of the priorities of research within specialized programs of NATO (NBC – Science for Peace) and European Union (Chemical Program Weapons, Monitoring and Protection, created in the OPCW).

The acute toxicity of organophosphorus compounds known as nerve agents mainly result from their action as irreversible inhibitors of acetylcholinesterase (AChE). They suffer some conformational and chemical changes, resulting a phenomenon known as "aging", whose speed of appearance is directly proportional to the toxicity of compounds.[5,6]

Accumulation of acetylcholine stimulation leads to persistent cholinergic muscarinic receptors that trigger the syndrome whose symptoms include miosis, salivation, bronchial hypersecretion, bradycardia, bronchoconstriction, hypotension and diarrhea.[7]

Another effect of organophosphate anticholinesterases is the desensitization of nicotinic receptors followed by overstimulation, translated by skeletal muscle twitching and subsequent paralysis.[8] Central nervous system toxic effects include anxiety, restlessness, confusion, ataxia, tremors, convulsions, paralysis, cardiorespiratory effects and coma [9].

The treatment of nerve agents poisoning is based on an antimuscarinic agent (atropine), and an acetyl-cholinesterase reactivator called oxime according to its chemical structure. Atropine blocks the effects of accumulated acetylcholine resulting overstimulation of muscarinic receptors.[9,10]

Acetylcholinesterase reactivators dephosphorylate the acetylcholinesterase – organophosphorus complex, reactivating the enzyme activity.[11] The early appeared seizures were counteracted by benzodiazepines.

Currently available oximes (pralidoxime, obidoxime), have been shown to be less effective against one of the most toxic nerve agents (soman tabun). There is a strong interest in developing new, more potent acetyl cholinesterase reactivators with oxime structure.[12]

The present paper represents an in vivo screening study in selecting more potent chemical compounds, active in counteracting the nerve agents acute toxicity. The paper describes an experimental test of antidotal efficiency of some compounds containing imidazolium, or quinuclidinium rings, which equimolar replace obidoxime chloride in the antidoltal formula.

The results were expressed as the protective ratio representing the ratio between the DL50 of the neurotoxic compound administred to the poisoned and treated rat study group and the DL50 administered to the organoposphate compound of the poisoned and untreated study group.

MATERIAL AND METHODS

Chemicals

- obidoxime chloride (CAS number 111-90-9);
- atropine sulphate (CAS number 5908-99-6) were purchased from Sigma Aldrich;
- diclorvos (PESTANAL CAS number 62-73-7) were purchased from Sigma Aldrich.
- the experimentally tested quinuclidinium-imidazolium oximes were synthetized in the chemistry department of Medical Military Research Center.

Animals

Male Wistar rats (150-200g) were maintained on rice husk in polypropylene cages. Wistar free access to water and rodent pellet food. The study was

approved by the Ethical Committee on Animal Experimentation.

Acetycholinesterase measurement method: Elisa kit.

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Acetylcholinesterase in human serum, plasma and other biological fluids.

Target Information: Acetylcholinesterase hydrolyzes the neurotransmitter, acetylcholine at neuromuscular junctions and brain cholinergic synapses, and thus terminates signal transmission. It is also found on the red blood cell membranes, where it constitutes the Yt blood group antigen.

Figure 1: The chemical structure of the in vivo tested compounds as acetycholinesterase reactivators

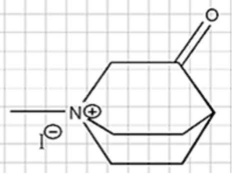

1methyl-quinuclidine-3-on-iodide

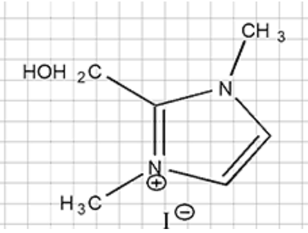

1,3-dimethyl-2-hidroxyethyl-imidazolyl-iodide

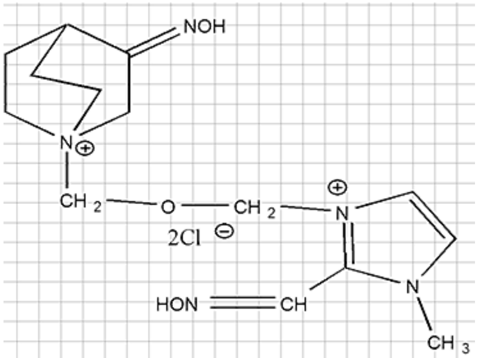

3oxim[3(2-hidroxy-iminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride

Treatments

120 Wistar rats were divided into 12 groups, each, including ten rats as follows:

- 1st group: control group unpoisoned, untreated
- 2nd group: poisoned with diclorvos (1,5 DL50) and untreated;
- 3rd group: poisoned with diclorvos (1,5 DL50,) after one minute, were administered the antidotal formula including atropine (2mg) and obidoxime chloride (250 mg);
- 4th group: poisoned with diclorvos (1,5 DL50), after one minute, were administered the antidotal formula including atropine (2 mg) 1methyl-quinuclidine 3-on iodide in an equimolar dose with obidoxime chloride (78,5 mg);
- 5th group: poisoned with diclorvos (1,5DL50,) after one minute were administered the antidotal formula including atropine (2 mg), 1,3- dimethyl-2-hidroxy-ethylimidazolyl iodide in an equimolar dose with obidoxime chloride (61,94 mg/kg);
- 6th group: poisoned with diclorvos (1,5 DL50), after one minute, were administered the antidotal formula including atropine (1,5 mg) and 3oxim[3(2-hidroxy-iminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride (118 mg/kg).

The above mentioned oximes were experimentally tested against 1.5, 1.75, 2, 2.5, DL50.

24 hours after poisoning mortality, protective ratio was registered and erythrocyte achetylcolinesterase activity values were measured.

RESULTS

The aim of the study is to evaluate the most active newly synthesised imidazolquinuclidine-oxime with the great efficacy in reactivating the phosphorylated acetylcholinesterase through some similar chemical compounds synthesized in our chemistry department, able to decrease acute neurotoxic compounds toxicity.

The protective ratio of the obidoxime and experimentally tested formulas correlated with their efficacy as acetylcholinesterase reactivators are represented in the Table 1.

The 3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2-oxapropyl] quinuclidine dichloride protective ratio correlated with its acetylcholinesterase reactivator activity is greater than the obidoxime, resulting a

better antidotal efficacy.

Statistical analysis Student's t-test probability associated with statistically significant differences between mean values of erythrocyte acetyl-cholinesterase between the control study group and the poisoned and treated study groups is represented in tables 2-7.

The poisoned and untreated group statistically significantly differs from normal in terms of the acetylcholinesterase inhibition.

Neurotoxic organo-phosphorous compounds cause in the intoxicated group and untreated study group an acetylcholinesterase inhibition of 75.08%, incompatible with survival.

Obidoxime and new synthesized imidazolium-quiniclidinium oximes highlighted new capabilities of acetylcholinesterase reactivation correlated with the protection index.

3-oxime compound [3-(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl]-quinuclidine-dichloride-quinuclidine 3-on iodide revealed a protection index bigger than other obidoxime and other imidazolium compounds studied.

Explanation of this is that the quinuclidine ring, by its position intensifies allosteric oxime group (Table 1).

Tables 2-7 reveals that the intoxicated untreated control group differs statistically significantly from normal and so the intoxicated study groups were treated with different therapeutic formulations.

Table 1: The protective ratio of the antidotal formulas containing the experimentally tested imidazolium oximes as AChE reactivators

Study group	**Acetylcholinesterase reactivator**	**Dose of reactivators** (mg/kg)	**Protective ratio** (DL_{50})	**Acetylcholinesterase reactivation due to oxime** (%)
2	-	-	-	
4	1methyl-quinuclidine 3-on iodide	78.58	1.5	47.82
8	1,3-dimethyl-2-hidroxyethyl-imidazolyl iodide	61.94	1.75	60.28
10	obidoxime chloride	100	2	73.33
12	3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride	118.88	2.5	82.31

Table 2: Statistical analysis of the probability P (T test) associated with average values of erythrocyte AchE of unpoisoned and poisoned and untreated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
1	3.45 ± 0.13	0.003	$P \leq 0.05$ statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 1: unpoisoned and untreated study group;
Study group 2: paraoxon poisoned and untreated study group

Table 3: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and obidoxime treated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
10	2.53 ± 0.15	0.007	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 10: paraoxon poisoned and obidoxime treated study group;
Study group 2: paraoxon poisoned and untreated study group.

Table 4: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and 1,3-dimethyl-2-hidroxyethylimidazolyl iodide treated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
8	2.01 ± 0.07	0.004	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 8: paraoxon poisoned and 1,3-dimethyl-2-hidroxyethylimidazolyl iodide treated study group;
Study group 2: paraoxon poisoned and untreated study group.

Table 5: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and 1methyl-quinuclidine 3-on iodide study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
4	1.65 ± 0.03	0.009	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 4: paraoxon poisoned and iodide treated study group;
Study group 2: paraoxon poisoned and untreated study group

Table 6: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and 3-oxim[3(2-hidroxyiminomethyl-1- imidazolyl)-2oxapropyl] quinuclidine dichloride- quinuclidine 3-on iodide treated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
12	2.84 ± 0.05	0.0002	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 12: paraoxon poisoned and 3-oxim[3(2- hidroxyiminomethyl-1- imidazolyl)-2oxapropyl] quinuclidine dichloride- quinuclidine 3-on iodide treated study group;
Study group 2: paraoxon poisoned study group

Table 7: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, obidoxime treated and 3-oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride-quinuclidine 3-on iodide treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
12	2.84 ± 0.05	0.01	P ≤ 0.05 statistically significant difference between groups
10	2.53 ± 0.15		

Legend:

Study group 12: paraoxon poisoned and 3-oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride-quinuclidine 3-on iodide treated study group;

Study group 10: paraoxon poisoned and obidoxime treated study group

Table 8: The correlation between administered dose of acetylcholinesterase reactivator and the pharmacodynamic effect

Study group.	Reactivator dose used in the antidotal formula (mg)	AChE Mmol/ml	Correlation coeficicient
4	61,94	1,65	0,9927
8	78,58	2,01	
10	100	2,53	
12	118,88	2,84	

Legend:

Study group 4: poisoned study group and 1methyl-quinuclidine 3-on iodide treated;

Study group 8: poisoned study group and 1,3-dimethyl-2-hidroxyethyl-imidazolyl iodide treated;

Study group 10: poisoned study group and obidoxime chloride treated;

Study group 12: poisoned study group and 3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride treated;

From a medical standpoint, an organophosphoric nerve poisoning that caused inhibition of acetylcholinesterase survival limit was applied.

This intoxication was further antagonized with different formulas containing the synthesized imidazoliumquinuclidinium oximes and obidoxime as antidotes.

They showed mean values of acetylcholinesterase significantly different from the poisoned and untreated group. The studied imidasolium quinuclidinium oximes showed average values of acetylcholinesterase statistically significantly different between them, thus emphasizing that the doses used may cause significant variations in therapeutic effect.

Table 8 highlights the so-called "dose finding", the correct correlation between dose and pharmacodynamic effect. The correlation coefficient of 0.9927 demonstrates that doses of acetylcholinesterase reactivators used in the antidotal formulas are correct, being those that cause maximum pharmacodynamic effect.

CONCLUSION

- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride synthesized in the Medical Military Research Centre Chemistry Department has shown a better protective ratio and a more prolonged surviving time than obidoxime considered as reference.
- It has shown the best acetylcholinesterase reactivation of all the synthetised compounds and obidoxime
- The very good correlation dose-effect highlights that the correct dose of acetylcholinesterase reactivator was chosen in order to obtain the better pharmacodynamic effect.

- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride can be considered an efficient antidote in neuroparalytic organophosphorous hazards.
- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride can be a cheaper and better option for replacing obidoxime in the antidotal formula active in nerve agent poisoning.
- Thus one can conclude that the result of the experimental study is consistent with the proposed object.

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ORIGINAL ARTICLES

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Dosimeters: measuring occupational UV exposure's involvement in skin cancer development

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Abstract: *Introduction: Solar exposure is a major causative factor for multiple skin pathologies. Even though UV radiation is the most important factor implicated in skin cancer pathogenesis, still, few European countries recognize UV occupational-induced skin cancer as an occupational disease, Romania not being part of this category. The objectives of this study were to evaluate the UV exposure doses in agriculture and construction workers and to establish efficient protective measures.*

Materials and methods: In our Department, a prospective, observational, analytical study was conducted, including six outdoor workers (in construction and agriculture), over a period of three summer months (July, August, September). Subjects were asked to wear personal dosimeters and the following parameters were analyzed: profession, duration of exposure, UV exposure doses, skin aspect, preexisting preventive measures.

Results: The wearing of dosimeters was well tolerated by all subjects, without any interference with daily professional activities. Daily UV radiation doses ranged from 1.79 SED (tractor driver) to 19 SED (agriculture worker). Maximum UVR doses were recorded between 10:00 am and 4 pm. Clinical examination was made after measurements, with no detectable skin changes.

Conclusion: Even though workers in agriculture and construction in Romania are exposed to significant UV doses, with 40% more than the general population, these professions are not recognized as high-risk professions for developing skin tumors. More prospective and retrospective studies are necessary for more significant statistical results and for demonstrating the necessity of introducing primary, secondary and tertiary preventive measures.

Keywords: *ultraviolet radiation, occupational exposure, skin cancer*

INTRODUCTION

Solar exposure is a major causative factor for multiple skin pathologies. Most frequently it is the case of cutaneous tumors, both precancerous and malignant lesions (actinic keratoses, basal cell carcinomas, squamous cell carcinomas and melanomas). [1]

There are numerous professions that imply working

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outdoors, from which we concentrated our study on agriculture and construction workers. Occupational ultraviolet (UV) exposure can be measured with UV dosimeters.

Personal UV dosimeters are electronic devices that are able to record data that permits analysis of UV exposure dose on working days. Modern dosimeters are small in size, therefore easy to wear at the workplace, permitting personal UV measurements. Even more so, multiple dosimeters can be used, recording data from different parts of the body. They are relatively new devices used more and more frequently in UV exposure-related studies. [2-5]

Ultraviolet radiations represent segments of the electromagnetic specter and are classified in UVC (100-290 nm), UVB (290-320 nm) and UVA (320-400 nm).

UV radiation is very important in dermatological practice, being able to determine both exacerbations and remissions of different skin disorders and of course playing a major role in skin cancer development. [6]

Even though UV radiation is the most important factor implicated in skin cancer pathogenesis and World Health Organization introduced UV radiation on the carcinogens agents list of group I, still, few European countries recognize UV occupational-induced skin cancer as an occupational disease, Romania not being part of this category. [7]

The lack of studies in this area in Romania suggests the fact that more analysis should be made for a better understanding of this aspect in our country.

Objectives: to evaluate UV exposure doses in agriculture and construction workers and to establish efficient prevention and protection measures.

MATERIALS AND METHODS

In our Department, a prospective, observational, analytical study was conducted, including six outdoor workers (in construction and agriculture), over a period of three summer months (July, August, September).

Subjects were selected based on their profession. Our study took place in Bordusani, Ialomita, Romania, with the geographical coordinates 44°28′21″N 27°53′59″E for the agriculture outdoor workers and in Bucharest, Romania, with the geographical coordinates 44°25′57″N 26°06′1 for the construction workers.

Subjects were asked to record in personal diaries information regarding: profession, location, date, start time and end time of their schedule, lunch breaks and weather conditions.

The following parameters were analyzed: profession, duration of exposure, UV exposure doses, skin aspect, preexisting preventive measures.

For the agriculture workers, we've selected irrigation system manufacturer, tractor driver and beans manufactures and for the construction site we've selected road workers.

Subjects were asked to wear personal dosimeters for several working days. Sun exposed areas involved the face, neck, forearms and dorsum of hands, the rest of the areas were covered by equipment.

Clinical examination was performed before and after measurements to detect any skin changes.

The personal dosimeter used in this study was X-2012-10 Personal UV Irradiance Dosimeter from Gigahertz-Optik and a "Velcro" fastening system was used to attach it on the subject's forearm.

Data was processed using Microsoft Office Excel 2013.

RESULTS

The wearing of dosimeters was well tolerated by all subjects, without any interference with daily professional activities.

Daily UV radiation doses ranged from 1.79 SED (tractor driver) to 19 SED (agriculture worker).

Maximum UVR doses were recorded between 10:00 am and 4 pm. Apart from standard equipment, no protective measures were applied.

Clinical examination was made after measurements, with no detectable skin changes.

DISCUSSION

Sunlight is composed of a continuous spectrum of electromagnetic radiation divided into three major groups: ultraviolet, visible and infrared. [8] Ultraviolet radiation is a major causative factor in skin cancer development. [9]

Even though UVA has less energy than UVB and penetrates deeper, into the dermis, UVB radiation is most implicated in carcinogenesis. UVA is responsible for carcinogenesis through immunosuppression: it determines production of reactive oxygen species that cause damage in the deoxyribonucleic acid, therefore gene mutations and damage in the epidermal keratinocytes and dermal connective tissue.

Examples of gene mutations include PTCH and p53 tumor suppression gene in basal cell carcinomas, activated mitogenic ras pathway and p53 in squamous cell carcinomas and ras pathway, inactive p16 and p53 in melanomas. [10]

Also, the duration and intensity of UV exposure are factors implicated in skin cancer development. For example, cumulative, long-term UV exposure is related to chronic actinic skin damage, therefore implicated in the appearance of actinic keratoses and squamous cell carcinomas, since intermittent, high intensity UV exposure especially at young ages would most likely cause basal cell carcinomas and melanomas. [11]

For measuring UV exposure doses on an outdoor worker, irradiance should be determined - the flux of radiant energy per unit (W/m2). But this applies only for one moment in time, so for a larger period (e.g. one working day) the term "exposure dose" (J/m2) is used. [12] For this, a standardized unit – SED – Standard Erythema Dose is applied, where 1 SED is equivalent with 100J/m2. [13]

The annual exposure dose for the general population in Germany is 130 SED. [14] Considering our results, with a daily UV exposure dose of 19 SED (15% daily of the annual exposure dose), we have to acknowledge the fact that both agriculture and construction professions in Romania are professions with high-risk of developing solar exposure related pathologies.

Even more so, by German standards, if the outdoor worker was exposed to a cumulative UVR dose with 40% higher than the general population, than the nonmelanoma skin cancer is considered to be of "occupational origin". [14]

Therefore these professions should be placed in the occupational skin diseases category.

It is very important to take into consideration that different parts of the body absorb different UV doses depending on body geometry, therefore involve different risks. Multiple personal dosimeters should be used, calculating UV doses on different body sites. [16]

Horizontal body locations seem to receive 2x - 2,5x more UV that the vertical ones. [17]

Also, UV exposure dose depends on the reflection of UV radiation on surrounding structures. For example, UV reflection for construction workers surrounded by metallic structures is different to that of fishermen that are surrounded by water. This is a significant additional benefit of personal dosimeters, being able to record UV doses from different parts of the body and not taking into consideration only the direct, vertical radiation.

For example, even if an outdoor worker has correct equipment, with a broad-brimmed hat, UV reflection from surrounding structures could still reach him. Therefore additional protective measures should be implemented, such as protective sun creams. Also, personal dosimeters, being able to measure UV radiation received by the subject, these measurements could be compared with those stated by meteorological departments. [18]

Even more so, occupational exposure to UV radiation can determine specific features of the tumors involved. For example, basal cell carcinoma appears more frequently in the "mask area" of the face in occupational exposed workers and also histopathologically appears to be more aggressive in evolution. [19]

All of this important features reinforce the necessity of implementing strict preventive and protective measures for outdoor workers, including protective sun creams (SPF 30 or more), special sun protective

equipment (long pants and long sleeves, broad-brimmed hats or helmets, sunglasses), multiple breaks in indoor areas and rotating shifts. [15] Also, considering the fact that the maximum UVR dose was detected between 10 am and 4 pm, outdoor workers should avoid working outside in this timeframe.

Even if by the end of our study no pathological skin changes were expected, we have to keep in mind that most UV skin related pathologies depend on a cumulative UVR dose with long term exposure. More interventional and observational studies are necessary for a better understanding of UVR effects on outdoor workers in Romania.

CONCLUSION

Even though workers in agriculture and construction in Romania are exposed to significant UV doses, with 40% more than the general population, these professions are not recognized as high-risk professions for developing nonmelanoma skin cancer or melanomas. More prospective and retrospective studies are necessary for more significant statistical results and for demonstrating the necessity of introducing primary, secondary and tertiary preventive measures.

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VARIA

Artificial Intelligence in cardiovascular medical imaging

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Abstract: *Artificial Intelligence (AI) has become an important tool for computer-aided diagnosis of medical imaging. This review aims to provide an overview for clinicians, explaining the relevant aspects of artificial intelligence and machine learning (ML) and presenting up-to-date applications of AI and ML techniques to medical imaging methods such as angiography, magnetic resonance, and echocardiography.*

For each imaging method, we present the acquisition process, the types of diagnostic test interpretation, and the challenges related to them, as well as how AI/ML techniques, have improved the process of decision making.

A summary of selected works applying AI/ML techniques to medical imaging is organized into a table, which highlights the scope of the study, the dataset used, the details of each approach as well as the measured results, including objectives and criteria. The overall benefits of AI in medical imaging are extracted based on the diverse applications and high evaluation scores. In the end, cardiologists should have an advanced understanding of using AI to integrate clinical data and making the final decision in diagnosis.

Keywords: *machine learning, cardiology, angiography, echography*

INTRODUCTION

The application of AI algorithms to a data set has the usual initial steps of developing the algorithm's ability to "learn" based on the training data and to apply the knowledge gained in making predictions. The “learning phase” of an algorithm consists of an incremental optimization of the model parameters with which it is configured, so that performance on a given training objective (e.g. classification of the most likely diagnosis, regression of numerical value of a health parameter) increases.

Machine Learning is a methodology used to recognize such patterns; the applicability of this can be extended very well in the context of medical imaging. Usually, this technique starts with the use of an ML-based system that determines those characteristics of the images that are considered to be important in making the prediction or diagnosis. As a result, the system identifies the best combination of these characteristics for the image classification and computes statistical data for the different regions identified.

The last decade demonstrates the huge potential of machine learning techniques in many domains along with the development of the graphical processing units (CPU/GPU),

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the availability of big data, and the growth of the learning algorithm efficiency [1].

Figure 1 shows the principal categorization of ML algorithms according to their main functionality. One can distinguish between Supervised Machine Learning (i.e. having an expert provide the so-called ground truth - the correct class or value - for each example in a dataset) and Unsupervised Machine Learning, where the objective is to find interesting patterns and frequent associations between examples in a dataset, without any human supervision (e.g. finding an automatic grouping of diseases by the similarity of symptoms).

Medical imaging applications mostly belong to the supervised ML category, because the correct interpretation (e.g. diagnosis, the classification of severity of a disease) of the result is provided by the medical experts who have analyzed it.

In the past decades, there was substantial growth in the healthcare applications one example is the deep learning algorithm that proved to have an outstanding accuracy in diabetic retinopathy identification [2].

Figure 1: Grouping the different types of machine learning algorithms according to the type of learning [3]

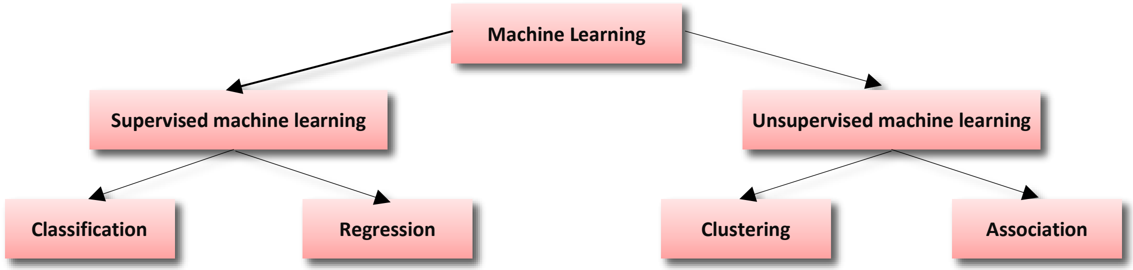

Yang et al [4] demonstrated the importance of AI about big data and parallel programming in medicine, especially in the field of cardiac imaging.

These last decades have also demonstrated the importance of medical imaging in the detection, diagnosis, and treatment of various conditions. In clinical applications, medical imaging is largely performed by a group of experts, such as cardiologists, radiologists, endocrinologists, and so on [5]. In this regard, the computer-assisted analysis enables a better interpretation of the medical image database available. Regarding the decoding of the parameters highlighted by the medical images, it is worth noticing the recent advances in deep learning techniques, which represent a considerable leap in the identification, classification, and quantification of medical conditions and/or health parameters based on input from different medical imaging techniques [6].

ARTIFICIAL INTELLIGENCE IN ECHOCARDIOGRAPHY

In the world of multimodal imaging in cardiology, echocardiography remains crucial for the diagnosis and management of patients with cardiovascular disease [7]. The advantages of the ultrasound of the heart compared to other imaging tools are low price, portability, the possibility of being repeated, short examination time, and the fact that the administration of contrast medium is unnecessary. However, errors in echocardiography quantification and conflict in the interpretation of echo images are frequent in everyday clinical practice.

Although it remains the main imagining modality used in cardiology, echocardiography has several shortcomings that can be improved. The echo machines have developed intensively during the last year (leading to accurate 2D and Doppler imaging and new technologies like tissue Doppler, speckle tracking, or 3D echocardiography). However, one of the main limitations of this method is high operator dependence. The learning curve in echocardiography is very steep and the knowledge, the experience, and sometimes even the mood of the echocardiographer are very important for the exam interpretation. For example, left ventricle ejection fraction is a crucial parameter for patient management but has a high inter- and intraobserver variability. Therefore, the result given by one observer can be different compared to another one or, even more, the same observer can give different interpretations when the reading is repeated. The purpose of AI is to overcome these issues.

Another important role of AI in this field is to solve the problem of a mismatch between the high number of echocardiography required in clinical practice and the number of cardiologists/echocardiographers available to interpret the studies. Moreover, a large volume of already acquired data is under-utilized during a routine echocardiogram and AI offers the possibility to interpret multiple datasets in a very short time [8].

The purpose of artificial intelligence in echocardiography is to detect the disease based on some specific echocardiographic criteria [9, 10]. AI can be used in the image-acquisition process as well and helps people with limited echocardiography training to obtain standard views by anatomic guidance.

Although the implication of AI in echocardiography is still less important compared to other imaging modalities, there are many examples of applications already being used.

Software algorithms are capable of correct identification of the ultrasound modality (2D, pulse wave Doppler or continuous wave Doppler) or the view (four-, two- or three-chamber view) [11]. Moreover, the identification of structures and even the quantification of some related parameters based on border detection are also possible. One of the first applications of AI in echocardiography was the assessment of left ventricular volumes and function initially based on 2D and subsequently on 3D [12]. Based on 3D echocardiography, the correct identification of the heart chamber borders offers results comparable to those measured by cardiac magnetic resonance (left ventricle volumes and function [13], right ventricle volumes, and function [14]. The new software can compute the chamber volumes not only at a maximum or minimum level but also dynamically through the cardiac cycle [15]. Some models for the automated diagnosis of regional wall motion abnormalities and myocardial infarction using deep learning algorithms are still developing [16].

AI is already aiding in valvular heart disease; there is automated software that offers the assessment of mitral [17] or aortic valve.

Some applications can already differentiate, with high sensitivity, between some pathological conditions that need an experienced human examination: left ventricular hypertrophy in athletic population vs. in hypertrophic cardiomyopathy [18], constrictive pericarditis vs. restrictive cardiomyopathy [19].

AI can change the way we read echocardiograms. Normally the interpretation of an echocardiogram is based on consecutive views analysis. For example, in aortic stenosis grading, the observer has to integrate information from multiple non-consecutive views (annulus diameter from the parasternal long axis, continuous wave and pulse wave Doppler data from apical 5 chamber or right parasternal). AI allows the classification of these images and the analysis of the ones related to aortic stenosis grading although they have not been acquired consecutively. Therefore, in the future, the reading of an echocardiogram will be based on the chamber or valve analysis in multiple views and not on the interpretation of consecutively acquired views.

A very important aspect is that using artificial intelligence in echocardiography can modify our understanding regarding the echocardiographic data. Our knowledge of echo interpretation is based on clinical trials that have included a target population. However, this population may not always be representative of the entire population. The large amount of data obtained by including artificial intelligence and machine learning in echocardiography can change our classifications, cut-offs, and interpretation of these data. For example, a recent study based on the NEDA registry (Australian New Zealand Clinical Trials registry) showed that patients with moderate aortic stenosis have similarly high rates of mortality compared to patients with severe aortic stenosis and, likely, we'll need to reevaluate our current data regarding the indications for intervention in aortic stenosis [20].

Therefore, in echocardiography, AI offers the possibility to interpret data from the real world and not from selected patients included in clinical trials and probably a better understanding of the diseases.

Recently, machine learning and deep learning have become the main direction of development in echocardiography, which is proved by the number of publications in PubMed [21]. The possible impact of artificial intelligence on echocardiography is impressive but for now, the human mind is still far too complex compared to AI [22].

Although we all dream of an automated program for echocardiogram interpretation, there is still a long journey ahead. Thus, cardiologists should have advanced knowledge and the capability to use artificial intelligence as extended intelligence.

ARTIFICIAL INTELLIGENCE IN CARDIAC MAGNETIC RESONANCE IMAGING

There is a concern among radiologists and medical students that AI will replace the radiologist. This is not likely to be the natural evolution, but radiologists who use artificial intelligence will replace those who do not or do not think they can use it.

The modern principles of medical imaging are based on the most efficient use of the techniques that bring the maximum amount of information in the shortest time possible, starting from the moment of clinical suspicion. This aspect makes it necessary for the clinician to know the value and the limits of each type of sectional imaging exploration, to optimize the indications of the examinations. Cardiac MRI has a very wide range of clinical applications and has been included in the guidelines for the assessment of congenital heart disease, heart tumors, pericardial disease, cardiac ventricular dysplasia, or myocardial ischemia - hibernating myocardium [23]. Other indications such as the evaluation of myocardial perfusion, ventricular or valvular function can be accurately evaluated by MRI, but compete with other imaging modalities, such as single-photon emission computed tomography (SPECT) and echocardiography, more commonly used in outpatient settings.

The major advantages of MRI are the absence of ionizing radiation and the very strong contrast and temporal resolutions (as opposed to CT, where the spatial resolution is very high [27]. Cardiac MRI provides a wealth of morphological and functional information about the heart. In some practices, the evaluation of the heart MRI requires a long global time, so the tendency is to standardize and reduce the time required for the exploration, but also for the analysis of the images obtained, by using the FAST MRI type techniques. To optimize these protocols for analyzing the data obtained from the scanning of the heart, AI techniques have been developed and used in clinical practice.

They are based on automatic machine learning techniques to enhance the ability to analyze images and data obtained by MRI scanning. The results are remarkable both in the optimization of scanning techniques and protocols, as well as in the analysis and integration of data and results. In an impressive achievement, the introduction of AI and the optimization through the machine learning algorithm can increase the speed of interpretation of the images obtained by cardiac MRI from an average of 13 minutes to about 4 seconds [24].

Cardiac MRI can establish the most appropriate plan for cardiac surgical planning, implantation of cardiac defibrillators, and assessment of cardiac toxicity in patients with oncological pathology. These decisions are based on the accuracy measurements made by MRI.

Multiple studies performed on significantly large numbers of patients have used the information to develop machine learning protocols [25]. These are based on neural network learning. For the time being, it is still necessary to validate the superiority of the efficient application of these AI-type protocols over the aspect of the presently considered examinations. At this moment, the cardiac MRI examinations intend to use a large database which, after a standardized examination and by using a minimum of subsequent maneuvers, the results will be offered in the form of measurements made automatically by the computer capable of filling an MRI result with multiple preformed fields. Also included in this result with preformed fields, the neural network algorithm also offers the diagnostic suspicions based on which differential diagnoses can be issued. The vision of those who work and create this advanced analysis and diagnostic software is to increase the speed of examination without sacrificing the accuracy and precision of interpretation.

The use of artificial intelligence is used to solve the following problems and challenges - the small number of radiologists, the long time required for each examination, the insufficient use of radiological equipment without corresponding efficiency [26].

Traditionally, CT and ultrasound are the most frequent techniques used for cardiac imaging examinations. However, MRI can occupy a very important place due to the very important contrast of tissue structures without ionizing radiation.

One of the major disadvantages of cardiac MRI is that the examination lasts for about an hour. For example, an existing software solution, called Heat Vista (available in the US), reduces the examination for suspected cardiac ischemia from 90 minutes to 15 minutes, and the solution is applied to existing equipment. The software recommends the protocol for about 10 seconds. All these technological advances also eliminate the need to examine through sequences with blocked breathing. This progress is achieved through sequences that recognize and detect artifacts and alert the technician if the sequence needs to be redone [28].

Another challenge is MRI examinations for patients with cardiac and vascular implants of various types of devices. It is necessary to adapt these protocols to the particular aspects of each patient and this aspect can be efficiently optimized by AI protocols depending on the type of implant and the manufacturer that produced them. Considering all the above, cardiac imaging and especially cardiac MRI will benefit from the integration of various AI solutions.

ARTIFICIAL INTELLIGENCE IN CARDIAC ANGIOGRAPHY

X-ray angiography is a medical imaging technique dedicated to the diagnosis and even treatment of vascular diseases. An angiography is obtained using an X-ray source and a detector located on both ends of a C-arm gantry. The imaging apparatus provides temporal images series for several seconds.

Table 1: Artificial Intelligence in cardiovascular medical imaging

Imaging Technique	**Scope**	**Dataset**	**Approach Details**	**Measured Results**
Cardiac MRI	Ventricle Segmentation [29]; Estimating Left Ventricle and Right Ventricle functioning	Cardiac MRI datasets, MICCAI 2012 RV segmentation challenge; 48 patients (16 training, 32 test)	Region of Interest (ROI) extraction using 960 cardiac MRI images with manually labeled RV and LV bounding boxes of size 150 x 150 px. Train CNNs for endocardium and epicardium semantic segmentation of RV on extracted bounding boxes.	Objective: contour overlap of segmented RV with ground truth Criteria: DM, HD, R, ME for EDV, ESV, and EF.
	Heart chamber segmentation [30]; Analyzing abnormality of wall motions	Cardiac MRI datasets, MICCAI 2012 RV segmentation challenge	Heart chamber ROI extraction using a deep learning approach for semantic segmentation. The segmentation approach was initialized with input from a prior edge detection algorithm.	Objective: contour overlap of segmented RV with ground truth Criteria: DM, HD, R, ME for EDV, ESV and EF.
Echocardiography	Regression task: left ventricular volume and ejection fraction computation; Decision making in valvular heart disease, heart failure and post-infarct care [31]	255 usable 2-dimensional echocardiography (2DE) studies	Local Ejection Fraction (EF) estimation. Comparative evaluation of automated EF measurements against manual inspection.	Objective: automated EF estimation Criteria: Intraclass correlation coefficients Bland-Altman analyses
	Regression task: left ventricular volume and Ejection Fraction estimation; [32]	218 patients including 165 with abnormal left ventricular (LV) function. 10,000 apical 4- and 2-chamber view images, from patients with cardiomyopathies, wall motion abnormalities, and dyssynchrony. Cardiac MRI for 21 patients	Identification of the Left Ventricle cavity. Manual tracing of the endocardial border. Left Ventricle volume estimation using modified Simpson's rule for Ejection Fraction computation.	Objective: automated EF estimation Criteria: 2-tailed Student t-test Bland-Altman analysis
Myocardial Perfusion Imaging (MPI) + Coronary angiography	Automatically predicting obstructive disease likelihood from myocardial perfusion imaging (MPI) [33]	1,638 patients without known coronary artery disease, undergoing stress 9mTc-sestamibi or tetrofosmin MPI in 9 different sites Coronary angiography performed within 6 months of MPI	Semantic segmentation of myocardium using a neural network method trained on raw and quantitative polar maps. Input for the network obtained using manual annotation of images obtained from nuclear cardiology software tools.	Objectives: obstructive disease prediction Criteria: area under the receiver-operating characteristic curve (AUC), sensitivity (SS)
SPECT+ computed tomography angiography	Estimating the likelihood of abnormality of coronary arteries in patients with ischemia [34]	1001 patients Coronary angiography within 60 days of myocardial perfusion SPECT for 59 patients with stable coronary artery disease	Semantic segmentation of artery zones with perfusion disorders. Use of segmentation to extract features such as shape, extent, location, count, perfusion homogeneity, regional motion, wall thickening, and sex	Objectives: obstructive disease prediction Criteria: SS, specificity, accuracy, AUC

DM – Dice metric, HD – Hausdorff distance, R – correlation coefficient, ME for EDV – mean errors for end-diastole volumes, ESV – end-systole volumes, EF – ejection fraction, MRI – magnetic resonance imaging

There are three types of angiograms: (i) monoplane angiography, which is the most common method and utilizes the single view principle, (ii) biplane angiography, which uses two C-arm systems for simultaneous acquiring of two different projections, and finally, (iii) rotational angiography, which can give the most accurate three-dimensional scenes by making a complete rotation around the patient.

In [33], the angiography and myocardial perfusion imaging are acquired from the same patient and used as training data on an artificial neural network (ANN) for an automatic prediction of obstructive disease. In [34], the coronary angiography is acquired within 60 days of myocardial perfusion SPECT for patients with stable coronary artery disease from a Japanese hospital. The purpose of ANN was to detect the coronary arteries responsible for the patients' cardiac ischemia. The algorithm accuracy to diagnose the culprit artery was similar to that of a medical expert.

Opportunities for AI applications at the peri-intervention stage are presented in [35] where diverse clinical investigations such as angiography, EKG, biomarkers are reunited for diagnosing STEMI patients. Some applications of machine learning techniques in angiography and other cardiac imaging methods can be found in Table 1.

CONCLUSIONS

The next challenge will be moving from focusing on technique and image analysis to tasks dedicated to physicians in the context of automation of other tasks. This may increase the time efficiency of the medical procedures, job satisfaction, better communication between patient-physician, and improvement of healthcare equality. AI-based solutions will contribute to fighting modern days' challenges, such as the decreasing number of skilled radiologists, leak of medical equipment used, and time-consuming diagnoses.

The final decision after integrating clinical data belongs to cardiologists. The role of artificial intelligence is not to replace humans but to make their work more accurate, fast, and efficient.

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VARIA

A simple approach for risk stratification in the plan of exercise in older adults

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Keywords: *aged; exercise; risk*

Letter to the Editor

The benefits of physical activity (PA) in decreasing morbidity and increasing life span have been proven at all ages, even in later life [1]. In addition, PA not only improves the quality of life but also contributes to maintenance of social and psychological well-being [2].

There are a lot of specific PA programs for adults aged over 65 years, with various recommendations and implementations. However, adherence and persistence in PA are generally not at desired levels in these individuals [3]. While numerous causes can decrease the compliance in PA, a set recommendations specific to person's condition and needs can successfully be used to individualize the exercise plan in older adults. Comorbid conditions and functional impairment can reduce engagement in PA. Therefore, these should be reviewed in the first step of a systematic approach by the physicians, especially in the primary care setting.

In order to improve the efficacy of PA plan offered to an older adult, we intended to test a novel approach in small sample of community dwelling elderly. The proposed tool classifies the subjects in three categories based on a geriatric evaluation that includes review of chronic diseases and 12-month fall history, assessment of cognitive status [assessed by Mini-Mental State Examination (MMSE), range 0-30], and screening for the functional status (assessed by KATZ index, range 0-6) [4, 5].

1. Low-risk individuals: Those with no or a single chronic disease, no cognitive impairment (MMSE score ≥ 28), no functional impairment (KATZ score ≥ 5), and no history of falls in past 12 months.

2. Medium-risk individuals: Those with two or more chronic diseases, mild cognitive impairments (MMSE score ≥ 24 and ≤ 27), mild functional disabilities (e.g., using a cane or walker) (KATZ index score 3 or 4) or positive history of falls without injury in past 12 months.

3. High-risk individuals: Those with two or more chronic diseases, significant cognitive impairment (MMSE score ≤ 23), advanced functional disability (KATZ index score ≤ 2), or positive history of falls with injury in past 12 months.

Table 1 suggests some exercise recommendations for the risk groups [6].

Encouraging older adults to participate in PA is an important issue at the primary care level. There are still problems to

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constitute an activity plan in this population. Therefore, comorbidities and physical activity status of older adults need to be assessed to recommend a suitable exercise plan and achieve reasonable outcomes.

Table 1. Examples of exercises according to the risk groups

Risk groups	Exercise types	Frequency	Intensity
Low	• Aerobic • Strength • Flexibility/Balance	• ≥ 30 min/day and ≥ 5 days/week • ≥ 2 days/week • ≥ 10 min and ≥ 2 days/week	• Moderate to high • Moderate to high • Moderate to high
Medium	• Aerobic • Strength (In selected subjects) • Flexibility/Balance (In selected subjects)	• ≥ 30 min/day and ≥ 5 days/week • 1-2 days/week • 1-2 days/week	• Low to moderate • Low to moderate • Low to moderate
High	• Active, active assistive, and passive (an individualized PA plan should be created)	• 20-30 min/day and ≥ 3 days/week	• Low

PA, physical activity.

Circle or check the boxes of the conditions that apply. "Low Risk" column refers to only individuals in Low Risk; "Medium Risk" column refers to only individuals in Medium Risk; "High Risk" column refers to only individuals in the High Risk (Please see the text).

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ORIGINAL ARTICLES

Assessment of complications and outcomes of mechanical bowel obstruction in a military hospital

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Abstract: *Aims: Mechanical bowel obstruction is a frequent surgical emergency and a frequently confronted burden in abdominal operation. This study aimed to find out the model of mechanical bowel obstruction in the Iranian population.*

Methods and materials: In this study, all patients with clinical and radiological evidence of mechanical bowel obstruction that admitted to the Department of Surgery of Baqiyatallah Hospital with a diagnosis of mechanical bowel obstruction from January 2012 to December 2015 were included. The age, gender, symptoms operative details, postoperative complications, outcome, and mortality, were recorded.

Results: 193 patients with mechanical bowel obstruction were admitted in our study. Regarding the clinical presentation of the patients, the absence of passage of flatus and/or feces with abdominal pain (65.8%) were the most common presenting symptoms. Adhesions, colonic volvulus, hernias, different types of colorectal cancer were the most frequent causes of obstruction (54.9%, 15.54%, 7.2%, and 9.8%, respectively). Twenty-one patients died, resulting in a mortality rate of 10.9%. In the 19 patients with colorectal cancer, 7 (31.6%) patients have died and cause of death in all them related to cancer disease.

Conclusion: The results showed that the most common mechanisms of intestinal obstruction were adhesions, volvulus, cancers, and hernias, respectively, and the most common risk factors were appendectomy history, herniation surgery history, and cholecystectomy history, respectively. About 21 patients (10.8%) underwent obstruction surgery with a diagnosis of malignancy, which was confirmed in 19 cases of tumor pathology, of which about one third had recurrence within 3 years.

Keywords: *mechanical bowel obstruction, adhesions, colorectal cancer*

INTRODUCTION

Mechanical bowel obstruction is a frequent surgical emergency and a frequently confronted burden in abdominal operation [1-3]. Intestinal obstruction relates to extremely severe circumstances, demanding a fast and accurate diagnosis as well as critical, rational, and efficient treatment [4].

The diagnosis of mechanical bowel obstruction is not always simple, and an implication of operation requires a vast indication of suspicion [5, 6]. Comprehensive past and precise clinical exams are important to obtain a practical diagnosis and preparation therapy [7]. There are several cardinal characteristics, i.e. colic, distension, vomiting, and constipation, but the influence of each of these is affected by the position and kind of obstruction [5-9]. The administration needs early diagnosis and therapy with meticulous fluid, electrolyte balance, and timely operational intervention [10]. The complications can be decreased by

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early diagnosis and immediate surgical intervention [11].

Emergency surgery for malignant bowel obstruction is correlated with a mortality incidence of up to 30 percent [12]. It also results in considerable morbidity with prolonged hospital stay, delay at the beginning of chemotherapy and many stoma organizations, with a disorder of quality of life [12-14].

Also, malignant bowel obstruction has a major affected on health costs given that these cases are hospitalized and may need multiple mediations [13]. Medical administration has lower short-term mortality but may be correlated with low resolve or recurrence of the obstruction [14]. Because of the enormous problem of MBO in cases with cancer and the meaningful discrepancies in the morbidity of surgical versus medical treatments, it is necessary to characterize the predictors of survival and the impact of therapy modality on case results [15]. However, MBO may be due to the primary tumor, metastatic disease, or past surgical intervention leading to adhesions or an internal hernia [16, 17]. Albeit, many studies showed that MBO in the setting of colon cancer, the actual incidence, and the results remain unknown [9, 12-16].

In a study showed that 10-50% of patients with advanced cancer stage would develop MBO during their illness and experience from intractable abdominal pain, vomiting, which result in a decreased quality of life, mental and emotional problems [18]. Various therapies, including operation, palliative radiotherapy, chemotherapy and total parenteral nutrition formulas, may be used to reduce the symptoms in MBO patients; however, there is currently no agreement about the optimal treatment approach and no substantial evidence supporting the effectiveness of any treatment in enhancing the quality of life and prolonging survival [16-18]. Varying the pattern of the disease from time to time needs periodic studies to assess the etiological factors and behavior of the disease [12-18]. Also, as well as regional differences in the pattern of bowel obstruction and changes in the illness pattern over the years, are well documented in the previous studies [18]. This study aimed to find out the model of mechanical bowel obstruction in the Iranian population.

METHODS AND MATERIALS

In this study, all patients with clinical and radiological evidence of mechanical bowel obstruction that admitted to the Department of Surgery of Baqiyatallah Hospital with a diagnosis of mechanical bowel obstruction from January 2012 to December 2015 were included. The enrollment of the patients in the study was approved by the ethics committee of the Baqiyatallah University of Medical Sciences and written informed consent was obtained from all patients. Patients with paralytic ileus were excluded from our study.

The age, gender, symptoms operative aspects, post-operative complications, result, and death were recorded. Following clinical investigations, every six hours with the same attending surgical unit were conducted in all cases to assess the patients' symptoms. All cases had been operated on by the authors in the general surgery departments of the Baqiyatallah hospitals.

The diagnosis was based on clinical judgments, history, and supported by ultrasonography and radiological studies (plain x-rays) in all cases. The final diagnosis was presented at exploratory laparotomy, which was endeavored following thorough first evaluation, examinations, and resuscitation. The operation was determined as required in those patients where the operation was conducted in thirty-six hours.

Chi-square test and Fisher's exact test was utilized for the relationship between qualitative variables. The non-parametric Mann-Whitney test made a comparison among the groups. The Kolmogorov–Smirnov test was used for the normality of the data. The data was used by SPSS 20 software. The significance level for the tests was considered less than 0.05.

RESULTS

193 consecutive patients with mechanical bowel obstruction were included. The average age was 58.1 ± 19.0 years while women comprised 32.64% of the group. The most of the patients (60.1%) presented in above 59 years old.

Regarding the clinical presentation of the patients, the absence of passage of flatus and/or feces with abdominal pain (65.8%) were the most common presenting symptoms, only abdominal pain (27.5%) was the second frequent physical finding on clinical examination abdominal.

Regarding the types of previous operations, 35 patients (23.33%) had undergone an appendectomy, 27 (18.0%) hernia procedures, 24 (16.0%) had a cholecystectomy, and 86 (43.3%) had other surgical procedures.

Adhesions, colonic volvulus, hernias, different types of colorectal cancer were the most frequent causes of obstruction (54.9%, 15.54%, 7.2%, and 9.8%, respectively). There was no significant difference in the cause of obstruction regarding gender and age levels. An evaluation of 193 cases of mechanical bowel obstruction from the standpoint of etiology, age, and sex distribution, is presented.

Types of colorectal cancer were overrepresented accounting for 8 (42.1%) with obstruction due to sigmoid cancer, whereas six (31.6%) patients had a colon cancer, 3 (15.8%) had a cecum cancer, and one (5.2%) had a rectum cancer.

Regarding the clinical presentation of the patients and cause of obstruction, Adhesions and abdominal pain (56.08%) with were the most common presenting symptoms and cause of obstruction. The absence of passage of flatus and/or feces and Adhesions (33.9%) were the second frequent presenting symptoms and cause of obstruction.

There was a significant association between the cause of obstruction and history of surgery (P<0.001).

109 patients (56.4%) of the total study group were safely and more efficiently treated.

Eighty-four cases of the 193 patients sustained complications; 28 (33.3%) suffered from recurrence adhesion along with obstruction, 15 (17.9%) had abdominal pain, 15 (17.9%) had constipation. In the 19 patients with colorectal cancer, six (31.6%) patients had a recurrence.

Twenty-one patients expired, following in a death rate of 10.9%. In the 19 patients with colorectal cancer, 7 (31.6%) patients have died and cause of death in all them related to cancer disease.

DISCUSSIONS

In the past, the most common cause of intestinal obstruction was a strangulated hernia, but today, due to the higher prevalence of surgery than in the past, the most common cause of intestinal obstruction (75-80% of cases) is adhesions due to previous surgeries [7, 9, 11, 12]. These patients present with clinical signs of obstruction including colic abdominal pain, abdominal distension, and nausea and vomiting, and constipation. The complete obstruction causes intestinal dilation with an accumulation of water and gas. Radiological signs are visible 3-5 hours before obstruction [11-13]. In the present study, the most common postoperative diagnoses were adhesions in 106 patients (54.9%), volvulus in 30 cases (15.5%), colorectal cancers in 19 patients (9.8%) and hernias in 14 patients (7.2%), respectively. While in the study of Saber, the diagnosis of the cause of obstruction was observed in most cases with sigmoid volvulus and inguinal hernia, each with 18% and the site of obstruction in the small intestine with 55%. The most common symptoms in the present study were abdominal pain, which was seen in more than 95% of cases, and the second most common symptom was lack of gas or stool, which was seen in more than 65% of cases. In the study of Markogiannakis et al., the most common symptoms were flatulence (90%) followed by bloating and abdominal pain (65.3%). Adhesion was observed in 64.8% of cases, incarcerated hernia in 14.8%, and colon cancer in 13.4% [3].

Füzün et al. (2005), which looked at the causes of mechanical intestinal obstruction, found that intestinal adhesions were the most common cause of obstruction with 44%, followed by hernias with 23.9% and volvulus with 12.7%. Colon carcinoma was 10.1% of the causes of intestinal obstruction [9]. Our study is consistent with the above study in terms of 4 common factors and only slightly different in order after the most common cause, which is adhesion in both studies. The prevalence of colorectal cancers is very close in the two studies.

Regarding the clinical presentation of our cases, the lack of passage of feces or/and flatus with abdominal pain were the many common presenting symptoms and abdominal pain was the second most prevalent physical conclusion on clinical research [12-14]. The results, even though some variations are marked, are in agreement with the studies. Especially, Cheadle et al. showed abdominal vomiting 82%, pain 92%, distention 59%, and abdominal tenderness 64% as the most frequent signs [19]. While abdominal distension, bilious vomiting, absolute constipation, and abdominal pain were the main signs and symptoms in another study. In research on 100 patients with adhesive small bowel obstruction and recognized that the most common symptoms were vomiting 77%, colicky abdominal pain 68%, and absence of passage of flatus and/or feces 52% while abdominal distension developed the most common clinical symptom with a rate of 56% [20]. In a study of patients with bowel obstruction due to large bowel volvulus, the most common symptom of sigmoid volvulus was distension 79% and the most common symptoms were pain 58% and obstipation 55%, while most patients with cecal volvulus presented with pain 89% [21]. Moreover, in a review study of patients with obstruction because of small and large bowel intussusception, abdominal pain, nausea, vomiting, and abdominal distension were the frequent symptoms and signs, respectively [22].

Adhesions, colonic volvulus, hernias, different types of colorectal cancer were the most common causes of obstruction [23]. These results were also mentioned in the current research.

Furthermore, adhesions were the most common etiology of obstruction in and the total study group. Several studies propose that adhesions are responsible for 32%-74% of bowel obstruction and are the leading cause of small intestinal obstruction representing 45%-80% of it [20-23]. The large majority of the cases with adhesive obstruction have experienced previous abdominal procedures [23]. In the present study, this was agreement with previous studies.

The model of mechanical intestinal obstruction shows a difference in various areas. The obstructed herniae were observed as the 3rd common condition in the current research, whereas it was the second common condition of the other research, which almost is comparable with the other investigations carried in the country [14-15]. As for the types of surgeries in our cases, appendectomies, and hernia surgeries, cholecystectomies were more prevalent. This results also in agreement with the literature [9, 16].

However, the proper administration of adhesive obstruction is still uncertain, a large portion of these cases, varying from 35% to 65% in some investigations, can reliably and efficiently be managed with operation administration as it was also noted in our cases [23, 24].

The growing performance of adhesions as a condition of acute intestinal obstruction requires a higher requirement for regular precautionary standards toward adhesion formation [25]. Some effective actions are now supported through elective abdominal surgery to reduce the increase of adhesions that might consequently produce intestinal obstruction [26].

In previous studies showed sigmoid cancer is the etiology of obstruction that cases with high intestinal obstruction with a percentage of 40%-90% [27]. A study by Baloch et al., of 252 patients with mechanical bowel obstruction, showed due to the regional epidemic, the most common cause of obstruction was abdominal tuberculosis, which was observed in 30% of cases, and unlike our study and most other studies, adhesion was the second most common cause of obstruction. Also, in this study, the hernia was observed in only 17.5% of patients [15].

In our research, complication and mortality percentage were almost small. In the previous studies, complication proportion varies from 6 to 47 percent while death ranges from 2 to 19 percent [25, 28]. In our study, there was the association between mortality rate and colorectal cancer disease.

CONCLUSION

The results showed that the most common mechanisms of intestinal obstruction were adhesions, volvulus, cancers, and hernias, respectively, and the most common risk factors were appendectomy history, herniation surgery history, and cholecystectomy history, respectively. About 21 patients (10.8%) underwent obstruction surgery with a diagnosis of malignancy, which was confirmed in 19 cases of tumor pathology, of which about one third had recurrence within 3 years.

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ORIGINAL ARTICLES

Assessment of patient satisfaction with primary health care in a military setting: A cross-sectional study from Turkey

Alper Tunga Kokcu

Abstract: *Background and Aim: Information on patients' experiences with and evaluations of health care is considered an essential outcome indicator to assess the quality of health care. This study aims to evaluate patient satisfaction with primary health care in a military setting.*

Methods: This cross-sectional study was conducted at the primary health care center located in a military setting, in Ankara, Turkey. A total of 3080 military personnel who received primary care in the last 12 months formed the population of the study. Turkish version of the European task force on patient evaluations of general practice care (EUROPEP) questionnaire was administered to the participants.

Results: In the study, 2677 participants were reached with a response rate of 86.9%. The mean satisfaction rate (MSR) of clinical behaviour (69.3%) was higher than the MSR of the organization of care (58.4%). The MSR of the overall questionnaire was 67.1%. Significant differences were observed in the mean satisfaction scores of patients according to their descriptive characteristics including gender, age level, troop type, and the doctor providing the care.

Conclusions: This study highlights the low patient satisfaction levels, which may have resulted from the fact that the study population was young and relatively healthy, and that there was no possibility of choosing the general practitioner. The EUROPEP questionnaire, which is an internationally validated and standardized instrument, can also be used to improve primary health care in military settings.

Keywords: *doctor-patient relationship, military medicine, patient satisfaction, primary care, quality of care*

INTRODUCTION

The World Health Organisation's definition of quality of care is "the extent to which health care services provided to individuals and patient populations improve desired health outcomes. To achieve this, health care must be safe, effective, timely, efficient, equitable, and people-centered." [1]. There are various parameters or indicators to judge the quality of health care. The measurement of patient satisfaction is considered as an essential outcome indicator to evaluate health care quality. Questionnaires are the most widely used instruments to assess patient satisfaction [2,3]. Although there is a controversy about measuring patient satisfaction or patient experience of the quality of care, many authors hold that satisfaction is linked to patient expectations of ideal care and their real experience of care [4].

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In 1997, it was stated by the Council of Europe that the continuous improvement of quality of care is a key priority for all member states. According to this decision, information on the needs, priorities, and experiences of patients at all levels of care provision had to be gathered through appropriate methods ensuring the active participation of patients [5]. A valid and easy-to-use instrument to gather information on patients' experiences with and evaluations of general practice care was developed by the European working party on quality in general practice in 1999. The European task force on patient evaluations of general practice care (EUROPEP) instrument was designed to assess the quality of general practice and to provide relevant feedback to general practitioners, patients, and health care policymakers at different levels [6]. The EUROPEP questionnaire, which is an internationally validated and standardized instrument for patients' evaluations on general practice care, has been used in many European countries, including Turkey.

The objective of this study was to evaluate patient satisfaction with primary health care in a military setting by using the EUROPEP questionnaire. The impact of the differences in patient characteristics on their satisfaction was also investigated. Besides, comparisons were made between this study and other studies, especially those conducted with the EUROPEP questionnaire.

METHODS

This cross-sectional study was conducted at the primary health care center (PHCC) located in Gendarmerie and Coast Guard Academy, in Ankara, Turkey. A total of 3080 military personnel who received care from the PHCC in the last 12 months formed the population of the study. Since the whole population was included in the study, a sample was not chosen. The data were collected between August and October 2018.

Using a five-point answering scale with the extremes labeled as 'poorest' and 'excellent,' the EUROPEP questionnaire is a multidimensional instrument comprising 23 questions on evaluations by patients of specific aspects of general practice care. The questionnaire has two dimensions: clinical behavior (items 1-16), and organization of care (items 17-23). Through this questionnaire, patients are asked to rate the quality of care that they have received in the last 12 months from the general practitioner that they usually visit [6]. In 2002, Akturk et al adopted the English version of EUROPEP questionnaire into Turkish and demonstrated that the Turkish version of EUROPEP questionnaire is a valid and reliable instrument that can be used for the Turkish population [7]. Three items of organization of care (making an appointment and contacting PHCC or doctor by phone) not applicable in this study were removed from the EUROPEP questionnaire. In the study, the remaining 20 items of the Turkish EUROPEP questionnaire (items 1-16: clinical behavior; items 17-20: organization of care) were administered to the participants. Descriptive characteristics of the participants were also obtained through the questionnaire.

Participants who scored the two most positive answering categories (good and excellent) were considered as satisfied patients. The rate of satisfied patients was used for the description of patients' views for each item. For the overall questionnaire and each dimension, the mean satisfaction rate (MSR) was used to assess patient satisfaction. Additionally, each item was scored on a five-point answering scale, ranging from one (poorest) to five (excellent). The mean satisfaction score (MSS) and the standard deviation of mean were used for each item. For the overall questionnaire and each dimension, MSS was used to assess patient satisfaction according to descriptive characteristics.

The internal consistency was assessed by using Cronbach's alpha. The data analysis was performed using the SPSS 22.0 software package. As the dataset was neither distributed normally nor was homogeneous, non-parametric statistical tests were used in the analysis. Mann-Whitney U test and Kruskal-Wallis test were used to determine whether there was any difference in the answers of patients according to their descriptive characteristics. If the Kruskal–Wallis test was found to be statistically significant, Dunn-Bonferroni test was performed for pairwise comparisons. A value of $p<0.05$ was accepted as statistically significant. The descriptive tables including numbers, rates, and means are presented and the results are discussed at the end of the paper.

The necessary administrative permission was obtained from the command of the military setting before the study. The ethical approval for this study was provided by the relevant University Human Research Ethics Committee on 4 July 2018 (No:3304). The completion of the questionnaire was voluntary and anonymous. Consent forms were obtained from the participants before the administration of the questionnaire.

RESULTS

In the study, 2677 participants were reached with a response rate of 86.9%. In subgroups according to the descriptive characteristics of the participants, response rates ranged from 81.2% to 92.3%. All of the participants were military personnel of four different troops and received care from

PHCC at least one time over the last 12 months. General practice care was given by two doctors working in PHCC. Most of the patients were unmarried (n=2251; 84.1%) and male (n=2472; 92.3%). The mean age of patients was 24.8±2.5 and ranged from 18 to 40 years. The descriptive characteristics of the patients are summarized in Table 1.

In the study, all items of the questionnaire were aggregated into two dimensions: clinical behavior (items 1-16) and organization of care (items 17-20). The Cronbach's alpha ranged between 0.975 for "clinical behavior," and 0.883 for "organization of care." For the overall 20 items of the Turkish EUROPEP questionnaire, the Cronbach's alpha was 0.974.

Table 1: Descriptive characteristics of the patients (n=2677)

Characteristics	n	(%)
Gender		
Male	2472	(92.3)
Female	205	(7.7)
Marital status		
Unmarried	2251	(84.1)
Married	426	(15.9)
Age level		
18-20	158	(5.9)
21-25	1408	(52.6)
>25	1111	(41.5)
Troop type*		
A	1997	(74.6)
B	347	(13.0)
C	176	(6.6)
D	157	(5.8)
Visit number		
1-2	1663	(62.1)
3-4	637	(23.8)
>4	377	(14.1)
Dr. providing the care*		
Dr-A	1436	(53.6)
Dr-B	1241	(46.4)

**Names of the troops and doctors were encoded because of confidentiality*

For each item, the MSS and the rate of patients who scored the two most positive answering categories (good and excellent) are presented in Table 2. The highest satisfaction rate and MSS were observed for "keeping your records and data confidential" (81.2%; 4.15±0.86), "thoroughness" (75.9%; 4.06±0.97), and "listening to you" (75.9%; 4.04±0.96). The lowest satisfaction rate and MSS were found for "waiting time in the waiting room" (47.2%; 3.30±1.25), and "the helpfulness of the staff (other than the doctor)" (56.5%; 3.56±1.16). MSR of clinical behavior (69.3%) was higher than MSR of organization of care (58.4%). The MSR of the overall questionnaire was 67.1%. While the MSS of clinical behavior was 3.89±0.85, the MSS of organization of care was 3.60±1.00. For the overall general practice, the MSS was 3.84±0.84.

It was observed that there were significant differences in the MSSs of patients according to their descriptive characteristics including gender, age level, troop type, and the doctor providing the care. The MSS of female patients was lower than the MSS of male patients ($p<0.05$). The MSS of patients in the 18-20 age group was higher than those in the other age groups ($p<0.05$).

Regarding the overall questionnaire and each dimension, the patients from troop-A had lower MSS, compared to the patients from troop-C and troop-D ($p<0.05$). Regarding the dimension of clinical behaviour, the patients from troop-A also had lower MSS, compared to the patients from troop-B ($p<0.05$). Patients consulting Dr-A reported more satisfaction regarding the organization of care and overall general practice than the patients consulting Dr-B ($p<0.05$). However, there were no differences among other descriptive characteristics of patients such as marital status and the number of visits. The detailed results of comparisons of MSSs concerning the descriptive characteristics are given in Table 3 and Table 4.

DISCUSSION

It can be said that patient satisfaction is related to the physicians' adherence to the four principles of biomedical ethics including autonomy, beneficence, non-maleficence, and justice. Physicians' adherence to the principles of biomedical ethics, especially of autonomy, can be evaluated by using the instruments for assessing patient satisfaction. Most of the time, patients are not aware of the quality of health care delivered to them. Therefore, valid and reliable instruments are used to evaluate patients' experiences of health care.

In this study, the EUROPEP questionnaire was used for the assessment of patient satisfaction with primary health care in a military setting for the first time. Having this study conducted with the EUROPEP instrument allows us to make direct comparisons between the results of this study and the results of previous studies conducted with the same instrument. Similar to other studies, the current study revealed very high values for Cronbach's alpha for the EUROPEP questionnaire and its two dimensions [8-10]. For the overall questionnaire and each item, this study reported low patient satisfaction levels. Significant differences were observed in the MSSs of patients according to their descriptive characteristics including gender, age level, troop type, and the doctor providing the care. On the other hand, no differences were determined among other descriptive characteristics of patients, such as marital status and the number of visits.

Consistent with the previous EUROPEP studies, the results of this study showed that the patients were more satisfied with "clinical behavior" compared to the "organization of care." [8,9,11-14]. Similar to other studies, the highest and lowest satisfaction rates were for the items ''keeping your records and data confidential'' (81.2%), and "waiting time in the waiting room" (47.2%) [8-10,12-14].

Table 2: Descriptive statistics for 20 items of the questionnaire (n=2677)

Questions	MSS (Mean±SD)*	Good or excellent response [n (%)]**	
Clinical behaviour			
1. Making you feel you had time during consultations	3.80±1.02	1768	(66.0)
2. Interest in your personal situation	3.85±0.99	1802	(67.3)
3. Making it easy for you to tell him or her about your problems	3.97±0.95	1953	(73.0)
4. Involving you in decisions about your medical care	3.82±0.99	1763	(65.9)
5. Listening to you	4.04±0.96	2032	(75.9)
6. Keeping your records and data confidential	4.15±0.86	2173	(81.2)
7. Quick relief of your symptoms	3.85±1.00	1809	(67.6)
8. Helping you to feel well so that you can perform your normal daily activities	3.89±0.97	1867	(69.7)
9. Thoroughness	4.06±0.97	2033	(75.9)
10. Physical examination of you	4.00±0.99	1942	(72.5)
11. Offering your services for preventing diseases	3.89±0.98	1823	(68.1)
12. Explaining the purpose of tests and treatments	3.80±1.05	1744	(65.1)
13. Telling you what you wanted to know about your symptoms and/or illness	3.87±1.01	1833	(68.5)
14. Helping you deal with emotional problems related to your health status	3.72±1.05	1646	(61.5)
15. Helping you understand the importance of following his or her advice	3.86±0.99	1810	(67.6)
16. Knowing what he or she had done or told you during contacts	3.75±1.06	1688	(63.1)
MSS and MSR (items 1-16)	3.89±0.85		(69.3)
Organization of care			
17. Preparing you for what to expect from a specialist or hospital care	3.83±1.05	1784	(66.6)
18. The helpfulness of the staff (other than the doctor)	3.56±1.16	1512	(56.5)
19. Waiting time in the waiting room	3.30±1.25	1264	(47.2)
20. Providing quick services for urgent health problems	3.71±1.17	1690	(63.1)
MSS and MSR (items 17-20)	3.60±1.00		(58.4)

MSS: Mean satisfaction score; MSR: Mean satisfaction rate; SD: Standard deviation.

* *Items were scored on a five-point answering scale, ranging from 1 (poorest) to 5 (excellent).*

** *Participants who scored the two most positive answering categories (good and excellent) were considered as satisfied patients.*

By using the EUROPEP instrument, Petek et al. conducted a study in eight European countries in 2009. The study aimed to describe evaluations of general practice care by patients with a chronic illness. The MSRs ranged from 82.1% to 93.0% in the European countries [15]. It may be questionable that the study was conducted with a single group of chronic care patients. While a study reported that better health status was associated with a more positive evaluation of care, another study showed that patients reporting a chronic condition were more positive in their assessment of the general practice than patients without a chronic condition [16,17]. However, conducted by Rosemann et al., a previous study utilizing the EUROPEP instrument revealed that patient satisfaction was not determined by the disease-specific quality of life, at least not in osteoarthritis patients [18]. The Turkish Ministry of Health conducted a study in 2010 that surveyed 34472 patients across Turkey. The study aimed to measure patient satisfaction with PHCCs. The MSR was found to be 77.8% with the EUROPEP instrument [19]. The MSR of the current study (67.1%) is lower than that of the previous studies conducted in European countries and Turkey with similar methodology.

In contrast to some other studies, this study found that female patients' satisfaction levels were lower than those of the males [12, 16, 20]. On the other hand, the gender results of this study were consistent with the previous studies conducted in the military settings that found male patients to be more satisfied than female patients [21, 22].

Similar to the study conducted by Petek et al., no difference was found according to marital status in this study [15]. On the other hand, Baltaci et al. observed that the patient satisfaction level was higher for married patients than it was for the unmarried [20].

Table 3: Comparisons of mean satisfaction scores concerning the descriptive characteristics (n=2677)

Characteristics	n	(%)	MSSs (Mean±SD)** and p-values***					
			Clinical behaviour	p	Organization of care	p	Overall general practice	p
Gender								
Male	2472	(92.3)	3.91±0.84	0.000[a]	3.60±1.00	0.022[a]	3.85±0.84	0.000[a]
Female	205	(7.7)	3.68±0.87		3.49±0.83		3.64±0.83	
Marital status								
Unmarried	2251	(84.1)	3.89±0.84	0.679	3.60±0.99	0.308	3.83±0.84	0.897
Married	426	(15.9)	3.90±0.84		3.55±0.99		3.83±0.83	
Age level								
18-20	158	(5.9)	4.09±0.83	0.003[a]	3.81±0.99	0.008[a]	4.03±0.83	0.004[a]
21-25	1408	(52.6)	3.89±0.83		3.60±0.99		3.83±0.83	
>25	1111	(41.5)	3.85±0.85		3.56±0.99		3.80±0.84	
Troop type*								
A	1997	(74.6)	3.84±0.86	0.000[a]	3.55±1.00	0.000[a]	3.78±0.85	0.000[a]
B	347	(13.0)	3.98±0.79		3.66±0.97		3.91±0.79	
C	176	(6.6)	4.09±0.68		3.80±0.89		4.04±0.67	
D	157	(5.8)	4.07±0.85		3.77±1.00		4.01±0.84	
Visit number								
1-2	1663	(62.1)	3.89±0.79	0.149	3.63±0.95	0.254	3.84±0.79	0.441
3-4	637	(23.8)	3.87±0.89		3.53±1.05		3.80±0.88	
>4	377	(14.1)	3.92±0.96		3.55±1.07		3.84±0.94	
Dr. providing the care*								
Dr-A	1436	(53.6)	3.92±0.83	0.052	3.64±0.99	0.004[a]	3.87±0.83	0.035[a]
Dr-B	1241	(46.4)	3.85±0.86		3.54±0.99		3.79±0.85	

MSS: Mean satisfaction score; SD: Standard deviation.

**Names of the troops and doctors were encoded because of confidentiality.*

***Items were scored on a five-point answering scale, ranging from 1 (poorest) to 5 (excellent).*

****Mann-Whitney U test and Kruskal-Wallis test were performed ([a] p<0.05).*

In contrast to other studies, this study found that younger age was associated with higher levels of patient satisfaction. [15, 16]. However, it is worth bearing in mind that the participants in this study were aged 40 or under.

A previous study conducted in a military setting revealed that waiting times were significantly correlated to overall patient satisfaction [23]. The current study found a difference in the satisfaction levels according to the troops the patients belonged to. In the military setting where the current study was conducted, according to the rules, the patient should visit PHCC with the other patients of his/her troop at the same time in the morning, except for emergencies. As the number of patients in the troop increases, the waiting time in the PHCC extends, which may be the underlying reason for the low patient satisfaction level in crowded troops like troop-A.

The current study couldn't confirm the results of previous studies that there is a positive association between the number of visits and the level of patient satisfaction [15, 16]. A majority of the participants of this study (85.9%) had a low number of visits (≤4), and the mean number of visits was 2.6. In Turkey, the total number of visits per person to a general practitioner in 2017 was 2.9 [24].

In this study, it was observed that there was a difference in the satisfaction levels depending on the doctor providing the care. While Dr-A is a military general practitioner, Dr-B is a civilian general practitioner, which may be one of the reasons for the low satisfaction levels of patients evaluating Dr-B.

A strength of this study is that it was conducted with a large population sample. On the other hand, there are relatively several limitations to the study. Because the study was conducted in a military setting, participants were younger than 40 and most of the visits to PHCC were due to acute health disorders. Due to the limited number of general practitioners working in PHCC where the study was conducted, the participants were able to evaluate two doctors in terms of general practice care.

Table 4: Pairwise comparisons of multiple groups whose comparisons were found to be statistically significant

Pairwise groups	p-values**		
	Clinical behavior	Organization of care	Overall general practice
Age level			
18-20 and 21-25	0.009[a]	0.019[a]	0.010[a]
18-20 and >25	0.002[a]	0.005[a]	0.003[a]
21-25 and >25	1.000	1.000	1.000
Troop type*			
A and B	0.034[a]	0.413	0.062
A and C	0.004[a]	0.010[a]	0.005[a]
A and D	0.003[a]	0.035[a]	0.005[a]
B and C	1.000	0.777	1.000
B and D	1.000	1.000	1.000
C and D	1.000	1.000	1.000

**Names of the troops were encoded because of confidentiality.*

***Dunn-Bonferroni posthoc test was performed ([a] $p<0.05$).*

This study highlights the low patient satisfaction levels, which may have resulted from the fact that the study population was young and relatively healthy, and that there was no possibility of choosing the general practitioner and PHCC. However, according to the results obtained through the questionnaire, taking necessary actions to promote patient satisfaction will improve the quality of primary health care in this military setting.

The EUROPEP questionnaire, which is an internationally validated and standardized instrument for patients' evaluations on general practice care, provides feedback to general practitioners and policymakers. The results of a study conducted with this instrument can be directly compared with other studies conducted with the same instrument. These comparisons can shed light on the similarities and differences between various PHCCs. This instrument can also be used to improve primary health care in military settings.

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Patient consent: Consent forms were obtained from the participants before the administration of the questionnaire.

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ORIGINAL ARTICLES

Assessment of traumatic injuries in 1028 patients referred to the Ayatollah Taleghani Hospital from the Sarpul-Zahab earthquake

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Abstract: *Introduction: Investigating and recognizing the epidemiological structure of earthquake damages in earthquake-prone countries is of great importance. The aim of this study was to evaluation of traumatic injuries in1028 patients referred to the Ayatollah Taleghani Hospital from the Sarpul-Zahab earthquake (November 12, 2017).*

Method: This study is a cross-sectional study that includes 1028 traumatic patients from the Kermanshah earthquake. The patients, including outpatients in the emergency department, admitted in orthopedic, general surgery and neurosurgery, or even patients who needed to be transferred to other treatment centers.

Result: Of the 1,028 patients, 488 were men (47.5%). The number of men with high injury severity was 51 (10.4%) and the number of women with high severity of the injury was 58 (10.7%). The average age of the injured was 33.04±15.01 years. The mean ISS measure in this study was 8.9±1.6. A total of 109 (10.6%) had scores above 17. The number of cases with head and neck injury was 328 (24%), 233 cases (17%), chest 114 (8.3%), The abdomen or pelvis is 190 (13.9%), the spine is 200 patients (14.6%). After examining the type of trauma in 1791 injuries, 1074 injuries (59.9%) had penetrating trauma and 717 injuries (40.1%) of blunt trauma were calculated. Of patients with severe trauma, 83 patients suffered severe injuries (7.7%) and in patients with blunt trauma, 68 patients suffered severe injuries (9.4%). The mortality rate was 5 (0.5%).

Conclusion: This study established a fracture pattern in the referred patient of the Sarpul-Zahab earthquake to the Ayatollah Taleghani Hospital and is practical for clinicians to increase understanding of this natural disaster and permits superior selection of the triage management and therapeutic process.

Keywords: *earthquake, disaster, trauma, Kermanshah*

INTRODUCTION

Trauma is the most common cause of morbidity and mortality in the first third of life in human societies [1-3]. In recent years, trauma has always been one of the three main causes of injury and general mortality [4]. The distribution of incidence of natural disasters is different in the various regions of the world. There are more than a few potent geographical regions for trauma experiences, such as earthquakes, organized violence, terrorism, combats, and other natural disasters [5-6]. In the past few decades, Iran has experienced more than a few earthquakes, which caused severe and massive damages. Therefore, investigating and recognizing the epidemiological structure of earthquake damages in earthquake-prone countries such as Iran is of great importance. On November 12, 2017, an earthquake

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take place in Sarpul-Zahab and Sallatha Babajani cities, Kermanshah Province, Iran, with a 6.9-magnitude on the Richter scale. The urban and population density areas were exposed due to this earthquake for significant severe and massive damages. The fracture pattern in injured patients due to earthquakes is different in the various regions of the world, for example, in the earthquake of Armenia, the head and neck injury rate was 22% [7]. In the Wenchuan earthquake, the highest number of injuries has been seen in those who suffered damage to the external surface of the body [8-9]. Generally, the fracture pattern of an earthquake can be including simple morbidity, to even mortality. Therefore, characterization of the mechanism and epidemiologic variables of these traumatic events is necessary for the management of proper initial triage [10].

The aim of this study was to evaluation of traumatic injuries in1028 patients referred to the Ayatollah Taleghani Hospital from the Sarpul-Zahab earthquake (November 12, 2017) regarding different levels of demographic variables (age and gender), pathology of the disease (anatomical location of the injury, type of trauma, triage level, etc.) and the stages of treatment (how the patient transmits, operating care, etc).

METHODS

This study is a cross-sectional study that includes traumatic patients from the earthquake in Kermanshah province on November 12, 2017, referred to the Ayatollah Taleghani Hospital of Kermanshah as the largest treatment center in the west of Iran. The study population included 1028 patients, including outpatients in the emergency department, admitted in orthopedic, general surgery, and neurosurgery, or even patients who needed to be transferred to other treatment centers.

Data analysis including frequency varying levels, the percentage of damage to the severity of the trauma for variables such as age, sex, type of trauma, and injury as well as the correlation between trauma severity and anatomical location of some of the variables. After the initial studies, the patients' medical records were filled in for two months.

The severity of trauma by ISS method according to the AIS criteria for traumatic patients with a proper pre-study was carried out. To calculate the patient ISS, the body is divided into six parts as follows:

Head and neck
Face
Chest
Abdomen or pelvis
Spinal cord
Limbs

To calculate the ISS, the highest severity of AIS is considered in the three areas of the body that have seen the most damage, then we squeeze three AIS scores together to calculate the severity of the ISS damage. AIS scoring is based on the amount of injury and the anatomical location of the damage between 1 and 6, which is 1 indicates minor damage and 6 is the number of fatal injuries. Hence, the severity of the ISS damage is between 1 and 75, since by eliminating deadly injuries with a score of 6, the sum of the squares of other AIS levels is 75 (25 + 25 + 25 = 75), 75 (25 + 25 + 25), the injuries that the IIS is 6, 75 are considered [6, 13, 14]. Registered demographic data allow the proportion of male and female patients or the age range of patients to be identified in terms of the severity of the injury. The structure of the damage is also known by its characteristics, such as trauma, triage, and anatomical location. The protocol of the study was approved by the ethical committee of Baqiyatallah University of medical sciences.

Data analysis was performed using SPSS-20 software. Descriptive statistics such as frequency, percent, mean and standard deviation were presented.

RESULTS

1028 patients transferred to Ayatollah Taleghani hospital were included in the study. Table 1 depicts patients' characteristics regarding transition status, sex, age levels, Anatomic location of the injury, Triage level, Type of trauma, Duration of admission, ISS, and outcomes (Table 1). Of the 1,028 patients, 488 were men (47.5%). The number of men with high injury severity was 51 (10.4%) and the number of women with high severity of the injury was 58 (10.7%). The ratio of women with high severity of the injury was 5.6% for all patients and 4.9% for men. The average age of the injured was 33.04±15.01, range from 1 to 95 years.

Table 1: Patients' characteristics regarding transition status, sex, age levels, Anatomic location of the injury, Triage level, Type of trauma, Duration of admission, ISS, and outcomes

Variable	N	%
Transition status		
Emergency	354	34.4
Personal car	420	40.9
Other ways	254	24.7
Sex		
Male	488	47.5
female	540	52.5
Age		
0-4	56	5.4
5-14	59	5.7
15-24	136	13.2
25-44	410	39.9
45-64	242	23.5
>65	125	12.3

Variable	N	%
Anatomic location of the injury		
Head and neck	328	18.3
Face	233	13
Chest	114	6.3
Abdomen and pelvis	190	10.7
Spine and Spinal cord	200	11.2
Limb	726	40.5
Triage level		
1	2	0.2
2	470	45.7
3	534	52.0
4	22	2.1
5	0	0.0
Type of trauma		
Penetrating	1074	59.9
Blunt	717	40.1
Duration of admission		
1 day	676	65.8
1-7 day	328	31.9
7-30 day	22	2.1
>30 day	2	0.2
Operational care		
Emergency clearance	527	51.3
Orthopedic surgery	349	33.9
General surgery	109	10.6
Neurological surgery	43	4.2
The outcomes		
Partial recovery	733	71.3
Die	5	0.5
Transfer to another hospital	189	18.4
Other	101	9.8
ISS		
1-7	578	56.2
8-16	341	33.2
17-25	71	6.9
>25	38	3.7

The mean ISS measure in this study was 8.9±1.6, ranging from 1 to 75. A total of 109 (10.6%) had scores above 17.

The total of injuries for 1028 patients was 1,791 injuries, according to the anatomical status of injury, the number of cases with head and neck injury was 328 (24%), 233 cases (17%), chest 114 (8.3%), The abdomen or pelvis is 190 (13.9%), the spine is 200 patients (14.6%). The organs are 726 cases (53.3%) (Table 2).

Table 2: Frequency of different anatomic location groups of damage in the ISS levels

		ISS				
		1-7	8-16	17-25	>25	Total
Anato	Head and neck	136	99	54	39	328
	Face	104	98	21	10	233
	Chest	43	50	14	7	114
	Abdomen or pelvis	66	62	30	32	190
	Spinal cord	83	62	25	30	200
	Limbs	489	161	51	25	726
	Total	921	532	195	143	1791

The division of patients in terms of the age group showed that the highest number of patients was in the age group of 25-44 years, and the lowest number of patients was in the age group of 0-4 years. This severity of injury shows that the severity of injury in the age group of 15 to 24 years has the highest proportion compared to other groups (Table 3).

Table 3. Frequency of different age groups and frequency of severe injuries in groups

		Frequency	Frequency percent	Percentage of damage
Age	0-4	56	5.4	3.5
	5-14	59	5.7	6.7
	15-24	136	13.2	12.5
	25-44	410	39.9	11.4
	45-64	242	23.5	8.6
	>65	125	12.3	14.4

After examining the type of trauma in 1791 injuries, 1074 injuries (59.9%) had penetrating trauma and 717 injuries (40.1%) of blunt trauma were calculated. Of patients with severe trauma, 83 patients suffered severe injuries (7.7%) and in patients with blunt trauma, 68 patients suffered severe injuries (9.4%). The mortality rate was 5 (0.5%).

DISCUSSION

Earthquakes are administered in different methods, according to particular regions and countries [11]. In the past few decades, Natural disasters have affected 800 million people, and have caused over $500 billion of economic costs [12-14].

In recent decades, various methods have been used to evaluate the severity of the trauma, including ISS, TRISS, etc. In this study, the ISS method was used and we examined the severity of trauma with other variables. Being the benchmark used in this article, ISS is a global traumatic survey criterion defined based on the sum of the three injuries with the highest AIS score. ISS is usually divided into 6 areas per person and is also used for patients with more than one injury. The numerical value of this numerical criterion is between 1 and 75, with scores less than 7.5 categorized in the group of weak injuries, scores between 7.5 to 17 categorized in the group of moderate injuries, and scores more than 17 categorized in the group of severe injuries. Traumatic studies and epidemiological structure of the trauma caused by the earthquake led to the classification of vulnerable groups and the identification of vulnerable anatomical parts of the body in injured people. This knowledge informs us about the necessary steps to prevent further problems and to deal with the damage that has been caused.

In this study, five mortality (2 female and 3 male patients) were seen that have multiple trauma and blunt abdominal trauma. 60 % of the dead were between the ages of 25-45. The severity of injury for all of them was higher than 25, with an average injury severity of 38.4. this mortality can reduce if the management of triage was done with the standard method, for example, Sonography in Trauma (FAST) can be regarded in the administration of disaster management, that it can reduce mortalities due to blunt abdominal and multiple traumas, as Kakaei et al. [15] reported on the value of FAST as a primary diagnostic method for revealing of blunt abdominal and multiple trauma. Therefore, conducting FAST is a primary screening with high speed that can use in situations of disaster. The impotence of the FAST as a primary diagnostic method has been confirmed in the previous studies for primary screening [16].

The administration of triage of patients is one of the most important episodes of disaster management, hence, it has been indicated that triage of injured patients in disastrous events, particularly earthquakes, can decrease considerably mortality and morbidity. The accessibility of hospitals is very significant in disaster administration after earthquakes, and this subject is related to the structure of the hospital and building standards [17]. In this study, we have few mortality (5 cases), because the administration of triage of patients was done correctly, and the hospital was accessible and all the patients were established on the same crisis day. Although, in the Bam earthquake, the patients were triaged during the night following the disaster, since the two main hospitals of Bam were destroyed [18].

In this study, the demographic results established that trauma was more common in females (52.5% for females and 47.5% for males), and 39.3% of the patients have 25-44 years. The several studies indicated comparable outcome with these in the different regions of the world however In the Pakistan earthquake, 60.7% of the patient was male and 39.3% were female and 49% of patients have 25-44 years [19] because the most population of this region are in the third decade of life.

In this study, the age range of the patients was 1-95 years with a mean of 33.4 years. In the Marmara earthquake, Turkey, the age range of the patients was 11-74 years with an average of 34 years.

Limb injuries are the most common trauma in earthquake injuries, in this study, 42% of patients have presented limb injuries that the previous studies indicated similar outcomes, as 39% of the patients suffered head and neck injuries in the Armenia earthquake [8-11]. In our study, the most frequent area exposed for injury was limbs (42%) and followed by head and neck (18%), face (12.7%), abdomen and pelvis (10.5%), chest (5.9%). Also, the most common area exposed for injury was limbs (38.5 %) and followed by chest (21.4%), abdomen and pelvis (15.2%), spine (9.5%), head and neck (7.9%), face (1.3%) in the Wenchuan earthquake. In the Bam earthquake, 68% of the organs were damaged, compared to 42% in our study. Because the Bam earthquake occurred more time than this earthquake, also the strength of the Bam earthquake was higher due to the surface of the earthquake center [12, 20-24].

CONCLUSION

This study allows us to assess and manage the crisis, trauma due to earthquake, epidemiology of earthquake trauma. The results of this study confirmed with studies of other earthquakes in the world, however, some results different since this study was conducted to determine the epidemiological structure of the trauma, the issues which haven't been discussed in the literature. Consequently, this study established a fracture pattern in the referred patient of the Sarpul-Zahab earthquake to the Ayatollah Taleghani Hospital and is practical for clinicians to increase understanding of this natural disaster and permits superior selection of the triage management and therapeutic process.

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Association of rare disease and acute intoxication – case report

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Abstract*: Benzodiazepine overdose has various clinical manifestation, mainly regarding the central nervous system (CNS), cardiac and respiratory side effects, but rarely results in significant morbidity and mortality. Acute benzodiazepine poisoning results in dizziness, ataxia, nystagmus, dysarthria, hypoxia, hypothermia, bradycardia, hypotension, apnea, pulmonary aspiration, respiratory depression, coma, cardiopulmonary arrest and death. Anyway, deep nonresponsive coma should be investigated for additional etiologies. On the other hand, human transmissible prion disease has a fatal outcome with no specific treatment.*

Keywords: *prion disease, benzodiazepine intoxication, opisthotonus*

Human transmissible prion diseases are neurodegenerative disorders characterized by infectious pathogens accumulation with a long incubation period and devastating outcomes. A prion is defined as a small infectious agent lacking of nucleic acid, but containing protein [1]. It has an important feature regarding resistance to a number of decontamination procedures including processes affecting nucleic acids such as hydrolysis [2]. Up to present, human pathology recognize five related entities: Creutzfeldt-Jakob disease (CJD), Kuru, variant Creutzfeldt-Jakob disease (vCJD also known as new variant CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) [3].

Prionopathies result from abnormal isoforms accumulation in the brain that leads to neuron apoptosis and important brain damage. Infective prion is a product resulting from defective folding of the normal prion, having the gene located on the short arm of the chromosome 20 [4]. Unfortunately, the prognosis is very poor in these patients, despite all efforts and sustained interventions.

Benzodiazepines are commonly used against various disorders such as insomnia and anxiety. They also are preferred agents for drug overdose, although their acute toxicity is low. Oral benzodiazepine intoxication rarely results in

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significant morbidity and mortality. The body develops tolerance against such effects in the course of time and most common side effects are sedation and ataxia. Anxiolytic effects is rarely implicated in tolerance development. Acute benzodiazepine poisoning may result in dizziness, ataxia, nystagmus, dysarthria, hypoxia, hypothermia, hypotension, bradycardia, apnea, pulmonary aspiration, respiratory depression, coma, cardiopulmonary arrest and death.

CASE PRESENTATION

We present the case of a young white female, aged 26, admitted in our Toxicology – Intensive Care Unit of Clinical Emergency Hospital Bucharest from Bucharest, the capital of Romania. She was referred to us after deliberate ingestion of 200 mg benzodiazepine associated with 500 mg metoprolol. Ten months before this event the patient experienced complaints such as mood disorders, memory loss, irritability, inability every day social behavior and insomnia; initially, the condition was assessed as depression but no certain treatment was recommended. We couldn’t find any history of visual hallucinations or tremor. Interview emphasized multiple study visits in the United Kingdom and occasionally intravenous abuse substances dependence. Any other past medical data were unremarkable. The first medical examination revealed a conscious patient, mild agitated, stable vital signs, with unusual hyperosmia and visual impairment. Toxicology assessment was positive for benzodiazepine in the urine, detected with fluorescence polarization immunoassay method; toxicological screening was negative for other drugs. She had a rapid unfavorable evolution the following hours after admission experiencing degenerative progression of psychomotor and cognitive functions, lack of hand-eye and alternating hand coordination, left hand tremor and left lower extremity weakness and in the end, ten hours after ingestion, she developed impressive opisthotonus and deep coma that required mechanical ventilation. The evolution was unexplained by her toxicological status and occurred unexpectedly. Brain tomography was in normal ranges (Figure 1). She was performed lumbar puncture and the cerebral fluid features included clear aspect, normotensive, with glucose and electrolyte levels within normal limits and no type of cell detection. Regarding highly suspicion of tetanus, due to her occasionally intravenous drug administration, the infectious disease evaluation indicated anti-tetanus serum therapy, but clinical evolution wasn’t improving. After 48 hours of nonresponsive evolution a new brain CT scan identified massive cerebral edema. Cranial MRI showed marked cerebral atrophy in frontoparietal areas, DWI hyperintensities (cortical ribboning) in basal ganglia bilaterally and diffusion restriction in these areas suggesting progressive multifocal leukoencephalopathy, whereas putamen and caudate nucleus were free of signal intensity (Figure 3). Considering these aspect, we performed PCR for the detection of JC virus which was negative. A prionic disease was highly suspected and 14-3-3 protein immunoassay on CSF was positive. The patient died after 2 months in Critical Care Unit due to multiple dysfunctions, with the diagnostic of Creutzfeldt-Jakob disease.

DISCUSSION

As a part of the prionic disease family, CJD evolution is characterized by rapid evolution and neurological deterioration. The majority of cases are represented by sporadic human prion diseases, while 1-2% of the remaining are infectious forms acquired from an established source with the prion disease and 5-15% is represented by the autosomal dominant type [1]. The presented case exhibits most of the sporadic CJD clinical features with rapid cognitive decline and mioclonus; MRI was an indispensable tool for differential diagnosis considering that DWI hyperintensities in the putamen, caudate and cortex have a high specificity for sporadic prion disease [6]. However, the short dramatic course of the disease and detection of protein 14-3-3 in CSF adds value to diagnostic criteria. The literature shows that the mean age of onset is around 65 years of age with a very short median survival time around four months [7], but we report an extreme age (eg. 26 years old).

CONCLUSIONS

The case presented in this paper was meant to describe the clinical progression of a rapid evolving encephalopathy in a patient admitted for benzodiazepine ingestion. It also underlines the importance of keeping an open mind when dealing with unexpected and unusual evolution of acute intoxication.

Figure 1. The first CT scan within normal ranges

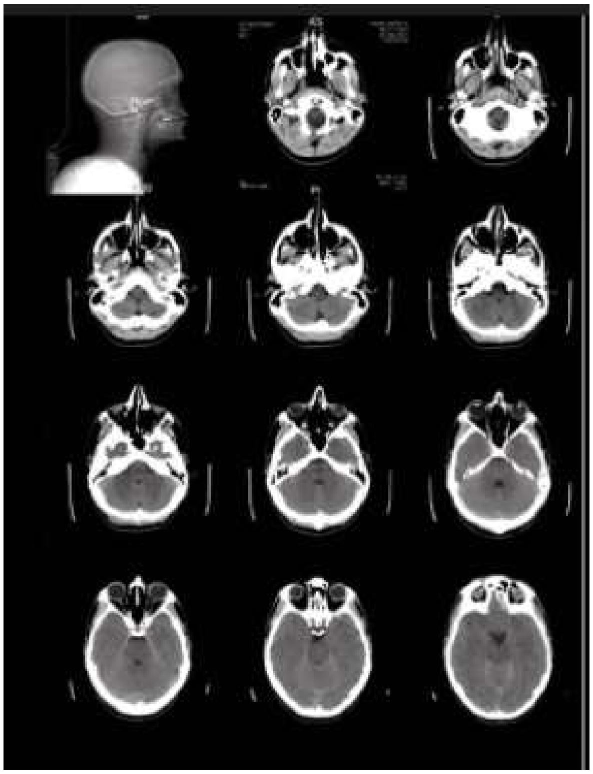

Figure 2. Cranial MRI showing cerebral atrophy, DWI hyperintensities (cortical ribboning) in basal ganglia bilaterally and diffusion restriction in these areas suggesting progressive multifocal leukoencephalopathy, whereas putamen and caudate nucleus were free of signal intensity

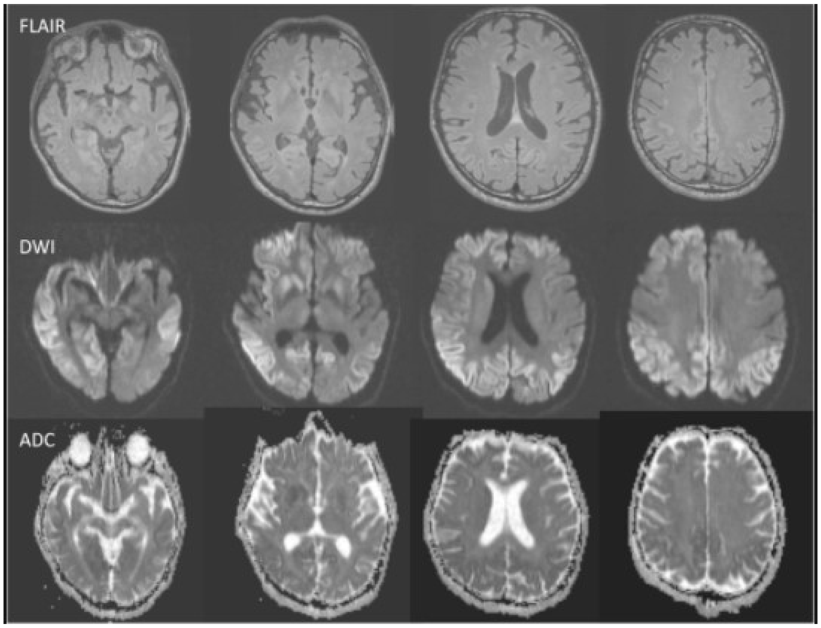

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REVIEW

A systematic review on a comparison between intravenous paracetamol and intravenous morphine sulfate for management of patients with trauma injuries

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Abstract: *Introduction – Pain is known the most common complaint in emergency departments. Several studies are administered many different protocols to control pain in trauma patients. The aim of the study was to review the major relevant articles in case of application of intravenous paracetamol and intravenous morphine sulfate for management of patients with trauma injuries.*

Methods – We searched the online databases such as PubMed, MEDLINE, EMBASE, ISI Web of Knowledge. We applied keywords included pain, acute, chronic, morphine sulfate, paracetamol, trauma, limb, wounds, and injuries, damage and etc.

Results – We included four eligible randomized clinical trials in this study. Three studies reported superior effects in intravenous paracetamol compared to intravenous morphine sulfate in controlling pain in trauma patients (limb trauma and trauma headache). However, a study reported no significant differences between the two groups in knee arthroscopy in trauma patients. All studies used from VAS methods for measurement of pain score.

Conclusion – The results indicated that IV paracetamol presents a superior analgesia than IV morphine sulfate particularly after acute limb trauma and trauma headache. However, further studies should be done that clinician could be have better decision for control pain in trauma patients.

Keywords: *intravenous, paracetamol, morphine sulfate, pain, trauma injuries*

INTRODUCTION

Pain is known the most common complaint in emergency departments [1, 2]. Studies have reported about 70% of all patients attending emergency departments to suffer different levels of pain. Several studies are administered many different protocols to control pain in trauma patients [3-7]. However, stand for an ideal suggestion for an analgesic to be administered as the main part of the treatment is a debatable issue yet [4, 7, 8].

Regarding control different levels of in both acute and chronic pain, opioids have many beneficial [9, 10]. Morphine sulfate has known the prototype utilized in patients suffering from different levels of pain because of trauma injuries [8, 11, and 12].

Morphine sulfate has an acceptable analgesia by several controlling modalities in the trauma patient for controlling acute pain [11]. However, several complications such as constipation sedation, confusion, miscellaneous neuro-psychological symptoms, nausea/vomiting and respiratory depression & hallucinations have been reported in patients taking morphine sulfate intravenously [13]. Considering some limits and side effects may result in applications in order to quest for more efficient and less disadvantageous alternative analgesics [4, 8, 14].

N-Acetyl-p-Aminophenol which is acknowledged as paracetamol is an inhibitor of cyclooxygenases which has been recognized globally as a safe and effective substance for pain drug and decreasing fever [12-16]. Paracetamol is one of the leading generally used analgesic and antipyretic factors. Since 2001, intravenous paracetamol has been available in Europe under the trade name Perfalgan and is accessible in many countries [15]. It is generally applied for reducing different rates of the pain of trauma patients from

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mild to moderate [15]. Also, it is used to control fever in neonates, children, and adults [8, 15, 17].

Studies reported paracetamol has fewer complications than opioids or nonsteroidal anti-inflammatory drugs (NSAIDs) [8, 12]. Studies have reported several complications such as opioid administration such as nausea, emesis, histamine release/pruritus, hypotension, sedation, respiratory depression, and constipation following administering this analgesic [16]. Also, hepatotoxicity may happen as a consequence of overdose or exceed therapeutic dosage medicine [15, 16].

Several investigations were carried to analyze the effectiveness of applying paracetamol and morphine either in separated groups or in combination with each other or alternative adjuvant therapy, including postoperative pain relief, cancer pain control or intravenous regional anesthesia [14-17]. A few studies are carried to study the comparative efficacy of prescription of morphine sulfate versus paracetamol in trauma injuries [8, 12]. So it should be realized that further studies may be required to confirm the findings. In this study, we aimed to review the major relevant articles in case of application of intravenous paracetamol and intravenous morphine sulfate for management of patients with trauma injuries to support clinicians to have a better understanding of these technologies and choosing the better healing choice.

METHODS

We searched the online databases such as PubMed, MEDLINE, Wiley, EMBASE, ISI Web of Knowledge and Scopus. We applied keywords included pain, acute, chronic, morphine sulfate, paracetamol, trauma, limb, wounds, and injuries, damage and etc. All relevant randomized clinical trial studies were included in this study. Two reviewers independently searched and evaluated the titles and abstracts of all articles.

RESULTS

In a randomized, clinical trial by Vahdati et al. (2014) evaluated the analgesic and safety of paracetamol compared to morphine in the management of a headache in patients with a trauma headache. Patients were randomly classified into two groups to get either intravenous paracetamol [Apotel 1 g (paracetamol 1 g made by Unipharm factory, Greece) normal saline (1 g/100 mL/10 minutes)] or morphine (0.1 mg/kg/100 mL/10 minutes NaCl). They concluded intravenous paracetamol is an effective and safe treatment than morphine [16].

Craig et al. (2012) compared the effect of intravenous paracetamol versus morphine in patients with moderate to severe traumatic limb pain. They included patients (16 to 65 years) with isolated limb trauma and in moderate to severe pain (pain score of 7 or more) received either 1 g intravenous paracetamol or 10 mg intravenous morphine sulfate over 15 min. They measured pain score via visual analog scale at 0, 5, 15, 30 and 60 min following beginning drug treatment. They reported there was no difference in analgesic effect and rescue analgesia between the two groups at any time interval. But there were significantly more adverse reactions in the morphine group than the paracetamol group. They concluded intravenous paracetamol seems to give a level of analgesia comparable to intravenous morphine in isolated limb trauma patients [17].

Jalili et al (2016) did a randomized double-blinded clinical trial for comparison of intravenous 0.1 mg/kg morphine sulfate with 1 g intravenous paracetamol in order to pain reduction in sixty patients with pain score of greater than 3/10 in the limb trauma. They reported the mean pain intensity scores for paracetamol (3.86) and for morphine (2.16) ($P < 0.001$). 15 patients (morphine group) plus four patients (paracetamol group) required rescue analgesia ($P = 0.05$). They concluded that IV paracetamol present superior analgesia than IV morphine in these traumatic patients. However, further studies should be done that confirms this issue [18].

Hashemi et al (2015) evaluated comparison of IV paracetamol with morphine for the pain administration after diagnostic knee arthroscopy in sixty traumatic patients in a randomized double-blinded clinical trial. They measured the nausea and vomiting, pain level, and sedation. They reported no considerable difference between the two groups regarding to the pain level. The complications were not seen in the paracetamol group; although, the postoperative nausea was seen in 22.3% of PCA morphine group. Hashemi et al presented clinical advantages for IV paracetamol including stable hemodynamic parameters, reduction of analgesics management, improved postoperative pain, no nausea and vomiting, lower complications, reduction length of hospitalization, and increased patient comfort and satisfaction [19].

Dijkstra et al (2013) did a systematic review in order to recognize efficient and safe primary pain interventions for traumatic patients with acute pain in the chain of emergency care, accessible in the Netherlands. They reported that Paracetamol (both IV and orally) and IV opioids (fentanyl and morphine) comfirmed to be efficient. They not recommended non-steroidal anti-inflammatory drugs (NSAIDs) for use in pre-hospital ambulance or emergency medical services because NSAIDs showed mixed results [20].

Table 1: Characteristics of the reviewed studies

Author(s) (year)	Medication	Design	Trauma	N	Setting	Pain score outcome measure	Pain relief results	Conclusion
Jalili et al (2016) [18]	intravenous 0.1 mg/ kg morphine sulfate with 1 g intravenous paracetamol.	randomized double-blinded clinical trial	Limb Trauma	60	trauma center of a university hospital (emergency department)	VAS	The mean reduction in numerical rating scale pain intensity scores at 30 minutes was 3.86 (± 1.61) for paracetamol, and 2.16 (± 1.39) for morphine	They concluded that IV paracetamol present superior analgesia than IV morphine in these traumatic patients. However, further studies should be done that confirms this issue
Hashemi et al (2015) [19]	intravenous infusion of 1 g paracetamol within 15 minutes after surgery and every 6 hours to 24 hours in the paracetamol group. The patient-controlled analgesia group received morphine through PCA infusion pump at 2 mL/h base rate and 1mL bolus every 15 minutes. Pain level, nausea and vomiting, and sedation were measured and recorded during entering the recovery, 15 and 30 minutes after entering the recovery, 2, 6, and 24 hours after starting morphine pump infusion in the morphine and paracetamol in the paracetamol groups.	randomized double-blinded clinical trial	knee arthroscopy in trauma patients	60	trauma center of a university hospital (tertiary hospital)	VAS	They reported no considerable difference between the two groups regarding to the pain level. The complications were not seen in the paracetamol group; although, the postoperative nausea was seen in 22.3% of PCA morphine group	Hashemi et al presented clinical advantages for IV paracetamol including stable hemodynamic parameters, reduction of analgesics management, improved postoperative pain, no nausea and vomiting, lower complications, reduction length of hospitalization, and increased patient comfort and satisfaction
Vahdati et al. (2014) [16]	intravenous paracetamol [Apotel 1 gr. (paracetamol 1 gr. made by Unipharm factory, Greece) normal saline (1 g/100 mL/10 minutes)] or morphine (0.1 mg/kg/100 mL/10 minutes NaCl).	randomized, clinical trial	Trauma Headache	60	trauma center of a university hospital (tertiary hospital)	VAS	After 15 minutes, pain was 31.7 ± 18.0 and 48.3 ± 14.1 mm in Paracetamol and Morphine Respectively (P<0.005).	They concluded intravenous paracetamol is an effective and safe treatment than Morphine
Craig et al. (2012) [17]	1 g intravenous paracetamol or 10 mg intravenous morphine sulfate over 15 min.	randomised, double-blind pilot study	traumatic limb	55	Emergency department	VAS	After 60 minutes, pain was 52.9± 27.4 and 44.0± 22.6 in Paracetamol (and Morphine Respectively (P=0.28).	They concluded intravenous paracetamol seems to give a level of analgesia comparable to intravenous morphine in isolated limb trauma patients

DISCUSSION

Efficient management of pain is an important concern for anesthesiologists. The patients still endure insufficient postoperative pain administration in spite of the progression of pain control methods. The management of pain by opioids has some complications such as vomiting, nausea, urinary retention, pruritus, and respiratory depression [21]. Consequently, the non-opioids have become more accepted to avoid of these complications [22]. Hashemi et al reported that paracetamol and morphine equally reduced the intensity of postoperative pain. Decreasing of opioids usage is crucial for the reason that it dues to a reduction in complications and side-effects. In addition, any method that reduces admittedly total opioid consumption dose is considered an advantage. It demonstrated that intravenous paracetamol be as successful as ketorolac, diclofenac, and morphine in the some clinical studies. Therefore, it is favorable for the management of moderate to severe pain after surgery [23, 24].

The correct mechanism of function of paracetamol has not been found. The paracetamol might cause activation of serotonin receptor II and subsequently prevention of cyclooxygenase pathway for the production of prostaglandins with double-barreled impacts [25-27]. The paracetamol can be related to bleeding complications through preventing the synthesis of prostaglandins and associated compounds [25]. Paracetamol may possibly reduce peripheral vascular resistance and cardiac output [28]. Hashemi et al presented clinical advantages for IV paracetamol including stable hemodynamic parameters, reduction of analgesics management, improved postoperative pain, no nausea and vomiting, lower complications, reduction length of hospitalization, and increased patient comfort and satisfaction.

Different studies discuss the advantages of IV paracetamol such as; Sinatra et al [29] reported that IV paracetamol is a valuable analgesic, although Haddow et al [30] commented that it is more readily accessible than morphine, which is an effective and restricted drug. Todd et al [31] demonstrated that no difference between IV morphine and IV paracetamol in the analgesic effects on patients. Also, Van Aken et al [32] demonstrated that IV paracetamol is superior tolerated after dental surgery. Craig et al [33] demonstrated that approximately no difference in pain scores for comparison of the effects of 10 mg of IV morphine with 1 g IV paracetamol in the UK ED patients. Paracetamol can reduce opioid supplies by between 20% and 30% because it is an efficient adjunct to opioids [34]. The researches indicated that IV morphine would have intensified the iatrogenic influences of fentanyl, especially its potential to cause respiratory depression and hypotension, while IV paracetamol be able to decrease the amount of fentanyl required [35].

Institute for Clinical Systems Improvement reported that paracetamol is extensively applied in clinical practice and is an effective safe, simple, and analgesic with few side effects. Hoogewijs et al and Viallon et al [36-37] reported that intravenous management of paracetamol in the ED seemed to have a quicker beginning, while paracetamol controlled p.o. does not need an i.v. catheter supplement and is consequently easier and less time-consuming for emergency care professionals. Feldman et al indicated rectal usage of paracetamol has a inconsistent bioavailability, and it cannot be simply appropriate in pre-hospital emergency conditions; consequently, this method of management is not suggested in emergency care. Dijkstra et al reported that Paracetamol and opioids including morphine and fentanyl proved and verified to be effective and safe analgesics for trauma patients [20].

Jalili et al demonstrate and suggested that acetaminophen is a safe and effective nonopioid analgesic for ED patients with acute traumatic limb pain. They showed IV morphine sulfate is efficient for pain administration in these patients, although 1-gram dose of IV paracetamol presented a superior level of analgesia comparison to morphine. In the other hand, the morphine consumption is considerably lower in the paracetamol group [18]. Several studies confirmed prospective efficiency of intravenous paracetamol in acute pain administration. Nevertheless, the majority of them demonstrated that the analgesic outcome of IV paracetamol is analogous to effect of IV morphine sulfate. Craig et al comprised IV paracetamol and IV morphine sulfate for pain administration of acute limb trauma in the ED, they showed no considerable difference in analgesic outcome between the morphine and paracetamol groups at any time point [33]. However, amount of morphine sulfate was greater than the used morphine dose by Jalili et al. Bektas et al. evaluated the analgesic outcome of IV morphine and IV paracetamol for the cure of renal colic, while were observed no considerable reductions in the pain intensity between paracetamol and morphine group [38]. Sinatra et al. assessed the safety and efficacy of paracetamol for pain administration following major orthopedic surgery [38]. They reported the pain intensity scores for consumption of paracetamol and morphine is considerably reduced while no complications were seen during 24 hours after surgery in comparison to the placebo group [38].

CONCLUSIONS

The results indicated that IV paracetamol presents a superior analgesia than IV morphine sulfate particularly after acute limb trauma and trauma headache. However, further studies should be done that clinician could be have better decision for control pain in trauma patients.

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REVIEW ARTICLE

Atherosclerosis in rheumatoid arthritis – the importance of imaging tests

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Abstract: *As reported by literature, rheumatoid arthritis is a rheumatic disease with an increased risk of developing cardiovascular complications. Early detection of atherosclerosis by non-invasive techniques represent a current challenge of patient management. Current EULAR guidelines recommend careful monitoring of cardiovascular risk factors by stratification of patients and strict control of rheumatic disease activity to reduce it. The classic evaluation of intima-medium thickness (IMT) was completed by quantifying the degree of inflammation of the carotid plate using positron emission tomography or the degree of coronary calcifications by calculating Agatston score using Coronary Computer Tomography. These modern investigation techniques allow identification of several subclinical stages of atherosclerosis, the interest in their early detection resulting from the fact that, in this stage, through specific measures, the cardiovascular complications can be prevented.*

Keywords: *rheumatoid arthritis, atherosclerosis, cardiovascular risk, imaging tests*

INTRODUCTION

Rheumatoid arthritis is a rheumatic disease with an increased risk of cardiovascular events due to advanced atherosclerosis [1]. The development of atheromatous plaque is based on chronic inflammation found in both rheumatoid arthritis and atherosclerosis[2]. Recent studies have shown a higher prevalence of plaque in patients with rheumatoid arthritis, which explains their negative predictive role[3,4]. The higher frequency of traditional risk factors in patients with rheumatoid arthritis (hypertension, type II diabetes, smoking, high cholesterol and obesity) is another factor contributing to the development of cardiovascular complications. Its impact is different from that of the population not suffering of this pathology [5,6]. Current EULAR guidelines recommend careful monitoring of cardiovascular risk factors by stratification of patients and strict control of rheumatic disease activity to reduce it [6]. The early detection of atherosclerotic changes by non-invasive techniques is a current concern of patient management. Collaboration between rheumatologist and cardiologist and prospects of a new specialty - cardio-oncology will be encouraged in future in order to improve the prognosis of patients affected by rheumatoid arthritis and atherosclerosis.

NONINVASIVE IMAGING OF INTRACRANIAL VESSELS

Atherosclerotic plaque is the trigger for cardiovascular events, its vulnerability being due to plaque composition, inflammatory cell content, age, and not necessarily plaque size [7, 8]. In rheumatoid arthritis, advanced inflammation causes a particular phenotype of the plaque, thus presenting an increased risk of myocardial infarction or stroke [9, 10]. Transcranial Doppler examination is a method of measuring cerebral blood flow in the main intracranial arteries. This method uses 2 MHz ultrasound probes to dynamically detect intracranial arteries through thinner areas of the skull called

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acoustic windows [11, 12]. This study has proven inexpensive, reproducible and provides information on the prognosis of cardiovascular events [13, 14, 15, 16]. Measurement of average mean cerebral arterial flow rate, pulsatility index, and calculation of the relationship between these two indices revealed prognostic factors of recurrent cardiovascular events [17, 18] In patients with rheumatoid arthritis, there was an increase in the prevalence of distal occlusions of the middle cerebral artery and basilar artery detected by transcranial Doppler [11].

Another investigation commonly used in the clinic is the Doppler ultrasound of the carotid arteries. This is a good way to identify patients with rheumatoid arthritis at high cardiovascular risk who can benefit from active treatment.

Proper stratification of cardiovascular risk is a key element in the management of patients with rheumatoid arthritis. It is well known the EULAR's adaptation of cardiovascular risk assessment based on coronary risk assessment (SCORE) multiplied by 1.5 in patients with rheumatoid arthritis that meet two of the following three criteria: disease> 10 years, rheumatoid risk factor (RF) or anti-cyclic cytoplankin peptide (anti-CCP) antibody and presence of serious extra-articular manifestations [19]. However, the authors recommend performing a carotid ultrasound scan when SCORE (Figure 1)[20] does not report high cardiovascular risk, considering studies in which patients with rheumatoid arthritis were not considered at high risk for cardiovascular events by calculating SCORE risk, but who had significant cardiovascular events: myocardial infarction and stroke [21].

Figure 1: Cardiovascular risk estimation using SCORE

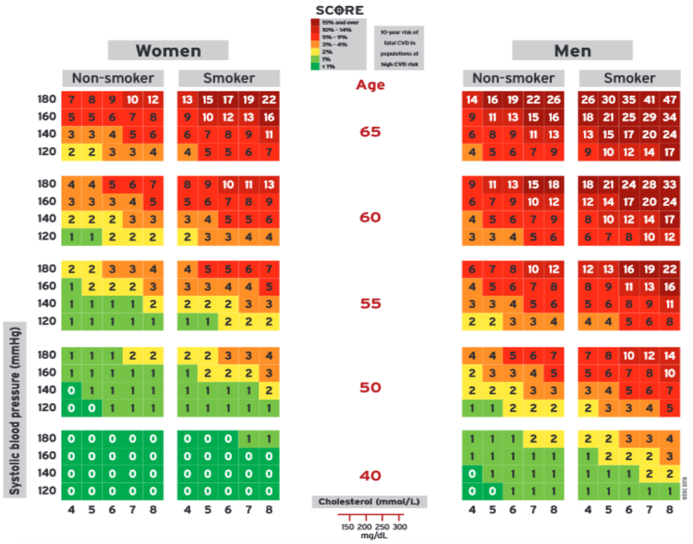
SCORE
15% and over
10% - 14%
5% - 9%
3% - 4%
2%
1%
< 1%
10-year risk of fatal CVD in populations at high CVD risk
Cholesterol (mmol/L)
150 200 250 300
mg/dL
©ESC 2016

Age	Systolic blood pressure (mmHg)	Women										Men									
		Non-smoker					Smoker					Non-smoker					Smoker				
		4	5	6	7	8	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8
65	180	7	8	9	10	12	13	15	17	19	22	14	16	19	22	26	26	30	35	41	47
	160	5	5	6	7	8	9	10	12	13	16	9	11	13	15	16	18	21	25	29	34
	140	3	3	4	5	6	6	7	8	9	11	6	8	9	11	13	13	15	17	20	24
	120	2	2	3	3	4	4	5	5	6	7	4	5	6	7	9	9	10	12	14	17
60	180	4	4	5	6	7	8	9	10	11	13	9	11	13	15	18	18	21	24	28	33
	160	3	3	3	4	5	5	6	7	8	9	6	7	9	10	12	12	14	17	20	24
	140	2	2	2	3	3	3	4	5	5	6	4	5	6	7	9	8	10	12	14	17
	120	1	1	2	2	2	2	3	3	4	4	3	3	4	5	6	6	7	8	10	12
55	180	2	2	3	3	4	4	5	5	6	7	6	7	8	10	12	12	13	16	19	22
	160	1	2	2	2	3	3	3	4	4	5	4	5	6	7	8	8	9	11	13	16
	140	1	1	1	1	2	2	2	2	3	3	3	3	4	5	6	5	6	8	9	11
	120	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4	4	5	6	8
50	180	1	1	1	2	2	2	2	3	3	4	4	4	5	6	7	7	8	10	12	14
	160	1	1	1	1	1	1	2	2	2	3	2	3	3	4	5	5	6	7	8	10
	140	0	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	4	5	6	7
	120	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	4	5
40	180	0	0	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2	3	3	4
	160	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2	2	2	3
	140	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	2	2
	120	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1

(Source: European Guidelines on CVD Prevention in Clinical Practice 2016 Eur J Prev Cardiol. 2016 Jul;23(11):NP1-NP96. doi: 10.1177/2047487316653709)

Carotid Doppler ultrasound evaluates intima-medium thickness (IMT) and highlights atherosclerotic plaques, making it an affordable, easy-to-use, reproducible, low cost study. A meta-analysis of 1384 patients with rheumatoid arthritis concluded that a carotid IMT increased from the control group population – 1147 patients (normal IMT <0.9 mm) [22]. Olah C. et al [11] also published in 2017 a study on the assessment of intracranial vessels in combination with carotid atherosclerosis and stroke in patients with rheumatoid arthritis in which more patients with rheumatic disease had at least a plaque in the right carotid artery, and its score was higher than that of the control group patients. Thus, we can say that carotid ultrasound remains an easy-to-use tool in practice, but different protocols for measuring the carotid plaque and operator-dependent, makes this investigation more and more replaced by carotid magnetic resonance.

Carotid magnetic resonance has the advantage of capturing the entire circumference of the carotid wall, including the adventitia and the outer layer, as opposed to carotid ultrasound, which only visualizes the intima of the carotid artery. In the multiethnic study of atherosclerosis, conducted in six US research centers, 698 patients were included and examined by carotid ultrasound and MRI. The results of the study showed that MRI could be a better predictor of cardiovascular events than carotid ultrasound, as the combination of wall thickness measured by magnetic resonance was stronger than that of ultrasound-measured IMT [23]. In addition, the arterial history revealed by MRI seems to be a better predictor of cardiovascular complications in a study that showed an increase in inflammatory cytokine expression in aortic adventitia in patients with rheumatoid arthritis who suffered from coronary artery bypasses compared to control patients [24, 25]. Another advantage of this method of non-invasive diagnosis is the strong correlation with the histological phenotype of atherosclerotic plaque, the dynamic contrast-enhanced MRI (DCE-MRI) allowing to evaluate the neovascularization of the atherosclerotic plaque, its vulnerability by the degree of inflammation and the degree of hemorrhage in plaque, and the effectiveness of therapeutic interventions [26, 27].

Another more modern technique capable of quantifying the degree of inflammation of the carotid plate is positron emission tomography. This exploration uses 18-F-fludeoxy-glucose, a glucose analogue, which has the ability to accumulate in inflamed atherosclerotic plaque, where exists a greater amount of marked pozitrons [28]. Thus, CT or magnetic resonance imaging is possible to map the affected area, the absorption of fludeoxoxiglucose being associated with the identification of lesions at high risk of embolization [29]. Another radioactive agent used in the detection of atherosclerosis is sodium fluoride (18-F-NaF), which is capable of binding to calcified unstable arterial zones. During the course of atherosclerotic disease, two types of vascular damage can occur: macrocalcification – conferring stability to plaque atheroma and microcalcification – associated with an increased risk of plaque vulnerability. Sodium fluoride PET-CT differentiates the two types of lesions presented above, the new technique being able to revolutionize the treatment of atherosclerosis, increasing the chances of preventing cardiovascular complications [30].

NONINVASIVE CARDIAC IMAGING

Advanced atherosclerosis in rheumatoid arthritis is also evident in the heart, manifesting itself in various forms: coronary ischemia - micro and macrovascular, inflammation or myocardial fibrosis (myocarditis) [31]. Rheumatoid arthritis should therefore be closely monitored using non-invasive techniques to identify patients at high risk of developing cardiovascular complications. A recent study performed by a Danish team, which included the entire population over 18 years old diagnosed with rheumatoid arthritis between 1978 and 2008, found an increased risk of heart failure in patients with rheumatoid arthritis, justifying the need for cardiovascular screening [32].

Coronary arteries can be assessed non-invasively by transthoracic or transesophageal ultrasound and magnetic resonance imaging computed tomography or computed tomography after endothelial-dependent vasodilation [33].

Transthoracic echocardiography is a classic method of evaluating cardiac function that, by its characteristics, can detect patients with a high cardiovascular risk, such as those with rheumatoid arthritis. Recent studies have demonstrated subclinical impairment of right and left ventricular function in patients with rheumatoid arthritis versus control, as demonstrated by the comparison of early ventricular (E-wave) filling, late ventricular (A-wave) filling, and the E/A ratio of the two lots [34]. A modern clinical application of transthoracic echocardiography is transthoracic stress echography with dipiridamol, able to evaluate the left coronary reserve. This represents the ratio of maximal coronary flow during stress to basal rate, which is a very sensitive diagnostic marker (> 90%) for coronary heart disease [35, 36]. In the literature, it has been reported that a value of coronary reserve flow <2 is associated with the presence of coronary stenosis [37]. In the absence of evidence of epicardial coronary stenosis, an abnormal coronary flow reserve may indicate a pathology of coronary microcirculation in patients with reperfused myocardial infarction, hypertension, diabetes mellitus, hyper-cholesterolemia, X syndrome or hypertrophic cardiomyopathy. Recent studies have also demonstrated a negative prognostic value of coronary reserve flow in patients with or suspected of coronary artery disease [38].

Transesophageal cardiac ultrasound is an investigation that can successfully complete transthoracic cardiac ultrasound, which has the disadvantage of visualizing the posterior structures of the heart, or which has the disadvantage of a difficult window of exploration in obese patients or affected by lung disease. Transesophageal cardiac ultrasound was used in a case-control study conducted on a group of 30 patients diagnosed with rheumatoid arthritis, which concluded that there was a high frequency of regurgitation of the mitral and aortic valves and obvious signs of cardiomyopathy in most patients [39].

Computer tomography is another method of assessing atherosclerosis present in the patient diagnosed with rheumatoid arthritis by completing the classical techniques mentioned above. A recent study aimed at examining the impact of rheumatoid arthritis on the prevalence and severity of coronary artery disease, concluded that the presence of coronary calcifications is common in patients with serum-positive rheumatoid arthritis [40]. This result was based on measurements of calcium score, an indicator of the degree of coronary artery calcification, by means of computer tomography. Figure 2 presents intense calcifications in the coronary artery walls, more evident on the LAD 2 (60-70%). The examination is non-invasive, without the use of a contrast substance, guided by the ECG; it does not require a long examination time and shows minimal irradiation.

Figure 2: Intense calcifications present in the coronary artery walls, more evident on the LAD 2 (60-70%). Normal detachment of coronary arteries.

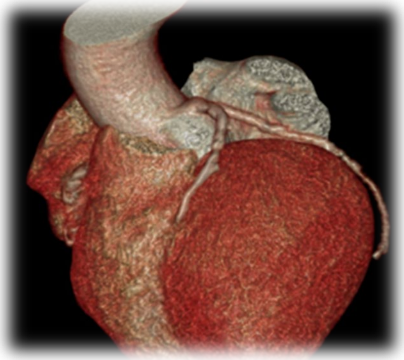

The patient is lying down during the exam, and the calcium score is measured in deep inspiration. Coronary CT scan provides information on cardiovascular risk by calculating Agatston score: a small risk is equivalent to a score below 100 Agatston units, an intermediate risk of 100 to 300 Agatston units, and an increased risk of more than 300 Agatston units (Figure 3). The presence of an elevated risk score (over 300 Agatson units) requires a myocardial scintigraphy and the presence of a score of over 600 Agatson Units to perform invasive coronarography.

Still in the field of noninvasive imaging techniques, cardiac nuclear magnetic resonance is increasingly used, which is the gold standard for the evaluation of myocardial viability.

This modern technique was used in a study comparing the structure and function of myocardial patients with rheumatoid arthritis compared to a control group included in the multi-ethnic Baltimore study on atherosclerosis, unaffected by rheumatic pathology.

Figure 3: Cardiac CT: Calcium Scoring 529.74 Agaston units (LAD 329 UA, LCX 70.62 UA, RCA 129.86 UA)

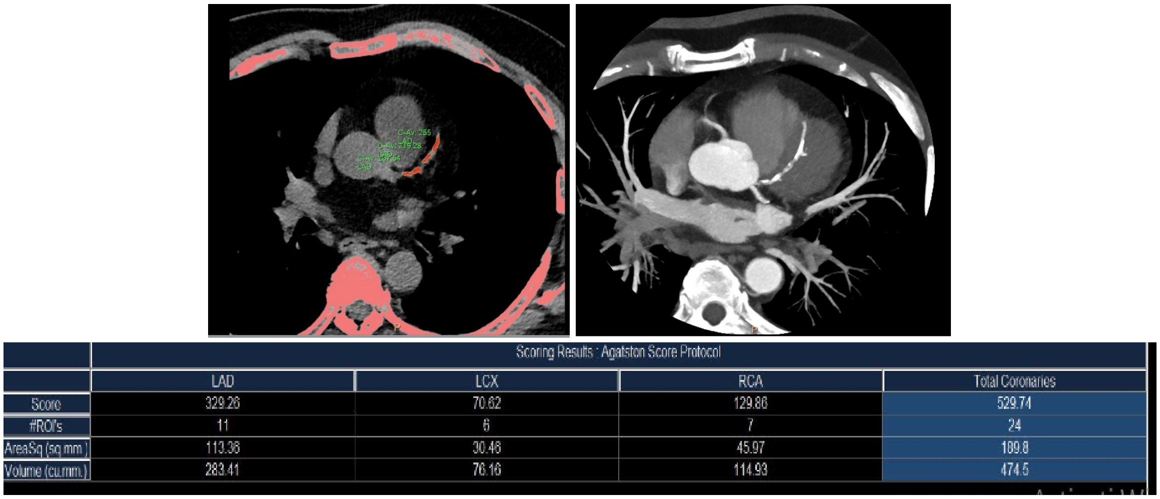

	Scoring Results : Agatston Score Protocol			
	LAD	LCX	RCA	Total Coronaries
Score	329.26	70.62	129.86	529.74
#ROI's	11	6	7	24
AreaSq (sq.mm.)	113.36	30.46	45.97	189.8
Volume (cu.mm.)	283.41	76.16	114.93	474.5

The findings showed that there was no statistically significant difference between the two volumes in the left ventricular and telediastolic volumes, but that the left ventricular ejection fraction and the cardiac output were lower in patients with rheumatic arthritis, indicating a progression of heart failure [41]. Cardiac Magnetic Resonance has also been applied to a group of rheumatoid arthritis patients with no history of cardiovascular disease (pulmonary hypertension, coronary artery disease, valvulopathy, atrial fibrillation or diabetes), and the results were detection of subendocardial infusion defects in correlation with disease activity, respectively with markers of inflammation – PCR and VSH [42]. These results confirm the major involvement of inflammation in worsening cardiovascular status in patients with rheumatoid arthritis.

NON-INVASIVE IMAGING OF PERIPHERAL CIRCULATION

Peripheral circulation can be investigated non-invasively by a variety of methods from the classical ankle-arm index, or measuring post-occlusive reactive hyperemia with a plethysmograph up to brachial artery mediated vasodilatation, measured ultrasonographically or by magnetic resonance. These tests are useful in assessing endothelial function and may have considerable potential in the future in assessing rheumatic patients.

CONCLUSIONS

In conclusion, we can state that the modern investigation techniques allow identification of several subclinical stages of atherosclerosis, the interest in their early detection resulting from the observation that in this stage, through specific measures, the cardiovascular complications can be prevented.

Systemic chronic inflammation in atherosclerosis and rheumatic diseases brings to the forefront the need for a multibiomarker approach in cardiovascular risk assessment. Biological markers correlated with imaging tests may be important prognostic elements capable of delaying the progression of atherosclerosis, providing the opportunity for early cardiovascular treatment.

Until now, atherosclerosis imaging has been more centered on the anatomical aspect, with current trends focusing on a more functional approach using multi-modal images. The ultimate goal would be to fuse between anatomical and functional data and to achieve a more faithful image of the atherosclerotic plaque. In the future, an exploration of molecular and cellular mechanisms of cardiovascular disease is expected through molecular imaging that is able to overcome various aspects of inflammation involved in atherosclerosis. Slowing or stopping the progression of the inflammation process will be the future target of patient management by improving their prognosis and decrease the rate of their cardiovascular complications.

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CLINICAL PRACTICE

Atypical case of achalasia

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INTRODUCTION

Esophageal cancer, represented by squamous carcinoma and adenocarcinoma, is ranked 8th in incidence among all the types of neoplasm in the world (456,000 new cases in 2012, 3.2% of total cancers), and is characterized by a significant lethal potential, being the 6th cause of cancer-induced mortality (400,000 in 2012, 4.9% of total cancers) [1]. 5 years survival after diagnosis is less than 5%, as esophageal cancer causes symptoms when more than 60% of the esophagus is infiltrated by tumor [2].

CASE REPORT

A 48-year-old patient presented for progressive dysphagia to solids, weight loss of about 15 kg and diffuse abdominal pain for the last 3 months. In addition, the patient noticed moderate dyspnea and intense pain localized at the right shoulder, without relation to movements. Two months before presentation, the patient performed a superior digestive endoscopy, which diagnosed him with ulcerative esophagitis, but without improvement of the symptoms despite recommended treatment. The patient is known with two surgical procedures, a Billroth I anastomosis for perforated gastric ulcer 12 years ago, and a cholecystectomy in 2018. Moreover, the patient had a stroke in 2014 and in 2015 was diagnosed with hepatic cirrhosis of unspecified etiology. The patient was a smoker with a 30 pack per year history and didn't work in a toxic environment.

The patient was underweight (BMI = 18.0 kg/m^2), but had a satisfactory overall condition. The abdomen was dense and painful on palpation.

The patient had hepatomegaly, was cardio-respiratory balanced and did not show any significant changes in the other organs and systems.

At the time of admission, stage diagnostics included dysphagic syndrome, abdominal algic syndrome, underweight, post-gastrectomy status, sequelae of stroke, liver cirrhosis in observation.

It is to be mentioned the inflammatory syndrome (fibrinogen = 484 mg/dL), cholestasis (total bilirubin = 1.47 mg/dL, direct bilirubin = 0.45 mg/dL, alkaline phosphatase = 145 U/L, GGT = 322 U/L), hyposideremia (Fe = 58 micrograms/dL) without anemia (Hb = 14.2 g/dL, Ht = 41.2%, RBC count 4.70 million/mm^3). During

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hospitalization, hemoglobin, hematocrit, and RBC count decreased but did not indicate iron deficiency anemia (hemoglobin = 13.6 g/dL, hematocrit = 39.5, number of erythrocites = 4.5 million/mm^3). The patient's hormonal profile indicated euthyroidism. The patient was tested for HIV infection, hepatitis B and C virus infection, Kohn bacillus infection, but the results refuted those diagnostics. CEA and CA 19-9 had elevated values (CEA = 58.21 ng/mL, CA19-9 = 47.53 U/mL) and α-fetoprotein was slightly above the normal range (AFP = 1.1 ng/mL).

Abdominal ultrasound and thoracic radiography did not reveal pathological changes in the first phase. At upper digestive endoscopy fluid debris were present in the esophagus and stomach that prevented visualization of the digestive tract. At the repeated upper digestive endoscopy, white deposits were attached to the normal mucosa at the level of the esophagus, characteristic of mycotic esophagitis. Initially, we couldn’t overcome the cardia with the normal endoscope, which is why we turned to babyscope. There were no pathological changes in the stomach and duodenum I and II, and the mouth of anastomosis from the perforated ulcer surgery was supple. Due to these endoscopic findings, the patient underwent esophageal manometry, and was diagnosed with type 2 achalasia (panesophageal pressurization 100%, IRP = 80.2 mmHg).

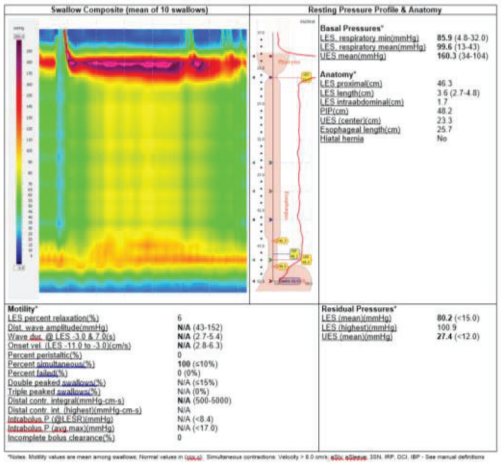

Swallow Composite (mean of 10 swallows)

Resting Pressure Profile & Anatomy

Basal Pressures*	
LES, respiratory min(mmHg)	**85.9** (4.8-32.0)
LES, respiratory mean(mmHg)	**99.6** (13-43)
UES mean(mmHg)	**160.3** (34-104)

Anatomy*	
LES proximal(cm)	46.3
LES length(cm)	3.6 (2.7-4.8)
LES intraabdominal(cm)	1.7
PIP(cm)	48.2
UES (center)(cm)	23.3
Esophageal length(cm)	25.7
Hiatal hernia	No

Motility*	
LES percent relaxation(%)	6
Dist. wave amplitude(mmHg)	**N/A** (43-152)
Wave dur. @ LES -3.0 & 7.0(s)	**N/A** (2.7-5.4)
Onset vel. (LES -11.0 to -3.0)(cm/s)	**N/A** (2.8-6.3)
Percent peristaltic(%)	0
Percent simultaneous(%)	**100** (≤10%)
Percent failed(%)	0 (0%)
Double peaked swallows(%)	N/A (≤15%)
Triple peaked swallows(%)	N/A (0%)
Distal contr. integral(mmHg-cm-s)	**N/A** (500-5000)
Distal contr. int. (highest)(mmHg-cm-s)	N/A
Intrabolus P (@LESR)(mmHg)	N/A (<8.4)
Intrabolus P (avg max)(mmHg)	N/A (<17.0)
Incomplete bolus clearance(%)	0

Residual Pressures*	
LES (mean)(mmHg)	**80.2** (<15.0)
LES (highest)(mmHg)	100.9
UES (mean)(mmHg)	**27.4** (<12.0)

*Notes: Motility values are mean among swallows; Normal values in (xxx.x). Simultaneous contractions: Velocity > 8.0 cm/s; eSlv, eSleeve, 3SN, IRP, DCI, IBP - See manual definitions

Taking into account the persistent symptoms and the changes of the above mentioned laboratory analyzes, computerized thoracic and abdominal tomography with contrast substance was performed. Computed tomography revealed pulmonary diffuse multiple micronodular lesions of millimetric dimension. At the eso-gastric junction a tumor block was described, with exophytic growth, with polylobate form and iodophyllic aspect, with a maximum diameter of about 5.5/3.8 cm, with small necrosis included, causing distension and stasis in the upstream esophagus. Additionally, adenopathy blocks in the small gastric curvature and celiac trunk were present, measuring up to 6 cm in diameter, the largest being without boundaries of demarcation with the celiac and pancreatic trunks. The liver was increased in size, with a maximum cranio-caudal diameter of 19.5 cm, with irregular contour, and irregular inhomogeneous structure due to the presence of multiple nodular lesions, randomly developed in the entire parenchyma that measured up to 1.8 cm, iodophyllic, some with a tendency to conflate, compatible with secondary lesions. Intrahepatic peripheral bile ducts in the left lobe exhibited slight dilation, most likely by infiltration generated by secondary lesions while extrahepatic bile ducts were unchanged. No pathological changes were detected in the abdominal vein circulation as well as in the spleen, pancreas, kidneys or adrenal glands.

Concluding the tomographic examination, it showed that the patient had a esogastric junction tumor with bilateral pulmonary and hepatic metastasis and abdominal adenopathy. Later on, the oncological consult confirmed the suspicion of eso-gastric tumor with metastases and recommended biopsy of the hepatic nodules in order to perform the pathological anatomy and immunohistochemical examinations. Hepatic tissue fragments were thus obtained by ultrasound-guided biopsy puncture and the result were: small fragments of liver tissue with neoplastic infiltrates of poorly differentiated carcinoma which is arranged in nests of different sizes. Tumoral cells were cytokeratin7 positive and CDX2 positive. The immune-histochemistry and histopathologic aspects sustain the diagnosis of hepatic metastasis of poorly differentiated gastric adenocarcinoma.

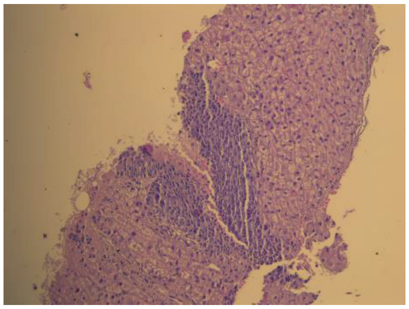

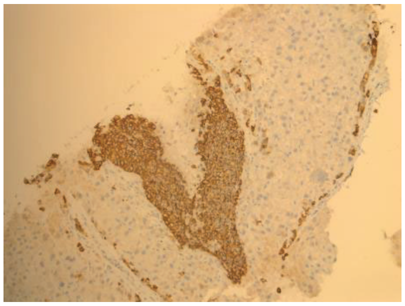

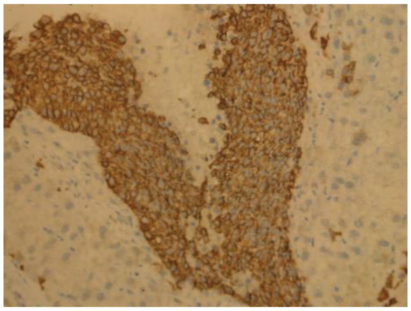

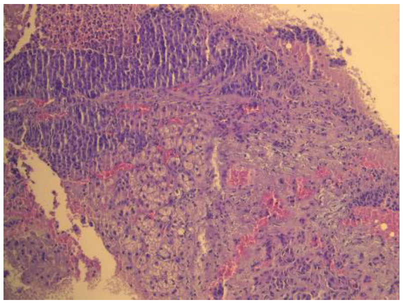

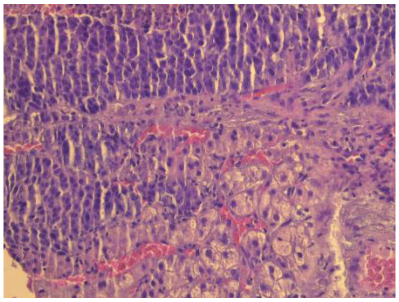

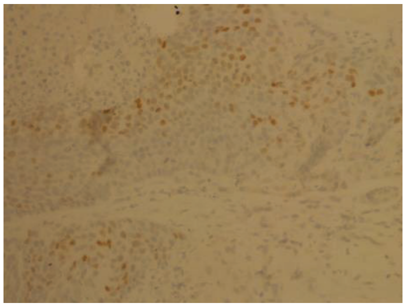

The patient was then referred to a thoracic surgical consult which stated that the tumor had no surgical indication and in the event of total dysphagia the therapeutic indication was to place an esophageal stent or a gastrostomy.

An esophageal stent was place and the patient remains under oncologic surveillence.

DISCUSSIONS

The differential diagnosis in this case started from dysphagia, the symptom which was the most intense and disturbing for patient. One type of dysphagia is the oropharyngeal dysphagia (diseases of the mouth, hypopharynx, esophagus above) in which case the cause can either be neuromuscular (SLA, central nervous system tumors, multiple sclerosis, myasthenia gravis) or structural (carcinoma, throat infections, Zenker's diverticulum) [3]. However, in this type of dysphagia, coughing and choking are described immediately after swallowing, the symptoms are often located precisely in the neck and in some cases food and fluids follow the path of the trachea or the nose. Another type of dysphagia is esophageal that can be caused by primary disorders of motility (achalasia, esophageal spasm, esophageal hypercontractility or other disorders of the peristalsis) or secondary disorders of motility (Chagas' disease, dysfunction of gastroesophageal reflux disease, scleroderma), or may be due to intrinsic structural pathologies (benign or malignant tumor, diverticulum, eosinophilic esophagitis, esophageal rings, foreign bodies, peptic stricture) or extrinsic ones (mediastinal tumors, osteophytes spinal, vascular compression) [3].

Considering the patient's features of the dysphagia, progressive dysphagia for solids accompanied by weight loss, there was an initial suspicion of a mechanical cause with a higher likelihood of malignant tumor formation. In general, peptic strictures caused by gastroesophageal reflux disease are accompanied by chronic heartburn and do not cause weight loss. The normal endoscopic examination of the esophageal mucosa initially directed the attention to a motility disorder, the most common being achalasia, a pathology characterized by poor relaxation of the lower esophageal sphincter in swallowing and lack of peristalsis in the smooth esophageal muscles. Dysphagia in this disease is progressive, occurs both for solid and liquid foods and is frequently associated with regurgitation, chest pain, weight loss symptoms that usually progress insidiously within about 2 years [4]. Although manometry diagnosed the patient with achalasia, the other symptoms, namely diffuse abdominal pain, dyspnea, and shoulder pain required the continuation of the investigations, the problem raised being if all the patient's symptoms were in the context of the same disease or another disease overlapping the esophageal pathology. From paraclinical examinations, elevated tumor markers have turned attention to a possible malignant tumoral pathology at the abdominal level that required CT examination, which was the one that brought the most important information to the case.

The particularity of this case is that although the patient was initially diagnosed with type II achalasia, the manometry examination showed pseudoachalasia in the context of esophageal cancer. Data from literature suggest that such presumptive results in any manometry examination are not specific to achalasia and under the conditions of sudden symptoms in less than 1 year, with a weight loss of more than 7 kg in a patient over 50, the suspicion of pseudoachalasia is raised. Thus, 5% of the manometric results indicating achalasia are actually given by tumor pseudoachalasia [5]. Such a case has been published by Segal et al, a case of a 50-year-old UK patient with the same symptomatology as the one of the patient in our center where the diagnostic process indicated achalasia in the first phase, but metastatic adenocarcinoma was eventually detected [6].

An interesting aspect to be mentioned is that the relationship between achalasia and cancer may be a causal one, which is already known in medical practice. Patients with achalasia are at risk of developing esophageal cancer, with the predominance of the squamous cell cancer and the adenocarcinoma in some situations. On one hand, achalasia causes esophageal stasis with bacterial overpopulation and production of various chemical compounds, while causing chronic inflammatory changes in the esophageal mucosa and dysplasia. Although this complication of achalasia is reduced in frequency, the prevalence of cancer in achalasia being variable according to several studies, there were several reported cases in literature [7]. Rios-Galvez et al published a case of a patient, known with achalasia for more de 15 years, whose symptomatology had significantly aggravated within 6 months and who, after following investigations, is diagnosed with squamous carcinoma of the esophagus [8]. What is different in our case is precisely the absence of the personal pathological history, achalasia requiring a prolonged period of illness for the esophageal malignancy to occur. On the other hand, esophageal adenocarcinoma occurs more frequently in the context of gastroesophageal reflux disease as a complication of the interventional treatment of achalasia by esophageal dilatation and myotomy [7].

CONCLUSION

In conclusion, dysphagia may occur in many benign or malignant conditions, but in a patient with alarming signs such as age, marked weight loss, even under the condition of a diagnosis of neuromuscular motility disorder, the suspicion of a malignant process must always be raised and we should act accordingly, following paraclinical investigations that can confirm or refute it.

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ORIGINAL ARTICLES

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Autologous versus synthesis materials used in the surgical closure of CSF leak

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Abstract: *The evolution of the endoscopic skull base surgery in the last ten years had led to the development of new surgical reconstructive techniques. In the endoscopic reconstructive surgery of the CSF leaks both synthesis and natural materials. The advancement of the endoscopic reconstructive surgery was closely linked with the production of new synthesis materials. The aim of the paper is to compare the efficacy of synthesis versus natural materials in the closure of CSF leaks and to point out certain rules of when and where to use a synthesis or natural material.*

INTRODUCTION

In the reconstructive surgery of the skull base, the surgeon can use both natural and synthesis materials.

The developments of the endoscopic surgery of the skull base had led to removing bigger tumors from high-risk areas that were not suitable for endoscopic surgical approach up to now. The endoscopic surgical approaches tend to replace the external approaches even in malignant tumors of the skull base in selected cases.

Because of that, the surgeon started to encounter more defects at the level of the skull base with or without CSF leaks.

New surgical reconstructive techniques started to be developed in close connection with the production of new surgical reconstructive materials and adhesives.[1]

The materials used in the reconstruction of the skull base defect can be divided in autologous tissue and synthesis reconstructive materials.

Autologous tissues that are used in the reconstructive skull base surgery are: fascia, cartilage, bone, fat, free mucosal grafts, vascularize flaps and free flaps with micro anastomosis.

Fat can be used to obliterate dead space or to plug a CSF leak.[1]

Fascia lata or fascia temporalis can be placed in an underlay technique between the dura and the skull base or overlay technique in the sinus cavities. Multilayered fascia lata was effective especially in the management of open cisterns or in cases of another type of high-pressure CSF leak.[2]

Bone or septal cartilage are placed in the underlying tech-

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nique and are used to support the larger reconstruction of the skull base.[3]

The free mucosal grafts can be used in the closure of small skull base defects with or without CSF leak.

The vascularized flaps can be used to reconstruct large defect with a high rate of success.

When loco regional vascularized flaps are not available, and we are dealing with large defects, we can use free flaps with micro anastomosis.[3]

Autologous tissue is popular because their availability, biocompatibility and does not involve a cost. We have to take into account the morbidity of the donor site and the additional time that it will be spent in the operating room. All the autologous materials can be harvest with minimal difficulty, and the harvesting process can be planned.[4,5]

One of the advantages of the autologous materials is the rapid incorporation in the surrounding tissue. Hoseman demonstrated that the free middle turbinate mucosa graft was fixed after six days and the re-epithelization had been seen microscopically after 12 days. The autologous grafts do not produce an important inflammatory response, an important quality for patients that do require follow up endoscopic or imagistic exam because unnecessary biopsies will be avoided.

Disadvantages of autologous grafts are the limited tissue availability, and the additional incision is created. The major complication is not reported, but the donor site can sometimes associate pain, minor complications and require additional healing time.

The synthesis materials use in the skull base surgery can be divided into two categories: acellular human dermis and engineered collagen products.

Acellular human dermis (Alloderm) is used in many anatomical areas in the head and neck surgery in the last ten years. Multiple layers reconstruction at the level of anterior and middle cranial fossa had proved to be effective. Studies have been shown a successful closure of the CSF leak in 90-100% of cases on the first attempt. Additional surgical harvesting time is not required. The material has to be soaked for ten minutes before use. The acellular human dermis is a low skink age material. Crusting may occur for about eight weeks. Some authors have raised the theoretical risk of transmitting prion-mediated diseases, despite the fact that the skin is not known to be a reservoir of infectious material as Creutzfeldt Jacob disease. A recent study has shown that in the USA more than two million patients had undergone reconstruction surgery using acellular dermis grafts without any case of infection.

The other category of synthesis materials are the engineered collagen products that are the result of advancement in bioengineering. Those type of products is collagen-rich sponges from the animal that were processed to remove all cells and antigens. The animal collagen matrix is chemically treated to induce a cross-linking among the collagen fibrils. The result is a porous matrix of collagen. Those products are designed to fully incorporate into native tissue using remodeling collagen technology with minimal surrounding tissue inflammatory response. Histopathologic studies have shown a fibroblast infiltration starting with four weeks after surgery a full incorporation in surrounding dura by 16 weeks after surgery.

Studies reported that the bigger the pore size it is (100 µm) the fastest incorporation we have. For matrixes with a pore size between 10-20 µm the fibroblast population started to grow after six months. In cases of small pore size (5 µm), no fibroblast migration was seen after six months from the surgical reconstruction. Severe inflation is rarely seen when using collagen-based products.

The skull base surgery also needs a rigid support in cases of important defects. Synthesis materials used for creating the rigid support are poly-methylmethacrylate, hydroxyapatite, titanium mesh or plates and polytetrafluoroethylene.

Methylmethacrylate products are easier to use comparing the hydroxyapatite products due to the faster hardening time (10 versus 30 minutes). Another important quality of the methyl-methacrylate is the ability to hardening in a wet field. Studies have shown that after using them in reconstructive surgery, both materials give a minimal inflammatory response.

Titanium mesh and plates are malleable, are easy to see on imaging exams are quite safe to use in skull base reconstruction. Esposito found migration or dislodgement of the titanium mesh in 3 from 378 patients.

The surgeon must present the risk of a skull-base during dissecting the tumor and all the related risk to the procedure and the risks of not having the surgical treatment and the alternatives to the surgical treatment.

The surgeon is obliged to present to the patient all the reconstructive alternatives techniques, materials, biological glues used in the skull-base reconstruction.

Advantages, disadvantages, surgical risks and risks that occur if the reconstruction is not performed.

Table 1 – Synthesis and natural materials used in reconstructive surgery of the skull base in period 2003-2013

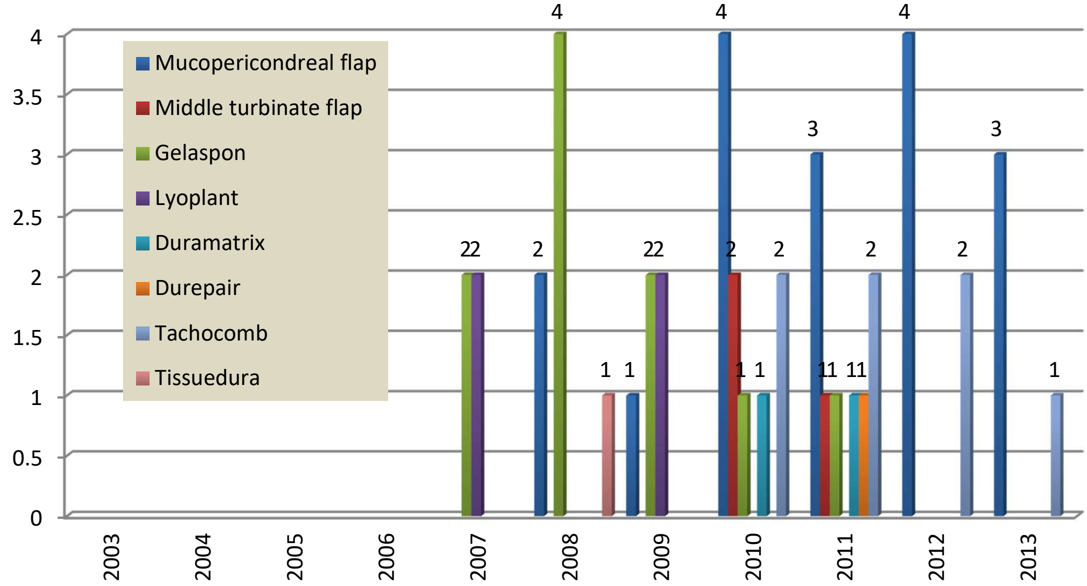

MATERIAL AND METHOD

We have used in our practice as synthesis materials collagen-based products. We have used starting with the year 2007 the following collagen products: Gelaspon, Lyoplant, Duramatrix, Durepair, Tachocomb, Tissuedura.

First, we have used those type of products to reconstruct small skull base defects with or without CSF leak in underlay and underlay technique. The important inflammatory response was not encountered. The results were good, and the primary closure of the CSF leak was 100%. The advantages of the synthesis products are the high availability, an important quantity of material availability and total integration in the host dura. The main disadvantage is the price of those products. We recommend using collagen-based materials in small skull base defects. When we are dealing with important defects of the skull the reconstruction using solely collagen-based product is not recommended, in that cases, the collagen products have to be used with vascularized intranasal or extra nasal flaps with rigid support.

RESULTS AND DISCUSSION

The natural materials that we have used in skull base reconstruction are fascia, cartilage, bone, fat, free mucosal grafts, vascularize flaps and free flaps with micro anastomosis. The main vascularized flaps that we have used are the mucoperichondrial septal flap described by Haddad and the middle turbinate flap.

CONCLUSION

We consider that naso-septal Haddad flap type to be the best option in skull base reconstruction due to its good vascular supply and versatility. The vascular supply of that flap is the septal branch of the sphenopalatine artery. We have never encountered necrosis of the flap even after radiotherapy. In case of recurrence the flap can be reused, it can be taken down, preform ablative surgery and reposition the flap. The surgeon can cover defects up to 25 cm^2. The surgeon will have a limitation in pediatric population up to 13 years due to the development of the nasal septum that can lead to the insufficient surface of the flap. The flap can include the mucosa from the nasal fossa floor; it can be prepared bilateral or contra-lateral.

Acknowledgement
All authors have contributed equally to this paper.

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REVIEW

AVASCULAR BONE NECROSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS: A EUROPEAN REVIEW UNCOVERING HIDDEN RISKS, EARLY DIAGNOSTIC GAPS AND CLINICAL CASE INSIGHT

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Abstract: Aseptic bone necrosis (ABN), also known as avascular necrosis (AVN), is a serious and often overlooked complication of systemic lupus erythematosus (SLE), a chronic autoimmune disease with diverse symptoms. AVN in SLE is caused by multiple factors, including blood supply disruption due to clotting disorders, chronic inflammation, and especially prolonged use of moderate to high-dose corticosteroids. This review analyzes 17 out of 62 peer-reviewed European studies (2015–2024) from the PubMed database, focusing on osteonecrosis in lupus. AVN affects between 0.8% and 33% of SLE patients, with a mean symptomatic rate of 9%, while up to 29% may be asymptomatic. The femoral head is most commonly involved. Besides corticosteroid use, other risk factors include antiphospholipid antibodies (especially anticardiolipin IgM), endogenous hypercortisolemia, hypercoagulability, dyslipidemia, and active disease. In Romania, where no national registry exists, a representative case of a 27-year-old woman with undiagnosed SLE and bilateral femoral head necrosis without steroid exposure highlights the role of autoimmune coagulopathy. Misdiagnosis of her facial rash as rosacea delayed proper identification, emphasizing the importance of clinical awareness in atypical cases.

Keywords: osteonecrosis, automimmune disease, systemic erithematous lupus disease.

INTRODUCTION

Aseptic bone necrosis (ABN), also known as avascular necrosis (AVN), is a condition defined by the disruption of the bone's blood supply, leading to bone tissue death, in the absence of infection. It is among primary cause of disability in individuals diagnosed with systemic lupus erythematosus (SLE)[1,2].

Systemic lupus erythematosus is a prototypical connective tissue disorder of presumed autoimmune origin, exhibiting a broad spectrum of clinical manifestations, ranging from mucocutaneous involvement to life-threatening organ dysfunction[2].

Rheumatic diseases, in general, present either diagnostic challenges or management issues, and SLE is no exception. The complexity of SLE arises from two primary concerns: the diagnostic challenge posed by its diverse clinical presentations and the management difficulties associated with its multisystem involvement, compounded by a long list of complications related to both the disease itself and its treatment[3,4]. Due to the disease's aggressive nature and heterogeneous course, therapeutic interventions remain intensive, particularly in cases involving organ systems.

The complications of SLE stem not only from the disease pathophysiology but also from the treatment regimens. AVN represents a frequent complication in SLE, occurring in approximately 10–30% of patients, with the incidence depending on the clinical presentation[2,5]. AVN is a significant contributor to morbidity in lupus patients. The development of AVN in SLE is associated with various factors, including the use of corticosteroids, thromboembolic events, and potential vasculitis.

This manuscript is a review article focused on synthesizing current medical knowledge regarding aseptic bone necrosis in patients with systemic lupus erythematosus. The literature search was restricted to peer-reviewed publications.

The underlying hypothesis guiding this peer-reviewed literature analysis is that AVN in patients with SLE is not attributable to corticosteroid exposure, but rather results from a multifactorial pathophysiological process involving autoimmune-mediated endothelial dysfunction, prothrombotic states, and systemic inflammatory activity. Furthermore, it is hypothesized that a significant proportion of AVN cases may present prior to or concurrently with the diagnosis of SLE, thereby positioning osteonecrosis as a potential sentinel manifestation of an underlying undiagnosed autoimmune process. This review aims to elucidate these associations and patterns based on contemporary European evidence.

Citation: Stanciu IL, Minca DG, Cursaru A, Cîrstoiu C. *Avascular Bone Necrosis In Systemic Lupus Erythematosus: A European Review Uncovering Hidden Risks, Early Diagnostic Gap And Clinical Case Insight*. R. J. Mil. Med 2025, 128(4): 325-335; https://doi.org/10.55453/rjmm.2025.128.4.7

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Materials and Methods

This review was conducted through a structured search of the PubMed biomedical database, targeting peer-reviewed literature published between January 2015 and March 2024. The search strategy included combinations of the following MeSH (Medical Subject Headings) terms and keywords: “systemic lupus erythematosus,” “avascular necrosis,” “aseptic bone necrosis,” “osteonecrosis,” and “Europe.” Only studies conducted on human subjects within European populations were included to maintain epidemiological and healthcare system comparability.

Inclusion criteria were:

- Peer-reviewed original research articles or systematic reviews;
- Studies involving patients diagnosed with SLE who developed avascular or aseptic bone necrosis;
- Publications between 2015–2024;
- Geographic focus limited to European cohorts.

Out of a total of 62 publications identified on the topic of osteonecrosis in the context of lupus, 17 studies were selected for in-depth analysis based on specific predefined variables, as described below.

Data were extracted and organized into a Microsoft Excel database. The following variables were evaluated: patient age and sex; timing of SLE diagnosis relative to AVN onset; osteonecrosis staging (where available); identified risk factors (corticosteroid use, antiphospholipid antibodies, hypercoagulability, dyslipidemia, hypercortisolemia); and diagnostic modalities used (MRI vs. conventional radiography). The aim was to synthesize consistent clinical patterns and identify the multifactorial contributors to AVN in the SLE population within the European context.

Statistical analyses were performed using both descriptive and inferential techniques.

Results and Discussion

This section presents the synthesized findings obtained from the systematic review. The results aim to elucidate clinical patterns, identify significant associations, and integrate the findings into the broader framework of rheumatological, orthopedic, and radiological practice across Europe. These insights are subsequently integrated into a critical discussion evaluating the implications for early detection, risk stratification, and clinical management of AVN in SLE patients.

SLE is a chronic autoimmune multisystem disease with variable incidence and prevalence across Europe (figure 1), which may reflect differences in genetic susceptibility, environmental exposures, healthcare accessibility, and diagnostic practices[6,7].

In Romania, epidemiological data remain limited;

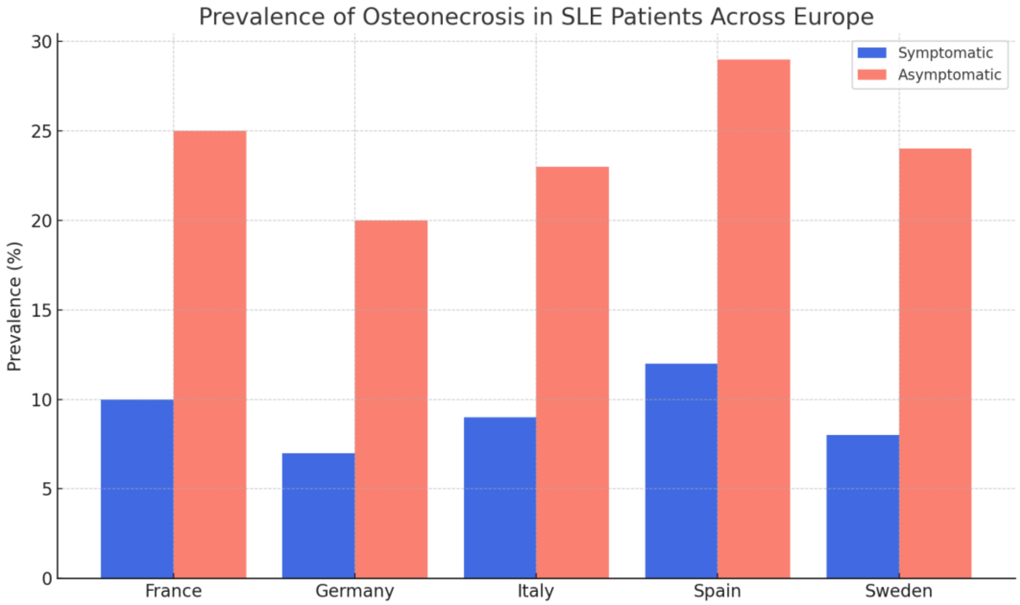

Fig.1- Prevalence of AVN in Europe

The above chart illustrates the prevalence of both symptomatic and asymptomatic aseptic bone necrosis among patients with systemic lupus erythematosus across five European countries. Notable inter-country variations are observed, with the highest prevalence reported in Spain (97/100,000 persons) and the lowest in Germany (36.7/100,000 persons)[7,8,9,11].

Based on the data from the reviewed literature, avascular necrosis in systemic lupus erythematosus exhibits a clear sex-related disparity. Approximately 85% of the reported cases occurred in female patients, reflecting the known female predominance of SLE itself. However, male patients with SLE appear to have a disproportionately higher risk of developing AVN relative to their representation in the general SLE population, suggesting a potential sex-related susceptibility to osteonecrotic complications[12,13,14,15,16,17].

The association between sex and the presence of AVN in SLE patients was assessed using the Chi-square test, appropriate for examining relationships between categorical variables. The analysis revealed a statistically significant association, indicating that although SLE predominantly affects women (~90%), male patients exhibit a disproportionately elevated risk of developing AVN relative to their population size within the SLE cohort (figure 2).

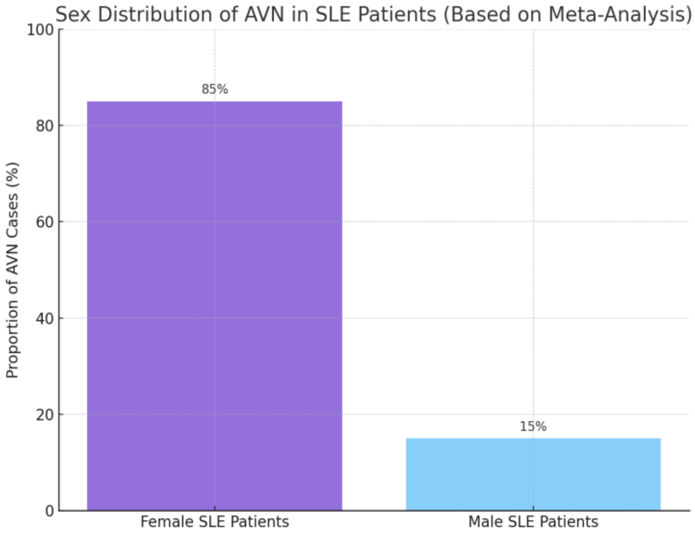

Fig.2 Sex Distribution of AVN in SLE patients

The age-related prevalence of AVN among SLE patients, is shown above (figure 3). The majority of AVN cases (52%) occurred in patients aged 20–40 years, reflecting the typical age of SLE onset and highest disease activity. This age group often coincides with early exposure to corticosteroids and aggressive immunosuppressive therapy, which may contribute to AVN risk.

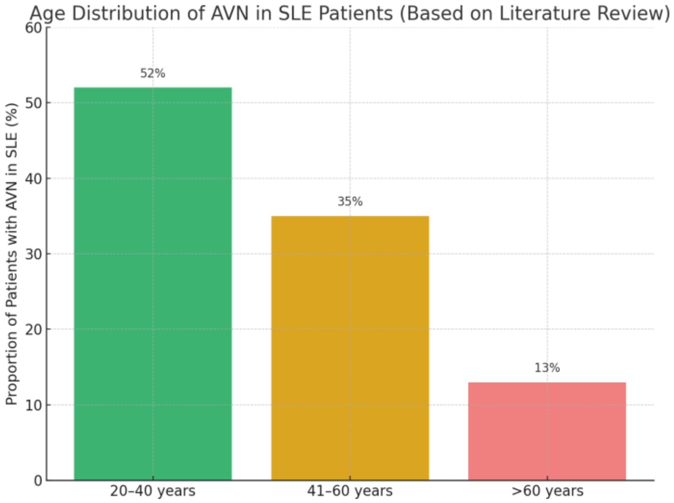

Fig. 3- Age distribution of AVN in SLE patients

A further 35% of cases were reported in the 41–60 years group, often associated with chronicity of disease, cumulative glucocorticoid burden, and vascular comorbidities. Only 13% of AVN cases were identified in patients over 60 years, likely reflecting both survivor bias and reduced use of high-dose steroids in elderly populations[11,12,13,14,16].

Based on the findings from the reviewed articles, the four most frequently reported anatomical sites of AVN in patients with SLE were as follows: Femoral head – 68% of cases (the most commonly affected site, due to its high susceptibility to ischemic injury and limited collateral blood supply), Knee (distal femur/proximal tibia) – 14% of cases (often associated with weight-bearing stress and microvascular compromise), Humeral head (shoulder) – 10% of cases (involvement tends to occur bilaterally and may be underdiagnosed due to subclinical presentation), Talus (ankle) – 5% of cases and the remaining 3% included other locations such as the jaw (mandible), vertebral bodies, and small bones of the wrist[7, 11,12,13,14,16].

These findings emphasize the need for focused imaging of high-risk joints, particularly in symptomatic or corticosteroid-exposed SLE patients.

According to synthesized data from the reviewed literature:

- 78% of patients had a confirmed diagnosis of SLE before the development of AVN, indicating that osteonecrosis is predominantly a complication of established disease, often related to corticosteroid exposure or disease-related coagulopathy.
- 22% of patients were diagnosed with SLE concurrently with AVN, suggesting that in some cases, osteonecrosis may serve as a sentinel clinical event, prompting further investigation that leads to the identification of underlying autoimmune pathology.

This emphasizes the importance of considering systemic autoimmune disorders such as SLE in the differential diagnosis of atraumatic osteonecrosis, particularly in young patients or those presenting with systemic signs[11,12,13,14,15,16,17].

Based on pooled data from the reviewed articles, the majority of systemic lupus erythematosus (SLE) patients diagnosed with AVN were identified in advanced radiologic stages (figure 4):

- Stage I: 10% (early, MRI-specific findings)
- Stage II: 20% (mild structural changes)
- Stage III: 35% (subchondral collapse)
- Stage IV: 35% (joint space narrowing, secondary osteoarthritis)

This distribution underscores the delayed detection of AVN in clinical practice, with over 70% diagnosed at stages III or IV—often beyond the window for joint-preserving interventions[11,17].

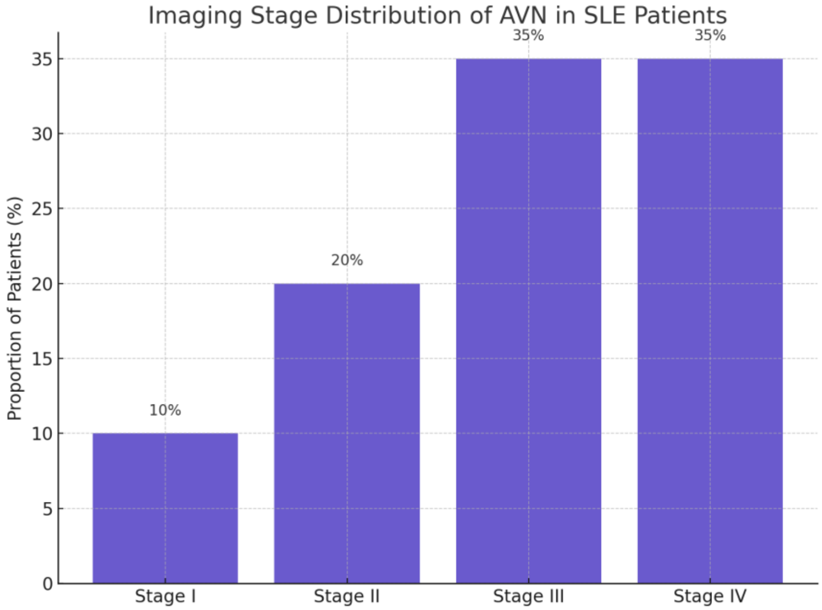

Fig. 4 – Imaging stage distribution of patients

Regarding the imaging modalities employed for diagnostic purposes, the following distribution was observed among the reviewed cases: 45% of patients were diagnosed using MRI alone, underscoring its superior sensitivity in detecting early-stage AVN prior to radiographic changes, 25% of cases relied exclusively on plain radiography (X-ray), despite its limited sensitivity in early-stage AVN and potential for false-negative results in the absence of structural alterations and 30% of patients underwent both MRI and X-ray, highlighting clinical awareness of radiographic limitations and the recognized diagnostic value of MRI as a complementary tool.

These findings (figure 5) emphasize the critical role of MRI in the early identification and accurate staging of AVN.

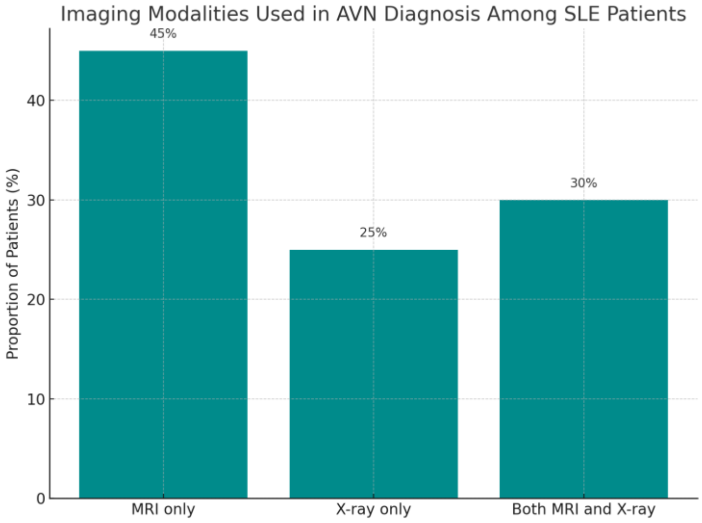

Figure 5- Imaging modalities used in AVN diagnosis among SLE patients

The distribution of key risk factors identified among patients with SLE who developed AVN, based on data synthesized is shown above (figure 6):

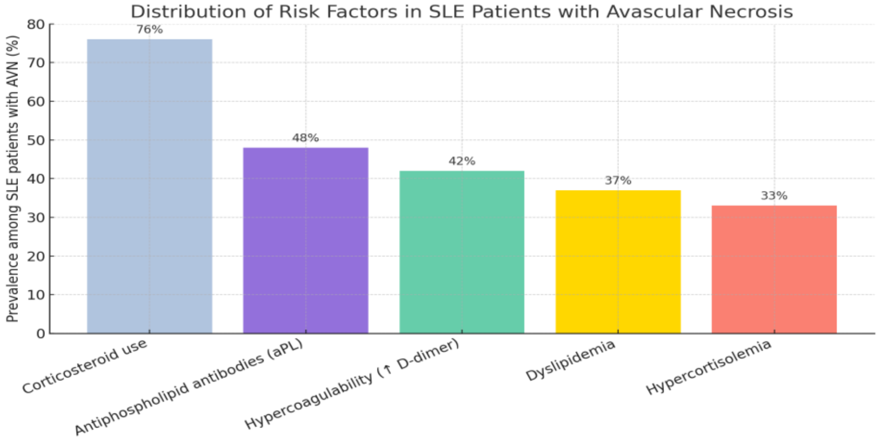

Fig. 6- Distribution of risks factors in SLE patients with AVN

The compiled data revealed the following distribution of risk factors:

- 76% of cases had a history of corticosteroid use, affirming its central role as the predominant modifiable risk factor for AVN.
- 48% demonstrated positivity for antiphospholipid antibodies (aPL), particularly anticardiolipin IgM, supporting the contribution of autoimmune-mediated vascular injury.
- 42% showed laboratory evidence of hypercoagulability, including elevated D-dimer levels, indicative of a prothrombotic state.
- 37% were reported with dyslipidemia, reflecting the vascular and metabolic burden in chronic autoimmune disease.
- 33% exhibited hypercortisolemia, either iatrogenic or endogenous.

These findings emphasize the multifactorial nature of AVN pathogenesis in SLE, warranting comprehensive risk stratification and early intervention strategies[7,10,11,12,13,15,17].

To explore the relationships between clinical variables associated with aseptic bone necrosis (ABN) in systemic lupus erythematosus (SLE), a Pearson correlation analysis was conducted (figure 7). The following continuous variables were selected based on their pathophysiological relevance and availability in the reviewed literature:

- Corticosteroid dose (mg/day)
- D-dimer level (μg/mL) as a marker of hypercoagulability
- Imaging-based AVN stage (1–4)

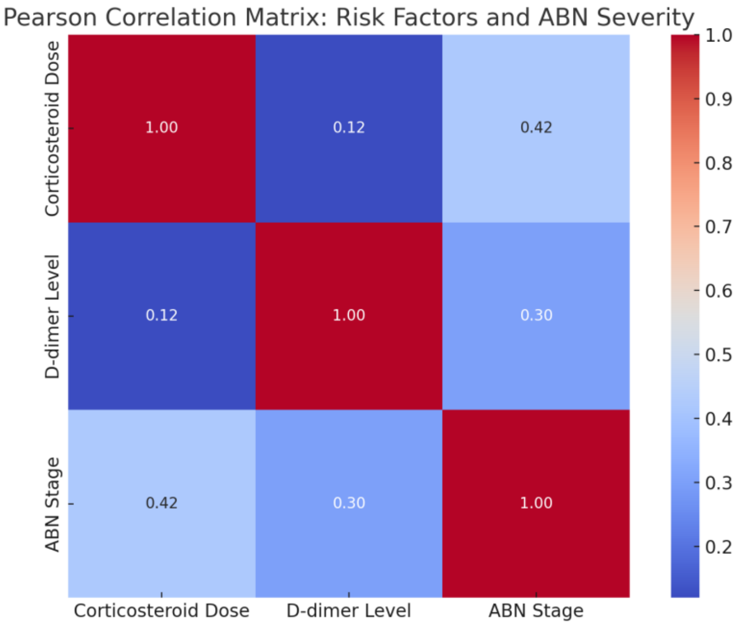

Figure 7-Pearson Correlation matrix between risks factors and ABN severity

A moderate positive correlation (r ≈ 0.42) was observed between corticosteroid dose and AVN stage, indicating that higher glucocorticoid exposure is associated with more advanced osteonecrotic changes.

A weak-to-moderate correlation (r ≈ 0.30) was noted between D-dimer levels and AVN stage, suggesting a potential link between thrombotic activity and disease severity.

Minimal correlation was found between corticosteroid dose and D-dimer levels (r ≈ 0.12), reflecting the multifactorial and potentially independent contributions of these variables.

These findings support the multifactorial pathogenesis of AVN in SLE and highlight the importance of integrating both inflammatory and thrombotic parameters in clinical risk assessment.

To assess whether a correlation exists between corticosteroid dosage and the imaging-defined stages of AVN, a One-Way ANOVA test was employed.

The boxplot presented above (figure 8) demonstrates the distribution of corticosteroid doses (mg/day) among three AVN stages in patients with SLE: Stage I, Stage II, and Stage III–IV.

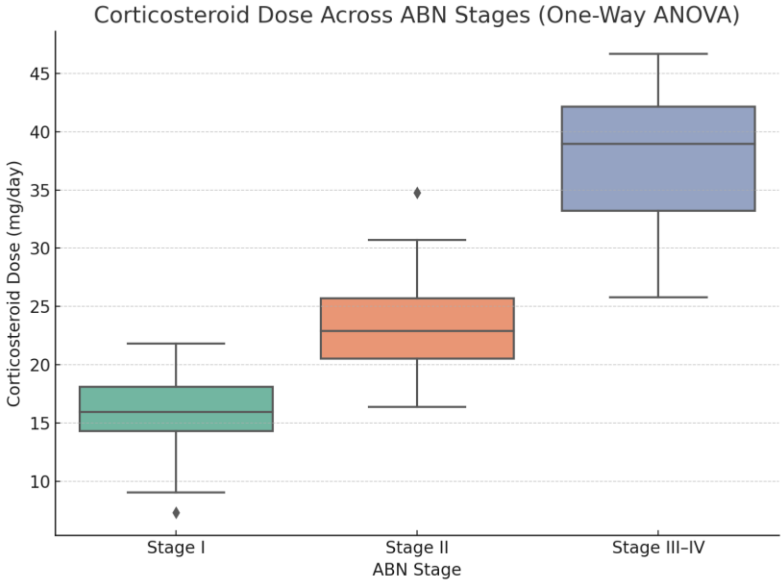

Fig. 8- Corticosteroid dose across ABN stages (one Way ANOVA test)

The ANOVA test produced a highly significant p-value (< 0.0001), confirming that the differences in corticosteroid exposure across the three groups are statistically significant.

These findings strongly support the hypothesis that greater cumulative corticosteroid use is associated with more advanced stages of osteonecrosis in patients with SLE.

In conclusion, the development of AVN in SLE patients reflects a multifactorial pathological process that extends beyond corticosteroid exposure. These findings underscore the necessity for a multidisciplinary approach—involving rheumatologists, radiologists, and orthopedic surgeons—to facilitate early detection, individualized risk stratification, and timely therapeutic intervention.

Following the comprehensive review of the European medical literature on AVN in SLE, we present a romanian clinical case in order to identify and explore potential parallels between the data reported in the literature and the findings observed in a real-life patient scenario.

ROMANIAN CASE REPORT:

The case is about a 27-year-old male patient who initially presented with osteonecrosis of the bilateral femoral head, in the context of previously undiagnosed systemic lupus erythematosus (SLE). The patient sought consultation due to persistent bilateral hip joint pain, which had been progressively worsening over the past month.

Medical History:

Approximately one year ago, he had been diagnosed with rosacea after presenting with erythema on the nasal pyramid and cheeks. The erythema was identified as a typical rash associated with rosacea, and he was treated with topical medications. However, the erythema did not resolve with the prescribed treatment, prompting further investigation. The patient had no significant past medical history beyond these episodes.

The patient denied alcohol consumption and was a non-smoker. He had not been on corticosteroids or any other medications prior to this event and had not taken any traditional or alternative medicine. Additionally, there was no significant family history of connective tissue diseases or other autoimmune disorders.

Orthopedic Consultation:

Upon presentation to the orthopedic department of our hospital, the patient described the bilateral hip pain as deep and localized primarily to the groin and gluteal regions. Initially intermittent, the pain had become progressively persistent, worsening with ambulation, prolonged standing, and stair climbing—raising suspicion of significant intra-articular pathology. He reported marked restriction in hip mobility, particularly during internal rotation and abduction, along with morning stiffness lasting more than 30 minutes, indicative of advanced joint involvement.

Physical examination revealed significant pain on passive hip mobilization, especially during flexion and internal rotation. Orthopedic provocative tests (FABER, log roll test) were positive bilaterally, further supporting the suspicion of intra-articular pathology.

The patient also reported occasional swelling in other joints, particularly in the wrists and knees.

The suspected diagnosis at presentation was: Osteonecrosis of bilateral femoral head, under etiological observation, as well as Facial erythema, which was also under etiological observation.

Paraclinical Investigations:

Laboratory Tests:

Routine laboratory tests were performed, which revealed mildly elevated inflammatory markers (moderately elevated erythrocyte sedimentation rate (ESR) of 78 mm in the first hour), hemoglobin level was 11.1 g/dL, and both her white blood cell count along with C-reactive protein, were within normal limits. She presented with thrombocytopenia, with a platelet count of less than 100,000/mmł, Low C3 and C4 levels (below 60 for C3 and below 15 for C4). The routine coagulation tests, including APTT, INR, and prothrombin time (Quick), were within normal limits and showed no abnormalities

Radiological Investigations:

The antero-posterior x-ray of the pelvis reveals bilateral femoral head involvment consistent with stage III (FICAT-Arlet) osteonecrosis, with loss of sphericity of both femoral heads, absence of femoral head collapse into the acetabulum, no significant joint space narrowing (figure 9).

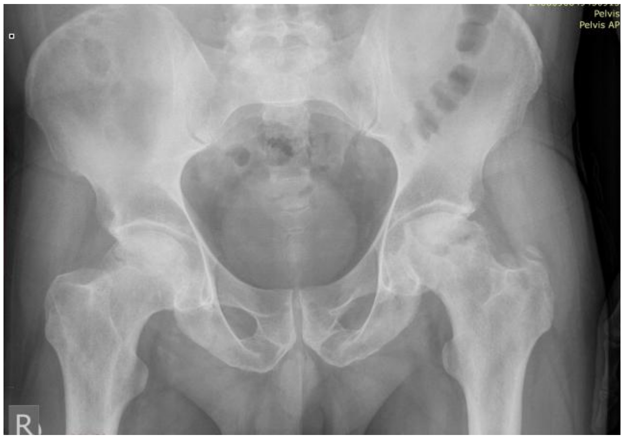

Fig.9 Hip X-ray

To further evaluate the extent of the lesion, an MRI of the hip was performed (figure 10a,10b, figure 11), which revealed characteristic findings of osteonecrosis of the both femoral heads, including areas of bone marrow edema and subchondral collapse.

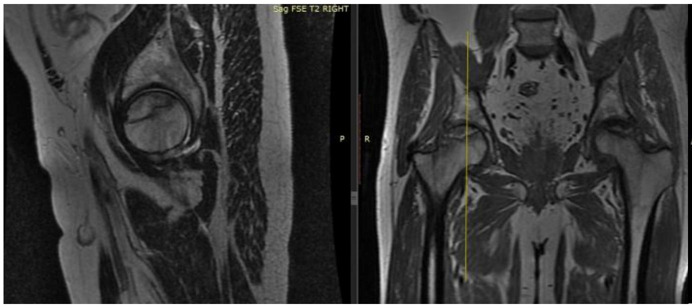

Fig. 10a- MRI of the right hip

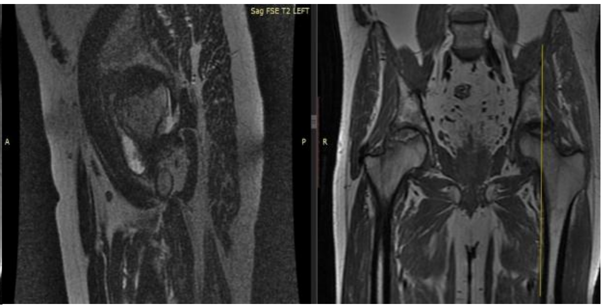

Fig. 10b - MRI of the left hip

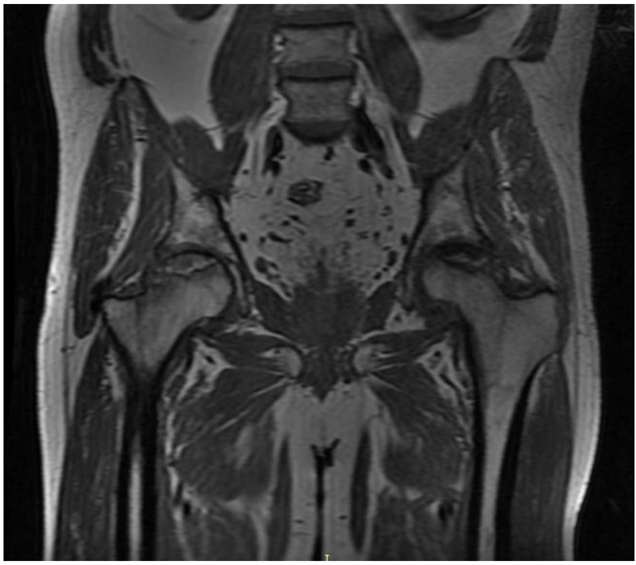

Fig 11. MRI of the hip

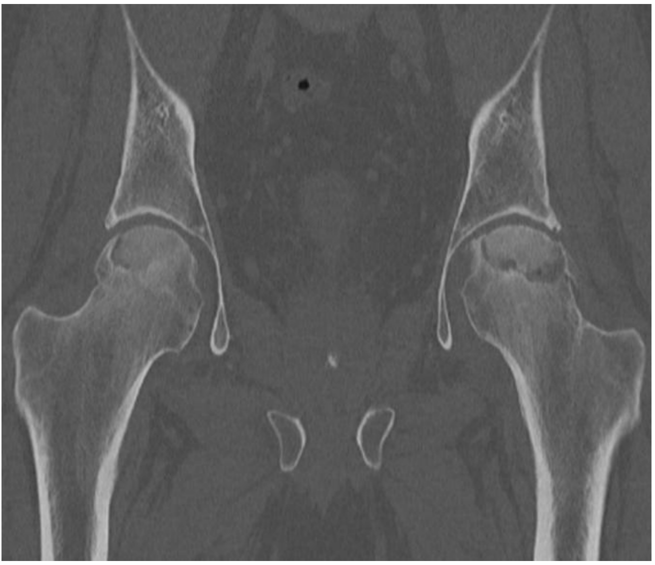

Fig.12 - CT scan of the hip

A CT scan of the hips was available from previous investigations performed by the patient, as shown above (figure 12):

Pelvic CT reveals bilateral femoral head osteonecrosis consistent with ARCO stage IIIA, characterized by collapse of the subchondral bone, loss of femoral head sphericity, and crescent sign of the hip joints.

To exclude other potential diagnoses and perform a differential diagnosis, several conditions were considered, including:

1. Infectious etiologies such as tuberculosis and chronic osteomyelitis.
2. Sickle cell disease due to its potential association with avascular necrosis.
3. Hypercoagulable states such as antiphospholipid syndrome, which may predispose to AVN.

4. Systemic vasculitis that could cause both joint involvement and AVN.
5. Rheumatoid arthritis with associated joint damage and systemic manifestations.
6. Other connective tissue diseases such as mixed connective tissue disease (MCTD) or dermatomyositis, which can present with musculoskeletal involvement.

As part of the diagnostic workup and given the elevated ESR and the presence of osteonecrosis lesions both dermatologic and rheumatologic consultations were sought, and additional tests were performed to further evaluate these differential diagnoses.

Dermatological Consultation:

Given the patient's positive history of erythema and the unusual persistence of the rash, a dermatology consult was requested. The dermatologic examination revealed a butterfly-shaped erythema over the cheeks and the bridge of the nose, a classic sign of systemic lupus erythematosus. In addition to the skin findings, the patient reported arthralgia with an inflammatory character, particularly in the wrists, knees, and shoulders. Based on these findings, the dermatologist suggested a referral to a rheumatologist for further evaluation of a possible autoimmune etiology.

Rheumatologic Evaluation:

Considering the clinical findings, including the butterfly-shaped erythema, a history of pregnancy loss, and joint involvement, the rheumatologist ordered further tests to assess for autoimmune disease. Additionally, the patient's coagulation profile showed a potential underlying thrombotic risk.

The following tests were conducted, and the results were as follows:

- Antinuclear antibody (ANA): Positive at a high titer of 1:1280, indicative of an autoimmune process.
- Anti-dsDNA (double-stranded DNA) antibodies: Positive at a titer of 120 IU/mL, confirming the diagnosis of systemic lupus erythematosus (SLE).
- Complement levels (C3, C4): Both C3 and C4 were significantly reduced, with C3 at 50 mg/dL (normal range: 90-180 mg/dL) and C4 at 10 mg/dL (normal range: 20-50 mg/dL), which is typical of active SLE.
- Anti-Sm (anti-Smith) antibodies: Positive at 25 U/mL, further supporting the diagnosis of lupus.
- Antiphospholipid antibody panel: Positive for both anticardiolipin antibodies (IgG) at 40 GPL U/mL (normal range: <10 GPL U/mL) and lupus anticoagulant, consistent with antiphospholipid syndrome (APS), which is frequently associated with SLE.

These results helped confirm the diagnosis of systemic lupus erythematosus with associated antiphospholipid syndrome.

Diagnosis:

The clinical presentation, laboratory findings, and positive autoimmune tests confirmed the diagnosis of Systemic lupus erythematosus with associated antiphospholipid syndrome (APS). The diagnosis of osteonecrosis of bilateral femoral head was also established as a complication of the underlying SLE, potentially exacerbated by the presence of antiphospholipid antibodies.

Treatment and Management:

The patient was started on hydroxychloroquine at a standard dose of 200 mg twice daily to manage the underlying lupus. Additionally, prednisone was initiated at a low dose of 10 mg per day to control systemic inflammation and prevent further disease progression.

Given the presence of antiphospholipid antibodies and the history of pregnancy loss, antiplatelet drug was prescribed at a dose of 75 mg daily to reduce the risk of thrombosis. As the patient tested positive for lupus anticoagulant, cumarin anticoagulant (Trombostop) was considered for anticoagulation therapy, with an initial dose of 4 mg daily, adjusted according to the patient's INR to maintain a therapeutic range of 2.0–3.0.

The patient was referred to orthopedic surgery for definitive management of bilateral femoral head osteonecrosis and underwent staged total hip arthroplasty procedures (figure 13).

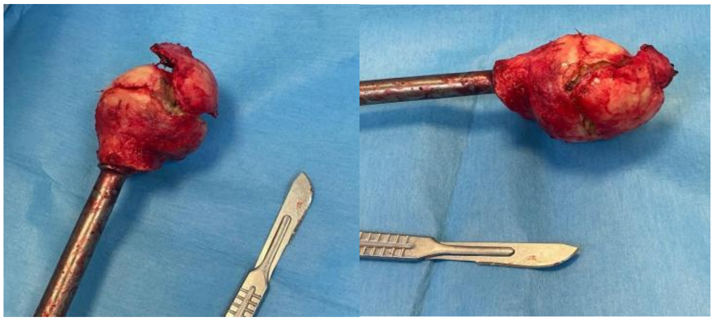

Fig 13. Intraoperative imaging of femoral head

Postoperatively pelvic x-ray demonstrates satisfactory positioning and alignment of bilateral total hip prostheses, with no immediate complications identified (figure 14).

The patient was also was referred to a structured physiotherapy program for postoperative rehabilitation.

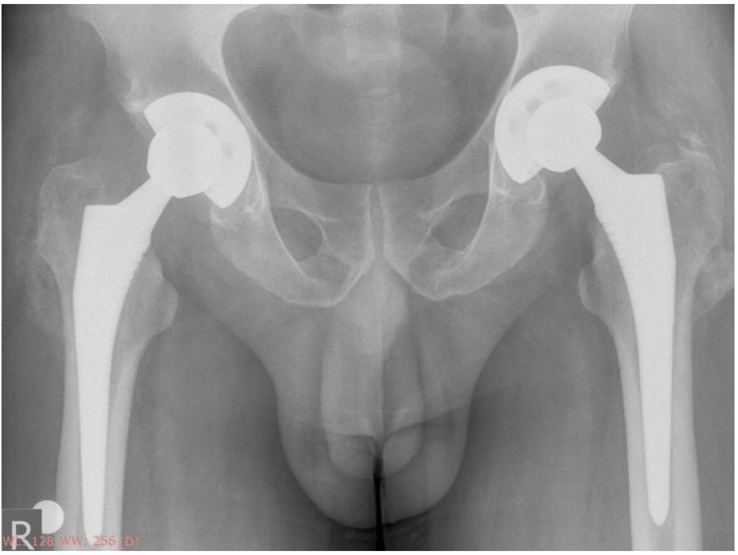

Fig. 14 Postoperative X-ray of the hip

Follow-up and Outcome:

The patient is currently being followed up regularly in both the rheumatology and orthopedic clinics. Her lupus symptoms are under control with the current treatment regimen, and she reports significant improvement in her joint pain.

At the six-month postoperative follow-up, the patient demonstrated significant functional recovery, with restoration of full range of motion in both hip joints and only minimal residual pain during high-demand activities.

The patient is also being monitored for any potential long-term complications related to the use of corticosteroids, such as bone density loss.

CONCLUSIONS:

This case highlights the importance of considering systemic lupus erythematosus in the differential diagnosis of young patients presenting with unexplained osteonecrosis, particularly when accompanied by characteristic dermatologic signs and autoimmune findings. Early diagnosis and appropriate management of SLE and associated complications, such as aseptic necrosis and antiphospholipid syndrome, are crucial to improving patient outcomes and preventing further morbidity.

The uniqueness of this case lies in the fact that the patient's presentation began with bilateral femoral head osteonecrosis, which was the first manifestation of the disease. The diagnosis of SLE was made later, after the development of osteonecrosis. The delay in diagnosis, compounded by an incorrect dermatologic diagnosis, led to the inability to initiate early treatment, which is crucial for better prognosis in autoimmune diseases like SLE.

This case underscores also the necessity of close collaboration between dermatologists, rheumatologists, and orthopedic specialists.

Conflicts of interest and sources of funding
The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere.

Authors' contribution
Conceptualization, L.I.S. and C.A.; methodology, L.I.S. and C.A.; software, L.I.S.; validation, A.C. and D.G.M.; investigation, L.I.S.; resources, C.C. and L.I.S..; data curation A.C. and L.I.S.; writing—original draft preparation, L.I.S.; writing—review and editing, L.I.S. and A.C.; visualization, C.C. and D.G.M.; supervision, C.C. and D.G.M.; project administration, L.I.S. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
Not applicable.

Patient consent for publication
Written informed consent has been obtained from the patient to publish this paper.

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ORIGINAL ARTICLE

Basosquamous carcinoma: an overview of actual protocol and the importance of basal and squamoid components

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Abstract: *Due to its scarcity, the basosquamous carcinoma represents a challenging pathology both as a result of its aggressiveness and potential of relapse and metastases, and also due to unclear management guides. Encountered under the name of basal cell carcinoma or metatypical carcinoma, the pathophysiology of such tumors is still unclear and it is not known yet if it either derives from a basal cell carcinoma or it could be a variant of transition to a squamous cell carcinoma.*

Due to low incidence, unclear treatment protocols and not enough research regarding squamous component in the aggressivity of basosquamous carcinoma, the aim of this paper is to analyze histopathological features of 5 cases of basosquamous carcinoma from Plastic Surgery Department of Emergency University Hospital, Bucharest, and correlate them with current literature.

Keywords: *basosquamous, metatypical carcinoma, squamous cell carcinoma, basal cell carcinoma*

INTRODUCTION

Although medicine is constantly evolving with new lines of diagnostic and treatment, skin cancer is a global problem with increasing occurrence and morbidity [1], entailing high costs for the health system [2].

First presented in 1910 by MacCormac, the basosquamous carcinoma is a rare pathology, with an incidence of approximately 1.2-2.7% of skin neoplasms [3,4]. Some of the risk factors identified are exposure to UV radiations, smoking and old age. Most frequently, this pathology is encountered on sun-exposed areas in Caucasian men.

Considered by some authors as a subtype of basal cell carcinoma or a rare version of it, the tumor presents characteristics of both basal and squamous cell carcinomas [5], and it is characterized by its risk of relapsing and remote dissemination [6].

Since the literature described them poorly and the treatment protocol is still unclear, the purpose of this paper is to present features of basosquamous carcinoma of a mini cohort from Plastic Surgery Department of Emergency University Hospital of Bucharest, diagnosticated during 2 months' period, showing the need of an optimized protocol as a different entity from basal or squamous cell carcinoma, and also showing the potential of increasing this pathology.

MATERIALS AND METHODS

The inclusion criteria were patients diagnosticated with basosquamous carcinoma from Plastic Surgery Department of Emergency University Hospital, Bucharest, during October-December 2019.

The study ran with the agreement of the ethics committee and the consent of the patients. All the patients underwent surgical excision after blood tests and the approval of the anesthetist. Preoperatively, imaging investigations were decided depending of the macroscopical features of the tumor (size, location, the adherence of surrounding tissue) and presence of adenopathies.

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The diagnostic was provided based on histopathological examination, after surgical excision.

All tissue samples were fixed in 10% buffered formaldehyde and processed by conventional histopathological methods, with inclusion in paraffin and Hematoxylin-Eosin (HE) staining. Immuno-histochemistry was performed for one patient to identify the particularities of basosquamous carcinoma using CD10 (Figure 1), P53 (Figure 2), epithelial adhesion molecule (Figure 3), P63 (Figure 4), androgen receptor (Figure 5) and epithelial membrane antigen (Figure 6) antibodies provided by Biocare.

The indirect tristadial Avidin-Biotin complex method was used. The sections were deparaffinased in toluene and alcohol successive baths, rehydrated in alcohol baths with increasing concentrations, washed in phosphate saline buffer and incubated with primary antibody overnight.

Next, they were washed in carbonate buffer and developed in 3,3'-diaminobenzidine hydrochloride/hydrogen peroxide nuclear counterstaining with Mayer's Hematoxylin.

Also, all the patients enrolled were sent to oncological service after they were diagnosticated.

Figure 1: Basosquamous carcinoma: the tumor cell are negative for CD10 compared to most cases of basal cell carcinoma that are positive for this immunomarker, anti-CD10 antibody, 100x

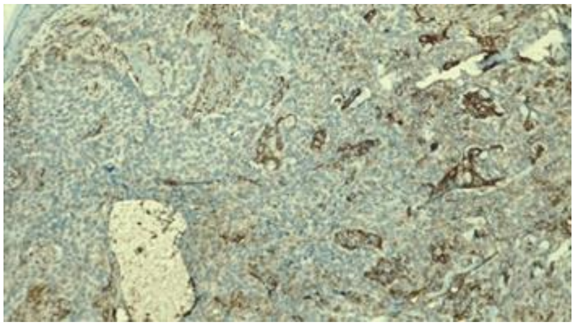

Figure 2: Basosquamous carcinoma: strong, diffuse nuclear immunoreactivity for P53 (hyperexpresion, mutant staining pattern). The expression pattern is similar to that found in squamous cell carcinomas of the skin, anti-P53 antibody, 100x

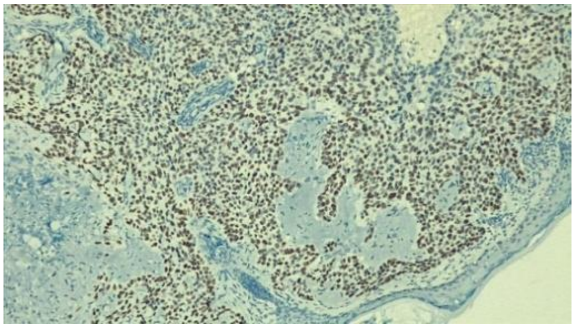

Figure 3: Basosquamous carcinoma: EP-CAM (epithelial adhesion molecule) negative in squamous component and positive in basal cell carcinoma (anti-EP-CAM antibody, 100x)

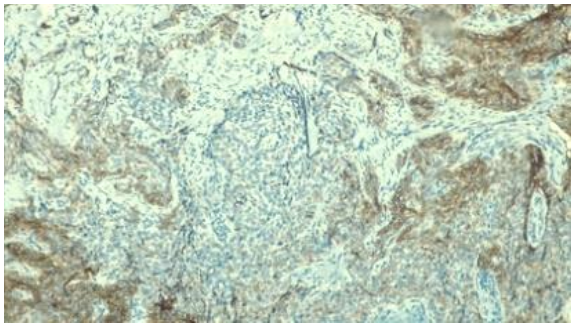

Figure 4: Basosquamous carcinoma: Positive P63 immunohistochemical reaction for both basal and squamous areas, anti-P63 antibody, 100x

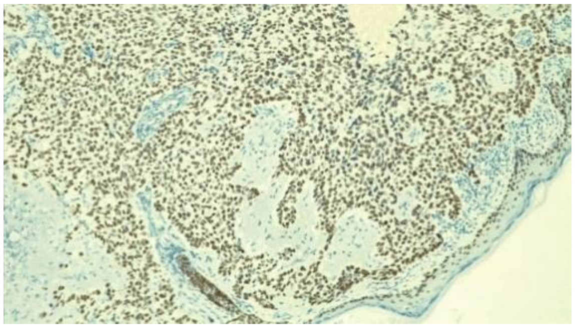

Figure 5: Basosquamous carcinoma: the tumor cell are negative for AR (androgen receptor), anti-AR antibody, 100x

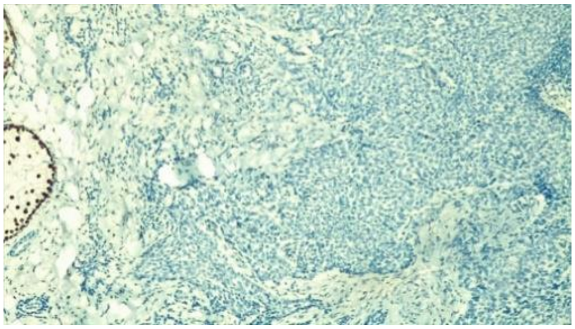

Figure 6: Basosquamous carcinoma: the tumor cells are EMA (epithelial membrane antigen) negative. Note the internal positive control (pilosebaceous unit), anti-EMA antibody, 100x

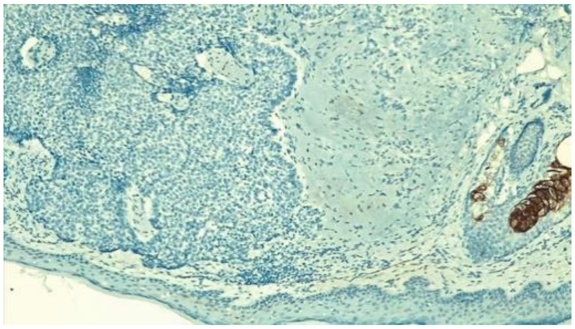

Case no. 1

The first case is a 75 years old man, with long term exposure to sun, who came to plastic surgery service with a growth of an endophytic tumor mass (Figure 7) on superior level of the left cheek measuring approximately 2/1.5 cm.

The tumor appeared about 3 months ago; it was ulcerated and presented perilesional telangiectasias which are a characteristic sign for basal cell carcinoma [7].

Considering the aggressiveness of the tumor and its evolution, surgical excision with pathology examination was decided.

Figure 7: Ulcerated endophytic tumor on superior level of the left check with perilesional telangiectasias, a characteristic sign for basal cell carcinoma

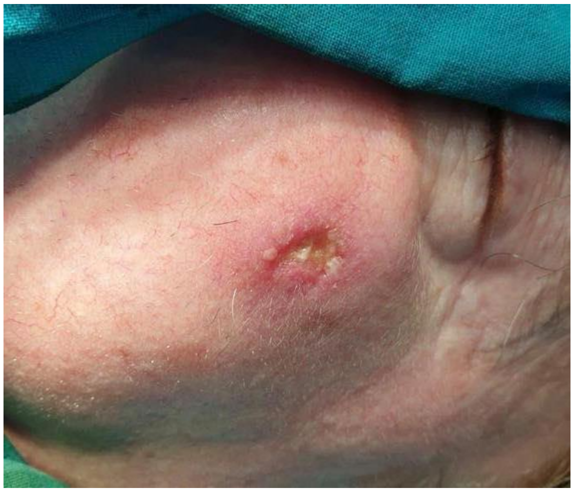

The histopathological examination (Figures 8 and 9) revealed an ulcerated tumor consisting of a superficial proliferation of malignant basal cell-type with basophilic cytoplasm and hypercromatic nuclei, with minimal atypia admixed with cells that have acidophilic cytoplasm, vesicular nucleoli and atypical mitoses infiltrating the deeper levels of the dermis.

The tumoral proliferation is surrounded by a lymphocytic inflammatory infiltrate.

Thus, a diagnosis of basosquamous carcinoma, with squamous predominance of 80% was made. The tumor infiltrated the deep dermis and was reported with a Clark index of IV, with perineural invasion >0.1 mm but without vascular invasion.

The tumor was completely excised.

Figure 8: Metatypical carcinoma, with solid nests of malignant cells with eosinophilic cytoplasm and vesicular nuclei, HE 10x

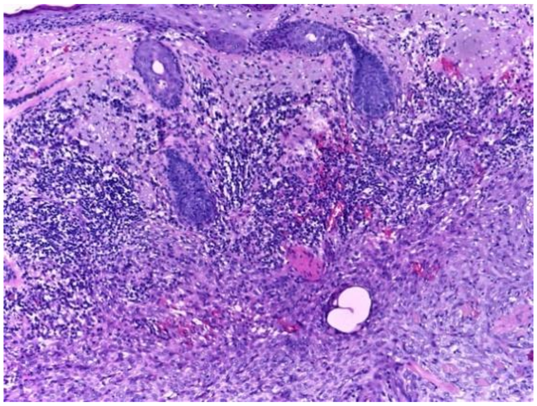

Figure 9: Basosquamous carcinoma with non-keratinizing squamous component of 80%, with solid and infiltrative pattern, reaching a nervous structure, HE 20x

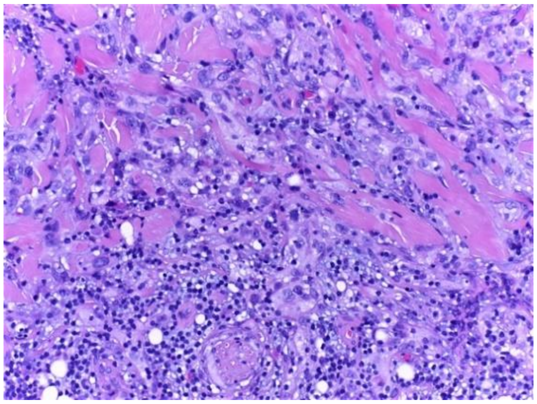

Case no 2

An 88-year old patient, former smoker, with long-term exposure to sun, presented with an ulcerated tumor located on the right auricular pavilion (Figure 10), covered with hemorrhagic crusts, measuring about 5/4 cm that appeared a few months ago. He came to our service because of local pain and tumor bleeding.

Figure 10: Ulcerated tumor on auricular pavilion

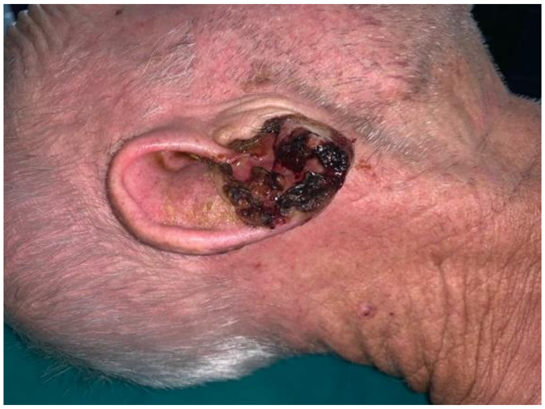

A preop cervical CT scan revealed multiple ganglia images at the superficial and deep laterocervical level, and also at the submandibular level, with the maximum size of 1 cm. The patient was also evaluated by the maxillo-facial surgery service, which recommended only the follow-up of those adenopathies. In this case, it was decided the partial amputation of the ear (Figure 11) with macroscopically tumor-free margins and reconstruction by fixing helix on auricular lobe (Figure 12).

Figure 11: Partial amputation of the ear

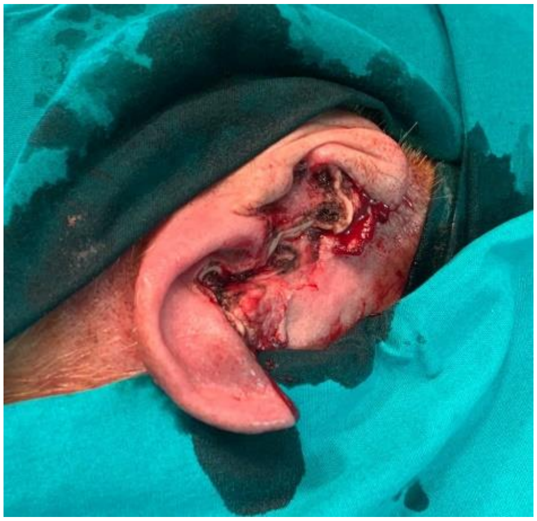

Figure 12: Day 3 postoperatively. Reconstruction of auricular pavilion

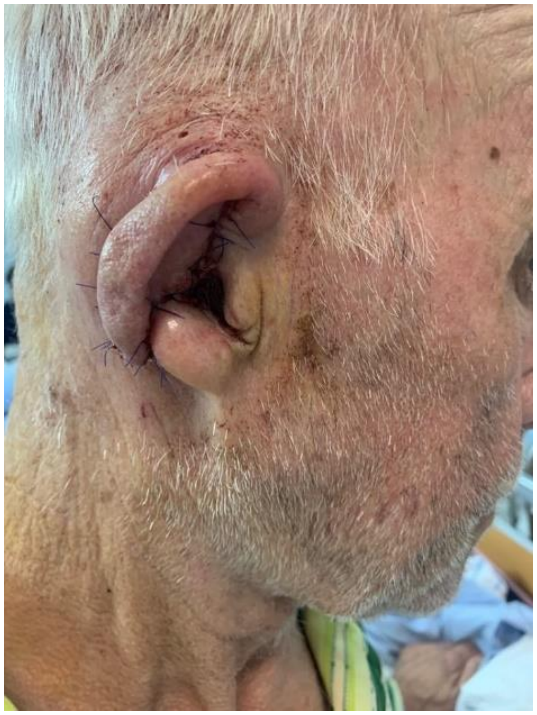

After conventional HE method processing and examination, a malignant proliferation of cells with intermediate aspects of both basal and squamous features was identified. The nuclei presented atypical mitoses, a hypercromatic and slighty oval nuclei. The solid proliferation infiltrated the soft tissue, and presented clear ulceration on the skin. A diagnosis of basosquamous carcinoma without cartilage infiltration was made. (Figures 13 and 14).

Figure 13: Ulcerated metatypical, invasive carcinoma, with a solid, non-keratinizing pattern, with intermediate aspects between basal and squamous cell; notice hyaline cartilage without tumor invasion, HE, 4x

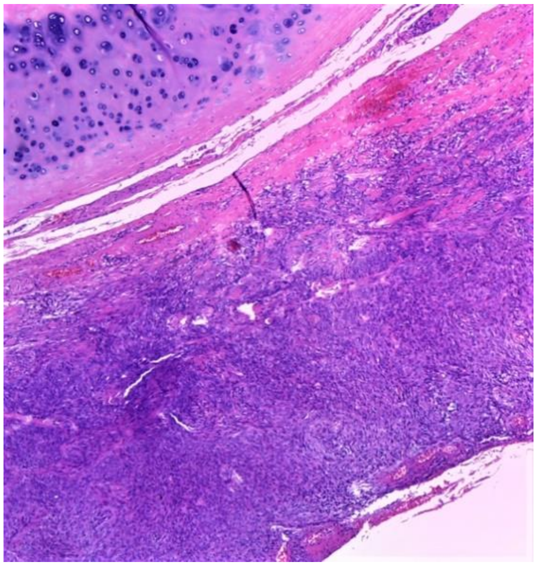

Figure 14: Ulcerated metatypical, invasive carcinoma, with solid islands of malignant cells, with hypercromatic nuclei and moderate pleomorphism, HE, 10x

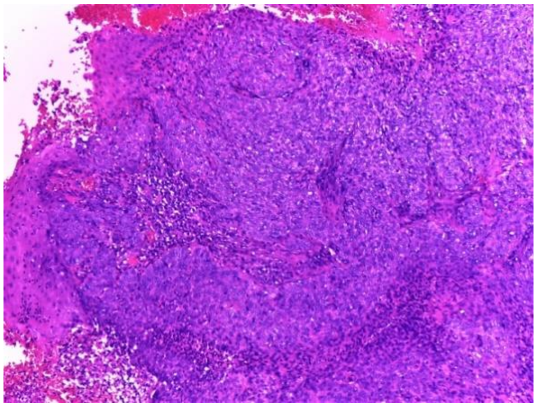

Case no 3

The 3rd case is of a 76 years old Caucasian woman who was transferred to plastic surgery service for a biopsy of a nasal pyramid mass (Figure 15). Her tumor appeared a few months ago after local trauma and sun exposure.

Figure 15: Ulcerated tumor of the nasal pyramid

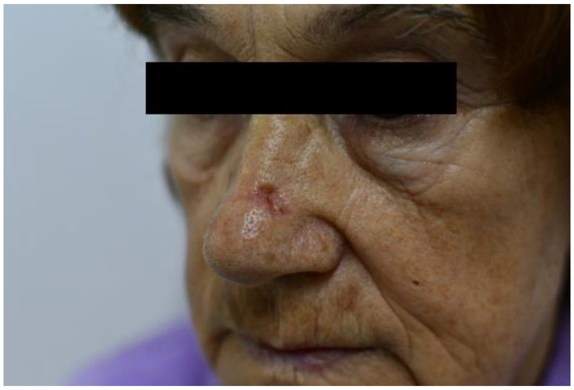
[redacted]

After the resection, the histopathological examination revealed a metatypical carcinoma consisting of a proliferation of intermediate cells invading the dermis, incompletely excised (Figure 16). The re-excision revealed a small island of basaloid cell proliferation and an important granulomatous foreign body reaction with multinucleated giant cell reaction (Figures 17 and 18). Surgical margins were tumor-free.

Figure 16: Island of metatypical carcinoma invasive in dermis, HE 10x

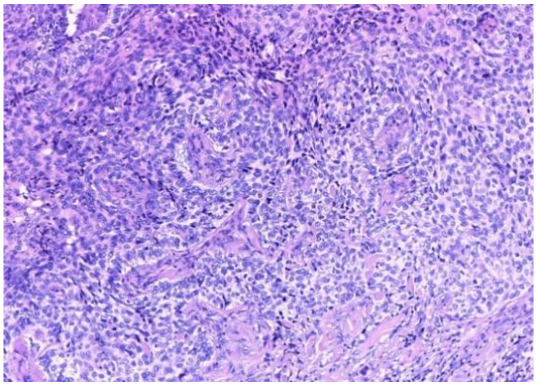

Figure 17: Re-excision of basosquamous carcinoma (granulomatous foreign body reaction with multinucleate giant cells, HE. 10x)

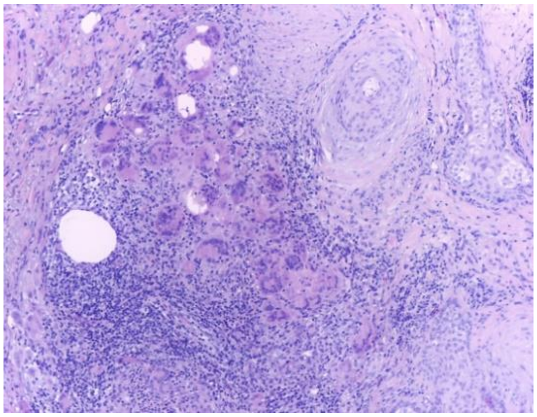

Figure 18: Re-excision of basosquamous carcinoma – isle of superficial basal cell carcinoma, HE, 10x

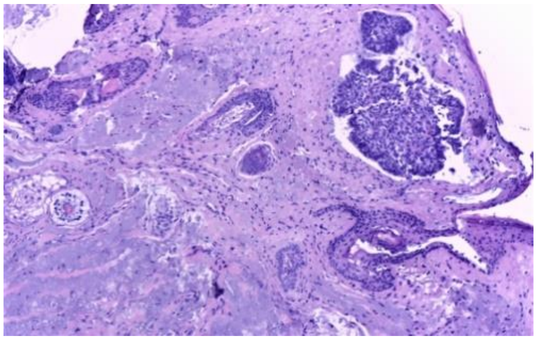

Case no. 4

The 4th case was a surgical challenge; the patient was a 68year old man, from rural environment, with an ulcerated giant tumor (Figure 19) on right nasogenian, malar and lower lid region, with an evolution of approximately 10 years. The dimension reached approximately 7.5/4 cm.

Figure 19: Ulcerated tumor covering the right nasogenian, malar and infraorbital area

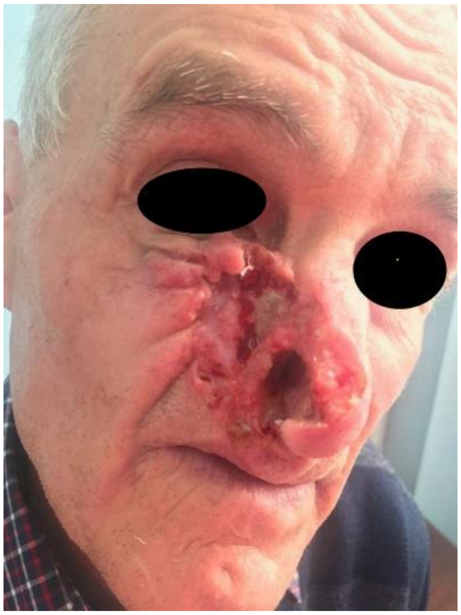
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Preoperatively, a cerebral-cervical-thorax CT scan to evaluate the degree of invasion of tumor and potential disseminations was performed, which revealed no local bone invasion and multiple multiple ganglia images, under 1 cm, at the level of mediastinal, axillar, submandibular and on bilateral carotid space.

The tumor was excised with macroscopically oncological safety margins. The defect remained needed cover with 2 local flaps, one right cervico-facial and one transposed frontal (Figures 20 and 21).

Figure 20: The defect after tumor resection

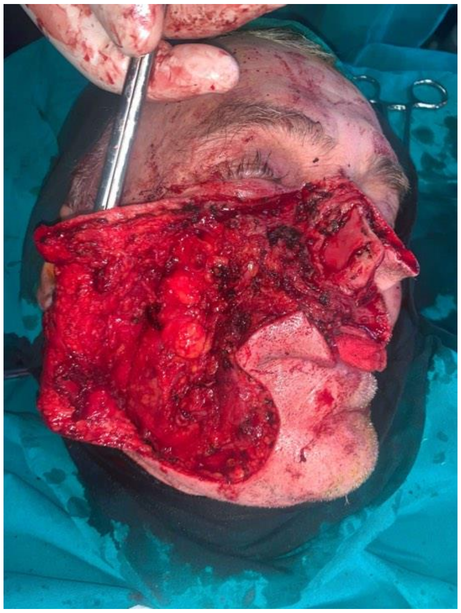

Figure 21: Three months post-surgery

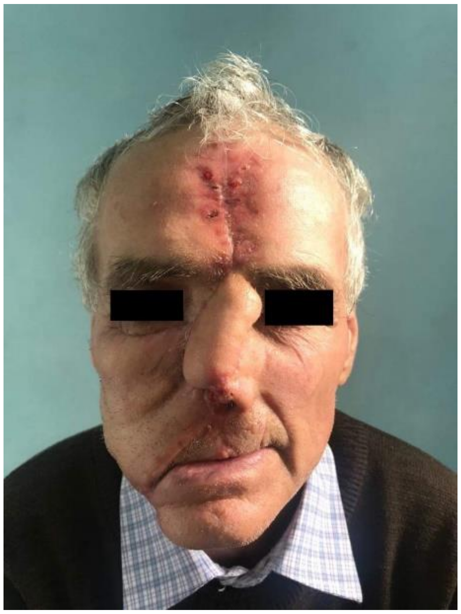

The histopathological exam confirmed the diagnostic of basosquamous carcinoma with a proliferation of intermediate and squamous cells with slightly spindle shape, with eosinophilic cytoplasm, infiltrating the deeper portions of the skin and ulcerating the epidermis (Figures 22 and 23).

The squamous component presented individual keratinization, with vesicular nucleoli and numerous atypical mitoses (Figure 24).

Figure 22: Basosquamous carcinoma (left side of photo) and respirator epithelium (right side of photo), notice the solid proliferation of intermediate cells with slighty spindle shape, with hypercromatic nuclei admixed with vesicular nuclei and eosinophilic cytoplasm, HE, 20x

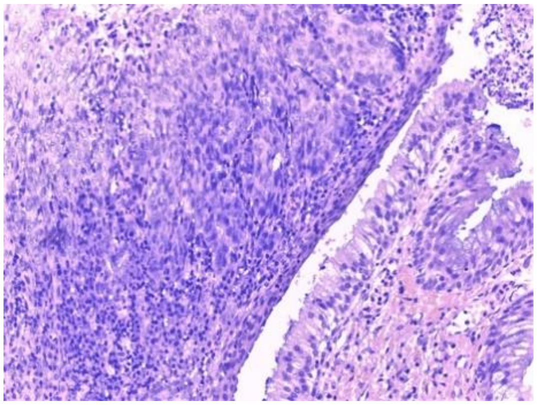

Figure 23: Basosquamous carcinoma (basal cell component) with epidermal ulceration and infiltration of the deeper dermis, HE, 10x

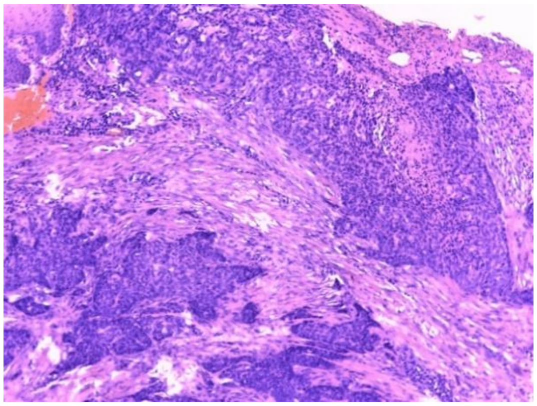

Figure 24: Detail of basosquamous carcinoma (squamous component) with individual keratinizing and several atypia, with pleomorphic vesicular nuclei, HE, 40x

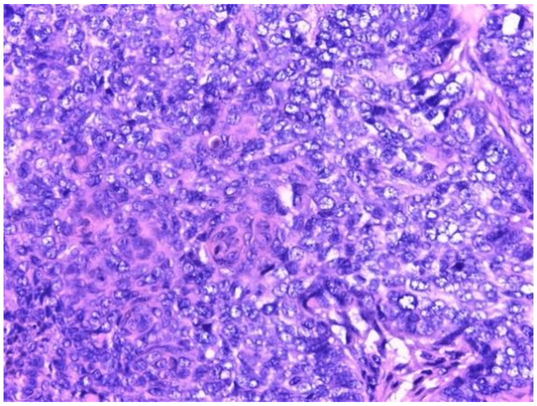

Case no 5

The last case is of a 76 years old woman, with insulin-dependent diabetes mellitus and obesity that presented with an ulcerated tumor (Figure 25) of approximately 2/2cm on the left nasal side.

Figure 25: Erosive tumor on left nasal side

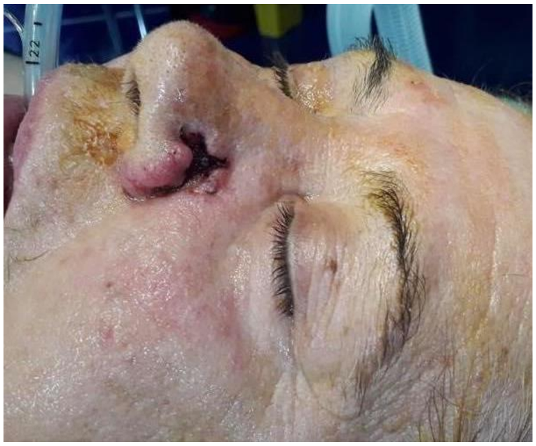

The tumor progressed to a full thickness skin erosion over a 2 years time period. The surgical excision skin defect (Figure 26) was covered with a local flap (Figure 27).

Figure 26: Defect remained after tumor excision

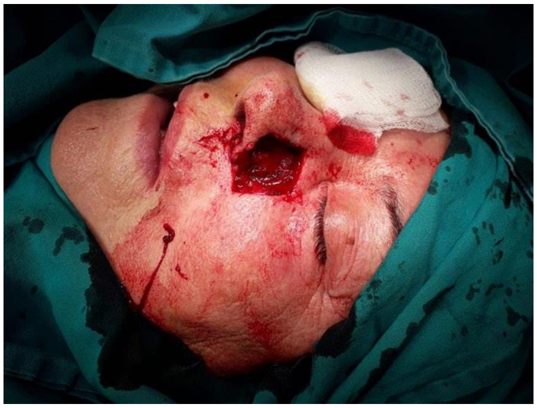

Figure 27: Immediately post-surgery, cover of defect by rotated nasolabial flap

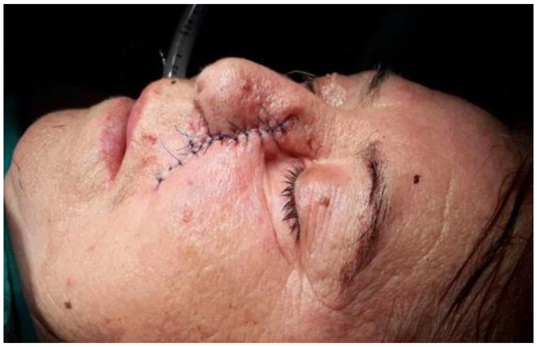

The pathology results under Hematoxilin-eosin examination revealed a malignant proliferation of polygonal squamous cells with polymorphism and eosinophil cytoplasm admixed with islands of basaloid cells and areas of dystrophic microcalcifications (Figures 28, 29 and 30). A diagnosis of completely excised metatypical carcinoma was made.

Figure 28: Carcinoma islands with squamoid aspect, formed of polygonal, pleomorphic cells admixed with basaloid cells, infiltrating the conjunctive tissue, HE, 20x

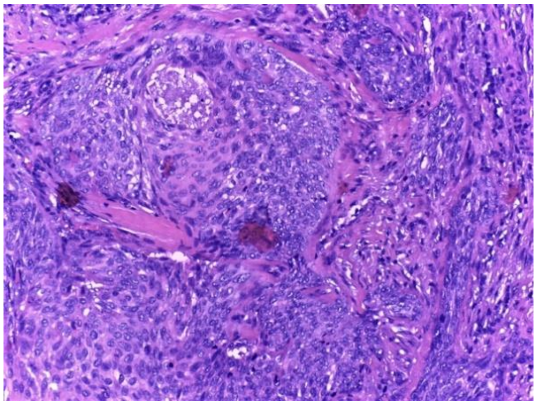

Figure 29: Islands of basal cell carcinoma with palisades and calcification, HE 10x

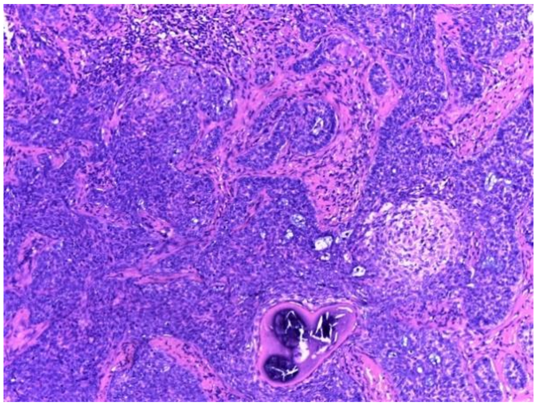

Figure 30: Association between component of squamous (cells with eosinophil cytoplasm, vesiculous nuclei) and basaloid cells (cells with basophile cytoplasm and tachychromatic nucleus) with calcification and incipient keratinizing, HE 10x

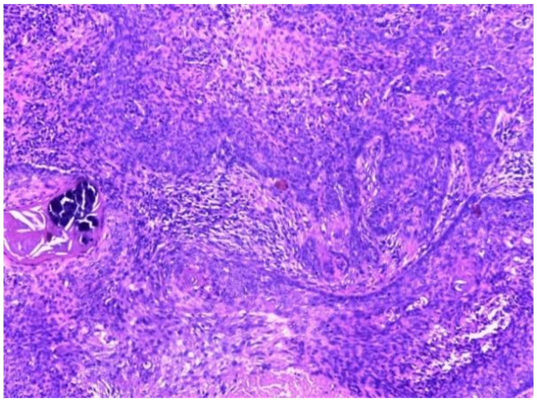

DISCUSSION

Among the skin neoplasms, two of the best known tumors are squamous cell carcinomas and basal cell carcinomas. Although the majority remain locally invasive, there is a risk of local-regional and remote dissemination. Considering the predisposition for basal cell carcinoma to appear on the head and neck in 70-80% of cases, and also the size of tumor, the metastasis rate is appreciated at around 0.0028-0.55% [8,9,10]. Regarding squamous cell carcinoma, it was demonstrated that the tumor size, depth and perineural invasion increased the risk of metastasis. The tumors measuring more than 2 cm in thickness that infiltrated into the deep skin structures or the bone, counted for 75% of the metastases [11]. The current guides target a much more detailed analysis considering the perineural invasion, thickness of tumor and dimensions of free margins of tumors for squamous cell carcinomas. It can be extrapolated that these prognostic factors can be applied to basosquamous carcinomas, especially for those with an important squamous component [12]. In our study, only the patient with perineural invasion was reported to have an increased risk of relapse.

Although few cases of basosquamous carcinoma are reported in literature, it is important to notice that all of the 5 presented cases were identified only in a 2 months' period. Hence, it is expecting to increase the incidence for this subtype of skin cancer.

To some researchers, the basosquamous carcinomas are presented with metatypical basal cells and histopathological characteristics of both basal cell carcinoma and of squamous cell carcinoma, with or without transition area between them. This neoplasm represents a controversial pathology since its first description in 1910, when McCormac reported a range of erosive ulcers classifying them in 3 types of tumors: pure, mixed (side by side basal and squamous cells) and intermediary subtypes. Subsequently, in 1912 Korbl presented a form of transitional skin cancer with a structure of basal typical cell carcinoma but with bigger, pallor cells, rich in cytoplasm and containing intercellular bridges.

The term of metatypical epithelioma was introduced in 1922 by Darier, presenting it as a typical basal cell carcinoma with conglomerates of squamous cells.

In 1928, the notion of epithelioma with basosquamous cells appears, pursuant to a study of Montgomery that reported a transitional area between squamous cell carcinoma and basal cell carcinoma. In 1951, Thackray accepted basosquamous carcinoma as an entity, although he did not define it. Multiple contradictions related to such neoplasms existed along the years. Some researchers considered them as a variant of basal cell carcinomas with keratinizing areas but without prognostic signification [13].

The terminology of such neoplasm still raises controversies since the literature does not clearly defines it. Currently, it's being recognized as being an aggressive form of basal cell carcinoma, with a risk of relapse ranging between 10-48% and a risk of metastasis between 5-7.4% [14].

In opposition, Martin et al. didn`t take in consideration the degree of differentiation and size of initial tumor as factors predisposing to relapse. The positive indicators considered were positive excision margins, lymphatic and perineural [15].

Leibovitch proved in a 178 patients study, that 95% of basosquamous carcinoma were located on head and neck regions having an increased predisposition for the nose (33,1%), auricular region (18,5%) and eye area [16].

It is interesting to notice that similar to the literature's data, all the tumors described in this paper were located on head and neck regions, on sun exposed areas, and affected patients with an average age of 76.6 years. Macroscopically, all tumors had an erosive nature with an aggressive growth, except for the case no. 4 which had an evolution of approximately 10 years. In case no 4, multiple ganglia images (one mediastinal, axillar, submandibular and on bilateral carotid space) were found. The literature presents the risk of metastasis in basosquamous carcinoma higher than that of basal or squamous cell carcinoma, with pulmonary prevalence [17]. Thus, correlating with the literature data, the presence of the adenopathies and the dimension of the tumor are associated with bad diagnostic and risk of relapse. Also, slow evolution of case no 4, during ten years, shows the necessity of studying the squamous component prevalence in the evolution of these tumors, as much as the predisposing factors or precursor lesions.

It is important to mention that some studies show an aggressiveness of basosquamous carcinoma higher even than that of squamous carcinoma [18].

Macroscopically, we notice that such tumors have ulcerative, erosive nature, a feature proven also by Cigna et al based on a retrospective study including 312 patients with metatypical carcinomas [19].

Another aspect that needs to be taken into account is the tumor's location, especially on head, which represents a challenge for both the surgeon and the patient. Many times, wide, meticulous excisions are needed, and several vascular-muscular-nervous important elements are encountered. Therefore, the complex reconstructions could be mutilating to the patient.

Paraclinical, the only common abnormal marker found at the patients included in the study was increased rate of blood sedimentation, which is a nonspecific marker of inflammation. Even if they have macroscopically similar tumor features like erosive and ulcerated characteristics, the evolution was impredictible, from a few months to ten years. This it increases even more the necessity of studying the role of the squamous component percentage in the basosquamous carcinoma evolution, together with the skin response at sun exposure and inflammation.

Although the treatment of basosquamous carcinoma is not optimized, we need a more carefully approach both surgically and imagistically to evaluate the existence of disseminations, as well as oncologically with a very strict follow up. In our study, one of 5 patients received radiotherapy (case no 4).

One year postoperatively none of the patients presented local relapse, confirming that the tumor free-margins are the key for a good management of this pathology.

For a relevant treatment, it is important that the correct diagnostic is made based on histopathological examination. In cases of basosquamous carcinoma, the immunohistochemical determination with EP-CAM and CD10 markers may be useful, basal cell carcinomas being positive and squamous cell carcinomas negative [20]. CXCR4, MMP-13 and b-catenin may also be considered as prognostic markers [21].

The particularities identified based on immunohistochemical reactions rise even more attention on the necessity of treating basosquamous cell carcinoma as a unique entity with an unpredictable evolution and an aggressive pathway. We noted that basosquamous carcinomas may also have overlapping immunohistochemical features found in both basal cell and squamous cell carcinomas.

CONCLUSION

Given the increasing incidence of basosquamous carcinomas and unpredictable evolution, sometimes even more aggressive than squamous cell carcinoma, there is a clear need for an optimized therapeutic protocol and a correct diagnosis based on histopathological and immunohistochemical examinations [22,23]. Treating as a different entity than typical basal and squamous cell carcinomas, understandying the pathology and approaching the role of squamous component in its evolution, should be considered. Resection with tumor- free margins and correct histopathological examination are the key features in the approach of basosquamous carcinoma, backed-up by oncological approach and a rigorous follow up. The development of non-invasive tests concerning modifications, surprising the carcinogenesis in situ, and the education of people regarding sun exposure and skin cancer, are also two important steps to decrease the incidence of skin carcinomas.

Acknowledgment

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CLINICAL PRACTICE

Bilateral pneumatization of middle and superior nasal turbinates

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Abstract: *The pneumatization of all nasal turbinates is an extremely rare finding. Several previous reports have presented the bilateral superior, middle, and inferior conchæbullosæ. We report the bilateral pneumatization of middle and superior nasal turbinates. The variant was documented in cone beam computed tomography (CBCT) in all three dimensions. The inferior concha bullosa was a bilaterally moderate subtype of lamellar pneumatization that presented as a conchal recess of the maxillary sinus. The concha bullosa media was bilaterally of the extensive or true type and associated unilaterally with an uncinate bulla. The concha bullosa superior was asymmetrical bilaterally, narrower on the right side, and larger on the left where it was also septated and tri-cameral. The supreme conchæ were rudimentary, as were their pneumatizations. For an accurate anatomic diagnosis of conchæbullosæ in computed tomography, sagittal, coronal, and axial cuts, as well as three-dimensional reconstructions, should be used to ensure adequate planning of functional endoscopic sinus surgery.*

Keywords: *agger nasi; ethmoid bone; nasal fossa; nasal concha; nasal turbinate; anatomic variation*

INTRODUCTION

The lateral nasal wall attaches the nasal turbinates or conchæ. The inferior one is an independent bone. The middle, the superior, and the inconstant Santorini's supreme turbinates are the ethmoidal turbinates that are attached on the nasal side of the ethmoidal labyrinth [1].

A concha bullosa defines the pneumatization of a nasal turbinate. Although the term is commonly used to indicate the pneumatization of the middle turbinate, other nasal turbinates (inferior, superior, and supreme) have also been found to be pneumatized [2-15].

Sagit et al (2008) reported the first case of the pneumatization of six turbinates [16]. Fidan (2012) also assumed that the "pneumatization of all turbinates in a person has not been published yet" and suggested the term "panconcha bullosa" for such pneumatization of all six turbinates [17]. Ozturan et al (2013) failed to document these two previous reports and presented a case with "bilateral triple turbinate pneumatisations," noting, "Despite an extensive search of the medical literature, we could locate only one description of extensive turbinate pneumatisation involving anywhere near as many conchae as in our patient" [18].

Moreover, Bergman's Comprehensive Encyclopedia of Human Anatomic Variation [19] indicated only that the "pneumatization of several turbinates in the same patient has been reported," citing the reports of Sagit et al (2008), Ozturan et al (2013), and a third paper in which Pittore et al (2011) reported only a case of concha bullosa inferior [10], and not a panconcha bullosa. The reports of Fidan (2012) and Toplu et al (2013) were omitted [15, 19].

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We therefore report the fifth known case in the literature of the pneumatization of all nasal turbinates. To our knowledge, it is the first such case being documented in cone beam computed tomography (CBCT) and not on coronal CTs, as well as the first case in which findings were equally documented with bi-dimensional multiplanar reconstructions (MPRs) and three-dimensional volume renderizations (3DVRs). Nevertheless, we hereby report the pneumatization of eight, and not six, turbinates, as well as Santorini's first supreme turbinate was also found to be bullous.

ANATOMIC VARIATION

A retrospective CBCT study of the archived files of a 52 years old female patient was performed.

The subject had been scanned using a CBCT machine – iCat (Imaging Sciences International), the DICOM file being further documented with the Planmeca Romexis Viewer 3.5.0.R software, according to a previously detailed protocol of study [20-23]. We evaluated the nasal turbinates in all the three anatomical planes, as well as the three-dimensional volume renderizations. Relevant anatomical features were exported as image files. The patient has given written informed consent for all medical data to be used for research, provided they are anonymized.

In the case hereby reported, a Type 5 Mladina nasal septal deformation was found, with a horizontal septal spur sticking laterally and deeply in the right nasal fossa between the middle and inferior turbinates (Figure 1). The postero-superior part of the nasal septum accommodated an anterior (septal) recess of the right sphenoidal sinus. However, the septum did not appear deviated.

The inferior turbinates were bilaterally pneumatized by conchal recesses of the maxillary sinuses (Figures 1A–E, 2, 3F, 4, 6). The conchal pneumatization penetrated the horizontal segment of the lamellar part of each turbinate (Figure 6), with a 6.10 mm depth on the right side, and 3.02 mm depth on the left side (on coronal slices). These inferior conchæ bullosæ were located between the ethmoidal and maxillary processes of the inferior turbinate (Figure 6).

We also found bilateral pneumatizations of the middle turbinates (Figures 1A, 3, 4). These were both extensively pneumatized (true or extensive concha bullosa media) and were draining in the middle meatus on that side. An uncinate bulla was found unilaterally on the outer side of the right concha bullosa media (Figure 1A). It was pneumatized from an anterior adjacent lacrimal cell located, in turn, posterior to the agger nasi cell.

Figure 1: Anterior to posterior series (A–F) of coronal MPRs identifying the eight turbinates' pneumatizations. The beaked-shape pneumatization of each inferior turbinate is identified (A–E, white double-headed arrows). The bilateral concha bullosa of the middle turbinates (A, white arrows) is of extensive type, lamellar and bulbous. A right uncinate bulla is found (A, *). The superior turbinates are bilaterally pneumatized (B, white arrowheads). A horizontal septum divides the anterior part of the left superior concha bullosa in two chambers, superior (C, black arrowhead) and inferior (C, black arrow). Both drain into the upper nasal meatus (C, D). A third posterior chamber of the left superior concha bullosa (E, black double-headed arrow) appears as a medially evaginated posterior ethmoid air cell. On both sides of an anterior (septal) recess („X") of the right sphenoidal sinus the rudimentary supreme turbinates also appear pneumatized (F, black arrowheads).

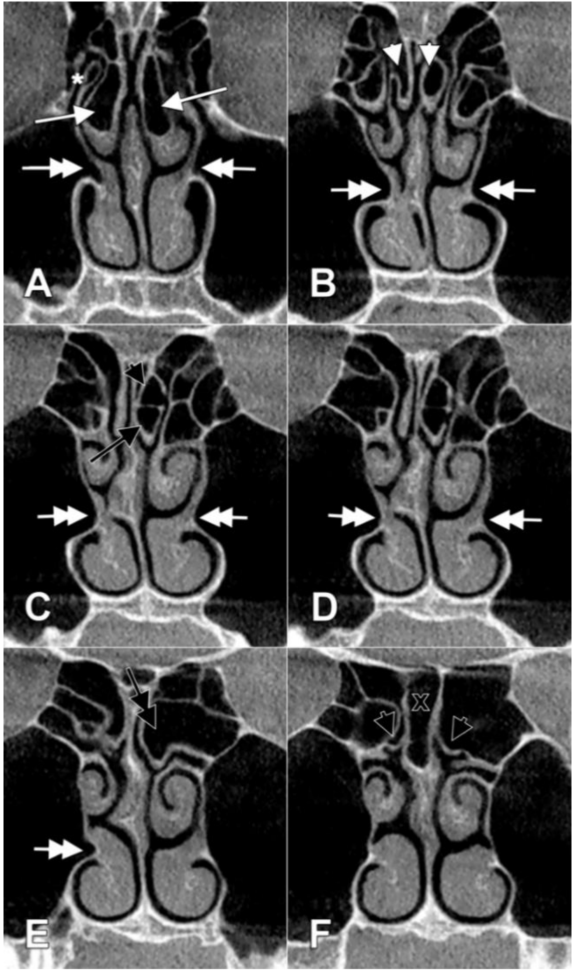

We also observed a bilateral concha bullosa superior (Figures 1B–E, 3-6). The conchal air space was narrow in the right side (1.75/4.01 mm on coronal slices). The left concha bullosa superior was larger (4.81/11.35 mm on coronal slices) and septated by an overturned "Y"-shaped septum (Figures 3A, 5).

Thus, it consisted of three chambers: antero-superior, antero-inferior, and posterior. The anterior chambers were draining in the left superior nasal meatus (Figures 1C, D).

Figure 2: Axial MPR demonstrating the bilateral inferior concha bullosa (double-headed arrows).

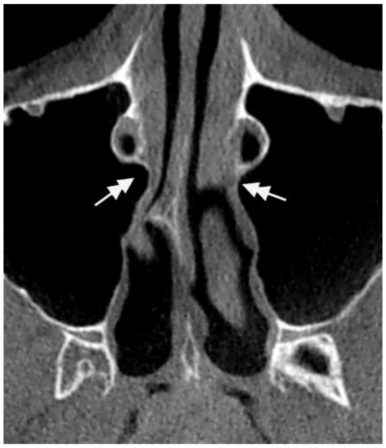

Figure 3: Lateral to medial series (A–F) of sagittal MPRs of the left lateral nasal wall. An agger nasi cell (A–F, *) is found anterior and superior to the anterior end of the middle nasal turbinate. Posterior to it lies the middle concha bullosa (A-–, white arrow). The inferior concha bullosa is indicated (F, white double-headed arrow). The superior concha bullosa is double-septated and consists of three chambers (A, B, white arrowheads): two anterior and one posterior. The posterior one is a conchal recess of a posterior ethmoid air cell (B–F, „X”), as is the supreme concha bullosa (D, black arrowhead). The superior (black arrow) and supreme (black double-headed arrow) turbinates are indicated (C–E).

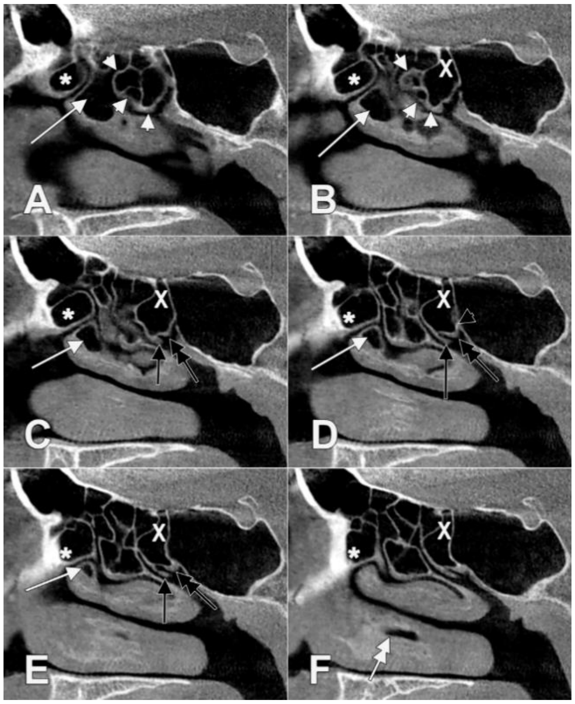

The posterior chamber appeared as the extension (conchal recess) of a posterior ethmoid air cell which, in turn, was also extending to form the left concha bullosa suprema (Figure 3).

As the concha bullosa suprema was also found on the right side (Figures 1, 3–5), we recorded this variation as bilateral. The rudimentary supreme conchæ bullosæ appeared as small diverticula of the respective posterior ethmoid air cells.

Figure 4: Sagittal MPR of the right lateral nasal wall crossing thorough all four conchal pneumatizations: inferior (double-headed arrow), middle (arrow), superior (white arrowhead), and supreme (black arrowhead).

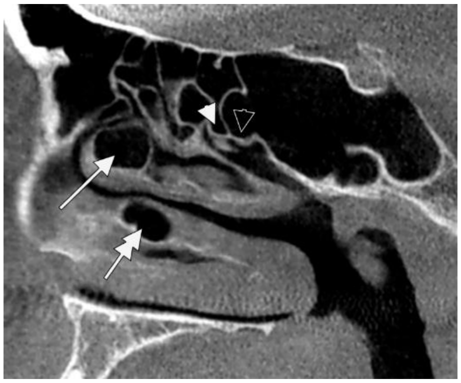

Figure 5: Three-dimensional sagittal volume renderization of the left lateral nasal wall. The superior concha bullosa is separated by septa in three chambers (diverging thin arrows). A posterior ethmoid air cell ("X") pneumatize the posterior chamber of the superior turbinate (white arrowhead) and the supreme turbinate (black arrowhead). The thick arrow indicates the middle concha bullosa and the double-headed arrow—the agger nasi cell. FS: frontal sinus; SS: sphenoidal sinus; IT: inferior turbinate; NS: nasal septum (partly).

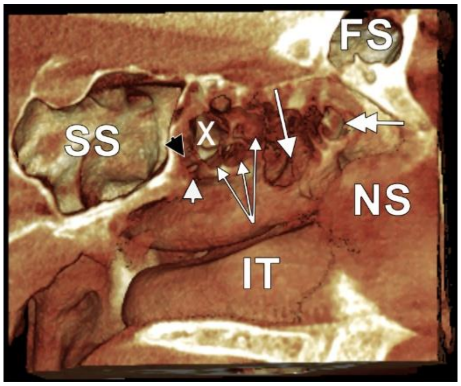

Agger nasi cells were found bilaterally. The right one was draining in the frontal recess and the left one in the ethmoidal infundibulum (Figure 7).

DISCUSSION

As was already discussed in the Introduction, reports of panconcha bullosa, such as this one, are rare. The nasal septum typically deviates away from an asymmetrically enlarged dominant concha bullosa [7].

Nasal septum deformities were classified by Mladina, the left-sided deformities being more prevalent than the right-sided ones [24]. According to these authors, type 5 indicates "an almost horizontal septal spur that sticks laterally and deeply into the nasal cavity," the opposite side of the septum being flat [24].

This corresponds to the septal morphology we found in the present case. The absence of an important septal deviation indicates that we encountered a case of bilateral pneumatizations balanced in size.

Figure 6: Three-dimensional coronal volume renderization of inferior turbinates (ITs). On each side, conchal recesses of the maxillary sinuses (MS) penetrate the turbinate roots (double-headed arrows) to form communicating lamellar inferior conchæ bullosæ. These pneumatizations are located on each side between the ethmoidal process of the ITs (1), which further articulates with the uncinate process (2), and the maxillary process of the ITs (3).

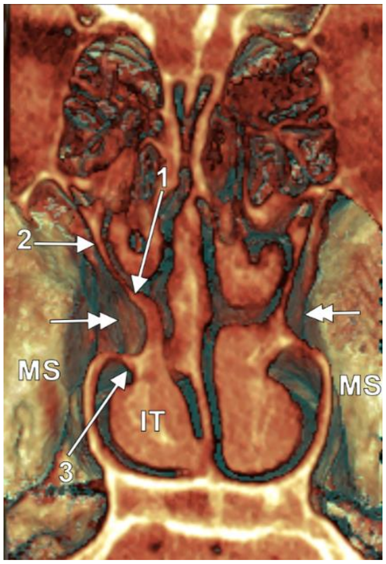

Figure 7: Sagittal MPRs demonstrate bilateral agger nasi cells (*). The right one communicates with the frontal recess (arrow), and the left one drains in the ethmoidal infundibulum (arrowhead).

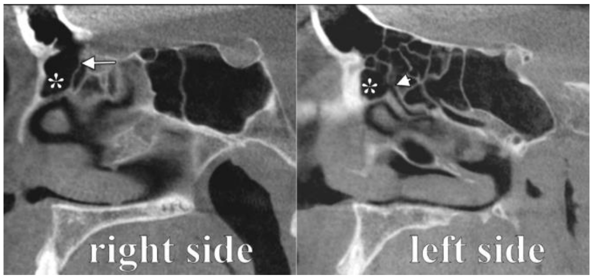

Figure 8: Reprinted from Hindawi – Turhan San, Selma San, Emre Gürkan, and Barış Erdoğan, "Bilateral Triple Concha Bullosa: A Very Rare Anatomical Variation of Intranasal Turbinates," Case Reports in Otolaryngology, vol. 2014, Article ID 851508, 2 pages, 2014. doi:10.1155/2014/851508, which is licensed under CC BY. We indicate (inset, digitally magnified) that the so-diagnosed left inferior conchal pneumatization is merely a strongly curved inferior turbinate, due to a septal spur that pushes the left inferior turbinate.

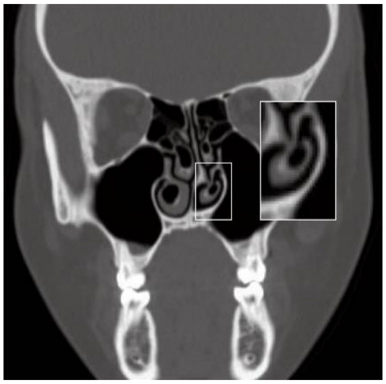

According to Kiroglu et al (2007), the pneumatization of the upper and middle nasal conchæ is commonly encountered, but a concha bullosa inferior is very rare [25]. Koo et al (2017) documented 1188 sides and found a unilateral concha bullosa media in 17.3%, a bilateral concha bullosa media in 36.4%, a unilateral concha bullosa superior in 11.3%, a bilateral concha bullosa superior in 27.4%, and concha bullosa inferior in only 1.0% [13]. A supreme concha bullosa was reported, to our knowledge, only once, by Măru et al (2015) [2].

The first report of an inferior concha bullosa as an anatomic possibility was that of Zinreich et al (1988), while the first paediatric case with a concha bullosa inferior was assumed by Kiroglu et al (2007).

Bolger et al (1991) evaluated in 202 patients the common anatomical variants of the middle nasal turbinate, such as its pneumatization and the paradoxical curvature [26]. In 53% of patients, the authors detected concha bullosa media [26]. They further classified the respective pneumatizations according to their location, within the lamellar part of the middle turbinate, or in its bulbous part, or in both these. It resulted in a classification of concha bullosa media, lamellar, bulbous, and extensive or true, respectively [26].

Further, Yang et al (2008) extended the classification of Bolger et al (1991) to the inferior turbinate [27]. Yang et al (2008) evaluated 59,238 sinonasal CTs and contributed 16 patients with concha bullosa inferior (pneumatized inferior turbinate) to the 28 cases they had already documented as reported at that time [5, 28-41]. They found that in relation with the maxillary sinus, a concha bullosa inferior can be either communicating or non-communicating [27]. This equally feeds the hypothesis of misinvaginated epithelium within the double chondral lamella of the developing inferior turbinate (the non-communicating type) and the hypothesis of maxillary sinus extension within the inferior turbinate (the communicating type) [27]. The communicating type of a concha bullosa inferior was found in 8/18 pneumatized inferior turbinates by Yang et al (2008), thus in 44% of the series. Yang further subdivided the communicating type of a concha bullosa inferior into three subtypes, according to the degree of communicative pneumatization (27). These are (a) a subtle type with a beaked shape of pneumatization, (b) a moderate type in which the pneumatization fills the superior part of the vertical plate of the inferior turbinate, and (c) a marked type, in which the pneumatization completely fills the vertical plate of the turbinate [27]. It is interesting to note in this context a study by Măru et al (2013) in which the authors investigated 100 cases documented by CBCT and found unilateral extensions of the maxillary sinus within the inferior turbinates in 6% and bilateral extensions in 16% , which they termed conchal recesses of the maxillary sinus. Although these last authors did not indicate these recesses as pneumatized turbinates, it is bona fide the same morphology previously described by Yang et al (2008) as pneumatized inferior turbinates. According to the Yang classification, in the case we report here, the inferior conchæ bullosæ we found are of the communicating lamellar type, i.e., the moderate subtype. They can be equally classified as conchal recesses of the maxillary sinuses, however. Such communicating conchal pneumatizations were also indicated as common cavities of the maxillary bone and inferior turbinates [42], thus antro-, or sino-conchal cavities.

One should keep in mind that, according to Yang et al (2008), pneumatized inferior turbinates could be either communicating with the maxillary sinus, or non-communicating. Therefore, a concha bullosa inferior that drains in the inferior nasal meatus is a false one. We support this comment with the report of a "bilateral triple concha bullosa" of San et al (2014), who documented such a concha bullosa inferior only on coronal CT in a four-reference paper in which the study of Yang et al (2008) was not quoted. This is a false concha bullosa inferior that communicates with the inferior nasal meatus, and not with the maxillary sinus; narrowing of the turbinate concavity is rather due to a septal spur on that side (Figure 8). The left nasal spur (Figure 8) corresponds to the apex of maximum left septal deviation and is higher than the right concha bullosa inferior. Therefore, the concha bullosa inferior was not bilateral, as is supported by Tomblinson et al (2016), who demonstrated that the apex of maximum nasal septal contralateral deviation is positioned more superiorly when a unilateral concha bullosa is present and that the severity of septal deviation increases in direct proportion to the concha bullosa's width [7]. Therefore, studies of conchal pneumatization should be evaluated in three dimensions and carefully documented.

In this study, we documented a septated concha bullosa superior that was not, to our knowledge, previously reported. There were, however, a few reports of septated concha bullosa media. The first septated concha bullosa media was found unilaterally and reported by Yanagisawa et al (2008) in a patient elected for functional endoscopic sinus surgery [43]. The second such case was reported by Perić et al (2009), who found a unilateral tri-cameral concha bullosa media [44]. A tri-cameral concha bullosa media was also reported later, by San et al (2013), and was also unilateral [45]. Septation of the middle turbinate is not significantly related to sinonasal pathologies [46]. As supported by our results, the septation of turbinates is not a specific feature of the middle one. Further studies should carefully observe whether the observed conchæ bullosæ contain septa that determine multiple drainage pathways of such pneumatizations.

CONCLUSION

In conclusion, the pneumatization of all nasal turbinates appears as a rare anatomic variation that sums the individual possibilities of the pneumatization of nasal turbinates. Conchæbullosæ are better documented in CBCT and in all three dimensions and, in turn, are of better use for surgeons dealing with functional endoscopic sinus surgery.

Conflict of interests

The authors declare that they have no conflict of interest.

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CLINICAL PRACTICE

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Black hairy tongue due to antibiotic intake

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A 65-year-old man presented to our clinic for a sudden onset of black hairy tongue. The patient who has no significant medical history was explored 9 days before for non-specific gastrointestinal symptoms which had progressively accentuated in the past two months. Physical examination showed epigastric tenderness and bloating. Blood tests were within normal ranges but stool antigen test revealed Helicobacter pylori infection. Triple therapy was initiated with 1 gram amoxicillin, 500 mg clarithromycin and 30 mg lansoprazole, all recomanded twice daily for 14 days. In the 9th day he presented with a black hairy tongue which appeared "overnight".

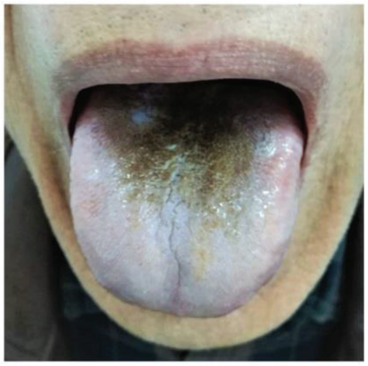

Black hairy tongue represents a defective desquamation of the filiform papillae on the dorsal surface of the tongue, usually due to a lack of mechanical stimulation and debridement [1]. A range of medications [2] have been linked to hairy tongue including amoxicillin, penicillin, bismuth, linezolid. This condition is reported more often in males and the main problem is its unsighty appearance [3].

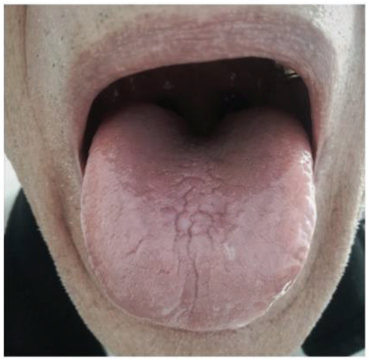

Sometimes patients may complain of a burning sensation on the tongue and halitosis. Black hairy tongue doesn't typically require medical treatment [4].

In this case the patient stopped the antibiotic intake on his own initiative and was encouraged to have a good oral hygene. He continued taking 30 mg of lansoprazole for three more weeks. One month later the tongue recovers its normal characteristics. Reevaluation of the stool test showed the efficiency of the short course of treatment, Helicobacter pylori being eradicated.

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VARIA

Bladder injury – A team challenge

Monica Cirstoiu[1,2], Oana Bodean[1], Octavian Munteanu[1,3], Darius Brinzan[4], Bogdan Cretu[5], George Pariza[6], Popescu Dan[5], Catalin Cirstoiu[5,7]

Abstract: *Bladder trauma is caused by a direct blow to the distended bladder, severe injury fracturing the pelvis, iatrogenic, or penetrating wounds. Early detection and diagnosis are key to successful management of cases, but omission or late reveal of bladder damage increase mortality and create long-term problems. A bladder injury may not always present with immediate obvious signs and symptoms, especially in a multi-trauma patient, who is also more difficult to investigate. Moreover, during the COVID-19 pandemic, delayed patients' access to tertiary, multidisciplinary hospitals, increases the risk for delayed diagnosis and also increases the need for more specialists from different surgical and non-surgical areas to raise their awareness of less common manifestations of bladder trauma. We present a review of literature and cases of less common bladder damage from the perspective of a multi-disciplinary team in the University Emergency Hospital in Bucharest.*

Keywords: *bladder injury, pelvic fracture, urinary fistula*

BACKGROUND

Bladder injuries

Based on etiology, bladder rupture and injury can be blunt, penetrating, iatrogenic, or spontaneous. Anatomically, bladder injury can be extraperitoneal (40-60%), intraperitoneal (15-30%) or mixed (10-25%) [1].

Most of these injuries are caused by road traffic accidents, work-related accidents, falls, crashes, violent crimes, military conflicts, and iatrogenic maneuvers, such as obstetric, gynecologic, and urologic procedures [1, 2]. Common signs and symptoms of blunt bladder trauma are gross hematuria, suprapubic pain, abdominal bruising, extravasation of urine into the perineum and genital organs and thighs [1]. Delayed diagnosis may lead to peritonitis, fistula, and septicemia [1, 2]. In patients with other associated pathology or other associated trauma, undetected bladder injury can even be fatal [1].

Bladder fistula

A less common complication of delayed bladder injury

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diagnosis is bladder fistula [1, 3]. Fistulas are abnormal communications within the genitourinary system or may involve other structures as the gastrointestinal tract, skin, vascular and lymphatic systems [1-3]. Bladder fistulas may involve the urethra, skin, gastrointestinal organs, female reproductive tract, and even pelvic joints [1, 2].

MATERIALS AND METHODS

Because bladder trauma and genitourinary fistulas are so diverse in their anatomy and clinical appearance, we present a review of literature about the less common occurrence of bladder trauma and our experience with this pathology.

Patients' data and imaging were collected from the University Emergency Hospital in Bucharest database.

RESULTS

Tertiary unit hospitals usually confront with multiple trauma patients who need a complex evaluation. Some of these patients come from isolated areas or after being initially partially evaluated in other healthcare facilities.

As described in the literature, most patients with bladder injuries present after obstetrics and gynecology procedures, intra-abdominal injuries (44-68.5%), or pelvic fractures [1-3]. The principal sign of bladder injury is visible haematuria, suprapubic pain, and urinary leakage, but according to the type of injury and the associated complications, bladder trauma diagnosis can be a real challenge for the specialists confronting with it. Usually, urologists and general surgeons are those who operatively manage the cases, but the nature and timing of intervention depend on additional complications and may require a differential diagnosis.

A complication of bladder rupture is a fistula, but rare types of fistulas, such as vesical-cutaneous fistulas associated with pelvic trauma have been sporadically described and no guidelines exist for their management. A particular type of posttraumatic bladder fistula with no immediate clinical manifestations is a vesical-vulvar fistula. As illustrated in the case below, these fistulas can often be mistaken for labial tumors or abscesses.

Posttraumatic bladder injury with vesical-labial fistula

A gynecological and urological consultation was requested for a non-functioning urethral-vesical catheter of a 60 years-old female patient admitted in the Orthopedic Department that underwent surgery with external fixation 2 days prior for a complex pelvic fracture (transverse fracture of the right acetabulum, left obturator ring fracture, left sacral fracture, fifth lumbar transverse apophysis fracture with paralysis of the external left sciatic nerve) (Figure 1).

Figure 1: Preoperative x-ray scan showing a complex pelvic fracture type Tyle C2.

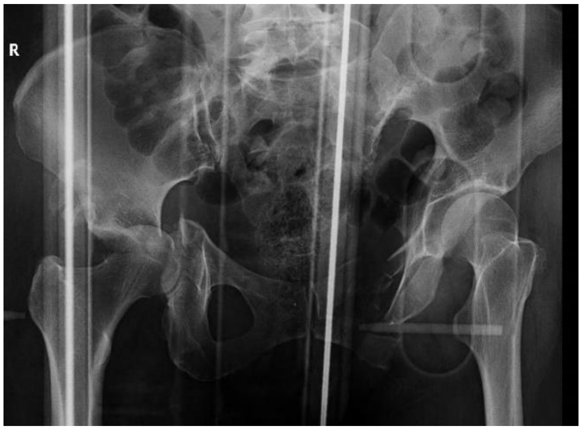

The patient presented with a left labial abscess with spontaneous exteriorization of content. Upon examination, the labial abscess raised suspicions due to the secretions exteriorized that were abundant and liquid, milky at first then clear (Figure 2).

Figure 2: Vesico-labial fistula in a patient with pelvic fracture and suspected bladder injury. The aspect could be easily misdiagnosed as a vulvar abscess.

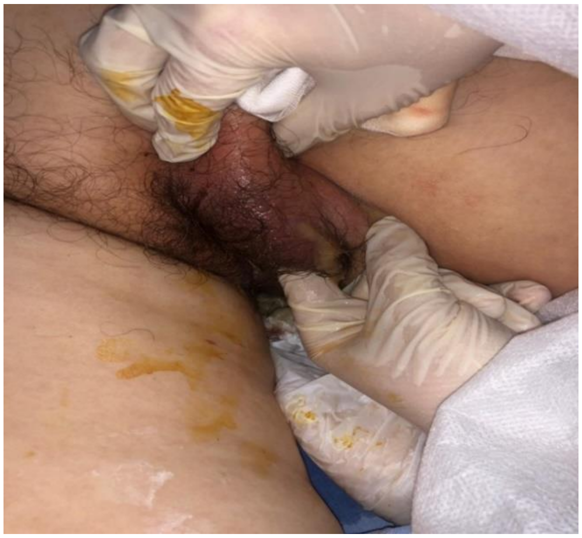

The suspicion of the vesical-cutaneous fistula was confirmed after administering methylene blue in the bladder via the functioning Foley catheter and observing the dye exteriorizing at the site of the labial orifice, therefore adding the diagnosis of extraperitoneal bladder injury complicated with a cutaneous fistula. The existing 14 CH Foley catheter was replaced with a 22 CH one and the external orifice of the fistula tract was drained with a draining tube.

The patient's recent history revealed the above-mentioned fractures with the left bladder wall tractioned laterally with no contrast extravasation 15 days before the consultation with surgical treatment delayed due to a positive RT-PCR COVID 19 test, the patient being transferred to a SARS-Cov 19 support Hospital. The onset or presence of hematuria in this time interval is not specified. The patient did benefit from bladder drainage, but with multiple clogging and rather unfunctional drainage before our consultation.

A second CT-scan was requested and confirmed the suspicion of vesical fistula (Figure 3) and revealed fluid accumulation (10/5.5/3cm) in the left external obturator space.

Figures 3: CT-scan showing complex pelvic fracture and the communication between the bladder and the left labia.

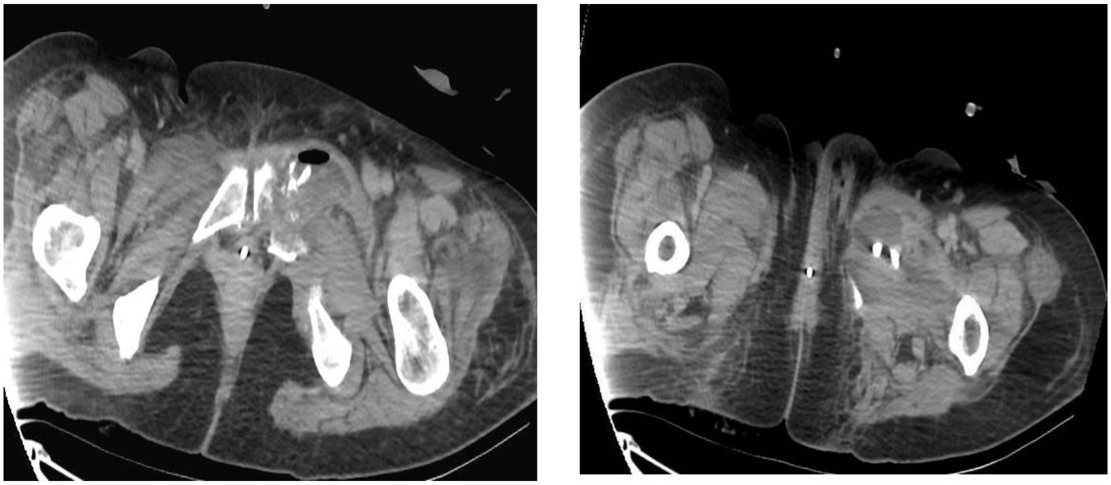

A second draining tube was inserted in this space with ultrasonic guidance to drain intramuscular collections (Figure 4).

Figure 4: Ultra-sonographic aspect of fluid and pus collection on the fistulous trajectory of the vesical-labial fistula.

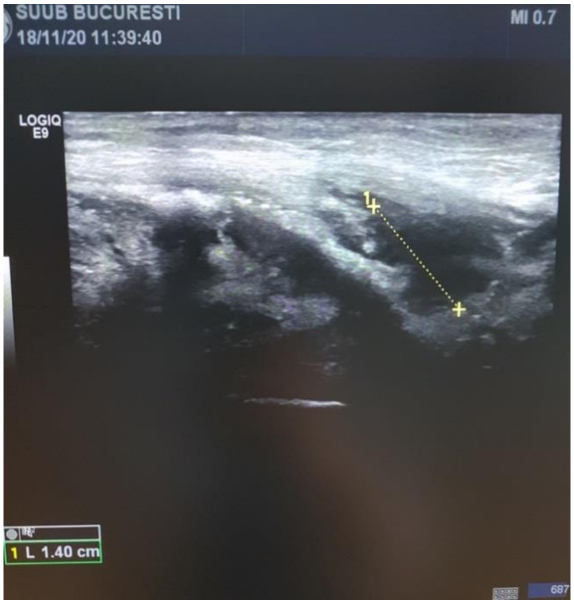

Since the urological consultation and the diagnosis of the fistula took place 2 days after the patient underwent external fixation surgery, the possibility for concomitant primary bladder repair was excluded, leading to the decision for conservative management, with antibiotic treatment, monitored diuresis, and drainage. In case of a favorable outcome, the conservatively treated bladder injury will be followed by cystography to rule out extravasation. On the other hand, if the conservative treatment fails, the patient will undergo abdominal surgery for bladder repair.

The particularity of this case is that the bladder rupture presented with unspecific signs and symptoms, with a rather unusual fistula. The nature of the pelvic fracture, the malfunctioning catheter drainage, and the postoperative status of the patient leads to a more difficult and delayed diagnosis and management of the bladder injury.

DISCUSSION

Researchers found that 85% to 100% of bladder injuries are associated with concomitant pelvic fractures [1-3]. The bony spicules of a fractured pelvis and the shearing forces are the underlying mechanisms of extraperitoneal bladder rupture. Extraperitoneal injury is almost always associated with pelvic fractures [2]. The highest risk of bladder injury was found in disruptions of the pelvic circle with displacement > 1 cm, diastasis of the pubic symphysis > 1 cm, and pubic rami fractures [3, 4].

Intraperitoneal bladder rupture most commonly occurs on the dome of the bladder because it is the least protected area. Direct blow and deceleration forces are the underlying mechanisms. Urine leaking into the abdomen can be absorbed by the peritoneum, causing increased urea and creatinine, metabolic alterations, and low urine output. Infected urinomas can cause pelvic abscesses and peritonitis. Mixed intra- and extraperitoneal bladder rupture are less common, occurring in 8% of bladder injuries associated with pelvic fractures [5].

Diagnosis of bladder injuries can be challenging due to the often vague and non-specific presentation. Literature studies found that up to 23% of all bladder and urethral injuries associated with a pelvic fracture are missed at initial evaluation [6]. Moreover, other studies highlight the delay in diagnosis since the patients were from isolated areas, with poor access to a tertiary trauma center [7].

Iatrogenic bladder injuries are not uncommon. The bladder is the most frequently injured organ during obstetric and gynecologic interventions, such as hysterectomies and cesarean sections. Frankman et al. reported an incidence of 13.8 bladder injuries per 1000 procedures in 2010, but as the incidence of cesarean sections has increased worldwide, the incidence of urologic injuries is expected to rise as well [8].

Vesicovaginal fistula is the oldest described type of fistula. It may occur as a complication of childbirth, hysterectomy, irradiation, and trauma. Patients with neoplasms of the cervix and bladder, especially those who underwent irradiation, are more prone to develop vesicovaginal fistulas [1,4]. Vesicocutaneous fistulas occur after surgical procedures or trauma. Those occurring at suprapubic cystostomy sites after catheter removal usually close spontaneously.

A rare complication of pelvic trauma with bladder injury is a vesical-vulvar fistula, which can be misdiagnosed as a labial tumor or abscess. Also, a vesical-articular fistula can involve the hip joint, causing septic arthritis [9].

Imaging diagnostic methods of bladder trauma and fistula

According to literature studies, conventional intravenous abdominal/pelvic CT will miss 40% of bladder ruptures [1, 10]. Absolute indications for bladder imaging include visible haematuria and a pelvic fracture or non-visible haematuria combined with high-risk pelvic fracture (disruption of the pelvic circle with displacement > 1 cm or diastasis of the pubic symphysis > 1 cm) or posterior urethral injury [4]. Cystography is the preferred diagnostic modality for non-iatrogenic bladder injury and a suspected iatrogenic bladder trauma in the postoperative setting [11]. CT cystography has a higher sensitivity and specificity (99-100%). Both the American Urological Association (AUA) and the European Association of Urology (EAU) recommend either plain-film or CT retrograde cystography for detecting bladder injuries in all suspect patients. In critically ill patients, when cystography cannot be initially performed, a Focused assessment with sonography for trauma (FAST) exam can be used. Bedside ultrasonography with intravesical saline instillation may assist in the early diagnosis of bladder rupture, but it depends on the ultrasonographer's experience [12, 13]. Ultrasonography can also detect intra or extraperitoneal fluid. In the case of pelvic fracture, CT-cystography is superior in detecting bony fragments within the bladder [2, 13]. Cystoscopy is the preferred method for the detection of intraoperative bladder injuries as it directly visualizes the laceration and can localize the lesion concerning the position of the trigone and ureteral orifices [14]. Large perforations are suggested by the lack of bladder distention during cystoscopy.

Imaging diagnostic methods of bladder fistula also include fistulography, cystoscopy, and voiding cystourethrography.

In large vesicovaginal fistulas, urine leaking into the vagina can be easily seen, or methylene blue dye can be used to mark the fistulous path [15].

Management options from international guidelines

The EAU states that the majority of uncomplicated extraperitoneal bladder injuries can be managed with catheter drainage alone and will heal in about 14 days. Conservative management, which comprises of clinical observation, continuous bladder drainage, and antibiotic prophylaxis is the standard recommended treatment in all uncomplicated extraperitoneal injury due to blunt or iatrogenic trauma [16, 17]. In any patient with penetrating or intraperitoneal injury, emergency exploration and repair are recommended even in the absence of pelvic fracture. Bladder neck involvement, bone fragments in the bladder wall, concomitant rectal, or vaginal injury, or entrapment of the bladder wall necessitate surgical intervention [5].

Orthopedic management of pelvic fractures includes internal fixation or temporary external fixation. Given the increased infection risk associated with prosthetic material used in orthopedic surgery, urologists are advised to consider primary repair of uncomplicated bladder injuries to reduce the risk of colonized urine infecting osteo-synthetic material (EAU guideline, Grade B recommendation). Recent studies comparing conservative management to concomitant bladder repair in the setting of internal fixation found an increased risk of infection in patients that did not benefit from concomitant bladder repair [18, 19]. Therefore, bladder repair is recommended at the same time with the internal fixation and exploration for other injuries to decrease the risk for complications and to reduce recovery time [20-22].

Bladder closure is performed with absorbable sutures. There is no evidence that a two-layer is superior to watertight single-layer closure [2, 23].

Follow-up of patients with bladder injury must always include bladder drainage to prevent elevated intravesical pressure and to allow the bladder to heal. Conservatively treated bladder injuries must be followed up by cystography to rule out extravasation and ensure proper bladder healing [4]. The first cystography is planned approximately ten days after injury. In case of ongoing leakage, cystoscopy should be performed to rule out bony fragments in the bladder, and a second cystography is warranted one week later [24, 25]. Cystography is always advised in cases with complex injury (trigone involvement, ureteric reimplantation) or with risk factors of impaired wound healing [13, 15]. In healthy patients with a surgical repair of a simple bladder injury, the catheter can be removed after 10 days without a cystography. Some authors recommend drainage catheter removal after 5 days in conservatively treated internal iatrogenic extraperitoneal bladder perforations and after 7 days for extraperitoneal perforations [11, 22].

Management options for vesico-vulvar and vesicovaginal fistula

The treatment of choice for vesicovaginal fistulas is surgical, although spontaneous closure of small fistulas with simultaneous permanent catheterization for 19-54 days is mentioned in the literature. Approximately 10% of vesicovaginal fistulas from hysterectomy resolve conservatively if the fistula is small (<1cm, <1.5cm), is recent and the Foley catheter is inserted as soon as possible [26-29].

Thus, if a fistula does not close spontaneously in 4 weeks, surgery is needed. Hillary et al. described high percentages of success for the vaginal approach of fistula repair (90.8%) as compared to the abdominal approach (83.9%) [30]. Studies about fistula approach and follow-up are generally scarce and report small numbers of patients. Kapoor et al., in a study on 52 cases, for which he used vaginal and abdominal approach, found that vaginal approach is superior in uncomplicated cases, with small fistulas, but an intraabdominal repair is mandatory for extragenital fistula, for highly situated fistula, when reimplantation of ureters is needed, or when the vaginal approach is impossible [31].

The best timing to proceed to surgical repair is between 3 to 6 months from the appearance of the fistula. Ideally, the best time would be in the first 2 weeks, before bladder inflammation and fibrosis appear, but unfortunately, it takes more than 2 weeks for the fistulas to be detected.

The surgical approach may be transvaginal, transabdominal, or transvesical. Bilateral ureteral catheterization before intervention is recommended, as illustrated in Figure 5. A Foley catheter will also be kept in place for 14 days and removed after a prior retrograde cystography [32].

Figure 5: Intraoperative image of a transabdominal repair of a vesical-vaginal fistula in a post-hysterectomy patient. Ureteral catheterization was also performed and the bladder was sutured.

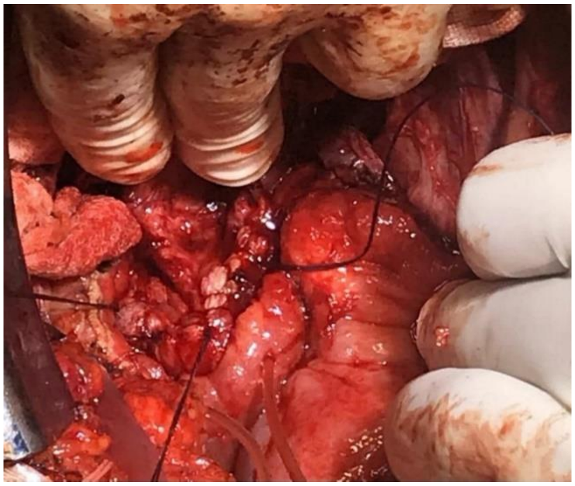

Vesico-labial fistulas are rare and have been scarcely described by literature. Their initial appearance which can lead to the misdiagnosis of vulvar abscesses and the need to primarily address the associated lesions makes this type of vesical-cutaneous fistula to have a later diagnosis and lead to a later approach by transabdominal repair.

CONCLUSIONS

Bladder rupture must be suspected in multi-traumatized patients with complex pelvic fractures, even in the absence of immediate onset of common symptoms like gross hematuria.

Because 40% of bladder injuries are missed at first emergency evaluation, repeating CT scans and using additional imaging techniques can reveal the bladder injury and other posttraumatic lesions.

Vesico-labial fistula is a rare complication of a delayed diagnosis of posttraumatic bladder rupture that can be easily misdiagnosed as a vulvar tumor or labial abscess, requiring a different approach to fistula repair.

Conservative management is the treatment of choice in a patient who already underwent surgical external fixation for pelvic fracture as long as the bladder injury is not complicated by infection and the bladder fistula is small. Bladder drainage with a large Foley catheter is mandatory, with permanent surveillance of catheter functionality and antibiotic prophylaxis. Concomitant bladder repair with internal pelvic fixation or other abdominal wound exploration is recommended to decrease complications and healing time.

The correct diagnosis and management of such cases always require a multidisciplinary team, including urologists, general surgeons, orthopedists, Ob&Gyn specialists, and radiologists.

Medical teams must raise their awareness of potential insidious complications of bladder injuries as some diagnosis can be delayed or incomplete due to the challenges that come with the COVID19 pandemic.

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Blending between occultism and scientism in Vasile Voiculescu's short stories

Mirela Radu[1]

Abstract: *Vasile Voiculescu (1884-1963), besides being a well-known writer, was also a prominent, much respected physician who practiced between the two world wars. He attended the courses of the Faculty of Letters and Philosophy in Bucharest for a year (1902-1903), but gave up on them in order to attend the Faculty of Medicine. The literary debut took place in 1912 in the journal Convorbiri literare. He published the first volume of lyrical poems in 1916 and in 1918 he was granted the Academy Award for lyrical volume From the Aurochs Country and other poems. In 1935 he became a member of the Academy of Sciences of Romania and in 1941 he received the National Poetry Prize. The following volumes of poems are: Ripeness (1921), Poems with Angels (1927), Destiny (1933), Ascent (1937), Gleams (1939). His work also includes a short story: The Demiurge (1943). Shakespeare's last imaginative sonnets in Vasile Voiculescu's translation (1964), and the novel Zahei-The blind (1966), Sentimental Gymnastics (1972) are published posthumously. The present article is aimed at revealing the world of Voiculescu's short stories.*

Keywords: *medical studies, abyssal psychology, scientism, mystery, obscurity*

Although the most analyzed part of Voiculescu's work seems to be his poetry, he proved his worth in the same manner in storytelling. His creative enthusiasm needed several means of expression as Voiculescu the poet was sometimes haunted by a "spirit of storytelling manifestted as a spontaneous eruption." [1]

His inspiration manifested itself as prose also because creative adaptability seems to grow with age: "As the man seemed to «bend under the weight of the years», the creator received new powers, as a sum of his life's and artistic experience by a high level of expression, in a veritable eruption if poetry, in verses and prose." [2] The surprise comes from the chameleonic nature of the author who took on prose with different motives than those in his poetry, coming out as a writer who was: "(…) a perfect master of his own means, not related in any way with the poet." [3] Voiculescu's stories are that much more appealing as they give the sensation of combining some fantasies from the folkloric supernatural, amusing stories, hunting adventures but also "(…) oddities to be told at midnight, parapsychological medical cases etc., which the author attributes a narrative density, emphasizes their equivoque and gives them a superior meaning through the means of literature." [4]

What characterizes the doctrinal physicist's stories are miraculous happenings populated by divine characters

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with an occult composition, “(...) in which «the efficiency of symbols» is not alien to the writer's anthropomorphic manner.” [5] Some of his stories seek to demystify believes: the conviction that the shadow building can lead to death (The shadow, 1935), even to the actualization of some pseudo-scientific procedures of rejuvenation fashionable at the time, among which the method of doctor Sergiu Voronoff for grafting of some glands called by him interstitial, a method that was widely debated in the periodicals of the time [6] (The Demiurge, 1943) [7]. The connection between certain life forms represents the main theme in the physician’s novellas. The influence of medical studies is present due to the fact that Voiculescu combines the folkloric preconceptions with scientific revelations and with: “the hypothesis and conclusions of modern experimental psychology (…). [8] The reason for his leaning towards folkloric heresies was the fact that, being in a permanent search for answers, the literate-physician did find them even in practiced medicine. Unlike other prose writers with folkloric roots, Voiculescu’s characters are fantastic with occult origins. Some critics noticed his short stories astonishing capability of entwining the new with the old, and of “discovering pathways of the archaic in the modern, some depths of existence itself, this causing Voiculescu to write some of his best prose.” [9]

Supernatural, mystery and obscurity were what fascinated him, ticking his interest even before his medical studies in :”(…) heresies, magical symbols, in one word the occult part of existence.” [10] Unavoidably, the permanent dispute in his soul between objectivity and belief can be seen in his work. A proof in this this sense is represented but the fact that in his short stories, the author uses characters that, if not belonging to the medical world, at least belong to the scientific one, meant to shed an accessible perspective of fantastic events to the reader. These characters can be considered an alter-ego of the author, his nature of undressing every fantastic element by finding a clear explanation, or are either introduced in order for the reader to decipher the message. In a world in which the occult loses ground to the prosaic, these characters anchored in the real world have the task of opening doors through which a new reality can be either seen or understood.

The separation of social and personal life is impossible. This can be seen in Voiculescu’s work. The contaminations from one to another: living scientifically but sensing aesthetically can be found in written pieces: “(…) the diagnostician and the therapist can be found, in way or another in writing (…) but the physician-writer relationship can reside in other places too and can be the subject of a study on man, as a professional in medicine but also in prose, who borrows aspects of the life and work of the former.” [11] This combination can only occur by presenting bizarre medical cases, almost always pathological.

To prove this deduction, Florentin Popescu brings the example of the short story The cursed cow (written by the literate-physician in only one day, 27th February 1947) which portraits the morbid case of zoophilia between a spoon maker who satisfies his sexual urges with the cow, Dumana. The short story Dead season (2nd June 1948) from the volume Magical love appeals to psycho-analysis. This time, the character dominated by rationalism- physician Charles and his dog Azor are preyed upon by a mysterious force. Azor mates with a fox and the medic with a peasant. Everything comes back to normal when the mystical element, the fox, is slain. The explanation that the rational man-Charles-tries to attribute to the semi-hypnotic event is insolation and the effect that the moon had on him: “Meaning that his brain was struck by the too powerful light of the moon. Like others get insolation, there are some more sensible to the magnetic waves of the moon.” [12] Some exegetes saw this piece as a case of “ecological Freudiansim” [13], a way of setting loose the sexual restraints. The same as in other stories of Voiculescu, the deeds are described by a narrator which seeks to be objective, doctor X, “… medic and poet in his free time.” [14] This same narrator has he role of arguer, even if he doesn’t acknowledge his ability to psycho-analyze the events “I am not a psychologist and less a Freudian (…) I never sought an explanation for humanism that erupted in him covering the self-imposed artificially to himself.” [15] The writer does not judge, and nor does he incline the scale towards cabalistic or rationalism, he just offers facts left for the reader to judge and decipher. "His interest goes towards a magical underlay in the peasant's spirituality, which he does not judge or condemn." [16]

Dramaturgy represents another aspect under which the inner torments of the Voiculescu, the physician-literate, developed on paper. Among his plays are The wanderer, Sentimental Gymnastics and The Demiurge. In Sentimental Gymnastics, a commoner, Ion Ionescu, comes in touch with psychological elements from William James' doctrine when he receives training, from an ex-colleague – Puiu Moldovian, in repressing affective emotions. The purpose of this training is that of freeing the character from doubts and boosting his self-esteem. If professionally this turn of events is favorable to him, sentimentally it ends in a failure as he becomes too attractive to the women in his town who will fall prey to his charm.

The influence of Expressionism can be seen in the short story On the miracle doorstep (1934) also named dramatic poem in which the issues of religion and fantasy are exposed. Itic Holzfleisch carries the burden of introducing a new religion to mankind. Because of his lack of credibility he is arrested. In prison he hangs himself behind the door where passers could not see him. Because of his supposed disappearance Itic is thought to indeed possess supernatural powers and can become invisible. The miracle though is not fully completed, hence the key in the title – "on the doorstep" – meaning on the edge. Ovid. S. Crohmălniceanu identifies a Verkundigung Drama in this short story. By not respecting any canons, this short story presents itself as an "authentic modern «mystery»." [17] Religious in appearance, the short story could be labeled in the template set by Gândirea, but by shedding light on the main character's inner feelings, the critic perceives it as expressionistic. The same Ovid. S. Crohmălniceanu considers another two short stories belonging to the same literary current, The shadow and The bear's girl, both published in 1930 and then included in 1943 into The Earth's spirit.

The short story The Demiurge (1943) precedes the nightmare that Europe would live three years later: the second worldwide conflagration and the Nazis' experiments for perfecting the pure race. This time, the main character is yet again a medic: Muşatin. Descendant of an old family he seeks to refresh his family's genealogy by gene amelioration. In order to do this, he focuses on experiments, at first on animals, and later on human beings. Lucian, his son, does not agree with his father's conceptions and becomes his victim, having his passion and sexual characteristics removed. The critics saw this short story not only as a S.F. one, but also as a mockery, from a scientific standpoint, of certain theories that would later become a nightmare, thus providing: "a virulent and convincing satire on the dangerous fascist theory of race amelioration and dramatically-beyond value, resistance in the face of critics and posterity- probes the lucid, realistic and healthy position of a physician in the face of some aberration which humanity has lived at a certain time in history." [18]

To narrow down The Demiurge to either the categorization of a satire or a S.F. short story is a narrow angle of analysis. The short story represents a new phase for the political drama. The utilization of science to the damage of mankind and the certainty that a scientist can replace God make this short story an outpost of Romanian novellas with political connotations, resembling with "The physicists by Durenmatt" [19] in its intrigue. Just like Faust, Muşatin defies morality, makes a pact in which his own son will fall: the physician transplants his son's genital organs to an elderly person. Other critics claimed to Romanticism the doctor's ability to fuse the real with the fantastic, by embracing revelry and creation. Belonging also to Romanticism is his approach on national history, as an"(…) altar on which he issued canonicals." [20]

Voiculescu's characters take on magical properties, through spells and calling upon magic being able to access a world separated from the perceivable knowledge. This can be seen in the short story In the middle of the wolves (written 2nd May 1947). This time the reasoning main character is a magistrate that collects folkloric elements. To his astonishment, people around him think greatly of him because of his belief of having supernatural powers. The character meets Lupar, the one that used wolves instead of dogs. The influence of the studies attended by the author can be seen in the characterization of Lupar, which resembles the physiognomy of the criminal noted by Cesare Lombroso, Italian physician and criminalist. In order to get closer to this bizarre character, the magistrate admits that he is no stranger to magical practices, as he practiced spiritism. The physician-literate, as Eugen Simion also noted, has the ability to reiterate mythical elements, through refinement, he refreshes them without fatiguing the reader: "V. Voiculescu uses folkloric syncretism which grants him the ability to update the myths without seeming outdated or suggesting their social death and without sociologism and ethnography." [21]

If it is natural to appreciate the pages which send you towards the abyssal psychology thanks to the knowledge Voiculescu gained in his years of medical study, other critics see those writing that forego scientism and appeal to the fabulous as having a real value: "The storyteller Voiculescu finds his own space in those stories in which he steps in not as a psychologist or a social-moralist but as a character in contingency with the unnatural and supernatural." [22] These short stories filled up with an electrifying combination of the profane and sacred, a world which the prose writer does not reveal to the reader but rather lets him discover it, make the author a homo religious. [23]

This effort to reveal the intangible needs to be appreciated even more so knowing that behind the author hides a rational nature, a man of science dominated by practicality and who values systems of rational thinking and deductive judgment: "The positivism of the physician and the rationalism as the love of beings and things of the spiritual man impregnated Voiculescu's stories with the resin that makes objects durable, giving them good clerical fragrance." [24]

Originating from an urban environment, the characters from Voiculescu's short stories are townsfolk which fight a battle of regaining the right to dream, opposing the civilized world which estranges them from their fabulous origins, as the society from which they originate alienates the human being "through the abolishing of humanity." [25] These urbanites fall prey to devouring love, as humanly as possible, and the magic is given off by "beings with a htonic power." [26] Voiculescu remodels reality enriching the means of sensible knowledge: "Sooner a systematic try of apprehending reality through abundancy of sensations, though the equity of perception." [27] As any men of science, Voiculescu is inclined towards explaining in a somewhat scientific manner, in a rational way all deeds that appear irrational." [28] And these explanations are given through the prism of psychoanalysis and psychology.

Treated by some critics as epigrammatic dystopia, Prefrontal Lobo coagulation appears in 1948, meant as a caustic response, a counterweight to the work of the American psychiatrist Benjamin Karpman, entitled Prefrontal Lobes, enemies of humanity. The work references a practice which would end up serving communism through the psychiatric reprogramming of individuals, of creating "new" people and through eugenics. Voiculescu's short story has the merit of constructing itself in a "revelatory text specifically for Voiculescu's harsh perspective and for the pioneering that is represented by his work." [29] Written on the 27th of July 1948, the short story will be printed only later in the journal Transylvania, in its 9th number of the year 1966. Another source of the short story is found in an article signed by Pierre Wertheimer published on the 10th of July 1948 in the French periodical Medical Presses. Voiculescu's short story is not just a S.F. story, even though many a people identified it as such, but also a ridicule of such deviant practices which were used by the Nazi as well.

The new social order is imposed by the Perfect Permanent Presidium of the Nations for Peace and the newborns are subject to an operations that have as a goal the removal of the prefrontal cerebral lobes, whose physiological function is the adjustment of personality. Initially, this intervention has as subjects, those who belong to certain classes, such as: "the sick, anxious, melancholic, suicidal, perfectionists, schizophrenics, mad men, which often times brought them back to normal." [30] The goal was that of neutralizing possible revolutionary elements though the "affective sterilization, aiming especially for the nervous centers of unrest and revolt." For a bigger impact the medic Voiculescu goes through with the description of the inhuman procedure: “But then it was a barbaric procedure. The medic would make two holes in the cranium and then search blindly and cut the roots of the required fibers.” [31] Later they would resort to a less invasive method, automated radio coagulation, which is applied to all people, striving for the numbing of emotions.

To the stupor of all scientists who saw the operation as the perfect way of relieving people from sentiments and emotions, the youth would first present physiological secondary effects such as: “dyspepsia, problems with digestion, lack of appetite, anemia, and especially constipation” followed by reactions that danger the procedure itself: “the disorders took a psychological turn among the youth, the cases of soul sufferings started to stain the once impeccable epithelium of the P.P.P.P.P and the wanderings started to multiply.” [32] People become more and more independent and are surprised by waves of feelings, daring to feel and even love.

The danger proved way greater: people try to be conscious of spirit. Although these manifestations of revolt are harshly punished, scientists try to answer the question if: “not somehow those blasted fibers had awakened some other hidden branch, untouched by the coagulator?” The answer seems to be found in the pineal gland, responsible for sexual impulses and as such it is the next organ which will be annihilated through radio coagulation. In vain, as people continue to be affected by affection among which “dreaming, sloth, glittered with names such as meditation, thinking, the work of inner searching, the inclination toward sadness, jealousy, the fight against competitors for a woman, for a job, all kinds of shortcomings, and most of all the fear for the future, all the metaphysical fears, and the sentimental complications took all kinds of shapes, as never seen before” [33] The search for the source of evil continues and end on the conclusion that the hearth modifies itself physically obtaining a new cerebelous structure because:” (...) it changed not only its aspect, form and weight, but also its histological architecture. It was heavier, redder, more globulous, less muscular, and full of nervous fibers and autonomous ganglions” and more striking is the fact that “in the upper part, on its globe, underneath the exit of the aorta, there were some curly buds that resembled brain buds.” [34] Voiculescu ends his story in a more optimistic way and once again proves the power of his medical studies even in building good quality literature.

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VARIA

Blockchain technology used in medicine. A brief survey

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Abstract: *Blockchain is the technology that allows people to make transactions without intermediaries. Bitcoin was the first cryptocurrency launched. Cryptocurrencies are digital tokens that can be used for transactions. They use cryptography to establish their authenticity and are not linked to a central authority. Blockchain has many advantages for the healthcare industry and can be used in various fields such as: implementation and improvement of electronic patient records, in clinical trials, neuroscience, pharmaceutical industry and research. Its security can help to improve the confidentiality of patient data and can also help secure the supply chain of medicines. The security and transparency of the blockchain will play a crucial role in the medical industry. This will allow companies to register their products and conduct secure transactions. QR codes can also be placed on the back of medicine containers to help customers identify the authenticity of the products they purchase. The exchange of health information through the blockchain will also have various challenges, such as maintaining the confidentiality of patient data. At the same time, due to the different regulations in different countries, it can be difficult to establish an efficient and secure exchange. Unlike other cryptocurrencies, blockchains are usually immutable, which means that the data added in the chain will always remain. This eliminates the risk of data loss.*

Keywords: *blockchain, cryptography, healthcare*

BLOCKCHAIN TECHNOLOGY – INTRODUCTION

Blockchain is the technology behind Bitcoin, an open peer-to-peer value transfer network that has solved the problem of double spending for the first time. With the launch of Bitcoin, cryptocurrencies became the first use of the blockchain. Cryptocurrencies are similar to fiat money, such as dollars or euros, facilitating the exchange of value, but they use cryptographic protocols as a basis for governance, rather than depending on a central authority, such as banks. In recent years, there has been a growing awareness that the blockchain has much more to offer than cryptocurrencies; the blockchain can be an ideal tool for creating solutions based on trust [1].

In Bitcoin, as in cryptocurrencies, a blockchain is a linear chain of blocks or register that applies only to a computer network. A block is a group of validated transactions in the network, and the two consistent blocks are cryptographically linked. However, lately there have been variations in the way the bloc kchain is defined. It is often used as an umbrella term for distributed registry technology used in any peer-to-peer network that tries to solve problems such as double spending. The blockchain can be considered a peer-to-peer distributed registry only for attachments, with immutable and tamper-resistant records. It is important to understand the concept and life cycle of a transaction in order to understand how blockchain works. In the blockchain, a transaction is a state change. For example, a transfer of money from a sender to a receiver is a transaction in which the account balance represents the status. When a new transaction is created, it is transmitted to the network where a mining node (computer) takes over the transaction and composes a block by combining one or more transactions and transmits the block to the network. Each blockchain has a set of rules that determine whether a block created by a miner is valid or not and only valid blocks are added to the chain. For example, a b lock is considered valid and exploited in Bitcoin if it has the correct cryptographic hash. This set of rules forms the basis of blockchain consensus [2].

A blockchain tracks and records transactions and assets. Its

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immutability is guaranteed by a network of peer-to-peer computers and not by a centralized authority. Assets can be tangible, such as property and money, or intangible, such as patents and intellectual property rights. Each data blockchain contains a hash (a fingerprint), packets with recent transactions marked in time and a hash of the previous block [3].

Through this architecture, each block is connected in chronological order, and the total number of connected blocks forms a blockchain. It is impossible to modify a block in the middle of the chain, because all the blocks that follow it must be modified at the same time. This mechanism is what provides immutability of data in a blockchain network [4].

Blockchain technology is constantly improving, but it may face some challenges that need to be addressed before it can be adopted in medical applications. The first challenge involves transparency and confidentiality, because all data in a blockchain is public. A second challenge is speed and scalability. Given that the number of transactions in the medical system is enormous, it is clear that a revolution in blockchain technology is needed. A final challenge is that of a possible attack and although this is only theoretical, a viable solution should be found [3].

Blockchain has many benefits for the healthcare industry, such as improving the security of patients' digital medical records, resolving issues related to drug authenticity and traceability of the drug supply chain, and at the same time enabling secure interoperability between healthcare organizations [5].

Every year, the medical industry makes about 30 billion transactions, more than half of which are just faxes, which, despite the high costs, do not always arrive on time in the hands of doctors, leading to an increased mortality rate due to mistakes, administrative, communication of patient-related data [6].

Despite the medical records management system, the lack of a transparent and reliable supply chain leads to huge losses for the industry, as an inefficient supply chain involves the inclusion and sale of counterfeit pharmaceuticals. All of this leads to a substantial loss of money and human lives, but blockchain can solve these problems very effectively, making data interoperable and providing doctors with real-time access to it [6].

Not only the drug supply chain benefits from blockchain technology, but supply chains in general. Counterfeit products are a problem everywhere, especially in the food and pharmaceutical industry. Implementing blockchain technology so that it replaces the traditional supply chain management system is the only way to counteract this situation and prevent the sale of counterfeit pharmaceuticals [7].

Transparency and security are two properties of the blockchain that will play a key role in the medical sector. All companies that want to register their products in the blockchain will have to be reliable, and this aspect will be decided by those who control the private blockchain. Once access is allowed to register their pharmaceuticals in a blockchain, they will be able to store all the data and information about them. Each block of data in the blockchain will be marked temporarily and therefore unchanged. Thus, no entity involved in the transaction of pharmaceutical products, from the company to the retailer will be able to modify the data, so as to introduce counterfeit products. At the same time, the movement of drugs from one entity to another can be tracked in real time, through data stored in the blockchain [6].

QR codes can also be attached to the backs of medicine containers, which provide data about their manufacturers and will help customers verify the authenticity of products, thus preventing them from purchasing counterfeit and therefore dangerous products.

When it comes to managing medical records, no matter how important a patient's medical data is, there is still no effective system for storing them all in one place. Assuming that there is such a system, however, different organizations have different types of systems, which lack interoperability, which makes them useless to some extent. A blockchain network for medical records management can bring order to the medical industry. Regardless of the medical centers and organizations that patients visit throughout their lives, blockchain networks will allow all data to be stored in one place, which allows them to verify and monitor their own information [5].

TYPES AND EXAMPLES OF BLOCKCHAIN

The blockchain can be public or private depending on the level of permission [7], but here we generally classify it on three different levels of granularity.

a)Public blockchain: does not require a permit, and anyone can easily participate and validate transactions. Transactions are public and anonymous / pseudonymous. The blockchain is maintained by the public community, so there is the highest level of decentralized trust. Bitcoin is the pioneer of the public blockchain. Bitcoin, Ethereum, Waves (2018), Dash (2018) and Bitshares (2018) are some examples of public blockchains.

b) Federal blockchain: is a licensed blockchain that operates under the leadership of a group often called a consortium. Predefined consortium nodes control consensus. Transactions may or may not be public. Some examples include R3 Corda (2016), EWF (Energy) (2018) and B3i (Insurance) (2018).

c) Private blockchain: is a centralized licensed blockchain in a government organization. Transactions are validated internally and can be read publicly or not. Private blockchains usually have faster blocking times and can process larger transactions. However, these blockchains are vulnerable to security breaches. The value of the private blockchain can be seen as a reliable transformer in which trust is based on an algorithm rather than an authority. Monax (2018), HyperLedger with Sawtooth (2018), private Ethereum are some examples of private blockchains.

The use of a private blockchain for monitoring and storing personal clinical data is one of the potential examples associated with the Healthcare Data Gateway (HDG) method, introduced by Yue et al.[8]. In this personalized healthcare method, patients are free to access, monitor and manage personal clinical data and healthcare summary, stored on a private blockchain (a centralized database system with restricted access control only for authorized or specific users).

Griggs adopted a private blockchain, based on the Ethereum protocol, to not only facilitate the safe and secure use of medical sensors, but also to eradicate the security risks associated with a remote patient monitoring system. Their blockchain-based strategy can facilitate real-time, remote monitoring, allowing practitioners to track the health of their patients in remote locations while maintaining a secure, safe, and up-to-date patient history [4].

Similarly, Ivan [9] demonstrated an approach to the secure storage of health data by implementing a public blockchain (a decentralized database system with open access control to anyone connected to the network) for encryption. In this method, encrypted healthcare data is stored publicly, developing a blockchain-based personal health record. Their proposed method has allowed patients to have better access to their clinical data, where they can freely access and not only monitor their data, but also contribute to their registration and share them with any associated care agency.

In another study, Chen and colleagues proposed an integrated framework based on cloud and blockchain storage, to manage and share the patient's personal medical data. The proposed scheme could be used to secure and secure the storage and exchange of personal medical data of the patient. The suggested approach is unique in nature, as it gives patients complete access and control over their personal medical data, thus excluding the involvement of any external third party [10].

Wang proposed a blockchain framework, based on parallel execution and artificial health systems, to assess the health status of patients' diseases. The proposed method evaluates the general condition, diagnosis and treatment process of the patient and analyzes the associated therapeutic procedures through parallel executions and computational studies for clinical decision making. The suggested system was tested on real as well as artificial health systems to assess the accuracy of the diagnosis and the effectiveness of the treatment [11].

The researchers developed BloCHIE, a unique blockchain-oriented platform for exchanging health information. The suggested platform assesses healthcare data sharing requirements, mainly for personal healthcare data and electronic medical records, and handles various other forms of data by implementing blockchains in different sources. To validate satisfactory authenticity and confidentiality requirements, they coupled the platform with in-chain and out-of-chain verification processes [12].

In 2017, Shubbar introduced a blockchain-based telemonitoring healthcare framework for the diagnosis and treatment of cancerous tumors in remote patients. The suggested protocol uses smart contracts, along with blockchains that can be widely used to ensure the validity and security of patient data in specialized medical centers as well as in patients' homes [13].

In another study, DermoNet was suggested, a blockchain-based method for assisting dermatology patients through an online dermatological consultation through a tele-dermatology monitoring system (“tele-dermatology”) [14].

ProActive Aging is a blockchain-based platform that helps support the active lives of older people. It should be noted that in extensive medical treatment processes, such as chronic diseases (eg cancer, etc.), surgery, and aging, blockchain technology can be an ideal and well-chosen choice. Moreover, it may be possible for the pharmaceutical industries, drug manufacturers and biomedical researchers to use DNA data stored on blockchains to conduct advanced transnational genomic research [15].

BLOCKCHAIN IN MEDICINE – ADVANTAGES, CHALLENGES, BARRIERS

In Table 1 is shown a SWOT analysis of Blockchain Technology.

Blockchain-based health information exchange will be an interesting use case that requires a balance between confidentiality, transparency and efficiency. Moreover, country-specific regulations will be another obstacle to an exchange that connects several regulatory regions. Because patients have full ownership of their healthcare data in many countries, the blockchain-enabled patient-centered healthcare data model may be an appropriate way to circumvent these regulatory challenges [16].

Blockchain also has use cases in managing the clinical trial process for pharmaceutical research. Recently, the Institute of Electrical and Electronics Engineers Standard Association organized a forum on Blockchain for clinical trials in order to use blockchain to innovate in patient recruitment, ensure data integrity, and make rapid progress in drug development. Scrybe, a blockchain project presented on the forum, enables an efficient and reliable mechanism to accelerate clinical trials and the research process. Among other things, it allows for an easy and transparent framework for legal and ethical validations of the audit process by auditors [17].

Table 1: Clinical characteristics and laboratory results of chronic inflammatory rheumatic diseases groups

Strong	Weak
security there is no need for a trusted third party reliability deals with falsification of immutability records more reliable health system control over healthcare journals	high complexity energy consumption the system is still in the prototype phase the integrity of the information is not guaranteed
Oportunities	**Threats**
safer health systems the patient manages his own data patient records with confidentiality more reliable monitoring of patients at home	resistance of the medical system to change privacy issues medical systems without the ability to support the blockchain structure

Blockchain can be used to manage the consent, data and outcome of a clinical trial in a confident and open manner. Such innovations in the implementation and management of clinical trials are crucial for advances in pharmaceutical research. A lot of clinical trials exceed the budget and deadlines. Competitive sharing of clinical data and studies can accelerate research and discovery. Such data sharing can be enabled with blockchain, using examples of approaches. Moreover, the research aspect of pharmaceuticals is quite broad and goes through the process of drug discovery to device manufacturers and the results of clinical trials. A solution in this spectrum is offered by BlockRX using so-called advanced digital registry technology (ADLT). The general goal is to interconnect the disconnected parts in silos [18].

There are many persistent interoperability challenges. Exchange between different institutions can be an operational challenge and requires significant collaboration between the entities involved. Data sharing agreements, complex patient matching algorithms, governance procedures and rules are just some of the issues that need to be agreed before data exchange can take place [19].

There are also many technical barriers. For example, transactional authentication and the entity must be robust (and repeated for each relationship between entities). Activity and threshold monitoring, together with some detection of anomalies, should also be in place. Finally, data exchange security is essential, and standards for data exchange (eg, Fast Healthcare Interoperability Resources (FHIR) or Confidential Disclosure Agreement (CDA) must also be agreed) [20]. In this context, there has been a recent explosion of energy to improve patients' ability to access their own health data. There is little ambiguity as to whether patients should be able to access their health data - the Health Insurance Portability and Accountability Act (HIPAA) requires covered entities to provide individuals with access to their health data upon request (with certain exceptions, such as would be psychotherapy notes).

The transition to patient-led interoperability will require new processes around security protocols, confidentiality configurations, electronic consent, and governance [21].

All transactions with clinical data have associated verification costs: there is the cost of securing data and complying with regulatory guidelines, such as HIPAA, along with the actual cost of maintaining a primary source of truth, the costs of authenticating different entities and transactions, and also the cost of matching patients (and the implicit cost of non-compliance, measured in clinical errors, manual effort and financial costs). Interoperability efforts absorb these costs through various mechanisms - security and confidentiality personnel, technical assistance, health information management offices, etc. associated with joining and operating a network [22].

Blockchain could provide an important catalyst for improving data exchange, especially for patient-driven interoperability [23].

At a high level, blockchain technology can be considered a platform for digital exchange, where the platform operates without a traditional intermediary. Health data can live in several systems, and data exchange requires many points of collaboration between entities. As interoperability becomes more patient-centered, there is an opportunity to use blockchain technology to facilitate this exchange and give patients greater control over their data [24].

Blockchain allows a centralized and shared mechanism for managing authentication and authorization rules around data. For example, a blockchain may have "Smart Properties" - an entity whose property is managed through a blockchain - to allow a certain form of digital property to have clean ownership. The custodian of the data (e.g., the patient) is clearly represented on the blockchain and can subsequently assign access rules and permissions around their data, allowing for easier sharing [25].

A second way in which blockchain technology could foster patient-driven interoperability is the availability of data. Clinical appointments could be securely broadcast on the network and linked to the patient's anonymous digital identity. Patients could publish their own patient-generated health data in a blockchain network. Such patient-generated health data could provide activity monitoring or other personal health data captured outside the formal health setting, provided that a patient authorizes release [26].

Rapid access to clinical information is a third major way in which blockchain technology could improve interoperability in the patient context. For data (or permissions) stored "in a chain", this is immediately clear - the immutable register clarifies the data permissions and, once this is established, the parties can exchange data. If the data is not stored on the blockchain, but there is metadata about the primary data - for example, calendar or location - then this also allows simplified access. Knowing that a patient has undergone an imaging study at a particular hospital in a given year is helpful, as the investigation may focus on that resource, even if the imaging study itself is not available. In these ways, the blockchain improves the liquidity and availability of data and makes it easier for patients to share their data with other entities [27].

Blockchain's use of the public key infrastructure provides a centralized method of identification - an individual's public key - that can be used to link that patient's records between institutions. If each institution knows a patient's public key and the key was linked to its own internal identifier (for example, the patient linked his public key to the patient's portal account when he registered), then subsequent clinical events broadcast on the blockchain network could includes that public key as a reference for the patient, facilitating patient matching [28].

Because blockchains are usually immutable, the data added in the chain will persist. This reduces the risk of loss, provides an audit trail (for example, in the case of a malicious actor), and ensures that a complete digital history is available to all parties (provided there are adequate access controls) [29].

Although the blockchain could allow for patient-led interoperability, there are several barriers that will require attention. The first barrier is related to the size and volume of clinical data. High volume and high frequency transactions are a cornerstone of clinical data, and the size of clinical data is growing exponentially with modern advances in technology [30].

Another challenge concerns privacy and security. Some implementations of blockchain technology are pseudonyms - the identity is usually hidden behind a public key, but other attributes of transactions are shared publicly. This is problematic for health data [31].

The General Data Protection Regulation adopted by the European Union (EU) includes a "right of erasure" and places health data in a special category of personal data. This further raises additional questions about the intersection of the blockchain, encrypted data and patient privacy, especially in the EU context [30].

Perhaps the biggest barrier to adoption is incentives. Although the legislation requires EHRs (Electronic Health Record) to have patient-oriented APIs, the same is not true for all healthcare data and stimulating institutions to build patient-oriented data connections without financial motivation to do so. Thing will be a challenge - the difference between compliance and true interoperability. For example, although a list of drugs may be a necessary output of a hospital's patient-oriented EHR API, it is not clear that a pharmacy benefit manager must structure and expose each approval or drug transaction without a clear commercial value. Other incentives in terms of data exchange will further strengthen the API economy and lead to greater autonomy of patient data [30].

Table 2: Blockchain network – oportunities and challenges

Oportunities	Challenges
Transparency	Interoperability
Short transaction time	Scalability
Sure	Storage
Cost effective	Social acceptance
Irreversible operations	Requires standardization

BLOCKCHAIN APPLICATIONS IN MEDICINE

Blockchain in Electronic Health Record (EHR)

A prototype called "MedRec" uses distinct blockchain advantages in managing authentication, privacy, integrity, and easy data exchange. It operates on a decentralized records management system and aims to provide patients with a detailed, immutable history and allows easy access to their healthcare information among various treatment providers and institutions [3].

During the implementation of EHR (Electronic Health Record), the sharing of medical data often faces critical limitations, such as loss of control over the data, source of the data, audit and security of the data on the medical data. MeDShare could be used to share medical data and maintain electronic medical records between cloud service providers, hospitals, and health research entities [5].

Dubovitskaya et al. proposed a blockchain-based framework for managing, maintaining and sharing electronic medical records of cancer patients. They have adopted a blockchain technology allowed to access, manage and store encrypted patient data. Such proposed frameworks can be used to practically implement blockchain technology for accessing and managing patient confidentiality and data and patient history in clinical practices [32].

In 2016, Estonia emerged with a global leadership in blockchain technology, when it proposed the idea of keeping millions of private medical records and, at the same time, making them available to healthcare providers and insurance companies [33].

Blockchain in clinical research

In the study proposed by Kevin et al., Ethereum allows for a protocol that provides intelligent contract functionality in the blockchain and is used in parallel with clinical data management systems. The main focus of the study was to address the issue of patient enrollment. The results of the study showed that Ethereum led to faster transactions compared to Bitcoin and, therefore, the derived conclusion proposed the use of Ethereum smart contracts for the transparency of data management systems in clinical trials. Therefore, enrolling patients using blockchains is one of the existing applications of this technology in clinical research [34].

Another research was conducted by Mehdi Benchoufi: A framework was implemented to obtain the informed consent of patients to track and store it in a way that is safe, publicly verifiable and non-falsifiable. They used a blockchain technology to create their workflow [35].

Blockchain in detecting medical fraud

Supply chains are vulnerable and consist of sensitive areas for fraudulent attacks, as they involve a number of moving parties and people. Blockchains provide a secure and secure platform to eliminate this problem and, in some cases, to prevent fraud, by introducing greater data transparency and improved product traceability. Because a blockchain record can only be validated and updated by a smart contract, manipulating the blockchain is not easy [34].

Blockchain in neuroscience

The special task of reading and interpreting brain signals is solved by neural interface devices equipped with several sensitive sensors, computing chips and wireless communications. They read the electrical activity of the brain, which is further deciphered and transmitted to the equipment under control [36]. Compex algorithms and big data will use the blockchain philosophy to store those brain signals on the neural interface. One of the companies that confirms that they will use blockchain technology is Neurogress. By recording user data on a decentralized blockchain, this data becomes "resistant to hacking attacks" and therefore more private. At the same time, the use of blockchain technology makes the Neurogress system "open and transparent to potential users of Neurogress platform services" (https://neurogress.com/).

Digitizing an entire human brain obviously requires an environment in which to keep it, and blockchain technology is raising its head again. One proposal is to store mental files, which would act as blocks of data in personal thought chains, which can be shared in a peer-to-peer network file system that allows historical versions. This type of blockchain thinking is proposed as an input-processing-output computing system, with several features that provide opportunities for artificial intelligence, human improvement and their potential integration. Blockchain allows an interconnected computer network to reach timestamp intervals to validate the source and truth of a register [37].

In the not-so-distant future, we can begin recording our sensory experiences using wearable technology, the current state of brain and nerve implants, biofeedback imaging, and any other sensors that allow a multifactorial fingerprint specific to a particular person's recording of temporal experience. Using these technologies as a starting point, research can be conducted in improving decision-making protocols, learning, recall, and rehabilitation [37].

Blockchain in the pharmaceutical and research industry

In common cases, pharmaceutical companies face the challenges of tracking their products in a timely manner, which sometimes leads to severe risks, allowing counterfeiters to compromise production or invade counterfeit drugs into the system. Consequently, the production and distribution of counterfeit medicines has become one of the major and global health risks, especially in developing countries [25].

During the production, as well as research and development of these drugs, the blockchain could be a technology that fits best, which can be used to evaluate, monitor and ensure the production processes of potential drugs. Recently, Hyperledger launched a medical project using blockchain technology to inspect and combat the production of counterfeit drugs. A digital drug control system (DDCS) could be a sustainable solution for the prevention of counterfeit drugs [38].

Many blockchain-based solutions for intellectual property management have been proposed in the generic space, which can also be applied to innovations in drug development. Bernstein (https://www.bernstein.io/) provides blockchain-based management of digital routes, with timestamping, to protect intellectual property priorities; this feature may be beneficial in collaborative pharmaceutical research. A solution from iPlexus (https://www.innoplexus.com/news/blockchain-and-ai-based-platform-iplexus-to-revolutionize-drug-discovery/) uses blockchain to make any unpublished and published data from drug development studies available for use. Blockchain solves the puzzle of maintaining trust and protecting intellectual property to enable such a revolutionary initiative and framework.

Using a digital drug control system based on blockchain, the major pharmaceutical industries (Sanofi, Pfizer and Amgen) have launched a joint pilot project for the inspection and evaluation of new drugs. Using the blockchain as an approach, it would be possible to aim not only at the production and location of medicines at a given time, but also at improving the traceability of counterfeit medicines, security of the drug supply system and guaranteeing the quality of medicines supplied to consumers or end users [39].

BRIEF CONCLUSION

Blockchain technology is very advanced and secure and could help store data in the medical system and share it much faster between medical institutions.

At a high level, blockchain technology can be considered a platform for digital exchange, where the platform operates without a traditional intermediary. Health data can exist in several systems, and the exchange of data requires many points of collaboration between entities. As interoperability becomes more patient-centered, there is an opportunity to use blockchain technology to facilitate this exchange and give patients greater control over their data.

Of course, there are certain barriers and challenges that need to be overcome.

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VARIA

Bucharest mobile military hospital – response to the COVID-19 pandemic

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Abstract: *The idea of using mobile hospitals - a fully functional hospital is a system with a high degree of complexity - in case of a pandemic crisis, is not new, but shows how much they are needed.*

Solutions belonging to the category of mobile architecture are increasingly common, technical and logistic difficulties are solved in more perfect way. This applies mostly to objects with simple layouts and functional relationships, with relatively little technical equipment. In the case of healthcare facilities there are several factors increasing the difficulty: multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure.

The Romanian military and civilian specialists from Bucharest Central Military Emergency University Hospital (SUUMC) showed that is possible to build a hospital as a modular and mobile structure in order to fight COVID 19, but the strategic actions and the effort needed to organize and run mobile hospital far exceeds the expenditure, protocols and specialized M.D.s necessary to run the stationary hospital.

Keywords: *mobile hospitals, COVID-19, strategic actions*

INTRODUCTION

SARS-CoV-2, the causative agent of COVID-19, is a newly discovered coronavirus. It is transmitted through respiratory droplets and direct contact; other modes of transmission (i.e. airborne and digestive) have been proposed, but they are still under investigation. The average incubation period is estimated at 5 to 6 days, ranging from 1 to 14 days [1]. There is currently no approved specific treatment or vaccine against COVID-19.

Business-as-usual service delivery approaches are not sufficient to respond in the fight against COVID 19, once cluster of cases or widespread community transmission is registered and superior capacity will be needed.

While World Health Organization (WHO) advises that aggressive measures to find, isolate, test, treat and trace are the best ways to stop the spread of this virus, public health interventions to "flatten the curve" may slow transmission and mitigate peak capacity needs. Modelling studies point to the impact of nonpharmaceutical interventions (NPIs), such as physical distancing, school and university closures, banning of mass gatherings, and remaining indoors, on spreading the number of cases over a longer period to give health systems the opportunities they need to cope with caseloads [1, 2, 3, 4]

These models have helped to reinforce the message from WHO about the implementation of such measures, alongside widespread testing, to detect cases and break chains of transmission. In addition to these measures, phased plans for creating surge capacity in acute and intensive care will help respond to COVID-19 cases while maintaining essential health services.

Surge capacity is the ability of a health system to manage a sudden and unexpected influx of patients in a disaster or

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emergency situation.

Surge capacity can be created from intrinsic and extrinsic resources. The former covers all local resources that can be used for the response, while the latter involves leveraging resources from outside the affected area (across geography or across specialty).

As the COVID-19 pandemic necessitates both in tandem in all countries, this note covers both these aspects together. Creating surge capacity involves a comprehensive approach linking the 4 S's of surge capacity: space, staff, supplies and systems. [5]

RECOMMENDATIONS AND STRATEGIC ACTIONS

In the following, are presented the recommendations and strategic actions to activate surge capacity in line with the 4 S's drawing on past experiences in emergencies and on the emergent experiences of world countries in the COVID-19 response.[6]

Strategic action 1. **Create and activate a stepwise plan to expand physical space to care for COVID-19 patients respecting infection prevention and control protocols**.

This action requires the following steps, and is schematically presented in the Figure 1.

Figure 1: Necessary steps for strategic action 1

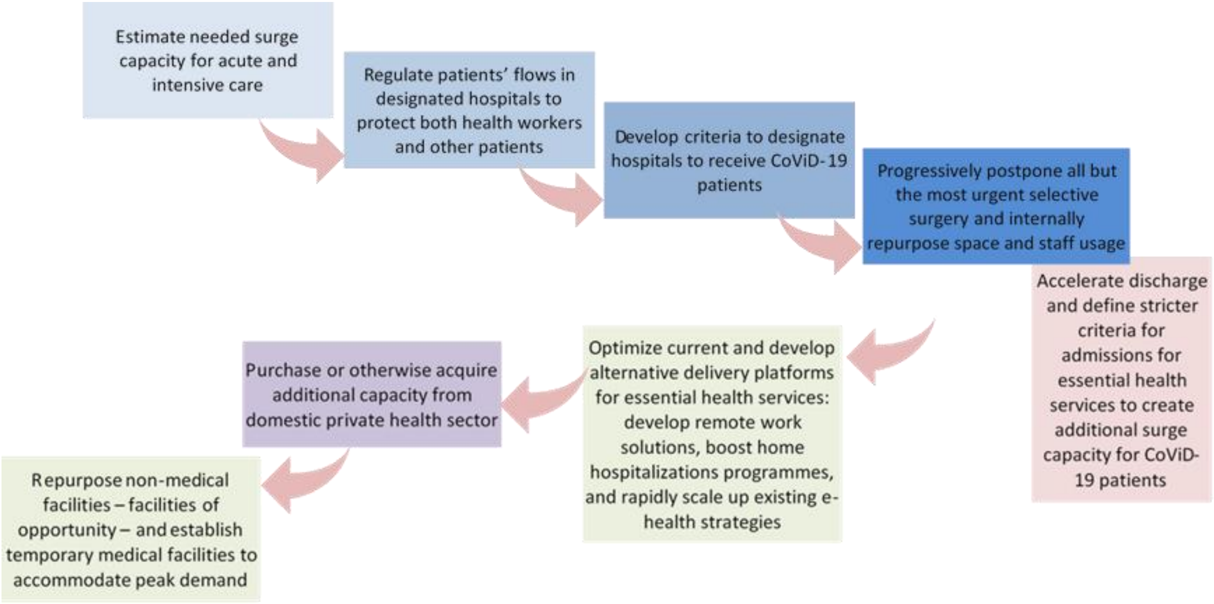
Estimate needed surge capacity for acute and intensive care
Regulate patients' flows in designated hospitals to protect both health workers and other patients
Develop criteria to designate hospitals to receive CoViD-19 patients
Progressively postpone all but the most urgent selective surgery and internally repurpose space and staff usage
Accelerate discharge and define stricter criteria for admissions for essential health services to create additional surge capacity for CoViD-19 patients
Optimize current and develop alternative delivery platforms for essential health services: develop remote work solutions, boost home hospitalizations programmes, and rapidly scale up existing e-health strategies
Purchase or otherwise acquire additional capacity from domestic private health sector
Repurpose non-medical facilities – facilities of opportunity – and establish temporary medical facilities to accommodate peak demand

Strategic action 2. Identify the health workforce available for surge capacity demands, and repurpose and upskill for rapid deployment to meet surge capacity needs

The steps necessary for this strategic action are depicted in Figure 2.

Figure 2. Necessary steps for strategic action 2

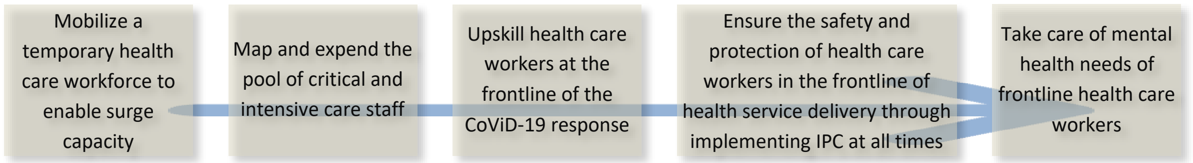
Mobilize a temporary health care workforce to enable surge capacity
Map and expend the pool of critical and intensive care staff
Upskill health care workers at the frontline of the CoViD-19 response
Ensure the safety and protection of health care workers in the frontline of health service delivery through implementing IPC at all times
Take care of mental health needs of frontline health care workers

Strategic action 3. Ensure adequate supplies to support surge in demand

The necessary steps for strategic action 3 are presented in Figure 3.

Strategic action 4. Establish systems to manage and align policies to meet surge in demand

The steps that are necessary for strategic action 4 are mentioned in Figure 4.

Figure 3: Necessary steps for strategic action 3

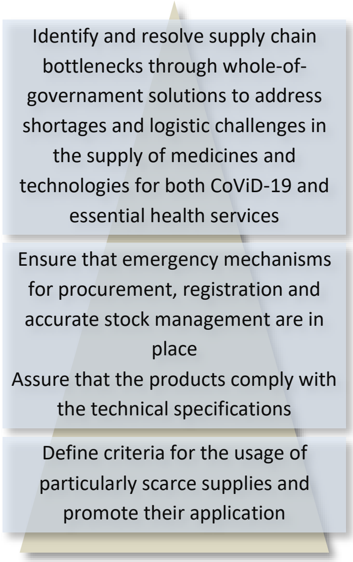
Identify and resolve supply chain bottlenecks through whole-of-government solutions to address shortages and logistic challenges in the supply of medicines and technologies for both CoViD-19 and essential health services
Ensure that emergency mechanisms for procurement, registration and accurate stock management are in place
Assure that the products comply with the technical specifications
Define criteria for the usage of particularly scarce supplies and promote their application

Figure 4: Necessary steps for strategic action 4

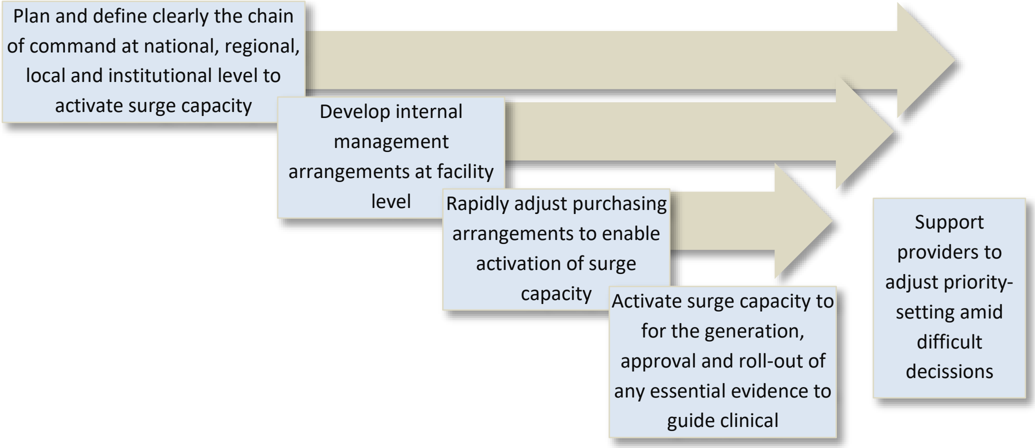
Plan and define clearly the chain of command at national, regional, local and institutional level to activate surge capacity
Develop internal management arrangements at facility level
Rapidly adjust purchasing arrangements to enable activation of surge capacity
Activate surge capacity to for the generation, approval and roll-out of any essential evidence to guide clinical
Support providers to adjust priority-setting amid difficult decissions

THE ROL 2 MILITARY HOSPITAL AT THE ANA ASLAN INSTITUTE IN OTOPENI, NEAR BUCHAREST – A CASE STUDY

The ROL 2 military hospital was installed at the Ana Aslan Institute in Otopeni city, near Bucharest, to support the Romanian government as a response to the Covid-19 pandemic. The ROL 2 was declared operational on Saturday, March 28, in the presence of President Klaus Iohannis and Prime Minister Ludovic Orban, the Ministry of Defense announced.

Once accredited by the Ministry of Health, the ROL 2 hospital will operate as an external facility of the Central Military Emergency University Hospital (SUUMC). The mild and medium cases of COVID-19 will be admitted and treated in this medical facility (Figure 5).

Figure 5: The ROL 2 first medical team

The ROL 2 mobile military hospital operate as a stand-alone entity (as in the American solutions), or in conjunction with the whole hospital – Bucharest Central Military Emergency University Hospital (but still autonomously, as in European solutions). These two variants are taking into account organisation of space or technological line flow. The difference between the two models comes down to one important aspect: in the European pattern patients' flow is unidirectional, almost linear, from the entrance of the patient to the segregation area (TRIAGE), through diagnostic and treatment facilities ending at the hospital bed wards). This is due to the principles of Emergency Department (DE) operation. ED is an integral part of the hospital and at the same time it is an emergency path of hospitalization. The American ED is more autonomous unit, operating often as an outpatient clinic compared to European standards: the TRIAGE and central nurse station become the most important areas of the ED. Other functions of the ED are grouped around the focal point of the nurse station. The flow of patients through the ED is less structured, without clearly separate movement paths, always through the nurse point.

In the case of building a mobile military hospital there are several factors increasing the difficulty: multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure, which includes both the military and civilian objectives.

The mobile military hospital is built as a reaction to disasters, both natural and those resulting from human factor, for "emergency" (acute and crisis situations) activities. An emergency medicine, no matter of the form of its organisation, is a set of medical actions, serving a life rescue.

The technical solutions are related to 2 terms: modularity and mobility. Modularity should provide a construction of possibly most numerous and diverse structures of various purposes. Mobility is to ensure a solution which enables the module (typical commodity container) to be loaded and transported by standard ways: truck or a ship, or even air-borne.

For this achievement, the management of the Bucharest Central Military Emergency University Hospital (SUUMC) conceived a procedure which has been developed to support hospital preparedness for the management of COVID-19 patients, based on European Centre for Disease Prevention and Control recommendations [7]. The procedure started with the procurement and stock management. A procurement procedure to acquire the necessary materials and supplies was activated on short notice and alternative suppliers have been identified. The acquisition team took into account that if main suppliers should run out of stock (especially for personal protective equipment (PPE)) a buffer stock of key supplies has been acquired (e.g. for hand and respiratory hygiene, PPE, isolation, ICU supplies, mechanical respirators). A procedure to monitor and regularly update the inventory and a stock inventory have been conducted. In order to avoid misuse or overuse, a plan was developed to keep track and custody of key supplies (e.g. PPE, ventilators, cleaning and disinfection material, alcohol solution etc.).

The maximum facility capacity, including the maximal number of ICU beds, mechanical ventilators, bins for infectious waste, and the required human resource capacities (M.D.s, healthcare workers and cleaning personnel) and supply capacity have been calculated.

Storage facilities which meet all demands with respect to temperature, humidity, cold-chain, logistics etc., for additional stock have been identified, also a proper amount of cleaning and disinfection products shown to be effective against coronaviruses is available. A procedure to ensure that equipment is in perfect working order and can be quickly replaced whenever necessary was developed.

All staff, healthcare and non-healthcare workers, have been informed and trained on the following topics: Case definitions, Hand and respiratory hygiene, How to use PPE's and know the documents outlining the procedures, Triage procedures, Placement and movement of patients in isolation, Internal and external communication lines and rules, Sick-leave policy and what to do if staff members show symptoms and Security plan.

Procedures for patient prioritization (e.g. triage, discharge criteria, triggers to postpone elective hospitalizations or interventions) are in place A patient' system before they arrive at the hospital is in place: phone/email/telemedicine services are in place for possible cases.

A procedure for the cleaning of the rooms on a regular basis and after a patient's discharge has been established.

Figure 6: The ROL 2 mobile military hospital – general view

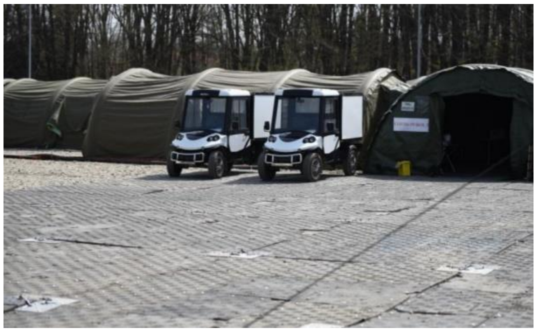

The ROL2 mobile military hospital, made up of medical tents and containers (Figure 6) is intended for the triage, diagnosis and hospitalization of patients confirmed with COVID-19. The structure of the hospital is composed of two parts: the medical and the administrative part. The medical part is structured in the following sections/laboratories/

compartments:
- Emergency Room Unit (ERU) with 20 beds
- Infectious Diseases Department (IDD) with 120 beds
- Intensive Care Unit (ICU) with 24 beds
- Imagistic Laboratory
- Medical Analysis Laboratory
- Pharmaceutical point

The hospital was equipped with medical equipment necessary for the diagnosis and treatment of patients with COVID-19, as follows: necessary RT-PCR for the identification of COVID-19, biochemistry analyzer, mobile radiology apparatus, ultrasound device, oxygen concentrators, pulse oximeters, laryngoscopes, intermittent mechanical compression devices, patient heating system, trolleys for the storage of sanitary materials and medicines, , air purification devices, wheel sprayers for car-trucks disinfection.

The Intensive Care Unit (ICU) was equipped with specific beds, secretion aspirators, oxygen pumps, automatic syringes with related stations, mechanical fans, and monitors for monitoring vital functions. Very important is the fact that telemedicine system has been installed with video cameras for the monitoring of patients, so the medical staff can keep them under observation, with minimal contact.

The Emergency Room Unit (ERU), adjacent to the patient triage area, is equipped with 8 beds and a stationary area featured with 6 compartments of 2 beds for patients waiting for lab results and for carrying out complementary investigations (interdisciplinary evaluations, imaging investigations). If the number of patients presented is greater than the capacity to take in the ERU, there are two waiting areas for patients featured with chairs located at a distance of at least 1 meter, in an adjacent tent (Figure 7).

The Infectious Diseases Department (IDD) is designated for the admission of patients confirmed with COVID-19. The section is provided with 120 beds equally distributed in 30 individual reserves with own sanitary group. Each reserve is provided with medical equipment and equipment for the permanent monitoring of patients, with the possibility of ensuring the oxygen requirement to patients who do not meet the criteria for admission to the intensive care unit. All IDD rooms are sterile environments; all personnel wear protective clothing (scrubs), shoe covers, masks, caps, eye shields and other coverings.

The inventory for 1 IDD reserve (Figure 8) includes: 4 beds, disposable linen, 1 TV set, 1 telephone and equipment for the medical supervision of patients (nurse-call system), 1 refrigerator, 4 stands of infusion, 1 acute care physiologic monitoring system (comprehensive patient monitoring systems that can be configured to continuously measure and display various parameters via electrodes and sensors connected to the patient), 4 pulse oximeters, 1 treatment table, 4 bedside tables. Lingerie is changed daily and whenever necessary.

Figure 7: The Emergency Reception Unit (ERU)

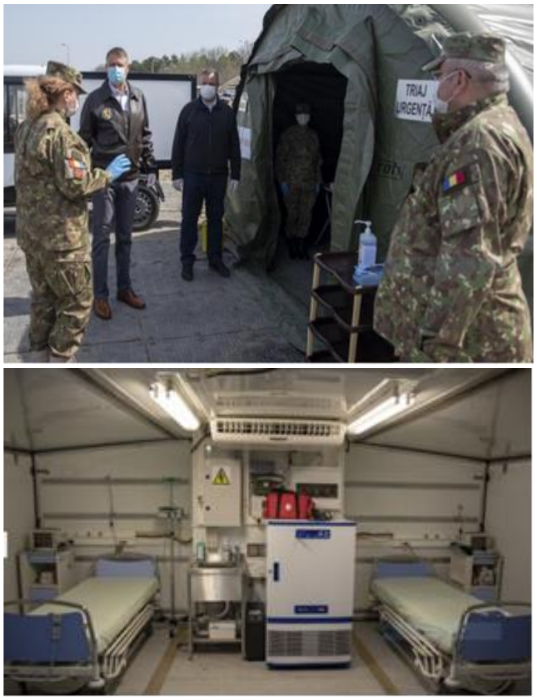

Upon admission to the IDD, each patient will receive a disposable kit containing: 1 disposable pajamas, 1 pair of disposable slippers, 1 toothbrush and toothpaste, 2 absorbent fields (towels) size 90/140 cm^2. These materials will be later distributed, whenever appropriate.

Figure 8: IDD reserve

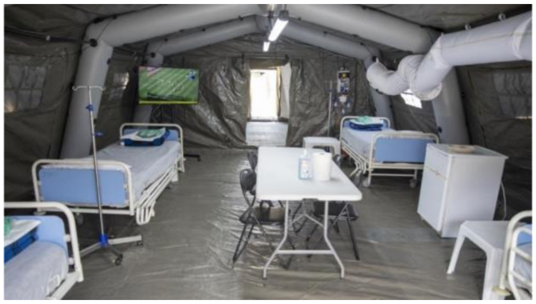

The medical part also comprises:

- **The Intensive Care Unit** (ICU) is undergoing an arrangement with 24 beds and it is located in the annex building of the ROL 2 hospital. Patients with severe respiratory disorders cannot be properly treated in tents, as they must benefit, for a certain period of time, of special care and connection to mechanical fans.

- **Two own laboratories** (Figure 9) where diagnostic tests (RT-PCR) and respectively hematological and biochemical investigations (complete blood count, CBC (hemoglobin), Erythrocyte Sedimentation Rate (ESR), coagulation test, myoglobin, D-Dimer, blood chemistry tests including Ferritin, troponin test, procalcitonin (PCT), presepsin (P-SEP), HBV, HCV, HIV Screening Tests, detection of SARS CoV 2 with RT-PCR) will be performed.

Figure 9: Laboratory for diagnostic tests (RT-PCR) and hematological - biochemical investigations

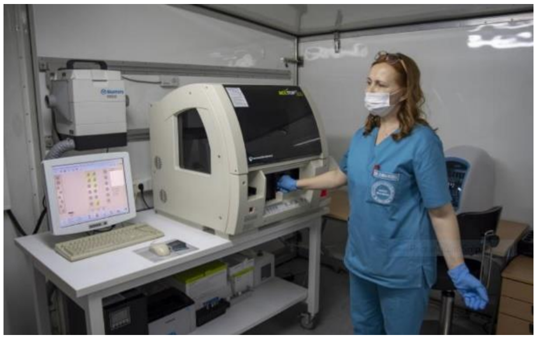

- **Imaging laboratory** provided with a mobile radiology device and Computer Tomography machine.
- **Pharmaceutical point** (Figure 10) where antiviral drugs necessary for the treatment of patients confirmed according to the national protocol adopted at the level of the Ministry of Health and taken over by this structure will be available.

Figure 10: Pharmaceutical point

At the ROL 2 hospital level, the functional circuits with the delimitation of the red and green areas, are ensured by providing separate flows for patients and medical staff, with rooms for equipping the medical and auxiliary staff and separate rooms with their own sanitary for undressing.

The sections and compartments of the ROL 2 hospital are computer interconnected between them and the command point, as well as with the computer network of S.U.U.M.C "Carol Davila".

The payroll comprises a total of approximately 270 positions distributed over each structure. For the operationalization of the ROL 2 Hospital, the personnel will be provided from the human resources of the S.U.U.M.C. and partly from the Medical Directorate.

The work program of the staff is structured on each separate compartment, in 12-hour shifts, over a 7-day cycle and is provided by working medical corps, made up of doctors, nurses, stretches. After this period of 7 days, the work team enters the period of self-isolation for a period of 7-14 days, a necessary measure for both the rest and the physical and mental recovery of the personnel and for the epidemiological supervision. The activity will be continued by other working medical corps, following the same structure.

At the entry and exit of the 7-day work cycle, the entire workforce will be clinically evaluated and the diagnostic test using RT-PCR for COVID-19 will be performed. Also, the staff will be tested as needed if it becomes symptomatic during the activity.

The work program is structured as follows:

- 7 consecutive days with 7 days rest for doctors and 14 days for nurses, nurses, stretches and medical administrators;
- Daily, there will be two shifts (day and evening) at 12 hours intervals; the ranges are from 08:00 – 20:00, respectively 20:00 – 08:00;
- Each 12-hour work program is provided by separate working medical corps.
- The structure and work schedule for a 7-day cycle of the IDD
- 2 specialist/consultant doctors (infectious diseases and pneumology or internal medicine);
- 6 residents (infectious diseases – 2 and internal medicine – 4);
- 12 nurses (also includes the head nurse);
- 4 orderlies;
- 2 stretches.

The procedure described may not be applicable to all military or civilian hospitals and may need to be adapted to the specific characteristics of the hospital, the individual national health system, legislation and community where the hospital is located.

“Today I visited the ROL 2 Military Hospital, a medical facility made in record time by the Army and the military medical personnel. Romania is prepared to cope as well as possible with this epidemic and I assure you that we will continue to

take all necessary measures to be even better prepared," Klaus Iohannis (Figure 11) said after visiting the hospital. [9]

Figure 11: The Romanian President Klaus Iohannis and Major General Assoc. Prof. Florentina Ioniță-Radu MD, PhD at the inauguration of the ROL 2 military hospital installed at the Ana Aslan Institute in Otopeni (Photo: Presidency.ro)

The same ROL 2 military hospital was deployed in 2015 when it was supposed to treat potential Ebola infection cases. It was also used in NATO military exercises in 2019, including Vigorous Warrior – the biggest medical military exercise ever organized by NATO, which was hosted by Romania.

"*I have entrusted the supreme ally commander of Europe with the task of coordinating all the means of supporting, coordinating and directing all the excesses at national level to the allies who need support at this particular moment, a first step taken by NATO, at this moment, is to provide the logistics of allies who need support in the fight against the coronavirus pandemic*." said Mircea Geoana, NATO Deputy Secretary General. [10]

CONCLUSIONS

The set up of the mobile military hospital installed at the Ana Aslan Institute in Otopeni, near Bucharest, to support the Romanian government as a response to the Covid-19 pandemic complies all the health requirements regulations defined by the National Authority of Quality Management in Health (Autoritatea Naţională de Management al Calităţii în Sănătate – ANMCS) throughout specific accreditation standards for medical units.

Further studies are necessary in order to determine the Covid-19 pandemic amplitude actions to end it, and future research projects destined to cover the need and development of these mobile hospitals, which involve a multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure is necessary.

Note

The ICU is arranged in the annex building of the ROL 2 hospital. Patients with severe respiratory disorders cannot be properly treated in tents, as they must benefit, for a certain period of time, of special care and connection to mechanical fans.

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ORIGINAL ARTICLES

Burnout syndrome in the Emergency Department of the Central Military Emergency Hospital before and during the COVID-19 pandemic

Florea Costea[1], Mihai Sălceanu[1], Iulia M. Staicu[2], Alexandru G. Andreescu[3]

Abstract: *Introduction: Public interest in burnout has grown recently in developed countries, as has media coverage. Burnout has been a topic of scientific research in recent years for psychologists and sociologists alike. They have published numerous articles on the identification and classification of burnout syndrome.*

The medical staff is quite exposed to burnout because they often experience strong emotions such as the desire to treat or save their patients, fear of failure, occasional failures in the treatment of diseases.

Materials and methods: This study aims to highlight the impact of the burnout syndrome in the Emergency Department of the Central Military Emergency Hospital during the COVID-19 pandemic, by conducting a comparative study based on the results of a questionnaire applied to the medical staff before and during the COVID-19 pandemic.

Results: The study interviewed 65 participants aged between 20 and 60 years. They had to anonymously answer 16 questions marked with scores from 1 to 4.

Comparing the results highlighted by the questionnaire, applied before the COVID-19 period, with those obtained in the questionnaire applied between May and June 2020, we noticed an increase in the level of burnout in all categories interviewed during the pandemic.

Conclusions: In conclusion, the high level of contagiousness and the lack of a vaccine or treatment against SARS-CoV2 infection are additional concerns for burnout syndrome among healthcare professionals.

Keywords: *COVID-19, burnout syndrome, healthcare professionals*

INTRODUCTION

What is burnout?

The term "burnout" appeared 25 years ago in the United States. One of the first scientific descriptions of burnout syndrome was made by the psychoanalyst Freudenberger who described it as manifesting polymorphic symptomatology that fluctuates in degree from person to person [1]. In 1981, Maslach introduced a far-reaching definition and psychometric tool for assessing burnout syndrome, which is still the most commonly, used today, The Maslach Burnout Inventory [2].

An alternative to assessing the burnout syndrome has become "The Oldenburg Burnout Inventory: A Good Alternative to Measure Burnout (and Engagement),"

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published by Prof. Dr. Evangelia Demerouti in 2007 at the Utrecht University in the Netherlands. She is well-known for her research in the field of occupational burnout and introduced this method to quantify the burnout syndrome based on two proportions: exhaustion and disengagement. Her annotations in the 2008 article define exhaustion as being a repercussion of intense and prolonged physical, affective, and cognitive pressure such as the long-term effects of protracted tedious job demands. On the other hand, she noted that disengagement can be identified as a breakaway, chiefly from work, the activity, or the object of the said workplace, for example, becoming uninteresting, challenging, or even disgusting [3].

Burnout has been a topic of scientific research in recent years for psychologists and sociologists alike. They have published numerous articles on the identification and classification of burnout syndrome, but the major problem that remains is that there is no generally accepted definition of burnout. Identifying potential causal factors and separating them from other health disorders is difficult, which represents the main reason why it is difficult to identify a generally valid definition. Therefore, an interdisciplinary approach would probably help a broader understanding of burnout syndrome, which has become a common pathology in modern occupational medicine (Figure 1).

Although hard to systemize and cut down to a single phrase definition, studying the medical literature, we found it is widely accepted that burnout is a syndrome defined by emotional debilitation, a loss of the sense of self, and personal accomplishment. Symptoms that may be identified in medical staff are anxiety, impatience, mood swings, and depression. Moreover, physical health may be impaired, with manifestations such as disseminated aches and pains, digestive problems, and increased cardiovascular risk. All in all, these symptoms are not specific enough to guide a person in seeking help [4].

Burnout syndrome was found more widely in careers that involve personal contacts with other people, specifically those that imply a high level of demand and pressure on the worker. Even though the medical staff represents the majority of those affected by burnout, it is important to note that occupational groups such as advocates, teachers, or human resources have been reported to also be prone to this syndrome [5].

Specific psychiatric literature has not yet classified burnout, for example in the “Diagnostic and Statistical Mental Disorder, 5th edition (DSM-5) [6]. In some European countries, such as Sweden, symptoms of burnout represent a justification for sick leave. Proof of the developing awareness of this syndrome is its inclusion as health altering and contact with medical services in the 10th revision of the “International Classification of Diseases” [7] (ICD-10) where burnout is coded Z73.0 and characterized as a state of vital exhaustion [8].

Figure 1: The burnout syndrome evolution ("Burnout Waterfall") [9]

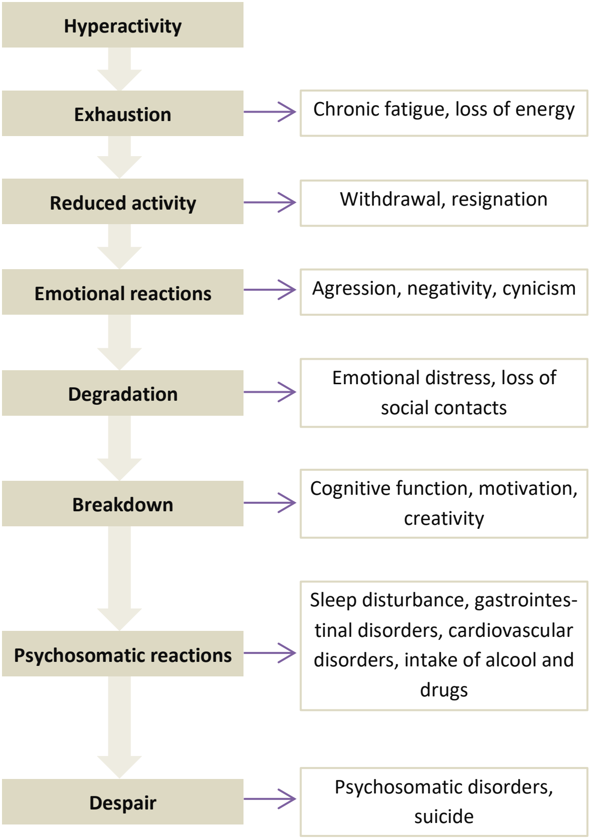
Hyperactivity
Exhaustion
Chronic fatigue, loss of energy
Reduced activity
Withdrawal, resignation
Emotional reactions
Agression, negativity, cynicism
Degradation
Emotional distress, loss of social contacts
Breakdown
Cognitive function, motivation, creativity
Psychosomatic reactions
Sleep disturbance, gastrointestinal disorders, cardiovascular disorders, intake of alcool and drugs
Despair
Psychosomatic disorders, suicide

Burnout on medical staff

In the last few years, there has been an increasing emphasis on the impact of professional activity regarding the health of medical staff, with numerous studies showing that medical staff is more affected by burnout syndrome than staff in other fields.

Healthcare workers have always had an active, routine life and have assumed from the beginning of their careers that they will have to make personal sacrifices for the good of patients. Besides, the daily decisions on which patients' chances of healing or their chance at life depend on them.

The factors that manifest a strong emotional impact on the medical staff have recently multiplied: there has been a risk of malpractice, the swift evolution of technology that requires continuous professional training (in detriment of free time), increased administrative burdens correlated with reduced consultation time for each patient.

Table 1: Factors that may influence a doctor's well-being [11]

Categories of factors	Examples
Chronic fatigue	Excessive workload Sleep deprivation Constant access demands (eg. Electronic availability) Decresed personal time
Perceived threats	Malpractice lawsuits Medical error Reduce compensation Research funding climate
Loss of autonomy	Practice environment Time to interact with patient constrained
Inefficiencies	Administrative requirements Lack of support staff Practice organization
Balancing needs	Suboptimal integration of work and life responsibilities Clinical service requirements and additional demands (teaching or administration)
Chronic stress	Work pace Practice setting
New technologies	Electronic medical health records Keeping up with technological advances in the practice
Physician factors	Perfectionism Internal drive and ambition Negligence regarding personal health and well being

All the aforementioned factors create an environment in which the practitioner is exposed to multiple high-stakes decisions, rendering the situation into a physically, psychologically, and emotionally stressful experience. As a consequence, medical professionals may show signs of emotional distress and avoidance behavior. Furthermore, long-term absence may be seen, with an alarming level of skill drain, leading to economical strain. The quality of medical services may also be affected [10].

All these factors have led to an increase in the number of medical staff exposed to burnout syndrome (Table 1).

The global effects of COVID-19

Coronavirus is a family of viruses that can cause diseases such as respiratory viruses, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS). In the winter of 2019, a new member of this family, SARS-CoV-2, was identified as originating in China, more precisely the source of the outbreak was the municipality of Wuhan. The disease caused by this virus is called COVID-19. In January of 2020 isolated cases were reported in some European Union states. By the end of February 2020, Italy reported an important increase in COVID-19 cases. [12] Following the accelerated evolution and spread of SARS-CoV2, the World Health Organization (WHO) declared COVID-19 as a global pandemic.

The rapid spread of SARS-CoV-2 globally had a strong psycho-emotional impact among the population, developing an increased level of stress and anxiety. The most affected by the psychological effects were the vulnerable groups directly involved in this infection, the elderly, the people from the placement centers, the staff in the front line. At the same time, this increase in the level of stress and anxiety was accentuated by the strict measures applied to prevent and combat the disease (quarantine, isolation, change of daily routine). Adding up to the ambiguity experienced by the population was a storm of catastrophic and sometimes sensational bits of information that was spread through various forms of media consumption platforms, rendering the citizens uncertain and helpless while sustaining a sense of distrust towards official information [13]. All these factors have led to an increase in the number of people affected by depression, an increase in alcohol and drug use, and an increase in the number of suicides, while in the long run post-traumatic stress disorder (PTSD) is anticipated (Figure 2) [11, 14].

Effects of COVID-19 on medical staff

The COVID-19 pandemic generated a major health crisis globally, which led to the reorganization of health services, with an emphasis on emergency services, intensive care units, and infectious disease and epidemiology departments.

At the national level, several support hospitals have been declared, for example in Bucharest, support hospitals – phase II – such units are the Central Military Emergency University Hospital-ROL2-COVID-19 Military Camp Hospital, Marius Nasta Institute of Pneumophtisiology.

Medical personnel is constantly exposed to the risk of becoming infected with viruses, bacteria, or parasites. This risk is accentuated during a global pandemic, thus increasing the predisposition for the development of burnout syndrome in the case of the health personnel. The fast pace of work, special conditions (requiring special protective equipment), the multitude of uncertainties since the beginning of the pandemic regarding the mode of transmission, the treatments, and prevention methods have created an additional level of stress and anxiety for the medical staff.

Figure 2: The psychological and emotional impact of COVID-19 [15]

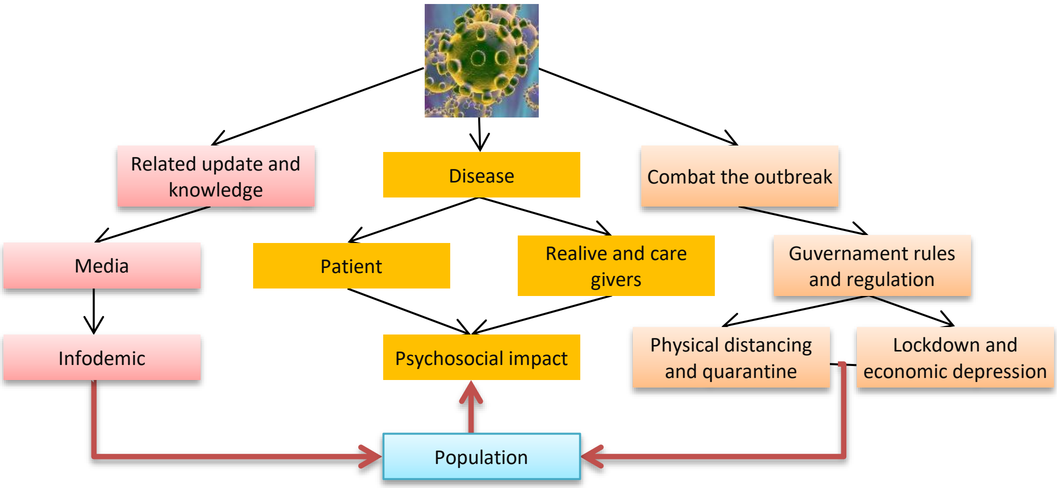
Related update and knowledge
Disease
Combat the outbreak
Media
Patient
Realive and care givers
Guvernament rules and regulation
Infodemic
Psychosocial impact
Physical distancing and quarantine
Lockdown and economic depression
Population

Medical teams in the emergency department were also at increased risk of contamination because at the time a patient was presented to the emergency department; health care staff could not tell if he or she was infected with COVID-19 until the PCR test was performed. This situation together with spending an increasing number of hours in the hospital to provide the best care to patients has led to a strong social and psychological impact on the medical staff.

Thus, many of those working in the health system during this period experienced conditions such as anxiety, fear, social marginalization, depression, and post-traumatic stress. Therefore, if we take into account the definition of burnout which involves long-term exposure to stress and exhaustion at the workplace, we can explain the increase in the intensity and impact of burnout syndrome during this period of medical staff.

OBJECTIVES

Healthcare workers are extremely vulnerable to stress and exhaustion due to the characteristics of the work performed. Numerous studies conducted so far highlight the presence of psychosocial impairment of health professionals in many countries, thus becoming a global problem. Furthermore, they may be exposed to additional factors in the work environment: that is physical, biological, chemical factors. The COVID-19 pandemic is an additional element that has amplified the risk of burnout syndrome among medical staff.

This study aims to highlight the impact of the burnout syndrome in the Emergency Department of the "Dr. Carol Davila” University Central Military Emergency Hospital during the COVID-19 pandemic, by conducting a comparative study based on the results of a questionnaire applied to medical staff before and during the COVID-19 pandemic.

MATERIALS AND METHODS

While writing the current study we have used the “Oldenburg burnout inventory scale” and the statistical data which have been obtained through a survey carried on the medical staff within the ER unit of the Central Military Emergency University Hospital "Dr. Carol Davila” in the May-June 2020 timespan.

The psychometric tool used in this situation – to assess the syndrome’s impact on the ER medical staff – has consisted of the “Oldenburg burnout inventory”. This involves a set of 16 queries (8 with a direct answer, the other 8 including indirect answers) which aim at measuring the burnout and professional involvement levels.

The clinical method was based on the application of a questionnaire to the medical staff from the Emergency Unit of the Central Military Emergency University Hospital „Dr. Carol Davila ”between May and June 2020.

The analytical method was used to interpret the results thus obtained. The data thus obtained were analyzed and shared with those resulting from the application of the same questionnaire in the period before the COVID-19 pandemic. For the statistic, we used the EpiInfo program and Excel.

RESULTS

The study interviewed 65 participants aged between 20 and 60 years. They had an anonymous answer to 16 questions marked with scores from 1 to 4 (1- Totally agree, 2- Agree, 3- Disagree, 4- Total disagree) (Table 2).

Table 2: Oldenburg Burnout Inventory in ER unit of the Central Military Emergency

	Questions	Totally agree	Agree	Disagree	Totally disagree
1	I always find new and interesting aspects in my work.				
2	There are days when I feel tired before I get to work.				
3	It happens more and more often that I speak badly to people.				
4	After the program, I need more time than before to relax.				
5	I can easily cope with the pressure at work.				
6	Lately, I tend to think less about work and do things more automatically.				
7	I find the service a positive challenge.				
8	During work, I often feel powerless.				
9	Over time, I may become disinterested in what I am currently working on.				
10	After work, I have enough energy left for my favorite leisure activities.				
11	Sometimes I feel disgusted with my tasks at work.				
12	After the program, I usually feel worn out and tired.				
13	I wouldn't see myself working on anything other than what I'm doing now.				
14	Usually, I can dose my work well.				
15	I feel more and more captivated by my work.				
16	When I work, I usually feel full of energy.				

In the questionnaire applied before the COVID-19 pandemic, 65 participants aged between 21 and 60 years were interviewed, of which 25 women and 40 men, distributed on the following personnel categories: 12 doctors, 31 nurses, 5 people belonging to other categories, 6 ambulances, and 8 stretchers.

At the same time, in the questionnaire applied between May and June 2020, 66 medical staff aged between 21-60 years participated, of which 32 women and 34 men, distributed as follows: 22 doctors, 19 nurses, 11 stretchers, 3 ambulances, and 5 people belonging to other categories.

We observe that in both questionnaires the doctors obtained the highest average scores of the burnout level, both in terms of the component of emotional exhaustion and the component of professional involvement. At the same time, we observe an increase in the level of burnout during the COVID-19 pandemic (in the study conducted before the pandemic, doctors obtained average scores of 40.66 out of 64 possible), while following the questionnaire applied during the pandemic the average scores were 43 of 64) (Figure 1).

Figure 1: Results obtained broken down by personnel categories

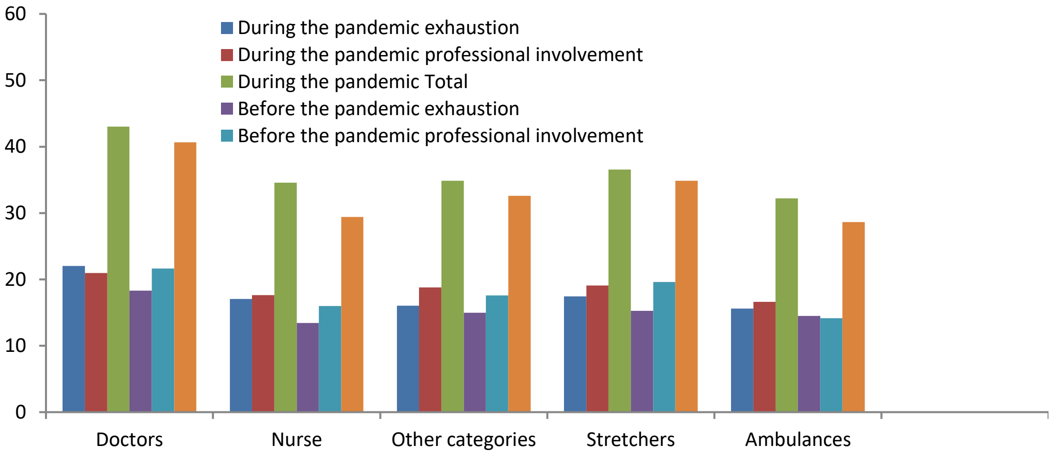

Also, we notice the biggest difference between the two periods among the nurses, before the pandemic the scores obtained were on average 29.43, unlike the current ones, which had an average of 34.57, the difference is due mainly to the marked increase of the exhaustion component. At the opposite pole, with the lowest level of burnout remain the outpatients, although comparing the results obtained in the two questionnaires, we notice among them a sharp increase in burnout.

If we talk about age, we observe the increase of the burnout level concerning the advancing age (Figure 2). This can be explained by the accumulation of fatigue due to the extended work schedule and the accumulation of problems at work with those in private life.

Figure 2: Results according to age

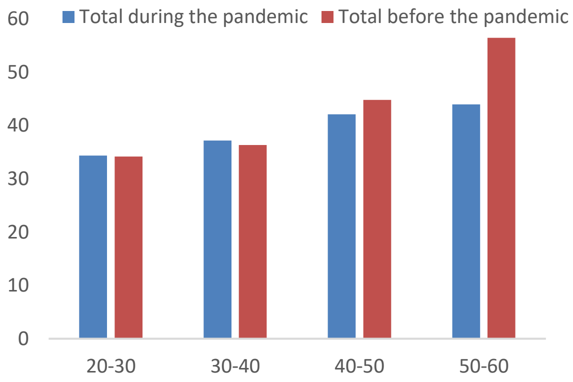

A perhaps surprising aspect was the fact that there were no notable differences in the degree of burnout between the men and women interviewed in any of the questionnaires. In the first questionnaire, the average values varied around 32 points, while in the second questionnaire the average values were 36.5 points (Figure 3).

Figure 3: Results according to sex

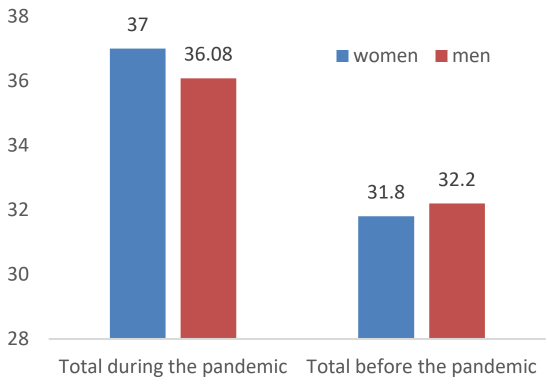

CONCLUSIONS

In recent years, interest in burnout has increased, being a syndrome more and more common in developing countries and with an increased impact in both personal and professional life.

Healthcare personnel represents one of the most exposed professional categories to this syndrome, due to overtime, desire to treat all patients, fear of failure, inability to suffer from certain pathologies, and exposure to additional factors in the workplace: physical, biological, chemical factors.

The COVID-19 pandemic was an additional stress factor for medical staff, on the one hand, due to the numerous unknowns related to treatment, prevention, disease evolution and on the other hand, due to the intensive work regime in special conditions, being forced to comply to a set of additional rules and the period in which they had to be separated from their loved ones to protect them.

According to the study conducted between May and June 2020, there is an increased level of burnout of health care personnel, the highest score being obtained by doctors.

Comparing the results highlighted by the questionnaire applied before the COVID-19 period with those obtained in the questionnaire applied between May and June 2020, we notice an increase in the level of burnout in all categories interviewed during the pandemic.

There is also a gradual increase in the level of burnout proportional to aging, but slower than before the pandemic.

The percentage of burnout reported by sex groups is close, with no significant differences between men and women. But even in this case, there is an increase in the incidence of burnout in the last 4 months.

Therefore, burnout syndrome remains a common problem in the health field, especially affecting doctors, probably because they represent a complex interface with patients, relatives, the rest of the ER staff, doctors from other specialties; have the responsibility of the performed medical act; working hours over time due to the need for continuous training.

To decrease the incidence of this syndrome, but also its effects, it is important to apply various means of prevention such as identifying and combating the triggering factors, observing and early intervention on the disease, and treating the effects produced by it.

On this basis, we conclude that stress, exhaustion and distress lead to the development of the burnout syndrome, which, in this period is conspicuously increased by the

COVID-19 pandemic, essentially breaking what should be the first and most important rule of any medical practitioner: the safety of the rescuer.

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REVIEW

Cannabinoids – a new therapeutic strategy in neurology

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Abstract: *Cannabinoids' usefulness in the treatment of neurological disorders (epilepsy, and various neurodegenerative diseases, such as Multiple Sclerosis and Alzheimer's Disease) has been demonstrated in a growing number of studies. Of the 11 known general types of natural cannabinoids, the focus has been mainly directed at cannabidiol (CBD) due to its specificity in stimulating cannabinoid receptors and the low rate of side effects, as well as on Δ (9)-tetrahydrocannabinol (Δ9-THC). The natural and synthetic analogs of CBD have been described as a potential treatment in neurological diseases, as they showed their therapeutic benefits in reducing the seizures from epilepsy and their neuroprotectivity in neurodegenerative diseases. First and foremost, CBD's neuroprotective properties are due to its capacity to act as an endogenous cannabinoid receptor agonist. Second, CBD enhances neuroprotection by interacting with many signal transduction pathways mediated indirectly through cannabinoid receptors. CBD also reduces the hyperphosphorylation of glycogen synthetase kinase 3 (GSK-3) induced by the buildup of Amyloid β in the physiopathology of Alzheimer's disease.*

Keywords: *cannabinoids, cannabis, endocannabinoid, marijuana, epilepsy, multiple sclerosis, Alzheimer's disease*

INTRODUCTION

Traditional medicine based on phytotherapy uses cannabis to treat various ailments and symptoms, such as arthritis, seizures, pain, nausea, and vomiting [1]. CBD is a phytocannabinoid found in the Cannabis plant, although only in trace amounts in western strains. CBD, unlike Δ9-THC, has neither euphoric nor hallucinatory effects [2]. CBD is also implicated in several pathological processes, including neurotransmitter release control, given that it is already known as a potent anticonvulsant, regulating pain, used primarily in patients with oncological pathologies, adjusting gastrointestinal function, which comes in aid of the side effects caused by the chemotherapy and it also possesses antipsychotic potential.

The Cannabis or Cannabaceae plant has been used in traditional medicine, beginning with the medicine practiced in the East, for about 5000 years as an analgesic, anxiolytic, anticonvulsant, sedative, as well as a hypnoinducer. Botanists used morphological criteria to organize Cannabis into three types: Sativa, Indica, and Ruderalis. The last one is the most studied subspecies, as it is most frequently encountered in Western countries [3]. Recently, pharmacological studies have indicated that the cannabis plant has many properties, such as antiepileptic, anti-parkinsonian effect [4], pain-killer [5], antiseptic [6], anti-inflammatory [7], antihistamines [8], blood pressure regulator and anti-coagulant [9] effects. In clinical settings, cannabis has shown its efficacy in oncological diseases, based on its antitumor impact, and in the therapeutic management of specific neurological disorders [10,11]. Current changes in the legal status of cannabis use allow the study of cannabinoids for treatment purposes in a secure and endorsed medical setting [12].

It is known that the diversity of the effects of cannabinoids is mediated by the activation of the G protein, which fulfills the function of a receptor, to which endogenous ligands called endocannabinoids usually are bound. The endocannabinoid system comprises endogenous fatty acid

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ligands for cannabinoid receptors, also known as endocannabinoids, and proteins involved in endocannabinoid biosynthesis and breakdown (ECS).

Cannabinoid receptors type 1 and 2 (CB1 and CB2), which have seven transmembrane domains and are found in various tissues, are this system's central receptors [13]. CB1 receptors are primarily found in the cerebellum, basal ganglia, and limbic system, including the hippocampus [13]. Also, the reproductive systems of both men and women include these receptors. CB2 receptors are mainly present on the cellular membranes of immune cells in the periphery, particularly in peripheral lymphoid organs like the tonsils and spleen [14]. CB2 receptors have been found in the central nervous system (CNS) and have a variety of effects, according to the most recent data [15]. Only two of the five endocannabinoids that bind to CB1 or CB2 are particularly important; they appear to play a role in CNS function, especially 2-AG (2-arachidonyl-glycerol) and AEA (anandamide).

We refer to cannabinoids as a category of natural chemicals found in marijuana plants (the most studied species being Cannabis sativa). Compounds that are physically or pharmacologically similar to (9)-tetrahydrocannabinol are among these chemicals, which bind to cannabinoid receptors [16].

The two main compounds in cannabis are Δ (9)-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). Each of them has exhibited different actions from a pharmacological point of view. The presence of psychotomimetic and addictive effects of the primary psychotropic component (Δ9-THC) has led to apprehension about using cannabis for medicinal purposes. On the other hand, CBD inhibits the absorption and hydrolysis of anandamide (AEA), a key endocannabinoid, while having antagonistic effects on 9-THC [17].

Table 1: The main categories of cannabinoids

1	(-)-Δ9-*trans*-Tetrahydrocannabinol (Δ9-THC) [22]
2	(-)-Δ8- *trans*-Tetrahydrocannabinol (Δ8-THC) [22]
3	Cannabigerol (CBG) [22]
4	Cannabidiol (CBD) [23]
5	Cannabielsoin (CBE) [24]
6	Cannabichromene (CBC) [25]
7	Cannabinodiol (CBND) [26]
8	Cannabicyclol (CBL) [27]
9	Cannabitriol (CBT) [28]
10	Cannabinol (CBN) [25]
11	Various types [18]

Over the last few decades, significant progress has been made in researching the natural compounds contained in the Cannabis plant. At least 120 compounds have been identified as natural cannabinoids, including their derivatives and metabolites [18]. Given their structure, these natural cannabinoids can be classified into 11 categories, as shown in Table 1. Due to their diverse and complex structure and multiple substituents, number 11 in Table 1 includes another 30 subtypes with different skeletal shapes [19–21].

Cannabidiol was first isolated in 1940 by Roger Adams and his team of researchers at the University of Illinois [31]. Its chemical structure was described in 1963 by Mechoulam [29, 30]. In 1977 Jones identified two optical isomers of this compound, (-) - CBD and (+) - CBD, the first form being found naturally in the Cannabis plant, while the latter can only be obtained through organic synthesis [31].

THE USE OF CANNABINOIDS FOR THERAPEUTIC PURPOSES

CBD has been shown to offer neuroprotective advantages in the treatment of multiple sclerosis (MS), epilepsy, Alzheimer's disease (AD), and other pathologies caused by degeneration or abnormal nervous system activity in preclinical and clinical research.

Epilepsy

This disease affects 65 million individuals globally [33] and significantly impacts seizures, mortality, comorbidities, stigma, and associated healthcare costs. Significant progress has been achieved in understanding the pathophysiological underpinnings of the disease and the factors that influence its prognosis over the last decade. New conceptual and operational definitions of epilepsy, as well as altered diagnostic and classification criteria and nomenclature, have all contributed to these advancements.

Even though the number of antiepileptic medications available has expanded dramatically over the last 20 years, about one-third of patients remain drug-resistant. Despite the efficacy of surgical operations, which have resulted in more than half of all operated patients experiencing long-term seizure remission, this treatment method is still reserved for a small subset of drug-resistant patients. The quality of life of most individuals with epilepsy continues to be negatively affected by deficiencies in knowledge regarding the pathogenesis, diagnosis, treatment, education, legislation, and research in this field.

The anticonvulsant effect of cannabidiol has been researched in multiple studies, conducted both in vivo and in vitro. Stimulation of CB1 receptors is followed as the primary target for antiepileptic effects in animal studies.

Neurophysiological studies have confirmed that glutamic acid is the major excitatory neurotransmitter in the CNS. Excessive glutamate release causes neurotoxicity, extreme excitability, and epileptic seizures in mice and primates. The direct result of activation of CB1 receptors is a reduction in glutamate release on a presynaptic level. Decreased glutamate release leads to an anticonvulsant effect and thus constitutes a target within antiepileptic therapy. In addition, endocannabinoids act upon specific ion channels by activating the G-receptor protein complex, which models the intracellular concentration of K+. The effect exerted upon K+ channels may decrease neuronal excitability, thus preventing seizures by stabilizing the membrane potential and reducing other epileptogenic factors [34]. When CB1 receptors in the neuronal body are engaged, the activity of an extracellular kinase, which impacts neural signaling and the synthesis of brain-derived neurotrophic factor (BDNF), has been shown to increase [35]. The latter plays a long-lasting protective role in the hippocampus [35].

CBD acts through endocannabinoids to activate CB1 receptors, stimulating a cascade of phenomena, which play a protective role against toxicity caused by excessive neuronal stimulation. Thus, the information mentioned above suggests that CBD can become an essential therapeutic instrument for neuron diseases, whose trigger mechanism consists of hyperexcitability and neurotoxicity [36].

In addition to acting on CB1 and CB2 receptors, CBD works at low doses on transient receptor potential (TRP) channels, affecting cationic channels and decreasing glutamate release in the presynaptic cleft. TRP is a group of channels that modulate ion entry and mediate various neural signaling processes [36,37]. Moreover, CBD will activate 5A-hydroxytryptophan 1A (5-HT1A) receptors and inhibit adenosine reuptake through multiple mechanisms, so that cannabidiol may have anti-inflammatory and antioxidant effects [37–41].

There is a long history of unofficial use of CBD in epilepsy and other neurological disorders because many preclinical studies have shown favorable neurological effects. Ever since the end of the 19th century, physicians in England and the United States have observed a reduction in the incidence of seizures in patients treated with cannabinoids [42]. Another study indicated that CBD reduced the susceptibility of seizures induced in laboratory mice by accompanying stimuli [43]. CBD is an adequate anticonvulsant with higher selectivity and fewer neurotoxic consequences compared to classical antiepileptics. Also, CBD can shorten the duration and amplitude of the post-critical phase through increased selectivity and reduced excitability acting on the central nervous system. In 2017, the Epilepsy Therapy Screening Program (ETSP) was established by the American National Institute of Neurology and Neurovascular Diseases to investigate the effects of CBD on a variety of epileptic seizure models. CBD has been found to have anti-seizure activities in both humans and mice with epileptic seizures after stimulation by ETSP. [44]. In June 2018, the FDA approved an anti-epileptic drug that contains CBD, named Epidiolex, for treatment-resistant epilepsy (Lennox-Gastaut syndrome and Dravet syndrome) [45].

Multiple sclerosis

Autoreactive T cells invade the CNS, followed by clonal growth, with a preference for myelin or other CNS proteins. Different autoimmune cells, such as microglia and B lymphocytes, are also initiated, and when combined with T cells, they develop into a "cytotoxic" cascade that demyelinates neurons in the brain and spinal cord. The presence of both CB1, as well as CB2 receptors has been noted on the surface of specific cells around the platelets accumulated in the demyelinated areas present in MS [46]. Increased CB1 and CB2 receptors activity were observed in some MS patients' bloodstream in a 2009 study, suggesting that activating the endocannabinoid system, both inside the brain and in the periphery, can restrict inflammatory, immunological responses [47].

In 2007, an elevated level of AEA (anandamide) was identified in conjunction with the number of inflammatory lesions noted in the neurological images [48]. It has been pinpointed that the level of multiple endocannabinoids within the cerebrospinal fluid has a low level in patients with remission, but an increased level in those with active inflammatory lesions, in a study conducted in 2008 [49]. These three studies illustrate a correlation between MS and the regulation of endocannabinoid activity and demonstrate the influence of inflammatory processes and neural activity on the endocannabinoid system.

Nabiximols, in the form of oronasal spray containing Δ9-THC and CBD, is a product licensed in Great Britain and some countries within the European Union to be used as a treatment for MS, and it has been shown to present a therapeutic effect on neuropathic pain and in the case of sleep disorders [50]. As the following studies have shown [51–53], Nabiximols could also be used to control spasticity. In 2007, a study carried out on 189 patients who were administered Nabiximols over six weeks, compared to the placebo group, showed a substantial reduction in spasticity in the active treatment group [51]. A meta-analysis indicated that these cannabinoids exhibit favorable effects for both neuropathies and pain symptoms caused by spasticity [52].

Another retrospective study [53], carried out in eight centers in Belgium, followed 276 patients with MS-specific spasticity who began treatment with Nabiximols-containing oromucosal spray on March 1, 2016, after other conventional treatment regimens for spasticity were exhausted. The conclusion reached was that more than 60% of patients reported a clinically relevant symptomatic improvement and continued treatment over 12 weeks [53].

Endocannabinoids play a role in limiting the cytotoxic processes which occur during the inflammatory cascade, which initiates the neuronal damage in the context of MS via stimulation of the CB2 receptors. Likewise, stimulation of CB1 receptors may also have beneficial effects by inhibiting glutamate release and the depolarization induced by it. The activation of CB2 receptors in T cells and CB1 receptors within neurons is thought to be responsible for 9-THC's neuroprotective action in multiple sclerosis. In addition to their shown capacity to lower inflammation and excitotoxic lesions in MS, cannabinoids may aid in the repair of myelin sheaths in the axons [54].

Multiple studies have shown the effect of cannabis on the modulation of the myelination process in the developing brain by stimulating CB1 and CB2 receptors [55], in the normal adult brain [56], and in the irritated brain in the TMEV (Theiler's murine encephalopathy virus) model of MS [57]. The TMEV model's accelerated myelination process could be related to the efficacy of cannabis in reducing inflammation which detained the remyelination. Alternately, cannabinoids may have an exhilarating response to myelination by improving survival [57] and cellular division of oligodendrocytes [55]. Further study of changes occurring in MS in conjunction with the endocannabinoid system may lead to the development of novel therapeutic methods.

Alzheimer's disease

This chronic neurological disease is currently the leading cause of dementia worldwide, facing an acceleration of diagnostic that will bring us to an epidemic with 150 million cases globally by 2050 [58].

The pathogenesis of the disease is highly correlated with the accumulation of extracellular aggregates of amyloid β (Aβ) plaques and intracellular neurofibrillary tangles of TAU protein located in a different region of the brain, which drives the dissolution of microtubules in neurons, spoiling the architecture of the cytoskeleton of the cell, fracturing the neural transport system and causing a significant loss of neurons firstly in the hippocampus, which clinically leads to memory loss. These actions initially bring some biochemical communication disengagement in the connections between neurons, followed by neuronal death [59–61]. This is our current knowledge of the pathophysiology of AD, but as the mechanism is not fully understood, the therapeutical resources are very limited. Even if it has been shown in some studies on animal models that THC can have a direct effect on amyloid β (Aβ) aggregation, the psychoactive properties limit the possibilities of being used as a medication. Although other non-psychoactive cannabinoids, such as CBD, show neuroprotective activity on animal models by reducing oxidative stress and playing a role in microglial inflammation. Cannabidiol’s effect on inhibiting neuronal apoptosis by regulating pro-caspase-3 levels and decreasing caspase-3 levels has shown that CBD exerts a protective effect on the nervous system [65]. In another study, CBD was successfully used to counterbalance Aβ-induced Ca2+ growth, adequately added to the already known antioxidant effect [66]. Furthermore, cannabinoids can stimulate the clearance of intraneuronal amyloid-β and induce a protective effect on old age-associated nervous cells. In addition, they function in the absence of cannabinoid receptors in some cases. CBD increases cerebral blood flow mainly in the areas involved in memory processing, like the hippocampus [66]. In conclusion, cannabinoid products can be an alternative compound for AD drug therapy, but more longitudinal studies must be conducted.

CONCLUSION

Even though higher usage of cannabis extracts is linked to an increased risk of psychosis, CBD can help with epilepsy convulsions and the symptoms of neurodegenerative diseases like AD and MS because of its neuroprotective effect. If the exciting discoveries related to the cannabinoid-mediated weakening of the inflammatory process, the enhancement of remyelination, and behavioral and symptomatic changes are confirmed, these compounds can be used as a treatment for multiple sclerosis as they progress from studies on animal models model systems to humans trials.

Even though various molecular signaling pathways and targets linked to CBD have been identified and summarized, many questions remain unanswered. Finally, natural analogs of Cannabidiol, as well as metabolites and synthetic derivatives enriched with strong biological effects [68] and low neuronal toxicity should be studied for their potential benefits in the treatment of neurodegenerative illnesses such as AD, multiple sclerosis, and epilepsy.

More research is needed to understand how CBD affects the nervous system's growth factors, neurotransmitters, and electrophysiological activity.

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VARIA

Can peritoneal carcinomatosis of colorectal origin be treated with oral everolimus alone or in combination with capecitabine? Preliminary results

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Abstract: *Background: We wanted to evaluate the effectiveness of oral everolimus alone and in combination with capecitabine treatment on colorectal cancer-induced peritoneal carcinomatosis animal model.*

Methods: Caco-2 colon adenocarcinoma cells were injected intraperitoneally to BALB/cOlaHsd-Foxn1nu mice to establish peritoneal carcinomatosis. Mice were divided into four groups (everolimus, everolimus plus capecitabine, capecitabine, control group) with 2 mice in each group. Treatment was initiated 5 days after inoculation of Caco-2 cells. 7.5 mg/kg everolimus was administered orally every 2 days. 2.1 mmol/kg capecitabine was administered orally every 5 days a week. 22 days after inoculation of Caco-2 cells, mice were sacrificed.

Results: The mean weight of the tumor was 25 ± 7.07 mg in the control group (n=2); 0.7 ± 0.7 mg in the everolimus group (n=2); 0.28 ± 0.36 mg in the capecitabine group; 0.48 ± 0.07 mg in the everolimus plus capecitabine group.

Conclusion: Tumor samples excised from BALB/cOlaHsd-Foxn1nu mice revealed that everolimus alone, and in combination with capecitabine suppressed tumor growth. However, a comparison of tumor weights revealed no statistically significant difference within the four groups (p=0.227). To reach statistically significant data, the sample size should be increased.

Keywords: *colon cancer, everolimus, peritoneal carcinomatosis*

INTRODUCTION

Peritoneal carcinomatosis secondary to colorectal cancer progression is characterized by the formation of solid tumor deposits on peritoneal surfaces [1]. Even today, chemotherapy protocols plus cytoreductive surgery and hyperthermic intraperitoneal chemotherapy applied for the treatment of metastatic colorectal cancer have been able to increase the median survival up to between 20-62 months at most [2-4]. Moreover, chemotherapy protocols used for the treatment of advanced colorectal cancer contain intravenous (iv) interventions and hours of chemo-therapeutic agent infusions, which reduce both patient compliance and comfort.

In search of novel treatment options for peritoneal carcinomatosis of colorectal origin, an article published by Wagner et al. in 2009 drew our attention, in which they claimed that effective treatment of advanced colorectal cancer by rapamycin and 5-fluorouracil (5-FU)/oxaliplatin was achieved [5]. Rapamycin is both an antibacterial macrolide group antibiotic and a mammalian target of rapamycin (mTOR) inhibitor [6, 7]. Phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR signaling pathway is a central regulator in cell proliferation, cell growth, and angiogenesis [8-10]. Inhibition of mTOR by rapamycin prevents tumor proliferation and growth [7, 11]. Everolimus is the derivative of rapamycin that can be used orally and it has a higher

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bioavailability than rapamycin [12, 13]. Moreover, oral capecitabine treatment in metastatic colorectal cancer is equivalent to iv 5-FU plus leucovorin treatment has given the time to progression of the disease and overall survival [14]. Wagner et al. revealed that when oral rapamycin was administered in combination with iv 5-FU, colorectal cancer-induced peritoneal carcinomatosis was suppressed in a mouse model. However, 5-FU can only be used intravenously. Therefore, we thought that the treatment of peritoneal carcinomatosis with chemotherapeutic agents that could be used orally would both facilitate the compliance of the patient with the treatment and increase the patient's comfort. Thus, this study aimed to administer everolimus and/or capecitabine orally to BALB/cOlaHsd-Foxn1nu mice with colon cancer-induced peritoneal carcinomatosis to examine whether tumor regression was achieved.

MATERIALS AND METHODS

Local ethics committee approval was obtained before the study (Approval number: 2013/60).

Cell Lines and Chemical Substances

Caco-2 human epithelial colorectal adenocarcinoma cells were purchased from American Type Culture Collection (ATCC®, Rockville, MD, USA). Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), Dulbecco's Phosphate Buffered Saline (D-PBS), 0.05% trypsin-EDTA, and trypan blue stain 0.4% from Gibco® was purchased. Everolimus (Certican 0.75 mg, Novartis) and capecitabine (Xeloda 500 mg, Roche) were supplied from the market.

Animal Model

Eight BALB/cOlaHsd-Foxn1nu mice, 14-18 week-old female, weighing 14.1-24.2 g were used within this study. BALB/cOlaHsd-Foxn1nu mice were purchased from Harlan (Israel). BALB/cOlaHsd-Foxn1nu mice have housed in individually ventilated cages at 12/12 hours light/dark cycle at 23° C, and mice were fed with Altromin brand 1414 TPF catalog feed.

Preparation of Caco-2 Cell Culture

Caco-2 human epithelial colorectal adenocarcinoma cells were kept and propagated in DMEM medium containing 10% FBS within a 5% CO_2 incubator at 37°C. Cells were trypsinized when the cells completely covered the culture flask base. Afterward, these cells were counted and the viability of the cells was checked. The trypsinization procedure was performed as follows: The medium in the flask was poured out and washed twice with 10 ml D-PBS. 3 ml of 0.05% trypsin-EDTA was added to the cells and the cells were kept in an incubator containing 5% CO_2 at 37°C for 5 minutes. Cells were collected in a 50 ml centrifuge tube with the help of a Pasteur pipette. Cells were centrifuged at a 1200 rate per minute (rpm) for 5 minutes. After centrifugation, the supernatant was discarded. The pellet was mixed with 1 ml medium. To evaluate the viability of Caco-2 cells, 10 µl of the pellet which was mixed with 1 ml medium was taken. In a 1.5 ml Eppendorf tube, 10 µl pellet and 190 µl, trypan blue stain 0.4% (dilution factor 20, 10 µl pellet in 200 µl mixture) were mixed. 10 µl of this mixture (10 µl pellet and 190 µl trypan blue) were taken and placed on Thoma slide. The viability and number of cells on Thoma slide were determined with a 5x magnification light microscope. (There are 64 squares in four different areas on Thoma slide, and cells in each frame were counted). The total number of cells counted was divided by four to find out the mean value. The mean number of cells x 104 x dilution factor x 1 (1 ml of medium mixed with pellet after centrifugation) formula was applied to determine the number of cells in 1 µl.

Intraperitoneal injection of Caco-2 cells

1,500,000 Caco-2 human epithelial colorectal adenocarcinoma cells were injected intraperitoneally into each BALB/cOlaHsd-Foxn1nu mouse.

Treatment Protocol

Mice were randomly divided into 4 groups (everolimus, everolimus + capecitabine, capecitabine, control group). There were two mice in each group. The mice were taken to laboratory conditions one week before the experiment.

Treatment was started 5 days after intraperitoneal injection of Caco-2 cells into mice. Everolimus was diluted with saline (0.9% NaCl) and administered to two mice orally by gavage every two days at a dose of 7.5 mg/kg [15]. Capecitabine was diluted with saline and the maximum tolerated dose of capecitabine was administered to 2 mice 5/7 days per week by oral gavage at a dose of 2.1 mmol/kg/day (1 mmol capecitabine is 359 mg) [16]. Only PBS was given to 2 mice in the control group by gavage (Table 1).

Mice were daily evaluated. 22 days after the injection of Caco-2 cells into the intraperitoneal cavity, 17 days after starting treatment, all mice were anesthetized with intraperitoneal 50 mg/kg ketamine HCl and 5 mg/kg xylazine, and the mice were sacrificed by cervical dislocation method.

Surgical Procedure

Abdominal skin was opened by protecting the peritoneum with a midline incision. Saline solution was injected into the intraabdominal cavity and aspirated. Intraabdominal peritoneal washing fluid samples were reserved for cytological examination (Figure 1). Afterward, the peritoneum was excised without damaging other organs and the tumor developed at the intraperitoneal injection site was excised. The dimensions of the total tumor tissue removed were measured and the tumor was weighed. Tumor tissue formed in the peritoneum, mesenteric lymph nodes, liver, spleen, kidney, small intestine, colon, stomach, and intraperitoneal injection site were placed in formaldehyde solution for pathological examination.

Table 1: Treatment protocol

	Everolimus	Everolimus + Capecitabine	Capecitabine	Control Group
Number of Mice	2	2	2	2
Dose	7.5 mg/kg	7.5 mg/kg + 2.1 mmol/kg/day	2.1 mmol/kg/day	PBS*

**PBS: Phosphate-buffered saline*

Figure 1: (A) 0.9% NaCl was given into the abdominal cavity intraperitoneally and aspirated. Cytological examination of the aspirated fluid was investigated for malignant cells. (B) Excision of the peritoneum.

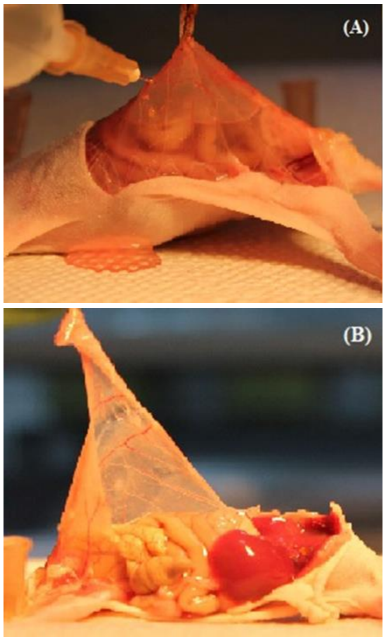

Statistical Analysis

Statistical analysis was performed using SPSS version 15.0. Kruskal-Wallis analysis of variance was used to evaluate the differences between continuous variables. P<0.05 was considered statistically significant.

RESULTS

Control Group

In the first mouse within the control group, a 1.5 cm lesion was observed where Caco-2 cells were injected in the left lower quadrant of the abdomen. The tumor which was located intraperitoneally was excised. The tumor size was 2x1.5x1.5 cm weighing 20 mg (Figure 2). No macroscopic tumor was found in other intraabdominal organs. Poorly differentiated adenocarcinoma was detected in the pathological examination of the tumoral lesion excised from BALB/cOlaHsd-Foxn1nu mouse.

Figure 2: (A) Tumor in the left lower quadrant of the BALB/cOlaHsd-Foxn1nu mouse within the control group. (B) The excised intraperitoneal tumor was 2x1.5x1.5 cm.

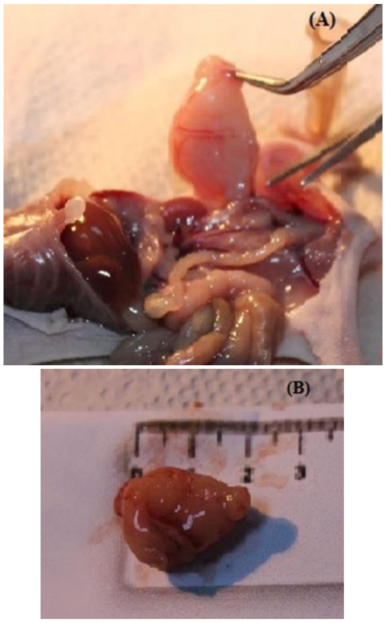

In the second mouse within the control group, the size of the lesion was 2.5x1.8x1.5 cm weighing 30 mg. The pathological examination of the excised tumor revealed poorly differentiated adenocarcinoma. No tumor, metastasis, or infiltration was detected in the peritoneum, mesenteric lymph nodes, liver, spleen, kidney, small intestine, colon, and stomach. No tumor cell was detected in the cytological examination of the peritoneal washing fluid.

Everolimus Group

During exploration, a tumoral lesion 0.2x0.4x0.4 cm in size, weighing 0.2 mg was detected in the first mouse. The cytological examination of intraabdominal fluid revealed no tumor cells. Tumor size was 1x0.6x0.4 cm weighing 1.2 mg in the second mouse. In addition, metastatic mesenteric, peripancreatic, and perisplenic lymph nodes were detected in the pathological examination. At the same time, two tumor deposits were detected on the small intestine surface. However, no tumor cell was detected in the cytological examination of the peritoneal washing fluid.

Capecitabine Group

In the first mouse given capecitabine, the tumor was associated with peritoneal tissue in exploration. Tumor size was 0.2x0.1x0.3 cm (Figure 3). No other macroscopic tumor tissue was detected. In the second mouse treated with capecitabine, a tumor 0.4x0.5x0.6 cm in size was detected. Apart from the other mice, poorly differentiated adenocarcinoma infiltrating diaphragm striated muscle was detected.

Figure 3: (A) Tumoral lesion detected on the peritoneum. (B) Poorly differentiated adenocarcinoma metastasis within the mesenteric lymph node (Hematoxylin&eosin; x40 magnification).

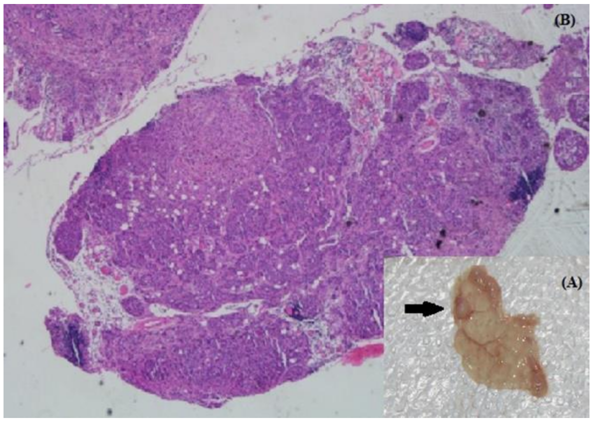

Everolimus plus Capecitabine Group

Tumor size was 1.2x1x0.8 cm in the first mouse which was treated with both everolimus and capecitabine. However, pathological examination revealed poorly differentiated adenocarcinoma metastases in mesenteric lymph nodes. Tumor size within the second mouse was 0.5x0.6x0.4 cm (Figure 4). No malignant cells were detected in other intraabdominal organs, peritoneum, and peritoneal lavage fluid.

Comparison of Tumor Weights

In the control group, tumor weights were 20 mg and 30 mg. In the group treated with everolimus tumor weights were 0.2 mg and 1.2 mg. Within the group treated with capecitabine, tumor weights were 0.03 mg and 0.54 mg. The tumor weights were 0.43 mg and 0.54 mg within the group treated with both everolimus and capecitabine (Figure 5).

Figure 4: (A) Significant tumor growth was not observed in the localization, where Caco-2 cells were injected. (B) Tumor size was 0.5x0.6x0.4 cm.

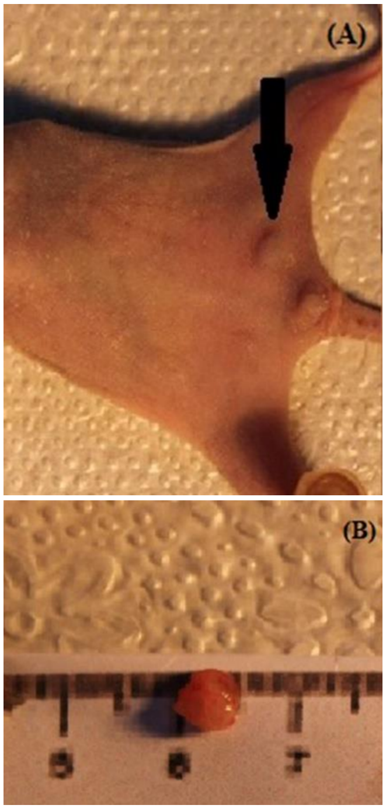

Figure 5: Weights of tumor samples excised from BALB/cOlaHsd-Foxn1nu mice. In mice treated with everolimus and/or capecitabine, tumor growth was suppressed compared to the control group. However, there was not a statistically significant difference between the four groups (p=0.227)

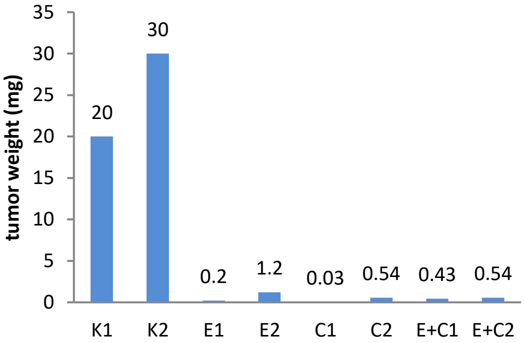

C: Capecitabine; E: Everolimus; K: Control

DISCUSSION

The mTOR (mammalian target of rapamycin) is a serine/threonine-protein kinase [17]. There are two mTOR complexes, mTORC1 and mTORC2 [18, 19]. mTORC1 includes mTOR, Raptor (regulatory-associated protein of mTOR), and mLST8. mTORC2 contains mTOR, Rictor (rapamycin-insensitive companion of mTOR), mLST8, and mSIN1 (mammalian stress-activated protein kinase interacting protein). The mTORC1 eukaryotic translation initiation factor controls protein synthesis through the ED (eIF4E) binding protein (4E-BP1) and p70S6 ribosomal kinase (S6K) [20]. mTORC2 is involved in cell growth. Rapamycin combines with FK506 binding protein (FKBP) 12 to block mTOR activity. The mTORC1 is sensitive to rapamycin, while mTORC2 becomes susceptible to rapamycin only if it is exposed to the rapamycin effect for a long time [21]. Rapamycin-like drugs such as everolimus, temsirolimus, and ridaforolimus which are called rapalogs, have been produced after the anti-tumoral properties of rapamycin were discovered [22].

Nowadays, everolimus is used because of its tumor suppression feature in patients with subependymal large cell astrocytoma, advanced breast cancer, progressive pancreatic neuroendocrine tumor, subependymal giant cell astrocytoma, and advanced renal cell carcinoma [22]. In addition, capecitabine is used for the treatment of colorectal cancer and metastatic breast cancer [12, 23-25]. The side effects of both everolimus and capecitabine are very well known in detail because they are included within the current treatment protocols [26-28].

The positive effect of everolimus on peritoneal carcinomatosis has been demonstrated in several experimental models. Taguchi et al. used 58As1 human stomach cancer cell series, which were prone to peritoneal metastasis, to demonstrate the effectiveness of everolimus in advanced gastric cancers with peritoneal spread, and showed that everolimus significantly reduced peritoneal spread [29]. Chu et al. tested everolimus on a human colorectal carcinoma cell line with KRAS mutation in a mouse model and revealed that everolimus alone had significant antitumor activity [30]. Mabuchi et al. reported that everolimus delayed tumor onset and progression in the transgenic mouse model in ovarian cancer [31]. Moreover, studies on humans investigated the effects of everolimus on tumor growth when used as a single agent. Okamoto et al. published the results of phase 1 studies on daily everolimus treatment for patients with advanced solid tumors and reported significant tumor shrinkage in patients with esophageal and gastric cancer with 10 mg/day everolimus treatment [32]. However, Yoon et al. stated that 10 mg/day of everolimus treatment had only a limited impact on advanced gastric cancer [33]. There have been also phase studies on human beings with everolimus and capecitabine. Deenen et al. published the results of the phase I study of oral capecitabine and everolimus treatment in patients with advanced solid cancers [34]. In this study, 18 patients with the primary pancreas, major duodenal papilla, esophageal, gallbladder, brain, osteosarcoma, and hepatocellular cancer were treated with capecitabine 1.000 mg/m^2 bid and everolimus 10 mg/day every 3 weeks for 14 days, and prolonged clinical efficacy was observed in 39% of patients with advanced solid malignancies. Lim et al. published the results of a phase I study of capecitabine and everolimus treatment for patients with previously treated metastatic gastric cancer [35]. Fifteen patients with metastatic stomach cancer who had progression after chemotherapy were included in the study. With 650 mg/m^2 capecitabine twice daily and 5 mg everolimus twice daily, the median time without progression of the disease was 1.8 months (range: 0.8–2.8 months). At the same time, the reduction in the longest diameter of the tumor was 28.7%.

In our study, tumors formed in mice within the control group were approximately x20 times larger than tumors excised from other groups, however, tumor growth in the control group was limited only to the inoculation site of Caco-2 cells. Everolimus and capecitabine treatment suppressed primary tumor growth in the region where Caco-2 adenocarcinoma cells were injected. No liver toxicity or mortality were observed during treatment with everolimus and/or capecitabine.

In light of these findings, the mTOR inhibitor everolimus suppressed tumor growth in the mouse model of colon cancer-induced peritoneal carcinomatosis when administered orally or in combination with capecitabine. However, the limitation of this study was the small sample size. Our study is unique because it has investigated whether peritoneal carcinomatosis could be treated with oral chemotherapeutic agents. However, to reach a definitive conclusion, it is necessary to work with a larger experimental group.

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Conflict of interests

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VARIA

Can we still manage the relationship with patients?

Liana Manolache[1]

Eighteen years ago, I started my out-patients' experience as specialist dermatologist. I worked from the very first day with National Health Insurance System. It was a chance to build a huge experience (more than 120,000 visits since then). Over time, relationships with patients have changed, influenced by many factors. To preserve good relationships we have to reflect and to use different approaches.

In 2001, my stamp and my signature were enough for a consultation. The cell phone era was at the beginning. Over the years, the process has become more and more complicated. Today, if the computer, internet, different types of applications, software, other devices (printer, card reader, etc.) are not working, my knowledge becomes absolutely necessary, but not sufficient for a consultation. So, the doctor is now, totally dependent on IT issues. Besides instant information, cell phones induce a lack of intimacy. Everyone could reach you everywhere, every moment. You are almost "forced" to give advice on whatsapp, e-mail, messenger and people expect you to answer on-site, otherwise you are not "reachable".

Time is the first pressure that we are feeling. Patients want and expect a consultation as soon as possible even you have appointments for the next few weeks. I try my best not to push later than 10 days, if it is a new ordinary consultation. Sometimes, it is possible for me to see the patient on-site (a child, an emergency, pregnant women, etc.). In the literature, time for appointment could vary from 7 working days in Brazil [1], to median 41 days in Canada (Ontario) [2] or median 45 days in US (Pennsylvania) [3] to a certain dramatic situation waiting list of 57 weeks in a unit in UK [4]. The number of consultations tends to increase. It is not only a perception, but a reality, revealed also by a French study (21% increase from 2000 to 2010) [5].

Time is also a burden for the doctor, because the real time for consultation has dramatically reduced in favor of bureaucratic issues. Official papers seem to be more important than people. It is a constant effort to remain emphatic and to really listen to patient's history. The stories that we are ignoring for lacking time could give us important clues for diagnosis, approach, could enforce the relationship and give trust, could relief the worries.

"You are the 5th dermatologist I am seeing"- a young woman told me the other day. I looked at her: she was around 20, some pimples on her jaw (not too many) and some tiny scars on her forehead covered by a lot of makeup. Not quite a good start for a relationship. Her expectations were not fulfilled by visiting the other doctors. Maybe the expectations were not realistic, maybe she was fed up trying different things. "How can I propose something new or miraculous when she tried probably "everything"? - I asked myself. I spent at least half an hour discussing about acne, therapeutic options and adjusting the expectations to our limits. It was more a counseling session than a dermatologic consultation.

Today, patients are coming in our office very informed. They "know" the diagnosis and sometimes even the treatment they want or need. After "Dr. Google" you can be a second opinion. Dermatology is underestimated even by other physicians that feel competent enough to prescribe medication inducing iatrogenesis [6]. So, what to expect from patients? It is a good thing to have an informed patient. It will save you a lot of time and energy in expanded explanations. But, there is also a lot of incorrect or

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misunderstood information, prejudices from other's experience shared on forums, incorrect auto-diagnosis or even incorrect auto-medication. It is our duty to listen and to correct, as much as we can. Patients have to be taught how to choose important information from the constant "soup of news feed".

The flux of information is huge, also for doctors as for patients. Thousands of opinions, articles, and new approaches invade our space daily. A good selection criteria for useful, relevant data is absolutely necessary.

A "happy" patient seems to be the one with a certain diagnosis, with clear treatment plan, including explaining side effects and with a contact number in case of recurrence [7]. The access of any information being so instant, patients expect a rapid response of the treatment. The result has to be now and definitive. The pressure of quick response is not very subtle, the doctor feeling it as a burden. It takes time to explain and to understand the progression of chronic illnesses.

Sometimes, the expectations are to get well with any treatment, eventually without any changes of their life style, even when doctor explains that some habits could aggravate or maintain the lesions. Taking responsibility for some adjustments is an important part to discuss with patient, as part of therapeutic approach. Paternal, omniscient doctor's image is no longer in actuality and patient is an important part of the relationship. Instead of an "infantile" patient, coming for any transitory rash or any mosquito bite, it is better to "grow" him/her as a self-confident "partner". The new and healthy bond has to transform patient from the passive, sometimes passive-aggressive role, into an assumed, informed, pro-active one. Cooperation is the key of healthy relation. Patient has the right to ask for explanations, to discuss therapeutic options, to refuse treatments. Patients have opinions that have to be respected and sometimes corrected if they are distorted. On the way to get therapeutic alliance and long-term cooperation, the doctor-patient relationship has to be personalized, giving value to it. This kind of relationship will make the difference in the end and even the direction is to involve more high tech and robots. Face-to-face relationship will not be replaced by anything and it will be highly appreciated after the "speed" condition passes. More social skills are often required, doctors not being known as the best communicators. Sometimes, doctors are not aware of patients' perception regarding communication [8]. I have got my social skills working day by day, no special courses were made during faculty, unfortunately. "We need fewer memorizers and more thinkers and communicators in modern medicine" is the most recent conclusion of Canadian Debate Series regarding medical students' selection [9].

Another pressure point is the constant fear of errors. With all the efforts of protocols for reducing the risk of mistakes or misconducts, unfortunately there are lots of gaps and debates. The fear of error and malpraxis leads to excess of medication and investigations, sometimes too expensive and useless.

Doctors should not be scared by the abundance of products, instruments, techniques, aggressively promoted. They have to be more flexible, more intuitive and more eager to try, making personal experience and not taking results for granted. Many of these products will not pass the test of time, even they are presented as "miraculous". Sometimes, patients' needs are the trigger for experimenting new methods and push us to progress faster.

Not only patients are in a rush, doctors too. The race for EMC points is making the doctor more informed, but we have to be careful not to become too superficial. Even a doctor is getting a diploma after a "3 days course", it doesn't mean that he/she will be an expert in that field, not even competent. It will take a lot of time and energy to really get the expertise, that course being only the very first step on the road. The "diplomas wall", real or virtual is a false goal. In the end, the real skills are more important than a sublime image and it will take time to get them.

Sometimes, vanity makes the teamwork harder. This will be unproductive for both doctors and patients. It is not a shame to refer the patient to an expert on a field when you feel that you have reached a limit.

People are hardly trying to change our state from patient to client, that new status being debatable. Being a client means to take some responsibilities as in a contract. That is the good part. But, fortunately, remaining a doctor means more than providing services. Practicing medicine is a state of knowledge, art, experience, intuition, with magic touches sometimes.

Fortunately, with all the changes during the last years, dermatologists seem to remain satisfied with their specialty. A recent Mexican study shows that 93% of dermatologists (with an average of 16 years of practice) were happy with their professional life, more than 98% choosing it once again [10].

Maintaining certain levels of professional and personal happiness, keeping informed and open-minded, avoiding burn-out, trying to fulfill patients' expectations, doctors are not in a battle, but in strong alliance with patients.

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VARIA

Cecostomy vs ileostomy for protection of anastomoses in colorectal surgery

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Abstract: *Background: The most frequently encountered complication of anastomotic surgery is fistula. In addition to the medical history of the patient, local conditions, the operative moment and surgical technique that were chosen, a series of some other factors can interfere, leading to suture disunity.*

Aim: The aim of the study was to analyze the existence of a relationship between the use of cecostomy (as a mechanism of temporary diversion) and the frequency of anastomotic fistulas.

Materials and methods: We conducted a retrospective study, over a period of 7 years, between 2011-2018 taking into consideration 194 patients who benefited from resections completed with anastomoses in the transverse colon, descending colon, sigmoid and rectum. They were divided into two categories: patients who benefited from cecostomy and patients for whom we did not use this technique. The frequency of fistulas was analyzed for the two main groups and in association with each type of surgery.

Results: It was observed the existence of a relationship between the appearance of fistulas and the use of a decompression mechanism such as cecostomy in the sense of a lower number of fistulas for the first situation (2.66%) compared to 11.76% for cases without cecostomy (p=0.034). The duration of hospitalization in the group with cecostomy was shorter than in those without this derivative stoma and the postoperative recovery was good.

Conclusions: Cecostomy is a useful protection for the anastomosis line, with the advantage that, when the tube is suppressed, the stoma will spontaneously close.

Keywords: *colon and colorectal anastomosis, intraluminal pressure, fistula, cecostomy*

INTRODUCTION

Colon and rectal surgery have encountered multiple complications over time, the most severe and frequent being the fistula. First fistula records come from antiquity (Old Testament and Hindu texts) and Middle Age with enterocutaneous fistulas being described after abdominal injuries. With the occurrence of anastomotic surgery, the first post-anastomotic fistulas were also described, with the initial frequency and mortality being at high levels. Risk of colonic anastomotic fistula continues to range between 1.5% and 23%, with low colorectal and coloanal anastomoses posing the highest risk [1].

Due to the need for achieving mechanical protection of the suture line, various ways of decompressing the perianastomotic colon were imagined. Colostomy, ileostomy and cecal stoma are thus used. These methods, however, have been associated with complications specific to the stomas in general, and often they remain in use for a long time. Anesthetic and surgical associated risk should also be considered, especially in elderly, due to the aged related frailty [2]. It is therefore necessary to find an anastomotic protection method that is effective, with as few as possible of the stoma-specific complications and which can be easily abolished, preferably without surgical reintervention.

MATERIALS AND METHODS

A retrospective study, over a 7-year period extending from January 2011 to December 2018, was conducted at Second Surgical Department at the County Clinical Emergency

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Hospital of Sibiu. The data of patients with this type of cancer were obtained from the electronic database, clinical files and operating registers. The aim of the study was to analyze the existence of a relationship between the use of cecostomy (as a mechanism of temporary diversion) and the frequency of anastomotic fistulas.

194 patients were included in the study, patients suffering from cancer located on transverse colon, splenic flexure, descending colon, sigmoid and rectum, on which various types of resections and anastomoses were used.

Data analysis was classified by age, gender, body mass index (BMI), ASA score, tumor location, type of resection and anastomosis, the presence or absence of cecostomy, frequency of fistulas, duration of surgery, and average time of hospitalization.

All the patients were positively diagnosed using colonoscopy, colonoscopic biopsy and computed tomography. There was no neoadjuvant treatment prior to surgery. Comorbidity factors included diabetes mellitus, cardiac pathology, obstructive pulmonary disease, chronic renal failure, liver cirrhosis, hypoproteinemia, anemia. Postoperative complications were followed according to the Dindo Clavien classification. The statistical data and analysis was organized and conducted using the Microsoft Excel Office 365 software. The differences were considered significant if p <0.05.

Surgical technique

After the anastomosis has been completed, the cecum is mobilized, released from adhesion (if any), a Pezzer tube with a caliber between 16 and 24 Fr is prepared and a purse string suture is applied on the anterior surface of cecum (Figure 1). In principle, the place of cecostomy should correspond to the appendicular stump, a maneuver that would keep the patient away from appendicular inflammatory complications. However, even if this was not done, we did not encounter local complications in our study. The cecum is pierced with the scalpel and the tube is inserted into the colon. The suture is tightened and knotted around the Pezzer tube (Figure 2).

A breach is made in the right iliac fossa through which the Pezzer tube is exteriorized. The cecum is fixed to the peritoneum and the probe to the skin (Figure 3). All cases were completed with peritoneal and perianastomotic drainage. In all cases we preferred a post-anastomotic decompression like manual anal dilatation or sphincterotomy. Intraluminal tube was not used (Figures 4-6).

Figure 1: Surgical intervention in relation with leakage site

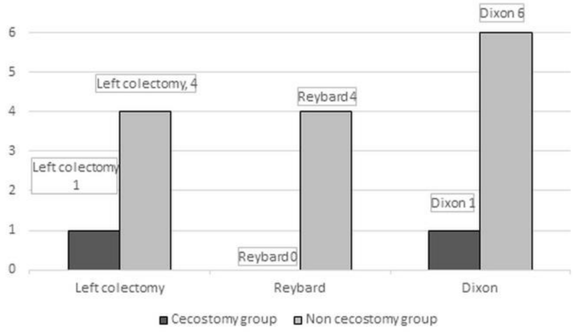

Figure 2: Anastomotic leak complication in Clavien-Dindo classification

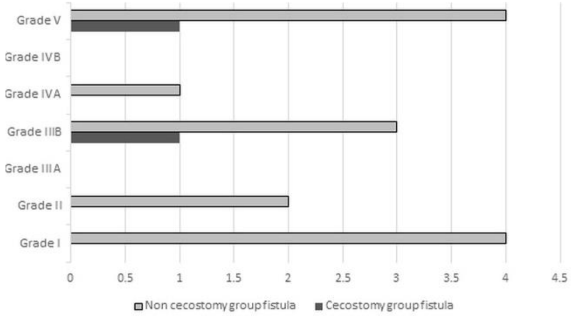

Figure 3: Fistula incidence in our study and in overall literature

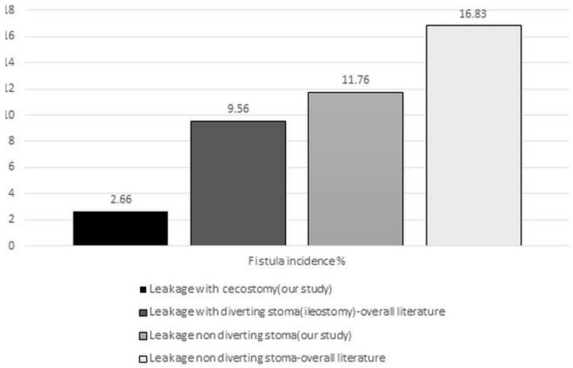

RESULTS

The distribution of cancer on studied colon segments was as follow: transverse colon (9.27%), splenic flexure (11.34%) descending colon (22.16%), sigmoid (39.69%) and rectum (17.52%). Gender distribution in general was 47.93% male and 54.06% women. For the cecostomy group, the distribution was 61.33% in women and 38.66% in male (Table 1).

Of the total number of patients, 75 patients (38.65%) had cecostomy, 119 patients (61.34%) were with no diverting stoma. In cecostomy group cancer repartition was 8% transverse colon, 9.33% splenic flexure, 16% descendent colon, 49.33% sigmoid and 17.33% rectal. Also 28% cases in the first group and 32.77% cases in the second one were anastomosis performed after a previous resection completed with stoma.

Table 1: Characteristics of the patients in the study group

	Cecostomy group	Non cecostomy group
Age (years) median range	62 (38-86)	64.5 (44-85)
Gender female/male	35/40	61/58
BMI kg/m²	27.75 (18.3-37.2)	28 (18.6-37.4)
ASA score (%)		
I	16%	22.68%
II	54.66%	59.66%
III	29.33%	17.64%
Tumor placement		
Transverse colon	6	10
Splenic flexure	7	12
Descendent colon	12	24
Sigmoid	37	48
Rectum	13	25
Blood transfusion	21	39

For the first group surgical techniques applied were segmental transverse colon resection (4%), left colectomy (25.33%), Reybard operation (46.66%) and Dixon operation (24.01%). The statistical analysis obtained was p = 0.042. In the second group the percentage for segmental transverse colon resection was 4.20%, 22.68% for left colectomy, 47.05% in Reybard operation and 26.07% for Dixon technique (p = 0.03).

All cases were resolved by laparotomy, the anastomoses being hand sewn. The average duration of surgery for the group in which cecostomy was used was 146 minutes and 133 minutes for the second group. Three hemorrhagic intraoperative complications were registered being identified and solved. In all cases, hand sewn anastomosis was performed with separate threads in one layer. The most common types of anastomoses for cecostomy group were end to end 77.33%, side to side 13.33% and end to side (9.33%), with a p value of 0.026. Repartition for the other group was end to end 50.42%, side to side 29.41%, and end to side 20.17% (p = 0.011).

The frequency of the fistula in the cecostomy group was 2.66%, with two cases, one with left colectomy status for splenic flexure cancer and the other one with Dixon low rectal resection.

Figure 4: Purse string suture applied on the anterior cecal wall

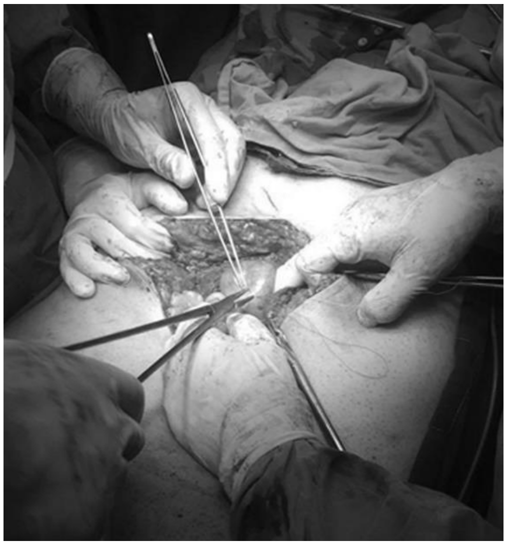

Figure 5: Cecum pierced and prepared for tube insertion

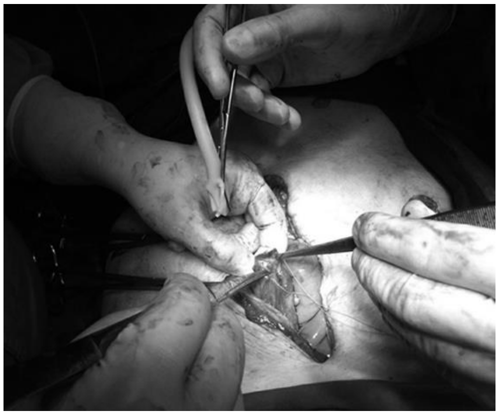

Figure 6: Cecum is fixed to the peritoneum

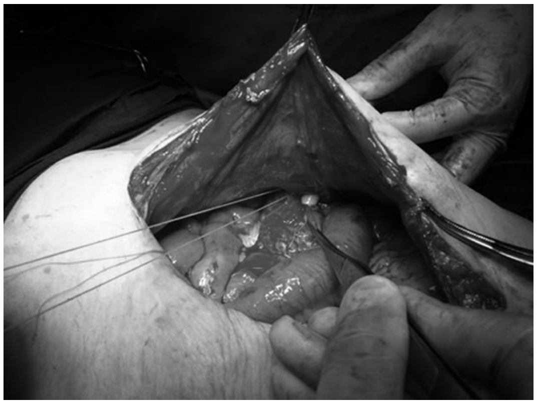

The first case was conservatively treated, the second one was surgically managed by practicing a stoma upstream of the dehiscence. Of the 14 cases with the fistula in the second group, 11 cases were resolved by conservative treatment, in 3 cases surgical approach was preferred, recording 4 deaths.

A statistically significant value p=0.034 was obtained, taking into account the standard value p <0.05. The duration of surgical technique for cecostomy alone was not significantly or statistically increased, the average duration being between 11 min and 5 min with an average of 7.2 minutes. Average length of hospital stay for the cecostomy group was 9.8 days by comparison with 10.3 days in the other group. In the group of patients who presented anastomotic fistula, with cecostomy previously performed the average period of hospitalization was 27.5 days, for the non-cecostomy group being 25.66 days.

The Pezzer tube was removed on average after 8.6 days. No local complications were encountered. The maximum duration of cecostomy drainage was of 14 days after extraction of the Pezzer tube, closing surgery procedures being unnecessary.

DISCUSSION

Colorectal anastomotic leak rates vary from 1.5 to 23% depending on numerous of risk factors such as malnutrition (due to the negative effect in fibroblast proliferation or collagen synthesis) and serum albumin level less than 3.5 mg/dl [1]. Immunosuppression, especially in patients with inflammatory bowel disease on the background of corticosteroid therapy for the underlying pathology and nonsteroidal anti-inflammatory drugs are also known to interfere with anastomotic consolidation [3-5]. Body mass index higher than 25 is associated with a higher risk of fistula.

Fat tissue, diabetes due to chronic complications, low blood levels of hemoglobin (ineffective tissue perfusion) and an ASA score higher than 3 is commonly met along with an increased risk for fistula [6-8]. Mechanical bowel preparation, a controversial topic, with studies that support the usefulness of this maneuver and studies that contradict it. We consider such a maneuver to be useful, it makes a reduction in septicity of the colon. Our experience recommends it to an unprepared colon mentioning that the intervention does not take place immediately after the mechanical preparation of the colon due to edema observed in the colorectal walls after a recent washout [9].

Suture line placement may be the most important factor in the occurrence of a fistula observing that the lower the anastomosis is located, the higher is the risk for leakage [10], many studies link a larger tumor size to a higher risk of fistula [7, 11]. The surgical technique differs according to the surgeon's experience and intraoperative conditions, but the following principles must be respected: achieving a tension free anastomosis and maintaining an effective perfusion on an unobstructed colon [10].

Surgical options include a handsewn or stapled anastomosis in various types, side-to-side, end-to-end, side-to-end, or end-to-side. Additional options include suture material, staple heights, number of layers. It has been demonstrated that regardless of whether the anastomosis has one or two layers, this does not affect the incidence of anastomotic leak, as long as the serosa is apposed and submucosa is incorporated [8]. Beside this particularities, intraluminal postoperative pressure has also its key role in the occurrence of anastomotic breaches.

Thus, postoperatively, the intra-abdominal pressure and the intraluminal pressure increase (by 10-15 mmHg for intraluminal pressure) especially because of paresis of the digestive tract during the period immediately following surgery. Frequently, the moment the fistula occurs corresponds to 5-8 days after surgery, at which time the swelling of the anastomosis is at maximum level, and the intraluminal pressure is at a high level, corresponding to the period in which the intestinal transit is not yet resumed.

Edema of the suture line may predispose to dehiscence, intraluminal pressure intervenes as a decisive factor in causing a further strain on an area of weak resistance with consequent production of leak [12-16].

To prevent pressure effects in the anastomotic area it has been argued that a diverting stoma is an effective and safe procedure to prevent anastomosis leakage complications after colorectal surgery, being imagined different ways of achieving a type of decompression through a diverting stoma such as loop or end ileostomy, colon stoma, cecal stoma, appendicostomy (using vermiform appendix as diverting stoma) or cecostomy [17]. Postanastomotic decompression using manual anal dilatation, sphincterotomy and intraluminal tube used alone are sometimes insufficient. Fistula rates when these procedures are used varies between 5-6% [12, 16, 18]. Intraluminal tube has the disadvantage that it can be mounted only in low colorectal resections. Manual anal dilatation or sfincterotomy are considered useful and had been used in all cases of the study.

The most widely used diverting stoma is ileostomy, more often the loop ileostomy type. Some studies have demonstrated a reduction in the fistula rate in those with ileostomy at values ranging from 3% to 10.9% [10, 11]. On a number of 41 prospective, retrospective and randomized control trials, between 2000-2017, researches that studied anastomotic leakage in patients with colon and colorectal anastomosis for stoma diverting group, the percentage of fistula was 9.56 % and for the non-diverting stoma group was 15.83% [16-22].

The benefits of this method have to be balanced with the specific complications of each type of stoma. The most common complications described were ileus, adhesion syndromes, wound infection and incisional hernias. Mortality associated with anastomotic leakage is raging between 2,1% to 22%. In addition to these, it must be recognized that this type of diverting stoma requires the patient to undergo two surgeries [13, 23-29].

Thus, cecostomy becomes superior to other ways of decompression. Intermittently used in surgery, it comes back in actuality based on an increasingly frequent intention to choose an anastomotic surgery instead of one ended through stoma. Technically it is easy to perform, does not require excessive extension of surgery time, it becomes functional quickly, reducing relating suture-pressure issues (especially the gas pressure) and during its use does not cause difficulty in handling, behaving like a drainage tube. It is recommended to maintain the Pezzer tube for 8-10 days, during which the adhesions process is complete, with the formation of adhesions between the cecum and the parietal peritoneum, the insulation of cecostomy from the abdominal cavity being meanwhile achieved. Suppression of the Pezzer probe does not require any special technique, a simple traction being enough. The area where the cecostomy is placed is covered with a colostomy bag, becoming progressively unproductive - at this time, the cecostomy is closed with a simple bandage on the area. Maintaining a good nutritional support and the use of probiotics improves the patients' quality of life [30, 31].

The advantages of cecostomy are that it is easy to perform, realizes a partial diversion, in transit it is easy to handle and cared by the patient, removal is done without the need for another surgical procedure, and local drainage stops spontaneously after a short time. Temporary diversion ileostomy, compared to cecostomy, implies in our opinion additional statements borne by both the patient and the medical system. In addition to the difficulties of management and care of an ileal stoma compared to a cecostomy, the risk of dysfunctionalities and stenosis of the anastomosis must be taken into account. Also, there is still no consensus regarding the time of instituting oncological treatment in relation to the abolition of ileostomy, the patient may be put in a position to delay chemotherapy. Possible absorption deficits in an ileostomy may increase during chemotherapy, leading to decreased compliance to therapy and increased duration of recovery, or may even affect the outcome of therapy.

The surgical gesture, as well as the anesthesia, especially the general anesthesia in the case of abdominal or pelvic surgery, is an aggression for the body [32]. It is the double exposure of the patient to the risks associated with surgical procedures and immunosuppression associated with additional surgery and anesthesia that we find inappropriate when it comes to two serial interventions. Those risks may be lowered if a minimally invasive, temporary option which does not interfere with cancer treatment and does not require reintervention, is considered [33].

CONCLUSION

We consider mini-invasiveness to be a must in the surgical approach whenever possible. Ileostoma has an additional disabling character, especially for a certain type of patients: the elderly, patients with neuro degenerative or neuro-mental disorders. In addition to all these elements, the cost of stoma care, stoma bags, a new hospitalization, the cost of anesthesia-surgery or postoperative care in the event of complications must be taken into account.

Although out of frequent surgical use, we believe that cecostomy has its advantages over other methods of diversion, and we believe that the tactical option and surgical technique should not be in line with a trend but should be strictly dictated by what brings the most benefits to the patient. The previously mentioned as well as personal experience make us recommend cecostomy as a protection mechanism in colorectal anastomotic surgery because of its safety, spontaneous reversibility and minimal invasivity.

Cecostomy is a safe and effective alternative for diverting stoma in colon and colorectal anastomosis, allowing a proper decompression that protects the anastomosis area, without requiring any surgical reintervention. Anastomotic colorectal surgery is thus safer with certain benefits for patients.

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EDITORS POINT OF VIEW

Challenges of the next normality

Victor L. Purcarea, Daniel O. Costache

At the beginning of last year, Professor Eliot Sorel continued to plead for a successful exit from the devastating pandemic, highlighting, among other aspects, the role played by the creative integration of the artificial and human intelligence in accelerating post-pandemic innovations (Sorel E. COVID-19 Vaccines, Accelerated Innovations, and 21st Century Social Contracts Challenges. World Soc Psychiatry. 2021; 3:7). At the same time, a study conducted by the McKinsey Global Institute (part of McKinsey & Company, involved in business and economic research) highlighted the *three groups of consumption and business trends accelerated by COVID-19*: distance working and virtual interactions, e-commerce, and digital transactions, as well as implementation of automation and AI (McKinsey Global Institute, The future of work after COVID-19, The post pandemic economy, Executive summary, February 2021).

In an article published by McKinsey & Company in November 2021 (Bujor, M., Cierkowska, A., Dorling, G., Dragan, R., Filip, A., Remes, J. and Vukojevic, M. How prioritizing health can be a prescription for Romania's prosperity, McKinsey & Company, November 2021), the aspects highlighted were: *the hard lessons learned in the context of the COVID-19 pandemic*, the opportunity of promoting health and welfare, taking into account the weaknesses of the health system and the indissoluble relationship between health and economy; a number of encouraging lessons on the ability to respond effectively in the context of the crisis (rapid development of COVID-19 vaccines, adoption of digital health tools and companies implementation of rapid operational changes in order to adapt to the pandemic).

In December 2021, in an episode from the McKinsey podcasting about health care, the executive director of Datavant company (which is about to become one of the world's largest healthcare data ecosystems, enabling patients, providers, payers, healthcare data analysis companies, patient applications, government applications and institutions dealing with living organisms and life processes *to exchange patient data safely*), Pete McCabe, talked about *current challenges: data fragmentation and access, the benefits of tokenization* (process of substituting a sensitive data element with a non-sensitive equivalent, called token; tokens can be individual words, phrases and even whole sentences; the process allows the division of a sequence into pieces, some characters, such as punctuation marks, being removed), *value-based care and the need for neutrality* (Reddy, P., 2021. How can healthcare unlock the power of data connectivity? McKinsey & Company, Healthcare Systems & Services Practice, December 2021). According to McCabe, for example, innovative technologies, such as tokenization, can close data connectivity gaps (this connectivity places together disparate data sets and applications, including data from different identity spaces, allowing collaboration between different parts with data control, ensuring secure and efficient activation across the wider ecosystem); the foundation of value-based care requires data and this can be as good as the basic data that supports it; value-based care is just the beginning of potential leadership, so, if the internet can recommend a piece of clothing based on consumer's buying history, why would not a doctor be able to recommend a treatment based on a holistic view of individual life data of a patient?

Also in December 2021, CB Insights highlighted the agile adaptation of technology giants such as Amazon, Microsoft, Google, and Apple to the challenges in the pharmaceutical industry caused by the COVID-19 pandemic, respectively, *the answer of these large technology companies materialized in building technology for drug discovery, clinical trials, production, and supply chains, as well as patients' remote monitoring and their health records* (CB Insights a, 2021. The Big Tech In Pharma Report: How Amazon, Microsoft, Apple, And Google Are Capitalizing On The Digital Transformation, December 14, 2021). Those who are familiar with the field are also accustomed to the developments that allow patients to manage their own health based on both data records from the Electronic Health Record/EHR, containing the medical history based on the physician's records (including lab results and care conclusions), and patient-generated data (such as symptoms). According to CB Insights, *the above-mentioned technology giants make the most of their expertise in software, data, and artificial intelligence to capitalize opportunities along the value chain* (its management considers the bottom-up flow, that is from the consumer/patient to the source; the value chain management 4.0 means generating and keeping value throughout the chain, using the technology usually associated with industry 4.0 everywhere, not just in the factory). CB Insights insists that *reducing the cost of drug development is a top priority, a way of making this possible by using digital solutions* (software), and, in this context, big technology companies pay close attention to the digital transformation of the pharmaceutical industry: Amazon builds the drugstore of the future using its supply chain in order to create a customer experience similar to its e-commerce business; Microsoft enters the pharmaceutical market with SaaS healthcare solutions and partnerships with the already existing players; Google uses patient data and artificial intelligence expertise to build drug research and development capabilities; Apple uses its hardware and user database to its advantage, in order to build a secure, centralized location for patient health data (CB Insights b, 2021. Apple's greatest contribution, Healthcare Insights, Thu 23/12/2021 14:04).

Obviously, the COVID-19 pandemic forced the reimagining of all aspects related to healthcare services, with *considerable changes in telehealth, digital therapy, and medical devices* (CB Insights c, 2021. Our Top 2021 Healthcare Reports, December 21, 2021). Thus, healthcare providers have considered innovative solutions to improve clinical workflows and decided for a wide range of remote care options (from consultations to therapies and mental health support). A confirmation of this concern in our increasingly digital world is the evolution of global funding for healthcare amid the continuing waves of COVID-19 pandemic (which reached $97,8 billion in the third quarter of this year, a figure that keeps on growing, breaking the total record of 2020 by 21%).

There is no doubt that the use of information and communication technologies in medicine (as well as in other medical professions, in order to manage disease and health risks and to promote well-being) will continue to play an increasingly critical role in improving health care. But, digital transformation (generally understood only as a shift to supporting more business functions with the help of technology) does not mean just switching to digital methods of communicating with potential clients (patients). "Digital" does not only refer to technology in business models, it refers just as much to people and mentalities, to humanization, as the effects of the COVID-19 pandemic have changed the habits of clients/users, and the providers of health care services have been forced to adapt their strategies on the continuous improvement of their relationship with the patient.

This improvement also depends on optimizing operational efficiency throughout the supply chain, to provide the client/ patient a frictionless, convenient, and personalized decision in all aspects, for a better understanding of all the moments (and even the micro moments) of the interaction in the "digital" space. The essence of an agile transformation is the reimagining of an organization as a network of high-quality teams, supported by an efficient and stable backbone of strategy, structure, processes, people, and technology. Digital adoption means reaching a state in which users gain the ability to use digital tools to the fullest and in the way they are intended, software adoption being just a part of the digital adoption process.

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VARIA – Letter to Editor

Challenging of COVID-19 crisis in the Emergency Department

Hassan Goodarzi[1], Sardollah Mahmoodi[1]

Dear editor,

The outbreak of coronavirus disease 2019 (COVID-19) is quickly spreading in the world [1]. The COVID-19 has negatively affected the standard services presented in the emergency department (ED). The ED must provide health services to large populations with various health problems. COVID-19 conditions raised pressure on the limited resources in ED and caused interruptions in preliminary treatment. Recently, evidence verified that there are increased rates in emergency healthcare services usage because of the raising traumatic injuries. In the COVID-19 crisis, the statistics and causes of trauma injuries patients were dramatically changed and because of the attendance of further family memberships at household domestic injuries and burning in children were increased [2].

Healthcare services overcrowding has negative consequences to the patients, the healthcare services, and the population. Overcrowding of the healthcare services could also hurt standards services preparation [2]. Moreover, in an outbreak of a communicable disease such as COVID-19, overcrowding has specific negative consequences on controlling and prevention of expansion of diseases. Overcrowding in ED could be the trigger to spread COVID-19 more rapidly. Furthermore, the structure in many EDs is insufficient for facing with overcrowding of patients, causing unsatisfactory situations while people are anticipated to have prolonged for suitable treatment in ED. Also, in many EDs maybe there are inadequate clinical spaces to facilitate evaluation and therapy of cases with confidentiality. Also, there are a high number of emergency patients and the seriousness of their conditions has hindered the timely admissions of emergency patients and cause overcrowding of healthcare services. The increased high non-urgent patients that were scared from coronavirus flow to the hospitals were other identified factors that contributed to the hospital crowding. These circumstances have adversely affected the quality of services and performance.

Following the quick spread of the COVID-19 crisis in the world, unfortunately, there is a limitation to access to supplies such as gloves, medical masks, respirators, hand washers, etc. However, a shortage of personal protective equipment could endanger the general population and as well as healthcare workers. Governments and healthcare administrators must performance rapidly to provide supplies.

Healthcare workers involved with COVID-19 cases in ED were commonly under high pressure. Maintaining the mental health of Healthcare workers in ED is very crucial to manage COVID-19 cases. Administrators must have care to the work stress and the associated causes of the Healthcare workers.

A suitable registry system in ED could help for better management and follow-up of COVID 19 cases and also it could help researchers for better understanding and studying about various aspects of COVID 19 during a long time follow-up after treatment.

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ORIGINAL ARTICLES

Characteristics and complications of supernumerary permanent teeth in a sample of patients examined in a university pedodontics clinic

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Abstract: *Aim. The assessment of distribution of supernumerary permanent teeth (SPT) and the disturbances caused by them.*

Material and method. Cross-sectional study upon a sample of 85 children (58 boys) aged between 3 and 18 years (mean age=9.08±3.09 years) with at least one SPT. Were analyzed: patients' distribution according to gender, age and number of dental units; SPT distribution according to arch and topography and also the disturbances noticed.

Results. a) boys:girls ratio=2.14:1; b) the 85 patients had 107 SPT, mean number=1.25; c) 76.47% of patients had one SPT; d) 89.72% of SPT were on maxillary arch, maxilla:mandible ratio = 8.7:1; d) 92.52% of SPT were localized in anterior region, anterior:posterior region ratio = 12.3:1; e) mesiodens –71.96%, supernumerary lateral incisor – 19.62%; f) disturbances caused by SPT: ectopic eruption of permanent teeth – 36.47%; delay or failure of eruption of permanent teeth – 21.17%; crowding – 20%.

Conclusions. 1. Supernumerary teeth were most frequent noticed in boys, in maxillary anterior region. 2. Most often they caused eruption disturbances of permanent teeth.

Keywords: *supernumerary permanent teeth, distribution, complications*

INTRODUCTION

SPT are defined as any tooth or odontogenic structure that is formed from a tooth germ in excess of the usual number of teeth for any region of the dental arch [1, 2]. In permanent dentition, this dental anomaly is 5 times more frequent than in primary dentition, the prevalence values reported ranging from 0.1 to 6% [2, 3].

In Romania, there are few recent studies have been carried out on the SPT [4]. These focused more on the distribution of SPT, but less on local disturbances caused by their presence. This study is important because it helps to identify early signs of the presence of SPT and thus prevent loss of permanent teeth or further complications.

The aim was to analyze the distribution of SPT and to assess the disturbances produced by them, in a group of children examined and treated in a University Pedodontics Clinic.

MATERIAL AND METHODS

A retrospective cross-sectional study was conducted on a sample of 85 children (58 boys, 27 girls) aged 3 to 18 years (mean age = 9.08 ± 3.09 years), with at least one SPT. Children were examined in the Pedodontics Department,

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Faculty of Dental Medicine, Carol Davila University, Bucharest. Criteria for choosing the study sample were: patients aged up to 18 years old, without genetic diseases, chronic diseases or lip-palate clift. Pre-existing conditions, such as cardiac pathology, were evaluated [5-8].

Data on dental status were obtained from dental records of patients examined and treated by a single dentist. The diagnosis of the number anomaly was made on clinical and radiological examinations. For each patient diagnosed with this anomaly were noted: age, gender, permanent teeth erupted, presence, position and shape of the SPT and disturbances produced by them.

The data obtained were collected and processed using SPSS-version 20.0 for Windows (SPSS Inc, Chicago, IL, USA).

The aim of the research was to analyze: the distribution of patients with SPT according to sex, age and number of affected teeth; the distribution of SPT according to the affected arch and the topography of the tooth, and the disturbances produced.

RESULTS AND DISCUSSION

Sex distribution of the sample

The percentage of boys diagnosed with this anomaly was 68.23% and for girls - 31.77%. The boys:girls ratio was 2.14:1.

It was noted that in 3 male patients it was observed that the SPTs were associated with the congenital absence of other permanent teeth (Hyper-Hipodontia syndrome).

Age distribution of the sample

Most often, the number anomaly was noticed at the age of 9 years (Figure 1).

Patients' distribution according to the number of affected teeth

The 85 children had a total of 107 teeth, with an average number of 1.25 teeth. Most commonly patients had a single permanent tooth in excess – 65 (76.47%), 18 (21.17%) had 2 SPT and only 2 patients (2.35%) had 3 SPT.

Figure 1. Age distribution of the sample according to the age at which the anomaly was diagnosed

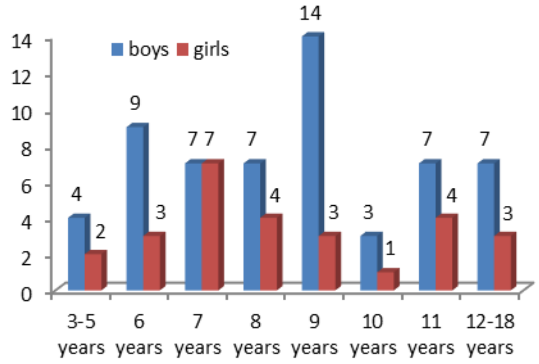

SPT distribution according to their topography

Most of the SPT were in the upper arch, in the front area (Table 1). Maxilla: mandible ratio was 8.7:1, and the anterior: posterior area ratio was 12.3:1. Note that multiple SPT have only met in the upper front area.

Table 1. Distribution of SPTs according to their topography

	Maxilla		Mandible		Total	
	n	%	n	%	n	%
Anterior	92	85.98	7	6.54	99	92.52
Posterior	4	3.74	4	3.74	8	7.48
Total	96	89.72	11	10.28	107	100

SPT distribution according to the type and shape of the teeth

The conical-shaped mesiodens was the most common oversized tooth, followed by lateral incisor with a similar shape to the normal-series tooth (Table 2).

Table 2. Distribution of SPT according to type and morphology

Shape	Supplementary	Conical shape	Tuberculate shape	Odontoma	Total	
					n	%
Mesiodens	9	47	17	4	77	71.96
Lateral incisor	19	1	1	0	21	19.62
Parapremolar	4	0	0	0	4	3.73
Paramolar	1	2	0	0	3	2.80
Distomolar	1	0	0	0	1	0.93
Canine	0	0	1	0	1	0.93
Total	33	50	19	4	107	100

Of the 77 mesiodens, 12 (15.58%) were inverted, being discovered by chance on the X-rays.

Distribution of the disorders caused by SPT

Most of mesiodens determined the deflection of the permanent tooth eruption from the normal series, while the lateral incisors caused or worsened the crowding (Table 3).

Table 3. Disorders caused by SPT

Disturbances	Mesiodens	Lateral incisor	Parapremolar distomolar	Para-molar	Canine	Total	
						n	%
Ectopic eruption	29	1	0	1	0	31	36.47
Failed eruption	16	2	0	0	0	18	21.17
Crowding	2	15	0	0	1	17	20
No consequences	8	2	0	0	0	17	20
It can't be appreciated	2	0	5	2	0	2	2.35
Total	77	21	5	3	1	85	100

DISCUSSIONS

In the literature there are numerous studies of this anomaly that analyze their frequency, shape variations and disturbances that can be associated with different population groups. Most studies were conducted on samples of patients from orthodontic clinics. But early diagnosis of SPT helps to achieve an appropriate treatment plan and to prevent further complications.

In permanent dentition, the prevalence of the disease varies between 0.1 and 2.1% in Caucasian, between 2.4 to 6% in Chinese, Japanese and black Americans [2, 5]. In Romania, the prevalence value was 3.04% [4].

Analyzing the distribution by sex, the boys:girls ratio of the SPT was 2.14:1. This result is similar to that reported by numerous studies conducted in international groups of children or adults [9-14]. Instead, Bäckman and Wahlin (2001) found a predilection of SPT in females [15], which was highlighted by Mitchell (1989), who found a female:male ratio of 2:1 [16]. Unlike them, Dominguez and Mendoza (1995) did not discover any difference between the sexes [17].

Rani et al. (2017) noted that SPT are more frequently diagnosed in people aged the first 3 decades of life [2]. In a study conducted by analyzing 2241 X-rays, Salcido-García et al. (2004) found that from the total number of patients with SPT 47.6% were in the third decade of life, followed by those in the first decade of life in a percentage of 28.5% [18]. This study, conducted on a group of children, showed that the average age to which this anomaly was diagnosed was 9 years. Also, Alberti et al. (2006) conducted a study on a lot of patients aged between 6 and 10 years and concluded that the interval for early diagnosis and prevention of malocclusions caused by hyperdontia is around the age of 9 [13]. More than ¾ between children had one extra tooth, resulting similar to values reported by other researchers [9-14].

Regarding the topography, the SPT were identified in the present study of approximately 90% in the upper jaw, a percentage similar to that reported by Zhu et al. [19] or Hattab et al. [20]. Instead, Fernández-Montenegro et al. (2006) [21] found that 74.5% of the SPT were at the maxilla, while Leco-Berrocal et al. (2007) – a percentage of 79.2% [9].

In the present study, 92.52% of the SPT were located in the front area. Other studies report in adult populations a higher frequency of SPT in the posterior jaw area. It is agree that in children the most frequent location is in the front area, and in adults in the posterior area [9].

In the group studied in the Pedodontics Clinic from Bucharest, mesiodens appeared in 71.96% of cases, followed by lateral incisor (19.62%). Also, Fernández-Montenegro et al. (2006) found that mesiodens was the most commonly diagnosed, followed by parapremolar and distomolar [21], Salcido-Garcia et al. (2004) found the order: mesiodens, parapremolar and lateral incisor [18] and Bereket et al.: mesiodens, distomolar, parapremolar [10]. On the other hand, in the study of Khandewal et al. (2018), parapremolars were most commonly met [14], and at Leco-Berrocal et al. (2007) [9] and Rani et al. (2017) – distomolars [2].

Most of the authors found that the conical shape is the most common, this fact also emerged in the present research [10-12, 14, 21, 22]. In second place, supplementary teeth were observed, as in the study conducted by Fernandez-

Montenegro et al. (2010) [21], while Shah et al., and Schmukli et al. (2010) found the tuberculate shape [11, 22].

As far as the consequences are concerned, in this study in most cases the deflection of the teeth eruption of the normal series was encountered, followed by the failed of permanent teeth eruption and crowding. Leco-Berrocal et al. reported that the most common complications were mechanical accidents, namely the deflection of adjacent teeth -39%, root resorption -12.5% and failed of eruption-4.1%, but also 19% follicular cysts [9]. Rajab and Hamdan were observed that 51.47% of meziodentes failed the eruption of permanent incisors [12].

No consequences were observed in 20% of cases. Instead, Fernández-Montenegro et al. (2006) and Bereket et al. (2015) found the lack of any consequences in a large percentage – 57.9%, respectively 61.64% [10, 21].

Most of the results from the present study were similar to the values reported in the literature. Differences may be caused by racial type and the mean age of the sample.

CONCLUSION

1. Hiperdontia was seen more frequently in boys compared to girls (2.14:1).
2. The SPT have been located most frequently in the upper front area.
3. The most common type of SPT was mesiodens (71.61%) followed by the upper lateral incisor (19.62%).
4. In most cases, the SPT resulted in the deflection of a tooth from the normal series.

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ORIGINAL ARTICLES

Chest CT-scan findings in COVID-19 patients: the relationship between the duration of symptoms and correlation with the oxygen saturation level

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Abstract: *Purpose: This study is carried out to evaluate the diagnostic value of using common features of computed tomography (CT) imaging in COVID-19 disease, and to assess the relationship between blood oxygen saturation level and severity of CT findings.*

Materials and Methods: In this retrospective study, the chest CT of 173 test-confirmed COVID-19 patients have been evaluated to determine the patterns of involvement in multiple phases of illness. Then, the correlation between the severity of lung involvement and oxygen saturation levels has been assessed.

Results: The chest CT results show that 87.6% of patients had GGO, which was the most common pattern in our findings. 83.8% of patients had bilateral lung involvement with the dominant multifocal and peripheral distribution. peribronchovascular involvement was also a common finding in our study (47.2 %). we found predominantly peribronchovascular view in 3 patients (1.7%), pleural effusion in 4 patients (2.3%), lymphadenopathy in 10 patients (5.8%), the tree in the bud in one patient (0.6%), and nodules in 4 patients (2.3%). We also found that GGO is the most common pattern during the early phase of the disease (97.4% of early phase cases). However, in the intermediate and late phases, consolidation and crazy paving patterns are more common. Moreover, our findings indicate that there is a significant relationship between oxygen saturation level and Total Severity Score, with the exclusion of the young adult patients (20-40 years).

Conclusion: Relying on chest CT-scan findings apart from the oxygen saturation level is sufficient for the diagnosis and management of patients with COVID-19 pneumonia.

Keywords: *COVID-19, pneumonia, spiral CT, diagnosis, treatment, Real-Time RTPCR*

INTRODUCTION

Coronaviruses as part of the coronaviridae family are nonsegmented, enveloped, positive-sense, and single-strand ribonucleic acid viruses [1]. This is the seventh known coronavirus to infect humans [2]. Two other notable examples of this coronaviridae family include severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). SARS began spreading in southern China

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and resulted in 774 deaths out of 8,098 infected individuals in 29 countries from November 2002 through July 2003 while MERS originated in Saudi Arabia and caused 848 deaths among 2,458 individuals in 27 countries through July 2019 [3, 4].

The number of confirmed COVID-19 cases continues to grow rapidly in the world. The recent outbreak of the novel coronavirus (COVID-19) began in December 2019 in Wuhan, the capital of central China’s Hubei province, and has led to a major pandemic [2, 5]. Widespread human-to-human transmission has resulted in 5,240,900 cases worldwide with 338,700 deaths as of May 23, 2020 [6].

Patients infected with this novel COVID-19 virus manifested with symptoms of severe pneumonia, including fever, fatigue, dry cough, and acute respiratory distress. 2019-nCoV caused clusters of fatal pneumonia with clinical presentation greatly resembling SARS-CoV. Patients infected with 2019-nCoV might develop acute respiratory distress syndrome, have a high likelihood of admission to intensive care, and might die. The cytokine storm could be associated with disease severity. More efforts should be made to know the whole spectrum and pathophysiology of the new disease [7].

According to available guidelines, the diagnosis of COVID-19 should be confirmed by reverse-transcription polymerase chain reaction (RT-PCR) or gene sequencing of respiratory or blood specimens. However, the RT-PCR has shown detection rates as low as 30% to 60% and also false-negative results at the initial presentation while this technique requires specific sample collection protocol, preparation, and particular facilities that have prevented accurate and fast results being available for further diagnosis [8]. These limitations in diagnosis and treatment subsequently result in a higher rate of infection demanding an effective strategy for the early diagnosis of COVID-19.

Radiological imaging is known as a critical assessment technique for the evaluation of severity and disease progression in upper and lower bronchial disorders such as COVID-19 infection. Among variable imaging techniques, Computed Tomography (CT) scans have shown promising potential in COVID-19 case ascertainment [9, 10]. Chest spiral CT is a crucial diagnosis test for COVID-19 disease, which is used to assess the severity of lung involvement in COVID-19 pneumonia. [10, 11]. A variety of imaging patterns in chest CT-Scan have been described in other studies such as bilateral lung opacities in infected patients and described lobular and subsegmental areas of consolidation as the most typical findings. Other investigators examined chest CTs in 21 infected patients and found high rates of ground-glass opacities and consolidation, sometimes with a rounded morphology and peripheral lung distribution [12, 13]. CT abnormalities might predate rtRT-PCR positivity in symptomatic and asymptomatic patients who were subsequently tested positive according to their rtRT-PCR results [14, 15, 16].

In this study, we characterize chest CT findings in 173 patients infected with COVID-19 and conformed with rt-RT-PCR in relationship to the time between symptom onset and the initial CT scan. Also, we evaluate the correlation between O_2 saturation and the severity of the lung involvement in chest CT scans.

MATERIAL AND METHODS

In this retrospective study from March 4, 2020, until March 10, 2020, 173 adult patients admitted to Baqyatollah hospital in Tehran province in Iran with confirmed COVID-19 infection and undergone chest CT scans were enrolled in our study. There have been no exclusion criteria considered in this research.

Confirmation of the disease was performed through laboratory testing for COVID-19 with real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) of respiratory secretions obtained by bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal swab, or oropharyngeal swab which were positive for all patients. The rRT-PCR test kit used in this study was provided by SinaPure TM viral (Tehran-Iran).

All patients were imaged with a 3.5-mm slice thickness CT on a Siemens Emotion 16 scanner (Siemens Healthineers; Erlangen, Germany). All scans were performed without intravenous contrast injections in the supine position during end inspiration.

In addition to the age [17] and gender parameters, other information such as the number of days between the onset of the symptoms and date of first chest CT-scan were recorded for further analysis. This disease changed rapidly at the early stages [18], so in this study, if the time interval between the first clinical symptom and CT was two days or less, the patient was considered to have been imaged in the “early” phase of the illness. If the time interval was between three and five days, the patient was considered to have been imaged in the “intermediate” phase of the illness. If the time interval was between six and 12 days, the patient was considered to have been imaged in the “late” phase of the illness.

Oxygen saturation of patients was also measured in the pressure of the air room once they were admitted to the hospital.

All CT images were reviewed by two trained and experienced radiologists with 10 years of experience. Imaging was reviewed independently and reported after the final decisions were made in consensus. Negative control cases were also examined.

For each patient, the chest CT scan was evaluated for the following characteristics:
(1) Presence of ground-glass opacities,
(2) Presence of consolidation,
(3) Laterality of ground-glass opacities and consolidation (unilateral or bilateral),
(4) Number of lobes affected where either ground-glass or consolidative opacities were present,
(5) Degree of involvement of each lung lobe in addition to the overall extent of lung involvement measured through a "total severity score",
(6) Presence of nodules,
(7) Presence of a pleural effusion,
(8) Presence of thoracic lymphadenopathy (defined as lymph node size of ≥10 mm in short-axis dimension),
(9) Distribution of the disease (categorized as a unifocal disease, multifocal disease, peribronchovascular disease, peribronchovascular predominant disease, or peripheral disease),
(10) Other abnormalities including opacities with a crazy-paving pattern, opacities with intralesional cavitation, the tree in bud appearance, and vascular enlargement were noted.

The ground-glass opacification was defined as hazy increased lung attenuation with preservation of bronchial and vascular margins, whereas consolidation was defined as opacification with the obscuration of margins of vessels and airway walls [19].

Regarding the total severity score, each of the five lung lobes was assessed for degree of involvement and classified as none (0%), minimal (1 - 25%), mild (26 - 50%), moderate (51 - 75%), or severe (76 - 100%). No involvement corresponded to a lobe score of 0, minimal to a lobe score of 1, mild to a lobe score of 2, moderate to a lobe score of 3, and severe to a lobe score of 4. An overall lung total severity score was reached by the sum of the five lobe scores (range of possible scores, 0 - 20). Then the correlation between "Total Severity Score "and O_2 saturation was calculated.

RESULTS

This study was conducted based on a CT-scan data set consists of 110 male and 63 female patients with an average age of 63± 3.94 years and the age range between 6 to 86 (Table 1).

Table 1: Patient characteristics

Gender	All patients N= 173	Early* (0-2 days) N= 39	Intermediate (3- 5 days) N=113	Late (6-12 days) N=21
Men	110 (63.5)	24 (61.5)	71 (62.8)	15 (71.4)
Women	63 (36.5)	15 (38.5)	42 (37.2)	5 (28.6)
Age (years)				
Mean	53.6	47.28	53.96	58.95
Range	7 - 86	21 – 85	7 – 86	36 – 85
SD	3.94	15.29	13.55	11.28
O_2 saturation				
Mean	89.44	93.05	89.27	83.67
Range	67 – 98	89 – 98	74 – 97	67 – 94
SD	4.76	2.82	3.58	6.88

**Early, intermediate, and late refer to time from symptom onset to time of the chest CT scan;*
SD – standard deviation.
Note: Numbers in parentheses are percentages.

According to the results, one-hundred and forty-four patients have shown multifocal bilateral lung involvement. Among these patients, 78 patients (54.2%) had only GGO (without consolidation), 14 patients (9.7%) had the only consolidation (without GGO) and 51 patients (35.4%) had both of them.

Figure 1: An axial CT image obtained without intravenous contrast in an 85-year-old male shows a "crazy-paving" pattern as manifested by bilateral ground-glass opacification with interlobular septal thickening with intralobular lines.

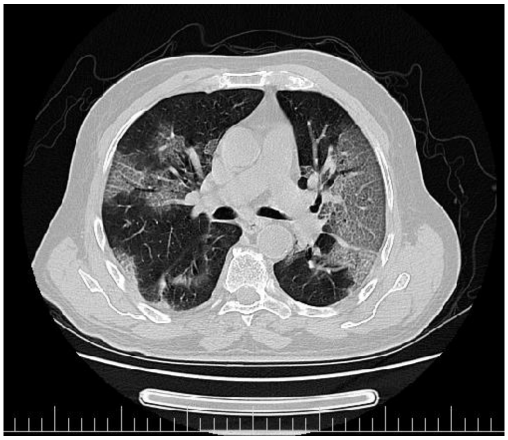

In this study, different types of lesions have been observed in the 173 patients with confirmed COVID-19 disease. These lesions are GGO (Figure 1, Figure 2, Figure 3), consolidation (Figure 3), crazy paving (Figure 1) and vascular enlargement (Figure 4), which are observed in 151 patients (87.6%), 77 patients (44.5%), 23 patients (13.3%) and 106 patients (61.3%), respectively.

Figure 2: An axial CT image obtained without intravenous contrast in a 52-year-old male shows bilateral peripheral and peribronchovascular ground-glass opacities with round morphology.

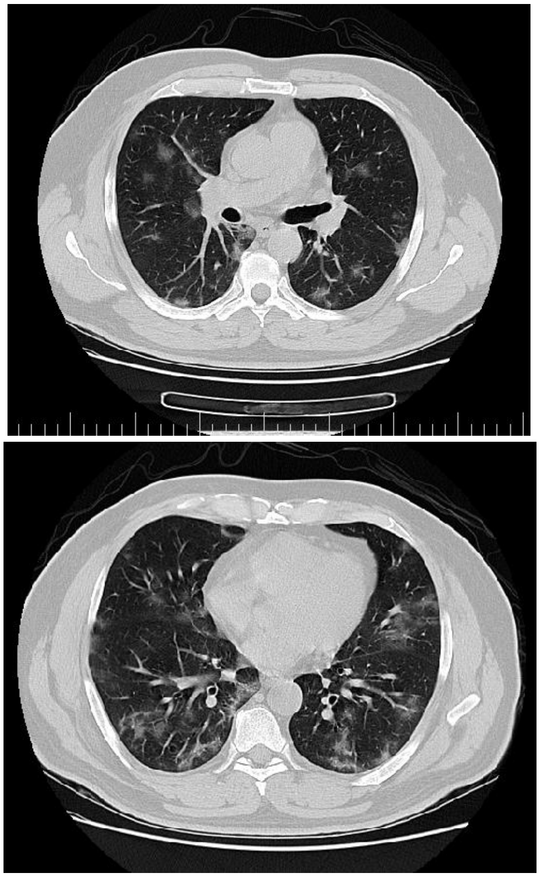

The number of affected lobes can vary in different patients. Among the 173 patients, 23 patients (13.3%) showed lung opacities in one lobe, 14 patients (8.1%) in two lobes, 13 patients (7.5%) in three lobes, 23 patients (13.3%) in four lobes, and 99 patients (57.2%) in all five lobes. Moreover, the right upper lobe of 133 (76.9%) patients, the right lower lobe of 151 patients (87.3%), the left upper lobe of 133 patients (76.9%), and the left lower lobe of 153 patients (88.4%) were involved.

145 patients out of 173 total patients (83.8%) had bilateral and 145 patients (16.2) had unilateral lung involvement as shown in Table 2. The average total severity score (ranged from 1 to 20) was 5.75±3.87. This study revealed that the relationship between the patient's age and the total severity score is statistically significant ($p<0.001$ and the correlation coefficient is 0.29).

Also, Table 2 shows the lesion distribution. According to this table, 21 patients (12.1%) had shown unifocal involvement, 153 patients (88.4%) had multifocal involvement, 170 patients (98.3%) had peripheral involvement and 82 patients (47.2%) had peribronchovascular involvement.

Figure 3: An axial CT image obtained without intravenous contrast in a 56-year-old male shows bilateral and peripheral ground-glass opacities with dominant consolidation.

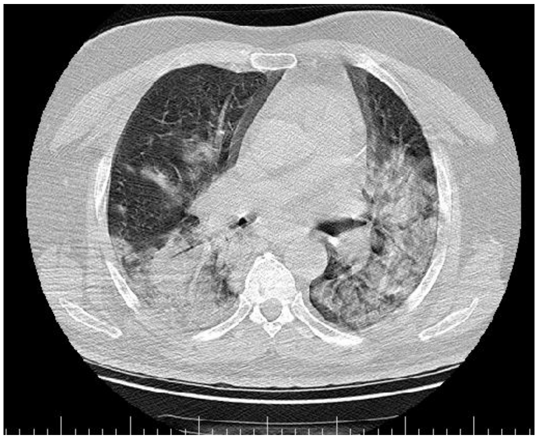

Figure 4: An axial CT image obtained without intravenous contrast in a 60-year-old female shows unilateral opacification with the tree in bud appearance which is a very rare presentation of COVID-19 pneumonia. Vascular enlargement is also noted which is a common finding.

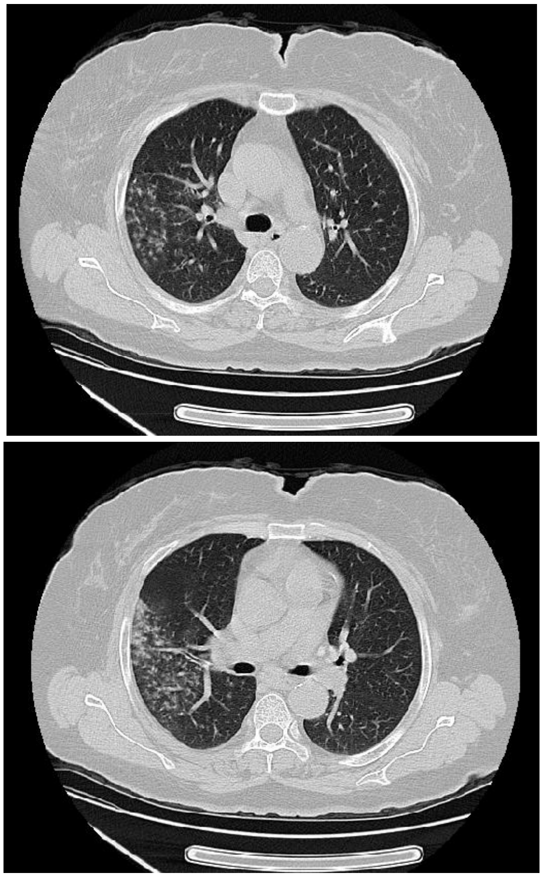

Table 2: Findings on chest CT in 173 patients

Ground-glass opacities and consolidation	
absence of both ground-glass opacities and consolidation	0 (0)
presence of either ground-glass opacities or consolidation	173 (100)
presence of ground-glass opacities without consolidation	96 (55.5)
presence of ground-glass opacities with consolidation	55 (31.8)
presence of consolidation without ground-glass opacities	22 (12.7)
Number of affected lobes	
0	0 (0)
1	23 (13.3)
2	14 (8.1)
3	13 (7.5)
4	23 (13.3)
5	99 (57.2)
more than 2 lobes affected	149 (86.6)
bilateral lung disease	145 (83.8)
Frequency of lobe involvement	
Right Upper Lobe	133 (76.9)
Right Middle Lobe	108 (62.4)
Right Lower Lobe	151 (87.3)
Left Upper Lobe	133 (76.9)
Left Lower Lobe	153 (88.4)

Note: Numbers in parentheses are percentages.

We also examined all patients to find any lesions unrelated to the COVID-19.

According to this examination, we found predominantly peribronchovascular view in 3 patients (1.7%), pleural effusion in 4 patients (2.3%), lymphadenopathy in 10 patients (5.8%), the tree in the bud in one patient (0.6%), and nodules in 4 patients (2.3%).

The cavity was not observed in any evaluated cases.

The results from the classification of patients based on the period between the early symptoms' observation and the CT-scan imaging showed that the number of ground-glass's opacity is significantly different within these three groups (p = 0.003), i.e., the GGO is observed much more in the early phase patient's group compared to the two other groups.

The GGO has been found in 97.4% of the early phase patient group, 87.6% of the intermediate phase patient group, and 66.7% of the late-phase patient group (Table 3, Figure 5).

The numbers of consolidation and crazy paving appearance are significantly different within these three groups (p <0.001). The number of consolidation and crazy paving appearance is increased by increasing the time elapsed from early symptom onset to CT-scan imaging.

Table 3: Findings on chest CT in patients categorized by infection time course

	Early (0-2 days)	**Intermediate (3- 5 days)**	**Late (6-12 days)**
ground-glass opacities	38 (97.4)	99 (87.6)	14 (66.7)
consolidation	3 (7.7)	60 (53.1)	14 (66.7)
lung opacities in 1 lobe	16 (41.0)	7 (6.3)	0 (0)
lung opacities in 2 lobes	8 (20.5)	6 (5.4)	0 (0)
lung opacities in 3 lobes	2 (5.1)	9 (8.0)	2 (9.5)
lung opacities in 4 lobes	7 (17.9)	16 (14.3)	0 (0)
lung opacities in 5 lobes	6 (15.4)	74 (66.1)	19 (90.5)
bilateral lung involvement	22 (56.4)	102 (90.3)	21 (100)
mean total severity score	2.59	5.87	10.95
"crazy-paving" pattern	1 (2.6)	12 (10.6)	10 (47.6)
peripheral distribution	37 (94.9)	112 (99.1)	21 (100)
pulmonary nodules	2 (5.1)	2 (1.8)	0 (0)
pleural effusion	0 (0)	3 (2.7)	1 (4.8)
lymphadenopathy	0 (0)	10 (8.8)	0 (0)

Note: Numbers in parentheses are percentages.

Figure 5: Frequency of selected chest CT findings as a function of time course from symptom onset

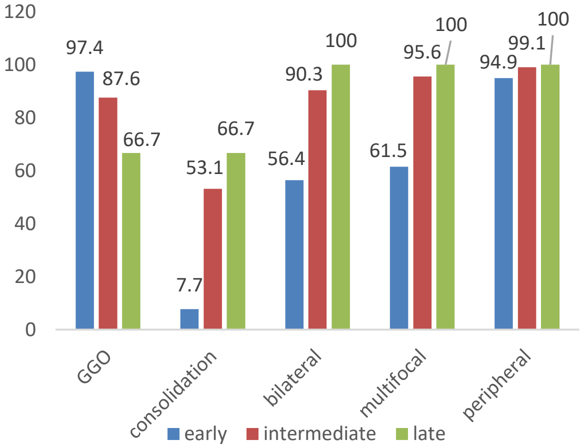

The observation within the early phase patient's group shows that consolidation and crazy paving appearance have been observed among 7.7% and 2.6% of the group, respectively. Besides, observation of the intermediate phase patient's group indicates that 53.1% and 10.6% of the patients showed consolidation and crazy paving appearance, respectively. Finally, in the late phase patient's group, 66.7% of consolidation, and 46.6% of crazy paving appearance have been observed (Table 3, Figure 5). Generally, there is a significant relevance between the time elapsed from early symptom onset to CT-scan imaging and the severity of lung involvement based on SST (p-value<0.001). Moreover, the severity of lung involvement is higher in the cases with the longest elapsed time from symptom onset to CT-scan imaging.

The level of oxygen (O_2) saturation has been measured for all patients before the CT-scan imaging. The average level of oxygen saturation was 89.01% (with a range of 67 to 97 and a standard deviation of 4.16). It is observed that there is a significant relevance between the oxygen saturation level and TSS ($p<0.001$ and correlation coefficient is -0.587); but there isn't any significant relevance between O_2 saturation and TSS within the age group of 20-40 years old.

DISCUSSIONS

COVID-19 has turned into a global challenge and a historic pandemic resulting in 2,019,088 cases worldwide with 131,886 deaths as of April 16, 2020 [6]. Although the rtRT-PCR testing of respiratory secretions or blood samples is the definitive diagnosis of COVID-19, the likelihood of false-negative cases has made chest CT-scan one of the important tests for relatively early diagnosis of COVID-19 pneumonia. Various imaging patterns have been reported in previous studies conducted on COVID-19 pneumonia, which varied in their importance.

In this study, we examined and classified the chest CT-scan imaging features of 173 patients with a definitive diagnosis of COVID-19 based on the rtRT-PCR test. The most common findings were ground-glass opacity (87.3%), vascular enlargement (61.3%), consolidation (44.5%) and crazy paving pattern (13.7 %). The majority of cases had bilateral (83.7%) and multifocal (88.4%) involvements. These findings are largely consistent with previous studies [12, 13], which indicates that the ground-glass view with or without pulmonary consolidation and in the forms of the bilateral peripheral and multifocal pattern are the diagnostic hallmarks of COVID-19 pneumonia.

Although the majority of patients had peripheral involvement, many of them had concomitant central or peribronchovascular involvement (47.7%). This distribution pattern was more common in the present study than in previous studies [13, 20], since the majority of our patients were in either the intermediate or the late-phase of the onset of the symptoms. It is worth noting that the public access to diagnostic-medical equipment, such as CT-scan, is lower in Iran than in the developed countries. Therefore, many infected individuals remained undetected in the early phases of the COVID-19 pandemic and thus had a late visit, which justifies more severe pulmonary involvement in the present study. Based on our findings, although the bilateral peripheral and multifocal involvements are the hallmarks of this disease, nevertheless, a portion of our patients had unilateral and even unifocal involvement, specifically in the early days of the onset of the symptoms. Besides, three patients and one patient have mildly and moderately pleural effusion, respectively. Previous studies highlighted that pleural effusion is detrimental to COVID-19 diagnosis. The present study confirms this finding since all of these four patients had congestive heart failure (CHF). Therefore, the authors recommend that in addition to examining CT-scan results for COVID-19, the underlying diseases of the patients should also be considered.

In total, 5.8% of the patients had mediastinal lymphadenopathy, which was higher than other studies [13, 21, 22]. It should be pointed out that lymphadenopathy can be seen in patients with heart failure, mediastinal infections, and/or cancer. In the present study, all 10 patients with lymphadenopathy had concomitant heart failure, based on their medical history and imaging results. Therefore, further studies with a more accurate design are required on a larger population for examining the underlying diseases to establish whether this type of involvement can be a marker of COVID-19.

In our study, the relationship between the imaging features and the elapsed time from early symptoms onset to CT-scan imaging shows that at the "early phase" of the disease the ground-glass has been the dominant imaging view, while by moving towards the intermediate and late phases of the disease, the intensity of the ground-glass opacity reduces, and instead the appearance of consolidation and crazy paving pattern increases. It is also observed that the consolidation view alone, without ground-glass opacities, is more common to have appeared at the late-phase of the disease. Moreover, pulmonary involvement increases with increasing the elapsed time. In total, these findings are consistent with the majority of previous studies [23, 24, 25].

Because it is very crucial to identify the O_2 saturation level at which a patient is needed to undergo a CT-scan test for measuring the severity of pulmonary involvement, we also considered the relationship between O_2 saturation level and the imaging's finding in our study. Our study showed a direct relationship between the severity of pulmonary involvement and decreased oxygen saturation, but this direct relationship was not observed among individuals aged between 20-40 years. This finding is important because it shows that younger patients with almost normal oxygen saturation can have severe pulmonary involvement. According to some studies in literature, patients with oxygen saturation < 93% should undergo imaging procedures [26, 27]. However, we argue this finding, because considering the recommend-dations provided in these works [26,27] may delay the proper treatment and makes the COVID-19 patients more vulnerable to the adverse events of the disease. Therefore, it is proposed that the oxygen saturation level alone is not an exclusive nor a decisive factor to decide on considering a chest CT-scan imaging, and yet patient's overall clinical conditions and all symptoms together should be considered more carefully to decide on offering chest CT-Scan and other diagnostic procedures.

CONCLUSIONS

This study aims to remind doctors involved in COVID-19 diagnosis and treatment around the world to start the treatments immediately and uninterruptedly for patients with chest CT-scan results similar to those highlighted in the present study and several other studies to prevent more losses. We believe that implementing these suggestive CT-scan patterns of COVID-19 patients, can accelerate the proper treatment and prevent more mortality and morbidity.

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Conflicts of Interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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ORIGINAL ARTICLE

Climate change and public health – past, present, future

Viorel Ordeanu[1,2], Lucia E. Ionescu[1]

Abstract: *The evolution of the planet's climate is multifactorial influenced and has a dynamic over time. Many scientists have approached this controversial field, and each relies on objective but divergent statistics.*

Geographers have established that planet Earth is in a period of slight slow cooling, not at the level of the four prehistoric ice ages, but only at the level of the Little Ice Age from the Middle Ages which probably caused the great migration of peoples from Asia to Europe. This slow cooling is only slowed down and may even be partially reversed by anthropogenic activities. The industry produces large amounts of carbon dioxide from burning fossil fuels and other greenhouse gases. Animals produce carbon dioxide through respiration and digestion, some also methane. The waste degradation, fires, volcanic eruptions, swamps, and thawing of permafrost release carbon dioxide, methane, and other greenhouse gases into the atmosphere.

Climate change exit and has always existed, but there is no scientific evidence for global warming or for climate risks other than those we already know. Public health is already facing danger, directly and indirectly, for multiple reasons, to which „climate change" is added.

Keywords: *global warming, climate change, public health, greenhouse effect, ecology, climate warfare*

INTRODUCTION

Towards the end of the twentieth century, the written and audiovisual media bombarded us with alarmist information about „global warming", but this did not come true. At the beginning of this century, the same media informs us about „climate change", the shortcomings it creates and the risks we will be exposed to. But as American writer and journalist Mark Twain humorously wrote, „If you don't read the newspaper, you're uninformed. If you read the newspaper, you're misinformed"

Is the climate changing?

It is known that the evolution of the climate on the planet is multifactorial influenced and has a dynamic over time, from one geological era to another, from one age to another, from one geographical region to another, from one year to another, according to the season, the circadian cycle and even from one moment to the next. Many scholars have approached this controversial field, some for, some against or simply trying to be impartial. Each relied on objective statistics, those mathematical tools about which the Germans say only half-jokingly: „Statistics is a science that can prove anything, absolutely anything...".

In our country, the geographer Prof. Elena Teodoreanu, who was also a scientific researcher in medical geography, tried to answer this question, systematizing the knowledge in the field in her book entitled „Is the climate changing? A question at the beginning of the millennium" [1]. She stated: „It is strange how a simple look at the climates of the Middle Ages shows us the existence of calamities in past centuries as well – droughts, floods, destructive frosts – and yet, nowadays, a certain state of unrest about climate anomalies, is made felt in almost all countries. It is obvious that the possibilities of information today are far superior to those of the historical past, which allows knowledge in the shortest time, of events of all kinds around the globe and especially of those that attract attention, disturbance, fear."

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Other scientists, some of our own, have asked the same question. Thus, the physicist Ştefan Hepites, who founded in 1884 the Romanian Meteorological Institute, published in 1898 the article „Has the climate changed?” in which it is well documented that it has not. Another head of this institution, the meteorologist Nicolae Topor, published in 1967 the article „Is the climate changing?” in which he demonstrates that the climate does not change and concludes that: „what we are commenting on today are aspects of the same climate, which can change only partially in millennia, and only after millions of years.”

CLIMATE WARFARE

The great catastrophes in Earth's history have not caused human casualties, because humans had not yet appeared on the planet. But the small local catastrophes that ensued left millions or even billions of human casualties in total: dead, wounded, sick, and refugees. In addition, man has created a series of anthropic or even artificial catastrophes: wars, revolutions, attacks, collective or mass accidents, changes in the environment, and much more...

At the time, Walter Orr Roberts, president of the American Association for the Advancement of Science, said: „We aim to put the entire globe under constant observation of the weather until 1970 with reasonable expenses. We assume that we will be able to obtain incomparably better forecasts on storms, frosts, droughts, and smog and we will be able to avoid disasters. But behind these advantages that science brings us lies the danger of a potentially terrible weapon of war - the deliberate manipulation of the weather in favor of a small number of people in power, to the detriment of the enemy and perhaps those who stand aside.” [1]. This conclusion does nothing but reminds us of the threats made by the heads of the Great Powers, Stalin and Bush jr., summarized in the expression: „He who is not with us is against us.”

In 2004, the French writer Jouzel Debroise published the book „Climate – a dangerous game”. The following year, the „Final Report, Air University of the US Air Force, AF 2025”, is published, stating: „Climate change will become part of domestic and international security and can be done unilaterally. The ability to generate rain, fog, hurricanes and to change or produce certain weather conditions is part of the same set of technologies that can weaken the opponent and allow access to domination, wealth and power.” [2]

It is estimated that the enemy's combat actions, logistics, and transport may be hampered, food crises may be created to starvation, the outbreak and spread of natural or biological-induced epidemics may be favored, and medical countermeasures may no longer act effectively. It seems that the United States and Russia already have the capacity for climate warfare, and politicians are interfering in climate research. In 2009, General Emil Străinu published the book „Geophysical Warfare. Techniques for modifying the environment for military purposes” [3] which draws attention to the risk following attempts to change the weather and presents some situations of alleged climate attack on some countries, including Romania.

Geophysical warfare, which also includes climate change or climate catastrophes, would also be a major challenge for military medicine and public health in general. New problems of pathology and epidemiology, diagnosis, treatment and prophylaxis, health logistics would appear, specialized forces and means would be needed, and in their absence, the situation could quickly degenerate into a biological crisis.

THE PHENOMENON OF „GLOBAL WARMING”

Although the natural change of the climate is imperceptible and the anthropic change is very discreet, the observation of seemingly frequent catastrophic consequences has launched the hypothesis of global warming, which should be stopped. It seems that initially, the fight against „global warming” was an attempt initiated by the US to slow down the boom of the Chinese R.P. economy, whose energy was based on coal. The Chinese have large deposits of coal, but no oil and gas, which they are currently looking for in the South China Sea. However, in the meantime, China has become the world's second-largest economic power, and the expansion of the fight against carbon dioxide (greenhouse gas) produced by burning fossil fuels (including oil and gas) is also hitting the US economy, largest producer and consumer of energy. As a result, Donald Trump, as US president, no longer supported scientific research on „global warming” and the United States withdrew from international organizations that oppose this controversial phenomenon. But at the same time, NASA publishes alarming studies showing that global warming tends to become irreversible, with serious consequences, including Arctic ice melting, rising sea levels, and flooding areas, with the cities of London, New York, Sydney, etc. [4]. The areas populated by „natives” did not get the attention of these world science leaders.

If we think about the planet's distant past, the atmosphere was much different: more oxygen, much more carbon dioxide than now, excess water vapor and heat, all in one place favoring the giant Carboniferous plants and the giant Jurassic animals. But even today the richest fauna and microfauna, as well as the richest flora and microflora are found in the equatorial jungles. So we can think that the danger is not so much for the thriving biosphere as for the business world that will have to adapt to the new conditions.

Due to the lack of scientific evidence on global warming (it can neither be clearly shown that it exists nor that it does not) another paradigm has been used: climate change. For now, specialists in the field are reluctant and do not express firm opinions, whatever is possible, in the near or distant future.

WHAT IS REALLY HAPPENING?

Geographers have established that planet Earth is in a period of slight slow cooling, not at the level of the four prehistoric ice ages, but only at the level of the Little Ice Age from the Middle Ages (when, in one winter, the Nile froze) which probably caused the great migration of peoples from Asia to Europe. This slow cooling is only slowed down and may even be partially reversed by anthropogenic activities. The industry produces large amounts of carbon dioxide from burning fossil fuels, as well as other greenhouse gases. Animals produce carbon dioxide through respiration and digestion, some also methane. The waste degradation, fires, volcanic eruptions, swamps, and thawing of permafrost release carbon dioxide, methane, and other greenhouse gases into the atmosphere. All this contributes to slight heating, especially in the most industrialized or zoo-intensive areas.

Meteorologists' measurements are very accurate, their thermometers measure air and water temperatures by tenths of a degree Celsius, and the records and reports are very rigorous, at least in recent centuries. But the place of measurement, the location of the weather station, remained in place although the anthropic area (housing and industry) is constantly expanding and warming the environment. As an example, the first Romanian weather station was in Bucharest, on Filaret Hill, where the Astronomical Observatory was located. As the area became more and more populated, and the station was now in the city, a second weather station was set up, in Băneasa, outside the city, in the North-West direction, where the predominant wind comes from. So the weather in the city could be compared with the weather outside the city. In the beginning, the recorded temperature differences were about 2°C, but now they are on average about approx. 1°C, due to the expansion of urbanization in this area as well. Almost all weather stations are currently included in urban areas, so they report very accurately measured temperatures, but with systematic error in addition to the respective geographical area, an involuntary falsehood.

For some, the mild winters of recent years, through early February, would be evidence of „global warming”. But I could give some examples from personal memories: after the winter of 1971-1972, when the Black Sea froze on the shore and seaports including Mangalia were blocked (river ports are the ones blocked every year), the old sailors said they had never seen anything like it since they were young. But in the winter of 1973-1974, it was spring-like weather; on February 11th (my birthday) I went for a walk along the seafront wearing just a suit. And ten years ago, when my niece was born, on February 9th, 2009, I went to the maternity ward in a suit, in spring-like weather. But that hasn't stopped the Black Sea from freezing again in 2011 as well as in other, more recent years. All this means that the climate is neither heating nor cooling, but that it is only a random variation between certain limits, a statistical dynamic with a sawtooth pattern. And the slightly ascending „regression line” could just be the result of the systematic error I pointed out before, or it could be the „sawtooth” pattern of statistics applied to another time scale.

ASSUMPTIONS AND EVIDENCE

Many see as objective evidence the partial melting of mountain glaciers and polar caps, especially in the Arctic, and the rising of the worldwide ocean levels. Anyways, every summer the polar caps fragment on the edges and partially melt, and during the polar winter they grow back. The phenomenon is so repeatable that nautical charts mark the minimum and maximum limits of the polar ice caps. If the alarm is real, major potential hazards arise from this. If the mountain glaciers would melt completely (the most important being in the Himalayas), the rivers and streams in Asia will no longer be supplied with water and there will be a shortage of freshwater, which will affect billions of people. This phenomenon could lead to water wars involving billions of people.

If the polar caps melt in time, the level of the Planetary Ocean will increase by approx. 10 m due to the Arctic and approx. 80 m because of Antarctica. [5]. It is believed that if all the solidified water on the planet melted, the sea level could reach almost +100 m isobaths, and the land area would be reduced by about a third. The land surface will be reduced from almost 30% to less than 20% of the globe's surface. But these major changes, which would take thousands of years, are hypothetical, just some apocalyptic scenarios imagined by some journalists (who want the public's attention) and some politicians (who want to show that their country could save the planet) or by others as such, which are not seriously taken by scientists.

For now, we see that the partial melting of the Arctic polar cap allows ships from the far North to move, during the boreal summer, on the Arctic Ocean (Northern Frozen Ocean), both through the East Passage, between the Atlantic to the West and the Pacific to the East north of Europe and Asia, as well as through the Western Passage, between the Pacific to the West and the Atlantic to the East (North of Canada). These new sea routes shorten freight routes and favor the great maritime powers (USA, Russia, China, etc.). Unfortunately, Romania, which was the 11th maritime power in the world Knowledge Horizons – Economics, no longer has many ships, so it will not be able to benefit from this opportunity [6].

The thawing in Northern Canada, Greenland, and Siberia also facilitates mining, oil, gas, etc., thus favoring economic development. But this is where the serious problem arises. The thawing of the Siberian permafrost (swamps frozen for thousands of years or still frozen since the last glaciation) allows the slow emanation of methane in the soil (natural biogas) and it has a greenhouse effect [7]. The complex industrial pollution in the area of the North Polar Circle, in continuous development, reduces the snow's albedo, which instead of reflecting most of the solar radiation, absorbs it and melts rapidly.

At present, this phenomenon may be intentionally potentiated by humans. The US military's High-Frequency Active Auroral Research Program (HAARP) aims to bombard the ionosphere with strong radio waves, heat it, and reflect it to the ground, causing heat and devastating phenomena. If this biophysical weapon becomes operational, it may also be used for peaceful purposes, for the benefit of the Arctic industry but aggravating the phenomenon of melting ice. It may not be a coincidence that specific US facilities are located in Alaska, Greenland, and Norway, and the Russian ones in Siberia [8, 9].

At the same time, it is observed that on the contrary, the Antarctic cap is not reduced there is an increase in area and thickness. And the Great Powers are already disputing the division of Antarctica to begin mining for minerals under the ice cap. Political, economic, financial, and military forces, state and supranational, as well as large multinationals, are repositioning themselves for the new negotiations on the Law of the Sea, the polar caps, and the outer space. The multipolar world is complex, unpredictable, and dangerous, especially for the little ones. As the poet Gr. Alexandrescu said: „We want equality, but not for puppies".

PUBLIC HEALTH CONSEQUENCES

Overall, the prosperity of a nation and its bio-psycho-social health are climate-dependent. The World Health Organization has defined the concept of human health as „physical, mental, intellectual and social well-being", or all these are interdependent and multifactorial conditioned. Holistically speaking, everything around us and everything that happens to us is reflected, directly or indirectly, in public health, in the broadest sense [10].

It is considered that it would be possible that if the average temperature increased by 4°C, the level of the Planetary Ocean would increase by 9 m. The areas with the largest population and the most developed economy would be flooded; all beaches, all low plains, all ocean and sea ports, most islands, and even entire countries such as the Netherlands, Bangladesh, and others would disappear. This hypothetical global catastrophe would lead to a population exodus of billions of people, with poverty, epidemics, and wars [11]. In our country, too, major changes would occur: the Romanian coast, as we know it, would disappear and return to its former form, the Danube Delta will once again be a bay of the Black Sea, and the Danube would flow into the sea through a long estuary from Calarasi to Galati, and northern Dobrogea could be an island or a peninsula. In this unlikely situation, the land area of Romania would be reduced to only approx. 200,000 km2 but the water area would increase [12].

If this global catastrophe were to occur in the coming centuries, the consequences for public health would be particularly serious for all states and all nations. It is already believed that climate change, with warming in some regions, such as Europe, favors the emergence of exotic and tropical diseases, which although at the beginning, only „import diseases", will spread and could become endemic diseases or epidemics in the temperate zone, so in populations that do not have natural protection against new pathogens. This creates a pandemic potential for exotic and tropical infectious diseases. Examples can be found permanently in the epidemiological communiqués of the World Health Organization (WHO), Center for Diseases Control, USA (CDC), European Center for Disease Control (ECDC), Ministries of Health (MoH), etc. and we will not insist on them. We know and sometimes we even face these diseases. But we will have to reconsider the entire public health monitoring system to deal, at least conceptually, with any present or future situation [13].

It should also be mentioned that if our Roman ancestors were afraid of the heat (heat wave – dog weather), we Romanians are afraid of the cold (dog weather), and jokers say that our Dacian ancestors, "the bravest of the Thracians", were not afraid of anything, only of draught and that's why they walked around wearing little hats covering their ears both in winter and during summer. Anyway, the

Romanian expressions “to stay warm”, “tucked up nice and warm”, and “someplace warm” express the preferences of Romanians, unlike those of Americans for whom being “cool” is a good thing. Let's not forget that the Romanian greeting formula “salut” comes from the Latin greeting of health (salus), which, as the Romanian people say, "there is nothing more important than good health". And the Romanian rulers used to welcome their equals and subjects with the greeting: "May all those present be healthy!" a wish that we also address to our readers. The international importance of climatic change for public health shows over 35 000 scientific articles indexed on the Web of Science [14].

A very special issue in this context, and which can become a global threat to humanity, is posed by biological agents of war and those of bioterrorism. Biological warfare was formally banned, meaning „with proper documents”. But this is only a formality. The Convention on the Prohibition of Biological Weapons, launched by the United Nations (UN) in Geneva (BTWC 1972) follows other conventions that prohibited, implicitly or explicitly, the use of biological weapons. But there are still no real control mechanisms and biological agents have been used repeatedly for biological attacks. The convention was accepted by approx. 90% of the countries of the world (including all the states of the European Union), which seems to be a very good situation. But the remaining 10%, who have not signed or ratified the Convention, represent about 20 states with about a quarter of the world's population [15].

Moreover, the Great Powers do not allow international control over their territory or military bases. On the contrary, Iraq, which had biological weapons but also destroyed them, was accused of possessing biological weapons, was attacked and invaded, almost destroyed, but no biological weapons were confirmed. The foreign invasion troops (peacemaking) were replaced by occupation troops (peacekeeping) and did not leave completely even after 17 years. Currently, Iraq is considered by the international community as one of the "failed states" but no guilt has been established so far and the "international community" and "public opinion" have not been effectively involved in resolving this critical situation.

Biological warfare agents (BWA) are living microorganisms (bacteria and viruses) and toxins obtained from living organisms, which are disseminated with the help of biological weapons: aircraft or spray aerosolization, explosive munitions such as missiles, air bombs, and artillery shells, or diversionary agents. In a broad sense, the disease itself, usually infectious-contagious and/or zooanthroponotic caused by biological agents is considered a biological weapon. Officially, 7 bacterial species, 3 groups of viruses, and 5 toxins are listed. This list is exhaustive due to the characteristics required for effective military use. In contrast, bioterrorists can also use any other pathogens for humans, animals, and/or plants (bacteria, viruses, prions, fungi, parasites, etc.) to make people sick, reduce agricultural or animal production, to create food crises. It should be noted that biological warfare viruses are not listed as species, but only as groups, which means that in hemorrhagic fevers, for example, Ebola, Marburg, and many more are implicitly included. This will technically (not legally) allow the use of exotic and tropical viruses in areas affected by global warming and explain the medical-military interest of some states for medical intervention in these epidemics [16].

THE NEW EUROPEAN UNION POLICY FOR ENVIRONMENTAL PROTECTION

The current President of the European Commission (EC), a kind of Prime Minister of the Government of the European Union (EU), and the former German Minister of Defense (Bundeswehr), Ursula von der Leyen, is a doctor by profession. In 2019, at the moment of her election, she published the document “A Union that strives for more. My agenda for Europe” in which she stated that “for the generation of my children, Europe is the only aspiration” [17]. Of the six priority goals she has proposed for the next 5 years, the first is A European Green Deal (an ecological Europe) and she said “I want Europe to strive for more by being the first climate-neutral continent”, partly by 2030 and partly by 2050. This requires investment in innovation and research, redesigning the economy, updating industrial policy, and a new industrial strategy. Some complied and immediately found a "minimum method" of application, without investment: they take some extra money from drivers and other small polluters, and that's about all for now [18].

But the new ecological conception was already rooted in the world for many years. We pay more for electricity because we also use more expensive, unprofitable, but environmentally friendly technologies. Electric cars are in vogue, since 1905 exceeded the speed of 100 km/h, but they were not imposed for technical and cost reasons, but there are fears that they will become mandatory. For the emotional awareness of the public opinion, at the end of 2019, an ecological skit was broadcast, in which the filmmakers did not shy away from using an autistic teenager. Was that politically correct? And maybe some were wondering, „What's next?” This was followed on February 1st, 2020 by the World Economic Forum (WEF) in Davos (Switzerland), with the main theme of the year:

„Stakeholders for a Cohesive and Sustainable World” (united shareholders for a sustainable and cohesive world), under the slogan „How to Save the Planet”. 3,000 delegates from the largest private companies participated. Work has been conducted to prepare for the massive reorientation of global capital flows, through which financial giants, including the World Bank and the European Central Bank, are repositioning themselves to maximize their profits. As an example, only the promoter of this initiative, the BlackRock investment fund, has to invest over 7 trillion dollars (i.e. thousands of billions), more than the annual budget of most countries. The Davos Forum has been strongly involved in the Climate Change Agenda. Although it is acknowledged that it is impossible to answer the question: „when will climate risk have an impact on the planet's economy?”. It is considered that climate risk is also a risk for investments and that there will be a massive reallocation of capital through the Green Finance Initiative. Thousands of billions of dollars of investment funds will be channeled to „green” projects, so funding is not for everything and especially not for everyone. But in the meantime, the outbreak of the Covid-19 pandemic partially canceled the old plans, replacing them with the Great Reset of the world economy for the benefit of the Great Powers, the world's great finances, and multinationals. The US has an ambivalent position [19].

It seems that the „climate change” paradigm will be imposed to become the „new religion” of the 21st century, with a God whom, although we do not perceive, His priests are threatening us in His name. There is a risk that money, not work, will become the engine of progress and well-being, including the source of public health. But the investment will be directed at „rule of law” states that stand upright in the face of great global finance. However, we have the advantage that we are members of the European Union, we shall rise with it and, God forbid, we will fall with it [20].

In 2019 the „international community” says that the present situation is a „climate emergency” and in 2020 Roumanian Academy publish a „point of view” about climate change [21].

CONCLUSIONS

Climate change exists and has always existed, but there is no scientific evidence for global warming or for climate risks other than those we already know. But we find them better and faster, and the damage is greater because the population is larger and the human environment is more extensive, including in risk areas. Public health is already facing danger, directly and indirectly, for multiple reasons, to which "climate change" is added. It seems that the problem is mainly political, with economic and financial implications, and will be used as financial leverage at the global level, for global interests. For now, let's remain optimistic, but also prepared and vigilant.

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CLINICAL PRACTICE

Clinical and dermatoscopical aspects of pigmented basal cell carcinoma – Case series and literature review

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Abstract: *The diagnosis of pigmented basal cell carcinoma can bring significant distress to patients and physicians alike. However, astute clinicians that can employ correctly the clinical and dermatoscopical clues can reach a correct preoperative diagnosis, and as such can avoid unnecessary worry for the patient, additional work-up, or overtreatment. We present a case series of pigmented basal cell carcinomas and a comparative case with pigmented cutaneous melanoma, together with a literature review, that will highlight these aspects of the management of pigmented tumours of the skin.*

Keywords: *dermoscopy, basal cell carcinoma*

INTRODUCTION

Pigmented tumours of the skin bring significant distress to patients and to physicians alike. While pigmented basal cell carcinoma is not a benign tumour, its prognosis is highly favourable if it is diagnosed and treated early. The dermatologist is also in the position to tell the patient that even if the tumour to be excised is a malignant tumour, the treatment, i.e the excision of the tumour, will result in the complete healing of the lesion, with a low probability of local recurrence.

We present a case series of pigmented basal cell carcinomas and a review of the literature in an effort to show that this particular pigmented tumour of the skin can be easily identified by the experienced dermatologist with the aid of clinical and dermatoscopical clues.

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REVIEW OF LITERATURE

Background

One of the oldest references [1] to pigmented basal cell carcinoma (pBCC) in PubMed involves a differential diagnosis with cutaneous melanoma. Indeed, the spectrum of pigmented cutaneous melanoma (PCM) can plague the management of a pigmented lesion of the skin leading to overtreatment, if a wide excision of the supposed PCM is intended, and/or to unnecessary worry of the patient and extended additional work-up, e.g. lymph-node ultra-sonography, lung radioscopy etc.

Goldenberg et al [15] describe the age-adjusted incidence of BCC in American population to be 226 per 100000 persons and the age-adjusted prevalence of BCC to be 342 per 100000 persons.

Maloney et al [13] and Betti et al [14] describe the histopathology epidemiology of pBCC to be of around 6.7% and 8.5% respectively of the total cases of BCC taken into consideration.

Altamura et al [17] found that actually the dermatoscopically non-pigmented BCCs can be as low as 15.1%, and the rest of the BCCs have some degree of pigmentation, from low to heavy. They [17] have not used the diagnosis of pBCC according to the histopathology, but according to the dematoscopical features pigmentation, albeit all lesions were confirmed with histopathology to be BCC.

Both studies, of Maloney et al [13] and Betti et al [14] found that pBCC has a better prognosis with clear excision margins than the other types of BCC. A similar conclusion is reached also by Ito T et al [6] by saying that narrow-margins excision of 3mm may be enough to obtain clear margins for pBCC, as opposed to 4mm margins required for classic non-pigmented BCC.

Clinical aspect

The clinical aspect of BCC should not be disregarded as we consider that an astute clinician can observe significant clinical clues that can lead him to an appropriate preoperative diagnosis.

Bolognia et al [11] describe the clinical aspect of a nodular BCC as “shiny, pearly papule with a smooth surface and the presence of arborizing telangiectasias”. They [11] also describe a “thin rolled border” in superficial BCC.

Wu et al [12] describe the clinical aspect of BCC to have a “pearly or translucent quality” with telangiectasias, the lesions having a “periphery more raised than the middle” – a rolled border. In addition, they [12] say that “a shiny quality” can be observed when a superficial BCC is illuminated.

Huang et al [10] used translucency as a clinical indicator to learn computer aided systems to detect BCC and achieved a 93% accuracy.

Dermatoscopy

Dermatoscopy, if correctly employed, can be an invaluable tool in diagnosing BCC and especially pBCC.

A very thorough article on the dermatoscopy of pBCC is the one of Menzies et al [5]. They established that there are several highly specific dermatoscopical patterns, e.g. “maple leaflike areas” and “spoke wheel areas” that have a specificity of 100%, albeit a low sensitivity of 17% and respectively 10% [5, 16]. Other dermatoscopical criteria with high specificity were “large grey-blue ovoid nests”, “multiple grey-blue globules” and “arborizing (treelike) telangiectasia” [5 , 16].

Marghoob et al [7] also describe several specific dermatoscopy aspects for pBCC: large blue-grey ovoid nests, multiple blue-grey globules, leaf-like structures, spoke-wheel-like structures, arborizing telangiectasias and ulceration.

Kittler et al [8] describe the dermatoscopic patterns of pBCC as “radial lines, dots, clods and structureless zones” and they say that a common pattern is blue clods of different shapes and sizes. In addition, other structures may appear, such as brown clods, grey, blue and brown dots, white structureless zones and radial lines. They [8] also describe patterns that are very strong indicators against BCC, e.g. non-radial lines (reticular, branched, curved and parallel), pseudopods and circles. Also, the description of the typical vessels found in BCC would be: “branched serpentine vessels that originate from thick stem” – usually found in nodular BCC, while in superficial BCC a polymorphous pattern of vessel can appear, e.g. thin, serpentine and coiled vessels. The pattern of dot vessels is a strong indicator against BCC [8], unless found within ulceration.

Cabo et al [9] considers the dermatoscopical pigmented structures, e.g. maple leaflike areas, spoke-wheel area, multiple blue-grey dots or small globules, blue-grey ovoid nests to be characteristic for pBCC, while “white shiny blotches and strands” are an additional criterion only seen with polarized dermatoscopy.

Felder et al [18] and Turkmen et al [19] describe a dermatoscopical blue-white variant of pBCC. This type of pBCC is included in the class of heavily pigmented BCC as described by Altamura et al [17]

Lallas et al [21] describe dermatoscopic features to differentiate between superficial BCC (e.g. maple-leaf like areas and short, fine telangiectasias) and nodular BCC (e.g. blue-grey ovoid nests, arborizing vessels, ulceration). Wozniak - Rito et al [20] agree with these findings.

Additional work-up

Usually additional work-up is not necessary for the diagnosis of pBCC. However, cytology i.e fine needle biopsy can be used [3] in certain cases that the clinical and dermoscopical aspects are not enough to establish a certain diagnosis. Usually this happens when the lesions are pure nodular, without a superficial part.

CASE SERIES

Case 1

A 59 years old female patient presented to our dermatology department for a 2/2 cm, ulcerated, pigmented lesion in the left axilla. The lesion had a history of about 8 years. The patient also had a personal history with another BCC lesion operated 10 years prior to this admission.

The clinical aspect of the lesion (Figure 1):
- central ulceration with serohematic crust on the surface,
- the non-ulcerated part of the lesion has a translucent greyish feature with superimposed dark-brown, blue, black punctate pigmentation
- the periphery of the lesion has a pearly pigmented aspect
- the lesion has a smooth-shiny surface that does not continue the adjacent normal skin architecture

Figure 1: The clinical aspect of Case 1 has a very specific pearly-pigmented aspect of the periphery of the lesion along with a translucent quality

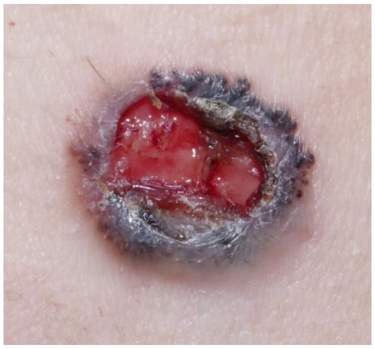

The dermatoscopical aspect of the lesion Figure 2:
- brown and blue-black-grey ovoid nests
- leaf-like areas (high specificity for pBCC)
- fine telangiectasias
- ulceration

Figure 2: The dermatoscopical aspect of Case 1 has leaf-like areas and brown and blue-black-grey ovoid nests

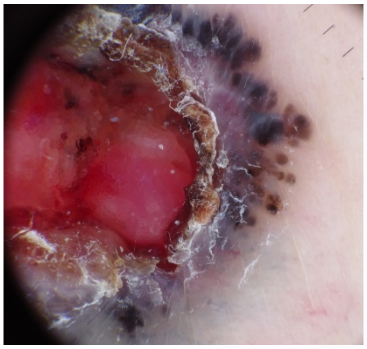

The lesion was excised with 4 mm margins and the histopathological examination established the diagnosis of pBCC, (Figure 3) with islands of basaloid cells with peripheral palisading, clefts between the islands and the stroma, and focal deposits of melanin within the basaloid islands.

Case 2

A 63 years old male patient presented to our dermatology department for a 1.5/2cm pigmented lesion on the posterior thorax. The lesion had a history of about 1 year. The patient had no significant medical history.

Figure 3: The histopathology of Case 1 established the diagnosis of pigmented basal cell carcinoma

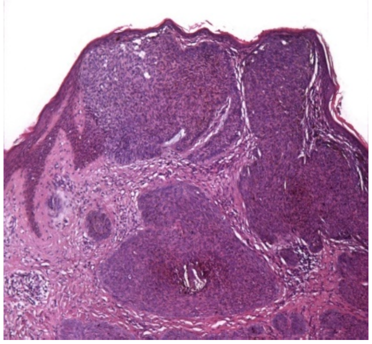

The clinical aspect of the lesion (Figure 4):
- small central ulceration
- translucent feature of almost the entire lesion with pigmentation – large pearly structures pigmented bluish-black
- shiny smooth surface that does not continue the architecture of the adjacent normal skin

Figure 4: The clinical aspect of Case 2 has an overall pigmented pearly and translucent feature with shiny smooth surface

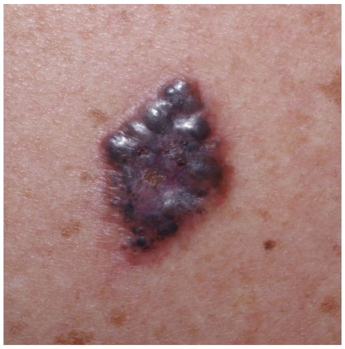

The dermatoscopical aspect of the lesion (Figure 5):
- blue-grey ovoid nests and globules
- arborizing telangiectasias
- the polarized dermatoscopy image showed white lines and blotches

The lesion was excised with 4mm margins and the histopathological diagnosis confirmed it was a pBCC.

Case 3

An 82 years old female patient, with no significant medical history, presented to our dermatology department for a 0.7/0.7 cm pigmented lesion on the left thigh. The lesion had a history of about 6 months.

Figure 5: The dermatoscopical aspect (polarized) of Case 2 has blue-grey ovoid nests, arborizing telangiectasias and white lines and blotches

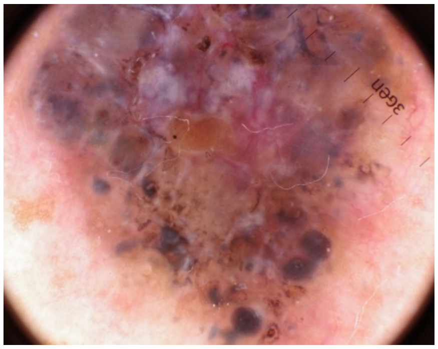

The clinical aspect of the lesion (Figure 6:

- central ulceration with serohematic crust on the surface
- translucent aspect the lesion with a general violaceous – bluish aspect and some brown pigmentation on the periphery of the lesion
- shiny smooth surface that disrupts the normal adjacent skin architecture (Figure 7)

Figure 6: The clinical aspect of Case 3 has a translucent violaceous-bluish aspect and a shiny smooth surface

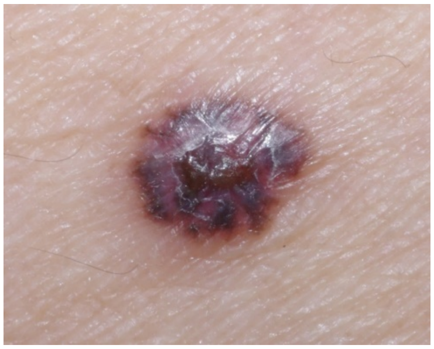

Figure 7: The particular aspect of shiny smooth surface that disrupts the normal adjacent skin architecture

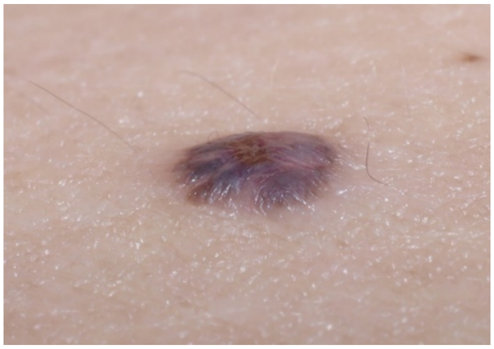

The dermatoscopical aspect of the lesion (Figure 8):

- blue-grey ovoid nests
- arborizing telangiectasias
- leaf-like structures (within the brownish periphery pigmentation)
- ulceration
- white lines and blotches (observed with polarized dermatoscopy)

Figure 8: The dermatoscopical aspect (polarized) of Case 3 has leaf-like areas and blue-black ovoid nests, arborizing telangiectasias and white lines and blotches

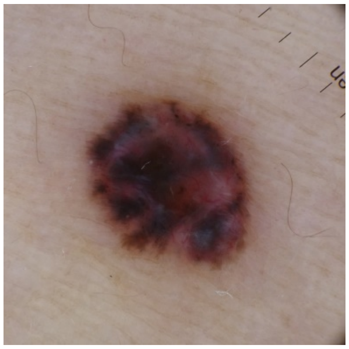

The histopathology of this lesion (Figure 9) confirmed the clinical and dermatoscopical diagnosis of pBCC, observing islands of basaloid cells with peripheral palisading, clefts between the islands and the stroma as well as deposits of melanin within the basaloid islands.

Figure 9: The histopathology of Case 3 observed islands of basaloid cells with peripheral palisading, clefts between the islands and the stroma, deposits of melanin within the basaloid islands reaching the diagnosis of pigmented basal cell carcinoma

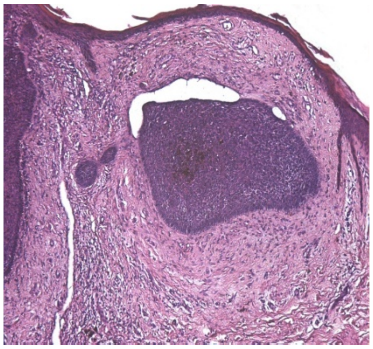

Case 4 – Comparative case

As our fourth case we present a lesion that highly resembles the clinical appearance of the Case 3 lesion (Figure 6), however the dermatoscopical aspect of this lesion has a rather conspicuous lack of clues for any specific diagnosis.

A 66 years old male patient, with no significant medical history, presented to our dermatology department for a 0.7/1.4 cm pigmented lesion on the abdomen. The lesion had a history of about 6 months.

The clinical aspect of the lesion (Figure 10):
- ulceration with serohematic crust
- pigmentation – pink – red – violaceous – black
- textured surface – tends to keep the architecture of adjacent normal skin, even if somewhat disorganized (the surface is not smooth - shiny)
- ovoid/rather rectangular shape

Figure 10: The clinical aspect of Case 4 observed rather similar type of pigmentation with Case 3, i.e. pink – red – violaceous – black, however with a textured surface (as opposed to Case 3, here the surface is not shiny)

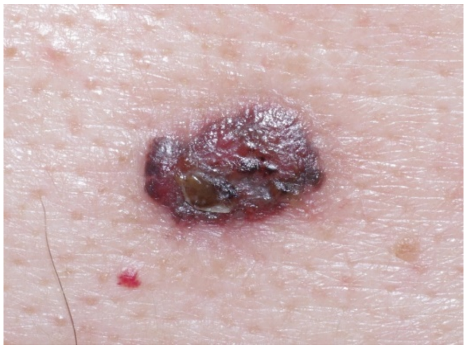

The dermatoscopical aspect of the lesion (Figure 11):
- ulceration
- pink – red – violaceous colour
- white lines and blotches on the periphery of the lesion (polarized dermatoscopy)
- lack of specific clues for pBCC (blue –grey ovoid nests, leaf-like structures, arborizing telangiectasias)

Figue 11: The dermatoscopical aspect of Case 4 has pink – red violaceous colour with lack of specific clues for pBCC

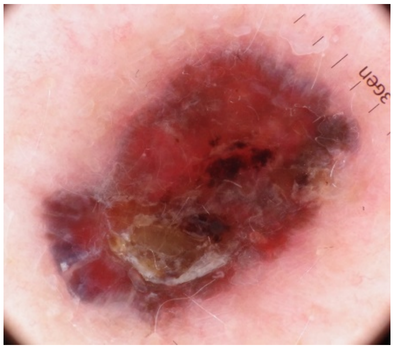

The histopathology of this lesion (Figure 12) reached the diagnosis of pigmented cutaneous melanoma (PCM), with a Breslow thickness of 0.8 mm.

As a discussion of Case 3 vs Case 4, Figure 13 presents a comparative view of both the clinical and dermatoscopical aspects of both lesions. On the clinical part, both lesions are ulcerated and have somewhat the same type of pigmentation.

Figure 12: The histopathology of Case 4 established the diagnosis of pigmented cutaneous melanoma, Breslow thickness of 0.8 mm, Clark IV

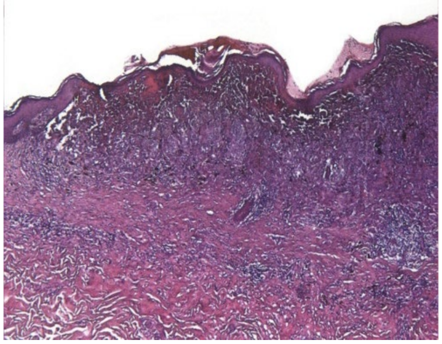

However the clinical clue of smooth – shiny surface that disrupts the normal adjacent skin architecture can be found in Case 3 and not in Case 4 where we find a textured surface of the lesion that tends to keep the normal adjacent skin architecture, even if somewhat disorganized. On the dermatoscopical part, both lesions present ulceration and similar type of pigmentation. However, while the Case 3 dermatoscopy image has conspicuous clues for pBCC, Case 4 dermatoscopy has a discomforting lack of clues for pBCC or for other pigmented lesion of the skin. Even though the polarized dermatoscopy clue of white lines and blotches are present in both Case 3 and Case 4, this comparative case shows that this particular clue cannot be used to differentiate between pBCC and PCM.

Figure 13: Comparative view of clinical and dermatoscopical aspects of Case 3 and Case 4

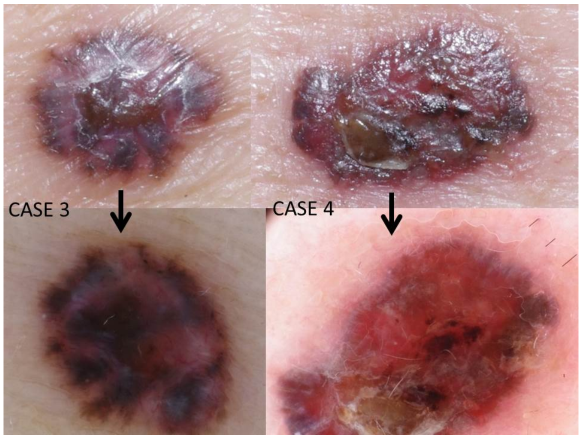

DISCUSSIONS

Regarding the epidemiology of pBCC we think that if the clinical and dermatoscopical aspect of pigmented structures within a BCC lesion are taken into consideration when establishing the pBCC diagnosis, the percentage may be significantly higher than what the studies of Maloney et al [13] and Betti et al [14] are suggesting, 6.7% to 8.5% . This may be because even in clinically non-pigmented BCC, the dermoscopy may reveal some pigmentation [17, 22], e.g. leaf-like structures, blue-grey ovoid nests or globules, and these structures may not appear in a given histopathology slide. To prove our point, Altamura et al [17] found that some degree of dermatoscopical pigmentation could be found in almost 85% of the BCC lesions. Therefore, the quantitative feature of the pigmentation may have an important role to play in reaching a histopathological diagnosis of pBCC.

To our knowledge, the clinical clue of disruption of the adjacent normal skin architecture within the lesion of a superficial pBCC has not been clearly described so far. There are papers that describe the “shiny quality” [12] or “shiny… smooth surface” [11] in superficial BCC and we think it refers to the same clinical quality. We consider this clinical clue important because superficial spreading cutaneous melanoma tends to conserve adjacent normal skin architecture within the lesion, as opposed to pBCC.

The clinical clue of pigmentation, even though is the most worrisome in the differential diagnosis of pBCC with PCM, should be observed within the overall aspect of the lesion, e.g. pearly translucent feature, rolled border.

If the clinical aspect of a suspected pBCC lesion is not enough to reach a correct diagnosis, usually dermatoscopy comes with supplementary clues. Moreover, some clues are highly specific for pBCC, e.g. leaf-like structures and spoke-wheel like structures [5] [7] [9]. Other clues that are indicative for pBCC are blue-grey ovoid nests, globules and dots, arborizing telangiectasias [5] [7] [9] (brown clods, grey, blue and brown dots, branched serpentine vessels that originate from thick stem [8]).

Even though the clinical and dermoscopical aspects of the pBCC are usually enough to diagnose a pBCC, there are papers [2 -4] that do not use dermoscopy as an aid.

On the other hand, papers focusing on dermatoscopy tend to overlook the clinical aspect of the lesion. As such, Turkmen et al [19], even though they present the clinical aspect of the lesions, the pictures are small and cannot be evaluated properly. In their article [19] the Case 3 and Case 6 seem to have a rolled border that may be a strong indicator for BCC, considering the fact that the dermatoscopical aspect, with blue-white veil cannot differentiate between pBCC and pigmented cutaneous melanoma (PCM).

While we consider the dermatoscopical aspect of pBCC of paramount importance, considering the highly specific clues that can simply establish the diagnosis of pBCC, we also consider the clinical clues very important, especially when the dermatoscopical clues are lacking, as we showed in our case series.

Considering the data regarding the clearing of margins in the pBCC management [6] [13] [14] we think that a careful evaluation, clinical and dermatoscopical, of a pigmented lesion can avoid unnecessary worry for the patient, additional work-up and overtreatment.

CONCLUSION

We consider that the clinical and dermatoscopical aspects, as well as the histopathological diagnosis should have a synergy within the management of pBCC.

Therefore, the clinical aspect of pearly border, translucency, and shiny surface – disruption of the adjacent normal skin architecture should be combined with the dermatoscopical aspect of “leaflike areas”, “spokewheel areas”, blue-grey ovoid nests and globules as well as arborizing telangiectasias and the diagnosis of pBCC can be reached in a swift manner. By doing so one can avoid unnecessary patient worry and additional work-up as well as one can adapt the treatment according to the diagnosis. Nevertheless, a biopsy should always confirm the clinical/dermatoscopical diagnosis.

Author Contributions:
The individual contributions of the authors is as follow: conceptualization, A.M.T., M.A.B.; methodology, A.M.T., B.D.; validation, G.S.T, V.T; formal analysis, A.M.T.; writing—original draft preparation, A.M.T.; writing—review and editing, D.O.C., R.S.C.; supervision, G.S.T.

Conflicts of Interest:
The authors declare no conflict of interest.

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VARIA

Clinical and therapeutic particularities of acute gallstones disease during COVID-19 pandemic

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Abstract: *Gallstones disease (GD) is a common pathology, with an incidence of approx. 20% in developed countries, a third of these cases being symptomatic. The paper aims to identify the diagnostic and therapeutic features of acute manifestations of gallstones during the COVID-19 pandemic.*

Materials and method: A retrospective, comparative study was performed of the cases presented to the emergency room (ER) and hospitalized for acute manifestations of gallstones in April-June 2020 vs similar period of 2019. The data regarding clinical form, associated pathology, therapeutic management were analyzed from the ER presentation register, hospitalization sheets and surgical protocols.

Results: During the COVID-19 pandemic, the number of hospitalized cases for acute cholecystitis and other acute manifestations of GD was significantly lower than in the same period last year (21 vs 49 patients). In both study groups the main form of presentation was acute catarrhal cholecystitis (52.4% vs 63.2%). During the SARS-Cov-2 pandemic, we report an increased incidence of complicated forms, mainly hydrops (23.8% vs. 4.1%, $p < 0.05$) and angiocholitis (19% vs. 4.1%, $p < 0.05$). Conservative treatment was chosen more frequently during the pandemic (48% vs. 29%). For operated patients, laparoscopic cholecystectomy was the main option, performed in 85.7% in both groups.

Conclusions: We maintain that laparoscopic cholecystectomy remains the gold standard for acute cholecystitis, even in the COVID-19 pandemic, as it guarantees the best outcomes for the patients. Conservative management may be an option for mild cases, but it carries the risk of recurrence of symptoms and increased incidence of severe complications.

Keywords: *laparoscopic cholecystectomy, gallstones disease, COVID-19 pandemic, acute cholecystitis*

INTRODUCTION

Gallstones disease (GD) is a common pathology, with an incidence of approx. 20% in developed countries, a third of these cases being symptomatic [1].

Laparoscopic cholecystectomy is the gold standard of treatment for this condition. Acute forms are of clinically variable severity, from possible regressive under conservative treatment to life-threatening, namely: biliary colic, acute cholecystitis, cholecysto-pancreatitis and acute angiocholitis. Early surgical treatment was recommended as

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standard in acute cholecystitis to prevent subsequent recurrent episodes, as well as migratory and infectious complications related to the displacement of stones in the main bile duct [2, 3].

The global spread of SARS-CoV-2 pandemic, with the declaration of a state of emergency in our country on the 16th of March 2020, for 60 days, followed by the state of alert since 15th of May, still in force today, had a profound impact on the surgical practice. The initial recommendations of the International and National Surgical Societies were to delay elective surgery, and to attempt first-hand a conservative management of mild and moderate forms presented to the emergency room. Another aspect was that of the aerosolization potential in laparoscopic interventions associated with the induction and dessuflation of the pneumoperitoneum, trocar leakage and laparoscopic smoke. Since the time of SARS-CoV, in 2003, there has been convincing evidence that aerosol-generating procedures (AGP) could potentially result in a wider human-to-human coronavirus transmission radius [4]. Bioaerosols range in size from 0.3 to 100 µm, and particles up to 5 µm remain airborne and can travel distances of more than 100 m, which may be a transmission path for SARS-CoV-2 [5]. Safety guidelines have been developed in laparoscopic surgery to prevent infection of medical personnel in patients suspected or infected with SARS-Cov2, which focuses on preoperative testing of patients, wearing appropriate PPE, consisting of FFP2 masks, goggles or faceshields, ultrafiltration of pneumoperitoneum and preventing accidental leakage of CO2 and plume during laparoscopic surgery [6-13]. Also, the addressability of the population and the desire for surgical treatment was lower for this pathology, partly explained by the fear of a possible contamination in the emergency room or hospital.

Aim

The paper aims to identify the diagnostic and therapeutic features of acute manifestations of gallstones during the COVID-19 pandemic.

MATERIALS AND METHODS

A retrospective, comparative study was performed of the cases presented to the guard room and hospitalized in emergency for acute manifestations of gallstones in April-June 2020 vs similar period of 2019. The data from the presentation register at the guard, hospitalization sheets and surgical protocols, on the particularities related to the clinical form at hospitalization, associated pathology, therapeutic management of patients with acute gallbladder disease, in the absence and during the SARS-CoV-2 pandemic. Changes in the surgical practice of patients with acute manifestations of gallstone disease imposed by international and national guidelines were also analyzed, as well as organizational changes in institutional circuits to prevent the spread of SARS-CoV-2 infection.

RESULTS

Establishing separate circuits within the department for the prevention of SARS-CoV-2 infection

During the pandemic COVID-19, our hospital, an emergency university center with many specializations, made sustained efforts to remain COVID-free, to be able to ensure the safe treatment of patients, and to prevent nosocomial contamination with SARS-Cov2.

In this regard, a strict protocol was implemented which involves testing by RT-PCR at the time of admission of all patients in the emergency room, their isolation respecting separate circuits until the result is obtained, simultaneously with the beginning of the appropriate treatment. If surgery is required immediately, it was performed by treating the patient as SARS-CoV-2 suspect, with all the precautions required by this: proper equipment of PPE in the operating room, avoidance as much as possible of aerosol-generating methods, including laparoscopic surgery, according to the recommendations of the national endoscopic surgery company ARCE in force during the analyzed period. When it was possible to delay the intervention for 24 hours until the result was obtained, in case of negative, the patient was operated laparoscopically, with standard PPE. If the test confirms the infection, the patient was transferred to one of the hospitals designated as a COVID-support hospital and further treated according to the guidelines in force.

Study group analysis

During April-June 2020, during the COVID-19 pandemic, the number of hospitalized cases for acute cholecystitis and other acute manifestations of GD was significantly lower than in the same period last year (21 vs 49 patients). Of the total presentations at the emergency room during the COVID-19 pandemic, only a percentage of 27.6.% agreed with the hospitalization (vs 48.5% in 2019), the rest expressing their request for conservative treatment.

The sex ratio was similar in the 2 groups (F: M = 2: 1), but the incidence of patients treated with rural origin was lower, this could be correlated with the travel difficulties imposed by the legislation during the state of expedite. The average age was higher in 2020, but statistically insignificant (64 vs 56 years).

Regarding the clinical forms, in both study groups the main form of presentation was acute catarrhal cholecystitis (52.4% vs 63.2%). We note, during the SARS-Cov-2 pandemic, the absence of mild forms of biliary colic, with increasing incidence of complicated forms, mainly hydrops (23.8% vs. 4.1%, $p < 0.05$) and angiocholitis (19% vs. 4.1%, $p < 0.05$).

From the analysis of associated comorbidities, we notice the increase of the share of cardiovascular diseases and diabetes in patients hospitalized during the pandemic, these diseases increasing the potential for aggravation of the general condition in patients with acute cholecystitis, making them unsuitable for conservative management.

Table 1: Comparative analysis of patients with acute manifestations of GD during the COVID-19 pandemic compared to the same period last year: demographic data, clinical forms at hospitalization, comorbidities

	April-June 2020	**April-June 2019**
Number	21	49
Male	33.3%	36.7%
Female	66.7%	63.3%
Urban/Rural	6:1	4:1
Age	64+/-3.2 years	56+/-2.7 years
Clinical form		
Biliary colic	0	8 (16.3%)
Acute cholecystitis		
- Cataral	11 (52.4%)	31 (63.2%)
- Phlegmonous	1 (4.7%)	2 (4.1%)
- Gangrenous	0	1 (2%)
Hydrops	5 (23.8%)	2 (4.1%)
Pio-cholecystitis	0	1 (2%)
Cholecistopancreatitis	1 (4.7%)	2 (4.1%)
Angiocholitis	4 (19%)	2 (4.1%)
Associated pathology:		
Obesity	6 (28.5%)	10 (20.4%)
Adherential Syndrome	5 (23.8%)	16 (32.6%)
Cardiac pathology	6 (28.5%)	6 (12.2%)
High Blood pressure	6 (28.5%)	16 (32.6%)
Diabetes	5 (23.8%)	3 (6.1%)
Gastritis/ Dyspeptic syndrome/Ulcer	3 (14.3%)	5 (10.2%)
Bronchopulmonary chronic obstructive disease	2 (9.5%)	0
Recent cerebrovascular accident	1 (4.7%)	0
Hepato-splenomegaly. Port vein thrombosis	1 (4.7%)	0
Hiatal hernia	0	1 (2%)
Neuropsychiatric disorders (epilepsy, drug addiction)	0	2 (4%)

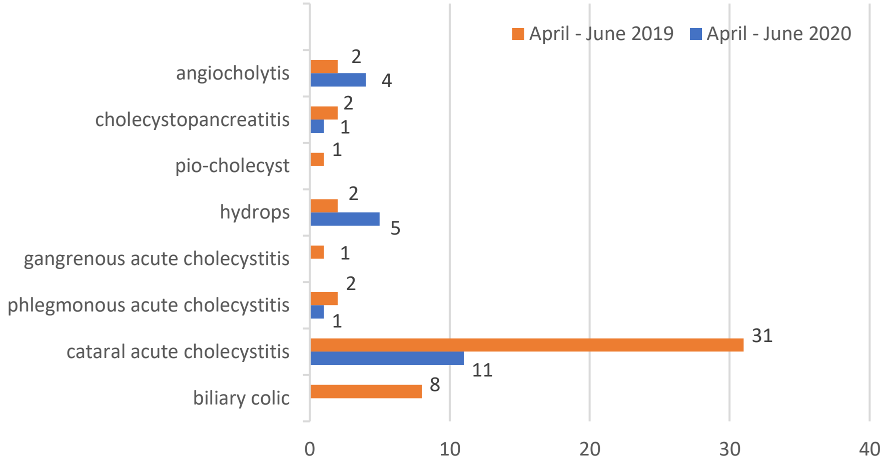

Figure 1: Comparative data for clinical forms during Covid-19 pandemic vs non-COVID period

After admission, all patients in the pandemic group were tested for Sars-CoV-2 infection and were hospitalized in a COVID-19 suspect area, with separate circuits from the rest of the department. Medical therapy was initiated, depending on the clinical form, with spasmolytics, analgesics, antibiotics and hydro-electrolytic rebalancing. In 20 of 21 cases, surgery could be delayed for up to 24 hours until the absence of COVID infection was documented. In one case, a piocholecyst with imminent of perforation was operated on in an immediate emergency, by open approach, and a cholecystectomy and subhepatic drainage were performed, with a slowly favorable evolution. In a case diagnosed with angiocholitis, SARS-CoV-2 positive, the patient was transferred for ERCP to a surgery department at a COVID-19 support hospital, evolving favorably under minimally invasive treatment.

Table 2: Therapeutic management and postoperative outcome in the COVID-19 pandemic group vs control group.

	April-June 2020	April-June 2019
% surgery	11 cases (52%)	35 cases (71%)
Type of surgery:		
LC	18 (85.7%)	30 (85.7%)
Conversion	0	2 (5.7%)
OC	1 (4.7%)	0
ERCP	1 (4.7%)	3 (8.6%)
ERCP+LC	1 (4.7%)	0
Hospital days	6.62	5.6
Postoperative complications:		
Deaths	2 (comorbidities)	0
Bleeding	1 (conservatory)	1
Infection of the wound	0	0

LC: laparoscopic cholecystectomy; OC: open cholecystectomy; ERCP: Endoscopic retrograde cholangiopancreatography

Regarding therapeutic management, there is an increase in cases that benefited from conservative treatment during the pandemic (48% vs. 29%). For operated patients, laparoscopic cholecystectomy was the main option, performed in 85.7% in both groups, except that during the pandemic, the intervention was delayed 24-48 hours to obtain confirmation of the absence of SARS-CoV-2 infection. There were no conversions for the pandemic group, a possible explanation being the better documentation of cases and the favorable effect of early medical therapy in cases with an increased risk of conversion due to local inflammatory anatomical changes.

The average hospital stay was slightly increased, but statistically insignificant, a possible explanation being the timing related to RT-PCR, but also the higher incidence of comorbidities and more severe forms of treated patients. The latter aspect also explains the higher rate of postoperative deaths (9.5% vs 0%), being related to cardiovascular and metabolic decompensation through the associated countries, in elderly patients.

DISCUSSION

The COVID-19 pandemic had a major impact on the management of acute gallstones. During this period, the proportion of cases treated conservatively increased, either outpatient, in the case of patients who refused hospitalization, or in hospitalized patients. This is communicated by similar studies conducted in Spain. Italy, UK or USA, also countries with a significant number of SARS-CoV-2 infections nationwide [1, 14-19].

One of the causes is the reluctance of patients to go to the emergency room or to consent to hospitalization for fear of infection. Harnett et al. estimates in this regard a decrease of up to 50% in the presentation of patients in the emergency room in the US during the pandemic [20]. On the other hand, given the increased pressure on health systems, but also of studies that show a bad outcome in surgeries performed on COVID + patients during the incubation period, international practice guidelines recommend conservative management and avoidance of surgery, if safe for the patient [6, 7, 12, 22-25].

Another important aspect is the reluctance to laparoscopic surgery, due to the possibility of aerosolization of surgical plume and CO2 at pneumoperitoneum desufflation [4]. Preoperative testing of patients is encouraged, as far as resources allow, however studies show a 30% chance of false-negative results, which can have unfavorable consequences for both the patient and the medical staff [26]. Considering possible presence of the infectious agent in blood, feces and intestinal wall and to avoid leakage near the trocar orifices, the use of low pneumoperitoneum pressure and low energy levels during electrocautery.

Regarding gallstone disease, laparoscopic cholecystectomy has established itself worldwide as a gold-standard, due to its indisputable advantages related to the reduction of operative complications, early recovery and reduced hospitalization time. Even during the COVID-19 pandemic, when the open approach was encouraged due to avoiding aerosolization, laparoscopic cholecystectomy remained the preferred technique in surgical practice in our department, being practiced in 87.5% of cases. Similar results are reported by Manzia, Narvaez and Kabir [1, 17, 27]. Moreover, Flemming and Kabir show that SARS-CoV-2 is not present in bile, gallbladder samples and peritoneal fluid [27, 28]. These preliminary findings suggest that the potential infectious risk for operating theater staff and surgeons during open and laparoscopic operations in SARS-CoV -2 patients is lower than initially expected.

Another observation found in the present study is the increase in the severity of cases hospitalized and operated during the SARS-Cov-2 pandemic compared to the same period last year. Gallstones disease, even if not an emergency, should receive surgical treatment when symptomatic. Neglecting this extremely common pathology can increase the incidence of severe, life-threatening complications such as cholangiopancreatitis or angiocolitis [28-33].

Additionally, a more advanced disease at the time of surgery may result in increasingly morbid operations, which are associated with prolonged hospital stay and higher costs. Since the global healthcare community has never faced a dramatic scenario such as the COVID-19 pandemic, it is difficult to forecast the effects of untreated GD and the development of its related complications during and after COVID-19 [1].

CONCLUSIONS

In light of recent findings, but changes to the surgical protocol are required to increased safety for medical personnel.

We maintain that laparoscopic cholecystectomy remains the gold standard for acute cholecystitis, even in the COVID-19 pandemic, as it guarantees the best outcomes for the patients.

Conservative management may be an option for mild cases, but it carries the risk of recurrence of symptoms and an increased incidence of severe complications.

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CLINICAL PRACTICE

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Clinical aspects in implant fixed prosthodontics oral rehabilitation. Case report

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Abstract*:* *Benzodiazepine overdose has various clinical manifestation, mainly regarding the central nervous system (CNS), cardiac and respiratory side effects, but rarely results in significant morbidity and mortality. Acute benzodiazepine poisoning results in dizziness, ataxia, nystagmus, dysarthria, hypoxia, hypothermia, bradycardia, hypotension, apnea, pulmonary aspiration, respiratory depression, coma, cardiopulmonary arrest and death. Anyway, deep nonresponsive coma should be investigated for additional etiologies. On the other hand, human transmissible prion disease has a fatal outcome with no specific treatment.*

Keywords: *implants, osseous integration, staging, fixed prosthesis*

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INTRODUCTION

Each year endoosseous dental implants gain greater acceptance among clinicians and patients. This has come about for several reasons, including excellent success rates published in long-term studies, improvements in fixture and abutment designs, more predictable surgical placement techniques, and increased consumer desire to have tooth replacement. Regardless of the implant system, the placement and functional success of endo osseous implants is greater than 90%) [1]. Fixed prosthodontic on implants is the most desirable treatment for the patients among the treatments on implants. When transforming a partially edentulous patient in a complete edentulous one, the transition difficult period can be avoided by keeping some teeth for fixed provisional, until the osseous integration of the implants is accomplished and the final prosthetic can be done [3]. In this regard, the importance of restoring patients with functionality during the implant healing period has grown in recent decades [7].

This paper presents a staged protocol of the patient's treatment that made the case easier to manage and offered a better comfort in the transition period.

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CASE REPORT

Material and method

T.S., a 66 years old female patient, with a general medical history of chronical C hepatitis, presented with a mucocell in the sinus cavity and a T,L-T maxillary edentation and L-T mandibular edentation, with inadequate fixed restorations, both in upper and lower jaw. An accurate and complex clinical examinations wad performed, including the prosthodontic, implant – surgical assessment, panoramic radiograph and computed tomograms for both jaws. The intermaxillary relationships were carefully examined and the OVD was recorded (5.7 cm).

A comprehensive treatment plan was proposed and decided, following the conclusions from the diagnosis findings. Thus, all the remaining teeth were in a poor dental status and needed to be extract, but in a staged approach, as in figure 1.

Figure 1: Initial dental status

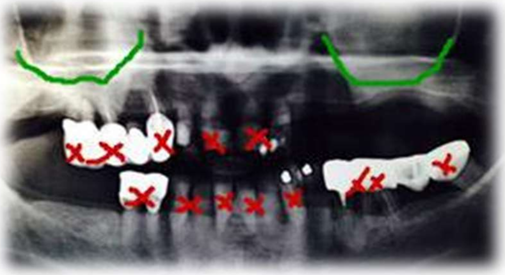

The 1st step was the surgical one on the maxillary arch. So, after CT, Rx measurements and evaluation of bone volume and density related to prosthetic planning dental implants position was decided the placement of 3 implants (Superline, Dentium) (2.2/2.4/2.6). Prior to this was performed the left sinus mucocel evacuation. Meantime was performed also the internal sinus lifting with mineral bone grafting (Osteon, Dentium).

The 2nd surgical step. After the healing period (2 months), right sinus lifting was performed with same materials and were placed 3 implants (1.4/1.5/1.6).

By the time of implants osseous integration, the 11 and 21 were kept in order to provide patient's minimal aesthetic and functional comfort. Provisionals, immediate loading – nor fixed or removable couldn't be used due to bilaterally sinus lifting with bone grafting materials and the bone density. (Figure 2)

Figure 2: The clinical situation before the 3rd step

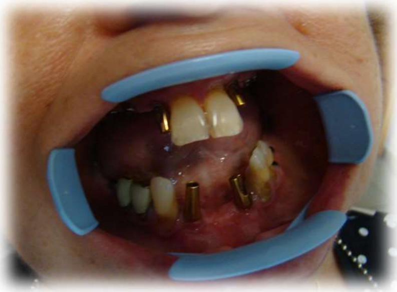

Meanwhile the 6 upper jaw implants osseous integration, the 3rd surgical step, consisted in the placement, on the lower jaw, of 2 implants (4.6/4.7) and 1 implant in the upper jaw (1.2), all of them prosthetic driven. After the evaluation of the occlusal vertical dimension (OVD) that we recorded and preserved, the vertical space was assessed as well as mandibular implants positioning in order to obtain correct occlusal relationships.

In the 4th surgical step the insertion of 2 frontal mandibular implants (3.2/4.2) and 2 left lateral mandibular implants (3.4/3.6) was performed. 3.3/ 3.4/4.3/4.4/4.5 for fixed provisional. The extracted teeth were: 1.2/1.3/1.5/1.6/2.2 initially and just before the implants uncovering, 1.1 and 2.1, in the upper jaw and 3.1/3.2/3.5/4.1/4.2/4.6/4.8 in the lower jaw and 3.3/3.4/4.3/4.4/4.5 were used for provisionals. (Figure 3)

Figure 3: Panoramic radiograph after the implant's placement

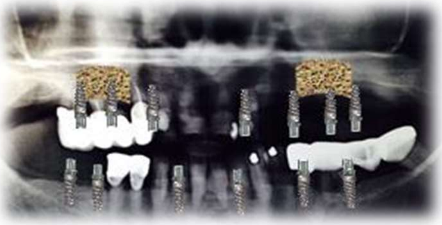

RESULTS AND DISCUSSIONS

Due to the case complexity, because of the surgical aspects (13 implants insertion), bilaterally internal sinus lifting with bone grafting and the evacuation of a mucocell, related to the prosthodontic ones, meaning the complete fixed bimaxillary rehabilitation, have made the treatment to last 1,5 years until the final prosthetic rehabilitation that we will describe next.

Due to the staged surgical approach, there was no possibility to split the final prosthetic work in more segments, nor in maxilla or mandible, so we produced 2 full – arch restorations on implants.

The idea of the final prosthetic treatment was to rehabilitate simultaneously the 2 jaws in order to split optimally the vertical prosthetic space. So, after all implant's uncovering and the last teeth extraction the healing abutments were applied and after 10 days the impression was taken. Monopren transfer (Kettehbach) was used as impression material, in an open individual tray technique, using transfer abutments.

The work casts were obtained with the analogue implants in their position. On this casts, occlusion rims were made for the intermaxillary relationship registration. All the steps were performed in order to establish accurately the occlusal and intermaxillary relationships: the vestibular curve of the upper occlusion rim was esthetical and phonetic established.The occlusal plane's level and direction were established using the usual methods and also the patient s youth photo, that provided a lot of useful information, such as the visibility of the 6 frontals. The initial correct OVD was kept and the 2 rims were fixed in the correct CR position. The information about the facial midline, the smile line and canine lines, together with other information regarding the teeth colour, size, form, arrangement and positioning were transmitted to the laboratory.

After the intermaxillary relationship registration, the dental lab produced the metal frame of the prosthetic work. The trial of the metallic part was done related to the axial and cervical marginal fit. With the patient s agreement, the last esthetical decision were taken, regarding the colour and form of the artificial teeth, to provide the dental lab all the information for the ceramic application. In the dental lab Vita ceramic layers were applied.

The try-in of the prosthetic work was done, aesthetic, phonetic and occlusal relationships and intermaxillary, horizontally and vertical. Also, occlusal adjustments were performed. Then the 2 fixed prostheses were glazed and polished.

The final work was provisional cemented and then definitively (Figure 4).

Figure 4: Final result

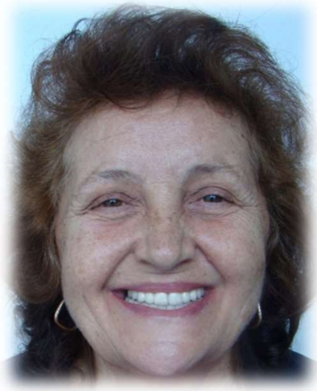

Figure 5: A 3 year follow up

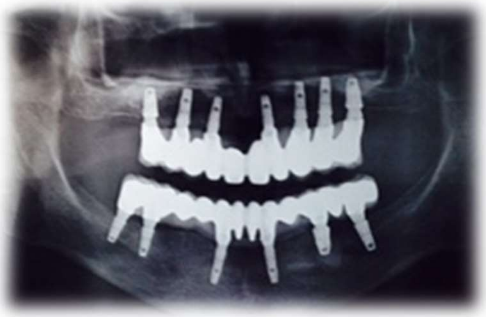

Figure 6: Final occlusal relationship

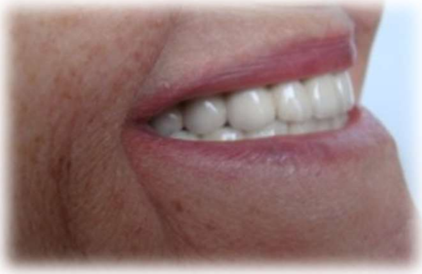

The follow-up was done, after 6 months and then after 3 years, and the results can be observed on the panoramic radiograph and indicates a very good situation.

CONCLUSIONS AND CLINICAL IMPLICATIONS

For many patients, as for the patient s case that we described previously, the transition between the dentate state and the completely edentulous state, with removable provisional is difficult to accept. That ⍰s why we choose a staged approach for our case, with strategic extractions and implants applied in 4 surgical different phases. The provision prosthetic was a fixed one, the major advantage of this treatment protocol. The treatment planning in such complex cases represents a challenge for the implantologist and prosthodontist and for the patient and our presented protocol facilitates the transition from a destroyed dentition to a full-arch reconstruction.

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VARIA

Clinical characteristics and management evolution of cholelithiasis in a pediatric population: 123 cases experience

Gabriel Stanciu[1], Ancuta Cardoneanu[1], Sorin Nedelea[2]

Abstract: *Introduction: The incidence of cholelithiasis has recently increased in the pediatric population, although it remains a rare disease among children. The purpose of this study is to highlight the management and the appropriate time to perform surgery.*

Materials and Methods: We performed a retrospective study over 10 years (2010-2010) and we found 123 patients who presented with biliary lithiasis and who underwent surgery in our department. We divided the study population into two groups depending on the time of admission and we found that in the 2010-2015 period we had a number of 33 patients, while in the last 5 years we admitted 90 patients with this diagnosis. Patients with other conditions such as bile duct atresia, choledochal cyst, hepatoblastoma that required a cholecystectomy were excluded from our study.

Results: After analyzing the two groups we concluded that the number of surgical procedures increased from 33 to 90, which represents a 2.6 times increase. There was no significant difference between the two groups regarding the average age on admission (12.6 +/- 3.37 in the first group vs. 13.91 +/-3.34 in the second group). Surgical management of lithiasis cholecystitis registered an important difference in the two groups. In the first group, 9 patients (27.3%) underwent classic cholecystectomy while 24 (72.7%) – patients benefited from the laparoscopic approach, while in the second group just one patient underwent open surgery with 89 (98.9%) patients undergoing laparoscopic surgery. The percentage of patients who benefited from surgery in the same hospitalization remained high in both groups studied with a statistically significant p-value (p = 0.0417).

Conclusion: The number of cases with cholecystitis and lithiasis is increasing in the pediatric population. Surgical management for these patients has become similar to that of adult patients, with minimally invasive surgery becoming the gold standard approach.

Keywords: *lithiasis, cholecystitis, cholecystectomy, pediatric*

INTRODUCTION

Gallbladder disease is one of the most common and costly digestive diseases that require hospitalization. Gallstones are more common in the adult population and remain relatively uncommon in children. However, the incidence of gallbladder disease in children has markedly increased over the last several decades. Although uncommon during the first years of life, it is being diagnosed with increasing frequency in neonates and infants, probably because of the widespread use of ultrasonography [1, 2].

There are a few well-known risk factors for pediatric cholelithiasis such as hemolytic conditions, prematurity,

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prolonged parenteral nutrition, obesity, and puberty (for girls) [1]. Although in the past, biliary lithiasis was most often associated with benign hematological disease, nowadays the prevalence of non-hemolytic gallstone disease has been shown to increase as the prevalence of obesity also increases [3].

Even if for adults cholecystectomy is a routine procedure, the same cannot be stated for pediatric patients. According to early reports over recent years, there has been a significant increase in pediatric cholecystectomies due to the rising incidence of lithiasis cholecystitis [4].

The primary pediatric indication for cholecystectomy is biliary dyskinesia or symptomatic cholelithiasis. One reason for the increase in the frequency of pediatric cholecystectomy may be that laparoscopic cholecystectomy is commonly accessible. Laparoscopic cholecystectomy has become the gold standard in the treatment of symptomatic gallstones. The major advantages of laparoscopic cholecystectomy include less postoperative pain, less time required for hospitalization and recovery, and better cosmetic results [5].

This study is a retrospective analysis of patients under 18 years old, following the characteristics, evolution, and surgical management of the patients diagnosed with lithiasis cholecystitis who underwent cholecystectomy at Grigore Alexandrescu Emergency Pediatric Hospital of Bucharest in the pediatric surgery department.

MATERIALS AND METHODS

Patients who were admitted to our department for cholecystectomy between January 2010 and December 2020 were taken into consideration and included in this retrospective study. We took into account patients who presented in our service either through the emergency room, as acute cholecystitis, or in a scheduled manner.

The main inclusion criteria for patient selection from our database were patients under 18 years old, who underwent cholecystectomy for lithiasis cholecystitis. Patients with hepatoblastoma, choledochal cyst, congenital biliary atresia, and other conditions that required cholecystectomies but the cause was not lithiasis were excluded.

Data for this review was obtained from the Pediatric Surgery Department's database. One hundred and forty-one cholecystectomies were performed during this period but after we applied the exclusion criteria the total amount of cases remained 123.

After we have considered the variables for all the patients, we divided them into 2 groups of interest depending on the period in which they presented in our department (group 1: 2010-2015 and group 2: 2016-2020) to investigate the trend over time.

Afterward, patients were divided according to their diagnosis into symptomatic and asymptomatic. Those symptomatic were further divided into uncomplicated (diffuse abdominal pain, biliary colic, nausea, and vomiting, without any inflammatory signs) and complicated (which associated choledocholithiasis, biliary pancreatitis, or acute abdominal pain in the upper quadrant in association with inflammatory signs like fever, leukocytosis, and high CRP). Patients who were referred to the emergency department with acute cholecystitis due to lithiasis have benefited from surgical treatment either during the same hospital stay (if the liver enzymes and amylase decreased) or less than a month after the acute event in most cases. For those who were diagnosed with gallstones accidentally by ultrasonography, the conservative treatment (ursodeoxycholic acid-dose 10 mg/kg/day) was the first choice and if during subsequent evaluation still presented lithiasis, cholecystectomy was taken into consideration.

Abdominal ultrasound was performed for all patients. If an impacted calculus in the common bile duct was found during an ultrasound, magnetic resonance cholangiography (MRCP) or endoscopic retrograde cholangiopancreatography (ERCP) were then performed.

We also took into consideration anatomical abnormalities as a risk factor for lithiasis and found 3 cases with abnormal anatomy of the gallbladder. Criteria like surgical approach, intraoperative cholangiography, postoperative complication, and days of hospitalization and rehospitalization were also taken into account. Postoperative follow-up ranged from 6 months to 2 years.

For data collection, analysis, processing, and filtering we used Microsoft Excel. Comparisons between both 1st and 2nd periods were made using a two-tailed Student t-test for continuous variables and a one-sample t-test for categorical variables with a p-value ≤ 0.05 defined as significant.

RESULTS

This study was conducted on 123 patients with lithiasis cholecystitis who underwent either open or laparoscopic cholecystectomy. Before dividing the cohort into two main groups, we can easily observe the ascending trend of lithiasis cholecystitis cases with cholecystectomy over the 10 years (Figure 1).

Figure 1: Number of lithiasis cholecystectomies

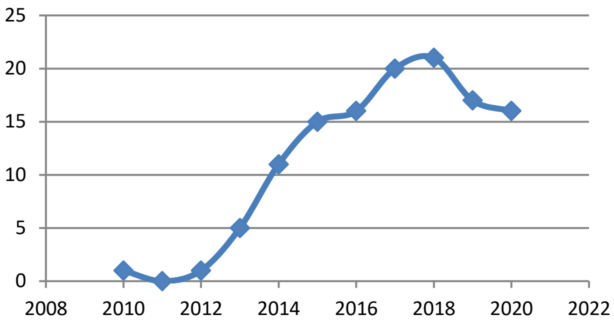

Figure 2: Mean age at the time of diagnosis in both groups

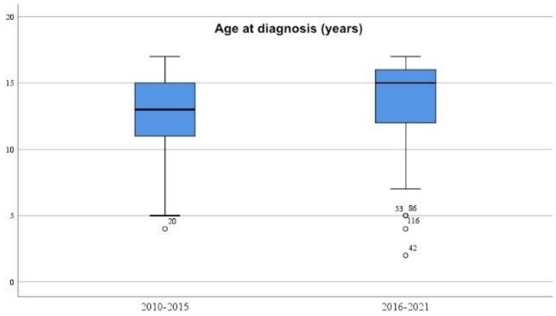

After the cohort was divided into two main groups according to the period, the median age registered a 2-year increase in the average age in group 2 compared to group 1 (p=0.2029) (range, 1 year to 17 years and 11 months) (Figure 2).

The vast majority of children were females in both groups (group 1 – 63.63%; group 2 – 66.66%). Taking into account the background, there is a slight increase in rural patients in group 2, but patients from the urban background are still the majority.

Table 1: Preoperative variables

Variable	**Category**	**Group 1 (G1) n=33 (100%)**	**Group 2 (G2) n=90 (100%)**	**P value <0.05** (One sample t-Test)	**P value <0.05** (Tow sample paired t-Test)
Gender	Male	12 (36.36%)	30 (33.33%)	0.0393	0.1266
	Female	21 (63.63%)	60 (66.66%)	0.0756	
Age (years)	Mean age (SD; Median)	12.6 (3.37; 13)	13.91 (3.34; 15)	-	0.2029
Background	Urban	20 (60.6%)	47 (52.22%)	0.0262	0.0005
	Rural	13 (39.4%)	43 (47.77%)	0.1239	
Comorbidities	Obesity	13 (39.4%)	37 (41.11%)	0.0744	0.002
	Hematologic diseases	1 (3%)	9 (10%)	0.4226	
	Neurologic diseases	0 (0%)	6 (6.66%)	0.6346	
	None	19 (57.6%)	38 (42.22%)	0.0054	
Clinical presentation	Simptomatic	23 (69.7%)	46 (51.11%)	0.0054	0.3454
	Asimptomatic	10 (30.3%)	44 (48.88%)	0.2245	
Simptomatic	Complicated	2 (8.7%)	10 (21.73%)	0.2672	0
	Uncomplicated	21 (91.3%)	36 (78.26%)	0.0003	
Pre- or intraoperative investigation	Ultrasonography	33 (100 %)	90 (100)	0.0619	-
	MRCP	1 (3%)	5 (5.55%)	0.2672	
	ERCP	0 (0%)	6 (6.66%)	0.6346	
	Colangiography	2 (6 %)	1 (1.11%)	0.0054	

Obesity is the most common comorbidity in both groups (p=0.0744), also the number of patients presenting with cholelithiasis associating comorbidities is higher, this being statistically significant (p=0.0054). In our study group, we took into consideration comorbidities like obesity (51), hematological (10), and neurological diseases (6). The main hematological disease was hereditary spherocytosis (10) and in the case of neurologic diseases were epilepsy (3) and spastic tetraparesis (3). One patient presented both epilepsy and spastic tetraparesis. A 20% increase in the number of patients diagnosed during a routine ultrasound examination can be observed, but the number of symptomatic patients is still high (group 2 - 51.11%; p = 0.0054). Even though the ultrasonographic examination is widely performed and asymptomatic patients are diagnosed early, the percentage of patients with complicated cholelithiasis has doubled (group 1 – 8.7% vs group 2 – 21.7%).

Ultrasonography is the main method of diagnosis of this pathology. However, there is a doubling of the percentage of patients who received MRCP (group 1 - 3% vs group 2 - 5.55%) and an increase in the percentage for ERCP (group 1 - 0% vs group 2 – 6.66%) in detriment of cholangiography which was preferred in the first period of our study (group 1 - 6% vs group 2 – 1.1%). Just the patients who associated choledocholithiasis symptomatology required the magnetic resonance cholangiopancreatography exam. Three patients with choledocholithiasis underwent ERCP before cholecystectomy and the other 3 has a cholangiography performed during cholecystectomy surgery.

Regarding the moment of surgery, the results are almost similar in both groups as can be seen in Table 2. There is an increase in the number of cholecystectomies (the difference between those two groups is about 2.6) taking into consideration the availability of radiological examination, especially ultrasonography and laparoscopic approach accessibility. Laparoscopic surgery was performed in 98.8% of patients in group 2 and 72.7% of patients in group 1 (Table 2).

Table 2: Surgical management

Variable	Category	Group 1 (G1) n=33 (100%)	Group 2 (G2) n=90 (100%)	P value <0.05 (One sample t-Test)	P value <0.05 (Tow sample paired t-Test)
Moment of surgery	Same hospitalization	19 (57.5)	48 (53.3)	0.0417	0.0004
	Less than a month	7 (21.2)	23 (25.5)	0.1216	
	More than a month	7 (21.2)	19 (21.1)	0.0605	
Surgical approach	Laparoscopy	24 (72.7)	89 (98.8)	0.1643	0.8698
	Clasic	9 (27.7)	1 (1.11)	0.4226	
	Converted laparoscopy	0 (0)	0 (0)	-	-
Priority	Urgent	19 (57.5)	48 (53.3)	0.0417	0
	Elective	14 (42.4)	42 (46.6)	0.091	
Postoperative complications	Bleeding or Local hematoma	1 (3.03)	4 (4.4)	0.1912	0.5294
	None	32 (96.9)	87 (96.6)	0.061	
Reintervention		1	2	0.0054	
Hospital stay (days)	Mean/Median	8.21/8	7.04/5		
Readmission	Yes	1 (3.03)	5 (5.5)	0.2672	0.4894
	No	32 (96.9)	85 (94.4)	0.0546	

The conversion was not considered in any patients. One patient underwent laparoscopic emergency surgery and a subtotal-cholecystectomy was performed due to the inflammatory process which did not allow safe dissection of the elements of the hilum. This patient underwent a second open surgery after 5 months during which the remaining gallbladder was removed.

In both groups, the number of emergency surgeries remains higher than the number of electively operated patients (Table 2). Postoperative complications were recorded in almost 7% of cases and they were only hemorrhagic complications. Three patients required a second surgery and in two cases the source of the active hemorrhage was the gallbladder bed, while in the third case a large hematoma was found without signs of active bleeding. Ultrasonography revealed hematoma in the gallbladder bed in 2 other patients, but they were treated conservatively. No complications such as bile leakage or bile duct injury, infection, or postoperative wound dehiscence were highlighted. No deaths were recorded in our study.

The median of hospitalization days in group 1 was 8 days, significantly higher when compared to group 2 where the median was 5 days (Table 2, Figure 3).

Figure 3: Mean hospital stay in both groups

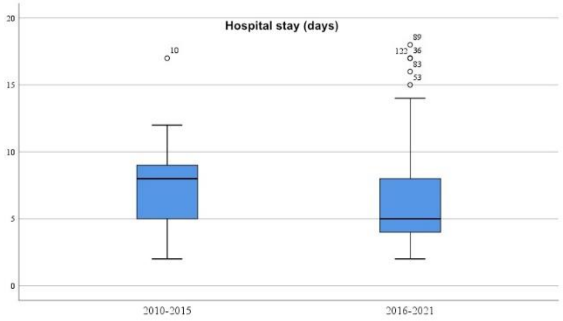

There is a slight increase in the percentage of patients readmitted in group 2 compared to group 1 (group 2-5.5%; group 1-3.03%), with the mention that from the patients readmitted in group 2 one needed surgical reintervention while the rest received symptomatic treatment. An overview of the time of intervention, depending on the patient's condition at presentation (acute or chronic), shows that most acute patients required surgery during the first presentation, compared to chronic patients who initially benefited from conservative treatment (Table 3).

Table 3: Moment of intervention depending on the general condition of the patient

	Same hospitalization	Less than a month	More than a month
Acute	39	10	4
Chronic	-	20	23

**27 of the chronic patients were previously diagnosed in other medical centers without being able to specify exactly the time of diagnosis, for this reason, they were excluded from this analysis.*

Considering the number of hospitalization days, we divided the patients into 2 groups, acute and chronic, and the results showed an average of 8.86 days for acute patients, compared to 5.58 days for chronic ones (Table 4).

Table 4: Mean of the days of hospitalization depending on the general condition of the patient

	Acute	Chronic
Days of hospitalization (mean)	8.86	5.58

Also, depending on the number of days of hospitalization, patients were divided into two groups regarding the surgical approach (classic and laparoscopic) resulting in a decrease of 2.55 of the average number of days of hospitalization for those operated laparoscopically (Table 5).

Table 5: Mean of the days of hospitalization depending on the surgery approach

	Classic	Laparoscopic
Days of hospitalization (mean)	9.7	7.15

DISCUSSION

Compared to adults, the prevalence of lithiasis cholecystitis is lower in children, so cholecystectomy is less common in this age group. Recently, several studies reported that cholecystitis and cholelithiasis are being diagnosed with increasing frequency in infancy, childhood, and adolescence and the incidence is reported to be between 0.15 & 0.22% [1]. As most cases of cholelithiasis are asymptomatic, the prevalence of this pathology cannot be correctly assessed. However, the symptoms that may occur are diffuse abdominal pain, biliary colic, nausea, and vomiting, without any inflammatory signs or by complications like choledocholithiasis, biliary pancreatitis, or acute abdominal pain in the upper quadrant in association with inflammatory signs like fever, leukocytosis, and high CRP. Comparing the two groups from our study, the number of cholecystectomies increased from 33 to 90, which represents an increase of 2.6 times higher in group 2, a result that is comparable to other studies [3]. This may be due to the frequent use of abdominal ultrasound in children with abdominal disorders which recently has resulted in the diagnosis of gallstones in a large number of asymptomatic children [1]. Also, the high number of cholecystectomies can be the result of the widespread use of laparoscopy.

As in the literature, there was a higher incidence of cholelithiasis in females (group 1 – 63.63%; group 2 – 66.66%). This can be explained by the hormonal changes that occur during puberty, with an increase in the saturation of cholesterol in bile [6, 7]. What strengthens our assumption is that the average age of cholelithiasis in both groups is around puberty (group 1 – 12.6 - group 2 – 13.9).

In contrast to other studies in which the incidence of cases of cholecystitis in patients with hematological diseases seems to decrease over time, in our study, there is a 7% increase in group 2 compared to group 1. However, the percentage of patients who have a hematological disease as a risk factor remains low compared to those presenting with obesity. Similar to other series, most of our patients were obese (group 1 – 39.4%, group 2 – 41.1%).

In general, the percentage of pediatric patients with symptomatic cholelithiasis is around 60% [2], similar to our data. However, even though the number of symptomatic patients is higher, our data showed a decrease of approximately 18% of symptomatic patients in group 2 compared to group 1, and an increase of 18% in asymptomatic patients. This is probably also the result of the widespread use of ultrasonography so that many patients with gallstones end up being diagnosed before the onset of symptoms.

The percentage of complicated cases was almost 3 times higher in the second group of our study, while the percentage of uncomplicated cases decreased. One theory that would explain this trend is that the diagnosis is established mainly in the asymptomatic phases of the disease, and in an attempt to treat them conservatively with ursodeoxycholic acid, the stones reach a diameter that allows them to migrate along the bile ducts and obstruct them.

In the adult population, ERCP has gained ground versus open exploration of the common bile duct, thus leading to a decrease in their risk of injury [8] and the same can be seen in our cohort, where in the last 5 years the number of pediatric patients undergoing ERCP has also increased. Gee et al. propose performing cholecystectomy and ERCP at the same time, arguing that this method is a safe way to treat choledocholithiasis, without increasing the rate of postoperative complications, and additionally it is a cost-effective method [9].

Acute cholecystitis due to lithiasis was considered a relative contraindication to laparoscopic cholecystectomy in the early years of the development of this technique but was later shown to be safe in the treatment of acute cholecystitis [10, 11]. Nowadays, the management of lithiasis cholecystitis in the pediatric population, as in the case of adults, consists in using laparoscopy as the main method of surgical treatment. This method is preferred to open cholecystectomy because it has many benefits such as reduced costs by reducing hospitalization days and the use of painkillers, as has been shown in other studies [12, 13, 14]. The fact that in the last decade laparoscopic treatment is used as the main approach in the case of cholelithiasis is also shown in our study.

The right time to perform the surgery in the case of patients presenting to the emergency room remains to be debated. While some studies suggest performing surgery after 6-9 weeks of conservative treatment in acute patients to reduce the acute inflammatory process, Lo et al. and Casillas et al. observed that conservative treatment of lithic cholecystitis for a long period leads to the formation of fibrotic tissue adhesions between the gallbladder and the surrounding structures due to inflammation and edema, which makes dissection difficult. They advocate that early laparoscopic cholecystectomy performed with appropriate timing will facilitate the dissection and reduce the possibility of late complications [10, 15, 16]. The indication for early cholecystectomy is supported by various publications, which report a 5% increase in complication risk for every 10 days surgical treatment is postponed [17-21]. Regarding postoperative complications in our cohort, no complications other than hemorrhagic ones were identified and even these were present in a small number of patients. Postoperative hospital stay was long (mean 8.21 days in group 1 and 7.04 days in group 2), although no major complications were noted.

CONCLUSION

The number of cases with cholecystitis and lithiasis is increasing in the pediatric population. Surgical management for these patients has become similar to that of adult patients, with minimally invasive surgery becoming the gold standard approach.

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VARIA

Clinical profile of Mediterranean fever – Study from an Indian tertiary care center

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Abstract: *Introduction: Brucellosis is a zoonotic disease which is also known as Mediterranean fever. It is acquired from the handling of infected animals or consuming contaminated milk or milk products. The incubation period varies from 1-3 weeks to several months before clinical disease appearance and it shows multiorgan involvement. This study focuses on the varied presentation of this disease and laboratory findings. Materials and Methods: This is a retrospective study, where the past 15 years data of cases diagnosed with brucellosis was studied in a tertiary care center in South India. Patient demographics, clinical presentation, duration of stay in the hospital, hematological and biochemistry profiles were collected and analyzed in SPSS. Results: More number cases were found among the male gender (70%), mostly in the 20-40 years age group (40%). 95% of cases reported fever, 40% with arthralgia (29% generalized in nature), and 32% with a headache. A Positive Pearson correlation between CRP and NLR, and CRP and PLR were found, which was statistically significant. Mean laboratory values were documented. 37% of patients were found to have hepatosplenomegaly on ultrasound Conclusion: Most of the patients were admitted as cases with pyrexia of unknown origin. Our study focused on the different clinical presentations and laboratory findings of brucellosis and will help give a better understanding and outline during brucellosis case diagnosis and management in a cost-efficient manner.*

Keywords: *brucellosis, undulant fever, Mediterranean fever*

INTRODUCTION

Brucellosis is a zoonotic disease caused by bacteria of the genus Brucella. It is also known as undulant fever or Mediterranean fever [1]. Caused by Gram-negative coccobacilli, non-spore-forming and non-motile and aerobic; B. melitensis, B. abortus, B. suisare the important human pathogens. It is acquired from the handling of infected animals or consuming contaminated milk or milk products. Exposure is frequently occupational. Human brucellosis is either an acute febrile disease or a persistent disease with a wide variety of symptoms like night sweats, asthenia, insomnia, anorexia, and headache.

The disease is controlled by the routine practice of pasteurizing milk and milk products, as well as by comprehensive campaigns to eradicate the disease by destroying domestic animals which exhibit positive serologic reactions to brucellae. Vaccines providing some protection to cattle, sheep, and goats are available [2]. The incubation period of this infection is between 1-3 weeks but can take several months before clinical disease appearance [1]. The disease shows multiorgan involvement including musculoskeletal, digestive, urogenital, hematological, cardiovascular, respiratory, and central nervous systems [3]. According to the duration of symptoms, it can be classified

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as acute (<8 weeks), sub-acute (8 to 52 weeks), and chronic (> 52 weeks).

Subacute brucellosis, typically presenting with undulant fever, described in endemic areas with localized infections such as epididymitis, orchitis, and osteoarticular complications, is most commonly seen. Osteoarticular involvement is the most common focal complication of brucellosis (10-85%) [4]. Sacroiliac joints are the most common site involved in younger patients; spondylitis and peripheral arthritis usually occur in older patients. Cardiovascular complications of brucellosis (endocarditis, myocarditis, and pericarditis) are rare (1% of cases) [5].

In humans, the most common route of entry of Brucella bacteria is through the gastrointestinal tract and it then localizes in the reticuloendothelial system. Hepatic enlargement is the most frequent gastrointestinal manifestation of brucellosis (32-63% of cases) [6]. Neurobrucellosis is the most serious complication of brucellosis with neither a typical clinical manifestation nor a specific cerebrospinal fluid (CSF) picture and mimics other neurological disorders leading to diagnostic dilemmas. The prevalence of Neurobrucellosis ranges from 1.7 to 10% of brucellosis worldwide [7].

This study aims to record the clinical features and cut off of certain laboratory values of human brucellosis cases in South India, for prompt diagnosis of the same.

MATERIALS AND METHODS

This is a retrospective observational study, where 15 years of data of patients diagnosed with brucellosis is collected in a pre-designed format. Details including demographics, clinical presentation, hematological and biochemistry profiles are collected and analyzed in SPSS. Inclusion criteria are patients diagnosed with brucellosis in our hospital, with positive IgM ELISA. Exclusion criteria include cases previously diagnosed from an outside hospital, patients who had taken the alternative form of treatment, etc to avoid bias in lab reports. Study design, data collection, and write-up were done in Amrita Medical College. Study analysis and review were done by V.S. Srikanth, using SPSS, where Pearson correlation and values of central tendency of the findings were recorded.

Data collection, analysis, and article preparation are done in accordance and with the permission of Amrita Institute's ethics review committee.

RESULTS

In our study, we found that 70% (54) were male and 30% (23) females, out of the total 77 cases. The age group of presentations were most in the 20-30 years group (22%); 30-40 years were 18% and remaining presentations were scattered across all other age groups (Table 1).

Table 1: Age distribution

Age	< 17 years	20-30 years	30-40 years	40-50 years	50-60 years	> 60 years
Number	13	17	14	10	11	12
% distribution	17%	22%	18%	13%	14%	16%

5 cases were reported outside Kerala. The hospital stay duration was a minimum of 3 days and a maximum of 30 days, with a mean duration of 10 days. Clinical presentation – Fever - 73 (95%) patients presented with fever, out of which 50 (68%) had a high-grade fever (102-104). The fever duration was a minimum of 5 days to a maximum of 120 days and a mean duration of 21 days. Arthralgia – 31 (40%) patients presented with severe arthralgia, rest had no features of arthralgia (Figure 1).

Figure 1: Arthralgia

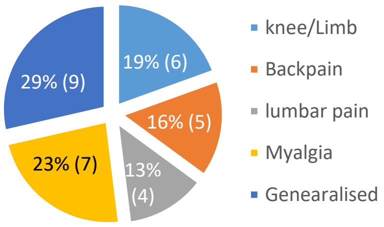

The most common GIT presentation was Constipation and vague abdominal pain and headache were present in 32% (25) of patients (Figure 2).

Figure 2: CNS symptoms

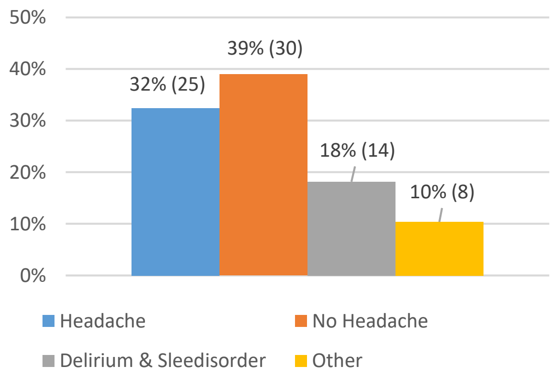

Mean values of neutrophil, lymphocyte, platelet counts, red cell distribution width, mean platelet volume, C reactive protein (CRP), procalcitonin, hemoglobin, SGOT, SGPT, serum protein, albumin, sodium, potassium, creatinine, and IgM Elisa values were found (Tables 2 and 3).

Table 2: Laboratory findings

Statistical Values	N (%)	L (%)	P (KU/ml)	RDW (%)	MPV (fL)	CRP (mg/L)	Procal (ng/mL)
Mean	56.32	31.79	303.01	14.95	8.39	61.58	0.78
Min Value	7.8	4.9	27.1	7	5	0.3	0.018
Max Value	90	72	687	27	25.8	306	5.3
Median	56	29.6	284.5	14.2	7.99	29.32	0.2
Std Deviation	17.33	16.12	147.01	3.09	2.99	72.46	1.42

Table 3: Laboratory findings – cont.

Statistical Values	Hb (g/dL)	SGOT (IU/L)	SGPT (IU/L)	Protein (g/dL)	Albumin (g/dL)	Na (mEq/l)	K (mEq/l)	Creat (mg/dL)	IgM ELISA (U/ml)
Mean	12.01	93.33	99.70	7.17	9.57	134.30	8.80	0.92	14.99
Min Value	6	9	9	5.33	1.77	120	2.7	0.05	0.8
Max Value	17.4	2142	2563	9.5	178.9	141.5	309	1.66	80.622
Median	12	36.35	51.5	7	3.7	134.55	4	1	5.5665
Std Deviation	2.31	253.51	293.64	0.84	27.77	4.12	37.82	0.32	20.65

Pearson correlation between CRP and neutrophil-lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) was found to be positive and statistically significant (Table 4, Figures 3 and 4 respectively). 37% (29) of patients were found to have hepatosplenomegaly on ultrasound.

Table 4: Pearson Correlation

		NLR	PLR
CRP	Pearson Correlation	0.476	0.579
	P value	0.001	0.001

Figure 3: Scatter Plot of Pearson Correlation between CRP and NLR

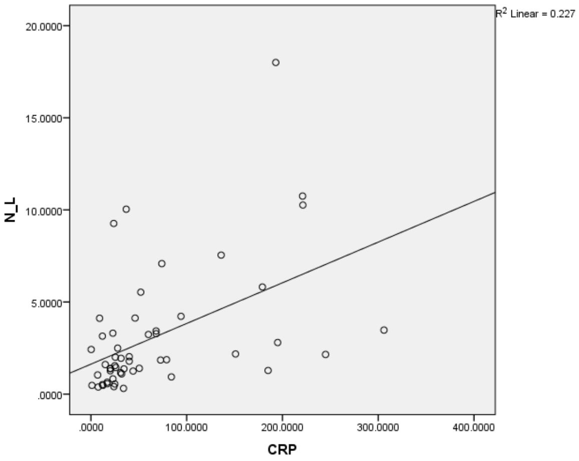

Figure 4: Scatter Plot of Pearson Correlation between CRP and PLR

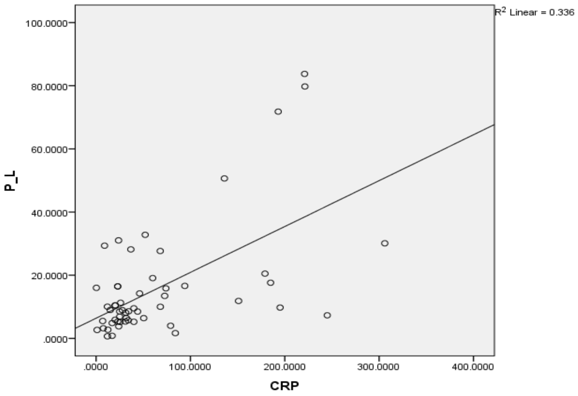

DISCUSSION

A higher incidence of cases was seen among males in our study (54, 70.1%), comparable to similar studies [9, 10, 18].

The mean age of brucellosis patients in our study was 36.95+19.22 years, similar to the findings of Huseyin S et al. [19]. In another similar study done by Mahmood Nabavi et al. [7], the mean age was 39.59 ± 17.28 years, and the most prevalent age groups was 31–50 (42.1%), while in another study done by Akhvlediani T et al. [9], mean age was 39.9 (standard deviation = 15.1) years and 45% of participants were 21–40 years old, which is comparable to our result.

Common clinical presentations in our study were fever (73, 94.8%), arthralgia (31, 40.26%), and generalized pain and swelling with myalgia (33, 43%), consistent with other studies [18]. Abdominal pain, loss of appetite, loss of weight, vomiting, etc were seen in 34 (44.1%) cases, while other symptoms like headache, altered sensorium, and fatigue were seen in 28 (36.3%) cases. In a similar study done by Akhvlediani T et al. [9], the most frequent complaints were sweats (92%), joint pain (91%), aches (90%), malaise (90%), fatigue (87%), and rigors (86%), arthralgia (72%). Loss of appetite, weight loss, and arthritis was also observed in 46%, 44%, and 24% of participants, respectively. The most prevalent clinical manifestations of brucellosis were fever, anorexia, weight loss, low back, and muscle pains in the study by Mahmood Nabavi et al. [7] and another similar study done by Kazanasmaz H [11], arthralgia (45.8%), fever (≥38.5 C0) (28.6%), and malaise and fatigue (11.5%) were the most common complaints at the time of presentation to the hospital [4].

29% of the patients with arthralgia presented with back pain, consistent with the findings of the study by Tasova Y et al. [15] and Turan H [16].

Mean neutrophil, lymphocyte, NLR, platelets, PLR, RDW, MPV, and CRP was found to be 56.3, 31.8, 3.22, 303, 15.67, 14.95, 8.39, and 61.58 respectively in our study. In a similar study done by Aktar F et al. {12], Neu – 71, Lym – 16, Plt – 282, NLR – 4.1, PLR – 154, RDW – 16.2, MPV – 8.2, and CRP-28.1. NLR can be determined from routine blood differentials at no additional cost, it increases in inflammatory conditions and this increase is considered as an indicator of systemic inflammation [13]. Studies showed that platelets also play an active role in inflammation while having regulatory effects on the immune system [14].

A similar MPV of 8.6 and a mean platelet count of 246 were also seen in the study done by Okan DH et al. [14]. Thrombocytopenia and lower MPV values found in our study are consistent with findings of other similar studies [12, 14]. Considering the same, MPV values in combination with platelet counts might be useful in patients with brucellosis [14].

C-reactive protein assay is a helpful adjunct in the diagnosis of patients with brucellosis [17]. High levels of CRP (61.58), SGPT (93.3), SGOT (99.7) was seen in our study, similar to the high CRP (39%), high ALT (33%), and high AST (20%) found in the study done by Shi et al. [18]. A positive correlation between standard agglutination titer and SGOT and SGPT was found by Huseyin S et al. [19].

AST, ALT, and CRP levels were found to be elevated in 27.6%, 21.6%, and 69.6% of the patients, respectively, and leukopenia (21.4%), thrombocytopenia (23%), and anemia (70%) in the study by Guler et al. [20]. 37% of patients were found to have hepatosplenomegaly on ultrasound, consistent with findings from a similar study done by Shi et al. [18].

Limitations

Only 77 cases could be included in the study. Not all the patients had all the laboratory tests done. Correlations were only weakly positive.

CONCLUSION

The most common clinical presentation of brucellosis was fever, arthralgia, abdominal pain, and headache. Elevated levels of CRP, procalcitonin, NLR, SGOT, and SGPT with thrombocytopenia and decreased levels of MPV were seen. A positive and significant correlation of CRP with NLR and PLR was also seen. Our study focused on the different clinical presentations and laboratory findings of brucellosis and will help give a better understanding and outline during brucellosis case diagnosis and management, in a more prompt manner.

Contribution of authors

Varsha Somakumar, V S Srikanth, Cibi Manoharan – concepts, design, the definition of intellectual content, literature search, data acquisition, data analysis, manuscript preparation, editing, and review; Vishal Marwaha, VR Mujeeb, M.G.K.Pillai – concepts, design, the definition of intellectual content, Manuscript editing and review; Anna Maria, Mehana Ashraf – data acquisition, data analysis, manuscript preparation, editing.

The manuscript has been read and approved by all the authors and each author believes the manuscript represents honest work. There is no conflict of interest among the authors.

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CLINICAL PRACTICE

Clinicopathological evaluation of Moyamoya disease. Case report and review of literature

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Abstract: *Moyamoya disease is a rare affliction, which progressively affects cerebral arteries, more frequently those in the circle of Willis and the internal carotid arteries. This condition has a genetic component, but the cause of the disease is unknown. It usually affects children but can affect adults as well, representing a health issue due to its mortality. Not in a few cases, Moyamoya disease has been difficult to recognize as a cause of ischemic or hemorrhagic strokes. In this article, we present the case of a young patient, with a medical history of cerebral hemorrhage, which was diagnosed with Moyamoya disease after clinical and cerebral angiographic corroboration in a specialized vascular center. The patient entered the Emergency Receiving Unit with a sudden comatose state. Paraclinical tests revealed massive intraparenchymal hemorrhage, with movement in the medial line and ventricular flooding.*

Keywords: *Moyamoya disease, stroke, cerebral death*

INTRODUCTION

Moyamoya disease is a rare progressive blood vessel disease characterized by progressive stenosis of cerebral arteries, more frequently those in the circle of Willis and the internal carotid arteries. A collateral blood flow is formed around the blocked arteries to compensate for the lack of blood flow, but those collateral vessels are small and fragile, thus increasing the risk of bleeding, aneurysm and thrombosis [1].

The name of Moyamoya disease comes from the Japanese language, where it means "cloud of smoke". This disease was discovered in 1957 as an internal carotid artery hypoplasia of the tunica intima [2].

This condition has a genetic component, but the cause of the disease is unknown. Patients may present chorea, headache, seizures, or cognitive impairment expression of transient ischemic attacks or ischemic stroke and a large proportion of patients, mostly adults presenting with intracerebral hemorrhage. Two age peaks are recognized: one during childhood and one in young adults [1, 3]. In most regions, women are more frequently affected than men [3].

Although Moyamoya is a vascular disease, it is associated with other medical conditions like autoimmune disease, Down syndrome, arteriosclerosis, neurofibromatosis type 1 or other multiple tumors developing in the spinal cord [4, 5, 6].

In this article we present the case of a young patient, with a medical history of cerebral hemorrhage, who was diagnosed with Moyamoya disease after clinical and cerebral angiographic corroboration in a specialized vascular center and we review the literature on this disease.

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CASE PRESENTATION

We present the case of a patient aged 39, from an urban environment, known with having a medical history of cerebral hemorrhage in the previous year (twice in 6 months). The patient was investigated in a specialized vascular center in western Romania, where she was diagnosed with Moyamoya disease and was recommended a course of treatment. She is brought into the Emergency Clinical County Hospital in Oradea, Romania, with a sudden comatose state which had come on several hours before. On physical examination, neurological testing reveals asymmetric mydriasis, bilateral flexion in the plantar cutaneous reflex, symmetrical dropping of the arms and feet on the bed and a Glasgow score of 3. Other examinations (skin, extremities, abdomen, musculoskeletal system) were within normal limits.

A cranial CT scan shows massive localized hemorrhage in the left temporoparietal region, of 5.6/4/1 cm, with ventricular flooding in the third and lateral ventricles and mass effect with movement in medial line of 8 cm (Figure 1).

Figure 1: Cranial CT image shows the hemorrhagic accumulation with fresh blood; intraparenchymatous temporo-parietal densities with 5.6/4.1 cm axial diameters; slight perilesional edema, ventricular flooding in the bilateral lateral and third ventricles, and mass effect, the median line is shifted to the right by approximately 8 cm.

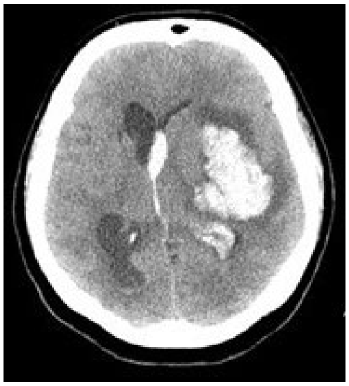

Laboratory results on admission showed hydro-mineral imbalance (hypokalemia and hypomagnesemia), which were corrected and remained within normal limits for the short duration of the patient's hospital visit. Complete blood cell count and examination of the peripheral smear showed normocytic anemia with normal leukocyte and platelet counts. Coagulation tests (D dimer, fibrinogen, partial thromboplastin time, prothrombin time, and thrombin time) were within normal limits.

The patient is then transferred to the Intensive Care Unit, where she receives orotracheal intubation and mechanical ventilation. Depletive and neurotrophic cerebral treatment is instituted, along with antibiotic therapy and acid-base and electrolytic balancing. The neurological state of the patient remains unchanged, with a Glasgow score of 3, fixed bilateral mydriasis and no cerebral reflexes. A control cranial CT scan is undertaken within another 48 hours, which shows the same collection of temporoparietal hemorrhage and no other modifications. The lateral ventricles have increased in size compared to the previous CT scan, with erased subarachnoid spaces (Figure 2). The suspicion of cerebral death is raised. After 72 hours, the suspicion of cerebral death was raised and the protocol in declaring cerebral death is followed, which is then confirmed by 2 EEG's, 2 apnea tests, ICU doctor, neurologist, neurosurgical assessment (Figure 3).

Figure 2: Cranial CT Image: the temporal-parietal intraparenchymal massive hemorrhagic left collection with ventricular intrusion in the right-side ventricle and ventricle 3 and relatively unchanged mass effect. The lateral ventricles have increased in dimension compared to the previous examination, bilaterally. Deleted subarachnoid spaces. Hypodensity of the cerebral parenchyma with deletion of the differentiation between the white subset and the newly developed gray substance which may suggest hypoxic ischaemia.

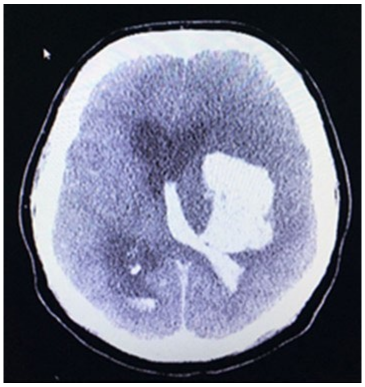

Figure 3: Evolution of patient

Day 1	- First hours, Glasgow 3 points - Emergency CT - Mass effect, with movements in medial line and compression of III and lateral ventricles
Day 3	- Glasgow 3 points - State of patient remains unmodified
Day 4	- Control CT - no modifications - Interclinical consultations for declaring cerebral death - Declared cerebrally dead

In the case we presented, the patient suffered a profound left cerebral vascular hemorrhagic accident, with three ventricular flooding and mass effect, as well as movement in the medial line by 8 cm, which made her comatose. Despite proper treatment, no signs of improvement were recorded. The family grants permission for transplant and investigations begin to facilitate organ transplantation. In the subsequent necrotic examination, a massive 7/6/3 intraparenchymal hemorrhage is observed, with erased channels and flattened circumvolutions, as well as reddish cerebrospinal fluid.

DISCUSSIONS

Moyamoya disease is a rare pathology, but its seriousness needs to make us aware of the risks that can appear. This disease is a medical problem from a variety of standpoints because it can appear in both children and adults, it progressively affects cerebral arteries through stenosis and occlusion and can lead to cerebral vascular accidents or repetitive transient ischemic attacks, which can kill the patient by themselves. Usually, children present symptoms attributed to slowly progressive cerebral hypoperfusion and repetitive ischemic symptoms, while adults have hemorrhagic or ischemic events [7-8].

The cause of Moyamoya disease is unknown, but it is believed that there is a genetic component involved [9]. According to some studies, this disease could be autosomal dominant, with incomplete penetration, depending on age and genomic factors [10]. Another study shows that the RNF213 gene is the main gene associated with Moyamoya disease [11]. In some studies, arterial stenosis was found to occur in not only the intracranial arteries but also renal, pulmonary, coronary, and pancreatic arteries. Renal artery stenosis is the most frequently reported associated with Moyamoya disease and the consequence is renovascular hypertension [12].

The walls of blood vessels have three layers in their structure: tunica intima, media and adventitia. From a morpho-pathological point of view, Moyamoya disease is characterized by the thickening of the tunica intima in internal carotid arteries bilaterally, which, at this level, can contain lipid deposits. The most frequently affected arteries are the anterior, middle and posterior cerebral arteries, which form the circle of Willis and can present with different degrees of stenosis and occlusion because of thickening in the tunica intima, curling in the internal elastic lamina and thinning of the tunica media.

The transversal sectioning of a basal artery in a patient with Moyamoya disease, with hematoxylin-eosin staining shows fibrosis and thickening of the tunica intima and proliferation of the smooth muscle cells (white arrows), as well as the folding and chronic contraction of the internal elastic lamina (blue arrows) and absence of inflammatory or atherosclerotic changes [13].

The incidence of the disease is greater in children around the age of five and adults around the age of 40. The condition is more frequent in women than in men, with a higher incidence in Asian population and a lower one in the European population. Women can present with transient ischemic attacks or cerebral hemorrhages in their medical history, or they can be asymptomatic. The recurrence in cerebral vascular accidents can be higher in these patients [14]. Clinically, patients with Moyamoya disease may present seizures, transient ischemic attacks, ischemic and hemorrhagic strokes. Headache, speech disturbances, visual deficits, intellectual deterioration, cranial nerve paralysis can also be seen in both children and adults. Mental retardation can be seen in children having Moyamoya disease.

Moyamoya disease is an important diagnosis to consider in children or young adults presenting stroke. Cerebrospinal fluid results of a lumbar puncture or electro-encephalography can be abnormal but are usually nonspecific. This pathology can be most frequently diagnosed in a quick fashion by using modern cerebral imaging methods such as an angio-CT, angio-MRI or 3D-DSA (rotational digital subtraction angiography), in a disease that is sometimes asymptomatic. Knowing all the radiological signs have a major influence on placing early and correct diagnosis, which can lead to prompt treatment and a possible increase in life expectancy in these patients.

Unfortunately, the therapeutic options are limited or inappropriate. Treatment in the acute phase is symptomatic for maintaining cerebro-vascular perfusion. Anticoagulant and antiplatelet agents, corticosteroids have shown no remarkable benefit. Surgical treatment procedures have been used to manage the hemorrhagic and ischemic consequences of Moyamoya disease to restore and maintain adequate cerebral perfusion, but there are serious adverse effects including cerebrovascular infarct or hemorrhage, motor or sensory dysfunction, cognitive decline, and death [15].

CONCLUSION

This pathology needs to be taken seriously because it presents a significant risk factor in creating cerebral vascular accidents, both ischemic and hemorrhagic, in young adults. Surgery can be beneficial, particularly if the diagnosis of moyamoya disease is made early. In patients with a history of cerebral accidents, angio-CT or angio-MRI and/or obtaining a protein RNF213 dosage would be recommendedto confirm or exclude Moyamoya disease in its early stages and institute proper specialized treatment.

Conflict of interests

None.

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VARIA

Cloning and surface expression of engineered enzyme of organophosphorus hydrolase in E. coli and evaluation of its function

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Abstract: *Nowadays, the chemical compounds of organophosphate cause biological contamination due to their presence in the environment and production of metabolites, which endanger the health of humans and organisms. Thus, it is crucial to use a safe method to eliminate the contamination of these compounds. The objective of the present study was cloning and surface expression of the engineered enzyme of organophosphorus hydrolase in E. coli and comparing its function with the non-engineered enzyme. The present study aimed at improving the function of the organophosphorus hydrolase enzyme by expressing this enzyme along with Ice nucleation protein (INP) on the surface of bacterial cells. For this purpose, the OPH enzyme coding sequence was cloned in the pET28a-InaV-N plasmid and sent into E. coli, the strain of BL21 (DE3) and protein expression was evaluated at 37° C after 16 hours of induction. To examine the site of expression of the recombinant protein, cell components (cytoplasm, inner membrane, and outer membrane) were separated and the results were analyzed by using SDS-PAGE. The results revealed that OPH enzyme protein was presented as a protein fusion with a molecular weight of 58 KDa next to the INP anchor on the surface of the outer membrane of the cell. The specific activity of the resulting protein was calculated at 3828.8 U/mg after incubation of this enzyme in the vicinity of the substrate at 37° C. based on the results of the present study, it seems that the expression of engineered enzyme and its presentation on the cell surface is an achievement in using the cell as a degrading agent of organophosphate toxins and eliminating the costs of downstream processes such as purification and refolding of protein.*

Keywords: *clone, expression, engineered enzyme of organophosphorus hydrolase, E. coli, function*

INTRODUCTION

Following developments made in a recent century in the chemical synthesis of materials, new compounds have been made, some of which are toxic. Releasing of toxic compounds into the environment such as pesticides, insecticides, solvents, explosives, and dyes in industrial and agricultural applications has increased over the last decade [1]. One of these synthetic compounds is organophosphate compounds that are used in pesticides, insecticides, and neurochemical agents. These compounds have three phosphoester bonds, so they are also called phosphotriester. Phosphorus is attached to oxygen P = O or sulfur P = S by a double bond. Paraoxon, parathion, and diazinon are considered important pesticides and insecticides made from organophosphate compounds [2]. War nerve agents include G-type and V-type. It should be noted that V agents are much more toxic than G agents. The sudden release or poor management of pesticide and insecticide residues has contaminated the soil and groundwater in the region. Also, residues of nerve agents after wars can have devastating effects on the environment

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and people. These compounds inactivate a large family of serine hydrolase enzymes (lipases and esterases) [3].

Acetylcholinesterase is one of the most important enzymes inactivated by these compounds, leading to the accumulation of acetylcholine in the nerve synapse and the continuation of nerve stimulation, and muscle dysfunction and impairment in the respiratory system respiratory failure, which results in coma and eventually death. Thus, it is essential to develop bioremediation technology to facilitate the degradation of organophosphate contaminants [4].

At present, most chemical and physical methods such as heat, superoxidase, carbon dioxide, etc. are used to remove contamination from organophosphate compounds, but they are associated with many shortcomings and disadvantages since they are often toxic, allergenic, corrosive, non-proprietary, and harmful to the environment [5]. Thus, safe technology is required to hydrolyze toxic compounds [5]. One of the useful methods for degrading the organophosphate compounds is the use of bacterial strains with enzymes that degrade these compounds with a wide substrate range.

Enzymes that as biocatalysts can degrade organophosphate compounds include organophosphorus hydrolase (OPH) derived from Pseudomonas diminuta and species of Flavobacterium, organophosphorus acid anhydrolase (OPAA) obtained from Alteromonas, and diisopropyl-phosphatase obtained from fish called Loligo vulgaris [6]. Expression of these enzymes in other hosts, including E. coli as recombinant, is one of the suitable methods for its production. OPH enzyme is present in Flavobacterium ATCC 75512 and P. diminuta.

The natural substrate for organophosphorus hydrolase is not known. This enzyme is a homodimer with a molecular weight of 72 kDa [7]. OPH can hydrolyze phosphotriester P-O bond in paraoxon, phosphonofluoride (P-F) bonds in DFP, sarin, and soman, phosphorothioate bond (P-S) in VX, and phosphoro amido cyanide (P-CN) bond in Tabun. The speed of OPH enzyme function is very high for a substrate with P-O and P-S bonds.

OPH is the only enzyme known for the degradation of both paraoxon and V-type war agents [8]. In a previous study, an engineered sample of this enzyme (creating a disulfide bridge to increase stability) was produced in the cytoplasm of E. coli. Despite changes in expression conditions, the enzyme was produced as an air inclusion and underwent difficult stages of unfolding and refolding [9].

In the present study, to solve this problem and express the enzyme in solution, by designing and synthesizing the anchor, the production of the desired enzyme was done as surface expression.

For this purpose, bioinformatics studies to design the best anchor were first considered, and then cloning, expressing, and finally measuring enzyme activity were performed.

MATERIALS AND METHODS

Preparation of materials required

Ecoli BL21 (DE3 (Novagen), pET32a (Biomatik), pET28-InaV-(Laboratory of Baqiyatallah University of Medical Sciences), LB-Agar and LB-Broth (Quelab), Yeast and Tryptone (Merck), Ampicillin, Kanamycin, Calcium chloride, XhoI, SacI (Restriction endonuclease), Agarose, DNA Safe Stain, Boric acid, Bromophenol Blue, 2-Mercaptoethanol, Taq DNA Polymerase, PFU DNA Polymerase, DNA Ladder-1Kb (# DM3200), T_4 DNA Ligase, Loading Dye, Ethanol 96%, Plasmid Extraction Kit, Fragment DNA Purification, Sodium dodecyl sulfate, Tris Base, Glycine, Glycerol, Coomassie Brilliant Blue G250, Glacial Acetic Acid, Tetramethylethylene-diamine, Isopropyl-β-thiogalactopyranoside, Acidleryed, B acrylamide Ethylenediaminetetraacetic acid, Triton X-100, Phenylmethanesulfonylfluoride, ammonium persulfate, Lysozyme, NaCl, Sucrose, and Urea (Sigma) were purchased.

pET28a-InaV-N plasmid

the pET system is one of the most powerful systems available for cloning and expression of recombinant proteins by E. coli. One of these systems is the pET-28a (+) expression vector, which includes the T7 promoter.

The gene can be cloned in the pET expression system and E. coli BL21 (DE3) can be transformed by the recombinant vector and expressed as a result of inducing by isopropyl β-D-1-thiogalactopyranoside (IPTG). pET28a includes an antibiotic marker of kanamycin. This plasmid has been used in previous studies and contains an anchor called InaV-N.

Figure 1 illustrates an image of this plasmid [10].

pET32a-OPH Plasmid

This plasmid has a T7 promoter for high protein expression and due to having a Thioredoxin tag sequence; it leads to secretion of the produced protein and has the antibiotic marker ampicillin.

This plasmid was used in a previous study conducted by Farnoush et al. The sequence of this plasmid can be seen in Appendix 1.

Figure 2 provides an image of the pET32a plasmid [11].

Figure 1: Plasmid pET28

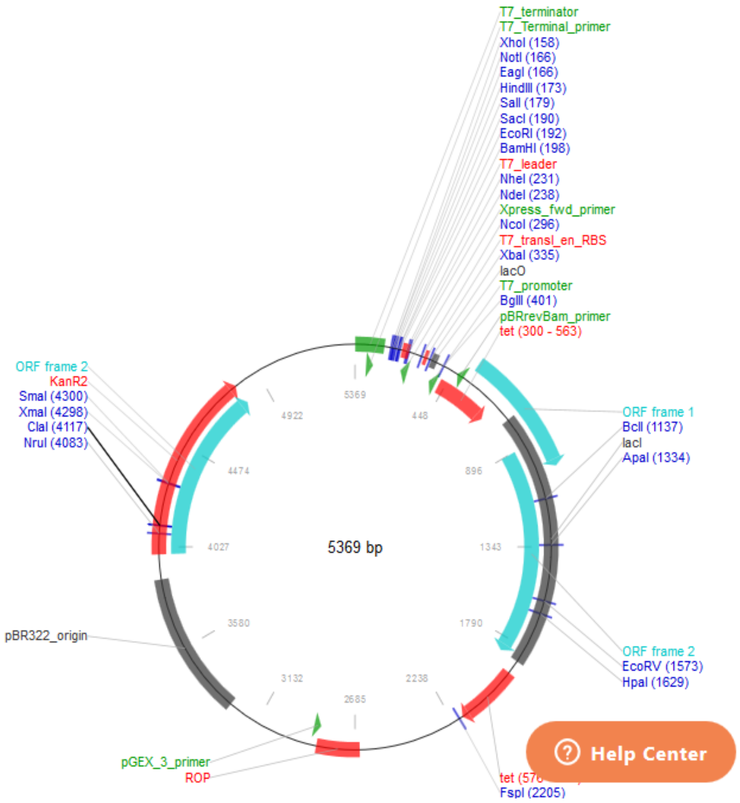
T7_terminator
T7_Terminal_primer
XhoI (158)
NotI (166)
EagI (166)
HindIII (173)
SalI (179)
SacI (190)
EcoRI (192)
BamHI (198)
T7_leader
NheI (231)
NdeI (238)
Xpress_fwd_primer
NcoI (296)
T7_transl_en_RBS
XbaI (335)
lacO
T7_promoter
BglII (401)
pBRrevBam_primer
tet (300 - 563)
ORF frame 2
KanR2
SmaI (4300)
XmaI (4298)
ClaI (4117)
NruI (4083)
ORF frame 1
BclI (1137)
lacI
ApaI (1334)
5369 bp
5369
448
896
1343
1790
2238
2685
3132
3580
4027
4474
4922
pBR322_origin
ORF frame 2
EcoRV (1573)
HpaI (1629)
pGEX_3_primer
ROP
tet (57
FspI (2205)
Help Center

Figure 2: Plasmid image containing OPH gene, The OPH gene is cloned between the two enzyme sites of BamHI and HindIII

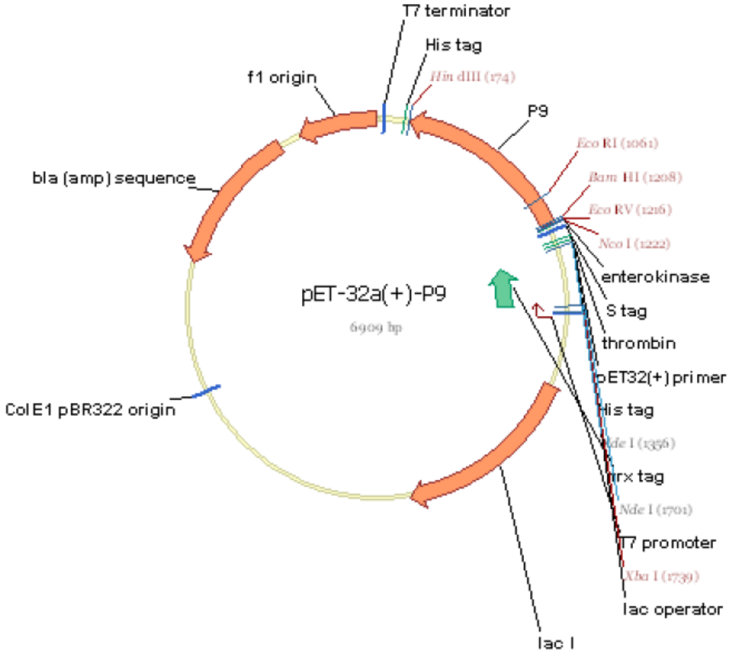
T7 terminator
His tag
Hin dIII (174)
f1 origin
P9
Eco RI (1061)
Bam HI (1208)
bla (amp) sequence
Eco RV (1216)
Nco I (1222)
enterokinase
pET-32a(+)-P9
6909 bp
S tag
thrombin
pET32(+) primer
ColE1 pBR322 origin
His tag
Nde I (1356)
trx tag
Nde I (1701)
T7 promoter
Xba I (1739)
lac operator
lac I

Plasmid purification

Plasmid purification was performed from strains containing plasmid pET28a and pET32a based on the standard protocol of the kit manufacturer. In short, 3 ml of the 18-hour culture of the strains was centrifuged and the supernatant medium culture was completely removed. The precipitate of cells was dissolved in 200 μl of FAPD1 buffer, 200 μl of FAPD2 buffer, and 300 µl of FAPD3 buffer and centrifuged at 250,000 rpm for 5 minutes. The transparent supernatant was poured on the columns for the kit and the columns were washed twice with 400 and 700 µl of W1 and Wash buffer and 50 µl of EB buffer was poured on each column and the desired plasmids were collected by centrifugation after 5 minutes [12].

Evaluation of purified plasmids by observing on agarose gel

Agarose gel electrophoresis is a standard method for isolating and identifying DNA fragments. A certain percentage of agarose is dissolved in the TBE buffer and, when cooled, forms a substrate that acts as a molecular sieve to separate DNA fragments based on their size in an electric field.

Polymerase chain reaction

For OPH gene amplification, two direct and inverse primers were required. These two primers were designed based on the initial and final sequences of the OPH gene. The direct primer is the complement at the beginning of the gene and the reverse primer is the reverse complement of the end of the gene. To insert this gene into the pET28a plasmid between SacI and XhoI sites, the cutting site of these enzymes was designed on direct and inverse primers and the synthesis order was given to the desired company (Table 1) [13].

Table 1: Primers of gene encoding OPH enzyme

Primer name	Primer sequence
Forward (p263)	5'_ CATGAGCTCTCTATCGGCACTGGTGAC_3'
Reverse (p264)	5'_ CATCTCGAGGCTTGCACGCAGAGTC_3'

Amplification of gene encoding OPH

To amplify the gene encoding OPH enzyme, the polymerase chain reaction was performed using designed primers according to the values presented in Table 2. PCR solution was prepared in 0.2 ml microtubes. In this reaction, the used model is pET32a, which contains the OPH gene, and a negative control sample was prepared according to previous values without the pET32a plasmid.

The microtubes were placed inside a thermocycler and reaction was done according to the temperature program presented in Tables 3-5 (Table 2) [14].

Confirming plasmid transfer into bacteria

To confirm the process of transfer of recombinant pET28-InaV-N/OPH plasmid into bacteria, PCR was performed for colonies grown on a solid culture medium. For each colony, some amounts were inoculated in 30 µl of sterile distilled water. Then, 15 µl of it was taken in 1 ml of liquid culture medium.

The remaining 15 µl was placed at 94° C for 15 minutes and then centrifuged at 13000 rpm for 2 minutes so that the broken cells precipitate. Supernatant media were used as a template in the PCR reaction.

The PCR reaction was prepared according to the values presented in Table 3 and performed according to the program of Table 4 [15].

Table 2: Values used in the PCR reaction to amplify the OPH gene

Material	Amount (µl)
Template (1 µg/µl)	10
Forward Primer (10µM)	2.5
Reverse Primer (10µM)	2.5
PFU SuperMasterMix 2x	25
Sterile deionized water	10
Total volume	50

Table 3: Values used in the PCR reaction for amplification of OPH gene

Material	Amount (µl)
Template (1 µg/µl)	2
Forward Primer (10µM)	0.25
Reverse Primer (10µM)	0.25
Taq 2x Master Mix RED	2.5
Total volume	5

Table 4: Values used in the PCR reaction to amplify the OPH gene

Cycle	Temperature (°C)	Time	Number of cycles
Primary denaturation	94	5 min	1
Denaturation Primer connection To lengthen	94 55 72	45 s 60 s 45 s	35
The final elongation	72	5 min	1

Enzymatic digestion

For cloning the OPH gene within the pET28a plasmid, both the amplified gene and the purified plasmid need to be cut using similar enzymes. It causes a complementary adhesive end on the gene and the plasmid and facilitates the binding of the gene to the plasmid.

Cutting both sides of the amplified gene was done using XhoI and SacI enzymes according to the values presented in Table 5.

The mentioned values were poured into 0.5 ml microtubes and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme [16].

Table 5: Values used for enzymatic digestion of gene amplified by PCR

Material	Amount (µl)
The amplified gene	50
Enzyme 10 Unit/µl XhoI	2
Enzyme 10 Unit/µl SacI	2
Universal buffer (10X)	7
Sterile water	9
Total volume	70

Cutting of plasmid pET28a using two enzymes of XhoI and SacI

The plasmid was cut according to the values of Table 3-10 by two enzymes of XhoI and SacI to linearize the pET28a-InaV-N plasmid and remove the previous fragment. The mentioned values were poured into a 0.5 ml microtube and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme (Table 6) [17].

Table 6: Values used in enzymatic digestion of plasmid pET28a

Material	Amount (µl)
Plasmid pET28a-InaV-N/OPH	5
Enzyme 10 Unit/µl XhoI	0.25
Enzyme 10 Unit/µl Sac I	0.25
Universal buffer (10X)	1
Sterile water	3.5
Total volume	10

Confirming entry of OPH gene into plasmid pET28-InaV-N by enzymatic digestion

To confirm the performed process and ensure the presence of the gene in the plasmid pET28-InaV-N, the plasmid was cut using two enzymes of XhoI and SacI with the values listed in Table 7. The mentioned values were poured into a 0.5 ml microtube and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme [18].

Table 7: Values used in enzymatic digestion of OPH

Material	Amount (µl)
Plasmid pET28a-InaV-N/OPH	5
Enzyme 10 Unit/µl XhoI	0.25
Enzyme 10 Unit/µl Sac I	0.25
Universal buffer (10X)	1
Sterile water	3.5
Total volume	10

Extraction of cut fragments from agarose gel using Kiagen kit

After cutting the gene and plasmid, it is necessary to separate these fragments from other extra fragments in the solution and then bind them together in a binding reaction. For this purpose, the product obtained from enzymatic digestion of plasmid was first taken on agarose gel and then extracted from the gel according to the kit protocol. The cut gene fragment was separated from the small cut fragments with the same kit without being placed on the gel. The gene was then purified by the clean-up method [19].

OPH enzyme protein expression

Plasmid gene-binding product was transferred into susceptible bacterial cells by the electroporation method. The culture was performed from colonies containing OPH gene plasmid in 5 ml of 2XY culture medium. When the optical absorption of the culture medium at 600 nm reached 0.7, after removing 1 ml of culture medium as an induction-free control, the promoter was induced by adding IPTG with a final concentration of 1 mM, and with the control sample, it was placed in an incubator shaker at 37° C for 18 hours. After the mentioned time, 1 ml of induced and control bacterial culture was precipitated and after adding 60 µl of 8 M urea, 16 µl of them were combined with 4 µl of sample buffer and heated for 5 minutes loaded on polyacrylamide gel [20, 21].

Primary evaluation of enzyme activity expressed on the surface

To evaluate the activity of the enzyme present on the surface, a precipitate of enzyme-expressing cells was collected and after twice washing with Tris buffer, 980 µl of cells with OD = 1 was poured into the microtube and 10 µl of cobalt chloride ion was added and 10 µl of parathion toxin was incubated for 1 h at 37° C [22].

Confirming surface expression of the enzyme

To locate the protein expression in the recombinant strain, it is necessary to first separate the cell components and then examine the fractions.

For this purpose, 20 ml of an overnight culture of control and test samples after protein expression, and their centrifugation and precipitate were dissolved in the lubricating buffer after washing twice in Tris buffer, and after half an hour of incubation at room temperature, the cell wall was broken with the sonicator device (4 30-second cycles with 80 power) was broken.

Then, centrifugation was performed at 2000 rpm for 3 minutes to separate the unbroken cells, and their supernatant was re-centrifuged at 14000 rpm for one hour. After emptying the supernatant into separate falcons, the precipitates were dissolved in Tris buffer containing Triton X-100 and 0.01 mM $MgCl_2$ and incubated at ambient temperature for 30 minutes to separate the inner and outer membranes.

Then, centrifugation was n performed at 13000 rpm for one hour. At this stage, the supernatant contains the inner membrane and the precipitate contains the outer membrane and the proteins attached to it. 500 µl of Tris buffer was added to both precipitates and dissolved.

Sampling was performed from each stage and examined using SDS-PAGE [23].

Evaluation of the specific activity of the enzyme in cell fractions

To evaluate the enzyme activity of the expressed OPH protein, a 50 mM Tris buffer was prepared.

The reaction medium containing 970 µl of Tris buffer, 20 µl of the sample (cytoplasmic samples, inner membrane, and outer membrane), 10 µl of cobalt ion with a concentration of 0.1 mM and 10 µl of parathion toxin with a concentration of 5 mg/ml were prepared.

After incubation at 37° C, the optical absorption of the samples was read at 405 nm and the specific activity of the enzyme was calculated using the following formula [24]:

$$\text{U/ml enzyme activity} = \frac{\text{dilution coefficient}\times\text{reaction volume}\times\text{absorption at 405nm}}{\text{time}\times\text{enzyme value}\times\text{PNP degradation coefficient}} \times 10^6$$

Reaction volume: 1 ml; Reaction time: 60 minutes; Dilution coefficient: 1; PNP degradation coefficient: 18000
Enzyme specific activity (U/mg) = normal activity (U/ml) / enzyme value (mg)

RESULTS

Amplification of OPH gene using PCR

In the first step, the OPH gene was amplified using p263 and p264 primers, and the PCR product was placed on agarose gel for confirmation. An observing band with a size of 1025 bp confirmed the amplification of the OPH gene. The OPH enzyme encoding gene is a 1008 bp sequence that, along with an enzyme site designed on primers, shows a length of about 1025 bp on the agarose gel (Figure 3).

Figure 3: OPH gene amplified by PCR.1- Marker # DM3200, DNA 1kb. 2. Negative control. 2. OPH amplified gene

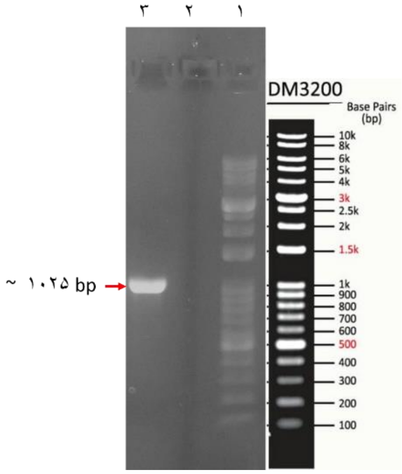

Evaluation of fragments obtained from PET28a-InaV-N plasmid cutting and PCR product on agarose gel

Plasmid pET28a-InaV-N and OPH gene PCR products were cut using XhoI and SacI enzymes and some of each reaction was loaded on an agarose gel to confirm the enzymatic digestion process. The 1017 bp band observed on the gel displaying the OPH gene and the 5978 bp band relate to the cut plasmid pET-InaV-N (Figure 4).

Figure 4: Fragments obtained from enzyme cutting of plasmid pET28a-InaV-N and PCR product. 1 and 2-fragments obtained from cutting OPH gene amplified by PCR.3-marker # DM3200, DNA 1kb. 4,5-plasmid pET28a- cut InaV-N

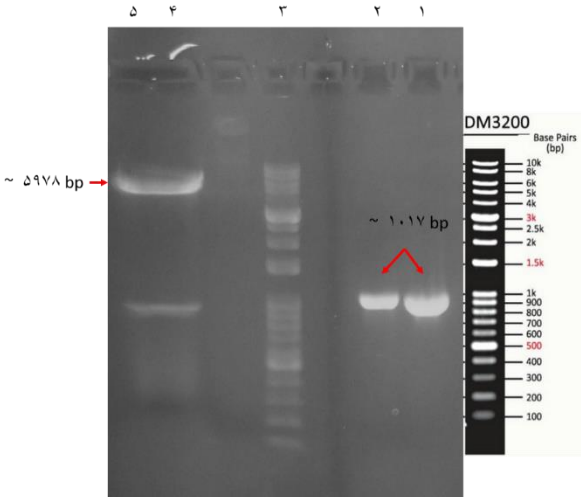

Extraction of plasmid and gene from agarose gel using Kiagen kit

After performing the enzymatic digestion process and examining the accuracy of cuts made with agarose gel, the area related to the desired fragment was cut and removed from the gel with the help of a special kit. The results of this purification were evaluated on a gel (Figure 5) and its concentration was measured with a nanodrop. The concentration of purified gene and plasmid was determined to be 144 ng and 82 ng, respectively.

Figure 5: Extraction of cut fragments from agarose gel. 1-marker # DM3200, DNA 1kb. 2. cut OPH gene

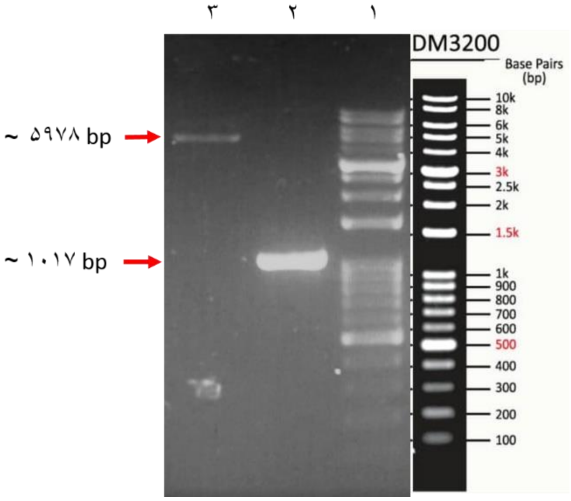
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Transfer of recombinant pET28a-InaV-N / OPH plasmid into E.coli BL21 strain (DE3)

After proximity of the OPH gene and pET28a-InaV-N at the presence of the enzyme ligase, the recombinant plasmid was transferred into a bacterium by electroporation E. coli BL21 (DE3) and cultured on an agar medium containing the antibiotic kanamycin.

As shown in Figure 6, plasmid-containing colonies grew overnight.

Figure 6: Colonies obtained from pET28a-InaV-N / OPH transforming into E. coli BL21 strain (DE3).

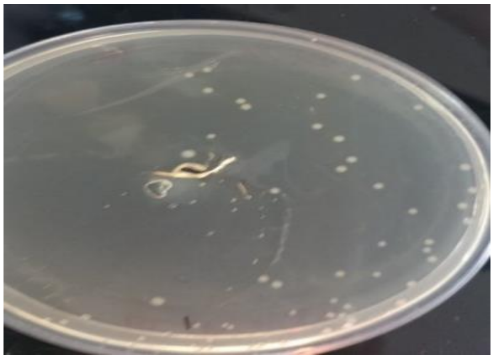

Confirming of colonies containing recombinant plasmid pET28a-InaV-N/OPH by direct PCR method from colonies

To evaluate the accuracy of transfer of the recombinant plasmid within the bacterial strain, 12 colonies grown on the medium were used for PCR. Among the tested colonies, the 1025 bp band, which is amplified by the OPH gene, was observed only in colonies 10 and 14.

The gene-free sample was considered as negative control and the sample containing plasmid pET32a-OPH was considered as a positive control (Figure 7).

Figure 7: Results of direct PCR of colonies grown on agar. Samples 1-10, 14, and 15 of PCR products of colonies. 11- DNA marker 1kb # DM3200. 12-Positive control. 13-Negative control

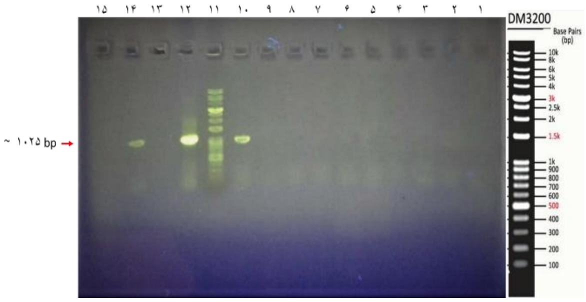
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Two-enzyme digestion of plasmid pET28a-InaV-N/OPH

The final confirmation of the presence of recombinant plasmids in BL21 strain (DE3) was performed by digestion with two enzymes of XhoI and SacI. The 1017 bp band obtained from the plasmid cut in Figure 8 indicates the presence of the gene.

Figure 8: Two-enzyme digestion of plasmid pET28a-InaV-N / OPH. 1. Uncut plasmid. 2-DNA1kb marker. 3. Cut plasmid.

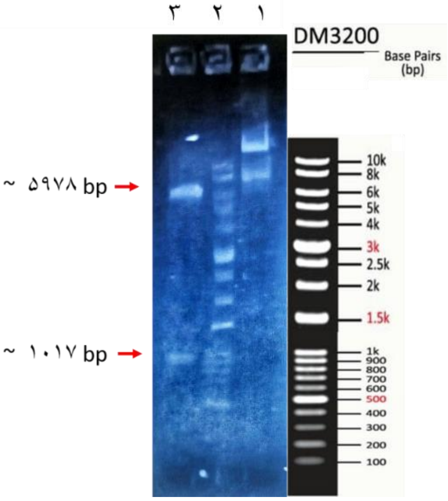
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Induction of expression for two confirmed colonies

Two confirmed colonies were inoculated in 5 ml of culture medium and expression was induced after reaching OD600 of culture medium to 0.7. The results obtained on polyacrylamide gel are as follows. As shown in Figure 9, the

58 kDa band is observed only in one of the test samples and is not observed in-band control sample.

Figure 9: Expression of OPH enzyme in BL21 strain (DE3) at 37° C. 1 and 5 - samples of the negative control. 2 and 4 - Induced samples after 5 hours of incubation at the presence of IPTG inductor. 3 - Protein marker # PM1700

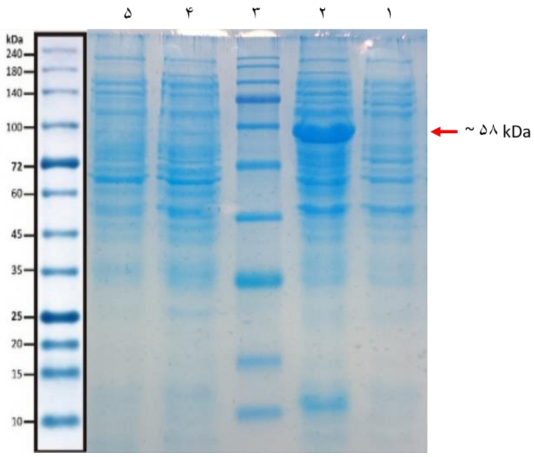

Evaluation of enzyme activity expressed on the surface

To evaluate the activity of the enzyme present on the surface at 37° C, 18 hours after adding IPTG, the precipitate of cells of test and control samples was collected and entered into the reaction medium. After one hour, the absorption of the reaction medium was read at 405 wavelengths and the enzyme activity was calculated. The samples include bacteria without the OPH gene and cytoplasmic enzyme-producing bacteria. As shown in Table 8, the activity of the enzyme expressed at the surface is lower than that of the enzyme produced as a solution in the cytoplasm.

Table 8: Comparison of enzyme activity expressed on the surface and sample expressed in the cytoplasm

Enzyme Activity (U/ml)	37^0 C
Gene without genes	0.74
Cytoplasmic expression	2.77
Superficial expression	7.67

Evaluation of the specific activity of OPH enzyme

After separating the cell components, the fractions related to each fraction of the cell were first examined by SDS-PAGE. Then, their activity was measured based on mentioned conditions, and the obtained data are shown in Figure 10. As shown in the figure, most of the activity is related to the outer membrane. It means the expression of enzymes on the surface of the outer membrane of the bacterium.

The specific activity of the OPH enzyme was estimated at 3828.8 U/mg using the formula mentioned in Figure 10 and Table 9.

Figure 10: Separation of cell fractions after protein expression at 37° C

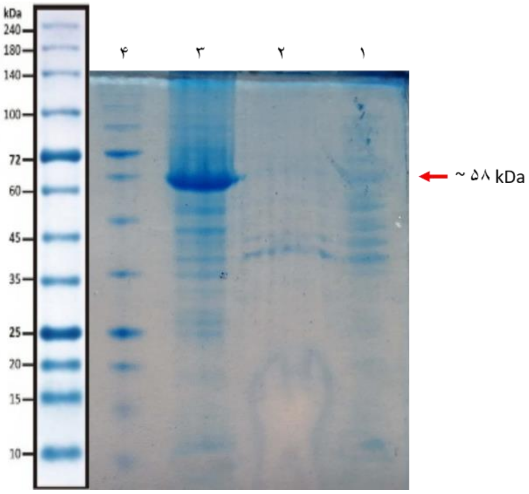

2. Cytoplasmic fraction. 2. Inner membrane fraction. 3. Outer membrane fraction. 4-Protein marker # PM1700

Table 9: Examining the enzymatic activity of each cell fraction

The activity of each cell segment (U/ml)	37^0 C
Cytoplasmic section	3.6936
Internal membrane section	1.2162
Outer membrane section	7.6576

DISCUSSIONS

Organophosphate compounds, including pesticides and neurochemicals, are very dangerous and insoluble in aqueous systems. Despite their high toxicity, organophosphate pesticides are still widely used to protect plants in agriculture. Due to the accumulation of these compounds in food products and water resources and its irreversible effects on human health and other organisms, it is essential to develop an advanced, appropriate, and cost-effective method to eliminate these compounds from the environment. Among the various physical, chemical, and biological methods, the use of natural strains as biodegraders has high importance. Strains such as Pseudomonas diminuta MG and Flavobacterium sp. ATCC 21881, which uses organophosphate molecules as food sources, can degrade a wide range of these compounds, including pesticides and neurochemical agents [25]. The degrading enzyme identified in these strains, called organophosphorus hydrolase (OPH), contains 336 amino acids and has a molecular weight of 36 kDa. The gene encoding this enzyme was used for expression in the cytoplasmic space of BL21 (DE3) bacterium after two mutations at sites 99 and 124 and altering two amino acids of threonine and glutamic acid to cysteine to create a disulfide bond, but its expression was mainly accumulated as protein masses in the cytoplasm and was dysfunctional and without proper structure [26, 27]. Thus, the bacterial cell must first be lysed and after extracting the accumulated proteins, they must be solubilized with a strong denaturator and the refolding process must be continued in the presence of appropriate stabilizers. All of these steps require much time and money, and finally, with the loss of a part of the protein, a protein is obtained that often does not regain its structure 100% and does not have good stability [28]. Another form of OPH enzyme expression is performed as periplasmic expression. In research carried out in 2015 by Latifi et al, the OPH enzyme encoding sequence in E. coli was synthesized and the OPH enzyme was expressed in the form of air inclusion using pET21a-opd and pET26b-opd in periplasmic space. Periplasmic expression of this enzyme also leads to limited production of this enzyme and requires complementary processes such as bacterial cell breakdown and protein extraction from the cell [25]. Besides the various methods proposed for the expression of recombinant proteins as a solution, including reducing the temperature, reducing the concentration of inducer, and using sequences that help solubilize the protein, the use of membrane anchors to provide protein on the bacterial cell surface is one of the most effective strategies used to express a wide range of proteins and has yielded promising results since molecule when attached to a matrix-like the cell surface is more accessible and much more stable than the free molecule and does not require long and costly purification steps [26, 27]. The use of natural integral proteins of the outer membrane as carriers is necessary for the goals of presentation on the surface. A variety of surface anchor motifs such as Lpp-OmpA, INP, and auto-transporters are used in this system due to their properties [31]. OPH enzyme expression in E.coli has been performed as a surface expression by various researchers. For example, in a study conducted by Richins et al in 1997, OPH enzyme expressed the OPH protein on the surface of E. coli using an Lpp-OmpA anchor system [32]. Shimazu et al in 2001 examined the expression of OPH protein by three anchors, including Inak-NC, InaV-NC, and Lpp-OmpA. The Inak anchor motif was derived from P. syringae KCTC 1832 and the InaV motif was derived from P. syringae INA [33]. Dong Kang et al in 2008 used only the second InaK amine to provide OPH on the surface [34]. In each of these studies, the OPH enzyme was expressed by different anchors on the cell surface. As a surface expression of OPH enzyme in previous studies in E. coli has resulted in the production of functional and highly stable proteins, and since the engineered OPH enzyme used in this study has been previously expressed in the cytoplasm due to its disulfide bond in the air inclusion and non-functional form, this sequence was re-expressed differently in E. coli in the form of surface expression using the InaV-N anchor. The results of investigating the activity of different parts of cell fractions showed that the fraction related to the outer membrane has the highest activity and has an activity more than twice as much as other cell fractions. Inav-N anchor not only can transfer OPH protein on the surface of bacteria but also has a positive effect on its activity.

CONCLUSIONS

The main objective of the present study was a surface expression and improving the efficiency of the engineered enzyme OPH (containing disulfide bridge) which was expressed in the previous study as air inclusion. The use of InaV-N anchors, which is one of the known anchors for surface expression, responded well to the desired enzyme and the enzyme could maintain its activity after surface expression. In a conclusion, this study could solve the problem of the insolubility of the protein, maintain its structure and its desirable activity, while surface expressing the engineered protein using the InaV-N anchor on E. coli bacteria.

Acknowledgment

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Colorectal cancer epidemiology and risk factors

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Abstract: *Colorectal cancer is an important health problem, being the third most frequent cancer pathology both in men and women. Programmes have been developed to decrease CRC incidence and mortality, including primary prevention strategies focusing on population education regarding diet and physical activity and secondary prevention programmes such as screening. Modifiable risk factors are known to be diet, obesity, smoking, alcohol and read meat consumption. Non-modifiable risk factors are age, inflammatory bowel disease, genetic syndromes and family history of colorectal cancer.*

Keywords: *colorectal cancer, risk factors, genetic syndromes, Lynch syndrome, diet, obesity, diabetes*

Colorectal cancer is an important cause for mortality and morbidity all over the globe and recent data from the United States of America report it as the third most frequent cancer pathology both for women and men. [1]

According to American Cancer Society, the risk for developing colorectal cancer during lifetime is 1:23 for men and 1:15 for women. [2] In industrialised western countries, the lifetime risk for such malignant pathology is approximately 5%. [3]

World Health Organization reports approximately 1.9 million new cases every year. [4] Incidence and morality due to colorectal cancer increase with age, most of the cases being diagnosed around 70 years of age. [5] During last years, it has been reported an increasing in incidence with 1 % for people 50 to 64 years of age and by 2 % for people younger than 50 years. [6] Because of increasing life expectancy, people migration and changes in daily habits, it is expected that by 2035, there will be 2.4 million new colorectal cancer cases world wide. [5]

In Romania, the incidence of CRC is 10.1/100000 in men and 7.3/100000 in women. [4]

All over the world, strategies have been developed in order to reduce colorectal cancer incidence and mortality. These strategies cover primary prevention such as educational programmes for healthy life style and secondary prevention, such as screening programmes. [7, 8]

Regarding the risk factors involved in colorectal cancer pathogenesis, they can be divided into two major groups:

1. Non-modifiable risk factors: age, sex, personal history of adenomas, personal history on inflammatory bowel disease, family history of colorectal cancer or genetic syndromes

2. Modifiable risk factors: diet, physical activity, weigh gain, medication, alcohol consumption and smoking

1. Age is an important non-modifiable risk factor, as colorectal cancer risk starts to increase after the age of 40, and most of cancers are diagnosed after 50 years, with a

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maximum of incidence between 60 and 70 years of age. [9] Latest reports show an increase in CRC incidence in 20-49 years of age persons and so it becomes one of 10 most frequent cancers for this group. [6, 10]

As for differences between sexes, it appears that colorectal cancer is more frequent in men that in women, regardless of the age group, with 4.4% risk precent in men and 4.1% in women. [11] There is an interesting discussion regarding the distribution of precursor lesions in men and women. In women, adenomas are more frequent in proximal colon while in men, adenomas have a distal grow pattern. [12] This information in useful in choosing the best method for screening both in men and in women. [13]

During lifetime, the risk of developing a tubular or villous colorectal adenoma is approximately 19%. [14] 95% of sporadic CRC develop from adenomas, as they are known to be precursor lesions. [15] Interestingly, not all adenomas evolve in colon cancer. Most of them will regress. It usually takes approximately 10 years for a polyp to transform in a malignant tumor. [16] So there is an opportunity window in order to discover precursor lesions through screening. This is why people with personal history of adenomas should be monitored with colonoscopy, in order to decrease the risk of developing colon cancer. [17] Also, an important topic to debate in this case is the role of modifiable risk factors that maybe influence in a positive manner by reversing the process of transformation from adenoma to carcinoma. If it is really so, it would be quite difficult to demonstrate it.

Another important risk factor for developing colorectal cancer is the personal history of inflammatory bowel disease, meaning Crohn disease and ulcerative colitis. The severity of endoscopic and histologic inflammation, the duration and the extent of colorectal lesions are important elements that increase the risks of neoplasia. [18, 19, 20] Patients with inflammatory bowel disease have a sixth time higher risk of developing colorectal cancer, comparing to normal population, and the risk is even higher for those with extensive colitis. [21]

Although approximately 75% - 80% of colorectal cancers are sporadic forms, the rest of 20-25% are diagnosed in persons with familial history of colorectal cancer [22] especially in first degree relatives of patients with colorectal cancer diagnosed before 60 years of age [23]. In these familial cases, genetic factors and life style work together.

Genetic syndromes are involved in 5-10% of diagnosed colorectal cancer. The best known and studied genetic syndromes are:

- Lynch syndrome - hereditary non-polyposis colon cancer (HNPCC)

- Familial adenomatous polyposis (FAP) with its subtypes: attenuated FAP (AFAP), Gardner syndrome and Turcot syndrome

- MUTYH - associated polyposis (MAP)

- Peutz-Jeghers syndrome (PJS)

Lynch syndromes is the most common inherited CRC syndromes as it accounts for approximately 2-4% of cases. [24] The risk of developing colorectal cancer during lifetime is about 70-80% and the median age at diagnosis is usually around 40 years of age. [25] This hereditary syndromes is causes by a mutation detected in the genes responsible for DNA repair (for example MLH1, MSH2 or MSH6). People with this condition can also develop gastric, pancreatic or uterine cancers. [26]

In the case of familial adenomatous polyposis (FAP), the risks of CRC cancer is given by the germinal mutation of APC gene from chromosome 5q21. [27] In this case, the patient is diagnosed with hundreds or even thousands of polyps in colon and rectum having a major risk of transformation in colon or rectal cancer before the age of 20 years. [28] This disease is diagnosed in less than 1% of population. [27]

People with AFAP are diagnosed with less than 100 polyps during colonoscopy and the higher risk of colorectal cancer is around the age of 40. [29] Regarding the other attenuated forms of FAP, the high risk for colorectal cancer remains and is associated with other forms of non-cancerous tumours of skin, bones or soft tissue as in Gardner syndrome or with medulloblastoma as in Turcot syndromes. [24, 28]

In MUTHY-associated polyposis, the risk of cancer is also reported at a young age. In this case, colon polyps appear because of MUTHY gene mutation, a gene that is involved in "proofreading" the DNA. People with this condition also have an increased risk of thyroid and other gastrointestinal cancers. [29]

Peutz-Jeghers syndrome appear because of STK11gene mutation. Is a very rare syndrome. It is associated with increased risk of hamartomas in digestive tract and breast, ovary and pancreatic cancer, beside colon cancer. [30]

2. Taking into consideration that 75-80% of colon cancer are sporadic forms, it is considered that environmental, dietary, cultural, educational and social factors play an important role in this disease occurrence and, by educational programmes, it is a preventable cancer. This statement is supported by population studies focused on health of migrants and their children. It was observed that in migrants that came from low incidence colorectal cancer countries,

the incidence of this neoplasia starts to increase during lifetime and their children have the same incidence of colorectal cancer as the people in the adoptive country [31, 32]. Also, the incidence is higher in urban area than in rural area. [32]

For many years, a large number of studies and meta-analysis tried to pinpoint the risk factors for colorectal cancer. The conclusion was that high consumption of read meat or processed meat, heavy alcohol consumption and smoking, high mass index and lack of physical activity are clear factor that can raise the risk ok colon cancer. [33-35] Data show that by consuming 5 portions of red meat per week, one can have a 13% increased risk of developing colon cancer. [28] In contrast with this data, fruit and vegetables can reduce the risk of colorectal cancer by 15% if consumed for as many as 3 portions per day. [36]

Alcohol and smoking are considered to produce inflammatory damage of normal colon mucosa, leading to formation of polyps. Although the exact mechanism by which alcohol affects colorectal mucosa is not completely understood, in the case of cigarette, it is considered that carcinogens are the ones to be incriminated in this process.[37] Studies showed that there is a 3 fold higher risk for smokers to develop colon polyps. [38]

Weight gain and maintaining a high body mass index can promote inflammatory state through inflammatory cytokines and adipokines. [39] This process favours angiogenesis and colon carcinogenesis. In this case, the protective role of NSAID, in reducing inflammation, especially Aspirin is quite easily to be understood. [40]

The EPIC study demonstrated that by adopting a healthy life style, meaning weight control, sustained physical activity, lack of smoking, low consumption of alcohol and read meat can actually decrease the risk of developing colorectal cancer. [41]

Given the important role of diet, constant exercise and maintaining a normal body index in preventing colorectal cancer and consistent with EPIC study results, World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR listed a number of recommendations for healthy population and also for colorectal cancer survivors [42] A summary of these recommendations is:

- Body mass index - one should maintain as thin as possible without becoming underweight
- Physical activity - at least 30 minutes per day
- Restrict as much as possible sugary drinks
- Consume vegetables and fruits
- Limit red meat and avoid processed one
- Limit alcohol intake
- Limit salt consumption
- It is preferred to have all nutrients mostly from diet and less from supplements
- Breastfeeding for women

An interesting discussion is linked to patients with diabetes. It appears that they have a higher risk of colon cancer, although this can be related to increased body mass and sedentary, studies demonstrated that even with correct diet and physical activity, they still have an increased risk of CRC than that of general population. [34]

Also, the use of insulin in the treatment of diabetes it appears to be related to a higher risk of CRC diagnosis and a worst prognosis than the patients with diabetes treated with metformin. [43]

Much discussion takes place in the last 10 years regarding microbiota role in colorectal cancer carcinogenesis. It is certain that in patients with colorectal cancer, colonic dysbiosis is reported. Several bacterial species might play a role in CRC such as Streptococcus bovis, Enterococcus faecalis, Fusobacterium spp or Bacteroides fragilis [44-46] Pro-carcinogenic results of the presence of these micro-organisms are described to be: genotoxicity, inflammation, oxidative stress, host defence regulation [47] More studies are in progress analysing the correlation between microbiota composition changes and molecular pathways of colorectal cancer

CONCLUSIONS

Taking into account all the modifiable risk factors, the need for primary prevention programmes is clear in order to decrease the burden of colorectal cancer incidence.World Health Organisation listed recommendations to be taken into consideration by general population regarding diet, physical activity and weight control.

Regarding non-modifiable risk factors, the secondary prevention programmes must be considered crucial for people with family history of colon cancer or for those diagnosed with inherited familial syndromes.

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VARIA

Common artifacts in myocardial perfusion imaging

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Abstract: *Myocardial perfusion imaging (MPI) is an important non-invasive imaging method, which allows the assessment of perfusion in the cells of the myocardial wall. The images are obtained after administration of a radiopharmaceutical at rest and/or after maximal stress (physical effort or pharmaceutical stress). Infarction areas or scars will not show any uptake of the radiopharmaceutical, neither in rest nor at stress (fixed defects), while the ischemic area will show reduced uptake at stress with recovery to normal perfusion at rest (reversible defects).*

MPI is a complex technique and it requires an experienced team to foresee, avoid, correct or minimize the artifacts that might appear in all the phases of the study

In this paper, we will present the most important categories of artifacts, based on the category of problem where they were generated. We will also present some solutions for limiting these artifacts or for recognizing and minimizing their effect for those which cannot be avoided.

Keywords: *myocardial perfusion imaging, artifacts, a non-invasive imaging method*

Myocardial perfusion imaging (MPI) is an important non-invasive imaging method, which allows the assessment of perfusion in the cells of the myocardial wall, after administration of a small quantity of radioactive material – a radiopharmaceutical. MPI is an imaging modality that helps identify the differences between myocardial perfusion at rest and stress (Figure 1).

The most used radiopharmaceuticals in MPI are 99mTc-MIBI and 99mTc-tetrofosmin as they distribute to the myocardial tissue proportional to the blood flow thus to the cell perfusion. The radiopharmaceutical is intravenously administered to the patient at rest and/or after a maximal effort – either physical effort or pharmaceutic stress. The pharmaceutical stress is achieved by administering substances that enhance blood flow in the healthy myocardium; this will lead to consequently decrease in variable degrees in ischemic territories. Therefore, the infarction areas or scars will not show any uptake of the radiopharmaceutical, neither in rest nor at stress (fixed defects), while the ischemic area will show reduced uptake at stress with recovery to normal perfusion at rest (reversible defects).

After the administration of the radiopharmaceutical, the patient is placed inside the gamma camera (SPECT scanner). With the rotation of the system detector around the patient, a series of planar images are obtained; these images (acquisition raw data) will later be used to obtain reconstructed views in the three-section plans of the heart: short axis, long horizontal axis, and long vertical axis. Two data sets of images are obtained in this way, illustrating

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myocardial perfusion at rest and stress.

Figure 1: MPI, 99mTc-tetrofosmin, one day protocol (stress-rest). Reversible defect in inferior wall

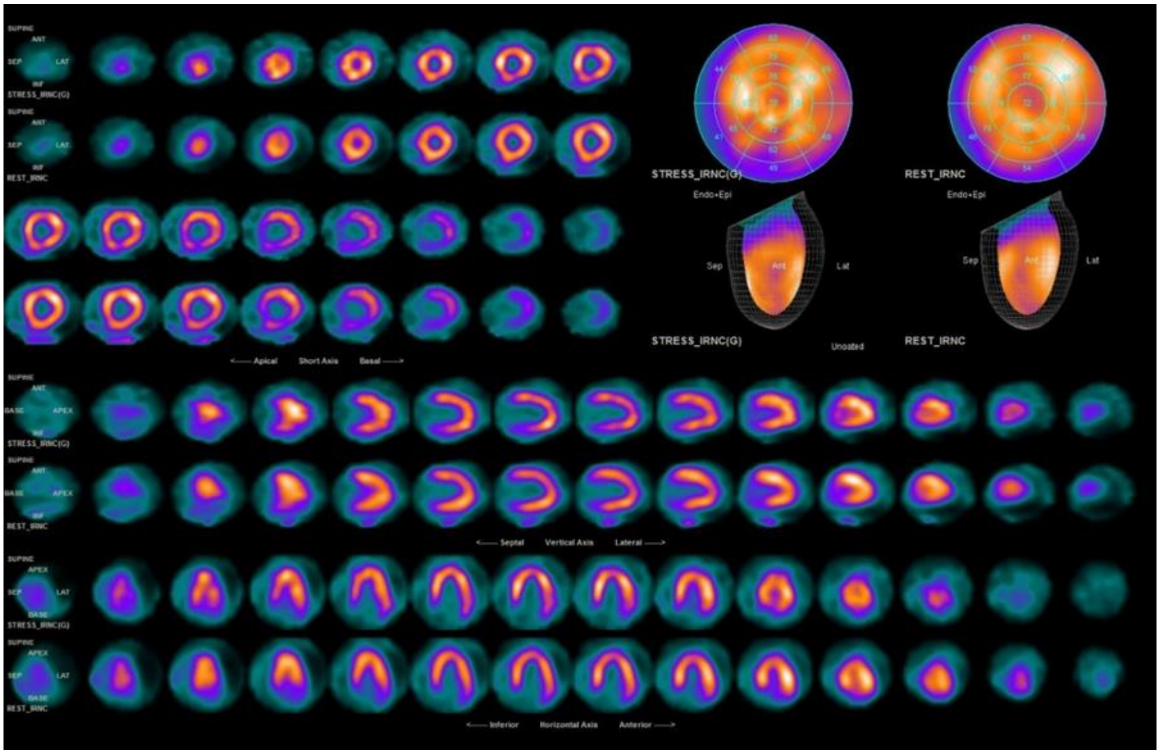

Modern systems allow image acquisition, synchronous with the ECG, resulting in a reconstruction that will provide information about the myocardial dynamic parameters like volumes (telesystolic and telediastolic), the systolic function, and systolic thickening.

In normal conditions, the myocardium of the right ventricle will not be seen on MPI. It can however show some degree of uptake in conditions that associate its hypertrophy.

MPI is a complex technique and it requires an experienced team to foresee, avoid, correct or minimize the artifacts that might appear in all the phases of the study.
Many types of artifacts can interfere with the MPI/ Most important of these artifacts can be classified according to the category of problem where they were generated (Table 1):
- problems related to the preparation of the investigation
- technical aspects involving scanning, processing, and the work protocol
- patient-related aspects: moving during the scan, attenuation of photons within the body, associated pathologies, intestinal radioactivity.

Problems within the preparation of the investigation

The investigation must be well prepared because neglecting some of the steps might lead to major artifacts or even compromise the study.

For the stress study, the patient is advised to stop medication containing beta-blocker or any other treatment that might prevent reaching the maximum heart rate [1]. Normally, the use of pharmacological stress is preferred for patients who can't perform physical effort that would lead to reaching a minimum of 80% of the maximum heart rate.

During the preparation of the radiopharmaceutical, it is necessary to follow a strict protocol to fulfill the radioprotection standards but also to avoid a higher percentage of free 99mTc (pertechnetate – 99mTcO$_4$). Free 99mTc - 99mTcO$_4$ has another physiological distribution compared to the 99m-Tc-MIBI/tetrofosmin. Furthermore, a higher free fraction will lead to a considerable shrinkage of the active doses.

The administered doses have to be sufficient and adjusted according to the patient's weight to obtain the awaited distribution, This is particularly important for one-day stress-rest studies that involve two injections. In this case for the second investigation is required a sufficient dose, because photons from the first administration might still be detected.

Table 1: Most common artifacts in MPI

Artifacts related to the investigation preparation)	Administering medication that limits the maximal heart rate at stress	
	Preparation of the radiopharmaceutical	
	Incorrect dose of administered radiopharmaceutical	
	The extravasation of substance during intravenous administration	
Artifacts due to technical issues	Equipment errors	Uniformity
		Rotation center
		Detector alignment
		Attenuation correction system
	Processing and postprocessing errors	Incorrect choice of section plans
		Choice of non-adapted filters
	Protocol errors	Non-compliance with the optimal post-injection acquisition time
		The short time between rest and stress studies (one-day protocol)
Patient-related artifacts	Movements during the scan	
	Photon attenuation within the body	Diaphragmatic attenuation
		Breast-induced attenuation
		Adipose-induced lateral defect in obese patients
	Associated pathologies	Left bundle branch block
		Arrhythmias
	Subdiaphragmatic Activity	
	Anatomical variations (apical thinness, shorter septum)	

The radiopharmaceutical is administered intravenously by trained medical personnel, avoiding extravasation.

Technical aspects involving scanning, processing, and the work protocol

Technical issues can be related to the scanning equipment (components of the scanner), image processing, or acquisition protocols.

The equipment requires daily check-up – both the gamma camera and the equipment required for measuring de radioactivity dose. There might be certain errors concerning the scanner's components that would lead to the appearance of artifacts and therefore to make the image interpretation more difficult.

Among the most frequent technical issues that can lead to image artifacts, we can name uniformity, misalignment of the rotation center, detector alignment, and desynchronized function of the attenuation correction system. All of these errors can be avoided or, at least, reduced by following a strict, periodical quality control protocol with systematic checking of the medical devices [1-3].

Processing of the acquisition raw data might be an important source of artifacts in the final images. Luckily, if the acquisition data were correctly obtained in a standard protocol – the processing procedure can be restarted and the artifacts corrected. The most frequent causes of artifact due to processing are related to incorrect filter application or mistakes in the reconstruction – heart orientation, choice of the section plans [4]. Processing errors can generate either false-positive or false-negative results.

Imaging acquisition at a shorter timespan after the administration of radiopharmaceutical than recommended might generate weaker statistics and faulty images. Additionally, for the one-day stress-rest protocol – the second investigation in a shorter timespan might considerably affect the quality of the study.

A good positioning within the scanner is required to get a full heart image.

Patient-related aspects

Artifacts resulting from patient movement during image acquisition might considerably influence the interpretation and in some cases even mimic a perfusion defect (false positive study).

The phenomenon of photon attenuation within the body is a common cause of artifacts. These are generally fixed defects visible both in rest and on stress images. Specifically, if there are no possibility of correcting the attenuation (e.g., through simultaneously transmission scanning as in CT) attenuation defects can be differentiated from real defects through gated SPECT. The territories which show attenuation defects will always present normal parietal kinetics [2, 5, 6]. Generally, there are three sources of soft tissues attenuation defects: the presence of big breast in women (anterior wall defects), diaphragm (especially in men – it determines false inferior wall defects), or in obese patients with important lateral adipose tissue (defects of the left ventricular lateral wall)

Some associated nonischemic pathologies can influence the appearance of the perfusion study. They are most commonly represented by arrhythmias and by the left bundle branch block.

Left bundle branch block can determine anteroseptal defect, either fixed or reversible, leading to a decrease in the specificity of MPI in this category of patients [4, 7, 8].

Other groups of pathologies that can influence and lead to artifacts are represented by arrhythmias, which might significantly influence ECG-gated studies.

Important subdiaphragmatic activity (hepatic or intestinal) is due to the excretion of the radiopharmaceutical through the hepatobiliary system. These phenomena can interfere with the radiopharmaceutical uptake on the inferior wall in two ways: (a) high uptake on the inferior wall (Compton effect) with the cover-up of a possible perfusion defect and (b) relative decrease of uptake on the inferior wall, next to the intestinal area of high uptake, a tributary to the use of reconstruction algorithms (false-positive results).

The subdiaphragmatic activity can be reduced through a low-intensity physical effort (resulting in a lower splenic activity), large quantities of fluids or even eating high-fat foods that might bias the radioactivity in the distal GI tract [5, 9].

Some anatomical variations might of course impact the visualization – e.g., the presence of a thin myocardial tissue layer at the apex might mimic a perfusion defect, as does a shorter sept (Figure 2).

Figure 2: MPI, 99mTc-tetrofosmin, one day protocole (stress-rest). Fix defect in apex (apexian thinning) and short septum

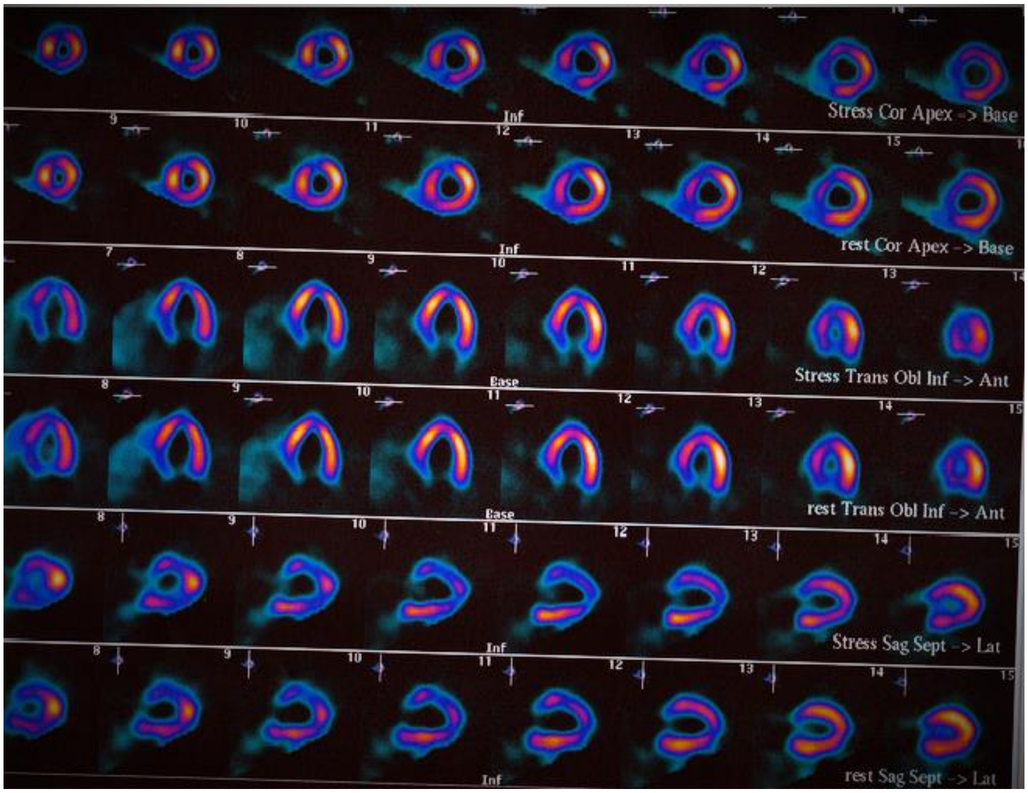

CONCLUSION

MPI represents a high-performance imaging modality that relates to advanced knowledge in pharmacology, pathology, and clinical cardiology combined with high-level technical aspects in medical devices and image processing systems. The role of a well-trained multidisciplinary team is mandatory for performing and interpreting the study. With the correct implementation of working protocols and the use of standardized methods, the presence of artifacts can be limited. However, there is several pitfall or artifacts that cannot be avoided due to particular conditions. In this case, it is important to recognize artifact changes with result in better accuracy of the procedure while maximizing the benefit of this imaging technique in routine clinical practice.

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VARIA

Comparative study on the independent learning activity of young people in the bachelor's and master's programs

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Abstract: *During the late adolescence, it can be reached the highest level of mental operative functions and other intelligent human manifestations. Abstract mental operations can be done at this level: synthesis of syntheses, generalization of generalizations, abstraction of abstractions. Students go through many attempts to find which is their personal style of learning new materials, new contents. The study was conducted on a number of 31 participants. The group was divided into two subgroups: 16 students in the 5th year at Faculty of General Medicine and 15 master students in the second year at University of Sports, Sports Performance program. The used instrument consists of 21 questions (11 questions are about the individual studying process of the participants and 10 are about their beliefs about the teaching process of academics in courses), along with 4 more questions related to personal data. It is an opinion questionnaire. The teaching style of academics differs: those of Faculty of General Medicine are focusing on a lecture type style (75%), and University of Sports on an interactive style (67%). We discovered differences in the process of self-learning: between continuous and discontinuous style. For medical students, there was a longer distribution of the self-taught learning process; instead, University of Sports students organize their learning time in the last part of the semester. University of Sports students are more concerned with on the development and refinement of their personal practical skills, but also of the subjects they work with (kids or athletes), their goal being to accumulate their own experience as rich as possible from working with as many individuals and less to take from the experience of other colleagues. The coach-athlete/sports teacher-pupil relationship is more personalized and longer-lasting than the doctor-patient relationship.*

Keywords: *laser Doppler vibrometry, middle ear*

INTRODUCTION

1. Learning activity and its role in summative assessment

1.1. Learning activity for young people - generalities

During the adolescence period (considered between 14/15 and 20 years), the individual seeks his own identity and begins the stage of abstract thinking. Hayford [1] highlights the student period of 18/20-24/25 years as a prolonged adolescence. The young adult age is between 20-40 years and assumes that the most important aspects are the intellectual skills that will be used in professional life and the foundation of intimate and personal life.

Around the age of 17-18, the highest level of mental functioning and the manifestation of human intelligence is reached. The operations of thinking are fully liberated from the informational contents to which they were initially applied, they are generalized, they are transferred and they become formal. Thus, the second-degree operations are

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successful: syntheses of syntheses, generalizations of generalizations, abstractions of abstractions.

Piaget describes the stage of concrete operations as ending around the age of 12-13 years old, when adolescents are able to perceive the constancy of objects in terms of transformations, regarding structure, volume, in a nutshell, the ability to understand the fact that objects are constant in relation to the notion of flexibility. After the age of 13, up to 17-18 years, the stage of formal operations is completed, when young people manage to think inferentially, including the possible, with the issuance of assumptions and the definition of specific pathways for each option seen separately. It defines the logic, the concepts of contradiction, certain grammatical structures of the “double negation” type, the detection of the grammatical concepts of circumstantiality and attributiveness and the optional-conditional, which could be seen differently. Also, an important role is played by the concept of “perceiving the absurd” or of the possibility to operate on the determinants, of the empty set, in the sense of superior mathematics.

During late adolescence, there is a strong conflict related to the balance between maturity and childhood, the balance between needs and how to meet them, due to the relationship between aspirations and possibilities and the conflict between dependence and independence.

Some authors [2] consider that adolescence is a period of confusion, but also a period of future projections that the adolescent makes. The desire for self-improvement appears, emotions and feelings are amplified, moral autonomy and self-awareness are developed, the adolescent has the feeling of independence. There are feelings of disagreement and rejection of ideas imposed by other people.

The period of youth, between 20-24 years [3] is considered a period of transition to adulthood. The acquisition of economic autonomy begins, leading to greater independence. Levinson [4] calls the period from 17 to 22 years - the period of young adulthood and considers that it is characterized by the coexistence of the adolescent and the young adult status. He considers that it is a period of transition dominated by the transition of the young man from the state of child-man to that of young man.

Young people begin to become autonomous, move away from the family environment, from the model imposed by their childhood, create their own social system and thus structure themselves as individuals and part of their generation. The aspirations, the ideal about the world and life, the value system begin to particularize and become constant, proper in the young person's life, giving coherence to his/her actions.

Learning is the main activity of young people. They have a very high capacity to assimilate information, to incorporate it. They also show an increased availability for this activity. The preoccupation for learning comes from an internal motive towards self-improvement, continuous development, search for existential meanings and senses. They are beginning to lose the importance of the school assessment, grading and ranking component, focusing on the activity of acquiring the necessary knowledge in the desired field. According to Erikson [5], the adult stage involves: amplifying social identity, employment and involvement in social life, intense feeling of love, the beginning of family life and the development of intimacy, the oscillation between isolation – intimacy - the tendency to link relationships with others groups.

Knowledge and, in general, the intellect has a wider notional area than the psychic process of memory. In turn, memorization is only the first part that defines memory as a high psychic function, the other two aspects being the preservation of information and updating (which can be in the form of recognition or reproduction). The intellect is the superior form to which the definition of the personality of young people accesses, this including the peaks of the "iceberg" represented by each function or psychic process. In the absence of memory, life would be a "ball of sensory impressions", structuring the continuity of the psyche leaving "gaps" in the network in which students submit their information. If we refer strictly to the psychic process of memorization, we can describe the concept of "buffer", in which many students run, more or less information, depending on individual abilities, some being those who learn early and cope with information exams stored in long-term memory, others having as a favorite profile the loading of the buffer (this being a capacity, an ability in itself) and having remarkable results with the information stored here. The persistence over time and the effectiveness of this type of long-term learning in relation to valid and valuable expression in the future profession is to be discussed.

The overall mental operations are developed and many specific algorithms are formed. The motivation for vocational learning is strong, especially practical work-related learning. Another important aspect is that learning becomes strongly selective, conscious and voluntary. The formation of hypothetical-deductive thinking appears when the young person has the capacity to abstract, to separate the notion of object from a concrete reality and the possibility to analyze his own thoughts. He/she begins to understand and deduce the cause-effect, singular-particular-general connections. The ability to apply in practice with an individual character is also formed - the young person has the tendency and ability not only to acquire new knowledge, but also to use it to create something new, integrates it much better [6].

Young people go through new challenges once they enter the university/vocational education system related to important exams, different classification system, different grading system, different type of examination, university structure and so on.

1.2. Self-training

Youth has an emphasis on the need and desire to learn more on their own, with autonomous effect. A style that focuses on the need for continuous renewal of one's knowledge through a constant personal effort is preferred, an effort that opens the perspective of developing the skills to learn alone, of autonomous and independent training. These self-taught systems will gain ground, because they respond better to self-motivations, an internal motivation for self-improvement and training, for self-completion, each individual having the opportunity to cultivate and better value their own abilities. and skills. Thus, the individual becomes his own "manager" of his intellectual, cultural and professional progress and plays an active role in the development of his cognitive function and his own training.

Self-training is defined as an integral part of self-education [7], which refers to the enrichment of knowledge and independent intellectual training. It involves a motivational impulse and the acquisition of tools of independent intellectual work. Individuals create their own self-training system, with personal, adapted techniques, which are based on his style. These involve: choosing the necessary information, systematizing it, processing the learning content, using the content to solve some learning tasks (exercises that help to integrate the learned content), self-control and so on.

Self-training is considered a form of manifestation of cognitive activism [8], a difficult and complex process that is not summarized only during university studies, but throughout the life of the individual. It is considered that the self-taught activity requires great efforts, ability to organize, to organize his/her own personal space, concentration on the task, developed motivational sphere, permanent control and self-improvement. This type of learning is common in high school. It is based on awareness and formation of personal appreciation of the domain taught. Metacognition is the theoretical foundation behind self-training. Self-regulated learning is based on self-responsibility.

The following question also arises: why a person cannot have a self-regulated learning throughout all learning experiences. Other theorists [9] believe that, when they are young, children cannot be self-regulators during learning in any formal way. In addition, comes Piaget's theory [10] that explains that against self-learning learning comes the egocentrism of the young child. And Vygotsky [11] emphasizes the importance of young children's inability to use inner language in guiding roles.

It is important to mention that the self-training activity must in turn be learned and repeated. Students go through many attempts in order to find out what is their personal style of deepening the teaching courses, their contents. Dordea [12] states that there are three sub-competencies that students must master in order to succeed in the process of self-teaching: self-appreciation of the personalized strategy, realization/implementation of that strategy, self-evaluation of results. Basically, the student has to "learn how to learn".

The individual learning project can be improved through two main ways: 1) indirectly, through experience; and 2) directly, through training. Thus, individual study can be encouraged through recurrent positive experiences (students notice that self-assessment is positive and that self-instruction increases the accuracy of learning), similar to the theory of positive reinforcement.

It is important that adolescents and young people acquire self-regulatory skills during their development. As the time passes, they need to process an ever-expanding volume of information and operate with knowledge at increasingly abstract levels. An essential feature of self-regulated learning is the dynamic approach of the topics to be learned. Thus, the student can analyze the teacher's comments and compare them with his own perceptions of the learning situation. Can self-learning lead to a burn-out syndrome due to the pressure and informational volume? Recent scientific research based on evidence-based medicine is highlighting the increased number of young people that are developing burn-out syndrome, sometimes due to medical conditions (neuroendocrine for example) that leads, in the severe form, up to depression [13].

The situation of students who become pregnant at a young age should also be discussed as a separate perspective (even if they have a lower frequency in the University of Medicine or Sports, given the specifics of learning or activities/ practical works). However, their number should not be neglected, especially in terms of pregnancy hormones, which cause mood swings, which can influence the ability of learning, given the motivational-volitional links with those of mnesic-prosexic processes and learning, especially in the amygdala area and the hippocampus area, brain areas significantly involved in both emotional motivational regulation and superior gnostic functions, so that they are active at the optimal level in terms of supporting the effort to complete a university curriculum [14, 15].

1.3. Adult education

The educational process of adults must respond to the challenges posed by today's society, which is changing its rhythm very quickly. Giddens [16] proposes a series of characteristics of late modernity, as typical elements for contemporary western society: industrial capitalism, reflexivity, individual responsibility. Modernization has accentuated digital education, with the help of digital technologies in many fields, from trade to education, medicine, teachers [17].

Adult education in Romania takes place in the context of another type of modernity than that of the western countries, namely tendentious modernity [18]. This concept refers to the fact that there are differences between the style in our country and the western one, due to some older concepts in our system, which makes the modernization to be partial and unfinished.

Adult education occurs only by virtue of personal own vision of the project of developing society, with all the implications of the coexistence of the old with the new, of progress with conservatism, of the modern with the traditional. It aims to improve existing skills and/or acquire new ones. It also aims to assimilate system values that have previously proven social performance and put them into practice. Adult education asserts itself in different forms of education: continuing education, non-school education, non-formal education.

There are a number of phenomena that prevent the formation of adults: inequality of opportunities in education, inequalities between levels of education (between educational units from the same area, between students), inequalities of access between faculties. In several cases these inequalities have a huge impact on the developing personality of an adult person even more if this personality closes the borderline type or reaches the psychiatric connotations [19]. There are several causes that are against unitary access to educational levels: poor material endowments, underfunding of educational units, differences between schools in urban and rural areas, problems related to human resources in schools, school dropout, repetition, the existence of a segment of the population that does not take advantage of the educational system. The higher the level of schooling, the higher the value of the additional costs of education that the family has to cover, discussing here for example: the fee for college, food expenses, textbooks, rent [20]. Nowadays, recent strategies involving the cost of education are trying to keep the step with full on-line education system, additional costs covered by the family have changed their levels: for example, a higher investment in modern communication devices. In the same time, the educational system redirects its costs based on this new on-line strategy.

Lifelong learning involves everything a person does throughout life to complete his/her knowledge, learn new things, acquire skills or develop his/her talents in formal, non-formal and informal contexts.

It is important to note that education, from one level onwards, is optional. Post-school training is often a personal choice. Unfortunately, there are no mechanisms in society to encourage adults to learn continuously. They may limit themselves to the knowledge gained in school due to routine, inability to cope with change, difficulty in economic and financial management, disinterest in performance.

It is observed that young people who have obtained a higher education have: information processing capacity that can be extended to other cognitive areas, the operability of thinking is kept at high levels, the young person's thinking is wide, deep, systematic, rigorous, very good mental adaptability to specific professional. Young people have critical thinking [21].

1.4. Evaluation - types of evaluation

Evaluation is a component part of the educational process. It informs us about the effectiveness of teaching-learning strategies and methods and appreciates the correct choice of operational objectives on lessons. The teaching-learning-evaluation relationship presupposes a cause-effect circle, in which each component conditions the other. If we are thinking at the western model, the evaluation should not be considered a control method that would punish those who fail it. Students must understand the learning content, not only memorize it, and the given task must be contextualized, in which the subject can express his/her learning style.

Evaluation can be of several types. It differs depending on the type of classification. According to the criterion of the moment at which it is performed, the evaluation can be:

- initial (in the initial phase of the instructive-educational process) - aims to see what is the level from which to start,
- continue (throughout the training program) - aims to observe the improvements along the way and to achieve the optimization of the educational process,
- summative (at the end of a session/module).

The evaluation can also be: formal and informal. The formal one presupposes well-defined criteria, previously outlined and it is associated with standardized tests, it presupposes a certain level of expertise of the one who performs it (License type exams, Baccalaureate, National Assessment etc.). Informal assessment is based on intuitive criteria and involves tests created by the teacher.

There is another classification of evaluation: internal or external. The internal one is made by the internal teacher (the one who designed the learning content is the one who evaluates it). In this case, the evaluator knows the particularities of the students, is familiar with them, knows their progress along the way. The external evaluation is performed by a teacher who has not been involved in the instructional-educational process and has the advantage that it is objective.

The evaluation can also be of normative and criterion type. The normative one has the following characteristics: the performance of the subjects is related to the group norm, to the reference group (class, year, respective series) and compares the students, it ranks them. Criterion evaluation presupposes that performance is related to an absolute criterion, to a standard. Evaluate absolute success in evaluation.

According to some other authors [22-24], evaluation can be: comparative (has the role of classifying subjects, relating them to each other, according to their level of success), based on a criteria (has the role of providing functional information, allowing subjects to be in in relation to achieving the objectives common to all subjects), corrective (wants to provide the subject with additional information depending on the difficulties he encountered), conscious or formative (oscillates between cognition and metacognition and promotes active participation and autonomy of the subject so that he/she, subsequently, takes control of his/her own transformation, becoming aware of its shortcomings and difficulties).

The traditional assessment methods are: oral tests, written tests and practical tests. There are also complementary, more modern methods, among which: systematic observation of behavior, investigation, project, portfolio, self-assessment [25].

Sometimes the psychological and physical overload based on a frail personality structure could lead to an interpretative phenomenon of key components of an evaluation, that, for the student, seems to be debatable. Risks will lead later on to anxiety and depression sometime at psychiatric levels [26, 27].

1.5. Summative evaluation

It is also called cumulative evaluation. The act of evaluation involves three stages: measurement, assessment of results and adoption of improvement measures [28]. It is performed at the end of a session/module. It has the purpose of accreditation/decision to improve future activities. It is performed by an internal or external evaluator, is done at long intervals and aims to see the final results of the students. It tries to test the final and accumulated knowledge that the students have acquired. It is important because of its dual role. It is useful for the trainer, to see if the chosen means and methods are useful, effective, if the information has arrived correctly and as quantitatively as possible. It is the feedback he receives from students. It is also useful for students to objectify what information they understood, what should have been clarified in more detail, etc.

It is a type of balance sheet assessment, global, at the end of relatively large activity segments. The disadvantage of this type of evaluation is that it does not allow the regulation/ improvement of the didactic process; it is possible only after relatively long periods or never. Thus, trainers cannot change the teaching-training style to improve the results of their students, because they cannot make a second summative evaluation. However, it highlights the level at which the activity took place and its results, recognizes the efforts made by the subjects and the interest shown by them. It also offers the satisfaction of the social recognition of the subjects' competencies. At the level of society, these cumulative assessments certify that subjects are able to cope with certain tasks and requirements and that they can play certain roles in social life.

It is well known that grades, often, do not exactly reflect the true level of performance of the subjects and as a result, are not stimulating and do not provide sufficient data on the effectiveness of the training program. It is advisable to use it together with other types of assessment, such as continuous assessment, to rely on continuous feedback to cooperate the teacher with the students [29].

MATERIALS AND METHODS

The present research aimed to highlight some differences in the learning-teaching process of young people at the level of students at the Faculty of General Medicine, compared to those at the National University of Physical Education and Sports. We tried to observe what is the time that young people aged 18-25 allocate for individual study in their preparation for the summative assessment and the level of awareness of the need to be involved in the self-taught process. We highlighted the perception of students in relation to the teaching style of academics. We also sought to highlight possible psychological motivational explanations for interpreting the results.

The tool used was a questionnaire that consists of 21 questions (11 questions are related to the individual study process of young people in the group, and 10 are about the teaching process sustained by the professors at courses), along with four more questions to highlight the factual data. It is an opinion questionnaire. The participants received the appropriate training to complete the answers and were assured of the confidentiality of the data, their results being used only for the purpose of this research. The questionnaire took about two minutes to complete.

The tool was uploaded in google forms and sent to the participants to form the database. The data were collected between April 15-25, 2020.

The study was performed on a number of 31 participants. The group was divided into two groups: 16 students in the 5th year at the University of Medicine and Pharmacy- Faculty of General Medicine and 15 master students in the second year at The National University of Physical Education and Sports, the Motor Performance program.

The ages of the participants ranged from 22 to 26 years. There was female (18) and male (13) participants.

RESULTS

Regarding the methods of education and training in young people, we highlighted the teaching style of the university staff from the two faculties and we noticed differences between the style of them, one activating in the field of health and the other one in the field of sports (Figure 1).

Figure 1: Teaching style of academics (percentage of teachers)

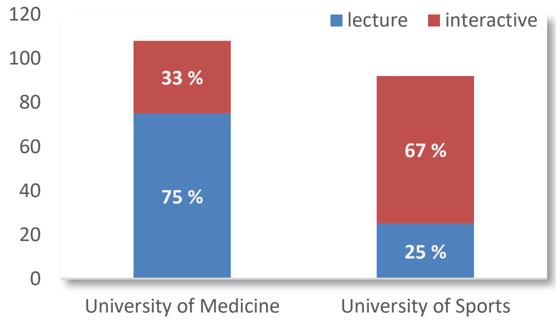

Although, currently, the teachers from the medical university are increasingly open to a more interactive teaching style, it is important that a well-structured lecture is based on a diagnostic tree, and the mastery of logical thinking by the medical student implies, in for the most part, the acquisition of diagnostic tree-type thinking. This is the reason why many medical teachers prefer a lecture style because it is better suited to the large learning content, compared to those in the field of sports that emphasize the practical part of the theorized content.

Medical professors focus more on teaching "thick materials" in a lecture-style method when they are addressing to a homogeneous group of young people, with preliminary and extensive concepts from the pre-clinical years and for whom the information presented makes sense and it is easy to understand. For more difficult subjects, students prefer a direct teaching method, in which they manage to write down the information as accurately as possible, in order to have the chance of a second or third passage through the materials represented by their own notes, in the idea that the information it was so abundant that the student (less familiar with information gaps from previous years) could not be assimilated. For such students, who have become more accustomed to the learning required to go to the University of Medicine, the lecture-method makes it less difficult for students to be less thorough, as they need accurate exposure of information and at a slower pace, so that the lesson can be more easily assimilated.

Regarding the presentation style of the information in the courses, the total distribution at the level of both faculties is presented in Figure 2 – presentation style. We consider that it is a too high percentage of teachers who read the presentations in the courses, being more a monologue type course, than a bidirectional one in which the information is incorporated by the student. It is true that of this 48%, 66% of respondents were medical students. This suggests, again, that the need for knowledge, the dictionary that future doctors must master, is much greater than that of sports specialists. Thus, it is absolutely necessary for health teachers to exhaustively expose, perhaps sometimes arid and dull, medical terms that students must master.

Figure 2: Presentation style

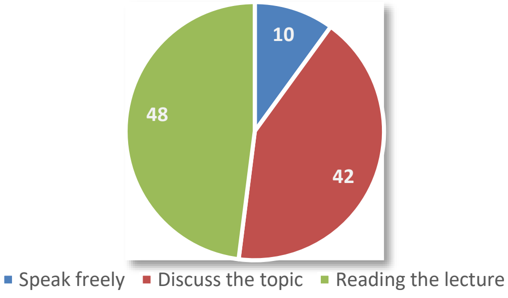

This research can show the need for certain teaching materials that must be presented in a readable and slow tempo. This need is related to a psychological concept regarding the teacher's ability to establish a connection between the unconscious mind of the students (who gradually enter in a learning state) and the unconscious mind of the teacher (the later having a more systematized information). The slow tempo prepares the student to enter the scientific atmosphere in a preliminary psychic state, kind of an awaiting state of mind for the "key" information, which comes gradually. The student must receive the information with the same curiosity, interest and respect for the simple information. These will represent clues for the future job, because the causal links cannot be understood exactly if the students expect only interesting and spectacular information and they pay attention only when these are exposed.

Most students (81%) of both groups estimated that a course has about 10-20 pages. All subjects accepted that they reopened the materials for the information taught in their practice.

To the question that probed the need to individually read and understand the content of the courses, the students from the sports university answered, in a percentage of 100%, yes. On the other hand, in the group of medicine students, the distribution was uneven. This shows us that at the university of medicine is a much more detailed and exhaustive content that requires explanations from teachers, while at the sports university is more emphasis on the practical side, students having the necessary knowledge to understand the content of the courses. The explanation can be seen in the Figure 3.

Figure 3: The need to extract the main information individually from the course content (number of students)

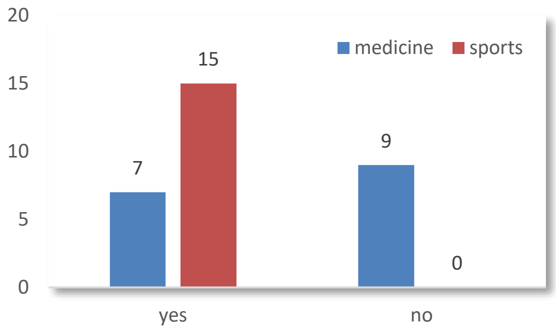

Regarding the information presented at either of the two faculties, if we refer to the high-level learning, more exactly, the preparation for master's or doctorate for UNEFS students versus the preparation for bachelor's or residency for medical students, it is necessary to explain the detailed information in time, for longer periods in terms of dosing effort. It is well known that for grade 7, students must go through the subject once, for grade 9, twice, and for grade 10, three times. This is especially important for difficult materials, which include arid parts (and less practical demonstrations), the preclinical subjects in the first years of the Faculty of Medicine or the theoretical parts of some less publicized sports. Also, the preparation of any important exam involves dosing the effort, thus, in the first days one learns about four-five hours a day, and in the last ten to fourteen days, in the memory has to run for the last time the whole subject, with the intelligence to focus on the key concepts and logical causal links.

The results showed that most medical students' courses have more than 7 pages, which makes them spend more time learning the content needed for summative assessment. It should be noted that the material to be learned from the Faculty of General Medicine follows a general pattern that involves a predetermined order: prevalence and incidence of the phenomenon/disease, etiological aspects, pathophysiological aspects, characteristic symptoms, their grouping in syndromes, main diagnosis, differential diagnoses, evolution and prognosis (positive factors, negative factors), treatment options. It is known that most of the time is spent understanding the pathophysiological mechanisms behind the diseases, but in the field of health it is absolutely necessary to go through these arid mini-chapters to understand the phenomenon in full, compared to specialists in sports, where the accent is on practical application. In addition to changing/developing learning skills in relation to the age and experience in student years, learning styles also change in relation to the years spent in a particular faculty, with the involvement in adapting to the specifics of that faculty. This capacity of adapting involves flexibility of thought and intellect and, of course, experience. Once familiarized with the most important concepts in the field of activity, after about five years in a faculty, students, master students, doctoral students or residents achieve the performance of reading about 20-30 pages of scientific content with which they are familiar, without feeling the effort, with pleasure, interest and the possibility of thinking in parallel for developing their own opinions. In the last category, the teaching-learning mechanism is reversed, starting from practice to conceptualization, which is more attractive for students because they enjoy the results of concrete work faster (Figure 4 – the learning processes).

At the level of both educational structures, students similarly reported the need to take notes during the courses, but two respondents from the medicine university noted that most courses receive written support which made them not need to take notes. We emphasize here the importance of distributing written course support to students.

Figure 4: The learning processes.

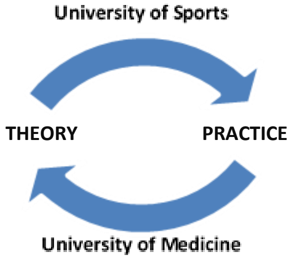

We consider that the educational instructional process is much more effective in the case of the existence of written materials after which the student can be guided later, during the individual study process. The main activity of the student during the course should be to focus attention on the topic of the course, on the information that the teacher has to offer. We are trying to adhere to a western education system, in which the modernization of the process with the help of technologies is desirable. We consider it appropriate to emphasize the link between taking notes and the facilitation of the active memorization, later learning and reproduction the material. There are students who manage to go through large materials (hundreds or thousands of pages) through active learning and using a specified type memory, the one from which the information drains unconsciously in a passive way. Others are focused on compiling their own notes, on taking notes, failing to store information if it is not processed and systematized in their personal way. We do not comment on the superiority of any of these techniques, but we want to emphasize different ways of how the mind function. The personal notes, as the individual dedicates himself to the profession, can become more and more schematic, becoming understandable only by the one who writes them. These personal notes can exclusively define causalities, "key" concepts, inferences, connections and fewer mnemonic formulas (these being typical for an earlier stage learning).

Another aspect to note is the desire for the lecture not to be completely similar to the course support and it proved to be more efficient to listen to the lecture, when the student does not focus on finding those notions in the written material, but on understanding the explanations given by the teacher and following the logical thread of the lecture, proving much more effective than the situation in which the student reads the learning content beforehand. The notions described in medical courses are difficult, imply new and unknown terms to the student, which makes it important to lecture and especially to be accompanied by explanations, examples that make the learning content more accessible. That said, the lecture is a material more understandable to the student than some course notes in medical terminology. In this case, the role of the lecture is to lower the level of understanding from abstract to concrete, from general to particular. Instead, in the sports environment, the provision of specialized materials is more easily accessible at first reading even to an audience unfamiliar with the field, but the difficulty lies in the practical applicability of the theoretical content.

In both Universities, students rated similarly on a Likert scale from 1 to 10 the "weight" – the difficulty of a course, somewhere at a grade of 7-8. This suggests that the teaching content is reported to the type of students taking part in the course. Thus, the participants do not have a comparison model, all the courses are of the same similar level within his university. At the both faculties we suggest the need to introduce courses with graded difficulty, thus the difficult subject is exposed at the, approximately, seventh-eighth meeting with students, after they have acquired interest in the studied information and have sufficient knowledge to be interested in deepening the subject.

We wanted to probe the students' conscientiousness about the self-instruction process for summative assessment. The Figure 5 shows the difference between continuous learning (throughout the year) and discontinuous learning (exhaustive period during the session, in which they only prepare for assessments). For medical students, there was a longer distribution of the self-taught learning process, instead, the sport university students organize their learning time in the last part of the semester.

Figure 5: Distribution of the self-training process over a longer period of time (number of students)

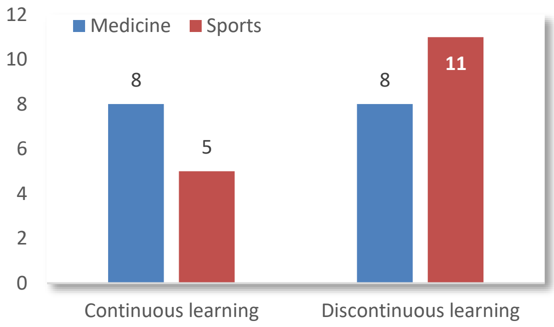

Figure 5 shows that the distribution of medical students' conscientiousness is divided equally between those who study continuously and discontinuously, while in the category of students at sports university, most of them learn the theory for the exam shortly before it.

Among the explanations, we thought about the aspect that, in both situations, the practice of that profession has two components: the practical part (working with patients versus working with students) and the conceptualization part which involves talking and theorizing what you do. For the doctor, we refer to the composition of the observation sheet, the possibility to explain the case and the scientific research; and for sport coaches and teachers, it involves the lesson plan, the training plan, the monitoring through observational feedback. Medical students, from year 3, go to hospitals, see clinical cases that arouse their interest, receive partial or incomplete explanations from their seminarians about solving the case. Thus, the interest to learn more is aroused, and with the help of modern forms of learning (internet, for example), their curiosity makes them look for information and learn more constantly through the challenges from practice to theory. This learning is not necessarily a systematized one, but it puts the student in a greater contact with the learning content.

The students at the sport University are concerned with everything related to the development and refinement of personal practical skills, but also of the subjects they work with, their goal being to accumulate their own experience as rich as possible from working with as many individuals and less to take from the experience of other colleagues, because the coach-athlete/teacher-student relationship is more personalized and longer lasting than the doctor-patient one (especially in the emergency medical branches and not in chronic diseases). However, there are also some long-term doctor-patient relationships, such as these in chronic diseases (such as psychosomatic, rheumatological, oncological diseases, which require time dosing the treatment regimens and proper management, both somatic and psychological). Such relationships are of the adult to adult type (when the patient is responsible for dose adjustment depending on the acute symptoms present), parent to child relationships (which are sometimes necessary when the patient himself is a doctor and such an attitude becomes useful for making the patient in question listen), friendship-type relationships (where the doctor goes from being an “enemy of the disease” to being a friend of the patient, a counselor, a trusted person who helps the patient not feeling the limitations imposed by disease). Similarly, the athlete-coach relationship can: define the coach’s domination (in terms of his personality and executive function) or transform the coach into an advisor of training, shifting the responsibility to the athlete. In the later type of relationship, the coach becomes guide for optimal and efficient development of his athlete. The coach can be a mentor, a friend, an advisor, an authoritarian superego, who controls the desires and objectives of his athlete and the dosage of efforts, but always maintains the high-performance standard.

We objectified that most medical students give for the learning process over 5 days before an exam, while those at the sports University give 1-5 days. The uneven distribution can be seen in the Figure 6.

Figure 6: Distribution of days granted for learning process, compared between the two universities (percentage of the students)

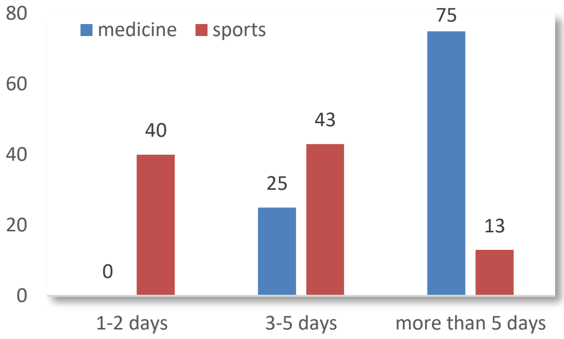

An explanation of the phenomenon could be that those medical students who give more than 5 days are those who, over time, have formed a structured thinking, to learn in steps, from micro aspects, to macroscopic aspects, from pathophysiology and etiology towards the logical understanding of the formation of the symptoms characteristic of the respective disease. In another category are those medical students who believe that diseases should be acquired from the clinic to understand the etiology and thus, they intentionally omit the arid part of the learning content, believing that it operationalizes learning. Many of these "more efficient" students can get high grades in practical exams, maybe even at oral ones, where the teacher requires only the clinical conceptualization of the case, but still a deeper approach will reveal the gaps in understanding the etiopathogenic phenomenon

We sought to probe the maximum time a young person can stay focused in a learning activity in late adolescence and whether there are differences between the training and the profession they choose, differences that will influence their ability to concentrate. From the collected data, we did not discover significant differences, which leads us to think that this capacity depends on certain internal factors and on the stage of personal development. This can be seen in the Figure 7.

The psychological explanation of this phenomenon can be in the concept of endurance and the development of long-term endurance in terms of staying longer and longer in contact with notions, concerns, problems, conceptualizations of the chosen profession. The faculty and the acquisition of information during it are only the preamble, endurance itself is obtained with the practice of the profession, important roles having curiosity, self-improvement, desire to be up to date with news, desire to interact and exchange information with other professionals. We consider that the age range of 18-25 years is the one in which the foundations of endurance are barely laid and still different students are looking for the right techniques for acquiring information, sweeping between what would be easy from theory to practice or to practice in theory ("learning to learn").

Figure 7: Concentration endurance

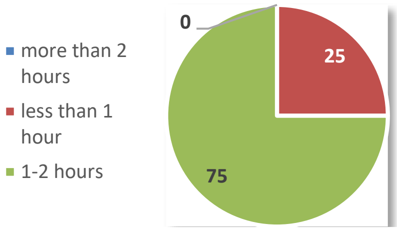

We found that there are significant differences between group samples in terms of the maximum number of hours per day spent for individual study. The percentage of those who spend more than 5 hours a day for individual study is in medical students' area. This demonstrates that they make greater efforts to self-train, have a greater ability to organize, focus on the task, a higher motivational motive, but also the desire for self-improvement more developed than in the field of physical education. The distribution can be seen in the Figure 8.

Figure 8: The maximum number of hours per day spent for self-training (percentage of the students)

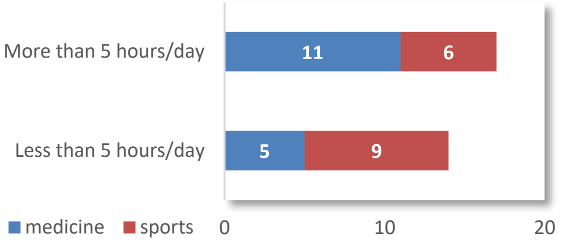

We also probed the importance of the self-taught process, by asking the need to study at home the material presented in the course to pass the final exam. We wanted to emphasize that medical students need a period of self-improvement, a period of in-depth assimilation of the information presented to the course or practical work, compared to those from a sports University, who managed to pass the exams based only on the knowledge assimilated to the courses (Figure 9).

Figure 9: The process of passing the exams based only on the knowledge assimilated to the course (percentage of students)

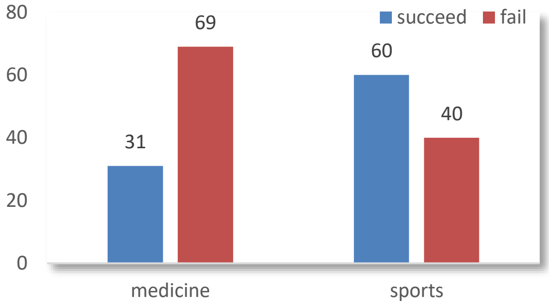

DISCUSSION

There is a major difference between the style of teaching knowledge for students in the two faculties - the mechanism for medical students is from theory to practice; while for students in the field of physical education, the learning mechanism is reversed, from practice to the formulation of theories.

The field of medicine has been developed for thousands of years, and during all this time, specialists have learned to work simultaneously: in one plan to diagnose and treat, and in the other to make explicit, theorize, generalize, argue, and develops an inferential and dichotomous thinking. They have developed their own style of communication between specialists to convey key information in operationalized and synthetic concepts. Thus, 66% of medical students perceive that the teaching style of teachers regarding the medical dictionary must be mastered as being much focused on the presentation of information and less on practical applicability. Instead, sports coaches or teachers have recently started to focus on the conceptualization of their work, on quantification, on operationalization, on ways of exchanging information (to external sources such as: psychologist, athlete's family, school leader, etc. or between them as specialists).

100% of the students from the sports University consider that they need to inspect the learning material themselves, but it is very important that they have the necessary information to understand it individually. In the field of coaches and sport teachers, the emphasis was on the practical side, on streamlining the educational-instructional process, on refining personal skills, on developing the means and methods of teaching the learning content.

Teachers in both faculties should focus on lecture-type teaching, but not identical to the written material; they have to include examples, follow a red thread (message to take home) and ensure that it has been passed on to the student.

Along these lines, students should be considered active partners in the teaching process even if their level of instruction is much lower than the teacher’s one and even if there are significant differences between participating students in terms of previous level of knowledge, involvement, curiosity and motivation. The teacher's role is to modulate the "global intelligence of the audience", encouraging the obviously participatory students and stimulating the less interested ones. It would be a didactic mistake to have the preconception that the overall intelligence of the audience is low, which may lead the teacher to lower the level of the lecture or consider that it is no longer necessary to provide complete information, omitting examples and features or not presenting parts considered more difficult. Students similarly rate the weight of the courses on grades 7-8, which demonstrates that the learning material is hard enough to keep them focused and grasp in their understanding, but easy enough not to lose them and to be at their level of knowledge.

Among the teacher's tasks should be to increase the student's endurance (making him forget how time passes), involving him in active listening in which the student's thinking becomes a partner of the teacher's thinking in understanding the chain of information explained (even if it not perceived). We notice that in young people aged 18-25, endurance is limited to 1-2 hours mostly, but it is acquired by stimulating logical thinking, memorization through vivid representations with reference to clinical cases and practical applications. It is also known that during this period, students are still learning how to improve themselves, how to learn information more effectively, learn how to learn. At the end of the lecture, the student must remain in a still active state, with the desire and curiosity to look for a minimum of personally discovered information regarding the topic of the course presented. Also, the student's thinking must remain during the lecture able to compare the newly received information with a previous knowledge database, which helps him to make his entire previous notional system richer and supported on a more solid basis.

According to the scientific literature, the period of late adolescence is considered a maximum in terms of the ability to assimilate new information, as well as curiosity and openness to it, the desire to filter and systematize it according to personal experience. Therefore, the teacher must teach them a material appealing to the maximum capacity that he intuits as being present in these young people, crediting them with motivation, life choices, determination for the profession, the belief that they will never give up self-improvement.

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Authors’ contribution:

AIT and RTB contributed in all the stages of the article, they designed the article and revised the manuscript for important intellectual content. AIT acquired the data and AIT and RVC drafted the work. RVC contributed to the conception of the work and revised the language.

All authors read and approved the final manuscript.

Ethics approval and consent to participate:

For this study, the agreement was obtained from the Ethics Committee of Teaching Staff Training Department of The National University of Physical Education and Sports. The subject data were completely confidential and they approved the publication of data.

Patient consent for publication: Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

The information generated and analyzed during the current study is available from the corresponding author on reasonable request.

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ORIGINAL ARTICLE

Comparison of complications of surgical repair in inguinal hernia with Bassini, Lichtenstein, and preperitoneal methods

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Abstract: *Introduction: Complication after inguinal hernia repair is one of the challenging problems after inguinal hernia repair. This study aimed to compare the complications of three common surgical methods in inguinal hernia repair, Bassini, Lichtenstein, and Preperitoneal methods (with anterior approach).*

Methods: In this prospective study, patients who underwent inguinal hernia repair surgery in Baqiyatallah Hospital from June 2019 to May 2020 were included. Patients were allocated in three groups: Bassini, Lichtenstein, and Preperitoneal (with anterior approach), based on surgeons' opinions. Postoperative pain, hematoma and seroma, wound infection, recurrence, and also we assed postoperative satisfaction after surgery.

Results: The mean age of patients was 49.90 ± 16.44 years, and 92.5% of patients were male. Of the total patients, 6.8% had an infection and there was no significant difference in the distribution of infection in the groups (P=0.230). The mean of pain in all patients was 2.37 ± 1.10. There was no significant difference between the three groups in the mean of pain (P = 0.253). There was no significant difference in the distribution of recurrence in the groups (P=0.356). There was no significant difference in the distribution of testicular swelling in the groups (P=0.421). No postoperative hematoma and seroma were reported in the groups. The mean of satisfaction in all patients was 93.61±19.63. There was no significant difference between the three groups in the mean of satisfaction (P = 0.156).

Conclusion: The results showed that infection, pain, and recurrence rate in the Preperitoneal group were lower than the other groups, although this difference was not significant. Also, testicular swelling, hematoma, and seroma as well as patients' satisfaction were similar between groups.

Keywords: *inguinal hernia repair, Bassini, Liechtenstein, preperitoneal*

INTRODUCTION

Operation of inguinal hernia is one of the most common procedures in the general surgery field. The prevalence of inguinal hernia is between 15-45% in different ages [1-3]. There are two categories for inguinal hernia, direct and indirect, that included 24 to 50% of all types of hernia, respectively [4-7]. Some studies reported chronic obstructive pulmonary disease, chronic cough, benign prostatic hyperplasia, chronic constipation, family history of hernia, previous right lower quadrant incision, smoking, collagen diseases, physical activity, and bearing the burden can trigger inguinal hernias [5-10].

The inguinal hernias can develop discomfort, pain, and dysfunction in the patient, and also it can cause a cosmetic problem for most patients [2-4]. Severe complications of this disorder, which often occur following hernia entrapment and suppression, require emergency intervention. These include obstruction, ischemia, and gangrene of the bowel or any organ trapped inside the hernia sac [4-7].

Surgical procedure is the standard treatment of this disease. Today, there are several techniques of surgery for it, and surgeons associated with circumstances chose a surgery

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strategy. The main purpose of surgery is to treat patients and decrease the recurrence chance of the disorder [11-15].

Inguinal hernia repair surgery has several potential complications such as hernia recurrence, pain, osteitis of the pubis, Damage to the cord and testicles, hematoma, and testicular swelling [1-3, 5]. There are two main approaches to operation including open surgery and laparoscopy. Also, there are several techniques to repair the herniation site such as classical and preperitoneal techniques. The classical method is a simpler procedure than other methods of repair conducted by most surgeons. Various conventional methods like Bassini's applying suture material are in usage despite the laparoscopic hernia repair [16-18]. Suture repair is related to a significant tension on the suture line. This is possible to induce ischemia of the tissues and final failure of repair causing recurrence [18-20].

Open surgical techniques include two categories of tissue repair and mesh repair methods. More traditional methods are based on initial tissue repair, but today with the production and presentation of industrial meshes, non-traction hernia repair with mesh is the standard method of inguinal hernias surgery [9-6]. Lichtenstein et al. reported the concept of tension-free hernia repair through a synthetic proline mesh that ensuring promising outcomes of hernia repair in the long term [1-3].

Preperitoneal repair is one of the pre-peritoneal methods, the mesh is placed under the transversal fascia and right on the peritoneum, and especially in cases of recurrent hernias after tissue repair methods. In this method, the peritoneal space is released through the anterior access, a 15 by 15 cm mesh is implanted on the peritoneum and fixed to the umbilicus and symphysis of pubis with non-absorbable thread, and then tissue repair with nylon loop thread is done [13-14].

This study aimed to compare the complications of Surgical Repair in inguinal hernia with Bassini, Lichtenstein, and Preperitoneal methods.

METHOD

In this prospective study, patients who underwent inguinal hernia repair surgery in Baqiyatallah Hospital from June 2019 to May 2020 were included.

Patients were allocated to Bassini, Lichtenstein, and Preperitoneal methods (with anterior approach) based on surgeons' opinions. Postoperative pain, hematoma, seroma, infection, recurrence, and postoperative satisfaction were assessed after surgery.

Sufficient information was given to patients about the type of different surgical techniques.

All cases were assessed and followed up according to the standard protocol. All the patients were followed up for 18 months postoperatively to assess complications. Excisional sac hernias were sent to a pathologist to examine their tissue for any malignancies. All cases were normal.

All operations were performed by surgeons in this research with a similar technique. The pain was measured according to the visual analog scale (VAS) post-operative. VAS was described for patients as a ruler that is calculated from 0 to 10. A score of zero shows painlessness and a score of 10 shows unbearable pain possible by the patients.

The protocol of the study was confirmed by the ethics committee of Baqiyatallah University of Medical Sciences with the IR.BMSU.BAQ.REC.1399.026 code. Consent forms were received from all participants.

Mean, standard deviation, frequency, and percent were reported to describe the data. Chi-square and Fisher's exact tests were used to determine the differences between qualitative variables. Data were analyzed by SPSS-20 software. The statistical significance level of all tests was considered less than 0.05.

RESULTS

Overall, 56 patients in the Bassini group, 50 patients in the Liechtenstein group, and 40 patients in the Reed-Reeves group were included.

The mean age of patients was 49.90 ± 16.44 years, and 92.5% of patients were male. The mean BMI of all patients was 25.52±3.56. The mean duration of surgery in all patients was 28.49 ± 75.97 minutes.

There was no significant difference between the groups in the sex distribution of patients (P=0.117). The mean age in the Bassini group was 18.59 ± 45.28 years and was significantly lower than the other groups (P = 0.003). The mean BMI in the Liechtenstein group was 26.95±4.13 and was significantly higher than the other groups (P = 0.002) (Table 1).

The mean duration of surgery in all patients was 28.49±75.97 minutes and there was no significant difference between the three groups in the mean duration of surgery (P = 0.226).

The mean length of hospital stay in all patients was 0.89. 1.02 days and in the Reed-Reeves group was 0.80 ± 0.50 days which was significantly less than the Bassini group (P=0.003).

There was no significant difference in the distribution of history of diabetes, COPD, and smoking between the three groups (P> 0.05) (Table 2).

Table 1: Patients' characteristics according to three groups of the study

Items	Bassini	Liechtenstein	Reed-Reeves	P-value
Age	45.28 ± 18.59	55.66±11.76	50.43±15.45	0.003
BMI	24.90±3.17	26.95±4.13	24.56±2.65	0.002
Sex	7 (12.1%)	1 (2.0%)	3 (7.5%)	0.002
	49 (87.9%)	48 (98.0%)	37 (92.5%)	

Table 2: Duration of surgery, length of hospital stay, diabetes, smoking, COPD and constipation in patients

Items		Bassini	Liechtenstein	Reed-Reeves	P-value
Duration of surgery		75.44±33.75	85.27±17.69	70.00±22.79	0.226
Duration of hospitalization		1.27±0.93	0.93±0.71	0.57±0.80	0.003
Diabetes	Yes	5 (9.1%)	4 (8.0%)	3 (7.5%)	0.002
	No	51 (90.9%)	46 (92.0%)	37 (92.5%)	
Smoking	Yes	11 (19.7%)	10 (20.0%)	7 (15.6%)	0.360
	No	45 (80.3%)	38 (76.0%)	31 (78.1%)	
	History	0 (0.0%)	2 (4.0%)	2 (6.2%)	
COPD	Yes	2 (3.1%)	6 (12.0%)	0 (0.0%)	0.050
	No	54 (96.9%)	44 (88.0%)	40 (100.0%)	
Constipation	Yes	1 (1.6%)	6 (12.0%)	8 (18.8%)	0.008
	No	55 (98.4%)	44 (88.0%)	32 (81.2%)	

The frequency of constipation in Reed-Reeves and Liechtenstein groups was significantly higher than Bassini group (P = 0.008).

Of the total patients, there was 6.8% postoperative wound infection and there was no significant difference in the distribution of postoperative infection in the three groups (P=0.230) (Table 3). No postoperative hematoma and seroma was reported in the groups (P=0.999). The mean of pain in all patients was 2.37 ± 1.10. There was no significant difference between the three groups in the mean of pain (P = 0.253).

Table 3: Infection, hematoma, postoperative pain, recurrence, and patients satisfaction

Items		Bassini	Liechtenstein	Reed-Reeves	P-value
Pain (VAS)		1.42±2.81	1.00±2.21	0.59±1.41	0.252
Satisfaction		96.96±11.22	91.42±26.45	89.65±20.61	0.156
Wound infection	Yes	5 (9.1%)	4 (8.0%)	0 (0.0%)	0.230
	No	51 (90.9%)	46 (92.0%)	40 (100.0%)	
Hematoma and seroma	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.999
	No	56 (90.9%)	50 (100%)	40 (100.0%)	
Testicular swelling	Yes	3 (6.1%)	2 (4.0%)	0 (0.0%)	0.421
	No	53 (93.9%)	48 (96.0%)	40 (100.0%)	
Recurrence	Yes	7 (12.1%)	5 (10.0%)	2 (5.0%)	0.356
	No	49 (87.9%)	45 (90.0%)	38 (95.0%)	

There was no significant difference in the distribution of recurrence in the groups (P=0.356). There was no significant difference in the distribution of testicular swelling in the groups (P=0.421). The mean of satisfaction in all patients was 93.61. 19.63. There was no significant difference between the three groups in the mean of satisfaction (P = 0.156) (Tables 3).

DISCUSSION

This research assessed the complications of Surgical Repair in inguinal hernia with Bassini, Lichtenstein, and Preperitoneal methods. Among different surgical methods for the repair of inguinal hernia, the rate of recurrence and complications in the three methods was low. Inguinal hernia is a common disorder in the field of general surgery. The results showed that infection, pain, testicular swelling, and recurrence rate were lower in the Reed-Reeves group than the other groups, although this difference was not significant. Also, there was no significant difference between the three groups in the mean of satisfaction.

Muldoon et al. (2004) conducted a clinical trial on patients with an inguinal hernia by Liechtenstein and Reed-Reeves methods. The average recurrence in the Liechtenstein method was 4.3% and in the Reed-Reeves method less than 1%, but this rate was not statistically significant [16]. The results of this study are in agreement with the results of the present study.

Akhavan Moghadam et al. (2011) in Iran compared the results between Liechtenstein and Reed-Reeves surgery in unilateral inguinal hernia repair. Postoperative pain and return to work time were significantly shorter in the Reed-Reeves group. The duration of surgery and postoperative recurrence were similar in both groups, but the rate of postoperative infection was higher in the Liechtenstein group [17]. The results of this study in terms of recurrence rate and wound infection rate confirmed the results of the present study.

In the study by Van Nieuwenhove et al. (2007), Which was performed on patients with inguinal hernia using the preperitoneal and Liechtenstein methods, the mean operation time in the preperitoneal method was 20±9 minutes and in the Liechtenstein method was 35±4 [18]. In another study by Nienhuijs et al. (2007), Compared Liechtenstein and pre-peritoneal methods, 41 and 54 minutes were reported for the preperitoneal and Liechtenstein methods, respectively. The recurrence rate was 1.1% in the Liechtenstein group and 2.3% in the peritoneal group [19]. In the present study, the mean duration of surgery in all patients was 28.49 ± 75.97 minutes and there was no significant difference between the three groups in the mean duration of surgery.

Asgari et al. (2013) conducted a study on patients with an inguinal hernia by Liechtenstein and preperitoneal methods. They found that the preperitoneal method was a shorter and more significant incision length and operation time than the Liechtenstein technique. They concluded that The peritoneal technique can be a suitable and safe alternative to the conventional Liechtenstein method [20].

A study by Ghoseiri et al. (2014) in a study On 240 inguinal hernia patients and the reconstructive method of Liechtenstein and preperitoneal showed that preperitoneal surgery is a suitable alternative to conventional Liechtenstein [21].

Jayesh Gohe et al. (2016) on 80 patients with an inguinal hernia in India examined Bassine, Liechtenstein, peritoneal, and the Shouldice method in terms of time and recurrence after surgery. The recurrence rate was reported at around 2.5% with no difference between the methods [22].

A study conducted by Gatabi et al. (2018) compared long-term complications of the two methods of Basini and Liechtenstein on patients with an inguinal hernia. They found that the results of the two methods Basini and Liechtenstein are very similar It is similar, so both methods can be used as a safe method for patients [23].

Mohebbi et al. (2009) compared the quality of life and complications after preperitoneal anterior approach (Rives), herniorrhaphy, and Lichtenstein methods of unilateral inguinal hernia surgery. They reported that herniorrhaphy had a lower quality of life and more complications than Liechtenstein and Reeves methods [24]. In the present study, it was shown that postoperative complications and pain in Liechtenstein and Reed-Reeves methods are less than Bassini surgery.

CONCLUSION

The results showed that infection, hematoma, pain, testicular swelling, and recurrence rate in the Preperitoneal group were lower than the other groups, although this difference was not significant. Satisfaction was similar between the three groups.

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ORIGINAL ARTICLE

Comparison of rinsing with chlorhexidine and matrica mouthwashes on the ventilator-associated pneumonia

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Abstract: *Introduction: The present study aimed to compare the effect of two methods of rinsing with chlorhexidine Oral hygiene in patients undergoing endotracheal intubation is one of the most important tasks of nurses, and Matrica mouthwashes on the incidence of ventilator-induced pneumonia (VAP) in intensive care unit (ICU) patients.*

Materials and Methods: This is a clinical trial carried out on 80 patients undergoing oral endotracheal intubation. Patients were included in the study using simple sampling and randomly divided into chlorhexidine mouthwash and the matrica mouthwash groups. Data collection was carried out using a demographic information questionnaire and clinical pulmonary infection score (CPIS).

Results: The results also showed that most participants in the chamomile group (75%) did not have pneumonia, but the chlorhexidine group had pneumonia in 77.5% of cases. The Chi-square test showed a statistically significant difference between the groups in terms of the incidence and non-incidence of pneumonia (p = 0). Also, the odd ratio of developing pneumonia in the chamomile mouthwash to chlorhexidine mouthwash was 0.30%.

Conclusion: Considering the advantages of herbal compounds such as their low price and low risk, matrica can be considered a suitable alternative to chlorhexidine.

Keywords: *ventilator-associated pneumonia, mouthwash, chlorhexidine, matrica, intensive care*

INTRODUCTION

Pneumonia is the second most common nosocomial infection in ICU patients, affecting 27% of all patients. Nosocomial pneumonia develops in hospitalized patients for more than 48 hours and its incubation period is usually not more than 2 days. Among nosocomial pneumonia, VAP develops in ICU patients undergoing mechanical ventilation for more than 48 hours. Various studies reported VAP-related mortality between 0 and 50% [1].

Risk factors for VAP are divided into three categories; the first category included host-related factors such as old age, physical conditions, immobility, underlying disease, nutrition, and level of consciousness. The second category includes factors related to devices such as endotracheal tube and tracheostomy and intubation time, ventilator route, and gastric-nasal tubes. Finally, the third category includes factors related to employees such as poor hand hygiene and non-use of gloves [2].

Aspiration, intubation due to low level of consciousness or cardiorespiratory arrest, cardiopulmonary resuscitation, emergency intubation, and Glasgow Coma Scale (GCS)< 9 are important risk factors for VAP in the first 48 hours of intubation [3]. In intubated patients, lack of eating, reduced salivary flow, and spontaneous cleaning properties of the mouth and teeth lead to an increase in plaque accumulation [4] as well as the probability of inflammation [5,6].

Adherence to oral hygiene and care in ICU patients is one of the main tasks of health care providers and one of the basic

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aspects of ICU nursing care [7,8]. Dental plaque is a good place for the accumulation of microorganisms. These plaques can be a good reservoir for pathogenic microorganisms in patients with poor oral hygiene (such as patients undergoing endotracheal intubation).

Fourier et al. demonstrated a significant relationship between dental plaque microorganisms and tracheal secretions. The plaque provides the opportunity for germs and other microorganisms to adhere to tooth surfaces. Bacteria in dental plaque act as pathogens when aspirated into the lower airways [9], and can cause VAP [10].

Pittet et al. believe that plaques increase during the first 7 days of intubation [11]. Therefore, oral care is considered one of the most important ways to prevent dental plaque formation and microbial contamination of the mouth [12].

There are two methods to eliminate dental plaque and its associated germs: the mechanical method (brushing) and the medical method (including antibiotics). One of the most effective methods to control plaque is to use mouthwash. Chlorhexidine is an antiseptic that is effective against a wide range of bacteria, some fungi, and some viruses [13].

Two studies have shown the effect of chlorhexidine mouthwash on reducing respiratory infections in patients after heart surgery, but it didn’t decrease the rate of VAP in other special departments of a hospital [14,15]. Although chlorhexidine has been recommended as the most effective plaque-killing agent in some studies [16], it is still not strongly recommended due to insufficient evidence for its effectiveness [17].

Various studies have investigated the effect of different chlorhexidine concentrations on the reduction of VAP, but the results have not been consistent [18]. Guideline from the Infectious Diseases Society of America also does not recommend routine use of chlorhexidine for VAP [4]. Besides, chlorhexidine has side effects that prevent its long-term use that incudes discoloration of teeth and mucous membranes, desquamation of oral mucosa, mouth irritation, stimulation of salivary gland stones (calculi), changes in taste, oral burning and dryness, peeling gums, and negative systemic effects on swallowing. Germ formation is also exacerbated by chlorhexidine due to the creation of an alkaline environment [12,19,20].

Therefore, to overcome the side effects of chemical drugs, World Health Organization (WHO) recommends research to find suitable natural substances such as herbal extracts [20]. For this reason, it is recommended to use non-chemical mouthwashes, including herbal mouthwashes [21]. Research has shown that the use of herbal mouthwashes such as chamomile has fewer side effects [22]. Chamomile extract has antibacterial, antiviral, and antifungal properties. The extract of this herb contains compounds that have strong antimicrobial properties against gram-positive and gram-negative bacteria [23].

Firoozian and Darvish Khezri (2015) showed that chamomile mouthwash not only reduces the number of oral bacteria colonies in healthy people but also affects the number of bacteria with different flora and higher resistance in patients undergoing mechanical ventilation than in healthy people [24]. VAP has been identified as a challenge for ICU personnel [25], therefore, prevention is a priority in ICUs [26].

Grap and Monro stated that oral hygiene and care as one of the most important components of nursing care should be taken into account [27]. Therefore, nurses who are in direct contact with the patient are expected to play an important role in preventing nosocomial infections, especially VAP [28]. Despite the high prevalence of VAP in ICU and the importance of mouthwash in its prevention, the prevalence of this dangerous complication has not been lower than expected despite the use of various solutions [26,29].

Also, considering the importance of using methods with minimal complications and risk that promote oral hygiene in the care of ICU patients, the widespread use of chlorhexidine mouthwash as a common solution in ICUs and due to its side effects and also the absence of these side effects in chamomile and instead its beneficial effects such as antibacterial, soothing and anti-inflammatory properties of this herb, it is recommended to use this herb if it is as effective as chlorhexidine in terms of antimicrobial performance.

It should be noted that antimicrobial effects of these mouthwashes on pathogens were investigated in vitro more frequently, and since there is a difference between ICU patients with outpatients in terms of the type of microorganisms, body resistance, and oral immunity, on the one hand, and despite the importance of preventing VAP in reducing mortality, reducing care costs, and reducing the length of stay [30], on the other hand, the present study aimed to compare the effect of two methods of rinsing with chlorhexidine and matrica (chamomile) mouthwashes on the incidence of VAP among patients admitted to ICU Department of Imam Reza Hospital in Birjand, Iran.

MATERIALS AND METHODS

This clinical trial was performed on ICU patients in Birjand in 2020 to compare the effect of rinsing with chlorhexidine and matrica mouthwashes on the incidence of VAP. The sample size was estimated at 36 people per group based on the formula $n = (Z_\alpha + Z_\beta) \times \left(\frac{(p_1+q_1)+(p_z+q_z)}{(p_1+q_1)^2}\right)$ and taking into account the result of Rakhshani et al.'s (31) study on the incidence of pneumonia before and after the intervention in chlorhexidine and matrica groups (p_1 = 0.93, p_2 = 0.80, α = 0.05, and β = 0.1). The minimum sample size per group was increased to 40 people to increase the accuracy and power of the test, considering the special conditions of patients, and the duration of the study.

Inclusion criteria included obtaining the consent of the patient's guardian to participate in the intervention, patients aged over 18 years of age and under 65 years of age, no oral and maxillofacial injury, no cancer or diabetes and chronic obstructive pulmonary disease, no immunosuppression by drugs or specific diseases, intubation in the first 24 hours and patients who are expected to remain intubated for at least 72 hours, CPIS< 6 upon admission, GCS < 9, no antibiotics before and during hospitalization, natural teeth and having no more than 6 artificial teeth, no use of anticoagulants, no history of allergies to herbal compounds. Exclusion criteria also included death or transfer of the patient from the studied ward, removal of the endotracheal tube before the end of the study, the occurrence of overt aspiration during the study period, reintubation due to the removal of the endotracheal tube for any reason during the first 5 days, sensitivity to mouthwash, the patient's guardian stated unwillingness to continue the study.

In this study, 80 anesthetized eligible ICU patients were selected by convenience sampling method and assigned to one of the two groups of chlorhexidine mouthwash (n=40), chamomile mouthwash (n = 40) in the order of time of admission using simple random sampling and a random number table. Oral care and rinsing were performed using chlorhexidine mouthwash in one group of patients and chamomile mouthwash in the other group. The frequency, duration, and manner of using mouthwash were the same in both groups. At the baseline, informed consent was obtained from the patient's guardian or legal guardian, after explaining the study process and informing him/her. Then the demographic information, medical history, cause of hospitalization, and drugs used were recorded in the information list. All patients were evaluated using CPIS and the results were recorded in the relevant form.

This instrument consists of 6 subscales including:

1. Temperature (score 0 if temperature 36.5 ≤ T ≤ 38.4, score 1 if temperature 38.5 ≤ T ≤ 38.9, score 2 if temperature T <36.5 or 39 ≤ T 2 points)

2. White blood cell (WBC) count (score 0 if 4000 ≤ WBC ≤ 11000, score 1 if WBC> 11000 or WBC <4000, score 2 if WBC> 1100 or WBC <4000)

3. Oxygenation (score 0 if 240 <or ARDS, score 2 if ≤ 240 or no ARDS)

4. Chest radiography (No infiltration (score 0), diffuse infiltration (score 1), local infiltration (score 2)

5. Tracheal secretion culture (pathogenic bacteria did not grow or there is a small amount of them (score 0), moderate to high growth of pathogenic bacteria (score 1), growth of pathogenic bacteria of the same type seen in gram staining (score 2)

6. Volume and appearance of tracheal secretions (absence of pulmonary secretions (score 0), presence of non-purulent pulmonary secretions (score 1), presence of purulent pulmonary secretions (score 2).

Each subscale is scored between 0 and 2, and the total score is 12. In this model, scores ≥ 6 at the baseline or after the entry of microbiological results were considered pneumonia.

Chlorhexidine gluconate mouthwash 0.2% (Behsa Pharmaceutical Co.) was used in the chlorhexidine group. In the chamomile group, chamomile solution (10% Matricaria Chamomila) (Adonis Gol Darou Co.) prepared from the flower of the plant was used according to the opinion of a counseling expert.

In both groups, the patients were placed in the supine position during each intervention, and if there is no prohibition, the head of the bed was raised by 30° and the face was placed to one side. Before performing oral care to ensure the suitability of occlusive endotracheal tube cuff pressure, it was set at 20-25 cmH_2O using a pressure manometer. The intervention was started using a soft pediatric toothbrush soaked with the desired mouthwash and all surfaces of the mouth including teeth and gums per quarter, tongue, inside of the cheeks, hard palate, and soft palate, and the endotracheal tube was brushed. Moreover, 2.5 cc chlorhexidine mouthwash 0.2 and 2.5 ccs chamomile mouthwash were used each time for 30 seconds on each of the aforementioned surfaces and a total mouthwash solution used was 20 cc (10 ccs in the first time and 10 ccs in the second time). The tongue was also brushed gently and away from the end of the tongue. For all patients in both groups, secretions were suctioned and performed at each stage of the intervention, if necessary, and at the end, the lips were cleaned and the mouth became dry. The endotracheal tube was removed and cleaned and placed in the patient's mouth again, the endotracheal tube strip was replaced and some Vaseline was swabbed to lubricate the mucosal surfaces and lips, and then the patient was returned to a comfortable previous state. It took a total of 6 minutes to perform the whole process of oral cleaning and rinsing in both stages. In addition, aseptic tips such as thorough and frequent hand washing and disinfection were observed by the researcher before, during, and after the intervention, and the use of examination gloves when rinsing the mouth and suctioning secretions.

Mouthwashes were used in both groups twice a day for 12 days. At the time interval between mouth-washing interventions, patients' mouth was rinsed with normal saline every 2-3 hours and was suctioned, if necessary. All patients were assessed on the fifth day after the intervention, based on the clinical lung infection assessment checklist that includes the following sections: 1. Temperature control with mercury thermometer (axillary temperature was measured in all patients due to intubation) 2. WBC was performed by CBC test. 3. Oxygenation which was measured by measuring arterial blood gases and pulse oximetry. 4. Chest radiograph was performed by a portable device and seen by an anesthesiologist. 5. Pulmonary secretions were cultured using sterile gloves and a special container by a researcher and another nurse to maintain the sterility of the procedure. 6. Examination of pulmonary secretions for presence or absence, purulence or non-purulence of endotracheal secretions.

The intervention duration was the same for patients in both groups and was performed by the researcher at an interval of 12 hours.

The collected data was entered into SPSS ver. 22 for data analysis. P-value <0.05 was considered the significance level. Independent t-test was used to compare the mean of quantitative variables between the two groups, such as age, and Chi-square and Fisher's exact test were also used to compare the frequency of qualitative variables between the two groups, such as comparing the mean frequency of positive endotracheal and oropharyngeal cultures.

RESULTS

The present study was performed on 80 ICU patients undergoing mechanical ventilation in chlorhexidine and chamomile groups (n= 40 people). There was no statistically significant difference between the two groups in terms of their mean age. The mean age was 39.85 ± 9.55 and 45.10 ± 9.29 years in the chlorhexidine and chamomile groups, respectively. There was also no statistically significant difference between the two groups in terms of the frequency distribution of sex, cause of hospitalization, marital status, place of residence, and insurance status (p> 0.05).

Regarding the prevalence of VAP, the results showed that most participants in the chamomile group (75%) did not have pneumonia and the chlorhexidine group suffered from this complication in 77.5% of cases. The Chi-square test showed statistically significant differences between the two groups in terms of the absence and presence of pneumonia (p = 0). Also, the odd ratio of developing pneumonia in the chamomile mouthwash to chlorhexidine mouthwash was 0.30%. In other words, the risk of pneumonia in the chamomile group was reduced by 30% compared to the chlorhexidine group, and chamomile reduced the risk of pneumonia by up to 70% (Table 1).

Table 1: Distribution of absolute frequency and frequency percentage of subjects in terms of presence and absence of pneumonia in chamomile and chlorhexidine groups

Pneumonia	Number(%)		Z score	p-value
	Cm	Ch		
Presence	10 (25%)	31 (77.5%)	<0.001	22.06
Absence	30 (75%)	9 (22.5%)		

Cm – Chamomile, Ch - Chlorhexidine

The most common microorganism in tracheal culture was Pseudomonas (12.5%) in the chamomile group and Enterobacter (27.5%) in the chlorhexidine group. There was also no microorganism in the tracheal culture of the chamomile group in 75% of cases. The Chi-square test showed statistically significant differences between the two groups in terms of the frequency of microorganisms (Table 2).

Table 2: Absolute frequency distribution and frequency percentage of research subjects in terms of microorganisms in chamomile and chlorhexidine groups

Microorganism	Number (n, %)		Z score	p-value
	Cm	Ch		
Enterobacter	2 (5)	11 (27.5)	26.29	0
Pseudomonas	5 (12.5)	6 (15)		
Klebsiella	0 (0)	7 (17.5)		
Aureus	1 (2.5)	2 (5)		
E-coli	1 (2.5)	3 (7.5)		
Acinetobacter	1 (2.5)	2 (5)		
Absence of microorganisms	30 (75)	9 (22.5)		

Cm – Chamomile, Ch - Chlorhexidine

DISCUSSION

The results of the present research show that chamomile mouthwash was more effective than chlorhexidine mouthwash in preventing VAP so that the CPIS was reduced significantly in patients of the two groups on the fifth day (p <0.001) and these differences were statistically significant.

In a study comparing the effects of chlorhexidine and tooth brushing on the prevention of VAP, Samani et al. found no significant difference between chlorhexidine and brushing or both of them in terms of incidence of VAP and they had no advantage over each other in reducing the incidence of pneumonia [32].

In a clinical trial on the effect of providing oral health services (including 0.12% chlorhexidine digluconate) with or without brushing on the incidence of VAP, Lorente et al. found no significant differences between the two groups in terms of incidence of VAP [33]. Khalifazadeh et al. also found that 0.2% chlorhexidine and swab in comparison with toothbrush and chlorhexidine solution had no significant effect on reducing the incidence of VAP in ICU patients [34]. The results of the study performed by Nideelman et al. showed that brushing 4 times a day with 0.2% chlorhexidine was not able to significantly reduce the incidence of VAP [35].

In a study, Salehi et al. reported that chlorhexidine was more effective on the number of oral bacterial colonies than persica and matrica, but matrica was more effective in reducing the number of bacterial colonies than persica [36].

In a study comparing the antifungal and antibacterial effects of persica, matrica, and Iral wax mouthwashes with chlorhexidine in vitro, Atai et al. showed that among herbal mouthwashes, matrica mouthwash had the strongest antimicrobial effects on Streptococcus sanguis, Streptococcus salivarius, Streptococcus sobrinus, Actinomycosis viscosus and Candida albicans [37].

In a study titled "Comparing the effect of chlorhexidine with chamomile and normal saline on oral bacterial colonization, Azimi et al. reported that chlorhexidine was more effective than both chamomile mouthwash and normal saline in reducing oral bacterial colonization, and also none of the mouthwashes was able to remove Staphylococcus aureus, Pseudomonas, Klebsiella, and Acinetobacter [38].

Another study aimed at determining the effect of brushing, chlorhexidine mouthwash, and brushing + and chlorhexidine on the incidence of VAP among patients undergoing mechanical ventilation showed that chlorhexidine reduced early-onset VAP. Brushing + chlorhexidine was not effective. However, chlorhexidine significantly reduced the incidence of pneumonia among patients with baseline CPIS< 6 on the third day, and brushing could not affect CPIS and increase the effect of chlorhexidine, which is not consistent with the results of the present study [26].

The researchers stated that the type of mouthwash solution is probably less important than using a toothbrush to clean the mouth, which has not been addressed in many studies. In the present study, in addition to mouthwash, toothbrushes were used for both groups of patients. Teeth were brushed using carefully using a soft children's toothbrush soaked in mouthwash. All surfaces of the mouth, gums, tongue, throat, and teeth were brushed. Moreover, all of the areas of the mouth, including both interior and exterior surfaces of the teeth (rotational movements), gums, and tongue (back and forth movements) were brushed. Brushing has a great effect on preventing plaque formation. In this regard, Estaji et al. conducted a study aimed at comparing the effect of chlorhexidine solution and toothbrush in the prevention of oral lesions of 30 ICU patients undergoing endotracheal intubation.

The results showed that toothbrushes and chlorhexidine are effective in the prevention of oral lesions, but a better improvement was observed in oral health in cases where toothbrushes have been used [39].

CONCLUSION

Considering the results of the present study and other relevant studies, we conclude that the use of toothbrushes with mouthwash may reduce the incidence of VAP to some extent directly or indirectly by acting on oral pathogens, however, it still cannot prevent its incidence. This may be because several factors are involved in the development of pneumonia in these patients. For example, there is a significant relationship between GCS< 9 and the incidence of pneumonia [14,40]. There are several other factors many risk factors for VAP in ICUs such as observing other aspects of care such as hand washing, open suction, patients with different immune systems, and the presence of resistant pathogens. Therefore, the limitation of the present study was the lack of control over all these aspects of care due to the limited time and small sample size.

Therefore, considering the advantages of herbal compounds over chemical compounds, the positive effect of chamomile as a mouthwash in critically ill or anesthetized patients can be considered a suitable alternative to chlorhexidine. However, there is a need to further studies on the effect of chamomile mouthwash, especially in the prevention of pneumonia among ventilator-dependent critically-ill patients.

According to the results of the present study, chamomile mouthwash was more effective than chlorhexidine mouthwash on the incidence of VAP in ICU patients and according to the benefits of herbal compounds, chamomile mouthwash can be considered a suitable alternative to chlorhexidine.

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Conflict of Interests:

There is no conflict of interest in the present study.

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ORIGINAL ARTICLES

Comparison of the effect of intravenous ketamine and midazolam as a premedication in children undergoing cochlear implantation at the time of separation from parents

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Abstract: *Background and aim: Preoperative anxiety and fear of separation from parents are some of the most important preoperative problems for children with deaf muteness. Here, the effect of intravenous administration of ketamine and midazolam was compared on sedation in children who candidates for choral cochlear implantation at the time of separation from parents.*

Patients and methods: This double-blind, prospective clinical study was performed on 74 children with deaf-muteness before cochlear implantation. Patients were randomly divided into two groups, midazolam (0.1 mg /kg) and ketamine (1 mg/kg). The sedation anxiety score scale (SASS) form was filled in to assess the anxiety and sedation status for each child. The children's relaxation score from recovery was assessed by the PACU sedation score scale (PACUSSS).

Results: The average of the children's anxiety score after the injection of both drugs during the separation from their parents was 3, and there was no significant difference in the mean of SASS between the two groups ($p>0.05$). The mean PACU sedation score scale in both ketamine and midazolam groups was 1.08 ± 0.89 and 1.32 ± 1.01, respectively ($p = 0.27$). The mean of extubation and recovery times in the ketamine group were 10.44 ± 4.47 and 30.27 ± 6.87, respectively, while the mean extubation and recovery times in the midazolam group were 11.83 ± 3.33 and 29.81 ± 6.31 minutes, respectively.

Conclusion: Both ketamine and midazolam caused equal sedation scores in children with deaf muteness at the time of separating from their parents.

Keywords: *deaf-muteness children, cochlear implantation, ketamine, midazolam, sedation, anxiety*

INTRODUCTION

According to the world health organization (WHO), more than 250 million people worldwide suffer from disabling hearing loss and 34 million of which are children [1, 2]. Pediatrics hearing loss, which often results from genetic defects, can be associated with multiple problems. Children with hearing impairment not only have difficulty talking, but also they do not respond to sounds [2]. Therefore, early diagnosis and treatment of this complication are very important for the disease improvement and quality of their life.

Cochlear implantation remains the only reliable therapeutic option for patients with hearing loss and deaf-mutism [3, 4]. However, this surgery is time-consuming and requires general anesthesia which may be associated with

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emergence agitation, restlessness, disorientation, and anxiety in children [5, 6]. Deaf-muteness children like healthy children recognize environmental events and preoperative anxiety and the fear of separation from parents are some of the most important preoperative problems for these children [5, 7]. Therefore, sedation or premedication is often needed for deaf-muteness children during their separation from parents and entrance to the waiting room.

Midazolam and ketamine are commonly used as premedication in children undergoing medical procedures [8]. Midazolam is an imidazole benzodiazepine that has depressant effects on the central nervous system and provides sedation, muscle relaxation, and amnesia before procedures [9, 10]. It has a rapid onset of action following oral, intravenous, intramuscular, nasal, and rectal administration [10]. A great number of studies have used midazolam as a safe premedication without any serious adverse effects for sedation of children during procedures [11, 12]. It has been also shown that preoperative oral midazolam reduces separation anxiety and emergence agitation in children following anesthesia [6].

Ketamine is a general anesthetic with sedative and analgesic properties which is commonly used as a premedication for sedation in children [13]. Its favorable effects on cardiovascular and pulmonary parameters are a reason by which physicians use this drug as a sedative [14, 15]. Therefore, these data indicated that both midazolam and ketamine are safe effective sedative premedication agents which can be used in children undergoing painful producers [16]. Although many studies have investigated the effect of midazolam and ketamine for sedation of children following medical surgery, no study evaluated the preoperative effect of these drugs on sedation in children with hearing loss and deaf mutism. Therefore, we designed this study, for the first time, to compare the preoperative effect of midazolam and ketamine as premedication on sedation in deaf-mutism children during their separation from parents and before cochlear implantation.

PATIENTS AND METHODS

Patients

This double-blinded, prospective clinical trial study was conducted on 74 children with deaf-muteness who were referred to Baqiyatallah Hospital for cochlear implantation from June 2019 to November 2019.

This study was registered in the Iranian Registry of Clinical Trials under no. IRCT20161022030421N3 cod. The protocol of the study was confirmed by the ethics committee of Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1397.031).

Written informed consents were signed by all parents. A questionnaire containing demographic data of all patients (e.g. age, sex, weight, and history of any previous illness) was filled before surgery. Children aged from 6 months to 5 years old, and American Society of Anesthesiology (ASA) Class I and II were included in the study. Children who met the following criteria were excluded from the study: (i) sensitivity to ketamine or midazolam, (ii) history of treatments with neuropsychiatric drugs, (iii) age less than 6 months, (iv) increased intracranial pressure (ICP), (v) history of seizure, and (vi) history of chronic diseases such as diabetes, liver disease, and upper and lower respiratory tract infection.

Intervention and measurements

After preliminary considerations and patient selection based on inclusion and exclusion criteria, using a computer-generated randomization list, children were divided into two groups of midazolam (n=37) and ketamine (n=37). Intravenous (IV) access was performed using local anesthetic cream 1 hour before their admission in the operating room. The sedation anxiety score scale (SASS) questionnaire was filled out by an observer who was unaware of the study groups. The questionnaire was filled out based on children's behavior in the pre-operative preparation room when they were with their parents. The SASS score ranged from 1 to 5 based on the child's status (Table 1).

Table 1: The SSAS scoring questionnaire

Score	Status	More details
1	Unconscious	Sleepy, no response to mild motor stimulations
2	Sleepy	Sleepy, may have closed eyes but react to mild stimulations
3	Normal	Calmly, comfortable sited or sleeping with eyes open
4	Anxious	Looks afraid, no adhesion to parents, without crying
5	Very rest less	Crying, stomping, or adhesion to parents

After SASS scoring, midazolam (0.1 mg/kg) or ketamine (1 mg/kg) drugs were intravenously injected into children by another person who was unaware of the study groups. Following the injection of the drug, pulse oximetry monitoring was provided for patients. Furthermore, the SASS form was filled out again following the drug's injection just after children felt calming and when they were separating from their parents. Children were then transferred to an operating room and the observer measured the extent of child tolerance during the anesthetic induction by SASS.

Pethidine (1 mg/kg) and atropine (0.2 mg/kg) were injected into children as premedication and then anesthesia induction was performed with propofol and atracurium. Sevoflurane was applied to maintain the anesthesia. Cefazolin, hydrocortisone, and dexamethasone were injected into all children. All children also received ondansetron (1 mg/kg). Children were transferred to the recovery room after surgery and the observer assessed the sedation rate by the post-anesthesia care unit (PACU) sedation score scale (PACUSSS) (Table 2). The PACUSSS score ranged from 0 to 4 based on the child's status. When children obtained the safe discharge criteria from the recovery room, they were transferred to the section. The observer also recorded any postoperative side effects or complications such as nausea, vomiting, emergence agitation, and decreases in arterial oxygen saturation below 92%. The duration of surgery, extubation time, and recovery time was also recorded.

Table 2: The PACU sedation score scale questionnaire

Score	Status
0	Slept, calmly, and without crying
1	Whining, Not rest less
2	Crying, Slightly rest less
3	Continuous crying

Statistical analysis

The results of this study were analyzed by SPSS software (IBM, version 19). Quantitative data were analyzed using the descriptive program and presented as Mean ± SD. Crosstabs and Chi-Square tests were used to compare the percentages or frequencies between the two groups. The comparison of the mean of parametric data between midazolam and ketamine groups was compared by independent student samples-test. In this study, p< 0.05 was considered statistically significant.

RESULTS

In this study, 74 patients were entered into the survey. 32 patients were boys (43.2%) and 42 patients (56.8%) were girls. There was no significant difference in the percentage of male and female genders between midazolam and ketamine groups (p=0.24; Table 3).

The mean age of all participated patients was 29.55 ± 16.92 months. There was no significant difference in the mean age of patients between the ketamine and midazolam groups (p=0.94; Table 3).

The mean weight of all participated patients was 13.18 ± 3.74 kg. There was no significant difference in the mean weight of patients in the ketamine and midazolam groups (p=0.72; Table 3).

Table 3: Demographic characteristics of patients in both groups

Parameters	Midazolam	Ketamine	p-value
Age (months)	29.7 ±17.68	29.4 ±16.36	0.94
Gender			
Male (%)	18 (48.64%)	14 (37.83%)	0.24
Female (%)	19 (51.35%)	23 (62.16%)	
Weight (kg)	13.33 ± 4.3	13.02 ± 3.13	0.72

A comparison of the mean of SASS between the ketamine and midazolam groups is shown in Table 4. There was no significant difference in the mean of each score between the two groups. The mean score of children status in the waiting room with their parents was approximately 4, indicating they were anxious. The mean score of children's status after drug injection, when separating from their parents was decreased to around 3, which suggests the normal status of the patients in both groups. Furthermore, the mean score of the patient’s condition at the anesthetic induction time ranged from 2.5 to 3, which indicates they had sleepy or normal conditions.

Table 4: Comparison of the mean of SASS between ketamine and midazolam groups

Parameters	Midazolam	Ketamine	p-value
In the waiting room with parents	4 ± 0.78	3.89 ±0.9	0.58
After drug injection, when separating from parents	2.91 ± 0.49	2.83 ± 0.55	0.5
Anesthetic induction time	2.62 ± 0.68	2.59 ± 0.68	0.86

The prevalence of each SSAS item in the waiting room before pre-medication is presented in Table 5. There was no significant difference in the frequency of each item in patients between the two groups (p=0.6). Most of the children (40.54% in the ketamine group and 48.64% in midazolam groups) had the anxious condition before premedication. Furthermore, 27.02% of children in the both ketamine and midazolam groups were very restless. The percentage of normal conditions in children in the midazolam and ketamine groups was 21.62% and 29.72%, respectively.

The prevalence of each SSAS item after drug injection, when separating from their parents is presented in Table 5. No significant difference was found in the frequency of each item between the two groups (p=0.68). Most of the patients (67.56% in the ketamine group and 75.67% in midazolam groups) had the normal condition. 24.32% of children in ketamine and 16.21% of patients in the midazolam group were sleepy. Only 8.1% of patients in both groups had anxiety conditions.

The percentage of each SSAS item at the time of anesthetic induction is shown in Table 5. There was no significant difference in the frequency of each item between the two groups (p=0.69). Most of the patients (54.05% in the ketamine group and 48.64% in midazolam groups) had the normal condition at the time of anesthetic induction, while 40.54% of children in ketamine and 45.94% of patients in the midazolam group were sleepy. Only 2.1% of patients in both groups were very resting less.

Table 5: The prevalence of pediatrics SSAS in different conditions

Parameters	Midazolam	Ketamine	p-value
Before pre-medication			
Unconscious	0	1 (2.7%)	
Sleepy	1 (2.7%)	0	
Normal	8 (21.62%)	11 (29.72%)	0.6
Anxious	18 (48.64%)	15 (40.54%)	
Very rest less	10 (27.02%)	10 (27.02%)	
Separating from parents			
Unconscious	0	0	
Sleepy	6 (16.21%)	9 (24.32%)	
Normal	28 (75.67%)	25 (67.56%)	0.68
Anxious	3 (8.1%)	3 (8.1%)	
Very rest less	0	0	
Anesthetic induction time			
Unconscious	0	1 (2.7%)	
Sleepy	17 (45.94%)	15 (40.54%)	
Normal	18 (48.64%)	20 (54.05%)	0.69
Anxious	1 (2.7%)	0	
Very rest less	1 (2.7%)	1 (2.7%)	

The prevalence of children's relaxation scores during the recovery time can be seen in Table 6. There was no significant difference in the prevalence of children's condition between the two groups (p=0.64). Most of the patients (37.83% in ketamine and 32.43% in midazolam groups) had to whine without rest less, whereas 29.72% of patients in ketamine and 24.32% in midazolam groups were kept without crying. Only 5.4% of children in ketamine and 13.5% in midazolam were continuously crying.

Table 6: The prevalence of children relaxation scores during the recovery time

Children status	Midazolam	Ketamine	p-value
Slept, calmly and without crying	9 (24.32%)	11 (29.72%)	0.64
Whining, Not rest less	12 (32.43%)	14 (37.83%)	
Crying, Slightly rest less	11 (29.72%)	10 (27.02%)	
Continuous crying	5 (13.51%)	2 (5.4%)	

There was no significant difference in the mean of extubation (p=0.2) and recovery times (p=0.76) between the two groups. The mean of extubation and recovery times in the ketamine group were 10.44 ± 4.47 and 30.27 ± 6.87 minutes, respectively, while the mean extubation and recovery times in the midazolam group were 11.83 ± 3.33 and 29.81 ± 6.31 minutes, respectively.

We didn’t observe any significant side effects from both studies' drugs. In the waiting room, no child showed a drop in SPO2 below 92% and there was no need for mask ventilation for these patients. Nausea and vomiting were the most important complications after recovery. Three patients (8.1%) in the ketamine group had nauseous and 10 (27.02%) in the midazolam group suffered from nausea (p=0.023). Also, one patient in the midazolam group had vomiting. None of the patients in both groups experienced emergence agitation and decreased arterial oxygen saturation in recovery time.

Interestingly, our findings showed that 37.03% of children in the midazolam group start laughing after receiving premedication (Figure 1), but this condition was not observed in patients in the ketamine group.

Figure 1: Children's reaction after premedication with midazolam. About 37% of children in the midazolam group start laughing after receiving this premedication, but this status was not observed for children in the ketamine group

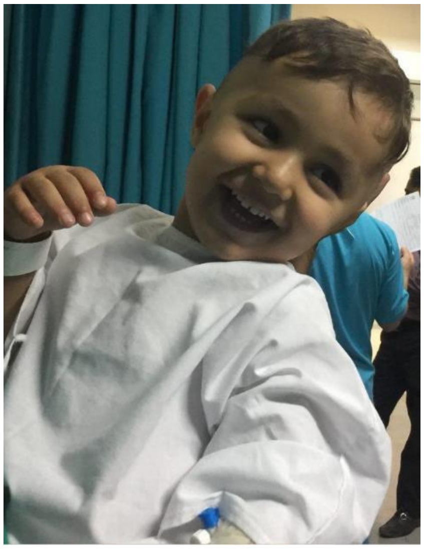

DISCUSSION

In this study, we compared the intravenous administration effect of ketamine and midazolam as a premedication in children undergoing cochlear implants at the time of separating from their parents. To the best of our knowledge, the use of intravenous midazolam and ketamine for sedation of children with deaf-muteness before cochlear implantation has not been investigated yet. Our data have revealed that there was no significant difference in the mean anxiety score between the ketamine and midazolam groups. Children were anxious (mean sedation anxiety score of 4) before premedication with ketamine and midazolam, while they reached normal status (mean sedation anxiety score of 3) after premedication. Furthermore, most children had sleepy and normal status after premedication at the time of anesthetic induction. There was no significant difference in the mean PACU sedation score between the ketamine and midazolam groups. Furthermore, we didn’t find any serious adverse effects in each group; however, nausea occurred more frequently in the midazolam group. More importantly, we found that 37.03% of children in the midazolam group started laughing after receiving the premedication, while this condition was not observed in patients in the ketamine group. However, this mechanism is unclear.

A great number of studies compared the effect of ketamine and midazolam as premedication in the prevention of anxiety, pain score, and emergence agitation in children and adults before entering the operating room. For example, Jeong et al. [17] demonstrated that intravenous administration of ketamine (1 mg/kg) before entering the operating room significantly reduces separation anxiety, postoperative pain score, and also the incidence of emergence agitation in children following ophthalmic surgery. Ozdemir et al. [18] reported that intravenous, oral, and rectal administration of ketamine and midazolam had equal sedation effects in children with malignancy. Several studies compared the combination of ketamine and midazolam as premedication in different groups. For example, Golpayegani et al. [19] compared the oral administration of ketamine and midazolam as sedative agents in pediatric dentistry. They found that the Ketamine-Midazolam combination provides sufficient sedative effect in children's behavior. In another study, Wathen et al. [20] found that intravenous ketamine and its combination with midazolam had equal effective sedation in children. They also found that vomiting occurred more frequently in the ketamine group, while oxygen desaturation occurred more frequently in the ketamine-midazolam group. Barkan et al. [21] evaluated the efficacy of oral midazolam alone and oral midazolam-ketamine combination in 60 children requiring laceration repair. They didn’t find a significant difference in the mean pain score between the two groups; however, midazolam and ketamine combination caused deeper sedation than midazolam alone. Additionally, no clinically significant adverse effects were found in either group which was in agreement with the results of our study. In another study, the effect of high dose oral ketamine (10 mg/kg) with midazolam (0.7 mg/kg) was evaluated in children during laceration repair [12]. The results showed better tolerance to local anesthetics and deeper sedation in children treated with ketamine with no serious adverse events in both groups [12]. Kim et al. [6] investigated the intravenous effect of ketamine (1 mg/kg) and midazolam (0.1 mg/kg) on emergence agitation in children at the postanaesthetic care unit. They showed that premedication with these drugs significantly decreases preoperative anxiety. However, they found that premedication with ketamine was more effective than midazolam in the prevention of early post-operative emergence agitation. Similarly, in this research, we used the same doses of ketamine and midazolam for the improvement of preoperative anxiety and sedation in children. Our data showed that premedication with ketamine and midazolam significantly improves anxiety and sedation in children without any serious side effects.

Our findings have also revealed that there is no significant difference in the mean of extubation and recovery times between the two groups. Similarly, Kim et al. [6] didn’t found a significant difference in the mean of extubation and recovery times between children in midazolam and ketamine groups. Ozdemir et al. [18] reported that ketamine and midazolam are equally effective in recovery time improvement. Recent evidence has proposed that the recovery time in patients receiving ketamine and midazolam is dependent on multiple factors such as drugs dosage, kind of surgery, and the physician on call. For example, previous studies didn’t observe a significant improvement in recovery times when children were treated with higher doses of ketamine (2.5-3 mg/kg) and midazolam (0.25-0.5 mg/kg). Several studies reported higher recovery times in children treated with higher doses of midazolam (0.5 mg/kg) and ketamine (5 mg/kg) [21]. Abdolahi et al. [22] reported that low dose administration of ketamine (0.5 mg/kg) during anesthesia induction did not affect postoperative pain score, postoperative nausea, and vomiting, as well as perioperative additional analgesic requirements in ophthalmic surgery. However, premedication with ketamine improved the extubation time. Based on these data, in our study, children were treated with 0.1 mg/kg midazolam and 1 mg/kg ketamine. Therefore, these data suggest that both drugs are equally effective for the prevention of anxiety in children before surgery and separating from their parents. However, the dose of drugs and kind of surgery are important factors affecting sedation and anxiety which should be considered before administration.

CONCLUSION

In summary, the results of the current clinical study indicated that both midazolam and ketamine are equally safe with desirable sedation effects on children with deaf-muteness before separating from their parents for cochlear implantation. Premedication with 0.1 mg/kg midazolam or 1 mg/kg ketamine decreases preoperative anxiety in these cases. Both drugs improved recovery and extubation times without any serious adverse effects. However, nausea occurred more frequently in the midazolam group. More importantly, children in the midazolam group start laughing after premedication with this drug which caused their parents felt well, while children in the ketamine group didn’t show this reaction. Further studies with a larger sample size should be conducted to determine the optimal dose and to establish the safety of the combination therapy.

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VARIA

Compliance of school doctors' practice with medical legislation

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Abstract: *School doctors' practice comprises specific aspects governed by legal rules. Knowledge and compliance with the applicable legislation are necessary in order to ensure respect of the patient's rights, to avoid claims of medical malpractice and to assure medical act's quality.*

The present study investigated compliance of school doctors' practice with the applicable legal requirements.

All respondent doctors violate the applicable legal requirements. Future quantitative research is necessary to measure the amplitude of the identified vulnerabilities.

INTRODUCTION

School doctors' assistance implies ensuring special conditions for children with chronic diseases, coordinating the optimal response during medical emergencies and managing school environment's health and safety.

Sports competitions for students represent another special feature in this field, given the school doctor's task of issuing a favourable notice for competition to students who participate in such competitions following a brief clinical examination, without always having the student's personal background checks or the possibility to send the student to a specialist for control, without having an appropriate emergency kit and under improper conditions for a medical act which can all generate problems related to malpractice.

Specific aspects of their activity expose them in case of ignorance and non-compliance of the applicable legal regulations.

RESEARCH OBJECTIVE

The assumption under which our research was initiated was that school doctors from Bucharest are not fully aware and do not fully comply with the legislation in force.

The research objective was to collect information regarding the compliance of school doctors' practice with the applicable legal requirements.

MATERIAL AND METHOD

The present research aimed to identity the existence of a phenomenon (the issue regarding compliance of school medical activities with the applicable legal provisions) and to establish the framework for possible future quantitative research addressed to the newly developed area of interest.

The target group included 25 general practitioners activating as school doctors in Bucharest. Sample was selected taking into account the qualitative objects of the research, in the sense that we aimed to identify possible specific tendencies among school doctors. The investigative techniques were represented by interviews, which led to structuring of a questionnaire comprising five closed questions destined to evaluate the risk of malpractice in the school doctors'

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activity. Developed models because of previous research in the field of knowledge and compliance with medical legislation by doctors were used when preparing the questionnaire.

The questionnaires were then circulated and filled in anonymously during a workshop organized by the Administration of Hospitals and Medical Services Bucharest (Administrația Spitalelor și Serviciilor Medicale București - ASSMB) and which took place in Bucharest, on 8.12.2017.

Participants were asked to answer the questions based on their daily practice and not on theoretical knowledge or personal opinions regarding the respective matters.

Moreover, respondents were informed that their answers would be used exclusively for scientific purposes, for performing an objective valuation of the malpractice risk resulting from non-compliance with the legal framework applicable to school doctors' practice.

Definition of the valuation criteria for answers to the questionnaire's inquiries

Thus, the interviews aimed to investigate if medical practice of respondent doctors respects the applicable legislation.

The doctors' answers were marked as "correct" if they indicated the fact that the practice of the respondent doctor respects the legal requirements involved by the question or "wrong" when the answer demonstrated non-compliance of the law by the respondent doctor during his daily medical activity.

In addition, the alternative answers selected by respondent doctors were analysed in detail with the purpose of identifying some models of practice and their justifications. Programme IBMSPSS Statistic, version 20 was used for processing the obtained data.

RESULTS AND DISCUSSION

General results

None of the respondent doctors chose the "correct" answer to all the questions. Therefore, we can consider that, in their current activity, respondent doctors do not fully respect the legal framework applicable to school doctors.

Specific results

Based on received answers we identified some domains of medical practice in which specific legal requirements are not fully respected: informed consent, confidentiality of medical data, patients' access to personal medical information, filming/photographing of patients and limiting the doctor's activity to one's own medical specialty.

Question no. 1

"How do you proceed when a patient requires health care for an associated disorder beyond the scope of competence of your specialty?"

Proposed alternatives to answer:
a) I do not provide health care, regardless the patient's status.
b) I provide health care after consulting a specialized doctor in the field of the respective disorder.
c) I provide health care in emergencies.

The correct answer (to be chosen between the proposed alternatives) is: c)

The general representation of answers to Question no. 1 is included in Table 1 and Chart 1.

Chart 1: Representation of answers to Question no. 1, according to valuation: correct/wrong.

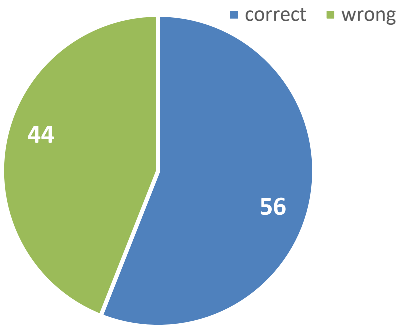

Table 1: Answers to Question no. 1

		Frequency	Percent	Valid percent	Cumulative percent
Valid	a	1	4.00	4.00	4.00
	b	4	16.00	16.00	20.00
	b, c	5	24.00	24.00	44.00
	c	14	56.00	56.00	100.00
	Total	25	100.00	100.00	

It may be noticed that 44% of answers were wrong. In order to identify the respondent doctors' attitude concerning limiting the medical practice to its own specialty we performed a detailed analysis of the received answers to each question, the results being included in Table 1 and Chart 1.

The correct action for the situation described by this question from the questionnaire is prescribed by legal texts: the medical practitioners are civilly liable for the damages caused during the exercise of their profession and when they exceed the limits of their competence. In case of some associated disorders', each of these conditions will have to be supervised/diagnosed/treated by a doctor specialized in the respective area of expertise/competence. Therefore, the legislation includes an interdiction for doctors to perform medical acts that are not covered by their specialty.

There is an exception to this rule in case of emergencies when there is an imminent danger/irreversible deterioration of patient's health state and when a medical practitioner with the necessary competence is not available. [3]

It may be noticed that 24% from respondents included the correct alternative c), but they also added alternative b) (which is wrong), while 16% from respondents mentioned only alternative b) as being correct.

The attitude of respondent doctors is to provide health care beyond their specialty in every situation after they ask a medical practitioner having the necessary area of expertise. These attitudes do not comply with the abovementioned legal requirements.

In situation when all 3 conditions are cumulatively met (urgency, imminent danger for patient's life/irreversible deterioration of patient's health state, lack of specialized medical practitioners) medical activities beyond one's area of expertise can be performed without the need to consult another medical practitioner.

Question no. 2

"*Can information regarding the treatment prescribed to a patient be communicated to third parties?*"

Proposed answers:
a) Yes, to non-governmental organizations which support the patients.
b) Yes, to patient's family members.
c) No.

The correct answer (to be chosen from the proposed alternatives) is: c).

According to the law, the doctor cannot disclose to any other person than the patient data regarding its health state. Information concerning patient's status, results of medical investigations, diagnosis, prognosis, treatment and personal data are confidential, even after the patient's death.

General representation of answers to Question no. 2 is included in Table 2 and Chart 2.

Wrong answers were generated by the doctors' attitude to communicate medical data to patient's family members. It may be supposed that doctors chose this answer given the fact that patients' majority (with the exception of students) are minors.

Chart 2: Representation of answers to Question no. 2, according to valuation: correct/wrong

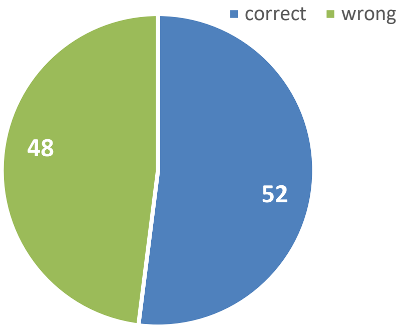

Table 2: Answers to Question no. 2

		Frequency	Percent	Valid percent	Cumulative percent
Valid	b	12	48.00	48.00	48.00
	c	13	52.00	52.00	52.00
	Total	25	100.00	100.00	

However, the question did not refer to minor patients. Even if we referred to minor patients, not every family member is a legal representative (there are various circumstances prescribed by law in which parents, relatives, other people can be legal representatives for a minor patient). Therefore, not even under this scenario, the alternative b) would have been correct. This represents more a vulnerability of respondent doctors' practice with respect to compliance of medical practice with the abovementioned legal requirements.

Question no. 3

"*The informed consent of the patient may be obtained*:"

Proposed answers were the following:
a) From the patient attaining the age of 18.
b) From a minor patient under some circumstances.
c) From one of the minor patient's parents.

Correct answer to this question: a), b), c).

According to law, the statutory age for giving an informed consent is 18.

Minor patients may give their consent in absence of parents or legal representative, in medical situations related to the diagnosis and/or treatment of sexual and reproductive problems, when specifically requested by a minor patient who is over 16 years old.

The important amount of wrong answers (96%) requires an in-depth analysis of answers to this question.

Chart 3: Representation of answers to Question no. 3, according to valuation correct/wrong

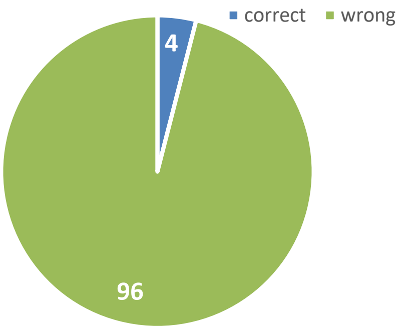

Table 3: Answers to Question no. 3

		Frequency	Percent	Valid percent	Cumulative percent
Valid		1	4.00	4.00	4.00
	a	3	12.00	12.00	16.00
	a, b, c	1	4.00	4.00	20.00
	a, c	20	80.00	80.00	100.00
	Total	25	100.00	100.00	

80% from respondent doctors only selected two of the three correct answers. Therefore, the legal prescription, which allows obtaining an informed consent from minor patients, which are at least 16 years old for sexual and reproductive problems, is not known and respected. Surprisingly, 12% of respondents did not identify the answers as being applicable to medical practice given the fact that these people consider that it is mandatory to obtain the informed consent from both parents of a minor patient.

Question no. 4

"Should medical data concerning undertaken investigations, established diagnosis and recommended treatment be made available to the patient?"

The proposed alternatives to answer:
a) It is not necessary; when discharging the patient from hospital, the treatment scheme and the necessity to come back for control should be explained to the patient.
b) Yes, always, in a complete manner.
c) Information related to diagnosis and established treatment should be made available to the patient"

The correct answer (to be chosen from the proposed alternatives) is b).

According to law no. 46/2003, the patient has unlimited access to all personal medical information and the doctor has to make it available to the patient and, in writing, if the patient requests it.

Almost half of respondent school doctors (44%) do not ensure unlimited access to personal medical information for patients/legal representatives.

Chart 4: Representation of answers to Question no. 4, according to valuation correct/wrong

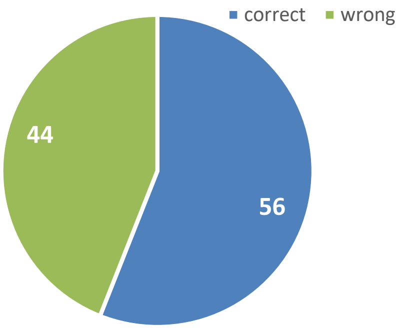

Table 4: Answers to Question no. 4

		Frequency	Percent	Valid percent	Cumulative percent
Valid		1	4.00	4.00	4.00
	b	15	60.00	60.00	64.00
	b, c	3	12.00	12.00	76.00
	c	6	24.00	24.00	100.00
	Total	25	100.00	100.00	

The analysis of chosen answers demonstrates that 24% from doctors consider that only information regarding diagnosis and treatment must be made available to the patient/legal representative.

This attitude may also be caused by an excess of legislation, which generates confusion. Thus, law no. 46/2003 was amended in 2016, the following article being added: "Article 12: the patient or the person expressly designated by the patient, according to articles 9 and 10, has the right to receive, when discharged from hospital, a written summary of investigations, diagnosis, treatment, medical care provided during hospitalisation and, upon request and once, a copy of high performance investigations' records.".

It may be generated the impression that information mentioned in this article could be accessed only by patients, but this interpretation would contradict article 9 of the norms for application with respect to Law no. 46/2003 regarding patient's rights which prescribes the following: "the hospital units must ensure unlimited access to personal medical data for patients ".

We believe that medical practice should take into account the most restrictive (unlimited access) legal requirement and this represents a correct attitude for a school doctor,

Question no. 5

"*May the medical personnel film the patient in the medical unit?*"

The proposed answers were the following>
a) After obtaining the patient's written consent.
b) By protecting the patient's identity.
c) For teaching and research purposes exclusively.

The correct answer (to be chosen from the proposed alternatives) is b).

The patient cannot be photographed or filmed in a medical unit without his consent, with the exception of the situation when the images are necessary for diagnosis and treatment and for avoiding the suspicion of medical malpractice.

Therefore, the medical personnel may film/photograph without the patient's or the legal representative's consent if the confidentiality of data which is obtained throughout this means is protected.

Chart 5: Representation of answers to Question no. 5, according to valuation correct/wrong

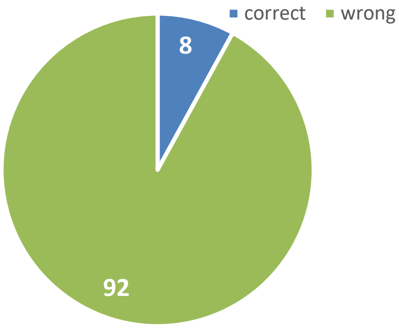

Table 5: Answers to Question no. 5

		Frequency	Percent	Valid percent	Cumulative percent
Valid		1	4.00	4.00	4.00
	a	11	44.00	44.00	48.00
	a, b	2	8.00	8.00	56.00
	a, b, c	6	24.00	24.00	80.00
	a, c	1	4.00	4.00	84.00
	b	2	8.00	8.00	92.00
	b, c	1	4.00	4.00	96.00
	c	1	4.00	4.00	100.00
	Total	25	100.00	100.00	

Most of wrong answers are generated by respondent doctors' conviction that they need patient's consent for filming/photographing him/her. It may be noticed that there are many alternatives of answers selected for this question that suggests the ignorance of the specific legal framework.

CONCLUSIONS

School doctors' practice does not respect entirely the applicable legal framework.

The consequences are that civil liability may be incurred against doctors and the protections of malpractice assurance ceases. Moreover, respecting patients' rights represents an element to measure medical act's quality, as defined by the National Authority of Quality Management in Health (Autoritatea Naţională de Management al Calităţii în Sănătate – ANMCS) throughout specific accreditation standards for medical units. Extensive further studies are necessary in order to determine this phenomenon's amplitude and configuring some education projects destined to cover the identified educational need is necessary.

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REVIEW ARTICLE

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Complications of systemic lupus erythematosus: A review

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Abstract: *Systemic lupus erythematosus (SLE) is a complex autoimmune condition, which often evolves with severe complications. In SLE, autoantibodies appear, with systemic inflammation and multiple tissue destructions. SLE is the most common form of lupus and is considered more severe than other forms. SLE can affect many parts of the body, including the kidneys, heart, lungs, brain, blood, and skin. Symptoms vary among patients. SLE can follow an unpredictable pattern of remissions (symptoms improve) and flares (symptoms worsen). Renal involvement is one of the most severe complications in SLE patients. The process of depositing the immune complexes in the kidneys could lead to the appearance of lupus nephritis. If the treatment is early and treat-to-target, it can change the course of the renal disease.*

Keywords: *systemic lupus erythematosus, renal complications, target treatment*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune condition, characterized by an exaggerated activity of the immune system, which often evolves with severe complications. In SLE, autoantibodies appear, with inflammation and multiple tissue destructions.

EPIDEMIOLOGY OF SLE

SLE is a disease that appears particularly in young people, aged between 15 and 45 years, mainly young women. 10-15 times more women than men have SLE. The black population is more commonly affected by the disease, 2-3 times more than the white population, and develop more aggressive forms [1]. The incidence of the disease has increased significantly over the last decades, probably due to the development of diagnostic methods. [2]

ETIOLOGY OF SLE

The etiology of SLE includes genetic, environmental, hormonal and immune factors, which have a strong importance in triggering the disease.

Genetic factors plays an important role in triggering SLE. The monozygotic twins` disease risk is about 57% higher compared to heterozygote twins' risk.[3]

The existence of genetic susceptibility is also supported

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by the presence of other autoimmune diseases, such as autoimmune thyro-iditis, hemolytic anemia, idiopathic thrombocytopenic purpura [4,5]. SLE is associated with a multigenic determinism. The correlation with the major histocompatibility complex (MHC) on the short arm of the chromosome 6 has a particular importance in the development of the autoimmune processes in SLE [6]. Clinical studies have demonstrated the association of certain haplotypes of HLA DR (DR2 and DR3), DQ (DQ6), B8, components of the complement system (C2 and C4) with various clinical and immunological manifestations. Other genes, like C1q, the Fcγ receptor gene may be involved.

The *environmental factors* involved in the SLE are: ultraviolet rays (UV), that can trigger the autoimmune processes, justifying the occurrence or exacerbation of clinical manifestations after exposure to sun; both viral and bacterial infections: antibodies anti Epstein-Barr virus are present in the serum of SLE patients; bacterial triggers (bacterial DNA, membrane lipopoly-saccharides, endotoxins) were described as factors that can induce an exaggerated immune response; medications that may cause SLE or "lupus-like" phenomena: hydralazine, procainamide, isoniazide, chlorpromazine, methyldopa, penicillamine, minocycline, TNF alpha inhibitors, alpha-interferon; other possible environmental factors: nutritional, vitamin D deficiency, smoking.[7-10]

Hormonal factors: the increased prevalence of the disease in women is supported by increased levels of estrogen and prolactin. Dihydroepiandrosterone has low values in SLE patients.[11] Birth control and substitution therapy hormone increases the risk of developing SLE in the women with a certain genetic susceptibility.[12]

Immune factors. Lymphocytes T and B, monocytes, macrophages and neutrophils have increased production of autoantibodies in SLE patients.[13] The formation of autoantibodies has a central role in the appearance of SLE complications. The main antibodies are antinuclear antibodies (ANA). Anti-DNA antibodies (Ac anti-ADNdc) are the most specific for diagnosis, being correlated with disease' activity. Anti-histone antibodies are associated with drug-induced SLE. Other types of antibodies are: anti-Chromatin; antibodies to RNA structures and ribonucleoproteins; anti Smith antibodies, only described in SLE; antiprotetic P ribosomal antibodies.[14]

Autoantibodies in SLE participate in lesions of various organs through the formation of immune complexes (by immune response of type III) or by the direct action of these antibodies (by type II cytotoxic reaction) [15]. Pathogenic alterations can be secondary to deposition of circulating immune complexes, but also the formation in situ of immune complexes, especially at renal, cutaneous, vascular, nervous system level, causing inflammatory phenomena because of activation of the complement system.[16]

Lupus has different forms: systemic, cutaneous, drug-induced, and neonatal.

The systemic form is the most severe and complex, leading to kidney, heart, lung, brain, blood and skin complications. Symptoms vary among patients. SLE can follow an unpredictable pattern of remissions (symptoms improve) and flares (symptoms worsen).

Cutaneous lupus is a form of lupus that involves only the skin and causes rashes. These rashes may appear anywhere, but are usually found on the face, neck, and scalp. When the cutaneous lesions had an atypical localization, a differential diagnosis is needed [17-19]. This type of lupus usually does not involve the internal organs, but a number of patients living with cutaneous lupus will develop systemic lupus.

Drug-induced lupus can occur after the intake of high doses of certain drugs. The symptoms are similar to systemic lupus, but usually disappear when the drug is stopped or can manifest six months to one year. The antinuclear antibody (ANA) test may remain positive for years.

Neonatal lupus is present in newborns of a woman with lupus or another autoimmune disease. This condition can cause skin rashes, anemia or liver complications. Symptoms usually disappear after a few months and do not cause permanent damage. Some babies with neonatal lupus can be born with a severe heart defect.[18]

COMPLICATIONS OF SLE

SLE is a complex, multifactorial and polymorphic disease that may lead to severe complications.

Muscular complications. Muscular pain, commonly encountered in many diseases, may occur frequently in SLE patients with progressive evolution and it can often lead to a misdiagnose [20,21]. It is often located in the scapular-humeral belt and symptoms like myalgia and muscle weakness often appear.[19] Finally, franc myositis is associated with the increase in serum muscle enzymes, aminotranspherases or creatine kinase levels.[22] The pain is, in many cases, very severe an requires multimodal treatment.

Osteoarticular complications. Arthritis in SLE resembles that of rheumatoid arthritis, symmetrically affecting the small joints of the hands. In the case of SLE, arthritis is non-erosive. A special form of SLE arthritis is Jaccoud arthropathy, with deformations “swan neckline” type. When erosive arthritis occurs in SLE, it is called rhupus. Osteoporosis can occur frequently in SLE patients and is directly related to the high risk of fractures [23]. The surgical management of these fractures is complicated, especially due to bone fragility, needing limited periosteum stripping in order to prevent further damage of the bone [24]. Osteoporosis can also be associated with the adverse effects of cortisone therapy in these patients.

Cardio-vascular complications. The most common are pericarditis, which may range from asymptomatic to accumulation of moderate fluid, and myocarditis, with global hypokinesia at echocardiography.[25] A typical lesion that may occur in SLE patients is Libman-Sacks verrucous endocarditis, with thickening of the mitral and aortic valve [26-28]. The presence of antiphospholipid antibodies appears to be directly related to valvular damage.[29]

Pulmonary complications. Pleural effusion is the most common, is bilateral, and associated with the presence of antinuclear antibodies. In patients with SLE, impairment may be at the level of parenchyma, with lupus pneumonia or alveolar hemorrhage, or at the pulmonary vessels level, with pulmonary hypertension.[30,31]

Gastrointestinal complications may vary from mesenteric vasculitis or intestinal obstruction syndromes to peritonitis, pancreatitis or inflammatory bowel syndromes [32,33]. The most common symptoms are abdominal pain, nausea, anorexia, and vomiting [34,35]. Hepatomegaly and hepatic enzyme elevations are found in 30% of patients, hepatorenal syndrome can appear [36], and Budd Chiari syndrome may also be associated with the presence of antiphospholipid antibodies.[37-39]

Neuro-psihiatric complications are the least understood complications. They may involve either the central nervous system, causing aseptic meningitis, seizures, anxiety syndrome, psychosis, or the peripheral nervous system, with myasthenia gravis, mononeuritis, autonomic neuropathy or polyneuropathy. Endothelial dysfunction may be responsible for the passage of autoantibodies to the nervous system and the occurrence of the lesions.[40]

Renal complications are one of the most serious complications in SLE patients [41-43]. The process of depositing the immune complexes in the kidneys could lead to the appearance of lupus nephritis.[44] The classification of ISN RPS 2004 of lupus nephritis is universally accepted:

- Class I: Minimal mesangial lupus nephritis, with normal glomeruli by microscopic evaluation, but mesangial immune deposits at immunofluorescence.
- Class II: Mesangial proliferative nephritis, with mesangial hyperplasia at the optical microscope, but mesangial immune deposits on immunofluorescence.
- Class III: Focal proliferative nephritis, involving less than half of the glomeruli, with or without mesangial involvement. Subgroups: IIIA (active lesions); IIIA/C (active and chronic lesions); IIIC (inactive lesions).
- Class IV: Diffuse proliferative nephritis, involving more than half of the glomeruli.
- Class V: Membranous nephritis.
- Class VI: Sclerotic nephritis with no active lesions.

Other less common forms of lupus renal disease include interstitial nephritis, drug-induced and vascular disease, when the renal vessels are affected.[40,44,45]

Paraclinical, the parameters evaluated are: 24-h proteinuria, with values above 0.5 g/24 h; active urinary sediment with diffractional red blood cells and cell cylinders; serum creatinine above the upper limit of the laboratory, low creatinine clearance, low complement and high titer of double-stranded DNA antibodies.[46]

Usually, about 25–50% of lupus patients have abnormalities of the renal function, starting early or during the evolution of the disease. The renal complications seem to be discovered in the first three years after the diagnosis of SLE.[47,48] Regarding the ethnic origin, different studies revealed that renal impairment is significantly worse in black subjects than in white patients with lupus nephritis.[49-51]

Proteinuria is considered to be the essential piece in the lupus nephritis structure. In a review on lupus nephritis, proteinuria was reported in 100% of patients, with nephrotic syndrome in 45–65%.[47] Microscopic hematuria was found in about 80% of patients during the disease course.[46]

A recent retrospective study, exploring the determinants of earlier renal disease in patients with SLE, found that features like young age, non-European origin and male sex have a great impact on the disease [52]. Approximately 10–15% of patients with lupus nephritis, despite treatment, progress to end-stage renal failure.[49] Features predictive of end-stage renal disease in patients with severe lupus nephritis included higher baseline serum creatinine level and failure to obtain remission, with increased level of potassium, which can lead to arrhythmic events53. In this stage of disease evolution, the anesthetic risk for this patients is high, being necessary a strictly perioperative management in order to prevent further complications.[54]

Anti-phospholipidic syndrome (APS) is a disorder associated with recurrent arterial or venous thrombotic events and pregnancy morbidity, along with the presence of antiphospholipid antibodies (anticardiolipin antibodies and/or lupus anticoagulant) [55]. Renal disease in APS is characterized by interstitial tubular or glomerular injury due to obstruction of the vessels. Renal manifestations include thrombotic microangiopathy, renal vein thrombosis, renal infarction, renal artery stenosis or malignant arterial hypertension.[56] APS nephropathy was found to be statistically correlated with arterial thrombosis, fetal loss, and the presence of lupus anticoagulant. These characteristics seem to be linked to an increased prevalence of hypertension, raised serum creatinine, and interstitial renal fibrosis.[57]

The therapy of renal disease in SLE is a challenge for the practitioner. An early and targeted intervention can influence the disease' course. The mortality of SLE decreased considerably after the introduction of cyclophosphamide as the major weapon against lupus nephritis, the renal disease no longer impairing the survival rates of these patients. The results of a recent study sustain the role of combined therapy with intravenous pulses of cyclophosphamide and methylprednisolone in the improvement of lupus nephritis [58,59]. However, the long-term use of cyclophosphamide can cause major side effects, such as bone marrow suppression, hemorrhagic cystitis [60-63], gonadal toxicity and, eventually, the development of neoplastic disorders or can complicate some surgical procedures [64-66], with subsequent infections.[67-70] On the other hand, studies have very good results on long-term remission rates and the ability to achieve a second remission with currently recommended intravenous cyclophosphamide regimens. Concerning the new therapeutic options [71,72], it seems that biological agents, such as blockers of other co-stimulatory pathways (for example, CTLA4-Ig), monoclonal antibody against CD20, anticomplement (anti-C5b) and anti-cytokine treatment, induction of T and B cell tolerance, and hormonotherapy, could be involved in the management of lupus nephritis, but these therapies still have no sustainable evidence.[73-75]

CONCLUSIONS

SLE is an autoimmune disease in which self-tolerance is lost, with polymorphic manifestations that represent a real challenge, both for diagnosis and for treatment. SLE often involves severe complication in the various systems of the body, causing a decrease in the patient's quality of life. Renal impairment is one of the most serious complications and could lead to end-stage renal failure. Lately, the focus is on early diagnosis and targeted-treatment for the good management of these patients.

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ORIGINAL ARTICLES

Concepts for the implementation of a technological platform for the production of specific antidotes for CBRN medical protection

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Abstract: *All large armies (EU and/or NATO) have pharmaceutical production facilities to provide the necessary antidotes for the troops and the population: The French Army Medical Directorate produces many military-specific pharmaceutical products in its own laboratory, the Turkish Army owns its own medicines factory, including CBRN antidotes, the US Army, in addition to a sustained drug purchase program in the pharmaceutical industry has launched a new concept: Pharmacy on demand.*

Providing the armed forces with antidotes is a necessity, the concept for their endowement in this sense can be based on imports (sometimes impossible to achieve) or on the national development of a specialized production structure.

The design or construction of a specific production capacity for antidotes can be accomplished on multiple variants, with a complexity proportional to the identified need. The total costs are high, but the objective and implementation of effective antidote supply mechanisms is a security guarantee for the armed forces and the civilian population (through commercialization to allied forces), given the risks of terrorist threats and hybrid warfare.

Keywords: *medical protection, antidote, production, technological platform, medical countermeasures, pharmaceutical technique, orphan drug production*

INTRODUCTION

The creation of a technological platform for the production of antidotes for CBRN medical protection is presented in synthesis, as an initial medical approach to a complex problem of the pharmaceutical industry, resulting from a practical need for therapeutic countermeasures in the field of CBRN protection and a strengthening the capacity of action.

The strategic need to create a technological platform for the production of specific antidotes for CBRN medical protection has led to the concept of a technological platform for the production of specific antidotes, on technical, constructive and product volume variants, depending on needs and possibilities [1].

1. Existing solutions in other countries

Worldwide, large armies (EU and/or NATO) have pharmaceutical production facilities: The French Army Medical Directorate produces many military-specific pharmaceutical products in its own laboratory. The Turkish Army owns its own medicines factory, including CBRN antidotes, the US Army, has a sustained antidotes purchase

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program in the national pharmaceutical industry [2].

The US has begun research for a new concept: Pharmacy on demand. Recent advances within the Defense Advanced Research Projects Agency (DARPA) show that it is possible to achieve complete, miniaturized and flexible platforms for the manufacture of pharmaceuticals. Current progress related to continuous flow synthesis, chemistry, biological engineering coupled with online analysis, automation and improvement of control measures may be elements that enhance the pharmaceutical supply chain and drug production. These new technologies, together with scientific advances, can be the prerequisites for authorizing the "on demand" manufacture of drugs on the battlefield and in other austere environments, increasing the readiness for CBRN threats, increasing the ability of medical authorities to respond to natural disasters and other catastrophic events, minimizing drug shortages, addressing gaps in the orphan drug market, supporting ongoing efforts towards personalized medicine, and improving access to needed medication in disadvantaged areas around the world. The modular platforms being developed through DARPA programs can in the future improve the safety, efficiency and timeliness of drug manufacturing [3].

2. General considerations regarding constructive variants of technological platforms for the production of antidotes for CBRN medical protection

In order to create an image regarding the international context, the Military-Medical Research Center through the Army Information General Directorate has obtained information on the antidote portfolio and how to provide them to different NATO armies [4].

The concept of providing specific antidotes for the protection of the armede forces and the population must meet the Security needs of Romania, and must take into account complex economic, social and legislative realities. Although many states have their own production facilities, they do not provide their own antidote portfolio, being approved for use in that state, based on special legislative regulations. If there are entities willing to sell antidote products (excluding the top ones), over time the company may modify the sales policy or may go bankrupt, the Romanian state remaining out in the open in regars to their provision.

Some CBRN Antidotes have a special regime of use, being necessary to be used in a very short time from the action of the causative agent, having to be in stock and distributed to the armed forces. Also, the preventive antidotes for counteracting the effects in the event of exposure must be in stock in sufficient quantities. It is therefore necessary to modify the regulations regarding the establishment and maintenance of the stocks of these products, assuming from the beginning a periodic, bearable financial loss, in exchange for obtaining the medical security of the troops and maintaining the action capacity.

In the same way that the Air Force provides Security and not revenues, the costs for the endowement with technological means, personnel training and operation of the technological means being important, with much lower costs the security of the personnel can be ensured by creating a specialized structure in the production of antidotes.

The concept of developing the Technological Platform for the production of antidotes for CBRN medical protection is presented in synthesis, as an initial medical approach to a complex problem of the pharmaceutical industry, resulting from a practical need for CBRN medical countermeasures in the field of CBRN protection. It should be noted that worldwide, the pharmaceutical industry, which is one of the most profitable in the world, was removed from the Health field and was included in the Industry field. This denotes the fact that the economic-financial function (the profit of the investors) prevails the social function, which ended up being considered secondary. Large producers bypass antidotes as orphan drugs, having a small retail market, due to the fact that the profits would not cover the investment. Thus, antidotes are difficult or impossible to obtain in crisis situations. Therefore, it is up to the State to guarantee the operability of the armed forces under the conditions of CBRN events, even with slight financial losses [5, 6, 7].

Depending on the need established by the competent bodies, several variants of CBRN antidote production facilities can be designed. These variants must have flexibility, depending on the situation, and their production will adapt immediately to the estimated volume for the given context. Whatever the risk, direct or indirect consequences with CBRN classification will be projected on the population requiring the adoption of specific and appropriate medical countermeasures, cascading with the management of epidemics or epizootics. It shall suffice to recall the aftermath of the earthquake in Haiti that led to the worst epidemic of cholera in recent history, as well as the civil war in Yemen that caused a catastrophic cholera epidemic. In both situations, multiple systems were affected that facilitated the outbreak of epidemics, the national health services were not properly prepared, and the international aid appeared late and in a volume that failed to cover the necessary.

Depending on the identified needs and the allocation of funds by the competent bodies, the solutions for the manufacture of specific CBRN and related antidotes extend over a wide spectrum. In the following are provided three variants: minimum level (galenic laboratories for antidote production), medium level (pharmaceutical laboratory for antidote production) or maximum level (drug factory or specialized department for antidote production).

The ideal solution is a new drug factory that complies with European Union GMP and GLP regulations. In principle, the Integrated Technology Platform for the production of specific antidotes for CBRN medical protection should include:

- Pharmaceutical laboratories for the production of antidotes, by categories;

- Laboratory for pharmaceutical control and quality assurance;

- Production laboratory for chemical synthesis of pharmaceutical substances;

- Microbiology laboratory for in vitro analyzes and tests of pharmaceutical microbiology, required by the regulations in force, such as the Romanian Pharmacopoeia X and the European Pharmacopoeia 10/2016 [8, 9].

- Biobase for in vivo analyzes and tests on experimental animals, required by the regulations in force, according to the Biosafety Guide for medical laboratories, MS 2006;

- Research and development laboratories for applying own research in pharmacology and/or pharmacy;

- Annex facilities and utilities.

The costs with the initial investment and the exploitation for such a production facility of the antidotes (orphan drugs) cannot be covered by sales, which is an impediment in making a political decision to implement such a solution. Given the major risk for public health in crisis situations and the possible catastrophic implications at national and international level, it is necessary to establish directions of action, depending on needs and possibilities, on variants. This pharmaceutical industry facility must be subordinated to a governmental structure for rapid coordination between central structures in order to limit the effects of the crisis situation.

The technological platform for the production of antidotes for CBRN medical protection, depending on needs and possibilities, can be developed in three variants: minimum, average or maximum.

For the medical protection against CBRN agents, the minimum necessary antidotes for the peacekeeping units belonging to the Ministry of National Defence, for the Ministry of Internal Affairs and for the secret services involved in response and the specific risk groups (personalities, medical service, etc.) must be calculated on the basis of a political-military decision, which we estimate at over 100,000 individual doses for each assortment.

3. Minimal variant: adaptation of existing spaces

If the risk and threat become imminent and there is not yet a facility in the country to produce CBRN antidotes, it would be possible based on a government approval with the partial suspension of the provisions of the Medicines Law, to prepare by micro-production (daily galenic lots) the antidotes necessary, for the possible emergency use of exposed, contaminated or ill personnel, as a result of the effect of CBRN attacks. Moreover, at the end of the Medicines Law there is a paragraph that provides this exception for crisis situations [10], respecting the basic rules for the operation of a pharmacy with its own laboratory (galenic), under the coordination of the College of Pharmacists of Romania and with the approval of the Pharmaceutical Inspection. The antidotes produced in such manner, would be from the spectrum of medical prototypes against chemical warfare agents (CWA): neuroparalytics, vesicants, asphyxiants, etc., against radiological warfare agents (RWA): radioactive isotopes and irradiation, as well as biological warfare agents (BWA): living biological agents, toxins [11]. In the event of force majeur, the necessary specialized personnel can be provided from the competent personnel belonging to the military scientific research structures, seconded for technical assistance to the facility that ensures the first phase microproduction. The benefits are represented by the minimization of costs and the deficiencies are represented by latency with which the affected personnel gets the antidotes, sometimes with disastrous effects and the creation of stocks of raw materials.

4. Medium variant: modernization of existing spaces

Since 2002, in the context in which Romania was to become a member of NATO and the EU, the idea of creating an infrastructure for the production of antidotes had been proposed, by modernizing some areas in the heritage of the Ministry of National Defence.

Thus, with the support of a specialized company from Austria, the necessity of using an industrial type construction or pavilions on reconfigurable reinforced concrete structure was proposed.

In the case of pavilions, on the ground floor could be arranged the department of self-injecting syringes for antidotes (manufacture, filling and storage), with the creation of clean room areas for an automatic line of Austrian origin. Upstairs could be created a department of solutions and powders as well as pharmaceutical control laboratories. Also, related spaces of the building (eg attic / bridge) could be adapted for storing raw materials, finished products or laboratory technique. The connections between the departments and the storage spaces, in order to comply with all the necessary flows, would be made wherever necessary and from light constructions outside the existing spaces.

The estimation made by the Austrian specialists for the plant for the manufacture of self-injecting syringes exceeded the value of 10 million euros, and for the whole "turnkey" project, with spaces, equipment, consumables, commissioning and training of the personnel amounted to about 19 million euros. It was suggested to include a financial reserve of 1 million euros for operation (raw materials, utilities, etc.) the general total amounting to 20 million euros. At the level of that period the investment seemed very high, but it was considered to be bearable by equipping the army with the avoidance of imports, ensuring the necessary for the protection of the population and recovering a part of the investment by the possibility that the surplus of products could be exported to the allied armies, following that Romania would become a supplier of antidotes (manufactured according to European Union standards) for the eastern flank of the alliance. The investment was likely to become profitable for the country.

Another explored work option was the creation of a facility in collaboration with the Turkish army's medicines factory, which would have turned out much cheaper, but which did not have approvals in line with European norms. This meant that after the accession to the EU the antidotes would no longer be used in Romania and, therefore, they could not have been exported to other EU countries. This option was eventually discarded.

The average variant could cover the need for specific antidotes for medical countermeasures against CBRN weapons/agents for the general mobilization military personnel (the Ministry of National Defence, The Ministry of Internal Affairs, the Secret Services), including for the risk groups and for the vulnerable population, with the capacity to produce over 1 million doses from each assortment.

5. The maximum variant: de novo construction of specific facilities

The facilities for the production of CBRN antidotes must comply with the CBRN protection requirements, as the manufacturing conditions for the drugs are more severe, according to the Medicines Law and must comply with the European norms in force.

Under these conditions, the old variants are no longer sufficient, except as a breakthrough formula in case of force majeure and therefore an approval for the establishment of a new building shall be needed, whose design, construction and equipment must comply with the minimum requirements for the pharmaceutical industry. According to the Medicines Law, it would result in a medicines factory, which should include: adequate space, unique flows, equipment, specialized personnel and procedures, according to the current requirements in the field.

This facility may be the only one able to ensure the complete protection of Romanian troops and of the civilian population, including of the military forces on the national territory and, in addition, an eventual export within the NATO or EU alliance. Depending on the political-military decision, this production could reach over 20 million doses from each assortment.

The space should in principle be presented as a miniature medicines factory or at least as a pharmaceutical manufacturing laboratory, in accordance with the WHO GMP and GLP standards [12, 13, 14]. The construction should be based on the concept of clean rooms in a separate or independent pavilion, resistant to an earthquake of 8 degrees on the Richter scale, with walls, ceiling and floor watertight and washable, with a height of approximately 5 m (for false ceiling and high floor for masking the pipes system), watertight doors and windows, divided into spaces for the main activities. The result is a total of at least 500 square meters, of which a minimum of 400 square meters inside the pavilion and a minimum of 100 square meters outside for the assembly of the technical installations. The clean rooms must comply with the provisions of the Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) guidelines, which are updated by the National Agency for Medicines and Medical Devices, depending on the biosafety level of the area: clean, aseptic, sterile.

The division of space and the access ways must allow the creation and the compliance of unique flows for the: personnel, equipment, materials and raw materials, for semi-finished products and finished products, solid and liquid waste, air (with a cascade of negative or positive pressure gradients, as applicable). This aspect is essential for the protection of the product and the operator. If they are to be protected from natural or intentional contamination of the environment, the enclosure will have positive pressure on the outside. If on the premises they are working with hazardous materials (biological, chemical, radioactive, etc.), a negative pressure must be maintained on a permanent basis, in order to avoid leaks that contaminate the outside. This is also one of the operating principles of highly secured laboratories (P4 or BSL4) [15].

The pavilion must have permanently complex systems of protection and alarm for the perimeter, for the building and for the workrooms. The construction and equipment works can only be performed by specialized and authorized companies in the field, that have the necessary experience, because the new objective must obtain manufacturing authorization for medicines, including injectables.

The endowment consists mainly of the purchase of a new plant for the manufacture and the filling of self-injecting syringes (about 10 million euros) and equipment for conditioning and filling the vials with powders, tablets, solutions, ointments, etc., of pharmaceutical control, packaging and of the mentioned technical installations, of the current pharmaceutical equipments: apparatus, inventory objects, consumables, protective equipment, raw materials, packaging, furniture, office supplies etc. (approx. 10 million euros) which are added to the construction costs, estimated at 23 million euros, of authorization, accreditation and training. Therefore, we are speaking of a total investment of about 50 million euros.

The necessary specialized personnel shall consist of a minimum of 23 specialists.

The procedures must describe each operation and must comply with the recommendations of the European Union’s Good Manufacturing Practices (GMP), Good Laboratory Practice (GLP), Good Clinical Practice (GCP) and must be endorsed by the National Agency for Medicines and Medical Devices of Romania (NADMR).

OBSERVATION

The need and the opportunity to design, build and operate this specific objective of pharmaceutical production is based on the realization of a prefeasibility study, which must be done with the multidisciplinary consultation of the specialists from qualified institutions, future operators and/or beneficiaries of this investment.

The need is conditioned by:

- The spectrum of threats in the current international political-military context, doubled by the threat of international terrorism, including with ADM CBRN;

- In order to ensure survival in CBRN events, endowment with antidotes is mandatory, in medical practice and especially in military medicine;

- In some crisis situations, the provision of antidotes may be deficient due to the lack of sufficient quantities and the impossibility of provision from import

- The first necessity is represented by the endowment with self-injection syringes of the active military staff, followed by the de-commisioned staff and the risk population, according to NATO norms.

Implementation costs: the total costs are important, but the objective can be amortized in a short time and the social benefit is very high; the average variant (proposed by the Austrian specialists) is estimated at 20 million euros; the optimal variant (the antidote medicines factory, in accordance with WHO, GMP norms) exceeds 50 million Euro, and the minimum variant (the adaptation of the existing one in case of force majeure) without self-injection syringes, under 1 million euro. Operating costs: depending on the variant and the production requirement, up to 1 million euros/year.

Conditions and legislation for certification/accreditation/ operation: Medicines Law, GLP, GMP, ISO 9001 norms.

It is possible to organize, within the military-medical system or in cooperation with the civilian system, a virtual structure for the production of specific CBRN antidotes, preventive and curative, and specific procedures that, in case of force majeure, can start producing small quantities of antidotes simple, necessary to the affected people.

Military history shows that, unfortunately, most of the time it is preferred to invest in offensive equipment and means at the expense of the defensive ones, ignoring the importance of protecting the troops and the civilian population. The human being is the most precious asset, both socially and militarily, since modern warfare uses sophisticated equipment whose use is only operated by highly qualified personnel, sometimes impossible to replace. Neglecting the protection of human capital can constitute the "Achilles heel" within a strategy of defense or army endowment, complex problems of the pharmaceutical industry, resulting from a practical need for CBRN medical countermeasures in the field of CBRN protection. This vulnerability is common to all armies, from all times.

CONCLUSIONS

The article presents brief elements regarding the development of a production/ microproduction facility of antidotes for CBRN medical protection, as an initial medical approach to a complex problem of the pharmaceutical industry, resulting from a practical need for CBRN medical countermeasures in the field of CBRN protection.

The problem is extremely complex, for choosing the optimum solution being involved many aspects (the doctrine on defense, the need for the military and population) established by the decision-makers taking into account the short and medium term threats to the security of Romania, the provision of funds and human capital etc.

It is possible to set up or build a specific production/ microproduction capacity for antidotes, depending on the complexity. The total costs may be high, but given the risk of terrorist actions of hybrid warfare in our geographical area, the military benefit of strengthening the capacity for action and the social one are very high.

The purpose of this article is to bring into discussion elements on issues of medical-military interest that can generate a constructive exchange of ideas between the decision makers that can lead to the identification of an optimal solution.

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ORIGINAL ARTICLE

Content, hierarchy, intensity of motives and their possibility to be implemented in servicemen with various levels of effectiveness of service activity

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Abstract: *In accordance with the results of conducted research there were figured out the content, hierarchy, intensity of motives and their possibility to be implemented in servicemen with various levels of effectiveness of service activity. 2435 male servicemen participated in the research, they equally presented all the operational and territorial military unifications and units of the National Guard of Ukraine. Each unit proportionately presented officers as well as military personnel under contract who included the participants of hostilities and those who did not have such experience. Examination of various aspects of servicemen motivation was conducted with the help of the following the methods which were standardized being based on the Ukrainian selection "Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs", "Questionnaire of Estimation of the Effectiveness of Military Tasks' Execution by Servicemen of the National Guard of Ukraine", questionnaire on "Sense-Bearing and Life Orientations", the methods of "Studying the Motivational Profile of Personality", and "Locus of Control". There was conducted the cluster analysis which gave the possibility to figure out six motivational types connected with the effectiveness of servicemen operational activity. Motivational types were located between the poles which were introduced by two dimensions: self-efficacy (autonomous and controlled motivation) and motivation of helping others (motivations of public service - selfishness). Those motivational types of servicemen were the following: motivated by public service, stagnant, prosocial, romantic, deficient, and dependent. Distinguished types included the content, hierarchy, intensity of motives and their possibility to be implemented.*

Keywords: *motive, motivational sphere, effectiveness of service, servicemen, public service*

INTRODUCTION

Motivation as a factor of professional as well as personal development was significant but was not completely discussed aspect of a complicated issue of professiographical examination of servicemen operational activity; though, thenecessity of examining the proposed topic was mostly caused by a need of practice (Prykhodko I., Matsehora J., Lipatov I., Tovma I., Kostikova I., 2019). Experience of using military units and formations of the Armed Forces of Ukraine (AFU) and of the National Guard of Ukraine (NGU) during combat operations in Ukraine since 2014 strongly convinced that with a considerable degree successful conduction of combat and operational tasks depended on the level of personnel's motivation. Its independent level during operations devaluated any advantages of military formations in logistics and professional development; moreover, it provoked combat stress (Prykhodko I., 2018). Before the combat conflict on the East of Ukraine daily service of servicemen faced difficult times including low level of logistics and salary, absence of prospects to receive their private accommodation, and plenty of other social and

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domestic problems which brought the situation to the line where servicemen made inappropriate choice (Prykhodko et alia, 2019). Insufficient level of motivation occurred in negative facts of refusal of some parts of the Ukrainian military formations' personnel to continue the service on the territory of the Autonomous Republic of Crimea, of Donetsk and Luhansk regions at the beginning of 2014 as well as in adequate efficiency of units' operation which were manned by mobilized servicemen, in significant quantity of suicides, crimes, and violations of military discipline among servicemen at the Anti-Terrorist Operation zone (further Joint Forces Operations) (Prykhodko et alia, 2019).

Besides the fact that there were many scientific researches of peculiarities of personal motivation concerning professional activity under extreme conditions, namely: combat operations constantly made adjustments to components of its diagnostics and formation. Especially that issue was related to the sphere of professional activity of the Ukrainian servicemen. Military service included participation in combat operations, execution of operational tasks connected with vital risks, combat trainings, and daily activities (Kolesnichenko, 2018). Motivation concerning the military activity included a range of various social and psychological conditions, and determined the complex of positive as well as negative aspects of military service (Kapliuchenko, Prykhodko, 2019). Motivation included the reveal and actualization of inner resources of servicemen and creation of positive motivation in combination with external conditions (Prykhodko et alia, 2019). Its specifics depended on personal peculiarities as well as on professional activity.

The vast majority of modern foreign researches on individual motivation concerning professional activity, namely, under extreme conditions, was focused on the paradigm of the theory of self-determination. In 1985 this paradigm was proposed by Deci and Ryan (1985) after that it was accepted as a reliable empiric theory. According to its core postulates human motivation was divided into external and internal, internal included three congenital universal psychological needs: competency (need of people to show their abilities), autonomy (right of choice to choose the profession), and interrelationship (need of feeling their own involvement into social groups and/or spheres of activity). This theory led to the conclusion that different regulating processes which, being the core of achieving goals, were correlated with the effective functioning and welfare. Different contents of goals were correlated with the quality of behaviour, mental health, and with the level of needs' satisfactionin different ways.

To prove the key notions of the theory of self-determination Gillet, Becker, Lafrenière, Huart, and Fouquereau (2017) analyzed interrelationship between motivating profiles of servicemen and civil workers. The results of their research showed that profiles were differently connected with accepted organizational support as well as with involvement into the activity of both selections includingcommunication and support from the side of management and with positive and negative effects in the second selection. Moreover, servicemen with the highest indicators of autonomous motivation presented the highest levels of organizational support acceptanceand involvement into activity. Nevertheless, the highestlevel of autonomous motivation was connected with the highest level of communication, support of commander, and positive influence.

Using the theory of self-determination as a theoretical basis, Chambel, Castanheira, Oliveira-Cruz, and Lopes (2015) created a model that included the servicemen's perception of organizational support on exchange between commanders and members of military staff, professional motivation and welfare. The results showed that motivation concerning autonomous activity was significantly connected with both contextual factors. Besides that, motivation concerning autonomous work was positively connected with its involvement into activity and was negatively connected with professional burnout while controlled motivation demonstrated the opposite reaction.

Foreign researchers (Griffith, 2008; Helmus et alia, 2018) to prove main aspects of the theory of self-determination distinguished institutional and pragmatic motivation as well as their combination. Internal motivation was included into the process of launching the activity because that was interesting and could self-satisfy despite the activities turned to the achievement of external goal. Though, Graffith (2008) using the analysis of motivation of the US National Guard servicemen included into the list of institutional type such internal motives as: patriotism, desire to test themselves, desire to be useful for country and society. Pragmatic motivation to serve was demonstrated through general attitude to army as if it was an employer who made delightful proposition.

In another research Helmus et alia (2018) from RAND center distinguished key motives of entering the US Army and revealed the specificity of army life reality. The results showed that the choice of future servicemen selection in the USA was influenced by two groups of motives which were interrelated with each other: institutional and pragmatic. The key motives to choose the profession of the US Army serviceman were "adventures and travelling", "advantages", "stability of the work and salary", "avoidance of negative surrounding", and "professional education".

Moreover, Helmus et alia (2018) figured out that on the contrary those servicemen who were motivated by pragmatic incentives tended to long-term military service in contrast with those who chose the service as a “call of their heart” being based on institutional motives. Thus, the most of candidates chose the military service in the US Army due to pragmatic (material) reasons, but for the majority of recruits the military service became the work and only after that – call of their hearts.

The research of Bury (2017) which was based onthe determination of motives of recruiting and retention in logistics’ divisions of the British Army reserve demonstrated that servicemen who served because of institutional motives were focused on longer service. Servicemen with pragmatic motivation were less satisfied with the conditions of their service and entered the military sphere to follow the contract only.

Woodruff (2017) had the same conclusion which pointed out that internal motives created stronger connections with army and military valuesthan external motives such as: salary, experience for future professional activity, finances for future education, etc.

The Norwegian experts Johansen, Laberg, and Martinussen (2014) proved that institutional and pragmatic motives could cohabit and a serviceman who was interested in salary, help and economic welfare was not obligatory a bad soldier. The authors pointed out that during the international mission beyond the borders of their own country it was hard to maintain the ideal images of military service; however, mainly the loyalty to traditional military ideals were the best motives of serving and factors of effective military organization.

Grigorov and Spirdonov (2018) confirmed the theses that there was institutional and pragmatic motivation. They conducted the research to distinguish core motives of choosing the military profession in the Republic of Bulgaria and suggested that the dominant motivational structures in the process of choosing the profession was the coexistence of leading factors which were connected with pragmatic motivation in comparison with internal factors which related to one definite work, independence, achievement, and recognition.

According to Taiyang (2010) the system of motivation to serve in the Chinese Army was determined with the factors of tradition and culture: national flag as a symbol of honour, courage, role of model caused the involvement into nobility and caring about other people; non-material motivated stimulation.

Analyzing the motivation of the Brazilian servicemen, Rodrigues-Goulart (2006) highlighted that it always had crucial importance for servicemen who executed tasks under combat (extremal) conditions which posed risks for their life and health. According to the researcherfor many situations motivation was a core to succeed in battle. Rodrigues-Goulart distinguished the following motives of military profession: sense of obligation, belonging to military sphere, responsibility, sacrificing, need of fate, desire of adventures, leadership, working abilities, tendency to recognition and awards, ideas of legitimacy of war, hope and victory, hatred of enemies, and need of self-preservation.

In accordance with Catignani (2004) “strong spirit” of military division was equal to high combat motivation. It was impossible to overestimate the importance of combat motivation to succeed in execution of military operations such as patrolling as well as full-scale war. Combat motivation was key factor that gave possibility to ordinary army to succeed in conflicts; talking about Israel it was called “secret weapon” of the army. Catignani (2004) pointed out that there was “a tough connection between cohesion, motivational level of servicemen and combat effectiveness”.

Analysis of modern scientific works indicated unbelievable urgency of the problem of servicemen motivation to act under extreme conditions in different countries all around the world. Systematization of analyzing the peculiarities of professional motivation of servicemen gave the possibility to conclude that motivation was one of the most significant factors for military division to achieve the posed goals in many situations which occurred in the process of executing combat operations under extreme conditions.

The aim of the research was to determine the content, hierarchy, intensity of motives and their possibility to implement them in servicemen with various levels of effectiveness of service activity.

MATERIALS AND METHODS

Respondents

The research was conducted from 2019 to 2021. There participated 2435 servicemen of the NGU who equally presented all the operational and territorial divisions and military units of the NGU. Each unit equally presented their officers – 29.84% (from Second Lieutenant to Colonel) and servicemen under contract – 70.16% (from Crewman to Warrant Officer), participants of hostilities and those who did not have such experience. The age of the respondents was from 20 to 55. All procedures carried out in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants gave consent for their data to be used in this research.

Instruments

To determine various aspects of servicemen motivation there were used the methods which were standardized being based on the Ukrainian selection.

1. “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” by Moskalienko (2002). This questionnaire was developed by the Ukrainian researcher for professional psychological selection for future policemen education, it gave possibility to evaluate: “Motives Related to Objective Socially Posed Tasks and to Content of Profession”, “Motives Related to Personal and Professional Development”, “Independent Profession Selection”, “Dependent Profession Selection”, “Motives Related to External Prestigious Professions and Financial Welfare”, “Motives Related to Romanticization of Profession”, “Motives Related to Eagerness of Compensation of Temper Flaws”, “Antisocial Motives (attitude to profession was as if it was a way to satisfy own antisocial needs)”. The instruction of this method proposed to answer the question concerning the reason of choosing this profession where the respondents using a scale made of 5 points could estimate 40 statements which described the profession of law enforcements.

2. “Methods of Studying the Motivational Profile of Personality”by Ritchie and Martin (1999). The use of this method gave the possibility to determine the urgent motivation of professional activity. It had the following scales: “Need of High Salary and Financial Benefits”, “Need of High Working Conditions and Comfortable Surrounding”, “Need of Accurate Structurization of Work and Feedback”, “Need of Social Relations”, “Need of Forming and Supporting Long-Term Stable Relations”, “Need of Obtaining the Recognition by Other People”, “Need of Posing Brave and Tough Goals and of Achieving Them”, “Need of Interesting and Socially Beneficial Work”, “Need of Self-Improvement and Development as Personality”, “Need of Being Creative, Thoughtful, and Open for further Ideas Employee”, “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”, “Need of Manipulation and Power, Eagerness to Control the Other People”. This questionnaire included 33 questions with 4 variants of answers where the respondents had to replace 11 points in accordance with the importance of the answers.

3. Method of “Will-Regulated Personality” by Hutkin and Mikhalchenko (2000) gave the possibility to determine general indicator which characterized willed organization and willed peculiarities of personality: organization of will and sense, organization of activity, determination, perseverance, self-control, and self-reliance. This method included 56 questions with 4 variants of answers.

4. Method of “Locus of Control” in modification proposed by Ksienofontova (1999) which gave the possibility to analyze internal orientation of serviceman personality, his/her ability to recognize and take responsibility for his/her decisions and actions. This method included 40 statements which respondent could agree or disagree with. This questionnaire determined 17 options of internality: general internality, internality in thoughts about life in general, internality in description of personal experience, internality in the sphere of achievements, internality in the sphere of fails, tendency to self-incrimination, internality in the sphere of professional activity, professional and social aspect of internality, professional and processual aspects of internality, internality in interpersonal communication, competence in the sphere of interpersonal relations, responsibility in the sphere of interpersonal relations, internality in the sphere of family relations, internality in the sphere of health, negation of activity, readiness for activities which was connected with difficulties overcoming, readiness for self-planning, acting and responsibility for their activities.

5. Test of “Sense-Bearing and Life Orientations”in adaptation made by Muzdybaieva (1998). This test was the adapted version of test “Goal of Life” by Crumbaugh, Maholick (1988). Within the further work with the method there was developed a scale of searching for sense-bearing and life goals (Crumbaugh and Maholick, 1969). It was planned as an additional scale for the test devoted to life understanding. According to the ideas of the authors the scale had to measure the strength of motivational tendency to search for sense of life; thus, it was opposite to the scale “Goal of Life” – the more goals of life there were the less need for their search.

Adaptation of this text into the Russian language was completed by Muzdybaiev (1998). The factor structure of the Russian version of the text was determined during special research. This research demonstrated that despite the small quantity (20 points) during the factor analysis there were distinguished six factors on thebasis of which there were marked out some subscales in the final variant of sense-bearing and life orientations.

6. “Questionnaire of Estimation of the Effectiveness of Military Tasks’ Execution by Servicemen of the National Guard of Ukraine” by Prykhodko (2013) gave the possibility to estimate the level of development of important professional qualities and peculiarities of executing the servicemen’s combat operations. Experts were the commanders of servicemen who participated in the

research.

The questionnaire included 37 criteria of estimation. In the process of estimation experts used the scale with 5 points: 5 points was the maximal possible level of development of estimated professional important quality or maximal level of effectiveness of the estimated aspect of service. 0 points was used to determine the absence, undevelopment of estimated important professional quality or full inefficiency of subordinate individual in estimated aspect of combat operations. Informative paragraphs of this questionnaire revealed all the processual aspects of work, peculiarities of professional communication and motives, professional skills, and possibilities of productive work.

Mentioned methods were standardized for the Ukrainian selections, they were often used to determine the peculiarities of professional motivation of servicemen and law enforcements of Ukraine.

Data Analysis

A cluster analysis was used to compare the data of psychodiagnostics scales in the study groups. To present the data, we used the main descriptive statistics (arithmetic mean M and standard deviation SD). To reliably detect significant differences between comparative groups, the t-Student criterion was used (significance level p<.1, p<.05, p<.01). Mathematical data were processed through SPSS 17.0

RESULTS

Conducted cluster analysis gave possibility to distinguish 6 groups which differeddue to their ways of estimation of combat operations effectiveness as well as typical sphere of motivation and will. The indicators for psychodiagnostic methods that determine the characteristics of the identified types of motivation in servicemen are showed in Table 1.

Table 1: Indicators for psychodiagnostic methods that determine the characteristics of the identified types of motivation in servicemen (in standard points)

Psychodiagnostic methods	MWE	MHE	HE	WE	MLE2	MLE1
“Questionnaire of Estimation of the Effectiveness of Military Tasks’ Execution by Servicemen of the National Guard of Ukraine”						
General indicator of the effectiveness of service and combat activities	19.43±3.22	20.43±2.16	21.05±2.59	18.52±1.93	20.23±1.88	20.40±1.71
“Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs”						
“Motives Related to Objective Socially Posed Tasks and to Content of Profession”	20.71±3.40	17.13±4.13	21.47±2.98	23.69±1.75	19.61±2.92	23.22±2.34
“Motives Related to Personal and Professional Development”	18.50±4.30	13.53±4.38	17.60±4.52	22.38±3.99	20.04±2.29	20.04±3.70
“Independent Profession Selection”	17.15±4.66	12.34±4.91	18.73±3.96	23.00±1.53	18.74±2.82	20.74±2.95
“Dependent Profession Selection”	13.20±4.97	9.87±4.53	10.41±4.34	21.31±4.94	18.17±3.07	11.98±4.89
“Motives Related to External Prestigious Professions and Financial Welfare”	18.20±3.58	13.51±3.76	17.00±3.28	23.62±1.89	19.26±3.66	18.93±3.27
“Motives Related to Romanticization of Profession”	15.71±4.51	11.00±3.99	13.68±4.41	22.69±2.72	18.96±3.35	14.83±5.01
“Motives Related to Eagerness of Compensation of Temper Flaws”	19.12±3.61	13.66±4.67	17.44±4.22	23.08±2.06	19.91±3.07	20.57±3.66
“Antisocial Motives (attitude to profession was as if it was a way to satisfy own antisocial needs)”	14.43±4.99	9.89±3.99	10.30±3.62	21.46±3.48	18.70±3.10	13.30±5.67
“Methods of Studying the Motivational Profile of Personality”						
“Need of High Salary and Financial Benefits”	32.49±6.74	36.11±9.65	32.67±8.12	30.69±4.13	39.17±7.62	34.56±7.53
“Need of High Working Conditions and Comfortable Surrounding”	30.72±5.40	31.70±5.99	28.96±7.36	31.08±2.56	30.26±4.27	28.50±5.80
“Need of Accurate Structurization of Work and Feedback”	33.58±7.92	31.30±6.81	33.17±5.53	32.00±4.00	33.52±4.33	32.70±6.53
“Need of Social Relations”	28.07±5.24	27.85±5.13	29.42±4.68	28.23±3.96	27.61±4.98	30.83±4.98

Psychodiagnostic methods	MWE	MHE	HE	WE	MLE2	MLE1
“Need of Forming and Supporting Long-Term Stable Relations”	28.41±5.45	28.02±4.55	28.64±6.66	31.23±2.74	28.83±3.65	29.89±4.47
“Need of Obtaining the Recognition by Other People”	31.29±5.93	30.87±5.03	30.95±5.76	29.38±4.13	34.09±6.16	32.39±6.96
“Need of Posing Brave and Tough Goals and of Achieving Them”	30.47±4.82	29.53±4.98	31.14±4.18	30.31±3.22	30.83±4.49	28.98±4.11
“Need of Manipulation and Power, Eagerness to Control the Other People”	26.66±7.54	26.85±5.42	26.35±5.28	28.85±2.51	26.61±5.24	26.70±4.45
“Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”	29.59±4.66	29.42±6.01	28.81±4.67	31.08±3.01	27.52±3.82	27.56±5.22
“Need of Being Creative, Thoughtful, and Open for further Ideas Employee”	29.34±3.93	29.02±5.09	29.23±4.12	30.62±6.21	31.30±4.35	28.59±5.00
“Need of Self-Improvement and Development as Personality”	31.27±5.03	30.60±4.09	30.87±4.59	30.62±1.85	28.43±3.81	31.22±4.11
“Need of Interesting and Socially Beneficial Work”	31.12±5.57	31.74±6.02	32.79±6.31	28.92±4.35	24.83±4.98	31.07±5.16
Test of “Sense-Bearing and Life Orientations”						
General indicator of “Sense-Bearing and Life Orientations”	90.41±11.21	95.23±14.75	116.60±12.03	105.38±20.48	78.91±12.02	120.09±9.84
“Goal”	27.76±5.08	29.62±5.23	36.35±4.39	29.08±8.90	24.13±5.89	38.17±2.95
“Process”	25.85±4.55	28.72±4.75	32.90±4.86	33.92±7.25	23.91±5.52	32.69±4.64
“Result”	22.92±4.20	24.26±4.19	30.17±3.27	26.54±5.90	19.17±4.99	31.72±2.68
“Locus of Control-I”	18.84±3.60	19.83±3.51	24.29±2.76	20.85±4.88	18.04±4.67	25.98±1.68
“Locus of Life Control”	27.42±4.69	28.30±5.57	35.73±4.23	28.85±6.73	24.57±5.81	37.81±3.24
Method of “Locus of Control”						
General internality	25.71±3.74	20.64±2.94	29.60±3.55	24.15±3.29	20.43±3.70	20.44±3.50
Internality in thoughts about life in general	12.07±2.33	9.81±2.39	14.05±2.24	10.31±1.49	9.87±2.51	9.37±1.83
Internality in description of personal experience	13.64±2.38	10.83±2.68	15.56±2.12	13.85±2.97	10.57±2.84	11.07±2.68
Internality in the sphere of achievements	5.76±1.68	4.55±1.29	6.82±1.21	6.46±1.45	4.17±1.44	4.76±1.53
Internality in the sphere of fails	4.59±1.24	3.40±1.46	4.68±1.47	5.15±1.41	4.04±1.19	3.04±1.44
Internality to self-incrimination	-1.16±1.91	-1.15±1.97	-2.15±1.72	-1.31±1.55	-0.13±1.74	-1.72±2.18
Internality in the sphere of professional activity	10.63±1.75	7.66±1.80	11.83±1.85	9.85±1.52	8.13±1.96	7.78±2.00
Professional and social aspect of internality	5.43±1.13	3.81±1.23	6.04±1.25	4.69±0.75	4.13±1.36	4.06±1.41
Professional and processual aspects of internality	5.20±1.23	3.85±1.34	5.79±1.33	5.15±1.28	4.00±1.65	3.72±1.46
Internality in interpersonal communication	9.34±1.88	7.75±2.08	11.15±1.90	8.00±1.83	8.09±2.09	7.74±1.67
Competence in the sphere of interpersonal relations	5.02±1.67	3.98±1.50	6.40±1.38	3.77±1.74	3.70±1.46	4.24±1.48
Responsibility in the sphere of interpersonal relations	4.31±1.46	3.77±1.22	4.76±1.16	4.23±0.83	4.39±1.47	3.50±1.46
Internality in the sphere of family relations	4.15±1.17	3.74±1.18	4.90±1.22	3.69±0.85	3.87±1.10	3.69±1.30
Internality in the sphere of health	5.60±1.28	4.28±1.26	6.29±1.17	5.54±0.66	4.30±1.40	4.37±1.57
Negation of activity	5.14±2.12	7.55±1.67	3.30±1.86	7.46±0.78	8.13±1.74	7.83±2.39

Psychodiagnostic methods	MWE	MHE	HE	WE	MLE2	MLE1
Readiness for activities which was connected with difficulties overcoming	5.19±1.63	3.96±1.37	6.63±1.25	2.54±1.45	3.22±1.62	4.13±1.89
Readiness for self-planning, acting and responsibility for their activities	5.67±1.58	4.49±1.09	6.07±1.45	6.00±1.78	4.65±1.87	4.04±1.67
Method of “Will-Regulated Personality”						
Value-semantic organization of personality	14.02±3.25	13.19±3.73	17.60±2.85	13.54±3.67	11.04±2.40	19.39±2.69
Organization of activity	14.56±2.37	13.32±2.41	15.53±2.17	13.69±1.89	11.39±2.10	16.00±2.64
Determination	15.16±3.21	14.72±2.75	18.56±2.99	13.85±3.89	11.22±2.26	20.11±2.56
Perseverance	15.85±2.97	14.72±3.77	19.25±2.81	14.15±4.04	10.87±2.26	20.46±2.45
Self-control	15.30±2.84	14.42±3.32	18.44±2.64	13.23±4.68	10.91±2.43	19.31±2.39
“Self-Reliance”	12.88±3.15	13.85±3.00	14.66±2.38	9.15±4.20	8.35±2.90	14.54±3.54
Lies index	10.72±2.38	8.79±3.32	11.70±3.04	11.08±3.12	10.13±2.62	13.43±2.98
General indicator characterizing the volitional organization of the personality	87.78±12.20	84.21±12.48	104.04±10.10	77.62±18.06	63.78±5.33	109.81±7.75

Notes: MWE – middle level of effectiveness with tendency to weak indicator of effectiveness; MHE – middle level of effectiveness with tendency to high indicator of effectiveness; HE – highly effective; WE – weakly effective; MLE2 – middle level of effectiveness; MLE1 – middle level of effectiveness

Table 2 show the significance of differences between the proposed types according to the scales of methods for describing the features of the motivation of military personnel.

The group of highly effective (HE) servicemen included 35% of the general selection of the research. This group consisted of 1/3 from every category which participated in the research: officers, military personnel under contract, and participants of hostilities (PH).

The group of weakly effective (WE) servicemen included 4% of the general selection of the research. This group consisted of 85% of military personnel under contract and 15% of officers. 85% of this group were the participants of hostilities.

There were two groups with middle level of effectiveness of operational activity. Group number 1 with middle level of effectiveness (MLE1) was more numerical – 15% of the general selection of the research. This group consisted of 2/3 of military personnel under contract and 1/3 of officers. The status of the participants of hostilities belonged to 3/4 of respondents.

Group number 2 with middle level of effectiveness (MLE2) was less numerical – 7% of the general selection of the research. The indicator of combat operations’ effectiveness was distinguished as 20.23±1.88. This group consisted of 87% of military personnel under contract and 13% of officers. The status of the participants of hostilities belonged to 48% of respondents.

There was distinguished one more group which took intermediate place between the groups with high effectiveness and with middle level of effectiveness. It was marked as a group with middle level of effectiveness with tendency to high indicator of effectiveness (MHE). The group included 15% of the general selection of the research. This group consisted of 70% of military personnel under contract and 30% of officers. The status of the participants of hostilities belonged to 50% of respondents.

Moreover, there was detected the group which indicator of combat operations’ effectiveness took intermediate place between the group with middle level of effectiveness and the group with weaklevel of effectiveness. It was marked as a group with middle level of effectiveness with tendency to weak indicator of effectiveness (MWE). The group include done-in-four participant of the research. This group consisted of 73% of military personnel under contract and 27% of officers. The status of the participants of hostilities belonged to 45% of respondents.

According to “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” (Moskalienko, 2002) there were highlightedthe motives of choosing military profession by group HE, but their indicators were significantly weaker than those of groups WE and MLE1.

The peculiarity of motives of choosing military profession in this group was determined by hierarchy indicators.

Thus, the most expressed were “Motives Related to Objective Socially Posed Tasks and to Content of Profession”.

Table 2: The significance of the differences between the proposed types according to the scales of methods for describing the features of the motivation of military personnel (in standard points)

Psychodiagnostic methods	$t_{MWE-MHE}$	t_{MHE-HE}	t_{MWE-WE}	$t_{MWE-MLE2}$	$t_{MWE-MLE1}$	t_{MHE-HE}	t_{MHE-WE}	$t_{MHE-MLE2}$	$t_{MHE-MLE1}$	t_{HE-WE}	$t_{HE-MLE2}$	$t_{HE-MLE1}$	$t_{WE-MLE2}$	$t_{WE-MLE1}$	$t_{MLE2-MLE1}$
Questionnaire of Estimation of the Effectiveness of Military Tasks' Execution by Servicemen of the National Guard of Ukraine															
General indicator of the effectiveness of service and combat activities	2.19*	3.89**	1.42	1.52	2.32*	1.65^0	3.12**	0.41	0.09	4.34**	1.81^0	1.99*	2.57*	3.21**	0.37
Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs															
Motives Related to Objective Socially Posed Tasks and to Content of Profession	5.30**	1.67^0	4.91**	1.55	5.17**	6.91**	8.78**	2.98**	9.35**	4.01**	2.80**	4.22**	5.24**	0.81	5.26**
Motives Related to Personal and Professional Development	6.54**	1.47	3.24**	2.32*	2.25*	5.60**	7.03**	8.48**	8.29**	4.06**	3.91**	3.77**	1.94^0	1.93^0	0.01
Independent Profession Selection	5.72**	2.56*	8.90**	2.05*	5.58**	8.37**	13.38**	7.16**	10.70**	7.72**	0.02	3.75**	5.88**	3.87**	2.81**
Dependent Profession Selection	4.06**	4.21**	5.51**	5.96**	1.42	0.74	7.60**	9.31**	2.32*	7.65**	10.36**	2.04*	2.07*	6.12**	6.71**
Motives Related to External Prestigious Professions and Financial Welfare	7.27**	2.47*	8.31**	1.24	1.24	5.87**	13.72**	6.24**	7.94**	10.98**	2.77**	3.61**	4.70**	6.81**	0.38
Motives Related to Romanticization of Profession	6.43**	3.24**	7.78**	3.81**	1.05	3.96**	12.54**	8.96**	4.38**	10.58**	6.57**	1.47	3.63**	7.73**	4.22**
Motives Related to Eagerness of Compensation of Temper Flaws	7.27**	3.08**	5.73**	1.06	2.31*	5.08**	10.96**	6.89**	8.51**	8.22**	3.32**	5.01**	3.69**	3.30**	0.81
Antisocial Motives (attitude to profession was as if it was a way to satisfy own antisocial needs)	5.91**	6.57**	6.36**	5.07**	1.21	0.65	10.43**	10.40**	3.60**	10.96**	11.62**	3.58**	2.38*	6.61**	5.37**
Methods of Studying the Motivational Profile of Personality															
Need of High Salary and Financial Benefits	2.40*	0.18	1.32	3.83**	1.65	2.28*	3.09**	1.48	0.93	1.46	3.72**	1.50	4.33**	2.51*	2.44*
Need of High Working Conditions and Comfortable Surrounding	0.97	2.00*	0.39	0.43	2.26*	2.59*	0.57	1.19	2.81**	2.18*	1.17	0.45	0.72	2.43*	1.48
Need of Accurate Structurization of Work and Feedback	1.80^0	0.42	1.13	0.05	0.71	1.76^0	0.48	1.71^0	1.09	0.96	0.34	0.46	1.06	0.49	0.65
Need of Social Relations	0.24	1.92^0	0.13	0.39	3.13**	1.91^0	0.29	0.19	3.05**	1.01	1.62	1.77^0	0.41	2.02*	2.60*
Need of Forming and Supporting Long-Term Stable Relations	0.45	0.27	2.94**	0.44	1.75^0	0.71	3.26**	0.82	2.14*	2.68**	0.20	1.47	2.23*	1.38	1.09
Need of Obtaining the Recognition by Other People	0.45	0.41	1.45	1.95^0	0.96	0.10	1.11	2.21*	1.30	1.25	2.27*	1.33	2.73**	2.02*	1.06
Need of Posing Brave and Tough Goals and of Achieving Them	1.09	1.05	0.15	0.34	1.94^0	2.06	0.69	1.12	0.62	0.86	0.31	3.20**	0.40	1.26	1.69^0

Psychodiagnostic methods	$t_{MWE-MHE}$	t_{MHE-HE}	t_{MWE-WE}	$t_{MWE-MLE2}$	$t_{MWE-MLE1}$	t_{MHE-HE}	t_{MHE-WE}	$t_{MHE-MLE2}$	$t_{MHE-MLE1}$	t_{HE-WE}	$t_{HE-MLE2}$	$t_{HE-MLE1}$	$t_{WE-MLE2}$	$t_{WE-MLE1}$	$t_{MLE2-MLE1}$
Methods of Studying the Motivational Profile of Personality – cont.															
Need of Manipulation and Power, Eagerness to Control the Other People	0.17	0.33	2.04*	0.04	0.04	0.56	1.96^0	0.18	0.15	2.96**	0.21	0.45	1.73^0	2.32*	0.08
Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine	0.18	1.20	1.52	2.20**	2.34*	0.66	1.42	1.65	1.71^0	2.43*	1.43	1.52	3.08**	3.21**	0.03
Need of Being Creative, Thoughtful, and Open for further Ideas Employee	0.39	0.18	0.72	1.96^0	0.93	0.27	0.86	2.00^0	0.44	0.78	2.11*	0.83	0.35	1.09	2.39*
Need of Self-Improvement and Development as Personality	0.85	0.58	0.87	2.94**	0.06	0.38	0.02	2.23*	0.78	0.39	2.72**	0.51	2.30*	0.80	2.87**
Need of Interesting and Socially Beneficial Work	0.61	2.03*	1.63	5.25**	0.05	1.05	1.92^0	5.21**	0.61	2.90**	6.73**	1.90^0	2.57*	1.54	4.98**
Test of Sense-Bearing and Life Orientations															
General indicator of Sense-Bearing and Life Orientations	2.04*	16.16**	2.58**	4.13**	16.46**	9.31**	1.68^0	5.06**	10.24**	1.94^0	13.81**	2.03*	4.26**	2.52*	14.49**
Goal	2.07*	12.75**	0.52	2.70**	15.34**	8.21**	0.21	3.86**	10.38**	2.91**	9.48**	3.22**	1.79^0	3.63**	10.87**
Process	3.51**	10.74**	3.90**	1.55	8.55**	5.33**	2.46*	3.63**	4.37**	0.50	7.31**	0.28	4.32**	0.59	6.68**
Result	1.84^0	13.44**	2.13*	3.30**	15.14**	9.14**	1.31	4.28**	10.95**	2.19*	10.18**	3.32**	3.80**	3.09**	11.39**
Locus of Control-I	1.60	11.85**	1.43	0.76	15.88**	8.22**	0.71	1.65	11.53**	2.50*	6.22**	5.02**	1.68^0	3.74**	7.94**
Locus of Life Control	0.96	13.12**	0.74	2.17*	15.49**	8.69**	0.27	2.61*	10.78**	3.61**	8.78**	3.59**	1.92^0	4.68**	10.27**
Method of Locus of Control															
General internality	8.89**	7.58**	1.56	6.06**	8.44**	17.44**	3.52**	0.24	0.32	5.64**	10.98**	15.98**	3.11**	3.61**	0.01
Internality in thoughts about life in general	5.47**	6.14**	3.64**	3.79**	7.64**	11.01**	0.94	0.09	1.07	8.12**	7.45**	14.62**	0.66	1.94^0	0.86
Internality in description of personal experience	6.26**	6.00**	0.24	4.76**	5.75**	11.40**	3.34**	0.38	0.47	2.02*	8.02**	10.88**	3.23**	3.08**	0.73
Internality in the sphere of achievements	4.76**	5.05**	1.60	4.52**	3.61**	10.92**	4.35**	1.07	0.77	0.87	8.32**	8.79**	4.56**	3.76**	1.61
Internality in the sphere of fails	4.97**	0.45	1.36	1.95^0	6.56**	5.34**	4.01**	2.03^0	1.28	1.16	2.26*	6.94**	2.41*	4.85**	3.19**
Internality to self-incrimination	0.03	3.81**	0.30	2.47*	1.55	3.20**	0.31	2.26*	1.43	1.83^0	5.11**	1.27	2.09*	0.79	3.40**
Internality in the sphere of professional activity	9.55**	4.79**	1.69^0	5.55**	8.62**	14.02**	4.48**	0.98	0.32	4.38**	8.39**	12.73**	2.92**	4.13**	0.72
Professional and social aspect of internality	7.78**	3.68**	3.06**	4.21**	6.05**	11.03**	3.29**	0.97	0.96	5.70**	6.27**	8.95**	1.60	2.25*	0.22

Psychodiagnostic methods	$t_{MWE\text{-}MHE}$	$t_{MHE\text{-}HE}$	$t_{MWE\text{-}WE}$	$t_{MWE\text{-}MLE2}$	$t_{MWE\text{-}MLE1}$	$t_{MHE\text{-}HE}$	$t_{MHE\text{-}WE}$	$t_{MHE\text{-}MLE2}$	$t_{MHE\text{-}MLE1}$	$t_{HE\text{-}WE}$	$t_{HE\text{-}MLE2}$	$t_{HE\text{-}MLE1}$	$t_{WE\text{-}MLE2}$	$t_{WE\text{-}MLE1}$	$t_{MLE2\text{-}MLE1}$
Method of Locus of Control – cont.															
Professional and processual aspects of internality	5.95**	3.32**	0.12	3.24**	6.17**	8.87**	3.26**	0.39	0.47	1.70^0	4.91**	8.93**	2.33*	3.52**	0.70
Internality in interpersonal communication	4.51**	6.85**	2.45*	2.60*	5.23**	10.20**	0.42	0.64	0.04	5.90**	6.56**	12.00**	0.13	0.47	0.70
Competence in the sphere of interpersonal relations	3.81**	6.27**	2.43*	3.75**	2.90**	10.04**	0.40	0.78	0.90	5.27**	8.22**	9.12**	0.13	0.90	1.49
Responsibility in the sphere of interpersonal relations	2.35*	2.35*	0.30	0.22	3.21**	4.99**	1.60	1.77^0	1.05	2.08*	1.13	5.59**	0.42	2.40*	2.44**
Internality in the sphere of family relations	2.02*	4.49**	1.71^0	1.08	2.14*	5.97**	0.15	0.48	0.21	4.64**	4.07**	5.85**	0.54	0.02	0.64
Internality in the sphere of health	5.97**	3.95**	0.29	4.04**	4.86**	9.90**	4.98**	0.06	0.32	3.56**	6.42**	8.06**	3.59**	4.15**	0.18
Negation of activity	7.44**	6.51**	7.40**	6.98**	6.78**	14.97**	0.27	1.36	0.72	15.28**	12.10**	12.39**	1.59	0.95	0.61
Readiness for activities which was connected with difficulties overcoming	4.75**	6.92**	6.03**	5.17**	3.39**	12.15**	3.21**	1.92^0	0.52	9.80**	9.57**	8.89**	1.29	3.33**	2.14**
Readiness for self-planning, acting and responsibility for their activities	5.22**	1.85^0	0.62	2.40*	5.76**	7.98**	2.93**	0.39	1.67^0	0.14	3.45**	7.76**	2.14*	3.61**	1.36
Method of Will-Regulated Personality															
Value-semantic organization of personality	1.35	8.23**	0.45	4.87**	10.58**	7.70**	0.31	3.00**	9.85**	3.87**	11.65**	4.01**	2.20*	5.41**	13.44**
Organization of activity	2.96**	3.03**	1.49	6.23**	3.27**	5.76**	0.60	3.51**	5.49**	3.29**	8.63**	1.15	3.37**	3.64**	8.13**
Determination	0.87	7.77**	1.16	6.76**	10.08**	8.31**	0.76	5.80**	10.50**	4.24**	13.57**	3.52**	2.23*	5.52**	15.20**
Perseverance	1.86^0	8.33**	1.45	8.73**	9.98**	7.86**	0.46	5.49**	9.33**	4.44**	15.66**	2.90**	2.70*	5.40**	16.61**
Self-control	1.62	8.09**	1.55	7.41**	8.99**	7.83**	0.86	5.14**	8.75**	3.95**	13.45**	2.19*	1.66	4.55**	13.96**
Self-Reliance	1.81^0	4.43**	3.07**	6.54**	2.81**	1.75^0	3.80**	7.52**	1.09	4.65**	9.84**	0.24	0.61	4.27**	8.00**
Lies index	3.68**	2.62**	0.39	0.98	5.63**	5.48**	2.33*	1.88^0	7.59**	0.69	2.58*	3.53**	0.92	2.46*	4.85**
General indicator characterizing the volitional organization of the personality	1.65	10.17**	1.96^0	13.94**	13.07**	10.23**	1.25	10.00**	12.73**	5.19**	28.08**	4.15**	2.70*	6.29**	30.06**

Notes: MWE – middle level of effectiveness with tendency to weak indicator of effectiveness; MHE – middle level of effectiveness with tendency to high indicator of effectiveness; HE – highly effective; WE – weakly effective; MLE2 – middle level of effectiveness; MLE1 – middle level of effectiveness; $^0p\leq .1$; $^*p\leq .05$; $^{**}p\leq .01$

The next stage of hierarchy was occupied by “Independent Profession Selection”, “Motives Related to Personal and Professional Development”, “Motives Related to Eagerness of Compensation of Temper Flaws”, “Motives Related to External Prestigious Professions and Financial Welfare”. Law there were “Motives Related to Romanticization of Profession”. The lowest stage of hierarchy was occupied by “Dependent Profession Selection” and “Antisocial Motives”. The indicators of “Dependent Profession Selection” and “Antisocial Motives” were the lowest among the compared groups (they were the lowest only in participants of group MHE).

According to “Methods of Studying the Motivational Profile of Personality” (Ritchie and Martin, 1999) there were determined the highest points of the scale“Need of Accurate Structurization of Work and Feedback” that demonstrated their responsible attitude to accurate realization of the goal as well as eagerness to satisfy the expectations. The second stage of the hierarchy was occupied by “Need of Interesting and Socially Beneficial Work” and “Need of High Salary and Financial Benefits”. The proximity of indicators concerning those indicators showed that for servicemen of the NGU socially beneficial work considered to be the source of income. The next stage was occupied by “Need of Posing Brave and Tough Goals and of Achieving them”, “Need of Obtaining the Recognition by Other People”, “Need of Self-Improvement and Development as Personality” which characterized those servicemen as brave, ambitious, and desiring to develop people.

In accordance with “Sense-Bearing and Life Orientations” (Muzdybaiev, 1998) servicemen of group HE had one of the highest indicators of “Life Understanding”. They had significant prospects of life and one of the highest indicators concerning the scale “Locus of Control-I” and “Locus of Life Control”.

During the use of the method “Locus of Control” (Ksienofontova, 1999) servicemen of group HE had the highest indicators of internality among all the compared groups: “General Internality”, “Internality in the Sphere of Achievements”, “Internality in the Sphere of Professional Activity”, “Internality in Interpersonal Communication”. Furthermore, they had high indicators of the scale “Internality in the Sphere of Health”, “Readiness for Activities which was Connected with Difficulties Overcoming”, “Readiness for Self-Planning”. This group had the lowest indicator of a return scale “Negation of Activity”. Servicemen of this group did not eager to shift the blame of fails on others “Internality in the Sphere of Fails”.

Due to the method of “Will-Regulated Personality” (Hutkin and Mikhalchenko, 2000) members of group HE also had one of the highest indicators they conceded only before group MLE1. The highest indicators of group HE were “Perseverance”, “Determination” and “Self-Control”. The lowest indicators were “Self-Reliance” and “Organization of Activity”. The latest indicators were the highest among the compared groups and had sufficient level of development.

The less effective group was distinguished on the basis of other significantly high indicators ofthe method which described the motivation. Thus, due to the data of“Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” (Moskalienko, 2002) the participants of group WE differed from all other groups on statistically significant level. The exceptions were the indicators of group MLE1 in the scale “Motives Related to Objective Socially Posed Tasks and to Content of Profession”, “Motives Related to External Prestigious Professions and Financial Welfare” and the lowest and minimal indicators were presented by the scales “Dependent Profession Selection”, “Antisocial Motives”. This data demonstrated thepassion of those servicemen and their high emotionality.

To the core of “Methods of Studying the Motivational Profile of Personality” (Ritchie and Martin, 1999) there was incorporated the mechanism of overcoming social eagerness to get answers. It gave more information concerning the hierarchy of motives of this group; however, the distance from the most significant motive to the least significant stayed the shortest among all the compared groups. The most expressed motives of this group as well as in group HE of servicemen were “Need of Accurate Structurization of Work and Feedback”, but the next stage was occupied by motives which described comfortable conditions of work including normal circle of communication: “Need of Forming and Supporting Long-Term Stable Relations”, “Need of High Working Conditions and Comfortable Surrounding”, “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”. The least important in that group were the motives which described the interest in society: “Need of Obtaining the Recognition by Other People”, “Need of Interesting and Socially Beneficial Work”, “Need of Manipulation and Power, Eagerness to Control the Other People”, “Need of Social Relations”.

According to the data proposed by “Sense-Bearing and Life Orientations” (Muzdybaiev, 1998) the participants of group WE had middle indicators of life overthinking and control localization (that level was higher than in groups MWE, MHE, and MLE2 and lower than in groups MLE1 and HE). Peculiarities of this group were in concentration on “Life Process”, in accordance with this scale there were distinguished the highest indicators among all the compared groups.

Moreover, there was a need to highlight the indicators of group WE concerningthe method of "Locus of Control" (Ksienofontova, 1999) and their combination with indicators on the method "Will-Regulated Personality" (Hutkin and Mikhalchenko, 2000). Thus, on the "General Scale" those servicemen with low efficiency had one of the highest indicators giving priority only to group HE. That showed absolute connection between their "I" and motives including desires and needs. However, the scale "Readiness for Difficulties Overcoming" demonstrated the lowest indicators among all the distinguished groups.

Participants of this group had one of the lowest indicators of the scale "Will-Regulated Personality", they gave priority only to group MLE2. Among all the compared groups the least effective servicemen had the lowest indicators of the scale "Self-Reliance" that way they developed the dichotomywith highly effective servicemen whose indicators of this scale were the highest among all the groups.

Participants of group WE as well as of MLE1 had the highest points of the scale "Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs" (Moskalienko, 2002). However, the hierarchy of motives of choosing the profession in group MLE1 differed from group WE. The most expressed and significant in comparison with other groupswere the "Motives Related to Objective Socially Posed Tasks and to Content of Profession". The next hierarchy stage was developed by three types of motives which were marked with the eagerness of self-regulation: "Independent Profession Selection", "Motives Related to Eagerness of Compensation of Temper Flaws" and "Motives Related to Personal and Professional Development".

"Motivational Profile of Personality" of this group was built around the vector: "Need of High Salary and Financial Benefits", "Need of Manipulation and Power, Eagerness to Control the Other People". That characterized those servicemen as performers, so called "employee".

The second hierarchy stage was formed by: "Need of Accurate Structurization of Work and Feedback", "Need of Obtaining the Recognition by Other People". Such a high position of desire to be recognized highlighted this group among all the other groups.

The third stage was developed with the help of the following motives: "Need of Self-Improvement and Development as Personality", "Need of Interesting and Socially Beneficial Work". Their combination demonstrated the importance of professional honour for those servicemen.

Specific stage was occupied by the motives of social cooperation "Need of Social Relations", "Need of Forming and Supporting Long-Term Stable Relations". Indicators of the scale "Need of Social Relations" of this group were the highest among all the compared groups (differences reached the level of statistic importance and tendency to these motives with other groups except group HE).

The next stage included four types of motives due to which servicemen of group MLE1 had the lowest indicators among all the compared groups: "Need of Posing Brave and Tough Goals and of Achieving Them" – 28.98±4.11, "Need of Being Creative, Thoughtful, and Open for Further Ideas Employee", "Need of High Working Conditions and Comfortable Surrounding", and "Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine".

The peculiarities of group MLE1 included incredibly high indicators of the general scale "Sense-Bearing and Life Orientations" which statistically differed from other groups. Sense of life in this group was focused on the future – those servicemen had the highest indicators of the scale "Goal". However, group MLE1 had the highest indicators among all the compared groups of the scale "Result" that highlighted the importance of recognizing their achievements by the participants.

Group MLE1 had an interesting combination of scales "Locus of Control" and "Will Regulation". Thus, group MLE1 had the highest indicators among all the compared groups of both scales of locus of control method of "Sense-Bearing and Life Orientations" (Muzdybaiev, 1998): "Locus of Control – I", "Locus of Control – Life". Although, according to the method"Locus of Control" in group MLE1 there were one of the lowest indicators of the general scale and according to the scales which differentiated that indicator: "Internality in Thoughts about Life in General", "Professional and Processual Aspects of Internality", "Responsibility in the Sphere of Interpersonal Relations", "Readiness for Self-Planning", and "Internality in the Sphere of Fails".

According to all the scales "Will-Regulated Personality" the servicemen of group MLE 1 had the highest points. The exception was made by the scale "Self-Reliance" which was the highest in group HE. Among the components of will regulation of servicemen from group MLE1 the most expressed were: "Perseverance" and "Determination". The least expressed indicators were: "Self-Reliance" and "Organization of Activity".

Such combination demonstrated the executive motivation in the sphere of professional activity and cooperation concerning the conscious perception of the role of “a hard-working performer”. Servicemen of group MLE1 realized their actions and were able to overcome difficulties to achieve the posed goals, they trusted the decisions of their commanders, and they were assured in their actions that gave them the possibility to express determination.

Group MLE1 also had the highest points of the indicator “Insincerity” that did not demonstratethe insincerity, but their being orientated on socially approved “correct” answer on the commander’s claims.

Participants of group MLE2 were the only individuals whose motives connected with socially posed goals and with the content of profession were not leading during the military profession selection. Other peculiarities included slight differentiated motives of profession selection. Thus, the most highlighted were: “Motives Related to Personal and Professional Development”, “Motives Related to Eagerness of Compensation of Temper Flaws”. The least expressed were motives “Dependent Profession Selection”. The second stage was occupied by “Motives Related to Objective Socially Posed Tasks and to Content of Profession”. Penultimate stage was obtained by “Motives Related to Romanticization of Profession”, “Independent Profession Selection”, and “Antisocial Motives”. Nevertheless, romantic and antisocial motives were significantly higher than in other compared groups, but they gave priority only to the indicators of group WE.

Received indicators of the method “Motivational Profile of Personality” (Ritchie and Martin, 1999) were protected from the effects’ activity which negatively influenced the differentiation of motives. According to the data of this method group MLE2 had the following significant indicators: “Need of High Salary and Financial Benefits”. The least expressed and lagged indicators were “Need of Interesting and Socially Beneficial Work”. Motivational vector, which they developed, depicted their expressive, useful, and selfish direction.

The second stage in hierarchy of motives was occupied by “Need of Obtaining the Recognition by Other People”, “Need of Accurate Structurization of Work and Feedback”. The data represented by the scale “Need of Obtaining the Recognition by Other People” showed that on statistical level servicemen of group MLE2 differed from other groups except group MLE1 with which they had almost identical indicators.

The next stage in hierarchy of motives “Need of Being Creative, Thoughtful, and Open for further Ideas Employee”, “Need of Posing Brave and Tough Goals and of Achieving them”, and “Need of High Working Conditions and Comfortable Surrounding”. Group MLE2 had one of the highest indicators of “Need of Being Creative, Thoughtful, and Open for Further Ideas Employee” due to which on statistic level they differed from all other groups except group WE.

On the next stage there was a combination of “Need of Forming and Supporting Long-Term Stable Relations” and “Need of Self-Improvement and Development as Personality” which demonstrated definite benefits of relations. Servicemen of group MLE2 wanted to choose the surrounding which could help their development.

The next stage was occupied by “Need of Social Relations”, “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”, it determined that social relations were used by the participants of group MLE2 for fun, rest, and spare time spending.

Servicemen of group MLE2 had the lowest indicators among all the compared groups on the scales of the method “Sense-Bearing and Life Orientations”. Despite that, the indicators of this group on the scale “Goal” were and “Process”, those results were almost identical that demonstrated their orientation on the nearest future.

Group MLE2 among the other groups washighlighted with the highest indicator of the scale “Negation of Activity” that demonstrated the confidence in futility of eagerness to achieve the goals of life.

Moreover, group MLE2 had the lowest indicators of all the scales of the method “Will-Regulated Personality” among all the compared groups. Extremely low indicators were inthe scale “Self-Reliance”.

Indicators of the motivation of choosing the military profession in group MHE were weakly expressed in the scale of the method “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs”. The motives which were diagnosed with the help of this method developed the vector from “Dependent Profession Selection”, “Antisocial Motives” to “Motives Related to Objective Socially Posed Tasks and to Content of Profession”. The second stage in hierarchy of motives of profession selection showed the struggle for professional development in the past: “Motives Related to Eagerness of Compensation of Temper Flaws”, “Motives Related to Personal and Professional Development” and “Motives Related to External Prestigious Professions and Financial Welfare”.

Due to the “Methods of Studying the Motivational Profile of Personality” (Ritchie and Martin, 1999) the group MHE had the most expressed indicators which made them significantly different from others in “Need of High Salary and Financial Benefits" and the least expressed were indicators of "Need of Manipulation and Power, Eagerness to Control the Other People".

The second stage in hierarchy of motives was occupied by "Need of Interesting and Socially Beneficial Work", "Need of High Working Conditions and Comfortable Surrounding", "Need of Accurate Structurization of Work and Feedback". Then there were motives which demonstrated their ambitions "Need of Obtaining the Recognition by Other People", "Need of Self-Improvement and Development as Personality".

Less important for group MHE were the motives which described the eagerness to get new and non-standard issues in their work: "Need of Posing Brave and Tough Goals and of Achieving Them", "Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine", and "Need of Being Creative, Thoughtful, and Open for further Ideas Employee". Even less important for them was the desire to be engaged into social cooperation: "Need of Forming and Supporting Long-Term Stable Relations", "Need of Social Relations".

Due to the scales of the method "Sense-Bearing and Life Orientations" (Muzdubaiev, 1998) servicemen of group MHE had statistically lower indicators than groups HE and MLE1, but higher than group MLE2. Besides that, they as well as the mentioned groups had almost identical indicators of the scales "Goal" and "Process". There was not any increase of the scale "Result".

Due to the indicators of the method "Locus of Control" (Ksenofontova, 1999) servicemen of group MHE had identically low quantity of general points as well as groups MLE1 and MLE2. The peculiarities of group MHE included the lowest indicators among all the compared groups "Internality in the Sphere of Professional Activity" which were formed because of weak indicators "Professional and Social Aspect of Internality". Moreover, other indicators related to the control in cooperation were decreased "Internality in Interpersonal Communication", "Competence in the Sphere of Interpersonal Relations" and "Responsibility in the Sphere of Interpersonal Relations".

In accordance with the data of the method "Will-Regulated Personality" (Hutkin and Mikhalchenko, 2000) servicemen of group MHE had indicators which were maximally close to group WE. However, their indicators of scales were weakly differentiated that did not demonstrate any urgency of will regulation, they substituted it with postarbitrary activity to make professional activities automatic.

Servicemen of group MHE had the lowest indicator of "Insincerity" that showed not only the desire to give completely honest answers, but also demonstrated slightly weaker social tolerance and communicative correctness.

In the structure of motivation of choosing the military profession group MWE had the most expressed motives such as: "Motives Related to Objective Socially Posed Tasks and to Content of Profession". Close to them there were "Motives Related to Eagerness of Compensation of Temper Flaws". Further there occurred "Motives Related to Personal and Professional Development" and "Motives Related to External Prestigious Professions and Financial Welfare". On the other side of hierarchy there were Dependent Profession Selection", "Antisocial Motives", "Motives Related to Romanticization of Profession". In group MWE intermediate position between values and motives which depreciated was occupied by the motives "Independent Profession Selection".

The participants of group MWE came close to the indicators of group HE: "Motives Related to Objective Socially Posed Tasks and to Content of Profession", "Motives Related to Personal and Professional Development". According to the scales "Dependent Profession Selection", "Motives Related to External Prestigious Professions and Financial Welfare", "Motives Related to Romanticization of Profession" and "Antisocial Motives" participants of group MWE moved closer to group MLE1.

Due to the data presented by the "Methods of Studying the Motivational Profile of Personality" (Ritchie and Martin, 1999) group MWE had the most expressed indicators of "Need of Accurate Structurization of Work and Feedback", "Need of High Salary and Financial Benefits". The next dense group of motives revealed in group MWE the urgency of needs of improving social status with their own powers "Need of Obtaining the Recognition by Other People", "Need of Self-Improvement and Development as Personality" and "Need of Interesting and Socially Beneficial Work".

The eagerness to change life for better and to make it more comfortable distinguished the place in hierarchy of motives of group MWE due to results received from the scales: "Need of High Working Conditions and Comfortable Surrounding", "Need of Posing Brave and Tough Goals and of Achieving Them".

Group MWE was quite low in hierarchy of motives "Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine", "Need of Being Creative, Thoughtful, and Open for further Ideas Employee".

Less important for group MWE were indicators of "Need of Forming and Supporting Long-Term Stable Relations", "Need of Social Relations” and “Need of Manipulation and Power, Eagerness to Control the Other People” that demonstrated the eagerness to avoid social relations.

According to the scales of the method “Sense-Bearing and Life Orientations” (Muzdybaiev, 1998) servicemen of group MWE had one of the lowest indicators (lower than in the 5th group). Their indicators of the methods “Locus of Control” (Ksenofontova, 1999) and “Will-Regulated Personality” (Hutkin and Mikhalchenko, 2000) moved indicators closer to group WE (it had the lowest quantity of statistically important differences). Unlike group WE they had significantly better indicators of the scale “Negation of Activity”, they had more readiness to act to overcome the differences, as well they had higher level of confidence in possibility to run their own lives, they did not tend to shift their responsibility on others due to results of cooperation, and they had significantly higher indicators of “Self-Reliance”. That indicated their high level of self-reliance and low level of dependency in professional and interpersonal relations in comparison with participants from group WE

DISCUSSION

The research demonstrated that servicemen with the lowest effectiveness of combat operations had the highest indicators through all the motives unlike those with higheffectiveness. However, this case wasnot the realization of the law of Yerkes and Dodson (1908). It was likely that the received data demonstrated the phenomenon of forming the tangentiality of awakening and of free motivation which were described by the Eastern European psychology by Lieontievym (1975) and Byhotskym (1983), by phenomenon of self-efficiency proposed by the American psychology Bandura (1994) who considered that the success of activity was determined not only by the attractiveness of result, but also by the trust in their personal powers.

Thus, to estimate the motivation appropriately there was a need to include servicemen of distinguished groups into motivational types which consisted of content, hierarchy, intensity of motives and their possibility to be implemented.

In that way highly effective servicemen choosing profession considered its content, expressed eagerness to realize themselves in their profession, comply with it, develop in profession, and acquire the professional skills. They rejected dependency and unlawful benefit as motives of profession selection. For relevant motivation of HEit was typical to have eagerness to execute posed tasks and to meet expectations. It was important for them to do socially beneficial work which was understood by them as the only source of income. Important peculiarity of their motivation was the preservation of high professional ambitions – they struggled for professional development, tough tasks, and high estimation of their achievements. Motives which described the conditions andregime of work, social cooperation were non-essential. Servicemen with high effectiveness were characterized as those who had high level of life understanding, significant life prospects, and considered themselves to be strong personality with sufficient will of selection. They were assured that people could control their lives, could make decisions by themselves, and could implement them. Existing content of motivation was supported by high level of internality and developed qualities of will. Thus, servicemen of group HE took ultimate responsibility for their actions having analyzed them, they did not avoid any work, could plan, and put efforts by themselves to achieve the results they struggled for and whichthey were not ashamed of. Their responsibility spreadon the results of professional activity and interpersonal communication as well as on the health. Their obtained level of internality was, first of all, their own experience of successes and fails. Strong-willed quality of servicemen from the group with high efficiency were well-developed and, first of all, met the requirements of their professional activity including tough and unsafe character of their profession as well as cruel normative settlement.

Servicemen with weak effectiveness, as it was already mentioned, had significantly high indicators of all the scales which described the motives of activity and its selection and slight differentiation of those motives. This data gave possibility to talk about insufficiently formed randomness of activity which was presented by inability to cope with desires, low level of self-reliance and reluctance to overcome difficulties. Servicemen from group WE depended on their desires as well as on their surrounding. They wanted to have simple, comfortable work which was usually occupied by them thanks to the patronage of people from their close surrounding.

Presented features of servicemen from groups HE and WE on the indicators of combat activity correlated with the situations concerning the connection between Public Service Motivation (PSM) with work effectiveness and its mediated self-efficiency (Breaugh, Ritz, and Alfes, 2018).

According to it servicemen from group HE was depicted as the type which was guided by motivation of public service, and servicemen from group WE as dependent motivational type.

Servicemen from group MLE1 had highly expressed motivation of a performer which was based on conscious trust in their commanders and marked qualities of will.

Those servicemen were orientated namely on military profession acquiring (high points for the motives of professional selection) and on making efforts to master it. They were responsible and determined concerning the execution of their tasks. They agreed to execute tough tasks, routine actions, and were undemanding concerning the working conditions. It was quite important for those servicemen to have high salary, recognition of their achievements, conformity of social expectations, and support of friendly relations.

Having compared motives of groups HE and MLE1 it was possible to conclude that they had plenty of common aspects concerning their motives of public service and prosocial motivation (Schott, Neumann, Baertschi, and Ritz, 2019). According to that data servicemen from group MLE1 were included into prosocial motivational type. Besides that, the description of this type was supported by the data received by Gillet, Becker, Lafrenière, Huart, and Foiquereau (2017). They determined that servicemen with the highest indicators of autonomous motivationfelt high organizational support, commander’s support, positive experience, and high level of communicationbecause they were engaged into professional activity due to their pleasure, personal choice, and personal will, demonstrated the highest level of well-being.

Servicemen of group MLE2 were the only participants among all the distinguished types who chose their profession being led by their own mercenary interests, possibility to work with things they liked and were interested in, those activities were full of romantic, but not of objective content of the profession. They used social relations to be “on good standing” with their commanders or to gain satisfaction from communication. Those servicemen did not want to realize their strong-willed qualities. Such servicemen required constant control from the side of commanders because they could be addicted to the “romantic” features of their profession neglecting its core sense. The description of this group of servicemen was close to the definition of motivational type proposed by Stéphanie Vincent Lyk-Jensen and Ane Glad (2018) – “Personal Benefit”. Thus, servicemen from group MLE2 were included into romantic (struggle for adventures) motivational type. Servicemen of this type searched for adventures, challenges; however, they were unstable concerning the hardships of service, the actions of combat factors of stress and, usually, these servicemen resigned after the first contract expired.

Servicemen of group MHE choosing the military profession considered its content and were ready to make everything possible to acquire it; however, this motivation lost its urgency. Acquiring the profession they concentrated on financial benefits, personal ambitions, and comfortable conditions of work; they were weakly motivated to put efforts to acquire new profession. It was useful for them to be orientated on the nearest future. Most of professional actions were executed automatically without any strong-willed efforts. They did not get along with their surrounding and showed low level of tolerance. This type was determined as a stagnated one. Nevertheless, even if this type had quite high indicators of effectiveness the general prospects for this type were pessimistic. In accordance with Voet, Steijn, and Kuipers (2017), who distinguished positive relations between the eagerness of changes and prosociality, group MHE was likely to decrease its altruistic behaviour.

Servicemen of group MWE chose the military profession because of its content as well as of his/her own desire to change the life and social status for better. In urgent situation it was important for them to have understandable and well-paid activity which would give them the possibility to improve their social status and conditions of lifeinternally. These servicemen did not feel themselves ready for intellectual and creative work. They did not cooperate with their surrounding and that fact detained the process of professional socialization. Despite the desire to help other people, work, endeavour, and change, they did not achieve high effectiveness in professional activity as a serviceman due to their concentration on defeating the difficulties of life. This type having analogy with Maslow (1943) was marked as a deficient type of motivation. The determination of deficient type of motivation of servicemen who joined the army to change their lives for better, to get new prospects, all those factors were not something new in military psychology. That was the way the American researchers pointed out that military service was often joined by Afro-Americans and people from Latin America, not so often it was joined by people who positively studied at school, planned to enter the college and by parents who graduated from the Higher Educational Establishments (Bachman, Segal, Freedman-Doan, and O’Malley, 2000).

CONCLUSION

Conducted research gave the possibility to determine 6 motivational types related to the effectiveness of servicemen’s operational activity. These motivational types were located between the poles which were introduced by two dimensions: self-efficacy (autonomous and controlled motivation) and motivation of helping others (motivations of public service – selfishness). Those motivational types of servicemen were motivated by public service, stagnant, prosocial, romantic, deficient and dependent. Distinguished types included the content, hierarchy, intensity of motives and their possibility to implement them.

Developmentof the motivation diagnostics of the applicants to the military service on the basis of their determinationto one of the distinguished motivational types would help to decrease the quantity of mistakes during the prediction of effectiveness of operational activity.

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Copper and its role in the human body – the importance of establishing copper concentrations in the body

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Abstract: *Copper is essential to all living beings, as a food mineral, being an essential component of the cytochromoxidase enzymes complex. It is also an essential component of several systems involved in the production of hemoglobin, of sugar metabolism, of catecholamines' synthesis and in the formation of cross-links between collagen, elastin and keratin fibers within the hair. The dysregulation of copper through impaired absorption or excretion, either genetically based or in acute or chronic poisoning, results in deficiency or toxicity. For this reason, the measurement of copper levels in various biological samples: urine, blood, or hair, provides the physician with information regarding contamination, poisoning, or a metabolic disease. Due to low concentrations in most heavy metals' and metalloids' cases, finding traces in biological samples is a difficult problem. Classical methods (staining, flame photometry, reagent kits, electrochemical methods) can only be used for a limited number of elements, such as sodium, potassium, calcium, magnesium, copper, iron and zinc. The analysis of biological samples is the most important use of graphite furnace atomization. The advantages of using this technique are its great sensitivity and the very low quantity of sample necessary for performing the measurement.*

In modern medicine, determining microelements is very important in diagnosing diseases and/or in treating them. The GF-AAS method is quick, reproductible and has very good sensitivity.

Keywords: *copper, toxicity, metabolism disorders, Wilson disease, determination methods, spectrophotometry, GF-AAS*

INTRODUCTION

A great number of chemical elements in nature are present in the body, either as standalone components, or bound one to another, forming organic and inorganic substances that facilitate or create various disorders in the human body. A part of them (Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, Sr and V) are essential to life.

Other elements (As, Ba, Be, Cd, Li, Hg, Pb, Sb, Sn) are considered toxic, and their concentrations in any given biological product is preferred to be close to nil. When such concentration is different from zero, the value is considered normal if it is lower than the limit established by toxicology authorities around the world.

Copper is a chemical element that has been used for thousands of years in alloyes. Its compounds are frequently found as copper (II) salts, which usually yield the blue or green colours of minerals, such as azurite or turquoiuse, and have been largely used as pigments. Copper sulfate forms are bright blue crystals containing five molecules of water [$CuSO_4 \cdot 5H_2O$]. It is commonly known as the "Blue Vitriol" or "Blue Stone" [1] and it is used mainly for agricultural purposes as a pesticide, and in the leather industry [2, 3].

Biological Roles of Copper

Copper is essential to all living beings, as a food mineral, as

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it is an essential component of the cytochromoxidase enzymes' complex. It is found in the liver in vertebrates. In high concentrations, copper compounds are toxic for evolved organisms, and are used as bacteriostatics and fungicides.

Copper alloys have intrinsical natural properties that kill a large number of microorganisms, such as E. coli O157: H7, methicillin-resistant Staphylococcus aureus (MRSA), Clostridium difficile, influenza virus A, adenovirus, fungi [4, 5]. Some copper 355 alloys have been shown to kill more than 99.9% of germs within two hours, when properly cleaned [4]. The United States Environment Protection Agency (EPA) has authorized these copper alloys as „antimicrobial materials with public health benefits" [4]. Recent biotechnological researches use copper ions coupled with indocyanine and proteins in targeted cancer treatments [6].

Proteins which contain copper have several biological roles in electron and oxygen transport, processes in which interconversion of Cu (I) and Cu (II) easily takes place [7]. The biological role of copper began the moment oxygen appeared in our planet's atmosphere [8].

In the cytochrome-c oxidase, which is necessary for aerobic respiration, copper and iron work together to reduce oxigen. Copper is found in superoxide-dismutase, which catalyzes the breakdown of superoxides, through conversion to oxygen peroxide and hydrogen:

$$2\ HO_2 \rightarrow H_2O_2 + O_2$$

Copper is an essential component of several systems involved in the production of hemoglobin, in sugar metabolism, in catecholamine biosynthesis, and in forming cross-links between collagen, elastin and keratin fibers within hair.

Copper deficits leads to growth and behavioural disorders, especially in children [9]. Copper deficiency is an etiology of anemia, neutropenia, and bone marrow dysplasia that may be under-recognized, being a possible cause of medical errors in clinical practice [10, 11]. The mechanism of anemia in copper deficiency may be related to the role of copper-dependent enzymes, such as ceruloplasmin and cytochrome-c oxidase, in iron metabolism and transportation [12]. Bone marrow findings of marked vacuolization of both erythroid and myeloid precursors have been consistently reported, and ring sideroblasts are also occasionally reported [13].

Kinetics of Copper

Copper intake is mainly achieved through diet : it is easily absorbed, but its absorbtion is limited by homeostatic mechanisms, after requirements have been met. The human body contains aproximately 1.4-2.1 mg/kg of copper [14]. Copper is absorbed in the gut, then transported to the liver bound to albumin [15]. After being processed in the liver, it is then distributed to other tissues. Copper transportation involves a protein called ceruloplasmin, which also transports copper excreted in milk [16]. The body is able to excrete excessive levels of copper, if necessary, through the bile. The excreted excess is not reabsorbed by the intestine [17, 18]. In catalytic oxidation processes, metalloproteins containing copper are also necessary, as they intervene in oxygen metabolism. Also, copper also acts in oxidative phosphorylation reactions. Copper intake from food can also prevent certain diseases or deficiencies, such as allergies, hair loss, AIDS, leukemie, osteoporosis and gastric ulcer. Together with iron, copper helps in red blood cells' synthesis.

Copper metabolism disorders

The dysregulation of copper through impaired absorption or excretion results in deficiency or toxicity, as illustrated by two rare genetically based diseases.

Wilson's disease was first described in 1912 by Kinnear Wilson as "progressive lenticular degeneration". Wilson's disease can manifest itself at birth, but signs and symptoms only appear when copper accumulates in the brain, liver, or another organ. The signs and symptoms vary with the body part that is affected by the disease and can include fatigue, loss of appetite or abdominal pain, jaundice, brown-golden Kayser-Fleischer rings, muscular stiffness, lack of motor coordination, speech impairment and others.

Menkes' disease, when the body cannot stock copper, leading to its deficiency; it is also known and the uncombable hair syndrome (hair lacks in color, is sparse, wiry and rough, with occasional nodosities) or Menkes' kynky hair disease. It is a rare hereditary X-linked transmitted disease, described by Menkes in 1962 as a severe degenerative disease of the nervous system. Biologically, serum copper and ceruloplasmin levels markedly decrease. Some of the clinical manifestations pertaining to this disease are facial dysmorphia, signs of neuropsychic involvement, hypothermia, skeletal manifestations similar to those in scurvy, and the irregular lumen of blood vessels.

Clinical signs in copper intoxication

In acute intoxication, serum copper reaches very high values, ceruloplasmin and hepatic copper are within normal limits, and urine copper is increased.

In chronic intoxication, serum, urinary copper and ceruloplasmin are increased, and hepatic copper reaches very high values. The acute toxicity of copper in humans is likely due to the generation of reactive oxygen species, which deteriorate DNA [10]. A minimal dietary value of at least 3 ppm was reported for healthy growth in rabbits [20]. However, high concentrations of copper (100 ppm, 200 ppm, sau 500 ppm) in their diets can favorably alter their growth rates [21].

Copper toxicity is determined by the balance between copper intake and excretion. Voluntary copper salts intake, copper intake from food and beverages, inhaling dust which contains copper or aerosols which contain copper salts, through manual spraying of insecticides or fungicides, represent the main risks of exposing the human body to copper contamination.

The main actions of copper on the human body are gastrointestinal irritation, liver and renal disorders, intravascular hemolysis and shock. Acute intoxication following ingestion of copper salts targets the GI tract, cardiovascular and circulatory systems, hematopoietic system, liver, kidneys and nervous system.

The respiratory effects following inhalation are: acute exposure to copper vapors targets the respiratory pathways and chronic exposure to copper salts, seen in those who spray plants, targets the lungs.

The local, irritating effects, through cutaneous or eye contact with copper salts, are seen at skin and eye level.

Other diseases in which abnormalities in copper metabolism appear to be involved include Indian childhood cirrhosis (ICC), endemic Tyrolean copper toxicosis (ETIC), and idiopathic copper toxicosis (ICT), also known as non-Indian childhood cirrhosis. ICT is a genetic disease recognized in the early twentieth century primarily in the Tyrolean region of Austria and in the Pune region of India [22].

DETERMINING COPPER CONCENTRATIONS IN BIOLOGICAL PRODUCTS

Due to low concentrations in most heavy metals' and metalloids' cases, finding traces in biological samples is a difficult problem. Classical methods (staining, flame photometry, reagent kits, electrochemical methods) can only be used for a limited number of elements, such as sodium, potassium, calcium, magnesium, copper, iron and zinc.

Modern methods replace these types of tests. Elements can be determined through atomic absorbtion spectrometry AAS with flame atomization FAAS or graphite furnace atomization GTAAS, inductively coupled plasma and atomic emission spectrometry ICP-AES or inductively coupled plasma and mass spectrometry ICP-MS. ICP-MS is the most sensitive method for determining metals and metalloids, but it also has the highest cost. For determining metals and metalloids in biological samples, the GTAAS method is recommended. For Na+, K+, Cl-, Ca2+ it is much more cost effective to use machines which only measure blood or urine concentrations of these ions, through electrochemical methods.

The analytical method used to determine the concentration of studied elements, from the analyzed matrices, is the atomic absorbtion spectrometry coupled with graphite furnace atomization GF-AAS (Graphite Furnace Atomiser – Atomic Absorbtion Spectrometry). Atomic absorbtion spectrometry is an analytical method used to quantitatively measure metals or metalloids in a sample, based on the quantity of light that is absorbed or emitted by atoms, from a line that is characteristic for the specter of light the atom emits in an excited state.

The first method that was used to bring atoms to an excited state was heat emanated by a flame. The temperature of this flame is approximately 1200 – 13000C. Due to the low temperatures that can be achieved with this method, the sensitivity of this type of atomization is not useful in determining the majority of elements in biological samples.

Graphite furnace atomization has become very useful in analytical chemistry, as a technique which is very good for determining trace elements in a wide variety of sample types. The principle of this method, of achieving a controlled temperature, using electrical current, makes it perfectly reproductible, compared to flame atomization. The greatest advantage, however, consists in the high atomization temperatures, which can never be attained with a flame, which makes analyzing trace elements in biological samples possible.

The number of replicates (repeatedly performing the same determination) is very important for obtaining quality results. Thus, the working program allow real-time quality control of obtained results, by verifying the standard deviation of replicates. If the value of the square mean deviation (standard deviation) of values measured in the same sample exceeds the pre-established value, the system performs the determination once more. The usually admitted standard deviation is 4-5%. The increase of replicates' number increases the duration of a given determination, wears down the graphite furnace, but improves the accuracy of the results. Due to the marked stability of the machine, it is possible to use the minimum number of two replicates.

The absorbance signal has its own noise due to the variations in emitted light, due to its transmission through the optical chain, due to variations caused by the photomultiplier and the electronic system which amplifies the electric signal. This type of noise can be removed by mathematical methods. The methods which may be applied are either in 3, 5, or 9 points. The calibration method is extremely important for the accuracy of the results. For this reason, choosing an optimal calibration method is one of the most important decisions in establishing the method. For standard calibration, the software automatically chooses the liniar calibration. The calibration curbe may or may not pass through point zero. When measured levels are very low, this curve must pass through point zero. Quality control is mandatory to establish the accuracy of the obtained results. Quality control also involves checking: the standard deviation of replicates; the correlation coefficient of the calibration curve; the detection limit of the method and the apparatus.

Table 1: Concentrations of some essential trace elements in blood and urine (mg/l).

Element	**Serum** (μg/L)	**Blood** (μg/L)	**Urine** (μg/L)
Chromium	0.5	0.5	10
Cobalt	0.2	2.0	100
Copper	1100	1200	60
Iron	1200	-	180
Magnesium	0.5	5	300
Molibdenum	1.0	100	100
Nickel	5.0	5.0	85
Selenium	100	100	30
Strontium	50	-	150
Vanadium	0.5	4.0	16

In urine spot samples, there was a great variation in copper content, which is why collecting 24h urine and sampling from the total volume is recommended. The conventional level taken as diagnostic of Wilson's Disease is >100 mg/24 hours in symptomatic patients [23].

A method to determine the concentration of an element in the matrix involves establishing the working conditions of the spectrometer, of the graphite furnace atomizer, of the sample dispenser and the conditions for the automatic quality control of the test.

The calibration curves of the elements, absorbance as a function of concentration, are calculated with the following equation:

$$A/C = a + b * A + cA^2$$

where: A – is the sample absorbance, C – is the sample concentration, A, b, c, d – are coefficients of the calibration curve.

The determination of copper in biological samples is usually carried out for diagnosing Menkes or Wilson disease or for monitoring the distribution and the amount of copper removed during dialysis procedures of patients with chronic renal disease [24, 25].

In order to develop a method for determining direct GF-AAS copper in biological samples (blood and urine) the establishment of operating parameters for atomic absorption spectrometer system – AAS 880 Spectra Varian and for graphite tube atomizer – GTA 100 is of major importance.

The utilized wavelength must be a part of the absorbtion specter of copper and depends on the type of sample. It may be 327.4 nm or as alternative wavelengths, 324.8 nm or 217.9 nm or 218.2 nm. The correct choice is made with the purpose of eliminating the non-atomic absorbtion which may appear during sample analysis. The width of the used diaphragm is of 0.2 or 0.5 nm and its size depends on the type of analyzed sample.

Method for determining the level of copper in whole blood samples

The samples were analyzed with a system formed from the following components:

- Atomic absorbtion spectrometer AAS-880.
- Graphite tube atomizor GTA-100.
- Programmable sample dispenser – PSD.
- Water cooler – Neslab CFT 22.
- Nitrogen generator - Dominick Hunter.
- Gas cylinder (Argon) 99.9999% purity

Optimized working parameters for determining copper levels in whole blood

In order to determine the Cu concentration in the blood samples taken from patients (2 ml of venous blood, taken on anticoagulants), the following method was used:

- Pipetting method – automixing;
- Quantity of injected sample – 10 µl;
- Measurement method – peak height;
- Concentration calibration;
- Smoothing – 9 points;
- Number of replicates - 2 for standard and for sample;
- Cathode tube current – 4 mĂ;

- Wavelength – 327.4 nm;
- Slit width - 0.5 nm;
- Background correction – deuterium lamp;
- Drying – 1200C;
- Calcination - 8000C;
- Atomization - 23000C;
- Cleaning - 25000C;
- Three-point calibration curve – 0, 50 and 100 µg/l;
- Recalibration rate – 15;
- Method detection limit <1.0 µg/l.
- Apparatus detection limit <0.04 µg/l.

Automatic quality control – dispersion <10 %; correlation coefficient of calibration curve >0.998.

The optimization of the temperature program must provide optimal conditions for the measurement of Cu signals by GF-AAS, free of interferences from the background which is a very common problem in the analysis of biological samples (26). The application of the optimized temperature program made possible to eliminate the whole matrix of the sample before the atomization step, as confirmed by the low background signals observed in the measurement of Cu. No chemical modifiers were added. However, biological samples must undergo a deproteinization process, which is meant to eliminate non-atomic absorbance.

Table 2: Established and optimized steps for GTA 100

Step	Temp C	Time s	Flow L/min	Gas Type	Read	Signal Storage
1	40	5	3	N_2	No	No
2	80	10	3	N_2	No	No
3	105	5	3	Ar	No	No
4	105	10	3	Ar	No	No
5	800	20	3	Ar	No	No
6	800	5	3	Ar	No	No
7	800	2	0	Ar	No	Yes
8	2650	0.9	0	Ar	Yes	Yes
9	2650	2	0	Ar	Yes	Yes
10	2650	3	3	Ar	No	Yes
11	40	30	3	N_2	No	No
12	40	5	0	N_2	No	No

Copper stock standard solution containing 1000 mg/L copper was diluted with a premixed solution of distilled water and analytical grade concentrated nitric acid to provide working standards of various concentrations in 0,1% (w/v) HNO3. The calibration blank solution used throughout was a 0.1% w/v HNO3 solution. For copper, the working standards were 100 and 50 µg/L.

Table 3: Performance parameters of linear regression analysis

Conc. ug/L	Abs	Abs. Average	STDEV	RSD %
0	0.0193	0.0183	0.0014	7.55
0	0.0167			
0	0.0188			
50	0.3996	0.4001	0.0009	0.23
50	0.4012			
50	0.3996			
100	0.7214	0.7178	0.0034	0.47
100	0.7147			
100	0.7174			

The whole blood sample (200 µL) was treated with 800 µL solution 5% antifoam B (Sigma) and with 1000 µL of 1.6 M HNO3 solution. The mixture was maintained for 20 minutes and centrifuged with 5000 rpm for 5 min. The supernatant was analyzed by GF-AAS. The results are obtained in µg/dL. A linear relationship was found between the absorbance at 327.4 nm and the concentration of cooper in the range of 0.0 to 100 µg/L. The representative linear equation was y = 0,007x + 0,029 where: y is the absorbance, x is lead concentration (µg/L), calculated by the least squares method. The regression coefficient (r) standard curve was 0.9972, indicating good linearity (r > 0.99).

The performance parameters of linear regression equation are presented in Table 3. The parameters of the GF-AAS analysis method of lead are presented in Table 4. LOD and LOQ were calculated based on the standard deviation and the slope of the regression line.

Table 4: Validation parameters

Standard error	0.0173
R Square	0.9972
Intercept	0.0290
Slope	0.0070
SE of intercept	0.0091
LOD (µg/L)	***4.29***
LOQ (µg/L)	***13.01***

LOD is the lowest concentration of an analyte that can be detected while LOQ is defined as the lowest concentration of an analyte that can be determined at the acceptable level of precision and accuracy and were calculated according to the formula below: LOD = 3,3*(SD of intercept/Slope) and LOQ = 10*(SD of intercept/Slope)

The established method can be easily used to perform copper measurements in the laboratory, both in urine and blood samples taken from patients suspected for copper intoxication. Copper deficiency and toxicity can be either of genetic or non-genetic origin. The study of copper's genetic diseases, which are the focus of intense international research activity, has shed insight into how human bodies use copper, and why it is important as an essential micronutrient.

The evolution of the copper concentration in urine or whole blood provides very useful information for poisoning diagnostic when the history is not clear [27, 28].

CONCLUSIONS

Microelements play an important role in the normal course of life. The variation of their blood levels within the body can lead to metabolic disturbances. For example, copper deficiency is found in Menkes syndrome or ,,uncombable hair disease”, and copper excess leads to Wilson’s disease. The rise in blood levels of copper can also be due to acute intoxication, for example, as a result of spraying plants with copper sulphate.

For these reasons, measuring Cu concentrations in various biological samples, urine, blood, or hair, provides the physician with information regarding contamination, intoxication or the existence of a metabolic disease.

The analysis of biological samples is the most important use of graphite furnace atomization. The advantages of this technique are its high sensitivity and the very low quantity of sample necessary for the measurement.

In modern medicine, determining trace elements is very important in diagnosing various diseases and/or in treating them. The GF-AAS is a quick, reproductible and highly sensitive method.

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ORIGINAL ARTICLES

Correlation between FibroScan and AST/ALT ratio and splenic size in NASH patients in a tertiary care center

Varsha Somakumar[1], V.S. Srikanth[2], Cibi Manoharan[1], Vishal Marwaha[1], V.R. Mujeeb[2], M.G.K. Pillai[1]

Abstract: *Background: Non-Alcoholic Steatohepatitis (NASH) is an aggressive form of Non-Alcoholic Fatty Liver disease (NAFLD), with liver inflammation and scarring. Due to a lack of clinical biomarkers and asymptomatic nature, NASH is often under-diagnosed. It is the most common cause of chronic liver disease in the USA. Liver biopsy is the gold standard to diagnose NASH, but it is invasive and life-threatening and histologic evaluation of a liver biopsy sample is imperfect as a reference because of sampling variability due to the irregular distribution of fibrosis. Aim: Validating AST/ALT ratio as a stand-alone scoring system in NASH is scarce and so this study aims to establish a correlation between FibroScan values and AST/ALT ratio. Methodology: All NASH patients, who underwent FibroScan were included. Their demographics, FibroScan, AST, ALT values were recorded in M S Excel and Pearson correlation between FibroScan values and AST/ALT ratios of 150 NASH patients was calculated using SPSS. Results: Out of 150 NASH patients, 72% were males and 57.33% belonged to 40-50 years age group. FibroScan values and AST/ALT ratio showed positive Pearson correlation of 0.245, (p value=0.003). FibroScan values and splenic size also showed a positive Pearson correlation of 0.289 (p value<0.001). Conclusion: Males of 40-50 years age group had higher distribution of NASH, so middle aged males should be screened routinely as they are at a higher risk. FibroScan value with AST/ALT ratio and splenic size showed a positive correlation, thus showing that AST/ALT ratio and splenic size increases with increase in liver stiffness.*

Keywords: *NAFLD, NASH, FibroScan, Elastography, AST/ALT*

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a broad term used for a range of liver conditions affecting people who drink little to no alcohol, and is the most common cause of chronic liver disease in the United States of America [1], affecting approximately 90% of the obese population and 15-40% of the general population [2]. As the name implies, the main characteristic of NAFLD is excessive fat stored in the hepatic cells of the liver [1]. It consists of a spectrum of conditions: benign steatosis or non-alcoholic fatty liver (NAFL), steatosis accompanied by inflammation and fibrosis or nonalcoholic steatohepatitis (NASH), and finally cirrhosis [3].

The rising prevalence of NAFLD globally may be accounted for by changes in dietary habits and an increase in sedentary lifestyle [4]. Insulin resistance and oxidative stress play an important role in NAFLD development and progression [5]. Due to the lack of clinical biomarkers as well as its asymptomatic nature, NASH is often under-diagnosed and most commonly found incidentally during routine Ultrasound of the abdomen [3]. No highly sensitive and specific tests are available to differentiate NASH from simple steatosis, but diagnostic accuracy can be improved by combining blood biomarkers [6]. It is an aggressive form of fatty liver disease, marked histologically by liver

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inflammation and may progress to advanced scarring (cirrhosis) and liver failure, which is similar to the damage caused by heavy alcohol use. The number of people at risk for NAFLD is even greater given the increasing prevalence of obesity, diabetes and metabolic syndrome [1]. Although steatosis can be a significant cofactor in progression of established CLD, or even hepatocellular carcinoma (HCC), only steatohepatitis indicates the presence of progressive liver disease in patients with NAFLD. Benign and malignant liver tumors have been more frequently reported with the increasing prevalence of obesity and diabetes [7]. Early identification and targeted treatment of patients with NASH are needed to improve patient outcomes, directing patients toward intensive lifestyle modification to promote weight loss as well as for referral for bariatric surgery as indicated [8]. In NAFLD, HCC frequently develops in a not-yet cirrhotic liver; ss there are no surveillance programs for these patients, diagnosis often occurs at a tumor stage beyond curative options, further marking the importance of its early diagnosis [9]. According to a recent study done by Edenvik et al. [10], NAFLD and alcoholic liver disease were more commonly associated with deficient surveillance for HCC, and therefore early diagnosis was missed.

Although liver biopsy is considered the gold standard to diagnose NASH, only those patients with a high risk of NASH or advanced fibrosis require this evaluation, since it is an invasive and potentially life threatening procedure. Non-invasive tools for diagnosis and disease staging are required, reserving liver biopsy for those patients where it offers clinically relevant additional information only [11]. The use of non-invasive tests to stage the severity of liver disease (ie. scarring) is now well established in the management of patients with chronic liver disease since the assessment of liver scarring provides prognostic information and establishes treatment priorities [12]. Techniques such as transient elastography, magnetic resonance elastography and acoustic radiation force imaging are becoming more established in detecting fibrosis in a variety of chronic liver conditions in addition to NAFLD, as reviewed by Fitzpatrick E et al. [13]

One such technique, transient elastography (TE), is a simple, safe and efficient way to estimate liver scarring which is done using FibroScan, and is the most popular non-invasive device used to assess liver 'hardness' (or stiffness) via TE. Liver hardness is evaluated by measuring the velocity of a vibration wave (also called a 'shear wave') generated on the skin [12]. The main clinical indication for liver elastography is fibrosis staging of chronic liver disease and detect advanced fibrosis. Histologic evaluation of a liver biopsy sample is an imperfect reference standard because some degree of sampling variability is inevitable due to the irregular distribution of fibrosis in chronic liver disease.

FibroScan can be easily performed at the bedside or in the outpatient clinic with immediate results for the diagnosis of significant fibrosis and cirrhosis in nonalcoholic fatty liver disease chronic hepatitis C, in recurrence of hepatitis C after liver transplantation, in co-infected HIV-HCV patients, in chronic hepatitis B, in chronic cholestatic diseases, in alcoholic disease [14]. FibroScan evaluates liver stiffness, inflammation and portal hypertension and the values have to be interpreted according to clinical, biological and morphological data. FibroScan® results range from 2.5 kPa to 75 kPa. Between 90–95% of healthy people without liver disease will have a liver scarring measurement <7.0 kPa (median is 5.3 kPa) [12].

The usual observed biochemical pattern in hepatic steatosis due to NAFLD is of increased levels of transaminases, with alanine aminotransferase (ALT) levels exceeding those of aspartate aminotransferase (AST). This classical pattern differentiates between hepatic steatosis from NAFLD and alcoholic liver injury, with the latter normally associated with a high AST/ALT ratio [15]. Generally, the AST to ALT ratio increases with the severity of the necroinflammatory and fibrotic changes [16].

Data validating the use of the AST/ALT ratio as a stand-alone scoring system in NASH is scarce. This study aims to establish a correlation between FibroScan values and AST/ALT ratio. Given the high prevalence of NAFLD in the general population, these noninvasive methods could be used in clinical practice to screen patients with NAFLD [17]. Lifestyle modifications to reduce weight are a major factor in the treatment of NAFLD. No pharmacological substances are as yet approved for the indication NASH [18].

MATERIALS AND METHODS

Out of a total of 2000 patients diagnosed with NASH over a period of 3 years (January 2017 to December 2019) in Amrita Institute of Medical Sciences and Research Centre, Kochi, 150 patients who underwent Transient Elastography Scan (FibroScan) is selected. Inclusion criteria consists of abnormal liver function tests, fatty liver infiltration on ultrasonography (US), undergone FibroScan etc. Exclusion Criteria are patients with a history of alcohol intake, hepatitis B and C, history of hepatotoxic drug intake and other liver diseases. Ethics Comittee of Amrita Institute of Medical Sciences has approved the study.

The mean of 10 elastography values is taken as the FibroScan value. Patient details like their demographics, USG findings, AST/ALT ratio, Neutrophil/Lymphocyte ratio, LFT etc were obtained using their hospital numbers from Amrita Health Information System and recorded in MS Excel.

Pearson correlation of FibroScan values with AST/ALT ratio and splenic size and Receiver Operative Characteristic (ROC) is used to find out optimal cut off for AST/ALT and Splenic diameter, as well as their sensitivity, specificity, and predictive values, using SPSS.

RESULTS

The majority of cases were seen among males (Figure 1). 74% (111) of cases were above 40 years (Table 1 and Figure 2).

Table 1: Distribution of age

Age Group (years)	**Frequency**	**Percent Distribution**
11-20	1	0.6
21-30	15	10
31-40	23	15.3
41-50	38	25.3
51-60	48	32
61-70	22	14.6
71-80	3	2
Total	150	100

Figure 1: Distribution of gender

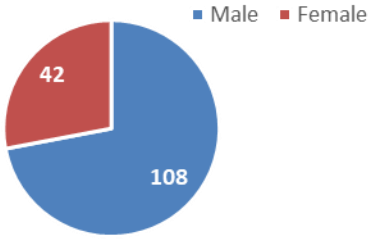

Figure 2: Distribution of age

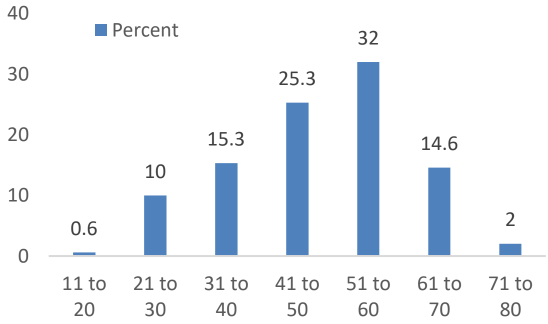

Mean laboratory values are given in Table 2.

Table 2: Demographics and laboratory characteristics

Mean Age	48.4 years
Gender (Males)	108 (72%)
Mean HDL	46.68 mg/dl
Mean LDL	127.34 mg/dl
Mean TG	136.52 mg/dl
Mean AST	72.18 IU/L
Mean ALT	103.72 IU/L
FibroScan	7 kPa
Splenic Size	9.7 cm
Neutrophil	54.12%
Lymphocytes	35.5%
NEU/LYM	2.02

In order check the correlation between FibroScan and AST/ALT (Figure 3), Pearson correlation was used. Positive correlation of 0.245 with a statistically significant P value of 0.003 was obtained. Positive correlation of 0.298 with a statistically significant P value of <0.001 was obtained between FibroScan and Splenic Diameter (Figure 4).

Figure 3: Scatter Plot of FibroScan and AST/ALT

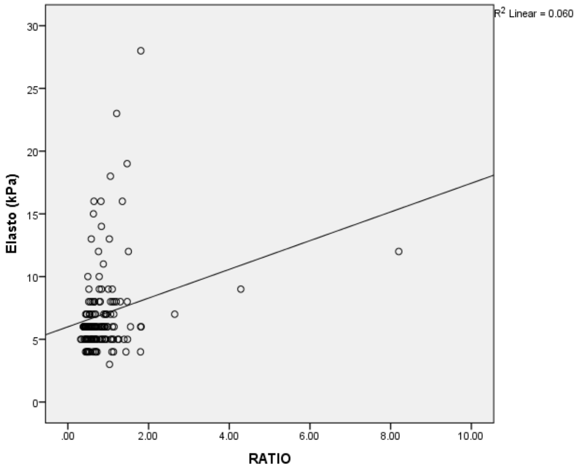

Figure 4: Scatter Plot of FibroScan and splenic size

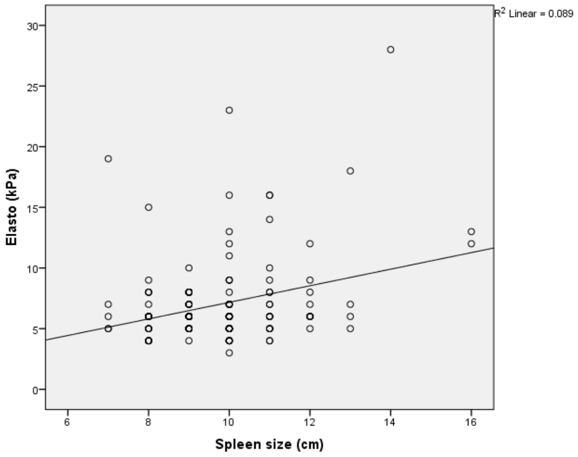

Table 3: Demographics and laboratory characteristics

		AST/ALT	Splenic size
Fibroscan	Pearson corrrelation	0.245	0.298
	p-value	0.003	<0.001

ROC curves were used to check the diagnostic accuracy of AST/ALT (Figure 5) which showed an AUROC of 0.641, ASTAST/ALT cut off 0.32 (P value 0.01), with a sensitivity and specificity of 97.3% and 29.2%. ROC for Splenic Diameter (Figure 6) showed an AUROC of 0.60, cut off of 9.5cm (P value 0.06), with a sensitivity and specificity of 62% and 51.3%.

Figure 5: ROC curve for AST/ALT

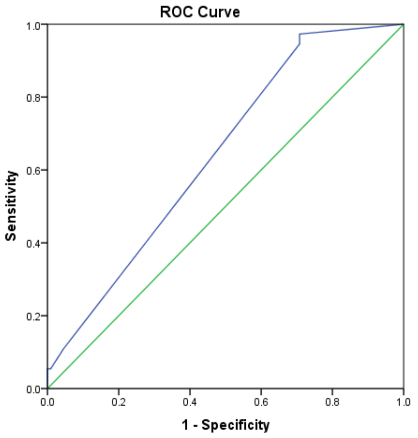

Diagonal segments are produced by ties.

Area	0.641
AST/ALT	0.32
P value	*0.01*
Sensitivity	97.3%
Specificity	29.2%

Figure 6: ROC curve for Splenic Size

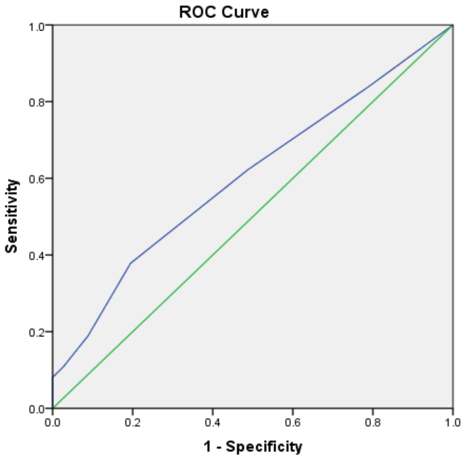

Diagonal segments are produced by ties.

Area	0.603
SS	9.50
P value	*0.061*
Sensitivity	62%
Specificity	51.3%

CONCLUSION

Males of age more than 40 years had higher distribution of NASH, so middle aged males should be screened routinely as they are at a higher risk for developing NASH. FibroScan value with AST/ALT ratio and splenic size showed a statistically significant positive correlation, thus showing that AST/ALT ratio and splenic size increases with increase in liver stiffness. Thus our study shows that AST/ALT ratio can also be used for prompt diagnosis and management of NASH patients.

Contribution of authors

Varsha Somakumar, V S Srikanth, Cibi Manoharan – Concepts, design, definition of intellectual content, literature search, data acquisition, data analysis, Manuscript preparation, editing and review; Vishal Marwaha, VR Mujeeb, M.G.K.Pillai - Concepts, design, definition of intellectual content, Manuscript editing and review.

The manuscript has been read and approved by all the authors, and each author believes the manuscript represents honest work. There is no conflict of interest among the authors.

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ORIGINAL ARTICLE

Costs for a hospital stay: another lesson learned from the COVID-19 pandemic

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Abstract: *Background and aim: After two years of pandemic, planning and budgeting for use of healthcare resources and services is very important. Inpatient COVID-19 hospitalizations costs, regardless of ICD-10 procedure codes, in a Covid-19 support military hospital were analyzed. Methods: The national protocol for the treatment of Covid-19 infection was applied. The costs for laboratory tests, drugs, protection equipment and radiological investigations (imaging techniques such as computed-tomography or radiography), hospitalization days and food were assessed. Results: In our hospital, from August 2020 through June 2021, 241 patients were hospitalized with COVID-19: mean age 59.92±7.8 years, 46% men, 26% military personnel, 11.57±3 days of hospitalization; two third of patients had moderate and severe forms of COVID-19. The main manifestations were: 69% respiratory (18% with severe pneumonia), 3.3% cardiac (2.9% with pulmonary embolism, diagnosed by computed tomography angiography), 28% digestive and 33% psychiatric (most commonly anxiety). The average estimated costs were about 3000€/patient, without significant differences based on disease severity. Equipment costs were 2 times higher than for drugs and 3 times than for laboratory tests. Conclusions: In a Covid-19 support military hospital that cared for patients with predominantly moderate forms of COVID-19, the costs for equipment were much higher than those for treatment. New criteria for hospitalization of these forms of COVID-19 deserve to be analyzed in order to avoid useless costs.*

Keywords: *COVID-19, pandemic, hospital stay, costs*

INTRODUCTION

SARS-CoV-2 is the most deadly and devastating virus of the last one hundred years, being more contagious than the Ebola virus, HIV, Swine Influenza virus (H1N1, H1N2), Severe Acute Respiratory Syndrome (SARS) or Middle Eastern Respiratory Syndrome (MERS) [1]. COVID-19 is a systemic disease determined by SARS-CoV-2, with a variable degree of severity and progression [2]. The evolution of this infection is mostly as pulmonary disease. Depending on the host's immune response, it may present as a severe form with multiorgan dysfunction, especially if the patient has comorbidities. COVID-19 can have cardiac, renal, digestive or cerebral manifestations. Therefore, it implies significant costs for diagnosis, drugs, hospitalization, protection equipment, etc.

Profiles of COVID -19 patients have been studied in various ways. Besides determining risk factors, influencing treatment protocols, setting priorities for vaccination, or inspiring further research, planning and budgeting for use of healthcare resources and services become very important given the duration of the pandemic. According to health policy researchers, many insurers claim that it is justified to charge patients for COVID-19 hospitalizations now that COVID-19 vaccines are widely available. For example, the hospitalization of a person with private insurance amounts to a total of $42.200 on average, whereas each hospitalization of a person with COVID-19 who has Medicare Advantage coverage averaged about $21.400 [3]. In comparison, the average cost for influenza hospitalization is around $1.000 [4]. Therefore, we emphasize that COVID-19 prevention is better than treating. Nowadays, vaccines targeting SARS-CoV2 are highly effective at preventing the severe forms of the disease, hospitalizations, and deaths

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from COVID-19 [5,6]. Despite the wide availability of safe and effective vaccines, vaccination rates have lagged in Romania.

The aim of this study was to analyze the costs of hospital stays in patients with predominantly moderate forms of COVID-19 in a small size military hospital located in Northeast Romania.

METHODS

This study included all patients hospitalized between August 2020 and June 2021 in a military hospital. Inpatient COVID-19 hospitalizations costs, regardless of ICD-10 procedure codes (U07.1), were retrospectively analyzed. The hospital's statistician offered all data about costs. We assessed hospital costs per patient, per severity degree of COVID-19 and per health service type.

Inclusion and exclusion criteria

According to National Center for Surveillance and Control of Infectious Diseases (CNSCBT) the definition of a confirmed COVID-19 case is: a person with laboratory confirmation of SARS-CoV-2 infection (by RT-PCR SARS-CoV-2), regardless of clinical signs and symptoms [7].

All patients with a positive RT-PCR test without complete vaccination were included in the study. Patients in the first 14 days after the first dose of anti-SARS-CoV-2 vaccine were not excluded. Patients with positive COVID-19 rapid tests were tested by RT-PCR. In accordance with the hospitalization protocol instituted after October 15th 2020, asymptomatic patients were no longer hospitalized and were managed in an ambulatory manner.

The local hospital ethics committee approved the study. All patients enrolled in the study gave their written informed consent.

All patients underwent a specific protocol for COVID-19 diagnosis and treatment, adapted by our hospital according to the national protocol.

Diagnosis protocol

Diagnosis protocol included the following exams:

1. RT-PCR for SARS-CoV-2

2. Monitoring of vital functions (temperature, blood pressure, heart rate, respiratory rate, oxygen saturation, diuresis, defecation)

3. Blood tests: complete blood cell count, C-reactive protein, urea, creatinine, blood electrolytes, ALT, AST, GGT, blood glucose, CK-MB, troponin, D-dimers, ferritin, other blood tests depending on comorbidities (presepsin, procalcitonin, TSH, fT4, etc.).

4. Urinary sediment analysis

5. Computed tomography (CT) examination (with or without contrast agent)

6. Electrocardiogram (the presence of sinus rhythm or new bundle branch block, maximum PR and QTc interval)

7. Sputum examination (if necessary)

8. Coproculture, Clostridium difficile toxins (if necessary)

9. Echocardiography (in case of suspected cardiac disease or complication)

10. Arterial gases (in case of impaired respiratory function)

Patient monitoring was done clinically and biologically, with daily biochemistry in patients with moderate or severe forms. Repeated imaging exams and biological tests were performed urgently in case of clinical worsening.

Radiological parameters used for the study were parenchymal involvement scores, CT score and CORADS score (COVID-19 imaging Reporting And Data System). Chest CT findings reported the COVID-19 imaging using CORADS score that divides the CT findings into six categories [8]. It ranges from CORADS category 1 with very low level of suspicion to CORADS category 5 with very high level of suspicion. If none of the five categories can be established due to incomplete or insufficient images, it is considered CORADS category 0. CORADS category 6 indicates proven COVID-19 by positive RT-PCR test results [8].

In addition, a semi-quantitative scoring system for estimating parenchymal abnormality based on the area involved has been used.

Each one of the five lung lobes was individually evaluated and scored based on parenchymal involvement on a scale ranging from 0 to 5, with 0 indicating no involvement, 1 for less than 5% involvement, 2 for 5–25% involvement, 3 for 26–49% involvement, 4 attributed to 50–75% involvement, and 5 indicating more than 75% parenchymal involvement. The total CT score was the sum of the individual lobar scores, which ranged from 0 (no involvement) to 25 (the maximum possible involvement) [9].

Patients were diagnosed with asymptomatic, mild, moderate, severe or critical COVID-19. Most patients with severe forms of the disease who evolved into critical condition with at least one of the following: acute respiratory distress, sepsis, impaired consciousness or multisystem organ failure, were transferred in hospitals with COVID-19 intensive care units.

Treatment protocol

Patients were treated according to the national protocol published by CNSCTB [7]. Asymptomatic cases were hospitalized for medical supervision and were symptomatically treated as mild COVID-19. Moderate forms of the disease, defined as no oxygen requirement, have been treated with antivirals (lopinavir/ritonavir or umifenovir, remdesivir), antibiotics and symptomatically (paracetamol, C vitamin, D vitamin, antitussive drugs). All severe COVID-19 forms required oxygen therapy; they had at least one of the following parameters: respiratory rate ≥ 30/min; $SaO_2 \le 93\%$; PaO_2/FiO_2 <300; pulmonary infiltrates that increased by more than 50% in 24-48 hours. Beside oxygen therapy, they received corticotherapy (dexamethasone), anticoagulation (enoxaparine or fraxiparine in prophylactic or therapeutic doses), antivirals, immunomodulatory drugs (tocilizumab).

The duration of treatment was decided by the physician and was prolonged or shortened according to the patient's clinical evolution, but no case has received medical treatment for less than 10 days (surveying for severe side effects or Covid-19 complications).

Personal protective equipment

All medical personnel used personal protective equipment against SARS-CoV2 and complied with WHO recommendations [10]. Besides medical facemasks, face shields, protective glasses, two types of gloves (examination and surgical) and gowns or aprons, disinfectants and other consumables were used to comply with the rules of aseptic and antiseptic environment.

The study was conducted in accordance to the Declaration of Helsinki and approved by the Ethics Committee of Hospital (protocol code 2021-01).

Statistical analysis

Continuous variables were presented as median and categorical as absolute values and percentages. Normality was verified using the Shapiro-Wilk test. The comparisons of groups were based on a student's two-sample t-tests. P-value ≤ 0.05 was considered statistically significant for all tests. Statistical analysis was performed using SPSS 10 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

In the specified time interval, 241 patients were hospitalized with COVID-19.

Patient population

In our study population, 46% were men and mean age was 59.92±7.8 years. Twenty six percent of patients were military personnel and 74% were civilians. The main COVID-19 manifestations were: 69% respiratory (18% with severe pneumonia, 2.9% with pulmonary embolism, diagnosed by angio-computed tomography), 3.3% cardiac, 28% digestive and 33% psychiatric (most commonly anxiety). Only two patients had a procalcitonin level >10 ng/mL (high probability of severe sepsis or septic shock) and 1 patient had moderate risk for septic shock (0.5-2ng/mL). Mean clinical, biological, electrocardiographic and radiological characteristics of patients included in the study were presented in Table 1.

Table 1: Mean clinical, biological, electrocardiographic and radiological characteristics of patients included in the study.

Characteristics	Value (n=241) n (%) or mean ± SD
CLINICAL	
Blood pressure (mm Hg)	130±21/78±19
Heart rate (/min)	82±21
SaO2 (mmHg)	93±17
Smoker (%)	11 (0.045)
BMI (kg/m^2)	32.9±17
Comorbidities	
Hypertension (%)	124 (51.4)
Diabetes mellitus (%)	53 (22)
Ischemic coronary disease (%)	82 (34)
Obesity (%)	133 (55)
Sleep apnea (%)	14 (0.06)
Neoplasia (%)	24 (0.1)
COPD (%)	17 (0.07)
BIOLOGICAL	
Leucocyte (/mm^3)	12750±7800
Lymphocyte (/mm^3)	3720±1230
Hemoglobin (g/dL)	14±2.7
Platelets (/mm^3)	163.000±78.000
D-dimers	613±430
CRP (mg/dL)	37±27
Ferritin (μg/L)	671±340
AST (UI/L)	35±4.8
ALT (UI/L)	65.5±17.8
Urea (mg/dL)	56±43
Creatinine (mg/dL)	0.9±0.8
Potassium (mEq/L)	3.7±0.7
CK-MB (UI/L)	18.4±7.6
Procalcitonin	0.4±0.03
ELECTROCARDIOGRAPHIC	
SR (%)	199 (82)
QTc max (ms)	431±43
PR max (ms)	165±34
RADIOLOGICAL	
Parenchymal involvement scored	3.4
CT score	14/25
CORADS score	5.6

ALT: alanine amino transferase; AST: aspartate amino transferase; BMI: body mass index; CK-MB: creatin kinase MB; CORADS: COVID-19 imaging Reporting And Data System; CRP: C-reactive protein; CT: computed-tomography; O2: oxygen; PR: interval PR; SR: sinus rhythm

According to COVID-19 severity (Table 2), most patients had moderate (104 patients – 43%) and severe (50 patients - 21%) forms. Seven patients with severe forms died because of multiple comorbidities and 30 patients were transferred to hospitals with COVID-19 intensive care units.

Table 2: Distribution of COVID-19 according to disease severity.

COVID-19 severity	Number of patients (%)
Asymptomatic	14 (6%)
Mild	73 (30%)
Moderate	104 (43%)
Severe	50 (21%)
Total	241

Hospitalization costs

Mean hospital stay was 11.57±3 days. In each day of hospitalization, at least 10 healthcare personnel members (4 nurses, 4 carers and 2 physicians) used protective equipment. Eight patients (3.3%) received Remdesivir and 2 patients (0,8%) Tocilizumab. In the first three months of the study, Plaquenil and Kaletra were not available in the hospital's pharmacy, therefore only 23 (9.5%) patients received these drugs. Antibiotics such as ceftriaxone were indicated in 152 (63%) patients. Dexamethasone was used in 190 (78.8%) patients. Prophylactic doses of low molecular weight heparin were administered in 220 (91.3%) patients and therapeutic doses in 7 (2.9%) patients. Asymptomatic patients did not receive anticoagulants.

The mean costs for patient according to disease severity are shown in Table 3, the average estimated costs were about 3000€/patient, without significantly differences depending on disease severity.

Table 3: The mean costs per patient according to disease severity.

COVID-19 severity	Total costs (€)	Mean costs/pts (€)
Asymptomatic	23410,65	1672,19
Mild	220949,62	3026,71
Moderate	318966,92	3066,99
Severe	148669,86	2973,39
Total	711997,05	10739,28

The costs for hospitalization days, food, laboratory tests, drugs, protection equipment and radiological investigations (imaging techniques such as computed-tomography or radiography) are presented in Figure 1.

Protection equipment costs were 2 times higher than for drugs and 3 times than those for laboratory tests (p<0.0001). However, the sum of hospitalization and protective equipment costs was almost nine times greater than medication costs (2436.76 € versus 604.12 €; p<0.0001).

Figure 1: Mean costs per patient according to each type of health services.

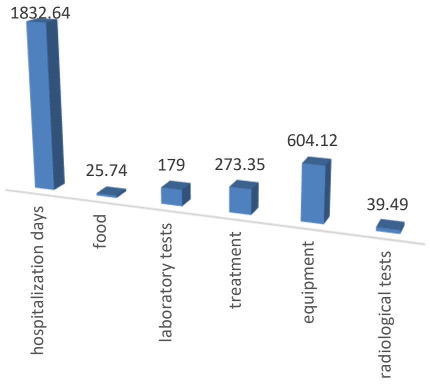

DISCUSSION

Two years after SARS-CoV-2 emerged, the coronavirus continues to affect every aspect of society, one of the major economic effects of the virus being experienced by the health care sector, with COVID-19 changing the economics of health care [11].

The results of our work are a summary of the hospitalization stay costs in patients with COVID-19, in a support hospital from Romania, before the full vaccination scheme was completed.

In August 2020 our hospital was designated as support unit in the battle with COVID. In accordance with this status and due to the fact that we did not have an intensive care unit available for COVID patients, predominantly moderate cases of COVID-19 were hospitalized. A number of patients had an unpredictable evolution to a severe form, some of them presenting exitus from cardiorespiratory arrest while awaiting transfer to an intensive care unit, given the high occupation rate of intensive care units across the country. However, a significant part of our patients (12.4%) was successfully transferred to an intensive care unit dedicated to COVID-19 patients. This explains why there were no differences in costs between moderate and severe forms. In intensive care units the mean costs for invasively ventilated patients in this first part of pandemic was about €10.000 (according to unpublished financial data from these hospitals in Romania).

Medical costs associated with COVID-19 have long been overlooked in previous reports and this is one of the few published studies estimating the costs associated with caring for patients with COVID-19 during hospitalization. In the first part of the SARS-CoV2 pandemic, even patients with asymptomatic forms of the disease were hospitalized. In this study, the cost for hospitalization for this type of patients were two times lower compared to that for symptomatic disease. However, one explanation for this fact could be the rising prices and high costs of personal protective equipment.

Real-time data on the costs of all COVID-19 hospitalizations are far from known. In the US, an average hospitalization cost is around $20.000 [11]. Data published by the Centers for Medicare and Medicaid Services showed that Medicare fee-for-service COVID-19 hospitalizations costs range from $21.752 to $24.033 [11]. Similarly, a FAIR Health analysis of private claims data including employer and private Medicare Advantage plans found that median in-network COVID-19 hospitalization costs ranged from $17.094 for people over the age of 70 to $24.012 for people in their 50s. However, the costs for pneumonia hospitalizations with complications averaged $20.292, but the costs for hospitalizations requiring a ventilator are much higher [12].

According to published data [12], in the US COVID-19 outpatient treatment costs can average $500-$1.000 per patient (average patient age: 39 years). Average treatment costs for patients treated in hospitals compared to patients managed in an outpatient setting for COVID-19 (average patient age: 54 years) was 45 times higher. In addition to this, for the same mean age, average treatment costs for patients treated in intensive care units without the need for ventilation, compared to those whose cases were managed in a medical ward were 2.5 times higher [13]. To our knowledge, costs for hospital stay have not been analyzed by category (personal protective equipment versus drugs, blood test, etc) in literature until now.

In our country vaccination started at the beginning of 2021, therefore the majority of patients were unvaccinated until June 2021. In the US, from June through November 2021, preventable COVID-19 hospitalizations among unvaccinated adults cost over $13 Billion [12]. Therefore, over 690.000 COVID-19 hospitalizations could have been prevented by vaccination between June to November 2021; this is a lesson that must be learned in order to cut down the hospitalization stay costs [12].

In many countries, the impact of COVID-19 on the public health budget was significant. For example, in Spain it comprised 12.3% of total public health expenditure, greater than multiple sclerosis, cancer and diabetes costs [14]. The mean cost per patient was significantly greater than in our study (€10.744). The main costs were staffing costs: for physicians (10.131 to 11.357 €/patient) and for nurses (10.274 to 11.215 €/patient) [14].

A multivariate model was developed to predict the length of hospital stay [15]. The prediction model included five variables: procalcitonin, heart rate, epidemiological history, lymphocyte count and cough [15]. The results showed that the overall length of stay was 11 days (5–15 days) and the median cost for a hospital stay was 7.388.19 RMB (Renminbi) (ranging between 5.085.39–11.145.44) or €1.024.77. Data about hospitalization duration published by Carrera-Hueso et al. [14] and Hong et al. [15] are similar to those in our study, but hospitalization costs were lower, probably due to differences in health service policies.

Another smaller retrospective study (70 patients), which assessed the cost factors and which was conducted at the very beginning of the COVID-19 pandemic, found that the cost for drug acquisition (that accounted for 45.1% of the overall cost) was the major contributor to the medical expenditure. The risk factors for higher costs were pre-existing diseases and severity of COVID-19 [16]. Comparing with this study, at that time, personal protective equipment was not an issue. However, clinical management of COVID-19 patients incurred a great financial burden to many national health systems. In addition, the treatment recommendations changed during this pandemic, each country having an agreed protocol.

Another study that used a Monte Carlo simulation model to estimate resource use and direct medical costs per symptomatic infection at national level found that a single symptomatic COVID-19 case could incur a median direct medical cost of $3.045 during the course of the infection alone [17]. However, a strong limitation of this study is the fact that all models, by definition, are simplifications of real life and cannot account for every possible outcome [18].

In Spain, the European country most economically affected by the pandemic, the gross domestic product cost accounted for 94.7% of the total cost of the COVID-19 and direct health-care costs were 2.14% [19]. This study was designed to analyze the cost-effectiveness of the test-tracking-quarantine strategy, which was the dominant strategy in Spain. Lopez-Valcárcel et al. [19] showed that for each € spent on it, 7 € would be saved in terms of health-care resources. Therefore, this is another important cost-effectiveness strategy in COVID-19 pandemic, similar to those used in an economic evaluation of health-care technologies [20].

Many countries made great efforts to support health-financing flows [21]. A patient with COVID-19 can have a higher probability of hospitalization and mortality than those for other common diseases, therefore hospitalization costs are higher [22-24]. However, before starting this study, on June 18th 2020, the number of confirmed cases across the world had surpassed 8 million. The European region accounts for 31% of cases and 43% of deaths globally [25].

There are some lessons to be learned from the COVID-19 pandemic about improving hospital care and healthcare delivery, both in day-to-day circumstances and future emergencies [26]. One of them is that many countries have learned of the disadvantages of no longer manufacturing certain medical equipment themselves. In addition to that, the reimbursement system of public hospitals seems not to reflect the real COVID-19 costs because the accounting systems of European countries vary widely by the share of reimbursement through DRG (diagnosis-related group based on ICD-10 procedure codes) payment and a reliable accounting system for calculating the actual expenditure per COVID-19 patient (based on actual costs and hospitalization data) is needed [27,28]. Given these issues, it is difficult to compare the costs for hospital stay in patients with COVID-19.

In conclusion, after the COVID-19 pandemic reduced global economic growth by an estimated 3.2% in 2020, with trade declining by 5.3% [29], hospital stay costs might be another lesson, especially given the fact that in Europe 90% of cases recovered in an ambulatory setting [30].

Study limitations

This study included all consecutive patients hospitalized in a single COVID-19 support hospital. In Romania, during the pandemic, hospitals were classified in units treating only COVID-19 cases, COVID-19 support hospitals and non-COVID-19 hospitals. Our hospital was a COVID-19 support unit without endowment of a COVID-19 dedicated intensive care unit. Even if patients were included consecutively, data from this study were analyzed retrospectively. The treatment of COVID-19 patients from this study was, mainly, symptomatic and supportive. These descriptive data provided some practice pattern in the treatment of COVID-19 for the first part of the pandemic.

CONCLUSIONS

In a small-size military hospital designated as COVID-19 support unit that cared for predominantly moderate forms of COVID-19, the costs for equipment were much higher than the treatment costs. Therefore, new criteria for hospitalization of these forms of COVID-19 deserve to be analyzed in order to avoid useless costs. Test-tracking-quarantine or other solutions like vaccination are very important to avoid important future health-care expenditure.

Aknowledgements

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Couvelaire-Uterus: literature review and case report

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Abstract: *Uteroplacental apoplexy or Couvelaire uterus is a rare complication in the spectrum of the life-threatening placental abruption.*

We report a 29-year-old pregnant woman who had a placental abruption complicated with the rare condition of Couvelaire uterus, with an delivery per uterus-sparring cesarean section.

The early diagnosis and proper medical management of placental abruption through uterus-sparring cesarean section present the best outcome for mother and child.

Keywords: *uterus Couvelaire, placental abruption, uteroplacental apoplexy*

INTRODUCTION

Utreroplacental apoplexy or Couvelaire uterus is a rare manifestation which complicates aprox. 5% of placenta abruptions and was described for the first time in 1911 by French obstetrician Dr. Alexandre Couvelaire [1, 2]. The placenta abruption complicates ~1% of pregnancies [3] and the maternal mortality can reach up to 5%. The uteroplacental apoplexy is a rare, nonfatal complication of the placenta abruption [4].

A literature search for the last 10 years was conducted on 25.11.2020 using the PubMed database and having the key words 'Couvelaire uterus'. This resulted in 27 articles published in the last 10 years. The Couvelaire uterus is often underreported and underestimated. The etiology is unknown and the diagnosis is done through inspection or histological examination [4].

This mechanism of placenta abruption can be seen as physiological after the fetus delivery and as pathological in the pregnancy with the fetus still in uterus. The acute condition of placenta abruption is the result of a compromise of the vascular structures supporting the placenta, having as outcome a retroplacental hematoma. This process may represent the starting point for Couvelaire uterus. The hemorrhage from pathologic vascular damage within the placenta seeps into the decidua basalis causing a separation of the placenta. Before the second stage of labour is finished, the blood created by bleeding resulting from the early placenta abruption infiltrates into the myometrium finding its way into the peritoneal cavity. Occasionally, such effusions of blood extend in the parametrium. As a result of the described mechanism we notice the macroscopic aspect of Couvelaire uterus which displays lesions characterized by violet to black ecchymoses [5, 6, 7]. The Couvelaire uterus sometimes loses its contractile power but usually responds well to intravenous oxytocin [6, 8]. The uterine ability to contract is preserved, particularly after amniotomy and decompression to allow constriction of spiral arteries [9].

Among the risk factors for placenta abruption are early

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pregnancy bleeding, hypertension or thrombophilia, or abdominal trauma [10]. The diagnose of placenta abruption is made clinical and is completed by the ultrasound scan and CTG. The patients present a painful, tender and rigid uterus, may have uterine contractions, vaginal bleeding is also common. The finding of a retroplacental clot on ultrasound scan is a risk factor for placenta abruption, the ultrasound scan plays an important role correlated to the clinical situation in the diagnose of the placenta abruption [3, 10, 11].

The delay of the diagnose of placenta abruption increase the risk of intrauterine fetal death, hypoxic brain injury, bleeding, coagulopathy with increased maternal and fetal morbidity and mortality [12, 13]. The incidence of coagulopathy in placenta abruption is reported to be 5.8% [3]. In the case of Couvelaire uterus the disseminated intravascular coagulation can be induced through the infusion of thromboplastic material in maternal circulation [14].

Couvelaire uterus was associated in different reports with placenta abruption, placenta previa, amniotic fluid embolism, and preeclampsia [4, 15]. Cesarean section in the patient’s history is a risk factor for uterine rupture, abnormal placental implantation, placental abruption and uterine scar dehiscence in subsequent pregnancies [16]. Some authors consider that the incidence of Couvelaire uterus is increased by the increase in cesarean deliveries [4].

The management of the antenatal and postpartum hemorrhage plays a key role, on one side in the control of the bleeding and blood transfusion, and on the other side aims to reduce fetal and maternal morbidity and mortality [4].

In the past hysterectomy was performed as a routine therapy of Couvelaire uterus. Nowadays, is recommended to opt for the conservative management [4, 8]. The proper management of this pathology reduces the high maternal and fetal risks. The literature reports cases of fetal demise, but also hysterectomy as a result of severe disseminated intravascular coagulopathy [3, 4, 17]. The early decision to perform a hysterectomy taken before the patient's condition is extreme serious is required in order to minimize the morbidity and mortality [18, 19]. On the other hand, before deciding for more aggressive procedures, like hysterectomy, B-Lynch technique should be taken into consideration in cases of postpartum haemorrhage as a result of abruptio placentae followed by development of Couvelaire uterus [19, 20].

In a study on placenta abruption poor perinatal outcome associated with Couvelaire uterus, pregnancy induced hypertension and disseminated intravascular coagulopathy is reported [18].

CLINICAL EXAMPLE

A 29-year-old gravida two, para one, in the 37th (36+6) week of an uncomplicated pregnancy, with a history of an uneventful spontaneous delivery, was admitted to the obstetric ward with symptoms of nephrolithiasis and urinary infection for further therapy. The obstetrical check-up was uneventful. The admission symptoms were alleviated, discharge could have been possible but she developed irregular and poorly coordinated uterine contractions. She developed uterine tenderness without relaxation between contractions, with wooden hardness by palpation. Vaginal examination revealed acute hemorrhage from the uterine cavity. The patient was hemodynamically stable. The cervix was 2 cm long and 1 cm dilated. CTG was normal. Cesarean delivery was indicated by suspicion of placental abruption. Intraoperatively massive hemoperitoneum and the diagnose of placenta abruption with Couvelaire uterus was confirmed (Figure 1).

A healthy boy was delivered. Uterus-sparring surgery was performed.

Postoperative recovery was uneventful.

Figure 1: Couvelaire uterus as a result of acute intradecidual hemorrhage produced by the rupture of the uterus–placental spiral arterioles which produces ecchymosis discoloration, secondary to extravasation of blood into the myometrium and serosa, can lead to hemoperitoneum.

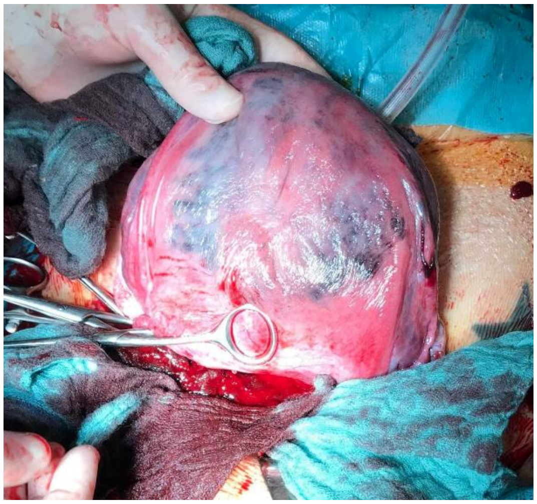

DISCUSSION AND CONCLUSION

Placental abruption represents the early separation of the placenta from the uterus wall developing a retroplacental hematoma which is an acute intradecidual hemorrhage produced by the rupture of the uterus–placental spiral arterioles. Couvelaire uterus or uteroplacental apoplexy is the result of blood extravasations in the tissues, occasionally the infiltrations which reach the peritoneal cavity creating hemoperitoneum [1, 2, 5].

In our case, the first intraoperative view after opening the peritoneal cavity was the massive hemoperitoneum and Couvelaire uterus with dark purple and copper color patches with ecchymosis discoloration and hemorrhagic infiltration in the lateral portions of the uterus.

Diagnosis is made through direct inspection or histological examination [2]. The management of this complication of the placenta abruption is conservative through uterus-sparring surgery, hysterectomy is usually not required and should be discouraged [2, 6]. Couvelaire uterus is a rare complication of placental abruption, with an underreported incidence and it is often associated with placenta previa, amniotic fluid embolism, and preeclampsia [4, 15] .

Often Couvelaire uterus is reported with massive placenta abruption requiring blood transfusion [4]. In our reported case blood transfusion was not required. Antenatal fetal and maternal monitoring, but also the delivery on time, plays an important role in the delivery outcome, otherwise due to bleeding it can come to fetal demise [15, 21]. Placental abruption has an increased maternal morbidity risk of haemorrhage, hysterectomy and consumptive coagulopathy [3]. The life-threatening condition of disseminated intravascular coagulation should not be underestimated [3, 18] .

In the reported case the patient was in the hospital under monitoring and the prompt medical decisions reduced the maternal and fetal risks.

The early diagnosis and proper medical management of placental abruption through the uterus-sparring cesarean section provide the best outcome for mother and child.

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ORIGINAL ARTICLES

CoViD-19 disease, Romanian health system response to outbreak and economic impact

Laura Bostangiu (Petcu)[1], Alexandru I. Iordache[2], Nicolae D. Garofil[3], Raluca S. Costache[3]

Abstract: *The pandemic called COVID-19 disease has an exponential course since the first cases were reported showing in 9th of May, 2020, more than 4 million cases of COVID-19. United States and European Council (EC), together with other European Union institutions, were closely monitoring the situation in order to take quick action.*

Objective: The aim of this article is to analyze the response to COVID 19 pandemic outbreak in term of strategy and money flow of Romanian health system in order to identify the strength and weakness and prepare for other similar provocative challenges.

Global and national strategy: United States Government is delivering a comprehensive package of services to support international partners around the world in combatting this disease adding American expertise for global benefit, saving lives by improving international partners abilities to respond to the COVID-19, and reducing secondary impacts. Meanwhile European Union is focused on 6 major coordinates limit the spread of the virus, ensure and provide medical supplies, decrease negative economic impact, support jobs, promoting research and help European citizens to repatriate. Despite all negative elements a proper and relatively quick response of Romanian Public Health System was noticed and the taken measures were harmonized and synchronized with the European coordinates, prioritize initiatives, build an action plan, establish a communication plan, workforce optimization and management strategy.

Conclusion: Global shock needs global response, with little or no government interventions, economic costs will be huge.

Keywords: *COVID-19, SARS-CoV-2, Coronavirus, pandemic financial impact, hospitalization cost*

BACKGROUND

COVID-19, coronavirus disease, is caused by a new type of coronavirus (SARS-CoV-2) and it was first identified in China in December 2019 when an outbreak in the municipality of Wuhan was described as quickly spreading to other regions of China and worldwide [1, 2]. By 7 of January 2020, isolated cases had appeared in some European Union (EU) member states. At the end of February 2020, Italy reported a significant increase of coronavirus disease (COVID-19) cases concentrated in two of the northern regions of the country [3]. By March 2020, all European Union (EU) member states had reported an exponential increase of COVID-19 cases. European Council (EC), together with other EU institutions, is closely monitoring the situation in order to take quick action [3]. This includes approving of relevant European

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Union legislation and ongoing coordination with member states to share information, assess needs and ensure a coherent European Union wide response under direct coordination of The World Health Organization (WHO) [3]. On 11 March 2020, the World Health Organization (WHO) declare COVID-19 as global pandemic with an important issue: in this declaration they were talking about geographic spread, and not about the severity of the disease [4].

The pandemic called COVID-19 disease has an exponential course since the first cases were reported showing in 9th of May, 2020, more than 4 million cases. Disease caused by the new coronavirus that was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, COVID-19, have been reported globally with more than 286,000 deaths in 180 countries, including all United States with 1,4 million persons recovered [3, 5].

As epidemiological pattern, all groups of ages are at risk for infection including here severe form of disease, even death. However, seems that the probability of fatal disease is highest in people aged more than 65 years, those with comorbidity and those living in a nursing home or long-term care facility [6, 7, 8]. Of underlying condition, especially when those are not controlled is appropriate to mention as major risk factor for severe or critical COVID-19 disease [3-7]: Hypertension, Obesity, Chronic respiratory disease, Cardiovascular disease, Cancer, Renal disease, Diabetes [9]. As for diagnose WHO recommends as gold standard nasopharynx samples to be used in order to detect SARS-CoV-2. Nasal swabs or oropharyngeal swabs may be acceptable alternatives [9]. Lower respiratory tract samples have a demonstrated higher sensibility than upper tract samples, but WHO recommend not to obtain because of concerns about aerosolization of virus during collection procedures. Regarding treatment, no drug has been proven to be safe and effective for treating COVID-19. There are no Food and Drug Administration (FDA) or EMEA -approved drugs specifically to treat patients with COVID-19. In the medical literature, reports have appeared claiming or promoting successful treatment of patients with COVID-19 using a variety of agents [6, 8, 9]. A single conclusion has emerging: definitive clinical trial data are needed to identify optimal treatments for this disease. General clinical management of patients with COVID-19 includes infection prevention, control measures and supportive care, including supplemental oxygen and mechanical ventilatory when indicated. In is also compulsory to mention that as in the management of any disease, treatment decisions ultimately reside with the patient and their health care provider [6, 7, 9, 10, 11, 12].

Behind the medical challenge generated by COVID-19 pandemic and all concerns, analyst anticipate an economic crisis. That is why worldwide governments are involved in generate measures in order to decrease negative economic impact.

OBJECTIVE

The aim of this article is to analyze the response to COVID 19 pandemic outbreak in term of strategy and money flow of Romanian health system in order to identify the strength and weakness and prepare for other similar provocative challenges.

GLOBAL ANALYSIS AND DISCUSSION

If we have to define the concept of support, probably in the last 50 years the United States (U.S.) will fit perfectly since they have been the largest contributor to global health security [4]. US built the foundation upon which the global health system is based, contributing more than $140 billion in global health assistance only in the last century and nowadays demonstrate leadership in fight to COVID-19 pandemic. From the beginning of the outbreak of COVID-19, the United States government provided more than $500 million in assistance specifically aimed to support anti-pandemic measures [3, 4, 13, 14].

But this is not all, US Government is delivering a comprehensive package of services to support international partners around the world in combatting this disease. The program should add American expertise for global benefit, saving lives by improving international partners abilities to respond to the COVID-19, and reducing secondary impacts [13, 14].

The strategy mentioned is focus to: Save lives by increasing international partners ability to respond to the pandemic; Reduce secondary impacts of the disease; Promote U.S. leadership for global benefit [13].

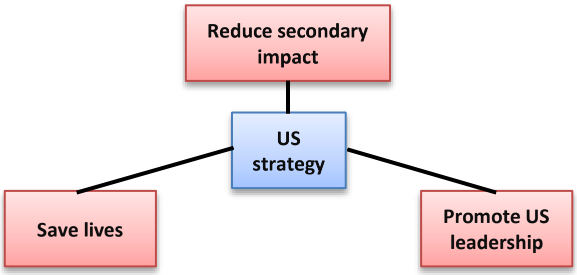

Figure 1: US Strategy for COVID-19 [13, 14]

The United States (US) was delivering a SAFE and more secure world from COVID-19 program as is state in the following table [13,14].

The European Union's (EU) response to COVID-19 outbreak focuses on 6 major coordinates as they are mentioned above [15,16].

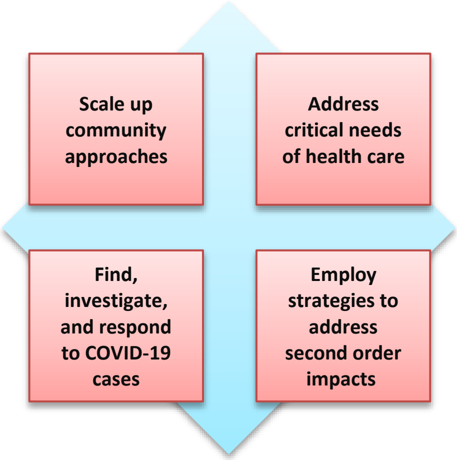
Scale up community approaches
Address critical needs of health care
Find, investigate, and respond to COVID-19 cases
Employ strategies to address second order impacts

Figure 2: US program SAFE for a safer and more secure world from COVID -19

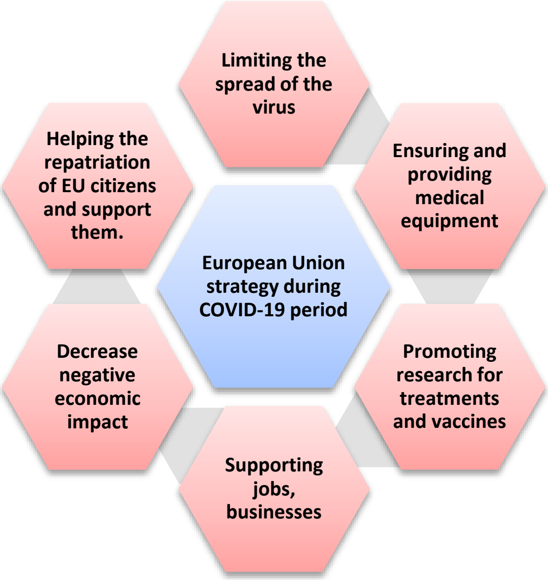
Limiting the spread of the virus
Helping the repatriation of EU citizens and support them.
Ensuring and providing medical equipment
European Union strategy during COVID-19 period
Decrease negative economic impact
Promoting research for treatments and vaccines
Supporting jobs, businesses

Figure 3: European Union strategy for COVID-19 disease [15, 16]

In order to slow down the transmission of the virus, European Union (EU) leaders agreed on a coordinated temporary restriction of non-essential travel to the EU for 30 days. The travel of European citizens outside the EU acquires negative recommendation. To coordinate the EU response to the COVID-19 outbreak the following measures were taken [15, 16, 17]:

- The Council activated the EU an integrated political crisis response (IPCR) with mandatory weekly roundtables gathering EU institutions, EU agencies experts and representatives of member states
- European Commission and the Council are gathering contact and coordination between relevant national ministries
- The European Centre for Disease Control (ECDC) quantify daily risk assessments and epidemiological updates for the EU population.

By using the union civil protection mechanism, the EU has: coordinated the deployment of medical teams to most affected areas, facilitated the sourcing for additional protective equipment, especially medical masks, activated the Emergency Response Coordination Centre, to coordinate support 24/7, created a new common European reserve of emergency medical equipment including also ventilators and lab supplies. More than €300 million was allocated for research and support initiatives in medical field and a European COVID-19 data portal was launched to ensure the rapid collection and sharing of available research data [15, 16, 18, 19].

The EU and its member states are taking action to minimize the negative impact of outbreak on the economy. On 9 April 2020, the Eurogroup generated, three immediate safety nets, worth €540 billion for: protect and generate new jobs and businesses in member states. The EU is putting forward temporary support to mitigate unemployment risks in an emergency (SURE) to help people keep their job during the crisis. The scheme consists of loans to member states of up to €100 billion to cover the costs related to the creation or extension of national short-time work schemes. European Investment Bank (EIB) Group to create a pan-European guarantee fund providing that way loans up to €200 billion for companies with a focus on small and medium-sized enterprises (SMEs) throughout the EU [15, 17, 18, 19].

European Stability Mechanism set up Pandemic Crisis Support based on an existing credit line, which will include loans available to all euro area member states up to 2% of their internal product estimated at €240 billion. The EU also has increased its budget for 2020, with €3.1 billion as response to the COVID-19 crisis [18, 19]. This additional fund will be used to purchase and distribute medical supplies, including protective gear and ventilators, boost the production of testing kits, build mobile hospitals, transfer patients for treatment in between member states, repatriate EU citizens stranded abroad [19].

The EU has also adopted measures to ensure additional flexibility in the use of structural funds. On behalf of

Coronavirus Response Investment Initiative Plus member states can transfer money between different capital lines in order to meet their needs, resources can be redirected to the most affected regions and the opportunity request up to 100% financing from the EU budget between 1 July 2020 and 30 June 2021 for programs involving measures with impact in the COVID-19 pandemic [15, 16]. The EU and its member states were working to repatriate more than 650.000 EU citizens stranded abroad. EU countries and EU delegations provide consular assistance to help EU citizens return home, providing 75% of the costs of the return flights and add measures in order to help them to cross EU borders [16].

Although Li at all [10] estimate that 86% of all infections were undocumented prior to 23 January 2020, when travel restrictions were beginning per person, the transmission rate of undocumented infections was 55% from all of documented cases. Due to their increasing factor, undocumented patients were the source for 79% of documented cases [12]. They conclude that two favorable condition are rising: existing estimates of case-fatality rates might be over-estimate and a grade of immunity is present in the system [12]. But they underline also that probably when interventions started in Europe and US the virus was already widely spread and simulation regarding on how measures of suppression will flatten the curve in the short period may be over-optimistic [12].

NATIONAL STRATEGY AND HEALTH ECONOMIC IMPACT

Geopolitical context

Romania is one of the countries that has to face the infection of the population with the new coronavirus. The most recent statistics shows that Romania has around 4.7 mil people that lives outside the country and they are spread all over the world [3, 19, 20].

According to statistics reported by the Ministry of Foreign Affairs from the total number of the Romanians that lives outside the borders in Italy lives 1.206.938 people which means that around 25% are located in the country that has the largest outbreak of the pandemic, and around 19% lives and works in Spain. This is the reason why Spain has become the second deadliest hotspot for COVID-19 after Italy with 27.967 cases and its death toll reached 24.824 on 1st of May 2020 [19, 20].

Having Italy as a “model “of how fast the virus spread among the citizens, medical stuff and hospitals and that no real actions were made until the spread was out of control, Romania introduced lockdown measures at an early stage in the outbreak: cancelled almost all flights in and out of the country, starting with the ones from the red zone, authorities were also quick to close bars, restaurants, cinemas and schools. Police were urging people to stay at home [19, 20].

The representatives of the Intersectoral Committee for the Prevention and Limitation of Diseases with the New Coronavirus (COVID-19), together with the representatives of the authorities responsible for this field, held a teleconference on the evening of February 22, 2020, taking into account the evolution of cases in Italy areas where many Romanian citizens are or traveling from [19].

After this point the public health authorities included persons arriving in Romania from the affected localities in the Veneto region and the province of Lodi / Lombardy or who have traveled to these localities in the last 14 days, in the category of those who must stay in quarantine for 14 days after entering the country. These measures were similar to those applied by the Italian authorities. At the same time, upon arrival in Romania, all asymptomatic passengers from the affected areas, respectively Hubei (China) or from 11 regions of Italy will be sent to quarantine for a period of 14 days [1, 2, 19].

New measures were sent by the Ministry of Health to the health units and family doctors, through the county and Bucharest public health directorates which included [21]:

- measures to control and prevent infection with the new coronavirus at land, sea / river and air border crossings. The Ministry of Health provided the necessary medical staff at the border crossing points, with specialists from the public health directorates, including by secondment from other counties and health units;
- all persons, who fall within the definition of a suspicious case, should be reported immediately by all health units where they appear (emergency units, hospitals, family doctors) to the public health departments and the Monitoring Service within Emergency Situation Department (DSU);
- Ministry of Health completed the legislative framework with necessary measures for quarantine and for the management of suspected and confirmed cases of coronavirus infection (COVID-19);
- public information campaign on the new coronavirus infection;
- individual and collective prevention measures were taken and extended with a daily information bulletin on the evolution of the situation at international level and any additional measures taken by the Romanian authorities;

Also, on March 10, 2020 the Ministry of Health operationalized the Operational Coordination Center, a unit that establishes in operational time the connection with the public health directorates and health units under the Ministry of Health subordination, to identify in real time the risk situations associated with COVID 19 infection and to establish measures that are required [20, 21].

The Operational Center had to provide up-to-date information and all the useful and vital information to establish the correct measures for the epidemic phases, real time stocks of medicines, medical equipment and disinfectants existing at national level in hospital units, the number of medical staff involved in the actions in order to limit the spread of infection, the number of free beds in infectious disease departments and Intesive Care Units [21].

Other important measure was to limit the scheduled hospitalizations in the hospitals coordinated by the Ministry of Health, in relation to the degree of bed occupancy (including ICU beds) and the local epidemiological evolution corroborated with daily report the situation of bed occupancy of the hospitals in the centralized electronic system of the Coordination Center. This measure was necessary both to ensure the capacity of hospitals to take over cases and to manage material and human resources correctly and efficiently [21].

Adequate public communication was ensured so that, during this period, the public could turn to hospitals only for emergencies. The aim was to avoid congestion in the sanitary units [21]. At that point was established a support network for infectious disease hospitals depending on the contagiousness of non-coronavirus cases. These hospitals are going to be support hospitals, in which patients will be transferred from the Infectious Diseases Hospital. In a first phase, was recommended that the support hospitals not be represented by the important emergency hospitals in the area, which can provide medical assistance for the other medical-surgical emergencies [21].

The Ministry of Health Plan also provides measures in case of extend of the crisis and in case of capacity exceed of Infectious Diseases Hospitals, as well regulations on the use of protective equipment [21].

On March 11, 2020 at the proposal of the Ministry of Health, the National Committee for Special Emergency Situations issued an order for including the temporary suspension, for a period of 6 months, of distribution, outside Romania, of medicines, medical devices and sanitary materials essential in prevention and treatment conditions associated with COVID-19 infection [21].

This measure is part of the action plan of the Ministry of Health which aims to provide adequate and continuous stocks of medicines, medical devices and essential medical materials in this situation. The list was going to be established together with the experts from the specialized commissions of the ministry, taking into account in particular, medicines such as: antivirals, anti-inflammatory, antibiotics, antipyretics, antifungals, anesthetics, sedatives, as well as other medicines considered necessary in the treatment of cases of COVID-19 and their complications [21].

The list also included medical supplies and medical devices such as overalls, gloves, syringes, masks, goggles, disinfectant solutions, as well as other medical supplies and devices identified as necessary for the management of COVID-19 cases [21].

The Centre for Supervision and Control of Transmissible Diseases issued recommendations which were published on 12 March 2020 [21].

- The preventive measures considered as most effective include: frequent hygiene of the hands with hydro-alcoholic substances through friction if hands are not visibly dirty, or with water and soap if the hands are dirty; avoid touching eyes, nose and mouth; practice respiratory hygiene by coughing or sneezing in the elbow, or use napkin/tissues and throw it away immediately; wearing a mask if the person has respiratory symptoms and ensuring in such case the hygiene of the hands after removing the mask; maintain the social distance of minimum 1 meter from the persons with respiratory diseases.

- Protective Personal Equipment (PPE) must be used based on exposure risk and based on the dynamic of transmitting the pathogen agent. The overall use of PPE will have a supplementary impact on the lack of supplies of PPE.

- The state office mentions that for the people with no symptoms, it is not recommended to use masks of any type. The wearing of the masks when it is not recommended can cause useless costs, a burden on purchases and can create a false feeling of security that can lead to the neglect of other essential prevention measures.

Threats and weaknesses

In order to analyze the response to COVID-19 outbreak, we identify a panel of weaknesses which conduct in early stage to quick spread of the virus and generate uncontrolled local outbreaks:

A panel of negative elements should be considered when we analyze the Romanian Health System response to outbreak.

- Low infrastructure in term of hospitals, circuits, Intensive Care Units and number of available PPE for health workers

- Administrative human resources with lack of management skills, unable to generate procedures and coordinates the Public Health Departments

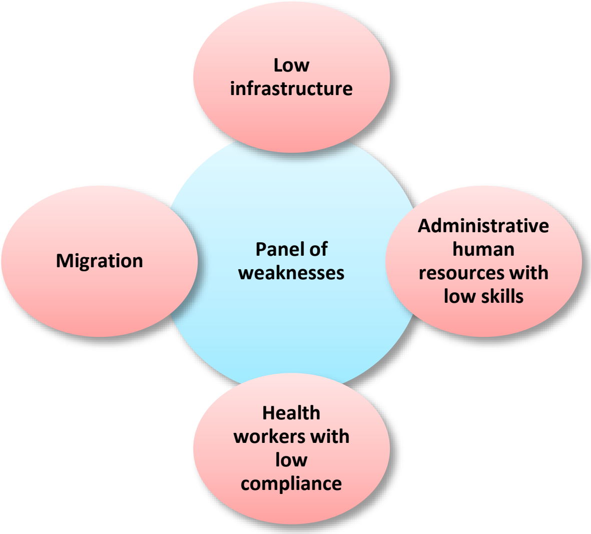
Low infrastructure
Migration
Panel of weaknesses
Administrative human resources with low skills
Health workers with low compliance

Figure 5: Panel of weaknesses regarding response to COVID-19 outbreak

- Health workers with low compliance at medical information and epidemiologic resources, unwilling in many situations to respect the new statement measures and legislation provided by Ministry of Health and National Health Institute

- Not as a last item, migration, the return of Romanian citizens from pandemic areas of Europe crossing the borders using in many situations false document of false declaration and being extremely low compliant of rules and measures, regarding quarantine or in self isolation

Ministry of Health strategy

Despite those negative elements a proper and relatively quick response of Public Health System and it could say that the taken measures were harmonize and synchronize with the European coordinates. Romanian Government through Ministry of Health established 5 directions as a strategy which enhanced the national response to COVID-19 disease.

When "everything is vital and important", Ministry of Health and the other public institution involved in fighting the pandemic had to prioritize the initiatives. In order to do that a Plan of measures for the preparation of hospitals in the context of the COVID-19 Coronavirus epidemic was approved. This plan was the base of the strategy framework regarding the hospitals that can treat patients infected with COVID19. In collaboration with the local public administration authorities, based on the local analysis of patient care capacities, was generated and approved the List of support hospitals for COVID - 19 positive patients requiring treatment for specific pathologies (eg heart disease, orthopedics, etc.)

Thus, a number of 57 hospitals/outpatient departments/ buildings, 44 maternity hospitals, 60 hospitals and centers for performing dialysis procedures in all counties of the country and Bucharest were identified and designated as support units.

Supportive cases of COVID 19 that require medical care for other pathologies are also treated in support hospitals.

As a first step, designated support hospitals are non-emergency medical units that can provide medical care for other medical and surgical emergencies. Included in the network of support hospitals, municipal/city units and hospitals in other counties mainly those with Infectious Diseases departments, as well as hospitals in other networks than the Ministry of Health (Ministry of Transport, Ministry of National Defense, private health units).

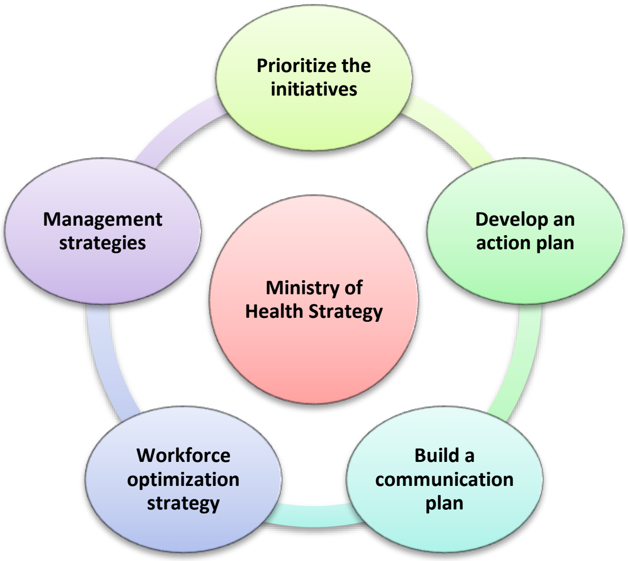
Prioritize the initiatives
Develop an action plan
Build a communication plan
Workforce optimization strategy
Management strategies
Ministry of Health Strategy

Figure 6: Romanian Ministry of Health direction strategy

"The plan of measures for the preparation of the hospitals was elaborated by the specialists within the specialized commissions ICU, infectious diseases and specialists within the Ministry of Health, based on the recommendations of the WHO and EC. I asked the managers of the health units, but also the public health departments, the ambulance services to inform the medical and auxiliary staff, repeatedly, the procedures to be followed in this crisis situation, all the existing measures in the plan of measures, especially the regulations. regarding the prevention measures and the correct use, depending on the exposure, of the medical staff, but also of the auxiliary" [21].

Also, all health units from public and private system have the obligation to ensure the provision of medical care to all patients, in accordance with the disposition of the approved plan of measures. Any refusal to ensure the provision of medical care according to the specific attributions it's going to be sanctioned according to the legal disposals [21].

Develop an action plan

In order to be able to control and establish the right measures Ministry of Health with the help of the Operational Center were able to collect in real time the stocks of medicines, medical equipment and disinfectants existing at national level from the hospitals, the number of medical staff involved and the number of free beds in infectious disease departments and Intensive Care Units.

As we could see the plan is to deliver the equipment or medication where is most needed, or to purchase the equipment or medication where is most needed.

In order to simplify the Purchases of materials, medicines and equipment necessary to combat the COVID-19 pandemic, during the state of emergency, are made directly by the central and local public authorities, the legal entities in which the state is the majority shareholder, the public health directorates, the health units, as well as the National Administration of Penitentiaries and the units subordinated based on the obligation to draw up a note justifying the necessity of the acquisition.

Regulations have been approved on the use of personal protective equipment (PPE) at the level of the health unit, rationally and correctly, depending on the department, staff and type of activity. Thus, in the context of COVID-19, personal protective equipment should be used based on the risk of exposure (eg type of activity) and the transmission dynamics of the pathogen (eg contact, drops or aerosols).

The type of PPE used when caring for patients with COVID-19 will vary depending on the situation, healthcare staff and work carried out. Medical personnel involved in the direct care of patients should use the following PPE: gowns, gloves, mask and eye protection (goggles or face shield).

Specifically, for aerosol-generating procedures for patients with COVID-19 (eg: intubation, noninvasive ventilation, tracheostomy, cardiopulmonary resuscitation, manual ventilation before intubation, bronchoscopy, gastroscopy, and COVID PCR testing) medical personnel should use as protection, gloves, gowns, masks FFP2 and FFP3; waterproof aprons will also be used if overalls/gowns are not waterproof.

Build a communication plan

Creating a communication plan to collect key information from stakeholders regarding the planned initiatives and the capacity building was an important step in order to manage media coverage of the topics related to the new coronavirus infection.

Therefore, by the Decision of the National Committee for Special Emergency Situations no. 2 from February 24 2020 The Strategic Communication Group was established and it consists experts from several institutions like Ministry of Health, Ministry of Defense, Emergency Situation Department, National Institute of Public Health, General Inspectorate for Emergency Situations.

In order to support the population a Free Direct Line (Telverde) has become operational for coronavirus information in coordination of National Institute of Public Health and people can request information on how the virus manifests itself, how to prevent the disease and other details related to preventive behavior. However, the experts informed the populations that if they have any health problems or suspect that you may be infected, to continue to call the emergency number.

Workforce optimizations strategies

The evolution of the epidemiological situation determined by the spread of coronavirus on the Romanian territory, as well as the declaration of the pandemic by the World Health Organization determined the public health risk assessment for the next period as a result of the increased number of active surveillance, early detection , isolation and treatment of positive COVID 19 cases, follow-up of contacts and prevention of the continuous spread of the infection which imposes the need for additional staff involved in these activities [3, 20].

Therefore, at the proposal of the Ministry of Health, the Government of Romania approved the supplementation with 2000 positions within the county public health directorates and county ambulance services for a determined period of 6 months with 2000 positions [21].

In the current situation, the operative crews of the ambulance services are involved both in the transport of patients confirmed with COVID-19, and in the collection of samples from people in quarantine or home isolation.

Also, the staff of the public health directorates ensures the epidemiological triage from the border crossing points, monitors the self-isolated persons at home, monitors the quarantined persons, collects field samples from the persons corresponding to the case definition for testing for COVID-19.

Currently, the number of posts allocated and fully distributed by ministerial order for the county and Bucharest public health departments is 3.862, and for the county ambulance services and the Bucharest-Ilfov Ambulance Service is 11.733 posts [21].

In order to prepare the health units and the health system and the emergency system to cope in case of an exponential increase of the cases with COVID and in case of signaling a personnel deficit in some areas it was necessary to detach the health personnel to cover the needs in the area. Therefore, specialized medical staff, primary care physicians, specialists or residents, medical staff, health auxiliaries and medical staff with higher education (chemists, biologists, biochemists, physicists, pharmacists and balneo-physiotherapists) employees of public health units are about to be seconded or delegated to other public health units that have a major staff shortage.

Management strategies

The socks which make up the pandemic cause a sharp drop in consumption and investment all over the world. With the previous experiences of pandemics, governments across the world have exercised a stronger caution towards outbreak by taking early measures, such as straitening the health infrastructure to prevent the outbreak.

With the measures taken by MoH regarding the fact that many hospitals were force to close outpatient departments and postponing or canceling elective visits and procedures, it's most likely that the financial viability to be threatened, especially for those with preexisting financial challenges and those heavily reliant on revenue from outpatient and elective services.

A 2018 report from the Agency for Healthcare Research and Quality suggested that elective admissions accounted for more than 30% of total inpatient hospital revenue [22]. Elective procedures, especially orthopedic and cardiac surgical procedures, are among the most profitable services for hospitals[23]. By one estimate, hospitals earn $700 more for elective admissions than for admissions through the emergency department [24]. Furthermore, for many hospitals, outpatient revenue now equals inpatient revenue, elective or otherwise [25].

Also in Romania though reduced outpatient and elective revenue may be partially offset by higher hospital and intensive care unit occupancy during the COVID-19 pandemic, and by an increase in services after the pandemic ends, this may not mitigate losses (particularly given the need for surge personnel and resources) or be evenly distributed across hospitals.

The COVID-19 pandemic represents an unprecedented medical and economic challenge for the Romanian health care system. In the absence of robust and sustained governmental support, almost all hospitals will experience financial difficulties.

But hospitals that are smaller, independent, rural, and have critical access status are particularly at risk. Policymakers provided dedicated support to these hospitals to access funds and allocated additional funding to them during the COVID-19 pandemic.

Table 1: Global Healthcare Systems Data

Country	Australia	New Zealand	Germany	UK	US	Romania
Healthcare expenditures per capita	AU $7845 ($5,612)	NZ $5,892 ($4,015)	€4,544 ($4,924)	£2,989 ($2332)	$10,612	€1,029 ($1,115)
Medical practitioners per 1,000 people	3,94	3,35	4,3	2,8	2,6	2,8
Beds per 1,000 people	3,9	2,6	6	2,5	2,8	6,8
Population aged +60 (%)	21,4	21,3	28,2	23,8	17	22,6

Source: https://insse.ro/cms/files/publicatii/Romania_in_cifre_breviar_statistic_2018.pdf, https://www.ibisworld.com/industry-insider/coronavirus-insights/effects-of-covid-19-on-global-healthcare-systems/ [26]

According to the Medical Group Management Association, 97% of medical group practices have experienced a negative financial impact directly or indirectly related to COVID-19. On average, these practices report a 55% decrease in revenue and a 60% decrease in patient volume since the start of the COVID-19 public health emergency [20, 24, 25].

In order to evaluate if Romania can face the expenditures due to COVID-19 outbreak we tried to compare data from different global healthcare system in their heroic and unprecedented ways to meet the challenges of COVID-19.

Hospitals and health systems face catastrophic financial challenges in light of the COVID-19 pandemic. We undertook four analyses to better understand and quantify these financial challenges.

Including:

- the effect of COVID-19 hospitalizations on hospital costs;
- the effect of cancelled and forgone services, caused by COVID-19, on hospital revenue;
- the additional costs associated with purchasing needed personal protective equipment (PPE); and
- the costs of the additional support some hospitals are providing to their workers.

a) Net Financial Impact of COVID-19 Hospitalizations

At this time there are no official estimates of the net financial impact of COVID-19 hospitalizations but for the over 6,800 patients tested positive until end of April, an expense of over 77 million lei (approximate 15 million euros) is estimated by the Ministry of Health [21, 26].

Table 2: Expenditure on protective equipment against COVID-19

Equipment	LEI	Euro
Protective coveralls	50,5 mil	10,4 mil
Disposable gown	15,2 mil	3,1 mil
Disposable caps	876,000	180,000
Disposable boots	3,1 mil	653,000
Disposable sort	732,000	150,000
Disposable gloves	7,7 mil	1,6 mil
Protective eyewear	2,2 mil	453,000
Protective visors	3,1 mil	640,000
FFP2 Mask	19,4 mil	4,1 mil
FFP3 Mask	2,4 mil	510,000
Surgical Mask	36,9 mil	7,6 mil
Disinfectant	21,8 mil	3,1 mil
Total	**153,9 mil**	**32,5 mil**

Source: Ministry of Health data collection from territory 30 April, 2020[27]

b) Total Revenue Losses from Cancelled Surgeries and Other Services

As a result of cancelled hospital services due to the COVID-19 pandemic, hospitals stand to lose approximately $200 million lei (approximate 40 million euros) in revenue over a period of four months, from March to end of April 2020. This includes cancelled surgeries, various levels of cancelled non-elective surgeries and outpatient treatment, and reduced emergency department services [26].

c) Additional Costs Associated with Purchasing Needed PPE

In order to estimate future expenditures and budget allocation Ministry of Health is collecting regularly data from territory regarding the consumption and demand for equipment and supplies, such as PPE, which considerably increased as a result of the COVID-19 pandemic. Hospitals have incurred additional costs as they struggle to acquire additional supplies to meet the needs of their patients and staff. Moreover, current guidelines require all hospital workers to wear some PPE, regardless of whether they are in direct contact with COVID-19 patients. These guidelines increase the need and expense for PPE relative to normal operations.

d) Costs of Additional Support Some Hospitals are Providing to their Front-line Workers

The govern could not yet estimate the cost of support for front-line hospital workers in COVID-19 hotspots for costs of providing child care, housing, transportation, and medical screening and treatment for COVID-19 for front-line workers but measures were taken to provide such services for them [27].

This report attempts to quantify these effects over the short-term, which are limited to the impacts over a two-month period from March 1, 2020 to April 30, 2020.

Although the government moved quickly to provide relief and the budget expenditures was increase by 2,5 billion euros in order to fight the financial impact, more help is needed [26, 27].

This includes:

- 120 million euros were added in staff costs, and this includes the payment of the risk incentive of 500 euros gross per month for doctors, medical staff, paramedical staff, including auxiliary staff directly involved during the emergency.
- Social security expenditures from National House increased by over 1,4 billion euros (technical unemployment and other health expenditures).
- Expenditures on the reserve fund increase by 3 billion lei.
- The Ministry of Labor also receives 1,6 billion euros and the Ministry of Health 785 million euros.

Following the immediate measures and reallocations of the government, the Ministry of European Funds obtained following the negotiations with the European Commission, a package worth 1.16 billion euros, European funds that will be injected urgently into the Romanian medical system to contribute to the socio-economic measures taken by the Government during this period [15, 16, 26, 27].

The allocation is going to cover expenditures as following [15, 16, 26, 27]:

- 100 million euros reimbursement of the risk incentive of 500 euros gross per month for doctors, medical staff, paramedical staff, including auxiliary staff directly involved during the emergency;

- 350 million euros for reimbursements of the purchases of medical equipment (ventilators, injectors, COVID-19 tests, etc.) and protective equipment (masks, gloves, coveralls, etc.), with the possibility of increasing the allocation to 500 million.

- 682 million euros for the purchase of medical equipment and equipment for the coordination system of the national emergency system, by financing an IGSU project.

The World Bank has allocated more than $1.4 billion to support the efforts of European and Central Asian countries to combat the effects of the COVID-19 pandemic. As of April 2, 2020, it has been approved to allocate approximately $ 490 million to be distributed for new emergency response programs [15,16]. In addition, it was decided to reallocate approximately $ 925 million, which will be redistributed from existing projects and loans, as well as additional funding, to support countries working to combat the effects of the COVID-19 pandemic [15]. Ministry of Health has purchased equipment for medical protection and treatment to fight against COVID worth 29 million euros from projects financed by the World Bank in Romania [27].

CONCLUSIONS

Global shock needs global response, with little or no government interventions, economic costs will be huge. For decrease negative impact, Government spending should be as large as the predicted economic costs, focusing directly on cash disbursement to firms and households. Central banks should support financial the government, not just through their own reserves but also by printing money if necessary. It is clear state that no country has fiscal capacity to stand alone [28].

Government priority should be on health expenditure but as the specialist anticipated is mandatory to generate a strategy to flatten the contagion curve that may rise back in the end of 2020. The tasks of test ‘at random’ representative samples of the population to identify the most contagious groups and isolate immediately the more vulnerable (e.g. the old) should be in front of strategy. A global expand of intensive care capacity (both beds and equipment) by building new units or convert available other type of buildings (e.g. hotel, barracks, etc.) is needed. Another imperative measures is to relocate patient who need intensive care to non COVID hospitals in order to increase capacity of that one placed in concentrated aria of contagion in terms of human resources and infrastructure.

Romania has harmonized with WHO and European Union requirements regarding preventive measures and legislation for COVID-19 disease and show a major and continuous concern in increasing testing capacity although the number of experts in that field was decreasing year by year. Despite the major weaknesses Ministry of Health adopted the best measures in order to combat the negative impact of pandemic. A perfect management strategy was setup in local outbreaks areas, introducing military management in hospitals in which wrong management decision were taken in early stage of COVID-19 disease.

Ministry of Health registered an extremely rapid reaction in support healthcare workers, purchase medical equipment and ventilators challenging the other health systems that faced with the same health problem forcing in a positive manner Public Health System for a simultaneous high inflow of SARS-CoV-2 positive patient in terms of human resources and infrastructure (mobile hospitals for patient with no and mild symptomatology, intensive care units etc.).

As part of European Nation, Romanian Government realized the harmonization of institution using emergency state providing this way an outstanding panel of experts in critical domains which initiate the best decision in a proper time, find the measures and optimal solutions for access to European available funds, cover the huge economic cost, helping Health System to fight against a complete new disease and providing the support for return of citizens in respect of European laws and Human Rights Declaration.

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All mentioned authors equally contributed.

JEL Classification: H51, I15, I18, I28

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CLINICAL PRACTICE

Cross-leg limb salvage solution: A case report regarding the management of a major defect in a patient with type IIIB open distal tibial fracture

M. Turbatu, C. Condrea, A. Oproiu, A. Cursaru, A. Lupu

Abstract: *Tissue coverage of lower limb defects after tibial fractures remain one of the hardest areas for a surgeon to deal with. The definitive management of Gustillo type IIIB fractures depends on a series of factors, which have a high impact in the multidisciplinary management of this severe trauma.*

Although free flaps have been used succesfully in the last two decades, there remains some cases where easier plasties using the contralateral limb tissue reserved could be indicated.

We describe our experience with a cross-leg flap in a severe-smoking patient for the reconstruction of a difficult leg defect.

Keywords: *Cross-leg plasty, Type IIIB fractures, External fixators*

INTRODUCTION

The tibia is reported to be the most commonly fractured long bone in the human body. Gustilo-Anderson IIIB injuries are a major trauma requiring aggressive excision, adequate fixation, and good coverage of tissue defects. They are open tibial fractures more difficult to manage than other I, II, and III A forms.

Stable skeletal fixation with immediate soft tissue cover has been the key to the optimal results in treating open tibial fractures, in particular, type IIIB fractures. The prognosis of these fractures depends on the degree of contamination of the wound, the devitalization of the soft tissue and area of the defect.

Pedicled cross-extremity flaps for calves wound coverage have been replaced by free tissue transfer in the last two decades. However, there are certain difficult situations where the free flap cannot be employed and alternative methods are needed.

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We describe our experience with a cross-leg gastrocnemius flap in a heavy-smoker patient with little tissue reserve necessary for the coverage of a defect in the middle to lower third anterior calf.

CASE PRESENTATION

We present the case of 58 years old patient who was admitted in the Orthopedics and Traumatology Clinic of the Emergency University Hospital of Bucharest with an injury after a direct trauma by axe and had pain in the calf, functional impotence of the inferior member and an open wound of about 10 cm on the inner side in the 1/3 middle anterior calf.

Following wound debridement (including post-debridement measurement) and skeletal fixation, the anterior defect was assessed [1].

The following days the patient underwent antibiotherapy and careful daily wound dressing until a proper defect coverage plan was decided.

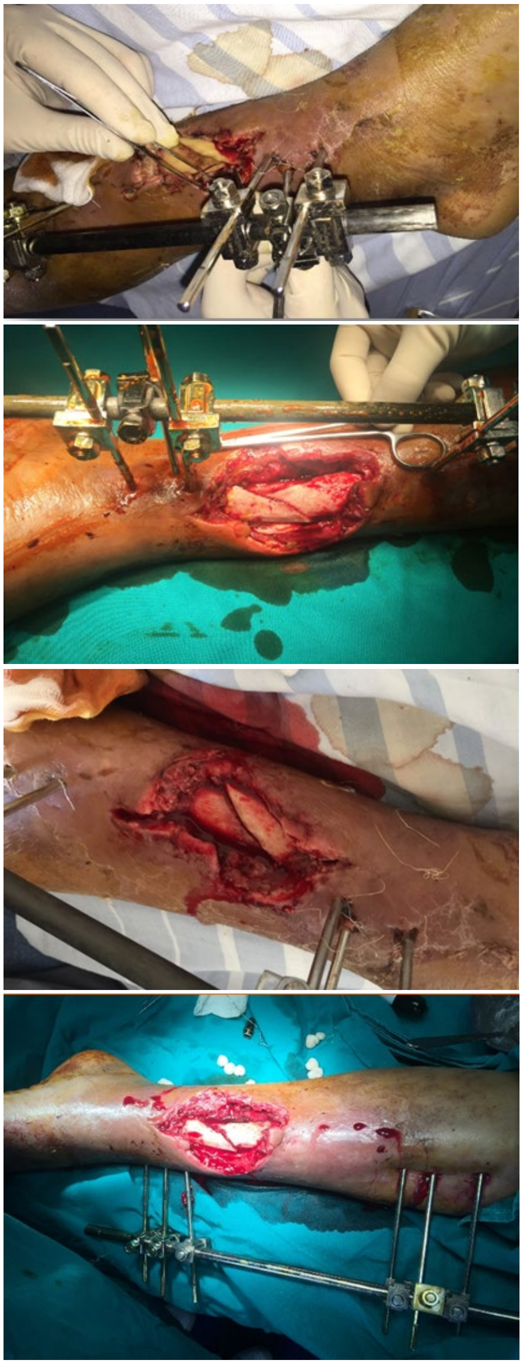

Free flaps are usually the first choice for soft tissue coverage in the distal leg. There continue to be, however, some clinical situations in which local fasciocutaneous and myocutaneous flaps are often not available [2].

Occasionally, a free flap may also have failed because of technical errors or damaged vasculature. In these situations, a cross-leg flap is the best choice.

In the end we decided to perform a pedicled cross-leg gasctrocnemius flap, with the utilization of an external fixator to keep both legs immobilized after the surgery.

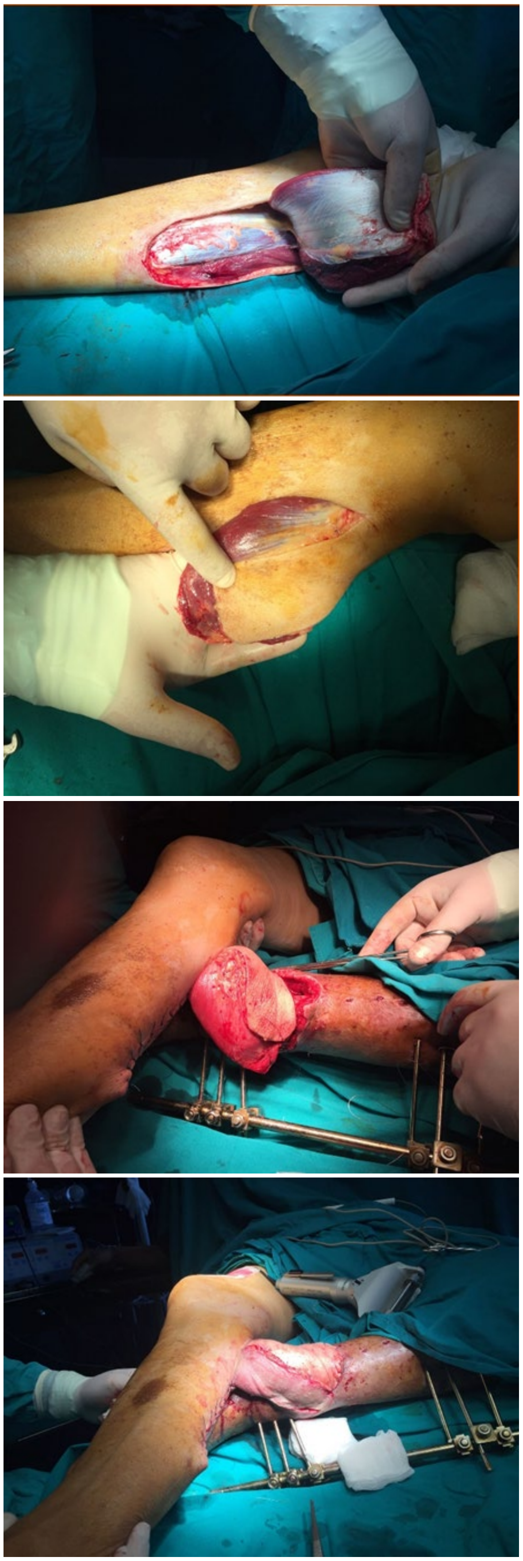

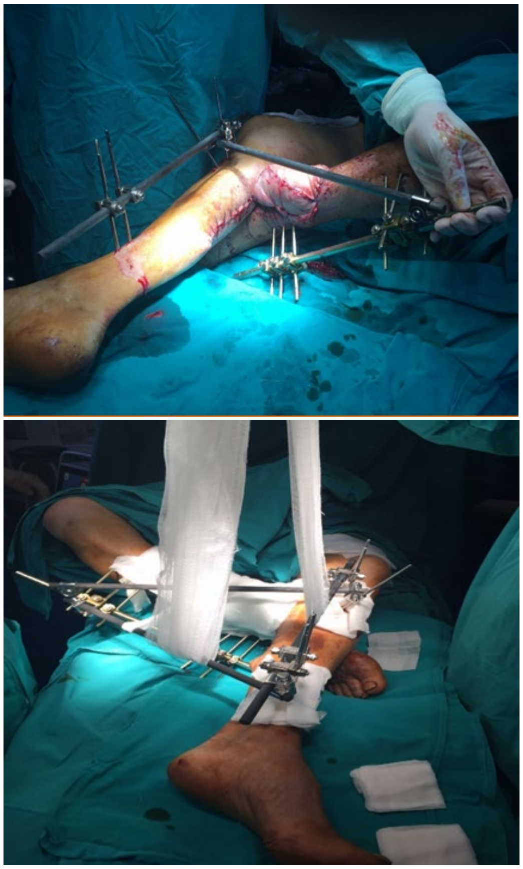

After proper preoperative markings, the flap was raised including the fascia. The inclusion of fascia in the flap makes the length-to-breadth ratio 3: 1 perfectly safe [3]. This allows much greater area of skin to be transferred with much more freedom of leg position. The donor site was covered using direct sutures and a split skin graft was also used to cover the donor muscle flap [4].

OUTCOME AND FOLLOW-UP

The release of the flap was performed at 21 days and the unaffected contralateral member was removed from fixation [5]. The patient continued treatment by monoplane external fixation for 8 weeks with progressive mobilization with the support of the unaffected limb. The patient evolution was satisfactory also making regular X-rays checks for the consolidation of the fracture.

At 4 months after the trauma, the ablation of the external fixation [6] was decided and the patient resumed progressive mobilization of both lower limbs. With the presence of the consolidated fracture, at 4 months postoperatively the patient successfully achieved a score of 62 according to Clinical rating system for the ankle and hindfoot [7].

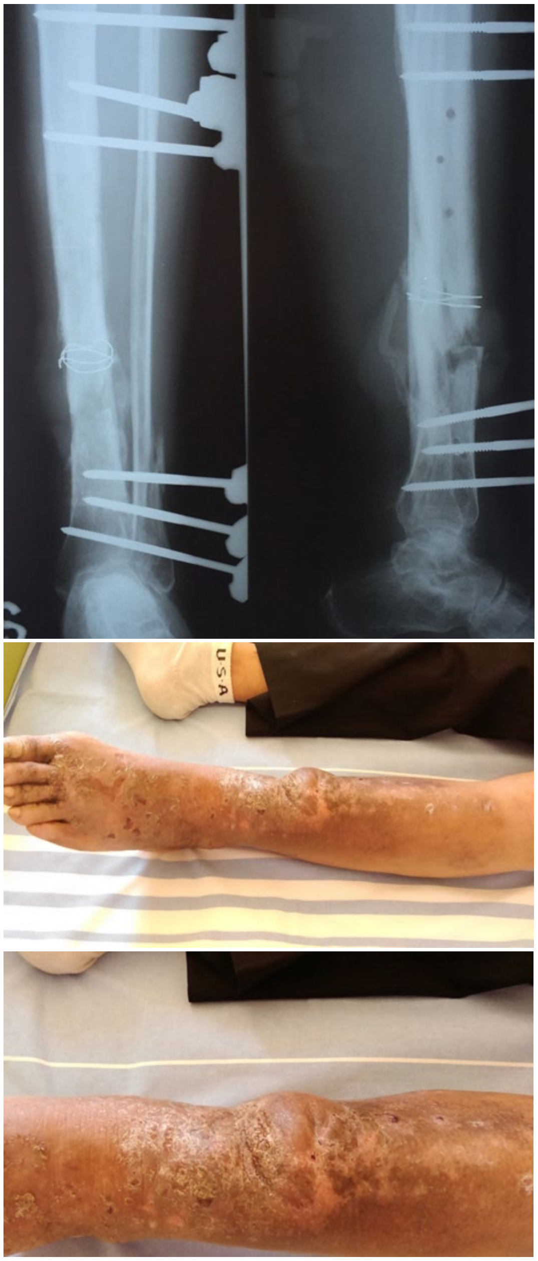

CONCLUSION

The cross-leg Filatov flap is still considered a safer and more reliable solution to free tissue transfer in certain situations of lower-limb trauma, especially open distal tibial fractures in which rotation flaps are not a viable solution.

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ORIGINAL ARTICLES

Crude venom of *Pseudocerastes persicus* snake: From the antibacterial to anticancer effects

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Abstract: *Antibiotic resistance has been reported as one of the world's most critical public health problems.*

Recent investigations have demonstrated that the venom of some species of snakes has antimicrobial and anticancer activities. The purpose of this study was to examine the antibacterial activity of Persian horned viper venom toward Escherichia coli, Bacillus subtilis, and Staphylococcus aureus bacteria and also investigate antitumor effects of concentrations of venom with higher antibacterial activity in a human hepatocellular liver carcinoma cell line (HepG2) to evaluate the potential use of its components as antimicrobial and anticancer agents. Bactericidal activity of crude venom in concentrations of 6.25-400 µg/ml was performed using MTT reduction, minimum inhibitory concentration (MIC), agar-well diffusion, and disc diffusion methods. Tetracycline (50 µg/ml) was used as a standard antibiotic. Cytotoxic effect in HepG2 cells was measured by MTT reduction assay and confirmed with neutral uptake assay following exposure of cells with different concentrations of venom (50-400 µg/ml). The apoptotic effect was investigated using the comet assay. Our findings demonstrated that venom displays higher inhibitory effects against Gram-positive bacteria as compared to Gram-negative. Furthermore, venom showed anticancer activity on the HepG2 cell line through induction of apoptosis and necrosis. The results of this study confirmed that the venom of Persian horned viper induces antibacterial and anticancer effects. These properties make the venom of this viper a potential source for isolation of effective molecule(s) having antibacterial and antitumor activity.

Keywords: *Pseudocerastes persicus, Antibacterial, Cytotoxicity, Snake venom, anti Cancer*

INTRODUCTION

In the world of pharmacology, along with the discovery and use of new antibiotics, pathogenic bacteria also have features that are gradually resistant to them. This has always been a problem for microbiologists, a constant research field for researchers to discover more effective and newer chemical antibiotics as well as antimicrobials derived from natural sources such as toxic plants and animals, including snakes (Park et al., 2012). Studies have shown that some of the active compounds in venomous animals are an important source for the separation of effective drug substances, some of which are naturally capable of destroying bacteria (Harvey and Robertson, 2004; Koh et al., 2006). Snake venom is a mixture of various biological substances, including various proteins with enzymatic and non-enzymatic properties (Chellapandi and Jebakumar,

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2008) which generally contain neurovenom, proteolytic, coagulant, phospholipase, cholinesterase, hyaluronidase, amino acid oxidase, and other enzymes (Sachidananda et al., 2007). Studies show that the effective ingredients in oral saliva or venomous snakes have long been considered by researchers and pharmacists for the treatment of some diseases, the production of various drugs, serums, and vaccines (Kuhn-Nentwig, 2003). The first reports of antibacterial activity in the venomous snakes of the family of Alpideh and Vipride in 1948 and 1968 were published (Ferreira, 1965) and Skarns also reported antimicrobial activity of some enzymes in Snake in 1970 for the first time (Aloof-Hirsch et al., 1968). Subsequently, many studies have been done about the presence of antibacterial agents in the venom of different species of snakes, which caused the isolation of some antibacterial peptides from them. Also, reports show that some of the molecules in the venoms of snakes have anti-tumor activity and prevent the growth and proliferation of cancer cells (Latifi, 2000). Chalmette's first study in 1933 examined the use of snake venom for the treatment of cancer in laboratory animals. It has introduced the results of snake venoms as an important natural source for the isolation and identification of effective molecules in the treatment of sudden illnesses like cancer in the world of pharmacology (Latifi, 2000). Pseudocerastes persicus snake is one of the most dangerous venomous snakes and a member of the Viperidae family. In addition to Iran, this Pseudocerastes persicus snake has been reported from Pakistan, Afghanistan, Oman, and Iraq, and in Iran, in the sandy, rocky and volcanic areas of Yazd, Khorasan, Kerman, Sistan and Baluchestan, Isfahan, Fars, Semnan, Central, Khuzestan, Zanjan, Tehran, Qom, and Hormozgan (Latifi, 2000). This study aimed to investigate the effect of Pseudocerastes persicus snake venom on the growth of gram-positive and negative bacteria and also the effect of probable concentrations of antibacterial effects of Pseudocerastes persicus snake venom on the growth of liver cancer cells in vitro conditions for evaluating the possibility of isolation of antibacterial and anti-cancer component.

MATERIALS AND METHODS

Venom Preparation and Protein content determination

To prepare the venom from the vein, while observing the safety considerations and placing its toxic teeth inside the glass of venom collection, and with brief pressurization to the toxic glands of the viper, the venom contained in them was injected into the glass (Lu et al., 2002). The lyophilized solution was stored and kept until it was used in experiments at -20°C. To carry out antibacterial tests, some of the venoms were pulverized in 250 µl of 50 mM sterile tris hydrochloride buffer, and a few of the venom was mixed in 150 µl water twice per sterilized distillation. The amount of protein in the homogenized toxic solution was determined using the Bradford method (Bradford 1976) at 595 nm (Bradford, 1976). To eliminate potential microbial contamination in the venom solution, the required 1% antibiotic-anti-smectic antibody (Antibiotic-Antimycotic: Invitrogen, USA) is required, and after one night at 4°C was used in experiments.

The bacteria and cell line used

Gram-positive bacteria Bacillus subtilis (nonpathogenic) (Bacillus subtilis subsp. spizizenii, ATCC 6633), gram-negative bacteria of Escherichia coli (ATCC 25922 Escherichia coli), and gram-positive strain of Staphylococcus aureus (pathogenic)(ATCC 25923) from the collection center of Persian Type Culture Collection (PTCC) and liver cancer cells (HepG2) were developed and used from the cell bank of Pasteur Institute of Tehran.

Antibacterial properties of crude venom

Culture media and antibiotics: For a culture of bacteria used from liquid and solid Mueller Hinton (MH) and for comparing the toxic effect of venom with a standard antibiotic used from tetracycline (Sigma, USA) at a concentration of 50 µg/ml.

MTT assay

MTT test is a colorimetric method based on the regeneration and breakdown of tetrazolium yellow crystals by the enzyme succinate dehydrogenase present in the cytoplasm and eventually the formation of insoluble blue crystals. These crystals are dissolved by adding DMSO (Wang et al., 2010). Test microorganisms were grown in MH broth for 5 h (exponential phase) and adjusted to 0.5 McFarland turbidity standard (A600 = 1.0), corresponding to 1.5×106 cfu/ml (Yalcın et al., 2014), 5µl was added in sterile plate 96-wells. Also, the final volume of each well was measured using a liquid culture medium (MH Broth) to 100µl using serial dilution. The bacteria were contacted at concentrations of 6.25, 12.5, 25, 50, 100, 200, and 400µg/ml of crude venom. In this experiment, tetracycline (50µg/ml) as a positive control, a culture medium containing bacteria as negative control, and a non-bacterial culture medium was used as blank. The plate was incubated for 23 hours at 37°C. Then, 5µl MTT (concentration 5µg/ml) was added to all wells and placed in dark conditions at 37°C for one hour. Then, 100µl of DMSO was added to each well. After 2 hours of incubation in dark conditions, the optical absorption of wells at 595 nm was measured using a plate reader (Biotek, USA). The above experiment was performed 3 times and at each time 3 wells (3 repetitions) were considered for each concentration. The percentage of bacterial survival after contact with different concentrations of the venom was calculated using the following formula (Yalcın et al., 2014).

Bacterial vital percent:

Viable Bacterial = (a/b) ×100
a = Optical Absorption Sample - Blank Optical Absorption
b = Negative optical absorption - Blank optical absorption

Minimum Inhibitory Concentration Test (MIC)

The steps of performing the minimum inhibitory test on plate 96-well were similar to the MTT assay but after placing the bacterium in a culture medium containing different concentrations of venom and incubating them for 24 hours at 37°C without adding any marker. Also, the absorbance of wells at 605 nm was measured using a plate reader (Biotek, USA). This experiment was repeated 3 times and at each concentration 3 wells (3 repetitions) were considered for each concentration. The inhibitory percentages of the effect of venom and standard antibiotics on the tested bacteria were calculated using the following formula (Shebl et al., 2012).

Percentage of inhibition of bacteria = [1-(a/b)]×100
a = Optical Absorption Sample - Blank Optical Absorption
b = Optical Absorption Control - Blank Optical Absorption

Disc diffusion assay

This experiment was carried out according to Bauer et al (Bauer et al., 1966).

The bacteria were first cultured in a culture medium Mueller–Hinton broth and incubated for 24 to 18 h at 37°C. A solid culture medium with a thickness of 4 mm was prepared in glass plates. 200µl of the 0.5 MacFarland suspension was placed on a plate surface and a cotton seedling was cultured using a sterile loop. Using sterile pin disks (prepared from antibacterial medicine) containing 40µl of venom in the concentration of 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml on the surface plate was placed. In this experiment, 50µg of tetracycline antibody was used as a positive control for 50 µM tetracycline, and 50 mM Tris hydrochloride was used as a negative control. Plates were incubated for 24-18 hours at 37°C, and then an inhibition zone was determined and its size was determined. This experiment was performed 3 times for each bacterial strain.

Well diffusion assay

This method is similar to the disk diffusion method, except that in this test, the venom solution was added to the well instead of being placed on the disk (Torres et al., 2010). In summary, after the cultivation of grass, using the end of the pasteurized pipette, the wells were transferred to the medium and cut into pieces of gel, and 20µl of liquid agar medium was used to block the bottom of the well and prevent leakage and solution leakage. The venom was poured into the well under the medium. After pouring 40µl of Crude venom in concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml, the plate was incubated for 24 to 18 hours in an incubator at 37°C. After the mentioned time, the presence of a halo around the wells was investigated and its diameter was determined. This experiment was performed 3 times for each bacterial strain in two replicates.

Evaluation of anti-cancer properties of crude venom

Cell culture

Liver cancer cell line (HepG2) was cultured using DMEM-F12 (Gibco, USA) containing 10% Fetal Bovine Serum (Gibco, USA). These cells were cultured in flasks of 25-50 ml cubic meters and stored at 37°C and 5% carbon dioxide. Replacing every 2-3 days of the culture medium and after providing the cell population required for each test, using a Trypsin-EDTA prepared from Sigma-Aldrich, the USA from a flask and after counting using a Neobar lam were placed.

MTT reduction assay

This experiment was carried out according to the method performed by Zargan et al. (Zargan et al., 2011). To perform this test, 3×104 cells/well were plated in 96 wells containing 100µl of non-serum culture media. After overnight incubation at 5% CO_2, moisture content of about 80% and temperature of 37°C were removed outside the culture medium and the cancerous cell was exposed to a new culture medium (lacking serum) containing different concentrations of venom (50, 100, 200 and 400µg/ml). After 24 hours, 5µl of MTT solution (concentration 5mg/ml) was added to each well. After the formation of crystalline (formalin) in living cells, the culture medium of each well was removed and after washing in the well with PBS, 100µl of DMSO was added. After 4 to 3 hours of incubation in dark conditions and dissolving purple crystals in DMSO, the optical absorption of wells at 570 nm was measured using a plate reader (Biotek, USA). In this essay, the culture media was used as a blank and control medium containing the cells as control. This experiment was repeated 3 times, and 3 wells (3 repetitions) were considered for each concentration. The percent survival of the cell after contact with different concentrations of the venom was calculated using the following formula (Shebl et al., 2012).

% Viable cell = (a/b) × 100
a = the absorbance of treated cells - the absorbance of the blank

b = the absorbance of the control - the absorbance of the blank

Cytotoxicity study by Neutral Red Uptake Assay

The steps of performing a neutral color red color test (Waheed et al., 2013) were similar to the MTT assay, but after incubating the cell with a toxic solution for 24 hours, a microplate of neutral red solution (5 mg/ml) instead of MTT was added to each well. After binding of neutral red matter to the lysosome surface of the living cell and the formation of red crystals in the living cytoplasm, the culture medium containing the neutral red of each well washed and after washing twice with each PBS, 100 µl of fixative buffer (37% formaldehyde, Calcium chloride (10%) was added. After one minute, the buffer was removed and 100µl of solvent buffer (0.5% acetic acid) was added. The plate was measured on a shaker for twenty minutes under microwave conditions and incubated at lab temperature and absorbed by wells at a wavelength of 540 nm using a plate reader (Biotek, USA). This experiment was repeated 3 times, and 3 wells (3 repetitions) were considered for each concentration at each concentration. The mortality rate due to different concentrations of venom on cell growth was calculated using the following formula.

Percentage of cell death = [1-(a/b)] ×100

a = the absorbance of treated cells - the absorbance of the blank

b = the absorbance of the control - the absorbance of the blank

Investigation of induction of apoptosis by venom using comet assay test

This test is one of the best methods for checking the damaged DNA in the cell (Sajad et al., 2013). To conduct this experiment, at first, in each well of the plate, 24 sterile wells containing 300µl of serum medium, cultured 12×104 cells. The plate was incubated overnight under conditions of 5% CO2, humidity 80%, and temperature 37 ° C. In this assay, the culture media was used as a blank and control medium containing the cells as control. After that, the initial culture medium of each well, evacuation, and cells was exposed to 300µl of a new culture medium (without serum) containing different concentrations of venom (50, 100, 200, and 400µg/ml).

After incubating the cell for 24 hours at 37 ° C, 80% moisture content, and 5% CO2, cells of each well were isolated using trypsin. Microtubes containing cells were evacuated for 5 minutes at 4 ° C with 1500 rpm of the centrifuge and aqueous solution. 400µl of PBS (pH = 7.4) was added to each of the tubes to wash the cells, and after centrifugation, the supernatant was evacuated. Then, 200µl of PBS was added to the tubes, and cells were separated from each other by the separate sampler and insulin syringe. Also, the slides needed for testing were coated with agarose with normal melting point (NMA% 1), test cell suspension and control with low melting point agarose (LMA% 1), one to two on the slides a flat layer was placed. To lyse the cell and nucleus membrane, all slides are placed in the refrigerator in a refrigerator buffer (NaCl 2.5M, EDTA 100mM, Tris 10mM, NaOH 0.2M, Triton X-100% 1 pH = 10) for 16-18 hours. After removing the buffer, the slides were washed for 20 minutes and 2 times with electrophoresis buffer (3M NaOH, EDTA 1mM, and pH>13), and then to open the DNA for 40 minutes in a cold electrophoresis buffer temperatures were maintained at 4°C. The slides were then extracted from the solution and placed in a buffer containing an electrophoresis tank and electrophoresed at 45°C for 45 minutes at a voltage of 25 and 300 mA. In the next step, to undo the playing environment, the slides were placed in a neutralizing buffer (Tris% 4 M, pH = 7.5) for 10 minutes. To staining cells, 100 µl of ethidium bromide solution (concentration 20 µg/ml) was added to each slide, and after 10 minutes incubated at the laboratory for 10 minutes, they were distilled twice with water. DNA of each slide cell was studied by fluorescence microscopy. For each sample, different slide positions and at least an image of hundreds of cells' DNA were prepared and the results were analyzed statistically.

Statistical analysis

The results of each test were reported as Mean±SD and the data were analyzed using software (GraphPad InStat). Different concentrations of venom were compared to the control group and compared to each other by one-way ANOVA and Tukey tests. P<0.05 was considered significant in all tests. Draw diagrams in the Microsoft Excel software (2013 version).

RESULTS

Evaluation of the Anti-Bacterial Properties of Crude venom

Test results MTT assay

The results showed that venom only at 400µg/ml concentration significantly inhibited the growth of Escherichia coli bacteria compared to the negative control (normal culture medium). The critical percentage of this bacterium in concentrations of 6.25-50 µg/ml was 100 and in 100, 200, 400 µg/ml concentrations respectively were 95.79, 96.29, and 83 (Figure 1).

In the case of the Bacillus subtilis strain, Crude venom in the concentration of 12.5 to 400µg/ml produced a significant inhibitory effect on bacterial growth compared to the negative control.

The percentage of vital bacteria in different concentrations of venom 6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml respectively were 89.62, 62. 49, 13.73, 6.56, 2.14, 1.63 and 4.6.

Figure 1: Antibacterial effect of different concentrations of Pseudocerastes persicus snake crude venom on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus using MTT assay. The effect of concentrations was evaluated in comparison with the negative control group.

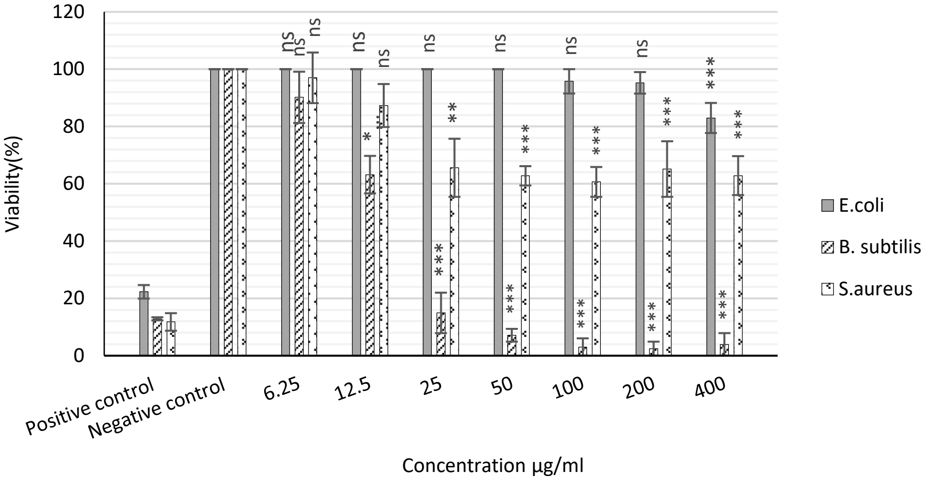

(: p<0. 05), (**: p<0. 01), (***: p<0.001), (ns: non-significant).*

Venom hurt the growth of this bacterium at a concentration of 6.25µg/ml, but this effect was not significant in comparison with negative control. In the range of 25-400, the inhibitory effect of the venom was greater than the positive control (tetracycline). Statistical analysis showed that the effect of venom in a concentration of 50-400 µg/ml was significant for this bacterium but it was not significant compared to each other. According to the results, IC50 of the venom for Bacillus subtilis was determined to be 15.32µg/ml. The results also showed that the vital percentage of Staphylococcus aureus bacteria in the concentration of 6.25, 12.5, 25, 50, 100, 200, and 400µg/ml of crude venom were 95.11, 85.29, 72.48, 62.23, 59.98, 63.31, and 63.96. Crude venom at a concentration of 25-400 µg/ml produced a significant inhibitory effect on the bacteria compared with the negative control. Venom in the concentration of 12.5µg/ml hurt the growth of this bacterium, but this effect was not significant compared to negative control. Statistical analysis showed that the effect of venom in concentrations of 25-400 micrograms per ml was significantly less than the negative control, but compared with each other, it is not significant (Figure 1).

Minimum Inhibitory Concentration Test results

Pseudocerastes persicus snake venom at concentrations of 6.25-100 did not inhibit the growth of Escherichia coli, but induced inhibitory effects at concentrations of 200 and 400 µg/ml, respectively were 12.15 % and 24.99 % which, compared to the intolerance of tetracycline, and the difference in their effect is significant (Figure 2).

The inhibitory effect of venom on Bacillus subtilis bacteria in the concentrations of 6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml was 23.22, 58.92, 92.80, 99.48, 99.65, 99.71, and 99.3, respectively. According to the results, the IC50 of the venom for Bacillus subtilis was determined by 10.81µg/ml. The statistical analysis showed that Crude venom at a concentration of 6.25-400 µg/ml had a significant inhibitory effect on Bacillus subtilis bacteria growth compared to the negative control. The inhibitory effect of venom was 25-400 µg/ml in comparison to the negative control, but compared with each other and in comparison with standard antibiotics was not significant (Figure 2).

Crude venom has an inhibitory effect on the growth of Staphylococcus aureus, and the inhibitory effect induced in it was 6.25, 12.5, 25, 50, 100, 200, and 400 were 13.95, 25.28, 29.13, 27.30, 30.52, 35.91, and 31.69 µg/ml, respectively. Statistical analysis showed that the toxic venom has a significant inhibitory effect on the growth of this bacterium at concentrations of 6.25-400 µg/ml, compared to the negative control. The losses caused by the effect of venom in different concentrations ranged from 25-400 µg/ml compared to the negative control but were not significant in comparison with each other (Figure 2).

Figure 2: Antibacterial effect of various concentrations of Pseudocerastes persicus snake crude venom on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus using MIC assay. The effect of concentrations was evaluated in comparison with the negative control group.

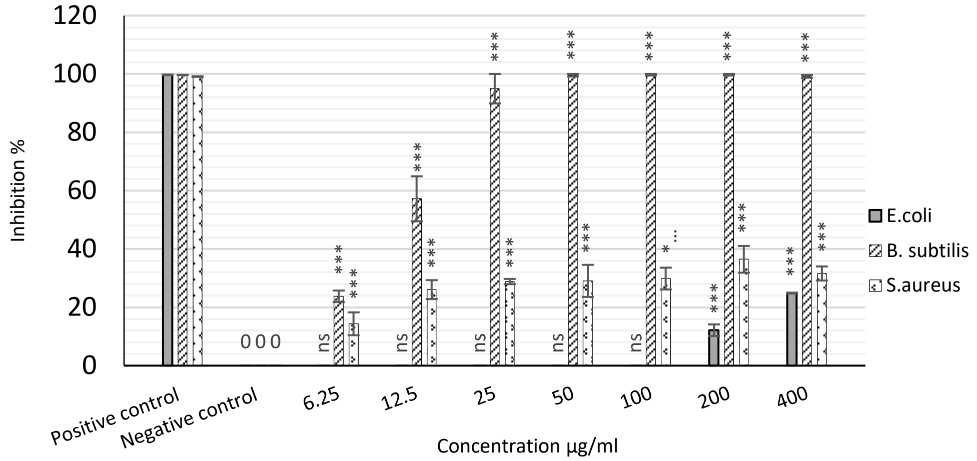

(: p<0. 05), (**: p<0. 01), (***: p<0.001), (ns: non-significant)*

Results of diffusion tests on disk and wells

The results of the effect of various concentrations of venom in the diffusion test on the disk and wells indicate that the results of these two tests, especially at low concentrations of venom, are not repeatable. However, according to the results of this study, it was found that the venom in these concentrations did not inhibit the growth of Escherichia coli (Figure 3).

Figure 3: Antibacterial effect of various concentrations of Pseudocerastes persicus snake crude venom on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus using MIC assay. The effect of concentrations was evaluated in comparison with the negative control group.

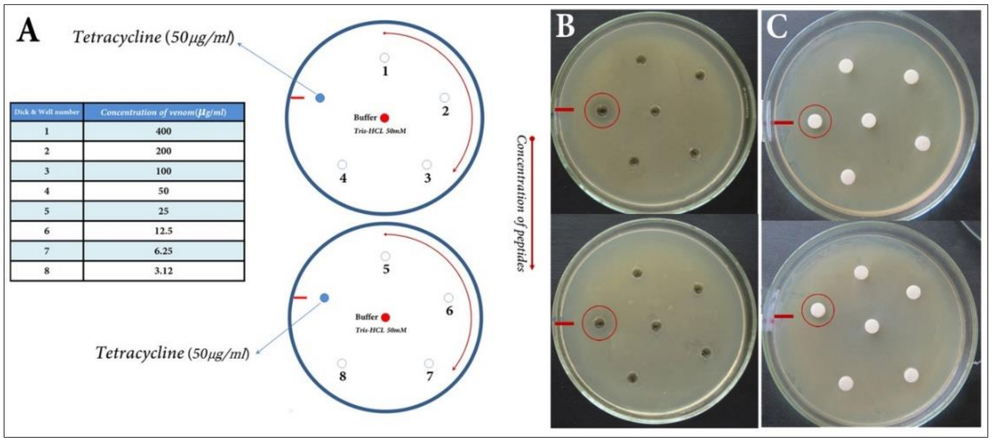

The results of these experiments on Bacillus subtilis bacteria showed that the venom had an inhibitory effect on bacterial growth at a concentration of 50-400 µg/ml, but did not have an inhibitory effect on the growth of the bacteria in the concentration of 6.5- 25 µg/ml (Figure 4).

Also, venom only prevented the growth of Staphylococcus aureus at concentrations of 200 and 400 µg/ml and did not inhibit the effects of lower concentrations (Figure 5).

Evaluation of anti-cancer properties of Crude venom

Results of cytotoxicity testing by MTT method

Venom at concentrations of 50, 100, 200, and 400 µg/ml causes high mortality in liver cancer cells, respectively, in which the vital percent of the cell at the above concentrations were 14.12, 10.97, 10.43, and 9.96% has dropped. The cells losses caused by the different concentrations of venom are not significant (Figure 6).

Cytotoxicity test results with neutral red colorimetric method

The mortality rate of liver cancer cells in the concentration of 50, 100, 200, and 400 µg/ml of crude venom was 60.13, 72.58, 81.61, and 77.48 %, respectively. Venom has a significant inhibitory effect at concentrations of 100-400 µg/ml in comparison with the control (containing cell and culture media), but the mortality rate is compared between all concentrations is not significant (Figure 7).

Figure 4: Antimicrobial effect of crude venom on diffusion and disk diffusion methods against growth Bacillus subtilis bacteria. A) map and concentrations used; b) well distribution; c) disk diffusion test.

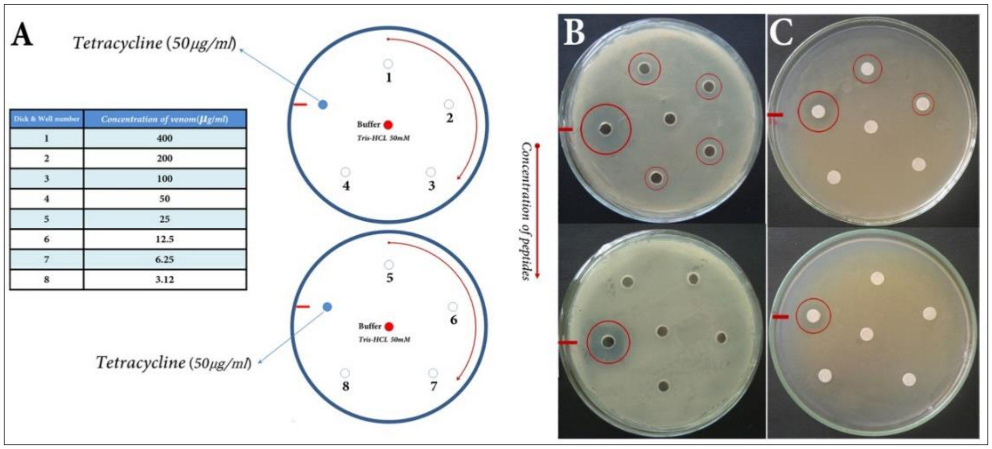
A
Tetracycline (50µg/ml)
Dick & Well number
Concentration of venom(µg/ml)
1 400
2 200
3 100
4 50
5 25
6 12.5
7 6.25
8 3.12
Buffer
Tris-HCL 50mM
1
2
3
4
5
6
7
8
Tetracycline (50µg/ml)
Concentration of peptides
B
C

Figure 5: Antibacterial effect of crude venom on diffusion and disk diffusion methods against growth Staphylococcus aureus bacteria. A) map and concentrations used; b) well distribution; c) disk diffusion test.

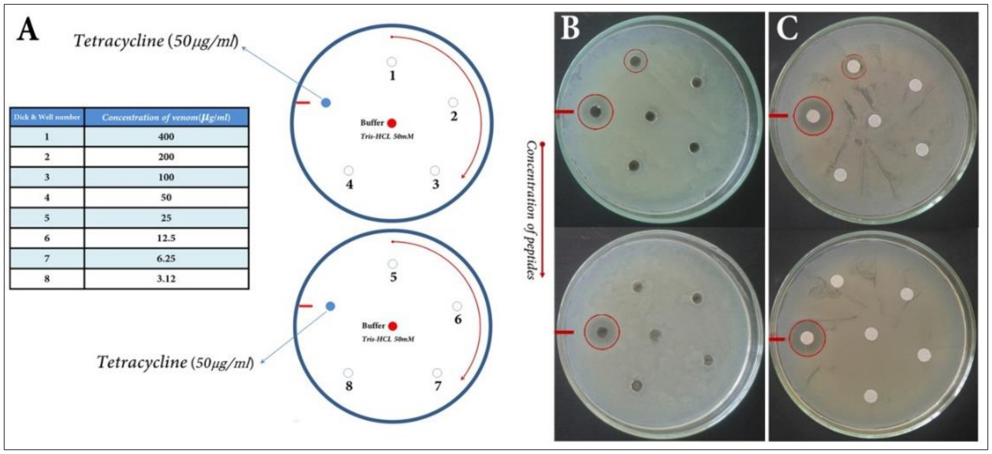
A
Tetracycline (50µg/ml)
Dick & Well number
Concentration of venom(µg/ml)
1 400
2 200
3 100
4 50
5 25
6 12.5
7 6.25
8 3.12
Buffer
Tris-HCL 50mM
1
2
3
4
5
6
7
8
Tetracycline (50µg/ml)
Concentration of peptides
B
C

Figure 6: Percentage of vital liver cancer cells after 24-hour contact with different concentrations of the crude venom according to the MTT assay method. The effect of concentrations was evaluated in comparison with the control group. (***: p<0.001).

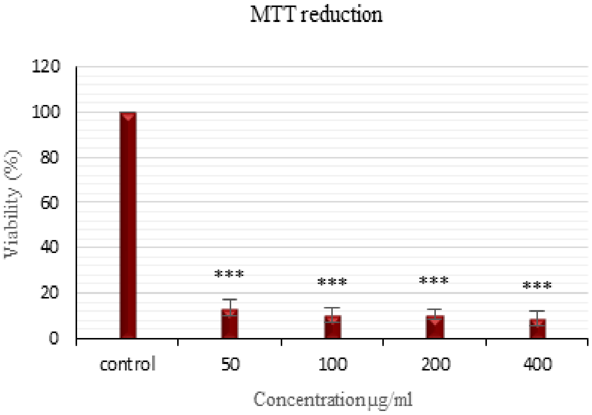

Figure 7: Percentage of mortality of liver cancer cells after exposure to different concentrations of crude venom Pseudocerastes persicus snake based on Neutral Red Color. The mortality due to the effect of each concentration is compared with that of the control group. (***: p<0.001)

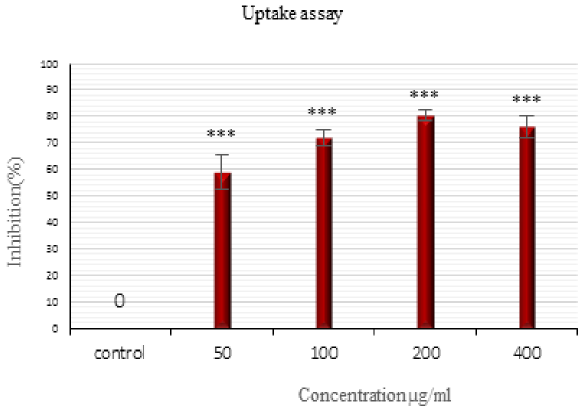

Investigation of venom apoptosis induction using comet assay test

The results of this study showed that Pseudocerastes persicus snake venom induces apoptosis and causes mortality in liver cancer cells. Venom at concentrations of 50, 100, 200, and 400 were 36.6, 43.28, 51.5, and 47.13%, respectively, induced apoptosis in the cell. The morphological study of venomous cells also showed that the venom of this aphid, in addition to apoptosis, induces necrosis in liver cancer cells, and this effect has an upward trend of more than 200 μg/ml in comparison with lower concentrations. These results indicate that mortality in cancer cells is due to the common effects of apoptosis and venous necrosis (Figure 8).

Figure 8: Rate of apoptosis induced by Pseudocerastes persicus snake crude venom in liver cancer cells based on the alkaline test.
A: The morphological changes in DNA from the concentration of each concentration compared to the control group.
B: Morphological changes in DNA of proximal cells with Pseudocerastes persicus snake crude venom compared to control cells
[1-3]: DNA image of the normal cell(×40), [4-6]: DNA image of apoptosis cell(×40).

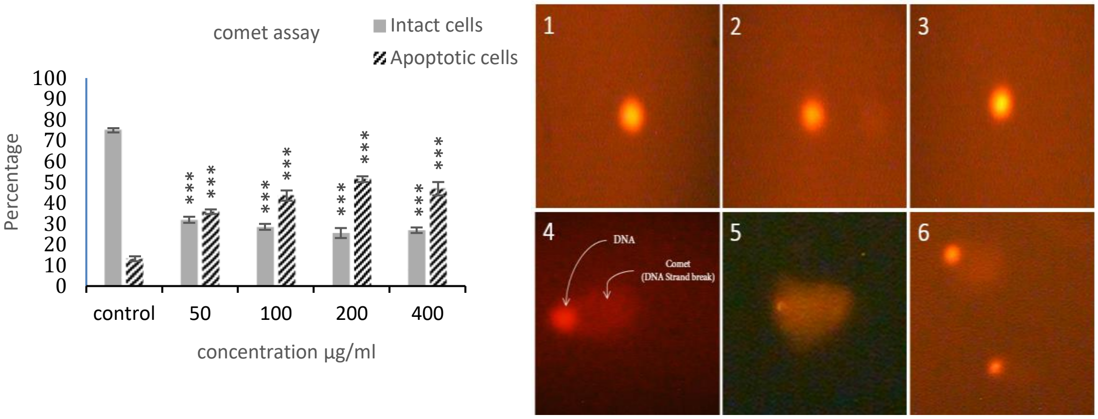

DISCUSSION AND CONCLUSIONS

According to the World Health Organization, 40% of the 50 million annual human mortality is due to bacterial contamination and microbial diseases (Ivnitski et al., 1999). Also, bacterial infections caused by resistant strains and problems due to the lack of treatment of people with them have become an important health problem. This has led researchers to investigate more in living organisms, including various venomous snakes, to discover more effective and new antibiotics. According to studies conducted in recent years, venomous snakes are a source of unknown molecules of active substances (25) that can be considered as an appropriate candidate for the production of antimicrobial and anticancer drugs. In this study, for the first time, the antibacterial and cytotoxic effects of Pseudocerastes persicus snake were studied in vitro conditions to evaluate the possibility of isolating molecules with antibacterial and toxic effects for cancer cells. MTT reduction, MIC assay, Disc diffusion assay, and Well diffusion test were used to study the antibacterial effects of crude venom. The results obtained from MTT reduction and MIC tests showed that Pseudocerastes persicus snake venom has an inhibitory effect on Escherichia coli bacteria as a representative of gram-negative bacteria only at the highest concentration (400 µg/ml). However, bacteria Bacillus subtilis and Staphylococcus aureus have been prevented from bacterial growth at concentrations of 12.5-400 and 25-400 µg/ml, respectively. The reason for the difference in the results of MTT and MIC is due to the difference in the calculation of the inhibitory effect of the venom in two methods, in the MIC method, the opacity is due to the presence of bacteria, which includes dead and living bacteria, is measured at a wavelength of 605 nm. While in the MTT method, the optical absorption of formazone particles formed in the cytoplasm of the living bacteria is determined after dissolution in DMSO at 570 nm. MTT testing seems to provide more accurate results than the MIC method. Also, the results of the study showed the antibacterial effects of crude venom using two methods of diffusion in wells and discs. In this study, snake venom has no inhibitory effect on gram-negative bacteria but has an antibacterial effect on two-gram positive bacteria. The results showed that these two methods qualitatively confirm the effects of MTT and MIC, but did not quantitatively produce repeatable results. One of the reasons for this issue, it seems, is that the proteins and toxic peptides are deposited at the bottom of the well, and the walls and around the wells act as a filter and penetrate the entire contents of the venom into the well prevents solid culture. In the case of a disk, it is also possible that the disk itself acts as a filter and prevents the outflow and full penetration of the components of the venom into the medium. The rapid absorption of water in the upper surface of the medium using a paper disk and the lack of complete absorption of toxic compounds that allow heterogeneous release in the medium is another disadvantage of this method. In this study, it was determined that Pseudocerastes persicus snake crude venom is more effective than gram-positive bacteria. This selective antibacterial activity may be due to several factors such as the difference between bacterial species such as density and lipopolysaccharide structure present in the wall of gram-negative bacteria or fat composition in the cytoplasmic membrane and electrostatic potential across the membrane in gram-positive bacteria Be (San et al., 2010). The results of studies by Husniye et al. (2012), Shebell et al. (2012), Stabley et al. (2004), Steels et al. (1991), and similar studies show that venomous affect gram-positive bacteria (Stiles et al., 1991; Lu et al., 2002; Yalcın et al., 2014; de Lima et al., 2005; Stábeli et al., 2004). Also, the results of the antibacterial effect of Jafri's venom (Jami et al., 2011) showed that snake venom has a better effect on Staphylococcus aureus bacteria than Escherichia coli bacteria. The results obtained in this study are consistent with previously reported results regarding the antibacterial effects of snake venom. On the other hand, the inhibitory effect of Pseudocerastes persicus snake crude venom in the highest concentrations studied in this study on E. coli is an effective component of this bacterium at very low concentrations in venom. The results of studies by Chalapandi et al. (2008), Bostley et al. (2008), Starker and Trierworth in 1986, and similar studies have reported the inhibitory effects of venomous organisms on gram-negative bacteria (Chellapandi and Jebakumar, 2008; Stiles et al., 1991; Shebl et al., 2012; Bustillo et al., 2008). Snakes venom are mainly composed of proteins and peptides that have diverse biological activities. Some of these molecules have been shown to have inhibitory effects on cancer cell growth in clinical trials and may find their way toward developing anticancer drugs in the future (Jain and Kumar, 2012). In this research, the effects of toxicity of venomous snake, which has antimicrobial properties induced by the use of MTT assay, Neutral red uptake assay, and Single cell gel electrophoresis (Comet assay) on liver cancer cells (HepG2 cell line) was taken into consideration in vitro conditions. Reports on the presence of cytotoxic effects in the venom snake have led to the use of HepG2 cells in this study (Latifi, 2000). The results of the MTT assay and uptake assay showed that crude venom at concentrations of 50, 100, 200, and 400 µg/ml has cytotoxic effects and causes the death of liver cancer cells. These results are consistent with findings from Bozemans et al. (2005), Yang et al. (2006), Depeca et al. (2012), Sang et al. (2012), to induce cytotoxic properties by venoms of a snake (Jain and Kumar, 2012; Bosmans et al., 2005; Cancer Control Office, 2005; Song et al., 2012; Yang et al., 2006). As well as the results of Shebel, et al suggesting that mortality in the cell would be increased by increasing the concentration of venom (Shebl et al., 2012). To investigate the possibility of inducing cell death through crude venom through apoptosis in HepG2 cells, this study was conducted using Comet assay. The results showed that crude venom at concentrations of 50, 100, 200, and 400 µg/ml caused apoptosis in cancer cells the liver causes it to die. It was also found that with an increase in concentrations of 50 to 200 µg/ml of crude venom, apoptosis has a tendency to increase, which is consistent with previous reports (Sajad et al., 2013). Generally, the results of this study showed that the crude venom of snakes contains molecules that can have anti-bacterial and anti-cancer activity. These results suggest that the venom of a snake is a suitable candidate for the isolation, purification, and introduction of valuable drug molecules to combat antibiotic-resistant bacteria as well as anti-cancer agents.

Acknowledgment

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REVIEW ARTICLE

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Management of Helicobacter pylori infection – new insights

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Abstract: *Introduction: Our objective is to review current international guidelines for Helicobacter Pylori treatment and our department`s experience in this field.*

Materials and methods: Helicobacter pylori is a Gram-negative, microaerophilic bacterium that can be found mainly in the gastric mucus or on the inner surface of the gastric epithelium, infecting up to 50% of the population. Colonization with this bacterium is not a disease in itself, but can cause chronic gastritis, peptic ulcer, gastric cancer and MALToma. Because of this, infection with H. pylori continues to be a major healthcare burden, especially in less-developed countries.

A multitude of non-invasive tests are available for the diagnosis of Helicobacter pylori infection (blood antibody, stool antigen or urea breath test), but the most reliable method of diagnosis is histological examination from two sites after endoscopic biopsy, combined with either a microbial culture or rapid urease test.

Treatment of Helicobacter pylori infection is becoming a challenge, as eradication following standard triple therapy is decreasing worldwide due to increased bacterial resistance against antibiotics, which has led to the development of newer therapies such as the sequential treatment in which a PPI and amoxicillin is given for 5 days followed by a PPI, clarithromycin and metronidazole for another 5 days, or the quadruple therapy based on a PPI, bismuth subcitrate, metronidazole and tetracycline for 10 days.

Results and conclusion: H. pylori infection remains one of the most challenging infectious diseases, causing high morbidity and mortality, mainly because none of the actual antibiotic therapies can provide successful eradication.

Keywords: Helicobacter pylori, ulcer, adenocarcinoma, antibiotics, PPI

INTRODUCTION

Helicobacter pylori (Hp) is a helix shaped, Gram-negative, microaerophilic bacterium that can be found mainly in the gastric mucus or on the inner surface of the gastric epithelium[1]. Hp infection is the most common chronic infection in humans, but colonization with this bacterium is not a disease in itself. Although most infected individuals remain asymptomatic for life, Hp is responsible for a number of pathological manifestations including chronic gastritis, gastric or duodenal ulcer, adenocarcinoma

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of the stomach or gastric mucosa–associated lymphoid tissue (MALT) lymphoma[2].

THE DISCOVERY OF HELICOBACTER PYLORI

The Helicobacter pylori bacterium was discovered by two Australian researchers by the name of Barry Marshall and Robin Warren, who also deciphered its role in gastritis and peptic ulcer disease (PUD)[3]. Their discovery was published in 1982, at a time when it was a long-standing belief that stress and lifestyle factors were the major causes of PUD. The scientific community of the time met their findings with skepticism and a lot of criticism, refusing to belief that any life-form can survive in the acid medium of the stomach. This is why it took a very long time and effort for their discovery to become widely accepted[4].

Marshall`s 1985 “self-help” experiment rebutted the “stress and life-factors” dogma. He first underwent gastric biopsy to show the absence of the bacterium, then, to the horror of his assistant, ingested a turbid, foul-tasting solution of Hp. Soon he developed a number of symptoms including nausea and vomiting and an endoscopy with biopsy was performed, which revealed signs of gastritis and the presence of Helicobacter Pylori[3].

EPIDEMIOLOGY

Transmission of Hp is believed to be by gastro-oral or fecal-oral routes as the bacterium can be cultured from vomitus or diarrhea stools. Therefore, the overall prevalence of infection can be influenced by lack of proper sanitation and basic hygiene, of safe drinking water, as well as overcrowding, making it a public-health issue in developing countries[1].

One of the most important risk factors is childhood socioeconomic status, meaning that infection is acquired at an early age, especially in developing countries. It is common for spontaneous clearance to occur during early childhood, but the chance of reinfection is greater in developing countries compared to developed ones – where it is estimated to occur in less than 0.5% of cases per year[5].

Due to these factors, prevalence of infection can reach up to 80 % by the age of 20-30 in developing areas, whereas in developed countries prevalence is less than 20 % in individuals younger than 30 years, but can reach up to 40-50 % in those 60 of age or older[2].

PATHOPHYSIOLOGY

Clinical outcome of Hp infection is dependent on sophisticated interactions between the bacterial, host and environmental factors. In order to promote chronic infection, H. pylori first has to survive in the harsh acidic environment of the gastric medium[6]. One of the most important bacterium survival technique is the “acid acclimation mechanism” that adjusts periplasmic pH by regulating activity of urease. Another factor on which successful colonizationof the gastric epithelium depends is bacterial motility provided by the presence of 4 to 6 functional unipolar flagella. Recent studies show that peptidoglycan-degrading enzymes are necesary for the proper assembly ofthese flagella[7]. After colonization, adherence to gastric epithelial cells is necessary and this is done thanks to a variety of outer membrane proteins (OMPs), several of which can serve as adhesins, the most important being BabA and SabA. H. pylori employs genetic diversification to adapt to the host immune response and promote persistent infection[8].

This pathogen possesses various virulence factors known to be significant in the induction of disease during infection. One of the most studied effector molecule is cytotoxin-associated gene A (CagA) which is injected into the host cell upon contact via the cag pathogenicity island (cagPAI)-encoded typeIV secretion system. Once intracellular, CagA localizes on the inner surface of the celular membrane and is subjected to a tyrosine-phosphorylation process by Src family kinases. The phosphorylated CagA subsequently induces a signaling cascade, causing proinflammatory responses in epithelial cells[9]. While the literature is contradictory, some studies, including those of Kang et al. and Papadakos et al., sugest that inflamation is induced via the nuclear factor (NF)-kB signaling pathway and subsequent interleukin (IL)-8 secretion[7]. Taking this into consideration, and also the fact that CagA translocation and phosphorylation are mediated by cholesterol-rich microdomains of the plasma membrane, Lin et al. found that methylantcinate B, a triterpenoid extracted from the Antrodia camphorata mushroom, attenuates CagA translocation and phosphorylation and inhibits CagA functions, including NF-kB pathway activation and IL-8 secretion; but further in vitro research is needed to assess the possibility of a new, antibiotic-free regimen[9].

DIAGNOSIS

The diagnostic procedure of Hp infection can be difficult due to the fact that up to 80-85% of infected individuals are asymptomatic, while the rest develop a series of non-specific symptoms, such as nausea, vomiting, abdominal pain, heartburn, halitosis or diarrhea[1]. This is why specific indications for testing were stipulated. These include active or documented history of peptic-ulcer disease, early-stage gastric MALToma, early gastric cancer, or uninvestigated dyspepsia in high prevalence areas[4]. Testing for the primary prevention of gastric cancer can be performed in individuals with high risk, such as those with family history of gastric cancer, or first-generation immigrants from a region with high-incidence for this malignancy. Some physicians recommend testing prior to starting long-term non steroidal anti-inflammatory drugs or proton pump inhibitors (PPI)[10].

There is also clear evidence that Hp can be involved in the pathogenesis of some extra digestive diseases, with a clear indication to test for infection in individuals with unexplained iron-deficiency anaemia, idiopathic thrombocytopenic purpura (ITP) and vitamin B12 deficiency[11]. Other studies have tried to find a link between Hp infection and some cardiovascular, lung, hepatobiliary or neurological disorders, but failed to produce an unequivocal causative association thus far[12].

Several methods are currently available for detecting Hp infection. Unfortunately, there is no gold-standard diagnostic test due to the fact that every method has it`s limitations and disadvantages [13] (as seen in table 1).

Table 1: Diagnostic tests for Helicobacter pylori infection [14]

Endoscopic Testing	Advantages	Disadvantages
A) Histology	Excellent sensitivity and specificity	Expensive. Requires infrastructure and trained personnel
B) Rapid urease testing	Inexpensive. Provides rapid results. Excellent specificity and very good sensitivity.	Posttreatment sensitivity significantly reduced
C) Culture	Excellent specificity. Allows antibiotic sensitivities determination	Expensive. Difficult to perform. Not widely available.
D) Polymerase chain reaction (PCR)	Excellent sensitivity and specificity. Allows antibiotic sensitivities determination	Not standardized across laboratories. Not widely available
Nonendoscopic Testing	**Advantages**	**Disadvantages**
A) Antibody testing (quantitative and/or qualitative)	Inexpensive. Widely available Very good NPV	PPV dependent upon background Hp prevalence. Not recommended after Hp therapy
B) Urea breath tests (^{13}C and/or ^{14}C)	Identifies active Hp infection. Excellent PPV and NPV regardless of Hp prevalence. Useful before and after Hp therapy	Reimbursement and availability remain inconsistent
C) Fecal antigen test	Identifies active Hp infection. Excellent NPV and PPV, regardless of Hp prevalence. Useful before and after Hp therapy	Polyclonal test less well validated compared to the UBT in posttreatment settings. Monoclonal test appears reliable before and after Hp therapy.

The sensitivity of all tests (endoscopic and nonendoscopic) that identify active Hpi infection is reduced by the recent use of PPIs, bismuth, or antibiotics
PPI = proton pump inhibitor; NPV = negative predictive value; PPV = positive predictive value; UBT = urea breath test.

Table 2: Possible reasons for Helicobacter pylori treatment failure.

1	resistance acquired by mutations
2	the low gastric pH limits antibiotic efficacy
3	antibiotic concentration can be insufficient if there is a high bacterial load
4	existence of sanctuaries to which antibiotics do not diffuse
5	existence of viable bacteria in dormant forms not accessible to antibiotics
6	impaired host mucosal immunity
7	risk of re-infection
8	lack of patient compliance (due to adverse effects or poor understanding of complex antibiotic regimen)

TREATMENT

Historically, various combinations of antibiotics have been used to eradicate the infection, however, no optimal treatment has yet been defined, as there is not a single drug regimen that can eradicate it. There are many factors that have increasingly compromised the effectiveness of most commonly used therapies (as seen in table 2)[1].

These factors have reduced the eradication rates to unacceptable levels (< 80% in some geographic areas). As a response, new treatment strategies have been studied and recently been validated to replace the standard ones[15].

Eradication therapies should be guided ideally by individual susceptibility testing. As this is not cost-effective, local antibiotic resistance patterns should dictate the regimen. Another factor that influences the treatment strategy is drug availability in different countries[16]. These regimens can be divided into first-line treatment and second-line or rescue treatment and are summarized in table 3[1].

Table 3: Treatment regimens for Helicobacter pylori infection

First-line therapies		
Regimen name	*General characteristics*	*Duration*
“Standard triple treatment”	simultaneous PPI + clarithromycin + amoxicillin (each twice daily)	7 days
10 or 14 days triple	simultaneous PPI + clarithromycin + amoxicillin (each twice daily)	10 or 14 days
Sequential therapy	5 or 7 days simultaneous PPI + amoxicillin, followed by 5 or 7 days simultaneous PPI + clarithromycin + 5-nitroimidazole (each twice daily)	10 or 14 days
Quadruple therapy	Simultaneous PPI (twice daily) + bismuth subsalicylate, metronidazole and tetracycline (each 4 times daily)	10 or 14 days
Second-line and rescue therapies		
Regimen name	*General characteristics*	*Duration*
Concomitant therapy	Simultaneous PPI + 3 antibiotics (often amoxicillin, clarithromycin, and 5-nitroimidazole) (each twice daily)	7, 10 or 14 days
Levofloxacin therapies	Levofloxacin will replace clarithromycin in triple, sequential or concomitant regimens	7, 10, 14 days
Hybrid therapy	7 days simultaneous PPI + amoxicillin, followed by 7 days simultaneous PPI + amoxicillin + clarithromycin + 5-nitroimidazole (each twice daily)	14 days
Rifabutin triple therapy	PPI + amoxicillin + rifabutin (each twice daily)	14 days

PPIs are mandatory compounds in every regimen and are administered twice daily at standard doses: omeprazole 20 mg, esomeprazole 40 mg, lansoprazole 30 mg or pantoprazole 40 mg (with studies showing higher eradication rates for the more potent second-generation PPIs – namely esomeprazole). Standard antibiotic dosages are used in all treatment regimens: amoxicillin 1 g, clarithromycin 500 mg, metronidazole 500 mg, tinidazole 500 mg, bismuth subsalicylate 524 mg or bismuth subcitrate 420 mg, tetracycline 500 mg, levofloxacin 500 mg, and rifabutin 300 mg[17].

A recently published meta-analysis by Li et al. has compared many of the available regimens for efficacy and tolerance. In terms of efficacy, the standard triple therapy was outranked by all other regimens, with the best eradication rates for the sequential therapy amongst first-line treatments and for the concomitant therapy amongst rescue treatments. The same study has shown that increased treatment duration improves eradication rates, but at the same time enhances the likelihood of adverse effects, lowering patient compliance – almost all rescue regimens were poorly ranked in terms of tolerance[18]. Because of the fact that none of these regimens is ideal, eradication should always be assessed, preferably using a non-invasive test (eg: urea breath test or stool antigen test). Confirmation of eradication can also provide an early image on the pattern of antibiotic resistance in a specific population[15].

All this effort in treating Hp infection is justified by the many benefits of eradication for patience with PUD, but also some malignant conditions. For example a cured individual has better ulcer remission rates for both gastric and duodenal ulcer and does not need maintenance acid suppression therapy after eradication and ulcer healing[10]. It is also more cost-effective and superior compared to maintenance acid suppressive therapy in preventing duodenal ulcer[1]. As for malignant pathologies, early stage low-grade MALT lymphoma can be cured by Hp eradication up to 80% of cases and there is also the possibility of regression or decrease in progression of precancerous gastric lesions (atrophic gastritis and intestinal metaplasia) after eradication[15].

Other studies have shown inverse associations between Hp infection and the prevalence of certain diseases. A recent study published by Rubenstein et al. confirmed prior studies that showed a strong negative association between Hp infection (particularly the cagA strain) and erosive esophagitis, Barrett's esophagus or adenocarcinoma of the esophagus. However, contrary to the prevalent hypothesis explaining this association, they were unable to detect a negative association between Hp infection and gastroesophageal reflux disease symptoms[19]. Lebwohl et al. found a strong inverse relationship between Hp presence and celiac disease (CD), but failed to establish the mechanism by which Hp might be protective against CD[20]. Other studies have postulated that Hp is protective against some extradigestive conditions, especialy asthma and other allergic pathologies in children[21].

THE ROLE OF PROBIOTICS IN HP MANAGEMENT

Probiotics are defined by the World Health Organization (WHO) as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Most commonly used in clinical practice are lactic-acid-producing micro-organisms (Lactobacillus spp. or Bifidobacterium spp.). Lactic acid produced by these probiotics can have a direct antimicrobial effect by lowering the pH, but can also inhibit the Hp urease. There is also increasing evidence in animal models that some strains of probiotics can inhibit H. pylori growth by competing with different adhesion sites in the gastric mucosae. Another mechanism being stipulated is that some probiotics can increase the expression of the MUC2 and MUC3 genes, which can subsequently lead to an increase of mucus thickness in both antrum and corpus after long-term probiotic intake.

Many studies have been conducted in this field and by analyzing some of them Enzo et al. have reached the conclusion that the use of probiotics as an adjuvant to PPI–antibiotic treatment could improve eradication rates, but the main benefit of this association is the prevention of side-effects, leading to better patient compliance[22].

FUTURE THERAPIES

Efforts to develop a vaccine against H. pylori began in early 1990`s with initial attempts to promote a localized mucosal immune response in the stomach via oral vaccines. Recently, an intramuscularly trivalent vaccine (recombinant Cag-A, Vac-A, and neutrophil-activating protein) was developed, but failed to induce immunity, despite the fact that these antigens were recognized by the host’s humoral and cellular immune systems. Although considerable progress has been made in understanding the innate and adaptive immune response against Hp infection, it is still uncertain how to promote the development of host immunity with the final goal of creating a successful vaccine[23].

DISCUSSIONS AND CONCLUSIONS

Despite the constant progress made in the management of Helicobacter pylori infection, it is still considered a major public health issue due to it`s high prevalence (especially in developing countries) and various conditions that can arise from it (even though 80-85% of infected individuals are asymptomatic). The main reasons for treatment failure is resistance acquired by mutations and lack of patient compliance and recent studies are showing that the standard triple therapy is out-performed in effectiveness by most other treatments. Probiotic adjuvant therapy appears to have a clear effect in reducing side effects, but insufficient data exists to conclude that their use improves eradication rates. Knowing that there is no “gold-standard” in Hp eradication therapy and falling short of the individual susceptibility testing goal, the regimen should be dictated by local antibiotic resistance patterns.

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SYSTEMATIC REVIEW

Current therapeutic strategies for erectile function recovery after radical prostatectomy – literature review and meta-analysis

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INTRODUCTION

Radical prostatectomy is the most commonly performed treatment option for localised prostate cancer. In the last decades the surgical technique has been improved and modified in order to satisfy both oncological safety and postoperative functional results. Urinary incontinence and erectile dysfunction (ED) are the main postoperative functional impairments in patients that undergo radical prostatectomy (RP). [1]

According to the EAU Guidelines erectile dysfunction is defined as "the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance". To this moment it is estimated that 25-75% of the patients who underwent RP develop ED with major implications in postoperative quality of life. [2] Various risk factors can be related to post-prostatectomy ED, among which the most important are the patient's age, comorbidities, preoperative erectile function, but also surgical approach (robot-assisted, laparoscopic, open) and nerve-sparing technique (inter/intrafascial). [2, 3] Among physiopathological modifications that concur to ED, the nerve damage (neuropraxia) appears to be the main domino piece that triggers other alterations like structural changes of the smooth muscle, arterial damage and veno-oclusive dysfunction which lead to cavernosal oxygenation impairments. [4–6] The clinical results of physiopathological alterations are shortening of penile size, impairment of orgasm and ejaculation achievement which have tremendous impact on psychological and mental health. [5]

Various treatment options include oral medication with phosphodiesterase 5 inhibitors (PDE5i), intracavernosal injections or intraurethral applications of prostaglandin E1 (PGE1), vacuum or vibratory treatments. Though there are many options for ED treatment, to this date there is no definitive protocol for penile rehabilitation (PR) after prostatectomy. [5, 7] In this regard we have made a literature review and meta-analysis of the most important randomised trials published in the last 5 years in order to find the best strategies of penile rehabilitation after radical prostatectomy.

MATERIALS AND METHODS

We have systematically reviewed and collected data from randomised trials published in the last 5 years involving patients diagnosed with prostate cancer who experienced ED after radical prostatectomy. In order to find the necessary trials we have searched PubMed and ScienceDirect databases and used several key words like: erectile dysfunction, penile rehabilitation, phosphodiesterase 5 inhibitors, erectile functional recovery, prostate cancer, prostatectomy and nerve sparing – in various combinations. The first aspect for our meta-analysis inclusion criteria was to identify randomized (prospective or retrospective) placebo-controlled trials involving patients with localised prostate cancer that benefited of nerve sparing radical prostatectomy and developed postoperative ED. The key aspect of our research was the penile rehabilitation strategy which included the therapeutic means used for erectile

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function recovery and the treatment duration. Other aspects followed by us regarded the means of ED assessment, the surgical means and invasiveness and the rate of treatment success reported to ED severity.

In this regard we have conducted our research for each year between January 2014 and December 2018 and reviewed a total of 631 articles published on this theme collected from both search engines. For a time-efficient assessment we have read the title and abstract of each article and included only those articles that matched our criteria. We have excluded the reviews and meta-analyses and also the trials of ED that was not related to prostate cancer surgery. That left us 194 publications from which to select. We have also excluded the trials withdrawn from database for various reasons and articles that didn't followed the therapeutic means of ED recovery. Finally we have selected 15 articles matching our criteria for meta-analysis that were thoroughly full text reviewed. Seven out of the 15 were prospective randomised trials, 2 of them being double-blind, placebo-controlled. Another 2 were nonrandomised prospective trials and the rest of 6 articles were retrospective review. Although some studies included by us followed-up both the ED and postoperative incontinence problem, we have concerned strictly on erectile dysfunction issue and collected the necessary data.

For statistical analysis we have used ReviewManager 5.3 and Microsoft Excel software with data of treatment strategies and drug dosages collected from the studies. Although there was less heterogeneity between the study designs of the included articles we found important data about postoperative EF recovery. These data allowed us to include the studies in subgroups in order to analyse them.

Table 1. Distribution of included studies

Trial author	Design	Patient no.	Mean age	Cardiovascular comorbidities	Diabetes
Vickers et al. [8]	Randomised prospective	2162	64.2	NoS	NoS
Montorsi et al. [9]	Randomised double-blind placebo-control	423	58	NoS	NoS
Seo et al. [10]	Retrospective review	92	67.9	40 (43.5%)	14 (15.2%)
Nakano et al. [11]	Retrospective review	103	63.4	NoS	NoS
Fode et al. [12]	Randomised prospective control	68	62	NoS	NoS
Stolzenburg et al. [13]	Randomised double-blind	422	59	NoS	NoS
Canat et al. [14]	Randomised prospective	112	63	42 (37.5%)	18 (16.07%)
Yiou et al. [15]	Retrospective review	75	59.4	30 (40%)	6 (8%)
Haglind et al. [16]	Prospective controlled nonrandomised	2625	63	NoS	NoS
Kim D. et al. [17]	Randomised prospective placebo-control	97	54	NoS	NoS
Capogrosso et al. [18]	Retrospective review	2364	61	NoS	NoS
Sooriakumaran et al. [19]	Prospective non-randomised	2545	63.3	556 (35.2%)	99 (6.2%)

RESULTS

A total of 11,831 patients were enlisted in the 15 studies we have included for analysis. Mean age of all patients was 61.91 years with a range between 54 and 67.9 years. (Table 1) Although not all the studies specifically mentioned the most encountered comorbidity was the cardiovascular disease (37.5% - 100%) followed by diabetes mellitus (6.2% – 16.07%). For most ED patients the presence of cardiovascular comorbidities creates the premises of disease trigger, followed by diabetes and obesity. [] All patients were diagnosed (by means of PSA, prostate biopsy and pelvic MRI) with localised prostate cancer mainly T1c - T2c, with Gleason score ranging between 6 and 7 and PSA values below 10 ng/ml. (Table 2) These data were correlated with the postoperative pathology findings which in some cases changed the actual diagnostic stage according to d'Amico criteria. As it can be seen in Table 2 in some cases based on the degree of invasion the cancer stage was changed from localised to locally-advanced (T3b-T4) and based on Gleason score from low or intermediate risk to high risk (Gleason 8).

All patients were proposed for radical prostatectomy using various approaches: open surgery (n = 4,460), conventional laparoscopy (n = 1,371) or robot assisted laparoscopic prostatectomy (n = 4,929). (Table 2) Most patients benefited of bilateral nerve sparing (BNS) dissection of the nerve bundles (n = 6,852) by al known technical procedures (interfascial/intrafascial), which represented 63.68% of the operated patients. When case did not impose BNS dissection, unilateral nerve sparing technique was attempted (n = 3,209) which was performed in 29.82% of the operated patients. The rest of 6.5% of the patients either had not benefited of NS dissection either the information about the NS status was not mentioned. [16, 18, 19] The NS status at the end of the surgery was crucial because it ensured the premises for postoperative ED recovery although most of the patients experienced postoperative neuropraxia.

Table 2. PCa status and surgical approach for each study

Trial author	PCa clinical stage	Gleason Score	Surgical approach			Nerve sparing	
			OP	CLP	RALP	BNS	UNS
Vickers et al. [8]	T2a – T3	6 - 8	859 (40%)	546 (25%)	757 (35%)	657 (30%)	1498 (70%)
Montorsi et al. [9]	T1c – T2c	6 - 7	68 (48.9%)	29 (20.9%)	31 (22.3%)	423 (100%)	-
Seo et al. [10]	T2a – T2c	4 - 8	-	-	92 (100%)	57 (62%)	35 (38%)
Nakano et al. [11]	T2 – T3	6 - 7	NoS	NoS	NoS	24 (23.3%)	79 (76.7%)
Fode et al. [12]	T2 – T3	NoS	7 (10.3%)	-	61 (89.7%)	37 (54.41%)	31 (45.58%)
Stolzenburg et al. [13]	T2-T3	≤ 7	189 (44.78%)	88 (20.85%)	115 (27.25%)	422 (100%)	-
Canat et al. [14]	T2a-T2c	5 - 8	NoS	NoS	NoS	112	-
Yiou et al. [15]	T2 - T3	6 - 8	-	75 (100%)	-	75 (100%)	-
Haglind et al. [16]	T1 - T3	6 - 8	778	-	1847	1318	750
Kim D. et al. [17]	T1c – T3	6 - 8	58 (61.7%)	-	36 (38.3%)	96 (98.96%)	1 (1.04%)
Capogrosso et al. [18]	T2 - T4	NoS	614 (26%)	633 (27%)	1117 (47%)	2253 (95%)	78 (3%)
Sooriakumaran et al. [19]	T2 – T4	NoS	1792 (70.41%)	-	753 (29.58%)	1283 (50.41%)	737 (28.95%)
Kim S. et al. [20]	T2a – T2c	4 - 8	95 (100%)	-	-	95 (100%)	-
Kwon et al. [21]	NoS	NoS	NoS	NoS	NoS	NoS	NoS
Jo et al. [22]	T2a – T2c	5 - 7	-	-	120 (100%)	NoS	NoS

NoS – no specification; OS – open surgery; CLP – conventional laparoscopic prostatectomy; RALP – robot assisted laparoscopic prostatectomy; BNS – bilateral nerve-sparing; UNS – unilateral nerve-sparing.

Table 3. Preoperative EF assessment and postoperative EF recovery means and results

Trial author	Type of EF questionnaire	Preop. mean EF score	Means of penile recovery	Subgroups	Trial duration	Recovery rates
Vickers et al. [8]	IIEF-6	≥22 - 937 (59%)	PDE5i (NoS) PGE_1 ICI	-	2 years	36% (NoS)
Montorsi et al. [9]	IIEF-EF	≥22 - 423 (100%)	PDE5i	Tadalafil 5 mg – OaD – 139 (32.86%) Tadalafil 20 mg – PRN – 143 (33.8%) Placebo – 141 (33.3%)	9 months	Tadalafil 5 mg – 25.2% Tadalafil 20 mg – 19.7% Placebo – 14.2%
Seo et al. [10]	IIEF-5	≥22 - 92 (100%)	PDE5i	Tadalafil 5 mg – OaD Non-Tadalafil	1 year	Tadalafil 5 mg – 13.2% Non-Tadalafil – 7.7%
Nakano et al. [11]	IIEF-5	17.7	PDE5i	Vardenafil 10 mg – OD – 30 (29.12%) Vardenafil 20 mg – PRN – 5 (4.85%) No treat – 68 (66.01%)	1 year	Vardenafil 10 mg + Vardenafil 20 mg – 21 (60%) No treat – 26 (38.2%)
Fode et al. [12]	IIEF-5	25	PDE5i PVS	PDE5i + PVS - 30 PDE5i - 38	1 year	PDE5i + PVS – 16 (53%) PDE5i – 12 (32%)
Stolzenburg et al. [13]	IIEF-5	≥22 – 422 (100%)	PDE5i	Tadalafil 5 mg – OaD - 102 Tadalafil 20 mg – PRN - 112 Placebo - 106	9 months	Tadalafil 5 mg – OaD – 29 (28.43%) Tadalafil 20 mg – PRN – 24 (21.42%) Placebo – 27 (25.4%)
Canat et al. [14]	IIEF-6	≥ 22	PDE5i	Tadalafil 5 mg – OpW - 38 Tadalafil 20 mg – PRN - 40 Non-Tadalafil - 34	1 year	Tadalafil 5 mg – OpW – 19.89 (mean IIEF) Tadalafil 20 mg – PRN – 15.8 (mean IIEF) Non-Tadalafil – 13.47 (mean IIEF)
Yiou et al. [15]	IIEF-EF EHS	≥ 24 ≥ 2	PDE5i PGE_1 ICI	PDE5i + PGE_1 ICI – NoS No treatment - NoS	2 years	M12 – 19.6 (mean IIEF – treat.) M12 – 18.07 (mean IIEF – no treat.) M24 – 4.63 (mean IIEF – treat.) M24 – 4.92 (mean IIEF – no treat.)
Haglind et al. [16]	IIEF-5	≥ 21	-	RRP RALP	1 year	RRP – 124 (15.93%) IIEF-5 ≥ 21 RALP – 339 (18.35%)
Kim D. et al. [17]	IIEF-EF	≥ 21 (100%) (Mean 28.1)	PDE5i	Sildenafil 50 mg - OaD + Sildenafil 100 mg - PRN Placebo + Sildenafil 100 mg - PRN	13 months	Sildenafil 50 mg - OaD + Sildenafil 100 mg – PRN – 15 (32.4%) Placebo + Sildenafil 100 mg – PRN – 13 (29%)
Capogrosso et al. [18]	IIEF-6	≥ 24 (Mean 27)	PDE5i PGE_1 ICI	12 months - 1779 24 months - 1095	2 years	12 mo – 483 (27%) 24 mo – 377 (34%)
Sooriakumaran et al. [19]	Penile stiffness Morning erection	1702 (66.9%) PPP.	PGE_1 ICI PGE_1 IUr	RARP RRP	2 years	RARP – 377 (21%) RRP – 106 (14%)
Kim S. et al. [20]	IIEF-5	22.4	PDE5i	Tadalafil 5 mg – OaD – 2 years Tadalafil 5 mg – OaD – 1 year No Tadalafil	2 years	Tadalafil 5 mg OaD – 2 yr. – 16.1 (mean IIEF) Tadalafil 5 mg OaD – 1 yr. – 13.5 (mean IIEF) No Tadalafil – 9.4 (mean IIEF)

Kwon et al. [21]	Penile lenght SHIM	NoS	PDE5i (Sildenafil, Vardenafil, Tadalafil)	Complete recovery Incomplete recovery	1 year	CR – 318 (60.2%) IR – 210 (39.8%)
Jo et al. [22]	IIEF - 5	≥ 17	PDE5i (Sildenafil 100 mg)	Early treatment - 58 (3 wk. postop.) Delayed treatment - 62 (3 mo. postop.)	1 year	ET – 24 (41.4%) DT – 11 (17.7%)

PPP – preoperative potent patients; IIEF – International Index of Erectile Function; OaD – oance a day; PRN – pro re nata (on demand); ICI – intracavernosal injections; IUr – intrauretral;

For pre and postoperative EF assessment the IIEF questionnaire was preferred in most of the studies in different forms: the classical 15 questions IIEF-EF or the shorter IIEF-5/IIEF-6 questionnaires.

Most authors preferred the IIEF-5 questionnaire due to its reliability and rapid way of determining the EF status. (Table 3) In most of the studies that used the IIEF questionnaire a cut-of value of 22 points was established for preoperative potent men [8–10, 13, 14] while others established close cut-of values. [16, 20] Only two studies used other means of EF assessment. Sooriakumaran et al. used two questions about penile stiffness and morning erections, both having single choice answer from 5 possible variants. [19] In a particular case Kim et al. compared the penile length both pre and postoperative and additionally used the SHIM questionnaire for ED appraisal. [20]

The most frequently used treatment method for penile recovery was the administration of PDE5i which was tested with various ways of administration and dosages and compared with placebo or no treatment control groups. The most commonly used drug in the included trials was Tadalafil [9, 10, 13, 14, 20], followed by Sildenafil [17, 22] and Vardenafil. [11] Some trials studied the single use treatment of PDE5i compared with combinations of PDE5 with PGE1 ICI [8, 15, 18] or penile vibratory stimulation (PVS) devices. [12] There were also particular studies that did not compare the method of PDE5i administration but rather the rate of EF recovery [8, 21], the moment of treatment initiation [22] or the length of treatment administration. [18] Haglid et al. compared the EF recovery based only on the surgical approach type (open vs. RALP) and not interfering with any medication in this regard while Sooriakumaran et al. compared the same surgical approaches using PGE1 treatment setting. [16]

Figure 1: Multivariate comparison of PDE5i OaD and PRN treatment compared to control group

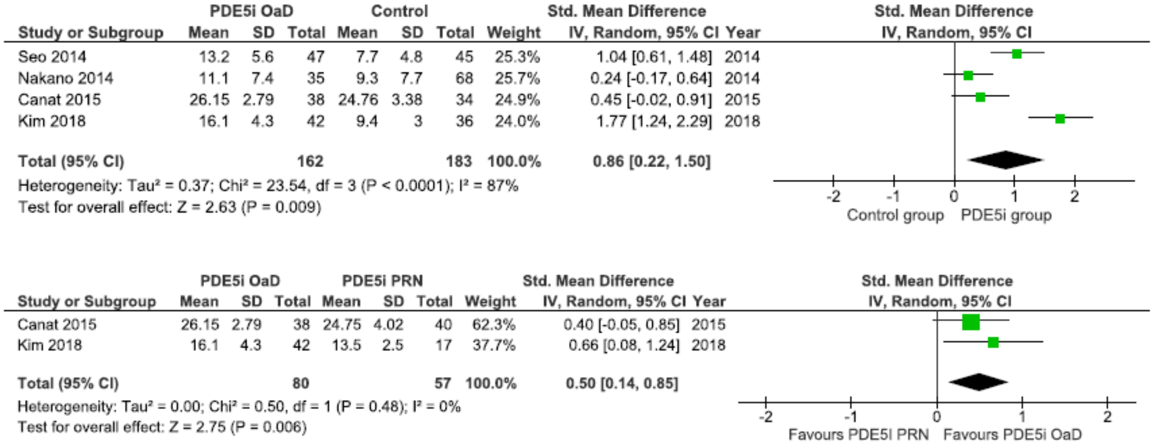

	PDE5i OaD			Control				Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	Year
Seo 2014	13.2	5.6	47	7.7	4.8	45	25.3%	1.04 [0.61, 1.48]	2014
Nakano 2014	11.1	7.4	35	9.3	7.7	68	25.7%	0.24 [-0.17, 0.64]	2014
Canat 2015	26.15	2.79	38	24.76	3.38	34	24.9%	0.45 [-0.02, 0.91]	2015
Kim 2018	16.1	4.3	42	9.4	3	36	24.0%	1.77 [1.24, 2.29]	2018
Total (95% CI)			**162**			**183**	**100.0%**	**0.86 [0.22, 1.50]**	

Heterogeneity: $Tau^2 = 0.37$; $Chi^2 = 23.54$, df = 3 ($P < 0.0001$); $I^2 = 87\%$
Test for overall effect: Z = 2.63 (P = 0.009)

	PDE5i OaD			PDE5i PRN				Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	Year
Canat 2015	26.15	2.79	38	24.75	4.02	40	62.3%	0.40 [-0.05, 0.85]	2015
Kim 2018	16.1	4.3	42	13.5	2.5	17	37.7%	0.66 [0.08, 1.24]	2018
Total (95% CI)			**80**			**57**	**100.0%**	**0.50 [0.14, 0.85]**	

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.50$, df = 1 ($P = 0.48$); $I^2 = 0\%$
Test for overall effect: Z = 2.75 (P = 0.006)

Although the articles included in our meta-analysis had dichotomous criteria of study design we could find similarities between some of them, which allowed us to organise subgroups of trials. On multivariate analysis we have included trials in which PDE5i was administered as OaD and PRN treatment compared to a pacebo control group. The variables included in analysis were the mean IIEF scores recorded at the end of the trial for each group (OaD, PRN and control). As it can be observed in fig. 1 the higher rates of recovery are in favour of PDE5i OaD group compared to control group (CI 95%, P < 0.0001). On a lower scale, the same result could be observed when PDE5i OaD treatment was compared to PDE5i PRN although in this analysis only 2 studies had the necessary data to be included (CI 95%, P =

0.48).

In a univariate analysis we also compared the trials were only the percentage of recovered patients after PDE5i treatment was given. (Fig 2) As in previous assessment the PDE5i OaD group had higher recovery rates compared to control group (CI 95% P = 0.40). Also when we have compared PDE5i OaD with PRN group the better results were slightly higher in favour of OaD group thus resulting that the benefit of PDE5i OaD compared to PRN treatment was minor (CI 95%, P = 0.95).

Figure 2: Recovery rates in trials using PDE5i OaD and PRN treatment compared to control group

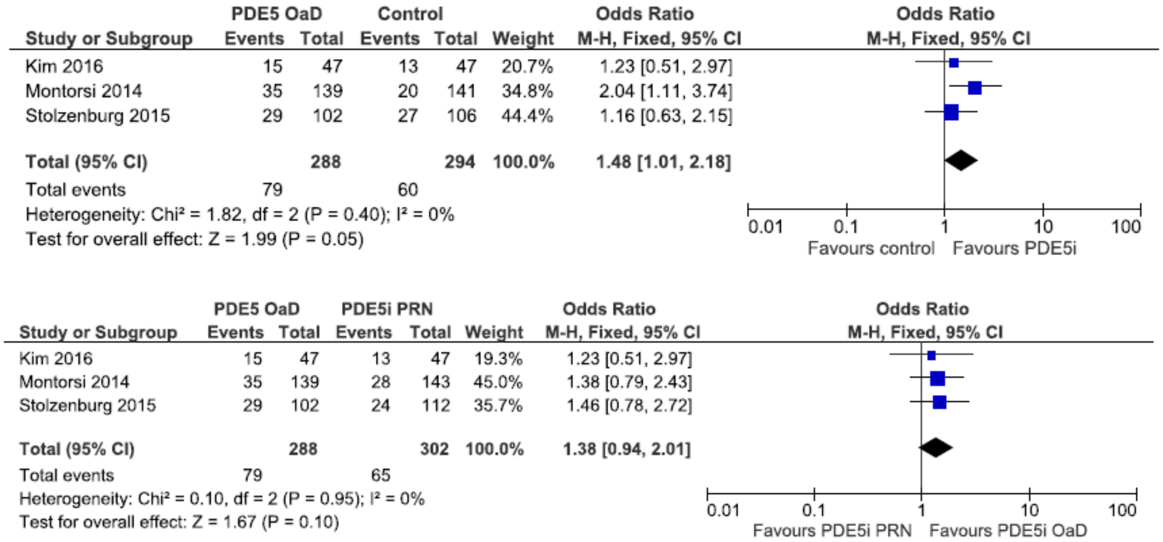

Study or Subgroup	PDE5 OaD Events	PDE5 OaD Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Kim 2016	15	47	13	47	20.7%	1.23 [0.51, 2.97]
Montorsi 2014	35	139	20	141	34.8%	2.04 [1.11, 3.74]
Stolzenburg 2015	29	102	27	106	44.4%	1.16 [0.63, 2.15]
Total (95% CI)		**288**		**294**	**100.0%**	**1.48 [1.01, 2.18]**
Total events	79		60			

Heterogeneity: Chi² = 1.82, df = 2 (P = 0.40); I² = 0%
Test for overall effect: Z = 1.99 (P = 0.05)

Study or Subgroup	PDE5 OaD Events	PDE5 OaD Total	PDE5i PRN Events	PDE5i PRN Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Kim 2016	15	47	13	47	19.3%	1.23 [0.51, 2.97]
Montorsi 2014	35	139	28	143	45.0%	1.38 [0.79, 2.43]
Stolzenburg 2015	29	102	24	112	35.7%	1.46 [0.78, 2.72]
Total (95% CI)		**288**		**302**	**100.0%**	**1.38 [0.94, 2.01]**
Total events	79		65			

Heterogeneity: Chi² = 0.10, df = 2 (P = 0.95); I² = 0%
Test for overall effect: Z = 1.67 (P = 0.10)

DISCUSSION

Erectile dysfunction is a major postoperative complication for men who undergo radical prostatectomy, alongside urinary incontinence. At this moment prostate cancer is the second most diagnosed neoplasia in men due to the systematic determination of PSA. [23] Radical prostatectomy remains the most important therapeutic procedure for localised PCa having a postoperative life-expectancy of 10 years. [2] Although the technical enhancements of prostatectomy reduced the rates of postoperative complication, ED still remains one of the most complex problem for PCa patient's quality of life.

Even if the nerve sparing dissection technique of the prostate bundles partially improved the ED rates, there are still problems regarding the postoperative penile rehabilitation. Neuropraxia is the main pathological alteration that leads to major structural changes of the penile tissue. This can also lead to veno-oclusive dysfunctions of blood drainage. Due to the constant state of hypo-oxygenation the penile smooth muscles suffers a process of apoptosis and fibrosis which in time are producing irreversible penile malformations such as penile shortening of 2-3 cm and Peyronie disease. [5, 24] In this regard it is advisable that urologists should discuss with their patients about the possibility of postoperative ED in various rates – from temporary to permanent. [25]

Two trials included in our meta-analysis studied the rate of ED occurrence and penile recovery degree after RALP compared to RRP in high volume centres from Sweden. In a 2015 prospective study, Haglid et al. found a slightly higher rate of penile recovery for RALP, but the differences were not significant for these two surgical approaches. [16] In a recently conducted prospective trial, Sooriakumaran et al. (2018) found earlier recovery rates of EF for RALP (21%) compared to RRP (14%) due to a more precise identification and preservation of nerve bundles during RALP. Also the rates of post-surgical positive margins were similar to open surgery which is an important aspect for oncological radicality. [19] Both studies were prospective non-randomised.

One key aspect of ED diagnosis is the use of pre and postoperative EF assessment questionnaires. As we have shown above the most frequently used questionnaire was IIEF-5 which is the short version of the IIEF-EF questionnaire. Other questionnaires like Sexual Health Inventory for Men (SHIM) or Expanded Prostate Cancer Index Composite (EHGS) have similar predictive results to IIEF and should be routinely used for postoperative ED diagnosis. [25, 26]

It is a known fact that older age, cardiovascular comorbidities and diabetes mellitus are the most important predictive factors for ED onset. [27, 28] On the other hand the younger age and the lower body mass index are demonstrated protective factors for ED recovery. [2] Even though not all the studies included in our meta-analysis reported the rate of cardiovascular disease and diabetes mellitus we could emphasize that in the post prostatectomy setting these two comorbidities could have significant impact on ED onset and the subsequent penile recovery. [10, 14, 15, 19, 20, 21]

Penile rehabilitation is the cumulus of therapeutically means which includes medication, medical devices or actions, simple or in various combination recommended for erectile function recovery. [5] PDE5i are the most frequently used drugs in clinical practice for penile rehabilitation and to this date is the most studied therapeutic method administered to ED patients. The main advantage of PDE5i therapy is that there are quick and easy to administer but it requires the integrity of at least 1 cavernosal nerve bundle. Also the treatment cost and side effects (headache, flushing and palpitations) are not negletable. [24]

In their common trial Montorsi et al. (2014) and Stolzenburg et al. (2015) studied the effect of Tadalafil administered once a day (OaD) and on demand (PRN) compared to a placebo-controlled group and found that the OaD administration of Tadalafil 5 mg has the best results for penile recovery after prostatectomy. [9, 13] Similar results for Tadalafil OaD administration were also found by Seo et al. (2014), Canat et al. (2015) and Kim S. et al. (2018) based on the mean IIEF score at the end of the trial. [14, 20] Nakano et al. (2014) and Kim D. et al. (2016) found similar results at the end of their trials although they have studied the effect of Vardenafil respectively Sildenafil. [11, 17] In our multivariate analysis the PDE5i OaD treatment has evident advantages over placebo and on demand PDE5i administration for postoperative penile recovery rate.

Regarding the choice of PDE5i treatment there are no specific trials that compare the efficiency of currently available substances in postoperative ED. In a 2005 open-label randomised trial Eardley et al. emphasized that Tadalafil is the preferred PDE5i used for ED treatment because of long-lasting effect in time compared to Sildenafil that allowed patients to have less concerns about spontaneity of erection. [29] Further more Hyndman et al. demonstrated in their randomised trial that Sildenafil could have major impact over urinary continence, thus impairing the overall recovery of PCa operated patients. [30]

Prostaglandin E1 intracavernosal injection (PGE1 ICI) was the first type of treatment for postoperative EF recovery, introduced and studied by Prof. Montorsi et al. [31] The most important advantage of PGE1 ICI is that is very effective in any type of surgical approach even if nerve sparing was not indicated, though the cost, the need to refrigerate the product, the invasiveness of the treatment and the side effects are a majos counterbalance for treatment initiation. [24] In our meta-analysis, Vickers et al., Yiou et al. and Capogrosso et al. have used PGE1 ICI in combination with PDE5i treatment for penile recovery. The results achieved by Yiou at 12 respectively 24 months of combined treatment were very similar in both drug-administered and control group. [15] On the other hand Capogrosso et al. found that at 24 months of combined PGE1 ICI and PDE5i treatment the rates of recovery were higher than at 12 months (34% vs. 27%). [18]

Vacuum and vibratory devices are non-invasive and very effective in EF recovery especially when combined with PDE5i treatment. Fode et al. demonstrated in their randomised prospective trial that EF recovery could be very effective when penile vibratory stimulation devices are used in conjunction with PDE5i (53% recovery rate) compared to PDE5i only group (32%). [12]

Regarding treatment initiation Jo et al. demonstrated that early penile rehabilitation commencement (at 3 weeks after surgery – immediately after urinary catheter removal) has better recovery results than delayed treatment initiation (at 3 months postoperative). [22]

CONCLUSIONS

The postoperative EF recovery of PCa patients can be achieved using a multitude of penile recovery strategies. For this purpose a preoperative assessment is very important, by using erectile function questionnaires. Patients should be informed about the postoperative ED risk and should be counselled in this area.

Nerve-sparing robot assisted laparoscopic prostatectomy is the most protective surgical approach for EF preservation. PDE5i is the main therapeutic approach for postoperative penile recovery.

Tadalafil 5 mg once a day was the most effective long term therapy for ED treatment both single use or in combination with PGE1 and other devices (vacuum or vibratory stimulation). Penile prosthesis implants should be regarded as last resort treatment option for cases when no penile recovery strategy has been successful.

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REVIEW ARTICLE

Current treatment of posttraumatic stress disorder – A review of therapeutic guidelines and good practice recommendations

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Abstract: *A unique approach to the treatment of posttraumatic stress disorder (PTSD) is difficult to support, because of the complexity and heterogeneity of this pathology. Multiple evidence-based guidelines for PTSD treatment exist, elaborated by prestigious institutions around the world, but a certain level of the inconsistency of their recommendations appears when these sources are compared with each other. Therefore, a review of current guidelines and extraction of the most supported by evidence recommendations is considered important for clinical practice, due to the need to approach such complex cases as PTSD-diagnosed patients in a stepwise manner. Structured monitoring of the clinical status during the treatment is also an important and rather neglected aspect of the case management in PTSD, and the necessity of follow-up visits for efficacy and tolerability should be taken into consideration. Psychotherapy (especially cognitive-behavioral oriented therapies and eye movement desensitization and reprocessing therapy) is supported by evidence as a first-line approach, as well as certain antidepressants, like the selective serotonin reuptake inhibitors and venlafaxine. Many second-line pharmacological agents and psychotherapies are also available, but there is an obvious need for more pragmatic trials with PTSD patients.*

Keywords: *post-traumatic stress disorder, psychotherapy, case management, antidepressants, therapeutic guidelines, good clinical practice*

BACKGROUND

While definitions of the diagnostic criteria for posttraumatic stress disorder (PTSD) are articulate and comprehensive in both the World Health Organization and American Psychiatric Association classifications of mental disorders [1-3], pragmatic recommendations for the treatment of this disorder are still lacking. Although there are several guidelines dedicated to this subject, they are based on randomized trials, and not on pragmatic, naturalistic studies, which leave some important questions unanswered. Even if the case management is based on these guidelines, there are several contradictions based on the different data they included. Therefore, we consider the first necessary step to be a critical analysis of these therapeutic guidelines, followed by the formulation of good practices and hierarchized recommendations, according to the GRADE level [4].

The diagnosis of PTSD could be formulated, according to ICD-11, if an extremely threatening or horrific event or series of events is followed by re-experiencing this trauma as intrusive memories, flashbacks, nightmares, accompanied

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by strong or overwhelming emotions and strong physical sensations; avoidance of thoughts and memories of the event(s), or avoidance of activities, situations, or people reminiscent of the events(s); persistent perceptions of heightened current threat, for example, indicated by hypervigilance or an enhanced startle reaction to stimuli such as unexpected noises [1]. These symptoms have to persist for at least several weeks and to cause significant impairment in personal, family, social, educational, occupational, or other important areas of functioning to support a PTSD diagnosis [1]. The ICD-10 mentions the capacity of the stressful event to cause pervasive distress in almost anyone who has been removed from the latest edition of this classification [1,3]. Also, differences in the structure of the symptoms list necessary to establish the PTSD criteria have been formulated between the two editions of ICD, from 13 in the tenth edition, to only 6, by removing the non-specific manifestations [1,3]. In the latest edition of the American Psychiatric Association classification of mental disorders (2013), there are 4 clusters of symptoms needed for the diagnosis: (1) intrusion symptoms associated with the traumatic event, (2) persistent avoidance of stimuli associated with the traumatic event, (3) negative alterations in cognitions and mood associated with the traumatic event, (4) marked alterations in arousal and reactivity associated with the traumatic event, and the duration of these manifestations are more than one month [2].

Complex PTSD may develop following exposure to an event or series of events of an extremely threatening or horrific nature, commonly prolonged or repetitive events from which escape is difficult or impossible [1]. Complex PTSD is characterized by severe and persistent problems in affect regulation, beliefs about oneself as diminished, defeated, or worthless, accompanied by feelings of shame, guilt, or failure related to the traumatic event; difficulties in sustaining relationships, and feeling close to others [1]. These symptoms cause significant impairment in personal, family, social, educational, occupational, or other important areas of functioning [1].

A high rate of comorbid major depressive disorder (up to 50%) has been reported in PTSD patients, a phenomenon which underlines the necessity for a thorough psychiatric evaluation, both initially and during the monitoring period [5,6]. Also, there has been described as an overlap between PTSD and psychosis that may raise important diagnosis challenges and treatment dilemmas [7, 8]. Substance-related disorders have also been described with relatively high incidence in this population, and specific psychotherapy algorithms have been developed for these patients with dual diagnosis, based on imaginal and in vivo exposure for PTSD, applied concomitantly with cognitive-behavioral techniques for substance use disorders [9]. Also, the pharmacological treatment should be adjusted according to the existing comorbidities, and the case manager should integrate all the data, taking into account the risk of pharmacologic interactions, contraindications, and side effects (e.g., avoidance of drugs with the risk of addiction, like benzodiazepines, is recommended in PTSD patients) [10].

ANALYSIS OF THE CURRENT THERAPEUTIC GUIDELINES FOR PTSD

Several prestigious institutions have elaborated evidence-based guidelines for the treatment of PTSD, therefore an important number of recommendations for approaching these patients to exist. While some therapeutic guidelines are focused on psychotherapy, others are based on pharmacological research, while still others encompass both types of treatment.

American Psychological Association Clinical Practice Guideline for the Treatment of PTSD (2017) strongly recommends as evidence-based psychotherapies in adults cognitive-behavioral therapy (CBT), cognitive processing therapy (CPT), cognitive therapy (CT), and prolonged exposure therapy (PE), and suggests the use of brief eclectic psychotherapy (BEP), eye movement desensitization and reprocessing therapy (EMDR), and narrative exposure therapy (NET) [11].

According to the **Australian Guidelines for the Treatment of Acute Stress Disorder and Posttraumatic Stress Disorder** (2013), individual-trauma focused CBT, including exposure and/or cognitive therapy, should be recommended if symptoms are consistent with acute stress disorder or posttraumatic stress disorder in the initial 4 weeks after a potentially traumatic event (level C evidence) [12]. Individuals with posttraumatic stress disorder benefit from trauma-focused CBT or EMDR (level A recommendation), and if the symptoms did not respond to these interventions, non-trauma focused approaches may be considered (e.g., stress inoculation technique) [12]. Also, there is some evidence that supports the efficacy of in vivo exposure therefore, by expert consensus, it is recommended to include this method in the treatment [12]. Group CBT (trauma-focused or non-trauma focused) may be offered as an adjunctive to trauma-focused individual therapy [12].

Medication should not be used for the treatment of acute stress reaction or posttraumatic stress disorder within the four weeks of symptom onset unless the severity of such symptoms- associated distress cannot be managed by psychological means alone [12]. The pharmacological approach may be continued while people are undertaking psychological treatments, but several agents, like benzodiazepines, may interfere with some effective psychological treatments [12]. SSRIs are considered the first line for the treatment of posttraumatic stress disorder [12].

According to the **Maudsley Prescribing Guidelines** (13th edition, 2018) patients diagnosed with posttraumatic stress disorder should be offered as the first line of treatment paroxetine, sertraline, fluoxetine (up to the maximum tolerated doses) or venlafaxine (37.5-300 mg/day) [13]. As second-line treatment the case manager could choose between antipsychotics (olanzapine 5-20 mg/day, risperidone 0.5-6 mg/day, quetiapine 50-800 mg/day), mirtazapine 15-45 mg/day, monoaminoxidase (MAO) inhibitors (phenelzine 15-75 mg/day), tricyclic antidepressants (amitriptyline 50-300 mg/day, imipramine 50-300 mg/day), and prazosin (2-15 mg nocte) for nightmare and sleep disturbances [13]. Possible choices if the first and second lines failed are duloxetine (60-120 mg/day), lamotrigine (up to 500 mg/day), phenytoin, valproate, or i.v. ketamine, and as non-drug therapies- eye-movement desensitization and reprocessing (EMDR) and trauma-focused CBT [13].

According to the **VA/DOD Clinical Practice Guideline for the Management of PTSD and ASD** (2017) pharmacotherapy in PTSD is classified as follows: sertraline, paroxetine, fluoxetine, venlafaxine as monotherapy (moderate evidence), nefazodone (low evidence), imipramine, phenelzine (very low evidence) [14]. The Work Group recommended against the use of atypical antipsychotics, benzodiazepines, and Divalproex as augmentation therapy due to low-quality evidence and potential adverse effects [14]. Also, prazosin is not supported by this guideline for the use in monotherapy or combination with other agents [14]. Individual trauma-focused psychotherapy for PTSD are Prolonged Exposure, Cognitive Processing Therapy, and EMDR, which are supported by the strongest evidence, but other therapies like specific cognitive therapies for PTSD, Brief Eclectic Psychotherapy, Narrative Exposure Therapy, written narrative exposure may be recommended [14]. If individual trauma-focused psychotherapy is not available, Stress Inoculation Therapy, Interpersonal Therapy, or Present-Centered Therapy may be recommended [14].

The **International Society for Traumatic Stress Studies new guidelines for the Prevention and Treatment of Posttraumatic Stress Disorder** (2019) has formulated the following recommendations according to their evidence-based status: (1) strong support for cognitive processing therapy, cognitive therapy, EMDR, individual CBT with a trauma focus, prolonged exposure; (2) standard recommendations for CBT without a trauma focus, guided Internet-based CBT with a trauma focus, narrative exposure therapy, present-centered therapy; (3) emerging evidence- couples CBT with a trauma focus, group and individual CBT with a trauma focus, reconsolidation of traumatic memories, single-session CBT, written exposure therapy, and virtual reality therapy [15].

Individual trauma-focused CBT intervention may be useful for adults who have acute stress disorder or clinically important symptoms of PTSD and have been exposed to 1 or more traumatic events within the last month- cognitive processing therapy, cognitive therapy, narrative exposure therapy, prolonged exposure therapy, according to the **NICE Guidelines** (2018) [16]. In adults with PTSD symptoms should be offered trauma-focused CBT, during 8-12 sessions, accompanied by psychoeducation about reactions to trauma, strategies for managing arousal and flashbacks, safety planning, or EMDR for patients with PTSD symptoms, if the patient has a preference for this method [16]. Trauma-focused computerized CBT for adults with a diagnosis of PTSD or clinically important symptoms of PTSD more than 3 months after a traumatic event, who do not want to be engaged in trauma-focused CBT or EMDR [16]. Symptom-specific CBT interventions for adults with a diagnosis of PTSD or clinically important symptoms of PTSD more than 3 months after traumatic events who are unable or unwilling to engage in a trauma-focused intervention that targets PTSD or have residual symptoms after a trauma-focused intervention [16].

Medication should not include benzodiazepines for the prevention of PTSD in adults, but venlafaxine or an SSRI may be chosen [16]. Antipsychotics may be offered if patients have disabling symptoms (psychotic) or if their symptoms have not responded to other drug or psychological treatments [16].

For patients with complex PTSD the case manager should help the patient control any symptoms (e.g. dissociation or emotional dysfunctions) that might interfere with engaging in trauma-focused therapies; ensure adequate time for the construction of the therapeutic relationship; to increase the number of trauma-focused therapy sessions according to the person’s needs; plan any ongoing support the person needs and has implications in the therapeutic management [16].

DESCRIPTION OF THE MAIN PSYCHOTHERAPEUTIC METHODS IN PTSD

Cognitive-behavioral therapy (CBT) in PTSD is focused on the relationship among thoughts, feelings, and behaviors that are present-oriented and target current problems and symptoms [17]. CBT is based on emotional processing theory and social cognitive theory, therefore it claims that changing associations between reminders of the traumatic event and responses will lead to healthier behaviors while reframing the relation between trauma and existing beliefs about self, others and the world will also help decrease self-blame and improve the processing of the trauma [17].

Cognitive distortions in PTSD include perceiving the world as dangerous, seeing him/herself as powerless, and feeling guilty about surviving while other significant ones have died [18]. Cognitive therapy fosters the development of more realistic and adaptive cognitions, by successful processing of the traumatic event, with engaging and organization of the trauma narrative [18, 19].

Cognitive processing therapy (CPT) is an efficient method for treating PTSD as demonstrated by a recent meta-analysis (n=11 studies, N=1130 participants), outperforming inactive control conditions on PTSD outcome measures at posttreatment and follow-up, but also other active treatments at posttreatment but not at follow-up [20]. Effect sizes of CPT were superior to 89% of the inactive control conditions at posttreatment and 82% at follow-up, which indicates a large and sustained effect of the CPT [20].

Veterans who were diagnosed with both PTSD and alcohol use disorder (AUD) completed a 6-week CPT-based program had significantly better outcomes, including trauma-related cognitive distortions, PTSD symptoms, depressive symptoms, and trauma-cued substance craving [21]. In another study that included veterans (n=465), enrolled in CPT plus a written trauma account either group format or individual, the PTSD and depressive symptoms decreased significantly, with medium effects for the first option, and large effects for the second treatment [22].

Cognitive therapy (CT) has proven efficient in a group of 20 patients diagnosed with PTSD, as it decreased core PTSD features, but also depressive and anxiety symptoms [23]. Good treatment outcomes were related to more changes in dysfunctional cognitions related to the trauma [23]. Intensive CT was applied in 14 patients diagnosed with PTSD, in an 18-hour format during 5-7 working days, followed by one session a week later and up to 3 follow-up sessions; intensive CT led to similar outcomes as weekly-administered CT, but the intensive treatment improved the key PTSD symptoms over a shorter period and led to greater reductions in depression [24].

Prolonged exposure therapy (PE) is another trauma-focused CBT effective for improving PTSD, with response rates in 60-65% of trauma victims suffering from this disorder [25]. PE is based on two types of exposure techniques, imaginal exposure, and in vivo exposure, and it is used for enhancing emotional processing through systematic confrontation with trauma-related stimuli [26-28]. Intensive PE therapy (12 sessions, 90 minutes each, during 4 days) followed by four weekly 90-minute booster PE sessions in an open trial (n=73) patients diagnosed with PTSD who presented multiple interpersonal trauma had positive results after repeated treatment attempts [29]. Exposure therapy has an impact on the fear and contextualization circuits by facilitating communication between the ventromedial prefrontal cortex, hippocampus, and the amygdala [30].

Brief eclectic psychotherapy (BEP) for PTSD is a manualized technique that combines elements from psychodynamic, cognitive-behavioral, and directive psychotherapy [31]. Psychoeducation with the participation of the spouse, and exposure used to help the patient to access his or her feelings related to the traumatic event are also used during BEP [31]. A 16-session protocol of BEP in PTSD includes the following steps: psychoeducation for the development of self-control and motivation for therapy (the patient is invited to call up his memories and related emotions one last time); imaginal exposure or guidance (brief periods of muscle relaxation, relieving of the traumatic experience by imaginal exposure of 20-30 minutes); writing tasks and mementos (for uncovering the difficult feelings that may be related to the traumatic experience); meaning and integration (exploring and accepting a new view of the world and the self); farewell ritual (symbolic ritual created to encourage the expression of the sorrow the patient may still feel, and then to leave behind these feelings) [31, 32].

The first randomized clinical trial for BEP in patients with PTSD included 42 police officers who received 16 weekly sessions (60 minutes each) of psychotherapy [32]. At posttest and follow-up (at 3 months) BEP had produced significant improvement in PTSD symptoms, work functioning, and several comorbid conditions (e.g., agoraphobia) [32].

Another clinical trial included patients diagnosed with PTSD (n=24) randomly assigned to BEP or a waitlist group for 4 months, with superior efficacy on PTSD core symptoms and general anxiety symptoms in the active intervention group [33]. In this trial, BEP consisted of 16 weekly sessions (45-60 minutes each), and a high rate of the dropout was recorded (25% for the treatment group) [33].

Eye movement desensitization and reprocessing therapy (EMDR) is a type of psychotherapy developed by F.Shapiro for intervention in emotional trauma and other negative life experiences [34]. This psychotherapy consists of a protocol with 8 phases and bilateral stimulation targeting patient desensitization from the discomfort induced by traumatic memories [35]. The final objective of the EMDR is to achieve the reprocessing of these memories and to integrate them within the autobiographical memory [35].

According to a review of 24 randomized controlled trials, EMDR is an evidence-based approach for this kind of disorder, with 7 out of 10 studies reporting EMDR to be faster acting and/or more effective than trauma-focused CBT [34]. Two meta-analyses supported the efficacy of EMDR in treating PTSD, with results superior to various interventions and control conditions, and showed the efficacy of this psychotherapy over symptoms of depression, anxiety, and subjective distress [36,37]. A review of the trials published between 2014 and 2017 regarding the efficacy of EMDR in PTSD further supported this method, because it improved core symptoms over time, compared to relaxation therapy and a waiting-list control group [38].

Narrative exposure therapy (NET) is a psychotherapeutic intervention dedicated to the treatment of survivors of multiple and severe traumas, and 3-6 sessions may be sufficient to provide significant relief [39]. It is a short-term approach based on CBT and testimony therapy [40]. NET was evaluated in a group of 59 political detainees, 18 of which had full PTSD criteria, who were allocated in a randomized manner to either one session of psychoeducation or five sessions of NET [41]. After 6 months, NET but not psychoeducation produced a significant reduction in PTSD symptoms and depression scores, although four out of the nine patients receiving NET and eight out of the nine patients undergoing psychoeducation still had PTSD symptoms at 6 months [41]. In another trial, NET was compared to supportive counseling and psychoeducation (4, 4 and one sessions, respectively) in a group of Sudanese refugees (n=43), and the results after one year of treatment were superior for NET (29% vs. 79% vs. 80% still had PTSD criteria) [40].

Interpersonal therapy (IPT) is a time-limited focused on the patient's actual events and reframing of the social functioning as a way to tackle the symptoms and may be recommended where the patient does not want to be exposed to exposure-based approaches [42]. In a case study, IPT proved itself efficient comparative to the other two psychotherapies, after 14 weekly sessions [42]. In a 14-week randomized trial, IPT was compared to prolonged exposure and relaxation therapy in 110 unmedicated patients with chronic PTSD and the response rates were 63% for IPT, 47% for prolonged exposure, and 38% for relaxation therapy, without significant differences between groups [43]. IPT and exposure therapy improved the quality of life and social functioning more than relaxation therapy [43].

Group Interpersonal Therapy (G-IPT) was evaluated in 13 patients diagnosed with chronic PTSD, in an 8-week treatment program, with favorable results [44]. The improvement was significant in social functioning, general wellbeing and depressive symptoms, and a moderate reduction in the avoidant symptom cluster of PTSD [44]. These positive effects were stable at 3 months and associated with perceived intra-therapy progress in solving problematic domains identified during IPT [44].

Present-Centered Therapy (PCT) for PTSD is a manualized psychotherapy originally designed as a treatment comparator in studies focused on the effectiveness of trauma-oriented CBT [45]. A systematic review of the randomized trials (n=12, N=1837 participants) states that moderate-quality evidence supports a superior efficacy of PCT in reducing PTSD severity compared to control conditions, and PCT was associated with lower drop-out rates comparative to trauma-focused CBT [45].

Stress inoculation training (SIT) is derived from CBT and proved itself efficient in veterans with PTSD [46]. According to this model, PTSD symptoms are maintained by an imbalance between perceived situational demands and perceived resources to fulfill the environmental and personal demands [47, 48]. The purpose of SIT is to increase the patient's feeling of self-confidence by helping him or her to control anxiety and related symptoms and to inoculate patients against possible future episodes of severe anxiety [48].

In a population with PTSD and traumatic brain injury (n=65 veterans), SIT was applied for 18 months in an outpatient clinic and the results supported the efficacy of this psychotherapy because significant reductions in PTSD and depressive symptoms were detected, as well as increases in perceived stress tolerance and performance in multiple life domains [46]. Improvements in social and occupational functioning were also reported [46].

In another trial, 96 female assault victims with chronic PTSD were randomized on prolonged exposure, SIT, combined prolonged exposure-SIT, or wait-list control [49]. All active treatments (9 twice-weekly individual sessions) decreased PTSD and depression symptoms compared to wait-list but did not differ significantly from each other, and these results persisted at follow-ups (3, 6, and 12 months) [49].

GOOD CLINICAL PRACTICE RECOMMENDATIONS

Therapeutic guidelines offer various and sometimes contradictory recommendations, therefore a critical analysis of the literature is granted to find common management strategies. While several guidelines are more cautious when recommending pharmacotherapy [12] and support a psychotherapeutic-based approach especially in the first stage of the PTSD, others are more focused on drug therapy [13], and still, others consider the type of approach should be selected according to the individual variables, including patient's preference, availability, etc [14].

We formulated GRADE recommendations according to 4 levels: A= high quality, defined as the level to what future research are very unlikely to change the validity of these recommendations; B= moderate quality, meaning that future research is likely to have an important impact on the appreciation of the recommendations; C=low quality, which means that it is very likely that future research will have an important impact over the confidence in the estimate of effect and is likely to change the recommendations; D= very low quality, defined by the uncertainty of any estimate of the effect [4].

A wide range of psychotherapies have been studied for patients with PTSD, but CBT and related techniques (CPT, CT, PE), as well as EMDR, are the most evidence-based approaches [11-15]. We consider a GRADE level A for these therapies is granted, based on the existing evidence. Level B evidence exists for BEP, NET, IPT, PCT, and SIT [31-33, 39-49].

The same level of evidence exists for SSRIs and venlafaxine, as these are the most supported by evidence pharmacological agents in PTSD patients [12-15]. If the first-line agents fail, then second-line agents may be used-mirtazapine, MAO inhibitors or tricyclics, or add-ons like atypical antipsychotics (level B of evidence) [13].

A structured diagnostic approach is strongly encouraged and adequate instruments should be used. Structured interviews (e.g., Mini-International Neuropsychiatric Interview, Composite International Diagnostic Interview, or Schedules for Clinical Assessment in Neuropsychiatry) are considered useful because of the high comorbidity rate met in PTSD patients [50-52]. Because the evolution of PTSD is not linear, even under treatment, detection of new-onset psychiatric disorders is also important because of their high impact over the treatment plan. Therefore, using structured clinical interviews during the first 6-12 months of the treatment is also recommended.

Monitoring treatment efficacy in PTSD using standardized instruments is strongly encouraged. For this purpose, we recommend validated scales, inventories, or structured interviews, like the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5), PTSD Symptom Scale Interview (PSS-I), Mississippi Scale for Combat-related PTSD (MISS) [53-55]. This is considered a good clinical practice because it may increase the objectivity of the psychiatric evaluation, although it is not specifically recommended in all the reviewed therapeutic guidelines.

We consider good clinical practice in the PTSD treatment to actively monitor the tolerability of the pharmacological treatment, to detect early adverse events that may decrease therapeutic adherence [56]. For this purpose, the administration of the Treatment Satisfaction Questionnaire for Medication (TSQM) [57] or a simpler visual analogic scale for self-evaluation is considered useful.

CONCLUSIONS

A review of the existing therapeutic guidelines focused on PTSD management detected several common recommendations. These refer to the use of CBT and related therapies and EMDR as a first-line approach and SSRIs or venlafaxine as the main pharmacologic agents [1-5, 11]. Our team has added as good practice recommendations the necessity to use validated instruments for initial and follow-up diagnostic evaluations, as well as the monitoring of treatment tolerability and efficacy. Because of the high rate of comorbid disorders in PTSD patients, the case manager should be alert to new-onset psychiatric disorders that may appear as complications or just comorbidities in this population.

An integrated, psychological, and pharmacological approach should be considered for these patients, with frequent follow-up visits, and emergent disorders have to be integrated into the case management plan as soon as they are detected.

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Demons-Meigs syndrome – Diagnosis and therapeutic conduct

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Abstract: *Demons-Meigs syndrome is a rare benign pathology that can be confused with other benign and malignant pathologies. The clinical picture is marked by an increase in abdominal volume, pelvic-abdominal pain, but it can also be associated with breathing difficulties due to excess ascites fluid and pleurisy. Imaging paraclinical examination easily reveals the three components: ascites, pleurisy, and ovarian mass.*

Surgical treatment helps to relieve symptoms in a relatively short time.

Keywords: *Meigs, ovarian tumor, imaging examinations*

INTRODUCTION

Demons-Meigs syndrome is represented by three elements: benign ovarian tumor – fibroids, ascites, and pleural effusion. Most often, it is a diagnosis of exclusion. Sometimes, the ovarian tumor can also be represented by thecoma, fibrothecoma, granular cell tumor, less often Brenner tumor. The prevalence and incidence of this syndrome are not fully known; an increase proportional to age was observed, the average of this pathology being around 50 years of age. At the same time, cases of pseudo-Demons-Meigs syndrome were reported in prepuberty in which the benign tumor was represented by another type of ovarian tumor formation (teratoma or cystadenoma). Of the ovarian masses surgically excluded, a percentage between 2 and 5% is represented by ovarian fibroids, and only 1-2% of them meet the criteria necessary for Demons-Meigs syndrome [1].

Demons-Meigs syndrome was first described in 1937 by gynecologist Joe Vicent Meigs, professor at Harvard Medical School, and by the pulmonologist John Class, in the American Journal of Obstetrics and Gynecology. The name Demons associated with the syndrome is given by Albert Jean Octave Demons, in 1887, when he communicates 9 cases of ovarian tumors that associate ascites fluid and hydrothorax [1].

Demons-Meigs syndrome involves the remission of ascites and pleural fluid when a benign ovarian tumor is surgically excluded – this fact is noticed and established by Albert Jean Octave Demons in 1903. Life expectancy after surgical treatment is the same as that of the general population [1].

Ascites was found in 10-15% of patients with ovarian fibromatous formations, while pleural fluid was identified in 1% of them. Pleural effusion is frequently located on the right side – in about 70%, while in 15% it is located on the left or bilateral side [1, 2].

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In addition to this syndrome, the pseudo-Demons-Meigs syndrome has been described, in which, along with ascites and hydrothorax, there is another benign ovarian tumor such as struma ovarii tumor, a teratoma, or a mucinous cystadenoma. The associated tumor may also belong to the salpinx, uterus, or may be a distant metastasis or a gastrointestinal tumor.

Ovarian tumors can be classified into germ cell tumors, gonadal germ cell tumors, and surface epithelial tumors [2].

Germ cell tumors account for 10-15% of all ovarian tumors and can be classified: (mature and immature) teratoma, dysgerminoma, gonadoblastoma, embryonal carcinoma, non-gestational choriocarcinoma. Only mature teratoma and gonadoblastoma are benign tumors [2].

The group of sex cord-stromal tumors includes granular cell tumors, fibroids-thecoma group (95% benign), Sertoli-Leydig cell tumors (5% malignant), gynandroblastoma (always malignant), and steroid cell tumors (70% benign).

Surface epithelial-stromal tumors come from the coelomic epithelium and account for 80-85% of all ovarian tumors. They include serous and mucinous cystadenoma (80-85 % benign), endometrioid tumors, clear cell tumors (which are 95-98% malignant), and Brenner tumor (benign in 98% of cases) [2].

PHYSIOPATHOLOGY

The etiology of ascitic fluid is poorly understood. A possible explanation for its appearance is due to the phenomenon of peritoneal irritation caused by ovarian fibroids. Also, the ovarian tumor could favor the appearance of ascites by the effect on the surrounding vessels represented by both the lymphatic vessels and capillaries. Another explanation would be the discharge of certain cytokines and interleukins that would increase capillary permeability, the result being ascites. Other hypotheses suggest that ovarian tumor torsion or hormonal stimulation may cause ascites. Many authors claim that tumors with an increased diameter of more than 10 cm could stimulate the secretion of ascitic fluid [2, 3].

The etiology of hydrothorax is unclear. The accumulation of pleural fluid is frequently on the right side, but can also be on the left or bilateral side. It is speculated that it was due to the transfer through the transdiaphragmatic lymphatics from the ascitic fluid. The amount of pleural fluid is directly proportional to that of ascitic fluid. The blockage of transdiaphragmatic lymphatics has been found to prevent pleural fluid from accumulating, while the amount of ascitic fluid increases [3].

It has been shown that both ascitic and pleural fluid can be exudative (rich in protein, caused by increased membrane permeability due to inflammation) and transudative (low in protein, by the increase of the hydrostatic pressure and decrease of the colloid osmotic pressure). However, pleural fluid is often exudative. Electrophoresis has shown that pleural fluid and ascitic fluid are of the same nature [3].

Figure 1: Giant ovarian fibroids. A 69-year-old woman who was brought to the Emergency Room of the Central Military Emergency University Hospital for altered general condition, dyspnea with orthopnea, loss of appetite, and enlarged abdominal volume, CA 125 is dosed – it's the value being 2317.7 U/mL

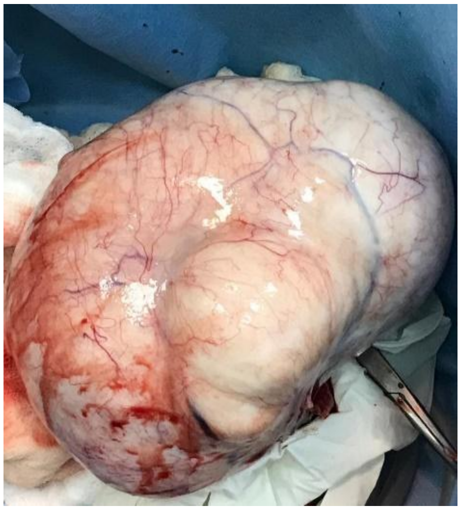

The signs and symptoms of this etiology include altered general condition, physical asthenia, fatigue, dry cough, weight gain, or loss. Among the gynecological signs and symptoms found in Meigs syndrome is amenorrhea, menstrual disorders (menometrorrhagia for women in the perimenopause period or metrorrhagia in the climax), iron deficiency anemia. A unilateral abdominal ovarian mass may be found, more often on the left or bilateral side, solid, asymptomatic, or symptomatic due to the presence of abdominal-pelvic pain, mass effect on surrounding organs [4].

The appearance of ascitic fluid generates abdominal distension, dyspeptic disorders (nausea and vomiting) due to increased intra-abdominal pressure, and constipation.

The appearance of pleural fluid leads to tachycardia, tachypnea, dyspnea with orthopnea, the abolition of the transmission of vocal vibrations, decrease or reduction of vesicular murmur, declivitous dullness to percussion. Pleural effusion is more common on the right side, may be exudative, protein-rich, or transudative. In the case of diagnosis or evacuation paracentesis or thoracentesis, it is found that the fluid does not contain malignant cells even if it is exudative or transudative [5].

The differential diagnosis of Demons-Meigs syndrome is broad and includes benign and malignant pathologies, and most often it is a diagnosis of exclusion.

The main differential diagnosis is with ovarian neoplasm, metastases from a breast neoplasm, colorectal, liver failure, nephrotic syndrome, Milroy's disease, tuberculosis, lung cancer, gastrointestinal tumors.

LABORATORY TESTS

The blood count shows information about hemoglobin, platelet count, and hematocrit. If the hemoglobin level is low, further investigations may be performed including syderemia, ferritin, total iron-binding capacity, and reticulocyte count. Iron deficiency anemia may require iron therapy before surgery or in more severe cases may be corrected by blood transfusion. Treatment with iron-containing supplements is continued postoperatively.

Carrying out clotting time is imperative before surgery. The ionogram is determined by checking and correcting any anomalies [1, 4].

Thoracentesis and paracentesis indicate whether the nature of the fluid is exudative or transudative type, and the cytological examination refutes the existence of malignant cells.

One blood biomarker used is CA 125. It is the antigenic representative of a 220-kD glycoprotein, and the physiological sources are represented by the coelomic epithelium and derivatives: pleura, pericardium, peritoneum as well as the epithelium of the fallopian tubes, endometrium, endocervix. Elevated values of this biomarker can be found in pregnancy, ovarian neoplasm, uterine fibromatosis, endometriosis, pelvic inflammatory disease, cancer of the fallopian tubes, but also in other non-gynecological pathologies (liver failure, kidney failure, autoimmune diseases) [5, 6].

In Demons-Meigs syndrome, there is an increase in the value of CA 125 which does not correlate in this case with the malignancy and which remits after the surgical exclusion of ovarian fibroids.

IMAGING EXPLORATIONS

Ultrasound is an investigation that detects tumor mass. The ultrasound appearance depends on the degree of compromise of vascularity, ovarian formations, and the presence or absence of adnexal hemorrhages. Ultrasound is the method of choice for symptomatic women but is limited in differentiating an ovarian lesion from a para ovarian one.

The radiography confirms and quantifies the existence of pleural effusion.

Figure 2: CT sections in the coronal plane. Tumor formation with probably left ovarian starting point, which includes tissue areas and areas of necrosis; ascitic fluid in increased amounts.

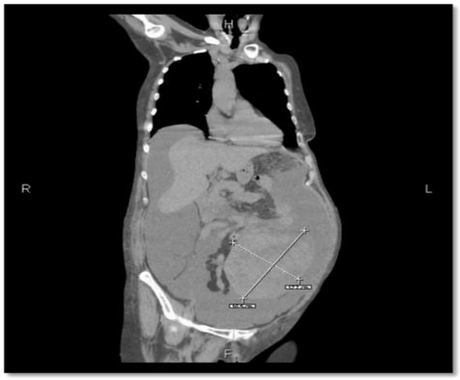

Figure 3: CT sections in the axial plane. Tumor formation with probably left ovarian starting point, which includes tissue areas and areas of necrosis; ascitic fluid in increased amounts.

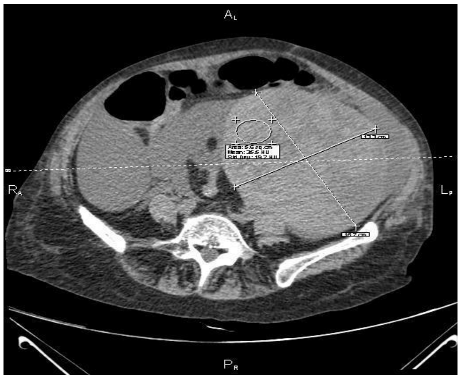

Figure 4: CT section of the thoracic region in the axial plane, parenchymal window. Accentuation of the interstitial lung disease and left pleural effusion.

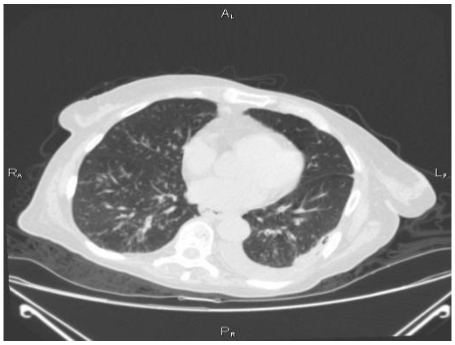

CT exploration of the abdomen and pelvis is a quick way to explore with great accuracy. Serial images formed during the exploration can be viewed on a screen, stored, and with them, three-dimensional reconstructions can be obtained, that provide precise details about the location of the tumor, vascular intake, but also useful information for the surgical approach. It can be done natively or after injecting an intravenous contrast-enhancing agent. CT examination may also be helpful when attempting tumor punch biopsy. The risks associated with this investigation are the cumulative effect of radiation. CT examination is contraindicated in pregnancy. Contrast enhancing agent allergy allows only the performance of native CT. Kidney failure is also a contraindication to the administration of the contrast-enhancing agent. In Demons-Meigs syndrome, CT examination confirms the existence of pleural fluid, ascitic fluid, ovarian tumor mass, and excludes distant metastases or the existence of other neoplasms [7, 8].

Figure 5: CT sections in the axial plane. Heterodense tumor formation with tissue structures and areas of necrosis included, clearly delimited, developed ilio-pelvic on the left side.

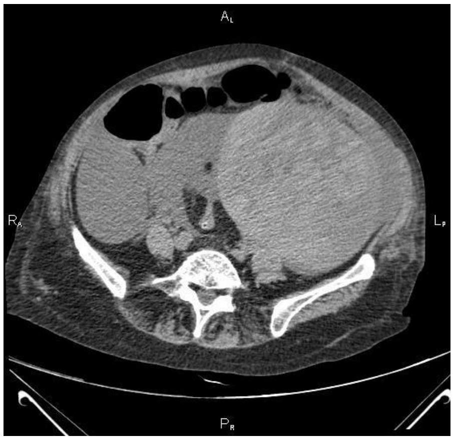

Figure 6: CT sections in the axial and sagittal plane. Heterodense tumor formation with tissue structures and areas of necrosis included, clearly delimited, developed ilio-pelvic on the left side.

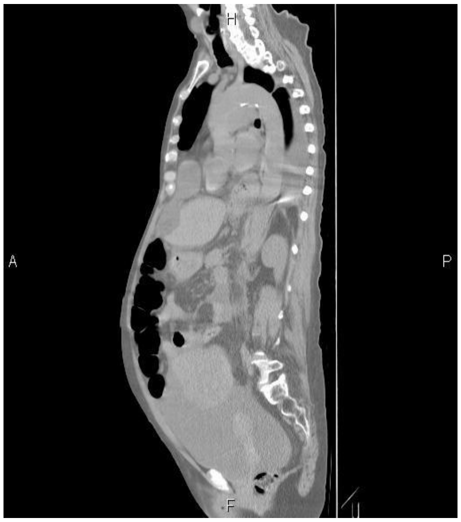

Figure 7: CT section of the upper abdomen in the axial plane. Fat densifications in the supramesocolic space.

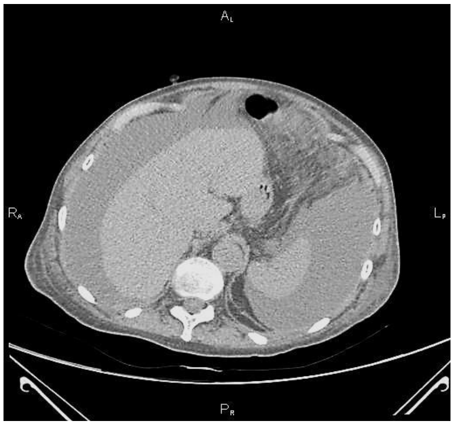

Magnetic resonance imaging characterizes suspicious adnexal masses, establishes ovarian affiliation in inconclusive ultrasound cases, argues in favor of benignity or malignancy of ovarian tumor mass, and specifies other associated abnormalities – peritoneal carcinomatosis, adenopathies.

Figure 8: CT section in the axial plane in the mediastinum. No mediastinal tumor adenomegalies; left pleurisy.

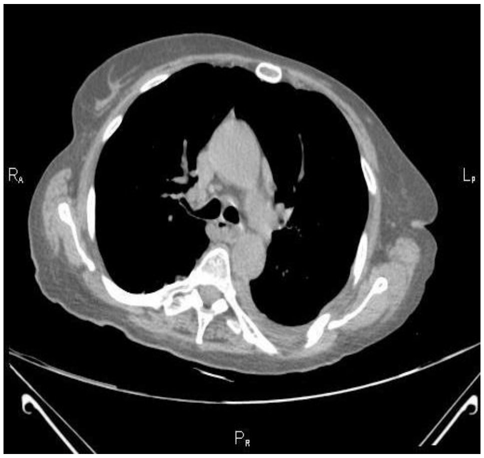

TREATMENT

The treatment of Demons-Meigs syndrome is surgical and is represented by exploratory laparotomy with surgical staging. Biopsy of ovarian mass, omentum, and lymph nodes that reveal benign nature can be performed [7, 9].

If the patient is in the prepubertal period, tumorectomy, partial oophorectomy, and unilateral salpingectomy are used. In women of childbearing potential, surgical treatment involves unilateral adnexectomy. Surgical treatment of menopausal patients may include total hysterectomy with bilateral adnexectomy [7, 8].

Figure 9: Woman 69-year-old – intraoperative: ascitic fluid is found (approximately 500 ml which is collected and sent for histopathological examination); fibroid uterus; right adnexa modified according to age, and at the level of the left adnexa a tumor formation with dimensions of 18/14/10 cm, firm consistency, white-yellowish color, irregular, pedunculated shape. Total hysterectomy with bilateral adnexectomy is performed (with the sending of the samples to anatomopathological examination).

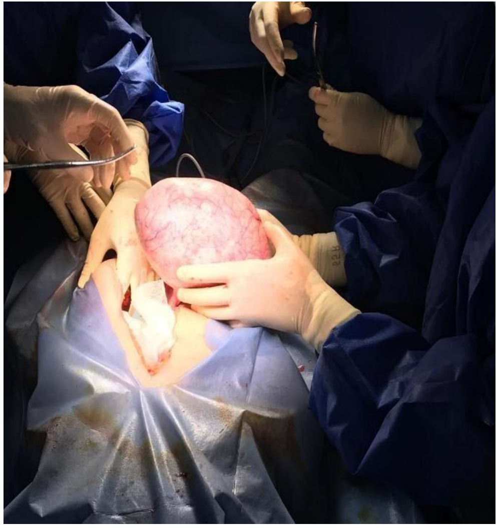

If the patient is in the prepubertal period, tumorectomy, partial oophorectomy, and unilateral salpingectomy are used. In women of childbearing potential, surgical treatment involves unilateral adnexectomy. Surgical treatment of menopausal patients may include total hysterectomy with bilateral adnexectomy.

The cure rate is high and no recurrences have been reported after the disappearance of the ovarian tumor. Ascites and pleural effusion disappear within a few weeks after surgical exclusion of the pelvic mass [7, 8].

Figure 10: Intraoperative image after total hysterectomy with bilateral abdominal adnexectomy for giant ovarian fibroids. The postoperative evolution was favorable under antibiotic, anticoagulant, anti-inflammatory treatment, and the treatment of the underlying pathology. Gradual remission of postoperative ascites and pleurisy was noticed.

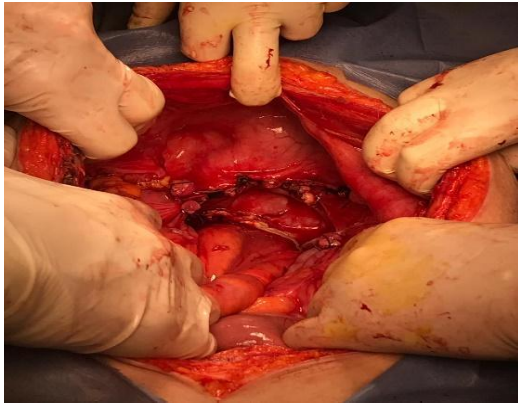

CONCLUSIONS

Demons-Meigs syndrome is defined by ovarian tumor with pleural effusion and ascites. Ovarian tumor is benign and is usually ovarian fibroids. After excluding the ovarian tumor, ascites, and pleural effusion remit. CA 125 tumor marker has elevated values similar to ovarian cancers, but values return to normal after surgical treatment. Even though this syndrome has been described for more than 100 years, it still poses problems in diagnosis, having an uncharacteristic picture. Molecular, hormonal, genetic, and mechanical factors are involved in its etiopathogenic mechanism.

The only treatment available for the remission of symptoms is surgery.

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VARIA

Desert malaria "Curse of the Thar Desert" – a unique geographical endemicity of malaria and combating it with modern technology

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Abstract: *Introduction: This study focuses on the Malaria endemic in the Thar Desert- The most populated desert in the world. Malarial endemicity in a desert environment is a unique and rare geographical presentation, as malaria is usually common in areas where there is rainfall and water stagnation - like ponds, fields, etc. In this study, we want to highlight the unique clinical presentation, lab findings, and reason behind the existence of malaria in the desert. Most importantly we also would like to share our innovative idea to eliminate breeding sites using Quadcopters and drones.*

Materials and methods: Study type – Prospective observation study, Study duration – October 2020 to December 2020, Sample size-103 cases, Inclusion criteria – all smear-positive Malaria cases, Exclusion criteria – Cases Referred to higher centers because of complications.Tools used for breeding site identification – Quadcopters and Drones with HD cameras. Since the area to be surveyed was vast (>30km), it was very difficult to do a manual survey in the harsh desert climate. The patients' demographics, Clinical Presentations, and laboratory parameters were collected systematically in a predesigned format and entered into an excel sheet. The finding of our study was compared with the malaria cases from different parts of the country.

Results: All malaria was identified as Plasmodium vivax. The mean age group was 36 years (min-21 and max-56). Clinical features- out of 103 cases, 78% of the patients presented with all 4 symptoms of fever, chills, headache, and bodyache.22% presented with atypical isolated symptoms of abdominal pain, vomiting, myalgia, severe joint pain. 77% of patients had mild hepatosplenomegaly, 7 patients presented with hypotension, and 2 were in sepsis. Lab parameters – 88% of the patients had anemia, of which 5% had severe anemia, 40% had leucopenia while 6% had leucocytosis.87%had thrombocytopenia, out of which 56% had a platelet count less than 50,000/ml. 2% had mild AKI, bilirubin levels were elevated in 53%, and 36% had transaminitis. Using technology, the survey of the entire area was done in 1 week and all the breeding sites were eliminated on the same day of identification and a drastic drop in malaria cases was noted.

Conclusion: We conclude that desert malarial presentation of Plasmodium vivax is unique in clinical presentation and lab derangements compared to other malarial cases. Usage of technology like quadcopters and drones for breeding site identification played a major role in curtailing cases drastically in a very short period in an effective way.

Keywords: *desert malaria, Plasmodium vivax, drone surveillance for breeding site*

INTRODUCTION

Desert Malaria, yes you have read it right! As commonsense dictates, malaria is common in tropical countries and areas, where the mosquito breeding site is more commonly seen in places where water gets stagnated during the rainy season, e.g., ponds, ditches, and marshy areas which serve as a

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temporary breeding ground for mosquitoes. Malarial endemicity in a desert environment is a unique and rare geographical presentation of this disease.

The Great Desert of Thar is the most widely populated desert in the world, with a population density of 83 people per km^2, which is located in the northwestern part of India [1]. Desert ecosystem, which has its peculiarities to offer, is yet another operating system for malaria.

Malaria is one of the oldest diseases known to mankind. In ancient India, malaria was described in The Compendium of Sushruta, a Sanskrit medical treatise. The World malaria report 2019 estimates that globally, there were an estimated 228 million cases of malaria in 89 countries. The estimated number of malaria deaths in 2018 and 2019 stood at 411,000 lives in 2018 and 409,000 lives in 2019 respectively [2]. In 2012, it was estimated that the total economic burden of malaria in India was around US$ 1940 million, with 75% from lost earnings and 25% from treatment costs borne by households. Besides saving lives, eliminating malaria in India would also avert these socioeconomic losses [3].

Malaria is caused by a protozoan parasite, Plasmodium, and transmitted by the vector anopheline mosquitoes. Malaria parasite in India has two major human malaria species, Plasmodium falciparum, and P. vivax. Approximately 65% of those at risk for becoming infected with malaria in Southeast Asia are individuals residing in India (WHO 2010). The central and eastern regions of India report the most malaria. An. culicifacies is responsible for 60–65% of the malaria burden [4]. The three most important challenges for malaria control in India are difficulties in control implementation and logistics, insecticide resistance, and antimalarial drug resistance. Being the world's second-most populous country, and having a faster rate of urbanization, adds to the challenges. India contributes 77% of total malaria in Southeast Asia. Multi-organ involvement/dysfunction is reported in both Plasmodium falciparum and P. vivax cases [4]. In this study, we did a detailed analysis of the unique presentation of desert malaria and compared it with other studies of malaria presentation in a tropical ecosystem.

MATERIALS AND METHODS

This is a prospective study done from Oct to Dec 2020, where 103 cases of malaria cases were studied.

Inclusion criteria – all smear-positive malarial cases

Exclusion criteria – cases referred to higher centers because of complications.

Patients' demographics, clinical presentations, and laboratory parameters were collected in a systematic method and the collected data was analyzed in windows excel sheet.

For malarial surveillance, we had used quadcopters and drones with HD cameras for regular and periodic surveillance, as it was very difficult to do breeding site surveillance manually due to the extreme temperatures and scattered sites.

The photos from the drone and quadcopters were collected and given to the respective head of units in the areas and necessary action was taken within 24 hours of target identification to eliminate the breeding sites.

RESULTS

103 diagnosed cases of plasmodium vivax malaria were admitted, out of which all were males. The minimum and maximum age of the patients reported was 21 and 56 respectively, with a mean age group of 36 years. Duration of the hospital stay ranged from a minimum of 2 to a maximum of 18 days.

The average temperature of the patients was 101.5 F, maximum temp was 103 F. 78% of the patients presented with all 4 symptoms of fever, chills, headache, and body ache. 22% presented with atypical isolated symptoms of abdominal pain, vomiting, myalgia, severe joint pain. 77% of patients had mild hepatosplenomegaly, 7 patients presented with hypotension, and 2 were in sepsis.

Lab parameters – 88% of the patients had anemia (Hb 9-11 g%), of which 5% had severe anemia (6.5-8.5 g%), 40% had leucopenia while 6% had leucocytosis. 87% had thrombocytopenia, out of which 56% had a platelet count less than 50,000/ml. 2% had mild AKI, bilirubin levels were elevated in 53%, and 36% had transaminitis.

Using technology, the survey of the entire area was done in 1 week and all the breeding sites were eliminated on the same day of identification and a drastic drop in malaria cases was noted. It was a very effective and time-saving modality.

Figures 2 and 3 show open tanks on the roofs of apartment buildings, as well as abandoned open manholes behind these buildings where people don't frequent and were therefore unnoticed by the residents.

One of the main breeding sites of Anopheles could also be the long canals that run for kilometers through the desert, providing a haven for the larvae, as shown in Figure 4.

DISCUSSION

Our results were comparable to that of a study done in south India by Madhu Muddaiahet al. [5], in which males (81%)

outnumbered females (19%) and Plasmodium vivax was the major parasite type (52.54%). In their study incidence of malaria increased from June onwards coinciding with monsoon season, contrary to our study, where caseload was more in October-December. A study by Pankajala Amritha et al. [6], also had 94.71% of P. Vivax cases, and the mean duration of hospital stay was 5.17±3.31 days.

Figure 1: The area identified using a quadcopter. The area seen in the photo was highly inaccessible; Red arrow marks show the sites of waterlogging and the Yellow outlines show the breeding sites of Anopheles mosquito where the larva develops, and the shady environment gives mosquito cover from the extremely hot weather

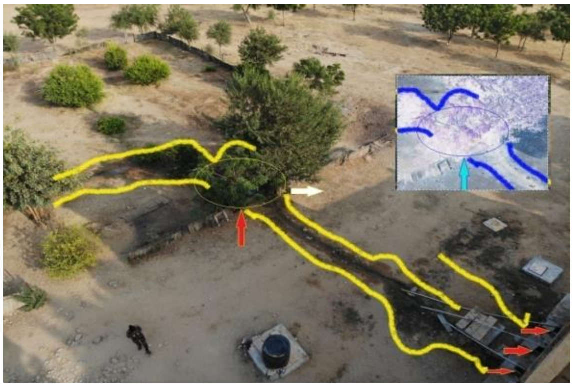

Figure 2

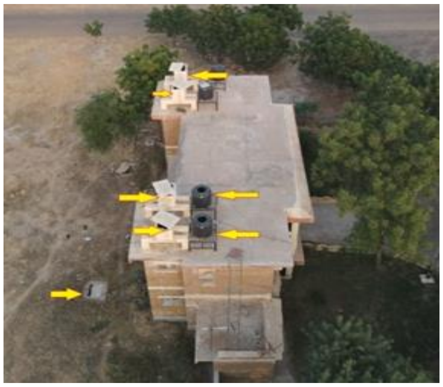

Figure 3

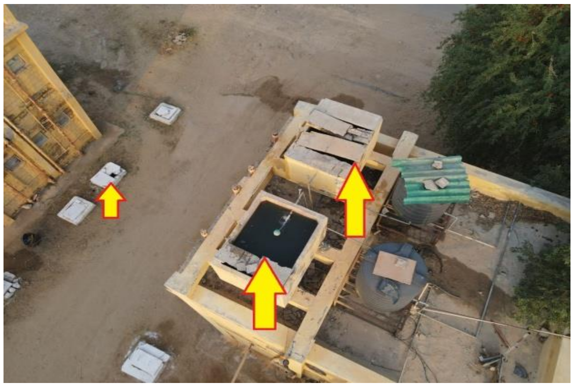

Figure 4

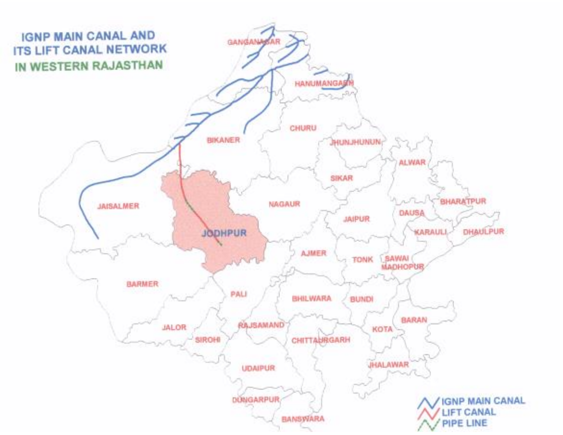
IGNP MAIN CANAL AND
ITS LIFT CANAL NETWORK
IN WESTERN RAJASTHAN
GANGANAGAR
HANUMANGARH
CHURU
BIKANER
JHUNJHUNUN
ALWAR
SIKAR
JAISALMER
NAGAUR
BHARATPUR
JAIPUR
DAUSA
JODHPUR
KARAULI
DHAULPUR
AJMER
TONK
SAWAI MADHOPUR
BARMER
PALI
BHILWARA
BUNDI
BARAN
JALOR
RAJSAMAND
KOTA
SIROHI
CHITTAURGARH
UDAIPUR
JHALAWAR
DUNGARPUR
BANSWARA
IGNP MAIN CANAL
LIFT CANAL
PIPE LINE

In a multicentre study done in Columbia [7], 92.5% of patients presented with fever, chills, headache, sweating, myalgia/ arthralgia, similar to our study. Mild hepatomegaly and splenomegaly as well as abnormal transaminases were mostly observed in P. vivax malaria patients.

A meta-analysis of malarial cases in Malaysia and Australia by Cho Naing et al. [8] provided evidence of the clinical relevance of severe thrombocytopaenia in P. vivax malaria which was in line with our result. In our study thrombocytopenia, anemia, and altered liver function were seen, contrary to study results found by Wajihullah Khan et al. [9] where anemia, jaundice, convulsions, and acute renal failure were significantly high (3-4 times) in subjects infected with P. falciparum than with vivax.

But a in study done in AIIMS Rishikesh by Jagdish Prasad Goyal et al. [10], there was no statistically significant difference found in complications such as anemia and thrombocytopenia between P. vivax and P. falciparum. Similarly in a Columbian study [11], anemia and thrombocytopenia were common in both.

A study was done in Aligarh Muslim University [12] in central India also showed that in P. falciparum infection, the incidences of anemia, splenomegaly, renal and liver dysfunction, and jaundice were higher compared to those in P. vivax infection. Several complications such as acute kidney injury, jaundice, severe anemia, metabolic acidosis, shock, hyperpyrexia, hypoglycemia, generalized tonic-clonic convulsions, etc. were found to be more prevalent in patients with P. vivax infection. These symptoms were until recently known to be associated with falciparum malaria [13].

Malarial surveillance programs by the government are adversely affected by an insufficient number of sanctioned posts of health workers and other program staff in different parts of the country. For instance, there are about 40000 multipurpose health workers (MPWs) against approximately 80 000 sanctioned posts for nearly 150000 sub-centers (SCs) across the country [14]. Over the past fifteen years, the fight against malaria improved dramatically. Insecticide-treated bed nets and indoor residual spraying resulted in an enormous reduction in the number of children dying of the disease [15]. Unfortunately, it is not enough.

For the first time, drones were tested to help fight malaria in Tanzania, on the island of Zanzibar, off the coast of Mozambique. High-tech 'Anti-Malaria Drones' drones were used to spray a silicone-based liquid gel (Aquatain) in endemic areas where malaria-carrying mosquitoes lay eggs, and where there are large concentrations of stagnant water with the hope that the new substance will stop mosquitoes' eggs from hatching. It is part of a program by the Zanzibar government, looking towards eliminating malaria by 2030 [16].

CONCLUSION

We conclude that desert malarial presentation of Plasmodium vivax is unique in its clinical presentation and lab derangements compared to other malarial cases. Doing manual surveillance for breeding sites is very difficult because of the large areas to be covered, the time involved, and the requirement of a lot of manpower. Using drones and quadcopters is a cost and time-effective method which helps in the very early identification and eradication of breeding sites.

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VARIA

Determine the medical causes and diseases which led to early and permanent medical disqualification of military cadets

Azade A. Farahani[1], **Hamze Shahali**[2]

Abstract: *Background and Aim: Efficient manpower is the most important of military capital, and many studies have shown the relationship between personal health and organizational performance. The preparation of military cadet volunteers to enter aeronautical training and the provision of optimal services in the form of well-trained pilots requires many material spiritual resources that disabilities, make them waste.*

Methods: This is a descriptive, cross-sectional, retrospective study with target population of Iranian air force cadets with early and permanent medical disqualification from 1986 to 2016.

Results: 80 cases were detected, including 25 kills and 55 early and permanent medical disqualification. The main causes were ear, nose and throat, psychiatry and cardiac, respectively. Common diseases include motion sickness, generalized anxiety disorder, adjustment disorder and epilepsy, respectively. The total number of lost service years was 1412 person years and the average of lost service years was 25.67 person years. Most of the lost service years were related to medical causes included ear, nose and throat, psychiatric and cardiac, and diseases included motion sickness, generalized anxiety disorder and adjustment disorder, respectively. Neurologic causes resulted in highest average of lost service years and high blood pressure was the major diseases, respectively.

Conclusions: Due to the lack of similar studies, the results of this study were compared with the data, which extracted from previous studies in the pilot's population. Because of occupational similarity between pilots and cadets, It was expected that the most common causes in pilots and cadets should be the same, but we found that the results are very different.

Keywords: *disabilities, medical disqualification, health, performance, prevention*

INTRODUCTION

Personal resources are the most important capital of armed forces, and many studies have shown the strong links between personal health and organizational performance. In order to increase productivity, organizations have anticipated numerous measures to evaluate pre-employment health and staff health during service. However, health risks remain the most important reducing factor for personal productivity. Disability is defined as the effect of a disorder on physical, mental, and social activity of a person and the work environment and family status are also effective elements. [1, 2, 3] Cadets are the future capital for professional aviation and the sufficient understanding of the medical causes and diseases leading to their early and permanent medical disqualification (EPMD), plays an important role in professional pilot training. According to statics, effective prevention of cardiovascular risk factors in aviation population has a significant role in improving the professional health index of pilots. The study of pilot's work environment and their lifestyle, active intervention, periodic

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regular examinations and screening test for early detection of diseases are among the basic measures. [9] Preparation of cadets to enter pilot's training courses and delivering desirable services in the form of expert military pilots requires 10 million dollars and extreme spiritual costs, and as a result disqualification, it will be a major loss of money and human budget for armed forces [21]. Thus, control of this dilemma has no way except of effective prevention. [1-5]

These days, the economic, mental and social problems caused by morbidity and mortality of armed forces are critical burdens. Regardless of the huge cost to run and maintain of powerful army, the financial and psychological burden of the victims can be devastating for any society. [20] In 1993, the United States army paid 500 million dollars to newly recruited armed forces. [6-7] In a study between 1980 and 1994 on the causes of disability in US air and sea forces, it has been shown that the disable armed forces has led to an excessive cost in about 1.5 million dollars for US army. [1] The federal aviation association(FAA) has conducted a great comprehensive study in 2014 on the causes of medical disqualification for pilots whose results indicate fifteen causes include pectoris angina, bipolar mood disorder, cardiac valve replacement, coronary artery diseases, diabetes mellitus, loss of consciousness with unknown reason, seizure, cardiac replacement, myocardial infarction, permanent pace maker, disabling personal disorder, psychosis, drug abuse and dependency and lack of neural control, respectively. [15] Cadets are the future maker of aviation and can become expert pilots in near future. Unfortunately, so far, there has not been a similar published study in the world, which makes it necessary to accomplish this research more effective and strategic.

The ultimate goal of this study was to determine the causes and diseases which led to EPMD of Iranian Air Force (IRIAF) cadets from 1986 to 2016. Other objectives include determining the exact numbers of cadet and aviation trainee kills in IRIAF, determining the lost service years (LSY) in IRIAF cadets with EPMD (with counting of seven years of aviation training course and a total service duration of 30 years in IRIAF), determination of the average LSY for IRIAF cadets with EPMD and the provision of scientific and practical solution to prevent the EPMD of IRIAF cadets.

METHODS

This study is a descriptive-cross sectional-retrospective research that its target population was the IRIAF cadets with EPMD from 1986 to 2016. IRIAF cadets with medical disqualification from 1986 to 2016, that their relevant official documents have been recorded in medical sessions and their medical profiles were included, but cadets with disqualification due to physical or mental illness and death in non-occupational accidents, non-medical reasons, personal requests or disciplinary punishments were excluded from study.

The ethical approval of this study was issued with registration No: 1016794 by the Ethics Committee of the Aerospace and diving medical faculty in Aja University of medical sciences. This study was conducted by the personal expense of the corresponding author and so, no governmental or military fund was not be used at all.

After obtaining administrative licenses (Allow access to information) and ethics approval (being ethical and maintaining the principle of patient privacy) in research ethics council of aerospace and diving medical faculty (which is subset of AJA University of medical sciences) and coordination with relevant authorities in IRIAF department of health, medical education and services for obtain permission of unlimited access to medical profiles and sessions, in order to maintain the medical confidentiality, the archivist assigned a secret and random code to each participant that was recorded on top of the electronic data forms. Required information including age, year of entry to and excite from service, main medical cause and disease which led to EPMD were inserted in an electronic form designed according to the study requirements. An individual archivist was used to enter data and in order to military and national information security, the total number of IRIAF cadets was not stated.

All of the IRIAF cadets were male and, in few cases, which there were more than one reason for EPMD, only the main cause was considered. Like other similar studies, all medical causes and diseases were classified based on the 10th version of international classification of diseases (ICD-10) and the causes in domain of nephrology and urology have been recorded as renal causes. However, possible differences in the statistics of similar studies may be related to their classification methods for causes and diseases. It should be noted that in any armed force, while training or real battle, casualties are inevitable and so killed cadets are not considered to be among the lost years of service. The data was collected and analyzed by the researcher using Microsoft office software (version 2016) and compared with the results of similar studies in pilot's population.

RESULTS

During the period from 1986 to 2016, 80 cases of early and permanent discharges were obtained from all the medical sessions and medical profiles, including 25(31.25%) kills and

55(67.75%) medical disqualification. The kills also included 17 cadets and 8 aviation trainees. This data are given in Table1.

Table1: Study Data

Occupation	Early and Permanent Discharge	Kills
Cadets	55	17
Aviation Trainees	0	8

Common causes of EPMD in IRIAF cadets included ear, nose and throat (ENT), psychiatric and cardiac, respectively. Their prevalence is depicted in Figure1. Common diseases resulted in EPMD of IRIAF cadets included motion sickness, adjustment disorder, epilepsy and, respectively and shown in Table2.

Figure 1: Causes lead to the EPMD of IRIAF cadets

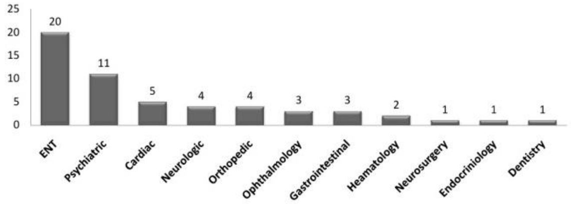

Table 2: Diseases lead to EPMD of IRIAF Cadets

Diseases	Motion Sickness	Generalized Anxiety Disorder	Adjustment Disorder	Epilepsy	High Blood Pressure	Others
N#	18	5	3	3	2	24

As 1412 LSY in IRIAF cadets, the most common causes include ENT, psychiatry and cardiac, respectively. More detail is on Figure2.Common diseases with most of LSY include motion sickness, generalized anxiety disorder, epilepsy, adjustment disorder, and high blood pressure, with detailed statistics in Table 3.

Figure 2: LSY of IRIAF cadets with EPMD according to causes

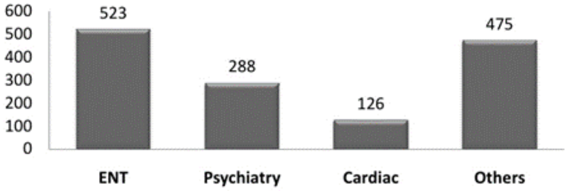

Table3: LSY of IRIAF Cadets with EPMD According to Diseases

Diseases	LSY
Motion Sickness	468
Generalized Anxiety Disorder	137
Epilepsy	82
Adjustment Disorder	74
High Blood Pressure	57
Others	594

The average of LSY in IRIAF cadets with EPMD was 25.67 person years (p/y), the frequency o which based on common causes, including neurology, psychology and ENT with comprehensive information shown in Figure3.

Common diseases resulted in the highest average of LSY in IRIAF cadets with EPMD including high blood pressure, occupational hearing loss, generalized anxiety disorder, epilepsy, and borderline personality disorder, as shown in Table4.

Figure 3: Average LSY of IRIAF cadets with EPMD according to causes

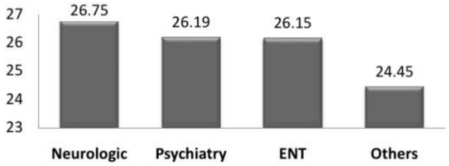

Table4: Average LSY of IRIAF Cadets with EPMD According to Diseases

Diseases	Average of LSY
High Blood Pressure	28.5
Occupational Hearing Loss	27.5
Generalized Anxiety Disorder	27.4
Epilepsy	27.33
Borderline Personality Disorder	26.5
Others	24

DISCUSSION

So far, there has been no investigation into the causes and diseases that lead to EPMD of military or civil cadets in Iran or the world. Therefore, in order to compare the results of this study, the studies in civil and military pilot's population were used, because cadets and pilots have similar occupational and social status. The main reasons for EPMD in IRIAF cadets during our study included ENT, psychiatric, and cardiac, respectively. In the study of Whitton-1984 on United State Air Force (USAF) pilots and navigators, common causes were cardiac and neurologic [16], while in the same study of Mc Crary-2002, the most common causes in 1995 to 1999, include cardiac, musculoskeletal, neurologic and endocrinology. [11] Also, in the study of Montazeri-2005, the most common causes of EPMD in IRIAF pilots included cardiac and musculoskeletal. [19] In the study of Ghazizade-2010 on the same population in 1992 to 2003, cardiac, nervous-skeletal and gastrointestinal were the most common causes. [18]

In the Dark study with the help of FAA, on medical disqualification of American airlines pilots in 1983 and 1984, the most commonly reported causes were cardiovascular and Neuropsychiatric events. [18] In the Nakanishi study on permanent disability of 260 Japanese crews, significant causes include malignancies, neurodegenerative, cardiovascular, gastrointestinal and musculoskeletal disorders. [8] In the review of trained pilots on Commonwealth countries from 1994 to 2004, the causes of cardiovascular were prevalent. [18] In the Arva study on 257 Norwegian civil pilots, the main causes were cardiovascular, neurologic, musculoskeletal and psychiatric disorders. [10] In the studies of Mitchell-2004 and Evan-2006 on sudden incapacitation of British civil pilots, 36 cases have been diagnosed and half of them were due to cardiac and cerebrovascular disorders and psychiatric disorders have been one of the leading causes. [12, 13] In the Nezami study on 200 civil pilots of Vnukovo airport in Moscow, cardiac, neurologic and digestive have been the main causes of medical disqualification. [9] Common diseases resulted in EPMD of IRIAF cadets, including motion sickness, generalized anxiety disorder, adjustment disorder, epilepsy and high blood pressure. In the Mc crary's review on USAF pilots and navigators in 1995 to 1999, more common diseases were ischemic heart diseases, high blood pressure, back pain, inter vertebral disc disorders and migraine. [11] In the study of Nezami on 200 civil pilots of Vnukovo airport in Moscow, ischemic heart diseases, myocardial infarction, high blood pressure, cerebrovascular sclerotic lesions and peptic ulcer have been common. [9]

The LSY was 1412 (p/y) and the highest was related to ENT, psychiatric and cardiovascular causes, respectively. Diseases with the highest LSY included motion sickness, generalized anxiety disorder, epilepsy, adjustment disorder and high blood pressure. The average of LSY was 25.67 (p/y) and the highest was related to neurologic, psychiatric and ENT causes, respectively. Diseases with the highest average of LSY included high blood pressure, occupational hearing loss, generalized anxiety disorder, epilepsy and borderline personality disorder. In the study of Montazeri-2005, the mean LSY of IRIAF retireds with EPMD was 6.5(p/y). [19] In the Ghazizade study on IRIAF medical disables from 1992 to 2003, the mean LSY was 6.14 (p/y) and the highest was 10 (p/y). [18]

This study, like other studies, has limitations that include: 1) the lack of a comprehensive electronic system for record of medical profile, 2) the possibility of oriented malingering in disorders which have not specific objective diagnostic methods and their detection are subjective, such as motion sickness, migraine, irritable bowel syndrome and etc, and 3) flying device factors(ergonomics and exhaustion), operational and airbase factors(work and rest schedule), Personal factors(life style, physical and psychological capabilities, socioeconomic state, use of legal and illicit drugs and habits), medical factors(medical and psychological advices and hygiene education) are important items which have effective influence on cadets health.

The present study has important benefits such as: 1) at yet, this is the first study on EPMD of military cadets in the world, 2) it has the longest cross-sectional period (35 years) that other longest studies has a period of 10 years, and 3) data presentation in order to kills of cadets and aviation trainees.

At the end, we are suggested that, while it is necessary to conduct similar studies in military and civil cadets and pilots (ever-in non-pilots' flight crew), this advises were recommended to prevent the recurrent medical dispensation of military cadets:

1) Facilitate the comprehensive electronic system for record military cadets' medical profile
2) Upgrading of on-the-job examinations of cadets based on the real requirements
3) Sufficient periodic in-service examination of cadets
4) Lifestyle modifications (habits, nutrition, smoking, use and abuse of drugs and substances, physical exercise etc)
5) Use of medical and psychological counseling (motivation, stress management, high-risk behaviors, risk aversion)
6) Hygiene and health education
7) Regular fitness-based training
8) Updating of flying systems, operational devices and air base facilities (ergonomics, work and rest schedule)
9) Effective and powerful preventive systems with regular updating
10) Improvement of services, which provide with occupational health centers.

List of Abbreviations

Early and Permanent Medical Disqualification (EPMD)
Permanent Medical Exchange in Service categories (PMES)
Iranian Air Force (IRIAF)
Lost Service Years (LSY)
Person Years (p/y)
Federal Aviation Association (FAA)
Myocardial Infarction (MI)

10th International Classification of Diseases (ICD-10)
Generalized Anxiety Disorder (GAD)
Post Traumatic Stress Disorder (PTSD)
Ear, Nose and Throat (ENT)
United State Air Force (USAF)

Declarations

Ethics approval and consent to participate:
The ethical approval of this study was issued with registration N# 1016794 by the Ethics Committee of the Aerospace and diving medical faculty.

Consent for publication:
I (Dr HamzeShahali as Corresponding Author), on behalf of the authors, expresses my satisfaction with the publication of this study in that valuable journal.

Availability of data and materials:
All the data of this study is available and could be accessible for publication by that valuable journal.

Competing interests:
I declare that there is no competing interest about the authors of this study, at all.

Funding:
This research was my special doctorate thesis and so, all the expenses were paid personally. I declare that I did not use any government funds to conduct this study, at all.

Authors' contributions:
Title Presentation, basic researching, approval the research subject, obtaining the necessary permits, collecting data and writing the paper was conducted by Dr HamzeShahali as Corresponding Author and Data analysis, editing and writing part of the article was done by Dr AzadeAmirabadi Farahani as Co-Author, too.

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VARIA

Developing a conceptual model for healthcare services branding in the military hospital

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Abstract: *Introduction: Given fast grow and competition in the healthcare industry, managers and health activists in this sector need to adopt strategies for branding. The purpose of the study was to develop a conceptual model for branding in the healthcare industry to build brand advocacy and positive brand image.*

Methods: The study includes patients referred to a military hospital in May 2018. Data gathered by questionnaire, and 162 surveys were collected by simple random sampling method. To test the hypotheses, the conceptual model was analysed by structural equation modeling with SmartPLS® software.

Results: The affective commitment and brand trust could predict brand advocacy. CSR significantly impacts brand image and affective commitment, furthermore brand attitude and COB significantly impact brand trust. There was no significant relationship between brand trust and affective commitment with brand image.

Conclusion Organizations can improve brand image and establish brand support through investing resources on branding. This study contributes insights for marketing managers in developing successful branding strategies for health care organizations.

Keywords: *branding, health care industry, hospitals, SEM*

INTRODUCTION

Healthcare services are dynamic, competitive and full of turbulence. The number of hospitals and healthcare providers rising continuously and with this situation, the atmosphere becomes more and more competitive. Furthermore, in Iran, hospitals can't advertise themselves (It's a law) for gaining more patients and customers (Maleki, 2016). In the other view, measuring the efficiency of healthcare services is depending on lots of quantitative and qualitative criteria (like capable employees, behavioural and technical interactions, treatment matter, type of patient, public and private services) and because of that, it is hard to measure (Thantry et al., 2006).

Due to the hard situation for hardware and material distinction, organizations prefer to be distinct in software (Kotler & Keller, 2012). Huge different exists between services and product usage and because of that, considering people who use them, is different and need other conceptualization (Wang et al., 2009; Van Riel et al., 2001). Moreover, a brand in the service sector corresponds with "organization" whereas in the production sector correspond with "product" and according to this, conceptualizing in the service sector is essential (Wang et al., 2009; Chahal & Bala, 2012). When a high value of satisfaction and emotional commitment is issue, branding in hospitals that refer to customer needs is crucial (Kemp et al., 2014).

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Health care is one of the most critical services that a patient receives (Kemp et al., 2014). Like brand in product sector that has socio-psychological attributes, for service sector, brand is same and can be inspiring. This means there is a bond between brand and customers (Kemp et al.). The relationship between brand and customer is binary. Developing relationships between brand and customer can be a challenging procedure and in a different pattern. For example, it is possible that nexus among brand and customer is according to simple or emotional cognition (Park et al., 2009; Thomson et al., 2005; Brakus et al., 2009; Grisaffe & Nguyen, 2011). When there is a strong relationship between brand and customer, the organization has a significant advantage in competitive atmosphere and also can immune organization from rivals (Kemp et al.).

The progress of healthcare services and economic development are closely linked; reaching one without other is impossible (Khodadad & Behboudi, 2017). Branding in healthcare services is a newfound phenomenon, but managers are gaining intuition from its competitive advantage. Meanwhile, hospitals as an expensive part of the healthcare industry that assign a high portion of GDP, need more attention and considering (Nasiripour et al., 2010). Branding plays a vital role in service organizations through increasing customer trust, make a better condition to illustrate services, acts as a mean of competitive advantage and gives value to the customers (Chahal & Bala, 2012). Branding in healthcare services requires a strong and organized commitment to presenting unique services. For having successful branding, managers should have a strategy for maintaining and guiding values for making trust and also managing emotion (Kemp et al., 2014; Mangini, 2002).

The purpose of the study was to develop a conceptual model for branding in the healthcare industry to build brand advocacy and positive brand image.

METHODS

This descriptive study surveyed patients who went to a military hospital in 2018 may, using simple random sampling. To determine the minimum sample size before gathering data Kock and Hadaya (2018) suggest the use of the inverse square root method, which estimates 160 samples. After distributing the questionnaires, 162 samples were gathered. The questionnaire that distributed had two parts, first demographic questions and then 28 questions that evaluate survey variables in Likert-5 scale. The research constructs were developed based on prevalidated measures (Table 1). Instrument items were modified by experts in any aspects and rephrased.

Table 1: Validity and reliability

Construct	Cronbach Alpha (α)	Composite Reliability (CR)	Average Variance Extracted (AVE)	Source
Corporate Social Responsibility	0.856	0.920	0.700	Kemp et al, (7)
Brand Attitude	0.849	0.906	0.710	Kemp et al, (7)
Customer-oriented Behavior	0.9	0.929	0.768	Kemp et al, (7)
Affective Commitment	0.883	0.936	0.831	Kemp et al, (7)
Brand Trust	0.890	0.932	0.821	Kemp et al, (7)
Brand Image	0.809	0.872	0.583	Wu (67)
Brand Advocacy	0.739	0.869	0.623	Kemp et al, (7)

After data gathering, single variable and two variables measurement analyses with SPSS® version 25 software and analysis in structural equation modeling calculated with Smart-PLS® version 3.2.7 (Ringle et al., 2015). Cronbach alpha (recommended threshold ≥ .7) (Cronbach, 1951) and composite reliability (CR) (recommended threshold ≥ .7) (Hair et al., 2016) estimated for reliability and average variances extracted (AVE) (recommended threshold ≥ .5) (Fornell&Larcker, 1981) estimated for validity with Smart-PLS®. Results of Validity and Reliability analysis (Table 1) exemplified acceptable value as exceeded recommended threshold, which is an indication of a validated and reliable instrument.

RESULTS

Researchers can figure out data essence with descriptive results. Results of descriptive analysis like gender, age, education, insurance and hospital familiarity (or use duration) are in Table 2.

Table 2: Demographic statistics

Measure		Frequency	Percentage
Gender	Male	94	53.4
	Female	82	46.6
Age	Under 20	8	4.5
	21-30	54	30.7
	31-40	47	26.7
	41-50	33	18.8
	Above 50	34	19.3
Education	Under diploma	40	22.7
	Diploma	50	28.4
	Bachelor	56	31.8
	Master	24	13.6
	Doctoral	6	3.4
Having Insurance	Yes	170	96.6
	No	6	3.4
Time of being hospital customer or (familiarity)	Under 1 year	78	44.3
	1 year	30	17
	2 years	21	11.9
	3 years	10	5.7
	Above 3 years	37	21

Most participants were male (53.4%), 30.7% were 21-30 years old, 31.8% had bachelor degree, almost every participant has insurance (96.6%), and 44.3% of participants were being hospital customer under one year. Constructs averages and standard deviations are in Table 3.

Table 3: Average and Standard Deviation of research constructs

Construct	Average	Standard Deviation
Corporate Social Responsibility (CSR)	3.806	0.971
Brand Attitude (BA)	4.117	0.514
Customer-oriented Behavior (COB)	3.458	1.239
Affective Commitment (AF)	3.448	1.266
Brand Trust (BT)	4.152	1.06
Brand Image (BI)	4.190	0.793
Brand Advocacy (BA)	4.024	0.743

In two variable analyses, researchers used Spearman coefficient correlation. Results of this analysis are in Table 4.

Results of assessing hypotheses using structural model in smart-PLS® (Ringle et al., 2015) (standard and T-values, values in parenthesis in the model) are in Figure 1 which shows H1a, H1b, H2a, H2b, H3 and H4 confirmed and H2c, H5 and H6 rejected. Results are in Table 5.

Figure 1

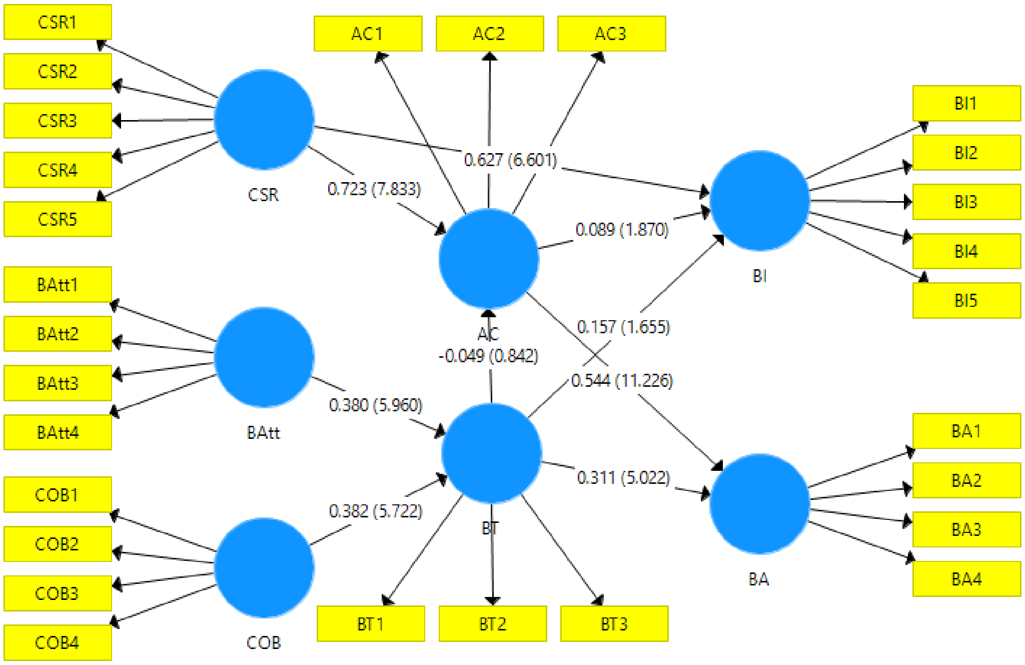
CSR1
CSR2
CSR3
CSR4
CSR5
AC1
AC2
AC3
BI1
BI2
BI3
BI4
BI5
CSR
0.723 (7.833)
0.627 (6.601)
0.089 (1.870)
BI
AC
-0.049 (0.842)
0.157 (1.655)
0.544 (11.226)
BAtt1
BAtt2
BAtt3
BAtt4
BAtt
0.380 (5.960)
0.382 (5.722)
0.311 (5.022)
BT
BA
BA1
BA2
BA3
BA4
COB1
COB2
COB3
COB4
COB
BT1
BT2
BT3

Structural model assessment: For assessing quality of structural model, R2 considered as criteria for assessing model (Hair et al., 2016) and results show that all variables are more than average (0.33 is the mid number). Table 6 shows the values.

Table 4: Spearman Correlation coefficient, all the numbers are significant at 0.01 level

Construct	CSR	BA	COB	AF	BT	BI	BA
Corporate Social Responsibility	1						
Brand Attitude	0.602	1					
Customer-oriented Behavior	0.501	0.262	1				
Affective Commitment	0.598	0.436	0.334	1			
Brand Trust	0.775	0.467	0.500	0.485	1		
Brand Image	0.712	0.477	0.463	0.481	0.637	1	
Brand Advocacy	0.666	0.445	0.379	0.701	0.627	0.571	1

Table 5

Hypothesis	Independent variable	Dependent variable	Path coefficient (β)	T-value	P-value	result
H1a	Corporate Social Responsibility	Brand Image	0.627	6.601	<0.01	Confirmed*
H1b	Corporate Social Responsibility	Affective commitment	0.732	7.833	<0.01	Confirmed*
H2a	Brand Attitude	Brand Trust	0.380	5.960	<0.01	Confirmed*
H2b	Customer-oriented behavior	Brand Trust	0.382	5.722	<0.01	Confirmed*
H2c	Brand Trust	Affective Commitment	-0.049	0.842	0.420	Rejected
H3	Affective Commitment	Brand Advocacy	0.544	11.226	<0.01	Confirmed*
H4	Brand Trust	Brand Advocacy	0.311	5.022	<0.01	Confirmed*
H5	Affective Commitment	Brand Image	0.089	1.870	0.059	Rejected
H6	Brand Trust	Brand Image	0.157	1.655	0.109	Rejected

* Significant at 99%

Table 6: Model assessment results

Construct	Average
Affective Commitment	0.446
Brand Trust	0.346
Brand Image	0.657
Brand Advocacy	0.553

Finally, according to Tenenhaus, the goodness of fit measured and according to formula

$$\mathrm{GOF} = \sqrt{\mathrm{Communality} \times \mathrm{R}^2} = 0.492$$

calculated that exhibits well goodness of fit (Tenenhaus et al., 2004).

DISCUSSIONS AND CONCLUSIONS

This research aimed to find a pattern for branding in the healthcare industry. Hence, variables like corporate social responsibility, brand attitude, customer-oriented behavior, affective commitment, brand trust, brand image, and brand advocacy recognized and the relationship between them through a conceptual model is investigated. Results showed corporate social responsibility has a significant effect on emotional commitment and brand image; these results are in accordance with recent researches (Kemp et al., 2014; Alrubaiee et al., 2017; Kolade&Ogunnaike, 2014). Brand image mostly previses corporate social responsibility. Therefore, by developing corporate social responsibility, brand image become better in customer mind, and marketing performance will improve. Besides, managers can determine policies and strategies in marketing more efficient due to this results and structures. Furthermore, it is crucial for hospitals to invest and help society, which can create an emotional bond and affective commitment.

According to the results, brand attitude and customer-oriented behavior predicted brand trust, and this is following Kemp et al. research (Kemp et al., 2014). Thus, creating a positive brand attitude and customer-oriented behavior from employees will improve brand trust. Educating employees for having better conduct and behavior with customers can improve customer-oriented behavior. Since the healthcare industry is a personal service and a close relationship exist between parties, trust is crucial due to personal information, mental and physical status exposure to healthcare providers (Kemp et al.).

Brand trust could not predict affective commitment that is in the opposite of previous surveys (Kemp et al., 2014; Patawayati et al., 2013; Feiz et al., 2016). However, it could predict brand support that is in accordance with previous researches (Kemp et al.; Moreira & Silva, 2015; Ansari &Mohammadi, 2016). Results showed with brand trust rise up; brand support will rise simultaneously. Hospitals should focus on high trust level in marketing (Kim et al., 2004). When a customer trust in healthcare service provider, it is possible that he/she continues to use that service provider and suggests it to others. Hospitals should use their sources to invest in developing more trust for creating more brand advocacy. Results showed affective commitment could predict brand advocacy and it is in accordance with previous surveys (Kemp et al.; Moreira & Silva; Feiz et al., Ansari &Mohammadi), so by developing an emotional relationship with customer, he/she becomes a brand advocator and finally, competitive advantage will be created.

This research demonstrates the advantages of branding and establishing a meaningful relationship with customers. Results of this research can help marketing managers and strategic planners. In the healthcare industry, vicinity with a hospital is a key factor for productivity (Beckham, 2000). Nevertheless, with increasing healthcare costs and more options for customers, marketers can act more strategic through creating competitive advantage, and distinguishing customers' needs (Brakus et al., 2009).

Healthcare organizations should effectively put their organization and its brand as a valuable factor in providing unique services. As proposed in this research, corporate social responsibility, affective commitment, customer-oriented behavior, customer engagement (like customer relationship management) and trust, are significant elements to reach this goal.

Although this study can help in creating and developing brands in healthcare services, future research and surveys in this field are imperative. Several limitations have existed in this study. First, as data gathered in a cross-sectional way, no cause and effect relationship can be established about research variables. Investing in a relationship between latent variables through longitudinal data is suggested. Future studies can investigate how the correlation between performance and actual satisfaction can create a connection between brands and consumers. Second, some discordance observed between the results of this research and literature, so the research results should expand cautiously. Third, respondents to questionnaires are from the same region so, gathering data from diverse regions is suggested. Forth, in this study, healthcare providers were limited to hospitals, more scopes like pharmacy, clinics, medical imaging, laboratories, and dental services should be investigated. Finally, respondents may somehow were not in their best physical and mental condition, so this maybe affects the data gathering and analysis.

At least once in a lifetime, people use healthcare services, and because of that, good experience and relationship with providers are crucial. As changing perspective in healthcare services in future years, research opportunity in strategical branding and marketing fields will be plentiful for healthcare service providers.

Conclusion Organizations can improve brand image and establish brand support through investing resources on branding. This study contributes insights for marketing managers in developing successful branding strategies for health care organizations.

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ORIGINAL ARTICLES

Development of quantitative real-time RT-PCR assay for detection and viral load determination of Crimean-Congo Hemorrhagic Fever (CCHF) virus

Mojtaba Sharti[1], Mohammad S. Hashemzadeh[1], Ruhollah Dorostkar[1]

Abstract: *Background and Aim: The CCHF (Crimean-Congo hemorrhagic fever) virus causes a severe disease in human with a case fatality rate of up to 50%. Since, there is no specific treatment or approved vaccine against CCHF viral infections, an accurate and early detection as well as a reliable surveillance and quantitative determination of viral load is necessary for patient improvement and case management. In this research, our aim was to develop a probe based one-step real-time reverse-transcription polymerase chain reaction (rRT-PCR) assay for in-house quantitative detection of CCHF virus.*

Methods: At first, the highly conserved S-fragment sequence of CCHF virus genome was adapted from GenBank and the specific probe and primers targeting this region were designed. Then, viral RNAs were extracted from 37 blood samples of different patients from east of Iran (Zahedan). The specificity and sensitivity of the probe and primers were also evaluated in positive blood samples, confirmed to have CCHF virus. A standard (PTG19-T vector containing S-fragment) for quantization was also constructed and the viral load was determined in some of positive samples.

Results: From a total of 37 suspicious blood samples, 15 samples were confirmed to be positive for CCHF virus by this probe based one-step rRT-PCR assay and no false-positive result was detected according to sequencing data. The predicted fragment of 176 bp was also confirmed in all positive samples by gel-based electrophoresis analysis. The assay was linear between 10 to 103 copy numbers per each microliter of extracted plasmid for this technique and the viral load determined in one of patient blood samples was 55,000 viral particles per each milliliter, for example. Bioinformatics and experimental evaluations approved the specificity of this assay. The LOD of the assay was 10 (or fewer) copy numbers of viral genome per each microliter of the extracted genome.

Conclusions: This research showed that the developed probe based one-step rRT-PCR assay is a specific, rapid, sensitive and the simple tool for detection and viral load determination of the CCHF virus.

Keywords: *CCHF Virus, Real-time RT-PCR, Quantitative, viral load, S-region*

INTRODUCTION

The CCHF (Crimean-Congo hemorrhagic fever) is a widespread disease caused by Nairovirus (a tick-borne virus) of the Bunyaviridae family. The CCHF virus causes a severe disease (a zoonotic viral hemorrhagic fever) in human and is endemic in Africa, the Balkans, Asia, and Europe [1-6]. In Iran, this virus has also caused serious challenges in the Sistan-va-Baluchestan province [7-15]. The fatality rate is about 10-50% [16, 17]. Since, there is no specific treatment or approved vaccine against CCHF viral infections, an accurate and early detection as well as a reliable surveillance and quantitative determination of viral load is necessary for case management, patient improvement, and protection of medical staffs.

Several different laboratory assays can be used for CCHFV diagnosis, including enzyme-linked immune sorbent assay (ELISA), serum neutralization, antigen detection, virus

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isolation by cell culture and reverse-transcriptase polymerase chain reaction (RT-PCR) assay [4, 18-21]. CCHFV RNA from different clinical samples is a proper diagnosis target during the acute phase of the infection or even before the beginning of disease when detection of antibody is untrustable or impossible [22-24].

Unlike the traditional two-step RT-PCR technique, the one-step real-time RT-PCR (rRT-PCR) assay does not need to pre-PCR (post-RT) sample handling, avoiding the PCR product dependent probable contamination transmission and resulting in more sensitive, rapid and also higher efficient assays. On the other hand, the probe based rRT-PCR assays have a very high specificity in addition to the mentioned features. Therefore, it has become an accepted recognition method for detecting many microbes [25]. However, in this research we developed a quantitative probe based one-step rRT-PCR assay for detection and viral load determination of the CCHF virus in suspected blood samples of the Sistan-va-Baluchestan prov¬ince of Iran. The aim of this experimental study was to develop a probe based one-step rRT-PCR assay for in-house quantitative detection of the CCHF virus.

METHODS

Collection of sample

To perform this study, we provided 37 blood samples of different CCHF suspicious patients from the Zahedan city in the southeast of Iran. A few samples had already been confirmed to be positive for CCHF viral RNA existence by rRT-PCR analysis (Pasteur Institute, Tehran, Iran) that were also used as the positive control and they had been stored at -70°C.

Extraction of viral genome

Viral genomes were extracted from 0.2 ml of blood samples using the High Pure Viral RNA Kit (Roche-Germany) in 30 minutes, according to manufacturer's instructions. Then, each one of the extracted genomes were dissolved in 50 µl of RNase free water and stored at -70°C until rRT-PCR analysis (of which only 5 µl was required in each assay). It is noteworthy that all of manipulations were performed in a biological hood with class II biosafety.

Designing and synthesizing the specific probe and primers

The nucleotide sequence of S-region of the available CCHFV strains was adapted from the NCBI database (GenBank) and the highly conserved sequences in this region were determinated and specified from the alignment data analysis obtained from MEGA 7 software. Then the specific probe and primers targeting this region were designed using the Oligo software, version 7.0 (Molecular Biology Insights, Cascade, CO, USA) and GeneRunner (Hastings Software, Hastings, NY, USA).

The probe sequence designed for S-region internal sequence was FAM-5'-CAA AAC AGG ATC TAC ATG CAC CCT GCC-3'-BHQ as well as the forward and reverse oligonucleotide primers for S-region internal sequence were 5'-TGG GGK AAG AAR CTY TAT GAG CT-3' and 5'-ATG GAC TTG GTR TGY CCA GAT CC-3', respectively. K, R and Y are the wobble bases. Eventually, the probe and primers were synthesized by the Bioneer Company (Korea).

One-step real-time RT-PCR assay

The probe based one-step rRT-PCR assays were developed by use of QuantiTect® Probe RT-PCR Kit (Qiagen, Hilden, Germany) using both the Applied Biosystem (ABI) 7500 (USA) and Corbet (Rotor-Gene) 6000 (Germany) real-time PCR instruments. The amplification process was performed in 60 minutes within 20 µl reaction mixtures including 5 µl of the viral RNA template, 1 µl (10 pmol) of both primers (0.5 µM in the reaction) and 0.5 µl of the probe (0.25 µM in the reaction). The conditions of real-time cycler were as follows: two single steps of 30 minutes at 50°C (required for reverse transcriptase activity) and 15 minutes at 95°C (required for HotStarTaq DNA polymerase activation); followed by 50 cycles of 15 seconds at 94°C; and 60 seconds at 60°C (collecting the fluorescence data was accomplished at the end of each 60°C step). So, the real-time PCR products were detected trough an increase in the fluorescence intensity from cycle to cycle. Cycling curves were evaluated with respect to negative and positive controls (as the quality controls of the process) in the real-time analysis. The rRT-PCR products were then approved by gel-based electrophoresis.

Evaluation of sensitivity and specificity of the assay

Analytical sensitivity

To determine the LoD (limit of detection) of the assay, serial dilutions containing 1, 10, 100 and 1000 copies of the positive control sample (plasmid) per each microliter of the extracted sample were analyzed. The highest dilution of sample at which 100% of rRT-PCR replicates were positive was considered as the LoD of the assay. In this assay, the LOD was 10 or fewer copy numbers of viral genome per each microliter of the extracted genome (2500 or fewer copy numbers of viral genome per each milliliter of patient blood sample).

Analytical specificity via in silico prediction and rRT-PCR assy

As previously mentioned, none of the probe and primer sequences demonstrated genomic cross-reactivity with other viruses, human genome and other likely interfering genomes in a BLAST search analysis and only detected a 176 bp fragment of the CCHFV S-region in bioinformatics analysis (data not shown). On the other hand, in the experimental rRT-PCR assay, some of the accessible RNA viruses such as norovirus, flu A, HCV, WNV and CCHF (as the positive control) were also evaluated in terms of specificity of the assay.

Linearity range and repeatability of the assay

Linearity of the assay was evaluated by analysis of serial dilutions containing 10 to 105 copy numbers of positive control sample per each microliter of extracted plasmid and the assay was linear between 10 to 103 copy numbers per each microliter of extracted plasmid for this technique (Figure 1 – *Ct was plotted against the quantity of plasmid DNA (common logarithmic scale). Measurement of the copy number of CCHF virus was estimated from Ct by quantitative rRT-PCR< 🟥 [in Ct = 30.69] represents unknown patient sample and ■ [in Cts = 35.36, 31.87 and 28.42] represents the standard dilution*). These assays were evaluated and repeated by at least three different users and three times by each one of them on different days and the repeatability of the assays was approved. Also the assays were developed using both the Applied Biosystem (ABI) 7500 (USA) and Corbet (Rotor-Gene) 6000 (Germany) real-time PCR instruments.

Quantitative determination of viral load in positive samples

Construction of a standard for quantization: The fragment of 176 bp within the S-region sequence of CCHF virus genome was amplified by rRT-PCR and the amplicon was cloned into the PTG19-T vector using a T/A cloning Kit (Vivantis, Malaysia). The plasmid was then purified using a plasmid purification kit (Qiagen, Hilden, Germany). After the performance confirmation of cloning process by PCR, the accuracy of this process and the sequence of cloned fragment was confirmed by sequencing. Determination of recombinant plasmid concentration was then performed by a NanoDrop.

For preparation of a standard curve, the plasmid was diluted serially from 103 to 10 copy numbers per microliter as a master of standard positive control. As soon as optimizing the assay conditions, we obtained a standard curve with a linear range across at least three logs of DNA (recombinant plasmid) concentration, from which the Ct values can be referred to the virus copy number (Figure 1).

Figure 1. The standard curve with a linear range across three logs of different concentrations of the recombinant plasmid DNA containing the S-region sequence of CCHFV genome.

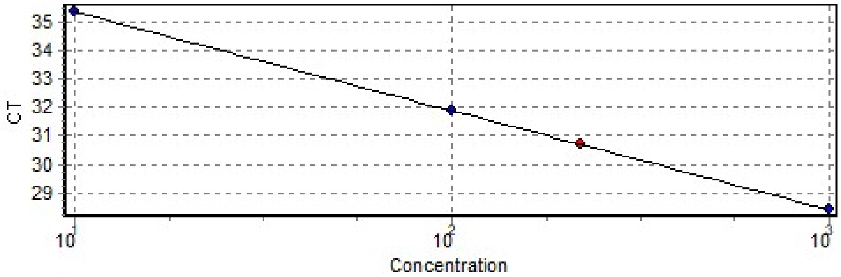

Determination of the Ct values and construction of the standard curve were performed using the Rotor-Gene 6000 software. In all real-time PCR assays, the correlation coefficient of the standard curve was greater than 0.980. Ct was plotted against the quantity of plasmid DNA (common logarithmic scale).

Measurement of the copy number of CCHF virus in unknown blood samples from different patients was estimated from Ct by quantitative rRT-PCR. Since, the viral genome was extracted from 200 µl of a patient blood sample, on the other hand, the extracted genome was dissolved in 50 µl of RNase-free water, as well as, 5 µl was only used in each quantitative assay, the final viral load in 1 ml of blood sample would be 50 folds.

RESULTS

Diagnostic assay of CCHF viral RNA in suspicious blood samples

From a total of 37 suspicious blood samples, 15 cases were approved to be positive and 22 were negative for CCHF viral RNA by use of this technique and no false positive was detected in the results of sequencing. The predicted length of 176 bp resulted by rRT-PCR amplification was approved in all the positive samples by electrophoresis analysis (Figure 2A). The amplification curve related to some of the suspicious blood samples evaluated is shown in Figure 2B.

Evaluation of analytical sensitivity of the assay

The serial dilutions prepared containing 1, 10, 100 and 1000 copies of the positive control sample (plasmid) per each microliter of the extracted genome were analyzed in rRT-PCR for the analytical sensitivity evaluation of the assay. As shown in table 1, the highest dilution at which 100% of rRT-PCR assays were positive (underlined) is considered as the LOD, which in this assay was 10 (or fewer) copy numbers of viral genome per each microliter of the extracted genome or 2500 (or fewer) viral particles per each milliliter of patient blood sample.

Figure 2. A) The predicted fragment with the length of 176 bp, resulted by rRT-PCR amplification. Lad is a 50 bp DNA Ladder (SinaClon, Iran).
B) The rRT-PCR amplification curve related to some of the suspicious blood samples considered (■ represents NTC [Not template control] or negative control sample, 🟥 [in Ct = 23.29] represents positive control sample, 🟦 and 🟪 represent negative patient sample, 🟫 [in Ct = 28.12] and 🟩 [in Ct = 26.85] represent positive patient sample)

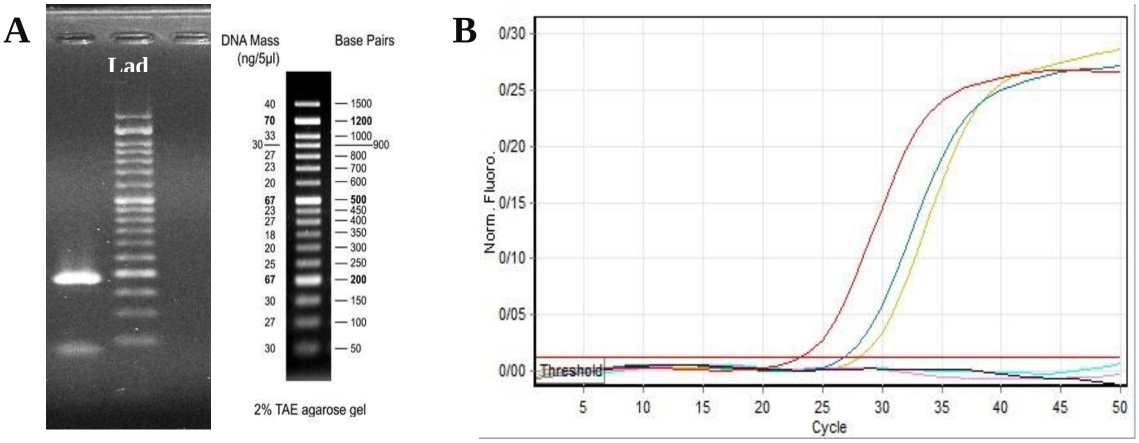

Table 1. CCHFV rRT-PCR assay limit of detection with positive control sample

Serial dilution *(copies/microliter)*	$\frac{\text{Positive tests } (\%)}{\text{s} - \text{region}}$
1	variable
10	100
100	100
1,000	100

Evaluation of analytical specificity via in silico prediction and rRT-PCR assay

As shown in Figures 3A and 3B, the highly conserved sequences (yellow) in the S-region were determinated from the alignment data analysis obtained from the MEGA 7 software which were quite specific for CCHF virus, as previously mentioned and the specific primers targeting this region were designed using the mentioned softwares (bioinformatics data for the probe is not shown).

Figure 3. The alignment data resulted from the MEGA 7 software related to the S-region of CCHFV. A) The Yellow zone is the highly conserved sequence used for designing the specific forward primer. B) The Yellow zone is the highly conserved sequence used for designing the specific reverse primer.

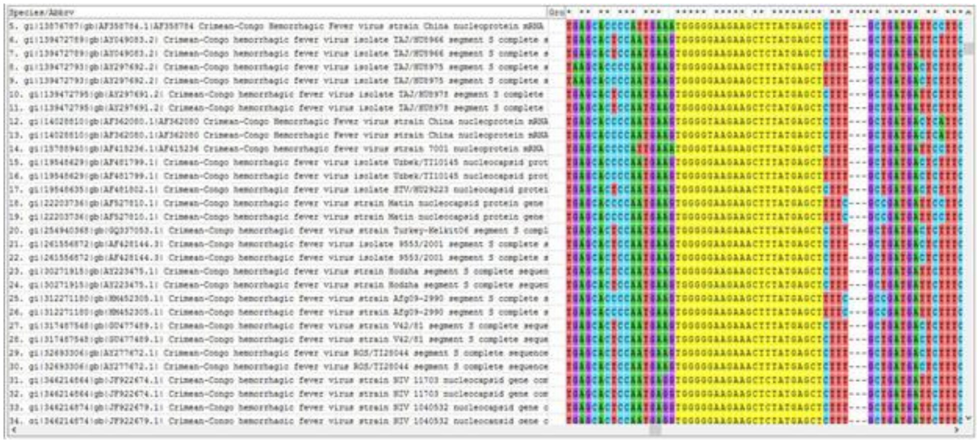

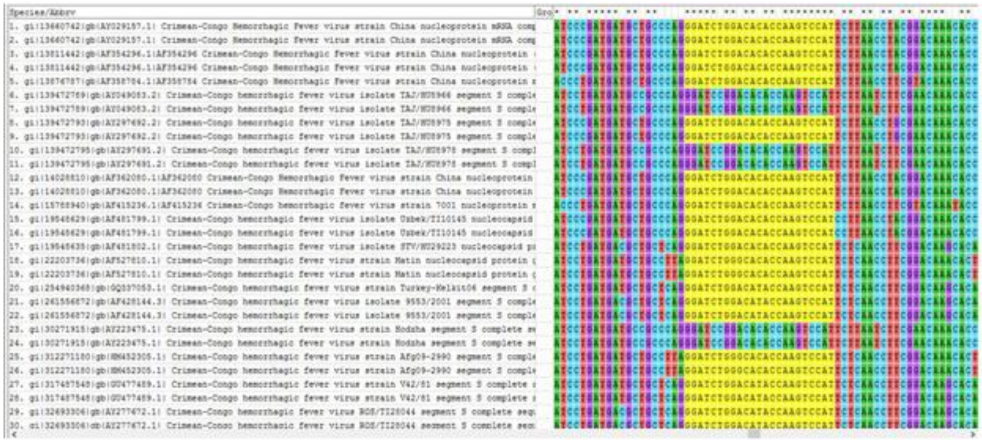

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Species/Abbrv                                                                                   Gro
1. gi|13660742|gb|AY029157.1| Crimean-Congo Hemorrhagic Fever virus strain China nucleoprotein mRNA comp
2. gi|13660742|gb|AY029157.1| Crimean-Congo Hemorrhagic Fever virus strain China nucleoprotein mRNA comp
3. gi|13811442|gb|AF354296.1|AF354296 Crimean-Congo Hemorrhagic fever virus strain China nucleoprotein
4. gi|13811442|gb|AF354296.1|AF354296 Crimean-Congo Hemorrhagic fever virus strain China nucleoprotein
5. gi|13876787|gb|AF358784.1|AF358784 Crimean-Congo Hemorrhagic Fever virus strain China nucleoprotein
6. gi|139472789|gb|AY049083.2| Crimean-Congo hemorrhagic fever virus isolate TAJ/HU8966 segment S comple
7. gi|139472789|gb|AY049083.2| Crimean-Congo hemorrhagic fever virus isolate TAJ/HU8966 segment S comple
8. gi|139472793|gb|AY297692.2| Crimean-Congo hemorrhagic fever virus isolate TAJ/HU8975 segment S comple
9. gi|139472793|gb|AY297692.2| Crimean-Congo hemorrhagic fever virus isolate TAJ/HU8975 segment S comple
10. gi|139472795|gb|AY297691.2| Crimean-Congo hemorrhagic fever virus isolate TAJ/HU8978 segment S compl
11. gi|139472795|gb|AY297691.2| Crimean-Congo hemorrhagic fever virus isolate TAJ/HU8978 segment S compl
12. gi|14028810|gb|AF362080.1|AF362080 Crimean-Congo Hemorrhagic Fever virus strain China nucleoprotein
13. gi|14028810|gb|AF362080.1|AF362080 Crimean-Congo Hemorrhagic Fever virus strain China nucleoprotein
14. gi|15788940|gb|AF415236.1|AF415236 Crimean-Congo hemorrhagic fever virus strain 7001 nucleoprotein
15. gi|19548629|gb|AF481799.1| Crimean-Congo hemorrhagic fever virus isolate Uzbek/TI10145 nucleocapsid
16. gi|19548629|gb|AF481799.1| Crimean-Congo hemorrhagic fever virus isolate Uzbek/TI10145 nucleocapsid
17. gi|19548635|gb|AF481802.1| Crimean-Congo hemorrhagic fever virus isolate STV/HU29223 nucleocapsid pr
18. gi|22203736|gb|AF527810.1| Crimean-Congo hemorrhagic fever virus strain Matin nucleocapsid protein
19. gi|22203736|gb|AF527810.1| Crimean-Congo hemorrhagic fever virus strain Matin nucleocapsid protein
20. gi|254940368|gb|GQ337053.1| Crimean-Congo hemorrhagic fever virus strain Turkey-Kelkit06 segment S
21. gi|261556872|gb|AF428144.3| Crimean-Congo hemorrhagic fever virus isolate 9553/2001 segment S comple
22. gi|261556872|gb|AF428144.3| Crimean-Congo hemorrhagic fever virus isolate 9553/2001 segment S comple
23. gi|30271915|gb|AY223475.1| Crimean-Congo hemorrhagic fever virus strain Hodzha segment S complete se
24. gi|30271915|gb|AY223475.1| Crimean-Congo hemorrhagic fever virus strain Hodzha segment S complete se
25. gi|312271180|gb|HM452305.1| Crimean-Congo hemorrhagic fever virus strain Afg09-2990 segment S comple
26. gi|312271180|gb|HM452305.1| Crimean-Congo hemorrhagic fever virus strain Afg09-2990 segment S comple
27. gi|317457548|gb|GU477489.1| Crimean-Congo hemorrhagic fever virus strain V42/81 segment S complete
28. gi|317457548|gb|GU477489.1| Crimean-Congo hemorrhagic fever virus strain V42/81 segment S complete
29. gi|32693306|gb|AY277672.1| Crimean-Congo hemorrhagic fever virus ROS/TI28044 segment S complete seq
30. gi|32693306|gb|AY277672.1| Crimean-Congo hemorrhagic fever virus ROS/TI28044 segment S complete seq
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The results of experimental rRT-PCR assay on the accessible RNA viruses such as Norovirus, Flu A, HCV, WNV and CCHFV (as the positive control) showed that this assay was quite specific for CCHFV and other viruses were not detected by the designed probe and primers (Figure 4 - ■ represents NTC [Not template control] or negative control sample, 🟥 [in Ct = 21.44] represents the positive control [CCHFV] sample, 🟦 represents the negative result from Norovirus sample assay, ■ represents the negative result from Flu A sample assay, ■ represents the negative result from HCV sample assay and 🟥 represents the negative result from WNV sample assay).

Figure 4. The rRT-PCR amplification curve related to some of the accessible RNA virus samples

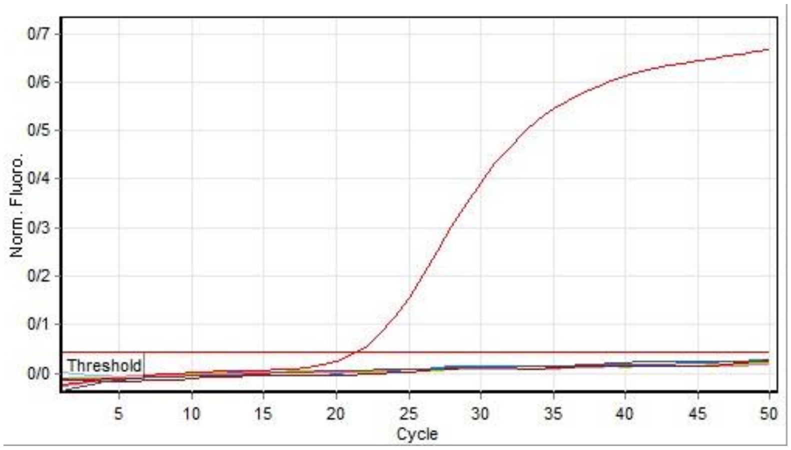

Figure 5. Representative amplification plot of the developed CCHFV quantitative rRT-PCR assay (quantitative curve) showing three ten-fold dilutions of the standards (Cts = 35.36, 31.87 and 28.42) and one unknown sample (Ct = 30.69).

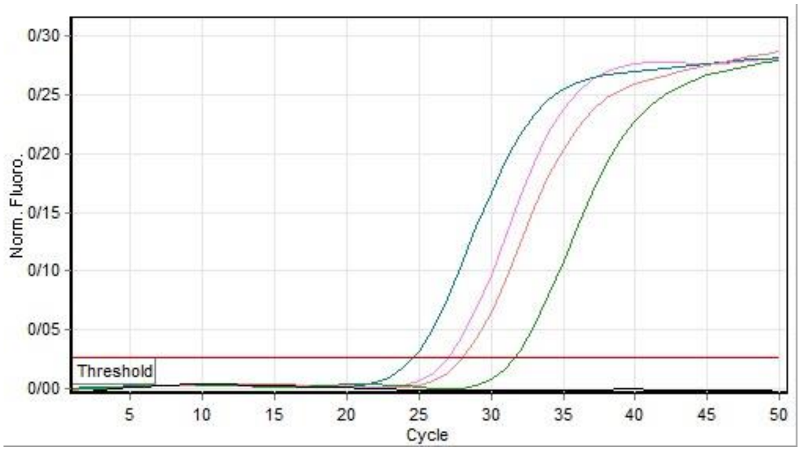

Quantitative determination of viral load in positive samples

The copy number of CCHF virus in different patient blood samples was estimated from Ct by the rRT-PCR assay (Table 2), based on the quantitative curve (Figure 5). For example, the viral load determined in patient blood sample-1 was 220 copy numbers of viral genome per each microliter of the extracted genome or 55,000 (250 folds) viral particles per each milliliter of patient blood sample.

It is notable that viral load decreased slowly in most of cases in subsequent samplings (severe form of disease) and in others (moderate form of disease) decreased very faster. The results of subsequent evaluations is not reported in this article.

Table 2. The calculated viral load of CCHF virus in several patient blood samples based on the quantitative curve and Ct, NTC is "not template control" or "negative control" sample

No.	Color	Name	Type	Ct	Given Conc. *(copy number/μl)*	Calc. Conc. *(copy number/μl)*	Calc. Conc. *(viral particle/ml)*
1	🟪	Sample 1	Unknown	30/69	------	220	55,000
2	🟦	Sample 2	Standard	28/42	1,000	994	248,500
3	🟥	Sample 3	Standard	31/87	100	101	25,250
4	🟩	Sample 4	Standard	35/36	10	10	2,500
5	⬛	Sample 5	NTC	------	------	------	------

DISCUSSION

The CCHF (Crimean-Congo hemorrhagic fever) is an acute widespread zoonotic illness, caused by Nairovirus (a tick-borne virus), which is characterized by a severe and often hemorrhagic course of disease, with a fatality rate of about 10-50% [16, 17].

The CCHF viral RNA from various clinical samples is a proper tracing target during the acute phase of the infection, or even before the beginning of the disease when detection of antibody is impossible or unreliable [22-24]. As there is no specific treatment or approved vaccine against CCHF virus, an accurate and early detection as well as a reliable surveillance and quantitative determination of viral load is necessary for case management, patient improvement, and protection of medical staffs.

Several different laboratory assays can be used for CCHFV diagnosis, including enzyme-linked immune sorbent assay (ELISA), serum neutralization, antigen detection, virus isolation by cell culture and reverse-transcriptase polymerase chain reaction (RT-PCR) assay [4, 18-21]. RT-PCR is the best molecular diagnostic techniques in clinical laboratories owing to its simplicity, high specificity and sensitivity [24]. Unlike the traditional two-step RT-PCR technique, the one-step real-time RT-PCR (rRT-PCR) assay does not need to pre-PCR (post-RT) sample handling, avoiding the PCR product dependent probable contamination transmission and resulting in more sensitive, rapid and also higher efficient assays. On the other hand, the probe based rRT-PCR assay has a very high specificity in addition to the mentioned features, compared with the SYBR Green based rRT-PCR assay that was also developed in our previous study [22]. Therefore, it has become an accepted diagnosis method for detecting many microbes [25] and also has greater repeatability when the need for post-amplification processing (analysis by agarose gel electrophoresis) is eliminated, thus avoiding the transfer contamination, difficulty and time-consuming [26, 27].

The assay was linear between 10 to 10^3 viral genomes per each microliter of the extracted genome for this technique and the bioinformatics and experimental evaluations approved high specificity of this assay. On the other hand, the LOD of the assay was 10 (or fewer) copy numbers of viral genome per each microliter of the extracted genome. Measurement of the CCHF virus copy number and quantitative determination of viral load in positive samples was estimated from Ct via the rRT-PCR on the basis of the quantitative curve. It is notable that construction of a standard for quantization of this assay is necessary and very difficult, which was devoted a lot of time in this study.

CONCLUSIONS

This research showed that the developed probe based one-step rRT-PCR assay is a specific, reliable, rapid, sensitive, repeatable and simple tool for detection as well as quantitative determination of viral load of the CCHF virus in various suspicious patient samples.

Acknowledgements

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ORIGINAL ARTICLE

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Diagnosis and management of cases with deep infiltrating endometriosis affecting the urinary tract

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Abstract: *Objective: The goal of this paper is to present the management of cases of deep endometriosis that affects the bladder, the ureters and the kidney.*

Materials and method: In this review we analyzed the sensitivity and specificity of imaging techniques for diagnosing deep infiltrating endometriosis, the optimal surgical technique and the surgical approach.

Results: In patients with pelvic endometriosis in advanced stages the urinary tract is frequently involved. Preoperative work-up of patients with deep infiltrating endometriosis is aimed at evaluating the extension of the lesions in order to assess the complexity of the intervention and to choose the optimal approach.

Conclusions: Laparoscopic and robotic management of these cases are good therapeutic alternatives. Ultrasound, sonovaginography with gel and MRI are useful imaging techniques for evaluating patients with deep infiltrating endometriois. The surgical management of patients with deep infiltrating endometriosis that involves the urinary tract consists of partial bladder resections, ureterolysis, ureteral resection and anastomosis and ureteral reimplantation..

Keywords: *bladder endometriosis, deep infiltrating endometriosis, ureterolysis*

INTRODUCTION

Endometriosis is a benign pathology of the female reproductive tract, characterized by the presence of endometrial glands or stroma outside the uterine cavity [1]. The presence of endometrial tissue at the level of the bladder will be stimulated during the menstrual cycle. In response to the cyclical stimulation of hormones throughout the menstrual cycle, some patients may even present with cyclic hematuria [2,3].

Bladder endometriosis can be defined as "primary" or "secondary". Primary bladder endometriosis is represented by lesions associated with deep infiltrating endometriosis.

Secondary bladder endometriosis is represented by lesions that have occurred iatrogenically after pelvic surgery, caesarean section or hysterectomy [4,5].

The distribution of the lesions is multifocal, affecting the bladder trigone and dome from the serosa to the mucosa [6,7].

The ectopic endometrial tissue is subjected to stimulation during the normal cycle creating hormonal changes that result in

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bleeding, inflammation and adhesions [8]. The two main manifestations of this disease are chronic pelvic pain of varying intensity and infertility [9,10].

Table 1: The prevalence of urinary tract endometriosis [11]

Kidney	4 % of all urinary tract locations
Ureter	9 % of all urinary tract locations
Bladder	85 % of all urinary tract locations
Urethra	2 % of all urinary tract locations
Prevalence	0,3 – 12 % of all cases of endometriosis

DIAGNOSIS

The diagnosis of bladder endometriosis is based on the presence of ectopic endometrial glands and stroma located at the level of the detrusor muscle. Patients presenting with bladder endometriosis often present nonspecific urinary symptoms. In some cases, patients will present with dysuria, urgency to void, frequent micturition and abdominal pain [12,13]. The main aspect that dominates the clinical picture is exacerbation of these symptoms during the menstrual period [14]. Due to the fact that the symptomatology of bladder endometriosisis is nonspecific, many women will not seek medical attention in the first few years. The symptomatology can vary depending on the site and the dimension of the lesion [15]. The various degrees of abdominal or pelvic pain associated with bladder endometriosis will not prompt patients to seek additional work-up for this syndrome [16]. Thus, an average of 6 years will pass from the onset of the symptomatology to the diagnosis of endometriosis [17].

Urinary tract endometriosis can present with a non-specific symptomatology [18-20]. The presence of endometrial tissue at the level of ureteral orifices can produce hydronephrosis, with subsequent renal failure [21,22]. If the ureteral obstruction is acute, the symptoms may mimic a renal colic that requires a form of drainage [23-25].In chronic urinary obstruction, the lumbar pain can be absent and the renal insufficiency could appear like arterial hypertension, due to a bad regulation of blood pressure, thus involving the delay of a right treatment [26-28]. The medical history and accurate identification of the patient’s symptoms can guide the clinician in choosing the correct imaging investigations based on the presenting symptoms.

Imaging diagnosis

Ultrasonography is the first line imaging investigation used in the work-up for patients with endometriosis. The bladder and the kidneys can be evaluated using transvaginal and abdominal ultrasound. The patient’s bladder should be in semirepletion for a correct evaluation. (Figure 1)

Figure 1: Vesical endometriosis

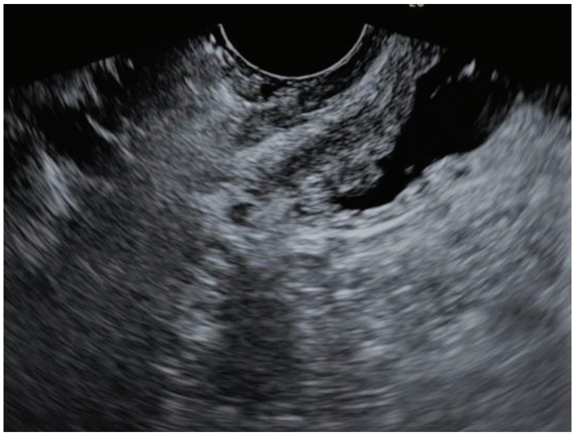

Abdominal ultrasound can be used in cases of suspected lesions of the bladder, abdominal wall and umbilical endometriosis [29]. During the initial work-up of a patient with deep infiltrating endometriois, an abdominal ultrasound must be performed to evaluate the impact on the ureters and kidneys. Endometriotic lesions can affect the ureter extrinsically or intrinsically. This can have an impact on the kidney causing hydronephrosis or ureterohydronephrosis. Any other causes of ureteral extrinsec compression must be excluded [30-33]. If surgical excision of the lesions is not performed this may affect the kidney leading to loss of function [19-25].

There are some authors that suggest that ureteral endometriosis should be suspected in all cases of deep infiltrating endometriosis [34].

Sonovaginography with ultrasound gel is a useful diagnostic option in cases with deep infiltrating endometriosis. This method is useful for identifying endometriotic lesions in the posterior compartiment as well as in the anterior compartment. An acoustic window is created by introducing 20 ml of ultrasound gel into the vagina. The technique initially used saline solution [35]. Using this technique, we observed a high diagnostic accuracy for the following locations (transvaginal ultrasound – TVS sonovaginography with gel – SVG) – Table 2.

Table 2. The diagnostic accuracy for transvaginal ultrasound and sonovaginography with gel in the anterior and posterior compartiment [36].

Endometriotic lesion	Ultrasound	Sensitivity	Specificity	PPV	NPV
Ovaries	TVS	94.8 %	95.6 %	92.5 %	94 %
	SVG	95.5 %	97 %	92.6 %	93 %
Urinary bladder	TVS	65.5 %	98.6 %	66.6 %	98.5 %
	SVG	67 %	97 %	98 %	98 %
Utero-sacral ligaments	TVS	69.7 %	95 %	84.2 %	87 %
	SVG	78.5 %	96 %	89 %	91 %
Vagina	TVS	58.5 %	99 %	94.3 %	95.4 %
	SVG	79 %	99 %	92 %	96 %
Recto-vaginal septum	TVS	64.8 %	99.4 %	81.5 %	97.8 %
	SVG	94 %	97 %	82 %	93.5 %
Pouch of Douglas	TVS	81.5 %	99 %	95 %	97 %
	SVG	81 %	98 %	91 %	97 %
Recto-sigmoid	TVS	91 %	97 %	95 %	96 %
	SVG	94 %	95.5 %	91 %	97.2 %

In the anterior compartment the transvaginal ultrasound and sonovaginography with gel have a similar sensitivity and specificity (80.1 % vs 81.2 % and 97.1% vs 97%). However, the positive predictive value is in favour of the latter (79.5 % vs 95.3 %). In cases of with deep infiltrating endometriosis the association between magnetic resonance imaging and computer tomography- based virtual colonoscopy has a sensitivity of 98.36 % for nodule identification. The association of these two investigations allows a superior mapping of the endometriotic lesions at the level of the posterior compartment. This aspect is important in the preoperative work-up and planning for the surgery [37].

TREATMENT

Medical treatment

Medical treatment using progestins, oral contraceptives and GnRH agonists can be used in the treatment of patients with endometriosis [38-40]. The contraindications of medical treatment in patients with urinary tract endometriosis are: evidence of the kidney being affected by hydronephrosis, the patient's desire and contraindications for the treatment [41,42]. The imunohistochemical aspects of patients treated with progesterone can be useful in choosing the optimal medical treatment after the surgical intervention [43,44]. The expression of the anti-apoptotic markers (Bcl-2), cell proliferation markers (Ki-67), estrogen and progesterone receptors offers anexplanation for the different outcomes after medical treatment for patients with endometriosis [45,46].

Surgical treatment

Surgical treatment of urinary tract endometriosis must be performed when the patient does not respond to medical treatment, if the patient suffers from infertility or if the lesion has an impact on the kidney (ureteral obstruction, hydronephrosis or uretero-hydronephrosis) [47,48]. Due to the fact that the obstruction of the ureter is due to fibrosis, patients will not respond well to medical treatment [49,50]. During the initial work-up and planning for the surgical intervention, the symptoms exhibited by the patient and the imaging investigations can guide the therapeutic conduct and anticipate the complexity of the case [51,52]. This is a key aspect in deciding the optimal intervention for the patient and anticipate whether a multidisciplinary approach is in order (gynaecologist, urologist and colorectal surgeon) [53,54]. The treatment for bladder endometriosis is represented by the surgical excision of the lesion.

Figure 2: Robotic ureterolysis (*Personal collection of Prof. Elvira Brătilă*)

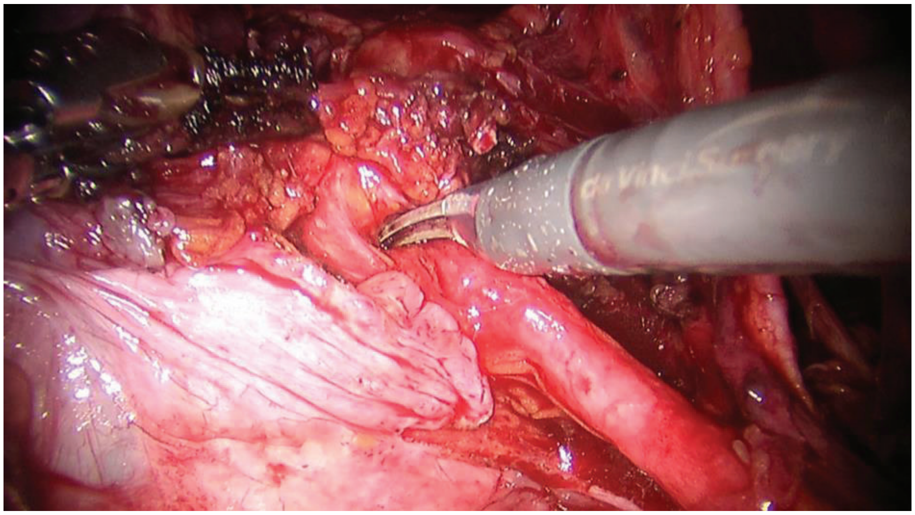

The surgical approach can be performed by laparotomy, laparoscopy or robotic surgery (Figure 2) [55-57]. The treatment for ureteral endometriosis consists of the surgical excision of the lesions involving the ureters in order to prevent ureteral compression (Figure 3).

Figure 3: Endometriotic lesion of that affected the ureter extrinsically at the level of the vesicoureteral junction (*Personal collection of Prof. Elvira Brătilă*)

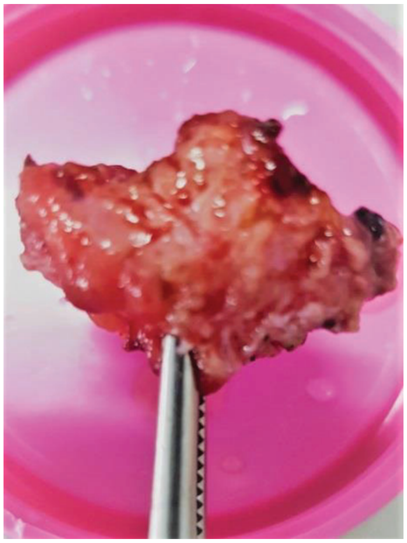

Ureterolysis is the first procedure performed when the lesion affecting the ureter is extrinsic. During the laparoscopic and robotic surgery, the transperitoneal approach way allows a thorough evaluation of the extension of the disease.

When the ureters are affected intrinsically, the ureteral segment can be infiltrated by the endometriotic lesions. In this case, a resection of the affected segment and reimplantation can be an alternative if ureterolysis cannot be performed [58,59].

After the completion of the ureterolysis (dissection of the tissue up to the level of the cardinal ligament), a thorough assessment of the remaining segment is required [60]. This must take into account the possibility of another obstruction of the ureter. The level at which the ureter is affected represents a factor for the intraoperative decision to perform ureterolysis, ureteral resection and anastomosis or ureteroneocystotomy [61,62]. If the lesion is located in the distal part of the ureter, the affected segment can be resected and the ureter can be reimplanted in the bladder. If the lesion is located in the upper third of the ureter, the affected segment can be resected and repaired with an uretero-ureteral anastomosis. The choice between ureterolysis and resection cannot be made preoperatively.

CONCLUSIONS

In patients with pelvic endometriosis in advanced stages, the urinary tract is frequently involved. Laparoscopic and robotic management of these cases is a good therapeutic alternative [63,64]. Preoperative work-up of patients with deep infiltrating endometriosis is aimed at evaluating the extension of the lesions in order to assess the complexity of the intervention and to choose the optimal approach [65,66]. Ultrasound, sonovaginography with gel and MRI are useful imaging techniques for evaluating patients with deep infiltrating endometriosis [67]. The surgical management of patients with deep infiltrating endometriosis that involve the urinary tract consists of ureterolysis, ureteral resection and anastomosis and ureteral reimplantation [68-70].

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ORIGINAL ARTICLE

Diagnosis of malignant neoplasms of the skin in servicemen of the Armed Forces of Ukraine

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Abstract: *Objective: Malignant neoplasms of the skin are diagnosed in high-risk groups, among which the military is special. The article is devoted to the analysis of diagnostics, and risk factors for malignant skin tumors in servicemen of the Armed Forces of Ukraine (AFU)*

Materials and methods: An analysis of 23 cases of diagnosed malignant neoplasms of the skin in servicemen of AFU for the period 1999-2021. The study included cases in which the diagnosis was confirmed histologically in two laboratories. Clinical, laboratory, and histological data of each case were analyzed, and further analysis took into account the length of military service and the risks of professional activity.

Results: Among the 23 cases, the majority - 22 were servicemen, and only 1 case of basal cell carcinoma in a 52-year-old employee. Among other servicemen, the age range was 25-55 years, with a mean age of diagnosis of 42 years. The period of military service at the time of diagnosis was 23.7 years, most cases were melanoma (15 of 23 cases), and in other cases - basal cell carcinoma. Patients were dominated by Air Force personnel (10 out of 23 cases)

Conclusion: The prevalence of malignant skin neoplasms in the Armed Forces corresponds to global trends. A group of increased risk of developing malignant skin neoplasms is servicemen of the Armed Forces of Ukraine with a long service life (more than 20 years), older than 40 years. However, there is a high probability of developing melanoma of the skin in servicemen of shorter service life, which requires enhanced dermatological supervision in servicemen of any age and service life.

Keywords: *malignant neoplasms of the skin, servicemen, armed forces of Ukraine*

INTRODUCTION

Malignant neoplasms of the skin are diagnosed in many age, national and occupational groups, due to numerous risk factors studied in great detail by dermatologists. Among them are insolation, contact with carcinogens, genetic factors, and many others [1]. However, among professional groups, the high-risk group – the military – attracts attention, which clearly demonstrates the acquired nature of the development of skin cancer [2]. After all, as a standard, at enlistment in military service, people with potential risk factors and precancerous diseases are eliminated and development in the future of such new growths is caused by features of professional activity.

It is proved [3] increased risk of malignant neoplasms of the skin and servicemen who had increased contact with ultraviolet radiation, and this trend has been observed over the past 100 years in participants of World War I and II, the Vietnam War, and other armed conflicts of the 20th century [4]. Among the military, two groups are known to have high levels of skin cancer: Caucasians and men over 50 [5]. Among the military, the Air Force has the highest rate of both melanoma and non-melanoma skin cancer [6]. Accordingly, officers, especially senior officers, have an increased incidence of melanoma and non-melanoma skin cancer compared to non-military personnel [7]. The significant role of ultraviolet radiation is indicated by the fact that pilots have the highest risk of developing malignant skin tumors among other groups of servicemen [8]. It is noted that the levels of ultraviolet radiation increase to 24 percent with each increase in altitude by 1000 meters [9]. The publications on the diagnosis and manifestations of malignant skin neoplasms are mainly based on data from the

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US or other NATO countries [2-9], we have not found publications on the analysis of diagnosis, risk factors for malignant skin tumors in the Armed Forces of Ukraine (AFU). Based on this, we set a goal – to analyze the features of diagnosis and manifestations of malignant neoplasms of the skin in the Armed Forces.

MATERIALS AND METHODS

We analyzed 23 cases of diagnosed malignant skin neoplasms in the Armed Forces of Ukraine for the period 1999-to 2021.

The data were obtained from the reports of surgeons and dermatologists in the hospitals of the Armed Forces of Ukraine. The study included cases in which the diagnosis was confirmed histologically in two laboratories. Clinical, laboratory, and histological data of each case were analyzed, and further analysis took into account the length of military service and the risks of professional activity. The study did not include suspected cases of malignant neoplasms not confirmed by repeated histological examination.

RESULTS

Among the 23 cases, the majority - 22 were servicemen and only 1 case of basal cell carcinoma in a 52-year-old employee.

Among other servicemen, the age range was 25-55 years, with a mean age of diagnosis of 42 years. There were 13 and 9 officers and sergeants, respectively, and servicemen, which does not allow to define of this indicator as a risk factor. The term of military service turned out to be a more predictable risk factor, as it amounted to 23.7 years, which practically corresponds to the average term of service of a serviceman of the Armed Forces. However, we note the presence of three cases of diagnosis of malignant neoplasms that occurred after 1-3 years of military service. These were cases of solid basal cell carcinoma in a 37-year-old soldier, a case of anonymous melanoma in a 25-year-old soldier, which was detected in the stage of widespread metastasis after 2 years of service, and primary multiple melanomas in a 44-year-old soldier, which was detected in 3 years of service. All these cases required radical treatment and led to dismissal with subsequent disability of patients.

Officers were dominated by skin melanoma in 9 of 13 cases, which was detected after 20 years of service and did not require urgent radical treatment, and in 5 cases did not lead to dismissal.

Further analysis of nosology and its prevalence revealed that most cases were melanoma (15 of 23 cases), in other cases – basal cell carcinoma. Other risk factors were predominantly in the Air Force (10 out of 23 cases), consistent with other studies on the increased risk of developing skin neoplasms in military personnel involved in flight missions and a significant increase in ultraviolet radiation.

DISCUSSION

Due to the prevalence of malignant skin tumors among APU servicemen relative to other population groups, it is difficult to make an adequate comparison. According to our data, the studied population of sick servicemen was in the age range of 25-55 years and mostly male, according to data for 2020 the incidence of melanoma in men was 7.4 per 100 thousand population, and non-melanoma malignancies 38.2 per 100 thousand population [10]. However, no data have been found on age, and it should be borne in mind that servicemen are a group of enhanced surveillance, in which early diagnosis is present and potential risk groups are excluded. In our opinion, it is not correct to compare the civilian population and the military.

In our opinion, it is the consideration of the peculiarities of the factors of supervision and closes medical examination of servicemen that should be taken into account when comparing the data on the incidence of malignant skin neoplasms in this category of the population and other populations. In particular, there are data [2] on the absence of a difference in the incidence of melanoma between the general population of the United States and the US Navy or Air Force, and even on reduced age-adjusted rates compared to civilians [5].

However, there is evidence of an increased risk of melanoma in US servicemen over the age of 45 [4], which is consistent with our data on a significant percentage of patients in this age group. The predominance of Air Force patients among patients is in line with the global trend and indicates the need for more careful supervision in this group of servicemen. Also noteworthy is the presence of a group of patients with relatively short service life in the Armed Forces and young people, who were diagnosed with malignant skin tumors with signs of disease progression, which is not a standard situation in this age group, but described the development of melanoma in 27-year-old US serviceman [11], which indicates the need for close medical examination of young servicemen and short service life.

CONCLUSIONS

The prevalence of malignant skin neoplasms in the Armed Forces corresponds to global trends. A group of increased risk of developing malignant skin neoplasms is servicemen of the Armed Forces of Ukraine with a long service life (more than 20 years), older than 40 years. However, there is a high probability of developing melanoma of the skin in servicemen of shorter service life, which requires enhanced dermatological supervision in servicemen of any age and service life.

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ORIGINAL ARTICLES

Diagnostic challenges in gastrointestinal infections

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Abstract: *Gastrointestinal infections are among the most common infectious diseases found all over the world, varying depending on the etiological agent. Symptoms usually include diarrhea, vomiting, and abdominal pain. Water and electrolyte imbalance is the main consequence of gastrointestinal infections. Most of them are cured or self-limited in few days, but at the same time, for a specific population such as immunocompromised, elderly patients or new-borns, these infections are potentially severe. In this context, it is very important to identify the etiological agents of acute diarrhea for the appropriate treatment and infection control measures. While routine laboratory diagnosis of parasitic diarrhea still depends largely on microscopic examination of fecal samples, immunological and molecular methods are becoming increasingly commercially available and, in well-resourced settings, will ultimately displace traditional methods. The present paper presents some of the most common and well-known pathologies of this type, being a brief presentation of the variety of gastrointestinal diseases, each with characteristic clinical manifestations and diagnosis.*

Keywords: *gastrointestinal infections, Rotavirus, Salmonella, Shigella, Helicobacter pylori*

INTRODUCTION

Worldwide, acute gastrointestinal infections are the second most common infectious diseases after respiratory tract infections and a major cause of morbidity and mortality among infants and children [1]. Gastrointestinal infections can be caused by a large number of microorganisms present in the water, food, or hospital environment, that cause inflammation of different segments of the gastrointestinal tract. The prevalence of the different microorganisms causing gastroenteritis depends on several factors, such as age (children, adults), community or nosocomial acquisition, country (developing or developed), patients who have traveled abroad and immune status [2].

The common clinical picture usually looks like acute gastroenteritis, with a sudden or insidious onset, immediately after or after a while from returning to the country of origin. At the first presentation, it is important to identify the patient's journey, with detailed information on particular aspects of the trip. Failure in identification of the epidemiological link may lead to delayed diagnosis and treatment, with increased risks of complications. Empirical intervention is often important when there is a strong possibility of a certain condition and confirmatory lab tests

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are delayed or unavailable, especially for infections that are rapidly progressive or with prolonged testing duration [3].

Traveling for business and pleasure is a small proportion of the total movement of people. Modern transport ways allow more goods and people to travel around the world at a faster pace; they also open up the airways to the transcontinental movement of vectors for infectious diseases. While traveling, people carry their genetic profile, immunological sequelae from previous illnesses, cultural preferences, traditions, and patterns of behavior. They are also accompanied by microbes, animals and other biological lives [4]. The range of infectious diseases present at a certain time and in a certain region has increased, with the increasing travel possibilities from one country to another and between continents. Transiting through different geographical areas, in a short time (as with long-distance flights), may increase the risk of contamination with foreign etiological agents. Cities are becoming important centers for the transmission of infectious diseases due to international travel and migration, as shown by recent pandemics. Several factors contribute to infectious disease emergence. Others commonly listed include microbial adaptation and change, demographics and behaviour, changes in the environment, technology and economic development, deterioration of public health and surveillance programs, and international travel and trade [5].

Many tropical diseases havea polymorphic clinical feature, making the diagnosis difficult, especially for clinicians from countries outside tropical areas. In developing and developed countries, doctors in urban settings need to be aware of developments in infectious diseases. Upon travel, an ill traveler's history must analyze exposure specifics, including visited destinations, activities, ingestion of infected food or drink, contact with insects, livestock, freshwater or blood and body fluids and other possible exposures. Information on the geographic dissemination of infectious disease is significant in creating the differential diagnosis and testing [6].

The purpose of this paper is to make a brief review of the most common gastrointestinal infections, with their relevant clinical features and diagnosis.

GASTROINTESTINAL INFECTIONS

In the geographical area of Romania, southeastern Europe, Rotavirus, Salmonella species, Shigella species, Clostridium difficile, Escherichia coli and Giardia lamblia are the most common etiological agents of gastrointestinal infections [7-9]. Infections with Helicobacter pylori, Campylobacter spp., and other viruses are also frequent. Several tropical infectious diseases, such as amebiasis, malaria, leishmaniasis, dracunculiasis, schistosomiasis, brucellosis, leptospirosis, dengue fever, yellow fever and viral haemorrhagic fever, can have a digestive onset, requiring increased attention from the clinician. Experimental animal studies and clinical research have shown that the virulence of infectious microbial agents can have variable severity, sometimes being involved even in deaths because of sepsis [10, 11].

Viral gastrointestinal infections

The most important etiological viral agents of diarrhea are rotavirus, norovirus, adenovirus, astrovirus, sapovirus and enterovirus. Gastroenteritis caused by norovirus, asingle-stranded RNA (ssRNA) viruses belonging to the family of Caliciviridae, may manifest with severe nausea, vomiting and diarrhea. The genogroup I (GI), II (GII) and IV (GIV) of Rotaviruses (double stranded RNA) are the main cause of diarrhea in children under 5 yo and responsible for the death of around 200,000 children/year. Rotaviruses belong to the Reoviridae family [12]. Major symptoms are usually abdominal pain, vomiting and waterydiarrhea (AWD). Rotaviruses serogroups A, B, and C are responsible for human infections.

Adenoviruses cause respiratory diseases, bladder infections, conjunctivitis. Adenoviruses belong to Adenoviriadae family of double-stranded (dsDNA) viruses. Taking into consideration 51 serotypes of human adenoviruses (classified into six groups, from A to F), gastroenteritis is mainly caused by Adenoviruses serotype 40 and 41.

Astroviruses discovered in 1975 are single-stranded (ssDNA) viruses and belong to the family of Astroviridae; the incidence of these infections is constantly increasing [13]. Clinical signs include vomiting and severe diarrhea, with moderate dehydration. In developed countries, the Astrovirus infection incidence is between 2-9%, affecting mainly children [14]. To date, serotypes 1 - 5 are the most relevant. Serological studies indicate that most children infected with Astroviruses develop antibodies to the virus early in life, which provide protection against future infections.

Sapoviruses are part of the family of Caliciviridae and are major causative agents of gastroenteritis worldwide, causing disease in humans of all ages. The highest incidence was described for children under 5 year-old, but infections are diagnosed in adults as well, with diarrhea, vomiting, and fever (similar clinical symptoms to those of norovirus infections). However, self-limited sapovirus infections lead to less severe gastroenteritis compared to norovirus-induced gastroenteritis [15].

There is a variety of different subtypes of human enteroviruses, such as human enterovirus 70/71, poliovirus, Coxsackie virus A and B, echovirus, with different degrees of tissue tropism and virulence, although retaining a basic antigenic identity. Infections are usually asymptomatic or present with mild cold-like symptoms. Severe enterovirus infections are poliomyelitis, meningitis and myocarditis. Coxsackie viruses are present worldwide and can cause the so-called “summer diarrhoea”. Other severe infections with Coxsackie virus or human enterovirus 70/71 can lead to conjunctivitis and myocarditis, whereas echovirus infections can result in aseptic meningitis, with echovirus 30 being the most common meningitis-causing serotype in Europe, America and Asia [16, 17].

Viral hemorrhagic fever (VHF) is characterized by severe multiple organ dysfunction syndrome. Etiologically, four particular RNA viral families (Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae) lead to VHF. The viruses that cause VHFs are spread across most of the globe. Haemorrhagic viruses are considered as potential biological warfare agents. Initially, clinical symptoms include fever, cephalgia, myalgia, arthralgia and chills, which can lead to massive haemorrhages, shock or even acute multiple organ dysfunction. Paraclinical parameters show an increase in liver enzymes and a decrease in albumin levels. The coagulation panel shows an increase in prothrombin time and a decrease in fibrinogen. These results of the paraclinical tests may be similar to diseases like disseminated intravascular coagulation, malaria, haemolytic uremic syndrome. Although the confirmation of the diagnosis requires detection of viral RNA, viral antigens, or specific antibodies, the clinician must raise the suspicion quickly, before laboratory results, because these diseases require strict isolation measures and biosafety level 4 laboratory facilities [18].

Dengue fever is a global arboviral infection spread by the mosquito genus Aedes (A) (primarily A. aegypti, but also A. albopictus, and A. polynesiensis), a mosquito found in the tropical and subtropical regions of the world, also involved in spreading Zika virus disease, yellow fever, and Chikungunya. About 40% of the world population, almost 3 billion people live in regions with a dengue threat [19]. In more than 100 countries, people are affected by dengue fever resulting in up to 22,000 deaths per annum globally [20]. In 2018, Romania reported 4 cases of dengue fever, all travel-associated [21]. With incubation of 3-14 days, 50-80% of patients are asymptomatic during the period of illness [22]. Classic dengue fever (break-bone fever) is distinguished by high fever, severe cephalgia, myalgia, arthralgia, retro-orbital pain, abdominal pain or tenderness with persistent vomiting, and hepatomegaly. The diagnosis is supported by the combination of thrombocytopenia, leukopenia, modifications of the liver function tests and serological tests for dengue virus.

Yellow fever virus etiologically belongs to Flaviviridae family, which causes acute viral disease transmitted by A. aegypti, and Haemagogus mosquitoes. The name's "yellow" refers to jaundice that affects most patients. In tropical regions of Africa and Central and South America, the virus is endemic. In 2018, one case of yellow fever was reported by Romania [21]. Severe cases can have a mortality rate of up to 50% [23, 24]. With an incubation period of 3-6 days, clinical manifestations in the second phase, which generally affect liver and kidney, consist in fever, vomiting, abdominal pain, jaundice, dark urine, hemorrhage, and even shock. Yellow fever, especially during early stages, is difficult to diagnose, it can be mistaken for diseases like leptospirosis, fulminant forms of viral hepatitis, or even severe malaria. Confirmation of the diagnosis requires the detection of the virus by identifying viral ribonucleic acid (RNA), specific antigen or immunoglobulins M.

Digestive infections caused by protozoans and parasites

Parasitic infections causing diarrhea are particularly important in young children, immunocompromised patients, and those living in conditions of poor sanitation and hygiene, with unsafe food and water supplies. Travelers from developed countries are another group at risk, particularly susceptible to parasitic pathogens, because they lack protective immunity acquired from previous exposure. Several of these organisms are of zoonotic origin, and there may be occupational or recreational exposure risks [25].

Giardia lamblia, Cryptosporidium spp., Entamoeba histolytica and Dientamoeba fragilis are the most important diarrhoea-causing protozoans. Helminths are less important as agents of diarrhea, but they may produce loose or even dysenteric stools in acute or heavy infections (for example, intestinal schistosomiases). One important exception is the nematode Strongyloides stercoralis, which has the unusual capacity to maintain subclinical infections for years or decades, being discovered only when the patient is immunosuppressed for various reasons, when severe or potentially fatal disseminated infection may occur [26].

Giardia lamblia infections (synonym G. Intestinalis, G. duodenales) in developed countries occur in 6-8% of children and 2% of adults, and around 33% of the population is infected with protozoans [27, 28]. In the US for example, CDC estimated 77,000 cases/year of Giardiasis [29].

One of the several species of the genus Cryptosporidium is

Cryptosporidium parvum, which, together with C. hominis, are the most common cause's ofcryptosporidiosis in humans [29]. Similar clinical symptoms could reveal infections by other Cryptosporidium spp. such as C. felis, C. meleagridis, C. canis, and C. Muris.Around 748,000 cryptosporidiosis cases/year occurred in the US [30, 31].

The causative agent of amoebiasis is Entamoeba histolytica - the only human pathogenic species of the genus Entamoeba. Amebiasis has a worldwide distribution, prevalent in Central and South America, Africa, and Asia, being produced by trophozoites of Entamoeba histolytica.

Clinical manifestations can appear up to 4 months from the contact with contaminated food or objects. In about 90% of cases, the disease may evolve asymptomatic, self-limiting, with recurrences. The rest of the patients may have different severity clinical forms, such as acute colitis (bloody diarrhea, fever, abdominal pain), severe colitis (fulminant, abscesses), and chronic form (ameboma, and inflammatory bowel disease). The etiological diagnosis is based on the microscopic identification of cysts and trophozoites in the stool of the patients, specific antibodies and antigens detection. Around 50 million people worldwide suffer from amoebiasis each year, resulting in 100,000 deaths each year [32].

One of the most underestimated diarrhea-causing protozoansis Dientamoeba fragilis and recent studies demonstrated the direct implication as a common cause of gastrointestinal disease and, in the meantime, his pathogenic potential.The prevalence of Dientamoeba fragilis varies from 0.3% to 52% and often exceeds that of Giardia lamblia [33].

Malaria is produced by infection with Plasmodium species, by the bite of Anopheles mosquito, being recognized as the second cause of death by infections in Africa. According to the European Centre for Disease Prevention and Control (ECDC), Romania confirmed and reported 18 cases of malariain 2018 [21]. The disease is endemic in sub-Saharan Africa and South Asia and has an incubation period between 8-25 days, with a clinical picture dominated by cyclical fever. The following manifestations: insomnia, weakness, arthralgias, myalgias, diarrhoea, abdominal pain, headache, and hepatosplenomegaly can be associated with the fever in case of acute malaria. Classical clinical triad present in malaria consists of fever, splenomegaly, and anaemia. The microscopic examination of thin or thick blood smears, the detection of the parasite nucleic acid by real-time polymerase chain reaction assay, the identification of the plasmodium antigen by immunochromatography are specific tests used to confirm the diagnosis of malaria [34].

Leishmaniasis is a neglected tropical disease, protozoan vector-borne infection, whose transmission is through the bite of an infected female sand fly (Phlebotominespecies). It is present in environments ranging from tropical forests in South and Central America to desert areas in West Asia, including North of Africa with cutaneous leishmaniasis, which is the most common form, mucocutaneous, and visceral leishmaniasis, the last one being the most severe form of the disease. The period of incubation varies widely, from a few weeks to several months after the initial bite, depending on the species. The wide spectrum of clinical manifestations in visceral leishmaniasis includes fever, anorexia, abdominal pain, emesis, diarrhea, hepato-splenomegaly. Diagnostic findings are based on skin biopsy, tissue culture, and presence of antibodies [35].

Dracunculiasis, also known as Guinea worm disease, can be found in rural areas of South Sudan, Chad, Ethiopia, and Mali, which relies on contaminated water supplies. Clinical symptoms can take up to a year to appear, and may include painful blister centered on the worm, usually localized in the lower extremity, fever, nausea, vomiting, and local lymphadenopathy [36].

Schistosomiasis is a disease caused by parasitic worms (Genus Schistosoma), also known as bilharzia, which is endemic in tropical and subtropical areas and considered as one of the neglected tropical diseases. With an incubation period of about 2-8 weeks, the acute clinical picture presents a flu-like syndrome, nausea, vomiting, abdominal pain, bloody diarrhoea, hepatosplenomegaly, and lymph-adenopathy. Laboratory findings include the direct detection of eggs by the microscopic examination or of the parasite's nucleic acid in patients'stool and urine, and serological tests for identifying the specific antibodies in patients returned from endemic areas, but without eggs demonstrated in faecal or urine samples [37].

Digestive infections caused by bacteria

One of the most common bacterial causes of gastroenteritis worldwide is Campylobacter spp. frequent especially in children under 2 year-old, causing diarrhoea, abdominal cramps, vomiting, fever and even bleeding. It is usually generated by the consumption of raw or undercooked meat (frequent/especially poultry) or contaminated milk.

Up to 25% of cases of antibiotic-associated diarrhea in healthcare institutions and hospitals are generated by Clostridium difficile. Elderly and immunocompromised patients have a higher risk. The most recent emergence of highly toxigenic and resistant C. difficile strains has led to more severe outbreaks, increasing mortality and morbidity [38].

In the developing countries, especially among children, Escherichia coli is a major cause of diarrheal disease and the leading cause of travellers'diarrhoea. The infections are produced by drinking water contaminated with human or animal feces. A special strain of this genus is Escherichia coli O157:H7, a Shiga toxin producing. These bacteria generate gastrointestinal infections with symptoms including vomiting and sometimes even bloody diarrhoea. Although it is usually cured after a few days, a percentage of 5-10% of infections lead to haemolytic uremic syndrome and its consequences (destruction of blood platelets, anaemia, kidney failure) [39].

Helicobacter pyloriis the main cause of gastritis and is associated with the development of gastric and duodenal ulcers or even stomach cancers. H. Pylori is the strongest etiological factor in both of these conditions, with the World Health Organization classifying it as a class I carcinogen. According to the CDC, approximately two-thirds of the population is infected with H. pylori, but less than 20% have a risk of developing ulcers or stomach cancer [40]. On the other hand, infection with H. pylori may protect against some cancers, such as oesophageal adenocarcinoma and gastroesophageal reflux disease [41]. It can cause stomach pain or nausea, but in many cases, there are no symptoms.

Salmonella and Shigella are food-borne GI illnesses. Salmonella is common and is found in raw meats, poultry, seafood and eggs, as well as milk and dairy products. Acute symptoms include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. Shigella is frequently found in water polluted with human feces. Symptoms of shigellosis or bacillary dysentery include abdominal pain, cramps, diarrhoea, fever, vomiting, and blood, pus, or mucus in stool.

Yersinia enterocolitica is a cause of diarrhea and abdominal pain relatively frequent after eating contaminated food, especially raw or undercooked pork products, milk or ice cream. Common symptoms are fever, abdominal pain and diarrhea, which is often bloody.

The most common cause of food intoxication, characterized by violent onset, severe nausea, cramps, vomiting, and diarrhea is Staphylococcus aureus. It is also associated with a large range of food, and could be found on humans (infected cuts, skin, throats and noses).

Brucellosis is a zoonosis caused by four different species of Brucella. It is considered as a major infectious disease in the Caribbean, the Middle East, Eastern Europe, Asia, and Africa. According to ECDC, one case of brucellosis was reported by Romania, in 2018 [21].The incubation period is between 2 weeks to 5 months. General brucellosis signs are usually vague and flu-like, with gastrointestinal symptoms such as dyspepsia, diarrhoea, constipation, vomiting, abdominal pain, hepatic abscesses. However, the clinical picture is dominated by fever, with undetected aetiology through routine laboratory investigations. The diagnosis of brucellosis, although easy to confirm based on serological tests, often requires a period of up to one year, due to the presence of a polymorphic, sometimes oligo-symptomatic clinical picture.

Leptospirosis is a global zoonotic infection with a high prevalence in Caribbean and Pacific islands, Central and South America, Southeast Asia, predominantly during the rainy period, caused by pathogenic spirochetes of genus Leptospira. In 2018, 51 cases of leptospirosis were confirmed in Romania [21]. Usually the incubation period is 1-2 weeks. It presents with a wide range of clinical symptoms, which could be easily mistaken for other diseases, including flu-like syndrome, nausea, vomiting, diarrhoea, rash, dry cough, jaundice, hepatomegaly and splenomegaly, oliguria/anuria, haemorrhages, with the involvement of multiple organs. Suspicion occurs in the case of association between digestive, liver, kidney manifestations with fever, and myalgia. Confirmation requires the determination of specific antibodies.

DIAGNOSIS

In the presence of symptoms of a possible gastrointestinal infection, the diagnosis could be confirmed by different old and modern laboratory tests using stool specimens first and others such as serum for specific antibodies (serological tests). The standard culture to isolate bacteria remains the gold standard for bacterial pathogens, even if it has a relatively low sensitivity and it is time-consuming (more than 24 h), but it may be followed by antibiotic susceptibility testing. In this way, the therapeutic options may be evaluated, but also the assessment of microbial resistance to antibiotic therapy [42]. Ova or parasite examination are ordered in case of suspicion of parasites. For this purpose, a direct examination by microscopy or a concentration method is used, but it has a low sensitivity and it is, once again, time consuming and requiring specific training of the laboratory staff. In addition, there are antigen tests used to detect Clostridium difficile (GDH, toxins), Giardia lamblia, Cryptosporidium spp., Helicobacter pylori, or Entamoeba histolytica. However, antigen tests can only detect a few specific parasites and therefore cannot replace ova and parasite examination. Antigen tests are useful to rapidly detect viruses causing gastroenteritis, such as rotavirus and adenovirus; however, these tests are not available for all entero-pathogens and show a variable sensitivity.

Taking into account the wide range of pathogens that can cause diarrhea and the similarity of the clinical manifestations produced by different pathogens; the molecular techniques seem to be the best tools for a rapid diagnosis. In the last years, some tools based on polymerase chain reaction (PCR) techniques have been developed to detect specific microorganisms or a panel of different entero-pathogens directly from stool samples [43-45]. Real-time PCR allows the simultaneous detection and differentiation of viral, bacterial and parasitic gastrointestinal pathogens. This assay also showed a high sensitivity for most pathogens included in the panel and efficient detection of coinfections. The main advantages of the molecular tools for the diagnosis of gastroenteritis are: high sensitivity, increased diagnostic spectrum, improved workflow (processing and diagnosis time is between 1 and 5 h), favorable impact on infection control and costs. Some disadvantages of molecular methods are the inability to distinguish between viable and nonviable microorganisms; they do not offer antibacterial susceptibility or epidemiological data; high cost; detection of the asymptomatic carriage could be difficult to distinguish from symptomatic/asymptomatic infections. Therefore, the application of these tools should be performed in patients selected based on clinical condition and/or the severity of gastroenteritis. Particularly in hospital settings, the rapid diagnosis provides important information for implementing infection control measures [46-48].

The clinical symptoms of all these diseases are nearly identical. Thus, it is difficult and almost impossible to determine the pathogenic cause based on clinical features alone. Rapid diagnosis, appropriate treatment, and infection control are important measures in these contexts. The early medical challenge is to include the possibility of tropical infectious diseases that are not common in the area, in the differential diagnosis. Not only the characterization of the symptoms but also the detection of infectious diseases based on a detailed history of the patient and a quick assessment can enormously help the clinicians in establishing a correct diagnosis and treatment.

Emerging evidence suggests that many gastrointestinal diseases are associated/caused by alterations in the gut microbiome. Specific microbiome signatures have been reported for irritable bowel syndrome and inflammatory bowel disease, and these have been associated with pro and anti-inflammatory effects, contributing to disruption or maintenance of gut homeostasis. Colorectal cancer is the third most common malignancy diagnosed worldwide, one of the leading causes for cancer-related mortality in both men and women. Different hereditary, environmental and inflammatory factors have been shown to increase the risk of colorectal tumorigenesis, but recently, bacteria and viruses have been described to be involved in colorectal carcinogenesis through direct modulation of epithelial cells, leading to genomic or chromosomal instability. Observational studies have found positive associations between gastric ulcers, periodontal disease, bacterial infections, and the risk of pancreatic cancer, the most rapid lethal cancer, with over 70% of patients dying within one year after diagnosis.

CONCLUSIONS

Concerning human mobility, several specific aspects must be considered, such as undiagnosed, atypical and subclinical types of diseases. These diseases are mistakenly treated as intercurrent diseases, and serious latent infections may develop. In the last few years, molecular methods have become readily available for many clinical laboratories and their utilization has resulted in a more rapid diagnosis and increased pathogens detection. As travel around the world continues, microbes will continue to evolve, adapting to new environmental conditions. The emergence of new infections will continue, and the distribution, severity and frequency of the identified infections will change, considering the new conditions. Travel or migration of people will continue to be an important factor in the occurrence and development of diseases. The analysis and monitoring of pathogens will focus the specialized actions and researches on the global movement and evolution of microbes. Integration of medical knowledge and multidisciplinary skills – social, biological and physical sciences – are therefore required.

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VARIA

Diagnostic challenges in late presenting diaphragmatic hernia

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Abstract: *Background: The incidence of congenital diaphragmatic hernia (CDH) has been reported to be 1 in 2000-5000 newborns. The late presentation can occur in 5-30% of all cases and it can be harder to diagnose, as symptoms are usually vague and non-specific.*

Methods: We performed a retrospective review of chart files of all patients admitted to our hospital with the diagnosis of congenital diaphragmatic hernia between 2010 and 2021.

Result: Of the ninety-two cases of CDH treated in our hospital between 2010 and 2021, twenty-six (28%) were late presenting. In the late-presenting CDH group, we found a slight female preponderance, with fifteen female patients (58%) and eleven male patients. A posterolateral left defect in the diaphragm was noted in fifteen cases (58%), a right defect was noted in just one case (4%) and ten patients (38%) were diagnosed with an anterior diaphragmatic hernia. The age at diagnosis varied from 2 months to 14 years. Associated anomalies were noted in just four cases. In all cases, a thoracoabdominal radiograph was performed and ten patients also had a CT scan. Primary repair was performed in 25 of the 26 patients. The mortality rate in our study population was 11%, we reported 3 cases with unfavorable evolution.

Conclusion: CDH presenting beyond the neonatal period is a usual finding and it represents a true diagnostic challenge.

Keywords: *congenital diaphragmatic hernia, late presentation, pediatric*

INTRODUCTION

One of the greatest challenges we face when diagnosing a pediatric patient is the constant concern that an unusual emergent condition, with non-specific symptoms, can easily masquerade as a common condition, and as such, they can be initially misdiagnosed.

Congenital diaphragmatic hernia is a congenital defect that occurs when the pleuroperitoneal canal does not close during the 8th week of fetal development, which allows the herniation of the bowel loops, stomach, solid abdominal organs, or the mesentery into the thoracic cavity. The incidence of CDH has been reported to be 1 in 2000-5000 newborns, accounting for 8% of all major congenital anomalies, with most cases being diagnosed prenatally or soon after birth (approximately 90%) [1–7].

CDH is a common cause of respiratory distress in the neonatal population and is associated with pulmonary hypoplasia, patients presenting with vomiting, tachypnoea, cyanosis, with diminished breath sound on auscultation after birth, but in some cases, it can go undiagnosed until later in life [3]. If CDHs are diagnosed later than 30 days they are considered late-onset CDHs [3, 8]. Late presentation in patients with CDH occurs in 5-30% of all cases, with patient age ranging between 2 months to 12.5 years [1, 3, 5].

Cases of CDH with delayed presentation are usually more subtle and because of that, they can be harder to diagnose [9]. Symptoms, diagnosis, and management of late-

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presenting CDH differ from those of neonatal CDH and are usually more diverse and non-specific. This usually leads to both clinical and radiological misdiagnosis, which can cause significant morbidity [4, 5, 10]. Massive pneumothorax and pleural effusion are the most common initial diagnosis on a chest radiograph in these cases and often, distinguishing between a CDH or a tension pneumothorax can be difficult and crucial, as the management of these two pathologies differs greatly [1, 3, 8]. Late-presenting CDH can cause acute or chronic respiratory or gastrointestinal symptoms such as dyspnoea, recurrent pulmonary infections, chest pain, postprandial fullness, or abdominal pain [2, 10].

This study aims to emphasize the fact that diagnosing CDH outside de neonatal period is often difficult and to help familiarize healthcare professionals with the clinical presentation that can occur in these cases to help facilitate early management and avoid misdiagnosis

MATERIALS AND METHODS

We performed a retrospective review of chart files of all patients admitted to our hospital with the diagnosis of congenital diaphragmatic hernia between 2010 and 2021. Patients who developed symptoms and were diagnosed in the first month of life were classified as having early presenting CDH, while those who were diagnosed after one month of age were considered to have a late-onset CDH. Only patients with late-presenting CDH age were included in our study. Patients with diaphragmatic eventration, hiatal hernia, or paraesophageal hernias were excluded from the study. Patient demographics, treatment, and outcome were then reviewed.

RESULTS

Of the ninety-two cases of CDH treated in our hospital between 2010 and 2021, twenty-six (28%) were late presenting. In the late-presenting CDH group, we found a slight female preponderance, with fifteen female patients (58%) and eleven male patients. A posterolateral left defect in the diaphragm was noted in fifteen cases (58%), a right defect was noted in just one case (4%) and ten patients (38%) were diagnosed with an anterior diaphragmatic hernia. The age at diagnosis varied from 2 months to 14 years. The patients were divided into 3 age groups: under 1 year, between 1 and 5 years, and over 5 years. We found that patients under the age of one presented with either left-sided defects or with an anterior hernia. In the case of patients with ages between 1 and 5 years, left-sided defects were more frequent than right-sided defects or anterior hernias, the same being the case for patients older than 5.

Associated anomalies were noted in just four cases: two patients had a history of esophageal atresia repair in the neonatal period, one patient presented with Down syndrome, and one patient presented with cardiac anomalies that did not require surgical correction.

Table 1 illustrated the relationship between age and location of the defect.

Table 1: The relationship between age and location of the defect

Age	Anatomical variant CDH		
	Left	**Right**	**Anterior**
< 1 year	2	0	5
1-5 years	9	1	3
> 5 years	4	0	2
Total number of patients	15	1	10

Clinical presentation in late-presenting CDH was mixed and various as can be seen in Table 2. For that reason, we dived the patients into four main categories as follows: 8 patients (31%) with respiratory symptoms, 6 (23%) with digestive symptoms, 6 (23%) with both, and 6 (23%) were asymptomatic.

Table 2: Classification of symptoms

Symptoms	Number of cases	Details
Respiratory	8	
Acute	6	Dyspnea 4 Cough 3 Febrile URTI 3
Chronic	2	Chronic respiratory distress
Gastrointestinal	6	
Acute	6	Vomiting 5 Abdominal pain 3 Loose stools 1
Chronic	0	
Both	6	
Acute	4	Dyspnea Vomiting Abdominal pain
Chronic	2	Failure to thrive + abdominal pain + chronic respiratory distress
Asymptomatic	6	

URTI – upper respiratory tract infection

Table 3 shows the site of the hernia concerning the presenting symptoms. Of the six patients presenting with gastrointestinal symptoms, five had a left-sided defect, and just one patient presented with an anterior hernia, while of the eight patients presenting with respiratory symptoms, four were found to have a left-sided defect, and four presented with an anterior hernia. The patient who was diagnosed with a right-sided defect presented with both respiratory and digestive symptoms. We found three patients with a left-sided defect and four patients with an anterior defect that were asymptomatic and were diagnosed incidentally when a chest radiograph was performed when monitoring another pathology.

Table 3: The relationship between symptoms and location of the defect

Symptoms	Right	Left	Anterior
Respiratory	0	4	4
Gastrointestinal	0	5	1
Both	1	4	1
Asymptomatic	0	2	4

In all cases, a thoracoabdominal radiograph was performed and it should be mentioned that almost half of our patients had previous chest radiographs performed routinely for other conditions that were normal at that time. (Figure 1, Figure 2) Ten patients also had a CT scan that confirmed the diagnosis of diaphragmatic hernia and four patients had upper gastrointestinal contrast studies performed. It bears mentioning that all the patients with upper GI contrast studies were admitted to our hospital at the beginning of the study period. In the last 7 years, upper GI contrast studies have not been performed to diagnose a late-presenting diaphragmatic hernia.

Figure 1: Chest radiograph showing left-sided diaphragmatic hernia

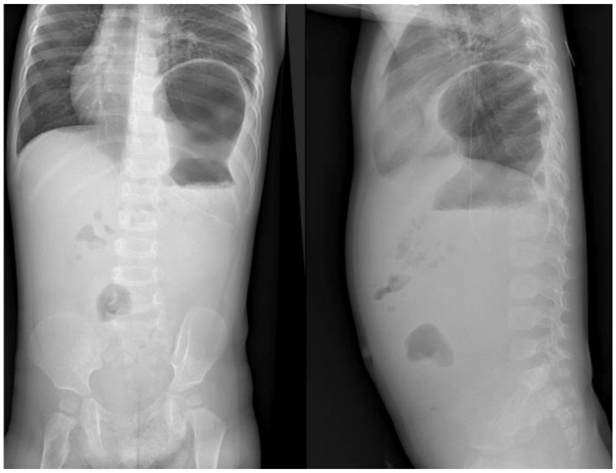

Primary repair was performed in 25 of the 26 patients. The open abdominal approach was favored in most cases (76%), while only six patients were operated on by minimally invasive techniques. Out of the six, four patients underwent laparoscopic approach, and two patients underwent thoracoscopic approach.

Figure 2: Chest radiograph showing anterior diaphragmatic hernia

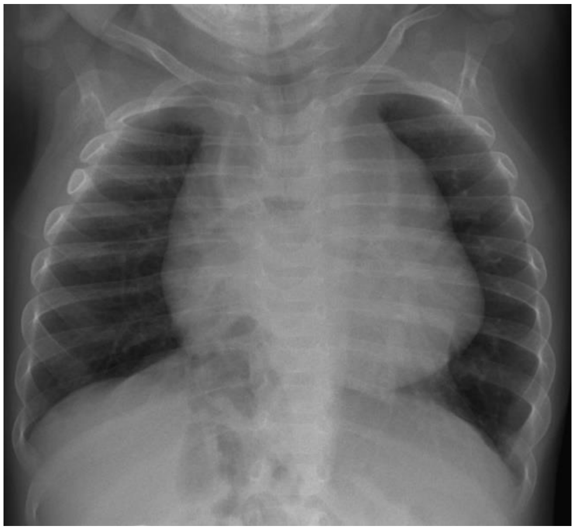

The mortality rate in our study population was 11%, with all three patients had presented with severe complications such as gastric volvulus. In the remaining patients, we identified just one case of pleural effusion after thoracoscopic approach that required drain placement, with no further complications being observed later on.

We report the cases of the three patients that had an unfavorable outcome.

The first case was that of a 14-year-old girl with a history of 3 days of diffuse abdominal pain and vomiting. On admission, clinical examination showed respiratory distress with diminished breath sounds on the left side. The patient went into cardiocirculatory arrest soon after admission that was successfully managed by external chest compressions and resuscitation drugs. Chest and the abdominal radiograph showed a hydroaeric image and the tip of the nasogastric tube situated in the left hemithorax, with no stomach bubble present in the abdominal area. A thoracoabdominal CT scan was performed to confirm the diagnosis of the left diaphragmatic hernia (Figure 3). Emergency surgery by laparotomy revealed a large left diaphragmatic defect with a herniated stomach. After stomach reduction into the abdominal cavity, a large quantity of blood was evacuated from the left thoracic cavity. The stomach was almost completely necrotic, perforated, and presented with thrombosis of the gastric vessels. Subtotal gastrectomy with oesophageal-gastric anastomosis and diaphragmatic defect suture was performed, but the postoperative outcome was unfavorable, the patient dying on postoperative day 10 due to multiple organ dysfunction syndrome and cardiac arrest.

Figure 3: CT scan showing a left-sided defect in the diaphragm with herniation of the bowel and stomach

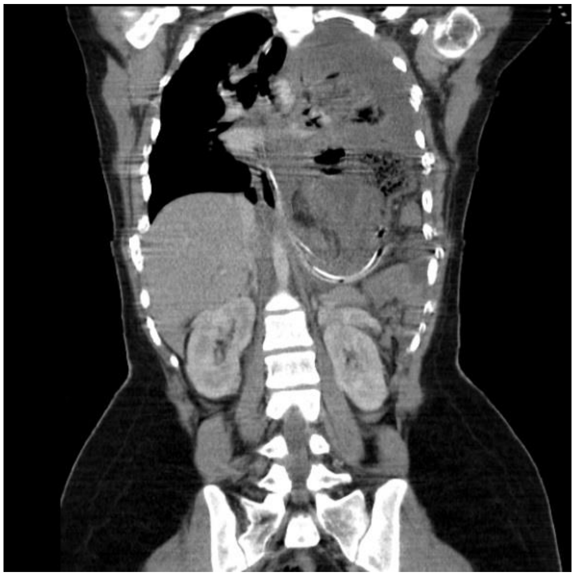

The second case was that of a 6-year-old girl with a history of abdominal distension, diffuse pain, and vomiting. On clinical examination, the patient presented with abdominal distension and diffuse abdominal pain with tenderness on palpation, dyspnoea, and paleness.

An abdominal radiograph showed a tension pneumoperitoneum, with an elevation of the left hemidiaphragm and collapsed bowel loops in the hypogastric region (Figure 4).

The patient was immediately taken into the operating room. She presented with severe bradycardia and went into cardiac arrest, which was managed by orotracheal intubation, external chest compressions, and resuscitation drugs. After 10 minutes, cardiac activity reappeared, and emergency laparotomy was performed. Intraoperatively, a large quantity of gastric liquid and chyme was evacuated from the peritoneal cavity. A large left diaphragmatic defect (10/6 cm) was found, with herniated stomach, spleen, and left hepatic lobe. After the reduction of the herniated content, a large perforation of the stomach was noted. A partial gastrectomy with diaphragmatic defect sutures was performed.

After surgery, the patient developed intravascular disseminated coagulation and acute renal failure, and 24h after admission she went into cardiac arrest that did not respond to resuscitation maneuvers.

Figure 4: Tension pneumoperitoneum with a left diaphragmatic hernia and gastric perforation

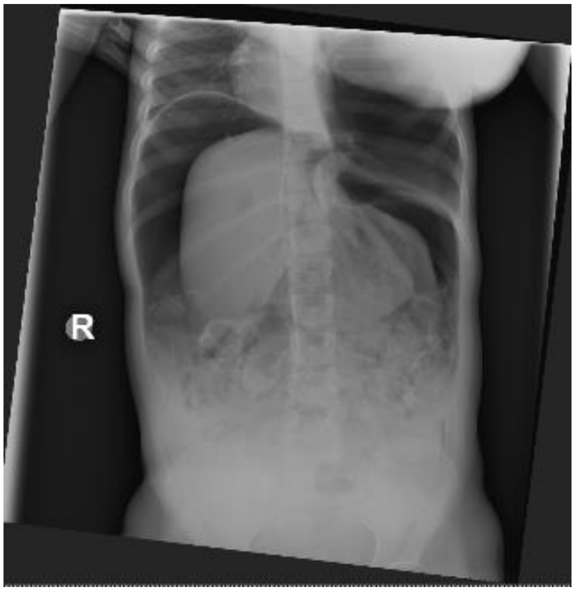

The third case was that of a 4-years-old patient who presented to the emergency room for diffuse abdominal pain and vomiting. An ultrasound was performed in the emergency room than normal. Due to the latest COVID 19 guidelines, the patient was admitted to the COVID ward, while awaiting her PCR test results. Six hours after admission, she went into cardiac arrest and the resuscitation protocol was started with external chest compressions and iv drugs administration. The patient was intubated, and a chest radiograph was performed that showed large hyper transparency in the left hemithorax with an important mediastinal shift to the right, with an imprecise contour of the left diaphragm (Figure 5).

Since the patient could not be ventilated after intubation and because of the image on the chest radiograph, the decision was made to insert pleural drainage. After the tube was inserted, the patient was stabilized.

A contrast substance was given through the nasogastric tube and showed extravasation of the contrast substance into the abdominal cavity, with an imprecise left diaphragm and a smaller hyper transparency in the left hemithorax. The diagnosis of gastric perforation was made and the patient was prepared for surgery. Before she could be taken into the operating room the patient went into cardiac arrest, but after multiple attempts of stabilization, she did not respond to resuscitation maneuvers.

Post mortem examination revealed a left diaphragmatic defect with a perforated stomach due to gastric volvulus.

Figure 5: Tension gastrothorax

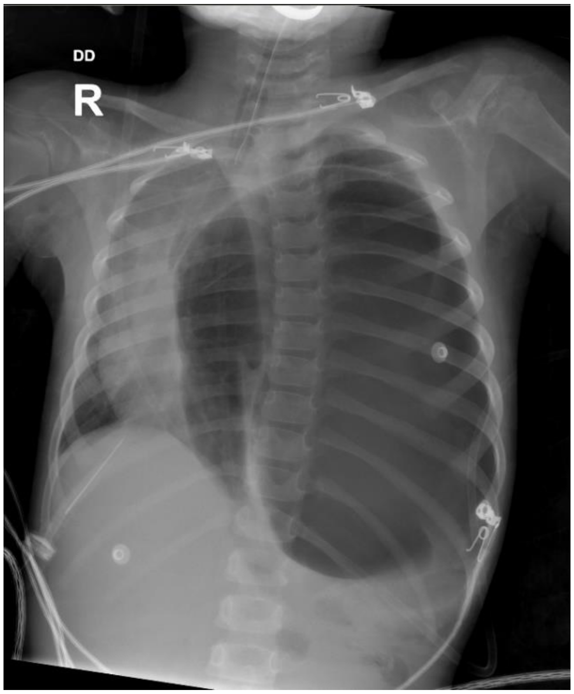

DISCUSSION

A congenital diaphragmatic hernia occurs because of the incomplete closure of the pleuroperitoneal canal during fetal development [11]. Diaphragmatic closure is usually complete by the ninth week of gestation, with the left side closing one week after the right side [12].

During the neonatal period, CDH is the most common cause of respiratory distress, and it can result in high mortality rates in this age group. In most cases, the diagnosis of CDH is established during fetal development or early in the neonatal period [11].

The classification of CDH includes 4 types of patients as follows:

- Type 1 – when the herniation occurs in the early period of fetal development, resulting in pulmonary hyperplasia and increased risk of perinatal death

- Type 2 – when the herniation occurs in the late period of rapid bronchial division, resulting in unilateral pulmonary hypoplasia and pulmonary hypertension

- Type 3 – when the herniation occurs during the late stages of gestation or early in the neonatal period; some patients can present with mild respiratory symptoms at birth, others may not present problems during the neonatal period

- Type 4 – when the herniation occurs after the neonatal period; these patients have no pulmonary hypoplasia [11, 13]

The incidence of late-onset CDH varies from 5 up to 45.5% [8, 9, 11]. There is a 2:1 male to female ratio in both left and right-sided hernias, with 32.7% of cases presenting with hernia sacs [11, 14, 15]. In our case series, we found a slight female preponderance.

Localization on the right side of the defect is more frequently encountered in patients with late-presenting CDH. Patients with right-sided CDH become symptomatic at a younger age compared to patients with left-sided defects [14]. In our study, most of the patients presented with left-sided defects (15 cases) and anterior defects (10 cases). There was just one case of right-sided hernia, in a 9-year-old patient.

Late-onset CDH is difficult and intriguing to diagnose due to various, nonspecific, vague, and misleading clinical presentations, with an array of symptoms that are not pathognomonic for this defect, making it a difficult diagnosis [4, 16–20]. Misdiagnosis of these cases is not uncommon (according to some reported occurring in 62% of cases) and can result in increased morbidity with herniated bowel necrosis, peritonitis, respiratory arrest, and, in some cases, even death [3]. A high index of clinical suspicion and a chest radiograph is essential in establishing a diagnosis [6]. Initial symptoms in patients with CDH can be respiratory (coughing, wheezing, frequent upper tract infections, dyspnea), gastrointestinal (abdominal pain, vomiting, nausea, constipation, failure to thrive), or a combination of the two [5, 21]. Symptoms of late presenting cases are often misleading, and patients are often treated for other conditions with similar symptoms [21]. There have been studies reporting a higher incidence of respiratory symptoms in younger children and right-sided hernias and gastrointestinal symptoms in older children as well as in those presenting with left side CDH [4, 18, 22–24]. 10-11% of patients may be asymptomatic at the moment of diagnosis [21, 25].

Our study population is too small to obtain statistical significance in this regard. Patients with left-sided defects presented with either respiratory symptoms, digestive symptoms, or both. Most patients with anterior defects presented with respiratory symptoms and, in the single case of right-sided diaphragmatic hernia we encountered, our patient presented with both respiratory and digestive symptoms. Four of the youngest patients in our study were asymptomatic, while one presented with respiratory symptoms and two presented with both respiratory and digestive symptoms.

Late-onset CDH presenting with gastrointestinal symptoms have a smaller hernia defect and the symptoms are usually caused by different degrees of obstruction secondary to incarceration [4].

A smaller defect can prevent herniation of the viscera, either due to the existence of a hernia sac that confines the viscera or secondary to occlusion of the defect by solid organs (such as the liver or the spleen), but in some cases, when the abdominal pressure increases (during coughing, sneezing or other strenuous activities) bowel may herniate into the thoracic cavity [1, 24, 26]. The patient may become symptomatic when the actual herniation occurs either after a hernia sac rupture or due to complications of the herniated content such as volvulus or strangulation [1]. The hernia may be intermittent and as such, a normal radiograph does not necessarily exclude the diagnosis [5, 6, 27]. In our cases series, more than half of our patients had normal previous chest radiographs.

Kim et al. reported that 23% of patients with late-onset CDH present with associated congenital malformations such as intestinal malrotation, cardiac defects, pulmonary hypoplasia, chromosomal anomalies, and urogenital anomalies, while others reported an increased prevalence between 8-80% [16, 24, 28-31]. Studies have shown that there is a lower incidence of associated anomalies in late-onset CDH when compared to early-onset CDH, diagnosed in the neonatal period [13, 31]. In our case, just two patients presented with a history of esophageal atresia repair.

Chest radiograph supplemented by additional imaging such as CT scan can be a useful diagnostic tool in the differential diagnosis of thoracic lesions such as lower lobe pneumonia, pneumothorax, pleural effusion, or other lung lesions [11, 22, 32].

There have been reports when CDH was misdiagnosed as pneumothorax and chest tubes were inserted into the thoracic cavity, which lead to morbidity in some cases. [6] Distinguishing between these two entities is extremely important as the management of these two conditions is different [3, 18, 24, 33]. Baglaj et al. reported that chest radiographs diagnose only 49% of cases, with almost 25% of them being diagnosed with other conditions [21, 34, 35].

If a chest radiograph fails to establish the diagnosis, upper gastrointestinal series or contrast-enhanced CT scans can be used to diagnose CDH [4]. Upper gastrointestinal studies are considered unnecessary in the neonatal period but have been recommended in older children [14]. Performing a CT scan means a high radiation burden for the patient, but this is a useful imaging modality that can bring important information regarding the anatomy of the defect, which helps in preoperative planning [3, 13]. Chest radiographs were performed in all cases and CT scans were performed in 10 cases to confirm the diagnosis. Contrast studies were not performed in four cases, but these patients were patients who had been diagnosed in the early years of our study period.

Once the correct diagnosis has been established, patients with late-onset CDH have a more favorable prognosis due to the less severe or the absence of pulmonary hypoplasia [8, 11, 18, 36]. In the case of late-onset CDH, a 3.6% postoperative complication rate was reported, with a 1.9% recurrence rate and a 1.95 mortality rate [37]. In our study, the mortality rate was higher, with patients having had an unfavorable outcome. Just one patient in our series presented with postoperative complications (pleural effusion) that was resolved by placing pleural drainage.

Late-presenting CDH is prone to rare complications such as tension gastrothorax, gastric perforation, splenic torsion or rupture, intestinal obstruction, or bowel necrosis [4, 27].

When the stomach herniates in the thoracic cavity, it can become distended resulting in tension gastrothorax. Acute gastric volvulus is a possible complication of patients with late-presenting CDH. Such cases are rare but can have important, life-threatening complications. The distended stomach leads to severe respiratory distress. Okuda et al. reported a case of a patient with acute tension gastrothorax with cardiopulmonary arrest and failed resuscitation. An acute gastric volvulus is a surgical emergency, with non-operative management being associated with an 80% mortality rate. There is also a risk of mediastinal spillage, with possible rupture of major blood vessels [13]. The first step in managing such patients is the immediate decompression of the stomach, reduction of the volvulus, and treatment of the underlying cause. The association of gastric volvulus and CDH is extremely rare, with only 27 pediatric cases having been reported thus far. This association can occur due to the absence of elongation of 2 of the 4 ligaments supporting the stomach (gastrophrenic and gastrosplenic) [13]. Two of our patients presented with this complication, with unfavorable outcomes [11, 27, 38, 39].

Surgical management is necessary to correct the defect and prevent further complications [20]. The type of approach varies in different centers and even in the same center among different surgeons. No consensus has been reached regarding the best approach for late-onset CDH. The current recommendation is for each surgeon to decide on the type of surgery based on one's experience and the patient's history [23, 40]. Thoracotomy allows for safer surgery when dissecting adherences between the abdominal viscera and the mediastinal contents. This approach is particularly useful in recurrent hernias, with some cases even requiring a combined thoracoabdominal approach [12].

For elective repair, the indication is to perform minimally invasive surgery [40]. Some studies have shown an increase in recurrence rate in minimally invasive surgery for the repair of CDH of 5-23.1%, with a higher rate in cases of thoracoscopic repair [9, 26, 41]. In their study, Obata et al. reported a higher recurrence rate after the laparoscopic approach, most likely due to difficult visualization of the posterolateral defect [23, 42].

Twenty-five of the twenty-six patients in our series underwent surgical repair of the diaphragmatic defect, nineteen by open abdominal approach, four by laparoscopic approach, and two by thoracoscopic approach. None of our patients presented with postoperative recurrence. There are some controversies when using the thoracoscopic approach regarding chest drainage placement, with some studies reporting good results with tube placement, while other authors suggest that the residual pneumothorax will spontaneously be resorbed, and the placement of the chest tube can cause negative intrathoracic pressure that can increase the risk of recurrence [9, 11]. In the two cases that were resolved by the thoracoscopic approach in our series, there was no tube placement, but one patient presented with postoperative pleural effusion that required a second surgery with fluid drainage and tube placement. After tube placement, the patient presented with no further complications

CONCLUSIONS

CDH presenting beyond the neonatal period is a usual finding and it represents a true diagnostic challenge, as the clinical presentation is non-specific, and misdiagnosis is a frequent occurrence and should always be considered as a differential diagnosis in different clinical settings. Raising awareness of late presenting diaphragmatic hernia may be key to improving prognosis and avoiding medical errors.

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CLINICAL PRACTICE

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Diagnostic difficulties in c-kit negative gastrointestinal stromal tumors: report of four cases

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Abstract: *Introduction: The gastrointestinal stromal tumors (GIST) are dominated by KIT and PDGFRA mutation. The immunohistochemical detection of CD117, a protein express by KIT gene, is essential for the diagnosis and those that are negative always represented a diagnostic challenge*

Case reports: In this article we present a series of 4 cases of CD117 negative GIST tumors, diagnosed and surgically resected in Fundeni Clinical Institute and an overview of the histogenesis, diagnostic problems and management of c-kit negative GIST. All patients were males and the tumors were located in the stomach and small bowel.

Conclusion: It is important for the pathologists to beware of the fact that a CD117 negative in the context of a typical morphological appearance does not exclude a GIST tumor and also the oncologist must be aware not to exclude the therapy with imatinib based on the negativity of CD117.

Keywords: *GIST, gastrointestinal stromal tumors, c-kit negative, CD117, DOG1, immune-histochemistry*

INTRODUCTION

GISTs are rare tumors accounting for less than 1% of all gastrointestinal tumors but they are the most common mesenchymal tumors of the GI tract.[1] The incidence in Europe and USA is 7-10 cases/1,000,000 [2] and in Korea is 16-22/1,000,000 [3]. Large population based studies from Iceland, Netherlands and Sweden found the incidence to be 11, 12.7 and 14.5 cases/1,000,000 [4,5,6]. However there are studies that explain this increased incidence by a better diagnosis using modern diagnosis criteria rather than a real increase [7].

The most common age of the diagnosis is between 50 to 60 years old (a mean age of 65) and a peak incidence between 70 and 79, before 20 years old being quite rare ≈ 1% [7,8]

GIST affects both sexes equally [6] though some authors found them to be more common in men [7,9]. They arise most often in the stomach (50-60%) and small intestine (30-35%) but it can occur in any segment of the gastrointestinal tract only with a lower frequency.[10]

Gain of function mutation in KIT or PDGFRA oncogenes lead to ligand-independent kinase activation [11] which gives rise to the majority of GIST. Immunohistochemistry is useful for diagnosis and

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typically detects c-kit protein expression, but if the tumor is negative for c-kit, a final diagnosis can be challenging.

In this article we present a series of 4 cases of c-kit negative GIST tumors, diagnosed and surgically resected in Fundeni Clinical Institute and an overview of the histogenesis, diagnostic problems and management of c-kit negative GIST.

CASE REPORTS

In the pathology archives of Fundeni Clinical Institute, between 2004 and 2017 we have traced four cases of c-kit negative GIST. We have examined available clinical data from the hospital database and also HE slides. Immunohistochemically investigations were performed with biotin-streptavidin method [12] in order to assess the phenotype and provide a definitive diagnosis.

Antibody suppliers were Novocastra, and Labvision/Thermo Fisher Scientific as previously reported [13] and the dilutions respected the manufacturer recommendation. The stratification risk according to Miettinen&Lasota (2006) was used [2]. For the mitotic rate we counted the number of mitosis on 50 high power fields (HPF). Tumor cellularity was divided in three categories: spindle, epithelioid and mixed.

Case no. 1

A 47 years old male who presented with lower gastrointestinal bleeding, was investigated with capsule-endoscopy procedure and found with a tumor on the ileum, close to the ileocecal valve. A segmental enterectomy was performed. The surgical specimen was 12 cm long and presented centrally a well circumscribed white nodule of 4/3/4 cm, expanded in the submucosa and muscularis propria with mucosal ulceration. Histologic examination revealed a tumor consisting of spindle cells with a mitotic rate of 1-2/50 hpf. Immunohistochemical stains showed negativity for CD34, CD117, PDGFRA, Desmin, a weak positivity for SMA and a diffuse cytoplasmic positivity for DOG1. The prognostic group was 2, and 7 years later the patient is alive with no recurrent disease.

Figures case 1

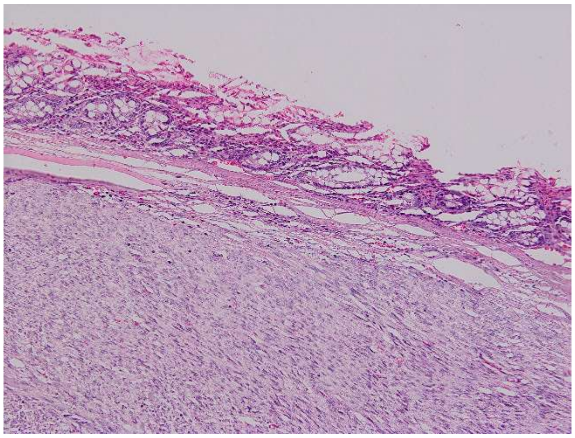

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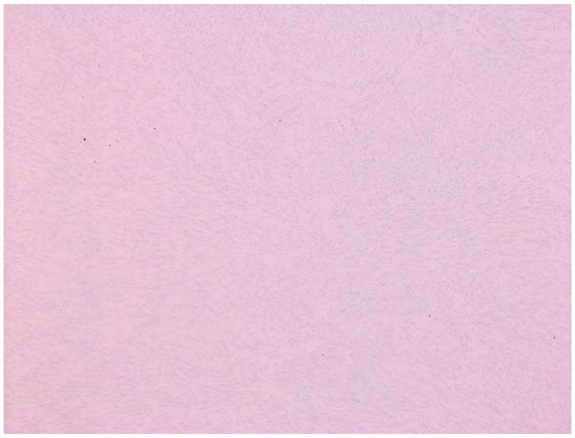

CD117x100

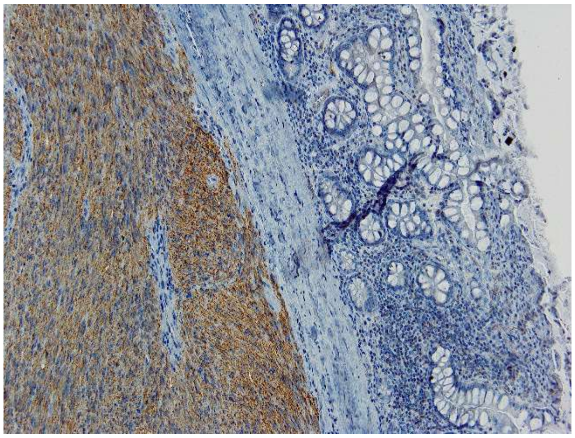

DOG1x100

Case no. 2

A 69 years old male presented with abdominal pain, nausea and vomiting for 12 hours before admission. The abdominal CT scan revealed a pelvic tumoral mass on the left paramedian side, developed behind the abdominal wall that involved a small intestinal loop. A segmental enterectomy was performed. The surgical specimen was 30 cm long and presented a solid white tumor with irregular contour 8.5/3.5/2cm with predominantly intramural and subserosal development and focal mucosal ulceration.

Microscopic examination reveal a mixt morphology with spindle and epithelioid cells, with mild pleomorphism, intratumoral hemorrhagic foci and a mitotic rate of 2/50 HPF.

Immunohistochemical stains were negative for SMA, Desmin and CD117 and positive for DOG1. CD34 and PDGFRA were not done.

Figures case 2

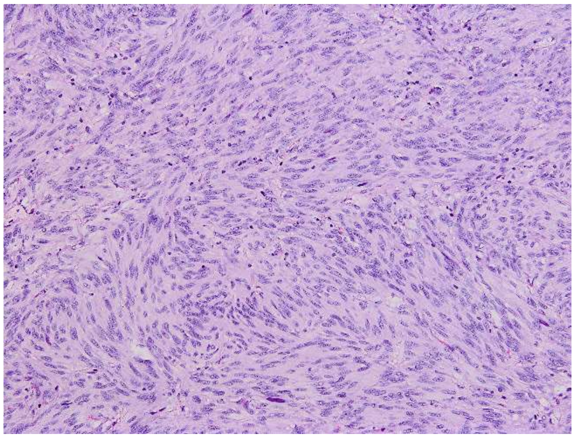

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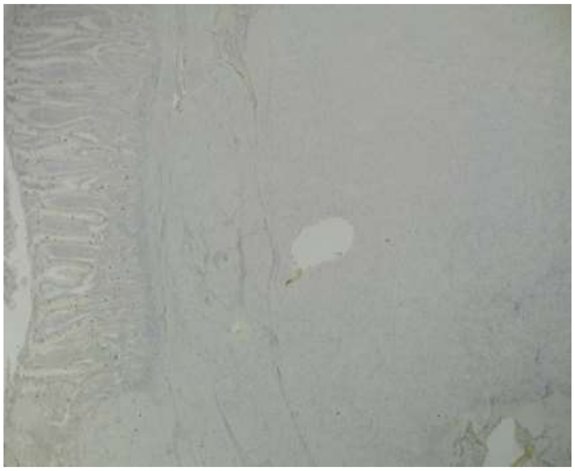

CD117x100

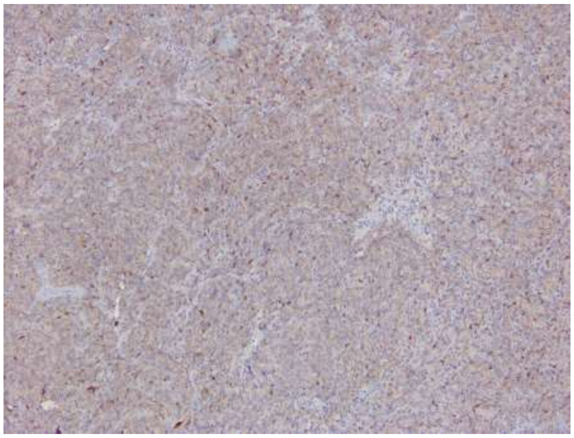

DOG1x100

On the bases of these results the tumor was diagnosed as a c-kit negative GIST with moderate risk (group 3a) and survival from the time of the diagnosis until death was 33 months.

Case no. 3

A 67 years old male was admitted to the hospital with melena, fatigue and weight loss. An esophagogastroduodenoscopy was performed, revealing a 1.5 cm lesion in the gastric fornix.

The tumor biopsy showed a stromal gastric hemorrhagic proliferation associated with a chronic gastritis with Helicobacter Pylori infection.

A local excision was carried out and on the surgical specimen was found a firm rubbery white intramural nodule with a diameter of 3/3/3 cm.

Microscopy revealed a tumor proliferation with spindle cell morphology, without mucosal involvement, with 1-2 mitotic figure/50HPF.

Immunohistochemistry showed negativity for CD117, Actin, Desmin and positivity for DOG1. CD34 and PDGFRA were not done.

The diagnosis of c-kit negative GIST with low risk (group 2) was established and the patient is alive with no recurrence.

Figures case 3

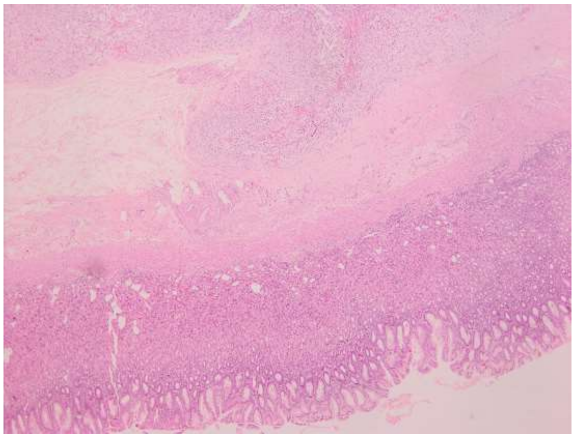

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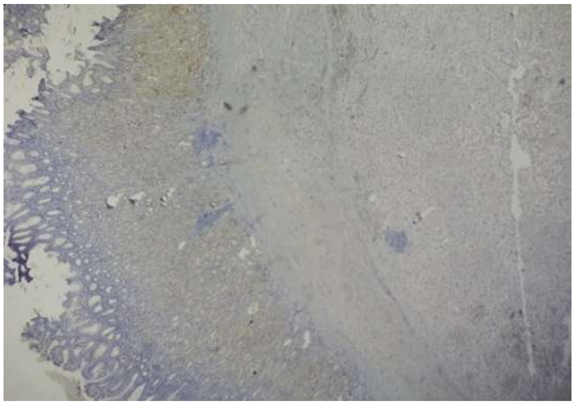

CD117x100

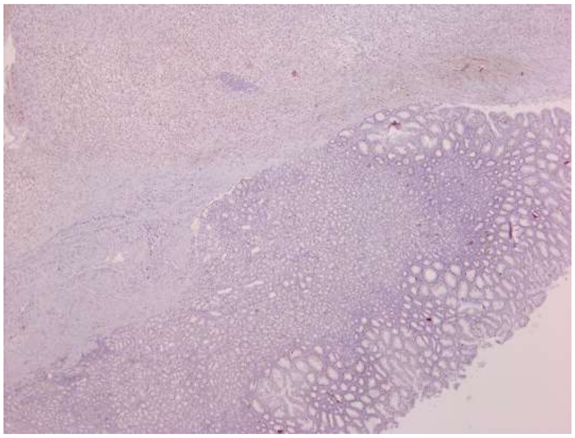

DOG1x100

Case no. 4

59 years old male with loss of appetite, abdominal pain, weight loss, is admitted to the hospital for investigations.

Esophagogastroduodenoscopy revealed an ulcerated tumoral proliferation on the body of the stomach and an ultrasound discovered multiple localized proliferations in left and right hepatic lobes.

A total gastrectomy was performed and the surgical specimen showed a12 cm white grey tumor with intramural development and cystic degeneration. On the microscopic examination it showed an epithelioid and spindle cell morphology with fascicles that intersect and intertwine at various angles, with more than 10 mitotic figures/50 hpf Immunohistochemistry showed negativity for CD117 and Desmin, and positivity for SMA and DOG1.

A diagnosis of GIST with high risk of recurrence (group 6b) was established and the patient survived time from the initial diagnosis was for 9 months.

Figures case 4

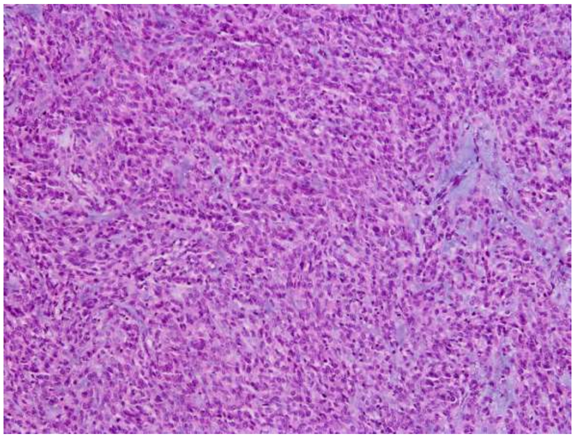

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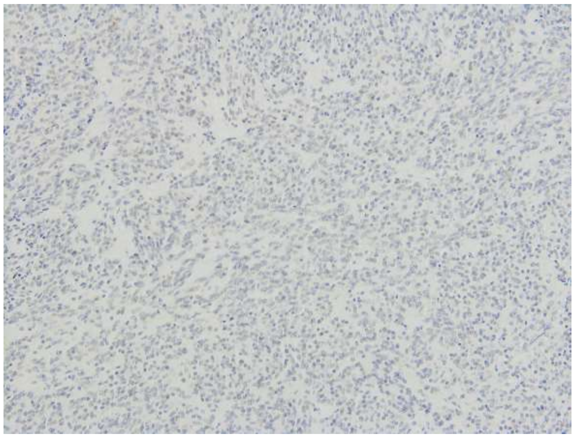

CD117x100

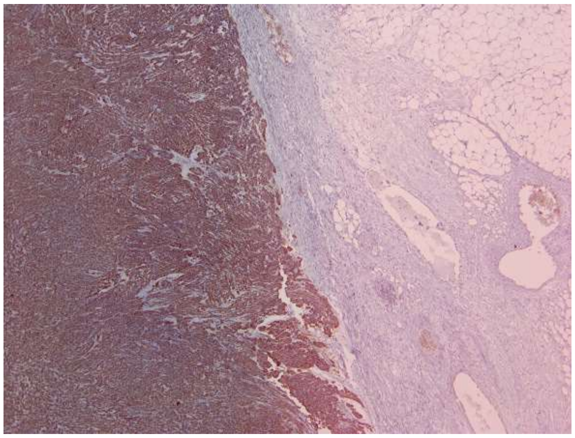

DOG1x100

DISSCUSSION

Arising in the interstitial cell of Cajal, GIST tumors are an entity dominated by mutations in KIT receptor tyrosine kinase which accounts for almost 80% of all cases. The histopathological diagnosis of such tumors is built upon the microscopic morphology and immunohistochemically stains.

The detection of CD117, a protein express by KIT gene, is essential for the diagnosis and we can find it in approximately 95% of GIST [8, 14].

Table 1: Clinical-pathological characteristics of the four cases presented

Case no.	Age	Location	Metastasis	Tumor diameter	Mitosis/ HPF	Cell type	Group	Death	Survival months	Resection
1	47	SI	no	4.0	<5	fusiform	2	no	84	R0
2	69	SI	peritoneum lung	8.5	<5	mixt	3a	yes	33	R1
3	67	S	no	3.0	<5	fusiform	2	no	135	R0
4	60	S	liver peritoneum	12.0	>5	mixt	6b	yes	9	R1

Table 2

	CD117	DOG1	Desmine	SMA
Clone Dilution	T595 1:40	K9 1:100	DE-R-11 1:100	αsm-1 1:50
Case no.1	negative	positive	negative	positive
Case no.2	Negative	positive	negative	negative
Case no.3	Negative	positive	negative	positive
Case no.4	negative	positive	negative	positive

However, there are 4-5% of GISTs that are negative for CD117 and in these cases immunomarkers like DOG1 (ANO1) are very useful for the confirmation of the diagnosis [9,15,16].

DOG1 shows higher prevalence of positivity in gastric epithelioid GISTs, which are often KIT negative.[17]

Depending of the antibody used, the positivity of DOG1 in KIT-negative GISTs varies: with clone DOG1.1, the positivity is 36% [18] and with clone K9, the positivity is 50–76%. [17,19]. We also used K9 clone in our cases, which we found very helpful.

Other immunohistochemical markers used, that can guide the diagnosis are CD34 (with a much higher positivity in the stomach-80% then in the small intestine-35%) together with SMA Desmin, Vimentin. CD34 was done only in one of our cases, a small intestine GIST and it was negative so the only reliable marker was DOG1.

Most of the GISTs are reported in the stomach (50-60%), jejunum and ileum (30-35%), duodenum (5%), colorectal (4%), and rarely in the esophagus and appendix (<1%) [10, 20]. Primary tumors outside the GI tract have been reported in small numbers in omentum [21,22], mesenteries and retroperitoneum [23,24]. The location of c-kit negative GIST is more frequently reported in the stomach [25, 26, 27] and less in the small intestine [28,29]. Two of our cases were located in the stomach but the other two located in the small intestine gave rise to diagnostic suspicion so we had to pay more attention at the differential diagnosis due to the rarity of the tumor site.

Location and size of the tumor influence the apparition of symptoms. Small tumors are usually silent and are being discovered incidentally during investigations or surgical procedures for other disease However if a GIST becomes symptomatic, it usually cause non-specific symptoms like weight loss, fatigue, nausea, bowel obstruction or overt or occult gastrointestinal bleeding due to mucosal ulceration or tumor rupture[30,9]. Two of our patients presented with nonspecific symptoms like abdominal pain, nausea, fatigue, weight loss, and the other two with bleeding signs. Non-specific symptoms delays the diagnosis and we can see from Table 1 that those two cases have also the largest diameter (8.5 cm in the small intestine and 12 cm in the stomach).

The prognosis and predictive factors for survival of patients with C-kit negative GISTs are still unclear and difficult to assess because of insufficient data. The tumor diameter, metastatic disease, incomplete resection is associated in our cases with a shorter survival rate (Table1).

DOG1 especially clone K9 is an invaluable marker of the diagnosis but mutational status comes in hand in the treatment management. Complete resection and inhibitors of tyrosine kinase are considered for the treatment as in c-kit positive GIST [31].

However when we encounter a c-kit negative GIST we need to take into consideration:
- The antibody that we use, the clone and the laboratory technique
- The fixation methods of the surgical specimen may influence the reaction
- The CD117 negativity does not exclude a GIST diagnosis and other markers like DOG1 are necessary to complete the immunohistochemical diagnostic panel; we also must think at GIST with other mutations like PDGFRA
- For a possible treatment with inhibitors of tyrosine kinase (imatinib) we must have in mind mutation analysis as well
- GIST wild type is a constantly changing concept, at first considered a KIT negative GIST, now is described as GIST with no identified gain of function mutation. Besides the KIT gene, GISTs may present mutations in another receptor tyrosine kinase - the PDGFRA which accounts for less than 10% of all GISTS [32].

According to most studies, activating mutations in KIT or PDGFRA are present in 85 - 90% and are mutually exclusive [33]. Other driver mutations studied, like BRAF, RAS, PIC3K, SDHA, NF1 need to have other genetic event in order to develop and progress [34,33, 35,36]

CONCLUSIONS

Kit negative GIST is a problematic entity which makes the diagnostic difficult.

In conclusion, we report four cases of CD117 negative, DOG1 positive GIST located in the stomach and small intestine, all being male patients.

It is important for the pathologists to beware of the fact that a CD117 negative in the context of a typical morphological appearance does not exclude a GIST tumor and also the oncologist must be aware not to exclude the therapy with imatinib based on the negativity of CD117.

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Conflict of interests
The authors declare no conflict of interests.

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ORIGINAL ARTICLES

Diagnostic predictability of using portal vein diameter in confirming portal hypertension

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Abstract: *Objective: To find an accurate cut-off value for Portal vein diameter as a diagnostic tool for Portal hypertension on comparing with Doppler ultrasound, CT abdomen, or endoscopy of collateral circulation.*

Materials and methods: This was a cross-sectional study, with a sample size of 100 patients. Inclusion criteria were patients above the age of 15 years who underwent routine USG abdomen for various conditions in the Radiology Department of a tertiary hospital. Distribution of age, gender, chronic liver disease, portal hypertension, and portal vein diameter of all the patients were measured and recorded. Portal hypertension was diagnosed using the presence of collaterals either on upper endoscopy (if done), Doppler USG, or CT multiphase abdomen.

Results: Portal vein diameter of 10.5mm was found to have a sensitivity of 61.22%, specificity of 60.78%, a positive predictive value of 60.00%, a negative predictive value of 62.00%, and accuracy of 61.00%. 45 out of 59 (76.3%) and 37 out of 49 (75.5%) patients of the 50-70 years age group had chronic liver disease and portal hypertension respectively. 45 out of 65 (69.2%) males 37 out of 65 (56.9%) males developed CLD and PHTN.

Conclusion: Using portal vein diameter as a diagnostic indicator of PHTN has an accuracy of %, so it can be used in routine USG abdomen of CLD patients to prevent the dreaded complications of PHTN and treat it early. Male gender and patients above the fifth decade were increasingly predisposed to both CLD and PHTN.

Keywords: *portal hypertension, chronic liver disease, portal vein diameter, collateral circulation*

INTRODUCTION

Portal hypertension is defined as portal venous pressure more than 12 mm of Hg [1]. It is measured as the hepatic venous pressure gradient (difference of wedge hepatic pressure and free hepatic venous pressure), which is normally 4-6 mm Hg. More than 10 mmHg is portal hypertension, and more than 12 mmHg causes clinically significant variceal bleeding. [2]

Portal hypertension is major pathophysiology in patients with cirrhosis. Portal pressure is the gold standard to evaluate the severity of portal hypertension, and radiological intervention is the only procedure for pressure measurement [3].

Causes of portal hypertension are pre-sinusoidal (portal vein thrombosis, extrinsic compression of portal vein, Schistosomiasis (S. mansoni or S. japonicum)), sinusoidal (cirrhosis) and post-sinusoidal (Budd-Chiari syndrome, hepatic venous-occlusive disease, congestive cardiac failure) [1]

Portal hypertension leads to dilatation of portal vein,

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splenomegaly, and formation of portal-systemic collaterals at different sites. The portal system and the systemic venous circulation are connected at several locations. Collaterals develop in areas where anatomic connections exist between the portal venous and systemic circulation. These are vascular channels that are functionally closed in normal conditions but become dilated in portal hypertension as a consequence of increased intravascular pressure and blood flow. These gastro-oesophageal varices are responsible for the main complications of portal hypertension and massive upper GI bleeding [4].

The complications of portal hypertension (gastrointestinal hemorrhage, hepatic encephalopathy, hepatorenal syndrome, and ascites) continue to be the cause of significant morbidity and mortality. Portal hypertension remains one of the most serious sequelae of chronic liver disease [2].

To prevent bleeding from occurring, an appropriate early diagnosis of oesophageal varices at risk of bleeding should be achieved. Upper digestive tract endoscopy is the gold-standard method for diagnosing the presence of gastroesophageal varices and identifying signs of risk of bleeding. Universal endoscopic screening of oesophageal varices was recommended in all patients newly diagnosed with cirrhosis until 2015. However, due to the increase in the proportion of patients with early cirrhosis achieved by non-invasive diagnostic methods, this strategy proved to lead to a large number of unnecessary endoscopies that would eventually decrease the patient's compliance and increase the health system costs. In the last 10 years, increasing evidence regarding non-invasive methods accumulated and proved useful for stratifying the risk of carrying varices and varices requiring treatment in patients with CLD [5].

60-80% of patients with cirrhosis of the liver are associated with esophageal varices and 25-30% of them have got a risk of bleeding. Every year incidence of esophageal varices increases by 5% and 5-10% of patients with small varices progress to large varices [6].

Endoscopy is the standard diagnostic approach in patients with portal hypertension. But it is an invasive technique. Noninvasive imaging techniques are preferred for early diagnosis and prevention of complications. Portal vein diameter, peak systolic velocity, presence of collaterals, splenomegaly, etc can be found using Doppler USG and CT multiphase abdomen. The overall sensitivity for detection of coronary-gastroesophageal collaterals using USG was 80% and 64%, respectively, in a study done by Subramanyam et al. [7]. Doppler USG is frequently obtained in patients with chronic liver disease, suspected portal hypertension, and known portal hypertension. Among the many findings of portal hypertension is the enlargement of the portal vein, decreased portal vein velocity, increased congestion index, development of portosystemic collaterals, and reversal of portal vein flow. Using all of these, it is possible to diagnose portal hypertension with a high degree of sensitivity and specificity non-invasively [8].

It is possible to rule out clinically significant portal hypertension in patients with cirrhosis (i.e., hepatic venous pressure gradient ≥ 10 mmHg) with a level of clinically acceptable accuracy by combining measurements of liver stiffness, spleen stiffness, and Doppler ultrasound evaluation. The combination of these methods allows for the identification of patients with the most serious degree of portal hypertension as seen by Bolognesi M et al. [9].

Despite the safety, affordability, and widespread use of ultrasound, there is a paucity of literature on ultrasonographic assessment of the portal vein (PV) diameter in adult patients in our local environment [10]. According to a study done by Nestaĭko OV [11], real-time ultrasound investigation could provide important information and should be used as a non-invasive and harmless investigation in hepatological studies. Splenic Stiffness can also be used for assessing and monitoring PHTN in hepatitis C induced cirrhosis according to the study by Colecchia et al. [12].

The cutoff point for portal vein diameter differs from study to study despite the existence of a normal mean portal vein diameter between 10–15 mm in different settings. This implies the existence of limited evidence on the exact portal vein diameter which can help diagnose portal hypertension early [13].

This study will help find an exact cutoff point of portal vein diameter which can help rule out false positives and find out false negatives and provide prompt detection and treatment and prevention of progression of a chronic liver disease patient to portal hypertension, using a routine non- invasive USG scan.

MATERIALS AND METHODS

This is a cross-sectional study carried out in the Radiology department of AIMS, Kochi. Since this is a pilot study, a total of 100 patients above the age of 15, referred from various other departments to the Department of Radiology for routine USG of the abdomen are included. Those patients who underwent imaging studies elsewhere, patients having thromboembolic problems, etc are excluded. Portal vein diameter and Doppler study of spleen for splenic collaterals are carried out. MRD numbers of these patients are collected to note down their age, history of CLD, Portal hypertension, and the presence of collaterals in the CT scan of the abdomen or upper endoscopy (if taken previously) is tabulated in MS Excel. The cut-off for Portal vein Diameter is found using Receiver Operator Characteristic Curve in SPSS. Qualitative variables are expressed as percentages and quantitative variables are expressed as mean with standard deviation. Informed consent was obtained from all the patients. The Ethics Committee of Amrita Institute of Medical Sciences has approved the study.

RESULTS

The mean age of the sample population was 54.9 +/- 13.89 years, median age 56 years (range of 17-84 years), and mode 67 years. The majority (61, 61%) of the patients were of the 50-70 years age group (Figure 1).

Figure 1: Distribution of age

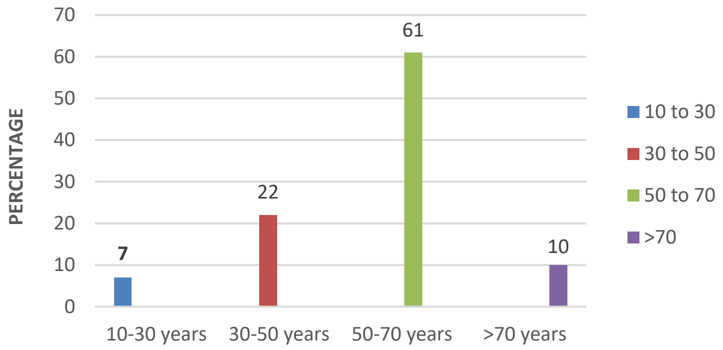

35 (35%) of patients were females and 65 (65%) were males (Figure 2).

Figure 2: Distribution of gender

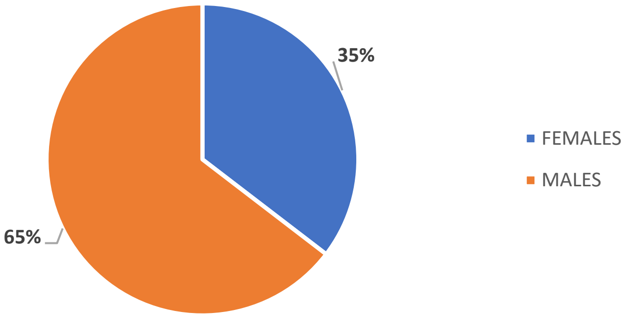

Of the 100 patients who underwent USG abdomen, 59 (59%) patients were found to have ultrasonographic features of chronic liver disease, while 41 (41%) patients did not have CLD.

49 (49%) patients were found to have collateral circulation, seen either on Doppler USG, or CT Multiphase abdomen, or Upper endoscopy (if done previously for any other indications), and 51 (51%) patients did not have any collaterals (Table 1).

Thus 49 (49%) patients had portal hypertension. Among the 50-70 years age group, 60.6% (37) of patients had PHTN, while 0 patients had PHTN in the 10-30 years age group, which clearly showed that PHTN was more common after the fifth decade of life (Figure 3).

Table 1: Distribution of portal hypertension (PHTN)

Collaterals	Frequency	Percent
Present	49	49.0
Absent	51	51.0
Total	100	100.0

More Males (56.9%) had PHTN as compared to females (34.2%), which was statistically significant, p-value <0.05 (Figure 4).

ROC curve to find out the portal vein diameter cut off had an Area 0.676, diameter 10.5 mm, with a sensitivity of 61.2%

and specificity of 60.8%, p-value of 0.002 (Figure 5).

Figure 3: Comparison of age and PHTN

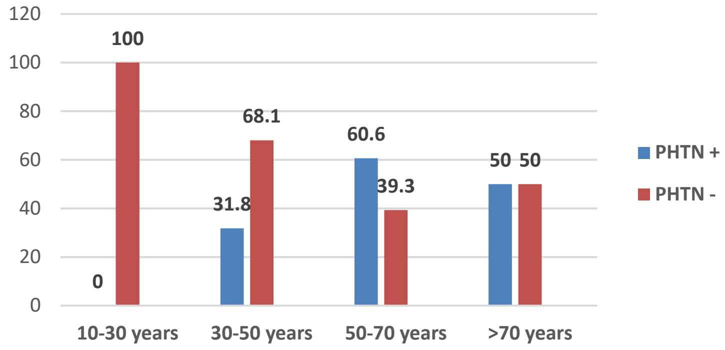

Figure 4: Comparison of gender and PHTN

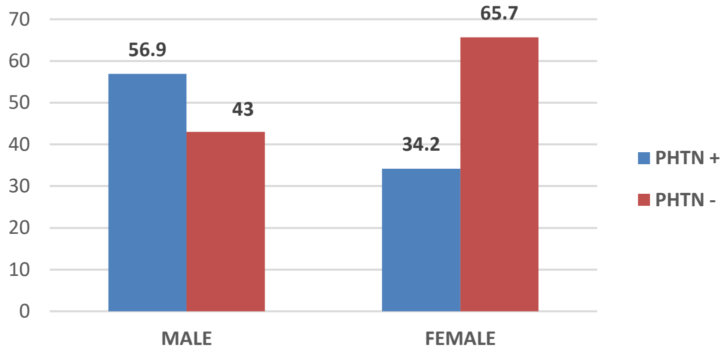

Figure 5: ROC curve of portal vein diameter and PHTN

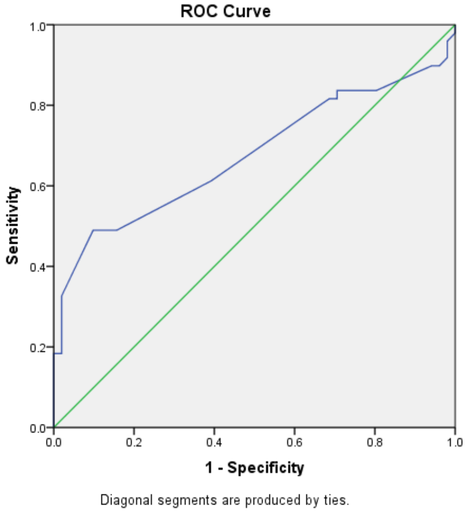

AUROC	0.676
PVD	10.5 mm
p value	*0.002*
Sensitivity	61.2%
Specificity	60.8%

DISCUSSION

Portal hypertension is the most common complication and also one of the important causes of death in chronic liver diseases. Increased resistance to portal blood flow due to alteration of the hepatic architecture leads to dilatation of portal vein, splenomegaly, and formation of oesophageal and gastric varices, variceal hemorrhage, ascites, hypersplenism, encephalopathy, etc [4]. The overall sensitivity for detection of coronary-gastroesophageal collaterals using USG was 80% and 64%, respectively, in a study done by Subramanyam et al. [7].

The mean age of the sample population was 54.9 +/- 13.89 years, the median age was 56 years (range of 17-84 years) and the mode of the age was 67 years. Most of the patients (61.0%) were in the 50-70 years age group, similar to the findings of Gaduputi et al. [14]. In a similar study done by Gunda et al. [15], it was found that the odds of having esophageal varices were independently higher among patients with increased age (Median age of 51 vs. 45 years; OR:1.02; 95%CI: 1.0–1.04; p = 0.030)

Out of the 100, 35 (35.0%) of patients were females and 65 (65.0%) were males. This pattern of male predominance is comparable to the study done by Sharma et al. [16] which had 86.1% of male patients. In the study by Akande et al. [17], 81% of males and a mean age of 44+12.6 years were seen among the cases.

More patients, i.e 73.7% and 60.6% of patients in the 50-70 years age group was found to have chronic liver disease and portal hypertension respectively; which clearly shows that both CLD and subsequently the development of PHTN is more common after the fifth decade of life. The same was the case with the male gender, i.e, 69.2% and 56.9% of males had CLD and PHTN respectively; which again shows that males have an increased association with CLD and subsequent development of PHTN. In a similar study done by Manik Mahajan et al. [18], it was found that the majority of the patients (32%) belonged to the 6th decade, with 66% of male and 34% of female patients having CLD and PHTN.

10.5 mm was found to be the cut off for Portal vein diameter in our study using ROC curve, with an area of 0.676, p-value 0.002, comparable to that found by Bhattarai S et al. [19], Sarwar et al. [20], and Guo et al. [21]. Collateral circulation was found in 49% of patients, which was seen either during Doppler ultrasound, CT multiphase abdomen, or endoscopy. Out of these 30, (60.0%) of the patients had a portal vein diameter of more than 10.5 mm. These were the true positive cases. Among these, 72% were in the 50-70 years age group. A similar study done by Gemecho et al. [13] revealed a normal mean portal vein diameter of 10.6 mm ±1.8 SD with a respirophasic variation of 25.6% and in the study by Ekta Gupta et al. [22] in North India, mean normal portal vein diameter measurement in male subjects was 10.33 mm ± 1.004 & in female 9.41 mm ± 1.16.

PVD > 10.5 mm was found in 20 (40.0%) of patients, but they did not have any collaterals; i.e these were the false-positive cases. CLD was found in 6 (30%) of these patients while 1 (0.5%) patient had portal vein thrombosis which explains the increase in diameter. Higher portal vein diameter was seen in those patients with esophageal varices in the study by Sangma MA [23] and Li FH et al. [24], as well as in cirrhotic children by Riahinezhad et al [25]. Diameter tended to increase in Child-Pugh scoring initially, before getting stenosed at higher scores, as found by Zhoy et al. [26].

The true negative cases were 31 (62.0%) of patients, who had a portal vein diameter less than 10.5 mm. The false-negative cases, i.e, 19 (38.0%) patients with PVD<10.5 mm but with the presence of collaterals, were long-standing chronic liver disease patients with well-established collateral circulation and a grossly shrunken liver, which led to the stenosis of the portal vein, leading to the decrease in PVD. Normal mean portal vein diameter in Ethiopian patients was found to be 10.0+1.8 mm (range 8.2 to 11.8) with a 21.5% reduction with the phases of respiration, in a similar study done by Scabel et al. [27]

In another study done by Lopamudra et al. [4], the average portal vein diameter of patients without gastro-oesophageal varices was 11.545 ± 1.514 mm and of patients with varices 13.998 ± 1.123 mm, which was slightly higher than the PVD found in our study, so was the normal mean portal vein diameter found in a study by Jefferey W et al. [28], which was 11 + 2 mm in patients aged between 21-40 years.

Thus, portal vein diameter of 10.5 mm had a sensitivity of 61.22%, specificity of 60.78%, the positive predictive value of 60.00%, the negative predictive value of 62.00%, and accuracy of 61.00%, in correctly diagnosing portal hypertension. In a similar study done by Johana Prihartini et al. [29], PVD of 11.5 mm had a sensitivity of 75% and a specificity of 54.5%. The sensitivity of using portal vein calibrations was found to be only 41.8% in the study done by Bolondi L et al. [30].

Limitations of our study: Portal vein diameter can vary in various physiological conditions, like inspiration, expiration, duration of liver pathology, etc. In our study, the diameter was taken at the hilum without taking into account the respiratory changes or the duration of CLD. Portal vein diameter can also vary according to age. There are wide variations in the age groups of the patients. More specific indicators like splenic size, peak systolic velocity, the direction of flow in the portal vein could have been included in the study.

CONCLUSIONS

Thus, portal vein diameter of 10.5 mm was the cut-off above which portal hypertension could develop, as was shown in the study. Though of limited sensitivity and specificity, it can be used to lookout for portal hypertension in chronic liver disease patients and prevent it as early as possible, before its dreaded complications set in. Both CLD and PHTN had a high incidence after the fifth decade of life, so screening for either disease is recommended after 50 years of age.

Contribution of authors

Varsha Somakumar, Cibi Manoharan, V S Srikanth – Concepts, design, the definition of intellectual content, literature search, data acquisition, data analysis, Manuscript preparation, editing, and review

Rajesh Kannan, Chitra Tomy, Anagha Vasant, Vishal Marwaha, VR Mujeeb - Concepts, design, the definition of intellectual content, Manuscript editing and review.

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ORIGINAL ARTICLES

Diagnostic value of ultrasound in comparison with the pathologic results of fine-needle aspiration of thyroid nodules for rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital

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Abstract: *Background and Aim: Various studies have reported that 4-7 percent of adults with palpable thyroid nodules that 50-70 percent of it's diagnosed with high-accuracy sonography. Although most thyroid nodules are non-cancerous and do not require treatment, a small percentage of them are associated with a potential risk of malignancy, which is an important early diagnosis by ultrasound or pathology. The present study aimed to compare the ultrasound with the pathologic results of fine-needle aspiration (FNA) of thyroid nodules for the rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital.*

Methods: The study population consisted of 100 (80 female, 20 male) patients with thyroid nodules in the age range of 19-82 years referred to the radiology ward of Baqiyatallah Hospital who were studied by the cross-sectional method. All patients were first scoring by ultrasound, then fine needle aspiration with thyroid nodule ultrasound was performed, and the specimens were referred to the pathology department for further evaluation.

Results: The pathologic results from fine-needle aspiration showed that only 8 of the 100 patients studied had malignant nodules that, by ultrasound scoring, 7 patients had 4 scores and 1 patient had 3 scores. However, the other 92 patients with benign nodules 57.60% of patients had score 1, 30.43% of patients had score 2 and 11.97% of patients had score 3.

Conclusion: According to the results of this study, it seems that ultrasound scoring is useful in the rapid diagnosis of thyroid nodules and prevent unnecessary thyroid nodule FNA.

Keywords: *fine needle aspiration, sonography, thyroid nodule, malignancy*

INTRODUCTION

The thyroid gland is one of the largest endocrine glands in the butterfly-shaped transverse section of the larynx. The units that make up the thyroid gland are a sinus or follicles, which are present in the central part of colloidal proteins as a source of thyroid hormone storage [1]. The thyroid gland has several key functions, such as iodine uptake and transfer, thyroglobulin secretion, iodine binding to thyroglobulin to make thyroid hormones, facilitating thyroid hormone secretion into the bloodstream, and interfering with body metabolism [2].

Thyroid hormones

Thyroglobulin, a large iodized glycoprotein, has been shown by researchers to be the first essential step in initiating the synthesis of thyroid hormones through iodine absorption through the food. Transmission of iodine to the thyroid gland occurs by binding to serum proteins, especially albumin, and

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unbound iodine is often excreted in the urine [3]. TSH, which is secreted by the anterior pituitary cells, plays a central role in the control and evaluation of thyroid function, which is regulated by the TRH hypothalamic hormone [4]. Various studies have shown that there is an inverse relationship between thyroid hormone levels and basal TSH production, indicating the role of thyroid hormones as a major regulator of TSH production [5].

Common disorders of the thyroid gland include hypothyroidism, hyperthyroidism, goiter or enlargement of the thyroid gland, thyroid cyst, thyroiditis (Hashimoto's) and thyroid nodules. According to the latest studies, 7-8% of the world population has one of the thyroid disorders [6].

The term thyroid nodule refers to a group of thyroid cells that grow abnormally and fall into a mass, nodule, or node, which are divided into two groups of benign nodules and malignant nodules [7]. Various studies have shown that most thyroid nodules have non-cancerous and benign cells and various reports have estimated 1 in 10 patients as malignant nodules [8].

The types of thyroid nodules are divided into 3 groups of benign (colloidal), malignant (papillary) and suspicious nodules based on pathological findings. Among the types of nodules mentioned, only malignant nodules require further surgery and care.

Symptoms of patients with thyroid nodules include neck, jaw or ear pain, difficulty swallowing, itching or tingling in the throat, shortness of breath, sore throat, hoarseness, and most studies have reported that most patients Thyroid nodules are without symptoms and are often discovered during examination for other causes [9].

Researchers have identified chronic follicular thyroid nodule (Hashimoto's), congenital malformations such as failure to form a lobe, thyroid abscess, simple cyst, and non-thyroid diseases such as lymphoma and teratoma [10].

Tests for the diagnosis of thyroid nodules include measurement of thyroid hormone and thyroid-stimulating hormone, ultrasound, scanning, fine needle aspiration, and pathological examination (cellular aggregation) [12, 11].

Since a rapid diagnosis of malignancy facilitates the process of thyroid nodule healing, therefore, finding the fastest method of diagnosis is important for initiating therapeutic measures.

The present study aimed to compare the ultrasound report only with the pathologic results of fine-needle aspiration of thyroid nodules with the aim of rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital.

MATERIAL AND METHOD

Following the approval of the research plan by the University Research Council, informed written consent was obtained from patients' participation and distributed to those who referred to the thyroid nodule interventional ultrasound department and the indication of fine-needle aspiration. No entry and exit criteria were considered for the study.

Finally, 100 patients (80 females, 20 males) from thyroid nodules in the age range of 19-82 years referred to the radiology ward of Baqiyatallah Hospital and the statistical population were selected by census method and studied by the cross-sectional method. After obtaining biographies and demographic information by the student, patients were referred to a skilled sonographer for ultrasound (using Philips Affinity fog) and fine-needle aspiration (with ultrasound guidance) and the specimens were referred to the pathology department for cytological examination.

In thyroid ultrasound, tissue is examined for ultrasound by echogenicity and rate and extent of calcification. 6 modes of Hyper echo nodule (white nodule in ultrasound), Hypo echo nodule (gray and pale nodule in ultrasound), cystic nodule (absolutely Echoless), solid nodule (uniform echo), microcalcification (calcification less than 2 mm) and macro calcification (calcification greater than 2 mm) was determined by scanning ultrasound for scoring from 1-4. The four nodule states were hypo echo, solid, microcalcification, and a 1 cm-high scoring criteria of 1-4. Each of the above was given a score of 1 and the nodule with all four attributes scored 4. In fine needle aspiration, the thyroid tissue is sampled using a fine needle for the microscopic examination of existing cells.

Among the characteristics of malignant nodules can be severe porcelain, cell size and shape variation, dense and double or multilayered cell masses, etc. These features were reported by a skilled pathologist.

Data were analyzed by spss23 software.

RESULTS

The mean age of patients was 48.26±08/13 and the results of this study showed that malignant thyroid nodules were more prevalent in 40-49 years old patients and women in our study ($p<0.05$).

Of the 100 patients with thyroid nodules, 8 (8%) had malignant nodules, indicating that a thyroid nodule may be about 12 times more likely to be benign.

Ultrasound reports showed that of the 92 benign nodules, 58 were hyper echogenic, 22 were hypo echogenic, and 12 were cystic nodular, whereas of the 8 malignant nodules, 5 were hypo and 3 were iso- echogenic.

It was observed that the ratio of solid in malignant nodules was more than benign. The calcification rate in benign nodules was 17% for microcalcification and 2% for macro calcification, which was 40% and 20% for malignant nodules, respectively.

Pathologic reports showed 53 patients with nodular goiter, 28 with adenomatous goiter, 1 with a follicular lesion, 8 with papillary carcinoma, 8 with thyroiditis, and two remained unknown due to insufficient sample that both nodules were cystic.

Of the 8 patients with malignant nodules reported by the pathologist from the side of ultrasound, 7 (87.5%) cases had score 4 and one patient (12.5%) had score 3. While 57.60% of benign nodules had score 1, 30.43% had score 2 and 11.97% had score 3.

From the side of ultrasound scoring, 53 cases received score 1, 28 cases received score 2, 7 cases received score 3 and 12 cases received score 4. In terms of thyroid nodule count were 47 single nodules and 53 multinodular nodules.

From hormonal tests, 4 cases were hypothyroid, 5 were hyperthyroid and 91 cases were normal, 3 cases were hypothyroid or hyperthyroid, and 5 cases were normal. Eleven of the patients had a family history of thyroid disorders, which was found to be 5 out of 11 people and 3 in people with no history of malignancy.

DISCUSSIONS

This study aimed to compare ultrasound only report with pathologic results of fine-needle aspiration of thyroid nodules with the aim of rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital. The pathologic results of fine-needle aspiration in the present study showed that only 8 of the 100 patients studied had malignant nodules which, by ultrasound scoring, 7 cases had score 4 and 1 case had score 3. However, the other 92 patients with benign nodules 57.60% had score 1, 30.43% had score 2 and 11.97% had score 3. In other words, by taking into account the pathologic results in this study, it can be concluded that ultrasound results can be used as a quick tool in predicting thyroid nodule malignancy. The reason for the high success of sampling accuracy in this study was the use of ultrasound guidance during biopsy and slide preparation by the pathologist.

In 2015, Siyadati et al investigated the diagnostic value of fine-needle aspiration compared to pathologic results in the diagnosis of thyroid nodules in Babol Shahid Beheshti Hospital, northern Iran, for 22 years. The researchers said that fine-needle aspiration of the thyroid is a selective method of examining thyroid nodules that have recently been questioned in the management of nodules. This cross-sectional study was performed on cytology specimens of 225 patients who underwent thyroidectomy in Shahid Beheshti hospital of Babol for 22 years (1989-91). Age, sex, physician performing FNA, type, and surgical site, FNA and pathology results were recorded. Pathologic FNA results were classified into four groups of inadequate, benign, malignant and suspicious and pathologic results were also divided into two benign and malignant and compared. The results showed that in the diagnosis of malignant thyroid nodules, sensitivity was 60%, specificity 96%, positive predictive value 65%, negative predictive value 95% and diagnostic accuracy of 81.8%. Of the 225 cytological specimens, 127 were benign (56.4%), 47 suspicious (20.9%), 27 malignant (12%), and 24 inadequate (10.7%). Final pathology was also benign in 150 patients (66.7%) and malignant in 75 patients (33.3%). According to the results of this study, fine needle aspiration can be used to diagnose thyroid nodules [13].

Rosario et al., 2013 in Brazil, designed a study to predict malignancies in thyroid nodules with non-diagnostic cytology and to evaluate the value of ultrasound. The results of this study showed that in patients with thyroid nodules and repeatedly diagnosed cytology findings, ultrasound findings are an excellent parameter for choosing to continue their treatment, which would be useful for periodic ultrasound [14].

In a 2005 study in Taiwan, Lin et al., in the study of thyroid nodules by ultrasound and fine-needle aspiration, concluded that ultrasound combined with fine-needle aspiration detected 10 years earlier than other methods of malignancy [15].

In 2006, in Italy, Baratolata et al. designed a study entitled Quantitative and Qualitative Evaluation of Thyroid Nodule with Ultrasound Contrast. These researchers concluded that quantitative data corroborated the subjective findings and contrast ultrasound of the thyroid gland was a feasible procedure but found other random events during ultrasound may interfere and are one of the reasons limiting the potential of this method for the detection of thyroid nodules [16].

In 2009, Tramaloni et al. evaluated ultrasound and Doppler ultrasound and fine-needle aspiration in thyroid nodules. In this study, microcalcification, hypoechogenic nodules, and intra-nodular vascularization were introduced as criteria for malignancy [17]. In our study, as the grayscale ultrasound was used, therefore, vascularization was not used in malignant scoring.

Rago et al. 2008 evaluated ultrasound and Doppler ultrasound and fine-needle aspiration for malignant patterns in thyroid nodules. In this study, microcalcification, hypoechogenic nodules, and intravascular nodular vasculature were introduced as criteria for malignancy [18]. In our study, as the grayscale ultrasound was used, therefore, vascularization was not used in malignant scoring.

CONCLUSIONS

According to the results of the present study, it seems that ultrasound scanning alone is useful in the rapid diagnosis of thyroid nodules and to suggest a more accurate result, the present study should be designed in a larger statistical population. More accurate and desirable results can be performed on specific types of lesions. Thyroid nodules with scoring 3 and 4 should also be subjected to the fine-needle aspiration to facilitate faster and more accurate diagnosis and to prevent the progression of malignancy. Among the properties of the nodule used in scoring, nodule size alone may not be a good criterion for nodule malignancy, whereas solidity, hypo echogenic, and microcalcification favor the malignancy of the nodule, and can exclude nodules that score 1 or 2 can be tracked by ultrasound and does not necessarily need to be sampled.

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CLINICAL PRACTICE

Differential diagnosis in case of overdose of antiepileptic treatment. Clinical case

Mihail S. Tudosie[2], Genica Caragea[1], Ana D. Radu[4], Ilenuţa L. Dănescu[3]

Abstract: *Acute drug-related acute intoxications or suicidal ideation following overdose with anticonvulsants may cause major morbidity, in many cases requiring intensive care and prolonged periods of hospitalization.*

The case of a 35-year-old patient known for a history of epilepsy with phenobarbital and carbamazepine treatment is brought to the Emergency Unit by transfer from another hospital in a comatose state with insufficient respiratory performance, bilateral active mydriasis. From the family members' claims, the patient discontinued treatment with phenobarbital two weeks ago. Following interdisciplinary examination, hospitalization in the Neurology Department with the diagnosis of epileptic status is decided. Due to the progressive aggravation of acute respiratory insufficiency, the ventilator support is constituted. Since the biological samples related to probability diagnosis and cerebral imaging were not conclusive, toxicological samples were collected. The GC/MS analytical toxicology screening in urine reveals the presence of carbamazepine and phenobarbital and their plasma levels (5.92 and 92.3 mg/l) as determined by the FPIA method indicate an overdose of phenobarbital therapy. The patient is taken over by the Department of Clinical Toxicology where specific intensive care measures are applied. Clinical evolution is favorable. The psychiatric examination highlights the depressive idea, suicidal ideas, and uselessness. The suicide attempt is confirmed. It also presents a review of the main information about acute poisoning with anticonvulsants, but also about the analytical laboratory methods that bring precious information in establishing the diagnosis of certainty.

Keywords: *phenobarbital, carbamazepine, GC/MS, urine, acute poisoning*

INTRODUCTION

Acute drug-related acute intoxications or suicidal ideation following overdose with anticonvulsants may cause major morbidity, in many cases requiring intensive care and prolonged periods of hospitalization. The evaluation and stabilization of the poisoned patient is similar to all cases presented at the Emergency Unit and aims at detecting vital risk disruptions and correcting them in the shortest possible time [1].

Phenobarbital ($C_{12}H_{12}N_2O_3$) is a drug of the barbiturates group used since 1912. It is generally administered to epileptic patients at doses of 60-200 mg/day, commonly in combination with other anticonvulsants [2]. In the chronic administration of 200 mg / day in epileptic patients, the concentrations had average values of 29 mg/l and 16-48 mg/l [3]. Plasma concentrations of 10-30 mg/l are generally considered appropriate for patients receiving phenobarbital as an anticonvulsant and may require 14-21 days to reach the level of equilibrium.

The compound had a plasma half-life of about 4 days [4]. Toxic reactions to phenobarbital at chronic doses occur at plasma concentrations exceeding 40 mg/l. Patients develop

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coma with reflexes present at plasma concentrations of 65-117 mg/l and reflexes loss occurs at concentrations of 100-134 mg/l [5].

The mechanism of action of barbiturates and implicitly of phenobarbital is related to their affinity for the GABAA receptor, which is fixed in distinct sites with both benzodiazepines and GABA (the main CNS inhibitor neurotransmitter), potentiating its effect. Barbiturates also block a glutamate receptor - the major excitatory neurotransmitter in the CNS [6].

In acute intoxication with barbiturates, because of the depression of central respiratory control mechanisms, following a general depression and anxiety, death may occur in the absence of artificial ventilation and vital functions support measures. In short-duration barbiturates intoxication, the cardiopulmonary arrest may be the cause of premature death and late: circulatory insufficiency, cerebral edema, acute pulmonary edema. Central nervous effects are related to the severity of intoxication. Because there is no specific antidote, the goal of therapy remains to increase the elimination of toxic and support vital functions. In determining the establishment of measures to increase the elimination of anticonvulsants (hemodialysis, hemoperfusion, plasmapheresis), it is also important to measure the plasma levels of these substances in relation to the clinical condition of the patient.

Carbamazepine is a classic anticonvulsant, largely used, with an iminostilbene nucleus, similar to that of tricyclic antidepressants, with a prolonged digestive absorbtion time. Its mechanism of action is partially known, acting on the neuronal membrane towards stabilizing its hyperexcitability, inhibiting repetitive neuronal discharges and reducing excitatory synaptic transmission by blocking voltage-dependent sodium channels. The reduction is glutamate discharge and the stabilization of the neuronal membrane contribute only supplementary to the antiepiletic effect [7]. Its main metabolite is 10,11-carbamazepine epoxide, an essential metabolite which possesses the same toxic effects as the basic drug. In pharmacological doses, the distribution volume of carbamazepine is of 1.4 l/Kg, and the half-life is of 15 hours.

The absorbtion of carbamazepine occurs slowly, 65-85% of it binds to plasma proteins and it is quickly distributed to all tissues. The maximum serum levels are reached within 6 to 8 hours following ingestion and steady-state plasma concentrations, considered to be therapeutic levels of carbamazepine, considerably vary between individuals; for most patients, the therapeutic level of carbamazepine is between 4 and 12 ml//l and the concentration of the active metabolite (10,11 epoxide-carbamazepine) is 30% of the plasma levels of carbamazepine [7]. In epilepsy, carbamazepine is administered in doses of 0.8 to 1.2 g / day, 2-3 times a day for an adult and 0.1-1 g / day (10-25 mg / kg / day) for children of ages from 1 to 15. Therapeutic doses can be optimized through therapeutic monitoring (TDM) due to a good correlation between plasma levels and the therapeutic effect [8]. As it is an enzymatic inducer, carbamazepine can induce its own metabolism, this phenomenon being responsible for the reduction of its half-life over repeated administrations.

In case of carbamazepine overdose, many of the observed effects can be tied to secondary mechanisms of its tricyclic structure. The symptomatology of an acute intoxication is characterized by coma, pyramidal signs, nausea/vomiting, hypotension, hypothermia, anticholinergic symptomatology (tachycardia, mydriasis, redness, urinary retention etc.) tremor, convulsions, respiratory depression, cyanosis, sinusal bradycardia or atrio-ventricular conduction disorders [9]. Plasma levvels of carbamazepine between 18-70 µg/ml have been measured in carbamazepine overdoses, while levels higher than 40 µg/ml have been associated with an increased risk of complications such as coma, convulsions, respiratory insufficiency, and cardiac conduction disorders [10].

CASE PRESENTATION

A.G, female gender, 35 years old, admitted for being comatose, with acute respiratory insufficiency and non-reactive bilateral mydriasis.

Personal medical history – known to have had previous comitial crises, treated with phenobarbital and carbamazepine

History – Comitial patient, brough from the Emergency Admission Unit in a coma, transferred from another hospital. From her family's affirmations, the patient had stopped taking phenobarbital 2 weeks before.

Interdisciplinary consults performed in the Major Emergency Department:

- neurosurgical consult: coma GCS = 5 points, no neurological signs;
- internal diseases consult: PA = 100 mmHg, AV = 76 b/min, ECG sinusal rhythm with no pathological alterations.

CT scan: no heterodense lesions are present at brain level, ventricular system situated on median line.

The patient is taken over by the Neurology Department with a diagnosis of epilepsy.

Objective examination upon admission: second degree coma; non-reactive; oro-tracheal tube, spontaneously breathes through the tube; TA = 120/70 mmHg, AV = 85 b/min; gastric statis = 200 ml; diuresis = 400 ml; positive bilateral Babinski bilateral; fixed mydriasis, pupil OD>OS, fixed eyeballs; no meningeal signs; spontaneously and discreetely mobilizes right superior limb. Due to the progressively aggraveted acute respiratory insufficiency, mechanical ventilation is applied (BIPAP, PEEP = 5 mbar, FiO2 = 40%, Pmax = 19 mbar);

In order to confirm the diagnosis, lumbar punction was performed and probes of cerebrospinal fluid were sent for biochemstry, bacteriology and cellularity.

A central subclavicular catheter was also applied.

The pulmonary radiography does not show evolving pleuro-pulmonary changes.

As the diagnosis could not be confirmed by the biological samples, toxicological samples were taken. The GC/MS examination of the urine shows the presence of carbamazepine and phenobarbital.

Biological table: biochemistry, hematology, coagulation within normal limits.

The patient is taken over by the Clinical Toxicology Department.

Intensive therapy measures: ventilation support; ensuring permeability of respiratory pathways – aspiration of tracheo-bronchial secretions through the IOT probe; naso-gastric drainage tube; hydro-electrolytic rebalancing; plasmatic and urinary alkalinization under paraclinical control (EAB).

Support therapy: monitoring of vital functions; ventilatory support; hemodynamic monitoring; diuresis monitoring; analytical control of urine.

Clinical evolution is favorable – the patient becomes conscious, reacts to stimuli; shows spontaneous breathing signs; is detubed and restarts spontaneously breathing. Physical recovery measures continue.

Psychiatric exam reveals depressive ideation, suicidal ideas and ideas of uselessness. A suicide attempt is confirmed.

She is tranferred to the specialized psychiatry clinic.

Analytical diagnosis of certainty in case of acute drug intoxication

One of the most used methods in identifying the substances incriminated in acute drug intoxications is gas chromatography - GC coupled with mass spectrometry - MS. Thus, by gas chromatograph injection of a completely unknown matrix, if the working conditions are well chosen, at the exit of the GC they will be placed in turn at the detector's disposal. If the detector is of the MS type and the injection matrix contains organic substances whose molecular weight falls within the working range of the mass filter, it will determine the mass spectrum for each substrate. In our case, the urine sample collected from the patient is follows to the liquid-liquid analytical procedure and injected into the GC/MS system, with ion trape detection, electronic ionisation 70eV. Midazolam is used as an internal standard. The method chosen for the separation and qualitative identification of analytes by GC / MS is called medicamente.mth, with a 45-minute acquisition time. Upon completion of the acquisition program, read the total recorded chromatogram. Apply the background correction and then proceed to the identification of each chromatographic peak. It establishes the mass spectrum that corresponds to it and these spectra are automatically compared with the existing spectra in the laboratory database. The database includes specialized spectral libraries (Nist98, PMW, Wiley6).

The temperature program used was:

- temperature injector: 280 ° C,

- for column oven: Start at 1400C, 2 minute range, increase temperature by 5° C / min to 290° C, 13 minutes (total time 45 minutes).

Following these investigations, the result is established. In our case, we separated the chromatographic column and identified the following substances: Phenobarbital and carbamazepine at the retention times shown in Table 1, based on the mass spectrum obtained by EI.

Table 1: Data on identified compounds (molecular weight, retention times and characteristic spectral lines m/z)

Compound	MW (g/mol)	Retention time (min)	CAS. No.	Spectral lines (m/z)
Phenobarbital	232	16.001	50-06-6	117, 204, 231
Carbamazepine	236	16,944	298-46-4	193, 236
Midazolam (SI)	326	26,951	59467-70-8	310, 327

In case of acute drug intoxications, measuring plasma levels is important both to establish the seriousness of the intoxication and to monitor the efficiency of the medical treatment.

Therapeutic drug monitoring is performed based on the principle of fluorescent light polarization on a Cobas Integra 400/800 system for serum samples taken from patients. It is a quantitative method which uses the the fluorescence of substances and is based on immunological properties (antigen- antibody) of the analyzed substances. The principle of this method is based on exciting the sample with a fixed wavelength and measuring the quantity of light emitted by a substance called a luminophore, on another wavelength. The specific antigens, present in the tracer, bind to the antibodies present in the sample [11]. Thus, the quantity of light emitted by the sample will be inversely proportionate to the quantity of searched substance.

Figure 1: Phenobarbital mass spectrum (top) and full scan chromatogram obtained from SD urine extract

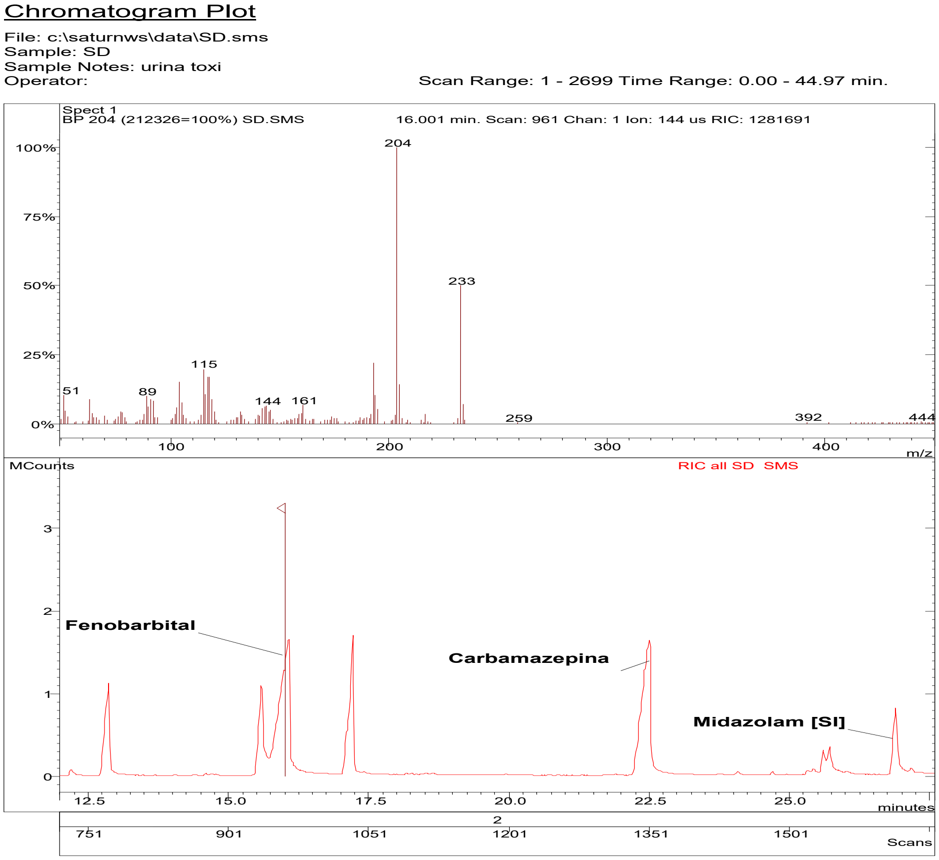

Figure 2: Calibration curve (in 6 points) for carbamazepine on the Cobas Integra 400+ System

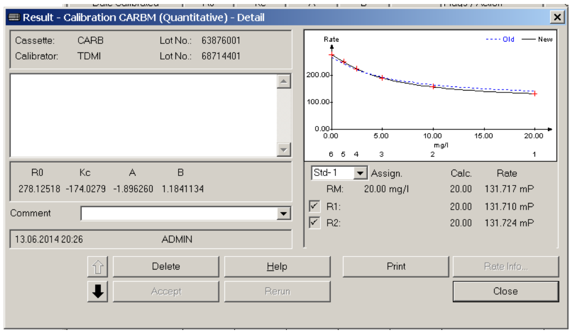

Cassette: CARB Lot No.: 63876001
Calibrator: TDMI Lot No.: 68714401

R0	Kc	A	B
278.12518	-174.0279	-1.896260	1.1841134

13.06.2014 20:26 ADMIN

Std- 1	Assign.	Calc.	Rate
RM:	20.00 mg/l	20.00	131.717 mP
☑ R1:		20.00	131.710 mP
☑ R2:		20.00	131.724 mP

In this case, determinations of the plasma levels of carbamazepine and phenobarbital in the samples were performed using the FPIA method, which implies determinations on a calibration curve in 6 points. The quality control is performed for three known concentrations.

The plasma concentration of carbamazepine on the first and second day of hospitalization was 5.92 and 2.90 mg/L, respectively. Because this value falls within the therapeutic limit, no measurements have been made during hospitalization. In contrast, the plasma level of phenorbarbital was increased and its variation in the first 7 days of hospitalization is shown in Figure 3.

CONCLUSIONS

Antiepileptic treatment based on selective anticonvulsant medication (carbamazepine and phenobarbital) follows the avoidance and treatment of epilepsy seizures, and at therapeutic doses these crises stop. Unfortunately, cases of overdose of treatment may occur either accidentally or for a suicidal purpose.

In the case presented, the crisis appeared could be interpreted in a double context, in the absence of correct anamnestic data, either in the case of baseline, in the absence of anticonvulsant treatment or in the case of overdosing of the medication.

Table 2: The therapeutic and toxic plasma levels for phenobarbital and carbamazepine (The Cobas Integra 400+ System)

Compound	Therapeutic levels (mg/L)	Toxic levels (mg/L)
Phenobarbital	10-30	>40
Carbamazepine	4-8 (associated with other anticonvulsants) 6-12 (unique treatment)	>8 >12

Compound	*Plasma levels (mg/L)*						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Phenobarbital	94.5	121.8	117.9	93.7	87.4	57.6	35.9
Carbamazepine	5.92	2.90					

Figure 3: Plasma levels of phenobarbital during the first 7 days of hospitalization

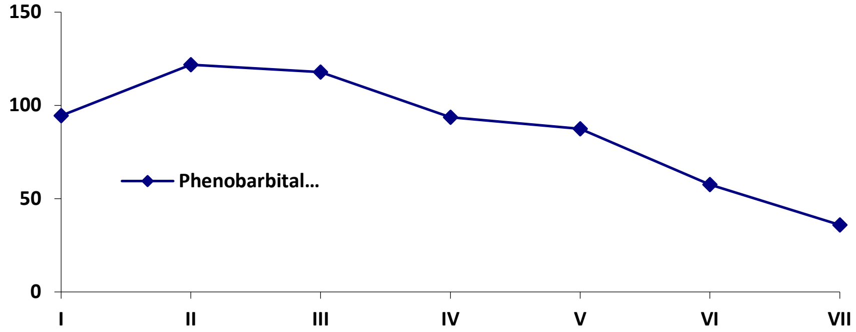

In establishing the differential diagnosis of certainty, toxicological screening of biological products was of great importance, both by identifying drug substances in the urine (by the GC/MS method), especially by measuring their plasma levels by the FPIA (Fluorescence Polarization Immunoassay). Measuring plasma levels of some drugs facilitates the optimization of therapeutic behavior but also reduces the toxic effects of these drugs in case of overdose

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Digital techniques for anterior teeth form selection in complete edentulism

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Abstract: *The aim of this study was to compare two computerized digital esthetic design methods (DSD and Visagismile) in order to properly choose the appropriate form of the maxillary frontal teeth for complete bimaxillary edentulous patients and to validate the use of these applications in complete edentulism. An online questionnaire was submitted to a group of 100 persons (dental doctors, dental medicine students and persons activating in other fields); the respective persons were requested to choose the form of maxillary frontal teeth for a group of four complete bimaxillary edentulous patients, whose esthetic design had been performed by using the two computerized progams. Following the respondents' analysis of the esthetic previews elaborated for the four patients 60% of the total answers were in favour of DSD while the remaining 40% were in favour of Visagismile; these percentages are identical with the ones obtained on the basis of the answers given by dental doctors and dental medicine students. In case of the respondents activating in other fields the percentages were 66% in favour of DSD and 34% in favour of Visagismile. Based on the results of the study the two computerized methods were validated also for cases of complete edentulism but further studies to confirm this validation are still required. These two methods improve the dental doctor/patient communication and certainly provide a higher predictability of the esthetic results of prosthetic restorations not only for dentate patients but also in cases of complete edentulism.*

Keywords: *esthetic preview, complete edentulism, DSD, Visagismile*

INTRODUCTION

Choosing the most appropriate form for maxillary frontal teeth has always represented a challenge in the prosthetic restorations of complete edentulism as doctors had available a limited number of parameters (facial profile, sex, age, personality), which were often subjective methods of analysis and, as a consequence, the esthetic results had a low degree of predictability. [1].

Modern dental medicine uses digital technologies also in the field of dental esthetics, thus ensuring that the esthetic results of the prosthetic restorations meet as many requirements as possible having, at the same time, a high degree of predictability, given the possibility to preview the results.

A considerable number of such digital applications for smile simulation have been developed, among which: DSD App, VisagiSmile, Photoshop CS6, Keynote, Planmeca Romexis Smile Design, Cerec SW 4.2, Aesthetic Digital Smile Design, Smile Designer Pro [2].

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Smile planning applications adjust the digital technology to the smile design process. They can be used as multi-use conceptual tools, diagnosis and treatment plan preview instruments and can facilitate the communication with the patient and the dental technician, enhancing the predictability of the final results and allowing a thorough analysis of the facial and dental features otherwise superficially evaluated through clinical diagnosis, photographic assessment or by using models [3]. Among the esthetic software programs used at present in dentistry Visagismile and DSD are considered to be the most powerful tools in assessing and modifying the design of deficient smiles. [4].

Apart from the well-known DSD (Digital Smile Design) concept a new one, Visagismile, has been developed in the last years starting from the Hippocratic four types of temperament (sanguine, choleric, phlegmatic and melancholy) and the Jungian archetypes and symbols (basic geometric shapes and primary and secondary colours) [5]. The artist Philipp Halawell explained and developed the Visagism concept, which implies the creation of an image expressing a person's (patient's) type of personality, concept that has been integrated in the dental medicine, thus obtaining a teeth form that ensures a correlation between dental-facial harmony and the personality type [3].

The Visagism concept analyzes the relation between the facial features, temperament and patient's harmonious teeth configuration. The concept has been developed in the last years and validated for a large number of clinical cases (over 800). The software program is user-friendly, the doctor uploading one standard photography of the patient on the application, where it will be calibrated and the points composing the facial map of the patient will be selected. Based on the facial map and one brief psychological questionnaire (named "personality interview") filled in by the patient the application will establish the patient's temperament and appropriate teeth design. Afterwards the doctor can edit the dental configuration and superpose it on the patient's teeth in the photography so that the patient can preview the esthetic aspect to be obtained at the end of the treatment. All details related to the future teeth will be sent to the laboratory directly from the application in order to obtain the final prosthetic restoration [6].

We identified a large number of studies regarding the application of the esthetic software programs on dentate patients while the number of studies regarding the application of these programs on edentulous patients is quite limited. Starting from this finding we applied the two digital methods for choosing the form of the maxillary frontal teeth to complete edentulous patients.

The purpose of our work is to establish which of the two computerized methods used for choosing the form of the maxillary frontal teeth is more appropriate in case of complet edentulism.

MATERIALS AND METHODS

We performed a comparative study on a group of four patients of the Complete Denture Department of the Faculty of Dental Medicine within the University of Medicine and Pharmacy, "Carol Davila", Bucharest, Romania.

We used the following criteria for including the patients in the studied group: complete bimaxillary edentulous patients (both with and without dentures) and patients who gave their consent for participating in the studied group and for being photographed. The criteria for excluding the patients from the studied group were: dentate patients and non-cooperating patients.

For each patient a full set of photographs was taken, according to the protocols of the two software programs and all the data and photographs thus obtained were loaded and processed using Visagismile, DSD Keynote and Microsoft Excel programs. Previews of the form of the future artificial frontal teeth were obtained for each patient using both software programs; in achiving these previews, different steps were followed, as described in Table 1.

The ideal digital form of the future artificial maxillary frontal teeth was achieved for each of the four patients of the studied group by using the two computerized programs.

Table 1. Comparison between the steps for obtaining the previews corresponding to DSD Keynote and Visagismile programs

Keynote DSD	Visagismile
- Load the photograph on the software program and position the photograph in accordance to the calibrating lines - Draw the smile line and the mesio-distal limits of the future frontal teeth - Choose the forms of standard teeth that are preset by the program - Selection of the best fitting teeth-form by the doctor together with the patient - classical criteria for selection are taken into consideration (reversed face, age, sex, personality etc.)	- Load the photograph on the software program - ”Auto face reading” command performs the automatic calibration - The facial map results automatically by joining 27 facial points and a “facial type” is obtained, according to the four types of temperament (choleric, sanguine, melancholy, flegmatic) - The patient is subjected to the psychological test (a short interview consisting of four questions) - The software automatically ranks the facial type in percentages depending on the statistical parameters of the facial map and the results of the interview - Patient’s preferences as well as design related information can be inserted in the program in order to be submitted to the laboratory - Based on all the information inserted so far the program proposes a teeth design

In order to comparatively evaluate the two computerized methods used for choosing the form of maxillary frontal teeth with the aim of establishing which one is more appropriate for patients with complete edentulism, we submitted a questionnaire to a number of 100 persons. Out of 100 submitted questionnaires, 2 were considered invalid and 98 valid; the 98 persons were represented by 12 dental doctors, 53 students at the Faculty of Dental Medicine and 33 persons activating outside the medical field. This self-administered questionnaire (SAQ) included several questions and the two previews generated by the software programs (Figure 1), allowing us to receive feedback regarding the results obtained by using the two computerized methods from persons both activating in the dental medical field and outside the medical field. The questionnaire was filled in under the anonymity protection as the persons included in the study were not requested to provide personal information such as surname, name or personal identification number.

Figure 1. The self-administered questionnaire submitted online

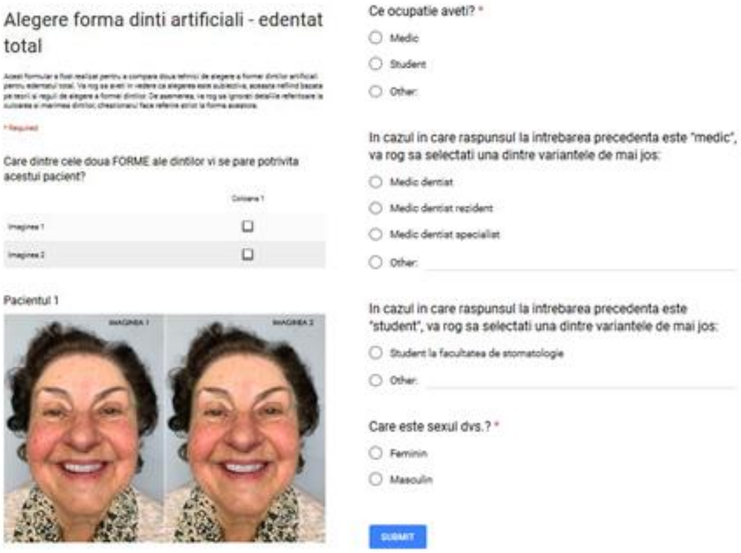

Alegere forma dinti artificiali - edentat total

* Required

Care dintre cele doua FORME ale dintilor vi se pare potrivita acestui pacient?

	Coloana 1
Imaginea 1	☐
Imaginea 2	☐

Pacientul 1

Ce ocupatie aveti? *

○ Medic
○ Student
○ Other:

In cazul in care raspunsul la intrebarea precedenta este "medic", va rog sa selectati una dintre variantele de mai jos:

○ Medic dentist
○ Medic dentist rezident
○ Medic dentist specialist
○ Other:

In cazul in care raspunsul la intrebarea precedenta este "student", va rog sa selectati una dintre variantele de mai jos:

○ Student la facultatea de stomatologie
○ Other:

Care este sexul dvs.? *

○ Feminin
○ Masculin

The questionnaire consisted of eight questions, of which the first four (closed-ended questions) regarded directly the four patients of the studied group while the remaining four were open-ended questions, referring to general information. The questionnaire, which was elaborated using Google Forms, was filled in online and it took sixteen days to receive all the answers. The information resulting from the answers to the questionnaires was further used, by employing Microsoft Office Excell 2018, to create statistical bases.

RESULTS

The results obtained after the questionnaire was filled in by the three categories of respondents are presented below:

- Dental doctors opted for DSD in proportion of 60% while 40% were in favour of Visagismile (Figure 2);

Figure 2. Options expressed by dentists

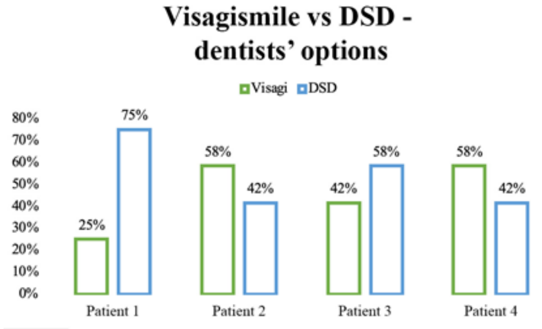

- Dental students were in favour of DSD in proportion of 60% while 40% chose Visagismile (Figure 3);

Figure 3. Options expressed by dental students

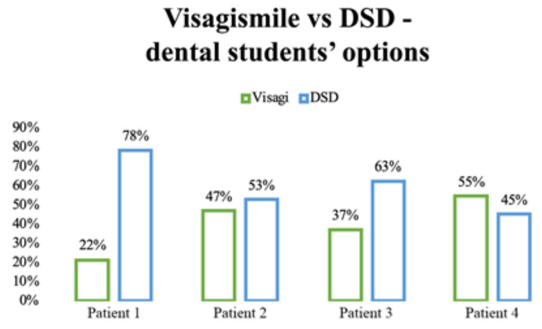

- The persons activating in non-medical fields were in favour of DSD in proportion of 66% while 34% opted for Visagismile (Figure 4).

Figure 4. Options expressed by persons from non medical field

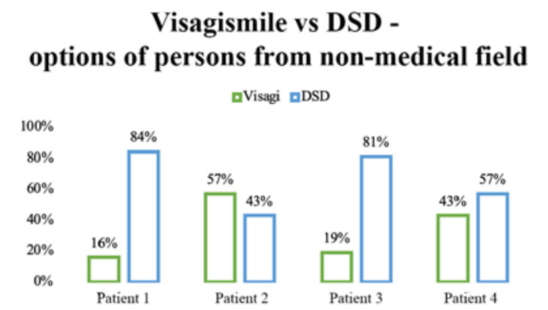

At the level of the whole group of respondents included in the study 60% opted for DSD while 40% were in favour of Visagismile (Figure 5).

Figure 5. General options

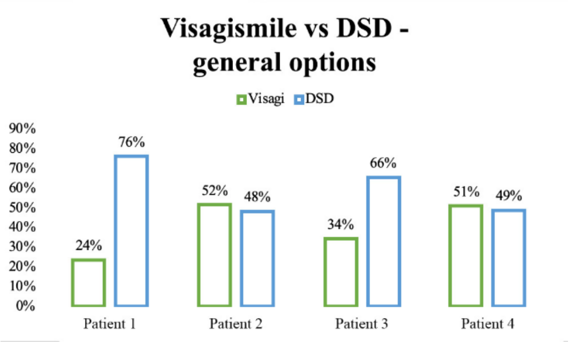

DISCUSSIONS

There are still insufficient articles and studies illustrating the use of the smile planning digital applications to patients with complete edentulism. The modern smile design techniques are more effectively controlled through data collection and different digital smile design tools, contributing to all treatment steps up to the fabrication of the definitive restorations, either fixed or removable. Utilizing these digital tools together with classical treatment-planning rules and principles facilitates a controlled and predictable therapy when redesigning a smile [8] both for dentate patients and for complete edentulism cases.

Visagismile concept supports both dentists and patients in deciding about the aesthetics of the final prosthetic restoration and it is mostly applied for dentate patients. Using a specific protocol, a smile design that corresponds to individual facial features, temperament and personal preferences of the patient can be achieved [7, 8]. This application can also facilitate to a great extent the conception of the complete denture design by allowing the preview of the final result and the choice of the form, colour, size and arrangement of artificial teeth depending on the facial typology and the desire of the patient, who is directly involved in elaborating the treatment plan.

In modern dentistry DSD programs are also successfully used as tools for diagnosis, treatment plan visualization and communication with the patient and the technician; all these elements can improve treatment outcome predictability. As regards the characteristics of the 3D functions, CAD/CAM connectivity and practicality, the DSD application proves to be even more performant than Visagismile. However, not all the DSD programs available today provide the same competency for the comprehensive analysis of the dentofacial esthetic parameters [8]. As in the case of complete edentulism, the actual DSD techniques can be used with certain limitations related to the following facts: dynamics of the lips when smiling; shooting of the initial photographs, which requires a good stability of the dentures; the wax occlusion rims that do not fit all the facial typologies. At the same time, the preview of the final prothestic result obtained by applying DSD offers the complete edentulous patient the possibility of having a realistic view on certain aspects related to teeth form, colour and size, which are not correlated with his/her age and facial typology.

On the other hand, the ability of the digital planning programs to perform an appropriate esthetic analysis is evaluated also depending on the number of the dental-facial esthetic parameters used. Thus, if Visagismile has the advantage of using additional elements related to the patient temperament, DSD uses more dental-facial esthetic parameters. [2] This could be the reason why the majority (between 60% and 66%) of the respondents to our questionnaire expressed their option for the DSD method.

CONCLUSIONS

Dental esthetics is a topical though subjective field and the techniques used for choosing the maxillary frontal teeth, either classical or modern (digital), have particular elements and, as a consequence, our study is a subjective one, based on the opinions expressed by the three categories of respondents.

DSD and Visagismile programs have proved their usefulness for dentate patients and our study could contribute to demonstrating their effectiveness also in case of complete bimaxillary edentulous patients. However, future studies of higher complexity and performed on larger groups of patients are necessary in order to validate the application of such programs in cases of complete edentulism.

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ORIGINAL ARTICLES

Drug allergies interpretation based on patient's history alone may have therapeutic consequences in hospital setting

Polliana M. Leru[1,2], Andreea Leontescu[3], Ion Stefan[1,4], Irena Nedelea[5], Iuliana Ceausu[6], Edu Antoine[1,3]

Abstract: *Background and aim. Adverse drug reactions (ADRs) are common events in medical practice, representing a matter of concern for both outpatient and in hospital care. Understanding and management of ADRs depend on their mechanism and clinical picture, which are complex and highly heterogeneous. Drug hypersensitivity reactions (DHRs) are immunologically-mediated ADRs, which are considered allergies after demonstrating evidence of either drug-specific antibodies or T-cells. The correct diagnosis of a drug allergy in hospital setting is essential for the outcome of treated disease and for the patient future in terms of therapeutic needs. Patients may be labeled as being drug allergic, based on their history and vague symptoms that do not correspond to a true allergic reaction. The aim of our study was to evaluate consequences of drug allergy labelling of female patients hospitalized in the department of obstetrics and gynecology from a university hospital during one year, in terms of medical attitude and therapeutic approach.*

Method and Results. We found that 159 out of 2395 patients hospitalized in the obstetrics-gynecology department declared a history of drug allergy on admission, but only 3 patients had medical documents and adequate recommendations from the allergist. Hospital medical attitude was significantly influenced in all declared allergic patients, mainly in terms of antimicrobial and anti-inflammatory therapy.

Conclusion. We concluded that overestimation of drug allergies in hospital care has important consequences in terms of current and future recommended therapy that should be based on standardized allergist evaluation.

Keywords: *drug allergies, hospital setting, patient's history, therapeutic errors*

INTRODUCTION

Adverse drug reactions (ADRs) are common events in medical practice, representing a matter of concern for both outpatient and in hospital care. The ADRs definition accepted by the World Health Organization is any noxious and unintended response to a medication that occurs at normal doses used for prophylaxis, diagnosis and/or treatment [1]. ADRs severity range from minor common side effects to potentially life-threatening medical conditions. The reported incidence of ADRs in general population varies between countries and may account for 2-3 % of all hospital admissions in some regions, as reported from Australia [2]. No reliable data about incidence of ADRs in Romania could be found. Understanding and management of ADRs depend on their mechanism and clinical picture, which are complex and highly heterogeneous [3].

The pharmacological classification of ADRs includes two major subtypes: type A which are dose-dependent and predictable reactions, with non-immunological mechanism,

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commonly termed intolerance and type B, which are unpredictable, not dose-dependent and have immunologic mechanism [4]. Drug hypersensitivity reactions (DHRs) are immunologically-mediated ADRs, considered allergies after demonstrating evidence of either drug-specific antibodies or T-cells [5]. Therefore it is recommended to use the term allergy only to describe reactions for which an immunological mechanism has been demonstrated. The majority of ADRs (>85%) are type A, while drug allergies comprise up to 15 % of ADRs [4]. It is considered that drug allergies affect more than 7 % of the general population [6]. The correct classification of an adverse drug reaction, by medical professionals, as allergy (due to immunological mechanism) or intolerance (non-immunological mechanism) has important medical implications [3]. Both underdiagnosis, due to underreporting and overdiagnosis, due to an overuse of the term „allergy" are common. There are still many controversies in drug allergy and recognized need for standardized and improved approaches of patients who report prior drug allergy [7]. Mislabeling of a drug allergy in patients records may result in unnecessary avoidance of an effective drug and medical recommendation of a second-line therapy, possibly less effective or more expensive. This situation is more frequent in case of antimicrobial drugs and may be also responsible for increased bacterial resistance to frequently used antimicrobials. Despite lack of reliable evidence, if an allergy label is noted into a patient medical record, it will remain for the rest of his life in most of the cases [8]. Maintaining the diagnosis of a drug allergy in hospital setting is essential for the outcome of treated disease and for the patient future in terms of therapeutic needs.

The aim of our study was to evaluate medical recording and consequences of drug allergy labeling of female patients hospitalized in the department of Obstetrics and Gynecology from a university hospital during one year, mainly in terms of current medical attitude, clinical outcome and therapeutic approach.

MATERIAL AND METHOD

We performed a retrospective analysis of hospital data over one-year period and we recorded diagnosis of drug allergies based on patient's history, as well as medical events considered as drug allergy and medical therapeutic attitude during hospitalization.

A total number of 2,395 female patients have been hospitalized during twelve months between January-December 2016 in the Obstetrics-Gynecology department of Nicolae Malaxa Clinical Hospital from Bucharest, with 1,817 patients hospitalized for childbirth and 578 patients hospitalized for gynecological diseases.

Patients were aged between 18-67 years, with a mean age of 33 years and about 80% lived in urban area. We recorded medical history documented in the patient file, including any type of reported drug allergies, written information regarding diagnosis confirmation and tolerated medication, concomitant diseases, other personal or familial allergies, current illness and medication, outcome and status at hospital discharge, particular recommendations regarding drug allergies. In cases who had medical events considered drug allergies during hospitalization, we noted type of reaction, severity, the possible culprit drug, relevant laboratory tests, therapeutic attitude and outcome.

RESULTS

We found that 159 out of 2395 patients, meaning 6.63% of female patients hospitalized in the Obstetrics-Gynecology department declared a history of drug allergy on admission. From this group, 116 were pregnant patients hospitalized for childbirth and 43 patients were hospitalized for gynecologic diseases.

We noticed that 26 patients out of 159 (16.35%) were current smokers. The possible culprit drugs based on patient's declaration were: antibiotics in 76 patients (47.79%), non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics in 41 patients (25.78%) and other drug allergies in 42 patients (26.41%) (Figure 1).

Only three patients out of 159 (1.88%) could show medical documents with diagnosis confirmation of drug allergy and clear recommendations to follow in case of future events.

Figure 1. Culprit drugs based on patient's declaration

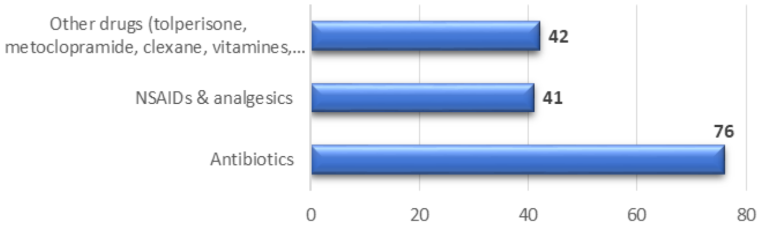

Clinical episodes considered drug allergies were recorded during hospitalization in another 9 patients from the total 2395 hospitalized group (0.37%), who have not declare any drug allergy on hospital admission. Eight patients had acute urticaria and one patient had respiratory symptoms. All these events have been successfully treated with antihistamines and systemic corticosteroids, based on allergist recommendation and resolved within short time. In all cases who declared history of drug allergy, the possible culprit drug was not administered and medication from another pharmacological class was considered. No allergological work-up could be performed during hospitalization, but this was recommended after hospital discharge. From the 76 cases who declared allergies to antibiotics, the incriminated classes were: beta-lactam antibiotics in 48 cases, macrolides in 9 cases, tetracyclines in 5, sulphonamides in 14 cases (Figure 2). The recommended antimicrobial therapy during hospitalization was quinolone in all cases (ciprofloxacin and levofloxacine).

Figure 2. Antimicrobials reported as culprit drugs

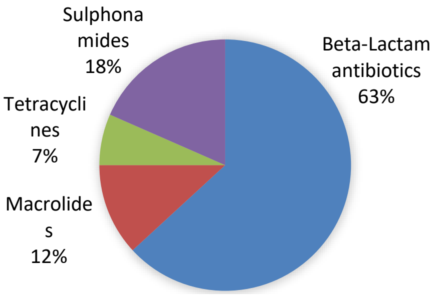

From the 41 cases who reported allergies to non-steroidal anti-inflamatory drugs (NSAIDs) and analgesics, the incriminated substances were: metamizole in 16 cases, aspirin in 8, acetaminophen in 3, diclofenac in 2, ibuprofen in 5, indomethacin in 3, nimesulide in 4 cases. (Figure 3)

Figure 3. NSAIDs and analgesics reported as culprit drugs

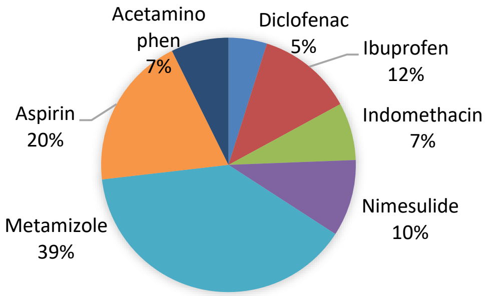

Other possible culprit drugs reported by patients who considered themselves to have drug allergies were: tolperisone, metoclopramide, clexane, vitamines, ergometrine, drotaverine.

The possible offending drug was avoided in all cases who reported drug allergies. No medical events attributable to anesthetics have been recorded.

In case of patients who declared allergy to NSAIDs, no drug from this class was administered, but also analgesics and antipyretics were avoided. No data referring to influence of drug allergy on duration of hospital stay could be found.

DISCUSSION

Our study showed that drug allergy is overestimated in hospital setting, based on patient's history alone and most of the patients have no evaluation or diagnosis confirmation from allergist. Hospital data referring to possible allergy were limited and some laboratory tests that might support atopy or certain symptoms of allergy, such as total serum IgE and blood eosinophilia were missing [9]. The main consequence for hospitalized patients who declare having drug allergy is change of the antimicrobial medication with a different class and refrain from any anti-inflammatory and painkiller medication. The situation might be more complicated and has to be more seriously considered for patients who declare multiple drug allergies to various and unrelated molecules.

The term „allergy" is frequently used and one study showed that more than 50% of reported allergies to antimicrobials have no immunologic pattern [10]. Generally, patients tend to overestimate adverse drug reactions and to interpret erroneously new symptoms or signs that occur during treating their disease.

Data from the literature showed that up to 25% of patient's self-report having drug allergies, most commonly induced by antimicrobials, mainly penicillins, followed by sulfonamides, macrolides and cephalosporines [11]. Beta-lactam allergy, recorded in up to 15 % hospitalized population and leading to increased use of broad-spectrum antibiotic use, is confirmed in only 5 % of patients [12].

Having a label of drug allergy or intolerance in the medical record may lead to withholding of first line therapy or even avoidance. It was proved that about 30% of patients having the label of drug allergy to at least one antimicrobial drug received another drug that did not reflect the standard of care [13]. Alternative antibiotic therapy may be less effective or may have less favorable safety profile, leading to treatment failure and increased risk of antibiotic resistance. Trends in antibiotic prescription rates show an increase use of broad-spectrum antibiotics, such as azithromycin and quinolones [14]. It was also proved that total hospital costs with medication were increased for patients caring the label of a drug allergy, with 63% higher antibiotic costs for patients with beta-lactam allergy [15]. Despite the potential deleterious consequences of selecting an alternate medication and the lack of validity of most allergy labels, many clinicians do not take time to complete a comprehensive drug allergy history before prescribing an antimicrobial. It was found that only 4% of patients who declared being allergic to antimicrobial medication on hospital admission had documentation detailing the specific reaction [16]. This finding may be due to the erroneous perception that therapeutic decisions based on an allergy label will not influence the patients' clinical outcome. The ability of health professionals to discriminate between allergy and intolerance, to evaluate the severity of an ADR and the degree of contraindication depends on their level of knowledge in the field of allergic diseases and was evaluated in recent studies and publications [17]. A published survey of physicians practice and knowledge in the field of drug allergies at a university medical center showed that those with any internal medicine training performed significantly better [18].

A recent study evaluating drug allergies in primary care practice in Romania concluded that the medical attitude and competence of family doctors in the field of drug allergies, as well as their collaboration with allergists are not standardized and updated according to guidelines and experts' recommendations [19]. Since primary care physicians play a key role in evaluating patients with possible drug allergies, there is a clear need for pre-and postgraduate allergy educational programs and training [20].

LIMITATIONS OF THE STUDY

Despite being the only study on drug allergy labeling performed in Romania, our study has some limitations. The main limitation is the retrospective analysis of medical records, with relative few and variable information about drug allergies, depending on the level of knowledge of doctors in training, students or nurses who filled in the patient's files. We had no way to verify the proportion of patients from our study with true versus false drug allergy label, based on standardized diagnosis criteria. No follow-up of the patients who experienced allergic reactions during hospitalization was performed.

CONCLUSIONS

We concluded that the impact of drug allergy label is underestimated in hospitalized patients and diagnosis is generally based on patient's history.

The allergy label is overused, most of the patients who self-report drug allergies have no confirmation from specialist and no medical documentation. The main consequences are switch of antimicrobial medication to another class, generally quinolones and avoidance of anti-inflammatory or analgesic medication.

We consider that information referring to drug allergy should be taken by medical staff with minimal training in this field and recorded more detailed in patient's files on hospital admission. Improved collaboration between specialists, including allergists and primary care physicians is needed.

Conflict of interest
The authors declare no conflict of interest in relation with this study and manuscript.

Disclosure statement
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SYSTEMATIC REVIEW

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Early in-stent restenosis after revascularization in patients with ST-elevation myocardial infarction

Alice E. Munteanu[1], Camelia Nicolae[2], Irina Florescu[3], Cristina Calcan[1], Daniel Niță[1]

Abstract: *Background and aim: Obstructive coronary disease is one of the most important causes of death worldwide. Percutaneous coronary intervention is one of the most important treatments of this pathology. Using of coronary artery stents represented a major advance in interventional cardiology. While bare metal stents (BMS) set the reference point for improved safety over balloon-angioplasty, first-generation drug-eluting stents demonstrated significant improvements in efficacy, but not necessarily safety, and further technologic developments have focused on optimizing both.*

Methods: We studied 228 patients with STEMI in the last 6 months, divided in 2 groups – in the first group are 122 patients (53.5%) who developed in stent restenosis and the second group has 106 patients had no signs of restenosis (46.5%). The mean time of performing the angiographic reevaluation was 111 days for the first group and 154 days for the second group. The clinical signs that indicate the need of coronary reevaluation was stable angina (116, 50.87%), acute coronary syndrome (58, 25.4%) and asymptomatic patients (64, 28.9%). The cardiovascular risk factors correlate with high risk of restenosis was diabetes (24.3% in first group and 12.2% in the second one, p=0.004), active smoking (p=0.010) and metabolic syndrome (p=0.003). The stent's length >28mm, stent's diameter ≤ 2.5 mm and chronic occlusion correlate with high risk of in-stent restenosis in BMS.

Conclusion: Understanding the importance of risk factors control, will reduce the risk of restenosis, of other cardiovascular event, even sudden death.

Keywords: *stent, early restenosis, STEMI, revascularization*

INTRODUCTION

Cardiovascular disease represents the first cause of morbidity and mortality world-wide.

ST-elevated myocardial infarction (STEMI) represents a challenge for any cardiologist interventionist team who has to decide the best treatment option based on short, medium and long-time prognostic and possible complications.

The first successful percutaneous transluminal coronary angioplasty (PTCA) was performed using a balloon catheter.[1] Due to high rate of coronary occlusion during PTCA and of high rate of in-stent restenosis bare metal stent (BMS) was developed. First studies have shown that BMS are very effective in treating or preventing acute blood vessel obstruction, thus avoiding emergency surgical bypass interventions. Two randomized trials – the Benestend and STRESS (Stent Restenosis

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Study) have shown that stenting lesions de novo on native coronary have reduced angiographic restenosis by about 30% compared to conventional balloon angioplasty.[1,2,3]

Implanting stents leads to a luminal diameter larger than balloon angioplasty, both right after the surgery and in the follow up, thus to a lower restenosis rate.

Using BMS was compared with coronary artery bypass grafting (CABG) in the treatment of obstructive multi-coronary disease in ARTS trial (Arterial Revascularization Therapies Study). The 1 year follow up showed that the mortality, myocardial infarct and stroke rate was similar in the two groups. Survival rate without cardiovascular events was higher in CABG group (87.8% vs 73.8%). Also less surgery patients needed a second revascularization procedure (3.5% vs 16.8%).[4]

Stone et al, studying the safety and effectiveness of BMS and DES on 3006 patients with STEMI with immediate PCI noticed that in DES group the restenosis in stent and recurrent ischemia needing revascularization procedure in 1 year follow up was significant lower than in BMS group.[5] Although, mortality and in stent thrombosis rate were similar for the 2 groups.

Drug eluting stents are a newer coronary stent category, superior to BMS due to a significant reduction of in-stent restenosis, a significant decrease of major adverse cardiac events, as well as target lesion revascularization in those patients which benefited from DES at 6 month follow up.[6, 7] Results at 5 years have demonstrated a similar rate of in-stent thrombosis between the two groups, but a significant lower incidence of major adverse cardiac events in the DES group.[6]

The beneficial using DES is not absolute. DES are superior in reduction of in-stent restenosis incidence especially in high risk lesions. For low risk restenosis patients or for those with high risk of acute in-stent thrombosis due to need of early dual antiplatelet therapy abortion the using of BMS has an important indication.[8,9] Although, lot of research show that using BMS is an alternative for uncomplicated patients or for those with long term dual antiplatelet therapy contraindication, in high diameter vessels, in venous graft lesions or in patient with acute coronary syndrome – STEMI.[10-14]

Despite last decades progresses, in stent restenosis stays an unsolved problem. Revascularization needs in 1 year is 6.7% in DES using, and 11% in BMS, frequently due to restenosis.[15]

METHODS

This study is a unicentric, retrospective, non-randomized trial. The patient from this study were patients of "Academician Vasile Candea" Emergency Clinical Center of Cardiovascular Diseases of the Army Bucharest. We included in the study patients diagnosed with STEMI who underwent myocardial revascularization in less than 24 hours from the appearance of chest pain, who followed the medical treatment according to ESC Guideline, and who underwent angiographic reevaluation in the first 6 months after PTCA. The patients were divided in 2 groups: the study group who developed in-stent restenosis in less than 6 months from PTCA, and the control group who has no signs of restenosis. We evaluate local factors (type of stent, the length and the diameter of stent, the affected vessel and the type of restenosis – focal or diffuse restenosis) and general factors (genetic factors, demographic factors, smoke, and comorbidities – arterial hypertension, dyslipidemia, diabetes, metabolic syndrome, history of stoke, peripheral arterial disease).

RESULTS

We studied 228 patients with STEMI in the last 6 months, divided in 2 groups – in the first group are 122 patients (53.5%) who developed in stent restenosis, from minimal restenosis (24 patients, 20%) to occlusion (16 patients, 13.3%), and the second group has 106 patients which had no signs of restenosis (46.5%). The mean time of performing the angiographic reevaluation was 111 days for the first group and 154 days for the second group.

Most patients came from urban areas (67% for both groups – without significant differences between groups). In the study group the most patients are men (82 patients, 68.3%), while in the other group only 54%

were men (OR=2.476; CI 95% 1.465-3.780; p<0.003). The clinical signs that indicate the need of coronary reevaluation was stable angina (116, 50.87%), acute coronary syndrome (58, 25.4%) and asymptomatic patients (64, 28.9%).

The cardiovascular risk factors correlated with high risk of restenosis were diabetes (24.3% in first group and 12.2% in the second one, p=0.004), active smoking (p=0.010) and metabolic syndrome (p=0.003). The influence of arterial hypertension and dyslipidemia over the risk of in-stent restenosis is hard to appreciate. Prior PTCA, peripheral arterial disease, genetic impregnation also have little correlation with restenosis risk.

The most frequent location of stent implantation in left anterior descending artery (LAD), followed by right coronary artery and circumflex artery, but the stent location doesn't correlate this risk of restenosis.

If we talk about stent's characteristics which can interfere with evolution and prognosis of the patients, the stent's length >28mm (OR 2.77; CI 95% 1.45-4.44; p<0.001), stent's diameter ≤ 2.5 mm (OR 2.45; CI 95% 1.54-3.46; p<0.001), and chronic occlusion (OR 3.78; CI 95% 2.03-7.35; p<0.001) correlate with high risk of in-stent restenosis in BMS. Although, the stent's diameter ≥3.25 mm (OR 0.34; CI 95% 0.24-0.49; p <0.001), the length ≤15 mm (OR 0.58; CI 95% 0.42-0.81; p=0.001) correlate with further restenosis.

DISCUSSION

Our results shows that diabetes mellitus, metabolic syndrome, prior interventional myocardial revascularization, longer stent length and smaller stent diameter are important factors that correlate with risk of in-stent restenosis. Prior PTCA, peripheral arterial disease and genetic factors correlate with restenosis risk. Our research was design to study the risk factors for early in-stent restenosis. Thus, we compared our result with many available researches that studied risk of long term and very long term in-stent restenosis, and results are similar. [16-19] It is known that diabetes, history of myocardial infarction and/or PTCA, different stent and lesion factors correlate with risk of long term and very long term in-stent restenosis, even on BMS or DES.[20-22] Despite rigorous risk factors control and correct medical treatment some patients develop in-stent restenosis, many factors could be incriminated in this phenomenon according with clinical trials. The factors incriminated in in-stent restenosis development, in both BMS and DES, are biological factors (drug resistance, hypersensitivity), mechanical factors and technical factors.[23]

The drugs in DES have a cytostatic (Sirolimus and its analogs) or a cytotoxic effect (Paclitaxel). Statistic data indicate that patients genetic mutations can develop resistance to Sirolimus or Paclitaxel.[24, 25]

Other biological factor is hypersensitivity of compound element in stents. For BMS, release of nickel and molybdenum, and for DES, cobalt releasing, are potential triggers for in-stent restenosis due to local allergic reaction, which can accelerate the atherogenic process.[26, 27]

The mechanical factor responsible of in-stent restenosis are stent underexpansion during the procedure, which is undetectable angiographically, nonuniform drug distribution due to local blood flow alterations, damage of the polymer, stent fracture resulting an important decrease in local drug delivery.[28-31]

Technical factors potentially responsible for increased risk of restenosis include barotrauma outside the stent, randomized clinical trials indicate that restenosis occurred at the proximal margin of stent and uncovered residual plaque, which correlate with increased risk of myocardial infarction at 1 year.[32-33]

Clinical presentation of patient with in-stent restenosis was represented by stable angina in more than half of patients and a quarter of patients were completely asymptomatic. Also, almost ¼ of patients presented acute coronary syndrome, in which smoking and prior myocardial infarction are clinical predictor factors.

Thus, is very important for our patients to understand the importance of risk factors control, reducing the risk of restenosis, of other cardiovascular event and sudden death. Also, there are some biological factors which are difficult to evaluate and some mechanical and technical issues that should be consider in patients with in stent restenosis. Further, we could study the impact of subclinical factors which could interfere with evolution and prognostic, early identifying technics and ways for prevention complications for increase the quality of live and decrease the costs of long term managing of patients.

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VARIA

Economic analysis of hospital/healthcare costs in patients with colorectal digestive anastomosis

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Abstract: *Intestinal anastomoses have always been a major problem in digestive surgery. With the use of mechanical suture devices, intestinal anastomosis techniques have improved, allowing standardization of methods and shorter duration of surgical procedures. In addition to shortening the operative time, reduced operating room utilization and length of stay (LOS), health financial strain is also reduced by a lower rate of postoperative complications, quicker recovery and socioprofessional reintegration of patients, and by lowering costs related to treating permanent disabilities such as permanent intestinal stoma. The aim of this study was to estimate the costs of surgical patients with colorectal anastomosis (mechanical or manual suturing) and to identify economically efficient surgical techniques. Material and method: Unicentric 10-year retrospective study in the Bucharest "Prof. Dr. D. Gerota" Emergency Hospital. The costs associated with manually constructed and stapled anastomoses in colorectal surgery were compared. Results: In the group with manual colorectal anastomoses (363 patients) the costs per patient were on average 5190 RON and the average hospital stay 14.95 days. In the group with mechanical colorectal anastomoses (97 patients) the average cost per patient was 5037 RON with an average hospital stay of 11.5 days. Conclusions: The use of mechanical colorectal suture devices resulted in a cost reduction of approximately 150 RON per case and a shortening of average hospital stay by 3.45 days. In addition to these direct benefits, we also list the increase in surgical volume (including the diversification of the range of operations) or indirect effects such as faster socioprofessional reintegration of patients.*

Keywords: *intestinal anastomosis, mechanical sutures, costs*

INTRODUCTION

The first gastrointestinal anastomosis was performed over 200 years ago. Since then, the continuous development of medical technologies was accompanied by a continuous improvement of anastomotic techniques with the goal of reducing the complication rate, standardizing the methods, and shortening the operative time. These goals have led to the emergence of mechanical suture devices and the improvement of surgical techniques. Until the use of circular staplers, most patients with rectal tumors underwent rectal resections with removal of the sphincter (rectal amputation). Mechanical sutures made possible low colorectal anastomoses to be performed, significantly

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improving patients' quality of life [1]. The use of mechanical sutures has become routine and has a major impact on intestinal sutures. In terms of efficacy, applicability and safety, numerous studies have shown that the use of mechanical suture tools is comparable to manual sutures [2]. Mechanical sutures tends to become indispensable in gastrointestinal tract reconstruction - mainly at the extremities of the gastrointestinal tract (esophagus and rectum) [3,4]. For elective gastrointestinal tract surgery studies that compared mechanical with manual anastomoses found no significant differences between the two methods [5-9].

MATERIAL AND METHOD

We conducted a unicentric retrospective study at the Bucharest "Prof. Dr. D. Gerota" Emergency Hospital between January 1, 2010 and December 31. 2019. All patients admitted to the General Surgery Department who were operated for a colorectal disease were included in the study. Inclusion criteria: age over 18 years, surgery performed during the hospitalization for a benign or malignant colorectal disease which involved performing an intestinal anastomosis to the colon or rectum. Exclusion criteria: intestinal anastomoses performed to resolve a surgical complication from a previous hospitalization. Medical expenses were obtained from medical records, patient discharge form, patient expense account from the HYPOCRATE software, and data from the hospital Accounting Department. The following expenses have been taken into account: accommodation, drugs and medical supply costs, medical tests, investigations from the patient's account, and the average cost of the mechanical suture devices used. The costs related to the training of medical staff regarding the use of the medical equipment used in colorectal surgery (courses, training, etc.) were not taken into account.

RESULTS

At the Surgery Department of the „Prof. Dr. D. Gerota" Emergency Hospital we started using mechanical sutures in colorectal surgery since the 2000s. As with any procedure, there is a learning curve - which is why since 2007 the technique has been used more frequently. Several types of staplers have been used over time - the most frequently used devices and agreed by the operating teams being the GIA two row circular stapler of 60 mm/80 mm/100 mm length, EEA two row circular suture devices 29 mm/32 mm, and Ta Linear Stapler 60 mm/90 mm. There was no experience in the ward (during the study period) regarding the "three-layer" suture or the use of mechanical sutures in laparoscopic interventions. Data on the patients with colorectal diseases who underwent surgery were collected from the Hippocrates database. The collected data were divided into two groups – manual anastomosis and mechanical anastomosis cases (each for malignant and benign diseases), and the following were compared: average LOS, the average cost for each disease, postoperative complications and their costs. Mechanical suture devices were used in the following interventions: colo-colonic anastomosis, ileotransverse anastomosis, colorectal anastomoses, ileal or colonic reservoirs, and appendectomy. Four hundred fifteen patients who underwent surgery for colorectal cancer and respectively 47 patients operated for benign colorectal disease were identified. Among patients with malignant conditions, a predominance of left colon and rectal cancers was observed (approximately 38% and 27%, respectively), and among benign diseases, a relatively similar frequency was found for colorectal inflammatory disease and ischemic intestinal disease (about 36% and 32%, respectively). Average LOS was 14.3 days for cancer and t 15.68 days for benign diseases. The longer hospital stay in patients with benign diseases is accounted for by more days of investigations required before surgery.

Table 1: Malignant cases distribution

Malignant diseases	Number of cases	Average LOS	Average cost/ diagnosis
Malignant tumors of cecum and right colon	46	15.32	5267.95
Malignant neoplasm of hepatic flexure of colon	15	13.23	5372.79
Malignant tumors of the transverse colon	14	15.22	7134.25
Malignant tumors of splenic flexure of colon	21	16.93	6544.93
Malignant tumors of the left and sigmoid colon	158	12.15	4033.84
Malignant rectosigmoid tumor	46	13.13	4284.48
Malignant rectal tumor	115	14.16	4092.12
	415	**14.30**	**4464.63**

In the case of malignant diseases, the cost of surgical therapy ranged between 4033.84 RON and 7134.25 RON, with an average cost per case of about 4464.63 RON. In the case of benign pathology, the cost per procedure ranged between 2723.75 RON and 10314.33 RON, with an average cost of about 5916.91 RON – which supports the above-mentioned hypothesis that these categories of patients required more investigations before surgery.

Table 2: Benign cases distribution

Benign diseases	Number of cases	Average LOS	Average cost/ diagnosis
Benign colon tumors	6	14.33	4569.68
Colorectal inflammatory disease	17	12	2723.75
Intestinal volvulus	9	22.89	10314.33
Ischemic bowel disease	15	13.53	6059.91
	47	**15.6875**	**5916.9175**

Figure 1: Average cost of benign diseases

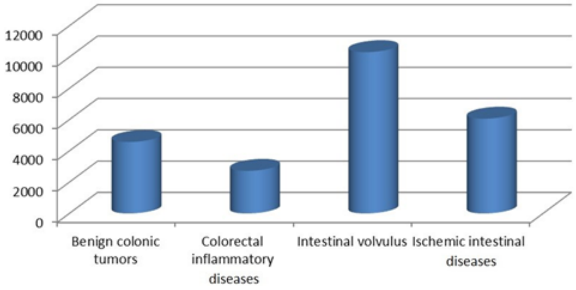

It should be noted that LOS in case of volvulus reaches about 22.9 days, which is associated with an increased cost per hospital stay. This results from an increased share in the medical care costs of accomodation expenses.

Figure 2: Number of cases

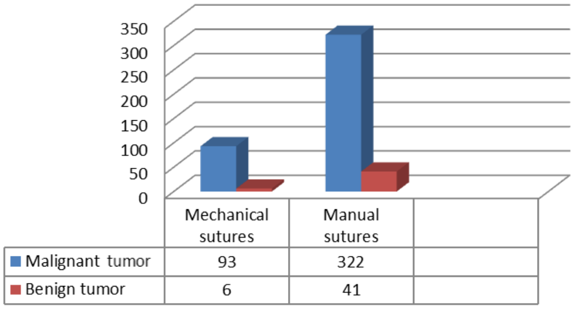

The 2 groups were divided as follows: 363 patients were treated with manual colorectal anastomoses of which 322 were operated for malignant tumors and 41 for benign tumors and a second group of 99 patients who underwent mechanical colorectal anastomoses of which 93 for malignant tumors and 6 for benign tumors (chart 2). As to the average LOS the following differences were found: in the group with mechanical sutures LOS was between 10.1-12.2 days, and in the group with manual sutures it ranged between 14.3 days for malignant tumors and 15.6 days for benign tumors.

Figure 3: Average lenght of stay

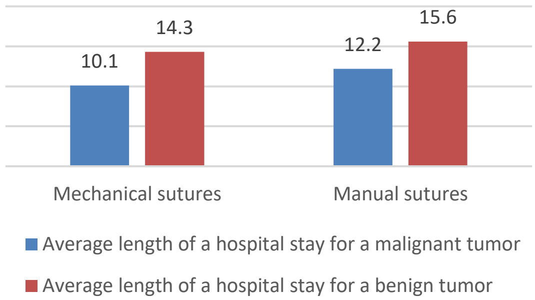

In the group with mechanical suture, the costs ranged between 4240 RON for malignant colorectal diseases and 5834.87 RON for benign colorectal diseases. In the group with manual suturing, the costs per case ranged between 4463 RON for malignant tumors and 5916.91 for benign colorectal diseases.

Figure 4: Average costs per pacient

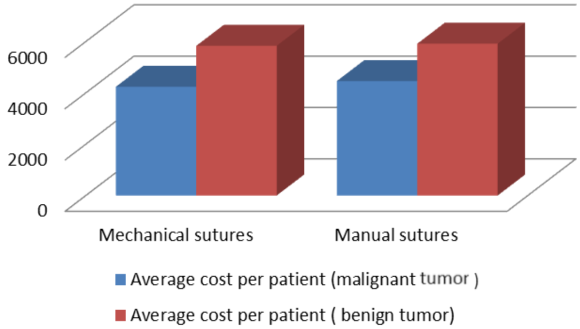

Of the total 462 patients operated for malignant and benign colorectal cinditions, 56 developed surgical complications that required surgical treatment in about half of the cases. The most common complications were intestinal fistulas (about 6%) - a common complication of both mechanical and manual anastomoses. There was no significant difference between the incidences of fistulas in mechanically sutured anastomoses compared to manual sutures.

Table 3: Case distribution according to age and sex

Postoperative complication	Number of cases	Average LOS (days)	Average cost/ diagnosis
Rectal stenosis	2	11,5	3089,1
Intestinal fistula	27	22,9	5532,49
Acute peritonitis	26	19	8930,32
Stoma stenosis	1	15	4447,09
	56	**17,1**	**5499,75**

In the case of anastomotic fistulas, LOS was longer, but the total cost was lower compared to the one identified in the case of septic complications which required broad-spectrum antibiotic therapy that increased expenses. There have also been several technical incidents associated with the use of mechanical suture devices (without them having been ratified or reported) – such as misfiriring or complete device failure during the procedure. Mac Rae et al. reported these incidents as common and associated them with significant morbidity [8]. In our study, no associations were found between intraoperative technical incidents and post-operative complications.

DISCUSSION

The mechanical suturing technique associates a series of benefits such as:

- faster healing of the anastomosis due to better vascularization, less inflammatory reactions of the anastomosis partners, less tissue manipulation, and better tissue alignment [10];
- reduction of septic intervals;
- reduction of operative time;
- technical advantages: can be used in low rectal anastomoses thus allowing the preservation of anal sphincter and significantly improving the patient quality of life
- shorter learning curve than in the case of manual sutures [11, 12];

Despite the above-mentioned advantages, the technique is not perfect and associates anatomical complications such as dehiscence, anastomotic fistulas, and anastomotic stenosis [13, 14]. The advantages and disadvantages of the 2 techniques should be discussed with the patient before signed informed consent is obtained [15]. On the other hand, it is important for health-care providers to report adverse events to manufacturers, to provide feedback helping the manufacturere to improve the design of these devices [13, 16]. The most commonly encountered complication remains the anastomotic fistulas. Reintervention and complications such as anastomotic fistulas are considered quality indicators in colorectal surgery. Anastomotic fistulas are among the most feared complications, especially after colorectal resections and are associated with increased morbidity and mortality, with a high reintervention rate, and increased LOS [17-21]. The prevalence of anastomotic fistulas after colon or rectal resections varies depending on anatomical location, with a lower frequency in the case of right-sided colon anastomoses. The incidence of radiologically identified anastomotic fistulas ranges between 0.5% and 21%, but of these only 1-12% are clinically significant anastomotic leaks after colorectal surgeries and up to 10-14% in low colorectal resections [10]. Overall, patients with anastomotic fistulas developed after colorectal surgery have a significantly higher risk of morbidity (56%) and mortality (32%) [10]. It is also associated with a significant economic burden because of multiple reinterventions, radiological procedures, the stomas needed to control fistulas and because of the increasing length of hospital stay [22]. Anastomotic fistulas are documented as a strong indicator that is associated with high costs in colorectal surgery and also with significant long-term costs for the patient and the health system due to the high rehospitalization rate (risk that is 1.3 times higher in the first 30 postoperative days in patients who have anastomotic fistula compared to those who do not have fistula after colorectal surgery). Also, high reintervention rates, increased incidence of postoperative infections, and longer LOS per admission increase the cost (in the literature was estimated on average at about 7 additional days) [23-26]. Factors of increased peritoneal aggression are known to cause adhesions, one of he most frequent causes of long term morbidity after abdominal surgeries [27-29]. The use of mechanical suture devices shortens the average surgical procedure by 15 minutes on average – from which benefits the patient by reducing anesthesia surgical stress and administrative costs, especially in hospitals with high workload. These have direct implications on the cost associated with anesthesia procedures and on increasing the utilization rate of the operating room, thus allowing more surgeries to be completed. The evaluation of the benefits related to shorter LOS and less medical care days due to a reduction in morbidity, in terms of the costs of using mechanical sutures, must be addressed to each situation, these being more obvious in clinics performing numerous esophageal and colorectal surgeries [12]. Reducing the costs for surgical patients and in particular for a patient with gastrointestinal surgery is a goal in the management of surgical patients.

CONCLUSIONS

Mechanical suturing technique must be mastered by each surgeon and the necessary devices must be found in each surgery department. Although the cost of these devices is high, overall, it seems that the cost/effectiveness ratio is in their favor. In addition to shortening the duration of colorectal surgeries, increasing the use of operating rooms, decreasing the LOS, the financial effort is reduced by a lower rate of postoperative complications, faster socioprofessional reintegration of patients, and lower costs related to treating permanent disabilities such as permanent intestinal stoma, directly influencing the decrease in costs associated with surgical patient care. An important component of surgical patient management is cost reduction, which can be achieved by reducing LOS and careful management of the case by selecting an appropriate surgical technique.

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REVIEW

Effect of Hydroxychloroquine, Favipiravir, Lopinavir/Ritonavir, Remdesivir, Umifenovir, and Interferon beta-1a in Covid-19 patients: A systematic review of Randomized Clinical Trials

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Abstract: *Background: Coronavirus disease 2019 (COVID-19) was recognized by the World Health Organization (WHO) as a global pandemic on March 11, 2020. Since then, researchers worldwide have focused their attention on identifying effective treatments and developing vaccines to combat this disease.*

Aim: To report the effectiveness of the drugs employed in the COVID-19 treatment protocols based on data from clinical trial studies conducted from the beginning of the pandemic until December 10, 2020.

Methods: Following the PRISMA guidelines, we conducted an advanced search in several electronic databases. A total of 13553 studies was screened by two people simultaneously and separately based on the article title, abstract and full-text. The quality of the studies was evaluated using the Cochrane criteria.

Results: Of the 13553 studies identified, 50 clinical trials were included in this systematic review. Of these, three studies explored the use of remdesivir, nine studies the use of hydroxychloroquine, five studies the use of lopinavir/ritonavir, six studies the use of favipiravir, one study the use of tocilizumab, two studies the use of interferon beta-1a and two studies the use of umifenovir.

Keywords: *Hydroxychloroquine, Favipiravir, Lopinavir/Ritonavir, Remdesivir, Umifenovir, Interferon beta-1a, Covid-19*

INTRODUCTION

Since December 2019, a new coronavirus, known as the novel coronavirus 2019 (nCoV-2019), has become the cause of a pandemic and a serious threat to global health. This coronavirus was first observed in Wuhan and has managed to spread all around China and ultimately worldwide [1]. The severe acute respiratory syndrome (SARS) Corona Virus 2 (SARS-Cov-2) is now recognized as the cause of coronavirus disease 2019 (COVID-19) and as a new member of the family of coronaviruses [2], being introduced by the World Health Organization (WHO) as the cause of the ongoing pandemic. The COVID-19 pandemic has spread rapidly around the world. By the end of 2020, nearly 100 million people have been diagnosed with this respiratory illness, of which about two million have already died [3]. COVID-19 can be present with no symptoms. The affected subjects may experience mild to severe symptoms of fever, chills, muscular pain, fatigue, cough, rhinorrhea, nasal congestion, and dyspnea, which can ultimately lead to life-threatening pneumonia. In addition, gastrointestinal symptoms, conjunctivitis, anosmia and ageusia have been associated with the diagnosis of COVID-19 [4]. This illness spreads by respiratory droplets and aerosols during coughing and (or) sneezing. The primary prevention protocol includes the use of face masks, environmental hygiene, and suitable hand washing. The majority of patients who died from COVID-19 had comorbid conditions, e.g., hypertension, diabetes, cardiovascular diseases, chronic obstructive pulmonary disease, malignancies, and chronic liver disorders [5]. On computed

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tomography (CT) of the chest, the most common patient findings include bilateral pulmonary changes, yet unilateral involvement has also been reported. Also, alterations of laboratory biomarkers like: leukocytosis, leucopenia or lymphopenia, an increase in the prothrombin time (PT), a decrease of the hemoglobin and an increase in the erythrocyte sedimentation rate (ESR) have also been reported. Moreover, an increase in the respiratory rate (>30 per minute), an oxygen saturation fall (≤93%), a decrease in PaO2/FiO2 (<300), and an increase in pulmonary infiltration (>50% of the lungs during 24 to 48 hours) accompany the clinical presentation of COVID-19 [4]. The mortality rate of COVID-19 is lower than the previous epidemics, namely 10% for SARS and 34% for Middle East Respiratory Syndrome (MERS). However, COVID-19 is more contagious and has a more extended incubation period, with SARS-CoV-2 infecting a larger population and causing public panic and an impressive economic impact [6]. Moreover, since there was no effective COVID-19 treatment and vaccines were not yet available, the pandemic has forced healthcare providers and regulatory organizations into repurposing current medicines aiming to test their potential efficacy in the management of COVID-19. For this reason, several clinical trials have been undertaken or are currently conducted worldwide. In some countries, physicians have prescribed medicines employed in the management of non-infectious diseases, such as chloroquine and hydroxychloroquine (used for malaria and rheumatoid arthritis), or antiviral Food and Drug Administration (FDA) approved drugs such lopinavir/ ritonavir, favipiravir, remdesivir, etc., incorporating the use of these pharmacological agents in the local COVID-19 therapy guidelines [7]. Thus, the purpose of this systematic review and meta-analysis was to assess the efficacy of these drugs in the treatment of COVID-19 patients.

MATERIALS AND METHODS

All procedures outlined in this review were performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [8]. The literature was searched with related keywords to ensure that no similar studies have been conducted recently. Subsequently, a structured study question was extracted and used to define keywords, possible combinations, and a search strategy. Then, the literature was systematically searched again in relevant databases. We systematically searched PubMed/MEDLINE, Cochrane Library, Scopus, and Embase from inception until December 10, 2020. The following keywords were used:

Drugs:

- Hydroxychloroquine, Plaquenil®, Hydroxychloroquine Sulfate
- Chloroquine, Chloroquine Sulfate, Resochin®, Dawaquin®, Lariago®
- Remdesivir, Veklury®, Covifor®, Remivir®, GS-5734®, Bemsivir®, BDREM-100®
- Favipiravir, Avigan®, Fabiflu®, Favicovid®, Avifavir®, Areplivir®, Favipira®
- Lopinavir/Ritonavir, Kaletra, Lopimune®, Aluvia®, LPV/r,
- Umifenovir, Arbidol, AR-1I9514, Arpeflu®
- Tocilizumab, Actemra®, Roactemra®
- Interferon beta-1a, Rebif®, ReciGen®
- Disease: COVID-19, SARS-CoV-2, Coronavirus disease 2019.

For each database, an appropriate search was computed, e.g., using MeSH keywords. To increase the sensitivity, we checked the reference lists of the identified clinical trials and of the review articles. Moreover, a manual search of relevant websites was carried out. All the identified articles were imported into the EndNote software (Version X8, Thomson Reuters, New York). After eliminating duplicates, we screened the articles based on their title, abstract, and full-text according to our inclusion and exclusion criteria. We included studies based on the following criteria: 1) Study population: adults receiving the drugs as mentioned above for COVID-19 treatment but not for COVID-19 prevention. 2) Intervention: the use of the aforementioned drugs 3) Comparator: standard of care, placebo, any other drug or combination 4) Outcome: efficacy, treatment rate, reduced length of hospitalization, viral load, and mortality reduction 5) Study design: published clinical trials, but not preprinted articles. Two reviewers screened the titles, abstracts, and full-texts separately based on the eligibility criteria. Any disagreements in scoring were resolved through discussions with the senior author. Two distinct reviewers assessed the quality of the randomized clinical trials (RCTs) using the Cochrane Collaboration tool [9]. The procedure used to evaluate the quality of clinical trials evaluated the following criteria: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of the outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases. We rated each trial as having a low, unclear, or high risk of bias. After inspecting the quality of the clinical trials, we designed an extraction data form based on previous review articles, in addition to employing the Cochrane extraction form for data gathering. Two separate authors extracted the data from the included articles. The extracted data included study specifications (design, duration of intervention, duration of follow-up), participants' specifications (number, age, and gender), intervention specifications, and measured outcomes.

RESULTS

Twenty-eight clinical trials were selected from the 13553 screened studies.

Table1: Included studies

Author	Country	No of pts	Severity	Intervention	Control
Cavalcanti	Brazil	667	Mild/Moderate	HCQ/HCQ+AZM	SC
Chen	China	30	Mild/moderate	HCQ	SC
Horby	UK	4716	NR	HCQ	SC
Huang	China	22	Mild/moderate	Chloroquin	SC
Mitja	Spain	353	Mild/moderate	HCQ	SC
Silva Borba	Brazil	81	Severe	Chloroquin	Chloroquin
Skipper	USA, Canada	491	Mild/moderate	HCQ	PL
Tang	China	150	Mild/moderate	HCQ	SC
Chen	China	240	Mild/moderate	FVP	UMF
Dabbous	Egypt	100	Mild/Moderate	FVP	HCQ
Lou	China	30	NR	FVP	Baloxavir
Zhao	China	14	NR	FVP	TCZ
Ivashchenko	Russia	60	NR	FVP	FVP, SC
DOI	Japan	88	Mild/Moderate	FVP	FVP
Cao	China	199	Severe	LPV/R	SC
Huang	China	101	Mild/moderate	LPV/R	Interferon-alfa and Ribavirin
Hung	China	127	Mild/moderate	LPV/R	Interferon-beta and Ribavirin
Wang	China	60	NR	LPV/R	SC
Li	China	86	Mild/moderate	LPV/R	SC
Yethindra	Kyrgyzstan	30	Mild/moderate	UMF	SC
Zhu	China	50	Mild/moderate	UMF	LPV/R
Beigel	USA	1063	Mild/Moderate, severe	RMD	PL
Goldman	USA,	402	Severe	RMD	PL
Wang	China	237	Severe	RMD	PL
Toniati	Italy	100	severe	TCZ	SC
Davoudi	IRAN	92	Severe	IFN b-1a	SC
Dastana	IRAN	20	NR	IFN b-1a	SC

HCQ: hydroxychoroquine, AZM: Azithromycin, FVP: Favipiravir, LPV/R: Lopinavir/Ritonavir, UMF: Umifenovir, RMD:Remdesivir, TCZ: Tocilizumab, IFN b-1a: Interferon beta-1a, SC: Standard Care, Pl: Placebo and NR: Not reported.

Chloroquine and Hydroxychloroquine with or without Azithromycin:

Both medications were formerly administered to treat malaria and rheumatoid arthritis. According to their mechanism of action, they could be potentially effective in the treatment of COVID-19. Hydroxychloroquine does not bind to the angiotensin-converting enzyme 2 (ACE-2) receptor directly but increases the acidity of the medium in which the COVID-19 protein-spikes interact with and attach to the ACE-2 receptors. The resulting acidity degrades the viral spike and reduces the infection rate, eventually impeding the spread of COVID-19 [1]. Moreover, azithromycin seems to constrain SARS-CoV-2 via direct binding to the ACE-2 receptors. Cavalcanti et al. analyzed hospitalized patients with mild to moderate COVID-19 and revealed that hydroxychloroquine alone or in combination with azithromycin was not associated with better outcomes within 15 days after treatment initiation as compared to standard care. Moreover, there was no significant effect on mortality or mechanical ventilation [3]. Similarly, Horby et al. showed that the treatment with hydroxychloroquine was not associated with a lower mortality rate in hospitalized patients with COVID-19 within 28 days versus conventional therapy [4]. Besides, after 3-7 days of treatment with hydroxychloroquine, the RNA viral load in the nasopharyngeal swab samples did not decrease. Also, this regimen did not reduce the duration of the complete resolution of the symptoms in patients with mild to moderate COVID-19 [5].

In other studies, hydroxychloroquine did not exert antiviral effects against SARS-CoV-2 and was not associated with symptom relief in outpatients with early, mild COVID-19 [7, 10-12]. In addition to their ineffectiveness in COVID-19 patients, treatment with hydroxychloroquine and chloroquine was associated with severe adverse drug reactions (ADR), i.e., depression, psychosis, insomnia, and increased risk of suicide [13]. These two drugs tend to cause QT-prolongation, which is a serious ADR. Thus, necessary cautions must be taken into account when are administered in combination with other medicines with similar side effects, such as azithromycin [3,14-16]. On the contrary, Chen et al. examined 48 patients with COVID-19 and indicated that hydroxychloroquine could reduce the duration of hospitalization [17]. Guerin et al. and Lagier et al. revealed that hydroxychloroquine combined with azithromycin contributed to symptom relief in COVID-19 patients [18,19]. Moreover, Arshad et al. demonstrated that hydroxychloroquine+ azithromycin reduced the mortality rate versus both medicines alone [19]. Overall, according to our review, hydroxychloroquine and chloroquine could not prevent or treat mild to moderate or severe COVID-19.

Favipiravir:

Favipiravir inhibits the RNA-dependent RNA polymerase enzyme, resulting in the inhibition of viral RNA synthesis [20]. Doi et al. showed that the viral load was not significantly different in patients who received early versus late treatment with favipiravir. Additionally, Chen et al. stated that favipiravir was not as effective as umifenovir in improving clinical symptoms [21]. Likewise, in a study by Lou et al., the therapy based on favipiravir was comparable to standard care [22]. However, in other studies, favipiravir was associated with a reduced time of recovery compared to the control group [23, 24]. Furthermore, Zhao et al. showed that the combination therapy of tocilizumab and favipiravir reduces pulmonary inflammation [25]. Hyperuricemia, hypertriglyceridemia, nausea, vomiting, and diarrhea are the reported ADRs for this medicine [21, 23, 26].

Lopinavir/Ritonavir:

Lopinavir/ritonavir inhibits the 3CL pro in vitro, a protease present in the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and highly conserved in SARS-CoV-2 [27]. Ye et al. showed that a combination of Lopinavir and Ritonavir was more effective in the resolution of fever and other clinical symptoms and signs in comparison to conventional therapy [28]. In contrast, Li et al. showed that lopinavir/ritonavir does not seem to improve the clinical outcome of adult patients with mild to moderate COVID-19 and has little benefit over supportive care [29]. Similarly, in Cao's study, there was no significant difference between the standard care and this regimen in hospitalized patients with severe COVID-19 [30]. Also, the UK Recovery Study compared 1596 patients receiving lopinavir/ritonavir with 3376 patients receiving standard care, and no benefit was observed [31]. In another study by Hung and Deng, the combination therapy of methylprednisolone or umifenovir with lopinavir/ritonavir seemed to improve clinical outcomes, e.g., shortening the duration of viral shedding and hospital stay [32,33]. All in all, at the moment, lopinavir/ritonavir cannot be recommended as mainstay therapy for COVID-19 since there is little supportive evidence regarding its efficacy against COVID-19.

Remdesivir:

Remdesivir is a monophosphoramidate adenosine analog that targets and inhibits the RNA-dependent RNA polymerase enzyme [34]. Grein et al. revealed that 68% of their patients with severe COVID-19 responded to treatment with remdesivir, which improved their health status [35]. Similarly, in a study by NIH, the treatment with remdesivir was associated with a reduction of the recovery time and a lower mortality rate [36]. Thus, remdesivir was authorized for emergency use by the FDA, the European Medicines Agency, and the National Health Services in the UK [37-39]. Moreover, in Goldman's trial, there was no significant difference between a 5-day course and a 10-day course of remdesivir in adults with severe COVID-19 not requiring mechanical ventilation [40]. Although hospitalized adults with severe COVID-19, remdesivir did not yield significant clinical benefits, early treatment in the course of the disease was associated with less recovery time [41]. Accordingly, remdesivir is a promising treatment option in COVID-19 and was the first drug to acquire FDA approval to treat COVID-19 [42].

Umifenovir:

Umifenovir protects the body against viral infections via blocking the viral entrance to host cells. Yethindra et al. revealed that patients with mild to moderate COVID-19 might benefit from umifenovir combined with standard care. Also, minimal adverse side effects were reported [43]. In addition, umifenovir monotherapy seemed to be superior to lopinavir/ritonavir in treating COVID-19 in a small sample size study [44]. Umifenovir, and supportive care, may relieve fever and other symptoms faster in COVID-19 patients, but it needs further investigation [45].

Tocilizumab:

A 3rd generation monoclonal antibody (humanized mAb), tocilizumab, inhibits interleukin-6 (IL-6) receptors and is used in the treatment of rheumatoid arthritis and other autoimmune diseases. Patients with severe cytokine release syndrome (CRS), and probably severe pneumonia, may also benefit from tocilizumab [46]. Toniati et al. recorded a rapid and sustained response to tocilizumab and observed a post-therapy clinical improvement [47]. However, Kewan et al. did not report significant clinical improvement with tocilizumab in patients with severe COVID-19 [48]. In addition, tocilizumab tends to decrease the mortality rate and the need for invasive mechanical ventilation in adults with severe COVID-19 pneumonia [49]. On the contrary, Colaneri et al. (2020) studied 112 adults with severe COVID-19 and showed that mortality or the number of intensive care unit (ICU) admissions was not reduced following the administration of tocilizumab [50]. Likewise, Kewan et al. did not observe a significant clinical improvement with tocilizumab in patients with severe COVID-19 [51]. Therefore, due to contradictory results, further studies are needed to establish the beneficial effects of tocilizumab in patients with severe Covid-19.

Interferon beta-1a:

Davoudi et al. reported that treatment with interferon beta-1a did not change disease duration. However, when this medication was added to the nationwide approved therapy regimens, an increased discharge rate on day 14 and a decreased 28-day mortality were detected [52]. Moreover, another study supports the use of interferon β-1a in combination with hydroxychloroquine and lopinavir/ ritonavir in the treatment of COVID-19 [53].

DISCUSSION

This systematic review provides a comprehensive analysis of clinical trials exploring the use of hydroxychloroquine, chloroquine, favipiravir, lopinavir/ritonavir, remdesivir, tocilizumab, and interferon beta-1a in COVID-19 patients. Despite the previous suggested benefits of chloroquine or hydroxychloroquine with or without azithromycin, these agents could not show favorable efficacy in the prevention or treatment of COVID-19. The occurrence of severe ADRs, i.e., QT-prolongation, which can enhance the interactions with other medicines and pose serious health threats, have obliged the National Institute of Health and the WHO to abolish these medicines from their trials. Lopinavir/ritonavir, formerly used to treat human immunodeficiency virus (HIV) infection, had no effect on the inhibition of virus replication, reduction of the duration of hospitalization or mortality, leading to the withdrawal of this drug from clinical trials. Favipiravir, a guanine analog, and umifenovir have been approved in some countries, e.g., Russia, but further research is warranted to clarify their effects in COVID-19. Some countries, despite their former approval of these medicines, later withdrew them from their national COVID-19 guidelines. Remdesivir is the first FDA-approved pharmacological agent for COVID-19, and its efficacy was observed in some multicenter, randomized, controlled trials. However, since these RCTs were funded by pharmaceutical companies, there is a risk of bias regarding their benefits. Moreover, there are some meta-analyses that described non-significant effectiveness on COVID-19 outcomes in comparison to standard care. Overall, more studies with a lower risk of bias are needed to enable confident statistical analyses. The IL-6 receptor blocker, tocilizumab, might be a robust anti-inflammatory agent in the management of severe COVID-19 patients during the inflammatory pneumonia phase. There are some other ongoing trials on tocilizumab that will be reported in the following updates. Although interferon beta-1a showed favorable efficacy in a phase 2 trial, more investigations are needed due to the high risk of bias and small sample size of this exploration. Our study analyzed only published RCTs, not observational studies (cohorts, case reports, and case series). According to different interventions, different doses, and different disease severity, more studies are needed to conduct reliable meta-analyses or dose-response studies.

CONCLUSION

The rapid transmission of SARS-CoV-2 has limited the opportunities to develop new treatment strategies. Thus, a try-and-error strategy was used to test medicines in the management of COVID-19 and develop standard treatment guidelines. Our findings suggest that hydroxychloroquine, chloroquine, and lopinavir/ritonavir are not favorable treatments for COVID-19. Favipiravir, umifenovir, and interferon beta-1a need investigation in more clinical trials as well. Tocilizumab and remdesivir might be effective in some COVID-19 patients, but more research is needed to draw conclusions.

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ORIGINAL ARTICLE

Effect of different physical stressors on soldier's psychophysiological response and marksmanship

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Abstract: *Shooting impairment may occur whereas there is either central or peripherical fatigue. However, the effects of different physical stressor stimuli on fine motor skills as shooting are yet not fully understood. The present research aimed to analyze the effect of endurance and resisted physical stressors on the psychophysiological response and pistol marksmanship of novel soldiers, and the possible differences in gender and BMI. Variables of heart rate, isometric hand strength, rate of perceived exertion, and pistol marksmanship were analyzed in 56 soldiers in endurance and resisted stress protocols. Isometric hand strength, heart rate, and RPE were significantly higher in both resisted and endurance physical stress protocols than in the basal sample. Gender differences were seen in strength, cardiovascular and RPE values. Also, subjects with larger BMI presented significantly higher isometric hand strength and higher marksmanship in all moments evaluated. We conclude that an endurance stress protocol produced a higher cardiovascular and perceived exertion than a resisted one, not affecting hand strength, shooting heart rate, and marksmanship. Females presented lower hand strength and marksmanship while higher rated of perceived exertion and heart rate during the endurance and resisted stress protocols than males. Overweight participants presented higher heart rates during both physical stress protocols, but higher hand strength and marksmanship while similar shooting heart rates than normal-weight participants.*

Keywords: *marksmanship; stress; heart rate; fine motor skills; fatigue*

INTRODUCTION

The effect of different stressor stimuli on fine motor skills is an area with wide applications, yet not highly developed. The stress effects may differ regarding the characteristics of the stressor stimuli. In the case of physical exercise, the intensity, duration, and type of exercise have a direct impact either on fine or gross motor skills [1].

Authors found how after a bout of high-intensity, lower-body resistance training passing accuracy was decreased [2]; after cycling in an ergometer at 100% of maximal capacity, significant attenuation on reaction times was found, like at lower intensities (70%) [3], suggesting an inverted-U function between exercise intensity and motor control.

Specifically, in combat, previous researchers found no effect on marksmanship despite the increased combat stress, neither with rifles and pistols independently of the caffeine ingestion [4,5]. The physiological mechanisms underlying the decrease of performance on fine motor skills may be related to i. the accumulation of hydrogen ions, which affect glycolytic enzymes due to low Ph, impairing ATP production and compromising the contractile capacity of the muscle [6]; ii. the high inorganic phosphate concentration, which inhibits Ca ++ reuptake increasing its concentration in the sarcoplasm and hindering muscle contraction; and iii. the ammonia concentration, which is related to the affection of neurobiological processes, affecting the quality of neural connections and electrical activity of the muscle [7].

In addition, all these acute metabolic responses are directly modulated by the intensity and type of exercise [8]. Related to shooting accuracy and marksmanship, previous authors showed that high-intensity exercise affected central aspects

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of shooting performance, shooting accuracy, shooting precision, and stability of hold, being accentuated when shooting in a standing position, compared with shooting in a prone position [9]. Acute protocols designed to fatigue the upper body flexor muscle groups (muscles involved in stabilizing and supporting a firearm), such as lifting, climbing, and pulling, presented significant decreases in rapid-fire rifle shooting performance in the standing position in soldiers immediately after these exercises [10]. In this line, independently of the type of exercise (concentric, eccentric, or a combination of both) or the location (knee, ankle, or entire lower extremity), acute fatigue may induce a loss of stability[11]. This fact, directly affects postural, standing, and gun stability, key factors connected to rifle shooting accuracy 12 since body sway and imbalance has been directly correlated with poor shooting score[13,14]. Superior shooting performance requires good postural balance, as well as, an ability to steady the gun while aiming[12]. However, the effect of different acute stress exercise-induced peripheral fatigue on fine motor skills such as shooting is yet not well documented. Most of the studies have focused either on soldiers or experienced subjects, showing how lower fitness levels, experience, and training increased fatigue and decrease marksmanship [15,16]. In addition, previous studies were the focus on the rifle, showing lower research on pistols, leading to greater muscular fatigue due to the farther center of mass to the shooter's body and the increase in joint angle [17]. Thus, we proposed the present research to analyze the effect of endurance and resisted physical stressors on the psychophysiological response and pistol marksmanship of non-experienced shooters. As a second aim, we aimed to analyze differences in gender and weight. We hypothesized that i. resisted physical stressor would produce a higher psychophysiological stress response, decreasing the marksmanship more than the endurance exercise stressor; ii. Subjects with higher body mass index (BMI) would present higher psychophysiological response and lower marksmanship; iii. There would be gender differences in the psychophysiological stress response and marksmanship.

MATERIALS AND METHODS

Participants

A total of 56 soldiers belonging to light military forces (23.6±3.7 years; 175.8±9.1 cm; 71.3±8.7kg; 23.1±2.2 BMI) were analyzed. Inclusion Criteria were: Professional Experience in the army. Exclusion Criteria for were: Presence of any medical condition or injury that would be a limiting factor for intense physical activity and the use of firearms (e.g. cardiovascular diseases) at the moment or during the protocol, intake of any dietary supplement, stimulants, or other ergogenic aids. Participants were informed of the characteristics of the study and the potential risks derived from those who were exposed. All the procedures were conducted following the Helsinki Declaration (as revised in Brazil, 2013), all the participants filled out an informed consent form previous to starting the research, and all the procedures were approved by the University Ethic Committee.

Methods

Procedures

Before the research started all the subjects received instructions/familiarization regarding the protocol and tests. Then 3 days of evaluation were distributed as follows: 1º Day: basal sample of all the variables measured in the study; 2º Day: 48h after, subjects performed randomly an endurance or resisted physical stress protocol; 3º Day: 48h after, subjects performed the remaining protocol.

For both physical stress protocols, participants conducted a warm-up composed by:

i) 2 min of joint mobility

ii)5 min of light aerobic running (50-60% Maximum Heart Rate)

iii) 2 series of 20 m of progressive running intensity

After the warm-up, participants performed the physical stress protocol, which was supervised and encouraged by sports science professionals:

Resisted Physical Stress Protocol

- 20 sets of burpees at maximum intensity

Endurance Physical Stress Protocol

- 5 min maximal running test in a track field.

Before the research, body weight was assessed with a SECA scale model 714 with a precision of 100 grams (range 0.1-130 Kg), located on a flat and smooth surface and calibrated at zero. Subjects were barefoot and with minimal clothes. Once located in the center of the platform, they remained without their body being in contact with surrounding objects, with the weight evenly distributed on both feet facing forward. Height, with a SECA scale model 714 with a precision of 0.1 mm (range 60-200 cm). The subject stood up, barefoot with the head oriented in the Frankfurt plane. Arms on both sides of the trunk and fully extended with palms touching the external face of the thighs, heels together touching the lower end of the vertical surface with the inner edge of the feet, occipital area, scapular, buttocks, posterior face of the knees and calves touching the vertical surface of the anthropometer.

Before and after the exercise stress protocols, we measured:

- Rating of perceived exertion (RPE), 6–20 scale.

- Isometric hand-grip strength using a TKK 5402 dynamometer (Takei Scientific Instruments CO. LTD). Each participant's grip strength was measured on the shooting hand. Participants were standing with 0 degrees of shoulder flexion, 90 degrees of elbow flexion, and the forearm in neutral. Idem, was done pre-post and among series.

- Heart Rate (HR) was measured before and during the entire protocol by a Polar V800 HR monitor (Kempele, Finland).

- Marksmanship was measured by the sum of three airsoft pistol shots to a target at a distance of 7 m, following procedures of previous research [4,5]. A gas blowback Beretta 92X Centurion airsoft gun (450 fps) was used.

Statistical analyses

To analyze the data, we used the SPSS statistical package (version 24.0; SPSS, Inc., Chicago, Ill.). Means and standard deviation (SD) were calculated using traditional statistical techniques. Normality and homoscedasticity assumptions were checked with a Kolmogorov-Smirnov test. To analyze differences between pre and post a T-test was administered, and to analyze differences between experimental groups (gender and BMI) and differences between moments of evaluation, an ANOVA test was conducted since variables presented a parametric distribution.

RESULTS

The results are reported as mean±SD. Isometric hand strength, HR, and RPE were significantly higher in both Resisted and Endurance physical stress protocols than in basal samples (Table 1).

According to gender differences, males present significantly higher isometric hand strength at all moments evaluated. HR at shooting at Basal and Endurance physical stress protocol of females was statistically higher than males. Female RPE was higher than males at Resisted and Endurance physical stress protocols (Table 2) (between parenthesis significant differences (p≤0.05) between males vs females).

Subjects with larger BMI (BMI ≥25 overweight) presented significantly higher isometric hand strength at Basal and Resisted physical stress protocol than subjects with lower BMI (BMI<25). Subjects with larger BMI also presented higher marksmanship in all moments evaluated (Table 3) (between parenthesis significant differences (p≤0.05) between BMI<25 vs BMI≥25).

Table 1: Psychophysiological parameters analyzed before and after the physical stress protocols

	Basal	Resistance Training	Endurance Training	F	P	η2	Moment comparison
Isometric Hand Strength	42.8 ± 8.4	44.9 ± 6.4	42.7 ± 2.1	8.481	.001	.239	2>1
Marksmanship	12.8 ± 9.5	14.5 ± 10.6	15.2 ± 10.1	1.844	.168	.068	-
Heart rate during protocol	-	166.1 ± 13.3	178.9 ± 11.7	68.156	.013	.711	3>2
Shooting Heart Rate	90.3 ± 17.5	125.2 ± 19.0	124.3 ± 17.6	50.122	.000	.650	2>1; 3>1
Rated of Perceived Exertion	6.6 ± 1.1	15.2 ± 2.0	16.8 ± 1.8	739.846	.000	.965	3>2>1; 3>1

Table 2: Psychophysiological gender differences of the parameters analyzed before and after the physical stress protocols

		Basal	Resistance based exercise	Endurance based exercise	F	P	η2	Moment comparison
Male	Isometric Hand Strength	44.8±7.8	46.3±6.2	43.9±9.5	3.973	.026	.153	2>1
	Marksmanship	13.6±9.5	16.0±10.8	15.7±10.7	1.940	.156	.081	-
	Heart rate during protocol	-	165.8±9.2	178.9±6.1	71.012	.021	.781	3>2
	Shooting Heart Rate	87.7±17.0	128.4±16.0	121.6±16.5	65.007	.000	.747	2>1;2>3;3>1
	Rated of Perceived Exertion	6.6±1.1	14.9±2.0	16.5±1.8	604.148	.000	.965	3>2>1; 3>1
Female	Isometric Hand Strength	33.4±2.4 (.000)	38.2±1.0 (.000)	36.9±2.1 (.025)	53.399	.000	.930	3>1; 2>1
	Marksmanship	9.2±9.1 (.011)	7.4±5.5 (.018)	13.2±7.3 (.024)	2.533	.141	.388	-
	Heart rate during Protocol	-	174.4±8.2 (.011)	191.2±7.1 (.006)	71.231	.000	.961	3>2
	Shooting Heart Rate	102.6±15.0 (.014)	113.6±27.1	136.6±17.9 (.014)	69.514	.000	.946	3>1; 3>2
	Rated of Perceived Exertion	6.6±1.2	16.2±1.8 (.009)	18.2±1.0 (.007)	392.302	.000	.990	3>1; 2>1

Table 3: Psychophysiological body mass index differences of the parameters analyzed before and after the physical stress protocols

		Basal	Resistance based exercise	Endurance based exercise	F	P	η2	Moment comparison
BMI <25	Isometric Hand Strength	41.7±3.5	43.7±7.0	42.9±7.7	1.197	.318	.238	-
	Marksmanship	11.4±9.7	12.4±10.6	12.5±8.8	.380	.686	.019	-
	Heart rate during protocol	-	163±8.5	178±7.2	67.145	.032	.631	3>2
	Shooting Heart Rate	91.8±15.2	125.9±14.7	126.8±17.2	28.322	.000	.685	1<2; 1<3
	Rated of Perceived Exertion	6.5±1.0	14.7±1.9	16.8±1.5	910.743	.000	.979	1<2; 1<3; 2<3
BMI ≥25	Isometric Hand Strength	44.5±2.8 (.019)	48.5±5.5 (.011)	39.7±12.9	22.960	.000	.793	1<2
	Marksmanship	16.8±7.8 (.001)	20.8±7.7 (.009)	23.4±9.8 (.000)	2.169	.157	.266	-
	Heart rate during Protocol	-	171±9.7 (.031)	188±9.1 (.021)	79.256	.038	.720	3>2
	Shooting Heart Rate	92.7±23.5	122.2±24.3	118.4±16.9	3.996	.047	.400	1<2; 1<3
	Rated of Perceived Exertion	7.0±1.3	16.4±1.7 (0.007)	18.8±2.4 (0.021)	112.325	.000	.949	1<2; 1<3

DISCUSSION

The present research aimed to analyze the effect of endurance and resisted physical stressors on the psychophysiological response and pistol marksmanship of non-experienced shooters. Our hypotheses were partially completed since i. There were no significant differences in stress protocols in the psychophysiological stress response or marksmanship. ii. A similar psychophysiological response was found independently of the BMI, but subjects with larger BMI presented higher marksmanship values. iii. There were gender differences in the psychophysiological stress response and marksmanship.

The cardiovascular response monitored at the basal time was higher than the considered standard for the population evaluated (60-70 bpm) [18]. This was consistent with an anticipatory anxiety response, a defense organic response modulated by the activation of the sympathetic nervous system that prepares individuals for any uncontrolled or new stimuli [19]. Previous authors reported a significant increase in the heart rate from a standing resting position to a standing position firing a gun, in line with the results obtained in the present research [20].

The cardiovascular response has a direct impact on fine motor skills as shooting, the higher HR shortens the diastolic phase and increases the systolic one, increasing body tremor [21], which in addition to the one produced by physical activity, marksmanship could be compromised [22,23]. After resisting and endurance physical stress protocols we found a significant increase in the cardiovascular response to shooting (38.6% and 46% respectively), however, marksmanship was not affected, suggesting no negative effect on shooting HR and fine motor skills.

In this line, previous authors obtained similar cardiovascular results, without a marksmanship compromise in archers [22] and pistol shooters [24]. The lack of significant differences in marksmanship between the two stress protocols highlighted the no effects of peripheral fatigue on shooting performance.

There are three possible hypotheses which would explain this: i) Subjects performed both resisted and endurance physical stress protocols with an optimal arousal level [25,26], in accordance with the Inverted-U hypothesis which predicts that performance is best at a moderate optimal level and becomes progressively worse with either increases or decreases in arousal, and comes to confirm that incorporating psychological factors in training (e.g. pressure), may enhance performance [27,28]; ii) Since subjects have none previous experience and despite performing a familiarization, as consequence of low total acquired experience, fatigue did not affect their marksmanship values; iii) Among the familiarization, breathing and holding techniques were taught to the subjects, which are essential to attenuate the postural tremors created by possible high HR at the moment of shooting, technique which has proven essential for rifle, pistol [15] and arch [22], the use of these techniques could be another possible explanation.

The ability to stabilize a gun is crucial for performance in pistol shooting, being related to the shooter's muscular strength [29]. The maintenance of isometric hand strength can play an important role in this fact, highlighting the importance of physical fitness for athletes and professionals that have to use fire weapons (such as biathlon athletes, or police and military respectively).

Previous authors proposed integrated operative high-intensity interval training as one of the best training tools for this aim since it allows participants to improve both, muscular and cardiovascular performance, two factors directly related to shooting performance [30,31]. In this line, both protocols elicited a significant increase in HR during its execution, being higher in the endurance stress protocol.

This cardiovascular response could be related to the continuous impact, load, and physiological demand of this type of work, a fact consequent with previous studies which compared similar exercise-based protocols [31,32]. The HR reached during the two stress protocols is associated with a decrease in fine motor abilities and specifically in shooting, a fact basic for any shoot actions independently performed by arch [33] pistol or rifle [24].

Nevertheless, at the shooting moment the HR decreased below 140 bpm, HR is not related to this fine motor abilities impairment.

These results highlighted the importance of cardiovascular stress response control in these activities, emphasizing the importance of a good aerobic physical condition for these activities. Females presented lower isometric hand strength values at all moments evaluated. This could be explained due to the female phenotype since they present lower body and muscle mass than males, lower baseline testosterone levels, and lower strength in the upper body muscles [34]. Previous authors also found similar results when comparing the hand grip of active handball and judo female athletes with healthy male subjects, presenting males with higher hand grip strength than females [35].

RPE seems to be also influenced by gender. Females presented higher RPE in the endurance and resisted physical stress protocols. These gender differences were also found in weight-bearing as treadmill [36,37,38], partial weight-bearing as ski machine [38], and non-weight-bearing as cycle [39]. Mechanisms underlying this apparent gender difference cannot be specified, however, there are several hypotheses as information processing [40] and physiological [41] differences as plausible explanations. In this line, previous authors suggested that gender differences in RPE may exist in the fact that females rate exercise as more effortful than males at absolute levels of power output [42].

Finally, a higher cardiovascular response during the protocols and while shooting at basal and endurance stress protocol was presented by females. These differences are related firstly to their smaller myocardium, beating slightly faster to make up for its size. While the average basal male heart beat is around 70-72 bpm, women's beats are 78-82 bpm [43]. Secondly, the increased basal heart rate would explain the aforementioned anticipatory anxiety response, which increases the heart rate due to perceived stress and novelty of the stimuli [19]. The response has been also evaluated in sports competition tasks [44] or novel paratroopers before jumping [45] with a similar physiological response. Thirdly, the lower recovery capacity after short burst high-intensity endurance activities of females [46], may explain the significantly higher shooting heart rate than males after the endurance stress protocol. BMI cutline was established at normal-weight (<25 BMI) and overweight (25 BMI >).

Overweighed subjects presented greater significant values of isometric hand strength, probably explained due to the larger body mass, consequent with previous studies comparing the strength of obese and non-obese subjects of either upper [47] and lower body [48] strength. In addition, overweighed subjects present greater marksmanship values evaluated at all moments, reinforcing the relationship between hand strength and shooting marksmanships [49].

However, the cardiovascular response of overweighed subjects was significantly higher during both physical stress protocols, explained due to inferior cardiovascular performance, since for the same stimuli and intensity greater cardiovascular output is needed compared to normal-weight subjects.

Nevertheless, during shooting actions their HR was similar to the normal-weight group, decreasing to HR values related with no negative effect on fine motor skills. In addition, RPE was also higher in overweighed subjects after both protocols, suggesting greater effort and exposing inferior fitness, explained due to worst physical conditioning, or the fact that being overweight is an added organic stressor 50. In this line, previous authors correlated larger BMI with reduced cardiovascular output and lower performance, showing greater sympathetic, stress, and RPE baseline values [51], supporting our present data.

CONCLUSIONS

An endurance stress protocol produced a higher cardiovascular and perceived exertion than a resisted one, not affecting hand strength, shooting heart rate, and marksmanship. Females presented lower hand strength and marksmanship while higher rated of perceived exertion and heart rate during the endurance and resisted stress protocols than males.

Overweight participants presented higher heart rates during both physical stress protocols, but higher hand strength and marksmanship while similar shooting heart rates than normal-weight participants.

Limitations of the study practical applications

The small number of subjects is one of the main limitations of the present study, however, there were no more available subjects at the moment. A second limitation was the lack of evaluation of hormones such as testosterone, alpha-amylase, and cortisol to control the hormonal stress response. These limitations were subjected to financial and technological lack.

Practical applications

The information of the present research could be used for specific training, allowing further individualization of the training stimulus according to gender, anthropometric and experience characteristics. In addition, soldiers, police, and other security crops, as well as those sport modalities in which shooting is present, for example, biathlon, could potentially benefit from this information.

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VARIA

Effect of pharmacist's patient education on patient compliance in the southeast of Iran: A quasi-experimental study

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Abstract: *Background: As an essential part of the treatment chain and also the link between physicians and patients, pharmacists play a major role in optimizing drug administration. Compliance with a therapeutic regimen is necessary for therapeutic success, while non-adherence can lead to disease progression and eventually treatment failure. This study aimed to evaluate the impact of pharmacist's patient education on different parameters of patient compliance.*

Results: The results showed the patients receiving the intervention compared to the control group, were significantly more adherence to the correct drug use dose (p = 0.016), time of use (p = 0.018), treatment duration (p =0.001), and frequency (p = 0.049).

Conclusions: This study indicates that detailed patient counseling at pharmacies can positively influence different parameters of patients' adherence.

Keywords: *pharmacists; monitoring drug therapy; disease management*

BACKGROUND

Medication adherence knowledge plays a crucial role in treatment success. Pharmacists' scientific skills to teach required information about drug administration, interactions, side effects storage is crucial to improve patient adherence and so treatment success [1, 2]. Patient education by pharmacists can decrease drug therapy problems arising from inappropriate drug administration or prescriptions and decrease overall health care costs [3-5]. Over the past 30 years, the scope of medication therapy management, as well as pharmaceutical care, focused on more specialized and patient-centered care through a more effective pharmacist-patient relationship [4]. Pharmacists also are one of the most important parts of rational drug use based on the World Health Organization (WHO) definition [6] and decreasing its burden [5, 7, 8]. On the other hand, inappropriate administration of antimicrobials may lead to drug resistance and treatment failure that is one of the most challenging problems today [7, 8]. Studies show that one of the main causes of irrational drug use is considering pharmacists only as drug distributors and ignoring their potential role as consultants and educators [9, 10]. In 2003,

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the WHO identified poor patient adherence to achieve all therapeutic goals as a major cause of treatment failure [4]. Although the impact of medication counseling by pharmacists on patient treatment outcome, has not been investigated truly, it has been shown that community pharmacists have a great impact on improving patient adherence especially in patients suffering from chronic diseases as well as improving overall community health [1, 11, 12]. Thus, growing an effective patient-pharmacist relationship is a key factor to optimize medication use and it seems necessary to focus on pharmacists consulting skills and supportive laws [13]. In the present study, considering the undeniable role of pharmacists in patient education and improving their compliance with therapeutic regimen, we evaluated the impact of medication consultation on the appropriate use of medication by patients referring to community pharmacies in Zabol city, southeast of Iran at 2019.

METHODS

Design and participants

This quasi-experimental study was conducted on 56 newly diagnosed patients in one governmental hospital (xxxx) affiliated to XXXX University of Medical Science in the southeast of XXXX from March 10, 2019, to August 30, 2019. Participants were selected using a simple random method and distributed to intervention and control groups. Patients were defined new to therapy if they had no evidence of filling prescriptions with their current medications during the past 6 months. Considering the mean scores for treatment belief for two control (5.04 ± 3.4) and intervention (2.52 ± 2.64) groups in pilot research, the type I error of 0.05, the test power of 0.80 as well as 20% sample loss, the required sample size was estimated as of 23 people in each group.

Intervention

The intervention group received their medications with written instructions on drug packages as well as face-to-face education by pharmacists mainly about dosage, regimen, and length of treatment of each drug in their prescriptions. In the control group received their medications only with routine written instructions on each drug package. Each patient in the control group who had a question that needs further education was excluded from the study.

Instruments

The patient’s demographic information was included in data collection forms. Two separate questionnaires designed based on literature review and pilot tested. Patients or family caregiver received the first questionnaire, before collecting their prescriptions and the second one 14 days after. The first questionnaire consisted of four questions about their risk of non – adherence [14] as well as eight questions about their belief about prescribed medicines [15]. The second one consisted of five questions about the patient's practice (correct dose, time, frequency, and therapeutic course) about each of their prescribed medications. The investigator who reviewed patient medications after 14 days and filled out the second questionnaire did not know whether patients are in sample or control groups. The face and content validity of the questionnaire was determined by collecting the six experts' opinions. The questionnaires were then distributed to 20 people in a pilot form. The final questionnaires were designed after identifying the bugs and fixing them. To assess the reliability of the questionnaire. Belief about prescribed medication score and total performance score was obtained from the sum of scores of the relative questionnaires.

Statistical analysis

Statistical analysis of the present data was carried out using an independent t-test and chi-square test using SPSS software (version 21). P-value < 0.05 was considered as significant.

Ethical consideration

The study protocol was approved by the XXXX University of Medical Sciences Ethics Committee (code: XXXXXX) and patients provided written informed consent before participating in the study.

RESULTS

After considering the exclusion criteria, 56 patients were eligible to enroll in this study, 28 patients in both the intervention and control group. Our participants consisted of 45 (80.4%) male and 11 (19.6%) female 7.1% of whom were uneducated. Their ages ranged between 15 - 75 years with the mean age of 33.46 (14.20) years. The mean scores of the patient’s primary risk of non-adherence and belief about prescribed medicines were 5.27 (0.92) and 23.89 (3.08) respectively.

There was no statistically significant difference between intervention and control groups in terms of age (p = 0.640), gender (p = 0.313), level of education l (p = 0.968), belief about prescribed medicines (p=0.180) and risk of non-adherence (p = 0.679) (Table 1).

Table 1: Demographic characteristics of sample and control groups

		Control	Sample	P-value
Age		30.03 (18.09)	24.43 (15.75)	0.640
Sex	Male	21 (75%)	24 (85.7%)	0.313
	Female	7 (25%)	4 (14.3)	
Level of education	Uneducated	2 (7.1%)	2 (7.1%)	0.968
	Under high school diploma	9 (32.2%)	7 (25%)	
	High school diploma	13 (46.4%)	14 (50%)	
	College degree	1 (3.6%)	3 (10.7%)	
	Bachelor degree	3 (10.7%)	2 (7.1%)	
Belief about prescribed medicines		24.11 (2.92)	23.68 (3.27)	0.679
Risk of non-adherence		5.11 (0.91)	5.43 (0.92)	0.180

Our results showed that patients receiving face-to-face education were significantly more adherent to the correct dose (p = 0.016), exact time (p=0.018), frequency of drug administration (0.049), and completing the therapeutic course (p <0.001), compared to the control group (Table 2).

Table 2: Comparison of patient's adherence to correct dose, time, frequency and completing the therapeutic course in control and sample groups

	Control No (%)	Sample No (%)	P-value
Correct dose	16 (57.1%)	25 (89.3%)	0.016
Correct Time	7 (25%)	17 (60.7%)	0.018
Correct frequency	14 (50%)	22 (78.6%)	0.049
Completing therapeutic course	3 (10.7%)	19 (67.9%)	<0.001

Finally, 100 % of the patients in the intervention group and 17.9% of the control group were satisfied with the pharmacist's services (p < 0.001) (Table 3).

Table 3: Comparison of participants' satisfaction in control and sample groups.

Satisfaction		Control	Sample	P-value
Agree	No (%)	5 (17.9)	28 (100)	<0.001
No Idea	No (%)	7 (25)	0 (0)	
Disagree	No (%)	16 (57.1)	0 (0)	
Total	No (%)	28 (100)	28 (100)	

DISCUSSION

Pharmacists as an essential part of the treatment chain and also the link between physicians and the patients play major roles in health care systems [16]. Studies have shown that pharmacists can improve patient adherence and the rate of treatment success. Noncompliance can lead to irrational drug use, expansion of drug therapy costs, the prolongation of therapeutic courses, and adverse drug reactions. In this study, we evaluated the impact of patient education by pharmacists in different aspects of patient compliance and concluded its positive impacts. Our study population is divided into two intervention and control groups. The intervention group received medication counseling especially about dose, time, and frequency of drug administration and duration of therapy besides routine written instructions on drug packages that were consistent in both groups. We observed that although sample intervention groups were not significantly different in terms of demographic data, risk of non-adherence, and belief about their prescribed medication but their final behavior about their prescribed medications were completely different. Adherence to the correct dosage, timing, frequency, and therapeutic course was significantly higher in patients receiving drug education by pharmacists. They were also more satisfied with the pharmacist's services. These results were in agreement with the previous results of previous studies [17-21].

They proved positive impacts of patient counseling at the pharmacies on accomplishing therapeutic goals, reducing adverse drug reactions, and administration drug errors in their researches. Our findings showed that a relatively high number of patients who did not receive counseling did not properly follow their treatment course, which can lead to therapeutic failure and its related complications. Especially, when patients are on antibiotics, non-adherence can lead to drug resistance and finally affect both patients and society. The small sample size was one of our limitations in this study, besides if we could monitor a patient's performance more often, we would be able to provide more accurate results and lower possible bias. One of the strengths of this study was that patients with a history of the same disease were excluded from the study so that their previous experiences did not affect the final results.

CONCLUSIONS

It can be concluded that detailed patient counseling by pharmacists, can positively influence patients' adherence. In this regard, it seems necessary to focus on communicating and counseling skills of pharmacists and even motivational tools as counseling fees.

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REVIEW ARTICLE

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Efficacy and safety of Ombitasvir/Paritaprevir/Ritonavir+ Dasabuvir and Ribavirin in patients with compensated HCV cirrhosis

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Abstract: *Background and aim: Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir in association with ribavirin was the only interferon-free regimen available in 2015 in Romania for compensated HCV cirrhosis. The aim of our study is to reveal our experience with interferon-free therapy in compensated HCV cirrhosis.*

Materials and methods: We conducted a multicenter prospective study including 90 patients with compensated (Child-Pugh A) HCV cirrhosis treated with Ombitasvir/Paritaprevir/Ritonavir + Dasabuvir and Ribavirin regimen for twelve weeks. The HCV infection was diagnosed using clinical, biological, and FibroMax tests. Each patient had follow-up visits at four, eight, twelve and twenty-four weeks after the initiation of the treatment.

Results: 44 (49% of) patients were female and 46 (51 % of) were male. The mean age of patients in the study was 61 years old. All the patients had HCV genotype 1b infection. 22 patients were naive and 68 had previous antiviral therapies based on pegylated IFN-α and ribavirin. At the initiation of treatment 64 (71%) of patients had cytolysis, 38 (42%) had thrombocytopenia, 19 (21%) patients had hyperbilirubinemia and 11 (12%) patients had anemia. It was noticed a decreasing of cytolysis, only 10% of 64 patients still having cytolysis after four weeks of treatment. One patient (1%) died after four weeks of treatment by complications of cirrhosis. 89 (99%) of patients had a virologic response (VR) at the end of treatment (EOT) and sustained virologic response (SVR) twelve weeks after the last dose administration.

Conclusion: The most common side effect among the patients in the study group was anemia (47% cases). Cytolysis disappeared after four weeks of treatment in 61% of patients. The interferon-free combination treatment was safe and highly effective in compensated HCV cirrhosis.

Keywords: *interferon-free regimen, HCV cirrhosis, anemia, Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir*

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INTRODUCTION

The World Health Organizations estimates that there are 150 million people infected with hepatitis C virus [1].

Hepatitis C virus (HCV) affects individuals worldwide with a high degree of morbidity and mortality. Chronic HCV can lead to cirrhosis, hepatocellular carcinoma, the need for transplantation and death. The annual incidence of hepatocarcinoma in patients with HCV cirrhosis is between 1% to 7% [2, 3, 4, 5]. Genotype -1 is the most common HCV genotype from all the 7 genotypes [6]. Genotype-1b is the most prevalent HCV subtype in Europe, Central America, the Middle, and Asia [7].

Therapy with pegylated interferon and ribavirin for 12 weeks to 72 weeks was associated with low SVR (only 60% of them achieved sustained virologic response) and with adverse events [8, 9]. It can be a challenge for patients with cirrhosis to achieve SVR.

Sustained virologic response is defined as undetectable HCV-RNA at 12 weeks, after the end of treatment (EOT) and it is associated with improved survival and reduced risk of HCC development in patients with cirrhosis [10,11,12].

New IFN-free direct- acting antiviral (DAA) therapies have greatly improved SVR rates in patients with cirrhosis [13, 14, 15, 16, 17]. In most regimens for maximizing the SVR rates in patients with cirrhosis, it was required the addition of ribavirin [18, 19].

The available DAA are classified according to the viral target protein as NS3/4A protease inhibitor, NS5B polymerase inhibitors (including non-nucleoside and nucleoside inhibitors) and NS5A inhibitors [20].

Direct-acting antiviral agents were approved for the first time in 2011 for the treatment of hepatitis C. Although the addition of the NS3/4A protease inhibitors boceprevir, telaprevir or simeprevir to pegylated interferon and ribavirin regimens increased SVR rates in patients with genotype-1 infection efficacy rates have remained low in patients with cirrhosis [21, 22, 23, 24, 25].

The regimen containing ombitasvir/paritaprevir/ritonavir and dasabuvir with or without ribavirin was approved by the European Medicine Agency and/or the Federal Drug Administration for the treatment of patients without and with cirrhosis Child-Pugh-Turcotte class A.

Ombitasvir (OBV) is an NS5A inhibitor co-formulated with NS3/4A protease inhibitor paritaprevir (PTV), and the pharmacokinetic enhancer ritonavir which increases peak through drug allowing for once daily PTV dosing exposure [26,27]. Dasabuvir is a non-nucleoside NS5B polymerase inhibitor. This 3-DAA regimen with or without ribavirin (RBV) is approved in many countries to treat HCV genotype-1 infection.

Most of prospective phase II and III trials included only a few patients with cirrhosis.

The regimen Ombitasvir/paritaprevir/ritonavir and dasabuvir were evaluated in two studies in patients with compensated HCV cirrhosis. One study-TURQUOISE II trial, an open-label phase III trial evaluated the 3D regimen with ribavirin for 12 weeks vs 24 weeks in patients with compensated HCV cirrhosis. The other study – TURQUOISE III phase III b, open-label study evidenced if it could be achieved a good SVR without ribavirin at 3D regimen in 60 patients with compensated HCV cirrhosis. Ombitasvir/paritaprevir/ritonavir + dasabuvir with ribavirin became available in Romania for compensated HCV cirrhosis from November 2015 to November 2017 as the only interferon-free regimen.

We reported the results of a prospective study designed to asses the efficacy and safety of the regimen ombitasvir/paritaprevir/ritonavir+dasabuvir with ribavirin in patients with compensated HCV cirrhosis genotype-1b, with or without experienced pegIFN/RBV.[28, 29, 30].

PATIENTS AND METHODS

Study Patients

We conducted a multicenter prospective study including 90 patients with compensated (Child-Pugh A) HCV cirrhosis treated with Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir and Ribavirin regimen for 12 weeks. Patients were diagnosed by clinical, biological, and FibroMax tests.

Patients 18 years or older were eligible for the therapy if they had HCV genotype-1b infection for at least 6 months prior to screening, an HCV RNA > 15 UI/ml. Cirrhosis had to be documented by liver biopsy (Metavir score >3) or FibroMax (available within 3 months before therapy) and a Child-Pugh Turcotte score 5 or 6.

We included in the treatment-naive patients or those with prior exposure to PegIFN/RBV based therapy.

Decompensated cirrhosis and hepatocarcinoma were exclusion criteria.

Each patient had follow-up visits at four, eight, twelve and 24 weeks after treatment initiation. Biological tests included: hemoglobin, ALT, AST, platelets, total bilirubin, serum albumin, INR. We defined anemia as Hb<12 g/dl in women and Hb< 13 g/dl in men. Cytolysis was defined as values of ALT or AST > at least 2 times upper limit of normal. Hyperbilirubinemia was defined as a level of bilirubin > than 1.2 mg/dl. HCV RNA level in blood was evaluated at baseline, end of treatment (EOT) and 12 weeks after EOT. Undetectable HCV RNA was defined as a viral load <15 UI/ml, assessment by PCR. Virological response (VR) was defined as undetectable HCV RNA at EOT and sustained virologic response (SRV) as undetectable HCV RNA at 12 weeks after EOT.

Study design

All patients received the therapy for 12 weeks. Co-formulated OBV/PTV/r was administered once daily (25/150/100 mg) and DSV 250 mg twice daily. Patients were followed up at four, eight, twelve, and twenty-four weeks after treatment initiation.

Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Efficacy, safety and pharmacokinetic assessments

Plasma samples were collected at screening and at each study visit and HCV RNA levels were determined processed by real-time polymerase chain reaction using the COBAS TaqMan HCV version 2.0 (Roche Molecular Diagnostics). Efficacy was assessed by the percentage of patients with SVR12, defined as an HCV RNA below the lower limit of quantitation of 25 UI/ml and a lower limit of detection of 15 UI/ml after the last dose of study drug.

The primary efficacy endpoint was SVR12 (HCV RNA level <25 UI/ml 12 weeks after the last dose of study drug. The rates of SVR 4 weeks after the end of treatment (SVR4) and rapid virologic response (RVR; HCV RNA level <25 UI/ml) were also determined.

Treatment adverse events were collected from the start of study drug administration until 30 days after the last dose. Laboratory tests and clinical parameters were assessed during the study. The severity of the adverse events and their relationship with treatment were evaluated by the investigator.

Statistical Analysis

Number and percentage of patients in different categories are reported. Categorical variables included in the analysis were: sex, the previous treatment regimen. Continuous variables included were: age, cytolysis, thrombocytopenia, anemia, hyperbilirubinemia.

Results

Baseline Patient Demographics and Characteristics.

We screened patients from November 2015 to August 2016 at five sites in Bucharest. 90 patients were enrolled and received the study drug. The majority of patients had prior treatment experience with PegIFN/RBV. 22 patients were treatment-naive and 68 patients had experienced Peg/IFN/RBV.

Demographics and clinical characteristics are presented in Table 1 and Table 2.

Table 1. Baseline demographic characteristics

Parameter	**Patients** (n=90) n/%
Age (years)	65
Female gender	44 (49)
Male gender	46 (51)
Treatment naïve	22 (24)
Treatment experienced	58 (76)

Table 2. Baseline clinical characteristics

Parameter	**Patients** (n=90) n/%
Cytolysis	64 (71)
Anemia	11 (12)
Hyperbilirubinemia	19 (21)
Thrombocytopenia	38 (42)

Efficacy Outcomes

89-99% of patients completed 12 weeks of treatment with ombitasvir/paritaprevir/ritonavir, dasabuvir and ribavirin. After 12 weeks of treatment, 99% of patients achieved SVR 12 (89/90, 95% CI, 80%-99.9%) (Table 3). One patient died after four weeks of treatment.

Another patient (female, 60 years old, known with diabetes mellitus) gave up ribavirin after four weeks of treatment because of severe anemia (level of hemoglobin was 6 g/dl). We hadn't any virologic failure.

Table 3. Efficacy and safety of DAA regimen

Parameter	**Patients** (n=90) n/%
SVR	89 (99)
Anemia	42 (47)
Death	1 (1)

Improvement in laboratory assessments

At baseline 71 % (64) of patients had cytolysis and 42 % (38) had thrombocytopenia (Table 4).

Only 10% (9) of patients had cytolysis at the end of treatment. Thrombocytopenia was improved at the end of treatment, thus it was seen at only 26% of patients.

Table 4. Characteristics of patients at the end of treatment

Parameter	**Patients** (n=90) n/%
Cytolysis	9 (10)
Anemia	2 (47)
Hyperbilirubinemia	6 (40)
Thrombocytopenia	3 (26)

Safety outcomes

Most of the patients had adverse events and one patient died after four weeks of treatment. Anemia was the most frequent adverse event. At baseline only 12% of patients had anemia, but at the end of treatment the number of patients who developed anemia was increasing up to 47%. Another laboratory abnormality was hyperbilirubinemia. At the beginning of the treatment, only 21% of patients presented a high level of bilirubin. At the end of treatment, hyperbilirubinemia was seen in 40% of patients. One patient developed severe anemia (hemoglobin level was 6 g/dl) after four weeks of treatment and thus ribavirin was stopped.

DISCUSSION

Patients with HCV infection and cirrhosis are at the highest risk for hepatocarcinoma, decompensation and, mortality. In this study treatment with OBV/PTV/r, DSV and RBV had the SVR 12 rate of 99% in HCV genotype-1b infected patients with compensated cirrhosis. Treatment was well tolerated with a low rate of severe AE.

The most side effect among the patients in the study group was anemia in 47% cases. Cytolysis disappeared after four weeks of treatment at 61 % of patients. 89 of 99 patients (99%) had sustained virologic response at twelve weeks. One patient died after four weeks of treatment (1%). The treatment was safe and effective in compensated HCV cirrhosis.

In conclusion the 12 week 3-DAA regimen of OBV/PTV/r, DSV and RBV achieved the SVR 12 rate of 99% experienced and naive treatment patients with compensated cirrhosis HCV genotype-1b.

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SYSTEMATIC REVIEW

Efficacy and tolerability of antibiotic augmentation in schizophrenia spectrum disorders – A systematic literature review

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Abstract: *Background: A large number of augmentation agents have been tried in patients with schizophrenia spectrum disorders, because negative and cognitive symptoms are difficult-to-treat with current pharmacological agents, and because the high percentage of treatment-resistant cases requires new therapeutic solutions. Inflammation is one of the supposed pathophysiological mechanisms in schizophrenia; therefore, antibiotics have been explored as add-on agents. No systematic review or meta-analysis about the efficacy of antibiotics in psychotic disorders has yet been conducted. Objectives: To assess if the use of antibiotics as add-on to current antipsychotic treatment may improve core symptoms of schizophrenia spectrum disorders, global clinical status, overall functionality, and if this augmentation strategy is well tolerated. Methods: A systematic literature review was conducted, including papers published between 1980 and 2018, which have been found in the main electronic databases (PubMed/MEDLINE, CINAHL, NCBI, Embase, Thomson Reuters/Web of Science). The keyword search strategy was formulated using the following paradigm: „schizophrenia spectrum disorders"/ „schizophrenia"/ „schizo-affective disorder"/ „schizophreniform disorder", AND „antibiotics"/ all the names of currently marketed antibiotics classes. This review was registered to PROSPERO database with the protocol number CRD42019119152. Results: Based on reviewing the selected trials regarding the efficacy of antibiotics over core symptoms of schizophrenia, only two agents were detected – minocycline and D-cycloserine. Minocycline as add-on to antipsychotic treatment was associated with mixed results, while regarding the efficacy of d-cycloserine either no significant difference, or superiority to placebo over negative and cognitive symptoms was reported. Minocycline had an overall effect over the clinical impression that was not distinguishable from placebo, although there was one trial supporting the efficacy of this antibiotic. The impact of minocycline over the global functioning when added to antipsychotic in patients with schizophrenia was mixed, with one positive and one negative trial (moderate to high quality designed trials). D-cycloserine had no significant impact over global clinical status or patients' overall functionality. Conclusion: From all the data reviewed and hierarchized resulted that larger trials are needed in order to confirm the efficacy of antibiotics as add-on to antipsychotics over negative and cognitive symptoms of schizophrenia spectrum disorders.*

Keywords: *antibiotics, augmentation strategy, negative symptoms, cognitive symptoms, tolerability, schizophrenia spectrum disorders*

BACKGROUND

Negative and cognitive symptoms are extremely challenging for clinicians who are treating patients with schizophrenia, and these clinical phenomena are important to approach because they are related to functional prognosis, real-world performance and quality of life. If currently marketed antipsychotics can control positive, behavioral and sometimes affective symptoms of schizophrenia, when negative and cognitive symptoms are analysed

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longitudinally, the results are still disappointing [1]. The prevalence of treatment-resistant schizophrenia is estimated to be approximately 30% (range 10-45%) [2] and an important number of patients have „ultra-resistant" schizophrenia, defined as resistance to clozapine [3]. In all these cases, a significant number of psychotic symptoms persist, although several trials of antipsychotics have been conducted for enough time in adequate doses [3]. Treatment-resistant cases require additional therapeutic agents, and a large number of solutions have been formulated without reaching a consensus (except for clozapine, but as previously mentioned, there are cases resistant even to this second-line atypical antipsychotic).

A significant number of symptoms detected in treatment-resistant patients are based on cognitive deficits [4], and the N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis attempts explaining why antipsychotic treatment may attenuate the striatal dopaminergic dysfunction without having an influence over the prefrontal cortex in treatment-resistant cases [5]. Negative symptoms have been demonstrated to explain the highest part of variation in disease severity in treatment-resistant schizophrenia, and these symptoms mediated the effects of verbal fluency dysfunctions and high-level neurological soft signs on disease's severity [6].

Many treatments from a large area of pharmacological, psychotherapeutical and other somatic approaches (e.g., deep brain stimulation) have been investigated for the management of negative and cognitive symptoms of schizophrenia, but there is no first-line therapy yet established for these cases. Atypical antipsychotics used at the lowest dose necessary to control positive symptoms, antidepressants as add-on agents, and cognitive-behavioral therapy have been suggested with limited efficacy in schizophrenia with prominent negative symptoms [7]. Many different pharmacological targets have been suggested for addressing cognitive symptoms in schizophrenia, from D1-agonists to glycine site agonists, and from type A gamma-amynobutyric acid (GABA-A) receptors agonists to alpha7-nicotinic receptors partial agonists, but without significant effects [8-10].

Antibiotics have been explored in clinical practice not only for their anti-microbial properties, but also for their neuroprotective and antiinflammatory effects. As schizophrenia has been associated with increased levels of circulating pro-inflammatory markers and microglial activation in both animal and human studies, researchers have become increasingly interested in the therapeutic potential of antibiotics [11]. Many β-lactams antibiotics have been associated with increased glutamate type 1 transporter (GLT1) expression in animal models, which can lead to a prevention of toxic neuroexcitation by excessive glutamate transmission [12]. Macrolides may confer neuroprotection against ischemic damage following cerebral ischemia without cerebral blood flow impairments [13]. Doxycycline was proven to prevent amyloid-β toxicity both in vivo and in vitro in a Drosophila melanogaster model of Alzheimer disease [14]. Minocycline is a second generation, semi-synthetic tetracycline that has been associated with neuroprotective, anti-inflammatory and anti-apoptotic properties, which also can inhibit proteolysis, angiogenesis and tumour metastatis [15-17]. D-cycloserine is not only an antituberculosis agent, but also a glycine site partial agonist studied for its properties to enhance neuroplasticity in schizophrenia [18].

While many data on the effects of antibiotics over neuroplasticity are derived from animal models, information on the clinical impact of these pharmacological agents in patients diagnosed with schizophrenia spectrum disoders are contradictory [18-22].

The objectives of this systematic literature review are (1) the evaluation of antibiotics efficacy as add-ons in the management of the core symptoms of schizophrenia spectrum disorders, global clinical status, overall functionality, and (2) to verify if this augmentation strategy is well tolerated. These objectives are considered of clinical interest for psychiatrists, but they also may be useful for researchers oriented on finding new, non-dopaminergic-based treatments for psychotic disorders.

METHODS

This systematic review is based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Table 1) [23]. The protocol of this review was registered in the PROSPERO with the protocol number CRD42019119152 [24].

2.1. Outcomes

The primary outcome for this review was the efficacy of any antibiotic in decreasing symptoms severity in schizophrenia spectrum disorders. We considered the impact of these agents over positive, negative, cognitive, behavioral, and/or affective dimensions of psychosis, as determined by validated psychometric scales for this specific pathology.

The secondary outcomes were (a) tolerability of antibiotics, assessed by either subjective reports or validated instruments, (b) general functionality of patients, and (c) global clinical status, assessed by structured instruments.

2.2. Search strategy

A keyword search strategy was formulated using the following paradigm: „schizophrenia spectrum disorders”/„schizophrenia”/„schizo-affective disorder”/„schizophreniform disorder”, AND „antibiotics”/all the names of currently marketed antibiotics classes. Electronic databases searched were Medline (PubMed), Embase, Cumulative Index to Nursing and Allied Health Literature (CINAHL), National Center for Biotechnology Information (NCBI), and Thomson Reuters/Web of Science. Papers included in the references of the analyzed articles were also reviewed, and their results were included if the quality of research corresponded to the pre-defined inclusion and exclusion criteria. We selected clinical trials that were published in any language, between January 1980 and January 2019.

Table 1: PRISMA-P 2015 Checklist [25]

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☒	☐	92-94
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☐	Not applicable
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	94
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	Title page
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	138-150
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☐	Not applicable
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	146-148
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☐	Not applicable
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☐	Not applicable
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	45-89
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☒	☐	135-136, Table 1
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☒	☐	124-133, Table 1
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	124-133
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	104-111
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	138-150

Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	138-150
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	138-150
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	138-150, Table 1
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☒	☐	96-102
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☒	☐	143-147
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☐	☒	
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☐	☒	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☐	☒	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☒	☐	156-164
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☐	☒	
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	52-57

This checklist has been adapted for use with protocol submissions to Systematic Reviews from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic Reviews 2015 4:1

Figure 1: Results of the PRISMA-based search paradigm

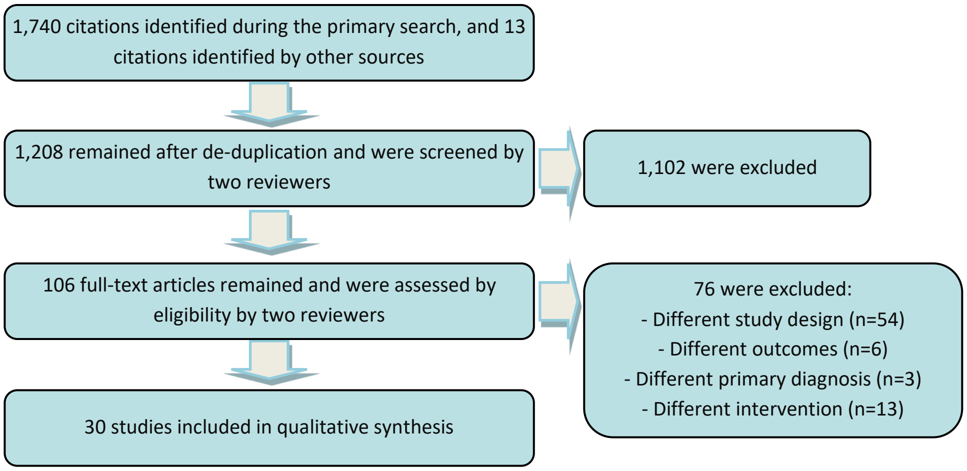
1,740 citations identified during the primary search, and 13 citations identified by other sources
1,208 remained after de-duplication and were screened by two reviewers
1,102 were excluded
106 full-text articles remained and were assessed by eligibility by two reviewers
76 were excluded:
- Different study design (n=54)
- Different outcomes (n=6)
- Different primary diagnosis (n=3)
- Different intervention (n=13)
30 studies included in qualitative synthesis

2.3. Inclusion and exclusion criteria

Studies were eligible for inclusion if they were randomized/non-randomized, open label, single blind or double blind, with or without active comparator, prospective/retrospective trials. These studies could be controlled with placebo, any other active drug, or usual care, and they must have specified diagnosis criteria for the explored clinical entity/entities. All patients included in these trials were over 18 years old at the initial visit, and no superior limit of age was established. Validated instruments for evaluation of the efficacy of the investigational product should be used at baseline and at least at end-point, e.g. Positive and Negative Syndrome Scale or Brief Psychiatric Rating Scale.

Only full-text articles were eligible for analysis, but if abstracts with outcomes corresponding to those defined by us were found, authors of the respective works were contacted to provide full-text of their papers. No limitation about the duration of the follow-up was included as a criterion of trials selection. A more detailed presentation of the inclusion and exclusion criteria is emphasized in Table 2.

Table 2: Inclusion and exclusion criteria

Operational criteria	Inclusion criteria	Exclusion criteria
Population	Minimum age of inclusion in the selected trial was specified as being 18, according to the study protocol. No superior age limit was specified. The main diagnosis was schizophrenia, schizo-affective disorder, schizophreniform disorder, or any combination of these entities. Diagnosis based on standardized criteria (DSM, ICD, or other compatible psychiatric classifications). Patients with resistant forms of schizophrenia were included (defined by resistance to at least 2 antipsychotic agents, each administered for a minimum duration and dosage, currently presenting symptoms of a minimum duration and severity determined by a standardized rating scale, and moderate or worse functional impairment) [15].	Patients with age under 18, except for cases where statistical procedures allowed for a separate evaluation of the adult vs. child population. The presence of severe somatic or psychiatric co-morbidities with significant impact over cognition, disposition, behavior, and overall functionality. The presence of infectious diseases, which require antibiotic therapy, as co-morbid or main diagnoses. Any co-morbidities which can create confusions in the domain of antibiotics efficacy over psychotic symptoms, either organic diseases (e.g., infections), or psychiatric (e.g., disorders induced or related to drugs or general medical conditions). Neurocognitive co-morbities were not allowed.
Intervention	Antibiotics used as add-on to any antipsychotic agent, either typical or atypical. Antibiotics from any class that is currently marketed, with no limitations related to the dosing regimen, adverse events profile, or any pharmacokinetic/pharmacodynamic property. The effects of the intervention over targeted symptoms of schizophrenia spectrum disorders were quantified using at least one validated instrument.	Trials controlled by psychotherapy only. Studies controlled by non-pharmacologic and non-psychotherapy interventions, e.g., deep brain stimulation, electroconvulsive therapy, transcranian magnetic stimulation. Over-the-counter products or dietary supplements as add-on or active comparators. Investigational pharmacologic agents used in phase II or III clinical trials. Administration of more than one antibiotic at a time.
Environment	Hospitals, day care, special care, permanent care, or intermediate care units. Both in-patient and out-patient regimen. A caregiver who is able to report on the patient's evolution under treatment and therapeutic adherence was considered optional.	Correctional environments. Unspecified environment.
Primary and secondary variables	Changes in psychotic symptoms (positive, negative, cognitive, behavioral, and/or dispositional) were evaluated by trained clinicians. Evaluation of the drugs tolerability, and patients overall functionality and global clinical status was also monitored. Studies were allowed to entered the analysis if they were focused on efficacy, tolerability, or both of these outcomes.	All trials using unspecified psychometric instruments, or only clinical observation were excluded.
Study design	Intervention was studied in randomized/non-randomized, open label, single blind, or double blind, with or without active comparator/placebo, prospective/retrospective trials. No limitation about the duration of the treatment was established. Only peer-reviewed studies were included.	Studies with unspecified or insufficiently specified design. Lack of methodologic specifications regarding statistic processing of the primary/secondary variables related to the efficacy. Case reports, case series. Animal model studies. Systematic literature reviews, meta-analyses.
Language	English, French, Spanish, Russian	Any other language, except for those mentioned in the „inclusion criteria”

2.4. Data extraction and analysis

Screening was performed by two independent reviewers, and the resulted material was filtered using inclusion and exclusion criteria mentioned above. Data regarding the main outcomes, the secondary outcomes, and other relevant information about the studies were extracted into the table 3. Data extraction and quality assessment of the studies were done independently by two reviewers (OV and either VAV or DV), and if any disagreement between the two appeared and could not be resolved by consensus, the third reviewer has been consulted.

Consensus was obtained regarding the methodological quality for the included studies after each study was independently rated using the Cochrane Handbook for Systematic Reviews of Interventions - Assessing risk of bias in included studies [26]. Each study was rated as having low, moderate or high risk of bias, based on the (1) randomization procedure, (2) blinding and treatment allocation, (3) use of placebo or an active comparator, (4) methods used for evaluation of outcomes, and (5) dimension of the study group.

All included trials were reviewed critically by authors of the current article, with consideration to the bias risk. A meta-analysis could not be performed because of the heterogeneity of the methods used within the selected trials.

RESULTS

A number of 1753 citations returned after the primary search, and only 1208 remained after de-duplication. After filtering-out articles non-compatible with our predefined inclusion/exclusion criteria, and safter excluding papers for which full text could not be found or provided by authors, a number of 30 trials resulted (Figure1).

3.1. Methodological quality assessment

Among the 30 articles included in this review, a majority were of moderate quality (n=15), while the high and low quality were almost equally represented (7 and 8, respectively). Most of these trials were randomized, placebo-controlled (n=28), one open-label, and one trial had a bi-phasic design, with a single-blind, followed by a double-blind period of treatment. Only 4 trials had a sample size over 100, while 19 trials included below 50 subjects, which reduced the power of the analysis for the poor-populated trials.

Frequently encountered problems were lack of information about concealment of the randomization, and about the intent-to-treat (ITT) vs. per protocol analysis. The mean percentage of agreement between the two reviewers for each trial was 90.7% (p<0.0001) for the validity criteria.

3.2. Evaluation of primary outcome: the efficacy of antibiotics in schizophrenia spectrum disorders

The only active interventions detected by our search were D-cycloserine (n=18 trials, most of them of moderate quality) and minocycline (n=10 trials, most of them of moderate and low quality).

The efficacy of minocycline as add-on to antipsychotic treatment was investigated in two large double-blind, placebo-controlled trials (n=200 and 207, respectively) and the results were negative [27, 28]. No improvement of negative (on medium and long term), cognitive and overall psychotic symptoms (on medium term) was detected by these two studies. A small trial (n=73 subjects) evaluated the impact of minocycline as add-on to clozapine over avolition and concluded that although higher plasma levels of clozapine were detected after minocycline initiation, there was no evidence that improvement in negative symptoms can be correlated with higher level of plasma antipsychotic [29].

Another small trial (n=52) concluded that minocycline did not improve significantly psychosis factor and overall BPRS (Brief Psychiatric Rating Scale) score, neither had it a significant impact over cognitive function, except for working memory [31]. The same trial reported a significant improvement in the BPRS anxiety/depression factor in the minocycline-treated patients [31].

Regarding the positive effects of minocycline, a small trial (n=24) detected significant improvements in patients treated with this drug over the positive and negative symptoms, compared to placebo, and a protective effect of this antibiotic over the gray matter loss in fronto-temporal areas involved in the pathophysiology of schizophrenia during 12-month of active treatment [32].

Another short-term trial (8 weeks, n=43 subjects) found that SANS (Scale for the Assessment of Negative Symptoms) total score decreased significantly compared to placebo, and the negative sub-scale of PANSS (Positive and Negative Syndrome Scale) also decreased, but not significantly compared to placebo [33].

A study that evaluated 92 patients with schizophrenia treated with risperidone who received minocycline as add-on for 16 weeks reported greater improvements on SANS total scores and PANSS negative subscale versus placebo [34]. This study observed no difference between groups in cognitive performances, except for the attentional domain [34].

Table 3: Qualitative analysis of the trials selected

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
Minocycline	Weiser M, Levi L, Burshtein S, et al. [27]	Efficacy of minocycline over positive, negative and cognitive symptoms of schizophrenia	16-week double-blind, placebo-controlled RCT; minocycline (200 mg/day) /pramipexole /acetyl- salicilic acid/ placebo as add-on to antipsychotic treatment	N=200 patients with schizophrenia or schizo-affective disorder	Primary outcome measurements - PANSS total score at the end of the trial. Secondary outcomes- positive, negative and general psychopathology scored by PANSS; CGI-S, CGI-I; BACS; rates of drop-outs	No significant differences between minocycline and placebo for PANSS total score, PANSS subscales, CGI, and BACS were detected. No significant difference between groups in the frequency of adverse events.	High
Minocycline	Deakin B, Suckling J, Barnes TRE, et al. [28]	Efficacy of minocycline over negative symptoms in schizophrenia with recent onset	12-month double-blind, placebo-controlled RCT; minocycline (200 mg/day for 2 weeks, then 300 mg/day for the remainder of 12 months) vs. placebo plus continuing treatment	N=207 patients with schizophrenia spectrum disorders that begun in the last 5 years	Negative symptom subscale score of the PANSS; prefrontal grey matter volume, dorsolateral prefrontal cortex activation during working memory task, and plasma concentration of IL6	No effect of minocycline was observed over ratings of negative symptoms. Biomarker outcomes did not change over time, and were not affected by minocycline. No difference in the appearence of serious adverse events was detected between groups.	High
Minocycline	Liu F, Xie L, Zhang B, et al. [30]	Effects of adjunctive minocycline over body metabolism in risperidone- treated patients with schizophrenia	16-week placebo-controlled, double-blind crossover RCT	N=63 patients diagnosed with schizophrenia,treated with stable dose of risperidone for ≥4 weeks	Body weight, BMI, waist circumference, fasting insulin, glucose and lipids serum levels	No difference between groups was detected ($P's>0.3$)	Moderate
Minocycline	Wehring HJ, Elsobky T, McEvoy JP, et al. [29]	Pharmacokinetic impact of minocycline over clozapine and correlation between modified levels of clozapine /norclozapine over negative symptoms in schizophrenia	10-week placebo-controlled RCT, minocycline vs. placebo as add-on to clozapine	N=73, DSM IV-TR-based diagnoses of schizophenia or schizo-affective disorder, currently treated with clozapine ≥200 mg/day and blood level of clozapine ≥350 ng/ml.	Serial plasma clozapine, norclozapine and total clozapine levels, SANS	Clozapine plasma levels are increased after the initiation of minocycline (21% increment to baseline) compared to placebo. However, smoking patterns were not analysed in relation to this plasmatic difference in concentration. Avolition improvement on SANS was not explained by changes in clozapine blood levels. There is no evidence that improvement in any symptom domain is due to higher clozapine levels.	Moderate
Minocycline	Kelly DL, Sullivan KM, McEvoy JP, et al. [31]	Efficacy of minocycline as adjunctive treatment to clozapine in chronic psychoses	10-week double-blind, placebo-controlled RCT; 100 mg p.o BID minocycline vs. placebo.	N=52 patients with schizophrenia and schizo-affective disorder with persistent positive symptoms	Positive and cognitive symptoms were primary outcomes. Avolition, anxiety/depression, and negative symptoms were secondary outcomes.	BPRS- psychosis factor and BPRS- total score were not changed significantly during treatment. Still, a change in total BPRS score of more than or equal to 30% was observed more frequently in minocycline treated patients (p=0.044). Global cognitive function (MATRICS) did not differ between groups, but significant	Moderate

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
						improvement in working memory favored minocycline. SANS total score did not differ, but minocycline improved significantly working memory (p=0.023). Significant improvement in the BPRS anxiety/depression factor was observed with minocycline (p=0.028). The overall tolerability of minocycline was good.	
Minocycline	Chaves C, Marque CR, Maia-de-Oliveira JP, et al. [32]	Effects of minocycline on brain morphology and cerebral perfusion in patients with recent-onset schizophrenia	12-month double-blind, placebo-controlled RCT of minocycline as add-on treatment (200 mg/day minocycline vs. placebo)	N=24 outpatients with recent-onset schizophrenia	SPECT and MRI were performed after 12 months of treatment for detection of morphologic changes.	Minocycline may have protective effect against gray matter loss and modulate fronto-temporal areas involved in the pathophysiology of schizophrenia. Significant lower gray matter volumes were observed in the midposterior cingulate cortex and in the precentral gyrus in placebo group, in comparison with minocycline group. Positive and negative symptoms were significantly decreased versus placebo.	Moderate
Minocycline	Ghanizadeh A, Dehbozorgi S, Omrani Sigaroodi M, Rezaei Z [33]	Effectiveness of add-on minocycline in schizophrenia	8-week placebo-controlled RCT; minocycline 200 mg/day + risperidone vs. placebo + risperidone.	N=43 patients diagnosed with DSM IV schizophrenia	Schizophrenia negative symptoms severity as primary outcome	SANS total score decreased significantly in the minocycline group at week 8, compared with placebo group. The decline of PANSS Negative score was more in the active treatment group than in the placebo group (not statistically signifi-cant). Tolerability of minocycline was good.	Moderate
Minocycline	Liu F, Guo X, Wu R, et al. [34]	Efficacy and safety of minocycline for the treatment of negative symptoms and cognitive impairments in patients with schizophrenia	16-week double blind, placebo-controlled RCT; minocycline 200 mg/day vs. placebo.	N=92 with early stage schizophrenia treated with risperidone	The primary outcome was represented by negative symptoms of schizophrenia, evaluated by SANS scores. Secondary outcomes were scores of response rate on SANS, PANSS, CGI, cognitive tests	Patients receiving minocycline had greater improvements on SANS total scores and PANSS negative subscale scores vs. placebo. Rates of treatment response in the minocycline group were significantly higher than those in the placebo group after 16 weeks of treatment. Cognitive performances were not improved significantly by minocycline, except for the attentional domain.	High
Minocycline	Khodaie-Ardakani MR, Mirshafiee O, Farokhnia M, et al. [35]	Efficacy and tolerability of minocycline add-on to risperidone in treatment of negative	8-week double-blind, placebo-controlled RCT; minocycline (titrated up to 200 mg/day) vs. placebo as add-on to	N=40 outpatients with chronic schizophrenia stabilized on risperidone for at least 8 weeks prior to baseline	Primary outcomes- negative symptoms evaluated by PANSS	Significant difference between groups on negative scale of PANSS favoured minocycline at the end of the trial (p<0.001). PANSS total score, positive score and general psychopathology score were also improved by minocycline compared to placebo.	Moderate

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
		symptoms in chronic schizophrenia	risperidone (maximum 6 mg/day)			No difference between groups regarding the severity of adverse effects was observed.	
Minocycline	Chaundhry IB, Hallak J, Husain N, et al. [36]	Efficacy of minocycline as add-on to treatment as usual over negative symptoms in early psychosis.	Placebo-controlled RCT, one year duration, minocycline (titrated up to 200 mg/day) vs. placebo.	N=144 patients with early psychosis	Primary outcomes- negative and positive symptoms evaluated by PANSS. Secondary outcomes- GAF, CGI, AIMS, neuropsychological outcome measures.	Minocycline improved negative symptoms (p<0.001) in the ITT population. No other outcomes were influenced significantly by minocycline. AIMS scores tend to improve more in the placebo group.	High
Minocycline	Levkovitz Y, Mendlovich S, Riwkes S, et al. [37]	Efficacy of minocycline as add-on treatment for alleviating negative and cognitive symptoms in early-phase schizophrenia	Double-blind, placebo-controlled RCT, 6 months duration, minocycline 200 mg/day vs. placebo + atypical antipsychotic (200-600 mg/day chlorpromazine-equivalent doses).	N=54 early-phase schizophrenia patients (DSM IV) - age 18-35 years	Primary outcome- negative symptoms severity evaluated by SANS. Cognitive measures evaluated by CANTAB. Functional measures and clinical evaluations by GAF, SOFAS, MCAS, CGI.	A significant time effect for SANS total scores was detected in the minocycline group, negative symptoms improved since week 14. CGI improved better with minocycline, as did cognitive functioning, mainly executive functions (working memory, cognitive shifting, and cognitive planning). GAF and SOFAS showed a better improvement with minocycline. Also, minocycline had a favourable effect in preventing weight gain during treatment.	Moderate
Minocycline	Miyaoka T, Yasukawa R, Yasuda H, et al. [38]	Efficacy and tolerability of minocycline as adjunct to antipsychotic medication in schizophrenia	Open-label, pilot study, minocycline 150 mg/day 4 weeks as adjunct to current antipsychotic medication	N=22 patients with schizophrenia	Psychotic symptoms severity determined by PANSS	All scales of PANSS improved during minocycline treatment. All patients tolerated the full dose during the study.	Low
D-cycloserine	Takiguchi K, Uezato A, Itasaka M, et al. [39]	Effects of d-cycloserine over positive, negative and cognitive symptoms of schizophrenia. To investigate if white matter integrity influences response to d-cycloserine.	6-week placebo-controlled, double-blind, cross-over design RCT; d-cycloserine (50 mg) vs. placebo as add-on to their current antipsychotic treatment	N=41 patients, in- and out- patients with DSM IV diagnostic criteria for schizophrenia	PANSS and SANS scores, BACS, EQS- interpersonal, intrapersonal and situational domains; correlations between d-cycloserine treatment and MR-DTI white matter integrity.	No improvement of positive, negative, or cognitive symptoms of schizophrenia. The better treatment effect of d-cycloserine on BACS was observed when fractional anisotropy was higher in sagittal striatum, cingulum, fornix stria terminalis, genu of corpus callosum, and external capsule, and the better treatment effect on PANSS- general psychopathology score was observed when fractional anisotropy of the splenium of corpus callosum was higher. The better treatment effect on PANSS- general psychopathology score and SANS-IV was observed when fractional anisotropy was lower in the posterior thalamic radiation.	Moderate

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
D-cycloserine	Forsyth JK, Bachman P, Mathalon DH, et al. [40]	Exploration of NMDA-receptor signaling on working memory and experience-dependent plasticity	Double-blind, placebo-controlled, between-group design RCT, 100 mg d-cycloserine vs. placebo; subjects were tested at one day after d-cycloserine or placebo administration	N=45 patients diagnosed with schizophrenia	Neuroplasticity changes evaluated by cognitive tests and EEG with high-frequency visual stimulation	D-cycloserine did not affect plasticity (similar neural potentiation in both active and placebo group), similar information integration and weather prediction tasks performances. Still, patients who received active drug and who were actively involved in memory task had superior 2-back performace compared to placebo-receiving patients. NMDA-receptor-signaling was not translated into synaptic plasticity change in schizophrenia.	Moderate
D-cycloserine	Cain CK, McCue M, Bello I, et al. [41]	Efficacy of d-cycloserine + cognitive remediation (CR) program in improving memory of patients with schizophrenia	8-week placebo-controlled RCT; 3-5 times per week CR program + once-weekly adjunctive treatment with 50 mg d-cycloserine or placebo administered before the first session within each week.	N=36 outpatients, stable medicated adults	Primary outcomes were performances on an auditory discrimination task, MATRICS battery composite scores, SANS total scores	Performance on the practiced auditory discrimination task significantly improved in the d-cycloserine group compared to placebo. Active treatment was associated with significantly greater negative symptom improvement for subjects symptomatic at baseline. Improvement on MATRICS was observed only in the placebo group.	Moderate
D-cycloserine	Gottlieb JD, Cather C, Shanahan M, et al. [42]	Efficacy of d-cycloserine as add-on to CBT for the management of delusions in schizophrenia	Double-blind, cross-over, placebo controlled RCT, single oral dose of d-cycloserine 50 mg vs. placebo, one hour post-drug administration patients received 2 CBT sessions focused on challenging patients' paranoid appraisals, with 7-day and 14-day re-evaluations	N=21 outpatients diagnosed with schizophrenia or schizo-affective disorder and moderately severe delusions	Delusional severity was assessed using SAPS; alternative explanations generated on the ABA; PSYRATS score	No significant effect of d-cycloserine on delusional distress or severity has been reported.	Moderate
D-cycloserine	Goff DC, Cather C, Gottlieb JD, et al. [43]	Investigation of d-cycloserine over negative symptoms and cognition in schizophrenia	8-week double-blind, parallel-group RCT; add-on of d-cycloserine 50 mg or placebo administered once-weekly.	N=50 adult outpatients treated with any antipsychotic except clozapine	Primary outcomes were change from baseline to week 8 on SANS total score and on a composite cognitive score.	D-cycloserine significantly improved SANS total scores compared to placebo at week 8. Cognitive performance did not improve with d-cycloserine at week 8. Delayed thematic recall was significantly improved with the first dose of d-cycloserine vs. placebo, but not immediate thematic recall and item recall.	High

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
D-cycloserine	Buchanan RW, Javitt DC, Marder SR, et al. [44]	Efficacy of glycine or d-cycloserine in patients with schizophrenia with moderate to severe negative symptoms and cognitive impairments.	16-week double-blind, double-dummy, parallel-group RCT of adjunctive glycine, D-cycloserine, or placebo	N=157 inpatients and outpatients diagnosed with DSM-IV schizophrenia or schizo-affective disorder and retrospective and prospective criteria for moderate to severe negative symptoms without marked positive, depressive, or extra-pyramidal symptoms	The primary outcome- average rate of change of SANS total scores and change in the average cognitive domain z scores	No significant differences in change of the SANS total score between glycine and placebo, or d-cycloserine and placebo. No significant differences between glycine and placebo or d-cycloserine and placebo were recorded on the average cognition z score.	High
D-cycloserine	Yurgelun-Todd DA, Coyle JT, Gruber SA, et al. [45]	Examination of patterns of cortical activation underlying d-cycloserine's therapeutic efficacy in patients with schizophrenia	2-week single-blinded, placebo lead-in phase RCT; after placebo phase, patients received 50 mg/day d-cycloserine or placebo in a double-blind manner for 8 weeks.	N=12 out- and in-patients with DSM-IV criteria for schizophrenia on stable dose of antipsychotic for at least 4 months and presenting prominent negative symptoms	fMRI patterns of cortical activation; SANS for severity of negative symptoms	Patients receiving d-cycloserine had a significant increase in temporal lobe activation, associated with reduction in negative symptoms.	Low
D-cycloserine	Goff DC, Herz L, Posever T, et al. [46]	Efficacy of d-cycloserine as augmenting agent for conventional antipsychotics in decreasing negative and cognitive symptoms of schizophrenia	Double-blind, parallel-blind RCT, 50 mg/day d-cycloserine or placebo for 6 months	N=55 patients with schizophrenia with prominent negative symptoms	Primary outcomes- negative symptoms and cognitive impairments evaluated by PANSS, SANS, cognitive battery	No difference between groups was detected on any primary outcome at 8 or 24 weeks (PANSS, SANS, cognitive tests).	High
D-cycloserine	Duncan EJ, Szilagyi S, Schwartz MP, et al. [47]	Efficacy of d-cycloserine over negative and cognitive symptoms of schizophrenia	Double-blind, parallel-group RCT, 50 mg QD d-cycloserine or placebo for 4 weeks	N=22 male patients with schizophrenia displaying prominent negative symptoms stabilized on typical neuroleptics	Symptoms severity (SANS, BPRS, Abrams and Taylor rating scale). Cognition was assessed by the Sternberg Memory Test and the Continuous Performance Test.	No significant differences between the d-cycloserine and placebo group on any symptom rating	Low
D-cycloserine	Evins AE, Amico E, Posever TA, et al. [48]	Effects of d-cycloserine when added to risperidone	Placebo-controlled RCT, 2 weeks of placebo and 4 doses of d-cycloserine	N=10 patients with schizophrenia treated with risperidone	Negative symptoms severity	D-cycloserine (50 mg/day) significantly reduced negative symptoms.	Low

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
		on negative symptoms of schizophrenia				Ratings of depression, extrapyramidal side effects, and cognitive function were unchanged.	
D-cycloserine	Heresco-Levy U, Ermilov M, Shimoni J, et al. [49]	To investigate the clinical effects of d-cycloserine when added to conventional antipsychotics, olanzapine, or risperidone for treatment-resistant schizophrenia	Double-blind, placebo-controlled, crossover study, 2 random-order 6-week treatment arms (d-cycloserine, 50 mg/day vs. placebo), separated by 2-week adjuvant treatment washout.	N=24 patients diagnosed with treatment-resistant schizophrenia, and they were on stable doses of medication for at least 3 months before study entry	Psychotic symptoms evaluated by PANSS, depression severity evaluated by HAMD, and adverse events quantified by SAS and AIMS	Significant reduction in negative symptoms in patients treated with d-cycloserine. No difference between patients treated with conventional neuroleptics or atypical antipsychotics was observed.	Moderate
D-cycloserine	Heresco-Levy U, Javitt DC, Ermilov M, et al. [50]	Efficacy of d-cycloserine over negative symptoms in schizophrenia	16-week, double-blind, placebo-controlled, adjuvant treatment RCT with 50 mg/day d-cycloserine	N=9 treatment-resistant chronic schizophrenia-diagnosed patients	Psychotic symptoms evaluated by PANSS, depression severity evaluated by HAMD, and adverse events quantified by SAS and SAS and AIMS	Symptoms changes between groups were not significant, except for a significant reduction in negative symptoms which was registered during treatment with d-cycloserine but not placebo. Greater reductions were registered in patients with lower baseline serum glycine levels. No side effects were registered.	Low
D-cycloserine	van Berckel BN, Evenblij CN, van Loon BJ, et al. [51]	Effects of d-cycloserine as add-on to typical antipsychotics in reducing negative symptoms in schizophrenia	Double-blind, parallel group, placebo-controlled RCT; 50 mg d-cycloserine BID or placebo for 8 weeks.	N=26 patients with DSM-IV schizophrenia	PANSS, CGI, ESRS	D-cycloserine slightly worsened positive symptoms and general psychopathology compared to placebo. No significant change in negative symptoms was detected or extrapyramidal symptoms. Tolerability of d-cycloserine was good.	Moderate
D-cycloserine	Goff DC, Henderson DC, Evins AE, Amico E [52]	Effects of d-cycloserine over negative symptoms in schizophrenia	6-week RCT, d-cycloserine 50 mg/day or placebo, crossover design separated by 1 week placebo washout for a total of 13 weeks	N=17 outpatients diagnosed with schizophrenia (DSM-IV criteria) treated with clozapine	PANSS, SANS, GAF, HAMD, AIMS, SAS, BAS	D-cycloserine significantly worsened negative symptoms compared to placebo (increased SANS scores and negative subscale of PANSS)	Moderate
D-cycloserine	Goff DC, Tsai G, Levitt J, et al. [53]	Efficacy of d-cycloserine over negative symptoms and cognitive function when added to conventional neuroleptics	8-week double-blind RCT, d-cycloserine 50 mg/day vs. placebo, added to their conventional neuroleptic	N=47 patients with schizophrenia meeting criteria for deficit syndrome	SANS total score	The mean reduction in negative symptoms with d-cycloserine was significantly greater than with placebo (SANS scores). No differences were found in performance on any cognitive test between groups or in changes in any other clinical measure.	Moderate

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
D-cycloserine	Goff DC, Tsai G, Manoach DS, et al. [54]	Efficacy of d-cycloserine as add-on over negative symptoms in patients treated with clozapine	2-week placebo-controlled RCT; 5, 15, 20, 50 and 250 mg/day d-cycloserine	N=10 outpatients with schizophrenia receiving clozapine who presented criteria for primary deficit syndrome	SANS	Significant dose effect of d-cycloserine on SANS scores, 50 mg/day produced a mean increase of 21% in SANS score. No improvement was detected on clozapine-treated patients with negative symptoms.	Low
D-cycloserine	Rosse RB, Fay-McCarthy M, Kendrick K, et al. [55]	Efficacy of d-cycloserine as add-on to molindone in schizophrenia	4-week placebo-controlled, double-blind, parallel-group RCT, 10 or 30 mg/day d-cycloserine as adjuvant to molindone (150 mg QD)	N=13 patients diagnosed with schizophrenia	BPRS, SANS, CGI	Neither dose of d-cycloserine had adjuvant therapeutic efficacy, although the tolerability of the d-cycloserine was good.	Low
D-cycloserine	Goff DC, Tsai G, Manoach DS, Coyle JT [56]	Efficacy of d-cycloserine over negative symptoms of schizophrenia	2-week trials of placebo and four doses of d-cycloserine; 5, 15, 50, 250 mg/day d-cycloserine	N=9 outpatients with schizophrenia (DSM III R criteria) treated with stable doses of conventional antipsychotics for at least 4 months prior to inclusion	SANS, Negative symptoms subscale of BPRS, SAS, AIMS, GAF, Sternberg's Item Recognition Paradigm	D-cycloserine at 50 mg/day produced significant reduction in negative symptoms and significantly improved reaction time (as a measure of executive dysfunction)	Low

RCT = randomized controlled trial; MR-DTI = Magnetic resonance - diffusion tensor imaging; PANSS = Positive and Negative Syndrome Scale; SANS = Scale for the Assessment of Negative Symptoms; EQS = Emotional Intelligence Scale; BMI = Body Mass Index; BPRS = Brief Psychiatric Rating Scale; ITT = Intent to treat analysis; GAF = Global Assessment of Functionality Scale; CBT = Cognitive Behavioral Therapy; SAPS = Scale for the Assessment of Positive Symptoms; ABA = Alternative Beliefs Assessment; PSYRATS = Psychotic Symptom Rating Scale; CANTAB = Cambridge Neuropsychological Test Automated Battery; SOFAS = Social and Occupational Functioning Assessment Scale; MCAS = Multinomah Community Ability Scale; HAMD = Hamilton Depression Rating Scale; SAS = Simpson-Angus Rating Scale; AIMS = Abnormal Involuntary Movement Scale; ESRS = Extrapyramidal Symptoms Rating Scale; BAS = Barnes Akathisia Scale; CGI-S = Clinical Global Impressions- Severity; CGI-I = Clinical Global Impressions- Improvements; BACS = Brief Assessment of Cognition in Schizophrenia

Another 8-week trial (n=40) confirmed significant differences between groups in favour of minocycline regarding the negative symptoms evaluated by PANSS, while positive and general psychopathology scores were non-significantly improved [35].

A large, long-term trial (n=144, 12 months) reported that minocycline improved negative symptoms in the ITT population (p<0.001) [36]. The efficacy of minocycline over negative symptoms was supported also by a study (n=54) which included early-phase schizophrenia patients, with difference between groups appearing at week 14 [37]. An open-label study confirmed a positive effect of minocycline over all scales of PANSS after 4 weeks [38].

D-cycloserine was associated with lack of efficacy over positive, negative and cognitive psychotic symptoms, in a small trial (n=41), but some interesting correlations were reported between MR-DTI (Magnetic resonance - diffusion tensor imaging) and score evolution of general and cognitive symptoms, i.e. fractional anisotropy in certain thalamic regions (where assymetric alterations in the white matter integrity were demonstrated) could be improved by this antibiotic and it may be a mediator of the therapeutic response [39].

No significant effects were observed in patients treated with d-cycloserine over neuroplasticity and cognitive performances, evaluated by cognitive tests and EEG with high-frequency visual stimulation, in a small trial (n=45) [40]. In a crossover design study, no significant effect of d-cycloserine on delusional distress or severity has been reported [42].

Data from a large trial (n=157) found no difference between d-cycloserine and placebo over the severity of cognitive and negative symptoms [44]. No significant differences were found between d-cycloserine and placebo in two trials, which included patients with prominent negative symptoms in the domains of negative and cognitive manifestations [46, 47]. D-cycloserine slightly worsened positive symptoms and general psychopathology compared to placebo, and it did not significantly change negative symptoms in an 8-week placebo-controlled trial (n=26) [51]. D-cycloserine significantly worsened negative symptoms compared to placebo in a 13-week trial (n=17) [52]. When added to clozapine, d-cycloserine worsened negative symptoms dose-proportionally in a small study (n=10) [54]. D-cycloserine added to molindone did not resulted in improvements of general psychopathology or negative symptoms (n=13) [55].

Regarding the positive results of d-cycloserine, this drug was significantly superior to placebo on reducing negative symptoms compared to placebo (n=36, 8-week trial) [41]. D-cycloserine significantly improved SANS total scores after 8 weeks in small trial with outpatients, but no effect was observed over cognitive functions (n=50) [43]. A small trial (n=12) found that d-cycloserine increased significantly temporal lobe activation, a phenomenon correlated with negative symptoms [45].

Table 4: Efficacy of antibiotics over symptom severity in schizophrenia spectrum disorder

Treatment	Clinical trials	GRADE Recommendations
Minocycline	[27-29,31-38]	Positive symptoms: ? Negative symptoms: D+ Cognitive symptoms: D+ Behavioral symptoms: ? Affective symptoms: D Overall symptoms: D+
D-cycloserine	[39-45,48,50-56]	Positive symptoms: ? Negative symptoms: D+ (but not as add-on to clozapine) Cognitive symptoms: ? Behavioral symptoms: ? Affective symptoms: ? Overall symptoms:?

GRADE recommendations for the level of evidence [57]: A=high quality= future research are very unlikely to change the validity of these recommendations; B= moderate quality= future research is likely to have an important impact on the appreciation of the recommendations; C=low quality= it is very likely that future research will have an important impact over the confidence in the estimate of effect and is likely to change the recommendations; D= very low quality= any estimate of the effect is very uncertain. Intermediate steps (+ or -) represent nuances of the recommedantions based on the quality of trials and tolerability of antidepressant agents, as resulted from these trials.

Another small trial (n=10) signaled reduced negative symptoms with d-cycloserine, but no effect of the same drug over cognitive function [48]. In a trial with treatment-resistant schizophrenia-diagnosed patients, adding d-cycloserine to current antipsychotic agent has lead to significant reduction in negative symptoms, without differences between patients treated with atypical or typical antipsychotics [49].

A small trial (n=9) reported significant improvements in negative symptoms versus placebo, especially in patients with lower baseline serum glycine levels [50]. A significant reduction in negative scores (evaluated by SANS) was observed in an 8-week double blind RCT (n=47), but no effect was detected over cognitive symptoms [53]. When added to conventional antipsychotics, D-cycloserine 50 mg/day produced significant reduction in negative symptoms and improved executive dysfunction in patients with schizophrenia (n=9) [56].

Table 5: Tolerability of antibiotics in patients diagnosed with schizophrenia spectrum disorders

Treatment	Clinical trials	GRADE Recommendations
Minocycline	[27, 28, 31-38]	Overall adverse events: B Extrapyramidal symptoms: B Body weight, waist circumference, lipid and glucose metabolism: B-
D-cycloserine	[44, 48-52, 55, 56]	Overall adverse events: B- Extrapyramidal symptoms: B- Body weight, waist circumference, lipid and glucose metabolism: ?

Table 6: Effect of antibiotics over global clinical status in patients diagnosed with schizophrenia spectrum disorders

Treatment	Clinical trials	GRADE Recommendations
Minocycline	[27, 34, 36, 37]	D-
D-cycloserine	[51, 52, 55, 56]	D-

Table 7: Effect of antibiotics over global functionality in patients diagnosed with schizophrenia spectrum disorders

Treatment	Clinical trials	GRADE Recommendations
Minocycline	[36, 37]	D
D-cycloserine	[51, 52, 55, 56]	D-

3.3. Evaluation of antibiotics tolerability in the studied population

Tolerability of minocycline was evaluated in a number of nine trials of moderate quality, most of them being double blind, placebo-controlled, that included 825 patients, and had variable duration (between 4 weeks and 12 months). Most of these trials included small groups, but there were also 3 large-group (over 140 patients each) studies [27, 28, 36].

No significant differences in the rate of adverse events between minocycline- and placebo-treated patients were reported [27, 28, 31, 32, 35, 36, 38].

No difference between minocycline and placebo was detected in a study focused on tolerability in the domain of body weight, waist circumference, fasting insulin, glucose and lipid serum levels [30]. Minocycline had a favourable effect in preventing weight gain during treatment in a trial with early-phase schizophrenia patients (n=54) [37].

Tolerability of d-cycloserine was evaluated systematically in 6 trials, of low-to-moderate quality, that included 99 patients, with a duration between 2 and 16 weeks [44, 48-51, 55]. All these trials reported a good tolerability of d-cycloserine, and the main adverse events monitored were extrapyramidal symptoms. However, none of the trials, which included scales for extrapyramidal symptoms, reported any significant changes during d-cycloserine administration [49-52, 56].

3.4. Evaluation of general functionality and global clinical status

Minocycline improved the global clinical impression in only one study (n=54) [37], while in other three studies (with a total of 436 subjects) it did not distinguished itself from placebo [27, 34, 36]. Trials included in this analysis were of high quality.

The global functioning was improved by minocycline more than by placebo in one trial (n=54) [37], but did not differ from placebo in another trial (n=144) [36]. The quality of these trials was high to moderate.

D-cycloserine had no significant impact over global clinical status or overall functionality, but data were derived from only 2 trials for each outcome, and they included low number of participants (39 and 26, respectively) and were of moderate to low quality [51, 52, 55, 56].

CONCLUSIONS

Based on reviewing the selected trials regarding the efficacy of antibiotics as add-on to antipsychotic treatment over core symptoms of schizophrenia, minocycline (n=10 trials, N=950 participants) was associated with mixed results regarding its efficacy, both positive [32-38] and negative [27-29, 31]. The large majority of these studies were of moderate and low quality, which further complicates the evaluation of their conclusions. Most of the data derived from these trials did not support a significant efficacy of minocycline over negative, cognitive, and overall psychotic symptoms, when it was added to various antipsychotic agents, clozapine included [27-29]. Other trials reported isolated efficacy of minocycline superior to placebo over anxiety/depression factor of BPRS, working memory, or significant improvement in negative and/or positive symptoms [31-38]. Non-significant superior efficacy of minocycline over placebo was also reported over positive and general psychopathology [35]. A definite conclusion about the efficacy of minocycline can not be formulated based on these data, and the GRADE recommendation could not be over the D+ level regarding the positive, negative, and overall psychotic symptoms, as well as D level for affective symptoms of schizophrenia (table 4).

D-cycloserine was investigated in moderate quality trials (n=18, N=626 participants) focused on its efficacy over symptoms of schizophrenia, when used as add-on to antipsychotics. No significant difference between d-cycloserine and placebo was observed in trials focused on negative and cognitive symptoms of schizophrenia [39-56]. A trend to the worsening of positive symptoms by d-cycloserine was also reported, as well as a trend to aggravation of negative symptoms, especially when added to clozapine [51, 52, 54]. D-cycloserine was also proven superior to placebo over negative symptoms [41, 43, 45, 48-50, 53, 56] and cognitive function [56], without difference between atypical and typical antipsychotics as main treatment [49]. Based on these contradictory data, d-cycloserine could be rated as D+ recommendation for the treatment of negative symptoms of schizophrenia, when added to typical or atypical antipsychotics, but not to clozapine. Thorough monitorisation of patients undergoing d-cycloserine augmentation should be initiated because there is a risk of worsening positive symptoms or negative symptoms (the last case has been reported in clozapine-treated patients).

After reviewing the trials focused on tolerability of antibiotics in patients diagnosed with schizophrenia as add-on to antipsychotics (n=9, N=825 participants), the data collected was considered as being of moderate quality (table 5). Almost all of them reported no difference in the rate of adverse events between minocycline and placebo-treated patients, and two studies showed a favorable effect of this antibiotic over metabolic status of the patients undergoing antipsychotic treatment [27, 28, 30-32, 35-38]. These data support a level B- for the tolerability of minocycline as add-on to antipsychotic regarding the metabolic profile, body weight and waist circumference, and level B for the overall adverse events (extrapyramidal symptoms included).

Tolerability of d-cycloserine was also evaluated as good in moderate to low quality trials (n=6 studies, N=99 participants), and the main adverse events were extrapyramidal symptoms [44, 48-51, 55]. These data support a level B- for the tolerability of d-cycloserine as add-on to antipsychotic regarding the overall adverse events (extrapyramidal symptoms included).

Minocycline had an effect over the clinical global impression that was not disguinshible from placebo [27, 34, 36], although there was one exception [37]. Data was derived from high quality studies (n=4, N=490 participants), and the recommendation for improving this outcome with minocycline as add-on is D- (table 6). The impact of minocycline over the global functioning when added to antipsychotic in patients with schizophrenia was mixed, with one positive and one negative trial (n=2, N=198 participants, moderate to high quality designed trials) [36, 37]. Based on these data a D level is formulated in this case.

D-cycloserine had no significant impact over global clinical status (n=2, N=39 participants) or overall functionality (n=2, N=26 participants) [51, 52, 55, 56]. These trials were of moderate to low quality and there was formulated a D- recommendation for each outcome based on the before-mentioned results (Tables 6 and 7).

From all the data reviewed and hierarchized it is evident that larger trials are needed in order to confirm the efficacy of minocycline and d-cycloserine over negative and cognitive symptoms of schizophrenia. The tolerability of these two antibiotics was good in almost all trials that were reviewed, which is encouraging in the perspective of designing new trials with these agents.

Limitations of this review are related to the heterogeneity of design in trials that were analysed, and the impossibility of contructing a meta-analysis. The use of different scales for monitoring the same cluster of symptoms made difficult the comparison of the results. In addition, most of the trials included in the current review were conduceted on small groups, which make the generalizability of data very difficult.

Disclaimer

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ORIGINAL ARTICLE

Ego-strength mediates the relationship between religious health behaviors and mental health symptoms during the COVID-19 pandemic in Iran

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Abstract: *The present study aimed to examine the mediating role of ego-strength in the relationship between religious health behaviors and mental health symptoms during the COVID-19 pandemic in Iran. A total of 346 university students (301 women, 45 men) completed the Ego-Strength Scale, Religious Health Promoting Behaviors Inventory, and Mental Health Inventory from May 2021 to August 2021. The structural equation modeling and the Pearson correlation method were used. The results revealed that religious health behaviors have a positive and significant relationship with, psychological well-being, and ego-strength, as well as a negative significant relationship with psychological distress. The results also showed ego-strength has a negative relationship with psychological distress. Ego-strength mediated the relationship between religious health behaviors and symptoms of mental health. Ego-strength can be considered as a very important variable in religious health psychology; reinforcing ego-strength may have some implications for mental health during the Covid-19 pandemic.*

Keywords: *ego-strength, health behavior, religious health behavior, mental health, Covid-19*

INTRODUCTION

COVID-19 pandemic is the widest public health crisis in the world. In addition to the huge physical health threat of the COVID-19 pandemic, long feelings of uncertainty and fear, grief and separation, upset of economic and social systems have led to mental health threats [1]. The impacts of COVID-19 on mental health are alarming [2]. As Erikson (1965) suggested, ego-strength may be defined as specified qualities that start to animate the individuals comprehensively within successive periods of life [3]. Ego-strength is related to self-regulation. The term self-regulation is generally understood to mean a process that is guided by goals and its aims to achieve and sustain one's goals [4]. When a person has mental health, there is a consonance between id, ego, and superego [5]. The ego-strength also is one of the personality characteristics, and is defined as the attitude of a person toward his problems, his general response to distress, and his adaptive ability in situations which are emotional [6]. In other words, ego-strength is the extent of power that the ego works in adjusting the impulses and adapting to the environment. The balance while postponing the gratification of needs can be prepared by ego-strength. When the ego-strength is low, people experience a lack of motivation and confidence and believing that they are incompetent. But elevated ego-strength helps the persons to be resilient, motivated, confident, and trusting themselves and their capabilities; thus, they are likely to adapt to challenges [7].

In a study that used group psychotherapy that included components of individual psychotherapy, the majority of participants showed an increase in their ego-strength [8]. Another study among students showed that some variables such as sports, student government, and volunteerism were associated with ego-strength. Also, religious attendance wasn't related to ego-strength [9]. In another study, authoritative parenting and extroversion were related to ego-strength. Also, extroversion has moderated the association between authoritative parenting and ego-strength [10]. In one study, aggressive alcoholics had weaker ego-strength than non-aggressive alcoholics [11]. One of the

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variables of the present study is ego-strength which despite its great importance, has received little attention in psychological research. As a result, addressing it can open up new horizons for the science of psychology.

Also, some studies have shown the important role of ego-strength in mental health [10]. Mental health is one of the most important psychological variables. increasingly, the rates of mental disorders in colleges across the world are rising [12]. One of the major researches carried out by WHO in 21 countries indicated that one-fifth (20.3%) of college students had 12-month DSM-IV/ICD-10 disorders [13]. Many research suggesting that religious/spiritual behaviors and beliefs are correlated with mental health [14]. A systematic review indicated that religious involvement is associated with higher mental health especially in the domain of substance abuse, depression, and suicide [15]. Mental health is also considered in the present study. In particular, mental health is very important for university students and according to the statistics mentioned above, it is very important to address it. As a result, the present study deals with the mental health variable in students.

One recent study found a correlation between religiousness and a lower probability of depressive symptoms, especially in individuals that attending religious service [16]. Lots of people benefit from religious beliefs and practices to cope with stressful life happenings and enjoy mental peace and purpose in life. A comprehensive systematic review has shown that there is an association between religion, psychiatry, psychology, and medical care [17]. The correlation between religiousness and health can be largely explained by lifestyle [18]. A healthy lifestyle is very essential for a good life. Adequate physical activity and a limited amount of passive behavior can prevent various chronic diseases [19]. To date, many studies suggest the relationship between religious involvement and a variety of health outcomes. Some of them hypothesize the influence of religion on "health behaviors". But the underlying processes of the connection of religion and health have been studied very little [20].

Religion is the most momentous component of human nature for attaining the meaning and purpose of life. It has a great role in the patient's health [21]. Religion is a serious protective effect on risky health behaviors [22]. Greater religious involvement is correlated with positive lifestyle practices [23]. A study indicated that there is a relationship between risky drinking and religion. The religious group in contrast to the non-religious group had a lower probability of risky drinking [24].

Some studies indicated that self-efficacy and self-esteem are mediators of the relationship between religious involvement and health outcomes. But there is mixed evidence about the relationship. For example, one of the studies indicated that self-esteem and self-efficacy mediated, at least in part, the relationship between religious beliefs and better vegetable and fruit consumption, and lower alcohol consumption. Also, religious behaviors have a direct impact on health behaviors [25]. Research outcomes suggested that self-regulation can be the expository mechanism for the association between religiosity and positive health behaviors. However, various religious motivations have different impacts on health-related outcomes. These can be the reason for the negative effects of religion that are found in some research. One study suggested that self-regulation mediated the association between religious motivation and health behaviors [26].

A study indicated that a religious intervention can improve the patient's health [27]. Another study suggested that an intervention based on religion can promote the health of patients. In several studies, religious interventions had a desirable impact on patients [28]. Health behavior components also can predict mental health levels [29]. Psychological health is related to physical health outcomes. One of the effective components of this relationship is health behaviors. Higher distress is related to less eating fruits and vegetables and physical activity, and higher sedentary behaviors and smoking [30]. Also, mental illness and health behaviors are associated strongly. But the causality of the link is challenging. A new study indicated the correlation between psychological distress and health behaviors. Furthermore, there is a causal relationship between psychological distress and health behaviors [31].

The underlying processes of the connection between religion and health have been studied very little [20]. Also, interest in the role of religion and spirituality in mental health is increasing [17]. Due to the remarkable burden of disease which is correlated to unhealthy behaviors, more research is needed to understand the causal links between mental health and health behaviors [31]. Given the ascendant rates of mental disorders in colleges across the world [12], the correlation between religious/spiritual behaviors and beliefs with mental health [14], the importance of research in this field, the relatively high levels of religious involvement among some cultures, and the important role that ego-strength may play in the relationship of religious health behaviors and mental health and illness, the present study examined the mediating role of ego-strength in the relationship between religious health behaviors and mental health symptoms and illness in a group of university students.

The research hypotheses in the present study are as follows: (1) there is a positive significant relationship between religious health behaviors and mental health, ego-strength, and all of its subscales, and a negative significant relationship with mental distress. Also, there is a positive significant relationship between ego-strength subscales and total score of it with religious health behaviors and mental health, and a negative relationship with mental distress; (2) The ego-strength can mediate the relationship between religious health behaviors and mental health symptoms and illness.

METHOD

In the present study, the mediating role of ego-strength in the relationship between religious health behaviors and mental health and distress was investigated using structural equation modeling from May 2021 to August 2021. The conceptual model is shown in Figure 1.

The data were analyzed using SPSS 26 and Amos software.

Figure 1: Conceptual model

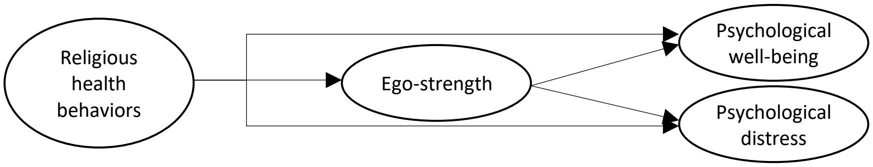

Participants and procedure

The present study is a descriptive one. The statistical population of the present study was university students. A random sample of 346 university students (M = 23.01, SD = 7.12) participated in the present study. The inclusion criteria were being a student, being literate, not suffering from serious psychiatric or physical disorders, and not having substance dependence. All of the students signed an informed consent document before completing the scales. The students' demographic characteristics showed in Table 1.

Table 1: Study sample characteristics

Variables		n	%
Gender	Male	45	13
	Female	301	87
Levels of education	Undergraduate	297	85.8
	Postgraduate	46	13.3
	PH.D. students	3	0.9
Marital status	Single	273	78.9
	Married	73	21.1
Job	Student	289	83.5
	Teacher	43	12.4
	Other jobs	14	4.1
Number of children	0	307	88
	1	13	0.03
	2	17	0.04
	3 +	8	0.02

Measures

Demographics. At the top of the page of the first measure, participants reported their gender, educational levels, marital status, job, and the number of children.

Religious Health Promoting Behaviors Inventory. Religious health-promoting behaviors in participants were measured by the Religious Health Promoting Behaviors Inventory (RHPBI; [32]). It is a 16-item measure that assesses the personal behaviors and habits based on religious doctrines and beliefs in everyday life on a 7-degree scale ranging from 0 (completely disagree) to 6 (completely agree). Cronbach's alpha coefficient for the scale questions was obtained in a sample of 93 individuals (0.88). Convergent and differential validity of the RHPBI was calculated through the simultaneous implementation of the Spiritual Well-being Scale (SWBS; Palutzin and Ellison 1982) and the Mental Health Inventory (MHI; [33]) in the mentioned sample. The results of Pearson correlation coefficients showed that there was a significant positive correlation between the scores of subjects in the RHPBI and SWBS and psychological well-being ranging from 0.37 to 0.49 (P < 0.001) and a negative correlation with psychological distress (-0.33, P < 0.001). These results confirm the convergent and differential validity of RHPBI [32]. In the present study, Cronbach's alpha coefficient for the scale questions was 0.721.

Mental Health Inventory. The mental health of participants was measured using the Mental Health Inventory (MHI-28; [33]). It is a short form of the 34-item Mental Health Scale (Veit and Ware 1983) and it is a 28-item test that has 22 psychological well-being and psychological distress items in a 5-Likert scale ranging from 1 (completely disagree) to 5 (completely agree). The psychometric properties of the 28-item form of the scale were studied. Cronbach's alpha coefficients of psychological well-being and psychological distress for the scores were 0.94 and 0.91. The correlation coefficients between the scores were calculated with a 2-week interval for test-retest reliability assessment. Coefficients for psychological well-being and psychological distress were r = 0.90, r = 0.89, and P < 0.001 respectively. The concurrent validity of the MHI-28 was measured by the simultaneous implementation of the General Health Questionnaire. The results of Pearson correlation coefficients showed that there was a significant negative correlation between the general score of the subjects in the general health questionnaire and the subscale of psychological well-being (r = -0.86, P < 0.001), and a significant positive correlation with the subscale of psychological distress (r = 0.89, P < 0.001) [33]. In the present study, the subscales were consistent internally and Cronbach's alpha coefficients were 0.888 for psychological well-being and 0.903 for psychological distress.

Ego-Strength Scale. The Ego-strength of participants was measured using the Ego-Strength Scale (ESS; [6]). It includes 25 items that have been validated to assess the power of ego in managing and controlling life situations that are difficult. The instrument's items are rated on a 5-degree Likert-type scale ranging from 1 (not at all) to 5 (very much). In several studies in the general (n = 1257) and clinical population (n = 372), the psychometric properties of ESS have been assessed and confirmed [6]. The scale includes 5 subscales (Ego control, ego resiliency, mature defense mechanisms, problem-focused coping strategies, and positive emotion-focused coping strategies). In one study, all subscales were internally consistent and the Cronbach's alpha coefficient ranged from 0.73 to 0.91 [34]. In the present study, all subscales were consistent internally and Cronbach's alpha coefficients ranged from 0.753 to 0.876.

RESULTS

In the present study, the mediating role of ego-strength in the relationship between religious health behaviors and mental health and distress was investigated using structural equation modeling. Using structural equation modeling requires observing the assumption such as normality, outlier data control, linearity, and multicollinearity that need to be met before examining the results of research questions. Kolmogorov-Smirnov test was used to test the normality of the data for each of the research variables. The results showed that the assumption of normality was observed.

The boundary data method was used to evaluate the outlier data. It was indicated that the assumption has been observed. Also, based on Table 2 and 3 data, the assumption of linearity and multicollinearity was found. Based on the assumption of linearity, there must be a linear relationship between the criterion variables and the predictors. So, the results of the correlation matrix indicated that there is a positive significant relationship between religious health behaviors and mental health, ego-strength, and all of its subscales (including ego-control (ECON), ego-resiliency (EROS), mature defensive mechanisms (MDEF), problem-focused coping strategies (PFOC), and positive emotional focused coping (PEMO)), and negative significant relationship with mental distress. Also, there is a positive significant relationship between ego-strength subscales and its total score and religious health behaviors and mental health, and a negative relationship with mental distress. As Table 3 shows, the correlation between all variables was significant (P < 0.001).

Table 2: Descriptive indicators of research variables

Variables	Scale	*M*	*SD*	*V*	Min. score	Max. score
Religious health behaviors	RHPBI	65.04	11.84	140.34	21	98
Psychological well-being	MHI	50.45	9.92	98.43	19	79
Psychological distress	MHI	35.04	10.75	115.74	7	62
Ego-strength	ECON	16.95	3.55	12.65	5	29
	EROS	16.55	3.88	15.12	6	25
	MDEF	15.91	3.81	14.54	6	47
	PFOC	17.33	3.15	9.96	7	25
	PEMO	16.79	3.22	10.43	8	25
	Total	83.55	14.27	203.81	35	120

According to the point that the entering of 2 predictor variables with a correlation coefficient of more than 0.70 in these types of analysis is not allowed, the correlation matrix results were observed. The results showed that there was a noticeable relationship between the criterion and prediction variables, but the relationship was not sufficient for multicollinearity. The highest correlation value was 0.671 for the EROS subscale of ego-strength. Accordingly, all variables were kept in the analysis.

Table 3: Correlation matrix between ego strength, religious health behaviors, mental health, and distress

Variables	1	2	3	4	5	6	7	8	9
Religious health behaviors	1								
Psychological well-being	0.22	1							
Psychological distress	-0.22	-0.48	1						
EROS	.025	0.48	-0.48	1					
ECON	0.32	0.38	-0.36	0.61	1				
MDEF	0.25	0.27	-0.36	0.58	0.55	1			
PFOC	0.28	0.34	-0.29	0.60	0.67	0.45	1		
PEMO	0.30	0.43	-0.30	0.58	0.49	0.42	0.56	1	
Ego-strength	0.35	0.47	-0.45	0.67	0.65	0.66	0.60	0.54	1

P< .001

In reporting the results of structural equations, the main component in examining the fitness of the hypothetical model is the necessary indicators for reporting and the scientific acceptable criteria for confirming these indicators. According to the experts, from all 3 groups of fitness indicators (absolute, adaptive, and purposive), some indicators must be reported (Figure 2).

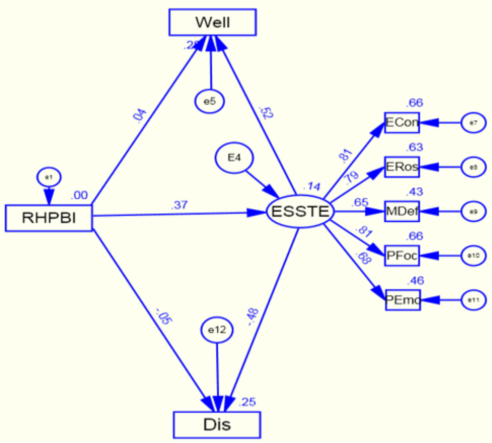
Figure 2: Unmodified hypothetical model of mediating role of ego-strength in the relationship between religious health behaviors and mental health and distress

According to Myers et al. (2006), the numerical value of 3 for the relative chi-square index, the numerical value of 0.60 for root mean square error of estimation, and the numerical value of 0.90 for adaptive indicators are acceptable. Also, for purposive indexes, numerical values greater than 0.50 are suitable. Given the results of Table 4 and the cut-off points of each index, the hypothetical model needed to be modified. The test for fitting the model with observed data, using stepwise selection model correction showed that the model can be corrected: In the ego-strength variable, by creating covariance between the residual error of "ECON and PEMO" indices and the residual error of "EROS and MDEF" markers as well creating covariance between the residual error of mental health and distress (Figure 3).

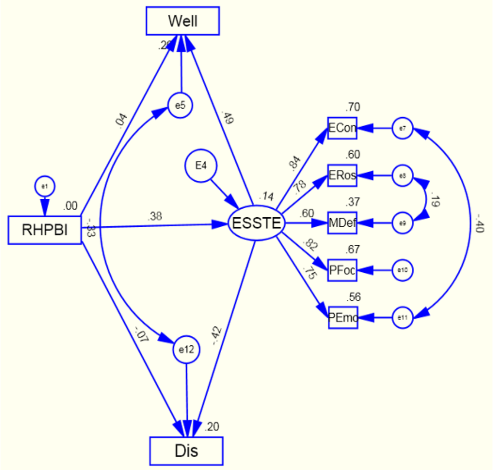
Figure 3: Modified hypothetical model of the mediating role of ego-strength in the relationship between religious health behaviors and mental health and distress

Table 4: Goodness of fit indices of unmodified and modified assumption model

	Absolute indicators				Adaptive indicators			Conventional indicators	
Statistics	Chi	df	Chi/df	RMSEA	GFI	NFI	CFI	PCFI	PNFI
Unmodified	135.80	20	6.79	0.130	0.896	0.880	0.895	0.639	0.628
Modified	72.507	17	4.265	0.210	0.950	0.936	0.950	0.557	0.568

As Table 4 shows, the modifications made on the hypothetical model resulted in a 3-degree decrease in the degree of freedom in the modified model, and a 63.294-degree decrease in the chi-square value, resulting in a 2.535-degree decrease in the relative chi-square value. Therefore, in the modified model, indicators of absolute fit indices along with other indicators such as root mean squared error of approximation with a confidence interval of 0.90, adaptive indicators (GFI, NFI, and CFI) with a cut-off point of 0.90, and the indicators (PCFI, and PNFI) with a cut-off point of 0.60, are in a desirable condition. It indicates that the hypothetical modified model is valid.

The results of the modified model show that the ECON subscale with a coefficient effect of 0.84, had the maximum contribution and the MDEF subscale with a coefficient effect of 0.60, had the minimum contribution in the score of ego-strength. The factor loadings of all paths were significant in the hypothetical model. The results also indicated that the direct effect of religious health behaviors on mental health and distress were respectively 0.4 and -0.5. Based on these results, it can be said that religious health behaviors have had a positive non-significant effect on mental health and a negative non-significant effect on mental distress.

To investigate the statistical significance of the indirect effect of religious health behaviors on mental health and distress through ego-strength (the mediating role of ego-strength on the relationship between religious health behaviors and mental health and distress), the Bootstrap method was used.

The results showed that religious health behaviors have affected mental health through ego-strength 0.185. The result is significant statistically (P = 0.001) and suggested that with one unit increase in religious health behaviors, 0.185 units will add to the mental health scores. The results also suggested that religious health behaviors have affected mental health and distress through ego-strength -0.159 The amount is significant statistically (P = 0.001) and suggested that with a unit increase in religious health behaviors, 0.159 units will decrease in the mental distress.

DISCUSSION

The COVID-19 pandemic is one of the risk factors for psychological diseases. COVID-19 related post-traumatic stress symptoms are correlated to distress, anxiety, and low sleep quality [35]. Furthermore, during and after traumatic events, depression has been increased [36], but during the COVID-19 pandemic, the increase is more than in previous traumatic events.

One of the mechanisms through which ego-strength can affect mental health and illness symptoms is resilience (one of the ego-strength components) during the COVID-19 pandemic. Resilience is the dynamic process of adapting positively to hard and negative experiences [37]. Using this feature probably enhances one's ability to manage conflicts.

Ego-strength has an important role in predicting health and reflects the attitudes toward problems, responding to failure, and the ability to adapt to one's emotional status. Also, ego-strength plays a predictive role in health conditions and the therapeutic alliance in chronic problems [38]. Ego-resiliency can moderate the association between cortisol level and emotional labor [39].

Another mechanism through which ego-strength can influence symptoms of mental health and illness is the use of enhanced defensive strategies in the COVID-19 pandemic. Defensive mechanisms are responsible for protecting the individual against anxiety and awareness of the dangers and causes of internal and external stress. Ego-strength enhances problem-solving skills [40], and using these effective strategies to cope with stress can increase mental health.

Maladaptive and adaptive coping can moderate the association between life events and mental health problems [41]. Coping strategies moderate the association between job insecurity and mental health [42]. Empowerment helps the individual to manage stressful situations to some extent by using adapted emotion regulation strategies [6].

There has been some research on the relationship between ego-strength dimensions and depressive disorders that support the finding of the present study [43]. In interpreting the finding, we can note the relationship between ego-strength and psychopathology. Ego-strength is one of the key components of psychodynamic models for explaining psychological trauma. Low ego-strength makes the individual unable to perform the reality test properly, increasing interpersonal and intrapersonal conflicts [44]. Therefore, the development of mental disorders seems to be related to low ego-strength. Low ego-strength is associated with dysfunctional defensive mechanisms, lack of adaptive capacity to defeat, and low level of arousal. One of the main syndromes reflected in the functioning of neurotic patients and personality disorders is the low level of ego-strength and poor defensive mechanisms [7].

Ego control, the ability to control and manage situations is another component of ego-strength [45]. Under controlled individuals have some characteristics such as self-dramatizing, unpredictable, and unable to delay gratification, assertive, moody, rebellious, and self-indulgent. Over controlled individuals have some characteristics such as being consistent, bland, calm, and dependable. Resilient individuals have some traits like having a high level of aspiration and wide interests, socially poised and skilled, assertive, and cheerful, and neither lacking personal meaning in life, emotionally bland nor self-defeating [46].

Limitations and Future directions

There are some limitations in the present study which are worth acknowledging. The findings are derived from a cross-sectional method that can influence the results, and causal interpretations of the findings are speculative. The issues of causality could best be investigated through longitudinal surveys. The present study was conducted only on university students (most of them were young), and the generalization of the findings to less-educated persons should be done with caution. Also, the findings may not fully generalize to other countries, because of the diverse cultural and socioeconomic factors. Furthermore, due to the higher number of female students in some universities, the number of women in the present study is much higher than men. Therefore, this point should be taken into account when generalizing the results. Future studies can explore similar questions in other samples such as subjects with various diseases, different group ages, various education levels, and different socioeconomic and cultural environments. The ego-strength is a novel variable that is not yet well known and needs a lot of research attempts to understand it and the various roles it can play. There may be many other variables that can mediate the relationship between religious health behaviors and mental health and illness and can be explored in future research. Finally, there may be other alternative hypothetical models that had not been evaluated which would fit the data well or even better. Future research can test alternative or more complex models for the present variables.

CONCLUSIONS

In conclusion, the current study provides evidence that ego-strength can mediate the relationship between religious health behaviors and mental health symptoms and illness. Also, the results showed that there is a positive relationship between religious health behaviors and mental health, ego-strength, and all of its subscales, and a negative relationship with mental distress. Furthermore, there is a positive relationship between ego-strength subscales and their total score and religious health behaviors and mental health, and a negative relationship with mental distress during the Covid-19 pandemic. The data provides new insights into the importance of ego-strength in the religious health psychology area. Mental health and religious health behaviors are both very important psychological variables. The present study helped to clarify the relationship between these variables. Any practical effort to reinforce ego-strength can help strengthen the relationship between mental health and religious health behaviors. Everyone can benefit from the results of the present study.

Ethical Approval

All procedures performed in studies involving human participants were under the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent was obtained from all individual participants included in the study.

Conflict of interest

All authors declare that they have no conflict of interest.

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CLINICAL PRACTICE

Elastofibroma – an uncommon cause of upper limbs pain

Natalia Dumitrescu[1], Bianca Sandu[2], Silviu V. Dumitrescu[2]

A female patient age 65 came to our clinic forcontinuous pain of the upper limbs (left more than right), increased when moving the scapulae. Patient declared that in the past few months the dorsal decubit became gradually painful and she cannot achive a good sleep. One year ago patient was diagnosed with bilateral suprascapular lipoma and a surgical removal was proposed.

At the visual examination, we found two well-circumscribed suprascapular tumor mobile with arms movements. At palpation, we noticed that each scapula was pushed from below from an underlying tumor with a very firm consistency.

Figure 1: Clinical aspects

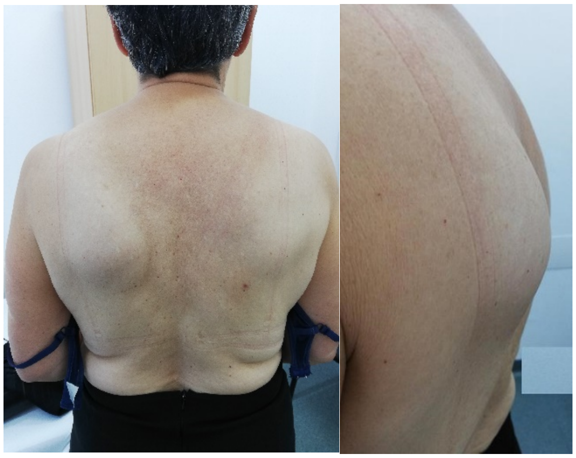

Chest radiography was normal.

A CT scan of torax was performed and revealed two subscapular unencapsulated, soft tissue masses with skeletal muscle attenuation interspersed with strands of fat attenuation [1, 2].

Figure 2: CT aspects

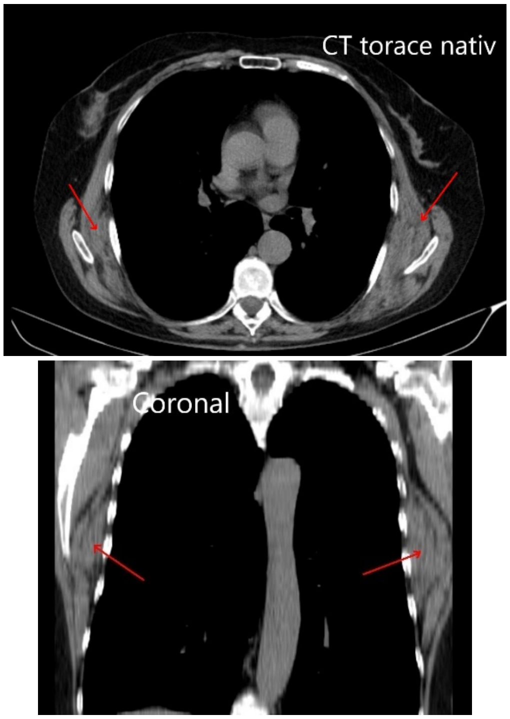

The patient was directed to thoracic surgery and the tumors were removed. The histological findings were consistent with the diagnosis of elastofibroma [3]. The recovery period was long, allmost two months passed before complete healing.

The long period needed for healing, usually burdened with local complications and the fact that the tumor is benign makes the surgery not recommendable for the patients with no discomfort or impared arms movement [4].

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ORIGINAL ARTICLES

Elastofibroma dorsi: clinical experiences of 19 cases

Hacer B. Yesilcay[1], Sencan Akdag[1]

Abstract: *In this study, symptoms, functions, and outcomes of patients who underwent surgery with the diagnosis of Elastofibroma dorsi between 2007-2019 in our clinic were discussed retrospectively.*

A total of 19 patients were operated on with the diagnosis of Elastofibroma dorsi in our clinic. The demographic characteristics of patients such as age and gender, symptoms, clinical findings, diagnostic and radiological features, surgical procedures, results of surgical treatments, and postoperative follow-up results were evaluated based on the records.

The mean age of patients who underwent surgery was 55.7 and there were 13 females and 6 males. The most common clinical complaint was swelling (61%). Seven of ED were located on the right side, 3 of them were located on the left side and 7 of ED were located bilaterally. The mass in all cases was over 5 cm in diameter, complete surgical excision was done via muscle-sparing technique. All patients were followed up postoperatively and there was no recurrence.

ED should be considered in terms of differential diagnosis when middle-aged patients present with a mass in the scapular region and shoulder pain. Total excision is surgically sufficient in symptomatic patients.

Keywords: *elastofibroma dorsi, shoulder pain, chronic back pain*

INTRODUCTION

Elastofibroma dorsi is a benign, rarely seen, slow-growing soft tissue lesion. First described by Jarvi and Saxen in 1961. The lesion is usually located at the inferior angle of the scapula., deep to the serratus anterior, and may be attached to the periosteum of the ribs [1]. The etiology is not known clearly [2]. Symptoms are usually chronic back pain, stiffness, swelling, snapping of the scapula, and increased pain during shoulder movement. Noninvasive imaging methods such as ultrasonography, computed tomography, and magnetic resonance imaging are used in the diagnosis of elastofibroma dorsi [3]. Total excision is the best treatment to prevent relapses and relieve symptoms [4]. In this study, we describe our experience of diagnosing and treating elastofibroma dorsi patients between 2007-2019.

METHODS

Between 2007 and 2019, 19 patients operated with the diagnosis of elastofibroma dorsi were retrospectively reviewed. The cases were evaluated in terms of age, gender, complaints, clinical findings, diagnostic and radiological features, surgical applications, surgical treatment results, and postoperative follow-ups. The radiological examination was applied by ultrasonography, thorax computed tomography (CT), and magnetic resonance imaging (MRI).

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The mass diameters of all cases were measured by radiological evaluation. None of the cases were directly diagnosed by biopsy. Excision was undertaken in all cases after pre-operative consent was obtained. A transverse or parabolic incision at the inferior pole of the scapula was used. All operative samples were sent for histopathological evaluation. The cases were followed up in six-month periods after surgery.

RESULTS

13 of the cases were female (69%), 6 were male (31%) and the mean age was 55.7 (31-68) years. The most common clinical complaint was swelling (61%). Other complaints were chronic back pain (31%), snapping of the scapula (23%), and increased pain with shoulder movement (19%). In our series, 27% of cases were found to be asymptomatic. The mean duration of symptoms was 20 months (3 to 96). The lesion was located in the subscapular region in all of the patients, being unilateral in ten and bilateral in nine. Of the unilateral tumors, three lesions occurred on the left side and seven on the right. The radiological examination was applied by ultrasonography and thorax computed tomography (CT) for all patients (Figure 1). Magnetic resonance imaging was applied in 7 cases that could not be evaluated adequately by thorax computed tomography.

Figure 1: CT scan image of a patient

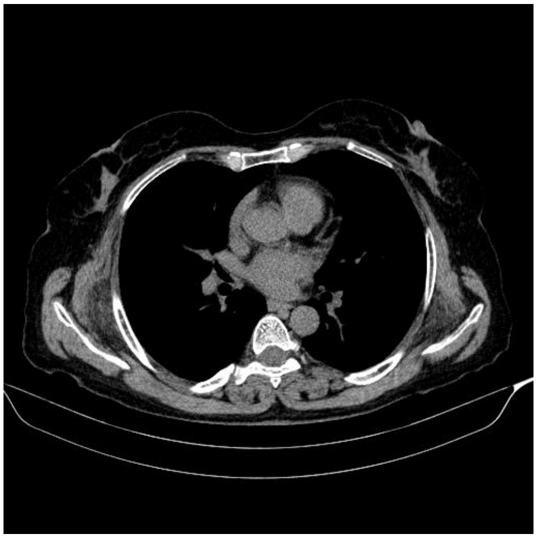

In all cases, total excision of the lesion was provided via muscle-sparing technique then, hemovac drain which was removed 24-72 hours after the operation was placed in the subscapular area and the layers were closed following the anatomy. All excised samples were sent for pathological examination. In the pathological examination macroscopically, solid lesions were observed with a yellow and white cross-section surface covered with fibrous capsule and containing adipose tissue (Figure 2). In the microscopic examination of hematoxylin-eosin sections, mature adipocytes and elastic fibrillar stained positive with Von Gieson were observed. Postoperative complications developed in four (21%) of operated cases; in one (5%) case antibiotic allergy was observed and in three (16%) cases seroma requiring needle aspiration was observed. The mean length of hospital stay was 4 days (2 to 8) and the mean follow-up was 5 months (3 months to 2 years). No recurrence was observed during the follow-up period.

Figure 2: Solid lesions with a yellow and white cross-section surface covered with a fibrous capsule and containing adipose tissue

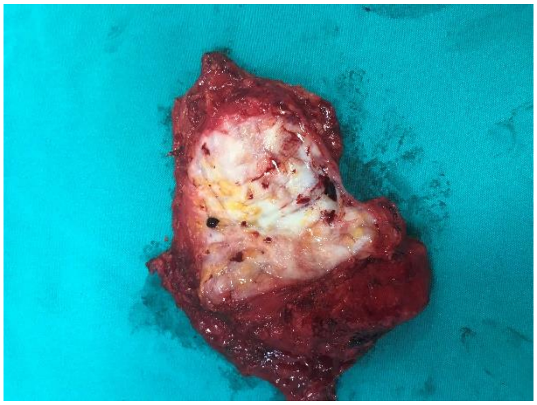

DISCUSSIONS

Elastofibroma dorsi is a benign, unencapsulated, slow-growing soft tissue tumor, is usually seen in women, in the 5th and 6th decade of life. First described by Jarvi and Saxen in 1961 [1]. Despite it is traditionally considered to be rare, Jarvi and Lansimies showed, in a series of 235 postmortems in patients older than 55 years, changes in the subscapular thoracic fascia similar to elastofibroma in 24.4% of women (29 of 119) and 11.2% in men (10 of 89) [5]. The age and gender of patients in our series were consistent with the literature.

ED is often localized in the subscapular region which is between the rhomboid and latissimus dorsi muscles and the sixth and eighth ribs. However, other localizations such as deltoid muscle, foot, greater trochanter, olecranon, cornea, stomach, greater omentum, ischial tuberosity, intraspinal space, and chest wall have been reported in the literature [2, 6, 7].

ED is often unilateral and right-sided. The number of bilateral cases is around 10-60%. Bilateral cases can be developed synchronously or asynchronously [8]. In our study, the lesion was located in the subscapular region in all of the patients, being unilateral in ten (52,7%) and bilateral in nine (47,3%). 16 of the 28 ED lesions were localized on the right side (57,1%). Lesions were synchronously detected in patients with bilaterally located elastofibroma dorsi.

The pathogenesis of ED is not clear, several hypotheses have been put forward. Repeated micro-injuries between the chest wall and the scapula, the source of excess elastin production, and collagen degeneration could play a physiopathological role in this rare lesion [9].

This view has been supported by the higher ED prevalence particularly among individuals who work at hard manual labors. However, patients who have never been involved in hard manual work, as well as those with elastofibromas in different locations, have undermined this view. In our study 3 patients (15,7%) were manual laborers. The rest of the cases did not have heavy labor history or heavy sporting activities in their anamnesis records.

In a study investigating genetic anomalies in ED cases, changes in DNA copy number were observed in tumor tissue, mainly in the chromosome Xq12-q22 and 19 regions [10]. In a cytogenetic study by Mc Comb et al., they detected genetic instability in chromosome number 1 and translocation in number 8-12, stated that they may be neoplastic, not reactive, due to these clonal abnormalities. In the largest reported series of 170 patients with the lesion, a familial predisposition was suggested with 32% having a positive family history for elastofibroma [8]. In our study, none of the patients had a family history of ED.

There are also opinions such as reactive fibromatosis, degeneration due to vascular insufficiency, elastotic degeneration, and enzyme defect [8].

Clinical findings are mostly related to the size of the lesion. They often grow slowly and are asymptomatic. As the lesion grows, there is swelling in the back, increased pain with shoulder movements, snapping of the scapula, and chronic back pain [11]. In our series, the most common clinical complaint was swelling (61%), 27% of cases were found to be asymptomatic.

Imaging modalities for diagnosis include ultrasonography, CT, and MRI. Solivetti et al. reported that the use of diagnostic USG is an adequate and inexpensive method [12]. Kransdorf et al. reported that radiological evaluation with MRI or CT is compatible with histopathological evaluation [13]. The most important imaging modality is accepted as an MRI. Malghem et al. reported that fibrous tissues within the mass have similar signal characteristics with the surrounding muscle tissues in the MRI examination, while fat tissue has higher signal characteristics than the mass, and these findings are pathognomic for the mass [14]. In our study, the radiological examination was applied by ultrasonography and thorax CT for all patients. MRI was applied in 7 cases that could not be evaluated adequately by thorax computed tomography. In our series, radiological findings were compatible with the literature.

Lipoma, neurofibromas, metastatic lesion, primary or metastatic sarcoma, fibrosarcoma, synovial sarcoma, the desmoid tumor should be considered in the differential diagnosis. Needle aspiration or incisional biopsy may be performed to eliminate the possibility of malignancy. However excisional biopsy is often preferred due to diagnostic radiological evaluation's sufficiency [15, 16]. In our series, a needle biopsy wasn’t performed.

Macroscopically, ED is in the form of a fibrous lesion of dirty white color, is non-encapsulated, and contains streaks of fat tissue. Some elastofibromas may have cystic degeneration. The histological appearance of the lesion is typical. In large areas, it contains hyalinized collagenous stroma and little amount of fat tissue in between. In hypocellular collagenous stroma, fibrils [6] and globules that show eosinophilic staining are striking. In sections of hemotoxylin-eosin, the presence of fibrils and globules is important for determining the location of the lesion and its diagnosis [4, 9].

Elastofibroma dorsi is treated with total excision, but surgery is not recommended for asymptomatic lesions smaller than 5 cm [17]. In our series, the mass in all cases was over 5 cm in diameter, complete surgical excision was done via muscle-sparing technique, which requires preparation of latissimus dorsi and serratus anterior muscle flaps.

The most common complications after surgical excision are hematoma or seroma. Therefore, after excision of the mass, the bleeding control should be performed cautiously [11]. In this study, we used hemovac drainage and a compression bandage to reduce these complications. Postoperative complications developed in four (21%) of operated cases; in one (5%) case antibiotic allergy was observed and in three (16%) cases seroma requiring needle aspiration was observed.

Local recurrence after total excision is rare and malignant transformation has not been reported. In the first recurrence, total surgical excision can provide a cure, but in subsequent recurrence, total excision may not be performed [9]. In our cases, no recurrence was detected during the follow-up period.

As a result, ED should be considered in terms of differential diagnosis when middle-aged patients present with a mass in the scapular region and shoulder pain. Total excision is surgically sufficient in symptomatic patients.

Declaration of conflicting interests

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VARIA

Electrodiagnostic studies in carpian tunnel syndrome

Hazim Elayan[1], Ana M. Oproiu[2], Radu C. Jecan[2], Ioan P. Florescu[2]

Abstract: *The diagnosis in Carpian tunnel syndrom is based on clinical symptoms and electrodiagnostic studies. Electrodiagnostic studies help to stage both motor and sensory nerve damage.*

Keywords: *electrodiagnosis, nerve conduction, motor nerve, sensory nerve*

INTRODUCTION

Electrodiagnostics represents a test or a paraclinical study of analysis of the muscular and nervous system, which evaluates the transmission of the motor and sensory nerve impulse, respectively, which helps the specialist doctor to obtain a diagnosis or prognosis, after the anamnesis and clinical examination have been performed [1].

When we talk about electrodiagnostics we refer to both Electromyography (EMG) and Electroneurography (ENG) [1].

Figure 1: Electrodiagnostics (https://share.upmc.com/2017/09/facts-electrodiagnostic-medicine/)

There are several advantages of electrodiagnostic [2]:

1. May specify the location of the neurological or muscular injury.

2. Can provide data on the type and severity of the lesion (demyelinating, axonal or mixed lesion).

3. It can specify the degree of axonal injury (neurapraxia, axonotmezis, neurotmezis).

4. It can stage affection (acute, subacute, chronic stage).

5. Can diagnose and monitor the progress of the disease over time.

6. It can formulate a prognosis of the disease and can be used in monitoring the effects of treatment (radiculopathy).

7. Represents an objective method of investigating the neuro-muscular system.

Limitations of electrodiagnostic studies

In chronic nerve compression, the first changes that occur in nerve fibers are changes in demyelinated nerve fibers, which cannot be evaluated with electrodiagnostic studies and tests [3]. Example: early symptoms of pain and paresthesia cannot be objectified with electrodiagnostic studies [4].

Another limitation of the nerve conduction study is the location of the nerve lesion in the extremity. Nerve problems occur very distally or very proximal and are difficult to assess [4].

Dynamic changes in blood flow that may intermittently cause changes in peripheral nerve function may not be detected with electrodiagnostic studies [4].

The timing of nerve conduction velocity studies also influences their usefulness because even complete sectioning of the nerves will not become evident for 26

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weeks after injury [4].

In situations with more than one level of injury or when systemic polyneuropathy is present, nerve conduction studies may be less reliable [4].

Electrodiagnostic studies in carpal tunnel syndrome

Electroneurography (ENG)

1. Nervous motor conduction:

The median nerve is electrically stimulated at the wrist and the motor response from the short abductor muscle of the thumb is recorded, with the help of surface electrodes; usually, the motor response occurs 4 msec after the application of the stimulus, an interval called motor distal latency (DL) (latency is the time required for the impulse to travel the distance from the stimulation site to the collection site); if a distal latency over 4.5 msec is observed, it means that we are dealing with a carpal tunnel syndrome [5, 6, 7].

Nerv conduction velocity ($NCV = s/t = S/PL - DL$)[1] in the motor nerve fibers is 45 - 65 m/s, in the nerve fibers, depending on the thickness of the nerve (Figure 2).

Figure 2: Compound muscle action potential (CMAP). Distal latency is measured from the stimulus to the onset of the negative response. The amplitude is measured from the base value to the negative peak (https://musculoskeletalkey.com/the-electrodiagnostic-examination/; Jinny Tavee, Kerry H. Levin)

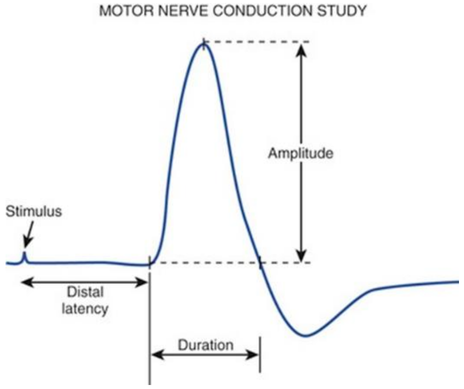

Observation: It can also stimulate the median motor nerve both palmar and at the wrist, evaluating the amplitude of the motor response thus providing information on the lesion: conduction block or axonal lesion [5, 6, 7].

The two-point stimulation method is preferred because it has the advantage that it eliminates the inaccuracies generated by the fact that the measured time represents not only the conduction in the nerve fiber, but also the conduction through the motor plate.

2. Nervous sensitive conduction:

Most frequently, it is measured antidromically, electrically stimulating the median nerve in the wrist and recording with surface electrodes in the fingers (most often, in the index and middle); a decrease in the sensitive conduction velocity (obtained by dividing the distance between the stimulator cathode and the active recording electrode at the sensitive distal latency) below 40 m/s is diagnostic for carpal tunnel syndrome [5, 6, 7].

Figure 3: Sensory nerve action potential (SNAP). The peak latency is measured at the beginning of the negative phase. The amplitude is measured from the base value to the negative peak (https://musculoskeletalkey.com/the-electrodiagnostic-examination/; Jinny Tavee, Kerry H. Levin)

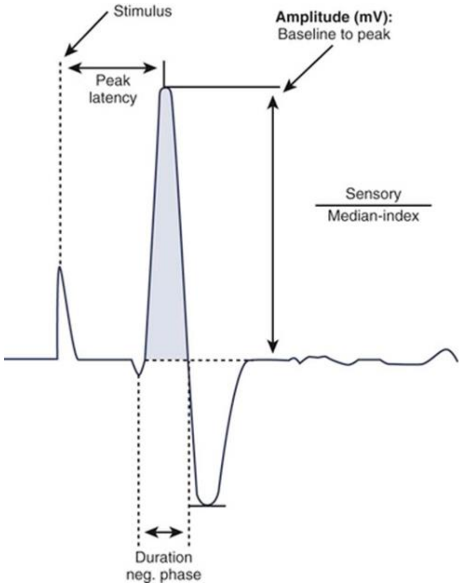

Another method applied when the changes are smaller is to stimulate from the same distance the ulnar and median nerves at the wrist, with the recording of the response from the IV finger – ring, which has mixed sensory innervation; a distal latency when stimulating the median nerve by more than 0.5 msec higher than when stimulating the ulnar nerve has diagnostic significance [5, 6, 7].

Electromyography (EMG)

When the existence of an axonal suffering of the median nerve is suspected, by prolonged compression, electromyography with needle is performed of the short abduction muscle of the thumb to detect early (before the installation of clinically obvious amyotrophy) the denervation changes; in this case, surgery is mandatory [5, 6, 7].

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[1] S = distance, t = time required for the impulse to move between the two stimulation points

Muscles are also tested electromyographically, when necessary for differential diagnosis (radial flexor of the carpus – median nerve injury above the carpal tunnel; adductor police – ulnar nerve injury, upper thoracic opening syndrome, etc.) [5, 6, 7].

CONCLUSIONS

Electrodiagnostic studies include electromyography (EMG) and nerve conduction studies.

Tests are not a substitute for a detailed clinical examination by a specialist.

Unfortunately, some doctors rely on these studies without recognizing the major limitations of electrodiagnostics examination [8].

Electrodiagnostic studies are useful to eliminate others associated problems such as cervical disc disease, motor neuron problems, myopathy or overlapping polyneuropathy.

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ORIGINAL ARTICLES

Emergency surgery for complicated colorectal cancer – What we choose: a retrospective cohort study

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Abstract: *Introduction: Despite the progress that has been made regarding the diagnosis, a worrying percentage of patients are still admitted in an emergency, with complicated colorectal tumors, which often require interventions without a curative visa. The study aims to analyze the factors involved in choosing the type of surgical treatment.*

Patients and methods: We included in the study a group of 431 patients admitted and operated in an emergency for complicated colorectal cancer in the Surgery II Clinic of the Clinical Emergency County Hospital "Sf. Ap. Andrei" from Galați, between 2008-2017. We retrospectively analyzed the patients' data and we made statistical correlations between the type of emergency surgery and epidemiological, clinical, paraclinical, and intraoperative factors.

Results: Colostomy was mainly practiced in older patients ($p<0.01$), from rural areas ($p<0.01$), with associated cardiac diseases ($p<0.01$), with hydro electrolytic disorders ($p<0.03$), rectal tumors ($p<0.01$), peritoneal carcinomatosis ($p<0.02$), frozen pelvis ($p<0.01$). Hartmann's operations were associated with patients with: leukocytosis ($p<0.01$), intestinal perforation ($p<0.01$), sigmoid tumors ($p<0.01$). Internal derivations were associated with patients with: liver metastases ($p<0.01$), splenic angle tumors ($p<0.01$), tumors invading other organs ($p<0.01$), and stage IV of the disease ($p<0.01$). Resections with anastomosis were associated with transverse colon tumors ($p<0.01$), well-differentiated tumors ($p<0.01$).

Conclusions: The type of emergency surgery performed correlated well with the age of patients, the area, the comorbidities, the history of the disease, cachexia, anemia, oligoanuria, hydroelectrolyte disorders, tumor complication, and location of the tumor, hepatic metastases, and tumors' invasion in other organs, tumor grading and stage of the disease.

Keywords: *surgical treatment, colorectal cancer, emergency, correlations*

INTRODUCTION

In Romania, according to the GLOBOCAN 2012 analysis, colorectal cancer was the second [1]. According to the data provided by the GLOBOCAN 2018 statistics, colorectal cancer ranks third in incidence globally (after lung and breast

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cancer) and at the 2nd place as cancer mortality (after lung cancer) [2].

The incidence is increasing both in developed countries and in those where until a few decades ago, the colorectal cancer was not a major concern [1].

Both sexes are affected in approximately equal proportions and the most affected decades of age are the sixth and the seventh [1].

Despite the improvements that have been made regarding the diagnosis and the possibilities of screening, a worrying percentage of patients are still admitted in an emergency, with complicated colorectal tumors, which often require interventions without a curative visa [3].

Of all the complications of colorectal cancer, the intestinal occlusion is the most frequent and it occurs especially in the case of tumors located in the left colon and rectum [4].

There are no clearly established protocols regarding the choice of the type of emergency surgery for colorectal cancer. Various standardizations have been tried, but no consensus has yet been reached [5]. The most debated issue is the choice of the optimal intervention for occlusive left colon cancers [6].

The choice of the surgical treatment method in an emergency is made according to several factors, such as the biological status of the patients, the duration of the complication, comorbidities, age, local invasion of the tumors, the presence of metastases, the appearance of the affected colon. Whenever possible, it is ideal to choose a method that involves the tumor resection or allows it in a second time [7].

The most recent recommendations of the WSES Scientific Committee indicate in the case of occlusive or perforated colon tumors to practice a right hemicolectomy with a terminal ileostomy. The right hemicolectomy with ileocolic anastomosis can be performed if there is no significant increase in operative time and good bowel vascularization. For left colon occlusive tumors, the Hartmann operation is considered the procedure of choice. In patients with severe instability, a colostomy should be performed. For perforations on the left colon, Hartmann's procedure is recommended, with the mention of considering the open abdomen [8].

PATIENTS AND METHODS

We included in the study a group of 431 patients admitted and operated in an emergency for complicated colorectal cancer in the Surgery II Clinic of the Clinical Emergency County Hospital “Sf. Ap. Andrei” from Galați, between 2008-2017.

The patients’ data were collected from observation sheets, operating protocols, and pathological exams. The following epidemiological and clinical factors were analyzed: age, sex, provenance area, hereditary-collateral history, abdominal surgical history, comorbidities, preoperative diagnosis, and clinical status at admission (weight, height, diuresis).

We analyzed the following paraclinical factors: the values of hemoglobin, hematocrit, leukocytes, platelets, and ionograms at admission. Among the therapeutic factors, we analyzed the tumor location, the invasion in other organs, the intraoperative appearance of the frozen pelvis, the presence of hepatic metastases and peritoneal carcinomatosis, the type of surgery performed in an emergency, lymph node dissection, liver biopsy, stoma reversal after Hartmann procedure.

The inclusion criteria were patients with colorectal malignancies hospitalized and surgically treated in the Surgery II clinic of the Clinical Emergency County Hospital “Sf. Ap. Andrei" from Galați, between 2008-2017, patients over 18 years old.

The exclusion criteria were patients with benign colorectal pathology, patients with malignant colorectal tumors operated electively, patients whose observation sheets no pathological reports were attesting the type of tumor, grading, and classification in the pTNM system were not found.

Study design

Epidemiological, clinical, paraclinical, and therapeutic factors were retrospectively analyzed, to correlate them with the four types of surgery (external derivations, Hartmann procedure, internal derivations, and resections with anastomosis).

Statistical analysis

Using the SPSS version 23.0, statistical correlations were obtained, indicating the p-value with the Pearson, Chi-Square, and Likelihood Ratio tests. Statistical conclusions were formulated using a statistically significant difference threshold the value $p < 0.05$ for all calculations performed.

RESULTS

In each of the 10 years included in the observation period of this study, an average of 43 patients was hospitalized and operated in an emergency.

There have been practiced 179 (41.5%) external derivatives (of which 158 colostomies and 21 Pezzer cecostomies), 134 (31.1%) Hartmann operations, 29 internal derivatives (of which 16 ileo-transverse, 9 transverse-descending, 2 ileo-sigmoid, 1 ileo-descending, and 1 transverse-sigmoid anastomosis) and 89 (20.9%) resections with anastomosis (of which 47 right hemicolectomies, 13 left hemicolectomies, 26 transverse/sigmoid/descending segmental colectomies, 1 subtotal colectomy, 1 ileo-transverse anastomotic resection for a relapsed tumor and 1 rectal tumor resection with mechanical anastomosis).

The sex distribution of patients in the study reveals the predominance of men, with a ratio M/F = 256/175. Comparing the types of surgery performed (1 - colostomy/ cecostomy, 2 - Hartmann, 3 - internal derivations, 4 - resections with anastomosis), no association with the sex of the patients is obtained.

In the studied group, the patients from the urban area predominated. Patients in rural areas were associated with a colostomy ($p<0.01$) (Table 1).

Most patients (55.92%, n = 241) included were in the 5th and 6th decades of age. An association of the advanced age of the patients with the colostomy is noted ($p<0.01$) (Table 1).

43 (9.97%) of the patients had a significant hereditary-collateral history, as follows: 19 had a brother/sister with colorectal cancer, another 18 patients had a father diagnosed with colon or rectal cancer, and six patients had a son with colorectal cancer. Patients with a significant heredocolateral history for colorectal cancer were associated with colostomy and with Hartmann's procedure ($p<0.02$) (Table 1).

174 patients (40.37%) had a personal history of abdominal surgery, as follows: appendectomy - 72 patients, hysterectomy - 31 patients, cholecystectomy - 29, gastric resections (for gastroduodenal ulcer disease) – 12, caesarean section - 8, splenectomy (post-traumatic spleen rupture) -6, nephrectomy (renal lithiasis) - 6, segmental colectomy (descending colon cancers) - 4, right hemicolectomy - 2, adnexectomy - 2, hepatic hydatic cyst operated - 1 patient and colon esophagoplasty (for accidental ingestion of sodium hydroxide in adolescence) -1 patient. Comparing the patients with or without a surgical history, no statistical associations are found with the type of surgery.

Most patients (51.04%) included in the study had comorbidities. 148 of the patients (34.33%) had associated cardiac pathology, of which 77 (17.86%) with high blood pressure, 38 (8.81%) with atrial fibrillation with chronic anticoagulant treatment, 8(1.85%) with a history of myocardial infarction, 13 (3.01%) with varying degrees of heart failure, 12 (2.78%) with ischemic stroke; 42 (9.74%) patients were diabetic; 9 (2.08%) patients were diagnosed with liver cirrhosis, with thrombocytopenia; 9 other patients had colonoscopically diagnosed colon pathology, 5 of whom had colonic polyposis, 3 with Crohn's disease and one with diverticulosis. 2 of the patients had active pulmonary tuberculosis at the time of admission (transferred from another hospital). We also found statistical association by correlating the type of surgery with the presence of heart disease and atrial fibrillation, but not with liver cirrhosis. Thus, in patients with cardiac disease or atrial fibrillation, type 1 surgery (colostomy) was performed ($p<0.01$), respectively ($p<0.01$) (Table 1).

The majority of patients (87.94%, n = 379) had as main symptoms at admission the abdominal pain and the disorders of intestinal transit, and the rest (12.06%, n = 52) presented with rectal bleeding. Regarding the beginning of the symptomatology, the majority of patients (58%, n = 250) had symptoms (intestinal transit disorders and rectal bleeding) that they neglected. In patients with neglected symptomatology, it was performed a Hartmann procedure or colostomies ($p<0.01$). Patients with abdominal pain and intestinal transit disorders were associated with colostomy/ Hartmann ($p<0.01$) (Table 1).

Figure 1: Perforated sigmoid tumor (Surgery Clinic II of the County Hospital of Emergency Clinic „Sf. Ap. Andrei „Galati)

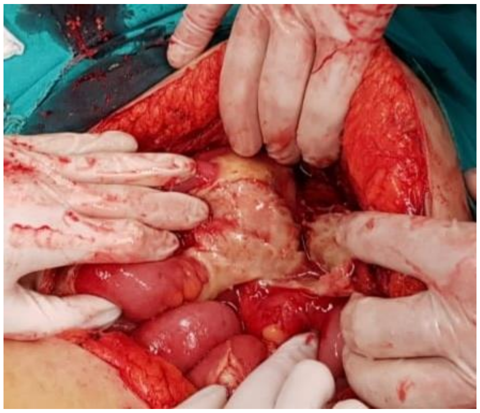

The preoperative diagnosis was intestinal obstruction in 332 patients (77.03%); 36 patients (8.35%) had intestinal perforation (tumor/diastatic) (Figure 1), 14 (3.25%) with imminent diastatic perforation (Figure 2) and 49 (11.37%) had rectal bleeding. Patients with hemorrhage or with imminent intestinal perforation (tumor/diastatic) at admission were associated with a colostomy; patients with intestinal occlusion were associated with internal derivations and resections with anastomosis. Patients with intestinal perforation were associated with the Hartmann procedure (p<0.01) (Table 1).

Table 1: Statistical correlations of the main types of surgery

	Type 1 (N=179 – 41.5%)	**Type 2 (N=134 – 31.1%)**	**Type 3 (N=29 – 6.7%)**	**Type 4 (N=89 – 20.9%)**	**p_value (test)**
Sex F M	 74/179 105/179	 47/134 87/134	 12/29 17/29	 42/89 47/89	0.341089 (1)
Age	72.35±12.5117	67.88±12.3811	62.72±10.2396	64.89±11.2426	0.000001 (2)
Area R U	 98/179 81/179	 52/134 82/134	 7/29 22/29	 20/89 69/89	0.000001 (1)
Number of days of hospitalization	10.0 [7.0, 15.0]	13.0 [10.0, 16.2]	14.0 [12.0, 16.5]	15.0 [12.0, 17.0]	0.000000 (3)
Abdominal pain=yes	135/179	133/134	29/29	83/89	0.000000 (1)
Intestinal transit disorders=yes	135/179	133/134	28/29	81/89	0.000000 (1)
HCA=yes	10/179	20/134	1/29	12/89	0.017821 (1)
Surgical abdominal antec.=yes	81/179	50/134	13/29	30/89	0.243466 (1)
History=yes	118/179	87/134	11/29	31/89	0.000001 (1)
Anemia=yes	134/179	76/134	22/29	67/89	0.002115 (1)
Anemia transfusions=yes	55/179	27/134	2/29	23/89	0.018961 (1)
Leukocytosis=yes	13/179	33/134	4/29	13/89	0.000342 (1)
Trombocytosis=yes	4/179	5/134	0/29	0/89	0.092185 (4)
Cachexia=yes	80/179	36/134	4/29	8/89	0.000000 (1)
HE disorders=yes	82/179	54/134	7/29	27/89	0.029122 (1)
Oligoanuria=yes	72/179	35/134	2/29	5/89	0.000000 (1)
Preop. diagnosis H I O P	 42/179 11/179 120/179 6/179	 1/134 3/134 106/134 24/134	 0/29 0/29 29/29 0/29	 6/89 0/89 77/89 6/89	0.000000 (4)
Tumor location Ascendant Descendant Rectum Sigmoid Transverse	 1/179 6/179 149/179 19/179 4/179	 0/134 23/134 0/134 102/134 9/134	 14/29 12/29 0/29 0/29 3/29	 45/89 16/89 2/89 12/89 14/89	0.000000 (4)
Tumor invasion=yes	69/179	26/134	22/29	9/89	0.000000 (1)
Synchronous tumors=yes	1/179	0/134	0/29	2/89	0.232949 (4)
Frozen pelvis=yes	38/179	5/134	0/29	2/89	0.000000 (1)
Hepatic MTS=yes	37/179	23/134	16/29	5/89	0.000000 (1)
Carcinomatosis=yes	9/179	2/134	2/29	0/89	0.019195 (4)
Cardiac diseases=yes	83/179	38/134	7/29	20/89	0.000133 (1)
FiA=yes	26/179	7/134	2/29	3/89	0.005035 (1)
HC=yes	4/179	5/134	0/29	0/89	0.092185 (4)
HP ADK GIST	 179/179 0/179	 134/134 0/134	 28/29 1/29	 89/89 0/89	0.142874 (4)

Grading					
1	60/179	26/134	1/29	28/89	0.002476 (1)
2	104/179	97/134	22/29	52/89	
3	15/179	11/134	6/29	9/89	
Mucoid=yes	6/179	3/134	1/29	6/89	0.411940 (4)
pTNM					
2	20/179	51/134	0/29	35/89	0.000000 (1)
3	120/179	59/134	12/29	50/89	
4	39/179	24/134	17/29	4/89	

Figure 2: Rectosigmoid tumor with necrosis and imminence of diastatic perforation (Surgery Clinic II of the County Hospital of Emergency Clinic „Sf. Ap. Andrei „Galati)

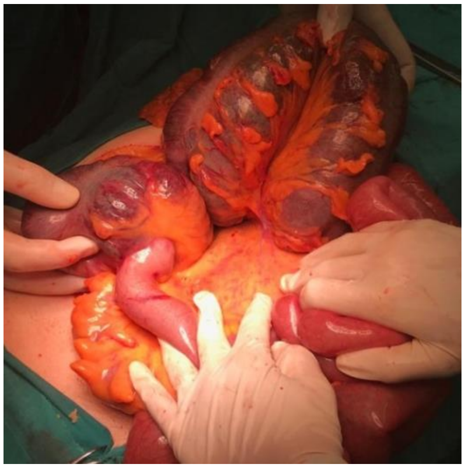

All patients benefited from preoperative imaging investigations, such as empty abdominal radiography and for those with a clinical diagnosis other than intestinal obstruction, abdominal ultrasonography was performed.

128 patients (29.69%) presented with cachexia when admitted (BMI <18.5). 114 patients (26.45%) had oliguria at admission (diuresis <= 500 ml). We found statistically significant differences in the cases of cachectic and oliguric patients at admission. In these patients, the colostomies were mainly practiced and only in very few cases the internal derivations/resections with anastomosis were chosen (p<0.01) (Table 1).

299 (69.37%) of all patients had anemia at admission. Of these, 107 required full blood transfusions or erythrocyte mass. According to the reference interval of our laboratory, we considered anemia values of hemoglobin less than 11g/dl and hematocrit less than 35%. Statistical analysis revealed the association of patients with anemia with a colostomy, and Hartmann's operations (p<0.01); as well as patients with anemia with blood transfusion with a colostomy, or internal derivations (p<0.02) (Table 1).

63 patients (14.61%) had leukocytosis at admission (a number of leukocytes> 10,000/µl). Thus, patients with leukocytosis were associated with Hartmann's interventions (p<0.01) (Table 1).

170 patients (39.44%) had hydro electrolytic disorders at admission (especially hypopotassemia). We considered that the patients with hydro electrolytic disorders were those whose ionograms on admission showed values of Na <136 mmol/l and potassium values <3.5 mmol/l. Patients with hydro electrolytic disorders were associated with a colostomy (p<0.03) (Table 1).

During the emergency surgery, in 13 cases (3.01%) the presence of peritoneal carcinomatosis was found (Figure 3). Peritoneal carcinomatosis has been associated with a colostomy (p<0.02). 81 (18.79%) patients had liver metastases, found either on preoperative ultrasound examination or the occasion of laparotomy (in most cases). Patients with liver metastases were associated with internal derivations (p<0.01) (Table 1).

Figure 3: Ascendant colon tumor, peritoneal carcinomatosis (II Surgery Clinic, Sf. Ap. Andrei Clinic Emergency County Hospital Galati)

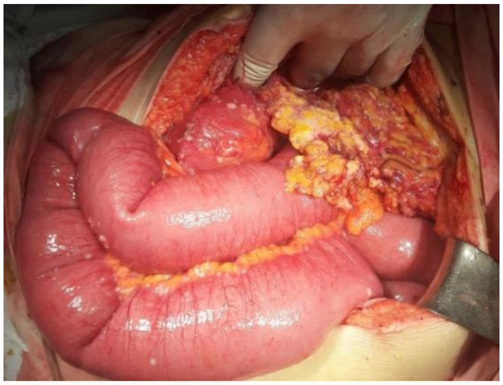

45 (10.44%) of the patients had "frozen pelvis" (tumor block comprising other pelvic organs, to which there is firm adherence, with very difficult or non-existent cleavage planes, to which small bowel loops adhere). Patients with frozen pelvis were associated with a colostomy (p<0.01) (Table 1).

Regarding the location of tumors, 60 (13.92%) were located at the level of the ascendant and hepatic angle of the colon, 30 (6.98%) at the level of the transverse colon, 57 (13.22%) at the level of the descending and splenic angle of the colon, 133 (30.85%) in the sigmoid colon and recto-sigmoid junction and 151 (35.03%) were rectal tumors.

For the tumors of the ascending colon and hepatic angle, in most cases, resections with anastomosis (right hemicolectomies) were practiced, followed by internal derivations and cecostomies (p<0.01), the patients with tumors located on the descending colon and the splenic angle mainly benefited from internal derivations or Hartmann's interventions (p<0.01), for patients with tumor location in the rectum, colostomies were practiced (p<0.01) and for the transverse colon locations, resections with anastomosis (p<0.01) were performed (Table 1).

In 126 cases (29.23%) we encountered tumors invading other organs: 27 invasive tumors in the prostate, 26 cases with tumors that invaded the small intestine, 18 invasive tumors in the gastrocolic ligament/large epiploon, 13 in the vagina, 11 in the abdominal wall, 10 in the sacrum bone, 6 tumors were invasive in the bladder, 4 in the duodenum (+/- head of the pancreas) and spleen, 3 invasive tumors in the right/left annexes, 2 in the left renal capsule and 2 tumors that invaded the pelvic ureter. The subset of patients with invasive tumors in other organs was associated with internal derivations (p<0.01) (Table 1).

Data from the histological reports revealed the predominance of adenocarcinomas (99.76% of cases) and only one GIST tumor. 16 (3.71%) of the patients had tumors with a mucoid component.

Regarding tumor grading, 115 (26.68%) were well-differentiated adenocarcinomas (grade 1), 275 (63.8%) medium differentiated tumors (grade 2) and 41 (9.52%) weak tumors differentiate (grade 3). Well, and medium differentiated tumors were associated with colostomies or with resections with anastomosis (p<0.01).

For postoperative staging, we used the classification recommended by The Union for International Cancer Control (UICC). Thus, according to pTNM staging, tumors in stages II and III predominated - 106 (24.59%), tumors in stage II, 241 (55.91%) in stage III, and 84 (19.5%) in stage IV. The patients in stage II were associated with resections with anastomosis or Hartmann operations (p<0.01), patients with tumors in stage III were associated with colostomies (p<0.01), and patients in stage IV were associated with internal derivations and colostomies (p<0.01) (Table 1).

DISCUSSIONS

According to literature data, up to 30% of colorectal cancer patients present with complicated tumors in emergency [9]. Despite many standardization attempts, the best surgical option for patients with complicated colorectal tumors is still under debate. [5, 10]

We have shown in this study that the type of surgery in patients with complicated colorectal cancer correlates with: age, provenance area, associated cardiac diseases, history of the disease, cachexia at admission, oliguria and hydro electrolytic disorders, diagnosis, presence of liver metastases, and frozen pelvis appearance, tumor location, tumor invasion in other organs and staging.

There is no consensus regarding the surgical treatment of the elderly affected by colorectal cancer, but it is generally accepted that patients over 80, who present in an emergency, compared to the younger ones, have a higher risk of postoperative complications and mortality, despite the progress made in preoperative care, surgical techniques, and intensive care.[11] Most authors reported significant correlations between the type of intervention and the age of the patients [6, 12], as we found in our study, but different from the data obtained by other authors.[13]

Several authors found significant correlations between the type of operation and the patients coming from the rural area [14], as we found in our study. Most authors agree that patients in rural areas have lower addressability due to more difficult access to medical services and therefore tend to show up later, with more advanced tumors.[15, 16]

The comorbidities, especially the cardiac ones, are associated in many studies with the practice of non-curative interventions in patients with complicated colorectal cancer.[17, 18] We also found this correlation in our study.

The pathophysiology of cachexia syndrome is characterized by negative energy and protein balance. In neoplastic patients, it is a major cause of morbidity and mortality [19-22]. A recent study, which included 235 patients with advanced colorectal cancer, reaffirms the negative impact of cachexia on the evolution of the patients with complicated colorectal cancer.

Oliguria is considered a specific and sensitive biomarker of renal dysfunction and a good predictor of mortality risk in critically ill patients, including complicated colorectal tumors.[24, 25] For patients with complicated colorectal cancer, with oligoanuria at admission, colostomies are performed, according to several reports [26, 27], as we found in our study.

Neoplastic patients frequently present with hydroelectrolyte balance disorders. These occur during tumor growth, because of inadequate absorption of electrolytes or because of the secretion of metabolically active substances by the tumor [28]. The hydroelectrolyte disorders present at admission are associated with the type of surgery performed in patients with complicated colorectal cancer [29], as we have shown in our study.

The liver is the most common location of secondary dissemination of primary colorectal tumors. Between 10% and 25% of colorectal cancer patients have liver metastases at the time of surgery [30]. Despite all the therapeutic progress made in recent years, the presence of liver metastases remains a negative prognostic factor.[31] Most of the time, these patients have advanced tumors and the only viable option in an emergency is a colostomy, which will allow them to survive for several months.[32] We made the same finding in our study.

The type of surgery is associated with the preoperative diagnosis, which, most commonly, is the intestinal obstruction. The therapeutic attitude in these cases depends on the location of the primary tumor, the stage, the general state of the patient, and the local changes (tumor resectability, colic distention, ischemic lesions of the colic wall, integrity of the ileocecal valve).[33, 34]

For perforated tumors, Hartmann's intervention is the recommended option in most published works [35-37], as we have shown in our study. For hemorrhagic tumors, the options are different depending on the location of the tumor.

In our study, most hemorrhagic tumors were rectal, and the emergency surgical option was most often a colostomy, after a local bleeding control. In the literature, there is also mentioned the practice of arteriographic embolization in these situations [38], a technical possibility that we did not have.

Patients with locally advanced colorectal tumors, with invasion in other organs, can be a therapeutic challenge [39-41].In most cases, in these situations, a terminal colostomy is practiced [42], which also happened in our study group. Other authors have considered the practice of plurivisceral resections in these situations.

The first such series was reported by Sugarbaker in 1946.[43] Croner et al. performed a study on 174 patients who underwent plurivisceral resections during the period 1978-2002 [44]. Most of the patients had sigmoid colon cancers and the most commonly invaded organs were the small intestine, the bladder, and the abdominal wall.

The type of emergency surgery performed for complicated colorectal cancer is significantly statistically correlated with the colorectal tumor location in all the analyzed studies. Most authors consider, as in our study, that single-phase procedures for left colon tumors are not indicated in emergency and that in these situations the Hartmann procedure is preferable.[45, 46] But some authors believe that, including for occlusive left colon tumors, the first option should be a resection with anastomosis [7, 13, 47, 48]. For the complicated tumors located on the right colon, there seems to be unanimity regarding the surgical option, in these cases the right hemicolectomy being the option.[8, 49]

The TNM staging proposed by the AJCC has remained a universal model since its first edition in 1977 [50]. Since then, the guides have been revised several times and the last update was proposed in 2018 [51]. In our study, stage II of disease correlated with Hartmann's operation and stage IV with a colostomy, as we found in other works.[52, 53]

CONCLUSIONS

1) The colostomy was associated with: older age, rural area, associated cardiac diseases, especially atrial fibrillation, neglected symptomatology, cachexia, oliguria, anemia, and hydro electrolytic disorders at admission, tumors complicated with hemorrhage or with imminent diastatic perforation, the intraoperative aspect of the frozen pelvis, rectal tumors, well or medium differentiated tumors, advanced stages of disease (III or IV pTNM).

2) Hartmann's operations were associated with the significant hereditary-collateral history, neglected symptomatology, intestinal transit disorders and abdominal pain upon admission, leukocytosis, intestinal perforation, sigmoid, and recto-sigmoid junction tumors, and stage II TNM.

3) Internal derivations were associated with the presence of intestinal occlusion at admission, liver metastases, and invasion in other organs, with tumors of splenic angle or descending colon and with stage IV disease.

4) Resections with anastomosis per primam were associated with the absence of comorbidities, tumors located at the level of the ascendant colon, hepatic angle, or transverse colon and well-differentiated tumors.

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REVIEW ARTICLE

Emerging concepts regarding the molecular profile of breast carcinoma: one-year experience in a University Center

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Abstract: *Breast carcinoma represents the leading cause of oncologic mortality for women. Due to therapeutic and diagnostic advances, the mortality and morbidity in the last decade declined, but breast cancer still has a great impact on quality of life and also on medical service cost. In the light of these facts, an integrated approach, considering histopathology features, molecular profiles and corroboration with clinical and imagistic data is necessary. A descriptive retrospective one-year study analyzed the breast cancer heterogeneity in 121 cases registered in the Pathology Department of the University Emergency Hospital in Bucharest, Romania. Our purpose was to evaluate histopathological, immunohistochemically, clinical and imagistic aspects of breast cancer considering the current molecular classification (Luminal A, Luminal B, HER2 enriched and triple negative/basal like).*

Our assay revealed that most prevalent histotype was NST (no special type) followed by invasive lobular carcinoma. Considering the molecular pattern, the most common was luminal B. Triple negative basal like and HER 2-enriched were correlated with an aggressive morphologic pattern and lymph-nodes positivity. Considering the imagistic acquisitions, mammography proved to be the most accurate technique for measuring the dimension of NST.

In conclusion, breast carcinoma is a heterogeneous disease that needs an integrated approach and personalized treatment based on the histopathologic and molecular features. Considering the great number of advanced stages as diagnosis, a national screening program for breast cancer is imperiously needed.

Keywords: *breast cancer, NST, ILC, IHC, molecular subtypes, biopsy*

INTRODUCTION

According to the most recent statistic database of WHO 2015, breast carcinoma represents the main cause of mortality for Romanian women [1]. The decline of breast cancer mortality is a trend that, according to the most recent epidemiologic estimation will continue [2]. This direction is mostly due to advancement in diagnosis and treatment [3].

A better understanding of pathogeny and a more accurate diagnostic approach of breast cancer led to several updates in guidelines for classification and treatment. Since 2003, for grading breast carcinomas, the Elston Ellis semiquantitative method modified score was used according to the 3rd edition of WHO guidelines for classification the breast tumours. The most recent WHO diagnos-

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tic guideline (4th edition, 2014) considers that for prognostic and predictive factors a panel of antibodies is needed for a further classification into molecular subtypes (luminal A, luminal B, Her2 enriched and triple negative basal-like). This approach is supported by multiple studies and summarized in the most important European or American conferences or guidelines. For example, the 14th St. Gallen International Breast Cancer Conference, 2015 [4], considers breast carcinoma a heterogeneous pathology according to its intrinsic molecular types whereas the 8th edition of AJCC (American Joint Committee on Cancer) states that breast malignant tumours should be staged according to the prognostic group, based on population that had been offered and mostly treated with appropriate endocrine or systemic therapy which includes anatomic T, N and M plus grade and status of HER2, ER, PR. [5]

MATERIAL AND METHOD

The aim of this retrospective analysis was to offer an exhaustive perspective of breast cancer considering clinical and imagistic presentation, morphological and molecular profiles of the main mortality factor for women. We analyzed the breast cancer heterogeneity in 121 cases registered as mastectomies, lumpectomies, quadrantectomies or biopsies in the Pathology Department of the University Emergency Hospital in Bucharest, performed over a period of 1 year (1st January 2017-31st of December 2017). The diagnostic varied from benign lesions (fibroadenoma, fibroadenomatoid mastopathy) to the most common malignancies, NST carcinoma and even rare cases like carcinoma with medullary features, mucinous, adenosquamous, papillary carcinoma or Phyllodes tumor.

For clinical data and imagistic interpretation, we used the hospital electronic database. The histologic specimens were studied using both conventional histopathological examination with HE stains and also immunohistochemically methods, classifying them further in the main 4 surrogate-molecular subtypes: luminal A, luminal B, HER2-neu non-luminal and basal-like/triple-negative. For diagnosing the main histologic subtypes, the last WHO guideline criteria were applied. For data collection and processing Microsoft Office Excel 2010 was used.

Table 1. Frequency of the surgical procedures used for the histopathological diagnoses in the analyzed population

Type of lesion	Mastectomy + axillary in dissection	Lumpectomy	Core needle biopsy	Total
NST	40	19	19	78
NST with papillary features		1		1
Invasive lobular carcinoma	4	1	3	8
Medullary carcinoma	1			1
Adenosquamous carcinoma	1			1
Phyllodes tumour	1			1
Adenomioepithelioma		1		1
Metastasis of B cell lymphoma		1		1
Benign lesions, non-inflammatory	5	5	1	11
Fibroadenomas	1	8	2	11
Benign lesions, inflammatory	1	3		4
Metastasis of breast carcinoma			3	3
Total	54	39	28	121

RESULTS

This one-year retrospective study analyzed 121 cases female patients registered in Pathology Department of the University Emergency Hospital in Bucharest with diagnostic of benign lesions and also with malignant pathologies. Those included carcinomas, with NST histotype being the most common (78 cases), ILC (8 cases) and also rare types. Detailed information is found in Table 1.

We focused our attention on the most common types of breast carcinoma: NST and ILC. In our study the most common histotype of breast carcinoma was NST, with a predominance of G2 grade of differentiation (59%), followed by an equal percentage of G1 and G3 (8% for each one).

In the absence of precise data about menstrual status, we considering the climax age at 50 years old. With this presumption, most women were postmenopausal (79%), and the average age at presentation was 61.48 years, varying from 35 to 86 years old. Distribution of the analyzed patients according to their age, histotype and molecular profile of their tumor is detailed in Table 2.

Table 2. Age distribution according to the main histotype, grade of differentiation and molecular profile of the most important histotypes of breast carcinoma

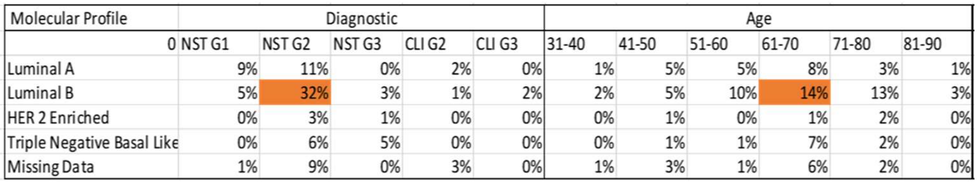

Molecular Profile	Diagnostic					Age					
0	NST G1	NST G2	NST G3	CLI G2	CLI G3	31-40	41-50	51-60	61-70	71-80	81-90
Luminal A	9%	11%	0%	2%	0%	1%	5%	5%	8%	3%	1%
Luminal B	5%	32%	3%	1%	2%	2%	5%	10%	14%	13%	3%
HER 2 Enriched	0%	3%	1%	0%	0%	0%	1%	0%	1%	2%	0%
Triple Negative Basal Like	0%	6%	5%	0%	0%	0%	1%	1%	7%	2%	0%
Missing Data	1%	9%	0%	3%	0%	1%	3%	1%	6%	2%	0%

Most patients addressed to surgery or Obstetrics-Gynecology Departments of the University Emergency Hospital in Bucharest for painless tumor masses. We also noticed sporadic cases of ulcerated bleeding tumors. A single patient presented for asymptomatic tumor incidental discovered with mammographic screening.

A demographic brief analysis revealed that most patient came from urban areas, with a percentage of 56% for the latter and 44% for rural areas; taking a closer look, we observed that the majority of advanced stages (III and IV) were more frequent in women with urban provenience (10.7% for stage III respective 4.7% for stage IV) in comparison to rural population that addressed to our hospital (1.2% stage III and 0.6% for stage IV).

Regarding clinical data obtained from hospital database, we noticed that the metabolic profile of a majority of analyzed women was impaired, most of them being overweight and obese, only a quarter bearing a normal weight, as the Figure 1 reveals. More than half of studied patients suffered dyslipidemia and experienced cardiovascular complications (essential hypertension and atherosclerosis were most noted). A subgroup also registered a history of gynecologic or endocrine pathology but not enough for a trustable correlation.

Figure 1. BMI of analyzed cases

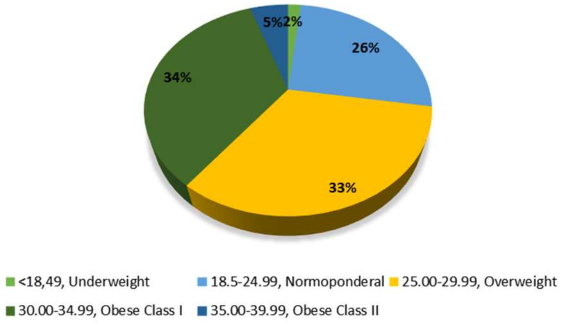

When we put side by side the matric data of different types of investigations (ultrasonography, mammography at CT) with histopathological measurement (macroscopically and microscopically evaluation) we noticed that the highest accuracy in appreciating the dimension of breast lesions is radiologic examination (mammography and CT), followed by ultrasonography which tend to underestimate most of lesions. Considering the main histotypes, the most difficult breast tumor to be precisely measured in conventional imagistic methods is invasive lobular carcinoma, in accordance with its manner of infiltrating the mammary tissue.

With the available data, we observed that invasive breast carcinomas were all scored with BIRADS 5 or 6, a single case of NST G1, luminal A, T1c being underappreciated as 3.

Figure 2. Molecular profile of breast carcinoma according to histotype (%)

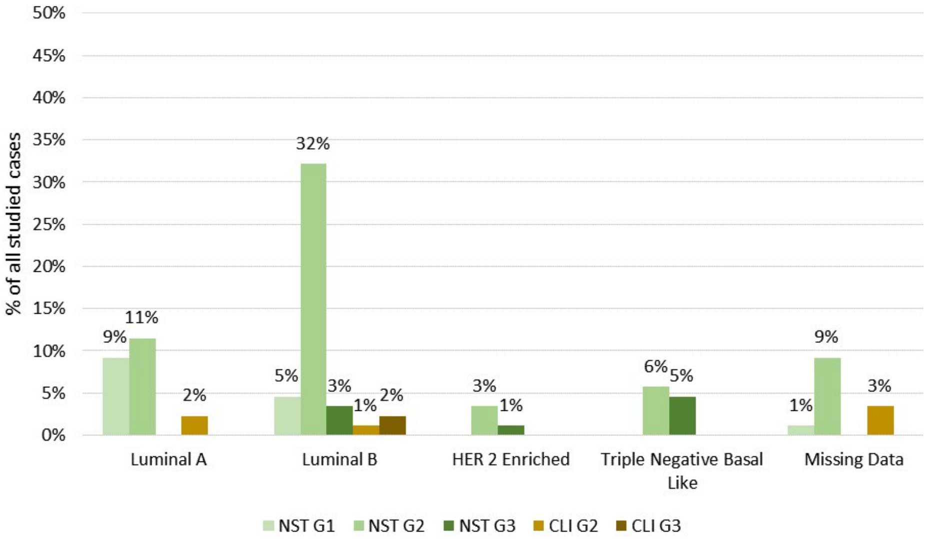

In relation to histologic examination of breast cancer, formalin fixed-paraffin-embedded tissue were studied using both conventional histopathological examination including HE stains and also immunohistochemically methods, classifying them further in the 4 main surrogate-molecular subtypes: luminal A, luminal B, HER2-neu non-luminal and basal-like/triple-negative. For diagnosing the main histologic subtypes, the last WHO guideline criteria were used.

When we evaluated breast cancer histotypes and their molecular pattern, we observed that the most prevalent is luminal B with a pick incidence in NST grade 2, followed by luminal A, also most prevalent for NST G2.

The oncologic stage according to WHO 2014 for HER 2 enriched and triple negative varied between IIB and IV. We did not find any single case in stage I. We also observed that for luminal A category the majority of cancer were in stage I and II. An interesting aspect is registered for luminal B, the most frequent molecular type of our statistic. We can easily observe that it has the highest frequency in most of cases, but not in stage I of disease. A more explicative illustration of the preponderance of tumor stages according to the 7th edition of UICC for TNM classification of malignant tumors, in relation to their molecular profile is highlighted in figure 3.

Figure 3. Molecular profiles of tumor stages related to the molecular profile

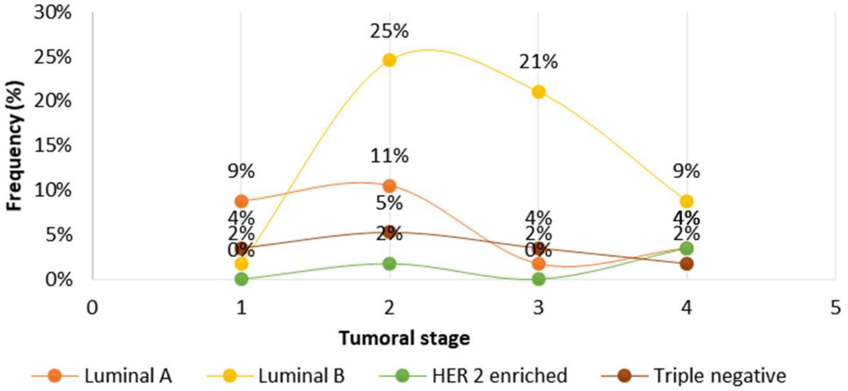

Figure 4. Regression between ER expression and KI 67 in luminal A and luminal B breast carcinomas

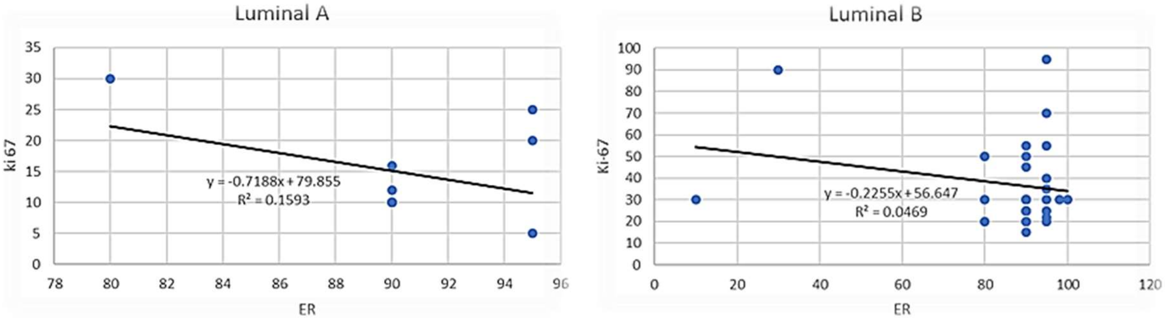

Considering the hormonal expression in the luminal type carcinomas and their proliferation index, we observed the regression between ER expression and the mitotic index evaluated by Ki 67 in luminal molecular profiles. For luminal A there is a better correlation between a higher expression of ER and a lower Ki 67 (R2=0.159) comparing to luminal B (R2=0.046), as in the Figure 4 can be easily seen. The juxtapose of the two types was not only useful for understanding the variation of hormonal variation regarding the proliferation rate, but also necessary considering the limited number of analyzed cases.

In our study, no invasive lobular breast carcinoma was identified among non-luminal carcinomas. We registered more triple negative than HER 2 enriched molecular subtype, and their prevalence were highest in climax women. As expected, their histologic features were more aggressive, with large areas of necrosis, infiltrative pattern of growth, and large numbers of mitoses, none of those being graded as well differentiated.

DISCUSSION

Breast carcinoma is not a single disease, it has a large panel of shapes, clinical manifestations, imagistic and histologic representations. The current WHO guidelines for breast carcinoma counts 37 different coded histotypes, but this classification it is far from being adequate for proper treatment [6]. Tumors with identical clinical and pathological presentations may have different behaviors. Beside the TNM classification and grading method (today the Nottingham histologic score system is universally accepted), the interest for treating in the most efficient and precise way each particular biological form of disease gave birth to the molecular subtype classification. This procedure evaluates the hormonal expression of tumor tissue (ER, PR), presence or absence of HER2 protein and levels of Ki-67.

We started our analysis by looking on the preferred diagnostic procedure, and in some cases, it was similar with the surgical treatment. The method of choice for the majority of invasive breast carcinoma clinically diagnosed is represented by mastectomies with axillary lymph node dissections were. Most of these procedures started with a frozen section for certifying the malignant nature of the lesion. After the frozen section, surgical interventions ended with mastectomy and lymph node dissection. Less than half of analyzed women received a neoadjuvant chemo-radio-therapy after core needle biopsy and came back for completing the surgical treatment.

The concordance of core needle biopsy and surgical excision specimen for diagnosing the histotype and hormonal profile of the tumor was proofed to be safe in large studies [7, 8]. According to ESMO, assessment of primary tumor includes: physical examination, mammography, breast ultrasound for lymph node determination, breast MRI and core biopsy with pathology determination of histotype, grade, ER, PR, HER-2 and Ki67 status. If preoperative systemic therapy is planned, a core needle biopsy is mandatory to ensure a diagnosis of invasive disease and assess biomarkers (recommendation IIIA) [9].

Figure 5. NST HER2 enriched carcinoma a. Numerous atypical mitosis HE 40X; b. perineural invasion, HE 40X; c. Intravascular tumor emboli; d. Lymph node invasion, HE 10X; e. HER2 intensely positive, scored 3+, 20X; HER2 negative, score 0, 20X.; f. Bone metastases with GATA 3 positivity suggesting breast origin, 20X.

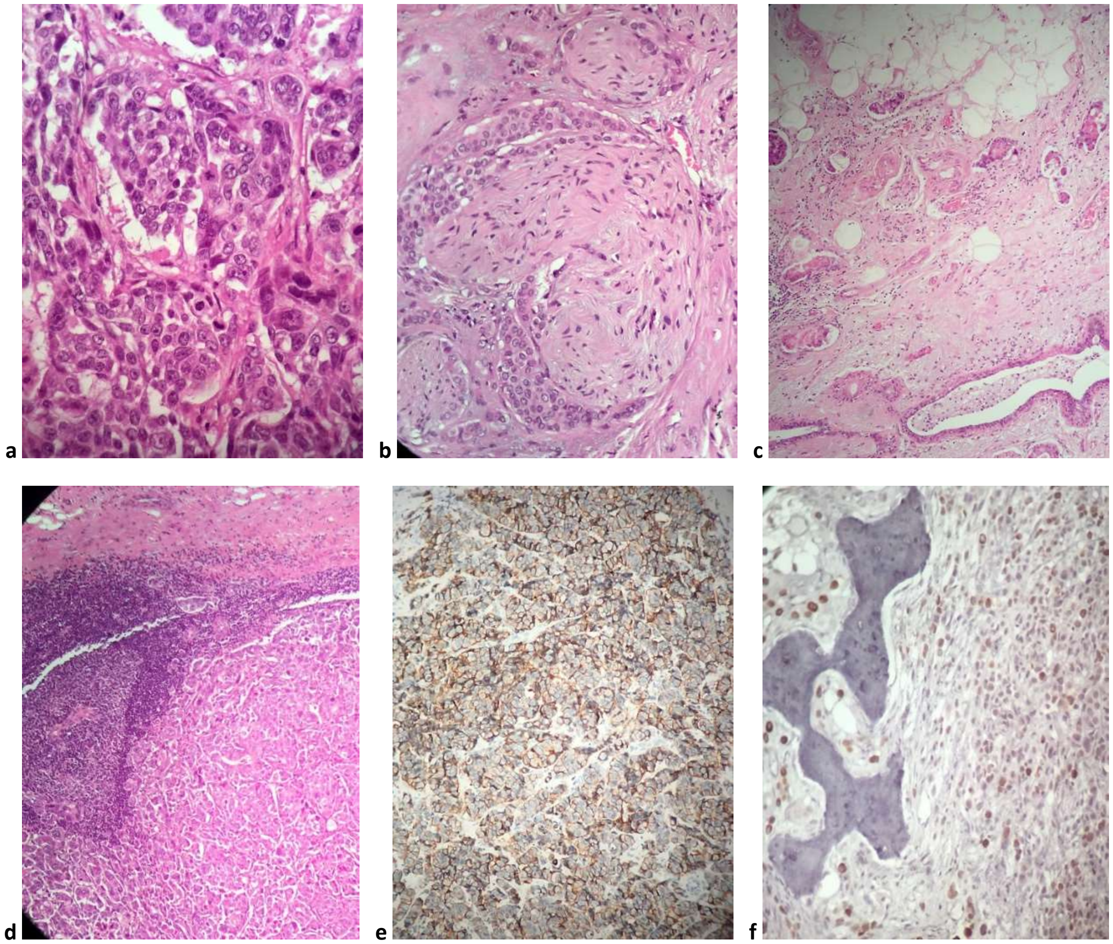

Since it is not yet a standardized method, CNB does not represent a IA indication for non-operative diagnostic. Even if the Royal College of Pathology [10], the WHO guidelines for breast cancer and AJCC 8th edition [5] enclose certain recommendation for reporting the results of a CNB, this practice is controversial regarding the rate of metastasis [11], the reliability of histological grade considering the dimensions of collected fragments [12], the limitation in diagnosing certain subtypes of carcinoma (e.g. mucinous carcinoma) [13], and even rise the problem of “vanishing carcinoma”, when tumor cells are present on CNB but absent in the surgical specimen [14];

We focused our attention on the most frequent histotypes of breast tumors, NST and ILC. The rare types of malignancies like papillary carcinoma, medullary carcinoma, Phyllodes tumor or metastasis to the breast are not well represented in this one-year study and for the sake of statistic relevance, we choose not to include them in our calculation. Also, the benign lesions (fibroadenoma, adenomyoepithelioma, abscesses or fibrocystic disease) were not relevant for the heterogeneity of breast carcinoma.

The interesting demographic results that we obtained underlines that the majority of severe stages of disease (stage III and IV) were addressed from urban population. This trend is not observed in other studies made in Australia [15], Africa [16] or even in multiethnic and multiracial population [17], but in our opinion, these unexpected results may appear because of many reasons: probably the most important one is the limited number of cases that were included in our analysis. Other reasons may consider the higher addressability of urban women to private health system, the migration of urban population to the country side or a better surveillance of patients from rural area by their family doctor. On a deeper assessment, it is easily noticed that most of our luminal B cases had a high Ki 67 index (above 20%), only a third of them being HER2 positive or equivoque on IHC. A possible explanation could be the absence of a -ISH method in our department for a precise assessment of HER 2 mutation. Anyway, considering the limited period and number of analyzed cases, for a more accurate distribution of luminal subtypes more studies are needed.

Taking into account hormonal expression, using regressions, we observed that for luminal A molecular subtype the higher the estrogen receptor expression is, the lower the proliferation index is (quantified by Ki 67); in comparison, for luminal B subtype p-value was only 0.0469.

Regarding the age, the highest amount of breast cancers appeared in the 6th decade of life, with a considerable delimitation between pre- and post-menopausal age. Even so, in the 3rd decade we registered only luminal subtypes of cancer (considered to have a better prognosis) but in the 4th decade we registered all the molecular subtypes.

Regarding clinical correlation, we became aware of the impaired weight of our studied group, most patients being overweight, with a medium BMI of 27.73. Also, we noticed that a great proportion of studied women who underwent a diagnostic biopsy and returned after approximately 3 months for surgical treatment gained extra kg, their BMI being augmented. This observation could be an alarm signal for a better nutritional surveillance and psychologic management of oncologic patients, considering that obesity it is an important risk factor for malignancy, comorbidities and mortality [18 - 20].

Unfortunately, our clinical correlation was limited because we did not had access to variables like family and history of menstruation, oral contraceptive use, age of first full-term pregnancy, number of children, duration of lactation or hormonal replacement therapy.

CONCLUSIONS

In our study, primary malignant epithelial tumors of the breast were NST with a moderate grade of differentiation with an age of incidence ranging from 3rd to 8th decade, being most prevalent in the 6th decade. Regarding molecular classification, luminal B was more common than others, with a wide clinical and histologic appearance; it was found in all analyzed histotypes and in all pathologic stages of disease. Because of its predominance, most of severe cases we registered (metastatic breast disease, ulcerated tumors) were also luminal B subtype. We admit the limitations of our study (few cases in a single year in a single hospital with emergency profile) but we are convinced that for a better breast cancer management, keeping in mind the most important aim, a lower mortality and morbidity, a proper national screening program is of an imperative necessity.

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ORIGINAL ARTICLES

Endoscopic treatment in carpal tunnel syndrome

Hazim Elayan[1], Jawad Latif[1], Ana M. Oproiu[2], Radu C. Jecan[2], Ioan P. Florescu[2]

Abstract: *Carpal tunnel syndrome is currently the most common peripheral nerve compression neuropathy that affects approximately 1% of the population [7,8,9]. The incidence of CTS seems to be increasing. The diagnosis is based on clinical symptoms and electrodiagnostic studies.*

Keywords: *endoscopic, carpal tunnel, median nerve, compression*

INTRODUCTION

Definition

Carpal tunnel syndrome is the compression of the median nerve in the wrist (radiocarpal joint and palm) and the most common neuropathy in the upper limb. Paresthesias, especially nocturnal pain, numbness and weakness of the hand, tingling, atrophy of the thenar muscles are the most common clinical signs of CTS in addition to the Tinel sign, the Phalen and Durkan test, ultimately leading to severe damage to the hand and forearm [1].

Anatomy

The Carpal tunnel consists of the carpal bones and the transverse carpal ligament that forms the roof of the tunnel and extends from the hamat bone (hook bone) and triquetrium (pyramidal bone) on the ulnar side to the scaphoid and trapezium on the radial side. The carpal bones are located in 2 rows (from radial to ulnar); proximal row: scaphoid, lunate, triquetrium, and pisiform bone, distal row: trapezium, trapezoid, capitate, and hamat. Through this canal pass the median nerve which is accompanied by the artery of the median nerve wrapped in a "perinerv" sheath and the flexor tendons which are wrapped by the synovial sheath which helps the proper functioning of the flexor apparatus under normal conditions. (long thumb flexor, four superficial flexors of the fingers, and four deep flexors of the fingers).

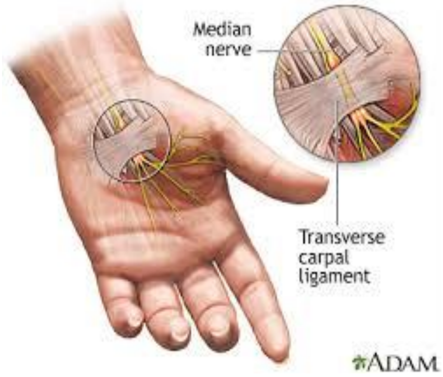

Also at the wrist, there is a canal where the ulnar vascular-nervous package passes called the Guyon canal, which may or may not be affected at the same time as the carpal tunnel syndrome.

Etiology

Among the causes of CTS are: idiopathic, hereditary,

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infectious, endocrinological, metabolic, hematological, renal, rheumatic, traumatic, tumor.

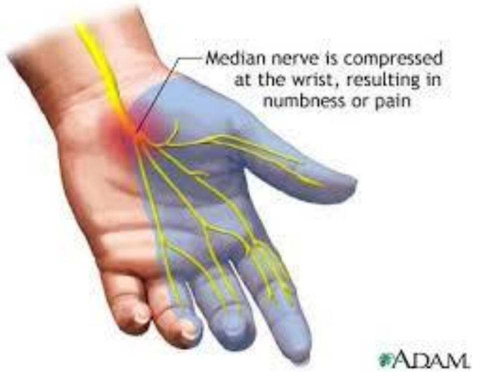
Median nerve is compressed at the wrist, resulting in numbness or pain
ADAM.

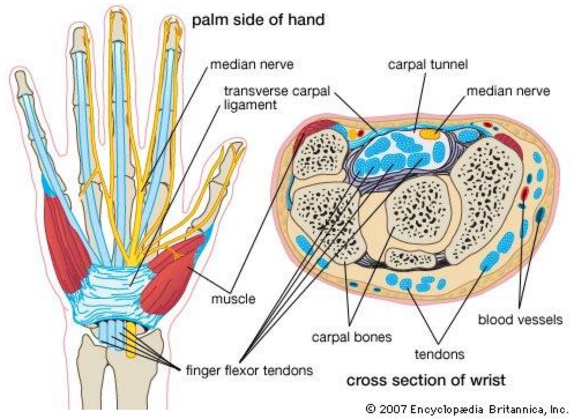

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Diagnostic

It is put in principle on the clinical signs, the Tinel sign, the Phalen and Durkan test, then it is passed to the paraclinical analyzes: electromyography (EMG), nerve conduction speed, radiology, ultrasound, MRI, laboratory blood tests.

The costoclavicular Adson and Wright tests are used to exclude chest compression syndrome that can mimic CTS. Cervical examination, including the Spurling test, is essential to exclude cervical radiculopathy. The percussion of all the primary peripheral nerves can lead us to an unexpected area of compression [2].

A study of nerve conduction speed and electromyography is performed. They help us to confirm the diagnosis of CTS and to exclude other patho-logy[3].

Basic x-rays of the wrist are useful in detecting unexpected pathology.

TREATMENT

Over time, several treatment methods have been tried; conservative by rest and immobilization, treatment with topical or infiltrated anti-inflammatory drugs in the carpal tunnel (in rare cases), and surgical treatment by a classical or endoscopic technique that have proven effective compared to conservative treatment which is considered a way of delaying surgery; Surgical treatment by classical technique involves a curved longitudinal incision parallel to the tenerian crease for prolonged sometimes proximal zigzag to forearm through the flexion creases of the wrist to relax the forearm fascia, external or internal neurolysis, sinevoectomy or remove any formations that can compress the median nerve, sectioning TCL from proximal to distal on the ulnar side to avoid injury to the thenar motor branch, hemostasis control, skin suture, dressing, and immobilization. Risks including pillar pain, weakness in the hand, sensitivity to scarring, delayed return work and slower return to office, relatively long recovery period, and the risk of adhesions. This technique has proven good over time, but because medicine is evaluating and we are continuing our search for new techniques and procedures to minimize risk and recovery time, we will present endoscopic surgical treatment.

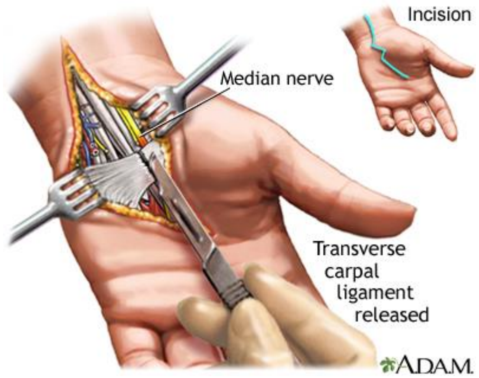

History of carpal tunnel endoscopy

Interest in less invasive open procedures for treating CTS developed in the early 1980s. Paine [4], a neurosurgeon, used a retinaculatom to divide TCL through a small transverse incision in one of the creases of the wrist. However, he found a 2.5% incidence of the incomplete division of the TCL. There were four cases of palmar hematoma, most likely developed due to superficial palmar arch injury. Pagnanelli [5] reported the use of retinaculatom in 577 patients and had only two incomplete divisions (0.3%) of TCL with no median or vascular nerve injuries.

In the history of carpal tunnel endoscopy, 2 main techniques for relaxing the carpal tunnel have been described; "One portal procedure" and "duel portal procedure". The first endoscopic technique was introduced in the relaxation of the carpal tunnel "one portal procedure" in 1986-1987 by Okutsu, a Japanese orthopedist, and in 1989 the technique

"Duel portal procedure" was introduced by Chow, an orthopedist. the two techniques had modifications and developments by other doctors (Agee, Menon, Worseg, Brown, Tsai Resnik, and Miller...) but remained in 2 categories: one portal procedure and duel portal procedure.

Indications

The indications are similar to those of classical technique and include the severity of symptoms, duration of symptoms, ineffective response to conservative treatment.

Relative contraindications

Include patients with severe CTS with evidence of muscle atrophy, rheumatoid arthritis, previous surgical treatment of repeated CTS !!, significant loss of wrist extension, proliferative synovitis, and lesions invading the carpal tunnel [2].

Endoscopic surgical technique

One portal procedure (Agee's technique modified):

Following the inflation of a tourniquet with the initial recommendation of general or regional anesthesia, then with the evolution of the surgeons' experience, they started to use local anesthesia with or without sedation, a 2-3 cm incision is made in one of the flexion creases of the wrist between ulnar carpal flexor and radial carpal flexor. Longitudinal subcutaneous dissection exposes the forearm fascia.

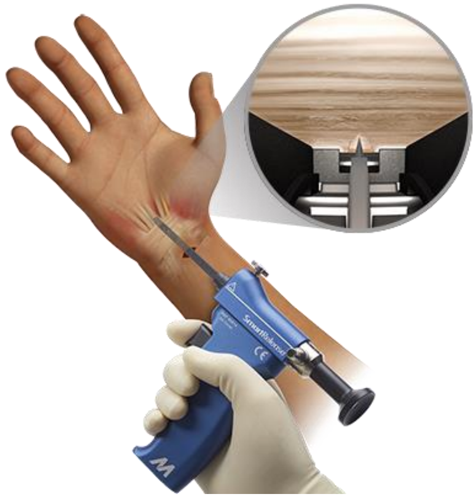

A U-shaped incision is made on the forearm fascia, creating a rectangular flap based distally on the TCL. While the flap is raised, a synovial lift is placed under the TCL [6]. An instrument (a kind of dilator) is inserted into the carpal tunnel to create a path for the blade assembly. The blade endoscope assembly is then inserted into the carpal tunnel and is passed distally to the distal edge of the TCL. A few steps are usually required to obtain an adequate definition of TCL. After defining the distal edge of the ligament, the tip of the instrument (along with the blade) is placed distal to the edge of the TCL. After checking the correct positioning, the trigger mechanism is activated and the blade is engaged and raised to 3.5 mm above the assembly at an angle of 80° [6]. The instrument is retracted, and under direct view, the TCL is split in a distal to proximal direction. With the blade retracted, several passes can be made to check the ligament transection. If necessary, the blade can be coupled and the remaining TCLs can be sectioned. Then, a proximal volar antebrachial fasciotomy is performed, followed by standard closure of the wound.

Dual portal procedure (Chow's technique and its modifications):

His original technique involved a transbursaral approach to the carpal tunnel. The surgeon and the nurse should sit in front of each other at the hand table. A TV monitor is placed behind each surgeon so that both can have a complete view of the procedure. A transverse incision of 1 cm is made at 0.25 cm proximal and 0.25 cm radially from the pisiform bone. Chow later modified his technique by changing the landmarks of the proximal incision [3, 7, 8]. He recommended that the proximal portal be made by drawing a transverse line of 1 to 1.5 cm radially from the proximal pole of the pisiform bone. A second line is longitudinally marked 0.5 cm from the end of the first line. A third line is transverse 1 cm to the radial from the end of the second line to indicate the entrance portal. A longitudinal incision is made on the forearm fascia, taking care to protect the artery and the ulnar nerve. The flexor tendons are exposed (trans-bursal) and retracted towards the radial part, finding the space between the ulnar neurovascular bundle and the flexor tendons. A trocar is inserted, and the wrist is placed and held in hyperextension by a special hand frame. Using the tip of the trocar, feel the base of the harness hook, then lift it and feel it, if you feel the cannula means it is above the TCL or in the Guyon channel and must be removed and reinserted into the channel under the TCL. A second incision (the palmar one) is made is distal to the distal edge of the carpal transverse ligament along the long axis of the ring finger. The endoscope is inserted proximally into the trocar, which has an end that is immediately below the TCL. Steps are taken to ensure that only the transverse fibers that makeup TCL are visible and not tendons or nerves. The endoscope is inserted into the proximal incision and advanced to the distal edge of the TCL. A knife inserted through the second incision (palmar incision) is used to cut the distal edge in a proximal direction up to half the ligament. To complete the release of the TCL, the endoscope is inserted into the distal opening. The knife is used to cut the proximal edge of the TCL, and the retrograde knife is inserted into the middle section and advanced proximally to complete the release. The wounds are sutured, a simple dressing is applied, and the sutures are removed after one week postoperatively. Because the transbursive technique frequently produced ulnar neuropaxia, either due to withdrawal pressure or the cannula, the technique was modified by Resnick and Miller [9] in a subligamental or extracurricular approach. In this technique, the forearm fascia is incised proximal to the TCL, and the dissection is made under the fascia and in the carpal tunnel under the ligament, avoiding the flexor tendon bursa. The rest of the operation is performed in a similar way to the Chow method.

In our clinic, we use the endoscopic technique "one portal procedure” (Figures 1 to 6).

Figure 1: Surgical instruments for carpal tunnel endoscopy @ byDr.HazimElayan

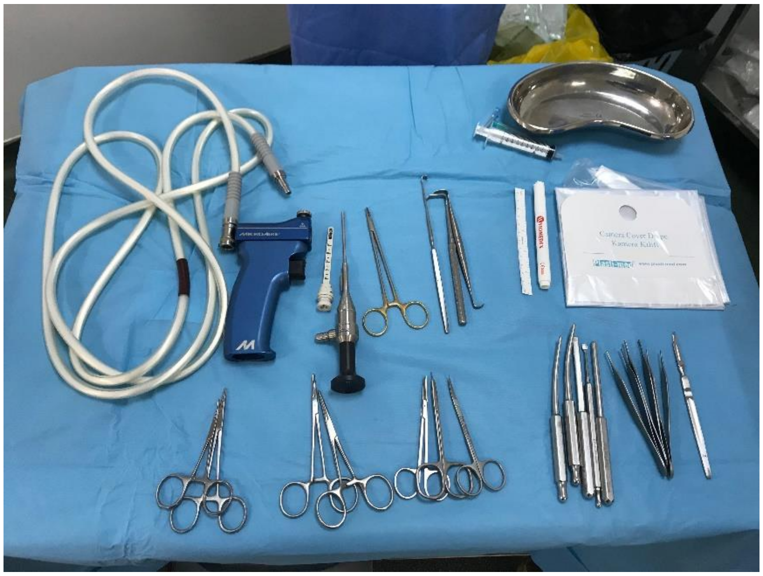

Figure 2: Preoperative marking lines @ byDr.HazimElayan

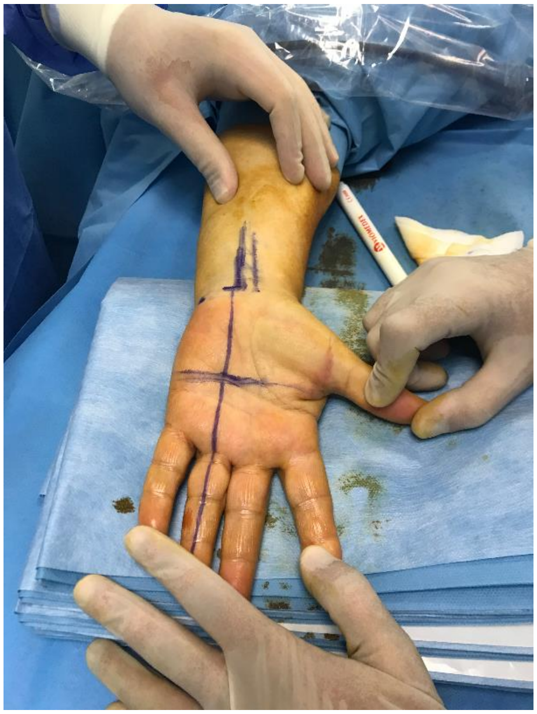

Figure 3: Endoscope inserted into the carpal tunnel through an incision of about 1.5 cm @ byDr.HazimElayan

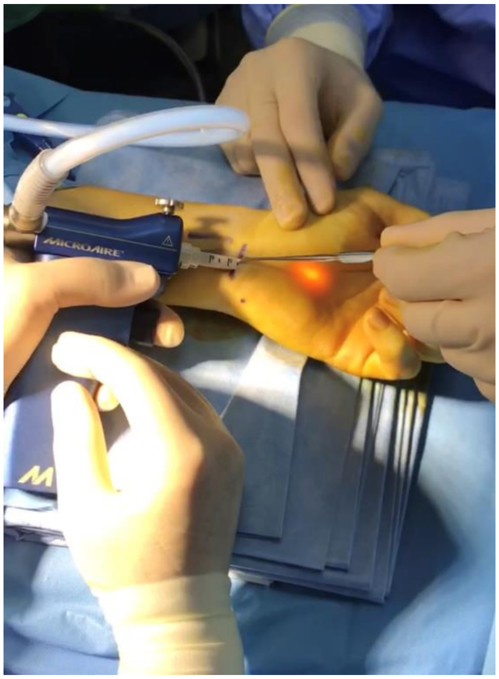

Figure 4: Transverse carpal ligament visualized on the monitor through the endoscope @ byDr.HazimElayan

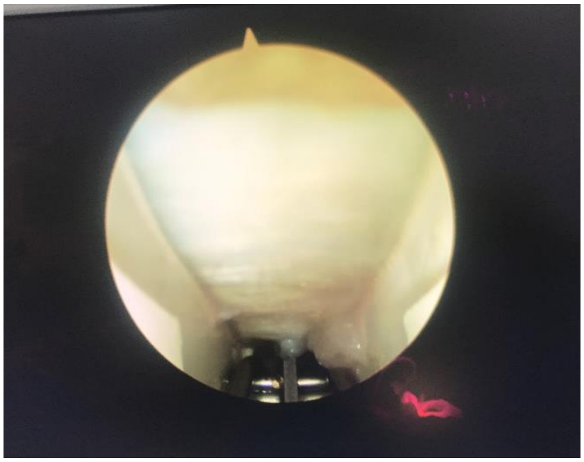

Figure 5: Sectioning the transverse carpal ligament, the remains of fibers that are sectioned by repeating the endoscope blade 2-3 times until complete sectioning @ byDr.HazimElayan

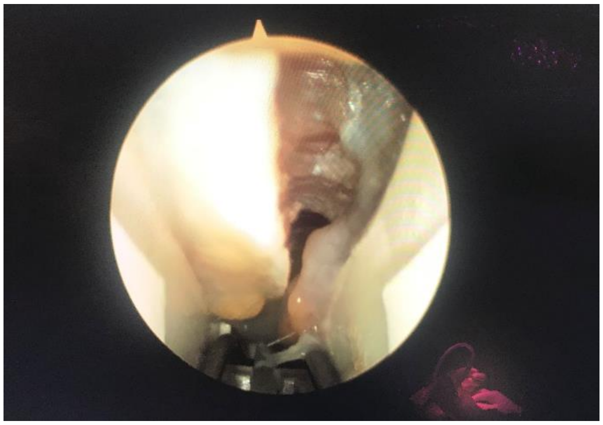

Figure 6: The end of the intervention, skin suture. @ byDr.HazimElayan

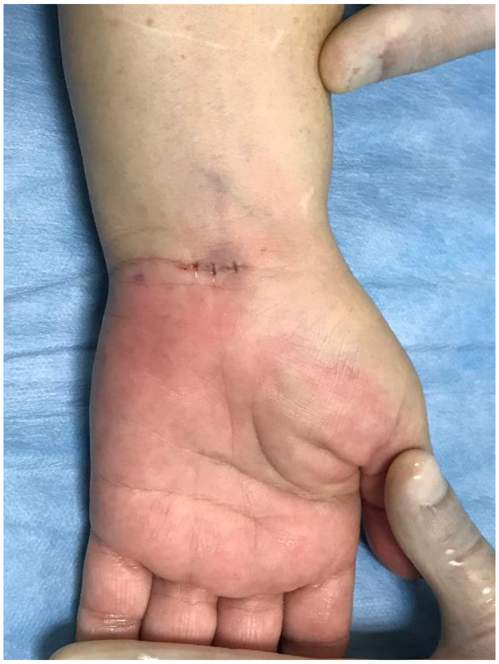

COMPLICATIONS

Endoscopic carpal tunnel release-specific adverse events have been described and are listed here to avoid these possible complications.

1. Nerve injury:

This usually involves the digital nerve of the middle and/or ring finger, which are in a straight line coinciding with the insertion of the endoscope [10, 11, 12].

2. Lesion of the superficial palmar arch:

Because the distance between the superficial palmar arch and the distal edge of the transverse carpal ligament is approximately 9.4±3.5 mm [11, 13], insertion of the endoscope too distally should be avoided.

3. Flexor tendon injury:

This is due to an absence of experience using the endoscope and most commonly affects the superficial flexor tendon of the ring finger, typically a risk in the one-portal procedure [11, 12].

4. Penetration of the Guyon Canal:

This can injury the nerve or the ulnar artery [11].

5. Incomplete sectioning of the TCL

The only recognition of this may be the persistence of carpal tunnel syndrome symptoms in the postoperative period [11].

Benefits (advantages)

Minor postoperative pain, faster recovery, no immobilization of the hand, minimal scarring, faster return to work.

Disadvantages

Higher cost. inability to excise the formation or other structures that may compress the median nerve.

CONCLUSIONS

1.It is a modern technique that requires a special professional experience of the surgeon.

2.It is a minimally invasive technique with the protection of noble elements (vessels, nerves, and tendons) at this level (from the radiocarpal joint and palmar).

3.Recovery of the hand-operated much faster with endoscopic technique (does not require immobilization, rapid socio-professional reintegration).

4.The duration of surgery in an experienced surgeon is much shorter than with the classical technique.

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VARIA

Evaluating the effects of medication reconciliation by pharmacists on patient safety in hospitalized patients

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Abstract: *Backgrounds: Medication errors are one of the most common errors that are associated with adverse effects on patients and may even lead to mortality. One of the methods for determining patient safety in hospitals is assessing the prevalence of medication errors. In this regard, medication reconciliation is used to improve drug safety in the treatment process. The present study was conducted on 7564 patients in the emergency department of one of the hospitals in Tehran, Iran.*

Methods: In total, 256 adult patients with a mean of 45 years, who had been admitted to the emergency department of a hospital in the past six months and had used at least three drugs before admission, were entered into the study. In this study, the effect of medication reconciliation on admitted patients was assessed to prevent drug errors at the time of admission by pharmacists. The main outcome measure was Type and Frequency Percentage of Drug Differences Observed during Medication Reconciliation were measured.

Results: According to the results, the presence of a trained pharmacist was significantly effective in medication reconciliation for enhancing patient safety and treatment process accuracy. In addition, it was concluded that age, the condition of hospitalization, and medical center personnel had a direct relationship with the frequency of medication discrepancies.

Conclusion: The presence of a trained pharmacist to perform the medication reconciliation process significantly affected the improvement of patient safety and the accuracy of the treatment process.

Keywords: *medication reconciliation; pharmacists; patient safety*

INTRODUCTION

Medication errors are quite prevalent and are the fourth leading cause of death all over the world. These errors may occur through dug prescription, preparation, distribution, or administration [1, 2, 3]. Some patients like the elderly, are at higher risk of medication errors because they always suffer from different comorbidities and use complex drug regimens [4]. A study in one of our teaching hospitals indicated the high rate of medication errors especially the missed dose errors [5]. It is one of the most important goals of all health care systems to prevent different types of medication errors.

The medication reconciliation process is a method to identify the most accurate list of all medications a patient is taking, to improve patient safety. It is always performed on

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admission, to compare patient drug history with what is ordered in the hospital, and discharge as well as transferring from one ward to another [6]. It was first incorporated as a National Patient Safety Goal (NPSG) in 2005 by the Joint Commission. WHO and collaborators have designed a project for its implementation in a range of countries [7]. This process can effectively reduce the rate of different types of medication errors in hospitals [8, 9, 10]. It can also decrease the rate of readmission and its related costs [10].

Medication reconciliation is known as an important tool to prevent drug-related problems and their related burden. This process includes four stages, as follows:

- Preparing and recording the medication history of patients upon admission
- Comparing the medication history of patients with drug orders in the hospital and identifying and resolving discrepancies
- Providing a written list of medications upon the discharge of patients
- Teaching drug regimens to patients upon discharge [11].

Evaluating the medication reconciliation process supports the role of pharmacists as key members dealing with patients' medications, and collaboration between pharmacists and other healthcare team members is crucial in preventing drug-related errors and promoting patient safety especially in high-risk patients [12]. Medication reconciliation is a difficult and time-consuming process and is affected by factors like drug resources, the discharge process, and hospital policies. Distinguishing different barriers will contribute to the improvement of the exact drug information available between different health care settings [13].

Considering the impact of the medication reconciliation process on patient safety and the fact that this process used to be managed by doctors, medical interns, or nurses in our hospitals and is recently reformed with emphasis on the role of pharmacists, we designed this study to evaluate the following objectives in one of the hospitals in Tehran:

- Determining the difference between drug history taken by pharmacists and nurses
- Determining the frequency of identified errors in the reconciliation process
- Determining the frequency of identified errors related to dangerous medications (high risk)
- Determining the time spent for each medication reconciliation (time of comparison of BPMH and the current medication prescription inserted into the patients' file by the physician), resources used for history taking, the mean amount of medications used before admission, and time spent for corrective measures.

Ethics Approval

This study was approved by the Guilan University of Medical Sciences Ethics Committee.

MATERIALS AND METHODS

The impact of admission medication reconciliation by pharmacists on the rate of medication errors was assessed in this study.

During the six months from September 2019 to March 2019, 256 patients who were admitted to the emergency department of one of the general private hospitals in Tehran, were included in this study. The hospital had 350 approved beds and 300 active beds with an admission rate of 50 % in the emergency ward.

Inclusion criteria:

- Age over 45 years old,
- History of taking at least 3 medications at home

Medication reconciliation was conducted by trained hospital pharmacists from 8 a.m. to 8 p.m. each day. Patients who were admitted after 8 pm were visited by pharmacists the following day.

During the study period, all patients who were admitted to the emergency department were referred to the pharmacist for medication reconciliation if met the inclusion criteria. The pharmacist collected the best medication history (BPMH) from the patients or their caregivers as well as other information sources like patient GP or local pharmacy if required, using effective communication skills. Information sources were specified in medication reconciliation forms (MRFs). Insurance company records were also used to determine the details about drugs that were covered by insurance. Patients were also asked to bring all the medications they were using at home, if there were any, to provide more details. All the following information were entered in medication reconciliation forms:

- History of chronic diseases (e.g., diabetes, hypertension, hyperlipidemia, metabolic and genetic disorders, seizure, history of myocardial infarction (MI), and stroke)
- Any weight loss or weight gain diet and related medications
- Any herbal drugs in the form of topical, tea, and distillates

- Any prescribed and non-prescribed drugs
- Any high-risk medication as an anticoagulant, chemotherapy, antic arrhythmia, anti-convulsion, immunosuppressant, insulin
- Generic and brand name, dosage, frequency, and route of administration as well as how long days were on that medication were specified about each medication.
- History of smoking, alcohol, and narcotics
- Pregnancy and breastfeeding status
- History of any drug and food allergy

Then MRFs were compared with the medication history recorded in patient's files who were routinely taken by nurses. It was also compared with doctor's drug orders on admission. Any differences observed at this stage that required correcting the doctor's order were considered as clinical interventions.

The time spent by pharmacists and nurses to provide the BPMH were also recorded for each patient,

Finally the following were evaluated

- The differences between medication histories taken by the pharmacists and nurses
- The frequency of medication errors during the reconciliation process
- The frequency of medication errors related to high-risk drugs
- The time spent on each medication reconciliation
- Information sources used to take the medication histories and an average number of medications used before admission.

RESULTS

About 46% of our study population were male and 54% were female with a mean age of 61 (±16) years, they were taking 3- 10 medications before hospital admission (average of 6 drugs). Our results showed that from 256 patients who entered the study, 92 patients required some kind of clinical intervention. These interventions were approved by medical doctors in 87 cases, that 15.2% of these interventions were related to high-risk drugs.

In addition, we observed at least one difference between MRFs and medication history by nurses in 98.7% of cases. (1025 cases) which 13.4 % of these differences were related to high-risk drugs. Details about these differences are described in Table 1. Drug omission was the most prevalent difference we observed in this study. The second and third most prevalent differences were about dose and frequency of drug administration.

Table 1: Type and Frequency of differences Observed between MRFs and nurses medication histories

Type of observed medication error	Frequency percentage of error
Drug omission	48%
Dose	21%
Drug regimen	20%
Drug name	5%
Drug duplication	3%
Dosage form	2%
Other cases	1%

Regarding the time spent taking BPMH, we observed that pharmacists spent an average of half an hour to feel an MRF compare to 3 minutes, for taking a medication history, by nurses. In addition, the average time spent on comparing MRFs with doctors' drug orders was 12 minutes and it took a median of 9 minutes for pharmacists to complete their clinical intervention.

Information regarding medication reconciliation was obtained from the patients in 84 cases (32.8%), their caregivers in 61 cases (23.82%), patients' home medication bags in 43 cases (16.79%), their insurance records in 58 cases (22.65%) and other sources in 4 cases (1.56%) (Figure 1).

Figure 1: Source of information for medication reconciliation

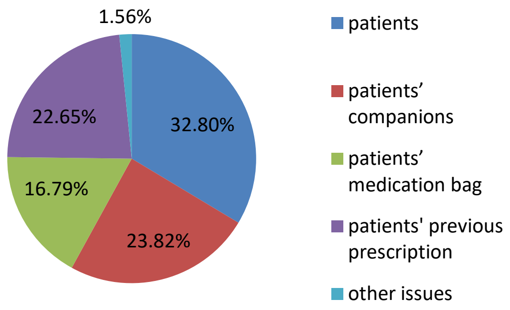

DISCUSSION

The impact of medication reconciliation by pharmacists on the rate of medication errors was evaluated in this study. Our results showed that medication histories taken by pharmacists were more comprehensive than routine drug histories taken by nurses. We also observed that pharmacist interventions can have a positive impact on the rate of medication errors.

Results of various studies indicate the high that rate of different types of medication errors and related burden in hospitalized patients especially through admission or discharge process as well as transferring from one unit or facility care to another. These errors are mainly the result of miscommunication and lack of required information and can lead to serious complications [5, 14, 15, 16]. Our results showed a high rate of medication errors in our study population, especially during the discharge process. We observed that errors related to the dose of patient medications were the most common errors. While in some other studies various types of medication errors, as well as errors in drug administration, were observed with almost the same frequencies [19].

Medication reconciliation, as a method for identifying and correcting medication errors, is widely used in health care settings in different countries [17]. The results of a systemic review showed that medication reconciliation can significantly decrease the frequency of different types of medication errors (17 from 17 studies), and drug side effects (2 from 2 studies) [18]. Different studies indicate that a large number of patients admitted to hospitals, experience medication discrepancies that can be prevented by careful medication reconciliation by pharmacists [20, 21]. We also observed in the present study, that different types of medication errors can be avoided by performing a careful medication reconciliation by a trained pharmacist especially when a patient is admitted to the hospital. This can prevent any discrepancies between what has been taken at home and what is ordered in the hospital.

About two-thirds of the complications that occurred after discharge are due to medication-related problems. Generally, medication reconciliation at discharge could be a strategy to reduce medication errors and improve patient safety even after discharge from the hospital [22].

CONCLUSION

In the present study, the impact of medication reconciliation by a pharmacist, on the rate of medication errors was evaluated. The results showed a significant difference between the history taken by the pharmacists and nurses. It should be noted that although the process of medication reconciliation by pharmacists is time-consuming and need considerable human and financial resources that may be difficult to afford now, its positive impact on direct and indirect costs of medication errors cannot be dismissed. Our findings also indicated that the involvement of a trained pharmacist in patients' treatment teams can significantly improve their safety profiles.

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Conflict of interests

The authors have no conflicts of interest to declare.

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ORIGINAL ARTICLE

Evaluation of muscle atrophy after sciatic nerve defect repair – experimental model

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Abstract: *Peripheral nerve injuries are one of the most important causes for disability generating considerable costs around the world. Appropriate assessment of the extent of peripheral nerve lesions and the choice of the right therapeutic protocol remain some of the biggest challenges. Non-invasive neuroimaging approaches are suitable in managing peripheral nerve repairs, providing in the same time accuracy details in structural neural detection, with minimal discomfort at a low cost. Medical imaging technology development has led to progress in examination of peripheral nervous system, using a series of tools and methods, such as: ultrasonography (US), positron emission tomography (PET), magnetic resonance imaging (MRI) and magnetic diffusion tensor imaging (DTI). In this study, we evaluated the regeneration process in Wistar rats after sciatic nerve defects repair with 4 different techniques (i.e. nerve graft, rat aorta used as a nerve conduit, rat aorta filled with platelet rich plasma (PRP) and rat aorta filled with stem cells) by using MRI investigation alongside the clinical evaluation. Our results showed that among the 4 batches, the PRP batch had the best results in muscle atrophy condition (both on MRI scan and on gastrocnemius index); on the second place the stem cell batch, then the nerve graft batch and finally the aortic conduit batch. MRI proved to be a reliable non-invasive monitoring method and showed good result in correlation with the footprint test (sciatic functional index) and the gastrocnemius index.*

Keywords: *muscle atrophy, sciatic nerve, platelet rich plasma*

INTRODUCTION

Nerve injuries can be extremely debilitating due to the many functions that a peripheral nerve may have. The sensitive nerves are responsible for acquiring information such as temperature, pressure, shape, while motor nerves are important in muscle contraction and movement. These injuries are even more severe when they translate into nerve defects, which require a special type of bridging in order to recover its original function.

After the nerve is repaired it takes a long period of time for regeneration. This is due to the fact that after the nerve is sectioned, Wallerian degeneration occurs, which represents a disintegration of the axon distal to the injury site [1]. Afterwards, the growth cone leads the regeneration process through the modified Schwann cells – the Bungner columns [2]. The nerve growth rate is considered to be 1 mm/day in humans and 1.5-2 mm/day in rats. Several imaging instruments and techniques are currently used in examination of peripheral nerves: ultrasound (US), positron emission tomography (PET), magnetic resonance imaging (MRI), diffusion tensor imaging (DTI).

Nerve ultrasound is capable of assessing not only the size, but also the morphology and it may be used in diagnosing different types of peripheral nerve pathologies. The most common findings using ultrasound examination are nerve enlargement, followed by other abnormalities such as increased hypo-echogenicity or increased intraneural vascularization [3,4]. Moreover, ultrasound investigation can also asses the surrounding environment, being helpful for determining the etiology of the neuropathy (compression, trauma).

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PET represents an imagistic investigation in which a radioactive tracer such as [18F] FTC-146 is injected into the bloodstream and it concentrates at the level of the nerve injury, [5] investigation which is used in order to detect the area of the neuropathy. Diffusion tension imaging (DTI) evaluates the movement of water molecules in different biological structures. DTI can evaluate a nerve injury both in terms of quantity and quality [6].

As its great similitude to the human nerve, the sciatic nerve of the Wistar rat is the perfect candidate for experimental nerve surgery. The sciatic nerve divides into 3 main branches – tibial, common peroneal and sural nerve.[7] The tibial nerve innervates the gastrocnemius muscle. Therefore, the regeneration process of the sciatic nerve can be evaluated by assessing the function, weight and diameter of this muscle.

Nerve defects can be solved by several techniques – either nerve grafts or different surgical protocols type. Defects up to 70 mm can be bridged by one of these options. [8] Nerve grafts can be either autologous or heterologous, while nerve conduits can be biologic or synthetic. [9,10] Better results were obtained by using the nerve protocol technique in combination with different substances which contain growth factors. [11] Among these, platelet rich plasma (PRP) has proven its efficacy in nerve regeneration. [12,13,14] Stem cells, similar to PRP, also have a positive impact in nerve regeneration. [15,16,17]

A footprint test in rats undergoing surgery, became a useful evaluation asset since Bain and MacKinnon introduced a formula which could calculate the sciatic functional index (SFI) based on 3 parameters-plantar length, intermediary interdigital length and the maximum interdigital distance. [18]

This study aims was to compare the regeneration process in Wistar rats after sciatic nerve defects using 4 different techniques (i.e. nerve graft, rat aorta as a nerve protocol, rat aorta filled with platelet rich plasma (PRP) and rat aorta filled with stem cells) by using MRI investigation and comparing it to the results obtained from the footprint test (sciatic functional index) and the gastrocnemius index.

MATERIAL AND METHODS

The research was conducted on 40 Wistar rats, from Carol Davila University (Convention no. 7491/21.03.2019). They were accommodated in plastic cages, receiving normative murine food and water.

The rats were divided into 4 groups – nerve graft group, aortic conduit group, aortic conduit with PRP and aortic conduit with stem cells. All rats underwent surgery with a right side approach. The biologic materials used (PRP and aortic conduits) were obtained by sacrificing 2 extra rats. The stem cells used, originated from human bone marrow mesenchymal stem cells (hBM-MSC cell line) were obtained from Life Technologies. All other chemicals were purchased from Sigma–Aldrich. The study was approved by the Ethics Committee of Veterinary Faculty (approval No. 1738/ 17.04.2019).

Preoperative assessment

All rats were weighed before surgery and they were anesthetized using a cocktail of 75-100 mg/kg ketamine and 10 mg/kg xylazine. The anesthetic injection was administrated intraperitoneal, maintaining anesthesia for one hour. If the rat presented signs of waking up, a second dose could be administrated.[19]

Surgical procedure

The surgical protocol was the same for all subjects. The rat was positioned in prone decubitus, than a surgical incision was performed along the right femur, 0.5 cm inferior to the bone. The biceps femoris was then dissected and right beneath it the sciatic nerve could be found.

Figure 1: Incision site (12 weeks postoperative scar)

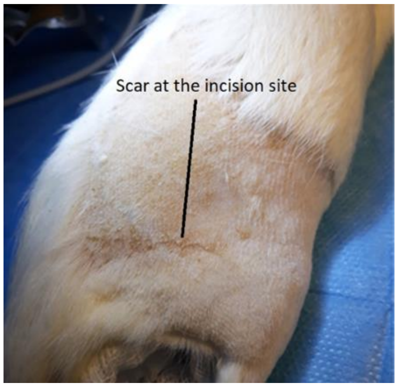

Nerve graft

After nerve exposure, the sciatic nerve was sectioned at 2 sites to create a 0.5 cm nerve graft. In the first batch this graft was sutured in place, while in the other batches the graft was discarded, leaving a 0.5 cm defect. In the first batch, the epineural vessels served as guide to ensure correct alignment between the nerve endings.

Simple aortic conduit

Two rats were sacrificed and their aortas were taken from the heart to the bifurcation into the common iliac arteries. The aortas were then divided into 6-7 mm long segments to serve as nerve material for the next interventions. In the 2nd batch, the aortic conduit was used to bridge the nerve defect by suturing the nerve endings 1 mm inside the vessel conduit.

PRP treatment

The 3rd batch used PRP inside the aortic conduit. After extracting 2 ml of blood directly from the heart, the blood was centrifuged for 5 minutes at 4000 rpm in order to obtain PRP material. The PRP was placed inside the aortic conduit and sutured to the nerve endings.

Mesenchymal stem cell treatment

In the 4th batch, mesenchymal stem cells from human bone marrow were used. These were transported in liquid nitrogen at -196oC and defrosted using the following protocol: cryogenic tube extraction, 500 µL phosphate-buffered saline (PBS) solution was added, then the solution was centrifuged for 5 minutes at 1500 rpm and the supernatant was disposed of; the last 3 steps were repeated and after obtaining the stem cells, they were suspended in Ringer solution. The stem cells were then included in the aortic conduit and this was sutured to the nerve endings.

The immediate postoperative treatment included antibiotic therapy using enrofloxacin at a dose of 0.003 mg/kg (Enroxil) and an anti-inflammatory drug (meloxicam 1 mg/kg) administered subcutaneously for 3 days.

Postoperative assessment

All subjects were evaluated at 2, 4, 6, 8, 10 and 12 weeks after surgery, including sensitive and motor evaluation, as well as a footprint test. At 12 weeks all rats were sacrificed using KCl after an overdose with the above mentioned anesthetic. The gastrocnemius muscles were evaluated using MRI imaging and then they were harvested and weighed (bilateral).

Walking footprint analysis

The SFI (sciatic functional index) represents a fairly objective test which evaluates the peripheral nerve regeneration in rats and mice. The method consists of recording, measuring and calculating the dimensions of the footprints of the studied rats after unilateral sciatic nerve injury. Monte-Rasso has demonstrated that this index is measurable and reproducible starting the 3rd week after the nerve lesion. [20]

The traditional method of recording the footsteps involves using paper and ink, but it can record many artifacts which could affect the results. The development in technology by using an infrared system eliminated this problem and reduced the effort of the investigator. [21]

The formula for the SFI introduced by Bain and MacKinnon takes into account foot values of both the operated and the non-operated limb. A value close to 0 indicates normal function, while -100 represents complete function loss:

$$SFI= 38.3 \times EPL-NPL/NPL + 109.5 \times ETS-NTS/NTS+ 13.3 \times EIT-NIT/NIT - 8.8.$$

where EPL – plantar length (operated limb), NPL – plantar length (non-operated limb), EIT – intermediary interdigital length (operated limb), NIT – intermediary interdigital length (non-operated limb), ETS – maximum interdigital distance (operated limb) and NTS – maximum interdigital distance (non-operated limb)

Magnetic Resonance Imaging (MRI)

The imagistic investigations were conducted at the Veterinary Medicine Faculty in Bucharest using an MRI scanner Vet-MR Grande Esaote de 0.2 T. A standard spin echo pulse protocol with T1 and T2W in sagittal, dorsal and transversal planes was performed. This protocol included a girth of 2mm/slice and 0,5mm distance between slices. Due to the small size of the rat, an articulation human coil was used.

The rat was examined in prone position in the MRI coil, with both inferior limbs in extension. The thorax and the head of the rat were sustained on 2 sponges during the investigation. Each rat required 15-20 minutes for the investigation and 5 minutes for preparation. On the transverse sections, the calf diameters were measured (right and left) and then a ration between the 2 values was calculated.

For the MRI calf muscle ratio (right/left), the measurements were performed on anaesthetized rats lying in prone position, with both inferior limbs extended. The image selected for measurement was the first imagine in transverse plane where the limbs appeared attached to the body.

Gastrocnemius muscle – operation and measurements

After incising the dorsal skin of the calf in the midline and dissecting through the superficial flat muscle, the gastrocnemius muscle revealed. It was then harvested from the level of Achilles' tendon to its origin on the femur. Using an electronic scale, the weight of the gastrocnemii muscles were measured and the ratio between operated and non-operated side was noted.

Statistical analysis

To process the data obtained, we used IBM Statistical Package for Social Science (SPSS) - Median test for the continuous variable analysis, where the median and the Inter Quartile Range (IQR) were reported. The threshold for statistical significance was $p<0.05$.

The correlations between continuous non-parametric variables were performed using Kendall Tau b test. The representation of the data was done using box-plot graphics and scatter-dot and line diagrams.

RESULTS

40 male Wistar rats of similar weight and age were used for research, divided into the 4 studied groups and 2 extra rats were used to harvest biologic materials. All rats underwent a surgery on the right sciatic nerve. 2 rats from the first batch were excluded from the study – one due to postoperative death and the other one due to a ruptured sciatic nerve. The rat who suffered a nerve rupture was kept until the end of the study (12 weeks).

The variables taken into account for nerve regeneration were:

- right and left gastrocnemius muscle weight and their ratio recorded at 12 weeks
- ratio between the right (operated) gastrocnemius muscle weight and the rat weight at 12 weeks (percentage)
- SFI at 6 and 12 weeks
- right and left calf diameters and calf diameter ratio recorded via MRI at 12 weeks

The MRI investigation could identify the nerve and the aortic nerve conduit in transverse, coronal and sagittal plane. The calf diameter could be measured bilaterally and the calf ratio could be calculated.

The images below describes the position of the sciatic nerve (lying inferior to the femur). One image shows the nerve graft in T1 sequence, while the other one shows the aortic conduit in T2 sequence. No image can however reveal the suture sites in any of the sequences.

Figure 2: T1 coronal plane - general view (A); Sciatic nerve (B); Distal femur (*) distal and proximal tibia (#)

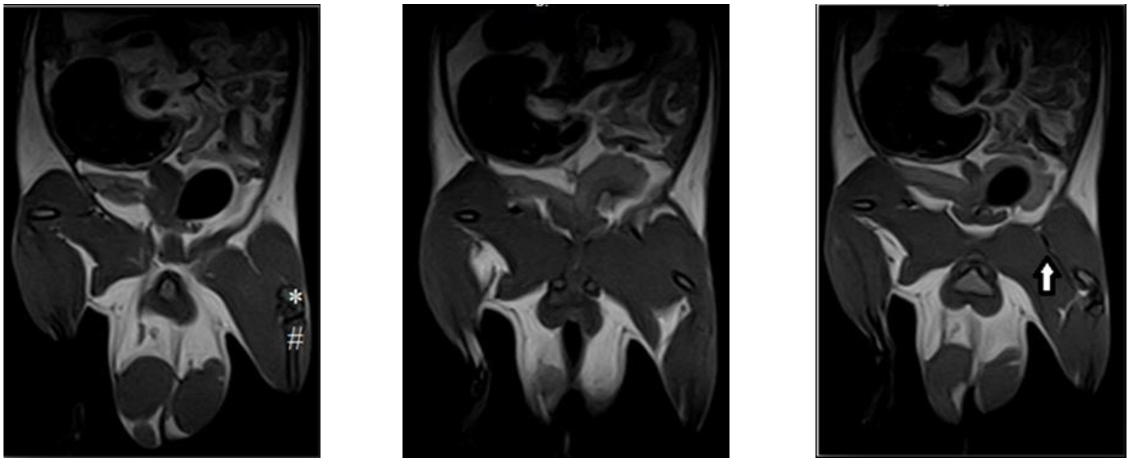

Figure 3: Nerve image in T1 sequence (A); Aortic conduit in T2 sequence (B)

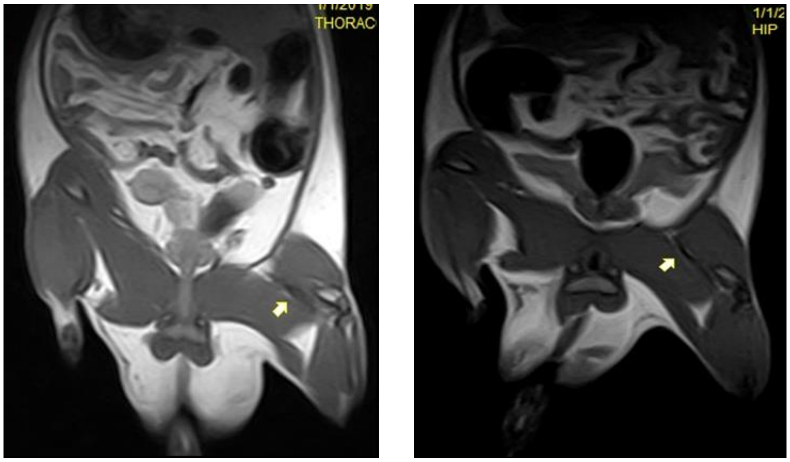

Figure 4: Coronal sections in T2 sequence of right femur - aortic nerve conduit and scarring (arrow); perilesional oedema (*)

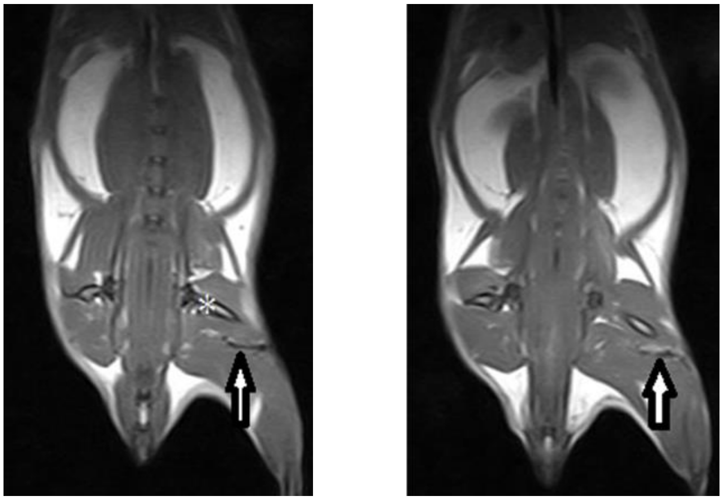

Figure 5: Series of MRI images T1 sequence transverse plan - calf muscles measurements

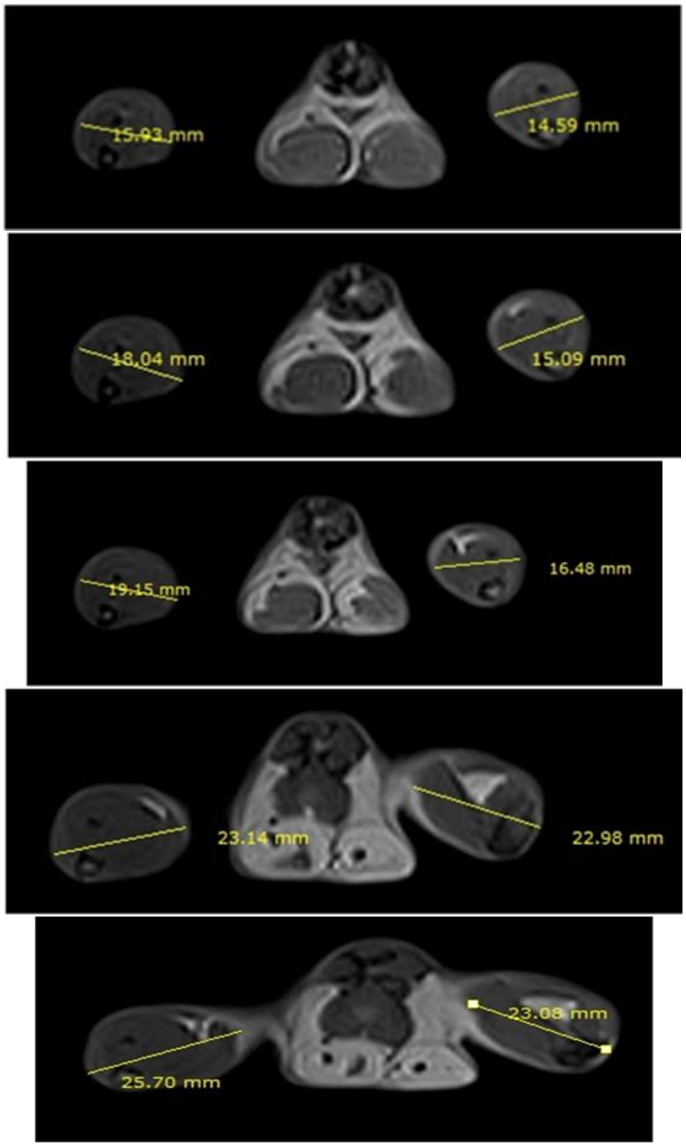
15.93 mm
14.59 mm
18.04 mm
15.09 mm
19.15 mm
16.48 mm
23.14 mm
22.98 mm
23.08 mm
25.70 mm

Figure 6: Series of MRI images coronal plane T1 sequence - calf muscles measurements

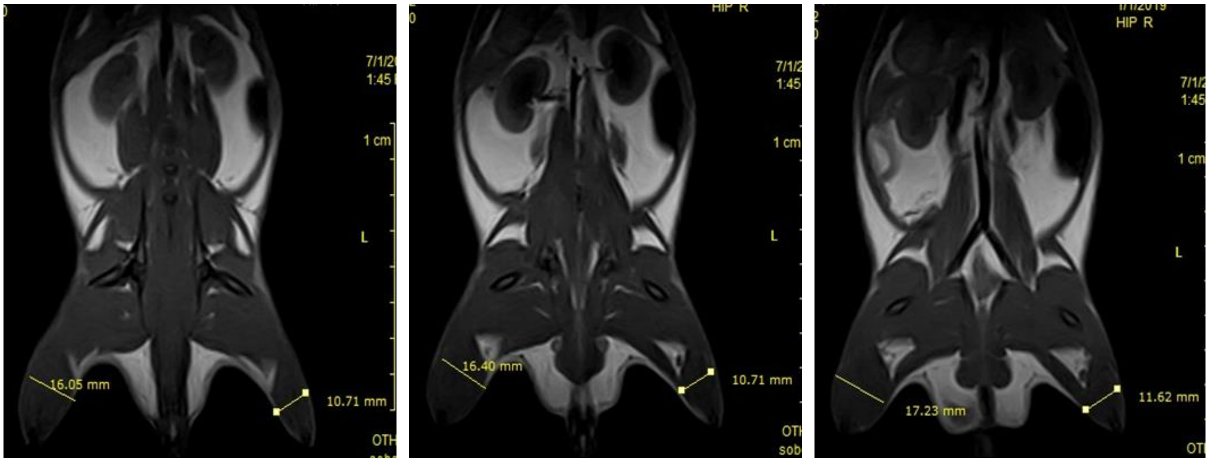

After completing the MRI scanning, the rats were euthanized and the gastrocnemius muscles were harvested bilaterally. A fine electric scale was used to weigh each muscle. These gastrocnemius ratios were compared to the MRI ratios for all batches.

Figure 7: Gastrocnemii muscles in pairs (left and right) – stem cell batch

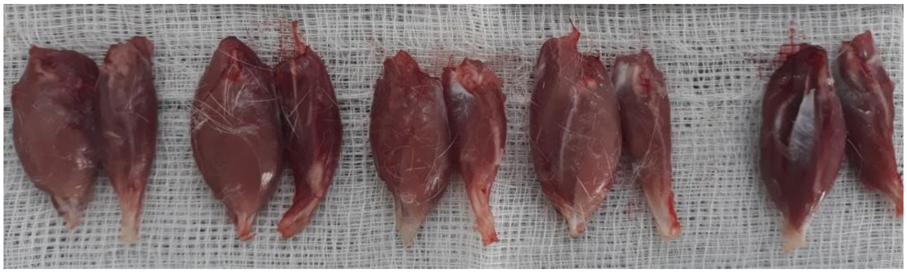

Table 1: Median analysis: weights of gastrocnemius muscles (right and left), gastrocnemius index

n=38	Batch 1	Batch 2	Batch 3	Batch 4	Total
Variable	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Right gastrocnemius	1.415 (0.25)	1.28 (0.45)	1.33 (0.31)	1.285 (0.13)	1.31 (0.21)
Left gastrocnemius	2.62 (0.55)	2.425 (0.24)	2.41 (0.26)	2.55 (0.30)	2.445 (0.29)
Gastrocnemius index	0.5 (0.15)	0.49 (0.18)	0.51 (0.17)	0.505 (0.03)	0.5 (0.07)
Rat final weight at 12 weeks (G6)	465 (59)	442 (61)	434.5 (28)	455 (57)	446.5 (39)
Percentage right gastrocnemius weight/G6	0.29 (0.09)	0.29 (0.07)	0.31 (0.08)	0.029 (0.02)	0.029 (0.05)

Table 2: Median test for gastrocnemius weight (right and left) and gastrocnemius index

Variable	Test Median (Degree of freedom)	P value
Right gastrocnemius	0.9 (3)	0.825
Left gastrocnemius	4.1 (3)	0.251
Gastrocnemius index	1.2 (3)	0.753
Rat final weight at 12 weeks (G6)	2.5 (3)	0.475
Percentage right gastrocnemius weight/G6	6.2 (3)	0.102

Table 3: SFI analysis at 6 and 12 weeks

n=38	Batch 1	Batch 2	Batch 3	Batch 4	Total
Variable	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
SFI at 6 weeks	-75.5 (8)	-76 (9)	-76 (7)	-76.5 (4)	-76 (4)
SFI at 12 weeks	-57.5 (9)	-58.5 (12)	-55.5 (10)	-57 (5)	-57 (6)

Table 4: Median test for SFI evaluation

Variable	Median Test (Degree of freedom)	P value
SFI at 6 weeks	1.08 (3)	0.782
SFI at 12 weeks	2 (3)	0.572

Table 5: Median analysis of the calf muscles diameters on MRI scan

n=38	Batch 1	Batch 2	Batch 3	Batch 4	Total
Variable	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Right calf diameter on MRI	17.06 (2.3)	16.4 (3.38)	16.39 (0.98)	16.94 (1.4)	16.67 (1.5)
Left calf diameter on MRI	21.45 (1.26)	20.82 (2.18)	20.63 (0.96)	21.38 (1.61)	21.1 (1.45)
Right/left calf diameters ratio	0.794 (0.064)	0.784 (0.072)	0.803 (0.066)	0.799 (0.017)	0.798 (0.049)

Table 6: Median test for calf diameters on MRI

Variable	Median Test (Degree of freedom)	P value
Right calf diameter on MRI	4.1 (3)	0.251
Left calf diameter on MRI	6.8 (3)	0.079
Right/left calf diameters ratio	1.2 (3)	0.753

Table 7: Correlation between MRI calf diameter ratio and gastrocnemius index

n=38	Total	
Corelation	***Kendall Tau Correlation coeficient***	***P Value***
MRI calf diameter ratio – gastrocnemius index	0.95	<0.001
MRI right/left calf diameter ratio– percentage right gastrocnemius weight/G6	0.57	<0.001
MRI right/left calf diameter ratio– G6	0.182	0.110
MRI right/left calf diameter ratio– SFI at 6 weeks	0.3	<0.010
MRI right/left calf diameter ratio– SFI at 12 weeks	0.485	<0.001

Peripheral nerve regeneration after nerve graft intervention

In the nerve graft batch, the SFI at 6 weeks varied between -83 and -69, while at 12 weeks it was between -60 and -50. The right calf diameter at the end of the study ranged between 15.35 and 17.97 mm. The left calf diameter had values between 19.96 and 22.04 mm. The calf diameter ratio varied from 0,76 to 0,84. The median values for the variables reported via MRI scanning are presented in tables 1 and 2.

The gastrocnemius muscles on the operated side weighed between 1.22 and 1.49 g while the non-operated muscles weighed between 2.27 and 3 g. The gastrocnemius muscle weight ratio varied from 0.45 to 0.65. The median values for the variables associated to gastrocnemius muscles are presented in tables 3 and 4 and those for the SFI values in Table 5.

Peripheral nerve repair with rat aorta graft

In the second batch, the SFI varied at 6 weeks between -83 to -73, while at 12 weeks between -63 and -49. The right calf diameter had values between 13.78 and 17.81 mm, while the left one was between 18.2 and 21.73 mm. The calf diameter ratio varied from 0.74 and 0.84. The median values for the variables reported via MRI scanning are presented in Tables 1 and 2.

The gastrocnemius muscles on the operated side weighed between 0.65 and 1.58 g while the non-operated muscles weighed between 2.06 and 2.86 g. The gastrocnemius muscle weight ratio varied from 0.29 to 0.69. The median values for the variables associated to gastrocnemius muscles are presented in tables 3 and 4 and those for the SFI values in Table 5.

The effect of platelet rich plasma (PRP) treatment on peripheral nerve repair

In the third batch, the SFI varied at 6 weeks between -80 to -71, while at 12 weeks between -62 and -48. The right calf diameter had values between 15.11 and 17.71 mm, while the left one was between 18.56 and 21.53 mm. The calf diameter ratio varied from 0.74 to 0.84. The median values for the variables reported via MRI scanning are presented in Tables 1 and 2.

The gastrocnemius muscles on the operated side weighed between 0.77 and 1.61 g while the non-operated muscles weighed between 2.28 and 2.73 g. The gastrocnemius muscle weight ratio varied from 0.34 to 0.67. The median values for the variables associated to gastrocnemius muscles are presented in Tables 3 and 4 and those for the SFI values in Table 5.

The effect of mesenchymal stem cells transplantation on peripheral nerve repair

In the third batch, the SFI varied at 6 weeks between -78 to -72, while at 12 weeks varied between -62 and -51. The right calf diameter had values between 15.65 and 17.88 mm, while the left one was between 19.38 and 21.73 mm. The calf diameter ratio varied from 0.78 to 0.81. The median values for the variables reported via MRI scanning are presented in Tables 1 and 2.

The gastrocnemius muscles on the operated side weighed between 1.17 and 1.46 g while the non-operated muscles weighed between 2.26 and 2.76 g. The gastrocnemius muscle weight ratio varied from 0.48 to 0.54. The median values for the variables associated to gastrocnemius muscles are presented in Tables 3 and 4 and those for the SFI values in Table 5.

Comparison of the 4 methods for the repair of peripheral nerve injury

SFI

The median values for SFI recorded at 6 weeks indicated better recovery in nerve graft batch, followed by the PRP and simple aortic conduit batches and the stem cell batch. At 12 weeks, the median values for SFI showed a very good recovery in the PRP batch, followed by stem cell and nerve graft batches, while the poorest results were recorded in the simple aortic conduit batch.

MRI test

The right (operated) calf diameter had the highest median value in the nerve graft batch, followed by the stem cell batch and simple aortic conduit and PRP batches with similar results. The same order was recorded on the left side as well. The highest calf diameter ratio (right/left) recorded via MRI scanning was in the PRP batch, followed by the stem cell batch, and the nerve graft batch, without statistical difference (p=0.753).

Gastrocnemius muscle

There were no statistical differences in the 4 batches when referring to the weight of the right gastrocnemius muscle. The highest median was in the nerve graft batch, followed by PRP batch, then stem cells batch and finally simple aortic conduit batch. The left one had the highest median value when referring to the weight variable in the nerve graft batch; followed by stem cell batch and then the PRP patch and last – the simple aortic conduit batch.

Figure 8: Distribution graphic of the MRI right/left calf diameter ratio for all batches

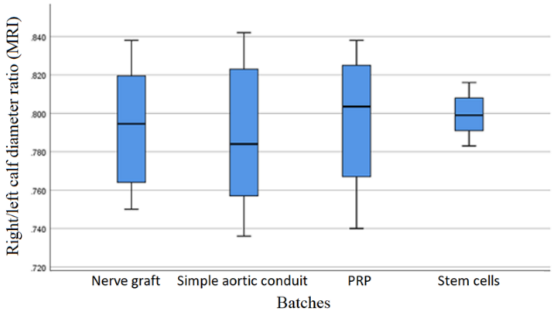

The median for the gastrocnemius index – the best results were in the PRP batch, followed by the stem cells batch, then the nerve graft batch and finally the simple aortic conduit batch. There were, however, no statistical differences between these batches.

The highest median when evaluating the percentage obtained from the right gastrocnemius muscle and the rat weight at 12 weeks was recorded in the PRP batch, all other batches obtaining similar results. Like the previous test, no statistical difference was achieved here either.

Using the Kendall Tau analysis, there were 4 positive statistical significant correlations (Table 7): MRI right/left calf diameter ratio – gastrocnemius index; MRI right/left calf diameter ratio – SFI at 6 weeks; MRI right/left calf diameter ratio – SFI at 12 weeks; gastrocnemius index (right/left) and SFI at 12 weeks.

The statistical analysis revealed a strong positive correlation between the MRI right/left calf diameter ratio and the gastrocnemius index. (r=0.95, p<0.001).

Figure 9: Correlation graphic between gastrocnemius index and MRI right/left calf diameter ratio

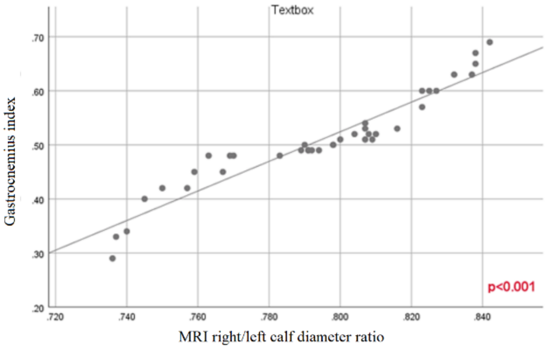

The statistical analysis revealed a strong positive correlation between the MRI right/left calf diameter ratio and the SFI at

6 weeks (r=0.3, p=0.010) and at 12 weeks (r=0.485, p<0.001).

Figure 10: Correlation graphic between MRI right/left calf diameter ratio and SFI at 12 weeks

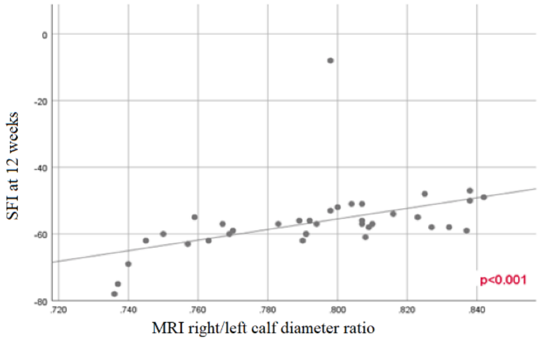

There was also a strong positive correlation between the gastrocnemius index and the SFI at 6 weeks (r=0.323, p=0.007) and at 12 weeks (r=0.487, p<0.001).

Figure 11: Correlation graphic between gastrocnemius index (right/left) and SFI at 12 weeks

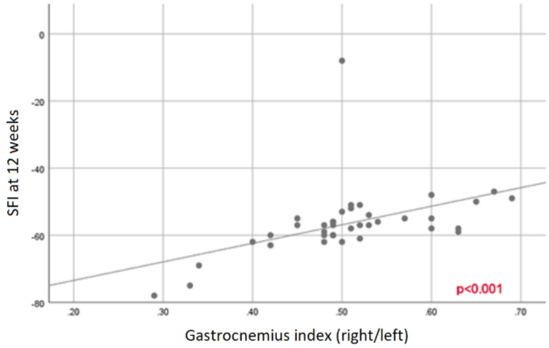

DISCUSSION

The MRI could not reveal the sciatic nerve in all the sections due to its small size. A high-performance MRI scanner (1,5 T or 3T) might be useful in identifying the extent of the lesions as well as small structures (fascicles of the human sciatic nerves), and a better and more detailed aspect of the suture sites.

Tremp et al used a 3T MRI scanner to assess regeneration in a rat sciatic nerve injury model. They were capable of visualizing the nerve graft by observing a small black outline, while the regenerating axon front could be seen as a small hyperintensity. The study also used the 3T MRI scanner to monitor the regeneration progress using the 2 types of stem cellular therapy (autologous and human stem cells). [24]

Muscle imaging and MRI in particular has become an important tool in the diagnosis of muscle diseases – both inherited and acquired. Subclinical progression of the different muscular diseases can be detected using quantitative muscle MRI techniques. [25]

Although MRI scanning could offer non-invasive information about nerve regeneration by observing the degree of muscle atrophy, this investigation is unlikely to become a gold standard as it reveals the negative effects at a late stage (when there is severe muscle atrophy). The gastrocnemius index can only be used in experimental studies and, similar to the previous investigation, it reveals the negative results only when it is too late (as the muscle atrophies progressively).

The MRI right-left calf diameter ratio could be correlated with the right-left weight gastrocnemius muscles ratio (gastrocnemius index) and with the SFI from the footprint test. The correlations between these indicators are justifiable, as all of them evaluate the degree of muscle sparing as a result of nerve regeneration process. Similar studies of MRI calf muscle evaluation after partial and complete denervation have been conducted on mice. The muscle atrophy was estimated by volume measurements on MRI, which were correlated to the post-mortem muscle weight examination. [26]

Nijhuis et al used a similar correlation to observe gastrocnemius muscle atrophy after sciatic nerve injury but instead of the MRI scanner, they used ultrasound imaging to asses muscle volume. Their study showed that the difference between the muscle thickness ratio (via ultrasound) and the muscle mass ratio was no more than 5%. [27] In clinical practice, ultrasound examination can therefore prove to be a reliable and accesible tool for muscle mass measurements. [28]

Another study conducted by Shen et al showed that the SFI parameter after sciatic nerve injury has positive correlations with indices such as electrophysiology, muscle strength and morphology. This research confirmed that the SFI can be a reliable index for nerve regeneration. [29]

A reliable investigation for nerve lesions is the electrophysiologic study. Vleggeert-Lankamp et al showed in a study using electrophysiological analysis that the ionic channels of both the Aalpha- and Abeta-fibers are altered after nerve injury and the correlation between nerve morphometry and electrophysiology is most suitable for evaluating nerve regeneration. [30]

Another study conducted by Shen et al used an electrical field for nerve regeneration purposes. They performed morphometric, electrophysiologic and functional evaluations on days 10, 20, 30 and 60 after the operation. Their research proved that direct current electrical stimulation facilitates peripheral nerve growth. [31]

Martins et al assessed recovery after sciatic nerve injury using several tests: electrophysiology, sciatic functional index (SFI) and histomorphometry. Using Pearson's analysis, they noticed 2 positive correlations with statistical significance (between the SFI and proximal nerve fiber diameter and between the SFI and distal nerve fiber diameter), thus concluding that there are few correlations between the 3 evaluation methods. [32]

With the evolution of technology, the imagistic evaluation of the peripheral nerves via MRI changed from the classical morphological studies to functional neurography. This new technique involves using diffusion tensor imaging and diffusion-weighted sequences and provides information (both functional and morphological) about the internal structure and physiopathology of the peripheral nerves. [33]

The magnetic resonance neurography (MRN) is the salvage solution for complex cases. The spacial distribution of the peripheric nerve helps with the classification of the peripheric nerve injuries in traumatic plexopathies or partial neuropathies of the fascicles. [34]

MRN has some important advantages: it is not operator dependent, it offers 3D representation of the entire nerve, it has great soft tissue contrast, and it provides detailed images of the nerve structure, fascicles and its surrounding environment. For these reasons, MRN is a suitable investigation for tumors, traumas (including the evaluation of brachial plexus injuries), as well as compressive lesions. [35]

Neurography is particularly useful in the cases where nervous lesions are less obvious (compression syndromes or partial nerve injuries); this investigation is capable of establishing the degree of the nervous injury, directing the therapeutic approach.

Microstructural defects of the peripheral nerves can nowadays be detected using quantitative diffusion tensor imaging. (DTI) The imagistic depiction on MRN of the smaller nerve branches can be improved by vascular suppression techniques and parallel imagining, which provides a higher spacial resolution. [36]

The gastrocnemius index was a quantifiable measurement in assessing nerve regeneration. A gastrocnemius index close to the value of 1 (meaning that the weights of the 2 gastrocnemius muscles – right and left - are similar) represents the normal value of a non-operated rat, with muscles that did not undergo atrophy. A very low value of this index indicates severe muscle atrophy, due to a poor nervous regeneration (or a lack of nerve regeneration).

The rat with the ruptured sciatic nerve in the first batch was taken out of the study in order not to influence the results. However, it was useful in order to observe the degree of muscle atrophy when no repair was performed and also as a reference in order to compare regeneration in the 4 batches.

While the median value of the gastrocnemius index for the 38 rats included in the study was 0.5, the gastrocnemius index for the rat with the ruptured nerve was 0.18. However, not having more rats in the category of the unrepaired sciatic nerve, no statistical analysis could be performed.

Figure 12: Two pairs of gastrocnemius muscles in 2 different rats

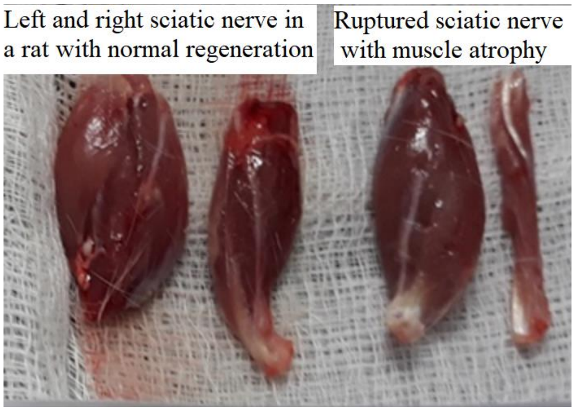
Left and right sciatic nerve in a rat with normal regeneration
Ruptured sciatic nerve with muscle atrophy

The positive correlation between the gastrocnemius index and the sciatic functional index at 6 and 12 weeks can be explained by the fact that both indicators are influenced by the degree of the calf muscle recovery.

CONCLUSIONS

Nerve regeneration can be properly evaluated only after a period of time. The MRI right-left calf diameter ratio represents a non-invasive investigation complementary to the gastrocnemius index as they both evaluate the same aspect in the process of nerve regeneration – the degree of muscle atrophy. Therefore, there was a strong correlation between these 2 investigations.

None of the repair methods tested showed a statistical significant advantage regardless of the test used to evaluate nerve regeneration.

The effect of muscle atrophy was reflected in the footprint test and therefore the sciatic functional index could be correlated to the MRI right-left calf diameter ratio.

Development in technology will provide better imagistic methods to monitor the regeneration stages after peripheral nerve injury both at the site of the injury, as well as at the site of the innervated organ.

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ORIGINAL ARTICLES

Evaluation of epidemiological characteristics, treatments, and prognosis of patients with COVID-19 hospitalized in the Intensive Care Unit

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Abstract: *Introduction: Intensive care facilities may play an important role in reducing corona mortality. This study aimed to assess the epidemiological features, treatments, and prognosis of corona patients that admitted to the Intensive Care Unit (ICU) of Baqiyatallah Hospital.*

Methods: All patients with a corona that admitted to the ICU of Baqiyatallah Hospital from March 5 to May 20, 2020, were included. The epidemiological characteristics, pharmacological, non-pharmacological, and supportive therapies and prognosis were assessed. Underlying diseases, smoking, and severity of lung involvement based on CT scan findings (mild, moderate, severe) and length of hospital stay in the ICU were recorded in a questionnaire. Non-invasive ventilation (NIV) or tracheal intubation, ventilator ventilation, and duration of endotracheal intubation, if needed, were recorded in the questionnaire. Meanwhile, whether tracheostomy was performed, the time of the procedure, and its duration were also recorded.

Results: Overall, 82 patients were included in the study that 68 (82.9%) of them were males. The mean age was 57.96±14.08 years. The mortality rate was 53 cases (64.6%). There were 57 subjects (73.1%) at a severe lung involvement and 21 subjects (26.9%) at a moderate lung involvement. There was a significant relationship between severity of lung involvement and mortality (Odds ratio= 14.3, P<0.001).

There was a significant relationship between mortality and Armadile use (Odds ratio= 2.9, P=0.04). There was a significant relationship between mortality and tracheal intubation (Odds ratio= 27.2, P<0.001). There was no significant difference between mortality and discharge of patients regarding the intubation period (P=0.26). There was not a significant relationship between tracheostomy, NIV, and mortality status in the patients (P=0.14 and 0.33 respectively). Also, there was no significant difference between mortality and discharge of patients regarding tracheostomy and NIV period (P=0.51 and 0.17). There was no significant difference in age between severe and moderate lung involvement cases (P=0.63). There was a significant difference in the duration of ICU hospitalization between severe and moderate lung involvement cases (P=0.004).

Conclusion: The results showed none of the factors of age, sex, underlying diseases other than hypertension, pharmacological methods other than Armadile, and non-pharmacological methods such as hemoperfusion, plasmapheresis, IVIG, hyperimmune plasma injection, and stem cell as well as Ozone therapy does not affect corona patient's mortality. Only the severity of lung involvement leading to endotracheal intubation and mechanical ventilation is related to patients' mortality

Keywords: *Covid-19, Mortality, ICU*

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INTRODUCTION

In December 2019, the world faced a great challenge. A respiratory disease caused by the coronavirus that causes severe acute respiratory syndrome (SARS) in patients and endangers the health of people around the world [1].

Treatment of these patients is supportive and usually respiratory failure is the main cause of death [2]. The clinical features and CT scan images help in the early diagnosis and treatment of this disease [3].

This disease’s mortality has been stated 3.6% in China and 1.2 to 1.7% outside of China. the difference between these statics is because of not considering patients with mild illness and taking diagnostic tests. The actual number of deaths is unknown [4].

Intensive care facilities may play an important role in reducing the risk of COVID-19 death [5].

In this retrospective study, we surveyed the epidemiological features, treatments, and prognosis of patients with coronavirus admitted to the ICU of Baqiyatallah Hospital, that we may identify preventable factors associated with the patient recovery and reduce mortality of these patients.

METHODS

In a retrospective study from March 5 to May 20, 2020, all patients with COVID-19admitted to ICU of Baqiyatallah Hospital were evaluated for their epidemiological characteristics, pharmacological, non-pharmacological, and supportive therapies, and prognosis. "Baqiyatallah Hospital is one of the important referral medical centers for corona patients in Iran."

The diagnostic criteria for Corona Patients are clinical symptoms, positive PCR test, and CT scan findings in this center. Hospitalized patients with respiratory distress or SPO2 with oxygen and storage bag below 90% in general wards and emergency departments were transferred to ICU.

Epidemiological characteristics of patients including age, sex, underlying diseases, smoking and Drug abuse, the severity of lung involvement based on CT scan findings (mild, moderate, severe), and length of hospital stay in the ICU were recorded in a questionnaire.

All drugs used in the treatment of patients, as well as non-pharmacological methods including antibody therapy, plasmapheresis, hemofiltration with Jeffron filter, hyperimmune plasma, IVIG, CRRT with cytosorb filter, stem cell, and other methods, were recorded in the questionnaire.

NIV or tracheal intubation, ventilator ventilation, and duration of endotracheal intubation, if needed, were recorded in the questionnaire. Meanwhile, whether tracheostomy was performed, the time of the procedure, and its duration were also recorded.

The data were analyzed by SPSS-22 software. Quantitative data were analyzed using descriptive software and displayed as Mean ± SD. Chi-Square tests were used to compare percentages or frequencies. A P-value less than 0.05 was considered statistically significant.

RESULTS

Overall, 82 patients were included in the study that 68 (82.9%) of them were males. The mean age was 57.96±14.08 years. The mortality rate was 53 cases (64.6%). Table 1 showed the distribution of age, sex, duration of hospitalization in the ICU, and history of diabetes mellitus (DM), hypertension (HTN), kidney disease, liver disease, rheumatic disease, smoking, opium addiction, corticosteroid use, and ARBS regarding mortality of the patients.

The mean age was 59.59±13.85 years in mortality cases and 55.03±14.24 years in discharge patients. There was no significant difference in age between mortality and discharge of patients (P=0.16). Also, there was not a significant relationship between gender and mortality (P=0.76(.

There was not a significant relationship between mortality and DM, kidney disease, lung disease, liver disease, and rheumatic disease in the patients (Table 1). However, there was a significant relationship between mortality and HTN (Odds ratio= 2.99, P=0.04) (Table 1).

There was not a significant relationship between mortality and smoking, opium addiction, corticosteroids, and ARBS in the patients (Table 1).

Table 2 showed the distribution of pharmacological treatment regarding the mortality status of the patients. There was a significant relationship between mortality and Armadile use (Odds ratio= 2.9, P=0.04) (Table 2).

Table 3 showed the distribution of tracheal intubation, intubation period, tracheostomy, tracheostomy period, NIV, NIV period regarding the mortality status of the patients.

There was a significant relationship between mortality and tracheal intubation (Odds ratio= 27.2, P<0.001) (Table 3). The mean intubation period was 3.50±2.83 days in mortality cases and 2.81±2.22 days in discharge patients. There was no significant difference between mortality and discharge of patients regarding the intubation period (P=0.26).

Table 1: Distribution of age, sex, duration of hospitalization in the ICU, DM, HTN, kidney disease, liver disease, rheumatic disease, smoking, drug use, corticosteroids, and ARBS regarding mortality status of the patients

Mortality status		Death	Discharge	Total	P-value
Number (%)		53 (64.6%)	29 (35.4%)	82	---
Sex	male	43 (81.1%)	25 (86.2%)	68 (92.9%)	0.76
	female	10 (19.9%)	4 (13.8%)	14 (17.1%)	
Age, years, mean (±SD)		59.59±13.85	55.03±14.24	57.96±14.08	0.16
Duration of ICU hospitalization, days, mean (±SD)		13.28±8.72	14.17±1.30	13.59±10.06	0.83
DM	Yes	22 (41.5%)	9 (31.0%)	31 (37.8%)	0.35
	No	31 (58.5%)	20 (69.0%)	51 (62.2%)	
HTN	Yes	23 (43.4%)	6 (20.7%)	29 (35.4%)	0.04*
	No	30 (56.6%)	23 (79.3%)	53 (64.6%)	
kidney disease	Yes	5 (9.4%)	3 (10.3%)	8 (9.8%)	0.58
	No	48 (90.6%)	26 (89.7%)	74 (90.2%)	
Lung disease	Yes	4 (7.5%)	1 (3.4%)	5 (6.1%)	0.65
	No	49 (92.5%)	28 (96.6%)	77 (93.9%)	
Liver disease	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	No	53 (100%)	29 (100%)	82 (100%)	
Rheumatic disease	Yes	0 (0.0%)	1 (3.4%)	1 (1.2%)	0.35
	No	53 (100%)	28 (96.6%)	81 (98.8%)	
Smoking	Yes	3 (5.7%)	0 (0.0%)	3 (3.7%)	0.54
	No	50 (94.3%	29 (100%)	79 (96.3%)	
Opium addiction	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	No	53 (100%)	29 (100%)	82 (100%)	
Corticosteroids	Yes	3 (5.7%)	2 (6.9%)	5 (6.1%)	0.58
	No	50 (94.3%	27 (93.1%)	77 (93.9%)	
ARBS	Yes	13 (24.5%)	4 (13.8%)	17 (20.7%)	0.35
	No	40 (75.5%)	25 (86.2%)	65 (79.3%)	

** P-value is significant at the 0.05 level, SD=Standard deviation*

Table 2: Distribution of pharmacological treatment regarding the mortality status of the patients

Mortality status		Death	Discharge	Total	P-value
Tamiflu	Yes	28 (52.8%)	16 (55.2%)	44 (53.7%)	0.51
	No	25 (47.2%)	13 (44.8%)	38 (46.3%)	
Interferon beta	Yes	12 (22.6%)	8 (27.6%)	20 (24.4%)	0.61
	No	41 (77.4%)	21 (72.4%)	62 (75.6%)	
Hydroxychloroquine	Yes	48 (90.6%)	27 (93.1%)	75 (91.5%)	0.52
	No	5 (9.4%)	2 (6.9%)	7 (8.5%)	
Remdesivir	Yes	6 (11.3%)	3 (10.3%)	9 (11%)	0.60
	No	47 (88.7%)	26 (89.7%)	73 (89%)	
Ribavirin	Yes	35 (66%)	18 (62.1%)	53 (64.6%)	0.71
	No	18 (34%)	11 (37.9%)	29 (35.4%)	
Favipiravir	Yes	20 (37.7%)	11 (37.9%)	31 (37.8%)	0.98
	No	33 (62.3%)	18 (62.1%)	51 (62.2%)	
Kaletra	Yes	51 (96.2%)	28 (96.4%)	79 (96.3%)	0.71
	No	2 (3.8%)	1 (3.4%)	3 (3.7%)	
Anticoagulant	Yes	47 (88.7%)	24 (82.8%)	71 (86.6%)	0.45
	No	6 (11.3%)	5 (17.2%)	11 (13.4%)	
Azithromycin	Yes	49 (92.5%)	28 (96.6%)	77 (93.9%)	0.41
	No	4 (7.5%)	1 (3.4%)	5 (6.1%)	

Mortality status		Death	Discharge	Total	P-value
Plasmapheresis	Yes	6 (11.3%)	4 (13.8%)	10 (12.2%)	0.73
	No	47 (88.7%)	25 (86.2%)	72 (87.8%)	
Corticosteroids therapy	Yes	47 (88.7%)	27 (93.1%)	74 (90.2%)	0.70
	No	6 (11.3%)	2 (6.9%)	8 (9.8%)	
Hemoperfusion	Yes	29 (54.7%)	25 (51.7%)	44 (53.7%)	0.79
	No	24 (45.3%)	14 (48.3%)	38 (46.3%)	
Naproxen	Yes	45 (84.9%)	25 (86.2%)	70 (85.4%)	0.57
	No	8 (15.1%)	4 (13.8%)	12 (14.6%)	
CRRT	Yes	0 (0.0%)	1 (3.4%)	1 (1.2%)	0.35
	No	53 (100%)	28 (96.6%)	81 (98.8%)	
Cinnora	Yes	3 (5.7%)	2 (6.9%)	5 (6.1%)	0.58
	No	50 (94.3%)	27 (93.1%)	77 (93.9%)	
IVIG	Yes	26 (49.1%)	9 (31.0%)	5 (42.7%)	0.11
	No	27 (50.9%)	20 (69.0%)	47 (57.3%)	
Armadile	Yes	20 (37.7%)	5 (17.2%)	25 (30.5%)	0.04*
	No	33 (62.3%)	24 (82.8%)	75 (69.5%)	
Actemra	Yes	14 (26.4%)	5 (17.2%)	19 (23.2%)	0.34
	No	39 (73.6%)	24 (82.8%)	63 (76.8%)	
AB therapy	Yes	45 (84.9%)	24 (82.8%)	69 (84.1%)	0.79
	No	8 (15.1%)	5 (17.2%)	13 (15.9%)	
Plasma hyper immune	Yes	20 (37.7%)	12 (41.4%)	32 (39.0%)	0.74
	No	33 (62.3%)	17 (58.6%)	50 (61.0%)	
Stem cell	Yes	6 (11.3%)	2 (6.9%)	8 (9.8%)	0.70
	No	47 (88.7%)	27 (93.1%)	74 (90.2%)	
Ozone therapy	Yes	2 (3.8%)	0 (0.0%)	2 (2.4%)	0.53
	No	51 (96.2%)	29 (100%)	80 (97.6%)	

** P-value is significant at the 0.05 level.*

Table 3: Distribution of tracheal intubation, intubation period, tracheostomy, tracheostomy period, NIV, NIV period regarding mortality status of the patients

Mortality status		Death	Discharge	Total	P-value
Tracheal intubation	Yes	50 (94.3%)	11 (37.9%)	61 (74.4%)	<0.001*
	No	3 (5.7%)	18 (62.1%)	21 (25.6%)	
Intubation period, days, mean (±SD)		3.50±2.83	2.81±2.22	3.37±2.72	0.26
Tracheostomy	Yes	29 (54.7%)	11 (37.9%)	40 (48.8%)	0.14
	No	24 (45.3%)	18 (62.1)	42 (51.2)	
Tracheostomy period, days, mean (±SD)		11.29±7.26	12.81±5.82	11.69±7.19	0.51
NIV	Yes	13 (24.5%)	10 (34.5%)	23 (28.0%)	0.33
	No	40 (75.5%)	19 (65.5%)	59 (72.0%)	
NIV period, days, mean (±SD)		3.63±2.20	2.54±1.43	3.09±1.90	0.17

** P-value is significant at the 0.05 level.*

There was not a significant relationship between tracheostomy, NIV, and mortality status in the patients (P=0.14 and 0.33 respectively). Also, there was no significant difference between mortality and discharge of patients regarding tracheostomy and NIV period (P=0.51 and 0.17) (Table 3).

Table 4 showed the distribution of mortality, age, tracheostomy, duration of ICU hospitalization, tracheal intubation, intubation period, tracheostomy, tracheostomy period, NIV, NIV period regarding the severity of the disease of the patients.

There were 57 subjects (73.1%) at a severe lung involvement and 21 subjects (26.9%) at a moderate lung involvement (Table 4). There was a significant relationship between severity of lung involvement and mortality (Odds ratio= 14.3, P<0.001).

The mean age was 58.05±13.43 years in severe lung involvement cases and 55.19±15.15 years in moderate lung involvement cases. There was no significant difference in age between severe and moderate lung involvement cases (P=0.63).

The mean duration of ICU hospitalization was 15.59±10.61 days in severe lung involvement cases and 8.85±5.78 days in moderate lung involvement cases. There was a significant difference in the duration of ICU hospitalization between severe and moderate lung involvement cases (P=0.004).

49 cases (86.0%) in severe lung involvement had tracheal intubation There was a significant relationship between the severity of diseases and tracheal intubation (P<0.001). The mean intubation period was 3.30±2.32 days in severe cases and 4.12±4.96 days in moderate cases. There was not a significant difference in the intubation period between severe and moderate lung involvement cases (P=0.86).

Also, 34 cases (59.6%) in severe lung involvement had a tracheostomy. There was a significant relationship between the severity of diseases and tracheostomy (P<0.001). There was not a significant difference in the tracheostomy period between severe and moderate lung involvement cases (P=0.74).

There was not a significant relationship between NIV and the severity of disease in the patients (P=0.15). The mean NIV period was 3.44±1.91 days in severe lung involvement cases and 1.50±0.57 days in moderate lung involvement cases. There was a significant difference in the NIV period between severe and moderate lung involvement cases (P=0.026) (Table 4).

Table 4: Distribution of morality, age, duration of ICU hospitalization, tracheal intubation, intubation period, tracheostomy, tracheostomy period, NIV, NIV period regarding the severity of lung involvement of the patients

The severity of lung involvement		Moderate	Severe	Total	P-value
Number (%)		21 (26.9%)	57 (73.1%)	82	---
Mortality	Yes	6 (28.6%)	43 (75.4%)	49 (62.8%)	<0.001*
	No	15 (71.4%)	14 (24.6%)	29 (37.2%)	
Age, Years, mean (±SD)		55.19±15.15	58.05±13.43	57.27±13.87	0.63
Duration of ICU hospitalization, days, mean (±SD)		8.85±5.78	15.59±10.61	13.78±9.98	0.004*
Tracheal intubation	Yes	8 (38.1%)	49 (86.0%)	57 (73.1%)	<0.001*
	No	13 (61.9%)	8 (14.0%)	21 (26.9%)	
Intubation period, days, mean (±SD)		4.12±4.96	3.30±2.32	3.42±2.79	0.86
Tracheostomy	Yes	4 (19.0%)	34 (59.6%)	38 (48.7%)	0.001*
	No	17 (81.0%)	23 (40.4%)	40 (51.3%)	
Tracheostomy period, days, mean (±SD)		10.50±4.79	11.75±7.08	11.62±6.84	0.74
NIV	Yes	3 (14.3%)	19 (33.3%)	22 (28.2%)	0.15
	No	18 (85.7%)	38 (66.7%)	58 (71.8%)	
NIV period, days, mean (±SD)		1.50±0.57	3.44±1.91	3.09±1.90	0.026*

** P-value is significant at the 0.05 level*

DISCUSSION

Overall, 82 patients were included in this study that 68 (82.9%) of them were males. The mean age was 57.96±14.08 years. The mortality rate was 53 cases (64.6%).

There was a significant relationship between mortality and history of HTN, Ardamadil use for treatment, the severity of lung involvement, and tracheal intubation. There was no significant relationship between NIV and the severity of lung involvement in the patients. There was a significant difference in the NIV period between severe and moderate lung involvement cases.

The mean duration of ICU hospitalization was 15.59±10.61 days in severe cases and 8.85±5.78 days in moderate cases. There was a significant difference in the duration of ICU hospitalization between severe and moderate cases

Daniel E L Promislow and colleagues concluded that aging is associated with increased complications of corona and that men of all ages have more serious complications than women [6].

Qirong Ruan and colleagues in a study of hospitalized corona patients concluded that age had a significant effect on mortality and gender had no significant effect. Underlying diseases, secondary infections, and high levels of inflammatory factors in the blood are predictors of high mortality [7].

However, in this study, patient's age and sex did not have a significant relationship with mortality. Maybe the reason that gender didn’t have a significant relationship with mortality in this study was that this study included patients admitted to the ICU and patients with critical situations were being transferred to this sector., and this statistic is different from the general mortality statistics in the society.

As in previous studies, Mandeep R. et al. Concluded heart diseases were associated with increased mortality in hospitalized corona patients. In their study, the use of ACEIs and ARBs was not associated with an increase in mortality [8].

In a meta-analysis of 1576 patients with COVID 19, JingYang and colleagues concluded that underlying diseases such as hypertension, heart disease, and respiratory disease were more common in critically ill patients than in less critically ill patients [9].

Marcello Covino et al. concluded that the use of ACEIs or ARBs in corona hypertensive patients was not associated with increased mortality [10].

In this study, in line with Mandeep R. and JingYang studies, blood pressure had a significant relationship with mortality. Also, in line with the study of Mandeep R. and Marcello Covino, the use of ACEIs and ARBs had no significant relationship with mortality.

Thomas C. Hanff and colleagues stated that corona hypertensive patients treated with ACEIs should not change their treatment regimen [11].

While Lei Fang and colleagues found that diabetes, heart disease, and hypertension were factors associated with corona disease severity, they suggested replacing calcium channel blockers with ACEIs or ARBs [12].

Li-Lin Liang and colleagues found that higher mortality rates could be due to fewer testers. Also, an older population, weak government, and a small number of beds can increase mortality [13].

A study by YUNPENG JI et al. In China concluded that the reason for the high mortality rate in HUBEI (2.9%) compared to other Chinese provinces (0.7%) was probably due to the rapid increase in the number of infections around the outbreak center. Which has led to insufficient health resources [14].

CONCLUSION

According to the results of this study, none of the factors of age, sex, underlying diseases other than hypertension, pharmacological methods other than Armadile, and non-pharmacological methods such as hemoperfusion, plasmapheresis, IVIG, hyperimmune plasma injection, and stem cell as well as Ozone therapy does not affect corona patient’s mortality. Only the severity of lung involvement leading to endotracheal intubation and mechanical ventilation is related to patients’ mortality, so according to studies by Li-Lin Liang and YUNPENG JI, it can be concluded that The most important factor in reducing patient mortality is the management of the disease epidemic, which makes adequate treatment resources available to patients and no patient dies due to lack of facilities in severe epidemics. And in epidemics, special attention should be paid to hypertensive patients.

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VARIA

Evaluation of fasting impact during Ramadan month on patients with a history of kidney stones in a military hospital

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Abstract: *Introduction: One of the cornerstones and common causes of kidney stone formation is the volume of fluid consumed during a 24- hour that influences on forming that by several mechanisms. Since fluid intake decreases during Ramadan, one of the common questions posed is whether Ramadan fasting is associated with the precipitating of stone formation. The present study was aimed to investigate the effect of Ramadan fasting on precipitating and Inhibitors of stone formation in patients with a history of renal kidney disease.*

Methods: The study population included all of the patients with kidney stone diseases who were seen at the Clinic of Urology and Nephrology affiliated with Baqiyatallah hospital during the month of Ramadan in 2014 and 2015. The number of 66 patients was chosen and included in the study through randomized simple sampling. All subjects underwent blood tests like serum uric acid, serum phosphate, serum calcium, and serum sodium. Samples were tested in terms of volume, magnesium, uric acid, Calcium, Phosphate, Sodium, citrate, oxalate, Calcium oxalate supersaturation, Calcium Phosphate supersaturation, Uric acid supersaturation.

Results: Sixty-six eligible patients were included that the mean age was 48.35±12.10 years. Fourteen men and 52 women were included. There were no significant differences in the mean pH of urine, urinary sodium, urinary oxalate, super-saturated levels of calcium oxalate in the urine, urinary phosphate levels, and super-saturated levels of urinary calcium phosphate between the two groups (P>0.05). The average volume of urine, urinary magnesium, urinary calcium, urinary citrate, the uric acid level in the non-fasting group was higher than the fasting group (P<0.05). There were no differences in the average blood calcium levels, blood sodium, blood phosphate levels, blood acidic levels between the two groups (P>0.05). There was no difference in the mean super-saturated level of uric acid between the two groups (P=0.035).

Conclusion: There was controversial evidence on the effect of Ramadan fasting on kidney stone formation and there is not enough evidence in the present study that Ramadan fasting precipitates risk factors associated with calculus formation

Keywords: *fasting, kidney calculi, inhibitor factors, and precipitating factors*

INTRODUCTION

Ramadan month is related to the Islamic calendar and is a holy month for Muslims. Healthy adult Muslims should be fasting during this month and the times spent on fasting maybe change from 12 to 18 hours depending on the seasonal and regional specialties. Any food and fluid eating are allowed only from sunset to sunrise in this month. Islam rules permit the break of fasting for particular people including unhealthy cases [1-5].

However, many Muslim patients reveal their willingness to keep the fast during Ramadan month to regard religious cultures. There are concerns regarding the influence of

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dehydration and the consequent renal hypoperfusion in Ramadan fasting for patients with renal illnesses [6]. This problem especially arises when Ramadan month happens during hot and dry summers with a long daytime span.

In the Ramadan month, fasting Muslims tend to alter their dietary habits. This involves changes in eating times and the content of food, in addition to fluid deprivation. It is fairly well known that fluid deprivation causes volume con-traction and renal hypoperfusion. The latter may cause renal dysfunction and aggravate existing renal impairment [7-9].

There is scarce scientific data about the safety of Ramadan fasting for patients with different renal diseases. The existing published reports are mainly focused on the impact of Ramadan fasting on patients with renal transplantation, patients with chronic kidney disease, and patients with the renal stone disease [10-12].

One of the cornerstones and common causes of kidney stone formation is the volume of fluid consumed during a 24- hour that influences on forming that by several mechanisms [13-15]. Since fluid intake decreases during Ramadan, one of the common questions posed is whether Ramadan fasting is associated with the precipitating of stone formation. The present study was aimed to investigate the effect of Ramadan fasting on precipitating and Inhibitors of stone formation in patients with a history of renal kidney disease.

METHODS

The study population included all of the patients with kidney stone diseases who were seen at the Clinic of Urology and Nephrology affiliated with Baqiyatallah hospital during the month of Ramadan in May 2016 and May 2017. The number of 66 patients was chosen and included in the study through randomized simple sampling. The sample size of the study consisted of 66 kidney stone patients (30 patients in the fasting and 36 patients in the nonfasting group). The case group was examined for three weeks after one week from the beginning of the holy month of Ramadan and their laboratory factors were recorded.

Inclusion criteria were included the presence of kidney stones at the time of referral or a history of kidney stones proven by ultrasound or CT scan, did not use drugs that affect the aggravation or treat of the kidney stones, did not use a diet that affects stone formation (Including frequent consumption of nuts such as nuts or frequent consumption of chocolate containing cocoa. Cola drinks, a high-salt or meaty diet, and plenty of citrus fruits). Also, patients with chronic liver disease, advanced cardiac disease, acute infection, or diabetes insipidus were excluded. All cases were fulfilled the consent form and they have consented to fast in Ramadan.

Collecting data was done by a Researcher-built Form. All subjects underwent blood tests like serum uric acid, serum phosphate, serum calcium, and serum sodium. Samples were asked to urinate for the first time at 9 a.m. and to be expelled. The urine is then poured into a special container until 9 pm and the entire urine collected within 12 hours is delivered the next day. Clinical evaluation of the patients included meticulous current and past medical history, and physical examination. Urine samples were tested in terms of volume, magnesium, uric acid, Calcium, Phosphate, Sodium, citrate, oxalate, Calcium oxalate supersaturation, Calcium Phosphate supersaturation, Uric acid supersaturation. Uric acid was calculated using the Tiselius formula in both groups. The evaluation of the patients was conducted near the end of the month of Ramadan.

After being collected, the data was inserted into SPSS software (Statistical Package for Social Science Version 20) and analyzed by using descriptive statistics and statistical tests such as t-Tests and the Mann-Whitney. A P-value of less than 0.05 was considered significant.

RESULTS

Sixty-six eligible patients were included. The mean age was 48.35±12.10 years. Fourteen men and 52 women were included. There were no differences between the two groups regarding age and gender (P=0.12 and 0.14 respectively).

Table 1: Demographic characteristics of all participants in the two groups

Groups	Non-fasting	Fasting	P-value
Age	48.3±13.37	43.4±11.06	0.118
Gender, M/F	8/28	6/24	0.452

There were no significant differences in the mean pH of urine, urinary sodium, urinary oxalate, super-saturated levels of calcium oxalate in the urine, urinary phosphate levels, and super-saturated levels of urinary calcium phosphate between the two groups (P>0.05) (Table 2).

The average volume of urine, urinary magnesium, urinary calcium, urinary citrate, the uric acid level in the non-fasting group was higher than the fasting group (P<0.05) (Table 2).

There were no differences in the average blood calcium levels, blood sodium, blood phosphate levels, blood acidic levels between the two groups (P>0.05) (Table 2).

There was no difference in the mean super-saturated level of uric acid between the two groups (P=0.035) (Table 2).

Table 2: Biochemical characteristics of all participants in the two groups

Groups	Non-fasting	Fasting	p-value
Blood Ca level	9.4±0.52	9.3±0.83	0.567
Blood Na level	141.80±2.01	140.80±1.91	0.101
Urine volume	1030.60±541.60	604.00±260.35	0.001
Urine PH level	5.60±0.67	5.80±0.86	0.317
Urine Mg level	105.70±27.14	43.20±14.86	<0.001
Urine Ca level	97.50±54.55	72.40±41.51	0.039
Urine Na level	97.30±43.46	77.10±40.66	0.066
Urine Sitrat level	1.90±1.02	1.40±0.88	0.034
Urine Exalarat level	0.31±0.81	0.30±0.80	0.36
Urine Extra ca Exalarat level	1.50±1.01	1.60±0.87	0.575
Blood Pa level	4.00±0.62	4.20±0.54	0.461
Urine Pa level	422.50±219.28	367.20±176.17	0.275
Urine Extra ca Pa level	477.90±433.29	2055.10±5856.15	0.159
Blood acid uric level	6.20±1.71	5.80±1.58	0.576
Urine acid uric level	208.59±73.49	154.90±66.57	0.004
Extra acid uric level	0.50±0.23	0.80±0.72	0.35

DISCUSSION

In this study, the effect of fasting during Ramadan month on the improvement of the urinary system was examined. The concept of ultra-stable saturation is due to the excessive increase in the constituent salts of the stone, such as calcium, phosphorus, oxalate, and uric acid in the urine, and the solubility of these minerals in the urine. It seems that the lack of saturation in non-stone individuals is due to the activity of crystallization inhibitors. These inhibitors include magnesium, citrate, phosphate ions, and coarse molecules such as Tamm-Horsfall protein. In the case of low inhibitors and the ability to form stone, the constituent salts of the stone adhere to the rock crystals and from these crystals, nodules or stone nuclei and finally from the enlargement of these nodules, one-millimeter pebbles and the enlargement and the accumulation of these pebbles form larger stones. Also, other conditions such as urinary saturation, increased deposition of kidney stones such as calcium, oxalate, uric acid, and phosphate, urine pH, decreased urine volume, the presence of crystallization facilitators such as uric acid, infection, acidity, and pointed to insoluble ions. The results of the present study showed that the level of uric acid in the blood, the level of calcium in the blood, the level of sodium in the blood, the level of urinary phosphate, the level of urinary sodium in non-fasting people is higher than in fasting people. On the other hand, urine volume, urinary magnesium, uric acid, calcium urine, total urine volume, and urinary citrate in non-fasting individuals were higher than in fasting individuals, which was statistically significant. Ultrasonic levels of calcium phosphate in the urine of fasting patients are higher than those of non-fasting patients but were not statistically significant.

In general, among the blood parameters involved in the formation of kidney stones, only the level of blood phosphate in the fasting group is higher than the fasting group, but statistically, this difference was not significant. According to the findings of this study, the average level of urinary citrate, urinary magnesium, urine volume in the fasting group is lower than the fasting group. The high levels of these three act as crystallization inhibitors and the low levels in fasting people provide the basis for the formation of kidney stones. But statistically, only urine volume and urine citrate among the above inhibitors were significantly lower in the fasting group than in the fasting group. They reduce the growth of crystals and their accumulations and prevent the formation of secondary nuclei. Facilitators refer to molecules that reduce the supersaturated surface needed to initiate the crystallization process. The imbalance between these two sets of factors is more important than other factors in the formation of kidney stones. These substances include organic compounds, proteins, and glycosaminoglycans.

In this study, the urine volume of fasting people was lower than that of non-fasting people. Excessive fluid intake has been a traditional treatment in recent decades, and doctors have been prescribed to prevent kidney stones in patients with kidney stones. The results of studies on the effect of urine volume on kidney stone formation are: Low volume of urine is considered as a risk factor for the onset of kidney stone formation and re-formation of kidney stone, an increase in urine volume due to excessive fluid consumption. It has a positive effect on the prevention of kidney stones and does not interfere with the normal activity of inhibitors. Mass consumption of liquids such as water, coffee, tea, beer has a deterrent effect on reversibility and kidney stone formation. Everything is in dispute and is always controversial. Therefore, high fluid intake, especially water, is considered an effective and economical method in preventing the formation of kidney stones, which is often ignored by people who have kidney stones [16]. In a study by Cheh et al., Which was conducted to investigate the effect of fasting on urinary excretion, the results showed that the volume of urine was reduced by 24 hours in resinous individuals, but this decrease was not significant. In this study, 24-hour urine volume was divided into three time periods, urine volume in the morning, evening, and night. In this study, the volume of nocturnal urine in the previous periods and the length of Ramadan have remained unchanged, and Ramadan does not affect the volume of nocturnal urine. But the volume of urine in the evening was less than in the pre-Ramadan period, and this decrease was significant. Which is consistent with the results of this study. Also, a decrease in urine in the second and third weeks was significant in fasting people, but in the last week of Ramadan, it was not significant, which is largely consistent with the findings of this study. Decreased urinary volume, indicating low fluid intake or loss of excess water, directly increases the risk of kidney stones by increasing the saturation of stone-forming salts in the urine. Fasting may limit fluid intake and, by affecting urine concentration and chemical composition of the blood, prepare the ground for the formation of kidney stones [17]. Magnesium is the fourth most abundant mineral in the body and is intentionally located in the bones. The magnesium in the diet is absorbed in the small intestine and excreted by the kidneys.

The normal amount of 12-hour urine magnesium is between 0.15 and 0.5. Only 0.1% of all magnesium in the body is mobile. Magnesium can combine with oxalate to reduce supersaturation. Oral magnesium intake reduces the absorption of oxalate and urinary excretion. Magnesium can also combine with calcium in the gut. Magnesium supplements increase the excretion of citrate in the urine during magnesium deficiency [18]. According to the findings of this study, the mean level of urinary magnesium in fasting people is significantly different from fasting people, so that its level is lower than in fasting people. In the study of Miladipour and colleagues, the level of urinary magnesium in fasting people Dar was higher than non-fasting people, but it was statistically insignificant, which differs from the findings of this study [19]. Zegal et al. investigated the effect of fluid and dietary restriction restrictions on fasting on the biochemical factors of kidney stone formation. In this study, 90 patients were divided into three groups of healthy fasting people (those without kidney stones), healthy people without fasting, and people with kidney stones without fasting. The results of this study showed that the mean inhibitory level of urinary magnesium among fasting groups without kidney stone disease is lower than non-fasting individuals, which is quite consistent with the results obtained in this study [19]. Another inhibitor of kidney stone formation is citrate, which is present in the form of trichloroxyl acid in the blood and combined with calcium, magnesium, and sodium at a pH of 7.4. Most citrate is derived from exogenous aerobic metabolism, which is freely cleared in the glomerulus. Approximately 75% of the filtered citrate is absorbed in the complex proximal open tubule. Hypothyroidism (low levels of citrate in the urine of 320 mg per day) is seen in more than 50% of people with kidney stones. The normal 12-hour urinary titrate is 0.6 to 4.8. Hypothyroidism is caused by a variety of factors, including medications such as acetazolamide and thiazides, renal acidosis, urinary tract infection, hypokalemia, low magnesium, and inflammatory bowel disease [20]. In the study of Zegal et al. Mentioned above, the level of citrate in fasting people was lower than in non-fasting people, which is consistent with the findings obtained in this field [19].

In this study, the mean urinary citrate level in the non-fasting group was higher than the fasting person, which was statistically significant. In the study, Miladipour et al. [19] in which 57 people participated, 37 had recurrent kidney stones and 20 had no kidney stones. Urine readings in three 24-hour urination periods before Ramadan (period 3), urination during Ramadan, and hours 19 to 15 (period 1), and urine was collected during Ramadan from 15 to 19 (4 hours urine as period 2). In this study, the amount of urinary citrate in people in the pre-Ramadan period (group 3) was lower compared to fasting people (group 1 and group 2), which contradicts the findings of this study. One of the facilitators of kidney stone formation in this study was uric acid. To form uric acid-containing stones, uric acid must be highly saturated in the urine. There are usually three main factors involved in the formation of uric acid-containing stones, including acidic urine, hyperuricemia, and low urine volume. Hyperuricemia is the excretion of more than 600 mg of uric acid per day in 24-hour urine. The degree of hyperuricemia is directly related to kidney stones [21]. High uric acid levels may be due to overproduction of uric acid in the body or reduced urinary excretion. In particular, the factors that increase the concentration of uric acid in the blood are diuretics, hypothyroidism, obesity, excessive alcohol and coffee consumption, genetic factors, etc. One of the findings of this study is The average uric acid level in the urine of fasting people was significantly higher than in fasting people. In the study of Miladipour et al. [19]. The mean uric acid level for 24-hour urine in non-fasting individuals was higher than the 24-hour uric acid level during Ramadan, but statistically, there was no significant difference following the findings of this study. Findings on uric acid and blood uric acid measurements show three major facts. 1. High concentrations of acid in the blood are due to decreased uric acid excretion in the urine, and low concentrations of urine are associated with increased uric acid. During fasting, a small portion of the uric acid that enters the nephrons from the blood is reabsorbed (reabsorbed.) There is a significant exchange of uric acid between the tissues and the blood for 24 hours a day.

12-hour normal urinary calcium is less than 250 mg. The results of this study showed that urinary calcium levels in fasting people were significantly lower than those without fasting. A study by Miladipour et al. [19] showed that the level of calcium in the urine of fasting people was significantly higher than that of fasting people, which is quite consistent with the results of this study. The concentration of calcium in the urine also indicates the concentration of calcium in the blood. The difference between urinary calcium levels in the two study groups appears to be due to the type of food and activity level, age, geographical location, and source of drinking water. Hyperoxaluria is an excessive increase in urinary oxalate (normal 12-hour urinary oxalate is between 0.23 and 0.68). In this study, urinary oxalate levels in the fasting and non-fasting groups did not differ significantly from each other. In the study of Miladipour et al. [19] the level of urinary oxalate in non-fasting and fasting people was not significantly different from each other, which is consistent with this study. Increased levels of oxalate in the urine of people are caused by factors such as diet or inflammatory bowel disease [22]. One of the major studies on the combination of calcium and oxalate is the cohort study conducted by Korhan et al. On 45619 men. After 4 years, 8.7% of the samples developed kidney stones. In this study, an inverse relationship was found between calcium intake and the formation of calcium-oxalate (CaOx) compounds [23]. Robertson's study found that there was a linear relationship between urinary oxalate levels and the formation of kidney stones, as well as the prevalence of kidney stones in different countries and the excretion of oxalate in the urine [24]. In general, 80% of calcium-based stones are oxalate calcium stones. In one study, randomized clinical trials of people with hypercalciuria were divided into two groups with low calcium and oxalate diets and one group with high calcium diets but oxalate, salt, and low protein. After five years, the renal stone recurrence rate in the second high-calcium group was reduced to 51% [25]. The results showed that the super-saturated levels of calcium oxalate in the urine were slightly lower in the fasting person than in the fasting person, but this difference was not significant. The amount of calcium oxalate in the urine may be altered by factors such as diet and fluid intake. Similar to the results of this study, in the study of Miladipour et al. [19] there was no significant difference between the mean calcium oxalate levels in fasting and non-fasting individuals, and also the desired variable was not significantly different in people with kidney stones during Ramadan and before Ramadan. In addition, there was no significant difference between healthy individuals during Ramadan and before Ramadan in terms of the average level of oxalate. Brochite ($CaHPO_{42}H_2O$) is the main cause of kidney stones of calcium phosphate origin. Urine in people who have kidney stones (which contain calcium) is saturated with super-high brushes due to the high concentration of calcium. Calcium is a type of calcium phosphate, but at least supersaturation is required for the formation and survival of rock containing calcium phosphate, because, in the lower levels of supersaturation, the primary nucleus of rock formation usually dissolves. All phosphates are calcium, containing calcium and phosphate ions. It is believed that the primary nucleus of calcium-containing rocks is calcium phosphate. Although calcium phosphate rocks may form in a supersaturated gaming solution, their formation in PH Urine is not known to be normal (26). In this study, the mean saturated level of urinary calcium phosphate in the fasting group was higher than the fasting population and this difference was statistically significant. In the study of Miladipour et al. [19], urinary phosphate is lower during fasting than before fasting, but it is statistically meaningless. The variability of calcium phosphate levels in the urine is influenced by factors such as diet and especially fluid intake, which is different for fasting and non-fasting days, but during Ramadan, there is not much difference for urine during different periods. In the study of rust, the amount of bruschetta was the same for healthy people fasting and people with kidney stones and without fasting, but for healthy people without fasting, this amount was less, which is consistent with our study. The excretion of uric acid in adults is 500 to 600 mg in 24 hours (the normal amount of uric acid in normal urine for 12 hours is less than 480).

The concentration of solution and pH plays an important role in the solubility of urate uric acid. However, the formation of uric acid stones is not common. Most people with an abnormal PH6 in their urine have urine saturated with uric acid, but they do not form a stone. More acidity (such as a pH of 3.5) or dehydration is needed to make a stone. Uric acid, which is not highly soluble, crystallizes at low pH and forms rock. The normal amount of uric acid in the blood is very important for the proper functioning of the body. The normal level of uric acid in the blood is between 0.3 and 0.7 mg / dL. Uric acid is formed in the human body system as a byproduct of purine metabolism and then excreted in the urine.

This means that the difference seems to be small due to various factors such as diet, urine volume, and dehydration, but this difference was not statistically significant. In the study of Miladipour et al. [19] the mean uric acid saturation level in the 4-hour evening urine (maximum concentrated urine) was higher than the pre-Ramadan uric acid saturation level and was statistically significant, which is consistent with our study. However, this rate is lower in the urine sample from 19:00 to 15:00 the next day than in the pre-Ramadan period. A small amount of sodium is absorbed from the stomach, but most are absorbed quickly through the small intestine. The absorbed sodium is carried by the bloodstream to the kidneys, where the sodium is purified and returned to the blood in the amount needed by the body. When the need for sodium increases, the action of some hormones stimulates the reabsorption of sodium by the kidneys. Conversely, when sodium intake increases, the secretion of these hormones decreases to reduce sodium retention. The amount of sodium in the urine depends on the daily diet, increases when consumption is high, and decreases when consumption is low. The increase or decrease in sodium in the blood should not exceed 0.5 to 1 mg/l. In the present study, the mean blood sodium level in the group of non-fasting people was higher than the fasting people, but this difference was not statistically significant. Also, the urinary sodium level of non-fasting people was higher than the fasting person, but this difference was not statistically significant.

In Miladipour's study, the amount of 24-hour sodium in non-fasting people was higher, but it is statistically meaningless, which is consistent with the present study. A study by Sakhai et al. showed that increasing the intake of sodium chloride from 140 to 310 mmol/L increases the amount of urinary calcium by 34% and reduces the amount of urinary citrate by 10%. Therefore, hyponitrite and calcification are among the main factors in the formation of kidney stones, which are caused by increased sodium chloride consumption [27]. In a study by Brutis et al., The results showed that sodium chloride salt consumption played a stronger role in increasing urinary calcium and the risk of stone formation compared to calcium and protein intake [28]. Consumption of sodium chloride salt is considered an indicator of the ratio of sodium to potassium in the urine [29]. The amount of normal 12-hour urinary phosphorus is between 1300-300. The average level of urinary phosphate in the non-fasting group was higher than in the fasting person, but this difference was not statistically significant. In Miladipour's study [19], the rate of 24-hour urinary phosphate in non-fasting individuals was higher and statistically significant, which is consistent with the present study. Decreased urinary phosphate levels in fasting people occur due to factors such as prolonged starvation during Ramadan.

The amount of phosphate reference in the adult blood is 2.7 to 4.5 milligrams per deciliter (mg/dL) or 0.87 to 1.45 milliliters per liter (mmol/L), which helps the kidneys control the amount of phosphorus in the body and excess phosphorus. It is filtered through the kidneys and excreted in the urine [30]. One of the serum factors studied in this study is the level of uric acid in the blood. The average level of uric acid in the blood of fasting people was higher than fasting people, but this difference was not statistically significant. In the study of Goma and colleagues, the serum uric acid level was compared with the serum uric acid level in the pre-Ramadan period at 6 am, 6 pm, and after iftar. Serum uric acid levels did not change at 6 a.m. during the first two weeks of Ramadan, but serum uric acid levels increased significantly during the second two weeks of Ramadan. Serum uric acid levels were significantly reduced at 6 p.m., then increased, and almost reached the pre-fasting period limit, which is consistent with the results of this study.

One study found that the average serum levels of calcium in fasting people were lower than in non-fasting people, but the difference was not significant. Also, the total amount of urinary calcium in the fasting group was lower than in the non-fasting group. This difference is statistically significant and is fully consistent with Miladipour's study [19]. Calcium balance is mainly achieved by the absorption of calcium in the intestine, its reabsorption in the kidneys, and the exchange and transfer between bone and other tissues. Serum calcium consists of ionic and protein compounds. Calcium reacts with compounds such as phosphate and citrate. In gaming environments, the amount of free ions in calcium serum decreases. 99.99% of the calcium ingested into the kidneys is reabsorbed, thereby regulating serum and urinary calcium levels [31]. No study found a link between fasting and serum calcium levels.

CONCLUSIONS

In recent years, it has been determined that the factors involved in the formation of kidney stones include urinary saturation levels with stone-forming components such as calcium, oxalate, sodium, etc., which were studied in this study. Fasting is important to prevent stone formation in the future. In general, by examining the results of this study and other studies, it seems that fasting during Ramadan has contradictory effects on urinary stone formation factors and there is not enough evidence about the effect of Ramadan on exacerbating exposure to stone-forming factors in this study.

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ORIGINAL ARTICLES

Evaluation of in-vitro bactericidal activity and anticancer effects of venom fractions of *Pseudocerastes persicus* snake

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Abstract: *Introduction: It was reported that snake venom contains a wide mixture of proteins and peptides with various toxicological and pharmacological effects. In recent years, the isolation of effective molecules from snake venom as a drug to treatment of some incurable diseases is considered by many biological research centers. This investigation aimed to determine electrophoretic and chromatographic patterns of Pseudocerastes persicus snake venom and in-vitro study of bactericidal and anti-cancer properties of its fractions in a human hepatocellular liver carcinoma cell line (HepG2).*

Materials and methods: The fractions of venom were isolated and collected using preparative RP-HPLC. The Bactericidal activity of fractions in 20 µg/ml of protein concentration was investigated toward Gram-negative and Gram-positive bacteria. Antibacterial susceptibility tests were done using MTT and MIC assays. Furthermore, cell viability was measured by MTT reduction assay and confirmed with Neutral red uptake assay following exposure of HepG2 cancer cells to 20 and 40 µg/ml of protein concentrations of fractions having more antibacterial properties. Apoptotic effects were investigated using the comet assay.

Results: SDS-PAGE pattern of the crude venom revealed 10 major bands with molecular weight ranging from 13.2 to 99.25 kDa and the single-step separation of different fractions from the venom by HPLC resulted in isolation and collection of 11 fractions. The results of this study showed that two fractions of snake venom have significant antibacterial activity against Gram-positive as compared to Gram-negative bacteria. On the other hand, our findings indicated that one of them is not toxic to the HepG2 cells.

Discussion: We for the first time reported bactericidal and anticancer effects of isolated fractions of Persian horned viper venom. These properties make components of these fractions a favorable source for isolation of molecule(s) with antibacterial and antitumor activities.

Keywords: *snake venom, Pseudocerastes persicus, antibacterial, cytotoxicity, cancer*

INTRODUCTION

One of the serious problems of the medical community in the treatment of infectious diseases caused by bacterial agents, many bacteria are resistant to common antibiotics. These bacteria are becoming a serious clinical problem around the world (Jami al Ahmadi et al., 2010). This has

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prompted pharmaceutical firms to explore new antibacterial drugs or optimize antibiotics to help them produce new and effective drugs. Nowadays poison of poisonous animals is considered by many researchers as one of the most important natural sources for the isolation and identification of antimicrobial molecules and it has been a great success.

Reports indicate that, despite that the oral and tooth smears of poisonous snakes in a wide range of pathogenic bacteria, there is a small incidence of bacterial infection in the bites of the injured patients due to snakebite (Talan et al., 1991). These observations have led the researchers to point out that antibacterial compounds are found in Snake (Deivy et al., 2005).

The first report on antibacterial activity in the venom poison the families of Alpidea and Vipride was published in 1948 and 1968 respectively (Ferreira, 1956). Also Skarnes in 1970, for the first time reported antimicrobial activity in some of the enzymes found in the venom of snakes (Aloof-Hirsch et al., 1968). Studies of Zey et al. in 2000 also led to the isolation of peptides from Naja atra, which in vitro inhibited the growth of resistant bacteria of Mycobacterium tuberculosis and showed inhibitory activity (Xie et al., 2003).

Studies show that molecular compositions and especially proteins present in the veniphil genus of the Viperidae variety are very diverse. Important compounds of these toxins include serine protease, metalloproteinase, phospholipase A2 (PLA2), C-type lectin, acetylcholinesterase, L-aminoacids, and detergents (Markland, 1997).

Recent studies have shown that many of these compounds have antimicrobial properties (Jami al Ahmadi et al., 2010; Stabeli et al., 2004; Lu et al., 2002).

In this study, the antibacterial effects of protein fractions isolated from Pseudocerastes persicus snake and the anti-cancer effects of fractions with antibacterial properties were studied.

MATERIALS AND METHODS

Venom preparation

To prepare the venom from the snake, the snake venom safety tips were placed directly into the venom collection jar, and the venom was briefly injected into the venous gland. The toxic solution was kept at minus 20 °C until lyophilization until use in the experiments.

Microorganisms and cell lines used

Bacillus subtilis subsp. spizizenii a gram-positive and non-pathogenic bacteria (ATCC 6633), bacteria Escherichia coli a gram-negative bacteria (ATCC 25922), and pathogen Staphylococcus aureus a gram-positive bacteria (ATCC 25923) were prepared from the Persian Type Culture Collection (PTCC) of Iran and liver cancer cells (HepG2, C158) of Pasteur Institute of Tehran cell bank.

Studying the crude venom electrophoresis pattern using SDS-PAGE method

To study the electrophoresis pattern of crude venom of Pseudocerastes persicus snake, 12-15% gel (Chellapandi and Jebakumar, 2008) was applied to each well of 5-10 microliters of toxic solution (containing 20 to 15 micrograms of protein) denatured with an appropriate volume of sample buffer was injected.

Standard protein was used to estimate the molecular weight of protein bands.

Electrophoresis first started at 50 V and after passing the dyeing gel through the concentrating gel, electrophoresis continued at 150V until the color of the tracing reached about 1 cm from the end of the gel cassette. After staining the gel with coma blue and removing excess dye from the gel surface, the estimated molecular weight of crude venom protein bands was determined.

Isolation of crude venom fractions using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

Fragile venom of Pseudocerastes persicus snake using RP-HPLC preparation with UV detector (220 nm absorbance), C18 SUPELCOSIC TM Pcc-18 (59185) 250mm×21.2mm, 12μm] column and Gradient Linear Application [buffer A (acetonitrile) 20% containing 0.1% TFA) and buffer B (80% acetonitrile containing 0.1% TFA)] separated in 70 minutes. In the reverse phase method, a polar mobile and non-polar stationary phase are used and the material is separated by hydrophobicity (Steuten et al., 2007).

For the separation of crude venom fractions, 40 mg of dried powder in 20 ml of buffer (water). Containing 0.01% TFA containing 5% acetonitrile and dissolved for 2 hours at 4°C. After centrifugation, the insoluble material was removed from the suspension for 5 min at 10,000 rpm at 4°C. The supernatant was used for chromatographic patterning and fractions separation by HPLC. The corresponding fractions of each peak were collected manually in perfectly clean glassware free of any protein contamination and stored at -80°C after lyophilization. The retention time of each peak was recorded for each peak.

Protein assay

For antibacterial and cytotoxicity tests, some of the lyophilized venoms were dissolved in sterile distilled water twice. The amount of protein in the toxic solution was determined using Bradford (Bradford 1976) method and the protein-free fractions were excluded from further studies.

Examination of antibacterial properties

Culture media and antibiotics: MH (Mulberry Hinton) Company, Quelab, Canada, was used for bacterial culture. Tetracycline (Sigma, USA) at a concentration of 50 µg/ml was used to compare the effect of the venom with standard antibiotics.

Evaluation of the antibacterial effect of fractions using MTT assay

MTT assay is a colorimetric assay based on the reduction and breakdown of tetrazolium yellow crystals by the succinate dehydrogenase enzyme present in the cytoplasm and ultimately the formation of insoluble blue crystals. These crystals are soluble by the addition of DMSO (Hengwei et al., 2010). The bacterium was cultured in the Muller Hinton broth medium and then after 5 h when it reached the absorbance was equivalent to the absorbance of 0.5-McFarland (at 600 nm with an absorbance of 0.08 to 0.1) (Husniye et al., 2013). 5 µL per 96 plate sterile wells were cultivated. Fractions were added to wells at a concentration of 20 µg/ml and the final volume of each well was made up to 100 µL using a liquid culture medium (Muller Hinton Broth). In this experiment, tetracycline (50µg/ml) was used as a positive control, bacterial suspension and culture medium as a negative control, and non-bacterial culture medium as blank. The plate was incubated in the incubator for 23h at 37°C. Then 5µl MTT (5µg/ml concentration) was added to all wells. The plate was incubated at 37°C under dark conditions for one hour. Then 100 µL of DMSO was added to each well. After 2 hours of incubation in dark conditions, the absorbance of the wells at 595 nm was measured using a plate reader (Biotek, USA). The above experiment was performed 3 times and 3 wells (3 times repeated) were considered for each concentration. Bacterial viability after exposure to different concentrations of venom was calculated using the following formula (Husniye et al., 2013).

$$100 \times (a/b) = \text{Bacterial vital percent}$$

a = Optical Absorption Sample - Blank Optical Absorption
b = Negative optical absorption - Blank optical absorption

Evaluation of antibacterial effect of fractions using MIC assay

In this study, we aimed to determine the inhibitory effects of crude venom on bacteria. The steps of the minimum inhibitory concentration test (Shebl et al., 2012) were similar to the MTT assay but after incubating the bacteria with different concentrations of the venom at 37 ° C for 24 hours without adding any biomarkers, the optical absorption of the wells at the wavelength of 605 nm was measured using a plate reader (Biotek, USA). The experiment was repeated 3 times and for each concentration 3 wells (3 times). The percent inhibitory effect of the standard venom and antibiotic effect was calculated using the following formula (Shebl et al., 2012).

$$100 \times (a/b)]-1[= \text{Inhibitory percentage of bacteria}$$

a = Optical Absorption Sample - Blank Optical Absorption
b = Negative optical absorption - Blank optical absorption

Anti-cancer properties of fractions

Cell culture:

To eliminate possible contamination in the toxic solution required by cellular tests, 1% antibiotic-antimycotic (Invitrogen, USA) was added and used in experiments at 4°C overnight.

DMEM-F12 medium (Gibco, USA) containing 10% FBS (Fetal Bovin Serum) prepared from the company (Gibco, USA) was used for HepG2 cell culture. These cells were cultured in flasks containing 25-50 ml cube and stored at 37°C and 5% carbon dioxide. Replace the old culture medium every 2–3 days, and after providing the cell population required for each experiment using trypsin-EDTA prepared from Sigma-Aldrich, USA, isolated from the flask and after counted with neobars lam were used.

Evaluation of cytotoxicity of fractions by MTT assay

This procedure was performed according to the method of Zargan et al. (Zargan et al., 2011). For this test, 3×104 cells were cultured in 96 well plates containing 100 µl serum-free culture. After overnight incubation at 5% CO2, humidity about 80%, and 37°C, the old culture medium was discharged and the new serum-free medium containing different concentrations of 20 and 40 µg/ml of the fraction (With antibacterial properties) were added to the wells. The plate was incubated for 23 hours after incubation with the venom cell. Then 5 µL of MTT solution (5 mg/ml) was added to each well. After the formation of dark blue crystals (Formazone), the contents of each well were removed and after washing with PBS, 100 µL of DMSO was added. In this test, the culture medium was used as control and the cell culture medium as control. Optical absorption of wells at 570 nm was measured using a plate reader (Biotek, USA). The experiment was performed 3 times and 3 wells (3 times) for each concentration. The percentage of cell viability after exposure to different concentrations of venom was calculated using the following formula (Shebl et al., 2012).

% Viable cell = (a/b) × 100

a = The absorbance of treated cells - The absorbance of the blank

b = The absorbance of the control - The absorbance of the blank

Evaluation of cytotoxicity of fractions by Neutral red uptake assay

The steps of Neutral red uptake assay (Abdul et al., 2013) were similar to MTT assay but after incubating the cell adhered to the toxic solution for 23 h, one microliter of Neutral red solution (5 mg/ml) was replaced with MTT solution. Ml) was added to each well. The plate was then incubated under dark conditions at 37°C, with 5% CO_2 and about 80% moisture until red crystals (neutral red with cell lysosome surface). Then, the solution in each well was drained and after washing each well with PBS twice, 100 µl of fixative buffer (37% formaldehyde, 10% purchase). After one minute, this buffer was evacuated and 100 µL of the solvent buffer (0.5% acetic acid) was added. Plates were then incubated on a shaker for 20 minutes in dark conditions at laboratory temperature and optical absorption of wells at 540 nm was measured using a plate reader (Biotek, USA). The experiment was performed 3 times and 3 wells (3 times) for each concentration. Inhibitory percentages induced by different concentrations of venom on cell growth were calculated using the following formula.

100× (a/b)]-1 [= Percentage of cell death

a = The absorbance of treated cells - The absorbance of the blank

b = The absorbance of the control - The absorbance of the blank

Evaluation of apoptosis induction by fractions using comet assay

Comet assay or single-cell gel electrophoresis is one of the best tests for DNA fragmentation in the cell (Mir et al., 2013). For this experiment, 12×104 cells were cultured in a sterile 24-well plate containing 300 µl of serum-free medium. Plates were incubated overnight under 5% CO2, 80% humidity, and 37°C. In this test, the culture medium was used as blank and the cell culture medium was used as control. The primary culture medium was then added to each well of the test well, drained, and 300µL of a new medium (without serum) containing different concentrations of 20 and 40µg/ml of fractions (with antibacterial properties). The plate containing the cell was incubated for 24 hours at 37°C, 80% humidity, and 5% CO2. After the end of the mentioned time, cells in each well were separated by trypsin and collected in a 1.5ml tube. The microtubes containing the cells were centrifuged for 4 min at 4°C at 1500 rpm and the supernatant was discharged. The cells were washed with 400 µl of PBS (pH = 7.4) and washed after centrifugation with the supernatant and then added to 200 µl of PBS. The slides required for testing were coated with normal melting point agarose (NMA% 1), and the cell suspension with low melting point agarose (LMA% 1) was compared to one to one. Two mixtures were poured onto the slides, one slide was placed on each slide to create a cell layer, and all slides were lysed in the lysis buffer to lyse the cell and nucleus membranes. (Fresh 2.5M NaCl, 100mM EDTA, 10mM Tris, 0.2M NaOH and Triton X-100 1% pH=10) were cooled and cooled in the refrigerator for 16–18 h. After removing the sliding buffer, slides 20 the cells were washed for 2 min with electrophoresis buffer (300 mM NaOH, 1 mM EDTA, and pH> 13) and then incubated in cold electrophoresis buffer for 40 min and then incubated in the refrigerator. The electrophoresis was carried out in an electrophoretic tank containing buffer and electrophoresis was performed at 4 ° C for 45 minutes under 25 V and 300 mA current. Samples were placed in a neutral buffer (Tris 400mM, pH =7.5) for 10 min to neutralize the playing environment. For staining, cells were added to each slide with 100 µL of ethidium bromide solution at a concentration of 20µg/ml for 10 min at room temperature. The slides were then washed twice with distilled water for 10 minutes and studied by fluorescent microscopy. At least 100 cells of DNA were obtained for each sample and the results were statistically analyzed.

Statistical analysis

The results of each test were reported as Mean ± SD and the data were analyzed using software (GraphPad InStat). Different concentrations of venom were compared to the control group and compared to each other by one-way ANOVA and Tukey tests. P<0.05 was considered significant in all tests. Draw charts in the Microsoft Excel software (2013 version).

RESULTS

Results of crude venom electrophoresis and chromatography results

The molecular weights of the bands were determined using standard protein diagrams. SDS-PAGE (15% gel) results for 10 protein bands with molecular weight of 13.2, 19.6, 24.2, 27.91, 39.8, 54.8, 65.35, 75.4, 98.32 and 99.25 KDa Showed (Figure 1). The results of RP-HPLC separation of crude venom fractions showed 11 distinct peak peaks (Figure 2). The fractions collected were named F1 to F11, respectively, at the time of departure from the column. The fractions corresponding to each peak were collected in perfectly clean and non-contaminated glass and stored at -80 ° C after lyophilization. The retention time of the product corresponding to each courier is given in Table 1.

Figure 1: SDS-PAGE pattern (15%) of venom of the Pseudocerastes persicus snake
Column 1: Standard Protein; Column 2: the venom of the Pseudocerastes persicus snake

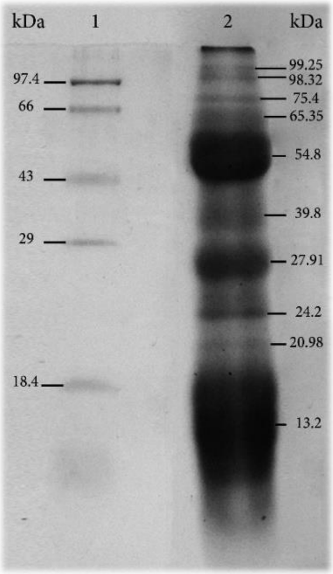

Protein assay: The results showed that fractions 1, 2, and 11 lacked proteins that were excluded from subsequent studies (Table 2).

Results of antibacterial tests

Results of antibacterial effect of fractions using MTT assay:

The results showed that fractions 3, 4, 7, and 9 had no inhibitory effect on Escherichia coli. But fractions 5, 6, 8, and 10 showed antibacterial effects, respectively, with the bacterial viability percentage being 81.55, 81.2, 69.87, and 71.45 % respectively. Statistical analysis showed that recent fractions had a significant inhibitory effect on bacterial growth compared to the negative control (normal culture medium containing bacteria) but their mortality rate was not significant compared to tetracycline as a positive control (Figure 3).

Figure 2: Chromatogram of unicorn raw venom of the Pseudocerastes persicus snake based on the results of R-HPLC Fractions of raw venom of Pseudocerastes persicus snake using R-HPLC preparation with a UV detector (220 nm absorbance), C18 column, and linear gradient program (buffer A: acetonitrile 20% containing 0.1% TFA and buffer B: acetonitrile 80% containing 0.1% TFA percentage was separated. The fractions corresponding to each peak were manually collected in the non-contaminated glass and the retention time of each peak was also recorded. The fractions collected were named F1 to F11 at the time of exit from the column, respectively.

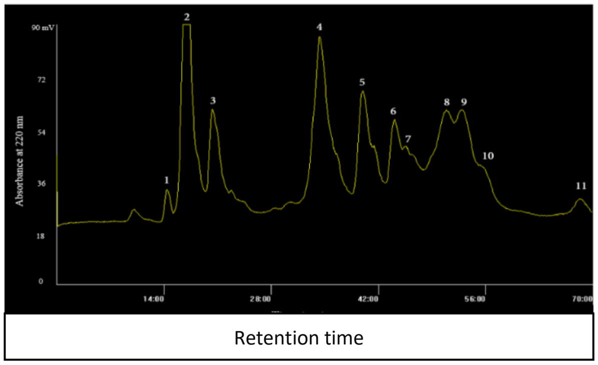

Table 1: Exit time of each component from column (in minutes)

Start time and end of peak	Fractions										
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Start	13:50	15:36	19:22	31:14	38:27	52:51	45:01	47:50	51:59	54:56	67:06
End	15:01	19:22	24:35	38:27	42:51	45:01	47:50	51:59	54:56	59:18	69:78

Table 2: Protein content of fractions isolated from the venom

Protein concentration (mg/ml)											
Fractions	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Protein concentration	0	0.0075	1.55	11.77	10.77	6.29	1.93	12.85	11.35	4.5	0

Also, test results on Bacillus subtilis showed that its vital percentage in media containing fractions 3, 4, 5, 6, 7, 8, 9, and 10 were 78.41, 0.35, 99.91, 81.27, 100, 98.94, 81.15, and 97.49 % respectively. Fractions 3, 4, 6, and 9 had a significant inhibitory effect on the growth of this bacterium compared to negative control of growth inhibition, among which Fraction 4 had more inhibitory effect than standard antibiotic (tetracycline) (Figure 3).

The viable percentages of Staphylococcus aureus in media containing fractions 3, 4, 5, 6, 7, 8, 9 and 10 were 91.58, 69.23, 70.47, 81.38, 100, 96, 99.76, and 95.76 % respectively.

Statistical analysis of data showed that fractions 4, 5, and 6 had a significant inhibitory effect on bacterial growth compared to the negative control. The highest inhibitory effect of fractions isolated from crude venom belonged to fraction 4 with a 30.77% inhibitory effect, which was significantly less than that of tetracycline (Figure 3).

Figure 3: Critical percentages of Escherichia coli, Bacillus subtilis, and Staphylococcus aureus after exposure to 20 µg/ml protein concentration from different raw venom Pseudocerastes persicus snake fractions based on MTT assay. The effects of fractions were evaluated in comparison with the negative control group

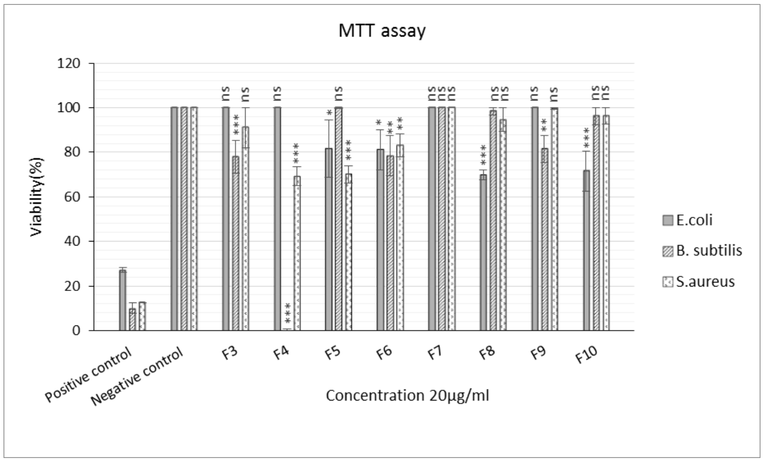

Results of antibacterial effect of fractions using Minimum Inhibitory Concentration Method (MIC assay)

The inhibitory effects of the fractions isolated at 20 µg/ml on bacterial growth showed that fractions 3 and 9 did not affect Escherichia coli, but fractions 4, 5, 6, 7, 8, and 10, respectively 5.21, 3.48, 25.2, 17.71, 17.8 and 13.06 % had an inhibitory effect on bacterial growth. Statistical analysis showed that fraction 6 had more inhibitory effect than other fractions but this effect was not significant compared to standard antibiotics (Figure 4).

Figure 4: Percentage of Inhibitory Effect of Protein Fractions (20 µg/ml Concentration) of raw venom Pseudocerastes persicus snake on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus by MIC Test. The effects of fractions were evaluated in comparison with the negative control group

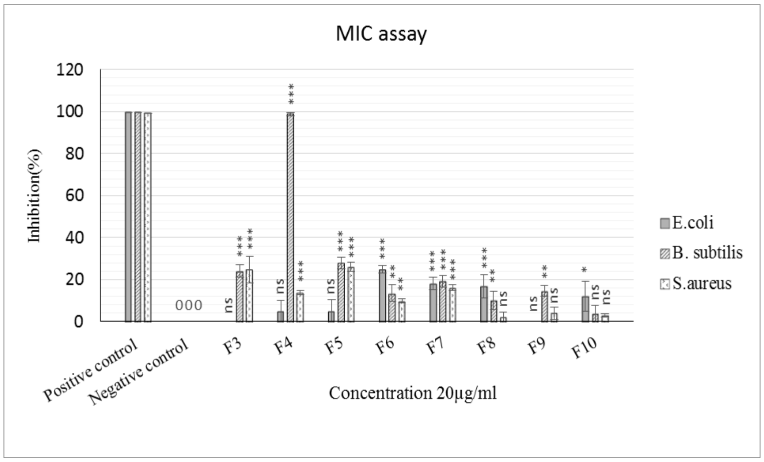

The results showed that fractions Nos. 3, 4, 5, 6, 7, 8, 9, and 10 in Bacillus subtilis were 24.06, 98.46, 28.77, 13.14, 17.88, 12.30, 13.76, and 2.61, respectively produced inhibitory effects. Statistical analysis showed that mortality caused by the effect of fractions 3, 4, 5, 6, 7, 8, and 9 on bacteria was significant compared to the negative control. It is noteworthy that the inhibitory effect of fraction 4 on the growth of this bacterium was much greater than that of the positive control (tetracycline), but this effect was not significant (Figure 4).

Also, fractions 3, 4, 5, 6, 7, 8, 9, and 10 were found to be effective on the growth of Staphylococcus aureus and were

25.46, 13.85, 25.88, 9.85, 15.88, 2.28, 4.74, and 3.03%, respectively. percent inhibitory effect on it. It is noteworthy that the inhibitory effect of fractions Nos. 3, 4, 5, 6, and 7 on this bacterium was significant compared to negative control but not significant compared to standard antibiotics (Figure 4).

Results of anti-cancer properties of fractions

Summary of the results of the antibacterial effects of the fractions isolated from the crude venom of Pseudocerastes persicus snake and statistical analysis of data showed that fractions 4 and 8 had more inhibitory effects on the tested bacteria compared to the other fractions. Therefore, in this study, the anticancer effects and their cytotoxicity were considered.

MTT assay for cytotoxicity test results

Evaluation of cytotoxic effects of fractions 4 and 8 isolated from crude venom on the growth of liver cancer cells at two concentrations of 20 and 40 µg/ml showed that viable percentage of cells affected by fraction 4 at two concentrations compared to control, respectively, 51.55% and 50.62%. However, cell viability after exposure to fraction 8 increased at above concentrations and reached 82.29 and 74.81, respectively. Statistical analysis of data showed that the effects of fractions Nos. 4 and 8 on cell growth inhibition at two concentrations of 20 and 40 µg/ml were significant compared to control, but the inhibitory effects of the two fractions were not significant (Figure 5).

Figure 5: Vital percentages of liver cancer cells after 24 h exposure to 20 and 40 µg/ml protein concentrations of raw venom Pseudocerastes persicus snake fractions based on MTT assay. The effects of fractions were evaluated in comparison with the negative control group

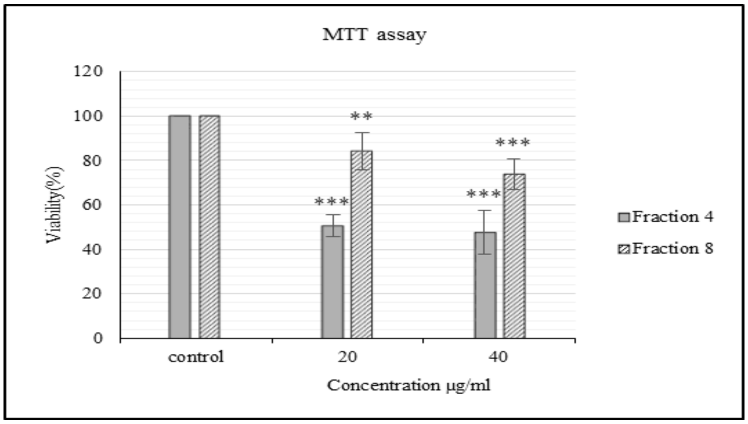

Evaluation of cytotoxicity of fractions by Neutral red uptake assay

The results of the cytotoxicity study of fractions 4 and 8 using Neutral red assay showed that mortality from fraction 4 at two concentrations of 20 and 40 µg/ml in cells was 35.19 and 50.93, respectively. The percentages in the case of fraction 8 were 82.29 and 74.81 percent, respectively, which were significant compared to the control (Figure 6).

Figure 6: Mortality rate of liver cancer cells after 24 h exposure to different concentrations of raw venom Pseudocerastes persicus snake based on Neutral red uptake dye method. Mortality from the effect of each concentration was compared to the control group

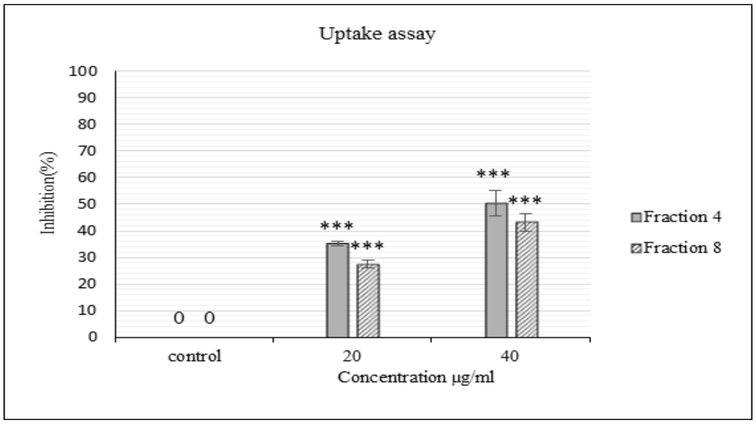

Results of fractions induction of apoptosis using comet assay

The results of the comet assay showed that fraction 4 in two concentrations of 20 and 40 µg/ml had 39.31% and 38.97% and fraction 8 in the above two concentrations was 24.73% and 25.34% apoptotic, respectively. Created liver cancer cell that was significant compared to control (Figure 7–9).

Figure 7: Apoptosis rate induced by Pseudocerastes persicus snake Fragment Fraction No. 4 in Liver Cancer Cells Based on Comet Assay Test Results. The effect of each concentration was evaluated in comparison with the control

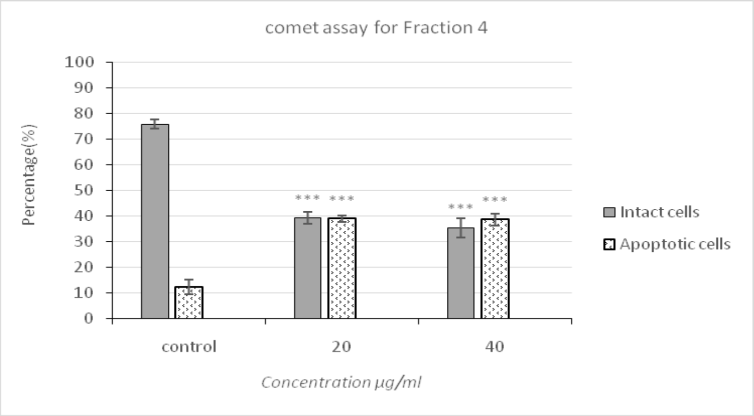

Figure 8: Apoptosis rate induced by a fraction of Pseudocerastes persicus snake number 8 in liver cancer cells based on Comet assay results. The effect of each concentration was evaluated in comparison with the control

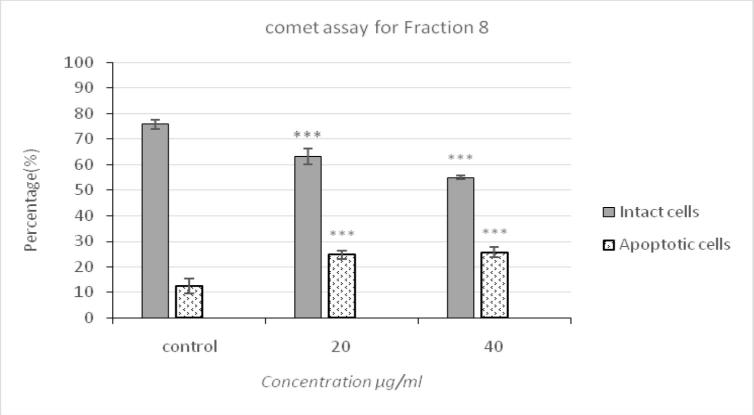

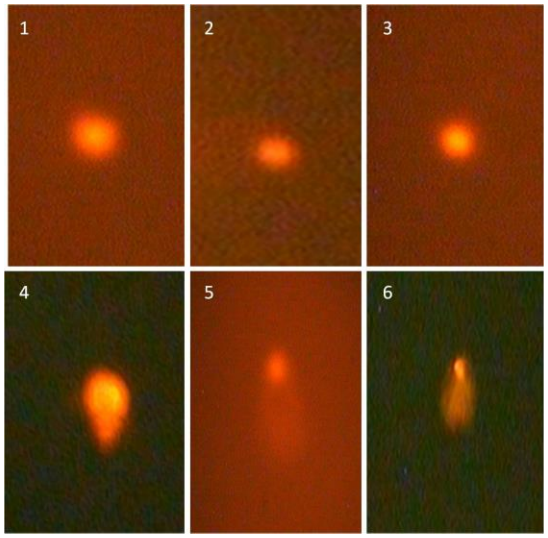

Figure 9: Morphological changes of DNA (40%) in liver cancer cells exposed to raw venom Pseudocerastes persicus snake fractions compared to control cells based on comet assay. [1-3: DNA image of healthy cells (40%), 4-6: DNA image of an apoptotic cell (40%)]

DISCUSSION AND CONCLUSION

Snake venom is a complex of molecules with different biological activities, which can be of great interest in medical and biological research (Suntravat et al., 2010). It was first carried out by researchers over 40 years ago (Master and Rao, 1961). Since the proteins and peptides present in snake venom have a wide range of molecular size as well as biochemical properties, proteomic analysis can be very important in the identification and isolation of these compounds (Phizicky et al., 2003). In the present study, a combination of electrophoretic and chromatographic methods was used to investigate the protein profile of venom. The chromatographic pattern of the venomous snake venom was studied in 2004 by Mehriyar Amini Inst using Sephadex G-50 gel filtration and 6 fractions were isolated from the raw venom (Amininasab et al., 2004).

In this study, the RP-HPLC method was used to evaluate the toxicity of the chromatographic pattern. Finally, 11 fractions were isolated and collected. Protein assay results showed that only 8 fractions of the separated fractions contained protein. In addition, our findings showed 10 protein bands in the electrophoretic pattern (SDS-PAGE). The discrepancy in the results of the electrophoresis pattern with the chromatography pattern is probably because some proteins are close to each other and are collected under a fractionation during separation.

Previous studies have shown that the pattern of electrophoresis and chromatography of different snakes is different (Nawarak et al., 2003; Rioux et al., 1998; Fry et al., 2003; Mehdizadeh-Kashani et al., 2012; Bhaskar et al., 2011). In many of these studies, by separating different components of the venom, proteins and peptides were isolated. Have been shown to have excellent therapeutic properties that can have broad implications for medicine (Barker et al., 2000; Zhao et al., 2001; Marcinkiewicz et al., 2003; Shuting et al., 2004).

The alarming growth of infectious diseases caused by bacterial resistance has prompted more research in natural resources, including the venom of various toxic animals such as snakes to discover more effective and newer antibiotics.

Based on the results of this study, it was found that the raw venom of Pseudocerastes has antibacterial properties. Also, the results showed that the raw venom of this snake against high gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus) has high inhibitory effects. And against Escherichia coli only the highest concentration used (400 µg/ml) has inhibitory effects. Therefore, in this part of the study, we tried to investigate the components of raw venom, isolation, and activity of each fraction against the mentioned bacteria. MTT redaction and MIC assay methods were used for this purpose.

The results showed that fraction 4 had the most inhibitor effects on the growth of gram-positive bacteria. The inhibitory effect of this fraction was 99% on Bacillus subtilis and 31% on Staphylococcus aureus. Fraction No. 8 with 30% inhibition had the most inhibitor effect on the growth of Escherichia coli.

These results are quite consistent with the data obtained from the study of the antibacterial effects of the raw snake venom listed on these bacteria. If these fractions were selected as antibiotics for use in animals or humans, their cytotoxicity on the cell should be assessed. Therefore, the cytotoxic effects of selected fractions (with antibacterial properties) were evaluated using MTT assay, Neutral red uptake assay, and Single cell gel electrophoresis (Comet assay) on liver cancer cell line (HepG2 cell line). This part of the study showed that fraction 8 induced the least toxicity in the cell. This property introduces this fraction as a suitable candidate for the isolation of effective molecules with antibacterial properties that have the least side effects on human cells. Fraction No. 4 also induced the greatest effect on cancer cells, which, according to Comet assay results, is most likely via induction of apoptosis. Therefore, this fraction is a suitable candidate for use in drug discovery. Will be anti-cancer. Previous studies on snake venom have also shown that their venom has anti-cancer properties (Yamileth et al., 2003).

In summary, it can be concluded that the results of the present study have the potential of fraction 8 for further study as an antibiotic and fraction 4 as an anti-cancer drug.

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VARIA

Evaluation of morbid obese patients with attitudes toward obese persons scale prior to bariatric surgery: Obesity from the perspective of real patients

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Abstract: *Background: The number of studies with the application of scales, such as Attitudes Toward Obese Persons Scale (ATOP), on morbid obese patients are limited. Therefore, we wanted to examine the attitudes toward obese people through the eyes of morbid obese patients.*

Methods: Between October 2019 - April 2020, 70 female and 20 male, morbid obese patients, who were admitted to the general surgery department to be evaluated for bariatric surgery were included in this study. These 90 morbid obese patients were asked to fill out ATOP, which included 20 statements.

Results: The mean ATOP score of all morbid obese patients was 63.44±18.58 (range:19-116). The mean ATOP score of female patients was 63.47±20.34 (range:19-116), whereas the mean ATOP score of male patients was 63.35±10.69 (range:43-80) (p=0.98). The frequency of the responses given by the male and female patients to the third statement, which was "Most obese people are more self-conscious than other people" pointed out a statistically significant difference (p=0.02).

Conclusions: Based on ATOP scores gained from morbid obese patients, 60% of morbid obese patients did not feel as happy as non-obese people, whereas 74.4% of the morbid obese patients thought that obese people were not as healthy as non-obese people. Moreover, ATOP scores which were slightly higher than 60 points in both female and male morbid obese patients indicated that morbid obese patients had neither negative nor highly positive attitudes toward obese people.

Keywords: *attitude, bariatric, morbid, obesity, surgery*

INTRODUCTION

Obesity is a chronic disorder that leads to an increasing public health problem worldwide [1, 2]. Obesity has a substantial psychosocial effect on a person's life [3]. It has been suggested that 20-60% of obese patients have a psychiatric disorder.

Depression, eating disorders such as binge eating, anxiety, low self-esteem, decreased quality of life, emotional neglect, stigma and discrimination have been associated with obesity. As weight loss improves the psychosocial functioning, psychosocial effects of obesity should be considered in the management of patients especially prior to bariatric surgery [4].

Obese patients may encounter stigmatization and weight bias not only at school and work but also within their social environment. Stigmatization refers to presence of negative perceptions toward obese patients, whereas weight bias refers to negative attitudes and beliefs toward obese patients without a reason. Attitudes of stigmatization and weight bias have been investigated in various study populations such as healthcare professionals, medical students and university students [5]. However, the number of studies with the application of scales, such as Attitudes Toward Obese Persons Scale (ATOP), on morbid obese patients are limited. Attitudes Toward Obese Persons Scale is used to evaluate whether people have favorable attitudes against obese patients or not [5]. Within this study, we wanted to examine the attitudes towards obese people through the eyes of morbid obese patients using ATOP.

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METHODS

Between October 2019 - April 2020, 70 (77.8%) female and 20 (22.2%) male, a total of 90 morbid obese patients, who were admitted to the general surgery department to be evaluated for bariatric surgery were included in this study. Local ethics committee approval was obtained prior to this study (2019/08-10). Patients with body mass index (BMI) greater than 40 kg/m^2 were regarded as morbid obese, and patients with BMI greater than 50 kg/m^2 were regarded as severe morbid obese [6]. These 90 morbid obese patients were asked to fill out ATOP, which included 20 statements. Each statement was valued between -3 to +3 points [7].

The participants were asked to mark each statement according to how much they agreed or disagreed with it, using a scale between, -3 to +3 points (-3: I strongly disagree, -2: I moderately disagree, -1: I slightly disagree, +1: I slightly agree, +2: I moderately agree, +3: I strongly agree).

These twenty statements are:
1. Obese people are as happy as nonobese people.
2. Most obese people feel that they are not as good as other people.
3. Most obese people are more self-conscious than other people.
4. Obese workers cannot be as successful as other workers.
5. Most nonobese people would not want to marry anyone who is obese.
6. Severely obese people are usually untidy.
7. Obese people are usually sociable.
8. Most obese people are not dissatisfied with themselves.
9. Obese people are just as self-confident as other people.
10. Most people feel uncomfortable when they associate with obese people.
11. Obese people are often less aggressive than nonobese people.
12. Most obese people have different personalities than nonobese people.
13. Very few obese people are ashamed of their weight.
14. Most obese people resent normal weight people.
15. Obese people are more emotional than nonobese people.
16. Obese people should not expect to lead normal lives.
17. Obese people are just as healthy as nonobese people.
18. Obese people are just as sexually attractive as nonobese people.
19. Obese people tend to have family problems.
20. One of the worst things that could happen to a person would be for him to become obese.

All of these statements were responded by the participants by the help of ATOP scale. In ATOP, points given to each statement by the participant are summed up. This final number is added to 60 in order to reach a total score, which varies between 0-120 [8].

Statistical analysis was performed using the SPSS 20.0 statistical package programme. Numerical variables were stated as the mean (±) standard deviation and medians (minimum–maximum). Categorical variables were stated as percentages. The Mann Whitney U test and independent samples t test were used to compare differences between two groups. The p-value <0.05 was considered significant.

RESULTS

This study included 90 – 70 female (77.8%) and 20 male (22.2%) – morbid obese patients. The mean age of the patients was 36.26±10.28 years (range: 18-65). The mean age of female patients was 36.77±10.22 years (range: 18-65), while the mean age of male patients was 34.50±10.54 years (range: 21-51). The mean BMI of the patients was 45.83±5.70 kg/m^2 (range: 40-65.3). The mean BMI of the female patients was 45.54±5.34 kg/m^2 (range: 40-64.3), while the mean BMI of the male patients was 46.84±6.86 kg/m^2 (range: 40-65.3) (p=0.57). The mean ATOP score of all morbid obese patients was 63.44±18.58 (range: 19-116). The mean ATOP score of female patients was 63.47±20.34 (range: 19-116), whereas the mean ATOP score of male patients was 63.35±10.69 (range: 43-80) (p=0.98). The responses of the patients to the statements within ATOP are depicted are in Tables 1, 2, 3.

The frequency of the responses given by the male and female patients to the third statement pointed out a statistically significant difference (p=0.02). Nevertheless, the frequency of the responses of the patients to the rest of the statements did not show any statistically significant difference (s1:p=0.95, s2:p=0.58, s4:p=0.40, s5:p=0.08, s6:p=0.38, s7:p=0.11, s8:p=0.40, s9:p=0.59, s10:p=0.98, s11:p=0.50, s12:p=0.68, s13:p=0.33, s14:p=0.97, s15:p=0.69, s16:p=0.44, s17:p=0.48, s18:p=0.66, s19:p=0.28, s20:p=0.73. s: statement)

Statement 1 was given -3 points by 42 (46.7%), -2 points by 4 (4.4%), -1 point by 8 (8.9%), 1 point by 11 (12.2%), 2 points by 9 (10%) and 3 points by 16 (17.8%) patients, respectively. The majority of morbid obese patients (60%) did not agree with the statement that obese people were as happy as non-obese people.

Statement 2 was given -3 points by 7 (7.8%) , -2 points by 6 (6.7%), -1 point by 8 (8.9%), 1 point by 10 (11.1%), 2 points by 12 (13.3%), 3 points by 47 (52.2%) patients, respectively. 76.6% of morbid obese patients agreed with the statement that most obese peopl felt that they were not as good as other people.

Table 1: Points given by the patients to the statements within attitudes toward obese persons scale. 60 (66.7%) patients strongly disagreed and 4 (4.4%) patients strongly agreed with the 8th statement, which was “most obese people are not dissatisfied with themselves”. In addition, most patients (58.9%) strongly disagreed with the 17th statement, which was “obese people are just as healthy as nonobese people” while only 7.8% of the morbid obese patients stated that they strongly agreed with this statement.

Score	-3	-2	-1	1	2	3
Statement	**Patients n (%)**					
S-1	42 (46.7%)	4 (4.4%)	8 (8.9%)	11 (12.2%)	9 (10%)	16 (17.8%)
S-2	7 (7.8%)	6 (6.7%)	8 (8.9%)	10 (11.1%)	12 (13.3%)	47 (52.2%)
S-3	17 (18.9%)	7 (7.8%)	3 (3.3%)	8 (8.9%)	10 (11.1%)	45 (50%)
S-4	28 (31.1%)	5 (5.6%)	6 (6.7%)	9 (10%)	13 (14.4%)	29 (32.2%)
S-5	13 (14.4%)	5 (5.6%)	10 (11.1%)	6 (6.7%)	21 (23.3%)	35 (38.9%)
S-6	17 (18.9%)	8 (8.9%)	7 (7.8%)	14 (15.6%)	20 (22.2%)	24 (26.7%)
S-7	19 (21.1%)	14 (15.6%)	11 (12.2%)	21 (23.3%)	15 (16.7%)	10 (11.1%)
S-8	60 (66.7%)	12 (13.3%)	5 (5.6%)	3 (3.3%)	6 (6.7%)	4 (4.4%)
S-9	25 (27.8%)	9 (10%)	11 (12.2%)	14 (15.6%)	12 (13.3%)	19 (21.1%)
S-10	18 (20%)	13 (14.4%)	15 (16.7%)	12 (13.3%)	15 (16.7%)	17 (18.9%)
S-11	20 (22.2%)	9 (10%)	11 (12.2%)	15 (16.7%)	14 (15.6%)	21 (23.3%)
S-12	12 (13.3%)	14 (15.6%)	15 (16.7%)	19 (21.1%)	8 (8.9%)	22 (24.4%)
S-13	24 (26.7%)	9 (10%)	7 (7.8%)	9 (10%)	6 (6.7%)	35 (38.9%)
S-14	11 (12.2%)	9 (10%)	6 (6.7%)	11 (12.2%)	12 (13.3%)	41 (45.6)
S-15	7 (7.8%)	3 (3.3%)	3 (3.3%)	17 (18.9%)	15 (16.7)	45 (50%)
S-16	27 (30%)	8 (8.9%)	9 (40%)	8 (8.9%)	14 (15.6%)	24 (26.7%)
S-17	53 (58.9%)	3 (3.3%)	11 (12.2%)	10 (11.1%)	6 (6.7%)	7 (7.8%)
S-18	47 (52.2%)	12 (13.3%)	10 (11.1%)	9 (10%)	3 (3.3%)	9 (10%)
S-19	16 (17.8%)	15 (16.7%)	12 (13.3%)	7 (7.8%)	11 (12.2%)	29 (32.2%)
S-20	12 (13.3%)	5 (5.6%)	7 (7.8%)	14 (15.6%)	13 (14.4%)	39 (43.3%)

Statement 3 was given -3 points by 17 (18.9%) , -2 points by 7 (7.8%), -1 point by 3 (3.3%), 1 point by 8 (8.9%), 2 points by 10 (11.1%), 3 points by 45 (50%) patients, respectively. 70% of morbid obese patients agreed that most obese people were more self-conscious than other people.

Statement 4 was given -3 points by 28 (31.1%), -2 points by 5 (5.6%), -2 points by 6 (6.7%), 1 point by 9 (10%), 2 points by 13 (14.4%) and 3 points by 29 (32.2%) patients, respectively. 56.6% of morbid obese patients belived that obese workers could not be as successful as other workers.

Statement 5 was given -3 points by 13 (14.4%), -2 points by 5 (5.6%), -1 point by 10 (11.1%), 1 point by 6 (6.7%), 2 points by 21 (23.3%), 3 points by 35 (38.9%) patients, respectively. The majority of the morbid obese patients (68.9%) thought that most non-obese people would not want to marry anyone who was obese.

Statement 6 was given -3 points by 17 (18.9%), -2 points by 8 (8.9%), -1 point by 7 (7.8%), 1 point by 14 (15.6%), 2 points by 20 (22.2%) and 3 points by 24 (26.7%), respectively. The majority of the morbid obese patients (64.5%) believed that severely obese people were usually untidy.

Statement 7 was given -3 points by 19 (21.1%), -2 points by 14 (15.6%), -1 point by 11 (12.2%), 1 point by 21 (23.3%), 2 points by 15 (16.7%) and 3 points by 10 (11.1%) patients, respectively. These results revealed that tendency of morbid obese patients was towards thinking that obese people were usually sociable (51.1%).

Statement 8 was given -3 points by 60 (66.7%), -2 points by 12(13.3%), -1 point by 5(5.6%), 1 point by 3(3.3%), 2 points by 6(6.7%) and 3 points by 4(4.4%) patients, respetively. 85.6% of morbid obese patients strongly disagreed with the statement that “most obese people are not dissatisfied with themselves”.

Table 2: Points given by the female patients to the statements within attitudes toward obese persons scale. 57.1% of the female patients strongly agreed with the 3rd statement, which was “most obese people are more self-conscious than other people”, while only 15.7% of them stated that they strongly disagreed with this statement.

Score	**-3**	**-2**	**-1**	**1**	**2**	**3**
Statement	**Female patients n (%)**					
S-1	33 (47.1%)	2 (2.9%)	6 (8.6%)	9 (12.9%)	9 (12.9%)	11 (15.7%)
S-2	6 (8.6%)	5 (7.1%)	7 (10%)	3 (4.3%)	11 (15.7%)	38 (54.3%)
S-3	11 (15.7%)	5 (7.1%)	2 (2.9%)	6 (8.6%)	6 (8.6%)	40 (57.1%)
S-4	21 (30%)	4 (5.7%)	4 (5.7%)	6 (8.6%)	11 (15.7%)	24 (34.3%)
S-5	8 (11.4%)	4 (5.7%)	5 (7.1%)	6 (8.6%)	18 (25.7%)	29 (41.4%)
S-6	15 (21.4%)	7 (10%)	6 (8.6%)	9 (12.9%)	14 (20%)	19 (27.1%)
S-7	16 (22.9%)	13 (18.6%)	9 (12.9%)	14 (20%)	11 (15.7%)	7 (10%)
S-8	48 (68.6%)	11 (15.7%)	2 (2.9%)	1 (1.4%)	4 (5.7%)	4 (5.7%)
S-9	18 (25.7%)	8 (11.4%)	10 (14.3%)	12 (17.1%)	10 (14.3%)	12 (17.1%)
S-10	14 (20%)	11 (15.7%)	11 (15.7%)	9 (12.9%)	11 (15.7%)	14 (20%)
S-11	16 (22.9%)	7 (10%)	9 (12.9%)	9 (12.9%)	9 (12.9%)	20 (28.6%)
S-12	9 (12.9%)	10 (14.3%)	14 (20%)	13 (18.6%)	5 (7.1%)	19 (27.1%)
S-13	21 (30%)	8 (11.4%)	6 (8.6%)	5 (7.1%)	2 (2.9%)	28 (40%)
S-14	8 (11.4%)	7 (10%)	6 (8.6%)	8 (11.4%)	9 (12.9%)	32 (45.7%)
S-15	7 (10%)	3 (4.3%)	3 (4.3%)	9 (12.9%)	11 (15.7)	37 (52.9%)
S-16	23 (32.9%)	3 (4.3%)	6 (8.6%)	5 (7.1%)	12 (17.1%)	21 (30%)
S-17	42 (60%)	2 (2.9%)	10 (14.3%)	8 (11.4%)	3 (4.3%)	5 (7.1%)
S-18	37 (52.9%)	11 (15.7%)	6 (8.6%)	7 (10%)	2 (2.9%)	7 (10%)
S-19	11 (15.7%)	12 (17.1%)	9 (12.9%)	6 (8.6%)	7 (10%)	25 (35.7%)
S-20	9 (12.9%)	5 (7.1%)	5 (7.1%)	9 (12.9%)	11 (15.7%)	31 (44.3%)

Statement 9 was given -3 points by 25(27.8%), -2 points by 9(10%), -1 point by 11(12.2%), 1 point by 14(15.6%), 2 points by 12(13.3%) and 3 points by 19(21.1%) patients, respectively. According to these results, half of the morbid obese agreed with the view that obese people were just as self-confident as other people, while the other half of morbid obese patients did not.

Statement 10 was given -3 points by 18(20%), -2 points by 13(14.4%), -1 point by 15(16.7%), 1 point by 12(13.3%), 2 points by 15(16.7%), 3 points by 17(18.9%) patients, respectively. As a result, 50% of the morbid obese patients were in favor of the statement that most people felt uncomfortable when they associated with obese people, while the other 50% disagreed with this statement.

Statement 11 was given -3 points by 20 (22.2%), -2 points by 9 (10%), -1 point by 11 (12.2%), 1 point by 15 (16.7%), 2 points by 14 (15.6%) and 3 points by 21 (23.3%) patients, respectively. 40 patients (44.4%) disagreed while 50 patients (55.6%) agreed with the statement that obese peopole were often less aggressive than non-obese people.

Statement 12 was given -3 points by 12(13.3%), -2 points by 14(15.6%), -1 points by 15(16.7%), 1 point by 19(21.1%), 2 points by 8(8.9%) and 3 points by 22(24.4%) patients, respectively. The majority of the morbid obese patients (54.4%) indicated that most obese people had different personalities than non-obese people.

Statement 13 was given -3 points by 24(26.7%), -2 points by 9(10%), -1 point by 7(7.8%), 1 point by 9(10%), 2 points by 6(6.7%) and 3 points by 35(38.9%), respectively. According to these scores, 50 (55.6%) morbid obese patients had a positive tendency toward the statement that very few obese people were ashamed of their weight.

Table 3: Points given by the male patients to the statements within attitudes toward obese persons scale. Most of the male patients (30%) strongly disagreed with the 3rd statement, which was “most obese people are more self-conscious than other people”. Statistically significant difference was observed between the frequency of the responses given by the male and female patients to the third statement (p=0.02).

Score	-3	-2	-1	1	2	3
Statement	**Male patients n (%)**					
S-1	9 (45%)	2 (10%)	2 (10%)	2 (10%)	0 (0%)	5 (25%)
S-2	1 (5%)	1 (5%)	1 (5%)	7 (35%)	1 (5%)	9 (45%)
S-3	6 (30%)	2 (10%)	1 (5%)	2 (10%)	4 (20%)	5 (25%)
S-4	7 (35%)	1 (5%)	2 (10%)	3 (15%)	2 (10%)	5 (25%)
S-5	5 (25%)	1 (5%)	5 (25%)	0 (0%)	3 (15%)	6 (30%)
S-6	2 (10%)	1 (5%)	1 (5%)	5 (25%)	6 (30%)	5 (25%)
S-7	3 (15%)	1 (5%)	2 (10%)	7 (35%)	4 (20%)	3 (15%)
S-8	12 (60%)	1 (5%)	3 (15%)	2 (10%)	2 (10%)	0 (0%)
S-9	7 (35%)	1 (5%)	1 (5%)	2 (10%)	2 (10%)	7 (35%)
S-10	4 (20%)	2 (10%)	4 (20%)	3 (15%)	4 (20%)	3 (15%)
S-11	4 (20%)	2 (10%)	2 (10%)	6 (30%)	5 (25%)	1 (5%)
S-12	3 (15%)	4 (20%)	1 (5%)	6 (30%)	3 (15%)	3 (15%)
S-13	3 (15%)	1 (5%)	1 (5%)	4 (20%)	4 (20%)	7 (35%)
S-14	3 (15%)	2 (10%)	0 (0%)	3 (15%)	3 (15%)	9 (45%)
S-15	0 (0%)	0 (0%)	0 (0%)	8 (40%)	4 (20%)	8 (40%)
S-16	4 (20%)	5 (25%)	3 (15%)	3 (15%)	2 (10%)	3 (15%)
S-17	11 (55%)	1 (5%)	1 (5%)	2 (10%)	3 (15%)	2 (10%)
S-18	10 (50%)	1 (5%)	4 (20%)	2 (10%)	1 (5%)	2 (10%)
S-19	5 (25%)	3 (15%)	3 (15%)	1 (5%)	4 (20%)	4 (20%)
S-20	3 (15%)	0 (0%)	2 (10%)	5 (25%)	2 (10%)	8 (40%)

Statement 14 was given -3 points by 11(12.2%), -2 points by 9(10%), -1 point by 6(6.7%), 1 point by 11(12.2%), 2 points by 12(13.3%), 3 points by 41(45.6) patients, respectively. Majority of the morbid obese patients (71.1%) accepted that most obese people resented normal weight people.

Statement 15 was given -3 points by 7(7.8%), -2 points by 3(3.3%), -1 point by 3(3.3%), 1 point by 17(18.9%), 2 points by 15(16.7%) and 3 points by 45(50%) patients, respectively. The vast majority of the morbid obese patients (80.6%) shared the idea that obese people were more emotional than non-obese people.

Statement 16 was given -3 points by 27(30%), -2 points by 8(8.9%), -1 point by 9(40%), 1 point by 8(8.9%), 2 points by 14(15.6%) and 3 points by 24(26.7%) patients. The vast majority of the morbid obese patients (78.9%) did not agree with the statement that obese people should not expect to lead normal lives.

Statement 17 was given -3 points by 53 patients by 53(58.9%), -2 points by 3(3.3%), -1 point by 11(12.2%), 1 point by 10(11.1%), 2 points by 6(6.7%) and 3 points by 7(7.8%) patients. 74.4% of the morbid obese patients did not agree with the statement that obese people were just as healthy as non-obese people.

Statement 18 was given -3 points by 47(52.2%), -2 points by 12(13.3%), -1 point by 10(11.1%), 1 point by 9(10%), 2 points by 3(3.3%) and 3 points by 9(10%) patients, respectively. 76.6% of morbid obese patients did not agree with the statement that obese people were just as sexually attractive as non-obese people.

Statement 19 was given -3 points by 16(17.8%), -2 points by 15(16.7%), -1 point by 12(13.3%), 1 point by 7(7.8%), 2 points by 11(12.2%), and 3 points by 29(32.2%) patients, respectively. 52.2% of the morbid obese patients agreed with the statement that obese people tended to have family problems.

Statement 20 was given -3 points by 12(13.3%), -2 points by

5(5.6%), -1 point by 7(7.8%), 1 point by 14(15.6%), 2 points by 13(14.4%) and 3 points by 39(43.3%). 58.9% of the patients agreed with the statement that becoming obese would be one of the worst things that could happen to a person.

DISCUSSIONS

Hereditary, social, economic and environmental factors have been included in the etiology of obesity [9, 10]. In accordance with increased obesity rates, bias about obese people has become more prevalent [11, 12]. Negative attitudes toward obese people which lead to prejudice and discrimination have a detrimental impact on the mental health of obese individuals (9). Obese people may experience discrimination in various aspects of life such as work, education and medical care [13-22].

In order to evaluate attitudes toward obese people, ATOP scale has been used in several studies [5]. However, ATOP scale which was used on different populations revealed conflicting results. Puhl et al. compared ATOP scores between female obese patients with and without binge eating disorder. The mean ATOP scores were similar in both groups. Puhl et a. suggested that obesity increased vulnerability to negative weight-based attitudes regardless of eating disorder [8]. Kadar et al. reported that obesity bias was common in chiropractic students and faculty members. Students and faculty members had anti-obesity attitudes according to ATOP. In addition, ATOP scores did not show significant difference between preclinical and clinical students [23]. Soto et al. reported more positive attitudes toward obese individuals in psychology students compared to medical students. Moreover, positive attitudes of the students according to ATOP were correlated with frequent contact with obese people [24]. Darling et al. investigated attitudes toward obese people in graduate and undergraduate nursing students, graduate education and graduate social work students. Darling et al. reported worse attitudes in nursing students, younger participants and participants without an obese family member or a friend [5]. Ambwani et al. reported that 92.5% of young adults aged 18 to 29 approved at least one negative attitude within ATOP. However, approval of negative attitudes did not show significant difference according to BMI [11].

It has been suggested that negative attitudes toward obese people might be observed in women more commonly than in men. However, Glenn et al. reported that women were significantly more positive in their attitude toward obese people compared to men [25]. On the other hand, Darling et al. reported similar ATOP scores in females and males, thus no gender difference in the attitudes toward obese individuals was identified [5]. Moreover, several studies revealed that individuals who had a relative or a friend with obesity or had contact to obese people commonly tended to have more positive attitudes toward obese people [5, 24, 26]. However, attitudes of obese people toward other obese people have not been investigated in depth, yet.

Since the ATOP score ranges from 0 to 120 and higher scores reveal more positive attitudes, the mean ATOP score of our patients (63.44±18.58) which was slightly higher than midpoint indicated that morbid obese had positive attitudes toward obese people. Previous studies reported the mean ATOP score was 60 in obese women, whereas 59.6 in obese women with binge eating disorder [8], 57.6 in preclinical chiropractic students, 59.7 in clinical chiropractic students, 49 in preclinical faculty members, 52.2 in clinical faculty members [8] and 68.5 in nursing students, 77.3 in social work students, 71.4 in graduate education students [5].

Within this study, the majority of the patients (77.8%) admitted to the general surgery clinic for bariatric surgery evalution were female. This result may point out two different findings. First of all, obesity is more common in female than male individuals. Secondly, the number of obese women seeking medical help to fight against obesity is more than the number of male patients. Within this study, no significant difference was observed in mean ATOP scores of the female and male morbid obese patients (p=0.98). Therefore, our results revealed that both women and men with morbid obesity had similar attitudes toward obese people. However, points given by morbid obese patients to the 3rd statement ‘’Most obese people are more self-conscious than other people’’ showed gender difference. More than half of female morbid obese patients (57.1%) stated that they strongly agreed to the 3rd statement while most of the male morbid obese patients (30%) stated that they strongly disagreed to the same statement. This result might reveal that morbid obesity might affect female and male patients differently in building relationship or in communicating with other people. Thefore, novel and gender specific scales about obesity may be helpful in evaluating obese patients before making an appropriate treatment plan prior to bariatric surgery.

Moreover, with the help of ATOP applied on morbid obese patients, we have gained insight into the attitudes of morbid obese patients on obese individuals. In addition, we had the opportunity to evaluate obesity from the perspective of morbid obese patients. Unfortunately, 60% of morbid obese patients did not feel as happy as non-obese people. 74.4% of the morbid obese patients thought that obese people were not as healthy as non-obese people. Moreover, 76.6% of morbid obese patients replied that obese people were not as sexually attractive as non-obese people. Nevertheless, 80.6% of morbid obese patients shared the idea that obese people were more emotional than non-obese people.

In conclusion, ATOP has been investigated in various studies in order to determine whether non-obese individuals had negative attitudes toward obese individuals or not. However, the data are scarce in the English medical literature about the attitudes of morbid obese patients toward obese individuals. Thefore, we evaluated ATOP in morbid obese individuals to gain a sight whether morbid obese patients had negative perceptions about other obese people. Any ATOP score above the midpoint, which is 60 points, has been reported to indicate that the individual had positive attitudes toward obese people [27, 28]. However, higher scores than 60 indicate more positive attitudes toward obese individuals. Therefore, ATOP scores identified within our study which were slightly higher than 60 in both our female and male patients indicated that morbid obese patients had neither negative nor highly positive attitudes toward obese people.

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All the authors agreed on the content of the manuscript.

Local ethics committee approved this study.

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VARIA

Evaluation of systemic second-line therapy in recurrent or metastatic esophageal cancer

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Abstract: *Background: More than half of patients with locally advanced esophageal cancer are unresponsive to first-line chemotherapy. To date, there is no standard second-line regimen; new agents, such as immune-checkpoint inhibitors or anti-Neurotrophic Tropomyosin-Related Kinas agents, have only been recently recommended. This study aimed to assess the benefits of classical second-line chemotherapy in terms of survival and tolerance. Materials and Methods: This retrospective analysis assessed the benefits and tolerance of different second-line regimens of chemotherapy in progressive, locally advanced or metastatic oesophageal cancer treated at the department of Radiotherapy-Oncology at the Institute of Oncology between January 2011 and January 2017. Results: The analysis found 87 patient files with a median age 59.5 years (ranging between 41 and 84), of which 40.2% (35 patients) failed the first-line platinum and 5-fluorouracil combination. Histology favoured adenocarcinomas (63.2%). The patients were in good clinical condition, with ECOG 0–1 in 86.1% (74 patients), and less than five percent had more than three metastatic sites (3.4%, 3 patients). The regimens were mostly two-drug cytotoxic (53.9%, 47 patients): FOLFIRI in 18 patients (20.7%), FOLFOX in 18 patients (20.7%), vinorelbine–platinum combination in 11 patients (12.6%), and docetaxel in 40 patients (38.7%). The median treatment duration was 7.74 weeks (range 1 to 12). The general response rate was 47.1% (41 patients), including stable disease. The median time to progression was 7.61 weeks, with a range between 1.6 and 8.6 weeks, with no differences in this respect among the fourth type of treatment received (p = 0.170). Grade 3–4 toxicities, including neutropenia, gastrointestinal problems, and metabolic disorders, were observed in 44 patients (50.6%). The median event-free survival was 2 months (range between 1 and 6.3), and 63.2% (55 patients) subsequently received a third line of chemotherapy. Conclusion: Second-line chemotherapy is still the first-choice recommendation in all fit patients with advanced oesophageal cancer. The new agents are efficacious only in cases with PD-L1 positive or high microsatellite instability.*

Keywords: *second-line chemotherapy, locally advanced/metastatic oesophageal cancer, survival benefit, toxicity*

INTRODUCTION

Esophageal cancer has two histopathological subtypes: squamous carcinoma found in the superior and upper-two-thirds regions [1], and adenocarcinoma, which develops at deep inferior and junctional mucosal glands. Both have high similarities in their pattern of metastasis [2]. Esophageal cancer is the sixth most common cause of specific deaths worldwide [3]. The peak of incidence is reported in the sixth decade of age, and cancer occurs more frequently in men [4].

Approximatively half of the patients have advanced disease at diagnosis. Three-quarters of resectable disease will relapse, of which more than half will be candidates for

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second-line systemic therapy [5]. Overall five-year survival is less than 10% [3].

Regarding the first line, for adenocarcinoma, the regimen used is similar to those used in gastric cancer, fluoropyrimidine-based chemotherapy while in squamous esophageal cancer the regimen is cisplatin-based and in general, regardless the histology, the median overall survival (OS) does not exceed nine months [6].

Currently, there is no established second-line systemic treatment for esophageal cancer [7]. Subsequent lines of chemotherapy have recently been proposed by NCCN (National Comprehensive Cancer Network) guidelines for locally advanced or metastatic esophageal and oesophagogastric junction cancer [8]. New treatment options include ramucirumab and paclitaxel which give a 2-month benefit in median overall survival (9.6 months versus 7.4 months for paclitaxel only, p = 0.017) [9]. Pembrolizumab is a category 1 recommendation anti-PD1 (programmed cell death protein) antibody for esophageal PD-L1 (programmed death-ligand 1) positive or for any histology displaying MSI-H (microsatellite instability-high) or d-MMR (deficient mismatch repair) status [8] based on a benefit of 1 month (8.2 vs. 7.1 months, HR = 0.78, P = 0.0095) versus classical chemotherapy taxanes or irinotecan in the squamous cell carcinoma subgroup [10]. With a 2B level of recommendation, entreticnib is proposed for a median 10 months' duration of response, 95% CI [7.1 - NE] [11], and larotrectinib is proposed in NTRK (Neurotrophic Tropomyosin-Related Kinase) fusion-positive esophageal tumours [12].

MATERIALS AND METHODS

Medical files from the institutional database of patients diagnosed with metastatic invasive esophageal squamous cell carcinoma (SCC) or adenocarcinoma (AC), referred to our institution for second-line chemotherapy were analysed. The main inclusion criteria were age >18 years, clinical-stage IV according to AJCC 8th edition [15], treatment by at least one regimen of chemotherapy or radiotherapy and assessed for response.

Patients were excluded if they were eligible for salvage surgery or had synchronous cancer or severe comorbidities that might impair our results. The study was approved by Ethycal Committee of Insitute of Oncology "Prof. Dr. Alexandru Trestioreanu", Bucharest, Romania no 1597/17th December 2010.

The primary outcome was time to progression, evaluated between the start of second-line chemotherapy and first progression or death by any cause. Secondarily, treatment benefit was evaluated using CT scan, and, in terms of tolerance, the rate and duration of grade III-IV adverse events (AE) classified according to CTCAE v. 4.03 [16].

Data were analysed using SPPS version 23 (IBM Corporation, Armonk, NY, USA). Non-parametric tests were used to compare subgroups. The chi-squared test determined the correlation between response to treatment and demographic and clinical variables. Fisher's exact test was employed when there were fewer than five cases in any cell of the 2×2 table. The associations between variables and survival parameters were analysed by logistic regression analysis, and survival curves were generated using the Kaplan–Meier method and compared using the log-rank test. Hazard ratio (HR) and 95% confidence interval (CI) for variables were calculated using the Cox proportional hazard model. All statistical tests were two-sided, and a p-value < 0.05 was considered significant.

RESULTS

3.1. Patient characteristics

During the period of analysis, a total of 87 patients were treated in our department by chemotherapy for esophageal cancer, with a mean age 59.5 (range between 41 and 84). Among them, 37 patients were treated for recurrent disease, 29 patients had metastatic disease at initial presentation, and 21 patients received the second-line treatment for a locally advanced disease with no indication of surgery or radiotherapy.

The principal histology was adenocarcinoma (63.2%, 55 patients), and the primary lesion was typically situated in the lower and junction of the esophagus (48.9%, 51 patients). One-third of patients had liver metastasis, but almost all had no more than two sites of secondary lesions (96.6%, 84 patients) and were in generally good condition, with ECOG 0–1 in 85.1% (74 patients) (Table 1).

Table 1: Patients characteristics in target population

Characteristics	n	%
Age (years): median 59.5; range [41–84]		
40–50	14	16.1
51–70	63	72.4
>70	10	11.5
Gender		
Male	58	66.7
Female	29	33.3
Location of primary lesion		
Upper	14	16.1
Middle	22	11.5
Lower	25	35.6
Junction	26	33.3

Characteristics	n	%
Histopathological sub-type		
Squamous cell carcinoma	32	36.8
Adenocarcinoma	55	63.2
The initial stage of disease		
I	17	19.5
II	10	11.5
III	31	35.6
IV	29	33.3
Metastatic site		
Lymph node	18	20.7
Lung	18	20.7
Liver	30	34.5
Bone	12	13.8
Other	9	10.3
Number of metastatic sites		
1	50	57.5
2	34	39.1
3 and more	3	3.4
ECOG		
0	44	50.6
1	30	34.5
2	13	14.9

Abbreviations: CHT = chemotherapy, ECOG = Eastern Cooperative Oncology Group Performance Status, FU-Pl: 5-fluorouracil–platinum regimen, LV5FU2CDDP =5-fluorouracil 1600 mg/m2 + leucovorin 200 mg/m2 + cisplatin 50 mg/m2q2w, 5FU-CDDP=5-fluorouracil 1000 mg/m^2 day 1-4 + cisplatin 100 mg/m^2 day 1 q3w, PR = partial remission , PD = progressive disease, SD = stable disease.

3.2. Treatment characteristics

The first-line combination of cisplatin plus 5-FU (5-fluorouracil) was most typical in localized and metastatic esophageal cancer (40.2%, 35 patients). This combination involves 5-fluorouracil 1000 mg/m^2 per day for 4 days and cisplatin 100 mg/m^2 on day 1 every 21 days (17) or, in the so-called LV5-FU2 – cisplatin two-week regimen, cisplatin 50 mg/m^2, calcium folinate 200 mg/m^2, 5-FU 400 mg/m^2 on day 1 and 5-FU 1200 mg/m^2 over a 46-hour continuous infusion (18). Other protocols used were FOLFIRI (39 pts, 44.8%) vinorelbine-cisplatine based (10 pts, 11.5%) and taxane based (3 pts, 3.4%).

Patients who received FOLFOX regimen, as second line treatment modified they represented 20.7% (18 patients), 46% (40 patients) received a docetaxel regimen, 20.7% (18 patients) received a FOLFIRI regimen, and 12.6% (11 patients) received a vinorelbine–cisplatin regimen. Details regarding the protocols used in second line are listed in the Table 2.

Table 2: Second line chemotherapy protocols summary.

Second line regimen	Protocol summary	Number patients	Response rate	Reference
FOLFOX6	oxaliplatin 85 mg/m^2, leucovorin 200 mg/m^2, 5-FU push 400 mg/m^2, 5FU 2400 mg/m^2 for 46 hours, q2w	18 (20.7%)	83.3% (15 pts)	(19)
NVB-CDDP	vinorelbine 25 mg/m^2 day 1+ 8, cisplatin 80 mg/m^2 q3w	11 (12.6)	45.5% (5 pts)	(20)
FOLFIRI	irinotecan 150–180 mg/m^2, 5-FU 400 mg/m^2 push, leucovorin/ calcium folinate acid, 125mg/m^2, 5-FU 2400 mg/m^2/46 hours, q2w	18 (20.7%)	50% (9 pts)	(21)
DTX	docetaxel 75mg/m^2, q3w	40 (46%)	12 (30%)	(22)

Abbreviations: FOLFOX6 = Oxaliplatin 85 mg/m^2+ leucovorin 200 mg/m^2 + 5 FU 2800 mg/m^2 q2w, FOLFIRI = Irinotecan 150–180 mg/m^2 + leucovorin 125 mg/m^2 + 5-FU 1600 mg/m^2 q2w, NVB-CDDP = Vinorelbine 25 mg/m^2 day 1 + 8 + cisplatin 80mg/m^2 q3w, DTX = docetaxel 75 mg/m^2 q3w.

The median time of chemotherapy was 7.74 weeks, with a range between one and twelve weeks. The mean number of cycles was 2.36, with a range between one and seven cycles and no significant differences was registered between the medians of any regimen ($p = 0.065$). Choice of chemotherapy regimen was correlated with the initial stage of disease, histological subtype, the number of metastatic sites, site of metastasis and performance status ($p < 0.0001$). More than half of the patients included in the study were able to benefit from the third line of treatment (55 pts, 63.2%) (Table 3).

3.3. Benefit

The response rates that were obtained were as follows: 28.7% (25 patients) had partial remission, 18.4% (16 patients) stable disease and 52.9% (46 patients) recorded either clinical or radiological progression.

Table 3: Second line chemotherapy protocols characteristics and toxicity.

Characteristics	n	%
Number of cycles: median 2.87, range [1–7]		
< 2	28	32.2
2–4	45	51.7
> 4	14	16.1
CHT duration (weeks): median 7.74, range [1–12]		
< 6	20	22.9
6–8	52	59.7
> 8	15	17.3
Grade 3–4 toxicities		
Without	44	50.6
Haematological	30	34.5
Gastrointestinal	10	11.5
Metabolic disorders	3	3.4

The difference between the response to different second line regimen of chemotherapy were not statistically significant (p=0.042).

We estimated that the median OS on the basis of the results of several studies of second-line chemotherapy [26-29].

Response was directly correlated with primary tumour location (p = 0.022) and histopathological subtype (p = 0.017) and with tumour burden and type of chemotherapy regimen (p < 0.0001).

The median time to progression was 7.61 weeks, ranging between 1.6 and 8.6 weeks, with no statistical difference between the fourth type of treatment received (p = 0.170).

Time to progression was associated with the number of metastatic sites (HR 2.886, p = 0.004) but not with the type of chemotherapy regimen (p = 0.373). By contrast, the log-rank test showed significant differences in time to progression by type of chemotherapy (chi-square 22.1852, p < 0.0001) (Figure 1).

Figure 1: Kaplan–Meier curves in locally advanced or metastatic oesophageal cancer patients according to the second-line chemotherapy regimen.

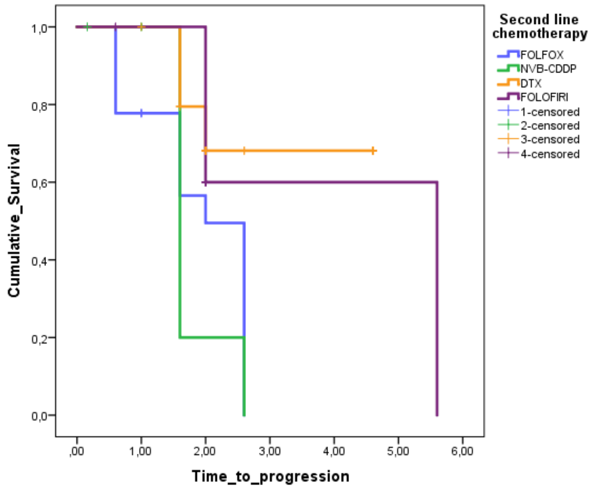

Fifty-five patients (63.2%) could benefit from a subsequent, third line of chemotherapy.

The median event-free survival for all patients was two months, with a range between 1 and 6.3 months.

3.4 Toxicity

Forty-four patients (50.6%) displayed no grade III-IV toxicities. Seven patients displayed haematological and gastrointestinal disorder together, and all patients presenting metabolic disorder also had febrile neutropenia.

DISCUSSION

Esophageal cancer are a challenging disease for which there are limited treatment effective options. Despite research, the only treatment with clinical benefit and more accessible in the second-line setting remains chemotherapy.

The population in our study was in relatively good clinical condition: 85.1% with ECOG 0 or 1, almost all without more than two sites of metastasis (96.6%), younger than 70 and, in more than 60%, bearing adenocarcinoma as histological subtype. In this analysis, the most-used (53.9% of cases, 47 patients) were two-agent regimens: FOLFIRI, or cisplatin–vinorelbine, and FOLFOX. The median treatment duration was 7.74 weeks, and most had received 5-fluoropyrimidine in the first line (85.1%, 74 patients).

The general benefit was reflected by a 47.1% (41 patients) response rate, including cases with disease stabilisation, the median time to progression of 7.61 weeks (range 1.6 to 5.6 weeks) and a median time to death of 2 months (range 1 to 6.3 months). Chemotherapy was generally tolerated, though almost half of patients developed grade III-IV toxicities; one-third of these were mostly non-complicated grade III anaemia or thrombopenia.

Several earlier retrospective or phase II studies have evaluated whether second-line chemotherapy might be useful for oesophageal cancer patients.

Regarding irinotecan, in a small phase II study, 29 patients (27 with adenocarcinoma) were given capecitabine 2,000 mg/m2 day on days 1–14 and irinotecan 250 mg/m2 every 3-week cycle. The study showed an objective response of 17% (five patients) and median progression-free survival of 3.1 months 95% CI [2.2–4.1] and median OS of 6.5 months 95% CI [6–7.1] with a 10% rate of febrile neutropenia [24].

In another phase II study, irinotecan combined with cetuximab in 63 cases of platinum-refractory gastro-esophageal carcinoma, led to an 11% (6 patients) partial response rate and 37% (23 patients) rate of stabilization, with median progression-free survival of 2.8 months, median overall survival of 7.1 months and a 32% rate of grade III-IV toxicities [25].

Even as a single agent, irinotecan showed a survival advantage, compared to best supportive care, in 40 patients with metastatic or locally advanced adenocarcinoma gastro-esophageal of the junction. Irinotecan given of 250 mg/m2 with the possibility of an increase to 350 mg/m2 if well-tolerated, gave a 53% rate of stable disease and 50% clinical benefit versus 7% and a median survival of 4 months 95% CI (3.6–7.5) versus 2.4 months 95% CI (1.7–4.9) and 0.48 HR for death 95% CI (0.25-0.92) [26].

In untreated metastatic gastro-esophageal adenocarcinoma (N = 220 patients), by substituting oxaliplatin for cisplatin in a FOLFOX-like regimen (fluorouracil 2,600 mg/m^2 over 24-hour infusion, leucovorin 200 mg/m^2 and oxaliplatin 85 mg/m^2 every 2 weeks) there was no statistically difference in terms of progression-free survival (5.7 vs. 3.9 months, p = 0.077), response rate (35% versus 20%) or median overall survival (10.7 vs. 9.8 months). Adverse events were significantly fewer (9% vs. 19%) and treatment showed better responses in patients aged over 65 years old compared to cisplatin: 41.3% response rate versus 16.7% (p = 0.012), time to treatment failure of 5.4 versus 2.3 months (p < 0.001) and progression-free survival of 6.0 versus 3.1 months (p = 0.029) [19]. A later meta-analysis (N =1294 patients in three randomized trials), regarding the meme subject, showed improved progression-free survival (HR = 0.88, p = 0.02) and overall survival (HR 0.88, p = 0.04) in advanced, unresectable gastric cancer [27].

In a monocentric retrospective study of 94 patients with metastatic esophageal cancer, a modified FOLFOX 6 regimen comprising fluorouracil 2400 mg/m^2 over 48 hours after a bolus of 400 mg/m^2, leucovorin 400 mg/m^2 and oxaliplatin 85 mg/m^2 gave an objective response rate of 41.4% (39 patients), of whom 6.3% (6 patients) with a complete response and 21.3% (20 patients) with stable disease. Any grade of hematologic toxicity was found in 69.1% of cases [28].

Regarding taxane-based chemotherapy, the UK COUGAR-2 phase III study(N=168) confirmed the benefit of second-line chemotherapy by docetaxel 75 mg/m^2 every 3 weeks compared to best supportive care by a median OS of 5.2 versus 3.6 months (HR 0.67, 95% CI [0.49–0.92]; p = 0.01) in patients with a good performance status [22].

In a retrospective study which assessed in 163 patients the clinical benefit of second-line taxane therapies, docetaxel at 70 mg/m^2 every 3 weeks versus paclitaxel 100 mg/m^2 weekly for 6 weeks of 8 weeks cycle, survival without progression was identical (2.3 months) for both regimens the overall survival was 6.1 versus 5.3 months, with 32.6% and 16.1% rates, respectively, of grade III-IV toxicities [29].

Only a few randomized studies have compared the benefits of different second-line chemotherapy regimens, mainly in gastric cancer and in Asian patients.

In 202 Korean patients with less than two prior lines of chemotherapy, randomized in a 2:1 ratio treated with docetaxel or irinotecan compared with supportive treatment,was recorded a superior overall survival (5.3 vs. 3.8 months; HR 0.66, p = 0.007) while no survival difference was recorded between docetaxel and irinotecan. The tolerance was good, the hematologic toxicities manageable and the rates of non-haematologic toxicities were comparable in both groups but there was with no significant differences in terms of survival between two regimens of chemotherapy [30].

In another Asian study, in 223 Japanese patients randomized to received paclitaxel alone or irinotecan alone as second line treament, the median overall survival was comparable (9.5 vs. 8.4 months, p = 0.38), although less toxicity appeared in the paclitaxel arm and more of those patients went on to receive third-line chemotherapy [31].

In a meta-analysis of 28 studies (N = 4810 patients), mostly retrospective, the second line showed no difference between taxane- and irinotecan-based regimens in terms of overall survival (HR 0.94, CI [0.78–1.13]) or survival without progression (HR 0.84, CI [0.69–1.03]), nor was there any difference for any single-agent regimen compared to best supportive care (HR 0.65, 95% CI [0.53–0.79]. Median survival gain ranged from 1.4 to 2.7 months among the individual studies considered. Doublet chemotherapy based on taxane or irinotecan plus platinum and fluoropyrimidine did not display different survival but showed increased toxicity compared to taxane or irinotecan monotherapy [6].

As far as new agents, despite the failures of several clinical trials involving molecularly targeted agents, several have shown some benefits worth mentioning that represent the basis of NCCN recommendations [8].

Ramucirumab, an antibody that targets the vascular endothelial growth factor receptor 2, the expression of which might be a mechanism of the aggressiveness of gastric and gastro-esophageal junction adenocarcinoma, led in pre-treated patients with esophagogastric cancer significant survival benefit, 5.8 months compared with 3.8 months for placebo (HR 0.776, 95% CI [0.603–0.998]), a similar benefit were seen in the COUGAR-02 trial assessing the benefit of docetaxel [32].

By adding paclitaxel to ramucirumab, in another phase III trial, it was reported a significant benefit in terms of median OS 9.6 months versus 7.4 months, HR 0.807, 95% CI [0.678–0.962] (p = 0.017). In terms of adverse events, the association was mainly marked by hypertension (14%, 46 patients vs. 2%, 8 patients), fatigue (12%, 39 patients vs. 5%, 18 patients) and abdominal pain (6%, 20 patients vs. 3%, 11 patients) [9].

Regarding the role of immune checkpoint inhibitors, in the KEYNOTE 181 trial (N = 628 patients), the response rates with pembrolizumab were double or even triple those achieved with chemotherapy at the investigator’s choice (paclitaxel, docetaxel, or irinotecan), though the median duration of response was longer in the group with PD-L1 CPS (Combined Positive Score) equal or higher than 10 (9.3 vs 7.7 months with chemotherapy HR 0.69; 95% CI [0.52–0.93]; $p = 0.0074$); 12-month overall survival rate in CPS ≥10 patients was 43% compared to 20%. With pembrolizumab, fewer patients had drug-related adverse events of any grade (64% vs. 86%) or grades 3 to 5 (18% vs. 41%) compared with chemotherapy. The most common events were fatigue (11.8%), hypothyroidism (10.5%), loss of appetite (8.6%), nausea (7.0%) and diarrhoea (5.4%) [10]. The phase III KEYNOTE-590 study (ClinicalTrials.gov identifier NCT03189719), assessing the efficacy and tolerance of pembrolizumab plus chemotherapy as first-line therapy for locally advanced or metastatic esophageal carcinoma, is ongoing [33].

HER 2 (human epidermal growth factor receptor) expression was not considered in the study due to the fact that it was not frequently found in the study population, and Trastuzumab treatment is not approved in Romania for second-line treatment [23, 34].

The time to disease progression was quite difficult to track due to the fact that several patients have continued their treatment at other institutions or have been transferred to a palliative care unit without the possibility to obtain their date of death, because in Romania there is not a computerized death reporting system and this is a limitation of the study.

The limitations of our study were the retrospective character, small number by each subgroup of treatment patients and lack of the subgroup of patients treated with both active and passive immunotherapy.

CONCLUSIONS

Immunotherapy using immune checkpoint inhibitors has revolutionized the cancer treatment and becomes the new standard of second line treatment in non-operable advanced or metastatic esophageal cancer.

Moreover, the new agents seem to be efficacious only in cases where the expression PD-L1 is positive or high microsatellite instability is present.

The old chemotherapy regimen has a similar efficacy and relatively good tolerance and still might the first-choice recommendation in fit patients

This analysis showed a generally good response to systemic treatment, comparable to the responses reported in other studies.

Our study did not show a difference in clinical benefit depending on the treatment regimen.

Moreover, the incidences of adverse events such as grade 3 or higher leukopenia and febrile neutropenia was observed in all patients. Forty-four patients (50.6%) displayed no grade III-IV toxicities. Seven patients displayed haematological and gastrointestinal disorder together.

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The study was approved by Ethical Committee of Institute of Oncology "Prof. Dr. Alexandru Trestioreanu", Bucharest, Romania.

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The authors declare that they have no competing interests.

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The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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VARIA

Evaluation of the accuracy of E-FAST ultrasound in blunt trauma patients referred to the Emergency Department

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Abstract: *Introduction: Trauma is one of the most common causes of death in the world. E-Fast ultrasound can detect the haemoperitoneum, intravenous pneumothorax, and hemothorax. In this study, we evaluated the accuracy of E-FAST focused ultrasound in blunt trauma patients referred to the Emergency Department.*

Methods: In this study 167 blunt trauma patients were included. E-FAST is performed from four standard spaces in terms of free fluid, chest in terms of hemothorax, and pneumothorax. Ultrasound is performed on the trauma patient and the obtained results were compared with the result of the standard gold CT scan test. All patient information including age, sex, mechanism of trauma, patient ISS, E-FAST result, and CT scan result were registered.

Results: Overall, 174 patients were included that 45 (25.9%) of them were females. The mean age of the patients was 38.39±12.31 years. E-FAST had a sensitivity of 66.7 and a specificity of 100% in the diagnosis of hemothorax. Also, a positive predictive value of 100% and a negative predictive value of 99.4% were detected for 120 people in both normal tests.

Conclusion: The results show E-FAST is a suitable alternative to CT scan in the diagnosis of hemothorax, pneumothorax, and free fluid in the abdomen, vulva, and pericardial cavity. Due to portability, safe body, no need for contrast, fast performance, as well as cheapness, and reproducibility, it will be a great help in diagnosing trauma-related lesions and prompt and timely treatment. CT scan should be used only in limited cases and special indications.

Keywords: *E-Fast ultrasound, blunt trauma, accuracy*

INTRODUCTION

Trauma is one of the most common causes of death in the world. Sometimes due to accompanying trauma and distracting pain, our findings in physical examinations are limited. In trauma patients, internal bleeding is life-threatening and requires prompt and timely action [1-3].

E-Fast ultrasound can detect the haemoperitoneum and intravenous pneumothorax and hemothorax. It can be performed in traumatic patients with symptoms of hemorrhagic shock or evidence of intra-abdominal injury [1]. The features of FAST have led to this practice being adopted as an international standard of care in most developed countries of the world [2].

The benefits of this method can be noninvasive, portable; the low cost can be done in less than 5 minutes, repeatable without the need for radiation and counter, and can be performed by an emergency medicine specialist or surgeon [3-5].

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Studies have shown that E-Fast has a sensitivity of between 99-86% compared to other imaging and diagnostic modalities in the presentation of haemoperitoneum [7]. Laparotomy requires early diagnosis to minimize mortality and morbidity, and FAST can also be a reliable surrogate for CT scans in the diagnosis of intra-abdominal fluid [8-13]. The use of CT is limited due to its high cost and its harms.

In this study, we evaluated the accuracy of E-FAST focused ultrasound in blunt trauma patients.

METHODS

The study was performed on 167 blunt trauma patients referred to the three emergency departments of Baqiyatallah, Shohadae 7th Tir and Imam Hussein Hospital. The statistical population was Multiple Traumatic Blunt Patients and referred to those three emergency departments. E-FAST is performed from four standard spaces in terms of free fluid, chest in terms of hemothorax, and pneumothorax. Ultrasound is performed on the patient's bed and the obtained results are compared with the result of the standard gold CT scan test.

All patient information including age, sex, mechanism of trauma, patient ISS, E-FAST result, and CT scan result are entered in the questionnaire form. In this study, patients are examined for the presence of free fluid in the pelvic abdomen, pericardium, and the presence of free fluid in the pleural cavity on both sides. Patients who are admitted to the emergency room as blunt triage trauma, which includes car accident, motor accident, and fall from a height, and a fight, all-penetrating trauma, were excluded from the study. At the beginning of hospitalization and in the primary survey stage, the E-FAST blunt trauma patients were operated on from the pelvic abdomen and chest (Morrison Patch, Splenorenal free fluid around the bladder, pericardium, and pleural cavity on both sides).

Its positive and negative results were recorded in a specific form. All patient characteristics by trauma time, "time of visit", age, sex, "trauma mechanism", are recorded in the patient's specific form and patient records.

ISS under 16 is excluded from the study because they do not need a CT scan. Patients with ISS above 16 are visited by a surgeon after the initial procedures. If the patient is hemodynamically unstable and undergoes laparotomy and thoracotomy without a CT scan, the patient's laparotomy and thoracotomy results are compared with the E-FAST result. For other patients, if they had a CT scan of the abdomen and chest, the results would be recorded in the desired form. Finally, only 176 patients who had a CT scan of the abdomen and chest, or a laparotomy and thoracotomy are included in this study. Their results are compared with the E-FAST result

Data presented as frequency, percent, mean and standard deviation. An Independent T-test was used for comparing data between various levels. Finally, accuracies indices such as sensitivity, specificity, positive and negative predictive values were assessed for determining the accuracy of E-FAST diagnostic value compared to CT scan as a standard gold test. P-value <0.05 was considered significant.

RESULTS

Overall, 174 patients were included that 45 (25.9%) of them were females. The mean age of the patients was 38.39±12.31 years. Among the total number of people, 61 (35.1%) referred to pedestrians due to a vehicle accident. The relationship between gender and the mechanism of trauma was statistically significant (P<0.001) (Table 1).

Table 1: Distribution subject respect to the mechanism of trauma and sex

			Mechanism of trauma						**Total**
			Car accident	**Vehicle accident with pedestrians**	**Car overturning**	**Falling from a height**	**Falling from a motorcycle**	**Conflict**	
Gender	Female	Count	5	29	2	4	5	0	45
		% of Total	2.9%	16.7%	1.1%	2.3%	2.9%	0.0%	25.9%
	Male	Count	24	32	26	13	33	1	129
		% of Total	13.8%	18.4%	14.9%	7.5%	19.0%	0.6%	74.1%
Total		Count	29	61	28	17	38	1	174
		% of Total	16.7%	35.1%	16.1%	9.8%	21.8%	0.6%	100.0%

The mean ISS in all patients was 29.65±10.25. The mean ISS was 28.15±10.93 in females and 30.05±9.99 in males

(P=0.28).

E-FAST showed normalized outcomes in 125 patients (711.8%), 2 patients (1.1%) hemothorax, 4 patients (2.3%) pneumothorax and 18 patients (3.10% morison pouch fluid, 13 cases (7.7%), 5% splenorenal free fluid, 11 cases (3.6%) of free fluid in pelvis.

One case of free fluid in the pericardial was observed that was not confirmed by CT scan. Two patients were diagnosed with hemothorax in both tests and 3 patients were diagnosed with pneumothorax in both tests.

Out of 176 patients, CT-SCAN results for 133 (76.4%) normal, 3 (1.7%) hemothorax, 4 (2.3%) pneumothorax, 31 (17.8%) free fluid in the abdominal and the pelvis, and 3 patients were found to have retroperitoneal fluid (Table 2).

Table 2: Distribution of results according to E-FAST and CT-SCAN tests

		CT.SCAN						Total (%)
		Hemothorax	Pneumothorax	Free fluid in the abdominal and pelvic		Retroperitoneal fluid	Normal	
E-FAST	Hemothorax	2	0	0		0	0	2 (1.1%)
	Pneumothorax	0	3	0		0	1	4 (2.3%)
	Pericardial effusion	0	0	0		0	1	1 (0.6%)
	Morison pouch fluid	0	0	14	31	0	4	18 (10.3%)
	Splenorenal free fluid	0	0	9		0	4	13 (7.5%)
	Free fluid in the pelvic	0	0	8		2	3	11 (6.3%)
	Normal	1	1	3		1	120	125 (71.8%)
Total (%)		3 (1.7%)	4 (2.3%)	31 (17.8%)		3 (1.7%)	133 (76.4%)	174

E-FAST had a sensitivity of 66.7 and a specificity of 100% in the diagnosis of hemothorax. Also, a positive predictive value of 100% and a negative predictive value of 99.4% were detected for 120 people in both normal tests (Table 3).

Table 3: Sensitivity, specificity, positive predictive value, and negative E-FAST predictive value

Items	Sensitivity	Specificity	Positive predict value	Negative predictive value
Hemothorax	66.7%	100%	100%	99.4%
Pneumothorax	75.0%	99.4%	75.0%	99.4%
Normal	90.2%	87.8%	96.0%	73.4%
Free fluid in the abdominal and pelvic	91.1%	88.0%	63.2%	97.7%

DISCUSSION

In this study, 174 patients were included. The mean ISS in all patients was approximately 30 and the mean was not significant in both sexes. The mean ISS indicates the severity of considerable. In this study, E-FAST had a sensitivity of 91% and a specificity of 88% in the diagnosis of normal and normal individuals. E-FAST obtained 66% sensitivity and 100% positive predictive value and 4.99% negative predictive value in the diagnosis of hemothorax. In this study, this test had a sensitivity of 75% and a specificity of 2.99% in the diagnosis of pneumothorax, and a positive predictive value of 74% and a negative predictive value of 4.99% were obtained. Results showed all items are almost equal and even better in some respects. The results indicate that FAST is a very good alternative to CT scan in the diagnosis of hemothorax, pneumothorax, free fluid in the abdomen, vulva, and pericardium. There were several cases of retroperitoneal bleeding that could not be detected by FAST but were detected by CT scan.

In a study by Porter et al. on a large number of patients (2576 patients), FAST achieved a sensitivity of 93% and a specificity of 90% in the diagnosis of hemoperitoneum. In another study by MT.MILLER on 359 patients Sensitivity was 68%, specificity was 98%, positive predictive value was 67% and negative predictive value was 93% in the diagnosis of hemoperitoneum [14].

In another study by Porter et al., It was observed that among 1631 people with abdominal trauma, fast ultrasound is one of the main methods with high sensitivity and specificity. This sonography can discuss the complications of the disease [15].

In another report by McKenney et al., 899 ultrasounds of abdominal trauma were performed, of which 101 were positive and 783 were negative. Among the negative cases, 768 patients were confirmed by CT scan, surgery, and subsequent follow-up. Among the positive cases, 95 patients were confirmed. In this report, 86% sensitivity and 99% specificity were calculated [16].

However, it seems that in our study, due to the high accuracy of E-FAST in intra-abdominal, pelvic, and thoracic lesions in blunt trauma patients, it is recommended that more training be given to emergency medicine students and surgeons by a radiologist. Now it is preferable to use high-quality methods and high resolution and at frequent and regular intervals without hasty action.

Also, if E-FAS are performed at regular intervals (4-6) hours for hemodynamically stable trauma patients, its results will be much better, but its false positive and negative cases will be minimized and its sensitivity will be more than 98. And due to its advantages such as portability, safe body, no need for contrast, fast performance, as well as cheapness and reproducibility will help in the diagnosis of trauma lesions and prompt and timely treatment. And CT scans should be used only in limited cases and special indications.

CONCLUSIONS

The results show E-FAST is a suitable alternative to CT scan in the diagnosis of hemothorax, pneumothorax, and free fluid in the abdomen, vulva, and pericardial cavity. The reason for decreased sensitivity and specificity of E-FAST in the diagnosis of abdominal bleeding in this study was mostly due to several cases of retroperitoneal bleeding that could not be detected by FAST but were observed by CT scan. Due to portability, safe body, no need for contrast, fast performance, as well as cheapness, and reproducibility, it will be a great help in diagnosing trauma-related lesions and prompt and timely treatment. CT scan should be used only in limited cases and special indications.

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ORIGINAL ARTICLES

Evaluation of the expression level of Sine oculis homeobox homolog 1 in cervical cancer tissue in comparison with healthy adjacent tissue

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Abstract: *Background and Aim: This study aimed to investigate the expression level of Sine oculis homeobox homolog 1(SIX1) in normal and cancerous cervical tissues. Methods: Real-time PCR was used to evaluate SIX1 expression level in fresh surgically obtained cervical biopsies. Also, cervical specimens were tested for HPV DNA detection by MY09/11, GP5+/6+ nested PCR method. Results: The expression level of SIX1 mRNA in Cervical cancer was markedly higher than in normal cervical tissue (p<0.0001). Moreover, HPV DNA was detected in 100% of cases and 21.05% of controls (P<0.0001). Conclusions: This finding indicates that increased SIX1 expression might be of relevance to the pathogenesis of cervical cancer. Perhaps, studies to find agents that specifically target SIX1, develop novel anti-cancer and anti-metastasis drugs. This idea has to be investigated in future clinical studies.*

Keywords: *cervical cancer, SIX1, molecular mechanism, pathogenesis, expression level*

INTRODUCTION

Cervical cancer (CC) is the fourth most common cancer in women with worldwide estimated 527,000 cases and 266,000 deaths in 2012 [1, 2]. Persistent infection with high-risk Human papillomaviruses (HPVs) known as the fundamental cause of CC; although, most of the infected women usually recover spontaneously and only a small proportion progress to CC [2, 3]. HPV E6 and E7 oncoproteins not only inactivate the tumor suppressors p53 and Rb respectively but also modulate a plethora of cellular proteins and signalling pathways related to DNA replication, cell survival, apoptosis, anoikis, epithelial to mesenchymal transition (EMT), anchorage-independent growth (AIG) and angiogenesis. Accordingly, alteration of these cellular cascades leads to cell immortalization, transformation and finally occurrence/development of CC and metastasis [4, 5]. Among these cellular factors, Sine oculis homeobox homolog 1 (SIX1) play a crucial role in CC onset and metastasis [6]. SIX1 is one of the important members of SIX family. This family, including a series of transcription factors, are homolog of the Drosophila sine oculis (so) gene and have a total of six members (SIX1 to SIX6) in vertebrates. Among them, SIX1 has attracted the attention of many researchers recently. SIX1 gene is located on human chromosome 14q23 and its structure composed of two conserved domains, the homeodomain (HD) and the SIX domains (SD). Many studies have shown that SIX1 is essential for the development of

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various human organs in physiological conditions and overexpressed in multiple human cancers, also associated with metastatic phenotype [7, 8]. It is indicated that SIX1 stimulates DNA replication, cell proliferation and maintains survival conditions in CC cells. In the other hand, HPV E7 oncoprotein can induce SIX1 overexpression. Consequently, HPV can obtain opportunity of effectively persistent infection in cervical cells and develop CC [9, 10]. Advanced stages of CC have not effective treatments and most of patients with metastatic CC die. Thus, the better understanding of HPV-induced CC molecular mechanism is indispensable and can assist to introduce more effective novel therapeutic approaches for HPV-induced CC prevention and treatment. In the present study, we evaluate the expression level of SIX1 mRNA by real-time PCR in CC biopsies in comparison with healthy adjacent tissues.

MATERIAL AND METHOD

2.1. Specimens

A total of 38 histopathologically confirmed fresh cervical biopsies consist of 19 cancer samples and 19 normal samples were surgically collected from the Department of gynecology at Imam Khomeini hospital in Tehran, Iran. Normal subjects were women referring to gynecology department of hospital for any cause (for example ovarian cancer), except for cervical involvement. No subjects received preoperative radiotherapy, chemotherapy, or other treatment. The study was approved by the medical ethics committee from Tehran University of Medical Sciences and written informed consent was obtained from all patients before enrollment (approval number 1395.1301).

2.2. DNA Extraction

DNA was extracted from biopsies by the High Pure Viral Nucleic Acid Kit (Roche, Mannheim, Germany) according to manufacturer's instructions. A Nanodrop spectrophotometer (Nanodrop Technologies) was used to determine DNA concentration and purity.

2.3. PCR Analysis

First, we confirmed the integrity of extracted DNA by amplification of a housekeeping gene β-globin (act as an internal control) using PCO3/PCO4 primers. The reaction conditions were as follows: 40 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min and a final extension at 72 °C for 7 min. Then, the presence of HPV DNA was examined by nested PCR assay in all of the positive specimens for the β-globin gene. For this purpose, we used MY09/11 outer followed by GP5+/6+ inner primers (are located within the L1 region of the HPV genome. The Sequences (5 to 3) of all PCR primers are listed in table 1. The temperature cycling program in the first round (MY09/11) and second round (GP5+/6+) PCR was identical as follows: 94°C for 4 min, 40 cycles of 94°C for 1 min, 42°C for 1 min, 72°C for 30 s and a final extension at 72 °C for 4 min [11].

2.4. RNA Extraction

The total RNA from tissue specimens was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted RNA was eluted in 50 µl Diethylpyrocarbonate (DEPC) water. The concentration and purity of RNA were determined by Nanodrop spectrophotometer (Nanodrop Technologies). In order to the prevention of genomic DNA contamination, RNA samples were treated with RNase-free DNase I (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.5. Real-Time PCR

One microgram of RNA samples was reverse transcribed to cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) following the kit's instructions. Real-time PCR reactions were carried out in duplicate with Applied Biosystems 7500 Real-Time PCR system using SYBRs Premix ExTapTM II (Takara, Japan) and specific primers that listed in Table 1 [12]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was applied as an internal amplification control. The 2−ΔΔCT method was used for relative quantification of SIX1 expression levels and results were normalized to GAPDH, as a housekeeping gene.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Variables were compared between groups using the Fisher's exact test or student's t-test as appropriate. Numerical results are expressed as the mean ± SEM. The p-values of <0.05 were considered statistically significant.

RESULTS

3.1. Demographic characteristics

The mean age of the women in the CC group and control group was 61.3 ± 10.2 and 36.0 ± 8.3 years, respectively. Based on statistical analysis, the mean age in the CC subjects was significantly higher than the control group (p-value <0.0001).

Table 1: Sequences of primers used for real-time PCR reactions in current study

Primers	Sequence (5′–3′)
β-globin	PCO3 (Forward) ACACAACTGTGTTCACTAGC PCO4 (Reverse) CAACTTCATCCACGTTCACC
HPV	MY09 (Forward) CGTCC(A/C)A(A/G)(A/G)GGA(A/T)ACTGATC MY11 (Reverse) GC(A/C)CAGGG(A/T)CTATAA(C/T)AATGG GP5+ (Forward) TTTGTTACTGTGGTAGATACTAC GP6+ (Reverse) AAAAATAAACTGTAAATCATATTC
GAPDH	(Forward) GGAGCGAGATCCCTCCAAAAT (Reverse) GGCTGTTGTCATACTTCTCATGG
*SIX*1	(Forward) AAGGAGAAGTCGAGGGGTGT (Reverse) TGCTTGTTGGAGGAGGAGTT

HPV, Human papilloma virus; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; SIX1, Sine oculis homeobox homolog 1

3.2. Prevalence and type of HPV infection

HPV DNA was detected in 100% (19/19) of the cancer cases and 21.1% (4/19) of normal cervical specimens (Table 2). There was a significant difference between the two studied groups (p<0.0001). After sequencing analysis of HPV-positive specimens, we identified HPV-16 in all of the CC samples. Also genotyping of 4 HPV-positive specimens in normal group was HPV-16: 2 cases, HPV-66 and HPV-68: each one was one case (Table 2).

Table 2: Demographic and clinico-pathological characteristics in patients with cervical cancer and without cervical cancer

Variables	Cases (n = 19)	Controls (n = 19)	p-value
Age (mean years)	61.3 ± 10.2	36.0 ± 8.3	<0.0001, t-test
HPV positivity	19 (100%)	4 (21.1%)	<0.0001, Fisher'sisher's exact test
HPV type	HPV-16 = 19	HPV-16 = 2, HPV-66 = 1, HPV-68 = 1	

HPV, Human papilloma virus

3.3. SIX1 mRNA expression

Relative real-time PCR results showed that the mean SIX1 mRNA expression in the CC patients was 2.61 ± 0.09 and in the normal subjects was 1.12 ± 0.03 (Table 3).

Table 3: Expression level of SIX1 in patients with cervical cancer and without cervical cancer

Gene		Cases	Controls
*SIX*1	Mean ± SE	2.61 ± 0.09	1.12 ± 0.03
	CI	2.42 - 2.79	1.06 - 1.18
	Minimum expression	1.94	0.92
	Maximum expression	3.47	1.39
	p-value	<0.0001 t-test	

SIX1, Sine oculis homeobox homolog 1; SE, standard error; CI, confidence interval

Then, statistical analysis revealed that SIX1 mRNA levels were significantly (p<0.0001) higher in CC tissue compared to the normal cervical tissue (Figure 1).

Figure 1: The expression level of SIX1 in CC and normal cervical group. Data are presented as mean ± SEM and the fold increase of transcripts for SIX1 mRNA normalized by GAPDH housekeeping gene. ****p<0.0001.

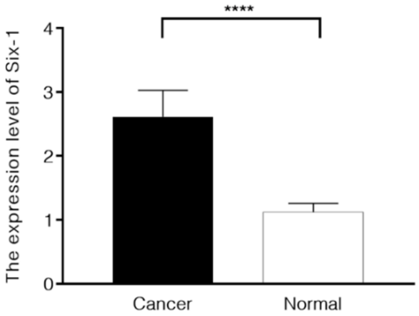

DISCUSSION

The sine oculis homeodomain (SIX) proteins are a family of evolutionarily conserved transcription factors that play a pivotal role in both embryogenesis and carcinogenesis. The homeoprotein transcription factor SIX1 is one of the members of this family that has attracted much attention from the scientific community in recent years [13]. Based on the previous studies, SIX1 upregulated in several human cancers such as breast, ovarian, cervical, liver, rhabdomyosarcoma and Wilms' tumor, also contribute to initiation and metastatic development of these malignancies. Researches have also observed the SIX1 overexpression strongly correlated with poor prognosis and shortened overall survival in cancer patients [14-16]. Consistent with previous studies, we demonstrated the SIX1 mRNA levels is significantly higher in CC than normal cervical tissues. The results of previous studies showed that SIX1 can drive cancer stem cell phenotype and EMT, also lymphangiogenesis, consequently metastasis via stimulation of the TGF-β signaling pathway and VEGF-C, respectively [17-19]. EYA2 act as a coactivator for SIX1, thus mediate its transcriptional activation. Previously it has been shown that inhibition of SIX1-EYA2 complex formation, may be a promising and more effective approach for treatment of cancer and tumor metastasis [20]. In CC cells, SIX1 act as principal regulator of DNA replication, as well as a key factor in metastasis process. Therefore, SIX1 upregulation can lead to malignant proliferation of cervical cells, also metastasis through stimulation of DNA replication-related genes and a5b1 integrin, respectively [10, 21]. Interestingly, high-risk HPV employ various mechanisms to promote metastasis in CC [4]. Anoikis, meaning homelessness, is a detachment-induced cell death. Anoikis resistance, EMT, AIG, and angiogenesis are important cellular events that closely associate with cancer metastasis [22]. Hr-HPV E7 oncoprotein can stimulate SIX1 overexpression in CC. SIX1 consider as an EMT-activating factor, thus its upregulation result in EMT through increasing of ZEB1 and decreasing of E-cadherin. Indeed, SIX1-induced EMT, increase cell survival in detached cancerous cells and drive anoikis overcoming and finally, metastatic dissemination [9]. In the present study, among the total of 38 fresh surgically obtained cervical biopsies, 100% (19/19) and 21.1% (4/19) were positive for HPV DNA, in CC and normal adjacent groups, respectively. Then, sequencing analysis revealed all of HPV-positive specimens in CC group were HPV type 16 that highlighted the indispensable role of HPV-16 in CC onset and progression. The previous studies demonstrated the high risk HPV-16 and HPV-18 account for about 70% of CCs [23]. In current study, four HPV-positive specimens in normal cervical group were: two samples of HPV-16 and one sample of HPV-66 and HPV-68. Based on international agency for research on cancer (IARC) classification, HPV-66 belongs to 2B category (probably carcinogenic). Also, HPV-68 belongs to 2A category (possibly carcinogenic) [24]. Our outcomes corroborated previous reports specifying that the SIX1 involved in the tumorigenesis of cervical cancer [13, 25]. The main limitation of our work was low sample size. In conclusion, SIX1 upregulated significantly in CC, which in turn induces TGF-β, VEGF-C and ZEB1 that collectively can have an association in the tumorigenesis and metastasis of CC.

CONCLUSIONS

We demonstrated SIX1 expression levels was frequently expressed in CC tissues. Also, HPV DNA was detected in 100% (19/19) of the cancer cases and 21.1% (4/19) of normal cervical specimens. HPV can upregulate SIX1 in CC. In addition, SIX1 can contribute to the development of CC and lymph node metastasis of cervical tumors. Nevertheless, the precise biologic role of SIX1 in CC is unclear yet and future works should study on the larger number of patients.

List of abreviation:

CC, Cervical cancer; HPV, Human papillomavirus; SIX1, Sine oculis homeobox homolog 1; EMT, Epithelial to mesenchymal transition; AIG, Anchorage independent growth; so, Drosophila sine oculis; HD, Homeodomain; SD, SIX domains; DEPC, Diethylpyrocarbonate; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; IARC, International agency for research on cancer.

Contribution Note:

EK, EF, MFP, TMA designed the study, EK, EF, MFP performed laboratory works, MFP, MF, MY statically analyzed the data, EK, EF, MFP, MF, MY, TMA wrote and revised the manuscript.

All authors read and approved the final manuscript.

Patient participation at the study respects ethical standards in medical research and GDPR.

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ORIGINAL ARTICLE

Evaluation of the protective effect of coenzyme Q10 on the growth process of embryos from in vitro fertilization in laboratory mice treated with cyclophosphamide

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Abstract: *Cyclophosphamide is an anticancer drug that acts as an alkylating agent after metabolism in the liver. Despite its many clinical applications in cancer treatment, this drug has toxic effects on the body's organs, especially the genitals. One of the most critical side effects is a change in the function of the female reproductive system, which can lead to infertility. This study aimed to investigate the antioxidant effects of coenzyme Q10 on cyclophosphamide-induced toxicity in vitro fertilized embryos in mice. In this experimental study, 24 female mice weighing 25 g 4 groups of 6 were divided and treated for 21 days. The first group (control group), solvent (cyclophosphamide) including DMSO and PBS (0.1 ml intraperitoneally), the second group (sham group), cyclophosphamide at a dose of 10 mg/kg was injected as a single dose, and the third group (experimental group), along with single-dose cyclophosphamide, coenzyme Q10 at a dose of 200 mg/kg/day was injected intraperitoneally and the fourth group (positive control group), only coenzyme Q10 at a dose of 200 mg/kg/day was injected intraperitoneally. At the end of the treatment period, ovulation stimulation was performed using PMSG and HCG hormones. Six adult male mice were used to prepare normal sperm. The animals were facilitated after anesthesia. After extraction of regular eggs and sperm and fertilization in HTF + 4 mg BSA medium, the fertilized eggs were incubated for 120 hours, and the embryonic developmental stages were examined during this period. Were analyzed by ANOVA and comparison of ratios. Cyclophosphamide significantly reduced oocyte quality, fertilization rate, pre-implantation embryonic development, and embryo quality. Coenzyme Q10 (Co Q10) significantly reduced the adverse effects of cyclophosphamide.*

The present study showed that crocin could protect the fertility of the female sex against damage caused by cyclophosphamide.

Keywords: *cyclophosphamide, ovary, coenzyme Q10, mice, fertility*

INTRODUCTION

Operation of inguinal hernia is one of the most common procedures in the general surgery field. The prevalence of inguinal hernia is between 15-45% at different ages [1-3]. There are two categories of inguinal hernia, direct and indirect, that included 24 to 50% of all types of hernia, respectively [4-7].

Some studies reported chronic obstructive pulmonary disease, chronic cough, benign prostatic hyperplasia, chronic constipation, family history of hernia, previous right lower quadrant incision, smoking, collagen diseases, physical activity, and bearing the burden can trigger inguinal hernias [5-10].

The inguinal hernias can develop discomfort, pain, and dysfunction in the patient, and also it can cause a cosmetic problems for most patients [2-4]. Severe complications of this disorder, which often occur following hernia entrapment and suppression, require emergency intervention. These include obstruction, ischemia, and gangrene of the bowel or any organ trapped inside the hernia sac [4-7].

A surgical procedure is the standard treatment for this disease. Today, there are several techniques of surgery for it, and surgeons associated with circumstances chose a surgery strategy. The main purpose of surgery is to treat patients and decrease the recurrence chance of the disorder [11-15].

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Inguinal hernia repair surgery has several potential complications such as hernia recurrence, pain, osteitis of the pubis, Damage to the cord and testicles, hematoma, and testicular swelling [1-3, 5]. There are two main approaches to operation including open surgery and laparoscopy. Also, there are several techniques to repair the herniation site such as classical and preperitoneal techniques. The classical method is a simpler procedure than other methods of repair conducted by most surgeons. Various conventional methods like Bassini's applying suture material are in usage despite the laparoscopic hernia repair [16-18]. Suture repair is related to a significant tension on the suture line. This is possible to induce ischemia of the tissues and final failure of repair causing recurrence [18-20].

Open surgical techniques include two categories of tissue repair and mesh repair methods. More traditional methods are based on initial tissue repair, but today with the production and presentation of industrial meshes, non-traction hernia repair with mesh is the standard method of inguinal hernias surgery [9-6]. Lichtenstein et al. reported the concept of tension-free hernia repair through a synthetic proline mesh that ensures promising outcomes of hernia repair in the long term [1-3].

Preperitoneal repair is one of the pre-peritoneal methods, the mesh is placed under the transversal fascia and right on the peritoneum, especially in cases of recurrent hernias after tissue repair methods. In this method, the peritoneal space is released through the anterior access, a 15 by 15 cm mesh is implanted on the peritoneum and fixed to the umbilicus and symphysis of pubis with non-absorbable thread, and then tissue repair with nylon loop thread is done [13-14].

This study aimed to compare the complications of Surgical Repair in inguinal hernia with Bassini, Lichtenstein, and Preperitoneal met Exposure to exogenous materials, whether natural or man-made, has a profound effect on vital processes and reduces or destroys reproductive capacity [1]. In the female animal, if it comes in contact with chemicals, it affects the oocyte cell, which is in various stages of primary, primary, secondary, or ovulation. Most chemotherapeutic agents are mutagenic and cause defects in sex cell chromosomes. Alkylating agents such as cyclophosphamide are among the significant chemotherapeutic agents used to treat cancers and autoimmune diseases [2]. This compound is a cytostatic drug from the oxazaphosphorine group and alkylated nitrogen mustard derivatives [3].

Cyclophosphamide is converted by its oxidase enzymes in the liver to its active metabolite, 4-hydroxy cyclophosphamide, which interacts with its totomer called aldophosphamide. Most aldophosphamide is oxidized by the enzyme aldehyde dehydrogenase (ALDH) to carboxy-phosphamide, and only a small amount of aldophosphamide is converted to mustard phosphor amid acrolein [4]. Despite its wide range of clinical applications, this drug causes reproductive toxicity. Studies have shown that cyclophosphamide disrupts the process of spermatogenesis by increasing sperm DNA damage [5]. Ovarian dysfunction decreased sex hormones, and reduced follicle count have also been reported with cyclophosphamide [6]. Cyclophosphamide also destroys primary follicles and, due to reduced follicular reserve, harms the reproductive system, and causes infertility and premature menopause in females [7]. Cyclophosphamide products chromatin and some of its effects have been observed in the next generation of F2 [8]. Injection of cyclophosphamide into pregnant mice on the thirteenth day of the fetus can cause a defect in the synaptic process as partial synapses or no bilateral synapses on the eighteenth day of the fetus [9].

Oxidative stress results from an imbalance between the production of oxygen free radicals versus the antioxidant defense capacity of the cell, leading to the production of free radicals, including superoxide anion (O_2), hydroxyl radicals (OH), and non-radical derivatives of oxygen such as hydrogen. Peroxide (H_2O_2). These free radicals are highly unstable and react rapidly and non-specifically with biomolecules, leading to the development and spread of cellular damage including plasma membrane peroxidation, amino acid, nucleic acid oxidation, apoptosis, and necrosis. Finally, it leads to a decrease in the viability and development of embryos in the culture medium [10,11]. Reproductive disorders in both males and females caused by oxidative stress have been reported. The effect of reactive oxygen species (ROS) levels on zygotes or early embryonic development has been identified. Oxidative stress may be responsible for fetal toxicity in the hydrosalpinx [12]. The addition of antioxidants such as vitamins C and E to the culture medium improves the blastocyst development ratio in mouse embryos [13]. It enhances the fertility ratio and increases implantation [11]. Cyclophosphamide, as an important and effective drug in the treatment of cancer, despite its improvement in cancer patients, acts as an oxidative stress-producing agent in the body and increases the production of ROS. Therefore, to modulate and prevent the effects of oxidative stress caused by cyclophosphamide, coenzyme Q10, which has an antioxidant role, can be helpful. Coenzyme Q10 (CoQ10), a fat-soluble molecule in almost all cell membranes, is located inside the mitochondrial membrane. CoQ10 is an essential component for transporting electrons in the mitochondrial respiratory chain to produce cellular energy. In addition, the reduced form of CoQ10, known as ubiquinol, acts as an antioxidant in cellular metabolism by inhibiting lipid peroxidation, protein, and DNA oxidation [14,15]. The amount of CoQ10 in tissues is often reduced in many system-related pathological conditions such as diabetes and Alzheimer's and prion diseases, as well as the use of chemicals. Although CoQ10 is synthesized in almost all normal tissues, CoQ10 tissue levels gradually decrease with age [16]. Defects in CoQ10 synthesis with specific clinical disorders associated with high-energy consuming tissues, including skeletal muscle, endocrine glands, and nervous system [17]. Studies have shown that CoQ10 supplementation causes a significant increase in CoQ10 levels in some tissues such as muscle, sperm, and plasma, and a substantial increase in the adrenal glands and ovaries [18,19]. Therefore, CoQ10 supplementation protects cells against ROS-induced damage due to its antioxidant properties, strengthening endogenous cell antioxidant systems.

Since no study has been performed on the effect of coenzyme Q10 as an antioxidant in reducing the effects of oxidative stress induced by cyclophosphamide on the growth of in vitro fertilization embryos, in this study, the protective effect of coenzyme Q10 on Improving the growth process of in vitro fertilization embryos was studied in cyclophosphamide-treated mice.

METHOD

In this experimental study, 24 female rats weighing approximately 20-25 g were randomly divided into four equal groups of 6 and treated for 21 days. Animals under standard conditions with a temperature of 22.2° C, humidity 30-60%, and the light cycle, 14 hours of light and 10 hours of darkness were maintained. Water and food were freely available.

The control group (cyclophosphamide) including DMSO and PBS (0.1 ml intraperitoneally), the sham group with a dose of 10 mg/kg was injected as a single dose of only cyclophosphamide, experimental group 1, with a single dose of cyclophosphamide, coenzyme Q10 at a dose of 200 mg/ kg/day was The control group (cyclophosphamide) including DMSO and PBS (0.1 ml intraperitoneally), the sham group with a dose of 10 mg/kg was injected as a single dose of only cyclophosphamide, experimental group 1, with a single dose of cyclophosphamide, coenzyme Q10 at a dose of 200 mg/kg/day was injected intraperitoneally into the positive control group, only coenzyme Q10 at a dose of 200 mg/kg/ day was injected intraperitoneally. Ovulation induction was required for this study. For this purpose, after the end of the treatment period and 72 hours before in vitro fertilization (IVF), 5.7 units of HCG (made by Folligon, Netherlands) in a volume of 0.1 ml was injected a volume of 0.1 ml and 48 ml. An hour later, 5.7 units of HCG (made by Folligon, Netherlands) in a volume of 0.1 ml was injected intraperitoneally. Ovulation was performed 13 hours after HCG injection.

The first step in IVF was to prepare normal sperm from 6 adult male laboratory mice by injecting ketamine (25 mg/kg body weight, intraperitoneally) under anesthesia with a high dose of easy ketamine. The skin of the abdomen was then sterilized with 70% ethanol. After making an incision in the abdomen and separating the connective tissues, we saw around the tail of the epididymis with some vas deferens from the testicle and into a 3 cm dish containing a medium. HTF culture, containing 4 mg/kg BSA (Sigma, USA), was previously transferred to the incubator for exchange. After making several incisions in the tail of the epididymis and pressure in the vas deferens to expel sperm, Petri the dishes were placed inside a carbon dioxide incubator. After 30 minutes, the sperm were released and spread in the environment. The sperms were then washed and separated using a flotation method. In this study, standard sperm samples with motility above 80% and one million per ml of culture medium were used. The second stage of work was ovulation in female mice. For this purpose, 13 hours after HCG injection (following day), animals after anesthesia with ketamine injection (25 mg/kg body weight, intraperitoneal) and injection. The high dose of ketamine was facilitated, then the fallopian tubes were removed and placed in HTF + 4mg/ml BSA culture medium at 37° C, which was prepared in advance, and the eggs were removed using an autopsy method. After washing, they were transferred to the fertilization medium under mineral oil containing HTF culture medium (containing 4 mg/ml BSA). Then the sperms were transferred to the culture medium in the amount of one million per ml of culture medium. It took about 3 to 5 hours after the sperm were added and thus, the zygotes were obtained. Zygotes from all groups were cultured in HTF medium for 120 h and embryonic developmental stages were evaluated under the inverted microscope to evaluate the effect of oxidative stress due to cyclophosphamide administration and the protective effect of coenzyme Q10. Fission rate was assessed 24 hours after culture and in zygotes in each group, embryos in terms of fragmentation, embryonic development, and the number of embryos stopped due to various factors such as embryonic slippage, necrotic, fragmentation, and the presence of cytoplasmic vesicles occurred. Embryo quality, number of embryos grown, and incision percentage were evaluated by examining the percentage of two-cell embryos, the number of stopped embryos, and the rate of blastocysts formed during 120 hours of incubation in each group.

RESULTS

The control group differentiated the percentage of embryos from blastocysts with appropriate quality after 120 hours of incubation, and the percentage of embryos that stopped at different stages of development. B, cyclophosphamide group, a small percentage of embryos have reached the blastocyst stage and the resulting blastocysts are not of good morphological quality. C, a cyclophosphamide-treated group with coenzyme Q10 (experimental) compared with the cyclophosphamide control group, a high percentage of embryos reached the blastocyst stage and the resulting blastocysts are of good morphological quality, and a small percentage of embryos are stopped. D, coenzyme Q10% higher percentage of embryos reach blastocyst and percentage of embryos are stopped at different stages (invert microscope, magnification × 400) (Figure 1).

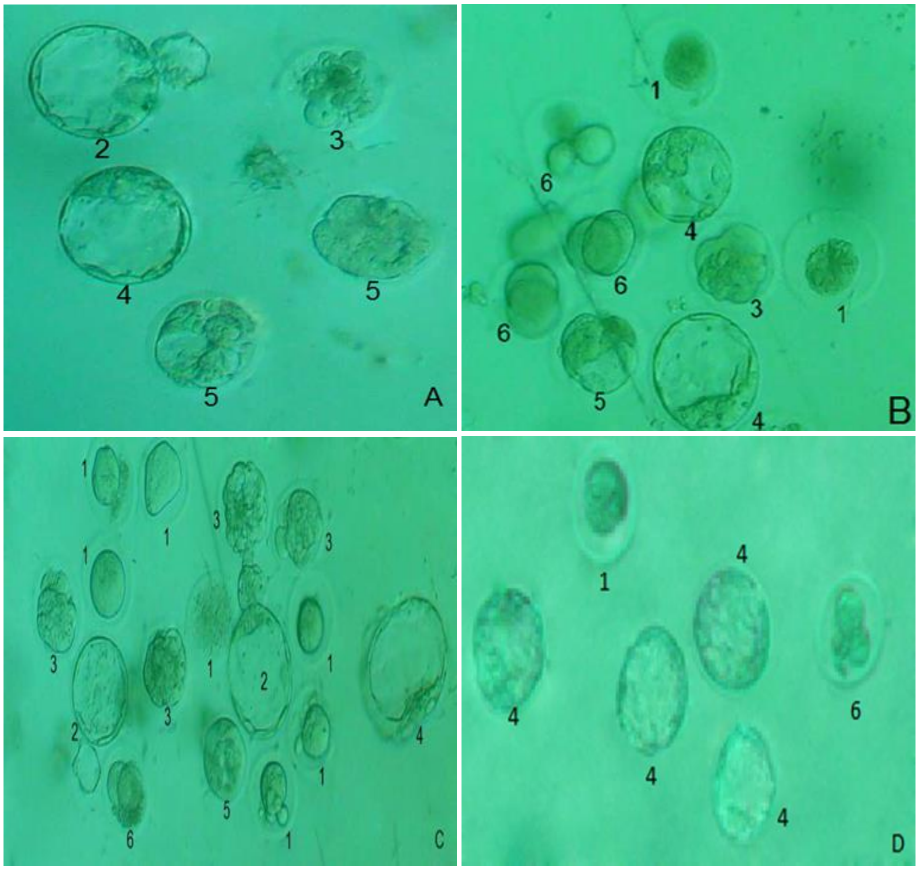

Figure 1: Fetal growth photomicrograph: A: Control group, B: Cyclophosphamide group, C: Cyclophosphamide with coenzyme Q10, D: Coenzyme Q10

1. Oocyst; 2. The fetus is being hatched; 3. Merola; 4. Blastocyst; 5. Primary blastocyst; 6. Fetal fetus

The interaction of different drug groups and antioxidants on the measured parameters showed that the effect of drug treatment on the number of oocytes was significant (P <0.05), so the highest number of oocytes was obtained from the control group and the lowest amount belongs to the cyclophosphamide group only. It was also shown that the mean number of oocytes in the cyclophosphamide group with coenzyme Q10 was almost similar to the control group, and there was no significant difference with this group (Figure 2).

The effect of antioxidant treatment in the cyclophosphamide group with coenzyme Q10 on the mean number of zygotes was significant compared to the cyclophosphamide group (P <0.05), but this increase was not significantly different from the control group among the treated groups. The highest number of zygotes belonged to the group that received coenzyme Q10 alone and was not substantially different from the control group; the lowest number of zygotes was obtained from the group that received only cyclophosphamide (Figure 3).

The mean of two-cell zygotes in cyclophosphamide-receiving groups with coenzyme Q10 was not significantly different from that of cyclophosphamide despite increasing the number of two-celled zygotes (Figure 4).

Figure 2

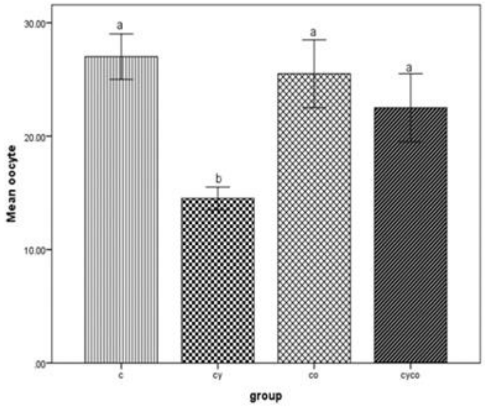

Figure 3

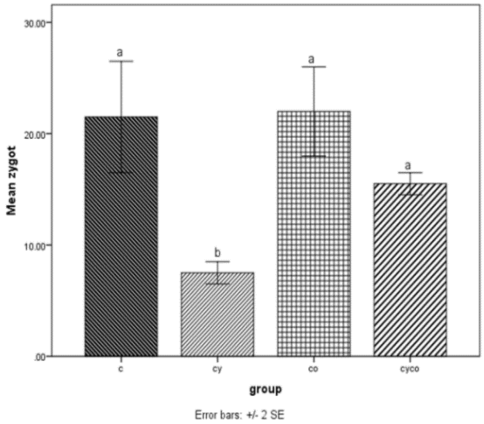

Figure 4

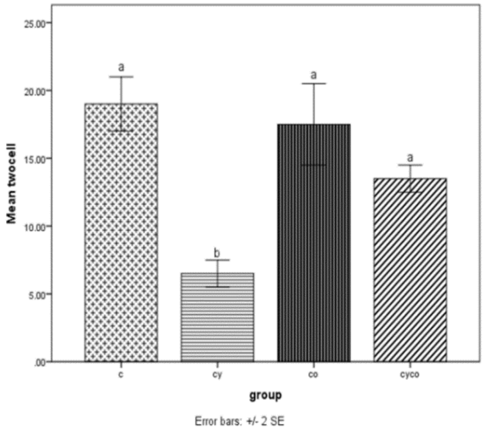

The effect of antioxidant treatments on the number of blastocysts was. The highest amount belonged to the control group and the lowest amount belonged to the group that received only cyclophosphamide (Figure 5).

The mean number of blastocytes in all groups receiving cyclophosphamide along with coenzyme Q10 was significantly different from the cyclophosphamide group (P <0.05). Regarding this parameter, the group receiving coenzyme Q10 alone did not have a significant difference from the control group (Figure 6).

Figure 5

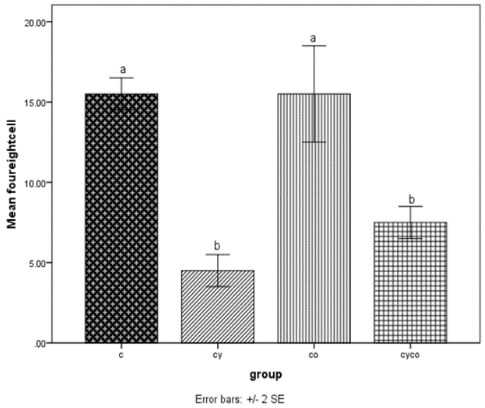

Figure 6

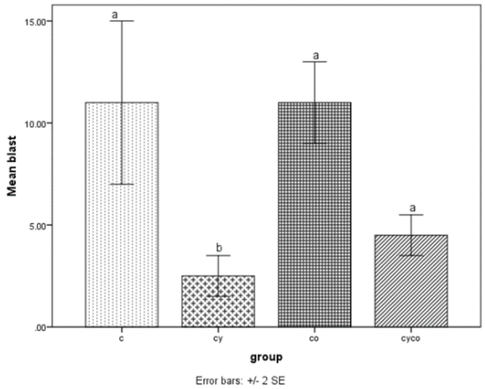

The highest number of discontinued embryos was observed in the cyclophosphamide group and the use of coenzyme Q10 with cyclophosphamide significantly reduced the mean number of discontinued embryos (P <0.05) (Figure 7).

Figure 7

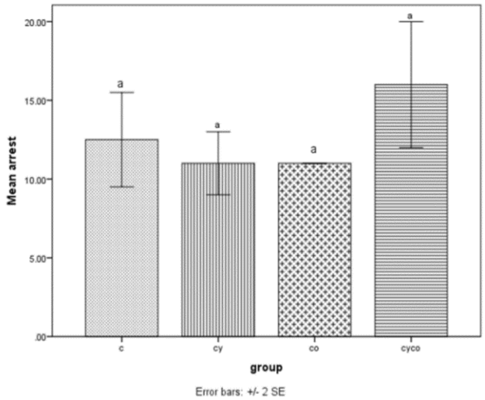

DISCUSSION

Cyclophosphamide causes reproductive toxicity by inducing oxidative stress, which is reduced by the concomitant administration of antioxidants. The ability of cyclophosphamide to produce free radicals, lipid peroxidation, and oxidative stress has been reported in laboratory mice [20].

Previous studies have shown that low doses and long-term use of cyclophosphamide in male and female mice impair fertility [21,22]. It changes the development of the next generation [23]. Intracellular thiol levels appear to play an essential role in determining cell susceptibility to cyclophosphamide-induced damage [24].

4-Hydroxycyclophosphamide can react with cellular thiols to produce a thio-alkyl derivative. Derivatives produced in equilibrium with 4-hydroxycyclophosphamide are considered alkylating agents [25] and can induce oxidative stress. Cyclophosphamide has also been shown to have immunosuppressive properties, which are related to its induction of oxidative stress [26].

Due to the effect of cyclophosphamide on chromatin of sex cells and DNA failure in the nucleus of oocyte cells, some of its effects appear in the next generation [8]. One study found that in cyclophosphamide-affected larvae, after extraction in a culture medium, the meiotic division was inhibited 48 hours later, whereas, in animals, fertility may continue in humans. As a result, under the influence of chemotherapy agents, the development of embryos is disrupted and anomalies develop in them [27]. This study was performed to modulate the side effects of oxidative stress exerted by cyclophosphamide on the growth process of in vitro fertilization embryos using coenzyme Q10 as an antioxidant. In this study, the growth rate and quality of embryos obtained from in vitro fertilization in the control group showed a significant decrease compared to the control group.

These observations indicate the toxic effect of cyclophosphamide on reproductive activity through oxidative stress, which is co-administered. Coenzyme Q10 (Co Q10), as an antioxidant, attenuated this toxic effect of the drug and increased the quality of fetal growth in the laboratory. Injection of high-dose cyclophosphamide into female mice mainly affects the primary follicles and disrupts other ovarian characteristics such as ovulation, egg transfer, and end endocrine regulation [28].

In a study conducted on the effect of Khuzestani Marzeh essential oil on in vitro fertilization of adult female mice treated with cyclophosphamide, administration of safflower extract with cyclophosphamide improved the growth of embryos obtained from in vitro fertilization.

Other studies on the modification or prevention of side effects of chemotherapy drugs by antioxidant agents have shown the toxic effects of cyclophosphamide on sperm morphology and ovarian tissue, are protected by lycopene and alginic acid as antioxidants [29].

The protective role of vitamin E against oxidative stress induced by cyclophosphamide has been demonstrated in mice. This action of vitamin E may be the result of the destruction of free radicals as well as the stimulation of the release of anterior pituitary gonadotropins [30]. Also, the presence of antioxidants in culture media improves the blastocyst development ratio in mouse embryos and improves the fertility ratio, and increases implantation [31].

CONCLUSION

Considering all the results obtained along with their interpretation, the present study reinforces the idea that the reproductive toxicity caused by cyclophosphamide administration occurs due to oxidative stress, and that a potent antioxidant such as coenzyme Q10 can regenerate the reproductive system. Protects against oxidative stress damage caused by cyclophosphamide, which often causes infertility. In the present study, coenzyme Q10 was able to increase the quality of germ cells, and fertilization rate, improve fetal growth in the laboratory, and reducing cessation at different stages of embryonic development. effective.

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REVIEW ARTICLE

Evidence based medicine: lessons learned from the NATO Military Medical Center of Excellence

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Abstract: *Evidenced-based medicine drives best practices. The Lessons Learned (LL) process in clinical medicine for deployed and military support services drive the NATO Military Medical Center of Excellence (MILMED COE). This review article focuses on the Lessons Learned process and its use in NATO and partner countries medical services. Organizational learning drives development and progress; capturing lessons from mistakes, colleagues' interactions and institutional experience can be lost without an organized lesson learned process. Therefore, in 2014, NATO institutions and Centers of Excellence across disciplines began to focus on the quality management, information sharing and evidence-based practices to maximize outcomes. Since this inception, NATO has implemented the Lessons Learned process and expanded the impact across the alliance in order to save life and prevent illness. The Lessons Learned process and sharing of experience is also a way to improve the quality of care in the military medicine, from preventive, epidemiological, trauma related, casualty evacuation and forward surgical care, among many others, to the medical standardization and organization of military medical services. The Lessons Learned process is an undertaking and an instrument that can also be used to achieve better international and civil-military cooperation. Finally, we deal with the current situation and use of the information obtained from the areas of military health care.*

Keywords: *evidenced-based medicine, knowledge management, lessons learned, Military Medical Service, NATO*

INTRODUCTION – APPLICATION OF THEORY OF LEARNING

Each scientific discipline must be driven by data. One of the areas from which information can be drawn is Lessons Learned, through analysis and a constant cycle of learning and sharing data. This is most applicable in the military medicine and across the healthcare sector, where shared information can be used from various areas, rooted in preventive medicine, epidemiology, traumatology, and others adding to military medicine and healthcare organization and administration.

Institutions that promote health act as microcosms of organism that can evolve through adaptation. As Charles Darwin posited, it's not the strongest survivor, not even the most intelligent, but the one who can best adapt. Every living organism adapts best by learning from past mistakes. In a broader sense, it is about adapting to the environment, as well as the active role of the individual and the choice based on challenges. But learning is not mere adaptation, but it is an active process in which an individual acquires (changes, adapts, and repeats) something that is new. Learning outcomes are actively created personal hypotheses and meanings of reality [1].

There are many theories of learning, but the process we describe in this paper focuses on the theory of Classical Conditioning (I.P. Pavlov), Thorndike's theory of Instrumental Conditioning, Skinner's theory of Programmed Learning, and the theory of Learning through Imitation. Simply put, we

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will do, repeat and develop what we have done and have some positive effect in a particular area. Conversely, we discuss what happened and had some negative consequences that should not be repeated. Based on applying these hypothesis listed above and core knowledge, every organization that wants to survive must behave to the basic strategy of learning from past mistakes.

THE WAY OF THE LESSONS LEARNED PROCESS TO MEDICAL SERVICES OF THE NATO ARMIES

In the early years of the 21st century, NATO understood the importance of transformation and has become a learning organization that has the ability to be constantly flexible and adaptive to remain the central core of European and North Atlantic Security structures in the promotion of safety and security across international communities. For this reason, NATO has issued guidelines outlining the process of learning and sharing experiences, with the clear aim of providing a standardized structure and process that enables this ability in all alliance command structures [2].

Moreover, as outlined within the Armed Forces Declaration, signed by the NATO Heads of State during the NATO Summit, Wales 2014, NATO was directed to enhance the sharing of best practices and lessons learned in support of its Armed Forces personnel and their families [3]. Through the Military Committee (MC), the Committee of the Chiefs of Military Medical Services in NATO (COMEDS) requested that the NATO Centre of Excellence for Military Medicine (MILMED COE) prepare a way ahead to be presented at the 42nd COMEDS Plenary. The proposal was approved and subsequently endorsed by the International Military Staff (IMS) and Military Committee (MC) [4].

LESSONS LEARNED PROCESS AS A PART OF KNOWLEDGE MANAGEMENT

Lessons Learned is defined as a major component of Knowledge Management (KM), not only in the military, but also in government and commercial organizations [5]. The priority objective of Knowledge Management is to achieve higher performance of the organization in fulfilling its tasks in a dynamic, asymmetric and unstable environment (i.e. across the domains of land, sea, air, space and cyber). Therefore, gaining knowledge and experience is a process whereby the Lessons Learned are explored in order to find the best way to deal with the situation and share the outcome to all stakeholders who can benefit from it, and preferably mitigate and reduce mortality and morbidity.

In addition, it is a process allows an organization to learn from its mistakes or use its achievements. Thus, Lessons Learned is an instrument for the continuous process of improving each organization based on evidence [6]. The essence of this process is that it reduces the risk of error repetition in the organization, and the likelihood of repetition of success increases. The introduction of this process within the military health service is important not only for updating the core doctrine that drives best practices, but also for the comprehensive preparation of units in both peacetime and deployed operations.

According to the NATO Bi-Strategic Command Directive 080-006 [7], which is a strategic document of the Lessons Learned process, the following components – the pillars through which the process is implemented – are needed (Figure 1):

- Processes – links between elements to implement the process, standardize the process to ensure its compatibility and universality both in the NATO structure and in collaboration with other partners.
- Structure – elements (bodies) that are responsible for individual subprocesses and which guarantee an efficient and smooth course.
- Tools – technology, selection and use of appropriate support tools and their optimization to support the Lessons Learned process and its subprocesses.
- Training and education – the main sponsor activities needed to understand the importance of this process.

Figure 1: Components of the Lessons Learned System (NATO, 2016, p. 9)

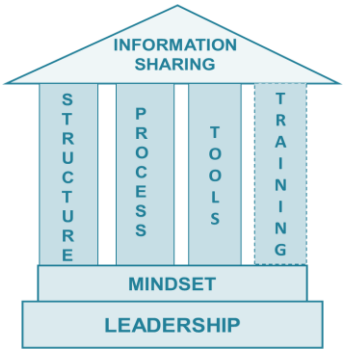

The ceiling of this process is the very important sharing of information that represents the culmination of the entire Lessons Learned process. However, the most important are the cornerstones of the whole process, without which this process could not work effectively. The base of the Lessons Learned process is made up of commander's support – leadership, with senior military management knowing and understanding the benefits of the process. Then it is the commitment and thought of all involved. It is therefore necessary to achieve a state where all the participants will understand the importance of this process and, in addition, the support of the commander will be clear to all [8].

The goal of the lessons learned proces

The general objective of the Lessons Learned process is to preserve the organization's knowledge, is to transform the individual's experience into the knowledge of the organization so that other members of the organization can use their experience in the absence of the expert [5].

Therefore, using lessons learned can positively influence other processes of the organization on a continuous cycle of improvement.

Scheme of lessons learned process

The NATO Bi-Strategic Command Directive 080-006 describes the individual parts and sequence of the Lessons Learned process, which is then illustrated in Figure 2.

Observation

This is an identified problem that requires action to be taken to improve the status or best practice for reuse. These are precisely described facts, events, claims, statements that are based on the evaluation of information that describes a certain activity of units, immediate causes, effects or effects.

Knowledge can be Raw Observation, which requires further study or analysis to understand the cause of the event and to supplement the information, or the Mature Observation, to which there is sufficient information, or the cause of the event is known and is not needed its further study and analysis [5, 6, 7].

Analysis

It is a study of the whole through careful examination of its individual parts and their interrelationships [5, 6, 7].

For the purposes of the Lessons Learned process, it is about describing a given phenomenon, its causes and consequences, including the initial design of measures to prevent repetition of inappropriate practices or to make repetition of the appropriate ones.

Lesson identified

Identified experience because of knowledge analysis. In addition to describing the event, the causes and the consequences, it includes a proposal for remedial measures, concrete steps and responsibilities for implementing these measures [5, 6, 7].

Lesson learned

It is the result of the implementation of measures that have increased the ability of a particular element [5, 6, 7].

Knowledge

Newly generated information, whether in the form of knowledge, identified or generalized experience has an individual value for the user corresponding to his/her knowledge (5). Interpreting such information in interaction with experience, facts, individual value, thought processes, and meanings can be understood as knowledge.

Share

One of the most important part of the Lessons Learned process, which is intertwined with the process, is sharing. If NATO really wants to work based on a learning organization, it is necessary for individual Member States to share knowledge with others.

Database of knowledge and experience

In addition, to centrally store, manage, share, and access the knowledge, national and international databases have been created to enable registered users to easily access the information they need.

IMPROVING THE QUALITY OF MEDICAL SERVICES ACCORDING TO THE NATO MEDICAL LESSONS LEARNED PROCESS

First, quality in healthcare can be elusive and very complex in the military medical paradigm. The World Health Organization (WHO) defines the quality of health care as the extent to which health services provided to individuals and patient populations improve health outcomes [9]. For this purpose, healthcare must meet these key properties for all. It must be safe, efficient, timely, effective, and fair and focused on people – all activities must be driven by data and evidence based clinical practice. Health care quality can also be explained as a continuous improvement in the quality of health services provided, the ultimate goal of which is to improve health, improve the quality of life and satisfaction of the people they care for [10].

Thus, the Health Care Quality System includes the entire process of creating procedures, collecting information, setting clinical standards and assessing health care, health services and most importantly healthcare outcomes. It is also worth noting that a quality system is an intrinsically interconnected concept of quality elements that should help health organizations meet the goals of their mission [11]. In general terms, this applies to both civilian and military healthcare that the objective of a particular quality system in healthcare is always to improve the quality of health and related care provided in accordance with individual needs and patient requirements and is rooted in evidence and best practice. In military medical operations, duty of care for warfighters and deployed personnel is the burden of the countries involved and the alliance; this is extended to the combatants and civilian population as outlined in the UN and Geneva Conventions and international norms. This includes the provision of healthcare to all at a standard of care as close to and in keeping with clinical standards provided at peacetime found in the participating countries [12]. This is an extremely difficult task for the provision of medicine for deployed personnel in the dynamic environment that is war, conflict and peacekeeping in remote, austere and resource challenged environment.

Figure 2: Scheme of Lessons Learned
(NATO, 2016, p. 11)

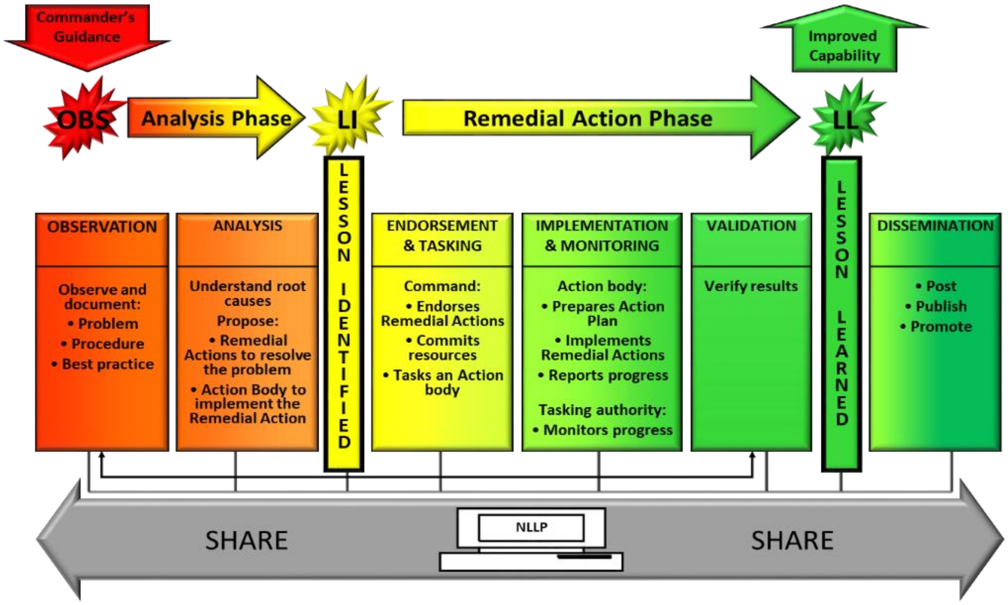
Commander's Guidance
Improved Capability
OBS
Analysis Phase
LI
Remedial Action Phase
LL
LESSON IDENTIFIED
LESSON LEARNED
OBSERVATION
Observe and document:
• Problem
• Procedure
• Best practice
ANALYSIS
Understand root causes
Propose:
• Remedial Actions to resolve the problem
• Action Body to implement the Remedial Action
ENDORSEMENT & TASKING
Command:
• Endorses Remedial Actions
• Commits resources
• Tasks an Action body
IMPLEMENTATION & MONITORING
Action body:
• Prepares Action Plan
• Implements Remedial Actions
• Reports progress
Tasking authority:
• Monitors progress
VALIDATION
Verify results
DISSEMINATION
• Post
• Publish
• Promote
SHARE
NLLP
SHARE

APPLICATION OF LESSONS LEARNED PROCESS IN NATO MILITARY MEDICAL SERVICES

In October 2014, the NATO Center of Excellence for Military Medicine (MILMED COE) hosted the International Security Assistance Force (ISAF), the NATO-led security mission in Afghanistan, Medical Lessons Learned Workshop. This was performed in order to preserve the knowledge gained in ISAF and discuss health care expertise in ISAF in Afghanistan. Examples of the use of appropriate combat application tourniquets during severe limb bleeding have clearly shown that collecting knowledge and sharing Best Practices saves lives and improves the clinical outcomes of those injured on the battlefield. Additionally, on the basis of Lessons Learned from Afghanistan, there was a demand for safe removal by a light armored medical vehicle for evacuation purposes. The MEDEVAC (Medical Evacuation) system was also created based loosely on this process, which can skip individual stages of health care and from point of injury for the warfighter or enemy combatant to ensure they are appropriately evacuated as soon as possible directly to a Medical Treatment Facility (MTF) at a ROLE 2E or ROLE 3 facility.

A number of shortcomings have also been identified in the quality system of medical support; these are of special interest when making the entire system more robust, more resilient and capable of fighting the next battle with potentially different weapon systems and challenges than the current.

The results were presented to the Committee of Military Health Service Chiefs (COMEDS) at their 42nd session. At this meeting, it was decided that NATO MILMED COE would become the main contact and coordination center for the Lessons Learned process within the NATO military health service. Lessons Learned Process for NATO Health Support Centers is illustrated in Figure 3 [13]. In effect, the MATO MILMED COE LL has become the clearinghouse and central crossroads for all thing's best practices in NATO Military Medicine across the alliance.

By positioning itself as the coordinator and collector of observations and lessons, the Lessons Learned Branch of the MILMED COE adds value to the NATO system, processing and sharing Lessons and best practices for all members of the Coalition across the alliance. The LL Branch are building a roadmap to the future of the delivery of Health Support

Services through best practices and rooted in clinical evidence. The members of the alliance have delivered amazing health care, and we need to collect and share the Best Practices across the organization to ensure that everything that has been learned can continue to save lives, financial resources and sparse clinical resources [14].

Currently, the NATO MILMED COE Lessons Learned Branch is developing an inclusive process to improve the collection, processing, analysis, storage and sharing of military health-related knowledge. All the information presented and analyzed is uploaded and stored in the Library of Medical Lessons Learned MILMED COE Information and Knowledge Management (IKM), which is located on the MILMED COE website (http://www.coemed.org/medlldb). An important achievement from the third Medical Lessons Learned Workshop, which took place in 2015, was the establishment of the Multinational Medical Lessons Learned Team (MMLLT), where the Czech Military Medical Service is represented and hosted the second meeting as well. Participating countries agreed to share national information and documents related to military health through the MILMED COE IKM portal. The MILMED COE KM Portal is a good starting point for sharing across the alliance. Each contributing nation should decide on a national level what kind of document they would share. COMEDS has requested to rename the MMLLT, because this name is too similar and thus be confused with Medical Lessons Learned Core Team. With these changes, the new name of the body is Medical Sharing Group.

Figure 3: Lessons Learned Process for NATO Health Support Centres (MILMED COE, 2015)

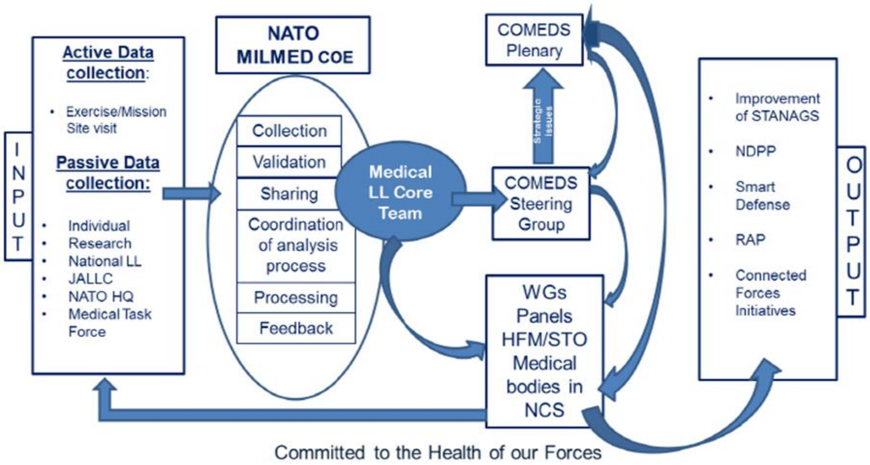
INPUT
Active Data collection:
Exercise/Mission Site visit
Passive Data collection:
Individual
Research
National LL
JALLC
NATO HQ
Medical Task Force
NATO MILMED COE
Collection
Validation
Sharing
Coordination of analysis process
Processing
Feedback
Medical LL Core Team
COMEDS Plenary
Strategic issues
COMEDS Steering Group
WGs Panels HFM/STO Medical bodies in NCS
Improvement of STANAGS
NDPP
Smart Defense
RAP
Connected Forces Initiatives
OUTPUT
Committed to the Health of our Forces

Moreover, the aim of the Medical Sharing Group is to establish the capability to share Lessons Learned information and knowledge across the Alliance [2]. Specifically, the Group members are responsible for reviewing Observations, Lessons Identified, and Lessons Learned information and knowledge within their national or organizational system and for forwarding any item to the NATO Medical Lessons Learned process that is considered beneficial to other Alliance Nations or to the Alliance as a whole. The intent is to empower the exchange of locally identified solutions or Best Practices with the goal of optimizing performance in current and future activities. The type and level of items to be shared will be established by the providing nation or organization and does not need to be the same for every participant. Additionally, the type and level of input provided by a participating entity does not have to remain the same over time and no formal process for change is required, enabling faster sharing of vital information [4]. The Medical Lessons Learned Process, an ongoing effort to improve process, is shown below in Figure 4.

Medical lessons learned conference on civilian – military interaction

A conference on civilian military cooperation has also been held several times. The main aim was to invite civilian and military organizations recently involved in Crisis Management, Humanitarian Assistance and relevant NATO, EU operations and share experiences, best practices and lessons learned. The last event was attended by 36 delegates from 12 nations and 13 organizations. A growing number of Crisis Management and Humanitarian Assistance missions/ events have been resulting in increasing demand for well-organized Civil-Military Interaction. Sharing individual perspectives on the situation and root causes of a crisis is the first step towards de-conflicting actions and complementing each other’s efforts, in effect to remove a duplication of effort. For the successful Civil-Military Interaction sharing of information is key importance to develop a common understanding about the situation and necessary measures.

Figure 4: Medical Lessons Learned Process
(MILMED COE, 2018)

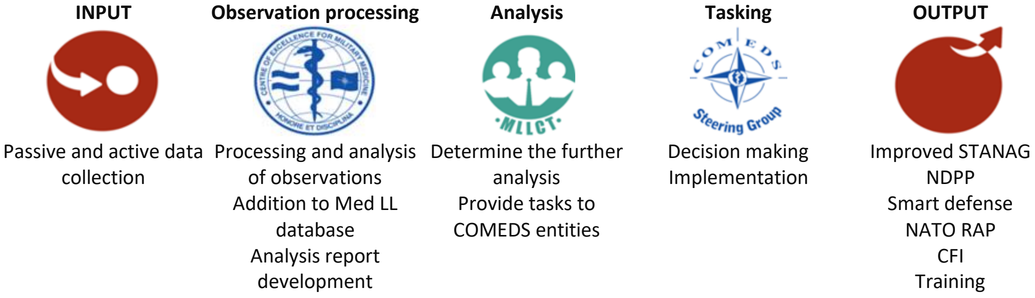
INPUT
Observation processing
Analysis
Tasking
OUTPUT
Passive and active data collection
Processing and analysis of observations
Addition to Med LL database
Analysis report development
Determine the further analysis
Provide tasks to COMEDS entities
Decision making
Implementation
Improved STANAG
NDPP
Smart defense
NATO RAP
CFI
Training

Knowledge and tools of information collection

During the multinational medical exercise Vigorous Warrior 2017 a total of 391 pieces of observations were collected and analyzed. Five focus topics for further analysis were identified: Exercise Development, Interoperability, Patient Evacuation Coordination Cell, Bio-responsiveness and Experimentation [15]. Furthermore, a new tool for collecting and retrieving observations via smartphones was also introduced – the Observation, Discussion, Conclusion, Recommendation (ODCR) application, which was one of the tools for gaining knowledge (also those responsible for collecting knowledge - Office of Primary Responsibility (OPR) LL and paper and electronic forms). This application is fully functional in ANDROID and Apple´s iOS system (free download from Google play and AppStore). The previously mentioned Medical Knowledge Management Portal is a very important tool for preserving and sharing of observations and lessons. Today, there are over 1234 lessons from various areas of military and civilian health care (Exercises, Operations, UN, Ebola and others). Currently, the observations from the latest large-scale international medical exercise, Vigorous Warrior 2019, where 38 NATO and partner countries participated, are being analyzed within these new frameworks. The total number of primary findings is 464, of course, not all of the observations will be uploaded to the KM Portal avoiding repetition, and analysis will follow a standard approach.

DISCUSSION AND CONCLUSION

Evidenced-based medicine drives best practices. Knowledge sharing is an important prerequisite for the functioning and improvement of quality at all levels of healthcare within the deployment of medical units and medical teams across NATO operations. The quality of planning, which often takes place under time constraints and in a hostile environment, is improving and maximizes efforts across the NATO Alliance. The Lessons Learned (LL) process in clinical medicine for deployed and military support services drive the NATO Military Medical Center of Excellence (MILMED COE). This paper describes the Lessons Learned process and its use in NATO and partner countries medical services. Organizational learning drives development and progress and capturing lessons learned improves the quality of care in military medicine on a preventive, epidemiological, trauma, casualty evacuation and forward surgical care basis. The Lessons Learned process is an undertaking and an instrument that can also be used to achieve better international and civil-military cooperation. Lessons Learned process is one of the key elements of developing medical services in line with the changing operating environment.

Acknowledgment
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Conflict of interests
Authors state no conflict of interests.

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REVIEW ARTICLE

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Evolution of mortality rates in ST elevation myocardial infarction

Alice E. Munteanu[1,2], Cristina M. Calcan[1], Irina Florescu[2]

Abstract: *The purpose of this paper is to provide information about mortality rate in patients undergoing primary angioplasty for STEMI. Cardiovascular diseases lead to one third of the deaths worldwide, surpassing the mortality rate produced by neoplasia, acute respiratory failure and diabetes mellitus all together. In the world, approximately 17 million die annually because of cardiovascular disease and every 5 seconds one is suffering from a myocardial infarction.*

In 2010, in our country was implemented a national program (RO-STEMI) to offer rapid interventional treatment for patients with STEMI, aligned to ESC Guidelines. At the beginning only small parts of the patients were transferred to the hospital in charge for STEMI, so, we chose to study patients from the second year of the program and from last year. We made a retrospective study, including patients with STEMI that reached our hospital for primary angioplasty in the first 12h after the debut of the symptoms, aiming to make a parallel between the in-hospital mortality rates at maximum one month after the primary PCI. In order to be included, the patients must present ST elevation and another 1 out of 3 criteria (clinical, echocardiographic and biological changes suggestive for myocardial infarction).

The main target was to evaluate the evolution of early mortality rates post primary PCI after the changes in the ESC Guidelines and the progresses in the medical field. A secondary target was to correlate mortality rates with the presence of comorbidities or risk factors.

INTRODUCTION

Alexander Muirhead is reported to have attached wires to a feverish patient's wrist to obtain a record of the patient's heartbeat in 1872 at St Bartholomew's Hospital.[1]

Another early pioneer was Augustus Waller, of St Mary's Hospital in London.[2] His electrocardiograph machine consisted of a Lippmann capillary electrometer fixed to a projector. The trace from the heartbeat was projected onto a photographic plate that was itself fixed to a toy train. This allowed a heartbeat to be recorded in real time.

An initial breakthrough came when Willem Einthoven, working in Leiden, the Netherlands, used the string galvanometer (the first practical electrocardiograph) he invented in 1901.[2]

This device was much more sensitive than both the capillary electrometer Waller used and the string Galvanometer that had been invented separately in 1897 by the French engineer Clément Ader.[3]

Einthoven had previously, in

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1895, assigned the letters P, Q, R, S, and T to the deflections in the theoretical waveform he created using equations which corrected the actual waveform obtained by the capillary electrometer to compensate for the imprecision of that instrument. Einthoven also described the electrocardiographic features of a number of cardiovascular disorders. In 1924, he was awarded the Nobel Prize in Medicine for his discovery.[3] In 1937, Taro Takemi invented the first portable electrocardiograph machine.[4]

Cardiovascular diseases represent the main cause of death worldwide, 30% of them being caused by coronary artery disease. Myocardial infarction (MI) is one of the five main manifestations of coronary heart disease, namely stable angina pectoris, unstable angina pectoris, MI, heart failure and sudden death.[5]

Myocardial Infarction (MI) is defined by myocardial cell necrosis due to significant and sustained ischemia. Most frequently it is an acute manifestation of atherosclerosis-related coronary heart disease. MI results from either coronary heart disease, which implies obstruction to blood flow due to plaques in the coronary arteries or, much less frequently, to other obstructing mechanisms (e.g. embolism, spasm of plaque-free arteries).[6]

The clinical presentation of MI varies from a minor coronary event to life-threatening clinical situations or sudden death.[2] The criteria for STEMI are well known – specific, prolonged >15-20 min pain, specific site, and it is not ameliorated by nitroglycerin intake. The ECG shows persistent ST elevation or new onset of LBBB. Specific biomarkers are represented by high-sensitive Troponin I, CK/CK-MB, which have a specific dynamic curve.[7]

In 2010 in our country debutantes a national program named ”RO-STEMI”. In this program all patients with STEMI, from a well-defined area, are send to a certain hospital for being coronarographic evaluated. Due to this program more than 63% of patients’ beneficiated of myocardial reperfusion and the mortality rate dropped all over the country from 13 to 7%.[8]

MATERIALS AND METHODS

It’s been realized a retrospective study in STEMI patients in our hospital, on all patients from 2012 and 2016. All the patients included in this study respected STEMI criteria for diagnosis. We took 252 patients from 2012, out of which 29% were women and 71% were men, and 136 from 2016, with a non-significant difference in gender balance (28% women and 72% men). The number of patients is smaller in 2016 than in 2012 because in the beginnings of the national program there were only a small number of hospitals being part of it. In present the number of STEMI hospitals has increased so STEMI patients are distribute to more STEMI centers than during the past years.

RESULTS

During this period of time, the median age of suffering an infarction was 60 years for men, with no significant difference between years, but for women the age evolved, so now, usually women suffer a myocardial infarction at about 63, compared with 66 years old in 2012.

The onset can be seen as a predictor for evolution and a gravity factor for STEMY patients.

A cardiorespiratory stop onset represents a high gravity risk factor. We noticed that in 2016 were twice more patients, than 2012, with cardiorespiratory stop on onset of STEMI. Knowing that, it is expected that the mortality rate being higher in 2016 that 2012.

Figure 1 Distribution of patients by cardiorespiratory stop onset.

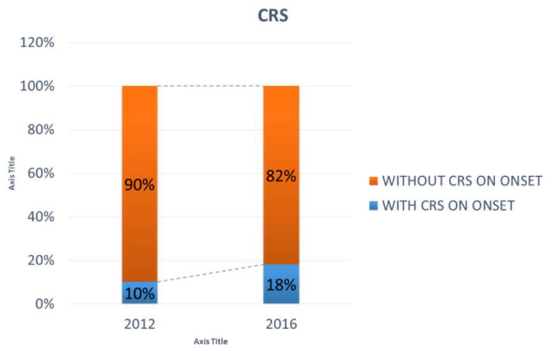

The distribution of modifiable risk factors is the same for the 2 groups, the change consists of the number of risk factors present at the same patient, which was definitely higher last year (in average 2 risk factors per patient in 2012 vs. average 3.5 risk factors/patient in 2016). Even if we strongly advise the patients to quit smoking, and there is also a huge implication of the government in this, the number of smokers increased, but the good news is that their mortality has a decreasing trend over the years.

The median time from pain to cath-lab has lowered from 12h in 2012 to 9h in 2016. This improvement could be in one side due to well function of RO-STEMI program, and in other side due to improvement in social health education, the patients being better informed about myocardial infarction symptom-tology which decrease pain-call time.

If we take a look to the coronary lesions, we can see that first affected artery is left anterior descending artery (LAD), followed by right coronary artery (RCA), circumflex artery and marginal artery.

A very important observation is the growth of the number of patients with right coronary artery involvement, with can be associated with right ventricle infractions and an extremely poor prognosis. Also right coronary artery involvement means a higher risk of malignant arrhythmias and sudden cardiac death. 45% of STEMI patients had only one affected vessel, 40% had 2 affected vessels and only 15% had three coronary arteries affected most of them with CABG indication.

Only 15% of the patients in 2012 were thrombolysed before PCI, while in 2016 the use of t-lyse increase to more than 30% of the patients, this measure helps physicians to buy time for them to reach the cath lab.

Figure 2 Rate of deceased after thrombolysis

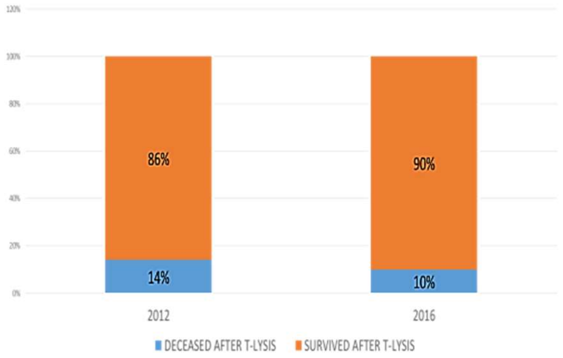

From the number of those thrombolised, only a small amount has died, but none because of a bleeding. All died because of malignant arrhythmias or asystolia secondary to the myocardial infarction. Notice that in 2016, even though the number of thrombolised patients was bigger than in 2012, the rate of deceased patients after thrombolysis was smaller in 2016 than in 2012. The reason for this difference could be a better triage of elected patients and the evolution of thrombolytic agents.

At the beginning, drug eluded stents were extremely rare and expensive, nowadays we use much more frequently DES then bare metal stents, in 2016 73% of used stents were DES and the target is using only DES, as the indication in ESC guideline.[7] This made the rate of early in stent restenosis to go from almost 20% to 0%.

Figure 3 Mortality rate in the two groups of patients

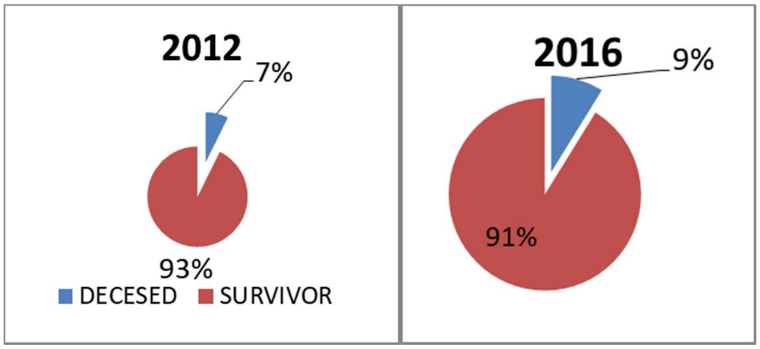

Due to the shortening of the time from symptoms-onset to revascularization and from associating more frequently thrombolysis with PCI, the number of those with ventricular dysfunction has dropped significantly.

Even though the number of thrombolysis raised, and the time to cath lab is shorter, the number of patients who didn`t make it to the cath lab is increasing, the mortality rate has increased from 2012 to 2016. If we consider that the rate of cardiorespiratory stop as onset as a high gravity factor and if we see that the patients in 2016 were more complex than in 2012, then the high mortality rate is expected. Another factor suggests that the gravity of cases was higher in 2016 is that the number of deceased patients without reperfusion was higher in 2016.

CONCLUSIONS

The National Program RO-STEMI became more effective by increasing the number of involved hospitals.

During time the mortality rate have had a slightly increasing trend due to a growth of the number of risk factors/patient, an increase in the severity of the coronary lesions, an increase in the number of the lesions in the right coronary artery, and a poorer prognosis in echocardiography.

The time from symptoms onset to cath lab decreased and by associating more frequently thrombolysis for “buying” more time, so the chances for the ventricle to remodel remain low.

By respecting the ESC guidelines for each period of time, even though the early mortality rate seems to have an ascending trend, the overall mortality rate and longtime prognosis of the STEMI patients are getting better.

It is very important to make such an analysis from time to time, in order to make thinks go better and see where some changes could be made.

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ORIGINAL ARTICLE

Exploring of clinical decision making in care of patients with acute care in critical nurses: A qualitative content analysis

Mohammad Aghajani[1], Neda M. Ajorpaz[2], Mohsen Taghadosi[3]

Abstract: *The appropriate clinical decision-making of the nurses can improve the patient outcome and reduce costs, otherwise delaying the recovery and preferably the death of the patient especially in acute care setting. The aim of this study was to describe the clinical decision making in care of patients with acute care in critical nurses.*

This study was a qualitative study conducted in 2020. Purposive sampling was used and continued until saturation. The study involved sixteen semi-structured interviews were conducted with critical nurses who had experience providing care for acute patients in critical care units. Interviews transcript and analyzed by qualitative content analysis. Two major themes were generated from the data included instantaneous decision-making and life-saving of the patient. This process was depended on the nurse's ability to summarize in the moment, to consider everything, to focus on the patient, to combine experience and knowledge, to concern the patient's life, to prevent the deterioration of the patient's conditions and responsibility to their patients.

Keywords: *clinical decision making, acute care, critical nurses, COVID-19*

INTRODUCTION

Clinical decision making (CDM) is a fundamental phenomenon in healthcare practice. While CDM influences the entire range of healthcare practitioners, nurses, as frontline clinicians, are faced with important clinical decisions on a daily basis [1, 2].

The appropriate decision-making of the nurse can improve the patient and reduce costs, otherwise delaying the recovery and preferably the death of the patient [3]. Based on studies, it is identified that up to 98,000 patients die each year as a result of poor decision-making in healthcare [4]. Also research shows that not making the right decision in a timely manner can prolong the treatment process and the situation of patients [5]. 4% of patients admitted to hospitals in the United States, about 16% and 6% in Australia and the United Kingdom have suffered injuries such as permanent disability and death due to lack of proper decision-making. While the right and timely decision making, team working and effective communication between staff can reduce half of these injuries [6].

Rapid and appropriate decision-making is a need of organizations and hospitals. However, complex issues surround the decision-making process, such as the past experiences, values, beliefs, skills and technical knowledge of the decision maker [7].

CDM in nursing is frequently based on two styles of thinking: analytical and intuitive thinking. Analytical thinking is a logical style of thinking based on objective data. Intuitive thinking is driven by intuition and experience [8]. In life-threatening conditions and crises, as well as situations wherein there is a need for creativity and innovation, nurses use intuition in their decision making. Therefore, in critical health conditions, CDM is essential for nurses to improve quality of care, manage crises, and diagnose patients'

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conditions, as well as to help reduce mortality rates [9].

Currently, nurses around the world are dealing with COVID-19, a disease that continuously demonstrates a variety of unknown and newly-known features. The global COVID-19 mortality rate is between 3% and 15% [10]. In this condition, due to lack of knowledge and clinical guidelines, nurses CDM have a vital role in managing the outcome of the disease and the health of clients. Decision-making in critical care nursing is a complex process [11]. Nurses must consider numerous, potentially competing factors when making decisions to meet patient and family needs [2]. This process is further complicated by the fact that nurses may care for five or more patients in a critical care unit [2]. The nurse's decision making in critical care is highly demanding, while critical nurses make a clinical decision almost every 30 seconds [12].

Therefore the question is how do nurses make decisions in acute situations. A better understanding of decision-making research in this context can help to guide future efforts to support nursing practice. Therefore, this study explores the experience of clinical decision making in care of patients with acute care in critical nurses.

METHOD

The present study was a qualitative descriptive study which was conducted to explore the clinical decision making in care of patients with acute care by critical nurses in 2020. Content analysis is a research approach for making inferences from data to their context, with the purpose of providing new insights, knowledge and the representation of the facts [13]. Qualitative content analysis as a research method provides an instrument for making inferences from data to their context [13], and in this process, the textual data explored and interpreted using the systematic coding process [14]

Participants and sampling

The purposive sampling was conducted among nurses who were providing care for patients in critical care units including the intensive care unit (ICU) and Emergency Departments (ED) of subspecialty Hospital of Kashan University of Medical Sciences. This comprehensive hospital allocated to care of COVID-19 patients. The inclusion criteria included the working experience in critical care units at least for two years and willingness to participate in the research. The exclusion criterion was reluctant to continue the study. The first participant was a nurse with independent and fast decision maker in the research setting, which had recommended by three nursing managers and was interested to speak about his experience.

Sampling continued until data saturation was done. Data saturation was identified through repetition of the participants' data in the interviews and that no new content is obtained [15]. In our study saturation occurred after interviewing with 16 participants.

Data collection

Prior to initiating interviews, main researcher was attempted to obtain the collaboration of the participants by creating a trust-based environment, explaining the aim of the study and ensuring the confidentiality of information. The data was collected with semi-structured interviews at the time and place that was appropriate for the participants. The process of interviewing initiated with open-ended questions based on the aim of the study. All interviews were recorded (Table 1).

Table 1: Interview techniques for data collection

Researcher activity
Introducing myself and the purpose of the study
Explaining the content of the consent form and answering the questions
Obtaining a verbal agreement and signing the consent form
Starting the voice recorder
Starting the interview with general open-ended questions such as: How do you make a clinical decision making in a critical situation? Would you please tell me about how do you of intuitive decision making in the care of COVID-19 patients? Would you please tell me about your experiences of clinical decision making for a COVID-19 patient without documentation? What conditions have you encountered in bedside that has caused you to use intuition? Please explain.
Asking for example: Please give me an example of your clinical decision making. Using active listening techniques: Eye contact, silence, head nods, use of statements such as "OK I see…". Watching for non-verbal cues: eye contact, facial expressions, tone of voice, gestures …
Using probing questions: Please tell me more about that. What else can you remember about that situation? Can you think of another example of this? What Exactly did you say? I'd be inspired.
Get more details: Tell me more about that. Can you tell me more about that?
Clarifying what the participants said: Please say what you mean by…. When you say … what are you actually doing or feeling? So you are saying...?
Summarizing the interview: It sounds like you are saying, "...". Is that a fair summary?

The participants were asked to discuss what they experience and understand as nursing CDM in moment in critical unit. The interview guide included the following questions:

- How to make clinical decisions in critical situations or acute care? Please explain your experience in this field.
- Why/how did you make such a clinical decision making?
- what are you actually doing or feeling?
- Tell me more about that. Can you tell me more about that?

Interviews were held in a private room, so that the privacy of the people was respected. The room was disinfected before and after each interview. The interviewer and the participant wore masks and gloves, and at least one meter separated the interviewer and the participant to observe social distancing.

The interview took between 40 to 80 minutes depending on the participants' circumstances. The interviews were conducted in Persian and analyzed and the results were translated into English only in the final report. The conversations were recorded on the audio file and then transcribed immediately.

Data Analysis

The transcribed interviews were analyzed through qualitative content analysis method following an inferential approach. The process of organizing the qualitative data in inferential content analysis included open coding, abstraction, and creating categories [16]. Elo and Kyngas pictured inferential categorization plan in qualitative content analysis [13]. Data analysis and data gathering were conducted simultaneously. Coding and classifying themes were done by the researchers

Trustworthiness

To ensure the trustworthiness of this qualitative research, confirmability, credibility, dependability, and transferability were established [17]. We used rewriting, member check, reviewing texts and using the participants' own words, monitoring translations, recording audio, the conducting of interview, coding, themes and sub themes and using of direct quotes to support the findings.

The researcher used an adequate amount of time to collect comprehensive data. The researcher informed them about the objectives, and established trust with participants throughout the interview and providing feedback to the research team for further interviews. We employed the MAXQDA 10 software for data management.

Ethical considerations

The study was approved by Ethics Committee.

The participants were informed about the study's objectives and consent form was obtained from all participants.

RESULTS

The participants were 16 critical nurses. Ten participants were women. Their nursing experience ranged from 4 to 25 years, and their academic degrees consisted of bachelors, masters, and Ph.D. (Table 2).

Table 2: Baseline characteristics of participants (n=16)

Characteristics	N (%) or mean ± SD	Median (range)
Gender		
Male	6 (37.5)	
Female	10(62.5)	
Age (year)	36.56±6.58	37.5 (26-52)
Working Experience	12.62±5.59	13.5(4-25)
Interview duration (min)	52.31±1.09	49.5 (40-80)
Education		
BSC	12 (75)	
MSC	3 (18.8)	
PhD candidate	1 (6.2)	
Marriage		
Married	11 (68.8)	
Single	5 (31.2)	
Current ward		
ICU	9 (56.2)	
CCU	6 (37.5)	
Nursing room	1 (6.2)	
Duty		
General nurse	14 (87.5)	
Head nurse	1 (6.2)	
Clinical supervisor	1 (6.2)	
Shift type		
Fixed	2 (12.5)	
Rotation	14 (87.5)	
Type of employment		
Official	8 (50)	
Contractual	8 (50)	

Based on data analysis, 362 primary codes, 7 subcategories, and two categories emerged (Table 3).

Table 3: The themes and subthemes of nurses caring for acute patients.

Themes	Subthemes
Instantaneous decision-making	Summarizing in the moment Considering everything Focusing on the patient combining experience and knowledge
Life-saving of the patient	The importance of the patient's life Deterioration of the patient's conditions Responsibility

From the point of view the participants and based on their experiences, the CDM of critical nurses had two major characteristics:

1. instantaneous decision-making (summarizing in the moment, considering everything, focusing on the patient, combining experience and knowledge) and

2. life-saving of the patient (the importance of the patient's life, deterioration of the patient's conditions, responsibility).

Instantaneous decision-making

Almost all nurses stated that they made instantaneous decisions in the face of critical situations, especially those where the patient's life was in danger. This DSM seems to be intuitive because it does not have the linear and logical features of evidence-based decision making.

A participant (P3) stated, “In a night shift, there was a 30-year-old patient in respiratory distress. I had an inner sense indicating that the patient’s health conditions would quickly deteriorate. I begged the physician, explaining that this was an emergency... there’s going to be a need for endotracheal intubation... we must intubate the patient right now. The physician said there was no need for intubation at the moment, and we needed to monitor the patient to see if the patient’s conditions would change. After a short while, the respiratory distress worsened very quickly, and the patient passed away. Unfortunately, we did not have the chance to intubate the patient and start mechanical ventilation on time. Later, the physician told me I was right...we should have intubated the patient sooner.” Indeed we see the perception of the inner sense, followed by anticipation and pursuit, and the inner revolution in the intuitive decision making.

Summarizing in the moment. Ten participants indicated that in critical situations in a short period of time, everything quickly summarized in their minds. In fact, at the critical moment and before the action, they summarized and concluded what they were receiving from the patient's signs and symptoms at the moment.

One participant (P15) said “….Everything is summed up in your mind until you decide what to do at that moment….”

Considering everything. Nine participants indicated that they unconsciously considered all conditions, possibilities, prognosis, and possible consequences for the patient.

The participant (P11) said: “A patient with a chest pain was brought to the emergency department by 115, I unconsciously compared and adapted all the patient conditions in my mind and I guessed the possibility of a heart attack that was happened.” Another participant pointed: “I consider the patient's history and prognosis, for example, that she had a history of heart disease based on my experiences.”

Focus on the patient. About half of the participants reported that when caring for acute patients, their minds were constantly engaged with the patient during resting and working time, and they focused on patient.

One participant (P4) reported “….even I thought about my patient while resting in the department”. Another participant believed that “I kept telling myself if something bad is going to happen what I should do, hence, I went to the patient's bed and by the way something happens to her at that moment”. Another participant indicated “…. concerned about my patient until morning”.

Combining experience and knowledge. Fourteen of the 16 participants reported combining their previous knowledge and experience in acute patient conditions. A participant stated, “In MI patients, I have seen that their skin color changes to whitish and blackish colors. Many COVID-19 patients also show these changes before a heart attack. In fact, I was reminded of previous experiences and the things that had read previously in books and combined them.” (P1).

Life-saving of the patient

Almost all the participants expressed reason for the CDM in patients with acute care were to save the patient's life. Participants indicated that the life-saving of the patient was a priority for them. The nurses believed that even if decision making was not in accordance with the usual and legal process of unit, but they did it if it was necessary to save the patient's life.

Some participants compared the COVID-19 condition to a battlefield. “I feel like I’m in a battlefield. When you have seven cases of death in just a few hours, you believe whatever you do, even if not necessary, might save your patient’s life.” (P5).

The importance of the patient's life. Participants considered life-saving of the patient to be the most important blessing from God and lack of appropriate and immediate clinical decision-making in critical situations was equated with patient loss.

“For me, the patient’s life is the priority. If I feel confident something is critical for the patient’s life and health, I will definitely do it”. (P8). Another participant indicated “…receiving Pack cell are very important for the patient and may save patient life”.

Deterioration of the patient's conditions. The participants reported that due to the importance of golden time in patients in critical situations, decision making is very important and lack of timely decision making will deteriorate the patient's conditions.

“I experienced in ICU that you put your hand on the patient and you see that it is cold, that is, lack of heart pulse needs 3 to 4 hours of care. So if we do not do something worse, we have to make a timely decision”. (P7)

Another participant considered the heavy workload and the related tensions as factors for relying to their clinical decisions. “Everything was novel. The whole staffs were feeling the same and were stressed. You felt extremely concerned about worsening your patients’ conditions. In these conditions, you feel like you have to trust your decisions.” (P5)

Responsibility

Almost all participants believed that they were responsible for their conscience, professional responsibility, patients, religious beliefs and society.

. “A sense of responsibility did not leave me alone. Based on your conscience, you feel responsible to do your best for your patient” (P15).

“To some extent, it is a kind of feeling of responsibility... About the COVID-19 crisis, we feel we are responsible for patients in all aspects.” (P3). Another participant (P7) believed that patients benefit from nurses’ decision making. “I felt like it was better for the patient to get discharged and go home. A hospital environment in this situation is not safe for the patient.”

DISCUSSION

Clinical decision making is a main role of professional nurses [18]. This study was conducted to clarify the clinical decision making in care of patients with acute care in critical nurses and two themes were categorized as the main characteristics for a critical nurse including "instantaneous decision-making" and "life-saving of the patient".

On the first category that emerged in the results, in the acute conditions of the patient, in which time plays an essential role in saving the patient, the CDM of the nurses and subsequent treatment-care actions are very important. CDM distinguishes the professional nurse from the non-professional care staff [18]. Critical nurses decided by focus on patients and considering everything related to their patient, then they summarized data in their mind and cared of the patients with integrating experience and knowledge. The research show that each nurse uses to make a decision depends more on the type of task and the field conditions than on her knowledge and experience [19-21]. In the other hand, effective decision-making requires a combination of knowledge and skills and close communication with the patient for a deep understanding of the situation, which has been interpreted by some authors as "seeing the holistic view" or "deep understanding of the clinical view" [22-24].

Two categories show that the most important item for deciding in a patient's acute conditions is life saving of patient. The participants in this research indicated that they make decision because of the importance of the patient's life and responsibility towards their conscience, profession, society and in order to prevent from worsening the patient's conditions. Other studies in intensive care unit nurses confirm these finding [5,11,25,26].

Overall, based on the findings and features extracted, CDM in acute care settings seems to be more intuitive than analytical. Today, in health novel and complex conditions, this type of decision-making has become even more important because of the care of patients with COVID-19. Recently, the use of intuition in the process of caring for the psychological aspect and its effects on decision-making has become a favorite subject for health researchers [11,22,27].

CONCLUSION

The findings of the study described how and why critical nurses in acute care made clinical decisions. Indeed critical nurses by instantaneous decision-making save life of the patient and prevent the deterioration of the patient's conditions. Nurses tried to provide the best outcomes of care and treatment through analytical and especially intuitive model in acute situations. This CDM was dependent on the nurse’s ability to summarize in the moment, to consider everything, to focus on the patient, to combine experience and knowledge, to concern the patient's life, to prevent the deterioration of the patient's conditions and responsibility to their patients. These findings can provide the basis to promote competency and practical guidelines for clinical nurses and health managers.

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Ethical Issues
This study research was approved from the Ethics Committee of Kashan University of Medical Science with the ethics code: IR.KAUMS.MEDNT.REC.1398.068.

Conflict of Interest
The authors declare no potential conflicts of interest with respect to the

research, authorship, and publication of this article.

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REVIEW ARTICLE

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Extra-pulmonary tuberculosis in immunocompetent patients: Case series and mini review of the literature

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Abstract: *Introduction: Tuberculosis (TB) is still one of the most frequent infectious diseases, causing 2 million deaths a year worldwide. The prevalence of TB has increased in both immunocompetent and immunocompromised and virtually, it can affect any organ. Up to 25% of tuberculosis cases present extra-pulmonary involvement. The diagnosis of extra-pulmonary TB (EPTB) is difficult, often requiring biopsy.*

Material and methods: We present two cases of EPTB in immunocompetent young women. The first case is about a 15-years old female who presented with persistent high fever, ascites and an increased CA-125 serum level. Biochemical analysis of ascites fluid revealed a high value of adenosine deaminase (ADA), negative Ziehl-Neelsen stain and undetectable Mycobacterium tuberculosis at PCR assay. The peritoneal biopsy confirmed the diagnosis of peritoneal TB. The second case, a 29-years old female with a seven-month history of cervical lymph nodes swelling, with drainage and excision biopsy performed, misdiagnosed as cats-scratch disease based on the histological description as granulomatous lymphadenitis, which turned out to be tuberculous lymphadenitis at the second histopathological opinion.

In conclusion, these cases highlight the need for tuberculosis consideration in the differential diagnosis of extra-pulmonary involvement, even in immunocompetent individuals, in order to decrease the misdiagnoses and complications rates.

Keywords: *extra-pulmonary tuberculosis, immunocompetent, peritoneal tuberculosis, lymphadenitis, challenging diagnosis*

INTRODUCTION

Tuberculosis (TB) can be considered an ancient disease which is still causing 2 million deaths a year worldwide. According to global statistics, one person is newly infected with TB bacilli every second world-wide and one third of the global population is currently infected with TB. TB ranks alongside HIV as a leading cause of death from an infectious disease. The prevalence of TB has increased in both immunocompetent and immunecompromised and virtually, it can affect any organ.

It is now seen that extra-pulmonary presentations form a major proportion of new cases. Between 2002 and 2011, extra-pulmonary TB (EPTB) accounted for 19.3% of all TB cases throughout Europe. EPTB is more common in children, women and minorities. Based on Romanian Tuberculosis Notification Data,

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12,498 new TB cases (a rate of 6.4 cases per 100,000 individuals) were registered in our country during 2014, which is higher compared with a rate of 5.2 cases per 100,000 individuals throughout Europe. Extra-pulmonary involvement occurred in 8% of the new cases. Among the extra-pulmonary TB (EPTB) cases, 30.1% were lymph node TB, 13.3 % osteo-articular TB, 6.3% genitourinary TB and 47.3% in other anatomical sites.

Lymphadenitis is the most common extra-pulmonary presentation of TB. Abdominal TB is also one of the most prevalent forms of EPTB. In particular, peritoneal TB is the sixth most common location of EPTB, with an incidence between 0.1% and 0.7% of all TB cases worldwide.

In EPTB, the diagnosis is challenging because of nonspecific symptoms and low sensitivity of classical diagnostic methods, such as microscopy and culture and it is often requiring biopsy. Delay in diagnosis and treatment initiations lead to poor prognosis and sequelae in up to 25% of cases.

MATERIAL AND METHOD

Case 1

A 15-year-old young female patient presented at the territorial hospital with abdominal distension, low abdominal pain, fever (40 degrees) with shiver, excessive sweating and loss of appetite. No other previous health problems were reported. All vaccines provided in universal vaccination schedule were performed. She denied smoking, drug use or alcohol consumption, and she was sexually inactive. The tests performed led to the diagnosis of ascites of unknown etiology. Despite the empirical antibiotic treatment with ceftriaxone, she continued to present fever, being subsequently referred to our clinic for further investigations.

At admission, the clinical status of the patient revealed a relatively good general condition, without fever, no abnormalities on pulmonary examination and normal vital signs. The abdomen was enlarged, with shifting dullness and positive ascitic wave, diffuse pain on palpation, without signs of peritoneal irritation. No masses or organomegaly were present on clinical examination. Blood tests showed lymphopenia (880/mm^3), mild microcytic anemia, elevated inflammatory markers (erythrocyte sedimentation rate of 58 mm/1h, Fibrinogen of 709 mg/dl, C-reactive protein of 112 mg/dl) with negative procalcitonin and normal biochemistry. Chest X-ray did not reveal any infiltrates. Abdominal ultrasonography showed the ascites in large quantity. Transthoracic echocardiography described minimal pericardial effusion (20-30 millimeters).

Further immunology and virology lab tests [rheumatoid factor, antinuclear antibodies, anti-double stranded DNA antibodies (Ab), complement fractions C3, C4, hepatitis B surface antigen, hepatitis C virus Ab, and Human Immunodeficiency Virus 1 and 2 Ab] were negative. The patient underwent paracentesis with ascitic fluid analysis. The gross appearance of the fluid was pale yellow, with exudative features, based on the biochemistry, a total nucleated cell count of 600/mm3 with predominance of polymorphic lymphocytes (72%). The ascites fluid ADA level reached a high value of 140 IU/l, normal value being <33 IU/L. The microscopy for non-specific bacteria and acid fast bacilli, using Gram and Ziehl-Neelsen stains, was negative. Moreover, we performed a broad-spectrum molecular microbial identification from the ascites (IRIDICA BAC BSI® Assay platform for bacteria and fungi) and MTB-DNA amplification (GeneXpert® platform), both of them being undetectable. The serum tumor markers such as CEA, CA19-9 and CA15-3 were within normal limits, apart from CA 125, which was significantly higher (189.5 IU/ml). Serum gamma-interferon specific to MTB antigens produced by T cells (QuantiFERON®-TB Gold) was positive.

In the meantime, we started empiric antibiotic treatment based on oral clarithromycin 1g/day for 3 days, with fever persistence. Then we switched to intravenous (iv) ertapenem 1g/day. After 5 days of ertapenem treatment, the fever has disappeared, with minimal ascites fluid regression. Abdominal and pelvic magnetic resonance imaging (MRI) showed the presence of ascites and described irregular masses with peripheral contrast enhancement near the ovarian fossa, similarly to epiploic fringes.

The patient was transferred to a pediatric general surgery department for exploratory laparoscopy and peritoneal biopsy. The laparoscopic findings described thickened peritoneum with disseminated miliary white nodules on the surface and intestinal wall congestion, being highly suggestive of PTB (Figure 1).

Figure 1. Laparoscopic findings with thickened peritoneum, diffuse congestion, disseminated milliary white nodules on the surface of peritoneum highly suggestive for TB.

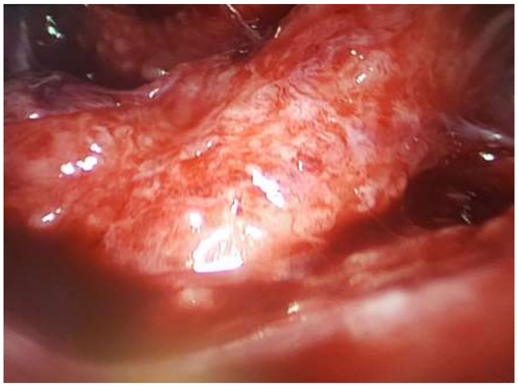

Figure 2. Multiple foci of granulomatous inflammation with multinucleated giant cells, epithelioid histiocytic cells and peripheral lymphocytes are seen on Periodic acid-Schiff (PAS) staining (100X).

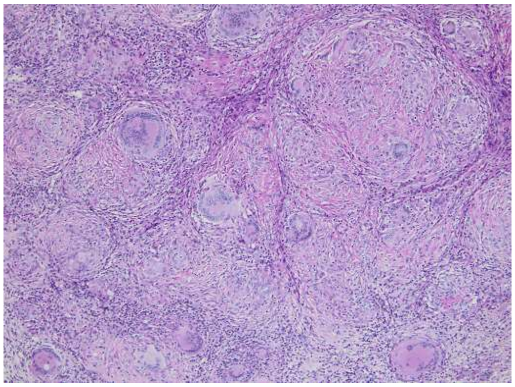

Multiple tissue biopsies were collected. Based on clinical and biological features, along with laparoscopic findings, we decided to start antituberculous therapy with isoniazid 200 mg, rifampicin 450 mg, pyrazinamide 1,500 mg, ethambutol 1,200 mg daily, and oral pyridoxine vitamin 250 mg/day. After 10 days of antituberculous treatment, the symptoms of the patient have regressed, serum C-reactive protein level decreased from 153 mg/dl to 8.6 mg/dl, with no ascites presence at the abdominal ultrasound.

The histopathological results confirmed the diagnosis of peritoneal tuberculosis (Figure 2). In addition, MTB-DNA from the biopsy sample was detected, with no detection of genetic mutations associated with rifampicin resistance.

Case 2

A previously healthy, 30 year-old Caucasian female, smoker (10 pack/year), presented to our department for laterocervical and supraclavicular lymph nodes enlargement, slightly painful on palpation. She also complained of fatigue and dry cough. She was not on any medication, no histories of allergies, all vaccines provided in universal vaccination schedule were performed and she has not travelled during the past year outside of Romania. She has a cat which is an indoor pet.

The symptoms began about 7 months ago with a red, painful swelling of about 4-5 cm, in her left laterocervical region, which she noticed several weeks ago and continued to enlarge. She went to the surgical department where was performed the incision of the lymph node with drainage and lavage. The cultures provided from the procedure were sterile at 48 hours.

5 months later, she presented to the thoracic surgery department with suppurated supraclavicular lymph nodes. All the laboratory tests performed were normal (blood count, coagulation, biochemistry (glucose, creatinine, ALT, AST, GGT, Alkaline phosphatase, sodium, potassium). The patient was evaluated by cervical and thoraco-abdominal CT and the images showed multiple extended outbreaks of pulmonary infiltrates with tree in bud pattern in the apical-dorsal segment of the superior left lobe and in the dorsal segment of the superior right lobe and some isolated centimeter nodal images and mediastinal, supra-clavicular, and bilateral laterocervical lymph nodes. They continued the investigations with bronchoscopy with GenExpert test, direct microscopic examination and culture for acid fast bacilli in bronchial aspiration and sputum. They performed an excision biopsy of the supraclavicular lymph node and the histopathological findings described as confluent epithelioid granulomas

with central clotting necrosis have been interpreted as specific lesions for cat scratch disease. The patient was referred to a department of infectious diseases for additional investigations and specific treatment. Meanwhile, the GenExpert test and the direct microscopic examination for acid fast bacilli were negative with cultures in progress.

When she came in our department, she had good general state. On physical examination we noticed in the left laterocervical area two scars with redness surrounding and several firm, tender, lymph nodes about 1 to 3 cm in diameter, palpable in the left laterocervical and supraclavicular area. The initial laboratory tests were normal, the test for antibody to human immunodeficiency virus (HIV) was also normal, with no other immunity issue found. The serology for Bartonella henselae infection was negative for both antibodies Ig M and Ig G. The patient was referred to the National Institute of Pneumophtisiology for reevaluation. Based on the high clinical and histopathological suspicion of tuberculosis, the biopsy blades were re-analyzed and by performing Ziehl-Neelsen coloration, were discovered few acid-fast bacilli (Figure 3). The histopathological description with necrotizing granulomatous epithelioid inflammation was also pathognomonic for tuberculosis.

Figure 3. Acid-fast bacilli on Ziehl–Neelsen smear (100X)

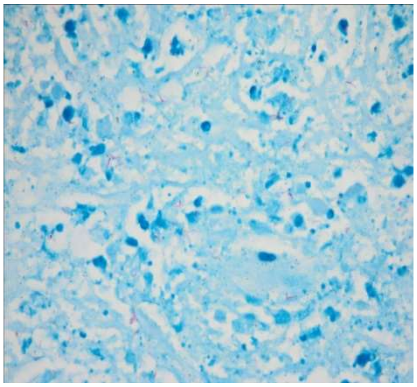

The patient was put on anti-tubercular drugs with good evolution. The cultures for mycobacterium tuberculosis read at 2 months were positive.

DISCUSSIONS

TB is still one of the most frequent infectious disease and worldwide strategies to eradicate it have met only with partial success. According to global tuberculosis report, in 2015, there were an estimated 10.4 million new TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. People living with HIV accounted for 1.2 million (11%) of all new TB cases. There were an estimated 1.4 million TB deaths in 2015, and an additional 0.4 million deaths resulting from TB disease among people with HIV. Although the number of TB deaths fell by 22% between 2000 and 2015, TB remained one of the top 10 causes of death worldwide in 2015.

Although it is well known that TB occurs mainly in immunocompromised host, it seems that the prevalence of TB has increased both in immune-competent and immunocompromised patients. Nowadays extra-pulmonary presentations form a major proportion of new cases of TB. Among them, lymphadenitis is the most common clinical presentation, with a major predilection for cervical lymph nodes.

Abdominal TB is also one of the most prevalent forms of EPTB and the peritoneal involvement has been observed in about 1-2% of all cases of TB. Pelvic TB is common in developing countries, mainly in young, fertile women aged between 15 to 25 years old. The common clinical features include ascites, fever, diffuse abdominal pain and weight loss. Therefore, peritoneal TB is often misdiagnosed, usually as carcinomatous peritonitis. The lacking of medical awareness of pelvic TB could lead to mutilating surgical treatment like adnexectomy for many young, fertile, women.

Our patient presented with a clinical picture highly compatible with peritoneal TB. However, we had to continue the investigations because the conventional methods, like microscopy and MTB-DNA amplification from ascitic fluid, failed to establish the positive diagnosis. In addition to clinic-biological findings and radiological characteristics, the accuracy of early diagnosis was increased by measuring the ascites ADA and serum CA125 levels.

The studies showed a significant correlation between

ADA and CA-125 in patients with peritoneal TB. The tests are simple, non-invasive, cheap, and it seems to have enough power to confirm or exclude the diagnosis of peritoneal tuberculosis and it is a good approach to begin the empirical treatment while waiting for the histopathological result or cultures.

The diagnosis of peritoneal TB can also be suggested by characteristics findings at laparoscopy or laparotomy; however, these are invasive examination methods and should be recommended with caution in clinical practice.

Lymphadenitis is the most common extra-pulmonary presentation of TB. It is seen in nearly 35 per cent of extra-pulmonary TB which constituted about 15 to 20 per cent of all cases of TB. Although previously considered a childhood disease, lymphadenitis has a peak age of onset of 20-40 years. The most common site of involvement is represented by cervical lymph nodes, reported in 60% to 90% patients, followed by mediastinal and axillary lymph nodes6.

Cervical lymph nodes can be involved via various pathological processes, including inflammatory diseases, lymphomas and even metastases. It is important that a high index of suspicion for tuberculous lymphadenopathy should be kept in mind because it mimics other pathologic processes and yields inconsistent physical and laboratory findings.

TB lymphadenitis usually presents with gradually painless swelling of one or more lymph nodes. Initially the nodes are firm, mobile, later they become matted with inflamed overlying skin and with abscesses formation in the advanced stages. Some patients may associate systemic symptoms such as fever, weight loss, fatigue, dry cough.

In our case, the patient had a seven-month history of cervical lymph nodes gradually swelling, which become matted with redness of the overlying skin. She also associated systemic symptoms represented by dry cough and fatigue.

The diagnosis of tuberculous lymphadenitis is difficult due to low sensitivity of mycobacterial isolation, prolonged time of the isolation process and multiple differential diagnosis that need to be ruled out. In endemic areas, for faster diagnosis, has been used fine-needle aspiration cytology (FNAC), combined with polymerase chain reaction for detecting Mycobacterium tuberculosis, in the absence of compatible cytological findings or negative acid-fast bacilli smears. Acid fast bacilli may be present in 25-50% of smears and the mycobacterium may be isolated in up to 70% of cases where there is a high suspicion of tuberculosis. Caseating granulomas are seen in almost all the biopsies and in 77% cases of the FNAC's. Typically, epithelioid cell granulomas, multinucleated giant cells and caseation necrosis are features of tuberculous lymphadenitis but these are neither specific nor sensitive and the diagnosis remains a challenge, necessitating a high index of suspicion, since granulomatous lymphadenopathy has an extensive differential diagnosis [13].

Cat scratch disease (CSD) is an infectious disease caused by gram-negative bacteria Bartonella henselae (B. henselae) and/or Bartonella quintana. It is represented by regional suppurated lymphadenitis, the lymph nodes affected are cervical (33%), axillary (27%) and inguinal (18%). The disease affects children and youths under 18 years old. Epidemiologic studies from the United States, Europe, Israel, Australia, and Japan, suggest that CSD has a worldwide distribution. In the United States, a retrospective study of patients younger than 65 years of age identified 13,273 patients with a diagnosis of CSD from 2005 to 2013. The diagnosis is based on the following: a) history of contact with a cat; b) checking of injury sites; c) regional lymph node swelling; d)characteristic histology of lymph node; e)skin test for CSD, replaced in the last years by immunohistochemically staining with antibodies to B. henselae in formalin-fixed paraffin–embedded tissue sections and polymerase chain reaction.

The histopathologic findings are nonspecific and depend upon the stage of the disease. Lymphoid hyperplasia is present initially, followed by the development of stellate granulomas. The centers are acellular and necrotic with histiocytes and peripheral lymphocytes surrounding these areas. Microabscesses develop and may become confluent at a later stage. Although these findings support a diagnosis of CSD, they should not be considered definitive.

Based on the granulomas found on the histological examination, our patient was first misdiagnosed as cat scratch disease without staining for acid-fast bacilli or PCR identification for Mycobacterium tuberculosis on the biopsy specimens.

CONCLUSIONS

Tuberculosis still remains a worldwide public health issue. It is still a common disease in developing countries, even in immunocompetent individuals. It should be kept in mind in the differential diagnosis of extra-pulmonary manifestations in order to avoid misdiagnosing and further complications. Surgical biopsy leaves unwanted scars, causing esthetic problems and also the possibility of long-term drainage problems through the incision line.

In young, fertile, women, presenting with febrile ascites, often mimicking adnexal malignancy, surgeons should be aware of peritoneal TB, avoiding unnecessary mutilating surgery leading to increase complications, such as infertility.

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***Conflicts of interest**: All authors – none to declare*

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Facial skin cancer: our surgical experience

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Abstract: *Skin cancer represents an important part of the plastic surgeon's practice, and surgical excision followed by reconstruction is the most frequently used procedure. The main objective of this paper is to report and evaluate our experience in the treatment of facial nonmelanoma skin cancer.*

Method. The study is based on 303 patients who were diagnosed with facial malignant tumors, between 2004 and 2015, in the Plastic and Reconstructive Surgery Clinic, Emergency University Hospital Bucharest Romania. We statistically analyzed the distribution by age, gender, facial location, the time from onset until the presentation, the type of tumor, the size of the tumor, the margin status, and the recurrence. Results were as follows: median age at 70 years, with an even gender distribution. The most affected areas were the cheek, followed by the nose, forehead, and eyelids. We calculated the dimensions of the tumors between 1.57 mm^2 and 1,846 mm^2, with a median value at 235 mm^2, and a mean value at 421.23 mm^2. Patients in whom safe margins were not obtained had a 4.15 times higher relapse rate versus the ones with safe margins at the first intervention, with a high statistical significance – p=0.002 (15% recurrence rate if safe margins were not obtained, versus 4.1% if margins were safe).

Conclusion. When discussing tumor excisions, one of the most controversial topics is that of safety edges. Currently, even if there are recommendations, a unified protocol is not formulated, which is why we found it useful to research this topic with extensive medical associations.

Keywords: *skin cancer, tumor excision, safety edge*

INTRODUCTION

Tumors represent an important part of the plastic surgeon's practice, surgical excision followed by reconstruction is the most frequently used procedure [1].

The current paper intends to present evidence-based information for such procedures, to establish the current status of cutaneous tumors approach in our clinic, in Romania, compared international guidelines, and to gather a set of recommendations for skin tumors surgery.

MATERIAL AND METHODS

The study is analytical observational and prospective – based on 303 patients who were diagnosed with facial malignant tumors, between 2004 and 2015, in the Plastic and Reconstructive Surgery Clinic, Emergency University Hospital Bucharest Romania.

The inclusion criteria were chronic malignant lesions of the face, with the complete histopathological result, which mentioned the surface and depth, the exclusion criteria

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were psychiatric disorders, epilepsy, patients who do not cooperate, incomplete histopathological result, tumors that affect other areas that the skin (ENT, ophthalmological, BMF).

Tumor surgical protocol, for malignant tumors, the excision aimed to encompass a safety margin of 3-6 mm. The excised pieces were sent for histopathological examination and the final and complete diagnosis was set after the result was available (7-21 days). The first postoperative evaluation was performed at a median of 3 weeks, and the patients were followed up every 3 months for 2 years.

The collected data was uniformly analyzed; statistical analysis was performed using Microsoft Office Excel for Windows, SPSS version 21.0 (Statistical Program for Social Sciences), and EpiInfo version 7. Non-parametric tests were used, as the studied population did not have a normal distribution. To establish risk rates, we computed the odds ratio (OR) and 95% confidence intervals using EpiInfo. Statistical significance was established for $p < 0.05$. To correctly interpret certain numerical variables and especially how these values may influence the safety margins, as well as the reconstructive procedures, we used ROC curves (receiver operating characteristic) – SPSS 21.0.

RESULTS

In the selected study group the distribution:
- by age was between 35-97 years, with the average age at approximately 71;
- by sex was 50,16% (152) vs 49,84% (151)-male vs female cases.

Analyzing the time since the first detection of the lesion and the moment of presentation to the doctor, we observed an average of 58 months and a median at 24 months; also, 19.3% of the patients had received different treatments for the lesions before presentation in our clinic.

The types of cutaneous malignant tumors were: 215 (70.95%) basal cell carcinomas, 56 (18.48%) squamous cell carcinomas, 5 (1.65%) malignant melanoma, 20 (6.6%) metatypical and mixed carcinomas, non-melanoma tumor relapses 5 cases (1.9%), and 2 cases of metastasis.

Depending on the location related to the face segments, we recorded: 94 lesions (31.03%) on the cheek, 52 lesions (17.16%) on the nose, 47 lesions (15.51%) on the forehead, 35 lesions (11.51%) on the eyelids, 17 lesions (5.61%) on the lip, 17 lesions (5.61%) on the ear, 2 (0.6%) chin lesions, 32 (10.56%) tumors with mixed locations and 7 (2.31%) tumors with an extended location.

Basal cell carcinomas were most commonly located on the cheek – 32.1%, nose – 18.1%, forehead – 14.9%, eyelids – 14%, ear – 4.7%, lip – 2.8%, and the squamous cell carcinoma were more commonly in cheek – 28%, lips – 22%, nose – 20%, forehead – 18%, eyelid – 6%.

We calculated the dimensions of the tumors between 1.57 mm^2 and 1,846 mm^2, with a median value of 235 mm^2, and a mean value at 421.23 mm^2.

Analyzing the tumor margins, we calculated that the safe margin (tumor-free) was obtained at 75.6% of the patients.

When the dimensions of the tumors were analyzed depending on the safe margin rate, we observed that the patients where safe margins were obtained had a median tumor dimension of 197.82 mm^2, significantly lower than the median dimension in patients where the margins were invaded (405.84 mm^2), p=0.0011 (Figure 1). Similar differences were recorded when comparing median depth of tumoral excisions for safe margins (5 mm), versus invaded margins (6 mm), p>0.05.

Figure 1: Free margin

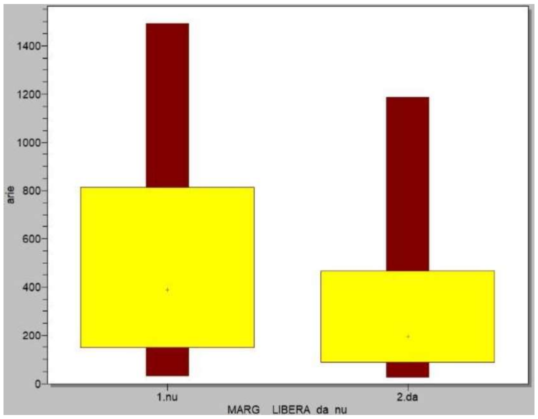

The rate of obtaining free margins depending on tumor location was as follows: for lips and chin were completely removed in all cases (100% rate of free margins) eyelid – 71.42%, nose – 73.06%, cheek – 72.33%, forehead – 72.34%, chin – 86.4%, ear – 64.70%, extended location – 57.1%, mixed location – 100%. For tumors with mixed locations, free margins were obtained in all patients (rate 100%), whereas for tumors with a unique location, the rate was 89.4% (p<0.05).

This apparent paradox is related to the lower medium dimensions of tumors with mixed location – approx. 146 mm^2 versus 247 mm^2 (for tumors with strict location), p>0,05. For tumors with extended location, on more than one esthetic unit, usually with a long history of evolution, free margins were obtained in only 62.5% of cases, presenting a 1.9 times higher odds ratio for lack of safe margin versus limited location tumors ($p>0.05$). The median dimensions of these tumors (extended location) was also significantly higher (1,041 mm^2 versus 219 mm^2, $p=0.0007$) for tumors limited to one esthetic unit.

Figure 2: Extensive location

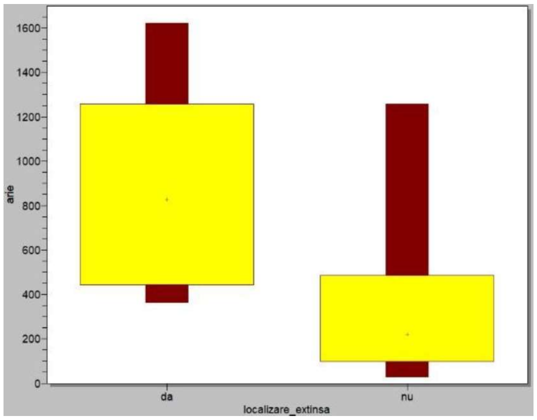

For malignant tumors, free margins were obtained in 64.20% of squamous cell carcinomas, 79.06% of basal cell carcinomas, 100% for melanomas, and 75% for metatypical and mixed carcinomas.

When analyzing the rate of safe margins depending on tumor dimensions for malignant tumors, we observed that the median dimension for basal cell carcinomas with free margins was 152.29 mm^2, versus 235.5 mm^2 for invaded margins, $p=0.017$.

For squamous cell carcinomas, median dimensions for free margins was 447.45 mm^2 compared to 706.50 mm^2 for non-tumor free.

For the most aggressive tumor, malignant melanoma, free margins were obtained for all patients (100% rate), due to the strict surgical protocol in these cases regarding macroscopic safety margins.

Figure 3: Free margin

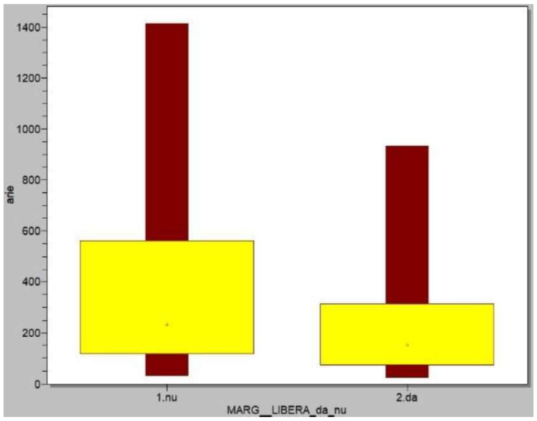

For more rare tumors (metatypical and mixed carcinomas) we obtained free safe margins in tumors with a median dimension of 663.53 mm^2 compared to 710.89 mm^2 for invaded margins.

We analyzed the recurrence rate for patients with malignant tumors and we observed that it appeared in 6.3% of the cases.

The recurrence rates differed on the type of tumor: 5.0% for basal cell carcinoma, 10% for squamous cell carcinomas, 0% for malignant melanomas, and 9.1% for mixed and metatypical carcinomas (Figure 4).

Figure 4: Extensive location (recurrence yes ●, recurrence no ●)

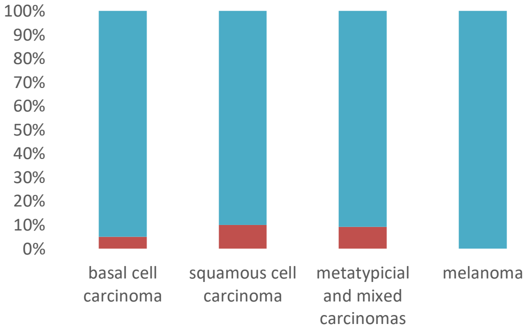

We made a separate analysis of the most frequent malignant tumors, respectively basal cell, and squamous cell carcinomas. Basal cell carcinomas had a very low relapse rate, with a relative risk of 0.48 ($p>0.05$) versus all other malignant tumors. The squamous cell carcinomas had a relapse risk of 1.90 ($p>0.05$) versus other malignant tumors.

Patients in whom safe margins were not obtained had a 4.15 times higher relapse rate versus the ones with safe margins at the first surgery, with a high statistical significance – $p=0.002$ (15% recurrence rate if safe margins were not obtained, versus 4.1% if margins were safe).

We did not observe any significant difference when we analyzed the recurrence rate by tumor dimensions.

Also, although the depth of the recurring tumors was higher than the depth of non-recurring ones (6 mm versus 5 mm), the difference was not statistically significant.

DISCUSSION

Skin tumors are the most common tumors, which is why reconstruction after tumor ablation is the most frequently performed procedure by the plastic surgeon and one of the most active areas of expertise [1]. More than in any other surgical area, the approach for facial tumors has to include a correct and complete tumor resection with a minimum area of excised tissue to preserve the function and shape of the reconstructed area. Our main interest in this study was represented by malignant facial tumors.

In our group, the median age was around 70 years and we observed an almost even gender distribution, consistent with the data reported for basal cell and squamous cell carcinomas [2-5]. Regarding the distribution by aesthetic areas, the most affected ones were the cheek, followed by the nose, forehead, and eyelids.

Regarding malignant tumor pathology, we found that basal cell carcinoma was most frequent, followed by squamous cell carcinoma; metatypical carcinomas – aggressive variants of basal cell carcinoma (they have squamous cell carcinomas behavior). This data overlaps the numerous reports that basal cell and squamous cell carcinomas are most common in the face [6-9, 21].

Analyzing the tumor size, we obtained a median size for the entire group of 303 patients, of 428.53 mm^2. The sizes of the tumors observed in our study are quite high, much larger than those reported by other studies. This was correlated with an increased positive margin rate, which in turn increases the risk of subclinical extension and recurrence [11].

When analyzing separately patients with basal cell carcinomas, we observed that free margin was obtained in 79.06% of cases. A meta-analysis of the literature led by Gulleth et al. on 16,066 cases, encompassing 106 articles on basal cell carcinomas, revealed an average diameter of basal cell carcinomas of 11.7 mm with an average percentage of free margins of 86% ± 12% [12]. Malik et al. in their analysis of 1,832 basal cell carcinoma obtained 86% negative margins excisions [13]. In a study on 2,016 cases for BCC with a diameter smaller than 10 mm, Breuninger obtained a probability of negative margin between 70%-95% depending on the size of the safety margin taken, 2, 3, or 5 mm [14]. In our group, the median dimension for basal cell carcinomas with positive margins was 235.50 mm^2, corresponding to lesions of approximately 23 mm/10 mm. The majority of available data on basal cell carcinomas report small lesions, respectively under 20 mm largest diameter [4, 5], whereas our series comprises patients with much larger lesions, as described above. Therefore, this could explain the lower rate of safe margins in our group, which is related to bigger lesions with a more prolonged evolution.

For squamous cell carcinomas, the median size for excisions with free margins was 447.45 mm^2, versus 706.50 mm^2 for invaded one, with 64.2% of negative margins. Studies on squamous cell carcinomas are much smaller, but we note the results of the prospective study by Peed-Yau Tan et al. in which although the authors do not mention the size of the lesions, they achieved a percentage of only 7.6% excisions with invaded edges [15]. Talbot S. and Hitchcock B. report an invaded edge of 14% in a study on basal cell and squamous cell carcinomas [16]. Again, the tumor size in our patients is quite high, much higher than the limits considered significant (respectively 20 mm largest diameter)[17], thus accounting for the lower rate of free margins.

Another variable tracked in our study was the depth of tumor invasion. There is no clear recommendation in the literature regarding how deep the excision should go; however, for basal cell carcinomas, tumor depth is correlated with the risk of recurrence, and respectively for squamous cell carcinoma, with the rate of metastases [10, 18]. Breuninger reported that, for over 6 mm depth, the metastases rate in squamous cell carcinoma is 15%, and Motley emphasized the depth of 4 mm as associated with recurrence [19]. In practice, deep excision is performed according to what is found intraoperatively and depending on the surgeon's experience. The average excision depth in our free margins lot was 5 mm, while for invaded edges it was 6 mm.

For basal cell carcinomas, recurrence occurred in 5% of patients with negative margins and 10% for positive margins, and for squamous cell carcinoma, respectively in 10% for negative margins and 15% for positive margins. In our study, we found no statistical relevance for recurrence concerning dimensions and tumor depth. In the literature, the BCC recurrence rate varies between 0-10.1% for complete excision, and for incomplete excisions, it is estimated at 27% but can reach as high as 35.5% [12, 20, 21, 23]. For SCC the local recurrence rate varies between 1.7-7% in some studies [24], but it can reach even 53.6% depending on location, the grade of differentiation, perineural involvement, and surgical excision method (standard or Mohs surgery) [25].

The first rule in nonmelanoma skin cancer surgery is to obtain a complete excision with negative margins. In our study, most of the cases had a long evolution period before the presentation, which explains why approximatively 57% were large tumors, above 2 cm. In the cases with positive margins, a re-excision was performed where it was possible, or the patient was referred to the oncology service.

CONCLUSIONS

When discussing tumor excisions, one of the most controversial topics is that of safety edges. What is the size of the safety edges required for complete excision, with free tumor margins, thus ensuring the lowest recurrence rates, and at the same time with a minimal defect for a successful reconstruction?

Currently, even if there are recommendations, a unified protocol is not formulated, which is why we found it useful to research this topic with extensive medical associations.

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ORIGINAL ARTICLE

Factors that influenced infection complications in combat related penetrating brain injuries during conflict in Ukraine

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Abstract: *Infection complication has a major impact on outcomes of patients with combat related penetrating head injuries. There is still a discussion around which factor influence outcomes and infection complication. In the beginning of war, in 2014, the evacuation system was not ready for a sudden start of hostilities because of limited medical resources, the lack of neuro visualization in the frontline, the lack of trained personnel provided first aid to the wounded.*

Aim: The aim of this study is to determine the factors that influenced the development of infectious complications in penetrating combat head injuries with limited medical capabilities on theatre

Materials and methods: This is retrospective study of 79 military cases with penetrating head injuries, which was received during the fighting in eastern Ukraine in the period from March 2014 to the end of December 2017. Information was collected on demographics, evacuation assistance, type of injury and infectious complications. R commander was used for statistical analysis. Statistical significance was defined as p<0.05.

Results: Infection complication of CNS were detected in 31 cases (39,24%). Factors that influence rate of infection are low GCS on first evaluation (p<0.001), CSF leak (p=0.002), ventricular injury (p<0.005), installation of tidal drainage (p=0.005), superficial wound infection (p=0.013) and wound dehiscence (p<0.001).

Conclusions: There are many controversies in managing penetrating gunshot wounds to the head. Still exist lack of information that neurosurgeon can trust. Data even about small samples still very informative.

Keywords: *infection complications; penetrating brain injury; military trauma; Ukraine*

BACKGROUND

Infection complications (IC) is one of most common reason for unfavorable outcomes after penetrating head trauma [1]. Infection is a reason for severe morbidity and significantly influences mortality rate. This complication prolongs patients' hospitalization time, as well as delays the recovery process with rehabilitation and prevents soldiers return to the battlefield [2]. The rate of IC in combat penetrating trauma higher than in civilian gunshot wounds. Mostly this is due to extended time to the first aid and extreme conditions existing at the war theatre [8]. The war in eastern Ukraine has been going for more than 7 years. In early 2014, the evacuation system was not ready for a sudden rise of hostilities. The most limitations included limited medical resources, the lack of computer tomography (CT) at the frontline, the lack of trained first aid personnel providers.

The purpose of our paper is to determine whether known risk factors for the development of infectious complications had influence to our sample group and what other possible factors could influence outcomes.

MATERIAL AND METHODS

This is a retrospective study of 79 patients with penetrating combat related brain injury. The study based on medical records of patients that were treated at National Military Medical Clinical Centre "Main Military Clinical Hospital" (NATO Role IV) from May 2014 until 31 December 2017. All

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patients participated in military conflict in Eastern Ukraine. All injured were evacuated through all echelons. Non-penetrating head injuries were excluded from the analysis.

Information collected from patient recordings include demographics, clinical examination findings during evacuation and in treating hospitals, laboratory results. Computer tomography scans were evaluated by neurosurgeon and radiologist separately. Surgical and therapeutical interventions after injury were noted.

Statistical analysis was performed using R version 3.6.3 (http://www.r-project.org). In univariable analysis, variables were compared between groups by Fisher's exact test for categorical variables and the Wilcoxon signed-rank test for numerical variables. Statistical significance was defined as $p<0.05$. Local ethics committee approval was obtained before the study.

RESULTS

From initial analysis of 109 patients with combat related head injury, 79 had penetrating brain injury. Type of injury included: penetrating (51 cases), perforating (8), ricochet (8), tangential (7), blast related penetrating injuries (5). All wounded were males. Age at presentation was 19.9 to 54.4 years (mean= 31.8, SD=8.4).

Level of consciousness was evaluated with Glasgow Coma Scale (GCS). We divided all patient into three groups according initial GCS. In first group GCS 3-5 were 6 patients, GCS 6-12 – 20 patients, GCS 13-15 – 51 patients, no information was available for two patients. Eight patients deteriorated during evacuation, 28 patients had the same level of consciousness, 32 improved, and in 11 cases information was not available. Loss of consciousness in battlefield for different periods of time was documented in 17 cases.

Bullet gunshot wounds were found in 16 cases, missile fragments injury in 58 cases, and 5 cases had injury related to blast wave impact. Isolated head injury was in 39 cases. Multiple injuries of different parts of the body were detected in 40 cases: 26 cases were combined with extremities, 19 cases with chest, 13 cases with face, 13 cases with abdomen, 13 cases with eyes, 4 cases with neck region. In 28 cases head injury was combined with more than 2 body regions. Injury severity score (ISS) were calculated in each case - average ISS was 26.6 (min 9, max 49, SD=9.1). In case of spine injury all patients evaluated with American Spine Injury Association (ASIA) score: grade A – two, grade D – four, grade E – four.

Head computer tomography performed in all cases (Light Speed VCT 64, General Electric, USA) during admission and when needed – in case of deterioration, appearance of new neurological deficit, routinely after surgery.

Initially retained bone fragments was in 63 patients, removed in 32 cases in previous echelon. Retained metallic fragments was in 58 patients, removed in 27 cases on previous stages. On last CT before discharge from hospital bone fragments detected in 26 cases, metallic in 28.

Patients operated because of epidural hematoma (EDH) in 11 cases (five combined with subdural hematoma (SDH), SDH was in 17 cases, intraventricular haemorrhage was in 13 cases, ventricular system weas injured in 17 cases.

Bullet or missile entrance wound was in 31 cases in frontal region, parietal in 22, temporal region in 13 cases, occipital in three, face in one, basal in one, transorbital in three cases. Exit wound was in frontal region in three cases, temporal in three, parietal in one, occipital in one case.

Paranasal air sinuses were injured in 23 cases: frontal sinus in 17 cases (two cases combined with maxillary), basal sinus in two cases (one with maxillary), one case maxillary, in three cases were injured mastoid air cells.

Infection complication of central nervous system (CNS) were detected in 31 cases (39.24%) – details in Table 1.

Table 1: Types of infection complications

Nosology	Number
Meningitis	17
Meningoencephalitis	6
Abscess	4†
Ventriculitis	2*
Empyema	2

*† one combined with empyema, two with ventriculitis, two with meningoencephalitis; * one combined with meningoencephalitis*

Infection complications were proved by clinical and laboratory data. Records of physical examination and local wounds status were also collected. Cerebrospinal fluid (CSF) cultures was analyzed in 12 cases. P. aeruginosa was found in three cases, S. cohnii was revealed in one case. Wound culture was performed in four cases, in one case grew S. aureus.

In 24 cases performed 24 reoperations: 17 patients underwent two surgeries, three patients underwent three surgeries, in one case four reoperations, in three cases five reoperations. Reasons for reoperations varied: CSF leak, removal of retained bone and metallic fragment, infection complications with mass effect, CSF diversion procedures.

Table 2 shows factors that influence rate of infection complications after combat related penetrating brain injury.

Outcomes at discharge was evaluated with Glasgow Outcome Scale (GOS) and present in Table 3. Six cases with GOS1 have different types of infection complications.

Table 2: Analysis of factors that influence rate of infection complications after combat related penetrating brain injury

	CNS infection N=31	No CNS infection N=48	P value
Year of admission			
2014	12 (38.7%)	16 (33.3%)	0.063
2015	14 (45.2%)	11 (22.9%)	
2016	3 (9.7%)	12 (25.0%)	
2017	2 (6.5%)	9 (18.8%)	
GCS on admission to main hospital*			
GCS 3-5	5 (17.2%)	1 (2.1%)	<0.001
GCS 6-12	15 (51.7%)	5 (10.4%)	
GCS 13-15	9 (31.0%)	42 (87.5%)	
Type of penetrating agent			
Bullet	8 (25.8%)	8 (16.7%)	0.615
Missile	21 (67.7%)	37 (77.1%)	
Blast wave	2 (6.5%)	3 (6.3%)	
Retained fragment before surgery	29 (93.5%)	39 (81.3%)	0.186
Bone	28 (90.3%)	35 (72.9%)	0.086
Metallic	24 (77.4%)	34 (70.8%)	0.607
Retained fragment after surgery	16 (51.6%)	23 (47.9%)	0.820
Bone	13 (41.9%)	13 (27.1%)	0.222
Metallic	11 (35.5%)	17 (35.4%)	1.000
Injured air sinuses	10 (32.3%)	13 (27.1%)	0.623
CSF leak	14 (45.2%)	6 (12.5%)	0.002
ISS on admission**	26 (25-30)	25(25-31)	0.357
Ventricular injury	15 (48.4%)	3 (6.3%)	<0.005
Tidal drainage	12 (38.7%)	5 (10.4%)	0.005
Repeated surgeries	15 (48.4%)	14 (29.2%)	0.099
Time to surgery			0.304
>24h	26 (83.9%)	44 (91.7%)	
<24h	5 (16.1%)	4 (8.2%)	
Combined injury	16 (51.6%)	23 (47.9%)	0.820
Brain compression	30 (96.8%)	42 (87.5%)	0.236
Subarachnoid hemorrhage	14 (45.2%)	12 (25.0%)	0.087
Wound infection	10 (32.3%)	4 (8.3%)	0.013
Wound dehiscence	13 (41.9%)	2 (4.2%)	<0.001

**Data was not available for 2 patients; **Median and interquartile range*

Table 3: Outcomes in patients with penetrating brain injury

GOS	5	4	3	2	1
group w/o complications	3	23	20	0	2
group with infection complications	0	9	14	2	6

DISCUSSIONS

Infection complication after penetrating combat related trauma one of the main factors that influence mortality and morbidity rate [1-3,6,13]. It can prolong staying in hospital and demand significant financial and physical resources, prolong rehabilitation process [1]. In our series infection complication was detected in 39.24% (31 cases), that is typical for centre which collect patients from previous levels [7], but this is significantly higher than in recently conflicts (from 5.2 to 31%) [1,7,8,10,11,14]. Structure of infection complications also changed – in pre – and early antibiotic era mainly were diagnosed abscesses and subdural empyema [15], now its meningitis and encephalitis [2,3,5-7].

Our results along with data from other studies showed several factors that influence rate of infection complications after combat injuries – low CGS on admission [1,13], CSF leak [1-4,11,12], ventricular injury [3,4,16] and wound dehiscence [6,13].

Retained bone fragment and repeated surgeries has some tendency to be influence factor but did not reach significance level (p=0.086, p=0.099 accordingly). These factors also described in literature as possible reasons for infection complication with additional circumstances for example CSF leak [5]. There are two different strategies for management retained bone fragments. First strategy includes an aggressive and complete removal of all retained fragment [3,6,7,9,10]. Second is so called «less aggressive» strategy that was proposed after Israeli Lebanese conflict. Authors maintain that less aggressive tactic in removal of fragments are better for outcomes without major influence on infection rate [1,4,6,8-11].

We didn’t detect in our study that injury through air sinuses can cause higher rate of intracranial infection complications (p=0.62) but some authors describe it as a factor that influence [3]

Also type of penetrating agent, ISS on admission didn’t influence rate of infection complication in our study, but other papers show opposite data [2,16].

Some authors believe that soldiers which were injured in the beginning of conflict have more chances to have an infection [12]. In our study we also can follow similar tendency of this but it did not reach a significance level (p=0.063). This can be explained by lack of organized an evacuation system at the beginning of the war when time of evacuation was extended sometimes up to 3 days.

Information regarding usage of tidal drainage difficult to find in modern literature. It was widely used during the Second World War by the Soviet Army [15,17], and still used in the so-called post-Soviet countries [2,18]. This method of drainage is not commonly used in our hospital. In our series, 12 out of 31 patients, who had IC, had such drainage during evacuation. Our hospital does not support such widespread usage of tidal drain, and in our opinion, this technique should be minimized as it is a predictor of infectious complications ($p<0.005$).

Time to surgery was hard to evaluate in our study. We can only divide surgery in two major groups – surgery that done in firs 24 hour and later. We cannot detect major difference between this groups. But some authors showed that patients which were operated in first 24 hours have better neurological outcomes and less IC [14,16].

CONCLUSIONS

We found factors associated with infectious complications that were earlier described in literature and also working in our sample as CSF leak, GCS on admission, injury reaching ventricular system, local wound infection and dehiscence. There are many controversies about retained fragments and their role in cause IC. Fast qualified medical care is likely to reduce the risk of IC. It is important to transform a gunshot wound from a penetrating to a closed non-penetrating one, which is the prevention of cerebrospinal fluid and IC. In the case of contaminated wounds, the possibility of installing tidal drainage should be avoided, as this increases the risk of infection.

Limitations

Our study has several limitations. Firstly, some of the data were missing due to its retrospective design, all date were collected from paper histories and sometimes not all hand written information can be right identified and probably missed. In addition, the relatively small sample group prevent us to analyze the contribution to infection risk of other important factors.

Authors’ Contribution:
MH, KK did study conception, data analysis, and manuscript writing. MH, KK did data collection and manuscript writing. MH, KK did data collection and manuscript writing. Both authors (MH, KK) contributed to all stages of the article.

Conflict of Interest:
The authors declare no conflict of interest.
The views in this article do not reflect the official position of the Ukrainian Army.

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P. Y., P. M., D. A., D. G. designed the study; P. Y collected and analyzed data; P. Y. wrote the manuscript; P. Y., G. O. performed the statistical analysis; All authors read and approved the final manuscript.

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VARIA

Fever of unknown origin

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Abstract: *Fever of unknown origin (FUO) is a diagnosis that describes a prolonged febrile illness without an established cause, despite investigations. From the first definition of the medical condition (given by Petersdorf and Beeson in 1961) the diagnostic criteria have changed over time, intending to improve the management and outcome of these patients. The main causes of FUO fall into 4 categories: infectious, non-infectious (inflammatory), neoplastic and diverse causes. The etiology of FUO has changed over time, due to ever-changing disease patterns and the development of diagnostic techniques. A significant percentage of patients with FUO remain without an etiologic diagnosis, despite advanced diagnostic tests. Gathering potentially diagnostic clues through history, physical examination and nonspecific key paraclinical abnormalities is the basis for the diagnosis of FUO.*

Keywords: *fever of unknown origin, infections, malignancies, systemic inflammatory diseases*

INTRODUCTION

Fever of unknown origin (FUO) was first defined in 1961 by Petersdorf and Beeson as a medical condition that has the following three characteristics [1]:

- Body temperature 38.3° C (101° F) at three or more successive measurements.
- A duration of at least 3 weeks.
- Uncertain diagnosis after one week of hospitalization.

In the following years, the definition of FUO changed. Immunosuppressed patients were excluded from this category because they have etiological peculiarities and require a different therapeutic approach. Thus, the current definition of FUO includes the following diagnostic criteria:

- a body temperature ≥38.3°C (101°F) at least 2 determinations;
- the duration of illness for ≥ 3 weeks or recurrent febrile episodes for ≥ 3 weeks;
- the absence of immunosuppression, defined as HIV infection or known hypogammaglobulinemia, treatment with prednisone 10 mg or equivalent for a duration ≥ 2 weeks in the previous 3 months before the onset of fever, and neutropenia for a period ≥1 week 3 months before the onset of fever;
- uncertain diagnosis, despite a rigorous case history and clinical examination and the following laboratory investigations: complete blood count, erythrocyte sedimentation rate, ferritin, three blood cultures, tuberculin skin test, C-reactive protein, electrolytes, total proteins,

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protein electrophoresis, creatinine, microscopic urinalysis, urine culture, creatine kinase, rheumatoid factor, antinuclear antibodies, alkaline phosphatase, lactate dehydrogenase, aminotransferases, chest X-ray, abdominal ultrasonography.

EPIDEMIOLOGY

Scientific and technological progress in the microbial diagnosis and classification of the pathogenesis of rheumatic diseases have led to a higher rate of etiological diagnosis among patients with fever. Thus, if in 1930, 75% of patients with fever had no identified causes, nowadays-true FUOs are uncommon [4, 5]. The causes of FUO vary greatly with age. Among children, the most common are viral infections, while among adults' multisystem diseases, such as rheumatic diseases or sarcoidosis, are more likely [5, 6]. Furthermore, geographical region and economic development have an important influence on the etiology of this disease. Thus, in developing countries, the most common causes of FUO are tuberculosis, typhoid fever, amebic liver abscesses and acquired immunodeficiency syndrome (AIDS). In developed countries, the travel of inhabitants to certain areas is a risk factor, resulting in the identification of rare diseases, such as malaria, filariasis, schistosomiasis, Q fever, relapsing fever, Zika virus, fever leash, etc. [5, 7]. Travelers may be infected with microorganisms to which local residents are not vulnerable, because of pre-existing immunity. The diseases contracted while abroad may have a very high incubation period, symptoms (such as fever) occurring after a period from the moment of return. This reduces the chance of an etiological diagnosis [5, 8].

A special category of patients is immunosuppressed patients, such as those with AIDS or known neutropenia. In the case of AIDS, the causes for fever are infections (79%) and malignancies (8%). In 9% of cases, the cause cannot be identified. Regarding the infectious agents, more than half of the cases were due to mycobacteria, especially atypical, the most common being the mycobacterium avium complex (MAC). Regarding malignancies among HIV-infected patients, lymphomas, particularly non-Hodgkin's lymphomas dominate [5]. Febrile episodes associated with neutropenia are most frequently caused by bacteremia, followed by the underlying disease, which has resulted in neutropenia, certain drugs, blood products, and in the case of transplanted patients, allograft rejection [5].

Usually, fever improves with an increase in neutrophils. When fever persists, although the neutrophil count has normalized, the diagnosis of hepato-splenic candidiasis can be considered [9].

ETIOLOGY

The etiology of FUO has changed over time, due to ever-changing disease patterns and the development of diagnostic techniques. The main causes of FUO fall into four categories: infectious, non-infectious (inflammatory), neoplastic and diverse causes [10]. There is also a significant percentage of cases when the etiology of fever remains unknown. A prospective multicenter study estimated the percentage of patients with FUO of 51% [11]. Over time, the frequency of malignancies and infectious diseases as causes of FUO has decreased in favor of noninfectious inflammatory diseases and cases without an etiological diagnosis [12]. From infectious diseases, the most common causes of FUO are tuberculosis and abscesses [13]. The most common malignancies presenting with fever are lymphoma, especially non-Hodgkin, leukemia, renal cell carcinoma, hepatocellular carcinoma, and liver metastasis [13]. Common noninfectious febrile inflammatory conditions correlate with patients' age. Thus, in young patients, systemic juvenile idiopathic arthritis and giant cell arteritis are frequent causes of fever [13]; in older patients, connective tissue diseases or granulomatous diseases are more frequently encountered. Table 1 presents the most common causes of FUO [10, 13, 14].

DIAGNOSIS

FUO remains a diagnostic challenge, considering that there are over 200 disorders that enter in the differential diagnosis. The diagnostic approach therefore should focus on a thorough history of the patient and finding hallmark clinical features that are characteristic of each disease, along with selective non-specific laboratory tests [13]. A detailed history of the patient can provide important diagnostic clues for FUO and should aim to orientate the paraclinical investigations necessary for the positive diagnosis [15]; it should include the history of present illness, data about recent travel, past medical history, drug history, social history and family history (Table 2) [16].

Every FUO category has clinical hallmarks the clinician should search during physical examination [15-18]. Searching potentially diagnostic clues (PDC) during the clinical exam is an important step in the diagnostic workup. PDCs are defined as "all localizing signs, symptoms, and abnormalities potentially pointing toward a diagnosis"[19]:

1. Infectious diseases:
- Spinal tenderness points: suggest spinal tuberculosis, subacute vertebral osteomyelitis;
- Hepatomegaly: Q fever, typhoid/enteric fever, rat bite fever, brucellosis;

- Splenomegaly: miliary tuberculosis, Epstein-Barr virus (EBV) infection, cytomegalovirus (CMV) infection, typhoid/ enteric fever, subacute bacterial endocarditis, Q fever;
- Regional adenopathy: characteristic of cat-scratch disease;
- Epididymo-orchitis/epididymal nodule: EBV infection, renal tuberculosis, brucellosis;
- Cutaneous findings of endocarditis: Osler nodes, Janeway lesions, splinter hemorrhages;
- Heart murmurs: suggest bacterial endocarditis.

Table 1: The most common causes of FUO.

Infections	Malignancies	Systemic inflammatory diseases	Miscellaneous
• Bacterial (tuberculosis, occult abscess, complicated urinary tract infection, osteomyelitis, Q fever, rickettsia infections, enteric fever, brucellosis) • Viral (Epstein-Barr virus, cytomegalovirus, human immuno-deficiency virus) • Parasitic (malaria, toxoplasmosis) • Fungal (histoplasmosis)	• Hepatocellular carcinoma (or liver metastasis) • Leukaemia • Lymphoma, especially non-Hodgkin's • Myelodysplastic syndromes • Multiple myeloma • Renal cell carcinoma • Sarcoma • Colon cancer • Lung cancer • Atrial myxoma	• Giant cell arteritis • Polyarteritis nodosa • Adult Still's disease • Takayasu's arteritis • Granulomatosis with polyangiitis • Mixed cryoglobulinemia • Systemic lupus erythematous • Sarcoidosis • Granulomatous hepatitis • Antiphospholipid syndrome • Behçet's disease	• Drugs (e.g. sulfone-amides, penicillin, nitrofurantoin, vancomycin, antimalarials, H1- and H2-blocking antihistamines, barbiturates, phenytoin, nonsteroidal anti-inflammatory drugs, hydralazine, methyldopa, quinidine, procainamide, anti-thyroid drugs, etc.) • Environmental (metal and polymer fume fevers) • Factitious fever • Disordered heat homeostasis • Alcoholic hepatitis • Venous thrombosis and thromboembolism • Hematoma • Hyperthyroidism and subacute thyroiditis • Pheochromocytoma • Adrenal insufficiency • Familial Mediterranean fever • Tumour necrosis factor receptor-1–associated periodic syndrome (TRAPS) • Hyper-IgD syndrome • Muckle-Wells syndrome • Familial cold autoinflammatory syndrome

2. Malignant diseases:
- Cachexia;
- Diffusely enlarged lymph nodes: lymphoma;
- Erythema nodosum: lymphoma;
- Sternal tenderness points: bone marrow disorders: preleukemia, myeloproliferative disorders;
- Eye examination: Roth spots - lymphoma, atrial myxoma; cytoid bodies - atrial myxoma; retinal hemorrhages - preleukemia.

3. Systemic inflammatory disorders:
- Skin rash: may suggest systemic lupus erythematosus (SLE), adult Still's disease [14];
- Unequal pulse: Takayasu's arteritis;
- Lacrimal gland enlargement: late-onset rheumatoid arthritis (RA), sarcoidosis, SLE;
- Oral ulcers: Behçet's disease, SLE;
- Lymphadenopathy: SLE, sarcoidosis;
- Heart murmur with negative blood cultures suggests a possible diagnosis of Libman-Sacks endocarditis.

4. Miscellaneous diseases. Miscellaneous fevers of unknown origin are often diagnosed based on historical clues rather than clinical findings. Relative bradycardia may suggest drug fever. Cirrhosis is often overlooked as a possible cause of FUO [15, 16]. Splenomegaly may be another important clue that may suggest regional enteritis, cirrhosis or hyper-IgD syndrome.

Paraclinical investigations

Before initiation of any diagnostic tests, treatments, such as antibiotics or glucocorticoids, must be stopped, as they can mask different signs and symptoms and thus delay the diagnostic process.

In any patient with FUO, the following basic laboratory tests are necessary: erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), hemoglobin, platelet count, leukocyte count and differential, electrolytes, creatinine, total protein, serum protein electrophoresis, alkaline phosphatase, aminotransferases (AST, ALT), LDH, creatine kinase, antinuclear antibodies, rheumatoid factor, urinalysis, together with blood cultures, urine culture, a tuberculin skin test or Interferon-Gamma Release Assays (IGRA), and imaging studies - chest X-ray, abdominal ultrasonography [19].

Table 2: Key clues to FUO derived from history.

Infectious Diseases	Malignant disorders	Systemic inflammatory diseases	Miscellaneous Disorders
Prior/invasive procedures or surgeries (abscess)	Significant weight loss (2 lbs/week)	Arthralgias	Fever periodicity (cyclic neutropenia)
Dentition (apical abscesses, subacute bacterial endocarditis)	Anorexia	Myalgias	History of lymphadenopathy (Rosai-Dorfman, Kikuchi's disease)
Antecedent/concomitant infections	Post-hot bath pruritus	Dry cough (Giant cell arteritis)	Neck/jaw pain (subacute thyroiditis)
Animal/pet contact (Q fever, brucellosis, toxoplasmosis etc.)	History of adenopathy	Visual disturbances (Giant cell arteritis)	Inflammatory bowel disease (regional enteritis)
Mosquito/tick exposure (malaria, babesiosis)	History of malignancy	Acalculous cholecystitis (SLE, peri-arteritis nodosa)	Alcoholism (cirrhosis)
Rodent exposure (leptospirosis, rat bite fever)	Pel-Ebstein fever-week long high fevers with week-long remissions (Hodgkin disease)	Family history of systemic inflammatory diseases	Medications (drug fever)
Blood transfusions (CMV, HIV)	-	Jaw claudication (Giant cell arteritis)	Prolonged immobility, car trips, flights (thromboembolic disease)
Immuno-suppressive drugs	-	-	-
Nocturia (prostatitis)	-	-	-
High-risk sexual practices	-	-	-
Travel to an endemic area	-	-	-

Complete blood count contains often-overlooked clues, for example, leukopenia, monocytosis, lymphocytosis-relative lymphopenia, eosinophilia, basophilia, atypical/abnormal lymphocytes, thrombocytosis, and thrombocytopenia. In FUO, an isolated alkaline phosphatase elevation may be a clue for lymphoma [20, 21, 22]. Eosinophilia may suggest polyarteritis nodosa, drug fever, or visceral leishmaniasis. Low levels of hemoglobin or hematocrit may point toward occult hemorrhage or hematoma (often retroperitoneal).

After all potentially diagnostic clues retrieved from the patient's history, clinical examination and paraclinical investigations have been identified, the clinician should make a list of the most probable diagnoses; further specific investigations should be guided by these diagnoses.

If the diagnosis remains elusive, more advanced paraclinical investigations may be necessary. In patients with hepatomegaly or splenomegaly, abdominal computed tomography (CT) scan may be useful in detecting abnormalities such as adenopathy, abscesses or masses [23]. Echocardiography is important in case of suspicion of endocarditis or atrial myxoma. Positron emission tomography-computed tomography (PET-CT) scans help detect obscure infectious/neoplastic causes of FUO, for example, lymphomas, Q fever endocarditis or aortic graft infection [24]. Magnetic resonance imaging (MRI) may be useful in case of osteomyelitis. Digestive endoscopy may become necessary in case of suspicion of inflammatory bowel disease or sarcoidosis [25].

Invasive tests, such as lymph node biopsy, bone marrow biopsy, epididymal nodule biopsy, liver or ileal biopsy may have a high diagnostic yield [26-29]. However, these tests should be ordered based on narrowed diagnostic possibilities [30, 31].

TREATMENT

Treatment should be directed toward the underlying cause once a diagnosis is made. Because they may mask, delay or obscure the clinical manifestations, empirical therapeutic trials with antibiotics [32, 33], glucocorticoids, or antituberculous agents should be avoided in FUO, except in immunocompromised, neutropenic patients, in case of severe systemic illness or those with a rapid deterioration (patients with central nervous system or miliary tuberculosis or giant cell arteritis) [34, 35].

CONCLUSION

FUO remains one of the most difficult diagnostic challenges, as the differential diagnosis consists of over 200 malignant/ neoplastic, infectious, rheumatic/inflammatory, and miscellaneous disorders. It is essential for the clinician to obtain potentially diagnostic clues through a detailed history of the patient, complete physical examination and guided paraclinical investigations. Interdisciplinary consultations may be appropriate in a majority of patients with FUO.

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ORIGINAL ARTICLE

Frequency and genotyping of human papillomavirus in patients with DNA tracking technique during 1996-99

Bahareh Panahi[1], Abbas A. Sepahi[1], Mahdieh Farzanehpour[2]

Abstract: *The prevalence of human papillomavirus (HPV) infections and their role in cervical cancer have been well documented in numerous articles, but it seems that a study in this field in developing countries and the Middle East, including Iran. It's not done. Therefore, the present study was designed and performed to investigate the frequency and genotyping of human papillomavirus in patients by DNA tracking technique. This study was a population-based study in which the frequency and genotyping of human papillomavirus in patients by tracking technique was designed and performed on 534 men and women living in Tehran by DNA tracking technique. Sequences obtained using the NCBI site were analyzed at (http://www.ncbi.nlm.nih.gov/BLAST/LaserGene) and Bio edit software. So that in three low-risk groups including 6 and 11 genotypes, Intermediate risk includes 26-31-33-35-39-40-42-45-49-51-52-53 genotypes -55-56-58-59-66-63-83 and High risk includes 16 & 18 genotypes were divided. According to the results, the prevalence of the papilloma virus in the study population was 21.16%, of which 19.46% were in the high-risk group, 69.89% were in the intermediate-risk group and 50.43% were in the low-risk group. According to the results of the present study, although the prevalence of HPV in the study population is less than in many countries in the world, it is suggested that conducting epidemiological studies in the country to extract the true prevalence and plan to prevent and control this disease.*

Keywords: *human papillomavirus, frequency, genotype, DNA tracking technique*

INTRODUCTION

Cervical cancer is the fourth most common malignancy and a major cause of death in women worldwide. Numerous reports show an association between a group of DNA oncogenic viruses and the development of malignancies in the human population, including human papillomavirus [1].

More than 200 different types of human papillomavirus have been found, of which about 40 are capable of infecting the genital tract. Human papillomavirus is classified into two low-risk and high-risk groups based on the degree of malignancy and cancer [2].

In the latest classification published by the World Health Organization, the International Agency for Research on Cancer, genotypes 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, and 59 as high-risk types, other cultivars including genotype 26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85 and 97 are also known to be carcinogenic types and genotypes 6 and 11, which are known to cause benign lesions of the genital tract. Introduced as low risk types [3].

The progression of cancer requires the expression of viral genes, the entry of viral DNA into host chromosomes, and changes in the genes and gene products of the host cell. This group of viruses requires the DNA polymerase of host cells to replicate the genome, and from the entry points created through fine scratches in the stratum corneum cell layers to reach the basal layer of the epithelium, the location of basal cells and pre-cells. They use instruments that can be reproduced [4]. The E6 and E7 oncoproteins in HPV are key players in carcinogenesis and target tumor-suppressing proteins in host cells. In high-risk HPVs, the E6 protein forms a complex with a ubiquitin ligase [5]. This complex binds to p53, which is followed by the breakdown of p53, which inactivates it. P53 acts as a transcription factor and induces

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the expression of genes involved in cell cycle arrest and apoptosis. When E6 is not inactive, the p53 protein increases, causing p53-dependent apoptosis and killing the infected cell before the virus multiplies [6]. E7 protein also exerts its oncogenic effect by inactivating retinoblastoma protein and two proteins related to retinoblastoma protein, p106 and p130 [7].

Retinoblastoma protein suppresses tumors and plays a key role in regulating the eukaryotic cell cycle. In the case of high-risk HPV infection, the E7 protein breaks down the E2F-Rb complex, releasing E2F, resulting in the cell entering the S phase outside the original time, which disrupts the cell cycle [8].

The human papillomavirus completes its evolution as it crosses the basal layer with the superficial cell layers, which increases the expression of the virus's L1 and L2 proteins, which play an important role in their identification. High-risk types of HPV cause lesions in the cervical epithelium, the severity of which varies depending on the extent to which squamous epithelial cells have been replaced by basaloid cells in infected species [9].

Grading CIN III, CIN II, CIN I High-risk HPV is a necessary and insufficient factor in the development of cervical cancer, so other factors associated with cervical cancer include smoking, age, nutrition, race, geographical location, having Multiple sexual partners noted long-term use of birth control pills, weakened immune systems, and host-related factors [10].

The global prevalence of HPV infections in 2012 is estimated at 440 million. In 2012 alone, these infections led to 550,000 cases of cervical cancer and approximately 288,000 deaths [11]. Also, according to published statistics on the diagnosis of human papillomavirus in various diseases, infection of this virus in 99% of cervical cancers, 97% of anal cancers, 70% of vaginal cancers, 47% of penile cancers, 40% of gastrointestinal cancers, 47 The percentage of urological cancer and 11% of cases of oral cancer have been observed [12].

In most of the results obtained from the studies of researchers inside and outside the country, the abundance of 6, 11, 16, and 18 is more than other genotypes. Therefore, the frequency and determination of HPV genotype due to the identification of low-risk and high-risk types have diagnostic and therapeutic value and can be prevented by screening and timely diagnosis of lesions that lead to malignancy. Therefore, it is recommended that women between the ages of 30 and 65 have a screening test every 5 years [13].

Human papillomavirus is known to be one of the most important causes of cervical cancer [14]. HPV infection is a sexually transmitted disease that the prevalence and type of virus that causes it varies depending on the area where people live. Studies in different human populations show that more than 50% of women become infected with at least one type of virus during their lifetime.

Human papillomavirus is a small, uncoated virus with double-stranded DNA of at least 200 different types. About 35 to 40 types of this virus infect the skin epithelium or mucous membranes in the female reproductive system [15]. Most viral infections do not cause specific symptoms or diseases and heal on their own. If the infection persists, the variety of lesions of the virus is high, ranging from benign skin warts that are self-limiting and often appear on the hands and feet to malignant and invasive lesions manifesting as fatal cervical cancer [16].

Condyloma acuminata is one of the most common sexually transmitted diseases. Mucosal types can be divided into high-risk and low-risk according to the risk of disease. High-risk types of human papillomavirus play a major role in causing cervical cancer. Among these viruses, at least 15 types of high-risk viruses cause more than 95% of cervical cancers and 80% of vaginal cancers [17]. Therefore, its accurate diagnosis will have a significant impact on the control and management of this cancer and the promotion of women's health in society.

Currently, 170 HPV genotypes are classified according to the type of biological wall and oncogenicity and their type of racial evolution, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are known to be high-risk, and types 26, 53, and 66 may also be oncogenic [18]. Abnormal cervical epithelial cells can be detected by Pap smear staining and microscopic observation. However, this diagnostic method is only used for women at risk for advanced disease, and about 50% of the lesions will not be detected if only a Pap smear is performed on them, as the HPV virus does not grow in culture and serological tests are of limited accuracy.

HPV infection triggers a humoral immune response against the capsid protein, which is an antibody that stays in the body for many years (serological tests are not suitable for diagnosing present and past infections [19]. As a result, accurate diagnosis of HPV infection relies heavily on molecular methods.

Molecular techniques can be used to accurately diagnose and identify HPV, as well as to inform the patient and follow up after treatment. Accurate HPV genotyping is essential to classify patients into high-risk or low-risk groups.

Evidence also suggests that the presence of multiple HPV types may reflect repeated exposure to the virus and may be associated with an increased risk of disease progression [20].

Prevalence and genotyping of human papillomavirus in precancerous and cancerous samples with DNA tracking technique from 1396 to 1399 in Tehran to evaluate the health of the female community.

This study aimed to investigate the frequency and genotyping of human papillomavirus in precancerous and cancerous samples by DNA tracking technique.

METHODS

This case-control study was performed by collecting 534 biopsy specimens from men and women. All biopsy specimens were examined for the presence of the human papillomavirus genome.

DNA extraction

DNA extraction was performed according to the protocol of a High pure viral nucleic acid kit from Roche (Germany). The steps are summarized in Figure 1.

Figure 1: General process of DNA extraction protocol by High pure viral nucleic acid kit

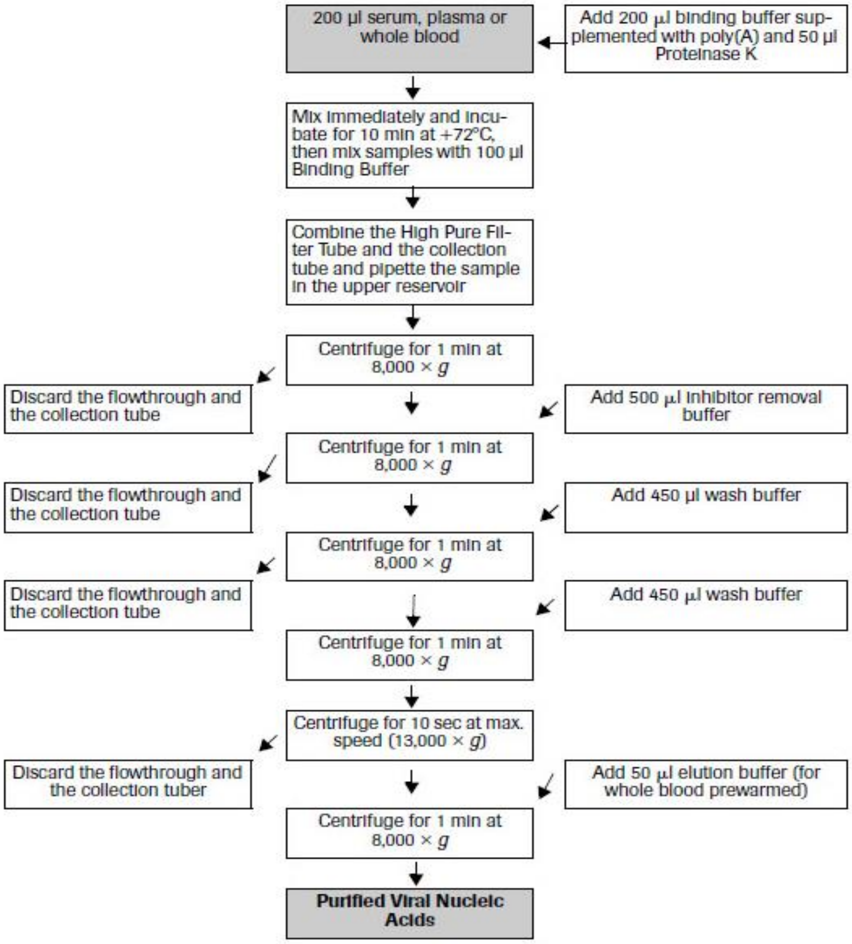
200 µl serum, plasma or whole blood
Add 200 µl binding buffer supplemented with poly(A) and 50 µl Proteinase K
Mix immediately and incubate for 10 min at +72°C, then mix samples with 100 µl Binding Buffer
Combine the High Pure Filter Tube and the collection tube and pipette the sample in the upper reservoir
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 500 µl inhibitor removal buffer
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 450 µl wash buffer
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 450 µl wash buffer
Centrifuge for 1 min at 8,000 × g
Centrifuge for 10 sec at max. speed (13,000 × g)
Discard the flowthrough and the collection tuber
Add 50 µl elution buffer (for whole blood prewarmed)
Centrifuge for 1 min at 8,000 × g
Purified Viral Nucleic Acids

Quality control of extracted DNA

At this stage, using the PCR technique and targeting the β-Globin gene as a housekeeping gene, quality control was performed, so that samples that were positive for the β-globin gene were considered suitable samples for testing in later stages. Were taken. Samples that were negative for this gene were retested, and if we did not respond again, we would have to retrieve the patient sample.

Table 1: β-Globin gene primers

Pco3	Forward	5′-ACACAACTGTGTTCACTAGC-3′
Pco4	Reverse	5′-CAACTTCATCCACGTTCACC-3′

Nested PCR for human papillomavirus genome

Nested PCR is a modified model of PCR that reduces nonspecific connections in the reaction product (due to propagation of components that are not expected). In this method, two pairs of primers are used to increase the sensitivity of PCR. First, with a pair of first primers over 15-30 cycles, specific pieces of target DNA are amplified. The resulting PCR product is then transferred to another tube and used as a template, and the second stage of PCR is performed by a pair of second primers. In the Nested PCR test to detect HPV, we first used the pairs of primers mentioned in Tables 2 and 3.

Table 2: Sequence of MY09/11 primers (Outer Primers)

MY09	Forward	5'-CGTCC(A/C)A(A/G)(A/G)GGA(A/T)ACTGATC-3'
MY11	Reverse	5'-GC(A/C)CAGGG(A/T)CTATAA(C/T)AATGG-3'

Table 3: Sequence of GP5+/6+ primers (Inner Primers)

GP5+	Forward	5'-TTTGTTACTGTGGTAGATACTAC-3'

The PCR product with MY09/11 and GP5+/6+ primers are 450 and 150 bp, respectively.

Sequencing

To determine the genotype of HPV positive samples, Biosystems ABI 911 automatic device was used using the cyclic sequencing method.

For this purpose, 4 separate PCR reactions are performed, each containing a similar primer and a different ddNTP terminator labeled with fluorescence dye.

Genetic analysis of HPV sequences

Sequences obtained using the NCBI site were analyzed at (http://www.ncbi.nlm.nih.gov/BLAST/LaserGene) and Bioedit software. So that in three low-risk groups including 6 and 11 genotypes, Intermediate risk includes 26-31-33-35-39-40-42-45-49-51-52-53 genotypes -55-56-58-59-66-63-83 and High risk includes 16 & 18 genotypes were divided.

RESULTS

The data obtained from 534 participants in the study were analyzed using descriptive statistical methods (mean, standard deviation, frequency, and percentage) and the results were obtained according to the objectives and hypotheses of this study, respectively.

Table 4 shows the age distribution of the research community in terms of quantitative variables.

The minimum age for men is 24 and the maximum age is 65, the minimum age for women is 19 and the maximum age for women is 75.

The mean and standard deviation of the age of men and women are equal to (35.12 ±77.44), (43.11 ±23.15).

Table 4: Descriptive statistics of quantitative variables

Variables	At least	Maximum	Mean	Standard deviation
Age of men	24	65	35.77	12.44
Age of women	19	75	43.23	11.15

Table 5 shows the PCR findings based on positive and negative criteria. Of the 18 men referred, 3 were negative for papillomavirus, and 15 were positive. Out of 516 female patients, 418 were negative for papillomavirus, and 98 were positive.

According to the results, the prevalence of the papilloma virus in the study population was 21.16%.

Table 5: Report of PCR findings based on positive and negative criteria

Variables	Positive number	Negative number	Percent
Age of men	15	3	13.27
Age of women	98	418	86.72

According to Table 6, 3 men were simultaneously High risk, Intermediate risk, and Low risk, 8 were only Intermediate risk, 3 were Intermediate risk and Low risk and 3 were Low risks.

According to Table 7, 34 women were Low risk, 36 were Intermediate risk, 7 were Intermediate risk and Low risk, 15 were High risk, 3 were High risk and Low risk, and 3 were High risk and Intermediate risk.

Table 6: Report PCR findings based on risk criteria – men

Low risk	Intermediate risk	High risk	Number of people
*	*	*	**1**
	*		**2**
	*		**3**
	*		**4**
	*		**5**
	*		**6**
	*		**7**
	*		**8**
	*		**9**
*	*		**10**
*	*		**11**
*	*		**12**
*			**13**
*			**14**
*			**15**

Table 7: Report PCR findings based on risk criteria – women

Low risk	Intermediate risk	High risk	Positive number
	*	*	1
	*	*	2
	*	*	3
*		*	4
*		*	5
		*	6
		*	7
		*	8
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*			94
*			95
*			96
*			97
*			98

Table 8 shows the frequency of different genotypes of papillomavirus in the study population.

Table 8: Report of PCR findings based on the frequency of papillomavirus genotype

Percent	Frequency	Genotype	Risk
34.51	39	HPV6	Low risk
15.92	18	HPV11	
0.88	1	HPV26	Intermediate risk
0.88	1	HPV30	
6.19	7	HPV31	
7.07	8	HPV32	
7.96	9	HPV33	
0.88	1	HPV34	
12/38	14	HPV35	
0.88	1	HPV39	
1.76	2	HPV40	
6.53	4	HPV42	
0.88	1	HPV43	
1.76	2	HPV44	
0.88	1	HPV51	
2.65	3	HPV52	
1.76	2	HPV54	
2.65	3	HPV56	
4.42	5	HPV58	
2.65	3	HPV66	
0.88	1	HPV68	
2.65	3	HPV70	
1.76	2	HPV81	
1.76	2	HPV87	
19.46	22	HPV16	High risk

DISCUSSION

Today, cancer is a dangerous and deadly disease that is one of the most important causes of death worldwide. In 2012, 14.1 million new cases of the disease were reported worldwide, and the disease has killed 8.2 million people [21]. While the mortality rate and cancer incidence in 2008 was 7.6 million and 12.7 million people, respectively [22]. Approximately 47% of new cancers and 55% of deaths from them occur in developing and less developed countries.

In Iran, cancer is the third leading cause of death and more than 30,000 people die of cancer every year [23]. With the increase in life expectancy and the percentage of aging in the country's population, the advancement of medical science and technology, and the departure of lifestyles from a clean environment free of industrial carcinogens, cancer incidence is expected to double in the next two decades. According to the World Health Organization, the incidence of cancer in Iran in 1399 will reach about 86,000 cases in the total population and the resulting mortality rate will reach about 63,000 cases [24].

Various factors involved in cancer include lifestyle, heredity, environment, host factors, and infectious agents. Infectious agents are one of the most important controllable factors in cancer. Studies show that 17.8% of cancers are associated with infectious agents, among which viral agents are of great importance because about 12.1% of cancers are associated with viral agents [25]. Annually, 1.3 million new cases of cancer are attributed to viruses, and cancers caused by viral agents lead to the death of 1 million people each year [26].

One of the viral families associated with cancer is viruses belonging to the papilloma viridae family. Cervical cancer is the fourth most common cancer in women worldwide [27] and has received much attention today due to the large social and economic costs imposed on society and the health system. Given that cervical cancer has a long and slow progression, early detection, especially in precancerous stages, can help reduce mortality from this important cancer. Current methods for screening, diagnosis, and follow-up of this disease are based on cytological and histological methods; for example, Pap smear, which has been used for many years and is unchanged [28].

Due to the limitations of these methods, including insufficient sensitivity in a study and the need for repeated studies, and high dependence on individual skills and sampling conditions, as well as relying on the principle of increasing our knowledge of this disease and understanding the causes of creating and Its development (mentioned in the introduction), and the invention of new diagnostic methods as a complement or even alternative to the methods currently used have been of interest to many researchers in this field. The study of the pathogenesis and the attempt to find biomarkers of cervical cancer have led to the emergence of new ideas for early diagnosis and treatment [29].

Recent studies in the field of cervical cancer

Scientific information is focused on the initial diagnosis and targeted treatment of cancer. HPV induces metastasis and related events by affecting cellular signaling pathways. Human papillomavirus increases the expression of many cellular genes, including genes involved in reproduction and survival, the transition from epithelial to mesenchymal, substrate-independent growth, and oxidative stress [30]. Research on cervical cancer shows that changes in the function of several genes lead to abnormal proliferation and invasion of tumor cells.

Cervical cancer has a global prevalence and is the most common type of cancer in developing countries. It is the fourth most common cancer among women and annually more than 400,000 cases of this malignancy are reported in the world, which accounts for approximately 12% of the most common cancers among women [31]. Researchers point to several risk factors for cervical cancer. In a case-control study conducted in Mexico, factors such as the number of deliveries, the age of onset of sexual activity, and the use of oral contraceptives were examined. The prevalence of this cancer was 5 times higher in women who had given birth 12 times than in women who had given birth up to 3 times. It was also twice as common in women who started having sex after the age of 19 than in women who were under 15 when they started having sex. In addition, the use of hormonal contraceptives has been shown to increase the chances of developing cervical cancer [32].

According to many case studies, HPV infection has been identified as a major factor in the development of neoplasia and malignancy in the genital epithelium, and this is especially evident when there is a persistent infection with high-risk types of the virus [33]. Research over the past two decades has shown a strong association between cervical cancer and some types of human papillomavirus. In more than 90% of cases of invasive cervical cancer, the HPV genome is detected by molecular methods [34].

Pap smear test is used as a common test for screening patients around the world due to its relative ease and cheapness, but unfortunately, the number of false negatives in this test is very high [35]. HPV infections are usually asymptomatic, so methods to improve the screening process are needed to diagnose the infection. Epidemiological studies and efforts to develop vaccines require reliable methods to diagnose HPV genital infections and to determine the common genotypes in the infection [36].

Methods such as PCR, which detect the DNA of the virus, actually identify the cause of the infection, while cytological tests, such as Pap smear, identify the lesions and complications of the infection [37, 38]. PCR technique is considered one of the most sensitive methods for detecting HPV in genital tissues due to its ability to amplify viral DNA using specific primers, and its combination with sequencing as a confirmatory test increases its accuracy by 100%. Therefore, its use along with cytological methods can be significantly effective in increasing the resolution and accuracy of clinical examinations of patients [39].

Determining the common genotypes of the virus in a geographical area can also be very important in epidemiological studies and HPV vaccination development programs [40]. Experimental findings on the diagnosis of HPV genome in the samples indicate that the prevalence of carcinogenic types of HPV in patients studied in this study was 19.46%, which is consistent with the results of studies in some other regions of Iran.

In a study conducted in northern Iran, the presence of HPV in cervical cancer samples was shown to be 81.4%. This study shows that the frequency of HPV infection in cases of cervical cancer in Iran is also significant and comparable to other parts of the world [41]. Type 6 was one of the most common genotypes found in this study. Since cervical cancer usually occurs after 10-12 years after HPV infection, it is known as a complication that occurs in middle age. Findings show that in Yazd province, the highest prevalence of HPV in age groups (31-50 years) is 60%, while in many other countries, the highest prevalence of this virus is usually observed in lower age groups [21]. This difference is related to the onset of sexual intercourse.

In Iran, sexual intercourse usually begins with marriage and usually occurs between 20-30 years, but in Western societies, sexual activity usually begins after puberty. This could explain the incidence of cervical cancer in older Iranian women with HPV compared to many countries where the age of HPV infection is usually lower and in the age group of 21-39 years [33].

In some studies, some statistical populations are diagnosed in terms of patient pathology but show negative results in the HPV PCR test. There are several possible justifications for this: One is that the formation of malignant and cancerous lesions in these people was due to a carcinogen other than the HPV virus. Another is that according to a series of studies, the cause of false-negative results in PCR tests, especially in cases where pathological tests indicate the presence of malignant and invasive cancers, may be related to how the virus genome is replaced in the host cell chromosome [36].

According to one hypothesis, the cyclic genome of the virus is replaced in the chromosome of the host cell in such a way that it transforms the region of the gene that was the target of the PCR process and therefore changes the PCR results. Although the E2 gene region of the virus genome is usually degraded during the replacement process, in some cases the PCR primer binding sequences in the L1 gene region may be contiguous with the degraded regions of the virus genome, resulting in HPV DNA detection by PCR.

Another factor that can negatively affect the falsification of PCR results is the problems that arise in the process of preparation and extraction of DNA from formalin and paraffin-stabilized samples [22]. It has sometimes been observed that in studies using fresh tissue samples for PCR testing, the amount and percentage of HPV reported are usually higher than that of formalin-stabilized samples, which is probably due to abnormalities.

In the present study, the nested PCR technique was used to evaluate the frequency of the HPV genome. The results showed a significant relationship in the presence of the human papillomavirus genome. The present study, confirming the reports of some researchers, showed that the selection of an appropriate extraction method is important in improving the quality and quantity of DNA obtained and is very effective in the reliability of diagnostic results. In the DNA extraction stage, the cross-sectional preparation of tissue is very important. Cervical epithelial tissue has a high cell density. Therefore, the thickness of the cut should be appropriate and appropriate. If the thickness of tissue sections is not appropriate, PCR may interfere. If the cut thickness is low, the DNA concentration is low and if the thickness is increased, the protein degradation is incomplete and the impurity increases [16].

DNA extracted from samples is often high in concentration and contains factors that attenuate PCR, so it is best to use multiple dilutions of DNA in each sample for PCR to reduce the likelihood of false-negative responses. The presence of false-negative reactions can be due to the presence of many inhibitors in biological samples that have the potential to interfere with the PCR reaction. Inhibitors inhibit cofactor activity in PCR. On the other hand, these inhibitors can bind to the target site of Taq DNA Polymerase enzyme or directly inhibit the activity of this enzyme [11].

Therefore, these inhibitors can cause slightly erroneous results or false-negative results. In this case, the first step is to dilute the sample to reduce contaminants. In preparing the dilutions, it is necessary to observe the sterile conditions. In this study, we faced this challenge and this problem was solved by diluting the sample. The use of Nested PCR, which increases the sensitivity and accuracy of diagnosis, especially in low-titer samples of the virus, and also reduces the concentration of inhibitors that interfere with PCR performance due to its two-step concentration, can be very efficient [8].

High concentrations of primers with non-specific bonding cause dimers and non-specific products. Low primer also reduces bandwidth. Differences in the results of human papillomavirus genome identification in different studies may be due to the different populations studied, the sexual habits of the people in that community and the age of onset of sexual intercourse, the rate of human papillomavirus exposure, laboratory techniques and methods. Be used [42]. According to Table 3-4 (a), 3 men were simultaneously High risk, Intermediate risk, and Low risk, 8 were only Intermediate risk, 3 were Intermediate risk and Low risk and 3 were Low risk. According to Table 7, there were 34 low-risk women, 36 Intermediate risk, 7 Intermediate risk, and Low risk, 15 High risk, 3 High risk, and Low risk, and 3 High risk and Intermediate risk.

The largest and most comprehensive study to investigate the presence of the HPV genome in cervical cancer specimens was performed according to a standard protocol by the International Agency for Research on Cancer (IARC). About 1,000 women with confirmed cervical cancer from 22 countries around the world were included in the study. Frozen biopsy specimens of tumors in the central laboratory were examined for the presence of human papillomavirus DNA by PCR. The results of this study proved the presence of HPV-DNA in about 99.7% of the samples, which is a reason for the causal role of HPV in the development and progression of cervical cancer [43, 44]. This study was performed on 66 paraffin blocks of cervical cancer tissue by the Nested Multiplex-PCR method. Type 16 human papillomavirus was detected in 60% of the samples [45]. In several other separate studies conducted in Iran, the presence of the HPV genome in cervical cancer samples were reported to be 71.8%, 83.7%, and 77.7% [46].

In a combined analysis and a meta-analysis performed by the IARC, 3,000 and 10,000 cervical cancer samples were examined for the distribution of HPV types, respectively. Eight types of the most common types of HPV were identified in both series of studies, which are in descending order: HPV types 16, 18, 45, 31, 33, 52, 58, and 35. Among these eight common types, HPV-16 is the most common. Outbreaks and HPV-35 have the lowest prevalence. These eight types are responsible for about 90% of all cervical cancers worldwide [47].

Persistent infection with the human papillomavirus is the most important cause of the development and progression of cervical cancer, but only a small percentage of women infected with HPV develop cervical cancer. Therefore, there may be other risk factors for having an independent or boosting role of HPV in the occurrence of cervical cancer, such as having multiple sexual partners, initiating sexual intercourse at an early age, contracting the human immunodeficiency virus (HIV), smoking, oral contraceptives, and other pathogenic factors, although the exact mechanism of each is still unknown [48].

Therefore, it is necessary to study the molecular mechanisms of cervical cancer and the role of HPV in creating and facilitating the process of this disease [49].

CONCLUSIONS

Several types of vaccines have been proposed to control cervical cancer, some of which have been licensed by the World Health Organization. HPV vaccination is common in countries such as the United States and Canada. The structure of these vaccines consists of L1 and L2 viruses of the virus and they are made using the common epitopes 6,18,16,11 HPV. Given that some types of papilloma viruses have a specific regional prevalence and are also identified in cervical cancer; therefore, some researchers believe that epidemiological studies can be an effective aid in determining the appropriate structure of the vaccine. Therefore, in Iran, such studies will be very useful in selecting or designing a suitable vaccine.

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ORIGINAL ARTICLE

From the discovery of combat gases to insecticides and bioterrorism – a brief history

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Abstract: *The identification of organophosphate (OP) compounds dates back to the 1800s. OPare largely used pesticides in the agricultural field and households but prior use as a chemical weapon was considered during wars. Their toxicity on humans has been previously studied and addresses both acute intoxication and low-level chronic exposure. Symptoms of acute exposure describe a cholinergic syndrome with bradycardia, miosis, confusion, fasciculations, tearing, sialorrhea, diarrhea, bronchorrhea, and bronchospasm. The intermediate syndrome causes paralysis of the gait muscles, neck flexors, and respiratory failure. Moreover, unrestricted access to OP has increased the rate of suicide, accounting for around 30% of cases, increasing concern about OP availability. Western countries have drastically restricted access to OP by imposing regulations regarding handling these hazardous substances, but efforts have to be made to globally reduce this struggle.*

Keywords: *organophosphates, pesticides, combat gases, bioterrorism, poisoning*

INTRODUCTION

Organophosphates (OP) are substances that contain carbon and phosphorus atoms in their structure (Figure 1), but not all compounds with this component inhibit acetylcholinesterase [1]. Depending on their chemical substrate, they can be divided into four classes with individual physical features. OP has been widely used as a pesticide, compounds being spread both in the agricultural field and in households [2].

Figure 1: Structure of organophosphate compounds (ro.wikipedia.org)

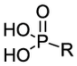

Their specific physical and chemical characteristics determine the duration of the pesticide effect, as well as the duration of persistence on applied surfaces or soil [3]. As a result, the degradation mechanisms differ from one OP compound to another [4].

Out of the OP, chlorpyrifos (Figure 2), is the world's most widely used organophosphorus insecticide, being effective against mosquitoes, and lice, while dichlorvos has shown beneficial in controlling plant lice (aphids), flies or spiders [1,5].

Figure 2: Chemical structure of Chlorpyryphos (cen.acs.org)

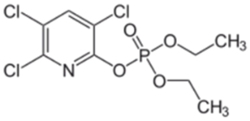

Diazinon (Figure 3), a thiophosphate compound, is effective against silverfish, fleas or ants, malathion is indicated against mosquitoes, wasps or insects that affect fruit trees [1]. Parathion (Figure 4) is a broad-spectrum insecticide, but its use has been restricted due to its toxic effects [6].

In addition to their use as pesticides, ester OP compounds are used as flame retardants or plasticizers in various industries. They are utilized for coating electronic instruments, such as antifoams or adhesives [7]. The OP

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esters are mixed with the other elements in the composition of the desired substances, without making any chemical bonds with them [8].

Figure 3: Chemical structure of Diazinon (en.wikipedia.org)

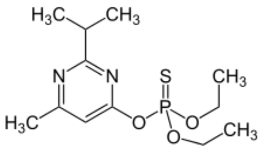

Figure 4: Chemical structure of Parathion (en.wikipedia.org)

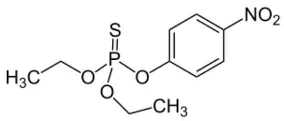

OP poisoning is a major cause of morbidity and mortality worldwide. Due to its widespread use in agriculture and domestic use, contact with OP substances has become very common. In countries where there are no imposed regulations on OP use in agriculture, the number of cases of intoxication is significantly higher [9].

A special aspect regarding the incidence of OP poisoning is their use as a method of suicide. Globally, this type of intoxication is considered one of the main methods of suicide, accounting for approximately a third of all cases, raising it as a top priority health problem [10].

HISTORY OF ORGANOPHOSPHATE COMPOUNDS – FROM DISCOVERY TO HAZARDOUS USE

With the perfection of ether synthesis methods by the chemist Alexander Williamson, the synthesis of organophosphates (OP) has been possible since the middle of the 19th century [11,12].

The initial discoverers of the OP class are traditionally considered to be the French chemist Philippe de Clermont and the Russian Wladimir P Moschnin, but the contribution of the Swiss Franz Anton Vögeli must not be forgotten [11]. In his laboratory, Gustav Magnus managed to synthesize two products resulting from the combination of ether with phosphoric acid [11]. Other sources claim that the Swedish Bo Holmstedt was the first to describe the structure of the first OP, namely tetraethyl pyrophosphate (Figure 5), in a letter to the French chemist Clermont [13].

However, there is evidence that indicates that several OP were synthesized by the French Jean Louis Lassaigne well before those mentioned above [13,14]. In the early 1800s, he succeeded to demonstrate the existence of phosphovinic acid, which was later synthesized by Jules Pelouze [12]. Later on, Anton Vögeli used these studies to elaborate on diethyl- and triethyl phosphate [11,12] (Figures 6 and 7).

Figure 5: Chemical structure of tetraethyl pyrophosphate (en.wikipedia.org)

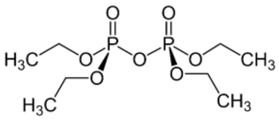

Figure 6: Chemical structure of Diethyl Phosphate (selleckchem.com)

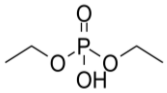

Figure 7: Chemical structure of Triethylphosphate (en.wikipedia.org)

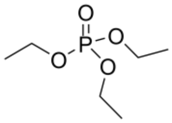

Although in recent research the French chemist Clermont has been invested with a major role in laying the foundations of OP, studies of the history of medicine indicate that W.P. Moschnin contributed to at least the same importance [15]. Moreover, there is evidence of joint activity of the two chemists in the same laboratory in Paris [13,15]. Despite being considered the parents of OP, Clermont and Moschnin were unable to understand and describe the effects of these substances [13]. After obtaining tetraethyl pyrophosphate, the first product that could inhibit acetylcholinesterase, Clermont's only description, obtained after tasting, distinguished that it is "a thick and sticky liquid, with an intense taste and a special smell" [11].

The chemists Ernst Ratzlaff and Adolf Schall identified, under the guidance of prestigious professor August Michaelis, a compound named diethylphosphoramidocyanate, similar to what would be called decades later "tabun"-diethylphosphoramidocyanate (Figure 8) [16].

Figure 8: Chemical structure of Tabun (researchegate.net)

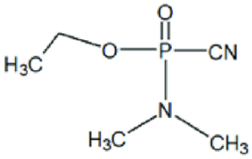

Research on the effects of OP was subsequently continued, at the beginning of the 20th century, by the Germans Willy

Lange and Gerda von Krüger, who first described the toxicity of these substances [17].

During the preparation of his doctoral thesis, Gerda von Krüger, together with his coordinating professor, Willy Lange, elaborated their most important scientific work: the description of toxic effects of OP, namely shortness of breath, photosensitivity, and disturbance of consciousness, which disappear within hours after ending exposure [17]. With the introduction of the Nazi regime, Willy Lange is forced to stop their study and reaches the United States, where he will devote himself to other research work [17].

The study of the toxic effects of OP was continued by the chemist Gerhard Schräder, who, in the attempt to obtain an OP-based insecticide, is exposed to it and needed medical care for several days [16]. When he returns to the laboratory, Schräder identifies the substance that caused the manifestations as ethyl-N-N-dimethylphosphoramido-cyanate, which he called “tabun” (from the German word "taboo") [16].

Throughout his activity Schräder successfully synthesized many OPs and created the first OP insecticide, octamethylpyrophosphoramide (Figure 9), marketed under the name E605 and used in agriculture [13]. With the end of World War II, this substance was taken over by the allied countries, where it was widely marketed under the name of parathion [13].

Figure 9: Chemical structure of Octamethylpyrophosphoramide (fischersci.com)

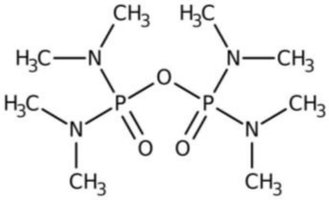

Although Nazi Germany secretly tried to create a chemical weapon based on OP, it has never been used on battlefields, despite repeated accusations from Stalin [13,18]. At the same time, other countries searched for chemical weapons based on tabun, sarin, or phosgene, but they were not used during World War II [13,18,19]. Although it has been proven that Japan used this type of weapon during World War II, it did not contain OP [19].

After the war, in the United Kingdom, the chemist Ranajit Ghosh, synthesized a new product called VX (Figure 10), thus creating a new class of OP (class V – from the English word "venomous") which was used as a chemical weapon [20].

Figure 10: Chemical structure of Vx (Wikiwand.com)

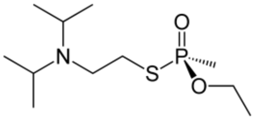

Meanwhile, the Soviet Union developed a similar compound VX, called VR or Russian VX (84). Despite international regulations regarding the use of combat gases (tabun, sarin, or phosgene), they have been used countless times during the First Gulf War between Iran and Iraq – tabun, Yemen – phosgene, Cambodia – phosgene, Angola – VX or Syria – sarin [21,22].

In addition to the armed attacks that used the combat gases, chemical terrorism has also developed, characterized by a series of attacks on the civilian population, the best known being the sarin attack on the Tokyo subway (Figure 11) which resulted in about 640 victims [23].

Figure 11: Sarin Attack in Tokyo (brittanica.com)

With the dissolution of the Soviet Union in the early 1990s, many documents were declassified, showing that during the Cold War, the Russian authorities secretly led a chemical weapon development program, called FOLIANT [20]. Thus it appears that VR served as a prototype for another class of OP called Novichok or class A [20]. This class of agents, with chemical weapon potential, came back to attention in 2018 when Russian spy Sergei Skripal and his daughter were attacked with a Novichok (Figure 12) compound [20].

Figure 12: Chemical structure of Novichok (chm.bris.ac.uk)

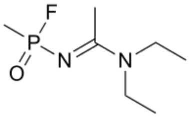

Beyond the development of chemical weapons, research continued to obtain insecticides that can be widely used. At the same time, the mechanism of action of OP has been discovered, as well as the possible antidotes that can be used in case of acute intoxication [13].

OP production continues to grow, with more than 200,000 tones of insecticides being used each year in the European Union [10]. According to Eurostat statistics between 2011 and 2016, pesticide consumption was steadily increasing, and only six countries reported a decrease in pesticide sales [24]. The largest decrease was in Denmark, at about 50%, followed by Ireland, Italy, Malta, Portugal, and Romania [24]. There are also countries in which the sale of pesticides has increased, the spike was in Austria (over 25%), followed by France, Slovakia, and Hungary [24].

The country with the highest pesticide production and consumption worldwide is China, where both imports and exports grow annually [10,25]. In the United States, the tendency to increase pesticide consumption is maintained, the most used compound being glyphosate [26]. In Brazil, pesticide consumption increased by more than 200% from 2000 to 2013 [27].

ORGANOPHOSPHATE TOXICITY AND CURRENT REGULATIONS

Toxicokinetics refers to the behavior of toxic substances in the body from the moment of penetration to elimination. Absorption, distribution, metabolism, and elimination of OP pesticides depend on the physical properties of the compounds, but also the dose involved [28]. Most OP compounds are fat-soluble and not ionized, which promotes rapid absorption [29]. OP is rapidly absorbed orally and by inhalation. The fat solubility of OP compounds favors the crossing of lipid membranes in the body, so they have a wide systemic distribution in all organs [29,30].

In the case of acute intoxication with pesticides, the onset of clinical manifestations depend on the characteristics of the agent involved, and the mode and duration of exposure [31].

Manifestations of OP poisoning are mainly characterized by a parasympathetic vegetative dysfunction, induced by the accumulation of acetylcholine [31]. According to the distribution of cholinergic receptors, specific signs and symptoms of acute intoxication correspond to the stimulation of nicotinic and muscarinic receptors [32].

Classically, manifestations of acute exposure are described as a cholinergic syndrome characterized mainly by bradycardia, miosis, confusion, fasciculations, tearing, sialorrhea, nausea, diarrhea, bronchorrhea, and bronchospasm [33].

The main features of the intermediate syndrome are paralysis of the gait muscles, neck flexors, cranial nerves, and respiratory failure caused by diaphragmatic paralysis and intercostal muscles [34].

Late poisoning manifestations manifest as polyneuropathy and occur more rarely than the intermediate syndrome [35]. Nevertheless, endocrine and metabolic dysfunction may occur even after low-dose OP exposure [36,37].

Considering that at present OP is widely used, we try to limit consumption by strict regulations regarding the quantities and concentrations applied to agricultural crops or in households [38,39]. For example, if in the early 1990s the number of pesticides exceeded 10 kg/ha in many Western European countries, European Union standards currently limit these quantities below 4.6 kg/ha [39]. Moreover, the use and marketing of OP are subject to the rules of the Rotterdam Convention which assessed, based on national reports, the risks associated with them [40]. In addition, the Convention had done a classification of these substances, which led to the development of worldwide use and labeling rules. Last but not least, substances considered to be at high risk for the general population were banned by the committee of the Convention [40].

Furthermore, in the European Union, there are regulations developed by the Parliament and the European Council, whose application has been mandatory in all member countries (Regulation (EC) No 1185/2009 of the European Parliament and of the Council of 25 November 2009 on pesticide statistics) [40].

The United States reinitiated regulatory processes of OP exposure since raising the possibility of OP’s risk to children. Thus, programs targeting to assess exposure risk to pesticides were made as well as considering the menace of co-exposure to pesticides with similar toxicity mechanisms [41].

The United Kingdom has revealed neuro-psychiatric disorders in participants of the Gulf War that were designated as OP poisoning. Moreover, identifying increasing rates of depression in farmers exposed to OP made new regulations eliminate OP and carbamates off the market [41].

Despite European efforts to decrease the accessibility to OP, Japan has only imposed restrictions on the most toxic compound, namely methamidophos. Regarding other OP compounds, indications are of correct use without imposing limitations in agriculture or domestic fields [42].

CONCLUSIONS

The discovery of OP compounds involved various scientists that would first describe their action, namely inhibiting acetylcholinesterase, and then describe toxic effects [43]. OP toxicity proved useful in acting as pesticides in agriculture but the risk of developing a chemical weapon based on OP compounds has dominated wars [44].

Exposure to OP is a significant public health problem associated with long-term repercussions like affecting human brain development [9]. Despite an incompletely known toxicity mechanism, several countries have issued strict rules regarding OP use. Although Romania is one of the countries that has reported a global decline in pesticide trade, the increased accessibility of this type of product for people untrained in handling and using it suggests the need for stricter regulations.

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ORIGINAL ARTICLE

Futurology and monitoring in the field of virology to deal with emerging diseases

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Abstract: *Every year we see many changes in the world around us. One of these changes is the climate and weather, and it could be one of the reasons for changing behaviors between humans and animals, which could cause zoonotic diseases. We usually face new diseases that infect a growing population leading to a pandemic. Different reasons are leading to an increase in the emergence of new infections. Some of these diseases can be associated with zoonotic diseases and the diverse world of viral genetics and its failures. Furthermore, properties like mutations and also unknown effects of these mutations on the virulence of the agents make it challenging to trace new diseases. As we know, preventing emerging diseases is inevitable and viral diseases are dangerous, and some viruses can spread quickly and cause an endemic or pandemic. As was noticed during the recent pandemic, the need to study emerging diseases in the field of virology has become more and more apparent to us. In this review, we want to look at how we could monitor and control these diseases with new approaches and what should be done when a disease emerges. Moreover, we will investigate risk factors, ways to deal with emerging viral infections, new treatments that make the condition better when a person gets infected, and the future of these diseases. We will also study the ways to deal with pandemics with the new generations of vaccines.*

Keywords: *emerging-disease, viral disease, futurology, vaccination*

BACKGROUND

The world has witnessed various emerging infectious diseases (EIDs) that reemerge and cause multiple health and financial issues. For instance, in 2010, an unusual clinical image showed severe inflammatory syndrome which was along with vascular conditions similar to transient ischemic attacks or venous thromboembolism. There were several reports of this issue, and almost ten cases were recognized. The patients having underlying autoimmune diseases or hematologic malignancies were the primary targets for this condition [1,2].

Emerging infectious diseases are recent phenomena that affect humans with a high rate of spreading in different geographical areas. We have experienced more than 50 EIDs in the last 40 years. Most of these infections are re-emerging after a period, which has led to the adoption of the term "emerging and reemerging infectious diseases." The term is used for infectious diseases that occur once in a region or among a population. It appears again with more virulence or with resistance to the drugs once used to eradicate them. We should also note that the re-emergence of infectious diseases happens at a higher occurrence rate than the initial emergence [3].

Various new and reemerging infectious diseases are threatening people all over the world, inflicting higher financial costs to societies along with increasing the risk of mortality. Some of the most recent infectious diseases include pneumonia, SARS, swine flu (H1N1), influenza, malaria, tuberculosis (TB), cholera, hepatitis, meningitis, food-borne gastroenteritis, chikungunya, Ebola virus diseases, campylobacteriosis, and salmonellosis. The most recent infectious disease threatening people all over the world is SARS-COV2 (Covid-19) [3–6].

There exist more than 50 EIDs in the world at the moment with varying levels of threat to public health. These infectious diseases can target human beings, animals, and plants. Studies indicate that climate change can also lead to the emergence of many of these diseases. Thus, controlling

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climate change and environmental factors can help control and eradicate infectious diseases[7].

Population growth has a fundamental role in the spread of diseases. It is also concurrent with the rise in speed and rate of travel across the earth which leads to the rapid dissemination of pathogens across the globe. This is partly due to globalization which has increased international trade, migration, and reduction in travel costs. Furthermore, an increase in leisure travels to previously unknown destinations is also one of the main factors in exposing people to various infectious agents [2,6–8].

During the last 20 years, researchers have encountered various species which were resistant to drugs, and some of them are classified as species that are resistant to several drugs, including methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci, multidrug-resistant or extensively resistant tuberculosis, carbapenemase-encoding Gram-negative bacteria, and extended-spectrum β-lactamase E. Coli. These are considered emerging pathogens and can rapidly disseminate among hospitalized patients and people of varying ages; hence, they require considerable attention [2].

These infectious diseases are a source of frustration among the general public. Notably, the emergence and re-emergence of all these diseases are dependent on the economic and geographical position of the region [3,9]. The good news is that most of these diseases are controlled through new chemotherapeutic methods, but that is not the case with all of them, since some of these agents are not controlled due to unknown etiology and lack of proper management methods [3,10,11]. Scientists have cited various problems as the reason for the onset of these infections, among which one can note population, improper food-processing methods, lack of awareness about hygiene measures, and problems in water distribution systems and sewage pipelines. Furthermore, the production of ineffective antimicrobial agents by pharmaceutical companies is another cause that makes it difficult to manage these infectious diseases. Infections acquired in the hospital and pathogens which are resistant to various drugs are also issues that can make the result of treatments uncertain [3,12,13].

Countries need to change their object of focus from healthcare and prevention to research on the determinants of health and primary healthcare provision. Political empowerment and creating a global initiative to increase the capacity of the health sector in developed and developing nations is another alternative that government officials need to consider to stop the spread and emergence of various infectious diseases [14,15].

Various drastic changes have been experienced during the last three decades in clinical virology laboratories. Direct diagnosis of the virus in clinical specimens is one of the main issues noticed in the last decades, which means the demonstration of virus particles (including viral antigens and nucleic acids) [16,17]. The application of new methods and reformulation of the old ones, demand for an easier test with higher accuracy and multiple applications, the trends existing in developing countries along with the detection of new concepts regarding microbial interactions are among the factors shaping molecular assays, development of diagnostic approaches, and changes in the interpretation of data obtained in the laboratory [18].

A considerable number of emerging infectious diseases are the result of zoonotic agents, and in some cases, vector-borne viruses are responsible for their emergence. Although resistance to antimicrobial drugs is another troubling issue, it is less likely to have bacterial or fungal pathogens as the cause of a pandemic [19].

In this study, we want to clarify how emerging diseases can be monitored and controlled and how we can deal with them in times of occurrence to stop or prevent the occurrence of a pandemic or endemic disease.

MONITORING AND OBSERVATION

Our local environment and global landscape is changing all the time. Due to the higher error rate of virus enzymes replicating their genomes, viruses containing RNA can adapt themselves to different conditions. That is the reason for the higher rate of emergence and re-emergence of diseases that are caused by RNA viruses [14]. For instance, the pandemics caused by influenza virus strains are caused by viruses that reside in host animals before infecting humans. Humans, horses, pigs, birds (domestic and wild), and sea mammals are among the species that can be infected with the influenza virus.

The present century has witnessed the death of over 20 million people in different countries due to Spanish influenza, which had a higher prevalence compared to HIV [18]. The ways to recognize the pathogens and distinguish test positive individuals from the negative ones are the first steps to controlling and monitoring emerging diseases.

New technologies and approaches to microbial interaction

CRISPR and next-generation sequencing (NGS) are among the recent technologies emerging in the market. A single reaction in NGS can parallel sequence a large number of DNA templates. It is quite fast and cheap at sequencing the complete genome of many microbial samples. Virology has various applications such as management of viral infections, figuring out the infection sources, investigating the immune response of the host, and selecting the proper intervention and treatment strategies [18,20,21].

NGS makes use of three methods to sequence the genetic material of the virus. The first one is identical to the Sanger method and is done through amplification and sequencing of the target. The second method is done through nonspecific amplification of the genetic content of the sample before NGS. The third approach enriches the genetic material of the virus and makes use of NGS to sequence them subsequently. NGS has higher throughput besides being fully automated and can find the genome mutations causing drug resistance. It is also able to target several viruses at the same time. The added benefit of NGS is that it can detect both known and new microbes despite being pathogen or commensal. NGS is mainly used for the detection of different drug-resistant variants of HIV and HBV in clinical virology.

The advantage of detecting the undetectable variants is not yet clear, but the evidence obtained from resistance tests of patients with low levels of virus and rare mutations (including patients with HIV) shows that they will have a great influence on routine clinical practice and platform for nucleic acid detection based on nucleic acid amplification and Cas13a-mediated cleavage of a reporter RNA which enables the detection of the target in real-time [22–24] SHERLOCK is among the best techniques which equal PCR essays in terms of sensitivity. It is used to detect one or several RNAs residing in the microbial strains. One of the best features of SHERLOCK is its ability to be combined with non-PCR methods used for amplification. SHERLOCK is widely used for the detection of nucleic acids from other viral threats, including the dengue virus and Zika. SHERLOCK is also important in terms of commercial usage since its reagents can be lyophilized and reconstituted on paper.

Researchers can also run it on body fluids without the need to be extracted before and provides the results in less than two hours [18,25,26]. DETECTOR (DNA endonuclease targeted CRISPR trans-reporter) is another technology that is similar to SHERLOCK in using Cas12a. The mere difference is that it works on DNA, and shows unspecific collateral single-stranded DNA cleavage activity once it recognizes the target sequence. DETECTOR has attomolar sensitivity in detecting DNA. Furthermore, it is widely used for detecting human papillomavirus 16 and 18, which are two human papillomaviruses that have a high tendency to neoplastic transformation. DETECTOR is easy to use and much faster than the existing methods of human papillomavirus detection. Moreover, you do not need to provide any special equipment for conducting DETECTOR. Hence, it can be considered a useful platform for clinics that lack resources, and it is a good option for conducting molecular diagnostics rapidly [18,27].

Previous multiplex assays combined the detected viruses according to various parameters, including primer and reagents, amplification reaction, sample processing compatibility, etc. Hence, the tests were mainly based on analytical methods.

Novel methods are capable of detecting a wide range of germs and viruses with the same clinical manifestations, entry method, and tissue tropism. This is the main reason for multianalyte tests known as syndromic tests.

There are some proprietary panels produced by pharmaceutical companies which are dedicated to infections of gastro intestine, respiratory system, central nervous system, and the infections that are transmitted sexually [18,28–30] Biocontainment laboratories that are located far from EVD treatment centers usually use RT-PCR as their main diagnostic test. Recent approaches make use of Point-of-Care ELISA-based assays for VP40 matrix protein. It provides considerable operational advantages and leads to the timely isolation of positive cases. Hence, it is a great tool to clear suspect cases with higher accuracy and sensitivity compared to the methods used earlier. However, the role it plays in field conditions is uncertain [31,32]

The existence of a centralized infrastructure for sequencing in areas that have a higher biological diversity which acts as the beginning sources of new human infections can help fast and timely detection of emerging pathogens. Furthermore, these centralized infrastructures can act as hubs to send resources to unknown locations in cases of new outbreaks. This infrastructure has the potential to search the environment regularly to figure out the spectrum of viral strains and species existing within phylogenetic families that can infect humans. Furthermore, it could be used for high-throughput sequencing to define all existing viruses [19].

An initiative of WHO named Research and Development (R&D) Blueprint works on decreasing the period from the initial reports of a public health emergency to the provision of test kits and diagnostic methods, treatments, and vaccines to rescue people’s lives and prevent the initiation of the health crisis. It also takes severe emerging diseases into account that lack any type of countermeasures to prevent their expansion. It was at the request of 194 Member States in May 2015 that WHO asked a group of specialists to develop a Research and Development (R&D) Blueprint for Action to Prevent Epidemics.

It was on 31 December 2019 that reports were sent to WHO about several pneumonia cases without a definite cause in Wuhan City, Hubei Province of China. Chinese authorities identified the coronavirus disease (COVID-19) as the causative agent on 7 January 2020 [4]. The R&D blueprint started its activity on the day it was informed of the pandemic to work on diagnosing, vaccinating, and therapeutic measures for eradication of the virus [4]. The infectious agents occurring for the first time pose various challenges due to a lack of knowledge about their identity, epidemiology, and pathogenesis, which are the case invariants of influenza or COVID-19. It is also difficult to identify the zoonotic reservoir which makes it more challenging to foretell the time, place, and possibility of an outbreak [33]. Most of the reports regarding emerging diseases are from developed countries. The United States accounts for more than a third of reports about emerging diseases [34] which shows the important role of geographical location in the emergence and spread of an infectious agent.

CONTROL, TREAT, AND FUTURE STUDY

Probable approaches to control and treat

Gene-based antigen-delivery technologies are the best methods to fight EIDs; however, some of these methods have not received the required licenses to be used in human medicine. Furthermore, in times of crisis when time plays an important role, these technologies can provide vaccines in the shortest time possible based on the information obtained from target-gene sequences compared to other methods which require the virus to be isolated and grown along with considerable containment [19]. Interference in the transmission of the virus from animals to human beings can help control zoonosis which is possible via vaccines that are self-disseminating. These vaccines can act on insects, animals, and also environmental reservoirs of the virus.

These methods work by the replication of the existing vectors of the virus that are engineered for the expression of the disease’s antigens. They are also used in cases when clinicians want to target specific groups of animals. The objective of animal vaccination is to keep pathogens of the disease in the animal reservoirs to prevent them from infecting humans. There are various issues including costs, possibilities, and the ensuing safety concerns that should be kept in mind during this method which are necessary for controlling the sources of these pathogens. Moreover, it is not possible to predict which animal pathogen can cause disease in humans. It should be noted that the usage of a CMV-based vector to vaccinate apes was suggested as a way to curb the transmission of the Ebola virus to people. The same method was also effective in protecting the animals against death [44,45].

Hospitals are the first place to have a measure to control the transmission of viruses. The staffs are at a higher risk in the initial outbreak; however, providing most centers with personal protective equipment and kits has prevented further transmission. It was also difficult to reduce the risk of transmission during the burial rituals. The main difficulty stemmed from making burial consistent with social and familial norms [31]. The world has witnessed different emerging and reemerging infectious diseases which have threatened it in terms of security, economy, and biodefense [46,47].

There were several diseases in the past few years including H1N1 (2009-Mexico) [48], SARS (2002-China) [49], MERS (2012-Saudi Arabia) [50], Ebola (EBOV) (2013-Africa) [51], Lassa virus (2018-Nigeria) [52], Zika virus (2015-Brazil) [53], and the COVID-19 pandemic (2019) [54] has made different scientists eager to devise strategies for quicker prevention, treatment, and/or controlling these epidemic viruses [44]. The number of fatal cases (case fertility rate) due to emerging and reemerging infectious diseases was high during the specified time, which indicated the need for immediate action to prevent their spread [44,55].

Various factors, including globalization, urbanization, travel, commerce, climate change, and the increasing elderly population are also influencing factors in the emergence of these diseases. Zoonotic diseases are also on the rise due to increasing contact with zoonotic sources. Traveling has also been on the rise due to rapid and comfortable means of traveling, and it makes it increases the distribution of pathogens all over the world. Furthermore, the elderly are far more at risk of these infections, which leads to a higher mortality rate. All these issues affect the way pathogens are distributed all over a previously endemic zone [56,57]. Due to the nonexistent nature of licensed antiviral drugs to prevent or treat emerging viruses, the treatment method is restricted to care and fluid- and electrolyte therapies. However, these prevention methods are also absent in most developing countries [58].

Cytokine-storm

Cytokine storm is regarded as one of the most lethal immune syndromes with a quick-spreading ability and highly activated T cells, natural killer cells, and macrophages. It also causes overproduction and release of many inflammatory cytokines and chemical mediators through immune or non-immune defense cells. Induction of hypercytokinemia, also known as cytokine storm (CS), is the main cause of the high fatality rate in immunocompetent individuals. It can also engender different pathological side effects such as shock, damage to the tissues, sepsis, the failure of several organs of the body, and edema. In some cases, lack of attention to these symptoms can lead to death.

Moreover, cytokine storm is concurrent with hemorrhagic fevers caused by a virus, and these immunopathological conditions can be due to several viruses including Lassa, Crimean, Congo hemorrhagic fever, Ebola, and Dengue fever (DF), and Marburg viruses [58,59].

Currently, there is no standard treatment to cure cases of cytokine storm and we lack drugs that are approved specifically to treat cytokine storm. Corticosteroids were not successful in treating cytokine storm in clinical testing. Furthermore, non-steroidal anti-inflammatory medicine was not able to treat cases of cytokine storm while used besides antiviral drugs. Hence, further randomized control is required in this regard. Some experiments are being conducted on new treatment options and they are in the preclinical testing stage. We hope that these methods prove effective in treating cytokine storm [58]. Although oseltamivir was being used in some countries of South Asia during the infection of the area with the HPAI virus, there is no report about how effective this method was. Furthermore, these clinical studies did not show a considerable reduction in the number of death cases due to using oseltamivir. The reports indicated that the rate of survival in patients receiving oseltamivir equaled 30% (13 of 34); while the survival rate in untreated cases was 25% (4 of 16). Hence, it can be said that the efficacy of oseltamivir treatment was dependent on timely administration. In cases when the treatment was within 5 days after the initiation of the symptoms, the rate of survival was 53%; however, postponing the oseltamivir administration to 6 days reduced the rate of survival up to 26% [60,61]. S

everal anti-inflammatory drugs (being administered alone or in combination with other therapies) have been assessed for the treatment of cytokine storm in influenza infections. However, it was seen in one of the studies that the utilization of anti-inflammatory drugs as a single treatment to cure cytokine storm comes with various challenges. The same study showed that curbing cytokine production by glucocorticoids did not lead to any form of protection for the mice infected with HPIA (A/Vietnam/ 1203/04 H5N1) [62]. The results obtained from another study indicate that combining COX-2 inhibitors with zanamivir has significant effects on survival since it acts by stopping the emergence of apoptosis and decreasing cytokine dysfunction [63,64]. Sphingosine-1-phosphate-1 receptor (S1P1R) agonists have been used for both suppressions of cytokine storm and treatment of pathogenic influenza virus infection in mice. The protection level was higher in this method in comparison to oseltamivir, and the rate of survival equaled 50%. However, the protection was the highest in cases receiving oseltamivir and AAL-R, and the reports delineate the survival rate equaling 96%. Being licensed, these drugs can help us in combating various pandemics, and they can be used to suppress various viruses including MERS, Ebola, and SARS which are the prominent causes inducing cytokine storm [65].

One of the novel approaches to treating cytokine storm involves the down-regulation of main signaling pathways or genes that are responsible for the regulation of various host responses including apoptosis or inflammation. The signaling pathway named C-Jun N-terminal Kinase plays a role in different mechanisms such as apoptosis, inflammation, and proliferation. The reports indicate that they are activated during the infection and replication of the influenza A virus [66]. Treatment of mice with short-term heat (39°C for 4 hours) led to a significant increase in HSP70, and it also increased the amounts of cytokines TNF-α, IFN-β, IFN-γ, and IL-6. The study showed that 57% of treated mice were alive after 14 days of being infected with HPVI. It is suggested that the positive effects of heat shock treatment on HPAI are correlated with the increased expression and protective effects of HSP70, which leads to transient down-regulation of the over-expressions of pro-inflammatory cytokines in the lungs [67]. It also makes interferon and TNF-alpha be expressed in response to infection and activates pathways to secrete specific proteins and antiviral cytokines. The results show that macrophages can be a good biomarker to assess the severity of influenza infection [68,69].

Following pathology identification, it is necessary to develop preclinical models to study the interactions between the virus and the host and select vaccination volunteers to define its features. Furthermore, the identification of the epitopes is required for the guidance of the vaccine and it has a role in the production of antibodies and lowering Nabs. All in all, these lead to antibody-dependent enhancement of the infection caused by the virus. Furthermore, the agencies, governments, and companies working in the field should share data and collaborate to have a strategic approach to the prevention of the next pandemic threats [70,71]. Due to the lack of effective drugs or vaccines to work against the COVID-19 pathogens, social distancing is the only method to hinder the spread of the virus. Hygiene measures are also necessary to prevent contact with patients. Using soap and water to wash hands along with using alcohol-based sanitizers are among the other preventive measures in the absence of effective drugs. It is noteworthy that COVID-19 pathogens can survive on surfaces for up to a few days;

hence, using disinfectants is usually effective in eliminating the pathogens residing on the surfaces [72].

The first step during a pandemic or endemic disease is to limit the contact between populations to stop transmission between human beings. In some cases, the diseases could be zoonotic and the animal vectors should be avoided. As said earlier about viral emerging diseases, we know that viral diseases mostly do not respond to drug-based treatments; hence, vaccination is the most effective choice to control emerging diseases [54].

Challenges in dealing with emerging diseases via vaccination

As noted earlier, vaccination remains the only effective method to stop the spread of viral emerging diseases. Vaccines can cause protective responses in the body against pathogens and reduce the causes of morbidity and mortality. Rapid vaccine production is essential to manage the outbreak and prevent the spread of the viral agent [44]. The history of using the vaccine to deal with viral emerging diseases dates back to 1938 when the U.S. army soldiers received influenza vaccines during World War II [73].

Vaccines have higher efficacy than other treatment options; hence, it is necessary to develop vaccines for all emerging infectious diseases. However, vaccine production is not readily possible for some viral infections due to the unknown identity of emerging pathogens, inadequate animal models, cost and time required for production, licensing, and global distribution, to name a few [74].

Vaccines are tested on young, healthy volunteers. This method was used for Dengue and Influenza virus; however, there should be an accepted treatment option if any problem occurs. Licensure and final approval of the vaccines are other issues that need to be considered. Rapid development and administration of a safe and effective vaccine is another challenge [75,76].

Long-lasting protection by making the body produce antibodies against the pathogens in question is the criterion to measure the efficacy of vaccines. The study conducted by Nakaya et al. analyzed the antibody responses following influenza vaccination on day 28 vs. 180. They emphasized finding pathways that were related to the waning or persistent reaction of the antibody [77].

The phrase ‘synthetic vaccinology’ is used in cases where only sequencing data are enough to start designing vaccine antigens and reagent production. Recent developments and advances in the field of protein engineering, understanding of the assembly of virus-like particles (VLPs), and the identification of other self-assembling molecules that improve immunogenicity by optimizing antigen display make it easier to adjust the vaccine-induced immune responses to specific antigens [19,78].

Some viruses can escape the trap of the immune system. The same strategies cannot be used for all cases of virus emergence, and there need to be new strategies for antigen design and formulation, pathway, sequence, and the timing of administration in some cases, such as extreme genetic plasticity and antigenic variability (HIV and Influenza virus), persistent infection and latency (HIV or Herpesviruses), or multiple distinct serotypes (rhinoviruses). Removing these biological obstacles of 'difficult' viral pathogens can provide methods to use in pre-pandemic vaccine development or rapid vaccine development in cases of emerging viral pathogens [19].

There are two types of mRNA vaccines developed so far. The first is a conventional non-replicating vaccine, and the other is a self-amplifying vaccine. A mature mRNA molecule is produced by the IVT method conducted a plasmid of the of a DNA template, which has the sequence for RNA polymerase that is dependent on DNA. It includes an open reading frame that can encode the required antigen, the 50 and 30 flanking UTRs, the terminal poly(A) tail, and the 50 caps. The host's cellular machinery translates the vaccine in vivo after receiving it and provides the corresponding post transnationally modified antigen. This is the process through which the vaccine mimics being infected naturally in vivo. These vaccines can activate the immune system by attaching to receptors, thus creating immune responses. The produced mRNA vaccines can make the body immune for a long time against various infections caused by viruses such as Ebola, Zika, influenza, Dengue, and other viral diseases [44,74,79].

Adenoviruses are mostly used viral vectors for priming the immune system. They can lead to both cellular and humoral responses. In clinical trials with adenoviruses, the existence of an anti-vector can challenge the vaccine response. To prevent immunity, the vaccine platforms for human beings are usually taken from adenoviral vectors obtained from animals. In some cases, rare species are also used for these purposes. Two vaccines are made of viral vectors: Imojev and Dengvaxia. The former is a vaccine used for JEV and the latter is a Dengue vaccine. Both these vaccines are produced by Sanofi Pasteur. Chimeric YFV is used as a vector in both of them. They work by propagating chimeric viruses inside the cell culture [44,80].

There are also protein-based recombinant vaccines that contain the immunogenic protein of the pathogen it aims to target. The production of these vaccines requires the identification of the pathogen. Bioreactors are used to produce these vaccines on a large scale depending on the posttranscriptional pattern of modification required by the use of heterologous expression systems, like yeasts, insects, bacteria, mammalian cell lines, or plants.

These vaccines are safe due to the lack of genetic information derived from the pathogen, and there is no need to live pathogen manipulation during the manufacturing process. They are suitable in times when a quick response is needed to an epidemic condition. It is because the production process can begin after the virus genome has been sequenced, and there is no need for virus isolation. Vaccines based on protein can be obtained during the production of recombinant virus subunits (SUVs). It is also possible to administer them along with adjuvants for enhancing the immune reaction of the host against the recombinant viral antigens [44,81].

CONCLUSION

It is estimated that zoonosis accounts for about 60% of diseases stemming from infections. The source of most infectious agents is wildlife, and increasing contact with wildlife can increase the occurrence of infectious diseases. Climate change is another issue leading to vector-borne diseases; however, it needs to be further evaluated to see the exact influence of climate alterations on the occurrence of the agents causing infections. Recently, various platforms are used to design vaccines which include viral-vector vaccines, nucleic acid vaccines, and recombinant protein-based vaccines.

Studies have revealed the overlap between animal and human ecosystems as one of the reasons for COVID-19 to pass the species barrier. Hence, there is a need for coordination with veterinary experts and the submission of laws to control the animal trade.

Our suggestion is the establishment of working groups and scientific consortia to govern the design process of vaccine antigens. The produced antigens should lead to the production of cross-reactive antibodies. These working groups should also look for novel vaccine administration methods to directly target the agents affecting immunity. Each pandemic enhances our scientific knowledge regarding the induction of cytokine storm by viruses. Identification of novel therapies for the treatment and control of infections requires enhancing our knowledge about the complicated interaction between antiviral immune responses of the host and the ability of the virus to change these responses and lead to immune dysfunction.

It is predicted that the world will witness the emergence and re-emergence of various zoonotic and mosquito-borne viruses in the future. The natural mutation will be the process through which these viruses evolve, and they will pose a real threat to public health. It will be difficult to protect against them due to the lack of proper therapeutic procedures including vaccines or drugs. Hence, it is necessary to be prepared for them and work on novel approaches for the treatment and control of these pathogens. In particular, the development of methods targeting the route taken advantage of by viruses provides the opportunity for effective treatment methods in different viral strains.

Almost all outbreaks have been with the nosocomial transmission, and there are several cases of transmission in the hospital. Hence, healthcare sector workers should be among the first groups receiving vaccination when a pandemic or endemic occurs. Our study showed that zoonotic diseases play the main role in emerging diseases, especially emerging viral diseases. Hence, the role of the veterinarian as the frontline confrontation with emerging diseases is quite clear in preventing the occurrence of a pandemic. Because there is no effective cure for viral emerging diseases other than prevention and control, all nations should have a group of veterinarian scientists to control and deal with the animal reservoirs and vectors to prevent a pandemic [19,78,82–88].

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Authors’ Contributions
Dr. H. Esmaeili and Mr. M. Baqerkhani both developed the concept, design, and definition of the intellectual content of this article, Dr. A. Soleimanzadeh and Mr. M. Baqerkhani both developed the literature search, data acquisition, data analysis and statistical analysis of this article. Dr. M. Farzanehpour and Mr. M. Baqerkhani both developed manuscript preparation for this article. Dr. M. Farzanehpour developed manuscript editing and manuscript review. Dr. M. Farzanehpour is in charge to take responsibility for the integrity of the work as a whole from inception to published article and is designated as 'guarantor'.

Ethics approval and consent to participate
This study was executed under the supervision of the ethical committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (ethical code IR.BMSU.REC.1399.555 (.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

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ORIGINAL ARTICLES

Gene therapy by using stem cells in the treatment of hepatitis

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Abstract: *Chronic hepatitis can resolve spontaneously or progress to more advanced stages, i.e. fibrosis, cirrhosis, and liver cancer, respectively. Two million deaths each year are attributed to liver diseases globally. Chronic liver disease and cirrhosis comprise 11th position in leading causes of death worldwide. Viral infections account for the etiologic factor of most cases of hepatitis. Almost the only available way for the treatment of end stages of hepatitis is liver transplantation. The biggest problem with this therapeutic approach is the number of liver transplant donors is less than the number of recipients. Therefore, more advanced methods must investigate. Recently, gene therapy by the utilization of stem cells yielded promising results in clinical and preclinical investigations to the treatment of liver diseases. Novel genome editing tools such as TALENs and CRISPR/Cas9 consider as powerful genome correction approaches and can employ for iPSCs gene editing. This paper highlights the various aspects of gene therapy through stem cells for liver diseases*

Keywords: *gene therapy, stem cell, hepatitis, CRISPR/Cas9*

INTRODUCTION

The liver is the real organ in the body complicated in the bile production, metabolism, toxins elimination, and immune responses, and several disorders of metabolism are of hepatic basis. There are many different types of liver diseases, comprising hepatitis, alcoholic liver disease, fatty liver disease, hereditary disease, cirrhosis, fibrosis, etc. These may result in liver failure and lead to irreparable liver impairment. Even though the liver is capable to repair itself, while the injury is beyond repair it is hazardous and lethal to patients [1]. Hepatitis consider as a condition of the presence of inflammation in the liver that divides into two categories, i.e. acute hepatitis and chronic hepatitis. The latter is defined as lasting for more than six months. This inflammation can resolve spontaneously or progress to more advanced stages, i.e. fibrosis, cirrhosis, and liver cancer, respectively [2-4]. Liver diseases are responsible for nearly 2 million deaths whole the world yearly. Chronic liver disease and cirrhosis ranked as 11th and 5th leading causes of death worldwide and for the non-Hispanic AIAN population of the United States, respectively [5, 6]. Most cases of hepatitis attribute to viral infections as an etiologic factor. Other important leading causes include infectious agents except for viruses, alcohol, drugs, and autoimmune reactions. The only available way for the treatment of end stages of hepatitis is liver transplantation. One of the main problems of this way is the discrepancy in the number of liver transplant donors and recipients. Furthermore, the recipient's immune system could reject the grafted organ [7]. Consequently, different methods must be considered and explored. Recent progress in gene therapy opens a promising new window to the treatment of a broad range of diseases. In the procedure of gene therapy, to reduce the symptoms or completely cure a disease, genetic material is delivered to a patient [8]. This approach tries to sustain express a transgene, as well as minimizes adverse side

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effects. One of the best profits of gene therapy is that a single treatment is sufficient, therefore is no need for repeated administration [9, 10]. Gene therapy utilizes in various fields such as the treatment of cancers, monogenic diseases, infectious diseases, heart diseases, neurological diseases, eye diseases, etc. [11, 12]. By 2017, about 2600 clinical trials initiate that some of which approves. The most common types of gene therapy include gene replacement therapy, CAR (Chimeric antigen receptor) T-cell therapy, and gene editing. In recent years, stem cells, as one of the top topics in the broad range of advanced scientific research, have attracted the attention of many researchers and become a good tool for gene therapy [13]. In fact, in vitro stem cell culture is straightforward and they can differentiate into hepatocytes and new liver cell sorts. The results of novel scientific investigations in stem cell gene therapy open new horizons in the treatment of hepatitis [14, 15]. It hopes that shortly, these approaches will enter clinical practice. There are several sources for stem cell therapy of liver diseases that are summarized in Table 1 [16-22].

Table 1: Sources for stem cell therapy

Liver-Derived Stem Cells	
	• Adult liver stem cells (oval cells) • Fetal liver stem cells (hepatoblasts)
Bone Marrow-Derived Stem Cells (BM-DSCs)	
	• Hematopoietic stem cells (HSCs) • Mesenchymal stem cells (MSCs)
Annex Stem Cells	
Embryonic Stem Cells (ESCs)	
Induced Pluripotent Stem Cells (iPSCs)	

In this review, we discuss gene therapy via manipulated stem cells for the treatment of hepatitis.

ANIMAL MODELS AND THERAPEUTIC GENE TRANSFER BY USING STEM CELLS

Today, animal models are considered a key tool in research fields. They have played a key role in the advancement of medical sciences, especially in cell and gene therapy for hepatitis. Although many efforts make in this regard, however, there are still differences between animal models and humans [23-26]. Among animal models, mice can be a good choice for the study of hepatitis pathophysiology as well as the development of novel, safe, and efficient drugs. A big problem is the mice cannot be infected with hepatitis viruses. Several strategies were developed to solve this problem: Humanized chimeric, transfection, and transgenic mice [27]. Gene therapy by using stem cells is a new version of previous gene therapy approaches. In this procedure, therapeutic genes are inserted into stem cells, and then the genetically modified stem cells are delivered to the patient's body [28, 29].

Currently, outstanding achievements in the development of stem cell therapy approaches have brought new hopes in treating liver diseases. Many studies have been carried out in this field and emerging evidence suggests that the utilization of stem cell-based-gene therapy is a good way for the treatment of liver diseases, especially hepatitis [30-32]. For example, a study conducted by Zhu et al. on a mouse model showed that the use of bone marrow-derived mesenchymal stem cells (BM-MSCs) led to the immigration of these cells to the liver that boosts immune system performance, and improves liver damage caused by (concanavalin A) ConA [33]. Gilsanz et al. in their experiment, examined the effect of human/rat adipose-derived mesenchymal stem cells (AD-MSCs) injection to the liver parenchyma of rats and observed that the biochemical serum analytes of the liver, hepatomegaly, splenomegaly, and ascites have remarkably improved [34]. Masalova et al. used modified mouse mesenchymal stem cells (mMSCs) of DBA/2J (H-2d) mice that expressed 5 non-structural proteins of the hepatitis C virus (HCV), including NS3, NS4A, NS4B, NS5A, and NS5B. Then the analysis of innate and adaptive immune responses showed this approach drives high phagocytosis, lymphocyte proliferation, and type I/II interferon production, as well as, IFN-γ secretion, Immunoglobulin G (IgG) production [35].

ADVANTAGES AND DISADVANTAGES OF STEM CELL-BASED THERAPY

The aim of stem cell-based therapy for liver disease is to substitute disease cells with healthy ones and let the healthy cells be efficient. Around the world, several research groups are conducting the expansion of stem cell-based therapy for liver diseases. The objectives are to recognize stem cell varieties that are appropriate for the applications and transplantation. To attain these aims, scientists should tackle significant challenges. Because adult tissues compose of many diverse cell sorts, the identification and trying to pinpoint the stem cells are the tasks for experts. Afterward, the cell isolation from the tissues, inducing cells to differentiate into favorite cell types is an additional impediment. As well as, cells might be rejected by patients' bodies after grafting, which is an additional concern [36].

One of the most significant advantages of Induced Pluripotent Stem Cells (iPSCs) over other stem cells is its application as autologous stem cell therapy with the minimum risk of rejection induced by the immune system of the host (allogeneic immune rejection). In addition, these cells can differentiate from hepatic cells and have the capability of self-regeneration and pluripotency [37, 38]. Observed that using Yamanaka factors including Oct3/4, Sox2, c-Myc, and Klf-4 can reprogram mouse fibroblasts into iPSCs [22]. On the other hand, iPSCs can be differentiated to hepatocyte-like cells (HLCs) and then delivered to the diseased liver, ultimately recovering their proper function [39].

Recent work demonstrates that gene transferring, genome editing, three-dimensional (3D) culture, and extracellular matrix approaches improved hepatic differentiation of human iPS cell-derived hepatocyte-like cells with more than 90% purity and a satisfactory level of liver functions that are comparable to primary human hepatocytes [40].

INDUCED PLURIPOTENT STEM CELLS AS PATIENT-SPECIFIC MODELS TO ASSESS EXOGENOUS GENE DELIVERY

Human iPSCs are a possible basis for the treatment of end-stage liver disease. Some research has stated the improvement procedure for differentiation of human iPSCs into the hepatocytes. Hannan et al. defined a procedure that organized the differentiation of human iPSCs into a close-homogenous population of HLCs by 25 days [41].

One of the critical aspects of gene therapy is selecting an appropriate method for transgene delivery to the target. A variety of vector systems are available for this purpose. For the genetic material transfer to the target cells, both viral and non-viral vectors can be utilized. The transduction efficiency of non-viral vectors is lower than viral vectors that confer a shorter duration of transgene expression. Properties of an ideal vector are high capacity, low antigenic potential, high transduction efficiency, providing conditions for targeted and controlled transgene expression, and being safe. Both ex vivo and in vivo gene delivery approaches are used for the liver.

The ex vivo process involves the removal of single cells from a host and transfers to the outside the body, gene transduction in vitro (gene modification), and then transmission back again to the body. The ex vivo has more aggression for the patient and more cell-specificity than the in vivo method [42, 43]. Interferon-alpha (IFN-α) gene transferring to the liver by using a sustained expression shuttle vector may lead to long-term production of IFN- α in the liver. In a study, researchers examined the effect of an adenovirus expressing IFN-α in preventing viral hepatitis in mice [44]. It has been demonstrated the anti-HBV ZFNs can disturb viral DNA in cell culture without any cell toxicity and off-target cleavage [45]. The TALENs are similar in arrangement to the ZFNs. Bloom and colleagues showed that the Anti-HBV TALENs have nearly 87% targeted mutagenesis and 80% HBV gene expression reduction in a murine model [46].

GENOME EDITING STEM CELLS

Genome editing in iPSCs generates a population of differentiated cells that are valuable for disease modeling, drug improvement, genetic editing, and finally, for autologous cell therapy. New advancements in the genetic engineering area introduce several strategies such as transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated system (Cas9) that are powerful genome editing tools for iPSCs gene correction. When the gene-editing process is complete, the iPSCs can differentiate into HLCs and, consequently, engrafted to the target [47, 48].

As noted above, viral and non-viral vectors are available for the delivery and expression of a transgene to the liver. Viral vectors showed the best results in the previous investigations. The viral vectors divide into two groups: integrating (adeno-associated viruses or AAVs, lentivirus, retrovirus) and non-integrating vectors (adenovirus or Ad, poxvirus, herpes simplex virus, or HSV). Transgene expression stability and infection transmission rate of integrating viral delivery systems is satisfactory, but there is a risk of insertional mutagenesis. If an integrating viral vector inserts in an aberrant location on the host genome, leading to high expression of a proto-oncogene or inactivation of a functional and essential gene, which, in turn, it may have unintended consequences like malignant cell transformation. For example, 5 patients in clinical trials performed to treat severe combined immunodeficiency (SCID), developed leukemia that the cause of this phenomenon consider to retroviral-mediated gene transfer [49]. Collectively, researchers should be careful about integrating vectors because there is a risk of mutation in these vectors. Unlike the integrating viral vectors, the non-integrating viral vectors as adenoviral, poxviral, and herpes simplex viral vectors cannot integrate into the chromosomal DNA and remain in the nucleus as an episomal form [50, 51].

TALENs and ZFNs strategies have a severe defect: off-targets side effects that lead to low specificity; additionally, the massive size of TALENs and epigenetic variations damaging access to the preferred chromosomal site are substantial challenges for use in a clinical setting, but recently discovered CRISPR/Cas9 technology show higher efficiency [52]. CRISPR/Cas9 genome editing is a regular RNA-mediated acquired protection mechanism found in some prokaryotic organisms [53]. It has quickly become the most hopeful genome editing tool with the pronounced potential to modernize biomedical treatment [54].

CONCLUSION

The treatment of liver diseases has been complicated, which needs and requests for novel and improved knowledge. Liver inflammation can resolve spontaneously or progress to more advanced stages, i.e. fibrosis, cirrhosis, and liver cancer, respectively. These three late stages were considered irreversible phenomenon. Still, recent advancements in gene therapy, stem cell therapy, and genome editing open a good road to treating liver diseases.

Regarding the other types of liver diseases, it is possible to provide potentially effective treatments. Although there are many obstacles, especially in the preparation of different systems in terms of safety and efficiency, different gene delivery pathways, the immunogenicity of vectors used, and improving cells type specificity, still, the potential to correct genetic defects and increase the quality of life in patients is very promising. It was merging advanced gene-editing techniques with methods for using viral and non-viral vectors that can revolutionize medical sciences and cure many disorders. Improving these methods in the future could target many polygenic conditions, and the ability of these methods to regulate gene expression could provide more effective treatments for a wide kind of disease.

Among stem cell types, IPSCs technology has provided unique chances to study, inhibit and treat a variety of human diseases, especially liver diseases. The ability of these cells to regenerate themselves and differentiate has led to the total production of hepatocyte-like cells for transplantation and research. For example, these differentiated cells can be used in cell therapy to repair, replace, and regenerate liver tissue efficiently. In addition, IPSCs consider valuable resources for evaluating drugs interactions, metabolism, and toxicity of the drug and biological factors [55-57].

However, there are still barriers and limitations to using these cells that need to address. For instance, evidence on IPSC cell lines and reprogramming procedures and their differentiation into hepatocyte-like cells should be enhanced to increase their effectiveness.

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REVIEW ARTICLE

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Gingival involvement of oral non-tumoral mucosal diseases

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Abstract: *Gingival conditions are frequently encountered in dental clinical practice and they can raise diagnosis and treatment problems. The diagnostic is variable from normal conditions to autoimmune diseases or even malignancy. Some of them cause disturbances such as pain, burning sensation and have an impact on patient's quality of life.*

INTRODUCTION

Although oral cavity is an area easy to explore by visual exam, there is a large variety of conditions and disorders which can be encountered. We present a general overview of gingival lesions frequently associated with oral mucosal diseases. Dental plaque-induced gingivitis and all gingival enlargements (reactive or tumorous with benign, cystic or malignant variants) are not the subject of this article.

DEFINITION OF SPECIFIC TERMS AND LESIONS

The gingiva is a "first line" mechanical barrier defined as the keratinized mucosa that covers the teeth and the alveolar bone. The periodontium is made of gingiva with the alveolar bone, periodontal ligament and cementum. The mucogingival junction separates the gingiva from alveolar mucosa [1]. In healthy persons the gingiva has a pink color (Figure 1), or it can have pigmented areas depending on the individual skin color (Figure 2).

Histologically, gingiva is composed of a thick stratified, keratinized epithelium and the underling connective tissue made of collagen and elastin fibers, fibroblasts, melanocytes and immune cells [2]. The epithelium cell-to-cell adhesion is done through desmosomes and adherence junctions while the adhesion to the basement membrane is done by hemidesmosomes and focal contacts [3].

Superficial lesions affect epithelium and underlying connective tissue. Depending on the clinical appearance, evidenced by visual examination, they can be divided into: keratosis or white lesion (Figure 3A and 3B), atrophic (Figures 4A, 4B, 4C), ulcers resulting from vesicles (Figure 5A) or bulla (Figure 5B).

The gingival diseases are presented in the periodontal diseases classification elaborated by Armitage in 1999 [4] which is universally

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accepted for both periodontal and gingival conditions.

Figure 1. Normal gingiva

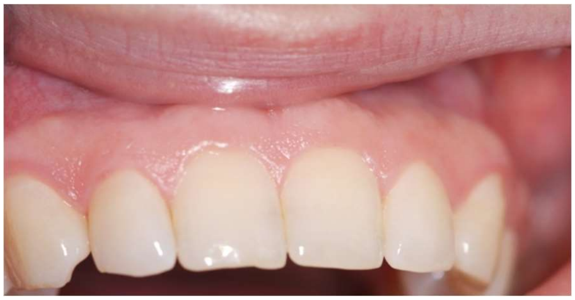

Figure 2. Pigmented gingiva

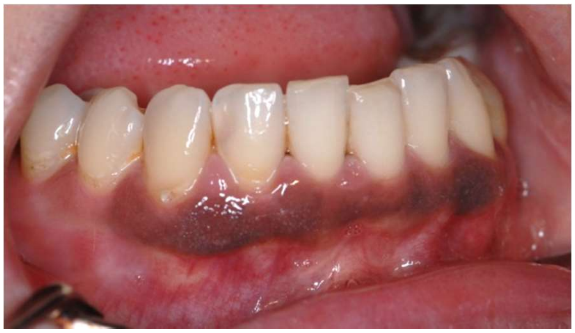

Figure 3A. Gingival white lesion. Extended homogenous plaque of unknown etiology. Diagnosis: leukoplakia

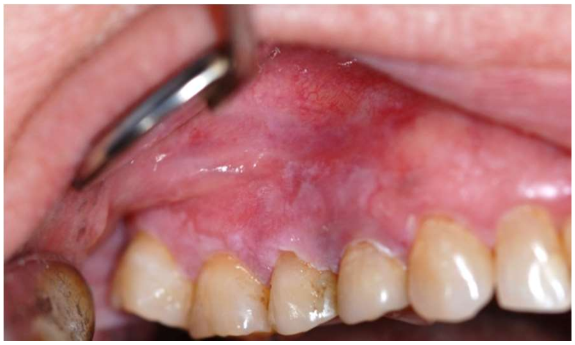

Figure 3B. Gingival white lesion. Diagnosis: traumatic keratosis (associated with aggressive tooth-brushing technique)

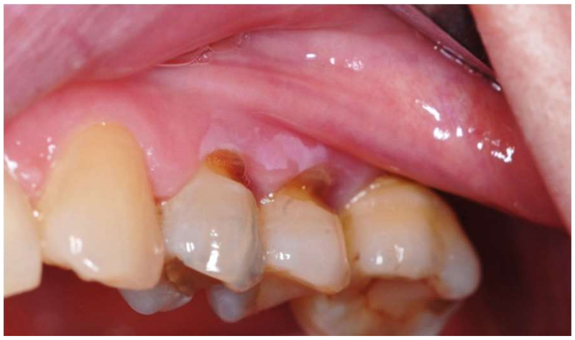

Figure 4A. Mild atrophy. Diagnosis: desquamative gingivitis in oral lichen planus

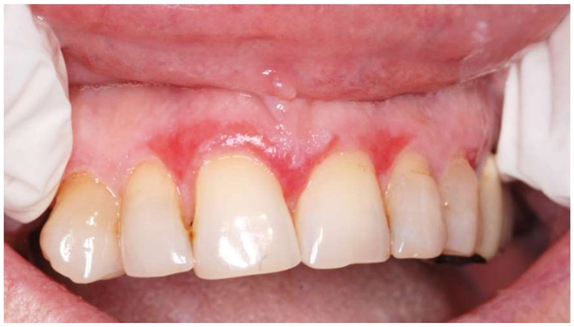

Figure 4B. Moderate atrophy. Diagnosis: desquamative gingivitis in oral lichen planus

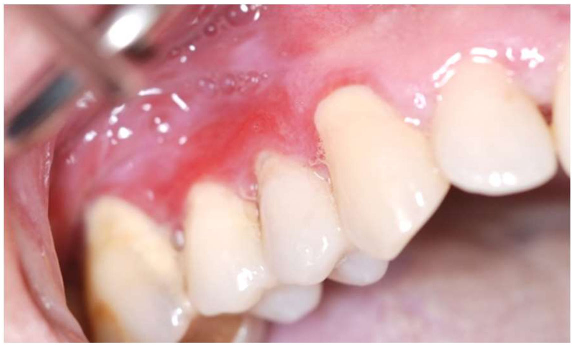

Figure 4C. Severe atrophy and reticular keratosis. Diagnosis: desquamative gingivitis in oral lichen planus

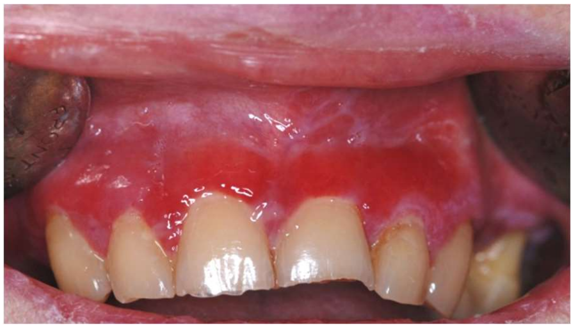

Figure 5A. Small gingival ulcers following vesicles. Diagnosis: primary herpetic gingivitis

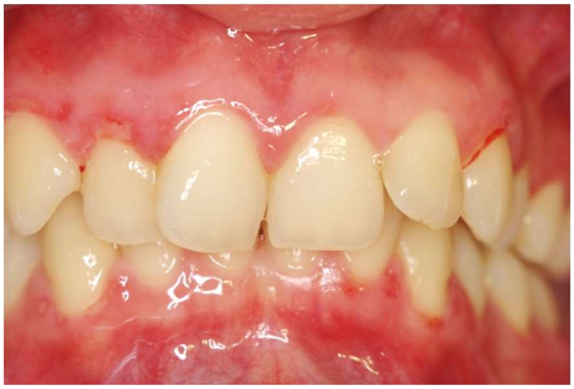

Figure 5B. Extended ulceration after bullae rupture. Diagnosis: desquamative gingivitis in bullous dermatosis

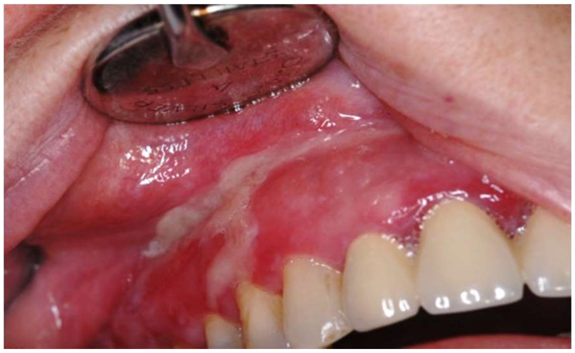

The gingival disorders are presented in Table 1.

CLINICAL FEATURES AND THE BASIC THERAPEUTICALLY APPROACH OF ORAL MUCOSAL DISEASES WITH GINGIVAL INVOLVEMENT

We reviewed and summarized the clinical characteristics of the most frequent oral diseases. The oral manifestations of general diseases cover a large range of conditions (Table 2). The mainly local signs usually affects more than one mucosal area, so the gingival lesions must be interpreted in the whole context.

Table 1. The gingival diseases classification [4]

Periodontal diseases classification. Section gingival diseases		
A. Dental plaque-induced gingival diseases	1. Gingivitis associated with dental plaque only 2. Gingival diseases modified by systemic factors 3. Gingival diseases modified by medications 4. Gingival diseases modified by malnutrition	
B. Non-plaque-induced gingival lesions	1. Gingival diseases of specific bacterial origin 2. Gingival diseases of viral origin 3. Gingival diseases of fungal origin	
C. Gingival lesions of genetic origin	1. Hereditary gingival fibromatosis 2. Other	
D. Gingival manifestations of systemic conditions	1. Mucocutaneous disorders	1) lichen planus 2) pemphigoid 3) pemphigus vulgaris 4) erythema multiforme 5) lupus erythematosus 6) drug-induced 7) other
	2. Allergic reactions	
E. Traumatic lesions (factitious, iatrogenic, accidental)	1. Chemical injury 2. Physical injury 3. Thermal injury	
F. Foreign body reactions		

Oral lichen planus is a disease with chronic evolution and immune mediated pathogenesis [5]. The clinical signs and symptoms are variable depending on the lesions associated [2]. The lesions are multiple, associated, merged and coexist and symmetrically distributed. The gingival involvement, commonly encountered in these patients is described as desquamative gingivitis. This shows various lesions from white, keratotic to atrophy, ulcerations and bullae. The diagnostic criteria universally accepted includes the histological confirmation (with hydropic degeneration and band-like T-cells infiltrate), although there are microscopic features similar to other mucosal diseases [6].

The therapy is not curative but it intends to control acute lesions and symptoms. The agents used cover a large spectrum of drugs: topical or systemic corticoids, hydroxychloroquine, azathioprine, mycophenolate mofetil, methotrexate, dapsone, thalidomide, biological agents etc. [6].

Mucous membrane pemphigoid is a chronic autoimmune blistering disease which presents subepithelial vesicles and bullae. Any area of the oral mucosa can be affected. The gingival lesions present as desquamative gingivitis which simulates oral lichen planus and are noticed in 60% of cases [6]. The diagnosis is established by histopathological analysis and direct immunofluorescence which shows along the basal membrane linear deposits of immune-globulin IgG, IgM, IgA and complement [6]. The therapy is mainly long-term immunosuppressive treatment used local and systemic.

Table 2. General data on oral mucosal diseases, clinical features [1-3]

Oral conditions	Disease	Oral mucosa manifestations
Autoimmune diseases	Oral lichen planus	Variable presentation, generally symmetrical lesions (reticular, popular, plaque form, atrophy, ulcers, bulla)
	Pemphigus vulgaris	Small intraepithelial bulla and secondary erosion
	Mucous membrane pemphigoid	Desquamative gingivitis, shallow ulcer after multiple blisters affecting the oral mucosa
	Linear IgA dermatosis	Annular vesicular-bullous lesions
	Allergic reaction to dental materials and/oral health products	Variable clinical features: inflammation, reddish or white areas
Infectious diseases		
Viral	Primary herpetic infection	Ulcerative lesions following short duration vesicles
	Secondary herpetic infection	
	Herpes zoster	Unilateral ulcers following vesicles, located along nerve distribution, mainly in older patients
Bacterial	Necrotizing ulcerative gingivitis	Painful, purulent ulcers of the gingival papillae; mostly in teenagers and young adults
Traumatic lesions	Caused by excessive tooth brushing	Ulcers, atrophy and keratosis
	Mucosal burn caused by substances held in close contact for a long period of time	
	Local radiotherapy	
Pigmented lesions	Rasial pigmentation	Pigmented areas on the gingiva
	Melanotic macule	
	Metallic tattoo	
White lesions	Leukoplakia	Predominantly white lesion of unknown or tobacco etiology

Oral pemphigus vulgaris is a lifelong autoimmune bullous disease which affects the epithelium with autoantibodies directed against the desmosomal cadherine – desmoglein 3 causing acantholysis and blistering [7]. Oral mucosa is the initial site of the lesions in 80% of the patients [7]. The painful, irregular-shaped erosions and blisters affect the buccal mucosa, the tongue and gingiva. In these patients the desquamative gingivitis shows ulcers following bullae. The diagnosis is established by clinical features, histopathology, immunopathology, and serology. Direct immunofluorescence shows intercellular deposition of immunoglobulin G and complement 3 [7]. Treatment is complex and includes a variety of immunosuppressive and anti-inflammatory steroids in order to get disease control.

In patients with desquamative gingivitis there is an increased risk periodontal tissue breakdown mainly caused by the plaque deposits and the pain associated with tooth-brushing [8].

Herpetic oral viral infection is caused by two types of herpes simplex virus. The primary infection which affects mainly children or young adults shows a wide range of manifestations varying from an extended oral vesicular-ulcerative eruption to a reduced, asymptomatic condition [2, 9]. The primary eruption may be associated with general signs and symptoms such as fever, malaise, lymphadenopathy and dysphagia. The secondary infective episode usually involve gingival mucosa and the hard palate. The lesions are vesicles which break quickly and leave painful ulcers. These heal in 7-10 days without scars [9] in healthy and immunologically competent patients. Infections diagnosed early show a good response to antiviral medication. Also local symptomatic suspensions will ease the discomfort in both primary and secondary herpetic eruption.

For **pigmented lesions of the oral cavity**, more than for other conditions the diagnosis is challenging even for an experienced clinician [10]. On the gingiva the physiological pigmentation and the melanotic macules are frequently seen but this does not exclude a melanoma or Kaposi sarcoma diagnosis. The physiological pigmentation is caused by an increased melanin pigmentation of the basal layer or incontinent melanin and/or melanophages in the superficial lamina propria and the melanotic macules are a result of a increased melanin production from the basal melanocytes. In these cases, the diagnosis is appreciated after size, shape, or color, location together with the anamnestic data [10]. Generally a biopsy is needed to determine a definitive diagnosis and to exclude risky conditions.

Oral leukoplakia is defined as a predominately white lesion of the oral mucosa, lesion of questionable risk having excluded (other) known diseases or disorders that carry no increased risk of cancer [11]. It can be met in any area of the oral mucosa, including gingiva. At present about this lesion, the literature states that neither histology nor clinical factors can reliably predict the behavior [12]. Proliferative verrucous leukoplakia is one of the most aggressive form described which shows a predominant gingival involvement.

CONCLUSIONS

These gingival conditions can share similar clinical features which may lead to misdiagnosis or underdiagnosed situations. Dentists and general practitioners need to be familial to the gingival disorders. In general a definitive diagnosis is based on biopsy of the lesional tissue but also the anamnestic data should be taken to account.

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VARIA

Golden hour of sepsis: Can we do more?

Sebastian Dogaru[1], Ciprian Jurcuţ[1], Caius B. Teușdea[1], Alexandru Rocşoreanu[2], Florea Purcaru[2]

Abstract: *Introduction: Sepsis is a life-threatening organ dysfunction due to a dysregulated host response to an infection1 and also an important worldwide health problem. Recent progresses in understanding pathogenesis are reflected in increasing emphasis put on time (Sepsis Update 2018 decreased the time for diagnosis and treatment from 3- 6 hs Sepsis-2 to 1 hour). The aim of the study was to combine an early warning score (increased sensitivity- NEWS2) and a biomarker (increased specificity- presepsin) in order to detect earlier an acute illness (infection) before becoming life-threatening.*

Materials and methods: It was a retrospective, single-center observational cohort study of 125 consecutive patients who were diagnosed with systemic inflammatory response syndrome between July 2016 and July 2018 at Emergency Department of "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania in order to determine sooner, which patient will further develop a sepsis. We used the area under receiver–operator characteristic curves (AUC) to assess the overall discriminatory power of NEWS2, MEDS and presepsin in detecting sepsis, septic shock and the probability of death on admission and compared them with combined scores between NEWS2 and presepsin.

Results: Using a combined score and a simple algorithm, the NEWS2 score had changed (12,8%) for 16 patients. For 10 out of 95 (10,52%) without sepsis and 6 out of 30 (20%) of septic patients, more points were added to NEWS2 meaning that more septic patients were diagnosed before an infection become life-threatening

Conclusions: In the present study, we have tried to assess the impact of a monitoring score and a rapid POCT biomarker, in a condition when time is of critical importance. Using a verified early warning score, NEWS2, with increased sensibility and low specificity and presepsin, POCT biomarker with increased specificity, we diagnosed sepsis earlier in a number of cases.

Keywords: *sepsis, early warning score, NEWS2, combined score, presepsin*

INTRODUCTION

Sepsis is a life-threatening organ dysfunction due to a dysregulated host response to an infection [1] and also an important worldwide health problem. Nowadays the global sepsis estimates are more than double than previous calculations, with 11 million sepsis deaths and 48.9 million incident sepsis cases in 2017 with a global trend of decreasing sepsis [2]. Although Sepsis-3 definition is designed to put a diagnosis in prehospital settings, battlefield and Emergency Department (ED) on arrival there is much to be done to further improve diagnostic of patients with sepsis. The specificity of the Sepsis-3 criteria is great, but sometimes the sepsis diagnostic is put too late for a specific patient.

Rapid clinical evaluation on arrival in ED and as soon as possible paraclinical evaluation are realized with POCT (point of care testing) devices which allow a more rapid targeted response (complete blood count, acid-base analysis, targeted biomarkers, etc.) to gain time for the patient and to prevent and fight against further deterioration of a patient in a not an optimal state. Sepsis-2 [3] emphasized the fact

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that time management is of critical importance. Early targeted therapy and management bundle put an algorithm at hand for doctors and 2018 Sepsis update [4] indirectly stressed out that rapid medical intervention is paramount because what it should have been done in the first 3-6 hours, from that moment on, it must be done in one hour. For greater accuracy and tailored treatment more exhaustive laboratory analysis usually arrive in at least 30-40 minutes.

By 2015 over 36 Early Warning Scores were used and evaluated for accurately forecast the prognosis [5]. Developed as specific tools, in different environments (different wards, ED, ICU), some scores are nowadays obsolete or of limited use [5, 6] but some are still used and revised (NEWS2).

A possible research direction in sepsis might be to combine an early warning sign/score with increased sensibility, low specificity and a specific biomarker which will increase the specificity to buy time for the patient and ultimately to save more lives with increased cost-results efficiency.

Conceived in 2012, verified on different wards for several years and revised in 2017, National Early Warning Score 2 is nowadays used in United Kingdom with great success [7].

Used in different environments, presepsin is one of the biomarkers which passed the proof the time, as a valuable tool in POCT, being used for more than a decade now [8, 9, 10, 11, 12, 13]. Presepsin increases in patients developing infections in a severity-dependent manner [14, 15, 16]. Previous studies showed that presepsin levels performed when used for early diagnosis, risk stratification and prognosis [17, 18].

The aim of the study was to find new ways in which to combine these recently developed tools, an early warning score (NEWS2) and a specific biomarker (presepsin) in order to diagnose more cases of severe infections, before developing sepsis, preventing further deterioration of an already altered patient with multiple comorbidities. The proposed score is easy to calculate, rapidly available and allowed us to markedly decrease the time for intervention for patients with altered medical status before becoming life-threatening.

MATERIAL AND METHODS

Study design

This was a retrospective, single-center observational cohort study of 125 consecutive patients who were diagnosed with systemic inflammatory response syndrome between July 2016 and July 2018 at Emergency Department of “Dr. Carol Davila” Central Military Emergency University Hospital, Bucharest, Romania in order to determine sooner, which patient will further develop a sepsis. We developed a simple algorithm for earlier diagnose of sepsis and compared the results with Sepsis-3 criteria, NEWS2 and MEDS scores. All patients were above 18 years old and have agreed to sign the informed consent. The study was approved by the ethical committee at “Dr. Carol Davila” Central Military Emergency University Hospital, Bucharest, Romania.

Blood sample analysis

Presepsin concentrations were measured using a chemiluminescent enzyme immunoassay (PATHFAST™ Presepsin), performed on the PATHFAST point-of-care analyzer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Measuring range is 20–20,000 ng/L; manufacturer's URL (95th percentile) is 320 ng/L. Correlation between heparinized and EDTA plasma was Y = 1.00× + 1.53 (r=0.999, n=23) [19].

Developing a tool for earlier diagnosis of sepsis

National Early Warning Score (NEWS) 2 is recommended by the NHS (National Health System) UK to determine the degree of illness of a patient, to prompt critical care intervention and recommends the NEWS 2 in the following settings: Emergency (for initial assessment, serial monitoring and assessment for triage), ward (for initial inpatient assessment and serial monitoring), prehospital (for communication of illness severity to receiving hospitals). Respiratory rate, hypercapnic respiratory failure/room or supplemental O_2, temperature, systolic BP (mmHg), pulse (beats per minute) and consciousness are assessed.

In order to try other combination of NEWS2 and PSP we have obtained other scorings to better predict the prognosis and earlier detection of sepsis.

We have created a new score NEWS2PSP: for PSP between 0-1000 pg/ml we granted 1 point; between 1000-2000 points=2 points, and so on. Adding those values to NEWS2 score lead us to the new score NEWS2 PSP.

Another compounded score was tested: for PSP<200 pg/ml we have given 1 point, 2 points for PSP=200- 300 pg/ml, 3 points for PSP=300-500 pg/ml, 4 points for PSP=500-1000 pg/ml and 5 points for PSP>1000 pg/ml. The new score was NEWS2PSP2.

NEWS2PSP3 resulted from granting 1 point if PSP>1000 and 0 points if PSP<1000.

Granting 0 points for PSP<5000 pg/ml, 1 point for PSP=5000-10000 pg/ml and 2 points for PSP>10000 and adding these points to NEWS2 lead us to NEWS2PSP4.

Statistical analysis

Descriptive statistics was used to summarize the study population. Baseline and follow-up characteristics were described by means and standard deviations (SD) or by median and interquartile range (IQR) for continuous variables, as appropriate, and by percentages for categorical variables. We performed Chi2 statistics or Fisher's exact tests when appropriate for qualitative variables, and the Wilcoxon/Mann–Whitney test for continuous variables with skewed distributions to compare baseline patient characteristics and study outcomes. Categorical variables are presented as counts and percentages. We used the area under receiver–operator characteristic curves (AUC) to assess the overall discriminatory power of NEWS2, MEDS and presepsin in detecting sepsis, septic shock of the probability of death on admission. The IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

RESULTS

There were 125 consecutive patients which were considered to have SIRS (systemic inflammatory response syndrome) and included in the study. When 2 or more SIRS criteria were found, presepsin (PSP) was assessed. From these 30 were diagnosed as having sepsis (Sepsis-3 definition, with 2 or more qSOFA criteria) and 11 were diagnosed as having septic shock. During the study 27 patients have died from different causes, some of them being sepsis or septic shock. The mean age was of 70.88 years with standard deviation of 15.74 years; they ranged from 18 to 96 years. Of the total study population, 79 (63.2%) were men. Table 1 provides the baseline characteristics demographic data, the calculated scores, selected comorbidities and the site of infection.

Table 1: Baseline characteristics, used scores, selected comorbidities and the site of infection

Demographics	All patients (n=125)	Sepsis (n=30)	Septic shock (n=11)	Exitus (n=27)
Age (mean, SD)	70.888 (15.74406)	73.4666 (15.3139)	72.2727 (15.3391)	75.6296 (14.4277)
Sex (male, %)	79 (63.2)	23 (29.11)	10 (12.65)	20 (74.07)
Weight (mean, SD)	71.472 (12.6835)	70.7333 (9.2805)	72.2727 (9.3525)	70.5185 (15.8637)
GCS (mean, SD)	14.264 (2.1841)	12.8666 (2.9970)	12.4545 (2.6064)	13.8148 (2.4651)
QSOFA (mean, SD)	0.672 (0.9779)	2.2666 (0.4422)	2.5454 (0.4979)	1.1481 (1.1123)
NEWS2 (mean, SD)	4.768 (3.6532)	9.7333 (2.8511)	12 (2.3741)	6.5925 (4.0208)
MEDS (mean, SD)	9.568 (4.3512)	14.0333 (2.9606)	15.8181 (2.5873)	12.9259 (4.2681)
PSP (mean, SD)	2594.608 (3063.226)	3582.8 (4306.524)	2961.545 (2163.594)	3272.593 (4373.756)
Selected comorbidities				
Malignancy (%)	35 (28)	10 (28.57)	2 (5.71)	8 (22.85)
Diabetes melitus (%)	27 (21.6)	9 (33.33)	4 (14.81)	6 (22.22)
Cardiac failure (%)	49 (39.2)	9(18.36)	4 (8.16)	13 (26.53)
Kidney failure (%)	36 (28.8)	7 (19.44)	3 (8.33)	11 (30.55)
Respiratory failure (%)	20 (16)	8 (40)	3 (15)	8 (40)
Institutionalized patient (%)	7 (5.6)	3 (42.85)	1 (14.28)	6 (85.71)
Site of infection				
Abdominal (%)	26 (20.8)	6 (23.07)	3 (11.53)	8 (30.76)
Cutaneous (%)	15 (12)	1 (6.66)	0	3 (20)
Endocard (%)	1 (0.83)	0	0	1 (100)
Pulmonary (%)	20 (16)	6 (0.3)	2 (10)	5 (25)
Kidney (%)	28 (22.4)	11 (39.28)	5 (17.85)	5 (17.85)
Unspecified (%)	35 (28)	6 (17.14)	1 (2.85)	5 (14.28)

GCS= Glasgow Coma Scale, qSOFA= quick Sequential Organ Failure Assessment, NEWS2= National Early Warning Score 2, MEDS= Mortality in Emergency Department Score, PSP= presepsin

Patients who met the Sepsis-3 criteria (2 or more qSOFA points) were treated accordingly to the current guidelines for sepsis. Only 30 patients were diagnosed with sepsis and 11 with septic shock.

Table 2 shows the Area Under the Receiver Operating Characteristics and confidence intervals for the different scores and compound scores.

NEWS2 9.7±2.9, MEDS 14.0±3.0 and PSP (presepsin) 3582.8±4380.1 showed increased values for sepsis with statistical significance for NEWS2 $p<0.001$ MEDS $p<0.001$; of note the statistical significance for PSP was $p=0.043$. For sepsis NEWS2 has the best AUROC (Area under ROC) 0.962

(95%CI 5.54-7.51), MEDS 0.875 (95%CI 4.39-7.36), PSP 0.603(95%CI 41.32-2559.17) according to Sepsis-3 criteria as depicted in Figure 2. ROC curves are the same for septic shock NEWS2 0.974 (95%CI 6.11-9.74), MEDS 0.913(95%CI 4.39-9.3) and respectively for PSP 0.626 (95%CI 1526.19-2330.88) (Figure 3).

Table 2: AUROC and 95%CI calculated for different combined scores in sepsis, septic shock and mortality

	Sepsis		**Septic shock**		**Mortality**	
	AUROC	**95%CI**	**AUROC**	**95%CI**	**AUROC**	**95%CI**
NEWS2	0,962	5,54- 7,51	0,974	6,11- 9,74	0,670	0,79- 3,85
MEDS	0,875	4,39- 7,36	0,913	4,39- 9,3	**0,762**	2,55- 6,01
PSP	0,603	41,32- 2559,17	0,626	-1526,19- 2330,88	0,561	-454,79- 2184,34
NEWS2PSP	0,z924	6,36- 9,45	0,949	5,66- 11,20	0,656	1,21- 5,39
NEWS2PSP2	0,953	5,69- 7,84	**0,975**	6,31- 10,19	0,676	0,94- 4,17
NEWS2PSP3	0,956	5,95- 7,65	**0,975**	6,22- 9,96	0,672	0,90- 4,03
NEWS2PSP4	**0,971**	5,69- 7,65	0,972	6,07- 9,76	0,670	0,82- 3,91

Figure 1: Receiver-operating characteristics (ROC) curves for NEWS2, MEDS, PSP and for derived scores (NEWS2PSP, NEWS2PSP2, NEWS2PSP3, NEWS2PSP4) in sepsis (AUROC 0,971 %95 CI 5,69- 7,65 for NEWS2PSP4)

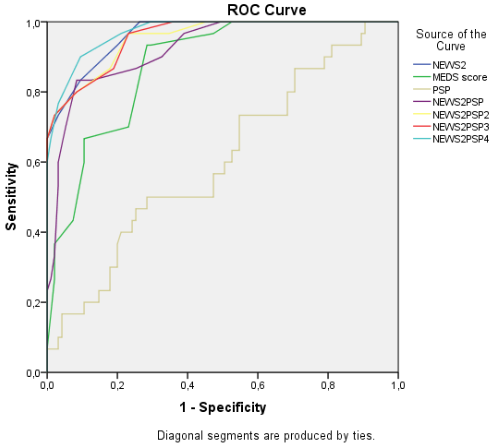

Figure 2: Receiver-operating characteristics (ROC) curves for NEWS2, MEDS, PSP and for derived scores (NEWS2PSP, NEWS2PSP2, NEWS2PSP3, NEWS2PSP4) in septic shock (AUROC 0,975 %95 CI 6,31- 10,19 for NEWS2PSP2 respectively AUROC 0,975 %95 CI 6,22- 9,96 for NEWS2PSP3)

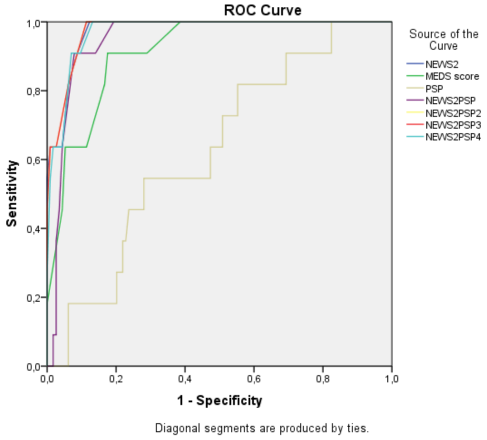

Figure 3: Receiver-operating characteristics (ROC) curves for NEWS2, MEDS, PSP and for derived scores (NEWS2PSP, NEWS2PSP2, NEWS2PSP3, NEWS2PSP4) in mortality (AUROC 0,762 %95 CI 2,55- 6,01 for MEDS score)

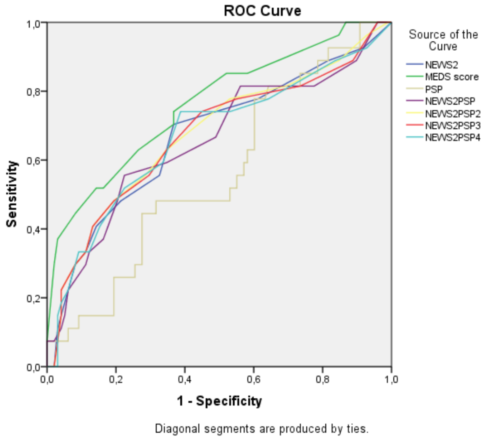

We created a new score, NEWS2PSP, transforming PSP values as follows: between 0-1000 pg/ml = 1 point; 1000-2000 pg/ml = 2 poits, and so on; adding these points to NEWS2 score resulted the new score NEWS2PSP; AUROC for sepsis and septic shock were not superior to NEWS2: AUROC NEWS2PSP in sepsis 0.924 (95%CI 6.36- 9.45), in septic shock 0.949 (95%CI 5.66-11.20), compared to AUROC NEWS2 in sepsis 0.962 (95%CI 5.54-7.51) or in septic shock 0.974 (95%CI 6.11-9.74). MEDS, a specific designed score was superior to NEWS2PSP in predicting mortality AUROC MEDS 0.762 (95%CI 2.55-6.01), AUROC NEWS2PSP 0.656 (95%CI 1.21-5.39).

Another compounded score was tested: for PSP<200 pg/ml we have given 1 point, 2 points for PSP=200- 300 pg/ml, 3 points for PSP=300-500 pg/ml, 4 points for PSP=500-1000 pg/ml and 5 points for PSP>1000 pg/ml. Those values were added to NEWS2 and formed NEWS2PSP2 which has no more added value to NEWS2 for predicting sepsis (AUROC NEWS2 0.962 (95%CI 5.54-7.51), AUROC NEWS2PSP2 0.953 (95%CI 5.69-7.84), minimal in septic shock (AUROC NEWS2 0.974 (95%CI 6.11-9.74), AUROC NEWS2PSP2 0.975 (95%CI 6.31-10.19). MEDS kept an advantage in predicting death over NEWS2PSP2 (AUROC MEDS 0.762 (95%CI 2.55-6.01), AUROC NEWS2PSP2 0.676 (95%CI 0.94-4.17).

NEWS2PSP3 resulted from granting 1 point if PSP>1000 and 0 points if PSP<1000- with no better prognostic value over NEWS2 for predicting sepsis ((AUROC NEWS2 0.962 (95%CI 5.54-7.51), AUROC NEWS2PSP3 0.956 (95%CI 5.95-7.65) and kept a slightly advantage over NEWS2 in septic shock AUROC NEWS2 0.974 (95%CI 6.11-9.74), AUROC NEWS2PSP3 0.975 (95%CI 6.22-9.96). MEDS kept an advantage in predicting death over NEWS2PSP3 (AUROC MEDS 0.762 (95%CI 2.55-6.01), AUROC NEWS2PSP3 0.672 (95%CI 0.90-4.03).

Granting 0 points for PSP<5000 pg/ml, 1 point for PSP=5000-10000 pg/ml and 2 points for PSP>10000 and adding these points to NEWS2 lead us to NEWS2PSP4 which is superior to NEWS2 (AUROC NEWS2PSP4 0.971 (95%CI 5.69-7.65) compared to AUROC NEWS2 0.962 (95%CI 5.54-7.51). Using a cut-off value of 9.5 for NEWS2PSP4 we can diagnosis of sepsis with a sensitivity of 60%. (for a NEWS2PSP4 compound score >6.5 sepsis is diagnosed with a sensitivity of 90% and 90.5% specificity).

Using this simple algorithm, for 16 patients the NEWS2 score had changed (12.8%). For 10 out of 95 (10.52%) without sepsis and 6 out of 30 (20%) of septic patients, more points were added to NEWS2 meaning that more septic patients were diagnosed before an infection become life-threatening.

For septic shock NEWS2PSP4 was a reliable score; AUROC values were as follows: AUROC NEWS2 0.974 (95%CI 6.11-9.74), and NEWS2PSP4 0.972 (95%CI 6.07-9.76) (very close), while MEDS kept the advantage in predicting mortality (similar AUROC for NEWS2 and NEWS2PSP4) (AUROC NEWS2 0.670 (95%CI 0.79-3.85), MEDS 0.762 (95%CI 2.55-6.01), NEWS2PSP4 0.670 (95%CI 0.82-3.91). Figures 1, 2 and 3 represent receiver-operating characteristics (ROC) curves for NEWS2, MEDS, PSP and the derived scores for sepsis/ septic shock/mortality as above.

DISCUSSION

In our study we proved that adding a specific biomarker, presepsin, to NEWS2 we can put a sepsis diagnosis earlier, reliable, with increased accuracy, avoiding further deterioration. Studies have proved that clinical deterioration is often forecasted by changes in vital signs up to 6 to 24 hours [20, 21]. This can buy time for a specific patient, but in fact also for the system to recover.

To be remembered, although an increased specificity sign, ST elevation, did not decrease mortality per se. Only after angina pectoris was combined with a specific biomarker, troponin, combined with proactive actions, coronary stenting, markedly decreased the mortality.

Immunity against a microorganism relies primarily on the activity of monocytes, macrophages and granulocytes that recognize pathogen-associated molecular patterns that activate downstream pathways and participate to bacteria clearance. This is partly done through a cluster-of-differentiation marker protein 14 (CD14), which has an immediate response against lipopolysaccharides (LPS) [14, 22]. After binding of LPS to CD14 through the LPS-binding protein (LBP), a subtype of soluble CD14 (sCD14-ST, or presepsin, a 13 kDa protein that is a truncated N-terminal fragment of CD14 [15, 16] is released to the blood flow.

Different scores were used as specific tools in specific circumstances. The more versatile ones are the ones that can be used in as many as possible situations, with minimum of resources, as fast as possible. National Early Warning Score 2 (NEWS2) is released after 5 years of trials after the first version of evaluation in hospitals in UK. This score advocates a system to standardize the assessment and response to acute illness and its efficacy was proven in time. Using six simple physiological parameters already recorded in routine practice, NEWS2 is a pragmatic approach with a key emphasis on system-wide standardization

Rapid diagnostic on ED saves lives. Acute myocardial infarction, stroke and sepsis are medical conditions when short time to diagnosis is of paramount importance, that cannot be overemphasized.

NEWS2 can be used as a point of departure for assessing an acute medical illness, but to accurately diagnose a patient a specific tool is needed (ST elevation for acute myocardial infarction on EKG, CT scan for stroke and nowadays qSOFA criteria for sepsis).

As Sepsis-3 stated, a sepsis is declared when an infection becomes life-threatening. This puts a rapid diagnose with minimal resources. Having some preliminary results can gain time to earlier diagnose a life-threatening condition and to prevent further deterioration. When we have the Sepsis-3 criteria, the diagnosis is obvious.

We proved that with minimal resources, monitoring vital parameters and assessing a score and when suitable a POCT biomarker, in 15 minutes we raised the suspicion of sepsis, before becoming manifest, with increased accuracy, gaining at least 45 minutes. We found that a biomarker, presepsin, can accurately earlier diagnose sepsis used in correlation with a verified score, NEWS2 which is used for a patient to access the appropriate level of care at a certain moment. We also took into account MEDS as a level of reference, for its power to predict the prognosis due to sepsis (Mortality in Emergency Department Score).

Of note is that the presepsin values were used to help diagnose sepsis after Sepsis-2 definition, but in our study, we tried to use the presepsin for earlier detection of sepsis, after Sepsis-3 definition, before the clinical life-threatening criteria to become manifest (SBP≤100 mmHg, ≥22 breaths per min, GCS<15).

With an altered mental state, incomplete anamnesis to be taken from relatives or the lack of proper documents, increasing number of patients on admission and too often not enough personnel, an accurate, proper assessment on admission is mandatory. After the initial evaluation, vital functions monitoring and blood samples are taken, before more complete laboratory analysis to be available – with a median of one hour, the Point-Of-Care-Testing devices can give you a more comprehensive understanding of the actual medical state in approximatively 15 minutes. POCT biomarkers, arterial gases analysis and complete blood count with multiple scores applied, offer a more comprehensive assessment of a patient and sometimes prioritize the subsequent investigations. While a MEDS score needs not so easy to get information (rapid terminal comorbid illness, neutrophil bands, lower respiratory infection or nursing home resident) NEWS2 is a reliable, verified score intended to be used for monitoring, with increased sensitivity. Based on initial evaluation, taking presepsin (available in 17 minutes) only to patients with SIRS and NEWS2 assessment, taken together increased the number of the patients to be earlier diagnosed with sepsis with 10%. Presepsin was the ideal biomarker due to the fact that it has earlier response compared with procalcitonin after the onset of an infection (peak level<2 h) plus a short half-time (0,5-1 h) compared with those of procalcitonin (2-3 h and 20-24 h, respectively) and CRP (6 h and 4-6 h, respectively) lead us to use this biomarker. The lack of specificity as procalcitonin increases also in surgical interventions, cardiogenic shock, thermal shock, graft-versus-host disease, immunotherapy, burns, acute pancreatitis lead us to consider using presepsin as specific biomarker [21, 23, 24].

To our knowledge this was the first report of PSP and NEWS2 score used in combination for sepsis diagnosis. Nevertheless, although this association is better for earlier detection of sepsis, there no added advantages in predicting septic shock or mortality.

Sepsis is not a completely understood pathology. Surviving Sepsis Campaign with the periodic reevaluation (even the Sepsis, Sepsis-2, Sepsis-3 definition suggests the dynamics and the major concern involved) still leaves room for refinement and improvement. Our study reflects our approach for earlier diagnose of sepsis. Having a patient with clear criteria for sepsis accordingly to qSOFA leaves no room for doubts and the nowadays guidelines stress the importance of earlier diagnosis and rapid measurements to be taken (2018 Sepsis Uptated) in the first hour.

Sepsis defined as life-threatening organ dysfunction caused by a dysregulated host response to infection does not shed light over the dysregulation. Is it due to the increased aggressivity of the pathogen, to the amount of the pathogen, to the particularities of the injured organ/system, of the comorbidities which decrease the defense mechanisms, of the defense of the defense mechanisms itself? In order to achieve timely treatment for a possible lethal condition, a sensitive screening tool is more important than a specific one [25, 26] and identifying the poor prognostic factors may have a role [27].

Although qSOFA has an increased specificity and minimal resources to be used, lacks the sensibility and consequently the diagnosis of sepsis is often put too late. Even one point granted on initial evaluation does not imply that subsequent evolution is not going to be worse, to further deteriorate without becoming life-threatening. So, more refined approach is sometimes necessary.

Study limitations and strengths.

The present study has some limitation. First, being a single center retrospective, observational study we may not have a comprehensive view of this complex pathology. Second, being a tertiary hospital, may have had more complex patients which may have biased in generalizing the conclusions. Future studies should include more patients from different sites. Third, sepsis defined as life-threatening condition, may alter the health status by itself- an out-control infection might bias the renal function by itself or by aggravation of an already altered renal function as a comorbidity in a more general health status. Fourth, the limited number of patients studied, although significant, are a mere part of the patients with sepsis, so the drawn conclusions should be further verified. Fifth, the size of the cohort might have not enough power requested to draw final conclusions. Finally, although already described in literature, the correlation between presepsin/ biomarker with renal clearance levels and kidney function in patients with life-threatening condition should be studied further.

As strengths in our study we assessed the impact of an earlier intervention over a life-threatening condition in which time is of critical importance. Although widely used, the scores are sometimes inadequately applied. Making distinction between the goals and the moment to be applied to, a score is not a goal in itself. Using a monitoring, verified score as NEWS2, high sensitivity, low specificity combined with a specific biomarker with short time until peak values are attained, lead us to suggest that before becoming dysregulated and life threatening, the sepsis might have an earlier, more optimized approach.

CONCLUSIONS

In the present study, we have tried to assess the impact of a monitoring score and a rapid POCT biomarker, in a condition where time is of critical importance. We acknowledged that in a not completely understood lethal pathology as sepsis, different approaches might be tried in order to earlier detect a life-threatening condition. Using a verified monitoring score, NEWS2, with increased sensibility and low specificity and presepsin, POCT biomarker result within 17 minutes, we diagnosed sepsis earlier.

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4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

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Accepted Articles

The accepted 'in press' manuscripts are published online very soon after acceptance, prior to copy-editing or typesetting. Accepted Articles are published online a few days after final acceptance, appear in PDF format only, are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. After print publication, the DOI remains valid and can continue to be used to cite and access the article. Given that copyright licensing is a condition of publication, a completed copyright form is required before a manuscript can be processed as an Accepted Article.

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Once the paper has been typeset, the corresponding author will receive an e-mail alert containing instructions on how to provide proof corrections to the article. It is therefore essential that a working e-mail address is provided for the corresponding author. Proofs should be corrected carefully; the responsibility for detecting errors lies with the author. The proof should be checked, and approval to publish the article should be emailed to the Publisher by the date indicated; otherwise, it may be signed off on by the Editor or held over to the next issue.

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Details are on the Copyright Agreement Form that must be completed and signed when the Article is accepted.

ADMINISTRATIVE ISSUES

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Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

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(1) Accessible to the public at no charge;
(2) Searchable by standard, electronic (Internet-based) methods;
(3) Open to all prospective registrants free of charge or at minimal cost;
(4) Validates registered information;
(5) Identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of the last follow-up, the target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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Parts of the Manuscript

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The title page should contain (i) a short informative title that contains the major keywords. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgments. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’ industrial links and affiliations. In the case of clinical trials or any article describing the use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

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As papers are double-blind peer-reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and keywords, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as a parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings, and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine’s Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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References should be listed in the following form:

Number references in the order cited as Arabic numerals in square brackets on the line. Only literature that is published or in press (with the name of the publication known) may be numbered and listed; abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Refer to only in the text, in parentheses, other material (manuscripts submitted, unpublished data, personal communications, and the like) as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication. Use Index Medicus as the style guide for references and other journal abbreviations. List all authors up to six, using six and "et al." when the number is greater than six.

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A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

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Accepted Articles

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Accepted Articles

The accepted ‘in press’ manuscripts are published online very soon after acceptance, before copy-editing or typesetting. Accepted Articles are published online a few days after final acceptance, appear in PDF format only, are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. After print publication, the DOI remains valid and can continue to be used to cite and access the article. Given that copyright licensing is a condition of publication, a completed copyright form is required before a manuscript can be processed as an Accepted Article.

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Once the paper has been typeset, the corresponding author will receive an e-mail alert containing instructions on how to provide proof corrections to the article. It is therefore essential that a working e-mail address is provided for the corresponding author. Proofs should be corrected carefully; the responsibility for detecting errors lies with the author. The proof should be checked, and approval to publish the article should be emailed to the Publisher by the date indicated; otherwise, it may be signed off on by the Editor or held over to the next issue.

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Details are on the Copyright Agreement Form that must be completed and signed when the Article is accepted.

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Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

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(1) Accessible to the public at no charge;
(2) Searchable by standard, electronic (Internet-based) methods;
(3) Open to all prospective registrants free of charge or at minimal cost;
(4) Validates registered information;
(5) Identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of the last follow-up, the target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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RJMM welcomes reviews of important topics across the scientific basis of medicine and advances in clinical practice. Most published reviews are in response to the editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

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Parts of the Manuscript

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Title page

The title page should contain (i) a short informative title that contains the major keywords. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgments. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing the use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer-reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and keywords, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as a parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings, and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments, and References*.

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References should be listed in the following form:

Number references in the order cited as Arabic numerals in square brackets on the line. Only literature that is published or in press (with the name of the publication known) may be numbered and listed; abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Refer to only in the text, in parentheses, other material (manuscripts submitted, unpublished data, personal communications, and the like) as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication. Use Index Medicus as the style guide for references and other journal abbreviations. List all authors up to six, using six and "et al." when the number is greater than six.

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SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at rjmilmed@yahoo.com

A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
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Accepted Articles

The accepted ‘in press’ manuscripts are published online very soon after acceptance, before copy-editing or typesetting. Accepted Articles are published online a few days after final acceptance, appear in PDF format only, are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. After print publication, the DOI remains valid and can continue to be used to cite and access the article. Given that copyright licensing is a condition of publication, a completed copyright form is required before a manuscript can be processed as an Accepted Article.

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Once the paper has been typeset, the corresponding author will receive an e-mail alert containing instructions on how to provide proof corrections to the article. It is therefore essential that a working e-mail address is provided for the corresponding author. Proofs should be corrected carefully; the responsibility for detecting errors lies with the author. The proof should be checked, and approval to publish the article should be emailed to the Publisher by the date indicated; otherwise, it may be signed off on by the Editor or held over to the next issue.

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Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

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It should also state clearly in the text that all persons gave their informed consent before their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects from being recognized (or an eye bar should be used).

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(1) Accessible to the public at no charge;

(2) Searchable by standard, electronic (Internet-based) methods;

(3) Open to all prospective registrants free of charge or at minimal cost;

(4) Validates registered information;

(5) Identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of the last follow-up, the target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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Parts of the Manuscript

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Title page

The title page should contain (i) a short informative title that contains the major keywords. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgments. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing the use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer-reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and keywords, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings, and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments, and References*.

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Number references in the order cited as Arabic numerals in parentheses on the line. Only literature that is published or in press (with the name of the publication known) may be numbered and listed; abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Refer to only in the text, in parentheses, other material (manuscripts submitted, unpublished data, personal communications, and the like) as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication. Use Index Medicus as the style guide for references and other journal abbreviations. List all authors up to six, using six and "et al." when the number is greater than six.

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1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly concerning studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
4. For research involving pharmacological agents, devices, or medical technology, a clear Conflict of Interest statement concerning any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.
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2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.
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Accepted Articles

The accepted ‘in press’ manuscripts are published online very soon after acceptance, prior to copy-editing or typesetting. Accepted Articles are published online a few days after final acceptance, appear in PDF format only, are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. After print publication, the DOI remains valid and can continue to be used to cite and access the article. Given that copyright licensing is a condition of publication, a completed copyright form is required before a manuscript can be processed as an Accepted Article.

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Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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(1) Accessible to the public at no charge;
(2) Searchable by standard, electronic (Internet-based) methods;
(3) Open to all prospective registrants free of charge or at minimal cost;
(4) Validates registered information;
(5) Identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published

reviews are in response to editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader’s task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

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Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’ industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine’s Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

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abbreviated in the style used in MEDLINE. Reference to unpublished data and personal communications should appear in the text only.

References should be listed in the following form:

Number references in the order cited as Arabic numerals in parentheses on the line. Only literature that is published or in press (with the name of the publication known) may be numbered and listed; abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Refer to only in the text, in parentheses, other material (manuscripts submitted, unpublished data, personal communications, and the like) as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication. Use Index Medicus as the style guide for references and other journal abbreviations. List all authors up to six, using six and "et al." when the number is greater than six.

Tables

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SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at rjmilmed@yahoo.com

A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.
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2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
4. For research involving pharmacological agents, devices or medical

technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

5. For clinical trials, that these have been registered in a publically accessible database.

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Accepted Articles

The accepted ‘in press’ manuscripts are published online very soon after acceptance, prior to copy-editing or typesetting. Accepted Articles are published online a few days after final acceptance, appear in PDF format only, are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. After print publication, the DOI remains valid and can continue to be used to cite and access the article. Given that copyright licensing is a condition of publication, a completed copyright form is required before a manuscript can be processed as an Accepted Article.

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Details are on the Copyright Agreement Form that must be completed and signed when the Article is accepted.

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Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

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It should also state clearly in the text that all persons gave their informed consent before their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects from being recognized (or an eye bar should be used).

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We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) Accessible to the public at no charge;
(2) Searchable by standard, electronic (Internet-based) methods;
(3) Open to all prospective registrants free of charge or at minimal cost;
(4) Validates registered information;
(5) Identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of the last follow-up, the target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader's task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

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Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major keywords. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgments. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing the use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer-reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and keywords, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings, and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

Text

Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments, and References*.

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References should be listed in the following form:

Number references in the order cited as Arabic numerals in parentheses on the line. Only literature that is published or in press (with the name of the publication known) may be numbered and listed; abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Refer to only in the text, in parentheses, other material (manuscripts submitted, unpublished data, personal communications, and the like) as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication. Use Index Medicus as the style guide for references and other journal abbreviations. List all authors up to six, using six and "et al." when the number is greater than six.

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A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly concerning studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
4. For research involving pharmacological agents, devices, or medical

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5. For clinical trials, these have been registered in a publically accessible database.

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2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.
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Accepted Articles

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To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review.

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Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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(1) Accessible to the public at no charge;
(2) Searchable by standard, electronic (Internet-based) methods;
(3) Open to all prospective registrants free of charge or at minimal cost;
(4) Validates registered information;
(5) Identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

Plagiarism Detection

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RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published

reviews are in response to editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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Abbreviations should be used sparingly and only where they ease the reader’s task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

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Parts of the Manuscript

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Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’ industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine’s Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

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abbreviated in the style used in MEDLINE. Reference to unpublished data and personal communications should appear in the text only.

References should be listed in the following form:

Number references in the order cited as Arabic numerals in parentheses on the line. Only literature that is published or in press (with the name of the publication known) may be numbered and listed; abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Refer to only in the text, in parentheses, other material (manuscripts submitted, unpublished data, personal communications, and the like) as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication. Use Index Medicus as the style guide for references and other journal abbreviations. List all authors up to six, using six and "et al." when the number is greater than six.

Tables

Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at rjmilmed@yahoo.com

A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
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3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.
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1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published

previously, in any language, in whole or in part, and is not currently under consideration elsewhere.

3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.

4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

5. For clinical trials, that these have been registered in a publically accessible database.

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Accepted Articles

The accepted ‘in press’ manuscripts are published online very soon after acceptance, prior to copy-editing or typesetting. Accepted Articles are published online a few days after final acceptance, appear in PDF format only, are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. After print publication, the DOI remains valid and can continue to be used to cite and access the article. Given that copyright licensing is a condition of publication, a completed copyright form is required before a manuscript can be processed as an Accepted Article.

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Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine.* Please read the complete Author Guidelines carefully prior to submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) Accessible to the public at no charge;

(2) Searchable by standard, electronic (Internet-based) methods;

(3) Open to all prospective registrants free of charge or at minimal cost;

(4) Validates registered information;

(5) Identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

Plagiarism Detection

The journal employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

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The journal subscribes to the principles of the Committee on

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Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader’s task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

References

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References should be listed in the following form:

Number references in the order cited as Arabic numerals in parentheses on the line. Only literature that is published or in press (with the name of the publication known) may be numbered and listed; abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Refer to only in the text, in parentheses, other material (manuscripts submitted, unpublished data, personal communications, and the like) as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication. Use Index Medicus as the style guide for references and other journal abbreviations. List all authors up to six, using six and "et al." when the number is greater than six.

Tables

Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at rjmilmed@yahoo.com

A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies

involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.

4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

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SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at daniel_costache@yahoo.com

A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.

2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.

3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research

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Two Word-files need to be included upon submission: A title page file and a main text file that includes all parts of the text in the sequence indicated in the section 'Parts of the manuscript', including tables and figure legends but excluding figures which should be supplied separately.

The main text file should be prepared using Microsoft Word, doubled-spaced. The top, bottom and side margins should be 30 mm. All pages should be numbered consecutively in the top right-hand corner, beginning with the first page of the main text file.

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ORIGINAL ARTICLES

Gut microbiota and obesity

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Abstract: *Worldwide, adults and childhood obesity are increasing alarmingly, being a major health problem. Obesity is correlated with an increased incidence of various systemic diseases including cancer, heart diseases, and diabetes mellitus type 2. Risk factors for obesity are dysbiosis, genetic, socioeconomic, behavioural and environmental. The gut microbiota has beneficial effects on human health, like host immune system stimulation, being influenced by pH, or nutrient intake. Studies performed on humans and animal models reported differences regarding microbiota at lean and obese individuals. The consumption of probiotics and prebiotics may balance the microbiota with positive effects in obesity. The main aim of this review is to reveal the interaction between dysbiosis and obesity.*

Keywords: *microbiota, diabetes, obesity, health problem, cancer, immune system*

INTRODUCTION

It is well known that obesity among children has long become a health problem at least among developed countries [1,2]. In the USA, childhood obesity involves children between 5 to 14 years, who started kindergarten overweight [3]. Obesity reflects the lifestyle of ‘‘developed’’ countries and reached epidemic proportions [4-6]. Compared to 1990 the incidence of obesity has increased by 1/3 in adults and almost double in children [7]. In 2017, UNICEF concluded in the case of children that "there has been no progress to stem the rate of overweight in more than 15 years" [8]. The main and the most common risk factors involved in obesity development are physical inactivity, high fat diet, sugar excessive intake and alcohol consumption [9-12].

Obesity is strongly correlated with an increased incidence of several systemic diseases, such as cardiovascular diseases, type 2 diabetes, and cancer [13-17]. Moreover, obesity can be defined as a complex and multifactorial disease. Over the past few years, gut microbiota plays an important role in obesity pathology [13,18]. Moreover, dysbiosis is implicated not only in intestinal diseases but also in autoimmune diseases, obesity-related diseases and even mental disorders [19].

THE GUT MICROBIOTA DYSBIOSIS

The gut microbiota represents trillions of microbial cells, which has the highest density in the intestinal compartment. Archaea, Bacteria, Eukarya, and viruses represent the gut microbiota family, which can establish complex trophic relationships with each other's and to their human host, leading from symbiosis to parasitism. The gastrointestinal microbiota may be affected by pH, oxygen levels, redox status, nutrient availability, water activity, and temperature [20]. Bacteroidetes and Firmicutes can be detected from healthy human faecal samples with the help of 16 S rRNA-targeted molecular analyses [21].

It is estimated that for every human cell, 10 micro-organismal cells colonise the whole surface of the human body. The gastrointestinal tract has a higher concentration

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of microorganisms, composed mostly of bacteria [22]. Therefore, the gut microbiota is a complex ecosystem that deeply affects the host health and can be modulated by environmental stimuli and genetic factors [23]. Currently, the ratio between human cells to bacteria should be closer to 1:1 (3.0x1013 to 3.8 x1013). Thus, this radio may be influenced by genetic and environmental factors. The gut microbiota has beneficial effects on human health such as host immune system stimulation, extra energy extraction, and host protection against pathogens [24]. Moreover, gut microbiota development starts with birth and is influenced by the host genome, nutrition, and lifestyle. Several factors such as pH, nutrient intake alteration (carbohydrates, proteins, and fats) perturb the microbiota composition [25]. Firstly, the gut microbiota is involved in the breakdown of substrates with resistant starch and dietary fibres, that are incompletely hydrolysed by the host enzyme at the small intestine level [25].

The fermentation products that result from the fibre breakdown are the short fatty acids such as acetate, propionate, and butyrate, which are additional sources of body energy. Therefore, thus acids can be used by the human body [25]. The propionate is taken up by the liver as a precursor for lipogenesis, gluconeogenesis, protein synthesis, acetate is used as a precursor for cholesterol synthesis, while butyrate is used as an energy source by the colonic epithelial cells [25]. The immunologist Elie Metchnikoff was the first who reported a causative link between microbes and human disorders, with the implication of compounds that result from microbial intestinal putrefaction. His research was the first to report the beneficial effects of consuming fermented food to avoid intestinal protein putrefaction in humans [8]. The axis, microbiota-gut-brain is a bidirectional axis involved in the regulation of body weight by balancing appetite, storage, and energy expenditure [26].

Taken together, the gut microbiota regulates, food digestion, nutrient uptake and metabolism, vitamins and bile acids synthesis, innate and mucosal modulation, layer development, epithelial growth, and host gene expression [27]. In humans, between obese and leans persons have been reported differences regarding the microbiota composition, functional genes, and metabolic activities [28]. Oxidative stress-induced by obesity is correlated with lipid peroxidation, DNA damage and decreased levels of antioxidants (enzymatic and non-enzymatic) [2,10-12].

Nowadays, the implication of intestinal microbiota in the progression and, prevention of metabolic dysfunction is more relevant [29]. Some bacteria may facilitate lipogenesis. Therefore, the gut microbiota of obese individuals has several genes that may be depleted or enriched. The genes related to vitamin, cofactor and nucleotide metabolism are usually depleted [21]. More than that, intestinal microbiota can regulate adipose tissue metabolism, homoeostasis, energy balance, appetite, and food uptake, playing a crucial role in obesity [30]. In obese-experimental models performed, some gut microbial strains may inhibit or attenuate the immune response related to chronic inflammation. These results suggest that microbiota plays a dual role, protective or pathogenic [31]. Targeting the gut microbiota using prebiotics, probiotics, and drugs (berberine and metformin) will have a beneficial effect on human health [32].

The immunomodulatory properties of gut microbiota have been observed especially in obese conditions. Therefore, this category of patients is characterized by an altered microbiota or microbial dysbiosis. Obese patients extract more energy from the diet compared with lean individuals leading to excessive energy stores. In obese or diabetic type 2 patients, an enrichment of the pro-inflammatory bacteria (Escherichia coli) and a decrease of anti-inflammatory bacteria (Fecalibacterium prausnitzii) had been reported. These findings suggest that these bacteria represent a source of inflammation-associated metabolic diseases [33]. Moreover, the fermentation products of gut microbiota influence the fatty acid oxidation process, fasting, bile acid production, satiety, lipogenesis, and even innate immunity [34].

A study conducted on C57BL/6 mice, which are genetically protected against obesity after consuming a diet rich in fat and carbohydrates, where colonise with obese mice microbiota. The research group reported a significant increase in body fat and insulin resistance development in C57BL/6 mice [34,35].

Gut microbiota dysbiosis is correlated with proinflammatory cytokines release, and further with increased risk of obesity. All the obesity-related factors will lead to the development of diabetes mellitus type 2 [36], liver disease [37-40], colon cancer [14,41] and even neurological pathologies (Parkinson’s disease, autism) [42]. Some studies reported that human gut microbiota may be affected by sex, so mean have lower microbial diversity the women. Regarding this aspect, women have a lower proportion of Bacteroidetes and increased levels of Akkermansia muciniphila. Instead in the case of men, Ruminococcaceae, Faecalibacterium, and Alistipes are the species detected to be higher, while Bacteroides has a decreased proportion and they do not have Prevotella [43]. The diets used for weight loss which involves high protein intake and decrease consumption of carbohydrates perturbates the microbiota. When these strategies are applied, protein fermentation by gut microbiota take place and metabolic products have released that lead to colon inflammation. Alteration regarding the proportion of Bacteroidetes and Firmicutes may conduce to obesity development [44,45].

PREBIOTICS AND PROBIOTICS IN DIGESTIVE HEALTH

Thus, probiotic supplement consumption may regulate the gut microbiota, improving the prognosis for diabetic patients [44]. Our diet habits play an important role in gut microbiota composition. Differences between intestinal microbiota among individuals fed with Western diet and those fed with a diet rich in fibres had been observed. Additionally, a diet rich in fat can promote the development of pro-inflammatory gut microbiota, leading to increased intestinal permeability and higher levels of lipopolysaccharides [46]. When we eat more high-fat and little fibres aberrant metabolites are produced, leading to the loss of tight junction integrity. This loss of integrity allows the metabolites such as lipopolysaccharides, trimethylamine to enter the circulation and to contribute to liver and adipose tissue chronic inflammation development [42]. Therefore, the relationship between gut microbiota dysbiosis and obesity can be regarded as a dual interaction [47,48]. Faecal metabolites evaluation provides information regarding the relationship between microbiota and host. Regarding the presence of metabolites, 14 compounds, including lysine, omega-muricholate, and glutamic acid, had been identified [49]. Therefore, a decreased proportion of bifidobacterial associated with the death of butyrate-producing bacteria leads to obesity development [50].

The consumption of probiotics, prebiotics or symbiotics may equilibrate the microbiome, with health benefits in obesity. Studies performed on a human, and animals reported that probiotics can promote weight loss in obesity [51]. Consumption of pickled and fermented foods, particularly vegetables and beans, are an important source of pre-, pro-, and synbiotics, with important benefits on human health [52]. ” Bifidobacteria, lactobacilli, and non-pathogenic fungi are the most used probiotics. Probiotics can confer protection against preeclampsia, gestational diabetes, vaginal infections, maternal and infant weight gain, and other diseases [53]. Therefore, manipulating gut microbiota with the help of probiotics as a supplementary therapy may enhance the immune system. This may be an important strategy to reduce side effects after a specific treatment like those with antibiotics [54]. Prebiotics and probiotics are the most used substances that may balance the microbiome when the microbiome homeostasis is perturbated in several diseases [55]. Therefore, probiotics stimulate the immune system, controlling the synthesis of pro-inflammatory and anti-inflammatory cytokines. Clinical trials which include patients diagnosed with systemic and respiratory infections reported that consumption of probiotics such as L. rhamnosus GG, improves intestinal and lung barrier and even homeostasis. L. rhamnosus GG consumption leads to increased regulatory T cells, ameliorates anti-viral defence, and decrease the pro-inflammatory levels [56]. Studies conducted on obese mice reported that bacteriocin-producing Lactobacillus salivarius UCC118 Bac+ altered the gut microbiota and may reduce the weight gain more effectively than the non-bacteriocin-producing strain L. salivarius UCC118 Bac-. Also, the research team reported elevated proportions of Bacteroidetes and Proteobacteria, and decreased proportions of Actinobacteria, while the proportion of Firmicutes remains the same. The beneficial effects of L. salivarius have been demonstrated even in clinical human studies. The Bacteroidetes–Prevotella–Porphyromonas–Firmicutes ratio, have been increased by the L. salivarius Ls-33. Regarding the effects on obese adolescents, L. salivarius Ls-33 did not have beneficial effects. Moreover, in mice who consume a high-fat diet, Lactobacillus, together with Bacillus amyloliquefaciens SC06, can decrease the Firmicutes/Bacteroidetes ratio, body weight, and hepatic steatosis [57].

Kong C and his research revealed that probiotic supplementation slows down weight Moreover, the study reported that both groups (HFD and HCD) had a reduced microbial diversity, an increased production of butyric acid-producing bacteria, an increased proportion of beneficial bacteria (Lactobacillus, Clostridium sensustricto, Prevotella, and Alloprevotella) and conditionally pathogenic bacteria (Bacteroides, Alistipes, and Anaerotruncu). Probiotics administration significantly balance the bacteria proportions in HFD and HCD groups [58]. Obesity left uncontrolled leads to metabolic syndrome installation, pathology associated with systemic and oral diseases in adults and children [39, 59-62].

CONCLUSIONS

The gut microbiota plays a dual role, having negative and positive effects on human health. In obese patients, perturbation of gut microbiota has been reported. Therefore, the obese patients' gut microbiota is characterized by an enhanced proportion of pro-inflammatory bacteria and a decrease of anti-inflammatory species, representing an important source of inflammation. Diet influences our intestinal microbiota, thus an unbalanced nutrient intake leads to dysbiosis, which is further correlated with proinflammatory cytokines release, and obesity development. In conclusion, the consumption of probiotics and prebiotics restore our intestinal microbiome, with health benefits to either lean or obese persons.

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REVIEW ARTICLE

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Gut microbiota – new insight in colorectal cancer pathogenesis

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Abstract: *Gut microbiota is a superorganism involved in homeostasis and in pathogenesis. Microbiota composition is influenced by several factors such as: type of birth (cesarean or natural), role of age, role of diet. Pathological consequences of certain type of diet (especially western type of diet) may, in fact, be mediated by gut microbiota alteration. Also few studies have investigated the issue of gut microbiota composition in patients suffering of colorectal cancer, several reports notice relevant differences. Certain pathogenic bacteria in gut microbiota have been extensively studied in relation to their role in colorectal cancer. Thus, Fusobacterium nucleatum, which is abundant in colorectal cancer patient's colon, may initiate the progression from adenoma to adenocarcinoma. A suggested pattern of oncogenesis in colorectal cancer may be represented by this: dysbiosis-inflammation-oncogenesis. A metanalysis that has been published in 2006 concluded a protective role of probiotics in colorectal cancer and in colonic adenoma.*

Keywords: *colorectal cancer, gut microbiota, probiotics*

Human gut harbors a complex and abundant microbial community which is symbiotically linked with host organism and plays a major role in maintaining the homeostasis by the way of fulfilling various functions such as: limiting the number of intraluminal pathogens, functionnal optimization of immunity system, and important contribution to maintaining energy balance.

It is estimated that there are around 1,014 microorganisms in the colon (mostly bacteria) which is 10 fold above the number of eukaryote cell of human body and that contains a 100 fold richer genome than human genome [1]. As a matter of fact we may acknowledge the existence of a true superorganism whose implications in physiology and pathology of human body are continuously revealed by a great pool of scientific data.

Microbiota composition is influenced by several factors such as: type of birth (cesarean or natural), role of age, role of diet.

Pathological consequences of certain type of diet (especially western type of diet) may, in fact, be mediated by gut microbiota changing [2].

It has been noticed that there are significant differences between European children that eat a western diet and African children that eat rural diet rich in vegetal fibers and poor in meat, as concerning the

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composition of gut microbiota.

Firmicutes genera is prevalent in European children and Bacteroidetes genera is depleted as opposed to African children in whom the ratio is reversed [3]. The real significance of those changes is to be better defined in the near future. Medical literature is abundant in information on this topic, especially about the composition of microbiota in various pathological situations, but the studies published are mostly animal studies and the implications in understanding of pathological mechanisms and in medical practice are ill defined.

The role of gut microbiota played in certain inflammatory ailments (such as inflammatory bowel diseases – IBD) is well known [1]. Important practical consequences may be derived from these data: stool transplant and probiotics treatment [4]. Chronic local inflammation is a risk factor for colorectal cancer (CRC). As a matter of fact patients suffering from IBD have a yearly risk of CRC of 1% after 10 years from the onset of inflammatory disease [4]. It is an answered question as to what extent the composition of gut microbiota is implicated in CRC susceptibility.

Also a few studies have investigated the issue of gut microbiota composition in patients suffering of CRC [1], several reports notice relevant differences. For instance, a study indicated an increased prevalence of Bacteroidetes and Prevotella genera than in general population [5]. Another study which investigated bacterial populations on the surface of colonic mucosa as opposed to luminal bacteria showed that there was an abundance of Faecalibacterium and Dorea species in patients suffering of adenomatous colonic polyps as opposed to those without polyps [6].

Certain pathogenic bacteria in gut microbiota have been extensively studied in relation to their role in CRC. Thus, Fusobacterium nucleatum, which is abundant in CRC patients' colon, may initiate the progression from adenoma to adenocarcinoma [7]. Rubinstein showed that the species of Fusobacterium which produced the virulence factor FadA stimulated epithelial cell proliferation, promoted inflammation and initiated oncogenesis [8]. Similar studies have been published about other components of gut microbiota such as: E. Coli, B. fragilis, S. Gallolyticus (former S. bovis particularly associated with CRC) [8,9,10,11]

Also it is obvious that there is a change in CRC patients gut microbiota composition, it is not clear if those changes are already present before the diagnosis of CRC suggesting a role of risk factor or those changes reflect the consequences of the disease.

From another point of view, the CRC risk factors modify gut microbiota and may elicit the oncogenic process by this route. Several studies have indicated that a meat diet, by the way of microbiota composition alteration, may play an important role in oncogenesis [12,13].

Obesity is a major risk factor in CRC. Many studies showed a certain pattern of gut microbiota change in obese subjects, in animal as well as in human studies [14,15]. Gut bacteria have an impact on peripheral resistance to insulin, systemic inflammatory status and on systemic adiposity by the way of interaction with intestinal epithelial cells. Those interactions are very complex and mutually beneficial. Thus, gut bacteria metabolize various nutrients such as digest-resistant carbohydrates which results in production of short chain fatty acids (mainly butyrate). Gut epithelial cells uses as a main source of energy those fatty acids. Moreover, butyrate may be involved in anti-oncologic effects [16]. Backend noticed, in a mice study, that germ-free subjects experienced a 60% increase in adiposity and a significant increase in peripheral resistance to insulin after 14 days from stool transplant performed from lean mice, in spite of a restrictive diet. Stool transplant from obese mice elicits an even greater increase in total fat mass, which may render a reasonable demonstration about the role played by microbiota in obesity and this may further suggest a role in colonic oncogenesis [15].

A suggested pattern of oncogenesis in CRC may be represented by this: dysbiosis – inflammation – oncogenesis. Alteration of gut microbiota (dysbiosis), as a response to an unidentified trigger, has been studied in animal experimental models with regard to collective or individual role of bacteria in development of inflammation and CRC [17]. The pathogenic

mechanism is still unclear but we may appreciate an involvement of certain receptors of innate immune system responsible for microbial detection [18].

Indirect data on the topic of gut microbiota in CRC are derived from the acknowledged protective role of probiotics. The term of probiotics has been used for several decades but World Health Organization has issued a definition only in 2001: „ live microorganisms that render health benefits when they are administered in proper doses” [19]. Lactic bacteria and Bifidobacteria are among the most known types of probiotics. A metanalysis that has been published in 2006 concluded a protective role of probiotics in CRC and in colonic adenoma [20]. The authors of this metanalysis suggested a cautious use of these data as the interventional type of studies were still not enough to render an unequivoque positive answer.

Recently, Faecalibacterium prausnitzii, a commensal bacterium in gut microbiota that has anti-inflammatory properties, has also been indicated as a probiotic to decrease the risk of CRC [21].

Mechanisms engaged by probiotics in the process of oncogenesis are multiple: cell cycle influences, oxygen reactive species, apoptosis, specific bacterial enzymes, influences on human host metabolome [1]. Probiotics have an important contribution to the development of mucosal associated immune system as it modulates unspecific inflammatory response and it dampens down mucosal inflammation. Probiotics exert also certain effects on dendritic cell, colonic epithelial cells, lamina propria T cells, thus having an impact on adaptive immune system. Perdigon noticed in a mice study, that joghurt (rich in lactic bacteria) hampered the growth of colonic tumors which had been previously induced by mice exposure to 1,2 dimetilhidralazine [22]. Urbanska reached a similar conclusion in another mice study. He showed that yoghurt might reduce the volume of the tumor. Induction of apoptosis may be credited as a major mechanism that mediates the beneficial effects of probiotics against oncogenesis of colonic tumors. Hence, the induction of apoptosis could be seen as a phenomenon of oncologic screening [23].

In spite of all these experimental data from animal models that indicate the fact that probiotics have the potential to stop the development of CRC, systematic studies to show the same benefits in humans are very limited. In a human study about the prophylactic effect of Lactobacillus casei in CRC the conclusion was that two years of continuous administration significantly reduced the incidence of CRC as compared to control group [24]. Recently, in a cohort study that included 45000 subjects over a period of 12 years it has been noticed that large daily amounts of yoghurt reduced the incidence of CRC, suggesting a major prophylactic benefit from long term administration of joghurt [25,26]. Future clinical studies should consider, also, the standardization of probiotics composition, as well as the control of diet, time and frequency of probiotics administration. They should identify the proper biomarkers that may allow monitor of long term effects.

In conclusion, a large and significant pool of data indicates a major role of gut microbiota in CRC oncogenesis as well as in CRC prophylaxis. The majority of studies are, still, animal studies. Those and the few human studies are, never the less, highly suggestive. Unfortunately, we do not know the definition of „normal” composition of gut microbiota, yet. The mechanisms that link dysbiosis and CRC are complex and are related to local and systemic chronic inflammation. Some of the modifiable risk factors in CRC (obesity, diabetes mellitus, and western type of diet) may act, also, through alteration of gut microbiota. The prophylactic role of probiotics may render a powerful mean to significantly reduce the incidence of CRC in the future.

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REVIEW ARTICLE

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Gut microbiota – new insight in colorectal cancer pathogenesis

Mihăiță Pătrășescu[1,2], Petruț Nuță[1], Raluca S. Costache[1,2], Săndica Bucurică[1,2], Bogdan Macadon[1], Vasile Balaban[1,2], Andrada Popescu[1,2], Roxana Călin[1], Florentina Ioniță Radu[1,3], Mariana Jinga[1,2]

Abstract: *Gut microbiota is a superorganism involved in homeostasis and in pathogenesis. Microbiota composition is influenced by several factors such as: type of birth (cesarean or natural), role of age, role of diet. Pathological consequences of certain type of diet (especially western type of diet) may, in fact, be mediated by gut microbiota alteration. Also few studies have investigated the issue of gut microbiota composition in patients suffering of colorectal cancer, several reports notice relevant differences. Certain pathogenic bacteria in gut microbiota have been extensively studied in relation to their role in colorectal cancer. Thus, Fusobacterium nucleatum, which is abundant in colorectal cancer patient's colon, may initiate the progression from adenoma to adenocarcinoma. A suggested pattern of oncogenesis in colorectal cancer may be represented by this: dysbiosis-inflammation-oncogenesis. A metanalysis that has been published in 2006 concluded a protective role of probiotics in colorectal cancer and in colonic adenoma.*

Keywords: *colorectal cancer, gut microbiota, probiotics*

Human gut harbors a complex and abundant microbial community which is symbiotically linked with host organism and plays a major role in maintaining the homeostasis by the way of fulfilling various functions such as: limiting the number of intraluminal pathogens, functionnal optimization of immunity system, and important contribution to maintaining energy balance.

It is estimated that there are around 1,014 microorganisms in the colon (mostly bacteria) which is 10 fold above the number of eukaryote cell of human body and that contains a 100 fold richer genome than human genome [1]. As a matter of fact we may acknowledge the existence of a true superorganism whose implications in physiology and pathology of human body are continuously revealed by a great pool of scientific data.

Microbiota composition is influenced by several factors such as: type of birth (cesarean or natural), role of age, role of diet.

Pathological consequences of certain type of diet (especially western type of diet) may, in fact, be mediated by gut microbiota changing [2].

It has been noticed that there are significant differences between European children that eat a western diet and African children that eat rural diet rich in vegetal fibers and poor in meat, as concerning the

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composition of gut microbiota.

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VARIA

Hashimoto thyroiditis amid COVID-19 pandemic: A clinical study

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Abstract: *During COVID-19 era, a special attention was considered for autoimmune disorders (AD), including at thyroid, which are regarded at higher risk knowing that stress and infections might aggravate the pattern of anti-thyroid antibodies as well as thyroid dysfunction. In this study, we aim to analyse how COVID-19 infection influenced the evolution of HT parameters versus patients with HT who did not go through infection. Study design: an observational, bi-centric study in Romania during first 14 months of pandemic. By the end of April 2022, Romania registered 1,047,520 total cases of COVID-19 infection (a morbidity rate calculated for entire Romanian population of 5.23%) with 27,267 total deaths (a lethality rate of 2.6%) and around 2000 new patients a day. From our database of 2210 HT patients, a number of 386 HT subjects were assessed (14 months). Among them, 33/386 patients had a different form of COVID-19 infection. The prevalence of COVID-19 infection among HT patients was 9.35% which is statistically significant higher when compare with official ratio for entire Romanian population (a morbidity rate of 5.23%, z = 3.162, p = 0.00033). A feminine prevalence was identified (89.37%, N=31/33), while age was not different between subgroups (average age over 50 y). At the moment of diagnostic, thyroid function was similar between the two subgroups (⍰2 = 2.02, p = 0.35). We checked ATPOs levels at every visits. Mean ATPO levels at the diagnostic time was 650 UI/mL in group 1 versus 840 UI/mL in group 2 (both groups had very high ATPO values, normal cut-offs below 34 UI/mL) with not statistical significance difference - student ttest (t⍰), p = 0.16. Group 1: the pattern of ATPO evolution was undulatory in 74.07% of patients, decreasing - 3.7%, increasing - 11.11%, unmodified (stationary) - 11.11%, similarly with group 2, meaning that ATPOs had the same profile, regardless the fact that some subjects went through COVID-19 infection. The rate of associated AD was 28.24% (N=2210), COVID-group of 42.42%, non-COVID group of 38.53% (z = 0.44, p = 0.66). In our COVID cohort of 33 patients with HT, we registered the following clinical stages of coronavirus infection: 4/33 patients who were asymptomatic; all women, average age: 65.65 years; 2/33 patients with lack of smell and taste; women, over 65 years old; 14/33 patients with mild form; from 31 years old to 70.5 years old (only one man); 10 patients with a moderate form; all women, from 34 to 71 years old; one 87-year-old male with a severe form; one 50-year old female with a very severe form in addition to a hematologic disease and a history of severe allergic reactions; and a single patient (a 68-year-old, obese female with a severe allergic background) died of COVID-19 infection (thus generating a lethality rate of 3.03%). Conclusion. Our study showed a higher prevalence of COVID-19 infection among HT patients. These individuals do not associate increased levels of ATPO or a elevated prevalence of AD when compare with non-COVID – HT group, and have similar age and sex ratio. Whether HT exposes the subjects to coronavirus infection is an open issue.*

Keywords: *COVID-19, thyroid, thyroiditis, Hashimoto, ultrasound, antibodies, endocrine, virus, pandemic*

INTRODUCTION

During COVID-19 (coronavirus-disease-year-2019) era, a special attention was payed for autoimmune disorders which are considered at higher risk amid this situation. [1] One of this autoimmune diseases is Hashimoto thyroiditis (HT), also known as chronic lymphocytic autoimmune thyroiditis; for what we know so far, stress and any type of infections are prone to either trigger or aggravate the clinical picture and associated hormonal imbalance of HT. [2] An analysis of chronic thyroiditis correlated with COVID-19 situation currently is approached with different levels of

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statistical evidence worldwide. [3-15]

The first patient confirmed with COVID-19 in Romania was registered in 26 February 2020. [16-19] When the first 139 cases were diagnosed, on the 16th of March 2020, it was declared an "Emergency State", which lasted for 2 months, until the 15th of May 2020. [16-19] During that period of time, there were registered 16,437 cases of COVID-19 infection with 1,070 deaths (a lethality rate of 6.5%). [16-19] This was considered as "First pandemic wave" with a peak on the 11th of April 2020 with 523 new cases/day. [16-19] Starting from that day, an "Alert State" was initiated in our country. [16-19] Under these conditions, we had a "Second Wave", from the 15th of September 2020, with a peak on the 18th of November 2020 (a total of 10,629 new cases per day, with a total of 324,094 COVID-19 cases and 8,389 deaths (a lethality rate of 2.59%). This wave followed by a "Third Wave", starting with the 3rd of February 2021, with a peak on the 25th March 2021 with 6,651 new cases per day, with a total of 919,794 cases and a total death number of 22,717 (a lethality rate of 2.47%). [16-19]

Aim

In this study, we aim to analyse how COVID-19 infection amid pandemic influenced the evolution of HT versus patients with HT who did not go through infection.

PATIENTS AND METHOD

Study design

This is an observational (non-interventional), bi-centric study in Romania. The endocrine patients were registered from two medical centres in Bucharest, Romania (Europe). We analysed the patients with HT who were diagnosed with COVID-19 infection compare with those who were disease free during the first 14 months of COVID-19 pandemic.

Evaluation of patients

Based on our files, we registered patients with HT for the last 25 years; these patients previously had a thyroid ultrasound performed on a daily bases and quantitative antithyroperoxydase antibodies (ATPOs) and antithyroglobuline antibodies (ATGs) assays which were performed in our laboratories. HT diagnostic was based on serum ATPOs levels above the normal upper limit (no restriction concerning the level of ATPO elevation in order to sustain the diagnostic of HT). Also, the patients with high ATPOs levels (cut-off of more than 34 UI/mL) and confirmed with HT were evaluated based on thyroid ultrasound to check of if they had specific inflammatory aspects which did not limit the HT diagnostic. Anterior neck ultrasound was performed with a linear probe, long of 4 cm, with 7.5 to 10 MHz.

Our records showed 2,210 patients with traditional diagnostic of HT (based on high ATPOs levels) within the last 25 years. From this database, we analysed the patients who were admitted (as outpatients) during COVID-19 pandemic (from the 16th of March 2020 to 22nd - 27th of April 2022). Overall, we analysed 386 patients with HT (during this mentioned period of 14 months). Some of prior patients were under levothyroxine therapy for associated hypothyroidism (a daily dose of 25 to 125 µg) in order to achieve a TSH (Thyroid Stimulating Hormone) within the normal lab limits. Patients with associated Graves' disease received daily thiamazole (from 5 to 20 mg per day depending on target TSH). Subjects with normal thyroid function did not receive any type of medication/ supplements. The decision of daily drugs was done by each individuals' current practitioner and did not restrict the enrolment of the patients.

The classification of type of COVID-19 infection

We used the WHO classification (Global COVID-19 Clinical Platform), with a few adjustments: Stage 1: asymptomatic; Stage 2: only lack of small and /or taste; Stage 3: mild symptoms (few hours of fever and/or cough); Stage 4: moderate (fever, cough, no lack of oxygen), Stage 5: severe meaning pre-ITU (Intensive Care Unit) hospitalization, oxygen saturation under 93%, above 85%, Stage 6: very severe form requiring an ITU hospitalization (oxygen saturation under 85%); Stage 7: death.

COVID-19 information for studied population

In order to assess the information concerning COVID-19 infection we analysed the medical records of the patients from other medical departments (which were provided by the patients themselves). Some of them were evaluated as outpatients in Endocrinology Department, and some through telemedicine. General information amid infected cases in our country were achieved by accessing public sources (universally free downloads). [16-19]

Statistical analysis

We used z Fisher test on ratios, ⍰2 test on discreet data, and Student t test on continuous data, as appropriate to analyse the differences studied between groups. Statistical significance was considered at $p < 0.05$.

RESULTS

Associated COVID-19 pandemic information (Romania)

Until the 22nd April 2022, Romania registered 1,047,520 total cases of COVID-19 infection (a morbidity rate calculated for entire Romanian population of 5.23%) with 27,267 total deaths (a lethality rate of 2.6%) and around 2000 new patients a day (see Figures 1-3, universally available free download from Public Source: https://www.worldometers.info/coronavirus/country/romania/.)

Figure 1: Total number of COVID-19 cases in Romania (from the 16th of March 2020 to the 22nd of April 2022) - universally available free download from Public Source: https://www.worldometers.info/coronavirus/country/romania/.)

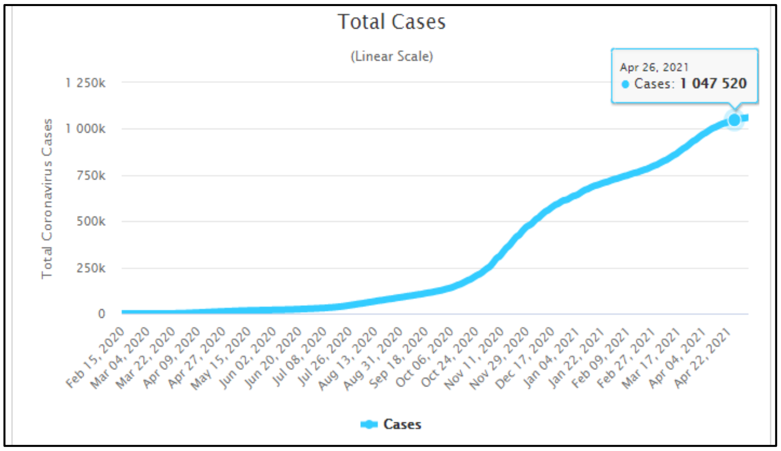

Figure 2: Total number of active cases concerning COVID-19 infection in Romania (from the 16th March of 2020 to the 26th of April 2022) - universally available free download from Public Source: https://www.worldometers.info/coronavirus/country/romania/.)

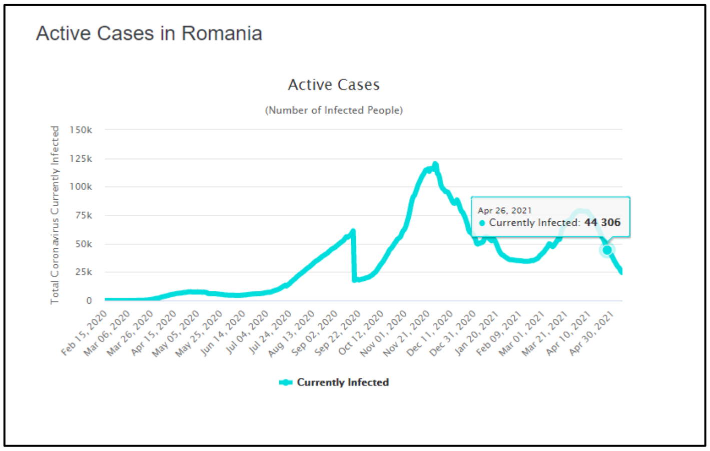

Figure 3: The report of daily new cases concerning COVID-19 infection in Romania (from the 16th of March 2020 to the 27th of April 2022) universally available free download from Public Source: https://www.worldometers.info/coronavirus/country/romania/.)

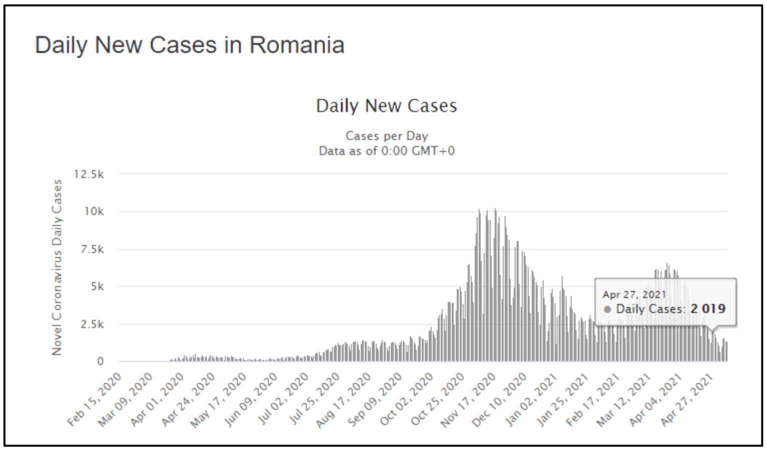

The impact of SARS-CoV-2 on patients previously diagnosed with TH

From our database of 2210 patients with HT, a number of 386 subjects were assessed during the first 14 months of COVID-19 pandemic. We registered 33 (out of 386) patients who had a different form of COVID-19 infection. All the data from these patients were compared with the data from our patients with HT who were evaluated during the same period of time but did not have COVID-19 infection. We the patients' profile in order to detect if there any differences between the two subgroups. The interval of time between COVID-19 infection and the moment we performed the endocrine evaluation varied from days to 6 months. (Table 1)

Table 1: Clinical and biological data from patients with Hashimoto thyroiditis: patients who had a COVID-19 infection versus subjects without infection

Parameter	Patients with COVID-19 infection	Patients without COVID-19 infection	Statistical significance
Number = 386	33 (9.35%)*	353 (90.65%)	z = 3.62, p= 0.00033*
Women = 345 (89.37%)	31 (93.94%)	314 (88.95%)	χ^2 = 0.42, p = 0.63, NS
Men = 41 (10.67%)	2 (6.06%)	39 (11.05%)	
Age (years) at COVID-19 diagnostic (mean) Current age at evaluation (mean)	52.82 58.43	51.36 55.99	p = 0.57, NS p = 0.36, NS
Euthyroidism at the diagnostic vs. current time	10 (30.30%) vs 24 (72.73%)	138 (30.09%) vs 205 (61.19%)	z = -0.74, p = 0.46, NS z = 1.3, p = 0.2, NS
Hypothyroidism at the diagnostic vs. current time	17 (51.52%) vs 8 (24.24%)	177 (50.14%) vs 124 (37.01%)	z = -0.22, p = 0.82, NS z = -1.42, p = 0.15, NS
Hyperthyroidism at the diagnostic vs. current time	6 (18.18%) vs 1 (3.03%)	38 (10.76%) vs 6 (1.79%)	z = 1.5, p = 0.14 z = 0.49, p = 0.62
ATPO levels (normal level < 34 UI/mL)	650 UI/mL	840 UI/mL	p = 0.16 (NS)
History of miscarriage	2 (6.45%)	30 (9.55%)	z = -0.58, p = 0.56, NS
Association with other immune/autoimmune conditions	11 (42.42%)	136 (38.53%)	z = 0.44, p = 0.66, NS

** applied to the morbidity in general population, NS = nonsignificant; vs=versus; ATPO= Anti-thyroperoxidase antibodies*

Prevalence analysis

The prevalence of COVID-19 infection among our patients which was registered during the studies 14 months was 9.35%. We compare this ratio with the official ratio of COVID-19 for entire Romanian population, the morbidity (see above), was 5.23%. For this comparison we used the z test from Fisher equation, which compare ratios. From this point of view, the prevalence is higher, the statistical significance was z = 3.162, p = 0.00033.

Gender prevalence

A feminine prevalence was identified in our cohort: 89.37% were women, and 10.67% were men. During the first 14 months of pandemic, 31 women and 2 men had a COVID-19 in addition to previously diagnostic of HT. When comparing gender prevalence with general data, there was no statistical significance (⍰2 test = 0.37, p = 0.79). (Table 2)

Age analysis

Mean age of 33 patients was 52.82 years at the diagnostic while the subgroup with HT without infection had an average age of 51.36 years at the diagnostic time and 55.99 years at the current time with not statistical significance difference. (Table 2)

Thyroid function

We analysed the thyroid function at initial diagnostic time and current moement, when patients were revised. Hypothyroid patients received levothyroxine and some become euthyroid; hyperthyroid patients received thiamazole, and mostly became euthyroid under this therapy. All these data were compared with the group without COVID-19 infections/14 months. All patients with high ATPO (anti-thyroperoxidase antibodies) who had hyperthyroidism had also positive TRAB (TSH Receptor Antibodies) according to a level higher than 1 UI/ml (co-occurrence of HT and Graves-Basedow's disease).

When analysing all cases in the same time by χ^2 test, there was no differences concerning the aspect of thyroid function both at the diagnostic time and the current time between patients from group 1 versus group 2 (χ^2 = 2.02, p = 0.35). Similarly, z analysis on thyroid function (separately for euthyroidism, hypothyroidism and hyperthyroidism distribution) showed no statistically significance difference. Therefore, the thyroid function, either at the diagnostic time or at the current visit, had no importance in catching the viral disease. (Table 2)

Table 2: The parameters of patients with chronic Hashimoto thyroiditis who had an episode of COVID-19 infection during the last 14 months

	Gender	Age at diagnostic moment	Current age	ATPO average UI/mL*	ATPO evolution	Thyroid function a)	Current thyroid function	Specific treatment for thyroid dysfunction	COVID-19 clinical form	Other observations. Anti-SARS-CoV antibodies (if available)
1	W	48	65	505	Undulatory	EUT	EUT	Substitution	No smell	
2	W	63	78.5	275	Undulatory	HOT	EUT	Substitution	Moderate	Husband – COVID-19 related orchitis
3	W	49	58.7	80	Undulatory	HOT	EUT	Substitution	Asymptomatic	ACAb = 2000 AU/mL
4	W	52	64.4	227	Undulatory	HYT	EUT	NT	Asymptomatic	ACAb = 250 AU/mL
5	W	45	53.5	847	Undulatory	HOT	EUT	Substitution	Asymptomatic	ACAb = 1000 AU/mL
6	W	46	53.5	1331	Undulatory	HOT	HOT	Substitution	Mild	
7	W	46	53.5	418	Undulatory	EUT	EUT	NT	Moderate	
8	W	42	49.6	66	Undulatory	EUT	EUT	NT	Very severe (ITU)	Von Wilebrand disease, hypophysitis, history of Quinke syndrome, ACAb = NA
9	W	80	86	969.5	Undulatory	HOT	HOT	Substitution	Asymptomatic (personal declaration)	ACAb= 2080 AU/mL, Husband (87y) died at ITU due to COVID-19
10	W	28	33	1150	Undulatory	EUT	EUT	NT	Mild	Endometriosis
11	W	54	61.2	1563.5	Undulatory	HOT	EUT	Substitution	Mild	
12	W	59	62	52	Undulatory	HOT	EUT	Substitution	Moderate	Institutionalized at local elderly centre
13	W	66	68.9	710	Undulatory	HOT	EUT	Substitution	Moderate	Institutionalized at local elderly centre
14	W	59	61	250	Unmodified	HOT	HOT	Substitution	Moderate	Institutionalized at local elderly centre
15	W	63	68	674	Undulatory	HOT	EUT	Substitution	Mild	She was vaccinated after 1 month
16	W	51	54	275	Increasing	EUT	EUT	NT	Mild	
17	W	39	41.8	281	Undulatory	HOT	EUT	Substitution	Mild	
18	W	23	31.5	3388	Undulatory	HYT	EUT	Thiamazole	Mild	TRAB history: 10-16 UI/mL
19	W	50	54.6	133	Undulatory	HYT	HYT	Thiamazole	Moderate	TRAB history: 1.5-17.5 UI/mL
20	W	70	70.5	40	Unmodified	HOT	EUT	Substitution	Mild	Primary hyperparathyroidism
21	W	34	34	53	-	EUT	-	NT	Moderate	
22	W	39	39	185	-	EUT	-	NT	Moderate	Nurse
23	W	71	71	40.5	-	EUT	-	NT	Moderate	ACAb = 3.65 AUI/mL
24	M	45	51.5	103.5	Decreasing	EUT	EUT	NT	Mild	
25	M	73	87	249.4	Undulatory	HYT	EUT	NT	Severe, Pre-ITU	Wife (85y) died at ITU due to COVID-19
26	W	37	37	250	-	HOT	-	Substitution	Moderate	ACAb qualitative = positive (qualitative assays)
27	W	68	73.5	524.5	Increasing	HOT	EUT	Substitution	No smell and taste	
28	W	50	50.12	640	Unmodified	HYT	HYT	Thiamazole	Mild	
29	W	63	70.4	266	Undulatory	HOT	HOT	Substitution	Mild	ACAb = 149 AUI/ml
30	W	68	68	5000	-	HOT	-	Substitution	Mild	ACAb = 4600 AUI/ml
31	W	65	68.8	505	Increasing	EUT	EUT	NT	Mild	ACAb = positive (qualitative assays)
32	W	41	41	125	-	HOT	-	-	Mild	
33	W	56	68	2772	Undulatory	HOT	HOT	Substitution	Death	

Abbreviations: EUT = euthyroidism, HOT = hypothyroidism, HYT = hyperthyroidism, NT = no specific treatment, ITU = intensive therapy unit, ACAb = anti-SARS CoV antibodies; NA = not available; "substitution" means levothyroxine replacement for hypothyroidism

** Upper normal limit = 34 UI/mL; a) Thyroid function at the moment of COVID-19 infection*

ATPO evolution

We checked ATPOs levels at every visits. Mean ATPO levels at the diagnostic time was 650 UI/mL in group 1 versus 840 UI/mL in group 2 (both groups had very high ATPO values) with not statistical significance difference according to Student test (t_α): p = 0.16.

We described the ATPOs evolution in 5 ways: undulatory, increasing, decreasing, non-evolutive (stationary) and normalization below mentioned cut-off of 34 UI/mL. Accordingly to this description, in COVID-19 patients, we observed the following ratio: undulatory = 74.07%, decreasing = 3.7%, increasing = 11.11%, unmodified (stationary) = 11.11%, and normalized values of ATPO = 0%. In non-COVID patients the ratios were: undulatory = 69.04%, decreasing = 12.13%, increasing = 8.37%, unmodified = 6.28%, normalized = 4.18%. ATPO evolution did not reach a statistical difference (χ^2 = 0.43, p = 0.1.67), meaning that ATPOs had the same pattern, regardless the fact that some subjects went through infection. (Table 2)

Infertility and miscarriage issues

In all our registered patients (N = 2210) the miscarriage was registered in the medical history of 150 patients (N= 7.33%). In non-COVID group, miscarriage was registered in 30 patients (9.55%). In COVID-19 group miscarriage was registered in only 2 patients (6.45%); z = -0.44, p = 0.66, NS.

Other immune/autoimmune associations

HT is frequently associated with other immune disturbances, which could be involved in an inadequate immune response to viral infections. In all our patients (N = 2210), the prevalence of immune/autoimmune associations were registered in 28.24% patients. In non-COVID group, the rate was 38.53%. In COVID-19 group, this rate was 42.42% patients (z = 0.44, p = 0.66).

COVID-19 infection: the clinical severity in HT patients

In our COVID cohort of 33 patients with HT, we registered the following clinical stages of coronavirus infection: 4/33 patients who were asymptomatic; all women, average age: 65.65 years; 2/33 patients with lack of smell and taste; women, over 65 years old; 14/33 patients with mild form; from 31 years old to 70.5 years old (only one man); 10 patients with a moderate form; all women, from 34 to 71 years old; one 87-year-old male with a severe form; one 50-year old female with a very severe form in addition to a hematologic disease and a history of severe allergic reactions; and a single patient (a 68-year-old, obese female with a severe allergic background) died of COVID-19 infection (thus generating a lethality rate of 3.03%) (Table 2)

SARS-CoV antibodies (post-infection or post-vaccine) in patients with HT

Only 17/386 patients had been vaccinated during the last 4 months of study protocol. Only 5/17 had been tested for antibodies against S-protein. The data were listed in AU/mL (1 AU/mL = 0.142 BAU/mL, according to WHO standards): 2500, 4200, 2000, 45300 and 5400. Only 2 patients with COVID-19 infection and chronic thyroiditis performed these antibodies after disease. The levels were of 4600 AU/ml, respectively of 18000 AU/mL, higher than seen in patients without a history of coronavirus infection.

DISCUSSION

For the first 14 months of pandemic, studied population showed a higher rate of COVID -19 infection when compare to entire Romanian population based on official records. [16-19] The fact that patients with thyroid conditions, including those antibodies - related, are at higher risk of contracting coronavirus infections is controversial up to the current level of scientific information. [20-30] As expected for a thyroid autoimmune condition, we had an overall female prevalence, including in COVID group. A higher susceptibility and a more severe coronavirus infection was suggested for males, unless autoimmune background or other particular sub-groups. [31-33]

We had an average age over 50 years on both subgroups. Generally, COVID-19 infection has been reported at any age, but seniors older than 65 years are at higher risk. [34-36]

We had a subgroup with individuals associating two autoimmune thyroid diseases: HT and Graves-Basedow's disease. Notably, in the COVID group there are slightly more patients with hyperthyroidism at the diagnostic time (18.18%) that in non-COVID patients at the diagnostic time (10.76%). Without reaching a statistical significance (p >0.05), these data suggest that hyperthyroidism (which means association of Graves-Basedow disease with HT) could add some pathogenic factors to increase the risk of SARS-CoV-2 infection. However, these factors do not seem to be related to thyroid function, but more to immunological association between HT and Graves-Basedow's disease. Coronavirus might be triggered by autoimmune hyperthyroidism. [37] We evaluated the function and the antibodies' profile concerning these two diseases; also, all the subjects had an ultrasound done but we did not introduce in final analysis the reports of ultrasound data. Generally, thyroid ultrasound is a key tool of evaluating the patients with these conditions. [38-44]

In COVID-19 group, the miscarriage rate was of 6.45%, which is not different from entire HT cohort. Some data suggested that females going through coronavirus infection might experience a higher rate of miscarriage which is not confirmed in our cohort. [45] Also, the group of persons experiencing the infection have the similar profile of autoimmune co-morbidities. The autoimmune background, however, influences the immune response amid infection. [46-48] Overall, our patients had a mild COVID-19 symptomatology, only 3 patients experiencing a severe form. Therefore, HT did not aggravate coronavirus evolution as other endocrine conditions (like diabetes mellitus or obesity).

As limits of the study we mention: one aspect involves the fact that we did not take into consideration the post-vaccine status which include a small number of subjects among studied subgroups; and, also, the small sample size of the cohort.

CONCLUSION

Our study showed a higher prevalence of COVID-19 infection among HT patients. These individuals do not associate increased levels of ATPO or an elevated prevalence of AD when compare with non-COVID – HT group, and have similar age and sex ratio. Whether HT exposes the subjects to coronavirus infection is an open issue.

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ORIGINAL ARTICLES

Health sector organization to respond to a bioterrorist attack

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Abstract: *Bioterrorism constitutes a major health threat to global communities, with many specific features that carry the potential to extraordinary stress the Public Health Sector (PHS) of any country, because of the needs of resources and of a coordination network required for a successful response to such incidents. Bioterrorism incidents of the near past prove that public mortality and morbidity can be further reduced if a functional protocol would allow first responders, hospitals, and officials of the PHS to steer more efficiently the public expectations. At the national level, the PHS director is responsible to mobilize and coordinate that network of the critical human, financial and logistical resources during the preparedness and response phases after a bioterrorist attack to mitigate its effects. Both these phases include many specific functions and responsibilities, which will be beneath analyzed.*

Keywords: *bioterrorism, public health sector, health preparedness*

BACKGROUND

Bioterrorism is the deliberate use of biological agents to disseminate life-threatening diseases to threaten or devastate a population [1]. The bioterrorist threat consists of a rapidly growing and perhaps the most frightening menace to world health security [2]. The weaponized application of pathogens to disorder populations has been established throughout history [3]. However recently, the profound development of bio-molecular engineering combined with the worldwide proliferation of high-level biosafety laboratories has aroused concern about the increased accessibility of biotechnology [4] and potential misuse of biological agents [5]. Bioterrorism manufacture requires just culture samples, the means to grow, purify, stabilize and disseminate them [6].

The motives underlying bioterrorist incidents of the last years have been nationalistic objectives, revenge, or a mix of paranoid – conspiratorial – apocalyptic prophecy [7]. Additionally, large terrorist organizations such Al-Qaida would always seek ways to acquire biological weapons [8].

The biological agents considered suitable for terrorism should have high inter-human infectiousness, high morbidity and/or lethality, ease of manufacture, storage, and delivery in adequate quantities, environmental stability after dispersion, and absence of effective treatment [9].

Most of the abovementioned characteristics are easily achievable and that raises concerns about the devastating power of biological weapons, because of various reasons: they disseminate in various ways, extremely small quantities of them are lethal, manufacture information is readily obtainable, and a bioterrorist attack can initially be covert since the appearance of symptoms and illness will lag for several hours or days [10].

Bioterrorism agents are classified into bacteria and viruses and yet into three categories; A, B, and C, depending on the ease of dissemination, severity of illness caused, and ability

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to cause death [11]. Of those, microorganisms of Category-A (i.e. anthrax, botulism, plague, smallpox, and viral hemorrhagic fevers), carry the potential to extraordinary stress the PHS, because:

1. The initial release of the bioterrorist agent could go undetected until the onset of disease, creating a sudden influx of severe illness;

2. Incidents that result in impairment of food and water supplies, as well as transportation and communication barriers, may gravely affect the national stability;

3. Contamination risks and subsequent fears of exposure may further complicate recovery operations and cause civil disorder [12].

Up to now, bioterrorism remains a challenge for the PHS of every nation, mainly due to the need for resources and a coordination network required for a successful response to an incident [13]. At the national level, the PHS director is responsible to mobilize and coordinate that network of the critical human, financial and logistical resources during the preparedness and response phases after a bioterrorist attack. Both these phases include specific functions and responsibilities of PHS, which will be beneath analyzed.

PREPAREDNESS PHASE

Risk Assessment

Among weapons of mass destruction, biological agents are much more destructive than chemical weapons, including nerve gases, whilst under certain conditions, biological weapons can be equally devastating to nuclear ones. For example, according to a traditional disaster simulation model [14], a few kilograms of anthrax in a dense urban area can kill as many people as a Hiroshima-size atomic bomb, or cause even more casualties when considering the current suburban living situation of a western Metropolis, combined with favorable upwind conditions [15].

Risk assessment is a multidisciplinary activity, aiming at identifying those who may wish to use biological weapons against the population, the agents that may be used, and the circumstances under which they may be used. Risk assessment of biological threats often involves modeling approaches based on certain risk assumptions. To understand these risks, planners assess them as a function of their likelihood to occur, their potential consequences, and the possible measures that could be taken to control them, based on an understanding of existing surveillance data, the literature, past experiences, and opinions of experts [16].

Intelligence

Preparedness for bioterrorist attacks includes international collaboration for the secure exchange of sensitive information regarding the capabilities of terrorists [2]. Accurately predicting potential biologic threats is difficult without the implementation of intelligence data, adequate funding [17], strategies of data- and cyber-security, and expanding use of electronic information, reporting and surveillance systems [18]. Furthermore, networked video cameras and advanced software designed to verify important intelligence information have become powerful tools for counterterrorism operations [19]. Notification to WHO is required for all cases of sufficient seriousness, such as diseases with severe public health impact, risk of international spread, cases of unusual or unexpected nature, and of course all cases of category A diseases, which constitute a "public health emergency of international concern" [20].

Disease Surveillance

The cause of a bioterrorist disease may be difficult to determine, especially if the initial cases are few. Because of rapid progression to illness and the potential for dissemination of bioterrorist agents, authorities of the PHS should apply accurate, sensitive, and specific surveillance systems to promptly recognize non-specific disease patterns that might indicate the early manifestations of a biological attack. That can be accomplished through two pillars; epidemiological and syndromic surveillance.

Epidemiological surveillance

Following a confirmed case of biological exposure or clusters of unusual symptoms or diseases detected by PHS surveillance, forensic epidemiology tracks back to identify the population at risk. This procedure is called the epidemiological investigation and uses descriptive, analytical, and molecular epidemiologic tools to identify the source and possibly to prevent further events [21, 22]. For that to be accomplished, epidemiological models encompass the potential agent to be used, the specific geographic location of an attack, the level of preparedness, and early intervention on a local and national level. Epidemiologic principles must be used to assess whether a patient’s presentation is typical of an endemic disease or is an unusual event that should raise concern. An epidemiological alert system should include multiple sub-systems regarding disease reporting, laboratory network surveillance for specific pathogens, sentinel hospital surveillance for infectious diseases based on primary healthcare data, and a rapid response system with a 24 h/7 d epidemic response team [23].

Syndromic surveillance

The concept involves real-time collecting and analyzing statistical data in a given population, focusing on symptoms rather than laboratory confirmation of diagnosis. Syndromic surveillance systems register suspicious or confirmed cases reported by physicians, and the data are used to monitor the progress of a bioterrorism outbreak [2]. The technique may be affected by various factors, such as data selection criteria, seasonality, size and dispersion of the infected population, the incubation period, selection of statistical detection thresholds, duration of follow-up, access to diagnostic practices or reference laboratories, and reporting procedures; all these results in potentially false alarms and overuse of resources to investigate them [24]. Specificity for distinguishing random variations of ordinary illnesses from bioterrorist attacks may be low when the early symptoms of bioterrorism-related illness overlap with those of many common infections, whilst respective sensitivity may also be low if detection thresholds are reduced to enhance specificity [25]. Lastly, syndromic surveillance is planned to enhance, rather than replace, existing diagnostic tools to epidemic detection [26].

Early Warning and Response System (EWRS)

Numerous biological agents may be used in bioterrorism; terrorists might use combinations of them, common or new, drug-resistant or genetically engineered pathogens, or attack in more than one location simultaneously. Early detection and response to highly contagious biological agents are hence crucial. That can be accomplished through an EWRS, i.e. a rapid web-based alert system used for exchanging information on health threats resulting from the deliberate release of a chemical, biological and radiological agents, but mostly for monitoring epidemics. A major benefit of global collaboration at a technical level is the development of trust networks among experienced scientists and clinicians, for both natural and intentional outbreaks [27].

Emergency Planning

Terrorist attacks are more frequently covert compared to bombings or even chemical attacks. That is an intrinsic feature of bioterrorism, due to the incubation period of pathogens that brings a delay between exposure and onset of symptoms and consequently, between the attack and the involvement of PHS and civil security agencies [28]. The importance of emergency planning lays in the fact that public health infrastructure should be prepared to confront a rapid sequence of emergency events, including a delayed massive wave of illness, rapid dissemination in the population, and the need for prevention strategies, analyzed below.

Laboratory Preparedness

A solid laboratory network would enable quick and accurate detection and subsequent response to biological threats. The response capacity of those laboratories is classified into four Biosafety Levels (BSL); from BSL-1 (clinical laboratories with minimal biosafety requirements), up to BSL-4 (implementing the highest biosafety standards), according to their scientific background, expertise, and infrastructure to support the diagnostic needs of a highly hazardous biological event [4]. During such, a proper laboratory network enables the following functions:

1. Activating all BSL laboratories nationwide.

2. Prioritizing and sharing information among sentinel laboratories and PHS officials on an emergency, 24/7 day basis, to ensure that the public received precise and prompt messages [18].

3. Recognizing and responding to any covert event involving the collection, preservation, transport, and testing of highly infectious human specimens according to uniform guidelines for handling samples of pathogens of public health interest [29].

Food and Water

Biological agents constitute a major concern regarding the safety of food [30, 31]; contamination of water with biological agents should be also considered, albeit that is improbable to be the major target of bioterrorism, due to chlorination, dilution, and the need for large quantities of the agent to cause a significant biological threat [32]. Nevertheless, quantities far less than a kilogram of botulinal toxin, for example, in the main water supply of a city may cause 28-40,000 deaths, depending on how early symptoms will be recognized and remedial measures will be applied [14].

Stockpiling

The strategic medical stockpile of a country is a national repository of vaccines, drugs (antibiotics, antidotes, etc), and general medical supplies that can be deployed to areas affected by various sorts of public health disasters, including bioterrorism. In the case of the dissemination and subsequent detection of a biological agent, strategic stockpiles of respective medication against the agent should be provided to the affected area within less than 24 hours; subsequently, the medical countermeasures should be appropriately dispensed in the affected community within another 24 hours [33]. Strategic stockpiling has to be considered within the framework of cost-effectiveness;

stockpiling of pharmaceuticals with a sort expiration date may be costly and problematic since the turnover inventory would require a significant monetary amount to maintain stockpiling facilities for continuous checking and renewing [34].

Training

Improved awareness and readiness during a bioterrorist attack include among others education of all medical personnel, especially primary-care providers and first responders to patients affected by a biological attack35. Training inserts knowledge about basic epidemiologic principles, disease patterns, clinical information on diagnosing and treating infected patients [36] and should be refreshed periodically with large-scale exercises to ensure that skills remain up-to-date [37]. Additionally, analysis of actual previous incidents provides precious information regarding proper response. Most importantly, the lessons learned should be incorporated into future planning to identify areas of improvement. Finally, education of health departments and the community on biosafety items, including information for crisis-phone hotlines, contributes to mitigating the negative health effects of a bioterrorist attack [38].

RESPONSE PHASE

Detection-Verification

Prompt diagnosis should be urgent in a probable bioterrorist incident, because for both health and security reasons [39]. The earlier a dissemination is detected, the faster the response and the lower the morbidity and mortality rates. Apart from significant awareness by the PHS, this also requires advanced diagnostic technology. Highly sensitive and specific and yet portable laboratory diagnostic systems are nowadays available to a greater extent than a decade ago, enabling a faster, cheaper, and more accurate verification of the infectious agent. Moreover, increased networking and collaboration of higher-level biosafety laboratories also improve response to biohazards [40]. The critical factors in reducing mortality following a biological incident were found to be early detection of exposure, registering of cases, and dispensing capacity at local and national levels [41, 42]. The verification of a possible bioterrorist attack should fulfill most of the following epidemiological criteria [43]:

1.Occurrence of a specific epidemic disease with similar symptoms in a discrete population;

2.Single case infection caused by an uncommon or class A biological agent;

3.Unusual disease for a specific geographic area or a specific age group;

4.Unusual season of transmission;

5.Many unexplained disease or death incidents;

6.Unusual high level of disease severity with resistance or response failure in standard treatment;

7.Unusual exposure routes of a “known” pathogen;

8.Higher incidence of occurrence in certain area zones, depending on whether the biological agent was released in- or outdoors;

9.Relevant claims by terrorists of a bioterrorism attack or existing intelligence for a potential threat.

Coordination

Biodefense is a complex procedure requiring a multidisciplinary approach. In any bioterrorist incident, interoperation mechanisms between major local, national and international, health- and non-healthcare agencies must be applied. Coordination should be reinforced and biodefense protocols effectively integrated into existing plans [10]. Government stakeholders called to react and collaborate to a bioterrorism incident include policy makers, law enforcement officials, emergency responders of PHS, special centers from scientific, research, forensic, and professional societies and technicians, along with the commercial branches of medical, safety, and decontamination equipment suppliers [28]. In any case, the management of a large-scale bioterrorism outbreak is well beyond the resources and capabilities of most countries; thus many lives can be saved in bioterrorist outbreaks by early mobilization of large-scale international assistance by W.H.O. Furthermore, many national armies afford special Chemical-Biological-Radiological (CBR) units, able to respond to a bioterrorism event; these have the means and often the war expertise to detect, verify and neutralize biohazard threats and to decontaminate victims and areas exposed to such agents.

Rapid Response

Following alleged or genuine dissemination of biological agents, a large number of people will probably seek medical care. Fear and panic are expected in symptomatic patients, yet also in involved health personnel of public hospitals and private clinics, who may be the first to recognize a biohazard outbreak and initiate a response. A broad network of communication towards many stakeholders must then be rapidly activated [44]. Moreover, a plan dealing in detail with the possibility of great numbers of people who concurrently might seek medical care, along with means for psychological support and medical treatment is of utmost importance in the triage rapid response process conducted by various health care professionals. These should operate according to the national SOPs regarding response to the outbreak of a biological agent [18]. Unfortunately, the mobilization of a specialized CBR unit can never match the 24-hour availability and general emergency-management experience of existing emergency response and public health services, whilst the use of CBR protective equipment also requires special training and the adaptation of existing procedures for emergency management [45]. Furthermore, in case of risk for international dissemination of the disease, the International Health Regulations (IHR) should be implemented to prevent the worldwide spread of diseases [46].

Mass Prophylaxis/Infection Control

For most biohazard agents, specialized testing is necessary by PHS specialists or laboratories. Then, vaccination and/or treatment with antibiotics or antitoxins must be started early for viral agents and bacterial diseases. Before undertaking a mass prophylaxis plan, the PHS should identify the population at risk, the sort and the availability of medication to be applied, and the mechanism of administration of such a plan. The decision to apply a mass prophylaxis plan is based on various factors, such as data from epidemiological analysis, hospital bed availability, availability and determination of the quantities of drugs required to support the plan, immunity delays till antibodies development, side-effects of a massive immunization and the estimated number of exposed or ill people [47]. For starting mass prophylaxis, the following admissions should be considered:

1. An adequate stockpile of vaccines and medications is maintained at the national level;

2. The biohazard situation is anticipated to (or already did) overwhelm the regular public health care system;

3. There are shortfalls in local capacity to dispense disease-specific health care services;

4. Multiple biohazard incidents in many locations are already involved with, or without parallel requests for specialized health care provision;

5. A request for international aid has already proceeded.

Vaccination or prophylactic antibiotic treatment of healthcare providers may also be sound, especially in the biological threat of category A biohazard agents, since the occurrence of infection in that cohort will not only severely compromise the ability of PHS to manage the incident, but will also lead the population to the erroneous perception that health care personnel constitute a high-risk source of infection [48].

Personal Protection/Decontamination Issues

During the spread of an aerosolized biological agent, the primary route of exposure will be via the airways and respiratory tract. Respiratory protection will then be the most important component of physical protection. Furthermore, all patients in healthcare facilities with suspected or confirmed bioterrorism-related illnesses should be managed to utilize standard precautions; these are designed to reduce transmission from both recognized and unrecognized sources of infection in healthcare units [49].

Adequate procedures for routine cleaning, disinfection, contaminated waste disposal, and sterilization of equipment and environment should also be available [50]. For category, agents additional precautions may be needed to reduce the likelihood of transmission. Since in most bioterrorism scenarios anthrax is considered the most likely agent to be dis¬seminated, it is worth noticing that anthrax spores can persist in the environment for months or even years, which requires careful evaluation of the decontamination process [51].

Protection of health-care personnel against infection remains particularly problematic; personal protective equipment should be affordable, effective, and available to use with minimal training and under harsh environments [52]. If agents of contagious diseases are released, standard precautions include basic hygiene and infection-control measures, e.g. gloves, hands-washing (whether or not gloves are worn), PPE masks with eye protection (or face shield), and disposable gowns. In parallel, avoiding as possible direct contact with secretions from infected individuals, keeping exposed persons away from public places, and isolating suspected or symptomatic cases, may be essential in limiting secondary spread. These guidelines may though be impractical in case the number of patients presenting to a healthcare facility is too large to allow routine isolation strategies. The fact that in a mass bioterrorism incident many victims may go to hospitals on their initiative has important implications for the application of triage and decontamination protocols. Grouping affected patients who present with similar syndromes into a designated section of a clinic may be a vital alternative to the dissemination of infection. Community containment strategies, including isolation and quarantine, are the ultimate measures to prevent further transmission. Isolation and quarantine are optimally performed voluntarily, but basic legal authority should be in place for mandatory isolation and quarantine of people and communities when necessary to protect public health [53].

Communication in crisis

Public preparedness and response capability relies greatly on efficient information dissemination in a clear, reassuring, and unemotional language. That chain of mostly non-confidential intelligence contains readily apprehensible, explicit, and comprehensive instructions of clinical and epidemiological information and communication channels, discouraging the parallel overreaction of the public after an incident. The population should also be told what signs and symptoms to watch out for and whom to call or where to go if they appear. If specific preventive measures are available to mitigate exposure and infection, the public must be clearly and rapidly informed. Failure to provide a clear public information flow-line may enhance anxiety, fear, and misinterpretations, mainly among citizens who feature non-specific symptoms of exposure to a possible biological agent and that may result in local health facilities becoming overwhelmed by uninfected or panicked patients. Convincing and trustable spokespersons, including respected clinicians, skilled scientists, and public authorities must be able to represent the PHS at all stages of the crisis, also taking into account that the global distribution of social networking increases the dissemination of false or misleading information [54].

Mass Fatality care

During a bioterrorist outbreak, laboratories that receive or dispose of suspicious human body specimens must be informed of relevant clinical and operational protocols. Autopsies should be conducted under full personal protective measures, especially when there is an increased risk of aerosolization or splatter of infectious liquids [44]. Concerning management of bioterrorism fatalities, morgue and funeral protective planning should also be implemented along with prompt and accurate electronic reporting details [18].

Psychological Aspects

The psychological impact, even from a minor bioterrorism-related event may be disproportionately strong55. After such an attack, panic can well affect both victims and healthcare personnel. Psychological reactions following a bioterrorist attack include fear, anger, panic, inappropriate or unrealistic worry about possible contagion or illness, social withdrawal, depression, or demoralization. To handle patient and public fears, the psychological preparedness for bioterrorism should aim at minimizing panic by offering a clear explanation of risks and medical support, and by avoiding unnecessary isolation or quarantine. Furthermore, excessive anxiety in unexposed individuals, experiencing objective but irrelevant clinical symptoms should be treated with non-medical psychological support or on occasion with anxiolytic medication administration. Concerning in particular the healthcare workers, their fears can be managed by bioterrorism readiness education, protection plans, and encouragement of participation in acting field-level roles (e.g. disaster triage volunteering) [56].

CONCLUSION

Increased preparedness, response planning, investments, training, and exercises constitute perhaps the best defense of PHS to mitigate the effects of bioterrorist attacks. Most importantly, citizens must always be aware of a possible deliberate biological contamination and how to respond appropriately. Bioterrorism incidents of the near past prove that public mortality and morbidity can be further reduced if a functional protocol would allow first responders, hospitals, and officials of the PHS to steer more efficiently the public expectations.

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REVIEW ARTICLE

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Heart failure with preserved ejection fraction: A review

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Abstract: *Heart failure is the final stage of evolution of a large number of cardiovascular diseases and represents a health problem worldwide, due to increased prevalence of cardiovascular diseases. The patients with heart failure with preserved ejection fraction (HFpEF) represent about a half of the patients with heart failure and the prevalence of HFpEF is on the rise. HFpEF is mainly a disease of the elderly patients, who have numerous cardiovascular diseases – hypertension, myocardial ischaemia, atrial fibrillation, valvular disease, and also non-cardiovascular comorbidities, such as obesity, diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, and obstructive sleep apnoea. HFpEF is more prevalent in women than in men, but women have a better prognosis than men. The pathophysiological changes that appear in HFpEF are: ventricular stiffening, cardiomyocite hypertrophy and hypercontractility, myocardial fibrosis and inflammation, which lead to abnormal diastolic function with delayed relaxation and inappropriate filling, in the presence of a normal systolic function of the left ventricle. The diagnosis of HFpEF is based on clinical, echocardiographic and biological criteria. In contrast to HFrEF, there is no effective treatment for HFpEF. The treatment of HFpEF includes diuretics for clinical improvement and treatment of comorbidities. The prognosis of patients with HFpEF is similar with those with HFrEF. Currently, the main objectives of the treatment in patients with HFpEF are to improve clinical status and decrease hospitalizations. Further studies are needed to establish an effective treatment to increase survival in these patients. Therefore, HFpEF continues to be a challenge for clinicians regarding the optimal therapy..*

Keywords: *heart failure with preserved ejection fraction, diastolic dysfunction, myocardial fibrosis, natriuretic peptides*

INTRODUCTION

Heart failure is the final stage of evolution of a large number of cardiovascular diseases. Heart failure is a major health problem worldwide, due to increased prevalence of cardiovascular diseases and prolonged survival of some categories of patients with cardiovascular diseases, after successful treatment of their conditions.

The most frequent causes of heart failure are: myocardial ischaemia, valvular diseases, arrhythmias, atrio-ventricular conduction disorders, myocarditis, pericarditis. Often, heart failure has more than one cause. It is extremely important to determine the aetiology of heart failure in order to establish the specific therapeutic options (for exemple to reduce

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heart rate in tachyarrhythmias, to replace a valve in valvular disease, revascularization of the coronary arteries in patients with myocardial ischaemia, etc.) [1].

Currently, heart failure is classified into heart failure with reduced ejection fraction (less than 40%) - HFrEF,mid-range ejection fraction (between 40-49%) – HFmrEF, and preserved ejection fraction (≥ 50%) – HFpEF1. HFmrEF is a new category of heart failure, first introduced in the classification of heart failure in 2016, in the European Society of Cardiology Guidelines [1].

HFmrEF is usually studied in trials along with HFpEF, so in this paper we will use the terminology of HFpEF, including both entities. Until now, the majority of clinical trials regarding the treatment of heart failure have focused on patients with HFrEF. While in the case of HFrEF, it had been proven that the treatment can reduce both morbidity and mortality, in the case of HFpEF there is no drug which can reduce mortality or morbidity. This is why HfpEF is more challenging for the clinician regarding the diagnosis and treatment.

Patients with HfpEF associate many comorbidities, such as hypertension, diabetes mellitus, vascular disease, atrial fibrillation, metabolic syndrome, chronic kidney disease, which complicate even more the evolution of HFpEF and increase the risk of mortality.

PREVALENCE OF HFpEF

In the last years, the prevalence of HfpEF increased, because of the demographic changes and the changes in the risk factors prevalence, such as the increasing prevalence of hypertension, atrial fibrillation and diabetes mellitus that are all risk factors for HFpEF [2]. HFpEF currently affects more than 7 million people in Europe and is the only cardiovascular condition that has increasing incidence and prevalence [3]. The increasing prevalence of HfpEF can also be a result of the changes in clinicians' awareness over time and of the fact that the diagnostic methods have evolved during the last decades.

The patients with HFpEF represent about a half or even more of the patients with heart failure and the prevalence of HFpEF is on the rise [4-8]. HFpEF is mainly a disease of the elderly, who associate numerous cardiovascular and non-cardiovascular comorbidities [9].

HFpEF is more prevalent in women than in men, with a ratio women to men of 2:1, explained by the different sex cardiomyocite remodeling pattern in women than men [10,11]. In men, the eccentric left ventricle remodeling is more frequent, while in women the concentric remodeling is more frequent, as a response to aortic stenosis or hypertension [10]. Due to the concentric remodeling, women have a smaller left ventricle cavity and an increased wall thickness, with less collagen deposition [10]. Consequently, women have a better contractility and ejection fraction of the left ventricle, with the predominance of HFpEF. Women with HFpEF are more likely to be obese, hypertensive or to have renal disease or diabetes mellitus, while men are more likely to have coronary artery disease, atrial fibrillation, chronic obstructive pulmonary disease, anemia [12]. Even if HFpEF is more common in women, they have a better prognosis, with a rate of hospitalization with 20% less than men and a lower risk of cardiovascular and non-cardiovascular events. [12]

RISK FACTORS

Traditional cardiovascular risk factors, such as smoking, obesity, diabetes mellitus, hypertension, precede the onset of both heart failure with reduced and preserved ejection fraction, but there are specific risk factors for each of them. While male sex, myocardial infarction, left bundle branch block or high potassium level represent major risk factors for HFrEF, the risk factors for HFpEF are female sex, higher systolic blood pressure, right bundle branch block and atrial fibrillation [13-15].

Hypertension is common in patients with HFpEF and increases the risk of developing heart failure. The optimal treatment of hypertension improves diastolic filling, leads to the regression of left ventricular hypertrophy and may reduce the progression of heart failure.

Atrial fibrillation (AF) and HFpEF are related, and each of the diseases predisposes to the other. AF occur in approximately two thirds of the patients with HFpEF and contribute to the progression of the illness, due to the uncontrolled ventricular rates and the loss of atrial contraction and its contribution to the left ventricle filling, leading to a shorter filling time and diastolic dysfunction of the left ventricle [16]. When present together, HFpEF and AF lead to a worse outcome than each condition separately [17]. Patients with HFpEF and AF have a higher risk of stroke and mortality. All patients with HFpEF and AF should receive an oral anticoagulant and should have a good control of the ventricular rate.

Myocardial ischemia may contribute to HFpEF, too, by altering the diastolic filling. Significant coronary artery disease is present in more than half of the patients with HFpEF. Patients with HFpEF and myocardial ischemia have a greater risk of ventricular dysfunction and mortality than those without myocardial ischemia. Coronary revascularization can improve the prognosis of patients with HFpEF and myocardial ischemia.

Regarding patients with diabetes mellitus, particularly patients with insulin-dependent diabetes mellitus have an increased risk to develop major adverse cardiovascular events, including cardiovascular mortality, hospitalization for heart failure, non-fatal myocardial infarction, non-fatal non-hemorrhagic stroke, aborted cardiac arrest. Therefore, patients with HFpEF and diabetes have a worse quality of life and poorer outcomes than those without diabetes mellitus.

Comorbidities such as obesity, diabetes mellitus, chronic kidney disease, anemia, chronic obstructive pulmonary disease, and obstructive sleep apnea are related to the worsening of heart function in HFpEF. The treatment of these comorbidities improves the prognosis of heart failure and reduces mortality [18-20].

PATHOPHYSIOLOGY OF HEART FAILURE WITH PRESERVED EJECTION FRACTION

HFpEF is characterized by a normal systolic function, but abnormal diastolic function, with inappropriate filling of the left ventricle in the presence of normal pressure, initially. In more advanced stages, it associates also elevated filling pressures, especially during physical exercise. High filling pressures are considered the primordial cause of dyspnea and exercise intolerance [21]. The abnormalities encountered in HFpEF include delayed relaxation, stiffening of the myocardium and inappropriate filling dynamics.

The changes that occur in the cardiomyocites are the key to understand the dysfunction that appears in HFpEF and consequently to discover a specific treatment. An important element is cardiac hypertrophy. The extent of hypertrophy is a major predictor for HFpEF outcome. Other elements that contribute to HFpEF are fibrosis, left ventricle filling pressure, left ventricle end-diastolic volume, systemic inflammation, and metabolic disorders [22].

HFrEF is characterized by a diminished contractile response of the cardiomyocite, reduced availability of calcium activator and inadequate storage of calcium in the sarcoplasmic reticulum [22]. Knowing these mechanisms, HFrEF may benefit of a specific treatment which acts at the level of renin-angiotensin-aldosterone system and beta-adrenergic receptors. The treatment for HFrEF is ineffective in HFpEF, because of the different pathophysiological mechanisms.

In the case of HFpEF, there are no animal models, which is a major problem to a complete understanding of the pathophysiological mechanisms, in order to establish a specific therapy.

A study published in June 2018 suggests a new theory regarding HFpEF: hypertrophic cardiomyocytes develop also hyperfunction and elevated availability of systolic calcium activator [23]. In vivo, it is present a diastolic dysfunction and in vitro, hypercontractility of the cardiomyocytes. The researchers also noticed discrete, focal areas of fibrosis and in vivo, propensity to arrhythmias, caused by the instability of the calcium from the sarcoplasmic reticulum, and sudden death [23]. Apparently, the hypercontractility due to the increase of the L-type calcium channel density is responsible for the normal systolic function [23]. On the other hand, the high level of calcium limits the relaxation and promotes the stiffness of the ventricle walls. It was noticed also a deficit in cardiomyocytes, which leads to the compensatory hypertrophy of surviving myocytes [23]. Therefore, when failure occurs, diastolic dysfunction is predominant, but ejection fraction is preserved and end-diastolic volume is normal. There have been also observed the hypertrophy of the cardiomyocytes, the stiffness of the ventricle walls and focal interstitial fibrosis.

Arrhythmias can occur in HFpEF and they are related with the high levels of operational calcium, which confer instability, and with spontaneous release of calcium from the sarcoplasmic reticulum during diastole. Arrhythmias increase the risk of sudden cardiac death [23].

Myocardial fibrosis contributes to diastolic dysfunction. Patients with HFpEF have a profibrotic state. Fibrosis is organized in focal areas, observed especially near the interventricular septum. Fibrosis increases the stiffness of the walls. Collagen type I and III are elevated in HFpEF, collagenase and metalloproteinase-1 are reduced, but tissue inhibitor of matrix metalloproteinase-1 is increased, changes which contribute to fibrosis [23]. In tissue biopsy of patients with HFpEF, inflammatory cells have been also found. Hypertrophy is related to a diminished population of cardiomyocytes at an early-stage of growth. Hypercontractility predisposes to cardiomyocytes rupture and substitution with fibrotic tissue. Consequently, the integrity of the cardiac muscle is affected by focal fibrosis and the change of the ventricle geometry. In time, as the failure progresses, the diastolic function is totally compromised, but the systolic function, measured by the ejection fraction, is conserved [23].

Ventricular stiffening is associated with vascular stiffening in HFpEF. Vascular stiffening increases with age and is related to hypertension, chronic kidney disease, diabetes mellitus and obesity [24-26]. Vascular stiffening requires increased cardiac output to fill the rigid arteries that contributes to ventricular stiffening. Thus, ventricular-vascular stiffening is a common association in patients with HFpEF.

Inflammation has an important role in HFpEF. Comorbidities, such as renal disease, diabetes mellitus, may contribute to the proinflammatory state [27]. Several proinflammatory cytokines are increased in HFpEF, such as interleukin-6, tumor necrosis factor α, pentraxin 3 and soluble ST2 [27]. These patients have systemic inflammation and endothelial dysfunction, caused by the elevated expression of vascular cell adhesion molecules: VCAM-1, E-selectin, reactive oxygen species (ROS) [20]. The high level of ROS leads to the reduction of nitric oxide and consequently aggravates cardiomyocyte stiffness, hypertrophy and fibrosis [20,26].

DIAGNOSIS OF HFpEF

Heart failure can be initially suspected based on clinical criteria. For a definite diagnosis, it is mandatory to perform an echocardiography.

At echocardiography, the ejection fraction of the left ventricle, the thickness of the ventricular walls, the cavities' geometry and dimensions, the associated pathologies, such as valvular stenosis or insufficiency, are evaluated.

The clinical and paraclinical elements necessary for the diagnosis of HFpEF/HFmrEF are:

- The presence of symptoms and signs of heart failure.
- A left ventricle ejection fraction ≥ 50% for HFpEF and 40-49% for HFmrEF.
- Increased levels of natriuretic peptides: BNP > 35 pg/mL; NT-proBNP > 125 pg/mL.
- Objective elements of structural and/or functional disorders of the heart as a possible cause for the clinical presentation.
- If the diagnosis is uncertain, a stress test or invasive measurement of increased left ventricle filling pressure may be necessary [1].

The specific symptoms of heart failure are breathlessness at variable degrees of effort or at rest and exercise intolerance. Dyspnea interferes with the daily activities and impairs the quality of life. On physical examination, patients with heart failure have pulmonary crackles, bilateral ankle edema, jugular venous dilatation, laterally displaced apical beat.

Echocardiography is the conclusive method for the diagnosis of heart failure. The left ventricle ejection fraction (LVEF) is the most important parameter used to determine the systolic function of the left ventricle and a powerful predictor of death in patients with reduced ejection fraction [27]. LVEF is an important predictor for fatal and nonfatal cardiovascular events in patients with heart failure, including heart failure-related death, sudden cardiac death, hospitalization, myocardial infarction, especially in patients with reduced ejection fraction [28]. A 10% reduction of the ejection fraction is associated with a 39% higher risk of cardiovascular mortality [27].

In patients with HFpEF, echocardiography reveals a normal or near-normal ejection fraction and inadequate diastolic relaxation and left ventricle filling.

The structural elements of HFpEF are the left atrial volume index > 34 mL/m^2, a left ventricular mass index ≥ 115 g/m^2 for males and ≥ 95 g/m^2 for females [1].

The diastolic dysfunction has 3 stages that can be evaluated by echocardiography. The first stage is delayed relaxation, with an E/A ratio under 0.8, deceleration time of E wave > 200 ms, E/e' medium ratio less than 8. The second stage is pseudonormal function, with E/A ratio between 0.8 and 1.5, deceleration time of E wave between 160 ms and 200 ms, E/e' medium ratio between 9 and 12. The last stage is the restrictive pattern, with E/A ratio more than 2, deceleration time of E wave less than 160 ms, E/e' medium ratio more than 13 [28].

If echocardiography at rest is inconclusive, the diastolic stress test, using a semi-supine bicycle to measure the pulmonary artery pressures and cardiac output changes with exercise, may be necessary [1]. Another paraclinical test is invasive measurement of the filling pressures – the pulmonary capillary wedge pressure ≥ 15 mmHg, left ventricular end-diastolic pressure ≥ 16 mmHg [1].

In the presence of atrial fibrillation, the echocardiographic diagnosis of HFpEF is more difficult, because the left atrial volume index is higher, the functional parameters of diastolic disorder are not well established. Also, the values of natriuretic peptides are higher in patients with atrial fibrillation. On the other hand, atrial fibrillation can be a mark of HFpEF. Patients with HFpEF and atrial fibrillation may have more advanced heart failure than those with HFpEF and sinus rhythm [1].

Biomarkers measured with highly specific and sensitive assay play an important role in the diagnosis of heart failure and its risk stratification [27]. The Brain Natriuretic Peptide (BNP) is higher in patients with HFpEF than in patients without heart failure, but lower than in patients with HFrEF [28]. BNP is associated with diastolic left ventricle walls stress and pressure. Biomarkers of extracellular matrix and fibrosis (soluble ST2, galectin-3, type I procollagen C-terminal propeptide, type I and II collagen amino-terminal propeptide, collagen telopeptides, matrix metalloproteinases – MMP-1, MMP-2, MMP-8, MMP-9, tissue inhibitor of metalloproteinases – TIMP-1, TIMP-4, osteopontin) can be increased in patients with HFpEF [6]. Renal biomarkers, such as cystatin C and urinary albumin, and cardiac troponins are also elevated6. All these biomarkers may sustain the diagnosis of HFpEF, but have a poor prognostic role and, excepting natriuretic peptides, cannot be used to guide the treatment [6]. The natriuretic peptides values are related to the severity of the disease, therefore higher levels of natriuretic peptides are linked with more advanced HFpEF [6].

TREATMENT OF HFpEF

There is no specific or effective treatment for patients with HFpEF, in order to reduce the mortality rate [29]. In clinical practice, fewer patients with HFpEF appear to receive beta-blockers, diuretics, angiotensin-converting enzyme inhibitors, angiotension receptor blockers and aldosterone antagonists than patients with HFrEF and if they receive these drugs, it may be to treat comorbidities, such as hypertension, coronary artery disease, atrial fibrillation [29,30].

Renin-angiotensin-aldosterone system blockers have been studied in patients with HFpEF, due to their effects on hypertension, fluid retention and fibrosis. Also, beta-adrenergic blockers were studied due to their effects of prolonging diastole and increasing the filling time of the left ventricle. Despite their well-known benefits in patients with HFrEF, in those with

HFpEF beta blockers offer only a modest symptomatic improvement and do not reduce mortality [31,32]. Some studies revealed a decreased hospitalization rate with angiotensin converting enzyme inhibitors/ angiotensin receptors blockers [6]. The study „The Effects of Candesartan in Patients with Heart Failure and Preserved Left-Ventricular Ejection Fraction" concluded that patients with HfpEF who received candesartan had fewer hospitalizations for decompensated heart failure compared with placebo, but without influence on the mortality rate [6]. Probably, renin-angiotensin-aldosterone-system does not have such an important role in HFpEF as in HFrEF, this is why its blockers have much less benefits [6].

The use of aldosterone antagonists was also tested in HFpEF. The results of the study ALDO-DHF, published in 2013, showed an improvement of the diastolic function, but no improvement was noticed in the maximal exercise capacity, clinical symptoms and quality of life [6]. The TOPCAT study, whose results have been published in 2014, also did not achieve its primary outcomes – cardiovascular mortality or aborted cardiac arrest, only a small decline in hospitalization rate has been observed [6,33].

Sildenafil, a phosphodiesterase-5 inhibitor, was tested in RELAX study and showed no advantage on mortality or symptomatic relief [6].

Statins can be prescribed in patients with HFpEF and have shown a significant impact on survival, due to their pleomorphic anti-inflammatory effects [6].

Considering the lack of results on mortality rate with beta-blockers use, ivabradine was also studied. Ivabradine slows the sinus node rate, but does not affect contractility and peripheral vasculature, in contrast to beta-adrenergic blockers [6]. Studies on mice revealed an improvement in the left ventricle function and lowering of aortic stiffness and fibrosis after four weeks of therapy with ivabradine [6]. A clinical trial, including 61 patients with HFpEF, has found an improvement in exercise capacity and decreased exercise-induced E/e' ratio, which represents diastolic pressure index, with ivabradine versus placebo [6]. No side effects appeared. Ivabradine cannot be used in patients with bradycardia or chronotropic incompetence and neither in patients with restrictive type of diastolic disorder, because filling occurs early and rapidly in these patients and decreasing heart rate would worsen cardiac output even more [6].

Introduction of device therapy was a great progress for patients with HFrEF, with pacemakers, implantable cardioverter defibrillators, and resynchronization therapy. In patients with HFpEF, asynchrony is rarer than in HFrEF, thus device therapy has no applicability in HFpEF [6].

No treatment has yet been found to diminish morbidity and mortality in patients with HFpEF and HFmrEF. An important objective of the treatment in these patients is to improve symptoms and the quality of life. Diuretics are prescribed to reduce pulmonary and systemic congestion signs and symptoms. As mentioned before, aldosterone antagonists and beta-blockers do not improve symptoms in patients with HFpEF, only a relative improvement in NYHA class with candesartan was observed [1].

Regarding hospital admission, patients with HFpEFin sinus rhythm may benefit from the use of nebivolol, spironolactone and candesartan, which decrease hospital admission1. For patients who associate atrial fibrillation, beta-blockers are ineffective and digoxin has not yet been studied [1].

Arterial hypertension should be carefully treated in patients with HFpEF/HFmrEF, who have often systolic arterial hypertension. The antihypertensive drugs recommended are diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and aldosterone antagonists [1]. Beta-blockers appear to be less effective [1].

The specific therapy for comorbidities is also important, for example losing weight in obese patients, oral anticoagulants for atrial fibrillation or other arrhythmias, medical and/or interventional treatment for coronary artery disease, treatment of diabetes mellitus, preferably with metformin [1], renal disease treatment [34-37], treatment of anemia, chronic obstructive pulmonary disease and obstructive sleep apnea.

The most challenging situation is the treatment of HFpEF when different kinds of surgical procedures are required [38-40]. Either is abdominal surgery [41-44] or pelvic interventions [45-47], in males [48-51] or females procedures [52-55], the management of these comorbidities involves complex treatment strategies from surgeons, cardiologists or anesthesiologist.

Thus, the management of HFpEF includes diuretics, which reduce systemic congestion and improve the filling pressure of the left ventricle, and treatment of comorbidities, which improves considerably the clinical outcome [56].

PROGNOSIS OF PATIENTS WITH HFpEF

Overall, the prognosis of patients with HFpEF is similar with those with HfrEF [57]. Patients with HFpEF have more frequent a non-cardiovascular cause of death or hospitalization, in contrast to the patients with HFrEF, who have commonly a cardiovascular cause for hospitalization or death [1]. Renal involvement in patients with HF is a redutable complication, especially in patients with increased blood pressure [58-61].

The left ventricle hypertrophy and elevated left ventricle filling pressure correlate with hospitallizations for heart failure [62,63]. Recurrent hospitalizations are as frequent in HFpEF as in HFrEF and are associated with higher mortality rate [62].

There are sex-related differences between the risk factors for HFpEF. Hypertension, obesity and renal disease are more common in women, while ischemia, atrial fibrillation, chronic obstructive pulmonary disease and anemia are more frequent in males [12]. Women have a 20% lower risk of hospital admission than men, and also a lower risk of cardiovascular and non-cardiovascular events [12]. Women have an overall better prognosis than men [12]. The prognosis is modified by the presence of atrial fibrillation, stable angina pectoris, and renal disease in both sexes [12].

CONCLUSIONS

Patients with heart failure with preserved/mid-range ejection fraction represent approximately a half of the patients with heart failure.

The diagnosis of HfpEF is based on clinical signs and symptoms, echocardiography, natriuretic peptides levels and stress tests or invasive measurement of filling pressures and left ventricular end-diastolic pressure, if necessary.

Overall, the prognosis of patients with HFpEF is similar with those with HFrEF, with the difference that in these patients the death of non-cardiovascular cause is more common, in contrast to the patients with HFrEF, in whom the cardiovascular death is more frequent.

There has not yet been found a specific therapy for HFpEF that may reduce morbidity and mortality, in contrast to HFrEF. The main objective of the treatment in patients with HFpEF is to improve clinical status and decrease hospitalizations.

Further studies are needed to establish an effective treatment, to increase survival in these patients. In conclusion, HFpEF continues to be a challenge for clinicians regarding the optimal treatment.

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ORIGINAL ARTICLE

High values of procalcitonin in non-septic patients with thermal and airway burns – an early severity predictor?

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Abstract: *Purpose – Combined airway burns with cutaneous burns is a further negative prognostic factor in burn patients. In this study we tried to identify the most relevant negative prognostic factors at admission in a relatively homogeneous population.*

Methods – In this study, ten young patients who suffered from skin and airway burns following a fire in a club, admitted to our ICU department, were included. At the intake, ABSI and APACHE II scores were calculated, and a series of biomarkers were also collected. The correlation and associations between gravity scores and biomarkers was then calculated using the Spearman correlation and Chi square tests.

Results – Based on data analysis, we found very high values of ABSI 8.9 ± 1.4 and APACHE II 25.4 ± 2.79 scores, procalcitonin (46.16 ± 68.18 ng/ml), white blood cells (41.26 ± 15.66 × 103/mm3), hematocrit (51.6 ± 7.31 %), and low corrected albumin values (22.08 ± 4.66 g/l). The statistical analysis revealed a good correlation between gravity scores and values of PCT and low albumin levels ($p< 0.05$). The mortality was also associated with the need for vasopressor support from the beginning ($p < 0.05$).

Conclusions – Adverse prognostic factors that we have identified in this group of young patients with skin burns and airway include: increased values of the severity scores and PCT, low values of corrected albumin and vasopressor support necessary at admission.

INTRODUCTION

Burns represent one of the most aggressive injuries of the human body known for high mortality, thus raising major public health issues such as increased length of hospitalization and recuperation, along with psychological and physiological traumas, all these leading to increased costs [1-4].

Burns-induced mortality rate is globally increased, and surprisingly high in the developing countries, including Europe's burn centers, where it ranges between 1.4% and 18% [5]. The high mortality is associated with a number of factors, including long-term severity of burn driven inflammatory response of the host, secondary human body frailty and abnormal response to further injuries like sepsis [6, 7]. The association of airways' and cutaneous burns induces a supplemental increase of the inflammatory response, difficult to quantify because of the inaccurate evaluation of the burns from the surface of the lungs and burn degree same time [8]. Consequently, in the case of mixed cutaneous-and-airway burns the mortality increases,

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despite the usage of advanced techniques to support lung function (Extracorporeal membrane oxygenation – ECMO) [9-12]. On the other hand, in the beginning, it is unclear whether a patient has both cutaneous and airway burns, as for the latter it might take up to 48 hours to observe any clinical manifestations. As such, the airways' burns assumption is made based on anamnesis and correlation between the high incidence of airways burns and the increased surface of cutaneous burns [11]. If one can clinically suppose an airways burn (in this case a bronchoscopy is usually performed for a certain diagnosis), there is no toll to quantify its' impact on the inflammatory response of human body. Currently, there is just an indirect estimation of their aggression on the human body using either dedicated markers or severity scores. Despite the wide availability of severity scores as Acute Physiology And Chronic Health Evaluation (APACHE II and APACHE III), Abbreviated Burn Severity Index (ABSI), and biomarkers (such as microalbuminuria, serum albumin, C-reactive protein, procalcitonin (PCT)), both used to estimate the severity of cutaneous burn injuries, they are not sufficiently standardized for the estimation of mortality risk in case of combined thermal and airway burns [13-16].

Considering the increased mortality and likeliness of combined burns complications, it is paramount to know the severity degree and the prognosis of the burn evolution from the beginning.

In the current study, we evaluated the absolute values of serological markers and severity scores upon admission to the Intensive Care Unit (ICU) in a group of young adult patients who presented both cutaneous and airway burns following the fire in a club in Bucharest, Romania and we studied their correlation with mortality.

MATERIAL AND METHOD

This study includes the victims of a fire at a club in Bucharest, in the night of 29-30 October 2015, all of them hospitalized that night in the Intensive Care Unit of the Emergency Hospital for Plastic Reconstructive Surgery and Burns, in Bucharest. All admitted patients underwent surgery (non-excisional debridement) and subsequently, they were connected to artificial breathing devices, central venous and urinary catheters being placed on them too.

Hydroelectrolytic rebalancing started from the operating room with a crystalloid solution (Ringer lactate), following the Parkland protocol (4 ml/kgc/Total Burn Surface Area-TBSA), while in patients who could not maintain a Mean Arterial Pressure (MAP) > 65 mmHg, epinephrine was administered in continuous perfusion from the beginning. Patients were admitted to ICU between 00:00 and 03:00 on 30 October, and the first set of blood tests was harvested at 6:00 on 30 October. To determine the quantitative procalcitonin (PCT) values, the testers arrived after about 48 hours from admission.

We estimated the severity of the patients' burns using ABSI and APACHE II scores.

In, order to estimate the appropriate level of serum albumin under hypovolemic conditions, we corrected it in accordance to the hematocrit (Ht) level, using the following formula:

$$corrected\ albumin\ level\ = measured\ albumin\ level\ \times \frac{normal\ Ht}{measured\ Ht}$$

where normal hematocrit (Ht) is 45% for men and 40% for women.

For the determination of the blood cells count, the Ac-T 5 diff CP (Beckman Coulter, Germany) was used. The biochemical markers were measured using the Konelab-20i/20XTI (Thermo Electron Corporation, Finland). Coagulation parameters were determined using the ACL-7000 instrument (Instruments Laboratory, Italy). PCT was assessed using the VIDAS, bio Merieux VITEK, France.

The conduct of this study was approved by the Ethics Committee of the Emergency Hospital for Plastic Reconstructive Surgery and Burns (number 5/10 July 2017).

Statistical analysis

The data is presented as the average ± standard deviation for quantitative variables, and absolute frequencies and relative frequencies, respectively for the qualitative variables. Batch variance analysis was performed with the Levene test. For the analysis of the difference between the environments the t-student test was used together with the calculation of the confidence intervals. For the correlation analysis Spearman correlation coefficient and chi-square nonparametric test were employed. In addition, for the correlation analysis we performed Percentile Bootstrap Correlation in order to calculate the Pearson parametric correlation coefficient. Confidence intervals for the value of the t test and the Pearson correlation coefficient were obtained through the bootstrap method with 10,000 and 1,000 replications, respectively. The results were considered statistically significant for a p value <0.05. For the statistical analysis IBM SPSS 18.0 and several R packages, such as wBoot, were used.

RESULTS

During the night of 29-30 October 2015, ten patients (seven male and three women) were admitted in our ICU. The deep cutaneous burn lesions (3rd degree) ranged between 5% and 50% of the body surface area and the age range of admitted patients was 18 to 40 years old. Their ABSI scores were between 7 and 11, associating a mortality between 10% and 60 - 80% and the APACHE II score ranged between 23 and 30, which is associated with a 46% to 70.3% mortality at admission. In Table 1 we report demographical data, several laboratory measures describing the admission conditions and, for some of them, the measures sampled 30 hours after admission.

Table 1. Characteristics of the patients included in the study

	Values
WBC (1000/ml)	41.26 ± 15.66
PLT (1000/ml)	142.5 ± 50
APTT (sec)	40.27 ± 10.76
INR	1.87 ± 0.45
pH	7.04 ± 0.11
Temperature (^{0}C)	35.95 ± 0.61
Albumin (g/l)	25.95 ± 4.52
Corrected Albumin (g/l)	22.08 ± 4.66
PCT (ng/dl)	46.16 ± 68.18
Creatinine (mg/dl)	1.39 ± 0.53
TGO (mg/dl)	70.8 ± 38.31
TGP (mg/dl)	54 ± 43.28
One month mortality (%)	80%
One year mortality (%)	90%
WBC (1000/ml)	41.26 ± 15.66
PLT (1000/ml)	142.5 ± 50
APTT (sec)	40.27 ± 10.76
INR	1.87 ± 0.45
pH	7.04 ± 0.11
Temperature (^{0}C)	35.95 ± 0.61
Albumin (g/l)	25.95 ± 4.52

Note: TBSA III = Total Burn Surface Area 3rd degree, WBC = white blood cells, PLT = platelets, APTT = activated partial thromboplastin time, INR = international normalized ratio, Corrected Albumin represents corrected values of albumin according to hematocrit, PCT = procalcitonin, TGO = glutamic oxaloacetic transaminase, TGP = glutamate-pyruvate transaminase.

For these variables, the values presented are mean ± standard deviation. Gender distribution and mortality after one month and one year are also provided in Table 1. Blood tests revealed: high levels of white blood cells (WBC) of 41.26 ± 15.66/mm3, elevated hematocrit (Ht) level51.6 ± 7.31%, albumin values of 25.95 ± 4.52 g/dl (uncorrected) and 22.08 ± 4.66 g/dl (corrected), creatinine level of 1.39 ± 0.53 mg/dl, glutamic oxaloacetic transaminase (TGO) values of 70.8 ± 38.31 mg/dl and glutamate-pyruvate transaminase (TGP) levels of 54 ± 43.28 mg/dl.

Forty-eight hour procalcitonin (PCT) values were extremely elevated: 49.85 ± 67.34 ng/ml (PCT was measured in eight patients). The coagulation parameters activated partial thromboplastin time (APTT), international normalized ratio (INR), and platelets (PLT) we report in Table 1 were measured at 30 hours from admission (the second set of analysis) because at the first set there were no changes (first set: APTT = 29.3 ± 3.01, INR = 1.15 ± 0.18, PLT = 306.2 ± 108.47). So we found: APTT = 40.27 ± 10.76 seconds, INR = 1.87 ± 0.45, PLT = 142.5 ± 50*103/ml. The pH values of 7.04 ± 0.11 presented in the table were obtained from central venous blood samples after the central venous catheters were placed. The temperature at admission had a mean value of 35.95 ± 0.61 Celsius degrees. Of the ten patients in ICU eight needed vasopressor support at admission and also during the next days.

Table 2 presents values of serological markers, Total Burn Surface Area 3rd degree in terms of mean ± standard deviation, and the number of patients who required vasopressor support with epinephrine (norepinephrine from the second day) from the admission, considering the total study group and the subgroup of those who did not survived.

Table 2. Values of serological markers, total burn surface area (TBSA) and norepinephrine status in the study group and subgroup

	Total	Non Survivors	p value
Albumin (g/l)	25.95 ± 4.52	24.63 ± 3.97	0.26
Corrected Albumin (g/l)	22.08 ± 4.66	21.25 ± 4.62	0.35
PCT (ng/ml)	46.16 ± 68.18	65.88 ± 71.52	0.35
Hematocrit (%)	51.6 ± 7.31	51.19 ± 5.07	0.43
WBC (1000/ml)	41.26 ± 15.66	44.00 ± 16.22	0.36
Norepinephrine	8 (10)	8	0.03
TBSA III (%)	25.7 ± 16.73	29.62 ± 16.27	0.30

Note: WBC = white blood cells, PCT = procalcitonin, Corrected Albumin represents corrected values of albumin according to hematocrit. TBSA III = Total Burn Surface Area 3rd degree. Data are presented as mean ± standard deviation.

In the case of norepinephrine requirements association chi-square test were performed.

Statistical significance is considered for a p value < 0.05. We performed parametric t-tests for the above variables in order to assess whether there are sizeable differences between the values recorded for all patients versus the values for those that died. Due to the existence of abnormal values for TBSA and PCT 48 hrs, we have eliminated TBSA values exceeding 8, and PCT 48H values under 200. Table 3 presents two versions of the t-tests: regular tests assuming unequal variances, and bootstrap tests with 10000 replications.

Table 3. T-tests and bootstrap confidence intervals

Parameter	T-test value	P-value	Bootstrap confidence interval limits			
			Lower 95%	Upper 95%	Lower 90%	Upper 90%
Corrected albumin	0.37421	0.7134	-2.90235	4.7619	-2.52655	4.1034
TBSA	-0.50211	0.6227	-17.32	9.86	-2.3798	4.0939
PCT	-0.38325	0.7093	-105.1441	8.8855	-100.0322	5.32
WCB (divided by 100)	-0.36157	0.7228	-16.404	8.845175	-13.9097	7.57075
TBSA>8	-0.28615	0.7793	-15.71875	11.5	-13.75	9.25
PCT48h <200	-0.4898	0.6367	-37.916	19.39814	-32.02486	14.458
Albumin	0.65438	0.5223	-2.195	4.57	-1.575	4.21
Hematocrit	0.16848	0.8683	-4.50495	5.85595	-3.6257	5.1709

All results show that differences between the two samples are non-significant at 90% confidence level. Further, we investigated the correlations between severity scores (ABSI and APACHE II), procalcitonin (PCT) and corrected albumin using the Spearman non-parametric (rank) correlation coefficient. Table 4 shows that these correlations are significant at 10% significance level. We pursued a more in-depth analysis of the correlations between the above variables calculating the Pearson parametric correlation coefficient using the Percentile Bootstrap Correlation Method available in the package wBoot of R. intervals obtained after 1000 replications.

Table 4. Spearman correlations

Correlation	Correlation Coefficient	p value
ABSI - APACHE II	0.54	0.053
APACHE II - Corrected Albumin	-0.59	0.036
ABSI - PCT	0.73	0.019
APACHE II - PCT	0.73	0.019

Table 5 presents the values of the correlation coefficient, the standard error as well as the 90% or 95% confidence

Table 5. Pearson correlations: results from the Percentile Bootstrap Correlation Method, 1000 replications

Variables	Mean coefficient	Standard error	Confidence interval		
			Lower Limit	Upper Limit	Level
ABSI , Apache II	0.4904211	0.2582198	0.008991	0.8477	90%
ABSI , PCT	0.5594826	0.1655465	0.2769	0.8614	95%
Apache II, PCT	0.5487429	0.208531	0.1969	0.9677	95%
Apache II, Corrected Albumin	-0.5645653	0.2334818	-0.8677	-0.151	90%

Bronchoscopic aspects at 48 hours did not reveal severe changes, most of them related to mild or moderate impairment of the lungs (Grade 0-2), only one exception with severe lesions (Grade 3) being identified. Following the Berlin criteria for the classification of acute respiratory distress syndrome (ARDS), nine patients presented mild ARDS and only one moderate ARDS upon admission to ICU.

We have obtained a good correlation between the APACHE II score at admission and the procalcitonin values (at 48 hours), ABSI score and procalcitonin and also between the

APACHE II score and albumin (Table 3).

One month after admission, two of the ten patients survived and after one year, one patient survived.

DISCUSSION

Upon arrival, patients showed signs of increased burn severity including high values of PCT (at 48 hours from admission), WBC, APACHE II & ABSI scores, and Ht, as well as low levels of albumin. Eight of the ten admitted patients required vasopressor support from the beginning.

It is known that PCT, a molecule secreted by the thyroid, intestine, liver, kidney, muscle and adipocytes under the action of TNFα and IL-1β has elevated values especially in bacterial sepsis [17] and not in viral infections [18] and also has a rapid kinetic growth from the first few hours of injury to a maximum of 12 hours [19]. PCT is also increased in septic burned patients. Several studies were performed for identifying a cut-off value for sepsis in burned patients, the yielded values ranging from 0.5 ng/ml to 1.5 ng/ml [20-23]. The highest PCT values reported for non septic patients were 2.3 ± 3.7 ng/ml [20].

Nevertheless, PCT was also found to be increased in non-septic states by systemic inflammatory response syndrome-SIRS, which is logical considering that its secretion is stimulated by TNFα and IL-1β. Additionally, it has been noticed that in complex visceral liver/spleen plus thoracic trauma the PCT values go up to 9.37 ± 2.71 ng/ml [24], consequently elevated values were also found in other SIRS states [25, 26]. In burns it was found that PCT grows even in the absence of infection, so patients who associate PCT values > 2 ng/ml within the first 48 hours of admission have a mortality of 65% at 50 days [27]. According to the American Burns Association criteria (ABA) [28], we had just one patient who presented sepsis criteria in the first 48 hours, so the high PCT values observed in our patients (46.16 ± 68.18 ng/ml) were explained by an extremely aggressive inflammatory response to burn. Our opinion is that this reaction was induced not only by skin burns but also by pulmonary injury so that high PTC values on admission may also indicate an associated airway burn in patients who have such a suspicion.

Serum albumin is a marker of severity in critical patients and is associated with an increased mortality [29, 30]. Low levels of serum albumin and urinary albumin occur by increasing vascular permeability induced by pro-inflammatory mediators in SIRS or sepsis [31, 32]. Patients with burn injuries lose their albumin by increasing the capillary permeability induced by pro-inflammatory mediators and the serum albumin value correlates with the burned surface, so at a burned surface of 60%, serum albumin (at admission) is around 1.95 ± 0.02 g/l [33, 34, 35]. In a recent study, it was found that in patients with burn driven injuries an albumin value < 2 g/l is correlated with a mortality > 80% [36]. For our patients, the value of serum albumin on admission was 25.95 ± 4.52 g/dl and corrected albumin was 22.08 ± 4.66 g/dl. We consider it more useful to report corrected albumin values considering that patients have high hematocrit variations as most of them are hemoconcentrated at admission. In all of the studies we considered, the serum albumin values were presented without being adjusted to the hematocrit value.

The APACHE II score is known as a severity score that is used to estimate mortality in intensive care units [37]. Its correlation with mortality is applicable to burn patients [38, 39] and also to acute respiratory distress syndrome (ARDS) patients [40, 41]. In our group, the high APACHE II scores have been interpreted as being induced by an extreme inflammatory response probably induced by thermal and airway burns. The ABSI score is a good indicator of the severity of a thermal injury and seems to estimate quite well the burning severity in patients with airway burns [42, 43]. ABSI score has a similar prognostic value compared to APACHE II in estimating the severity of patients with both cutaneous and airway burns. ABSI and APACHE II predict a similar mortality and the correlation of their scores is at the p = 0.05 limit. In our patients, the WBC count at admission was extremely high 41.26 ± 15.66 *103/ml. These elevated values can be only partially explained by hemoconcentration because the mean value of the hematocrit was just slightly elevated. WBC count is also increased in other situations with a systemic inflammatory response but not to those values reported in our group [44, 45].

The mean serum creatinine value, at 6 hours after admission, was 1.39 mg/dl, which may be due to hypovolemia induced by renal hypoperfusion. On the other hand, elevated transaminases with predominantly TGO/TGP increased ratio may be explained by their release from the burn cutaneous area rather than by hepatic hypoperfusion. The triad: acidosis, hypothermia and coagulopathy has a poor prognosis in polytraumatized patients and in burn patients [46]. This triad is particularly presents in patients with cutaneous and airway burns. All the patients with skin and airway burns showed severe metabolic acidosis at admission. Coagulopathy did not immediately appear but emerged in the next 30 hours. Hypothermia (temperature under 35.5°C degrees) was present in just two patients at admission, and the mean value was about 35.95 Celsius degrees. The absence of hypothermia can be explained by the very short time until the patients were brought to the hospital, the fire being located in a club in Bucharest, relatively close to the hospital. Thus, out of the three parameters of the triad, in our patients, only 2 of them were present.

These changes of PCT, WBC and high APACHE II and ABSI scores can be explained by an extreme inflammatory reaction or septic shock. This inflammatory reaction could be caused by thermal cutaneous lesions and/or airway burns. Although we found a good correlation between the APACHE II score & PCT & corrected albumin, and also between ABSI score & PCT, we did not find a statistically significant correlation between the burn lesions surface or depth and the biological parameters. So we can state that the inflammatory reaction was determined by a mixed cause represented by the thermal cutaneous lesions and airway burns. For patients with airway burns, the etiology may be thermal or chemical. In most of our cases, bronchoscopy did not reveal significant structural changes in the upper airways, which led us to the idea that pulmonary injury was mixed, thermal and chemical. Although we did not obtain statistically significant changes, we found an increase in WBC, PCT and TBSA III values in the case of non-survivors compared to the mean values obtained in the entire group of patients. Yet, a statistically significant difference was obtained with regard to vasopressor support, so all eight patients requiring vasopressor support at the admission died in the first month.

The patients who survived one month from the incident, had PCT values at 48 hours of 3.14 ng/ml and 0.41 ng/ml, respectively, corrected albumin of 22.3 g/l and 28.45 g/l, respectively, while the APACHE II values were 24 and 23 respectively, and ABSI scores were 8 and 7 respectively. These were also the only patients who did not require vasopressor support at admission.

CONCLUSIONS

The association of airway burns to the cutaneous thermal lesions is linked to an increased mortality even in young patients. There is no dedicated marker or score for the assessment of the combined cutaneous and airways burns aggression on the human body but an association of markers and scores could improve the evaluation of the burn severity. The negative prognostic factors we identified in our group were high PCT values at 48 hours, low corrected albumin levels, vasopressor requirements and elevated APACHE II and ABSI scores. The WBC count and TBSA could be also considered in establishing the severity of the cases.

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REVIEW ARTICLE

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Hyphema – from the effect to the cause

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Abstract: *The purpose of this review is to describe the etiology (traumatic, iatrogenic, spontaneous causes), clinical and paraclinical findings and the management of hyphemas.*

The clinical appearance of hyphema is variable and is influenced by the volume of blood and the amount of time erythrocytes are present in the anterior chamber. When hyphema is evident, a complete history should be obtained and a thorough physical examination performed to direct the initial selection of diagnostic tests. Secondary complications of hyphema include glaucoma, synechiae, cataract formation, blood-staining of the cornea, and blindness.

Early diagnosis and treatment can minimize or prevent the installation of secondary complications and improve the prognosis.

INTRODUCTION

Hyphema is blood collection in the anterior chamber of the eye (delimited anteriorly by the cornea, posteriorly by the lens and iris and circumferentially by the angle between iris and cornea).[1]

Depending on the blood volume present in the anterior chamber, hyphema is classified as (Figure 1):

- Grade 0: circulating erythrocytes (microhyphema)
- Grade 1: <1/3 anterior chamber volume
- Grade 2: 1/3 -1/2 anterior chamber volume
- Grade 3: >1/2 anterior chamber volume
- Grade 4: total anterior chamber volume (”eight ball hyphema”)

The symptoms that bring the patient to the doctor may vary depending of etiology, but the most common symptoms are blurred vision, pain and photophobia.

The most important thing in finding the etiology of hyphema is a detailed medical history of the patient. It’s important to ask the patient if he suffered a trauma, what was the mechanism and if an object was involved, because hyphema in some cases may be accompanied by the presence of an intraocular foreign body. Other questions will be related to certain diseases such as diabetes, sickle cell anemia or other blood disorders and treatment with antiplatelet agents or anticoagulants.

After a complete medical history, physical exam will include: biomicroscopy, FO exam, gonioscopy, ocular ultrasonography, ultrasound biomicroscopy, radiographic examination or CT (when there is severe orbit damage or the doctor suspects the presence of an intraocular

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foreign body).[3]

Figure 1: Hyphema grading system
(American Academy of Ophthalmology)[2]

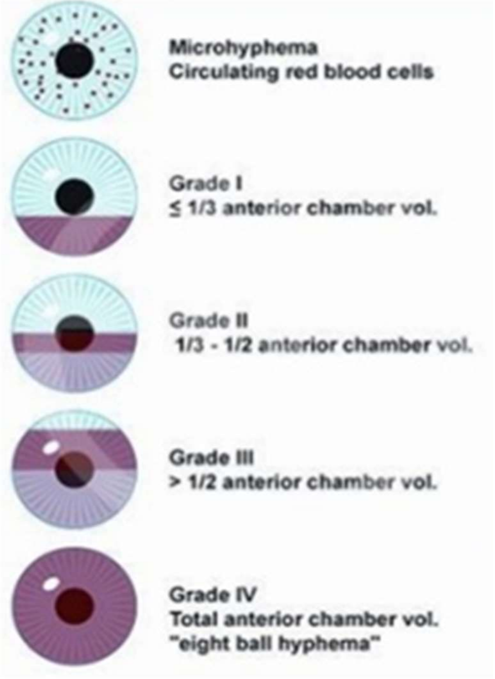
Microhyphema
Circulating red blood cells
Grade I
≤ 1/3 anterior chamber vol.
Grade II
1/3 - 1/2 anterior chamber vol.
Grade III
> 1/2 anterior chamber vol.
Grade IV
Total anterior chamber vol.
"eight ball hyphema"

ETIOLOGY

Most frequently hyphema is caused by trauma, followed by intraocular surgery and spontaneous hyphema: abnormal growth of blood vessels – neovascularization (diabetes, ischemia), intraocular tumors (iris melanoma, juvenile xanthogranuloma, retinoblastoma), infections (herpetic keratouveitis), blood dyscrasias (sickle cell anemia, leukemia), anticoagulant therapy.[4]

Traumatic hyphema is most common in young males. Ocular trauma may be blunt or lacerating. The mechanisms of developing traumatic hyphema are:

- In blunt trauma occurs a rapidly anteroposterior compression of the eye with equatorial expansion and increased intraocular pressure, which leads to shearing forces that tear the vessels of the iris and ciliary body with accumulation of red blood cells within the anterior chamber.

- In lacerating trauma, traumatic agent causes direct damage of the vessels

Studies have shown that more than 50% of the cases, hyphema occupies less than 1/3 of the anterior chamber volume and less than 10% of the cases hyphema occupies the total volume of the anterior chamber.

In the case of a trauma, hyphema may be accompanied by other injuries such as angle recession (seen in 85% of traumatic hyphemas and can be associated with the development of glaucoma), traumatic iritis (presence of inflammatory cells in the anterior chamber and presence of pigment on the anterior capsule – Vossius ring – Figure 2), fixed dilated pupil in approximately 10 % of the cases, iridodialysis (disinsertion of the iris from the scleral spur), cyclodialysis cleft (separation of the ciliary body from the scleral spur, creating a direct connection between the anterior chamber and the suprachoroidal space, leading to ocular hypotony), corneal changes (from minor abrasion to corneal endothelium damage), cataract, lens subluxation, posterior segment injuries (vitreous hemorrhage, retinal edema/hemorrhages/ holes/tears, choroidal rupture etc.).[1]

Hyphema caused by ocular surgery can occur both intraoperatively and postoperatively. Intraoperatively, has been described in cataract surgery by direct damage of iris vessels, in peripheral iridectomy, cyclodialysis, and intervention on the posterior pole. Amsler sign is the presence of blood in the anterior chamber in patients with Fuchs heterochromic cyclitis, following rapid pressure change after anterior chamber paracentesis.

Postoperatively, the Uveitis Glaucoma Hyphema syndrome may occur after cataract surgery, caused by mechanic trauma of the iris by the IOL, more common in anterior chamber IOL.[5]

Spontaneous hyphema may occur secondary to iris neovascularization (proliferative diabetic retinopathy, ocular ischemic syndrome, retinal vein occlusion, chronic choroidal detachment), chronic uveitis (herpes zoster), tumors (uveal melanoma, retinoblastoma, lymphoma, leukemia, metastasis etc.), drugs (both anticoagulant and antiplatelet agents), blood dyscrasias (hemophilia, thrombophilia, von Willebrand disease, immune thrombocytopenic purpura).[6,7]

Figure 2: Vossius ring [11]

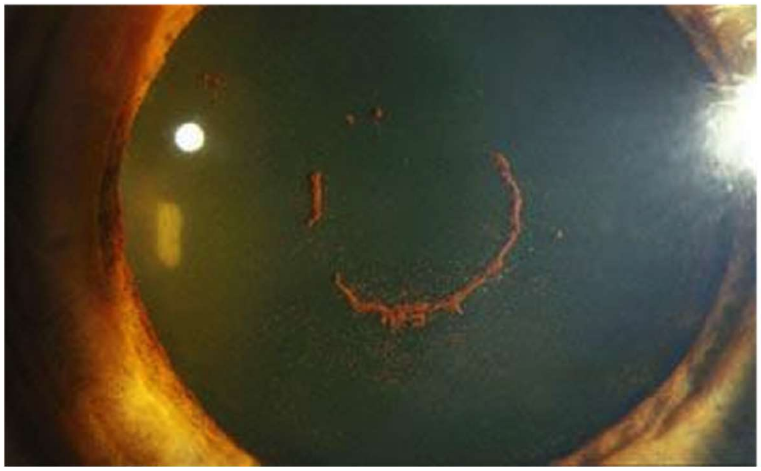

COMPLICATIONS

Obstruction of the trabecular meshwork with increased intraocular pressure is one of the complications that can result from untreated or improperly treated hyphema. This occurs in about 1/3 of hyphema cases. Studies have shown that 25% of patients develop intraocular pressure >25 mmHg and 10% of patients develop intraocular pressure > 10 mmHg. Usually, patients with a larger volume of blood in the anterior chamber have an increased risk in developing elevated intraocular pressure. Another risk factor would be the appearance of the recurrent bleeding. Intraocular pressure should be monitored daily. An untreated intraocular pressure > 35 mmHg for more than 5-7 days can lead to irreversible optic nerve damage.[8]

Recurrent bleeding or secondary hemorrhage (Figure 3) is another complication that can occur. It is described as increase of blood volume or appearance of a new layer of fresh blood in the anterior chamber. Most commonly occurs 3-5 days after the initial injury as a result of blood clot lysis. Recurrent bleeding increase the risk of other complications such as: increased intraocular pressure, corneal blood staining, optic atrophy, and peripheral anterior synechia. This complication is often associated with a weak prognosis or need for surgery.[9]

Figure 3: Recurrent bleeding [12]

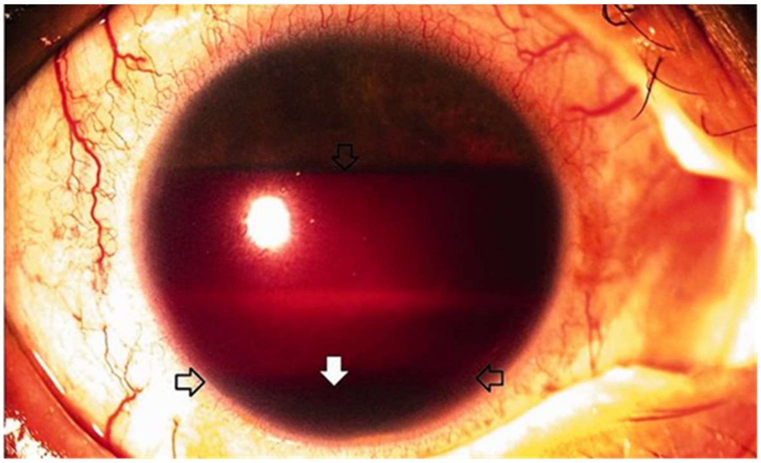

Glaucoma followed by optic atrophy occurs in 10-20 % of patients with traumatic hyphema due to trabecular meshwork damage or due to peripheral anterior synechia which leads to secondary open angle glaucoma.

Another complication that can occur in an untreated hyphema is synechiae formation (Figure 4), both posterior synechia and peripheral anterior synechia. Peripheral anterior synechia are more common and occur after inflammation or persistent blood clot. Patients with hyphema dating more than 7 days have a higher risk of developing synechiae.[10]

Figure 4: Peripheral Anterior Synechiae [13]

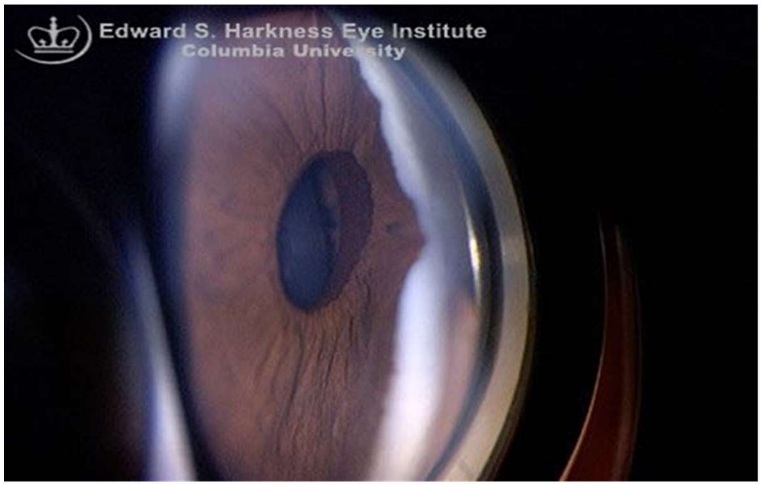

Corneal blood staining (Figure 5) is the corneal impregnation with intra and extracellular hemoglobin and hemosiderin particles. This occurs in 2-11% of cases, particularly in cases of total hyphemas, increased intraocular pressure, recurrent bleeding, long persistent of a blood clot, corneal endothelial cell dysfunction. The first sign of corneal damage is the presence of fine, deep, yellow line in the stroma, especially central, followed by extension in 24 -36 hours. After the resolution of hyphema, cornea gradually clarify, in months or even years, from the periphery to the center.[15]

Figure 5: Corneal blood staining [14]

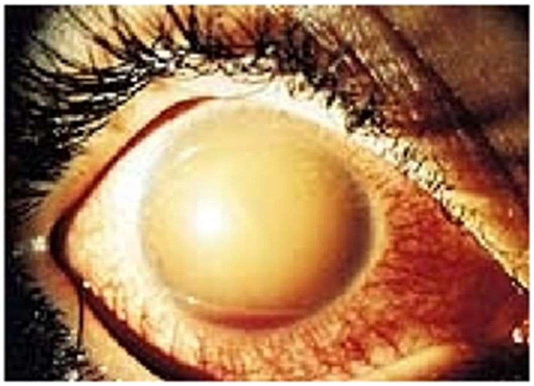

MANAGEMENT

The goals of the treatment are:

- clearing the hyphema
- reducing the bleeding rate
- treating the associated tissue lesions
- minimizing the long-term sequelae

Hyphema, unaccompanied by any complications, should be treated conservatively with a protective patch, limited activity, elevate the head of the bed at an angle of 30-45 degrees, so that the hyphema can settle out inferiorly and avoid obstruction of vision and limit the exposure of cornea to blood. The patient should be closely monitored during the first days when the risk of bleeding recurrence and increased intraocular pressure is higher.

Hospitalization is recommended in cases of noncompliant patients, patients with blood dyscrasias, with severe ocular and orbital damage, and patient with an increased intraocular pressure and sickle cell anemia.[16]

The medical treatment consists of administration of topical corticosteroids or, in severe cases, systemic corticosteroids to decrease inflammation associated with hyphema, topical cycloplegics (atropine 1% for 5 days: 1 drop/day in children, 1 drop x 3/ day in adults) to stabilize the blood-aqueous barrier, to decrease pain by relaxing the ciliary spasm and prevent the formation of posterior synechiae, topical aqueous suppressants (beta-blockers and alpha-agonists) or systemic carbonic anhydrase inhibitors and hyperosmotic agents (acetazolamide or mannitol – 1,5 mg/body weight) if topical management fails to control the pressure, antifibrinolytic agents (aminocaproic acid – 50-100 mg/body weight every 4 hours, up to a maximum of 30 g/day, for 5 days, tranexamic acid) to reduce the risk of recurrent bleeding.[4,15,17]

Surgical intervention is required in approximately 5% of cases with the following indications: IOP elevation > 50 mmHg for 5 days, IOP elevation > 35 mmHg for 7 days to avoid optic nerve damage, IOP elevation > 25 mmHg for 5 days in cases of total hyphema to prevent corneal blood staining, IOP elevation > 25 mmHg for more than 24 hours at patients with sickle cell anemia, large stagnant clots persisting for more than 10 days to prevent peripheral anterior synechia formation.[16]

Surgical techniques are paracentesis/ AC washout for liquid blood – is the safest and simplest method able to evacuate circulating red blood cells, can be repeated, avoid affecting the conjunctiva in case of a possible future filtration surgery, provide control of intraoperative bleeding and fast reduction of intraocular pressure, and bimanual cutting/aspiration for clotted hyphema, using the vitrectomy probe. Other surgical interventions that may become necessary include: peripheral iridectomy and trabeculectomy for glaucoma, cyclodiathermy and ultrasonic emulsification and aspiration.[10]

PROGNOSIS

Prognosis for visual recovery is related to the following factors: amount of associated damage to other ocular structures (choroidal rupture, retinal detachment, posttraumatic macular edema, lens opacification, vitreous hemorrhage, and angle-recession glaucoma), whether secondary hemorrhage or complications such as glaucoma, corneal blood staining or optic atrophy occur.

Studies have demonstrated that more than 75% of patients have a good prognosis, so approximately 80% of patient with hyphema <1/3 AC regain visual acuity of 20/40 (6/12) or better, 60% of patient with hyphema >1/2 AC but less than total of AC regain visual acuity of 20/40 (6/12) or better, while only 35% of those with total hyphema have good visual results. Poor visual outcome in traumatic hyphema can be directly attributed to the hyphema in 11% of patients; the poor visual outcome is usually the result of secondary hemorrhage associated with optic atrophy or corneal bloodstaining.[10,16]

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VARIA

Immunopathogenesis and treatment of ulcerative colitis: from modern medicine to herbal medicines

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Abstract: *Ulcerative colitis is one of the idiopathic inflammatory diseases of the large intestine with an unknown cause, which occurs in recurrent attacks of inflammation in the lining of the mucosa and below the intestinal mucosa. Impaired mucosal immune responses, the involvement of inflammatory cytokines such as IL-1, TNF-α, and IL-6, and other inflammatory mediators such as arachidonic acid metabolites play an important role in the immunopathogenesis of ulcerative colitis. Based on medical findings on the pathogenesis of the disease, new therapies have focused on the responses of the intestinal immune system and the inflammatory process. However, the widespread use of treatments such as aminosalicylates and corticosteroids continues. Most of these drugs have severe side effects. Some patients do not respond well to these treatments and sometimes have to undergo surgery and colectomy. Therefore, extensive studies are being conducted to control and prevent disease to use natural treatment methods such as the use of antioxidants with the approach of removing free radicals that prevent the destruction of cells and tissues in the body. Today, various studies have pointed to the effects of herbal medicines as a complementary treatment in inflammatory diseases. Their low cost and high effectiveness have attracted the attention of researchers in this field. The aim of the present study is a comprehensive review of modern and traditional treatments based on herbal medicines in the field of ulcerative colitis.*

Keywords: *inflammatory bowel disease, ulcerative colitis, immunopathogenesis, modern medicine, herbal medicine*

INTRODUCTION

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) characterized by uncontrolled inflammation of the large intestine and rectum. The prevalence of UC has increased in the last two decades [1]. Due to the unknown causes of the disease, the high risk of recurrence, and the poor prognosis, UC has become a clinical challenge in terms of treatment. One of the prominent features of this disease is excretion in the form of bloody diarrhea. The disease is characterized by periods of recovery and relapse that may occur spontaneously or in response to treatment [2]. However, conventional therapies for UC treatment, in addition to being successful and preventing recurrence, may lead to a variety of side effects [3]. UC is characterized by innate immune responses. Neutrophils, the first line of innate immune cells, are responsible for intestinal tissue damage by releasing large amounts of toxic components and free radicals during stimulation during UC progression [4, 5]. Meanwhile, the response of type 2 T (Th) helper cells, including over-activity of normal T cells and non-classical killer cells and Th2 cells, and significant production of cytokines, e.g. IL-5 and IL-3, have been reported in the pathogenesis of UC. Higher cytokine levels have been reported in patients with UC, including IL-5, IL-13, and other

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proinflammatory cytokines such as tumor necrosis factor (TNF) (Figure 1).

Figure 1: Cytokines involved in ulcerative colitis.

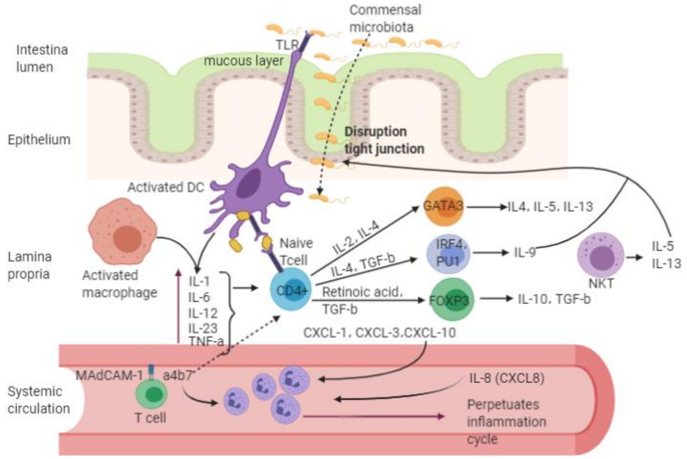

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After the release of immune cells, cytokines act to stimulate immune responses further, induce epithelial cell apoptosis, and regulate expression (claudin-2), here as damage to the epithelial barrier leading to disruption of the epithelial barrier. They become tightly connected to the intestinal epithelial cells [6-8]. Nuclear factor-kappa B (NF-κB) is a transcription factor responsible for the expression of a variety of genres, for example, TNF-α, in response to extracellular inflammatory stimuli [9]. Because high levels of TNF have been reported in the blood [10], fecal samples [11], and mucosa [12] of patients with active UC, it is widely accepted that NF-κB plays a pivotal role in UC progression. The association of NF-κB inhibition in IBD has been further demonstrated by the experimental treatment of colitis with an anti-NF-κB oligonucleotide that improves inflammation in the colon [13]. Infiltration of neutrophils and macrophages into the mucosal tissue of the colon is an important feature of ulcerative colitis. Activated neutrophils in the intestinal mucosa produce and secrete reactive oxygen species, including superoxide ions, hydroxide radicals, and hydrogen peroxide [14]. These factors cause lipid peroxidation, increase the permeability of mucosa and blood vessels, increase the entry of neutrophils into the mucosal tissue, and the development of inflammation [15]. Reactive oxygen species damage the intestinal wall, cause ulceration, bleeding, and diarrhea by affecting cytokine genes and enzymes involved in the inflammatory response. Oxidative stress also reduces antioxidant factors as a result of increasing disease severity [16, 17]. On the other hand, patients with ulcerative colitis are at risk for colon cancer for two reasons: lack of inflammation control and drugs such as anti-tumor necrosis alpha. The risk of cancer in these patients begins to increase after 8-10 years and increases by half to one percent per year [18-21]. For this reason, research is ongoing to find effective treatment with few side effects. The use of antioxidants can be effective in controlling the disease and preventing its recurrence. Antioxidant compounds can prevent cell damage by neutralizing free radicals [22]. Herbal medicine has been used for centuries as one of the most traditional medicine methods and clinical treatment of many diseases such as infections and functional disorders. Recently, the potential effect of herbs has been suggested as a UC treatment. But drugs such as aminosalicylates, corticosteroids, and immunosuppressants continue to be widely used. Amino salicylates are administered orally or topically (suppository and enema) and are safe and effective drugs and are always one of the first treatment options in the treatment of patients with mild to moderate forms of the disease and also for persistence. They are used to relieve symptoms. Corticosteroids are one of the most common therapy inactive diseases. These drugs can be given topically, orally, or intravenously. They have a great effect on active disease control but are not suitable for reducing symptoms in the long run (or maintenance treatment). Immune-suppressing drugs or immunosuppressants are also used to treat colitis. These drugs reduce inflammation but do so by suppressing the immune system response (which initiates inflammation) [23]. However, there is a constant effort to find better drugs. The aim of this study is a comprehensive review of modern and traditional therapies based on medicinal plants in the field of ulcerative colitis.

DISCUSSIONS

Modern medicine in the treatment of ulcerative colitis

As mentioned, the etiology of inflammatory bowel disease is unknown. Still, the influence of genetic predisposition, environmental factors, and immune system on each other has been evaluated, and the use of corticosteroid anti-inflammatory drugs, aminosalicylates, antibiotics, immunomodulators, and biological materials. They are effective methods in the treatment of ulcerative colitis [24]. Most of these drugs have severe side effects, including diarrhea, vomiting, fever, rash, growth retardation, anemia, and osteopenia, increased risk of infection, liver disorders, kidney disorders, pancreatitis, and gastrointestinal ulcers. Also, besides not all patients respond ideally to these therapies, and sometimes there is no choice but surgery and colectomy [25]. Prevention of colectomy, prevention of disease complications and death; and controlling inflammation with the least side effects of drugs are some of the important goals in treating ulcerative colitis [26]. In this part of the article, we describe modern medicine in the control and treatment of ulcerative colitis.

Amino salicylates

Amino salicylates have always been one of the first drug choices in treating patients with mild to moderate disease activity.

Sulfasalazine

Sulfasalazine is a combination of an antibacterial agent (sulfapyridine) and a salicylate (5-aminosalicylic acid). It was originally designed to treat rheumatoid arthritis and was first introduced in 1942 as a drug to treat inflammatory bowel disease (IBD). The effective therapeutic agent in treating IBD is 5-aminosalicylic acid and sulfapyridine is the only inactive carrier that allows this effective substance to be released in the colon. Despite the relative absorption of this drug in the small intestine, approximately 90% of the drug reaches the large intestine. After absorption in the large intestine, 20% of 5-aminosalicylic acid undergoes hepatic acetylation and is excreted in the urine. Approximately 15% of patients treated with sulfasalazine experience significant side effects that necessitate discontinuation of the drug [27-29].

Pentasa

One of the most common products is mesalazine, which contains micro granules coated with ethyl cellulose. The release of mesalazine in this product starts from the duodenum it continues throughout the small intestine and colon so that 50% of the 5-aminosalicylic acid of this product is released in the small intestine [28, 30]. Pentasa is a type of mesalazine released at the end of the ileum and colon, in a pH-dependent manner. The release of 5-aminosalicylic acid in this product, which occurs in the small intestine (release at pH above 7), is about 15-30% [28-32].

Closal

Used to treat active disease, it contains the 5-aminosalicylic acid molecule, which is attached to a benzoic acid derivative by azo. In the colon environment, 5-aminosalicylic acid is released by the bacterial enzyme reaction. Like sulfasalazine, almost the entire drug is transported intact to the colon, and its metabolites are rapidly excreted in the urine [33, 34].

Corticosteroids

Corticosteroids are one of the most common therapy inactive diseases. Prednisolone at a daily dose of 40 to 60 mg effectively treats severe to moderate disease the use of such systemic steroids should be limited to severe and refractory cases. Studies have shown that long-term use of corticosteroids does not affect the treatment of this disease. Although no study has directly compared the therapeutic effect of oral corticosteroids with injectable therapy, the injectable form is commonly used in more severe cases. Importantly, corticosteroids are not effective in maintaining improvement in ulcerative colitis, in addition to the side effects associated with corticosteroid use [35, 36].

Budesonide

Initially used to treat asthma and allergic rhinitis, it is structurally different from prednisolone. This structural difference has resulted in more local anti-inflammatory activity and a higher affinity for receptors than prednisolone. Budesonide has a metabolism of about 90% in the first hepatic passage. This low systemic bioavailability reduces the toxicity of the drug. The effect of oral budesonide in treating endoscopic findings similar to prednisolone has been reported [37, 38].

Antibiotics

Bacterial flora plays an important role in the pathogenesis of inflammatory bowel disease. In the case of infectious complications of inflammatory bowel disease, antibiotics have a therapeutic role in cases where the disease is active. But unlike Crohn's disease, these drugs are not very effective in treating an active disease or maintaining recovery in patients with ulcerative colitis [39, 40].

Immune-regulating drugs

The use of these drugs in the clinic for patients with inflammatory bowel disease is mainly due to the following reasons: 1 - Chronic active disease; 2 - Steroid-dependent disease; 3 - Long-term maintenance of disease recovery after treatment with biological agents; 4 - Prevention of the immune response to biological agents. Patients treated episodically with infliximab show a greater immune response to these agents, and concomitant use of immunomodulatory drugs reduces the immune response to Infliximab [41, 42].

Methotrexate

It is a folic acid antagonist that has anti-metabolic and anti-inflammatory activity. It is used in patients with Crohn's disease. Intramuscular injection of MTX at a weekly dose of 25 mg for 16 weeks is effective in patients with active Crohn's disease. A controlled study reported that a low oral dose of MTX of 12.5 mg per week was not effective in treating active ulcerative colitis. However, no controlled studies have been performed on the role of subcutaneous or intramuscular MTX in the active disease of ulcerative colitis [43, 44].

Cyclosporine A

This drug has been considered due to its immunomodulatory properties and therapeutic effect in patients with inflammatory bowel disease. Cyclosporine A may be appropriate in severe, fulminant, as well as steroid-resistant cases. For example, continuous infusion of cyclosporine can be effective in patients with severe colitis who have not responded to steroid therapy after 7 to 10 days. In one study, 82% of patients with active disease who did not respond to steroid therapy responded to intravenous cyclosporine-4 therapy. After six months of follow-up, 44% of respondents eventually underwent colectomy despite continuing maintenance treatment with oral CYA (45, 40). It seems that immunomodulators' use is effective in avoiding colectomy after induction of recovery by cyclosporine [45].

Immunosuppressive

Immunosuppressive drugs are commonly used when treatment with aminosalicylates and steroids is not effective or when steroids cannot be stopped to reduce inflammation without reactivating the symptoms and relapse. If the patient is dependent on treatment with steroid drugs, this method can be considered to receive immunosuppressive drugs, and then, slowly reduce or even stop using steroids without recurrence of inflammation. Slowly, for this reason, immunosuppressive drugs are sometimes called "steroid-sparing" drugs. In this group, azathioprine (Imuran, 2 to 2.5 mg/kg per day) and its active metabolite (6-mercaptopurine 6-MP); (1 to 1.5 mg/kg per day) as well as methotrexate and cyclosporine. Azathioprine and 6-mercaptopurine are effective treatments for persistent remission in both Crohn's disease and ulcerative colitis. Although their onset is slow (months), they are generally uncomplicated and well-tolerated. Other treatment regimens include intramuscular methotrexate to induce remission (15-25 mg weekly) and persistence remission (15-25 mg per week) in active Crohn's disease, as well as intravenous cyclosporine (2-4 mg/kg daily). Title Facilitators for severe ulcerative colitis are steroid-resistant. Due to the possibility of short-term or long-term side effects, and the need for careful follow-up, patients who need these drugs should be treated by a gastroenterologist [46].

Biological therapies

Recent advances in medical knowledge regarding the pathogenesis of inflammatory bowel disease have led to the introduction of new therapies that target specific aspects of the immune system and inflammatory processes in inflammatory bowel disease. One of these included in clinical therapy is treatment with anti-TNF-α. This cytokine drug is a key factor in the pathogenesis of inflammatory bowel disease. To date, only Infliximab (Remicade®), Certolizumab (Cimzia®), and Adalimumab have been shown to have a significant effect on inflammatory bowel disease. Recent data have shown that Infliximab is effective in inducing and maintaining recovery and the gradual reduction of steroids in patients with ulcerative colitis It should be noted that due to the increased risk of infection in patients using these drugs, examination of infectious foci such as chest mammography test before starting treatment is necessary [47-49].

Traditional medicine with a focus on herbs in the treatment of ulcerative colitis

Different types of herbs are used to control inflammation or reduce the symptoms of colitis. The herbal medicine includes a wide range of therapies within and outside the realm of modern medicine. However, the effectiveness of medicinal plants has limited evidence. Potential benefits of medicinal plants include high acceptance of these drugs by patients, good efficacy, relative safety, ease of use, and relatively low cost.

The effect of herbs on hundreds of people has been tested in ulcerative colitis. However, it seems that all over the world, herbal medicine has been widely used to treat and control the disease. In this part of the article, we will discuss some studies on medicinal plants concerning the control and treatment of ulcerative colitis.

Aloe vera

Aloe vera is a tropical plant used in traditional medicine around the world. Aloe vera has anti-inflammatory activity and has been used by some doctors for patients with UC. Aloe vera gel is widely used orally by patients with inflammatory bowel disease and is undergoing therapeutic evaluations for this disease [50]. This study aimed to investigate the effects of aloe vera in vitro on the production of active oxygen metabolites, eicosanoids, and interleukin-8, all of which can be pathogenic in inflammatory bowel disease. The antioxidant activity of aloe vera in free cell systems, radical generator, and luminescence was evaluated in a colorectal mucosal biopsy sample. The production of eicosanoids was measured by sampling and measuring interleukin eight by Caco-2 epithelial cells in the presence of Aloe vera by enzyme-linked ELISA. The study's findings showed that aloe vera gel had a dose-dependent inhibitory effect on the production of reactive oxygen metabolites. Aloe vera inhibited the production of prostaglandin E2 but so far had no effect on the production of B2 thrombosis and also reduced the release of interleukin-8 by Caco-2 cells. The results of this study show the anti-inflammatory effects of aloe vera gel in vitro and confirm that aloe vera gel can have favorable therapeutic effects in the treatment of inflammatory bowel disease [51]. In another study aimed at investigating the effect of aqueous extract of aloe vera gel on gastric acid secretion and colonic histopathology in the model of induction of acute ulcerative colitis by acetic acid enema on 32 Wistar rats (200-250 g); The animals were randomly divided into four groups: healthy group, acetic acid-induced colitis group, aloe vera treatment group (for eight days) and sulfasalazine treatment group. After ether anesthesia, the animals were induced with acute colitis using acetic acid. After treatment, colon biopsies were removed from the distal 10 cm section for histopathological studies. The scoring system (Bita 2012) was used to compare histopathological features quantitatively. Stomach acid concentration was measured by the titration method. The results were considered as the mean and standard deviation of expression, and $P < 0.05$ was significant. The results showed that treatment with an aqueous extract of aloe vera did not reduce gastric acid. Histological results showed an inflammatory response in the ulcerative colitis group. Aloe vera extract reduced the severity of histopathological symptoms of ulcerative colitis, although it was less effective than sulfasalazine. Thus, it can be concluded that according to the improving effects of this extract, it seems that this plant can be used to eliminate colon disorders. However, further studies are needed to determine the effective dose of this plant in reducing gastric acid secretion [52].

Jujube

Jujube (Ziziphus Vulgaris Lam) has been known as a medicinal plant in East Asian countries since ancient times. It is used in traditional medicine in these areas to treat diseases such as liver disorders, anemia, shortness of breath, vomiting, and cardiovascular diseases. Hypertension and gastrointestinal disorders are used [53, 54]. Phytochemical studies on different jujube species have shown the presence of compounds such as cyclopeptide alkaloids, flavonoids, sterols, tannins, and Triterpenoid saponins, and the key role of these compounds in scavenging radicals and proven, free. Numerous effects, such as antioxidant and anti-inflammatory effects have been attributed to this plant [55]. In a study by Yue et al. (2015) on the protective effects of jujube on inflammatory bowel disease, they induced colitis by injecting TNBS (Trinitrobenzene sulfonic acid) into the rat colon and found that jujube polysaccharides They significantly reduced the severity of colitis, which was characterized by less weight loss, decreased score of disease activity index, and improved mucosal damage in rat colitis. Jujube polysaccharides also inhibit inflammatory responses and reduce tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 IL-6), and myeloperoxidase (MPO) activity in rat colitis. They also found that by examining tight junction proteins, jujube improves intestinal wall activity, thus protecting against inflammatory bowel disease by enhancing intestinal wall function [56]. In a study conducted by Karawya et al. (2016), regarding the effects of jujube on acetic acid-induced colitis, they concluded that increased levels of myeloperoxidase and caspase-3 are associated with changes in the crypts and destruction of goblet cells. By administering jujube extract, a significant decrease in myeloperoxidase and caspase three was observed compared to the control group. Jujube extract was also found to act as a treatment for acetic-acid-induced colitis in rats [57].

Turmeric

Turmeric is known by its scientific name (Curcuma longa). Curcumin is the active ingredient in the rhizome of the turmeric plant with the chemical name (diferuloylmethane). In addition to Curcumin, it contains several chemical compounds, including volatile oils, zinc, aqueous acids, and aqueous acids, oats, oats, and oranges in the yolk sac [58-60]. In traditional medicine, turmeric is known as anti-flatulence, and its consumption with food improves digestion and reduces bloating. Also, its effect on stimulating and producing bile and thus digesting fats improves digestion. Therefore, this substance in people with chronic gastrointestinal disorders and people who feel tired and bloated after eating is recommended. In laboratory studies, oral administration of aqueous and methanolic turmeric extract to rabbits significantly reduces gastric acid secretion and Ingredients of gastric juice, Oral administration of turmeric methanolic extract reduces gastric juice secretion. It prevents damage caused by histamine, indomethacin, rasping, mercaptamine, methanol, hydrochloric acid, sodium hydroxide, and sodium chloride on the stomach and duodenum [61-63]. In one study, administration of 300 mg of turmeric twice daily to patients with duodenal ulcers showed that after four weeks, about 50% of patients recovered. After 8 and 12 weeks, all patients achieved complete recovery without any side effects [64]. Also, in a study performed on 98 patients with ulcerative colitis, the effect of this herbal product at a dose of 2 grams per day in the treatment and prevention of recurrence of the disease was significantly better than placebo. In another clinical study performed on ten patients with gastric ulcers, administration of 1 gram of turmeric per day accelerated wound healing and reduced gastric pain [65].

Curcumin

In a study to determine the therapeutic effect of Curcumin on ulcerative colitis caused by sodium dextran sulfate (DSS) and discover the relevant mechanism, 60 mice were randomly divided into 6 groups. A was the normal control group, B was the model group, and group C was the 1.5 mg/kg dexamethasone group. Groups D, E, and F received 15, 30, and 60 mg/kg of Curcumin, respectively. Mice were killed seven days after treatment. The expression of TNF-α and MPO in colon tissue was determined by ELISA and the expression of P38MAPK mRNA and P.P38MAPK was evaluated by immunohistochemistry and RT-PCR. The results obtained in groups F, D, C, E, TNF-a, and MPO levels decreased significantly. P38MAPK mRNA expression was decreased in groups F, E, C, D, and there was a significant difference between E and F. As a result, Curcumin has therapeutic effects, which play an important role in the treatment of UC by inhibiting the P38MAPK pathway and thus reduce TNF-α. Because there is a risk of recurrence and cancer in UC patients and Curcumin has beneficial effects in preventing cancer, Curcumin can prevent cancer. It should also be noted that Curcumin is inexpensive and has no specific side effects, and can lead to a new generation of anticancer drugs [66].

Silymarin

Silybum is a flavonoid compound extracted from the sage plant. This plant is an annual or biennial of the chicory family distributed in Europe and some parts of the United States and Iran [67, 68]. The substance in the seed extract of this plant includes silybin A and B, silibinin, apigenin, dihydrosilibin, dioxycylcysteine, silycrestin, dioxylcydianine, etc. Dried seed extract contains 1 and 4% of silymarin, which contains flavonoids such as silybin A and B, silybinine, silycristine, and dihydroxycylin [69]. This plant has antioxidant and anti-inflammatory properties [70]. Silymarin also acts as a cell membrane stabilizer and cell glutathione enhancer, responsible for detoxification and free radical scavenging in the body. Laboratory data indicate that silymarin stimulates neutrophils and chemo toxic and phagocytic activity. It does not affect. But when neutrophils are stimulated, silymarin prevents the release of myeloperoxidase. Inoculation of neutrophils with silybin prevents the movement of leukocytes (FLMP) [71]. In a double-blind, placebo-controlled trial in patients with alcoholic cirrhosis, treatment with silymarin increased leptin secretion, which induced lymphoblast deformity. Also, the number of T8 cells was reduced, and lymphocytosis was significantly suppressed compared to the control group [72]. In a study to evaluate the effect of silymarin on acetic acid-induced colonic ulcers in mice, 32 adult, Blab/C mice, weighing approximately 36 4 4 g were divided into four groups of 8. The face was intact and without induction of disease, and the control group had an intestinal ulcer (colitis) and was not treated. The wound treatment groups were treated with 40 and 80 mg/kg silymarin. Rats in the treatment group received silymarin daily for 14 days before the onset of the disease and then for one week after the disease´s onset. The drugs were given orally. To make a wound, 1 ml of 4% acetic acid was injected intrarectally. One week after the wound was diagnosed, part of the colon was removed for histological studies, and colonic lesions were examined by the Morty method. Alkaline phosphatase concentration was measured by ELISA. The extent of tissue edema was also assessed. Based on the results, the concentration of alkaline phosphatase enzyme in the wound group showed a significant increase (P <0.05) compared to the control group. In contrast, in the silymarin groups, this increase was significant (0.05). P <0) was controlled compared to the colitis group. As a result, it can be said that the use of silymarin can be considered as a proposed method in the treatment of disease [73].

Chamomile

Chamomile, with the scientific name of Matricaria chamomilla, is an annual, fragrant plant with a height of 20 to 40 cm that goes by car on farms and roadsides. The plant is now widely distributed in Europe, West Asia, North Africa, North and South America, and Australia. Chamomile is one of the oldest medicinal plants known to man, and its use dates back to ancient Greece [74]. In to investigate the effect of aqueous this plant in the animal model of colitis induction by acetic acid, five groups of 7 adult male NMRI rats in the weight range of 280-230 g were tested. Three groups received different doses of aqueous extract of chamomile (30 mg/kg, 20, 10), and the fourth group received the solvent orally. The last group was considered the control group. To induce colitis, rats were kept on an empty stomach for 36 hours and then subjected to mild anesthesia with ether.

2 ml of 4% acetic acid was injected into the colon. Twenty-four hours later, macroscopic observations of the stool showed signs of colitis. The results showed that in this study an aqueous extract of chamomile at doses of 30 and 20 mg/kg significantly reduced the ratio of weight to colon length. Also, the highest dose of the extract (30 mg/kg) reduced the severity and extent of inflammation. Histopathological studies of the colon showed that the therapeutic effect of chamomile aqueous extract at doses of 30 mg/kg and 20, 10 is more complete with increasing dose. As a result, it can be concluded that aqueous chamomile extract is effective in the treatment of ulcerative colitis in this model and has significantly reduced inflammatory and ulcerative parameters of ulcerative colitis [75].

Ginger

Ginger, which is used as a spice in various parts of the world, is a complementary treatment for some diseases, especially gastrointestinal diseases such as nausea and vomiting, and its anti-inflammatory, antioxidant and anti-ulcer properties have already been proven. It is classified in the Emonographs Valley as a plant with its properties and drug interactions [76]. A study aimed at investigating the effect of ginger as an anti-inflammatory and antioxidant food on quality of life, disease activity and serum levels of some inflammatory factors and oxidative stress in patients with mild to moderately active ulcerative colitis 46 patients with colitis Mild to moderate active took four capsules daily containing 500mg of dried ginger root powder or similar placebo for six weeks with meals. Use of accurate and valid questionnaires and serum levels of total antioxidant capacity (TAC), malondialdehyde (MDA), Tumor necrosis factor α (TNF-α), high sensitivity C-reactive protein, and nuclear factor kappa B (NF-kB) were evaluated before and after the intervention. The results showed that ginger consumption reduced the level of serum MDA factor in the experimental group compared to the placebo group ($P = 0.04$). Also, Besides TNF-α and disease activity in the ginger group decreased after six weeks of intervention compared to the beginning of the study. There was an increase in urinary incontinence from 1% to 3% ($P > 0/05$). It was concluded that consumption of 2 g of dried ginger root powder for six weeks reduces oxidative stress in patients with mild to moderately active ulcerative colitis [77].

Turpentine (Pistacia atlantica)

One of the plants used in cancer treatment prescriptions is turpentine. It is known as Bane and grows in the cities of central and western Iran. The scientific name is turpentine (Pistacia atlantica) and from the family Pistachio or Anacardiaceous [78, 79]. This study aimed to evaluate the therapeutic and protective effects of turpentine oleoresin in acetic acid-induced colitis in rats. Turpentine oleoresin dissolved in twine 80 (1% v/v) at doses of 100, 200, and 400 mg Kg was administered orally to animals. In this treatment, three days after induction of colitis, mice were treated for six days. After determining the most effective dose in the treatment, in this method, the animals were treated for six days with the optimal dose of gum. Finally, for microscopic and macroscopic examinations, the animals were anesthetized with ether, and the colon was removed. Based on the results obtained in turpentine oleoresin treatment in a dose-dependent manner, it showed significant effects in inducing recovery in acetic acid-induced colitis in the rats ($P < 0.05$). But in the protective method, no significant difference was observed between the treatment and negative control groups ($P > 0.05$). The results of this study showed the beneficial anti-inflammatory and wound healing properties of turpentine oleoresin in the treatment of acetic acid-induced colitis in rats [80].

Frankincense (Boswell serrate)

Frankincense extract has been used for many years to treat arthritis, heal wounds and strengthen the female hormonal system. According to previous studies, frankincense can positively affect brain development and possibly the formation of dendrites and axons and improve the relationship between them [81]. Frankincense has been used as an anti-inflammatory, anti-arthritis؛ analgesic in treating diseases related to chronic intestinal diseases, asthma, cerebral edema, and other diseases. Several clinical studies have confirmed their biological activity, and studies that have specifically investigated the mechanism of action of this plant have confirmed their anti-inflammatory and anti-tumor activity. Bosolic-acids are considered the main active ingredient in frankincense and are responsible for its therapeutic effects. In a study aimed at investigating the therapeutic effects of frankincense, based on the results of studies reviewed in this article, frankincense with anti-inflammatory, anti-arthritis, antimicrobial, and analgesic effects and with the least side effects in traditional and modern medicine in the recommended therapeutic doses can be effective in treating many diseases [74]. In vitro, animal studies and models have shown that boswellic acid can inhibit selective 5-lipoxygenase with the effects of inflammation and arthritis [83]. Studies show that Bosolicacid derivatives have strong anti-inflammatory properties and do not have the known side effects of steroids. Among these derivatives, AKBA and KBA are the strongest inhibitors of the 5-LOX enzyme with IC IC502.8 and M1.5 In different clinical studies, the efficacy of frankincense extract at a dose of 300 mg 3 times a day has been comparable with sulfasalazine and mesalazine to treat Crohn's disease and colitis [84].In a study of 30 patients with chronic UC, 20 patients were given 900 mg of frankincense gum daily in 3 doses for six weeks, and ten patients were given 3 grams of sulfasalazine daily in 3 doses for six weeks. This study showed that frankincense has beneficial effects on the disease because 14 patients out of 20 patients were directed to recovery and 18 patients out of 20 patients showed improvement in one or more parameters. In the sulfasalazine group, 4 out of 10 patients showed improvement, and 6 out of 10 patients showed improvement in one or more parameters [85]. In animal models, studies have shown that frankincense is effective in the treatment of inflammatory bowel diseases, including colitis and Crohn's disease [86].

Licorice

Licorice (Glycyrrhiza glabra L) is a perennial herb [87]. Licorice root contains various compounds such as flavonoids, sterols, amino acids, starches, essential oils, and saponins. Its major saponin is glycerin or glycyrrhizin [88, 89]. In addition to the above compounds, licorice root contains other compounds such as beta-glucuronosyl-glucuronide, asparagine, goose, sucrose, resin, volatile oils, flavonoid compounds as well as Coumarin compounds [90, 91].

One of the most well-known therapeutic properties of licorice rhizome has been its use in gastric ulcers [92], and its flavonoids are anti-Helicobacter pylori [93]. Licorice derivatives increase the prostaglandins concentration, inhibit gastrin secretion, and increase the lifespan of gastric epithelial cells, thereby healing gastric ulcers [94]. Some studies indicate the effects of some compounds of licorice extract on the gastrointestinal tract.

Evidence has also reported that glycerin in licorice can improve intestinal mucosal inflammation in mice by reducing the expression of NF-κB, TNF-α, and ICAM-1 in inflamed mucosa [95]. Clinical studies on licorice combined with other herbs have shown that licorice can be effective in controlling UC [96]. The anti-estrogenic function of glycerin has been proven to be high in concentration. Glycerin binds to estrogen receptors, thus acting as an antagonist for estrogen. However, estrogenic activity has also been reported for licorice and attributed to its isoflavone compounds [97]. Studies show that glycyrrhizin may inhibit the effect of mineralocorticoids by acting on 11b-hydroxysteroid dehydrogenase. Evidence has shown that glycerin can also suppress plasma renin activity and aldosterone secretion. Also, besides studies have shown that licorice has chemoprotective effects through its effect on Bcl-2 /Bax and is also effective in inhibiting cancer [98, 99].

Ulmus fulva

Ulmus fulva is a plant that can naturally prevent inflammation and help heal damaged and injured gastrointestinal tissue. It also helps to facilitate excretion naturally. Ulmus fulva extract has a beneficial role in maintaining the health of the gastrointestinal tract, especially the stomach. It should also be noted that this plant plays an important role in eliminating nutritional deficiencies and electrolyte imbalances in the body. This effective herbal substance can help control possible anemia by stopping bleeding. This herbal substance is a supplement that is made from the bark of the Ulmus fulva tree and is in powder form. This plant has long been used by Native Americans to treat coughs, diarrhea, and other gastrointestinal ailments. A recent study on slippery elm as a supplement for the treatment of IBD confirmed the antioxidant effects of slippery elm for use in patients with IBD. Research so far has been promising, but the available evidence is insufficient for the widespread use of slippery elm in the treatment of IBD [100,101].

Triticum aestivum

Triticum aestivum is used to treat various gastrointestinal problems. In a study in which Triticum aestivum was used for one month in patients with UC with conventional colitis medications, clinical outcomes improved in 78% of patients. Those who received a placebo improved 30% of clinical outcomes. The dose of Triticum aestivum is initially increased to 20 ml per day and 20 ml per day to a maximum of 100 ml per day. Triticum autism appears to be effective and safe as an adjunct therapy in the treatment of UC [102].

Bromelain

One of these supplements has long been used as a wound healer in traditional medicine, one of which is pineapple [103]. Pineapple contains phytonutrients, vitamin C and Bromelain. Bromelain is derived from the root of the pineapple fruit, and according to laboratory and clinical studies, several properties have been mentioned for it [104, 105]. Human and animal studies demonstrate the beneficial effects of Bromelain in reducing pain, wound healing and burn debridement, as well as its anti-inflammatory, anti-edematous, anticoagulant and cumulative platelet inhibitory properties. The major pharmacological effects of Bromelain are related to its proteolytic properties [104-106]. Studies have shown that oral Bromelain decreases dose-dependently levels of bradykinin, plasmacins, prostaglandin E2, and thromboxane B2 in patients with inflammation [105, 107]. Bromelain, Bromelain selectively inhibits thromboxane and changes the ratio of thromboxane to prostacyclin in favor of anti-inflammatory prostacyclin [108]. Also, besides it has been shown that Bromelain stimulates leukocytes to increase interleukin-1, 6, 8 levels and alpha tumor necrosis factor in the inflammatory region. Increased cytokines, especially interleukin 8, lead to the uptake of granulocytes and monocytes into the inflammatory region and increase phagocytic and chemotactic, chemotaxis activity [104, 105, 109, 110]. The anti-edematous properties of Bromelain are due to increased vascular permeability following fibrinolysis and increased circulating edema fluid reabsorption. Fibrinolysis activity of bromelain has been attributed to increased conversion of plasminogen to plasmin. Plasmin also breaks down fibrin, thereby increasing vascular permeability [106]. Other studies have shown that nutritional supplements, including Bromelain, trypsin, and papain, are effective in wound healing [103]. Other experimental results also show that the combination of bromelain and trypsin is effective in wound healing and reduction of edema and inflammation in those who have undergone major abdominal laparotomy [111]. Bromelain has anti-inflammatory properties and is used as a facilitator of gastrointestinal excretion and blood thinner. It is also used to treat sports injuries, sinusitis, osteoarthritis, and swelling from bromelain. Bromelain has been studied for use as an adjunct in the treatment of IBD, especially treating ulcerative colitis. Emerging research on pineapple shows that bromelain is an active ingredient in pineapple that may help treat UC by relieving inflammation. The mechanism that is primarily responsible for its anti-inflammatory effects is still unknown. However, proteolytic activity has been identified for the anti-inflammatory effects of bromelain on T cell activation and cytokine secretion in vitro and mouse models of inflammatory diseases [112, 113]. The major anti-inflammatory mechanism of action of bromelain appears to be proteolytic activity. However, there is also evidence of hormonal activity, such as acting through the intracellular signaling pathway. Bromelain has been reported to help reduce cell surface receptors, such as the hyaluronic acid receptor CD4, which is involved in leukocyte migration and the induction of inflammatory mediators [114, 115]. Also, besides bromelain has been reported to significantly reduce CD4 T cells, which in animal models are the primary inflammatory factors in the gut [116]. In a study performed on a clinical trial of bromelain in the treatment of mild ulcerative colitis, clinical and endoscopic evidence showed improvement [117].

Qing Dai

Qing Dai herbal medicine is traditionally used in Chinese medicine to treat patients with UC, but there is a lack of published information about the effects of Qing Dai in the treatment of UC. In a study of the mechanisms of action of Qingdai, nine patients with active UC who received common medications but were willing to take Chinghai as an alternative drug were studied. UC severity was determined based on the clinical activity index (CAI). Also, besides 5 out of 9 patients were evaluated endoscopically based on the Mats grading system. Each patient received 2 grams daily of Qing Dai orally and continued with other UC medications as prescribed. Electron rotation resonance was used to discover the working mechanisms of Qingdao. After four months of treatment with Qingdai, the CAI score decreased. Similarly, the degree of endoscopic mats decreased. Six of the seven patients treated with prednisolone at the time of enrollment were able to discontinue the corticosteroids. Electron rotation resonance showed that Qingdai has a strong hydroxyl radical scavenging activity. Qingdai showed significant clinical and endoscopic efficacy in patients who failed to respond to conventional medications. Modification of the activity of hydroxyl radicals seems to be a potential mechanism by which this drug acts. Still, the final decision on the ability to produce therapeutic effects of Qingdai in the treatment of colitis is due to the anti-inflammatory effect of this drug in the treatment of UC patients has shown that further research is needed on the therapeutic effects of this herbal medicine [118].

Tea grass

Tea grass (Hypericum perforatum), a perennial plant with yellow flowers belonging to the Clusiaceae family, is used to treat anxiety, depression, ulcers, and as an antioxidant, analgesic, and nervous system protector [119]. This plant has antibacterial and antiviral effects [120], which acts by inhibiting the transcription factor NF-Kb [121] and with serine/threonine kinase) which belongs to the protein kinase C family (PKC) [122]. Tea grass has a significant effect on wound healing and an anti-inflammatory effect [123]. The beneficial effect of tea grass on the healing of oral mucosa in the golden hamster has been investigated [119]. In a study, the effects of tea grass on histopathological changes and Malondialdehyde (MDA) levels obtained from colonic tissue of rats with colitis were evaluated. Seventy male Sprague Dawley rats were divided into seven equal groups. Colitis was caused by acetic acid in them. Groups 1 and 2 received one cc of oral solution at 600 and 300 mg, respectively. In groups 3 and 4, 1 cc of the gel was enema 20% and 10%, respectively. In group 5, 1 cc of Asacol was injected as a positive control group, and one cc of normal saline was injected as a negative control group in group 6. Group 7 received only the base gel via enema. Rat colon samples were evaluated for histopathological changes and tissue MDA after seven days of treatment. Based on the results, tea grass extract in both oral and enema forms had reduced therapeutic effects on the rats' tissues in which colitis had developed by reducing MDA activity. In the enema form, 20% gel showed a better effect than 10% gel. In the oral form with a dose of 600 mg/kg, a better therapeutic effect was observed than 300 mg/kg. Therefore, it can be concluded that tea grass can be used as a treatment of choice, especially in the form of enema in colitis [124].

Other treatments under consideration

As medical knowledge of the pathogenesis of inflammatory bowel disease progresses, the search for more effective treatments with fewer side effects continues. Some of these new therapies for Crohn's disease include Certolizumab and Adalimumab.

The treatments under consideration for the treatment of ulcerative colitis include peroxisome proliferator-activated receptor gamma (PPAR-anti-visilizumab, gamma) integrin therapy [32].

CONCLUSIONS

At present, medical treatment for ulcerative colitis is not entirely desirable, and none of the drugs that are now available and commonly used to treat this disease completely cure the disease. Continuous advances in the basic sciences related to ulcerative colitis provide the basis for further understanding of the cause of this disorder. Studying herbal medicines and using them in combination with other medicines used in the treatment of ulcerative colitis can open bright horizons for doctors to control this disease, which of course for the use of herbal medicines along with modern medicine. Extensive studies are needed.

We hope that these studies will lead to more specific and effective treatments for the complete cure of these diseases.

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Conflict of interest

No conflict of interest is reported by the authors.

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CLINICAL PRACTICE

Improving the outcomes for pregnancies in the context of kidney disease

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Abstract: *Renal disease in pregnancy is a rare pathology, but it raises several problems in terms of normal pregnancy evolution. Among pregnant women with renal injury, the rate of complications such as spontaneous abortion, preeclampsia, hypertension, intrauterine growth restriction, premature birth, fetal and neonatal mortality are much higher.*

For a good understanding of the pathology and a correct management of pregnant women with renal disease, it is necessary to know the normal pregnancy changes and the differentiation of chronic kidney disease from the acute injuries occurring during pregnancy.

It is useful to ensure counseling prior to conception and to perform all prophylactic measures in order to prevent pregnancy complications and subsequent decline in renal function in all cases of pregnant women with chronic renal insufficiency.

Treatment of acute renal failure in pregnancy involves therapy of the underlying condition that caused renal injury, prevention of irreversible kidney injury and pre-vention of maternal and fetal secondary complications. Monitoring carefully this cate-gory of pregnant women, although additional risks strike, often results in favorabl pregnancy evolution.

Keywords: *pregnancy, chronic kidney disease, acute renal failure, complications, preeclampsia*

INTRODUCTION

Chronic kidney disease (CKD) affects 3% of pregnant women and represents an isolated risk factor for the occurrence of complications such as preeclampsia, arterial hypertension, premature birth and intrauterine growth restriction [1]. Such complications are not the only challenges that women with kidney disorders face during pregnancy, adding the potential teratogenicity of medications and the risk for disease progression. The management of chronic kidney disease in the context of pregnancy has lately become increasingly relevant to the practicing obstetrician, women becoming pregnant at more advanced age. Challenges brought by the presence of an underlying disease in pregnancy, such as autoimmune disease or diabetes mellitus, raise the necessity of interdisciplinary medical team to ensure satisfying maternal and fetal outcomes. The presence of acute kidney injury in pregnancy is generally declining worldwide, but still remains a significant health concern in developing countries, where pregnancy might be the first time to diagnose the kidney disease or the hypertension. Acute kidney injury affects a very small number of pregnant women and in this case the renal function decreases rapidly. The etiologies of acute kidney failure are diverse and require specific treatment [2-4]. It is critical to understand and evaluate normal physiologic changes in pregnancy in order to establish the proper diagnosis. The most common causes of chronic kidney disease are diabetes mellitus and hypertension [5]. Preconception counseling is required for women known with renal insufficiency, so patients would

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understand the potential risks of pregnancy and make various changes of the treatment for kidney disease, so the therapeutic agents won't affect the pregnancy's normal course [2, 5]. There are cases in which, through serum and urinalysis investigations carried out at the beginning of the pregnancy, patients are diagnosed for the first time with chronic kidney disease [5, 6].

PHYSIOLOGICAL CHANGES OF THE RENAL FUNCTION DURING PREGNANCY

The pregnancy changes in the renal and urinary tract are both anatomical and functional [2, 7]. Anatomically, the renal volume increases up to 30 %, attributed to the expansion of kidney vascular and interstitial volume, by approximately 1-1.5 cm, predominantly at the level of the collector system. A certain degree of ureteral and renal pelvis dilation occurs, mainly secondary to progesterone-induced relaxation of the muscular layers and due to mechanical compression on the ureters themselves [2, 8]. In almost 86% of pregnant women is a right-sided preponderance of hydronephrosis, attributed to the right ureter peculiar anatomy, crossing over the iliac and ovarian vessels at an angle before entering the pelvis, while the left ureter passes at less acute angle and travels in parallel with the ovarian vein [9]. As a result, these changes should not be interpreted as hydronephrosis during an ultrasound [2, 10].

From a functional point of view, during pregnancy, blood volume increases by up to 50%, systemic vascular resistance decreases, cardiac rate increases by up to 30%, blood pressure decreases in the first two trimesters, with a compensatory increase in heart rate, and returns to the values before the pregnancy in the third trimester. [5, 11, 12]. These cardiovascular changes lead to increased renal blood flow by up to 50%, increased filtration rate up to 30%, and serum creatinine levels decrease up to 20%.

PREGNANCY AND CHRONIC KIDNEY DISEASE

Chronic kidney disease is a pathophysiological process that ultimately results in end-stage renal disease through a progressive loss of nephron number and function that persist 3 or more months [5]. It affects up to 6% of women of childbearing age and is estimated to be associated in 3% of pregnancies. Although the etiology does not significantly influence the evolution of pregnancy, we underline the most common causes: diabetic nephropathy, chronic focal glomerulonephritis, systemic lupus erythematosus, scleroderma and nodulous periarteritis, chronic pyelonephritis, reflux nephropathy, polycystic kidney disease, chronic nephrolithiasis, post-nephrectomy status and congenital unique kidney [13-17] Women with chronic kidney disease are frequently impaired to make the renal adaptations needed for a normal course of preg-nancy. These patients often show the inability to increase levels of renal hormones leading to reduced expansion of plasma volume, normochromic normocytic anaemia and vitamin D deficiency [16]. Advanced renal dysfunction with poorly controlled underlying primary renal disease, associating proteinuria, frequently causing hypertension, are all significant risks for adverse maternal, fetal and postpartum renal outcomes. These are the main reasons to plan the pregnancies, whenever is possible, thus achieving the best outcomes, allowing the opportunity to counsel the future parents in advance and to optimize these risks.

Chronic kidney disease in pregnancy is classified according to serum creatinine levels in three categories:
- Mild-serum creatinine level < 1.5 mg/dl
- Moderate – serum creatinine level 1.5-2.5 mg/dl
- Severe – Serum creatinine level > 2.5 mg/dl [18].

Another important marker of renal function in pregnancy is proteinuria/24 h, also being a diagnostic factor for preeclampsia [18].

The pregnancies in patients with mild or moderate renal disease, normotensive, generally evolve favorably, although these patients' prone higher risks for complications compared to pregnant women with normal renal function. Pregnant women with severe chronic kidney disease are more exposed to complications such as intrauterine growth restriction or premature birth, but these cases are extremely rare, the fertility rate at this stage of the disease being low [1]. Although of great importance, the kidney function is not the only predictive factor for the pregnancy outcome, changes in endothelial function and active systemic disease are more likely to be involved.

It is considered that the pregnancy accelerates more rapidly the renal dysfunction due to proteinuria and hypertension, but there are insufficient data in this matter [1], moreover, several small single centre studies suggested that the rate of progression of renal disease to end stage is unaffected by gestation, despite the known fact that many women experience a temporary decline in renal function during pregnancy, given the pathophysiological effects of hyperfiltration [19].

MATERNAL AND FETAL RISKS

Preeclampsia

The risk of developing preeclampsia for pregnant women with renal disease is significantly higher compared to the general population. In mild renal dysfunction, the risk is 20%, and in the severe one increases up to 60-80%, compared to the 5% risk in the general population [5]. The main fetal risks generated by preeclampsia are the restriction of intrauterine growth and premature birth [14]. A reduction of the risk for preeclampsia by 25% was demonstrated by daily administration during pregnancy of 150 mg of aspirin [5, 20].

Renal function

The decline in renal function rarely occurs in women with mild disease, but the risk for moderate illness is 25% in terms of definitive loss of 25% of renal function [5].

Hypertension

Once placental development is fully achieved, by 16 weeks' gestation, a 50% rise in plasma volume occurs, and in the vast majority of women blood pressure falls, due to the vasodilator response accompanied by an elevation of the cardiac output, arterial underfilling, subsequent ADH release and the stimulation of the renin-angiotensinogen system. Relaxin may also play a role in this chain of changes, because it has been linked to ADH secretion and water drinking, while levels are increased during pregnancy. In the third trimester, blood pressure starts to rise, reaching almost the pre-pregnancy values.

Among patients with renal disease, preexisting pregnancy hypertension is common, but during pregnancy it becomes much harder to control, on the one hand, due to the adaptive changes in pregnancy in the second and third trimester [5,10] and on the other hand, by the inability to use therapeutic agents of election, as inhibitors of the conversion enzyme and receptor blockers for angiotensin [5].

To reduce maternal and fetal complications it is necessary to maintain blood pressure below 140/90 mmHg, but an important decrease below 120/70 mmHg was correlated with increased risk of fetal demise [21]. Therapeutic agents that can be used in pregnancy are Methyldopa, Labetalol, Nifedipine, and Hydralazine (Table 1) [1, 5, 10, 22-24].

Table 1: Drugs used in pregnancy for women with kidney diseases, diabetes or kidney transplant

Drug	FDA	Indication	Fetal/neonatal effects	Use in pregnancy	Teratogenicity
ACE inhibitors/ARB	D	Hypertension	Cardiovascular, central nervous system defects in first trimester, FGR and neonatal anuria in second and third trimesters, fetal renal tubular dysplasia, oligohydramnios, perinatal acute renal failure, and other major congenital anomalies	Stop at conception	Yes
Beta-blockers	C	Hypertension	FGR in first trimester Atenolol may cause fetal bradycardia in the first trimester	Labetolol often used first line Atenolol can be used safely later in pregnancy	No
Ca channel antagonists – nifedipine, amlodipine	C	Hypertension	Nimodipine is prohibited	Used second line with methyldopa or labetolol	No
Hydralazine	C	Hypertension		Used for more severe hypertension Used in combination with methyldopa or beta-blockers	No
Methyl-Dopa	B	Hypertension		Often used first line	No
Biguanides – Metformin	C	Diabetes		Administered from first trimester	No
Second generation oral sulfonylurea hypoglycemic agent – Glyburide	B	Diabetes	Neonatal hypoglycemia higher when compared to insulin	Administered from first trimester Does not cross the placenta	No
Isophane (NHP) insulin	C	Diabetes	Can cause fetal macrosomia, and due to increased oxygen requirements is a risk factor for fetal death	First choice long-acting insulin The most natural agent for hyperglicemia	No
Rapid-acting insulin analogues – Aspart, Lispro	C	Diabetes		May be preferable to start pre-pregnancy	No
Longer acting insulin anlogues: Detemir,Glargine	C	Diabetes		Few studies, but encouraging results	No

Prednisolone/IV methylprednisolone	C	Kidney transplantation	Rare – except at large doses (cataract, adrenal insufficiency and infection) Increased frequency of premature rupture of membranes	maternal side effects: glucose intolerance, hypertesion, weight gain, hirsutism, acne, cushingoid features, impaired wound healing, osteoporosis, mood changes	Possible increase in oral clefts and mental retardation
Azathioprine – Imuran	D	Kidney transplantation		Maternal increased risk of infection and neoplasia.	Crosses the placenta as an inactive form. No congenital abnormalities have been observed.
Calineurin Inhibitors	C	Kidney transplantation	Transient immune alterations in the neonate (Cyclosporine) Hyperkalaemia and renal impairment (Tacrolimus)	Increasingly used - they have decreased rejection episodes and improved survival in transplant recipients.	No
Mycophenolate mofetil	D	Kidney transplantation	Increased risk of miscarriage and congenital abnormalities. Congenital malformations of the external and middle ear, cleft lip and cleft palate, and other major structural malformations involving the eye, hands and fingers, cardiac, bowel and skeletal systems.	Should be discontinued before pregnancy	Yes
Sirolimus (Rapamune)	C	Kidney transplantation	Small-for-gestational-age infants and delayed bone ossification	Immunosuppressant that could be used during pregnancy	Has not been implicated in specific fetal malformations. Further investigations are required

Proteinuria

The gold standard to diagnose proteinuria of pregnant women is the 24-hour urine collection and quantification of urine proteins level. Even so, errors in timing and urine retention in the dilated systems make urine collections incomplete, because of the large volumes contained at one time in the upper urinary tract.

The increase of the excretory level of urinary proteins is associated with important risk for intrauterine growth restriction and increased risk for renal dysfunction. [18, 20, 21].

In patients developing nephrotic syndrome during pregnancy (proteinuria > 3g/day, albuminuria > 30 mg/L and edema) there is a high risk for thromboembolic accidents and maternal death, even if blood pressure is maintained at satisfactory levels. In this category of patients, anticoagulant prophylaxis with low molecular weight heparins is recommended, up to 6 weeks postpartum. [5, 25].

Preconception Counseling

As general rule, the conception should not be discouraged as long as possible risks are understood and careful monitoring of the pregnancy is available. [1, 14, 20]. It is necessary to discontinue and substitute therapeutic agents with teratogenic potential (Table 1).

Patients with active vasculitis or lupus should be advised to wait for the episode to end prior to the conception. [5, 26].

RENAL TRANSPLANTATION AND PREGNANCY

Maternal status and fetal development in posttransplant renal patients is favorable, significantly better when compared to pregnant women with advanced renal disease requiring dialysis, especially if the following conditions are fulfilled [1, 8]:

- Renal function is stable
- Minimum one year after renal transplantation; latest recommendations ex-tend the waiting period at 2 years, thus reducing the risk for allograft failure
- Proteinuria maintained under 1g/day
- Controlled hypertension
- Minimal doses of immunosuppressants required (prednisone, tacrolimus, azathioprine, ciclosporin) (Table 1)

- No prior or pending rejection of renal transplantation [5].

Thus, women with diagnosed advanced kidney disease are often encouraged to wait until after successful kidney transplantation to obtain a pregnancy. The reason for this recommendation is that fertility is improved after transplantation and the risks for pregnancy complications, such as preterm birth and hypertension, are much lower. Pregnancy is considered a sensitizing event, which can result in the formation of anti-HLA antibodies that may impend finding a future suitable donor.

The incidence of premature childbirth, intrauterine growth restriction and preeclampsia is higher compared to the general population and 30% of pregnancies will be affected by these complications.

Vaginal childbirth is not contraindicated in case of mothers with renal transplantation. When Caesarian section is preferred, the approach of the lower segment may be difficult due to the trajectory of the transplanted ureter. [1, 5].

DIABETIC NEPHROPATHY AND PREGNANCY

Lately, as women do tend to procreate later in life, diabetic nephropathy is probably becoming the most common CKD seen in pregnancy. Pregnancy in women with diabetes type 1 or type 2 is associated with a two- to four-time increased risk of pre-term delivery, preeclampsia and perinatal mortality compared with the general population [27]. Pregnancies in women with CKD associate poor placentation and fetal growth restriction, with an incidence of small for gestational age newborns of 31% [19]. It is rather unclear if growth restriction is based on poor placentation or on fetal hyperinsulinamia, but women with more severe renal impairment are exposed to a greater risk for pregnancies with poorly grown infants, despite maternal hyperglycaemia, thus antenatal documentation of fetal growth restriction has been linked with worse fetal outcomes than macrosomia.

The first clinical sign of kidney malfunction is microalbuminuria, and urinary albumin excretion of 30–300 mg/24 h, corresponding to a spot urine albumin/creatinine ratio of 30–300 μg/mg. The progression towards diabetic nephropathy is seen in all cases left untreated, when microalbuminuria becomes persistent proteinuria, associating hypertension and an unavoidably decline in GFR [28].

Diabetic nephropathy adversely affects the course of pregnancy by the following three mechanisms: development of severe hypertension superimposed on the deterioration of kidney function in the mother, preterm delivery due to high maternal blood pressure and preeclampsia, and fetal intrauterine growth restriction and fetal distress caused by placental insufficiency.

The prepregnancy preparations in women with diabetic nephropathy or microalbuminuria would comprise intensive glycaemic control, low-dose aspirin, and intensive antihypertensive treatment. The treatment with ACE inhibitors followed by strict metabolic control for at least 6 months, usually results in low levels of albumin excretion and it has been found to be associated with a high rate of successful pregnancy outcome [27]. On the onset of pregnancy, early in the first trimester, ACE inhibitors are linked to increased risk of congenital malformations [29], as for the last part of the pregnancy, are associated with abnormal fetal renal development and neonatal renal failure [30] therefore treatment with ACE inhibitors or AngII antagonists should be stopped before conception. In the event of an unplanned pregnancy, women with diabetic nephropathy which necessitates continuous treatment with blockers of the RAS (renin angiotensin system), can benefit from a shift to other antihypertensive drugs successfully in early pregnancy. Severe congenital malformations have been linked to poor glycemic control before pregnancy, therefore the goal is strict glycemic control and HbA1c as close to normal as possible, at least <7% is recommended [31, 32].

In all women with high risk for pre-eclampsia, treatment with low-dose aspirin is proven to have some preventive effect, thus, at least theoretically, the same treatment could be of benefit in women with diabetic nephropathy [20] and is recommended in American and British guidelines.

The goal for CKD and diabetic pregnacy should be BP <135/85 mmHg and urinary albumin excretion <300 mg/24 h, obtained with antihypertensive treatment with pregnancy-friendly drugs, strict glycemic control (HbA1c <6.0%) and low-dose aspirin.

For many years, a pregnancy in women with diabetic nephropathy was related to a decline in maternal kidney function and important perinatal mortality, but recent improvements in pregnancy management substantially reduced those risks.

Cesarean delivery should be considered for every case where fetal weight is estimated for more than 4500 g. Vaginal birth at 39 weeks gestation is safe whenever blood glucose levels are well controlled and kidney function is normal. In those cases with vascular complications and poorly controlled blood glucose, delivery should be considered at 36 0/7 to 38 6/7 weeks or even earlier, depending on maternal and fetal status [33].

ACUTE RENAL INSUFFICIENCY IN PREGNANCY

Acute renal insufficiency is defined as the sudden degradation of renal function, ranging from the slight decline in renal function to the necessity of renal function replacement therapy [3, 34].

In the first part of the pregnancy, infectious pathology and hypovolemia are the most common causes of renal injury, and in the second part of the pregnancy, the most frequent etiological factors are preeclampsia, placental abruption or severe anemia.

The best management of this pathology is possible using the classification of the acute renal insufficiency by the cause, as pre-renal, renal and post-renal. Pre-renal injury occurs predominantly secondary to hypovolemia, hemorrhage, congestive heart failure, sepsis. Renal insufficiency has as main etiologies acute tubular necrosis, renal cortical necrosis, thrombotic microangiopathy, preeclampsia, acute interstitial nephritis, and glomerulonephritis. Post-renal insufficiency occurs due to ureteropelvic block, but rarely causes bilateral renal injury [3, 5].

Clinically, pregnant women with acute renal insufficiency present oliguria, de-fined as a urine production < 0.5 ml/kg/h. [3, 35]. Another marker of renal injury is the increased level of serum creatinine, which is difficult to be appreciated due to its low level in normal pregnancy. Secondary to renal injury, as a compensatory mechanism for renal hypo-filtration, an increase in systemic blood pressure and intravascular volume appears and changes of urinary electrolytic values due to the action of the system renin-angiotensin-aldosterone [36-38].

Treatment of acute kidney failure involves treating the underlying condition that caused renal injury and focuses on the restoration of urine production, avoiding secondary injury and increasing renal infusion. The effects of this pathology on the fetus are diverse, specific to each etiology, and may require delivery, as in the case of preeclamsia or placental abruption. [39, 40].

CONCLUSIONS

Although the risk of complications in pregnancy in patients with chronic kidney disease is higher than in the general population, in most cases, with the correct management of pregnancy and kidney disease, the pregnancy has a favorable evolution. There are insufficient data to certify the pregnancy as a potential factor in the decline of the renal function.

Intensive pre-pregnancy antihypertensive treatment in patients with diabetic nephropathy and good glycaemic control results in preservation of kidney function and favorable maternal and fetal outcomes.

Acute renal insufficiency, occurring during pregnancy, may benefit from specific etiological treatment, but may also require emergency delivery.

Close obstetric surveillance and screening for kidney function is important to improve pregnancy outcomes in these high-risk pregnancies.

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ORIGINAL ARTICLE

Inadequate perioperative control of modifiable risk factors in total knee arthroplasty and implications on functional results

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Abstract: *Diabetes and increased body mass index are two of the most associated comorbidities in patients undergoing total knee arthroplasty. With the number of patients undergoing knee arthroplasty increasing and with the incidence of diabetes and increased BMI, we can expect that in the coming decades the association of the two comorbidities will be frequent. An ethical issue arises when the patients who are refused for total knee arthroplasty are instructed to change the risk factors, and they fail either because of a mental problem or because of a physical problem.*

This study analyzes the effects of controlled, uncontrolled, uncomplicated and complicated diabetes on the rates of post-knee arthroplasty complications. The study proposes to evaluate obese patients in terms of functional outcomes and postoperative complications after total knee arthroplasty.

Keywords: *total knee arthroplasty, diabetes, body mass index, total knee arthroplasty complications, periprosthetic joint infection*

INTRODUCTION

Diabetes affects 8.5% of the Europe's population, and its prevalence increases with age [1]. It is one of the common comorbidities associated with patients over 65 years old undergoing total knee arthroplasty. The literature data suggest that one of 10 patients that are admitted for a total knee arthroplasty has associated diabetes, but the prevalence of diabetes is constantly increasing [2, 3]. This has important implications in the perioperative management of the total knee arthroplasty, with an increase in the number of patients with associated diabetes [4].

The risks of the association between total knee arthroplasty and diabetes are periprosthetic infections, deep vein thrombosis and stroke [5-9]. The risks are even higher for stroke and death when the diabetes is uncontrolled [10]. The risk of complications increases directly proportional to the value of glycated hemoglobin (HbA1c) [11]. This information allows the surgeon to change the risk even from the preoperative period.

Approximately 48% to 56% of patients with diabetes are developing osteoarthritis, which leads to decreased functionality, decreased physical activity and high mortality rates [12]. The current standard of treatment for knee osteoarthritis is total knee arthroplasty [13, 14, 15].

Given the increasing prevalence of both diabetes and osteoarthritis of the knee as they age, these patients are forced to coexist with these associated pathologies, but also with developed complications, which may result in the postoperative period [16, 17].

Obesity is one of the most important modifiable risk factors and at the same time is one of the most discussed in terms of increasing postoperative complications in total knee arthroplasty. Regarding the postoperative complications, there are data available that indicate the fact that an increased body mass index (BMI) will increase the risk of total knee arthroplasty, but there is no cut-off value to help

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us make the decision to perform surgery on a patient or not [18-21]. Literature data indicate that patients with high BMI benefit from a functional outcome and an improved quality of life more than normal-weight patients [22].

An ethical issue arises when the patients who are refused for total knee arthroplasty are instructed to change the risk factors, and they fail either because of a mental problem or because of a physical problem.

This study analyzes the effects of controlled, uncontrolled, uncomplicated and complicated diabetes on the rates of post-knee arthroplasty complications. The study proposes to evaluate obese patients in terms of functional outcomes and postoperative complications after total knee arthroplasty.

MATERIALS AND METHODS

The study ran over a period of three years from January 2017 until Decembre 2019 and included a number of 446 patients. The following patient data were collected: age, sex, body mass index, preoperative blood glucose, preoperative HbA1c, diabetes status (controlled/uncontrolled, complicated/uncomplicated), WOMAC score (preoperative, 3 months postoperatively and 12 months postoperatively), history of periprosthetic infection or complications of the wound.

In the statistical processing, the data were classified into quantitative, such as WOMAC score, and qualitative, respectively, sex, age range, controlled or uncontrolled diabetes. The qualitative or nominal data were represented as graphs. The graphs were of the column type (Column) and point cloud (Scatter Plot). In addition, Box Plots were used to represent the WOMAC score at different periods of time, preoperatively, at 3 months and 12 months depending on the patient's BMI.

The inclusion criteria in the study were patients with total knee arthroplasty, patients with and without diabetes and those with known BMI. In addition, the exclusion criterion from the study was the loss of the patient's records.

RESULTS

The study included a number of 446 patients, 20 of whom had periprosthetic infections.

The distribution of patients BMI shows that 173 were normal weight, 234 overweight and 39 obese. The analysis of the data shows that the mean WOMAC score was:

- at onset according to the patients' BMI was not variable. Specifically, the normal weight patients had a mean WOMAC score of 51, overweight patients had an average WOMAC score of 51, and obese patients had an average WOMAC score of 47.
- at 3 months according to the patients' BMI was for normal weight 74, overweight patients had an average WOMAC score of 69, and obese patients had an average WOMAC score of 60.
- at 12 months according to the patients' BMI was for the normal weight 88, the overweight patients had an average WOMAC score of 80, and the obese patients had an average WOMAC score of 69.

The distribution of patients with periprosthetic infection according to BMI shows that 1 normoponderal patient, 7 overweight patients and 12 obese patients presented with periprosthetic infection.

The distribution of patients with controlled and uncomplicated diabetes according to periprosthetic infections shows:

- The category of overweight patients with controlled and uncomplicated diabetes also had periprosthetic infections. The evolution of the WOMAC score for patients with controlled and uncomplicated diabetes and who presented periprosthetic infections is 38.5 preoperatively, 43 at 3 months and 53 at 12 months.
- The category of obese patients with controlled and uncomplicated diabetes also had periprosthetic infections. The evolution of the WOMAC score for patients with controlled and uncomplicated diabetes and who presented periprosthetic infections is 45 preoperatively, 50 at 3 months and 55 at 12 months.

The distribution of patients with controlled and complicated diabetes according to periprosthetic infections presents:

- The category of overweight patients with controlled and complicated diabetes did not show periprosthetic infections. The evolution of the WOMAC score for the patients with controlled and complicated diabetes and who did not have periprosthetic infections is 45 preoperatively, 66 at 3 months and 75 at 12 months.
- The category of obese patients with controlled and complicated diabetes also had periprosthetic infections.
- The evolution of the WOMAC score for the patients with controlled and complicated diabetes and who presented periprosthetic infections is 41 preoperatively, 45 at 3 months and 50 at 12 months.

The distribution of patients with uncontrolled diabetes according to periprosthetic infections shows:

- The category of overweight patients with uncontrolled diabetes also had periprosthetic infections. The evolution of the WOMAC score for the patients with uncontrolled diabetes and who presented periprosthetic infections is 38 preoperatively, 46.5 at 3 months and 50 at 12 months.

- The category of obese patients with uncontrolled diabetes also had periprosthetic infections. The evolution of the WOMAC score for the patients with uncontrolled diabetes and who presented periprosthetic infections is 42 preoperatively, 48.5 at 3 months and 57.5 at 12 months.

The ANOVA test with repeated measurements was used to investigate the evolution of the WOMAC score according to BMI. The Mauchly test showed that there are statistically significant differences both in BMI, WOMAC and in the evolution of BMIx WOMAC. Thus, for BMI, the Mauchly test had the value 0.97, p = 0.03, for WOMAC, it had the value 0.21, p = 0.001 and for WOMAC x BMI it had the value 0.04, p = 0.001.

The differences in BMI were as follows:
- Normal weight Vs. overweight (p = 0.001, 77.55 vs. 66.57);
- Overweight vs. obese (p = 0.001, 66.57 vs. 92.17);
- Normal weight Vs. obese (p = 0.001, 77.55 vs. 92.17)

The differences at the WOMAC level were as follows:
- WOMAC at baseline vs. WOMAC 3 months (p = 0.001, 68.11 vs. 80.34);
- WOMAC 3 months vs. WOMAC 12 months (p = 0.001, 80.34 vs. 87.84);
- WOMAC at baseline vs. WOMAC at 12 months (p = 0.001, 68.11 vs. 87.84).

The average ANOVA score varies significantly depending on the BMI, respectively the WOMAC score at 12 months reaches the highest point for all types of BMI (Figure 1).

Figure 1: The evolution of the WOMAC score according to patient's BMI

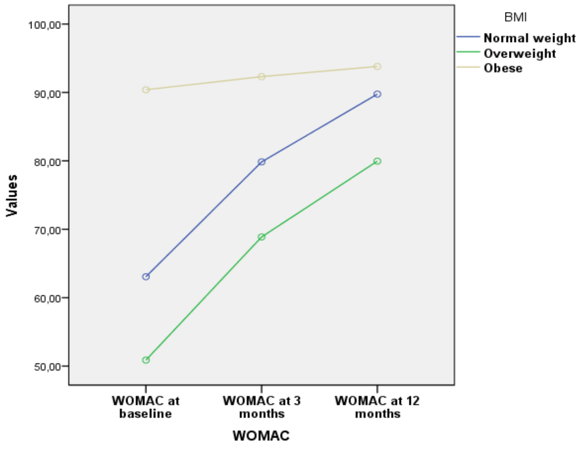

The distribution of patients' blood glucose according to BMI and WOMAC score reveals that the increased blood glucose levels at the WOMAC score time points were of obese patients (Figure 2).

Figure 2: The evolution of the WOMAC score according to patient's BMI

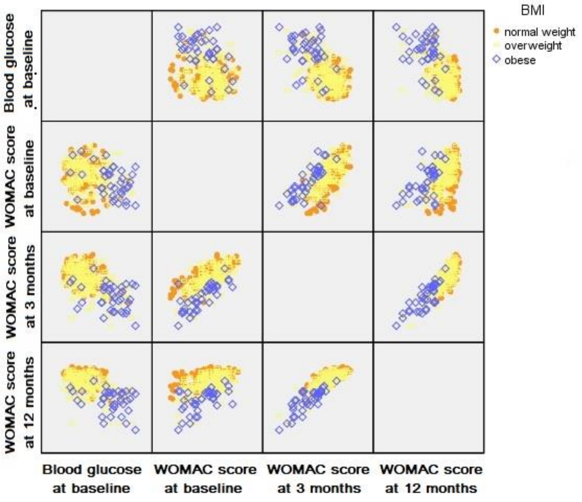

The distribution of glycated hemoglobin of patients according to BMI and WOMAC score reveals that the increased level of glycated hemoglobin at the time points of the WOMAC score were of overweight and obese patients (Figure 3).

Figure 3: The distribution of glycated hemoglobin of patients according to BMI and WOMAC score

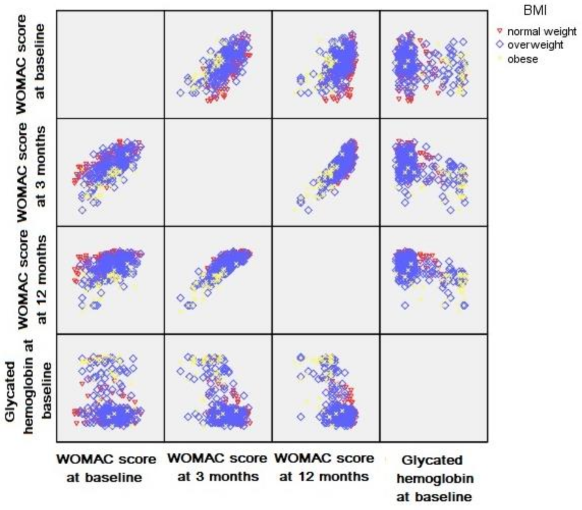

DISCUSSIONS

Perioperative risks assessment is a standard in arthroplasty procedures. Careful exposure of the risks to the patient is essential for physician-patient communication. Total knee arthroplasty is not a risk-free intervention, as risks can range from the smallest (e.g. a small wound dehiscence) to complications with major functional impact such as periprosthetic infections.

Given that the incidence of gonarthrosis increases from year to year and the incidence of diabetes reaches alarming levels, the association of the two is inevitable. Diabetes is associated with a number of important complications for the patient. When a diabetic patient is proposed for arthroplasty, the risks are much higher than in a non-diabetic, which must be fully understood by the patient. In this discussion, an increased BMI, another associated risk factor, often occurs.

Most often, these patients are advised by current orthopedic doctors to follow a hygiene and diet regimen for weight loss along with trying to control diabetes and then move on to a knee arthroplasty. When providing such indications, it should be borne in mind that increased BMI along with uncontrolled diabetes are the result of many years of negligence and any attempts to change the weight or level of glycated hemoglobin will fail in most cases. The patient will return for arthroplasty with a level of glycated hemoglobin above normal, with BMI unchanged from the last visit, but with gonarthrosis that advanced due to the passage of time, which will lead to a possible arthroplasty with a risk of complications higher than initially.

It is ideal for these modifiable risk factors to be kept under control through multiple and careful consultations by patients with their specialists in diabetes. However, it should not be overlooked that sometimes this is impossible to achieve at an optimal level. An elderly patient, with a high BMI and uncontrolled diabetes, in the best-case scenario, after an average period, they will manage a moderate control of diabetes and the absence of its complications.

Adapting surgical techniques and perioperative care along with the assumption of potential risks, both by the attending physician and by the patient in such cases, is the optimal conduct.

CONCLUSIONS

Early diagnosis and correct treatment of diabetes are essential for optimal postoperative results after total knee arthroplasty. Immediate postoperative functional outcomes are also influenced by glycemic control. Optimal methods of preoperative glycemic control are needed to maintain a high level of functional outcomes as well as to limit the complication rates.

The functional results analyzed using the WOMAC score showed low values at 3 months postoperatively for overweight and obese patients compared to normal weight, but at 12 months they showed a significant improvement in functionality. The increases scores remain important compared to the preoperative level for these patients.

Periprosthetic infections occurred mainly in overweight and obese patients, obviously associated with elevated glycemic levels and elevated glycated hemoglobin.

The WOMAC score was low in all categories of patients with periprosthetic infection regardless of diabetes status. Overweight and obese patients with periprosthetic infection had a significantly higher level of glycated hemoglobin.

It can be said that the incidence of periprosthetic infections was predominantly higher in overweight and obese patients who associated diabetes regardless of status, but the functional improvements of these patients are remarkable compared to the preoperative level.

These perioperative and modifiable risk factors are becoming more common. The obese patient, with diabetes in various forms and with gonarthrosis is a current reality of the times and cannot be ignored. The decision to operate on such a patient should not be made by highlighting the potential complications that may occur postoperatively, the potential for a possible arthroplasty to improve the patient's quality of life and functionality are in the forefront and should dictate the operability decision.

Improving surgical techniques and the use of specific perioperative care techniques specific to an obese and diabetic patient should be considered to minimize the risk of a possible periprosthetic infection.

The diabetic, overweight, or obese patient with gonarthrosis will represent in the future an important share of all gonarthrosis patients. Thus, adapting the surgical approach of these patients to reduce the rates of periprosthetic infection represent one of the main solutions that can be applied.

The attempts to modify the lifestyle and eating behavior of an overweight or obese and diabetic person often fail for their diabetologists, which will lead to an advance of gonarthrosis, resulting in an arthroplasty grafted with a much greater potential for complications.

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ORIGINAL ARTICLES

Indian experience of tetanus – A study from south India

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Abstract: *Introduction: Tetanus is an old world disease where 2 centuries ago people had realized the link between wound leading to muscle spasm and fatality, Even today there are many cases of fatalities of tetanus being reported from different parts of the country even after viability of a tetanus toxoid and Immunoglobulin injections. This one of very few recent studies done in India on tetanus, as there is very little data available on tetanus so we are trying to share our experience on tetanus, so it will help the physicians to get a better understanding.*

Materials and Methods: This retrospective study has done collecting the patients' detail from 2017- 2019, detailed case sheet review was done and the patients' clinical presentation and the prognosis were noted in predesigned format. Inclusion criteria – all diagnosed cases of tetanus, exclusion criteria – patient already received treatment from the local hospital. The patient details were kept confidential during all times.

Results: The total of 58 cases – 35 males and 23 females, The average duration of hospital stay was 15 days. The most common occupation were farmers (barefoot workers). The site of injury was the foot in 65% cases followed by injuries to the fingers or the hand in 30% and 5% cases due to injury while tooth picking with a pin, splinter removal using pins. Clinical symptoms – trismus "lockjaw" (41), difficulty in walking (2), limb pain/stiffness (17), back muscle pain/stiffness (12), dysphagia (7), 72% autonomic dysfunction. Opisthotonus position and risus sardonicus developed after 7-8 days of infection. 20% of cases were vaccinated still developed diseases. 18% mortality was noted most cases were unvaccinated cases.

Conclusion: Tetanus is preventable diseases if TT vaccination and IMMUNOGLOBIN are administered on time. In all primary health care levels, the cold chain should be maintained for vaccines. The patients should be made sensitized about the consequence of the disease process.

Keywords: *tetanus, bacterial infection*

INTRODUCTION

Tetanus is an old world disease, where 2 centuries ago people had realized the link between wound leading to muscle spasm and fatality. It was a disease that the ancient physicians of Egypt and Greece dealt with often, however, now its prevalence in developed countries has decreased significantly due to improvements in wound care and hygienic practices [1]. Arthur Nicolire isolated tetanus toxin from the soil in 1884 [2]. Even today it's a major health problem in developing countries and developed countries

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(Anuradha 2006; Ogunrin 2009) [2, 3].

In 1884 the etiology was further understood. The first transmissibility was demonstrated by Antonio Carle and Giorgio Rattone who were pathologists in Turin They produced tetanus in rabbits by injecting pus from a person with fatal tetanus into their sciatic nerves [4].

Tetanus is a non-communicable, potentially fatal disease contracted by exposure to the spores of Clostridium tetani. It is a gram-positive, anaerobic spore-forming bacteria that produces an exotoxin called tetanospasmin which is responsible for the lethal effects of the disease. It is more commonly seen in developing countries as a result of low vaccination coverage, poorer medical care, and more risk of exposure [5].

Tetanus spores are present in the environment irrespective of specific geographical locations. Once the disease is contracted, it is difficult to manage despite even intensive advanced medical care if timely medical intervention is absent. The high infection and fatality rates in India could be a result of incomplete vaccination coverage, lack of awareness of the protocols to be followed after sustaining a wound, lack of resources in hard reach areas, lack of medical facilities in such areas [6].

Spores are present everywhere in the environment, more commonly in the soil of warm and humid areas. The dormant spores develop into active toxin-producing bacteria in the presence of a favorable environment, ie devitalized, dead or necrotic tissue. They enter the human system via open or infected wounds, or even through unclean delivery practices, burns, dental procedures, or surgeries. The toxin, tetanospasmin thus produced by the active bacteria results in widespread sustained contraction and spasm of the muscles in the body, i.e dystonia. This is via the prevention of the release of the inhibitory neurotransmitter Gamma-aminobutyric acid (GABA) into the synaptic cleft. This results in manifestations such as pain, headache, trismus, stridor, laryngeal spasm, rigidity, opisthotonus, and stiffness [7].

Dystonia can manifest in various ways, such as with tortipelvic, torticollis oculogyric, buccolingual, or opisthotonic [8].

The prognosis of a case can be assessed via the Phillips scoring system (Table 1).

The spasms are most in the first 2 weeks, the autonomic disturbances following them by a few days and peaking in the second week. The muscle spasms and convulsions are often precipitated by even minor stimuli, such as light, touch, or noise. The severe spasms and muscle rigidity often necessitate paralysis in cases of tetanus. Autonomic disturbances occur in the form of labile hypertension, sweating, tachycardia in severe cases.

Table 1: Prognostic Scoring Systems in Tetanus Phillips Score

Factor	Score
Incubation Time	
< 48 hours	5
2-5 days	4
5-10 days	3
10-14 days	2
> 14 days	1
Site of infection	
Internal and umbilical	5
Head, neck, and body wall	4
Peripheral proximal	3
Peripheral distal	2
Unknown	1
State of protection	
None	10
Possibly some or maternal immunization in neonatal patients	8
Protected > 10 years ago	4
Protected < 10 years ago	2
Complete protection	0
Complicating factors	
Injury or life-threatening illness	10
Severe injury or illness not immediately life-threatening	8
Injury or non-life-threatening illness	4
Minor injury or illness	2
ASA Grade 1	0
Total Score	

The diagnosis of tetanus is clinical for the most part. The WHO definition of adult tetanus requires at least one of the following signs: trismus or rhisus sardonicus; or painful muscular contractions [6].

To manage this condition: isolation in a dark and quiet room, with heavy sedation and muscle relaxant administration to prevent spasms. Benzodiazepines are most commonly used; it's favored for its combination of antispasmodic, muscle relaxant, anxiolytic and sedative effects, which are particularly useful for tetanus patients. Diazepam modulates GABA-A transmission and increases presynaptic inhibition. Dantrolene is a muscle relaxant that is effectively used in the treatment of malignant hyperthermia and neuroleptic malignant syndrome [9]. Studies have proven that magnesium sulfate has significant efficacy in the management of autonomic symptoms [10].

Antibiotics are also administered in tetanus, penicillin, and metronidazole being favored. It reduces the proliferation of the bacteria at the inoculation site.

For neutralization of circulating toxin, tetanus immunoglobulin is used. There is no certified fixed-dose; for prophylaxis in susceptible wounds, it is 250 IU of TIG administered intramuscularly For active tetanus case a dose ranging from 3000 to 60000 IU may be administered, 500 IU at a time [11].

Recurrent tetanus is a possibility as infection does not provide immunity to subsequent infections [12].

Tetanus being a potentially fatal disease, the medical fraternity must emphasize its prevention rather than its treatment. Despite it being an “old world” disease, it is surprisingly prevalent in India, along with the other developing nations. This could be attributable to many factors, such as inadequate knowledge of wound hygiene, irregular immunization, lack of awareness on what the disease is, and how fatal it can be, unequipped health centers, unavailability of vaccine, etc. However, the annual mortality rate per 100,000 people from tetanus in India has decreased by 86.3% from 1990 to 2017 [13]. We are doing this study to understand the clinical profile of tetanus in our region.

MATERIALS AND METHOD

This is a retrospective hospital-based study done during the year 2017-2019 in Epidemic Diseases Hospital, Bangalore. All the cases presenting to the hospital and which were diagnosed with tetanus were included in the study. The patients who were started on treatment and then referred to the hospital were excluded from the study.

The patient’s demographics and clinical data were collected and compiled in an excel sheet and analyzed. The patient data which was collected maintained patient confidentiality throughout.

RESULTS

The total number of patients who meet the inclusion criteria were 58 cases. The majority around 87% of the cases presented from the rural area and 13% were urban areas. The sex distributions were 35 males and 23 females. The majority of the patients were in the age group of 55-60 years; the minimum and maximum age being 5 years and 89 years respectively.

The average duration of hospital stay was 15 days, the minimum duration being 1 day and the maximum is 45 days. Out of these cases, Discharge against medical advice was done in 11 cases due to patients stating personal reasons.

The site of injury was an injury to the foot in 65% of the patients, followed by injuries to the fingers or the hand in 30% and 5% were cases of injury due to tooth picking with a pin, splinter removal using pins. Out of the injuries which occurred, the majority of them happened in agriculture fields summing up to 70%; and 10 % were from carpentry works; 10% due to fall/trauma/accidents.

In 25 cases the wound was noted and in the others, it was a healed wound.

The mean incubation period was around 14.5 days from the time of injury to the development of symptoms in the patients who survived. Totally 14 deaths noted, the cause of death was a late presentation to the hospital, taking alternative medicine treatment; ignorance also contributed to delayed presentation to the hospital. The fatal cases also had multiple comorbidities like type 2 diabetes mellitus, hypertension, almost all the cases were in the age group of 65-75 years. The progression of illness was rapid and they passed away within 5 days. The patients with nil comorbidities have a better chance to recover. Overall 36 patients had NIL co-morbidities. 95% of death cases hadn’t received tetanus immunoglobulin.

During the initial presentation of symptoms:

- lockjaw/inability to open mouth: 41
- neck stiffness/pain: 25
- difficulty in walking: 2
- limb pain/stiffness: 17
- back muscle pain/stiffness: 12
- dysphagia: 7

Towards the peak of the diseases, most of the symptoms were present for all the patients.

72% of the cases were noted to have autonomic dysfunction like fluctuation in pulse, blood pressures, sweating, and altered respiratory pattern on and off, postural fall of BP.

DISCUSSION

After compiling the epidemiological and clinical profiles of these patients we concluded that in a majority of the cases, the disease was introduced via injuries sustained in agricultural fields. This is in accordance with a paper published by Arijit Sinha, Bikash Chandra Seth et. In this study, they found that 81.34% of their patients hailed from rural areas [14]. The agricultural population constitutes a major part of the country’s society, therefore we must focus on preventative and awareness promoting methods to significantly reduce the risk as well as improving prognosis.

Another inference reached was the predisposition of tetanus to develop in males; according to our study 35 of the 58 cases were male which comes up to 60.34%. This is in line with a study conducted by Anuradha et. al [2].

Both of these inferences can be explained by the fact that the male population of the lower socio-economic strata who spend most of their day in the fields will inadvertently be at much higher risk of acquiring tetanus. Also, the female population whose attire covers more body surface, are additionally protected by the antenatal dose of tetanus vaccine that they receive as part of the routine immunization schedule prescribed by the government. These factors, along with the generally lower frequency of going into the fields, put them at lesser risk compared to men.

In the study conducted by us, it was also found that the majority of the patients were in the age group of 55-60 years. This is in accordance with the studies done by V G Marulappa [15] and Chalya et al. [16] which concluded that the majority of patients were above 40 years old.

Another finding of the study we conducted was that the average duration of hospital stay was 15 days, which is almost in accordance with to study done by S Chaudhary in which the mean duration of hospital stay was 12 days, ranging from 1-32 days [17].

In the collected data of patients presenting to our hospital, the site of injury the foot in 65% of the patients. This could be because field workers and people in rural areas do not use adequate foot protection when working in the fields or elsewhere and thus are more susceptible to sustaining tetanus prone wounds. This is in accordance with the findings of other studies [14, 17, 18], one being a paper by K.V.L. Sudha Rani [18] in which 79% of the cases included in their studied involved wounds on the lower extremities.

In 25 cases the wound was noted and in the others, it was a healed wound. In other studies however it was noted that commonly they presented with acute forms of injury rather than old ones [18,19]; in a study by V G Marulappa, they recorded that 47.9% of their patients presented with acute trauma, with 14.6% presenting with older wounds [15].

The mean incubation period was around 14.5 days from the time of injury to the development of symptoms in the patients who survived, which is in accordance with other studies conducted, the study conducted by AHM Feroz et al reported it to be 10.8±2.1 days with a range of 3-28 days [18].

According to the compiled data of the clinical presentations of the cases that came to the hospital, the most common presenting feature is lockjaw, i.e. inability to open the mouth, which was present in 70.68% of the patients. The others were neck stiffness and pain (43.1%), limb pain (29.3%), back pain (20.68%), dysphagia (12.06%), and difficulty in walking (3.4%) in decreasing order of frequency. This is in line with the findings of a study by Pornchai S in Thailand, in which 87.2% of the cases had presented with trismus [20]. Other studies also infer trismus to be the most common presenting feature [14, 21]. In a study conducted by Muhammad Saleh Khakheli et al., they reported patients to be clinically diagnosed with tetanus if they had the following symptoms- trismus, neck or abdomen rigidity, and reflex spasms; and were accordingly classified as generalized or cephalic tetanus [22]. According to a study carried out in Pakistan by Mahsud I U et.al, the most common symptoms tetanus patients presented with were lockjaw, dysphagia, and trismus [23].

Out of the 58 cases, 14 deaths were noted, which sums up a case fatality rate of 24.1%. Other studies yielded similar values, for example in a study conducted in Ethiopia by Amare A. they had a case fatality rate of 27% [24]. In a study conducted in Solapur by A B Pawar el at., they found that in 26.3% of the cases complications such as respiratory failure, cardiac arrest, septicemia, etc arose, and in those with complications, there was a 75% fatality [25]. Autonomous complications have been reported in various other studies which significantly worsened the prognosis [1, 26]. Case fatality can be attributed to several factors, such as the presence of comorbidities, advanced age, the degree and mechanism of the wound, the time of presentation, lack of awareness of preventative measures, and immunization. 95% of the fatalities had never received immunoglobulin. Also, mortality would seem to be inversely proportional to the duration of the incubation period according to various studies [27, 28, 29].

72% of the cases were noted to have autonomic dysfunction like fluctuation in pulse, blood pressures, sweating, and altered respiratory pattern on and off, postural fall of BP. In other studies also autonomic dysfunction was found to be a frequent complication [18, 30]. In a paper in which the study was carried out in Mysore, it is reported that most of their cases too died as a result of cardiorespiratory arrest [15].

Out of the total number of patients, 11 of them went DAMA, i.e. had to be discharged against medical advice. This could be due to several reasons. A simple lack of awareness as to the seriousness of the condition, financial constraints, customs and beliefs, faith in alternate methods of non-medical treatment, etc, etc. The only way to minimize such cases is to make admission financially more feasible, increase the availability of immunoglobulin, ensure the adequate counseling of patient and attenders, and to spread awareness in the community as to how this disease must be treated at a fully equipped hospital, as well as the potential fatality of the disease.

CONCLUSION

Tetanus is a treatable disease if there are timely precautions and interventions, such as taking tetanus toxoid injection, followed by Tetanus immunoglobulin and wound care. Through our study, we want to promote awareness among the doctors about the current situation of tetanus and emphasize that it's a still ongoing and ever-prevalent problem and we have to address it by working together. We need to create awareness among the general public regarding basic wound treatment, the prescribed immunization schedule. We must also emphasize the symptoms and signs of the disease to them so that they may identify it early and allow for timely intervention, which significantly improves the prognosis. Most of all, it needs to be made known to the public that this is an ailment, that if left untreated, has a very high chance of fatality and must not be underestimated.

Disclosure statement

This is a retrospective study, where there was no involvement of any human subject or any intervention, it was just an observational study, and only data collected from the case sheet was done and patient details were kept confidential at all times.

The institutional ethics committees approved was this research complied with acceptable international standards (such as the Declaration of Helsinki)

Acknowledgments

Author contribution: *VSS was responsible for the idea, and conduct of the study; SN was responsible for the organization and fieldwork; AA was responsible for data collection; VRM was responsible for paper writing and coordination.*

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In memoriam – Brigadier General Mihai Nicolae Bînă

Dan Mischianu[1]

Now, in February 2017 as I write this, I find it appropriate to revive the dormant memory of the reader to remember about Prof. Mihai Nicolae Bina, MD.

The future General, Physician and Professor of Urology Mihai Nicolae Bina was born on April 2, 1942 in the city of Craiova and passed away on the day of Saturday, March 3, 2012, when he still had certainly much to say and decide in our profession.

He did not like to remember and to celebrate his birthday, because, unfortunately, he had lived in his youth, as a medical student, a memorable drama: on the day of April 2, on the same ward of the Central Military Hospital, in the same clinical unit which, over the years, he will lead with professionalism, skill and dedication, his mother died – the distinguished professor Olimpia Bina. I remember that sometimes on the eve of that day, he avoided to enter in that reserve.

His father, General Professor Nicholas Bina, was a distinguished professor at the Military Academy ("Carol I" National Defense University, new style).

He came, therefore, from a family with an application for the study and the profession of arms so that, from the outset, the stars lined up to become an excellent professional of military medicine.

After graduating "Dimitrie Cantemir" high school from Bucharest, in 1959 he became a student of the Faculty of Medicine of the Medical and Pharmaceutical Institute in Bucharest and also the "pupil" of the Medico-Military Institute (this was the denomination for those who do not know). He once told me with sorrow and frustration as he went, in boots, from the Military Medical Institute Street near North railway Station to the college. There were incomprehensible times...

He completed his university studies, he graduated first of his generation in 1965.

His professional excursus was, from the outset placed under the best possible circumstances and almost obvious under today's ARU slogan: "Labor omnia vincit improbus" – Virgil, Georgice.

He was "extern" by competition (1963-1964), "intern" by competition (1964-1967), a brief period of teaching assistants at the Military School in Focsani, then secondary physician urologist, managed with the highest scores in the country.

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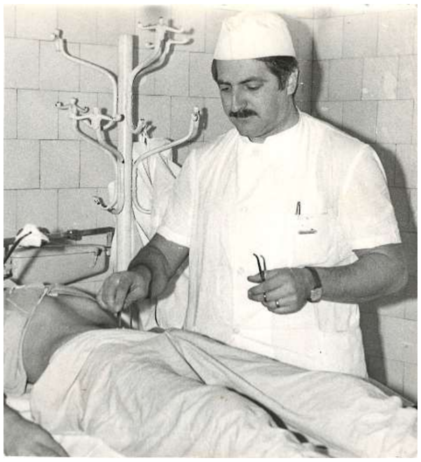

I got to make a confession.

Military doctors came, then and now from the same "strain" – the School of General Professor Carol Davila. After the Second World War, given the times and their understanding and approach their respective professional elite, felt, certainly some frustration in relation to their "civil" academic peers.

This "embarrassment" was not shared by the elite of the Romanian military medicine: Generals dr. Ion Atanasiu, Scarlat Longhin, Iuliu Şuteu, Gheorghe Niculescu, Traian Oancea, Academicians Victor Voicu and Ioanel Sinescu and many others, which through perfect and fair relationships with civilian colleagues have proven their worth and have integrated in the academic medical landscape of Bucharest as an unshakable symbiotic part. Future General Professor dr. Mihai Nicolae Bina had the same status!

Dr. Mihai Nicolae Bina became a specialist urologist (1971-1979) and consultant urologist in 1979 in one of the last competitions of its kind conducted in Communist Romania, because, then things have broken down altogether.

In fact, until 1991 the contest for "primary physician"/ consultant (new style!) was no longer conducted.

After a brilliant internship in general surgery in which he learned much and already mastered this "type" of specialty he confessed with some nostalgia the "impact" it had on him the first "seen" prostatectomy in Fundeni Hospital which was performed by his first urologic "mentor" – Professor Dr. Gheorghe Olanescu. Particularly bleeding, not followed by rigorous hemostasis as in abdominal surgery, continued by placing a hemostatic mesh, he seemed quite marked after three decades by this motivational experience.

Professor Bina was, and has been throughout his terrestrial existence, a great fighter.

And to dispel the "embarrassment" that we mentioned earlier he elaborated under the leadership of Professor dr. Docent Valentin Neagu – incomparable personality of Romanian urology (2017 is the 100th anniversary of his birth: who knows this?) his hard worked PhD thesis, entitled 'Readjustment of the upper urinary tract after dezobstruction of the bladder neck in benign prostate hyperplasia" – held in 1976.

In 1979 he became consultant urologist.

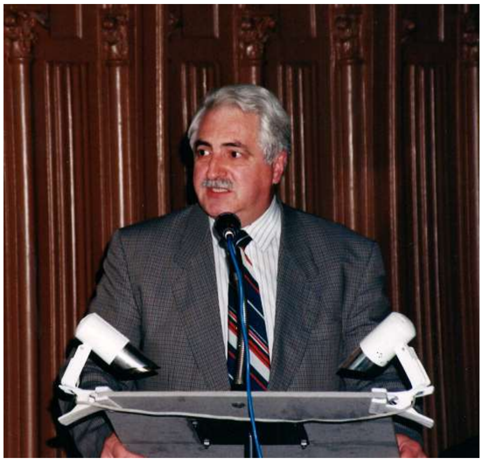

Meanwhile, in an absolutely dumb decade, he had carried out studies of “Emergencies in Urology" and "Urologic Oncology" (1979), under the mentorship of the Academician Eugen Proca and an International Course for young military doctors (Macolin, Switzerland), which he told me about often.

Post-revolutionary era found him prepared for future missions. He was the head physician of Clinical Urology Department of the Central Military Hospital between December 1990 and November 2004.

I was a doctor on duty on the day of November 30, 1991 when, he arrived from the Ministry of Defense, and he told me that on December 1, 1994 will be raised to the rank of Brigade General. I kissed him on both cheeks and I was proud that urology in this regard has its continuity.

This is a unique moment in the life of a man – after a lifetime of work and struggle.

This memory can certainly be connected to those written by the great writer Marin Preda, in a novel, once: "What has meaning for a civilian, for a soldier has a totally different one!"

In 1994 he accomplished his universitary dream: he became Senior Lecturer and later, in 2000, Professor of Urology of the Department of Urology, Faculty of Specialization and Training, Medico-Military Institute, University of Medicine and Pharmacy "Carol Davila" Bucharest.

Since 1995 he was a member of the Academy of Medical Sciences.

As I wrote five years ago in an ever undesirable "In Memoriam": "Hollow data fail to surprise and to outline the man that was Mihai Bina".

At Professor Mihai Bina I think everyone who knew him admired his sharp observation spirit, his gentleness, surgical talent, intelligence and diligence in all that which he has undertaken in earthly life.

Without Professor Mihai Bina, without his delicacy when advising or criticizing, without his professional experience and humanity, life looks very sad!...

To me, I confess, the work effort and way of thinking of Professor Mihai Bina was an example and an impulse.

The article was received on March 10, 2021, and accepted for publishing on June 13, 2021.

ORIGINAL ARTICLES

Innovative solution for SARS-COV2 epidemic management

Silviu Stanciu[1], Magdalena Gurzun[1], Diana Iancu[1], Valeriu Gheorghita[1], Cristian Lupascu[2], George Gugulea[2], Armand Ropot[2]

Abstract: *The SARS-CoV-2 pandemic challenged the authorities into taking measures that limit the spread of the virus in the community. TAMEC project (Advanced Community Approaches to Epidemic Management) developed a system that detects the contacts of patients infected with the SARS-CoV-2 virus and alerts them using mobile applications. It allows real-time analysis of confirmed patients and identified contacts, early detection of new outbreaks, and provides decision-making information to prevent viral transmission. The system uses its patent for homomorphic encryption, a method that allows to process and manipulate data in an encrypted format, without having access to the original data. Thus, the solution proposed in the TAMEC project fully respects the privacy rules of citizens imposed by the EU, using the concept of privacy preservation. Our solution offers the possibility to identify and validate a risk score that could become an extremely helpful tool in the stratification of COVID-19 patients. The TAMEC system is innovative in its simplicity and ability to facilitate the prevention of the spread of the SARS-CoV-2 virus in the community*

Keywords: *SARS-CoV-2 virus, prevention, privacy, artificial intelligence*

The SARS-CoV-2 virus pandemic challenged the authorities into taking measures that slow and control the spread of the virus and limit the disease among the population. Prompt identification of contacts and possible outbreaks is essential so that normal activities could be resumed. Researchers from Google and Oxford [1] concluded that if 15% of the population would benefit from a digital alert system, the number of infections and deaths could be reduced by 8% and 6%, respectively. Their studies concluded that 90% of people alerted would voluntarily start quarantine for 14 days, with an abandonment rate of 2% per day. One could assume that the benefits would be even greater if the participating population in such a system were more than 15%.

Epidemiological research is crucial in limiting the spread of the SARS-CoV-2 virus. It aims to identify and alert people at risk who have been in contact with confirmed infected patients. Currently, these surveys are carried out by Public Health Care (DSP) employees and include interviewing each infected person. Data collected are used in compiling a list of people in contact with the infected person within a period determined by epidemiologists. Each possible contact is then alerted and informed of the following procedure by a Public Health Care employee (DSP). This is time-consuming and involves significant human resources. Also, the collected data are sometimes incomplete.

Thus, we have developed a system that detects the contacts of patients infected with the SARS-CoV-2 virus and alerts them using mobile applications [2, 3, 4]. It aims to help limit the spread of the disease among the population and to reduce the health care employees' epidemiological research. This system can be extended for use in other

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contagious diseases, such as seasonal influenza.

An essential part of the TAMEC project (Advanced Community Approaches to Epidemic Management) [5] is the TAMEC Mobile Application which enables the detection and rapid warning of people who had been in contact with patients infected with the SARS-CoV-2 virus. The application provides a high degree of confidentiality for all users' data, thus protecting their privacy [6, 7].

The TAMEC project developed two systems that limit the spread of the virus in the community in two different effective ways:

- TAMEC Mobile System is responsible for early detection and notification of people who have been in contact with an infected person and who are possibly infected with contaminants.
- TAMEC Map System which is a real-time display of the distribution of confirmed cases and possible infected contaminants according to different parameters such as age, gender, profession, geographical location. This system analyses case distribution in different geographical areas and the time evolution of cases in different subgroups. This analysis allows early detection and geographical location of new outbreaks and provides decision-making information to prevent viral transmission.

To date, many such mobile applications rely on collecting location data or interactions of Bluetooth users. These approaches have drawn much criticism and concern for the safety of users' privacy. To solve this problem, the TAMEC Mobile application has been designed using the concept of privacy preservation. Thus, CERTSIGN has used its patent for homomorphic encryption [7]. This is a method that allows a third party to process and manipulate data in an encrypted format without having access to the original data (unencrypted data). This method allows a server to directly evaluate the encrypted data and obtain a result that is also encrypted, without the ability to decrypt the result or initial data. Thus, users will be assured that private information will not be misused, nor accessed without their explicit consent. If a user of the TAMEC mobile application has contact with an infected person, it is entirely their decision to inform the health care employees and to follow specific procedures such as testing or treatment. Also, a user notified by the mobile application as being in contact with an infected person will not be able to identify the infected person, nor the time of contact.

With all measures to secure data and preserve privacy, we are confident that users will be convinced the application is safe. The way the application is launched and promoted might determine the number of people who choose to use it. In addition to alerting the population through a mobile application such as the TAMEC Mobile system, it is also necessary to inform them to increase awareness in combating the spread of the SARS-Cov-2 virus. By knowing the evolution of the virus, one can have feedback and be motivated in adhering to the epidemiological strategies issued by the authorities.

Researchers such as epidemiologists and specialists from public health departments need well-structured and detailed data to determine key factors of the epidemic trend in the community. Accurate, concise, and easy-to-interpret data available for health care specialists enable the identification of outbreaks and risk areas. Thus, the authorities will be able to adopt prompt measures to prevent the spread of the virus from possible outbreaks.

TAMEC Mobile device application uses a system for detecting and alerting the contacts of confirmed infected patients. The system can preserve data privacy, revealing both authorities and users only the minimum information required. The core of this solution is generated by a homomorphic encryption scheme patented by CERTSIGN partner [7], the only Romanian patent of its kind. The system also includes existing digital contact tracing solutions already in use worldwide [8, 9].

The developed system is a modular one that includes six important components, namely:

- TAMEC mobile application. The TAMEC mobile application prototype is dedicated to Android operating system devices with 8 or newer Bluetooth mode versions. The detection of the contact was done using only Bluetooth technology, not GPS location, following the European Commission recommendation published shortly after drafting the project. The mobile app TAMEC is intended to be used by citizens, whom we will also call users. The application allows identifying contacts with confirmed infected patients, with sufficiently good precision in terms of distance and exposure time, using Bluetooth technology available on users' devices. The list of identified contacts that meet the case definition criteria is stored in the phone's memory (14 days for each contact). Periodically, this list is compared to the list of infected people from the Public Health Care System (DSP), using the backend components for anonymization. If a person is identified on both lists, the user is notified, without knowing who the infected person was or when the contact occurred in the last 14 days.
- The DSP web service is a backend component that has the role of ensuring the management of infected people and providing to the Computing Server component the list of

anonymized IDs of infected users. In the case of a production system, this component should be located and managed by the National Centre for Contagious Disease Surveillance and Control/Ministry of Health.

- The KMS web service (Key Management System) is also a backend component that has the role of managing the cryptographic keys necessary to ensure the confidentiality of the data from the system. In the case of a production system, this component should be located and managed by another state entity (e.g. STS);

- CS web service (Computing Server) is another backend component that has the role of performing the cryptographic calculations required by the system, for example, the intersection of anonymized mobile devices users list with the anonymized list provided by the DSP server to identify contacts with infected people. In the case of a production system, this component could be located and managed by a civil entity. This entity has also the role of giving trust to the users and ensuring that no state entity will be able to access, use, and manipulate confidential data of users.

- The CC web service (Client Configuration) is a component of the backend that has the role of centralizing mobile application configurations, depending on the phone type on which it is installed.

- The DSP-WebApp application is a web application that will be used by DSP employees in the territory for contacts management, respectively of the persons notified by the mobile application on the fact that they came in contact with an infected person, and report this and follow the necessary steps (isolation, testing, treatment, etc.). This web application can be hosted by the DSP, even on the DSP server that hosts the corresponding backend component (see the TAMEC Mobile application diagram in Figure 1).

Figure 1: TAMEC Mobile application graphic

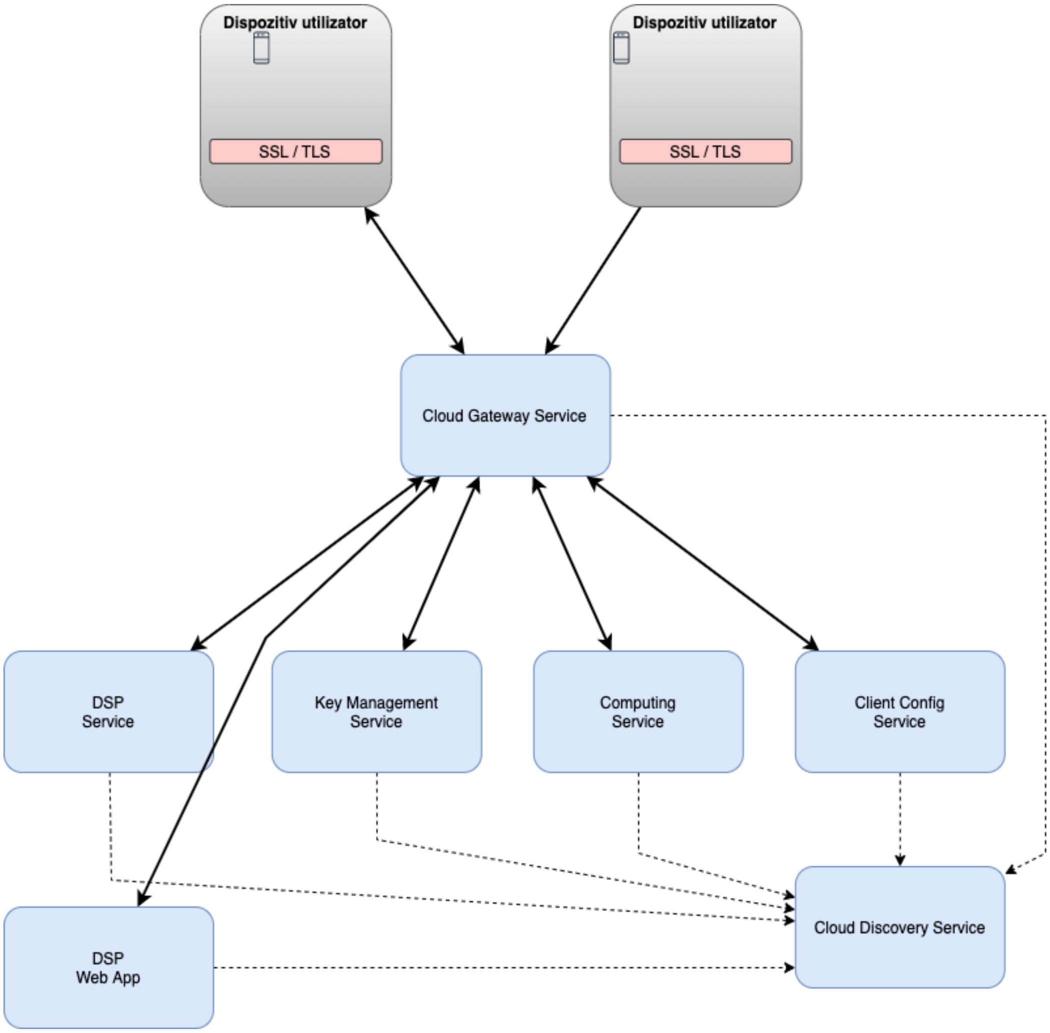

- The management system of all confirmed cases and possibly infected contacts detected by the system for detecting and warning the contacts of patients confirmed with SARS-CoV-2, TAMEC Mobile, was named TAMEC Map.

- The developed TAMEC Map system uses the software infrastructure already created in the first phase for the TAMEC Mobile system. The main components of the system are:

- TAMEC Map system database required to present the detailed map with the real-time distribution of all confirmed cases and possibly infected contacts. For each confirmed case or contact this includes the location, form of disease and severity, comorbidities, age, sex, professional category, etc. External data sources are used for this database, such as data available in the Corona-Forms system for confirmed cases, as well as data stored in the DSA WebApp database for contacts. From a technical point of view, the TAMEC Map system database is an extension of the DSA WebApp database developed in the first phase of stage I.

- The graphical interface of the TAMEC Map system is a web application that extends the DSP-WebApp application previously developed with the components necessary for the management and real-time analysis of confirmed cases and contacts and the display of this information, both on a map and in other graphical formats as well. This graphical web interface includes a public area used for dissemination related to the evolution of the pandemic in Romania, as well as a private area used by DSP and MS specialists to analyze the geographical evolution of the virus according to selected parameters, early identification of potential new outbreaks and making the best decisions based on the local situation. This web application can be hosted by the DSP, even on the DSP server that hosts the corresponding backend component.

Geolocations for confirmed or potential cases are obtained from the addresses provided by the Corona-Forms application using a service provided by Google, the Geocoding API. https://cloud.google.com/maps-platform/terms/maps- service-terms # 3.-geocoding-api.

This database is consumed by a graphical interface (called a dashboard) that allows real-time analysis of confirmed patients and identified contacts according to available criteria. Among the information available on this map, a series of necessary data will be identified and counted, such as the location of the diagnosed persons, age group, sex, comorbidities, occupational category, and severity of the disease. This database will not contain personal data of individuals, but only anonymized IDs that can allow only the relevant authority (DSP) to find the identity for a given ID, if necessary. From the graphical interface, it will be possible to easily generate and export reports regarding all the information from the database, depending on the search and reporting criteria. This information can be very helpful for the Public Health Care System to analyze the population epidemic impact for quick and adequate epidemiological measures initialization and to allocate the hospital resources where hospitalization and treatment are required according to medical recommendations.

Also, this type of representation allows real-time mapping of infected population characteristics in each territory, rapid triage, and classification into a risk category according to certain variables (age, sex, associated diseases, stage of disease, pathogenic type of the virus, biological and immunological status of the host). Thus, our solution opens a wide field of epidemiological and clinical research, offering the possibility to identify and validate a risk score that would become an extremely helpful tool in the stratification of COVID-19 patients.

The application also includes tools that allow the dissemination of certain types of reports on social media and indicators that measure their audience.

To conclude, the solution proposed in the TAMEC project is innovative in its simplicity and ability to facilitate the prevention of the spread of the SARS-CoV-2 virus in the community and fully respects the privacy rules of citizens imposed by the EU. The application will allow the development of statistical analysis components, adaptive filtering, and automatic prediction based on artificial intelligence. Starting from the existing information in the system database this can perform their automatic analysis, discover and predict critical developments (e.g. the emergence of new outbreaks), and generate alerts to epidemiologists, accompanied by intervention recommendations.

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ORIGINAL ARTICLES

In-silico evaluation of the potential of a viral protein (Apoptin) as an anticancer agent

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Abstract: *As a significant immunosuppressive virus, chicken anemia virus (CAV) is common among natural poultry hosts and newborn chickens. It contains a tiny single-stranded negative circular DNA genome, encoding three proteins vp1, vp2, and vp3. Apoptin, encoded by the vp3 gene of the CAV virus, is a small protein whose subcellular localization appears to be crucial for tumor-selective activity. Residing in the cytoplasm of normal cells, it translocates into the nucleus in cancerous cells. Apoptin has attracted much attention because of causes specific death in transformed and cancer cells and has toxic effects on cancer cells. In-silico analysis of apoptin revealed the unknown structural and functional features. Physicochemical features and sequence analysis and secondary structure prediction were also conducted using Expasy server tools. Furthermore, the global structure of apoptin was modeled using the I-TASSER server and antigenic features were obtained via the application of the IEBD web tools. The results of in-silico analysis indicated that this protein had a stable structure, was a suitable choice for therapeutic goals, capable of acting as a good anti-cancer agent.*

Keywords: *apoptin; in-silico analysis, chicken anemia virus; cancer*

INTRODUCTION

Cancer is defined as the Irregularity, growth, and proliferation of the cell cycle and sometimes the abnormal proliferation of cells in the body (metastasis). Since the onset of cancer is associated with mutations in some of the natural cell genes, it is considered a genetic disease. Tumor cell mass which is different in appearance and function from normal cells can be either benign (progressive and invasion free with slow growth) or malignant (with rapid growth, metastatic potential), ultimately leading to patient death [1]. Due to the high cost of the medications and cancer treatment facilities, numerous studies have concentrated on finding methods to prevent the disease or effectively treat it [2]. In previous studies, different viruses and their proteins have been used to find a treatment for cancer [3].

Viruses' ability to attach to the cancer cells has attracted the researchers' attention as a novel strategy comparable with conventional therapies, including surgery, radiation therapy, and chemotherapy [4]. The application of recombinant proteins has recently given rise to an interest in understanding and recognizing the physiology of tumor cells. In particular, the identification of these proteins as a new drug and therapeutic cancer vaccine which can use different mechanisms to defeat cancerous cells is crucial for cancer researchers. Unlike prophylactic vaccines, which are generally prescribed to healthy people, cancer vaccines are mostly prescribed to cancer patients. These vaccines are designed to kill cancer cells by boosting the patient's immune response. They kill different immune effects or

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mechanisms created by therapeutic vaccination, especially cancer cells and normal cells. The apoptin protein is one of these proteins encoded by the chicken anemia virus (CAV) genome [5, 6].

Infectious anemia virus is one of the most significant immunosuppressive viruses in newborn chicks which are regarded as the natural host of this virus. The virus has three protein sequences as follows: The 50kDa viral protein (VP1) which is the only protein isolated from virus components the 30kDa non-structural VP2 protein functioning as a scaffold protein during the vision accumulations, and the third viral protein VP3 weighing 14kDa, which is located the nucleus of infected cells, but not found in the purified virus components [7]. VP3 is called apoptin, a potent stimulant of apoptosis in and lymphoblastoid cell lines and chicken thymocytes [8]. VP3 gene product due to its apoptotic effect was renamed apoptin [9]. This protein causes apoptosis to be independent of the p53Bcl-2-dependent tumor inhibitor. Apoptosis-induced apoptosis included the induction of caspase, leading to mitochondrial changes and dispersion of cytochrome C [10]. The C-terminal region of apoptin has a bilateral nuclear localization sequence (NLS). NLS1 amino acids from 82 to 88 and NLS2 amino acids remain in the range 111 and 121 [11]. In addition, in the range of 75-107 amino acids, apoptin has a putative nuclear export sequence that transfers the apoptin through the nucleus. Apoptin proteins cause apoptosis in the altered cells (transformed cells), while they leave no effect on normal cells [12, 13].

Bioinformatics refers to the information technology used in molecular biology. Bioinformatics tools can easily identify and predict the primary and secondary structures in a new sequence. This diagnostic tool is applied to find patterns, detect several protein families and show the oligonucleotide primers for a polymerase chain reaction. It also serves as an essential prelude to molecular evolutionary analysis [14]. To the best of the author's knowledge, not much is known about the structural characteristics of Apoptin as an anticancer agent. The main objective in the current study is to evaluate the physicochemical features and determine the secondary and tertiary structure and antigenic features of the apoptin protein of CAV, using bioinformatics tools and databases.

MATERIALS AND METHODS

Sequence analysis

The primary sequence of a given protein (Apoptin) was retrieved from GenBank (ACC. No. AEB91668.1). The first structure of the protein in terms of aliphatic index, extinction coefficient, molecular weight, instability index, isoelectric point, amino acid composition, and half-life in vitro and in vivo are considered as physicochemical features which can be evaluated using various bioinformatics tools.

Physicochemical Features of Apoptin were evaluated using ProtParam, Predict protein, Compute PI/MW tools, and ExPASy server (http://expasy.org). ProtParam database calculated the parameters, such as extinction coefficient, estimated half-life, amino acid composition, theoretical PI, instability index, molecular weight, atomic composition, aliphatic index, and hydropathic mean (GRAVY).

Predict Protein tool can integrate feature prediction for globular regions, B-values, disorder regions, subcellular localization, transmembrane helices secondary structure, intra-residue contacts, structural switch regions, coiled-coil regions, solvent accessibility protein-protein, and protein-DNA binding sites, disulfide bridges, protein-protein, and protein-DNA binding sites, metal-binding sites, domain boundaries, beta-barrels, cysteine bonds.

Structural analysis

The secondary structure components include Alpha Helix, B-Sheet, Turn, Loop, and Coil. The tertiary structure is also formed by the twist of the protein chain caused by the bonding between amino acids which gives rise to functional domains.

Secondary structure was performed to identify Alpha Helix, B- strands, etc, using the Gor, Jpred tools from ExPASy server (http://expasy.org/cgi-bin). According to its amino acid composition and similarity to the sequences of the known secondary structure, the possible secondary structure of a protein can be predicted. RAMPAGE software (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) was used to determine the validity, stability, and quality of the spatial structure of the protein. Then, the stability of protein structure was evaluated based on the PDB model.

A pattern of comparative modeling was used by the I-TASSER server predicting the third structure based on Homology modeling. I-TASSER provides the biological function of protein molecules from amino acid sequences and the prediction of high-quality models of 3D structure. Energy minimization of the tree-dimensional structure obtained from I-TASSER was performed by the Swiss-Pdb Viewer 4.1.0 software.

Antigenicity

The http://www.iedb.org server was employed to identify antigenic fragments of the protein. IEDB contains experimental data on antibodies and T cell epitopes studied in the fields of infectious diseases, allergies, autoimmunity, and organ transplants in humans and other animal species. Predictions of B cell space epitopes were determined with ElliPro and Discotope servers.

We employed vaxijen server to estimate the antigenicity of the chimeric protein.

RESULTS

Primary sequence analysis

Table 1 shows the results obtained from the Prot param database, the number, and type of amino acids, and atomic components of Apoptin. This protein has 121 amino acids, most of which are Arg and the least are Met and Tyr. The molecular formula of the protein is C566H945N179O180S5. The protein's extinction coefficient is assumed to be 1740 if the number of cys is reduced to 1490. The protein's extinction coefficient is calculated using the amino acid composition (Table 1) of the protein. The estimated half-life span in mammals is 30 hours, in yeast 20 hours, and E. coli 10 hours. The protein instability index was calculated at 68.45.

The aliphatic index of protein was 65/37. The hydropathic mean (GRAVY) was also estimated to be -0.748

Table 1: Proteins parameters database

Number of amino acids	**121**
Molecular weight	13298/12
Theoretical pI	10/05
Formula	$C_{566}H_{945}N_{179}O_{180}S_5$
Total number of atoms	1875
Estimated half-life	30 hours (mammalian reticulocytes, in vitro)
	>20 hours (yeast, in vivo)
	>10 hours (Escherichia coli, in vivo)
Instability index	The instability index (II) is computed to be 68/45
Aliphatic index	65/37
Grand average of hydropathicity (GRAVY)	-0/748

The mean pI/Mw was calculated based on the Compute PI/MW database (PI=10/05, MW=13298/12)

According to the Ramachandran map obtained from RAMPAGE software, the protein is the apoptotic protein (Figure 1).

Figure 1: A (Image of 3D forms of the apoptin protein obtained from the i-Tasser database). B (prosaweb result). C (Image depicts the Ramachandran map of apoptin protein). D (Indicates the composition of the secondary structure of the protein)

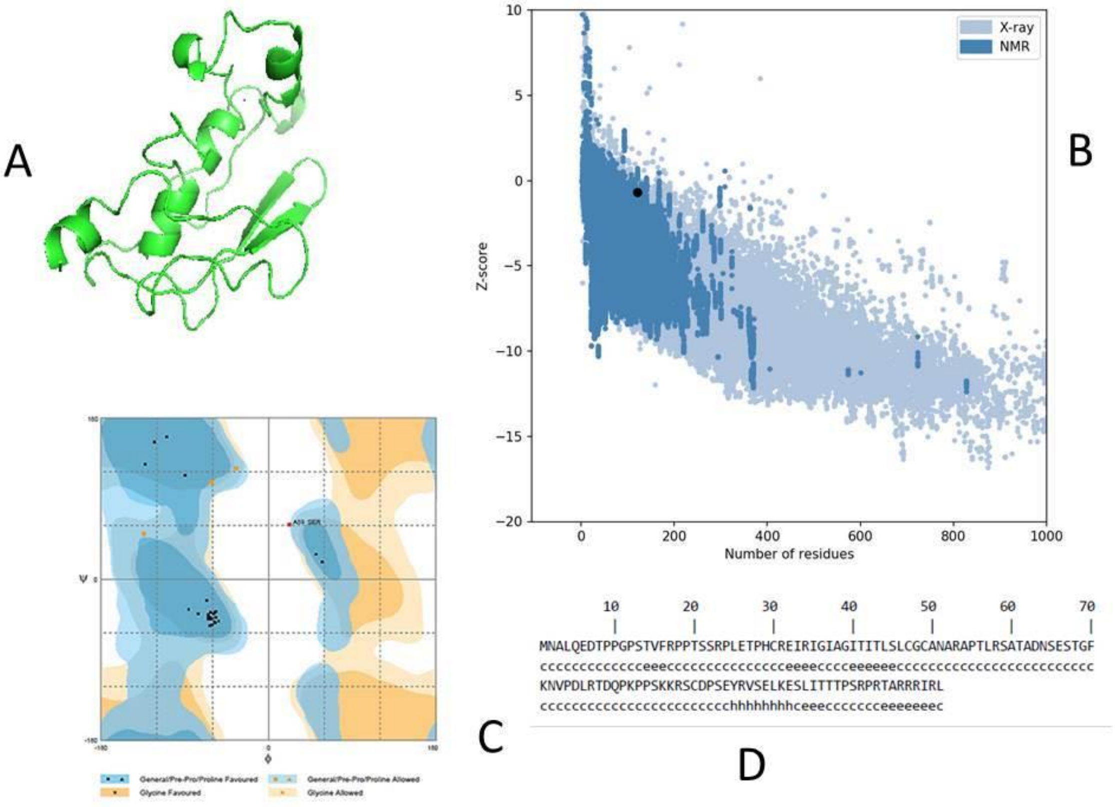

Prediction of tertiary structure

As shown in Figure 1, i-Tasser protein simulated models were calculated quantitatively by a C score based on the importance of pattern rules and convergence parameters of the structure assembly simulation. The energy minimizer of apoptin protein was obtained by using simulated models by the i-Tasser database in spdbv software indicating that apoptin is a stable protein.

Prediction of linear and discontinuous epitopes

According to Figure 2, the spatial structure of apoptin was obtained from the IEDB database, predicting an antigenically active peptide in the apoptin protein.

Figure 2. A (The Linear Epitopes spatial structure obtained from Ellipro). B (Discontinuous Epitope)

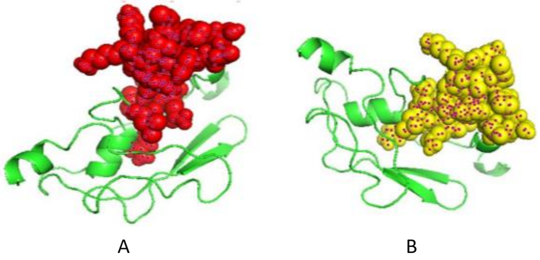

DISCUSSION

CAV is a small non-enveloping virus that has a single-stranded circular DNA genome measuring 2.3 KB. The major structural protein (VP1) of viruses is encoded by the genomic strand, while at least two other proteins (VP2 and VP3) are encoded by the antigenomic strand. VP3, called apoptin, is a non-structural protein made up of 121 amino acids. Apoptin contains some residues of proline, serine, and threonine and has a positive charge at the C- terminus [15].

Apoptin protein induces apoptosis just in altered cells (cancer cells) but does not affect normal cells. The specificity of the effect of Apoptin depends on its location in the cell. Its transfer from the outside to the nucleus of the cell in the attachment process to the cancer cells increases its specific effect compared to other normal cells. This protein, in cancer cells, is located in the nucleus and regulated by phosphorylation. Apoptin can induce specific apoptosis of tumor cells in a p53-independent pathway. Apoptosis is moderated by intracellular cysteine proteases (caspases) as the initiator and executor of the apoptosis process [16].

Zhuang et al. indicated that apoptin is capable of inducing apoptosis in human osteosarcoma cells. Subsequent studies found evidence that apoptin induces apoptosis in various human hematologic tumor cells. Also, another study on the effect of apoptin protein by bacteriophage carriers on human breast tumor cell line (BT-474) showed that it influences the cancer cells, while it has no inhibitory effect on normal cells [8].

Traditional laboratory-based cancer research consists of costly experimental trial and error strategies applied to humans, animals, and their harvested tissues. “In-silico experimentation,” combines computational technologies with the mathematical or theoretical properties of cancer bio cells and presents a new approach to advancing cancer treatment [17].

This study showed a simulated 3D model of apoptin generated by using homology modeling as a backup alternative to the conventional, crystallography- or NMR-based methods. In addition, bioinformatics tools for different purposes such as primary and secondary structures and computational modeling of protein structures can help predict new choices for cancer therapy [18, 19].

The results of physicochemical features of apoptin protein including instability index, GRAVY index, solubility index, and aliphatic index evaluated by Prot param database help predict desirable apoptin protein. Since no 3D structure has been identified to date, the present study aimed to evaluate the structure of apoptin using the I-TASSER server. The results stored in pdb were assessed for stable and appropriate energy conditions by Swiss-Pdb Viewer 4.1.0 software, and then energy minimization was analyzed. The image obtained from the server along with the prediction results compared to the Ramachandran graph prediction generally showed structural stability as a fusion protein. Lee et al. (2012) demonstrated that direct engineering of apoptin protein was a suitable and cost-effective strategy to enhance the expression of TAT-Apoptin protein in E. coli [20].

Despite having drawbacks targeting only one epitope or several epitopes of tumor-associated antigen (TAA), protein/ peptide-based vaccines are more cost-effective than autologous or personal vaccines. It is believed that the induction of antigen-specific CTLs and antigen-specific helper CD4 + T cells is essential for the optimal efficacy of the cancer vaccine. Some polypeptide vaccines (eg, Stimovax) potentially contain the CD4 and CD8 epitopes. Altering the peptide sequence of TAAs to introduce agonist-boosting epitopes is another method to increase the immunity of its antigen, which enhances the binding of the peptide to the MHC molecule or T cell receptor, resulting in a higher T cell response and/or T cells with higher or higher power [21]. In the present study, epitope mapping apoptin showed that this protein can be an appropriate candidate for therapeutic cancer vaccines.

To the best of the authors' knowledge, not much is known about the structural characteristics of apoptin. The main objective of the current study was to assess the potency and specificity of small apoptin protein in cancer therapy. However, more studies are needed for designing new small apoptin-like peptides to improve the anti-tumor activity of apoptin and avoid their undesirable side effects.

CONCLUSION

As the in-silico results indicate, apoptin has a stable structure and can be regarded as a suitable candidate for cancer treatment. Furthermore, the appropriate structure of apoptin may lead to its selection as a therapeutic cancer vaccine capable of stimulating both the humoral and cellular immune systems.

Acknowledgments

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Ethical Statement

Ethical approval was granted by the Medical Ethics Committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1397.360).

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REVIEW

Intention to vaccinate against COVID-19 in patients with autoimmune diseases

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Abstract: *Understanding COVID-19 vaccination attitudes is of paramount importance in order to estimate and influence the willingness to vaccinate.*

A cross-sectional study was performed in the spring of 2021 in Romania by sending a web-based questionnaire to patients with rheumatic autoimmune diseases in order to assess their willingness to vaccinate against SARS CoV-2 and to identify factors that might influence their decision. Variables derived from the questionnaire were analyzed in univariable and multivariable analysis.

71 patients, of which 60 (84.5%) women, were included in the study, with a 53.5% (38 subjects) willingness to vaccinate. A positive association was observed between the willingness to vaccinate and the patients' trust in authorities, trust in the healthcare system and the choice of scientific sources of information in their decision making. A positive correlation was observed between the intention to vaccinate and the global VAX score, as well as the subscale VAX scores (p<0.05). Global VAX score emerged as a predictive factor for the intention to vaccinate on logistic regression (p<0.05).

The present study underlines the importance of reliable scientific sources of information for pa-tients with autoimmune diseases and how the general attitude about vaccination becomes a pre-dictive factor for accepting COVID-19 vaccine.

Keywords: *COVID-19, vaccination, autoimmune diseases*

INTRODUCTION

The SARS-CoV-2 infection accounts for a high morbidity and mortality, having caused the death of more than 3 million people all over the world since the onset of the pandemic [1]. The COVID-19 disease particularly endangers people with comorbidities, leaving patients with autoimmune diseases in a place of uncertainty and vulnerability, enhanced by the fear of an increased risk for infection in the context of immunosuppression.

The current increasing prevalence of coronavirus worldwide will unfortunately provide the necessary data on the follow-up of autoimmune conditions. From an endocrinological point of view, patients with autoimmune thyroiditis have a higher risk of developing other antibody-related conditions such as vitiligo, alopecia areata, dermatitis herpatiformis, hypophysitis, autoimmune hepatitis, Sjogren's syndrome, Raynaud's syndrome, premature ovarian failure, primary adrenal insufficiency, type 1 diabetes mellitus, rheumatoid arthritis, atrophic gastritis, lupus, sclerodermia, vasculitis, and celiac disease [2].

The rapid development of vaccines against the SARS-CoV-2 infection provides the opportunity of acquiring immunity without the need of going through the disease. However, the novelty of the vaccine and of the technological platforms were paralleled by a gap in knowledge regarding the potential side effects of vaccination. Patient with autoimmune diseases thus struggled in a battle where on

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one hand was the desire for protection against a virus that might aggravate a serious, chronic disease and, on the other hand, was the concern regarding safety associated with vaccination. In this light, our aim was to assess the intention to vaccinate among patients with rheumatic diseases.

MATERIALS AND METHODS

A cross-sectional study was performed in the spring of 2021 in Cluj-Napoca, Romania. A web-based questionnaire was sent via a Google docs platform to patients with autoimmune diseases before a patient-dedicated virtual event, in order to assess their willingness to vaccinate against SARS CoV-2 and to identify factors that might influence their decision.

The questionnaire comprised a small section dedicated to general data, including a personal and family history of COVID-19 infection; a section evaluating the anxiety regarding COVID-19 as elaborated by Lee [3], a section evaluating the general attitudes toward vaccination, addressed by the means of the Vaccination Attitudes Examination (VAX) scale, developed in English by Martin and Petrie [4] and validated in Romanian by Huza [5]; VAX scale is comprised by the following four subscales: mistrust of vaccine benefit, worries about unforeseen future effects, concerns about commercial profiteering and preference for natural immunity.

Supplementary questions regarding the attitudes of patients towards the government, healthcare system and guideline compliance were imported from an article that published the data of the UCL COVID-19 Social Study [6]. One question evaluated whether the decision to accept the COVID-19 vaccine was due to information drawn from non-scientific sources (the opinion of a public person, opinion of friends, possible travel restrictions, others) or from scientific sources (scientific books and journals, opinion of an expert in the field).

Participants received no compensation for completing the questionnaire, however, they had enhanced access to communicate with the physician to address further inquiries regarding their autoimmune pathology or COVID-19 disease, including vaccination.

For the statistical analysis, variables were derived from the questionnaire and included in a bivariate analysis. Chi square test was used to assess the relationship between the intention to vaccinate and most of the variables considered for analysis. Fisher test was used to assess a relationship between the intention to vaccinate and COVID-19 guideline compliance and Freeman-Halton test for the variables type of immunosuppressive therapy and COVID-19 disease experienced by the subject, the family, close friends and acquaintances. We used Fisher and Freeman-Hallton instead of Chi-Square when the minimum of 5 expected cases was not met, with Fisher being used for 2x2 matrices and Freeman Gallton for 2x3 matrices. Significance threshold was considered under 0.05. Multivariable analysis was subsequently performed by the aid of logistical regression. Statistical analysis was performed using SPSS v21.

RESULTS

Of 75 answers that were submitted, 71 were included in the study after a quality control check. The study sample consisted mostly of women, 60 (84.5%), participants having a mean age of 46 years, mostly from urban areas 58 (81.68%), with various autoimmune diseases, such as dermatomyositis 4 (5.6%), systemic lupus erythematosus 13 (18.3%), rheumatoid arthritis 28 (39.43%), systemic sclerosis 12 (16.9%), Sjogren syndrome 3 (4.2%) and other autoimmune diseases 13 (18.3%). The diverse forms of autoimmune diseases necessitate diverse treatments, ranging from hydroxychloroquine 10 subjects (14%) and biologic therapies such as belimumab, rituximab, tocilizumab 7 (9.8%) to other immunosuppressive drugs such as prednisone, methotrexate, mycophenolate mofetil 54 (76%).

The proportion of patients with autoimmune pathology willing to get vaccinated was 53.5% (38 subjects). Upon statistical analysis, a positive association was observed between the willingness to vaccinate and the patients' trust in authorities, their trust in the healthcare system and the choice of scientific sources of information in their decision making ($p<0.05$). A positive correlation was observed between the intention to vaccinate and the global VAX score, as well as the subscale VAX scores ($p<0.05$).

No association was found between the intention to vaccinate and a history of in-fluenza vaccination. Furthermore, the intention to vaccinate was not influenced by a prior vaccination refusal, by the preference for a certain type of vaccine technology (ARN-based, viral vector, inactivated virus or no preference) nor COVID-19-related anxiety.

No statistically significant relationship was noted between the intention to vaccinate and whether the subject or the family/friends of the subject experienced the burden of the COVID-19 disease or whether they are compliant to COVID-19 prevention guidelines recommended by authorities.

Upon multivariable analysis, global VAX score emerged as a predictive factor for the intention to vaccinate ($p<0.05$).

DISCUSSION

The current pandemic day's prescription is marked by access through digital medicine. The access to investigations might be reduced but drug administration including vaccines should not be postponed, if considered necessary [7]. The willingness to vaccinate in our study (53.5%) was similar to the one reported in a cross-country study (54.2%) with more than 1500 participants with systemic autoimmune or inflammatory rheumatic diseases [8], but slightly lower compared to a willingness of 61% in a cohort of patients with rheumatic diseases in The Netherlands [9].

Our results are significantly lower compared to a 92.9% preparedness for vaccination against COVID-19 in Danish patients with systemic lupus erythematosus and rheumatoid arthritis [10]. The association between pancreatitis, panniculitis and polyarthritis, entitled PPP syndrome, is a special category that need to be included in future studies to see if the percentage is the same like the others categories [11].

The scarcity of clinical trials studying the adverse effects of vaccination on rheumatic diseases, at the time of the study, and the time gap from publication to patient information may be important factors that account for the lack of willingness to vaccinate.

Studies like the one published by Achiron et al [12] revealed the fact that disease relapse in another autoimmune disease, multiple sclerosis, was similar in patients compared to controls; the rates of adverse events was higher in younger patients and in subjects with lower disability, orienting the physician toward a possible subgroup of subjects that might possibly be at risk of developing unwanted side-effects [13].

Hypercortisolemia found in glucocorticoid osteoporosis is a well known cause of obesity-related type of secondary osteoporosis more frequent in obese subjects without Cushing’s syndrome that display chronic high values of basal cortisol which seems suppressible at dexamethasone inhibition test and the chronic autonomous secretion of cortisol is identified in patients with adrenal incidentaloma with an age-dependent incidence and this has been reported to increase the risk of osteoporosis independent of age, so, this category can be affected by the fear of getting the infection because they are immunosuppressed by their pathology, so the vaccination will be more wished [14].

Data reported in a short-term observational study assessing the safety of SARS-CoV-2 mRNA vaccines in patients with rheumatic and musculoskeletal diseases indicates mainly mild local and systemic adverse events [15,16]. Patients with regression melanomas, a phenomenon that occurs as a result of host immune response that attacks the primary melanocytic tumor cells through lymphocytes determining a fibrotic process, can be also a difficult category of persons that can not wish the vaccination, because they can fear of a faster evolution of the regression and feel the a faster dissemination of the neoplastic cells [17].

Patients with an autoimmune disease are known to have high rates of anxiety and depression [18]. In our cohort, the proportion of subjects with COVID-19-related anxiety was slightly higher than the one obtained from a questionnaire distributed to students enrolled in the healthcare system: 33.8% (24 subjects) vs 28.5% (article submitted). In our sample, choosing scientific sources of information correlated with the positive intention to vaccinate, in agreement with previously published data stating that not relying upon social media for virus information is a significant predictor for the intention to vaccinate [19].

We note that the majority of subjects in our cohort chose scientific resources, 47 subjects (66.19%); this may be attributed to a selection error, participants having been recruited with the occasion of a patient-dedicated virtual event.

In our opinion, the association between the intention to vaccinate and the global VAX score, as well as subscale VAX scores is an important finding since it reveals the way the general attitude toward vaccination influences a particular vaccination choice, in our case the SARS-CoV-2vaccine. The relationship between general vaccination attitudes and the intention to vaccinate against COVID-19 was highlighted in a study having students as a target group (Candrea et al, submitted), but also in a large study involving more than 32.000 participants of various social, ethnical and educational backgrounds [6,20].

The above-mentioned association has a broader impact on the healthcare of patients with autoimmune diseases since they have recommendation for the administration of other types of vaccines, including pneumococcal immunization [21].

Limitations of our study can be considered the psychiatric conditions in these patients, for example the diagnosis of depression as well as the treatment for depression were significantly more frequently associated with psoriasis, an immune mediated disease. Patients with mild psoriasis can experience psychiatric comorbidities; however, depression is more common in patients with severe psoriasis or psoriatic arthritis.

So, father research is needed to se if any psychiatric comorbidities can influence the patient decision to be vaccinated against COVID-19 [22].

In conclusion, the present study highlights the importance of reliable scientific sources of information for patients with autoimmune diseases and shows how the general attitude about vaccination becomes a predictive factor for the de decision to vaccinate against COVID-19. Therefore, it is recommendable to make efforts to shift patients' general attitude toward vaccination.

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Authors' contributions

Conceptualization, EC, CP, VJL; methodology, EC, CP, VJL, SR; validation, all authors; formal analysis, EC, CP; investigation, CP, CM, AB, SR.; data curation, EC, CP, AB; writing—original draft preparation, EC, CP, CM, TRM; writing—all authors; supervision, SR. All authors have read and agreed to the published version of the manuscript.

Ethics approval

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the 'I. Hatieganu' University of Medicine and Pharmacy, approval number 99.

Informed consent

Statement: By completing the questionnaire, subjects consented to participate in the study.

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ORIGINAL ARTICLES

Intraoperative parathyroid hormone assay in patients with primary hyperparathyroidism

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Abstract: *Introduction: Currently, parathyroidectomy represents the elective surgical treatment and the only one curative that relieves the patient of metabolic complications and improves the quality of life of the patient. Intraoperative confirmation of PTH is required to reflect the fact that the correct parathyroid gland has been extracted. Material and method: The study is retrospective. The period on which the data was gathered over a period of 2 years. The values of the PTH assay were obtained from blood drawn from the ipsilateral cephalic vein of the hyperproductive parathyroid gland: 1. at the moment of anesthesia induction; 2. at identification; 3. at 10 minutes after the extraction; 4. at 20 minutes after extraction. Results: We identified a number of 25 patients operated for primary hyperparathyroidism. Preoperative values of PTH were 380pg/mL with a maximum value of 821 pg/mL. PTH values decreased by 55% at 5 min, 68% at 10 minutes and 74% at 15 minutes. A marked reduction in PTH values occurred after the first 5 minutes while the interval between 10-15minutes associated a variation of only 8-10% decrease.*

Conclusion: Intraoperative PTH assay is a useful technique to predict the success of the intervention. The technique is standard although some issues have been raised and are still under discussion.

Keywords: *PTH, hyperparathyroidism*

INTRODUCTION

The first reported intraoperative dosage of the parathormone or PTH was reported in 1988 by Nassaum et al. [1] The level and the extension of the cervical dissection in patients with primary hyperparathyroidism is still under discussion. In the absence of any preoperative imagistic or laboratory investigations and using only the intraoperative exploration of the parathyroid glands Satava et al. confirmed a rate of succes up to 96% in identifying and extracting the correct parathyroid gland responsible of primary hyperparathyroidism [2].

However, this technique has its limitations which can lead to surgical failure if one encounters a case of multiglandular hyperplasia, multiple parathyroid adenomatous nodules or hyperfunctional parathyroid ectopic tissue.

Primary hyperparathyroidism is an endocrinological disorder commonly found to be produced in 87% of cases by a single adenoma [3, 4]. Hyperparathyroidism associated with the presence of two hyperfunctional adenomas associates an incidence of up to 15% [3, 4].

Currently, parathyroidectomy represents the elective surgical treatment and the only one curative that relieves the patient of metabolic complications and increases the quality of life. To achieve this goal, a minimally invasive

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surgical procedure, aimed at a single parathyroid gland that houses the hyperfunctional nodule, is now successfully used, thus replacing bilateral exploratory cervical dissections. This procedure was quickly adopted due to the effectiveness of paraclinical investigations in the field of scintigraphy that allow the exact identification of the parathyroid adenoma during the preoperative evaluation [5].

However, an intraoperative confirmation is required to reflect the fact that the correct parathyroid gland has been extracted. In this regard, the intraoperative PTH dosage is used which due to the reduced half-life of 2-5 minutes coupled with a 50% reduction of the circulating intraoperative values compared to the preoperative values confirms the correctness of the procedure or the opens up the possibility to a cervical exploration to identify other parathyroid glands or parathyroid ectopic tissue [6].

The current protocol involves 4 dosages of PTH: at the time of induction of anesthesia (which will be used as a threshold value) at the moment of identification, at 10 and 15 minutes after the gland has been extracted. Success rates when this technique is used can reach 94% [7].

However, literature has begun to bring into discussion the efficacy of PTH evaluation when multinodular hyperplasia is encountered and the cost-effectiveness of the technique [8].

The study represents a retrospective evaluation of the effectiveness of this technique incorrectly identifying the hyperfunctional parathyroid gland in patients operated in our clinic for primary hyperparathyroidism by a minimally invasive technique.

MATERIAL AND METHODS

The study is retrospective. The period on which the data was gathered over a period of 2 years (01.01.2017-01.01.2019). Data were obtained from the clinic of general surgery of the Clinical Emergency Hospital "Prof. Dr. Agrippa Ionescu," Bucharest, Romania. The following variables were obtained: age, sex, preoperative values of PTH, intraoperative PTH values, postoperative complications, preoperative scintigraphy, surgery time, and length of hospital stay.

The patients were operated using the standard technique of minimally invasive parathyroidectomy.

PTH values were obtained from blood drawn from the ipsilateral cephalic vein of the hyperproductive parathyroid gland: 1. at the moment of anesthesia induction; 2. at identification; 3. at 10 minutes after the extraction; 4. at 20 minutes after extraction.

Laboratory PTH values were obtained using the Radioimmunoassay method.

Inclusion Criteria: Diagnosis of primary hyperparathyroidism and surgery for primary hyperparathyroidism.

Ethics: The retrospective analysis of the data has been approved by an Ethics Committee of the institution.

RESULTS

We identified 25 patients, which were operated for primary hyperparathyroidism with a minimally invasive technique on which intraoperative PTH hormone assay was done (Figure 1).

Figure 1: Right parathyroid adenoma operated with a minimally invasive technique – the incision had a length of 5 cm and was focused on the parathyroid gland. The adenoma is clearly visible.

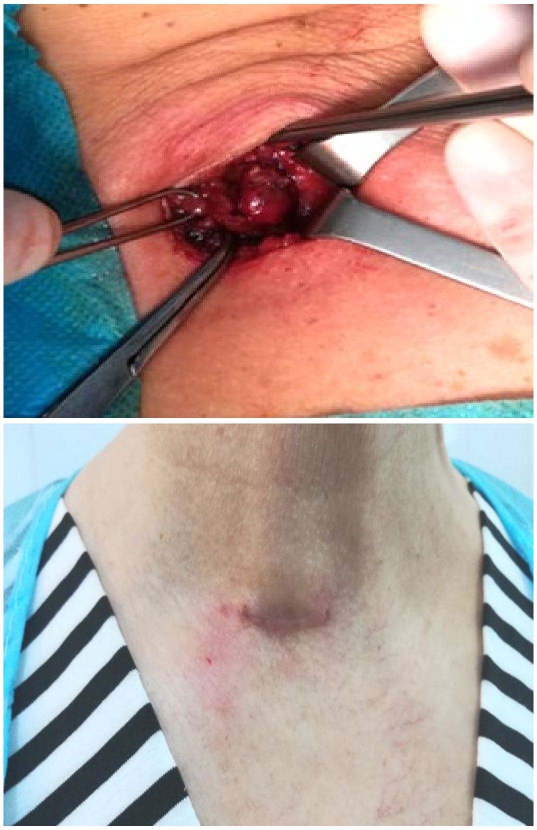

The average age at diagnosis was 54 years with a range of 32-68 years.

The study group was composed of 16 women and 9 men.

A number of 20 patients were diagnosed with singular parathyroid adenoma while multiple glandular adenomas were identified in five patients.

There were no mortalities reported in the studied group. There were no recurrent nerve lesions.

All of the patients were investigated before surgery with Tc 99m Scintigraphy to identify the location of the hyperproductive parathyroid gland. Only three cases did not correctly associate the preoperative scintigraphy with intraoperative exploration.

The average hospital stay was 2.3 days while a number of 8 patients were discharged on day 1 after surgery.

All of the patients were operated under general anesthesia.

Average preoperative values of PTH were 380pg/mL with a maximum value of 821 pg/mL.

The mean value (SD) for LVEDV was 144.37 (69.2) ml by 2DE and 143.6 (67.2) ml by 3DE, p=0.8, Spearman's coefficient 0.86. For LVESV the mean value (SD) was 106.26 (59.53) ml by 2DE, 103.24 (55.02) ml by 3DE, p=0.051, Spearman's coefficient 0.85 (Figure 2).

Figure 2: Decrease of intraoperative PTH values after parathyroidectomy at 5 minutes, 10 minutes and 15 minutes

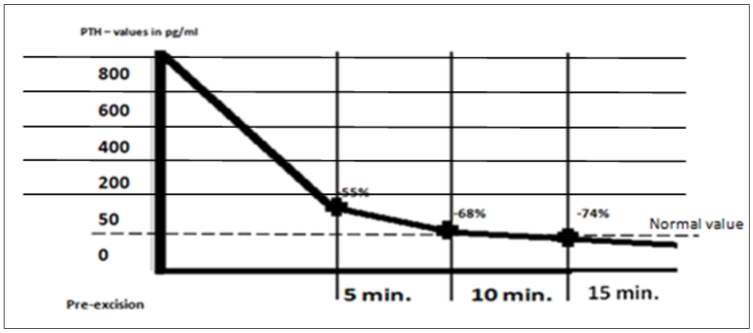

Average PTH values decreased by 55% at 5 min, 68% at 10 minutes and 74% at 15 minutes.

From the above graph, we can observe that a marked reduction in PTH values occurred after the first 5 minutes while the interval between 10-15 minutes associated a variation of only 8-10% decrease of PTH values.

There were no extended lateral cervical dissections done.

Two cases required local explorations by lifting and exploring the posterior region of the thyroid gland. In these cases, the PTH values decreased by 10% in one case at 20 minutes and the other they did not change.

DISCUSSIONS

The methods/techniques one uses to locate the parathyroid gland responsible for hyperparathyroidism has long been a topic of debate. Some surgeons advocate for preoperative investigations such as scintigraphy or cervical ultrasonography while others have fought for bilateral cervical exploration as a standard technique.

Today in order to identify the parathyroid gland, the scintigraphic examination is recognized as the gold standard, while other imaging solutions such as cervical ultrasound allow for a complete examination. Computer tomography and Magnetic Resonance Imaging are widely used to explore the suprarenal gland [9].

Ultrasound and scintigraphy are synergistic. When used together, they achieve a 95% sensitivity in identifying the hyperproductive parathyroid gland [10].

All of these investigations allow for a minimal-invasive targeted approach that translates into reduced hospitalization time, offer the possibility to use local anesthesia and ambulatory surgery, reduced operative time and low failure rates of only 1.5% [11, 12]. All of these advantages have led to a global reduction in costs [11, 12].

Surgical extraction of the parathyroid gland responsible for primary hyperparathyroidism produces a decrease in circulating PTH values thus confirming in real time the success of surgery or indicates towards a rapid change of tactics with the patient still on the operating table and under anesthesia. Failure of primary hyperparathyroidism surgery is largely related to correctly identify the parathyroid gland as it can be confused with lymphatic tissue or thyroid tissue [13].

Another cause of failure is multinodular hyper-parathyroidism, which, in the absence of advanced diagnostic techniques, will force the operator to do a cervical exploration – to identify at least four thyroid glands, extraction of the macroscopically affected glands and to wait for the extemporaneous investigation. This technique associates increased comorbidities and a significant increase in operative time. In literature, so-called false-negative results in intraoperative PTH assay were reported which were due to increased PTH values after extraction of the gland or a delay of up to 30 minutes until the values were reduced. These are mainly due to manipulation of the parathyroid tissue at dissection [14].

No such cases have been encountered in the studied group.

In these situations, the extemporaneous examination of the resected parathyroid gland is of real use. Good communication with the anatomopathological and the laboratory physician is mandatory in order to establish the optimal therapeutic course.

Intraoperative PTH assay has seen its share of discussions. We used this technique with great success and it has proven to be effective in the majority of our surgical interventions. The reproaches are of economical nature, cost-effectiveness and the half-life of PTH, which seems to differ in cases of a single parathyroid adenoma and multiglandular parathyroid hyperplasia. Other issues raised are related to the technique: intraoperative time of PTH harvesting and the PTH value set as the baseline to which a reduction of more than 50% after extraction of the gland is considered effective [15].

Therefore, some authors recommend as the baseline the PTH values from the preoperative day while other surgeons use the PTH value as baseline obtained at anesthesia induction. On the studied group, we used as the baseline the PTH values obtained before induction although some authors consider this value to be 60% higher than the previous day dosing. Subsequent PTH assays after gland extraction showed a 30-40% drop in values and a reduction in of more than 50% at 15 minutes in the cases of correct parathyroid removal.

In cases of multinodular hyperparathyroidism, the extraction of the parathyroid gland can lead to so-called false-positive. In these cases, one can observe a more than 50% reduction in PTH values from baseline although there is incomplete removal of all the hyperproductive parathyroid tissue [16]. To avoid these situations when there is a suspicion of multiglandular hyperparathyroidism, it has been proposed that one should pursue a more than 70% reduction in PTH values from the baseline [17]. A reduction of over 70% of intraoperative PTH values to 10 minutes is associated with healing with a sensitivity of 85% and specificity of 100% (18). When multiglandular hyperparathyroidism exists, preoperative investigations imaging investigations in particular scintigraphy allow for a complete evaluation.

As we observed in our studied group, of great importance is the place where the blood is obtained for the PTH assay. It should be obtained from the ipsilateral cephalic vein of the hyperproductive parathyroid gland. Contralateral blood harvesting delays the decrease in PTH values to more than 10 minutes due to the circulatory time of the hormone. Such contradictory values can raise false doubts as to the fairness of the procedure. In order to increase the sensitivity of the PTH assay, one can harvest blood the upper, middle or inferior thyroid veins [19].

Study limits:

However, the study has limits. It was difficult to appreciate the evolution of the disease until surgery. We could not follow-up, in the long run, the effectiveness of the surgical procedure.

In conclusion, although medical treatment in primary hyperparathyroidism has evolved in time, surgery has remained one of the pillars of treatment. Direct PTH evaluation is a useful real-time technique and can predict utility of the surgical procedure. The technique is standard although some issues have been raised and are still under discussion. As demonstrated in our study a reduction of more than 50% of PTH values when compared to baseline at anesthesia induction can predict the success of the intervention in agreement with the literature. Preoperative scintigraphy plays a key role if one decides to use this technique as it can decrease surgery time and hospital stay.

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ORIGINAL ARTICLES

Introducing two effective techniques for the detection of adenovirus respiratory infection in human sample

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Abstract: *Recently, respiratory viral infections have witnessed substantial diagnostic evolution, with the advent of emergent pathogenic agents and the improvement of new diagnostic approaches. Acute viral respiratory infection (ARVI) commonly causes illness and fatality, particularly in pediatric patients. Adenovirus has the highest prevalence in upper and lower respiratory tract infections (RTIs) amongst respiratory pathogens of importance. The purpose of this research was to introduce two effective techniques for the detection of adenovirus respiratory infection in Human samples, cell culture, and real-time PCR. Samples from patients aged less than ten years were obtained from Baqiyatallah (as) Hospital and cultured on HEK cells after preparation. After observing the cytopathic effects of the virus, a molecular test (Real-time PCR) with the help of specific primers was done on samples taken from the cell culture. Both assays detected viruses in clinical samples, and results of cell culture and real-time PCR confirmed infection with adenovirus in samples. According to our findings, simultaneous use of tests (cell culture and PCR) based test real-time PCR is a somewhat reliable method for detecting adenovirus in clinical specimens.*

Keywords: *Adenovirus, detection, cell culture, real-time PCR*

INTRODUCTION

Adenoviruses were first discovered and characterized in the 1950s by two groups who searched for the etiologic causes of acute respiratory disease (ARD) [1]. Adenoviruses as icosahedral viruses that have no enveloped with an approximate size of 70-90 nm. The viruses were found epidemiologically to be the main causative agent of ARD in recruited militarians [2]. Human adenoviruses are causing merely a minor percentage of ARD in the general population and for almost 5% to 10% of pediatric respiratory disease. Respiratory tract infections by viruses affect children [3]. Adenoviruses are responsible for epidemic outbreaks in soldiers [4]. In addition to respiratory infection, diseases such as conjunctivitis and infantile gastroenteritis caused by adenoviruses can induce fulminant fatal pneumonia, hepatitis, and/or encephalitis in mankind [5]. The respiratory diseases consist of both upper respiratory (pharyngitis and colds) and lower respiratory (bronchiolitis and pneumonia) syndromes [5, 6].

Around 7% of upper RTIs in youngsters aged below five years result from an adenovirus [3]. The respiratory indications are often associated with systemic demonstrations (e.g., generalized malaise, fever, chills, myalgia, and headache). The usual serovars are Ad 1, 2, 5, and 6 (species C or HA

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group III) and, sometimes, Ad3 and Ad7 (species B or HA group I), being indigenous in the majority of populations [7]. Sporadic cases might be indistinguishable from other viral respiratory contagions (e.g., influenza, parainfluenza, and respiratory syncytial virus) [8]. If conjunctivitis comes with the signs and symptoms denoted above, the illness is known as pharyngoconjunctival fever. Ad3 is the adenovirus serovar with the uppermost involvement, but Ad7 and Ad14 isolates among the same HA group have been reported in these patients [9]. Besides, adenoviruses induce lower respiratory tract contagions in youngsters and are a possible cause of nearly 10% of pediatric pneumonia cases [10]. The majority of patients recover from such lower respiratory contagions. However, some pandemics have led to a remarkable fatality, respiratory symptoms duration of almost one week, ocular symptoms for 2–8 weeks, and gastrointestinal symptoms up to 10 days [11]. Adenoviruses are detectable through isolating in nasopharyngeal and conjunctival discharges and throat washes and are visualized via electron microscopic assay of feces in the course of the acute disease phase [12]. Respiratory infections typically represent an elevation in CF antibodies to a common antigen. Virus-related antigens are also detectable in samples clinically through various immunologic methods and PCR [13]. The current research aimed to evaluate the detectability of adenovirus respiratory infections by two techniques, cell culture, and real-time PCR.

MATERIALS AND METHODS

Origin of samples: In this study, samples from patients less than ten years old were received from the Baqiyatallah (as) Hospital and prepared as oral swabs from patients with the common cold and pharyngitis symptoms. In most cases, NP swabs are obtained from elder children and adults, whereas newborns and youngsters are the common sources of NPAs. Since most patients cannot tolerate NP sampling well, other samples were obtained as well. Clinical specimens were transferred to the laboratory on wet ice to preserve viral vitality. These samples were placed in a transport media.

Cell culture: Human embryonic kidney cells (HEK) were cultured in three T25 flasks grown in Dulbecco's altered Eagle medium (DMEM (1X)) complemented with 10% fetal bovine serum (FBS). After inoculation, cell culture tubes underwent incubation at 37 °C and 5% CO2. Samples of patients in the two flasks were inoculated, followed by adding the maintenance medium (DMEM (1X) supplemented with 2% FBS) under the above conditions. Up to a week daily, flasks were examined microscopically to record the viral cytopathic effect (CPE) [14].

Nucleic Acid Purification and Real-Time PCR: A pair of primers were designed for the hexone locus, with forward (5-ACCTGAGTCCGGGTCTGGTGC-3) and reverse (5-GTCAACGGGCACGAAGCGCA-3 primer sequences, as well as a probe sequence of GATGTGACCACCGACCGTAGCCAGCG. Extraction of nucleic acids from respiratory specimens was done by a DNA kit (High Pure Viral Nucleic Acid Kit - Roche) following the manufacturer's instructions. In the next step, a pair of primers was designed for the hexon gene of the adenovirus for specific detection of the virus according to the melt curve. Each reaction had a total volume of 20 µL, including 10 µL of Mastermix PCR probe base, 5 µL of template, 1 µL of primer (0.5 µM), 1 probe (0.5 µM), and 3H2O. The cycling conditions included a primary denaturation step at 95 °C (2 min), followed by 60 cycles (each for 20 sec) at 95 °C, and at 57 and 72 °C for 40 and 30 sec respectively. The annealing temperature was 57°C. A melting curve analysis program was used to identify specific PCR products. After the last cycle, the temperature was increased to 95 °C, then decreased to 75 °C, and slowly elevated to 95 °C at a rate of 0.1 °C per second. Positive and negative controls were added in each set [15, 16].

RESULTS

The basis of this study was to show that the involvement of viruses can be an important factor in the upper respiratory system of patients. In this study, adenovirus was studied as one of the causes of conflict in the upper respiratory tract of children.

The samples used in our study were taken in Baqiyatallah (as) Hospital from patients with ages of fewer than ten years who had symptoms such as common cold, pharyngitis, and fever. As permissive cells for adenovirus, HEK cells infected with the virus show typical CPE that resembles grape-like clusters. HEK cells were cultured in three T25 flasks grown in Dulbecco's altered Eagle medium (DMEM (1X)) enriched with 10% FBS and 1% Pen Strep (10000 Unit/ml penicillin (P-4687) + 10000 µg/ml streptomycin (S-1277) Bio-Idea made from SIGMA powder).

When the cell confluence in the flask was approximately 100%, the patient samples were inoculated by the cells in an incubator with CO2 for one hour, after which samples were washed with PBS and added to each flask containing maintenance medium (DMEM (1X) enriched with 2% FBS and 1% Pen Strep).

Flasks were incubated in an incubator at 37 °C and 5% CO2. Flasks were checked daily to observe CPE. On the third day, CPE started formation in the two flasks infected with the virus, which included rounding of cells to clustering. Almost on the seventh day, grape-like clusters, which are typical CPE for adenovirus, were visible (Figure 1).

Figure 1: The cytopathic effects in cells after inoculation. A) Control cells that had not been inoculated with the virus. B & C) Patient samples 1 and 2 in the two inoculated flasks. Cytopathic effect was evaluated daily. The result was observed associated with adenovirus infection including grape-like clusters by separating the cells from the bottom of the flask

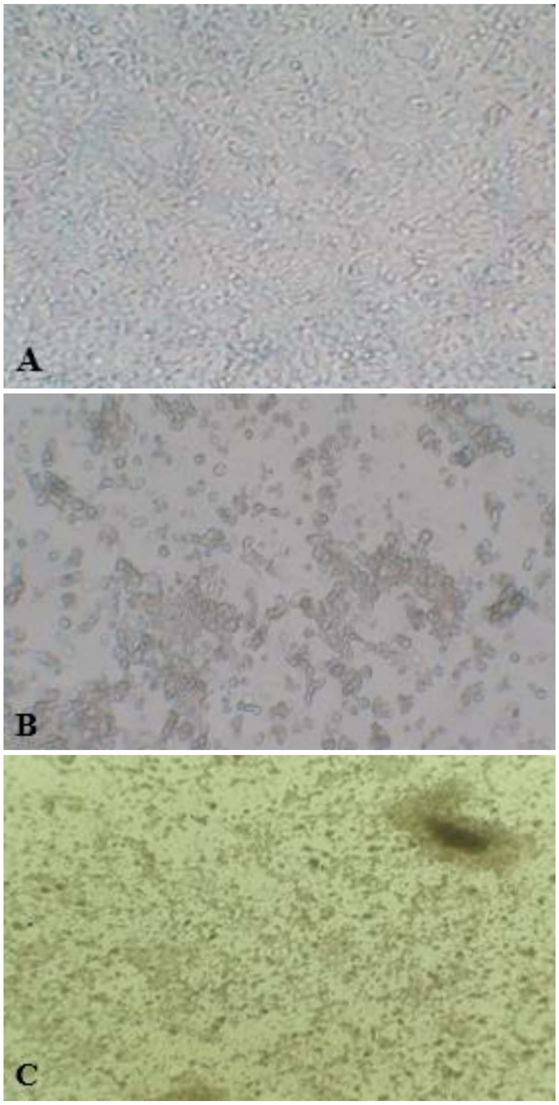

According to the figure of real-time PCR, clinical samples were positive for adenovirus in the form of double. The diagram shows that the amount of virus in sample 1 is greater than that of sample 2 (Figure 2A). Figure 2B shows real-time PCR results and related explanations.

DISCUSSION

Virus-related respiratory infections are prevalent during the year in all age clusters and all populations worldwide. Respiratory viruses can induce moderate ailments, including the common cold, or acute sickness (e.g., pneumonia), which affect both immunocompetent and immunocompromised hosts [17]. Despite the infection of both the upper and lower respiratory tract by every respiratory virus, special syndromes (e.g., croup or laryngotracheobronchitis) happen clinically by particular viruses. Both DNA and RNA viruses are reported to induce respiratory infection [18]. Researchers have described several novel viruses that have relationships with respiratory illnesses [19]. Pandemics of adenovirus respiratory infection are prevalent during winter, spring, and early summer, with protracted (a length of 1–3 years) durations [20]. Almost 80% of upper RTIs occur by viruses etiologically and can exacerbate asthma and acute otitis media [21].

Figure 2. A) Adenovirus molecular test results (real-time PCR) confirming the presence of adenovirus. In this test, a sequence-specific primer pair and a probe were used for the viral hexon sequence. B) Shows real-time PCR results and related explanations. In this test, other viruses were used as a negative control, the results of which are obvious.

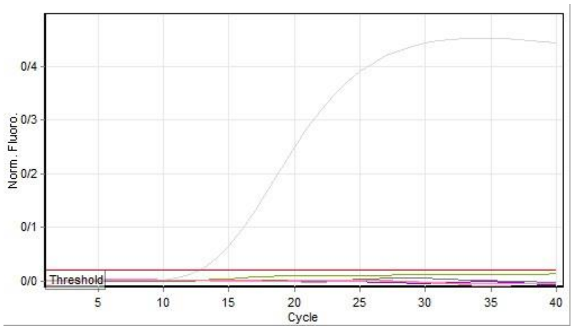

No.	Colour	Name	Type	Ct
22	🟩	HSV1	Negative Control	
26	🟪	NTC	NTC	
28	⬜	Adenovirus	Positive Control	12/69
31	⬛	HSV2	Negative Control	
37	🟪	CMV	Negative Control	
40	🟥	BKV	Negative Control	

Adenoviruses are the leading respiratory pathogenic agents and account for 5-8% of all RTIs in newborns and youngsters aged two years [22]. HAdV infections have clinical similarities to those of other respiratory viruses, and it is difficult to decisively detect and swiftly diagnose the virus type, which is not achievable clinically [23]. Due to the persistency and protracted casting of adenovirus, methods of diagnosis should have sufficient sensitivity for detecting small amounts of virus in clinical samples. Several laboratory methods are available to detect adenoviruses, including nested PCR, real-time PCR, PCR, IF, and cell culture, of which molecular and cell culture techniques are sensitive enough to detect the virus [24]. The viral antigen is swiftly detected by IF but is mostly not sensitive to detect some viruses;

hence it is occasionally required to be confirmed by viral culture [25]. Molecular analyses based on polymerase chain reaction (PCR) are gaining ground for diagnosing virus-related respiratory infections and conducting epidemiologic examinations [26]. As a benchmark, viral culture was well-known to test viral respiratory pathogens. Even so, the technique suffers from slowness in general, which usually requires over 14 days to deliver results [27]. Although the number of positive results can be increased by combining these two procedures, existing reports indicate that a considerable number of samples remain negative despite suspicious viral infections both clinically and epidemiologically [28]. During the last years, a growing body of evidence has been presented on the development of molecular biology diagnostic methods for viral infections, which will undoubtedly be the benchmark approaches presently applied in ordinary laboratory assays [29]. Molecular procedures significantly contributed to the fast detection of pathogenic viruses accompanying acute respiratory infections, which allow for rapid implementation of therapeutical actions and preemptive policies to prevent disease dissemination with high transmission ability [30]. Studies in Iran, Kenya, Brazil, China, Australia, and Hong Kong showed that 5-22% of samples were affected by adenoviruses [31].

Given the importance of cell culture and real-time PCR methods in recognition of respiratory adenovirus, cell culture was confirmed by real-time PCR in this study. Their grape-like clusters identified the CPE of the adenovirus in the cell culture. From the results of real-time PCR, it is concluded that the upper respiratory tract symptoms in children of our study might have arisen by adenoviruses. Suppose a large number of patients could be studied epidemiologically on this subject and in case of obtaining statically significant results. In that case, the season of adenovirus infection frequency can be verified in the studied district to demonstrate possible differences in the season frequency of infection.

CONCLUSIONS

This study showed that simultaneous use of tests such as cell culture and PCR-based tests, including the Real-time PCR, are somewhat reliable methods for detecting adenovirus in clinical specimens.

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Conflict of interest:

The authors report no conflicts of interest.

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ORIGINAL ARTICLE

Investigating the effect of methylprednisolone pulse on the treatment of back pain

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Abstract: *Background: Pain is a common problem afflicting humanity. Back pain is a musculoskeletal disorder with a high prevalence; this pain may occur to anyone at least once during their lifetime.*

Aim: the present study was performed to investigate the effect of the use of methylprednisolone pulse on the pain status of patients suffering from back pain in Ilam city.

Methods: This study is a clinical trial conducted as double-blind in 2021 in Ilam city with pretest and post-test groups plus a control group. The patients referred with vertebral disc herniation diagnosis suffering from diffuse pain in the right leg were assigned into test and control groups. A methylprednisolone 500 mg IV would be administered to the patient as pulse inside DW 500cc serum. The extent of pain was compared before and after the intervention at one, two, three, and seven days later. Placebo was applied for the control group patients. The data were analyzed by SPSS 16 as well as based on descriptive and analytical tests.

Results: According to the findings, no statistically significant difference was observed between the demographic characteristics of patients receiving methylprednisolone and patients in the control group ($p<0.05$). Also, the result showed the comparison of pain scores of patients in the two groups. According to the findings, the M (SD) pain score in the experimental group was 6.59 (0.88) before the intervention and 1.76 (0.69) in the week after the intervention, which was statistically significant ($P=0.000$). Also, the changes in the control group's score before intervention were 6.82 (0.71) and after the intervention was 6.77 (1.12) ($P>0.05$).

Conclusions: Considering the effect of methylprednisolone on mitigating the pain of patients suffering from back pain, it is suggested to use injective methylprednisolone for reducing the pain of patients suffering from back pain.

Keywords: *methylprednisolone, back pain, pain*

BACKGROUND

Pain is a common problem afflicting humanity [1]. Pain causes the development of numerous problems and constraints for the patient including reduction of quality of life, psychological health, life expectancy, daily activities, spiritual health plus related factors [2-5], as well as the development of depression, disability, and stress [6,7]. Back pain is a musculoskeletal disorder with a high prevalence; this pain may occur to anyone at least once during their lifetime [8]. Today, with changes that have occurred in the lifestyle of people, different groups may experience this pain, and this disease may happen in response to ergonomic-mechanical, as well as individual and social factors [9]. Back pain is a common cause of visits and medical interventions. Chronic back pain has considerable effects on functional status, constraining occupational activity, and certain socioeconomic consequences [10,11]. Among skeletal disorders, the damage related to back pain claims the largest share and in addition to healthcare aspects, it has also attracted the public attention economically [12]. The causes of this disease are numerous and its precise identification is sometimes difficult. Back pain is considered an important health problem that is common in all countries, especially industrial ones. Musculoskeletal disorders are typically

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associated with symptoms such as pain as well as permanent disorders in muscles, joints, nerves, and tendons. The effects of back pain may go beyond pain, which can be very serious and profound, causing incurrence of indirect costs, diminished capacity of individual ability, and increased risk of suffering from other medical conditions [13-15].

In pain etiology, various factors are involved, and one of the common features in all of them is neck movements; as these movements increase, so does the pain in that region [16]. Radiculopathy refers to nerve root disorders that may develop in response to structural injuries such as disk, tumor, trauma, vascular disorders, or infection such as viruses [17]. The radicular back pain is a kind of neuralgia that develops following sensory root stimulation or the dorsal root ganglion of the spinal nerve. Radicular back pains occur because of the generation of abnormal impulses in the dorsal root ganglion and its transfer through the peripheral nerve axon [18,19].

Different studies have been performed for the treatment of back pain and various therapeutic protocols have been criticized and examined. These methods include nonpharmacological methods such as educational intervention [20], exercise interventions [21], spiritual care [21], distraction [22], as well as pharmacotherapy [23]. There are different drugs for mitigating the pain in patients suffering from radicular pain including methylprednisolone. Prescription of this drug can help treat spinal nerve injury given its anti-inflammatory properties, immunosuppression, and improving neurological recovery [24].

OBJECTIVES

Identification of pain mitigation methods and especially attempts in choosing the best pain alleviation method in neurosurgery patients is one of the most important duties of neurosurgeons. This can be effectuated only through clinical research and performing interventional studies in this regard. Accordingly, the present study was performed to investigate the effect of the use of methylprednisolone pulse on the pain status of patients suffering from back pain in Ilam city.

METHODS

Study Design

This study is a clinical trial conducted as double-blind in 2021 in Ilam city with pretest and post-test groups plus a control group. The patients referred with vertebral disc herniation diagnosis suffering from diffuse pain in the right leg were assigned into test and control groups. A methylprednisolone 500 mg IV would be administered to the patient as pulse inside DW 500cc serum. The extent of pain was compared before and after the intervention at one, two, three, and seven days later. Placebo was applied for the control group patients.

Study Population

The statistical population in this study consisted of patients referring to hospitals, clinics, and practice offices of physicians in Ilam where the radicular pain. The sample size consisted of 94 patients; after the intervention, 12 of them were excluded, and eventually, the data were analyzed by 42 patients in the experimental group and 40 patients in the control group.

Inclusion and Exclusion Criteria

Inclusion Criteria

Informed consent for participation in the study, aged older than 20 years, at least three months past the disease, definite diagnosis of the disease by a neurosurgeon, and patients suffering from diffuse pain in the right leg were the inclusion criteria.

Exclusion Criteria

Desire to leave the study at any time, history of consuming medications other than the drugs of the intervention, history of any other disease affecting pain, and requiring surgery for radicular pain treatment constituted the exclusion criteria. Furthermore, patients with underlying problems, spondylolisthesis, spinal tumors, and congenital lesions were also excluded.

Ethical Approval

Receiving ethics code in the research with the code IR.MEDILAM.REC.1400.118, voluntary participation or lack of participation in the study, freeness of all visits, medications, and costs related to the interventions, confidentiality of patient information, explaining the objectives of pharmacological intervention with an understandable language for patients.

Study Tools

Demographic characteristics form and pain scale were used. VAS pain scale was applied to determine the severity of patients' pain. This scale is a 10 cm graduated ruler, which was used here for scoring the most severe pain and zero score according to the patient's report. In this instrument, acquiring a score 1-3 represents mild pain, a score 4-7 moderate pain, and a score 8-10 severe pain (25).

Statistical Analysis

The data were analyzed by SPSS 16 as well as based on descriptive and analytical tests.

RESULTS

According to the findings of Table 1, no statistically significant difference was observed between the demographic characteristics of patients receiving methylprednisolone and patients in the control group (p<0.05).

Table 2 showed the comparison of pain scores of patients in the two groups. According to the findings, the M (SD) pain score in the experimental group was 6.59 (0.88) before the intervention and 1.76 (0.69) in the week after the intervention, which was statistically significant (P=0.000). Also, the changes in the control group's score before intervention were 6.82 (0.71) and after the intervention was 6.77 (1.12) (P>0.05).

Table 1: Comparison of demographic characteristics of patients under study

Pain		Experimental Group	Control Group	P
		N (%)	N (%)	
Gender	Male	23 (54.8)	25 (62.5)	0.48
	Female	19 (45.2)	15 (37.5)	
Marital status	Married	29 (69)	28 (70)	0.92
	Single	13 (31)	12 (30)	
Economic situation	Weak	19 (45.2)	10 (25)	0.11
	Medium	18 (42.9)	24 (60)	
	Excellent	5 (11.9)	6 (15)	
Age, M (SD)		51.87 (18.46)	51.52 (9.40)	0.91

Table 2: Comparison of demographic characteristics of patients under study

Pain	Experimental Group	Control Group	P, F
	M (SD)	M (SD)	
Pain pre intervention	6.59(0.88)	6.82(0.71)	P=0.2, F=2.38
pain after 1 days	4.88(1.08)	6.72(0.59)	P=0.000, F=9.34
pain after 2 days	3.83(0.62)	6.45(0.95)	P=0.000, F=11.43
pain after 3 days	2.50(0.63)	6.35(1.23)	P=0.000, F=14.04
pain after 7 days	1.76(0.69)	6.77(1.12)	P=0.000, F=4.66

DISCUSSIONS

This study was conducted to explore the effect of methylprednisolone on pain mitigation among patients suffering from radicular back pain. The group of patients suffering from back pain claims a considerable percentage; in the meta-analysis by Azizpour et al., it was found that the prevalence of back pain in 20 papers with a total sample size of 5670 subjects was 51%, mostly afflicting 15-50-year-old age group [26] as the largest prevalence. As such, this group of patients requires special attention.

In this study, the usage of methylprednisolone drug could lead to a reduction of patient back pain. In a retrospective study by Dobohue et al. to compare methylprednisolone and dexamethasone drugs on pain mitigation, it was found that although both drugs could reduce the patient's pain, there was no significant difference between the two drugs against the patient pain mitigation. Further, methylprednisolone at two months following the intervention had caused 65% pain reduction for patients, which was 75% for dexamethasone [27]. In the study by Li et al., to explore the effect of methylprednisolone on neuropathic pain, they found that methylprednisolone could lead to improvement of quality of life and mitigation of pain. In that study, the extent of pain reduction was reported as about 50% or more [28].

The findings of other studies have also concurred with the present study results. In the meta-analysis by Liu et al. analyzing six clinical trial studies, 350 patients met the inclusion criteria. The results of this meta-analysis showed that usage of methylprednisolone at 6 and 24 h post-injection could result in pain alleviation for patients. Further, injection of this drug contributed to reduced nausea and vomiting for the researched patients [29]. Also, in the study by Lunn et al. on patients with total knee arthroplasty, again methylprednisolone had led to a reduction of pain and fatigue as well as an improvement in sleep quality [30]. The results of the mentioned studies [29,30] have been in line with the findings of the present study regarding the effect of methylprednisolone on pain alleviation in patients.

CONCLUSIONS

Considering the effect of methylprednisolone on mitigating the pain of patients suffering from back pain, it is suggested to use injective methylprednisolone for reducing the pain of patients suffering from back pain.

Authors' Contribution:
MH, KK did study conception, data analysis, and manuscript writing. MH, KK did data collection and manuscript writing. MH, KK did data collection and manuscript writing. Both authors (MH, KK) contributed to all stages of the article.

Conflict of Interest:
The authors declare no conflict of interest.

Ethical Approval:
The Ethics Committee of the Ilam University of Medical Sciences approved (IR.MEDILAM.REC.1400. 118).

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ORIGINAL ARTICLES

Laparoscopic sleeve gastrectomy and esophageal motility

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Abstract: *Laparoscopic sleeve gastrectomy (LSG) is becoming popular between the bariatric procedures. There are few studies available in the literature about LSG impact on esophageal motility, with debatable results. Technically, it modifies the anatomy of the esophago-gastric junction; it can damage the phreno-esophageal ligament and decrease the lower esophageal sphincter pressure that will predispose to reflux. Gastroesophageal reflux disease (GERD) is already common in obese patients due to frequent hiatal hernia, high intraabdominal pressure, increased transient lower esophageal sphincter relaxations (TLESR), altered esophageal motility and poor esophageal clearance. LSG can worsen gastroesophageal reflux disease, but also the weight loss achieved after it and the decrease of acid production in the small gastric pouch will probably reduce the GERD symptoms. Dysphagia and odynophagia were reported after sleeve gastrectomy, but usually associated with anatomical narrowing of the gastric sleeve, caused by esophageal dismotility or secondary to diabetes mellitus, low tiamine level, the use of opioids and non-steroidal anti-inflammatory drugs, even hypothyroidism. In addition to upper GI tract endoscopy, high-resolution esophageal manometry (HRM) should be required for all patients planning to undergo a LSG, and in the follow-up period according to the contractility pattern.*

Keywords: *obese, sleeve gastrectomy, esophageal motility, esophageal clearance, reflux*

INTRODUCTION

According to World Health Organization (WHO), overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. If the Body Mass Index (BMI weight in kilograms divided by the height in square meters) is between 25-30 kg/m^2 a person is overweight, and if BMI > 30 kg/m^2 obese [1]. Unfortunately due to behavioral and nutritional changes more than 20% of the worldwide population is overweight and 10% obese, and in the next 20 years the estimation is that 2.16 billion people will be overweight and 1.12 billion obese [2].

Morbid obesity, defined by BMI greater than 40 kg/m^2 or between 35 and 40 kg/m^2 with any obesity-associated conditions such as diabetes, high blood pressure, cardiovascular disease, stroke, sleep apnea, osteoarthritis, is a serious invalidating health condition that can interfere with basic physical functions such as breathing or walking [3].

The first step to obtain weight loss is through behavioral and lifestyle changes, with low-calorie diet and exercise, but usually previous attempts of weight loss are followed by rapid weight regain. Many obese associate to diet and exercise pharmacological drug therapy, achieving maximum 10% weight loss [5]. Unfortunately, a lot of patients fail to achieve long-term weight loss through these methods, so they are good candidates for surgical treatment.

Bariatric surgery cause weight loss by restricting the amount

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of food in the stomach, or by a combination of gastric restriction and malabsorption[6]. The bariatric surgical procedures can be purely restrictive, such as laparoscopic adjustable gastric band (LAGB) or laparoscopic sleeve gastrectomy (LSG), or malabsorptive-restrictive: Roux-en-Y gastric bypass (RYGB) and biliopancreatic diversion (BPD)[3, 6]. All the bariatric surgical procedures may have local complications (stenosis at anastomotic sites, stomal ulcers, band erosion, fistulae) but they can be managed with proper follow-up and close monitoring.

Table 1: The known risk factors [4] for morbid obesity are:

1. Genetic factors (familyhistory of obesity)
2. Dietaryhabitsandsedentary lifestyle, **q**uittingsmoking, microbiom
3. Stressandanxiety (highlevel of cortisol, high-calorie food)
4. Sleepdisorders
5. Female (post pregnancyweightgain or menopause)
6. Aging (slowingdown metabolism)
7. Somemedications (antidepressants, steroidsand beta-blockers)
8. Cushing'ssyndrome, Prader-Willi syndrome

The laparoscopic adjustable gastric band (LAGB) involves an inflatable band that is placed around the upper portion of the stomach, creating a small gastric pouch and reducing the amount of food. Is a reversible procedure, with shorter hospital stay and low mortality rate, but the band can slip or create erosion into the stomach, requires a foreign device to remain in the body and strict adherence to the postoperative diet and follow-up visits. The weight loss achieved is slower than the other bariatric procedures, with low risk of vitamin/mineral deficiencies, but a high rate of re-operation [6].

The Roux-en-Y gastric bypass was considered the “gold standard” of weight loss surgery, restricting the amount of food consumed and modifying hormonal level of ghrelin, decreasing appetite an enhance satiety. But is technically more complex than LAGB or laparoscopic sleeve gastrectomy (LSG), has longer hospital stay with possible long-term vitamin/mineral deficiencies (vitamin B12, calcium, folate, iron). Nowadays, remains an effective method to alleviate symptoms of GERD and many bariatric procedures are finally converted to Roux-en-Y bypass [6].

Laparoscopic sleeve gastrectomy (LSG) consist of a vertical division on the larger curvature of the stomach, removing a large portion of the body and all of the fundus, the effect being both restrictive and endocrinologic – reducing ghrelin. LSG is very popular among the bariatric procedures, it induces rapid and sustainable weight loss of > 50% for 3-5 years, has relatively short hospital stay (48h), but is a non-reversable procedure with potential long-term vitamin deficiencies [6]. Also, transforming the stomach into a cylindrical structure and altering the anatomy of the esophago-gastric junction will probably influence the lower esophageal sphincter pressure and esophageal motility.

OBESITY AND ESOPHAGEAL HEALTH

Gastroesophageal reflux disease (GERD) is common in obese patients, and they can be even more sensitive to the presence of acid in the esophagus than a normal weight person. Also, hiatal hernia, a risk factor predisposing to GERD is more prevalent among the obese [7]. Obesity may trigger vagal abnormalities and a higher level of bile and pancreatic enzymes, worsening the effect on esophageal mucosa [8].

According to a meta-analysis published by Halmpel H. et al. in 2005 [9], obesity may increase the risk of GERD symptoms by 50%, and also cause a 2-fold increase in esophageal adenocarcinoma [9]. The pathophysiological mechanisms of increased GERD in obese are: high intraabdominal pressure, frequent hiatal hernia, increased transient lower esophageal sphincter relaxations (TLESR), lower esophageal sphincter (LES) pressure, altered esophageal motility with poor esophageal clearance [10, 11].

The esophageal transit time in obese has been in the researchers preoccupation from1985, when Mercer CD et. al [11] measured it by radionuclide scintigraphy using a swallowed technetium sulfur colloid bolus. The data obtained were compared from obese patients with GERD to normal weight with and without GERD. The esophageal transit time was significantly prolonged in the obese group compared with both lean groups (p less than 0.001). Also performing conventional esophageal manometry theyconfirmed that in obese patients gastroesophageal pressure gradient is elevated [12].

Furthermore, in a study published in Obese Surgery in 1999, Jaffin BW et al. revealed a high prevalence of asymptomatic esophageal motility disorders among morbidly obese patients. During a 3-year period, they evaluated manometric and completed a questionnaire of symptoms, medications, demographic data, other medical conditions associated, 111 obese patients for bariatric surgery. Abnormal manometric findings were observed in 61% patients: 14% hypertensive contractions of the esophageal body, 7% diffuse esophageal spasm (DES), 1% achalasia, 25% hypotensive lower esophageal sphincter defined by LES pressure < 10 mm Hg, and 14% were nonspecific esophageal motility disorders [13].

Similar results have been obtained by Koppman's team [14], after performing esophageal manometric evaluation in 116 obese patients selected for laparoscopic gastric banding in 2003. 41% of the patients had abnormal manometric findings: 23% nonspecific esophageal motility disorders, 11% hypertensive contractions, 1% diffuse esophageal spasm, 1% achalasia, 3% isolated hypertensive LES pressure (>35 mmHg), 3% isolated hypotensive LES pressure (<12 mmHg). Only one patient with abnormal esophageal motility reported noncardiac chest pain [14].

LAPAROSCOPIC SLEEVE GASTRECTOMY AND ESOPHAGEAL MOTILITY

In a review of the impact of laparoscopic sleeve gastrectomy on gastrointestinal motility, published in 2018 by Eleni Sioka et al. [15], after a search of the medical literature with two independent reviewers, they selected only 9 eligible studies that focused on esophageal motility [16]. The esophageal motility data were debatable, with differences regarding the follow-up timing (from 6 weeks to median 13 months), the study design, the surgical technique, the bougie size, and the distance from pylorus. To clarify this subject we need more data from future prospective studies on larger cohorts.

Del Genio G et al. [17] studied the impact of LSG on esophageal physiology, performing high-resolution impedance manometry (HRiM) combined 24-h pH and multichannel intraluminal impedance (MII-pH) before and after the procedure, in 25 patiens. After a median follow-up of 13 months, HRiM showed increased ineffective peristalsis and incomplete bolus transit, but normal LES function.

In a retrospective analysis of 53 cases of HRiM performed after LSG, Mion F. et al. [18] found in 77% of patients an increased intragastric pressure (IIGP), even if they were asymptomatic with no specific manometric or impedance changes, and this could be assimilated as a manometric marker of LSG.

An interesting study of Petersen WV et al. [19] published in 2012, proved that LSG increased lower esophageal pressure independent of weight loss achieved after the procedure, thus protecting obese patients from gastroesophageal reflux.

Dysphagia was reported after laparoscopic sleeve gastrectomy, but usually associated with narrowing of the gastric sleeve which means inability to pass a 9.6 mm gastroscope due to stenosis or a sharp angulation [19]. In a study published by A. Nath et al. in september 2016, from 352 patients included, 22.7 % had dysphagia, 9.3% narrow sleeve, 7.1 % sharp angulation, 2.3% stenosis, and they responded well to ballon dilatation (1-3 sessions). Other medical conditions were associated with post-operative dysphagia: esophageal reflux, diabetes, low tiamine level, opioids and non-steroidal anti-inflammatory drugs or hypothyroidism, so dysphagia is not a specific esophageal motility disorder sign [20].

PRACTICAL CONSIDERATIONS

We performed a prospective study in Carol Davila University Central Emergency Military Hospital to determine the manometric pattern of obese patients, the prevalence of esophageal motility disorders prior and after gastric sleeve and the upper GI complications post sleeve.

The aim was to do high-resolution esophageal manometry (HRM) to all obese patients proposed for LSG, before and 6 to 12 months after the procedure, plus upper GI tract endoscopy and GERD questionnaire (GERD-HRLQ). Starting from January 2017, we included in the study 61 morbid obese patients evaluated both endoscopically and manometric. The exclusion criteria were: previous gastric surgery, another bariatric procedure surgical or endoscopically, the use of medications that could affect esophageal contractility or lower esophageal sphincter pressure (prokinetics, calcium-blockers, nitrates). Half of the patients are now in the post-operative evaluation period, because of surgical intervention timing, and the final results will be later published.

Esophageal manometry was performed with a 36-channel High-Resolution Solid State Catheter from Sierra-Given, after 6 h of fasting and previous upper GI tract endoscopy evaluation. The manometric data were obtained through 10 single test swallows of 5 ml of water. The HRM results were then analyzed according to the Chicago Classification v3.0 of esophageal motility disorders, using the ManoView ESO software program (component of the ManoScan system), providing visual images and quantitative physiological parameters.

The principal manometric variables analyzed [21] were summarized in the Table 2.

Diffuse esophageal spasm (DES) causes chest pain and sometimes mixed dysphagia for both liquids and solids. The substernal chest pain may waken the patient from sleep or is associated with exercise and often confused with angina pectoris. Hot or cold liquids may aggravate the pain. From the current data available, it is possible that in time this disorder to evolve into achalasia with impaired esophageal peristalsis and lack of lower esophageal sphincter relaxation during swallowing. Usually barium swallow may show poor progression of bolus plus simultaneous contractions [22]. Fragmented peristalsis and ineffective motility are minor esophageal motility disorders according to Chicago Classification v3.0 which can be seen also in normal person.

A particular manometric pattern was found during our study, in a 42-year-old female with morbid obesity BMI = 44.81 kg/m^2 (W= 122 kg, H= 165 cm), obstructive sleep apnea and minor GERD symptoms prior to the surgical intervention. The upper gastrointestinal endoscopy revealed erosive esophagitis grade A, duodenitis with Helicobacter pylori infection both treated preoperative.

Table 2: Principal manometric variables analyzed

Lower esophageal sphincter(LES) resting pressure	Normal 13-43 mmHg
Deglutitive LES relaxation = Integrated Relaxation Pressure (IRP)	For Sierra-Given solid probe the normal value < 15 mmHg
Esophageal peristalsis = Distal Contractile Integral(DCI)	Normal 500 – 5000 mmHg-cm-sec; > 5000 Hypertensive > 50% contractions weak (DCI < 500) or failed (DCI < 100) = IEM Ineffective motility
Duration of contraction waves in seconds= Distal Latency (DL)	Normal value > 4.5 sec, if 2 contractions are premature < 4.5 sec the diagnose is Diffuse esophageal spasm(DES)
Peristaltic break > 5 cm	More than 50% of swallows = Fragmented peristalsis(FP)

Figure 1: The upper GI tract endoscopy image preoperative – erosive esophagitis grade A Los Angeles Classification

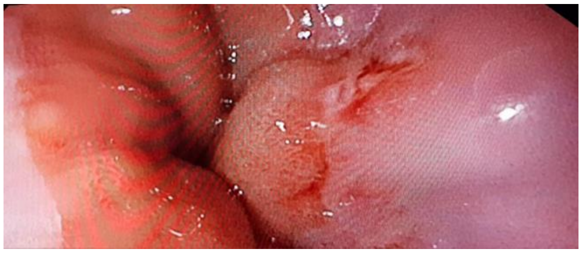

On the preoperative high-resolution esophageal manometry (HRM) she had normal lower esophageal sphincter (LES) basal pressure, LES length = 3 cm, normal deglutitive LES relaxation, with IRP median value of 6.6 mmHg, good esophageal contractility (DCI median= 956.1 mmHg-cm-sec and DCI maximum=1305.7 mmHg-cm-sec) but > 20% premature contraction (distal latency value < 4.5 seconds), aspect of diffuse esophageal spasm according to Chicago Classification v3.0.

Figure 2: Wet 5-ml test swallows recorded with HRM preoperatory – shorten distal latency

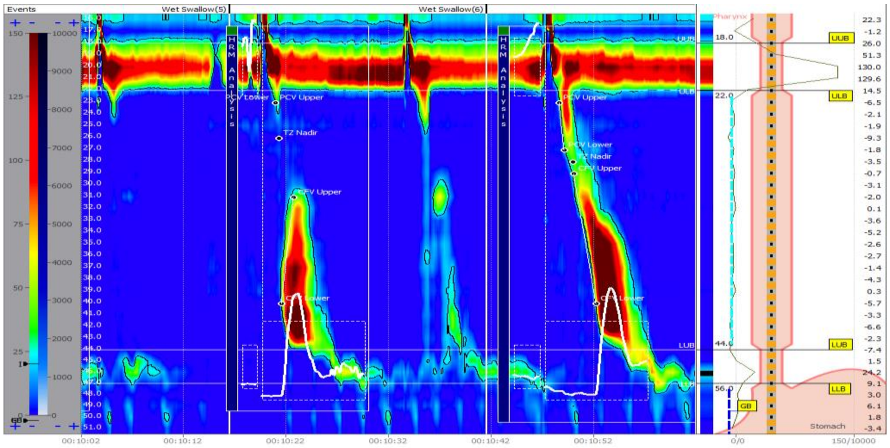

Considering the high cardio-vascular risk in a young patient, severe obstructive sleep apnea, the failure of diet and exercise and the need for sustainable weight loss, we didn't contraindicated the laparoscopic sleeve gastrectomy, but we've decided to closely follow the patient.

The laparoscopic surgical intervention went well, with good recovery after the procedure, good tolerance of food intake implementing the diet and exercise protocol. One year after LSG, the patient lost 40 kg, achieved a BMI of 30 kg/m^2 and had 82 kg weight. Clinically, now she had mild dysphagia, early satiety and fullness (that can be related to the low volume of the stomach), and less reflux symptoms compared to baseline.

The result of upper gastrointestinal endoscopy was encouraging: no evidence of erosive esophagitis, a normal gastric scar on the posterior wall of the stomach after LSG, as you can see in Figure 3.

Postoperative data from high-resolution esophageal manometry (HRM) examination revealed normal lower esophageal sphincter basal pressure (mean 20.2 mmHg, min -0.8 mmHg) with normal deglutitive relaxation of LES > 60%, mean IRP value 2.2 mmHg, the same LES length of 3 cm, but more than 50% of the esophageal contractions ineffective, with a DCI < 500 mmHg-cm-sec and normal distal latency, diagnosis of IEM ineffective motility according to Chicago Classification. Increased intragastric pressure (IIGP) after 5-ml water swallows was observed in 30% of the ten swallows examined.

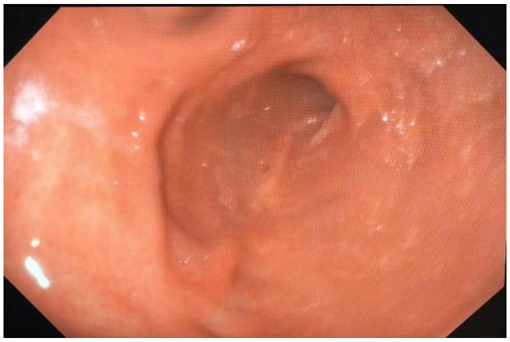

Figure 3: The longitudinal scar on the posterior wall of the stomach after LSG

Figure 4: Wet 5-ml test swallows recorded with HRM postoperatory – ineffective motility

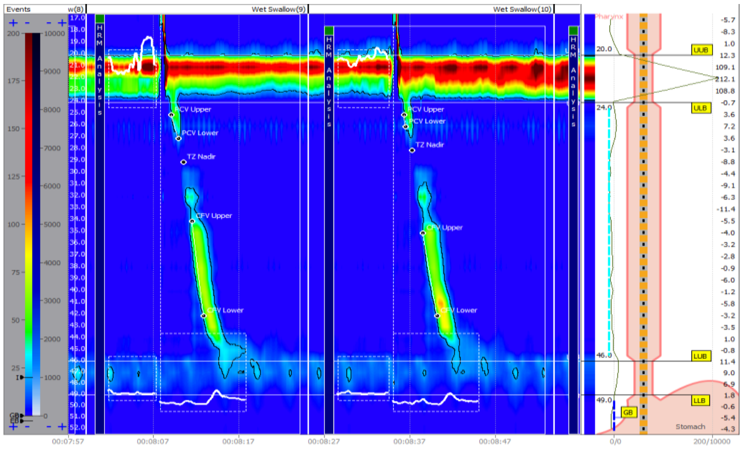

Table 3: The manometric data of LES 1-year after the LSG, no criteria of SED or achalasia as expected

Pressure meas. method	eSleeve	Normal
Basal (respiratory min.)(mmHg)	– 0.8	4.8-32.0
Basal (respiratory mean)(mmHg)	20.2	13-43
Residual IRP (mean)(mmHg)	2.4	<15.0
Residual (highest)(mmHg)	17.9	
Percent relaxation(%)	91	

Table 4: The manometric data of distal esophageal contractility 1-year after LSG (low mean value of DCI = hipocontractility)

High Resolution Parameters		Normal
Distal contractile integral(mean)(mmHg-cm-s)	444.4	500-5000
Distal contractile integral(highest)(mmHg-cm-s)	666.7	
Contractile front velocity(cm/s)	2.9	<9
Intrabolus pressure (@LESR)(mmHg)	– 0.5	<8.4
Intrabolus pressure (avg max)(mmHg)	3.4	<17

Table 5: Detail of all 10 wet test swallows recorded 1-year post LSG
(3 failed with DCI < 100 mmHg-cm-sec, 4 weak DCI < 500 mmHg-cm-sec).

	N	Med	1	2	3	4	5	6	7	8	9	10
DCI distal contractil integral (mmHg-cm-sec)	500-5000	444.4	666.7	N/A	422	182.6	N/A	375.7	557	515.2	391.3	N/A

Our patient experienced dysphagia after laparoscopic sleeve gastrectomy, but the GERD symptoms ameliorate. The HRM showed ineffective esophageal motility (IEM) and not achalasia as expected [16]. IEM is considered a minor motility disorder which can be seen also in asymptomatic patients according to Chicago Classification v3.0, but it was also cited by Del Genio G et al. after LSG [17]. We found 30% increased intragastric pressure (IIGP) after water swallows, corresponding to Mion F et al. data [18]. Further manometric follow-up is needed in this patient to see the contractility evolution pattern.

CONCLUSIONS

High resolution manometry (HRM) was already indicated for the preoperative assessment of peristaltic function in patients who needed antireflux surgery, but we consider that it is necessary to extend the HRM recommendation to bariatric surgery for better management of complications.

Studying the manometric changes in obese patients prior to laparoscopic sleeve gastrectomy is important, because it helps to select those patients who could develop postoperative esophageal motor complications and refractory reflux. In addition to upper GI tract endoscopy, high resolution esophageal manometry (HRM) should be required for all patients planning to undergo a LSG.

A proposed alternative to pharmacological treatment to relieve post laparoscopic sleeve gastrectomy GI symptoms is usually the Roux-en-Y gastric bypass, which is considered an effective method to alleviate symptoms of GERD, but it will not resolve the poor esophageal contractility. In conclusion, conversion to gastric bypass is not an universal method for managing GI complications, especially esophageal dysmotility after LSG, so we need to choose wisely the patient eligible for sleeve.

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REVIEW

Leadership skills in surgeons: a review of the literature

Alexandra Sopu[1], Juergen Apel[1]

Abstract: *Clinicians, professors of medicine in general, students, and professionals from the health sciences tend to focus their efforts on technical training related to their discipline in practice as well as activities that produce some benefit direct or personal satisfaction. It is unusual to devote time and effort to training in areas such as teaching or expand their skills in sociological and organizational aspects. Would you like to take a course on strategic planning if not a director or officer of the hospital? Manage time in your schedule for a workshop on communication and conflict management? Have room in your schedule for educational activities on leadership, organizational change, and diffusion of innovations, science or science complexity of implementation? Generally, doctors and teachers do not spend much time on these activities (at least voluntarily) because we feel that what we have learned from these topics on personal experience is enough [1].*

Effective teamwork, not only in the operating room but also throughout the perioperative route is considered an important and critical component of the safety and effectiveness of surgical care and leads to good results and quick recovery of surgical patients. Therefore, considerable efforts have been made to understand how teams work within the route of surgical care and to improve teamwork [2].

The simple act of bringing together some experts does not guarantee that turned out an expert team. Given the evidence suggests that teamwork in the operating room sometimes is far from harmonious and effective, the importance of training the surgical team to work effectively is receiving international acceptance as a key strategy to maximize safety in surgery.

This systematic literature review aims to present a review of literature that focuses on the importance and role of leadership skills in the surgeon and how these skills can play a significant role in increasing the quality of care and patients' safety in operating room (OR) settings.

Keywords: *leadership, surgeons, operating room, qualities, leader, surgical team, conflict*

INTRODUCTION

Clinicians, professors of medicine in general, students, and professionals from the health sciences tend to focus their efforts on technical training related to their discipline in practice as well as activities that produce some benefit direct or personal satisfaction. It is unusual to devote time and effort to training in areas such as teaching or expand their skills in sociological and organizational aspects. Would you like to take a course on strategic planning if not a director or officer of the hospital? Manage time in your schedule for a workshop on communication and conflict management? Have room in your schedule for educational activities on leadership, organizational change, and diffusion of innovations, science or science complexity of implementation? Generally, doctors and teachers do not spend much time on these activities (at least voluntarily) because we feel that what we have learned from these topics on personal experience is enough [1].

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The way to see the errors and patient safety has changed radically over the last 15 years. Effective teamwork, not only in the operating room but also throughout the perioperative route is considered an important and critical component of the safety and effectiveness of surgical care and leads to good results and quick recovery of surgical patients. Therefore, considerable efforts have been made to understand how teams work within the route of surgical care and to improve teamwork [2].

During the past 15 years, the conceptualization of security in surgery has changed substantially. There is a growing awareness that the skills required to consistently achieve security in surgery go far beyond the knowledge and technical skills of the surgeon [3]. Teamwork, defined in terms of "a set of behaviors, actions, cognitions, and attitudes that facilitate the work to be done" [4], is increasingly seen as an essential component of safe surgery. Work crews are essential when "errors can result in serious consequences; when the complexity of the activity exceeds the capacity of one person; when the working environment is ill-defined or ambiguous or stressful; when you need to make many decisions quickly, and when the lives of others depend on the collective view of the members'. The importance of teamwork to the safety and efficiency of operations has been recognized for some time and, therefore, is an integral part of the education, training, and evaluation of workers in many industries. It seems that, finally, the health community begins to embrace the concept of "work teams are a source of security" [5].

The simple act of bringing together some experts does not guarantee that turned out an expert team. Given the evidence suggests that teamwork in the operating room sometimes is far from harmonious and effective, the importance of training the surgical team to work effectively is receiving international acceptance as a key strategy to maximize safety in surgery. The development of training interventions to provide surgical team knowledge, skills, and attitudes that serve as a foundation for effective performance has become a dominant theme in the literature of surgery, anesthesia, and nursing.

OBJECTIVE

This systematic literature review aims to present a review of literature that focuses on the importance and role of leadership skills in the surgeon and how these skills can play a significant role in increasing the quality of care and patients' safety in operating room (OR) settings.

METHODS

A systematic review of the available literature was performed on leadership skills in the operating room. The search was made on the PubMed, JSTOR, and Cochrane library databases on June 10th, 2020 using the following formula: (Leadership skills AND Surgeons AND Operating Room). First, the articles were screened by title and abstract. The following inclusion criteria for relevant articles were used during the initial screening of titles and abstracts: must focus on leadership skills of surgeons, must focus on models of leadership for surgeons, and must focus on skills required by surgeons to be good leaders for the OR team. Other exclusion criteria were: studies published before 2010, studies other than the English language, studies conducted in under-developed countries, studies focusing on non-technical skills other than leadership skills.

In the second step, the full texts of the selected articles were screened, with further exclusions according to the previously described criteria. Moreover, articles not reporting clinical results were excluded. Relevant data were then extracted and collected in a single database with a consensus of the two observers to be analyzed for the present manuscript.

Table 1 provides the list of articles that are included in the review.

Table 1: List of articles included in the review.

Author (year)	Title
Yue-Yung Hu, Sarah Henrickson Parker, Stuart R Lipsitz, Alexander F Arriaga, Sarah E Peyre, Katherine A Corso, Emilie M Roth, Steven J Yule, Caprice C Greenberg (2016).	Surgeons' Leadership Styles and Team Behavior in the Operating Room
Arora, S., Hull, L., Fitzpatrick, M., Sevdalis, N. and Birnbach, D. (2015).	Crisis Management on Surgical Wards.
Balch, C., Shanafelt, T., Sloan, J., Satele, D. and Freischlag, J. (2019).	Distress and Career Satisfaction Among 14 Surgical Specialties, Comparing Academic and Private Practice Settings.
Culley, D., Fahy, B., Xie, Z., Lekowski, R., Buetler, S., Liu, X., Cohen, N. and Crosby, G. (2014).	Academic Productivity of Directors of ACGME-Accredited Residency Programs in Surgery and Anesthesiology.
Danis, D., Klinkner, G., Malec, A. and Rees, S. (2007).	Improving Patient Safety by Standardizing Handoff Communications.

Author (year)	Title
Dominguez, D., Garcia, C. and LaFrance, K. (2013).	Developing A New Graduate Program In Healthcare Management: Embracing The Transformation Of Healthcare Management Education On A Pathway To Success.
Doumouras, A., Keshet, I., Nathens, A., Ahmed, N., and Hicks, C. (2012).	A Crisis of Faith? A Review of Simulation in Teaching Team-Based, Crisis Management Skills to Surgical Trainees.
Graafland, M., Schraagen, J. and Schijven, M. (2012).	Systematic review of serious games for medical education and surgical skills training.
Hudspeth, R. (2008).	Complex Healthcare Regulation.
Khatrouch, I., Kaddes, M., Kermad, L., Boujelbene, Y. and ElMhamedi, A. (2013).	New approach for building multidisciplinary teams: case of surgical teams.
Kothari, S., Brethauer, S., Rosenthal, R., Nguyen, N. and Morton, J. (2015).	Response to “Ensuring Excellence in Centers of Excellence Programs”.
McKean, S. (2012).	Principles and practice of hospital medicine.
Mitchell, L., Flin, R., Yule, S., Mitchell, J., Coutts, K. and Youngson, G. (2011).	Thinking ahead of the surgeon. An interview study to identify scrub nurses’ non-technical skills.
Mumghamba, E. (2013).	Integrating a Primary Oral Health Care Approach in the Dental Curriculum: A Tanzanian Experience.
Parker, S., Flin, R., McKinley, A. and Yule, S. (2019).	The Surgeons' Leadership Inventory (SLI): a taxonomy and rating system for surgeons' intraoperative leadership skills.
Parker, S., Yule, S., Flin, R. and McKinley, A. (2012).	Surgeons' leadership in the operating room: an observational study.
Patel, V., Warren, O., Humphris, P., Ahmed, K., Ashrafian, H., Rao, C., Athanasiou, T. and Darzi, A. (2010).	What does leadership in surgery entail?
Siassakos, D., Fox, R., Bristowe, K., Angouri, J., Hambly, H., Robson, L. and Draycott, T. (2020).	What Makes Maternity Teams Effective and Safe? Lessons From a Series of Research on Teamwork, Leadership, and Team Training.
Sittig, D. (2011).	Defining Health Information Technology–Related Errors.
Stevens, L., Cooper, J., Raemer, D., Schneider, R., Frankel, A., Berry, W. and Agnihotri, A. (2017).	Educational program in crisis management for cardiac surgery teams including high realism simulation.
Thomas, E., Sexton, J. and Helmreich, R. (2003).	Discrepant attitudes about teamwork among critical care nurses and physicians*.
Valentine, M., Nembhard, I. and Edmondson, A. (2014).	Measuring Teamwork in Health Care Settings.
Winlaw, D., Large, M., Jacobs, J. and Barach, P. (2011).	Leadership, surgeon well-being and non-technical competencies of pediatric cardiac surgery.
Yeung, J., Ong, G., Davies, R., Gao, F. and Perkins, G. (2012).	Factors affecting team leadership skills and their relationship with quality of cardiopulmonary resuscitation
Yule, S. and Paterson-Brown, S. (2012).	Surgeons’ Non-technical Skills.

RESULTS

Importance of Leadership and Teamworking for Surgeons

From the technological point of view, surgery is highly complex and is a practice that faces a constantly changing scenario. The surgeon works in the Department of Surgery, often in a specialized unit, and participates with interdisciplinary teams, and develops their daily clinical practice with other specialists and technicians working in different settings (such as operating room, ward) [6]. The primary objective of a surgeon is to provide the best patient care, with greater safety. When required today in most prestigious forums, safe surgery for the patient, it must be remembered that the complications that arise during treatment (about 45% in surgical patients) are rarely the result of the failure of a single individual, but in over 80% of cases they are related to poor planning of patient care systems, or with failures in teamwork [7].

A significant number of studies draw attention to the importance of effective teamwork, which by nature must be interdisciplinary, to get quality treatment over the surgical patient stays. Among them are two: the one entitled “To Err is Human: Building a Safer Health System” [8] published by the Institute of Medicine in 2000, in which the high percentage of errors detected was evaluated in the Health System of the USA and the well-known Kennedy Report in May, which analyzed the very high mortality of pediatric cardiovascular surgery service in Bristol, UK. As a result of the Surgery Quality Improvement Project with which has reduced mortality by a third and two-fifths in morbidity in all the services of surgery [9]. They participated in June. The overall analysis has led to a clear conclusion: the causes of human errors and their adverse consequences for the patient are deficiencies related to teamwork, as well as the communication between its members.

While the topical figure of an individualist and arrogant surgeon, and even violent with his surgical environment, has already been exceeded, it is not uncommon that surgeons think they work together, when in fact, most do in the group [10]. Team working requires leadership essentially, to ensure that decisions making is done by consensus, analyze the reasons for the disagreements and try to fix them, ensure that the objectives are understood and accepted by the team, encourage all members to contribute their ideas, require the operation of the equipment is periodically reviewed [11]. It can only be made possible by the work of each of its members to appreciate, and finally, that leadership is shared, as necessary. Teamwork is not easy, but with good leadership and proper training of its members in cognitive abilities, and personal relationships, it can be achieved.

The results of recent research, advise that surgical services training programs to develop teamwork in specific tasks (operating room, emergency department, hospital ward), as has already been done, and successfully in other high-risk professions, such as aviation, by applying the methodology called crew resource management (CRM). The key elements of this methodology that can be applied to the work of the surgeons are: the "briefing" (a previous discussion of the action plan), the question between senior member and juniors team of decisions, and take "de-briefing" sessions (understood as the discussion of what happened afterward). Compliance with these requirements can only be achieved in an atmosphere of work and communication between members of a team in which everyone is always willing to learn [12].

The "Non-technical skills for surgeons' project, designed by the University of Aberdeen, has identified several cognitive skills and personal relationships, which must be the essential complement to the expertise of the surgeon, to keep the OR, high levels of quality and safety [13]. These skills include the surgeon's ability to exercise leadership, the state of permanent alert, the ability to take the right decision at all times, and the ability to stimulate communication and teamwork. It is currently available instruments to assess, on stage at the theatre, the teamwork, and skills that are not just technical.

Several studies have shown a relationship between teamwork (surgeons, anesthetists, and instrumentalists) [14], the "performance" and safety in the operating room. It is estimated that up to 1/3 of the duration of the activity in an operating room, communication failures occur in the team. 33% of these failures originate delays, workarounds an unsolved problem ("workaround"), increased environmental stress, unnecessary expense, and disturbance to the patient. A recent study assessed for the first time, the impact that a training course in non-technical skills (CRM methodology) had on the results of two types of surgery, showing that the improvement observed in the teamwork produced the best technical results. These data confirm, albeit in a different field, the results of the multicenter project MedTeams [15], in the US, where the application of CRM in the emergency services model was evaluated and found to a reduction in clinical errors (30.9 to 4.4%) and improved teamwork and attitudes of its members [16]. This is the clearest example of how improved teamwork can avoid complications. It is possible that system-based training, as well as patient simulators that allow the practice of specific behaviors, can avoid the need for real instructors.

However, since efficient teamwork does not arise spontaneously, should be encouraged as soon as possible the development of new hospital culture, for the hospital administration and the Department of Surgery must provide the training tools to help its implementation. But above all, it is essential to have well-trained surgical leaders, able to motivate and change, for the good behavior of team members, with the ultimate goal, to always be the best possible result for the patient. It would be desirable that scientific societies and the NHS itself develop programs similar to the Royal College of Surgeons of England, aimed at the specific training of leaders, and their corresponding surgical equipment, improving, no doubt, patient safety [17].

Leadership in Surgeons

Leadership is one of the most important qualities for a successful surgeon. Surgical outcomes depend critically on a surgical team rather than on an individual; therefore, the role of surgeons is essential for optimal management of patients in a surgical service [18]. There are different kinds of leadership: one who asks the group to follow him, the group encouraged to follow you, or those who are forced to follow. It is important to remember that the most effective leader is using different styles at different times. One should be aware of the strengths and weaknesses in him/her as a leader and recognize what kind of leadership is most often used, as must implement leadership strategies in line with the situation [19].

Several points should be present in every surgeon in assuming the leadership role within a surgical team and in OR [20]:

- One must learn the art of conflict resolution: it is important to remember that there is a conflict over a point of view and always more of a perception of truth. The surgeon should try to understand each different point and perspective, but it has not always solved them, has to determine the ideal to maintain the cohesion of the group and its mission.

- Role model: is defined as a guide; they are people showing the right path to be followed, through their actions and behaviors. It is a huge responsibility and a great opportunity to demonstrate their attitudes and skills. They can demonstrate such using a suitable treatment with the patient, excellent academic foundation, and good relationships.
- Counsellor: young interns and residents come daily to the surgeon with conflicts, patient care questions, personal problems, etc. In these situations, the surgeon should be a director and confidant. He/she should be affable, confident ear, who can provide advice on mental and physical health for the residents. Also, he/she helps monitor the stress level of the residents.
- It is the right program director to indicate the stress level of the residents; remember the old phrase of "praise in public and criticize in private". Feedback should attentively, timely, and honest, but critical. Compliments are an effective way to encourage positive behavior, academic, interpersonal, and professional effort among residents.

CONCLUSIONS

In conclusion here are some final tips for excellent leadership development for surgeons:

1. Be seen as someone approachable and trustworthy; residents gain a better understanding as people.
2. Time Management: schedule daily activities; new things appear daily, so the surgeon should learn to prioritize the moment they happen.
3. Surgeons must take time for personal growth and professional development.
4. Surgeons must create a relationship with their boss, additional close work with the program director.

Limitations of this Study

Since this is a systematic literature review, therefore, the main limitation of this study is that it lacks primary research. This is a purely secondary research study although the research reviewed in this review does contain primary research evidence.

Also, the author of this review could only conduct searches in only three databases. Another limitation of this review is that it includes studies in English only and therefore studies published in another language have been excluded.

The scope of the review is also limited because it focuses on the leadership skills of surgeons only and does not focus on the leadership skills of other staff in the operating room. Although there are several limitations in this review yet as explained in previous sections the search strategy is comprehensive and systematic which ensures reliability and validity of the results and findings.

Implications of this review

The main implication of this review is for surgeons and students. They can understand the importance of leadership skills and include the development of leadership skills in their development plans.

Besides, the management and administration of hospitals can also benefit from this review and motivate surgeons and other staff to develop leadership skills and ultimately maximize the quality of care and patients' safety in their hospitals.

The review stresses the importance of leadership skills and therefore it recommends that administrations should plan and implement training courses for surgeons and OR staff to enhance their leadership skills.

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Maintenance pharmacologic therapies for opioid use disorders: beyond opioid agonists

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Abstract: *Opioid use disorder is a worldwide challenge for an increasing number of patients, and still the therapeutic options presented in clinical guidelines for this condition are very few. The first line pharmacological therapies for opioid use disorder continue to be opioid agonists as substitution treatment, but the need to find new treatments without the effect of prolonging opioid dependence become more and more obvious. This systematic review presents the most supported by evidence pharmacological agents that can be used as monotherapy for opioid use disorder patients (i.e., naltrexone, extended release/implant and oral formulation), or as adjuvants to substitution therapy (e.g. olanzapine for aggressive behaviours, memantine or dextromethorphan for reducing inflammatory processes related to opioid use). More good quality data derived from larger, well designed trials are needed in order to find solid recommendations for non-opioid agonists in the treatment of opioid use disorder.*

Keywords*: opioid use disorder, naltrexone, memantine, dextromethorphan, olanzapine, maintenance treatment*

INTRODUCTION

The importance of opioid dependence could not be overemphasized, and during the last decade a continuously increasing number of alerts have appeared both in public media and scientific environment regarding the negative functional impact, increasing societal costs and mortality risk associated with this pathology.

Opioid dependence is a significant contributor to the global disease burden, as between 24 and 35 million people globally used an illicite drug from this category in 2010 [1,2]. North America, Eastern Europe and Southern sub-Saharan Africa are the most strongly affected by the premature mortality related to opioid use disorder [1]. United Nations Office on Drugs and Crime 2017 Report shows an overall increase of the heroin and synthetic opioids use (oxycodone, hydrocodone, tramadol, and fentanyl) since 2009 and the trend to more diversification of the opioid market [3]. Disability-adjusted life years (DALY) attributable to opioid dependence in 2015 reached the value of 12 million, or 70% of the global burden of disease attributable to drug use disorder [4]. Relapse rate after detoxification is very high, ranging from 72% to 88% after 12-36 months [5].

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Given consideration to the importance of opioid use disorder both at individual and societal level, worldwide efforts have been constantly made in order to find treatments able to prevent opioid use relapse and to maintain patients in remission as long as possible. The most validated treatments for the maintenance phase are methadone and buprenorphine, both with agonist actions at μ-opioid receptors (the first being a partial agonist, while the second is a full agonist). Unfortunately, due to their mechanism of action, both these drugs have abuse potential (less in case of buprenorphine) and associate withdrawal symptoms (although milder than in case of heroin or morphine). Therefore, the need to find non-addictive agents which can be used during the maintenance phase is considered urgent.

OBJECTIVE AND METHODS

This systematic literature review has as primary objective finding non-opioid agonist treatments for the maintenance phase in opioid use disorder patients, based on good quality data. The secondary objective is to formulate evidence-based recommendations for this indication, starting from existing data in the literature.

Table 1. Inclusion/exclusion criteria.

Operational criteria	Inclusion criteria	Exclusion criteria
Population	Minimum age for inclusion was 18 years, no superior age limit was established. The main diagnosis was opioid use disorder. Secondary diagnoses were allowed, if the study design permitted statistical analysis for at least one outcome regarding opioid use disorder. Diagnosis was established according to DSM-IV, IV TR, 5 or ICD-10 criteria.	Patients under age of 18. Presence of severe somatic or psychiatric co-morbidities that may have impact over patients' outcomes, or due to their treatment may lead to confusion about the outcome by pharmacokinetic or pharmacodynamic interactions.
Intervention	Any pharmacological agent without agonism (either partial or complete) over opioid receptors, either as add-on or as monotherapy. Combined pharmacological and psychological treatment. Treatment duration over 4 weeks.	Psychotherapy or psychological counselling as only type of intervention. Non-pharmacological and non-psychotherapeutical interventions, OTC, investigational products used in phase I, II or III clinical trials.
Environment	Inpatients, outpatients. Hospitals, day care, special care, intermediate care units. Carceral environment.	Unspecified environment.
Primary and secondary outcomes	Changes in opioid use- related parameters, e.g. days of abstinence, number of toxicology tests negative, rate of rehospitalisation for opioid consumption. At least one outcome is related to the opioid use.	All trials with outcomes not related to the severity of opioid use disorder.
Study design	Prospective or retrospective clinical trials. Randomized, open label, single blind, or double blind. Case control studies. Epidemiological studies. Observational trials.	Trials with unspecified or insufficiently specified design. Variables referring to opioid use disorder were not included in statistical analysis, or not presented in the published paper. Systematic review, narrative reviews, meta-analyses, case reports, case series.
Language	English, French, German, Russian	Any other language, except for those already specified.

Methods were based on the qualitative review of data found in the main electronic databases- PubMed, Complete MEDLINE, EMBASE, Cumulative Index to Nursing and Allied Health Literature (CINAHL), National Library of Medicine and the National Institute of Health (NCBI), PsychInfo and Thomson Reuters/Web of Science. All clinical trials and epidemiological studies published between 1998 and 2018 in English, French, German, and Russian were included in the primary review. The search paradigm used was „opioid dependence", „opiate dependence", „opioid/opiate use disorder", combined with „non-opioid agonists" or „non-opioid pharmacological treatment". This literature review is based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P) principles of research [6].

Inclusion and exclusion criteria were applied to all papers found during primary search (table 1).

Therapeutic intervention was focused on drugs with non-opioid agonistic properties. If any psycho-therapeutic intervention was administered, than it was only as an add-on to the pharmacologic management, and not the main method of treatment. Because this review was focused on maintenance therapy, brief interventions were not included. The outcomes of therapeutic intervention had to be periodically evaluated, and statistically analysed. Only trial which produced inferential, statistically-based data were included in the final step of the review.

During the secondary analysis, papers were reviewed and their results were analyzed. Only papers to which full text was found were included in the final analysis.

All studies were ranked according to their level of significance (LOS). This level was determined based on the quality of the study design: (5) double-blind randomized controlled trial, with/without an active comparator/placebo; (4) single-blind trials, with/ without an active comparator/placebo; (3) open-label trials with/without an active comparator; (2) retrospective studies, observational trials; (1) any other study design.

GRADE recommendations for the level of evidence [7] were formulated based on the evidence found in the secondary analysis. GRADE recommendations included 5 levels: A= high quality= future research are very unlikely to change the validity of these recommendations; B= moderate quality= future research is likely to have an important impact on the appreciation of the recommendations; C=low quality= it is very likely that future research will have an important impact over the confidence in the estimate of effect and is likely to change the recommendations; D= very low quality= any estimate of the effect is very uncertain. Intermediate steps (+ or -) represent nuances of the recommendations based on the quality of trials and tolerability of intervention, as resulted from these trials [7].

RESULTS

After the primary search 2560 papers surfaced, but only 65 remained when the inclusion and exclusion criteria were applied (figure 1). A number of papers were excluded because full text was not found, so that 29 studies remained to be reviewed in the final step. All trials included in the secondary review are presented in table 2.

These 29 trials included in the secondary analysis had a total number number of participants of 3,159, and a mean LOS of 3.58. A number of 13 trials were found to be high quality designed trials (LOS=5), meaning double-blind, either placebo-controlled or with an active comparator, randomized clinical trials.

Analysis of interventions corresponding to the inclusion/exclusion criteria previously mentioned showed that non-opioid agonist drugs used in the maintenance phase in patients with opioid use disorder who have been previously detoxified were: (1) naltrexone implant; (2) oral naltrexone; (3) topiramate; (4) memantine; (5) dextromethorphan; (6) dronabinol; (7) olanzapine; (8) baclofen; (9) ibudilast; (10) buspirone; (11) sodium valproate; (12) fluoxetine; (13) paroxetine; (14) clonazepam; (15) gabapentin. The last five drugs were used only in combination with another active medication or as active comparators, so they were not evaluated separately in this review.

Figure 1: Stepwise filtration of titles found during electronic databases search

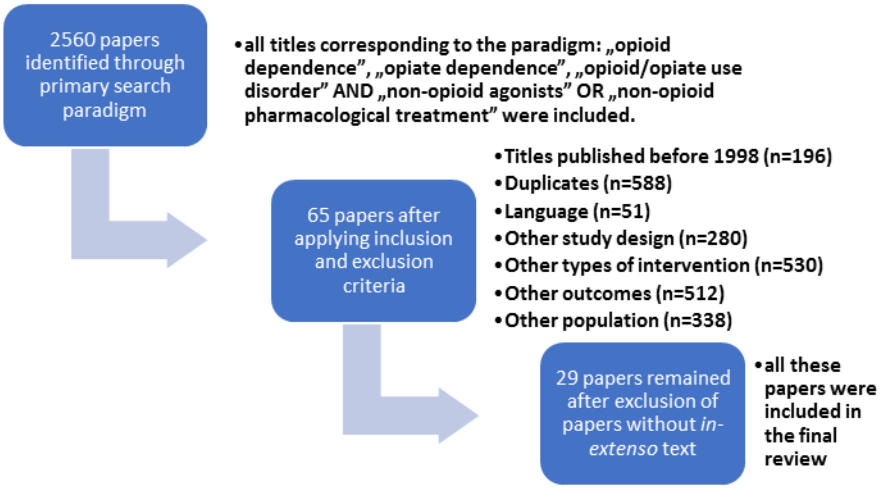
2560 papers identified through primary search paradigm
•all titles corresponding to the paradigm: „opioid dependence", „opiate dependence", „opioid/opiate use disorder" AND „non-opioid agonists" OR „non-opioid pharmacological treatment" were included.
65 papers after applying inclusion and exclusion criteria
•Titles published before 1998 (n=196)
•Duplicates (n=588)
•Language (n=51)
•Other study design (n=280)
•Other types of intervention (n=530)
•Other outcomes (n=512)
•Other population (n=338)
29 papers remained after exclusion of papers without *in-extenso* text
•all these papers were included in the final review

Naltrexone was the most investigated non-opioid agonist medication for the maintenance therapy in opioid use disorder, and 15 relevant trials focus on this drug's efficacy have been found during the secondary search. Naltrexone is an opioid antagonist, and both the parent drug and its active metabolite (6β-naltrexol) are competitive antagonists of the μ-, and to a lesser extend to κ- and δ-opioid receptor (10-25 times lower affinity for κ- and δ-receptors compared to the μ-receptors) [8]. Because oral naltrexone is extensively metabolized during first-pass metabolism, and because therapeutic adherence of opioid users is known to be very low, various implant formulations of this pharmacological agent have been developed.

Oral formulation of naltrexone has been investigated in comparison with buprenorphine/naloxone in the post-detox phase [9], methadone in stabilized patients [10], buprenorphine and placebo in detoxified patients [11], methadone in opioid dependent patients who were stablized on methadone maintenance treatment for controlling depressive and anxiety symptoms [12]. Oral naltrexone as monotherapy was compared with naltrexone plus gabapentin (1200 mg/day) for the management of muscle pain and craving [13], and naltrexone plus placebo was compared with naltrexone plus sertraline for controlling depression severity and opioid craving [14]. Another trial compared naltrexone (50 mg/day) + fluoxetine (20 mg/day) with naltrexone + placebo, fluoxetine + placebo, or placebo for 6 months plus psychotherapy for all groups for the monitoring of remission rate during 6 months [15]. The mean LOS of these studies was 3.85, and an overall number of 657 patients were included.

These trials supported a moderate efficacy and tolerability of oral naltrexone: buprenorphine/naloxone was superior to naloxone in the reduction of opioid-positive urine test and preserving patients participation in the treatment [6], naltrexone induced similar weight gain during 6 months of treatment when compared to methadone [16], naltrexone may improve depression in patients with opioid use disorder at 6 months [12], and naltrexone was inferior to buprenorphine (not statistically significant) in several outcomes regarding the rate of relapse in detoxified heroin dependent patients [11].

Naltrexone plus fluoxetine and individual psychotherapy has been proven a useful combination especially in women with heroin dependence, because it increased the remission rate and maintenance of remission in this population [15]. Naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine [13]. Naltrexone plus sertraline was a well-tolerated combination and increased on short-term treatment retention of recently non-depressed opioid abstinent patients, but the difference between this combo and naltrexone alone disappeared in time [14].

Naltrexone extended-release has been investigated in comparison with buprenorphine/naloxone [16], treatment as usual [5], oral naltrexone plus psychosocial therapy [17], and methadone [18]. Naltrexone implant was investigated also in several trials as single pharmacological agent, without an active comparator [19-22]. The mean LOS of these trials was 2.8, and the global number of participants was 1982.

The overall efficacy of naltrexone extended-release was good, although data are derived from poor and medium-quality designed trials. Its efficacy and tolerability were similar to buprenorphine/naltrexone in patients who wanted to switch from this combination of drugs to naltrexone implant [16], and it induced a lower rate of relapse when compared to treatment as usual, at least on short-term [23]. Heroin dependent patients who received extended release formulation of naltrexone had better outcomes than those treated with oral formulation of the same drug [17], and naltrexone implants but not methadone were associated with long-term benefits in decreasing opioid-related hospital morbidity [18].

In trials without an active comparator, naltrexone extended-release was efficient in preventing opioid overdose [22], improved depression severity at 4 weeks post-baseline, but it may also be responsible of worsening late insomnia [21], improved quality of life, rate of retention, opioid-negative urines, opioid craving, and rate of re-employment [20]. Also, naltrexone plus psychological counselling improved the acceptance of opioid dependence pharmacotherapy and prolonged the duration of abstinence to opioids, when compared to placebo plus counselling [19].

Topiramate is an anticonvulsant with GABA-enhancing properties and AMPA and kainate-inhibitory actions, involved in the modulation of extracellular dopamine release in the mesocortical and limbic system dopaminergic system, properties which recommended it as a potential treatment for cocaine dependence [24]. Patients with opioid use disorder who presented also cocaine dependence did not benefit from the addition of topiramate to their methadone maintenance therapy, because no difference to placebo could be found after 12 weeks [25].

Ibudilast is an anti-inflammatory drug with phosphodiesterase inhibition properties (especially PDE4) which has been associated in animal studies with the capacity of alterating several opioid-mediated effects [26, 27]. When compared with placebo in a 6-week RCT in patients detoxified from morphine, ibudilast treated patients presented lower rates of drug liking (oxycodone), lower craving to heroin/ tobacco/cocaine and lower subjective ratings of pain vs. placebo [26].

Memantine is a noncompetitive NMDA-receptor antagonist that blocks the effects of excessive concentrations of glutamate, currently used for Alzheimer Disease- related neurocognitive disorder and researched intensively for other dementias, ADHD, and as an agent able to reverse tolerance to opioids [28]. Memantine was administered in patients who were on buprenorphine/naloxone maintenance treatment, and compared to placebo [29], or in patients who were on methadone-maintenance-therapy, compared to placebo plus methadone-maintenance therapy [30, 31]. Memantine was also investigated as maintenance treatment in detoxified non-treatment seeker heroin dependent patients during 8 weeks of in-patient regimen [32].

These trials investigating the efficacy of mematine had a mean LOS of 4.25, and an overall number of 350 participants. It can be observed that the quality of trials focused on memantine efficacy was higher than previously mentioned research for other investigational products.

Memantine has been proven as a useful adjunctive medication for patients treated with buprenorphine/naloxone and it may provide an easier transition to buprenorphine-free therapeutic regimen [29]. Low dose memantine attenuated chronic opioid-dependence-induced cognitive impairment, decreased the daily dose of methadone, and had positive effect over probably NMDA-mediated inflammatory processes [30, 31]. Memantine decreased the subjective, but not the reinforcing effects of heroin in non-treatment seeker heroin-dependent patients [32].

Dronabinol is a synthetic form of 9-tetrahydro-cannabinol, with similar affinity for cannabinoid 1 and 2 receptors, currently approved for use in anorexia associated with weight loss in AIDS and for the treatment of nausea associated with chemotherapy resistant to other anti-nausea treatments [33]. A double-blind, placebo-controlled RCT investigated the effects of dronabinol in patients with naltrexone extended release and a significant effect of this drug in decreasing the severity of opiate withdrawal has been proven [34].

Dextromethorphan is an antitussive drug with N-methyl-D-aspartate (NMDA) receptor antagonist properties, involved also in protecting monoaminergic neurons against inflammation-mediated degeneration [35, 36]. Chronic opioid administration produces systemic inflammation, and for this reason the use of a drug with anti-inflammatory properties seems to be granted [37]. Based on its neuroprotective and anti-inflammatory properties, dextromethorphan was investigated as adjunctive agent in patients with opioid use disorder. Dextromethorphan 60 mg/day and 120 mg/day were compared with placebo as adjunctive to methadone treatment in opioid-dependent patients [37, 38], and in another trial dextromethorphan 240 mg/day was compared with placebo in methadone-stabilized patients [39].

These trials with dextromethorphan had a mean LOS of 5, and a total of 268 patients.

Dextromethorphan was useful in low doses as adjuvant treatment in methadone-treated patients both at inflammatory level and clinical level [37, 38], but in another trial the difference versus placebo was not significant in the otcome defined as mean methadone consumption [39]. The quality of life was superior in patients who reeived dextromethorphan as adjuvant to methadone versus placebo plus methadone [39].

Olanzapine is an atypical antipsychotic investigated in patients with opioid dependence who were on substitution therapy, as a drug targeting the aggresive behavior [40, 41]. The mean LOS of these trials was 3.5, and the total number of participants was 268.

In the first trial, olanzapine (2.5-15 mg) was compared with sodium valproate (600-1000 mg) in patients stabilized on methadone, and olanzapine was slightly superior in controlling aggressivity but did not differed from valproate in reducing the number of positive urine samples [40]. In the second, smaller-sample size, olanzapine was compared to fluoxetine/paroxetine and clonazepam and reduced aggressive/hostile behavior in heroin addicted patients undergoing substitution therapy [41]. In this second trial, olanzapine was superior to its comparators in the direct aggressiveness, overall hostility, verbal aggressiveness, and aggressive incidents; also, 70% of the completers achieved full remission, without significant differences between groups [41].

Buspirone is a non-benzodiazepine anxiolytic which possesses 5HT1A agonistic properties, acting as full agonist at 5HT1A presynaptic receptors and partial agonist at postsynaptic 5HT1A receptors [42]. A single trial that compared buspirone with placebo in patients with anxiety undergoing methadone therapy has been identified during the literature review [43]. Buspirone had no significant efficacy over anxiety symptoms in these patients, but it decreased depression severity and slowed down the return to substance abuse [43].

Baclofen is a GABA-B receptor agonist that may be helpful in promoting abstinence from a variety of drugs [44]. A double-blind, placebo-controlled, 12-week RCT with a LOS of 3, which included 40 patients with opioid dependence, recently detoxified, found a significantly better treatment retention in the baclofen-treated patients (60 mg/day) [45]. This trial had a small sample size and a high attrition rate, which make difficult to generalize its results.

Table 2. Trials focused on non-opioid agonist therapies during the post-detoxification phase in patients with opioid use disorder

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Pirnia B, Soleimani AA, Malekanmehr P, et al. [25]	Placebo-controlled RCT; topiramate (25-300 mg/day), 12 weeks, escalating doses; patients were treated also with methadone 20-140 mg/day and brief behavioral compliance enhancement treatment for cocaine dependence.	n=50 patients with dual cocaine and opiates dependence	Weekly urine drug screens.	Topiramate was no better than placebo in reducing cocaine use, but was superior to placebo in reducing cocaine craving.	The efficacy of topiramate in cocaine use reduction in patients with dual cocaine and opiates dependence was not supported.	5
Solli KK, Latif ZE, Opheim A, et al. [16]	Prospective cohort study, naltrexone-extended release vs. buprenorphine-naloxone 3 month + participants' choice of study medication for an additional 9 months; the active drug was administered every 4 weeks (380 mg).	n=54 opioid-dependent adults continuing naltrexone-extended release and n=63 opioid dependents inducted on the same treatment	Use of heroin and other illicit substances, opioid craving, treatment satisfaction, treatment retention, adverse events, addiction-related problems	Follow-up after 9 months showed completion rates of 51.9% among participants continuing naltrexone-extended release in the follow-up and 47.6% among those inducted on the active treatment, with opioid abstinence rates of 53.7 and 44.4%, respectively.	Opioid-dependent individuals who elect to switch from buprenorphine-naltrexone after 3 months to extended release naltrexone 9 months were experiencing similar results (treatment completion and abstinence rates) and similar rate of adverse events to those who had been on extended-release naltrexone from the start.	4
Metz VE, Jones JD, Manubay J, et al. [26]	6-week RCT focused on ibudilast (a nonselective phosphodiesterase inhibitor) effects over craving, reinforcing, analgesic effects of oxycodone in human volunteers diagnosed with opioid dependence; patients received maintenance treatment with ibudilast (50 mg bid) or placebo	n=11 patients detoxified from morphine addiction	VAS for measurement of subjective effects of oxycodone.	Patients receiving active ibudilast presented decreased rates of drug liking after 15 mg of oxycodone; heroin/ tobacco/cocaine craving was reduced under active ibudilast vs. placebo; mean subjective ratings of pain were lower in the active ibudilast condition.	Ibudilast may be useful in treating opioid use disorder and may enhance the analgesic effects of oxycodone.	3

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Earley PH, Zummo J, Memisoglu A, et al. [20]	Single-arm, multisite, open-label study; extended release naltrexone injections for up to 24 months, combined with counselling via intensive outpatient substance abuse treatment programs.	n=38 enrolled opioid-dependent healthcare professionals who had been detoxified for at least 2 weeks	Opioid drug tests monthly, measurements for quality of life, opioid craving, and treatment satisfaction	At 24 months, mean opioid craving fell by 45.2% and quality of life (SF-36) -mental component score improved by 31.1% from baseline; of the 22 unemployed subjects at baseline, 45.5% improved employment status at 24 months.	Long-term extended release naltrexone was associated with better quality of life, rates of retention, opioid-negative urines, opioid-craving reduction, re-employment	3
Lee JD, Friedmann PD, Kinlock TW, et al. [23]	Open-label RCT, 24-week extended-release naltrexone vs. usual treatment (brief counseling and referrals for community treatment programs);	n=308 criminal justice offenders with history of opioid dependence, abstinent from opioids at the time of randomization	Time to an opioid-relapse event (defined as 10 or more days of opioid use in a 28-day period).	Participants assigned to extended-release naltrexone had a longer time to relapse than did those who received usual treatment, lower rate of relapse, and higher rate of opioid-negative urine samples. After one year, the rates of opioid-negative urine samples were equal. During one year, there were no overdose events in the extended-release naltrexone treated participants, while in the usual-treatment group there were 7 cases.	Extended-release naltrexone was associated with a rate of opioid relapse lower than that with usual treatment, but after one year the protective effects waned.	3
Mokri A, Chwarski MC, Taherinakhost H, Schottenfeld RS [9]	Single-site, two-parallel groups, double-blind RCT, 12-week; oral naltrexone vs. buprenorphine/ naloxone.	n=102 participants with opioid use disorder in the post-detox phase	Initial duration of opioid abstinence (urine toxicology tests).	Mean number of days of initial duration of verified abstinence was higher with buprenorphine/naloxone (p=0.205); mean number of opioid-negative urine tests was higher in the buprenorphine/ naloxone-treated subjects (p=0.049); the mean number in treatment was also greater in buprenorphine/naloxone group (p=0.013); the rate of sustained, 12-week opioid abstinence was double in the buprenorphine/ naloxone group.	Sublingual buprenorphine/ naloxone was associated with a greater number of opioid-negative urine tests and treatment retention, but not significantly greater initial abstinence duration or proportions with sustained abstinence.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Gonzalez G, DiGirolamo G, Romero-Gonzalez M, et al. [29]	Double-blind placebo-controlled RCT, 13 weeks; patients already received treatment with buprenorphine/naloxone 16/4 mg/day and they were randomized to memantine 15 mg or 30 mg vs. placebo.	n=80 young adults with opioid dependence	Change in the weekly mean proportion of opioid use, and cumulative abstinence rates after rapid buprenorphine discontinuation at week 9	Memantine 30 mg/day significantly improved short-term treatment with buprenorphine/ naloxone by reducing relapse and opioid use after buprenorphine discontinuation, during the last 2 weeks of study participation.	Memantine could be an useful adjunctive treatment at 30 mg daily for patients treated with buprenorphine/ naloxone and helpful in the transition to buprenorphine-free therapeutic regimen.	5
Bisaga A, Sullivan MA, Glass A, et al. [34]	Double-blind RCT, dronabinol 30 mg/day (n=40) vs. placebo (n=20); patient underwent inpatient detoxification for opioid dependence and extended-release naltrexone induction; dronabinol was administered while inpatient and 5 weeks afterwards.	n=60 patients recently de-toxified from opioids	Severity of opioid withdrawal and retention in treatment.	The severity of opioid withdrawal during inpatient phase was lower in the dronabinol group vs. placebo; rates of successful induction onto extended release naltrexone and completion of treatment were not significantly superior for dronabinol vs. placebo.	Dronabinol reduced the severity of opiate witdrawal but had no effect on rates of extended-release naltrexone treatment induction and retention.	5
Chang YH, Chen SL, Lee SY, et al. [30]	RCT, memantine plus methadone-maintenance-therapy (n_1=57) vs. placebo plus methadone-maintenance therapy (n_2=77); healthy controls were matched with methadone-treated group; 12 weeks duration of monitoring.	n=134 patients stabilized on methadone	Cognitive performances	Both treatment groups showed improvements in their cognitive performances. Memantine add-on was superior to placebo in the post-treatment cognitive improvement.	Low-dose memantine (5 mg/day) may attenuate chronic opioid-dependence-induced cognitive decline.	5
Lee SY, Chen SL, Chang YH, et al. [37,38]	12-week RCT, dextrometorphan 60 mg/day (n_1=65), 120 mg/day (n_2=65) vs. placebo (n_3=66) as adjunctive to methadone treatment in opioid-dependent patients.	n=196 opioid dependents	Methadone dose required, plasma morphine level, retention in treatment.	Patients who received 60 mg/day dextromethorphan had significantly longer treatment retention and lower plasma morphine levels than did the placebo group. Plasma TNF-alpha was significantly decreased in the 60 mg dextromethorphan group compared to placebo.	Low dose dextromethorphan is useful as concomitant therapy in methadone-treated patients both at the pathogenic-inflammatory level, and at the clinical level.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Lee SY, Chen SL, Chang YH, et al. [31]	Double-blind RCT, placebo-controlled, 12-week duration; memantine (5 mg/day) (n_1=53) vs. placebo (n_2=75); patients with opioid dependence undergoing methadone maintenance therapy.	n=128 patients stabilized on methadone	Inflammatory bio-markers, overall dose of methadone	Memantine-treated patients required a somewhat lower methadone dose than did placebo-group (p=0.039), and they also had significantly lower plasma TNF-α and higher TGF-β1 levels.	Low-dose memantine may help patients during methadone substitution treatment because of the overall methadone dose decrease and positive impact over inflammatory process possible related to the NMDA-receptors.	5
Krupitsky E, Nunes EV, Ling W, et al. [19]	Double-blind, placebo-controlled multicentric RCT, 24 weeks; 380 mg extended-release naltrexone vs. placebo; counselling sessions were provided for both groups.	n=250 patients with opioid dependence, who underwent detoxification	Confirmed abstinence (urine drug tests and self-report).	Extended-release naltrexone-treated patients had significantly longer periods of confirmed abstinence (p=0.0002), more opioid-free days (p=0.0004), less craving (p<0.0001), more treatment-retention (p=0.0042).	Extended-release naltrexone in conjunction with psychosocial treatment improve the acceptance of opioid dependence pharmacotherapy and prolongs the duration of abstinence to opioids.	5
Krupitskii EM, Zvartau EE, Tsoi-Podosenin MV, et al. [15]	RCT, patients were randomized on naltrexone (50 mg/day) + fluoxetine (20 mg/day), naltrexone + placebo, fluoxetine + placebo, or placebo for 6 months; individual psychotherapy for the maintenance of remission was applied for all gropus (one session).	n=280 patients with heroin addiction	Remission rate, maintenance of remission	Naltrexone + fluoxetine was more effective than fluoxetine + placebo (p<0.01) and placebo (p<0.001) in the remission percentage. Naltrexone was superior to placebo and fluoxetine in the efficacy of maintenance of remission and preventing relapse in patients with heroin addiction. The combination of naltrexone and fluoxetine was more effective compared to naltrexone monotherapy only in women.	Naltrexone and fluoxetine could be useful in patients with heroin dependence, especially in women.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Zarghami M, Sheikhmoonesi F, Ala S, et al. [40]	12-week RCT, olanzapine (2.5-15 mg) vs. sodium valproate (600-1000 mg).	n=201 patients on methadone maintenance treatment	Aggressive behavior, urine toxicology.	Olanzapine and sodium valproate reduced the overt aggression and subscales of irritability, aggression, and suicidality. Olanzapine-treated patients presented more improvement in aggressivity. The percentage of positive urine samples (morphine, cannabis, methamphetamine) were not significantly different between the study groups.	Olanzapine and sodium valproate may be useful as adjunctive agent in decreasing aggressive behavior in heroin dependent individuals during methadone substitution treatment, with a slightly superior effect of olanzapine.	4
Salehi M, Zargar A, Ramezani MA [39]	Double-blind RCT, dextromethorphan vs. placebo; 6-month follow-up.	n=72 opiate abusers with methadone maintenance therapy	Methadone consumption dosage, quality of life, withdrawal symptoms.	The mean methadone consumption in the dextromethorphan-treated patients vs. control group were 62.7 mg/day vs. 70.4 mg/day. Total mean score of the quality of life was superior in the intervention group ($p>0.05$).	Dextromethorphan might be useful for opioid dependence treatment, but the differences vs. placebo were not significant.	5
Brooks AC, Comer SD, Sullivan MA, et al. [17]	Quasy-experimental comparison, long-acting injectable naltrexone (n_1=42) vs. oral naltrexone (n_2=69). Psychosocial therapy was also initiated for these patients.	n=101 patients on naltrexone	Days retained in treatment, severity of drug use	Long-acting injectable naltrexone had been associated with better outcome than oral naltrexone on days retained in treatment and other measurements for opiate use, while still other measurements for opiate use were not significantly different; heroin users with more severe baseline use showed better retention with oral naltrexone maintenance therapy, while less severe heroin users evidenced better outcomes when treated with long-acting injectable naltrexone.	Superior outcomes for heroin-dependents who received long-acting injectable naltrexone compared to oral naltrexone.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Mysels DJ, Cheng WY, Nunes EV, Sullivan MA [21]	Prospective evaluation using Hamilton Depression Rating Scale in patients treated with naltrexone depot maintenance and relapse prevention therapy; 2 and 4 -week post-baseline evaluations.	n=34 patients on naltrexone	Depression severity	Significant improvement of depression scores after 4 weeks post-baseline ($p<0.0004$), somatic and cognitive-affective subscales scores significantly decline; late insomnia increased significantly at 2 weeks post-baseline.	Naltrexone induction and maintenance does not worsen depression, but it may cause sleep impairments	2
Myesels DJ, Vosburg SK, Benga I, et al. [10]	Retrospective chart review; patients were maintained on methadone (n=16) or naltrexone (n=20);	n=36 opiate dependent patients stabilized on pharmacological treatment	Change in body weight from baseline to 3 months and 6 months into treatment.	No difference in weight was detected at baseline, 3 or 6 months between the two treatment groups.	No statistically difference over the weight between methadone and naltrexone maintenance treatment.	2
Schottenfeld RS, Chawarski MC, Mazlan M [11]	Double-blind RCT, 24 weeks; oral naltrexone vs. buprenorphine vs. placebo	n=126 detoxified heroin-dependent patients	Days to first heroin use (urine drug tests), days to heroin relapse, maximum consecutive days of heroin abstinence, reductions in HIV risk behaviours over 6 months.	Days to first heroin use, days to heroin relapse (3 consecutive positive tests), and maximum consecutive days abstinent had all supported the superiority of buprenorphine (significant to placebo). Differences between buprenorphine and naltrexone were not significant for days to heroin relapse and maximum consecutive days abstinent. Differences between naltrexone and placebo were not significant for any outcomes. HIV risk behaviours were significantly reduced by all three treatments.	Buprenorphine was superior to naltrexone, although for the most variables monitored the differences were not statistically significant.	5
Ngo HT, Tait RJ, Hulse GK [18]	Retrospective longitudinal follow-up; methadone (n_1=522) or naltrexone implant (n_2=314) as maintenance treatment for opioid- dependent patients. Evaluations at 6 months and 3.5 years post-treatment.	n=836 patients treated for opioid dependence	Hospital admissions rates, adjusted changes in risks and rates of overdose-related and non-overdose-related hospital morbidity associated with opioid vs. nonopioid drugs.	Naltrexone implant decreased risk for overdose and non-overdose-related events at 3.5 years, but not methadone; 5 drug-related deaths after methadone and 1 after naltrexone implantation.	Naltrexone implants, but not methadone, has been associated with long-term benefits in decreasing opioid-related hospital morbidity.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Gerra G, Di Petta G, D'Amore A, et al. [41]	12-week prospective, observational trial; open-label study; olanzapine vs. fluoxetine/ paroxetine+ clonazepam for controlling aggressiveness.	n=67 patients with heroin dependence undergoing substitution therapy	Hostility scores, incidence rates of aggressive incidents and attacks.	Direct aggressiveness, overall hostility scores, verbal aggressiveness scores, aggressive incidents rates were significantly lower compared to baseline in patients treated with olanzapine who comple-ted the treatment. Among completers, almost 70% achieved full substance abuse remission, 30% partial substance abuse remi-ssion, without signifycant differences between groups.	Olanzapine may be useful as adjunctive treatment in reducing aggressive/hostile behavior in heroin addicted patients who undergo maintenance substitution therapy.	3
Dean AJ, Saunders JB, Jones RT, et al. [12]	Open-label RCT, naltrexone 50 mg/day (n_1=42) vs. methadone (n_2=38) in opioid dependent patients who were stablized on methadone maintenance treatment; 6-month duration.	n=80 patients stabilized on methadone	Depressive symptoms severity, anxiety symptoms severity.	Patients who received naltrexone did not exhibit worsening of depressive symptoms; in patients attending follow-up assessments, those who received naltrexone presented an improvement in depression over time, compared to the control group; adherence to naltrexone correlated with lower depression severity scores.	Naltrexone has a positive impact over depressive symptoms during maintenance treatment in opioid dependent patients.	3
Hulse GK, Tait RJ, Comer SD, et al. [22]	Cohort study, pre-post design, data were prospectively collected; patients were evaluated before and after treatment with a sustained release naltrexone implant; a subgroup (n=146) had previously received treatment with oral naltrexone.	n=361 heroin dependent persons	Primary outcome was overdose events.	21 overdoses involving 20 persons in the 6 months pre-treatment; none was observed in the 6 months post-treatment; blood naltrexone levels at or above 3 ng/ml for approximately 6 months after injecttion; reduced number of opioid overdoses were also observed 7-12 months after implant; for those previously treated with oral naltrexone, more opioid overdoses occured in both 6-month prior to and after oral compared to the 6-month post-implant treatment.	Sustained-release naltrexone implant is efficient in preventing opioid overdose.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Comer SD, Sullivan MA [32]	Detoxified, non-treatment-seeker-heroin dependent patients; 8 weeks; 0, 30, 60 mg/day memantine as maintenance treatment; intranasal heroin (0, 12.5, 50 mg); inpatient regimen.	n=8 patients with heroin dependence	Subjective ratings of drug quality, liking, willingness to pay for drugs, craving.	Memantine produced modest reductions in subjective ratings of drug quality, liking, willingness to pay for the drug, and craving for heroin. It had little influence over the reinforcing heroin effects.	Memantine could be a useful agent for decreasing the subjective but not the reinforcing effects of heroin.	2
McRae AL, Sonne SC, Brady KT, et al. [43]	RCT, 12-week, placebo-controlled study; patients on methadone who presented also anxiety symptoms; buspirone vs. placebo.	n=36 patients receiving methadone maintenance treatment	Anxiety symptoms	Buspirone did not significantly decrease anxiety symptoms in opioid-dependent patients.	Buspirone had no significant efficacy over anxiety symptoms in subjects undergoing methadone maintenance treatment, but it was associated with reduction in depression scale scores and slower return to substance use.	5
Assadi SM, Radgoodarzi R, Ahmadi-Abhari SA [45]	Double-blind, placebo-controlled, 12-week RCT; patients received fixed-schedule baclofen 60 mg/day or placebo.	n=40 patients with opioid dependence, recently detoxified	The primary outcome was retention in treatment.	Treatment retention was significantly higher in the baclofen group (p=0.01); 30% of patients treated with baclofen completed the trial vs. 10% patients on placebo. The rates of opioid-positive urine samples did not differ significantly between the two groups. Baclofen was superior to placebo in terms of opiate withdrawal syndrome and depressive symptoms. Intensity of opioid craving and self-reported opioid and alcohol use were more favourably influnced by baclofen, although not statistically significant. The drug side effects of the two groups were not significantly different.	Baclofen could be useful in the maintenance treatment of opioid dependence, but the sample size was small, and the attrition rates were high.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Kumar P, Jain MK [13]	Open label trial; patients received clonidine (for detox) and later naltrexone p.o. (50 mg BD) as maintenance therapy vs. naltrexone plus gabapentin (1200 mg/day) for 3 weeks.	n=20 opioid dependent patients (pentazocine)	Muscle pain and craving (rated on visual-analogue scale), psychological dependence severity.	Patients receiving naltrexone + gabapentin scored significantly lower on both parameters- pain score and craving score (p<0.001), and psychological dependence was significantly lower in this group (p<0.01).	Gabapentin is efficient in controlling muscle aching, and no tolerance effect was observed after 3 weeks. Gabapentin may reduce pain in opioid dependent patients when added to naltrexone, signaling a possible synergistic effect. Gabapentin may help in controlling the opiate craving and potentiated the effect of naltrexone in this regard.	3
Farren CK, O'Malley S [14]	Double-blind, placebo-controlled trial, naltrexone 50 mg qd plus placebo vs. naltrexone 50 mg plus sertraline 50 mg qd; 12-week monitoring duration.	n=13 recently non-depressed abstinent opiate addicts	Depression severity, opiate craving, adverse events	Similar side effect profile in both groups, and similar retention rates. Significant negative correlation between Beck Depression Inventory scores and duration in treatment in the combined therapy group. Opiate craving decreased over time in both groups.	Naltrexone plus sertraline was well tolerated and initially successful in increasing treatment retention, but the difference to the naltrexone alone diminished in time.	4

Based on these results a number of recommendations can be made in patients who need maintenance treatment for opioid use disorder, if a drug without opioid agonistic properties is preferred (table 3). These recommendations have been hierarchized according to the GRADE system [35].

Regarding the limitations of current review it can be mentioned the overall low quality design of the clinical research, only 13 articles included double-blind, controlled, randomized trials. Another limitation is related to the low number of drugs investigated, and except for naltrexone (oral and extended-release), data regarding the efficacy or tolerability of other pharmacological agents were derived from few good quality trials. Only one reviewer was involved in the processing of data collected, so a bias related to this aspect can not be excluded. More research is needed, and larger, well designed trials focused on a more diverse range of drugs are also expected to be launched in order to provide more accurate recommendation in a continuously extending pathology, like the opioid use disorder.

Table 3. Recommendation based on the literature review for patients with opioid use disorder with non-opioid agonist pharmacological agents

Treatment	Clinical trials	Advantages/disadvantages	GRADE recommendations
Naltrexone- oral administration	[9-15]	(+) naltrexone improve depressive symptoms in patients with opioid use disorder at 6 months (+) naltrexone plus fluoxetine and individual psychotherapy has been proven useful in women with heroin dependence (+) naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine (+) naltrexone plus sertraline increased short-term treatment retention of recently non-depressed opioid abstinent patients (-) buprenorphine/naloxone was superior to naloxone in the reduction of opioid-positive urine test and preserving patients participation in the treatment (-) similar weight gain during 6 months of treatment when compared to methadone (-) naltrexone was inferior to buprenorphine (not statistically significant) in several outcomes regarding the rate of relapse in detoxified heroin dependent patients	B
Naltrexone-extended release	[16-23]	(+) efficacy and tolerability were similar to buprenorphine/naltrexone (+) lower rate of relapse when compared to treatment as usual, at least on short-term (+) better outcomes than those treated with oral formulation of the same drug (+) naltrexone implants but not methadone were associated with long-term benefits in decreasing opioid-related hospital morbidity (+) efficient in preventing opioid overdose (+) improved depression severity at 4 weeks post-baseline (+) improved quality of life, rate of retention, opioid-negative urines, opioid craving, rate of re-employment (+) prolonged the duration of abstinence to opioids, in combination with counselling (-) worsened late insomnia (-) requires implant of the active drug (usually subcutaneous)	B+
Topiramate	[25]	(-) negative results in patients with opioid and cocaine use disorder	D-
Ibudilast	[26]	(+) induced lower rates of drug liking (oxycodone), lower craving to heroin/tobacco/cocaine and lower subjective ratings of pain vs. placebo	C
Memantine	[29-32]	(+) may provide an easier transition to buprenorphine-free therapeutic regimen (+) induced attenuation of chronic opioid-dependence-related cognitive impairment (+) decreased the daily dose of methadone (+) it had positive effect over probably NMDA-mediated inflammatory processes in opioid users (+) it decreased the subjective effects of heroin in chronic users (-) it did not decreased the reinforcing effects of heroin in non-treatment seeker heroin-dependent patients	C+

Treatment	Clinical trials	Advantages/disadvantages	GRADE recommendations
Dronabinol	[34]	(+) it decreased the severity of opioid withdrawal (-) no effect over rates of naltrexone-treatment induction or retention	D
Dextro-methorphan	[37-39]	(+) it was useful in low doses as adjuvant treatment in methadone-treated patients both at inflammatory level and clinical level (+) the quality of life was superior in patients who received dextromethorphan as adjuvant to methadone versus placebo plus methadone (-) the difference versus placebo was not significant in the otcome defined as mean methadone consumption	C
Olanzapine	[40,41]	(+) it reduced direct aggressiveness, overall hostility, verbal aggressiveness, and aggressive incidents (+) improved the rate of treatment retention	C
Buspirone	[43]	(+) positive effect over depressive symptoms associated to methadone maintenance treatment (-) no effect over anxiety	C-
Baclofen	[45]	(+) significantly better treatment retention	C-

From the data collected in this review only extended release naltrexone seems to achieve a B+ level of recommendation, based on 8 trials, and the benefits of using extended-release naltrexone tend to overcome its potential disadvantages in patients with opioid use disorder who underwent successful disintoxication. This naltrexone formulation had positive impact over a large number of outcomes related to opioid use disorder, including rate of relapse, quality of life, opioid-related hospital morbidity, and depressive severity [16-23]. As an alternative, oral naltrexone may be tried, although negative trials exist – level B recommendation, based on 7 trials [9-15]. Naltrexone plus fluoxetine and individual psychotherapy has been proven a useful combination especially in women with heroin dependence [15]. Naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine [13]. Naltrexone plus sertraline were well-tolerated and increased on short-term treatment retention of recently non-depressed opioid abstinent patients [14].

Memantine has a level C+ recommendation for its use as adjuvant treatment in opioid use disorder because there more data to support its efficacy are needed. Low dose memantine attenuated chronic opioid-dependence-induced cognitive impairment and decreased the daily dose of methadone [30, 31]. However, information have been gathered about its positive effect over cognition, inflammatory processes, and decrease of methadone daily dose [29-32].

Olanzapine may be recommended for controlling aggressivity in patients with opioid use disorder, as adjuvant treatment (level C) [40, 41].

Dextromethorphan was associated with some positive results, although it did not decrease the necessary daily dose of methadone when used as adjuvant agent [37-39]. This drug was however useful in improving both clinical and inflammatory outcomes, and improved patients' quality of life [37-39].

Dronabinol did not prove itself efficient in the maintenance phase of treatment, although it may be of some help in alleviating withdrawal symptoms in opioid users [34].

Data for ibudilast, baclofen, and buspirone are insufficient to allow for stronger recommendations in the targeted population, but further research may change this perspective.

CONCLUSIONS

Data showing a continued increase of illegal opioids use are overwhelming, and it became obvious that clinicians must find new solutions for treating a continuously growing population affected by opioid use disorder. Opioid agonists like buprenorphine and methadone are considered now the first line for treatment of opioid dependent patients, but non-opioid agonist drugs should be investigated more, either as adjuvant agents or as monotherapy. These non-opioid agonist agents can lead to the administration of lower doses of opioid agonists (in case of adjuvant treatment), or even to their complete replacement, in both cases diminishing the risk for a new dependence.

From the data reviewed, extended-release naltrexone is the most supported by evidence recommendation, followed by oral naltrexone, in patients who were successfully detoxified from opioids. Naltrexone had positive results not only on the opioid use-related outcomes, but also over associated symptoms, like depression.

As adjuvant agents, olanzapine may be efficient for aggressive behaviours in opioid dependent patients, memantine could improve cognitive function and decrease the inflammatory processes associated with substance use disorders, and dextromethorphan may also help decreasing the inflammatory processes. Further research is needed in order to verify the efficacy and tolerability of olanzapine, memantine and dextromethorphan as adjuvant agents.

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Major radiological threats and emergencies requiring public health preparedness (the example of Greece)

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Abstract: *Radiological emergencies carry the potential for major disruptions to public health care provision, due to their infrequency, unpredicted nature, delay in appearance of adverse effects and the grave psychological impact on sufferers and society. This study aims to describe and analyze the main categories of radiological hazards and emergencies that require hospital response and preparedness at a nation-wide level, taking into specific account the example of Greece. These radiological threats are either accidental, like reactor emergencies and accidents in hospital or industrial units implementing radiological sources of high activities, or deliberate, like detonation of nuclear devices and malicious use of various radionuclides.*

Keywords: *public health, radiological incidents, radiation emergencies, preparedness, nuclear terrorism*

INTRODUCTION

Even though natural disasters and biological hazards are currently the most frequent hazards for massive casualties, recent data suggest that radiological emergencies from various nuclear incidents may not be a reassuringly remote possibility. Till the year 2015, more than 420 radiation incidents of various degrees of severity have occurred, thus surging the global preparedness to a steady ascent [1], whilst specific deficits in training and experience may lead to devastating increase in the victims and mortality rate in the case of a major radiological event, either accidental or malicious. Furthermore, there are roughly ten million sealed sources of radionuclides used for peaceful applications worldwide; only between 1995 and 2008, more than 600 of them were either lost or stolen and less than the half were eventually retrieved [2].

COMMONEST TYPES OF RADIATION EMERGENCIES

Greece does not have a nuclear power industry; thus, Greek radiation preparedness is directed mainly to radiological scenarios of low probability, with rather minor health and social impact compared to major accidents from power reactors. However, a wide variety of radioactive sources is used in medicine, industry and research. Several types of severe radiological emergencies that would necessitate a special medical response are bellow analyzed in detail.

Nuclear detonation

The detonation of a nuclear weapon constitutes the most catastrophic radiological scenario; the enormous number of casualties from the shock-wave, the thermal burns and the ionizing radiation would probably lead the health network of any urban target in total collapse. Although the probability of a terrorist attack involving the detonation of a nuclear device is considered rather unlikely, the scenario of a single, low-yield nuclear weapon, stolen from a military facility, for a major attack in a densely populated modern metropolis, appears as a quite feasible and thinkable perspective of the 21st century's terrorism, according to US strategic analysts [3].

Reactor Emergencies

Following a reactor emergency, it is expected that a radiological contamination will occur to the surrounding environment and also external exposure and contamination through inhalation or ingestion may affect the general

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public. Although many radionuclides are produced in a nuclear accident, the most important to human health are cesium-137 (Cs-137), strontium-90 (Sr-90) and iodine-131 (I-131) [4]. Apart from radioactivity, reactor emergencies may disperse long-lasting psychological consequences to enormously large populations [5].

The two most catastrophic reactor emergencies were the case of Chernobyl, Ukraine (April 1986), when enormous quantities of radioactive contaminants were dispersed across all continents [6], causing an extensive increase in neoplastic diseases and genetic malformations to the affected population [7], and that of Fukushima Daiichi, Japan (March 2011) which affected heavily the pacific ocean [8]. The size of the consequences of this kind of radiological incidents is vastly influenced by the meteorological conditions (mainly winds) prevailing in the area that would facilitate the movement of the contaminated cloud and deposits; in 1986 disaster, it took only ten days for the radio-contaminated cloud from Chernobyl nuclear power plant to cross southern Europe and arrive in Greece. Therefore, all nuclear power plants and radiological waste storage sites in Europe pose an existing threat. Yet, seismic activity is another factor that needs to be considered, especially concerning the seismogenic arc of the Asia Minor coast. Regarding the Balkan neighbors of Greece:

a. There are currently no active nuclear power plants in Greek territory and the low-power experimental reactor supervised by the Greek Institute of Nuclear & Radiological Sciences and Technology is under permanent non-operation state since 2001.

b. Bulgaria operates one of the oldest commercial nuclear reactors in Europe in Kozloduy since 1974 and according to the Belgium report [9] the vulnerability to failure and accidents of such units has steadily grown in the last years. In Germany, an accident in a poorly maintained nuclear facility out-borders is regarded the most probable major radiation incident [10]. Yet, as the effects of nuclear disasters have no borders, that old Bulgarian unit located only 260 km far from the northern borders of Greece, poses a significant radiological threat for all Balkan countries. The construction of a second nuclear unit in Belene was officially terminated in 2012 [11].

c. Romania operates a nuclear power plant in Cernavodae, located 325 km far from the northern borders of Greece (Figure 1), and in the indefinite future it plans to construct a second, larger unit probably in Transylvania.

d. Turkey's first nuclear power plant, at Akkuyu, commenced construction in April 2018. Although rather remote from the Hellenic inland, a severe radiological accident in Akkuyu will heavily affect Cyprus (Figure 2). Plans for two more power stations in Sinop (Northern Turkey, by the Black Sea) and İğneadain (in the European part of Turkey) have currently stuck, mainly due to economic reasons [12].

Nonetheless, since there are so many nuclear power plants, nuclear weapons and nuclear waste storage facilities within EU member states, advanced preparedness and countermeasures are necessary, under the threat of a European version of the 9/11 scenario, presumably in a more sophisticated implementation, e.g. a cyber-sabotage that would affect or interfere in the function of a nuclear plant (e.g. in the cooling or control sections).

Figure 1: Nuclear power plants neighboring to Greece; the green islets illustrate seismogenic locations during the period 1972-1997

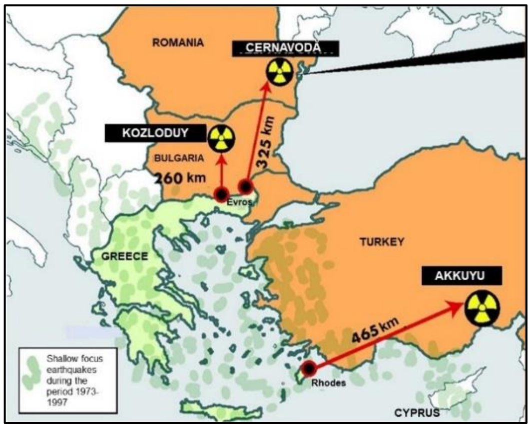

Figure 2: In the scenario of a severe radiological accident in the planned-to-operate nuclear power plant in Akkuyu, Cyprus would be inside the red zone of the disaster.

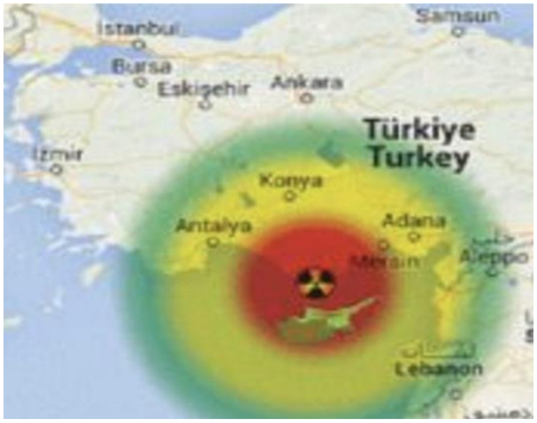

Accidental Medical overexposure

Medical teletherapy devices, though currently outdated by linear accelerators, are still used in many hospitals worldwide for cancer radiotherapy, by applying high energy gamma radiation focused on tumor targets. These devices typically use a single Co-60 source in a solid metal form, though a limited number of teletherapy devices use Cs-137 sources, which are in the form of a water soluble chloride salt, a much more dangerously prone to dispersion chemical form, thus making easier the contamination of people and environment [13]. The teletherapy device is securely encapsulated in heavy shielding and only highly trained personnel may operate it, because it poses extreme hazard if not properly handled. According to the decay energy and the physical half-life of the radionuclide, the equivalent dose for that radionuclide is 13 mSv per Ci per hour at 1 meter from the source; that may hypothetically lead a person to receive a lethal dose from a full 10,000 Ci unshielded Co-60 source when standing at one meter distance, within only a few minutes [14]. More realistic are of course scenarios of accidental medical overexposure from Co-60 teletherapy devices due to miscalculation of the radiotherapy treatment planning or improper function of such devices, or even when higher activities of radionuclides are accidentally administered in Nuclear Medicine departments. Although all above situations are considered as serious medical problems, they may not be classified as radiological incidents requiring hospital preparedness, because medical and radiation protection guidelines are regularly provided by health and physics specialists in each hospital.

Abandoned, lost or stolen radiation sources

Radiation sources may be improperly discarded, accidentally lost or even stolen for financial or malicious reasons. In many historical cases, decommissioned teletherapy units were unshielded, as a rule by people irrelevant to radiological education; the radioactive source was then stored or discarded and the metal shielding was scrapped. As a consequence of that, the perpetrators and sometimes the public, were either externally exposed to various levels of radiation or/and were heavily contaminated, resulting in serious injuries, illness or even death.

The worst of that kind of accidents occurred in 1987 in Goiânia, Brazil when an abandoned teletherapy source of a long-lived beta and gamma emitter, Cs-137 with activity of 1375 Ci, was unshielded by a group of metal scrapers. Four people received lethal doses of radiation, 28 suffered radiation injuries and 249 had been contaminated [15]. The delay in the verification of the radiological incident led to an uncontrolled and widespread radiological contamination in the urban district of Goiânia, including buildings, vehicles and soil [16].

Another potential radiological hazard comes from blood irradiators; these heavy-shielded devices contain Cs-137 with activity of 3,000-7,000 Ci [13] and are used in hospitals for the irradiation of blood prior to infusion. The most significant radiological incident concerning this type of device occurred in 2011 in a hospital in Finland where a blood irradiator containing 60.1 Tbq Cs-137 was exposed in high temperatures after a fire outbreak. Although no dangerous radioactivity was unleashed, quite a lot shortages in the flow of crisis information among the first responders, the radiation safety officer and the radiation safety authority were afterwards ascertained; furthermore defective preventive, storage and shielding protocols of such dangerous radiation sources were among the many lessons learned in the aftermath of a nearly disaster [17].

Incidents from dangerous industrial sources

Apart from medical sources, there are currently several hundreds of large industrial facilities worldwide that utilize high activities of ionizing radiation. The most dangerous of such industrial facilities is the gamma sterilization plant; such irradiation plants are usually located in industrial areas and are used to sterilize various products, most often food and medical devices, by using extremely high doses of ionizing radiation. A typical irradiation plant includes a large number of high activity Co-60 rod sources, housed in an array immersed in a water pool inside a shielded chamber.

The product to be irradiated is exposed by remote control to the sources for the time required to kill bacteria. Indicative of the high activities that are used in industrial irradiation is that a typical such facility contains from hundreds of thousands, up to some millions curies of Co-60 [13], that is roughly a thousand times more than the typical activity of a Co-60 medical teletherapy source.

Therefore, health emergencies occurring in industrial irradiation plants often lead to lethal levels of whole body absorbed doses after exposure of just some seconds [18]. Although such plants are highly secured and physically inviolable, operating according to globally standardized specifications and their safety to be established through decades of operational experience, training, supervision and safety systems technology, some sporadic serious accidents have taken place, like in Italy, 1975; Norway, 1982; [19]; El Salvador, 1989 [20]; Soreq, Israel, 1990 [19] and Nesvizh, Belarus, 1991 [18].

The thorough evaluation of all foreseeable hazards resulting from industrial facilities that use ionizing radiation needs to consider natural and anthropogenic disasters, failures in installation, operation procedures, supplies, machinery, technological infrastructures and safety education. Importantly also, users of radioactive material with longer experience possessed a surprisingly lower degree of nuclear security awareness compared to less experienced ones [21].

Malicious use of radioactive materials

Nowadays, it is widely accepted that international terrorism may have acquired the resources and technical capabilities to obtain or manufacture improvised nuclear devices or other types of radiological dispersal devices coupling conventional explosives and radioactive material [22]. The latter are known as "dirty bombs" and are much easier to be prepared than a nuclear detonation device and yet the raw materials can be found in industry, hospitals or research institutes. The appropriateness of a radiological source for malevolent applications depends on various factors like the size, accessibility and ease of portability of the source, the total radioactivity and physical half-life of the radionuclide it contains, the type of the radiation emitted (for example gamma emitters are suitable for external exposure, whilst alpha emitters only for radiological contamination) and also the physical and chemical form of the radioactive material [5]. For practical reasons the radioactive sources suitable for nuclear terrorism are classified into two general categories:

a. *Radiation exposure devices (RED)*; these usually involve small-sized sources with radioactive material emitting gamma radiation. In RED, the primary radiation source(s) is/are in a relatively intact physical state and shape, thus the radiological threat comes rather from external radiation exposure and usually raises minimal or no aspects of disseminated contamination. When in large quantity, like for example in the case of an unshielded medical teletherapy Co-60 source, the RED raises hazards of acute or chronic radiation syndromes to the victims. When of small quantity, the exposure dose to individuals is expected to be too low to allow for acute effects; however, the psychosocial fear of future cancers raises concerns of diffuse or uncontrolled public anxiety.

b. *Radiation dispersal devices (RDD)*; these involve larger quantity of radioactive material which is dispersed in micro-granular, airborne or water soluble form (as is usually the case for the radio-cesium chloride salt) over large areas, often by the use of conventional explosives, coupled with the radioactive substance. In this way, the contamination risk, either external (at the environment, skin, or cloths), or internal (by ingestion or inhalation) usually overwhelms the exposure threat. Furthermore, RDD carry the potential to cause complicated injuries, like blast and burn traumas from a chemical explosion combined with radiological contamination. And again, the psychological impact of such an attack, especially when affecting a large urban area, might provoke extensive economic and social disruption to a whole country.

Various radionuclides require enhanced nuclear security measures, since even gram or milligram quantities of some, assuming perfectly dispersed, might be enough to contaminate large areas above acceptable levels for habitation. The number of affected people in such a scenario also varies, according to the type of the bomb, the quantity of the nuclear material, the time and location of the attack. According to a scenario of U.S. Homeland Security Council [23], the concurrent blast of Cs-137 "dirty bombs" at three different locations in a densely populated urban area might cause 180 fatalities, 270 injured and 20,000 contaminated people at each site; hundreds of radiation induced cancers would also occur in the future unless tens of thousands people were relocated from the city [10]. The most common radionuclides that might be used in a RDD with corresponding thresholds to contaminate 1 square km are presented in Table 1.

Table 1: Common radionuclides that might be used in a RDD

Radionuclide	Threshold (grams) to contaminate 1 square km*
Co-60	0.01
Ir-192	0.01
Sr-90	0.37
Cs-137	0.48
Ra-226	13
Am-241	22.7
Pu-239	220

** refers to the theoretical amount of radionuclides in pure and perfectly dispersed form required to contaminate that area to the level that a person living there for one year would receive a cumulative dose of 20 mSv*

Concerning Po-210, there is always the threat of targeted assassinations like the case of Alexander Litvinenko, the first recorded victim of Po-210 radiation poisoning [24]; much scarier though is the theoretical scenario of a massive food poisoning, e.g. in grand hospitals, congress venues, cruise ships, etc. with lethal or pathogenic doses of radionuclides. Radio-contamination of the water is a far remoter perspective, due to both the sophisticated control mechanisms in central water supply reservoirs of the modern Metropolises and the vast quantities of radiological factors required to cause significant contamination of the water reservoirs. However, that is not always the case in smaller tanks irrigating small communities or sensitive infrastructures (e.g. hospitals) In any case crisis communication will play a significant role in dealing with such incidents, which may well overwhelm the health capacity and capability of the whole national medical network.

Furthermore, the enormous financial disaster that would result from a long-lasting radiological contamination of critical infrastructures, such as ports, train stations, government buildings, central squares, etc, and the economical loss of the consequent shutdown after such an incident for a large city has been estimated to tens of billions U.S. dollars [25]. Table 2 illustrates the theoretical probabilities for deterministic effects (acute or chronic radiation syndromes), concerning the threats described above whilst Table 3 presents the medical and public health characteristics of the three major categories of radiological events.

Table 2: Theoretical probabilities for deterministic effects (acute or chronic radiation syndromes), for radiation exposure and for radiological contamination

		Theoretical probability	Source of radiation	Acute radiation syndrome	Chronic radiation syndrome	Radiation exposure	Contamination	
							External	Internal
Accidental Incidents	Reactor Emergencies	√	Cs-137, I-131	no	no	no	no	√
	Industrial Steriliz. Accidents	very low	Co-60	√	√	√	no	no
	Co-60 Brachytherapy Accidents	√	Co-60	√	√	√	no	no
	Blood Irradiator Accidents	√	Cs-137	√	√	√	no	no
	X-ray Medical Accidents	√	X-rays	√	√	√	no	no
	Medical Radionuclide accidents	√	Med. Radionuclides	no	no	√	√	√
Malicious Incidents	Nuclear Detonation	very low	Fission products	√	√	√	√	√
	Co-60 RED	√	Co-60 RED	√	√	√	no	no
	Co-60 RDD	√	Co-60 RDD	no	no	√	√	√
	Cs-137 RED	√	Cs-137 RED	√	√	√	?	?
	Cs-137 RDD	√	Cs-137 RDD	√	√	√	√	√
	Po-210 RDD	√	Po-210 RDD	√	√	no	√	√
	Am-241 RDD, etc.	very low	Am-241 RDD	no	no	no	no	?

RED: Radiation Exposure Device, RDD: Radiation Dispersal Device

Table 3: Medical and public health characteristics of the main categories of radiological events (modified from Canestra, 2012)

Type of incident	Relative casualty-type distribution			
	Lethal injuries		Non-lethal injuries	
	Prompt	Delayed	Exposed individuals	Psychologically affected non-exposed individuals
Nuclear weapon	+++	+++	+++	+++
RED	+	++	+++	+++
RDD	+	+	++	+++

+++/++/+: *high/intermediate/low probability; RED: Radiation Exposure Device, RDD: Radiation Dispersal Device*

Contamination of food and water

The radiological contamination of food and water or any other goods of public consumption is another health emergency radiation scenario. That could result by two main reasons:

a. As a consequence of an accidental radiological accident, e.g. from a reactor emergency, that might release radiological contaminants to the environment and then to the food chain, or from a damaged radiological source, which might then be dispersed and contaminate the surrounding area. In the reactor emergency scenario it is expected a widespread contamination, leading to low level absorbed doses to a large population. Yet there is usually expected to be time for precautionary measures for the public protection, whilst as regards accidents involving radiological sources, these occasionally may be latent for a longer period of time [16].

b. As a malevolent act; that is the intentional contamination of public supplies with radioactive materials, or the attempt to assassinate specific individuals. In the case of a massive deliberate contamination of consumable public supplies, it is expected that the public would receive a rather low dose of radiation per person due to the dilution of the radioactivity load into a large pool of mass or consumed volume and consequently, due to the extremely large amounts of radioactive material required to achieve high levels of large-scale contamination. However, targeted assassinations [24] are another gloomy perspective of radiological terrorism; high doses of extremely dangerous radionuclides (Po-210, in that case), incorporated in the human body can cause genuine lethal deterministic radiation effects.

In any such case, fast actions from the public authorities involving radiological experts and the media are mandatory to quickly assess the health impact and reduce the consequences. Concerning the level of radionuclides in water supplies, the World Health Organization (WHO) has developed detailed guidelines [26]. On the social level however, an uncontrollable public concern or even panic is to be expected in such cases, as past experience has shown [27]. Furthermore, massive national or international financial consequences are expected concerning the distribution chain of food merchandise.

CONCLUSIONS

The key point concerning all above described cases of radiological emergencies, is that appropriate assessment of potential public threats and required preparedness or response actions should be applied before the appearance of the consequences of such incidents. Thus, continuous awareness and preparedness for such hazards is crucial for all public health stakeholders.

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ORIGINAL ARTICLE

Management of hepatitis C virus (HCV) infection: an update

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Abstract: *Million people worldwide are affected by the hepatitis C virus (HCV). The highest incidence of illness was between 1945 and 1975. It was also estimated that 70% of those people were not tested for the disease. Most recent treatment concepts are safe, highly effective and have a vital public health influence by achieving a viral constant response in a significant proportion of treated patients. It helps reduce liver fibrosis, liver cancer risk and dissemination. With its increased population incidence, HCV becomes a serious public health problem. This review discusses the current literature in this field in terms of the importance of screening of HCV, follow-up, treatment and includes considerations in specific populations such as patients with cirrhosis, with HIV/HCV co-infection, patients with HBV/HCV co-infection and with renal damage.*

Keywords: *hepatitis C virus, Direct-acting antiviral therapy, co-infection*

INTRODUCTION

The primary cause of final-stage liver disease in the Western world is the Hepatitis C virus (HCV). Notably, HCV is the only chronic viral infection in humans that can be healed [1,2]. The consequences of chronic infection (HCV) include cirrhosis (compensated and decompensated), portal hypertension, hepatocellular carcinoma and eventually liver-related death.

Fibrosis progression may vary due to multiple factors such as age, hepatitis B, alcohol consumption or human immunodeficiency virus (HIV) co-infection [2].

After cirrhosis is established, a yearly incidence of hepatic decompensation such as jaundice, episodes of ascites, variceal bleeding and encephalopathy of 3.9-5.7% and a yearly incidence of hepatocellular carcinoma of 1.4-3.4% has been reported [2,3].

The percentage of seropositive antibodies to HCV increased from 2,3 to 2,8 % between 1990 and 2005 [3] and the disease complications are increasing due to the ageing population and the percentage of liver fibrosis, which in time leads to hepatic insufficiency risk [4].

The key to the long-term benefits in HCV infection is to arrive at constant serologic feedback which is the lack of viremia 12 weeks after the end of the therapy [5].

This sustained viral response (SVR) decreases the prevalence of hepatocellular carcinoma, morbidity related to the risk of hepatic insufficiency and mortality of infected individuals [6-8].

HCV INITIAL TESTING AND FOLLOW-UP

Diagnosis of chronic C virus infection is done by testing anti-HCV antibodies. HCV RNA polymerase chain reaction is only required if the anti-HCV is positive and it confirms the diagnosis of chronic C virus infection [9,10].

There is a group of people (about 15-30%) who have a positive anti-HCV test and negative RNA HCV polymerase chain reaction [11] test. Those are people who can naturally clear out the infection. Spontaneous removal of the C virus is particularly rare after 6 months in which the person has acquired chronic infection [11].

People born during 1945-1975 [12,13], should perform

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screening for Hepatitis C annually. From 2020 universal HCV screening is recommended for all adults aged 18.

Annual HCV testing is required for all individuals injecting drugs, pregnant women, people with an immunodeficiency [14], persons with various pathologies, individuals with ongoing risk behaviours or exposures and persons who have unprotected intercourse with men [15,16] according to the American Centers for Disease Control and Prevention (CDC) and the American liver disease Survey Association (AASLD).

People with known or suspicious cirrhosis have an increased risk of liver complications and need to be frequently followed. Individuals who are immunodepressive (e.g.: chronic hemodialysis) [17] or who may have been exposed to HCV in the last 6 months and have not developed antibodies should be tested for HCV RNA even if they have had negative anti-HCV testing.

CLINICAL CARE FOR PERSONS WITH ACTIVE HCV INFECTION

Once diagnosed with active HCV infection, patients need counselling and specific clinical assistance before the initiation of antiviral therapy.

The most important thing is the prevention of further liver damage [18]. First of all, counselling patients and sustaining them not to consume alcohol, because there is a tight connexion between alcohol use, progressive fibrosis and the development of hepatocellular carcinoma (HCC) [19,20]. Moreover, people have to be trained on how to prevent the transmission of this infection.

This training can greatly reduce the hepatitis C rate. Some of the rules are: to avoid the sharing of different tools (toothbrushes or razor blades) and to avoid the possibility of others coming into contact with the blood of an infected person [18-20].

Assessment of hepatic fibrosis, hepatitis B virus [20], immunodeficiency and existing pathologies is required to determine the treatment strategy for HCV infection.

Every person with HCV should get vaccinated against hepatitis A and B and for persons with cirrhosis, vaccination against pneumococcal infection is recommended.

DIRECT-ACTING ANTIVIRAL (DAA) THERAPY

The physical exam conducted on the liver, routine blood tests (e.g. complete hemogram, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), albumin, bilirubin), elastography indicating cirrhosis (Fibroscan), liver imaging (ultrasound or CT), can indicate the severity of liver damage and aid for a previous assessment of the treatment scheme to follow for hepatitis C infection [21].

Recommended pretreatment laboratory tests are pursued to confirm chronic HCV infection and to rule out decompensated liver disease, HBV, HIV co-infection and renal disease, and/or current pregnancy previous therapy initiation, considering that they need more subtle care [22].

During antiviral therapy, it is recommended to inform patients about potential drug-drug interactions and to monitor adverse events. It is important to schedule some clinic visits or to have telephone contact to check medication compliance [23].

Patients suffering from diabetes can have a hypoglycaemia drop and blood glucose monitoring is recommended.

Patients undergoing anticoagulant treatment (warfarin) should be carefully monitored because they may experience changes in the anticoagulation status [24,25].

Clinical trials have proved the safety [26] of strong therapeutic success of Glecaprevir (300mg)/Pibrentasvir (120 mg) [27] taken for 8 weeks and Sofosbuvir (400mg)/ Velpatasvir (100mg) [28] for 12 weeks for treatment persons without cirrhosis no matter of HCV genotype.

To evaluate therapy response, HCV RNA and hepatic aminotransferase testing is counselled 12 or more weeks after finishing DAA treatment. Undetectable HCV RNA represents virologic cure and sustained response to treatment.

Numerous benefits for the health and general condition of the patient, including improved AST levels and reduced inflammation with decreased progressivity of liver fibrosis [29].

Patients healed by HCV, those with a sustained viral response (SVR), show a decrease of up to 70% of the risk of HCC development [30,31]. In the absence of cirrhosis, individuals with SVR do not demand liver-specific monitoring, but for those with constant HCV risk factors or an increase of hepatic aminotransferase, counselling and HCV RNA testing once a year is recommended [32].

Between 1% and 7% is the annual rate of hepatocellular carcinoma in patients with HCV cirrhosis, and for this reason, is recommended an ultrasound exam and periodic upper endoscopic monitoring.

The most frequent HCV genotype from all 7 genotypes is Genotype-1 [33]. In Europe and Central America Genotype-1, b is the most common HCV subtype [33].

Patients should be handled by clinicians with expertise in managing HCV therapy failure. Persons with persistent elevated hepatic aminotransferase levels and achieved SVR demand reevaluation for other causes of liver damage [34,35].

Assessment for liver damage progression every 6-12 months is counselled for patients with no treatment response. Ultrasound monitoring for HCC and alpha-fetoprotein [36] every 6 months is recommended for persons with cirrhosis. AASLD guidance on portal hypertensive bleeding in cirrhosis recommends upper endoscopic monitoring for oesophageal varices [37].

HCV AND COMORBIDITIES

Liver lesions can progress faster in the case of HCV-HIV coinfection [38,39]. Careful evaluation and observation of potential interactions between medicines are required.

Guidelines recommended the same therapy in the treatment of HCV mono-infection and HCV-HIV co-infection [39]. For patients with genotype 1a and 1b, with no cirrhosis, therapy for patients with mono HCV infection is with ledipasvir/sofosbuvir for 8 weeks, and 12 weeks in patients with co-infection [40].

For genotype 1a, 1b, 2, 3 and 4 individuals with compensated cirrhosis, AASLD-IDSA HCV proposes glecaprevir/ pibrentasvir for 8 weeks in persons with HCV and 4 weeks more in patients with co-infection [40]. And for genotypes 5 and 6, with or without compensated cirrhosis, glecaprevir-pibrentasvir for 8 weeks in patients with HCV infection and with 4 weeks more for those with co-infection, is recommended [40].

The particular examination should be given by controlling and managing HIV antiretroviral and HCV DAA drug interaction.

Measurements of serum creatinine and creatinine clearance are included in the patient assessment with HCV before treatment, and the stage of renal disease may be determined based on these values.

The normal value of GFR is 130 and 120 mL/min/1.73 m^2, and kidney failure is defined by a GFR less than 15mL/min/1.73 m^2. HCV is associated with the rapid development of kidney damage and renal carcinoma [41]. Hemodialysis is considered to be a risk factor for acquiring HCV infection [42].

The selection of DAAs should be carefully considered and based on patient characteristics, concurrent drugs and HCV genotype [43]. For all six major HCV genotypes, glecaprevir-pibrentasvir have shown to be effective and safe in persons with kidney chronic disease and HCV infection.

Sofosbuvir-based therapy does not have the safety and efficacy data to support its use in patients with chronic kidney disease [44].

Co-infection HBV-HCV has an approximate worldwide incidence of 1-15% [45]. In some cases of co-infection, there may be various effects on the progression of the disease, clinical manifestations and patient supervision, so the liver profile should be observed together with the level of transaminases [46,47].

The anti-viral activity against VHB and HCV is the Peg IFN-based regime. Its efficiency increases when combined with ribavirin: 35% efficiency in HBV and 50–60% efficiency in HCV. This combined therapy with ribavirin reduces the risk of HCC development by 34% [48,49].

In patients with chronic kidney disease and chronic C-virus infection, the DAA treatment works well, the sustained rate of virological response is substantially increasing [50-51].

CONCLUSIONS

The rate of viral eradication and the increase in the tolerance of adverse reactions have been sustained by the emergence of DAA agents, which have counted heavily in the treatment of patients with HCV, co-infections with HBV, HIV and CKD. Interrelations between drugs should be carefully checked before delivery of antiviral therapy.

The anamnesis should contain questions about the medical history of the patient (surgical interventions, diabetes, infections), other factors that may accelerate chronic HCV infection (alcohol, obesity), transmission risks and the approximate duration of infection, signs and symptoms of disease or other extrahepatic manifestations.

The treatments aim to achieve a sustained viral response and to keep disease manifestations under control. For each genotype of HCV, there are multiple therapies approved with quite high effectiveness.

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Management of infectious complications in diabetes mellitus mellitus patients

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Abstract: *Diabetes mellitus is a serious condition, causing multiple health problems due to the disease itself, but also to the complications that these patients are predisposed to. Being a current health problem, many patients tend to disregard the doctor's recommendations concerning the necessary measures to maintain adequate and continuous control of the glycaemia levels. This, in turn, may cause complications, which include neuropathy, microangiopathy and a predisposition to infections due to weak local defence mechanisms.*

In otorhinolaryngology, the infectious pathology is one of the most often causes for presentation to the physician. Ranging from common otitis, acute pharyngitis or laryngitis and sinusitis, these different entities will cause significant discomfort for the patient, prompting the need for medical care. In patients with diabetes, the natural course of the disease is altered, with more severe symptoms, a more extended period of recovery and the predisposition for aggravated cases with the tendency to complications. Such complications may be life-threatening if we take into account the complications associated with acute sinusitis for example, which include orbital or cerebral abscesses.

Our paper aims to present the authors experience on the complete approach of patients with infectious pathology concerning the sinuses associating diabetes mellitus.

Always, we must keep in mind that patients with diabetes represent a particular class of patients that require a personalized approach. They are predisposed to a vicious circle, in which the infection causes diabetes imbalance, which in turn decreases the defense mechanism and predisposes to complications. The correct management must always include a proper control of diabetes, a complete evaluation that will determine if complications are already present and aggressive management of the infection, with regular check-ups to determine the evolution of the patient.

Keywords*: infections, diabetes mellitus, local complications*

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INTRODUCTION

Due to the high prevalence of diabetes mellitus in the general population, this group of patients has always benefited from practical guides concerning the proper management of the disease and the therapy protocol in case of imbalances or complications of the disease. The same principle applies for the complete approach of any secondary problems, such as electrolites imbalances in the normal body functions or associated infections [1, 2].

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It is accepted that patients with diabetes are predisposed to infectious complications due to the depressed local defense mechanisms, the body's incapacity to limit the infection and the high risk of unbalanced glycaemia levels [3-5]. However, studies that show the specific cellular dysfunctions that cause this problem are still limited. Some even stated that the link between diabetes and infections is difficult to establish [6], while other studies reinforce this concept [7]. However, the number of patients included remains limited and the conclusions scarce.

One fact remains unanimously accepted, and that is that the management of a patient with diabetes that associates an active infection is different and must be more thorough than that of a non-diabetic patient with the same illness. The guides clearly state that the infection must be limited, while the diabetes is kept under control with normal glycaemia levels, even if the use of insulin becomes necessary. In this manner we can avoid further complications that can prove to be life-threatening.

The mechanisms by which diabetes mellitus affects the normal immune capacity of the body are still unclear. Studies so far have demonstrated an association between the presence of diabetes mellitus and a decreased function of polymorphonuclear cells and macrophages [8, 9]. These cells involved in the natural processes of chemotaxis and phagocytosis present with a decreased response, thus lowering the host’s capacity of self-defence. This was demonstrated when comparing the function of these cells in patients with diabetes compared to non-diabetic subjects [10].

Another mechanism that was described in order to explain the predisposition to infectious complications of diabetic patients is represented by disturbances in the humoral immunity, such as a low response of cytokines following stimulation or low levels of complement factor 4 [11].

In order to provide accurate and efficient management of these patients, it is essential to establish which mechanisms play the most significant role. Studies so far suggest that humoral adaptative immunity remains efficient also in patients with diabetes mellitus, as proven by their capacity to respond well to vaccines [12, 13]. As far as the cellular immunity is concerned, dysfunctions at this level have already been demonstrated, although at a lower level than in cases of HIV infections or other immunosuppressant conditions. Of this, one of the most important aspects is represented by lymphocytes dysfunctions in diabetes patients and delayed hypersensitivity reactions [14].

Diabetes mellitus may influence the onset and evolution of infectious diseases by other means as well. High levels of blood glucose create an environment in which a microorganism is more virulent and presents an increased adherence to the cells. This is especially true in patients with long-term unbalanced diabetes that present constant high levels of blood glucose.

Another problem that may occur in patients with diabetes and infections is the risk of diabetic ketoacidosis [3]. This complication may prove to be fatal in a large number of patients when it occurs, and it requires intensive care and aggressive management [15].

Due to all these factors, the management of the diabetic patient with an infection must be a multidisciplinary one. The team must include diabetes and nutrition specialist, an infectious diseases specialist, an intensive care unit doctor if the condition of the patients requires it, and specialist that treats the affected organ. Only with such a complex approach can these patients be balanced and healed in safe conditions.

Infections in diabetic patients can appear at any level and can involve primarily any organ. Starting from common cutaneous infections and up to severe ones, such as meningitis or myocarditis, these infections can appear alone or as part of a more severe condition and can lead to sepsis. The source of the primarly infection must be identified, and proper care measures must be ensured as soon as possible. These include broad-spectrum antibiotics as a first measure, followed by an antibiotic treatment according to an antibiogram after samples have been sent for testing. Other measures include anti-inflammatory drugs and the surgical drainage of any collection

MATERIALS AND METHODS

This paper aims to describe the proper management protocol used in our clinic for diabetic patients presenting with infections in the ENT field, more precisely the management of rhinosinusitis in diabetic patients.

Taking into account the septic state of ENT cavities, we must keep in mind that patients with diabetes are more predisposed than the general population to develop infectious conditions. These may include otic infections, laryngeal of pharyngeal problems, but one of the most common conditions is represented by sinusitis.

Patients diagnosed with sinusitis usually associate one or more of the following complaints: purulent nasal discharge, headache, cranio-facial pressure and vertigo. In acute cases, high fever may occur. The causes of sinusitis are divided into nasal and odontogenic. The last one may represent a severe complication in patients with diabetes due to their predisposition for dental problems.

In such patients, the primary protocol is similar to that of non-diabetic patients. Antibiotic treatment associated with anti-inflammatory drugs, antihistaminics and proper hydration are the most common measures and must always associate nasal decongestants.

Complete blood work must be performed in order to asses the toll this episode inflicts on the organism. Efficient monitoring of the patient is mandatory, especially in diabetic patients, due to the risk of rapid-onset complications.

Figure 1: CT images of a patient with diabetes and sinonasal mucormycosis.

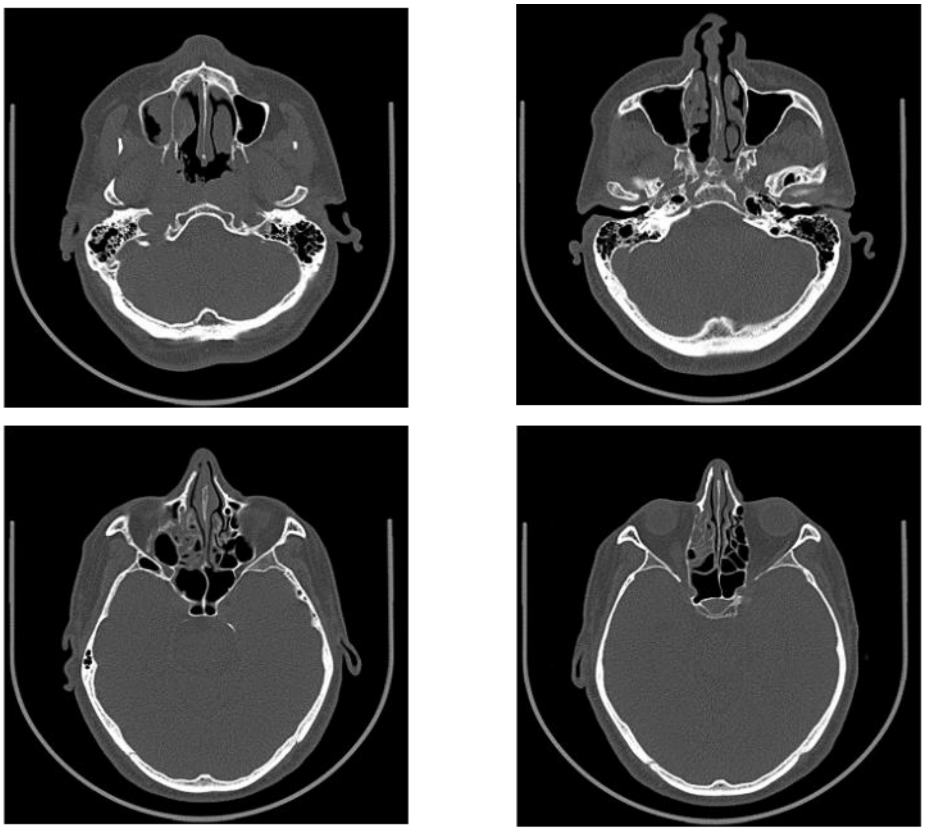

The complete diagnosis of these patients should include a computed tomography, which reveals the specific extension of any sinusitis. This is not routinely performed in the acute period, except for the cases that raise the suspicion of complications, such as an orbital abscess or meningitis. If the patients associate a positive response to therapy, the imagistic examination should be performed at a period of time following the acute infectious episode.

Taking into account the agent that causes the infection, sinusitis can be either bacterial or fungal. Each can occur at diabetic patients, but they are at risk of developing a more significant number of fungal infections than the general population. This may be caused by both low local defense mechanisms, but also to the high incidence of dental problems in these patients.

Figure 2: Mucormycosis lesion of the mucosa of the rhinopharynx in a diabetic patient – intraoperative view.

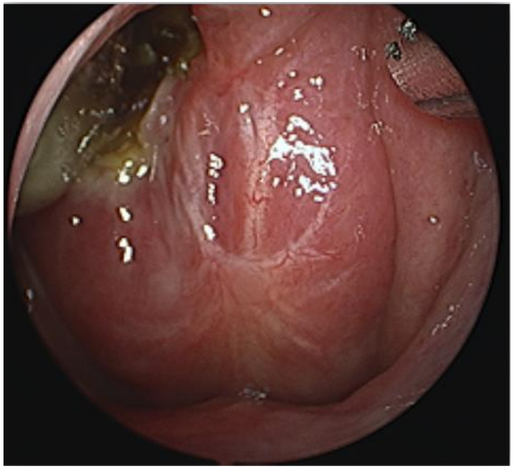

Figure 3: Intraoperative view of the same patient following an extensive debridation up to healthy tissue.

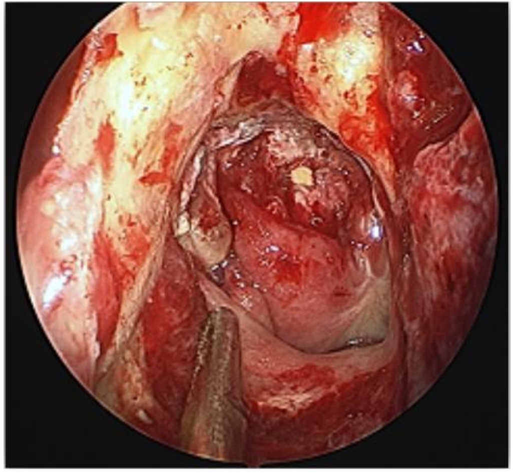

A significant aspect of the management of patients with sinusitis is the necessity to drain any purulent collection that prevents complete healing. The principle must be applied for all patients, but in diabetic patients, it is extremely important. Oral medication is more often inadequate in completely controlling the course of the disease. The surgical drainage may prove to be one of the simplest gestures that can be performed, with low risks. This principle applies to all patients but can be the only viable option in particular cases, such as Mucormycosis infections. In such cases, the surgical debridation of the affected area up to healthy tissue may be the only way to control the spread of the fungi.

The need for a multidisciplinary team remains, especially in patients with unbalanced diabetes that may require an aggressive approach [16]. Without adequate control of glycaemia levels, the healing process will be longer and less efficient. Also, an acute infection associates the risk of fluctuant glycaemia levels, with secondary complications. These patients have a higher risk of sepsis due to the decreased power of the body to contain the infection.

The surgical approach must be thorough and ensure adequate drainage of the infected sinuses. The same principle applies to bacterial or fungal sinusitis. Only the complete removal of the infectious reservoir will allow for a complete recovery.

RESULTS AND DISCUSSIONS

Patients with diabetes, especially if poorly controlled, represent a surgical challenge. Although the intervention itself follows the same principles as in non-diabetic patients, the surgeon must be even more thorough during the intervention and ensure that all infected sinuses have been drained and, in cases of fungal sinusitis, all affected tissue has been removed. The weak local defense mechanisms are not reliable in such cases, and any infectious reservoir remaining can cause a relapse in the disease [17].

These patients represent a challenge from the point of view of the anesthetist as well [18, 19]. The glycaemia levels are more difficult to control during the infectious process, they associate a higher risk of complications, including sepsis, and the response to usual therapy may be altered.

In our experience, the surgical gesture of draining the infected sinuses can be salvatory. Antibiotic or antifungal treatment is mandatory, depending on the type of sinusitis. However, therapy alone can be insufficient and unable to limit the infection. This is even truer in diabetes patients, due to their body's low defense mechanisms. In cases of sinusitis, after a complete diagnosis that must in some cases include an imagistic evaluation, the surgical intervention is advisable if the response to treatment in the first days is insufficient or if any complications appear. The imagistic examination of choice is represented by a CT scan, which can be completed with an MRI if complications, such as orbital or cerebral extension, are suspected.

The functional endoscopic sinus surgery is a minimally invasive one and ensures a good visualization of the affected area. It allows the surgeon to control the patency of every sinus and verify if all sources of infection have been removed.

The postoperative care is crucial, being a key element in the complete recovery of the patient. Especially in a diabetic patient, maintaining appropriate glycaemia levels in the postoperative period will increase the patient's chances of a full and uneventful recovery.

CONCLUSIONS

Diabetes remains a challenging disease, despite the advancements that are continuously made concerning the therapy. The diabetic patient remains a particular one, requiring a more thorough approach, especially when an infectious process is present. People with diabetes are at higher risk to develop complications and more susceptible to inadequate and incomplete healing. Another frequent problem is caused by the multiple problems caused by diabetes, even concerning the local defense mechanisms. In such patients, a vicious circle may occur, based on the relationship between infection and unbalanced diabetes, where diabetes favors the infection. This, in turn, causes difficulties in maintaining normal glycaemia levels, which paves the way for infectious complications.

The authors recommend a multidisciplinary approach of patients with ENT infections and diabetes. This applies even more for patients with sinusitis, which is a common disease but can develop even life-threatening complications if incompletely treated. If the disease does not respond well to medical therapy, it is advisable to ensure the surgical drainage of the affected sinuses and the ENT surgeon must do so before complications. For patients with diabetes, we must also bear in mind that the onset of complications may be more rapid and dramatic.

Once the infection has been drained, the healing process will have a normal course only if the patient is well balanced, with normal glycaemia levels. Insulin may be necessary, even in patients that did not require this prior to the event. Only the proper management of diabetes allows a normal recovery. Even so, these patients must be carefully observed to ensure no secondary complications or relapses occur, and if such cases occur, the necessary measures must be quickly taken, including revision surgery if needed.

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ORIGINAL ARTICLES

Management of war-related vascular injuries: A civilian surgeon experience in the treatment of war casualties at a secondary care hospital

Bariş Akça[1]

Abstract: *Background: This study presents the management of patients with war-related injuries and early results of vascular injuries treated at a secondary care hospital neighboring to civil war.*

Methods: Between December 2013-December 2014 ninety-six war-related injury patients evaluated by a single vascular surgeon and participating disciplines according to components of injury in polytrauma patients. Thirty-six patients who underwent vascular surgery were retrospectively registered to the study. Demographics, treatment and early results of patients reviewed.

Results: Mean age was 26.69±11.80 (7-66) years and 91.66% (33/36) were males. Injuries were caused by shrapnel wound due to bombing 61.11% (22/36), stab wound 8.33% (3/36), gunshot wound 30.55 % (11/36). The number of patients with polytrauma was 29, seven patients had isolated vascular injury. Among 42 arterial and 31 venous injuries primary suture repair, autologous vein graft interposition and prosthetic graft interposition performed to 20.54% (15), 28.76% (21), 19.17% (14) respectively. Amputation and all-cause mortality were %8.3 (3/36), 13.8% (5/36) respectively.

Conclusions: Vascular injury with concomitant trauma is the main issue of war-related injuries. Surgeons should be cautious about patients who were initially deemed not to need surgery for vascular injury and then were found to need it. Repetitive physical examination is very important in case of any suspicion, especially in limited conditions for diagnostic tools like CT angiography. A rapid, careful multidisciplinary evaluation of experienced surgeons for the diagnosis and the treatment priorities in war-related injury patients may lead to increase life and limb salvage rates with a better quality of life.

Keywords: *vascular system injuries, war-related injuries, mortality, amputation, multiple trauma*

INTRODUCTION

Vascular injuries reported as varying rates of 2-3% of civilian trauma [1, 2], while about 7% of war-related trauma [3]. War-related vascular injuries frequently result in life-threatening hemorrhage or limb loss, and while the amputation rate was 60 % in the past, with ligation being the preferred technique for arterial injuries, today this rate has decreased to as low as 1 % through developments in early transport, damage control methods providing hemodynamic stability, diagnosis and complex treatment techniques [4, 5]. Early intervention is considered crucial for a successful outcome, although the management of vascular injuries is clinically challenging even for experienced surgeons when resources are limited, particularly in polytrauma patients, as imaging methods like computed tomography angiography (CTA) or angiography is necessary for prompt diagnosis, as well as for prompt and appropriate treatment. Ideally, war-related injuries should be treated by surgeons with military surgery experience, although civilian surgeons may find themselves in practicing military surgery without prior

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training or experience in this field [6].

As a result of ongoing civil war in Syria, an increasing number of patients with war-related polytrauma have been managed in a frontier secondary care hospital which neighboring the civil war, in Kilis, Turkey. This study presents the experiences of a single civilian surgeon with war-related injury management strategies and the early results of vascular injuries treated at a secondary care center with limited resources.

METHODS

Between December 2013 and December 2014, 96 patients with war-related injuries were evaluated for suspected vascular injury through a multidisciplinary approach involving a single vascular surgeon and other disciplines, who were involved depending on the components (cranial, chest, abdomen, bone fracture and soft tissue loss) of injury in polytrauma patients. Data of patients were recorded for retrospective analysis in real time. This retrospective study was conducted after obtaining the Ethical Committee approvals of Inonu University and where the work was carried out.

All patients were transferred to our hospital from the place where the injury occurred. In accordance with Advanced Trauma Life Support protocols and damage control principles [7, 8], tourniquets were routinely applied to all patients to achieve rapid hemorrhage control, and permissive hypotension (massive red blood cell, plasma, and minimal crystalloid infusion) was used to initiate a hemostatic resuscitation plan aimed at correcting metabolic imbalances. Subsequently, patients were evaluated for vascular and other injuries. In the absence of angiography and CTA, diagnosis of vascular injury was made almost entirely through physical examination findings, with a Doppler ultrasound (DUS) used when possible. If a patient presented with only hard signs of vascular injury, such as active pulsatile hemorrhage, absent distal arterial pulse, ischemia, expanding or pulsatile hematoma with no concomitant injury, immediately transported to the operating theater for vascular repair. After the multidisciplinary agreement about the benefit of vascular repair and concomitant surgical interventions, and an initial surgery decision according to clinical status at admission, patients with polytrauma were sent immediately to the operating theater. However, patients presenting with soft signs of vascular injury (non-pulsatile and non-expanding hematoma, palpable but diminished or unequal distal arterial pulses, bone fracture, peripheral nerve deficit and history of bleeding that subsided spontaneously) and with no immediate threat to life or limb, were preferred to be referred to a center where further investigations could be made. If there was no such opportunity, these patients were evaluated by DUS, if possible, or mostly through repetitive physical examinations, for clinical findings of ischemia, cyanosis, the absence of arterial pulse and hemorrhage. CTA or angiography was essential for diagnosis and the treatment options in 19 (19.79 %) polytrauma patients with uncertain physical examination findings, hence they referred to the relevant centers.

Among 96 patients evaluated for vascular injury, a total of 36 (37.5%) patients operated by vascular surgeon enrolled in the study. All patients received a tetanus vaccine and dual antibiotics prior to surgery. The initial surgical exploration was usually performed by the vascular surgeon, both for better bleeding control and arterial and venous repair, especially in injuries of the extremities, based on hemodynamic status and duration of ischemia. The exploration of injured vessels was performed via standard incisions if in the same course as the injury area; otherwise, proximal and distal control of the vessels was obtained directly from the injury area. In patients with extremity injuries (bone, nerve, and soft tissue damage, whether individually or together) vascular repair was performed immediately after proximal and distal control of the vessels to achieve better bleeding control and stable hemodynamic status in cases where extremity-threatening critical ischemia existed. Otherwise, vascular repairs were made after bone stabilization with external fixators.

Systemic Standard heparin was used intra-operatively, based on the level of bone and soft tissue damage/loss in non-coagulopathic patients. Thrombectomy was performed routinely with an embolectomy catheter after the routine assessment of proximal inflow and distal backflow. Also, the distal segment of the artery was cleansed with heparinized saline in patients for whom systemic standard heparin could not be used.

Vascular injuries were managed via simple repairs, end-to-end anastomoses, interpositions of the autologous saphenous vein graft or polytetrafluoroethylene (PTFE) graft and simple ligation methods. The vascular repair method was determined based on an intraoperative assessment of the injured vessels. The decision of ligation was based on life-threatening hemorrhage and distal arterial circulation with more than one artery (Ulnar/radial and one of the tibia arteries). Venous injuries of large veins, especially to the femoral, popliteal and axillary veins, were repaired via simple repair, vein graft or PTFE graft methods, when possible. Other minor venous injuries were ligated.

During the initial evaluation in the emergency room, soft tissue defects and necrotic tissues in patients with limb injuries were scrubbed with 2000–3000 cc of Ringer solution and cleansed from foreign bodies prior to the operation. Subsequently, the necrotic tissue debridement, foreign body removal and soft tissue reconstruction performed after forceful irrigation with Ringer solution in the operating theater.

Low molecular weight heparin was given to all patients postoperatively for anticoagulation, and a dual or triple antibiotic regimen was applied postoperatively, based on the contamination and severity of the injury.

Fasciotomy was performed by orthopedists intra operatively or during intensive care unit follow up, according to the follow up of patient and operative management of injury. Nerve injuries were repaired either in the same session or delayed for two to three weeks, by orthopedists or neurosurgeons.

The demographics, treatment and early results (in hospital period; up to postoperative seven days) of patients were reviewed, and the studied variables included age, gender, clinical signs and symptoms on admission, anatomical region of vascular injury, mechanism of injury, concomitant injuries in polytrauma patients, Mangled Extremity Severity Score (MESS) [9], type of vascular repair and clinical outcome.

The data were analyzed using PASW Statistics software (SPSS Inc, USA), and was presented as mean (SD), range or numbers and percentage (%), as appropriate.

RESULTS

The mean age was 26.69±11.80 (7-66) years and 33 (91.66%) were male. The mean MESS score was 8.47±2.64 (3-14), and extensive tissue defect/loss was observed in 12 (33.33%) patients. The cause of injuries were shrapnel wound due to the bombing, stab wounds and gunshot (mostly rifle) wounds in 22 (61.11%), 3 (8.33%) and 11 (30.55%) patients respectively.

The anatomical localization of injuries presented in Table 1.

Table 1. Anatomical localization and repair methods of injured 73 vessels in 36 patients

Repair method **Vessel**	**n** *(73)*	**Primary repair** *(15)*	**Prosthetic graft** *(14)*	**Vein graft** *(21)*	**Ligation** *(23)*
Carotid artery	1				1
Subclavian artery	1		1		
Brachial artery	4	1		3	
Radial artery	3			2	1
Ulnar artery	3	1			2
Common Femoral artery	5		2	3	
Superficial femoral artery	8	2	3	3	
Profunda femoral artery	3	1		1	1
Popliteal artery	5		3	2	
Anterior Tibial artery	5	1		3	1
Posterior Tibial artery	4			3	1
Internal jugular vein	1				1
Subclavian vein	1	1			
Common femoral vein	7	4		1	2
Profunda femoral vein	3	1			2
Superficial femoral vein	7	3	3		1
Popliteal vein	4		2		2
Anterior tibial vein	4				4
Posterior tibial vein	4				4
Total (%)		20.54	19.17	28.76	31.50

Of the patients, high-energy injuries were observed in 24 (66.6%), medium-energy injuries in 2 (5.55%), low energy injuries in 3 (8.33%) patients and 7 (19.44%) patients had crush injuries (rescued from beneath the wreckage of collapsed buildings after the bombing). The number of patients with polytrauma was 29 (80.55%) and concomitant injury distribution of patients presented in Table 2.

In the study, 7 (19.44%) patients had isolated vascular injury; 12 (33.33%) patients underwent vascular surgery with concomitant active bleeding; 17 (47.22%) patients presented with hypovolemic shock upon arrival at the emergency room; ischemia signs were observed in 24 (66.66%) patients, and the mean ischemia time was 5.45 ± 5.20 (1-24) h.

Table 2. Concomitant injury distribution of 36 patients enrolled in the study

	n	%
Cranial injury	3	8.3
Neck injury	1	2.7
Chest İnjury	5	13.8
Abdominal injury	7	19.4
Pelvic injury	4	11.1
Lower extremity injury	28	77.7
Upper extremity injury	11	30.5
Large tissue defect	12	33.3

Of the patients, 25 (69.44%) had single extremity injuries, 9 (25%) had double extremity injuries and one patient had three extremity injuries. In 20 (55.55%) patients, extremity injuries were accompanied by bone fractures, of which 17 (47.22%) were open fractures.

Initial physical examinations revealed motor loss in 20 (55.55%) patients. Intraoperative nerve injury was mostly determined as a full-thickness incision in 12 (33.33%) patients. Three (8.33%) patients with polytrauma had postponed surgery decision after a follow-up period for repetitive physical examinations due to uncertain initial physical examination findings.

A total of 42 arterial and 31 venous injuries were observed in the operating theater. The most common repair method was graft interposition with autologous saphenous vein and PTFE. Ligation was the second preferred method, especially for venous injuries, and 31.50% of total injuries were ligated. The details of repair methods presented in Table 1.

Postoperative complications are listed in Table 3.

Table 3. Postoperative complications of patients

	n	%
Graft Thrombosis	4	11.1
Amputation	3	8.3
Mortality	5	13.8
Reperfusion injury	1	2.7
ARDS	1	2.7
MODS	1	2.7

ARDS: Acute respiratory distress syndrome, MODS: Multiple organ dysfunction syndrome

Thrombectomy and/or embolectomy performed for all postoperative graft thrombosis patients. Three patients had autologous vein graft thrombosis and one had a prosthetic graft thrombosis. The amputation rate was 8.3% (3/36). Two (5.55%) patients had secondary amputation after vascular repair and one patient with mangled extremity underwent primary amputation via an intraoperative multidisciplinary decision in order to save life. All three patients who underwent amputation had injuries of the popliteal artery including trifurcation.

The mortality rate was 13.8% (5/36), four of them had high-energy injuries and one had medium-energy injury. Furthermore, two patients with concomitant abdomen injuries died from sepsis, one patient with concomitant cranial injury died due to intracranial hemorrhage. Two patients in hypovolemic shock with concomitant large soft tissue defect underwent surgery with preoperative early intubation and died from acute respiratory distress syndrome and multiple organ dysfunction syndrome. A fasciotomy was applied to three patients, of which two were closed and one was open.

DISCUSSION

As reported variously, the incidence of war-related vascular injuries has been increased gradually from World war II (0.2-4%) to the wars in Afghanistan and Iraq (6.8 %), also a recent study from the Lebanon war reported that the rate of vascular injuries increased to 7.6% of all casualties, and to 10.8% in the soldiers [4, 10-12]. This increase is possibly correlated with shortened evacuation time, improved immediate resuscitation, damage control by the progressive and effective use of tourniquets and temporary shunts. Furthermore, these developments over years have brought improved life and limb rescue rates in war-related injuries [13-15]. In this civilian study, however, the distance to the war zone (15 min. away by road to the hospital) was the most important factor affecting the increasing admittance of war-related trauma patients.

Recent experiences have triggered the management of such injuries, assessing especially the effect of explosives on injury patterns and treatment strategies [3]. Explosive weapons are designed to increase the number and energy of casing fragments, leading to multiple penetrating wounds [16]. In the present study, most common injuries were shrapnel wounds from bombing and rifle wounds, hence these kinds of high energy injuries includes both penetrating and blast effects on tissues as designed. Therefore, vascular injuries associated with multiple trauma often lead to high mortality rates, unless prompt and appropriate surgical management is made [17], hence war-related injury treatment should be provided by surgeons with military surgery experience [6].

Vascular injuries of extremities are the most common type of injury in war-related trauma patients [3, 12-14], as is the case in this study and it may lead to limb loss or mortality. Bleeding may lead to hypovolemic shock, as was the case with 47.2% of the patients in the present study at the admittance. Consequently, the primary aim is to avert exsanguination and death, and then to restore blood flow to prevent amputation.

The initial physical examination of patients who present with hypovolemic shock may be misleading about vascular injury due to diminished or absent distal arterial pulse and inadequate perfusion of extremities. In patients suffering from hypovolemic shock, recurrent physical examinations are important, as in patients with soft signs, and also should be carried out after appropriate resuscitation of hypovolemic shock. Three polytrauma patients of this study underwent surgery through recurrent physical examination findings.

If the initial evaluation of a patient revealed hard signs of vascular injury with a single entry and/or exit wound, and with no concomitant injury, there is consensus on the decision to make an immediate vascular exploration after resuscitation in the emergency room. However, there is a lack of consensus regarding the management of patients with only soft signs in polytrauma patients complicated with concomitant injury, multiple extremity injuries or single extremity multiple segment injuries with soft or no signs of vascular injury [18]. For this grey zone of patients with soft signs, additional diagnostic methods such as angiography or CTA are required to be applied prior to surgery in hemodynamically stable patients, both for diagnosis and choice of treatment [19-21]. Of these, angiography is the optimum diagnostic method, although CTA has gained increasing value in diagnosis and treatment planning, therefore some studies reported that CTA should be considered as the first line modality [19].

In this study, patients underwent immediate vascular surgery on the basis of physical examination findings and hard signs of arterial injury with the goal of shortening the ischemic period. As neither angiography nor CTA was present, hemodynamically stable patients with soft signs of arterial injury were referred to a center where these examinations could be made. In a very small number of patients DUS could be used. In the absence of contrast imaging, physical examination findings with hard signs of vascular injury seem to be an effective means of diagnosis, although the need for contrast imaging methods for patients with uncertain physical examination findings of vascular injury is undisputed.

It should not be forgotten that the possibility of arterial trauma still exists, particularly in the upper extremities, with palpable distal arterial pulses [18-22], also impaired perfusions with absent distal arterial pulses may not be noticed due to of collateral circulation.

In this study, ligation of vessels was considered as the final option especially in hemodynamically stable patients, although ligation is a good strategy in selected vessels that do not cause distal extremity ischemia, and in cases of life-threatening bleeding.

Similar to other studies [3, 23, 24], superficial femoral, common femoral, popliteal and brachial arteries were the most commonly injured arteries in this study. Vein injuries are mostly accompanying with the adjacent artery injuries. The most common repair method of this study was saphenous vein graft interposition, as recommended in most studies [3, 25]. Applying a prosthetic graft is still a controversial issue, increased risk of thrombosis and infection, thus poor outcome with a high secondary amputation rate [17, 23, 26]. In this study, contrast with the previous studies the absence of early infection despite the high ratio use of prosthetic grafts may be attributed to the factors like; a large amount of wound irrigation as an initial response in the emergency department and also in the operating room, careful and aggressive debridement of necrotic and contaminated tissues, additionally use of dual or triple antibiotic regimen for the follow up. In contrast with expectations, the number of autologous vein graft thrombosis was higher than prosthetic grafts. Revascularization of these polytrauma patients was carried out initially, followed by bone and soft tissue operations. Therefore, thrombosis developed due to inadequate length (short/long) of the graft. No temporary vascular shunting was used in this study.

The amputation ratio relies on many variables, including the severity of limb injury, mechanism of the injury, ischemia time, existence of accompanying injuries, and disaster conditions when dealing with mass injuries [13]. In this study, three patients with popliteal artery injuries underwent amputation, one pediatric patient who had an ischemia time of 2h with MESS score of 9 underwent primary amputation by an intraoperative multidisciplinary decision in order to save life. The ischemia times of these three patients were found to be 2h, 4h, and 8h. The MESS scores of patients who underwent amputation were 9, 10 and 11 while the mean MESS score of this study was 8.47±2.64. During the treatment of mixed skeletal, vascular and soft tissue injuries, limb ischemia specifies the treatment primacy, and also high limb loss rates related with popliteal artery involvement [19]. The amputation rate of this study is close to previous studies reported in varying rates (3-20%) [27, 28]. Mortality rate (13.8%) of this study was higher than the varying rates (1.5% - 6%) have been reported in previous studies [28, 29].

Retrospective design of the study, short follow up period of patients and relatively small number of patients are the basic limitations of this study.

CONCLUSIONS

When dealing with war-related injuries, especially in polytrauma patients, occult vascular injuries have been possibly missed due to uncertain initial physical examination findings of vascular injury and frequently presents later. Therefore, the definite diagnosis of vascular injury should be revealed by angiography or CTA. Following the diagnosis, some critical decisions arising such as: which patient should be operated first, among who applied at the same time and requiring emergency surgical intervention; which one of the injury components should be operated initially in patients with polytrauma; which type of injuries of polytrauma patients should be operated by two different disciplines at the same time, in the same session; and also which type of injury can be delayed. These are challenging situations for even most experienced surgeons in patients with complex polytrauma accompanying with systemic effects. Therefore, a multidisciplinary approach should be used when establishing treatment priorities.

Civilian surgeons unexpectedly finding themselves acting war surgery with no background, hence may tend to make the same traditional mistakes that military surgeons have already learned from previous wars, nevertheless a rapid, careful multidisciplinary evaluation of experienced surgeons for the diagnosis and treatment priorities in war-related injury patients may lead to increase life and limb salvage rates.

Acknowledgement

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ORIGINAL ARTICLES

Managing radial nerve injuries associated with humeral fracture

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Abstract: *: The overall prevalence of radial nerve injury after humeral shaft fractures is up to 18% representing the most common peripheral nerve injury associated with long bone fractures.Spiral or oblique fractures of the junction between the middle and distal third of the humeral shaft are at greater risk for radial nerve injury. Iatrogenic damage to the radial nerve may also occur during manipulations of closed reduction.*

Material and methods: A prospective study between 2017-2019 following 5 patients (all male, with ages between 30 and 56 years old), who were treated at our clinic for complete radial nerve palsy after a high-energy fracture of the humerus

Results: All the patients were followed-up for a mean of 6 months (range 4 - 15 months) post op. The first 3 cases showed the initial signs of recovery 4 weeks after the operation. In the last 2 cases, a palliative radial intervention was required.

Conclusions: The consequences of the radial nerve palsy associated with humeral fractures is strongly related to trauma mechanism. In high-energy fractures, severe contusion or transection of the nerve must be expected. In this case, nerve recovery is unlikely and the patients should be informed of the poor prognosis and the need of tendon transfers.

Keywords: *humeral fracture, compression, upper limb, radial nerve, callus*

INTRODUCTION

Fractures of the humerus are often complicated with radial nerve palsy. The overall prevalence of radial nerve injury after humeral shaft fractures is up to 18% representing the most common peripheral nerve injury associated with long bone fractures.[1-5] This is caused by the relatively fixed position of the radial nerve and the direct contact with the periosteum of the humerus as the nerve courses through the middle and distal thirds of the upper arm and by the nerve's diminished mobility where it pierces the lateral intermuscular septum. As such, at this level the bone ends can easily entrap, contuse, or even lacerate the radial nerve, varying from neurapraxias to complete neurotmesis.

Spiral or oblique fractures of the junction between the middle and distal third of the *humeral shaft are at greater risk for radial nerve injury.[6] Iatrogenic damage to the radial nerve may also occur during manipulations of closed reduction or at the time of surgical intervention, during internal fixation with a intramedullary nailing or compression plate.[7, 8, 9]

Treatment is often expectant (non-operative), though surgery is also sometimes necessary by a variety of often imaginative procedures. Because radial nerve injuries are the least debilitating of the upper limb nerve injuries, results are usually satisfactory.[10]

PATIENTS AND METHODS

A prospective study between 2017-2019 following five patients (all male, with ages between 30 and 56 years old), who were treated at our institution for complete radial nerve palsy after a high-energy fracture of the humerus. In all cases, the fracture was stabilized and no other complications except the radial palsy. Baseline electrophysiological studies (i.e. nerve conduction velocity studies and electromyography) conducted before admittance showed a complete motor paralysis of the nerve and all had an absent Tinel's sign at each clinical examination.

Operative Procedure

Case 1: A 41-year-old male, with a 4 months old high-energy transverse diaphyseal fracture of the left humerus and faulty soft tissues following a car accident was treated with plating.

The same anterolateral approach used for plating was used during our surgical exploration where we found contusion of the radial nerve and perineurial adhesions of a small segment at the level of the humeral fracture (Figure 1).

Figure 1: Perineural adhesion of the radial nerve to the humerus

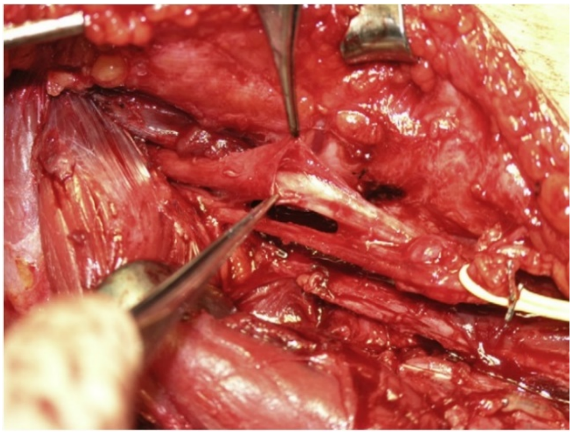

External neurolysis was practiced (Figure 2).

Figure 2: Neurolysis of the radial nerve

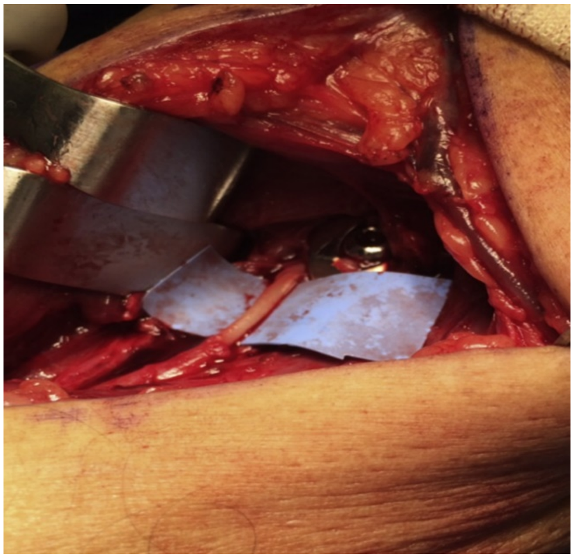

Case 2: A 38-year-old male, with 3 months old closed fracture of the right humeral shaft following a recreational arm wrestling match was treated with two non-staggered plates.

Upon surgical exploration via dorsal approach, a partially lacerated nerve draped over a loose compression plate.

Treatment associated the refixation of the plate and embedment of the nerve in the triceps muscle.

Case 3: A 40-year-old male, with a 3-month-old fracture of the humeral shaft following a car crush was treated with external fixator.

During surgical exploration, the distal pin of the external fixator was found to run through the brachioradial muscle into the body of the radial nerve destroying 15% of its circumference (Figures 3 and 4).

Figure 3: Distal pin winded up in the radial nerve

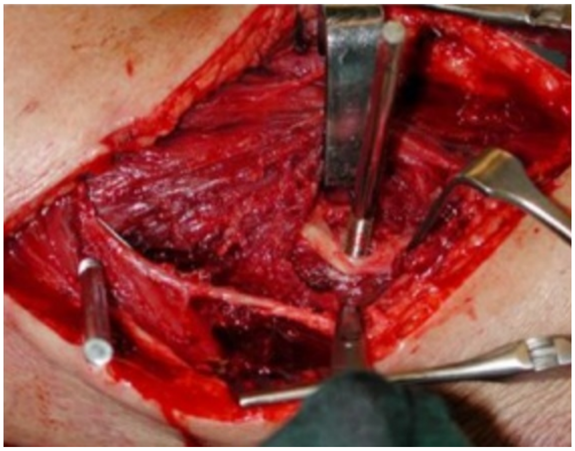

Figure 4: Radial nerve buried in the fracture callus

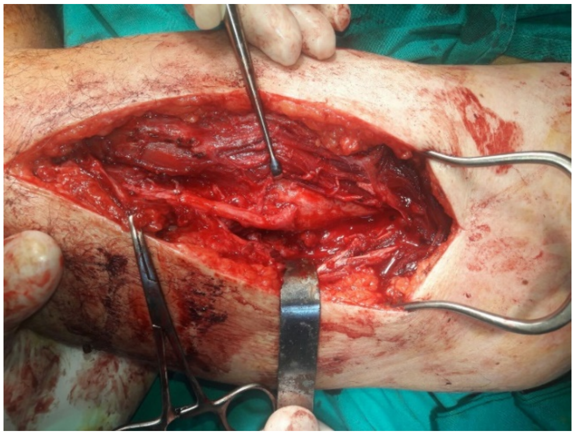

The pin was removed and reattached after releasing the nerve and embedding it in the triceps muscle.

Case 4: A 56-year-old male with a six-month-old fracture of the left humeral shaft was treated with internal fixation after crush in the conveyor belt.

Surgical exploration had a posterior approach through a lateral para-tricipital window revealing the nerve enveloped in the fracture callus more than 8 cm with the impossibility of finding the proximal end (Figure 5).

Figure 5: Presenting the donor tendons. From left to right: palmaris longus, flexor superficialis middle finger and flexor ulnaris carpi

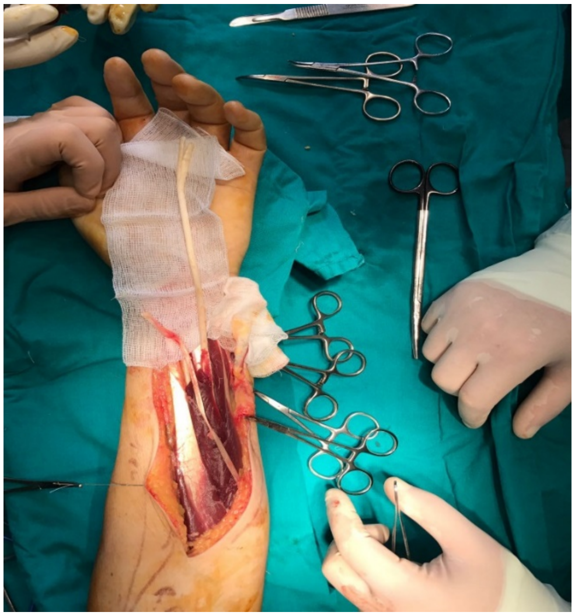

A follow-up tendon transfer procedure was carried out flexor ulnaris carpi to extensor ulnaris carpi, flexor superficialis of middle finger to extensor digitorum communis, palmaris longus to extensor pollici longus (Figures 6 and 7).

Figure 6: Tenoplasty of the extensor digitorum communis and extensor pollici longus by Pulvertaft technique

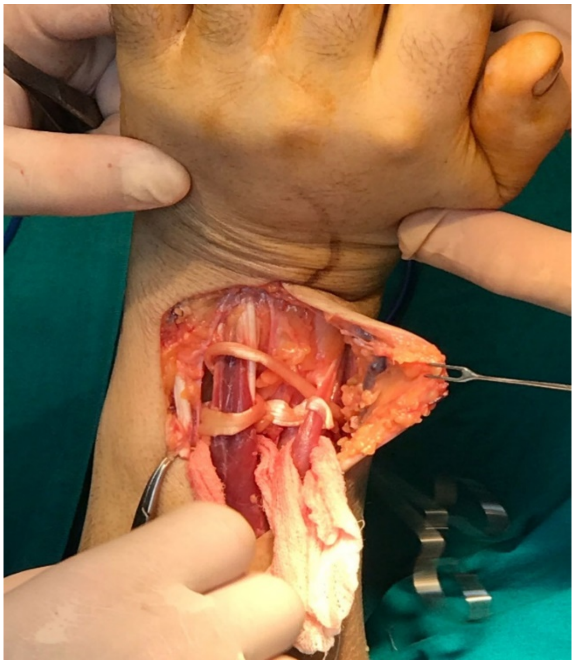

Figure 7: Active extension of the wrist, thumb and fingers 6 weeks post op

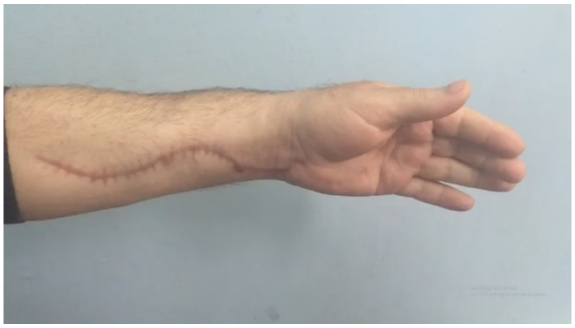

Case 5: A 30-year-old male, with a 12-month-old chainsaw trauma, which required open reduction and fixation of the humerus, anastomoses of the brachial artery with venous grafting and neurorrhaphy of median nerve and ulnar nerve, with the impossibility of finding the radial nerve ends, presented for second intervention in our clinic.

A palliative radial intervention practicing tendon transfer was used.

We transferred the pronator teres tendon to extensor carpi radialis tendon, flexor carpi radialis tendon to extensor digitorum communis and plamaris longus to extensor pollicis longus (Figures 8 and 9).

Figure 8: Presenting the donor tendons. From left to right: pronator teres, flexor radialis carpi and palmaris longus

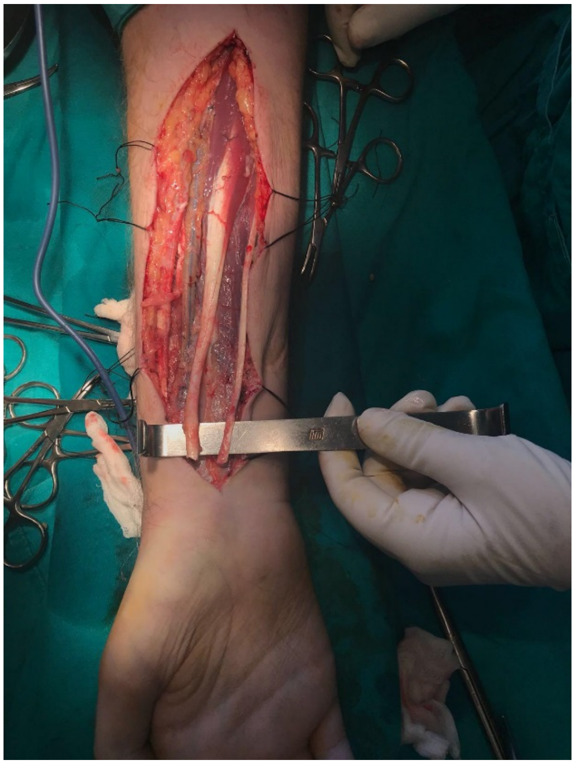

Figure 9: Tenoplasty of the extensor carpi radialis (over the scissors tip), extensor digitorum communis (forceps) by Pulvertaft technique

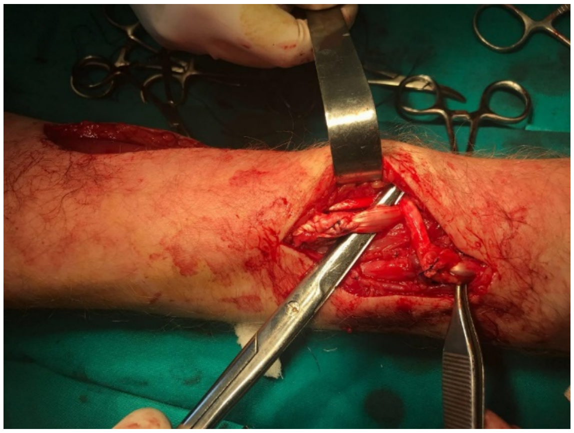

Postoperative follow-up examination included radiographic evaluation of the fracture, physical examination (i.e. Tinel's sign), and monthly electrophysiological studies for motor and sensory nerve recovery.

RESULTS

All the patients were followed-up for a mean of 6 months (range 4-15 months) post op. The first three cases showed the initial signs of recovery 4 weeks after the operation. Full nerve recovery was achieved in a mean of 19 weeks (range 16-22 weeks) with a slight sensory discomfort in the third case. An advancing Tinel's sign was a good predictor for nerve recovery.

In the last two cases where palliative radial intervention practicing tendon transfer was used, the imobilization was removed after approximately 4 weeks and a volar custom splint made by a hand therapist was applied with extension slings for the MP joint of the fingers. Active range-of-motion exercises of the fingers were begun at 3 to 4 weeks, and the wrist at 6 weeks.

Splinting as protection yielded excellent results and was done for 8 to 12 weeks postoperatively.

DISCUSSIONS

These cases highlights the necessity of a postreduction examination of the fracture. Nerve function should always be thoroughly assessed before and after reduction, with complete documentation in the medical record. To ensure accuracy, any patient who requires conscious sedation for reduction must return to full consciousness and alertness before obtaining the postreduction exam.

Radial nerve injury associated with a humeral shaft fracture is a very important injury pattern among trauma patients with an incidence up to 18%. [3] These injuries can be either primary or secondary depending on the timing of the palsy presentation. Early surgical exploration of the nerve is generally indicated for those cases, in which there was an open fracture, fractures that cannot achieve an adequate closed reduction requiring open reduction, fractures associating a vascular injury or a severe soft tissue injury [11].

Patients who demonstrate a primary radial nerve palsy have a reported spontaneous recovery rate of over 70% [12]. In the management of nerve palsy, nerve conduction examination with electrophysiological studies could be obtained at three months post injury, as motor potential should be recovered by this point, and these nerve conduction data will enable the clinician to determine if the injury should require surgical intervention. Early exploration revealing a nerve injury that can be repaired can lead to a quicker return to function and better nerve recovery. Degeneration of the motor endplate and irreversible muscle atrophy occurs if sufficient reinnervation is not present within 12–18 months after injury. [13] Poor functional recovery has been demonstrated in experimental studies when nerve repair is delayed for 6 months due to decreased motor neuron regeneration capacity. [14, 15]

Tendon transfer remains an essential technique in irreparable or long-standing radial nerve palsies for hand function restoration. Because the first signs of nerve recovery can be quite delayed, patience is merited before considering tendon transfers. A waiting period of 12 months is usually sufficient to see any evidence of nerve regeneration. Following a radial nerve injury, the extrinsic extensor function of the hand and wrist is lost, resulting in an inability to extend the fingers and wrist. It affects grip strength and has a large influence on thumb usage. Tendon transfer surgery must restore wrist, thumb and finger function without inflicting other motor deficits on the hand. [15] It is important that the joints have achieved good passive mobility in order to achieve optimal function post-tendon transposition. The donor muscles are tested for strength before surgery, and must come from a region unaffected by the pathology. [16]

Modern medicine sheds light on biological therapies and nerve regeneration. Research is being conducted with pluripotent stem cells and nerve conduits with promising developments using human amniotic membranes loaded with human umbilical cord mesenchymal stem cells in radial nerve repair. [17]

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REVIEW

Manipulation of the gut microbiome may be a promising pathogenic approach in rheumatic inflammatory diseases – A review

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Abstract: *A symbiotic association between humans and microbes has been established over time. Disruptions in gut homeostasis have been linked to immune-mediated inflammatory diseases.*

Factors such as genetics, environment, diet, age, and medication play a major role in shaping the intestinal microbiome. An altered gut microbiome seems to modulate the progression and severity of inflammatory rheumatic diseases. Disruptions in the eubiotic state of the intestinal microbiome might promote intestinal and extraintestinal autoimmune and inflammatory disorders, although the mechanisms involved are not well understood.

In this review, we provide a general overview of several studies concerning intestinal microbiome perturbations and their implications in rheumatoid arthritis, ankylosing spondylitis, and systemic lupus erythematosus. Furthermore, we discuss the relationship between diet, intestinal microbiota, and inflammation.

Keywords: *intestinal microbiome, intestinal dysbiosis, probiotics, rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus*

INTRODUCTION

The human digestive tract is inhabited by somewhere between 300 and 1000 different species of bacteria. Microorganisms like bacteria, yeast, and viruses compose the gut microbiota. Bacteria are classified into phyla, classes, orders, families, genera, and species. [1]

There are four dominant bacterial phyla: Bacteroides spp., Firmicutes spp., Actinobacteria spp., and Proteobacteria spp. The Firmicutes phylum contains more than 200 genera such as Lactobacillus spp., Bacillus spp., Clostridium spp. (95% of the Firmicutes), Enterococcus spp., and Ruminicoccus spp. The Bacteroidetes phylum consists of predominant genera such as Bacteroides and Prevotella. Actinobacteria phylum is less abundant and represented by the Bifidobacterium genus. [2]

The relationship between humans and gut flora is a symbiotic one. The microbial colonization occurs before birth and stabilizes around the age of three. The intestinal microbiome has various roles: fermentation and degradation of dietary fibers, modulation of antibody production, defense against pathogenic bacteria colonization, production of vitamins, and maintenance of intestinal epithelium integrity.

The most important end products of microbial fermentation processes are short-chain fatty acids (SCFA), acetate, propionate, and butyrate. These molecules promote the immunity of the intestinal mucosa and protect it from pathogens. [3]

Only 1% of bacteria can be grown in cultures. New

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techniques like the extraction of DNA and the amplification of the 16S ribosomal RNA gene (rRNA) allowed researchers to analyze nucleic acids (DNA and RNA) from the stool. The Human Microbiome Project (HMP) focused on identifying, studying, and cataloging uncultivated bacteria.

The intestinal microbiome is influenced by diet and the environment. People who eat more carbohydrates and simple sugars have more Prevotella, while Bacteroides are associated with saturated fats and proteins. Plant-based diets are associated with a greater abundance of Prevotella and a greater diversity of microbes [4, 5].

It is believed that high-fiber diets ameliorate autoimmune, metabolic, and allergic diseases mainly through their microbiota dependent fermentation to SCFAs that promote tissue barrier integrity, mucus production, immune serum globulin (IgA) secretion, and regulatory lymphocyte T (Treg) differentiation, sustaining an anti-inflammatory environment [6].

Some microbes, such as Bacteroides fragilis, can drive the immune system to create anti-inflammatory cytokines while some segmented filamentous bacteria favor the production of pro-inflammatory cytokines. Bacteroides promote the production of immuno-regulatory T cells that suppress immune responses to self and bacterial antigens and stimulate epithelial repair. [7]

Manipulation of the intestinal microbiota through diet and probiotics may be the target for future strategies and preventive interventions in rheumatic diseases.

METHODS

Relevant literature relating to the gut microbiome and autoimmune diseases was selected through searching online medical databases. Search terms used were: “intestinal microbiome”, “gut microbiota”, “intestinal dysbiosis”, “manipulation of the gut microbiome”, “rheumatic diseases and intestinal microbiome”, “probiotics”. Only English-written studies published from 2011 to date were selected and analyzed.

RHEUMATOID ARTHRITIS (RA)

Rheumatoid arthritis is a chronic, autoimmune disease that primarily affects joints and the first study that links intestinal microbiome to arthritis was published in the late 1970s, suggesting that bacterial flora may be a stimulus for induction of cell-mediated immunity and may also have suppressive effects on the disease evolution. [8]

The patient microbiome plays a big role in the progression of rheumatoid arthritis. Together with the lymphoid tissue, the microbiome helps maintain immune homeostasis. Changes at this level can damage the intestinal mucosa and the systemic immune system, triggering the onset of autoimmune diseases.

Using zonulin as a serological marker, some recent studies have demonstrated the increased gut permeability in rheumatoid arthritis patients. This finding has been shown in about 1/3 of pre-RA patients. [9]

In rheumatoid arthritis, studies have shown that patients present a dysbiotic state, with the depletion of gram-negative bacteria and an increase of gram-positive bacteria. Furthermore, these patients present a higher concentration of Collinsella and Eggerthella lenta than normal individuals. [10]

As well, these patients have a high level of Prevotella copri and a low level of Bacteroides. P. copri pathogenicity was explained through its capacity of stimulating Th1 cells. Some patients produce antibodies against P. copri (IgA, IgG). Moreover, an over-abundance of P. copri has been identified in individuals at risk of rheumatoid arthritis (patients without arthritis but anti-citrullinated protein antibody – ACPA/ rheumatoid factor – RF positive). [11, 12, 13]

A study showed that a Prevotella strain, Prevotella histicola, suppresses arthritis in humanized HLA-B27 mice. [14] P. histicola treatment can reduce pain and improve motor function as a response to an increase in T regulatory cells and a decrease in IL-6 levels. [15]

Lactobacillus salivarius is more abundant in rheumatoid arthritis patients but its effects are unclear. Administration of L.salivarius in mice showed a significant improvement of collagen-induced arthritis and it may help reduce interleukin-17 and increase anti-inflammatory interleukin-10. Thus, it may help reduce clinical manifestations in RA patients. [16]

A study conducted on 60 women with rheumatoid arthritis, showed the beneficial role of Lactobacillus casei. Used as adjuvant therapy, it reduces the symptoms, lowers the pro-inflammatory cytokines, and lowers serum C - reactive protein and DAS28 score. [17]

A more recent study, conducted on P. histicola treated mice, showed a decrease in the incidence and severity of arthritis. P. histicola decreases the intestinal permeability and lowers the pro-inflammatory IL-17 cytokine and increases IL-10. [14] Because P. histicola is normally present in the gut, the treatment as a probiotic in humans is beneficial, with minimum adverse effects.

A study conducted in China found a greater abundance of

Enterobacteriaceae spp. and Klebsiella spp. and a lower abundance of Bifidobacterium spp. in rheumatoid arthritis patients with high levels of TNF-α or IL-17A. Moreover, ACPA-positive patients had a greater abundance of Blautia spp., Akkermansia spp. and Clostridiales spp. then patients with negative ACPA. In conclusion, patients with RF/ACPA seropositivity exhibited a different microbial composition than RF/ACPA negative patients, suggesting an association between intestinal microbiome alteration and disease activity. [18]

Porphyromonas gingivalis, a periodonthopathogenic bacteria that exist in dental plaque can be swallowed with saliva and can affect the composition of the gut microbiome. Several studies have shown a strong correlation between P. gingivalis infection and systemic disease. Intestinal dysbiosis by oral inoculation of P. gingivalis has been shown to contribute to the progression of arthritis. [19, 20]

Figure 1: Associated microbiome in rheumatoid arthritis

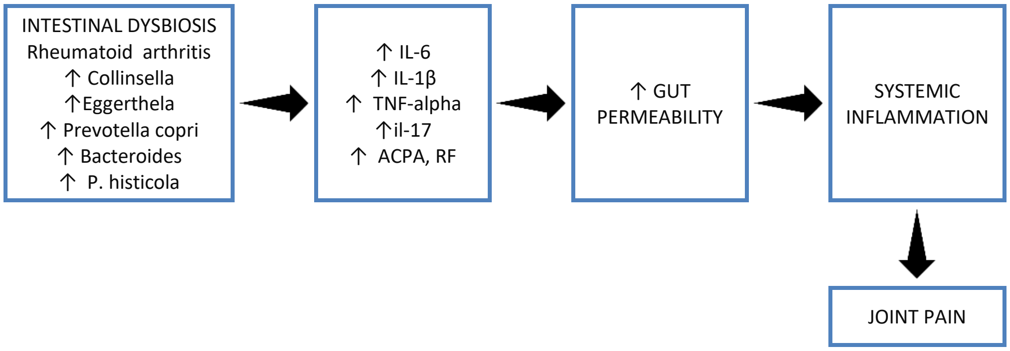

Rheumatoid arthritis treatment and intestinal microbiota

The use of disease-modifying anti-rheumatic drugs (DMARDs) can modulate the intestinal microbiome and has the potential to shift the intestinal flora towards a healthy microbiome.

Two studies showed a significant decrease in Bacteroides fragilis and an increase in Prevotella maculosa after treatment with Methotrexate (MTX). Dysbiosis associated with RA was partially resolved after treatment. [21, 22] Another study suggests that patients that were treated with Methotrexate showed increased species richness and diversity compared to other patients receiving different treatments. [23] These studies suggest that MTX could correct intestinal dysbiosis.

Some studies concluded that pharmacological treatment in rheumatoid arthritis increased the Firmicutes and Bacteroidetes phyla. MTX induced a significant growth in Firmicutes over the Bacteroidetes that was associated with improved drug response and reduced inflammation. [24, 25] In a study conducted in 2018, the microbial profile of Etanercept treated patients (in monotherapy or combination with Methotrexate) was compared to normal individuals. There has been reported a significant decrease of Faecalibacterium compared to healthy individuals. In Etanercept treated patients there has been reported a great abundance of Cyanobacterium compared to patients without treatment.

Some species of Cyanobacterium produce some metabolites and bioactive substances that have cytotoxic properties and are involved in gastroenteritis. On the contrary, some metabolites have anti-inflammatory and immunosuppressive properties that are beneficial in rheumatoid arthritis. [26]

ANKYLOSING SPONDYLITIS (AS)

Ankylosing spondylitis is a chronic, autoimmune disease characterized by long-term inflammation of the sacroiliac joints and spine with or without HLA-B27. But the most important genetic factor in ankylosing spondylitis, HLA-B27, could change the intestinal microbiome, contributing to multisystemic inflammation and the production of IL-23 and other proinflammatory cytokines.

HLA-B27 positive patients had changes in Faecalibacterium prausnitzii, Bacteroides vulgares, and Akkermansia muciniphila. An abundance of Bacteroides vulgatus has been discovered in HLA-B27 rats, suggesting that HLA-B27 may alter the intestinal microbiome composition. [27]

In ankylosing spondylitis, 87% of the patients have an altered microbiome (dysbiosis). Their microbiome was characterized by an abundance of species of Proteobacteria spp., Enterobacteriaceae spp., Bacilli spp., Streptococcus spp., Actinobacteria spp. and a low number of Bacteroides and Lachnospiraceae. [28] Dysbiosis is also associated with disease activity.

Akkermansia muciniphila, of the phylum Verrucomicrobia spp., resides in the mucus layer of the large intestine. It is implicated in maintaining intestinal integrity and can cause the degradation of the mucin in the gut epithelium. [29]

Prevotella can increase Th17-mediated immune response in the intestinal mucosa. Increased Prevotella abundance stimulates the production of IL-1, IL-6, IL-8 and also stimulates neutrophil recruitment. The mucosal inflammation caused by Prevotella leads to systemic dissemination of inflammatory mediators and promotes chronic inflammation. [30]

Some microbes, such as Faecalibacterium prausnitzii, administered to mice, have been found to protect them against induced intestinal inflammation, thus concluding that F. prausnitzii has anti-inflammatory effects. [31] A decreased abundance of F. prausnitzii was observed in fecal samples of patients with ankylosing spondylitis. [28, 32]

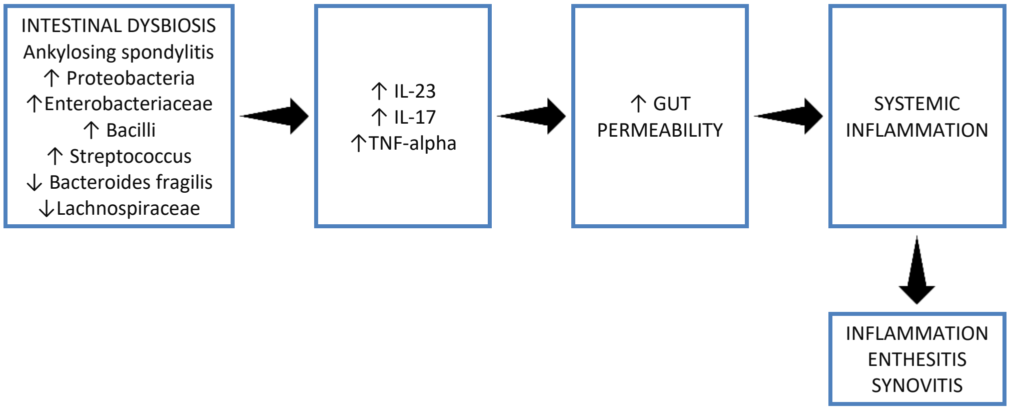

Figure 2: Associated microbiome in ankylosing spondylitis

Ankylosing spondylitis treatment and intestinal microbiota

Patients with ankylosing spondylitis treated with biological treatment have shown a greater abundance in Proteobacteria spp. (especially the Enterobacteriaceae family) and Veillonella genus compared to patients without treatment. [33]

A perturbation of the intestinal fungal microbiome (mycobiome) has been observed in patients with ankylosing spondylitis after biological treatment. An increase in the abundance of Saccharomycetales was found after anti-TNF and anti-IL17 treatment. [34]

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Systemic lupus erythematosus is an autoimmune disease that is characterized by severe inflammation that can affect almost all organs of the body. Lopez et al. observed the influence of the microbiota over T lymphocyte differentiation in healthy and SLE groups. These authors found a rise in lymphocyte activation and differentiation toward Th17 in SLE patients. [35]

There were some studies on SLE patients which demonstrated that the abundance of Firmicutes is decreased and that of Bacteroidetes is increased compared to healthy control groups. If the intestinal epithelial barrier is damaged, Enterococcus gallinarum and Lactobacillus reuteri, possible gut pathobionts, can translocate into the systemic tissue, inducing autoantibody production. In addition, Ruminococcus gnavus can trigger an anti-double-stranded DNA antibody response. [36, 37]

A study conducted in Spain has proved that there is a gut microbial dysbiosis related to SLE in Caucasian female patients. SLE patients developed intestinal dysbiosis with a decrease within the Firmicutes spp. and a rise in the Bacteroides spp. It also showed that SLE patients with inactive lupus disease had significantly lower Firmicutes/ Bacteroidetes ratios than healthy control individuals did. [38]

A study conducted by Van der Meulen et al also showed that in SLE patients, the intestinal microbiota composition is characterized by lower bacterial richness, lower Firmicutes/ Bacteroidetes ratio, and a greater abundance of Bacteroides species. [39]

Similarly, a study by Greiling et al concluded that the Firmicutes/Bacteroidetes ratio in SLE patients decreased compared to healthy controls. In addition, commensal bacteria which contain Ro60 orthologs could induce the production of autoantibodies that cross-react with human Ro60, therefore triggering autoimmune diseases like SLE. [40, 41]

On the contrary, a study concluded that Firmicutes/

Bacteroidetes ratio is not significantly different between SLE patients and healthy controls. Also, this study concluded that Gram-negative bacteria, like Proteobacteria spp. and Blautia spp. were increased and Odoribacter spp. was decreased in SLE patients. [42]

A study explored whether the alteration of the intestinal microbiome in SLE patients from China was according to the intestinal dysbiosis of SLE patients from Spain. The depletion of Firmicutes spp. and the abundance of Bacteroidetes spp. in SLE patients from China was in keeping with the SLE patients from Spain. This study also showed that nine genera of intestinal microbiota were SLE-related microorganisms: Rhodococcus spp., Eggerthella spp., Klebsiella spp., Prevotella spp., Eubacterium spp., Flavonifractor spp. and Incartae spp. sedis were found in great abundance but Dialister spp. and Pseudobutyrivibrio spp. were depleted in SLE subjects. These nine genera have the potential to distinguish SLE patients from healthy controls. Also, the gut microbiome profiles of SLE patients were found to be more influenced by disease than ethnicity. [43]

Another study conducted in China concluded that the genera Streptococcus spp., Campylobacter spp., Veillonella spp. and the species Anginosus spp. and Dispar spp. were in direct correlation to lupus activity, while the genus Bifidobacterium spp. was in negative correlation to the disease activity. [44]

A study conducted in New York, on SLE female patients, investigated the fecal microbiota for possible pathobionts. Patients with a high disease activity index had a greater abundance of Ruminococcus gnavus. The highest levels of serum anti-Ruminococcus gnavus strain-restricted antibodies were found in patients with active nephritis. These findings showed evidence of altered fecal commensal taxa and increased immunoglobulin levels and other biomarkers, as evidence of altered gut barrier function. The study concluded that some strains can contribute to the immunological pathogenesis of lupus nephritis. [45]

Treatment with probiotics composed by Lactobacillaceae members has been used as anti-inflammatory therapy. Studies found the up-regulation of anti-inflammatory cytokine genes and down-regulation of the pro-inflammatory ones.

Moreover, the probiotic-treated group exhibited higher anti-oxidant activities and low levels of lipid peroxides. CRP levels were found to be in the normal range in comparison with the untreated group which levels were elevated.

Immunohistochemistry found high levels of IL-10 and low levels of IL-6, demonstrating the anti-inflammatory properties of L. mucosae and L.fermentum. [46, 47]

Lactobacillus rhamnosus and Lactobacillus delbrueckii have been found beneficial in the management of newly diagnosed SLE patients. [48] L. rhamnosus stimulates IL-12 and IL-10 production. Moreover, it secretes soluble factors that decrease TNF-α production, a potent pro-inflammatory cytokine. [49]

Although there is still no clarity about the role of microbiota within the development of SLE, the evidence suggests that the dysbiosis observed within SLE patients might be associated with this disease.

Figure 3: Associated microbiome in systemic lupus erythematosus

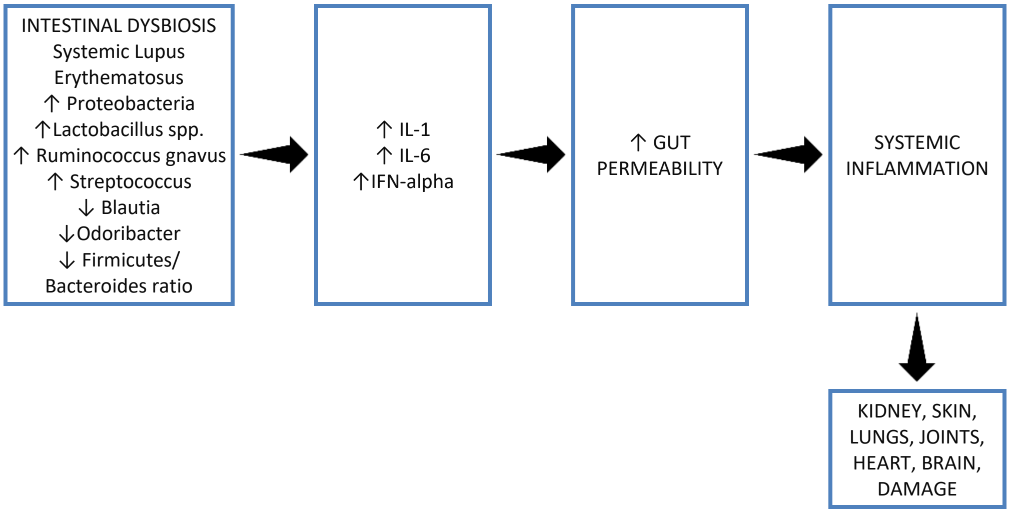

Systemic lupus erythematosus treatment and intestinal microbiota

Nonselective immunosuppressive therapies, like Dexamethasone and azathioprine, in SLE patients, may have vast effects on the intestinal microbiome. It is believed that at the same time as immunosuppressive medications are acting directly onto suppressing inflammation, these medications can also help in the formation of a microbiome that promotes the regulation of local inflammation within the bowels and potentially at distant sites within the body. A study showed that the gut microbiota tended to be more diverse after Dexamethasone treatment. Also, a greater abundance of lactobacilli was found after the treatment with Dexamethasone. [42]

On the contrary, no significant results were found between gut microbiota and methotrexate use in a recent study within the British TwinsUK cohort. [39] Chloroquine and hydroxychloroquine are reported to inhibit the intracellular growth of bacteria in vitro, but these drugs do not seem to own any antibacterial activity or effect on extracellular bacterial growth. Regarding the use of NSAIDs, a study observed no significant effect on the gut microbiome. Also, oral corticosteroid use didn't significantly affect the gut microbiome. [50]

DIET

Diet and the intestinal microbiota may be the target for future treatments and preventive interventions in rheumatic diseases. Diet can modulate the symptoms in rheumatoid arthritis through the metabolites produced by the intestinal microbiome. The intestinal microbiome has the capacity of modifying the circulating pro and anti-inflammatory mediators. [51]

Western diets are characterized by increased consumption of refined carbohydrates, red meat, and salt, which can induce a pro-inflammatory state and trigger the onset of autoimmune diseases. [52, 53]

Patients with a positive rheumatoid factor or anti-citrullinated protein antibody had a decreased concentration of omega-3 fatty acids in the red blood cell membranes, suggesting the potential beneficial effect in individuals with genetic susceptibility to rheumatoid arthritis. [54]

Some studies suggest that there might be a link between high salt consumption and the increasing incidence of autoimmune diseases, especially rheumatoid arthritis. A high concentration of sodium chloride can induce the activation of some signaling pathways, the activation of Th1, the overproduction of pro-inflammatory cytokines (IFN-gamma, IL-17), and the differentiation of macrophages. [55, 56]

For example, trimethylamine-N-oxide, a pro-inflammatory metabolite that derives from choline and carnitine, found in red meat, eggs, and dairy, is produced by Prevotella copri. [57][58]. Prevotella copri was found in great concentrations in newly diagnosed patients with rheumatoid arthritis. [59]

Probiotic use of Lactobacilli spp. improves intestinal immune function and stimulates anti-inflammatory cytokines (IL-10).

In general, Lactobacillus spp. and Bifidobacterium spp. are considered beneficial. Both species produce catabolites that decrease intestinal permeability and have anti-inflammatory and antioxidant properties, with the enforcement of the intestinal barrier function. Fermented foods that contain Bifidobacterium spp. and Lactobacillus spp. genera can have a protective role against immune-mediated diseases. [60]

A study evaluated the effects of L. casei supplementation on disease activity and inflammatory markers levels in rheumatoid arthritis females. A decrease in CRP, DAS28, and inflammatory cytokines was observed. [17]

The microbial degradation of complex carbohydrates will increase short-chain fatty acids that are beneficial for the intestinal immune response. [61] Several foods (highly refined flours, gluten, saturated and trans fats, red meat, dairy) have been identified as proinflammatory foods. Some vegetables as tomatoes, eggplants, and potatoes, contain solanine and increase intestinal permeability.

Foods high in omega 3 (chia seeds, flax seeds, sesame seeds, avocados), fruit that contain enzymatic proteins (pineapple, mango, papaya), turmeric, black pepper, green tea, and legumes offer multiple benefits.

To decrease inflammation, an ITIS diet has been suggested. It consists of a modified Mediterranean diet with several changes: the separation of carbohydrates and proteins, gluten-free foods, fruit with enzymes, and other anti-inflammatory foods mentioned above. [62]

A study regarding intestinal microbiome and Mediterranean diet observed that an increased ratio of Firmicutes/ Bacteroides was related to lower adherence to the Mediterranean diet. A lower animal protein intake was associated with an abundance of Bacteroides. Moreover, high consumption of sugars, saturated fats can affect the intestinal microbiota diversity. A plant-based diet was related to higher bifidobacterial counts. [63]

A case report discussing a fecal matter transplant (FMT) for a rheumatoid arthritis patient found that HAQ-DI (Health

Assessment Questionnaire – Disability Index) dropped seven days after FMT. [64] Therefore, fecal matter transplant may be an effective treatment for rheumatoid arthritis through the restoration of beneficial microbiota.

DISCUSSIONS

The treatment in rheumatology has made great advances with the potential to significantly impact the evolution of inflammatory diseases. A promising area of active research is the targeted manipulation of the microbiome to improve the outcome of rheumatic diseases.

The Fecal Matter Transplant in rheumatic diseases is not studied enough and more data is needed to achieve durable responses. Extensive donor testing is mandatory to guarantee patients' safety.

The use of prebiotics and probiotics can promote the growth of beneficial microbes. Probiotics supplements may be used as adjunctive therapy for rheumatic patients.

The microbiome composition can be used for prognosis and diagnosis, but most importantly, the manipulation of the intestinal microbiome through diet may be a promising future therapeutic approach. Large, long-term clinical trials are needed for specific dietary recommendations to rheumatic patients.

CONCLUSIONS

Current literature shows the link between the intestinal microbiome and the activity and severity of rheumatic diseases.

The imbalance of bacterial phylum, especially Firmicutes spp. and Bacteroidetes spp., is believed to be one of the most important causative factors for autoimmune diseases.

Although a lot of progress had been made in the past years regarding the intestinal microbiome, the research still requires more data. Targeted manipulation of the intestinal microbiome is needed for better treatment and management of inflammatory rheumatic diseases. Although 16S rRNA sequencing provides information about microbial identity, its function remains unknown.

Fecal matter transplant may have a good therapeutic effect on rheumatic diseases through its capacity of restoring and maintaining eubyosis.

Diet has been shown to influence the evolution and severity of autoimmune diseases. The use of pre and probiotics may be useful. The use of Lactobacillus species, as an adjuvant treatment, has been found beneficial.

Dietary or targeted manipulation of the intestinal microbiome may restore homeostasis and restrain disease-promoting pathobionts.

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ORIGINAL ARTICLES

Mass shooting incidents: evolution of preventive procedures, preparation, treatment, and medical care supply

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Abstract: *Mass shootings incidents occur with increasing frequency over time. Studying these cases proved that, despite their diversity, several common features could be taken into account in the early detection and possible prevention of certain future cases. Accepting that such incidents may occur anywhere and anytime, societies need to be prepared for their more effective response. Informing citizens about the best way to react to a mass shooting event is considered crucial and essential. The "Run-Hide-Fight" guideline/directive seems to be the most appropriate guideline given to the public. Proper training, of both the Suppression Forces and the emergency medical care providers, is considered of utmost importance. The role of the Incident Commander, regarding the incident management, as well as the external bleeding control of the injured people, are factors of paramount importance in trying to mitigate the casualties from such an incident. The alertness of both citizens and organizations/structures may lead to early detection of potential perpetrators and thereby averting a mass shooting incident. To achieve increased survival and a reduced number of casualties from a mass shooting event it is vital proper education be present at all levels. The response to a mass shooting event should be imprinted in an Emergency plan. Such a plan should have been decided and made, by the Security and Suppression Forces, the Healthcare Institutions, and the Public Safety Answering Point.*

Keywords: *mass shooting incident, first responder, active bleeding, tourniquet, Incident Commander*

INTRODUCTION

Although mass shootings incidents have taken place throughout recent world history, the literature has been relatively limited, as such incidents were more sporadic before the 21st century [1]. A mass shooting incident is usually the result of one or more people killing or attempting to kill people within a limited and inhabited area. In most cases, the perpetrators use firearms/guns and there are no specific models for victim selection [2]. The immediate response as well as the perpetrators' neutralization are required to mitigate the consequences and casualties. However, because it will be some time before specialized forces arrive at the scene and take action, the way surrounding people react to such an event is of equal importance.

The purpose of this review is to present both the most appropriate ways of prevention and preparation, as well as the optimal procedures for responding and dealing with possible mass shooting incidents. Informing, preparing, and training citizens and stakeholders such as the Suppression Forces and emergency medical care providers, is crucial in reducing the casualties in a mass shooting incident in an

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urban environment.

DEFINITIONS

A mass shooting event involves multiple incidents of violence with a firearm/gun. However, there is not a widely accepted definition of the term "mass shooting incident". The US Federal Bureau of Investigation (FBI), for instance, does not use the term "mass shooting incident", but refers to these incidents as "active shooter incidents" [2]. FBI, therefore, defines as an active shooter "a person who is actively involved in a murder or attempting to kill people in a residential area", while it also defines as mass murder "four or more murdered during an event without time distinction (cooling-off period) between murders" [3]. The United States Congressional Research Service (CRS) acknowledges that there is not a universally accepted definition and describes as a mass shooting incident "an event in which a perpetrator selects four or more individuals and kills them indiscriminately", adding to the FBI's definition the word "indiscriminately" [4]. The Gun Violence Archive (GVA), a non-profit organization that has been monitoring gun violence in the United States since 2013, describes as a "mass shooting event" four or more people killed in a single incident at the same time and location, not including perpetrator [2,3]. Moreover, this organization does not exclude situations such as domestic violence, gang activities, etc., nor does it differentiate victims depending on the circumstances under which they were shot, which means that its definition is broader than of other organizations and therefore includes incidents that are not recorded in other databases. Meanwhile, in 2013, the United States Congress defined mass murder as "three or more murders in a single incident" as part of a bill to enable the Department of Justice to assist local authorities during the investigations [2].

STATISTICAL DATA

The vast majority of mass shooting incidents have taken place in the United States of America [5]. According to GVA, from 1st August 1996 until December 2019, a total of 172 cases have been reported [6]. In just a few of these cases, the perpetrators were two, while no incidents involving robberies or domestic violence were reported. A total of 1228 people were killed, coming from almost all races, religions, socioeconomic status, and age. Of these, 196 were children and adolescents. Also, thousands of survivors with permanent disabilities and serious physical and mental health problems should not be omitted [6]. Perpetrators often used or carried more than one weapon, while in a case one perpetrator was found carrying 24 weapons. Of the total 326 weapons used in the above-mentioned incidents, 179 were legally acquired (55%), 61 were illegally acquired (19%), while it is not clear how weapons were acquired in 86 cases (26%). Of a total of 179 perpetrators, some were known to have violent tendencies or criminal history records, but there were also several who had not presented any criminal behavior at the time of the incident. The vast majority (99%) were men and most were 20-50 years old. More than half (55%) lost their lives on or near the scene of the incident, often by committing suicide. Lastly, incidents in schools and places of worship tend to come first in the public's memory but are a relatively small part of mass shooting incidents. The most common are those in offices, shops, and restaurants with the State of California having the most such attacks (29) of any State [6].

According to the FBI, after 2011 the frequency of mass shooting incidents has increased, with the period 2011-2014 occurring almost three times as many as in previous years [7]. During 2000-2013, a total of 160 cases (according to the wider definition than the GVA's definition) were recorded, with an average of 11.4 cases per year. According to the same report, 16.9% of attacks took place in schools and 45.6% in shops and markets. 90 of the above mentioned 160 incidents, were completed before the Suppression Forces even intervened, most often with the perpetrator's suicide. Of the 64 cases in which the duration of the incident was ascertained, at 44 (69%) the incident was completed in less than five minutes, while at 23 (36%) in less than two.

Mass shooting events are expected to occur worldwide. Two of these incidents received worldwide publicity, evolving many victims: on November 13th, 2015, a total of nine perpetrators launched an attack in Paris, killing a total of 130 people and injuring more than 350 [8], while on New Year's Eve 2017 in Istanbul, a perpetrator stormed a nightclub, killing a total of 39 people and injuring another 79 [9]. In both cases, there was a religious or racist motive.

PERPETRATORS' CHARACTERISTICS

To increase the chances of an early potential perpetrators' detection, both mass shooting events and perpetrator's physiometric characteristics have been studied [10]. According to an FBI study, regarding the pre-attack behavior of perpetrators in the United States for incidents that happened in 2000-2013 [11], the majority of perpetrators had exhibited 4 to 5 worrying-suspicious behaviors before the attacks. For perpetrators under the age of 18, these behaviors were more likely to have been noticed by classmates or teachers than by the family environment. Each perpetrator was exposed to an average of 3.6 stressors during the year before the attack. Of the cases in which some kind of suspicious behavior was observed, 54% did not take any action, while 41% of them were reported to authorities. According to the same study, in most cases (64%) at least one of the victims was pre-selected by the perpetrator [11].

As far as the age of the perpetrators is concerned, the youngest reported to be 12 years old and the oldest 88, with the average age of 37.8 years (94% were men and 6% women). Of the adult perpetrators, 20% were high school graduates, while 34% had completed at least an Institute of Technology or University. Of the latter, 5% were Master's Degree or Ph.D. holders. However, there was a significant percentage (36%) where the level of education could not be ascertained. 38% of the adult perpetrators were unemployed while 44% were workers. The remaining 19% included students, retirees, people with disabilities, etc. The 24% of adult perpetrators, at the time of the attack or in the past, were involved in some way with the Armed Forces or Security Forces, while 14% of perpetrators had one or more convictions before the attack [12].

The above-mentioned study also investigated two related but distinct time variables: a) the time the perpetrators spent planning the attack and b) the time they spent preparing it. The first category concerns the time from when the perpetrator began to think about a possible attack until its implementation. Because it is often difficult to determine its onset, in many cases this period could not be determined by the researchers. The second category is more specific, usually less than the first, and concerns the time from taking any kind of action by the perpetrator (eg gathering of relevant material, a supply of weapons and other equipment, etc.) to the implementation of the attack. In terms of design, 26% of the perpetrators took 1-2 months, 18% 3-5 months while 24% less than a week, with half of them less than just twenty-four hours. In terms of preparation, 28% needed a maximum of one day, 26% 1-7 days and only 4% consumed 6-12 months. The study also explored how perpetrators supplied the weapons they used. In 40% of cases, the perpetrator had obtained the weapons of the attack legally, 2% bought the weapons illegally, in 6% of cases the weapon was stolen, while in 11% it was borrowed. Besides, it seems that the majority of the perpetrators acted in a place known, if not familiar, to them.

This study also investigated the presence of stressors in perpetrators' lives. These included financial problems, physical and mental health issues, interpersonal conflicts with family, friends, colleagues, as well as alcohol abuse or drug use. The study acknowledges that many people occasionally face similar issues, but most have mechanisms (personality, psychological flexibility, interpersonal relationships) to cope effectively. Although the issue is multifactorial, it appears that the perpetrators usually experienced multiple stressors during the period before the attack (an average of 3.6 within the last year). The most common stressor detected was a mental health disorder in 62% of perpetrators. About half (49%) had financial problems, while 35% had work-related problems. In 34% of cases, serious family issues were reported, while 22% of the perpetrators were addicted to alcohol or drugs. Only 2% of the operators did not detect any of the studied stressors. Given that mental health disorder was the most common stressor, as well as the fact that it is a fairly broad and varied term, it is worth mentioning that of all cases in which mental health disorder was detected, only in 41% of cases, perpetrators had a previous official diagnosis by a health professional, with the 75% being diagnosed as a “mood disorder”.

Another interesting aspect of the study was the research for cases in which people from the perpetrator’s environment had found some kind of worrying behavior during the period before the attack. Thus, in 62% of the cases, deviant behaviors were observed from a psychological standpoint, in 57% interpersonal disorders, and in 54% of cases a problematic way of communication. Moreover, in 46% of perpetrators who were workers, there was a decline in performance at work and in 42% of those who were students, there was a corresponding decline in school performance. However, it is worth noting that, contrary to the common belief that the perpetrators of such attacks tend to be isolated, the study found that 68% of adult perpetrators lived with other people immediately before the attack. Additionally, 86% of the perpetrators were found to have a significant social relationship and contact with at least one person in the year before the attack.

So that research data be used for prevention, not only was it investigated when some deviant behavior began to be observed, but also by whom. Thus, it appeared that in 56% of cases the initial observation was made at least 2 years before the attack and in 29% between 1-2 years. Only in 2%, it was observed the week before the attack. Also in 87% of the cases, the deviant behavior was observed by the perpetrator's partner, in 68% by another family member, while among the perpetrators who were students in 92% of the cases the person who perceived some kind of behavior change was a classmate and in 75% a teacher.

Another particularly interesting finding was the fact that the majority of perpetrators (79%) appear to have been exposed to a mass shooting event in response to an injustice they experienced or believed they were experiencing. The reason for this feeling (injustice) varies from case to case, with the most common causes being injustice at the interpersonal level (33%) and in the work environment (16%). The fact that many people experience similar situations every day, the vast majority of whom choose to react in a non-violent way, proves that in the case of perpetrators of such attacks there is a synergy between various motives. Although in almost 4/5 of the cases the perpetrator was affected by some kind of injustice, the manifestation of the attack did not happen with the same percentages against the “target” people. This, according to the study, accounted for about 64% of cases (in 27% the victims that got murdered were pre-selected and in the rest 37% there were both pre-selected and random victims), while in about 37% of cases the victims were either completely unknown to the perpetrator, or known, without, however, being linked to his "complaints". This is explained by the fact that many times the perpetrator tries to act against a specific organization, such as a company, a school, etc. and not against specific people.

Another element of the study that could also be used in the field of prevention, is the fact that about half of the perpetrators of such attacks had committed suicide, several of whom had even attempted suicide within the year before the attack. If we exclude the cases in which, due to lack of sufficient evidence, the perpetrators were not registered in either of the two categories (suicidal ideation or not) then the percentage of those who had such behaviors is 85%.

In trying to outline the profile of a future perpetrator, it is also worth noting that more than half of the perpetrators (55%) had, to some extent, made their intentions known by making threats, mostly in person or, more rarely, in other ways such as in writing or by electronic means.

CITIZENS’ RESPOND TO MASS SHOOTING INCIDENTS

a) Before Suppression Forces intervention

It is a fact that the reaction of citizens who have been present in a mass shooting incident varies and depends on multiple factors, including the individual judgment regarding which action will better protect their lives, their characteristics, any previous education and information (or not), as well as the available means and space where the event happens [13].

However, although there is no appropriate response to all possible scenarios, specific actions have been defined to reduce casualties, the consequences of a mass shooting event, and increase survival [14].

Citizens should be aware of these guidelines to evaluate the situation and be able to react in the best possible way [15].

These instructions/guidelines, to be quite memorable, have been coded as "Run - Hide - Fight" [16, 17].

- Run

The initial instruction given to citizens is that in a mass shooting incident their first action should be to run away from the perpetrator. The move should be made to a predetermined meeting point if they are aware of it-or moving as far away from the firing point as possible until they are in a safe place. Despite the complexity of the situation, those who can move safely are advised to do so [12].

Citizens are advised to run-move, leaving behind their personal belongings so that they do not spend time and be more flexible. They should also raise their hands, making a signal to security forces that they are unarmed. Rolling stairs and elevators should be avoided. During their run-move, citizens are advised to help their fellow citizens to leave together, but not to stay behind. Finally, when citizens feel safe, it is recommended to call and inform the authorities, giving as much information as possible. The information that would be useful concerns the exact location of the incident, the number of perpetrators as well as their description, type and number of weapons or the possible use of explosives, possible number of victims, and, finally, whether shots continue to occur at the time of the call.

- Hide

If quick removal is not considered safe, the next option should be to try hiding. If possible, the place/building with the thickest walls and the fewest windows should be chosen. Doors should be locked and windows closed where possible. It would also be wise to place heavy objects/furniture behind doors. The instructions also include maintaining absolute silence from citizens who remain in hiding. For this reason, electronic devices, as well as mobile phones, should be silenced and lights should be off. As long as citizens remain hidden, they will have to consider ways to escape, looking for possible escape routes, as well as ways to deal with the perpetrators, if necessary. Attempts to communicate with authorities or with Suppression Forces should only be made when this is considered safe.

- Fight

When neither citizens’ safe removal nor their hiding is an alternative, it is recommended that citizens fight the perpetrators. According to an FBI investigation, in 17 of the 51 shooting events, the attack was stopped by civilians who were at the scene. Citizens may try to deal with the perpetrators mainly by throwing objects at them or using improvised weapons. In these cases, the energy must be as aggressive as possible. Naturally, trained individuals, such as security personnel, military, self-defense technicians, or professionals who may carry a weapon (eg security guards) are more likely to be able to deal with the perpetrator.

b) After Suppression Forces interference

A mass shooting event can take place long after the Suppression Forces have reached the scene. At this time, citizens must, on the one hand, remain as safe as possible and, on the other hand, not obstruct the work of authorities [13]. For this reason, citizens should try to stay calm and follow the instructions of the security forces. They should have their hands up and avoid screaming. This will help them to be easily distinguished from the suspects/perpetrators. Citizens should also move in the direction indicated, lying on the ground and generally being as cooperative and obedient as possible. Finally, it is useful that citizens provide relevant information that will facilitate the work of the Suppression Forces. Citizens will have to show similar behavior, remaining calm and following the instructions they receive, even after the attack has passed. This will help to better deal with the injured, identify/record the victims and not to alter the information required to investigate the incident and locate the perpetrators.

Apart from the widely used "Run - Hide - Fight" guideline, there are many other similar guidelines/instructions, also standardized in a way that is easy to remember that are proposed. One such is the "Avoid - Deny - Defend" [17-19]. This has to do with the directive, a) avoid danger (either by acknowledging the threat before the attack occurs, or moving away, b) deny access (preventing the perpetrator from accessing a safe place, if possible), c) take action (as a last resort, defending your life in any way possible by dealing with the perpetrator).

A similar process of four steps coded as "4As" also aims to reduce casualties in a mass shooting incident [20]. These steps are:
Accept: Accept that an emergency occurs
Assess: Evaluate what you need to do to increase your chances of survival
Act: take action following the "Run - Hide - Fight" direction
Alert: alert the Suppression Forces

Finally, the “ALICE” direction [21-23] is a five-step acronym that also aims to increase survival in such events:
Alert: be alert
Lockdown: if you decide not to try to escape, stay in a locked-secure area
Inform: if possible, forward the information to the authorities
Counter: If there is no other option, deal with the perpetrator
Evacuate: get away from the danger zone as soon as possible

It seems that the latest direction may be confusing, as the range of actions proposed is not in line with the rest and especially with the "Run - Hide - Fight" guideline, which has now been established as the optimal instruction for citizens to mass shooting events.

SUPPRESSION FORCES: FIRST RESPONDERS

The actions of the scene first responders are of utmost importance in a mass shooting incident [24, 25]. The first respondents are involved in both managing the incident and providing care to the injured [26]. The term "first responder" does not imply an official certification, restriction, or ability. If the attending citizens who may attempt to be involved are excluded, the first respondents are the first officials arrived at the scene and could be the police, military, firefighters, or even emergency medical personnel.

Moving on to the incident area, the first responder should ask for as much information as possible from the agency available (usually police). This information will allow access to the incident area from the safest and most convenient route, as well as the closest possible approach to perpetrators. In case that the first respondent does not have the authority to enforce order, he/she must remain safe and make an immediate request to the competent authority. Upon arrival at the scene, from the information already collected and the first scene assessment, first officials will identify the danger zone and install a point as an administration area. Depending on his training and the characteristics of the case, such as the number of perpetrators or the time needed to receive advanced help, the first responder will decide whether to operate on his own (or with the existing forces) or will wait for reinforcements to deal with the perpetrators. The first correspondent automatically undertakes the management of the incident (Incident Commander-IC) [27,28]. IC’s purpose is to develop a plan to deal with the incident based on the personnel and resources available, as well as to seek resources for the best response to the demands of the situation [29]. The first responder’s primary purpose is to stop the perpetrator, so even though he/she may assist in removing survivors outside the danger zone, IC should not be involved in helping injured people at this stage. Finally, the first responder should transmit, as soon as possible, all the information gathered to be used by the Forces that will be called to assist.

When additional Forces arrive in the incident area, they must contact the first correspondent who will have taken on the role of IC. As long as the mass shooting incident persists and the perpetrator has not been neutralized, all forces will work together IC’s common plan and guidelines to deal with [30].

As soon as the danger passes, all forces should assist the injured by applying the techniques and instructions mentioned below.

It should be noted that both IC and Command Post (the point where the administration is exercised) may change as the incident progresses. Once Suppression Forces have arrived and have started operating at the scene, the IC is usually the superior authorized officer. However, IC may be the most experienced or may be determined by other criteria, but in any case, IC should always be clearly defined to the forces involved.

Accordingly, the Command Post should be installed in a place known to all, which can be relocated, if necessary.

So that the main purpose of managing a mass shooting incident (which is minimizing casualties) be served, the incident scene is divided into three zones [31]. Within each of them, there is a different regime of security, accessibility, and actions that can take place. The direct intervention Forces must be aware of the characteristics of these three zones, while the determination of the zones should be clear to all the people involved. As a mass shooting incident is a dynamic situation, the boundaries of these zones may change over time.

- Hot zone

It is the area where there is still a risk of gunfire. Neither medical personnel nor Fire Service personnel (except for specially trained units) are not allowed to enter this zone because their entry would endanger their lives or the lives of others. Also, they may obstruct Suppression Forces' efforts, whose personnel are the only ones authorized to enter and operate in this zone, following IC's plan and instruction.

- Warm zone

It is the area where there is no immediate risk of gunfire, however, it cannot be characterized as a safe area. This implies specific restrictions on both entering the area and operating actions. Firefighters and medical/paramedic personnel may enter under IC's permission for only critical and extremely necessary actions. Medical/paramedic personnel, for example, will only participate in vital operation (life-threatening situations) to save injured people's lives [32]. The point of entry and exit as well as the possible assistance by Security Forces will also be determined by the IC. If allowed, there must be certification and registration of the people entering the warm zone. The time spent in the zone should be the minimum necessary.

- Cold Zone

This area is considered safe, without the risk of a shooting. Access is free for all emergency forces as well as their length of stay. Healthcare personnel can provide any kind of medical care required, operating based on purely medical criteria, having ensured their safety [33].

NOT ADVANCED MEDICAL CARE PROVISION

The experience of dealing with the injured during the US military operations, formerly in Vietnam and later in Iraq and Afghanistan [33], combined with similar medical research on wound care, has led to a large percentage in the establishment of modern procedures – instructions, regarding gunshot wound management. Particular emphasis has been given to tackling life-threatening active external bleeding.

The experience of war injuries in the late 1990s led to the standardization of a series of wound care procedures on the battlefield, called Tactical Combat Casualty Care (TCCC), focusing on the most common causes of death that could be treated infield, even by medical staff with no special skills [34].

Recognizing how this military experience could be useful and applicable to non-military operations, an independent group of citizens set up a Committee in 2011 (Committee for Tactical Emergency Casualty Care, C-TECC) [35]. The purpose of the Commission was to develop guidelines that would adapt the battlefield military experience to similar situations in an urban environment, taking into account the differences and peculiarities between the two operational applications [36].

In 2013, a group of Public Institutions, including police, Fire Department, the Army, Medical Institutions, pre-hospital care, etc. was convened (known as Hanford Consensus) [37,38] and the result was a consensus regarding strategies to increase survival in mass shooting events. The Committee developed the following acronym "THREAT", to standardize the process:

- **T**reat suppression
- **H**emorrhage control
- **R**apid **E**xtrication to safety
- **A**ssessment by medical providers
- **T**ransport to definitive care

The Committee argues that survivors of such an incident or the slightly injured may act as potential rescuers by following the instructions above. The Committee also considers that the control of active external bleeding should be a key skill of all the personnel of the Security and Emergency Forces. This training concerns, on the one hand, the use of a tourniquet [39] and, on the other hand, the placement of hemostatic gauzes and hemostatic agents in places when it is not possible to place a tourniquet [40].

Some of the TCCC-TECC skills that apply to mass shooting injuries in urban environments and that could also be applied by trained personnel of the Security Forces [41] to increase survival rates are:

- Use of a nasopharyngeal airway for injuries without maxillofacial trauma
- Placing the unconscious injured person in a prone position, in case of facial injury or airway bleeding
- Spinal immobilization for people with blunt trauma
- Ensuring an intravenous or intraosseous line for fluid management

ADVANCED MEDICAL CARE PROVISION

Gunshot wounds [10] can be quite deadly and therefore the most immediate medical care possible is vital to increasing victims' survival. The usefulness of an immediate intervention of specialized health care providers comes into contrast with the necessity for non-exposure of such personnel to danger. However, weighing the situation data each time, the general rule is to provide as much care as possible to the injured, while ensuring health personnel safety. At this point, the determination of action zones in the event scene is proved quite useful and crucial, since the provision of specialized health care begins before the perpetrators are even neutralized and the threat is completely stopped [42].

Healthcare providers should also follow the guidelines set out in Harvard Consensus [36, 38] and are concentrated in the THREAT algorithm. The medical care system can be divided into:

- Direct threat care: providing care while shooting or under adverse conditions
- Indirect threat care: providing care while the threat has been suppressed, but may reappear at any time
- Evacuation: providing care during the evacuation from the incident scene

The first line of care takes place within the hot zone. However, the entry of health personnel into this zone is only allowed under certain conditions. Providing care in the hot zone is restricted to only limited manipulations such as airway management and controlling active external bleeding by placing a tourniquet or applying pressure to the appropriate points. It also includes verbal instructions to victims to take care of themselves or others, if possible.

The second line of care is evolving in the warm zone [43], where the immediate threat has been suppressed for the time being and consequently, the health personnel may proceed with more invasive operations, without worrying about their personal safety, but also without complacency. In this zone, health care providers will provide advanced medical support aimed at maintaining life and stabilizing the injured before being transferred. Operations that take place at the warm zone may be: ensuring the airway access even in an invasive way, controlling bleeding using (except for tourniquet) hemostatic gauzes and agents, stabilizing fractures, as well providing fluids or blood factors (usually not at pre-hospital level), treating pneumothorax and, finally, preventing or treating hypothermia [44].

The final line of care takes place in the cold zone. This area is considered safe and has access to all health personnel involved in the incident. The priorities regarding the treatment of the injured remain the same, but there is the possibility of using additional means to achieve them. In this zone, the network for transporting the injured to the appropriate structures for the advanced medical care provider will also be established.

The limited resources available concerning the number of people that need medical support is a quite likely scenario in mass shooting incidents [45]. This may create the need for a screening system for the medical care process (triage) [42, 46]. "Triage" is defined as the process of separating the injured based on the need for immediate medical support, taking into account the possibility of benefiting from it. The triage process is applied when the needs for medical care exceed the available resources and aims to maximize the number of survivors. In other words, it aims to offer the greatest benefit to the largest number of victims with the resources available.

From time to time, various triage systems have been proposed, none has been universally accepted so far. In the case of gunshot wounds, the challenges for an effective triage system are even greater. These have to do with the nature of these injuries, where superficial injuries with minimal obvious bleeding may obscure extensive internal bleeding, and conversely, severe superficial injuries may not always indicate internal organ damage of similar severity [45].

Despite limitations, a widely used triage system, which has occasionally been used in mass shooting events, is the START system (Simple Triage And Rapid Treatment). It is a system developed in 1983 by the Fire Services and the Hoag Hospital personnel in California, and modified in 1996 [47]. Using a simple algorithm (RPM: 30-2-Can Do) evaluates respiration (Respiration, respiratory rate), circulation (Perfusion, capillary refilling time - in the presence of a radial pulse), and the level of consciousness (Mental status, execution of simple commands) [48]. Depending on the abovementioned criteria, the injured are classified into four categories:

- Immediate (marked in red): they are the injured who require immediate medical support at the scene
- Delayed (marked in yellow): refers to less serious trauma patients whose lives are not directly threatened and who require medical treatment, but not immediately
- Minor (marked in green): they are the least wounded with injuries that are not life-threatening and may be treated when possible
- Deceased/Expectant (marked in black): refers to the dead and those who have fatal injuries whose condition is considered irreversible

The triage process with the START system allows during sorting, the intervention only with simple maneuvers to open airway (jaw thrust, Tilt-chin lift) and applying pressure at the site of an active external hemorrhage [49]. The great advantage of this system is that it is relatively simple so that it can be used by properly trained non-medical staff. On the contrary, one of its disadvantages is that it usually leads to “over triage” [50].

The triage system and the injured classification will determine the priority of transfer. A key factor in the injured person transfer is time and the avoidance of unnecessary delays in assisting in the field [51]. However, at the same time, it is important to choose the destination (hospital-trauma center) that the injured should be transferred, a decision that should be made by the field healthcare providers. In cases where there is more than one possible option, the best one should be decided, taking into account both the health condition of the injured and the distance (and therefore the time) of the possible destinations, as well as the facilities of the various Health Institutions.

TRANSFER TO HOSPITAL

When a hospital’s emergency department is notified to receive victims of a mass shooting incident, appropriate preparatory actions must be taken immediately [52-54]. These actions would best be included in a pre-existing protocol for such cases [55]. The key to the best response to such events is the mobilization and recall of staff (especially doctors of surgical specialties, staff from the emergency department, operating room (OR) and laboratory personnel), informing the blood bank, providing the emergency department with crucial materials from hospital’s warehouses, as well as, decongesting the emergency department and OR, as possible [56].

Regarding the care of the wounded, initially, if needed (e.g. non-sufficient resources available) a triage procedure is also applied. At this point, there are again various triage systems, without any common acceptance. The Triage Revised Trauma Score [57, 58], even though is used mainly in a pre-hospital environment to determine the priority of injured that should be transported to a hospital/trauma center, may also be used when the injured arrive at the hospital in order to be reassessed. The parameters evaluated are the Glasgow Coma Scale, systolic blood pressure, and the respiratory rate. Depending on the score, each injured person can be categorized into one of four categories (again marked in the same colors as in the START system) which indicates the priority in terms of the need for medical care [59]

As for the injury types, the most common life-threatening situation is blood loss (active bleeding) [60]. For this reason, in case of trauma, the classic ABC life support algorithm (Airway - Breathing - Circulation) is converted to CBA, giving priority to hypovolemic shock treatment [61,62]. The need for blood, (or blood derivatives/substitutes) are expected to be high in a mass shooting incident with several injured. In the emergency department, regular assessment of the wounded should be made in order to adjust their priority. Finally, analgesia should not be omitted [63], including the trauma patients with low priority due to prior control of their bleeding by placing a tourniquet, as a significant degree of pain is expected approximately 15-20 minutes after its application [64].

In the hospital, the need for psychological assistance should not be skipped [65]. Psychological support will also be needed both to the relatives of the victims and the staff who will be in charge of dealing with a very difficult, demanding, and particularly stressful situation [66].

CONCLUSIONS

Although a mass shooting incident can occur anywhere and anytime, its prevention and deterrence must be pursued. Analyzing the perpetrator’s characteristics, it seems that, despite their great diversity, in many cases, there are precursors that can be detected. The alertness of both citizens and organizations/structures may lead to early detection of potential perpetrators and thereby averting a mass shooting incident. It turns out that it is essential citizens be informed about the best way to react to a mass shooting event. All citizens should be aware of the steps they need to take to improve their chances of survival. The "Run - Hide - Fight" directive is the most appropriate.

To achieve increased survival and a reduced number of casualties from a mass shooting event it is vital proper education be present at all levels. Security personnel must be trained in how to act in such an event. Security personnel should be trained in basic life support (first aid) and trauma skills, neutralizing the threat, and ensuring the staff's safety.

Healthcare personnel, especially those serving in crucial positions, such as pre-hospital life support and Emergency Department personnel, should also receive appropriate training. This training should include trauma management skills, knowledge about the recent guidelines, as well as familiarity with the relevant equipment. It would be useful that the critical healthcare personnel would be appropriately certificated, obtaining the required training/education to be considered suitable for involvement in a mass shooting incident.

Ordinary citizens should not be excluded from training/education. Educational programs can take place both in workplaces and in structures such as schools, places of worship, municipalities, public organizations.

The administration of a mass shooting event by the Incident Commander seems to be the most effective way to manage such an incident. For this reason, all those involved in a mass shooting incident should be aware of this management model and act accordingly.

In a mass shooting incident, the need for medical care may exceed the available resources. In this case, a triage process is needed. All emergency medical personnel should be trained in the triage process, as well as, a specific triage system has been pre-determined to achieve the best possible coordination and communication among all medical care providers involved.

The response to a mass shooting event should be imprinted in an Emergency plan. Such a plan should have been decided and made, by the Security and Suppression Forces, the Healthcare Institutions, and the Public Safety Answering Point (e.g 9-1-1). These operational plans must be updated regularly and adapted to the new data of each organization involved, by the time they are changed. All personnel involved in such an event should be aware of these plans. Ideally, the various stakeholders involved in a mass shooting event should have already worked together to draw up the plans, so that there is a clear division of roles and responsibilities, as well as common terminology and language of communication. Finally, at regular intervals, exercises should be carried out on these plans all the stakeholders become familiar with their implementation, as well as possible shortcomings/weaknesses may be identified and improve their completeness and effectiveness.

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ORIGINAL ARTICLES

Media, medical intelligence and hybrid war

Oana M. Ciobanu[1]

Abstract: *Contemporary hybrid war is a novelty in that it is taken to populated conflict zones, with the participation of the population, under the eyes of the international community. Thus, according to many authors, the most serious threats to the United States, including to its NATO dimension and to its partnership with Europe, would come from extremist groups and their ideology, fueled by poor urban areas of the world, where youth faces great economic and social problems, and is militating for a change. These unconventional actors can operate beyond state control by circumventing international borders, norms and agreements. Some of these belong to global terrorist networks, and others co-operate with criminal elements to destabilize certain areas. The hybrid confrontation with these groups will become "a clash beyond the physical elements of the conflict, in which media manipulation, the use of the internet and the integration of information operations with strategic communication programs are as important as weapon systems on the battlefield."*

Crises generated in the context of a hybrid war are events characterized by low probability and important consequences that threaten an organization for its most profound purposes. Because of their low probability, these events defy any interpretation and require a sustained effort to understand and adapt the reactions. Crises affecting social groups differ in their causes and duration. Obviously, because of the multitude of types of crises, human reason can only play a limited role in their development. However, they trigger human action, which can turn small deviations into major crises.

Crisis and conflict management involves very complex systems of political, diplomatic, social, economic, cultural, international, military and other actions.

Such a system could be structured on political, diplomatic, social, economic, informational, military, psychological and cultural components and could include the following types of action: on the causes; on effects; on the phenomenon; on value systems; on crisis-generating strongpoints (critical areas); on vulnerable sites; on the leaders.

METHODS

The analysis of open sources of information and of the experience gained during the activity in the field of communication and public relations, concretized through the elaboration and implementation of both the communication management strategies and of the projects included in the field of defense and national security.

In hybrid war, opponents (states, state-supported or independent movements) will exploit access to modern military capabilities and support insurgent, terrorist and criminal movements as well as the intervention of states that can combine high-tech military capabilities with terrorist actions and cyberwar operations against financial targets.

The analysis of available sour-

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ces suggests that the likelihood of a hybrid warfare is in direct connection with local population's satisfaction and the stability of states. Experts believe that, in failed or failing states, population security and services are often provided by dissident groups, armed factions, and criminal gangs. These areas become a fertile ground for terrorist, fundamentalist, extremist groups with regional or global influence and seriously affect the world economic system by facilitating illegal activities.

Contemporary hybrid war is a novelty in that it is taken to populated conflict zones, with the participation of the population, under the eyes of the international community. Thus, according to many authors, the most serious threats to the United States, including to its NATO dimension and to its partnership with Europe, would come from extremist groups and their ideology, fueled by poor urban areas of the world, where youth faces great economic and social problems, and is militating for a change. These unconventional actors can operate beyond state control by circumventing international borders, norms and agreements. Some of these belong to global terrorist networks, and others co-operate with criminal elements to destabilize certain areas. The hybrid confrontation with these groups will become "a clash beyond the physical elements of the conflict, in which media manipulation, the use of the internet and the integration of information operations with strategic communication programs are as important as weapon systems on the battlefield."

The issue emerging in the hybrid war resides in the fact that the enemy is trying to circumvent the military victory by focusing on long-term political victory by encouraging and maintaining tensions, crises and conflicts on a small scale, with implications at regional and sub-regional level, using the media handling concept with increasing intensity. Almost every person has come to have their own perception about manipulation, but without a complete conscious understanding of its content.

In the public debate, confusion is often made between information, adhesion, influence and manipulation.

Through the means of mass communication, the meaning of the war creates, metaphorically, a war of the meaning, of information, that conveys and / or prevents opponents from accessing information to influence their emotions, motives or goals, aiming at obtaining behaviors and actions favoring initiators.

The influencing action – whether by coercive or non-coercive strategies – has the purpose of altering the behavior of the people it is exercising upon. Regardless of the type of influence exerted on the individual, it produces its effects only through the human psyche.

In other words, any action of influence on an individual – be it propaganda, threat, promise, persuasion, intimidation, blackmail, noise, annoyance, surprise or harassment, etc. – it achieves its effects through the human psyche.

All definitions given to communication, regardless of the schools of thought to which they belong or the guidelines they enclose, present at least the following common elements: communication is the process of transmitting information, ideas, opinions, opinions from one individual to another, or from one group to another; conscious communication is an attribute of the human species; activities, events, processes and decisions involving people cannot be conceived beyond communication.

Any communication process has the following components (Zorlentan, T., 2001): Issuer; Receiver; Message; Context; Communication channels. An efficient communication process calls for both categories of channels to be considered, knowledge of how they operate, and the benefits and drawbacks that exist in their use and control [1].

Any human behavior, however, cannot be determined directly, but indirectly by actions on the elements that determine its appearance.

Misinformation consists of any intervention on the basic elements of a communicative process that deliberately modifies the messages being circulated in order to trigger in the target groups certain attitudes, reactions, actions desired by the opponent.

Vladimir Volkoff [2] defines misinformation as a "manipulation of public opinion for political purposes, using information subjected to hijacked means", as well as several ways in which it could be used: discrediting; undermining leaders' reputation; ridiculing traditions.

He also sets out twelve ways of effectively using misinformation to change the attitude of public opinion and to force it to act in the proposed direction of the manipulators: denial of deeds; reversing the facts; the mixture of truth and lies; changing the motif; changing circumstances; blurring; camouflage; interpretation; generalization; illustra-tion; unequal parts; equal parts. [3]

Disinformation as a weapon carefully conceals its origin and camouflages its intention of transforming the target into a manipulated object, refusing its right to specific traits and behaviors.

"Manipulation is the action of determining a social actor (person, group, community) to think and act in a way that is compatible with the interests of the initiator, instead of his interests, by using persuasion techniques that deliberately distort the truth, leaving the impression of freedom of thought and decision (...) The true intentions of the person who conveys the message remain insidious to the recipient. From a political point of view, manipulation is a form of imposing the interests of this class, group, community, not by means of coercion, power, but by ideological ones, by deception." [4]

In defining the notion of manipulation, it is necessary to specify a few specific elements [5]:

- The dissemination of a large amount of detailed information that cannot be verified while also concealing the real aspects of a case;

- The obsessive repetition of information with a negative trend, to be fixed in the memory of the listeners;

- <embargo> on the real information;

- Exploiting the fact that people are hungry with rumors.

New information technologies often separate the information as such from the identity of the person who initiated the news, and thus from the particular social context that would give it a certain credibility and a certain meaning. From this point of view, the power is directed towards credible communicators, towards those who can also offer a frame in an understandable context, easily recognizable by public opinion.

The context in which the news is located is very important – the reputation and credibility of the communicator matters. Type/brand becomes extremely important as a result. If 50 years ago, the battle was to gain control over the means of transmitting information, today the battle is being done to build/destroy credibility. The illusion of the need for information to be provided "readily interpreted" has been created, that is why the credibility of the source is a guarantee of a good interpretation. Global transparency, driven by new media opportunities and low-priced technologies, is now setting the stage for media outreach.

Thus, it is necessary to communicate very clearly to the target which will be its personal benefit by adopting a certain behavior or attitude, instead of the national, pan-national or regional benefit it will receive. The promised benefit in the future for that person must be personalized.

As for the brand image (it can be, for example, the US one), particular attention should be paid to the five basic dimensions of a brand: presence; relevance; differentiation; credibility; image and target. [6,7]

Crises are events characterized by low probability and important consequences that threaten an organization in its most profound purposes. Because of their low probability, these events defy any interpretation and require a sustained effort to understand and adapt reactions.

As a consequence, we can state that the crisis is an acute phenomenon which, left uncontrolled, can become chronical – that is, it can degenerate into conflict, therefore the dynamics of the decision-making process is particularly complex and it is carried out in most situations under uncertainty and stress.

Analyzing the phenomenon of crisis, we express the opinion that any crisis of any nature, has certain characteristics, such as: Threat; Expedite; Surprise; Time pressure; increased uncertainty and intensity.

Crisis and conflict management involves very complex systems of political, diplomatic, social, economic, cultural, international, military and other actions.

Such a system could be structured on political, diplomatic, social, economic, informational, military, psychological and cultural components and could include the following types of action: on the causes; on effects; on the phenomenon; on value systems; on crisis-generating strongpoints (critical areas); on vulnerable sites; on the leaders

Case Study: Ebola, Romania, 2015 [8]

The hypothesis of biological warfare, using the hemorrhagic fever virus, Ebola, as a weapon, was brought to the attention of the specialized public by military intelligence analysts, but also by the medical academics.

Although insignificant in size and proportions, the media crisis caused by the presence of Ebola virus in Romania raised concern at the level of state structures actively involved in the early detection, isolation and treatment of these patients, which led to the development of an action and communication strategy, following the premises mentioned in this article, which has also been concretized through an evaluation mission of the European Union experts.

Thus, during March 19-21, 2015, at the invitation of Dr. Raed Arafat, State Secretary and Head of the Emergency Situations Department of the Ministry of Internal Affairs, an expert team led by ECDC visited Romania. The main objectives of the visit were to provide specialized support to public institutions with tasks and responsibilities in the field of health, in order to review and verify the effectiveness of operational emergency intervention procedures, to verify the mechanisms of national inter-institutional cooperation in the context of management, under maximum safety and security conditions, of the cases imported by Ebola.

The main finding of the report was that Romania took the threat of Ebola seriously and initiated and established a wide range of measures as soon as possible, being able to ensure the rapid detection of infected persons, transport and specialized medical care, under conditions of maximum security for the staff involved in this procedural chain. Immediately after the World Health Organization Statement of October 2014 disclosing the threat to the Ebola virus, which was categorized as an emergency of international public health, the National Emergency Situation Committee of Romania created a national network, dedicated to managing this threat.

The role of the Ebola National Committee is to coordinate the interventions and measures needed to prevent and combat Ebola virus infections. It is chaired by the Head of the Department for Emergency Situations (Secretary of State within the Ministry of Internal Affairs), Vice-President - Secretary of State within the Ministry of Health.

In the Committee there are governmental decision making actors, namely within the Ministry of Health, the Ministry of Internal Affairs (Department for Emergency Situations), the Ministry of Foreign Affairs, the Ministry of National Defense, the Ministry of Education and Research, the Ministry of Transport, as well as key institutions, subordinated to these ministries, such as the Public Health Institute, the National Institute of Infectious Diseases "Matei Balş" and the Central Military Hospital "Dr. Carol Davila".

Throughout this period, the Ebola National Committee has worked intensively to develop a coherent national strategy for preparing for the safe identification and management of possible cases of Ebola and has consequently adopted seven decisions in this area, covering various issues related to early detection, triage, management and prevention of future spread of the Ebola hemorrhagic fever virus, as well as the creation of a dedicated budget to support this activity.

According to the Report, public health activity at the local level is carried out at the level of 42 public health authorities (VATA), and centralized data are provided to the National Public Health Institute (NPHI), which works under the aegis of the Ministry of Health. The Institute has five branches in the main cities:

Timişoara, Iaşi, Cluj-Napoca, Târgu-Mureş and Sibiu, and monitors all epidemiological situations with potential for national and international impact, along with other relevant institutions, being involved in activities aimed at increasing the degree of awareness. The site of the institute is the most complete and complex source of information, updated for both professionals and the general public. Although not mentioned in the Ebola National Committee's decisions, the NPHI also has the role of conducting and implementing population training campaigns, while maintaining institutional dialogue with regional public health authorities (VATA) as regards the evolution of relevant epidemiological issues, as well as contributing to the training and professional development of public health specialists.

In addition, CNAS and the Ministry of Health provide the early warning and response system to threats to national and, implicitly, European health security, assuming its responsibilities and commitments to WHO to address issues related to the implementation of the International Health Regulation (IHR).

At European level, key actors from other sectors of activity (e.g.: international affairs, civil protection, etc.) have been actively involved in discussions and in the start of inter-sector collaboration protocols that have taken place. In addition, a number of EU/EEA Member States have extensively participated in international efforts to counter the African epidemic by providing expertise, logistical and financial support.

Although for many countries the danger has passed, some European countries may still be at risk of exposure to the Ebola threat, given the migration phenomenon of residents from countries on the African continent, especially those in the West, severely affected by this disease.

The EU Health Security Committee continues to work to ensure effective communication at Member State level, in particular as regards the development and establishment of a joint action plan of addressing this challenge, further enhancing the dimension of the concept of preventing the spread of the disease by examining cross-sectoral and cross-border issues, as well as by checking capacities and capabilities for responding to biological incidents and accidents with extremely violent public health impact.

The lessons learned by Romania in this context have brought back a number of issues on the public agenda, such as the fragility of the healthcare and public health infrastructure; the high exposure to infection, given that our country is a gateway to Europe for the African population.

National vulnerabilities: lack of specialized medical personnel (in 2007 out of a total of 45 000 doctors, only 16 000 remained in Romania, others preferring other Member States, the phenomenon of labor migration is acutely felt in the intensive care, epidemiology and infectious diseases departments), the management of bio-waste, the lack of joint training drills in case of biological danger, the possible difficulties in the organization of medical evacuation - at this moment in Romania, there is no generic preparedness plan for a rapid response to eradication of outbreaks of infectious diseases, although it would be relatively easy to establish one as a result of the Pandemic Influenza Preparedness Plan used during 2009-2010-, the blocking of public procurement for advanced products/devices to support activities in the medical field as well as research-innovation and health system development.

An important aspect is also the need to ensure the functionality of a public health lab, of vital importance both for the national preparedness to respond to the threat of infectious diseases and for mandatory reporting at EU level through the ECDC. Not even today did the analyzes carried out by the Ion Cantacuzino National Research and Development Institute for Microbiology and Immunology surpass the threat of the uncertainty of activity financing, a key component of the public health system, which entails a whole chain of vulnerabilities that converge significantly to creating a state of health insecurity.

CONCLUSIONS

That is why medical intelligence, defined in Joint Pub 1-02 as "a type of information products resulting from the collection, evaluation, analysis and interpretation of medical, biological and scientific data as well as from the climate-related data, represent a special category, essential to national strategic planning, whether we are talking about the military or civilian environment, the planning of the fighting force conservation operations or the performance of medical capabilities assessments of rapid intervention."

"The role of medical intelligence structures is often confused with that of preventive medicine. Although the two disciplines have no common ground, they have become complementary over time, even in a relationship of interdependence.

While preventive medicine attempts to identify those events that constitute medical threats to subsequently assess the risks posed by exposure to such threats, while ensuring at the same time that decision-makers are advised on the adoption of preventive strategies, the medical intelligence is the component which ensures the permanent upgrading of the prevention process by identifying new types of non-conventional medical risks and threats, described in the published literature, in the possession of potential aggressors, depending on the changes in the security climate and the geostrategic map of influence (state actors or terrorist militarized formations), assesses new trends and medical technologies, especially in the dual-use industry." [9]

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VARIA

Medical causes and diseases leading to early permanent disqualification in IRIAF pilots based on their service categories

Mohammad Darvishi[1], Hamze Shahali[1]

Abstract: *Background and Aim: Efficient manpower is the most important capital and studies have shown the relationship between health and performance.Understanding the factors leading to disability has preventive importance.Our aim is determine the causes and diseases leading to early permanent medical disqualification of the Islamic Republic of Iran Air Force pilots based on their service categories.*

Methods: This is a descriptive, cross-sectional, retrospective study with target population of the Islamic Republic of Iran Air Force pilots with early and permanent medical disqualification from 1986 to 2016.

Results: Out of 508 cases of early medical discharges, 239 items were considered medical disqualification, 17 war captives, 34 service exchange and the remaining 218 was for on service killed or missed persons.The main medical causes were neurosurgery, cardiovascular and psychiatric, while common diseases include lumbar discopathy, myocardial infarction and cardiac arrhythmia.The lost service years was 3233 person year and the average was 12.63 person year per individual.

Conclusions: Based on the previous similar studies, it was expected that the common cause include neurosurgery, cardivascular and psychiatric with more prevalence in fighter, transportation, and helicopter service categories.At the end, we found that our results were in line with initial expectations.

Keywords: *disabilities, medical disqualification, health, prevention, performance*

INTRODUCTION

Human resources are the most important capital of armed forces, and many studies have shown the strong links between personal health and organizational performance. In order to increase productivity, organizations have adopted numerous measures not only to evaluate pre-employment health status, but also monitor staff health during their service life. Nonetheless, health risks remain the most important reducing factor of personal productivity. Disability is defined as the effect of a disorder on physical, mental, and social activity of a person where the work environment and the family status are also effective elements [1, 2, 3, 4]. According to statistics, effective prevention of cardiovascular risk factors in aviation population has a significant role in improving the professional health index of aviators (pilots). The study of aviator’s work environment and their lifestyle, active intervention, periodic regular examinations and screening tests for early detection of diseases are among the basic measures [10].

These days, the economic, mental and social problems caused by disability in armed forces are critical burdens. Regardless of the huge cost to run and maintain a powerful army, the supportive burden of victims can be devastating for any society [22]. In 1993, the United States (U.S) army paid 500 million dollars to compensation newly recruited armed forces [7, 8] In a study between 1980 and 1994 on the

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causes of disability in U.S. air and sea forces, it has been shown that disability in armed forces had led to an excessive cost of about 1.5 billion dollar in form of a compensation [2].

The preparation of military pilot volunteers to enter professional trainings and the provision of optimal services in the form of well-trained pilots require a huge amount of resources, both physical and intellectual [1]. The ultimate goal of present study was to determine the causes and diseases which led to early permanent medical disqualification (EPMD) of Islamic Republic of Iran Air Force (IRIAF) pilots based on their service categories from 1986 to 2016. Other objectives include; determine the causes and diseases which led to EPMD in IRIAF pilots who captured during the war, killed or missed in actions or have been forced to change their service category due to medical reasons, their lost service years (LSY) and its average and provision of scientific and practical solution to prevent the EPMD of IRIAF pilots, all based on their service categories.

METHODS

This is a descriptive-cross sectional-retrospective research which ethically approved by the ethics committee of the Aerospace and Sub-aquatic Medical Faculty in Aja University of Medical Sciences (with registration No# 10167118) and was conducted by the personal expenses of the corresponding author. However, like other up-to-date military articles, we are reluctant to disclose a number of military confidential information (i.e. total number of assessed cases). All the IRIAF pilots(which all are male) with EPMD who had relevant official records from 1986 to 2016 was included in the study and those who had medical disqualification due to physical or mental illness and death in non-occupational accidents, non-medical reasons, personal requests or disciplinary punishments were excluded. In this study, all pilots were captured during the war, killed or missed in actions, and those who confronted with service exchange were classified as permanent disqualification. However, this is one of the study advantages and all data was illustrated in detail separately. In order to observe the principle of confidentiality a non-disclosed random ID code were assigned for every person.

Main disease of the participants was classified based on the ICD-10 (10th version of international classification of diseases) and registered in to the Microsoft excel-based pre-designed electronic data sheet. Other recorded information included; date of entry into the service, date of departure from the service and service categories. Indeed, the total lost service years and average lost service years were calculated for each service category. Finally, this data was analyzed and displayed as graphs and figures for better presentation.

RESULTS

During the period of 1986 to 2016, 508 cases of early permanent discharges occurred in IRIAF pilots which contained; 239 medical disqualification, 218 killed or missed in actions, 34 service exchange and 17 war captives (Table 1).

Table 1: Study data

Service pilots categories / **Status**	**Fighter**	**Transportation**		**Helicopter**	**Total**
		light	**heavy**		
Medical disqualification	171	20	26	22	239
War captives	16	0	0	1	17
Killed or missed in actions	159	7	30	22	218
Service exchange	28	0	3	3	34
	374	27	59	48	508
		86			

The most prevalent medical causes of EPMD in IRIAF pilots were neurosurgery, cardiovascular and psychiatric (Figure 1).

A gynecological and urological consultation was requested for a non-functioning urethral-vesical catheter of a 60 years-old female patient admitted in the Orthopedic Department that underwent surgery with external fixation 2 days prior for a complex pelvic fracture (transverse fracture of the right acetabulum, left obturator ring fracture, left sacral fracture, fifth lumbar transverse apophysis fracture with paralysis of the external left sciatic nerve) (Figure 1).

The most common diseases led to EPMD in IRIAF pilots included; lumbar discopathy, myocardial infarction, cardiac arrhythmia, generalized anxiety disorder and post-traumatic stress disorder (Table 2).

Figure 1: Causes lead to the EPMD of IRIAF pilots based on their service categories

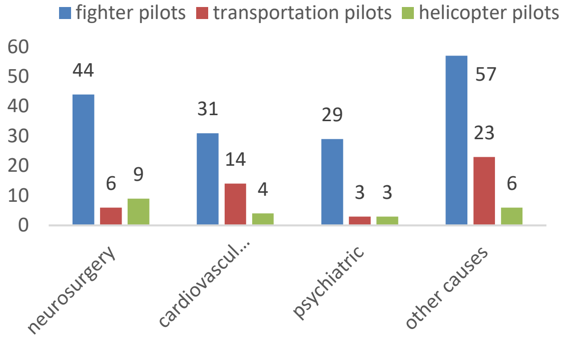

Table 2: Diseases lead to the EPMD of IRIAF pilots based on their service categories

Service pilots categories Status	Fighter	Transport	Helicopter
Lumbar discopathy	18	5	5
Myocardial infarction	12	6	2
Cardiac arrhythmia	10	2	1
Generalized anxiety disorder	10	0	1
Post-traumatic stress disorder	10	0	0
Other diseases	111	33	13

The most prevalent medical causes of EPMD in war captive IRIAF pilots were neurosurgery, psychiatry and orthopedics (Figure 2).

Figure 2: Causes lead to the EPMD of war captive IRIAF pilots based on their service categories.

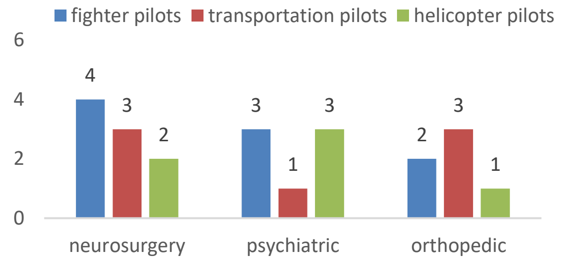

The most common diseases led to EPMD in war captive IRIAF pilots included; post-traumatic stress disorder, lumbar vertebral fracture with fusion and complicated bone fracture (Table 3).

The most prevalent medical causes of permanent medical service exchange (PMSE) in IRIAF pilots were ear, nose and throat (ENT), neurosurgery and orthopedics (Figure 3).

Table 3: Diseases lead to the EPMD of war captive IRIAF pilots based on their service categories

Service pilots categories Status	Fighter	Transport	Helicopter
Post-traumatic stress disorder	3	1	1
Lumbar vertebral fracture with fusion	2	0	1
Complicated bone fracture	1	0	1
Lumbar discopathy	1	1	0
Cervical discopathy	1	0	1
Other diseases	3	0	0

Figures 3: CT-scan showing complex pelvic fracture and the communication between the bladder and the left labia.

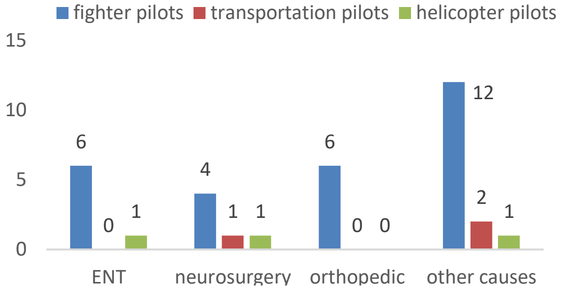

The most common diseases led to PMSE in IRIAF pilots included; motion sickness, complicated bone fracture, lumbar discopathy (Table 4).

Table 4: Diseases lead to the PMSE in IRIAF pilots based on their service categories

Service pilots categories Status	Fighter	Transport	Helicopter
Motion sickness	6	0	0
Complicated bone fracture	4	0	0
Lumbar discopathy	2	1	0
Cervical discopathy	2	0	1
Generalized anxiety disorder	2	0	0
Other diseases	12	2	2

As 3233 LSY, 2457 (%76) was belong to fighter pilots, 459 (%14.4) to transportation pilots and 317 (%9.8) to helicopter pilots. The most prevalent medical causes of LSY in IRIAF pilots with EPMD were neurosurgery, psychiatry and cardivascular (Figure 4).

The most LSY in IRIAF pilots with EPMD were belong to lumbar discopathy, generalized anxiety disorder and cardiac arrhythmia (Table 5).

Figure 4: Causes lead to the most LSY in IRIAF pilots with EPMD based on their service categories.

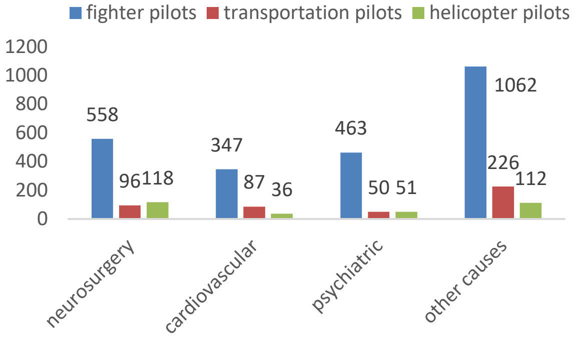

The average of LSY in IRIAF pilots with EPMD was 12.63 person year per individual (13.14 in fighter, 9.98 in transportation and 13.78 in helicopter pilots).

The most prevalent medical causes of LSY average in IRIAF pilots with EPMD were psychology, ophthalmology and neurosurgery (Figure 5).

Table 5: Diseases lead to the most LSY in IRIAF pilots with EPMD based on their service categories

Service pilots categories **Status**	**Fighter**	**Transport**	**Helicopter**
Lumbar discopathy	225	80	56
Generalized anxiety disorder	187	0	12
Cardiac arrhythmia	136	19	18
Complicated bone fracture	151	0	0
Post-traumatic stress disorder	151	0	0
Other diseases	1067	360	231

Figure 5: Causes lead to the most LSY in IRIAF pilots with EPMD based on their service categories.

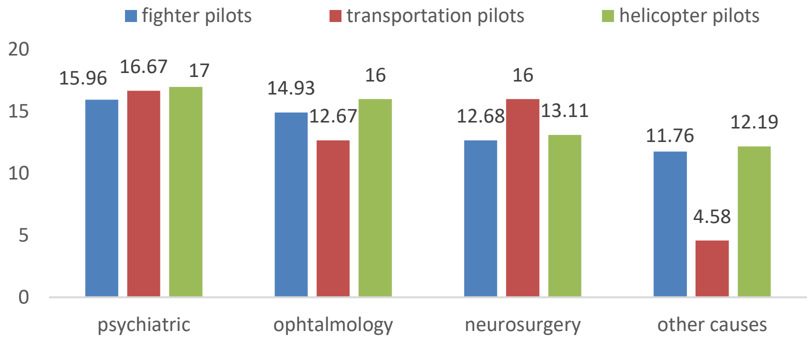

The diseases resulted in the highest average of LSY in IRIAF pilots with EPMD included; migraine, cardiac arrhythmia and lumbar discopathy (Table 6).

Table 6: Diseases lead to the most LSY average in IRIAF pilots with EPMD based on their service categories

Service pilots categories **Status**	**Fighter**	**Transport**	**Helicopter**
Migraine	13	16	23
Cardiac arrhythmia	13.6	9.5	18
Lumbar discopathy	12.5	16	11.2
Generalized anxiety disorder	18.7	0	12
Epilepsy	14	12	0
Other diseases	11.92	6.58	11.56

DISCUSSION

In the study of Whitton-1984 on United State Air Force (USAF) pilots and navigators, the common causes were cardivascular and neurologic [17], while in the same study of Mc Crary-2002, the most common causes in 1995 to 1999, include cardivascular, musculoskeletal, neurologic and endocrinology [12]. Also, in the study of Montazeri-2005, the most common causes of EPMD in IRIAF pilots included cardivascular and musculoskeletal [20]. In the study of Ghazizade-2010 on the same population in 1992 to 2003, cardivascular, nervous-skeletal and gastrointestinal were the most common causes [19].

In the study of Dark-1986 with the help of The Federal Aviation Administration (FAA), on medical disqualification of American airlines pilots in 1983 and 1984, the most commonly reported causes were cardiovascular and Neuropsychiatric events [18]. In the Nakanishi study on permanent disability of 260 Japanese crews, significant causes include malignancies, neurodegenerative, cardiovascular, gastrointestinal and musculoskeletal disorders [9]. In the review of trained pilots on Commonwealth countries from 1994 to 2004, the causes of cardiovascular were prevalent [19]. In the Arva study on 257 Norwegian civil pilots, the main causes were cardiovascular, neurologic, musculoskeletal and psychiatric disorders [11]. In the studies of Mitchell-2004 and Evan-2006 on sudden incapacitation of British civil pilots, 36 cases have been diagnosed and half of them were due to cardiovascular, cerebrovascular and psychiatric disorders [13, 14]. In the Nezami study on 200 civil pilots of Vnukovo airport in Moscow, cardivascular, neurologic and digestive have been the main causes of medical disqualification [10].

In the Mc crary's study, the more common diseases were ischemic heart diseases, high blood pressure, back pain, inter vertebral disc disorders and migraine [12]. In the study of Nezami, ischemic heart diseases, myocardial infarction, high blood pressure, cerebrovascular sclerotic lesions and peptic ulcer have been common [10]. FAA has conducted a great comprehensive study in 2014 on the causes of medical disqualification of pilots listing fifteen major causes, including; pectoris angina, bipolar mood disorder, cardiac valve replacement, coronary artery diseases, diabetes mellitus, loss of consciousness with unknown reason, seizure, cardiac replacement, myocardial infarction, permanent pace maker, disabling personal disorder, psychosis, drug abuse and dependency and lack of neural control, in order of significance [16].

In the study of Montazeri, the mean LSY of IRIAF retired with EPMD was 6.5 person year per individual [20]. In the Ghazizade study, the mean LSY was 6.14 and the highest was 10 person year per individual [19].

The present study has limitations that include: 1) missing details due to the lack of presence of a comprehensive electronic system for recording personal medical records; 2) the possibility of oriented malingering in disorders where specific objective diagnostic methods are not available and were mostly diagnosed subjectively, such as motion sickness, migraine and etc, and 3) flying device factors (ergonomics and exhaustion), operational and airbase factors (work and rest schedule), Personal factors (life style, physical and psychological capabilities, socioeconomic state, use of legal and illicit drugs and habits), medical factors (medical and psychological advices and hygiene education) are important items which have effective influence on pilots health.

The current investigation has important advantages such as: 1) so far, in terms of quantity and quality, there is a limited number of similar studies have been conducted about military pilot's health; 2) it has the longest cross-sectional period (35 years), the closest was performed during a 10-years period [18]; 3) express the statistics of IRIAF pilots which captured during the war, killed or missed in actions and those who confronted with service exchange, and 4) Records given were sorted according to the service categories.

CONCLUSIONS

More studies should be conducted on military and civil aviator's (pilots) health in the future. We believe that the life style modifications, regular fitness training, hygiene and health education and use of medical and psychological counseling for preventing the EPMD in military pilots are necessary preventive steps to take. Also, use of up-to-date comprehensive electronic system, upgrading on-the-job examinations, sufficient periodic in-service examination in occupational health centers are recommend by authors.

List of Abbreviations

U.S = United States; IRIAF = Islamic Republic of Iran Air Force; EPMD= Early and Permanent Medical Disqualification; LSY= Lost Service Years; ICD-10 = 10th International Classification of Diseases; PMSE= Permanent Medical Service Exchange; ENT = Ear, Nose and Throat; USAF= United State Air Force; FAA= Federal Aviation Association

Authors' contribution:

MD – data collector and supervisor, statistical analyzer and literary editor; HS – main owner of the idea, scientific supervisor, executive author and editor.

Ethics approval and consent to participate:

The ethical approval of this study was issued with registration No#10167118 by the ethics committee of the Aerospace and Sub-aquatic Medical Faculty in Aja University of Medical Sciences.

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Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

All the data of this study is available and could be accessible for publication by that valuable military journal.

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ORIGINAL ARTICLES

Medical devices in current medicine

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Abstract: *The treatment of diseases is based on pharmacological and non-pharmacological means, but adequate devices are needed for their application. Medicine uses a wide range of medical devices. Currently, many medical devices are used, many of them being disposable. As recognition of the importance of medical devices, the national authority of the drug is the ANMDM (National Agency for Medicines and Medical Devices).*

Medical devices are indispensable for therapy, and their evolution has led to an extraordinary diversification of the field and the emergence of industry related to the pharmaceutical industry, with specific laws and regulations.

Keywords: *medical device, medicine, pharmacological action, non-pharmacological action, health legislation*

INTRODUCTION

The treatment of diseases is based on pharmacological and non-pharmacological means, but for whose application different devices are needed, suitable physical objects, without which treatment cannot be applied. Modern medicine uses a wide range of medical devices, some derived from classical forms, and others that are new and more complex, which revolutionize therapy. Although their use is very old, the term as such has been newly introduced in medical terminology, from the English terminology medical device. Another neologism, related to this, is disposable = available, which, together with a noun, also means "disposable". Currently, many medical devices are used, either small or large, simple or complex, many of them being disposable. As a recognition of the importance of medical devices for the medical and pharmaceutical field, as well as for the medical and pharmaceutical industry, the current national authority of the medicines (not a drug, apud WHO) has been renamed the ANMDM (National Agency for Medicines and Medical Devices) [1, 2].

GENERALITIES

Definition: "Medical device" means any instrument, apparatus, machine, appliance, or other item used alone or in combination, including the software necessary for its correct application, intended by the manufacturer to be used for human beings, and which does not achieve its primary intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its intended function by such means, for one or more of the specific medical purpose(s) of:

- diagnosis, prevention, monitoring, treatment or alleviation of the pain,
- diagnosis, monitoring, treatment, alleviation of or compensation for an injury,
- the investigation, replacement, modification, or support of

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the anatomy or a physiological process,
- control of conception (Law 176/2000).

Legislation

The specific legislation, including regarding medical devices, also appeared later on - in 1976, the Regulatory System for the MD - US. In 1993, the EU Regulatory System 93/42/EEC and Romania: Law 176/2000 on medical devices and Law 434/2004, with the Government Decision and Orders of the Ministry of Health. Example: HM Order 253/2010 regarding the reclassification of medical devices and 75/2010 for the Rules for Good Pharmaceutical Practice.

Within the HM, operate specific structures such as the Commission for Medical Devices and the National Agency for Medicines and Medical Devices. We mention that in the European Union, the pharmaceutical field was removed from the Health and included in the Industry, which caused some controversy in the medical world [3-23].

Authorization

Medical devices can only be marketed if they are effective, meet the intended purpose, and ensure patient safety. The certificate of conformity issued by the manufacturer guarantees the attestation of the product for being placed on the market and the classification in the corresponding risk class. The inscription must comply with the European Conformity Marking "CE" (conformitè europèene), which allows free movement in the EU space and should not be confused with "C E" (China Export), which has a space between letters. It turns out that a new medical device, being a product of the scientific research of development-innovation, will go through the following stages: Experimental model, Functional experimental model, Prototype (technological demonstrator), and zero series (at the manufacturer). The technical file of a medical device, which is subject to the approval of the NAMMD, contains information regarding the: device description, type of raw materials used for manufacturing, stability study, analysis bulletin, validation, description of the packaging, and instructions for use. The serial products must be manufactured with the technical assistance of those who have the patent for the product.

CLASSIFICATION

There are many types of medical devices and they can be classified according to several criteria:

1. Risk level
- Class I, Low risk: non-invasive medical devices (eg patches, bandages) and invasive (eg surgical gloves);
- Class II, Medium risk (use 1h-30 days) invasive (eg needles, catheters);
- Class II b, potentially high risk (use - more than 30 days) invasive (eg: probes, devices for repeated administration) and non-invasive (eg: modern dressings that come in contact with the damaged skin);
- Class III, High risk: surgically invasive, a tissue of animal origin, implantable or an integral part of a drug (eg dressings with antibiotics).

2. Access path
- Urinal,
- Digestive,
- Surgical,
- Respiratory,
- Parenteral.

3. Duration of use
- Transitional action, less than 1 hour,
- Short term, less than 1 month,
- Long term, over 1 month.

4. Frequency of use
- Single-use,
- Repeated,
- Permanent,
- When necessary.

5. Impact on the body
- Non-invasive,
- Invasive,
- Surgically-invasive,
- Implantable.

6. Method of manufacture
- Sterile (disposable or re-sterilizable),
- Nonsterile/aseptic.

7. Mode of operation
- With power source (eg electric battery),
- Without a power source.

8. Place of use
- Medical institution,
- Home,
- Portable.

After use in different activities:
Medical devices for:
- Diagnosis and monitoring,
- Administration of medicines,
- Parapharmaceutical (ex: dressings),
- Damaged tissue (orthopedics, surgery, burns),
- Oral hygiene,
- Sexual protection,
- Childcare,
- Medical imaging.

Categories of users:
- Manufacturing companies,
- Suppliers and distributors,
- Sanitary units,
- Medical technical workshops,
- Patients and clients.

QUALITY

The quality requirements are set out in the specific Reference Standards. Starting with 01.03.2019, ISO 13485: 2016 is mandatory. This standard is designed for organizations involved in the design, production, installation, and maintenance of medical devices and for risk management.

• Sterility

All devices and technical-medical products that come into direct contact with tissues and biological fluids are required to be sterile.

Sterilization by heat, gas, or radiation is chosen depending on the nature of the material to be sterilized and the efficiency of sterilization is checked according to FR X unless otherwise provided.

• Storage

The storage and transport of medical devices are done according to the general provisions of FR X unless otherwise provided. Some materials must be stored under special conditions: rubber, plastics, metal tools, dressing materials, and utensils.

LABELING

Markings

Medical devices must be labeled, on the product, on the packaging and the instructions for use with the information essential to the safety of the user:
- The product's name,
- Identification of the manufacturer,
- Lot number and series, including the date,
- Storage conditions,
- Method of operation.

In some cases, additional data is required for special labeling:
- Sterile,
- Disposable,
- Exclusively for clinical investigations;

Different graphic symbols or warnings can be added.

RISK MONITORING

The safe handling of health products is called vigilance:
- Pharmacovigilance for pharmaceuticals and pharmaceutical raw materials,
- Materiovigilance for medical devices,
- Reacto-vigilance for in vitro diagnosis,
- Biovigilance for temporary-use cellular products,
- Hemovigilance for blood products.

Materiovigilance pursues the incidents arising from the use of the technical-medical products, to ensure the safety of the medical procedures, permanent monitoring of the incidents, and the risks of their occurrence. On the Materiovigilance Data Sheet are reported the essential information related to erroneous indications, omissions, or incomplete instructions. On their basis, studies and expertise are also carried out for the continuous evaluation of the risk level after being launched on the market.

RAW MATERIALS AND PACKAGING

The raw materials can be natural or artificial, single or in combinations: cellulose, cotton, metals, and alloys, natural and synthetic rubber, ceramic materials, glass, plastics, etc. or combinations thereof. All materials used must be compliant for medical use, from pharmaceuticals to the finished product, including packaging. As an example, medical devices for the application of innovative antibacterial and/or antibiofilm drugs fall within the general definition, according to the legislation in force. They must meet all the conditions for authorization, both as a package and/or applicator, and as a medicine administered for the treatment of the patient; they must come accompanied by a Technical File, Analysis Report, and a Conformity Certificate endorsed by the HM through the NAMMD. They fall into Class II b of Potentially High Risk, non-invasive as modern dressings, which are exposed to damaged skin or Class I Low risk, non-invasive. All components must be sterile or aseptic and must be stored and used properly. The labeling must be complete, accompanied by the appropriate markings and with the follow-up on the Materiovigilance. The pharmaceutical forms and the packages (applicators) in which they can be presented may differ, depending on the particularities of the treatment, being required several experimental models for choosing the prototype. Each of the components must come accompanied by a medical opinion for use, and if it does not, it must be requested from the NAMMD, which means time and money for taxes [3, 4, 9, 12].

As examples of medical devices for the application of pharmacologically active drugs may be listed the following:

1. Paper sachets, amylaceous, etc.
2. Capsules: soft or hard, gastro-soluble or gastro-resistant, with compartments or concentric (delayed), etc.
3. Pencils: liquid or powder dosage, etc.
4. Ampoules: glass or plastic, brittle, compartmentalized (lyophilized powder and solvent).
5. Bottles: glass or plastic, perforable, dropper, jet, sprayer (passive, active)
6. Bottle/dose atomizer under pressure (spray)
7. Patches: fast or delayed (for different areas) etc.
8. Implants: in an orifice, subcutaneous, submucosal, and visceral (eg: ovules, suppositories, spark plugs, cones, etc.)
9. Impregnated dressings: solutions, powders, solubles, ointments, absorbable or non-absorbable, hydrogel, etc.

COMMENTS

Currently, our research team is studying medical devices for the application of pharmacologically active drugs, for the substitute, adjuvant or complementary treatment to the anti-infective treatment, especially against resistant bacteria.

There are different medical devices, suitable for types of substances and dosages, authorized for medical use, from which we can choose the most suitable for the purpose; creating a new device, simple or complex, would require a complicated and costly procedure, but unnecessary in this case. The range of medical devices that can be filled with the appropriate pharmaceutical preparation is currently provided by the pharmaceutical industry upon request.

By combining the newly proposed drug with the chosen medical device, experimental model variants are achieved [24]. After experimenting with these under different conditions, the functional experimental model is obtained, as a masterful and/or galenic pharmaceutical preparation for the testing provided by the Law on medicinal products, and will subsequently enter under the pharmaceutical authorization procedures.

CONCLUSIONS

Medical devices are indispensable for therapy, pharmacological, or non-pharmacological. Their evolution has led to an extraordinary diversification of the field and the emergence of industry related to the pharmaceutical industry, with specific laws and regulations. Medical devices play a very important role in modern medicine.

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VARIA

Medical intervention in the biological attack with Bacillus anthracis

Viorel Ordeanu[1], Lucia E. Ionescu[1], Diana M. Popescu[1], Marius Necșulescu[1], Simona N. Bicheru[1], Gabriela V. Dumitrescu[1], Manuel Dogaru[2], Adrian A. Andrieş[3]

Abstract: *The biological agent most likely to be used as a biological weapon is Bacillus anthracis, which causes the anthrax infectious disease. During the Cold War, it was present in large quantities in the military arsenals of some states, and at the beginning of the 21st century was used in a series of small bioterrorist attacks. In case of occurrence of events using Bacillus anthracis, urgent and adequate qualitative and quantitative response measures are needed to manage the consequences of the attack and to reduce the effects on public health. These include complex and well-coordinated activities to ensure medical intervention for the prophylaxis, treatment and recovery of patients. General prophylaxis is achieved by vaccination, individual and collective CBRN protection, decontamination, partial and total sanitary treatment, microbiological analysis for the detection, identification and confirmation of the biological agent. Therapy for disease induced by weaponized B. anthracis differs from classic antrax disease regarding antibiotics, doses and time, resulting a large antibiotic consumption per case. Complete recovery of the patients is achieved not only in terms of restoring normal biological parameters, but also in terms of the organism's "sterilisation" regarding the causal biological agent.*

Keywords: *biological agent, antibiotherapy, anthrax, Bacillus anthracis, pharmacoeconomics, medical intervention*

INTRODUCTION

The biological attack, as an act of biological warfare or bioterrorism, occurs by attack with biological weapons, the biological agent being living (bacteria, viruses, etc.) or nevi (toxin, intermediates, etc.).

The most used biological agent, also confirmed by the existence of military arsenals or bioterrorist attacks in the 20th and early 21st centuries, is Bacillus anthracis, (sporogenous gram-positive bacillus) that causes anthrax infectious disease (Figure 1).

Anthrax spores can be disseminated by various means to produce a military attack with biological warfare agents (BWA), a limited bioterrorist attack, or a bio-crime against established targets (as they were in the US and EU in 2001 and 2002).

In order to reduce the effects on public health, urgent and adequate qualitative and quantitative response measures are needed to manage the consequences of the attack.

Figure 1: Bacillus anthracis 1119 R Stamatin – culture examined under a microscope x1500, (original)

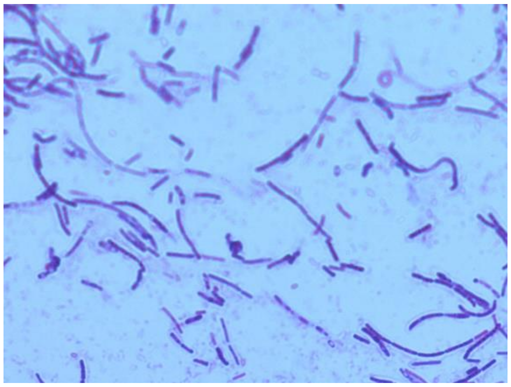

These include medical intervention for the prophylaxis, treatment and recovery of patients [1, 2].

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The general prophylaxis is achieved through health education, hygiene, vaccination, individual and collective CBRN protection, decontamination, partial and total health treatment, surveillance of suspects and contacts and, as the case may be, evacuation or isolation. It means that the medical service must collaborate with the veterinary service, the specialized military units, law enforcement agencies, local authorities and media representatives to disseminate accurate information, in order to reduce the epidemic and panic in the targeted community.

In practice, after a biological attack, the decontamination of the environment on large areas does not become an issue in terms of the excessive costs in using important forces and means, but only in terms of the personnel, the objects of strict necessity and of the access routes. The experience of the British army, on the island of Gruinard, is illustrative in this regard [3].

Although some authors consider (including in military textbooks) anthrax to be a non-contagious infectious disease, the risk of secondary contamination by manure, coughing and objects contaminated with bacteria in vegetative or sporadic form should not be overlooked. As a result, general precautions for anti-infective prophylaxis should be applied.

The diagnosis involves, as the case may be, an environmental stage with microbiological analysis for the detection, identification and confirmation of the biological agent in the air (if aerosolized), water, food, soil or different objects. This stage involves not only sanitary units (eg public health) but also military defense units against chemical, biological, radiological and nuclear weapons of mass destruction (WMD-CBRN), emergencies, secret services, etc. If contamination and disease are suspected, the task is of the human and/or veterinary medical service, which collects biological samples, cultivates, isolates and identifies with classical and/or modern means as well as confirmation by Polymerase chain reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA) and Mass Spectrometry (Figure 2). Immediately after shall be established the pattern of susceptibility/resistance of the vegetative bacteria to antibiotics, for the orientation of the antibiotherapy. Some anthrax strains may be less pathogenic, anthrax-like or vaccine strains, which would lead to false alarms and the loss of credibility of the CBRN health and surveillance/defense service [4].

Figure 2: Bacillus anthracis - mass spectrometry diagram (original)

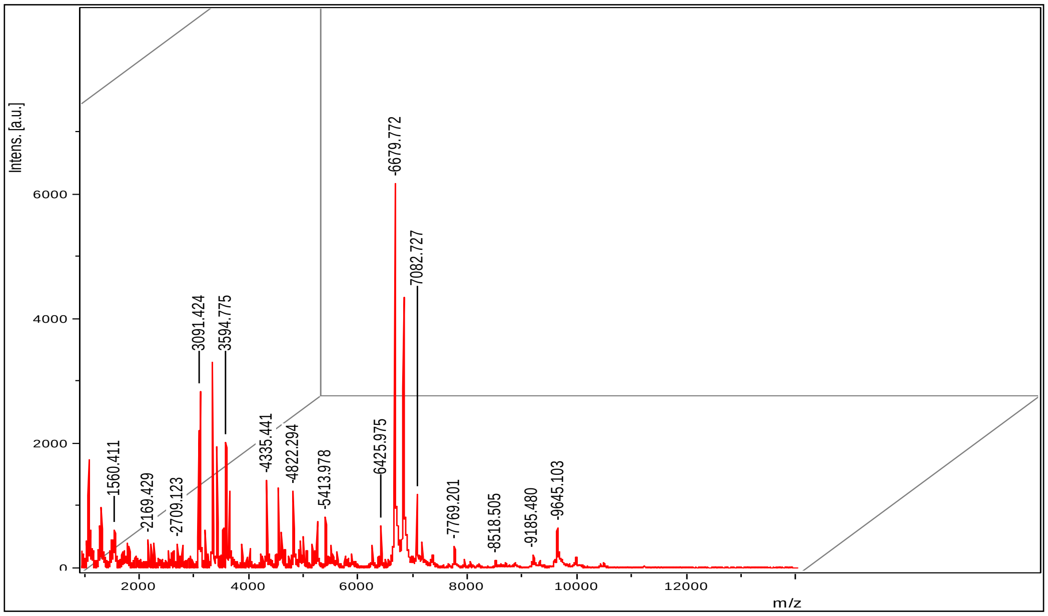

CLINICAL

Anthrax, also called charcoal, pustule, black sore, etc. is an acute infectious disease that naturally affects herbivores (horses, cattle, sheep, etc.) as zoonoses and epizootics, but can also be transmitted to humans, such as zooantroponosis and epidemic. Because the etiological agent B. anthracis is a telluric bacterium, a gram-positive bacillus that spores, therefore highly resistant to the environment, B. anthracis spores are considered the most likely biological agent for biological weapons and for bioterrorism, being confirmed by recent attacks. The spore itself is not pathogenic, because it does not have a metabolism and does not multiply, but it finds favorable living conditions (humidity, nutrients, heat, etc.) in the body that it contaminates and passes into vegetative form, which is pathogenic and causes the infection. For the biological attack on humans, domestic and wild animals or for the environment's contamination, it is estimated the use of strains of B. anthracis that are highly pathogenic, virulent, toxigenic, resistant to the environment and to the treatment with antibiotics (multiple antibioresistance), obtained either by selection either by genetic manipulation (Figure 3). As a result, biosafety measures must be respected, and treatment is intensive and prolonged [5, 9, 11-17].

Figure 3: Simulation of the dispersion of the biological agent Bacillus anthracis, 40 minutes report after aerosolization (original)

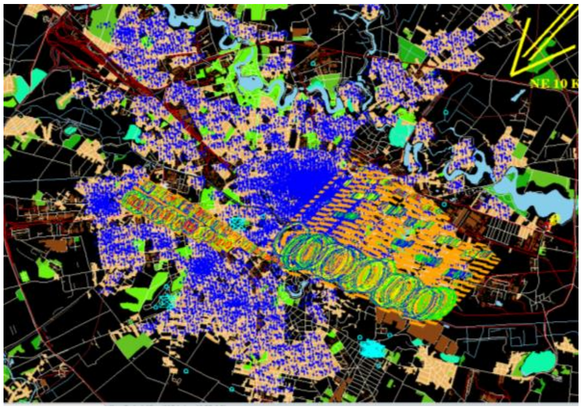

Depending on the pathway into the body and the location of the infection, the cutaneous anthrax (which has average severity) and the visceral anthrax are differentiated, with four clinical forms: gastrointestinal, pulmonary, meningeal and septicemic, which have a very serious prognosis.

Cutaneous anthrax the most common form of natural infection, most often occurs in animal breeders, is manifested by one or more characteristic "black sore", with a "charcoal" appearance (red-hot coal) with a red area and edematized around, painful, with medium-serious infectious and toxemic manifestations, but with reduced interhuman contagiousness. Classical antibiotherapy is applied (according to the infectious diseases manuals), symptomatic medication (anti-inflammatory, pain relievers, antipyretics), local antiseptics, dressing, possibly minor surgery.

Gastrointestinal anthrax (intestinal) caused by the ingestion of bacteria or spores with contaminated food or water that infects the gastrointestinal tract, with infectious and toxemic manifestations of high severity, with reduced human contagiousness, but with risk of environmental contamination. Classical antibiotherapy (according to infectious diseases manuals), symptomatic medication (anti-inflammatory, pain relievers, antipyretics), intestinal antiseptics, antidiarrheal and antiemetics are applied with caution. The waste will be collected and decontaminated energetically with conventional sporicidal disinfectants (chloride of lime, chloramine or formaldehyde).

Pulmonary anthrax (inhalation), caused by the inhalation of spores, in professional context or in aerosolization attacks for the deliberate release of spores. It is the most effective form of the biological attack, with infectious and toxemic manifestations of extreme severity: it is considered that morbidity is 99%, mortality over 90% in the untreated patients and about 50% in the treated patients. It is considered that it is not transmitted interhumanly, but we must also consider secondary aerosolization (from the patient) that can spread bacteria and spores through coughs, sneezes, saliva, etc. therefore precautions for air transmission must also be applied.

Other clinical forms of anthrax, more serious but rare, are actually complications of untreated disease, insufficiently treated or in immunocompromised organisms: meningeal anthrax and septicemic anthrax, in which mortality can reach 99%.

The incubation period for pulomonary anthrax varies between 1 and 60 days, depending on the host and the biological agent, and the patients often present: fever, discomfort, salivation, fatigue, low productive cough, nausea, vomiting and asfixia.

TREATMENT

The therapy for the disease resulting in a biological attack differs greatly from the classic recommendations, such as antibiotics (AB), doses and duration, achieving a very high consumption for each treated case. Our intention is to explain and complete the therapeutic procedures in the cases of anthrax resistant to treatment, as it can be the case in regards to BWA, bioterorist and biocrime attacks, with multi-resistant bacteria, in the patients with complications or immunosuppressed (by irradiation, intoxication or infections) etc. The old treatments were replaced or supplemented with antibiotherapy (ABT), which was a real breakthrough for the infectious disease clinic. But in the case of BWA, although this therapy is not wrong, it may not be effective: the bacterium is multi-resistant (AMR antimicrobial resistance), the clinical form is more serious, there should be a mass application treatment, so the therapeutic protocol differs. The causative agent of anthrax can persist in the body as a harmless spore and may become vegetative, thus pathogenic, later or even after the completion of the classic treatment. For this reason, after the attack with anthrax spores, post-exposure prophylaxis to contaminants and the anti-infective treatment in patients shall be continued without interruption for 60 days, according to the recommendations of the European Medicines Agency [6, 7, 8]. There is a risk of relapse and fallback, so treatment must be complex and individualized, but sometimes under mass therapy conditions. These conflicting requirements require continuous medical supervision and adequate health logistics [9].

There are no studies on humans for post-exposure prophylaxis with antibiotics, but experiments on guinea pigs and monkeys show that antibiotics from the cyclin and fluorinated quinolone groups, respectively Doxycycline and Ciprofloxacin, are the most effective in the prophylactic and curative treatment of anthrax [10]. However, any anti-infective treatment is important, because in the event of an epidemic, reserves of a certain antibiotic will be quickly depleted.

In the case of natural pulmonary anthrax (with "wild" bacillus) can be applied a complex treatment with classical antibiotherapy (according to the infectious diseases manuals), an adjunctive therapy (general or specific immunotherapy), complementary, symptomatic medication (anti-inflammatory, pain relievers, antipyretics), antiseptics, supporting vital functions (oxygen therapy, prosthetic respiration, etc.) and hospitalization in the AIC ward for infectious diseases.

In the case of the attack with aerosolized BWA, will be applied the principles of modern antibiotherapy, with antibiotic of choice or alternatives, for 60 days according to the recommendations of the EMA 2014 and the complex treatment depending on the specific situation. It turns out that in the case of an epidemic caused by a biological attack, the large number of patients who require the same special medical care and the same medicines creates a great problem of medical logistics.

Medical countermeasures may not be effective due to insufficient forces and means, which requires interinstitutional cooperation at local, national, European or international level. It should be noted that where the therapeutic recommendations state that "there is no recommended treatment" this does not mean that there is no treatment, but only that there is no specific treatment yet. So the doctor will apply any available treatment, which according to the medical logic and his experience, will heal his patient or will improve his condition, but in compliance with the classic principle "Primum non nocere" (Hippocrates). Regardless of the situation, no patient should be left without treatment.

Ciprofloxacin is recommended in the first line of treatment in two daily doses for 60 days [8], with all the pharmacological disadvantages it presents but the WHO has recommended caution with high and/or prolonged doses of ciprofloxacin treatment due to side effects such as tendon damage [18].

Other quinolones, such as Ofloxacin and Levofloxacin, offer options for alternative treatment, but dosage recommendations exist only for adults. Doxycycline and penicillins may be alternative therapies only if confirmed by susceptibility tests; Penicillin is not a safe bactericide on B. anthracis, but oral Amoxicillin is an alternative or continuation option if tolerated by the patient and if in vitro susceptibility is confirmed. B. anthracis can produce betalactamases, enzymes that hydrolyze penicillins [19].

Broad-spectrum antibiotics in prolonged treatment cause intestinal dysmicrobism and the microbiota should be protected with probiotics and the administration of useful bacteria if necessary.

The same antibacterial chemotherapics are recommended for the post-exposure prophylaxis, but Amoxicillin will be the drug of choice for pregnant women and young children, but only if the susceptibility tests are positive for penicillins.

Due to the high mortality in pulmonary anthrax, it is recommended to combine two or more antibiotics considered to be effective, the "therapeutic umbrella", but we do not have clinical trials for the multimedication therapy. Other active in vitro antibiotics that could be used in combination with Ciprofloxacin and Doxycycline are: protein synthesis inhibitors (Rifampicin, Chloramphenicol, Clindamycin, Clarithromycin, Erythromycin, Gentamicin, Streptomycin, etc.) and Vancomycin, but there is insufficient data to confirm their therapeutic efficacy in pulmonary anthrax [19]. Penicillins will not be used in combination with Ciprofloxacin. B. anthracis has a natural resistance to Sulfamethoxazole, Trimethoprim, Cefuroxime, Cefotaxime, Aztreonam, Ceftazidime etc., so these antibiotics cannot be used in the prophylactic or curative treatment of anthrax [10].

The injectable anti-infective treatment, although more effective, cannot be applied practically in the case of mass disease caused by a biological attack. Oral treatment is recommended if the patient can swallow and bioavailability is appropriate. As an example, Ciprofloxacin and Doxycycline are recommended for oral use [8].

Contraindications are those provided in the package leaflet, and as recommended by Prof. Ludovic Păun: "When prescribing an antibiotic, at least read the package leaflet!"

In pregnant and lactating women, shall be avoided the adminstration of risk products, breastfeeding will be discontinued and the treatment schedule shall be adapted accordingly. When applying any treatment, the axiom "There are no diseases, but patients" (Hippocrates) will be taken into account, and the complexity of the possible situations reminds us that medicine is equally science and art.

The present synthesis proposes therapeutic schemes for patients confirmed or suspected of pulmonary, gastrointestinal or cutaneous anthrax, and for the post-exposure prophylaxis in the case of confirmed or suspected exposure to B. anthracis, according to the studied bibliography and to the personal experience of scientific research of in vitro and in vivo anti-infective medical protection [19] and are presented by: International Common Denominator (ICD), role in therapy and prophylaxis, contraindications, dosage in treatment of suspected or clinically confirmed cases, post-exposure prophylaxis in confirmed or suspected cases of exposure to B. anthracis and duration of curative or prophylactic treatment. Depending on the clinical manifestations and situation, other anti-infective treatments, therapeutic serum (anti-carbonation serum, Cantacuzino I.), for supporting vital functions, immunomodulators, adjuvants, hygienic-dietary, psychotherapy, etc. will be applied.

In cutaneous anthrax with mild or medium forms, in which there are cases of spontaneous healing, the treatment will be done with the same dosage, but only for 7-10 days, and if there is a risk of inhalation of spores, the prophylaxis will last 60 days. In severe cases of cutaneous anthrax, with signs of systemic impairment, locally pronounced edema, with injuries to the head or the neck, a combined treatment will be applied [8].

The contraindications are those provided in the package leaflet, and in pregnant women and in lactation, the risk products will be avoided, breastfeeding will be discontinued and the treatment schedule adapted.

The dosage for children is calculated in mg/kg body weight, corresponding to a pharmacological reality, but without exceeding the adult dosage. From this it can be deduced that in adults with body weights significantly different from the average weight of 70 kg, therapeutic doses should be recalculated and we propose, for example, that individuals over 140 kg (without being obese) should be given double doses, if possible.

Antibiotherapy schemes according to EMA 2014

In the case of the BWA anthrax attack, the classical ABT is unlikely to be effective, due to changes in the "militarization" of the biological agent. As a result, AB will be used according to the EMA 2014 recommendations for biological agents, but also the antibiogram (ABG) will be used to confirm the spectrum of the isolated strain susceptibily/resistance from the patient.

Quinolones are the most important antibacterial chemotherapics in the treatment of anthrax.

Ciprofloxacin is the treatment of choice and is an alternative to continuing oral treatment, and is the first choice in prophylaxis until confirmation of the susceptibility of other antibiotics.

The dosage for the curative treatment of pulmonalry and gastrointestinal anthrax in adults (over 15 years) is initially 400 mg i.v. twice daily, continued with 500 mg p.o. twice a day for 60 days, totaling 60 g of the active substance. In children (under 15 years) is initially 15 mg/kg body weight i.v. twice a day, continued with 15 mg/kg body weight p.o. twice a day, (the daily dose should not exceed the adult dose) for 60 days.

Posology for the prophylactic post-exposure treatment in adults is 500 mg p.o. two times a day, for 60 days, totaling 60 g active substance, and in children 15 mg/kg body weight daily twice a day, (the daily dosage shall not exceed the adult dose), for 60 days.

It is known that antibiotics are among the most widely used drugs, and their costs are not very high. But in countries where compulsory health insurance operates, costs per dose can be increased by an order of magnitude. As a result, the costs per treatment must be compared, because for a large number of patients the total costs can be very high, so the principles of pharmacoeconomics will apply.

Ofloxacin is an alternative to Ciprofloxacin. The dosage for the curative treatment of pulmonary and gastrointestinal anthrax in adults (over 15 years) is initially 400 mg i.v. twice a day, continued with 400 mg p.o. twice a day for 60 days, totaling 48 g of active substance. The dosage for prophylactic post-exposure treatment in adults is 400 mg p.o. twice a day for 60 days, totaling 48 g of active substance.

Levofloxacin is an alternative to Ciprofloxacin. The dosage for the curative treatment of pulmonary and gastrointestinal anthrax in adults (over 15 years) is initially 500 mg i.v. once a day, continued with 500 mg p.o. once a day for 60 days, totaling 30 g of active substance. The dosage for prophylactic post-exposure treatment in adults is 500 mg p.o. once a day for 60 days, totaling 30 g of active substance.

Modern cyclins represent the optimal alternative antibacterial treatment.

Doxycycline is the alternative choice treatment and continuation of oral treatment, and is the first choice in prophylaxis if the sensitivity of the B. anthracis strain is confirmed.

The dosage for the curative treatment of pulmonalry and gastrointestinal anthrax in adults (over 15 years) and in older children 8-15 years, over 45 kg) is initially 100 mg i.v. twice a day, continued with 100 mg p.o. twice a day for 60 days, totaling 12 g of the active substance. In older children (8-15 years, under 45 kg) and in younger children (under 8 years) is initially 2.2 mg/kg body weight i.v. twice a day, (the daily dose should not exceed the adult dose) continued with the same oral dosage for 60 days, totaling 12 g of active substance.

The dosage for prophylactic post-exposure treatment in adults (over 15 years and in older children 8-15 years, over 45 kg) is initially 100 mg i.v. twice a day, for 60 days, totaling 12 g of the active substance and in children 15 mg/kg body weight twice a day (the daily dose should not exceed the adult dose). In older children (8-15 years, under 45 kg) and in younger children (under 8 years) is initially 2.2 mg/kg body weight i.v. twice a day, continued with the same oral dosage for 60 days, totaling 12 g of active substance.

Some beta-lactams may be used as an alternative antibacterial treatment, depending on the susceptibility of the B. anthracis strain.

Penicillin G is an alternative to first-line treatment if bacterial susceptibility is confirmed and penicillin allergy is excluded.

The posology for the curative treatment of pulmonary and gastrointestinal anthrax in adults (over 15 years and in older children 12-15 years) is 3 g (approx. 3 MUI) i.v. (preferably brannule infusion) 6 times a day (every 4 hours), for 60 days, totaling 1,080 g in 360 sessions (!?). In children (under 12 years) 30 mg/kg body weight, 5 times a day, for 60 days. It is not administered for post-exposure prophylaxis.

Amoxicillin is an alternative to first-line treatment and oral prophylaxis if bacterial susceptibility is confirmed.

Posology for the curative treatment of pulmonary and gastrointestinal anthrax in adults (over 15 years) 1 g i.v. 3 times a day, followed by 500 mg p.o. 3 times a day for 60 days, totaling 91.5 g of the active substance. In children (under 15 years), 80 mg/kg body weight per day, i.v. divided into 3 doses, followed by 80 mg/kg body weigh per day, p.o. divided into 3 doses (without exceeding the adult dose) for 60 days.

Posology for the prophylactic post-exposure treatment in adults (over 15 years) is 500 mg p.o. three times a day, for 60 days, totaling 90 g active substance, and in children is given 80 mg/kg body weight daily, p.o. divided into 3 doses, without exceeding the adult dose, for 60 days.

The recovery of the patients shall be considered not only in terms of restoring health but also in terms of the "sterilization" of the causal biological agent, so it is long lasting even in the case of an apparent cure.

Pharmacoeconomics

In case of a mass contamination or illness, very large quantities of specific antibacterial chemotherapics are needed, and the supply and replenishment of the sanitary units involved will impose significant financial costs, sometimes prohibitive. It turns out that, in addition to the pharmacological requirements, it is compulsory to take into account the criteria of pharmacoeconomics, for which we present below some examples, which can be found and/or updated on dedicated sites, on the Internet [20, 32, 33, 34].

“The wholesale cost of Ciprophloxacine in the developing world is between US$0.03 and US$0.13 a dose” [14]. In the United States it is sold for about US$0.40 per dose.

“Ofloxacin was patented in 1980 and approved for medical use in 1985 [21]. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system”. Ofloxacin is available as a generic medication [22]. The wholesale cost in the developing world is about US$3.27 per month [23]. In the United States a course of treatment costs about $50–100. The wholesale cost in the developing world is about US$3.27 per month [24]. In the United States a course of treatment costs about $50–100.

“Levofloxacin was approved for medical use in the United States in 1996. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system [25]. It is available as a generic medication” [24]. “The wholesale cost in the developing world is about US$0.44–0.95 per week of treatment. In the United States a week of treatment costs about $50–100. The wholesale cost in the developing world is about US$0.44–0.95 per week of treatment” [26]. In the United States a week of treatment costs about $50–100.

Doxycycline is available as a generic medicine and is generally inexpensive [27, 28]. “The wholesale cost in the developing world is between US$0.01 and $0.04 per pill. In the United States 10 days of treatment is about US$14; however, some in 2014 were selling it for more than $3.00–10.00 per pill. The wholesale cost in the developing world is between US$0.01 and $0.04 per pill” [29]. In the United

States 10 days of treatment is about US$14; however, some in 2014 were selling it for more than $3.00–10.00 per pill.

Benzylpenicillin was discovered in 1929 by Alexander Fleming and came into commercial use in 1942 [30]. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system [31]. The wholesale cost in the developing world is about US$0.24–2.72 per day [32]. In the United States a course of treatment costs $100–200.

Amoxicillin is available as a generic medication [33]. It has a wholesale cost in the developing world of between US$0.02 and 0.05 per pill [34]. In the United States, ten days of treatment costs about US$16 ($0.40 per pill).

OBSERVATION

The medical intervention for the prophylaxis, treatment and recovery of the patients includes an antibiotherapy that differs much from the classic recommendations, like antibiotics, doses and duration, realizing very high consumptions for each treated case and indeed huge for the winding up of the consequences of the biological attack. The biological agent must be detected, identified and confirmed as soon as possible, in the environment, in humans and animals and in patients for etiological diagnosis and antibiogram. Depending on the epidemiological triage, it is necessary to determine exactly who needs curative treatment, who is taking prophylactic treatment and who should be kept under medical supervision.

The anti-infective treatment should be personalized and supplemented as appropriate with other medicines: specific biological products (anti-carbon serum for the seriously ill, vaccine for those at risk of exposure), non-steroidal anti-inflammatory and pain relievers (aspirin, paracetamol etc.), antipyretics, antitussive, oxygen with or no assisted breathing, antiseptics, disinfectants, etc. Currently, there is a big problem at national level: the main producer in Romania, of biological products for treatment and diagnosis, INCDMM Cantacuzino, no longer has the marketing authorization for prophylactic and therapeutic vaccines, therapeutic and immunomodulatory sera, being undergoing re-technologization.

The seriously ill should be hospitalized, preferably in hospitals with existing infectious-disease wards or improvised in civilian sanitary units through civil-military cooperation (CIMIC) or by transforming some field hospitals (specialized in the management of polytrauma). The other patients, suspects and contacts, if large in number, will be treated and supervised by existing CIMIC forces and means, and if these shall not suffice, with international support from EU, NATO, WHO, etc.

The therapeutic schemes presented in this paper have been extracted from the specialized literature, being suggested as treatment alternatives in the case of pulmonary anthrax with resistant germs, or for the situations in which the resources have been exhausted and other therapeutic strategies should be used to avoid high risk situations for the public health, in the case of biological attack with B. anthracis. Pharmacoeconomic comparisons are presented, both for the guidance of clinicians and health decision-makers [35-49].

It should be noted that in the case of other bacterial biological agents, including BWA, the therapeutic schemes (post-exposure prophylaxis and curative treatment) range between 10-20 days, with usual antibiotics, ensuring medical countermeasures being much easier to achieve from a logistical standpoint.

CONCLUSIONS

The biological attack with B. anthracis is possible, as much as biological warfare, bioterrorist attack or biological accident. The management of the consequences also includes medical intervention, which is essential in combating and eliminating the effects of the attack with B. anthracis. The biological agent must be detected, identified and confirmed as soon as possible, in the environment, in humans and animals and in patients for etiological diagnosis and antibiogram.

The medical intervention for the prophylaxis, treatment and recovery of the patients includes an antibiotherapy that differs much from the classic recommendations, like antibiotics, doses and duration, achieving very high consumption per treated case and indeed huge for the liquidation of the consequences of the biological attack. Specific therapeutic schemes should be known by physicians and pharmacists for practical application, but also by the decision makers for setting up health stocks and could be integrated in the management of the consequences of the biological attack.

We believe that by applying the specific complex measures presented, the morbidity and mortality following the biological attack with B. anthracis can be reduced to an acceptable level, common for infectious diseases.

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ORIGINAL ARTICLES

Metformin – old treatment for diabetes, new treatment for psoriasis

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Abstract: *Metformin was a revelation in the treatment of patients with type 2 diabetes, being widely used, with remarkable outcomes. Lately, research has greatly expanded the indications of metformin treatment, which can be used not only in diabetes but also in many other conditions. Although still debatable, several studies have shown the beneficial effects of metformin treatment in patients with psoriasis. This article is a review of the latest data of scientific literature about the effects of metformin administration in patients with diabetes and psoriasis, but also in nondiabetic patients with psoriasis.*

Keywords: *metformin, diabetes, psoriasis*

INTRODUCTION

Psoriasis is a chronic inflammatory disorder characterized by hyperproliferation and inflammation of keratinocytes. Psoriasis is a complex disorder, both from the standpoint of the disease and from the many associated comorbidities. Obesity, diabetes, metabolic syndrome, arthritis and cardiovascular disease are the most common comorbidities in patients with psoriasis [1, 2, 25]. Pathophysiologically, patients with psoriasis present changes of the tumor necrosis factor alpha and interleukins [6, 8, 12, 17, 19 and 23], highlighting the pathophysiological link with the metabolic syndrome [3]. There is a two-way link between psoriasis and diabetes, patients with psoriasis are at risk of developing diabetes, while patients with diabetes are at risk of developing psoriasis [4, 5].

Numerous therapies are available for the treatment of psoriasis, from topical to biological. Many of the current therapies for psoriasis are expensive and/or may cause toxicity; new and more accessible therapies with reduced adverse effects are needed.

Metformin, a herbal remedy, was first synthesized in 1922, and subsequently Jean Sterne highlighted the properties of the metformin as Glucophage compound (glucose eater) (6). Metformin is largely used in the treatment of diabetes, being accessible, easy to administer, with few side effects and with potential to decrease the body weight in obese patients. Since 2009, metformin has been recommended by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) in patients with type 2 diabetes as first-line oral therapy [7]. Many studies focused on the potential benefits of metformin administration in patients with diabetes and other conditions.

Although generally well tolerated, metformin has a number of contraindications to administration (Figure 1).

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Figure 1: Contraindications to metformin administration

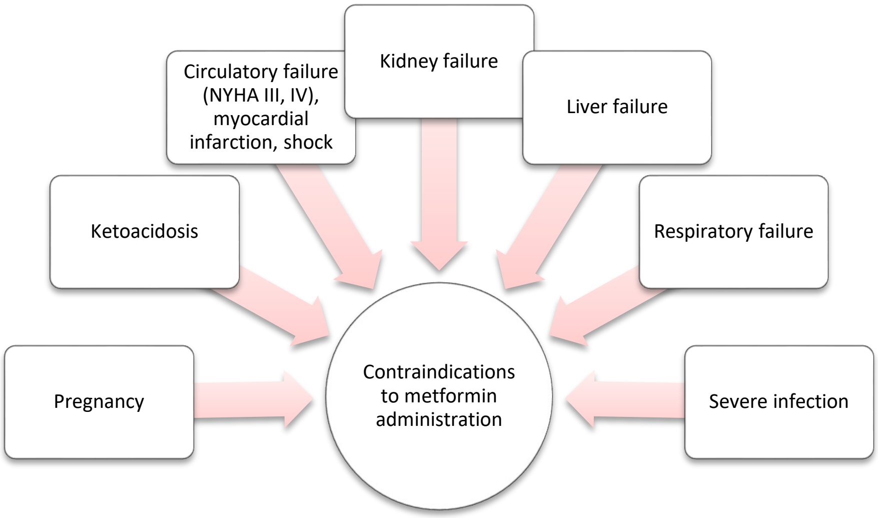
Circulatory failure (NYHA III, IV), myocardial infarction, shock
Kidney failure
Liver failure
Ketoacidosis
Respiratory failure
Pregnancy
Contraindications to metformin administration
Severe infection

METFORMIN IN THE TREATMENT OF DIABETES

Metformin is widely used for the treatment of type 2 diabetes. It is a first-line treatment as monotherapy or can be used as a combination therapy when monotherapy is no longer effective; insulin (regardless of the number of insulin injections/day) can be combined with a sulphonylurea, incretin or flozin [8]. In addition to the potential of lowering high blood glucose, metformin has a favorable effect on body weight, a very important aspect in diabetic patients.

Metformin does not cause hypoglycemia, because it does not stimulate the insulin secretion by the beta cells of the pancreas, the effect of lowering the serum glucose is due to inhibition of hepatic glucose production and sensitization of peripheral tissue to the effect of insulin.

Kirpichnikov et al emphasized the ability of metformin to reduce hemoglobin A1c by 1.3% - 2%, and may have a higher degree of reduction in patients with higher blood glucose levels during pre-treatment [9]. Although lifestyle changes seem to be more effective in the prevention of type 2 diabetes in people at risk, metformin can reduce the progression of diabetes and can be administered from the age of 10 years [10].

Following the administration of metformin in diabetics, in addition to the glucose-lowering effects, an improvement not only in the cardiovascular morbidity and mortality has been noticed, but also in the general health [11]. From the cardiovascular point of view, in patients at risk, the benefit of metformin administration is observed by the reduction of the body stress consequently to lower glucose levels, reducing the risk of myocardial infarction or arrhythmias.

In addition to the administration of metformin in type 2 diabetes, recent studies focused on its administration in type 1 diabetes. Nadeau et al, following the administration of metformin in patients with type 1 diabetes, observed a decrease in insulin dose, an improvement in body mass index and a decrease in waist circumference, especially in women [12]. Libman et al, in their study, have found that HbA1c was not significantly decreased following metformin administration, although the decrease in insulin dose was noted, and the patients complained of gastrointestinal distress [13]. Other observations following metformin administration in type 1 diabetes were improved outcomes of heart failure (regardless of decreased hemoglobin A1c) and decreased androgen levels in women with type 1 diabetes and hyperandrogenism [14, 15].

The benefits of using metformin in pre-diabetes were highlighted by the study of Capitanio et al, who have noticed a reduced risk of type 2 diabetes (by 31%) and a reduction of the body weight by average 2 kg in those who used metformin [16]. People with prediabetes should reduce their body weight and increase their daily physical activity; in addition, metformin can be considered for the prevention of type 2 diabetes in people at risk.

METFORMIN IN THE TREATMENT OF PSORIASIS

Starting from the pathophysiological mechanisms of psoriasis, with hyperproliferation and abnormal differentiation of epidermal keratinocytes, the main method of treatment of psoriasis focuses on inhibiting the excessive proliferation of keratinocytes [17}. HaCaT are cell lines derived from keratinocytes in normal adult skin, and excessive proliferation of these keratinocytes results in psoriatic lesions, with HaCaT cells being used as an in vitro model for the study of anti-psoriasis agents [18].

He et al investigated the effects of metformin on the proliferation of human immortalized keratinocytes. Metformin treatment regulated the level of p-AMPK and p-ERK1/2 in HaCaT cells and significantly inhibited the proliferation of HaCaT cells in vitro, by a mechanism associated with activation of the protein signaling pathway, mitogen-activated kinase [19].

Xuan et al have found differences in the quality of life of patients with psoriasis treated with metformin plus methotrexate, compared with patients treated with methotrexate alone. Combined metformin + methotrexate therapy contributes to a significant improvement in the quality of life of patients with psoriasis [20].

Because many psoriasis patients have also metabolic syndrome, Singh and Bhansali studied the effects of metformin (1000 mg/day) on the metabolic syndrome in psoriatic patients. These authors showed a marked improvement of the metabolic syndrome, following metformin administration, at 52.4%, compared with placebo (metformin versus placebo – OR (95% CI), 5.2 (1.3–20.7), P value 0.019) [21}. The proven efficacy of metformin on the metabolic syndrome is a potential axis for improving psoriasis. Because of metformin administration, 14.3% of patients compared with placebo (10%) had no changes or increases in IL-6 and TNF-α, followed by recurrence of psoriasis within the next 6 months [21].

Brauchli et al estimated a low risk of developing psoriasis (as a new diagnosis) using metformin compared to controls [OR = 0.77 (95% CI - 0.62-0.096)] [22].

Regarding the safety of metformin administration in patients with psoriasis and diabetes, it did not cause increased mortality, did not increase the severity of psoriasis or the rate of hospitalization for any other reason, metformin being safe to administer to patients with psoriasis [23].

Wu et al looked at the risk of developing psoriasis in patients with diabetes depending on the antidiabetic medication; they observed an increased risk of developing psoriasis in people with severe diabetes under insulin treatment, while the risk of developing psoriasis was not associated with metformin [5].

Starting from the cases of lichenoid rash and erythema multiforme that appeared following the administration of oral antihyperglycaemic drugs, we have found in the scientific literature only a case published in 2003 (possibly the first of this type reported), who suggests the occurrence of psoriatic rash following the administration of metformin, which eruption recovered after stopping the drug and reappeared with the reintroduction of the drug [24].

In addition to the proven beneficial effects of metformin on psoriasis, it seems that it has other applications in dermatology. Among the dermatological disorders studied from the point of view of metformin administration are acanthosis nigricans, acne, disorders of increased pigmentation, eruptive xanthomas, suppurative hidradenitis, hirsutism, skin cancer, squamous cell carcinoma, and melanoma.

CONCLUSION

The more extensive the metformin research areas, the more therapeutic applications appear. From the point of view of psoriasis, the use of metformin is safe and beneficial. Further studies are needed to determine the optimal dose of metformin recommended in patients with psoriasis, depending on the associated comorbidities.

Acknowledgement

All the authors have equal contribution.

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ORIGINAL ARTICLE

Mg-1Ca alloy intramedullary nailing influence on bone callus formation and on vital organs functions, as an alternative to bioplastics

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Abstract: *Magnesium alloys represent a new research direction in the field of orthopedic resorbable metal materials, because it has several important properties that recommend it as an excellent resorbable biomaterial for implants. From in vitro studies, Magnesium has osteoconductive properties, promoting the formation of new bone by stimulating the adhesion of osteoblastic cells, this leading to the idea that it will induce formation of hard callous at fracture site. The present study followed the in vivo evaluation of the biodegradation of pins made of Mg-1Ca alloy introduced centromedulary in rabbit tibiae, with previously induced experimental fractures. The influence of the biodegradation process on the formation of the bone callus was evaluated through radiological images and histopathological examinations. At the same time, we evaluated the function of vital organs through histopathological examinations and blood tests. We chose intramedulary nailing (IMN) with pins, because in orthopaedic surgery this is the golden standard for fractures of tibia or femur. In the radiological images obtained, we notice callus formation at the level of the experimental fracture at 6 weeks after surgery. The histological sections made from the samples collected from the fracture zone adjacent to the Mg1Ca alloy implant, confirm the formation of the bony callus. The trichrome Masson sections show a normal bone development with newly formed bone tissue at the tissue - alloy interface. In the sections harvested from the vital organs, no pathological changes are observed. These observations suggest that the biodegradation process of the Mg-1Ca alloy do not interfere negatively with the biological process of bone formation, allowing the formation of the bone callus and do not confirm the suspicion of pathological changes induced by systemically released corrosion products and gas embolism in vital organs.*

Keywords: *magnesium, alloy, implant, pins, biodegradation*

INTRODUCTION

Magnesium alloys represent a new research direction in the field of orthopedic resorbable metal materials. Use ofbioabsorbable materials will eliminate the need for a second ablation intervention leading to reduction of patient stress, reduction of postoperative infection risk and medical cost reduction. Also, it will have the advantage of reduction of bone/implant contact stress effect (stress shielding effect) and load transfer, gradually during resorption, toward the newly formed bone in the same time with resorption, decreasing the risk of fracture after surgery, as in the case of metal one

Main requirements of bioabsorbable metal materials are biocompatibility and biological functionality, physiological material without rejection reaction, that will assist the bone repair. In the same time it will need to have physicochemical properties consistent with the physiology of the region, meaning, no toxicities when resorption occurs, bone adhesion and allowing bone cell proliferation (osteoconductive), resorption by biodegradation and bone remodeling. Other recent studies in biomaterials have yet to take into account the possibility of bioabsorbable metal as an option [1], while others rely on biocompatibility without bioresorbtion [2, 3].

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Mg1Ca alloy

Mg-1Ca alloy consists in two natural elements of the human body, being able to dissolve in the body without inducing toxicities and foreign body reactions. Magnesium has osteoconductive properties, promoting the formation of new bone by stimulating the adhesion of osteoblastic cells, this leading to the idea that will induce formation of hard callous at fracture site.

Calcium is an important component of bone tissue and is an essential element in biochemical processes. It have a low density, 1.55 g/cm^3, which enables Mg-Ca alloys to achieve a density similar to bone, wich is 1.8 – 2.1 g/cm^3. Also, Magnesium has the potential to improve the incorporation of calcium into bone. Mg-Ca alloys are the most used and researched alloys, the solubility of Ca in Mg is 1.34 wt% [4].

The main drawbacks of Mg alloys are rapid biodegradation, increased rate of corrosion in the body, at a physiological pH of 7.4 to 7.6. Rapid corrosion will lead to loss of mechanical capabilities, the bending strength being able to decreasein a first stage of erosion from 652 MPa to 390 MPa [5].

Also, rapid corrosion will increase ph in the corrosion environment, thus producing a alkalizing effect of peri-implant tissues, with cytotoxic effect. According to ISO 10993-5:2009, a reduction of cell viability more than 30% it is considered a cytotoxic effect [6].

During corrosion process of Mg-Ca, hydrogen gas will be released. High hydrogen evolution rate corresponding to a high corrosion rate, will lead to gas bubbles formation into the surrounding tissues, leading to tissue necrosis, surgical incision dehiscence [7] and risk of gas embolisation in vital organs [8].

Mg-Ca alloys have a binary microstructure, consisting of a primary phase, α Mg, and an eutectic secondary phase, with lamellar structure, composed of Mg_2Ca [9]. Mg-Ca alloys corrosion process is the result of a galvanic circuitformation, due to the electrochemical potential difference between the primary phase α Mg and the secondary phase Mg_2Ca. Mg-Ca alloys undergo in SBF the corrosion process shown in figure 1:

a) galvanic corrosion of Mg and Mg_2Ca phase;

b) partially protective layer covering the surface of the Mg-Ca alloy;

c) absorption of chlorine ions for the conversion of $Mg(OH)_2$ to $MgCl_2$;

d) hydroxyapatite formation by consuming Ca^{2+} and $PO4^{3-}$ ions;

e) particulate residues decay emerging from the substrate volume.

Figure 1: Mg-Ca alloy corrosion in SBF (adapted after 8)

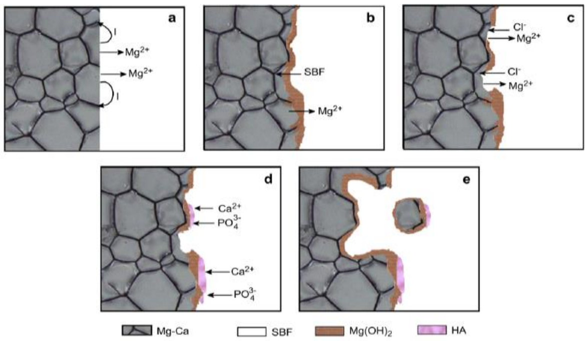

Based on this type of corrosion, literature studies concluded that controlling the amount of calcium in the alloy will influence corrosion rate and mechanical properties. Thus results obtained on alloys with low calcium concentrations, 1, 2 and 3% were better than those with high concentrations (5% - 20% Ca) both in terms of mechanical properties and corrosion rate [4, 10, 11], Mg-Ca 1% alloy being considered to be optimal for orthopedic implants [12].

Another method of improving the mechanical properties of Mg-1Ca alloys in the molded state is secondary mechanical processing. The tensile strength of the alloy has recorded, initially, a value of 71.38 ± 3.01 MPa. Through the process of hot rolling it reached a value of 166.7 ± 3.01 MPa, and by hot extrusion it was improved up to a value of 239.63 ± 7.21 MPa [12]. In the same time, this method improved hydrogen emissions meaning that corrosion rate is improved (high corrosion rate equals high hydrogen release). Diminished corrosion rate of the Mg-1Ca alloy, processed by hot extrusion has been expressed by a low hydrogen emission rate, from 35 ml/cm^2 to 10 ml/cm^2 [12].

During corrosion process, a hydroxyapatite layer is formed on the surface of the alloy, having a positive role on bone cell adhesion. Also, magnesium ions resulting from the degradation of the Mg-1Ca alloy, Zreiqat [11], could increase cell adhesion of HBDC (Human Body Derived Cells) in cell cultures. At the level of HBDC cells grown on the surface of the alloy, increased levels of α5b1 and b1 integrin receptors were discovered, which play a role in mediating cell adhesion to the surface of biomaterials. Thus, it is assumed that the increased viability expressed by Mg-1Ca alloys in cell cultures can be explained by facilitating the early response of osteoblasts, and it can be inferred that the release of Mg^{2+} ions from the alloy can stimulate bone cell adhesion and proliferation, being raised the hypothesis of the positive role of Mg-1Ca alloys in bone healing.

This research comes in suite with other areas of development in material science for orthopedic use in the local geographic area focusing on the interaction between the organic and the inorganic and also focusing on the impact of this research on the patients' lives [13, 14].

MATERIAL AND METHOD

Four specimens of domestic rabbit "Oryctologus Cuniculus", adult, minimum weight 3.5 kg, were used, divided in two groups. Group 1, included 3 specimens, with experimental fractures. Two rabbits with experimental fracture al anterior tibial tuberosity level and one with tibial diaphysis accidental fracture, during Mg-1Ca implant insertion. Fracture site was stabilized by the cylindrical implant followed by spica cast for three weeks. Group 2, control one, included one rabbit with a subtrochanteric fracture, occurred during a Mg-1Ca implant insertion (another experiment). In order not to lose the specimen, we extracted the magnesium alloy and continued with the hip osteosynthesis, plate and screws, austenitic steel.

We had used cylindrical implants of Mg-1Ca alloy, cast, with a density of 1.78 g/cm^3 and dimensions: L 40 mm, diameter 3.2 mm (Figure 2).

Figure 2: Mg-1Ca nail

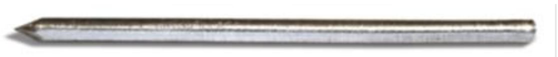

We chose intramedulary nailing (IMN), pins, because in orthopaedic surgery this is the golden standard for fractures of tibia or femur. This hypothesis is supported by biomechanical and biological factors.

Because of its design and mode of insertion, in the center of the bone, respecting the anatomical and mechanical axis of the bone, IMN is a load sharing implant compared to plates that are load bearing implants. This will increase the load bearing capacity of the implant, in the same time decreasing the stress shielding effect, fractures around the implant being less. Considering these characteristics, load bearing will be allowed earlier compared to plates osteosynthesis.

Regarding the biological factors , IMN will lead to less soft tissue damages compared to plates osteosynthesis. Fracture healing is a periosteum depended process and IMN will preserve it and also it will preserve the hematoma formed around the fracture site, that is a source of mediators for the bone callus formation process. Also, IMN is less invasive and the infection rate is low.

Another reason why we chose the intramedullary nailing system method is that the main objective of the study is to investigate the interaction of Mg-1Ca biomaterial with bone tissue. As we have shown in preliminary studies, the corrosion rate of Mg 1Ca alloy in contact with bone tissue is different from that in contact with muscle tissue. Unlike IMN which provides contact of the alloy only with bone tissue, plate and screw osteosynthesis will lead to two areas of contact of the alloy, with bone tissue and muscle tissue. This can generate false negative or false positive data, which can alter the results of the study.

The study was carried out with approval of the Ethics Committee for Scientific Research, no 92/20.04.2016, code PO-35-F-03. Animal care and clinical evaluation were performed according to FELASA (Federation of European Laboratory Animal Science Associations) guidelines and recommendations [15].

Preop procedures: Lower limbs radiographs, in two incidents, anterior-posterior and laterally, Figures 3 and 4. The animals were dewormed using Ivermectin, at a dose of 0.1 ml/body kg, subcutaneously (SC). Preoperative antibiotic therapy , one dose of Enrofloxacin (Boytril 5%) at a dose of 10 mg/body kg, subcutaneous.

Figure 3: Preoperative Xray

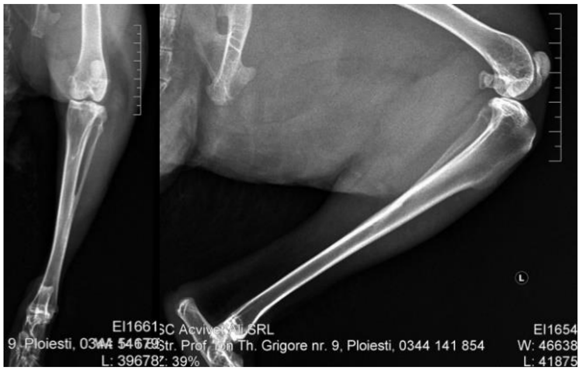

Figure 4: Preoperative Xray control group

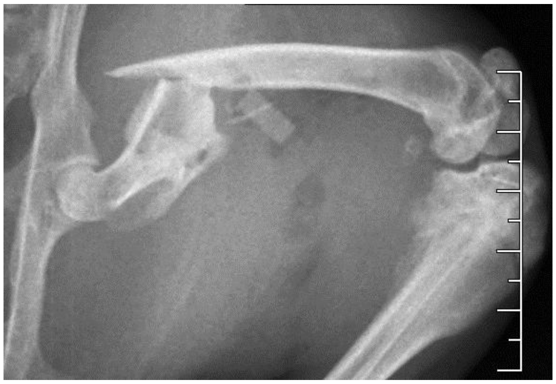

Blood tests: are needed to evaluate the possible impairment of the function of the vital organs, kidney, liver or ionogram by the corrosion of the alloy.For the blood samples we used as a source the auricular artery. All determinations of blood parameters were performed using the services of accredited laboratories. The determined parameters and their physiological values are represented in the following Tables (1 and 2).

Table 1: Ionogram

Element	Normal value
Magnesium	2.19 – 4.14 (mg/dL)
Calcium	12.4 – 15.6 (mg/dL)
Sideremia	111.7 – 329.6 (μg/dL)
Sodium (Na^+)	138 – 155 (mmol/L)
Potassium (K^+)	3.7 – 6.3 (mmol/L)

Table 2: Vital organ tests

Element	Normal value
Serum urea	12.6 - 50.3 (mg/dL)
Creatinine	0.38 - 1.88 (mg/dL)
Alanaminotransferase (ALT)	<61 (IU/L)
Aspartataminotransferase (AST)	<28 (IU/L)

Surgical procedures: The implants were chemically sterilized by ethylene oxide sterilization method, thus avoiding damage to the alloy.

The type of anesthesia chosen was, mask inhalation anesthesia, veterinary use (Figure 5). For induction Ketamine 20mg/body Kg, subcutaneous (s.c.) was used. The anesthetic agent used was: Sevoflurane.The anesthesia was maintained by sevoflurane delivered in oxygen (1.5–3.5% oxygenmixture; 1.5 L/min) Throughout the surgery we monitored cardiac activity and respiratory movements. Infusion of Ringer’s lactate solution(10 mL/kg/h) during the entire surgical procedure ensured cardiovascular function.

Figure 5: Anesthesia

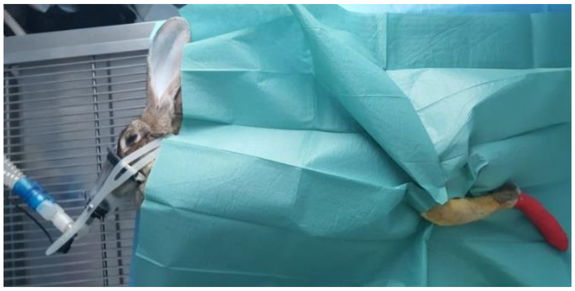

The surgery consisted of centromedular implantation, proximal tibia, cylindrical implant Mg-1Ca alloy, nail type by approach over the anterior tibial tuberosity (Figures 6, 7), followed by an experimental fracture, with oscillating saw, in tibial diaphysis at a distance of 1 cm distal of anterior tibial tuberosity (Figure 8).

Figure 6: Mg alloy implant insertion

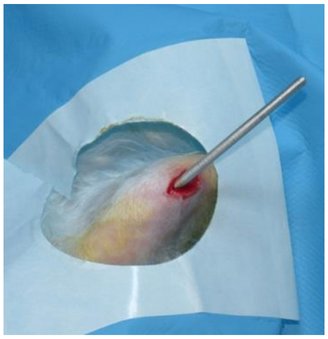

Figure 7: Mg alloy implant insertion

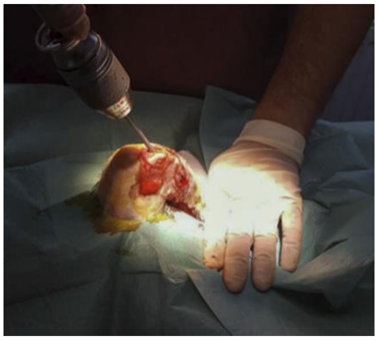

Figure 8: Experimental fracture

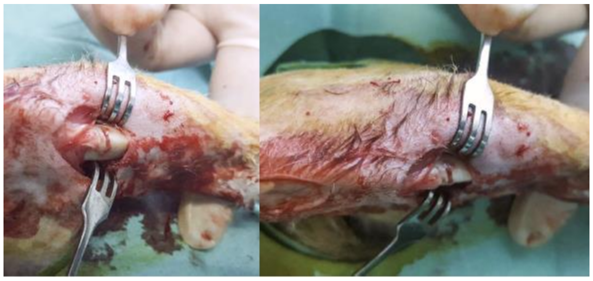

Postoperative procedures: Prophylactic antibiotic therapy, two days post-operatively. Enrofloxacin (Boytril 5%) at a dose of 5 mg/body Kg/12 hours, (s.c.) was used. Postoperative pain was controlled using Meloxicam at 0.2 mg/body Kg/24 hours (s.c.).

Two incidents radiographs, anterior-posterior and lateral, were used, with the same radiological device and the same technical data as in the case of pre-operative radiographs. Radiological follow up was for 6 weeks, and it is necessary to highlight the resorption of implants, and bone callus evolution.

At 6 weeks postoperatively, blood samples were collected.

Blood parameters and harvest conditions were similar to those performed preoperatively.

Euthanasia of rabbits was performed 6 weeks after implantation. In order to eliminate the suffering of the animals, we chose as an initial procedure their anesthesia, performed by s.c. Ketamine injection at a dose of 20 mg/body Kg. After this, euthanasia was performed by injecting 1 ml T61 (Etambutramide + Mebezonium Iodide + Tetracaine hydrochloric) intravenous (i.v.).

Post-euthanasia procedures: proximal tibialhalf, wasblock harvested, including bone callus formation area, and Mg-1Ca implant included insidethe bone piece, directly after sacrifice and fixated in 4% formaldehyde for postmortem histological analysis. We aimed to highlight the formation of bone callus in the fracture site, to highlight the cell viability, the morphology of the bone cells in the immediate vicinity of the Mg-1Ca alloy implant and those from the bone callus. The slides were analyzed using a Niko Eclipse E200 optical microscope, connected to a computer for the extraction and analysis of the obtained images. Bone tissue samples were decalcified in 10% EDTA solution. After decalcification, 4 µm thick sections were fixed in paraffin blocks, from which microtomes were cut. The sections were fixed on examination blades and colored. The staining method was Hematoxylin-Eosin, Van Gieson and Trichrome Masson. We also harvested tissue samples for histological examination from vital organs such as lungs, kidney and liver due to the suspicion of gas embolization in the vital organs and the influence of the resorption process of the Mg-1Ca alloy on the function of these organs, the same that I assessed through the laboratory tests. The slides were analyzed using the same type of microscope as before, and the staining method was Hematoxylin-Eosin.

RESULTS

The radiological follow-up after surgery in shown in Figure 9.

Figure 9: ATT fracture group, Mg-1Ca implant marked with no 1, fracture site marked with no 2.

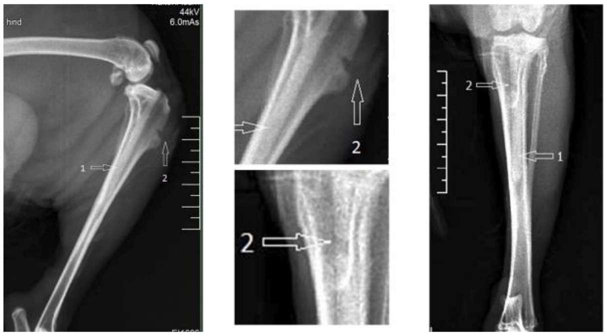

Figure 10 shows the accidental fracture of the tibial diaphysis that occurred during the introduction of Mg alloy implant. Figure 11 shows the control specimen, austenitic steel plate and screws osteosynthesis, marked with no 1.

Figure 10: Accidental fracture

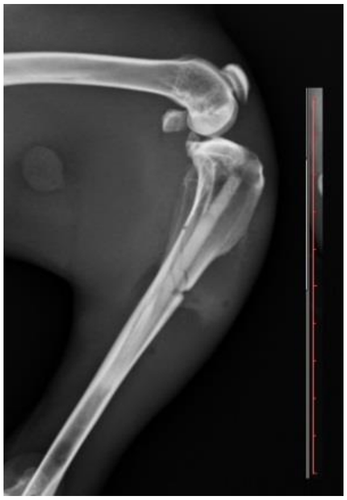

Figure 11: Control specimen

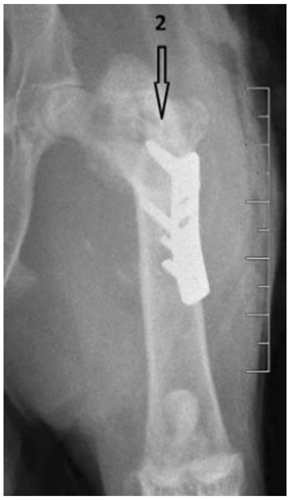

Six weeks after surgery

The situation of the experimental fracture at 6 weeks is presented in Figure 12. The fracture is healed, bone callus is noted with no 2. The implant from the Mg-1Ca alloy, Figure 1, is partially resorbed, especially in the metaphyseal area, but the shape is still preserved. The gas bubbles, no 3, are present in a small quantity, especially at the joint level. In the area adjacent to the implant, in contact with its surface, opaque areas, no 4, are observed, signs of mineralization. Figure 13 shows the accidental fracture, healed with a large callus formation.

Figure 12: ATT fracture

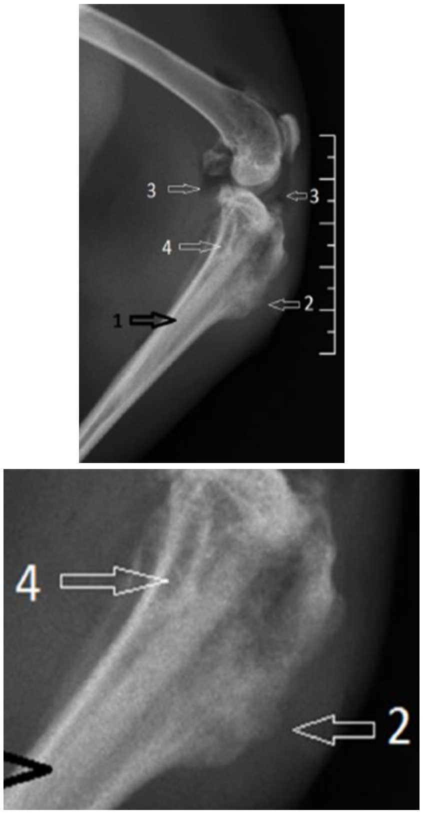

Figure 13: Accidental fracture

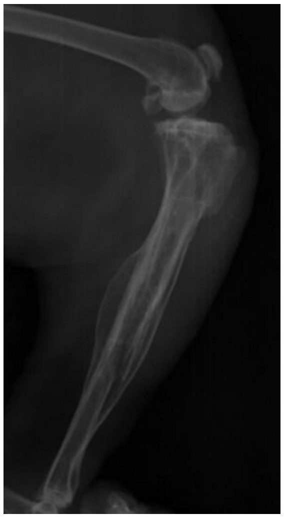

Histological results

Callus formation

Figure 14 shows a section from ATT callus, Hematoxylin-Eozine dye, magnitude 10X10. Bone tissue with nonuniform disposition, viable osteoblasts, with present intra-cytoplasmic nucleus and cartilaginous areas with chondroblasts with intracytoplasmic visible nuclei are observed, specific to bone callus formation. Figure 15 shows an overview of bone callus, Hematoxylin-Eosin dye, magnitude 4X10, from the accidental fracture callus site. Bone areas are irregularly arranged, alternating with cartilaginous areas, image of bone remodeling, specific to bone callus such as in Figure 14. Figure 16, is a detailed image of bone callus remodeling process, Hematoxylin-Eosin dye, magnitude 40X10, digital zoom. Multinucleate osteoclasts are visible, no 1, surrounded by bone tissue, with viable osteoblasts, intracellular nucleus visible, no 2. Figure 17 shows bone callus from the control specimen, Hematoxylin Eosin dye, 4X magnification. Bone remodeling areas are identified, being visible osteoclasts surrounded by newly formed bone tissue, with viable osteoblasts, similar to the previously presented samples.

Figure 14: ATT fracture

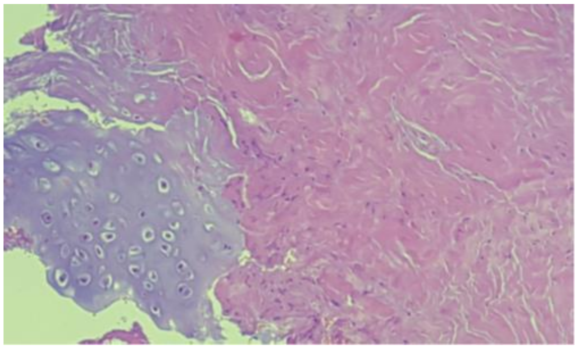

Figure 15: Accidental fracture callus

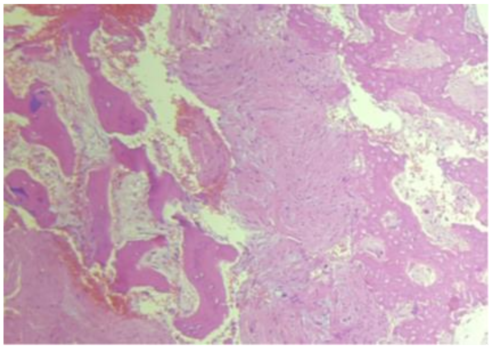

Figure 16: Bone remodeling

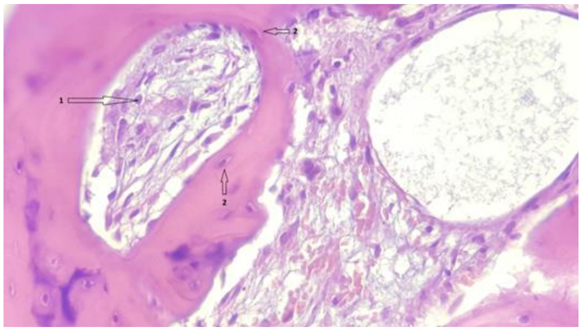

Figure 17: Control specimen callus

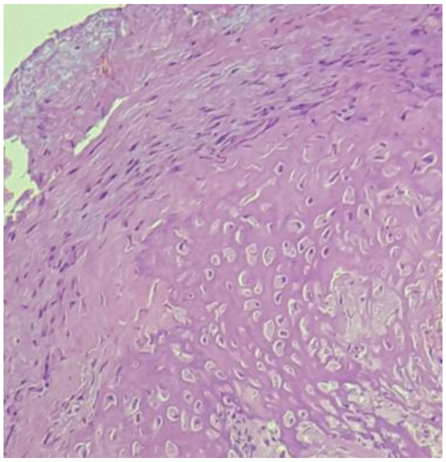

Bone alloy interface, it is showed in Figure 18, Tricrome Masson dye, magnitute 10x40. In this section we highlight viable bone cells, with intracytoplasmic nucleus, number 1 and collagen fibers, colored in blue, noted with no 2, arranged both pericellularly and in bone mass, sign of osteoblastic activity.The Mg-1Ca alloy, noted with no 4, which is in the process of resorption, is directly adherent to the bone surface, without identifying interposing tissues at the bone-alloy interface, no 5. In the immediate vicinity of the implant, the bone tissue is disposed in parallel lines, no 3, remineralization sign, bone formation.

Figure 18: Bone tissue and Mg-1Ca alloy, 6 weeks, Tricrome Masson dye

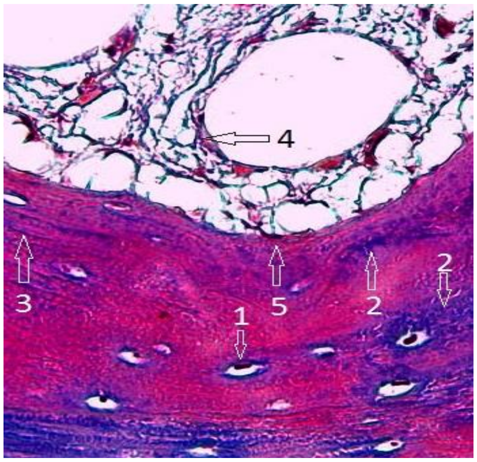

Vital organs

Figure 19 shows a section through the lung tissue. The alveoli appear in the form of empty spaces, marked with number 1. No intra-alveolar secretions or alveolar collabs are observed. Interstitial spaces, marked with no 2, have a normal appearance, without interstitial infiltration. A bronchioleis marked with no 3, with normal appearance and structure, and blood vessels are identified with no 4. No signs of gaseous embolism are observed.

Figure 20 shows a section through the renal tissue. It looks normal, with no pathological signs. The glomeruli of normal appearance and structure are marked with number 1, and with number 2 the collecting channels.

Figure 21 shows a section through the liver tissue. Liver cells, marked with number 1 have a viable appearance, with a visible nucleus inside. These are placed in columns delimiting the biliary drainage channels, marked with no 2. The tissue, does not have pathological aspects.

Figure 19: Lung, 6 weeks

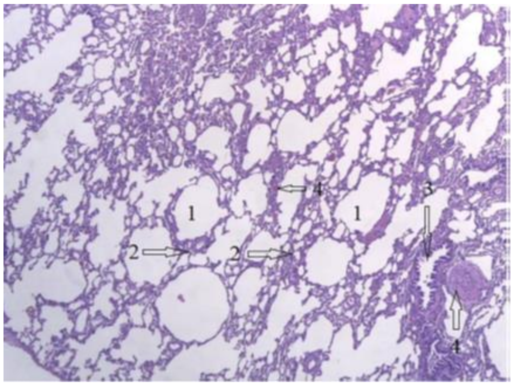

Figure 20: Kidney, 6 weeks

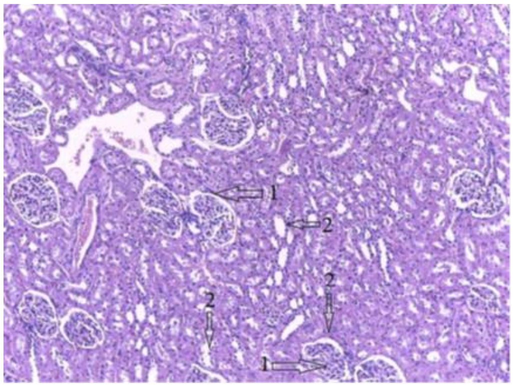

Figure 21: Liver, 6 weeks

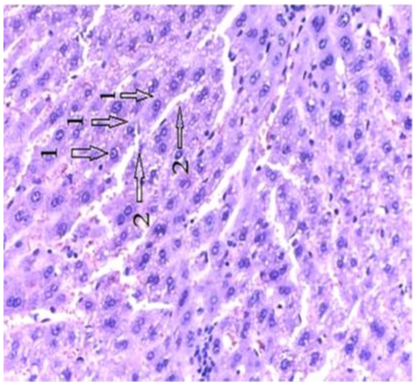

Blood tests results

The values obtained by monitoring the physiological blood parameters, preoperatively and at 6 weeks, are summarized in the following Table and Figures 22-25.

Table 3: Ionogram evolution

No	Magnesium (mg/dL)		Calcium (mg/dL)		Sideremia (µg/dL)		Sodium (mmol/L)		Potassium (mmol/L)	
	Preop	6 week	Preop	6 week	Preop	6 week	Preop	6 week	Preop	6 week
1	2.41	2.72	14.75	13.75	185.51	180.2	146.31	151.8	7	3.43
2	2.99	2.2	13.78	16.8	109.86	175.2	150	151.2	4.24	4.47
3	2.55	2.67	18.41	17.49	111.41	120.8	147.95	150.7	6.13	5.89

Figure 22: Mg evolution

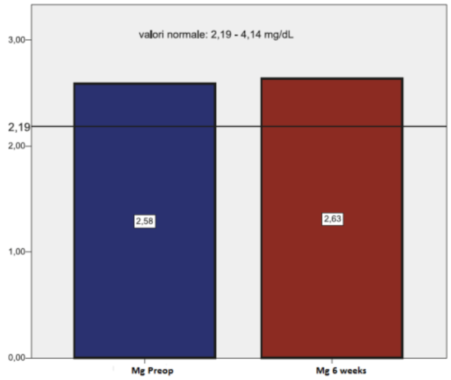

Figure 23: Ca evolution

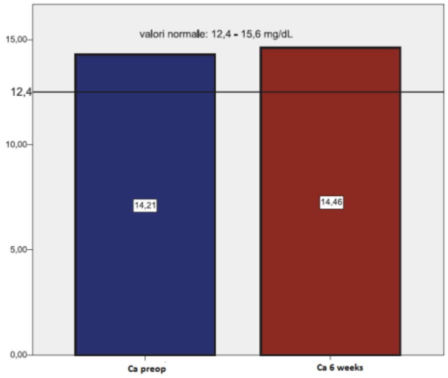

Figure 24: Na evolution

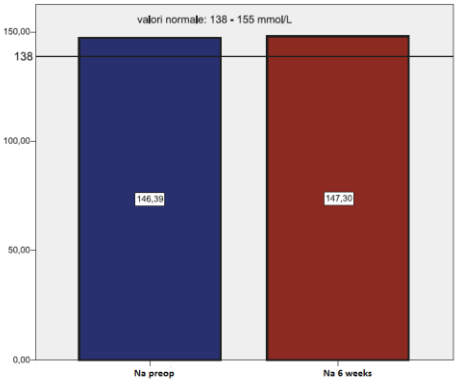

Figure 25: K evolution

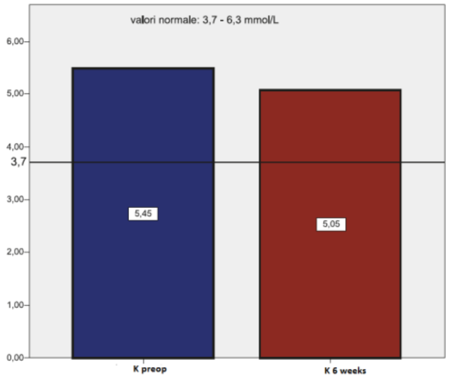

The studied parameters, magnesium, calcium, sodium and potassium, had registered average values preoperatively and at 6 weeks, within the range of normal, physiological values, characteristic to their species.

Urea has a slightly higher preoperative mean value than normal, 24.09 mg/dL compared to 23.50 mg/dL, but at 6 weeks we recorded an average value of 23.35 mg/dL, which is within the the normal, physiological limits of the species.

Creatinine has a preoperative mean value of 0.73 mg/dL and 0.74 mg/dL at 6 weeks, both values being within normal range.

Regarding the evolution of hepatic function indicators, both ALT and AST have preoperative average values and at 6 weeks, within the normal range, being from 25.8 IU to 23.2 IU for ALT and from 23, 3 IU to 24 IU.

Table 4: Vital organ function evolution

No	Serum urea (mg/dL)		Creatinine (mg/dL)		ALT (µg/dL)		AST (mmol/L)	
	Preop	6 week	Preop	6 week	Preop	6 week	Preop	6 week
1	16.7	17.2	0.56	0.59	21	23	22	23
2	28.6	27.5	0.69	0.72	18	19	27	25
3	33	16.1	0.53	0.7	17	23	25	26

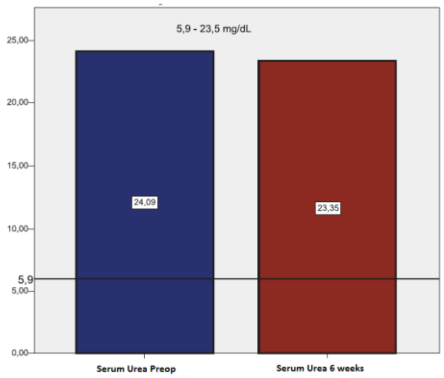
Figure 26: Serum urea evolution

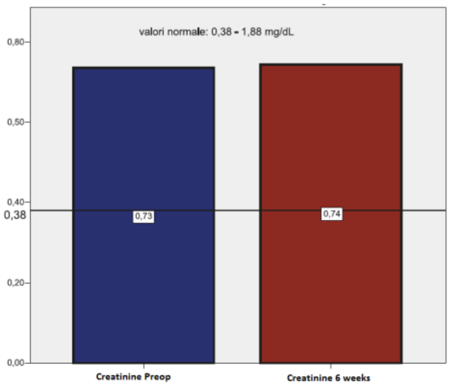
Figure 27: Creatinine evolution

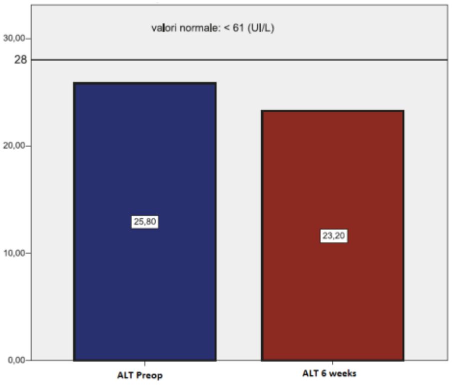
Figure 28: ALT evolution

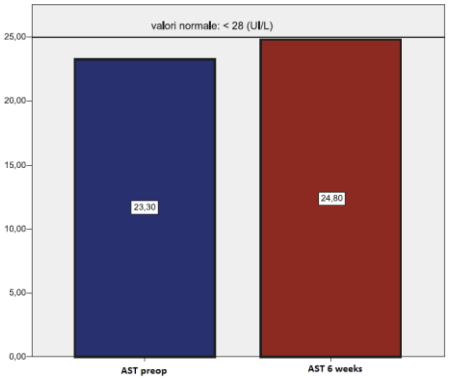
Figure 29: AST evolution

DISCUSSIONS

In vivo studies represent a superior stage of research required to certify the results obtained through in vitro studies. The present study, in vivo, performed on laboratory animals, rabbits, aimed at evaluating the biocompatibility of a Mg-1Ca binary alloy by evaluating the influence on the formation of bone callus, cell viability and systemic effects, impairing the function of vital organs.For this, we radiologically followed the evolution of the implant and the adjacent bone, we performed blood tests and histological sections

In the radiological images obtained, we notice callus formation both, at the level of the experimental fracture and in accidental fracture, at6 weeks after surgery. No radiologically transparent lines were identified at the bone implant interface, suggesting that there is no local osteolytic process or development of fibro-cartilaginous tissue at the bone implant interface. This observation suggests that the biodegradation process of the Mg-1Ca alloy does not adversely interfere with the biological process of bone tissue formation.

Histological examination is an important element in determining the biocompatibility of the Mg-1Ca alloy. Sample collection was performed at 6 weeks postoperatively.

The histological sections made from the samples collected from the fracture zone adjacent to the Mg-1Ca alloy implant, confirm the formation of the bony callus, showing areas of endochondral ossification, newly formed bone with disordered disposition, areas of bone remodeling, in which many osteoclasts and osteoblasts are identified. We can notice a cellularity specific to the formation of a normal bone callus, with no tissue necrosis. The trichrome Masson sections, shows a normal bone development with newly formed bone tissue at the alloy interface.

In the sections harvested from the vital organs, no pathological changes are observed.

This observations suggests that the biodegradation process of the Mg-1Ca alloy does not interfere negatively with the biological process of bone formation, allowing the formation of the bone callus in the fracture site, in a normal time period.

The study of blood parameters plays an important role in evaluating the systemic effects of the corrosion process of the Mg-1Ca alloy. Magnesium and calcium release from the alloy during the corrosion process did not lead to pathological changes in the serum values of the two ions. At the same time, we do not encounter pathological changes in serum sodium and potassium, ions with a very important physiological role.

Renal function is not influenced during the corrosion process, which is supported by the evolution of urea and creatinine values. Similarly, the liver function was normal, during the study, the ALT and AST transaminases recording average values within normal limits, at both evaluation times.

These data do not confirm the suspicion of pathological changes induced by systemically released corrosion products and gas embolism in vital organs.

The shape of the implant (pin) is basic, but reproducible; 3D printing of a patient-specific bioresorbable implant may be an option, as in modern knee surgery [16].

CONCLUSIONS

Thus, as a final conclusion, I can say that the Mg-1Ca alloy has been proven to be:

- Safe, with a high degree of biocompatibility
- It shows good capacity for osteointegration and osteo-conductivity
- With no local toxic effects, no impairment with bone callus formation
- No systemic toxic effects, no impairment with vital organs function
- A promising future solution for orthopedic surgery.

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ORIGINAL ARTICLES

Military psychological distress-virtues model

Azizeh Alizadeh[1], **Seyed H. Salimi**[2]

Abstract: *Background and Aim: Military personnel, parallel to appropriate personality traits to the military environment, need virtues and character strengths(CS) that contain values appropriate to the environment. We designed a model that examines the impact of comprehensive virtues and CS in reducing psychological distress.*

Methods: The present study is an SEM study. The sample community is an available example of 410 persons of military men in Tehran. We used a demographic information questionnaire, Kessler Psychological Distress Scale (K-10), and Virtues Inventory in Action (VIA-120).

Results: findings showed virtues and CS of wisdom, justice, humanity, and transcendence (except for spirituality) are significantly related to psychological distress. With increasing CS, psychological distress in employees decreased, but with increasing virtues, psychological distress in employees increased. The virtues and CS of temperance and courage were not significantly associated with psychological distress.

Conclusions: According to the results of this study, military services should pay more attention to the virtues and CS effective in reducing staff distress and strengthening these traits in them.

Keywords: *Military personnel, Psychological distress, Virtues, Character Strengths*

INTRODUCTION

Psychological distress is a mental health outcome embodied by psycho-physiological and behavioral symptoms that are not specific to a given mental pathology. It includes symptoms of anxiety, depressive reactions, irritability, declining intellectual capacity, tiredness, sleepiness, and may lead to premature morbidity and mortality and Low-efficiency organizations [1]. A review of texts related to the psychological distress in military personnel showed that studies conducted in this area are more focused on personality traits and behavior. Such as hardiness and adjustment [2], sleep, resilience [3], perceived support, substance use, suicidal ideation [4], self-compassion and morale injury [5], negative life events [6], and like that. In military psychology, analytical and practical intelligence, motivational factors, and personality (for example that low conscientiousness and high extraversion in the big 5-personality model) are considered a predictor of the outcomes of performance in military colleges. Personality is about differences between individuals and how one reacts to circumstances, but these characters do not address values and have limitations in predicting successful military personnel [7]. So, other variables have been incorporated into the network of predictive variables because of theoretical advances in military psychology and the evolution of the institutional function of the military [8]. One of that is positive military psychology with the assumption that the military being a positive institution consists of

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pathology-free mentally and physically fit soldiers who share positive traits, positive emotions, and positive relations. Therefore, it is imperative to identify, nurture and optimize all those positive aspects of a soldier which can strengthen him for better operational effectiveness, adjustment, and optimal functioning [9]. According to positive military psychology, Virtues and strengths can be more suitable predictors for successful military personnel and institution [7]. Military doctrine has affirmed that character and values are critical for successful military environments [10]. An individual expresses his or her values through one's CS. This has been found to play an important role in leadership, adaptability, and achievement [11]. Therefore, CS is an important factor for being resilient applicants to high-risk organizations [12].

A soldier must not only have the courage and aggressiveness for combat operations but also cultivate the empathy and compassion necessary to treat noncombatants and enemy prisoners humanely [13]. In addition, virtue ethics as being the best way of enhancing the chances of soldiers behaving morally because the rules or codes of conduct imposed from above, lack flexibility, and leave less room for personal integrity. changes in the military’s environment have led to a shift from traditional tasks to more complex tasks, and especially in today’s missions, one could expect that the proper virtues are not solely the martial ones [14].

Gordon Alport’s theory argued that character belonged to philosophy and not psychology, and denied any connection between the character's strengths and the personality concept. Later, Peterson and Seligman´s research on positive psychology from 2004 sparked a renewed interest in the concepts of character and CS (15). Peterson and Seligman formulated a taxonomy of 24 “CS within six different virtues” based on Confucian, Taoist, Buddhist, Hindu, Greek, Judeo-Christian, and Muslim writings concerning virtue [16].

Wisdom and knowledge – cognitive CS that entail the acquisition and use of knowledge (creativity, curiosity, open-mindedness, love of learning, perspective), Courage - emotional CS that involve the exercise of will to accomplish goals in the face of opposition, external or internal (authenticity, bravery, persistence, zest), humanity – interpersonal CS that involve tending and befriending others (kindness, love, and social intelligence), justice – civic CS that underlie healthy community life (fairness, leadership, citizenship), temperance-CS that protect against excess (forgiveness, modesty, prudence, self-regulation) and Transcendence – CS that forge connections to the larger universe and provide meaning (appreciation of beauty, gratitude, hope, humor, spirituality) [17].

CS are personality traits that are positively valued, and represent different routes to the superordinate virtues [18]. The relationship between virtues and PTG in different countries demonstrated these three virtues (Interpersonal, Vitality, and Cautiousness) were positively related to life with satisfaction and flourishing, as well as negatively related to depression, anxiety, psychological distress, and pathology Internet use [19].

A review of the literature on virtues and CS showed that many studies have been done on the relationship between psychological well-being and virtues. We found no study to examine the role of a complete list of virtues and character strength in causing psychological distress. More subjective/ psychological well-being can be achieved by applying CS [20].

Studies explored a significant positive relationship between the three virtues (Interpersonal, Vitality, and Cautiousness) and life satisfaction [21] and psychological well-being and enhanced mental health among diverse populations in western and eastern cultures. In a study, CS use is a unique predictor of subjective well-being, but not the health-related quality of life. The VIA strengths of hope and zest were significant positive predictors of life satisfaction [22].

A Structural equation modeling study that specifies the predictive effects of some CS (Seven CS of critical thinking, emotional intelligence, gratitude, forgiveness, hope, spirituality, and zest) in a depression on individuals with major depression, demonstrated an indirect effect on depression through the mediation of dysfunctional attitudes, negative affect, and happiness. CS had negative effects on dysfunctional attitudes and positive effects on happiness [23].

According to Leontopoulou & Triliva (2012), Wisdom, courage, and transcendence were each highly and positively correlated with the wellbeing subscales of environmental mastery, purpose in life, and self-acceptance. Good physical and mental health were positively predicted different aspects of wellbeing and CS; nevertheless, mental health was a stronger predictor of wellbeing and CS than physical health.

Positive psychology interventions that involve the enhancement of strengths are found to increase well-being and decrease depression effectively, as suggested by a meta-analysis of 51 studies (Sin and Lyubomirsky, 2009). Indeed, empirical findings suggest that having a good character through the use of strengths is inversely related to depression, suicidal ideation, and substance abuse, whilst the use of strengths can have a buffering effect against stress, reduce the disability caused by mental disorders and contribute to their prevention. Wright and Lopez (2005) proposed that strengths constitute important resources for mental health improvement and as such their enhancement should be incorporated into the health and well-being interventions [24].

Based on an international sample of 238 adults, general CS underuse and overuse were related to negative outcomes, while optimal use was related to positive outcomes. The overuse of social intelligence and modesty, and underuse of zest, humor, self-regulation, and social intelligence were associated with social anxiety. Using discriminant analysis, this combination successfully resorted 87.3% of the participants into those that do and do not have clinical levels of social anxiety. These findings suggest that strengths are multifaceted, providing novel insight into the role that sub-optimal-use facets play in undesirable outcomes, providing a glimpse of psychopathology through the lens of positive psychology [25].

Exploring psychological distress through the lens of virtues and CS allows for understanding individual differences and kinds of virtues and characteristics that help decrease psychological distress in people. As mentioned, we did not find a comprehensive study that examines the wide range of negative emotions (distress) based on all the virtues and strengths. Studies have focused more on the role of some of the strengths and ignored the role of the six virtues of Seligman and Patterson. The concept of psychological distress without any diagnostic label can help identify and assess people at risk for psychological problems. Therefore, the authors decided to examine the role of each of the virtues and characteristics in reducing psychological distress. The purpose of this study is to investigate the model of psychological distress based on virtues and CS.

METHOD

The present study is a cross-sectional study. The sample community is an example of all the military men in Tehran. Sample in the present study, the military organizations of Tehran have been selected by available sampling. The sample size in this study is 384 people, which has been done by available sampling based on the Cochran sample size formula:

$$n=\frac{Z^2\,pq}{d^2}$$

In this formula: z= 1.96; p = q = 0.5; and d (error value) = 0.05. To compensate for possible errors and increase the test power, the sample size was increased by 410 units [26].

Demographic information questionnaire: This questionnaire will be designed by the researcher and includes information such as age, education, work experience, marital status.

Kessler Psychological Distress Scale (K-10): The Kessler Psychological Distress Scale (2002), identifies mental disorders in the general population. Questions on this scale are scored as a Likert scale from always (0) to never (4). This scale doesn’t target a specific psychological disorder, but generally, identify the level of anxiety and depressive symptoms that a person has experienced over the past few weeks. Various studies have shown that the K-10 questionnaire has good validity and reliability. Studies on K-10 show a strong correlation between high scores on the Kessler Psychological Distress Questionnaire and the diagnosis of mood and anxiety disorders by the International Composite Diagnostic Interview Form (CIDI). There is also a less but significant relationship between questionnaire scores and other psychiatric disorders [27].

Virtues Inventory in Action (VIA-120): Seligman and Patterson prepared a short form of 120 that includes 6 virtues of wisdom, courage, humanity and love, justice, temperance, and transcendence. Each of the moral virtues contains several CS that is measured by 5 questions, and are complemented on a Likert scale from very similar to me (5) to opposite to me (1), and dimensionally each point Show the strength of character. The 120-item scale is fully consistent with the original English form VIA-240 in terms of reliability, validity, and factor structure [28]. The reliability and validity of this scale were assessed and confirmed by Namdari [29].

RESEARCH PROCESS

To implement the questionnaires, first, the necessary permits were obtained from the relevant centers and then the command of each unit was coordinated. Questionnaires were distributed among the staff and the necessary explanations were given on how to answer and confidentiality of the answers. After completing the answer, questionnaires were collected.

Result

Descriptive findings of demographic variables showed the mean and standard deviation of the age and work experience (years of work in the organization) of the participants, respectively are 36.72± 7.02 and 17.35±6.19. In addition, 20% were married and 30% were single. 32% had one child, 20% had two children and the rest had more than two children. Almost 14% had a diploma, 56% had a bachelor's degree, 20 % had a master's degree, and 10% had a doctorate. 60% worked in the morning shift, 30% in variable work shifts, and 10% in the evening shift.

Measurement Model

In measurement model a): Internal consistency (Cronbach's alpha and Composite reliability), convergent validity (Indicator reliability, Average Variance Extracted) (in Table 1), and discriminant validity were suitable. According to Table 1, all values CR and AVE are in the appropriate ranges. Discriminant validity showed that all variables had appropriate HTMT. Also, according to the Fornell-Larcker scale, each variable had more variance in common with its variable than the other. In Figure 1 the model of psychological distress of military personnel based on the virtues and CS is shown.

Table 1: Cronbach's alpha, Composite reliability and Average Variance Extracted for virtues and CS

Virtues (second order variables)	CS (First order variables)	Cronbach's alpha	CR	AVE
Wisdom	----		0.893	0.736
Wisdom	Creativity	0.618	0.796	0.566
	Curiosity	0.706	0.819	0.532
	Open-mindedness	0.700	0.816	0.529
	Love of learning	0.762	0.841	0.516
	Perspective	0.618	0.796	0.566
Courage	----		0.893	0.672
	Orginity	0.731	0.832	0.553
	Persistence	0.786	0.854	0.541
	Bravery	0.579	0.778	0.541
	Vitality	0.764	0.841	0.515
Humanity	----		0.900	0.695
	Kindness	0.719	0.826	0.543
	Love	0.629	0.777	0.563
	Social intelligence	0.728	0.831	0.55
Justice	----		0.901	0.770
	Fairness	0.615	0.795	0.564
	Leadership	0.515	0.755	0.507
	Teamwork	0.705	0.819	0.531
Temperance	----		0.893	0.736
	Forgiveness	0.742	0.836	0.561
	Modesty	0.534	0.763	0.517
	Prudence	0.716	0.824	0.54
	Self-regulation	0.401	0.765	0.621
Transcendence	----		0.955	0.782
	Appreciation of beauty	0.668	0.818	0.601
	Gratitude	0.753	0.835	
	Hope	0.769	0.839	0.511
	Humor	0.755	0.835	0.506
	Spirituality	0.821	0.873	0.579

Table 2 shows path coefficients of virtues and CS with the psychological distress of military personnel. According to this table, virtues, and CS of wisdom, justice, humanity, and transcendence (except for spirituality) are significantly related to psychological distress. With increasing CS, psychological distress in employees decreased, but with increasing virtues, psychological distress in employees increased. The virtues and CS of temperance and courage were not significantly associated with psychological distress.

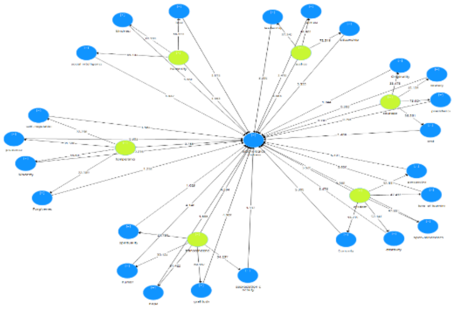

Figure1: Military psychological distress-virtues model

Table 2: The path coefficients of virtues and CS with the psychological distress

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	T Statistics (|O/STDEV|)	P Values
originality -> psychological distress	-0.287	-0.389	0.275	1.044	0.297
curiosity -> psychological distress	-5.938	-0.579	1.100	5.399	0.000
forgiveness -> psychological distress	-0.160	-0.136	0.127	1.258	0.209
modesty -> psychological distress	-0.028	0.014	0.128	0.216	0.829
appreciation of beauty -> psychological distress	-3.357	-0.812	0.774	4.337	0.000
bravery -> psychological distress	-0.091	-0.158	0.232	0.393	0.694
citizenship -> psychological distress	-5.834	-0.789	1.750	3.333	0.001
courage -> psychological distress	0.774	1.072	0.970	0.797	0.426
creativity -> psychological distress	-6.579	-0.796	1.201	5.476	0.000
fairness -> psychological distress	-4.621	-0.742	1.352	3.418	0.001
gratitude -> psychological distress	-5.333	-1.328	1.221	4.367	0.000
hope -> psychological distress	-5.516	-1.622	1.200	4.595	0.000
humanity -> psychological distress	10.598	2.516	3.123	3.393	0.001
humor -> psychological distress	-4.111	-0.885	0.991	4.148	0.000
justice -> psychological distress	12.501	1.852	3.717	3.363	0.001
kindness -> psychological distress	-3.981	-0.826	1.219	3.266	0.001
leadership -> psychological distress	-3.935	-0.527	1.193	3.299	0.001
love -> psychological distress	-3.887	-1.081	1.075	3.615	0.000
love of learning -> psychological distress	-7.009	-0.987	1.247	5.620	0.000
open-mindedness -> psychological distress	-6.131	-0.775	1.101	5.568	0.000
perspective -> psychological distress	-4.680	-0.527	0.866	5.401	0.000
persistence -> psychological distress	-0.268	-0.359	0.337	0.796	0.426
prudence -> psychological distress	-0.064	-0.011	0.163	0.393	0.695
self- regulation -> psychological distress	-0.170	-0.181	0.109	1.562	0.119
social intelligence -> psychological distress	-4.270	-0.979	1.276	3.347	0.001
spirituality -> psychological distress	-0.073	-0.069	0.071	1.029	0.304
temperance -> psychological distress	0.274	0.146	0.363	0.755	0.451
transcendence -> psychological distress	14.920	3.529	3.483	4.284	0.000
wisdom -> psychological distress	25.036	3.165	4.535	5.520	0.000
zest -> psychological distress	-0.421	-0.531	0.283	1.489	0.137

The most common measure used to evaluate the structural model is value R2 and Stone-Geisser's Q^2. The value of R2 equal to 0.75, 0.50, or 0.25 for the endogenous latent variables can be described as a general rule as strong, moderate, and weak, respectively. For Stone-Geisser's Q^2 if the value of Q2 for an endogenous structure achieves three values of 0.02, 0.15, and 0.35, it indicates the weak, medium, and strong predictive power of the structure with its related exogenous structures [30]. Table 3 shows these values for virtues and CS.

Table 3: Values of R^2 and Q^2 for virtues and CS

Virtues (second-order variables)	CS (First order variables)	R Square	R Square Adjusted	Q^2
Wisdom	----	0.78	0.78	0.27
	Creativity	0.74	0.74	0.34
	Curiosity	0.67	0.67	0.39
	Open-mindedness	0.67	0.67	0.33
	Love of learning	0.66	0.66	0.31
	Perspective	0.59	0.59	0.32
Courage	----	0.84	0.84	0.33
	Originality	0.65	0.65	0.38
	Persistence	0.77	0.77	0.35
	Bravery	0.68	0.68	0.36
	Zest	0.67	0.67	0.33
Humanity	----	0.79	0.79	0.31
	Kindness	0.78	0.78	0.39
	Love	0.73	0.73	0.35
	Social intelligence	0.77	0.77	0.39
Justice	----	0.76	0.76	0.26
	Fairness	0.72	0.72	0.39
	Leadership	0.64	0.64	0.31
	Citizenship	0.83	0.83	0.41
Temperance	----	0.74	0.74	0.23
	Forgiveness	0.62	0.62	0.27
	Modesty	0.63	0.63	0.31
	Prudence	0.58	0.58	0.30
	Self-regulation	0.48	0.48	0.28
Transcendence	----	0.85	0.85	0.29
	Appreciation of beauty	0.59	0.59	0.34
	Gratitude	0.77	0.77	0.36
	Hope	0.76	0.76	0.36
	Humor	0.58	0.58	0.26
	Spirituality	0.47	0.47	0.24

DISCUSSION

Findings of this study showed, the CS of wisdom, including, creativity, curiosity, love of learning, open-mindedness, perspective, played a mediating role in reducing psychological distress. The research literature is consistent with our findings. Creativity led to looking at problems from different perspectives, establishing new solutions and connections, flexibility in the thought process, increasing their connection to the unknown [31], brainstorming phenomenon, and reducing workplace stresses [32]. Studies show that curiosity and open-mindedness with the tendency to tolerate potentially different opinions [33], new knowledge and information, the choice of diverse and different perspectives, the ability to use a balanced and objective mentality when participating in problem-solving [32]. Open-minded people think logically because they can accept all possible logical perspectives and come up with a better solution [34]. Curiosity with openness to the experience is associated with self-regulation, cognitive development, school and university learning, job performance, interpersonal communication, personal growth, and well-being [35].

In this study, justice's CS, including citizenship, fairness, and leadership had a significant role in reducing distress. "good citizens" have higher levels of social trust and a more positive view of human nature, score low on alienation and ethnicity [36], organizational citizenship behavior is likely to be formally associated with organizational rewards and helps the organization promote a positive social and psychological climate. Beyond their duties, employees find it more attractive to engage and inform about the organization, to talk and encourage others, to volunteer to take on additional responsibilities in the workplace, with the help and guidance of co-workers [37]. Also, supervisor-nurse relationships, teamwork, and wellbeing explain almost half of the nurses' commitment to the hospital, as well as half of the intention to leave the hospital [38]. About fairness, people with higher fairness in career advancement show more parental role, respect, and commitment to others, care, and understanding in relationships [36]. Employees' perceptions of fairness lead to more positive variables such as job satisfaction, organizational commitment, productivity, and organizational citizenship behavior, work results, or attitudes and work behaviors [39]. Also, according to Seligman and Peterson (2004) people with leadership power are more likely to be promoted in their jobs and feel more well-being [36].

In addition, findings of this study indicated a significant role of transcendence CS (except spirituality) including appreciation of beauty, hope, humor, and gratitude with psychological distress. The use of these CS led to reducing psychological distress. This finding is consistent with the research literature. Research has shown that appreciation of beauty is positively correlated with openness to experience, extraversion, and agreement [40], life satisfaction, positive impact, and post-traumatic growth [41].

Depression was negative and gratitude was a positive predictor of psychological well-being [42], and negatively related to several negative emotional [43]. Also, expressed higher mood gratitude with fewer depressive symptoms, and PTSD. About humor, higher levels of humor were associated with fewer depressive symptoms [44] low anxiety and depression, and anti-production behaviors in the workplace, and increased organizational citizenship behaviors, moderating role between organizational stressors and organizational citizenship behaviors. The use of humor leads to mental flexibility and intellectual stimulation and reduces the threat by restoring the situation against the destructive effects of stressors [45]. Our findings showed spirituality had not been significantly associated with psychological distress. Spirituality is positively associated with happiness and meaningful living. Many studies have shown that spirituality and religiosity are more closely linked to greater physical and mental health. For example, a meta-analysis concluded that more religiosity was associated with fewer depressive symptoms, with no evidence that age, gender, or ethnicity influenced the relationship. According to Einsliecht et al., belief in religion modulates anxiety [8].

Also, our study showed there is a positive and significant relationship between CS of love and humanity including kindness, love, and social intelligence, and psychological distress. This result is consistent with the literature. In the study of the effect of parental attachment and the language of love on resilience, it was found that secure attachment and receiving love from parents lead to more resilience. Parents' loving language, affirmative words, and supportive actions significantly build resilience by creating emotional, motivational, and practical resources [46]. The results of a study showed that spiritual care with the faith-hope-love model has had positive results for the patient [47]. Also confirmed that there is a significant relationship between health and love and faith. Multidisciplinary research evidence argues that different religions have one thing in common, at least in terms of one main message: that life means loving one another. The definition of love, in its essence, is an expression of generosity and altruism. Likewise, love can influence not only positive psychology and existentialism but also the fanatical and fundamentalist views and practices that still exist in the world today [48]. Regarding social intelligence, the results of a study showed that social intelligence can protect young people from the psychological damage of being victimized by peers [49]. Findings [50] showed that there is a significant relationship between social intelligence and depression. Also, a significant relationship was found between social skills and social awareness, but no statistically significant relationship was observed between social information processing and the level of depression. Also, important relationships between social desirability and social intelligence in smoking status have been found [51].

Other CS in the virtues of courage (courage, originality, perseverance, and vitality) and temperance (forgiveness, modesty, prudence, and self-regulation) were not significantly associated with psychological distress. The results of the most relevant research showed that each of these positive characteristics can affect psychological well-being and positive emotions and results. Research in these studies has shown, for example, that high self-control leads to thought control, emotion regulation, psychological well-being, greater academic achievement, better interpersonal relationships, and ultimately greater adaptation and low self-control, in contrast to many social problems, obesity, and Drug use, crime, shopping, and procrastination [52]. The constructive relationship between forgiveness and health through direct mechanisms may be accomplished by controlling the cognitive process of rumination, and the resulting negative emotions, such as anger or resentment or indirect effect, may be mediated through distinct variables such as health behavior, performance between Individuals develop social support and mental health and are associated with low suicidal behavior and depressive symptoms [53]. We reason that the virtues of temperance and courage and the CS of them, can logically and intuitively be incorporated into the nature of the military job and be considered as an inseparable feature of this job. Therefore, increasing it cannot affect reducing psychological distress.

In the present study, all six virtues (significant including wisdom, justice, and transcendence) and non-significant (humanity, courage, and temperance) were positively associated with psychological distress. That is, with the increase of these virtues, the level of psychological distress of the employees increased. The positive association of virtues with psychological distress is a strange consequence, given that according to Seligman and Peterson, virtues lead to well-being and ultimately self-fulfillment [36]. Based on our hypotheses, the relationship between virtues and psychological distress was expected to be negative. According to these findings, while the results of the first-order variables of CS were negative with psychological distress, but the increase in the virtues of wisdom, humanity, justice, and excellence in the sample led to more distress and suffering. According to Rosch et al. (1976), CS has a "natural classification" that manifests itself in practice conveniently and clearly. Experimental studies support this idea and show that cognitive processes also have a more specific efficiency when they have a primary and natural surface content. While CS at the abstract levels is called virtues and their performance is more complex [36]. According to early texts in this field, if we consider distress or suffering as opposed to happiness, any person who has a virtuous life will be happy. But according to Bloomfield, virtue does not guarantee happiness, and the ethics of virtue involves worries about oneself and others [54]. Suffering is included in the theoretical framework of virtue and is an inseparable part of virtue. Virtues have the appropriate structure to maintain different types of values. The connection between suffering and virtue already exists in the history of philosophy and theology, albeit sporadically. Suffering plays a key role in the proper functioning of groups or groups and their wisdom. In addition, suffering is very important in a group or social virtues such as justice and love and individual virtues such as faith [28].

Limitation

Our sample in this study included only men. These people mostly had administrative work and were somewhat away from the hardships of military life, such as being sent to war zones. Of course, they experience some limitations and difficulties of working in military services.

CONCLUSION

According to the results of this study, military organizations should pay more attention to the ethical virtues effective in reducing staff distress and strengthening these traits in individuals.

Ethical considerations

This article is a part of a doctoral dissertation approved with IR.BMSU.REC.1399.341 Ethics Code. The online version of the application is available at the following address and is open to the public: https://ethics.research.ac.ir/IR.BMSU.REC.1399.341. Target individuals were informed about the purpose of the study and their right to withdraw from the study at any point. Participants were free to withdraw from the study at any point. All interviews were kept confidential.

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Modern methods to differentiate benign thyroid nodules from malignant ones

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Abstract: *Objective: The purpose of this study is to compare the capsular structure appearing in follicular adenoma and papillary thyroid carcinoma to differentiate the benign and malignant thyroid nodules.*

Methods: Second Harmonic Generation (SHG) Microscopy was used to image collagen distribution in the capsules of several types of nodules. The tissue fragments were formalin-fixed, paraffin-embedded but without H&E staining, with 4-5 microns thick sections. From the same sample, two consecutive sections were made. The first was colored with H&E and capsule images were acquired with an optical microscope. The second was not colored with H&E. On the uncolored sample, type I collagen from the capsule was visualized by SHG microscopy. In addition, a parameter was used to study the orientation of collagen fibers, obtained from the Fast Fourier Transform (FFT) analysis of 2D-images.

Results: SHG microscopy images were acquired to assess the collagen organization of tumoral capsular thyroid nodules previously diagnosed as benign or malignant by conventional H&E staining. Different degree of collagen fibers orientation was observed from the two kinds of capsules and quantified using FFT analysis.

Conclusion: These above described microscopy method can be used to distinguish between benign and malignant thyroid nodules, based on different degree of the capsular collagen fibers orientation.

Keywords*: follicular adenoma, papillary thyroid carcinoma, benign and malignant thyroid nodules, capsular collagen fibers*

INTRODUCTION

The clinical and imagistic diagnosis of malignancy must be confirmed in pathology departments.

Although histopathology and cytopathology are the main tools for diagnosing cancer, there are some dificult cases that pathologists may encounter during routine diagnosis procedures, where the differentiation between a benign tumor proliferations from a malignant one is very difficult to achieve. In such situations complementary examination procedures are required (eg. serial sections from formalin-fixed, paraffin-embedded tissue blocks, mounted on glass slides and usual stained with H&E, immunohistochemistry, molecular studies, electron microscopy, etc.).

Another method which can be used in thyroid pathology for diagnostic purposes is Second Harmonic Generation (SHG) Microscopy.

SHG microscopy is a label-free laser scanning

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microscopy technique that exploits a nonlinear optical effect – the generation of the second harmonic in samples illuminated by an intense laser field [1, 2]. SHG can occur only from materials that do not have a center of symmetry, thus analyzing absolute molecular structure in organized organic environments, such as collagen fibrils, microtubules and the myosin chain of muscle cells. SHG microscopy has been proven to be a useful tool in biomedical tissue imaging [2] because it can be used for imaging endogenous structures like type I collagen [1] as well as for the characterization of collagen organization [3-5] and has been recognized as a promising approach for the diagnostics of different cancer types [6-8], by detecting collagen structure modifications [7].

The thyroid gland is wrapped in a thin fibrous capsule, from which originate numerous fibrous septae that penetrate the thyroid parenchyma and divide it into incomplete lobules (the so-called thyromeres). Macroscopically, the thyroid capsule seems complete, but it is incomplete from the microscopic point of view, as it adheres tightly to adjacent structures (laryngeal thyroid cartilage and trachea) [9].

Nodular enlargement of the thyroid gland comprises those situations in which one or more nodules are present. This presence of nodular enlargement is the most common in the case of non-toxic nodules, also known as adenomatous goiter or colloidal goiter.

Polinodular goiter with different-sized follicles can be a clinical manifestation of a wide range of different pathologies and not the expression of a single disease [10].

The usual classification of neoplasms of follicular epithelial cells are in benign (adenomas) and malignant (carcinomas) tumor proliferation. The benign ones are called follicular adenomas [11], which are encapsulated, non-invasive tumors showing evidence of thyroid follicular cell proliferation without the specific nuclear characteristics of thyroid papillary carcinoma [12].

Papillary Thyroid Carcinoma (PTC) is a malignant epithelial tumor, usually invasive, showing evidence of abnormal proliferation of thyroid follicular cells with characteristic nuclear changes: irregular contours and nuclear pseudoinclusions, nuclear membrane irregularities, and chromatin marginalization. For a correct diagnosis of the classical PTC, the papillary fibrovascular axes, the invasive character or the characteristic cytological features are required. [12].

The encapsulated variant of PTC is a type of proliferation with typical cytological and architectural changes, entirely surrounded by a thin, fibrous capsule that may be intact or infiltrated only focally by the tumor. If the capsule is invaded by the proliferation of malignant tumors, we are dealing with an aggressive form of PTC.

The encapsulated variant represents about 10% of all cases of PTC and has an excellent prognosis. Differential diagnosis is made with follicular adenoma with papillary hyperplasia [12].

Over the years, diagnostic criteria for follicular and papillary thyroid carcinomas have changed, but the two names have been retained. There have been several variants of papillary carcinoma for which the primary diagnostic criterion is based on nuclear features [11].

Some thyroid encapsulated neoplasms may present diagnostic difficulties due to the fact that only nuclear changes are sufficient to justify and diagnose PTC but there is the uncertainty about the capsular or vascular invasion. Encapsulated follicular tumors lacking the PTC nuclear characteristics are difficult to differentiate. For example, follicular carcinoma (a malignant tumor) can be distinguished from follicular adenoma (a benign tumor) only by demonstrating invasive character in the nodule capsule or capsular vessels [12].

We started from the idea that SHG can be used to observe three-dimensional structures of a tissue sample without the need for staining.

In this way, this method helps us in the correct diagnosis of encapsulated tumor entities that require complementary examinations from the pathologist or in order to determine a possible capsular structural change before the onset of malignant transformation of a benign encapsulated tumor.

OBJECTIVE

The purpose of this study was to use the collagen structure in the nodule capsule to obtain information on the follicular adenoma capsule and encapsulated papillary thyroid carcinoma (PTC) capsule for differentiating between benign and malignant thyroid nodules. The study was based on optical microscopy images and SHG microscopy, with the latter used for the visualization of type I collagen. We also show that the FFT analysis of the SHG images can provide information about the collagen fiber orientation.

MATERIALS AND METHODS

A batch composed of encapsulated-PTC and follicular adenoma tissue from 24 sections tissue samples (12 for Encapsulated-PTC capsules and 12 for follicular adenoma capsules) was used for this study. Sections of 4-5 µm were made from each paraffin tissue block. They were mounted on glass blades and stained with Hematoxylin and Eosin (H&E) and visualized with a light field microscope (Leica DM3000). The tissue architecture of the normal, benign and malignant regions of interest and the capsule surrounding the nodules have been identified. Unstained pairs of the H&E stained tissue sections were used for SHG imaging for collagen organization assessment.

SHG imaging was performed using a Leica TCS-SP confocal laser scanning microscope modified for nonlinear optical imaging [13]. We have used a mode-locked Ti:Sapphire laser operating at 780 nm and with ~150 fs pulses. A 40X, 0.75 numerical aperture objective was used to focus the laser beam on the sample, while the SHG signals were collected in the forward direction through a condenser lens. The average power reaching the sample plane was kept less than 15 mW and no photodamage was observed on the samples.

SHG images were used to estimate the collagen fiber orientation by FFT analysis using ImageJ software. For an image containing aligned fibers, we expect an FFT image with higher values along the direction orthogonal to the direction of the fibers and with an elliptic intensity plot. On the other hand, for an image with randomly oriented fibers, the FFT image intensity plot should be close to a circle. The FFT power spectra images were first binarized and then fitted with an ellipse. A collagen orientation index was calculated based on the length of the minor (S) and major (L) axes of the ellipse: $N = 1 - S/L$ [14]. Collagen orientation can thus be represented by an index ranging from 0 (random fibers) to 1 (aligned fibers).

RESULTS

Optical microscopy images of H & E stained sections do not give us information about the ultrastructure of the studied tumoral nodules capsule. The capsule appears as a fibrous structure surrounding the tumoral nodule. It looks identical to the follicular adenoma nodule and to the Encapsulated-PTC nodule. So this optic method is limited in terms of capsule study.

On the other side, the SHG helps to study the ultrastructural changes of the capsule. SHG highlights type I collagen in the capsule structure. So we can see if there are qualitative changes of this type of collagen from the two kinds of capsules. In addition, using FFT analysis of SHG images we can bring precious differentiation information of the two types of capsules: the follicular adenoma nodule or the Encapsulated-PTC nodule. We have noticed that in the Encapsulated-PTC nodule capsule there appears to be a collagen fiber organization as if they were trying to isolate malignant epithelial proliferation.

We acquired images with Second Harmonic Generation Microscopy which were used to assess the collagen orientation of tumoral capsular thyroid nodules previously diagnosed as benign or malignant by conventional H&E staining.

We compute the FFT spectra for the SHG images acquired for the considered pathologies. One can see that the average collagen orientation (N) computed from the FFT spectrum is higher for the follicular adenoma capsule ($N = 0.69$) than for the encapsulated-PTC capsule ($N = 0.4$). The FFT spectra were calculated for the entire images. We consider that if the spectra are computed for smaller regions of interest (ROIs), a distribution of values can be obtained, depending on the degree of alignment for the collagen fibers in the considered ROI. Averaging over a larger area, which is also the case of the whole area analysis, it results in a lower N. Even though N was calculated on whole areas, the obtained results show a difference in collagen organization between the two capsules.

Figure 1. Follicular adenoma capsule

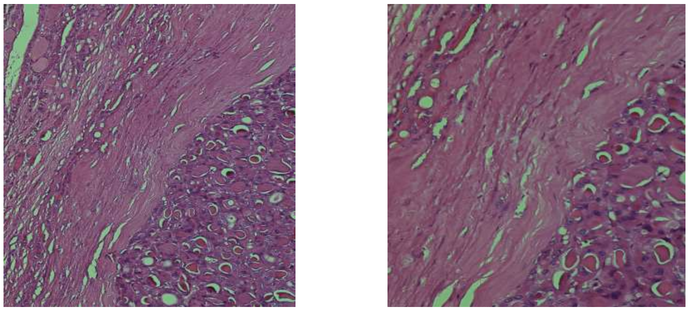

a). H&E 100x b). H&E 200x

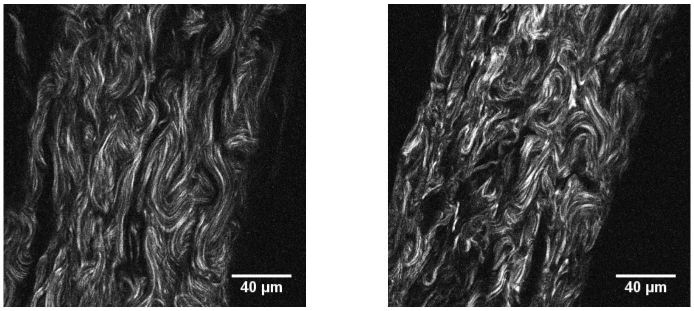

c). SHG image 1 d). SHG image 2

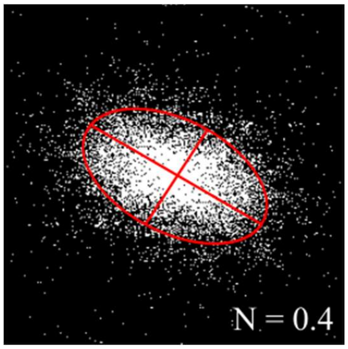

e). The FFT power spectra images - Follicular Adenoma Capsule

Figure 2. Encapsulated-PTC capsule

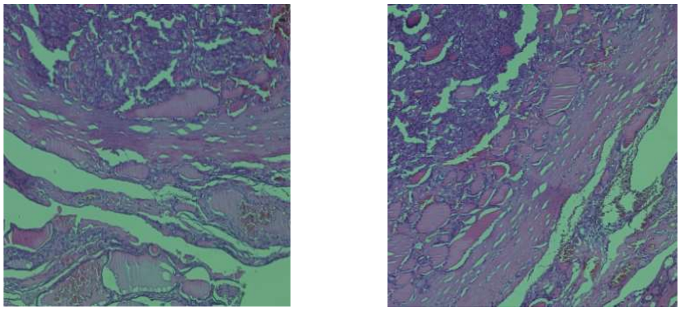

a). H&E 100x b). H&E 200x

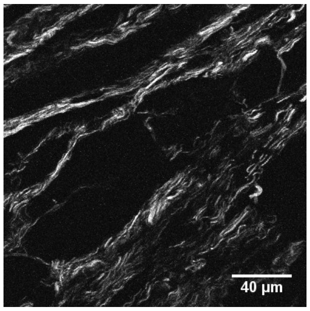

c). SHG image 1

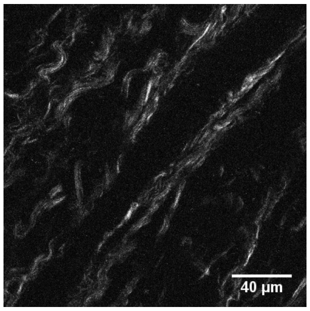

d). SHG image 2

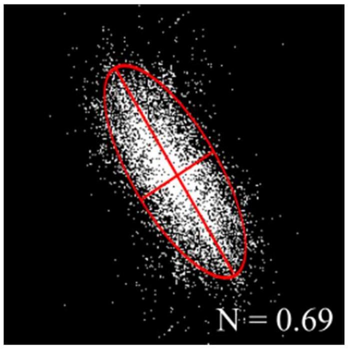

e). The FFT power spectra images – Encapsulated-PTC Capsule

CONCLUSIONS

We found differences in the orientation of collagen fibers between the capsules surrounding benign nodules (follicular adenoma) and malignant ones (Encapsulated-PTC) by using SHG microscopy images and FFT analysis of these images.

Our research indicates that different collagen structures can be detected in the malignant nodules capsule (PTC) compared to the benign nodule capsule and the thyroid capsule.

Higher orientation was detected for the malignant capsule but that does not mean the collagen fibers are organized or that the malignant tissue organizes the fibers. We believe it is rather a defense mechanism that goes from the level of the thyroid adjacent stroma of tumoral nodules.

Future plans

The present study deals with the differential diagnosis between follicular adenoma versus encapsulated – PTC, since both are clear cases, thus making it easier to prove the validity of the theory regarding the reorganizing of the collagen fibers within the two types of nodules, as proven with SHG Microscopy. Having completed that, the research may advance to analyzing less obvious situation:

1. Differential diagnostic between:
- Follicular adenoma versus follicular carcinoma;
- "Non-invasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) versus encapsulated papillary thyroid carcinoma with capsular invasion;
- Adenomatous nodule vs follicular adenoma.

2. Early detection of papillary carcinomas developed on follicular adenomas.

The SHG images can be developed as markers for use in clinical applications – a complementary method to early detection of suspected malignant nodules.

These above described microscopy methods help us distinguish between benign or malignant thyroid nodules, based on the capsular collagen organization.

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ORIGINAL ARTICLE

Musculoskeletal sports injuries of the upper limb in children

Michail Kotsapas[1], Apostolos Gantsos[1], Symeon Naoum[2]

Abstract: *The participation of children and adolescents in sports is advantageous in many ways regarding their physical and mental health, promotes cooperation and socialization between them, prevents diseases, such as obesity, and also sets the base of a healthy lifestyle in adulthood. In addition, childhood per se is characterized by an increased level of physical activity. Nevertheless, despite the multiple benefits of physical activity, it does not lack the possibility of unfortunate events or harmful conditions. The inner need of the athlete for excellence and recognition, the effort to reach or keep on an elite level of sports, early sports specialization, and pressure derived from family and coaches often lead to sports injuries-diseases either acute or chronic. Many of these conditions sometimes are underestimated or undiagnosed in the Emergency Department, especially if the main complaining symptom does not correlate with the disease. This non-systematic literature review aimed to identify the most common upper limb sport related-injuries and conditions among pediatric athletes. These injuries are described into groups depending on the anatomical area e.g shoulder girdle, elbow joint, and wrist.*

Keywords: *sport-related injuries in children, sport-related injuries, pediatric athletes, musculoskeletal injury in sports, sports medicine, little league shoulder*

INTRODUCTION

Nowadays, children are encouraged to participate in sports at higher levels at a much younger age than before [1]. As a consequence, there are increasing numbers of young athletes visiting their pediatricians because of sports-related injuries or asking for a piece of advice regarding injury prevention [2]. However, children are not "small adults". Growth plates act as stress risers in the distal upper extremity, resulting in injuries that may have a serious impact on a child's overall growth, joint alignment, and/or extremity length [1]. Injuries of the ligaments in children usually present as avulsion injuries. Moreover, attention should be paid to comprehension of the soft tissue attachments of any bony spot seen in imaging studies.

From an economic standpoint, the economic burden of the above-mentioned injuries is quite considerable, especially among children and adolescents. Additionally, these injuries may have a long-term impact on the musculoskeletal system, resulting in decreased levels of physical activity [3].

This non-systematic literature review aimed to identify the most common upper limb sport related-injuries and conditions among pediatric athletes. The inclusion criteria for the selection of the articles were concerning pediatric athletes, musculoskeletal sport-related injuries, English language, published until October 2021 and access to the full text. The PubMed, Scopus and Google Scholar database were searched with the terms "musculoskeletal sport-related injuries in children", "youth" or "pediatric athletes", "musculoskeletal injury in sports". Both review articles and original research articles were used to synthesize this review with a focus on contemporary and seminal papers.

SHOULDER GIRDLE

Little League Shoulder

Most often seen in the dominant shoulder, this injury refers to an undisplaced type 1 Salter-Harris fracture (epiphysiolysis) of the proximal humerus and affects mostly young athletes involved in throwing sports (e.g. baseball pitchers) or in excessive overhead activity (e.g.

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tennis)[1,2,4,5]. Young males are more commonly affected than females, while the peak prevalence of the disease presents at the time of the most rapid skeletal growth gain (11-16 years old for males) [4-6]. This injury is the result of the repetitive rotational and traction forces applied on the epiphysis, especially during the late cocking phase, with the humerus at maximum external rotation and abduction, and acceleration phase, where strong internal rotation and adduction forces are applied [7]. The increased overall number of throwing events is considered the most important risk factor for the prevalence of the disease. The patient's history may be remarkable for shoulder pain during sports that are alleviated at rest or correlated with an increase in practice intensity. Another complaint may be the deterioration of their athletic performance due to a decrease in throwing accuracy or velocity. On physical examination, glenohumeral internal rotation deficit (GIRD) may be noted. Radiographs are remarkable for physeal widening [5]. Sometimes, a radiographic evaluation of the contralateral shoulder is essential for the diagnosis [4]. At the late stages of the disease, premature fusion of the physis is noted, accompanied by angular deformity of the proximal humerus. Magnetic Resonance Imaging (MRI) of the shoulder is necessary for the diagnosis of the disease in patients with normal radiographs. In addition, MRI is useful for the evaluation of other soft tissue lesions, such as a rotator cuff (RC) tear, subacromial impingement, Superior Labrum Anterior to Posterior (SLAP) lesions, labral tears, etc [4,8]. The first line of treatment should be the cessation of throwing for at least 3 months. If symptom resolution is achieved, physical therapy is recommended with an emphasis on the strengthening of the shoulder girdle and posterior capsule stretching. Return to the sport should be gradual, and only after improvement of throwing technique through the appropriate practice sessions. Complications are rare thought serious, because of the developmental deformities of the physis that are included (premature physeal closure, growth arrest, upper limb length discrepancy, angular deformity, humeral head osteonecrosis) [4,5,8].

Glenohumeral Internal Rotation Deficit – GIRD

Young throwing athletes, such as baseball pitchers, even as young as 10 years old, are susceptible to this disease [9]. By definition, GIRD results in loss of internal rotation of the affected shoulder (more than 20°), compared to the contralateral shoulder [10]. Responsible for this range of motion (ROM) loss is considered the posterior capsular-rotator cuff tightness, which in turn causes abnormal shoulder deceleration during throwing [9,10]. At first, tightness results in a posterosuperior migration of the humeral head, as well as anterior capsule stretching, and, if left unaddressed, it leads to increased humeral head retroversion and abnormal shoulder functionality [5]. This condition predisposes young athletes to serious conditions, such as rotator cuff tears, internal impingement, labral tears, SLAP lesions, scapular dyskinesia, and Ulnar Collateral Ligament (UCL) injury of the ipsilateral elbow [5,9,10]. The patient's complaints include vague shoulder pain, stiffness, and the necessity for extended warmup or deterioration of their athletic performance. Physical examination should include meticulous shoulder ROM assessment and comparison with the contralateral limb in every thrower, who reports shoulder pain [6]. Furthermore, the physician should verify that the internal rotation ROM loss compensates with an equivalent external rotation ROM increase [4,10]. Plain radiographs rarely reveal abnormal findings, while MRI assessment of the soft tissue may reveal concomitant lesions. Management of GIRD requires rest from throwing activities for 6 months and physical therapy focused on posteroinferior capsular stretching, as well as, rotator cuff strengthening, and, fortunately, the prognosis is excellent for the majority of young patients [5][6]. Conservative treatment failure may require surgical management. Surgical management is, also, indicated, if concomitant lesions (e.g. rotator cuff tear) are present.

Rotator Cuff Disease

Rotator cuff tear is considered mainly an adult disease due to its rareness in underaged patients. Only 1.4% of children addressing a physician for shoulder pain are affected by rotator cuff tear [11]. Nevertheless, increased sports participation and early specialization in overhead activities result in a rising tendency of rotator cuff pathology at these ages [4,5,11,12]. Swimmers, baseball pitchers, throwers, tennis and volleyball players are particularly vulnerable to rotator cuff internal impingement, tendinitis, and, ultimately, tear because of the repetitive overhead and abduction-external rotation motion [5,11]. As far as swimmers are concerned, the term "swimmer's shoulder" is used to describe an overuse condition associated with impingement of the humeral head and the rotator cuff on the coracoacromial ligament [5]. It is difficult to make management recommendations on the rotator cuff tear in young patients because of the rarity of the disease. Even in the best available literature, information derives from case reports or small case series [11,12]. Each patient should be managed individually, and it is essential to take into consideration the level of the athlete, the necessity of a return to the pre-injury level, the injury as an entity (e.g. number of tendons affected, partial or full-thickness tears, concomitant soft tissue lesions, etc) [11]. Excellent functional outcomes in rotator cuff surgical repair are reported in limited case series studies although overhead athletes may not be able to play in the same position after surgery [11,12].

Shoulder Instability

Anterior shoulder dislocation is much more common (95%) than posterior dislocation or luxatio erecta [13,14]. About 1.7% of the general population is affected and 20% of the patients are younger than 20 years old, with a higher incidence in male patients [15]. Recurrence chance increases as the age of first dislocation decreases. The recurrence rate is as high as 90-95% for the age of the first dislocation under 25 years old [16], and in individuals with open physes escalates to almost 100% [13]. Nevertheless, some studies report significantly lower recurrence rates (33%) in ages of the first dislocation under 13, while for ages 14-17 recurrence exceeds 90% [17]. Risk factors for re-dislocation are age as abovementioned, male sex, contact sports, ligamentous laxity, and glenoid bone loss [4, 6, 13, 16, 17].

Patients with shoulder dislocation on examination present with asymmetry due to loss of the normal round shape of the shoulder, moderate to severe pain, and numbness, while the dislocated arm is usually supported by the healthy arm. The physical examination should always include the neurovascular assessment (especially the axillary nerve) of the affected arm. Plain radiographs are essential not only to confirm the diagnosis but also to exclude the fracture, before the reduction [6]. Many reduction manipulations have been described, such as the Hippocrates method, Kocher, or Stimson [16]. Some of them include the use of hard objects (e.g. the doctor’s heel in the Hippocrates method) to apply the necessary reduction force on the humeral head in the patient’s axilla [14]. Those techniques present an increased risk for neurovascular iatrogenic injuries due to the compression-traction of the axillary vessels, as well as the brachial plexus, and should be approached with caution [14]. The patient requires adequate analgesia and muscle relaxation medication, accompanied by psychological support, to achieve an effortless and painless reduction as soon as possible, with the fewest required manipulations. In case of failure to achieve a reduction in a conscious patient, general anesthesia might be necessary, especially in patients with heavy musculature. Reduction is usually felt by both the patient and the physician, by palpation, hearing, and, also, visually as the shoulder regains its normal round shape. Post-reduction neurovascular assessment should be performed, and a plain radiograph is necessary to confirm reduction and exclude any manipulation-related fracture [14]. After the reduction, a shoulder sling is used [6,16]. Position of immobilization in external rotation and adduction, despite support by many authors in the past, seems to have similar results in soft tissue healing and recurrence rate compared to immobilization in internal rotation and adduction [13,16]. The duration of immobilization is, also, a matter of debate [13,16].

Existing literature supports surgical stabilization after the first episode of shoulder dislocation, as far as active young adults are concerned. On the other hand, such a literature unanimity does not exist, regarding underaged active patients, who experience their first dislocation episode. According to Franklin et al. [15], conservative management of underaged patients leads to a higher recurrence rate (approximately 70-73). In addition, return to play rates are significantly higher for the athletes who underwent surgery (95.3% operative vs 41,3% nonoperative, Zaremski et al.) [18]. In conclusion, young active underaged patients are more prone to re-dislocation, so the benefits and drawbacks of both operative and nonoperative management should be thoroughly presented and discussed with the patient and the parents, to choose the best available method of treatment [15].

ELBOW

Ulnar Collateral Ligament Injury

Growth plate injuries are much more common in skeletally immature patients than injuries of the attached ligaments [4,5,19]. However, due to single-sport specialization and the intensity of throwing programs at an early age, ulnar collateral ligament (UCL) injury rates seem to rise among pediatric and adolescent overhead athletes population [20,21]. In pediatric patients, injuries to the anterior bundle of UCL are more common than in the posterior bundle [21] and might refer to partial or full tears, attenuation, or insufficiency of the ligament [5,22]. In cases of acute injuries, the patient usually experiences a popping sensation associated with swelling, ecchymosis, and pain on the medial or posterior elbow aggravated during late cocking and acceleration phases of throwing [5,13,22]. The valgus stress test of the elbow may indicate instability [22]. Patients with chronic injuries complain of vague medial elbow pain and deterioration of their throwing performance [22]. Plain radiographs are necessary to exclude pathologic findings, such as fractures-epiphysiolyses or avulsion of the medial epicondyle. MRI is indicated in the evaluation of the UCL in the case of normal radiographs. Initial treatment consists of cessation of throwing, ice therapy bracing and analgesics, followed by physical therapy and gradual return to sports [5,21]. In elite adult athletes, in case of conservative treatment failure, UCL reconstruction is preferred over UCL repair. Nevertheless, optimal surgical management is less clear regarding adolescent patients even if athletes between 15-19 years represent the largest patient population, in which UCL reconstruction is performed [22]. The newest available data present an 80% and 84% rate of return to play at the same or higher level of competition for javelin throwers and baseball players respectively, following UCL reconstruction [20].

Little League Elbow

Little League Elbow is an umbrella term used to describe medial elbow painful pathologic conditions that affect skeletally immature overhead athletes, such as medial epicondyle apophysitis, avulsions or stress fractures, UCL injuries, and flexor-pronator mass strains [4-6,8, 19, 23]. Tension overload of the medial elbow, as a result of the repetitive valgus stress experienced by these athletes during throwing, is the responsible mechanism [19]. Pitching despite the symptoms, high-speed fastballs, throwing breaking pitches, increased pitches per game, and year-round play are considered risk factors for the prevalence of the disease [19]. Apart from medial elbow pain, the young throwers complain of deterioration of their throwing accuracy or velocity. On plain radiographs, the medial epicondyle may be displaced. In case of doubt, an examination of the contralateral elbow may be required [6]. Management of flexor-pronator muscle strains and apophysitis of the medial epicondyle is conservative and includes cessation of throwing and physical therapy [19]. Avulsion fractures with a displacement of less than 5mm should be initially immobilized and follow an early range of motion physical therapy protocol. Displacement of more than 5mm may require open reduction and internal fixation of the affected medial epicondyle [5,6,19].

Osteochondritis dissecans (OCD)

Repetitive microtrauma, associated with axial compression forces or valgus stress, is considered to be the main etiologic factor for the development of the disease. This notion is amplified by the fact that OCD is more common in skeletally immature throwers and gymnasts. The elbows of skeletally mature athletes are rarely involved, probably due to the greater blood supply of the mature distal humeral epiphysis. The capitellum of the dominant upper limb is affected more often and males are more prone to elbow OCD than females [5,23-25]. Patients usually complain of lateral elbow pain and, as the disease progresses, mechanical symptoms may present, such as locking, clicking, decreased ROM, and swelling [25,26]. Early diagnosis is essential to avoid chondral fragmentation and loose body formation, which may lead to degenerative lesions at a young age [26]. Late-stage OCD may appear as increased radiolucency of the capitellum or loose intraarticular bodies on plain radiographs [19,25]. In the case of radiographs with normal findings and strong suspicion of OCD, an elbow MRI should be obtained, which provides the optimal evaluation of the lesion stability [27, 28]. In general, the stability of the lesion indicates roughly the conservative or surgical management of the disease [28]. Most of the early-stage lesions in patients with open physes are best managed nonoperatively [5,25,28]. Unstable symptomatic lesions (pain, decreased ROM, locking, fragmentation, closed physes) require surgical intervention. Although a consensus does not exist due to insufficient reliable data and many treatment modalities have been reported (arthroscopic debridement, abrasion chondroplasty, microfractures, fragment fixation, osteochondral autograft transplantation-OAT, costal osteochondral transplantation-CAT), it seems that osteochondral autografting techniques may be superior in restoring the local anatomy and elbow function.

OCD of the elbow should not be confused with Panner's disease, which is a self-limiting osteochondrosis of the capitellum, with an excellent prognosis. It affects more commonly children of younger age (4-9 years old). On physical examination, symptoms may be mimicking those of the OCD. Conservative management (cessation of activities, analgesics, and immobilization) is sufficient [19].

Olecranon apophysitis

Similar to the Osgood-Schlatter (tibial tubercle apophysitis) disease, olecranon apophysitis is the result of traction microtrauma due to the repetitive contraction of the extension mechanism, in skeletally immature athletes. Apophysitis may progress to avulsion fractures in younger patients or stress fractures in older individuals. Posterior elbow tenderness, swelling, and decreased ROM are the most common complaints. Conservative management with rest, activity modification, and analgesics is often the treatment of choice [5,19,29]. In the case of olecranon apophyseal nonunion surgical management may provide satisfactory results [30].

WRIST

Distal Radius Fractures

These fractures are the most common children's fractures (20% of all fractures), with a predominance in male patients, and happen usually in contact sports, such as soccer [1, 5,31]. A developing skeleton is characterized by unique fracture patterns, such as buckle fractures, greenstick fractures, plastic deformation, and epiphysiolysis [1,31]. Children's fractures, also, present a great healing and remodeling potential. Remodeling is more eminent near the most active physes, in the plane of joint motion, and younger skeletal age [31]. The pediatric trauma surgeon should consider the abovementioned, as well as the patient's and parents' capability of compliance, before applying the proper treatment. Surgical intervention must be performed with minimal physeal trauma. Growth arrest is a devastating complication of physeal injuries, affecting up to 10% of physeal fractures and may cause deformities, such as length discrepancy, angulation, and joint incongruency, ultimately leading to lifelong disability [1,31].

Gymnast's Wrist

The distal radial physis of the young gymnast not only does suffer from fractures or epiphysiolyses, but also chronic overuse injuries, such as the "gymnast's wrist" [4,5]. Due to the needs of this particular sport, the wrist of the pediatric athlete is forced to bear heavy axial loads with the wrist in full extension multiple times during practice or competition [1,23]. The repetition of this abnormal loading pattern is responsible for microtrauma to the physis, which may even lead to physeal arrest [1,5]. Plain wrist radiographs in young gymnasts with wrist pain and contralateral comparison, regardless of the physical examination signs, are suggested by some authors [1]. Abstaining from gymnastics may be a necessity if any abnormality, such as irregularity or physeal widening, is noticed on radiographs. A cast may also be applied [1,5]. In the case of normal x-rays, an MRI may reveal bone marrow edema on both the proximal and distal side of the physis [4]. Management of patients with physeal arrest might be quite challenging and depends on the years remaining until expected physeal closure, the portion of physis with physeal arrest, and the existence of deformity (ulnar positive variance, bowing), and the articular congruency. Surgical treatment options include recurrent radial lengthening, both radial, and ulnar lengthening, radial osteotomies, and ulnar epiphysiodesis. Despite the good results reported, most of the patients will not be able to return to their previous level of gymnastics [1].

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VARIA

Myocardial work – are we ready for a new parameter in clinical practice for evaluating left ventricle function?

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Abstract: *Left ventricle systolic function is an essential parameter in different clinical scenarios and the usual methods available for its assessment are sometime suboptimal, depending on the filling conditions of the heart. Therefore, the development and implementation in clinical practice of a new parameter for assessing left ventricle contractile function is desirable. Mechanical work defined as energy transferred to or from an object via the application of force along a displacement seems to be a promising method for myocardial performance estimation. Although initially this parameter was calculated on the left ventricle volume-pressure curve estimated by cardiac catheterization, an easier method considering non-invasive left ventricle pressure and global longitudinal strain assessed by speckle tracking echocardiography was developed recently. Myocardial work offers information regarding the segmental and global function of the left ventricle, and it is considered a more sensitive marker for assessing left ventricle performance compared to ejection fraction and global longitudinal strain. Its applicability in various cardiac pathologies was demonstrated recently in several studies and its use in every day practice may bring important additional information for clinical decision making.*

Keywords: *myocardial work, mechanical work, global work, systolic function*

INTRODUCTION

Left ventricle systolic function is an essential parameter in different clinical scenarios and its accurate estimation is mandatory in every day clinical practice. The methods available for assessing left ventricular (LV) contractile function, such as ejection fraction (LVEF) or global longitudinal strain (GLS) are dependent on the filling conditions of the heart. Myocardial work (MW) emerged as a promising option for assessing LV systolic performance, as a more reliable and independent technique. This method removes afterload dependence by incorporating it into the analysis of the GLS [1,2,3].

Mechanical work is defined as energy transferred to or from an object via the application of force along a displacement. This formula is not well suited for the heart because it is difficult to calculate the myocardial force applied during the cardiac cycle. Therefore, the idea of using pressure as a substitute for force arose. This is defined as the force applied to the LV walls divided into several segments. Usually, myocardial work is determined based on the volume-pressure curve in the LV, obtained by cardiac catheterization. This method was introduced in 1979 by Saga and reflects myocardial oxygen consumption and LV contractile function. However, the clinical utility is reduced as it requires an invasive maneuver [1,2,3].

An easier method of approximating mechanical work takes into consideration non-invasive LV pressure and GLS, a concept developed by Russel and colleagues. The LV pressure curve is estimated by incorporating the systolic blood pressure measured at the brachial level and the phases of the cardiac cycle (isovolumic relaxation phase, ejection phase, isovolumic contraction phase), established using echocardiography. In their study, the LV volume-pressure curve correlates with the pressure-strain curve in patients with left bundle branch block and intra-ventricular contraction asynchrony or in those with ischemic heart disease. Therefore, myocardial work is a promising method

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for non-invasive exploration of LV myocardial performance, already validated for several clinical situations [1,2,3].

ACQUISITION AND ANALYSIS

Myocardial work is obtained by using a speckle-tracking technique. The necessary ultrasound acquisitions include the apical four, two and three chamber views, to generate the global longitudinal strain. It is necessary to acquire images at 60-80 frames/second, to properly visualize the edge of the endocardium and to establish an adequate width of the region of interest at the level of the myocardium, to allow the correct evaluation of GLS and, thus, of myocardial work [1,2].

Blood pressure measurement using a standard cuff placed at the brachial level should be performed simultaneously with the acquisition of echocardiographic images. This is an essential parameter used as a surrogate for LV systolic pressure for the calculation of the global work index [2].

After obtaining the GLS and the characteristic bull's eye image, the opening and closing of the aortic and mitral valves will be selected in an automatic or manual manner, guided by the three-chamber apical view and the concomitant electrocardiogram. The LV volume-pressure curve is generated from an empirical curve, modified according to the duration of the isovolumic contraction phase and the ejection phase. These phases are determined by echocardiography after selecting the moments attributed to the opening and closing of the aortic and mitral valves. Thus, it is possible to define the phases of the cardiac cycle. The maximum LV systolic pressure is equal to the systolic blood pressure, in the absence of a gradient in the LV ejection tract or at the aortic valve [1].

Subsequently, systolic, and diastolic blood pressure are introduced into the formula and the calculation of myocardial work is approved (Figure 1) [2].

Myocardial work is measured by the area of the LV pressure-strain curve, generated based on filling pressures and GLS1. Myocardial work is composed of several parameters - global work index, global constructed work, global wasted work, and global work efficiency [1].

Figure 1: The first step in the MW method involves acquiring four-, two-, and three-chamber apical views, followed by GLS calculation. The myocardial work option is then selected, followed by the introduction of blood pressure values (systolic, diastolic) and the selection of the opening and closing moments of the aortic and mitral valves, guided by the concomitant electrocardiogram. Based on the pressure-strain curve, myocardial work is calculated, along with its components, the bull's eye image is generated for the myocardial work index (with the values of this parameter on a 17 segments model, along with the color code) and the relationship between constructed work and wasted work through the bar chart.

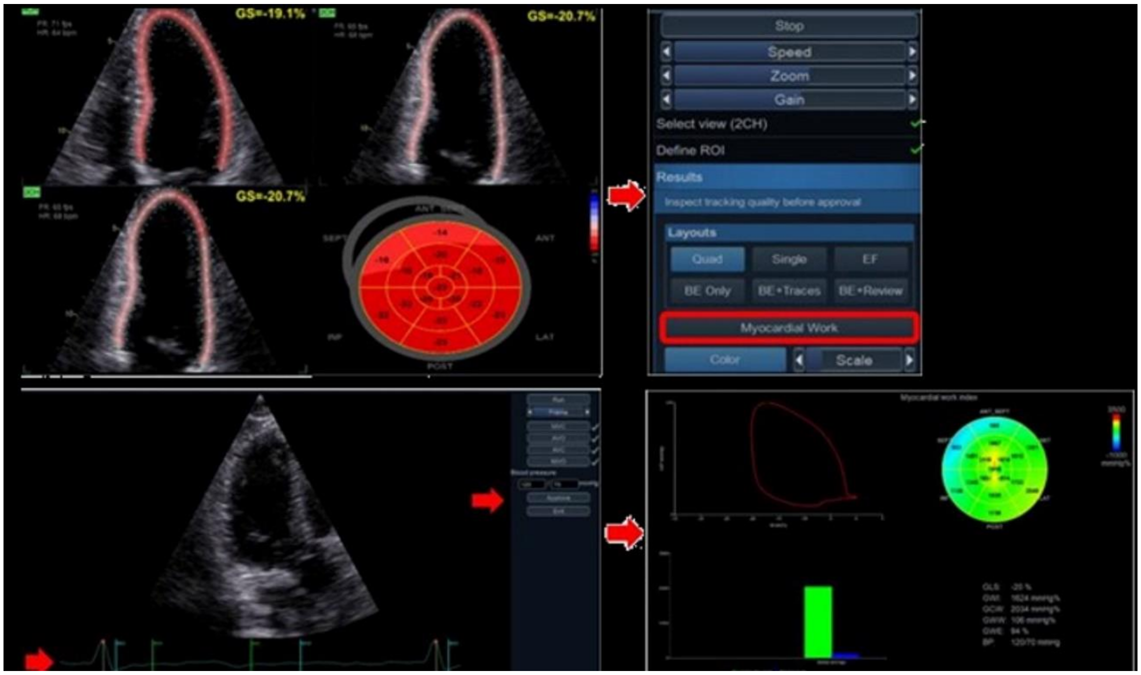

GLS: -20 %
GWI: 1624 mmHg%
GCW: 2034 mmHg%
GWW: 106 mmHg%
GWE: 94 %
BP: 120/70 mmHg

Global work index is calculated by the area of the pressure-strain curve resulting from the closing and opening of the mitral valve (Figure 2), representing the mechanical systole, including the phase of contraction and isovolumic relaxation. Given that pressure is used instead of force, it is not possible to calculate myocardial work, but an index of it (GWI) [2].

Figure 2: The pressure-strain curve, along with the phases of the cardiac cycle and the moments of closure and opening of the aortic and mitral valves. Adapted from (1). AVC - aortic valve closure, AVO - aortic valve opening, MVC - mitral valve closure, MVO - mitral valve opening.

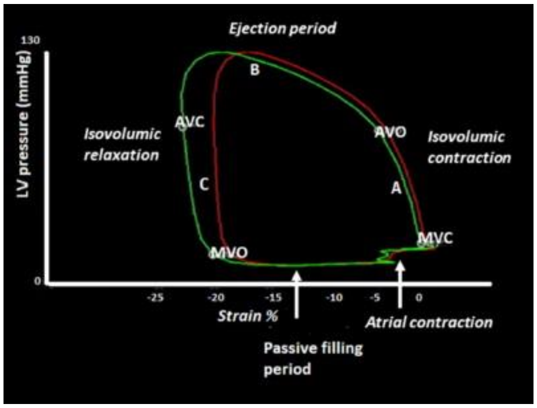

Normally, the myocardial segments contract and relax synchronously to participate constructively in achieving the stroke volume.

Global constructed work (GCW) represents the productive work submitted by the LV for achieving systolic shortening and elongation during the period of isovolumic relaxation, the physiological processes carried out between the opening of the aortic valve and the mitral valve [1,3].

Consumed work (global wasted work) represents the unproductive effort made to elongate the myocardial fibers in the LV systole and shorten them in the isovolumic relaxation phase. It occurs in cases with asynchronous intraventricular contraction, such as patients with bundle branch block or myocardial ischemia.

Global wasted work (GWW) increases the metabolic burden of LV and may contribute to its remodeling [1,2,3].

Global work efficiency (GWE) is the constructed work divided by the sum of the constructed and consumed work. This parameter expresses how efficiently energy was invested during the cardiac cycle and is reported as a percentage.

Therefore, the value for a normal heart with synchronous contraction and relaxation, this value should be close to 100% [1]. This parameter is reported in the form of a bull's eye numerically and by color code (green indicates areas with a high degree of efficiency and red indicates areas with a low degree of efficiency). Possible clinical applications for determining this parameter include improved selection criteria for cardiac resynchronization therapy (CRT) or for revascularization of patients with myocardial infarction [1,2,3].

The parameters described above are evaluated globally and segmentally (17 segments), generating a bull's eye, like GLS. The global work index can be evaluated visually by means of a color scale - red represents an intense work area, green normal work, and blue negative work. In the case of negative work, energy consumption exceeds production, due to elongation of fibers during systole or shortening during the period of isovolumic relaxation.

NORMAL REFERENCE VALUES

The NORRE study conducted by the European Association for Cardiovascular Imaging was the first prospective, multi-center study to include a large group of 226 healthy volunteers between the ages of 20 and 78, to establish reference values for myocardial work and its parameters [1,2]. The values obtained in this study are presented in Table 1.

Other studies on healthy volunteers resulted from the STAAB cohort or the study led by Galli5, with the results shown in Table 2.

The results obtained in the two studies were similar, indicating normal values above 95% for GWE. GWW has low values in healthy subjects, but not equal to zero.

Table 1: Reference values for myocardial work parameters according to the NORRE study. Adapted from [2].

	Total number (n=226)	**Male (n=85)**	**Female (n=141)**
GWI (mm Hg%)	1,896 ±308	1,849 ± 295	1,924 ±313
GCW (mm Hg%)	2,232 ± 331	2,228 ± 295	2,234 ±352
GWW (mm Hg%)	78,5 (53-122,2)	94 (61,5-130,5)	74 (49,5-111)
GWE (%)	96 (94-97)	95 (94-97)	96 (94-97)

Table 2: Normal reference values of myocardial work from three studies with healthy subjects. Adapted from [1].

Variabile	GWI	GCW	GWW	GWE
NORRE study	1,896 ±308	2,232 ±331	78,5 (53-122,2)	96 (94-97)
STAAB cohort	2,209 ±307	2,430 ±351	74 (54-101)	96 (95-97)
Galli et al.	1,926 ±247	2,224 ±229	90 (62-123)	96 (94-97)

An example of myocardial work for a healthy subject is shown in Figure 3A**.**

PRACTICAL APPLICATIONS OF MYOCARDIAL WORK

MW is considered a more sensitive marker for assessing LV performance compared to ejection fraction and GLS. Its applicability in various cardiac pathologies was demonstrated in several studies [4].

Bundle branch block

In a normally functioning heart, all 17 segments contract at the same time and contribute to the ejection of the blood into the aorta, resulting in a very low GWW value. In the presence of a bundle branch block, certain LV segments elongate in the systole or contract against a closed aortic valve, resulting in a significant increase in GWW. GWW does not contribute to LV ejection, which explains why MW is more sensitive in assessing LV efficiency, even when LV contractility is normal [4].

Cardiac resynchronization therapy (CRT)

Galli et al demonstrated a potential role of MW in assessing post-CRT response [5]. Thus, at baseline, GCW and GWW were significantly higher in CRT responders compared to non-responders. Following a multivariate analysis, values of GCW > 1057 mmHg % and GWW > 384 mmHg % were considered predictive of positive response post-CRT.

The 6-month reassessment after the intervention showed that responders had an increase in GCW and a decrease in GWW associated with reverse LV remodeling.

Figure 3: A – Normal heart - GWI and GWE within normal limits with GCW predominance over GWW, GLS with normal value; B – Arterial hypertension - GLS is normal, while MW bull's eye shows elevated values of GWI and GCW, with a normal value of GWE

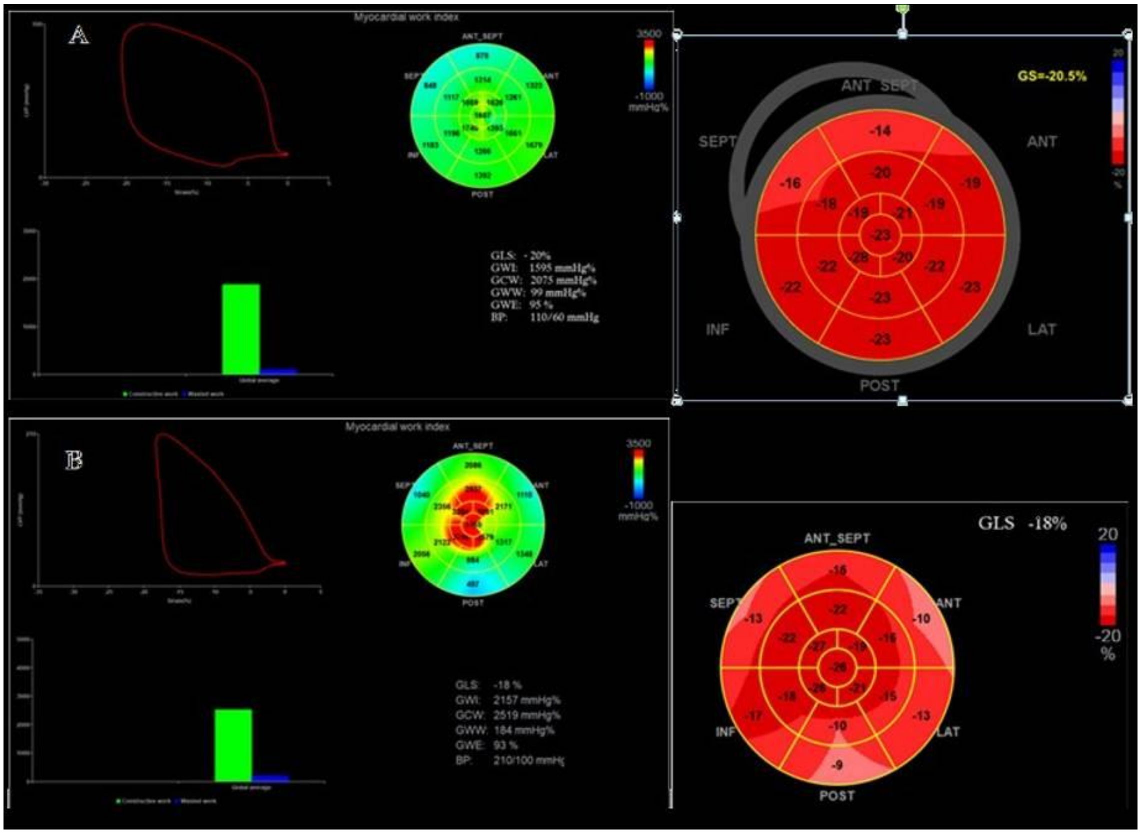
A
Myocardial work index
ANT_SEPT
SEPT
ANT
INF
LAT
POST
3500
-1000 mmHg%
GLS: -20%
GWI: 1595 mmHg%
GCW: 2075 mmHg%
GWW: 99 mmHg%
GWE: 95 %
BP: 110/60 mmHg
GS=-20.5%
20
-20 %
B
GLS: -18 %
GWI: 2157 mmHg%
GCW: 2519 mmHg%
GWW: 184 mmHg%
GWE: 93 %
BP: 210/100 mmHg
GLS -18%

Van der Bijl et al also evaluated patients with resynchronization indication, concluding that another predictor for CRT response is GWE, with lower values before CRT being associated with a better prognosis [6].

In another study, Galli et al analyzed the effect of a preserved GCW value regarding echocardiographic response and post-CRT mortality, showing that GCW > 888 mmHg% correlated with a 15% reduction in LV telesystolic volume and a significant decrease of mortality compared to lower values [7].

Arterial hypertension

Loncaric et al have shown that under acute pressure overload, the LV ejection fraction remains unchanged, while the GLS decreases and the MW increases.4 In these patients, the MW increases, as each myocardial segment must produce additional energy to pump blood against an increased afterload.

Under conditions which involve chronic exposure to pressure overload, LV remodeling occurs, with myocardial hypertrophy and fibrosis, and in this situation the MW values decrease (Figure 3B).

Chronic heart failure (HF)

a) *with preserved LV ejection fraction*

Chronic heart failure with preserved LVEF is becoming an increasingly diagnosed entity, the main pathophysiological mechanism being the presence of diastolic dysfunction. Paolisso et al followed the role of MW in predicting the first hospitalization caused by HF in patients with preserved LVEF, showing that an increased value of GWW with a cut-off of 170 mmHg % was associated with a higher percentage of patients being hospitalized [8].

b) *with decreased LV ejection fraction*

Hedwig et al demonstrated a good correlation between GWI and traditional echocardiographic parameters (LVEF, GLS), but also with the NT-proBNP value and the maximum O_2 consumption in the cardiopulmonary exercise test, a GWI value < 500 mmHg % having a negative predictive role [9]. An example is presented in Figure 4A.

Figure 4: A – Nonischemic dilated cardiomyopathy - severely reduced GWI and GCW and high GWW; B – Hypertrophic cardiomyopathy - low GWI and GCW and high GWW, noting a scratchy pattern of MW distribution due to the presence of asymmetric hypertrophy, especially in the lateral LV wall and in the mid and apical segments; C – Coronary heart disease - the red curve shows the overall value of MWI, while the green curve shows the pressure-volume curve at the level of a normal myocardial segment (C1) compared to an akinetic segment (C2). There is a lower value of GCW and a higher value of GWW in the akinetic segment compared to the normokinetic segment.

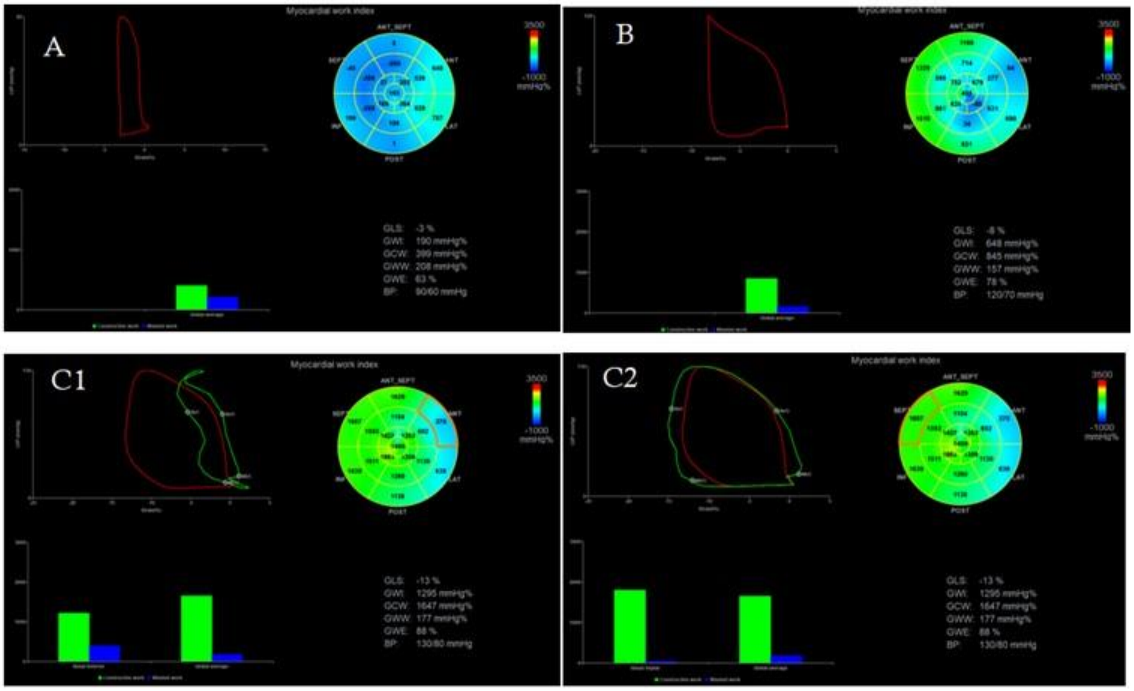
A
Myocardial work index
ANT_SEPT
SEPT
ANT
INF
LAT
POST
3500
-1000 mmHg%
GLS: -3 %
GWI: 190 mmHg%
GCW: 399 mmHg%
GWW: 208 mmHg%
GWE: 63 %
BP: 90/60 mmHg
B
Myocardial work index
GLS: -8 %
GWI: 648 mmHg%
GCW: 845 mmHg%
GWW: 157 mmHg%
GWE: 78 %
BP: 120/70 mmHg
C1
Myocardial work index
GLS: -13 %
GWI: 1295 mmHg%
GCW: 1647 mmHg%
GWW: 177 mmHg%
GWE: 88 %
BP: 130/80 mmHg
C2
Myocardial work index
GLS: -13 %
GWI: 1295 mmHg%
GCW: 1647 mmHg%
GWW: 177 mmHg%
GWE: 88 %
BP: 130/80 mmHg

Wang et al showed that patients with a GWI < 750 mmHg % had a significantly higher risk of mortality and a higher rate of hospitalization due to HF compared with those who had a GWI > 750 mmHg % [10].

In another study by Bouali et al, in patients treated with sacubitril/valsartan there was an improvement in both conventional echocardiographic parameters and GCW and MWE at reevaluation, 12 months after initiation of treatment. Instead, the GWW remained unchanged. A GCW value ≤ 910 mmHg at baseline was associated with more advanced disease, higher LV telediastolic volume, and lower LVEF, and was also a predictor of major cardiac adverse events [11].

Coronary heart disease

Evaluation of patients with coronary heart disease is difficult in the absence of LV regional dysfunction. Echocardiographic evaluation is based on subjective methods, to establish the segmental and global systolic LV function. MW has shown better sensitivity and accuracy compared to LVEF and GLS in identifying patients with coronary heart disease. Edwards et al demonstrated that GWI is a strong predictor. A GWI cut-off value of 1810 mmHg % had a specificity of 92% and a sensitivity of 51% [12]. An example is presented in Figures 4C1 and C2.

Boe et al analyzed a cohort of patients with acute coronary syndrome without ST-segment elevation, showing that GWI is significantly lower in the territory supplied by the coronary artery at risk of acute occlusion, being an important parameter that could guide the indication for prompt intervention [13]. Meimoun et al studied MW in patients with anterior ST-segment elevation myocardial infarction, showing that GCW is the best parameter to predict segmental and global LV recovery [14].

The use of MW was also attempted in the echocardiographic stress test, with Borrie et al demonstrating a significant decrease in GWI among LV segments with inducible ischemia, in contrast to healthy subjects in which a 54% increase in GWI was obtained [15].

Hypertrophic cardiomyopathy

There is several studies that have observed the evolution of MW in patients with hypertrophic cardiomyopathy (HCM). Hiemstra et al showed that GCW, GWI and GWE were significantly reduced, while the value of GWW was increased among patients with HCM [16]. An example is presented in Figure 4B.

GCW was the only predictor for the presence of myocardial fibrosis confirmed by cardiac magnetic resonance, with values < 1623 mmHg% proving a good sensitivity and specificity (82% and respectively 67%) [17].

Figure 5: A – Aortic stenosis- The average aortic transvalvular gradient was added to systolic blood pressure to estimate LV afterload. We can observe that, although the GLS value is similar for two patients with aortic stenosis, the first patient has lower values of GWI and GCW (A1) compared to the second one (A2), the first one having more severe symptoms; B – Mitral regurgitation - normal values of GCW and GWI are observed in a patient with severe asymptomatic mitral regurgitation; C – Aortic regurgitation - normal values of the parameters in a patient with asymptomatic chronic severe aortic regurgitation

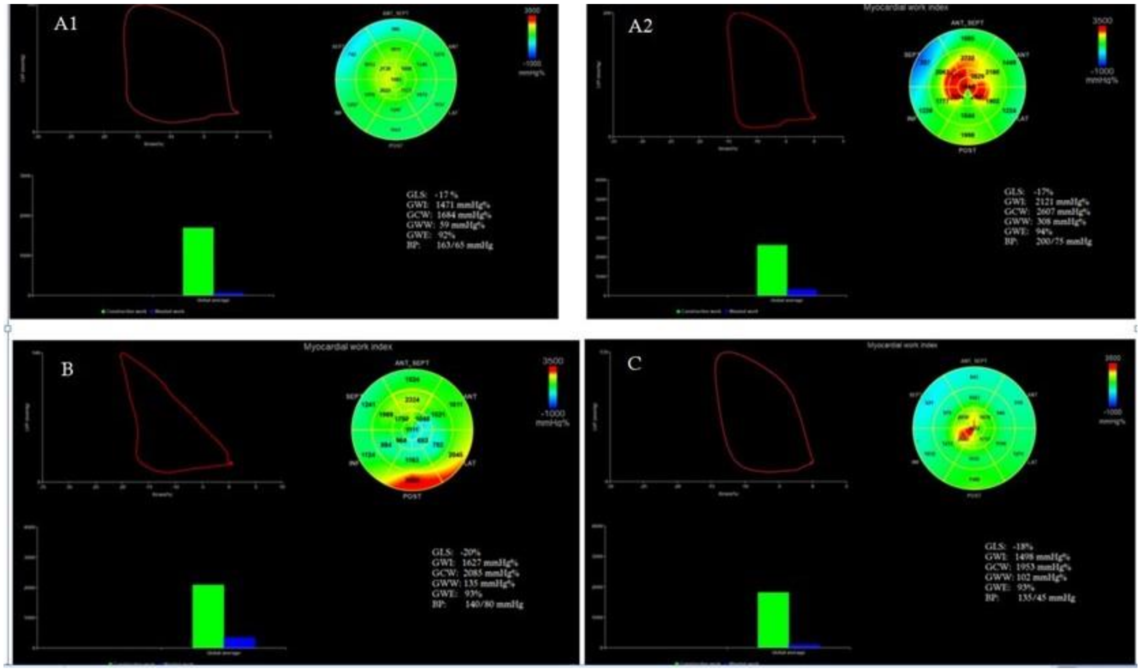
A1
GLS: -17 %
GWI: 1471 mmHg%
GCW: 1684 mmHg%
GWW: 59 mmHg%
GWE: 92%
BP: 163/65 mmHg
A2
Myocardial work index
ANT_SEPT
SEPT
ANT
LAT
INF
POST
3500
-1000 mmHg%
GLS: -17%
GWI: 2121 mmHg%
GCW: 2607 mmHg%
GWW: 308 mmHg%
GWE: 94%
BP: 200/75 mmHg
B
Myocardial work index
ANT_SEPT
SEPT
ANT
INF
LAT
POST
3500
-1000 mmHg%
GLS: -20%
GWI: 1627 mmHg%
GCW: 2085 mmHg%
GWW: 135 mmHg%
GWE: 93%
BP: 140/80 mmHg
C
Myocardial work index
GLS: -18%
GWI: 1498 mmHg%
GCW: 1953 mmHg%
GWW: 102 mmHg%
GWE: 93%
BP: 135/45 mmHg

In patients with asymmetric LV wall hypertrophy, a scratchy pattern of MW distribution was observed. In this case, segmental MW values were more useful in predicting the presence of myocardial fibrosis compared to global ones [18].

Different segmental values were also identified among patients with other HCM phenocopies. Clemmensen et al also showed that in patients with cardiac amyloidosis the MW values were significantly lower compared to healthy subjects. At the same time, lower values of GWI were observed in the basal segments compared to the apical ones, and GWI was associated with a lower effort capacity and a worse prognosis [19].

Valvular heart disease

a) *Aortic stenosis*

To estimate more accurately LV afterload, Jain et al added the mean aortic gradient to systolic blood pressure, demonstrating a good correlation with invasive measurements of systolic blood pressure. In the same study, the authors demonstrated an immediate decrease of MW in post-TAVR patients [20].

Another study found that in patients with aortic stenosis, the presence of more severe symptoms of heart failure correlated with lower values of GWI and GCW, consistent with the presence of a higher degree of myocardial fibrosis secondary to chronic exposure to increased afterload [21].

A practical example is shown in Figure 5 A1 and A2.

b) *Aortic regurgitation*

Evaluation of MW in patients with chronic severe aortic regurgitation and preserved LVEF could provide additional information on myocardial performance. Meucci et al recently published a study showing that patients with severe aortic regurgitation and preserved LVEF have elevated or normal GWI and GCW values and normal GWE and GWW values [22]. An example is shown in Figure 5C.

c) *Mitral regurgitation*

Tunyan et al analyzed MW in patients with primary mitral regurgitation. LVEF, GLS, and GWI did not differ between symptomatic and asymptomatic patients, but symptomatic patients had lower GCW and higher GWW values [23]. An example is shown in Figure 5B.

MW was also evaluated in patients with HF with reduced LVEF and severe functional mitral regurgitation in whom Mitra-Clip was performed. The study showed that GCW was the only predictor for LV reverse-remodeling one year after surgery, a GCW value > 846 mmHg % being associated with a decrease of > 10% in the LV telesystolic volume [1].

CONCLUSIONS

MW is a new method, less dependent on the LV loading conditions compared to LVEF and GLS, which provides important information about the segmental and global function of the LV. In recent years, promising results have been obtained on the applicability of MW in various cardiac pathologies, vouching the significant additional role MW could play in daily practice.

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ORIGINAL ARTICLES

Negative-pressure wound therapy - advantages and disadvantages

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Abstract: *The applications of negative-pressure wound therapy (NPWT) have achieved remarkable diversification. Initially used in the therapy of soft tissue wounds related to open fractures, this technique led to the understanding of the complex mechanisms underlying it. This paper aims to analyze the evolutionary scenarios of a group of patients with heterogeneous lesions under negative pressure aspiration treatment. We aimed to identify and analyze various associated pathologies that have the potential to negatively influence the healing process under negative pressure treatment and implicitly analyze the particularities of representative cases for the group whose evolution under treatment proved to be unfavorable. Retrospective, observational study, type series of cases focused on a group of 17 hospitalized patients, who received treatment by negative aspiration, between 2015-2019 in the Surgery Department of the University Emergency Hospital Bucharest. Regarding the obtained results, diabetes mellitus, obliterating arteriopathy, and anemia had a prevalent distribution in the group with unfavorable evolution. The glycemic value can predict 17% of the evolutionary scenario of a patient (favorable/unfavorable) being necessary an integrative therapeutic conduct. Concluding, although an extremely promising therapeutic option, NPWT can prove to be disappointing given its impact in the background of suboptimally managed comorbidities.*

Keywords: *negative pressure therapy, negative prognosis, comorbidities, amputation, wound complications, lower limb obliterative arteriopathy*

INTRODUCTION

The negative-pressure wound therapy (NPWT) method involves the application of a negative (subatmospheric) pressure to the wounds in a controlled manner [1]. Using a system consisting of a vacuum source, a porous material (filler) arranged inside the wound that respects its limits, a polyurethane adhesive film, which is transparent, permeable to water vapor, and impermeable to bacteria, this therapy method ensures tightness [2]. The first indications of negative pressure aspiration therapy cited in the literature were in the management of soft tissue lesions associated with open fractures and for closing dermatofasciotomy wounds [3]. Currently, after numerous studies have elucidated many complex gears underlying the mechanism of action and it was possible to understand how the overall therapeutic effect is influenced by these mechanisms, the range of therapeutic indications has gained remarkable proportion [4].

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There are many ways in which negative wound pressure suction therapy promotes healing [5]. The suction force acting on the lesion drains the exudate and causes mechanical stress at the edges of the wound [6]. This mechanical stress alters the local tissue perfusion, consequently stimulating angiogenesis and the development of granulation tissue [7].

The changes in tissue perfusion induced by NPWT are a consequence of multiple determinism. Angiogenesis is responsible for a part of the perfusion changes [8]. In addition, pressure modifications from the wound and the physical changes of the existing blood vessels, which in turn initiate alterations in blood rheology, are other important components of the angiogenesis process [9]. Various studies have concluded that these perfusion changes induced by NPWT depend on several factors [10]. The first factor is the regime of application of negative pressure (continuous or intermittent) [11]. Then, the pressure regime used (-50mmHg - -200mmHg), the condition of the tissue (healthy/ ischemic), the type of tissue, as well as its proximity to the area on which the negative pressure acts [12].

The healing process can be influenced by modifying various functional and material parameters [13]. Thus, the pressure level, the application of the pressure regime (continuous/ intermittent), the type of filler material (foam/gauze), the presence of additional layers in contact with the wound, installation, etc., represent the therapeutic variables that must be agreed with the patient’s particularities. Respectively, the patient’s pathology, the type of wound, even its shape are factors to consider [14]. All these data play a key role in the therapeutic equation, whose ultimate goal is to obtain the maximum therapeutic benefit and minimize the disadvantages as much as possible [15].

Numerous studies on different types of wounds have revealed that NPWT is a more economical treatment method compared to conventional ones [16]. This involves the use of fewer dressings, and the frequency with which other reconstructive options are used is lower [17]. The healing time of wounds is shorter, and the overall duration of treatment and hospitalization is remarkably reduced [18]. Although the materials used in NPWT are at the beginning more expensive than those required for conventional treatment, in the long run, the costs of conventional therapy outweigh those of NPWT [19].

NPWT was introduced as a preventing technique for post-cesarean delivery wound morbidity, especially for obese women. The reported results are controversial; the heterogeneity of the results being summarized in two suggestive meta-analyses [20]. The background for using preventive NPWT consists in the actual rising trend of obesity and cesarean delivery among pregnant women and the compelling data regarding the results of this therapy in non-obstetrical surgical specialties [21]. However, at this moment, there are no valid studies to demonstrate that the use of NPWT is beneficial as a prevention strategy for wound infections and complications in the obstetric population; it is also not justified in the matter of economical features [22].

MATERIALS AND METHODS

The retrospective study used data collected from the computer database of the hospital, respectively the discharge summaries of the patients. The data collection process focused on the following: demographic data, hospitalization duration, and the main diagnosis. Special attention was given to the associated pathologies that may influence the evolution of lesions of interest under treatment with negative pressure (lower limb obliterative arteriopathy – LLOA, anemia, atherosclerosis, type II diabetes). In addition, other factors were registered, such as laboratory tests (complete blood count, biochemistry analyses, laboratory tests for hemostasis), general clinical examination data, paraclinical investigations (radiography, Doppler ultrasound, angiography, CT (computed tomography), angio-CT), the nature of the primary and subsequent intervention, clinical evolution on the period of hospitalization under negative pressure therapy, the post-vacuum aspect of the lesions, the clinical condition of the patient and the aspect of the lesions of interest at the post-discharge controls.

The centralized data were analyzed descriptively using Microsoft Excel 2019. SPSS Statistics software was used to perform logistic regressions. After the first session of NPWT, the division of patients into the two groups reflecting the general evolution (favorable/unfavorable) and the post-vacuum aspect of the lesions of interest was considered.

Objectives

We aimed to analyze the prognosis of a group of patients with heterogeneous lesions undergoing NPWT. Our objectives included the identification and division of cases according to the favorable/unfavorable evolution of lesions of interest. It also included the appreciation of the distribution among patients of various associated comorbidities significant for the targeted population; according to literature, they have the potential to negatively impact the healing process, even under NPWT (diabetes, obliterating arteriopathy, anemia). We also looked into the analysis of the particularities of the representative cases from the study group whose evolution under treatment proved to be unfavorable.

RESULTS

According to the inclusion criteria, a group of 17 patients was identified (5 female patients and 12 male patients), aged between 47 and 80 years, the average age being 62 years between 01.01.2015 - 31.12.2019, within the General Surgery III Department of the University Emergency Hospital of Bucharest. The situation of inpatient diagnoses, as well as the prognosis balance, are presented in a more schematic framework in Table 1.

Table 1: Inpatient diagnoses of the studied group

Diagnosis at admission	Patients no.	Prognosis
Gangrene in the lower limbs with various locations	7	Favorable – 5 Unfavorable – 2
Foot trophic ulcer	2	Unfavorable
Secondary purulent peritonitis	2	Unfavorable
Local complications of postoperative wound	5	Favorable – 4 Unfavorable – 1
Necrotizing fasciitis and cellulite of the abdominal wall	1	Favorable

Regarding the prognosis scenarios under NPWT, 7 patients (2 women and 5 men) showed an unfavorable course, the remaining 10 patients (3 women and 7 men) presenting an improved aspect of the lesions of post-vacuum interest (Table 2) (Figure 1).

Following the centralization of the number of hospitalization days, it was possible to outline a distribution of patients, respectively, the minimum number of hospitalization days (six), as well as the maximum number of days of hospitalization (seventy-five). Intuitively, the minimum duration of hospitalization was recorded in the favorable outcome group. Somewhat more edifying, it turned out to be the average number of days of hospitalization, calculated for each group. Therefore, an average of 22 days of hospitalization corresponded to the group with a favorable outcome, while for the group with an unfavorable prognosis, the average number of days of hospitalization was 40 days, with an increase of approximately 45% of the average time spent in the hospital and all its related costs.

Regarding the associated pathologies studied, respectively type II diabetes, anemia and LLOA, were identified in both groups, but overall there was a greater aggregation of these comorbidities within the group with an unfavorable prognosis.

Figure 1: A. Myxofibrosarcoma of the thigh; B. Wound dehiscence after hip disarticulation; C. Wound dehiscence treated with NPWT; D. improved lesion post vacuum therapy

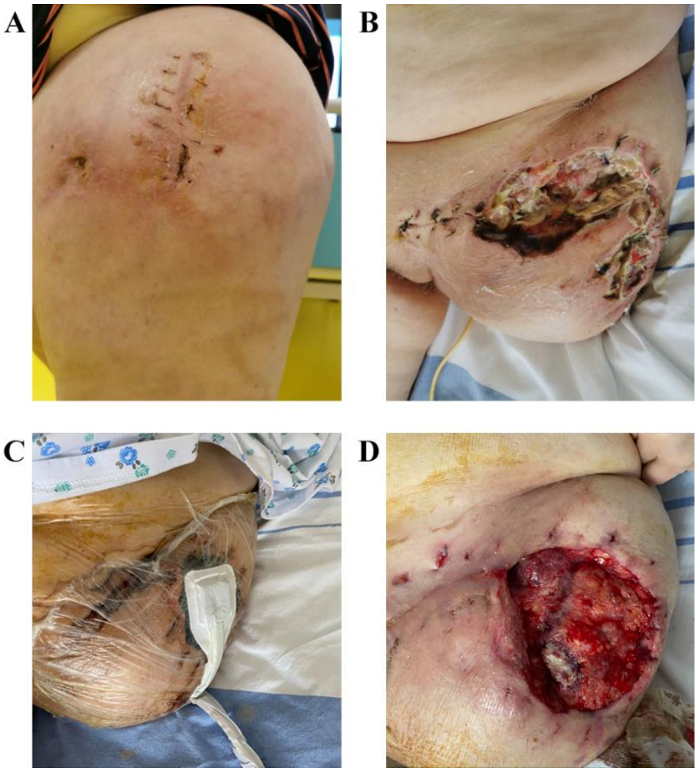

Table 2: The detailed situation of the study group situation regarding the evolution of the diagnosis and the number of hospitalization days.

Diagnosis at admission	Post NPWT	Hospitalization days
Post-operatory wound suppuration	Improved	6
Leg gangrene	Improved	11
Gangrene at the level of the stump after amputation of the toe	Gangrene at the level of the stump	11
Forefoot gangrene	Improved	12
Post-operatory wound seroma	Improved	15
Stump gangrene after amputation of fingers II-V	Improved	15
Post-operatory wound suppuration	Improved	19
Secondary purulent peritonitis (strangulated eventration)	Wound seroma	24
Finger V gangrene	Improved	25
Calf gangrene	Improved	27
Post-operatory wound suppuration	Wound suppuration	31
Necrotizing fasciitis, abdominal wall cellulite	Improved	36
Heel trophic ulcer	Trophic ulcer	40
Leg gangrene	Gangrene at the level of the stump	52
Secondary purulent peritonitis (perforated gastric ulcer)	Wound dehiscence	53
Post-operatory skin necrosis	Improved	56
Toe trophic ulcer	Gangrene at the level of the stump	75

The particularities of the distribution of comorbidities in the studied group of patients are as follows:

- 5 patients diagnosed with LLOA; 4 belong to the group with unfavorable evolution;
- 9 patients diagnosed with anemia; 5 belong to the group with unfavorable evolution;
- 12 patients diagnosed with type II diabetes; all 7 patients constituting the group with unfavorable evolution had type II diabetes.

Regarding the blood glucose values at admission, out of the 12 known patients with type II diabetes, the only values corresponding to the normal range (70 - 110 mg/dl) were recorded in 2 patients belonging to the group with favorable outcome, respectively 85 and 93 mg/dl. The rest of the patients, more precisely the 3 remaining patients of the group with favorable outcomes and the totality of the patients that formed the group with unfavorable prognosis registered glycemic values higher than the normal range.

The tendency was of aggregation of high glycemic values, with small exceptions, in the group with unfavorable evolution.

Upon admission, 9 of the 17 hospitalized patients presenting with the inflammatory syndrome. Of these, 5 patients had a favorable follow-up.

DISCUSSION

The heterogeneity of the studied group lies both in the variety of types of lesions for which vacuum therapy was chosen, and their pathological personal history.

It was noted that the shortest duration of hospitalization, as well as the lowest value of blood glucose at admission, were found in patients belonging to the group with favorable evolution, while their maximum values belonged to patients in the group with unfavorable evolution. This similar pattern of distribution of maximum and minimum values can offer a prediction of the number of hospitalization days based on blood glucose at admission. However, the result obtained was not statistically significant, the value of blood glucose at admission explaining only 0.5% of the number of days of hospitalization and the hypothesis that the value of blood glucose at hospitalization is a predictor of the prognosis of patients has not been confirmed. On the other hand, the statistical results obtained suggest that the glycemic value explains 17% of a patient’s evolution.

For a better understanding of the factors involved in guiding the evolution towards a favorable or unfavorable outcome, the second part of the study focused on a succession of 5 case presentations. All the selected 5 patients presented lesions with persistent or even worse follow-up under NPWT, the evolution as a whole of each case lying on the background of various comorbidities.

Regarding the first 3 cases, we present the outcome of 3 male patients with the ages of 53, 58, and 47 years, respectively. All these cases presented chronic lesions located in the lower limbs, as well comorbidities with significant resonance on blood perfusion: type II diabetes, LLOA, anemia (case 1 and case 3). In all 3 cases, the presence of LLOA is an important issue in the healing process of the lesions, which is why its solution by intervention or surgery plays a crucial role in the management process along with other surgical interventions and NPWT.

Unlike the cases presented above, case 4 is characterized by the vital risk of the associated pathology with which the patient presents, namely generalized peritonitis secondary to a revived, imprisoned multisacular eventration, complicated by strangulation, torsion, and necrosis of the intestinal loops. The patient's prognosis is impacted by the purulent post-operative wound, with a dragging character despite the NPWT applied. Regarding the unfavorable outcome of this case, we have to mention that in addition to the multiple comorbidities (type II diabetes, stage II hypertension, osteoporosis, bilateral gonarthrosis) and multiple personal pathological surgical histories (thyroidectomy, appendectomy, myomectomy, intestinal occlusion) in this case, the particularity emerges from the coexistence of short bowel syndrome and anorexia nervosa. Short bowel syndrome is the prerogative of an extensive surgical bowel section that involved subtotal enterectomy [23]. Anorexia nervosa is a complex behavioral and psychiatric pathology [24]. It is impressive how the two pathologies are intertwined and maintained an altered biological status of the patient, burdened by dehydration and electrolyte and acid-base disorders. All these overlapped with metabolic disorders caused by an unbalanced type II diabetes lead to a status of severe malnutrition. This context, associated with anemia, deprives the whole body, including the postoperative wound of nutrients needed for healing. One mechanism by which NPWT promotes the healing process is by increasing local blood flow, so implicitly the supply of oxygen and nutrients, but the beneficial effect is canceled if the blood flow to the wound is poor in oxygen and deprived of nutrients [25].

In selected case 5, the patient presents for a parietal suppuration in the right inguinal region after surgery with alloplastic materials for the right inguinal hernia. The patient's personal history includes an evacuated inguinal parietal abscess, which occurred after the surgical correction of the right inguinal hernia. In terms of comorbidities, the pathologies representative for the group with unfavorable evolution, respectively diabetes and anemia were noted. In addition, cardiovascular abnormal findings and a prostate adenoma were diagnosed. In the management process, an abscess underlying the pubic symphysis is identified. Surgical treatment aimed to remove all the debris and a cartilaginous fragment, subsequently applying NPWT to the semi-open wound. The patient was discharged healed and in good general condition.

At the first follow-up visit, the patient presents with a dehiscent wound with a visible granulation process and with a good general condition. However, at the second reevaluation, the biological and clinical condition of the patient was significantly worse (presence of fever and inflammatory signs), the right inguinal region appeared pasty and edematous, with the suppuration area located at the inferior area of the wound. Imaging results (inflammatory phenomenon extended to the muscle groups adjacent to the suppurative process, multiple lymphadenopathies, densification of subcutaneous inguinoscrotal and bilateral gluteal tissue, the osteolytic appearance of pubic bones) corroborated with other clinical and paraclinical investigations outlined the diagnosis of pubic osteitis. This diagnosis placed the case on a higher hierarchy in terms of gravity. It is to note that under conservative treatment the symptomatology diminished.

In an attempt to understand the unfavorable evolution of the patient, certain aspects should be discussed. First, the patient was the oldest from the study group (80 years) and the specialty literature mentions the alteration of healing processes in the advanced senescence process [4, 20, 21]. Secondly, there were various comorbidities, of which poorly controlled diabetes, which is responsible for the management difficulties of traumatic wounds. In addition, the infectious factor must be brought into question - staphylococcus aureus was isolated from the patient's wound – a virulent pathogen that causes treatment difficulties, even in people without a significant amount of comorbidities.

There are several limitations of this study that should be mentioned. One of these is the small number of patients included. Also, the study was retrospective and the data were collected from indirect sources, namely the computer database of the hospital, with no information available regarding the materials (filler material, wound contact layers) and the functional parameters applied (pressure level, pressure regime, vacuum source). Certain biological parameters crucial for documenting the prognosis of the case could not be identified, for which reason it was not possible to include them in the statistical analysis.

CONCLUSIONS

According to our study results, the majority of patients for which negative pressure wound treatment was applied presented a favorable outcome.

In order of frequency, the diagnoses at admission for patients included in our study were as follows: gangrene with various localizations in the lower limb, local complications of postoperative wound, legs trophic ulcers, secondary purulent peritonitis, necrotizing fasciitis, and parietal-abdominal cellulitis.

The average number of hospitalization days for the group with unfavorable outcomes was almost double compared to that of the group with favorable evolution, which engaged higher costs.

Pathologies such as diabetes, lower limb obliterative arteriopathy, and anemia had a prevalent distribution in the group with unfavorable evolution. According to the statistical analysis, the glycemic value can predict 17% of a patient's prognosis scenario (favorable/unfavorable).

The cases presented in the study mean to highlight the importance of an immediate, integrative and exhaustive therapeutic approach. Although an extremely promising therapeutic option, NPWT can prove to be disappointing, given its impact in the background of suboptimally managed comorbidities.

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Authors' contribution:

GP - design of the paper; CIM - drafting of the paper; VAB - analysis of data; NT - interpretation of data; MMC - revising of the paper; BC - final approval of the paper; CC - final approval of the paper All authors read and approved the final manuscript.

Ethics approval and consent to participate:

The study was done following the ethical standards stipulated by the Helsinki Declaration.

Patient consent for publication: Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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ORIGINAL ARTICLES

New anatomical landmark for rotational assessment of total knee arthroplasty

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Abstract: *One of the most controversial topics in total knee arthroplasty is rotation of the femoral component. The current gold-standard in total knee arthroplasty consists in positioning the femoral component in 3 degrees of external rotation to the epicondylar axis, having as reference the tangent to the posterior condyles. Achieving the correct rotation of the femoral components is one of the main goals during total knee arthroplasty. Multiple complications can result from internal femoral rotation, such as lateral patellar tilt, patellar subluxation or dislocation, mobilization with movement on pain, and low survival rates of the femoral components. Postoperative rotational assessment of protective components can only be performed correctly using computed tomography. The known evaluation methods are related to anteversion of the femoral neck, femoral trans-epicondylar line, insertion of the posterior cruciate ligament from the tibia and tibial tuberosity. The purpose of this study was to evaluate the rotation of the prosthetic components in the group of patients with the methods validated by studies and to find alternatives to evaluate the rotation of the femoral component. Thirty-four patients diagnosed with gonarthrosis, proposed for total knee arthroplasty, were included in the study. This is a prospective cohort study in which applied statistics consisted of analyzing data using frequency and percentage for qualitative and mean variables and standard deviation for quantitative variables.*

Keywords: *total knee arthroplasty, femoral component rotation, posterior condylar angle, linea aspera*

INTRODUCTION

Rotation of the femoral component is one of the most controversial topics in total knee arthroplasty [1]. From the preoperative assessment required to determine the rotation of the femoral condyles relative to the hip and knee followed by the intraoperative decision to position the femoral component in a slight external rotation to the trans epicondylar axis and to the postoperative evaluation of the rotation, all these are subject to controversies of analysis and decision that may represent another factor of error in the postoperative evolution of the patient [2].

The current gold-standard in total knee arthroplasty consists in positioning the femoral component in 3 degrees of external rotation to the epicondylar axis, having as reference the tangent to the posterior condyles. This is a surgical move that will place the femur in a slight external rotation. It is said that this surgical move will minimize the possible complications as patello-femoral/femoro-tibial movement and arthroplasty survival over time.

Postoperative rotational assessment of protective components can only be performed correctly using computed tomography. The known evaluation methods are related to anteversion of the femoral neck, femoral trans-epicondylar line, insertion of the posterior cruciate ligament from the tibia and tibial tuberosity. If the two femoral epicondyles are difficult to identify on CT scans even with software for removing metal artifacts, it was decided to introduce new signs of rotational CT analysis, which materialized through the linea aspera as a radiological landmark.

Achieving the correct rotation of the femoral components is one of the main goals during total knee arthroplasty [3].

The proper alignment of the femoral component during total knee arthroplasty is a crucial step that influences postoperative results. The position of the femoral

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component in the axial plane is responsible for flexion stability, knee joint kinematics, flexion alignment, and patellar tracking.

Complete axial plane measurement before and after total knee arthroplasty can only be performed using CT scans. For a proper assessment of total knee arthroplasty, the spread of metal artifacts should be reduced and anatomical signs should be properly identified, which is sometimes difficult to achieve mainly for the two femoral epicondyles. Linea aspera is an anatomical sign that is constant and it is not influenced by metal artifacts.

The purpose of this study was to evaluate the rotation of the prosthetic components in the group of patients with the methods validated by studies and to find alternatives to evaluate the rotation of the femoral component.

The study focused on comparing the standard evaluation of femoral component rotation was performed using the posterior condylar angle (PCA) with a new sign that is using the linea aspera ridge and the Akagi line was used for the rotational evaluation of the tibial component [4]

MATERIALS AND METHODS

Thirty-four patients diagnosted with gonarthrosis, proposed for total knee arthroplasty, were included in the study. All the patients analyzed were from the orthopedics and traumatology clinic of the Bucharest University Emergency Hospital during 2018-2019. The study was approved by the hospital's ethics commission in compliance with international research ethics standards. The following angles were measured using CT scans: the angle between a tangent to the posterior condyles and the surgical trans-epicondylar axis (surgical TEA) called the posterior condylar angle (PCA) (Figure 1), the angle between a tangent to the posterior condyles and the tibial component axis (Akagi line) (Figure 2) for rotational assessment of the tibial component.

The posterior condylar angle was compared with a new sign (Figure 3) measured preoperatively and postoperatively as follows: 15 cm proximal to the joint line, is identified and circumscribed in a circle linea aspera, the femoral shaft is also circumscribed in a circle, and the center of the two circles describes a line and the angle between this line is called the linea aspera and a tangent to the posterior condyles was called the aspera condylar angle (ACS) preoperative and postoperative.

This is a prospective cohort study in which applied statistics consisted of analyzing data using frequency and percentage for qualitative and mean variables and standard deviation for quantitative variables. The ANOVA test with repeated measurements was also used to compare the different variables, as well as the Pearson test to verify the correlation between the variables.

Figure 1: Tangent to the posterior condyles

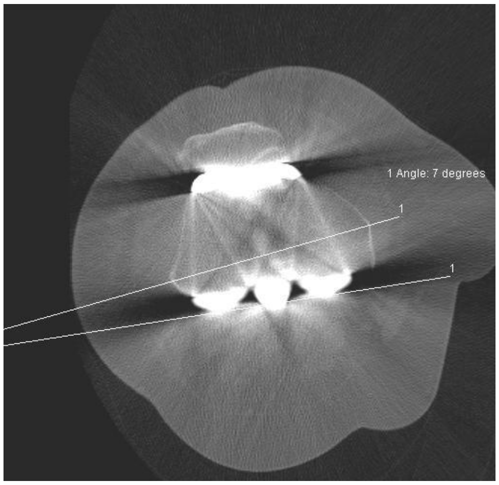

Figure 2: Akagi line

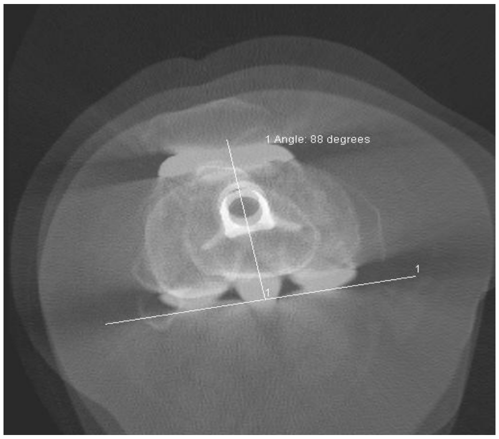

Figure 3: New landmark

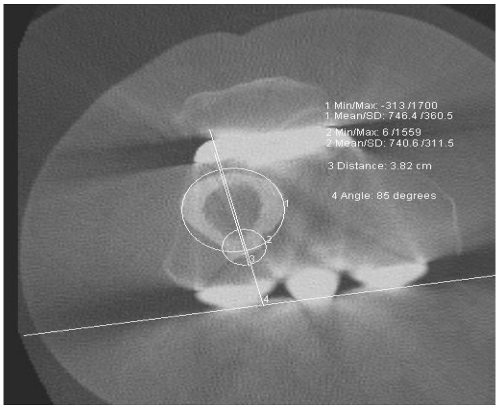

The landmark proposed for validation was evidenced following the steps: 15 centimeters proximal to the joint line, the linea aspera and the femoral shaft were identified. Both were framed in a circle and the centers of the two circles were identified, later joining the two identified points by a line, called the linea aspera. The purpose was to evaluate the angle made by the tangent to the posterior condyles (standard and easily identifiable sign) with this line in the preoperative and postoperative phases.

The main hypothesis of this study was represented by the complex evaluation of the rotation of the prosthetic components in a patient undergoing total knee arthroplasty to understand the clinical effects of malrotations.

RESULTS

Regarding to the degree of reliability of radiological measurements, it was rated from 1 to 5, 5 representing the highest degree of confidence. There is a percentage of 45.71% of specialists who gave a degree of confidence 5, followed by a degree of confidence 4 (37.14%) and a degree of confidence 3 (17.14%).

Regarding to the quality of the acquisition of radiological images, it was rated from 1 to 5, 5 representing the highest degree of confidence: a percentage of 51.43% gave 5, equal to 3 and 4 (20%) and 8.57% gave 2.

Table 1: Average values of the new landmark in the preoperative-postoperative and PCA phases

		New preoperative reference	**New postoperative reference**	**PCA**
No.	Valid data	35	35	35
	Missing data	0	0	0
Average		89.3400	93.1309	3.1034
Standard deviation		12.28395	12.37648	1.70943

The average values of the quantitative variables are depicted in Figures 4, 5 and 6.

Using the Pearson test, there is a statistically significant and strong correlation between the new preoperative reference and the new postoperative reference (r = 0.98, p = 0.001). There were no statistically significant correlations with PCA.

Applying the Anova test with repeated measurements, it was concluded that there is a statistically significant difference in the evolution of the new sign in the preoperative phase and the postoperative phase (p = 0.001, 89.34 vs. 93.13).

Using the ANOVA method, there were no statistical differences in the level of specialists who awarded the confidence levels and the new preoperative sign, the new postoperative sign and PCA, who had similar averages.

Figure 4: Distribution of the new preoperative reference. The average value of the new preoperative reference is 89.34 (12.28)

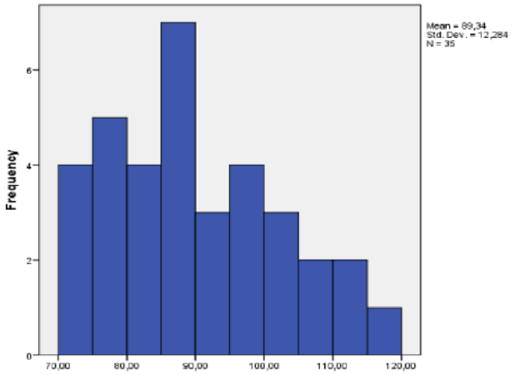

Figure 5: Distribution of the new postoperative reference. The average value of the new postoperative reference is 93.13 (12.37)

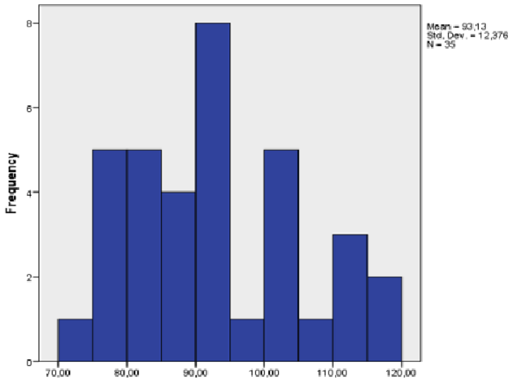

Figure 6: PCA distribution. The average PCA value is 3.10 (1.70)

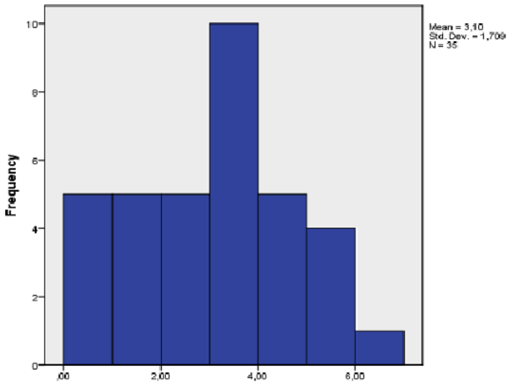

Using the ANOVA method, there were no statistical differences in the level of specialists who assessed the quality of the acquisition and the new preoperative sign, the new postoperative sign and PCA, which had similar averages.

DISCUSSION

Establishing the proper rotation of the femoral component is unanimously accepted as an important point to achieve in total knee arthroplasty. As Mochizuki pointed out more than

30 years ago, the correct rotational positioning is in a slight external rotation [5].

Table 2: Correlations between the new preoperative and postoperative references and PCA

		New preoperative reference	New postoperative reference	PCA
New preoperative reference	Pearson correlation	1	0.982 **	-0.274
	p		0.000	0.111
	No.	35	35	35
New postoperative reference	Pearson correlation	0.982 **	1	-0.218
	p	0.000		0.209
	No.	35	35	35
PCA	Pearson correlation	-0.274	-0.218	1
	p	0.111	0.209	
	No.	35	35	35

Multiple complications can result from internal femoral rotation, such as lateral patellar tilt, patellar subluxation or dislocation, mobilization with movement on pain, and low survival rates of the femoral components [6].

Also, an internal femoral rotation will increase the laxity in the lateral compartment during flexion, varus deviation and unfavorable clinical results [7].

Conversely, excessive external femoral rotation will result in poor functional results, and the patient will experience some degree of flexion instability.

Internal rotation of the femoral component combined with internal rotation of the tibial component are the leading cause of anterior pain after total knee arthroplasty [8].

Soft tissue rebalancing and femoral component rotation can be performed by the two known techniques: the measured resection technique and the “tension gap” technique (extension space equal to flexion space) [9,10]. A rotation of 3 degrees to the tangent to the posterior condyles is unanimously accepted in the technique of measured resection but not always perfect [11, 12].

In the case of the technique of measuring the extension and flexion spaces, it can be performed by two mechanisms: 1. the use of a conventional spacer, but there is no consensus on the tension that need to be applied; and 2. the use of blocks with predetermined dimensions that must be introduced in the two spaces, a maneuver that is strictly subject to the surgeon's experience and is not providing objective information regarding to the tensions in the two compartments [13, 14].

A randomized study comparing the two techniques states the superiority of using a mechanical spacer (laminar spreader) in a resection measured to establish an appropriate femoral rotation [15].

Intraoperative technique of rotational positioning of the femur is relatively standardized and well supported scientifically with prospective and randomized clinical trials. The superiority of one technique over another is not proven. Both techniques will position the femur in a slight external rotation which has proven beneficial for the vast majority of patients for both immediate and long-term results.

The rotational positioning problem for a small number of patients with low satisfaction rates, results from standardization itself, not taking into account the rotation of the femoral condyles relative to the femoral neck and the entire axis of the pelvic limb in the preoperative stage.

In the case of patients in whom a significant deformity of the entire pelvic limb can be observed during the standard preoperative assessment, radiological examination of the pelvis may reveal changes of coxa vara or valga type, an examination of the orthopantogram reveals an exaggerated femoral or tibial curvature, clinical examination indicates altered gait due to deformities and a CT scan of the entire pelvic limb may be recommended. This will evidence rotational changes that do not fall within the preoperative standard assessment and may impose intraoperative decisions specific to each patient.

Starting from the fact that the two femoral epicondyles needed to trace the trans epicondylar axis to measure the rotation of the femoral component are both difficult to identify in the preoperative stage but especially after prosthetic implantation due to metal artifacts, we searched and identified another anatomical sign, unchangeable for rotational assessment and specific to each patient.

CONCLUSIONS

Following the computed tomography analysis of the first patients admitted to the study, an initial conclusion was reached, namely that the evaluation of the rotation of the femoral component should be performed after the trans-epicondylar line, which is difficult to identify correctly on CT scans, even with removal of artifacts. It was concluded that it is necessary to find a new radiological sign for the rotational assessment of the femoral component.

For all cases included in this pilot study, the femoral component was placed intraoperatively at 3 degrees of external rotation relative to the posterior condyles. The analysis of the new radiological sign evidences the external rotation of the femoral component, when it is performed taking as a sign the tangent to the two posterior femoral condyles.

Linea aspera has proven to be a reliable and useful anatomical sign for measuring the rotational positioning of the femoral component in total knee arthroplasty due to its constant position and because its identification is not obstructed by metal artifacts.

This new sign can be used in the same patient in the preoperative and postoperative phase, using CT examination. However, no correlation could be identified with the established assessments between the values obtained from the measurements, a correlation possible only in much larger studies, with larger patient groups.

It has been proposed to use this sign for radiological analysis of total knee arthroplasty in patients in whom preoperative CT examination is performed due to major axis deformities or bone stock deficits, followed by postoperative CT analysis.

The high variability of the linea aspera compared to the posterior condyles in the preoperative evaluation evidences the rotational complexity of the knee and the need for in-depth knowledge of its kinematics in relation to the entire pelvic limb.

The results of the measurements with the help of the proposed new sign show that it can be used in extreme cases in the rotational assessment of the femoral component.

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ORIGINAL ARTICLES

New approaches regarding the protection forces’ health against the effects of some toxic substances

Pavel Otrisal[1], Camelia Diaconu[2], Ovidiu Bratu[3], Florentina Ionita Radu[3], Zdenek Melicharik[4], Simona G. Bungau[5]

Abstract: *In the past, finding out the chemical resistance of the barrier materials designated for the production of individual protective equipment ofthe protection forces (policemen, firefighters, soldiers) was based on the need to respond to protection against weapons of mass destruction. Simple chemical methods based on indicating the colorimetric reactions were used. It was necessary to switch to more universal methods due to a change in the security of the environment and the need to focus on toxic industrial materials. The universality is due to using sensitive QCM sensors equipped with a special detection layer and some special carbon electrodes. The paper deals with new methods that are used to determine the chemical resistance of the barrier materials against the permeation of toxic substances, primarily in field conditions. However, they can also be used in stationary chemical laboratory conditions.*

Keywords: *Permeation, barrier material, PIEZOTEST, KONDUKTOTEST, SORPTEST*

INTRODUCTION

Public health is and must be a major interest for the policies and legislation of a country. Maintaining and improving it must be done by implementing appropriate measures and programs, by educating the population and by raising the standard of living. A particular sector within this field of health is represented by the health insurance of the protection forces (police, firefighters, soldiers). In addition to psychological training, physical training, proper nutrition, etc., they must be provided with the optimal equipment to cope with specific interventions. Exposure to various harmful factors (fire, extreme heat, chemicals, pollutants of all kinds, etc.) must take place in the optimum parameters, ensuring the best performing materials, with maximum level of protection [1]. Direct contact with these substances (drugs, chemicals, flammable substances, poisons, narcotics, various medical and pharmaceutical waste) [2-4] which can usually be found in nature and especially in cases of emergency (fire, war, explosions) can often be lethal, through harmful compounds that are released or which, in direct contact with the skin can cause severe burns, major infections, dizziness, fainting, headache, choking, serious pathologies as cancer [5, 6] etc., and finally – death.

The interest to produce protective materials impervious to possible contamination agents has increased in recent years. The political decision makers, as well as the economic agents and the manufacturing companies (that are producing harmful substances, pollutants, as well as those who's

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activity field involve obtaining the performant barrier materials) concentrate their activity more and more intensely on this aspect of the development of the protective equipment with high quality, reduced cost, with high demand on the market and which generate waste as little as possible following the production process [7-14].

In the last decade, there has been a shift from relatively simple chemical colorimetric methods for testing the resistance of the barrier materials against the permeation of toxic substances to sophisticated methods based on a principle of sensor technologies [15-21]. This change was called by a gradual re-evaluation of the security threats experienced by military professionals in military operations [22, 23]. Representatives of the North Atlantic Treaty Organization declared that high use of weapons of mass destruction is currently unlikely [24]. Industrial chemicals are now considered to be a significantly more important security threat because their leakage can cause many losses and can cause contamination of large areas of interest [25, 26]. New methods that enable the testing of the chemical resistance of the barrier materials against the permeation of toxic substances find their practical application also in testing of already established final products and also of the barrier materials that may find their use in the future [27, 28]. New methods also allow very fast evaluation of the permeation data found and meet all requirements set by military and European standards [29]. The concept of research, development, and production of permeation devices using sensor technologies has been designed to meet the requirements for their use in field conditions and to enable obtaining permeation data with minimal attendance and service [30-32].

All these aspects have led to a gradual decrease in the efficiency of work with colorimetric methods, applicable only for determining the chemical resistance of toxic substances (used in wars) under simulated laboratory conditions. These methods did not allow a detailed analysis at the level of the North Atlantic Treaty Organization nor at the European Union level.

PIEZOTEST method

The PIEZOTEST method uses a QCM (Quartz Crystal Microbalance) based piezoelectric sensor to detect permeated toxic substances. These sensors, which find their application in many technical and industrial applications, have been modified to detect the permeation of the toxic substances through the barrier materials by applying the appropriate detection layer to guarantee the functionality of this sensor as a gravimetric detection device. The 1 Hz sensor's response is reported to correspond to the detection of 1 ng of a toxic substance. This sensitivity is quite enough for operating the measuring device in both field and laboratory conditions. Application and software equipment, which is an integral part of the measuring equipment, allows on-line monitoring of a wide range of standardized parameters. The most important data is the breakthrough time (or also the resistance time) when the permeation mass's (cumulative amount) value is reached for a particular (subtracted) value based on the permeation mass (cumulative mass) versus time graph. However, it can also be specified as the normalized breakthrough time, but only if it relates to the normalized permeation weight (cumulated amount) specified by the relevant standard. For fast data evaluation without the need for application and software equipment, it is possible to use the value of protection time (chemical resistance time) expressed as lag-time, to which the value of diffusion coefficient is automatically recalculated. The time to reach the maximum permeated concentration, which is sometimes referred to as the time of half diffusion flow, is also significant for the purposes of assessing the capture efficiency of the test chemicals through barrier materials. The permeation calculator automatically recalculates the value of the respective diffusion coefficient to this value, which creates a space for comparing the penetration rate of the test chemical through the tested barrier material. The time to reach the maximum permeation rate (when the lag-time is reached) and the maximum reached permeation rate (when the steady state permeation rate is reached) is very important for the evaluation of the breathable barrier materials is very significant. Results of the calibration measurements showed that it is possible to achieve high-quality dependencies of the QCM sensor working frequency increase, thus the concentration of test chemical over time, even though this method was not primarily intended as analytical.

KONDUKTOTEST method

The KONDUKTOTEST method is based on the basic analytical method working on electrochemistry, thus dissociation of ions. It introduces practical knowledge of conductometry into the implementation testing practice and adapts them to the conditions for determining the chemical resistance of barrier materials against the permeation of toxic substances. A significant benefit of the used measuring device is the replacement of very expensive platinum electrodes with glass electrodes. These electrodes are characterized by a very good sensitivity and minimal maintenance and operation requirements. Their further advantage is that they do not experience problems with memory effect, which is an important and limiting factor for the electroanalytical methods. The elimination of this effect is based on the actual needs of measurement when working with very dilute solutions. In practice this means that a very low concentration of dissociated ions is sufficient to form a permeation curve, which serves as a basis for the reading of standard values. This concentration is so low that it does not produce the memory effect of the used carbon electrodes even after their repeated use. The KONDUKTOTEST method uses the dependence of electrical conductivity increaseover time to generate the resulting permeation data and evaluate the effectiveness of barrier materials to withstand to a direct contact with toxic substances. The other standard values are the same as in the previous case. The results of the calibration measurements confirmed the theoretical considerations associated with classical conductometry, which in practice means that high quality results have been achieved.

SORPTEST method

The SOPRTEST method suitably complements the abilities of the above two methods. Its primary use can be seen to determine the chemical resistance of barrier materials under dynamic conditions. Due to the combination of the conductometric measurement and the measurement based on the use of QCM sensors, a unique device has been constructed. It enables the combination of methods and used barrier materials. The permeation cells can be adapted to the particular type of measurement and the test material. In addition to breathable and insulating barrier materials, the sorption properties of the bulk sorbent layers can also be tested. By partial modification of the measuring system it is also possible to characterize filters of protective masks and thus to study their dynamic and static sorption capacity. The source of the gas phase of the test chemical is a test tube fitted with a Peltier´s cooler (condenser). The device enables continuous regulation of air flow during its use within calibration, actual measurement and desorption of QCM sensor. Before starting and during the measurement, it is possible to adjust the temperature, which ensures the transfer of the test chemical into the gas phase and the temperature of the Peltier´s cooler. Permeation measuring cells are tempered separately. A biological incubator has proved to be a suitable device. The SORPTEST measurement device allows the generation of the same standardized permeation data. This approach respects the principle of comparability of the observed permeation data across the older used measurement methods. Application and software equipment of this device enables creation of an output report from measurements. It includes results of calibration measurements and individual graphs characterizing the course of monitored standard quantities. The report also includes data concerning the chemical test that was used, its basic tabulated characteristics and toxicological data.

CONCLUSIONS

Obtaining permeation data characterizing barrier materials in accordance with European standards enables their unambiguous description not only at the national level but also at the European level.This approach will allow describing the barrier properties not only during their use, but also during their development and quality verification. The combination of methods for the implementation the knowledge of physics, sensor technologies, classical analytical chemistry and toxicology has resulted in the construction of very modern and sophisticated measuring systems that meet the highest demands on the quality of the obtained data while maintaining economic rationality.

Compliance with Ethics Requirements

The authors declare no conflict of interest regarding this article

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ORIGINAL ARTICLES

Non-melanoma skin cancer (NMSC): Extramammary Paget ’s disease

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Abstract: *Non-melanoma skin cancer (NMSC) comprises squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and severalrare skin tumors (i.e., Extramammary Paget’s disease), being the most common malignancy-affecting people worldwide. Itrepresents the majority of skin cancers and a significant percentage of all malignancies. Despite increasing public awarenessand scientific interest, the incidence of NMSC is constantly increasing. Even though most NMSC`s are associated with lessaggressive behavior, they can still be invasive locally and cause extensive damage to neighboring structures, inducingsignificant morbidity. In addition, different types and subtypes of NMSC tend to have frequent recurrences and may have significantmetastatic potential. As a direct consequence, NMSC has become an important issue for healthcare systems with a significantsocio-economic impact.*

Keywords: *Extramammary Paget’s disease; World Health Organization; keratinocyte stem cells; EMA*

INTRODUCTION & BACKGROUND

Non-melanoma skin cancer (NMSC) comprises squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and several rare skin tumors (i. e. Extramammary Paget’s disease), being the most common malignancy affecting people worldwide. It represents the majority of skin cancers and a significant percentage of all malignancies. Despite increasing public awareness and scientific interest, the incidence of NMSC is constantly increasing. Even though most NMSC`s are associated with less aggressive behavior, they can still be invasive locally and cause extensive damage to neighboring structures, inducing significant morbidity. Also, different types and subtypes of NMSC tend to have frequent recurrences and may have significant metastatic potential. As a direct consequence, NMSC has become an important issue for healthcare systems with a significant socio-economic impact.

EXTRAMAMMARY PAGET’S DISEASE (EMPD)

EMPD localized to the vulva is a rare disease that canmimic different types of dermatosis and only four cases havebeen reported in our practice in the last 5 years.

Definition

EMPD, according to World Health Organization, is a rare intraepithelial neoplasm characterized histopathologically by a prominent growth of large cells, with apocrine or eccrine glandular-like pattern, originating in the skin (primary EMPD) or representing intraepithelial spread of a hidden visceral carcinoma (secondary EMPD). This type of disease is represented by the International Classification of Diseases for Oncology with the following code: 8542/3. [1, 2]

EMPD is a marginated plaque that clinically and histologically

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resembles Paget's disease, but occurs in sites rich in apocrine glands, such as vulva, anogenital region and axilla. Currently, there is controversy as to how often this condition appears against on the background of an underlying carcinoma and how often it occurs primarily in the epidermis or apocrine ductal tissue in the affected area. This gave rise to the concept of primary and secondary extra-mammary Paget's disease [3, 4].

Epidemiology and Etiology

EMPD accounts for about 1 % of all ano-genital neoplasms. It develops more frequently in Caucasian women in the postmenopausal period [1] and usually begins in the fifth decade or later. Current opinion is that, in about 75% of cases, extracellular Paget's disease appears as a primary intra-epidermal neoplasm, possibly from ductal cells of the apocrine gland or keratinocyte stem cells. Another possible origin may be the Toker's cells [3, 5]. In the remaining 25% of cases, there is a hidden primary adenocarcinoma. These cases are referred as secondary Paget's disease.

Only a few cases of association between mammary and vulvar Paget's disease have been described. Under extraordinary circumstances, EMPD is a secondary event caused by extension of an adenocarcinoma either of the rectum to the perianal region, or of the cervix to the vulvar region. On the other hand, genital Paget's disease with a long evolution, can extend into the cervix and urinary tract [6].

Localization

The labia majora is the most widespread site, followed by the labia minora and clitoris. The perineum, perianal area, scrotum, penis, lower abdomen and inguinal folds can also be affected [1].

Based on origin, several classification schemes have been proposed [22] :
Type 1: primary vulvar cutaneous origin
Type 2: secondary to primary anal or rectal adenocarcinoma
Type 3: secondary to urothelial carcinoma

OR

Type I: Paget disease of primary cutaneous origin
Ia: as a primary intraepithelial neoplasm
Ib: as an intraepithelial neoplasm with invasion
Ic: as a manifestation of underlying adenocarcinoma of skin appendage or vulval glandular origin
Type II: Paget disease of noncutaneous origin
IIa: secondary to anorectal carcinoma
IIb: secondary to urothelial neoplasia
IIc: as a manifestation of another noncutaneous carcinoma: endocervical, endometrial or ovarian adenocarcinoma, etc.

Clinical features

The most common presentation is a relatively well-demarcated flat or slightly elevated, scaly, erythematous patch or plaque, accompanied by itching or burning, often simulating eczema. Unusual forms include lesions with hyper- or hypopigmentation and multi-centric bruising [1].

EMPD lesion has many features in common with Paget's disease of the nipple. The edges are sharp, rounded and slightly raised, and includes an area that is pink or red. The affected area may be tiled, and small, gray-colored crusts may cover erosion. Itching is an essential feature and there may be excoriations or lichenification. Areas of fluctuating hyperpigmentation may be present, causing confusion between extra-mammary Paget's disease and superficial spread melanoma. In some cases, leukoplakia may be present [3, 7, 8, 9].

The clinical presentation of the disease varies by site, the most frequent involved being vulva. The first symptom, especially in vulvar lesions, is itching and burning, which can be persistent and growing. It is often seen as dermatitis and may be disturbed by topical therapy. The surfaces of the lining of the labia are periodically redder than the skin when both areas are involved, and the change may proliferate towards the thighs, mons pubis and vaginal introitus. There will be a limited papillomatous area. Characteristic clinical features include fierce advancement, challenging all local applications, and the whetted margin. Eventually, one area may become thickened and ulcerated as evidence of downwards invasion. Lymph nodes involvement or distant metastases can appear. Although most cases of primary carcinoma result from a hidden adenocarcinoma of the sweat gland, it is essential to examine the patient for evidence of a later adenocarcinoma hidden in the cervix and rectum [3].

Pathogenesis

Histogenesis of primary EMPD is unknown [1].

Paget cells in the epidermis may appear as an in situ upward extension of an adenocarcinoma located in the deeper glands. Alternatively, EMPD may have a multifocal primary origin in the epidermis and skin appendages, and does not represent an epidermotropic spread or metastasis from an intrinsic carcinoma of the sweat gland. It can be shown that almost 25% of EMPD originates from primary adenocarcinoma of the secretory glands. Primary tumor and Paget cells are usually mucus secretory [10].

At one point, it was widely accepted that in primary EMPD, Paget cells in the epidermis are due to an in-situ upward extension of an in-situ adenocarcinoma of the sweat glands along the eccrine or apocrine ducts. This theory is comparable to the generally accepted view that secondary EMPD shows an extension of a cervical, rectal or urinary adenocarcinoma and that the progression of Paget breast disease is an extension of a lactiferous canalicular epidermotropic carcinoma. The fact that a hidden adenocarcinoma often could not be demonstrated, can be explained by the different anatomic characteristics between the breast, which has only 20 large, conspicuous lactiferous ducts, and the genital skin, which has thousands of small apocrine and eccrine glands, making the localization of this particular small gland invaded by carcinoma increasingly difficult [6].

Histopathology

EMPD disease has in common with Paget's disease the presence of varying numbers of Paget cells within the epidermis (Figure 1). In some cases, the Paget cells are found to be limited to the acanthotic epidermis, accompanied by hyperkeratosis, parakeratosis, erosion or ulceration. Frequently, Paget cells can be identified within the epithelium of hair follicles or eccrine sweat ducts. This types of in situ malignancy associated with EMPD has the same favorable prognosis as EMPD limited to the epidermis. However, the prognosis is much worse in cases in which the Paget cells have invaded the dermis from the epidermis or from an underlying sweat gland carcinoma. Glandular clusters with a central lumen, absent in mammary Paget's disease may be identified in the lower epidermis in extra-mammary Paget's disease [6].

Figure 1: EMPD. Paget cells in epidermis (photomicrograph taken from our cases), hematoxylin &eosin, magnification 200x

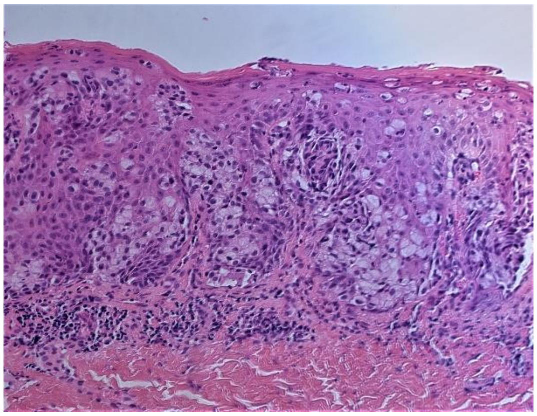

Serial sectioning of excised lesions of vulvar EMPD demonstrated that the lesions within the epidermis and its appendages have a multifocal origin. In the cases that have extensive epidermal lesions, the involvement of eccrine glands and ducts is occasionally, and the involvement of apocrine glands and ducts is absent, even if some Paget cells form apocrine glandular structures in the epidermis. Despite the existence of cohesion between the epidermal and the ductal foci, no one can definitive say that the extension is in an up or down direction. The dermal invasion in EMPD has its beginning point in the epidermis and not ductal or glandular structures.

The origin of the Paget cell may be the poral portion of an apocrine duct, or the pluripotential germinative cells within the epidermis [6]. Paget cells have an abundant basophilic to amphophilic cytoplasm and vesicular nuclei with pronounced nucleoli. They are generally distributed in a solitary fashion or may form gland-like structures or well-demarcated solid nests. Involvement of adnexa (hair follicles and eccrine ducts) is seen in most cases. Rarely, single cells, glands and solid nests can be found in the dermis; this is considered to be microinvasion. Dermal invasion is rare in vulvar EMPD, more common in perianal region. However, this type of microinvasion cannot be applied in cases that are associated with an underlying adenocarcinoma with a ductal in-situ component [1].

Immunohistochemistry

Immunohistochemical staining shows that Paget cells stain consistently positive for CK7 (cytokeratin 7) [1] (Figure 2) and EMA (Epithelial membrane antigen) – used by pathologists like a marker of Paget disease [11] (Figure 3).

Figure 2: Paget cells showed positivity for CK7 (cytokeratin 7also called as keratin 7, CK7, KRT 7, K7) (photomicrograph taken from our cases), magnification 200x

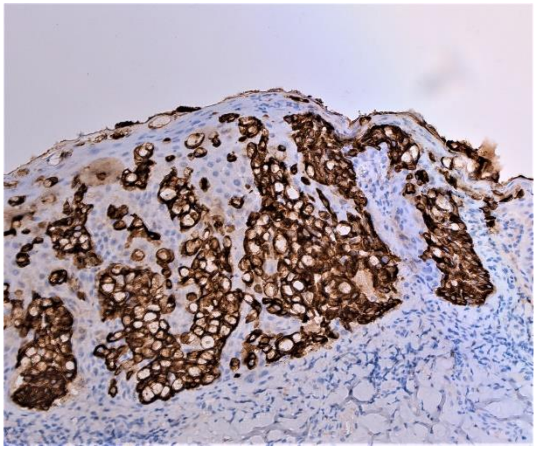

Paget cells often express GCDF-P15 (gross cystic disease fluid protein) (Figure 4), ERBB2 (HER2/neu) (Figure 5), CEA (carcynoembrionic antigen), CA125 (cancer antigen 125/ MUC16), and AR (androgen receptor) [1].

Figure 3: Paget cells showed positivity for EMA (epithelial membrane antigen also known CD227, MUC1 or episialin) (photomicrograph taken from our cases), magnification 200x

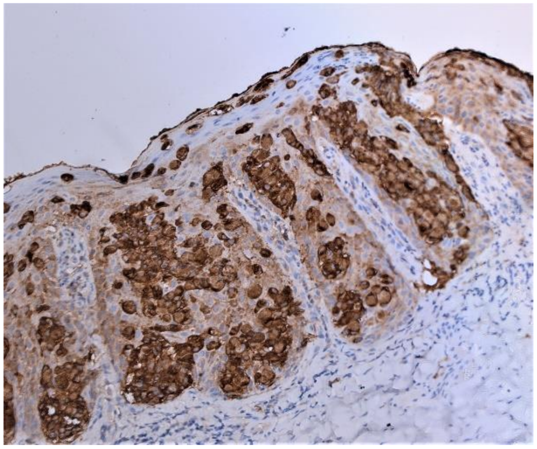

Figure 4: Paget cells showed positivity for GCDFP-15 (gross cystic disease fluid protein also known BRST2, PIP-prolactin induced protein) (a photomicrograph taken from our cases), magnification 200x

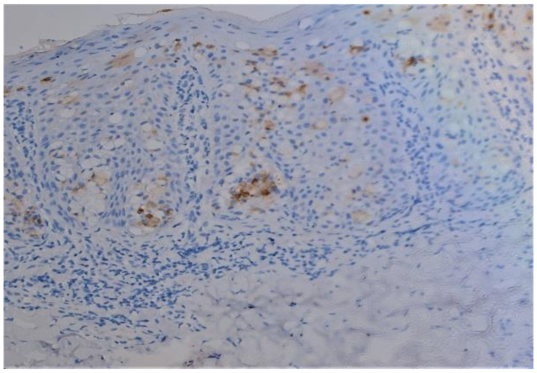

Figure 5: Paget cells showed positivity for ERBB2 (human epidermal growth factor receptor 2, also known HER2/neu, c-erbB2, CD340)(photomicrograph taken from our cases), magnification 200x

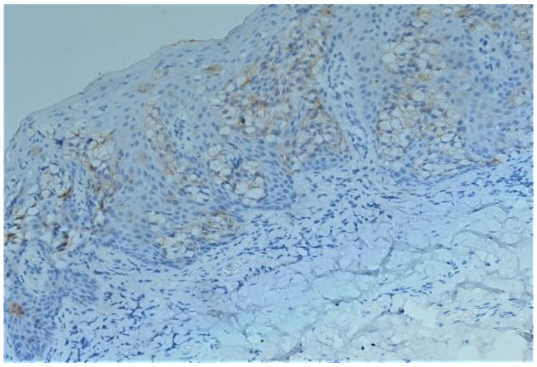

Differential diagnosis.

For correct diagnosis, immunohistochemistry and clinico-pathological correlation are essential [1].

The most pressing matter is distinction between primary and secondary EMPD (Table 1). Potential imitators of EMPD are Toker vulvar cells, vulvar and penile analogues of cervical stratified mucin-producing intraepithelial lesion, high-grade squamous intraepithelial lesion, melanoma, pagetoid dyskeratosis, and clear cell papulosis. [1]

Table 1: Differential diagnoses of Extra-mammary Paget's disease (Immunohistochemical Markers). [12]

Immuno-histochemical marker	Primary extra-mammary Paget's disease	Secondary extra-mammary Paget's disease	Bowen's disease	Superficial spreading melanoma
CK7	+	+	+ or -	-
EMA	+	+		
CEA	+	+	-	-
GCDFP-15	+	-	-	-
Melan-A	-	-	-	+
P63	-	-	+	
S100 protein	-	-	-	+
HMB45	-	-	-	+

For differential diagnosis with superficial spreading melanoma we currently use as immunohistochemical markers S100 protein and HMB45 (Figure 6), and we do not use Melan-A because it has often given false positive results (Figure 7).

Figure 6: Paget cells showed negativity for HMB45 (Human Melanoma Black) (photomicrograph taken from our cases), magnification 100x

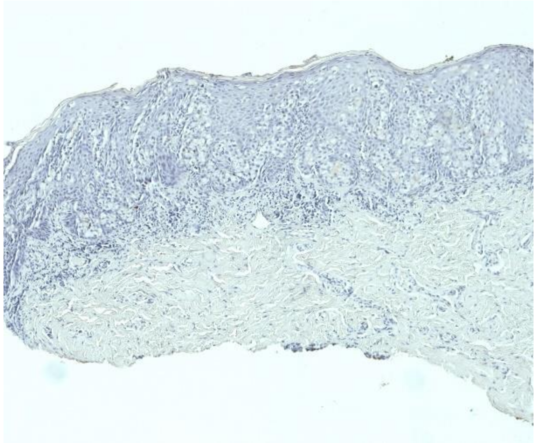

For proper localization of a large perineal lesion (vulvar or perianal) we usually use GCDFP-15 (positive for vulvar localization) and CK20 (positive for perianal localization) [13]

Prognosis and treatment

Primary EMPD's prognosis generally is better than that of mammary Paget's disease [6].

Local recurrence is frequent, even in cases with a wide surgical extraction, the recurrence rate being 12-58%. This may be related to positive surgical margins, skip areas, and multicentricity.

Figure 7: Paget cells showed false positivity for Melan-A (Melanoma Antigen, also called MART-1 – Melanoma Antigen Recognized by T cells 1)(photomicrograph taken from our cases), magnification 100x

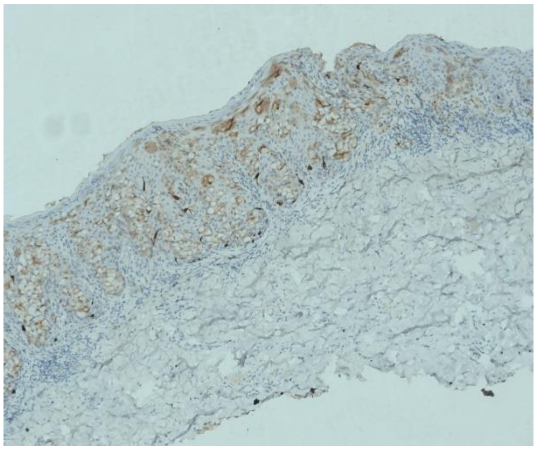

Adverse prognosis is associated with increased serum levels of CEA and the depth of invasion. Proper tissue sampling and other investigations are vital to determine if there is an associated underlying malignancy that requires surgical excision. If such a malignancy exists, it should be excised along with all clinically abnormal epithelium. If no underlying malignancy is clinically and surgically detected on careful examination, the entire affected area of skin should be excised.

In the case of primary EMPD associated with an invasive component, the prognosis is significantly worse, and it can undergo lymph node metastasis. Cases with gene mutation of PIK3CA and AKT1 are often invasive. [1]

An important method is “Mohs surgery” (microscopically controlled surgery) that minimizes the recurrence rate by carefully controlling excision margins of the tumor [14, 15, 16].

Photodynamic therapy is another important tool in the treatment of EMPD, but it requires larger series and longer periods of follow-up [17, 18].

Topical imiquimod has been used with good results in cases with limited disease [19].

Radiation therapy has been supported either as an adjuvant to surgical therapy [20] or as an alternative therapy for elderly patients in whom surgery may have a greater risk [21].

DISCUSSIONS

Because of the resemblance of the lesions in the EMPD with other dermatological conditions, it is important that the clinician always consider a possible diagnosis of EMPD, especially in the case of lesions in the genital and anal spheres.

Due to the false positive results that may appear at various markers used for the diagnosis of EMPD, the pathologist cannot always rely on immunohistochemical tests for differential diagnosis with other disorders. Therefore, a correlation between clinical, paraclinical and histopathological data is very important for a correct diagnosis.

CONCLUSIONS

EMPD is a very rare skin malignancy that needs to be diagnosed and treated accordingly [23]. Because it can mimic a lot of benign or malignant diseases, a correct differential diagnosis is crucial for the treatment and prognosis of the patient. Also, it is imperative to differentiate primary EMPD from a secondary type, in the latest the identification of the primary site of the tumor being vital for the patient.

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Conflicts of Interest: The authors declare no conflict of interest.

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REVIEW ARTICLE

Nonoperative management of high-grade splenic injury. A review of the literature and case report

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Abstract: *Splenic injuries causedby traumatic events are common in the emergency departments, mostly because the spleen is frequently affected in trauma. The lesions of the spleen can be diagnosed using a contrast enhanced computed tomography and based on the results, the injury of the spleen is graded. The management of the patient can be operative or nonoperative. The choice of treatment is decided by the hemodynamic stability of the patient.*

INTRODUCTION

Blunt abdominal trauma mostly due to sports activities is common in the emergency departments in Europe and 30% of the polytrauma patients have abdominal organs injuries [1]. The most frequently injured organ in blunt abdominal trauma is the spleen, which is involved in about 32% of abdominal injuries [2, 3].

The splenic injuries could be treated surgically or by a nonoperative management. In cases of hemodynamically stable patients, nonoperative management of the blunt splenic injuries is preferred and the recognition of the overwhelming post splenectomy infections also sustains the nonoperative management [2, 4].

The splenic injury in trauma is diagnosed based on computed tomography (CT) and it can also show other injuries associated with trauma [5]. The sensitivity of the CT examination in detecting splenic injuries has been reported to be as high as 95% [6].

On trauma patients should be performed FAST to find the evidence of intraabdominal hemorrhage. The patients with positive FAST and unstable hemodynamically, with or without cardiovascular associated disorders [7-9], are rushed to the operating room and the stable ones are planned for contrast-enhanced computed tomography [10].

Based on the CT results, various grading systems were introduced to help physicians to take the best decision regarding the management of the patient [6].

The best known and used splenic and hepatic grading system is the one established by the American Associationfor the Surgery of Trauma (AAST) [11] (Figure 1).

Vascular injuries in splenic trauma with active bleeding could be detected on a contrast-enhanced helical CT scan as perisplenic material extravasation [6, 12].

The selection of the treatment, operative or nonoperative, is difficult, but there are some factors such as hemodynamic status, age, grade of splenic injury, quantity of the

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hemoperitoneum and associated injuries useful to decide the optimal standard of care for the trauma patient [11].

The rate of success in the nonoperative management of splenic injuries is very high in many published series, between 80-94% in the cases of adult trauma patients[2, 11].

There is some evidence that older patients might have a worse prognosis with nonoperative management than younger patients, and several series have reported thatpatients older than 55 years are less likely to have a successful nonoperativemanagement [11, 13, 14].

Other option of treatment in splenic lesions is the splenic artery embolization, which is classified as nonoperative treatment. Splenic artery embolization is used to reduce the incidence of reccurent bleeding, factor that would lead to hemodynamic instability [15-17]. Studies confirm a high rates of splenic salvage after the combined treatments such as splenic artery embolization and nonoperative management [3].

Table 1: The splenic organ in Jury Scaling System of the American Association for the Surgery of Trauma, 1994 revision

Grade*	Injury description	
I	Hematoma	Subcapsular, <10% surface area
	Laceration	Capsular tear, <1 cm parenchymal depth
II	Hematoma	Subcapsular, 10–50% surface area, <5 cm in diameter
	Laceration	1–3 cm parenchymal depth that does not involve a trabecular vessel
III	Hematoma	Subcapsular, >50% surface area or expanding; ruptured subcapsular or parenchymal hematoma; intraparenchymal hematoma >5 cm or expanding
	Laceration	>3 cm parenchymal depth or involving trabecular vessels
IV	Laceration	Laceration involving segmental or hilar vessels producing major devascularization (>25% of spleen)
	Laceration	Completely shattered spleen
V	Vascular	Hilar vascular injury that devascularizes spleen

**Advance one grade for multiple injuries up to grade III*

The operative treatment is preferred in all cases of instable hemodynamically patients with/without dyselectrolytemia [18, 19]. There are two surgical options that the operator can take after examining the injury: splenectomy or spleen preserving surgery [20, 21].

There are some types of spleen preserving surgery including spleen packing, partial splenectomy or splenorrhaphy [11].

Spleen packing is used in selected cases with isolated splenic injuries and stable patients.

If the patient is unstable with a grade IV or V spleen injury splenectomy should be performed. Another surgical technique that can be performed is the partial splenectomy and this is possible because of the segmental nature of the splenic blood supply [20].

One of the most dangerous complications that could appear after a splenectomy performed in trauma is OPSI (overwhelming postsplenectomy infection). OPSI is an emergent condition which at first may develop as a mild infection and then rapidly progress to sepsis [22, 23] that has a 50-70% risk of mortality with or without treatment [24-28].

Vaccination against S. pneumoniae, N. meningitidis, and HIB are recommended in addition to a yearly influenza vaccine to avoid OPSI, but the administration of these vaccines is recommended after the postoperative day 14 to increase the immune response in patients who have undergone splenectomy due to trauma [29-31].

MATERIAL AND METHOD

We highlight the key aspects of the nonoperative management of blunt trauma splenic injuries by doing a review of the literature.

We researched the PubMed and Cochrane datebases for studies involving nonoperative management of the splenic traumatic injuries. The studies we analyzed were published between 2017 and March 2019.

CASE REPORT

We present the case of a 25-year-old woman, who came into the emergency room after a motocycle accident. The clinical examination showed tenderness in the left upper quadrant and the right arm was painful, the patient was stable hemodynamically. An X-ray of the right arm showed fracture of the radius and cubitus that were immobilized in a cast [32].

Point of care abdominal ultrasound was performed and it suggested a splenic lesion – non-homogeneous echostructure and hypoechogenic areas (Figure 1), and free fluid in Morrison and Douglas pouches (Figure 2).

Figure 1: Ultrasound finding of splenic lesion

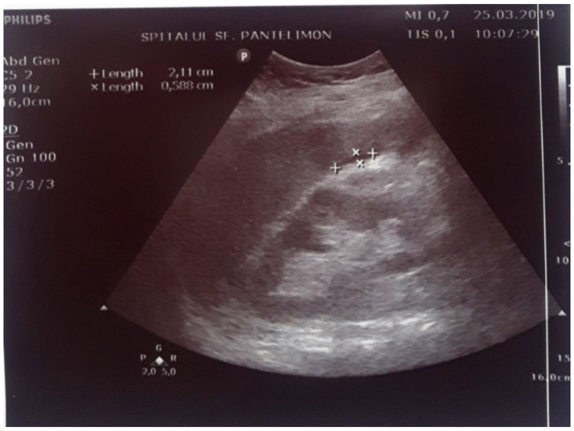
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Figure 2: Hemoperitoneum: hypoechoic, nonhomogeneous paravesical image

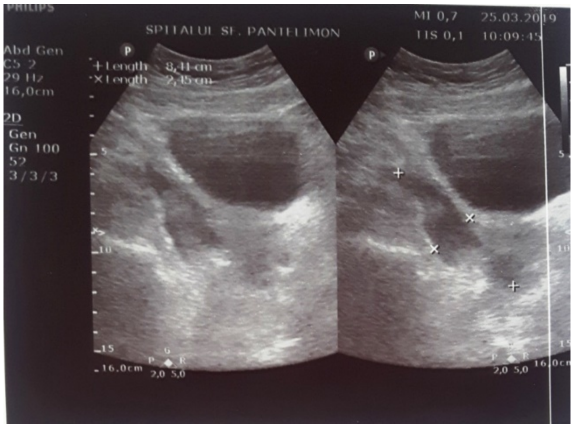
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Figure 3: Native CT image showing grade III splenic lesion (hypodense zone – densities between 34 and 41 Hounsfield Units – from hilum to capsule)

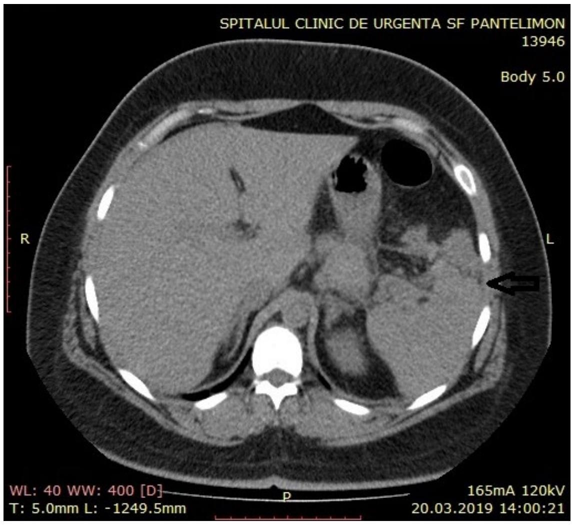
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Figure 4: Ultrasound finding of splenic lesion

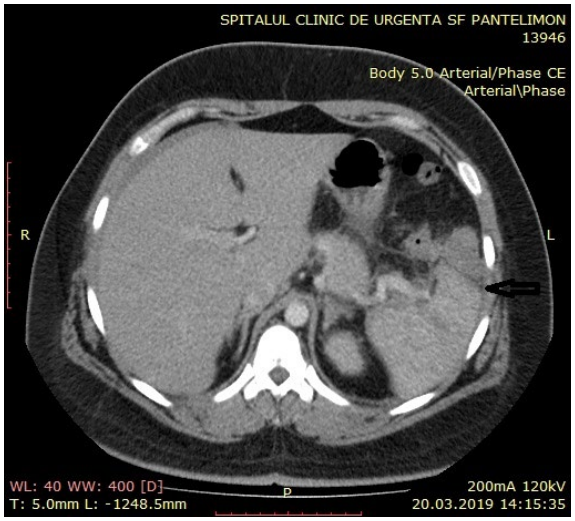
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Figure 5: Contrast-enhanced CT scan - spleen hilum involvement

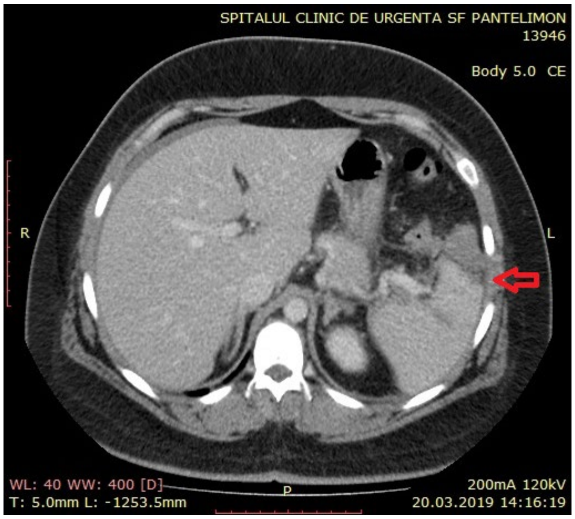
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Figure 6: Blood accumulation in Douglas' pouch

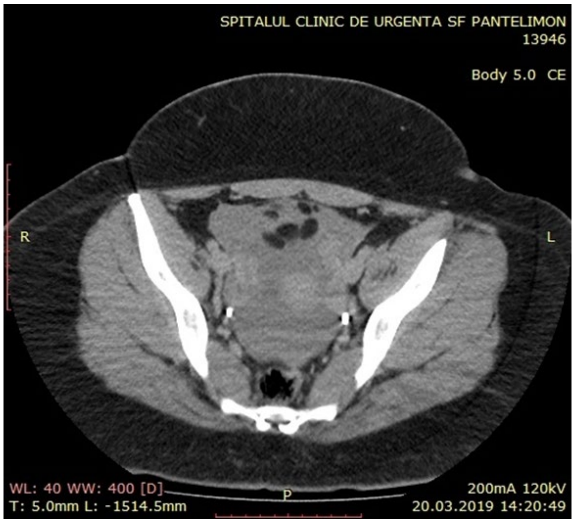
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The blood tests revealed anemia (hemoglobin 9.30 g/dL) and leukocytosis (14.510/uL).

Contrast-enhanced CT scan of the abdomen and pelvis revealed a grade III splenic injury (5.6 cm hematoma and 2 cm laceration) (Figures 3, 5), fluid surrounding the liver, in the Morrison pouch, in the right and left paracolic gutters (Figure 6). No contrast extravasation at level of the spleen was detected, no other traumatic injuries (Figure 4).

Hypodense zones of splenic tissue should be differentiated from cystic lesions of spleen, even hydatid cyst that, in case of rupture, could mimic acute abdomen [33].

We decided to treat the patient conservatory, to monitor her clinically every 6 hours, the blood pressure and pulse were measured also every 6 hours and she was monitored ultrasonographic daily. There were no modifications during the admission into the hospital and the patient was discharged in the fifth day. She was informed about the signs of hemorrhage (abdominal pain, light-headedness, syncope, difficulty breathing and chest pain) and she was instructed to call immediately a surgical emergency center if any of these symptoms appear.

One week after the discharge the patient came to follow up, she was examined clinically, ultrasonographically and biologically and everything was normal.

At this moment, there is no information about the nonoperative management of the splenic trauma regarding how long should the patient be admitted, how long should it be between the discharge and the follow up, how frequently should the level of hemoglobin and hematocrit be measured and how frequent should the patient be examined ultrasonographically [34, 35].

CONCLUSION

Splenic injuries are common and the patients stable hemodynamically who have grade III splenic lesions due to trauma could be managed nonoperatively. There are no protocols about the nonoperative management of the splenic injuries and we think their existance could influence the morbidity and mortality of these patients. Prospective trials could be performed to establish a protocol of treatment and follow up.

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VARIA

Normal pressure hydrocephalus – Diagnosis and therapeutic challenges

Alin V. Chirtes[1], Teofil Mures[1], Marian Mitrică[1]

Abstract: *Normal pressure hydrocephalus (NPH) is a rare pathological condition that generally affects older people (>65 years old), and can take one of two forms: idiopathic or secondary. It is a reversible cause of dementia, and it manifests itself through a very specific triad: gait impairment, urinary incontinence, and dementia. The clinical diagnosis of NPH is particularly difficult to establish due to the association between these symptoms and old age, as well as due to the absence of specific paraclinical markers. Nevertheless, a holistic application of clinical, imagistic, paraclinical criteria, as well as of the various developed scales may, in time, lead to a diagnosis. Moreover, the evolution of magnetic resonance imaging has certainly made easier to establish the condition. The sole therapeutic method proven to be efficient is shunting surgery which has good results in the majority of the cases, showing clear signs of improvement from the very first-day post-surgery.*

Keywords: *normal pressure hydrocephalus, ventriculoperitoneal shunt, aqueductal stenosis, cerebral ventriculomegaly, communicating hydrocephalus, obstructive hydrocephalus*

INTRODUCTION

Normal pressure hydrocephalus (NPH) was firstly recognized by Adams et al. in 1965, by identifying a number of patients suffering from hydrocephalus, but having a normal pressure in the cerebrospinal fluid (CSF) at the lumbar puncture (LP) [1]. This represented a potentially reversible syndrome. The pathology is most often present in older people, and it manifests itself through a gait disturbance, urinary incontinence, and cognitive deficit. It has also been determined that gait impairment, along with one other characteristic element is essential for diagnosis. The clinical assessment demands further examinations, such as brain imagery or draining the CSF, to properly confirm the condition [2].

Traditionally, hydrocephalus was divided into two types: communicating hydrocephalus or obstructive hydrocephalus (non-communicating). The former type is characterized by an increase in the volume of CSF without any stenotic lesions in the drainage. In this case, the classification of NPH is still the one being used since 1965: idiopathic NPH (iNPH) – in roughly 50% of all cases, and secondary NPH (sNPH), which comes as a result of either subarachnoid hemorrhage (SAH), intracerebral hemorrhage (ICH), meningitis, intracranial tumors or trauma [1]. While iNPH is generally noticed at adults, sNPH may be present at any age, with both genders being affected equally [3]. Apart from the ventriculomegaly, there are no other observable radiological signs.

Obstructive or non-communicating hydrocephalus comes after the stenosis of the drainage between ventricles and the subarachnoid space, either congenital or acquired. The stenosis of the cerebral aqueduct is a common cause in young adults, although the symptoms are not visible until

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adulthood [4].

In the largest study ever conducted on NPH, in Sweden, the results estimated a 0.2% prevalence in the 70-79 age group, and 5.9% in those aged over 80 [5]. In Japan, the results estimated a 0.01% prevalence in the general population and 0.03% in those aged over 60 [6]. In another study on a Finnish cohort, the incidence was 1.58 for every 100.000 persons [7].

The comorbidities associated with NPH are: Alzheimer disease (15%), chronic high blood pressure (50%), hyperlipidemia (14%), and diabetes mellitus (20%) [6, 7]. The latter two factors increase the risk of developing NPH by doubling it, as compared to a normal patient [8]. Similarly, obesity and psychological stress, heart diseases, and strokes have also been named as contributory to the development of this pathology [8, 9].

Unfortunately, the criteria for NPH diagnosis remain uncertain. While some specialists consider it the most common form of hydrocephalus in adults, others contest its very existence [3, 10, 11].

NPH is difficult to diagnose due to its broad and general symptomology, commonly associated with old age. Thus, in individuals aged over 75, urinary incontinence is present in 20 to 30% of them, whereas 20% develop some kind of dementia and 20% show signs of ventriculomegaly [4]. Implicitly, the management of NPH is equally difficult, due to the complications associated with shunting. As such, the morbidity and mortality rates are 20% and 0.2%, respectively [12].

PHYSIOPATHOLOGY

The CSF is mainly secreted by the cells of the choroid plexus, other regions playing mainly a rather insignificant role. CSF's volume is between 125 and 150 ml, and its secretion varies between 400 and 600 ml daily, renewing itself 4 to 5 times every 24 hours in a healthy adult [13]. Its role is vital, namely, the protection and nutrition of the parenchyma [14]. Furthermore, its dynamic is highly dependent on the rate of production and absorption, as well as on the blood-related pulsatile flow through both the subarachnoid and Virchow-Robin spaces to the cerebral parenchyma [15, 16]. According to Adams et al. original theory, NPH develops when the level of CSF's reabsorption diminishes, thus resulting in intracranial hypertension, leading to ventricular dilation as a compensating mechanism [1]. Hence, a larger volume of CSF is redirected to the Virchow-Robin spaces, giving birth to a compression of the parenchyma and ischemia of the white matter.

The specific pathophysiology of iNPH is unclear, yet various mechanisms have been put forward as possibly involved in developing the pathology, such as [2]:

- Reduced compliance of the subarachnoid space
- Reduced reabsorption of CSF in the venous system
- Inadequate expression of TNF-alpha in CSF
- The hyperdynamic flux of CSF in the cerebral aqueduct
- The higher pressure of the CSF
- The reabsorption of CSF through abnormal mechanisms

CLINICAL PRESENTATION

iNPH must be suspected in older patients suffering from abnormal walking, sometimes combined with dementia or urinary incontinence [3].

Symptomatology can vary massively between patients. They may show signs with the classic triad in percentages of 30 to 50 (gait disturbance, urinary incontinence, and cognitive deficit), or only a combination of these: only gait impairment (5%), only dementia (2%), both combined (28%), gait abnormality together with urinary incontinence (4%) and dementia accompanied by urinary incontinence (1%) [6][17]. Approximately half of the patients with iNPH only show signs of a gait disorder, the other two characteristic symptoms being absent [6].

DIAGNOSIS

According to international guides, the imagistic diagnosis is based on the following elements [18]:

- Ventricular dilation with an Evans index > 0.3 [19]
- The absence of a macroscopic obstruction of the dynamics of the CSF
- At least one of the following [20]:
    - o Callosal angle exceeding 40° [21]
    - o Temporary dilated temporal horns of the lateral ventricles, but not fully due to the atrophy of the hippocampus
    - o Changes in the CT and MRI signal caused by the alteration of the content of cerebral water, not fully owed to the microvascular ischemic modifications or demyelination.
    - o Signs of a "flow void" in the MRI in the Sylvian aqueduct

The following imagistic methods can be engaged as well: MR elastography (MRE), glymphatic MRI, or the Silver index [22, 23, 24].

Regarding the Evans index, it is established based on a CT scan in the axial incidence. An index exceeding 0.3 indicates a pathological condition of the ventricles (sensibility of 80%) [19]. The threshold for indicating a pathological component varies by age, namely: between 65 and 69 years – 0.34; between 70 and 74 years – 0.36; between 75 and 79 years – 0.37; between 80 and 84 years – 0.37 [19].

Callosal angles are measured based on a CT or MRI scan, at the median point of the corpus callosum [21]. Identifying this point necessitates using the sagittal plane, placed parallel to the floor of the fourth ventricle [21]. The limit was 105.4° (42% sensibility, 87% specificity) [21]. It is deemed that, with the decrease of every degree in callosal angles, a patient has a 4% greater chance of benefitting from a shunting surgery [21].

The Japanese guidelines for diagnosing NPH do not regard periventricular changes as relevant. However, they do include two new criteria of diagnosis, i.e. Sylvian fissures and enlarged basal cisterns, as well as the narrowing down of the subarachnoid space and the cerebral sulci at the median level of the brain [25]. In 2017, the efficiency of the two guides was debated, since there are significant discrepancies from the international medical practice, the former being substantially more specific, demanding the presence of at least two symptoms of the classic triad to confidently identify the diagnosis [26]. Furthermore, the study has underlined the necessity of a common system for diagnosis, more objective than the current one [26].

Clinically, several procedures could be executed to diagnose NPH. Firstly, "tap test" or CSF's evacuation test consists in removing 30-50 mL of CSF through a lumbar puncture, to improve the symptomology, the association of the gait function with the frontal assessment battery score, the improvement of verbal fluency and gait [25, 27, 28, 29]. Secondly, different weights can be given to the various markers from CSF, such as the expression of certain isoforms of transferrin, of the tyrosine phosphatase Q-type receptor, or has-miR-4274. Similarly, the correlation between slow vasogenic waves (SWV) and intracranial pressure must be investigated [30].

DIFFERENTIAL DIAGNOSIS

The differential diagnosis must be performed with Alzheimer disease (AD), frontotemporal dementia, dementia with Lewy body, the one from Parkinson disease (PD), the one associated with AIDS, dementia caused through corticobasal degeneration and any other type.

Generally, the level of proteins in the CSF is measured, considered alongside biomarkers of the AD. In this particular case, the level of p-tau and t-tau are high, whereas the level of a β-amyloid peptide (Aβ) is low [4]. In the particular case of NPH, all these parameters are high, going back to normal values after shunting [31].

Apart from the AD, the PD, as well as other parkinsonian syndromes cause gait impairment [4]. Numerous investigations can be carried out, such as SPECT (Single Photon Emission Computed Tomography), this being limited through detecting an exhaustive set of neurodegenerative diseases, or FDG-PET (Fluorodeoxyglucose – Positron Emission Tomography), which can identify caudate hypometabolism, a potential marker of NPH [4].

TREATMENT

The clinical presentation of NPH is insufficient to recommend ventriculoperitoneal shunting surgery (VPS), because either one of the symptoms of NPH may have multiple potential etiologies. The diagnosis must be established clearly through the abovementioned methods in order to recommend surgical treatment.

VPS is the only efficient method of treatment for iNPH, by implanting a ventriculoperitoneal shunt. The international and Japanese guides, as well as AAN (American Academy of Neurology), confirm VPS as the only efficient method of treating iNPH, as opposed to the rather supposedly inefficient endoscopic approach of the third ventricle [25, 32, 33].

The purpose of a shunt is to drain CSF towards space where it may be reabsorbed. A shunt has three components: a proximal catheter, inserted at the level of the right lateral ventricle, a distal catheter in the peritoneal cavity, and a valve of shunting which contains a mechanism opening the valve up when the difference in pressure between the two cavities reaches a pre-established value [3]. Once the valve is open, CSF is drained in the peritoneum. Another approach is represented by lumboperitoneal shunt (LPS), which has the proximal end in the lumbar zone. In a study from Japan, it has been stated that LPS is used in 55% of the cases, whereas VPS is used in 43% of the surgical cases of NPH [6]. Another option of VPS is placing the distal end of the shunt between the two sheets of the greater omentum, the technique is considered as having good results and a low rate of post-surgery complications [34].

Concerning the efficiency of the shunting surgical treatment, in the first three months, in most cases, the headache is gone, and the gait deficit has improved in 85% of the cases, urinary incontinence in 70%, and cognitive functions in 65% [35].

In a study on the efficiency of LPS, it has been concluded that the method is comparable with VPS for patients showing signs of NPH. Nevertheless, the revision of the shunt took place more frequently in patients with LPS than in those with VPS [36].

Regarding the prognostic, 60% of the patients have benefitted from an improvement in symptomatology after the shunting surgery, and 40% have shown signs of significant improvement. These were measured using the mRS scale [9]. Both pre-surgery and post-surgery, patients must be evaluated using the following systems: Japanese NPH scale, Berg scale, DGI (Dynamic Gait Index), FIM (Functional Independence Measure), MMSE (Mini-Mental Status Examination), and TUG (Timed Up and Go) [2].

A series of post-surgery factors can suggest a good prognostic, such as: an aqueduct flux exceeding 42 µl, the absence of lesions of the white matter on MRI, resistance to the CSF debit exceeding 18 mmHg [2]. Antagonistic factors are severe dementia, dementia as a symptom of presentation and MRI abnormalities such as cerebral atrophy or lesions of the white matter [2].

Complications that may arise include:

- Surgical complications of the shunt: the failure of the shunt (3%), under- or over-drainage, subdural hematoma (3-4%), infections (1%) [37];
- Surgical complications unrelated to the shunt: seizures and intracerebral hemorrhage (under 5%) [2];
- Schizophrenia – 3 to 4 times more likely in patients with iNPH than in the healthy population [38];
- Hypertension and diabetes type 2 are frequent in iNPH patients, the latter risk factor causing increased mortality in affected patients [7].

CONCLUSIONS

NPH is a rare, yet treatable disease, and the diagnosis may be difficult to establish due to the rather broad symptoms. Both iNPH and sNPH cause similar features.

The difference between the idiopathic and secondary hydrocephalus has led to a difference in approach in a similar pathology, the latter being labeled as a surgical emergency. Both the concept of NPH, as well as the therapeutic approach retain a status of controversy. The diagnosis of iNPH must be considered when a patient shows signs either of the classic triad, or of some elements of it, or in the case of suggestive imagistic criteria.

Following this evaluation, treatment must be sought only when the risks and benefits of the shunt are carefully balanced. Recent innovations in the field of imaging have greatly improved the identification of patients who may benefit from shunting surgery. Furthermore, it should be also keep in mind that patients suffering from iNPH also present associated comorbidities, such as PD, AD, or other neurodegenerative diseases. Hence, the diagnosis must include tests that draw a clear distinction between NPH and these potentially present diseases. For the same reason, the patient's response to the drainage of CSF can be unpredictable.

NPH must be seen as a reversible cause of dementia. In the future, ample studies must be made to demonstrate the efficiency of shunting surgery.

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REVIEW

Nuclear Medicine Techniques in the Diagnosis of Cardiac Amyloidosis

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Abstract: Cardiac amyloidosis (CA) represents the accumulation and deposition of misfolded protein fibrils in the myocardium, resulting in progressive restrictive cardiomyopathy. Light chain (AL) and transthyretin (TTR) amyloidosis are the most common types of CA. While endomyocardial biopsy remains the gold standard for diagnosing cardiac amyloidosis, its invasive nature and associated risk of complications have led to increased reliance on clinical suspicion and noninvasive imaging modalities as alternative diagnostic tools. Over the last decade, radionuclide imaging studies have become a widely accepted tool in diagnosing ATTR CA, whereas its diagnostic utility in AL CA detection remains limited. Recent advances in PET-CT radiopharmaceuticals have further expanded the potential of nuclear imaging as a comprehensive tool for diagnosis, prognostication, and therapy monitoring. This literature review appraises the current applications of nuclear imaging in the clinical management of cardiac amyloidosis.

Keywords: Cardiac amyloidosis; Transthyretin amyloidosis; Light-chain amyloidosis; Radiolabeled bisphosphonates imaging; Amyloid-specific PET tracers; Non-invasive cardiac imaging

INTRODUCTION

Amyloidosis is a heterogeneous systemic disease characterized by the aggregation and extracellular deposition of misfolded protein fibrils in various tissues and organs [1]. The term ‘amyloid’, derived from the Greek άmylon and Latin amylum – both meaning ‘starch’, in reference to its iodine staining properties – was first introduced into the scientific literature in the 19th century to describe iodine-positive deposits observed in the brains of patients with dementia [2]. Cardiac amyloidosis (CA) refers to myocardial infiltration by amyloid fibrils, leading to progressive restrictive cardiomyopathy and associated cardiovascular manifestations. Although the type of CA cannot be differentiated based on patterns of deposition, there seems to be a prevalence of diffuse, pericellular, endocardial, and arterial or arteriolar deposits in AL amyloidosis, with nodular deposits in TTR amyloidosis [3].

The most frequent clinical forms – accounting for more than 95% of cases – are light-chain (AL) and transthyretin (TTR) amyloidosis [3]. AL (or primary) amyloidosis results from the deposition of misfolded immunoglobulin light chains, aggregated as amyloid fibril precursors. These last can damage the tissues directly – through their

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proteotoxicity – or form stable fibrils – causing organ damage through impaired histological architecture [2]. TTR amyloidosis is characterized by pathological deposits of transthyretin, a normally occurring protein used in transportation of thyroid hormones and retinol-binding [4]. Wild-type transthyretin amyloidosis (ATTRwt) – formerly known as senile systemic amyloidosis – is an age-related form of cardiac amyloidosis, most often presenting in the seventh decade of life, with a marked male predominance [2,5]. In contrast, variant transthyretin amyloidosis (ATTRv), which is inherited in a dominant autonomic fashion with incomplete dominance, may present as early as the third decade of life, depending on the specific mutation [2,3,5].

With divergent prognoses, treatment strategies, and therapeutic responses, early and precise diagnosis of the distinct forms of cardiac amyloidosis is therefore essential to guide appropriate and timely management. While endomyocardial biopsy remains the gold standard for diagnosing cardiac amyloidosis, its invasive nature and associated risk of complications have led to increased reliance on clinical suspicion and noninvasive imaging modalities as alternative diagnostic tools [4,6].

This paper aims to present current nuclear imaging modalities of diagnosing cardiac amyloidosis and to synthesize recent evidence regarding their prognostic significance and role in evaluating therapeutic response in patients with CA.

CLINICAL SPECTRUM, BIOMARKERS, AND CONVENTIONAL IMAGING

Clinical manifestations

Cardiac amyloidosis is associated with a spectrum of clinical manifestations reflecting the gradual myocardial infiltration and consequent restrictive physiology. Amyloid infiltration leads to progressive thickening of the atrial and ventricular walls, culminating in diastolic dysfunction and heart failure (HF) – often with preserved ejection fraction. Systolic dysfunction typically occurs in the later stages of the disease. While atrioventricular block and tachyarrhythmias also become increasingly clinically apparent as the disease advances, common cardiac symptoms include fatigue, shortness of breath, and syncope [2,3].

AL amyloidosis, caused by clonal plasma cell dyscrasias, typically presents as a multisystemic disease, most frequently involving the heart and kidneys (60-70%), but also the liver (15%) and peripheral nerves (10%) [3]. The amyloidotic heart shows symmetrical increase in biventricular wall thickness (pseudohypertrophy) with markedly increase LV stiffness and filling pressures – in the presence of nondilated or small ventricles [2]. Due to restrictive cardiomyopathy and fibril proteotoxicity, patients with AL amyloidosis can present with dyspnea, fatigue, arrhythmias, severe fluid retention, hepatomegaly and ascites [1,2]. Circulating light chains exert direct cardiotoxic effects, contributing to rapid disease progression and disproportionately elevated cardiac biomarkers relative to amyloid burden [3]. Late-stage diagnosis, when cardiac and renal damage is advanced, correlates with a poor survival – measured in weeks [1,3].

ATTR amyloidosis also presents with a cardiac-dominant phenotype – manifesting as left ventricular hypertrophy (LVH) and HF [5] – particularly in the wild-type form (ATTRwt), which affects elderly men and follows a slow progression. Early extracardiac manifestations, such as bilateral carpal tunnel syndrome, lumbar stenosis or biceps tendon rupture, may precede cardiac symptoms by several years [1,3]. Severe aortic stenosis (SA) has been associated with cardiac amyloidosis, especially in the ATTR variant, as amyloid fibril deposition within valvular tissue contributes to leaflet thickening and progressive stenosis. The coexistence of CA and SA has been reported to have a poorer prognosis; therefore, concomitant diagnosis is essential in guiding appropriate therapeutic management [7]. ATTRv manifestations vary by mutation: Val122Ile leads to predominant cardiac involvement, while Val30Met causes early-onset neuropathy and Thr60Ala produces mixed features [3]. Late-onset ATTRv and ATTRwt share similar cardiac presentations and fibril compositions, often involving both full-length and fragmented transthyretin [2].

Serum biomarkers

When cardiac amyloidosis is suspected, initial evaluation should include serum measurements of N-terminal pro-B-type natriuretic peptide (NT-proBNP), troponin, creatinine and free light chains, along with calculation of estimated glomerular filtration rate (eGFR). A study by Ionescu et al. [8] found the burden of cardiac amyloidosis on eGFR to be more pronounced in ATTR patients compared to AL, regardless of the method used (either by MDRD formula or the Cockcroft-Gault equation).

Assessment of serum and urinary free light chains should be performed to aid the differentiation between ATTR and AL amyloidosis. An abnormal kappa-to-lambda free light chain ratio may be indicative of AL amyloidosis. For further evaluation of the differential diagnosis, bone marrow biopsy is recommended to distinguish between AL amyloidosis and multiple myeloma [2].

Electrocardiography

As one of the simplest yet highly informative diagnostic tools, electrocardiography (ECG) can reveal several 'red flags' – particularly when interpreted in conjunction with imaging findings – allowing for a refined prognosis and stratification. ECG appearances suggestive of cardiac amyloidosis are defined by a pseudoinfarction pattern and disparity between the LV wall thickness and low QRS voltage. Due to myocardial infiltration of non-conductive material, the direct proportionality correlation between QRS voltages and LV mass is therefore abolished. The pseudoinfarction pattern consists of the presence of Q waves measuring at least 1mV in at least 2 contiguous leads, in the absence of ischemic heart disease and/or regional kinetic abnormalities of the LV [2,5]. In AL CA, low peripheral voltage is more common due to proteotoxicity of misfolded light chains, whereas ATTR CA presents more frequently with conduction disease (incomplete or complete bundle branch block), arrhythmias (atrial flutter or fibrillation) and bradycardia [2,3].

Echocardiography

Echocardiography signs suggestive for cardiac amyloidosis consist of increased LV wall thickness (>12 mm) with relative apical sparing ("cherry on top"), low tissue doppler velocities, restrictive filling pattern, increased biatrial volume, atrial septal thickening (>6 mm) and pericardial effusion. LV wall thickness is usually more pronounced in ATTR CA (>15 mm), which also presents more commonly with concomitant aortic stenosis [3,9]. Combining ECG and echocardiographic findings has been shown to provide higher diagnostic accuracy, with 91% sensitivity and 89% specificity in cases with a ratio<0.4 between R-wave amplitude in lead I and posterior wall thickness [2].

Cardiac magnetic resonance (CMR)

Cardiac magnetic resonance imaging, particularly late gadolinium enhancement (LGE) and T1 mapping techniques, allows for noninvasive tissue characterization, therefore aiding the differential diagnosis of cardiomyopathies with hypertrophic phenotypes by detecting amyloid deposits in the myocardium [9]. Morphofunctional quantification reveals concentric and symmetric left ventricular hypertrophy with preserved ejection fraction but reduced indexed stroke volume – often in the context of diastolic dysfunction. Associated findings include thickened valves, left atrial appendage thrombi, atrial enlargement, interatrial septum hypertrophy and mild pericardial and pleural effusions. Increased native T1 mapping is particularly sensitive for early myocardial involvement, often detecting abnormalities before late LGE becomes apparent. Especially during early to intermediate contrast phases, LGE typically demonstrates diffuse subendocardial enhancement in a non-coronary distribution with relative apical sparing. In advanced stages of CA, transmural or intramyocardial, patchy enhancement may be observed, extending into the right ventricle or atria. Characteristic features include myocardial nulling preceding blood pool nulling and early blood pool darkening. Additionally, elevated extracellular volume (commonly>40%) serves as an indirect marker of amyloid burden and correlates with disease severity and prognosis [2,10].

NUCLEAR MEDICINE IMAGING IN CARDIAC AMYLOIDOSIS

Radiopharmaceuticals

Non-amyloid specific radiopharmaceuticals currently used in cardiac scintigraphy are bone-avid radiotracers. These radiopharmaceuticals are labelled with 99mTechnetium: 99mTc-diphosphono-1,2-propanodicarboxylic acid ([99mTc]Tc-DPD), 99mTc-pyrophosphate ([99mTc]Tc-PYP) or 99mTc-hydroxymethylene diphosphonate ([99mTc]Tc-HMDP) [11]. A comprehensive study by Gilmore et al. [12] has shown high specificity and sensitivity of 99mTc radiotracers for TTR amyloidosis, which correlates with negative monoclonal proteins in blood and/or urine samples. It is not yet fully understood why phosphonate compounds accumulate in cardiac tissue at the sites of amyloid deposition. It is hypothesised that this is because amyloid fibril deposits have a higher concentration of microcalcifications in the cardiac TTR, together with slow disease progression and therefore the potential for greater accumulation of deposits in the myocardium and/or other systemic structures. [4,13,14]

A study conducted by Park et al. [15] comparing 99mTc-PYP and 99mTc-DPD showed that the latter may be more useful in evaluating the severity of the disease. They found a more significant relationship between uptake and visual grading, as well as with biological parameters in [99mTc]Tc-DPD scans. Further studies are necessary in order to formulate a proper conclusion.

Quarta et al. [16], in their study of [99mTc]Tc-DPD uptake in AL CA, found a high percentage of patients with bone tracer uptake, almost a third of them with moderate cardiac uptake. This emphasizes the necessity of serological exclusion of plasma cell dyscrasia in case of bone tracer uptake in the myocardial tissue.

There has been a growing interest in recent years for the applicability of amyloid specific radiotracers, used in positron emission tomography – computed tomography (PET/PET-CT) studies in the diagnosis of cardiac amyloidosis [17]. Their primary use is for detection of Alzheimer disease [18]. These radiotracers bind directly to the beta amyloid, thus identifying any other systemic sites of amyloid aggregates. They are not yet approved for clinical use in diagnosis of CA but have shown promising results [19]. Rosengren et al. [20] were able to show high accuracy of 11C-labeled Pittsburgh compound B ([11C]PiB) in the detection of AL CA. Patients with documented systemic amyloidosis and only cardiac wall thickness also had [11C]PiB uptake on PET-CT scans, suggesting a prospective use of it in preliminary stages of the disease. Furthermore, Choi et al. [21] showed that PET-CT with [11C]PiB is a reliable independent factor for 1 year survival in patients with AL CA, alongside other established bio-indicators. The survival rate after 1 year in their 35 patients with [11C]PiB uptake was a little over 50%. Lee et al. [22] determined that PET scans with [11C]PiB are useful in quantifying the degree of amyloid burden by grade of uptake. Their prospective study reflected the usefulness of [11C]PiB in predicting overall outcome. A reduction in the uptake after treatment was observed in a small number of patients, raising the possibility of [11C]PiB PET as a non-invasive tool of assessing treatment response.

Similarly, Genovesi et al. [23] tested the feasibility of [18F]Florbetaben PET-CT for detection of AL amyloidosis. Their results showed that it may be able to detect light chain amyloid burden and to discriminate between forms of CA and other restrictive cardiomyopathies. Along with a previous pilot study by Law et al. [24], both may endorse the use of [18F]Florbetaben as a non-invasive imaging technique to diagnose AL amyloidosis, yet further studies are necessary. It may represent an aid to scintigraphy, which cannot discriminate from other than TTR cardiac amyloidosis.

Apart from the amyloid-specific radiotracers, Wang et al. [25] showed the potential use of 68Ga-labeled Fibroblast Activation Protein Inhibitor ([68Ga]FAPI) PET-CT imaging for the diagnosis of AL CA, due to fibroblastic activity – a possible new instrument in heart failure and its cardiac implications [26].

Radionuclide imaging techniques

Planar acquisition

Interpretation of the cardiac planar images is based on a relatively simple visual scoring and a semiquantitative analysis. The visual grade consists of the Perugini score (Table 1), comparing the intensity of the cardiac uptake with rib cage uptake, and is more useful in case of [99mTc]Tc-DPD or [99mTc]Tc-HMDP [13, 27]. Grade 0 represents no cardiac uptake of the 99mTc radiotracer – and normal uptake of the ribs – being considered negative for CA. Grade 1 – equivocal for the diagnosis of ATTR amyloidosis – consists of mild cardiac uptake, less than rib uptake. Grade 2 represents cardiac uptake of the radiotracers equal to the rib uptake, while Grade 3 translates to a cardiac uptake greater than that of the rib cage. Grades 2 and 3 on planar studies are considered ATTR positive – after the exclusion of plasmatic dyscrasia [28].

Table 1: Visual grading system of myocardial radiotracer uptake on bone scintigraphy according to the Perugini score

Perugini score	Cardiac uptake
Grade 0	absent
Grade 1	cardiac uptake < rib uptake
Grade 2	cardiac uptake = rib uptake
Grade 3	cardiac uptake > rib uptake

Semiquantitative indicators consist of several methods of evaluation, including the heart-to-contralateral ratio (H/CL), most valuable in the case of bone scintigraphy with [99mTc]Tc-PYP [13]. The H/CL measures the ratio between the counts in the two regions of interest (ROI) – the heart and the contralateral lung. With a value greater than 1.5 on one-hour acquisitions, in correlation with a Perugini score of 2 or 3, the cardiac study is highly suggestive of TTR cardiac amyloidosis [28]. However, in cases where the Perugini score is classified as grade 1, histological confirmation of amyloid deposits (potentially located in the extracardiac tissues) is required to establish the diagnosis and determine the amyloid type [29].

The current standard time between injection and acquisition is between one (1h) and three hours (3h) [30]. Images at one hour have a higher sensitivity due to peak myocardial uptake, yet it is of higher specificity in the case of delayed images [17]. Saitou et al. [31] confirmed this remark by a comparison between planar acquisitions at 1h and 3h post-injection on radiolabeled bisphosphonate scans with [99mTc]Tc-PYP. Their study showed a higher specificity in planar 3h studies – results corroborated with single photon emission computed tomography with computed tomography (SPECT-CT). Moreover, a 3h scan can provide a more accurate diagnosis, as proven by their differences in positive predictive values (almost 80% after 3h versus 57% after 1h). Similarly, Nichols et al. [32] emphasized the advantages of using 3h planar and SPECT-CT images for diagnosis. Three-hour acquisitions are advantageous due to lesser blood pool activity and peak bone uptake [11].

SPECT and SPECT-CT

SPECT-CT, a hybrid imaging technique that evaluates function through SPECT and anatomy through CT, provides a useful three-dimensional assessment with the possibility of segmental evaluation and attenuation correction [13].

The visual scoring on planar images is a well-established and reliable method of analysis. Nevertheless, it is prone to misinterpretation because of blood pool activity, osseous uptake that overlaps the cardiac region or inexperience of the interpreter [30]. SPECT-CT images are able to confirm the diffuse left ventricular, biventricular, interventricular septum and/or atrial myocardial uptake of the radiotracers, highly suggestive of cardiac amyloidosis.

Quantification of cardiac uptake on SPECT-CT using standardized uptake values (SUV) was shown to have greater accuracy than visual assessment on planar-only acquisitions. SUV is a semiquantitative index that reflects the uptake of radiotracer in the tissue, but it has yet to be included in standard practice. It is the ratio between radioactivity in a region of interest (ROI) and the dose administered per the body weight of the patient. Kessler et al. [33] compared quantitative SPECT and SPECT-CT results with those obtained through Perugini score in suspected CA patients that underwent [99mTc]Tc-DPD scintigraphy. A value of SUVmax greater than 6.1 showed a higher specificity for CA and particularly for ATTR type – where a 99% specificity value has been reported. Furthermore, in a study by Taha et al. [34], the use of SPECT-CT fusion was able to enhance diagnostic accuracy by adding the anatomical element of the low-dose CT in order to differentiate between myocardial and ventricular diffuse uptake (blood pool uptake). Scully et al. [35] showed that the use of SPECT-CT and SUV values offers a more accurate means of distinguishing between Perugini scores of 2 and 3.

PET-CT

PET-CT imaging can be a more precise tool in describing anatomical and functional characteristics of the disease. PET-CT scans offer more accurate quantifying methods of the biological and physiological processes through SUV values, retention index, target-to-background ratio, as well as visual grading [30]. Amyloid-binding PET radiotracers have accumulated a substantial amount of evidence; however, they are not yet licensed for clinical application in CA. Further investigations are needed to optimize acquisition procedures and validate results in broader groups. In contrast to bone-avid scintigraphy and SPECT radiotracers, PET radiopharmaceuticals have been explicitly designed to attach to the amyloid fibrils. Consequently, PET is the sole imaging modality that is unequivocally selective for amyloid deposits and highly selective for amyloid variants. Moreover, PET is capable of detecting ATTR CA, AL CA, as well as uncommon hereditary variants. Advancements in the quantification of myocardial uptake in both SPECT and PET have produced more detailed and consistent results, facilitating improvements in early diagnosis, risk stratification, and treatment response assessment [36].

DISCUSSION AND FUTURE PERSPECTIVES

Atrial and right ventricular uptake: a new perspective in radionuclide cardiac imaging

The diagnostic role of scintigraphy with bone-avid radiotracers in the diagnosis of CA – specifically of TTR form – is almost in all cases unequivocal [12,37]. However, their role in the prognosis of CA remains still unclear. Recent years' studies have focused on this perspective.

The amyloid burden can be easily identified in the left ventricle alone – yet in most cases, biventricular and/or atrial strain is present, leading to restrictive myopathy [3]. Two promising fields of research regarding the role of radionuclides in prevention and prognosis assessment are the increased uptake in the atrium and in the left ventricle on scintigraphy. Porcari et al. [38] studied the implications of right ventricular uptake of bone radiotracers on scintigraphy. Their results showed a biventricular uptake in 100% of their total of 1422 patients with TTR amyloidosis, identified on SPECT and inconsistently on planar “whole-body” images. It is known that right ventricle involvement in heart failure is linked to an unfavorable disease evolution [5]. This study [38] identified a diffuse right ventricle uptake in more than half of their patients, which was an independent mortality predictor.

Atrial involvement is a part of the onset of cardiac alterations [37]. It appears as part of global amyloidosis (AL or TTR) or as regional impairment of the atrial function due to pathological natriuretic peptide accumulation age related [39]. Hussain et al. [40] reported a high percentage of patients with TTR amyloidosis and atrial fibrillation (AF) to have atrial uptake on [99mTc]Tc-PYP planar images and SPECT-CT. Scintigraphy with 99mTc derivatives is unequivocally useful in assessing the ventricular uptake. Nonetheless, this study raised awareness for atrial implications – with or independent of that of ventricular – related to prevalent high-risk cardiovascular manifestations (AF, thrombi, pulmonary embolism).

National and International Guidelines

Adopting a uniform, standardized protocol, locally and internationally, for nuclear medicine studies for CA, would ensure the best accuracy of disease detection, thus improving the therapeutic approach and prognosis of the patient.

A recent study done by Stan et al. [41] showed the necessity of implementing national standardized protocols – including acquisition times and interpretation algorithms – for accurate reporting of CA scintigraphy studies.

CONCLUSION

As of today, nuclear medicine imaging studies represent a viable and reliable non-invasive imagistic method to diagnose cardiac amyloidosis. Furthermore, what was once an incurable disease with scarcely any treatment prospects is now more easily and promptly diagnosed with the courtesy of vast improvements in the field of radiopharmaceuticals used in cardiac nuclear imaging. While the diagnostic value of bone-avid radionuclides is unequivocal in TTR cardiac amyloidosis, novel indicators of their use in earlier stages or cardiac particularities (atrial or right ventricle uptake associated with unfavorable outcomes) are highlighted through recent studies, persuading specialists to study more this field. Furthermore, with promising results in cardiac imaging, PET may be considered and approved as a viable means of diagnosis, assessment of prognosis, and response to therapy.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization G.S.P.,M.M.M. and S.M.S..; methodology, G.S.P., L.E.M., M.G.; validation, D.M. and S.M.S.; data curation, D.M., G.S.P., M.M.M.; writing—original draft preparation, G.S.P, M.M.M, B.C.; writing—review and editing, L.E.M.,C.M., V.C.M..; supervision, S.M.S.; project administration, S.M.S..; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Not applicable .

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REVIEW

Detection of Acupuncture Points and Meridians Based on their Electrical Properties: Current Evidence and Future Research Perspectives

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Abstract: Acupuncture is one of the main components of Traditional Chinese Medicine and has been applied as a therapeutical method for centuries. As a therapeutical system, it is based on the use of specific lines in the human body, called meridians, and specific acupuncture points, for choosing the locations where the needles will be applied, depending on the specific problem. In the last few decades, there have been increasing efforts to detect and document the existence of such meridians and points based on at least some of their electrical properties being different when compared to the surrounding skin. A number of researchers have designed experiments, which have had varying degrees of success. Since different researchers have used different experimental setups, and the sample size is usually low, there are issues of validity and comparison between different research results. On the other hand, the majority of available evidence is in favor of there being points and lines characterised by distinct electrical properties, corresponding to acupuncture points and meridians respectively. At the same time, several other theories have been proposed to explain the biological basis of acupuncture points and meridians, while their connection to specific cellular components or neural sequences is an intriguing possibility. While comprehensive research is required to fully document the electrical properties of acupuncture points and meridians, a connection between such properties and their biological basis must also be established.

Keywords: acupuncture points; meridians; electrical properties; TCM; impedance; resistances

INTRODUCTION

Acupuncture is an integrative part of Traditional Chinese Medicine, and has been practised in China [1] and Japan and also in Korea [2, 3] for centuries. It is certain that acupuncture appeared in China although it is difficult to trace its exact historical era of emergence [4]; much later it would be disseminated in Japan, during the 5th century AD [5]. The practises of acupuncture would later become known to Europe by the end of the 17th century, through the works of Willem Ten Rhijne and Engelbert Kaempfer [6]. Following a hiatus of almost a century, after 1850, it would be again be advocated fervently by George Soulié de Morant in 1940s France, and gradually spread to Europe [7].

In the West, there is an ongoing disagreement regarding the efficacy of acupuncture in particular and of alternative therapies in general, both within and outside of the medical community, despite there being numerous studies which are in favour of acupuncture’s effectiveness [8-16]; iatrogenic complications and side-effects are also very rare [17]. Even veterinary acupuncture has been gaining traction as of late [18-21], although we will not be dealing with that in the present article; notably, research on acupuncture points has already began in earnest since the 1990s [22,

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23] – there have even been some reports of the effects of acupuncture in plants [24-29]. It has long been accepted by numerous physicians and researchers that acupuncture can be integrated with modern Western medicine [11,30].

In contrast to the methods of Western medicine, also known as “evidence-based medicine”, the exact definition of acupuncture points and meridians and the associated “flow of qi”, which constitute the principal theory behind acupuncture therapy, is a concept that cannot be quantified and determined accurately by available technical means, at least for now. There are numerous research efforts which have attempted to describe the different electrical properties of acupuncture points.

The different electrical properties of acupuncture points compared to non-acupuncture points in the human body form the basis for the development of special acupuncture point locators, both for somatic and for auricular acupuncture points. It is important to note here that the existence of differences, at some physiological level, between random points and acupuncture points, lends credence to acupuncture being a legitimate therapeutic method more so than mere psychological suggestion [31] or some type of a placebo effect. A research by Vickland et al. [32] suggested that acupuncture changes the electrical properties of the acupuncture points themselves. Even so, the general difficulty in obtaining consistent and reproducible readings in certain points in the skin and the absence of any known physiological mechanism does not allow for a definite determination of the electrical characteristics of acupuncture points [33]. Recently, the advances in machine learning and artificial intelligence [34-39] may allow for different approaches and yield more conclusive results, although nothing definitive is available as of yet. It is possible that a part of the difficulties in establishing a clear-cut model for detecting TCM meridians and acupuncture points has to do with the biological structure of the skin.

The human skin comprises four successive layers, from the exterior to the interior: stratum corneum, living epidermis, dermis, and hypodermis. Detailed investigations into the structure and specific properties of the skin have been made by a number of authors [40-42]. Each of these layers has different conductive, capacitive and dielectric properties; predominantly these depend on the applied voltage although both age [43] and the relative health condition affect them [44]. In essence, parameters such as resistance, impedance and capacitance are a reflexion of the physical properties of the skin [45].

There exist a number of studies, detailing the different skin dielectric properties, depending on the applied voltage, at different frequencies, both for the entirety of the skin layer [46] and for each layer in particular [47-49]. In general, the living epidermis has higher resistivity than the dermis, while the resistivity of the dermis is not as variable. The higher the number of available ions the lower the resistivity values [50]; since the dermis is more hydrated it is reasonable that it has a higher ion content and therefore a lower resistivity [51]. The main component of impedance is the stratum corneum, the most hydrophobic of the skin layers [45]. Another important factor is the relevant permittivity, which reflects the amount of bound charge displacement or polarisation, in a given area, under the influence of an applied electric field [52]. This property is frequency-dependent and is influenced by the existing conditions at a cellular and molecular level [53-55].

In this review, we will present the most important research regarding the determination of acupuncture points and meridians, mostly based on the measurements of electrical parameters, in an effort to interpret their different and sometimes contradictory results. We will also try to interpret these results in the context of the general biological properties of the skin and underlying tissues. As it will become evident, there are a multitude of different studies and methods and we allude to as many of them as reasonably possible, but we have chosen to present in tables only those researches which have focused in measuring electrical properties using electrodes, as this is the focus of our paper. Finally, we will offer possible explanations for the observed phenomena, based on recent discoveries, and directions for future research.

TCM MERIDIANS AND RESEARCH ON THEIR EXISTENCE

There are numerous types of meridian groups, but here, for research purposes we will focus on the 12 principal meridians and the 2 most important extraordinary meridians (Table 1) – to our knowledge no research on locating any other type of meridian, such as collateral or connecting meridians, has been performed using modern methods.

As limb localisation, based on TCM, we define the limb on which most of a meridian’s points can be located; most often it also contains the point of origin of the meridian. The time of maximum activity for each meridian indicates when its most active for therapy, and may correspond to the modern concept of circadian rhythms, which has been discussed in many clinical contexts [59-62].

The 12 principal meridians are associated with organs of the human body, although the properties of these organs do not always correspond to those attributed to them by Western Medicine, and medical acupuncture research. For example, in TCM, the Pericardium Meridian corresponds to the ‟organ” of pericardium, which is however recognised only as an anatomical structure surrounding the heart, and not an organ proper [63,64]. Moreover, Du Mai and Ren Mai do not correspond to any organ but are associated with the regulation of other processes and functions [65,66].

In another example, the Lung meridian is associated with skin health in TCM, a seemingly paradoxical connection by Western medicine standards; however, according to a number of studies, pulmonary pathologies have skin-related manifestations, as is the case, for example of chronic obstructive pulmonary disease [67,68], and of recurring pulmonary capillaritis [69]. In a reverse example, some skin disorders in children have pulmonary manifestations [70]. While these remarks are interesting and point to a corroboration of many of the principal tenets of TCM, the mainstay of research on verifying the existence of meridians is concerned with identifying them based on their different electrical properties.

Table 1: Principal TCM meridians and their principal characteristics
(based on Soulié de Morant [56], Liangyue et al. [57], Tilikidis [58], and Periferakis [6]).

Western Meridian Name	Chinese Meridian Name	Limb Localisation	Point of Origin	Acupuncture Points in Meridian	Time of Maximum Activity
Lung	Taiyin	Hand	Upper lateral chest, approximately at the level of the 1st intercostal space	11	0300-0500
Large intestine	Yangming	Hand	Radial end of the distal phalanx of the index finger, near the corner of the nail	20	0500-0700
Stomach	Yangming	Foot	Under the eye, directly on the line traversing the pupil, between the eyeball itself and the infraorbital ridge	45	0700-0900
Spleen	Taiyin	Foot	Medial side of the big toe, near the corner of the nail	21	0900-1100
Heart	Shaoyin	Hand	Apex of the axillary fossa	9	1100-1300
Small Intestine	Taiyang	Hand	Ulnar side of the distal phalanx of the little finger, near the corner of the nail	19	1300-1500
Urinary Bladder	Taiyang	Foot	Near the eye in the depression near the inner canthus	67	1500-1700
Kidney	Shaoyin	Foot	Sole of the foot, in the depression former with the foot in plantar flexion	27	1700-1900
Pericardium	Jueyin	Hand	In the 4th intercostal space, by the nipple laterally	9	1900-2100
Triple Boiler	Shaoyang	Hand	Ulnar side of the distal phalanx of the ring finger near the corner of the nail	23	2100-2300
Gall Bladder	Shaoyang	Foot	Lateral to the outer canthus, on the lateral side of the orbit	44	2300-0100
Liver	Jueyin	Foot	Lateral side of the big toe near the corner of the nail	14	0100-0300
Governor Vessel	Du Mai	-	Under the tip of the coccyx	28	-
Conception Vessel	Ren Mai	-	Perineum	24	-

Previous studies on the identification of meridians based on their electrical properties

In general, and compared to the studies on individual acupuncture points, which will be presented on the next section, meridian studies are characterised by a larger sample size and are thus of a higher statistical significance. A number of different measuring techniques were used, and this raises the issues of the comparability and reproducibility of results (Table 2). Probably the earliest research on the different electrical properties of meridians was conducted by Nakatani [71], in the 1950s.

The research of Reichmanis et al. [72] calculated electrical parameters between two different acupuncture points of the same meridian, and determined, using Laplace analysis, that the electrical resistance values were lower. Between the line connecting the two acupuncture points studied, the 14th and 12th of the Large Intestine meridian, there was no other acupuncture point, which could potentially alter the measurements. In the same year, the same authors used the same experimental setup to calculate electrical resistance between the 3th and the 4th acupuncture point of the Heart meridian, with similar positive results [73].

The research of Reichmanis et al. [74] this time measured the resistance between two non-acupuncture points on the Heart meridian; again skin impedance was significantly lower, compared to the control area. This indicates that the presence or absence of acupuncture points in a given meridian area does not affect the clearly different electrical properties of the meridians.

The study of Ogata et al. [75], while not focused on finding the location of meridians based on their electrical properties – indeed it took for granted that the meridians are located on the trajectories described by Traditional Chinese Medicine – detected that there were significant differences in the electrical resistance values of meridians during the different stages of general anaesthesia. We consider the fact that all 12 of the studied meridians had such variations is an, at least indirect, corroboration of meridian location based on different electrical properties, and this is why we included this research. Moreover, we have also included this research in the acupuncture points section of this paper, given that the meridian resistance values were calculated as averages from a number of distinct points in each meridian. Similar results, albeit of a lesser magnitude, were noticed by Ogata et al. [76] in patients under local anaesthesia. Due to the smaller extend of the electrical resistance fluctuation we classify the findings of this research as ambiguous.

It is important to note that the electrical parameters of the skin during post-surgical resuscitation are known to vary, based on the research of Bubnova et al. [77], but it is uncertain if this phenomenon has any bearing on the two aforementioned researches.

Following electrical stimulation at the Jing point of the Stomach (45th of the meridian), 98% of the patients, from a sample of 51 patients tested in an acupuncture clinic, were found to exhibit altered impedance values across the Stomach meridian [78]. The research of Hu et al. [79], which used a larger sample, compared to most of the research efforts examined in this paper, determined that the majority of low impedance points, in the areas examined, where found within a distance of less than 5 mm from acupuncture meridians. Interestingly, they performed this test for all 14 main meridians. A year later, Hu et al. [80] used an electrode moving at an even velocity over the forearm, and computer plotting to demonstrated that most of the detected low skin impedance points were located within the traditional courses of TCM meridians. Similar results were produced by Huang et al. [81], although few details exist on their experiments.

During the research of Zhang et al. [82], impedance was found to be lower in meridians, compared to other skin regions, and to change during needling, a process potentially associated with interstitial fluid fluctuation caused by neuronal reflexes. The research of Martinsen et al. [83], performed using a sample of 20 healthy subjects, did not discover any low resistance trajectories on the volar aspect of the forearm. However, the accuracy of the measuring method of this research has come into question [84]. A subsequent research by Johng et al. [85] yielded positive results, while the research of Ahn et al. [86] had ambiguous findings, in that it demonstrated that the impedance along the Pericardium meridian was decreased, but this was not the case for the Spleen meridian.

The research of Spaulding & Chamberlin [87], who performed acupuncture in order to measure the conductivity across the Large Intestine meridian, yielded statistically significant differences in favour of the meridian, but these were not pronounced enough; thus, we regard these results as being ambiguous.

Table 2: Basic characteristics and outcomes of research of the location of meridians based on their electrical properties

Authors & Reference	Year of Study	Sample Size	Healthy Individuals	Control	Current Used	Experimental Setup	Results	Evaluation
Reichmanis et al. (73)	1977	10	Yes	1-1.5 cm parallel lines medially and parallelly	DC, 1 V	1 cm carbon-impregnated conducting rubber electrodes (modified LIDC electrodes by Ritter Co.)	The electrical resistance values in the line connecting the two acupuncture points were lower compared to the control	Positive
Reichmanis et al. (72)	1977	10	Yes	1-1.5 cm parallel lines medially and parallelly	DC, 1 V	1 cm carbon-impregnated conducting rubber electrodes (modified LIDC electrodes by Ritter Co.)	The electrical resistance values in the line connecting the two acupuncture points were lower compared to the control	Positive
Reichmanis et al. (74)	1979	10	Yes	1.5 cm parallel lines medially and parallelly	DC, 1 V	1 cm carbon-impregnated conducting rubber electrodes (modified LIDC electrodes by Ritter Co.)	The electrical resistance values in the line connecting the two non-acupuncture points were lower compared to the control	Positive
Ogata et al. (75)	1983	10	No	None	AC*	Standard ECG electrodes taped over the chosen acupuncture points in each meridian; values measured during various intervals from preanaesthesia to postanaesthesia	There was a decrease of up to 36 % in skin resistance in 10 of the 12 studied meridians, and by a maximum of 100 % and 73 % on the kidney and bladder meridians respectively	Positive
Ogata et al. (76)	1983	6	No	None	AC*	Standard ECG electrodes taped over the chosen acupuncture points in each meridian; the values were measured before and after the application of local anaesthesia	There were fluctuations of a rather small scale in all of the meridians studied	Ambiguous

Xiang et al. (78)	1984	51	No	None	AC, 0-100 V	Stimulation of the Stomach Jing point using a bipolar electrode and measurements based on the process of Zhu et al. (88)	A distinctly localised meridian line for the Stomach was located in the overwhelming majority of subjects after the initial stimulation	Positive
Hu et al. (79)	1992	68	Yes	Areas laterally removed from the meridians	DC, 0-50 V	1 mm pure Ag tip electrode	The majority of the studied points within less than 5 mm from all 14 main meridians was found to have low impedance	Positive
Hu et al. (80)	1993	12	Yes	Areas not belonging to established meridians	AC†	Exploratory electrode moving at a standard velocity over the forearm – computer system used for plotting	Low skin impedance points (LSIPs) were discovered and were generally distributed along known meridian courses	Positive
Huang et al. (81)	1993	25	Yes	Areas not belonging to established meridians	AC†	n/a (perhaps similar to the setup of Hu et al. (80))	Low skin impedance points (LSIPs) were discovered to lie mostly within or very near to known meridian courses	Positive
Zhang et al. (82)	1999	12	Yes	Non-acupuncture points in surrounding skin	AC	4 electrode technique – 4 mm diameter pipes with saline cotton	There was a decrease in impedance in the meridian area studied, which fluctuated during needling	Positive
Martinsen et al. (83)	2001	20	Yes	None	AC	16 electrode (0.5 mm diameter) linear array	No linear low resistance trajectories were detected	Negative
Johng et al. (85)	2002	30	Yes	Lateral parallel control at a distance of 6-7 cm from the tested meridian	AC	4 electrode technique similar to that of Zhang et al. (82)	Higher capacitive effects at acupuncture meridians, compared to the control	Positive
Ahn et al. (86)	2005	23	Yes	Control segments 0.8 cm distant, medially and parallelly to the tested meridians	AC	4 electrode method (0.25 mm Au plated needles serving as the electrodes) supplemented by ultrasound imaging of the connective tissue planes along the tested meridians	While impedance in the case of the Pericardium meridians was indeed lower, the same cannot be said for the impedance of the Spleen meridian	Ambiguous
Spaulding & Chamberlin (87)	2011	20	Yes	Non-meridian tissue	AC†	A test configuration involving a data acquisition hardware, while processing was performed in Matlab; acupuncture was performed during the measurement	There is a somewhat better conduction along the Large Intestine meridian, compared to non-meridian tissue; while there is a statistical significance of the values, these are not dramatic	Ambiguous

** For these researches the exact data on the type of current used are not available; therefore, based on their use of ECG electrodes and apparatuses we have assumed that it is AC current (this being the more common modality in such machines)*

† Similarly, no data on the current type were mentioned and we have assumed it to be AC, which is the most common setting

The particular research parameters of the aforementioned studies on meridian locations are presented in the table below (Table 3).

As can be seen, meridian studies have a higher mean sample size, compared to the acupuncture point studies, which will be presented in the following chapter. In general, there is a greater homogeneity of in research methods and subject selection compared to the following studies on acupuncture points presented in the next section.

The studies with control have also used healthy subjects and there is about an equal number which used AC and DC; most of them have results which can be evaluated as “successful’’. The fewer and more diverse studies with no control have also had a relatively positive total outcome. Of course, it is easier to locate meridians compared to acupuncture points, as acupuncture point location is a more difficult and precise process.

In the next subsection, a number of studies on locating meridians using other methods were presented. The association between meridians and interstitial fluid has also been explored by a number of authors along with a number of other proposed alternatives

Table 3: Data on the presented research on meridians

Study Type	N	Mean Sample Size	Subjects' Health			Type of Current Used			ReportedOutcome		
			Healthy	Not Healthy	Mixed	AC	DC	Both	Positive	Ambig.	Negative
Studies with control	10	22.00	10 (100%)	0 (0%)	0 (0%)	6 (60%)	4 (40%)	0 (0%)	8 (80%)	2 (20%)	0 (0%)
Studies with no control	4	21.75	2 (50%)	2 (50%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)	2 (50%)	1 (25%)	1 (25%)
Total studies number	14	21.90	12 (85.7%)	2 (14.3%)	0 (0%)	10 (71.4%)	4 (28.6%)	0 (0%)	10 (71.4%)	3 (21.4%)	1 (7.2%)

For example, Li & Zhao [89] proposed that meridians may coincide with the courses of some blood vessels, while Zhu [90] proposed that the meridians as explained by the TCM are, in fact, an erroneous representation of the nervous system. Several hypotheses were proposed by Zhang [91] while the current research consensus focuses on the meridians representing low hydraulic resistance channels associated with the movement of interstitial fluid and specific metabolites [92]. The association of meridians with stem cells was also proposed by Pawitan [93]; bearing in mind that the stem cell potential varies with a number of factors [94-100], this could have a bearing on the differences in meridian studies across individuals.

Other Studies and Theories on the Properties and Location of Meridians

Apart from the more traditional method of detecting and/or identifying meridians based solely on their different electrical properties, a number of authors have tried a variety of different approaches (Table 2). The most probable explanation is that the different electrical properties of meridians are based on a neural electrical field [101].

The tentative research of Jiang et al. [102] indicates that along meridians, the microcirculatory response to moxibustion therapy is different; thus it may be inferred that there may be well be some level of difference in the microvasculature of meridians, compared to other locations on the body. Research suggests that, in rat experiments, moxibustion therapy seems to modulate gene expression [103]. A clinical trial protocol on the same principle was proposed by Hu et al. [104]. On a different note, the research of Lin et al. [105], indicated that the electrical flow across meridians changes depending on acupuncture needle manipulation during therapy.

Crucially, as is also the case for certain acupuncture points, the electrical properties across meridians seem to differ between pathological and healthy conditions [106,107]. On a related note, a potential association between electrical resistance changes and pain after orthopaedic surgery, which however warrants further investigation, was proposed by Rezvani et al. [108].

The most currently prominent avenue of research on defining the morphofunctional characteristics of meridians centres on them being supposedly channels where a higher amount of interstitial fluid is found. A gel model based on this concept seems to suggest that there is some merit in this theory [91]. An earlier research by Zhang et al. [109] in rats, had not produced any corroborating results.

Finally, the different electrical properties of meridians may be associated by an ion drift theory, as proposed by Liu et al. [110]; notably the research of Lee et al. [111] also mentioned that the special properties of acupuncture points may be associated with different ion concentrations.

VARIATION OF SKIN ELECTRICAL PROPERTIES AT ACUPUNCTURE POINTS

The classical Chinese theory postulates that there exist over 300 acupuncture points, distributed across the 12 principal and 2 extraordinary meridians mentioned above, all over the human body.

The stimulation of these points, based on the view of traditional acupuncturists, adjusts the “qi flow” along the human body; the most frequent proposed interpretation for this notion of “qi energy” is electrical energy [112] and this has been the focus of the majority of meridian detection-associated research.

Electrical properties of acupuncture points – Research history

While, based on the traditional theory, the location of points is determined based on the anatomical parameters and locations of the human body, it was though also possible that these points exhibit distinct electrical properties; based on this speculation, relevant research was performed in the 1950s ([33,113]; and references therein), documenting that at least some acupuncture points where characterised by specific electrical properties.

Table 4: Basic characteristics and outcomes of research of the location of acupuncture points based on their electrical properties

Authors & Reference	Year of Study	Sample Size	Healthy Individuals	Control	Current Used	Experimental Setup	Results	Evaluation
Reichmanis *et al.* (122)	1975	7	Yes	Anatomically similar location within 1 cm^2	DC, 8 V	1.5 mm measuring electrode and a specially designed wheel electrode; both made from stainless steel	Most of the acupuncture points measured were identified based on their electrical parameters in all subjects	Positive
Reichmanis *et al.* (123)	1976	10	Yes	Suitable nearby anatomical location	DC, 2 V	36 stainless steel rods in a grid pattern over the acupuncture point	Over 75% of the acupuncture points were electrically different compared to the control areas	Positive
Hyvärinen, & Karlsson (124)	1977	5	Yes	Surrounding skin	DC, 6.7 V	0.5 mm circular electrode and 2.5 mm reference electrode	Points of consistently low electrical resistance, resembling acupuncture points, were identified	Positive
		5	Yes		DC & AC	0.5 mm silver wire	Massively lower, about 50-100 times, electrical resistance was identified in the same points as above	Positive
McCarroll & Rowley (125)	1979	8	Yes	None	AC (V is a measured variable)	5 x 5 grid with the acupoint in the centre	The decreased electrical impedance detected at a few points was attributed to temporal variations and the pressure of the probe on the stratum corneum	Negative
Poon et al. (127)	1980	9	Yes	Surrounding skin	AC, 0-18 V	Special epiductive tape electrode	Numerous spots of increased conductance were identified, many lying across the meridians, but a lot differing significantly from conventional acupuncture locations	Ambiguous
Jakoubek & Rohlícek (129)	1982	n/a	n/a	n/a	DC & AC	Multiple electrodes	Many points with increased electrical conductance, corresponding to acupuncture points, were detected	Positive
Ogata et al. (75)	1983	10	No	None	AC†	Standard ECG electrodes taped over the chosen acupuncture points in each meridian; results measured during various intervals from preanaesthesia to postanaesthesia	There were marked electrical resistance variations during the different stages of anaesthesia, in most of the acupuncture points studied	Positive
Ogata et al. (76)	1983	6	No	None	AC†	Standard ECG electrodes taped over the chosen acupuncture points in each meridian; the values were measured	There were fluctuations of a rather small scale in all of the individual points measured	Ambiguous

						before and after the application of local anaesthesia		
Margolin et al. (131)	1996	34	No	Auricular control zones not comprising any acupuncture points	DC	Specially developed device with a 1 mm Ag/AgCl electrode composition; use of biogel as a conducting medium – the measurements were performed using auricular acupuncture points; subsequent acupuncture of both control and active points was performed	The therapeutic effects of acupuncture in low impedance points were not very much different from the effects on non-acupuncture points	Ambiguous
Zhang et al. (82)	1999	12	Yes	Non-acupuncture points in surrounding skin	AC	4 electrode technique – 4 mm diameter pipes with saline cotton	There was a decrease in impedance in the meridian area studied, which fluctuated during needling	Positive
Falk et al. (130)	2000	34	No	Control zones on the helix of the ear	AC	An Ag/AgCl probe of 1 mm diameter in a 2 x 2 grid	The sites of lowest impedance were identified as being within active zones	Positive
Zhang et al. (138)	2004	12	Yes	Two points on either side of the low impedance points at a distance of 0.5 cm	AC	Four-electrode impedance instrument (Korean SPAC instrument) based on the methodology described by Zhang et al. (82)	It was found that 71 % of the acupuncture points tested had a lower impedance compared to the control points	Positive
Lee et al. (139)	2005	20	Yes	One non-acupuncture point	DC, 1.28 V	Electrodes affixed to two acupuncture points and one non-acupuncture point; constant pressure applied in the 4th point of the Large Intestine Meridian	Meridian points exhibit a greater conductivity compared to non-meridian points	Positive
Pearson et al. (141)	2007	20	Yes	Nearby control sites no more than 5 cm away from the acupuncture points	DC & AC	Prognos instrument (4.57 mm probe tip) along with a specially designed PT probe	None of the acupuncture points measured exhibited lower impedance values compared to the control points	Negative
Kramer et al. (142)	2008	43	Yes	Surrounding skin	DC, 4.8 V (mean)	Specially designed array comprising 64 electrodes covering an area of 36 cm^2	Only about 1/3 of the measured acupuncture points exhibited significantly decreased electrical resistance values	Negative
Kramer et al. (143)	2009	53	Yes	Surrounding skin	DC, 4.8 V (mean)	Specially designed array comprising 64 electrodes covering an area of 36 cm^2	Some acupuncture points displayed elevated and some reduced impedance values compared to the surrounding areas; it is therefore considered possible that acupuncture points possess different transient electrical properties compared to the surrounding skin	Ambiguous
Rezaei et al. (144)	2012	18	Yes	Parallel series of four control needles placed 0.8 cm	AC	4 electrode technique specifically designed for the study in question	The point studied (PC4) was found to be electrically distinct from other non-meridian points and similar	Positive

				medial to the meridian needles			to other points of the same meridian	
Wong (145)	2014	65	Yes	A neighbouring non-acupuncture point, 2 cm distant	DC, 0.5 V	Bipolar electrode (3 mm in-between electrodes) and a sensing probe	The point studied (LI14) was not found to be electrically distinct from the control point	Negative

** For these researches the exact data on the type of current used are not available; therefore, based on their use of ECG electrodes and apparatuses we have assumed that it is AC current (this being the more common modality in such machines)*
† Similarly, no data on the current type were mentioned and we have assumed it to be AC, which is the most common setting

Some decades earlier, in the 19th century, August Weihe (1840-1896), a medical doctor and homeopathy practitioner came to the conclusion that specific points in the human body were found to be more painful compared to the others, following the ingestion of a toxic quantity of certain homeopathic remedies [114]; it has been mentioned that Weihe knew the notion of specific therapeutic points from a relative in China [115]. It is also notable that Soulié de Morant mentioned that these points coincided with Chinese acupuncture points [116]. The theory of homeosiniatry was proposed by Roger de la Fuye (1890-1961), in 1932, to account for the physiological similarities between homeopathic remedies and acupuncture points; notably, he impregnated some acupuncture needles with homeopathic remedies in a number of his acupuncture therapies [116]. Today, both Weihe and de la Fuye are considered as the ancestors of the theory of electroacupuncture [117]. In 1953, probably the first electroacupuncture apparatus was designed and built by R. Voll (1909-1989) and F. Werner [113].

Despite many studies having taken place on this subject (Table 4), some of them are deemed as being of poor quality, due to a number of factors [118]. A particular point in researching the electrical properties of acupuncture points is the standard to which the data obtained are compared with; more precisely, while acupuncture points may not present minimal electrical resistance at an absolute level, the issue is if they exhibit a different electrical resistance compared to their surrounding tissues. Dedicated research to for the particular, if any, electrical properties of acupuncture points began in the 1970s; notable research efforts from this era are those of Frost & Orkin [119] and Kaslow [120]. Contemporary views on the existence and function of acupuncture points in particular and acupuncture in general can be found in McCarroll [121].

The research of Reichmanis et al. [122] was based on the principle that acupuncture points exhibit a reduced electrical resistance [30]; accordingly, they aimed to prove that acupuncture points exhibited a distinct resistance minimum compared to the surrounding tissue. They employed direct current and used some of the most easily accessible points of the Large Intestine (LI) and Pericardium (Per) meridians; they concluded that all of the studied points exhibited an increased electrical conductance, relevant to the surrounding tissues and most could be located in the majority of subjects. In a subsequent research [123], using a 36 rod grid, on skin disinfected with 90 % alcohol and moistened with tap water, over selected acupuncture loci; suitable control areas were also selected. The points selected were again easily accessible, from the Triple Boiler (TB) and Lung (Lu) meridians. Most, but not all, points were detectable based on different electrical parameters. Further promising results were obtained by Hyvärinen & Karlsson [124], who detected a number of low electrical resistance points on the forearm, hands and ears, which resembled acupuncture points, albeit in a small sample of five healthy individuals. Similarly, in these points the electrical impedance values were found to be massively lower compared to the surrounding skin [124].

Despite these positive results, McCarroll & Rowley [125], who measured the electrical conductance in certain acupuncture points in the hands and arms, came to an unfavourable conclusion. It is noteworthy that this study considered extensively the probable consequences of the pressure applied by the electrode on the skin, to the measurements; in addition, it considered whether any break in the stratum corneum may influence the results. The authors mentioned that, based on their measurements, the time-dependent nature of skin conductance, as asserted by Geddes & Valentinuzzi [126], applied to their measurements as well. They concluded that any detected variations were the result of differing electrode pressure on the stratum corneum and the temporal variability of skin conductance [125].

In an effort to minimise the effect of the electrode itself, Poon et al. (127) used a special epiductive tape electrode, based on the method of Burton & Maurer (128). They determined that a number of points which had different electrical properties corresponded with acupuncture points, but other such points did not; moreover, no statistical analysis demonstrated that across all subjects no significant coincidence between electrically "special" points and acupuncture points was revealed. Even more strikingly, the points of good conductance differed between the two arms of the same subject (127). In addition, a number of low resistance points coinciding with acupuncture points were detected in both humans and rats, and also some such points – not corresponding to acupuncture points – were found in areas of referred pain (129). The research of Ogata et al. (75), to which we have already referred to in the meridians section, also noted that electrical properties of acupuncture points changed during different stages of anaesthesia. For the same reason, the other research of Ogata et al. (76) is included in this chapter too.

A four-electrode method was employed by Zhang et al. (82) to evaluate a single acupuncture point, the 3rd of the Pericardium meridian, during, before and after needling, compared to a number of control points. It was noted that electrical impedance changed in all points, but the impedance reduction in acupuncture points was greater; it was theorised by the researchers that the impedance decrease was owed to a transient increase of interstitial fluid during needling, which is most probably associated with an axonal reflex and/or blood capillary expansion (82). We have already included this research in the section on meridians, but we also place it here because of its focus on the area around a single point.

Meanwhile, Falk et al. (130), using as test subjects patients under treatment for cocaine addiction, detected significantly lower impedance values in the active zones of the ear, i.e. those corresponding to auricular acupuncture points. A similar research had already been conducted by Margolin et al. (131), who tested the differential efficacy of acupuncture treatment in high and low impedance points in the ear; the results in this clinical trial had been ambiguous, in that while needling in low impedance points – corresponding to acupuncture points – had somewhat better effects, this difference was assessed as not being significant enough. Again, this therapeutic approach concerned auricular acupuncture in cocaine addicts. Interestingly, during auricular acupuncture it proved impossible for the subjects to identify, based on pain perception, when acupuncture points were needled, or when needle placement was random (132). Incidentally, there is as of yet no consensus regarding the efficacy of auricular acupuncture in treating cocaine addiction; the results range from mildly encouraging (133, 134) to inconclusive (135, 136) or even negative (137).

Using the four-electrode method, Zhang et al. (138) determined that in their research on a small number of heathy subjects, 71 % of the acupuncture points had a significantly lower impedance compared to the control points on either side. The research of Lee et al. (139) suggested that between two acupuncture points the conductance was greater than between a non-acupuncture and an acupuncture point. Since the second pathway did not belong to an established meridian, they concluded that their experiment perhaps corroborated the theory of Chen (140), who proposed that the dielectric constant along meridians is lower compared to other non-meridian trajectories. Contrary to this, the research of Pearson et al. (141), which was one of the most well-controlled studies, did not detect any significant differences in electrical impedance between acupuncture and non-acupuncture sites.

The research of Kramer et al. (142), who studied the variations of electrical skin resistance in the 34th point of the Gall Bladder meridian in 43 healthy volunteers concluded that, compared to the surrounding skin, only about 33% of the studied points exhibited statistically significant differences; they rightly considered such a small percentage to not be in agreement with most other performed research. A subsequent clinical trial (143) yielded ambiguous results.

The research of Rezaei et al. (144) aimed to determine if a certain acupuncture point, the 4th of the Pericardium meridian, had any different properties compared to its surrounding ones and other points in the same meridian; it was found that it was electrically distinct compared to non-meridian points, but not so much compared to meridian ones. The four-electrode method employed was based on the research of Zhang et al. (82).

The research of Wong (145) is particularly important, as it strived to do stringent lab-controlled measurement of skin resistance of an acupuncture point (14th point of Large Intestine Meridian) on a larger sample (65 participants) than other studies. In their larger sample, they found no statistically significant difference in the electrical resistance between acupuncture and non-acupuncture points.

The results from all the studies in Table 4 are presented in the following table (Table 5), in order to enable a more complete view of the current research consensus.

Table 5: Data on the presented research on acupuncture points

Study Type	N	Mean Sample Size	Subjects' Health			Type of Current Used			ReportedOutcome		
			Healthy	Not Healthy	Mixed	AC	DC	Both	Positive	Ambig.	Negative
Studies with control	15	23.1	13 (86.7%)	2 (13.3%)	0 (0%)	5 (33.4%)	8 (53.3%)	2 (13.3%)	9 (60%)	3 (20%)	3 (20%)
Studies with no control	3	8	1 (33%)	2 (66%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)	1 (33%)	1 (33%)	1 (33%)
Total studies number	18	20.6	14 (77.8%)	4 (21.2%)	0 (0%)	8 (44.5%)	8 (44.5%)	2 (11%)	10 (55.6%)	4 (22.2%)	4 (22.2%)

As it can be easily seen from the aforementioned concise table on research results, the studies with control have a considerably larger sample size compared to the studies with no control, where its absence along with the limited sample size further undermines their statistical significance.

In assessing the more significant first group of studies, the overwhelming majority used healthy subjects, while half of them used direct current. Only a small percentage were negative, while the majority was positive or ambiguous. The results were equally distributed in the second group of studies; overall, about half of the studies presented in this section were unambiguously positive, although we would suggest that this statistic has been negatively skewed due to results from the studies with no controls. The majority of studies selected healthy subjects only, while the most frequent current used was alternating current by a small margin, followed by direct current; very few studies opted for both currents.

The relative discrepancy in results can be partly, in our opinion, explained by the fact that the majority of studies have limited their measurements in the upper limbs. The most recent research indicates that acupuncture point resistance values are non-linear [146] and this may influence the results, because on one hand some researches may have focused in non-optimal areas, and on the other few data on the lower parts of the body, and the head exist, if any at all. It is, however, a fact that electrical resistance measurements exhibit great variations depending on anatomical locations [118].

A curious phenomenon, termed the “drift” effect, was discovered by Mayer-Gindner et al. [147], where, if alternating current (AC) is used by the electrode, in the same point, gradually the electrode-skin resistance will decrease. They proposed that at least 20-30 min, if not 40-50 min should pass between two consecutive measurements, even if the point measured is not the same but an adjacent one. They suggested a new method for locating acupuncture points using alternating current. However, this does not seem to be the case, at least to our knowledge, as 5 out of 6 control-group AC studies yielded a positive result.

Presumably, the same influence of the circadian rhythm on meridians, if confirmed, should apply also to acupuncture point measurements. It is known for certain that the circadian rhythm affects skin physiology [148]. The circadian rhythms are also known to affect skin hydration [149], a factor which for sure influences the electrical resistance of the skin and potentially the detectability of acupuncture points.

In addition, there are evidence which suggest that the partial oxygen pressure values in the tissues underlying the meridians are higher than the tissues under random anatomical sites [150]. We mention this research here, instead of the previous meridian chapter, since it was performed on acupuncture points but in essence concerns the whole meridians trajectories. The extensive scientific literature in matters other than electrical properties per se, as outlined in the previous section, seems to corroborate the existence of acupuncture points as distinct morphofunctional entities.

We may conclude that regardless of whether the loci of low electrical resistance can be considered as being the same as the acupuncture points of TCM or not, it is by far more probable that such distinct loci do exist. Their diameter ranges from 1 mm to about 4 mm [120,151,152]. A factor which complicates matters is the relative uncertainty in locating acupuncture points [153], as based on textbooks there may be slight variations [154]; taking into account the tiny diameter of the loci of decreased resistance, it is conceivable that some negative research outcomes were influenced by a slightly erroneous choice of measurement sites. Research on whether needling of the exact location is necessary for a successful treatment outcome is still ongoing [155]; perhaps a general location with a diameter of some mm with the locus of minimum impedance at the centre may be considered as an acupuncture point. What is more certain is that the stimulation of acupuncture points, or of areas around them at least, is by far more effective that the stimulation of random, non-acupuncture points, based on a number of studies [156-160].

Other studies and theories on the properties of acupuncture points

There are a number of other studies, which are associated with the particular properties of acupuncture points, but which do not adhere to the pattern thus described, i.e., the comparison between acupuncture and non-acupuncture points in terms of their electrical properties. The effort of morphologically defining acupuncture points has begun since the late 1970s [161] and it is still an ongoing field of research, where many suggestions and research results must be taken into account. From a structural standpoint, it has been proposed that the microvasculature is capable of providing a larger blood supply in acupuncture points [162].

For example, Liang et al. [163] have asserted that the NO (nitric oxide) concentration in the skin, in acupuncture points, is higher compared to non-meridian and non-acupuncture points. According to their experiment, a NO-noradrenaline system is associated with the different electrical resistance at acupuncture points. Their experiment was based on the previous findings of Chen & Ma [164]. In the same year, Ma [165] asserted that, in addition to the NO-noradrenaline system, the reduction of nitrates by bacteria at acupuncture points was also contributing to the different electrical properties. Other relevant studies, with quite favourable results also exist [166-169].

A limited research effort by Khorsand et al. [170] used an entirely different setup, in that it evaluated the changes of electrical properties of acupuncture points before and after acupuncture was performed – none were detected. A study by Turner et al. [171] aimed to ascertain whether, between arthritis patients which experienced pain and pain-free arthritis patients, there would be any difference between the electrical properties of certain acupuncture points. They concluded that patients which felt pain had different resistance values at acupuncture points, and ventured to suggest that the different electrical energy flow in these patients might be seen as a connection between Western and Chinese medicine concepts.

Ambiguous results, regarding menstrual pain, were yielded by She et al. [172]; the authors do remark, however, that it is a promising field for further investigation. Indeed, following a recent thermographic analysis, Xisheng et al. [173] suggested that a number of acupuncture points is sensitised in patients with primary dysmenorrhea. Interestingly, based on Xu et al. [174], the electrical resistance of specific acupuncture points changes with the variations of blood flow in the uterus, during the menstrual cycle, a result also previously yielded by the research of Wei et al. [175]. At any rate, a nerve-related model to explain acupuncture analgesia was put forward by Silberstein [176]. The values of the electrical properties of a number of acupuncture points were also found to be different in encephaloma patients undergoing surgery [177].

It is also compelling to consider the implications of the research of Weng et al. [178] who suggested that electrical conductance was different between healthy and obese patients, and in fact changed as those obese patients started losing weight.

Recently, an emerging theory suggested that at least some acupuncture points may be thermically distinct between healthy and unhealthy states [179]; this is also a promising field for future research. An earlier review on the subject [180] was more assertive in acupuncture points having a distinct thermal profile, but this is not a unanimously supported conclusion. The infrared profile of acupuncture points may also be of interest in determining the best course of acupuncture therapy [181]. Another study, focusing on asthma patients, suggests that it may be possible to use the values of electrical impedance at certain acupuncture points for screening and treatment effectiveness purposes [182].

The individuality, so to speak, of acupuncture points maybe also be attributable to their differing, compared to the surrounding tissue, ion concentrations, as suggested by the research of Lee et al. [111]. A number of inorganic ions, such as Ca2+ and Mg2+ are distributed prominently on the upper delimitation of the stratum granulosum [183-185], while the distribution of other ions varies in the other layers [184-187]. We might thus suggest, that from a morphological point of view, the distinct acupuncture points represent areas where, for some reasons, there is a differential horizontal and lateral ion distribution across the skin layers. Interestingly, given that the upper stratum corneum is permeable to external substances [188-191], its ionic profile can theoretically be externally modified; thus, by modifying the electrical resistance of acupuncture points it may be possible to modify treatment efficacy.

It must also be noted that, according to the review of Colbert et al. [192], there is an important clinical utility in assessing the variations of electrical properties in acupuncture points, given that they might reveal latent pathologies or be associated with a state of physical exhaustion. The stimulation of acupuncture points with chemical means, also has therapeutical effects as suggested by a number of researchers [193-196]. Regarding this aspect, it is interesting to remark that drug administration in acupuncture sites seems to be more effective compared to non-acupuncture sites [197]. Furthermore, the research of Periferakis & Periferakis [198], in a sample of 40 patients, both healthy and not, using the Pointo Select DT device, for detection of auricular acupuncture points, determined that in the majority of cases, most, but not all points are easily detectable.

An interesting concept is that of neuronal acupuncture [199], which is based on the premise that in acupuncture points, specific neuronal and neuroactive components are stimulated; these loci could comprise the 361 WHO-accepted acupuncture points [200]. The concept of neuronal acupuncture could perhaps be used to explain how or why acupuncture points exhibit different electrical properties, compared to the surrounding skin.

Finally, Cho et al. [201] measured biopotential differences between acupuncture and non-acupuncture points during acupuncture treatment; acupuncture points seemed to have greater variability which seems to be related to the classical concept of the "flow of Qi". Even the practicing of Qigong, which is theorised to affect Qi circulation, results in significant changes of the electrical potential of acupuncture points [202]. It has also been documented that with needle placement in certain acupuncture points it is possible to observe electrocardiogram and electroencephalogram changes [203,204]; changes where affected during stimulation of non-acupuncture points also, but needle placement in acupuncture points produced more pronounced changes [204].

DISCUSSION

Research on the properties of acupuncture points and meridians has been one of the most prominent topics amongst relevant scholars [205]. It is obvious from the presented studies, that many different researchers have evaluated the identification of acupuncture points based on their electrical parameters, but many of these methods differ significantly, and it is little wonder that their results are not comparable. Of particular interest, however, is the fact that the voltage selected each time seems not to affect the results, i.e., a different voltage will not turn a low impedance point into a high impedance one [206], so at least this offers a modicum of result comparability even if the experimental parameters differ.

Nonetheless, it must be stressed that a host of factors from the parameters of the electrodes, to the physiological and psychological status of the subjects, to the frequencies used, affect the detection of low impedance points [85,207]; therefore, many research results may be rendered inaccurate, and this may also explain the discrepancies in-between the values even of positive-outcome research efforts. Another research by Prokhorov et al. (208) suggest that between racially different populations, the DC measurements of skin resistance are not comparable, a factor which must also be taken into account in future studies. A useful summary of confounding factors in acupuncture point detection research is provided by Luo et al. [209]. It should be noted here that considerable variations in the localisations of acupuncture points is a fairly common occurrence even amongst experienced acupuncturists [210], i.e., the discrepancies are not limited to apparatus-based location methods.

Current research efforts and their significance

Based on the cumulative research results on both the meridian and acupuncture studies (Table 6) we can see that the majority of them where positive, in that it was possible to identify electrically distinct locations and lines on the surface of the human body. Most of the studies used DC, although an almost equal number used AC, and only 2 used both types of current. Finally, the mean sample size was 21.2 subjects. It is difficult to conclude here what the optimal sample size should be for such a research, as there are quite a number of factors which influence its size [211]; moreover, the statistical significance of a sample does not directly in itself validate the experimental results [212]. However, since meridians and acupuncture points, should, in principle, be detectable in any individual, for this particular type of studies, even a small sample should suffice to determine the outcome. However, as mentioned by Reichmanis et al. [123], not all acupuncture points are detectable at all times, and this may be important for the therapeutic efficacy of acupuncture in different patients, or for different protocols used on the same patient. Anomalies in the detectability of acupuncture points, specifically for auricular acupuncture, have been reported by Periferakis & Periferakis [198].

Table 6: Combined and comparative data on the detection of acupuncture points and meridians based on their electrical properties

Study Type	N	Mean Sample Size	Subjects' Health			Type of Current Used			ReportedOutcome		
			Healthy	Not Healthy	Mixed	AC	DC	Both	Positive	Ambig.	Negative
Studies with control	25	21.9	23 (92%)	2 (8%)	0 (0%)	11 (44%)	12 (48%)	2 (8%)	17 (68%)	5 (20%)	3 (12%)
Studies with no control	7	20.6	3 (42.9%)	4 (57.1%)	0 (0%)	7 (100%)	0 (0%)	0 (0%)	3 (42.8%)	2 (28.6%)	2 (28.6%)
Total studies number	32	21.2	26 (81.3%)	6 (18.7%)	0 (0%)	18 (56.3%)	12 (37.5%)	2 (6.2%)	20 (62.5%)	7 (21.9%)	5 (15.6%)

A number of meridian and point locator devices have entered the market after the 1970s [138]. Given the differences in approaches and the fact that even with complicated experimental setups results cannot always be obtained, it is no wonder that the efficacy of early acupuncture point detectors, based on the properties of the points themselves, was called into question [213]. Newer and more sophisticated devices and sensor arrays, such as those proposed by a number of authors [214-216] may improve detection results and eliminate ambiguity.

Regarding research purposes, the delayed luminescence method [217] and the low energy laser (LEL) coherent light interaction with skin [218] may be of future use in evaluating the dielectrical properties of acupuncture points. The more modern method of electrodermal mapping may prove even more useful [219]. Indeed, in the recent years measuring reliability seems to have improved considerably [220].

Finally, the variations and importance of electrical resistance will prove useful even in considering the physical composition of the needles themselves; recent evidence suggests that by some specific material treatment methods it is possible to affect electrical resistance and therefore the therapeutic potential of the treatment [65]. At any rate, as discussed elsewhere, the electrosensitivity of acupuncture points may be a useful clinical and treatment marker [221]. Bearing in mind that there are a number of proposed mechanisms for the action of acupuncture [222], there exists a host of potential modifying factors in assessing the function and potential of acupuncture points and meridians.

Potential Future Directions of Research

Regarding attempts at a structural and biological interpretation for the existence of acupuncture points and meridians, there exists a number of recent research attempts regarding evidence on a cellular level. The most recently discussed theory is that telocytes may be the underlying biological bases of meridians. This is a specialised type of interstitial cells [223], with cytoplasmic extensions; such a structural particularity enables the communication with other distant cells [224]. Telocytes have been found to reside in a number of different tissues [225-232]. Although appearing similar to some other specialised cells types, both telocytes themselves and their extensions, the so-called telopodes are structurally different [233]; the telopodes are expressed both in the body and in cell cultures [234]. There are many theories on what their exact function may be [235-238], but as of yet there is no consensus. A detailed investigation into the potential association between telocytes and TCM meridians was performed by Yonghong et al. [239], on tissues of different animals. Indeed, it was proved that there exist both homocellular and heterocellular connection between telocytes and telocytes and other cell types respectively [239]. Since telocytes interconnect smooth muscle cells, glandular cells and cells for different organs, along with nerves and other structures, this could be the long-elusive network enabling the stimulation of superficial skin structures to have an effect on distant and apparently unrelated morphofunctional units. Notably, as Yonghong et al. [239] assert, the existence of telocytes could be a basis for the interpretation of all the current meridian-associated theories proposed by numerous authors [240-244]. Despite the promising aspects of telocyte research, it must be noted that, as of yet, and to the best of our knowledge, it has proven impossible to interpret all the physiological and pathological connections mentioned in TCM using the notion of telocytes. This of course might be traced down to the fact that telocytes are dynamic cells, so that static imaging techniques may not be always able to trace the full extent of their network. Alternatively, there might be telocytes that are sometimes overlooked in research for a number of reasons [245]. Or, apart from the two aforementioned constraints, it may just so happen that telocytes are not the only answer to this conundrum. Interestingly, a telocyte-based mechanism for the cure of ulcerative colitis by acupuncture was recently proposed by Bai et al. [246].

Another theory on what is the biological basis for meridians and acupuncture points involves keratinocytes, the most dominant cell type of the epidermis [247]. Regarding their electrical activity, it is known that they possess physiologically, a variety of transient receptor potential channels [248-253], along with a number of other channels and receptors [254, 255]. Based on this aspect, and on the experimental research of Denda & Tsutsumi [256], it is hypothesised that it is possible for keratinocytes to generate spatially restricted signalling patterns, which they can then transmit to the unmyelinated nerve fibres of the epidermis [256]. While this may not explain the exact localisation of the points and meridians as proposed by TCM, it does however propose a mechanism by which the stimuli by the needle puncture can be transmitted to the unmyelinated nerves present in the skin [257] and hence affect even spatially distant areas (256). In addition, other researchers have linked acupuncture to the concept of neuroimmunology (e.g., [258-262]].

One additional research avenue is the study of the location of acupuncture points or meridians, based on the effects of phytochemicals when applied at such locations. The most prominent such efforts concern the application of capsaicin, a phytochemical with numerous beneficial properties [263-267], in acupuncture points which was found to have favourable results [268-270]. Based on the past history of use of certain compounds at acupuncture points [116], other phytochemicals, with demonstrated health-related properties, such as kaempferol [271,272], curcumin [273,274], piperine [275], pinosylvin [276], and a host of natural compounds used in the field of oncology [277], could be used experimentally, to verify or dismiss such claims. Furthermore, it is known that acupuncture can measurably influence certain hormone levels in body fluids [278,279]; therefore, we propose that clinical trials could be designed, especially in cases where hormones or their analogues could be of therapeutical benefits, such as in the case of somatostatin [280, 281].

CONCLUSION

Studies on the detection of TCM meridians and acupuncture points have been ongoing, intermittently, during the last decades, by numerous different research teams, using different experimental setups. Whereas the majority of results is positive, i.e., TCM meridians and acupuncture points can be distinguished from the surrounding skin, due to theirs having different electrical properties, there exist a number of shortcomings in most experimental efforts. To be more precise, the different parameters used in most experiments do not permit an easy comparison of results, while the small sample size oftentimes used raises questions of statistical validity; in a number of cases there is also a lack of control factors. Even though we can conclude that it is possible to detect TCM meridians and acupuncture points using electrical properties, more research efforts are needed in order to standardise the experimental approaches and find a structural or functional basis for their existence.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, A.P., K.P.; methodology, A.P., K.P., A.I.; validation, A.P., and D.O.C.; formal analysis, A.P.; investigation, A.P., K.P., A.I., L.T., C.M.; resources, C.M.; writing—original draft preparation, , A.P., K.P., A.I., L.T., A.-T.P., C.M., D.O.C.; writing—review and editing, A.P., K.P., A.I., L.T., A.-T.P., D.O.C.; visualization, A.P.; supervision, A.P., K.P., D.O.C.; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

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ORIGINAL ARTICLE

The Career Decision of Medical Students: A Tool for Organizational Psychology in the Healthcare System

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Abstract: Background: The deficit of medical staff in various health care systems points out the need for a more detailed strategic management toward sustainability in human resources. The purpose of this study is to explore the relationships linking self-esteem, self-efficacy, general attitudes, and career preferences among medical students, aiming to provide insights that could support sustainable career development. Methods: We performed a monocentric observational study that included 157 students. The participants completed four standardized questionnaires evaluating the self-efficacy, the self-esteem, the general attitudes and beliefs, and the perception of medical career specialty. We assessed the relationships between the psychological variables and career preferences. Results: Significant correlations emerged between the students' year of study and the need for achievement, the importance of mentorship, and career decision-making. Clinical students, in particular, placed greater emphasis on gaining practical experience in their chosen field. Conclusions: Our results reaffirm the impact of occupational psychology in the health care system, providing more sustainability in career decisions. Further research involving larger and more diverse samples is recommended to gain a more comprehensive understanding of how the study of various psychological factors could impact human resources management in the field.

Keywords: human resources, sustainability, health care, self-esteem, self-efficacy, medical students, career perception, occupational psychology

INTRODUCTION

The healthcare systems experience, nowadays, an important deficit of human resources, achieving health care quality very challenging, especially in developing countries. Thus, the strategic management of human resources should use various psychological tools in order to reach stability in various medical specialties, considering the psychological characteristics of the medical students [1].

Medical students often experience chronic stress during their academic development. Thus, self-esteem, self-efficacy, and general attitudes and beliefs are

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of paramount importance in the process of psychosocial growth and have a marked effect on thoughts, feelings, values, and professional goals [2]. Furthermore, future career decisions should rely on occupational psychology as a guarantee for the sustainability of the human resources in health care systems worldwide [3]. In this regard, the perception of self-esteem, self-efficacy, and general attitudes and beliefs among medical students serve as tools for occupational psychology in the field [4].

The integration of organizational psychology into the training of medical students is essential for the sustainable development of human resources in the health field. This approach contributes to preparing the future doctors for professional challenges and promoting an efficient work environment.

Self-esteem refers to the totality of the value judgments of an individual regarding their person. Both very low self-esteem and very high self-esteem can lead to psychological problems. Thus, the self-esteem should be at an average level. Self-efficacy refers to the beliefs of an individual about his or her ability to mobilize his or her cognitive, behavioral, and motivational resources to complete a task, respectively, to his/her ability to organize and execute purposeful actions. People with a high level of self-efficacy optimally allocate their resources to solve tasks successfully, whereas people with a low level of self-efficacy avoid initiating actions, withdraw from tasks, fail, and reinforce their beliefs about their incompetence in the respective tasks.

The general attitudes and beliefs refer to four dimensions: achievement, approval, comfort, and justice. With respect to cognition, it measures the global assessment of one's own person and the global assessment of the value of others, rationality, and preferences, considering the choice of students with regard to the medical specialty.

Given that medical students experience high levels of stress and are at increased risk of psychological disturbances during the learning process, self-esteem and self-efficacy may be important variables in their well-being and performance [5].

In the literature, self-esteem and self-efficacy have been associated with academic performance and job satisfaction. Recent studies have shown that these variables are interconnected and influence the perceptions and career choices of medical students [6,7].

Social perfectionism, defined by the need to meet the expectations of others, as a part of general attitudes and beliefs, can negatively affect academic self-efficacy and lead to academic burnout [8,9]. Educational interventions that support competence, learning from mistakes, and self-efficacy can reduce these negative effects [10].

Using four well-established questionnaires, which quantify the self-efficacy [11], the self-esteem [12], the general attitudes and beliefs [13], and the perception of medical career specialty [14], this study aimed to: (1) compare differences in self-esteem, self-efficacy, general attitudes and belief scores with respect to the career preferences of medical stu-dents; (2) correlate self-esteem, self-efficacy, general beliefs and attitudes scores, and ca-reer-related perceptions among medical students; and (3) identify how these variables in-fluence the career preparation and orientation of the students at different stages of their academic training.

MATERIALS AND METHODS

This research consists of a monocentric observational study that includes medical students from Carol Davila University of Medicine and Pharmacy, Bucharest-Romania, who were interviewed between March and July 2024. We enrolled 157 participants (118 females, 39 males). The mean age was 21.6 years (+/- 2.16). They were asked to answer a survey consisting of 4 scales: the Self-Efficacy Scale (SES) (10 items) [11], the Self-Esteem Scale (SS) (10 items) [12], the General Attitudes and Beliefs Scale (GABS-SV) (26 items) [13], and the Perception of Medical Career Specialty Questionnaire (PMCS) (24 items) [14]. Table 1 reveals the main items included in each scale.

Table 1: The main items included in each scale

Name of the Scale	Items included in each Scale
Self-Efficacy Scale	I always manage to solve difficult problems if I try hard enough/Even if someone opposes me, I manage to identify ways and means to get what I want/It is easy for me to be consistent with my goals and achieve my goals/I have confidence that I could successfully cope with unforeseen events/Thanks to my resources I know how to deal with unexpected situations/I can solve most problems if I put in the necessary effort/I can stay calm when faced with difficulties because I can rely on my coping skills/When faced with a problem I can usually identify several solutions/Usually, when I have a problem, I can think of a solution/I can usually handle any situation and any kind of problem I am faced with
Self-Esteem Scale	I am generally satisfied with myself/Sometimes I think I'm good for nothing/I feel that I have many qualities/I am able to do things as well as other people/I feel like I do not have much reason to be proud of myself/Sometimes I feel useless /I feel that I am as valuable a person as anyone else/I wish I had more respect for myself /I generally tend to think of myself as a loser/I have a positive attitude toward my person
General Attitudes and Beliefs Scale	The need for achievement, the need for approval, the need for comfort, the absolutist demand for justice, the overall assessment of others, overall assessment of self-worth, rationality, irrationality

Perception of Medical Career Specialty questionnaire	Financial importance, perception of opportunities for further specialization, challenging domain perception, perception of less stressful field, the importance of flexible schedule, important establishment in an urban environment, important research opportunity, didactic opportunity, short working hours, combination of hobby and job, comfortable lifestyle, the importance of the sense of vocation

The data were analyzed via GraphPad Prism 6.00 (GraphPad Software Inc.). For the comparative analysis, we used one-way ANOVA followed by post hoc Tukey's test for interactions between groups. With respect to career perception, we divided the students into 4 groups: surgery (students who might choose a surgical field after graduation), non-surgery (students who might choose a medical field, nonsurgical field after graduation), paraclinical (students who might choose a medical field with no patient contact after graduation), and N/A (students who did not decide about a specific field after graduation) and compared the main scores among them.

Considering the study year, we divided the students into two groups, namely, the preclinical group (students in the first two years of medical study) and the clinical group (students in the last four years of medical study), in accordance with the curricula of the general medicine studies, and compared various items between them. The data are expressed as the mean ± SEM. A two-sided P value <0.05 was considered statistically significant. For binary data, we used the chi-square test.

For the correlational data, we used a correlation matrix that included all the items independently. The Spearman correlation coefficient, r, was determined, considering the non-normal distribution of the group. We considered a two-sided P value <0.05 to indicate statistical significance for each correlation. We assigned r values under 0.4 to have negligible correlative effects. Finally, we excluded nonsignificant correlations.

RESULTS

Figure 1 shows the comparative analyses of self-esteem, self-efficacy and general attitudes and beliefs scores with respect to career perception. We observed relatively homogenous score values among the groups regardless of the career perception.

Considering the self-efficacy score, we observed the following mean values among the groups: surgery group vs. non-surgery group, 31.58±0.67 vs. 29.05±0.52 (p=0.018); paraclinical group, 30.48±0.82; and N/A group, 28.25±1.48, without any other statistically significant changes (Figure 1A). The mean self-esteem score with respect to career perception did not differ significantly among the groups: 28.6±0.79 in the surgery group, 27.16±0.56 in the non-surgery group, 27.9±1.08 in the paraclinical group, and 25±2.67 in the N/A group (Figure 1B). Furthermore, we revealed relative homogeneity among the groups in terms of the general attitudes and beliefs score: 9±0.49 in the surgery group, 9.77±0.33 in the non-surgery group, 10.29±0.65 in the paraclinical group, and 12±1.08 in the N/A group (Figure 1C).

Figure 1: Comparative analysis of self-efficacy (A), self-esteem (B), and general attitudes and beliefs (C) scores between students who choose one of the following medical fields after graduation: surgical, non-surgical, paraclinical, or no option yet. The values are expressed as the means ± SEMs. * represents a p-value between 0.05 and 0.01

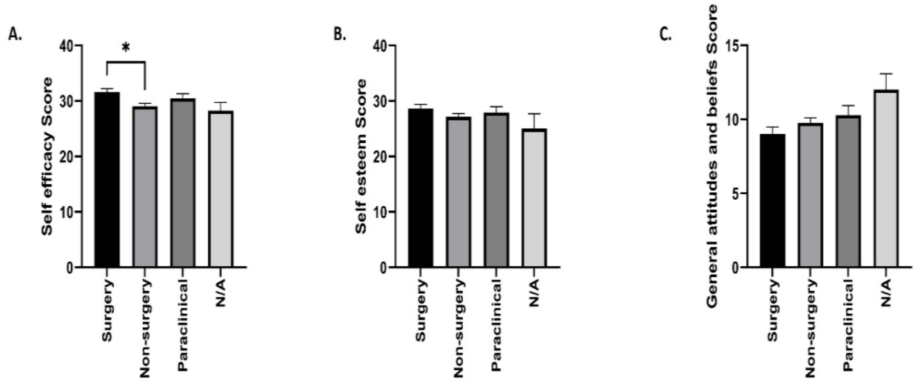

The correlations between self-esteem, self-efficacy, individual general beliefs and attitudes, and career-related perceptions among medical students are shown in Figure 2. The positive correlations are marked in blue, whereas the negative correlations are red.

Self-esteem correlated positively with rationality (r=0.51, p<0.001) and negatively with the overall assessment of self-worth (r=-0.56, p<0.001) and the need for comfort (r=-0.48, p<0.001). The rationality correlates negatively with the need for approval (r=-0.44, p<0.001). Additionally, the link between the overall assessment of self-worth and the need for comfort suggests a strong positive correlation (r=0.71, p<0.001). Moreover, the need for achievement correlates positively with the need for approval (r=0.53, p<0.001), comfort (r=0.51, p<0.001) and absolutist demand for justice (r=0.54, p<0.001). We also observed a positive correlation between the need for approval in student perception and the need for comfort (r=0.48, p<0.001) and absolutist demand for justice (r=0.51, p<0.001). The need for comfort also positively correlates with the absolutist demand for justice (r=0.42, p<0.001), which, further, correlates positively with the overall assessment of others (r=0.54, p<0.001). We revealed a weak positive correlation between financial importance and lifestyle (r=0.41, p<0.001) and between perceptions of opportunity for further specialization and perceptions of the challenging domain (r=0.41, p<0.001).

Figure 2: The main correlations between various items included in the 4 scores

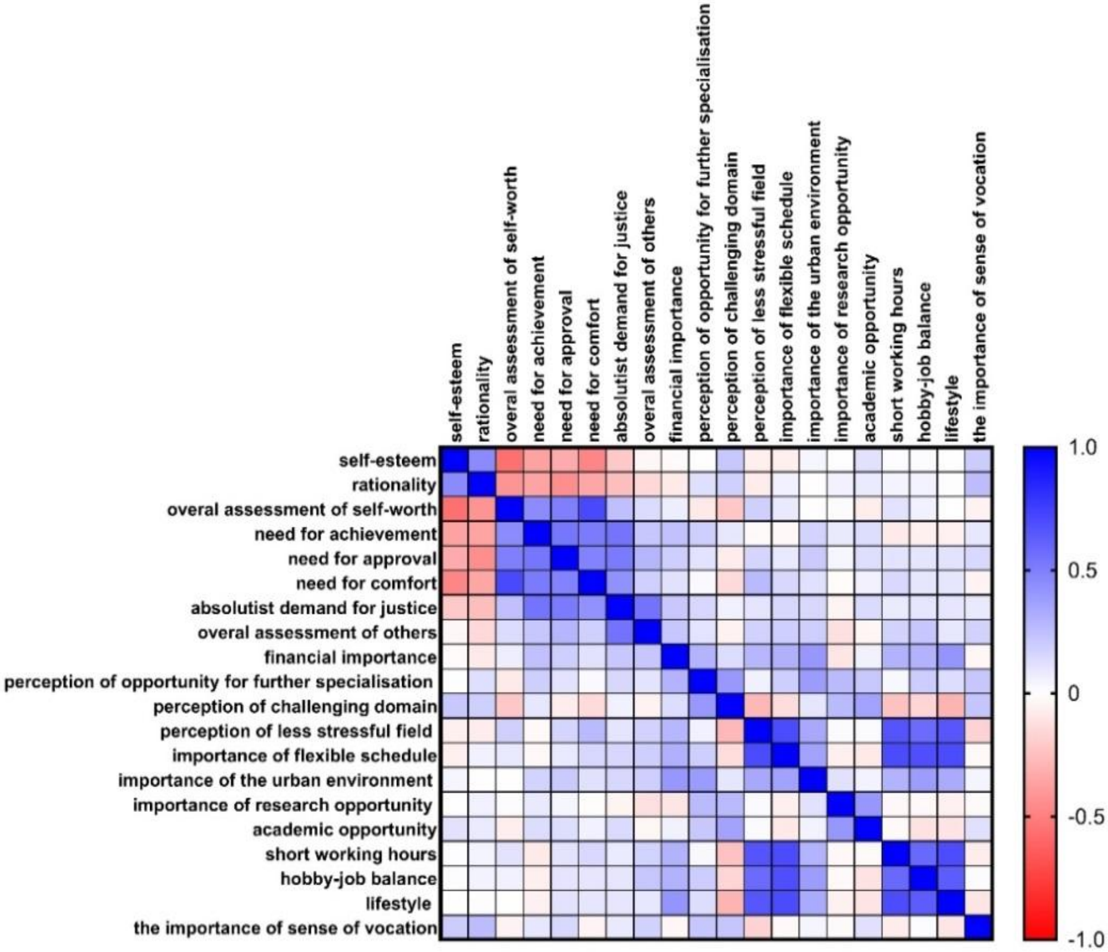

The perception of a less stressful field strongly correlates with the importance of a flexible schedule (r=0.7, p<0.001), short working hours (r=0.66, p<0.001), hobby-job balance (r=0.57, p<0.001), and lifestyle (r=0.65, p<0.001). Furthermore, we observed positive correlations between the importance of a flexible schedule and short working hours (r=0.7, p<0.001), hobby-job balance (0.68, p<0.001), and lifestyle (r=0.69, p<0.001), between short working hours and hobby-job balance (r=0.58, p<0.001) and lifestyle (r=0.69, p<0.001), and between hobby-job balance and lifestyle (r=0.63, p<0.001).

The comparative analyses between the years of study and the need for achievement, academic opportunity, opportunity for patient care, and importance of the models are shown in Figure 3.

Our study revealed a significantly greater score for need for achievement in the preclinical years than in the clinical years: 15.14±0.49 vs. 13.67±0.38 (p=0.03) (Fig. 3A). With respect to the academic opportunity, we observed a statistically significant increase in the perception score among the students from the clinical compared with those from the preclinical years: 3.29±0.14 vs. 2.69±0.17 (p=0.008) (Fig. 3B). Furthermore, the opportunity for patient care was better highlighted in the clinical group than in the preclinical group: 3.29±0.11 vs. 2.72±0.15 (p=0.008) (Fig. 3C). Finally, the importance of the models was greater for the students in the clinical years than for the students in the preclinical years: 3.8±0.12 vs. 3.38±0.15 (p=0.03) (Fig. 3D).

Figure 3: Comparative analysis between clinical and preclinical years of study and mean values of need for achievement (A), academic opportunity (B), opportunity for patient care (C), and importance of models (D). The values are expressed as the means ± SEM. The bars represent the SEM. * represents a p value between 0.05 and 0.01, ** represents a p value between 0.01 and 0.001

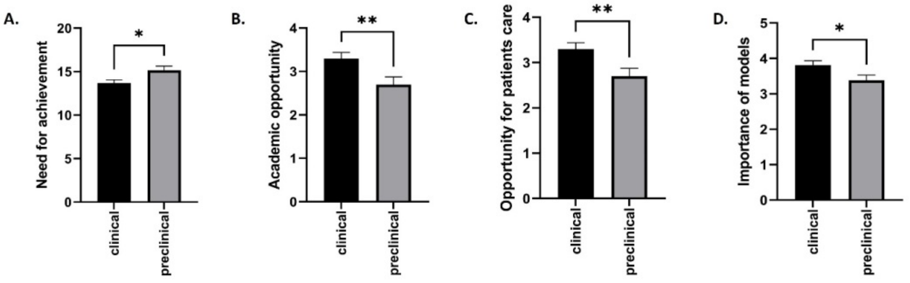

DISCUSSION

Modern management strategies in human resources in health care organizations should consider the sustainability potential of the providers, based on their self-efficacy, self-esteem, and general attitudes and beliefs perception. Thus, further organizational stability could be achieved and qualitative health care procedures guaranteed [15].

Our study reveals the role of occupational psychology among medical students, which better tailors the decisional tree for future medical subspecialties. Among the groups of students in different specializations, the analyzed variables, such as self-esteem, self-efficacy, general attitudes and beliefs, and career perceptions, are relatively homogeneous. Our results are in accordance with the literature. A recent study highlighted how self-esteem and self-efficacy are critical factors in career decision-making among medical students. Research indicated that medical students across various fields (surgery, general medicine, etc.) tend to show relatively uniform levels of self-efficacy and self-esteem, which are essential for their career maturity and decision-making abilities [16]. This is consistent with studies focusing on specialty identity and self-efficacy, where students from various medical disciplines demonstrated similar levels of career maturity and engagement [17].

Moreover, the importance of psychological factors such as self-esteem and career perception has been highlighted by a study examining flourishing and well-being. These studies revealed that regardless of their specialization, medical students benefit similarly from positive psychological constructs, which mediate their career decisions [18].

According to our results, the self-esteem of the medical students is closely related to rationality, suggesting that those with high self-esteem tend to think logically. Positive self-evaluations are associated with the need for justice, support, and approval, whereas irrationality is correlated with a desire for comfort and a critical evaluation of others. The need for absolute justice is linked to the importance of social status and a comfortable lifestyle, especially in the urban environment. Current research underscores the importance of self-esteem in rational decision-making and professional development in young adults, including medical students. For example, Harris and Orth highlighted in 2020 that high self-esteem is associated with rational behavior and an increased ability to face academic and professional challenges, which can positively influence students' career paths [19].

In terms of beliefs and attitudes, various data from the literature suggest that cognitive rigidity and irrational thinking can negatively affect professional adaptability. A study from David et al. discussed the negative impact of irrational beliefs on academic and professional performance, confirming observations that such beliefs can limit career success [20]. Career motivations, such as the need for specialization and the preference for a comfortable lifestyle, are critical to job satisfaction. Another study revealed that aligning career goals with personal values can increase motivation and career satisfaction [21].

Self-efficacy, defined as confidence in one's ability to achieve goals, plays a critical role in academic and professional success. Zajacova et al. identified that academic self-efficacy is a significant predictor of academic performance and the adjustment to university demands among young adults, highlighting the importance of this skill for medical students [22]. The results of our study show a significant correlation between interest in the specialty and the importance of a sense of duty. This suggests that students who show a strong interest in a particular medical specialty are motivated by a strong sense of duty to the profession and patients. Moreover, the importance of vocation and responsibility is related to the choice of medical specialties [23].

Global self-evaluation is a central construct in cognitive theory and is related to various forms of irrational demands, such as the need for absolutist justice and the need for approval, and research. Dryden and David suggest that absolutist demands and emotional needs are predictive factors for negative global self-evaluations [24]. Specifically, in our study, irrationality was correlated with the need for comfort, the absolutist demand for justice, and the global evaluation of others. Our results are in accordance with those of Ellis et al., in which irrationality might reflect a cognitive tendency to adopt extreme or unrealistic perspectives on the world and others [25]. This correlation suggests that when students face uncomfortable situations or perceive that justice is not respected, they may exhibit irrational tendencies, thus influencing their learning process and interpersonal relationships.

A statistically significant positive correlation between specialty interest and the importance of sense of duty suggests that students who are strongly interested in a particular specialty also have a well-developed sense of duty to the profession in that type of specialty and to their own patients, and these results are in accordance with the literature [26]. They noted that the perception of difficulty and complexity of a field can stimulate interest in in-depth specialization, as students seek to push their limits and acquire advanced skills. The importance of vocation and responsibility in choosing medical specialties integrates this psychosocial context [23].

Our study revealed that students who value teaching opportunities also appreciate the possibility of engaging in research activities. Our results are in accordance with those of Taylor et al., who revealed a link between the involvement of students in didactic activities and the research interest, considering it a natural extension of the academic learning [27].

Significant correlations between the year of study and the variables such as need for achievement, didactic opportunity, and opportunity for patient care, in our study, suggest a progressive development of the priorities of students and motivations as they progress through the degree program. These findings are consistent with another research showing that as students progress through medical education, they become more achievement-oriented and increasingly value role models of career success [28]. The importance of model perception with the progress through medical education could help further develop new sustainable management strategies in human resources.

Moreover, preclinical students often focus more on academic achievement and didactic learning. Some studies have demonstrated that role models significantly influence students' self-efficacy, particularly when these role models are seen as relatable and their success attainable [29,30]. Additionally, expectancy-value theory suggests that students who find mentors similar to themselves are more likely to value the associated career path, reinforcing the importance of mentorship in clinical education. This aligns with more recent research emphasizing the critical part that role models play in shaping the students' perceptions of their future careers [31]. Furthermore, achievement motivation and self-efficacy have been found to fluctuate on the basis of the type of mentorship and patient exposure students receive, which further highlights the transitional priorities from the preclinical to the clinical years [32]. These studies underscore the evolving nature of the motivations of the medical students and the influence of practical exposure and mentorship as they progress through their education.

The findings of this study emphasize the substantial influence of lifestyle factors on medical students' career preferences, which aligns with the literature. Many medical students today prioritize work–life balance, flexible schedules, and reduced working hours when selecting their specialties. A survey by Dorsey et al. revealed that the increasing importance of a controllable lifestyle has led to a shift in specialty preferences among U.S. medical students, with fewer students opting for time-demanding fields such as surgery and more favorable fields that offer a more predictable schedule [33]. Thus, in order to achieve stability of the human resource in the health care area, regardless of the medical sub-specialty, the medical organization should adhere to a predictable work program [34].

Furthermore, a study by Lambert and Holmboe revealed that medical students are increasingly focused on selecting specialties that allow them to maintain a balance between their professional and personal lives, contributing to their overall career satisfaction [35]. These findings mirror those of our study, where a flexible schedule and hobby-job balance were key factors for students aiming to pursue less stressful specialties.

Another study by Dyrbye et al. demonstrated that burnout among medical students and physicians is closely linked to lifestyle factors, with specialties offering better work–life balance associated with lower burnout rates [36]. These findings support our results, highlighting the need to consider lifestyle when guiding students in their career decision-making.

Finally, we observed an absence of a significant correlation between the importance of the sense of vocation and the other analyzed variables. This suggests that a sense of vocation, although considered an essential element in choosing a medical career, may function independently of other factors, such as teaching or research opportunities. This result is supported by the research of Newton and Grayson, who highlighted that vocation can be a strong intrinsic factor but is not necessarily related to external circumstances or other professional motivations [37].

Study limitations

Our research presents some limitations. Firstly, the lack of significant correlations does not necessarily indicate the absence of differences between groups but may be influenced by sample size. Secondly, the study was monocentric and did not consider the importance of intercultural variability among different societies. Finally, we did not perform a separate analysis of the enrolled international students.

CONCLUSION

Our study highlights the need for applying various occupational psychology tools for a more sustainable human resources in the health care system, considering the medical students as future providers. Further studies that explore other aspects or use alternative measurement methods are needed to confirm our results.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, E.N and S.I.; methodology, S.A.C., A.C.C., and E.N. ; software, S.I., and M.L.; validation, A.C.C., M.M.M, and T.I.; formal analysis, C.V.A., and S.I.; investigation, E.N., and G.D.; resources, T.I., and S.I.; data curation, M.C.P., and M.L.; writing—original draft preparation, M.C.P., S.A.C., and E.N.; writing—review and editing, T.I., S.I., and C.V.A; visualization, G.D., and M.M.M.; supervision, S.I.; project administration, G.D.; funding acquisition, E.N. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Fundeni Clinical Institute (10337/07.03.2024).

Participant consent for publication

The informed consent for publication was included in the questionnaires and signed by each participant.

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SYSTEMATIC REVIEW

New Insights in Microcirculation Research in Sepsis

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Abstract: Sepsis remains a leading cause of morbidity and mortality worldwide, with certain high-risk populations—including elderly patients, neonates, immunocompromised individuals, those with chronic diseases, and pregnant women—experiencing worse outcomes. These groups exhibit distinct pathophysiological responses, which complicate diagnosis, treatment, and prognosis. Despite advancements in sepsis management, challenges persist in early risk stratification, individualized therapeutic strategies, and long-term recovery. Aim: This study aims to evaluate the impact of sepsis on high-risk populations, identify prognostic factors influencing clinical outcomes, and explore personalized treatment approaches to optimize patient management. Methodology: A systematic narrative review was conducted, following PRISMA guidelines, by analyzing peer-reviewed literature from 2015 to 2025. This study included the analysis of 80 scientific articles from eight international databases. Studies focusing on sepsis pathophysiology, microcirculatory dysfunction, diagnostic techniques, therapeutic interventions, and post-sepsis outcomes in high-risk populations were included. Results: Elderly patients demonstrated the highest sepsis-related mortality rates (>40%), while neonates and immunocompromised individuals exhibited delayed inflammatory responses, complicating diagnosis. Prognostic biomarkers such as IL-6 and IL-10 showed potential utility in these populations. Personalized fluid resuscitation, antimicrobial stewardship, and immune-modulating therapies were identified as critical to improving outcomes. Post-sepsis syndrome (PSS) was prevalent, particularly in survivors with chronic comorbidities and prolonged ICU stays. Conclusions: Optimizing sepsis management in high-risk populations requires a multidisciplinary, personalized approach, incorporating early biomarker-based diagnostics, precision-guided hemodynamic support, and tailored rehabilitation programs. Future research should focus on novel immunotherapies, antimicrobial resistance strategies, and long-term functional recovery in sepsis survivors.

Keywords: sepsis, microcirculatory dysfunction, prognostic biomarkers, post-sepsis syndrome, antimicrobial resistance

INTRODUCTION

Microcirculatory Alterations in Sepsis

Sepsis is a life-threatening condition characterized by a dysregulated host response to infection, leading to systemic inflammation, multi-organ dysfunction, and, in severe cases, death. One of the critical pathophysiological hallmarks of sepsis is microcirculatory dysfunction, which plays a pivotal role in tissue hypoxia and organ failure [1]. The microcirculation comprises small blood vessels, including arterioles, capillaries, and venules, responsible for oxygen delivery and metabolic exchange at the cellular level [2]. In sepsis, widespread endothelial activation, increased vascular permeability, and impaired autoregulation disrupt normal microvascular perfusion, exacerbating cellular distress and metabolic imbalance [3].

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Microcirculatory alterations in sepsis are driven by complex interactions between inflammatory mediators, coagulation disturbances, and endothelial dysfunction. Inflammatory cytokines such as TNF-α, IL-6, and IL-1β induce endothelial cell activation, promoting leukocyte adhesion and microvascular thrombosis [4,5]. Additionally, nitric oxide (NO) dysregulation contributes to an imbalance between vasodilation and vasoconstriction, further impairing tissue perfusion [6].

From a diagnostic perspective, bedside techniques such as sublingual videomicroscopy and sidestream dark-field imaging (SDF) have emerged as valuable tools for assessing microcirculatory abnormalities in critically ill patients with sepsis [7]. These imaging modalities allow real-time visualization of capillary flow, providing essential insights into the extent of microvascular dysfunction and guiding therapeutic interventions. Advanced imaging approaches, including contrast-enhanced ultrasound (CEUS) and near-infrared spectroscopy (NIRS), offer additional perspectives on micro-circulatory heterogeneity and oxygenation deficits in septic patients [8,9].

Understanding microcirculatory impairments in sepsis is crucial for developing targeted therapeutic strategies aimed at restoring tissue perfusion and mitigating organ dysfunction [10]. Recent research has explored the potential benefits of fluid resuscitation strategies, vasoactive agents, and adjunctive therapies such as corticosteroids and endothelial-targeted interventions in optimizing microvascular function.

Fundamental Pathophysiological Mechanisms – Microcirculatory Dysfunction and Endothelial Abnormalities in Sepsis

The pathophysiological mechanisms underlying microcirculatory dysfunction in sepsis are complex and involve a cascade of inflammatory, coagulative, and endothelial disturbances that ultimately lead to impaired oxygen delivery and tissue hypoxia [11].

Microcirculatory impairment is a defining feature of sepsis, characterized by altered capillary perfusion, increased heterogeneity in blood flow, and endothelial dysfunction [12]. Unlike systemic hemodynamic failure, which is typically reflected in macrovascular changes (e.g., hypotension), sepsis-related microcirculatory alterations occur independently of blood pressure changes and directly contribute to organ failure [13].

Inflammation plays a central role in disrupting microcirculatory homeostasis. The excessive release of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β leads to endothelial activation, increased vascular permeability, and leukocyte adhesion to the endothelium [14]. This inflammatory cascade results in endothelial swelling, capillary plugging by activated leukocytes, and microthrombi formation, all of which impede oxygen diffusion and exacerbate tissue hypoxia [15]. Additionally, dysregulated nitric oxide (NO) production contributes to vascular tone abnormalities, creating an imbalance between vasodilation and vasoconstriction that further disrupts blood flow distribution at the microvascular level [16].

From a structural perspective, capillary rarefaction and endothelial glycocalyx degradation are key pathological features of microcirculatory dysfunction in sepsis. The glycocalyx, a protective layer covering endothelial cells, plays a crucial role in vascular permeability and mechanotransduction [17]. In sepsis, glycocalyx breakdown due to enzymatic degradation (by heparanase and matrix metalloproteinases) results in increased vascular leakage, impaired shear stress sensing, and loss of anticoagulant properties, contributing to sepsis-induced coagulopathy (SIC) [18].

Endothelial dysfunction in sepsis is a result of direct cellular injury, oxidative stress, and immune activation. Endothelial cells, which normally maintain vascular homeostasis through the balanced release of vasoactive substances, become dysregulated, shifting towards a pro-inflammatory and pro-thrombotic phenotype [19].

One of the key mechanisms involved in endothelial dysfunction is increased expression of adhesion molecules such as ICAM-1, VCAM-1, and selectins, which facilitate leukocyte adhesion and transmigration into tissues, further amplifying inflammation [20]. This process leads to capillary plugging and increased diffusion distance for oxygen, creating a mismatch between oxygen delivery and cellular demand [21].

At the same time, mitochondrial dysfunction in endothelial cells exacerbates metabolic failure, reducing the ability of microvascular endothelial cells to regulate vascular tone and respond to local oxygen needs appropriately [22]. The resulting endothelial metabolic shutdown contributes to an irreversible cycle of hypoxia, organ dysfunction, and cellular apoptosis in severe sepsis cases [23].

Sepsis-induced coagulopathy (SIC) is a well-recognized phenomenon in which systemic inflammation disrupts the balance between coagulation and fibrinolysis, leading to widespread microthrombosis and perfusion defects [24]. The overactivation of the coagulation cascade is primarily driven by excessive tissue factor (TF) expression, leading to thrombin generation, platelet aggregation, and fibrin deposition within the microcirculation [25].

Additionally, antithrombotic mechanisms become suppressed due to reduced production of key anticoagulant proteins such as antithrombin, protein C, and thrombomodulin, further promoting a prothrombotic state [26]. The resulting microvascular thrombosis impairs capillary perfusion, leading to local ischemia, tissue necrosis, and multi-organ failure in severe septic shock cases [27].

Furthermore, fibrinolytic dysfunction exacerbates these complications. Sepsis induces the release of plasminogen activator inhibitor-1 (PAI-1), which suppresses fibrinolysis and prevents the clearance of microthrombi, further worsening microvascular obstruction

[28]. This shift towards a hypercoagulable state with impaired clot resolution represents a critical therapeutic target in sepsis management.

The long-term impact of microvascular dysfunction in sepsis extends beyond the acute phase of the disease. Persistent endothelial damage and capillary loss contribute to post-sepsis syndrome, characterized by prolonged inflammation, immune suppression, and residual organ dysfunction [29]. Chronic microvascular dysfunction has been linked to long-term cognitive impairment, cardiovascular complications, and persistent fatigue in sepsis survivors [30].

From a therapeutic standpoint, interventions targeting endothelial protection, micro-vascular flow optimization, and early coagulation modulation have shown promise in experimental and clinical studies [31]. Strategies such as glycocalyx preservation (e.g., fluid therapy with albumin, heparan sulfate analogs), endothelial stabilizing agents (e.g., steroids, angiopoietin modulators), and anticoagulant therapy in select patient populations are under investigation for their potential to mitigate sepsis-induced microvascular injury [32].

Prevalence of Microcirculatory Dysfunction in Sepsis

The prevalence of microcirculatory dysfunction in sepsis varies depending on disease severity, patient population, and the diagnostic techniques used. Studies using sublingual videomicroscopy and sidestream dark-field (SDF) imaging have shown that nearly 90% of patients with septic shock exhibit significant microvascular abnormalities, including reduced capillary density, increased flow heterogeneity, and impaired oxygen extraction [33,34]. Even in patients with less severe sepsis, microcirculatory alterations are observed in approximately 50–70% of cases, highlighting the early involvement of the microvasculature in disease progression [35].

The extent of microcirculatory dysfunction is closely linked to the severity of sepsis. In its early stages, microvascular perfusion changes may be subtle while overall hemodynamics remain stable. As sepsis advances to septic shock, severe capillary perfusion defects become more pronounced, leading to increased lactate levels, tissue hypoxia, and multi-organ failure [36].

Certain patient populations are particularly susceptible to profound microcirculatory disturbances. Elderly patients often experience more severe endothelial dysfunction due to age-related vascular changes and a diminished ability to compensate for inflammatory stress [37]. Patients with chronic diseases such as diabetes or chronic kidney disease face a heightened risk due to pre-existing endothelial dysfunction and oxidative stress, which exacerbate sepsis-induced microvascular damage [38]. Immunocompromised individuals, including cancer patients and transplant recipients, frequently exhibit atypical microcirculatory responses due to an altered inflammatory cascade, making early detection more challenging [39].

Microcirculatory dysfunction is significantly more prevalent in sepsis compared to other critical illnesses. In patients with non-septic shock, such as cardiogenic or hemorrhagic shock, global hemodynamic parameters may be compromised, but microvascular function remains relatively preserved in the early stages [40]. In contrast, sepsis induces early and widespread microvascular failure even when macrocirculatory parameters, such as blood pressure, appear normal [41].

In non-septic intensive care unit (ICU) patients, studies indicate that microcirculatory alterations affect only 10–30% of individuals, whereas in sepsis, this prevalence is three to five times higher [42]. This contrast highlights the unique impact of sepsis on the endothelium and microvascular network, making microcirculatory dysfunction a key prognostic indicator.

Special Categories of Patients with Sepsis

Sepsis represents a major challenge in medical practice, but certain categories of patients are at significantly higher risk of complications and mortality. These patients require tailored therapeutic approaches, as they have distinct physiological and immunological characteristics that influence their response to treatment and overall prognosis [43].

Older adults are particularly vulnerable to sepsis due to multiple physiological changes associated with aging. Their immune system undergoes a progressive decline, known as immunosenescence, which is characterised by a reduced ability to respond to infections and impaired production of pro-inflammatory cytokines [44]. Additionally, a state of chronic low-grade inflammation (inflamm-aging) contributes to dysfunctional immune responses, increasing the severity of sepsis.

From a cardiovascular perspective, elderly patients often exhibit increased vascular stiffness and reduced cardiac reserve, limiting their ability to compensate for septic shock [45]. Furthermore, frailty and sarcopenia negatively impact prognosis, increasing the risk of multiple organ failure.

The treatment of sepsis in elderly patients should be individualised, considering their increased risk of cardiovascular overload during fluid resuscitation and their predisposition to acute kidney injury. Close monitoring of haemodynamic status and careful adjustment of antimicrobial therapy based on renal function are essential to improving outcomes.

The presence of chronic diseases affects both the progression and management of sepsis, often complicating treatment strategies and patient outcomes.

Diabetic patients are more vulnerable to severe infections because persistent hyperglycaemia weakens neutrophil function and encourages the growth of resistant pathogens. Additionally, diabetes contributes to endothelial dysfunction, worsening the microcirculatory disturbances typical of sepsis [46].

Patients with chronic kidney disease experience a heightened risk of sepsis as uremia-induced immunosuppression weakens their immune response. The frequent use of vascular catheters further increases infection risks, while the accumulation of uraemic toxins disrupts immune function and slows antimicrobial clearance [47]. These factors make precise antibiotic dose adjustments essential, considering the patient's glomerular filtration rate.

Patients with chronic obstructive pulmonary disease are prone to respiratory sepsis because impaired mucociliary function and chronic airway inflammation promote recurrent infections. Prolonged corticosteroid use, a common treatment for COPD, can further suppress the immune system, making patients more susceptible to opportunistic infections [48].

Managing sepsis in patients with chronic conditions requires a careful approach that balances aggressive therapy with the risks posed by pre-existing diseases. Optimising antimicrobial therapy and fluid resuscitation plays a key role in preventing further deterioration of organ function [49].

Sepsis in children differs significantly from adult sepsis in terms of pathophysiology and clinical presentation. Neonates and infants have an immature immune system, making them more susceptible to severe bacterial and viral infections. Furthermore, infants have a limited ability to mount an effective inflammatory response, potentially delaying the diagnosis of sepsis [50].

Beyond immunological differences, children exhibit major variations in drug metabolism, necessitating careful adjustments in antibiotic and vasopressor dosing based on body weight and organ maturity [51].

Early recognition of sepsis is critical in paediatric patients, as hypotension—a hallmark of sepsis in adults—tends to appear late in children. Identifying early signs of impaired tissue perfusion is therefore essential for the timely initiation of treatment.

Immunosuppressed individuals face a significantly higher risk of severe sepsis and life-threatening complications, often with atypical clinical presentations that can delay diagnosis and treatment [52].

This group includes oncological patients, organ transplant recipients on immunosuppressive therapy, individuals with advanced HIV infection, and patients with autoimmune diseases undergoing biologic treatments. Due to a weakened immune response, sepsis in these patients is frequently caused by opportunistic pathogens, and the usual inflammatory signs may be absent. In cases of severe neutropenia, fever might not be present, making early detection more challenging [53].

Effective management relies on the prompt administration of broad-spectrum antibiotics, close monitoring of inflammatory markers, and immune support when necessary. Granulocyte-colony stimulating factor may be considered to enhance neutrophil recovery and improve outcomes in selected cases [54].

Pregnancy induces significant physiological changes that increase a woman's susceptibility to severe infections and septic complications. Increased placental blood flow and alterations in immune responses can influence both the progression of sepsis and the response to treatment [55].

Sepsis during pregnancy can have devastating consequences for both the mother and the fetus. Intrauterine infections, such as chorioamnionitis, may trigger preterm labour or result in fetal demise. Moreover, maternal hypotension and placental hypoperfusion can severely impact fetal development [56].

The management of sepsis in pregnancy must be prompt and aggressive, with rapid administration of antibiotics that are safe for the fetus and careful monitoring of maternal haemodynamic status. The timing and mode of delivery should be determined through a multidisciplinary approach, balancing maternal and fetal risks.

MATERIALS AND METHODS

This study employed a comprehensive approach to investigate the impact of chronic comorbidities on the progression and management of sepsis. A systematic narrative review methodology was chosen to integrate the structured rigor of a systematic review with a narrative synthesis approach, ensuring a nuanced analysis of a complex, multi-disciplinary topic. The review synthesizes existing literature on the interactions between sepsis and chronic conditions such as diabetes, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and immunosuppressive states, emphasizing clinical implications and future research directions.

The analysis focused on three key aspects:

Pathophysiological mechanisms underlying increased susceptibility to sepsis in patients with diabetes, CKD, COPD, and immunosuppression, highlighting immune dysfunction, endothelial damage, and microbiome alterations.

Impact of chronic diseases on sepsis outcomes, including prolonged hospitalization, increased mortality risk, and the need for individualized therapeutic strategies.

Optimization of sepsis management in these high-risk populations, exploring antimicrobial stewardship, fluid resuscitation strategies, immunomodulatory therapies, and personalized treatment approaches based on disease-specific factors.

A key question that shaped the direction of this review was: How do chronic comorbidities influence the pathophysiology, clinical presentation, and management of sepsis, and what strategies can optimize patient outcomes in these high-risk populations?

To ensure a systematic synthesis of findings, studies were selected based on predefined inclusion criteria, prioritizing clinical trials, observational research, and meta-analyses that explored sepsis in patients with chronic comorbidities. A comparative evaluation of treatment strategies was conducted, examining the effectiveness and safety of current therapeutic approaches while identifying gaps in knowledge and areas requiring further investigation.

Approach to Identifying Relevant Articles

A systematic literature search was performed across multiple academic databases, including PubMed, Scopus, Web of Science, Elsevier, Springer, Wiley Online Library, MDPI, and Frontiers, to investigate the role of microcirculatory dysfunction in sepsis. The search process adhered to PRISMA guidelines, employing Boolean operators (AND, OR) to enhance search precision and optimize the identification of relevant studies on microvascular changes associated with sepsis.

Key search terms included: "microcirculatory dysfunction in sepsis" (17,100 results), "sepsis-induced endothelial dysfunction" (17,800 results), "capillary perfusion in septic shock" (16,700 results), "microvascular alterations and organ failure" (18,300 results), and "sepsis-associated mitochondrial dysfunction" (10,800 results).

Structured search strings were applied to maximize relevance and retrieve high-quality studies, such as: "(microcirculatory dysfunction OR endothelial dysfunction) AND (sepsis OR septic shock) AND (capillary perfusion OR mitochondrial dysfunction)."

To ensure the inclusion of the most relevant and up-to-date studies, several selection filters were applied. The search was restricted to publications from 2015 to 2025, allowing for the incorporation of both recent developments and foundational research that contribute to the understanding of microvascular dysfunction in sepsis. Only full-text articles available in English were considered to ensure accessibility to studies with broad international relevance.

The review focused exclusively on peer-reviewed literature, including clinical trials, systematic reviews, and meta-analyses. Priority was given to review articles offering a comprehensive synthesis of current knowledge, while clinical studies were evaluated for their insights into the relationships between microvascular dysfunction, disease progression, and treatment outcomes.

Search queries were tailored for each database to account for variations in indexing and search functionalities. Beyond standard database searches, citation tracking and reference analysis of key studies were conducted to identify additional relevant research that may not have been captured in the initial search. This method helped enhance the dataset by incorporating essential studies that might have been overlooked through automated search strategies.

Eligibility Criteria for Study Selection

Inclusion Criteria

The selected studies needed to address the challenges and treatment approaches for sepsis in high-risk patient groups. Included publications were required to provide relevant information on these aspects:

Pathophysiological mechanisms, such as immune dysregulation, endothelial dysfunction, and metabolic alterations in neonates, elderly patients, immunocompromised individuals, and those with chronic diseases.

Diagnostic and monitoring strategies, including biomarkers, advanced hemodynamic assessment tools, and molecular diagnostics for early detection and risk stratification.

Therapeutic approaches, addressing personalized treatment strategies, the role of immune modulation, tailored fluid resuscitation, and organ-support therapies such as renal replacement therapy and extracorporeal membrane oxygenation (ECMO).

The review incorporated original research, systematic reviews, meta-analyses, and clinical studies published in peer-reviewed, indexed journals to ensure the selection of high-quality, reliable sources.

Exclusion Criteria

The following types of studies were not included in the review:

Research that did not adhere to PRISMA guidelines, as standardized reporting is crucial for ensuring methodological reliability.

Case reports that lacked generalizability or did not provide adequate data on sepsis treatment in high-risk patient populations.

Studies that focused only on general aspects of sepsis without addressing the specific challenges faced by neonates, elderly individuals, immunocompromised patients, or those with chronic illnesses.

Articles that were not based on original research or were published in non-peer-reviewed sources were excluded, ensuring that only high-quality, evidence-based studies were considered.

Selection Process

A structured approach was used to evaluate all identified articles based on predefined inclusion and exclusion criteria, ensuring the selection of high-quality, methodologically sound studies relevant to the research objectives. The PRISMA framework was implemented throughout the process to maintain transparency and consistency in study identification. The final selection offers a comprehensive analysis of sepsis patho-physiology, diagnostic methods, and treatment strategies in vulnerable patient populations.

The selection process was carried out in two main stages. In the initial stage, titles and abstracts were screened to determine relevance, prioritizing studies that focused on sepsis in neonates, elderly individuals, immunocompromised patients, and those with chronic conditions. Studies that did not specifically address these populations or lacked original research data were excluded at this point.

During the second stage, a full-text review was conducted on the remaining articles. Preference was given to studies that examined the relationship between sepsis severity, microvascular dysfunction, immune response alterations, and organ failure. Additionally, research discussing tailored treatment approaches—such as immune modulation, hemodynamic stabilization, and organ-support therapies—was prioritized.

To ensure the credibility and reliability of findings, all selected studies underwent a rigorous quality assessment to confirm adherence to PRISMA guidelines and alignment with the research scope. This approach helped minimize potential biases and strengthened the validity of the conclusions drawn.

Despite efforts to create a comprehensive selection, certain limitations must be acknowledged. The exclusion of grey literature may have led to the omission of valuable studies not indexed in major academic databases. Restricting the review to English-language publications may have excluded relevant research available in other languages. Additionally, limiting the search to specific databases may have overlooked studies published in other sources. Lastly, the decision to focus on research published between 2015 and 2025 may have resulted in the exclusion of older foundational studies that could provide historical insights into sepsis mechanisms and treatment advancements.

RESULTS

Overview of Selected Studies

To ensure transparency and traceability in the selection process, a PRISMA diagram has been included in the material, illustrating the number of studies identified and filtered at each stage of the analysis. This diagram initially presents the total number of articles retrieved through the search, followed by the number of those excluded after reviewing titles and abstracts, and finally, the number of studies selected for in-depth analysis. Additionally, the reasons for excluding certain articles are specified, providing clarity regarding the decisions made during the selection process.

The use of the PRISMA diagram is essential for maintaining transparency, as it allows readers to understand the decision-making process behind article selection and ensures compliance with the criteria established at the outset of the review [57,58].

The PRISMA flow diagram in Figure 1 outlines the selection process for studies examining microcirculatory dysfunction and sepsis management in vulnerable patient populations. A systematic search was performed across eight major databases, yielding a total of 80,700 results.

Following the removal of duplicate records (22,000) and studies excluded due to lack of relevance, incomplete data, or methodological limitations (502), a total of 700 articles remained for screening.

During the initial screening phase, 532 records were excluded based on predefined criteria, primarily for not specifically addressing sepsis in neonates, elderly individuals, immunocompromised patients, or those with chronic diseases. In the first review phase, 168 studies were assessed for eligibility, with articles evaluated for their relevance to sepsis pathophysiology, diagnostic tools, and treatment strategies.

In the second phase, a more detailed evaluation was conducted, applying stricter inclusion criteria related to immune dysregulation, microvascular dysfunction, and patient-specific therapeutic approaches. Studies that focused solely on general aspects of sepsis without addressing unique challenges faced by high-risk patient groups were excluded.

The final selection included 80 studies, covering a wide range of topics, including the pathophysiological mechanisms of sepsis, personalized treatment strategies, and emerging diagnostic technologies. These studies, published between 2015 and 2025, provide valuable insights into optimizing sepsis management in vulnerable populations.

Figure 1: PRISMA Flow Diagram of articles related to Microcirculatory Dysfunction and Sepsis Management in Vulnerable Patient Populations

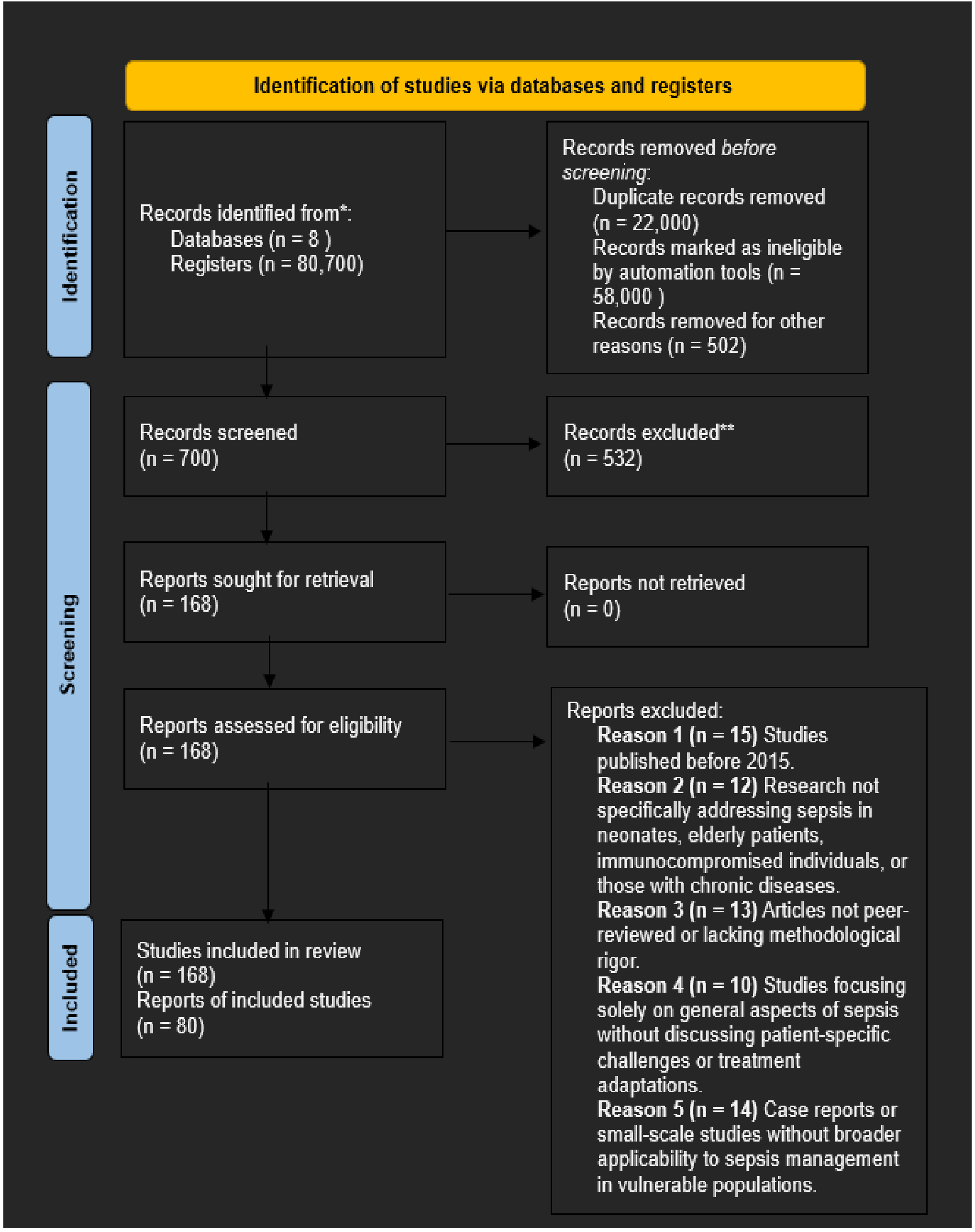
Identification of studies via databases and registers
Identification
Screening
Included
Records identified from*: Databases (n = 8) Registers (n = 80,700)
Records removed *before screening*: Duplicate records removed (n = 22,000) Records marked as ineligible by automation tools (n = 58,000) Records removed for other reasons (n = 502)
Records screened (n = 700)
Records excluded** (n = 532)
Reports sought for retrieval (n = 168)
Reports not retrieved (n = 0)
Reports assessed for eligibility (n = 168)
Reports excluded:
Reason 1 (n = 15) Studies published before 2015.
Reason 2 (n = 12) Research not specifically addressing sepsis in neonates, elderly patients, immunocompromised individuals, or those with chronic diseases.
Reason 3 (n = 13) Articles not peer-reviewed or lacking methodological rigor.
Reason 4 (n = 10) Studies focusing solely on general aspects of sepsis without discussing patient-specific challenges or treatment adaptations.
Reason 5 (n = 14) Case reports or small-scale studies without broader applicability to sepsis management in vulnerable populations.
Studies included in review (n = 168) Reports of included studies (n = 80)

This systematic review was conducted following the PRISMA 2020 guidelines. The PRISMA checklist and flow diagram are provided as supplementary materials.

This methodology enabled a comprehensive analysis of the specialized literature, synthesizing key findings on microcirculatory dysfunction and sepsis management in vulnerable patient populations. By incorporating data from multiple disciplines, this approach provided a well-rounded understanding of sepsis pathophysiology, diagnostic innovations, and personalized treatment strategies.

The studies included in the results section strictly followed the predefined inclusion criteria, ensuring the selection of high-quality and relevant research aligned with the core objectives of this review. These studies were systematically analyzed and synthesized to explore the relationship between microvascular alterations, immune dysregulation, and patient-specific therapeutic approaches, offering objective data and clinically significant insights to enhance sepsis management in high-risk groups.

Sepsis in High-Risk Patient Populations: Unique Challenges and Management Strategies

Patients at increased risk of sepsis belong to special categories, each with specific physiological, immunological, and clinical characteristics that significantly influence disease progression and response to treatment. In these groups, sepsis can progress rapidly, present with atypical symptoms, and have a more severe prognosis, requiring personalized management and close monitoring (Table 1).

Table 1: Categories of Patients at Increased Risk of Sepsis and Their Specific Characteristics

Patient Category	Specific Characteristics	References
Elderly Patients	- Immunosenescence: weakened immune response - Blunted inflammatory response (atypical symptoms) - Compromised cardiovascular and renal function - Increased risk of multi-organ dysfunction	[59], [60]
Patients with Chronic Comorbidities	- Impaired immunity due to preexisting conditions (diabetes, kidney disease, COPD, heart failure) - Increased oxidative stress and chronic inflammation - Risk of hemodynamic imbalances and fluid overload	[61]
Pediatric Patients	- Immature immune system - Increased risk of bacteremia and multi-organ dysfunction - Different drug metabolism (requires careful dose adjustment) - Nonspecific symptoms (lethargy, hypothermia, feeding difficulties)	[62], [63]
Immunocompromised Patients	- Weakened inflammatory response (atypical symptoms) - Increased susceptibility to opportunistic infections - Requires aggressive antibiotic therapy and intensive monitoring	[64], [65]
Pregnant Women	- Physiological changes increase infection susceptibility and excessive inflammation - Early signs may be masked by cardiovascular adaptations - Specific risks: chorioamnionitis, postpartum endometritis - Therapy must be carefully selected to ensure fetal safety	[66], [67]

As we can see in Table 1, elderly patients face a range of physiological and immunological changes that increase their vulnerability to sepsis. With aging, the immune system undergoes immunosenescence, characterized by a weakened innate and adaptive immune response. This leads to a reduced ability to combat infections and a delayed activation of defense mechanisms [59]. Additionally, the inflammatory response may be blunted, meaning classic symptoms of sepsis, such as fever and tachycardia, may be absent or less pronounced. Cardiovascular and renal functions are often compromised in elderly individuals, making hemodynamic balance and fluid management more challenging. Their overall frailty, combined with a higher risk of multi-organ dysfunction, necessitates a carefully tailored therapeutic approach to prevent rapid deterioration and severe complications [60].

Patients with chronic comorbidities represent another high-risk group, as preexisting conditions directly impact infection response and the body's ability to maintain homeostasis during sepsis. Diabetes mellitus, for example, impairs immune function by increasing oxidative stress, altering neutrophil function, and reducing phagocytic capacity, making severe infections and associated complications more likely [61]. Chronic kidney disease leads to the accumulation of uremic toxins that suppress immune function and increase susceptibility to opportunistic infections. Additionally, patients with chronic obstructive pulmonary disease or heart failure exhibit persistent inflammatory states and limited functional reserves, causing sepsis to exacerbate these conditions rapidly. For these patients, treatment strategies must balance infection control, organ protection, and the prevention of adverse effects, such as fluid overload from aggressive resuscitation.

Pediatric patients exhibit significant physiological and immunological differences compared to adults, affecting both the presentation and management of sepsis. The immune system of neonates and infants is underdeveloped, resulting in a reduced ability to mount an effective response against infections, which increases the risk of bacteremia and multi-organ dysfunction [62]. Additionally, drug metabolism differs in children, requiring careful dose adjustments for antibiotics and other therapies based on body weight and organ maturity. Clinical signs of sepsis in this group are often non-specific, including lethargy, respiratory distress, hypothermia, or feeding difficulties, making early diagnosis challenging. Close monitoring and rapid intervention are crucial to preventing severe complications and reducing mortality in pediatric sepsis [63].

Immunocompromised patients, including those with cancer, autoimmune diseases, or those undergoing immunosuppressive therapy, are highly vulnerable to sepsis due to an impaired immune system that cannot effectively control infections. The absence of a robust inflammatory response allows infections to spread quickly, and classic sepsis symptoms such as fever and leukocytosis may be absent, leading to delayed diagnosis [64]. These patients are also prone to opportunistic infections caused by pathogens that are typically non-threatening to immunocompetent individuals, complicating the choice of antimicrobial therapy. Sepsis management in immunocompromised patients requires an aggressive approach, including the rapid administration of broad-spectrum antibi-otics, intensive monitoring, and, in some cases, adjunctive therapies to support immune function [65].

Pregnant women represent a unique high-risk category for sepsis, as physiological changes during pregnancy can increase both infection susceptibility and exaggerated inflammatory responses. The immune system undergoes adaptations to tolerate the presence of the fetus, potentially reducing the ability to fight off infections effectively [66]. Simultaneously, increased plasma volume and cardiac output alter the body's response to infection and can mask early signs of sepsis. Obstetric infections, such as chorioamnionitis and postpartum endometritis, are common causes of sepsis during pregnancy and can have severe consequences for both the mother and the fetus. Treating sepsis in pregnant women requires careful consideration of fetal safety, necessitating the selection of appropriate antibiotics and close hemodynamic monitoring to prevent maternal and fetal complications [67].

Each of these special patient categories requires a personalized approach to sepsis management, tailored to their unique physiological and immunological characteristics. Early diagnosis, intensive monitoring, and prompt therapeutic interventions are essential to improving outcomes and reducing mortality in these vulnerable populations.

Tailored Therapeutic Approaches for Special Patient Populations in Sepsis

Managing sepsis in high-risk patient populations requires a personalized therapeutic approach that considers age, comorbidities, immune status, and physiological differences. Standard treatment protocols may not be fully applicable to these groups, necessitating dose adjustments, targeted antimicrobial strategies, and specialized supportive interventions to optimize outcomes while minimizing complications.

Table 2 presents the key therapeutic considerations for each of these high-risk patient groups, highlighting the necessary adjustments in sepsis management to optimize clinical outcomes and reduce complications.

Table 2: Tailored Therapeutic Approaches for Special Patient Populations in Sepsis

Patient Category	Key Therapeutic Considerations	References
Elderly Patients	- Adjust antibiotic dosages due to reduced renal and hepatic function - Lower doses or extended intervals for renally excreted antibiotics (e.g., aminoglycosides, vancomycin) to prevent nephrotoxicity - Monitor for fluid overload and hypotension with advanced hemodynamic tools (e.g., echocardiography)	[68], [69], [72]
Pediatric Patients	- Use weight-based dosing due to immature liver enzymes and different drug metabolism - Close monitoring of oxygenation, perfusion, and fluid balance - Early noninvasive ventilation (e.g., high-flow nasal cannula) to prevent respiratory failure - Adjust antibiotic regimens based on age-specific pharmacokinetics	[73], [74]
Patients with Chronic Comorbidities	- Modify medication regimens to prevent interactions (e.g., fluid overload in heart failure) - Adjust diabetes management to avoid sepsis-induced hyperglycemia - Consider early renal replacement therapy in patients with kidney disease - Balance infection control with organ protection strategies	[71], [75]
Immunocompromised Patients	- Use prolonged or higher-dose antibiotic courses if needed, balancing toxicity risks - Consider antifungal and antiviral coverage for broader protection - Utilize immunotherapies such as G-CSF for neutropenic patients - Monitor for opportunistic infections and tailor treatment accordingly	[70], [76], [79], [80]
Pregnant Women	- Select antibiotics carefully to avoid teratogenic risks (prefer beta-lactams, macrolides, cephalosporins) - Adjust dosages based on increased plasma volume and altered metabolism - Monitor fetal well-being and placental perfusion in maternal hypotension - Involve obstetric and neonatal specialists for optimal maternal-fetal outcomes	[83], [84], [85], [86], [87]

According to Table 2, antibiotic and medication dosages must be carefully adapted based on age, underlying conditions, and overall patient status. In elderly patients, reduced renal and hepatic function can significantly alter drug metabolism and clearance, increasing the risk of toxicity [68]. Lower doses or extended dosing intervals may be required for renally excreted antibiotics such as aminoglycosides and vancomycin to prevent accumulation and nephrotoxicity [69]. Pediatric patients have immature liver enzyme systems and differing body compositions, necessitating weight-based dosing and close monitoring to ensure therapeutic efficacy while avoiding adverse effects. In immunocompromised individuals, higher or prolonged antibiotic courses may be required due to impaired immune clearance of pathogens, but this must be balanced against the risk of toxicity and resistance [70]. Patients with chronic diseases, such as diabetes or heart failure, may require modifications in medication regimens to avoid interactions or worsening of underlying conditions, such as fluid overload with aggressive intravenous hydration [71].

Supportive interventions play a critical role in managing sepsis in these vulnerable populations. Elderly patients often have diminished cardiovascular reserve, making them prone to hypotension and fluid overload, requiring careful hemodynamic monitoring using advanced tools such as echocardiography or dynamic fluid responsiveness tests [72]. Pediatric patients, particularly neonates, have immature cardiovascular and respiratory systems, necessitating closer monitoring of oxygenation, perfusion, and fluid balance [73]. In critically ill patients with underlying respiratory diseases such as chronic obstructive pulmonary disease or in neonates with underdeveloped lung function, early implementation of noninvasive ventilation or high-flow nasal cannula therapy can help prevent respiratory deterioration [74]. Patients with renal impairment may require early initiation of renal replacement therapy to prevent worsening metabolic derangements and fluid overload [75].

Targeted antimicrobial therapy is essential to optimizing treatment outcomes while minimizing the risk of antibiotic resistance, particularly in patients with recurrent sepsis [76]. Broad-spectrum antibiotics should be initiated promptly but de-escalated based on culture results and antimicrobial susceptibility testing to avoid unnecessary prolonged exposure to broad-spectrum agents [77]. In patients with frequent hospitalizations or prior antibiotic exposure, local resistance patterns should guide empirical therapy to ensure adequate pathogen coverage [78]. Immunocompromised patients, such as those undergoing chemotherapy or organ transplant recipients, are at higher risk for fungal or viral infections in addition to bacterial sepsis, necessitating a broader diagnostic approach and potential inclusion of antifungal or antiviral agents in their treatment regimen [79].

Immunotherapy has emerged as a potential adjunct in managing sepsis, particularly in immunocompromised individuals. Strategies such as the administration of granulocyte colony-stimulating factor (G-CSF) can enhance neutrophil function in patients with chemotherapy-induced neutropenia, reducing the risk of severe infections [80]. Intravenous immunoglobulins (IVIG) may be beneficial in certain septic patients with antibody deficiencies, such as those with primary immunodeficiencies or hypogammaglobulinemia secondary to chronic diseases [81]. Additionally, emerging therapies targeting immune modulation, such as checkpoint inhibitors and cytokine blockade, are being explored to balance excessive inflammatory responses and prevent immune paralysis in sepsis [82]. However, these approaches require further clinical validation to determine their efficacy and safety across different patient populations.

Special considerations are necessary when treating sepsis in pregnant women to minimize risks to both the mother and fetus. Physiological changes in pregnancy, including increased plasma volume, altered renal clearance, and changes in drug metabolism, require careful selection and dosing of medications [83]. Certain antibiotics, such as tetracyclines and fluoroquinolones, should be avoided due to potential teratogenic effects or adverse fetal outcomes [84]. Beta-lactams, macrolides, and selected cephalosporins are generally considered safe and are preferred when treating infections in pregnancy [85]. Management of sepsis in pregnant patients also involves close fetal monitoring, particularly in cases of maternal hypotension, as reduced placental perfusion can lead to fetal distress [86]. Early involvement of obstetric and neonatal specialists is crucial to ensuring optimal maternal and fetal outcomes, particularly in cases requiring preterm delivery due to worsening maternal condition [87].

A tailored therapeutic approach is essential in managing sepsis in high-risk populations, as a one-size-fits-all strategy may not be effective or safe. Personalized dosing regimens, targeted antimicrobial selection, intensive supportive care, and emerging immunomodulatory therapies must be carefully integrated into treatment plans to improve survival and reduce complications in these vulnerable patient groups.

Outcomes and Prognostic Factors in Sepsis Among High-Risk Patient Populations

The prognosis of sepsis varies significantly among different patient populations, with special categories such as the elderly, pediatric patients, immunocompromised individuals, those with chronic diseases, and pregnant women facing distinct challenges that influence survival rates, recurrence risk, and long-term health outcomes. The interaction between individual patient characteristics, severity of illness, and therapeutic interventions plays a crucial role in determining overall outcomes.

Mortality and morbidity rates in sepsis differ across patient groups, with elderly individuals exhibiting the highest case fatality rates due to pre-existing frailty, immune senescence, and a diminished physiological reserve. Studies indicate that sepsis-related mortality in elderly patients can exceed 40%, particularly in those with multi-organ dysfunction or underlying cardiovascular disease [88]. Pediatric patients generally have lower mortality rates due to their greater physiological resilience and the ability to recover from severe infections when treated promptly [89]. However, neonates and premature infants remain highly vulnerable, with significantly higher sepsis-related mortality due to their immature immune systems and increased susceptibility to bloodstream infections [90]. Immunocompromised individuals, including cancer patients, transplant recipients, and those undergoing immunosuppressive therapy, also face elevated mortality risks, as their inability to mount an adequate immune response allows infections to progress more aggressively [91]. Patients with chronic diseases such as diabetes, chronic kidney disease, and chronic obstructive pulmonary disease experience worse outcomes due to baseline organ dysfunction, which limits their ability to compensate for sepsis-induced metabolic and inflammatory disturbances [92]. Pregnant women with sepsis generally have lower mortality rates compared to other high-risk groups, but severe maternal sepsis remains a leading cause of maternal morbidity and can result in adverse fetal outcomes, including preterm birth and stillbirth [93].

The success of treatment and the likelihood of sepsis recurrence depend on multiple factors, including early diagnosis, appropriate antimicrobial therapy, hemodynamic stabilization, and organ support. Patients in high-risk categories often require more aggressive and prolonged interventions, yet they may also experience higher rates of treatment failure. In elderly patients, delayed recognition of sepsis due to atypical presentations contributes to increased mortality, and even those who survive the acute phase frequently develop post-sepsis complications such as cognitive decline and functional impairment [94]. Pediatric patients, despite their potential for recovery, may experience long-term immunological and developmental consequences following severe sepsis. Immunocompromised individuals often require extended antimicrobial treatment due to persistent infection risks, but this increases their susceptibility to antimicrobial resistance and recurrent sepsis episodes [95]. Patients with chronic diseases frequently experience sepsis relapses, particularly when their underlying conditions are poorly controlled, leading to recurrent hospitalizations and a progressive decline in overall health status [96].

Prognostic factors vary based on patient category and the therapeutic approach used. In elderly patients, markers of frailty, pre-existing comorbidities, and the presence of multi-organ dysfunction strongly predict poor outcomes. In pediatric sepsis, factors such as gestational age, birth weight, and early initiation of antibiotics influence survival rates [97]. Immunocompromised patients with profound neutropenia or severe lymphopenia are at the highest risk of mortality, particularly when infections are caused by multidrug-resistant organisms or opportunistic pathogens [98]. The effectiveness of immunotherapy, such as granulocyte colony-stimulating factor (G-CSF) or intravenous immunoglobulin (IVIG), in improving survival in these patients remains an area of ongoing research. In patients with chronic diseases, glycemic control in diabetics, renal function stability in those with kidney disease, and optimal management of pulmonary disease play critical roles in determining outcomes [99]. Pregnant women with sepsis have better prognoses when infections are detected early and treated promptly, but complications such as septic shock, disseminated intravascular coagulation, and fetal distress significantly worsen both maternal and fetal outcomes [100,101].

The long-term impact of sepsis on quality of life has become an increasingly important topic of discussion, particularly with the recognition of post-sepsis syndrome (PSS), a condition characterized by persistent physical, cognitive, and psychological impairments following recovery from sepsis [102]. Many sepsis survivors experience chronic fatigue, muscle weakness, neuropathy, and reduced exercise tolerance, which significantly impact their ability to perform daily activities [103]. Cognitive dysfunction, including memory impairment and difficulty concentrating, is particularly common in elderly patients and those who require prolonged intensive care unit (ICU) stays [104]. Anxiety, depression, and post-traumatic stress disorder (PTSD) are frequently reported, further diminishing quality of life [105]. Pediatric patients who survive sepsis may experience developmental delays and long-term neurocognitive impairments, especially if they suffered from hypoxia or severe inflammation during the acute phase [106]. Immunocompromised patients often face ongoing infection risks and require long-term prophylactic antimicrobial therapy, increasing their burden of care. Individuals with chronic diseases who survive sepsis often experience accelerated disease progression, leading to increased dependence on healthcare resources [107]. Pregnant women who recover from sepsis may face complications such as placental insufficiency, preterm labor, or long-term cardiovascular effects, emphasizing the need for close postpartum monitoring [108].

The impact of long-term sepsis management on patient health and functionality underscores the importance of comprehensive post-discharge care. Early rehabilitation programs, including physical therapy and cognitive training, can help mitigate some of the adverse effects of post-sepsis syndrome [109]. Ongoing monitoring for secondary infections, organ dysfunction, and psychological distress is essential to improving the long-term well-being of sepsis survivors. Targeted interventions, such as personalized nutrition plans, immune-modulating therapies, and infection prevention strategies, may help reduce the risk of recurrent sepsis and promote a better quality of life for high-risk patients [110].

The prognosis and long-term outcomes of sepsis vary widely depending on patient characteristics, timely intervention, and post-sepsis care. While advances in early recognition and targeted therapies have improved survival rates, the high burden of post-sepsis complications highlights the need for continued research and multidisciplinary approaches to optimize long-term recovery in vulnerable populations.

Microcirculatory Dysfunction in Sepsis and Its Clinical Implications

Microcirculatory dysfunction plays a critical role in the progression and severity of sepsis, significantly influencing patient outcomes. Various diagnostic techniques are employed to assess alterations in microvascular perfusion, endothelial integrity, and oxygen delivery at the tissue level. Sublingual video microscopy, using techniques such as sidestream dark field (SDF) imaging, allows for direct visualization of capillary flow abnormalities [111]. Near-infrared spectroscopy (NIRS) is utilized to evaluate tissue oxygenation, while biomarkers such as lactate levels and endothelial activation markers provide additional insights into microvascular impairment [112].

The characteristics of microcirculatory dysfunction in sepsis differ significantly from those observed in other critical illnesses. Septic patients frequently exhibit extensive capillary heterogeneity, increased leukocyte-endothelial interactions, and impaired oxygen extraction, even when macrocirculatory parameters such as blood pressure appear normal [113]. Comparing microvascular alterations between septic and non-septic critically ill patients helps elucidate underlying pathophysiological mechanisms and guides the development of targeted interventions [114].

Table 3 summarizes key diagnostic techniques used to assess microcirculatory dysfunction in sepsis, highlighting their advantages, limitations, and clinical relevance in managing critically ill patients.

Table 3: Diagnostic Analysis of Microcirculatory Dysfunction in Sepsis

Category	Aspect Analyzed	Description	Advantages	Disadvantages	Examples	References
Diagnostic Techniques Used	Sublingual Video Microscopy (SDF/OPS Imaging)	Direct visualization of microvascular flow abnormalities and capillary perfusion deficits.	Real-time, bedside assessment of microcirculation.	Operator-dependent, limited standardization.	Identification of capillary heterogeneity and sluggish flow in sepsis.	[115], [116]
	Near-Infrared Spectroscopy (NIRS)	Measures tissue oxygenation to assess microvascular dysfunction in real-time.	Non-invasive, continuous monitoring.	Limited spatial resolution, indirect measurement.	Detects impaired tissue oxygen extraction in septic shock.	[117], [118]
	Plasma Biomarkers (Lactate, Endothelial Activation Markers)	Biochemical markers reflecting endothelial dysfunction and microvascular impairment.	Easily accessible, quantitative assessment.	Nonspecific, affected by multiple factors.	Elevated lactate and angiopoietin-2 levels indicating sepsis severity.	[119], [120]
Key Microcirculatory Features	Capillary Perfusion Deficits	Impaired blood flow distribution at the microvascular level, leading to tissue hypoxia.	Helps explain oxygen delivery failure in sepsis.	Difficult to correct solely with macrocirculatory support.	Decreased proportion of perfused capillaries in septic patients.	[121], [122]
	Endothelial Dysfunction and Glycocalyx Damage	Disruption of endothelial barrier function, increasing vascular permeability, and inflammation.	Critical factor in sepsis progression and organ failure.	No direct bedside measurement is currently available.	Degradation of the endothelial glycocalyx leading to capillary leakage.	[123], [124]
	Oxygen Extraction and Mitochondrial Dysfunction	Sepsis-induced alterations in cellular oxygen utilization and mitochondrial energy production.	Key insight into metabolic failure in septic shock.	Challenging to measure in clinical settings.	Reduced oxygen consumption despite adequate oxygen delivery.	[125], [126]

According to Table 3, the assessment of microcirculatory dysfunction in sepsis highlights the necessity of a multimodal approach for accurate diagnosis and patient management. Sublingual video microscopy remains a valuable bedside tool for directly visualizing microvascular perfusion abnormalities [115,116], while near-infrared spectroscopy provides non-invasive, real-time insights into tissue oxygenation deficits [117,118]. Biomarkers such as lactate and endothelial activation markers offer an accessible means of assessing microvascular impairment [119,120], though they lack specificity and require correlation with other clinical parameters.

The distinct microcirculatory alterations observed in sepsis, including capillary perfusion deficits [121,122], endothelial dysfunction [123,124], and mitochondrial impairment [125,126], underscore significant pathophysiological differences compared to other critical illnesses. These findings suggest that conventional macrocirculatory-focused management strategies may be insufficient, emphasizing the need for personalized interventions targeting microvascular restoration.

Integrating microcirculatory assessment with inflammatory and metabolic biomarkers can enhance early risk stratification, optimize therapeutic strategies, and improve patient outcomes. This underscores the importance of a multidisciplinary approach, incorporating intensivists, infectious disease specialists, and hemodynamic monitoring experts to refine sepsis management and reduce mortality in high-risk populations.

Challenges

The treatment of sepsis in high-risk patient populations presents significant clinical and scientific challenges. Despite advancements in early diagnosis, antimicrobial therapies, and supportive care, difficulties persist in tailoring treatment strategies to meet the unique needs of vulnerable groups. Two major challenges are the identification of reliable prognostic markers to predict disease progression and the absence of standardized protocols for the personalized management of sepsis in special populations [127].

A primary obstacle in managing sepsis in these high-risk groups is the lack of robust prognostic markers that enable early risk stratification and guide treatment decisions. In the general sepsis population, biomarkers such as procalcitonin, C-reactive protein, and lactate levels are commonly used to assess disease severity and response to treatment [128]. However, in elderly patients, these markers may be less reliable due to an altered inflammatory response and atypical sepsis presentation. Similarly, in immunocompromised patients, the absence of a strong inflammatory response can lead to falsely low biomarker levels, delaying diagnosis and appropriate intervention [129]. Pediatric patients, especially neonates, have distinct immune responses that differ from adults, making it difficult to apply standard sepsis biomarkers to this population [130]. Recent studies have explored cytokines such as IL-6 and IL-10 as potential markers for sepsis severity in immunocompromised and pediatric patients, but further research is needed to validate their clinical utility. The absence of well-defined biomarkers complicates early detection, treatment optimization, and outcome prediction in these vulnerable groups [131].

Another key challenge is the lack of standardized protocols for the individualized management of sepsis in special populations. Although general sepsis guidelines exist, they do not always account for the unique physiological and immunological characteristics of elderly patients, pediatric patients, those with chronic diseases, or immunocompromised individuals [132]. For instance, fluid resuscitation strategies must be carefully adjusted in elderly patients with reduced cardiac and renal function to prevent fluid overload. Pediatric patients require weight-based adjustments for antibiotics and vasoactive medications, but there is no universal protocol guiding optimal dosing in neonates with sepsis [133]. Immunocompromised patients often require broader anti-microbial coverage, yet prolonged exposure to broad-spectrum antibiotics increases the risk of resistance and secondary infections, making antimicrobial stewardship particularly complex [134]. Similarly, in pregnant women, balancing maternal treatment with fetal safety is a significant challenge, as some life-saving antimicrobial and vasopressor therapies pose potential risks to fetal development. The absence of tailored sepsis management guidelines for these groups leads to variability in clinical practice and inconsistencies in patient outcomes [135].

Standardizing diagnostic and treatment protocols is essential for improving sepsis management in high-risk populations. Without clear evaluation and treatment criteria, clinical decision-making often relies on physician experience rather than evidence-based guidelines, leading to disparities in care across healthcare settings [136].

DISCUSSION

The findings from this analysis highlight the complexity of managing sepsis in high-risk patient populations and underscore the need for a personalized, multidisciplinary approach. The strength of evidence supporting various therapeutic interventions, prognostic indicators, and post-sepsis outcomes varies across patient groups, influencing decision-making for healthcare providers, researchers, and policy-makers.

Early and targeted antimicrobial therapy remains the cornerstone of sepsis treatment, with strong evidence (Level A) supporting its role in improving survival rates. The literature consistently emphasizes the importance of initiating broad-spectrum antibiotics within the first hour of suspected sepsis to reduce mortality, particularly in immunocompromised patients and neonates who are highly susceptible to rapid disease progression [137]. However, antibiotic stewardship remains a critical concern, as prolonged or inappropriate antimicrobial use contributes to resistance, especially in patients with recurrent sepsis [138].

Supportive interventions such as hemodynamic monitoring and organ support therapies play a crucial role in managing sepsis in elderly patients, pediatric populations, and those with chronic comorbidities [139]. Evidence suggests that goal-directed fluid re-suscitation and vasopressor therapy improve outcomes in patients with sepsis-induced hypotension (Level A). However, the literature also indicates that fluid overload can worsen prognosis, particularly in elderly patients with cardiac or renal impairment, necessitating individualized resuscitation strategies [140]. Pediatric patients require weight-based hemodynamic adjustments, yet a lack of standardized protocols for fluid management in neonatal sepsis creates challenges in optimizing treatment [141].

The role of immunotherapy in sepsis treatment remains an evolving area of research, with emerging evidence suggesting potential benefits for immunocompromised patients. Studies exploring the use of intravenous immunoglobulins (IVIG) and cytokine-targeting therapies indicate possible improvements in immune function and infection control (Level B) [142]. However, data remain inconclusive, and the heterogeneity of immune responses in different patient groups complicates the identification of optimal candidates for such therapies [143].

Post-sepsis outcomes and long-term patient recovery have become increasingly recognized as critical aspects of sepsis care, particularly with the identification of post-sepsis syndrome (PSS) [144]. Studies show that sepsis survivors, particularly elderly patients and those with pre-existing comorbidities, frequently experience persistent physical, cognitive, and psychological impairments that reduce their quality of life (Level A) [145]. The literature highlights the need for early rehabilitation programs, including physical therapy and mental health support, to improve post-sepsis functional outcomes [146]. Pediatric patients, though generally more resilient, may experience long-term developmental delays, while immunocompromised individuals remain at high risk for recurrent infections [147].

The analysis of sepsis treatment and outcomes in high-risk populations underscores the need for a more personalized approach to management. While early antimicrobial therapy, hemodynamic support, and organ protection strategies are well established, optimizing treatment for specific patient groups remains a challenge [148,149]. Further research into immunomodulatory therapies, targeted resuscitation strategies, and post-sepsis rehabilitation programs could improve outcomes for vulnerable populations. For healthcare systems and policy-makers, prioritizing research funding and developing population-specific guidelines will be crucial in advancing sepsis care.

CONCLUSION

The findings of this analysis reinforce and expand upon the existing research on sepsis management in high-risk patient populations, emphasizing the distinct pathophysiological mechanisms and therapeutic challenges that differentiate these groups from the general sepsis population. The evidence strongly supports the fundamental role of age-related immune changes, comorbid conditions, and immune suppression in altering sepsis progression, response to treatment, and long-term recovery. These differences explain the variability in treatment outcomes and underscore the necessity for patient-specific, tailored therapeutic approaches.

From an antimicrobial therapy perspective, the literature confirms that early and targeted antibiotic administration remains the cornerstone of sepsis treatment, particularly in immunocompromised patients and neonates, where delayed intervention significantly worsens prognosis. However, the growing concern of antimicrobial resistance in patients with recurrent sepsis highlights the urgent need for enhanced antimicrobial stewardship programs and the integration of rapid molecular diagnostics to optimize therapy selection. The challenge of balancing broad-spectrum coverage with the risk of resistance remains a key consideration in long-term sepsis management.

The role of hemodynamic support and organ protection strategies in sepsis treatment is well established, with evidence favoring individualized fluid resuscitation and vaso-pressor therapy based on patient-specific factors such as age, renal function, and baseline cardiovascular status. While goal-directed resuscitation strategies improve survival, the risk of fluid overload in elderly and chronically ill patients necessitates a more precise, dynamic approach to fluid management. The increasing use of bedside ultrasonography and advanced monitoring techniques presents an opportunity to refine resuscitation protocols and improve patient outcomes.

The growing recognition of post-sepsis syndrome (PSS) highlights the need for a more comprehensive approach to sepsis care, extending beyond the acute phase to address long-term health complications. Studies confirm that sepsis survivors, particularly elderly individuals and those with preexisting conditions, frequently experience persistent physical, cognitive, and psychological impairments that significantly impact their quality of life. Post-sepsis rehabilitation programs, including structured physical therapy, cognitive assessments, and mental health support, are increasingly recognized as essential components of recovery. However, gaps remain in the availability and accessibility of these services, underscoring the need for healthcare systems to prioritize long-term sepsis care.

These findings highlight the importance of integrating personalized therapeutic strategies, advanced diagnostic technologies, and long-term rehabilitation programs into sepsis management.

Implications for Future Research

Given the limitations of standard sepsis management in high-risk populations, future research should focus on developing personalized treatment strategies that account for immune dysfunction, comorbid conditions, and age-related physiological changes. Studies investigating immunomodulatory therapies, such as IL-6 and IL-10 inhibitors, could provide new therapeutic avenues for immunocompromised patients who fail to mount an adequate immune response. Additionally, research into host-directed therapies, including immune checkpoint inhibitors and cell-based treatments, may help restore immune balance in patients with immune exhaustion or dysregulated inflammatory responses.

The growing threat of antimicrobial resistance, particularly in patients with recurrent sepsis, underscores the need for novel antibiotic stewardship strategies. Future studies should explore rapid molecular diagnostics and point-of-care pathogen identification tools to facilitate early, targeted antibiotic therapy while minimizing unnecessary broad-spectrum antibiotic use. Additionally, research into alternative antimicrobial approaches, such as bacteriophage therapy and antimicrobial peptides, could provide effective solutions for multidrug-resistant infections. The development of nanotechnology-based drug delivery systems to enhance antibiotic penetration into infected tissues and biofilms should also be a priority.

Hemodynamic monitoring and resuscitation strategies in sepsis require further refinement to improve outcomes in specific patient populations. Research into precision-guided fluid resuscitation, incorporating real-time hemodynamic assessment tools such as microcirculatory imaging and noninvasive cardiac output monitoring, could help tailor fluid therapy and prevent complications such as fluid overload in elderly and chronically ill patients. Additionally, studies comparing restrictive versus liberal fluid strategies in different patient populations could provide insights into optimizing volume resuscitation while minimizing adverse effects.

The long-term impact of sepsis, particularly post-sepsis syndrome (PSS), remains an area requiring further exploration. Future research should focus on identifying bi-omarkers that predict long-term cognitive, physical, and psychological impairments following sepsis. Large-scale longitudinal studies evaluating the effectiveness of rehabilitation programs, including physical therapy, neurocognitive interventions, and mental health support, are needed to establish standardized post-sepsis care protocols. Investigating the role of gut microbiome alterations in post-sepsis recovery may also provide opportunities for microbiome-targeted therapies to improve immune homeostasis and overall health outcomes.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, G.P.G. and D.R.T.; methodology, G.P.G and D.R.T; software, M.B.N and I.S.C.; validation, G.P.G and L.P; formal analysis, R.M.S and C.P.; investigation V.T.G and I.S.C.; resources G.P.G, L.P and V.T.G..; data curation, R.M.S., L.P. and M.B.N.; writing—original draft preparation G.P.G. and C.P.; writing—review and editing, G.P.G,. D.O.C. and V.T.G..; visualization, M.B.N and R.M.S.; supervision, G.P.G.; project administration, V.T.G and I.S.C. All authors have read and agreed to the published version of the manuscript.

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ARTICLE

Expectations vs. Appropriate Testing in Preventive Care: A Discussion Based on Insights from Romanian Responders

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Abstract: Preventive medicine plays a crucial role in ensuring the efficiency of healthcare systems, and laboratory investigations represent one of its main pillars. However, achieving an optimal balance between the benefits and costs of testing remains a challenge. Caught between public expectations, appropriate test utilization, and the need for adequate physician training in test ordering, enhancing patients' understanding and knowledge becomes essential. To explore this aspect, a general online survey was conducted, completed by 321 respondents. The results show that, while respondents generally followed physicians' recommendations, many also sought laboratory testing on their own initiative. Direct access to laboratory services - facilitated by the widespread availability of private laboratories - has become a common practice. This raises concerns about the potential overuse of laboratory testing and underscores the importance of further investigating the factors driving patient self-empowerment. Enhancing shared decision-making between patients and physicians is essential to optimize the benefits of testing, minimize unnecessary costs, and reducing the risk of health-related anxiety.

Keywords: laboratory investigations; routine testing; public health; medical education; healthcare overuse

INTRODUCTION

Laboratory testing plays a critical role in the diagnosis, monitoring, prognosis, and treatment of diseases. In modern healthcare, best practice emphasizes the principle of shared decision-making. Although this approach is more commonly applied to therapeutic decisions, it is equally relevant to laboratory investigations and diagnostic testing, as highlighted by Watson et al. While patients rarely request blood tests directly, they often perceive laboratory investigations as a meaningful step in the management of their health condition and as an indication that their physician is taking their concerns seriously. For patients, laboratory results are expected to provide clear answers or solutions, whereas physicians typically use them as tools to rule out serious conditions and to guide clinical reasoning. Due to the discrepancy between patients' expectations and the lack of adequate explanations regarding the actual purpose of the investigations, some patients are disappointed when the tests do not provide a clear diagnosis or concrete solutions for their medical condition [1].

In the context of individuals' tendency to absolutize the role of laboratory testing - as either a means of validating their health status or resolving medical issues - direct-to-consumer laboratory testing emerges as a form of self-empowerment. This is a challenge for the safety and efficacy of healthcare because it allows patients to obtain test results without physician referral, clinical interpretation, or a clear understanding of the limitations related to the test's analytical and diagnostic performance or its overall quality [2].

In the context of an increased volume of test requests, there is a potential for negative consequences. When performed by physicians, ordering unnecessary tests may lead to overdiagnosis, which can result in unwarranted treatments, potential adverse effects, and increased costs for the healthcare system. Contributing factors

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include patient expectations and pressure, as well as physicians' fear of legal liability, all of which may influence the decision to order tests that are not clinically justified [3].

Furthermore, the education and training of physicians in ordering adequate laboratory investigations might be a challenge. According to a study conducted by Fung et al., the majority of residents in family medicine ordered appropriate tests from a list of 38 laboratory investigations for prevention and screening purposes; however, the majority of the participants ordered at least one inappropriate test per patient (average from 3.3 to 5.7 per patient), leading to excessive costs. Several factors have contributed to the tendency to order unnecessary laboratory tests, including patient requests, fear of overlooking important clinical details, working in solo practice, and concerns about potential litigation [4].

While the main reason why healthy individuals visit the primary care facilities is preventive check-ups, and in this context, the family physicians order a high number of screening tests, the overuse of laboratory investigations has decreased from 1978 [5].

Considering the major influence in balancing overuse and effective screening of individual perceptions, understanding, and emotional responses regarding the role of laboratory testing, this study aims to provide general insights from individuals living in Romania. It explores their opinions, habits, behaviors, and knowledge related to laboratory testing, intending to inform the design of future representative studies.

MATERIALS AND METHODS

A survey was conducted using an online data collection tool (Google Forms), which included questions with predefined answers. Respondents selected the answers they deemed most appropriate. The questions addressed various topics related to medical laboratory investigations, including knowledge, opinions, habits, and behaviors. Participation in the survey was voluntary; the link to the form was accessible to anyone, and respondents' completion of the survey was automatically considered as consent to participate in the study. An introductory section provided information about the study's purpose.

Data were collected using a general data collection instrument rather than a validated questionnaire, aiming to capture a broad perspective and highlight insights and new areas for research, rather than yielding quantitative and generalizable results.

The data were centralized and analyzed using Microsoft Excel and Google Sheets. Relative frequencies were calculated and presented in tables and charts. This study employed descriptive statistics only, reflecting its exploratory nature and the self-selected sample. Therefore, no inferential analyses were performed, and causal or generalizable conclusions cannot be drawn. Further research with representative samples and appropriate analytical methods is needed to investigate potential associations.

RESULTS

1. Characteristics of the study respondents and health status

A total of 321 respondents participated in the survey, with an average age of 29 years (ranging from a minimum of 18 to a maximum of 81 years). Among the participants, 85% identified as female and 15% as male. Geographically, 22% of respondents resided in rural areas, and 78% in urban settings. The educational and professional backgrounds of the respondents are detailed in Table 1. Notably, 27% reported having no relatives or close friends working in the healthcare field. Regarding self-reported health status, 77% of respondents considered themselves healthy, while 23% indicated they suffered from chronic conditions, as illustrated in Figure 1.

Table 1: Educational and professional profile of the respondents

Level of studies	Middle school	High school	Higher education
	1.5 %	27%	72%
Occupation	Student	Employee	Other
	45%	50%	5%
Study or work in one of the following fields: health, medicine, veterinary medicine, dentistry, pharmacy, biology		No	Yes
		55%	45%

The majority of respondents rated their overall health status as good or very good; however, most also reported being concerned or very concerned about their health, as shown in Figure 2.

Several behaviors were reported by respondents as being associated with their health-related concerns, as presented in Table 2.

Figure 1: The relative frequency of chronic diseases among respondents who reported having illnesses

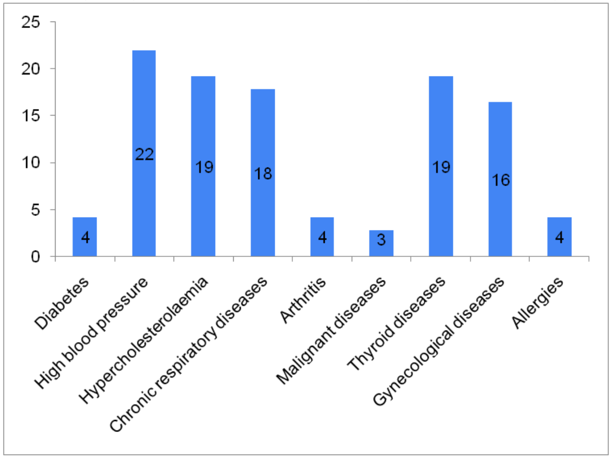

Figure 2. Self-perception of the overall health status (a) and level of concern related to the health status (b)

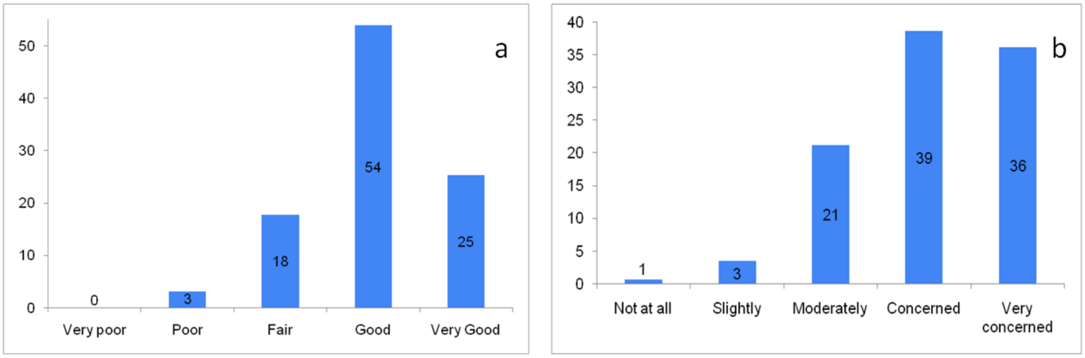

Table 2. Self-reported behaviors associated with health concerns – statements and percentages of respondents in agreement

Behavior linked to health concerns	Relative frequency
I don't consider myself concerned about my health.	4%
I frequently see a doctor for preventive purposes.	28%
I visit the doctor immediately when symptoms appear.	47%
I get medical tests done frequently.	50%
I get laboratory tests done as soon as I notice symptoms.	25%
I go directly to the pharmacy to request treatment if I notice something is wrong.	21%
Concern about my health makes me think about it all the time or frequently.	15%
Concern about my health makes me anxious.	20%
I adopt a healthy lifestyle to be in the best possible health.	36%
I'm afraid of catching infectious diseases in various everyday situations.	26%
I generally postpone going to the doctor for as long as possible.	11%
When I should go to the doctor, I prioritize it over other professional or personal matters.	38%
When I should go to the doctor, I first deal with other professional and personal matters and only then make an appointment.	13%

Opinions about laboratory investigations

Table 3 summarizes respondents' views and knowledge about medical laboratory investigations, their importance, and the recommendations they reported being familiar with regarding laboratory testing.

Table 3: Opinions about laboratory investigations – statements and percentages of respondents in agreement

How important do you consider routine laboratory tests to maintaining health?	
Very important, laboratory tests are essential for detecting and monitoring health problems.	87%
Moderately important, laboratory tests may be useful in certain situations, but are not always necessary.	12%
I do not consider laboratory tests essential for maintaining health.	1%
Do you think it is important to have regular laboratory tests, even if you are healthy?	
Yes, to detect potential health problems in their early stages.	90%
No, if I don't have symptoms, I don't need tests.	2%
It depends on each person's age and lifestyle.	7%
I am not sure.	1%
How well do you think laboratory test results indicate health status?	
The tests show exactly what health problems I have.	25%
Most of the time, the analyses are indicative.	41%
The tests cannot accurately indicate what health problems I have, regardless of their nature.	4%
Certain tests can indicate the exact disease, while others assess the general state of health.	29%
What do you consider to be the ideal approach?	
We should have all kinds of tests done frequently to make sure we are healthy.	51%
We should perform several more important types of tests frequently to ensure we are healthy.	48%
I do not believe that it is necessary to perform routine tests frequently to detect possible health problems.	1%
Do you think that your doctor recommends enough tests for you?	
I didn't need to see my family doctor/specialist.	8%
Yes, I believe I am receiving the necessary recommendations for my health.	63%
I am not sure.	8%
No, I think I am being recommended too many tests.	2%
No, I think that too few tests are recommended for me.	16%
I don't do laboratory tests.	2%
How frequently do you think laboratory tests are recommended for someone your age?	
3-4 times a year	6%
1-2 times a year	83%
Once every 2-3 years	2%
Less often	1%
No specific time frame is recommended	3%
What is your level of confidence in the results of laboratory tests?	
Very high	28%
High	61%
Moderate	11%
Low	0%
Absent	0%
How do you see the doctor's role in interpreting laboratory test results?	
As an essential guide in understanding the results.	90%
Like someone who should only be consulted in case of problems.	6%
I do not consider it necessary to have a doctor interpret it, as there is enough information available on the internet.	1%
No opinion.	2%

Do you think laboratory tests should be used more in prevention?	
Yes, it could help prevent some conditions.	62%
Yes, it could help detect diseases in their early stages.	31%
No, it could cause unnecessary worries.	1%
It depends on each individual case.	5%
How do you evaluate the experience during sample collection for laboratory analysis?	
Comfortable and without problems.	55%
Acceptable, there is potential for improvement.	40%
Uncomfortable and unpleasant.	4%
I have no personal experience in this regard.	1%
Which of the following aspects do you think could improve your experience while performing laboratory tests?	
Reducing waiting time for results.	12%
Lower costs or covered by health insurance.	60%
Better communication and explanation of results.	13%
Reducing waiting time for sample collection.	2%
Do you consider that laboratory results are managed with confidentiality in mind?	
Yes, I trust that they are treated confidentially	77%
I am not sure	15%
No, I have doubts about their confidentiality	2%
I have no personal experience with this	3%
No opinion	2%
Do you think there is enough public education about the importance and usefulness of laboratory tests?	
Yes, there is enough public education.	7%
No, more education would be beneficial.	87%
Not sure.	4%
I don't think public education would make a difference.	2%
No opinion.	1%
When you receive the results of laboratory tests, do you consider it useful to automatically receive explanations of the results in a formal setting?	
No, because I understand all the content and I don't need any other information.	7%
No, because I can find the necessary information by asking people I know or searching the internet.	1%
I do not know.	1%
Yes, I think it would be useful to receive explanations of the results every time I have my lab tests.	88%

Behavior of respondents

Regarding how the respondents deal with the results of medical laboratory investigations, most respondents reported either seeking medical advice or requesting feedback about their health, as shown in Figure 3.

Table 4 presents self-reported adherence to physicians' recommendations regarding laboratory tests, as well as respondents' motivation, testing frequency, and behaviors related to receiving laboratory results.

The majority of the responders reported that the fear of blood or the fear of blood collection does not prevent them from having their laboratory tests done, as presented in Figure 4.

With regard to the entity involved in laboratory investigations, the majority of the responders preferred private laboratories to have their tests done, as shown in Figure 5.

Access, availability, barriers and knowledge related to laboratory testing

In the group of responders who were employed, 48% mentioned that their employer does not provide access to routine laboratory testing, 47% that they have access to routine laboratory testing once a year or more often, and 4% that they have access to routine laboratory testing from their employer every few years.

The responders considered costs and lack of time as the main factors that prevent them from having their routine laboratory testing done, as presented in Figure 6.

Figure 3: Respondents' attitudes toward normal (a) and abnormal (b) laboratory results

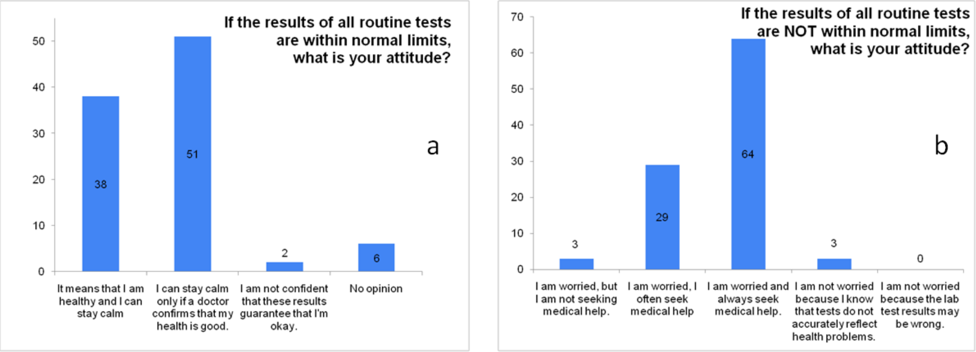

Table 4: Behaviors related to undergoing laboratory investigations, compliance with physicians` indications and dealing with lab test results – statements and percentages of respondents in agreement

Do you do laboratory tests on your own initiative or on the recommendation of a doctor?	
On the recommendation of a family doctor/specialist.	23%
On my own initiative.	24%
Both on the recommendation of a doctor and on my own initiative.	51%
I don't do laboratory tests.	2%
How often do you do your routine lab tests?	
Twice a year or more often	23%
Once a year	61%
Once every few years	6%
Only when I have a health problem	8%
Never	2%
When you do laboratory tests on your own initiative, what is the motivation? (multiple choice)	
I do not do my laboratory analyses on my own initiative.	14%
I want to make sure that my health remains good.	63%
I want to know if I have a health problem, even if I don't have symptoms.	52%
I have chronic illnesses or other conditions and for this reason, I want to have tests done.	13%
When you performed laboratory tests on the doctor's recommendation, for what purpose were you guided in this direction? (multiple choice)	
To establish a diagnosis.	46%
To follow up a disease.	24%
To perform routine tests without having a health problem.	69%
I did not do any tests recommended by a doctor.	6%
Do you always do your lab tests when your doctor recommends them?	
Always	74%
Often	21%

Rarely	3%
Never	2%
When you don't do lab tests following your doctor's recommendation, what is the motivation? (multiple choice)	
No medical tests were recommended to me.	26%
I was healthy and it was just routine tests.	30%
I did not have enough time.	19%
I did not have access to a collection point/medical analysis laboratory.	7%
I didn't see the usefulness of the analyses in that context.	6%
The costs were too high.	17%
What would you do if your tests are outside normal limits, and the doctor tells you that everything is fine with your health status?	
I turn to another doctor.	35%
I inquire into other sources and make my own decisions for diagnosis and treatment.	7%
I continue the investigations and repeat the tests.	40%
I trust the doctor and do nothing extra.	17%
What is your main source of information regarding laboratory tests? (multiple choice)	
My physician	89%
Acquaintances who work in the medical field	33%
Healthcare related websites	13%
Family, friends and acquaintances who do not work in the medical field	11%
I did not request/seek information about the analyses	3%
Books, magazines and specialized articles	17%
Websites of laboratories and clinics	28%
Social media groups	1%

Figure 4: The impact of the fear of blood or blood collection on having laboratory testing done

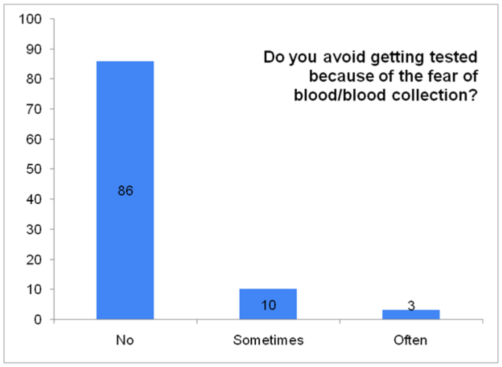

The knowledge and opinions about the laboratory investigations reimbursed by the public healthcare insurance system are presented in Table 5.

The majority of the responders have a sample collection facility or a medical laboratory within a reasonable walking distance from their home and reported that the distance from it does not influence their decision to have their routine tests done (Figure 7).

DISCUSSION

Overall, respondents were optimistic about their health status, with most rating it as good or very good. Nevertheless, the majority also reported being concerned or very concerned about their health. This particular perception may influence their attitudes and behaviors related to laboratory investigations. For example, health anxiety has a major impact on healthcare use [6].

Despite expressing concern about their health, fewer than one-third of respondents reported seeking preventive medical care. Approximately 40% reported adopting a healthy lifestyle, and less than half indicated that they consult a physician promptly upon noticing symptoms. More than 60% agreed that laboratory testing could be used more extensively for prevention, and over 30% believed that laboratory tests could be better utilized for early disease detection. However, half of the respondents considered that they already undergo laboratory tests frequently, while only a quarter reported getting tested when symptoms occur.

In general, respondents demonstrated awareness of the importance of laboratory testing for disease diagnosis (including early detection) and monitoring. They also acknowledged that the variety of available laboratory tests serves different purposes in healthcare. While less than one-third expressed very high confidence in laboratory test results, more than 60% reported having confidence in them overall. Additionally, nearly 80% of respondents stated that they trust laboratory investigations are handled confidentially.

Figure 5: Preferences for public and private laboratories

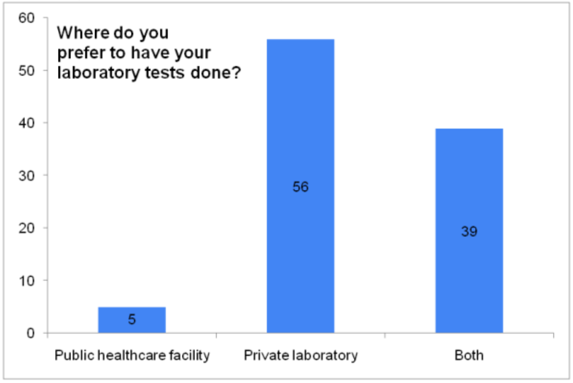

Figure 6. Main barriers to routine laboratory testing

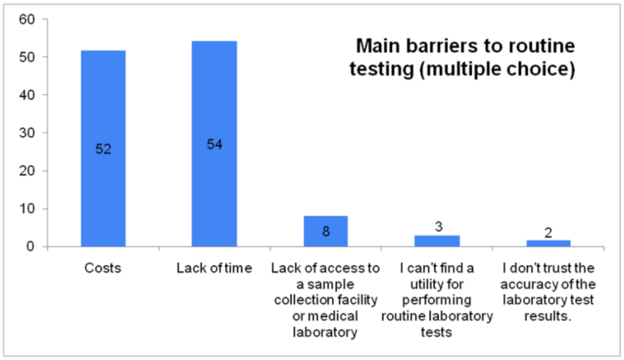

Table 5: Opinions and knowledge of the responders about the reimbursement of the costs for laboratory investigations – statements and percentages of respondents in agreement

Are laboratory tests reimbursed by the public healthcare insurance system?	
I don't know this information.	30%
Routine tests are reimbursed once a year.	26%
Laboratory tests are reimbursed, regardless of whether they are routine or not, once a year.	4%
Routine tests are reimbursed whenever a referral from the family doctor is used.	33%
Lab tests are reimbursed, regardless of whether they are routine or not, whenever needed.	3%
Lab tests are reimbursed only for those with an established diagnosis.	2%
Lab tests are reimbursed only for hospitalized persons.	1%
Have you ever benefited from laboratory tests reimbursed by the public healthcare insurance system?	
Rarely	15%
Every time/almost every time	21%
Often	36%
I do not know	10%
No	17%

Patients often perceive laboratory tests as inherently accurate and confidential, yet studies suggest gaps in their understanding of these concepts. Sankar et al. highlighted that misconceptions about confidentiality can undermine trust, with some patients overestimating privacy safeguards [7]. Educating patients about the accuracy limits of tests and existing privacy protections may strengthen their engagement and promote informed participation in healthcare decisions [8].

With regard to healthcare received from physicians and the medical system, most respondents considered that, in general, they are adequately recommended for laboratory testing. Overall, routine laboratory testing appears to be driven equally by the respondents' own initiative and by physicians' recommendations. Patients who undergo laboratory testing on their own initiative are motivated by various factors, including the desire to confirm their perceived good health status or personal experiences—such as witnessing family members or acquaintances being diagnosed or saved through timely laboratory testing [3]. The growing availability of direct-to-consumer testing has enabled patients to access laboratory analysis without a physician's mediation, but some studies highlight potential risks, including misinterpretation of results and unnecessary anxiety [9]. According to the results of Shaked et. al, in the case of patient-requested laboratory investigation, even after the doctor explained why the tests were unnecessary, the majority of patients expected to receive a referral for laboratory testing, likely due to the perception that blood tests are objective and reliable tools for validating one's health status [3].

Figure 7: The availability of sample collection facilities or medical laboratory close to the residence of respondents (a) and its impact on decisions regarding laboratory testing (b)

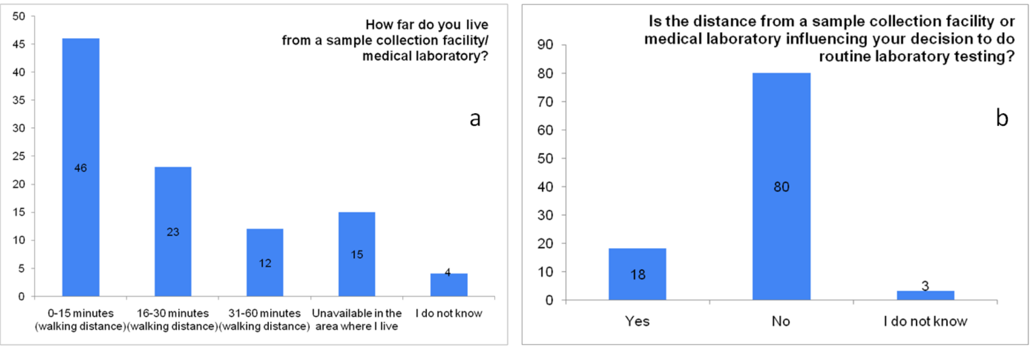

The main reasons for undergoing laboratory tests without a medical recommendation are to ensure good health and to detect early or subclinical diseases. Balancing preventive strategies with patient education about the limitations and risks of indiscriminate testing is essential to avoid fostering a culture of overmedicalization [10]. Approximately 90% of the respondents of this study acknowledged the importance of physicians in explaining laboratory test results. Similarly, nearly 90% believed it would be beneficial to receive explanations of their results in a formal setting each time they undergo laboratory investigations.

Around half of the participants reported that, when laboratory results fall within the biological reference interval, they feel reassured only after receiving confirmation from a physician that their health status is adequate. When routine test results fall outside the reference interval, more than 60% of respondents stated that they always seek medical help, while nearly 30% reported doing so often. According to a study performed by Marloes et al., patients overestimate the performance of blood testing, and results within normal limits were automatically seen as proof of an optimal health status. However, this study included only patients who voluntarily presented to the general practitioner for blood tests [11].

Furthermore, the vast majority of respondents (approximately 90%) identified physicians as their primary source of information regarding laboratory tests. Random online, non-professional sources were not commonly used. Instead, acquaintances working in healthcare and official websites of medical institutions were reported as the second and third most frequently cited sources of information, respectively. On the other hand, a survey by Cocco et al. revealed that an important part of respondents searched for explanations of lab results online before consulting a clinician, sometimes resulting in inappropriate self-diagnosis [12]. McMullan et al. found that patients using online resources for interpreting laboratory results often encounter conflicting or inaccurate information, leading to confusion and heightened anxiety [13].

When routine testing is recommended by a physician, the majority of respondents comply with the recommendation and undergo testing. Among those who do not comply, the main reasons reported are the perception of being in good health due to the routine nature of the check-up, lack of time, and associated costs. Adherence to recommended laboratory testing is essential for effective clinical management, but can be suboptimal. In patients with diabetes, a large registry study found that approximately 1 in 7 did not complete essential lab tests (such as HbA_1c or creatinine) within six months of ordering. Key predictors of better adherence included older age, female sex, and orders placed during face-to-face visits [14].

The majority of respondents considered that routine laboratory testing should be performed once or twice per year. Over 60% reported undergoing routine testing once annually, while more than 20% reported doing so twice a year or more frequently. The optimal frequency for routine blood testing depends on individual health status and clinical context. For healthy adults, many guidelines recommend annual basic panels during preventive visits, but more frequent testing is justified in chronic conditions or medication monitoring [15].

Although fear of blood or blood withdrawal is generally common [16], only 4% of respondents in this study described the sample collection procedure as uncomfortable or unpleasant. Additionally, nearly 90% reported that such fears do not influence their decision to undergo laboratory testing.

From the respondents' perspective, reducing waiting times for sample collection or results, as well as receiving more information and explanations about laboratory investigations and their outcomes, were not considered priorities for improvement. The primary issue identified was the cost of laboratory investigations and their coverage by health insurance.

Cost represents a significant concern, especially given that the majority of respondents reported preferring to have their laboratory tests performed in private laboratories rather than in public healthcare facilities. Additionally, lack of time and financial constraints were reported as the main barriers to undergoing laboratory testing.

Due to the widespread availability of private laboratories in Romania and access to their services without the need for a physician's referral, two specific aspects emerge: firstly, the healthcare system lacks control over potential overuse of testing, and secondly, the costs are often entirely supported by the patient. This situation may differ in other countries, where the overuse of laboratory investigations is potentially driven by the extensive coverage provided by public or private health insurance. When testing is widely accessible and patients and the healthcare provider are not directly burdened by the costs, the likelihood of overuse may increase [17].

Respondents appeared to have limited knowledge regarding the coverage and conditions for laboratory investigations under the public health insurance system, and few reported utilizing reimbursed laboratory tests.

Overall, respondents expressed the view that there should be increased public education regarding the importance and benefits of laboratory testing. Patients often struggle to understand the need for certain laboratory tests and their results, highlighting the importance of public education in laboratory medicine [18].

Overall, the responses to questions regarding laboratory medicine suggest that the general public in Romania may have high expectations for routine testing. Currently, there are no regulations in Romania that restrict access to direct-to-consumer testing. While direct-to-consumer access to routine laboratory investigations is generally permitted across countries, some nations have imposed restrictions on direct access to genetic testing [19]. Although most genetic conditions are rare, for example, familial hypercholesterolemia is a relatively common genetic disorder [20]. In the absence of regulatory limitations, such conditions may be perceived by the general public as suitable for direct-to-consumer testing - particularly given the high prevalence and awareness of hypercholesterolemia. However, this raises the risk of misinterpretation, since direct-to-consumer tests for this condition often lack optimal performance for accurate detection [21].

The current study has several limitations. First, the data collection instrument was not a validated questionnaire, which limits the generalizability of the findings. Additionally, respondents were self-selected, resulting in a sample that is not representative of the general population. Moreover, the sample did not uniformly or comprehensively cover all demographic backgrounds of Romanian citizens, with most respondents holding higher education degrees and residing in urban areas. Finally, all data were self-reported, relying on respondents' subjective perceptions and, in some cases, their memory and ability to estimate the frequency of events (e.g., how often they underwent routine laboratory testing).

However, it is essential to conduct this type of exploratory research, which is less quantitative in nature, to identify key areas for further investigation. This study provided a rapid assessment of various aspects related to patients and laboratory testing, offering a foundation for more detailed future research.

Future studies should build on the exploratory findings of this research by employing validated questionnaires and representative samples to enhance the generalizability of results. Further investigation is needed to understand the multifaceted factors influencing patients' adherence to physician recommendations for laboratory testing, including psychosocial, economic, and cultural determinants. Additionally, evaluating the impact of public education campaigns on patients' knowledge, trust, and behavior regarding laboratory investigations could provide valuable insights. Qualitative research methods, such as in-depth interviews and focus groups with both patients and healthcare providers, would offer a deeper understanding of the perceptions, fears, and informational needs surrounding laboratory testing. It is also important to explore disparities in access to laboratory services across urban and rural settings and among different socio-economic groups. Finally, examining the role of cost and health insurance coverage in shaping patients' decisions to undergo laboratory testing would contribute to the development of targeted interventions to reduce barriers and improve utilization.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

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Authors' contribution

Conceptualization, M.D., T.C., I.A.B and E.P.; methodology, M.D., T.C., I.A.B. and E.P.; investigation, M.D., T.C., I.A.B., I.I., R.M.U., G.T.L., R.R., A.S., M.M.C., E.P.; data curation, M.D., I.A.B., E.P.; writing—original draft preparation, M.D., T.C., I.A.B., I.I., R.M.U., G.T.L., R.R., A.S., D.O.C., E.P.; writing—review and editing M.D., T.C., I.A.B., I.I., R.M.U., G.T.L., R.R., A.S., M.M.C., E.P.; supervision, M.D., T.C., E.P. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

This study was conducted using an online questionnaire that was publicly available and open to the general population. Participation was voluntary, self-selected, and anonymous. No personal or identifiable data were collected, and no recruitment of patients was involved. Information about the purpose and nature of the study was provided at the beginning of the form. Completion and submission of the form were considered as an indication of informed consent to participate in the study. Considering all these aspects, the study was conducted in compliance with ethical guidelines.

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REVIEW

Combatting HIV/AIDS in the Armed Forces: A Literature Review

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Abstract: The Human Immunodeficiency Virus (HIV) and the Acquired Immunodeficiency Syndrome (AIDS) remain significant public health concerns worldwide. This review seeks to present an updated perspective on the spread, consequences, and control measures of HIV/AIDS, focusing on military personnel as a particular at-risk group. It starts by outlining general information about the transmission of the virus and summarizing recent global epidemiological trends. This review further explores the difficulties that HIV/AIDS presents in military settings, including policies regarding the management of service members living with the virus. Additionally, it examines several prevention strategies implemented by militaries around the world, such as educational programs, provision of condoms, and efforts to reduce the stigma associated with the infection.

Keywords: HIV; AIDS; military population; prevention; treatment; management

INTRODUCTION

The Human Immunodeficiency Virus (HIV) was first presented in 1983 as a non-transforming retrovirus [1]. This virus targets CD4+ T lymphocytes and is responsible for Acquired Immunodeficiency Syndrome (AIDS) in individuals with the infection [2]. This condition is characterized by two human lentiviruses: HIV-1 and HIV-2, with transmission occurring exclusively from human to human. Typically, in the absence of a treatment, HIV-1 infection can lead to AIDS, a syndrome associated with significant mortality rates [3]. Both HIV-1 and HIV-2 are RNA viruses that carry the necessary information for the synthesis of the viral proteins essential for the replication and assembly of the virus [4].

HIV transmission occurs either horizontally or vertically [5]. Vertical transmission is the most common way for children to acquire the infection, either in utero, during childbirth, or through breastfeeding [5,6]. Horizontal transmission involves needle sharing or unprotected sexual contact [5,7,8].

One population that has been identified as vulnerable to acquiring HIV infection is military personnel, due to frequently reported high-risk sexual behaviour [9,10] or to their high mobility and exposure to biologically foreign environments [11,12].

Aim

This literature review explores the challenges faced by military populations in the context of HIV/AIDS and presents prevention strategies and proposed solutions for effective prevention and control in this high-risk group.

MATERIALS AND METHODS

This paper examines the medical literature concerning HIV/AIDS, as well as the public information disseminated by state agencies regarding programs and strategies aimed at preventing HIV transmission among military personnel.

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RESULTS

HIV/AIDS: General Overview and Key Statistics

At the end of 2022, the World Health Organization reported that 39 million people were living with HIV, including 1.5 million children. That year, 1.3 million people contracted the virus, reflecting a 38% decline compared to 2010, when 2.1 million new cases were reported [13].

Concerning HIV-related mortality, 2022 saw 630,000 deaths attributed to the virus, down from an estimated 1.3 million deaths in 2010. This represents a 51% decrease over a 13-year period.

In terms of treatment, around 29.8 million people received antiretroviral therapy (ART) in 2022. Among those infected that year, 86% were aware of their HIV status, 76% had begun treatment, and 71% had successfully achieved viral suppression [13].

The African continent remains the most affected globally by HIV, with an estimated 25.6 million individuals living with the virus. Within this group, 90% are aware of their status, 82% receive antiretroviral therapy (ART), and 76% have achieved suppressed viral loads. The region has also seen a significant drop in incidence per 1,000 uninfected individuals. Along with a 56% decrease in AIDS-related deaths since 2010, down to an estimated 380,000 annually [13].

The European Region has an estimated 3 million people living with HIV. Of these, 72% know of their status, 63% are undergoing treatment, and 60% have achieved suppressed viral loads. Roughly 1.9 million individuals are on ART. However, Europe has experienced a rise in HIV incidence over the past decade, from 0.16 to 0.20 per 1,000 uninfected people, alongside approximately 180,000 new infections reported in 2022. AIDS-related mortality has also increased by 37% since 2010, with an estimated 52,000 deaths recorded in 2022 [13].

In Romania, according to the latest data, 716 people were diagnosed with HIV/AIDS in 2023. Over the period from 1985 to 2023, a total of 18,389 individuals have been recorded as having the disease, with 8,556 losing their lives due to HIV-related causes [14].

Epidemiology of HIV in Military Populations

Military populations represent a unique demographic in the global HIV epidemic [11,12], being predominantly composed of young adults [15,16] and often deployed to environments where risk behaviors may increase - factors shown to contribute to new infections among U.S. Army Soldiers [17].

HIV incidence differs substantially across regions, with particularly high rates observed in certain areas of sub-Saharan Africa and Southeast Asia. In the United States, while overall HIV prevalence is lower, military personnel are not exempt. Despite strict screening protocols, they are not exempt [18]. As of March 2021, over 170,000 U.S. service members were deployed globally, marking the largest international military presence at that time[19]. By June 2024, approximately 166,000 active-duty U.S. troops- representing 12.8% of the total force- were still stationed overseas [20].

In 2021, the HIV incidence in active-duty U.S. military was 21 cases per 100,000 service members, slightly higher than that of the general U.S. population, which was approximately 13.9 per 100.000 [21].

Between 1990 and 2024, the Department of Defense conducted over 46 million HIV screening tests, reflecting an extensive and ongoing surveillance effort aimed at early detection and prevention. A total of 1,581 active-duty service members were diagnosed with HIV between January 2017 and June 2022, with 62% remaining on active duty following their diagnosis [22].

These trends are particularly relevant given the global mobility of U.S. forces and their deployment in diverse environments, often under operational stress or during humanitarian missions, which may complicate prevention efforts [23].

In sub-Saharan Africa, HIV/AIDS has disproportionately impacted military personnel compared to civilians. This increased vulnerability is linked to demographic factors- mostly young, sexually active men- and the specific conditions of military service, such as extended time away from home, deployment to unstable regions, and social dynamics that encourage risky sexual behavior [15,16,24].

In 2014, Lloyd et al. reported that the prevalence of HIV among military men from sub-Saharan Africa and other low-income settings was notably higher compared with civilian men of reproductive age, and even higher among longer-serving service members [25]. In 2024, Grillo et. al revealed that military members were frequently involved in dense HIV transmission networks, alongside the civilian population and female sex workers [26].

The Perspective on HIV/AIDS in the Romanian Army

Military personnel infected with HIV but without any functional deficiency (e.g., those who are asymptomatic, experiencing acute retroviral syndrome, or presenting with generalized persistent lymphadenopathy) or who have only low functional impairment (e.g., clinical manifestations due to HIV infection; constitutional symptoms; herpes zoster; idiopathic thrombocytopenic purpura; or peripheral neuropathy) are not classified as having a disability and are allowed to continue performing their military duties [27].

Risk Factors for HIV in the Military

Military populations are considered to be at an elevated risk for acquiring HIV infection [12] due to exposure to a multitude of risk factors. The composition of military forces- mostly young, sexually active males- places them in a higher-risk demographic compared to the general population [25]. Frequent deployments that often result in extended separation from stable partners may further increase the tendency to engage in unsafe sexual practices [28]. The mobility of military troops and exposure of personnel to high-prevalence settings also contribute to this risk [11, 26].

Sexual risk behavior is an essential driver of transmission. In 2015, Hakre et al. reported that among HIV seroconverters in the U.S. Army between 2012 and 2014, most reported multiple sexual partners, inconsistent condom usage, and casual sexual contact or anonymous partners in the year before diagnosis [10].

A culture of masculinity and peer pressure has been reported to contribute to the encouragement of sexual risk-taking behaviors among military personnel [29], influencing HIV-related practices and increasing vulnerability to infection [30].

Tran et al. reported in 2019 that in the Armed Forces of the Republic of the Congo, alcohol consumption, commonly reported among active-duty soldiers, has also been shown to encourage risky sexual habits such as a reduction in condom usage; an increase in transactional sex and multiple sexual partners compared to the lower risk peers [31]. Similar patterns were reported on by Harbertson et al. in 2019, in the U.S. Marine Corps, where a reduction of condom use was associated with alcohol abuse or drug use to enhance sexual experiences [32].

Institutional barriers that may delay diagnosis, prevention, and linkage to healthcare within the military population include concerns over confidentiality and fear of stigma or professional consequences [33], furthering a decrease to essential social and medical care, such as ART [34].

HIV Prevention Strategies and Program Implementation in Military Settings

HIV epidemiology and prognosis have improved substantially due to the universal use of ART, the availability of medications that are more effective and better tolerated by patients [35], and the introduction of preventive measures such as pre-exposure prophylaxis (PrEP) [36]. With a timely diagnosis followed by early initiation of ART, the life expectancy of individuals living with HIV has significantly increased [37, 38].

The Department of Defense (DoD) in the United States of America offers the TRICARE program as part of its efforts to reduce the spread of HIV. This program includes a range of services aimed at both HIV prevention and care. Key initiatives include coverage for pre-exposure prophylaxis (PrEP), as well as promoting access to treatment options such as ART. Additionally, TRICARE provides counseling and support services, and it covers HIV testing for beneficiaries identified as being at risk for contracting the infection [23, 39].

Nonetheless, public health initiatives still encounter obstacles. Misconceptions about HIV transmission are prevalent among people of reproductive age in developing countries and represent a barrier to effective prevention efforts [40,41]. Promoting medical education and raising awareness around HIV-related stigma may have a positive impact on lowering risky sexual behaviors, particularly among military populations [42, 43].

The Department of Defense HIV/AIDS (DHAPP) of the United States of America implements an extensive HIV/AIDS prevention program with various strategies and interventions. These include index case HIV testing, periodic updates to military HIV policies, promotion of condoms, and facilitating access to them. DHAPP also conducts training to prevent stigma and discrimination related to HIV/AIDS [44].

In 2011, Singh and Banerjee conducted a survey among regimental officers regarding hiv prevention in the armed forces and found that there were difficulties in program implementation, especially in the promotion of condom use [45]. In 2015, Tran et al. reported that distributing free condoms to military personnel with improved packaging and a more pleasant scent led to an increase in usage among servicemembers, thereby enhancing efforts to prevent sexually transmitted infections within the military community [46].

Anastario et al. conducted in 2012 a pilot study in a military unit in Barbados, where a modified version of the Popular Opinion Leaders (POL) intervention was used. This intervention involved selecting and training Popular Opinion Leaders within the military personnel to promote conversations on condom use. As a result, there was a noticeable rise in condom use among military personnel after the intervention [47].

CONCLUSION

HIV/AIDS continues to pose a serious health challenge on a global scale, even as progress has been made in recent years. The prevalence of the disease remains a significant social and public health concern, with millions of individuals affected worldwide.

In military settings, targeted prevention strategies are particularly important due to the specific occupational and behavioral risks associated with service. Various armed forces have adopted structured programs aimed at limiting transmission, supporting personnel living with HIV/AIDS, and addressing stigma within military environments.

By focusing on education, encouraging risk-reduction behaviors, and ensuring access to services such as PrEP and ART, military institutions play a key role in HIV control. Sustained implementation of these measures can help transition HIV/AIDS from a widespread public health threat to a manageable chronic condition.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

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ARTICLE

The impact of combat stress on the coping strategies selected by mobilized military personnel with differing family statuses

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Abstract: Since February 24, 2022, military personnel of the Ukrainian Defense Forces have been resisting the military aggression of the Russian Federation. The fighting and the mobilization of men for military service complicated the well-being of Ukrainian families. The purpose of the article is to identify the relationship between the choice of coping strategies and post-traumatic stress disorder (PTSD) and maladaptation indicators in married and unmarried mobilized military personnel. Ukrainian Defense Forces military personnel (n=171 males, between 20 and 60 years of age) took part in this study. The "Strategic Approach to Coping Scale", "Mississippi Scale for Combat-Related Posttraumatic Stress Disorder", and "Disadaptation Express Questionnaire" were used. Correlation and exploratory factor analysis made it possible to identify the structure of coping strategies in mobilized servicemen with different family statuses and to determine the relationships between coping strategies, PTSD, and maladaptation indicators. The pre-war experience of using coping strategies by married and unmarried mobilized military personnel was not a sufficient barrier to the emergence of PTSD symptoms and maladaptation manifestations. Married and unmarried mobilized servicemen had a deficit structure of coping strategies characteristic of war.

Keywords: military personnel; coping strategies; PTSD; family; combat stress; mental health; mobilized soldiers; Russian-Ukrainian war

INTRODUCTION

The large-scale war in Ukraine, which began on 24.02.2022, has exacerbated the problem of mobilizing civilians and preparing military personnel to participate in hostilities to protect the sovereignty and lives of Ukrainians [1]. However, mobilization for military service has also complicated the well-being of families, who are forced to overcome problems arising from threats to the health and lives of family members, changes in family lifestyle, and everyday difficulties on their own, without men/women. This was also due to military actions on the territory of the country: experiencing threats of air raids, loss of housing, forced displacement, distance learning for children and their permanent stay at home, remote work or its absence, etc. [2,3]. These circumstances were factors that destroyed families, as well as additional stress for military personnel who, while defending their country, cannot protect and preserve their families.

Coping with potentially traumatic situations, including combat stress, involves the use of coping mechanisms and stress management strategies [4]. One of the models of stress management is the "Multiaxial Model of Coping", which comprises three axes: active–passive, prosocial–antisocial, and direct–indirect [5]. These axes represent dimensions of general coping strategies that allow for broader application across individualistic and collectivist cultures. The model assumes that active and avoidant strategies are not socially indifferent, and ignoring this fact may lead to a misinterpretation of their effects [6]. Prosocial coping involves strategies that positively utilize social resources. Antisocial coping includes behaviors that, while

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satisfying the individual's needs, can be detrimental to others [7]. Aggressive coping is also effective as long as it does not become outright antisocial. Antisocial coping does not denote psychopathological behaviors, but rather coping methods that can be potentially harmful to others, involving the use of others or even exploiting them for personal gain [8].

Research has detailed the negative impact of combat deployments on service members and their families, the devastating effects of maladaptation and post-traumatic stress disorder (PTSD) symptoms on families, including violence against spouses and children [9,10]. PTSD couples reported clinically significant levels of relationship distress several times more frequently than comparison couples, both for general distress and across all specific problem areas (e.g., aggressive behavior, quality of leisure time together, sexual functioning, conflicts about finances and child rearing) [11]. Another study found that greater unit support, marital satisfaction, and psychological hardiness were associated with less anger, anxiety, depression, and PTSD symptomatology in soldiers [12]. The positive effects of intimate partner [13] and unit support reduced negative emotionality and traumatic stress only for veterans with less severe combat experiences [14].

However, the conducted studies did not examine the features of PTSD manifestations and maladaptation to the choice of coping strategies of mobilized servicemen with different family statuses. For such people, military service was not their own choice, and they were forced to adapt their civilian traumatic experience to overcome the effects of combat stressors and wartime stress factors.

The purpose of the article was to identify the relationship between the choice of coping strategies and indicators of post-traumatic stress disorder and maladaptation in married and unmarried mobilized military personnel.

MATERIALS AND METHODS

Study design and participants

This study was an exploratory descriptive study. All participants provided their informed consent for inclusion before engaging in the study. Ukrainian Defense Forces military personnel (n=171 males, aged 20 to 60 years, with a mean age of 41.84±6.49 years) participated in this study. The participants included 71% privates and 29% non-commissioned officers, all of whom participated in the Russian-Ukrainian war and had combat experience ranging from 6 to 10 months (8.75±2.58 months). Before the war, 62% of the participants had urban origins, while 38% had rural origins; 24% had completed secondary education, 57% had completed secondary specialized education, and 19% had attained higher education. The military personnel were sent to the rehabilitation center from combat positions to partake in a psychological recovery program lasting 14 days [15]. Two groups were formed to conduct the study: group 1 included 76 unmarried participants, while group 2 consisted of 95 married mobilized military personnel. The participants were identified based on various manifestations of acute stress reactions, significant negative experiences—including signs of depression and suicidal ideation—presence of PTSD symptoms, sleep problems (more than 50%), somatic complaints (more than 80%), and wounds and contusions (more than 75%); they also faced difficulties in returning to combat missions due to the consequences of illness, injury, and wounds. Female military personnel were excluded from this study because less than 0.5% of female combatants participated throughout the program period. Officers were also not included in the study due to their small number in the psychological recovery program (less than 1%). Participants were randomly selected for the study.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the National Academy of the National Guard of Ukraine, Kharkiv, Ukraine (protocol code 2024/12, dated September 24, 2024).

Instruments

To determine the relationship between the choice of coping strategies and indicators of PTSD and maladaptation in mobilized military personnel, the "Strategic Approach to Coping Scale" (SACS), "Mississippi Scale for Combat-Related Posttraumatic Stress Disorder" (MSCRPTSD), and "Disadaptation Express Questionnaire" (DEQ) were used.

The SACS was used to determine coping strategies and behavior patterns of military personnel to overcome stressful situations, adapted into Ukrainian [16,17]. The SACS consists of 54 statements, the answers to which were given on a 5-point Likert scale (Cronbach's α = 0.846). The scale allowed for the choice of coping strategies (behavior models) such as "Assertive Actions" (Cronbach's α = 0.627), "Social Joining" (Cronbach's α = 0.736), "Seeking Social Support" (Cronbach's α = 0.746), "Cautious Actions" (Cronbach's α = 0.676), "Instinctive Actions" (Cronbach's α = 0.691), "Avoidance" (Cronbach's α = 0.669), "Indirect Actions" (Cronbach's α = 0.654), "Antisocial Actions" (Cronbach's α = 0.687), and "Aggressive Actions" (Cronbach's α = 0.724). The results were analyzed by comparing the data on the degree of expression (low, medium, high) of a specific person's coping behavior for each subscale with the average values of the coping stress models.

The MSCRPTSD [18] was used to diagnose PTSD in military personnel on missions in the war zone, adapted into Ukrainian [17, 19]. The scale consists of 35 statements (4 subsets), the answers to which were given on a 5-point Likert scale (Cronbach's α = 0.88). Subset 1 (11 statements) describes the symptoms of the "intrusion" group when the traumatic event is constantly repeated in the experience in one (or more) ways. Subset 2 (11 statements) relates to the symptoms of the "avoidance" group when there is a constant avoidance of stimuli associated with trauma, blocking of emotional reactions, and numbness, which was not observed before the trauma. Subset 3 (8 statements) describes the symptoms of “excitability” when persistent symptoms of arousal increase that were not observed before the injury. Subset 4 (5 statements) describes symptoms associated with guilt and suicidal tendencies. Despite the grouping of statements into 4 subsets, one general indicator was calculated taking into account the conversion of the answer into a score for direct and inverse statements, reflecting the severity of PTSD symptoms (range from 35 to 175 points, where 35-80 points is a variant of the norm; 81-114 points - separate symptoms of PTSD; 115-175 points - clinical manifestations of PTSD, a psychiatric examination and inpatient examination were recommended).

The DEQ was used to identify signs of a violation of the adaptability of the soldier’s personality: violation of the regulatory function of the emotional-volitional sphere and self-esteem; lack of prospects for continuing life and the ability to overcome life’s difficulties (probability of committing suicidal attempts); loss of moral convictions, the likelihood of committing addictive and delinquent acts; loss of communicative potential (comradely support, reduced ability to accept the help of one’s team) [19]. The DEQ consists of 45 statements included in 5 subscales (Cronbach’s α = 0.848): “Sincerity of answers” (Cronbach’s α = 0.719), “Violation of behavioral regulation” (Cronbach’s α = 0.736), “Probability of committing suicide attempts” (Cronbach’s α = 0.743), “Violation of moral normativity” (Cronbach’s α = 0.702), and “Loss of communicative potential” (Cronbach’s α = 0.709). Each positive response was worth 1 point, and each negative 0 points. The overall DEQ scale was calculated as the sum of scores on 4 scales (values on the “Sincerity of answers” scale were not included). The results of the overall DEQ scale were evaluated as follows: 1-10 points - high adaptation to combat operations, sufficient tolerance to adverse mental and physical stress, including under conditions of severe combat stress; 11-14 points - average adaptation, unstable level of performance, especially in combat conditions; 15 points or more - low adaptation (distress and adjustment disorders) that does not meet the requirements for soldiers in combat conditions.

Information on the implementation of a formal translation or back-translation protocol for SACS, MSCR-PTSD, and DEQ is unavailable. The authors of the article did not pilot test or validate the design of the psychodiagnostic instrument used in this study; we used data from the aforementioned Ukrainian sources. We are not aware of any published or internal references that describe the psychometric properties of the Ukrainian versions of these instruments in detail.

Statistical Analysis

The study was conducted in two stages. At the first stage, the use of correlation analysis allowed us to determine the relationships between the indicators of coping strategies, PTSD, and maladaptation in unmarried (group 1) and married (group 2) participants. At the second stage of the study, the indicators of coping strategies, PTSD, and maladaptation were compared in two participant groups. For the data presented, basic descriptive statistics were used (arithmetical mean M, standard deviation SD). The reliability of differences in the results of the mean values in two interrelated groups was determined using Student’s t-test. For the assessment of the statistical significance of differences, we used the level of significance from $p = 0.05$ to $p = 0.001$. No correction for multiple comparisons was applied.

Exploratory factor analysis was used to determine the structure of coping strategies in each participant’s group. Exploratory factor analysis of matrices was carried out using the extraction method (principal component analysis) and the rotation method (Varimax with Kaiser normalization). We did not perform the Kaiser-Meyer-Olkin (KMO) statistic and Bartlett’s test of sphericity for each group. Correlation analysis allowed us to determine the relationships between the identified factors in married and unmarried participants. The study results were statistically analyzed using SPSS 22.0 (IBM, Armonk, NY, USA).

RESULTS

Tables 1–2 present the results of the correlation relationship between the indicators SACS, MSCRPTSD, and DEQ scales by the group 1 and group 2 participants.

Table 3 presents the results of the coping strategies by the group 1 and group 2 participants.

Tables 4–5 present the exploratory factor analysis results of the coping strategies structure by the group 1 and group 2 participants.

Tables 6–7 present the correlation results between the factor structure of coping strategies and indicators of PTSD and maladaptation by the group 1 and group 2 participants.

Table 1: Correlation relationship indicators between the SACS, MSCRPTSD, and DEQ scales by the group 1 participants

Scale name	SACS								
	Assertive Actions	Social Joining	Seeking Social Support	Cautious Actions	Instinctive Action	Avoidance	Indirect Action	Antisocial Actions	Aggressive Actions
MSCRPTSD									
General indicator of PTSD	-0.10	-0.30**	-0.19	0.01	0.19	0.16	0.17	0.21	0.58**
DEQ									
Sincerity of answers	-0.02	0.06	0.03	0.21	0.06	-0.02	0.23	0.12	0.37**
Disruption of behavioral regulation	-0.13	-0.16	-0.14	0.12	0.27*	0.29**	0.25*	0.13	0.50**
The likelihood of committing suicide attempts	-0.15	-0.15	-0.18	0.09	0.30**	0.18	0.17	0.18	0.51**
Violation of moral norms	-0.08	-0.12	-0.19	0.12	0.18	0.18	0.26*	0.26*	0.36**
Loss of communicative potential	-0.07	-0.19	-0.12	0.15	0.17	0.20	0.30**	0.11	0.43**
General indicator of maladaptation	-0.13	-0.19	-0.18	0.14	0.28*	0.26*	0.28*	0.19	0.53**

*Note. *p = 0.05, **p = 0.01*

Table 2: Correlation relationship indicators between the SACS, MSCRPTSD, and DEQ scales by the group 2 participants

Scale name	SACS								
	Assertive actions	Social joining	Seeking social support	Cautious actions	Instinctive actions	Avoidance	Indirect actions	Antisocial actions	Aggressive actions
MSCRPTSD									
General indicator of PTSD	-0.42**	-0.31**	-0.24*	-0.10	-0.06	0.09	0.03	-0.02	0.42**
DEQ									
Sincerity of answers	-0.21*	-0.13	-0.16	-0.01	-0.20*	0.12	0.21*	0.09	0.14
Disruption of behavioral regulation	-0.40**	-0.27**	-0.29**	-0.10	-0.05	0.11	0.08	0.03	0.35**
The likelihood of committing suicide attempts	-0.34**	-0.30**	-0.34**	-0.06	-0.12	0.07	0.05	-0.02	0.31**
Violation of moral norms	-0.29**	-0.19	-0.28**	-0.05	-0.01	0.18	0.15	0.13	0.26*
Loss of communicative potential	-0.38**	-0.26**	-0.33**	0.02	-0.14	0.03	0.11	0.13	0.35**
General indicator of maladaptation	-0.43**	-0.31**	-0.38**	-0.06	-0.09	0.12	0.11	0.08	0.39**

*Note. *p = 0.05, **p = 0.01*

Table 3: Coping strategies indicators by the group 1 and group 2 participants (points, Student's t-test, Cohen's d)

Coping strategies name	Group 1	Group 1	t	p	d
Assertive actions	19.82±3.01	20.32±3.52	1.00	0.32	0.15
Social joining	21.96±4.86	23.56±4.66	2.17	0.03	0.34
Seeking social support	21.12±5.09	23.00±5.30	2.36	0.02	0.36
Cautious actions	21.34±4.08	21.80±4.19	0.72	0.46	0.12
Instinctive actions	18.47±3.66	18.61±3.47	0.25	0.74	0.05
Avoidance	17.14±3.99	16.89±4.36	0.39	0.73	0.05
Indirect actions	17.01±4.35	17.74±4.33	1.08	0.29	0.16
Antisocial actions	15.01±4.66	14.55±3.73	0.71	0.47	0.11
Aggressive actions	17.49±4.99	16.03±5.18	1.86	0.05	0.29

Table 4: Factor structure of coping strategies by the group 1 participants

Coping strategies name	Factors		
	1 (33.98%)	2 (24.92%)	3 (11.97%)
Assertive actions	0.393	0.047	0.776
Social joining	0.807	-0.058	0.210
Seeking social support	0.904	-0.040	-0.009
Cautious actions	0.755	0.226	0.032
Avoidance	0.481	0.359	-0.602
Indirect actions	0.167	0.862	0.107
Antisocial actions	-0.012	0.865	-0.018
Aggressive actions	-0.016	0.655	-0.281

Table 5: Factor structure of coping strategies by the group 2 participants

Coping strategies name	Factors		
	1 (31.61%)	2 (24.43%)	3 (13.40%)
Assertive actions	0.651	0.169	0.062
Social joining	0.812	-0.126	-0.172
Seeking social support	0.829	-0.238	0.095
Instinctive actions	0.167	0.154	0.815
Avoidance	-0.162	0.151	0.798
Indirect actions	0.013	0.874	0.178
Antisocial actions	-0.121	0.846	0.145

Table 6: The correlation relationship indicators between the factor structure of coping strategies and the scales MSCRPTSD and DEQ by the group 1 participants

Factors name	PTSD and maladaptation indicators					
	General indicator of PTSD	**Disruption of behavioral regulation**	**Likelihood of committing suicide attempts**	**Violation of moral norms**	**Loss of communicative potential**	**General indicator of maladaptation**
Factor 1 "Limited search for socially significant assistance"	-0.18	-0.06	-0.11	-0.09	-0.06	-0.09
Factor 2 "Destructive methods of gaining an advantage over others"	0.38**	0.36**	0.34**	0.37**	0.35**	0.41**
Factor 3 "Self-affirmation as a demonstration of decent behavior"	-0.21	-0.28*	-0.24*	-0.13	-0.17	-0.25*

*Note. *p = 0.05, **p = 0.01*

Table 7: The correlation relationship indicators between the factor structure of coping strategies and the scales MSCRPTSD and DEQ by the group 2 participants

Factors name	PTSD and maladaptation indicators					
	General indicator of PTSD	**Disruption of behavioral regulation**	**Likelihood of committing suicide attempts**	**Violation of moral norms**	**Loss of communicative potential**	**General indicator of maladaptation**
Factor 1 "Socially significant methods of attracting external resources"	-0.37**	-0.34**	-0.35**	-0.26*	-0.36**	-0.41**
Factor 2 "Socially inconsistent methods of attracting external resources"	0.01	0.09	0.07	0.19	0.19	0.16
Factor 3 "Uncertainty in one's resources and careful attitude towards them"	0.10	0.12	0.06	0.14	-0.04	0.08

*Note. *p = 0.05, **p = 0.01*

DISCUSSION

At the first stage of the study, the use of correlation analysis allowed us to determine the relationships between coping strategies and indicators of PTSD, maladaptation in mobilized military personnel with different family statuses. Thus, productive coping "Assertive actions" (p = 0.01), "Social joining" (p = 0.01), and "Seeking social support" (p = 0.01) were associated with lower indicators of PTSD and maladaptation only in married participants (group 2). Such unproductive coping as "Instinctive actions" (p = 0.05), "Avoidance" (p = 0.05), "Indirect actions" (p = 0.05), "Antisocial actions" (p = 0.05) had relationships with the indicators of PTSD and maladaptation only in unmarried participants (group 1). The coping "Aggressive actions" (p = 0.01) was interrelated with the PTSD and maladaptation indicators in both groups of participants. The obtained results allow us to assume that such civilian experience as life in marriage influenced some features of the choice of coping strategies that determine the ability of mobilized servicemen to withstand combat stress factors. Similar studies showed that problem-solving coping reduced PTSD symptoms, while coping strategies aimed at avoiding stressful situations increased them [20]. Our results showed that this relationship is also be influenced by the family status of a serviceman.

As previously noted, social relationships such as intimate partners and military unit support reduced PTSD symptoms and negative emotionality and had a positive impact on military personnel's well-being [14]. In our study, we assumed the possibility of other variants of these relationships: the maladaptation indicators could have been by receiving specific social assistance ("Seeking social support"), and the symptoms of PTSD were could reduced while maintaining or restoring the ability to engage in general activity ("Social joining"). We found that in group 2, the indicators of choosing such coping strategies as "Social joining" and "Seeking social support" were the highest among all coping strategies, and they differed from the indicators of group 1 (p = 0.05 for both values).

In the second stage of the study, exploratory factor analysis and correlation analysis were used to identify the tentative structure of coping strategies among mobilized military personnel with different family statuses, as well as to determine the relationship between coping strategies and indicators of PTSD and maladaptation.

In group 1, the identified tentative three-factor structure of coping strategies described 70.72% of the variance of features. The factor 1 content was determined by the coping strategies "Seeking social support," "Social joining," and "Cautious actions." This factor was associated with the involvement of additional external resources. Perhaps, when asking for assistance, the group 1 participants were afraid of making a mistake, which could become an additional source of stress. This factor was designated as "Limited search for socially significant assistance." The factor 2 content was determined by the coping strategies "Antisocial actions", "Indirect actions", and "Aggressive actions". These coping strategies were associated with the abuse of actions to create one's superiority over others, to create conditions for using the resources of colleagues for one's purposes. This factor was designated as "Destructive methods of gaining an advantage over others". The positive pole of factor 3 was determined by the coping strategies "Assertive actions", and the negative pole "Avoidance". These coping strategies were associated with one's confidence and readiness to take responsibility. However, unlike assertive actions in group 2, where they may have been part of the interaction to overcome the consequences of stress factors. In group 1 assertive actions were possibly a component of self-affirmation. This factor was designated "Self-affirmation as a demonstration of decent behavior".

It should be noted that the group 1 participants showed a tendency where destructive ways of gaining an advantage over others rather increased mental trauma than contributed to protection from the effects of stress factors. In this group, the factor "Self-affirmation as a demonstration of decent behavior" to a certain extent contributed to the reduction of "Disruption of behavioral regulation" (p = 0.05) and "Likelihood of committing suicide attempts" (p = 0.05). Indeed, the self-confidence and assertiveness that characterized the participants in this group may have been characteristics that allowed them to control themselves in a stressful situation.

In group 2, the identified tentative three-factor structure of coping strategies described 69.43% of the variance in features. The content of factor 1 included the coping strategy "Seeking social support," "Social joining," and "Assertive actions." These strategies emphasized the importance of seeking external resources while considering the interests of fellow soldiers to mitigate the effects of combat stress factors. We referred to this factor as "Socially significant methods of attracting external resources." The content of factor 2 was determined by the coping strategy "Indirect actions" and "Antisocial actions". This factor was designated as "Socially inconsistent methods of attracting external resources" and had a statistically significant negative relationship with the consequences of the stress experienced: PTSD indicators (p = 0.01) and all maladaptation indicators (p = 0.01). Factor 3 was associated with the coping strategy "Instinctive actions" and "Avoidance". This factor was designated as "Uncertainty in one's resources and careful attitude towards them". The main goal of the described factor structure of coping strategies in group 2 was associated with to attract additional resources and avoid unfavorable circumstances.

In studies that examined the factor structure of coping strategies, three main factors have often been identified: problem-focused coping, getting social support, and avoidance [21,22]. However, we found that all mobilized servicemen had a deficit structure of coping strategies. Moreover, married servicemen had a desire to obtain additional external resources by any means to overcome the effects of combat stress factors, as well as a careful attitude towards their resources. It can be assumed that married servicemen were more oriented towards interaction to overcome stressful situations, while unmarried servicemen were forced to rely more on their strength.

It is a common assertion that the effectiveness of stress attenuation is limited because it is often not possible to avoid stressors, and avoiding or minimizing stress can lead individuals to miss opportunities for performance and growth. Thus, during stressful situations, a more efficacious approach is to optimize stress responses (i.e., promote adaptive, approach-motivated responses) [23]. Another study concluded that an avoidant-focused coping style acts as a vulnerability factor for PTSD symptoms, whereas hardiness acts as a resilience factor against symptom development [24]. Thus, when interpreting coping strategies such as "Cautious actions" and "Instinctive actions", it is necessary to take into account their role in the overall structure of coping and how personal resources for coping relate to the strength of stressful impacts. Our results partially confirm the conclusions that: (1) a robust latent variable named personal resources (indicated by social support, unit cohesion, and trait resilience) was negatively associated with PTSD severity; (2) personal resources were negatively associated with negative posttraumatic cognitions; (3) negative posttraumatic cognitions fully mediated the association between personal resources and PTSD severity [25].

Study limitations

This study certainly had limitations. 1) The study served as a generalization of experiences from the rehabilitation center focused on the psychological recovery of military personnel following participation in hostilities. This significantly influenced the amount of available empirical data (including sample size and psychodiagnostic survey methods), the study's structure, and the mathematical processing methods used. 2) The research was exploratory, aimed at generalizing the outcomes of surveys of military personnel involved in the psychological recovery program, as well as identifying potential hidden resources and methods for preventing PTSD and maladjustment. 3) Information on the implementation of a formal translation or back-translation protocol for SACS, MSCR-PTSD, and DEQ is unavailable. The authors of the article did not pilot test or validate the design of the psychodiagnostic instrument used in this study; we used data from the Ukrainian sources. We are not aware of any published or internal references that describe the psychometric properties of the Ukrainian versions of these instruments in detail. 4) The risk of false positive results was increased by the fact that no correction for multiple comparisons was applied. 5) Data on resources for building resilience against combat stress factors was limited among mobilized military personnel who did not choose military careers and received very brief training. 6) Existing data on family support for servicemen relates to scenarios where families are safe, a situation not observed in Ukraine, creating an ambivalent role for the family that can serve as both a source of anxiety and a resource. 7) The research was conducted on a sample of servicemen who participated in the psychological recovery program, specifically those exhibiting signs of mental and physical exhaustion, PTSD symptoms, and maladjustment. Consequently, the results related to the characteristics of coping structure deficits in both established variants—typical for married and single mobilized servicemen—should be specifically attributed to the corresponding samples. As a result, it is inappropriate to extend the findings of this study to servicemen who cope more effectively with the impact of combat stress. 8) The results obtained possess low practical value (Cohen's d); however, they allow us to formulate valuable hypotheses for further research. For example, PTSD prevention may be linked not only to direct family support but also to shared life experiences such as joint problem solving, which remain relevant even in the absence of direct family support, highlighting its value. Additionally, an intriguing hypothesis is the assessment of individual coping: its position within the overall coping strategy framework (which may exhibit signs of collective or individual culture) and the general nature of this structure concerning the situation (for instance, a deficiency in the overall structure indicating a reliance on external assistance). 9) Female military personnel were excluded from this study because less than 0.5% of female combatants participated throughout the duration of the psychological recovery program. 10) The participant sample consisted solely of enlisted military personnel and sergeants; officers did not take part in this study. 11) This study faced limitations due to the brief duration of the psychological recovery program and the need to avoid overloading participants with additional activities that did not align with the program's objectives, which limited the application of research methods and the repeatability of the survey, among other factors. Finally, the current study was constrained by the absence of an active comparison condition and a longitudinal follow-up. Thus, by summarizing some of the outcomes from the rehabilitation center's work on the psychological recovery of military personnel following military operations, we aimed to attract the scientific community's attention to addressing new urgent challenges in military medicine and psychology, particularly in the unique area of psychological support for mobilized military personnel.

CONCLUSION

The pre-war experience of using coping strategies by married and unmarried mobilized military personnel was a rather weak preventive measure for counteracting combat stressors, and not a sufficient barrier to the emergence of PTSD symptoms and maladaptation manifestations Assertive actions could be associated as a preventive factor for the negative consequences of combat stress only in married military personnel. Perhaps this was due to their structure of coping strategies, in which assertive actions were an integral part of interaction, while in unmarried servicemen it was self-affirmation. The creation of a wide range of social relationships had a positive impact on the preventive power of social interaction, which helped to overcome the consequences of PTSD and social maladaptation manifestations for both groups of mobilized military personnel. Aggressive actions, which became a source of additional stress and a method of responding to stress, were linked to higher PTSD scores.

Psychological support of the family among married mobilized servicemen was associated with the formation of resilience to the action of combat stress factors. In addition, compatible actions of married servicemen in stressful situations due to feedback formed an adequate stress response, reduced the likelihood of meta-experiences. Unmarried mobilized servicemen did not have family experience, so their coping strategies mainly came down to reacting and individual resilience to combat stress factors.

Married and unmarried mobilized servicemen had a deficit structure of coping strategies characteristic of war. They realized that their resilience to the effects of combat stress factors depended on the possibility of receiving help from others. And if married mobilized servicemen were more inclined to exercise caution when spending their resources, then unmarried servicemen tried to use self-assertive behavior as a method to raise their authority and thus attract additional external resources.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, Y.M., and N.Ku.; methodology, N.Kr.; software, V.F., and V.K.; validation, Y.M., O.H., and N.Ku.; formal analysis, Y.M.; investigation, N.Kr.; resources, V.F., and O.H.; data curation, Y.M., and N.Ku.; writing—original draft preparation, Y.M.; writing—review and editing, N.Ku., and Y.M.; visualization, V.K., and O.H.; supervision, N.Kr., and V.K.; project administration, N.Ku.; funding acquisition, V.F. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the National Academy of the National Guard of Ukraine, Kharkiv, Ukraine (protocol code 2024/12 and date of approval 24/09/2024).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

New Study of the antimicrobial activity of natural extracts of Taraxacum officinale and Chelidonium majus

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Abstract: The rise of antimicrobial resistance poses a significant challenge to global health. This study investigates the antimicrobial properties of Taraxacum officinale and Chelidonium majus. Taraxacum officinale, commonly known as dandelion, has been utilized for centuries in traditional medicine for its various therapeutic benefits, including treating cholecystitis, enhancing hepatic function, regulating blood pressure and cholesterol levels, and exhibiting diuretic effects. Additionally, research has indicated that infusions derived from this plant offer favorable outcomes in managing renal diseases. This study aims to assess the antimicrobial activity of extracts from Taraxacum officinale and Chelidonium majus employing qualitative screening, quantitative determination, and in vitro evaluation of anti-biofilm properties. In vitro evaluation of the antibiofilm activity of plant products revealed their ability to inhibit the development of biofilms on inert substrate; plant extracts of C. majus were found to inhibit biofilm formation in all bacterial strains tested, while tincture of T. officinale showed very good activity on gram-negative and fungal strains. The results obtained are promising, supporting further research into the biochemical characteristics of these extracts in order to formulate strategies for the control of bacterial and fungal infections in humans.

Keywords: extracts, antimicrobial effect, antibiofilm, Taraxacum officinale, Chelidonium majus

INTRODUCTION

The modern era of antimicrobial chemotherapeutics began with the discovery of sulfonamides and penicillin, and in the following decades the spectrum of antimicrobial substances was broadened by the discovery of new active substances capable of preventing and/or treating infections caused by pathogenic microorganisms. Thus, the introduction of antibiotics was a crucial moment that underpinned the saving of millions of patients, while at the same time leading to significant advances in medicine [1].

However, less than a decade after the clinical use of antibiotics, as a result of inappropriate prescribing or overuse of antibiotics both in humans and in veterinary and zootechnical medicine, without certainty of the infectious agent determined by laboratory tests or to promote animal husbandry [2], the gains from the development of this treatment are threatened by increasing rates of pathogen resistance to antibiotics, now leading to the Antimicrobial Resistance Crisis (AMR) [3].

The global increase in the incidence of infectious diseases that can no longer be eradicated with the substances developed to date represents the evolving response of microorganisms to the widespread administration of antibiotics. It has been declared by multiple organizations, such as the World Health Organization (WHO),

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to be a public health problem of concern. Therefore, the AMR crisis has led to the need to investigate and discover new potential antimicrobial agents [4,5,6].

A new research direction is to exploit the antimicrobial properties of large varieties of medicinal plants, which represent a natural reservoir providing many compounds useful in the fight against multidrug-resistant microorganisms [7,8].

Taraxacum officinale (dandelion), is a medicinal plant that has been used for centuries for its benefits in the treatment of conditions such as cholecystitis, improving hepatic function, lowering blood pressure and blood cholesterol levels, or for its diuretic effect. In addition, studies in the literature have shown that infusions made from this plant have beneficial effects on renal diseases [8].

Chelidonium majus (celandine), one of the oldest and most widely used plants in traditional medicine, presents manyfold benefits, being often used in the treatment of eczema and condyloma caused by Human Papilloma Virus (HPV) infection, hepatic and gastric diseases, as well as having anti-inflammatory and analgesic effects. [9,10,11,12,13,14,15].

The aim of this work is to study the antimicrobial activity of extracts of Taraxacum officinale (tincture), Chelidonium majus (tincture). The following objectives were proposed to achieve this aim: qualitative screening of the antimicrobial activity of plant extracts, quantitative determination of the antimicrobial activity of plant extracts and in vitro investigation of the anti-biofilm properties of plant extracts. [16,17,18].

MATERIALS AND METHODS

Two plant extracts were analyzed in this study: Taraxacum officinale (dandelion) tincture and Chelidonium majus (tumbleweed) tincture.

Reference microorganisms were tested, two gram-negative bacterial strains: Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853, two gram-positive strains: Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212 and one fungal strain: Candida albicans ATCC 10231, according the American Type Culture Collection.

Qualitative evaluation of plant extracts was carried out by the drop method on solid agar medium by adapting the diffusimetric antibiogram method. Technique is: a 5µl volume of each plant extract was dropped onto the surface of a Plate Count Agar (PCA), non seleciv medium, inoculated with a standardized microbial suspension obtained from the strain to be tested by the cloth seeding technique. Sensitivity to a particular plant extract can be observed by inhibition of microbial growth around the deposited droplet. Interpretation of results: antimicrobial activity of the extracts was determined by assessing the formation of an inhibition zone around the deposited extract droplet and measuring its diameter using a millimeter ruler.

Quantitative determination of the antimicrobial activity of plant extracts was carried out by the microdilution method in liquid medium. Principle of the method: in 96-well ELISA microplates with liquid culture medium, 1/100 diluted microbial suspensions of the test strains are inoculated with an initial density of 0.5 McFarland in a gradient of plant extract concentrations. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values are determined by drop seeding on agar medium the first 8 wells corresponding to 50%- 0.195% concentrations of each plant extract.

Interpretation of results: antimicrobial activity of the extracts was determined by evaluating the growth of microbial cultures on agar medium. The MIC value represents the concentration at which the plant extract inhibits the growth and multiplication of microorganisms, having a bacteriostatic effect, and the MBC value represents the concentration at which the plant extract has a lethal action on microorganisms, having a bactericidal effect.

Evaluation of the antibiofilm activity of plant extracts was tested by the 1% crystal violet microtitration method: following incubation of sterile 96-well ELISA microplates with liquid culture medium in which microbial suspensions are inoculated in a gradient of concentrations of the tested plant extracts, the in vitro antibiofilm activity of different concentrations of tinctures and alcoholic extracts is analyzed on the basis of absorbance values of the 1% crystal violet dye at 492 nm. Interpretation of the results: the antibiofilm activity of the plant extracts could be evaluated by determining the absorbance value from the spectrophotometric reading at 492 nm. The concentration of the plant extracts required to inhibit biofilm growth corresponds to the last well at which the absorbance value is less than half the absorbance value of the positive control corresponding to the same plant extract.

RESULTS

In this study we aimed to investigate the antimicrobial properties of some plant extracts of Taraxacum officinale (tincture) and Chelidonium majus (tincture). Qualitative evaluation of the antimicrobial activity of T. officinale plant extract determined an inhibition of microbial growth against E. faecalis ATCC 29212, P. aeruginosa ATCC 27853 and C. albicans ATCC 10231 strains, showing inhibition halo values of 9 mm, 8 mm with weak microbial growth within the microbial inhibition zone and 8 mm with the presence of a large number of colonies within the inhibition halo, respectively. Resistance to the action of the plant extract was recorded for strains of S. aureus ATCC 25923 and E. coli ATCC 25922, with the presence of T. officinale tincture not inhibiting their microbial growth.

Microbial growth of the fungal strain was inhibited in the presence of the plant extract from C. majus, resulting in an inhibition zone value of 7 mm. The same growth inhibition zone diameter was also recorded for the E.coli strain ATCC 25922, and a value of 11 mm was observed for the gram-positive strain E. faecalis ATCC 29212 (Table 1). This extract showed no antimicrobial activity against S. aureus ATCC 25923 and P. aeruginosa ATCC 27853 strains.

The qualitative determination study of the antimicrobial activity of S. marianum tincture on reference strains reveals that on both gram-negative strains, E. coli ATCC 25922 and P. aeruginosa ATCC 27853, and fungal strain, C. albicans ATCC 10231, the plant extract showed no antimicrobial activity (Table I). Inhibition of microbial growth could however be observed for the gram-positive strains S. aureus ATCC 25923 and E. faecalis ATCC 29212, with growth inhibition zone values of 9 mm and 8 mm respectively.

Table 1. - Quality determination of antimicrobial activity.

Strain tested	**Growth inhibition zone diameter (mm) and CMI**					
	***T. officinale* (tincture)**	**CMI**	**Interpretation**	***C. majus* (tincture)**	**CMI**	**Interpretation**
S. aureus ATCC 25923	0	N/A	resistant	0	N/A	resistant
E. faecalis ATCC 29212	9[1]	52µg/ µL	sensitive	1[1]	4298 µg/ µL	intermediate
E. coli ATCC 25922	0	N/A	resistant	7[1]	87 µg/ µL	sensitive
P. aeruginosa ATCC 27853	8[1]	66µg/ µL	sensitive	0	N/A	resistant
C. albicans ATCC 10231	8[2]	66µg/ µL	sensitive	7[1]	87 µg/ µL	sensitive

Legend: [1]weak/slight microbial growth within the inhibition zone. [2]presence of a large number of colonies within the inhibition zone.

Formula: $CMI=Q/V$ $Q=10000\mu L$ $V=\pi R^2 G$ (1)

The method of quantitative determination of the antimicrobial activity allowed to highlight the concentrations of the plant extracts at which the growth and development of the tested reference strains are inhibited, as well as the concentrations determining the bactericidal action. (Fig. 1)

Figure_1 - Results of antimicrobial activity testing: Agarized, drop-seeded medium from 96-well microplates with strains of *S. aureus, E. faecalis, E. coli, P. aeruginosa* and *C. albicans* after incubation for 18-24h at 37ºC

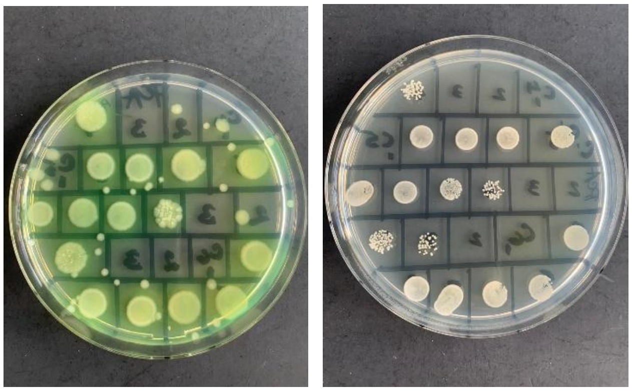

The plant extract of T. officinale shows a strong inhibitory effect on gram-negative P. aeruginosa ATCC 27853 and fungal strains, the bacteriostatic effect being achieved at a concentration of 3.13%, while on gram-positive strains and E. coli ATCC 25922, the concentration of the plant extract required to inhibit microbial growth is 12.5-25%. On gram-negative strains and E. faecalis strain ATCC 29212, the 12.5% concentration of C. majus extract has a bacteriostatic effect, thus a medium inhibitory effect was recorded, but a weak and a strong inhibitory effect of this extract was observed on C. albicans ATCC 10231 and S. aureus ATCC 25923 strains, with MIC values of 50% and 3.13% respectively.

The tinctures tested in the study showed lethal action on the reference microbial strains at an average concentration of 20.8%. For the plant extract of T. officinale, a concentration of 12.5% was required for bactericidal effect on S. aureus ATCC 25923, E. coli ATCC 25922 and C. albicans ATCC 10231 strains, and for E. faecalis ATCC 29212 and P. aeruginosa ATCC 27853, concentrations of 50% and 6.25%, respectively, were required. In the presence of C. majus tincture, the lowest CMB value recorded was 6.25% for S. aureus strain ATCC 25923. Bactericidal action on E. faecalis ATCC 29212 and P. aeruginosa ATCC 27853 strains was observed at a concentration of 12.5% of the plant extract, while concentrations of 25% and 50% were required for the gram-positive E. coli ATCC 25922 and fungal strains, respectively.

Analysis of spectrophotometric data revealed that T. officinale tincture shows little effect on Gram-positive strains, inhibiting biofilm growth at a concentration of 25%. The effect of this tincture was higher on gram-negative and fungal strains, with inhibition of biofilm growth observed at concentrations of 0.78%, 1.56% and 3.13% for C. albicans ATCC 10231, E. coli ATCC 25922 and P. aeruginosa ATCC 27853, respectively. The minimum concentration of C. majus tincture required to inhibit biofilm growth of C. albicans strain ATCC 10231 is 1.56%, while for E. faecalis ATCC 29212 and E. coli ATCC 25922 antibiofilm activity was achieved at a concentration of 12.5%, and for S. aureus ATCC 25923 and P. aeruginosa ATCC 27853 at 6.25%.

The chromatograms obtained in this study provide detailed information about the phytochemical composition of the extracts from Taraxacum officinale and Chelidonium majus. These analyses are essential for identifying the active compounds involved in the observed antimicrobial activity and for correlating them with the experimental results.

The attached figures present representative chromatograms for each extract, highlighting the main absorption peaks and specific compounds. This information contributes to a better understanding of the mechanisms through which the substances in the studied plants act against various microbial strains. The presence of active compounds is clearly demonstrated, supporting the qualitative and quantitative results obtained in the antimicrobial activity tests.

The chromatogram represents the analysis of hydroalcoholic extract of Taraxacum officinale, (Fig. 2) highlighting major phytochemical compounds such as luteolin glucoside (the main peak, indicating the dominant compound), caffeoyl derivatives, and luteolin. Additionally, chlorogenic acid and quercetin are present, known for their antioxidant and anti-inflammatory activities. This composition suggests a high therapeutic potential, supporting the antimicrobial activity observed in the study.

Figure_2 Chromatogram of hydroalcoholic extract of *Taraxacum officinalis*

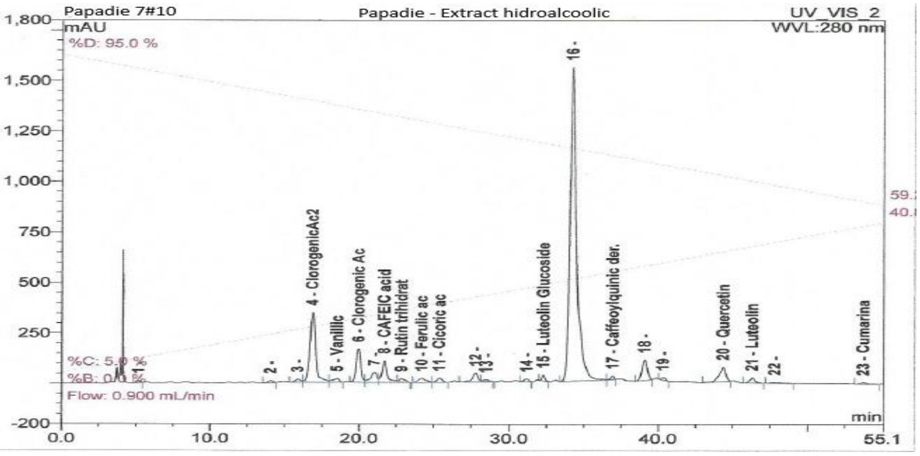

The chromatogram analyzes a hydroalcoholic extract of Chelidonium majus (Fig. 3), highlighting chelidonine as the main compound (retention time 6.667 minutes), suggesting a high concentration. Chelidonine is an alkaloid known for its antimicrobial and antitumor properties. The smaller peaks indicate the presence of other alkaloids, such as sanguinarine or berberine.

Figure 3_ Chromatogram of hydroalcoholic extract of *Chelidonium majus*

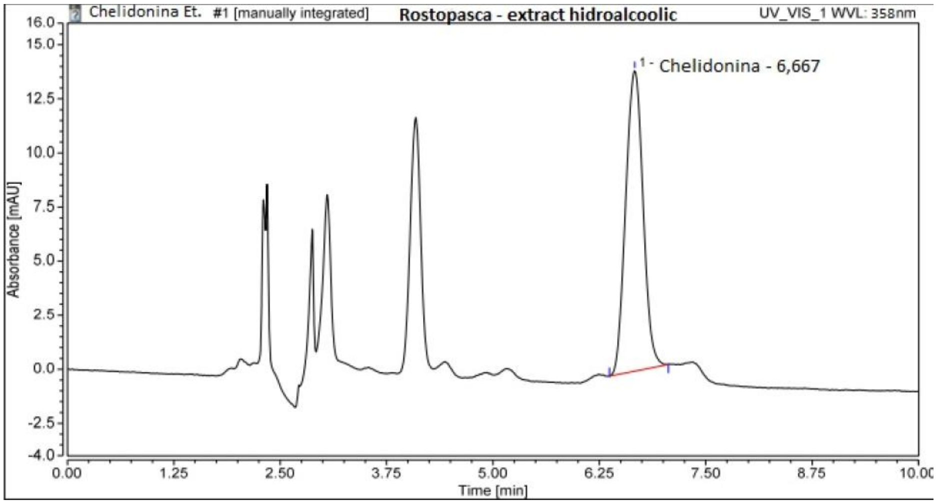

DISCUSSION

The present study investigated the antimicrobial properties of plant extracts tinctures from Taraxacum officinale and Chelidonium majus. The qualitative and quantitative evaluations of antimicrobial activity revealed differential effects of these plant extracts on different strains, shedding light on their potential therapeutic applications.

Taraxacum officinale L. is a flowering herbaceous perennial plant of the Asteraceae family. Interest in researching the biochemical characteristics as well as the pharmacological properties of this species increased considerably in the 1950s, and numerous active substances have now been identified, including flavonoid compounds, phenolic acids such as cyclonic, chlorogenic and caffeic acid, alkaloids, and terpenes. In modern medicine, this plant is marketed and used as an herbal treatment to relieve symptoms of conditions such as arthritis, hepatic disorders and to suppress oxidative stress [17,18, 19,20,21,22,23]. Dandelion exhibits a diverse array of pharmacological effects, making it a valuable medicinal plant with potential therapeutic applications. The study by Trinh et al. 2020, reported that ethanol-based plant extract from T. officinale exhibits bactericidal effect on S. aureus strain ATCC 25923 at a concentration of 20 mg/ml [24]. T. officinale extract tested in the study by Sangeetha and Ezhilarasan 2016, showed lethal action on E. faecalis strain ATCC 29212 at a concentration of 200mg/mL [25]. The study by Shah et al. (2011) [26] showed that S. marianum extracts did not exhibit antimicrobial activity against the tested gram-negative strains E. coli ATCC 25922 and P. aeruginosa ATCC 27853.

Chelidonium majus is a medicinal plant belonging to the Papaveraceae family that is found in the wild flora of Europe, Asia, North America, as well as in some regions of Africa. It is widely used in traditional herbal medicine to treat conditions including biliary and hepatic dysfunction, jaundice and to relieve pain caused by edema. It has antidiuretic, spasmolytic, sedative, and antimicrobial properties. The dried aerial part of C. majus is an official medicine in the pharmacology of European countries, but it is necessary to administer it under the supervision of authorized medical personnel due to the presence of a rich concentration of isoquinoline alkaloids. [27,28,29,30,31]

The antimicrobial activity of the studied plant extracts varied significantly depending on the microbial strain and the type of extract used. For instance, T. officinale exhibited inhibition against E. faecalis and P. aeruginosa strains but not against S. aureus and E. coli. Similarly, C. majus showed inhibition against E. faecalis and C. albicans but not against S. aureus and P. aeruginosa. These findings suggest that the efficacy of plant extracts may be strain-specific, highlighting the importance of considering multiple strains in antimicrobial studies. [32,33,34,35]

The results of this study are consistent with some previous findings but differ from others. For example, the antimicrobial activity of T. officinale against S. aureus strain ATCC 25923 reported by Trinh et al. (2020) aligns with our findings, whereas Tunç et al. (2019) reported no antimicrobial activity against E. faecalis strain ATCC 29212, contradicting our observations. Such discrepancies may arise from variations in extraction methods, microbial strains, or growth conditions, emphasizing the need for standardized protocols and comprehensive strain selection in antimicrobial research.

The determination of MIC and bactericidal concentration provided insight into the potency of the plant extracts against different microbial strains. Higher MIC values were often observed for gram-negative bacteria compared to gram-positive bacteria and fungi. This differential susceptibility underscores the complex interplay between microbial cell wall structures and the mechanisms of action of plant compounds.

The ability of plant extracts to inhibit biofilm formation is of particular interest due to the role of biofilms in antimicrobial resistance. Our study demonstrated varying degrees of biofilm inhibition by the tested extracts, with some showing promising activity against both gram-positive and gram-negative bacteria as well as fungi. These findings suggest the potential of plant extracts as adjuncts in combating biofilm-associated infections.

Despite the valuable insights provided by this study, several limitations should be acknowledged. The use of reference strains may not fully represent the diversity of clinical isolates, warranting further investigations with clinically relevant strains. Moreover, elucidating the underlying mechanisms of antimicrobial activity and conducting in vivo studies are crucial steps toward clinical translation.

The chromatographic analysis of the hydroalcoholic extracts of Taraxacum officinale and Chelidonium majus revealed the presence of major bioactive compounds, each contributing to the specific therapeutic potential of the plant. The chromatogram of Taraxacum officinale indicated Luteolin glucoside as the main compound, known for its antioxidant and anti-inflammatory activities, alongside caffeoyl derivatives. For Chelidonium majus, chelidonine (retention time 6.667 minutes) was identified as the major compound, recognized for its antimicrobial and antitumor properties.

These results emphasize that each extract possesses a distinct phytochemical composition that may synergistically contribute to the observed antimicrobial activity. The identification of specific compounds through chromatographic analysis supports the understanding of the mechanisms of action of the extracts and justifies further investigations into their use in antimicrobial, antioxidant, and hepatoprotective treatments. This study underscores the diverse antimicrobial potential of plant extracts against a range of microbial pathogens. Further research focusing on standardization, mechanism of action, and clinical efficacy is warranted to harness the therapeutic benefits of these natural products effectively.

CONCLUSION

In this experimental work, the antimicrobial properties of two plant extracts of Taraxacum officinale (tincture) and Chelidonium majus (tincture) were studied on 5 reference bacterial strains, two gram-positive (Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212), two gram-negative (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) and one fungal strain, Candida albicans ATCC 10231.

Qualitative testing of antimicrobial activity revealed that the plant extracts inhibited the growth and development of the tested microbial strains.

Quantitative determinations of antimicrobial activity allowed the determination of the concentrations of plant extracts responsible for the bacteriostatic and bactericidal effect, and a higher sensitivity of microbial strains could be observed in the presence of tinctures.

In vitro evaluation of the antibiofilm activity of plant products revealed their ability to inhibit the development of biofilms on inert substrate; plant extracts of C. majus were found to inhibit biofilm formation in all bacterial strains tested, while tincture of T. officinale showed very good activity on gram-negative and fungal strains.

The results obtained are promising, supporting further research into the biochemical characteristics of these extracts in order to formulate strategies for the control of bacterial and fungal infections in humans.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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Authors' contribution

Conceptualization, R.C.S., and V.O.; methodology, C.T and D.C.; software, M.C.R.; validation, M.M., V.O and D.C.; formal analysis, R.C.S. and M.C.R.; investigation, M.C.R..; resources, V.O.; data curation, D.C.; writing—original draft preparation, D.C and F.D.U.; writing—review and editing, M.C.R. and C.E.M; visualization, C.E.M.; supervision, V.O.; project administration M.M. .All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ARTICLE

Five-year Analysis of Head and Neck Non-Melanoma Skin Cancer Incidence, Demographics, and Site Distribution in a Tertiary Care Center

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Abstract: Introduction: Non-melanoma skin cancers (NMSC) represent a significant global public health burden, particularly in high-income countries. The increasing incidence of head and neck NMSC contributes to rising morbidity and necessitates multi-disciplinary management. Objectives: This study aims to evaluate the incidence, demographic distribution, histological subtypes, and seasonal variation of NMSC over a five-year period at a tertiary center in Romania. Materials and Methods: A retrospective observational study was conducted from January 2020 to December 2024, including 180 patients diagnosed and treated for malignant skin lesions. Data were extracted from clinical and histopathological records. Chi-square tests assessed seasonal distribution, and Mann-Whitney U tests compared lesion depth and surface area between demographic groups. Results: A total of 388 malignant lesions were recorded. Basal cell carcinoma (BCC) was the most common subtype (n = 168), followed by squamous cell carcinoma (SCC) (n = 25). Most patients resided in urban areas, particularly Bucharest. Males presented with significantly deeper lesions (mean: 1.5 mm) compared to females (mean: 1.25 mm; $p = 0.009$). Seasonal analysis revealed a significant variation in incidence, with the lowest number of cases in spring ($\chi^2 = 10.31$, $p = 0.016$). Conclusion: The incidence of malignant skin lesions increased from 2020 to 2023, followed by a decline in 2024. Seasonal and sex-related differences were observed, with the face being the most frequent site of involvement. While the sex distribution was relatively balanced, males exhibited significantly greater lesion depths compared to females.

Keywords: non-melanoma skin cancers; squamous cell carcinoma; basal cell carcinoma; seasonal variation; epidemiology; retrospective study; head and neck cancer; Romania

INTRODUCTION

Cutaneous malignancies accounts for most cancers across the world and they are primarily divided into melanoma (MSC) and non-melanoma (NMSC) skin cancers [1]. According to the Globocan (Global Cancer Observatory) 2022 data, non-melanoma skin cancer ranks as the fifth most commonly diagnosed cancer worldwide, with over 1.2 million new cases reported [2]. However, despite its high incidence, NMSC ranks only 22nd in terms of cancer-related mortality, highlighting the generally favorable prognosis of NMSC compared to other malignancies [2].

The incidence of head and neck MSC and NMSC is rising all over the world and can be the source of significant morbidity and mortality [3]. The necessity of a multidisciplinary team that includes dermatology, plastic and reconstructive surgery, medical and radiation oncology remains a financial problem in many countries, due to the comprehensive care that needs to be provided for patients with head and

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neck cutaneous malignancies [3,4].

The vast majority of non-melanoma skin cancers include basal cell carcinoma (BCC), followed by squamous cell carcinoma (SCC), both showing a rapidly increasing trend in their incidence in the last years and a significant health burden in many countries [5,6]. Although the exact global incidence of these two is unknown, an overview of the last years carried out by World Health Organization concluded that BCC comprises about 80% of all NMSC, while SCC comprise about 20%, with a BCC to SCC ratio ranging from 2 to 4:1 [5,6]. While this is a classic agreed ratio, the recent studies suggest a possible changing in the pattern due to aging of the population, with equal numbers of SCC and BCC being treated in the future [6,7].

Multiple etiological risk factors related to the individual or the environment were studied for understanding the cause of NMSC, but the main driving carcinogen remains ultraviolet radiation (UVR) [5,6]. This explains why over 80% of these malignancies occur on the sun exposed skin of the head and neck (followed by the hands and forearms), with higher prevalence of BCCs in the nasal area, while SCCs are preferentially located on the auricle [5,8,9]. UVR are responsible for the induced dinucleotide mutations at pyrimidine bases, exhibiting strong transcription strand bias and also showing a high mutational burden with the mutant genes varying between BCCs and SCCs [6].

Although its mortality is low and it rarely results in metastatic disease, BCCs can cause significant health burden through his great morbidity, generated by the destructive local spread [9]. These tumors are slow growing but can, however, develop large, causing locally tissular destruction and spreading into vital structures such as the central nervous system or important blood vessels [10]. Traditionally, the five most commonly accepted histological subtypes of BCC, described by Lang and Maize, include superficial, nodular, micronodular, infiltrative and morpheaform or sclerosing BCC [10,11]. Of note, a particular form of BCC, called the basosquamous cell carcinoma (BSCC) or the metatypical BCC, may arguably be a SCC subtype, as it shows both basal cell and squamous cell cancer differentiation in a continuous fashion [10,11]. The metatypical BCC, together with morpheiform and infiltrative BCC belong to the aggressive-growth subsets, while the others are considered indolent-growth variants [11]. Regarding their epidemiology, in one large retrospective study on 1039 consecutive cases of BCC, the histological analysis demonstrated 21% were nodular, 17% superficial, 15% micronodular, 7% infiltrative and just 1% morpheic [12].

In contrast with basal cell carcinoma, the squamous cell carcinoma has metastatic potential, and when present, the general prognosis is rather poor due to the mortality associated with regional and nodal metastases [9]. It is the most common skin cancer after BCC and generally has a good prognosis, the overall metastasis rate varying from 1% to 4% [13]. There are several risk factors attributed with the regional metastatic potential, like a poor differentiation, a perineural invasion, the presence of recurrency, a depth of invasion greater than 6 mm or a size bigger than 2 cm [14]. The SCC typically develop in a progressive manner, beginning with premalignant lesion -actinic keratosis (AK)-to in situ SCC (Bowen's disease) and eventually to invasive squamous cell carcinoma [13]. Usually, only a small proportion of the precursors develop into invasive cutaneous SCC [13,15]. Despite that, the high mortality of the metastatic variant and the high frequency of SCC in general remain of great concern and represent a financial burden for the health care services, as it needs a close clinical surveillance for many years [15].

Unlike the BCC variants, where the biological behaviors show little significant difference, there are a wide histopathological subtype of SCCs which demonstrates not just microscopic differences, but also a very diverse clinical behaviors [16]. There are several histologic subtypes of SCC and each of them carry an important prognostic significance [16,17]. The generic type (the simplex or conventional) has the greatest risk of metastasis, followed by lobular (acantholytic), Bowenoid, spindle (pleomorphic)-all three with a similar risk- and the last, verrucous carcinomas, has the most favorable prognosis [16,17]. Another predicting cancer behavior in cutaneous squamous cell carcinoma are the histopathologic parameters, which stage tumors into four categories primary based on their nuclear pleomorphism [17]. According to Broder, the tumors can be well-differentiated (less than 25% undifferentiated cells), moderately- well differentiated (less than 50% undifferentiated), poor differentiated (less than 75% undifferentiated) and anaplastic or pleomorphic (more than 75% undifferentiated) [17].

Nevertheless, the spectrum of head and neck cutaneous malignancies ranges from fairly well-behaved tumors (most of the BCCs) to potentially very aggressive cancers with high metastatic rate (usually melanoma, but also some histologic types of SCC) [3]. Their increasing incidence, the necessity of a multidisciplinary team and the need of a well-developed early screening evenly distributed among countries and regions, make the topic current and highly important in the present context.

The aim of this paper is to present the incidence of non-melanoma skin cancers of the head and neck in a single tertiary center in a retrospective study, from 2020 to 2024. Also, we'll describe the correlation between malignancies and gender, age, type of cancer, and its dimensions, together with trends in their frequency. Understanding temporal trends and potential fluctuations in incidence is essential for effective healthcare planning and resource allocation. Seasonal patterns in skin cancer diagnoses suggest that environmental factors, such as ultraviolet exposure, may influence disease occurrence and warrant further investigation. Additionally, variations in lesion characteristics between different demographic groups, including sex and geographic location, highlight the importance of exploring underlying biological, behavioral, and healthcare access factors. Investigating these aspects can contribute to improved prevention strategies, early detection, and tailored treatment approaches for skin cancer patients.

MATERIALS AND METHODS

A retrospective observational study was conducted on a database of patients diagnosed and treated for malignant skin tumors at a tertiary care center in Romania between January 2020 and December 2024. Due to the COVID-19 pandemic, there is a notable gap in data collection between April 2020 and June 2021, during which clinical activity was significantly reduced. The goal was to assess demographic distribution, histological types, anatomical localization, and treatment patterns of skin cancer patients in Romania in the last 5 years. Data were extracted from the institution's electronic medical records and physical registries, curated by the dermatology department.

The patients were selected using the following inclusion criteria: Patients with a clinical diagnosis of a skin lesion suggestive of malignancy; Hospital admission (i.e., full inpatient hospitalization, not outpatient consultation or day-case admissions); Histopathological confirmation of a malignant skin tumor.

Exclusion criteria consists of: Benign skin lesions and suspected malignancies for which no biopsy was performed (e.g., due to patient refusal or other contraindications).

Following the application of the inclusion criteria, a total of 388 malignant non-melanoma skin lesions were identified across 180 patients. While most patients presented with an average of approximately 2–3 lesions, a small subset exhibited over 10 malignant lesions each. Although not all lesions underwent histopathological confirmation, those that were not biopsied shared highly similar macroscopic features with confirmed cases, allowing for a reliable clinical diagnosis based on visual assessment.

The dataset includes both male and female patients residing in Bucharest as well as in 19 other counties across Romania. All patients sought medical evaluation and treatment at the tertiary facility. Of the 180 eligible patients, 125 (69.4%) originated from urban areas, while 55 (30.6%) were from rural settings. Sex distribution was relatively balanced, with 52% of the cohort being female and 48% male. Patient ages ranged from 35 to 93 years, with a median age of 69 years for females and 71 years for males.

The standard clinical pathway involved an initial dermatologic evaluation, during which a provisional diagnosis was made based on the morphological characteristics of the lesion. Patients then underwent a biopsy for histopathological confirmation. Upon receiving the diagnostic result, patients returned for definitive treatment, which typically consisted of complete surgical excision or electrocauterization, depending on the lesion's type, size, and location.

The following variables were systematically collected for each patient: demographic information (including age, sex, and place of residence); clinical characteristics (lesion location, provisional clinical diagnosis, year and month of diagnosis); histopathological data (tumor type and size); and treatment details, specifically the definitive procedure performed—either surgical excision or electrocauterization—as well as the occurrence of recurrence or the need for re-excision.

All tissue specimens were processed and analyzed by board-certified pathologists within the same department, using standard hematoxylin-eosin (H&E) staining tech-niques.

Data base was created using Microsoft Excel 2019. Statistical analysis was per-formed using JASP 0.19.3 program. Statistical analyses included Chi-square tests to as-sess seasonal distribution differences and Mann-Whitney U tests to compare lesion depth and surface area between demographic subgroups.

RESULTS

When analyzing the annual incidence of malignant skin lesions, a progressive in-crease was observed over the first four years of the study period: 18 cases in 2020, 25 in 2021, 40 in 2022, and a peak of 63 cases in 2023. This upward trend was followed by a decline in 2024, with 34 cases recorded (Fig. 1). A linear regression analysis revealed an average annual increase of approximately 7 cases (slope = 7), with a coefficient of determination (R^2) of 0.64, suggesting a moderate correlation between time and case number. However, the trend did not reach statistical significance ($p = 0.244$), likely due to the limited number of years analyzed and the variability introduced by external factors such as the COVID-19 pandemic in 2020–2021, the post-pandemic rebound in 2023, and the subsequent decrease observed in 2024.

The average hospitalization duration remained relatively stable over the past five years, starting at 4.2 days in 2020, slightly increasing to 4.5 days in 2021, then gradually decreasing to 4.3 days in 2022, and further declining to 4.1 days in both 2023 and 2024 (Fig. 2). The longest hospital stay was recorded in 2020, lasting 19 days, while the shortest stays of 2 days were observed in 2023 and 2024.

Figure 1.The trend line of malignant skin lesions

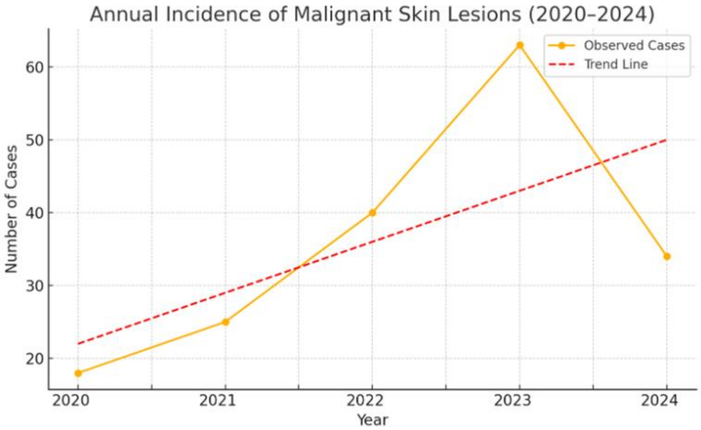

Figure 2. Evolution of the average hospitalization days (2020-2024)

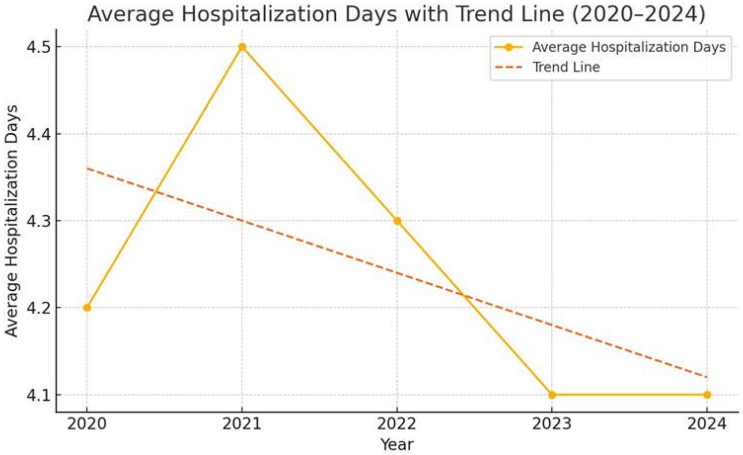

Of the 180 patients, 95 were residents of Bucharest, with the highest concentration coming from Sector 3 (n = 35) and Sector 4 (n = 32). Among patients from outside the capital, the neighboring counties of Ilfov (n = 16) and Giurgiu (n = 14) were the most represented.

When assessing seasonal distribution over the five-year period, the lowest incidence of diagnosed malignancies was observed in spring, with only 27 cases recorded. The other seasons showed relatively similar frequencies, with 47 cases in summer, 55 in autumn, and 51 in winter (Table 1, Fig. 3). In order to determine whether there is a significand difference between the observed frequencies and expected frequencies, we have applied the Chi-squeare test (χ^2). The test revealed a statistically significant difference in the seasonal distribution of skin cancer diagnoses ($\chi^2 = 10.31$, $p = 0.016$).

Table 1. Presentation period by month, year and season

Presentation period	2020	2021	2022	2023	2024	TOTAL	No. of cases per season
Jan.	6	Pandemic	1	9	5	**21**	**51 winter**
Feb.	9		1	5	5	**20**	
Mar.	3		2	3	6	**14**	**27 spring**
Apr.	Pandemic		2	1	3	**6**	
May			3	1	3	**7**	
Jun.			5	3	2	**10**	**47 summer**
Jul.		1	7	7	2	**17**	
Aug.		10	2	5	3	**20**	
Sept.		11	4	8	2	**25**	**54 autumn**
Oct.		1	6	6	3	**16**	
Nov.		1	4	9	0	**14**	
Dec.		1	3	6	0	**10**	
TOTAL	**18**	**25**	**40**	**63**	**34**	**180**	**180**

Figure 3. The percentage distribution of cases by season

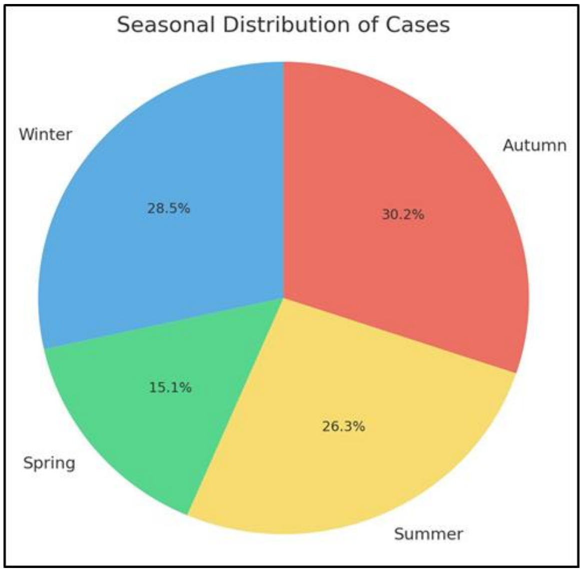

The malignant skin lesions identified in this study were categorized into basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. Out of the total 388 lesions, 168 were classified as BCC (Fig. 4). Histopathological evaluation allowed for further subtyping of BCC cases. The nodular subtype was the most prevalent (n = 68), followed by the adenoid type (n = 43), superficial BCC (n = 26), and the infiltrative variant (n = 23). Rare subtypes included basosquamous, pigmented BCC, Pinkus fibroepithelioma, and infundibulocystic BCC, each represented by one or two cases. Additionally, a single case associated with Gorlin syndrome was documented. Notably, no cases of micronodular BCC were observed (Fig. 5). Regarding squamous cell carcinoma, a total of 25 cases were identified. The majority were well-differentiated SCCs (n = 16), followed by moderately differentiated SCCs (n = 8) and poorly differentiated SCCs (n = 3). No anaplastic SCCs were detected during the study period.

Figure 4. Distribution of non-melanoma skin cancers cases

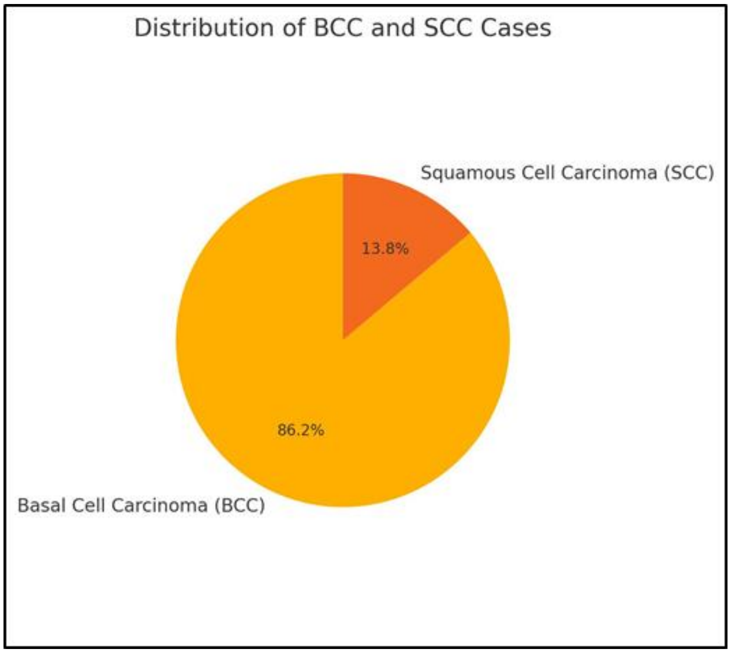

Figure 5. The incidence of histological subtypes of BCC

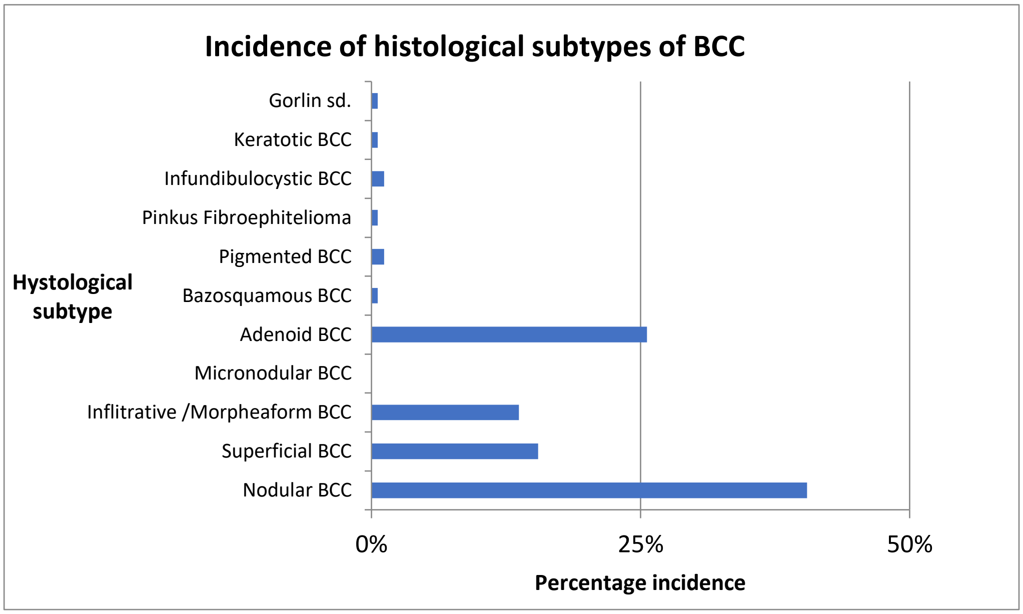

Based on the collected data, the majority of malignant lesions were located on the face (n = 152), a significantly higher number compared to those on the neck (n = 16) and scalp (n = 12). A more detailed anatomical breakdown of facial lesions revealed 20 cases on the forehead (10.3%), 23 on the nasal pyramid (11.8%), and 18 on the zygomatic region (9.2%). Additionally, 23 patients presented with multiple lesions on various facial subregions. Beyond the face, 24 other patients were identified with multiple malignant lesions distributed across the head and neck areas. Regarding the neck, the posterior cervical triangle showed a higher incidence (n = 11) compared to the anterior cervical triangle (n = 5). As for the scalp, lesion distribution was as follows: 2 cases on the vertex, 2 in the temporal region, 4 in the parietal region, 1 occipital lesion, and 3 patients with multiple scalp lesions.

When comparing the anatomical distribution of BCC and SCC, distinct patterns were observed. Several regions—including the anterior cervical triangle, vertex, temporal, , parietal, occipital, external canthus, upper and lower eyelids, chin (menton), perioral region, nasal area, auricle, and mastoid region—exhibited only BCC lesions, with no SCC cases recorded in these locations. On the forehead, 11% of all BCCs were located, compared to only 4% of SCCs, indicating a preferential distribution of BCC in this region. In contrast, the zygomatic region accounted for the highest proportion of SCC cases (20%), suggesting a predilection for SCC in this area. Within the posterior cervical triangle, SCC represented 8% of all cases found there, while BCC accounted for 5%, indicating a slightly higher occurrence of SCC in this particular region (Fig. 6).

The vertical dimension of malignant skin lesions showed notable variation across histological subtypes. Among basal cell carcinoma (BCC) types, the infiltrative/morphea form subtype had the highest mean depth (1.46 mm), followed closely by the superficial type (1.35 mm) and adenoid (solid adenoid) variant (1.30 mm). Less invasive subtypes such as nodular BCC had a mean depth of 1.21 mm, while rare variants like pigmented, Pinkus fibroepithelioma, and Gorlin syndrome-associated BCC displayed moderate vertical dimensions (ranging from 1.1 to 1.75 mm). Micronodular BCC was not reported in the dataset. In contrast, squamous cell carcinoma (SCC) subtypes tended to show greater vertical extension. Well-differentiated SCCs had the greatest average depth at 1.92 mm, while moderately differentiated SCCs averaged 0.97 mm, and poorly differentiated SCCs presented an intermediate value of 1.08 mm. These findings suggest a potential correlation between SCC grade and tumor depth.

Figure 6. Distribution of NMSC by anatomical location

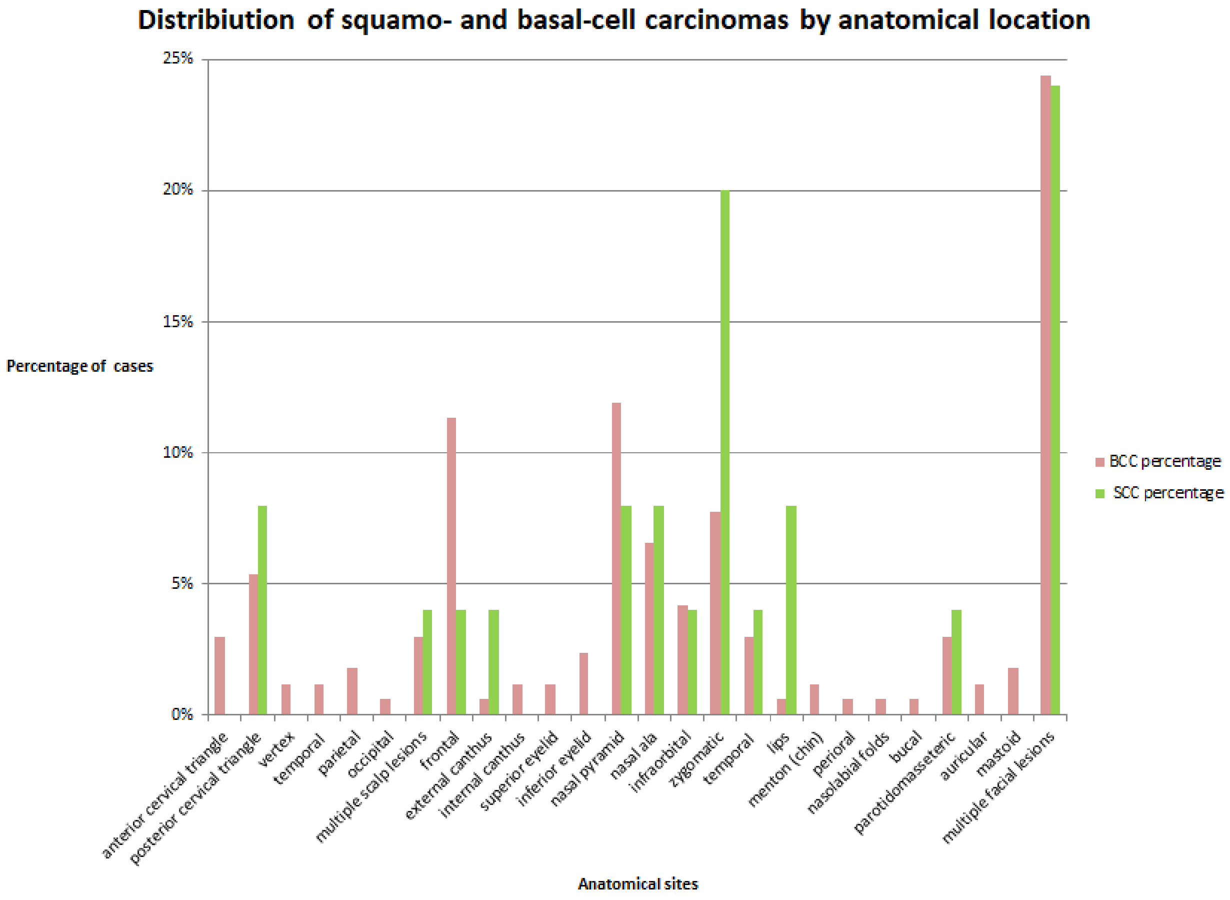

Temporal analysis revealed fluctuations in lesion depth over the five-year period. For nodular BCC, the vertical dimension ranged from 1.0 mm to 1.49 mm, indicating no clear increasing or decreasing trend. A more pronounced variation was observed in the superficial BCC subtype, which reached a peak of 1.85 mm in 2022 and dropped to 1.06 mm in 2023 before rising again to 1.61 mm in 2024. Infiltrative BCCs peaked at 2.52 mm in 2022—suggesting a possible diagnostic delay or more aggressive presentation in that year. Regarding SCC, well-differentiated lesions consistently displayed greater depth than other SCC subtypes, with a maximum of 2.5 mm recorded in 2022. However, sample sizes for some years and subtypes are limited, warranting cautious interpretation.

A Mann–Whitney U test was performed to compare the vertical depth of BCC and SCC. The median depth in BCC subtypes was 2.20 mm, while SCC subtypes showed a median of 1.32 mm. The test yielded a U statistic of 129.5 and a p-value of 0.797, indicating no statistically significant difference between the two groups.

In order to assess whether there is a correlation between age and the vertical di-mension of the lesion, we applied Spearman's rank correlation test. The results (Spear-man's ρ = 0.023, p = 0.744) suggest that there is no significant association between the two variables, indicating that lesion thickness does not appear to vary meaningfully with patient age in our dataset.

A Mann–Whitney U test (which is non-parametric) was conducted to determine whether there was a difference in vertical dimension between males and females. The results indicated a statistically significant difference between the groups (U = 6473.0, p = 0.009), suggesting that sex may influence vertical dimension. The vertical dimension mean in men was 1.5 mm, while in female was 1.25 mm.

When analyzing the lesion surface area across basal cell carcinoma (BCC) subtypes, we observed considerable variability. The infiltrative/morpheiform subtype demonstrated the largest mean area, with an average of 2.47 mm², followed by the adenoid type (1.58 mm²) and nodular type (1.43 mm²). The superficial BCC subtype had a slightly higher average area (1.85 mm²) than nodular, which may reflect the lateral spread characteristic of this variant. Rare subtypes such as basosquamous/metatypical carcinoma presented a notably high mean area of 74.46 mm², although this finding is based on a single case and should be interpreted cautiously. Other rare variants, including keratotic, fibroepithelioma of Pinkus, and infundibular-cystic subtypes, exhibited relatively small areas ranging from 1.2 to 1.43 mm². The Gorlin syndrome-associated lesion had an average area of 1.55 mm².

For squamous cell carcinoma (SCC), the mean lesion areas were generally larger than most BCC subtypes. Well-differentiated SCCs demonstrated the highest average area, reaching 2.41 mm², while poorly differentiated tumors averaged 1.07 mm². Moderately differentiated SCCs had the smallest mean area among SCC subtypes at 0.77 mm².

A Mann–Whitney U test was also conducted to compare the lesion surface areas between BCC and SCC tumors. The analysis yielded a U statistic of 133.5 with a p-value of 0.60, indicating no statistically significant difference in lesion surface area between the two groups. This suggests that, despite observed differences in average lesion sizes, such as larger surface areas in some well-differentiated SCCs. These differences may be attributed to sample variability rather than a true underlying disparity in tumor size between BCC and SCC in this cohort.

There was a significant difference in lesion area between males and females (U = 6290.0, p = 0.0095) (Fig. 7). While the median lesion area was higher in males (1.0 cm² vs. 0.7 cm²), a rank-based comparison showed a small-to-moderate effect (rank-biserial r = −0.21), likely influenced by high outliers in the female group. A lesion measuring 74.46 mm in a female case was excluded from the analysis to minimize the impact of outliers and ensure that the results more accurately reflect the central tendency and variability within the dataset.

Figure 7. Grouped bar chart showing lesion area distribution by sex

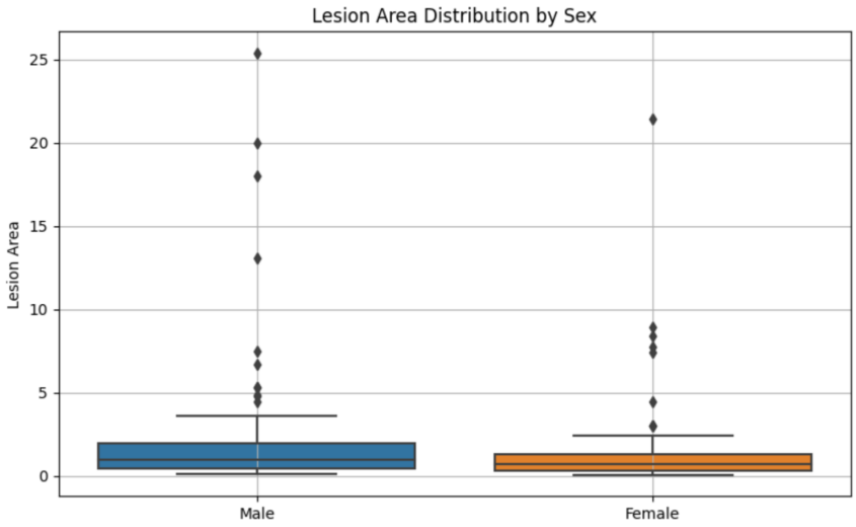

DISCUSSION

Comparing the data we collected with other clinical studies conducted in recent years in different regions of Romania, a consistent national trend in the incidence of non-melanoma skin cancers can be observed. Both a one-year 2022 study from South-Western part of the country (Craiova city) and a five-years 2014 study from the North-Eastern region (Iasi city) support and validates our findings, confirming their reliability and broader applicability. Thus, both studies indicate a prominence of basal cell carcinomas among the identified NMSC cases, with reported proportions of 55% in the 2014 study and 75,5% in the 2022 study [18,19]. Notably, the 75,5% figure closely aligns with our own findings, where BCC accounted for 86% of cases [18]. This may reflect either an increasing trend in the incidence of BCC over time, or differences in exposure to specific risk factors depending on region. Other similarities to our study include the predominance of the nodular subtype of BCC, which was the most frequently encountered variant in both comparative studies [18,19]. In our study, nodular BCC accounted for 40% of cases, closely matching the 40,4% reported in Iasi [19]. Additionally, all three studies identified the face as the most common anatomical site for tumor occurrence, which may support the correlation between UV exposure and lesion development [18,19]. Moreover, a slight female predominance was observed consistently: women represented 52% of cases in our study and 53% in the Craiova one [18]. These very similar findings highlight the potential role of common environmental and behavioral risk factors and support the need for continued epidemiological monitoring in Romania.

The observed seasonal variation, with higher incidence on non-melanoma skin cancers during autumn and lower numbers in spring, is consistent with the findings of previous studies. A 2017 study from North Carolina concluded that the diagnosis rates of BCC and SCC were highest in October and August, so at the end of the festival season, that confirmed the idea of delayed diagnosis after summer UV exposure [20]. According to Bianconi et al., the Umbria region of Italy had also a higher incidence of NMSC during autumn (mostly October), which was explained as a late cancer progression effect of UV exposure [21]. These similarities support the hypothesis that medical consultation is often delayed due to the clinical appearance of suspicious skin lesions following prolonged summer sun exposure. Consequently, many lesions are diagnosed in the following season- autumn- after cumulative sun damage becomes clinically. Given the seasonal pattern of delayed diagnosis, it is crucial to examine potential preventive measures, such as public health campaigns targeting periods of high UV exposure months. These interventions should aim to raise awareness about the risks of sun damage, promote early skin examinations, and encourage timely medical consultations.

The lack of statistical significance regarding the vertical dimension and area may be due to sample size limitations or uneven subtype representation within groups. For example, rare or extreme outliers like the metatypical BCC area (74 mm²) can skew averages and impact variability. Future studies with larger, more balanced samples may provide clearer insight into surface area differences between malignant skin lesion subtypes.

The recent Covid-19 pandemic impacted in a negative way the worldwide healthcare systems, leading to disruptions in routine medical services. As observed in Table I, there were no admissions of dermatology cases in our department between April 2020 and June 2021, which indicates that dermatological services were not operational through this period. There are several existing factors which contributed to the activity disruption. Firstly, the pandemic's health care focus likely caused resource redirection. Secondly, institutional policies mandating restriction consults of any case that is not an emergency severely capped support for patients. Another reason may be the fact that considerable sections of the medically unwell population opted not to attend medical facilities due to the high likelihood of contracting the virus, which adds even more weigh. This local experience aligns with global trends observed during the pandemic. A study analyzing data from the American Academy of Dermatology's DataDerm registry reported a significant decrease in in-person dermatology visits between April 2020 and June 2021, with a corresponding increase in tele-dermatology appointments [22]. However, expanding tele-dermatology services did not ascertain timely diagnosis and prompt treatment for a significant number of patients. Research shows lockdowns led to a diverse range of disruptions that caused cancer diagnoses to be missed on a global scale. The World Health Organization's International Agency for Research on Cancer estimated that about one million cases of cancer went undetected globally during the lockdown because of Covid-19 [23]. This statement emphasizes the need for strategies to maintain essential healthcare services even during public health crises. The increasing number of diagnoses of cutaneous cancer in the post-pandemic period, could stem from the backlog of cases that went undiagnosed when routine dermatological checkups were either postponed or unavailable.

In the present study, a statistically significant difference was observed in the vertical thickness of non-melanoma skin cancers (NMSC) between male and female patients, with mean measurements of 1.5 mm and 1.25 mm, respectively. This disparity, though numerically modest, holds substantial clinical relevance, as increased tumor thickness is associated with a higher risk of local invasion and recurrence.

Several factors may contribute to this gender-based difference in tumor presenta-tion. One of them could be considered the delayed medical consultation among men. Men are generally less likely to engage in preventive healthcare practices. According to a 2009 study, the white middle-class men were the most affected by melanoma because of the delay in seeking medical attention for skin lesions [24]. This tendency may result in the progression of tumors to greater thickness before diagnosis. Another contributing factor to the gender difference seems to be the sun protection behaviors. The American Association for Cancer Research reported in a 6 years study, that women are more likely to adopt behavioral changes as using sun protection measures like including sunscreen and protective clothing, to prevent future cutaneous cancers [25]. They would also limit more the outdoor activity and when outside, they would seek shade more than men [25]. This may contribute to earlier detection and thinner tumors at diagnosis.

Although our study shows a higher incidence of NMSC in urban areas, this finding is most likely explained by the location of the study center- a tertiary hospital in Bucharest. The majority of patients included in the study were residents of Bucharest, which leads to an overrepresentation of urban cases. This does not necessarily reflect a higher exposure to risk factors in urban settings, but rather the geographic bias introduced by easier access to specialized dermatological care in the capital. As shown in a retrospective U.S. study with patients recruited from two primary care practices in one rural Maine regional health system, rural patients often face significant barriers to dermatological evaluation and treatment [26]. That may include longer travel distances, limited availability of specialists and socioeconomic constraints.

Another point to discuss is the predominance of basal cell carcinoma in our studied population, with a higher incidence of the nodular subtype (40% of the BCC cases). These findings are aligning with the worldwide trend, as the BCC is well-known the most frequent skin cancer in adult patients with fair skin, the nodular lesions accounting for more than 60% of all BCCs [27]. Nodular subtype are usually slow growing, non-aggressive tumors, belonging to the indolent-growth subset [10]. Thus, its predominance in the studied population may indicate that most cutaneous neoplasms in fair-skin adults are having a favorable outlook. This may be explained by the commonly less aggressive nature of the nodular variant, or by the fact that they are being detected in an early stage, before becoming invasive. The last one is not a well-supported hypothesis, as we have to emphasize the frequent delay in making a definitive diagnostic due to several contributing factors like the still existing need for access to a good dermatological care for all socioeconomic groups.

Regarding squamous cell carcinoma, our study revealed a greater average vertical dimension of well-differentiated lesions (1,92mm), compared to all the mean depths of the different subtypes of BCCs. The bigger vertical growth of SCC can have potential implications in the tumor outcome, as may be an indicator for the cancer aggressiveness. According to a 2024 literature review, the cutaneous squamous cell carcinoma of the head and neck can present metastasis in approximative 2,5% of patients, most often localized in the level II lymph nodes and the parotid glands [28,29]. Unlike BCC, cSCC can spread both nearby and distant locations, one of the high-risk criteria for the metastasis being represented by a depth invasion greater than 6 mm [28,29]. Although none of the studied lesions were deeper than 6 mm, it is necessary to highlight the importance of correlating depth patterns of cSCCs with their metastatic rates and with the right choice of treatment. The primary method of treatment remains the surgical resection, but the tumor depth and size can dictate the type of therapy need to be used. The vertical dimension of cSCCs influences also the follow-up protocols, as patients with greater tumor depths (so high-risk groups) requires a tighter follow-up [29].

Lastly, although most NMSC cases are treated successfully with local excision, ad-vanced or recurrent head and neck lesions (particularly large or deep infiltrating squamous cell carcinomas) may require complex surgical resections. In such cases, reconstruction becomes critical not only for functional and aesthetic restoration but also for maintaining the patient’s quality of life. Free flaps and other advanced reconstructive techniques, such as the radial forearm or anterolateral thigh flap, offer valuable options. The success of these procedures underscores the importance of surgical expertise and a multidisciplinary approach, as emphasized in recent scoping reviews. [30]

Our study's possible limitations should be taken into account when interpreting the results as they may have introduced bias. Since only 180 patients were chosen from a single tertiary medical center, the study had a relatively small sample size and was carried out over a five-year period. Since it ignores possible variations in clinical, histo-pathological, or demographic characteristics among various populations or geographical areas, this may lower its statistical power and have an impact on the findings' generalizability. Furthermore, the COVID-19 pandemic coincided with a portion of the study, which disrupted data collection and resulted in fewer patients having access to medical services. Clinical activity was interrupted by these contextual factors, resulting in missing or insufficient data. Notwithstanding these drawbacks, our research still provides insightful information.

Nevertheless, we emphasize the very actual need of further prospective studies which should assess the targeted screening programs influence in early cancer detection and diagnosis, and the effectiveness of different treatment strategies in patients with head and neck non-melanoma skin cancers.

CONCLUSIONS

The study identified a progressive increase in the incidence of malignant skin lesions from 2020 to 2023, followed by a decrease in 2024. This trend could be attributed to factors such as the impact of the COVID-19 pandemic on healthcare access and a possible post-pandemic surge in cases. Continued monitoring is essential to validate whether this trend persists in subsequent years.

The analysis revealed a statistically significant variation in skin cancer diagnoses across seasons, with the lowest incidence observed in spring and relatively similar fre-quencies in summer, autumn, and winter.

The face was the most common location for malignant lesions, emphasizing the significant role of sun exposure in the etiology of NMSC. BCC was the most frequent histological subtype, with nodular BCC being particularly prevalent. In contrast, SCC, although less common, demonstrated greater metastatic potential and depth, especially the well-differentiated subtype.

Our analysis revealed statistically significant differences in lesion size between sexes, with both area and vertical dimensions being greater in males compared to fe-males.

The majority of NMSC cases were recorded in urban areas, particularly Bucharest, suggesting a potential link between urban living and higher skin cancer incidence or, conversely, a lack of adequate healthcare access in rural regions. Additionally, while the sex distribution was relatively balanced, males exhibited significantly greater lesion depths compared to females.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, M.M., M.D. and A.C.; methodology, I.A.T. and G.M.; software, A.Z.C.A. and D.V.; validation, M.M., S.R.G. and A.C.; formal analysis, M.D. and I.A.T.; investigation, M.M. and S.R.G.; resources, G.M. and A.Z.C.A.; data curation, M.D. and D.V.; writing—original draft preparation, M.M. and M.D.; writing—review and editing, M.M. and I.A.T.; visualization, G.M. and D.C.; supervision, S.R.G. and D.C.; project administration, A.C. and D.V.; funding acquisition, A.Z.C.A. and D.C. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Carol Davila University of Medicine and Pharmacy (protocol code 16341 from 1st July 2025).

Patient consent for publication

Not applicable, because it is a retrospective study.

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ARTICLE

Development and Implementation of Checklist Management in Manned Space Medical Rescue Transfer and Handover

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Abstract: Background Medical rescue transfer and handover in manned spaceflight is a critical component of space rescue operations, directly impacting the safety and well-being of astronauts. Objective This study aims to develop a comprehensive checklist for the medical rescue transfer and handover processes associated with manned spaceflight, thereby enhancing the efficiency of these procedures. Methods The checklist was designed and implemented during practical training sessions focused on manned space medical rescue. Participants were divided into two groups: one utilizing the checklist for transfer handover and another relying on verbal communication. Key metrics such as transfer time, quality of handover, and satisfaction scores were collected and analyzed for both groups. Results The group employing the checklist demonstrated significantly improved outcomes compared to the verbal group in terms of transfer time, quality of handover, and overall satisfaction scores (P < 0.001). Conclusion The implementation of checklist management has been shown to enhance both the efficiency and quality of transportation and handover processes, establishing a robust foundation for successfully executing manned space medical rescue missions.

Keywords: Manned Space Medical Rescue; Transfer; Handover; Checklist Management

INTRODUCTION

China's manned space program has advanced to the stage of conducting missions involving a space station, resulting in significantly extended durations for astronauts' stays. These missions are increasingly arduous and complex, which elevates the likelihood of accidental injuries and illnesses [1,2,3]. Space medical rescue is a critical component in ensuring astronaut safety and constitutes an essential aspect of manned space system engineering [4,5]. The efficiency and quality of transfer and handover during medical rescue directly impact the stability and prognosis of astronauts. Research indicates that standardized transfer handovers can reduce handover time, enhance accuracy and efficiency, and decrease the occurrence of adverse events. Checklist management has emerged as an effective strategy to mitigate errors arising from oversight; it offers advantages such as rapid comprehension, straightforward implementation, and precise handovers [6,7]. Currently, checklist management has been successfully employed in emergency evacuation and transfer protocols by foreign Marine Corps units, yielding positive outcomes in reducing adverse incidents while improving the quality of transfers and handovers [8].

This study aims to implement checklist management within nursing transfer processes during aerospace medical rescue training. A statistical analysis will be conducted on the time taken for these transfers, the quality achieved therein, as well as satisfaction levels among nursing staff compared with traditional oral handovers. This approach seeks to provide insights into methodologies for enhancing both the quality and efficiency of aerospace medical rescue operations alongside nursing transfers and handovers.

One population that has been identified as vulnerable to acquiring HIV infection is military personnel, due to frequently reported high-risk sexual behaviour [9,10] or to their high mobility and exposure to biologically foreign environments [11,12].

MATERIALS AND METHODS

1. Design of transfer handover list

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By convening a panel of eight experienced experts in the field of space medicine, we developed a comprehensive checklist for manned space rescue, transfer, and handover procedures. In designing the content, we prioritized the timeliness of first aid interventions prior to astronauts exiting their cabin. This checklist encompasses critical information including the arrival time of medical rescue personnel, the timing of astronaut cabin egress, on-site emergency treatment duration, astronaut evacuation timing, as well as vital signs monitoring and condition changes throughout the evacuation process [8]. As illustrated in Table 1, the handover list is categorized into four dimensions comprising fifteen items:

(1) Basic Information: This section includes data such as astronaut identification number, gender, age, exit time from the cabin, and entry time into the medical transport vehicle.

(2) Preparation Before Transport: This dimension covers essential vital signs of astronauts prior to transport along with safety measures for astronauts themselves; it also addresses preparations by medical staff and necessary equipment.

(3) Observation During Transport: Key observations during transportation include monitoring astronauts' vital signs—such as consciousness level, blood pressure readings, heart rate fluctuations, oxygen saturation levels—and noting any sudden changes in their condition.

(4) Transfer and Handover: The final category consists of data regarding consciousness levels and vital signs at transfer points; skin status assessments; past medical history including allergies; venous access details; artificial airway management; and medications administered during transit.

The signature section was duly signed off by both nursing staff involved in pre-cabin treatment and transport as well as receiving nursing personnel at the destination hospital. Additionally, precise records were maintained detailing each step of an astronaut's transfer to a ward down to minute-level accuracy. The transfer handover documentation exists in duplicate copies that are stored separately to serve as formal handover references.

Table 1: Astronaut transfer handover record sheet

<table>
<tr><td colspan="5">Astronaut transfer handover record sheet</td></tr>
<tr><td>Basic Information</td><td colspan="4">Number: Gender: Age: Astronaut out time: Into the medical carrier time:</td></tr>
<tr><td rowspan="4">Preparation</td><td colspan="4">Personnel Preparation: Doctor ☐ Nurse ☐ Departure time:</td></tr>
<tr><td colspan="4">Vital Signs: T: ℃; P: times/min; R: times/min; BP: mmHg; SPO$_2$: %</td></tr>
<tr><td colspan="4">Safety Protection:
Head and Neck Fixator ☐ Limb Protection ☐ Transport Equipment Good Performance Mouth ☐</td></tr>
<tr><td colspan="4">Equipment:
ECG Monitoring ☐ Oxygen Bottle ☐ Defibrillator ☐ Sputum Suction Equipment ☐ First-aid Case ☐</td></tr>
<tr><td rowspan="4">In transit (Observation)</td><td colspan="4">Vital Signs: T: ℃; P: times/min; R: times/min; BP: mmHg; SPO$_2$: %</td></tr>
<tr><td colspan="4">State of consciousness:
Awake ☐ Somnolence ☐ Lethargy ☐ Light Coma ☐ Deep Coma ☐ Delirium ☐ Agitation ☐</td></tr>
<tr><td colspan="4">Pupil Condition: Left ( ) Right ( )
Light reflex: Sensitive ☐ Slow ☐ Absent ☐</td></tr>
<tr><td>Changes in Condition</td><td colspan="3">No ☐ Yes ☐</td></tr>
<tr><td rowspan="3">Transfer & Handover</td><td colspan="4">Vital Signs: T: ℃; P: times/min; R: times/min; BP: mmHg; SPO$_2$: %</td></tr>
<tr><td>Past history</td><td>No ☐ Yes ☐</td><td>History of Allergies</td><td>No ☐ Yes ☐</td></tr>
<tr><td>Pipe line</td><td>Artificial Airway</td><td colspan="2">No☐ Oxygen Tube ☐ Mask ☐ Oropharyngeal Airway ☐</td></tr>
</table>

			Tracheotomy ☐ Tracheal intubation ☐ Depth of intubation: Ventilator parameters:
		Venous Channel	No ☐ Yes ☐ Indwelling Needle ☐ Bone Marrow Puncture ☐ Site: Puncture Time:
		General	No ☐ Abdominal Cavity Drainage Tube ☐ Thoracic Cavity Drainage Tube ☐ Others:
			Catheterization Time: Leakage Length: Pipe Clamp End: Yes ☐ No ☐
	Medication	Special Drugs	Yes ☐ No ☐ Name/Dose：
		General Drugs	Yes ☐ No ☐ Name/Dose：
	Skin	No ☐ Yes ☐ Site:	

Signature of Pre-cabin Emergency Nurse: Signature of Post-cabin Hospital Nurse: Handover Completion Time:

2. Training of transfer handover list

After the completion of the design for the transfer and handover checklist, nursing staff training was conducted by the organizers in accordance with expert consensus on astronaut treatment before the re-entry module of the landing site of space station mission[4]. This training also adhered to the Standard Operating Procedure (SOP) for emergency medical care for astronauts[9,10,11], as well as operational specifications pertaining to medical care and support during space station missions[12,13]. Methods such as group discussions and scenario simulation demonstrations were employed to elucidate each item on the checklist systematically. The effectiveness of this training was assessed through on-site questioning, ensuring that participants comprehensively understood each item and minimizing potential errors in documentation.

3. Group study on the transfer handover list

To implement the transfer and handover checklist in manned space medical rescue training, a study was conducted with two groups: a control group utilizing traditional oral handover methods and an experimental group employing the checklist handover approach. Each group consisted of 15 participants. The metrics evaluated included transfer preparation time, transfer handover time, and total transfer time.

Transfer preparation time refers to the duration required for the simulated astronaut to enter the transport carrier, during which the medical team counts personnel, consumables, and equipment while confirming both the condition of the injured individual and the status of all equipment. Transfer and handover time encompasses the period needed for the simulated astronaut to exit from the transport carrier and be transported to the astronaut ward. This phase includes transferring essential information such as basic details, vital signs, level of consciousness, and any changes in condition between pre-cabin rescue nursing staff and posterior support hospital nursing staff.Total transit time is defined as the cumulative sum of both transit preparation time and transit handover time.

4.Evaluation

The handover process with the posterior branch hospital was systematically evaluated by the training team leader during each transfer. The quality of handover for both the experimental group and the control group was assessed based on three key components: preparation prior to transfer, the actual transfer and handover process, and comprehension of the patient's disease status. Each component was assigned a score ranging from 1 to 3 points, where 3 points indicated excellent performance, 2 points denoted good performance, and 1 point represented fair performance.

Concurrently, a satisfaction questionnaire was distributed to nursing staff at the backward branch hospital to gauge their satisfaction regarding the transfer and handover process across six dimensions: vital signs monitoring, level of consciousness, changes in condition, venous access management along with other pipelines, medication administration, and skin conditions. Each aspect received a score between 1 (minimum) and 10 (maximum).

5.Statistical analysis

All data were analyzed using IBM SPSS Statistics 24 . Measurement data that conformed to a normal distribution were presented as mean(M) ± standard(SD) deviation. Count data were expressed in terms of frequency (percentage). The comparison of normally distributed measurement data between the two groups was conducted using an independent samples t-test.

RESULTS

1. Transit time

The transfer time was significantly reduced in the experimental group compared to the control group. In the experimental group, the transfer preparation time averaged 13.27±1.10 minutes, while the transfer handover time was 9.73±1.67 minutes, resulting in a total transfer time of 23.00±1.89 minutes. Conversely, in the control group, the average transfer preparation time was 18.13±2.07 minutes and the transfer handover time was 14.80±1.97 minutes, leading to a total transport time of 32.93±2.84 minutes. The difference between these two groups was statistically significant (P <0.001). Detailed results are presented in Table 2.

Table 2: Comparison of transit times in different groups

Groups	Number	Transit Preparation Time (min)	Transfer Handover Time (min)	Total Transit Time (min)
Control group	15	18.13±2.07	14.80±1.97	32.93±2.84
Experimental group	15	13.27±1.10	9.73±1.67	23.00±1.89
T		10.21	13.65	15.1
P		<0.001	<0.001	<0.001

2. Quality of transfer handover

The scores of the experimental group surpassed those of the control group across three dimensions: preparation prior to transfer, the transfer and handover process, and mastery of disease status. As illustrated in Table 3, the mean scores for preparation before transfer, transfer handover process, and mastery of disease status in the experimental group were 3.00±0.00, 2.53±0.51, and 2.60±0.50, respectively. In contrast, the corresponding scores for the control group were 2.40±0.13, 1.67±0.49, and 1.60±0.63. The differences between these two groups were statistically significant (P < 0.001).

Table 3: The quality of transfer handover was compared in different groups

Groups	Number	Prepare Before Transfer	Transfer Handover Process	Mastery of Disease Status
Control group	15	2.40±0.13	1.67±0.49	1.60±0.63
Experimental group	15	3.00±0.00	2.53±0.51	2.60±0.50
T		-4.58	-6.5	-5.12
P		<0.001	<0.001	<0.001

3.Satisfaction

Table 4 presents the satisfaction survey scores of medical staff at the posterior branch hospital. The satisfaction scores for the experimental group regarding vital signs, state of consciousness, condition changes, venous access and various pipelines, medication, and skin conditions are as follows: 9.83 ± 0.27, 9.41 ± 0.37, 9.56 ± 0.38, 9.41 ± 0.37, 9.66 ± 0.38, and 9.67 ± 0.38 respectively. In contrast, the control group's satisfaction scores in these same categories were: vital signs (8.73 ± 0.45), state of consciousness (8.57 ± 0.39), disease changes (8.75), venous access and each pipeline (8.63 ± 0.46), medication (8.79 ± 0 .41), and skin conditions (8 .59± 0 .50). The results indicate that the experimental group exhibited significantly higher satisfaction levels compared to the control group (P < 0 .001).

Table4: Comparison of disability among soldiers with different reasons

Groups	Number	Vital Signs	State of Consciousness	Changes in Condition	Pipe Line	Medication	Skin
Control group	15	8.73±0.45	8.57±0.39	8.75±0.41	8.63±0.46	8.79±0.29	8.59±0.50
Experimental group	15	9.83±0.27	9.41±0.37	9.56±0.38	9.41±0.37	9.66±0.38	9.67±0.38
T		-9.39	-8.82	-8.84	-6.24	-8.56	-7.63
P		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

DISCUSSION

To ensure the life and health of astronauts is fundamental to manned space projects. A review of the history of manned space flight worldwide reveals a number of accidents that have resulted in significant losses. In the United States, there have been 19 incidents and failures during manned space flights, leading to 18 fatalities. The former Soviet Union conducted numerous manned space missions, which also resulted in injuries among astronauts, including six deaths [14,15]. To date, all of China's manned space missions have been successfully completed without any serious injuries. As our country embarks on a new phase with its space station program, the frequency of manned launch missions is increasing, while records for in-orbit flight durations continue to be extended. This rise introduces unforeseen factors and maintains an ongoing risk of accidental injury to astronauts. In the event of such an accident, medical rescue serves as the final safeguard for astronaut safety.

The transfer and handover process is a critical component of aerospace medical rescue, significantly influencing the prognosis of injured individuals and patients. The occurrence of adverse events during transfer and handover is closely associated with various factors, including equipment issues, patient conditions, communication barriers, and other challenges faced by medical personnel [16,17]. Implementing an appropriate transfer and handover checklist can enhance communication efficiency among medical staff, enabling them to quickly and effectively comprehend the patient's condition while minimizing the risk of overlooking essential information. This ultimately ensures the safety of patients during their transfer [18,19].

By utilizing a standardized checklist for transfer handovers that delineates key points and critical content in written form, a unified standard is established. This approach improves both the accuracy and efficiency of the transfer process while preventing omissions of vital items. Consequently, it facilitates evidence-based practices in transfer handovers and safeguards patient safety throughout this procedure [20]. Research indicates that employing a checklist for handovers can reduce the incidence of adverse events during transfers from 13.8% to 5.4%, thereby significantly enhancing both quality and efficiency in these processes [21].

Checklist management was implemented in the transfer and handover process of aerospace medical rescue training. Utilizing a transfer and handover checklist, each item was marked off systematically, resulting in a rapid and concise documentation process with accurate and clear recorded content. The critical links that are often overlooked during handovers were consolidated into a standardized format to mitigate the risks associated with oral communication, thereby reducing the likelihood of omissions or repetitions. This approach not only saves time during transfers but also ensures that medical personnel at receiving hospitals have comprehensive access to vital signs and overall injury assessments of simulated casualties throughout the entire evacuation process [22]. The findings from this study indicated that the checklist-based handover group (experimental group) outperformed the oral handover group (control group) significantly across two evaluation metrics: transfer handover time and quality. Furthermore, medical staff at the receiving hospital expressed markedly higher satisfaction regarding six key aspects of the transfer process compared to their counterparts in the oral handover group (control group).

The practice of aerospace medical rescue training has demonstrated that checklist management offers several advantages:

(1) Ensuring the accuracy of transfer and handover information. Traditional oral handovers often suffer from inaccuracies in information transmission, ambiguity regarding key content, omissions of critical disease data, and sometimes deficiencies in the objectivity, accuracy, and completeness of the conveyed information. These unclear handovers and existing shortcomings directly impact the quality of care following transfer. By utilizing a tabular format, a nursing transfer and handover checklist can effectively reflect the basic situation and condition of simulated astronauts in a simple, comprehensive, and intuitive manner. This checklist provides complete information—including vital signs, illness details, rescue efforts, and nursing considerations—for medical personnel at posterior support hospitals. It serves as both a reference point for handover tasks and contributes to making the process more standardized and evidence-based.

(2) Improving the quality and efficiency of transfers and handovers. The checklist facilitates an item-by-item review according to its contents during handover sessions; this approach significantly reduces omissions related to key content or data due to limitations in energy levels or memory capacity among medical staff—factors exacerbated by misunderstandings or time constraints. Consequently, it shortens overall handover duration while substantially enhancing its quality. Research findings indicate that groups utilizing checklists for handovers performed significantly better than those relying on oral communication concerning both transfer times and overall quality of transfers. Following implementation of checklist-based procedures for handing over responsibilities, there is clear evidence that both efficiency and quality are markedly improved while ensuring safety and timeliness throughout the process.

(3) To ensure the safety of astronaut transportation, space medical rescue is critical to both the life and safety of astronauts as well as the overall success or failure of the mission. In the event that an astronaut sustains an accidental injury, there are heightened risks associated with the transfer process. The implementation of a checklist handover serves as a safe nursing practice designed to facilitate effective communication before, during, and after the transfer based on a predetermined transfer handover list [23]. The self-designed transfer handover checklist plays a vital role in conducting risk assessments prior to transfer, thoroughly evaluating timing considerations, and emphasizing careful monitoring of conditions along with emergency interventions throughout the transfer process. Upon safely reaching their destination for transfer, this checklist is utilized once more to convey essential information

regarding vital signs, consciousness levels, venous access status, various drainage tubes in place, medication administered, and any changes in condition experienced by the astronauts. This systematic approach proves beneficial for medical personnel at receiving facilities by enabling them to swiftly assess conditions and execute necessary procedures effectively.

CONCLUSION

With the successful completion of the inaugural in-orbit rotation involving the astronaut teams of Shenzhou 14 and Shenzhou 15, China's space station has transitioned into a long-term operational mode. Consequently, space medical rescue and support missions will now be conducted under standard working conditions. This study demonstrates that effective checklist management can enhance both the efficiency and quality of the transfer handover process. The objective of this approach is to normalize, standardize, and systematize the procedures for space medical rescue transfers and handovers, thereby establishing a robust foundation for the successful execution of astronaut medical support and rescue operations.

Conflicts of interest and sources of funding

The authors declare no conflict of interest and this research received no external funding.

Authors' contribution

The initial draft was prepared and revised by JL, while GH, PS, XG, and LD contributed to the collection of information and the preparation of the dataset. YW further refined the analyses and provided essential support for the manuscript. LS and YT supervised all aspects of the study, including the design of the data analysis plan, oversight of the data analysis process, validation of results, and writing of the manuscript based on the first draft. All authors participated in reviewing and finalizing the manuscript; furthermore, all interested parties have read and approved it.

Ethics approval and consent to participate

This study does not consider pertinent ethical issues related to individuals.

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EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Academic Editor. The Editor-in-Chief reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

ETHICAL CONSIDERATIONS

Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed following the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at: http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent before their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects from being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

– Accessible to the public at no charge;

– Searchable by standard, electronic (Internet-based) methods;

– Open to all prospective registrants free of charge or at minimal cost;

– Validates registered information;

– Identifies trials with a unique number; and

– Includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of the last follow-up, a target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

Plagiarism Detection

The journal employs a plagiarism detection system. By submitting your manuscript to this journal, you accept that your manuscript may be screened for plagiarism against previously published works.

Committee on Publication Ethics

The Journal subscribes to the principles of the Committee on Publication Ethics (COPE).

MANUSCRIPT CATEGORIES AND SPECIFICATIONS

All articles, except for Editorials, must contain an abstract of no more than 200 words. Abstracts for original articles should be formatted as detailed in the word form. Titles must not be longer than 120 characters (including spaces). In every article, there must be three to ten pertinent keywords specific to the article yet reasonably common within the subject discipline.

Editorials

These are invited by the Editor-in-Chief or their delegated editor and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure, and cite up to 15 references, including the source article. This should be cited as Rom. J. Mil. Med. (year); (vol): [issue].

Review Articles

RJMM welcomes reviews of important topics across the scientific basis of medicine and advances in clinical practice. Most published reviews are in response to the editorial invitation, including thematically related “miniseries” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this

avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are suggested to limit to 3500–5000 words, with an abstract of up to 200 words and up to 100 references and figures or tables.

Meta-Analyses or Systematic Reviews

RJMM particularly welcomes the submission of Meta-Analyses and Systematic Reviews, which underpin evidence-based medicine. Guidelines for the preparation of Meta-analysis and Systematic Reviews are similar to other reviews, and articles are subject to the usual peer review process. Meta-analyses and Systematic Reviews are suggested to be limited to 3500–5000 words, with an abstract of up to 200 words and up to 100 references and figures or tables.

Original Articles (including clinical trials)

RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and/or historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Original articles are suggested to be limited to 3000 words, with an abstract of up to 200 words and up to 100 references and figures or tables.

Education and Imaging

The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Executive Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In Part 1, a case will be described briefly, including a summary of the presentation, clinical features, and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to a diagnosis or are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, the presentation of analogous cases or related images from a similar case might be appropriate.

Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

MANUSCRIPT PREPARATION

Style

Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication, as presented at http://www.ICMJE.org/.

Spelling

The journal uses US spelling and authors should, therefore, follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

Units

All measurements must be given in SI units as outlined in the latest edition of Units, Symbols, and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations

Abbreviations should be used sparingly and only where they ease the reader's task by reducing the repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: the title page; and the main text file according to the word model.

Title page

The title page should contain (i) a short informative title that contains the major keywords. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgments. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or an article describing the use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer-reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented according to the word model. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 200 words or less the purpose, basic procedures, main findings, and principal conclusions of the study. If appropriate, divide the abstract with the headings: Background and Aim, Methods, Results, and Conclusions. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to ten keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser – http://www.nlm.nih.gov/mesh/meshhhome.html.

Text

Authors should use the word form in editing the submitted material.

References

The Vancouver system of referencing should be used. In the text, references should be cited using Arabic numerals in square parentheses in the order in which they appear. If cited only in tables or figure legends, number them according to the first identification of the table or figure in the text. In the reference list, the references should be numbered and listed in order of appearance in the text. Cite the names of all authors when there are six or fewer; when seven or more list the first three followed by et al. Names of journals should be abbreviated in the style used in MEDLINE. Reference to unpublished data and personal communications should appear in the text only.

Use Index Medicus as the style guide for references and other journal abbreviations.

Tables

Tables should be self-contained and complement, but not duplicate, the information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table and legend must be

understandable without reference to the text. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, •, ‣ should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure legends

Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

Figures

All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals, and cited in consecutive order in the text. Each figure should be supplied as a separate file, with the figure number incorporated in the file name.

Preparation of Electronic Figures for Publication:

Although low-quality images are adequate for review purposes, publication requires high-quality images to prevent the final product from being blurred or fuzzy.

SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at office@rjmm.ro. A cover letter containing an authorship statement should be included. The cover letter should include a statement covering each of the following areas:

– Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author. It is not accepted the idea of equal contribution.

– Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.

– A statement outlining how ethical clearance has been obtained for the research, particularly concerning studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.

– For research involving pharmacological agents, devices, or medical technology, a clear Conflict of Interest statement about any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

– For clinical trials, these have been registered in a publicly accessible database (see more under 'ETHICAL CONSIDERATIONS (Further Information)' later in these guidelines).

If the above items are not included in the cover letter, manuscripts cannot be sent for review. Please also note that the cover letter does not require a detailed or lengthy description of the content or structure of the manuscript itself.

Two Word files need to be included upon submission: A title page file and the main text file that includes all parts of the text in the sequence indicated in the section 'Parts of the manuscript', including tables and figure legends but excluding figures which should be supplied separately.

The main text file should be prepared using the word model that is to be found on the web page (http://revistamedicinamilitara.ro/wpcontent/uploads/2022/07/Macheta-articole.docx).

Each figure should be supplied as a separate file, with the figure number incorporated in the file name. For submission, high-resolution figures (at least 300 d.p.i.) saved as .eps, .jpg or .tif files are required.

PUBLICATION PROCESS AFTER ACCEPTANCE

Accepted Articles

Accepted Articles are published online after final acceptance, and appear in PDF format only.

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ORIGINAL ARTICLES

Obesity and osteoporotic fractures

Florica Şandru[1,2], Mihai C. Dumitraşcu[2,3], Simona E. Albu[2,3], Mara Carsote[2,4], Ana Valea[5,6]

Abstract: *Obesity correlates with a higher risk of fractures in some sites as vertebras, upper arm, and legs. This is a narrative mini-review focused on several key points that link obesity with osteoporosis. Waist circumference may a better predictor of fractures than body mass index (BMI) in obese females. Even obese menopausal women have an extra source of estrogens in fat tissue this actually is not enough to offer a complete protection against osteoporotic fractures. Low levels of testosterone in males induce a higher risk of fall dependent or independent of sarcopenia (which is more evident in elderly). High BMI is correlated with vitamin D deficiency. Obesity is a contributor to muscle damage and decline due to fat accumulation and inefficient fuel utilisation by the muscle, namely sarcopenic obesity, regardless the age. Associated type 2 diabetes mellitus involves bone damage and fracture risk due to glycated proteins of the matrix, the changes of adipokines, increased cortical porosity, high risk of fall because of blood pressure and glycaemia anomalies, visual disturbances, renal and peripheral nerves diabetes-associated conditions etc. The fracture risk is also augmented via fat derived cytokines and chronic inflammation. Even it seems logical that the weight correction though bariatric surgery reduces the fracture risk in obesity, actually data from longitudinal studies pointed that, despite the procedure is life saving, it does not actually protect against osteoporosis which is regarded as a potential long-term complication. A high fracture burden in obesity should increase the level of awareness for practitioners of different medical areas due to multiple levels of complications and to the epidemiological impact of obesity.*

Keywords: *obesity, osteoporosis, fracture*

INTRODUCTION

Obesity currently represents a pandemic issue and every medical and surgical speciality includes different complications of obesity from cardiovascular conditions, type 2 diabetes mellitus and insulin-resistance, some types of cancer to infections at any level including skin, lungs, urinary tract, gastrointestinal infections, respective infertility, sleep apnoea, non-alcoholic fatty liver disease, depression, and finally any of these or their combination may increase the overall mortality. [1, 2, 3, 4] The increasing epidemiological impact has brought a large area of concern worldwide. [1, 2, 3, 4] The endocrine frame of obesity perception is related to hypothyroidism, hypercortisolemia, male hypogonadism, female hyperandrogenemia, and lately to osteoporosis and fragility fractures, including hypovitaminois D. [5] A systematic review associated with a meta-analysis published in 2019 by van Hulsteijn et al. included 68 studies (meaning 19,996 obese subjects). [6] The study identified that 28% of the individuals had reduced thyroid function of different severities, while 0.9% of them had hypercortisolemia. [6] When it comes to males the prevalence of hypogonadism was between 32 and 42%

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depending on criteria. [6] Contrary to historical records that consider obese women protected against osteoporosis because of the estrogens excess at the level of adipose tissue, last two decades brought enough proves that actually this hypothesis is not entirely true. [7]

AIM

Our purpose is to introduce data related to the risk and prevalence of osteoporotic fractures in obese persons.

MATERIAL AND METHOD

This is a narrative mini-review of the literature based on a research focused on several practical key points that correlate obesity with osteoporosis. A number of 69 articles have been included in the selection.

RESULTS – GENERAL DATA

The influence of abdominal obesity versus body mass index

The link between obesity and fractures may be expressed as BMI (Body Mass Index), either as abdominal obesity. A cross-sectional study published in 2019 showed that among 669 elderly women 65 had fragility fractures of different sites, with an average age of 73.9 years. [8] BMI was not associated with fragility fractures but abdominal obesity suggested by a waist of more than 88 centimetres (cm) was, regardless the presence of different clinical risk factors as smoking or age etc. [8] Some authors suggested that actually the waist circumference is a better predictor of fractures than BMI in overweighed and obese females. [8,9] It seems that low body mass index as well as high content of body fat, respective increase BMI both are associated with high fracture risk, especially at vertebral site. [9] Generally the fat content of the body has been reported as an accurate predictor factor for several conditions including cardiovascular and metabolic morbidities. [10] On the other hand, the influence of BMI on osteoporotic fractures risk may include the fact that low and high BMIs are considered at risk for fractures despite Bone Mineral Density (BMD) values which are directly correlated with BMI and it might not reflect the real fracture risk in obese individuals. [11, 12]

Postmenopausal women

The female individuals in menopause suffer of estrogens deficiency related to menopause thus causing bone loss and low trauma fractures. [13, 14, 15] Even obese menopausal women have a second source of estrogens in fat tissue which is superior to normal weighted subjects this actually is not enough to offer a complete protection against osteoporotic fractures. [13, 14, 15] Some studies pointed a similar rate of fractures in menopausal women regardless they were obese or not and others showed a different distribution depending on site, for instance: a high risk is for vertebral fractures, humerus, upper arm, leg and ankle while a low risk has been described for hip and wrist. [13,14,15] This aspects are most probably related to the physics of fall depending on weight. [13, 14, 15]

Male hypogonadism

Even osteoporosis is less frequent in males, the prevalent fractures are found in obese men through associated hypogonadism independent of the BMI values. [16, 17, 18] Low levels of testosterone induce a higher risk of fall dependent or independent of sarcopenia (which is more evident in elderly). [16, 17, 18] Testosterone therapy may reduce the fracture risk. [16, 17, 18]

Hypercortisolemia

Glucocorticoid osteoporosis is a well known cause of obesity-related type of secondary osteoporosis. [19, 20, 21] The obese subjects without Cushing’s syndrome display chronic high values of basal cortisol which seems suppressible at dexamethasone inhibition test. [19,20,21] Chronic autonomous secretion of cortisol is identified in patients with adrenal incidentaloma with an age-dependent incidence and this has been reported to increase the risk of osteoporosis independent of age. [22, 23, 24]

Hypovitaminosis D

The interest in the area of hypovitaminosis D has been explosive during the last decades. A higher BMI is positively correlated with a high risk of vitamin D deficiency based on large epidemiological studies. [25, 26, 27[However, there is still a matter of debate regarding which is the cause and which is the effect. [28, 29, 30] The potential causes of vitamin D lack include: the relative immobility and decreased sun exposure, lower dietary intake, decreased intestinal absorption, high accumulation of vitamin D at the level of adipose tissue since it is a liposoluble vitamin thus a lower bioavailability of the vitamin D. [31, 32, 33] Decreased levels of 25-hydroxivitamin D are contributors to impair muscle resistance and to sarcopenia, thus increasing the risk of fall, and finally the risk of fracture. [34, 35, 36] Also, hypovitaminosis D is a contributor to metabolic disturbances as insulin resistance, hyperlipemia etc. [37, 38] The doses of vitamin D required for supplementation are usually two to three times higher in obese versus non-obese individuals due to fat accumulation. [39, 40]

Sarcopenic obesity

The loss of muscle mass and function is progressive since the age of 40 and accelerated after 60 years. [41, 42] Obesity is a contributor to muscle damage and decline due to fat accumulation and inefficient fuel utilisation by the muscle, namely sarcopenic obesity, regardless the age. [41, 42] The collateral contributors to this pathological status is represented by low 25-hydroxivitamin D levels and the presence of glucose metabolism anomalies. [43, 44] Overall, the weakness of muscles causes a higher risk of fall thus of fractures. [43, 44]

Type 2 diabetes mellitus

The metabolic anomalies are associated as seen in metabolic syndrome where obese individuals are also diabetics. [45, 46] The risk of fracture caused by type 2 diabetes mellitus is more evident in menopausal women. [45, 46] Type 2 diabetes is associates bone damage and high fragility fracture risk due to glycated proteins of the matrix, the changes of adipokines as leptin, increased cortical porosity, increased risk of fall due to blood pressure anomalies, visual disturbances, renal and peripheral nerves diabetes-associated conditions. [47, 48, 49, 50]

Chronic inflammation

The network between fat and bones includes anatomical aspects like high BMI associates high BMI or mechanical loading is protective for fracture, but also changes of the skeleton micro-architecture in obese subjects that differs with the skeleton site thus some types of osteoporotic fractures are more exposed. [51, 52] Moreover, the fracture risk is augmented via fat derived cytokines and chronic inflammation as seen in metabolic syndrome or abdominal adiposity. [51, 52]

Bariatric surgery

Even it seems logical that the weight correction though bariatric surgery might reduce the fracture risk in obese individuals, actually data from longitudinal studies pointed that, despite the procedure is life saving, it does not actually protect against osteoporosis. [53, 54] Why? Because of the changes involving the mechanical loading due to the weight decrease, of lower intestinal surface for nutrients absorption (including potential malabsorption), anomalies of gut-produced calcitropic hormones, and restrictive diets after surgical procedure etc. [55, 56] Even osteoporosis after bariatric surgery seems a small price to pay, the post-operatory fractures are currently regarded as a long term complication on patients of different ages. [57, 58]

Depression

A higher risk of depression has been reported in obesity. [59] This may further involve reduced physical activity, including decreased sun exposure with risk of hypovitaminosis D and other factors leading to higher risk of fall and fractures. [60] Some mediators anomalies (as well as antidepressants-related) including serotonin have been reported at risk of bone loss through peripheral changes of its concentration despite the fact that circulating 5-hydroxytriptamin has not been yet indicated as a useful bone turnover marker in daily practice and conditions with pathological increase of the molecule as neuroendocrine tumours are not conventionally on the list of secondary osteoporosis. [61, 62]

DISCUSSIONS

Fracture risk assessment

DXA (Dual-Energy X-Ray Absorptiometry) does not particularly reflect the fracture risk in obese individuals since high BMI is correlated with high BMD. [63] FRAX also uses as input BMI. [64] Bone turnover markers might be blunted in associated diabetes and dramatically increased after bariatric surgery. [65, 66] Also type 2 diabetic menopausal women may beneficiate from TBS (Trabecular Bone Score) analysis. [67] The assays of 25-hydroxivitamin D might also be useful. [68]

Current limits

Obese individuals are less likely to receive anti-osteoporotic drugs since they already have several non-osteoporotic complications requiring different therapies. [69, 70, 71] Also, if a specific advisable drug in these subjects should be used in order to treat osteoporosis and/or to prevent fractures is not clearly understood up to this moment which is that drug, and probably the therapy for osteoporosis should be done based on similar protocols as in normal weight subjects with osteoporosis. [69, 71] Moreover, higher doses of vitamin D are necessary in obesity. [69, 71] The importance of therapy in order to provide an adequate primary and secondary intervention regarding osteoporosis is essential since the complications of fractures may become more severe due to obesity co-morbidities. [69, 71, 72]

CONCLUSION

A high fracture burden in obesity should increase the level of awareness for practitioners of different medical areas due to multiple levels of complications and to the epidemiological impact of obesity.

Abbreviations:

BMI = Body Mass Index
BMD = Bone Mineral Density
cm = centimetres
DXA = Dual-Energy X-Ray Absorptiometrry
TBS = Trabecular Bone Scor

Acknowledgment:
None

Conflict of interest:
The authors have nothing to declare.

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ORIGINAL ARTICLES

Obesity impact on symptomatology associated with pelvic floor dysfunction

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Abstract: *The increasing incidence of obesity, a significant health problem worldwide, represents a major socio-economic burden with a high impact on the quality of life. It is considered a risk factor for multiple medical conditions. More than half of women from developed countries are overweight or obese, this comorbidity can affect them from puberty through the reproductive period and then in the menopausal period, involving multiple medical and surgical specialties. It has a significant impact on the onset of puberty, the menstrual cycle, ovulation, fertility rate, and pregnancy outcome. In postmenopausal women, obesity represents an important risk factor for breast cancer, endometrial cancers or polyps, fibroids, pelvic organ prolapse, or stress urinary incontinence in addition to a higher risk of post-operative complications and also to some types of fragility fractures, especially if diabetes mellitus is associated. Pelvic floor disorders include pelvic organ prolapse, urinary or fecal incontinence. Obese patients seem more affected compare with normal-weighted females. We introduce a single-center, retrospective study on 74 patients (admitted between 2016 and 2019) with pelvic organ prolapses that were referred for surgical correction, and further on they were evaluated after one month, respective after 6 months since procedure. On admission, the subjects, aged between 41-82 years (median of 57 years) showed a strong correlation between the symptomatology caused by the prolapse degree and obesity (40.54% of them), as it appears that the discomfort and symptoms are significantly higher in the women with higher body mass index. In the reevaluation form, the patients with obesity complaint more frequently compared to the non-obese females about urinary and colorectal distress and low sexual desire and interest. The use of surgical treatment for pelvic organ dysfunctions is a contributor to improved quality of life in adult obese females.*

Keywords: *pelvic organ prolapse dysfunction, obesity, prolapse degree, body mass index, menopause, reproductive age*

INTRODUCTION

Obesity is one of the most severe health issues in developed and developing countries contributing to more than 4 million deaths each year [1]. It is by far the most frequent comorbidity severe pathologies and represents a major socio-economic burden with a high impact on the quality of life because of the disease itself, associated conditions, and impair the outcome of apparently non-connected conditions like abdominal surgery or procedures for gynecological diseases [2]. Excessive weight in the female population of reproductive age interferes with puberty, menstrual cycle, ovulation, fertility rate, abortion rate, pregnancy outcome, perinatal complications, and increased risk of severe infections [3-6]. Also, it is a risk factor for endometrial cancer, breast cancer, and uterine fibroids [7, 8]. It might

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represent a poor prognosis factor in other malignancies like ovarian or color-rectal types [9, 10]. Most cases of obesity are associated with elements of metabolic syndrome like dyslipidemia, high blood pressure, and type 2 diabetes mellitus, also associating high serum uric acid, chronic inflammatory syndrome, anomalies of bone turnover markers, damage of coagulation profile, and mineral metabolism including hypovitaminosis D [11-14]. Obesity also may be secondary to endocrine tumors like adrenal – and pituitary neoplasia (for instance, Cushing syndrome, acromegaly, etc.), also associating anomalies of glucose profile, higher risk of some other tumors, poor wound healing, etc. [15]. Other causes of obesity and glucose profile anomalies are related to pancreatic tumors (somatostatinoma, glucagonoma, etc.) with heterogeneous manifestations [16-18]. Recently, obesity has been linked with a higher risk of fragility fractures, even though estrogens (including those derived from adipose tissue, not only of ovarian origin) are traditional elements of bone protection and that bone mineral density is directly correlated with body mass index [19, 20].

Pelvic floor dysfunction affects both females of reproductive age and post-menopausal, and it includes pelvic organ prolapse and urinary incontinence are significantly influenced by obesity [21]. Urinary incontinence is associated with increased intra-abdominal pressure caused by central obesity and is related to the period that women had had excessive weight [22]. Systematic studies regarding the effect of obesity on urinary incontinence and the results of surgery on this pathology showed that one of the main causes of decreased surgery effectiveness is represented by excessive weight [23,24].

Similarly, many studies had found a higher prevalence of pelvic organ prolapse among women with excessive weight especially morbidly obese and severely obese patients [25]. Unfortunately, the tissue damage from the pelvic floor caused by excessive weight is irreversible, as the cystocele, rectocele, or uterine prolapse does not reduce after weight loss [26]. Pelvic organ prolapse progresses with aging and has a low progression rate after menopause [27]. Some authors consider that the incidence of pelvic disorders will increase by 46% by 2050 [28].

Several surgical treatments for pelvic organ prolapse and stress urinary incontinence are known but many variables can influence the outcome. These are represented by prolapse degree, symptomatology, lifestyle, age, body mass index, sexual activity. This type of pathology, the treatment required, and the correlated risk factors have an important impact on the quality of life.

Obesity is particular comorbidity that is found in many patients with pelvic floor disorders. Obese patients can associate severe life-threatening pathologies but in the meantime, they can present sexual dysfunctions, pelvic organ prolapse, modified bowel movement, or urinary incontinence [29]. Pelvic floor dysfunctions are correlated by themselves with poor quality of life, modified bowel movement, modified urinary pattern, or sexual dysfunction. Obesity is correlated with an increased risk of urinary incontinence between 20 and 70% proportional with the body mass index [30]. Urinary stress incontinence is, without doubt, more frequent in obese women and it is demonstrated that bodyweight loss will lead to decreasing in the number of episodes [31, 32]. In the literature are reported studies in which up to 40% of patients admitted for pelvic organ prolapse presented with a high body mass index [33, 34]. Persisting and progressive obesity were associated with progressing prolapse degree but weight loss was not associated with significant improvement of the prolapse degree [35].

Studies have shown that obesity influences postoperative evolution. A poor outcome with anatomical and functional failure after surgery was associated with obesity, multiple vaginal deliveries, and the intensity of the symptoms before surgery [36].

MATERIALS AND METHODS

This study aimed to evaluate the influence of obesity on women with pelvic floor dysfunction that underwent surgery in our clinic between January 2016 and December 2019. It was designed as a retrospective study in women with pelvic organ prolapses who had clinical post-operative follow-up at 1 month and 6 months. The patient data collected included age, body mass index, number of births, hormonal status. The patients agreed to anonymously use their medical data during hospitalization for surgical procedures and during follow-up.

The pelvic floor disorder was evaluated before intervention for every patient with the Pelvic Floor Impact Questionnaire (PFIQ 7) which evaluates three aspects: Urinary Impact (UIQ-7), Colorectal-Anal Impact (CRAIQ-7), and Pelvic Organ Prolapse Impact (POPIQ-7) and with Pelvic Floor Distress Inventory-20 (PFDI-20) [19]. The women responded to the questionnaire in the presence of the medical team and the responses were corroborated with the patient’s history.

RESULTS

There were evaluated 74 patients that underwent surgery and had completed the questionnaires before the procedure. We included all 74 women aged between 41 and 82 years with a median of 57 years to participate in this study. Most of the patients were above 50 years at the moment of surgery (70.27%) and only 29.73% under 50 years. Only 6 of the 74 patients were nulliparous, 27 had one child and 33 had 2 children. Only 8 patients had more than 3 children. The majority of the women had one or two children (44 patients). Of these women, 59.46% had a body mass index lower than 30 and 40.54% were obese with a body mass index higher than 30. The majority of women reached menopause (48 patients), of which 30 were non-obese. Stage I and II pelvic organ prolapse was presented in 26.67% of the obese patient and 56.81% of the non-obese patients. Stage III and IV were present in 73.33% of the obese women and 43.18% of the non-obese women. The mean body mass index for patients with pelvic organ prolapse III or IV was 30.95 (±0.83) in obese patients and 25.64 (±1.96) in non-obese patients. The demographic data are presented in Table 1.

Table 1: Demographic data

	Non-obese n=44	Obese n=30
Age	40 – 77 (mean 59)	41 – 82 (mean 52.5)
IMC	20.65 – 29.39 (mean 25.33)	30.07 – 33.26(mean 30.83)
Menopause	30	18
Parity		
- nulliparous	4	2
- 1 birth	17	10
- 2 births	19	14
- 3 and more than 3 birts	4	4
Pelvic organ prolapse		
- I or II	25	8
- III or IV	19	22

The symptomatology was evaluated with PFIQ-7, PFDI-20, and FSFI for sexual life evaluation. A high result in PFIQ-7 and PFDI-20 represents an increased discomfort (scales 0 to 300). These patients also have poorer sexual function. For the PFIQ-7 the mean value was 191.73 (±60.00) for obese women and 152.72 (±69.16) for non-obese women (p = 0.0034, t = 3.0248, df = 72). The PFDI-20 the mean value was 192.5 (±43.12) for obese women and 156.84 (±64.33) for non-obese women (p = 0.0097, t = 2.6535, df = 72). These values suggest a strong correlation in our patients between the symptomatology caused by the prolapse degree and obesity, as it appears that the discomfort and symptoms are significantly higher in women with higher body mass index. Women with high scores on PFQI and PFDI had high scores on sexual function FSFI. Sexual desire and interest were low on 56%, sexual arousal reduced by 61% and 71% reached orgasm less than half of the time or rarely. The data collected is shown in Table 2.

Table 2: Pelvic Floor Distress Inventory-20 and Pelvic Floor Impact Questionnaire – scores in obese and non-obese patients

Variable	Non-obese (n=44)	Obese (n=30)	P value
PFIQ-7	Mean = 152.72 S.D.=69.16	Mean = 191.73 S.D.=60.00	p = 0.0034 t = 3.0248 df = 72
PFDI-20	Mean = 156.84 S.D.=64.33	Mean = 192.5 S.D.=43.12	p = 0.0097 t = 2.6535 df = 72
Prolapse degree	I-II = 25 III-IV= 19	I-II = 8 III-IV= 22	

PFIQ = Pelvic Floor Impact Questionnaire (scale 0-300)
PFDI = Pelvic Floor Distress Inventory (scale 0 -300)

The 74 women were evaluated 1 month after surgery, with a period of 4-6 weeks of sexual abstinence. The reevaluation was realized 6 months after surgery. At the reevaluation, the patients with obesity (30%) complaint more frequently compared to the non-obese females (18%) about urinary and colorectal distress and low sexual desire and interest.

There was 55.41 % with high pelvic organ prolapse degree (III or IV), from which most were women with body mass index above 30 (22 patients, compared to 19 non-obese patients). The high pelvic organ prolapse degree does not correlate with the body mass index (p=3.7, df =39).

DISCUSSION

The prevalence of obesity in developed and developing countries is continuously increasing. Although it can be managed with many options, it remains one of the most difficult comorbidity to correct. In our study, obesity was not correlated with stage III or IV prolapse, but pelvic floor symptoms were associated with a body mass index higher than 30.

The literature that evaluates the association between pelvic floor symptoms and obesity contains various results. The main cause of different results can result from the methodology of symptoms evaluation and defining the prolapse degree. In our study, one of the limits is represented by the small lot of patients that reduces the power of the study.

Hendrix et all [34] included in their study 16,616 women, from which 14.2 had uterine prolapse, 34.4% cystocele, and 18.6% rectocele, and found a strong association between this pathology and parity and obesity. Whitcomb et al [37, 38] enrolled 1,155 women in their study and concluded that the prevalence of any type of pelvic organ dysfunction was highest in morbidly and severely obese patients (57% and 53%) compared to obese women (44%), but the degree of discomfort was not influenced by the degree of obesity. Another large study [26] that included 16,608 patients concluded that obesity is associated with the progression of the pelvic organ prolapse and the damage might be irreversible. Both obesity and pelvic floor dysfunctions are associated with low quality of life.

CONCLUSIONS

Although the data can be conflicting, the practitioner must evaluate the patient and discuss the optimal approach to obesity-related complications. Managing obesity, even if it is not always a priority, it must be taken into consideration. Results take time, but in correlation with surgical treatment of pelvic organ dysfunctions, the overall quality of life of these patients will be significantly improved.

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Authors' contribution:

MCD drafted the manuscript and critically revised the final form, AEN is the corresponding author and revised the literature, NA researched the literature, AMO critically revised the manuscript, AP drafted the manuscript and revived the references, CGN researched the literature and approved the final form. All authors read and approved the final manuscript.

All authors read and approved the final manuscript.

Ethics approval and consent to participate:

For this study, the agreement was obtained from the Ethics Committee. The subject data were completely confidential and they approved the publication of data.

Patient consent for publication: All patients consented that the data declared could be used for educational purposes and statistical studies.

Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

The information generated and analyzed during the current study is available from the corresponding author on reasonable request.

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ORIGINAL ARTICLE

Obesity is not related to individuals but to environmental conditions; from the perspective of obese patients

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Abstract: *Background: The Beliefs About Obese Persons Scale (BAOP) is used among non-obese people in English speaking countries to evaluate their opinions on obese people. Nevertheless, the data are scarce from the view of obese people. Therefore, we wanted to test BAOP on obese patients in order to evaluate their opinions from the perspective of obese patients.*

Methods: Between August 2019-June 2020, 115 (76.7%) female and 35 (23.3%) male, a total of 150 obese patients who were admitted to the general surgery department to be evaluated for bariatric surgey, were included in this study. Local ethics committee approval was obtained (2019/08-10). These obese patients were asked to fill out BAOP, which included 8 statements in which each statement was valued between -3 to +3 points.

Results: Mean score of BAOP within all obese patients was 34.87 ± 8.12 (range: 5-48). The average score of BAOP was 34.33 ± 8.60 (range: 5-48) in females and 36.62 ± 6.06 (range: 21-48) in males (p = 0.14). The frequency of the answers given by the male and female patients to the 5th statement, which was "Most obese people eat more than non-obese people", showed a statistically significant difference (p = 0.03).

Conclusions: Obese patients had higher BAOP scores compared to previous BAOP studies performed with non-obese people. Within this study, obese patients considered obesity as a condition which was not under their control.

Keywords: *bariatric, belief, obesity, surgery*

INTRODUCTION

Obesity is a worldwide public health problem causing predisposition to diabetes, heart disease and malignancy such as breast and colon cancer [1-3]. In the United States, more than 20% of the population has obesity [4]. Moreover, the number of people with obesity is rapidly increasing in Turkey. Patients who suffer from obesity not only have health related problems but also experience weight-related discrimination [5]. Patients with obesity often claim that they are accused of lacking self-discipline, uncontrolled binge eating, sedentary lifestyle, inadequate exercise and laziness [6].

Until now, different kinds of measuring scales have been introduced to evaluate the thoughts and beliefs of non-obese people over obese people [7-14]. Allison et al. published the article entitled "The measurement of attitudes toward and beliefs about obese persons" in 1991 [15]. One of the aims of Allison et al. was to develop reliable measures of beliefs about obese people, thus The Belifs About Obese Persons Scale (BAOP) was generated. Furthermore, Dedeli et al. tested and confirmed the validity and reliability of BAOP to measure beliefs about obese people in Turkish speaking countries [16].

Most of these previous studies with BAOP were performed on non-obese people. Nevertheless, the data are scarce from the point of view of obese people. Commenting on somebody else without having the same or similar conditions might be easy, however comments of obese people are of utmost importance to identify and solve obesity related problems within the society. Moreover, medical doctors should not only present a patient-oriented

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approach to investigate the causes of obesity and treatment options but also include patients in the decisions about their health [3, 17]. Therefore, we wanted to test BAOP on obese Turkish patients in order the understand their beliefs about obesity and obese people. For this purpose, we asked 150 obese patients, who were admitted to the general surgery department to be evaluated for bariatric surgery such as sleeve gastrectomy, to fill out BAOP.

METHODS

Between August 2019-June 2020, 115 (76.7%) female and 35 (23.3%) male, a total of 150 obese patients who were admitted to the general surgery department to be evaluated for bariatric surgey, were included in this study. Aksaray University Ethics Committee approval was obtained (2019/08-10). The patients with body mass index (BMI) between 25 and 29.9 kg/m^2 were regarded as overweight and patients with BMI 30 kg/m^2 and above were regarded as obese [5, 18]. In addition, BMI between 35–39.9 kg/m^2, 40-49.9 kg/m^2 and over 50 pointed out severe obesity, morbid obesity and severe morbid obesity, respectively [19].

These 150 obese patients were asked to fill out BAOP. The Beliefs About Obese Persons Scale is an 8-statement questionnaire [15, 16]. The participants were asked to mark each statement according to how much they agreed or disagreed with it, using a scale between, -3 to +3 points (-3: I strongly disagree, -2: I moderately disagree, -1: I slightly disagree, +1: I slightly agree, +2: I moderately agree, +3: I strongly agree). These eight statements are: “1. Obesity often occurs when eating is used as a form of compensation for lack of love or attention., 2. In many cases, obesity is the result of a biological disorder., 3. Obesity is usually caused by overeating., 4. Most obese people cause their problem by not getting enough exercise., 5. Most obese people eat more than non-obese people., 6. The majority of obese people have poor eating habits that lead to their obesity., 7. Obesity is rarely caused by a lack of willpower., 8. People can be addicted to food, just as others are addicted to drugs, and these people usually become obese.”

All of the statements should be responded by the participants by the help of BAOP scale. In BAOP, points given to each statement by the participant are summed up. This final number is added to 24 in order to reach a total score, which varies between 0-48 [9, 20].

Statistical analysis was performed using the SPSS 20.0 statistical package programme. Numerical variables were stated as the mean (±) standard deviation and medians (minimum–maximum). Categorical variables were stated as percentages. The chi-square test and independent samples t test were used to compare differences between two groups. The p values <0.05 were considered significant.

RESULTS

This study included 150 obese patients, 115 (76.7%) were female and 35 (23.3%) were male. The mean age of the patients was 38.68 ± 12.09 years (range: 18-66). The mean age of women was 37.81 ± 11.53 years (range: 18-66), while the mean age of men was 41.51 ± 13.59 years (range: 21-66) (p = 0.11). The mean BMI of the patients was 42.03±7.24 kg/m^2 (range: 30.10-65.30 kg/m^2). Average BMI was 42.29±7.20 (range: 30.10-64.30 kg/m^2) in women and 41.16±7.41 (range: 30.16-65.30 kg/m^2) in men (p = 0.42).

The mean score of BAOP within all obese patients was 34.87 ± 8.12 (range: 5-48). The mean score of BAOP was 34.33 ± 8.60 (range: 5-48) in females and 36.62 ± 6.06 (range: 21-48) in males (p = 0.14). The answers given by all patients, female patients, and male patients separately to the questions on the scale of beliefs about obese people are shown in Table 1, Table 2 and Table 3, respectively.

Table 1: The frequency of the answers given by the patients to the questions of the Beliefs About Obese Persons Scale

	Patients (n/%)							
Score	**Q-1**	**Q-2**	**Q-3**	**Q-4**	**Q-5**	**Q-6**	**Q-7**	**Q-8**
-3	23 (15.3)	10 (6.7)	12 (8)	8 (5.3)	19 (12.7)	4 (2.7)	14 (9.3)	14 (9.3)
-2	13 (8.7)	11 (7.3)	7 (4.7)	7 (4.7)	13 (8.7)	4 (2.7)	8 (5.3)	4 (2.7)
-1	8 (5.3)	16 (10.7)	10 (6.7)	13 (8.7)	9 (6)	7 (4.7)	11 (7.3)	12 (8)
1	34 (22.7)	31 (20.7)	23 (15.3)	28 (18.7)	21 (14)	21 (14)	37 (24.7)	25 (16.7)
2	24 (16)	37 (24.7)	29 (19.3)	32 (21.3)	20 (13.3)	28 (18.7)	29 (19.3)	29 (19.3)
3	48 (32)	45 (30)	69 (46)	62 (41.3)	68 (45.3)	86 (57.3)	51 (34)	66 (44)

†Q: Question

48 (32%) patients gave 3 points to the first question, while 8 (5.3%) patients gave -1 points. (The first statement with “Obesity often occurs when eating is used as a form of compensation for lack of love or attention” has been answered as “I strongly agree” by 48 (32%) obese patients). 45 (30%) patients gave 3 points to the second question, while 10 (6.7%) patients gave -3 points. However, the patients gave most frequently 3 points ‘’I strongly agree’’ to all questions.

Table 2: Replies of female patients to questions about Beliefs About Obese Persons Scale

Female Patients (n/%)								
Score	**Q-1**	**Q-2**	**Q-3**	**Q-4**	**Q-5**	**Q-6**	**Q-7**	**Q-8**
-3	17 (14.8)	8 (7)	10 (8.7)	8 (7)	15 (13)	4 (3.5)	13 (11.3)	11 (9.6)
-2	9 (7.8)	9 (7.8)	6 (5.2)	5 (4.3)	13 (11.3)	4 (3.5)	7 (6.1)	4 (3.5)
-1	5 (4.3)	13 (11.3)	7 (6.1)	12 (10.4)	5 (4.3)	7 (6.1)	10 (8.7)	10 (8.7)
1	26 (22.6)	23 (20)	18 (15.7)	21 (18.3)	16 (13.9)	17 (14.8)	25 (21.7)	18 (15.7)
2	20 (17.4)	26 (22.6)	17 (14.8)	25 (21.7)	19 (16.5)	19 (16.5)	21 (18.3)	22 (19.1)
3	38 (33)	36 (31.3)	57 (49.6)	44 (38.3)	47 (40.9)	64 (55.7)	39 (33.9)	50 (43.5)

†Q: Question

38 (33%) of the female patients gave 3 points, while 5 (4.3%) patients gave -1 points to the first question. Most of the female patients gave 3 points ''I strongly agree'' to all eight questions.

Table 3: Replies of male patients to questions within Beliefs About Obese Persons Scale

Male Patients (n/%)								
Score	**Q-1**	**Q-2**	**Q-3**	**Q-4**	**Q-5**	**Q-6**	**Q-7**	**Q-8**
-3	6 (17.1)	2 (5.7)	2 (5.7)	0	4 (11.4)	0	1 (2.9)	3 (8.6)
-2	4 (11.4)	5 (5.7)	1 (2.9)	2 (5.7)	0	0	1 (2.9)	0
-1	3 (8.6)	3 (8.6)	3 (8.6)	1 (2.9)	4 (11.4)	0	1 (2.9)	2 (5.7)
1	8 (22.9)	8 (22.9)	5 (14.3)	7 (20)	5 (14.3)	4 (11.4)	12 (34.3)	7 (20)
2	4 (11.4)	11 (31.4)	12 (34.3)	7 (20)	1 (2.9)	9 (25.7)	8 (22.9)	7 (20)
3	10 (28.6)	9 (25.7)	12 (34.3)	18 (51.4)	21 (60)	22 (62.9)	12 (34.3)	16 (45.7)

†Q: Question

While 10 (28.6%) of the male patients gave 3 points to the first question, 3 (8.6%) of them gave -1 points. Most patients (31.4%) gave 2 points to the second question. Twelve patients gave 2 points to the third question and the other 12 patients gave 3 points. None of the male patients gave -3 points to the fourth question and -2 points to the fifth question. None of the patients gave -3, -2 and -1 points to the sixth question. The seventh question was answered as 1 point and 3 points by 12 patients equally. Most of the male patients (45.7%) gave 3 points to the eighth question.

The frequency of the answers given by the male and female patients to the 5th question showed a statistically significant difference (p = 0.03). However, the frequency of their answers to the 1st, 2nd, 3rd, 4th, 6th, 7th and 8th questions did not show a statistically significant difference (p = 0.82, p = 0.89, p = 0.18, p = 0.35, p = 0.28 p = 0.32).

DISCUSSION

BAOP scale is used to evaluate the beliefs about obese people. A low score on BAOP suggests that obesity is controllable as obese individuals cause their obesity by themselves. Nevertheless, a high score on BAOP scale indicates a stronger belief that obesity is not under the control of the obese person [20, 21]. Moreover, lower BAOP score points out tendency towards anti-obese attitudes such as accusing obese individuals as lazy and deprived of self-control [22]. Mean score of 17.9 with BAOP scale was reported to be associated with anti-obesity attitude [21].

Sonneville et al. explored weight bias among 322 public health trainees by an cross-sectional online survey assessing explicit beliefs about the controllability of obesity using BAOP [8] The mean BAOP scores within public health trainees was 22.9. Relative to male students, female students had a stronger belief that obesity was not within the control of the individual (p = 0.03) [8]. Soto et al. performed BAOP scale on 528 students (250 medical students, 278 psychology students), where 56.3% of the participants were women. The average age was 20.7 ± 3.0. The mean BAOP scores were 18.6±5.7 and 16.6±5.5 in psychology and medical students, respectively. No differences were observed between BAOP scores of the participants related to age, gender or BMI [23]. Darling et al. investigated BAOP scale scores of 403 nursing students, 35 graduate education students, and 88 graduate social work students. BAOP scores were inbetween 14.35-25.39 which indicated that there was room for improvement for the belief that obesity was not under the individual's control. BAOP scores were not signifficantly different between male and female students. Moreover, Darling et al. reported that perception of one's own body as obese and having a friend or family member who was obese did not lead to more positive beliefs that obesity was not under the individual's control [3]. Usta et al. aimed to determine beliefs of 658 nursing students about obese people [9]. The 76.6% of the students was female and the mean age of the students was 20.63 ± 1.52. The percentage of the students who were overweight was 15.2%. In addition, 18.2% of the students claimed to have had discrimimation related to their weight. More interestingly, 17.5% of the nursing students did not want to get involved in daily care of obese patients. The mean BAOP score was 23.36 ± 0.83 (minimum 21.38, maximum 26). Usta et al. found out that nursing students who had a previous history of diet and regular physical exercise believed that obesity was controlled by individuals [9]. Kadar et al. tried to assess the obesity bias among 143 preclinical and clinical chiropractic students and 30 faculty members [24]. Average scores of BAOP scale were 31.68±5.19, 31.26±4.83, 27.93± 4.85 and 30.07±7.85 in the student and faculty member groups, respectively. BAOP scores of preclinical faculty group was significantly higher than each student groups. As a result, obesity bias was common among students and faculty members [24]. Yilmaz et al. aimed to assess whether there was prejudice towards obese people among 190 student nurses and 189 registered nurses using BAOP [14]. The most of both student nurses and registered nurses had negative beliefs about obese people. The mean scores of BAOP scale were 17.4± 4.8 and 15.7±5.8 in student nurses and registered nurses, respectively which revealed that both groups had negative beliefs about obese people [14].

Within our study, the mean score of BAOP within all obese patients was 34.87 ± 8.12 which revealed that obese patients considered obesity as a condition that was not under their control. In addition, we identified that majority of obese patients gave 3 points to each question of the 8-item survey as they strongly agreed. Therefore, obese patients within this study had higher BAOP scores compared to BAOP studies performed with non-obese people. The results obtained from this study revealed that non-obese and obese people had different opinions about the reasons of obesity. Morever, mean BAOP scores within our study did not differ statistically in relation to gender as male and female. Nevertheless, the fifth statement within BAOP which questioned if most obese ate more than non-obese people was replied by female patients statistically different from male patients (p = 0.03). Thus, this might be a clue pointing out the different eating habits of male and female obese patients.

Previous BAOP scale-related studies performed on non-obese people revealed low BAOP scores which indicated that obesity was related solely to individuals. Therefore, obese people were accused of lacking self-control, for example not controlling enough their eating habits. However, in our study, obese individuals reached high BAOP scores which indicated that obesity was not related to individuals but to environmental conditions. This discrepancy within the BAOP scale points of obese and non-obese individuals require detailed investigation of the beliefs towards obese people and reasons of obesity. We hope these results encourage our colleagues not only to present a patient-oriented approach to investigate causes of obesity but also include obese patients in the decision process of their further medical or surgical treatment options.

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Occipital radionecrosis and neuroplasticity in astrocytoma

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Abstract*: Background: Late-delayed complications after radiotherapy (RT) results from an imbalance between cell lesions and the protective capacities of the CNS. Temozolomide (TMZ) after RT may represent a potent radiosensitizing regimen. Although radionecrosis (RN) of the anterior visual pathway has been documented, in our opinion this is the first report of reversible visual field loss associated with occipital lobe radionecrosis.*

Case observation: We report a patient who suffered a left lateral homonymous hemianopia one year after radiochemotherapy (RCT) for an infiltrative low grade fibrillary astrocytoma. The visual field deficit was completely reversible after one month. Visual field defects have been described after the use of conventional external beam therapy for lesions near the anterior visual pathway. The cortex is relatively spared after RT.

Conclusions: Given the new scientific data, we suppose that neuroplasticity may play a role in the reversibility of visual field deficit. At this time there is no proven treatment of radionecrosis. Homonymous hemianopia caused by occipital lesions are attributable to vascular disease and tumors, but take into account radiation effects. The risk of neurogenic visual loss must be factored into the decision to irradiate the whole brain.

Keywords: *fibrillary astrocytoma, temozolomide, radiation necrosis, visual recovery, neuroplasticity*

INTRODUCTION

Radiation therapy is an integral part of modern therapy in gliomas. Early or delayed side effects are taken into account in determination of the therapeutic algorithm of each patient. The combination of radiotherapy and chemotherapy further increases the vulnerability to side effects. In this context, we present the case of a young woman diagnosed with fibrillary astrocytoma. One year after combined radiotherapy and chemotherapy with Temozolomide, right occipital lobe radionecrosis with impaired vision appeared.

Although such cases cited in literature are irreversible, in this case, both visually campimetric deficit and occipital brain lesion were fully reversible.

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MATERIAL AND METHODS

A 21 years old, right-handed Caucasian female with mild idiopathic thrombocytopenia presented to the hospital with right sensory-motor hemiparesis. MRI revealed a subcortical low grade glioma in left rolandic region, extended to basal frontal and ascending parietal white matter regions, invading the dominant arcuate fasciculus, confirmed by brain biopsy.

Given the infiltrative character of the tumor and its deep location, a surgical intervention is considered risky (the tumor invades left pyramidal fibers, thalamocortical fibers and superior longitudinal fascicle), especially without perioperative functional cortico-subcortical cartography procedures. Our patient undergoes 64Gy (photon teleradiation) radiotherapy for 6 weeks, followed by chemotherapy with Temozolomide (TMZ), 250 mg/day, 5 days/month for 6 months. Conventional MRI control reveals stationary aspect of the tumor (figure 1).

Figure 1

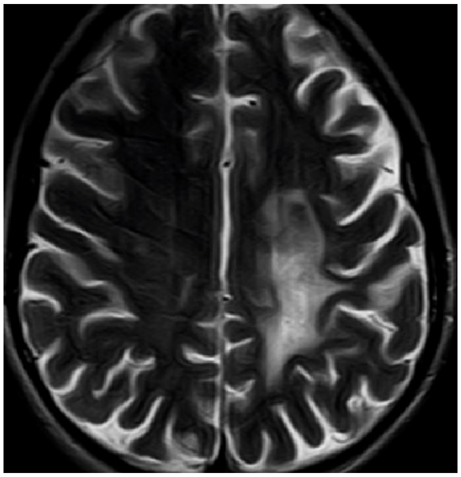

One year after chemotherapy, the patient insidiously develops left lateral homonymous hemianopia. A new conventional brain MRI shows a new 26/10 mm, irregular, non-homogenous, imprecisely defined right occipital lesion with hyperintense T2/Flair and isointense T1 signals, without contrast media enhancement. Fluorodeoxiglucose (FDG) PET-CT scan allows the differential diagnosis between tumor growth or radionecrosis and it reveals significantly decreased FDG uptake in the right occipital region, including the visual cortex (figure 2).

Figure 2

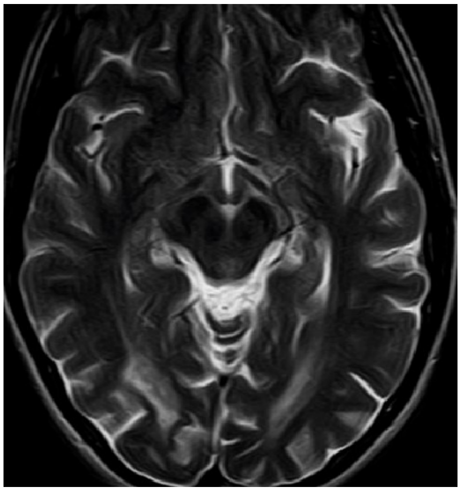

RESULTS

We conclude that the visual field deficit is the result of cerebral radionecrosis. The patient started a short period of corticoid treatment, with no improvements. One month from the first appearance of the hemianopia, it completely remitted and occipital right lesion were not visible.

Worsening deficit in patients with cerebral tumors may suggest either tumor progression or its recurrence [1]. Imagistic methods reveal edema and enhanced lesions near the tumor area. On the other hand, radiotherapy may determine effects after months or years and these are called late-delayed reactions [2].

Figure 3

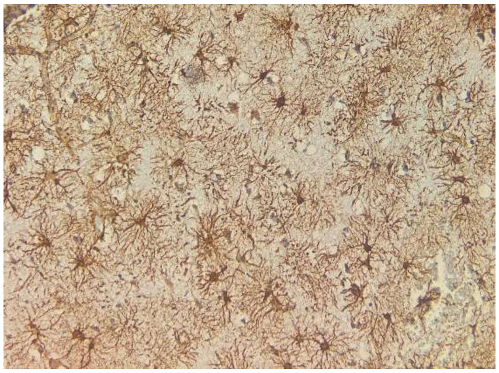

One of them is radionecrosis that occurs by vascular changes and ischemia, increase of capillary permeability and cerebral edema. Due to capillary permeability disturbances, adverse reactions of chemotherapy appear earlier and are more severe (figures 3, 4). Necrosis can be a result of radio and chemotherapy combination [3].

Figure 4

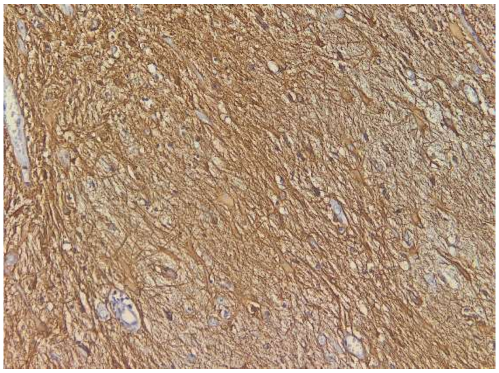

Temozolomide (TMZ) amplify the answer to radiations through DNA modifications and apoptosis [4]. TMZ combined with radiotherapy, enhances blood-brain barrier damage, leading to increased concentrations of therapeutic agents [5].

DISCUSSIONS

Few things are known about radionecrosis because it is difficult to differentiate from the tumor relapse with imaging techniques [6] and because the rate of surgical secondary interventions and brain autopsies are low in these patients [7].

In a study of 426 patients presenting glioma, Ruben and co. prove that the average time for the first appearance of necrosis is 11.6 months [8]. Also, adjuvant chemotherapy rises to over 4 times the cerebral necrosis risk. Peterson and co. reported a 2.5% incidence of radionecrosis, 8-31 months post-radiation, in a study including 200 patients with radiated and chemo-treated cerebral tumors [9]. So, as many have already demonstrated, chemotherapy has an amplifying effect in cerebral radionecrosis development. The incidence of radionecrosis is slightly raised in anaplastic oligodendroglial tumors than anaplastic astrocytomas and this is an argument for glial theory and its role in radionecrosis occurrence.

Things are even more complicated because the edema associated to this pseudoprogression is as symptomatic as the tumor actually progresses [10]. Conventional MRI (T2 and T1 with contrast) is limited in differentiating between tumor relapse and RT induced necrosis. Unfortunately, in many Gad positive lesions that occur after combined treatment, there are necrosis lesions and viable tumor cells and molecular magnetic resonance imaging of endogenous proteins and peptides are not for current use [11]. Diffusion MRI measures the mobility of water from tissues into the cells. After treatment, tumor cells are dehydrated and have a high apparent diffusion coefficient (ADC). Decreases of ADC indicate high cellularity [12]. Magnetic Resonance Spectroscopy detects tumor metabolites from tissues (choline, creatinine, lactate, lipids, N-acetyl aspartate) and is more sensible [13]. Dimensional spectroscopy has superior sensitivity indicating a relapse based on raised choline/NAA and choline/creatinine ratios [14].

18-Fluorodeoxyglucose PET scan is useful for differentiating between radionecrosis and recurrence and is 81-86% sensitive and 40-94% specific [15]. Still, false positive results have been encountered in non-malignant inflammatory processes, electric epileptic seizures and healing processes up to 3 months post-surgery. Even though, radionecrosis activates healing mechanisms and raises the glucose metabolism. This is why MRS is superior. There are some mechanisms that can explain postradiation necrosis: DNA modifications, coagulation factors imbalance with thrombosis facilitation [16]. The main mechanism of radionecrosis is associated with oligodendroglia injury or vascular endothelial damage (figures 5, 6).

Figure 5

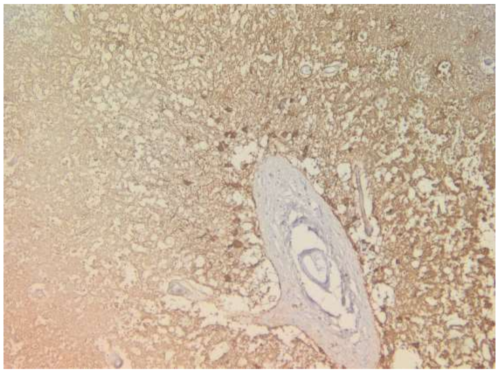

As a result, white matter tissue is often affected than gray matter tissue. Cytotoxic edema and tissue necrosis are the consequence of imbalance between tissue plasminogen activator and urokinase plasminogen activator.

But an autoimmune mechanism may be also involved.

Figure 6

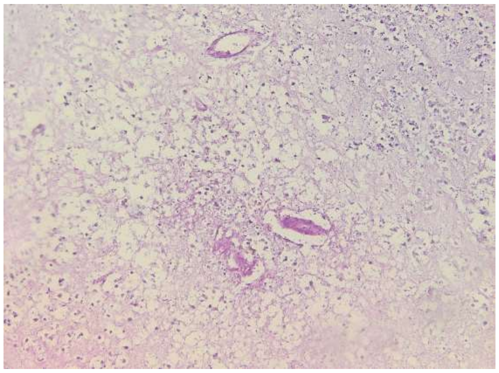

Anterior visual pathways necrosis was well documented, especially in pituitary tumors, craniopharyngioma, parasellar meningiomas or optic nerve tumors [17]. In generally, visual field defects occur after one or more years after the radiotherapy stopped. Corticosteroids, surgery, bevacizumab and Hyperbaric Oxygen Therapy (HBO) are all possible good treatment options for symptomatic RN. The treatment goal is to provide the patient with resolution of neurologic symptoms, with the least toxicity and invasiveness [18]. Corticosteroid therapy was used to diminish the cerebral edema and cytokine release after radiation. Hyperbaric oxygen is considered to be useful in raising the tissular oxygen supply and for initiating tissue healing.

Diffuse, infiltrative, low grade gliomas (LGG) represent a clinical, histopathological and molecular distinct group with a very controversial management [19]. LLG patients may survive up to 20 years from the moment they were diagnosed, but there is the risk of tumor evolution into higher grades, eventually leading to neurological deficits and death [20]. Diffuse astrocytomas include the fibrillary, gemistocytic and protoplasmic types [21]. The fibrillary one is the most widespread and it presents a homogenous cellular population and moderate uncharacteristic nuclear modifications. Mitosis rate in grade II OMS astrocytomas is very small [22]. Clinically, the patient presents epileptic seizures, focal deficits occurred after many years and intracranial high pressure, especially in the subtentorial and intraventricular location. Conventional MRI is useful for guiding the biopsy and the resection, for radiotherapy and treatment monitoring. The usual method is MRI without contrast media, but when we add contrast it reveals changes in tumor grade transformation, better. In LGG spectroscopy shows high choline (high turn-over) and low NAA, reflecting neuronal loss [23]. Even though, similar results can be seen in non-tumor lesions. MRI is used rather for guiding the biopsy in an intense active area than for monitoring the evolution. 18 Fluorodeoxyglucose PET scan has a limited value as long as LGG indicates a low caption comparative to normal cortex [24]. The role of PET-FDG is, however, demonstrated in differentiating radionecrosis from tumor recurrence. More frequently used are [11C] methionine-PET scans given the fact that methionine is captured by tumor cells showing their proliferation process, thus giving a better contrast. PET-MET is also used for differentiating LGG from non-tumor lesions [25].

Neuroplasticity defines the ability to regain function after a neuronal lesion due to neuronal structures that take over the function of the damaged area and to nervous tissue reorganization (that is based on formation of new connections between neurons and/or new cell synthesis). The theory imposed in 1920 by S. Ramon y Cajal (which stated that once the development stopped, growth and regeneration of the axon and dendrites irrevocably stop) is torn down discredited by present scientific data [26]. Altman and co. showed the ability of some brain areas (such as hippocampus and olfactory bulb) to generate new neurons and they called this process neurogenesis [27]. Later, proves appeared that shown this process is influenced by factors like age, stress, physical condition [28].

Multipotent stem cells divide periodically in our brain giving birth to other stem cells or to adult cells. The newly formed stem cells move into areas that allow maturation. Approximatively 50% of them will die without reaching maturity. The others will become glial cells or neurons depending on the necessities of the brain area where they migrated. Fred H. Gage, emphasized in his review, that the necessary time for a stem cell to become functional and adapted to brain is about a month [29]. In this way we could explain full recover of the patient with left lateral homonymous hemianopia a month after the debut. A series of growth neuronal factors play an important role in keeping stem cells alive, mature and functional. Regaining cortical functions can be somehow explained by reorganization of cortical areas [30]. This process takes also place in the areas near the lesion or near the contralateral homologous cortex. Visual cortex reorganization after focal lesions implies plasticity mechanisms accompanied by excitation/ inhibition imbalances and changes in dendritic and axonal divisions [31]. Studies demonstrated a decrease in GABA inhibition and a growth of glutamate answer mediated by NMDA receptors in adjacent areas with the afflicted visual cortex [32].

CONCLUSIONS

Combined radiation and chemotherapy rises many times the cerebral necrosis risk, which may occur months or years later. Patient management should use specific and sensitive diagnostic tests. The differential diagnosis between tumor recurrence and radionecrosis is very important. Based on this differentiation, therapeutic attitude is different. For diagnosis certainty, tumor molecular biology tests are very useful, especially that current imaging techniques still cannot make a clear distinction. Low-grade gliomas should be better supervised by complete excision of the tumor, where the situation allows. Other therapeutic interventions, like chemo and radiotherapy, especially in the early years of evolution, should be used with caution, given their unpleasant effects. Radionecrosis should be treated promptly with corticosteroids, surgery, hyperbaric oxygen or bevacizumab.

Also, we need a plan for neurorehabilitation that assist in the neuroplasticity. All these compete to increase life expectancy and quality of life.

Conflict of interests

The authors declare that they have no conflict of interests.

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CLINICAL PRACTICE

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Oncological follow-up after radical prostatectomy

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Abstract: *Radical prostatectomy is the gold standard treatment for patients with localized prostatic cancer, but often the pathological diagnosis reveals a locally advanced stage.*

The aim of this paper is to present the oncological follow-up after radical prostatectomy on a group of 42 patients, patients which have been diagnosed after surgery with a more aggressive stage, locally advanced PCa. Over a period of four years 136 patients have undergone radical prostatectomy. The pathological examination has established that 42 patients presented extracapsular invasion and/or lymph node invasion. 18 patients (pT3-4N0M0) have undergone radiotherapy alone and 24 patients (8 pN1 and 16 pT3-4N1M0) have received androgen deprivation therapy in association with radiotherapy. Over the next two years 15 patients presented biochemical recurrence. For these patients chemotherapy was initiated, but in two cases PSA increase has been observed during periodic evaluation. Although it is not uncommon that postoperatively the histopathological examination establishes a more aggressive disease stage than that established before surgery, radical prostatectomy remains the solution for the localized prostate cancer, solution that can be boosted by radiotherapy and/or androgen deprivation therapy.

Keywords: *prostate cancer, oncological follow-up, complications*

INTRODUCTION

Prostate cancer is one of the most frequent, if not the most frequent neoplasic pathology encountered in men with ages over 60 years, its incidence increasing with age. The steady growth of life expectancy, due to a healthier life-style that more and more people tend to adopt, will automatically lead in the fallowing decades to a higher rate of prostate cancer detection [1-3].

Since its introduction, more than 20 years ago, prostate specific antigen (PSA) screening has significantly increased the number of patients diagnosed with localized prostate cancer.

The discovery of this marker at the beginning of the 1970s by Dr. Ablin and his colleagues has radically changed, in a good manner, the prognostic and the lives of the patients who suffer from this disease, but it took almost two decades to approve its use as a screening marker.

Before the FDA approval and introduction of the PSA as a tool to evaluate the treatment response and disease recurrence in 1986 and later in 1994 as a screening test for prostate cancer, the patients were usually diagnosed in advanced stages, with metastatic disease, associating a poor prognostic, less than 5% of the patients being curable [4].

Currently, due to early detection of prostate cancer,

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the curability rate of this pathology has significantly improved, the patients being diagnosed more frequently with lower disease stages, thanks to PSA screening. Radical prostatectomy among other therapeutical options (radiotherapy, brachytherapy) offers good disease control and even good cure rates, with low rates of local recurrence.

Radical prostatectomy is the most frequent therapeutical option used in the management of patients diagnosed with localized prostate cancer and with a life expectancy that is estimated to be higher than 10 years, being considered the gold standard treatment for this stage of disease [5]. Often, despite the fact that the preoperative evaluation establishes a localized stage, the postoperative pathological examination reveals a more advanced stage of prostate cancer.

The purpose of this article is to present the oncological follow-up in a group of patients diagnosed after radical prostatectomy with a locally advanced prostate cancer stage, after initially establishing a localized stage during the preoperative evaluation.

MATERIAL AND METHODS

We have retrospectively analyzed the experience of our clinic regarding open radical prostatectomy for localized prostate cancer and we have encountered that over a period of four years, from January 2013 to January 2016, we have performed 136 radical prostatectomies. Only the patients diagnosed during the preoperative evaluation with localized prostate cancer have been included in this study.

We have analyzed the PSA value, the result of the pathological examination after prostatic biopsy, pre- and postoperatory staging and Gleason score and as well as the short and long term oncological results and complications.

The preoperative diagnostic of prostate cancer was established by transrectal ultrasound prostate biopsy, this procedure being done in patients who presented repeated PSA values above the upper limit of the PSA reference interval or for the patients who presented a clinical suspicion of prostate cancer during digital rectal examination. After the pathological examination confirmed the cancer diagnostic all the patients have undergone pelvic contrast MRI and bone scan.

The prostatectomy was performed 6 to 8 weeks after the transrectal biopsy and 12 weeks after transurethral prostatic resection (TUR-P), for the patients diagnosed with prostate cancer after the pathological examination.

The postoperative evaluation was done at one month after the surgery and afterwards periodically at three months in order to evaluate the PSA level, the urinary continence status and erectile function. Biochemical recurrence was defined as two consecutive increases of the PSA level over 0.2 ng/mL.

RESULTS

The mean age of the patients included in the study was 63.09 years, varying between 51 and 78 years. The number of the prostatic tissue fragments harvested during prostatic biopsy puncture ranged between 6 and 12, being higher in saturation biopsy (24 to 32 fragments).

The mean operative time was 130 minutes, ranging between 100 and 300 minutes. Intraoperative bleeding has decreased over time, due to increased surgical experience, from 1500mL (in the first prostatectomies) to less than 200 mL in more recent surgeries. No major complications have occurred during the procedure.

As early postoperative complications we report macroscopic hematuria in less than 10% of the cases, wound infection (4%), prolonged lymphatic drainage in 8% of the cases and anastomotic fistula in 3 patients. Regarding the late postoperative complications (one year after the surgery) we have encountered: urinary incontinence, erectile dysfunction and lower urinary tract symptoms (LUTS).

The degree of urinary incontinence was evaluated periodically at one month after the surgery and afterwards at 3, 6 and 12 months using the International Continence Society male questionnaire. The degree of urinary continence was established by the number of urinary pads used during 24 hours. Out of 136 patients who have undergone radical prostatectomy only 3 patients presented total urinary incontinence, during the postoperative follow-up, 38 patients being partially continent and 95 patients being completely continent. Complete urinary continence was defined as the absence of the need for urinary pads during 24 hours and as well as the absence of urinary leakage during daily activities.

Erectile function was evaluated using the IIEF- 5 questionnaire. It was noticed that 78% of the patients for whom we have practiced bilateral nerve sparing surgery and 30% of the patients with unilateral nerve sparing presented satisfactory erections. For the patients with erectile dysfunction we have recommended phosphodiesterase 5 inhibitors.

9% of the patients presented moderate or severe LUTS, due to urethral stricture or due to bladder neck sclerosis. These postoperative complications were managed endoscopically.

42 patients presented after the pathological examination extracapsular invasion and/ or lymph node invasion. Therefore these patients presented in fact locally advanced prostate cancer and not the localized stage that was established during the preoperative evaluation. 18 patients out of 42 with extracapsular invasion (pT3-4N0M0) have undergone radiotherapy alone and 24 patients with lymph node invasion (8 patients with pN1 and 16 patients with both extracapsular and lymph node invasion) have undergone radiotherapy and androgen deprivation therapy (complete androgen blockade for two years).

15 patients presented successive increases of the PSA value at more than 2 years after treatment initiation, cases for which chemotherapy was recommended. 2 patients presented castration-resistance prostate cancer. Their therapeutical management consisted in bilateral orchiectomy and anti-androgen therapy. Two patients out 15 were resistant to the chemotherapy.

DISCUSSIONS

PSA detection has proven over the years to be a vital, cheap and reliable investigation for the detection of prostate cancer. Early PSA screening has led to a significant increase of the rate of patients diagnosed with localized prostate cancer, nowadays approximately 90% of this patients being curable [6].

Several studies have compared over the years the efficacy of radical prostatectomy and radiotherapy in patients with localized prostate cancer and have concluded that both therapeutical options provide good results regarding the recurrence free survival, cancer specific survival, overall survival and biochemical recurrence, especially for the low risk and intermediate risk patients [7-10]. In a study comparing the efficacy of radical prostatectomy versus intensity-modulated radiotherapy, Aizer concluded that for the high risk patients the radiotherapy approach provided better results when compared to the radical prostatectomy group. No significant differences were found between these two types of treatment for the low risk and intermediate risk patients [11].

Schreiber et all reported a better biochemical control for the patients with high risk localized prostate cancer for whom radiotherapy was the treatment of choice when compared to the radical prostatectomy group after a follow-up period of 6 years (67.4% RT vs 41.3% RRP). Regarding the low and intermediate risk groups he reported no significant differences between radiotherapy and radical prostatectomy, despite the fact that in both risk groups radiotherapy presented initially better results. The rates of biochemical control after 6 years for low risk and intermediate risk were 90.3% and 82.6% without any salvage therapy, while for the radical prostatectomy group the rates were 85.6% and 59.7% without salvage radiotherapy and 90.3% and 74% with salvage radiotherapy [7]. Petrelli reported in a 2015 meta-analysis, a better overall and cancer specific survival in high risk patients treated with radical prostatectomy when compared to radiotherapy [12].

In a 2015 study regarding the comparative outcomes between radical retropubic prostatectomy, high dose rate brachytherapy (HDRBT), cryoablation and high-intensity focused ultrasound (HIFU) for localized prostate cancer it was concluded that HDRBT presented the worst outcomes (cancer control, biochemical recurrence, salvage treatment free rate and metastases free rate) when compared to the alternatives [13].

CONCLUSIONS

Radical retropubic prostatectomy is the gold standard in the treatment of localized prostate cancer and in some cases, in the locally advanced prostate cancer.

Radical prostatectomy provides a significant improvement of the survival rate, for the cases where the surgical indication and technique have been correctly established and carried out.

Although it is not uncommon that postoperatively the histopathological examination establishes a more aggressive disease stage than that established before surgery, radical prostatectomy remains the a solution for the localized prostate cancer, solution that can be boosted by radiotherapy and/or androgen deprivation therapy.

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EDITORIAL

One health

Victor Purcărea

Prof Eng VICTOR PURCĂREA

Executive Editor

Life, freedom, and pursuit of happiness are mentioned in the Declaration of Independence and engraved in the quote "***think big, you can do anything***" that defines the American spirit. Health is the element without which (sine qua non) obtaining all these would not be possible, and which is essential in the longevity and quality of life. Health is a priceless good in the economic and social development and in supporting the economies and powerful democracies, both at the individual and at the population level. Moreover, health is important for the security and prosperity of the nation. "We hope that, during the elections for the Congress, in 2018, the candidates will first think about the American citizens, thus relaunching the American tradition "think big, you can do anything", by applying the spirit of the winner in the reform of the authentic healthcare services", affirmed the famous professor Eliot Sorel (Senior Scholar Healthcare Innovation & Policy Research, GWU School of Medicine & School of Public Health and Founder Conflict Management Section WPA) in his article "*Health: its importance in Life, Freedom and Pursuit of Happiness*", which was published online on April, 14 2017.

Health of people represents a priority both for the European Union. The EU's main objective regarding the public health is the improvement of the health state of the population and the prevention of the diseases. This objective is mentioned in article 168 of the Treaty regarding the Functioning of the European Union (the Treaty of Lisbon), which also offers examples of activities which the EU cannot undertake in order to achieve the objectives. Among these, the following can be mentioned: controlling epidemics, studying their causes and their prevention. EU also supports drugs abuse control, as well as the efforts of the member states in the fight against smoking and of excessive alcohol consumption. Another objective is the increase in the degree of complementarity of the healthcare services in the border areas.

The remarkable progress of healthcare doubled by better life conditions continuously lead to a raise in the life expectancy of the people everywhere worldwide.

In contrast to this positive evolution, the diseases threatening the health of the people keep on developing, diversifying and, implicitly, the expenses and the effort to preserve an appropriate state of health continually raise and use a huge amount of resources that are hardly sustained.

In this context, a new global medical concept, "One Health", started being organized worldwide, at the same time trying to find the causes for the evolution of the diseases and their spreading, looking to recommend the adequate solutions in the fields in which the human health is more and more assaulted.

Among the accomplishments in this field, the International Center for Food Chain and Networks

Research – **FoodNetCenter Bonn**, founded in 2006 in the University of Bonn, can be reminded. The center's main objectives are the following: interdisciplinarity, contribution to the evolution of the university profile, research, and development.

Moreover, it has three *Focus Groups*, which are base units for the organizational structure, fully integrated in the University: *Food Waste*, *"One Health"* and *Responsible system innovation.* The main common problem of all the three Focus Groups is the fact that the food request will double until 2050 and, the interdisciplinary groups of interest could try to solve the problem by contributing to a responsible and solid production, processing, and food consumption.

The University of Bonn (the International Center for Food Chain and Networks Research – FoodNetCenter Bonn) has involved from the beginning in founding, in 2015, the Education and Qualification Alliance – ***EQA,*** which acts at the level of the network by supporting the knowledge suppliers, the standardization and certification organisms in promoting quality, food safety, sustainable development, social and corporate responsibility and, ***"One Health",*** only one Health for People, animals and the Environment, represents the new global concept that reunites preoccupations of the specialists in all the fields that threaten health, including Food, Nutrition and Occupation, with two main action directions: Primary Prevention of Life and Life Post-Therapy.

From the initiative, giftedness, and generosity of some important personalities in the social, cultural, and scientific life, the Association **"ONE HEALTH-New Medical Concept"** Romania was founded in Romania in May 2015, being an associated member of the ***Federation*** of the ***European Academies of Medicine*** (FEAM) (www.onehealth.ro).

In our country, as well as in others, the *"One Health"* concept implies the summing of the efforts and the achievements that *Human Medicine, Veterinary Medicine, Environment Medicine, Food and Nutrition Medicine, Occupational Medicine, Compared Medicine (zoonoses and compared oncology) record with the Primary prevention of life, promoted as a major vector*, together with their application in medicine, while desiring a strong health state with costs as low as possible.

The objectives of the activity are the following: creating a national network of research and development and implementation in the field of One Health, which is internationally affiliated; promoting and developing scientific research projects; establishing professional and organizational relationships with research-development and education structures; educating the people who work in the field of compared medicine, food safety, environment medicine and bioeconomy, as well as cooperation in the scientific academic field; organizing events and activities that would favor the exchange of scientific ideas in the field of compared medicine, food safety, environment medicine and bioeconomy; implementing new scientific guidelines of the involvement of "life environment" in the "one health"-human medicine-veterinary medicine-environment medicine relationship, in order to achieve the concept of eco-prevention and eco-therapy in the initiative of developing the strategy of primary prevention of diseases in humans and animals; promoting postgraduate education in the field of compared medicine and stimulating the activity of technological transfer in the field of compared medicine.

In order to achieve these goals, an efficient and continuous implementation and international recognition, the Association founded departments on specialties such as *Department of Management, Bioeconomy and Law; Department of Compared Medicine; Department of Human Medicine; Department of Veterinary Medicine; Department of Environment Medicine; Department of Food Safety; Department of Zoonoses; Department of Pharmaceutical Production and Biological Drugs; Department of Scientific Research; Department of Education; Department of Non-Conventional Therapies; Department of Compared Oncology; Department of Radiology in Medicine; Department of Phytopathology* and *Department of Accreditation*.

ONE HEALTH New Medical Concept Association has already organized its first prestigious scientific meeting, One Health National Conference 2017, in Târgu-Mureș, with the support of the University of

Medicine and Pharmacy in Târgu-Mureș, the Academy of Medical Sciences in Romania, Spiru Haret University, Mureş County Council, College of Physicians in Romania and ***Romanian College of Veterinarians***, the President of Honor being Andre Laurent Parodi – Academy of Medicine in France, and, the executive presidents being Acad. Nicolae Manolescu – President of OHNMC Romania, Prof. Leonard Azamfirei, MD, PhD – Rector of Târgu-Mureș University of Medicine and Pharmacy and Prof. Irinel Popescu, MD, PhD – President of the Academy of Medical Sciences in Romania. Next year, the Association will also organize "ONE HEALTH - 2nd European Inter-regional Conference, 5–8 September 2018, under the high patronage of the Government of Romania. The organizers of the event will be Spiru Haret University, Bucharest; ONE HEALTH - New medical concept Romania and the University of Veterinary Medicine Hungary – Budapest. The area of interest will be the following: Environmental medicine; Antimicrobial resistance; Comparative medicine; Food safety and nutrition medicine; Comparative oncology and Zoonoses. The Presidents of Honor will be Prof. George GRIFFIN, MD, PhD, President of the Federation of European Academies of Medicine (FEAM) and Member of the British Academy of Medicine; Prof. Bernard CHARPENTIER, MD, PhD, Past President of the Federation of European Academies of Medicine (FEAM) and Member of the French Academy of Medicine, Prof. André-Laurent PARODI, DVM, PhD, Honorary President of the French Academy of Medicine and Honorary Director of Alfort National Veterinary School, France, Prof. René CARLSON, DVM, PhD, Past President of the World Veterinary Association AVMA and Director of International Affairs, and the Executive presidents will be Prof. Nicolae MANOLESCU, DVM, PhD, Correspondent Member of the Romanian Academy, Correspondent Member of the French Academy of Medicine and President of the Romanian One Health New Medical Concept Association; Prof. Stefan CONSTANTINESCU, MD, PhD, Correspondent Member of the Romanian Academy, Professor at the Université Catholique de Louvain, Brussels, Belgium, Vice President of the Federation of European Academies of Medicine (FEAM) and Vice President of the Romanian One Health New Medical Concept Association; Prof. Peter SOTONYI, MVD, PhD, Rector of the University of Veterinary Medicine, Budapest, Hungary; Assoc. Prof. Aurelian A. BONDREA, PhD, Rector of Spiru Haret University, Bucharest, Romania; Cheryl STROUD, MD, PhD, Executive Director of One Health Commission, and Prof. Florian POPA, MD, PhD.

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VARIA

Orthodontic treatment and periodontal disease – between advantages and disadvantages

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Abstract: *Orthodontic treatment and periodontal disease can have an inter-conditional relationship, particularly when the accumulation of bacterial plaque, favoured by orthodontic appliances and poor oral hygiene can cause periodontal inflammation. The objective of the study is to highlight the influence of the risk factors' polymorphism on the therapeutic response in periodontal disease. 64 patients with mixed or definitive dentition and poor oral hygiene were included in the study, of whom 22 patients followed treatment with fixed orthodontic dispensers, 22 patients were treated with mobile orthodontic braces and 20 were non-attendants. From all patients gingival crevicular fluid was collected and assessed, before and after applying a chlorhexidine-based gel antiseptic agent.*

Keywords: *periodontal disease, orthodontic treatment, chlorhexidine gel, gingival fluid*

INTRODUCTION

Periodontal disease inflicts a wide range of oral changes, ranging from gingival tissue inflammation to tooth loss. During some orthodontic treatment protocols patients may not to compliant to keeping a good oral hygiene, possibly due to the difficulties that certain orthodontic appliances may impose on efficient teeth brushing [1]. Microbiological patterns involved in the genesis of periodontal disease are more common in orthodontic treatment patients, so the bacterial plaque is left undisturbed for a long period of time around the orthodontic appliances and can grow in bacteria number and pathogenic potential. Microbial periodontal pathogens, especially Gram-negative bacteria, are more frequently found around fixed/mobile orthodontic appliances [2]. Periodontal disease may have different clinical forms, with a wide range of manifestations, from gum tissue damage to periodontitis, which can even lead to tooth loss in some cases [3].

The clinical manifestations and evolution of periodontal disease may vary in accordance with the intensity of the bacterial challenge. In orthodontic treatment patients, the periodontal tissues are also challenged by the orthodontic forces which generate teeth movement. During teeth movement, all periodontal structures, including the gingival, the periodontal ligament and the alveolar bone are under constant mechanical stress [4]. This mechanical stress can cause a decrease of the tissue's defensive capabilities. Combined with the increased bacterial challenge, caused by poor oral hygiene, this creates very favourable conditions for the onset of periodontal disease [5]. Consequently, a good understanding of the periodontal disease's existing aetiology and risk factors is required for a correct diagnosis and

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treatment plan for a patient undergoing orthodontic therapy.

The objective of this study was to highlight the influence that certain risk factors have on the therapeutic response in periodontal disease in orthodontic patients and to assess the local impact that the use of an anti-plaque agent has in these patients.

MATERIAL AND METHOD

The study included a group of patients who were clinically examined and expressed similar symptoms during the first visit to the dental office. In order to collect the data obtained from the anamnesis, observation charts were prepared for each patient. The inclusion criteria used for the study were the following: (1) patients who were treated or not with orthodontic braces/mobile dispensers; (2) age 12-20 years; (3) the presence of mixed or definitive dentition; (4) the presence of gingivitis subjective and objective clinical signs (gingival bleeding, gingival oedema, halitosis). Following the clinical examination, a bacterial plaque index was calculated for the participating patients, its values being directly proportionate with the degree of gingival inflammation.

A total of 64 patients with mixed or definitive dentition and poor oral hygiene were selected, being divided into 3 study groups: (1) 22 patients carrying a mobile orthodontic device; (2) 22 patients with fixed orthodontic appliances and (3) 20 patients who did not require or have refused orthodontic treatment.

Before initial periodontal therapy (airflow cleaning and professional brushing) gingival crevicular fluid was sampled from the gingival sulcus of the participating patients. For sampling sterile paper, cones of 20 mm were kept for 30 seconds in the periodontal pocket/gingival sulcus, after the pre-removal of the supragingival bacterial plaque, in order to avoid contamination of the sample with supragingival bacteria. The collection and transport of the biological products and the stages of microbiological diagnosis were performed by the clinician and their assistant.

The results of microbiological investigations depend to a large extent on the quality of the sampling procedure, which needs to follow specific rules:
(a) the patient was informed about the aimed analysis and about the correct way of sampling procedure;
(b) the sampling instruments were sterile, disposable and adapted to the investigation;
(c) the pathological product was harvested prior to administration of any topical or general topical treatment; it is harvested from areas where it is most likely to find the bacterium that produced the infection;
(d) the outside of the container was disinfected if it has been contaminated during sampling procedure;
(e) the name of the patient and his provenance, the type of biological product, the date and time of collection was written on the container;
(f) each pathological product was accompanied by a bulletin containing information about the sample and the patient;
(g) transport was as quick as possible to the laboratory to prevent the degradation of the pathological product;
(h) transport media used ensured good survival of bacteria;
(i) for the oral cavity, the sampling procedure consisted in carefully cleaning the edge of the gum and tooth surface to remove saliva and food debris, afterwards a periodontal scalpel was used and the lesion secretion was removed;
(j) transport was carried out using an anaerobic transport system in less than 2 hours from sampling.

Microbiological tests were performed to detect aerobic and anaerobic gram-positive and gram-negative bacteria. After the initial periodontal treatment, the patients were randomly re-divided into two groups, as follows: (1) 12 patients with orthodontic devices (six fixed devices, six mobile devices) and (2) 12 appliance donors, who received applications of a chlorhexidine-based gel (Elugel 0.02%, Pierre-Fabre, France). The other 24 patients were only instructed for good oral hygiene and monitored. The ethical standards of medical research and GDPR (Global Data Protection) and the approval of the Ethics Committee of the Faculty of Dentistry of the “Titu Maiorescu” University in Bucharest were followed by the present study.

RESULTS

The distribution of the participating patients regarding orthodontic treatment type was similar between the patients with mobile and fixed orthodontic appliances (Figure 1).

Figure 1: Selection criteria: fixed/mobile orthodontic handlers/ carriers

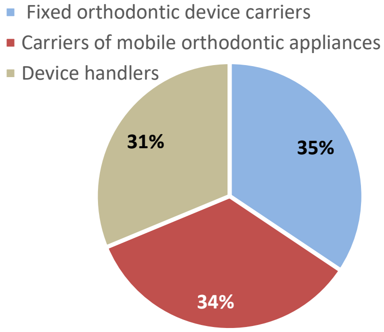

The microbiological assessment revealed bacteria of the violet periodontal complex: (Veillonella parvula, Actinomyces odontolyticus), yellow complex (Streptococcus spp), green complex (Eikenella corrodens, Capnocytophaga spp.) red complex (Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola) and of the orange complex (Prevotella intermedia/nigrescens, Micromonas micros, Campylobacter rectus, Fusobacterium nucleatum) (Figure 2). Compared to the microbial flora normally found in gingival tissue of healthy individuals with no periodontal problems, microbiological tests revealed the exaggerated growth of germs in the case of fixed carriers and a significant increase in the case of mobile carriers, as shown in Table 1.

Streptococcus spp, P. gingivalis, and F. nucleatum predominantly occured in fixed-bearer carriers, while Treponemma is constantly present whether or not the patient is treated with fixed or mobile devices. The Actinomyces spp were present within the saprophytic flora and does not develop during orthodontic treatment.

Figure 2: Bacteria type frequency in periodontal heath vs. gingival inflammation (%)

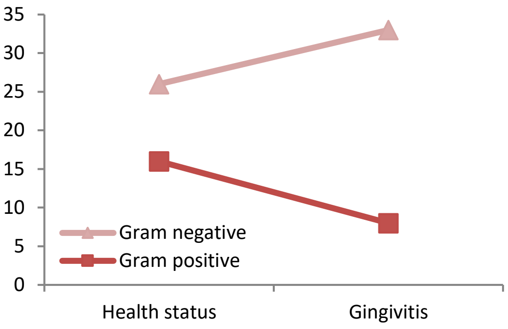

Table 1: Distribution of bacterial pathogens according to study groups

Pathogen	Health	Not carriers of orthodontic braces	Carriers of orthodontic braces	Carriers of mobile devices
Streptococcus	+++	+	+ ++	+ +
Actinomyces	+++	+	+	+
Veillonella	+	+	+	+
Aggregatibacter actinomycetemcomitans	-	++	+	?
Capnocytophaga	+/-	+/-	+/-	+/-
Treponeme orale	+/-	+++	+++	+++
Porphyromonas gingivalis	-	+	+++	++
Prevotella intermedia	+/-	+	+++	++
Tannerella forsythia	-	++	++	?
Fusobacterium nucleatum	+	++	+++	++

Figure 3: Patients treated with chlorhexidine-based gel antiseptic substance

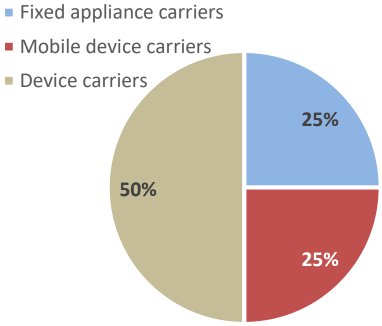

Following the application of the chlorhexidine-based gel antiseptic substance to orthodontic patients, it was found that after two weeks the results of the microbiological analysis of the gingival sulcus exudate revealed a reduction of the number of pathogenic bacteria in the orthodontic treated subjects and their disappearance in the non-attendant devices (Figure 3).

For the patients who did not benefit from the application of chlorhexidine-based gel substance, it was found that for the orthodontic handlers the microbial flora was still highly present. After 30 days, when the patients were reassessed, it was discovered that the pathogenic flora disappeared in all patients treated with chlorhexidine-based gel antiseptic substance, both with orthodontic treatment or not.

DISCUSSION

According to the literature, a study by Al-Bazi et al. from 2016 demonstrated that the formation of dental biofilm is a result of the increase in the number of microorganisms on the tooth surface, which was found to be 2-3 times higher in patients under orthodontic treatment than in young people without orthodontic treatment [6]. Previous studies have shown that regular dental brushing and the use of dental floss alone do not decrease levels of S. mutans in patients treated with fixed orthodontic appliances [7]. In our study, subjects treated with mobile orthodontic devices, compared to healthy individuals without periodontal problems, revealed an exacerbated growth of germs in the case of fixed-bearer carriers and a significant increase in the case of the mobile-bearer carriers. Some bacterial species (Streptococcus, Porphyromonas, Prevotella and Fusobacterium) are found mainly in fixed-bearer carriers, while Treponemma is constantly present whether or not the patient is a carrier of fixed or mobile devices and Tannerella forsytia is absent in patients' exudates.

Different protocols have shown the ability of CHX (Chlorhexidine) to inhibit the formation of dental biofilm and to reduce plaque development [8]. Randomized, controlled clinical trials demonstrated the efficacy of 0.50% and 0.75% CHX to control gingivitis and bleeding in orthodontic patients, concluding that lower concentrations of chlorhexidine may reduce the risk of tooth colouring, without compromising its effectiveness level against pathogenic bacteria [9]. In our study, applying the chlorhexidine-based gel antiseptic substance significantly reduced the number of bacterial colonies in all patients of the study. However, antiseptic treatment should not replace dental mechanical treatment, but be applied in a complementary manner [10]. Combining mechanical treatment with local application of antiseptics gives statistically better results than each of them taken separately does.

CONCLUSIONS

Given the results of the study, we can conclude that for the patients treated with orthodontic appliances, either fixed or mobile, it is impossible to completely clean all oral surfaces and to decrease the number of microbial colonisation without the application of local antiseptics during the treatment. The results of the study can be used to prevent periodontal disease in patients with orthodontic treatment.

Conflict of interest:
Nothing to declare.

Contribution Note:
All the authors equally contributed to the drawing up of the present paper.

Patient participation at the study respects ethical standards in medical research and GDPR.

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VARIA

Osteoporosis and the fracture risk in systemic lupus erythematosus

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Abstract: *Osteoporosis is a systemic skeletal condition, characterized by a microarchitectural degradation of bone density and quality, giving low resistance and an increased risk of fracture. Osteoporosis is an important public health problem, with considerable medical, social and economic impact. Data accumulated in recent years demonstrate a significant incidence of osteoporosis in the group of patients with major collagen diseases; of these, Systemic Lupus Erythematosus (SLE) is the prototype of autoimmune disease in which osteoporosis and associated complications have a significant clinical impact.*

Although additional studies are needed to deepen the relationship between SLE - osteoporosis - associated fractures, we believe that these patients should be managed from the early stages of the disease.

Keywords: *systemic lupus erythematosus, osteoporosis, risk factors, fragility fracture, therapeutic principles*

INTRODUCTION

Osteoporosis is a systemic skeletal condition, characterized by a microarchitectural degradation of bone density and quality, conferring low resistance and an increased risk of fracture [1, 4]. Osteoporosis is a significant public health problem, with considerable medical, social and economic impact, through the associated costs. The prevalence of osteoporosis in Romania in the population over 50 years of age is 20.5% in women and 6.2% in men [1].

Osteoporosis is an asymptomatic disease. The main clinical manifestation and at the same time complication of osteoporosis is the fragility fracture. Worldwide, it causes over 9 million fractures annually, approximately one osteoporotic fracture every three seconds [2].

Classification of osteoporosis [1, 3, 4]:

- Primary osteoporosis: type I (postclimax) and type II (elderly).
- Secondary osteoporosis: occurs in association with other conditions or with the use of certain drugs.

Causes of secondary osteoporosis [3]:

- Endocrinological: thyrotoxicosis, hyperparathyroidism, hypogonadism, Cushing's syndrome, insulin-requiring diabetes, Addison's disease, sarcoidosis.
- Gastrointestinal: severe liver damage, primary biliary cirrhosis, gastrectomy, malabsorption syndromes.
- Metabolic and nutritional: hypophosphatemia, hypercalciuria, hemochromatosis, amyloidosis, parenteral

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nutrition.

- Hematological disorders and neoplasms: hemophilia, thalassemia, myelomatosis, lymphoma and leukemia, tumors secreting PTH-like peptides.
- Inflammatory diseases: rheumatoid arthritis, systemic lupus erythematosus (SLE), etc.
- Others: chronic kidney disease, cardiovascular, neurological diseases, etc. [4].
- Medications: glucocorticoids (over 5 mg/day prednisone or equivalent, more than three months), anticonvulsants, proton pump inhibitors, heparin, antidepressants, etc.

This classification in primary and secondary is not delimited. Sometimes the causes of secondary osteoporosis can coexist with post-menopausal status or with aging.

Classical risk factors for osteoporosis

Modifiable risk factors:

- Nutritional deficiencies: Diet influences bone mass and risk of fracture. Nutrients with an important role in bone metabolism are calcium, vitamin D and, last but not least, proteins. Low protein intake is an essential determinant of bone mass. Malnutrition can increase the risk of falling both by affecting coordination and reducing the muscle strength [5-9].
- Physical inactivity: A significant cause of bone loss. It has been observed that forced immobilization in healthy volunteers or in patients with neurological disorders such as paraplegia, leads to decreased bone mineral mass. The negative effect of immobilization is much greater than the potential benefit of exercise on an already outpatient subject [10].
- Weight: A low body mass index correlates with a low bone mineral density (BMD), probably due to decreased peripheral estrogen production by adipose tissue and a lower mechanical load on the skeleton that metabolically influences bone composition [3].
- Smoking: After menopause, bone mineral density in women who smoke decreases by two percent/decade compared to non-smokers [12]. A much more significant loss of bone mass was observed in smoking men, especially in the femoral head. The risk decreases with smoking cessation but becomes significant only after ten years of withdrawal [13].
- Excessive alcohol consumption: Indirectly, influences the bone metabolism by decreasing protein and calcium intake, impaired gonadal function, mobility (increases the risk of fracture by falling), and directly, through the toxic effect on the osteoblast [3].

Non-modifiable risk factors:

- History of falls: Falls are the most common cause of osteoporotic fractures. The annual risk of falling increases by about 20% in women between 35 and 49 years and is over 30% in eldery men [14]. The risk of falling is directly correlated with the number of comorbidities (e.g., neurological disorders, cardiovascular diseases, urinary incontinence, depression, etc.). Also, medications such as hypnotics, sedatives, or antidepressants increase the risk of falling [14-17].
- Age over 65 or under 65 who associate several risk factors.
- Women: a higher risk of osteoporosis. Bone loss is 0.86-1.21% and increases after menopause, unlike men, where the loss is about 0.04-0.90% [18]. Despite this, secondary osteoporosis appears to be more common in men [19, 20].
- Fragility fractures in personal history: the presence of an osteoporotic fracture increases the risk of subsequent fractures. Thus, in both men and women who have suffered a distal fracture of the forearm, the risk of subsequent fractures of other skeletal regions is approximately doubled [21-24].
- Genetic predisposition: about half of the total bone mass, as well as bone architecture and geometry (elements relevant for bone strength), can be genetically determined [25, 26].

Preliminary data studied reveal a significant incidence of osteoporosis in patients diagnosed with collagen diseases. Of these, SLE is the prototype of autoimmune disease that predominantly (but not exclusively) affects young women, but also men. For this group of patients, osteoporosis is a frequent long-term complication.

With the progress of therapeutic strategies and the introduction of new effective treatments in SLE, organ complications are much better controlled, which has led to a significant increase in life expectancy. In this context, osteoporosis and associated complications have a greater clinical impact and the therapeutic attitude must focus on this aspect, from the early stages of the disease.

Regarding the method of objectification and osteoporosis diagnosis, there are several techniques for measuring bone mineral density, but the most common is using dual X-ray absorption (DXA). Bone densitometry provides two results: the T score, which provides the result by comparing the patient's bone density to a person with maximum bone density – a healthy 30-year-old, and the Z score, which compares the patient's bone density with that of a person of the same age to assess the degree of bone loss.

PARTICULARITIES OF OSTEOPOROSIS IN SLE

Osteopenia, defined as a T score between 1.0 and −2.5 in the spine and femoral head, has been reported in 25–74% of patients with SLE, while osteoporosis, defined as a T score less than −2.5, in 1.4–68% of patients with SLE in cohort studies [27].

Despite the large variability of epidemiological data, a significantly increased prevalence of low bone mineral density is recognized in SLE patients versus the general population.

Particular risk factors and mechanisms of occurrence

The etiology of bone density reduction and fractures is multifactorial, involving classical, disease-independent risk factors and particular risk factors. Among them, we mention:

- Inflammatory factors: Systemic inflammation contributes to bone loss by influencing the balance between bone resorption and osteosynthesis, causing an increase of osteoclasts activity. In addition, in the active forms of the disease, an increased level of tumor necrosis factor (TNF) and oxidized low-density lipoprotein (LDL) was observed, the latter interfering with the maturation of osteoblasts and activating T lymphocytes [27, 57, 58]. T lymphocytes involved in the synthesis of TNF and nuclear factor kappa B ligand receptor activator (RANKL) stimulate osteoclast differentiation [58]. A low complement C4 level (an indicator of disease activity) was considered a predictor of decreased bone density in the lumbar spine (Hopkins Lupus Cohort) [28]. A 5-year study showed a correlation between the frequency of disease outbreaks and the impact on bone density, confirming the hypothesis that systemic inflammation contributes to bone loss in SLE. In addition, more than half of diagnosed patients develop inflammatory nephritis during the disease, which can progress to renal failure [30]. In severe forms of renal impairment, the appearance of secondary hyperparathyroidism causes an increase in osteoclastic activity and bone resorption [29, 30]. Another mechanism may be a low level of 1.25 dihydroxy vitamin D by decreasing alpha1 hydroxylase activity in the kidney [59].

- Serological factors: Although autoantibodies role in influencing bone metabolism is unclear, anti-Ro may increase the osteoporosis risk [27, 31]. Up to date, no correlation between the presence of anti-dsDNA antibodies and bone mass has been demonstrated. The anti-Sm could have a protective role [31].

- Metabolic factors: Vitamin D deficiency in SLE can be determined by several factors such as photosensitivity, involving avoidance of sun exposure and use of SPF protection screen, kidney failure, disease activity, prolonged glucocorticoid therapy, the presence of anti-vitamin D antibodies (approx. 4 % of patients) [59].

- Hormonal factors: Unlike the general population at risk for osteoporosis, which has an estrogen deficiency (especially women over 50 years), in SLE, there is a predilection for females in the 15-45 age group, with high levels of estrogen. In those patients, low levels of androgen hormones appear to have a stronger influence on bone metabolism than relatively high levels of estrogen [32].

- Genetic factors: The density of bone structures could be influenced by the genetic polymorphisms of the vitamin D receptor - FOK I (FF, Ff, ff). However, a prospective study conducted during 5.3 years did not show a significant difference between the three genotypic groups [33].

The impact of medication on osteoporosis in SLE

Corticosteroid therapy influences in a dual way the bone mineral density in patients with SLE. On the one hand, its risk of inducing osteoporosis is well known, but it may also play a beneficial role by reducing systemic inflammation's effects on the bone. Two extensive prospective studies [29, 34] have shown that bone loss in SLE occurs predominantly in patients treated with at least 7.5 mg prednisone per day.

Hydroxychloroquine. The contribution of this treatment in osteoporosis prevention is not yet well defined. Despite this, the control of systemic inflammation with immunosuppressive drugs is important both by reducing inflammatory outbreaks and also by reducing the dose and duration of corticosteroid therapy. In women with SLE, HCQ use was associated with higher BMD in the lumbar spine [31, 61, 62] and hip [62]. However, more extensive studies are needed in patients with SLE and other rheumatic diseases in which hydroxychloroquine is used.

The risk of fracture in SLE

Fractures can be an important complication in the course of patients with SLE. The data accumulated in recent years demonstrates an increase of 1.2 - 4.7 times the risk of symptomatic fractures than the general population (Echblorn 2013). In the case of vertebral fractures, which can frequently be clinically inapparent, morphometric analysis is important. Morphometric identification of vertebral deformities is also significant for the attending physician because it is associated with significant impairment of quality of life and an increased risk of symptomatic fractures (e.g., hip) [35]. Current studies showed the presence of at least one vertebral fracture in 3.7-50% of patients with SLE [35, 36, 37-42].

The etiology of fractures is multifactorial in patients with SLE, including bone-dependent factors and factors associated with the risk of falling [36].

The main factors associated with the increased risk of symptomatic fractures in SLE are:

- Age;
- Menopause;
- Smoking, alcohol consumption;
- Duration of the disease;
- Kidney damage;
- Decreased BMD;
- Duration of treatment and cumulative dose of glucocorticoids.

In addition, patients with SLE have an increased risk of falling and associated conditions: physical asthenia, joint damage, neurological complications, impaired visual acuity or decreased muscle strength (which may be associated with both inactivity and corticosteroids or vitamin D deficiency) [8, 17].

A particular aspect of SLE patients is the relationship between bone mineral density and fracture risk. The idea of reduced BMD in patients with SLE is generally accepted, but not all studies showed a clear correlation with an increased risk of fracture. In addition, about 1/3 of patients with SLE and vertebral fractures have normal BMD [43, 44]. These data demonstrate the limited value of BMD in estimating fracture risk and the multifactorial etiology of SLE fractures.

PREVENTION AND TREATMENT OF OSTEOPOROSIS ASSOCIATED WITH SLE

SLE is a chronic condition that predominantly affects young women. Therapeutic regimens used to control the disease generally involve extensive and long-term use of glucocorticoids. Thus, decreased bone mineral density and increased fracture risk appear as natural problems in managing this condition. Lifestyle measures and control of risk factors are general principles in the management of osteoporosis and fracture risk. The current recommendations are based mainly on adopting a healthy lifestyle and preventing falls (as a central risk factor in fragility fractures).

Important aspects are: avoiding smoking, alcohol consumption, maintaining an optimal weight and regular physical activity. From a nutritional point of view, the measures are based on optimizing the intake of calcium, proteins and vitamin D, essential for bone mineralization and maintaining an adequate neuromuscular function. Data from the literature recommend a daily calcium intake of at least 1000 mg (1200-1500 mg in patients with corticosteroids), the dosage of serum level and supplementation with 800-1000 IU vitamin D per day and a protein intake of 1 g/kg per day [45].

Pharmacological therapy in SLE is addressed primarily to osteoporosis patients with a history of fragility fracture and glucocorticoid treatment.

The 2010 American College of Rheumatology (ACR) recommendations for preventing and treatment of glucocorticoid-induced osteoporosis include bisphosphonates as the first-line medication for postmenopausal women and corticosteroid patients [45]. This therapeutic class presents consistent evidence of efficacy in increasing BMD and reducing the risk of fracture. The antiresorptive agents inhibit bone resorption by attaching to the active remodeling sites causing apoptosis of osteoclast cells. The main representatives of the class currently used are the second generation drugs (e.g., alendronate, ibandronate, pamidronate) as well as those of the third generation, with much higher potency (e.g., risedronate and zoledronic acid). They are recommended for patients with normal renal function [45] to prevent and treat glucocorticoid-induced osteoporosis. Limited data on the teratogenic effects of bisphosphonates require their administration only to postmenopausal women [46]. In addition, patients should be informed of the risk of complications such as osteonecrosis of the jaw or atypical femoral shaft fractures, especially in patients on corticosteroid therapy [47-49].

Studies have identified new therapeutic targets in osteoporosis, such as cathepsin K, sclerostin, or the RANKL system. Of these, denosumab, an anti-RANKL monoclonal antibody, has entered and prevailed in clinical practice being an option for premenopausal women with glucocorticoid-induced osteoporosis, as well as for patients with SLE and renal impairment [27].

The risk of osteonecrosis of the jaw and atypical fractures remains in this category of therapeutic agents. Potential complications are the rebound fractures phenomenon and the greater tendency towards hypocalcemia [60].

Estrogen replacement therapy to prevent osteoporosis in patients with SLE is not recommended, as it is associated with an increased rate of inflammatory flares and thromboembolic events [50]. Raloxifene, a selective modulator of estrogen receptors, is an option for postmenopausal women with inactive lupus and without a history of thrombotic events or antiphospholipid antibodies [51].

Another treatment, the recombinant human PTH analogs (e.g., teriparatide), may be an option for men and postmenopausal women who have not responded to bisphosphonate therapy and are at increased risk of fracture [53]. The experience in the young adult population, including premenopausal women, is limited, so this treatment should be initiated only when the benefits outweigh the risks. Premenopausal women with glucocorticoid-induced osteoporosis should be considered at high risk of fracture if they have a pre-existing fracture or a combination of risk factors such as low bone mineral density, intensive treatment with glucocorticoids in high doses (≥7.5 mg/day) for at least six months or background disease in acute onset [52]. In patients with glucocorticoid-induced osteoporosis, this drug was associated with an increased BMD and a significant reduction of vertebral and non-vertebral fractures, but not of hip fractures, compared to the group treated with bisphosphonates [53, 52]. The administration must be made with caution in patients with moderate renal impairment [54].

SCREENING AND MONITORING OF TREATMENT

Patients should be monitored during the therapy by periodic reassessments, both biologically (alkaline phosphatase, blood calcium levels, phosphatemia, urine calcium levels, etc.) and imaging (bone densitometry, skeletal radiographs to document fractures). The inadequate response, low adherence, or adverse reactions to treatment, call into question the decision to discontinue or change it [55, 56].

Regarding the direct assessment of fracture risk, we mention that there is no current algorithm accepted and adapted to patients with SLE. The most widely used in practice is the Fracture Risk Assessment (FRAX), proposed by the World Health Organization [55]. FRAX has important limitations in SLE: it was developed for the postmenopausal period (and thus does not cover a significant subgroup of patients), does not take into account the cumulative dose of glucocorticoids (but only their use or not), and does not include fall risk assessment and the presence of vertebral deformities [27].

CONCLUSIONS

The last decades have made significant progress in the evaluation and treatment of patients with SLE, allowing current therapeutic strategies to control and stabilize affected organs. In this context, associated osteoporosis and fracture risk have become important in the medium and long term management of the disease.

At the moment, the practitioner has consistent data on the epidemiology, pathogenic mechanisms, and clinical impact of osteoporosis and fractures in patients with SLE. Current recommendations include: optimizing calcium and vitamin D intake, lifestyle adjustments and drug treatment. Special attention is directed to the group of women at fertile age, for whom treatment options are still limited.

Additional data on screening methodology and algorithms, morphometric assessment and fall risk are expected. In spite of this, there are currently several arguments for rheumatologists in order to turn their attention to the management of these complications from the early stages of the disease.

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ORIGINAL ARTICLES

Ovarian cancer under the magnifying glass

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Abstract: *Cancer is a pathology generating problem to the public health systems all over the world.*

The etiology and pathogenesis of ovarian cancer are still partially unknown. It is still a challenge when it comes to the origin, the molecular basis of ovarian carcinogenesis, tumor progression, and therapy options of ovarian cancer.

This paper brings an overview of ovarian cancer, starting with the pathogenesis, histopathological forms, and molecular biology of ovarian cancer, and follows the growth factors as molecular targets for specific therapy.

Keywords: *ovarian cancer, pathogenesis, molecular biology, growth factors*

INTRODUCTION

Ovarian cancer is a pathology that throughout history has sparked both controversy and questions, some of which remain unanswered to this day. Over time we have been working hard towards laying the foundation for the current therapeutic standard in addressing existing ovarian tumors.

Following the epidemiological data and the steps taken gradually by our forerunners in their pioneering work in both surgical as well as drug therapy, but also the new findings in the field and the literature developed, overall representing a rich scientific dowry, we realize that we are faced with pathology that over time rose many difficulties for clinicians, researchers, and patients under therapy.

EPIDEMIOLOGY AND PATHOGENESIS DATA

Cancer is a pathology generating problem to the public health systems in Romania, its incidence being among the lowest in Central and South-East Europe, whilst having a higher mortality rate [1]. Both body functions and immune response are involved in the pathogenesis of cancer and angiogenesis, hypoxia and inflammation can be identified at the cellular level [2].

The etiology and pathogenesis of ovarian cancer are still partially unknown [3, 4].

When determining the origin of ovarian cancer, there are several theories including the theory of the de novo ovarian cancer [5], the hypothesis of the inclusion of cysts [6, 7, 8] based on a Mullerian phenotype causing the malignant transformation of the mesothelial ovarian cortex corresponding to the serous, endometroid, mucinous, clear cell, transition types, but also to the Mullerian epithelium found outside the uterus, fallopian tube, cervix [7, 8, 9, 10, 11]. The fallopian tube may be more or less involved in the origin of serous ovarian cancer, both through the proliferation of fallopian secretion cells and through its vicinity, causing dysplastic lesions, the inclusion cysts formed at the time of ovulation near the fimbria causing the

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formation of endosalpingiosis [6, 12, 13], the transformation of epithelial ovarian inclusions, of the serous adenomatous cyst into an ovarian borderline serous tumor which may become invasive carcinoma [14, 15, 16].

PECULIARITIES OF HISTOPATHOLOGICAL FORMS OF OVARIAN CANCER

Ovarian cancer has the following histological types according to WHO (World Health Organization): serous, endometrioid, mucinous (most common), clear cell, transitional cell, squamous cell, and undifferentiated and mixed epithelial. Tumors can be benign, malignant, or borderline, and, depending on the subtype, they can be low grade or high grade (depending on nuclear atypia and mitosis) [17, 18]. Borderline tumors are considered potentially indolent and weakly malignant [19, 20, 21].

Ovarian cancer can be classified as follows: type I consisting of low-grade carcinomas -serous, endometrioid, clear cell, mucinous, and Brenner tumors, they are generally indolent, may have originated in borderline tumors and endometriosis, showing molecular entities such as KRAS, BRAF, ERBB2, PTEN, CTNNB1, PIK3CA, and type II consisting of high-grade carcinomas, serous, endometrioid, mixed mesodermal malignant tumors (carcinosarcomas) and undifferentiated carcinomas, they are generally aggressive, characterized by genetic instability, may have originated in intraepithelial carcinoma of the uterine tube, their molecular entity presented as mutations at the TP53 level [19, 20, 21, 22, 23, 24]. Both types may have originated in the extra ovarian tissue that can be implanted in the ovary [25].

Following the changes made in the FIGO staging, a new staging resulted. As recommended by FIGO, it is indicated that histology, including the grading, should be part of the staging process and also that the primary site (ovary, fallopian tube, peritoneum) should be mentioned where possible, and if this is not possible, this also must be mentioned. The damage to the retroperitoneal lymph nodes must be proven histologically or cytologically and stage I tumors with dense adhesions can be classified as stage II if malignant cells are proven to be histologically present [6, 26]. According to the International Agency for Research on Cancer (IARC), from histology's point of view, we can differentiate between carcinomas (serous carcinomas, mucinous carcinomas endometrioid carcinomas, clear cell carcinoma, NOS adenocarcinoma, other specific carcinomas, nonspecific carcinomas), sex-cord stromal tumors, germ cells tumors, other specific cancers (these including malignant Brenner tumors, carcinosarcomas, mixed Mullerian tumors), non-specific cancers [27, 28].

MOLECULAR BIOLOGY OF OVARIAN CANCER

The molecular basis of ovarian carcinogenesis and progression are not yet fully elucidated, considering the genetic and epigenetic alterations that translate into alterations of the molecular signaling pathways involved in survival, proliferation and cell death, adhesion, and angiogenesis.

Carcinogenesis involves inadequate adjustment mechanisms of the signaling pathways governing cell cycle, differentiation, apoptosis, expression of oncogenes and/or antioncogens, the action of transcription, and growth factors [29, 30, 31].

Amongst the RB family members, the RB gene (retinoblastoma gene) is involved in ovarian carcinogenesis. The majority of malignant ovarian tumors have had a strong expression of the pRb about a normal ovary where the protein is barely detectable [32, 33]. It was demonstrated that overall survival was positively influenced by the positive expression of p21 and p27 in patients with negative p53 only [34]. The p53 is the most relevant oncosupressor gene. Inactivation of the p53 oncosupressor gene's function appears to be an early event in hereditary ovarian cancer induction characterized by germ mutations of the BRCA1 tumor suppressor gene. The somatic mutation of p53 is the most common molecular change that occurs in epithelial ovarian cancer. The immune-histochemical expression of p53 protein was associated with the highly differentiated serous type, positive cytology, residual tumor, and stage of disease [4, 35, 36, 37, 38].

Studies in the specific literature suggest that the expression of Bcl-2 and Bax is an important prognostic marker [39].

The ovary is subject to cyclical changes where the angiogenesis process is continuous [3]. VEGF (vascular endothelial growth factor) is a growth factor that stimulates the angiogenesis process and increases microvascular permeability [40]. Ovarian cancer causes the release of VEGF in response to conditions of hypoxia and acidity, the serum and ascitic fluid VEGF values being elevated with malignant tumors as opposed to benign [40, 41, 42 43]. Type I and II ovarian tumors differ in molecular-genetic evidence just as much as they differ morphologically [44].

High-grade serous ovarian cancer, which is part of type II tumors, has frequently known mutations of TP53 (in more than 80% of cases) and amplification of CCNE1, which are characteristic of type II tumors, but rarely mutations that characterize type I tumors such as KRAS, BRAF, ERBB2, PTEN, CTNNB1 and PIK3CA [44, 45, 46, 47]. Type I tumors are more stable genetically and show distinctive pattern mutations that happen in a particular cell type, as opposed to type II which present morphological and molecular homogeneity, are genetically unstable with a high-frequency mutation of TP53, thus suggesting that ovarian tumors develop on different molecular pathways [44, 48]. The mutations in the KRAS or BRAF lead to subsequent activation of the MAPK signaling pathway (mitogen-activated protein-kinase) [49, 50]. It was shown that some inflammatory markers, glucose protein transporter 1 (Glut-1), iNOS, cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), nuclear factor kappa B (NF-kB) are involved in the initiation and progression of cancer [51, 52]. Immunohistochemical analyses showed statistically increased levels of expression of Glut-1, iNOS, COX-1, COX-2 in type II ovarian tumors, but no statistical difference of expression of NF-kB in ovarian tumors type I and II [52].

Researchers have identified five molecular subtypes presenting clinicopathological characteristics and survival rates for ovarian epithelial cancer, which are: Epi-A, Epi-B, Mes and Stem-A, and Stem-B. Epi-B and Stem-A subtypes are independent prognosis factors [53].

The BRCA1 gene was often studied because of the proven implications in the transmission of hereditary breast and ovarian cancer. In molecular terms, we distinguish as follows: low-grade serous ovarian carcinoma presents KRAS and BRAF mutations, and the high-grade present mutations of TP53 and abnormalities of BRCA. Serous high-grade ovarian carcinoma shows an increased nuclear expression of Ki-67 and has immunoreactions for p53, BRCA1, WT1, p16. Mucinous ovarian carcinoma presents KRAS mutations in its tumorigenesis, which are immunoreactive for CK7 and CK20. Endometrioid ovarian carcinoma shows mutations of the β-catenin (CTNNB1), PTEN, and ARID1A. Endometrioid ovarian carcinoma is immunoreactive for vimentin, CK7, CK20, the epithelial membrane antigen, estrogen, and progesterone receptors. Ovarian clear cell carcinoma presents ARID1A mutations, lack of protein BAF250, is positive for HNF1-β, and is negative for ER and WT1 [5, 54, 55, 56, 57, 58].

GROWTH FACTORS AS MOLECULAR TARGETS FOR SPECIFIC THERAPY

Knowing that VEGF is involved in tumor growth, invasion, and dissemination to the lymph nodes, liver, lungs, numerous drug agents are being used with the target role occupied by VEGF in ovarian cancer, especially when peritoneal carcinomatosis and/or ascites are present. One of the agents used in neutralizing the VEGF is bevacizumab, a recombinant humanized monoclonal antibody that stops the binding of VEGFRs on all the VEGF isoforms, thus preventing tumor growth and ascites production. Paclitaxel can be used in combination with bevacizumab acting synergistically and with the same effects. Bevacizumab used in combination with cisplatin increases the survival rate [59, 60, 61].

Agents acting as inhibitors of tyrosine kinase receptors are counted as VEGFR antagonists (Ramucirumab, Cediranib, Semaxanib) and agents that have as target multiple tyrosine kinase receptors (Sunitinib, Sorafenib, Vatalanib, Intedanib, Pazopanib, Motesanib, Vandetanib, AEE788) [62, 63].

VEGF overexpression in ovarian cancer makes it a therapeutic target, thus aiming at the inhibition of neo-vascularization, but also anti-angiogenic agents. VEGF is a dimeric protein, a member of the VEGF/PDGF, including VEGF-B, VEGF-C, VEGF-D, PIGF, and PDGF, involved in angiogenesis, proliferation, survival, migration of endothelial cells and stimulated by hypoxic conditions [12, 60, 64, 65].

Anti-VEGF therapy has been shown to play an important role in female patients with primary and recurrent ovarian cancer [60, 61, 66]. The use of bevacizumab can have aggressive complications that can lead to death, the risk is even higher when used in combination with chemotherapy, particularly platinum-based (cisplatin, carboplatin, oxaliplatin) or taxanes (paclitaxel, docetaxel) [66, 67, 68, 69]. As standard therapy in advanced ovarian cancer, cytoreduction surgery followed by platinum-based chemotherapy is used with a fairly good response, but sometimes resulting in tumor recurrence within 5 years and therapeutic resistance [66]. In case of resistance to platinum other chemotherapeutics are used such as metronomic paclitaxel, docetaxel, topotecan, liposomal doxorubicin, etoposide, gemcitabine, but the response is quite limited with a fairly low survival rate [50]. Attempts have been made to develop different therapies aiming at the interruption of angiogenesis, molecular target, inhibition of the Poly- (ADP) Ribose polymerase function (PARP), growth factors such as insulin-like growth factor (IGF) [70, 71]. Treatments often target the vascular endothelial growth factor pathway (using bevacizumab or aflibercept), the mechanisms of DNA repair (using iniparib or olaparib), or are guided against folate ways (using pemetrexed farletuzumab, vintafolid) [61].

Oral therapy with olaparib is currently being used as maintenance therapy, this being a PARP inhibitor used on patients with recurrent serous ovarian cancer sensitive to the platinum-based therapy, where the existence of mutations in BRCA has been proven and where the patient had two or more courses of platinum-based chemotherapy and showed a partial or complete response to the most recently used platinum-based chemotherapy [22, 71, 72, 73, 74, 75, 76].

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ORIGINAL ARTICLES

Overview of bilirubin as criterion for higher military education admission

ViorelM. Oniciuc[1]

Abstract: *This article was written because of the necessity of clarifying the recent biomedical selection criterion for the medical exam of the students who are applying for higher military education. In order to screen for or monitor liver disorders or hemolytic anemia the bilirubin is measured. The increased values of the bilirubin can be caused by multiple factors and they are presented in the following chapters.*

Keywords: *Bilirubin, liver, gall, alimentation, genetic diseases*

WHAT IS BILIRUBIN?

Bilirubin is a linear liposoluble tetrapyrrole derived from the enzymatic catabolism of the hem fraction from various hemoproteins in the body. Understanding changes in plasma levels of bilirubin under pathological conditions and differentiation of icteric syndromes involves a prior knowledge of the mechanism of formation, agglutination and elimination of this compound.

The normal values observed in the laboratory analysis are:
- Total bilirubin = 0.3 - 1.2 mg / dl, (or 0.1 - 1.2 mg / dl)
- Direct bilirubin = 0.1 - 0.35 mg / dl
- Indirect bilirubin = difference

Linear tetrapyrrole is the result of the enzymatic mechanism that catalysis the oxidative breakdown of the tetrapirolic (porphyrinic) ring of the hemoglobin heme molecule, primarily of the aged erythrocytes.

The process leads to hemoglobin formation in at least two stages. Bilirubin is mainly formed in the liver (about 80-85% up to 90% according to some authors), the rest having extrahepatic origin, in part reticuloendothelial (spleen, bone marrow).

Involvement of the liver in bilirubin production

The main degradation product of porphyrins is bilirubin, the red-orange bile pigment, which still possesses the four pyrrole rings. In the plasma, the pigment circulates as total bilirubin, consisting of two components: bilirubin bound to unconjugated albumin, indirectly referred to as free glucuronide bilirubin (mono- and diglucuronate), related to the diazotation reaction, Hijmans Van Den Bergh. Total bilirubin level serum concentration is 0.1-1.2 mg/dL or 17 μmol/l. Under normal circumstances, bilirubin accounts for 85% of the erythrocyte hemoglobin degradation and the remaining 15% originates in the degradation of other hemproteins such as myoglobin, cytochromes and other hemic enzymes (catalases, peroxidases, cytochrom-oxidases).

Direct bilirubin (agglutinated with glucuronic acid) circulates freely in the blood. Indirect bilirubin (primary, unconjugated) is transported by binding to albumin, partially in the form of the albumin-phosphatide complex and in small amounts bound to both metals, amino acids, peptides or other small molecules. In the gall there are bilirubin esters with sulfuric

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acid and other acids. By binding the bilirubin to the elastic fibers of the skin and the conjunctiva the symptoms of jaundice appear (yellowing of the skin and conjunctiva).

Bilirubin production is 35 mg per gram of hemoglobin, yielding 250-300 mg daily bilirubin. Hemoglobin degradation in bilirubin is performed in Kupffer stellate cells in the hepatic reticulo-histocithic system (RHS), which is the primary location of the process, the spleen and bone marrow being organs involved in this activity.

The secretion of the gall

One of the most important functions of the liver is the production of its external secretion, the gall, which reaches the duodenum through the biliary tract. The gall fulfills two major functions:

• elimination of endogenous substances – end products of metabolism and foreign substances such as bromsulfonaphthalein (BSP) drugs and dyes;

•intervention in lipid digestion and reabsorption – the liver secreted by hepatocytes, the hepatic ball, differs in appearance and concentration of vesicular bile. The first one is clear, yellow-gold and almost isotonic with blood, and the second is of brownish green (oxidized bilirubin), cloudy and filthy. Vesicular vesicle is more concentrated than the liver due to water reabsorption through the bladder wall.

INCREASE IN BILIRUBIN VALUES

Without entering into anatomical and physiological details and the claim of accurately discovering the causes that cause the increase of bilirubin values, applying filters such as the elimination of neo-natal casuistry, serious diseases (cancerous disease, cirrhosis etc), which have a very low incidence at the age of the students, we can focus on two main directions that could underpin the rise of increased bilirubin at our students:
1. Physiological-behavioural and social.
2. Inherited, triggering some hereditary disease syndromes.

Prior to the two directions, two further specifications are required:
• The triggering of the studied phenomenon belongs mainly to the age of adolescent-premature.
• In specialty work, the possibility of recording normal values occurs once in 3-5 laboratory tests, at intervals of at least three weeks, repeated bilirubin tests can be associated with other investigations, to determine normal hepatic functionality.

1. On the liver tract, intrahepatic and extrahepatic, gall bladder, biliary bladder, we narrowed down the multitude of possibilities with pathological manifestation (even anicteric, cholestasis mild hepatitis, confirmed by Dr. Anton).

However, it is worth mentioning that hyperbilirubinemia has been reported in a different institution, namely the "Football Academy" (according to A. Constantinescu – Fundeni University Clinic)

We noticed an increase of bilirubin in the cases of:
• Automedication (we advise that medication is administered and supervised by the medic):
- Paracetamol, even a slightly increased dose,
- Oral contraceptives,
- Consumption of food supliments, protein for muscle mass.
- Energy drinks, sweet juices, fast food
• Alimentation (canteen, home or other sources):
- Lack of food diversity
- Lack of lipids in the diet

The most important aspects of nutrition belong to food adjuvants mainly preservatives:
- Sodium metabisulphite (E223) in biscuits, cakes, fast food, juices, pickles, medications.
- Monosodium glutamate (called "good taste") is less directly incriminating, but it induces fatigue, headaches, sometimes aggravating breathing, causing stress. It is omnipresent in soups, sausages, vegetable preserves, flavors etc. This heat-treated (E621) adjuvant gives the browning reaction resulting in very toxic compounds.
- Sodium benzoate, preservative (E211).
- Carbon tetrachloride, hepatotoxic.

An important factor is "pernicious anemia" - ineffective erythropoiesis, lack of vitamin B12 due to lack of food, or more severe, due to the lack of intrinsic Castle's factor in gastric juice, necessary for absorption. B12 or perhaps stress-related somatostatin secretion that inhibits the secretion of the Castle's factor.

Sure, the theme is vast. Surveillance over time and complementing with specific analyzes, not just laboratory (CT, eco) will explain the causes.

2. On the track from the liver, intra-, extracapsal, bile duct to gall bladder several hereditary transmission syndromes can be manifested with precise localization and with a slightly variable pathological manifestation. They are triggered at adolescent age, according to the algorithm for the interpretation of the functional - liver test results (according to Henry J.B.). The most common genetic diseases are:

- *The Gilbert syndrome*

It is characterized by chronic, benign, intermittent, familial hyperbilirubinemia found in a routine control. It is caused by a defect in the transport and conjugation of unconjugated bilirubin. The jaundice is accentuated in feverish states, physical exertion or consumption of oral contraceptives.

- *The Crigler-Najjar syndrome*

Type I may be a homozygous of Gilbert's disease. Type II is characterized by partial glucuronide-transferase deficiency that bilirubin conjugates to the liver cell. Usually at adolescents, complications are rare and occur in the case of poor eating habits or by excluding lipids from the diet.

- *The Dubin- Johnson syndrome*

It is characterized by the inability to transport bilirubinuriuronide from hepatic cells to the tubule while the conjugation is normal. The stress periods are a factor. The jaundice is harmless and reversible and occurs after eating estrogen and contraceptives. It can be confused with mild viral hepatitis.

- *The Rotor Syndrome*

It is asymptomatic, benign; withdrawal and storage of conjugated bilirubin defect, and possibly related to transfer from liver to bile. Usually it is reported in adolescents (medication, exercise-physical stress). Other liver functions are normal.

- *Epstein-Barr virus infections (human herpesvirus 4)*

It induces bilirubin increases in about 30% of the patients, but if liver function abnormalities are not found, another diagnosis should be sought.

CONCLUSIONS

After analyzing the studied material together with the medical laboratory specialists (Chem. Stanciulescu C., Synevo, Biochem. Apircioaei O., former SMC), the two physicians mentioned above, Dr. Stanciulescu P., internal specialist and I tried to highlight some aspects of hyperbilirubinemia:

- Self-medication - forbidden.
- Nutrition - the main recommendations:
  - Regular fresh fruits and vegetables consumption
  - Reduced fast food and unhealthy drinks consumption.
  - Reducing fast at the first deviations from the normal functioning of the body, especially during the body-growing period.
- Elimination, at least limitation of stress, in all forms of manifestation: physical, food, intellectual, psychosocial.
- Avoid fatigue of any kind (not enough sleep at night)

These landmarks must be all considered, taking care of only one recommendation cannot bring immediate positive results.

As for hereditary transmission diseases, unfortunately, the possibilities of reaction are very limited in Gilbert and Crigler - Najjar syndromes, phenobarbital brings a certain decrease (Jacques Wallach, p. 341, confirmed by Dr. Anton) only under the supervision of the doctor. The most important factor is however a varied diet, avoiding starvation and tiredness.

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VARIA

Overview of telemedicine use aspects in natural and technical disaster and combat situation

Hamid R. Rasouli[1], Karim S. Kondor[2], Fathollah Ahmadpour[1], Mohammad Nobakht[2], Ali A. Esfahani[2], Mohsen A. Farajzadeh[2]

Abstract: *Background: Preparation and hospital development estimated an essential part of the policies and strategic purposes of healthcare in each government. Today, applications of management to reduce the burden of natural and technical disasters appear are very necessary.*

Objectives: The study aimed to review the major relevant articles in the case of the application of telemedicine for trauma injuries in disaster and military situations.

Methods: Original clinical trials investigating the effects of telemedicine on trauma injuries published before April 2018 were aggregated, and used in this study. We search the scientific sources such as PubMed, MEDLINE, Wiley, EMBASE, ISI, and Scopus. The keywords that applied to identify these articles combined: Telemedicine, Mobile Health, Health, Mobile, mHealth, Telehealth, eHealth, Trauma, War, military, medicine, Wounds and Injuries, injuries.

Results: The text of the articles and abstracts were examined from various aspects, the 21 articles were evaluated for quality, and then 10 papers entered in a systematic review. We found that the use of telemedicine in trauma injury, rural and the military is very helpful for high-quality trauma care; also the telemedicine and telephone consultations can be were demonstrated reducing the risk of physician-related medicine mistakes. The economic evaluation of telemedicine was demonstrated cost-saving and cost-effective of telemedicine consultations. We also realized the importance and practicable of telemedicine for improving rural trauma care.

Conclusions: Ultimately, the telemedicine can help patients and health government organizations economically, the telemedicine consultations due to cost-saving and cost-effective. Further studies to evaluate the running of telemedicine need to abating the practical and high-efficiency method, that can be done everywhere and it's free from mistakes and defects.

Keywords: *telemedicine, trauma, disaster*

BACKGROUND

Preparation and hospital development estimated an essential part of the policies and strategic purposes of healthcare in each government. Today, applications of management to reduce the burden of natural and technical

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disasters appear are very necessary. [1, 2]. Telemedical technology is increasingly moving into the medical system. Teleconsultation between crisis positions with a lack of specialists' physician and a trauma center in surgical and trauma patients could save interventions [3-5]. In recent years, Telemedicine is growing more prevalent in intensive care medicine, but its impacts and the excellent organizational pattern is also ambiguous [6-8]. Particularly in situations that there is a shortage of extremely educated healthcare workers on-scene such as rural EMS, disaster, and military conditions, conditions teleconsultation could improvements in patient care via consultation of a specialist [3-7].

Telemedicine is a valuable method of implementing remote diagnoses such as ophthalmology, dermatology, and radiology [8]. Telemedicine represents the use of medical knowledge transferred from one position to another by electronic connections to enhance patients' health situation and care [2-5]. Crisis management in natural and technical disasters should decrease the harmful impacts of trauma events, mortality, and morbidity applying a designed program of development and mobilization. Notwithstanding, there are a lot of experiences in crisis management, it has limited application of telemedicine in disaster and military situations [2-6].

Application of the telemedicine in disaster and military situations management is the principal subject to maintain the health of accident patients [7]. Regarding the destruction created in the health services infrastructure, infrastructure has become a significant issue, the existence, and utilization of telemedicine technology and according to continuous development in electronic technology, communication to deliver health services in remote regions [6-8].

We try to review the major relevant articles in case of application of telemedicine for trauma injuries in disaster and military situations to help clinicians and patients to have a better understanding of these technologies and choosing the better therapeutic option. A highly standardized evidence-based review can be one of the best references for clinicians to decide about their treatment plan.

MATERIALS AND METHODS

Original clinical trials investigating the effects of telemedicine on trauma injuries published before April 2018 were aggregated, and used in this study. We search the scientific sources such as PubMed, MEDLINE, Wiley, EMBASE, ISI Web of Knowledge, and Scopus.

The keywords that applied to identify these articles combined: Telemedicine, Mobile Health, Health, Mobile, mHealth, Telehealth, eHealth, Trauma, War, military, medicine, Wounds and Injuries, injuries. Two authors have independently assessed the titles and abstracts of all papers obtained by the searching method outlined above.

The studies with the following criteria were excluded: did not evaluate the performance of Telemedicine; were not English-language study, and were review studies and letters to editors or conference abstracts. The two reviewers who carried out the literature search also individually made decisions and selected studies considering inclusion criteria. The following details were extracted from included studies independently by two reviewers, study location, year of publication, the first author's surname, study design, and outcomes.

Figure 1: Flow chart the inclusion studies

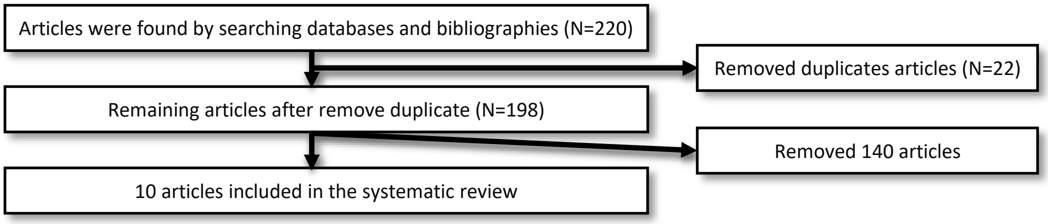
Articles were found by searching databases and bibliographies (N=220)
Removed duplicates articles (N=22)
Remaining articles after remove duplicate (N=198)
Removed 140 articles
10 articles included in the systematic review

RESULTS

Ten studies matched the criteria for analysis and discussion.

Daniel et al. (2013) in a randomized trial study examined emergency care between two groups. Doctor-staffed EMS teams compare to paramedic teams that were established telemedical by an EMS doctor. Overall 16 teams that each team contained one EMS physician and two paramedics randomly divided into the control and telemedicine group. Telemedical functionalities included 2 methods of audio communication, transportation of vital information, and video streaming from the situation place to the remotely placed EMS doctor. There were no significant differences in the case-specific items and, no potentially hazardous mistreatments were reported. They showed telemedical supported paramedic care as possible and at least not poor respect to conventional EMS teams with a doctor on-scene in these situations [9].

Table 1: Author's name, type of study and summaries of outcomes in the studies

Author's name	Type of study	Outcomes
Daniel et al. (2013) [9]	Randomized trial study	They showed telemedical supported paramedic care as possible and at least not poor respect to conventional EMS teams with a doctor on-scene in these situations
Brennan et al. (1998) [10]	Randomized trial study	They concluded that telemedicine was observed to be a satisfying system for pre-chosen emergency department patients and was observed by the doctors as an admissible way of complementary application.
Bowman et al. 2003 [11]	Cross-Sectional	Their results showed Telemedicine was a precise, reliable, and effective way of diagnosing and controlling these patients, particularly if slit-lamp images were applied.
Yang et al. 2015 [12]	Cross-Sectional	They concluded that a healthcare payer aspect, telemedicine to health care workers of severely sick and pediatric patients performing to rural EDs are cost-saving or cost-effective regarding the telephone.
Mohr et al. 2016 [13]	Observational cohort study	The telemedicine consultation was requested for severely injured rural traumatic patients (burns, penetrating trauma, and those with abnormal vital signs) in the Emergency Department, but Telemedicine consultation was not decreased interhospital transfer.
Oeveren et al. 2017 [14]	Cross-Sectional	They demonstrated a success rate of 96% of telemedicine during emergency intubation. Telemedicine-assisted intubation can be performed in rural hospitals practically.
Gilmore et al. 2014 [15]	Cross-Sectional	HBT can provide mental health services in a preferable procedure of location in military sexual trauma with PTSD patients and help to them for reducing mental pains and increasing readiness.
Dayal et al. 2016 [16]	Retrospective cohort study	The performance of a telemedicine program is designed to assist in the seriously ill children at admission to the PICU. They provided evidence for improving clinical outcomes in pediatric critical care with telemedicine programs.
Shah et al. 2016 [17]	Prospective cohort study	High-intensity telemedicine can be alternative to traditional severe disease care and it must be included in population health programs.
Dharmar et al. 2018 [18]	Retrospective chart reviews	They were demonstrated reducing the risk of physician-related medicine mistakes among critically sick and traumatic children of 8 rural EDs from academic children's hospital.

Brennan et al. (1998) conducted a randomized clinical study in the emergency department. Overall 104 patients were included. Fifty-four participated in the telemedicine group and 50 to the control group. There are no differences between the groups for an incident of 72 hours emergency department return visits; the requirement for further care; doctor communication; nurse communication; satisfaction. The mean patient throughput period for the telemedicine group was 106 minutes; the mean for the control group was 117 minutes. They concluded that telemedicine was observed to be a satisfying system for pre-chosen emergency department patients and was observed by the doctors as an admissible way of complementary application [10].

Bowman et al. (2003) assessed the precision and effectiveness of telemedicine in diagnosing and treating eye injuries. The methods were a clinical trial with a face-to-face group (40 patients) and a telemedicine group (40 patients). In the face-to-face group, consultations were in person; in the telemedicine group, researcher one handled video conferencing technology at 384 kbit/s, and observer two observed the patient face to face. In this study, 80 patients were included in the department. Principal result measures were agreement levels between the two observers for every phase, time of consultation, and several unnecessary recalls. There were more important conflicts applying telemedicine, in per sample the telemedicine diagnosis and management failed on the side of safety; consequently, no patient would have experienced via the improper administration because of the consultation using telemedicine. Their results showed Telemedicine was a precise, reliable, and effective way of diagnosing and controlling these patients, particularly if slit-lamp images were applied [11].

Yang et al. (2015) carried a financial assessment to determine the price, effect, and return on investment of telemedicine consultations presented to health care workers of severely sick and pediatric patients in rural EDs confronted with phone consultations from a healthcare payer prospect. The cost-effectiveness investigation was according to program effectiveness, obtained from change choices after telemedicine and telephone consultations. They concluded that a healthcare payer aspect, telemedicine to health care workers of severely sick and pediatric patients performing to rural EDs are cost-saving or cost-effective regarding telephone [12].

Nicholas M. Mohr et al. evaluated factors associated with telemedicine and interhospital transfer as a cohort study of all adult treated traumatic patients between 2008 and 2014 in North Dakota critical-access hospital (CAH). They demonstrated telemedicine consultation was requested for severely injured rural traumatic patients (burns, penetrating trauma, and those with abnormal vital signs) in the Emergency Department, but Telemedicine consultation was not decreased interhospital transfer [13].

Lucas Van Oeveren et al. evaluated intubations which done by telemedicine in rural EDs with ED telemedicine network. They were collected probable data of all patients with video telemedicine-assisted intubation from May 1, 2014, to April 30, 2015, and telemedicine involvement/intervention was evaluated using statistical analysis. Lucas Van Oeveren et al have involved 206 intubations with respiratory failure as most common. They demonstrated a success rate of 96% of telemedicine during emergency intubation. Telemedicine-assisted intubation can be performed in rural hospitals practically. This telemedicine-assisted intubation was done at first in emergency tracheal intubations via a live telemedicine link [14].

Amanda K. Gilmore et al. was described a randomized controlled trial comparing Prolonged Exposure (PE) delivered face-to-face and delivered via Home-based telemedicine (HBT) in female veterans, they focused on female veterans with military sexual trauma with PTSD. Their hypothesize was more be effective in improving the quality of life in PE delivered via HBT than SD at the post, 3-, and 6-month follow-up. Ultimately, Amanda K. Gilmore et al concluded HBT can provide mental health services in a preferable procedure of location in military sexual trauma with PTSD patients and help to them for reducing mental pains and increasing readiness. Generally, Home-based telemedicine can reduce systemic barriers for treatment [15].

Parul Dayal et al have compared the effects of access to a telemedicine program (pediatric critical care) on the intensity of disease and results for referral children to children's hospital PICU in the cohort study. The 582 patients with telemedicine that were younger and less sick than 524 patients from without telemedicine were compared during the study period. Parul Dayal et al was indicated the performance of a telemedicine plan for the care of seriously ill children. The performance of a telemedicine program is designed to assist in the seriously ill children at admission to the PICU. They provided evidence for improving clinical outcomes in pediatric critical care with telemedicine programs [16].

Manish N. Shah et al have evaluated the effect High-intensity telemedicine programs on emergency department use for the care of Six senior living community (SLC) residents in a cohort study over 3.5 years. They were indicated reducing emergency department use in High-intensity telemedicine care of SLC residents. Manish N. Shah et al was presented High-intensity telemedicine can be alternative to traditional severe disease care and it must be included in population health programs [17].

Madan Dharmar et al was evaluated the rate of physician-related medicine mistake on seriously 234 patients receiving 85 telephone consultations, 73 telemedicine consultation, and 76 no consultations in rural emergency departments, then outcomes of the study were compared. They were performed retrospective reviews critically sick and traumatic children of 8 rural EDs from academic children's hospital. Ultimately, Madan Dharmar et al was demonstrated reducing the risk of physician-related medicine mistakes among critically sick and traumatic children of 8 rural EDs from academic children's hospitals [18].

DISCUSSION

Traumatic injury is a common cause of death for those under the age of 44 years. On the other hand, accessing sufficient and adequate high-quality trauma care is a secondary problem after the trauma, and one of the main mechanisms associated with bad results in trauma patients. Generally, the improvement of trauma results is under the influence of triage of seriously traumatic patients. Telemedicine is a strategy to improve trauma care, with the aim of given tertiary level services locally. Using telemedicine is particularly increasing and improving the care of trauma patients, it can be valuable when the time-sensitivity is palpable in trauma interventions [19-30].

This study reviews several strengths aspects of telemedicine such as generally, treatment in a high-risk (population, condition, and location) and removal of barriers to care of traumatic patients.

In this systematic review, the literature searched in important databases for telemedicine in various fields such as trauma injury, war, rural, and military. The text of the articles and abstracts were examined from various aspects, the 21 articles were evaluated for quality, and then 10 papers entered in a systematic review. This manuscript is in the first study in various fields of telemedicine.

We found that the use of telemedicine in trauma injury, rural and the military is very helpful for high-quality trauma care; also the telemedicine and telephone consultations can be were demonstrated reducing the risk of physician-related medicine mistakes. Also, telemedicine can useful, practical, and preferable procedure of location for care military sexual trauma patients with PTSD and help to them for reducing mental pains and increasing readiness. The economic evaluation of telemedicine was demonstrated cost-saving and cost-effective of telemedicine consultations. We also realized the importance and practicable of telemedicine for improving rural trauma care.

Duchesne et al. [31] were reported that the performance of a telemedicine plan decreases trauma transfers to 89%. Many rural EDs have not board-certified physicians in emergency medicine [32] despite, the increasing of traumatic injury and trauma-related mortality in rural populations.

In a study that uses tele-emergency services, they were indicated to improve 95% quality of care by using tele-emergency services [33]. The clinical care is improved by using of telemedicine because access to specialized physicians [33]. in the other study, they found the utilization of expert physicians via telemedicine helped stabilize trauma patients during early care [34]. This study demonstrated a limit of 8% of patients have received care that may survive through using telemedicine consultation [34]. On the other hand, the levels of task-based communication of care services can be increased through telemedicine care [35]. Lucas Van Oeveren et al results were illustrated telemedicine improves care and enhances accessibility to treatment.

MST-related PTSD as one of the subjects for telemedicine was investigated by Gilmore et al. in particular; HBT increases treatment access women and even men with their MST histories which suffering from other physical or mental difficulties.

Although quality of care is fairly suitable in most EDs to children, it may behave rates of poor events, such as medication errors in non-children's hospitals for seriously ill children [36–38], nevertheless, the telemedicine can provide access to pediatric expertise for a decrease of physician mistakes [39].

The telemedicine programs significantly increase the quality of care and reduce mortality in traumatic injuries, also the telemedicine is the importance and practicable for improving rural trauma care. This producer can be a suitable alternative to traditional care, in other words, this method causes access to skilled and experienced physicians in far-off areas, subsequently; telemedicine can decrease the risk of physician-related ED medication errors particularly in a pediatric case. Besides, telemedicine can useful and practical producer for PTSD patients; and home-based telemedicine access for these patients in their home.

CONCLUSIONS

Ultimately, the telemedicine can help patients and health government organizations economically, the telemedicine consultations due to cost-saving and cost-effective. Further studies to evaluate the running of telemedicine need to abating the practical and high-efficiency method, that can be done everywhere and it's free from mistakes and defects.

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CLINICAL PRACTICE

Crossed renal ectopia

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Abstract: *The case of 73 years - old male patient with inferior crossed renal ectopia diagnosed upon imaging workup using a GE LightSpeed 16 Slice CT, with urothelial carcinoma, at the level of the left kidney, nephrolithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery. The upper lobe of the right kidney fused with the lower lobe of the left kidney, on the left side of the midline. There were 6 renal arteries (4 on the left and 2 on the right) originating from the left side of the abdominal aorta, at the level of the termination of the abdominal aorta, the left common iliac artery and the right internal iliac artery. The patient presented with 6 renal veins (3 on the left and 3 on the right) that drained anteriorly on the left side of the inferior vena cava into the left common iliac vein and into the right internal iliac vein.*

Keywords: *crossed renal ectopia; arteries – veins*

INTRODUCTION

Crossed ectopic kidney (CFE) is an anomaly within the fused kidneys (FKs) group of malformations, consisting of the fusing of the upper pole of one kidney with the opposite lower pole of the other kidney traversing the midline, with a reversed L-shaped view. [1] describes 6 types of fused kidneys (FKs). [2], quoting Sousa Escandon and Aguilar Tubet, mentions that the first case of crossed renal ectopia was described by Pamarolus in 1654 and the classification of fused kidneys was done in 1957 by McDonalds and McClellan. According to [3], most frequently, the left kidney is the one fusing on the right part, aspect met in the case described by [1]. In our case, as well as in the first two cases described by [4] and in the case described by [5], it was the right kidney that traversed the midline in order to fuse with the left kidney. Numerical variation are frequently met in crossed fused ectopia of the renal veins, comporting 1 to 6 arteries originating from the aorta or the iliac veins (common and internal), and one or more veins that terminate into the inferior vena cava or in the iliac veins (common or internal). The ureters usually do not present variants or anomalies, being just one for each kidney. The renal hilum is anterior or anteromedial. Frequently, crossed fused ectopia is asymptomatic [2, 3, 5, 6] and incidentally discovered during a consult for different other medical reasons, although cases of crossed fused ectopia may present a pathology associated with congenital malformations. As associated pathologies, we have : hematuria, dysuria, urinary tract infections, renal failure, nephrolithiasis, obstruction of the ureteropyelic junction, hydronephrosis, urinary stasis, ectopic ureterocele, urothelial carcinoma [2, 3, 5, 6, 7]. As for congenital malformations, we may encounter the following: cardiac malformation, skeletal-muscle malformation, genital malformations, gastrointestinal malformation [3, 6, 7, 8, 9]. The case presented in our review was diagnosed with urothelial carcinoma of the left kidney, nephrolithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery.

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CASE STUDY

73-year old male patient, admitted at the Emergency Clinical County Hospital “Sf. Andrei” in Constanta, diagnosed with urothelial carcinoma of the left kidney, renal lithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery. After the CT scan that was performed using GE LightSpeed 16 Slice CT, the patient was diagnosed with crossed renal ectopia. The right kidney crossed the midline – its upper pole fused with the lower pole of the left kidney, forming a 129,4° angle, showing a reversed L-shape aspect. The diagnosis of inferior crossed renal ectopia – type D was made, according to the classification of [1].

The left kidney was located vertically in the upper part and presented with the upper pole laterally to the costal processes of the L2 vertebra, covering the inferior top of the 12th rib. Its inferior pole reached the pelvis, at the level of inferior half of the S1 vertebra, at the level of sacral ala and the upper medial of the left iliac fossa. The renal isthmus, slightly oblique-inferior to the right, was located above the superior margin of the L5 vertebra and at the level of the L5 – S1 intervertebral disk and at the level of the left lateral part of the S1 vertebral body, at the level of the sacral ala. The inferior pole of the left kidney was positioned anteriorly to the sacral ala and also anteriorly to the upper medial part of the left iliac fossa. The left renal hilum was positioned anteriorly. The right kidney was horizontal (slightly oblique-lateroinferior to the right) with its superior pole corresponding to the inferolateral part of the L5 vertebral body, to the right lateral part of the L5 – S1 intervertebral disk and to the left sacral media ala, and also corresponding to the anterolateral sides of the sacral S1 – S2 vertebras. Its inferior pole overpassed the midline of the sacrum corresponding to the right lateral side of the S3 vertebra, above the anterior third sacral hole (Figure 1). The right hilum was positioned anteroinferiorly.

Figure 1: Our case of CFE in relation with vertebral column

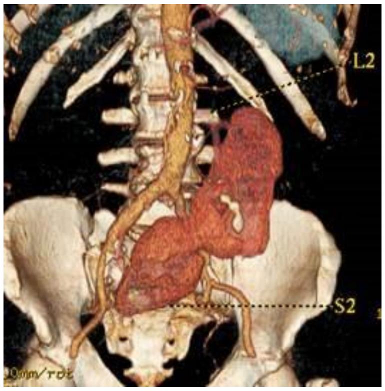

The complex of the 2 kidneys had a longitudinal diameter of 190.6mm, a transverse diameter of 113.8mm and with a volume of 295,788 cm^3. The left kidney (Figure 2) had a bipolar diameter of 122.9mm, a transverse diameter of 65.3mm and an anteroposterior diameter of 36.5mm, with a parenchymal thickness of 20mm and a volume of 160 cm^3.

Figure 2: Left kidney diameters

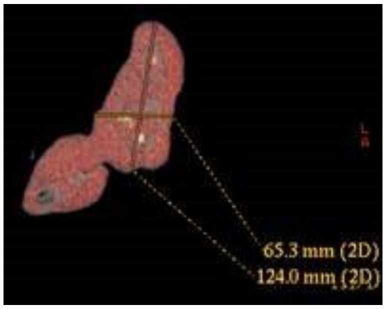

The renal isthmus (Figures 3, 4) had an anteroposterior diameter of 18.1mm, a length of 31 mm, located next to the L4 – L5 intervertebral disk, 30 mm laterally to the midline.

Figure 3: Isthmus antero-posterior diameter (18.1 mm)

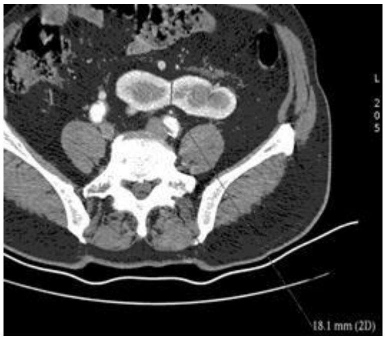

Figure 4: Isthmus lenght (30.6 mm)

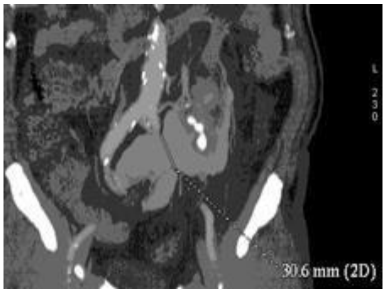

The right kidney (Figure 5) had a bipolar diameter of 105.7 mm, a transverse diameter of 58.8 mm, an anteroposterior diameter of 35.8 mm, with a parenchymal thickness of 20.3 mm and a volume of 136,295 cm^3.

The right ureter had a diameter of 6.0 mm and the left ureter presented with a diameter of 5.0 mm.

The 6 renal arteries of the cross fused ectopia (CFE) were positioned one under the other, originating at the level of the abdominal aorta and at the level of the internal and common iliac arteries, each artery with its own vascularization territory.

Figure 5: Right kidney diameters

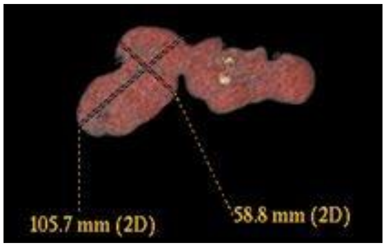

The first renal artery originated on the left side of the aorta, approximately 19mm inferiorly to the origin of the superior mesenteric artery, at the inferior 1/3 of the L1 vertebral body, with a diameter of 1.7 mm that penetrated the upper pole of the left kidney giving birth to a branch the descended anteriorly to the bassinet.

The second renal artery also originated from the left side of the aorta, approximately 55mm inferiorly to the first renal artery, next to the right upper part of the L3 vertebra, with a diameter of 4.3 mm penetrating the renal sinus from behind the bassinet of the left kidney.

The third renal artery originated at the level of the aortic termination, next to the L4 vertebral body, approximately 40 mm inferiorly to the second renal artery, with a diameter of 4.6 mm. It bifurcated into an anterior branch penetrating the renal sinus behind the bassinet and into a posterior branch penetrating the renal parenchyma of the inferior pole issuing a branch that penetrated the renal isthmus and branches for both renal poles.

The fourth artery originated in the left common iliac artery, approximately 60 mm from the bifurcation of the aorta, with a diameter of 2.6 mm. The artery bypasses the lower left pole of the kidney, penetrating the renal sinus anterior to the bassinet flowing into the inferior mesorenal.

The fifth renal artery originated in the right internal iliac artery, at the level of its origin, approximately 62mm from the bifurcation of the aorta, with a diameter of 6.8mm; it penetrated in the right renal sinus where it bifurcated into a prepyelic and retropyelic branches.

The sixth renal artery originated in the posterior trunk of the right internal iliac artery, approximately 62 mm inferiorly to the fifth renal artery, with a diameter of 2.2 mm, perforating the renal parenchyma next to the left inferior kidney.

The left testicular artery originated from the left side of the abdominal aorta, 28 mm under the first renal artery, next to the middle of the L2 vertebral body, with a diameter of 1.7mm and in its ascending route passing behind the left bassinet, issuing a branch for the tumor formation.

There were 6 renal veins (Figures 6, 7, 8), 3 veins for each kidney – for the left kidney we had V1, V2 and V4 and for the right kidney, we had V3, V5 and V6.

Figure 6: CFE 6 renal arteries (R1, R2, R3, R4, R5, R6) and vascular territories

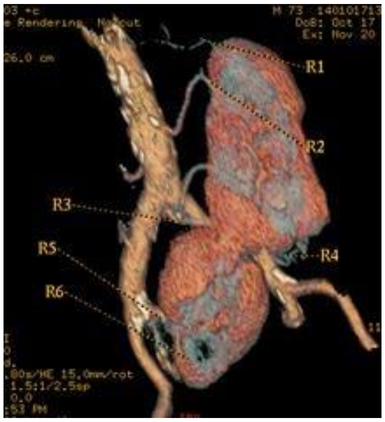

Figure 7: CFE 6 renal veins (V1, V2, V3, V4, V5, V6)

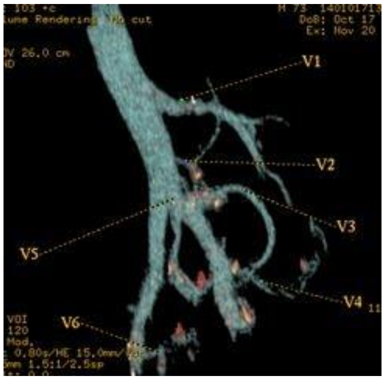

Figure 8: CFE veins and renal drainage territories

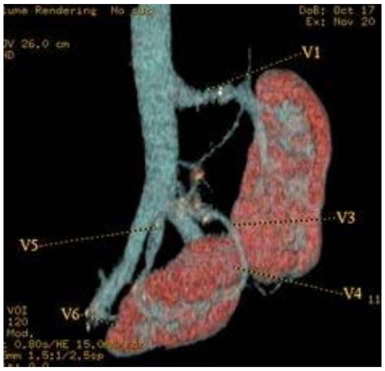

The first renal vein ended into the left side of the inferior vena cava, next to the L2 vertebra, it drained the left kidney, with a retropyelic and retroaortic trajectory and with a diameter of 9.3 mm.

The second renal vein formed by the fusing of an anastomotic branch with the first renal vein with a left lumbar branch, also ending into the left side of the inferior vena cava, next to the L3 vertebra, with a retroaortic trajectory, draining the left kidney and having a diameter of 4.8 mm.

The third renal vein originated from the right renal sinus anterior to the bassinet (also draining the upper pole), ending into the left common iliac vein, 19 mm from the formation of the inferior vena cava, with a diameter of 8.7 mm, draining the right kidney.

The fourth renal vein originated from the left renal sinus next to the inferior pole, accompaning the fourth renal artery, ending into the left common iliac vein, approximately 68 mm from the formation of the inferior vena cava, with a diameter of 5.4 mm.

The fifth renal vein originated from the renal sinus on the posterior side of the right bassinet along with the fifth renal artery was bypassing anteriorly the right common iliac artery, ending on the anterior side of the inferior vena cava, approximately 10 mm above the IVC origin and approximately 22 mm inferiorly to the second renal vein, with a diameter of 8.3 mm.

The sixth renal vein originated from the right inferior pole along with the sixth renal artery, ending in the right internal iliac vein at 33 mm inferiorly to the formation of the right common iliac vein, with a diameter of 5.2 mm.

DISCUSSION

Within the fused kidneys (FKs) group of malformations, the most frequently met is the horseshoe kidney (HK), the crossed fused ectopia (CFE) being the second anomaly in terms of frequency. The horseshoe kidney has a bigger rate of incidence: 1/400 cases according to Lawsley, Eisendrath, Bauer (quoted by 10), 1/600 according to Moore, or 1/1,200 according to Rubin (both quoted by [4]). According to [7], CFE is a rare anomaly of the kidneys, with a prevalence rate of 1/1000 – 1/7500 of cases (Wilmer, Abeshouse, quoted by 10), although a renal anomaly, according to [3] and Bauer (quoted by [7]) incidence rates of 1/300 – 1/7500 of cases. [10] considers the ratio between the frequency of CFE/HK is of 1/7.7. CFE is most frequent in male cases, i.e. double the cases in relation to female cases [9], Bauer (quoted by [7]), 3/1 Boyan (quoted by [5]) or 2.3/1 according to Muttarak (quoted by [5]). [2] describes 3 cases of CFE, 2 of which are female cases and 1 male case.

[4] describes 2 cases of CFE: in one case he finds 6 right renal arteries (2 arteries originating from the lateral side of the aorta and were destined to the left kidney; 1 artery originating from the posterior side of the aorta, vascularizing the isthmus and the left inferior kidney; 1 artery originating at the level of the bifurcation of the aorta that penetrated the left kidney on the posterior side and issued a voluminous branch for the right kidney; the right kidney was vascularized by 2 arteries – one originating on the anterolateral side of the aorta and the other originating from the right common iliac artery). In this case, he finds only 3 renal veins (2 right renal veins and 1 left renal vein). In the second case, he also finds 6 renal arteries: 2 left renal arteries originating on the lateral side of the aorta and 4 right renal arteries - 3 originating on the lateral side of the aorta and one artery originating at the level of the terminal bifurcation of the aorta. In this case, he finds only 2 renal veins – right and left.

[5] describes the case of a CFE that presented 4 renal arteries – 1 artery for the superior renal parenchyma (left kidney) and 3 renal arteries for the inferior renal parenchyma (right kidney). De venous drain was composed of 2 renal veins.

According to Papin, quoted by [7], fused renal anomalies may present with 2 renal arteries (in 20% of the cases), 3-5 renal arteries (in 66% of the cases) and more than 5 renal arteries (in 14% of the cases).

[3] quoting Bauer and bailey, considers that the total number of the arteries for a crossed fused ectopic kidney varies from 1 to most frequently met cases ranging from 2 to 4 arteries; he discovered 3 renal arteries for the left kidney and 1 renal artery for the right kidney.

[6] describes a case of CFE that presented 2 renal arteries originating in the common iliac arteries.

[9] describes one single case presented with only 2 renal arteries (originating on the sides of the aorta), the right renal artery having a precaval trajectory and 2 renal veins – the left renal vein with a retroaortic trajectory.

The origin of the CFE arteries varies, usually the main arteries or in the case where there are only 2 renal arteries originating in the abdominal aorta. [6] finds the origin of the 2 renal arteries in the common iliac arteries.

We found that out of the 6 renal arteries, 2 renal arteries (R1 and R2) originated on the left side of the abdominal aorta, one renal artery originating at the level of the terminal ramification of the aorta (R3) and into the left internal iliac artery (R4), the last 2 arteries originating in the right internal iliac artery – 1 at the level of the unique trunk of the artery (R5) and the other at the level of the posterior trunk of the right internal iliac artery (R5).

[34] finds that most frequently the origin of the CFE arteries is at the level of the abdominal aorta (lateral right side or left side, posterior side and aortic bifurcation), considering the origin of the artery at the level of the right common iliac artery.

In the case of [5], he finds the origin of the 4 renal arteries only at the level of the lateral sides of the aorta – on the right side, 1 artery and on the left side, 3 arteries.

According to [3] the origin of the fused ectopic kidney is at the level of the superior abdominal aorta (in 25% of the cases), whereas for the rest of the cases, he considers the origin at the level of the inferior aorta and the iliac arteries.

The 6 arteries penetrated the kidney at its superior pole (R1), at the level of the renal hilum (R2, R4 and R5), or at the level of the inferior pole of the kidney (R6). R3 terminated into a bifurcation that issued a hilar branch and another branch that inserted in the kidney through its inferior pole. R2 penetrated the hilum posteriorly to the bassinet whereas R4 anteriorly to the bassinet and R5 issued one prepyelic branch and another retropyelic branch.

In two of his cases, [4] finds the CFE arteries penetrating the kidneys about the same levels as ours and he also describes a renal artery for the left kidney that bifurcated into one hilar branch and into an isthmic branch, whereas for the right kidney he finds 2 renal arteries that penetrated the kidney through its anterior side, respectively at the level of its posterior side.

The most voluminous of the arteries was the fifth renal artery that vascularized the right kidney and the less voluminous artery was the sixth renal artery also destined to the right kidney.

There was no renal artery for the renal isthmus. It receives a branch through an artery destined to the inferior pole of the left kidney or through an artery destined to the right kidney.

Out of the 6 renal veins of the CFE, 3 of them terminated in the inferior vena cava, 2 in the left common iliac vein and 1 in the right internal iliac vein. The veins of the left kidney left the kidney through the upper pole (V1) and through the renal hilum (V2 and V4) whereas the veins of the right kidney left the kidney through the renal hilum (V3 and V5) and through the lower pole of the kidney (V6). Out of the veins of the right kidney, the first 2 renal veins had a posterior trajectory to the bassinet, presenting also with a retroaortic trajectory, and out of the veins of the right kidney, the fifth renal vein had a posterior trajectory to the bassinet. The veins of the left kidney terminated on the left side of the inferior vena cava (V1 and V2) and into the left common iliac vein, and the veins of the right kidney terminated into the anterior side of the inferior vena cava (V5) into the left common iliac vein (V4) and in the right internal iliac vein (V6). The first renal vein (V1) was the most voluminous one and belonged to the left kidney, and the less voluminous one was the second vein and originated also from the left kidney. There was no vein to serve exclusively the renal isthmus. Out of the 3 veins of the left kidney only the renal vein had a retroaortic trajectory.

In one of the cases [4] described, he met 3 renal veins: 2 right renal veins – one collecting the blood at the level of the left pole of the left kidney and from the level of the isthmus and flew into the left internal iliac vein, and the other collecting the blood of the upper part of the isthmus ending in the inferior vena cava, above its termination into the cava of the left renal vein. The left renal vein was normally positioned but it received an anastomosis from the left common iliac vein. The other case of CFE of [4] presented a normal left renal vein and 2 right renal veins: one originated at the level of the left pole of the kidney and terminated into the inferior vena cava, 2 cm under the termination of the left renal vein and the left pole of the right kidney with the isthmus presented a vena that terminated in the left iliac vein.

In the case described by [5] there were only 2 renal veins: the upper renal parenchyma was vascularized by a vein that terminated in the left side of the inferior vena cava and the lower renal parenchyma was drained by the second renal vein that terminated also in the left side of the inferior vena cava.

CONCLUSIONS

The case we presented is special due to its uniqueness, as we have not found a similar case of crossed renal ectopia presented with 6 renal arteries (4 left and 2 right) and 6 renal veins (3 left and 3 right) in the specialist literature.

There was no artery – vein correspondence in all 6 cases, such a correspondence was met only at the level of the pole vessels and there was only one case at the level of the hilum vessels. Regarding arteries, the most voluminous was a hilum artery, whereas the veins; the most voluminous, was a superior polar one.

We consider MDCT examination to be the best method of describing vascular and uretheral abnormalities in case of crossed fused ectopia, being able to indentify also, other associated malformation, aspect noted also by [3, 5, 7].

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ORIGINAL ARTICLES

Patients' knowledge and attitudes regarding human papillomavirus disease and the effectiveness of education in patients referred to the Dermatology Clinic of Baqiyatallah Hospital in 2020

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Abstract: *Introduction: Human papillomavirus infection considers one of the leading causes of infection-related cancer worldwide. The general public's range of knowledge and understanding of human papillomavirus is low; the present study was done intending to evaluate the knowledge and attitude of patients with human papillomavirus and the effectiveness of educational intervention in those patients.*

Methods: This was a quasi-experimental study. The study population included patients with genital human papillomavirus referred to Dermatology Clinic of Baqiyatallah Hospital in 2020. The patients filled out a pre-test questionnaire. In the next step, they were trained by the treating physician about the disease, transmission ways, complications, treatment methods, and laboratory tests, and available vaccines. After that, an educational pamphlet was given to the patient to complete and review the information. Moreover, a post-test was performed in the patient's next visit. Data were analyzed using SPSS software version 22.

Results: 92 patients were included in the study. The levels of knowledge and attitude of patients were between 50 to 65% before training and training had a significant impact on improving the level of knowledge and attitude of patients ($P < 0.001$) so that the level of knowledge and attitude of patients reached above 95%. Demographic factors such as age, gender, marital status, and level of education were not related to the initial knowledge and attitude of individuals based on the statistical analysis ($P < 0.05$). However, the knowledge of married people about the complications of the disease was more than single ones ($P = 0.032$).

Conclusion: the level of knowledge about human papillomavirus and its complications, transmission ways, prevention and treatment is low, which has increased with training significantly.

Keywords: *human papillomavirus, education, knowledge, attitude*

INTRODUCTION

Human papillomavirus infection as one of the main causes of infection-related cancers worldwide also causes other diseases like genital warts and respiratory papillomatosis [1]. According to the International Agency for Research on Cancer, the high-risk genotypes of Human Papillomavirus include types 16, 18, 31, 33, 45, 52, and 58 are responsible for about 90% of anogenital positive Human Papillomavirus cancers worldwide, however, Human Papillomavirus types 6 and 11, low-risk genotypes, are responsible for 90% of genital warts [2]. Human papillomavirus, besides anogenital cancers, is responsible for some cancers of the head and neck [3]. About 80% of sexually active people become infected with human papillomavirus during their lifetime. The immune system of the body controls most of these infections within one to two years. If the infection persists, however, it can cause cellular changes that may lead to

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certain types of cancer [4]. The overall prevalence of human papillomavirus in a global meta-analysis of women with normal cervical cytology is estimated at 10.4%, which varies by geographical area [5]. Most human papillomavirus infections do not cause a problem and the body can clear them within two years. This is recommended for specific clinical manifestations of human papillomavirus-related disease (e.g., anogenital warts, precancerous lesions, or cancers [6].

The human papillomavirus vaccine is for the prevention of this disease, which reduces the progression of cervical cancer by up to 70%, and for better effects should be administered before or shortly after sexual intercourse [4]. According to the European Center for Disease Control and Prevention, 19 European countries have started vaccinating against human papillomavirus in women, as of 2012, and ten countries have initiated initiatives in this regard [7]. The coverage of human papillomavirus vaccination programs, despite these advances, varies greatly between countries [7]. Further, the least aware groups of the infection include racial and ethnic minorities, low-income women, with low education levels and no insurance, which are at significant risk for morbidity and mortality from the disease consequences [8]. The general public has little knowledge and understanding of human papillomavirus. It seems that the stress of diagnosing human papillomavirus infection is related to the lack of understanding of human papillomavirus [9].

Researches in Western countries suggest that most women, adolescents, and young people, even those with higher education, are not much aware of Human Papillomavirus infection [10]. In Iran, there is no public education program for human papillomavirus and vaccination for the general public.

The patients who have experienced the disease play a vital role in maintaining the disease transmission chain, and raising patients' awareness of disease transmission ways. Moreover, the treatment approaches can be very effective in decreasing disease transmission. Moreover, the relationship between human papillomavirus and cervical cancer considers important information which people must be aware of which may not even be taken seriously by the medical staff [11]. A study was conducted for surveying the attitude of patients with genital warts towards their disease in patients referred to the dermatology clinic of Razi Hospital in Iran, which indicated that patients' attitudes about various dimensions of the aforementioned disease are impaired [12]. The evolution of current knowledge and attitudes about human papillomavirus in patients is vital in trying to improve it.

This study was conducted with the aim of evaluating the knowledge and attitude of patients with Human Papillomavirus, transmission ways, treatment and its complications, training of patients moreover re-evaluating the knowledge and attitude of patients.

METHODS AND MATERIAL

The present study was a quasi-experimental one. The study population was patients with genital papillomavirus referred to the skin and hair clinic of Baqiyatallah Hospital in the year 2020. The sample size was obtained according to the prevalence of 38.6% [13].

Moreover the alpha of 5%, the beta of 20% and the sample size of 92 was obtained. The inclusion criteria of the study of human genital papillomavirus affection was confirmed and the satisfaction of participating in the study was related to the age range of 14 to 65 years. The exclusion criteria of the study was dissatisfaction of continuing the study. A researcher-made questionnaire was according to the desired trainings for patients that their validity of was questioned by referring questions to five experts. The reliability of this questionnaire was calculated using SPSS and Cronbach's alpha statistical method, which was higher than 70%, so the reliability of the aforementioned study was confirmed. The patients were asked about having satisfaction participating in the study before starting the study. At first, the patients filled out a pre-test questionnaire. Then the patients were trained about human papillomavirus in two parts. First, The doctor explained the cause of the disease, transmission ways of the disease, ways of prevention and treatment of the disease moreover its complications for the patients through face-to-face training. Second, A pre-designed pamphlet was provided for the patients, which contained complete explanations of the mentioned cases. Moreover, a phone number was given to the patient to ask any probable questions. the post-test questionnaire was filled out again after one month of revisit. Patients were included in the study in the form of encoding. The patient information was confidential. The individuals in the project adhered to all Helsinki’s moral principles.

Data were analyzed using SPSS software version 22. The results were expressed as the mean ± SD for quantitative variables and as a percentage for qualitative variables. The Chi-square and McNemar tests were used.

RESULTS

In this study, 92 infected patients with human papillomavirus were assessed about the knowledge and attitude of the disease before and after training in patients referred to the skin and hair clinic of Baqiyatallah Hospital in the year 2020. Table 1 indicates the distribution of demographic variables in patients.

The factors such as age, gender, marital status, and level of education were not related to the initial knowledge and attitude of individuals based on the statistical analysis (P <0.05), although, the knowledge of married people about the complications of the disease (P = 0.032), was more than single ones (70.8% vs. 45.5%).

The level of knowledge and attitude of patients was between 50% and 65% before training. The results indicated that training had a significant impact on improving the level of knowledge and attitude of patients as it reached above 95 at all levels after training (P <0.001) (Table 2).

Table 1: Distribution of demographic variables of age, gender, marital status and literacy level in the patient

Leadership Productivity	Factor Loading	Percentage	Frequency
Age	<30	33.3%	29
	30-40	52.9%	46
	>40	13.8%	12
Gender	Female	18.4%	16
	Male	71.6%	71
Marital status	Single	25.3%	22
	Married	76.4%	65
Education level	Less than diploma	22.9%	19
	Diploma	42.2%	35
	More than diploma	34.9%	29
History of STDs	Yes	3.4%	3
	No	96.6%	85

DISCUSSION

Human papillomavirus by silencing apoptosis (program cell death) and producing the inhibitor proteins of p53 and Retinoblastoma genes (inhibitor genes of cell growth) provides the conditions for the production of cellular cancer masses [14]. The human papillomavirus is involved in 30% of oropharynx cancers, 45% to 95% of anal cancers, 60% to 65% of vaginal cancers moreover 40% to 60% of vulvar cancers [15, 16]. The incidence of uterine and cervical cancers is about seven in one hundred thousand, based on the reports of the Cancer Institute. This cancer normally occurs between the ages of 30 and 55, even though there have been numerous reports among young women recently. [17] For this purpose, the existence of sufficient knowledge about the aforementioned virus and methods of its prevention and treatment can have a significant impact on promoting public health.

The present study surveyed the basic knowledge about human papillomavirus, its prevention, and treatment among patients referred to the dermatology clinic of a reference hospital and tried to indicate that the best solution for improving patients' knowledge and attitude is training them by conducting face-to-face training using educational pamphlet moreover providing some pieces of training. The findings indicated that the level of knowledge and attitude of patients was between 50% to 65% before training which was accompanied by improvement of knowledge and attitude of clients in all of these cases using training (P<0.001).

The present study indicated that the age factor does not have any impact on the knowledge and attitude of individuals about the causative agent, transmission ways, complications of the disease, the role of vaccines in disease prevention moreover the treatment way. Another study surveyed the information of people about this disease in various age groups. The amount of people's information has increased with the increment in the range of age (up to 40 years) [18]. The aforementioned study was not in line with the present study, which could be because of the wide age range mentioned in the study.

It was indicated in the current study, that education was not associated with knowledge and attitude of people about the cause of warts, transmission ways of disease, complications, disease, vaccine role in disease prevention moreover ways of treatment. Such an issue indicates that there is not any difference in knowledge and attitude of patients with human papillomavirus, even among highly educated individuals. On the other hand, it indicates a lack of adequate information or education in the community and at the general level too.

The present study surveyed the impact of gender on attitudes toward the disease, but any difference was not seen in this case. Another study conducted in this field which indicated that the gender of individuals doesn’t have any impact on their awareness of Human Papillomavirus moreover its related factors. [19]

63% of patients were aware of the cause of genital wart before being trained. This rate reached about 98% after revision (P <0.001). A similar study was conducted by Zu et al during which it was determined that the awareness of people of human papillomavirus was 15.5% while the awareness of people was 18.9% for human papillomavirus vaccines (20). Another study, conducted on Italian students to learn about human papillomavirus and its vaccination, indicated that 60% and 70.3% of pre-school students have relative and little knowledge about these cases, respectively. [21]

Table 2: Frequency of answers to questions before and after training

Questions	Answer	Pre-test	Post-test	P value
Identifying of the cause of the disease	Right	58 (63.04٪)	90 (97.9٪)	<0.001
	Wrong	34 (36.96%)	2 (2.1٪)	
Understanding the ways of disease transmission				
Direct and indirect skin contact	Right	57 (60.8%)	91 (99.0٪)	<0.001
		35 (38.2)	1 (1.0٪)	
Virus-infected instruments	Right	57 (60.8%)	89 (96.8٪)	<0.001
	Wrong	35 (38.2)	3 (3.2٪)	
Blood and blood products transfusions	Right	57 (60.8%)	91 (99.0٪)	<0.001
	Wrong	35 (38.2)	1 (1.0٪)	
The gyms	Right	57 (60.8%)	89 (96.8٪)	<0.001
	Wrong	35 (38.2)	3 (3.2٪)	
Hairdressers	Right	50 (54.3%)	90 (97.9٪)	<0.001
	Wrong	40 (44.7)	2 (2.1٪)	
Sexual contact	Right	55 (59.8%)	91 (99.0٪)	<0.001
	Wrong	37 (40.2)	1 (1.0٪)	
Shared facilities	Right	53 (57.6%)	90 (97.9٪)	<0.001
	Wrong	39 (43.4)	2 (2.1٪)	
Disease complications and treatment				
The complications of the disease	Right	54 (58.7%)	89 (96.8%)	<0.001
	Wrong	38 (41.3)	3 (3.2%)	
The vaccine for this disease?	Right	52 (56.5%)	90 (97.9%)	<0.001
	Wrong	40 (43.5)	2 (2.1%)	
Treatment methods	Right	46 (50.0%)	91 (99.0%)	<0.001
	Wrong	46 (50.0%)	1 (1.0%)	
Identifying of prevention				
vaccination	Right	48 (52.1%)	88 (95.6%)	<0.001
	Wrong	44 (47.9%)	4 (4.3%)	
Education	Right	46 (50.0%)	89 (96.8%)	<0.001
	Wrong	46 (50.0%)	3 (3.2%)	
Boosting of immunity system	Right	49 (53.3%)	91 (99.0%)	<0.001
	Wrong	43 (46.7%)	1 (1.0%)	
Prevention by medication	Right	47 (51.1%)	90 (97.9%)	<0.001
	Wrong	45 (48.9%)	2 (2.1%)	

The knowledge of the subjects in the present study about vaccination was 52% before training which reached 95% after training. The amount of training and advertising about Vaccines in our country has been poor. The present study is designed to indicate the importance of training about vaccines while the amount of training about this vaccine in other countries is much more extensive and is being conducted with a variety of tools.

Clients were informed about the transmission ways of the disease. Various factors including direct and indirect skin contact, virus-infected equipment, blood transfusion, and blood component, gyms, hairdressers through waxing, sexual contact, and common equipment in the dormitory were examined during conducting this study. The knowledge of people was between 57-50% before training which reached 91-89% after training.

Other studies have surveyed the amount of awareness of people of transmission ways of the disease, which indicated a variety of various results [22]. A study by Joshi et al. found that 44.3% of subjects were aware of the transmission ways of the disease [18]. Moreover, it was concluded in this study that both the level and type of education of individuals have significant impacts on their awareness of the aforementioned virus as well as its vaccination and treatment.

CONCLUSIONS

The knowledge and attitude of patients with human papillomavirus disease, despite its importance, seem not to be enough. However, effective and well-planned education can have a significant impact on the knowledge and attitude of patients with this disease. Thus, it is suggested that in public education programs, the important educational points about Human Papillomavirus must be given priority.

Acknowledgment

Researchers thank to guidance and advice from the Clinical Research Development Unit of Baqiyatallah Hospital.

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ORIGINAL ARTICLES

Peptide nucleic acid (PNA) as a novel tool in the detection and treatment of biological threatening diseases

Mohammad S. Hashemzadeh[1]

Abstract: *Peptide Nucleic Acids (PNAs) are nanostructures similar to nucleic acid molecules (synthetic DNA/RNA analogs) wherein the negatively charged backbone (sugar-phosphate) present in DNA/RNA molecules is replaced by a backbone without polyamide or peptide charge. Later, it was found that PNAs containing both purine and pyrimidine bases form highly stable duplexes with DNA and RNA. Although it is not as stable as 2PNA/DNA triplexes containing a homopyrimidine strand, it is still more stable than DNA/DNA and/or DNA/RNA duplexes. The unique characteristics of PNAs add new aspects to these nanostructures relative to conventional analogs to make them appropriate for molecular biology studies. The most important applications include the use of these nanostructures in the detection and treatment of diseases caused by threatening biological agents using the antisense/antigen technology and as genetic regulator drugs.*

Keywords: *Peptide Nucleic Acids (PNAs), synthetic DNA analog, genetic regulator drugs, antisense-antigen technology*

INTRODUCTION

Nielsen et al. (1991) were the first who reported the synthesis of a molecule, called Peptide Nucleic Acid (PNA), as a novel, completely synthetic analog of nucleic acids, which was commercialized in 1993 [1]. PNA molecules are considered as DNA/RNA synthetic analogs where the negatively charged backbone (sugar-phosphate) present in DNA/RNA molecules is replaced by a backbone without polyamide or peptide charge [2].

Biochemically, the main backbone of PNA structure forms by repetitive units of N-(2-aminoethyl) glycine connected by peptide bonds, and different nucleobases (purine and pyrimidine) are attached to the main backbone through methyl carbonyl linkers [3]. The same peptides are also drawn as the C-terminus and N-terminus, a state corresponding to 3′ and 5′ termini in DNA [4]. These characteristics render PNA to be stable biologically, making it usable in therapeutic applications [5].

IMPORTANT CHARACTERISTICS OF PNA MOLECULES

1) PNAs have extremely high affinity, specificity, and sensitivity to interact with nucleic acids (RNA/DNA), which is 50-100 times stronger than a typical DNA/DNA or DNA/RNA hybridization.

2) They possess a very high hybridization ability.

3) The occurrence of a mismatch or single nucleotide polymorphism (SNP) in their hybridization with DNA or RNA leads to a very strong destabilizing effect in the hybrid

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formation, which strongly reduces the annealing temperature.

4) PNAs are very stable molecules both chemically and biologically.

- Extremely high chemical stability to temperature and pH.

- Extremely high biological stability to nuclease and protease enzymes, meaning that they are resistant to enzymatic digestion and hydrolytic processes.

5) Their hybridization with such nucleic acid molecules as DNA is independent of environmental salt concentrations.

6) They attach to DNA or RNA strands the same as common Watson-Crick attachments.

7) The formation of a triplex with DNA is possible when PNA containing two homopyrimidine oligomers interlinked by a flexible linker reacts with the DNA homopyrimidine fragment. In such a case, a PNA strand through Watson-Crick pairing and the other via Hoogsteen pairing interact with DNA, with a DNA strand extruding the triplex structure, which will have many applications in gene expression regulation.

8) They have an asymmetric, peptide-like, and uncharged structure, which is hydrophilic and water-soluble.

- Although the uncharged nature of PNA facilitates its delivery through the cell membrane, this transfer into the cell is a difficult process requires using a variety of carriers.

- The uncharged nature of PNA molecules results in their much stronger interconnection (than DNA/DNA and/or DNA/RNA) due to the lack of electrostatic deterrence with DNA and RNA molecules.

9) Tm is much higher for PNA hybrids than other hybrids (DNA/DNA and/or DNA/RNA), with Tm values of 50 and 70 °C for PNA hybrids of 10mer and 15mer, respectively.

It is noteworthy that triplex structures with DNA are better formed in acidic pH (4.5-6.5) in vitro, but the main prerequisite is the presence of PNA pyrimidine.

As mentioned above, the PNA-PNA hybrid is extremely thermostable and is independent of environmental ionic potential, such that PNA-PNA > PNA-RNA > PNA-DNA > DNA-DNA. This stability, however, is strongly affected by basic compositions in the PNA sequence, such that Purine PNA >> pyrimidine PNA; among pyrimidines, on the other hand, homopyrimidines show very high stability.

Due to the very high binding ability of PNA molecules with DNA, it is not necessary to design long PNA oligomers hence fragments of 20-25 nucleobases are appropriate [6-10].

PREPARATION AND STORAGE OF PNA SUSPENSION

PNA can be easily dissolved in 0.1% trifluoroacetic (TFA) and the prepared stock can be stored frozen for a long time [7].

PNA hybridization and the salt effect

As denoted previously, the Tm of the PNA molecule does not change significantly with changes in environmental ionic potential. As such, the Tm of a 15mer PNA duplex only decreases 5 °C with an increase in NaCl concentration from 10 mM to 1 M, but the altered concentration has a very strong effect on the Tm of DNA/DNA hybridization. As a result, PNA can serve as a very suitable probe for the identification of target sequences at low salt concentrations.

Tm drops significantly due to a mutation in the allele of a gene and the incidence of a mismatch with PNA. In mismatch detection, therefore, DNA probes are the winners in competition with PNA probes, and this hallmark can be used in the detection of allele type [8].

Advantages of PNA molecules with very stronger pairing characteristics

PNA fragments with very shorter lengths can be used as probes, with lengths of 20-25 meres for DNA probes, but those of 13-17 meres long also operate well as PNA probes.

2) This hybridization is over 100 times faster than DNA/DNA and/or DNA/RNA hybridization. For example, if DNA hybridization lasts about 3 h to overnight, PNA hybridization will last 30-45 min.

3) As mentioned above, the incidence of a mismatch in this hybridization will result in more destabilization than typical DNA/DNA duplexes. This will significantly reduce Tm, leading to a decrease in the specificity because of a reduction in the annealing temperature as well [9-13].

Some disadvantages of using PNA

1) It takes a high cost for mass production as it is produced synthetically.

2) In the PCR process, the polymerase primer cannot detect its C-terminus and only detects the 3' terminus.

3) It cannot be amplified by the cloning process [14].

SOME PROBLEMS OF PNA IN CLINICAL APPLICATIONS

1) The dose and toxicity problem

A dose that responds properly in vitro is toxic to the body (both PNA itself and the associated molecules such as cationic peptides); hence, dosimetry should inevitably be done to determine the toxicity level.

2) The delivery problem: It is difficult to deliver it into target tissue cells and has a low efficiency [15].

PCR clamping

This feature can be used in the detection of point mutations or SNPs in which an allele-specific primer competes with an SNP-containing PNA primer. If the SNP occurs in the relevant allele, PNA will win the competition (due to its higher Tm in normal conditions) and PCR will cease. If SNP does not occur, PNA will have a mismatch, Tm drops, and consequently, the primer will be the actual winner, leading to the occurrence of PCR (due to a higher Tm), which will be visible on the PCR agarose gel (Figure 1) [16].

Figure 1: The use of PNA in the PCR process

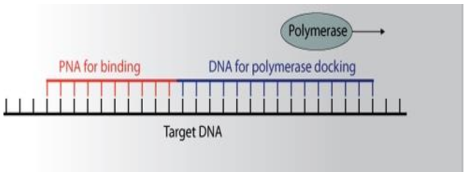

VARIOUS APPLICATIONS OF PNA MOLECULE

PNA molecule can be applied:

1. As a probe for the diagnosis and detection of biological threatening diseases, including:

1.1 Fluorescent In Situ Hybridization (FISH) probe

In this method, PNA labeled probes are utilized to detect certain telomeric reigns of the human chromosome or other specific sequences in various organisms. An example of this application in the detection of a conserved sequence on the rRNA of Staphylococcus aureus is depicted in Figure 2.

Figure 2: The use of PNA molecule as a specific probe in FISH

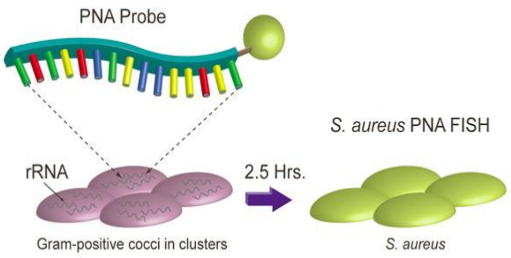

1.2 Detection of SNPs as described above
1.3 Light UP probe (or Q-PNA probe)

PNA is used as a probe in real-time PCR (RT-PCR) so that fluorescein and a quencher can be placed on each terminus of the molecule. A molecular beacon model of RT-PCR is shown in Figure 3.

Figure 3: The use of PNA as a probe in RT-PCR

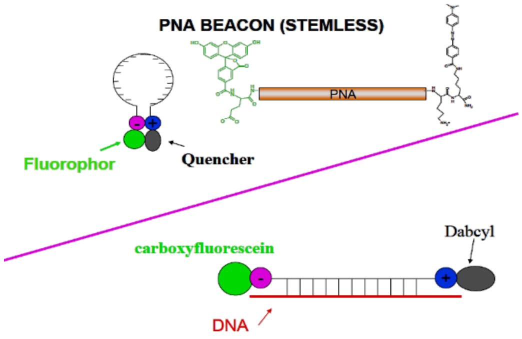

2. As a molecular tool in functional genomics research, including:
2.1 A probe for the northern and southern blot processes
2.2 PCR Clamping
2.3 In the separation and purification of the target DNA molecule
2.4 PNA array, which can be used in the PNA-based microarray technique.

3. The use of PNA as regulator drugs, including the antisense and antigene effects and so on, is one of the most important applications of PNA that has been the focus of worldwide research [17], which is discussed further in the following.

In the antisense application, PNA is designed against cellular RNAs, and in the antigene application, PNA is designed against the gene fragment (DNA). This indicates that many genetic diseases can be treated that are caused by defects in a gene function and/or overexpression; it can also be effective in cancer treatment, which often results from disrupted gene expression. In other groups of diseases, PNA is designed against cellular RNAs, such as in AIDS, where cDNA is synthesized from cellular RNAs through the action of reverse transcriptase. If PNA can react with RNA, it will inhibit the action of this enzyme on RNA and ceases the above reaction, thereby controlling the disease [18].

GENETIC REGULATOR DRUGS

1. The antigene technology

In this application, PNA is used as a gene inhibitor that needs to form a triplex with a double-strand DNA. Based on nucleobase combinations in DNA, this PNA is designed against external gene enhancer fragments that play a key role in the onset of transcription to inhibit the transcription process by RNA polymerase. This will occur when the designed PNA is attached to the non-coding strand of the gene enhancer as it plays the role of a template. PNA can be designed against the promotor region (−10 and −35) to further inhibit the non-coding strand and is not accessible to the enzyme. PNA can also be designed against the internal gene enhancers or other internal gene sequences containing homopurine sequences and their strong hybridization with DNA results in incomplete transcription of the gene and no expression of the target gene; it can also be very effective in cancer treatment as mentioned above [19].

2. Direct activation of gene

The gene is activated in this application unlike the antigene effect aiming at inhibition of gene expression as described above. The triplex forms in such a way that will extrude the non-coding strand. PNA is designed against the coding strand concerning nucleobase combinations and will be used in many genetic diseases caused by a defect in the functional expression of a gene. The target gene, which has become part of the heterochromatin, will occasionally return to the euchromatin by the effect of PNA and become accessible to transcription enzymes [20].

3. The antisense technology

This application uses synthetic PNAs against the transcribed gene product (sense-RNA) such that the PNA oligomers are started against the Shine-Dalgarno sequence, the codon region, or the internal mRNA sequences; in the latter case, it results in the inhibition of protein elongation process by the ribosome. In most cases, antisense PNA is simultaneously designed against the Shine-Dalgarno sequence and the codon region, as these two are not much apart. In such cases, the strong PNA/RNA hybrid becomes a stable duplex and is prevented from the translation process [21].

4. Inhibition of 16S rRNA

In this case, PNA is designed against rRNAs in the ribonucleoprotein (RNP) structure, the most important of which in bacteria is the 16s as it plays a role in the attachment of ribosome to the Shine-Dalgarno sequence and inhibition of 16S rRNA, i.e. inhibition of ribosome attachment to mRNA, thereby inhibiting protein synthesis. Existing evidence indicates that PNAs designed against 16S rRNA and 23S rRNA segments play a bacteriostatic role (cessation of bacterial growth and protein synthesis). These PNAs are considered effective drugs and genetic antibiotics (instead of chemical antibiotics) [22].

5. Inhibition of microRNAs

These RNAs play a regulatory role in gene expression. A PNA oligomer can be designed to inhibit the function of a given mi-RNA in which mi-RNA, as an inhibitor of certain gene expression, is bounced out to resume the expression of such genes. This application can be utilized in many diseases caused by the aberrant presence of mi-RNAs (where they must not be present) [23].

6. Inhibition of telomerase function

A reason for cancer cell immortality can be the continuous activity of this enzyme and its re-expression due to the activity of a series of oncogenes. If the PNA fragment is designed against the RNA part of this enzyme, it inhibits the enzyme function and cures cancer [24].

7. Alternative splicing

Here, a specific PNA is designed against splicing-specific sequences in pre-mRNA to change the mRNA product and ultimately achieve target gene expression. Accordingly, PNAs can play a role as regulator drugs in the treatment of bacterial and viral infections, as well as many other diseases [25].

SOME IMPORTANT CLINICAL APPLICATIONS OF PNA

1. Cancer therapy

As described above, this application can be achieved by the suppression of telomerase. Thus, PNA can also be considered as an anti-cancer drug, or exert antisense and antigene effects on such genes as Bcl2. This begins by designing a PNA probe against the codon region, the Shine-Dalgarno sequence, or homopurine sequences on the coding DNA strand to reduce the expression level of Bcl2 and prevent cancer cells from using this mechanism for their survival [26].

2. Treatment of biological threatening diseases

Research has shown that the PNA molecule can effectively inhibit the function of reverse transcriptase that has a contribution to disease development. Indeed, this enzyme synthesizes a cDNA by the detection and transcription of template RNA. An appropriate and specific PNA oligomers can be designed to effectively improve these viral infections (27).

PNA DELIVERY STRATEGIES TO THE CELL

As denoted before, it is very difficult to deliver PNA to eukaryotic and prokaryotic cells, with low efficiency. For this reason, strategies are employed to facilitate the delivery process, some of which are described below.

1. Making some modifications in their main backbone structure

2. Pairing them with peptide delivery groups

3. Paring them with cationic peptides (lysine and arginine

residues)

4. Coupling them with some DNA oligomers

5. Coupling them with specific ligands (e.g., antibodies) linking to cell surface receptors

6. Delivery using liposomes for which typical liposomes should be used instead of cationic liposomes due to their toxicity despite higher efficiency.

7. By the use of cationic polymers

Figure 4 illustrates a schematic of the delivery of a PNA molecule using liposomes.

Figure 4: Delivery of PNA molecule to a eukaryotic cell using liposomes

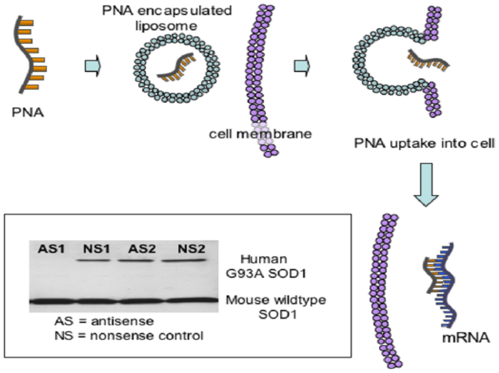
PNA
PNA encapsulated liposome
cell membrane
PNA uptake into cell
AS1 NS1 AS2 NS2
Human G93A SOD1
Mouse wildtype SOD1
AS = antisense
NS = nonsense control
mRNA

RESULTS AND DISCUSSION

As noted above, the main backbone of PNA structure forms by repetitive units of N-(2-aminoethyl) glycine connected by peptide bonds. As with peptides, these are drawn by C and N terminus that correspond to the 3′ and 5′ terminus in DNA (Figure 5).

Figure 5. Comparison of PNA, DNA, and protein structures

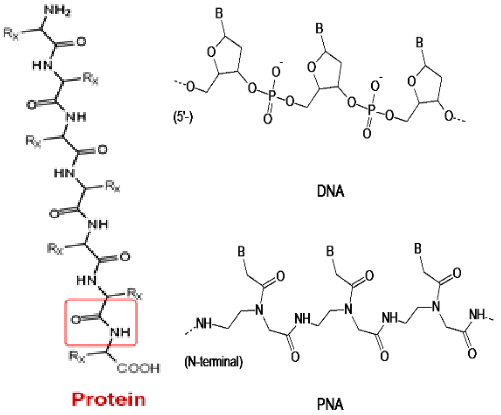
NH2
Rx
HN
NH
COOH
Protein
B
(5'-)
DNA
(N-terminal)
PNA

Tm changes are insignificant for PNA with fluctuations in environmental ionic potential in comparison to DNA and RNA. Thus, PNA can serve as a very appropriate probe for the detection of target sequences at different salt concentrations (Figure 6) [28].

Figure 6: Ionic and thermal changes of PNA/DNA and DNA/DNA

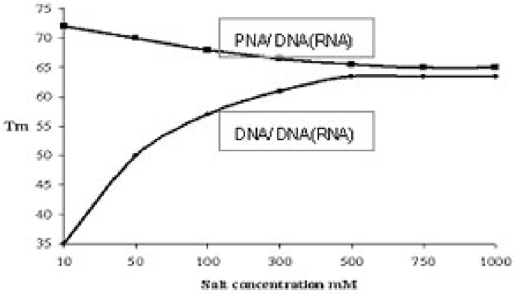

As mentioned above, PNA is designed against the gene fragment (DNA) and cellular RNAs in the antigene and antisense techniques, respectively (Figures 7 and 8).

Figure 7: Inhibition by the antigene technique

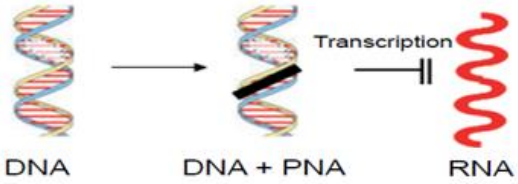
Transcription
DNA
DNA + PNA
RNA

Figure 8: Inhibition by the antisense technique

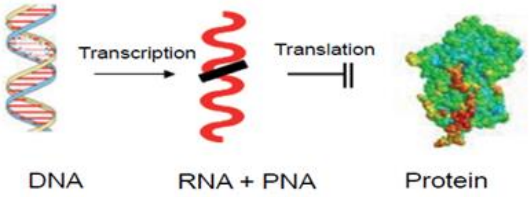
Transcription
Translation
DNA
RNA + PNA
Protein

Figure 9: Changes in β-galactosidase activity with rising inhibitory PNA concentrations

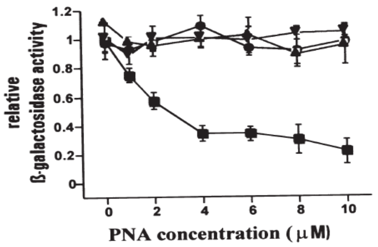

Anti-β-galactosidase PNA was reported to inhibit the expression of the β-galactosidase reporter gene in E. coli AS19. As shown in Figure 9, the activity of this enzyme decreases with an increase in PNA concentrations, indicating the decreased expression of this gene by a specific PNA. Control samples are presented in the diagram (Figure 9) showing a change in the enzyme activity.

Therefore, these two critical technologies can help to effectively improve diagnostic and therapeutic goals.

CONCLUSION

Overall, it can be concluded that PNA nanostructures are currently considered by scientists to have a wide array of applications.

Accordingly, further investigations on these nanostructures will provide an effective tool to achieve the above diagnostic and therapeutic goals.

Medical and molecular biotechnology has been revolutionized with the synthesis of PNA.

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VARIA

Planning a hospital to respond to the COVID-19 outbreak: experience from a Romanian reference unit

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Abstract: *During the COVID outbreak, many hospitals were nominated as a support hospital for the treatment of positive tested patients infected with SARS CoV-2 virus. "Dr. Nicolae Ruşdea" Pneumophtiziology Hospital Baia Mare carried out an exceptional activity compared to the previous year considering the current epidemiological context. Major changes were made in strategies, procedures and protocols. The staff had to adapt to the new epidemiological situation through dedication and devotion. In 2020, there were no COVID outbreaks within the unit, as separate compartments were made, intended to protect the medical personnel, but also to ensure the health of the patients.*

Keywords: *COVID plan, hospital, Romanian reference unit*

INTRODUCTION

"Dr. Nicolae Ruşdea" Pneumophtiziology Hospital Baia Mare is a specialized hospital providing medical services in the specialties of pneumology for adults and children. Starting with April 2020, it provides hospital care for patients tested positive for the SARS-CoV-2 virus, including intensive care. In 2020, the hospital served the entire county of Maramureș for patients tested positive for SARS-CoV-2 virus. In the intensive care department, patients from Maramureș County received health care, mainly from the country, depending on the places available at the request of the Committee for Emergency Situations. Throughout this period, the fight against pulmonary tuberculosis continued through the national control and surveillance program as well as ensuring the continuity of integrated ambulatory care services for patients with non-Covid pneumological diseases and the follow-up of post-Covid patients. Compared to the previous year, considering the current epidemiological context, which led to major changes in the way of working, procedures and protocols. In order to provide the resilience of health systems during the COVID-19 pandemic, we describe, at the hospital level, the key elements to provide the medical staff sand patients safety, including the challenges and issues to anticipate following the experience of our center

CLINICAL EXAMPLE

The aim was to establish synergies between health worker safety and patient safety policies and strategies, developing linkages between occupational health and safety, patient safety, quality improvement, infection prevention and control programmes [1]. It was necessary to review and upgrade the standards, guidelines and codes of practice in order to assure the health worker and patient safety [1]. The executive board consisting of a multidisciplinary team: general manager, epidemiologist and the technical-administrative staff, planned the separation of the hospitals' circuits in COVID and NON-COVID. During the pandemic, the hospital changed his usual structure, consisting of 2 departments of medical specialties (pneumology, intern medicine, cardiology, urology, metabolic diseases,

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neurology), one section of surgical specialties, one pediatric department, a department ICU (intensive care unit), blood transfusion units, palliative care and an operating room. The rest of the structure was maintained: farmacy, sterilization, medical analysis laboratory, radiology department, tuberculosis dispensary, reception desk, evaluation and medical statistics department (Figure 1).

Figure 1: Plan for new structure of the hospital to augment the health system during the pandemic.

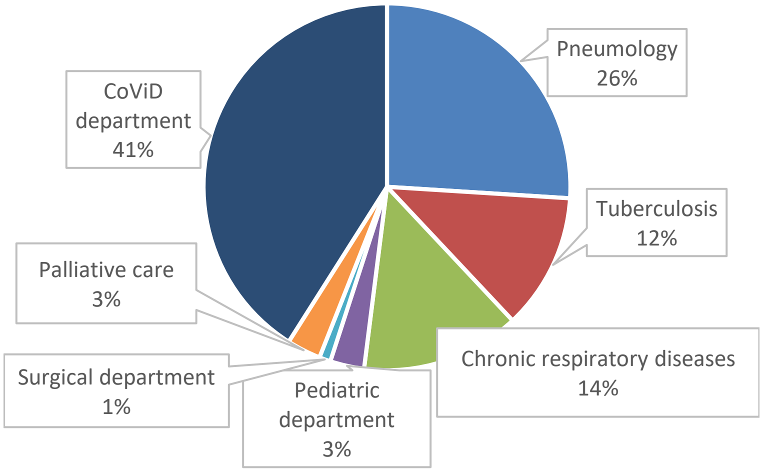

After the State of emergency in the coronavirus pandemic, depending on the local epidemiological evolution, the hospitalizations and the scheduled surgical interventions can be resumed, as well as the activity in the outpatient clinics. Being a 4-storey Hospital, a level was created for the green zone, non-COVID, and the 2nd and 3rd floor were allocated for the COVID section - red zone, served by an elevator: at each level a separate filter for dressing and undressing the protective equipment; the 4 elevators and 6 exits of the hospital had separate destinations: a red zone entrance, one for staff, one for drugs and one for food. A special elevator for patients, one for the staff and the elevators on the opposite side of the building are used for waste and medical staff. A separate circuit was intended for the ICU section, and a separate one for the rest of the staff. In the green area, the staff was separated, depending on the section, so as not to be conglomerated by people. Radiology department: provides a separate circuit for non-COVID patients hospitalized, and a dedicated department for tuberculosis dispensaries. Also a sequential circuit for computer tomography access. The organization of the COVID departments provides a separate circuit for access to the Covid patients section with a dedicated elevator only for COVID-positive patients. The medical personnel circuit with clothing filter area, decontamination area, green area for rest and document preparation (common for COVID and ATI section). The contaminated waste had a special circuit - collection and transport on a circuit intended only for waste (all waste generated in the COVID section are considered hazardous waste). The food circuit, consisting in disposable dishes, are collected as infectious medical waste. Assistance to acute and chronic non-COVID pneumology patients was assured. When entering the hospital, all people will go through an epidemiological triage, depending on the symptoms presented, the suspicious cases will be hospitalized in special isolation areas (buffer-zones), organized at the 1st floor, to be tested by RT-PCR for SARS-CoV-2 infection (according to the recommendations of the National Institute of Public Health). Separate circuits are provided for these patients.

DISCUSSIONS

With all the circuits, red-zone and green-zone, the hardest part was teaching the staff to overcome fear, but the idea that you have some completely separate safe circuits made the staff trustful and work, and accept the new rules imposed. Moreover, having 4 training teams that teaches the use of the protective equipment, the existence of a supervisor in the first 3 months to monitor the dressing and undressing the equipment, in order to avoid mistakes. As a result, there was no outbreak in the hospital for 12 months, with only isolated cases coming from outside of the hospital.

CONCLUSIONS

The coronavirus disease (COVID-19) outbreak is a global public health problem. Medical personal is an important at-risk population that can get COVID-19 due to close contact with patients2. The healthcare reform, with subsequent amendments and additional changes, caused a new reorganization and restructuring of health units, depending on the needs, in order to ensure the isolation conditions and the functional circuits that aim at the prevention and control of the SARS-COV-2 infection3,4. The reorganization and restructuring of the health units subordinated to the local public administration authorities will be done with their consultation. The reason for these changes is mostly because of the epidemiological evolution of the SARS-COV-2 virus pandemic. In addition, there is an increased addressability of patients with pneumological problems including patients with pulmonary diseases after SARS-COV-2 infection that need recovery. Also, the need to carry out the activities of the tuberculosis prevention and control programs.

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ORIGINAL ARTICLE

Post-traumatic stress disorder and cardiovascular disease

Corneliu A. Moraru[1], Elena D. Năfureanu[1,2], Adelina Proca[1,2], Iuliana Zavadovschi[1], Mariana Floria[1,2], Dragoș M. Popescu[3]

Abstract: *Posttraumatic stress disorder (PTSD) is a psychological-psychiatric state caused by exposure to stressful, traumatic events that manifests as a vivid reminiscence of them by flashbacks, nightmares, reccurent memories, emotional and physical manifestations. Cardiovascular diseases (CVD) are of interest in patients suffering from PTSD because there is an increasing body of evidence that these pathologies are linked. Hypertension, dyslipidemia, diabetes, and other cardiovascular risk factors are common in patients with PTSD and all of them increase the incidence of coronary syndromes, both chronic and acute. Posttraumatic stress disorder has a major impact on the lifestyle and health of subjects exposed to trauma or stress. Cardiovascular diseases tend to appear sooner in PTSD diagnosed patients due to a variety of reasons: poor habits, low addressability to health care systems, chronic inflammation status, changes at molecular levels, etc. Otherwise, PTSD tends to be induced by CVD, thus inclining the balance towards whis association. According with data published until now, there is a strong pathophysiologic relatonship between PTSD and some CVD; there is also outlining a vice versa relationship, from some CVD to PTSD.*

Keywords: *posttraumatic, stress, disorder, cardiovascular, disease*

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a psychological-psychiatric state caused by exposure to stressful, traumatic events that manifests as a vivid reminiscence of them by flashbacks, nightmares, recurrent memories, emotional and physical manifestations.

Cardiovascular disease (CVD) is of interest in patients suffering from PTSD because there is an increasing body of evidence that these pathologies are linked.

Posttraumatic stress disorder seems to be associated to an increased risk of CVD due to its psychosocial consequences that become cardiovascular risk factors and due to its ability to cause cardiovascular structural changes and alter the neuroendocrine mechanisms involved in maintaining body functions. This disorder is mainly associated with hypertension and coronary artery disease but it can also lead to arrhythmia or stroke, with recent studies bringiging into attention the pathological link at molecular and genetic levels. The shortcomings of existing studies are that they do not report the incidence rate of each CVD after exposure to stressful events but rather tell us about the incidence rate of cardiovascular risk factors in populations suffering from PTSD (mostly military field workers, either veterans or active soldiers).This systematic review of the literature aims to bring forward the extent to which PTSD can cause damage to the cardiovascular system and to present the pathological link between PTSD and CVD as well as to present the vice-versa relationship: PTSD induced by CVD.

MATERIALS AND METHODS

We conducted the present systematic review in concordance with updated Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [1]. A literature search using a prespecified MeSH combination of words (“posttraumatic stress disorder " AND "cardiovascular disease”) was performed in PubMed and Google Academic. Two essential inclusion criteria were defined and applied for retrieved citations which guided the eligibility assessment: (1) randomized controlled and non-randomized clinical trials

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with available abstract (for PubMed) and containing ”the exact phrase” (for Google Academic); (2) studies which enrolled at least 50 patients or more (due to the high probability of not having a significant statistical power). Additionally, studies were excluded from the analysis if they met at least one of the following criteria: articles that could’t be retrieved because of access to the editorial, doubles, articles written in languages other than english, other types of literature (books, e-books, disertation thesis, article in press), studies conducted on animals and articles that have not treated the issue of our search. All studies without PTSD diagnosis defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-III), DSM-IV, DSM-5 or International Classification of Diseases (ICD-10) were excluded. We taking into consideration the reviews, systematic reviews and meta-analysis only in discussions, to avoid duplications and bias selection.

Two independent investigators decided if studies fulfilled the inclusion criteria and any disagreements were solved by discussion and consensus.

RESULTS

The flow-chart of the search process as well as complete databases search strategies were provided in Figure 1. We retrieved a total number of 244,976 articles from both databases out of which were removed 244,589 due to automation tools presented above, 11 doubles and other 118 articles that were other types of literature or whose abstract was written in other language than english, resulting in 258 articles for screening.

Figure 1: Systematic search of the literature: PRISMA flowchart

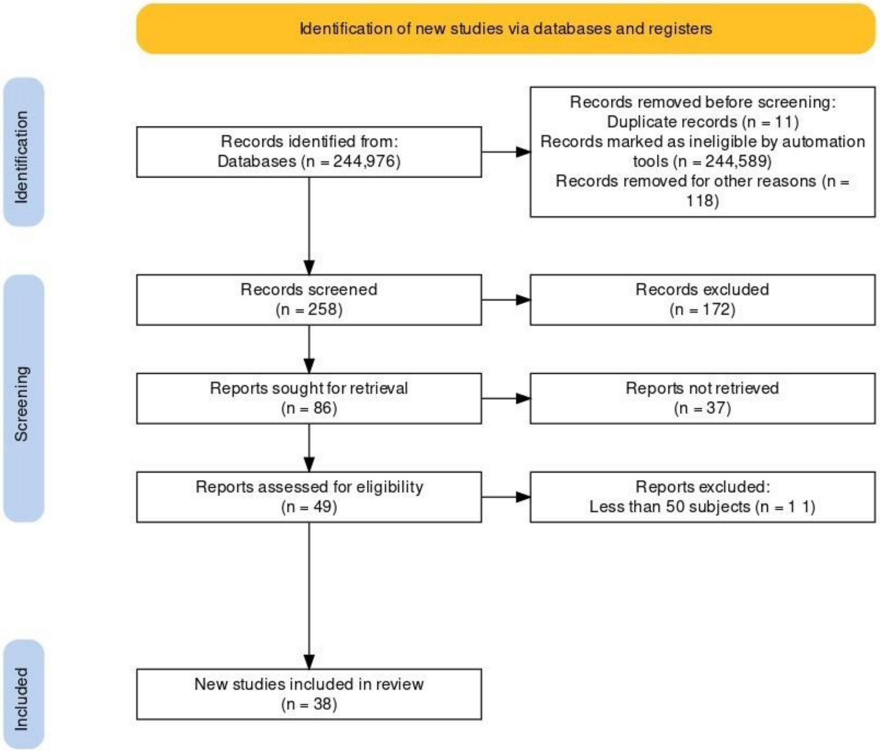
Identification of new studies via databases and registers
Identification
Records identified from: Databases (n = 244,976)
Records removed before screening: Duplicate records (n = 11) Records marked as ineligible by automation tools (n = 244,589) Records removed for other reasons (n = 118)
Screening
Records screened (n = 258)
Records excluded (n = 172)
Reports sought for retrieval (n = 86)
Reports not retrieved (n = 37)
Reports assessed for eligibility (n = 49)
Reports excluded: Less than 50 subjects (n = 1 1)
Included
New studies included in review (n = 38)

After exclusion of 172 articles (19 presenting studies conducted on animals and the rest not being of interest because they did not debate the relationship between PTSD and CVD), another 37 could not be retrieved and 11 not being taking in consideration because they used less than 50 subects in trials.

The final number of articles used in our systematic review was 38 (n=2,507,724 patients): 25 prospective (n=1,451,924 patients) and 13 retrospective (n=1,055,800 patients) studies.

Study types and their aims, along with demographic, methodology of each and results are presented in Table 1 [2-39].

Twenty three prospective (n=1,449,992 patients; 99.9%) and nine retrospective (n=1,043,266 patients; 98.8%) studies showed a relationship between PTSD and CVD.

Table 1: Studies taken into consideration.

Author	Study type and aim	No. of patients	Methodology	Results
Scherrer JF et al., 2020 [2]	Prospective study Primary objective:estimate the risk of incident CVD (CVD) in patients with vs. without a clinically meaningful PTSD (PTSD) improvement. Secondary objective: if results differed by depression status and age group.	1079	Eligible patients, were 30–70 years old, diagnosed with PTSD and used Veterans Health Affairs PTSD specialty clinics. Patients had a PTSD Checklist score (PCL) ≥ 50 between 2008 and 2012 and a second PCL score within 12 months and at least 8 weeks after the first PCL ≥ 50. Clinically meaningful PTSD improvement was defined by ≥20 point PCL decrease between the first and second PCL score. Patients were free of CVD diagnoses for 1 year prior to index. Index date was 12 months following the first PCL.	Patients with vs. without PTSD improvement did not differ in CVD risk (HR = 1.08; 95%CI: 0.72–1.63). Results did not change after stratifying by age group or depression status.
Kubzansky LD et al., 2014 [3]	Prospective observational To determine if women who develop PTSD symptoms are more likely to gain weight and become obese relative to trauma-exposed women who do not develop PTSD symptoms or women with no trauma exposure	54224	Trauma and PTSD Screening Questionnaire was used to determine whether women present traumatic exposure according to the DSM-IV PTSD diagnosis. These women were divided in 2 separate groups for analysis depending on when trauma exposure/PTSD occurred. Data on weight and height was reported biennially. The BMI was calculated.	If trauma leads to the development of PTSD symptoms, the patient has an increased risk of weight gain. Women with PTSD should be monitored or undergo screening for development of adverse cardiometabolic outcomes. It is important to expand PTSD treatments to attend to behavioral alterations.
Bourassa KJ et al., 2020 [4]	Retrospective study A secondary analysis of a larger intervention study of active duty soldiers with PTSD The aim of this study is to see if first-line exposure therapies are associated with improvements in participants cardiovascular functioning and reactivity compared to not receiving treatment patients.	104	Active duty soldiers with PTSD were randomized to an exposure therapy—either prolonged exposure or virtual reality exposure (VRE) or a waitlist control condition. The interest variables were: change in participants' resting heart rate (HR) and HR reactivity from baseline (prior to randomization) to mid- and post-treatment.	Decreased resting HR (B = -5.06, p = .024) and HR reactivity (B = -2.46, p = .005) from baseline to post-treatment for PE and VRE relative to waitlist. Exploratory analyses found that change in resting HR and HR reactivity were not significantly correlated with either self-reported or clinician-rated PTSD symptom change.
Burg MM et al., 2017 [5]	Prospective study The aim is to investigate the prospective relationship of PTSD to incident hypertension risk and the effect of PTSD treatment on hypertension risk.	194319	Data from a nationally representative sample of 194,319 veterans were drawn from the Veterans Administration roster of United States service men and women. The variables were: incident hypertension, PTSD treatment	The incident hypertension risk independently associated with PTSD ranged from HR=1.12 (95% CI 1.08 – 1.17, p<0.0001) to HR=1.30 (95% CI 1.26 – 1.34, p<0.0001). The interaction of PTSD and treatment revealed that treatment reduced the PTSD associated hypertension risk (e.g., from HR=1.44 [95% CI 1.38 – 1.50, p<0.0001] for those untreated, to HR=1.20 [95% CI 1.15 – 1.25, p<0.0001] for those treated)
Dennis PA et al., 2014 [6]	Prospective study The aim o this study is to demonstrate that: 1) PTSD symptoms would be associated with lower HRV; 2) PTSD symptoms would be associated with greater smoking, higher rates of lifetime alcohol dependence, higher rates of abdominal obesity, and more sleep disturbance; and 3) each of these behavioral risk	227	Short-term laboratory-based and 24-hour ambulatory measures of HRV were collected from 227 young adults (18-39 years old), 107 of whom were diagnosed with PTSD. They assessed the relationship of PTSD symptoms with HRV along with potential behavioral health mediators.	PTSD symptoms were associated with reduced HRV, β = -.21, p = .002. This association was reduced in models that adjusted for cigarette consumption and history of alcohol dependence, and was rendered non-significant in a model adjusting for sleep disturbance. Abdominal obesity was not a

Author	Study type and aim	No. of patients	Methodology	Results
	factors would partially mediate the association between PTSD symptoms and HRV.			significant mediator.
Chang JC et al., 2017 [7]	Prospective study The present study estimated incident PTSD and comorbid diseases for over a decade in a cohort from a community-based integrated screening program.	76417	The cohort was derived from a population of about 390 thousands adults (aged 30 years and above) in the northeastern part of Taiwan. The enrolled participants were interviewed to collect social demographics, health behaviors (e.g., smoking or alcohol drinking), and family history of major illnesses (e.g., psychiatric disorders).	Compared with non-PTSD participants, those with PTSD tended to be female, younger, more highly educated, non-smokers, and non-drinkers. Furthermore, participants with PTSD had a higher proportion of CVD.
Meli L et al., 2018 [8]	Prospective study They tested whether younger age was associated with greater threat perceptions during ACS evaluation in the ED and greater posttraumatic stress symptoms (PSS) in the frst week after ED enrollment.	871	Participants completed measures of demographics, including age, sex, and race/ethnicity, and perceived threat during evaluation for suspected acute coronary syndrome in the ED. During inpatient stay or by phone after discharge (median 3 days after enrollment), participants reported PSS (i.e., symptoms of re-experiencing and/or avoiding reminders of the trauma, and hyperarousal) in response to the event that brought them to the emergency department.	Younger, versus older individuals are at risk for greater PSS after acute coronary syndrome evaluation, and elevated perceived threat partially mediated this association.
Rosman L et al., 2019 [9]	Retrospective study They assessed the association between PTSD and incident transiemt ischemic attack (TIA) and ischemic stroke	987855	They assessed the incidence of TIA and ischemic stroke in a cohort of 987855 young and middleaged Veterans (mean age of 30.29±9.19 years; 87.8% men, 64.4% white) who first accessed care through the Veterans Health Administration from October 2001 to November 2014 and were free of TIA and ischemic stroke at baseline.	PTSD was significantly associated with new-onset TIA (hazard ratio [HR], 2.02; 95% CI, 1.62–2.52) and ischemic stroke (HR, 1.62; 95% CI, 1.47–1.79). The effect of PTSD on ischemic stroke risk was stronger in men than in women (HR, 0.63; 95% CI, 0.47–0.86; P=0.003), but no effect of sex was found for TIA.
Giesinger I et al., 2020 [10]	Prospective study To assess whether 9/11-related probable PTSD is associated with increased mortality risk	63666	PTSD assessments at baseline (wave 1: 2003-2004) and 3 follow-up time points (wave 2: 2006-2007, wave 3: 2011-2012, wave 4: 2015-2016). PTSD was defined using the 17-item PTSD Checklist–Specific (PCL-S) self-report measure (score≥50) at each wave (waves 1-4	Comparable findings between responders and civilians suggest that 9/11-related PTSD is associated with an increased mortality risk.
Lima BB et al., 2020 [11]	Prospective study. To evaluate the association of PTSD with mental stress–induced myocardial ischemia among individuals who survived a myocardial infarction (MI).	303	Participants had a verified history of MI within 8 months. Patients received technetium 99m–labeled sestamibi myocardial perfusion imaging at rest, with mental stress (ie, a speech task) and conventional stress (ie, exercise or pharmacologic)	PTSD was associated with the development of myocardial ischemia with mental stress.
Lima BB et al., 2019 [12]	Prospective study The aim is to demonstrate that survivors of a myocardial infarction (MI) who have PTSD would show an enhanced inflammatory response to acute psychological stress compared to those without PTSD.	271	Individuals with a verified history of MI within 8 months and a clinical diagnosis of current PTSD underwent a mental stress speech task. Inflammatory biomarkers including interleukin-6 (IL-6), high-sensitivity C reactive protein (HsCRP), matrix metallopeptidase 9 (MMP-9), intercellular adhesion molecule	MI patients with current PTSD exhibit enhanced IL-6 response to psychosocial stress, suggesting a mechanistic link between PTSD and adverse cardiovascular outcomes

Author	Study type and aim	No. of patients	Methodology	Results
			(ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and monocyte chemoattractant protein (MCP)-1 were measured at rest and 90 min after mental stress.	
Britvic D et al., 2015 [13]	Retrospective study The aim of this study was to investigate the differences in the prevalence of somatic diseases among combat veterans and their contemporaries who were not exposed to the traumatic experience at the battlefield, and to determine whether socio-demographic factors, exposure to war-time trauma and/or injury might predict individual somatic diseases	1326	501 male combat veterans with posttraumatic stress disorder (PTSD) and the corresponding control group of 825 men who were not exposed to combat experience. All participants were assessed systematically.	Veterans with PTSD suffered more often from cardiovascular, dermatological, musculoskeletal, pulmonary and metabolic diseases than corresponding control subjects who were not exposed to combat experience. PTSD as a result of exposure to war trauma increases the likelihood of developing somatic diseases.
von Känel R et al., 2021 [14]	Retrospective study This study explored whether a preventive behavioral intervention leads to a decrease in myocardial infarction-induced PTSS between two follow-up assessments.	104	104 patients with MI were randomized to a 45-min one-session intervention of either trauma-focused counseling or stress counseling (active control). Sociodemographic, clinical, and psychological data were collected at baseline, and PTSS were assessed with the Clinician-Administered Post-traumatic Stress Disorder Scale 3 and 12 months post-MI.	PTSS severity showed no change over time from 3 to 12 months post-MI, either in all patients or through the intervention.
Agarwal S et al., 2019 [15]	Prospective study Compared 1-year all-cause mortality and major adverse cardiovascular events in cardiac arrest survivors with and without PTSD symptomatology at hospital discharge.	114	Compare ACM or MACE— hospitalization for nonfatal myocardial infarction, unstable angina, congestive heart failure, emergency coronary revascularization, or urgent implantable defibrillators/permanent pacemaker placements within 12 months of discharge, in case of cardiac arrest suvivors with/without PTSD.	PTSD symptomatology is common after cardiac arrest (CA), and CA-induced PTSS was associated with significantly higher risk of death and cardiovascular events.
Goetz M et al., 2014 [16]	Retrospective study They examined whether PTSD and combat exposure were associated with CIMT (Carotid Intima-Media Thickness) in VietnamWar–era twins after controlling for shared genetic and childhood factors.	465	465 middle-aged twins from the Vietnam Era Twin Registry who were free from CVD were included. PTSD was diagnosed using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition and CIMT was measured by ultrasound.	Among Vietnam War–era veterans, combat exposure and PTSD are associated with CIMT, though the associations are largely mediated by shared childhood factors
Jiang C, 2018 [17]	Prospective study Revealed the prevalence, associated risk factors, and long-termoutcomes of PTSD in patients after intracerebral hemorrhage (ICH)	64	64 patients with a first ICH were included. PTSD was determined with Diagnostic and Statistical Manual of Mental Disorders (fifth edition; DSM-5), and questionnaires on mental and physical status were used. Patients were followed at 3 and 12 months after ICH	Patients with PTSD were more likely to be female (67% vs. 29%, p ¼ 0.01), and had higher rates of receiving minimally invasive surgery (MIS; 47% vs. 18%, p ¼ 0.04) and and had poorer quality of life and worse stroke-related disability.
Rosman L et al., 2019 [18]	Prospective study The study assessed the incidence of atrial fibrillation over a 13-year period among 988 090 young and middleaged veterans	988090	988090 young and middleaged veterans (mean age, 30.299.19 years; 87.8% men, 64.5% white) free of AF, atrial flutter, or atrial tachycardia at baseline were included and followed for 13 years	Patients with PTSD had a higher overall incidence of AF (P<0.0001) and were more likely to develop AF at a younger age than those without PTSD (P=0.004).

Author	Study type and aim	No. of patients	Methodology	Results
von Känel R et al., 2018 [19]	Prospective study Assessed if trauma-focused counseling prevents the incidence of ACS (Acute Coronary Syndrome)-induced PTSS.	190	190 patients with ACS were randomized to a single session intervention of either trauma-focused counseling or an active control intervention (in first 48 hours) and additional health outcomes were assessed at 3 months of follow-up.	Self-rated PTSS indicated less beneficial effects with trauma-focused (6.54; 4.95–8.14) versus stress counseling (3.74; 2.39–5.08; p = 0.017).
Seligowski AV et al., 2020 [20]	Prospective study Assessed the cardiovascular response to extinction in a sample of trauma-exposed individuals.	51	51 trauma-exposed women underwent a fear conditioning paradigm. Heart rate variability (HRV) was calculated at baseline and during the extinction session.	Higher baseline HRV was significantly associated with greater fear bradycardia during early extinction. Fear bradycardia may be a relevant biomarker in predicting how individuals will respond to extinction-based psychotherapy.
Chen YY et al., 2021 [21]	Prospective study Investigated the associations of PTSD (PTSD) manifestation and early risk factors for cardiovascular physiological measures among adolescence.	606	606 adolescents aged 14-19 years old were included. They were asked to rate their possible trauma experiences and PTSD symptoms via questionnaires. The measured variables were: blood pressure, heart rate and body mass index.	PTSD symptoms had a significant effect on blood pressure and heart rate
Cave B et al., 2019 [22]	Retrospective study Determined, based on prior arterial blood pressure monitoring (ABPM) studies, if PTSD diagnosed veterans have abnormal nocturnal dipping patterns compared with the general veteran population	470	Retrospective chart review was performed on all archived ABPM studies and classified by nocturnal dipping status and BP control rates.	Veterans with PTSD had similar distributions of dipping patterns and rates of overall, awake, and nocturnal hypertension compared with the general veteran population.
Campara TM et al., 2014 [23]	Prospective study The aim of this study was to determine how lipid component act after a certain time distance after effects of multifactorial war stress, in the postwar period among the members of the police department.	300	300 police officers were divided in 2 groups :150 as experimental and 150 respondents as a control group. They were investigated the same way: detailed history, physical examination,complete laboratory evaluation, lipid electrophoresis, ultrasound of the abdomen and Color Doppler of the neck vascular structures.	A significant increase in levels of cholesterol and triglyceride levels was noticed in experimental compared to the control group. The ultrasound of the abdomen showed fatty infiltrated liver in 16% of respondents from the experimental and 2% of the respondents in the control group.
Yu S et al., 2018 [24]	Retrospective study The aim of this study was to investigate the association between 9/11-related PTSD (PTSD), dust cloud exposure, and subsequent development of stroke.	42527	Using data from the World Trade Center Health Registry surveys, they employed Cox proportional hazards regression models to assess the associations.	Incidence of stroke was higher among those with PTSD or intense dust cloud exposure than those without, and it was even higher for those who had experienced both
Baranyi A et al., 2021 [25]	Prospective study 1) Determine whether blood levels of trimethylamine N-oxide (TMAO) vary immediately after acute myocardial infarction (AMI) in patients with/without AMI-induced PTSD symptomatology (2) Investigated whether TMAO is a potential biomarker that might be useful in the prediction of PTSD.	114	114 AMI patients were included. The Clinician Administered PTSD Scale for DSM-5 was used to explore PTSD-symptoms at the time of AMI and 6 months after AMI. To assess patients' TMAO status, serum samples were collected at hospitalization and 6 months after AMI.	Participants with PTSD-symptomatology had significantly higher TMAO levels immediately after AMI than patients without PTSD-symptoms. An elevated TMAO level might be a biological marker for severe stress that is associated with vulnerability to PTSD.
Eslami B et al., 2017 [26]	Prospective study Compared the level of PTSD between adults with and without congenital heart disease and examined the correlates of PTSD	853	Patients were divided in two groups: 347 patients with congenital heart disease and 353 without. PTSD was assesed using PTSD symptom scale	52% of patients with congenital heart disease had PTSD while 48% of those without congenital heart disease had PTSD; p=0.183

Author	Study type and aim	No. of patients	Methodology	Results
Ratcliff CG et al.. 2017 [27]	Retrospective study Examined the association between COPD and CHF in patients with mental health disorder, among them PTSD (based on patient electronic medical records and self-report data)	180	Patients with COPD and CHF were assessed for PTSD (and other mental health issues) using Beck Anxiety Inventory and Patient Health Questionnaire -9	13% of patients with COPD or CHF were diagnosed with PTSD
Jane Irvine J et al., 2011 [28]	Prospective study Assessed the relationship between ICD (implantable cardioverter defibrillator) and PTSD (implantable defibrillator carriers may develop PTSD)	193	96 patients with ICD had received cognitive behaviour therapy while 97 were adressed to standard clinical care; both groups were assessed regarding PTSD and other mental health issues at baseline, 6 and 12 months	Use of cognitive behavioural therapy showed an improvement in PTSD symptoms in patients with ICD (p<0.01)
Shemesh E et al., 2006 [29]	Retrospective study Evaluated the symptoms of PTSD and adherence to aspirine in patients with PTSD which received cognitive behavioral treatment	65	14 patients with a previous MI and diagnosed PTSD: 6 patients received cognitive beahavioral treatment while 8 had received standard care	PTSD symptoms improved in those following cognitive behavioral treatment; adherence to aspirine improved in patients submited to standard care
Gilsanz P et al., 2017 [30]	Prospective study Assessed the association of PTSD symptom severity and trauma exposure to the risk of CVD	49859	The subjects (female nurses enrolled in 1989) were followed up by biennial questionnaires regarding risk factors and health conditions and two supplementary questionnaires assessing exposure to violence, trauma, PTSD symptoms onset, severity and remision	Women exposed to trauma and those with PTSD presented a higher risk of CVD (OR 1.69, 95% CI1.08-2.63) Minimizing the symptoms of PTSD attenuates the cardiovascular risk
Gradus JL et al., 2015 [31]	Prospective study Examined the relationship between PTSD and adjustment disorder and four CVDs: myocardial infarction, stroke, ischaemic stroke and venous thromboembolism	4724	Data from Danish national registries were analysed	There is an association between PTSD and adjustment disorder and all four types of CVD
Remch M et al., 2018 [32]	Prospective study Determined PTSD as a risk factor for myocardial infarction and stroke	6481	Subjects were involved in cleaning the World Trade Centers debris PTSD and depression were self reported, and a 4 year follow up sought for myocardial infarction and stroke among subjects using interview or hospitalizations	The cummulative incidence of myocardial infarction or stroke was higher in PTSD patients
Vaccarino V et al., 2013 [33]	Prospective study Determined the association between PTSD and coronary heart disease	562	Subjects were monizygotic and dyzigotic male twins who were military active in Vietnam era subjected for a 13 years follow up At least one twin of each pair should have had PTSD or depresion or none of them for both but in either case, no previous CVD	In twins with PTSD the incidence of coronary heart disease was higher (p<0.001)
Vidal C et al., 2018 [34]	Retrospective study Determined the risk for CVD in different specific groups	10165	Two datasets that form part of the Collaborative Psychiatric Epidemiology Surveys - the National Latino and Asian American Study and the National Comorbidity Survey Replication were analyzed	Cardiovascular events rate was higher in patients with a diagnosis of PTSD: OR=2.10, 95% CI, 1.32-3.33; all ehnical groups, except for asian responders, had a higher likelihood of cardiovascular events associated with PTSD
Chen MH et al., 2015 [35]	Prospective study Assessed PTSD induced strok	5217	-	PTSD subjects have higher risk for any stroke (HR 3.37, 95% CI 2.44-4.67) and ischaemic stroke (HR=3.47, 95% CI 2.44-4.67)

Author	Study type and aim	No. of patients	Methodology	Results
Goldfinger JZ et al., 2014 [36]	Retrospective study Measured the prevalence of stroke induced by PTSD	535	PTSD Checklist Specific for stroke was applied to adult patients who suffered a stroke or a transient ischaemic attack within 4 years; a PTSD was diagnosed based on score of 50 or more	18% patients had a score of 50 or more Stroke induced by PTSD was more frequently in young patients
Scherrer JF et al., 2019 [37]	Retrospective study Sought for the relationship between PTSD and CVD based on other comorbid associations	11856	Data for 5940 patients with PTSD and 5916 without PTSD, aged 30-70 years, were obtained from electronic health records	PTSD depends on other factors to determine CVD: it was of signifficant statistic value just the association adjusted based on age (HR=1.41; 95% CI: 1.21-1.63) and metabolic conditions (HR=1.23; 95 CI:1.06-1.44)
Cyr S et al., 2021 [38]	Retrospective study Assessed the type of trauma inducing PTSD in patients with CVD	148	37 patients with PTSD and CVD were compared with 111 patients with adjustment disorder and CVD	In 51% cases PTSD was induced by a medical trauma
Spitzer C et al., 2020 [39]	Prospective study Assessed the relationship between PTSD and traumatic stress to carotid plaque and intima-media thickness	3119	General population based study with the patients subdivided in five age groups; each subject underwent a carotid arteries ultrasound PTSD and trauma exposure was assessed by interview	Carotid intima media thickness was higher in subjects with trauma experiences compared to the group without trauma exposure (p<0.001) In the group age 40-49 years carotid intima media thickness was higher in the PTSD group (p=0.005)

ABPM: arterial blood pressure measuement; ACS: acute coronary syndrome; AMI: acute myocardial infarction; CA: cardiac arrest; CHF: congestive heart failure; CIMT: Carotid Intima-Media Thickness; COPD: chronic obstructive pulmonary disease; CVD: CVD; ED: emergency department; HR: heart rate; HRV: heart variability rate; ICD: implantable cardiac deffibrilator; ICH: intracerebral hemorrhage; MACE: Major Adverse Cardiovascular Events; MI: myocardial infarction; PTSD: PTSD; PTSS: posttraumatic stress symptoms; VRE: virtual reality exposure.

DISCUSSIONS

1. PTSD and cardiovascular disease – mechanisms and pathways

1.1. General mechanisms

Emerging literature (Table 2) postulates that there are several mechanisms that contribute to the occurrence of cardiovascular disorders in people suffering from PTSD, none of them acting alone but rather together (Figure 2). It is already known that anxiety and depression as well as other psychosocial factors are modifiers of the calculated total cardiovascular risk as we find in the CVD prevention guidelines [41]. These eventually lead to poor health behavior: sedentary life, obesity, smoking and alcohol consumption that contribute to hypertension, diabetes mellitus and dyslipidemia.

Figure 2: Mechanisms of the PTSD-cardiovascular disease relationship. IL6: interleukin 6; PTSD: Posttraumatic stress disorder

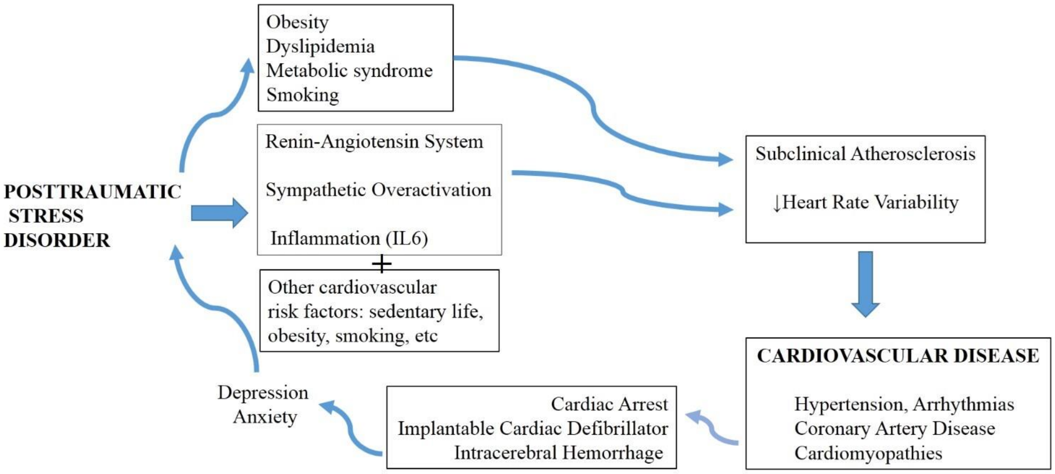
Obesity
Dyslipidemia
Metabolic syndrome
Smoking
Renin-Angiotensin System
Sympathetic Overactivation
Inflammation (IL6)
+
Other cardiovascular risk factors: sedentary life, obesity, smoking, etc
POSTTRAUMATIC STRESS DISORDER
Subclinical Atherosclerosis
↓Heart Rate Variability
CARDIOVASCULAR DISEASE
Hypertension, Arrhythmias
Coronary Artery Disease
Cardiomyopathies
Cardiac Arrest
Implantable Cardiac Defibrillator
Intracerebral Hemorrhage
Depression
Anxiety

Table 2: Studies for general mechanisms and pathways in PTSD generating cardiovascular disease

Author, year	Conclusion
Lima BB et al., 2019, (12)	MI patients with PTSD exhibit an increased inflammatory response to stress (enhanced IL-6 response), suggesting a mechanistic link between PTSD and adverse cardiovascular outcomes.
Brudey C et al., 2015 (40)	This study demonstrates a link between PTSD and CVD. The renin-angiotensin system, sympathetic overactivation and inflammation contribute to compromised cardiovascular health in PTSD.

CVD: cardiovascular disease; MI: myocardial infarction; PTSD: Posttraumatic stress disorder; IL-6: interleukin 6

Chronic systemic inflammation associated with PTSD is the cause for accelerated atherosclerosis that causes vascular disease (peripheral, cerebrovascular) as well as coronary artery disease. Studies have shown that in patients with PTSD there is an imbalance between proinflammatory and anti-inflammatory cytokines in favor of the first ones, with high levels of IL-1β, TNFα, IL-6 and CRP and low levels of IL-4 and IL-10 [42]. In accordance to this pathway, Lima and collaborators measured the levels of inflammaftory markers in survivors of a myocardial infarction with and without PTSD and found out that, after using a mental stress test (public speaking), levels of IL-6 were 68% higher in the PTSD group (p=0.01) [12].

Exposure to a chronic stress and inflammatory state as that in PTSD induces cardiac structural changes at the level of gap junctions. Studies have shown a decrease in the gap junction protein connexin 43 (Cx43) that is physiologically involved in intercellular communication and thus in action potential conduction through the heart [43]. Decreased levels of Cx43 may be involved in triggering cardiac arrhythmia since this protein is present in both atrial and ventricular cardiomyocytes. Neuroendocrine disturbances are also involved in increasing the risk for CVD. In people suffering from PTSD there is an increased activity of renin-angiotensin system as well as overactivity of sympathetic nervous system, these two together contributing to structural cardiovascular changes, hypertension and its consequences [40].

1.2. PTSD as a two edge sword – PTSD induced by cardiovascular disease

More recently, PTSD is seen as a two edge sword because emerging literature is determining that CVD are acting as its triggers (Table 3). Vice versa, in cardiac patients suffering from an acute coronary event, PTSD can occur after the event. Meli L and her collaborators evaluated the association between PTSD symptoms and age during acute coronary syndrome evaluation in the emergency department.

Table 3: Studies debating cardiovascular disease induced stroke

Author, year	Conclusion
Schultebraucks et al., 2020 [44]	Screening for stroke-induced PTSD in the emergency department has an important role in the potential implementation of primary and secondary interventions to improve patient outcomes.
Chang J-C et al., 2017 [7]	Cardiovascular heart disease, bipolar disorder and majore depressive disorder are associated with an increased risk of developing PTSD.
Meli L et al., 2018 [8]	Younger patients are at risk for greater posttraumatic stress symptoms than older ones, after acute coronary syndrome evaluation in the emergency department.
Lima BB et al., 2020 [11]	In myocardial infarction survivors, PTSD is associated with developing ischemia with mental stress. Traumatic reminders can provoke frequent episodes of mental stress-induced myocardial ischemia, aspect that can lead to worse cardiovascular outcomes in these patients.
von Känel R et al., 2021 [14]	The preventive one-session intervention of trauma-focused counseling had no effect in myocardial infarction survivors (posttraumatic stress symptoms were persistent in the first year after the event).
Agarwal S et al., 2019 [15]	PTSD symptoms are frequent after cardiac arrest. Cardiac arest -induced PTSS is associated with elevated mortality and cardiovascular events.
Jiang C, 2018 [17]	Intracerebral haemorrhage is associated with an increased risk for PTSD in the chinese population, especialy in those who are female, more severely disabled, or received minimally invasive surgery.
von Känel R et al., 2018 [19]	In myocardial infarction survivors, trauma-focused counseling had no effect in preventing acute coronary syndome-induced PTSD.
Greenman PS et al., 2018 [45]	Mental health history, the tendency to stifle negative emotional experiences (known as ‘‘type D personality’’), and social support in patients with heart disease are related to the onset of or protection from depression, anxiety, and posttraumatic stress.
Baranyi A et al., 2021 [25]	An elevated TMAO level might be a biological marker of acute myocardial infarction-related severe stress that is associated with vulnerability to PTSD.
Eslami B et al., 2017 [26]	Half of the adults with congenital heart disease present PTSD diagnosis, which is associated with elevated depressive symptoms and history of cardiac surgery.
Ratcliff CG et al., 2017 [27]	Veterans with cardiopulmonary disease experiencing mental health problems are

	underrecognized and improvment of menthal health screening is necessary.
Jane Irvine J et al., 2011 [28]	The cognitive behavior therapy intervention in combination with usual cardiac care is associated with a greater improvment in psychological functioning of implantable cardiac defibrillator patients.
Shemesh E et al., 2006 [29]	PTSD symptoms and cardovascular risk improved in individuals who received trauma-focused cognitive-behavior treatment.
Edmondson D et al., 2012 [46]	Symptoms of acute coronary syndrome induced PTSD are moderately prevalent and are associated with increased risk for recurrent cardiac events and mortality.

PTSD: Posttraumatic stress disorder; PTSS: posttraumatic syndrome symptoms; TMAO: Trimethylamine N-Oxide

They found that younger versus older subjects are at risk for greater posttraumatic stress symptoms after acute coronary syndrome evaluation and elevated perceived threat partially mediated this association (p < .0001) [8].

An observational prospective cohort study (114 subjects) compared prognosis (1-year all-cause mortality and major adverse cardiovascular events) in cardiac arrest survivors with and without PTSD symptomatology at hospital discharge. After adjustment for demographic and medical covariates, they concluded that cardiac arrest-induced PTSD is associated with greater risk of death and significant cardiovascular risk (HR=3.2; CI, 1.7–6.1). Cardiac arrest induced-PTSD may be considered a distinct type of medical-event induced PTSD, because cardiac arrest survivors rarely remember the event or early post-event period [15].

Recent studies are focusing on acute coronary syndrome-induced PTSD prevention. von Känel and his collaborators concluded that self-rated posttraumatic stress symptoms indicated less beneficial effects with single session trauma-focused counseling versus active control intervention [19]. Implantable cardiac defibrillator (ICD) carriers may develop PTSD and a study conducted on 193 ICD patients showed that cognitive behavior therapy intervention in combination with usual cardiac care is associated with a greater improvment in psychological functioning of these patients [28].

Cerebrovascular pathology can induce PTSD. A study on 64 chinese subjects showed that intracerebral hemorrhage is associated with increased risk of PTSD and the more likely profile of these patients is: female, more severely disabled and subjects that received minimally invasive surgery [17]. Goldfinger measured the prevalence of stroke induced PTSD in patients with a history of stroke or transient ischemic attack within 5 years (mean age was 63 years) and defined the portrait of stroke-induced PTSD patient: young, disabled, with recurrent strokes and comorbidities [36].

A study conducted by Eslami compared the prevalence of PTSD among adults with (347 adults) and without (353 adults) congenital heart disease and found that half of the congenital heart disease patients presented a likely PTSD diagnosis, which was associated with greater depressive symptoms (OR=1.27) and history of cardiac surgery (OR=2.02) [26].

2. PSTD and cardiovascular disease

2.1. PTSD and hypertension

As we know hypertension is a cardiovascular risk factor and also a disorder itself that results from the addition of other risk factors (obesity, sedentarism, smoking, dyslipidemia, alcohol abuse) that, in subjects suffering from PTSD prevail due to bad habits that this people have as a mechanism of coping. Hypertension is the second most studied cardiovascular associated disease in PTSD, after coronary artery disease.

Table 4: PTSD and hypertension

Author, year	Conclusion
Burg MM et al., 2017 [5]	Patients with untreated PTSD have a greater risk to develop incident hypertension.
Chen YY et al., 2021 [21]	PTSD may be a risk factor for elevated blood pressure and heart rate.
Cave B et al., 2019 [22]	There were no differences in distribution of dipping patterns and rates of overall, awake,and nocturnal hypertension in veterans with PTSD compared with the general veteran population.
Sumner JA et al., 2021 [47]	Significant effects of PTSD symptoms were detected for SBP and DBP.

DBP: diastolic blood pressure; PTSD: Posttraumatic stress disorder; SBP: systolic blood pressure

The main studies attributed to hypertension as a consequence of PTSD or a stressful event are presented in Table 4. Burg MM et al. investigated the relationship between PTSD and incident hypertension in a large, nationally representative sample of almost 200,000 Veterans of Operations Iraqi Freedom, Enduring Freedom, and New Dawn and proved that patients with untreated PTSD have a 24-46% greater risk for incident hypertension [5]. Interestingly, another study showed that systolic blood pressure and heart rate modifications present a linear relation with the number of trauma episodes (p =0.003 and p=0.046 respectively), aspect that strengthens the supposition that PTSD is linked to CVD [21]. Despite these evidences, the study of Cave found that there were no differences in distribution of dipping patterns and rates of overall, awake and nocturnal hypertension in veterans with PTSD compared with the general veteran population, but this study had important limitations (retrospective design; variable dipping statuses were not evaluated) [22].

2.2. PTSD and coronary artery disease

Coronary artery disease is the most studied associated pathology to PTSD (Table 5). Proinflammatory status and hyperlipidemia associated with chronic stress contribute to accelerating the process of atherosclerosis thus leading to coronary events. Remch and collaborators found that World Trade Center attack–related PTSD is a risk factor for myocardial infarction and stroke with the same impact in men and women, independent of depression and traditional cardiovascular risk factors [32]. Behavioral mechanisms (smoking, obesity, alcohol overuse) may represent the link between PTSD and elevated risk of coronary artery disease, but the majority of the included studies made adjustments for these variables. All selected studies concluded that PTSD is independently associated with increased risk for incident coronary heart disease and it may be a modifiable risk factor for this pathology [32, 33, 48].

Table 5: PTSD and coronary artery disease

Author, year	Conclusion
Akosile W et al., 2018 [49]	This study demonstrates a possible association between PTSD and incident coronary artery disease.
Gradus JL et al., 2015 [31]	Stress-related psychopathology (including PTSD) is associated with CVD events (myocardial infarction, stroke, ischaemic stroke, venous thromboembolism).
Edmondson D et al., 2013 [48]	PTSD is independently associated with increased risk for incident coronary heart disease and it may be a modifiable risk factor for coronary heart disease.
Remch M et al., 2018 [32]	WTC attack–related PTSD is a risk factor for myocardial infarction and stroke of similar magnitude in men and women, independent of depression and traditional cardiovascular risk factors.
Vaccarino V et al., 2013 [33]	Among Vietnam-era veterans, PTSD is associated with an increased risk of coronary heart disease. This risk is not explained by adverse health behaviors, established risk factors for coronary heart disease or by familial risk factors.

PTSD: posttraumatic syndrome disorder; WTC: World Trade Center

2.3. PTSD and other cardiovascular disease

Stroke represents the leading cause of long term disability and in the same time the leading preventable cause of disability. The mechanisms of the relationship between PTSD and incident stroke are not completely understood until now in the literature (Table 6). One theory is that PTSD implies chronic inflammation, endothelial dysfunction, platelet activation and aggregation, autonomic dysregulation and all of these may have a significant role to atherothrombotic manifestations [9]. The association between PTSD and incident transient ischemic attack (TIA) and ischemic stroke was assessed in a cohort of 987855 veterans (mean age of 30.29±9.19 years) which found that PTSD is associated with a significant increased risk of early incident TIA (HR, 1.61; 95% CI,1.27–2.04) and ischemic stroke (HR, 1.36; 95% CI,1.22–1.52), risk that persisted after adjustment for established stroke risk factors, coexisting psychiatric disorders and healthcare utilization. Also, the same study concluded that PTSD may be a risk factor for stroke at younger ages and also a target for stroke prevention strategies in this category of patients [9].

Table 6: PTSD and other cardiovascular disease

Author, year	Conclusion
Rosman L et al., 2019 [9]	PTSD is independently associated with increased risk for early incident TIA and ischemic stroke in young and middle-aged Veterans. Sex moderated the relationship for adults with ischemic stroke (the effect of PTSD on ischemic stroke risk was stronger in men than in women) but no effect of sex in case of TIA.
Yu S et al., 2018 [24]	9/11-related PTSD and intense dust exposure were each independently associated with an increased risk of stroke.
Kastaun S. et al., 2016 [50]	Studies supporting a link between psychiatric disorders and Takotsubo syndrome. Anxiety and depressive disorders seemed to be more common in patients with Takotsubo syndrome than patients with ST-segment elevation myocardial infarction. Major depression and generalized anxiety disorder were also diagnosed more often in the Takotsubo syndrome cohort than in patients with acute coronary syndrome. Optimal management and treatment of Takotsubo syndrome should involve integration of the psychoneuroendocrinological aspects of the syndrome with the well-established functional abnormalities of the disease.
Edmondson D et al., 2013 [51]	Stroke-induced PTSD is common (1 in 4 stroke/ transient ischemic attack survivors in the first year develop PTSD symptoms, and after that 1 in 9 may experience chronic PTSD) and may represent a new risk factor for recurrent stroke.

Gradus JL et al., 2015 [31]	Stress-related psychopathology (including PTSD) is associated with CVD events (myocardial infarction, stroke, ischaemic stroke, venous thromboembolism).
Remch M et al., 2018 [32]	WTC attack–related PTSD is a risk factor for myocardial infarction and stroke of similar magnitude in men and women, independent of depression and traditional cardiovascular risk factors.
Chen MH et al., 2015 [35]	Patients with PTSD have an increased risk of developing any stroke and ischaemic stroke.
Goldfinger JZ et al. [36]	Stroke-induced PTSD is related with younger age, recurrent strokes, greater disability, and comorbidities.
Perkins JD et al., 2021 [52]	PTSD independently predicts stroke and is seems to be linked to many major stroke risk factors.

PTSD: Posttraumatic stress disorder; PTSS: posttraumatic syndrome symptoms; TMAO: Trimethylamine N-Oxide

Another cohort study that included 6481 subjects who were involved in cleaning the World Trade Centers debris, concluded that PTSD is a potent determinant for myocardial infarction and stroke of similar importance in men and women, independent of depression and traditional cardiovascular risk factors [32]. A longitudinal follow-up study (5217 patients with PTSD and 20868 age – and gender-matched controls; mean age 36.65 years with 79.1% women) aimed to determine the temporal association between PTSD and stroke and found that the PTSD goup presented an increased incidence of any stroke (3.82 versus 1.00 per 1000 person-years, P<0.001) or ischemic stroke (2.07 versus 0.53 per 1000 person-years, P<0.001) at a younger age compared to control group [35].

2.4. PTSD and arrhythmia

The relationship between PTSD and potential arrrhythmia is yet an unknown field because most studies are focusing on other aspects of CVD related to PTSD (Table 7). Trials looking forward to bring in attention this link are rather aiming for changes in heart rate variability (HRV) as a consequence to PTSD than to look for specific arrhythmia. As presented by Dennis and his collaborators in their study conducted on 227 subjects, 107 of whom were diagnosed with PTSD (49 of which were veterans), those in the late category presented a decreased HRV (p=0.002). Interestingly, despite the fact that sleep disorders, abdominal obesity and poor health behavior (alcohol consumption and smoking) were more frequently encountered in the PTSD group compared to nonPTSD group (p<0.001 for each), when adjusting for each of it, analysis was either non-significant (as in the case of sleep disorders and abdominal obesity) or the relationship between PTSD symptoms and decreased HRV was attenuated (for smoking and alcohol consumption) yet, when taken together (smoking, sleep disorder and alcohol consumption), they were responsible for 94% of the effects of PTSD symptoms on decreasing HRV [6].

Table 7: PTSD and arrhythmias

Author, year	Conclusion
Bourassa KJ et al., 2019 [4]	The exposure therapy (prolonged exposure or virtual reality exposure) for PTSD may reduce cardiovascular risk in these patients, improving cardiovascular functioning.
Dennis PA et al., 2014 [6]	The behavioral health risks (smoking, alcohol overuse, sleep disturbance) mediate the association between PTSD and HRV. A healthy lifestyle may enhance the benefits from psychiatric and psychological interventions in PTSD.
Ge F et al.. 2019 [54]	The reduced HRV may be regarded as an endophenotype in PTSD research.
Rosman L et al., 2019 [18]	In this cohort of young and middle-aged veterans, PTSD is associated with an increased risk for early incident atrial fibrillation.
Seligowski AV et al., 2020 [20]	Fear bradycardia may be indicative of greater vagal control (i.e., HRV), both of which are psychophysiological biomarkers that may influence cardiovascular and autonomic disease risk in trauma-exposed individuals.

PTSD: Posttraumatic stress disorder; HRV: heart rate variability

More recently, a prospective study of middle age veterans sought for atrial fibrillation and found out that those with a diagnosis of PTSD had a higher incidence of it (p<0.0001) in unadjusted and adjusted models. Oddly, development of atrial fibrillation was associated with younger ages (p=0.0004) [18].

Arrhythmia may be more frequently in people suffering from PTSD due to a few aspects which are substracted from the former studies: first, there is an increase in sympathetic activity as the PTSD was proved to be associated with increased plasma levels of norepinephrine [53]; secondly, PTSD may generate an unfavorable lifestyle with increasing obesity which favors the appearance of obstructive sleep apnea that, in turns, is also responsible for increasing the sympathetic activity and thirdly, this poor health behavior along with sympathetic overactivity increases the risk of coronary artery disease which can generate life threatening arrhythmia.

3. PTSD – modifiable cardiovascular risk factors and prophilaxy

Posttraumatic stress disorder is associated with a greater risk of cardiometabolic disease (Table 8) and the involved mechanisms are complex: increased inflammation, dysfunction of autonomic nervous system and of hypothalamic-pituitary-adrenal axis, poor behavioral factors (alcohol overuse, smoking, sedentary lifestyle, increased caloric intake) [55]. Rosenbaum's study reinforces this supposition by founding that PTSD patients have a greater risk to develop metabolic syndrome compared to age and sex matched controls (almost twice) and a routine screening for behavioral factors plus an adequate management is needed [56]. Obesity in relationship with PTSD was evaluated in a group of 54224 subjects (ages 24-44 years in 1989) which concluded that women with at least 4 PTSD symptoms had a greater risk of becoming overweight ($p < .001$) [3]. The authors concluded that PTSD treatment should include correction of behavioral alteration and women with a PTSD diagnosis may undergo screening for development of adverse cardiometabolic outcomes [3].

Table 8: PTSD – modifyable cardiovascular risk factors and prophilaxy

Author, year	Conclusion
Bartoli F et al., 2020 [57]	PTSD may be considered a systemic condition and assesment, monitoring and treatment of metabolic variations may determine a significant improvement in its management and prognosis.
Kubzansky LD et al., 2014 [3]	PTSD is associated with an elevated risk of becoming overwight/obese.
Bartoli F et al., 2013 [58]	PTSD is associated with a greater risk of metabolic syndrome.
Goetz M et al., 2014 [16]	Among Vietnam War–era veterans, PTSD is associated with carotid artery intima-media thickness (measure of subclinical atherosclerosis), though the association is largely mediated by shared childhood factors.
Campara TM et al., 2014 [23]	Elevated levels of cholesterol and triglycerides were found in the blood serum of the policemen who have spent the entire war in Sarajevo (experimental), compared to the police officers who spent the war in other areas of Bosnia and Herzegovina (control group). The war determine changes in lipid profile of persons employed at risk professions, such as police officers.
van den Berk-Clark C et al., 2018 [55]	PTSD is associated with reduced healthy eating and physical activity, and increased obesity and smoking. The relation between PTSD, metabolic and CVD may be due to poor diet, sedentary lifestyle, high prevalence of obesity and co-occurring smoking in these patients.
Rosenbaum S et al., 2015 [56]	PTSD is associated with metabolic syndrome (PTSD patients are almost twice as likely to have metabolic syndrome compared to age and sex matched controls).
Spitzer C et al., 2020 [39]	Association between PTSD and atherosclerosis is inconclusive.
Chen YY et al., 2021 [21]	PTSD may be a risk factor for elevated blood pressure and heart rate.
Solter V et al., 2002 [59]	Patients with PTSD have a higher risk of atherosclerosis.

CVD: cardiovascular disease; PTSD: Posttraumatic stress disorder

CONCLUSIONS

Posttraumatic stress disorder has a major impact on the lifestyle and health of subjects exposed to trauma or stress. Cardiovascular disease tend to appear sooner in PTSD diagnosed patients due to a variety of reasons: poor habits, low addressability to health care systems, chronic inflammation status, changes at molecular levels, etc. Otherwise, PTSD tends to be induced by CVD, thus inclining the balance towards whis association. Prevention of PTSD by proper psychological and/or psychiatric intervention in subjects exposed to trauma or stress, including that generated by CVD, might be of help in preventing the appearance or worsening of cardiovascular disorders in this population. Regarding arrhythmic events, we suspect that, PTSD patients may be at risk of developing arrhythmic events, atrial fibrillation probably being the most common. In the light of the above mentioned facts, looking for arrhythmic patients in people suffering from PTSD may be of particular interest in future studies.

Study limitations

Most of the studies analyzed CVD events (defined as myocardial infarction, stroke, ischaemic stroke, venous thromboembolism) not separately on each CVD. Due to the inhomogeneity of the data about CVD type associated with PTSD and the limited data on each type of CVD type (hypertension, coronary artery disease, arrhythmias, or cardiomyopathies like Takotsubo syndrome) and the parameters that was analyzed for this CVD, there was not possible to make an extensive statistical analysis.

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ORIGINAL ARTICLES

Prediction of anxiety, depression and social function among mothers of NICU-hospitalized infants compared to healthy infants' mothers in Baqiyatallah and Imam Hossein Hospitals

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Abstract: *The main objective of this research is to predict anxiety, depression and social function of NICU-hospitalized infants' mothers compared to healthy infants; mothers. The methodology of research is descriptive in type of correlative and causative-comparative. Population included all mothers of NICU-hospitalized infants and healthy infants' mothers in two Tehran Hospitals from June 2018 to September 2019. The main sample size was randomly selected. Sample size was calculated as 120 at this level based on Cochran's table. The instruments were Beck's (1969) anxiety and depression inventories, and Lekomet et al. (2014) social functioning questionnaire (SFQ). Data was analyzed using analysis of variance, multivariate and step-wise regression. The results implied that those NICU-hospitalized infants' mothers experienced higher levels of depression and anxiety than healthy infants' mothers. Moreover, formers had lower social functioning than latters. On the other hand, depression and anxiety were suitable predictors of mother' social functioning and there was a significant relationship between depression and social functioning mediated by mother's anxiety.*

Keywords: *anxiety, depression, social functioning, infants' mothers, NICU ward*

INTRODUCTION

Low-weight and immature infants are at-risk groups socially and they would experience more physical, psychological and mental diseases than natural infants. Because of physiological specifications they have, immature infants need regulated care to survive and regain their natural growth [1]. On average, 9% of the infants need NICU caregiving processes [2] most of whom are immature infants [3].

Pregnancy, giving birth and postpartum periods are sensitive instances during the woman's life. Thisperiod of time incudes natural and mental transformations in woman's both social roles and familial role [4]. Pre-term parturition is usually traumatic and a source of the parents' distress. This may be recognized as an emotional crisis which often is diagnosed by sense of lacking and sorrow. Sometimes, these statuses remain for a long time after the child birth. For many mothersadaptation to having preterm infants resembles the adaptation to having disabled child [5].

The potential stressor that affect the parents of preterm infants include problems associated with infants' health in hospital as well as after being discharged, reduced responsiveness and concerns about the infants' mental development [6].

Approximately, 28 to 70 percent of preterm infants' mothers experience high levels of the psychological pressures. The damaging influence of preterm infant on mother disrupts her subjective abilities increasing other tensions of having high-risked child. High levels of stress transform and disrupt mother-child interactions. On the other hand, parents with preterm child -may experience wrong and sad ideas as their infant is disabled and is vulnerable against any type of diseases and damages. This parents' idea causes undesirable parents-child interactions and, as a result, leads to their depression and anxiety disorders causing disruptions in

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parents cannot play their parenting role well. Consequently, this adversely affects the child's growth and interactive abilities.

One of the variables studied associated with mothers is anxiety. Anxiety is a resisting and chronic phobia of a particular situation or objects which spontaneously is not fearful and this phobia leads the individual to avoid from that situation or object; while individual understands his/her phobia is illogical [7]. Anxiety is referred to as a part of every human's life and everyone experiences it to some balanced extent. To this extent, anxiety is deemed a compromised response [8]. Lack of anxiety may expose individuals to significant risks and issues but anxiety may reach chronic and constant aspects. In this case, this can no longer be a compromised response but it has to be taken as a failure, uncompromised reaction or extensive despair which established a large spectrum of cognitive and physical disorders rather than unjustified phobias [9]. There have been various definitions provided for anxiety. In definition, "excessive phobia in real or imaginative situations" is anxiety. However, anxiety and phobia are similar in many aspects, they indicate apparent differences. Phobia signifies adverse event occurring or experience which will occur soon while anxiety signified an unjustified phobia. Main feature of anxiety is concern including worrying excessively in situations with uncertain outcomes. Excessive concern is harmful since it disrupts the problem-solving capability [10]. Generally, anxiety means resisting and chronic phobia of something or of situations which in fact are not damaging. Such phobialeads to acute tendency to avoid anxious situations; however, individual understands his/her phobias and avoidance are illogical [11].

Studies on epidemiology of this disorder implied that about 8-12% of children and 5-10% of adolescents are exposed to one of the criteria of anxiety disorder diagnosis to the extent that their natural and daily life and functionality are disrupted. This is while girls experience higher levels of anxiety than boys [12]. These issues are often manifested more from pre-school years and highlightedduring the school years behaviorally [13]. Typically, anxiety accompanies with other problems such as eating disorders and substance abuse. Anxious children are less academically achieved and successful, experience interpersonal relationship with same-aged children and are weak in social abilities. Excessive absences during school years and defective relationship with same-aged children will result in future in weak job adaptation and issues associated with self-image as well as psychological disorders [14].

General forms of anxiety disorders are separation anxiety which is diagnosed by excessive level of anxiety due to being distanced from main caregivers, generalized or extended anxiety, phobia of open spaces, social phobia which includes excessive anxiety due to being judged by others or behaviors in a group, phobia disorders which are diagnosed by unpredictable fearful reactions, particular phobias like phobia of objects, animals or particular situations, OCD disorder which is diagnosed by constant and iterative thoughts and habitual behaviors, post-trauma stress disorder which is the result of a shocking event like disasters, sexual rape or death of someone [15].

Howeverall disorders share the anxiety as their main feature, contextual or particular situational factors make them isolated. Particular phobias as King, Hin and Elending (2005) put refer to a wide range of phobias including the five groups mentioned below:

- Zoophobia
- Nature phobia
- Ampule phobia
- Situational phobias
- Other types of phobia [16]

The other variable studied in this research is depression. Depression is a prevalent illness to the extent which is called psychiatry cold. At any certain time section, 15-20% of adults suffer from depressive signs significantly and at least, 12% have got depression to the extent they need therapy. It is guessed that about 75% of hospitalized cases in Psychiatric hospitals consist of depressed cases. Due to reasons not clarified yet, depression among women is approximately two times more than men in western societies [17].

It seems impossible that only one factor can explain depression, but depression is result of several factors interactions. It is shown that depression starting point and development depends on various variables like biology, background of depression, socio-psychological variables and environment among which followings are mentioned:

- Neurotransmitters dysfunction
- Depression background or alcoholism in family
- Losing parents during childhood
- Being neglected by parents
- Current negative life events
- Marrying a criticizer or aggressive spouse
- Lack of closed and reliable relationship
- Lack of social support
- Lack of long-term sense of being valuable

Scientists believe that acute depression is highly prevalent among children. About 2% of children in 6-12 years and 4% of adolescents are depressed. Based on DSM TR-IV criteria for children and adolescents substantial depression, although increasing among children, they are not enlisted among having depressive disorders due to lack of particularity among these age groups. Australian international psychological health and safety provided a checklist of children's behavioral assessment which classified behavioral issues in two general groups and 7 categories. In this classification, anxiety and depression among children are listed as specialized disorders [18].

Practically, four sets of signs are introduced for depression. In addition to behavioral or emotional signs, the intellectual or cognitive signs, motivational signs and physical signs are also mentioned:

- Emotional signs: sense of disappoint, dissatisfaction, lack of values, humility are emotional depression sign but sorrow is the most important and frequent emotional sign of depression.

- Cognitive signs: depressed individual thinks negatively about him/herself. These negative ideas toward him/herself underlie his/her present and future self-image. Depressed individual often experiences low self-esteem. He/she believes in his failure and knows him/herself responsible for the failures. He/she believes in his/her humiliation, incompetence and unworthy.

- Motivational signs: depressed individual face many difficulties to start their activities. In chronic type, lack of response is willingness paralysis. Such patient cannot even take necessary actions for his life. In acute depression, there may be psycho-motor slowness.

- Physical signs: This includes weight loss, reduction or increase of appetite and sexual tendency reduction. Depressed individual is isolated and his body does not attract anything and increasing concerns occur about pains and suffrages [19].

However, there are not sufficient studies on stress and anxiety among NICU-hospitalized infants' parents in Iran and present resources are mostly focusing on effect of psychological diseases on mother due to preterm parturition. Therefore, based on the significance of early diagnosis of anxiety and depression and low social functioning, interventions to increase the preterm infants' parents' coping power, effect of these disorders on mother and infants' health and lack of sufficient studies in Iran, present study aims to compare the anxiety and depression and social functioning of preterm infants' mothers hospitalized in NICU with healthy infants' mothers.

Fliz Arlan &Koel Koyko (2011) in a research in Anadolu University, Turkey, studied the issues of public hospital NICU-hospitalized infants' families and found out that unreliability of adaptation with child's status, executive processes on child and instruments used, lack of visiting or limited time of visiting, lack of sufficient information from medical team, lack of parenting role implementation when child is hospitalized are variables which lead to anxiety among the families of these children and infants [20].

Beheshti Pour et al. (2014) studied compared the stress level of parents with preterm infants hospitalized in NICU and implied that mothers' stress level was higher than that of fathers. Therefore, providing more information and paying more attention to and supporting for parents when their infants are hospitalized should be more focused by medical team particularly the NICU nurses [21].

Jebrayili et al. (2009) in their study investigated the stressors from hospitalized infants' mothers' perspective in Tabriz and indicated that most stressors for these mothers were appearance and behavior of their infants and their parenting role changes [22].

Bastani & Haghighi (2012) in a research found that parents with hospitalized infants in NICU experience depression, disappoint, sense of isolation, stress, anxiety, emotional duality and reduced control on situations [23].

Greene et al. (2015) analyzed depression, anxiety due to prenatal (PPTS) situation among mothers with low-weighted infants hospitalized in NICU and concluded that psychological screening is important in NICU and PPTS symptoms indicated that there may be need for Primiparas therapy for intervention [24].

Bozkurt et al. (2017) studied whether mothers' psychological disorder are effective on preterm infants' birth in pregnancy over 32 weeks and found that mother's depression is negatively related to preterm infants' nervous development during the pregnancy over 32 weeks and during the long-term infants' follow-up of preterm infants, mothers psychological health has to be taken into consideration [25].

METHODOLOGY

Present research is a descriptive study in term of methodology and since the research predicts the anxiety, depression and social functioning of NICU-hospitalized infants' mothers compared to healthy infants mothers, itis a non-pilot study because the inter-variables associations are not changed. This research compares the anxiety, depression and social functioning of NICU-hospitalized infants' mothers with healthy infants' mothers; therefore, this study is a causative-comparative one. Population included all mothers of NICU-hospitalized infants and healthy infants' mothers in Baqiyatallah and Imam Hossein Hospitals from June 2018 to September 2019.

Because the population studied was extensive and this was not possible to provide a list of all members of this population, 120 mothers and their infants were selected as sample size using available random sampling in 2 hospitals, Tehran. The sample size was obtained using Cochran's formula used for large populations with reliability coefficient of 0.95 and accuracy coefficient of 0.05 and the final sample size was 120 participants. The information required for this research was collected from primary resources like theoretical studies for theoretic discussions including specialized books, articles, journals and specialized researches and secondary recourses including information and comments obtained from healthy and NICU-hospitalized infants' mothers

To find these comments, a questionnaire -for this purpose was distributed among the participants. The questionnaires included Beck's anxiety inventory (BAI) involving 21 items. The participants answer multiple choice questions as their level of anxiety. This questionnaire includes scoring and scores interpretation table.

This scale measures particularly the chronic signs of clinical anxiety among individuals with high reliability and validity. The internal homogeneity coefficient (Alpha-coefficient) is 0.92 whose validity was confirmed to be 0.75 in a reexamination after one week and items correlation was from 0.30 to 0.76.

Beck's depression inventory (BDI): this scale is a self-assessment 21-item questionnaire which measures the depression symptoms. Each item represents illness sign based on four levels from zero to three as scores. The score zero indicates the lowest level and score three indicates higher level of individuals experiencing depressive signs. The sum of scores of each scale is 0-63. This is administrated for measurement if depression level among individuals older than 13 years.

Social functioning questionnaire: this was administrated in 2014 by Lekomet et al. with 34 items. The main objectives of this questionnaire is to analysis and assess the social functioning dimensions (friendships and social activities, independence in life skills, interaction with others, intimacy, family, communications and social activities at work, work competences, communications and social activities at school/university, educational competences). The reliability of this questionnaire was assessed in SPSS by internal homogeneity (Cronbach's Alpha coefficient). The reliability calculated for this questionnaire was 0.8 which is desirable (Cronbach's Alpha coefficient>0.7).

Demographic information questionnaire: this scale aims to measure and study human population and its changes over time which includes age (age distribution), number or population of a group, growth rate, distribution, gender, level of education, urban/rural population ratio and the basis of this questionnaire is statistics and data. In this research, this information includes mothers' age, child birth status, number of children and infants' gender. To analyze data the SPSS was used.

RESULTS

Demographic information

The results implied that 50% of the participants were healthy and 50% were hospitalized.51.7% of mothers had bachelor's degree and 10.8% of mothers had master and PhD degrees. Most of the participants (72.5%) were first child and 5% were 4th child. 23/3% were mothers with physical or mental illnesses and 76.6% had no background of physical or mental illnesses and in sum, 20% and 80% of participants were hospitalized in women's ward before parturition and after parturition, respectively.

Most of the participants (55%) were hospitalized for oxygen therapy and 5% had genetic problems (physical issues). Generally, 52.5% of mothers were 20-30 years old and 15% were 25 years or older.

H1. There is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of anxiety.

To test three first hypotheses, one way ANOVA was used.

Results of ANOVA (Table 1) indicated that there is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of anxiety in significance level lower than 0.05.

H2. There is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of depression.

Results of ANOVA (Table 2) indicated that there is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of depression in significance level lower than 0.05.

H3. There is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of social functioning.

Results of ANOVA (Table 3) indicated that there is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of social functioning in significance level lower than 0.05.

Table 1: One-way analysis of variance to compare anxiety between NICU-hospitalized and healthy infants' mothers

Variable	Group	Mean	SD	F	P
Anxiety	NICU-hospitalized infants' mothers	35.36	10.11	394.62	0.00
	Healthy infants' mothers	7.60	5.4		

Table 2: One-way analysis of variance to compare depression between NICU-hospitalized and healthy infants' mothers

Variable	Group	Mean	SD	F	P
Depression	NICU-hospitalized infants' mothers	30.9	8.48	278.97	0.000
	Healthy infants' mothers	8.86	5.69		

H4. Anxiety is a desirable predictor of social functioning of mothers.

H5. Depression is a desirable predictor of social functioning of mothers.

To test hypotheses 4 and 5 the Pearson's correlation test and multivariable regression analysis were used. Tables 4 and 5 present that 0.52 of the changes in mothers' social functioning can be justified by anxiety and depression.

Results of the social functioning regression analysis based on depression and anxiety (table 6) implied the significance of regression equation, i.e. the predicted share of social functioning by depression and anxiety is significant.

Based on regression coefficients tale (Table 7), each predictive variable, i.e. depression and anxiety, can significantly predict the social functioning changes.

Table 3: One-way analysis of variance to compare depression between NICU-hospitalized and healthy infants' mothers

Variable	Group	Mean	SD	F	P
Social functioning	NICU-hospitalized infants' mothers	64.96	23.61	197.32	0.001
	Healthy infants' mothers	115.95	15.25		

Table 4: Correlation matrix of anxiety and depression with social functioning of mothers ($P<0.01$, $N=120$)

Variable	Anxiety	Depression	Social functioning
Anxiety	1		
Depression	0.73	1	
Social functioning	-0.63	-0.7	1

Table 5: Results of social functioning regression based on anxiety and depression

Criterion variable	R	R^2	Adjusted R^2	se
Mothers' social functioning	0.73	0.53	0.52	22.32

Table 6: Results of social functioning analysis of regression based on anxiety and depression

Variable	Source of changes	Sum of Squares	Df	Mean Squares	F	Sig. level
Social functioning	Regression	6631.40	2	33158.20	66.56	0.000
	Residue	58293.38	117	498.23		
	Total	124609.79	119			

Table 7: Results of social functioning regression coefficient based on anxiety and depression

Model	Non-standardized coefficients		Standardized coefficients	T	Sig. level
	B	Se	Beta		
Constant	126.90	3.76		33.75	0.000
Anxiety	-0.51	0.18	-0.25	-2.75	0.007
Depression	-1.27	0.22	-0.52	-5.62	0.000

H6. There is a significant relationship between social functioning and depression mediated by the mother's anxiety.

To test this hypothesis, multiple regressions in the hierarchical procedure were used. Here, to achieve the goal of hypothesis 6 i.e. analysis of mediating role of mothers' anxiety, first the anxiety was introduced into the regression equation to predict the social functioning and second, anxiety and depression were introduced into the multiple regressions equation. The results of hierarchical regressions (Table 8) indicated that first, anxiety explained 40% of the social functioning variance and then, anxiety and depression were entered into the regression. The results of multiple correlations changes indicated that 52% of the social functions was predicted by depression*anxiety.

Table 8: Results of social functioning regression based on depression mediated by anxiet0079

Stages	Predictive variable	R	R^2	Moderate R^2	se	Changed R^2
1	Anxiety	0.63	0.40	0.40	25.05	0.40
2	Anxiety*depression	0.73	0.53	0.52	22.32	0.12

DISCUSSION

The results of this research implied that difference of mean scores of two groups of mothers (with healthy infant and with NICU-hospitalized infants) was significant in term of anxiety and equal mean of two groups is rejected. It can be stated that there is significant difference between mothers with healthy infant and with NICU-hospitalized infantsin term of anxiety and anxiety was higher among mothers with NICU-hospitalized infants. In a research in Anadolu University, Turkey, that studied the problems of - NICU-hospitalized infants' families- it was found out that unreliability of adaptation with child's status, executive processes on child and instruments used, lack of visiting or limited time of visiting, lack of sufficient information from medical team, lack of parenting role implementation when child is hospitalized are variables which lead to anxiety among the families of these children and infants.

Difference of mean score of two groups (with healthy infant and with NICU-hospitalized infants) in term of depression was significant and the equal mean score of two groups was rejected. It can be stated that there is significant difference between mothers with healthy infant and with NICU-hospitalized infants in term of depression and depression was higher among mothers with NICU-hospitalized infants. Jebrayili et al. (2009) in their study investigated the stressors from hospitalized infants' mothers' perspective in Tabriz and indicated that most stressors for these mothers were appearance and behavior of their infants and their parenting role changes. In a study on pregnant mothers in Dezfoul, it was observed that it is 3.1 times more probable among depressed women to give low-weighted child than healthy women which was a significant difference statistically. In this research, mean weight and head perimeter were lower significantly among depressed mother's infants than other group [23]. Mean score difference of mothers (with healthy infant and with NICU-hospitalized infants) in term of social functioning was significant and the equal mean score of two groups was rejected. It can be stated that there is significant difference between mothers with healthy infant and with NICU-hospitalized infants in term of social functioning and social functioning was lower among mothers with NICU-hospitalized infants.Explaining this finding, it can be stated that hospitalized infants' mothers are more exposed to the communicational risks. Level of socio-psychological interactions of hospitalized infants' mothers was lower than that of healthy infants' mothers which reduces their social functioning. Explaining why of hospitalized infants' mothers were significantly different from healthy infants' mothers in term of social functioning, it can be stated that based on the formers' situation if vulnerability is addressed, first the individual is non-functioning and then others play role in her lack of desirable functioning. Weak social functioning is associated with limited opportunities of learning, self-esteem, social knowledge and social restrictions. Obviously, socialization process and social functioning obtaining are complicated processes and numerous factors are influential on its transformation. Pregnancy and maternal status are recognized as potentially vulnerable instances for women's social functioning.

There was significant and negative relationship between social functioning and anxiety; therefore, it can be stated that increase in mothers' anxiety reduces their social functioning. So anxiety is a desirable predictor of social functioning among mothers. The results of Beheshti Pour et al. (2014) were consistent with this result.

Beheshti Pour et al. (2014) studied compared the stress level of parents with preterm infants hospitalized in NICU and implied that mothers' stress level was higher than that of fathers [22].

There was significant and negative relationship between social functioning and depression; therefore, it can be stated that increase in mothers' depression reduces their social functioning. So depression is a desirable predictor of social functioning among mothers.

Bastani & Haghighi (2012) in a research found that parents with hospitalized infants in NICU experience depression, disappoint, sense of isolation, stress, anxiety, emotional duality and reduced control on situations [24].

There was significant relationship between social functioning and depression mediated by anxiety and research hypothesis is confirmed. Therefore, it can be stated that it is not simple to distinguish between depression and stress symptoms. These signs sometimes overlap and do not let understanding whether you are depressed or under stress and pressure. Although there are similarities between these symptoms, symptoms of depression are often more intensive and deeper and they may last for some weeks - while stress is experienced only some days. During pregnancy and parturition, deep changes occur in woman's moods due to highly sensitive psychological drivers and they sometimes lead to psychological issues. Postpartum depression can accompany with reduction of mother's activities and disrupts the mother-child relationship and familial activities and constant disruption and lack of diagnosis and early therapy will lead to ineffective adaptation of mother with child, spouse and family and as a result, lack of ability in taking maternal duties. High level of anxiety and stressleads to psychological vulnerabilities. Therefore, hospitalized infants' mothers' anxiety in social functioning after giving birth causes lack of desirable relationship with child and relatives and results in negative reactions and establishes the grounds of social communication reduction among mothers. Behavioral problems prevalence such as weak social behaviors, aggressive behaviors and behavioral disorders are some of the issues the hospitalized infants' mothers experienced.

CONCLUSIONS

Based on the results, parents with chronic child go through predictable emotional stages. First stage is shock of unpredicted disease which generally changes all their life. Next, sadness, sorrow, anger and disappointment occurabout the child who didn't meet his parents' expectations and hopes. Following to these outcomes, parents' social functioning changes particularly among mothers as a predictable outcome and the variables life depression, anxiety and social functioning, as studied in this research, resulted in statistical analysis and significant relationships with NICU-hospitalized infants' mothers.

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CLINICAL PRACTICE

Pregnancy outcomes in a patient with Fontan circulation for single ventricle congenital heart disease and aberrant praevia placental lobe

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Abstract: *Single ventricle heart (SVH) is a very rare congenital heart disease. However, since the introduction of Fontan's intervention, the survival rate of patients with SVH has been significantly improved, increasing the number of women who may become pregnant in this condition. It has been already reported, in isolated case presentations or in some series of patients, that the pregnacy in the context of SVH and Fontan circulation carries a very high risk for first trimester miscarriage, maternal cardiovascular or obstetric complications and for an increased rate of pre-term birth. Therefore, it is an open field of debate weather or not to allow pregnancy in this category of patients. We are presenting the case of a 22 years old patient who underwent two surgeries for SVH: a Glenn procedure when she was 2 years old and the implantation of a Fontan circuit at the age of 16 years. Since the last surgery she was under permanent internal cardiac stimulation. During the pregnancy the patient was hemodynamically stable, with a normal resting oxygen saturation and a good ejection fraction of the single ventricle. However, the evolution of this patient has become even more challenging due to an aberrant praevia placental lobe. This condition brought her to hospital for several episodes of metrorrhagia and uterine contractions. She delivered at 33 weeks of gestation by emergency Caesarian section with a good evolution until discharge, for mother and the newborn. This case is supporting the opinion that pregnancy can be tolerated without important cardiovascular events in patients with SVH and Fontan circulation, unless heart failure or cardiac arrythmia are associated. Nevertheless, the obstetrical complications remain an important problem in pregnancy overcome of patients with Fontan circulation for SVH that can be superimposed with other unfavorable conditions, like aberrant praevia placental lobe in this case. During the management of such particular clinical settings difficult decisions must be taken, including those addressing the antithrombotic therapy that will be covered in the presentation of this case.*

Keywords: *Pregnancy outcome; Cardiovascular pregnancy complications; Fontan procedure; Delivery, obstetrics*

INTRODUCTION

A single ventricle is a heart defect in which one lower chamber does not develop resulting in a one pumping ventricle only and cyanosis. Single ventricle heart (SVH) defects are rare. They occur in about five out of every 100 000 live births [1]. There have been distinguished more features of SVH, but the most frequently encountered is double-inlet left ventricle [2]. Since its description for the surgical management of tricuspid atresia in 1971, the Fontan procedure has become the definitive palliative surgical treatment of SVH. Its principal consists in the deviation of the systemic venous return directly to the pulmonary artery without passing through a subpulmonary ventricle. Contemporary cohorts of surviving patients with SVH in adulthood have undergone modified procedures compared with the original version, of which the most common is a Dacron by-pass connecting the inferior caval vein to the inferior aspect of the right pulmonary artery [3]. Since the

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introduction of Fontan's intervention, the survival rate of patients with SVH has significantly improved, increasing the number of women who may become pregnant in this condition. Overtime, patients with SVH and Fontan circulation are developing complications characterised by atrial arrhythmias, thrombo-embolic events and myocardial and even hepatic dysfunction [4-7]. In particular, the lack of subpulmonary ventricle predisposes to a low cardiac output state with pulmonary blood flow dependent on adequate preload [8]. This fragile balance can be overcome during pregnancy, when basal oxygen consumption increases by 50 mL/min at 40 weeks, requering a 30-80% increase in cardiac output [9]. Patients with Fontan circulation have limited resources to take over a volume overload or to increase adequately their cardiac output [10]. Moreover, it has been shown that pregnacy in the context of SVH and Fontan circulation carries a very high risk for first trimester miscarriage, maternal cardiovascular or obstetric complications and for an increased rate of pre-term birth [11]. Therefore, it is an open field of debate wether or not to allow pregnancy in this patients with SVH and Fontan circulation.

Patients with Fontan circulation have a high rate of thromboemebolic events, recorded in about 20% of cases, which seems to be similar in all variants of this operation, with most of the thrombs located in within the venous circulation [12]. However, current guidelines of the European Society of Cardiology adressed to cardiovascular diseases in pregnancy consider that therapeutic anticoagulation should be considered in pregnant women with Folan circulation, but balanced with the risk of bleeding [13].

CASE PRESENTATION

We are presenting the case of a 22 years old patient who underwent two surgeries for SVH: a Glenn procedure in order to connect the superior vena cava to the right pulmonary artery when she was 2 years old, and the implantation of a Fontan circuit at the age of 16 years. Since the last surgery she was under permanent internal cardiac stimulation.

Due to the fact that patients with Fontan circulation are considered as having high and very high risk in pregnancy (WHO risk class III or IV) (13) the patient was clearly informed of the possible complications that might occur. However, she gave her written consent for keeping the pregnancy, despite the repeated warnings of the cardiogists and obstetritions.

The patient had a double-inlet left ventricle with an ejection fraction of 57% and a small regurgitation of the right atrio-ventricular valve (Figure 1). Throughout of pregnancy the patient remained hemodynamically stable, with a normal resting oxygen saturation and a good function of the left ventricle, including during delivery. No arhythmia have been recorded. She received antiplatelet therapy with acetylsalicylic 100 mg per day, up to 31 weeks of pregnancy, in order to prevent thromboembolic events.

Figure 1. Transthoracic echocardiography indicating the one pumping chamber with double inlet left ventricle and a part of the Glenn-Fontan circuit

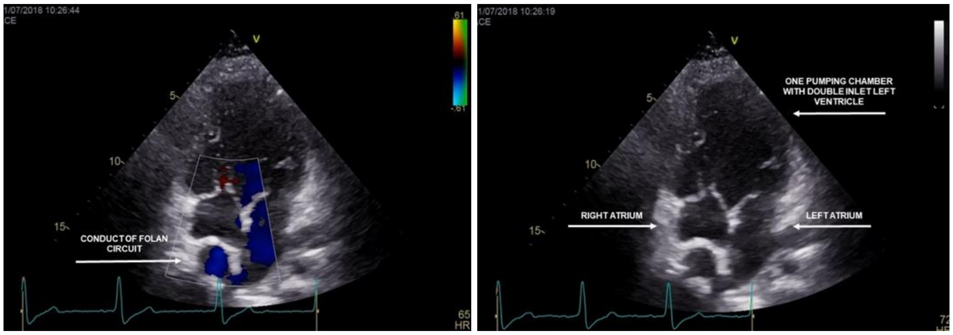

Based on echographic parameters in conjunction with maternal serum biochemistry, the estimated risk for trisomy 21, 18 and 13 was low. The thrombophilic work-up revealed MTHFR C677T homozygote mutation associated with PAI-1 4G/5G. An infection with Ureaplasma Urealyticum was detected, that required antibiotic treatment at 17 weeks of pregnancy.

However, the pregnancy overcome has become more challenging due to an aberrant praevia placental lobe. This condition brought her to hospital for several episodes of metrorrhagia and uterine contractions.

At 33 weeks of gestation the patient was admitted for uterine contractions and vaginal bleeding. She delivered by emergency Caesarean section for placenta praevia and pelvic presentation. The intervention was particularly difficult considering the emergency state of the procedure and the massive bleeding, but during delivery and afterwords, in the Intensive Care Unit, sufficient volume replacement was added in order to maintain adequate preload of Fontan circulation. The postoperative outcome was satisfactory, without further bleedings under prophylactic anticoagulation with low molecular weight heparines. After 12 days from the Cesarian section the patient was suspicioned for an intrauterine residual mass confirmed at echography (Figure 2) for which a guided instrumental revision was performed. The preterm neonate, weighting 1700 g, was registered with 7 on Apgar scale, but with good evolution until discharge.

Figure 2. Ultrasonographic aspect of intrauterine residual mass (transverse section), measuring 27 mm

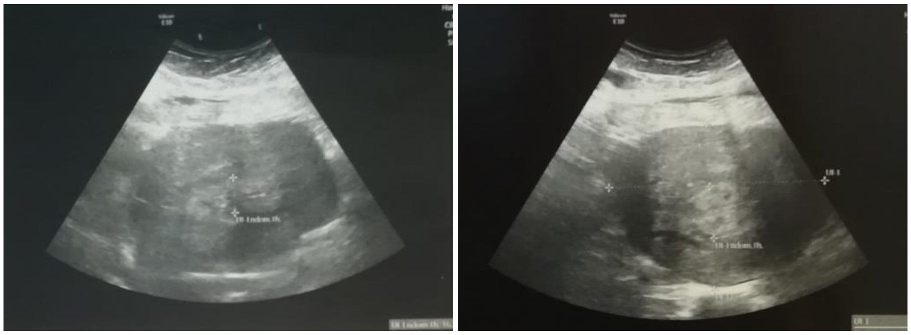

All the fragments extracted underwent an extensive histopathological analysis which concluded their placental origin with postpartum endometritis and decidual abcesses (Figures 3-5).

The patient received antibiotic therapy with Carbapenems for 10 days. She was discharged on post-operative day 25 in a good condition and without cardiovascular manifestations.

Figure 3. Acute villitis and intervillositis diffuse neutrophilic infiltration of intervillous space. HE 10x

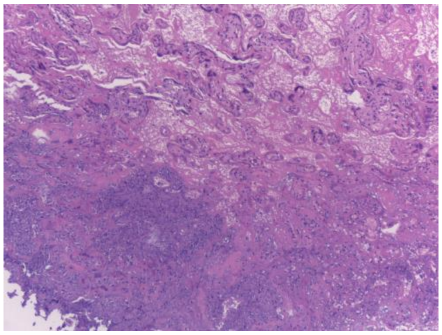

Figure 4. Massive inflamation and infection of the decidua. HE 20x

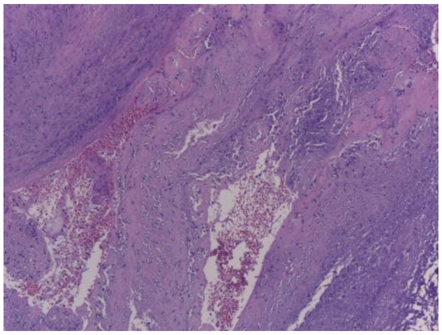

Figure 5. Postpartum endometritis leading to decidual abscess, massive inflamation and necrotic debris. HE 10x

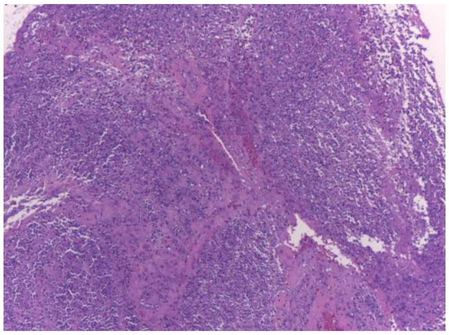

DISCUSSIONS

Since the introduction of Fontan's intervention, the survival rate of patients with SVH has significantly improved, increasing the number of women who may become pregnant in this condition. Based on a report from UK it is expected in the next decade a rise of the adults' number with this condition by 60% [14]. It has been already reported, in isolated case presentations or in some series of patients, that the pregnacy in the context of SVH and Fontan circulation carries a very high risk for first trimester miscarriage, maternal cardiovascular or obstetric complications, including perinatal haemorrhage and for an increased rate of pre-term birth or small for gestational age [15-20]. Due to these reports many authors advised against pregnancy in women with Fontan procedure for SVH. However, in some series of patients with this condition have been identified some categories of patients which might safely evolve during pregnancy, in particular those without heart failure or arrythmias and with a normal resting oxygen saturation [11, 21-23]. The presented case is supporting the opinion that pregnancy can be tolerated without important cardiovascular events in patients with SVH and Fontan circulation in selected situations. Age might have an important role in pregnancy overcome, as long as the risk of cardiovascular complications increases with the duration of time from the palliative intervention.

Antepartum haemorrhage complicates 2–5% of pregnancies, of which approximately one-third are caused by abnormal placental implantations [24]. Placenta previa is characterised by the partial or total covering of the internal os [25]. In certain situations, like in the presented case, only an aberrant lobe may have this aberrant location. Placenta praevia is an obstetric condition that can induce massive antepartum vaginal bleeding. The risk is even higher when accreta and percreta invasion are occuring [26]. It has been reported that the incidence of placenta praevia and abnormal placental invasion has increased in the last years, probably as a result of the large number of C-sections worldwide [25]. However, in our case the patient was primiparous and did not undergo any other previous surgical interventions, but the risk of antepartum bleeding was augmanted by the cardiovascular condition of this patient. Data accumulated over the two past decades are indicating that the maternal and fetal obtetrical complications are high in patients with Fontan circulation, even in those with an estimated low score of cardiovascular complications during pregnancy: a rate of about 50% for obsterical complications and between 70-80% of preterm delivery [11].

Patients with Fontan procedure have a high-risk of thromboembolic events. In pregnancy this risk can be augmented. For this reason our patient had an indication of therapeutic anticoagulation with low molecular weight heparine [13], but her pregnancy overcome was superimposed with an aberrant praevia placental lobe and repeated episodes of metrorrhagia. In this context, the obstetritions and the cardiologists involved in the management of this patient have chosen to give her acetylsalicylic 100 mg per day, because the risk of hemorrhages was too high under heparin therapy. This decision was taken according to the single multicenter, randomized, controlled study which has revealed no differencies in the thrombosis rate of Fontan patients between the use of aspirin and heparin/warfarin [27]. Moreover the postpartum period was complicated by the expulsion of an intrauterine mass. The extracted fragments proved to be placental and infected. Following the prompt guided instrumental revision and the antibiotic cure with Carbapenems, septic complications that could particularly affect Fontan's circulation have been avoided.

CONCLUSIONS

This case is supporting the opinion that pregnancy can be tolerated without important cardiovascular events in patients with SVH and Fontan circulation, unless heart failure or cardiac arrythmia are associated. Nevertheless, the obstetrical complications remain an important problem in pregnancy overcome of patients with Fontan circulation for SVH that can be superimposed with other unfavorable conditions, like aberrant praevia placental lobe in this case. During the management of such particular clinical settings difficult decisions must be taken, including those addressing the antithrombotic therapy, that must be carried on by a multidisciplinary team in order to reduce as much as possible the maternal and fetal morbidity and mortality.

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All authors have participated equally in developing this study.

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ORIGINAL ARTICLES

Prevalence of osteoarthritis disorders in Iranian chemical injured patients: A cross-sectional study using the COPCORD approaches

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Abstract: *Introduction: Osteoarthritis is considered as the most common joint disease. Sulfur mustard (SM) is a toxic compound, which was applied as a chemical warfare agent by Iraq against Iran veterans during 1980-1988. Chemical veterans suffer from chronic implications caused by sulfur mustard that not only adversely affect their quality of life, but also restrict their working abilities and their participation in social activities. This study aims to consider the prevalence of osteoarthritis disorders among Iranian SM-exposed veterans.*

Methods: In this cross-sectional study, 248 Iranian SM exposed patients were evaluated from 2013 to 2014. Veterans filled a special questionnaire for COPCORD studies and then general and specific rheumatology examinations were applied under the supervision of internists and rheumatologists. The obtained data from 245 filled questionnaires were analyzed by SPSS software and the results were reported as a percentage or mean ±SD.

Results: Bone and joint pain (72%), knee joint pain (52.5%), Joint stiffness (49.2%), and shoulder joint pain (45.8%) were the most common type of complications in these patients. Osteoarthritis in at least one joint was the most common type of osteoarthritis in veterans. 69.5% of veterans had osteoarthritis in at least one joint. Knee osteoarthritis was the second most common type of osteoarthritis, which has been found in 58.4% of all veterans. The prevalence of neck osteoarthritis in all veterans was 23.9%.

Conclusion: The total prevalence of musculoskeletal disorders among Iranian veterans was significantly higher than the average of society.

Keywords: *chemical warfare, osteoarthritis, prevalence, COPCORD*

INTRODUCTION

Osteoarthritis (OA) is considered as the most common joint disease and its main pathologic indication at the tissue level is joint cartilage breakdown. This disease is classified into two main groups: (1) primary or idiopathic osteoarthritis in

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which no underlying disease causes the disorder, and (2) secondary osteoarthritis in which an underlying predisposing factor imposes the disease locally or systemically [1]. However, another classification based on the number of involved joints is considered which includes generalized osteoarthritis in which more than 3 joints are involved and also localized osteoarthritis in which less than 3 joints are involved [2, 3]. Identification of predisposing factors gives better insights for the disease pathogenesis. There are various factors such as age, gender, race, genetic, bone density, sex hormones, nutritional status, and environmental factors that can affect the prevalence of OA. Age is the most important risk factor that is not controllable; as it increases, osteoarthritis prevalence rises [4, 5]. The type of joint involvement before 55 years is the same among both sexes; however, when age increases hand joint involvements more likely occur among females but hip joint involvements are more prevalent among men [6, 7]. The prevalence of OA can be increased along with aging and obesity. According to the United Nations, 130 million people will suffer from OA by 2050, of which 40 million will be severely disabled by the disease. The burden associated with OA includes costs for adaptive aids and devices, medicines, surgery, and time off at work. Osteoarthritis prevalence among black and white races is different [8]. The risk of osteoarthritis among individuals who have parents suffering from the disease is significantly higher [9]. Some studies have proposed a reverse relation between osteoporosis and osteoarthritis and some have suggested that bone density increscent is correlated to thigh and knee osteoarthritis but not hand osteoarthritis [10-12]. Endocrine or metabolic disorders such as diabetes, hyperparathyroidism, and acromegaly are in association with osteoarthritis [13]. Nutrition status and antioxidant materials in diets may prevent or postpone osteoarthritis. The damage caused by reactive oxygen species (ROS) is determined through the osteoarthritis trend [14]. Knee osteoarthritis progress among people suffering from vitamin C deficiency is significantly more than those consuming a high amount of this vitamin [15]. Obesity is one of the most important causes of osteoarthritis progress especially in the knee [16]. High pressure on joints due to sport or job, congenital disorders, and inflammatory or infectious diseases are the other affecting factors [16].

The chemical contamination from the wars is one of the main causes of various diseases in veterans. Sulfur mustard [17] is one of the warfare agents which has been utilized during various chemical wars and it was widely applied during the imposed war of Iraq against Iran (1980-88)[18]. The first report on the late implications of SM toxicity among 236 Iranian chemical veterans revealed 78% respiratory, 45% central nerve system, 41% skin, and 36% eye complications. These abnormalities were reported 2-28 months after SM exposure [19].

Chemical veterans are still suffering from the late effects of SM toxicity which negatively impacts their quality of life. More importantly, the progression of their disease restricts their working abilities and participation in social activities. There is little information about the prevalence of osteoarthritis or musculoskeletal disorders among chemical veterans. Since the status of nutrition, medicine, economy, mobility, and generally the lifestyle of the veterans are different from other parts of the society, low bone density or suffering from endocrine and metabolic disorders or chronic infectious disease is more likely to occur among this community [20]. Therefore, the purpose of this study was to evaluate the frequency of osteoarthritis disorders among Iranian chemical veterans who previously exposed to SM using the COPCORD approach.

METHODS

This cross-sectional study was conducted on Iranian chemical veterans in Tehran from 2013 to 2015. For sampling, the initial list of chemical veterans was provided from the lung clinic and medical commission of Baqiyatallah University of Medical Sciences, and individuals who fulfilled the inclusion criteria were entered into the study. Furthermore, according to the coordination of the Veterans and Martyrs Affair Foundation (VMAF), a list of patients who fulfilled the inclusion criteria were selected and invited to participate in the study. The Including criteria were as follows: having documented exposure to sulfur mustard (confirmed by related authorities), male gender, being over 40 years old, moderate and negligible involvement of lungs, and giving consent for participating in the study.

The special questionnaire for COPCORD studies was filled by veterans and the general and specific rheumatology examinations were applied under the supervision of internists and rheumatologists. This questionnaire consisted of demographic questions, complaints about osteoarthritis in the past 7 days, questions about the restrictions, accidents, damages, and external joint symptoms related to musculoskeletal diseases, and also questions in the scope of the treatment detailed examination by specialists and assessing medical histories elucidated the prevalence of different osteoarthritis complications for the mentioned population.

The final part of the questionnaire contained the recorded probable and final diagnosis of the patients who complained about at least one joint (pain, inflation, and stiffness) or external joint pain. If the patient needed more examinations such as radiology in the opinion of the physician, this information was obtained and attached to the questionnaire. Finally, the filled questionnaires were analyzed and the obtained data were revised and after excluding the defaced cases, the questionnaires data were entered into the SPSS software version .20 and the results were described as percentages and mean. The confidentiality of the information of the subjects studied by researchers and project implementers were considered as the ethical considerations of this study. The COPCORD questionnaire used in this study was provided by the Rheumatology Research Center at COPCORD website.

RESULTS

In this study, 245 subjects were evaluated in which 97 patients lived in Tehran and 148 subjects lived in Alborz Provinces. The mean age of the studied population was 51.1 years. Data on demographic information and smoking status can be seen in Tables 1 and 2, respectively.

Table 1: The frequency distribution of studied veterans based on demographic variable

Variable		Number (%)
Marriage status	Single	1 (0.4)
	Married	237 (98.8)
	Spouse dead	1 (0.4)
	Separated or divorced	1 (0.4)
Race	Fars	149 (62.1)
	Azeri	67 (27.9)
	Kurd	11 (4.6)
	Lor	7 (2.9)
	Other	6 (2.5)
Occupation status	Employee	76 (34.1)
	Worker	6 (2.7)
	Retired	76 (34.1)
	Self-employed	49 (22)
	Unemployed	15 (6.7)
	Disabled	1 (0.4)

Table 2: The smoking status of studied veterans

Smocking	Frequency	Percentage	Use average daily	Average of Time (year)
Never	178	75.4	--	--
I am smoking	23	9.7	10.9	19.9
I used to	35	14.8	12.7	6.8

The prevalence of bone and joint complaints in each age group is presented in Table 5. There was no significant difference in the percentage of bone and joint complaints between different age groups. Overall, bone and joint complain (78.4%), bone and joint pain (72%), knee joint pain (52.5%), Joint stiffness (49.2%), and shoulder joint pain (45.8%) were the most common types of bone and joint complains in studied patients.

The percentage of disease intensity and restriction following pain is summarized in Table 3. Most veterans have complained about the intensity of annoying and intolerable pain (73.9%). Furthermore, musculoskeletal pain is getting worse in 66.9% of veterans during activities. Approximately 68.8% of veterans wake up due to musculoskeletal pain.

Table 3: Prevalence of intensity and restriction following pain for complaining veterans

Variable	Percentage
Musculoskeletal pain going worse in the morning	54.1
Musculoskeletal pain going worse during activities	66.9
Waking up because of musculoskeletal pain	68.8
The intensity of annoying and intolerable pain	73.9
Restrictions due to the type or the number of activities in the present or past because of pain, sensitivity, etc.	55.7
Perfect adaptations with problems (pain, sensitivity, etc.)	55.2

Table 4: The relative prevalence of osteoarthritis based on different age groups

Type of the Disease	Age Group (year)			Total
	40-49	50-59	≥ 60	
osteoarthritis at least in one joint (%)	82(69.5%)	72(69.2%)	15(71.4%)	169(69.5%)
dorsal dick osteoarthritis	1(0.8%)	0(0%)	0(0%)	1(0.4%)
spinal osteoarthritis	2(1.7%)	2(1.9%)	0(0%)	4(1.6%)
shoulder osteoarthritis	1(0.8%)	1(0.8%)	0(0%)	2(0.8%)
wrist osteoarthritis	0(0%)	2(1.9%)	0(0%)	2(0.8%)
foot osteoarthritis	1(0.8%)	0(0%)	0(0%)	1(0.4%)
neck osteoarthritis	23(19.5%)	30(28.8%)	5(23.8%)	58(23.9%)
hand osteoarthritis	0(0%)	1(0.8%)	0(0%)	1(0.4%)
hip osteoarthritis	1(0.8%)	3(2.9%)	0(0%)	4(1.6%)
knee osteoarthritis	70(59.3%)	57(54.8%)	15(71.4%)	142(58.4%)

The relative prevalence of osteoarthritis based on different age groups is shown in Table 4. Osteoarthritis in at least one joint was the most common type of osteoarthritis in each age group. 69.5% of veterans had osteoarthritis in at least one joint. Knee osteoarthritis was the second most common type of osteoarthritis in each group. It has been found in 58.4% of all veterans. The prevalence of neck osteoarthritis in all veterans was 23.9%.

The prevalence of osteoarthritis is also considered in men with different age groups (Table 6). Similarly, osteoarthritis in at least one joint was the most common type of osteoarthritis in each age group. Knee osteoarthritis was the second most common type of osteoarthritis in each group.

Table 5: The prevalence of bone and joint complaints in the last 7 days based on the age group.

Type of implication	Percentage in age groups			Total
	40-49 years	50-59 years	≥60 years	
Bone and joint complain	77.1	81	72.2	78.4
Bone and joint pain	73.7	72	61.1	72
Joint inflation	28.8	32	27.8	30.1
Joint stiffness	47.5	50	55.6	49.2
Shoulder joint pain	46.6	47	33.3	45.8
Shoulder joint stiffness	21.2	26	16.7	22.9
Pain due to pressure in the shoulder	30.5	29	16.7	28.8
Elbow joint pain	26.3	22	22.2	24.2
Elbow joint inflation	6.8	8	5.6	7.2
Elbow joint stiffness	10.2	12	11.1	11
Pain due to pressure in Elbow	12.7	17	22.2	15.3
Wrist joint pain	24.6	27	27.8	25.8
Wrist joint inflation	7.6	10	11.1	8.9
Wrist joint stiffness	8.5	14	22.2	11.9
Pain due to pressure in the wrist	14.4	20	16.7	16.9
Pain in the hands and fingers joints	23.7	25	16.7	23.7
Inflation in the hands and fingers joints	12.7	6	11.1	9.7
Stiffness in the hands and fingers joints	9.3	14	11.1	11.4
Pain caused by pressure in the hands and fingers joints	13.6	17	16.7	15.3
Pelvic joint pain	33.1	28	16.7	29.7
Pelvic joint stiffness	11.9	19	11.1	14.8
Pain caused by pressure in the pelvic	19.5	28	22.2	23.3
Knee joint pain	50.8	55	50	52.5
Knee joint inflation	18.6	22	27.8	20.8
Knee joint stiffness	16.9	29	38.9	23.7
Pain due to pressure in the knee	28	34	33.3	30.9
Ankle joint pain	22.9	25	22.2	23.7
Ankle joint inflation	11.9	14	16.7	13.1
Ankle joint stiffness	10.2	18	22.2	14.4
Pain due to pressure in the ankle	15.3	22	16.7	18.2
Toes joint pain	16.9	21	16.7	18.6
Toes joint inflation	5.1	8	16.7	7.2
Toes joint stiffness	5.1	12	16.7	8.9
Pain due to pressure in the toes	12	18	27.8	15.7
Neck joint pain	37.3	39	16.7	36.4
Neck joint stiffness	18.6	21	16.7	19.5
Pain due to pressure in the neck	20.3	29	16.7	23.7
Spine joint pain	47.5	46	27.8	45.3
Spine joint stiffness	22	21	16.7	21.2
Pain due to pressure in the spine	29.7	32	16.7	29.7

DISCUSSION

Musculoskeletal implications negatively affect individuals' health, sanitary, and treatment systems [21]. In a previous study by Joshi and Chopra (2009), they considered the prevalence of osteoarthritis types in 8145 adult subjects in the Pune area located in India. Their results revealed that musculoskeletal pain was the most prevalent type of osteoarthritis in these individuals [22]. The results of the current study demonstrated that the prevalence of musculoskeletal complaints during the past 7 days among veterans is significantly high (78.4%). The most prevalent complaints from the veterans were related to Bone and joint pain, Knee, shoulder joint pains, and joint stiffness. The results of previous studies from different parts of Iran showed that the prevalence of joint complaints in the past 7 days is 54.13%, 41.9%, 42.8%, and 44.7% [23-26]. The comparison of the approached global studies indicated that the prevalence of musculoskeletal complaints in Iran is generally higher than in other Asian countries [27].

Table 6: Prevalence of osteoarthritis among men (Tehran COPCORD results)

Type of the Disease	Age Group (Year)		
	40-49	50-59	≥ 60
osteoarthritis at least in one joint (%)	39%	27%	16.6%
neck osteoarthritis	3.35%	2.41%	1.14%
hand osteoarthritis	7.14%	1.39%	0.5%
hip osteoarthritis	1.99%	0%	0.14%
knee osteoarthritis	36.32%	24.46%	14.49%

Based on the obtained results and final diagnosis of the following study, osteoarthritis, back pain, and pre-arthritis (69.9%, 30.5%, and 27.2%) had the highest frequency of the musculoskeletal diseases among the studied population. This finding confirmed the results of Tehran's study [27]. The results of a previous study on the rural population in Iran showed that approximately 20% of individuals suffered from osteoarthritis in at least one joint. The comparison of COPCPRD results in 17 countries revealed that Iran is second place in regards to osteoarthritis complications (with a frequency of 16.9%) [26[.

The results of this study demonstrated that the most prevalent type of osteoarthritis among veterans is knee (58.4%) and neck osteoarthritis (23.9%), respectively. Interestingly, knee osteoarthritis has been reported as one of the most prevalent rheumatic disorders among the Asian-Oceanic population [23]. The prevalence of knee osteoarthritis was previously calculated to be 5.8% in the rural areas of India, 22-28% in the urban areas of India, 25% in the rural populations in the north of Pakistan, 7.5, 9.2 and 10.6 in the rural, urban slumps and developed urban areas of Bangladesh, and 7.5% in China respectively [28-31]. The results of other studies on the Iranian population demonstrated that the prevalence of knee osteoarthritis in the southeast areas, rural areas, Sanandaj, and the whole country is 18.66%, 19.34%, 26.3%, and 15.5%, respectively [24-26, 28]. Recent studies have indicated that Iran is r ranked third for knee pain among 17 investigated countries [25]. According to previously accomplished data and results of the current research, the incidence of knee pain in veterans is significantly higher than that in the general population of Iran.

In this research, the prevalence of Gout arthritis in veterans was 0.4%. In previous studies, the prevalence of gout arthritis among individuals who lived in Tehran and Sanandaj was 0.13% and 0.12%, respectively [27, 32]. Dawatchi et al. indicated that Iran is ranked 11 among 17 countries regarding the prevalence of rheumatic diseases [25]. Further statistical analysis revealed that Indonesia (1.6%) and Australia (1.5%) had the highest frequency for this disease [25].

CONCLUSIONS

The results of this study described the frequency of musculoskeletal disorders and associated complaints among Iranian chemical veterans. Our data have revealed that the total prevalence of musculoskeletal disorders among Iranian veterans is significantly higher than the average of society. Due to the restrictions of these individuals and specific treatment needs, periodic screening tests, effective and proper nutrition, athletic and therapeutic interventions are strongly suggested to improve musculoskeletal disorders among chemical veterans.

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Declaration of interest

No competing interest was declared by any of the authors.

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ORIGINAL ARTICLE

Prevalence of rheumatic disorders in Iranian chemical injured patients: a WHO–ILAR COPCORD study

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Abstract: *Background: Rheumatic disorders are one of the most prevalent human diseases which commonly become chronic and cause different types of disabilities. Chemical veterans may suffer from chronic difficulties caused by rheumatic disorders that can lead to decrease of their quality of life. The main purpose of this study was to evaluate the prevalence of rheumatic disorders among Iranian chemical warfare patient's long-years after sulfur mustard (SM) exposure.*

Methods: This cross-sectional study was conducted from 2013 to 2014 on 245 Iranian chemical veterans. In order to evaluate rheumatic disorders, a special questionnaire for COPCORD studies were filled by veterans and then general and special rheumatology examinations were applied under the supervision of internists and rheumatologists. Data from all questionnaires were analyzed by SPSS software and the results were reported as percentage or mean ±SD.

Results: Osteoarthritis (69.9%) was the most common complications diagnosed in these patients. After that back pain (30.5%) and pre-arthritis (27.2) were the other popular complications among veterans. The percentage of frozen shoulder and moderate carpal tunnel syndrome was 13.2% and 11.5%, respectively. Tennis elbow and fibromyalgia (3.7%), rheumatoid arthritis (0.8%) and gouty arthritis with 0.4% were the lowest complications among these patients.

Conclusion: Rheumatic associated complications in Iranian chemical injured veterans are higher than the mean of whole community. This data provided the possibility of calculating the disease's burden and making efficient decisions in the sanitary and treatment systems.

Keywords: *chemical veterans, rheumatic disease, prevalence, COPCORD*

INTRODUCTION

Rheumatic disorders are among the most prevalent human diseases. One of seven referring patients to physicians suffer from rheumatic diseases [1]. Several factors such as bacterial infections, life style, and environmental factors can be correlated to rheumatic disorders [2, 3]. This disease can cause different types of important disabilities which in turn adversely influence the quality of life, physical and social activities in affected individuals [4]. It was estimated that ~40% of all chronic diseases and ~54% of long term disabilities are caused by rheumatic diseases [5]. For instance, Disability Adjusted Life Year (DALY) in cases of osteoarthritis was increased from 13275 patients in 1990 to 14230 patients in 2000 [6]. These patients are significantly susceptible to other disorders [7] and diseases like hepatitis B [8].

Back into the 7th decade, the manuscript of the community oriented program for the control of rheumatic diseases (COPCORD) was provided in the non-communicable disease section of WHO [9]. This manuscript describing a model of consumable agenda in rural and urban areas of developed and developing countries, was evaluated in the first meeting of the ILAR and WHO in January 1981 in Genève and formed as COPCORD [10].

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COPCORD is one of the most comprehensive epidemiologic studies worldwide. The first stage of the agenda was approached in Iran which was applied by the Rheumatology Research Center of Tehran University of Medical Sciences on the adult population of Tehran's urban areas via cluster sampling method. It was also followed by other studies from other areas of the country in the recent years [11].

A joint study by the Iranian and Bangladeshi researchers with the objective of providing and improving a questionnaire consisting of identification of knee osteoarthritis risk factors in developing countries, reported a pilot survey in the phase III of the COPCORD questionnaire. They suggested that these questionnaires must be validated for identifying knee osteoarthritis among Asian and Oceania populations [12].

Mustard gas (Sulfur mustard) is one of the warfare agents which has been utilized during chemical wars and used by Iraq against Iran during 1980-1988. During Iraq-Iran war, SM injured more 100,000 veterans that many of whom are still suffering from its late effects. Chronic infection, purulent bronchitis, spread tightened trends, bronchial hyper activity, severe asthma, bronchitis obliteran, psychophysiological asthma and vascular-pulmonary problems are the most prevalent implications caused by sulfur mustard exposure in veterans [15, 16]. Chemical veterans may suffer from some chronic difficulties such as rheumatic disorders which can lead to decrease of their quality of life, their working abilities, social relationships and physical activities. However, there is little information about the prevalence of rheumatic disorders among chemical veterans.

Since nutrition, medicine, economic, activity status and lifestyle of the chemical veterans are different from other communities, the frequency of rheumatic diseases among this population may be different.

Therefore, the aim of this study is to evaluate the prevalence of rheumatic diseases among chemical veterans exposed to SM during the imposed war of Iraq against Iran using a community oriented program for the control of rheumatic diseases.

METHODS

This cross-sectional survey was conducted on the 245 Iranian chemical veterans from Tehran and Karaj cities in 2013 to 2015. The veterans who fulfilled the following criteria were included: (1) having a documented history of sulfur mustard exposure (confirmed by related authorities), (2) male gender, (3) being over 40 years old, (3) moderate and negligible involvement of lungs and (4) giving a consent letter for participating in the study.

Sampling in Tehran city was accomplished by introducing the initial list of certain chemical veterans from the hospitalization sections of Baqiyatallah Hospital, lung clinic and the medical commission of the Baqiyatallah University of Medical Sciences. Due to the restrictions of veterans visiting, only the information of 97 chemical veterans from Tehran was obtained successfully.

For sampling in Karaj city, a list of 1050 chemical veterans was prepared by the coordination of the Alborz province Veterans and the Martyrs Affair Foundation (VMAF) primarily. From the initial list, 148 subjects who fulfilled the inclusion criteria were invited to participate in the study. In order to evaluate rheumatic diseases, special questionnaire for COPCORD studies were filled by veterans and general and special rheumatology examinations were applied under the supervision of internists and rheumatologists.

All data from 245 filled questionnaires were analyzed by SPSS software and the results were reported as percentage or mean ±SD. The COPCORD questionnaire used in this study was provided by the Rheumatology Research Center at Baqiyatallah University of Medical Sciences.

RESULTS

Among the 245 studied veterans, 97 subjects were resident in Tehran and 148 patients lived in Alborz Provinces. The mean of age and BMI of all patients was 51.1 years and 26.7 kg/m^2, respectively. Other demographic data such as smoking habitat, race and marriage status are presented in Table 1.

After examinations by specialists and assessing medical histories, diseases which were responsible for the mentioned implications were diagnosed. Figure 1 shows the percentage of complaints associated with rheumatoid arthritis in SM-exposed veterans. Osteoarthritis (69.9%) was the most common complications diagnosed in these patients.

After that back pain (30.5%) and pre-arthritis (27.2) were the other popular complications among veterans. The percentage of frozen shoulder and moderate carpal tunnel syndrome was 13.2% and 11.5%, respectively. Tennis elbow and fibromyalgia (3.7%), rheumatoid arthritis (0.8%) and gouty arthritis with 0.4% were the lowest complications among these patients.

The frequency of rheumatic disease associated complications for each age group and the whole studied population can be seen in Tables 2, 3 and 4.

Table 1: The frequency distribution of marriage status, race, occupation status and smocking background in veterans

Variables				Frequency	Percentage
Marriage status	Single			0.4	1
	Married			98.8	237
	Spouse dead			0.4	1
	Separated or divorced			0.4	1
Race	Fars			62.1	149
	Azeri			27.9	67
	Kurd			4.6	11
	Lor			2.9	7
	Other			2.5	6
Occupational status	Employee			34.1	76
	Worker			2.7	6
	Retired			34.1	76
	Self-employed			22	49
	Unemployed			6.7	15
	Disabled			0.4	1
Smoking		**Average of time (years)**	**Daily average use**		
	Never	--	--	75.4	178
	I smocked	19.9	10.9	9.7	23
	I used to	6.8	12.7	14.8	35

Table 2: The relative frequency of pre-arthritis (at least one type) based on different age groups

Age Group (Years)	COPCORD Males Tehran	Veterans	
	%	%	Frequency
40-49	4.19	23.7	28
50-59	7.21	31.7	33
Over 60	10.78	23.8	5
Total	---	27.2	66

Table 3: The relative frequency of moderate carpal tunnel syndrome based on different age groups

Age Group (Years)	COPCORD Males Tehran	Veterans	
	%	%	Frequency
40-49	0.69	11.7	14
50-59	0.69	12.5	13
Over 60	0.28	4.8	1
Total	---	11.5	28

Table 4: The relative frequency of rheumatoid arthritis based on different age groups

Age Group (Years)	COPCORD Males Tehran	Veterans	
	%	%	Frequency
40-49	0.25	0.8	1
50-59	0	0	0
Over 60	0	4.8	1
Total	---	0.8	2

The percentage of pre-arthritis in veterans with 51-59 years-old was higher than that in other age groups (Table 2). Veterans over 60 year-olds showed higher percentage of rheumatoid arthritis (4.8%) and moderate carpal tunnel syndrome (4.8%) compared to the other age groups (Table 3 and Table 4).

DISCUSSION

With the objective of identification, prevention and controlling rheumatoid diseases in the developing countries, WHO and ILAR coordinated in 1981 in order to organize COPCORD [9]. This program has been performed in many countries such as Australia, Bangladesh, Brazil, Chili, China, Cuba, Indonesia, India, Kuwait, Malaysia, Mexico, Pakistan, Egypt, Guatemala, Lebanon, Tunisia, Philippines, Tailed, Vietnam, as well as Iran [9, 12, 17-30].

Figure 1: The frequency of diagnosed joint diseases in chemical veterans

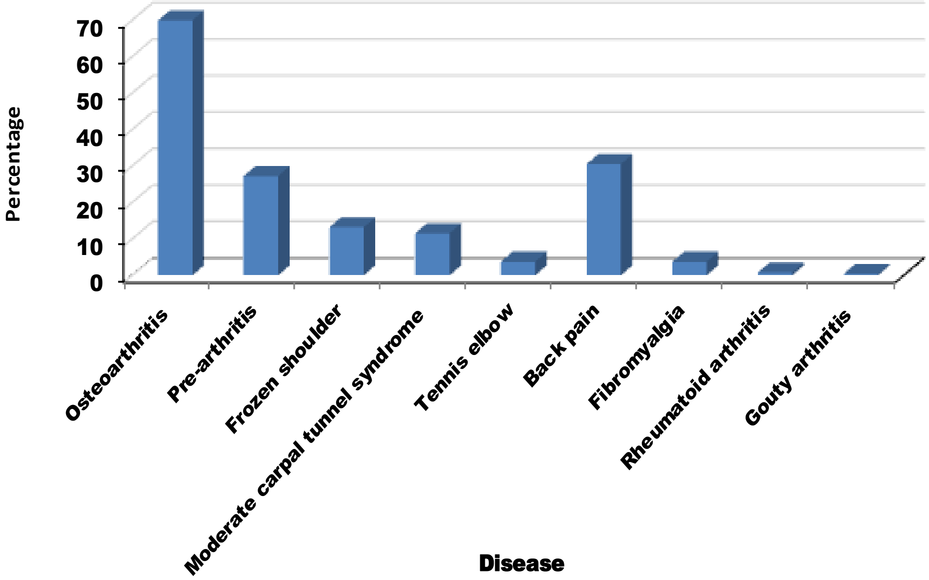

Degenerative joint diseases are among the most prevalent rheumatoid disorders in human populations worldwide [31]. Studies indicating the relationship between age and knee osteoarthritis have demonstrated that along with aging, some levels of degenerative changing occur in joints such as knee joints [32]. In spite of several studies which emphasized rheumatoid disorders in Iran, our study is the first survey in which the percentage of rheumatoid disorders was evaluated in Iranian chemical veterans. Our data have revealed that osteoarthritis and pre-arthritis with the prevalence of 69.9% and 27.2% were the most prevalent among the studied population, respectively. The results of a study conducted in Tehran confirmed these results [11]. The results of a study on rural areas of Iran revealed that about 20% of patients suffered from osteoarthritis in at least one joint. Also, the COPCORD results of 17 countries indicated that with the prevalence of 16.9%, Iran stands in the second level of osteoarthritis prevalence [33].

Our results showed that the prevalence of pre-arthritis or soft tissue rheumatoid among veterans was remarkably higher than the general population of Iran. Similarly, previous studies in Iran showed that the prevalence of pre-arthritis in Sanandaj, Tehran and for the whole country was 5.5%, 4.62% and 4.6%, respectively [11, 24, 34].

The prevalence of rheumatoid arthritis in our study was 0.8%, which was close to the results reported previously in the southeast areas of Iran (0.98%) [35]. Meanwhile, the prevalence of rheumatoid arthritis among veterans was higher than the population of Tehran (0.33%) and Sanandaj (0.51%) [11, 24]. The comparison of the following results with the general population of other countries revealed a higher prevalence of the mentioned diseases among veterans.

The prevalence of gouty arthritis in this study was 0.4%, while it was previously reported as 0.13% in Tehran and 0.12% for Sanandaj [24, 36]. Dawatchi et al. indicated that Iran is ranked 11th among 17 countries in regards to the prevalence of this disease [34]. The other statistics revealed that Indonesia (1.6%) and Australia (1.5%) had the highest frequency for this disease [34].

CONCLUSIONS

This study evaluated the prevalence of rheumatic complications and disorders among Iranian chemical veterans community. Our results have demonstrated that total rheumatic complaints and disorders among this population are higher than the mean of the whole community. These data provided the possibility of calculating the disease's burden and making efficient decisions in the sanitary and treatment systems. Due to the restrictions and special treatment needs of this community, approaching periodic screening tests, designing beneficial nutrition, athletic and treatment interventions can improve and meliorate rheumatic diseases among chemical veterans. Therefore, this study has performed the initiating steps in the case of identifying mentioned complications among chemical veterans and following steps must be taken to investigate the preventing applications of rheumatoid disorders among this population. Also, medicine prescription as well as the intervention of the prescribed drugs must be evaluated through a widespread study.

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REVIEW ARTICLE

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Primary retroperitoneal tumors – A brief classification and differential diagnosis

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Abstract: *According to literature the primary retroperitoneal malignant pathology is rare, representing less than 0.5% of all malignancies. In spite of its low incidence, this pathology often proves challenging in terms of diagnosis and treatment. The most com¬mon retroperitoneal malignancy is represented by lymphoma that accounts for approximately 33 % of the retroperitoneal malignancies. Other frequent retroperitoneal tumors are sarcomas: liposarcomas, leiomyosarcomas and malignant fibrous histiocytoma (in order of incidence). We have analyzed the existing data regarding the most common primary retroperitoneal tumors in order to realize a brief classification and their differential diagnosis considering their clinical, imagistic, histopathological and molecular characteristics.*

The technological developments that have been made over the years in terms of imaging investigations, as well as in biomelecular and cytogenetic studies have offered new possibilities for of assessing a retroperitoneal mass in order achieve more precise informations that can guide physicians to better distinguish between different types of retroperitoneal tumors and therefore their therapeutical protocol.

Keywords: *retroperitoneal tumors, diagnosis, imaging investigations*

INTRODUCTION

According to literature, approximately 70% – 80% of the primary retroperitoneal neoplasms it is estimated that are malignant, these tumors accounting for 0.1%–0.2% of all malignancies [1, 2]. These retroperitoneal masses can be divided into solid and cystic tumoral masses [3-5]. Because the treatment options vary, it is useful to be able to distinguish these masses and to diagnose them clearly in order to choose the correct therapeutical protocol. Imaging investigations and biopsy play a crucial role in establishing the tumors characteristics in terms of tumour content, size, shape, wall thickness, the presence of intratumoral septa, calcification, fat, necrosis, detecting the extension of the disease in nearby structures and also distant metastases, as well as in predicting the patients prognosis [1, 6, 7].

SOLID RETROPERITONEAL TUMORS

Solid retroperitoneal neoplasms can be split into four groups: 1. mesodermal neo-

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plasms; 2. neurogenic tumors; 3. germ cell, sex cord, and stromal tumors; 4. lymphoid and hematologic neoplasms [8].

Mesodermal Neoplasms

Retroperitoneal neoplasms are predominantly of mesodermal origin, the most frequent tumors located in the retroperitoneum being liposarcomas, leiomyosarcomas and malignant fibrous histiocytoma.

Retroperitoneal sarcomas represent approximately 0.2% of all malignancies, being more frequently discovered during the 6th decade of life. Quite often these retroperitoneal masses are found incidentally during imaging investigations, especially in early disease stages when the patient has no alarming sings, nor symptoms that could alert the patient and raise a question mark. Otherwise, a great percentage of retroperitoneal tumors are detected in advanced stages, when the tumor has important dimensions, often being associated with symptoms related to the nearby organ compression or invasion and therefore being less possible to achieve a complete and oncological surgical resection. The rate of relapse in such cases is high, often these patients will develop distant metastases, more frequently in the lungs, liver, bones and brain [1, 2].

Liposarcoma

Liposarcoma is the most common soft tissue sarcoma encountered in adult patients.This fatty cell tumor usually develops in middle-aged individuals as a mass of submucosal tissue that grows easily without pain and ulceration, yet some lesions can grow rapidly and become ulcerated. The most recent classification of sarcomas identifies five categories of liposarcomas: well differentiated, with adipocytic, sclerosing, and inflammatory subtype, dedifferentiated, myxoid, round cell, pleomorphic.

The histological type appears to be closely related to the anatomical distribution, so the well-differentiated liposarcoma appears more frequently in the soft tissues of both limbs and retroperitoneum, whereas the mixoid/round-cell liposarcomas and the pleomorphic liposarcomas have a striking predilection for limbs.

The tumor usually grows slowly over time, leading to progressive and diffuse abdominal distention and to other symptoms that appear to be secondary to the adjacent organ compression. Physical examination can prove itself to be inconclusive (especially in early stages when the tumor is small and without secondary signs nor symptoms), but in advanced stages (when the tumor has important dimensions) it may prove to be a useful tool in terms of raising the suspicion of a retroperitoneal mass.

Laboratory studies have demonstrated that cytogenetics have an important role in diagnosing lipomatous tumors based on the differences of chromosomal abnormalities between tumor types. The lipoblastoma usually exhibits modifications of bands 8q11-13 and the gene PLAG1 has been involved as the target of chromosomal changes [9].

Another important method used for evaluating these types of tumors is represented by imaging investigations. For example, computer tomography (CT) scanning has proven to be superior to magnetic resonance imaging (MRI) when we are investigating a bone erosion and demineralization, whereas MRI provides information's about the limbs characteristics, describing the fatty nature of the tumor. Liposarcomas usually have well-defined and mostly lobulated margins. The well-differentiated liposarcomas are formed of mainly fat tissue with septa or nodules. Myxoid liposarcomas are described as having a pseudocapsule with a homogeneous or heterogenous aspect. Pleomorphic tumors have a markedly heterogeneous structure. Another imagistic technique that can be used for the diagnosis process is angiography. This invesigation can demonstrate tumor malignancy on the basis of a prominent vascular component, therefore having an important value in planning the therapeutical protocol. The diagnostic procedure for liposarcoma is biopsy. For large (>3 cm) and deep tumors, the diagnosis implies open incisional biopsy accompanied by definitive resection. Fine-needle aspiration (FNA) should be followed by immunohistochemical and histological examination [10, 11].

Immunohistochemical examination is used for excluding other types of sarcoma. Lipid staining may be helpful, although oil red O stains or Sudan black are insufficient for diagnosis. Helpful stains include the following [12]:

- S-100 – Positive results in fat cells and lipoblasts;
- Alpha-1-antitrypsin – Positive results in malignant fibrous histiocytomas;
- Desmin – Positive results in leiomyosarcomas;
- Myoglobin – Positive results in rhabdomyosarcomas;
- Adipophilin stain to document lipid content in the cytoplasm of the neoplastic cells using formalin-fixed paraffin-embedded issue.

Histologically the identification of lipoblasts is decisive for the diagnosis of liposarcoma. A lipoblast is capable to produce and accumulate non–membrane-bound lipid within its cytoplasm. The most important morphologic features in diagnosing the liposarcoma are the demarcated cytoplasmic lipid that shifts the indentations in an irregular hyperchromatic nucleus and the characteristic scalloping of the nuclear membrane.

Well-differentiated liposarcomas usually contain a predominance of mature fat cells with relatively few, widely scattered lipoblasts. In the sclerosing subtype of a well-differentiated liposarcoma, collagen fibrils that encircle fat cells and lipoblasts make up a prominent part of the matrix.

Myxoid liposarcoma, the most common type, is diagnosed by the observation of a delicate plexiform capillary network that is associated with both primitive mesenchyme-like cells and a variable number of lipoblasts. In the round-cell type, lipoblasts are interspersed among sheets of poorly differentiated round cells.

Poorly differentiated pleomorphic liposarcoma is recognized by a mixture of bizarre, often multivacuolated lipoblasts and atypical stromal cells, many of which contain highly abnormal mitotic figures. Hemorrhagic and necrotic areas are common. Lipoblasts are present [12].

Leyomiosarcoma

Leiomyosarcoma derives from smooth muscle tissue, Wolffian duct remnants or blood vessels, being the second most frequently primary retroperitoneal tumor. This type of sarcoma can grow and reachenormous sizes (>10 cm), being more frequently encountered in female patients, usually in the 5th or 6th decade of life. Histopathologically, it is characterized by large areas of cystic degeneration and necrosis, but calcification is uncommon. Immunohistochemistry (IHC) experimental tests have showed that the tumor cells were significantly stained by desmin and actin antibodies, with some variation in staining intensity in different tumor cells. Relatively frail vimentin staining has been observed, especially if it is compared with the intense vimentin - specific decoration of endothelial cells and fibroblasts. Leiomyosarcoma can manifest itself predominantly as extravascular (62%), intravascular (5%) in the retroperitoneum or it can have a combination of extra- and intravascular components (33%). At CT examination, small tumors are usually homogeneously solid, although large tumors have areas of necrosis and occasional hemorrhage.

Extremely rare, leiomyosarcoma can appear as a cystic tumor. The MRI highlights these tumors to have intermediate to low signal intensity on T1-weighted images and intermediate to high signal intensity on T2-weighted images, depending on the amount of necrosis. So in conclusion, the existence of necrosis in a retroperitoneal tumor, with contiguous involvement of a vessel, is suggestive for leiomyosarcoma. Distant metastases usually occur late in the course of the disease, the most common locations being the liver, the lungs and/or lymph nodes [1, 13].

Approximately 6% of these retroperitoneal tumors arise from the inferior vena cava (IVC) [1]. These tumors have a large extravascular component that makes it difficult to distinguish them from a secondary involvement of the IVC. The most commonly affected location is the segment between the diaphragm and the renal veins. Tumors involving the superior segment of the IVC (above the hepatic veins) may manifest with Budd-Chiari syndrome, those of the middle segment (between the hepatic and renal veins) manifest with right upper quadrant pain, discomfort, nephrotic syndrome, or arterial hypertension [14-17] and the tumors of the inferior segment of the IVC (below the renal veins) can manifest with pain and lower extremity edema [13]. The differential diagnosis includes benign thrombus, angiosarcoma and a tumor extending to the IVC from adjacent organs [13, 18]. Tumors that develop from the middle segment of the IVC seem to have a better prognosis than those that arise from other IVC segments [18].

Malignant Fibrous Histiocytoma

This type of tumor is the third most common retroperitoneal sarcoma, but overall is the most frequently sarcoma in the body [19]. Malignant histiocytoma derives from primitive mesenchymal elements and it is estimated that approximately 15% of these tumors appear in the retroperitoneum [1]. In terms of gender distribution is it reported that male patients have higher risks for developing this type of tumor (75% male patients versus 25% female patients), being discovered especially in the fifth decade of life. Imaging techniques are nonspecific and usually describe a large, intrusive and heterogeneously enhancing mass with large areas of necrosis and hemorrhage, with invasion of the nearby organs, like other pathologies look alike [20]. Different patterns of calcification can be seen in the peripheral areas of these tumors. A cystic variant with peripheral calcification has been reported. The presence of calcification may help to distinguish leiomyosarcoma from malignant fibrous histiocytoma [1].

Germ cell, sex cord, and stromal cell tumors

Primary extragonadal Germ Cell Tumors

Even though germ cell tumors are present commonly in the testes or ovaries, a slight percent of germ cell tumors arise from an extragonadal location. These tumors are supposed to appear from abnormal primordial germ cell remnants that are cause of: germ cells distributed physiologically to liver, bone marrow, and brain or faulty migration of germ cells from the yolk sac or endoderm to the urogenital ridge [21]. Before an analysis of primary extragonadal germ cell tumor is made, a metastasis should be excluded. Sometimes the primary gonadal tumor could not be detectable because tumor relapse has left only a scar, with asynchronous or synchronous metastasis. This type of tumors is more frequently found in men. From the histopathological point of view, these tumors are classified in nonseminomatous germ cell tumors or seminomas, which involve teratoma, yolk sac tumor, choriocarcinoma, mixed germ cell tumors, and embryonal carcinoma. Increased levels of the beta subunit of human chorionic gonadotropin (choriocarcinoma) and of alpha-fetoprotein (yolk sac tumors, embryonal carcinoma) may be found with laboratory tests [21].

Extragonadal germ cell tumors are frequently seen near the midline, especially between the T6 and S2 vertebrae, the retroperitoneum being the second most common site after the mediastinum, a midline mass being more evocative for a primary extragonadal germ cell tumor than of metastasis. At imaging, the data collected are nonspecific for primary extragonadal germ cell tumors. The imaging characteristics are similar to those of gonadal germ cell tumors. Seminoma is occasionally located in the retroperitoneum and it can be recognized as a lobulated, large, well-defined homogeneous solid mass with fibrous septa and ring-like or speckled calcification. Non-seminomatous germ cell tumors are illustrated as heterogeneous tumors with areas of necrosis, hemorrhage and heterogeneous enhancement. The tumors in the extragonadal sites have a worse prognosis than that of their gonadal counterparts [22].

Lymphoid and Hematologic Neoplasms

The most common retroperitoneal malignancy is represented by lymphoma that accounts for approximately 33% of the retroperitoneal malignancies [1].

Lymphoma can be generally categorized into non-Hodgkin lymphoma and Hodgkin lymphoma. Hodgkin lymphoma usually occurs in patients during their second and sixth decade of life, having a bimodal age of distribution, often involving the spleen and mediastinum, manifesting itself with limited disease. Non-Hodgkin lymphoma is manifested in the forth-seventh decade of life, often associating extranodal disease in the spleen, bowel or liver. It is estimated that paraaortic lymph nodes are affected in 55% of the patients with non-Hodgkin lymphoma, as well as in 25% of the patients with Hodgkin lymphoma. A retroperitoneal mass can be discovered in approximately 15% of the subjects with non-Hodgkin lymphoma. Mesenteric lymph nodes are also frequently involved in non-Hodgkin lymphoma [1, 2].

At CT, lymphoma is identified as a circumscribed homogeneous mass, with moderate homogeneous contrast enhancement, that disseminates between normal structures without compressing them. The IVC and aorta can be anteriorly modified, producing the imagistic sign called “floating aorta”. Necrosis and calcifications are exceptional before therapy [23]. According to literature approximately 23% of the non-Hodgkin lymphomas are heterogeneous and they cannot be differentiated from other primary retroperitoneal tumors on the basis of their enhancement characteristics alone.

Ureterohydronephrosis, with secondary urinary tract infections [24, 25] and IVC obstruction may be found [1].

Image-guided needle biopsy is a first-line investigation when facing such cases, being useful in the diagnosis of lymphoma present in the retroperitoneum, especially if we consider its simplicity, as well as its noninvasiveness, often sparing the patients from radical procedures. Lymphoma comprises a heterogeneous group of lymphoproliferative malignancies, with varying morphologic features depending on the specific subtype [26, 27]. The abnormal lymphocytes in the lymph node, bone marrow, or in extranodal sites can be small, cleaved or noncleaved, intermediate or large cells and they can have a follicular or diffuse pattern. In contrast to reactive follicular hyperplasia, lymphomas usually alter the lymph nodes architecture, the capsule being usually involved [26, 28].

However, needle biopsy encounters several limitations; in few cases, core needle biopsy offers an amount of normal tissue or tissue that has lost its normal architecture, making diagnosis impossible [28, 29].

Immunophenotypic analysis aids to determine neoplastic from reactive lymphoid infiltrates, nonlymphoid from lymphoid malignancies, and specific lymphoid neoplasms. Although bcl-2 expression differentiates reactive follicular hyperplasia from follicular lymphoma, bcl-1 expression strongly supports a diagnosis of mantle cell lymphoma. CD30 expression is also important for the identification of anaplastic large cell lymphoma and it can also be found in the majority of Hodgkin lymphomas. Lymphoma is treated with chemotherapy or radiation therapy [30]. Soft-tissue masses can persist after treatment due to fibrosis and cannot be dif¬ferentiated from viable tumors [31].

Cystic Neoplastic Masses

Retroperitoneal cysts are infrequent, these tumors rarely producing symptoms, usually these symptoms being related to the cysts location and size, as well as to their complications. Differential diagnosis should be made between retroperitoneal cysts and omental, mesenteric, splenic and enteric duplication cysts.

Serous Cystadenocarcinoma

Serous cystadenocarcinoma is a rare primary epithelial retroperitoneal tumor that develops through mucinous or serous metaplasia of retroperitoneal coelomic mesothelium, which is located along ovarian descents [32]. Other approach of expansion includes a supernumerary ovary, an enterogenous cyst and a teratoma. Serous cystadenocarcinoma is discovered in women with normal ovaries. Histopathologically, the mass is multicystic and encapsulated, full with serous fluid and extruding papillary nodules. Imaging techniques are nonspecific and display a defined cystic mass with solid intramural nodules. Increase of the wall and solid components may be found. Other important diagnostic characteristics include: an elevated carcinoembryonic antigen level in the serum andglandular epithelial cells at biopsy [32]. This type of neoplasia needs surgical excision with wide margins [33]. Other rarely primary retroperitoneal epithelial neoplasms are mucinous cystadenocarcinoma, borderline mucinous tumor and borderline serous tumor [32].

CONCLUSIONS

Retroperitoneal masses can appear from different tissues and are a heterogeneous group, including some rare neoplasms.

Histopathology, immunohistochemistry, imaging techniques and other diagnostic exams are valuable in the evaluation of the retroperitoneal masses, particularly in staging as well as in assessing the nearby invasion. Although for now a specific diagnosis may be difficult to determine due to a lack of diagnostic information, the identification of certain characteristic features, along with clinical and demographic information, may help in narrowing the differential diagnosis.

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Primary thyroid lymphoma – case report

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Abstract*: Introduction. Even though, primary thyroid lymphoma (PTL) is a rare thyroid malignancy and extranodal non-Hodgkin lymphoma, this type of disease should not be disgraced in order to achieve an early diagnosis and multimodal treatment.*

Materials and method. We selected for discussions a primary thyroid lymphoma patient with absent constitutional (B-type) symptoms, who undergoes a multidisciplinary approach of the disease, chemotherapy and radiotherapy. In this clinical case report a 84 yo male patient with stage IE diffuse large B-cell histological type (Ann Arbor staging) was treated using R-CHOP chemotherapy, followed by involved field radiotherapy, with an excellent treatment response and disease control.

Results and Discussion. Usually this type of extranodal non-Hodgkin lymphoma is present in the seventh decade of life, with males being affected earlier than females. One of the most common presentations of thyroid lymphoma is an enlarging neck mass and a history of Hashimoto's thyroiditis, which is the major risk factor. The most common PTL subtypes are diffuse large B-cell lymphoma (the most aggressive histological subtype, representing 70% of all PTLs) and MALT lymphoma (30% of the PTLs). Advances in both diagnosis and treatment in the last years have improved the early diagnosis and shortened the time for treatment implementation.

Keywords: *primary thyroid lymphoma; non-Hodgkin lymphoma; thyroid cancer; radiotherapy*

INTRODUCTION

Even though lymphoma is one of the most common hematological malignancies, primary thyroid lymphoma (PTL) is very rare and accounts for 0.5-5% of all thyroid malignancies and about 2% of extranodal lymphomas [1]. Chronic Hashimoto's thyroiditis it is one of the main risk factors known to increase incidence of this type of malignancies [2]. PTL is a rare extranodal lymphoma which is defined as lymphoma that involves just the thyroid or the thyroid and the regional lymph nodes, without metastasis to other areas at the time of diagnosis [3].

Treatment and prognosis varies according to history and clinical stage. Surgery, chemotherapy, radiotherapy or their combination are the treatment of choice in this particular type of Non-Hodgkin Lymphoma (LNH). Such a case, with PTL, is presented in this article [4].

CASE REPORT

An 84 years old man, known with Hashimoto's autoimmune thyroiditis, with no other relevant personal or family history, visits the oncology department for recent-onset gross neck mass, throat

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discomfort, dysphagia and hoarseness (noticed about 1 month before presentation). The patient had no fever, night sweats or weight loss. The physical examination reveals firm, immobile and painless mass descending in the anterior chest wall. Thyroid hormones at presentation revealed high antithyreoglobulin antibodies, an increased thyrotropin level and normal thyroxin. The ultrasound showed a macronodular goiter and chronic lymphocytic cells. On the CT examination of the head and neck region, showed an enlarged thyroid gland, with a nodular structure, starting from the hyoid cartilage and ending in the upper mediastinum (Figure 1). The endotracheal biopsy and immune histological analyses led to the diagnosis of a Non-Hodkin diffuse large B-cell lymphoma (DLBCL), CD20+, Ki-67>90%. A bone biopsy was requested for disease staging, which showed no evidence of neoplazic cells on the examined tissue. Also, the CT scan of thorax, abdomen and pelvis showed no other signs of evolution.

Figure 1 (explication in text)

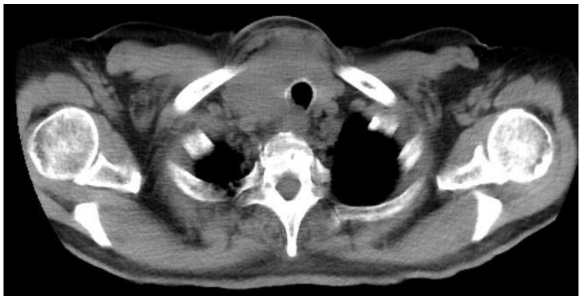

Regarding the fact that only the thyroid was involved (which is an extranodal site, on the same side of the diaphragm) and the fact that the patient had no B symptoms (fever, night sweats or weight loss), the stage of the disease, at the time of the diagnosis, was stage I AE.

After the diagnosis, the patient underwent 4 cycles of chemotherapy with R-CNOP chemotherapeutic protocol, i.e. rituximab (anti CD-20 antibody) 1400 mg, mitoxantrone 14 mg, cyclophosphamide 1350 mg, vincristine 2 mg and dexamethasone 16 mg. The treatment, after 4 cycles of chemotherapy, was stopped because of low tolerance. At the PET-CT scan, following chemotherapy was highlighted high metabolic activity in the right thyroid lobe.

Radiation therapy (RT) was recommended after chemotherapy. A total dose of 36 Gy (18 fractions, 2Gy/fraction) was administrated, using involved field technique to delineate the clinical target volume (CTV). For treatment preparation a fusion between PET-CT and planning CT-scan had to be used for accurate CTV delineation. Three months after radiotherapy, another PET-CT scan was made, to evaluate treatment response, showing low metabolic activity in the right thyroid lobe. The follow-up at 9 months post-chemoradiation was done using clinical examination and a CT scan, which showed a marked regression of the tumor mass (Figure 2).

Figure 2 (explication in text)

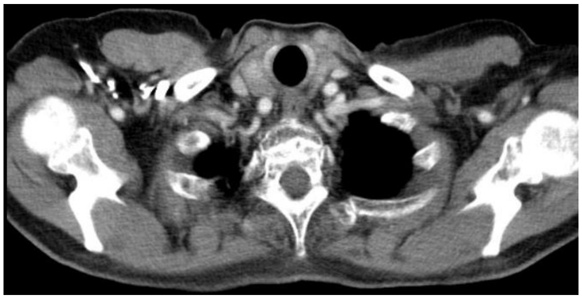

DISCUSSIONS

In general population this type of disease is rare, with an annual incidence rate of 2.1 persons per milion [6]. Also, it is known that PTL affects mainly women (5x times more common in women than men) between 50 and 80 years of age, whereas its incidence is low in individuals younger than 40 years of age [7].

We presented a patient with chronic lymphocytic thyroiditis (Hashimoto's thyroiditis), high levels of antithyroglobulin antibodies in different blood tests. These types of patients, with Hashimoto's disease, have a much higher risk of PTL than the general population. It has been presumed that abnormalities in immunity due to an approximately 20-year history of Hashimoto's disease increase the risk of developing PTL [8].

Clinical findings in our patient on presentation, made us consider an anaplastic carcinoma of the thyroid gland, because of the overlapping symptoms. After tumor and bone biopsy, the initial assumption was changed into primary thyroid B-cell lymphoma. The bone biopsy was made to exclude systemic involvement [9].

The current standard of care is chemotherapy followed by radiotherapy. The role of surgery has been diminished, because an aggressive debulking surgery would not have a more successful response over chemo-radiotherapy [10]. The recommended treatment was 6 cycles of R-CHOP followed by adjuvant RT [5]. In our case it was used R-CNOP chemotherapy because of mitoxantrones lower cardiotoxicity than doxorubicin, but with the same therapeutic outcome as the R-CHOP scheme [11]. RT treatment plan was realized by applying involved field radiotherapy technique, which included in the CTV the whole thyroid gland. A total dose of 36 Gy (2 Gy/fraction, 18 fractions) was administered at the end [7, 12].

An excellent prognosis for early stage PTL (stage IE and stage IIE) is achieved with proper modern treatment management and a multidisciplinary approach. A 5-year survival rate of 87.5% has been observed in patients with DLBCL type. Using modern treatment (combination of chemotherapy plus rituximab agent and radiotherapy), a good response is observed in this type of patients [7].

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CLINICAL PRACTICE

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Principles of antimicrobial therapy in urosepsis: an approach from the perspective of the infectious diseases physician

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Abstract*: Background: This topic is aimed to review the treatment approach in adult patients diagnosed with urosespsis, in order to decrease mortality and to maximize the outcome of the antibiotic stewardship efforts.*

Methods: We present an approach of antibiotic therapy in urosepsis from an infectious diseases physician perspective, based on the recently published data integrating the epidemiology of the urinary tract infections in a multidisciplinary hospital from Romania.

Results: The principles of therapy in urosepsis should have five goals, but not limited to rapid diagnosis, specific intensive care unit treatment, obtaining urine and blood cultures, anti-infective treatment and identifying and correction of underlying risk factors. Due to the higher prognostic value, the antimicrobial therapy in urosepsis should consist in "two steps" regimen: initial antimicrobial therapy, based on "empirical" approach for the first 48 - 72 hours and directed or "definitive" antimicrobial therapy adapted to the susceptibility profile of the isolated pathogens, the penetration into the urinary tract and the patient clinical response. Clinicians should be aware of the different antibiotic susceptibility profile of the bacteria from the hospital compared to the community. Thus, the initial choice of antimicrobial agents depends on several criteria like the severity of illness and the risk factors for multidrug resistant pathogens: the immune status of the patient (age, debilitating chronic illnesses, immunosuppressive treatment or uncontrolled malignancies), broad-spectrum antimicrobial use and health care environment exposures in the previous 3 to 6 months, susceptibility of prior urinary isolates, and local community resistance prevalence of bacteria.

Conclusions: Our important messages highlighted the need of a holistic approach of urosepsis, in a multidisciplinary team, with integration of risk factors for AMR, focusing on the first phase of antibiotic treatment as the main driven of the survival rate.

Keywords: *urosepsis, antimicrobial therapy, infectious diseases, antibiotic resistance*

INTRODUCTION

In about 30% of the sepsis cases, the underlying condition is related to the urinary tract infections (UTIs). The main risk factors are obstructive uropathies and most frequently, urosepsis develops in the context of health care–associated (HCA) UTIs [1]. The prognosis of urosepsis is more favorable compared to that of sepsis in general, with a mortality rate about 20–40% for severe cases. Unlike etiology in all-cause sepsis, dominated by gram-positive bacteria (GPB), the most common pathogens isolated

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in urinary sepsis are gram-negative bacteria (GNB) [2]. In March 2018, the Centers for Disease Control and Prevention released the "Hospital Toolkit for Adult Sepsis Surveillance", a guidance useful for hospitals to identify sepsis outcomes and evaluate the effectiveness of sepsis prevention efforts [3].

In our perspective, one of the main causes of failure to antibiotic treatment in sepsis is related to the antimicrobial resistance (AMR) and inadequate spectrum of the prescribed antibiotics at the beginning. Therefore, this topic aims to review the treatment approach in adult septic patients with acute complicated UTIs, in order to decrease mortality and to follow the antibiotic stewardship recommendations.

ETIOLOGY AND SUSCEPTIBILITY OF ISOLATED STRAINS

Worldwide, the etiology of urosepsis is dominated by enterobacteriaceae, with Escherichia coli (E. coli) accounting for about 52% of the cases, followed by Klebsiella species (spp), Pseudomonas aeruginosa, Enterobacter spp and GPB, such as enterococci (5%) [4]. In 2017, among the strains isolated from the urology department of our Romanian tertiary hospital, E. coli was encountered in 59.2% of all cases of UTIs, followed by other GNB like Klebsiella pneumoniae (K. pneumoniae) [unpublished data].

It has been reported that the resistance rates of pathogens in urosepsis are higher than 10% for almost all antibiotics. The European Antimicrobial Resistance Surveillance Network for 2016 showed that more than half (58.6%) of the E. coli isolates were resistant to at least one of the antimicrobial groups under regular surveillance (aminopenicillins, fluoroquinolones, third-generation cephalosporins, aminoglycosides and carbapenems) [5].

According to the European Centre for Disease Prevention and Control, the level of E. coli resistance to carbapenems remained rare in 2016 (< 0.1%), with a national percentage ranging from 0% to 1%, meanwhile for K. pneumoniae, data showed large differences, ranging from 0% to 66.9% [6]. Carbapenem-resistant enterobacteriaceae (CRE) represents a significant threat to patients and healthcare systems in all European countries, being associated with high mortality, especially due to the delay in administration of effective treatment and limited treatment options [6]. Despite the fact that in 2016 the E. coli carbapenem resistance level in Romania has dropped from 1.9% to 1%, the reported rates of AMR are very high and rising in comparison to other European countries [7].

MANAGEMENT

In the management of septic patients, we must consider that sepsis is a life threatening condition with a directly dependent prognosis on the timeliness and accuracy of therapeutic intervention. In the latest update of the Surviving Sepsis Campaign (SSC) bundle from 2018, the most important change of the 2016 guidelines was that the 3-h and 6-h bundles have been combined into a single "hour-1 bundle" [8]. Clinicians should initiate antibiotic treatment immediately, especially in patients with hypotension, rather than waiting and extending resuscitation measures. Other measures such as obtaining blood samples, urine and blood cultures, administration of fluids and antibiotics, should all begin immediately [8].

The principles of therapy in urosepsis should follow five rules [9]. First of all, we have to establish a rapid diagnosis based on clinical and paraclinical tests: classical symptoms (dysuria, frequent urges to urinate, flank pain and/or costovertebral angle tenderness, fever, chills, pyuria), quick Sepsis Related Organ Failure Assessment score (qSOFA), biomarkers of sepsis and imaging findings [9].

In addition, a specific intensive care unit (ICU) treatment should be start with fluid replacement, supportive and adjunctive therapy as needed [9]. Obtaining urine and blood cultures prior to antibiotic administration represent another important rule [9].

On the other hand, initiation of antibiotic treatment must be adapted to the patient profile (usually broad spectrum antibiotics) after the quick assessment by the clinician and/or infectious diseases specialist [9]. The last but not least rule is addressed to identification and correction of risk factors (calculus, obstruction, renal or perinephric abscess) by the urologist [9].

ANTIBIOTIC TREATMENT

Broad-spectrum antibiotic therapy aiming to cover all likely pathogens should be started immediately, ideally in the first hour. Data from the literature have shown an association between the rapid administration of antibiotics and improved survival in patients with sepsis. Initiation of empiric antibiotic treatment within the first hour of hypotension is associated with a survival rate of about 80% [10]. Each additional hour of delay was found to decrease the survival rate by 7.6% [10].

The initial choice of antimicrobial agents depends on several criteria like the severity of illness and the risk factors for multidrug resistant pathogens: the immune status of the patient (age, debilitating chronic illnesses, immunosuppressive treatment or uncontrolled malignancies), broad-spectrum antimicrobial use and health care environment exposures in the previous 3 to 6 months, susceptibility of prior urinary isolates, and local community resistance prevalence of bacteria. Clinicians should be aware of the different antibiotic susceptibility profile of the bacteria from the hospital compared to the community. Therefore, an accurate classification of the UTIs according to the place of acquisition of the infection is mandatory. There have been defined two major categories of infections comprising of community-acquired (CA) UTIs or health-care associated (HCA) UTIs with the following scenarios: community onset HCA UTIs or hospital onset HCA UTIs. In CA-UTIs, the possible pathogens are limited usually to susceptible enterobacteriaceae, ampicillin-susceptible Enteroccous faecalis or Staphylococcus saprophyticus. In HCA UTIs, in general we are dealing with resistant strains of bacteria such as extended spectrum beta-lactamases (ESBL)-producing enterobacteriaceae or CRE. It has been shown that inappropriate antibiotic treatment in critically ill patients was associated with decreasing in survival rate of at least 5 times in septic shock [11].

Table 1. Choice of antibiotic therapy in urosepsis

"Empirical" phase of antimicrobial therapy *(combination therapy (ß-lactam antibiotics plus the second antimicrobial agent) is preferred especially in critically ill patients with high risk of death*		
Community-acquired UTIs[#] [#] *in some regions the risk of ESBL enterobacteriaceae has to be considered also in community-acquired UTIs*	**Health-care associated UTIs**	
	without risk of non-fermenting GNB or hemodynamically stable patient	*risk of non-fermenting GNB or hemodynamically unstable patient*
Aminopenicillins +/- beta lactamase inhibitor (BLI) (Amoxicilin/ clavulanate) 3rd generation cephalosporins (e.g. Ceftriaxone) Fluoroquinolones (e.g. Ciprofloxacin, Levofloxacin) Aminoglycosides (e.g. gentamicin)	Carbapenems (e.g. Ertapenem) Ureidopenicillin plus BLI (e.g. Piperacillin/tazobactam) Aminoglycosides (e.g. Gentamicin, Amikacin, Netilmicin, Tobramycin) Glycopeptides (e.g. Vancomycin)	Carbapenems (e.g. Imipenem, Meropenem, Doripenem) Carbapenems plus BLI (e.g. Meropenem/vaborbactam) Ceftolozane/Tazobactam Ceftazidime/Avibactam Monobactams (Aztreonam) Colistin Glycopeptides (e.g. Vancomycin) Aminoglycosides (e.g. Gentamicin, Amikacin, Netilmicin, Tobramycin)
"Directed" phase of antimicrobial therapy[±,¥]		

[±] *according to the antibiotic susceptibility profile of the detected pathogens*

[¥] *monotherapy is preferred (exception of critically ill patients with high risk of death and infections with CRE)*

The antimicrobial therapy in urosepsis consists in "two steps" regimen:

a) Initial antimicrobial therapy, based on "empirical" approach, should starts ideally, after obtaining blood and urine cultures, during the first hour of the patient presentation in the emergency room or after the diagnosis of urosepis in case of hospitalized patients. Due to the prognostic value of this treatment phase, as a general rule, the broad-spectrum antibiotics with good penetration into the urinary tract are preferred for the first 48-72 h [8];

b) Directed or "definitive" antimicrobial therapy represent an adaptation of the initial regimen (e.g. de-escalation) according to the antibiotic susceptibility profile of the isolated pathogen, taking into account the clinical evolution of the patient [8]. The duration of definitive therapy should have a minimum duration with maximum efficiency, usually between 10 to 14 days [2];

Based on our clinical experience and the evidence base medicine, we present a personal approach, not validated in clinical trials, regarding the choice of antibiotic therapy in urosepsis (Table 1).

In addition to antimicrobial therapy, an underlying condition such as urinary obstruction, anatomical or functional urinary tract abnormalities (neurogenic bladder, bladder catheters, nephrostomy tubes, and ureteral stents), should be rapidly identified and managed [12]. One of the important issues is related to the appropriate collection of the urine sample in case of urinary tract obstruction. In case of kidney drainage (e.g. percutaneous nephrostomy) we must collect also the urine from the inserted tube in addition to the usual urine sample.

ASSESSMENT OF TREATMENT RESPONSE

Usually, symptoms improve rapidly when antimicrobial treatment is effective, but the fever can persist at least 3 days despite the appropriate antibiotic treatment. The response to treatment is assessed by the following criteria: clinical (fever remission), biological (decreasing levels of inflammatory markers, sepsis biomarkers, alleviating organ dysfunction) and bacteriological (negative urine and blood cultures). In the PRORATA trial, a multicentre randomized controlled trial, it has been evaluated the use of procalcitonin (PCT) to reduce the patient's exposure to antibiotics in ICU. It has been shown that a strategy consisting in measurement of PCT concentration on a daily base allowed for a reduction of antibiotic consumption in ICU [13]. Also, other studies have been proposed a PCT-based algorithm for starting, continuing or stopping antibiotics in patients with sepsis in the ICU [14]. A Swiss randomized controlled trial showed that an algorithm based on PCT concentration and quantitative pyuria measurements, significantly reduces antibiotic exposure, with about 30%, without apparent negative effects on clinical outcomes [15]. In case of persistent or worsening symptoms after 72 hours of appropriate antimicrobial therapy, an additional evaluation is required in order to identify potential factors that might interfere with the favorable clinical outcome. Dosing reevaluation, especially for aminoglycosides and glycopeptides, surgical drainage of the new abscesses, assessment of the inserted tube position and permeability, repeating urine and blood cultures and readjusting treatment to the antibiotic susceptibility profile of other isolated pathogens, should be considered.

CONCLUSIONS

Our important messages highlighted the need of a holistic approach in urosepsis, provided by a multidisciplinary team, with integration of risk factors for AMR, focusing on the first phase of antibiotic treatment as the main driven of the survival rate. The new sepsis "hour-1 bundle" and the principles of antimicrobial therapy should be followed in all emergency departments as a clinical tool intended to improve the general prognosis in urosepsis.

Author Contributions

Concept: VG, AMR; Design: VG, IS, AMR; Supervision: VG, IS, AMR; Materials: CG, FA, AMR; Data collection and/or processing: CG, FA, AMR; Analysis and/or Interpretation: CG, FA, IS; Literature Search: CG, FA, AMR; Writing Manuscript: VG, AMR; Critical Review: VG, IS;

Disclaimer

The authors have no conflict of interest to declare.

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ORIGINAL ARTICLES

Prioritisation in delivering health services: A military health system example

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Abstract: *Objectives: The aim of this study is to use of available resources effectively and efficiently, to determine the situation of recource allocation, priority setting and to offer an insight into future planning in delivery of health services in Turkish Armed Forces (TAF).*

Study Design: This study was conducted using qualitative focus group method and it is a descriptive and qualitative study. Questions to be asked in the focus group discussions, the location and the time of discussions and the participants was determined by review of the literature.

Methods: Focus group interviews lasted between approximately 90-120 minutes, respectively, to each focus group, 9 chief phsycians, 13 doctors /dentists, 6 nurses, 8 health noncommissioned officers, 7 officers, 6 noncommissioned officers and 6 health administrators were attended.

Results: The result of focus group discussions; priority settings in military health services must be done and to preventive health care would be prioritised. The military hospitals in Internal Security Operation Area should be strengthened in terms of medical personnel and medical supplies and be full-fledged hospital. There was found that secondary care should be met from civilian hospitals in the other regions.

Conclusion: It is evaluated that the study will be contributed to set of priorities in TAF military health services take the investments in the public or private health sectors and operational requirements into consideration, the studies carried out by TAF Medical Command in order to restructure of the military hospitals, to be employed the recources allocated for health services and health personnel graduated from Gulhane Military Medical Academy such as physicians, health noncommissioned officer and nurses in real requirement positions.

Keywords: *Military hospital, prioritisation, primary health services, focus group, health personnel*

INTRODUCTION

Health services have great importance in protecting human health and according to the World Health Organization, they include all services necessary for diagnosis and treatment of all diseases, maintenance and improvement of health [1].

Primary purpose of health services is to protect the society from diseases and to ensure all individuals are healthy during their lifetimes within the scope of preventive health services; when it is not possible, to provide diagnosis and treatment services which are the secondary purpose of health services; and the third purpose is to give rehabilitation services to individuals who have not fully recovered [2].

Facilitating the access to health services, improving the scope and quality depend on resources such as money, personnel, material and medicine.

During the last 50 years, GPD expenditure on health in OECD countries has increased from 3.7% to 9.4%. Growth in health expenditures has nearly exceeded the economic growth

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nearly in all OECD countries in the last 15 years. Since current resources are insufficient compared to this growth and it is difficult to meet all health needs in an optimum level with current means, it is concluded that health services need to be limited and prioritization concepts in health services have begun to be studied [3].

Prioritisation in health services is a complicated decision process which includes many dynamics and decision-makers such as plans/plannings regarding which services, regions, programs, hospitals, patients or diseases the resources allocated to health service should be divided into primarily.

Prioritisation in health services is realized by receiving opinions of all sides and analysing data regarding service demands. These are findings regarding politicians and health managers, service providers (doctor and other health personnel), service recipients (society) and cost and efficiency of health needs and proper responses/treatments [4].

Methods such as public opinion survey, opinion researches, public meetings, focus group studies are used to determine the priorities in health services.

Prioritisation and resource allocation in health service can be used in many criteria such as respect for humans, necessity, individual responsibility, efficiency, the number of persons affected as well as fundamental criteria such as lifesaving, cost- effectiveness, cost- benefit and equality [5].

Determination of priority studies have been realized in many countries for many years. National Health Accounts (NHA) and National Burden of Disease (NBD) studies were realized for prioritisation in health services in Turkey [6], but no study regarding the prioritisation in national and international military health services has been found.

Military health services include controls for compatibility and suitability to the task and periodical or mandatory controls, health certificate control, prevention and removal of medical threats, discharge, treatment, health logistics, blood services and other needs with regards to military health [7].

The aim of military health system is to provide sustainability for soldiers to be healthy, prevent the weakening of combat power due to epidemics, discharging the wounded in time and successfully and saving the limbs and life of the injured. In short, it is to increase the desire and confidence for warfare of each soldier by facilitating well-coordinated and not-failing health system, keeping militant power fit [8].

Purpose of this study is to determine priorities regarding health services by taking military operational requirements and features into consideration, shedding light on studies conducted with regards to restructure of military hospitals, revealing data to take a good turn in terms of resources allocated for armed services. Also, it is to contribute to the employment of military health personnel, especially doctors, medical non-commissioned officers and nurses, in the areas where there is actual need.

MATERIALS AND METHODS

Study of prioritisation in delivering health services is realized through focus group meeting which is a qualitative method. Focus group meetings were realized from September 2014 to April 2015. The universe of this study consists of sides of health services which are service providers (doctors, nurses and medical non-commissioned officers), service recipients (commissioned officers, non-commissioned officers, specialized sergeants/privates and their families) and health managers (hospital chief surgeons, health administrators and headquarters planners). Participants are selected from chief surgeons and hospital administrators who work in different military hospitals; doctors, nurses and medical non-commissioned soldiers who have worked in different garrisons and have experience in terms of troop and headquarters, willing personnel of commissioned officers and non-commissioned officers who received health service from primary care and secondary care health organisations – who accept to participate to focus group meeting.

Focus group meetings were realized in four stages in this study. In the first stage, the subject of prioritisation in health services is presented through a wide scale literature review. In the second stage whom to be interviewed was decided. Groups who participated to the focus group meeting and their numbers are presented in Table 1.

After reviewing the literature, the questions to be asked in focus group meetings were prepared with contributions from the Department of Military Health Services, the Department of Biostatistics and Epidemiology faculty members who have worked in different positions including administration in different levels of health system. Which titles were to be used and which questions to be asked in which order during the meeting were determined beforehand, the questions were prepared by abstaining from directing the interviewee.

In the third stage, the place and time of the meeting was determined. Meetings were minimum 60 minutes and maximum 120 minutes. 9 chief surgeons, 6 doctors, 6 nurses, 8 medical non-commissioned officers, 7 commissioned officers, 6 non-commissioned officers and 7 doctors/dentists participated to the meeting, respectively.

Table 1. Focus groups and number of participants in group consultation.

Status		Female		Male		Total	
		N	*%*	*N*	*%*	*N*	*%*
Healthcare Providers	Chief Surgeon	0	0	9	100	9	100
	Administrative Chief	2	33.0	6	67.0	8	100
	Medical/Dentist Officer	2	15.3	11	84.7	13	100
	Nurse	7	100	0	0	7	100
	Health Non-commissioned Officer	0	0	8	100	8	100
	Total	**11**		**34**		**45**	**100**
Healthcare Takers	Officer	0	0	7	100	7	100
	Non-commissioned Officer	0	0	6	100	6	100
	Total	**0**	**0**	**13**	**100**	**13**	**100**

In the final stage, the meeting was summarised, content analysis was performed on the acquired data and the meeting results were presented. Data acquired in this study is reviewed and interpreted by way of content analysis method, researchers compared data acquired from each focus group with other focus groups. Opposing and similar views are tried to be distinguished by benefitting from the perspective of each group meeting.

FINDINGS

Distribution of participants who participated to focus group meetings by force are presented in Table 2 and Table 3.

Table 2. Distribution of participants who provide health service by force (*there is no force separation regarding nurses*).

Status	Land Forces		Naval Forces		Air Forces		Gendarmerie		Total	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Chief Surgeon	7	78.0	1	11.0	1	11.0	0	0	9	100
Administrative Chief	5	62.5	1	12.5	1	12.5	1	12.5	8	100
Medical/Dentist Officer	7	53.8	2	15.3	2	15.3	2	15.3	13	100
Health Non-commissioned Officer	3	37.5	1	12.5	3	37.5	1	12.5	8	100
Nurse									7	100

Table 3. Distribution of participants who received health service.

Status	Land Forces		Naval Forces		Air Forces		Gendarmerie		Total	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Officer	5	71.4	1	14.3	1	14.3	0	0	7	100
Non-commissioned Officer	3	50.0	0	0	1	16.6	2	33.4	6	100
Total	8	61.8	1	7.6	2	15.3	2	15.3	13	100

When the answers of participants are reviewed in focus group meetings, most participants stated that there is “a requirement for prioritization” to be able to present available resources and means in an effective and efficient way in terms of delivering health service, 1 of the nurses and 2 participants who receive health service stated that “all services must be provided without any prioritization”. Chief surgeons stated health care needs of armed forces as prioritization criteria; administrators and doctors stated operational requirements, civil health care means, cost-effectiveness status; nurses stated regional needs; medical non-commissioned officers stated regional needs, disease burden, frequent diseases whereas service recipients put emphasis on efficiency and cost-effectiveness.

When asked who should play a role in prioritization, people who provide healthcare service stated that especially doctors should play a role, opinions of those who receive service should be obtained, also opinions of patients who receive healthcare service and their families as service recipients must be received and they also stated that opinions of health personnel are important. Hospital administrators stated that the prioritization is needed to be realized according to operational requirements and opinions of generals who decide the operation are also needed to be received. A participant in the service recipient group stated that prioritization should be done according to data in the database by establishing a council for health science.

When asked if preventive health service, diagnosis-treatment services or rehabilitation services should be prioritized in terms of health care delivery of armed forces, health care providers stated their opinions that “preventive health service” and “primary health service delivered within unit” should be at the forefront by taking operational requirements into consideration since armed forces personnel mostly consist of able-bodied, young men and health service is given to a group healthier compared to the society. The opinion that duty of armed forces is to deliver primary health service and secondary and tertiary health are possible to receive from civil health organisations is stated especially by doctors and medical non-commissioned officers. Nurses who mostly work in hospitals and healthcare recipients stated that diagnosis-treatment services should be prioritized.

When the current structure of military hospitals is considered, chief surgeons stated that the most suitable thing is “to reduce the number of military hospitals and turn them into a general structure in terms personnel and medical devices” and to enable the existence of military hospitals only in the regions where there are operations against terrorism and “to enable other regions to receive secondary health service from civil hospitals”. Hospital administrators stated that the hospitals are currently inadequate and they are in no state to compete with civil health organisations in terms of service quality and delivered healthcare. Doctors, nurses and medical non-commissioned officers stated that actions should be in accordance with operational requirement, thus military hospitals outside of the region where there are operations against terrorism should be closed since secondary health service can be received from civil hospitals and military hospitals in the regions where operations against terrorism are realized should be fully-organised and in a level enough to compete with civil hospitals. Service recipients pointed out that military hospitals are not currently cost efficient and there is no high-quality health service in these hospitals, they also stated that there are only need to be military hospitals in the regions where operations against terrorism are realised so as to provide high-quality health service.

When asked which areas of expertise should be available in military hospitals, chief surgeons stated that “psychiatrists are essential” due to the increase in the number of personnel and specialized sergeants/privates who have psychological problems in the recent period, they also stated that there is a need for branches such as orthopaedics, general surgery, anaesthesia for operations; administrators presented their opinions that there should be evaluations specific to the regions, there is a need especially for psychiatry, emergency medicine, surgical branches and anaesthetists; doctors/dentists and nurses stated that there is a need for internal medicine, psychiatry, emergency medicine, anaesthesia and surgical branches; medical non-commissioned officers stated that there is no need for gynaecology and obstetrics and paediatrics, instead there should be surgical branches; some service recipients stated that all branches should be available and the others stated that there should be branches that can treat the wounded personnel.

RESULTS

When studies on this subject realized in countries such as USA, England, Germany, Holland and Sweden, it is determined that a basic benefit package is created to provide a balance between limited health resources and healthcare needs, to prevent parameters such as treatment costs, medicine and surgery expenses within the scope of general health insurance from imposing an excessive burden on national economy. Committees are formed to determine health services which are to be included in basic benefit packages. These committees consist of the representatives of healthcare providers (such as doctors, nurses) and healthcare recipients (society). Even though these committees differ in each country, they prepare guides in which priorities in delivering health services are determined by meeting with people from all levels of society and taking health data of countries into consideration. Whether disease-treatment (medicine, surgery etc.) expenses are to be covered by health insurances is decided according to these guides. Within this framework, studies such as International Health Accounts Household Health Expenses and Disease Burden Projects are realized in our country in the recent time.

Military health services are primary health services provided especially for operation. It is determined by all participants in the focus group meetings that primary health service should be prioritized. It is pointed out in the focus group meetings that there must be doctors and assistant health personnel in primary health organisations; it is not enough to have only health personnel and laboratory facilities and medical devices and materials should be re-enforced. Hospital administrators and doctor's point to the increase in the number of personnel and specialized sergeants/privates who have psychological problems in the recent years in troops, psychological counselling and guidance services which are mostly given by reserve officers could be more efficient if they are provided by regular personnel or civil psychologists.

In the studies realized by Wiseman and Rosén, transparency, equality, justice, efficiency, responsiveness of health system and health personnel's approach towards patients are stated as priority determination criteria in health services. In the study realized by Mamas, priority determination criteria in health services is stated as maintaining trust and accountability between patients and doctors and establishing patient-centered approach [9, 10, 11]. In some studies, criteria are determined as benefit of patient, cost effectiveness and severity of disease [12, 13]. In the PHD thesis by M. Top, the principles of equality, health need, cost- effectiveness are placed near the top [5]. In this study, prioritization criteria are listed as operational requirements of the region, status of civil health facilities in the garrison, benefit of patient and cost-effectiveness.

Parties who are required to take part in delivering health service are stated as doctors, patients and their families, respectively, by the doctors and as patients and their families, doctors and health authorities, respectively, by the patients who receive service in a study realized in Greece. Both groups stated that politicians should play no part [11]. In the study realized by Wiseman and others, it is stated that doctors, health service administrators and patients and their families should especially take part in determination of priorities [9]. In the studies realized by Bowling and Kneeshaw, the result was that doctors, local health authorities and public should be a great part of the prioritization studies [11]. In harmony with the literature, the opinion regarding health personnel and especially doctors is stated by service providers whereas service recipients had the opinion with regards to patients and their families and doctors in this study.

When military hospitals are evaluated according to their current state, it is seen that there are no specialists in many branches of many hospitals and there is only one doctor when there is a specialist in a branch. All participants who participated to the focus group meeting stated that secondary health service delivered in many military hospitals fall behind of civil health services and it would be suitable if military hospitals outside of the region where there are operations against terrorism should be closed and secondary health service is received from civil hospitals. It is emphasised that military hospitals in operation areas where fights against terrorism happen should be fully-organised with sufficient personnel, medical device and equipment and can provide service for 24 hours in a day with ambulance helicopters and health teams. The fact that many military hospitals are currently in an idle state and they can only provide service as polyclinics, the number of in-patients and surgeries are very low is exemplified. There are similar problems in the United States of America. Existence of military hospitals and how efficient and effective they are in their current state have become highly questionable. Pentagon military health administrations state that primary mission of hospitals is military needs, doctors and nurses must develop their abilities for combat requirements. Two thirds of USA Military Hospitals treated 30 or less patients daily in 2013 and less than one-third of them treated as many patients as civil hospitals treated. Nine hospitals treated 10 or less patients [14]. The military worked in more than half of domestic hospital network until today. Pentagon analysts suggested that 15 of 25 hospitals are to be reduced, but the list has been reduced to eight due to complaints by Land, Naval and Air Forces [15].

When criteria such as operational requirements of military hospitals, status of civil health organisations in the region are considered, it is stated that areas of expertise that are required to have in military hospitals first of all must be to meet the operational needs; afterwards, psychiatry, internal medicine and anaesthesia, surgical branches such as orthopaedics, general surgery are listed by the participants. Common opinion of all groups who participated to the focus group meeting is that there is no need for branches such as paediatrics, gynaecology and obstetrics in military hospitals in the regions where there are operations against terrorism and these services can be delivered by civil health organisations. When the literature is examined, it is seen that 66% of injuries happening in theater of operations are orthopaedics, 21% is general surgery, 6% are of head and neck injuries, 5% are of burn injuries [16]. In another study based on the experiences of English military health personnel got in Afghanistan, it is determined that 93 of 124 people who had been wounded in battle lost their lives because of injuries in upper thigh, inguen and pelvic. Most of the casualties are because of pelvic injuries (17). Almost 11% of 7,856 trauma patients who applied to English Role-3 Sahara Hospital in Afghanistan from 2003 to 2011 applied because of chest trauma and almost 14% of them lost their lives. Most of the wounded people whose systolic blood pressures were detected very low had head and abdominal injuries and cardiac arrest and they caused mortality [18]. In another study, 83% of 271 people who lost their lives because of war injuries died because of brain injuries. 80%

of 287 people who were potentially survivable, haemorrhage caused by major trauma caused mortality. When bleeding site was examined in these people, it is determined that 48% of bleeding happened in torso, 31% extremity and 21% neck, axilla and inguen [19]. In a study realized by Owens and others, extremity injuries in 1281 soldiers are examined and it is seen that 53% of them have penetrating soft tissue injury, 26% have fractures and these fractures are localized similarly in upper and lower extremities [20]. Of injuries except thoracic injuries of 258 patients treated for thoracic injuries happened with weapons with high kinetic energy between 1996 and 2005, 13% are intra-abdominal injuries, 5% are fractures, and 3.5% are major vascular injuries [21]. The most frequent injuries of patients who are brought to an emergency ward in a hospital in Turkey after getting injured in the civil war in Syria are extremity injuries, internal diseases; chest, abdominal and head injuries with similar rates and eye injuries, respectively [22].

In the light of this information, as stated by the participants, it is important to have specialists on surgical branches such as orthopaedics, general surgery, brain surgery, cardio-vascular surgery, thoracic surgery and branches such as anaesthesia, internal medicine, radiology to be able to treat the wounded people effectively and increase the motivation of the personnel in the military hospitals in the regions where there are operations against terrorism.

DISCUSSION

The following results are obtained through this study realized with focus group meetings to determine priorities in delivering health service to armed forces.

Almost all parties and participants who participated to the study pointed out the necessity for determining priority in military health system to use resources allocated to armed forces effectively and efficiently since resources are limit, requirements are many. While making prioritization, it is stated that criteria such as operational requirements, structure of civil health organisations in the region and military health organisations being utilized by personnel and their families is needed to be considered because of unique structure of armed forces.

As a result of focus group meetings, it is determined that the opinions of doctors especially are needed to be at the forefront during prioritisation as well as opinions of health personnel, hospital managers such as chief surgeon, assistant chief surgeon, hospital administrator, privates who receive service and civil patients.

It is emphasised that the priority of armed forces should be primary health services and preventive health services and primary health service is a must for armed forces. Considering regional features and operational requirements, it is common opinion of all parties that secondary health service can be received from civil health organisations. Health boards in military hospitals stated that specific health services such as health service for operational requirement must be focused on and it is more appropriate for rehabilitation services to continue as they are currently.

It is determined that military hospitals cannot be utilized in an efficient and effective way due to the fact that personnel and their families prefer civil health organisations, there is not sufficient specialists, medical devices and materials are lacking, physical conditions of hospitals are worse compared to civil hospitals, there are problems in logistics and the system responds to the needs very slowly.

The importance of providing efficient, correct and quick primary health service is emphasised. Within this scope, it is pointed out that there must be a doctor regarding primary service, but it is not enough only to have doctors, there should also be laboratory facilities which consist of complete blood, urine, roentgen devices and primary health services must be supported.

Recently, there is an increase in the number of personnel and specialized sergeants/privates who apply to Psychological Counselling and Guidance Centers and it is determined that to deliver more productive service to these patients, regular personnel or civil officers should work instead of reserve officers so that the service becomes more effective and efficient.

When military hospitals are evaluated according to their current state, it is seen that there are no specialists in many branches of many hospitals and there is only one doctor when there is a specialist in a branch, especially in the surrounding hospitals. Participants are in agreement regarding the fact that the military hospitals in the regions where there are operations against terrorism must be re-enforced and turned into hospitals which have a fully-organised structured and several doctors in each branch to continue delivering service, hospitals except this must be closed or turned into health centers suitable to the unit needs.

Parties that participated to the group meetings emphasised the importance of quick and efficient patient/wounded discharge regarding operations is emphasised. Afterwards, it is concluded that having an ambulance helicopter which have regular health personnel who provide service in accordance with 24-hour principle within military hospitals and having first aid and emergency medical technician working in units which take part in operations improve the efficiency of health services.

The following suggestions are developed in the light of these results:

Health system of armed forces is required to be restructured in a way that efficiency and economy criteria are taken into consideration, medical board processes specific to armed forces can be realized, diagnosis-treatment abilities and capacities focused on unit and operation needs, in accordance with national health legislation are increased without losing specific features and by re-evaluating vision, mission, current situation and the point the national health system has reached.

Health Services Policy Determination and Assessment Board should be organised to give scientific support to restructuring studies of Health System, one member from the Departments of Public Health, Military Health Services, General Surgery, Internal Medicine, Psychiatry, Infection Diseases and Family Medicine and Health Services Management and the Department of Epidemiology, 5 Chief Surgeons from Military Hospital and 3 general practitioners should be assigned.

Inpatient treatment facility and laboratory facilities which consist of complete blood, urine, roentgen devices should be provided especially for trainings units to deliver more efficient and effective primary health services and to make these services more suitable to unit needs.

For health board needs, regional hospitals, military hospitals outside of the region where there are operations against terrorism should be transformed into proper health centers, other military hospitals should be structured in accordance with the criteria of the Ministry of Health through personnel and medical device savings with this transformation.

As specialist quota is determined, planning should be made by considering operational requirements of armed forces, current status of staff in health board branches, especially branches such as general surgery, orthopaedics, anaesthesia, internal medicine, psychiatry, and radiology should be focused on.

When the fact that there is an increase in the number of personnel and specialized sergeants/privates who have psychological problems in the recent periods, regulations regarding regular personnel recruitment should be made to solve problems arising from conducting psychological counselling and guidance services in the troops especially with reserve officers.

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ORIGINAL ARTICLES

Procedure and effectiveness of Basic Cardiopulmonary Resuscitation during the landing of an airplane: an exploratory study on military flights

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Abstract: *Introduction: The incidence of cardiorespiratory arrest (CRA) in aircraft is estimated at one per 5-10 million flights. The early initiation of cardiopulmonary resuscitation (CPR) can double or quadruple survival rates after CRA, and is associated with a better prognosis in terms of decreased brain damage and better quality of life. The aim of the present study is to evaluate the effectiveness of basic CPR procedures carried out by the flight crew in a clinical simulation performed in a real environment, during the landing of a military training flight.*

Methodology: The clinical simulation was carried out with 20 members, grouped in ten pairs, of the Méndez Parada Military Parachuting School at the Alcantarilla Air Base (Murcia, Spain). At an altitude of approximately 1,100 feet, each pair simulated CPR on a Little Anne® manikin fitted with a SimPad® PLUS. The results obtained were recorded by SkillReporter ™.

Results: The mean duration of the procedure was approximately five minutes. During the flights, the compression depth achieved with CPR was correct in only six of the ten cases. In only one case was the "patient" checked for consciousness and respiration. In 50% of the simulations, the forehead-chin manoeuvre was not performed (i.e., the airway was not opened).

Conclusions: The lack of standard recommendations in current guidelines on CPR during aircraft landing may provoke discrepancies in the management of this critical situation and reduce the quality of the treatment provided.

Keywords: *cardiopulmonary resuscitation; aircraft; cardiac arrest; special environments; in-flight medical emergencies; landing medical emergencies*

INTRODUCTION

Although commercial aircraft are safe and passenger flight is one of the most widely used means of transport, the millions of persons carried, the fact that some will have pre-existing medical conditions and the long distances often flown all increase the chances of an in-flight emergency happening [1]. It has been estimated that cardiorespiratory arrests (CRA) occur in-flight once in every 5-10 million flights [2-4]. The prompt initiation of cardiopulmonary resuscitation (CPR) can double or quadruple the odds of survival after CRA, and is associated with a better prognosis in terms of decreased brain damage and better quality of life [5-7].

In-flight CPR is a challenging task. Some studies have suggested that the use of a mechanical resuscitation device produces a higher rate of chest compression than is obtained by standard basic life support. However, such devices are not always available [8]. The current guidelines of the American Heart Association (AHA) and the European Resuscitation Council (ERC) only offer general recommendations for in-flight CPR, without addressing aspects such as how to act if an emergency occurs during landing [9-11]. In the context of the armed forces, the role of critical care air transport teams and other trained personnel is essential for the safe transport of unstable patients and combat victims [12].

The aim of the present study is to evaluate the effectiveness

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of the basic CPR procedure employed by the flight crew in a clinical simulation in an otherwise real environment, during the landing of a military training flight.

METHOD

The participants in this exploratory clinical simulation study were 20 members of the Méndez Parada Military Parachuting School at the Alcantarilla Air Base in Murcia (Spain), grouped into ten pairs of jump commanders. Ten clinical flight emergency simulations were created in a real environment during training for parachute jumps with automatic opening. This exercise took place in 2019, using T-12 Aviocar military aircraft (Figure 1A). The CRA simulation began five minutes before landing, at an altitude of approximately 1,100 feet (Figure 1B), when the crew was informed that a CRA had occurred. The simulation ended once the pilot had landed the aircraft and brought it to a halt. During this simulation, CPR was performed on a Little Anne® manikin fitted with a SimPad® PLUS and SkillReporter™, and wearing a helmet, TP2Z parachute, and RTP-26Z reserve (Figure 1C). The simulation was evaluated by two members of the research team (RG and LL), who also formed part of the crew during the training exercises.

Figure 1: Images of the CPR study context during landing. A: T-12 Aviocar military aircraft; B: Altimeter recording 1,100 feet, beside the Little Anne® manikin fitted with a SimPad® PLUS, a helmet, a TP2Z parachute and an RTP-26Z reserve; C: CPR simulation during flight and landing.

All participants gave prior signed informed consent and authorized the video recording and photographing of the simulation. All had basic life support training as part of their combat training and annual refresher courses.

RESULTS

Among the results obtained, the frequency of compressions per minute exceeded ERC recommendations [median ± IQR = 123 ± 32] and the mean depth (mm) was just within the recommended range [median ± IQR = 55.5 ± 11]. Figure 2 summarises the main data obtained in the quantitative evaluation of the quality of external chest compressions. The mean duration of the procedure was approximately five minutes (Figure 2a). The number, frequency and depth of compressions were similar in all the flights except number 7, in which the crew decided to secure the manikin; the difficulty involved in this process limited the effectiveness of their CPR actions (Figures 2b and 2d).

We also conducted a qualitative evaluation of CPR performance, by reference to the corresponding ERC guidelines [10]. Table 1 lists the results of this evaluation, quantifying the instances in which each aspect of CPR was performed correctly or otherwise. Notably, the depth of the compressions obtained was only correct in six of the ten cases considered.

DISCUSSION

Our qualitative assessment of the data on CPR performance reveals that chest compressions were performed better than ventilation. In the latter case, certain aspects were clearly open to improvement. This shortcoming has been reported previously, in the executive summary of the 2015 ERC guidelines [10]. In our case, the difference may have arisen from the fact that only basic life support was performed, that is, without the availability of material for the airway and within the confined space of an aircraft, which prevented good positioning for the mouth-to-mouth ventilation technique.

As recommended in Section 4 of the 2015 ERC guidelines ("Cardiac arrest in special circumstances"), the CPR was performed in a suitable place in our simulation exercises, within the limitations imposed by the characteristics of the aircraft9. Specifically, it was carried out in the middle of the fuselage, where there was most space, with the head of the simulated victim positioned towards the tail of the plane. Although the 2015 ERC guidelines do not indicate the most appropriate position for the victim's head, this question should be considered because the head is likely to suffer tilt variations during landing [10]. In each CPR simulation (in accordance with the ERC guidelines) the patient's chest was uncovered and, in this military tactical environment, the parachute and helmet attached to the manikin were also removed [10]. In one flight, the inappropriate positioning of the manikin reduced the quality of the compressions performed.

Figure 2: Quantitative characteristics of the external chest compressions performed. (a) duration of the session, in seconds [median ± IQR = 269 ± 68]; (b) number of compressions per session [median ± IQR = 265 ± 94]; (c) frequency of compressions per minute [median ± IQR = 123 ± 32]; (d) depth of compressions in millimetres [median ± IQR = 55.5 ± 11]. The solid line represents the median and the dashed lines represent the interval ± IQR.

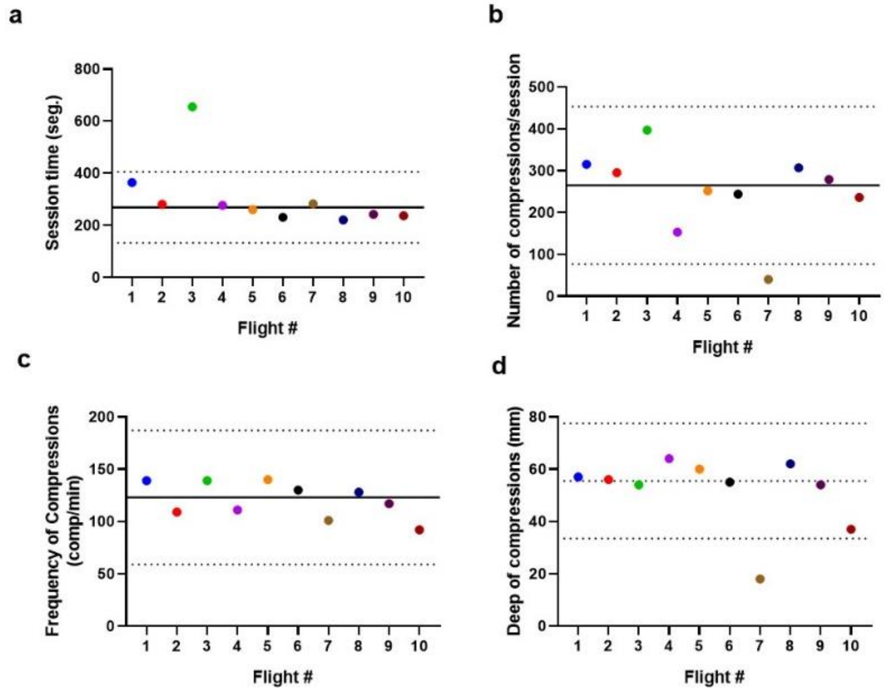

Table 1: Qualitative aspects of the ten simulations considered

Item	Performed	Not performed
Check/ask state of consciousness	1	9
Declare emergency situation	7	3
Check respiration	1	9
Place victim lying face up	10	0
Clear airway (forehead-chin manoeuvre)	5	5
Place hands on the centre of the chest	4	6
Interlock fingers	3	7
Extend arms	0	10
Vertical position over the victim	4	6
Compression: 5-6 cm	6	4
30 compressions at 100-120/min	4	6
Hyperextension of the head	6	4
Pinch the nose	7	2*
Lips-mouth seal	6	-**
Insufflation for 1 second	9	1
Raise the chest	2	7*
Remove mouth, maintain forehead-chin manoeuvre and observe chest elevation	0	10
Maintain ventilation manoeuvres for no more than 5 seconds	10	0
30:2 ratio	10	0

** In one landing, this variable could not be determined by the evaluator; ** In four of the landings, this variable could not be determined by the evaluator.*

In six of the ten flights, CPR was suspended as the aircraft was landing, but the victim was secured in only four cases. Furthermore, the treatment team was not secured in any of the landings, although they did remain seated in six cases. Our review of the literature revealed no specific considerations on the question of safety precautions as the aircraft is landing [9].

In the present study, for which the simulations were conducted within military aircraft, the chest compressions were performed by crew members. Forti et al. [13] have observed that the use of mechanical chest compression devices should also be evaluated since these mechanisms can provide high-quality chest compressions in the confined space of an air ambulance. However, in the present case, this option was not feasible, as no such devices are incorporated in the aircraft considered. A positive factor in our study is that the military personnel who provided CPR attention all had basic life-support training and took annual refresher courses, thus complying with the recommendation that pilots and cabin personnel should be trained in emergency procedures [9].

As a proposal for improvement, the standard recommendations for CPR in aircraft, both in flight and during landing, should be more fully implemented. In this respect, we note that the current AHA and ERC guidelines lack specific recommendations for the treatment of cardiorespiratory arrest in aircraft.

CONCLUSION

In conclusion, we find that the absence of standardized recommendations in the current guidelines on CPR during aircraft landing can provoke discrepancies in the management of this situation and/or reduce the quality of the CPR operations performed. This outcome is due, in part, to the need to balance the safety of the patient against that of the treatment team (i.e., ensuring that everyone is properly secured when necessary and that CPR maneuvers are suspended in certain flight situations), or minimizing pauses in resuscitation, even if this endangers the physical safety of all involved.

Ethical approval and consent to participate

This study was approved by the Ethics Committee of the Catholic University of Murcia (UCAM) and by the Méndez Parada Military Parachuting School at the Alcantarilla Air Base (Murcia). All participants gave signed informed consent to take part in this study. All of the authors undertake to maintain data confidentiality and respect the rights of the participants. These undertakings are stipulated in the author responsibility document, the publication agreement, and the transfer of rights.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

All authors conceived and designed the study. MPR conducted the experiments and analyzed the data. SNC has participated in data collection, analysis and interpretation, and writing. MGV has participated in the data collection; MLI has participated in the data collection; JHM has participated in the interpretation of the data and the writing of the manuscript; ECL has participated in the analysis and interpretation of the data and writing and CCE has participated in the design of the study, data collection, analysis, and writing.

All authors contributed to manuscript revisions.

All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

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ORIGINAL ARTICLES

Psychological consequences of the Covid-19 pandemic. A study carried out on the psychiatric medical staff

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Abstract: *Motivation for the choice of topic: The covid-19 pandemic has restructured the entire health care system. As systems of care were overwhelmed, many health professionals in related professional areas became increasingly involved in providing medical aid. The present paper aims to highlight a comparative analysis between two psychiatric wards of the same hospital, with qualified medical staff, with similar levels of competence, one of the wards caring for patients exclusively with major psychiatric pathologies, and the other patients with associated SARS-COV2 infection.*

The objective is to highlight links between different variables, such as motivation versus amotivation, anxiety and/or burnout, work experience, and resilience, all of which also relate to decisions to accept and provide quality medical care to this particular category of patients.

Methodology Application of specific questionnaires and scales on a group of 60 medical workers in two psychiatric wards, one of which was declared a ward working with psychiatric patients with covid.

Results: Resident doctors, then nurses, followed by hospital attendants, attending physicians and lastly psychologists, cope in descending order with professional stress related to covid-19 anxiety. This is high and correlates positively with depression and burn-out - which, in the case of young people, risks demotivating them to the point of destroying their future plans and the hope of successful professional self-esteem.

Conclusions: The covid-19 pandemic in a psychiatric hospital has raised anxieties even among medical staff, who have moved from somatic to psychiatric care over the years, and the comorbidity of mental illness and SARS-COV2 infection has raised particular problems. Among staff, anxiety, risk of depression, burnout, with depletion of compensatory resources have increased. Resilience calls for the use of mature defence mechanisms and professional experience protects against anxiety and burnout.

Keywords: *burnout; Covid-19 anxiety; stress; resilience; professional status*

INTRODUCTION

The particularity of the study lies in the fact that mental health workers are health professionals who routinely care for psychiatric patients, who are known to have certain characteristics such as: absence of insight into illness, loss of reality testing, productive phenomenology requiring particulars of supervision, suicidal risk, which may be amplified by somatic comorbidities. In other words, the group on which the research was carried out was a highly specific one - the motivation at work of employees in the "psy" area being a special one, involving dedication and training courses in the field, as well as an intrapsychic development developed over years, of a certain nature [1,2].

The research hypotheses were:

1) We assume there are statistically significant correlations between the psychological constructs analysed.

2) We can predict the level of employees' motivation according to the level of depression, resilience and healthcare experience.

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3) We assume that there are statistically significant differences in levels of resilience and intrinsic motivation between employees in a Covid-support medical ward versus employees in a non-Covid medical ward.

4) The occupational status variable has a moderating effect on the relationship between resilience and motivation.

5) The occupational status variable has a moderating effect on the relationship between anxiety and burnout.

6) The healthcare experience variable has a moderating effect on the relationship between amotivation and fear of Covid [3].

METHODOLOGY

Participants

The research was carried out on 60 people, employees of the "Prof. Al. Obregia" Clinical Hospital of Psychiatry, Bucharest, working on two different wards, one declared by the hospital management as a non-Covid medical ward and one ward treating exclusively Covid positive patients. Participants filled in their status on the medical ward: consultant/ registrar (7%), resident doctor (12%), nurse (21%), hospital attendants (26.7%), clinical psychologist (6.7%). The mean age of the group was 39.83 (with SD = 10.47). The inclusion criterion for the study was the employment contract in the hospital and the participants' involvement in medical and psychological activities.

Sampling procedure

The research was carried out in an online environment. The study was carried out on participants employed at the "Prof. Alex. Obregia" Psychiatric Hospital in Bucharest and the completion of the instrument was done through Google forms. Participants were presented with a full description of the study and received written informed consent in accordance with the research standards for human subjects study and confidentiality of personal data was guaranteed. Participants had several professional occupations: consultants or registrars, medical residents, nurses, hospital attendants, clinand ical psychologists [4].

Instruments, measurements, variables

1) The DASS 21-R test [5], which consists of 21 questions with 4 response options on a 0-3 Likert scale (0 = "Did not fit me", 1 = "Fit me to a certain extent or sometimes", 2 = "Fit me quite a lot or quite often", 4 = "Fit me very much or most of the time"). The 21 questions measure 3 subscales (each with 7 items). Items 1, 2, 10, 13, 15, 18, 20 measure the Depression dimension. Items 4, 5, 8, 9, 12, 14, 21 measure the Anxiety dimension. The remaining items measure the Stress dimension. The final score is calculated by summing the items for each.

2) The Maslach Burnout Inventory (Schaufeli, Leiter, Maslach & Jackson, 1996) [6] is composed of 16 items with responses on a Likert scale from 0 to 6 (0 = "Never", 6 = "Daily"). The questionnaire consists of 3 subscales: Exhaustion, Cynicism and Professional Inefficiency The total score can also be calculated. A higher score means a higher burnout.

3) Connor-Davidson Resilience Scale (CD-RISC) [7], which contains 25 items with responses on a Likert scale from 0 to 4 (0 = "not at all true", 1 = "rarely true", 2 = "sometimes true", 3 = "often true", 4 = "always true"). The scale is based on how subjects have felt in the last month. The scale has scores between 0 and 100, with higher scores explaining higher levels of resilience.

4) Multidimensional Workplace Motivation Scale (MWMS) [8], which contains 19 items, with responses on a scale of 1 to 7 (1= "not at all", 2= "very little", 3= "a little", 4= "moderate", 5= "strong", 6= "very strong", 7= "total"). The scale measures 6 subscales: Amotivation, Social extrinsic motivation, Material extrinsic motivation, Introjection regulated motivation, Identification regulated motivation, Intrinsic motivation. A higher score is associated with a higher level of motivation.

5) Fear of COVID-19 scale [9] containing 7 items, with responses on a scale from 1 to 5 (1= "strongly disagree", 2= "disagree", 3= "neither agree nor disagree", 4= "agree", 5= "strongly agree"). Scoring is established according to the summative scale, with sums ranging from 7 to 35. The higher the score, the greater the fear of COVID-19.

In addition to the administered questionnaires, participants filled in factual data on: gender, age, education level, marital status, personal pathological history, health care experience, current work experience, education level, professional status [7].

Research design

The research took place in an online environment and was an observational, cross-sectional study. The database was processed in SPSS version 22, also using the PROCESS extension version 3.5. The variables we worked with were:

- Non-parametric variables: gender, education level, marital status, personal pathological history, education level, professional status;

- Parametric variables: Age, health care experience, current work experience, depression, anxiety, stress (measured with DASS 21), burnout, cynicism, professional inefficiency, total burnout (measured with Maslach Burnout Measurement

Inventory), resilience (measured with CD-RISC), amotivation, extrinsic social motivation, extrinsic material motivation, introjection regulated motivation, identification regulated motivation, intrinsic motivation (measured with MWMS), fear of COVID;

- Moderator variables: professional status, health care experience [10].

RESULTS

Descriptive analysis for the data collected is set out in Table 1.

Table 1: Descriptive analysis

	Minimum	Maximum	Mean	Std. Deviation	Skewness	Kurtosis
	Statistic	*Statistic*	*Statistic*	*Statistic*	*Statistic*	*Statistic*
Age	23.0	59.0	39.833	10.4770	-.010	-1.364
Health experience		43	11.12	10.183	.902	.252
Current experience		30	8.10	8.691	1.060	-.073
Depression	0	19	3.92	4.571	1.595	2.127
Anxiety	0	17	3.57	4.159	1.350	1.276
Stress	0	18	4.45	4.781	1.225	.768
Exhaustion	0	24	6.33	6.299	.793	-.373
Cynicism	0	22	5.20	5.713	1.106	.372
Professional inefficiency	0	27	6.90	7.498	.919	-.374
Burn-out	0	73	18.43	18.671	.954	-.076
Resilience	35	100	75.43	16.532	-.529	-.581
Amotivation	3	19	5.43	4.048	1.840	2.758
Extrinsic social motivation	3	21	9.42	5.782	.402	-1.014
Extrinsic material motivation	3	20	8.48	5.407	.394	-1.277
Introjection motivation	4	28	19.93	6.913	-.689	-.484
Identification motivation	3	21	17.33	4.501	-1.524	2.116
Intrinsic motivation	5	21	17.37	4.084	-1.147	.658
Motivation	33	111	77.97	19.749	-.566	-.346
Covid fear	7	26	12.83	4.705	.670	-.063

To operationalize the variables, the level of education that was noted among the participants was divided into three classes: postgraduate and bachelor studies (graduates of bachelor's, master's, and postgraduate programmes), high school and college of nursing (this included nurses who had graduated from the college of nursing or hospital attendants who had graduated from high school), and secondary school or 10-grade education (for those with less than 10 grades completed), as depicted in Table 2. The distribution by professional status is showed in Figure 1.

Table 2: Level of education

		Frequency	Percent
Valid	Bachelor studies and postgraduate	29	48,3
	High school and college of nursing	17	28,3
	High school	14	23,3
	Total	60	100,0

To test Hypothesis 1, which seeks to see if there are significant correlations between the psychological constructs analyzed on the group of subjects, we used Pearson correlation. Among the important correlations to mention, we list:

- There is a statistically significant negative correlation between experience in the health field (and experience in the current job, p=0.031, r=-0.279) and introjected motivation (p=0.018), with a weak correlation intensity (r=-0.305), which explains that as experience in the health field (implicitly the one from the current job) increases, the level of motivation through introspection decreases.

Figure 1: Distribution by professional status

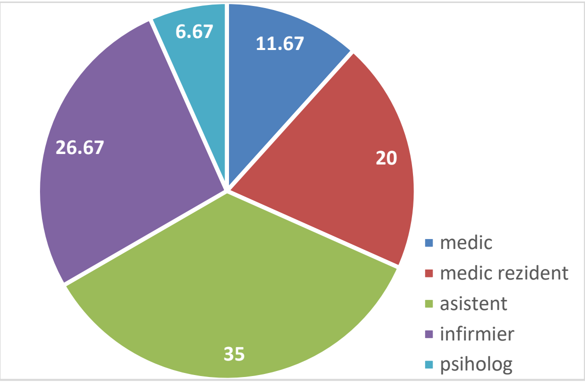

- There are positive correlations of the depression scale with the exhaustion (r=0.744, strong to very strong intensity), cynicism (r=0.698, strong intensity), job inefficiency (r=0.644, strong intensity), burnout (r=0.732, strong intensity), amotivation (r=0.263, weak intensity), extrinsic social motivation (r=0.294, weak intensity), extrinsic material motivation (r=0.427, moderate intensity) scales.

- There are positive correlations of the anxiety scale with the exhaustion (r=0.703, strong intensity), cynicism (r=0.688, strong intensity), job inefficiency (r=0.688, strong intensity), burnout (r=0.724, strong intensity), amotivation (r=0.289, weak intensity), extrinsic material motivation (r=0.314, weak intensity) scales and a negative correlation with intrinsic motivation (r=-0. 28). These data explain that when anxiety levels increase, intrinsic motivation levels decrease.

- There is a positive correlation of stress level with all burnout scales (burnout, cynicism, job ineffectiveness), which denotes that when stress level increases, burnout level increases with its subscales. Stress also correlates positively with amotivation level (r=0.269, low intensity), which explains that when stress level increases, amotivation level increases.

- There is a positive correlation of fear from Covid level with anxiety level (r=0.368, low intensity) and stress level (r=0.263, low intensity), exhaustion level (r=0.384) and cynicism level (r=0.259), work inefficiency level (r=0.356, low to moderate intensity), burnout level (r=0.352, low to moderate intensity) and amotivation level (r=0.312, low intensity).

- Resilience level correlated negatively with the level of the scales: cynicism (r=-0.305), job inefficiency (r=-0.358), burnout (r=-0.322), amotivation (r=-0.327), identified motivation (r=-0.372). Level of resilience correlates positively with level of intrinsic motivation (0.603) [8].

To test Hypothesis 2, which sought to predict the level of motivation of healthcare employees according to predictor variables, we ran multiple linear regression analysis, where the criterion variable was motivation score (measured with MWMS). The motivation variable was included in the regression analysis as a dependent variable and several predictor variables were introduced from the beginning: age, healthcare experience, experience in current workplace, depression, anxiety, stress, exhaustion, cynicism, job inefficiency, burnout, resilience. As a method, we used the Backward Analysis method, a method that, in the end, provides the correct statistical model, after trying, one by one, all possible models, eliminating one by one a variable that is not relevant. In Table 3 we follow the value of the adjusted R2, which indicates the relevance of the model. The highest value indicates model relevance and is 0.192 in model 5. Eight models were generated.

Table 3: Model summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.537[a]	0.289	0.143	18.278
2	0.537[b]	0.288	0.160	18.096
3	0.534[c]	0.285	0.173	17.956
4	0.529[d]	0.280	0.183	17.848
5	0.524[e]	0.274	0.192	17.752
6	0.503[f]	0.253	0.184	17.840
7	0.492[g]	0.242	0.187	17.809
8	0.476[h]	0.227	0.185	17.825

The standardized Beta coefficients indicating the influence of the predictor variables on the dependent variable are included in Table 4.

Table 4: Standardized Beta coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
8	(Constant)	47.024	11.825		3.977	0.000
	Experience in the health field	-0.401	0.232	-0.207	-1.732	0.089
	depression	1.693	0.523	0.392	3.236	0.002
	Resilience	0.381	0.147	0.319	2.602	0.012

Following the presentation and analysis of the table with the Beta coefficients of the regression equation, the corresponding multiple regression equation is as follows:

$$\text{Motivation} = 47.024 - 0.401*\text{health experience} + 1.693*\text{depression} + 0.381*\text{resilience}$$

To test Hypothesis 3, which seeks to highlight differences between the Covid ward employee group versus the non-Covid ward employee group in terms of the variables analyzed, we ran One Way Anova analysis.

Hypothesis 4: The occupational status variable has a moderating effect on the relationship between resilience and motivation. In this way, to investigate the moderating relationship, we used the PROCESS program. The dependent variable was motivation. The predictor variable was resilience. The moderator variable was occupational status [11].

The results explain the occupational status variable as a negative moderator of the relationship between resilience and motivation at work. As shown in Figure 2, the intensity of resilience and motivation is stronger for the category of physicians, medical residents, and psychologists, as resilience is a factor strongly influencing the increase of motivation at work. This result helps us know which psychological constructs should be acted on to increase motivation at work, depending on professional status.

Figure 2

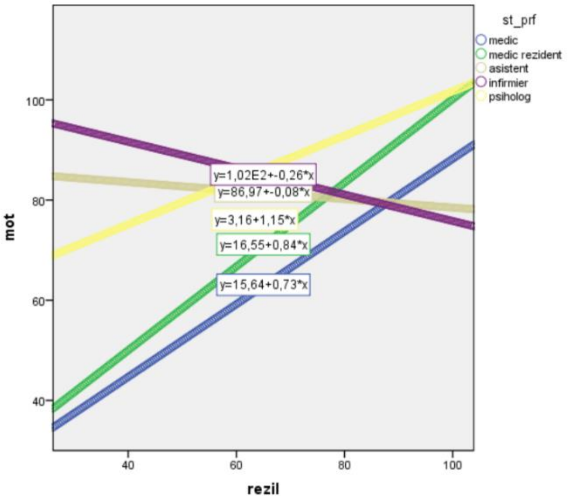

Hypothesis 5: The occupational status variable has a moderating effect on the relationship between anxiety and burnout. Thus, to investigate the moderating relationship, we used the PROCESS program. The dependent variable was burnout. The predictor variable was anxiety. The moderator variable was occupational status. As can be seen in Figure 3, the intensity of anxiety and burnout is stronger for the category of physicians, residents, hospital attendants, and nurses, with a downward slope for the category of psychologists. Thus, for the 4 categories of employees in the "psy" health care field, it is observed that an increase in the level of anxiety leads to a substantial increase in the level of burnout, with maximum intensity in the category of resident doctors, then nurses, then hospital attendants and, finally, on the attending physicians. This result helps us know which employee is more likely to develop burnout as anxiety increases [6].

Figure 3

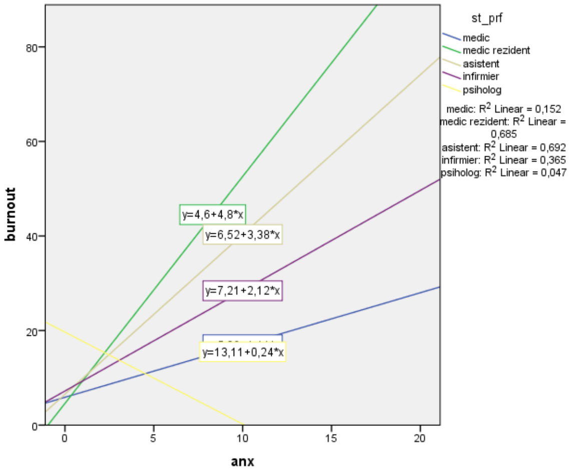

For Hypothesis 6, we tested whether the health care experience variable has a moderating effect on the relationship between amotivation and fear of Covid. Thus, to investigate the moderating relationship, we used the PROCESS program. The dependent variable was fear of Covid, the predictor variable was amotivation, and the moderator variable was health care experience [5]. Due to some clear manifest factors (like lack of taste and smell) that could be due to some other diseases associated with Covid [12], we have had to distinguish between a pathological status versus a psychological fear.

These results explain the occupational status variable as a positive moderator of the relationship between anxiety and burnout at work.

CONCLUSIONS

A first conclusion that stands out in the present study is the regression equation for predicting the motivational level of employees in the "psy" health care field. We have shown that the regression model explains 18.5% of the motivation scale variance. The percentage is a low predictor of motivation according to the scales: depression, resilience and current work experience, however, using the Backward analysis method, which looked for the model that predicts the best, taking each variable in turn and eliminating those that were not significant. The importance that these predictor variables have for the criterion variable is demonstrated. Specifically, depression, resilience and work experience are significant predictors of motivation for several reasons [13].

Depression among staff employed in a psychiatric hospital is less obvious, as those concerned have developed adaptive coping mechanisms for dissimulation, which in crisis situations makes it difficult to highlight emotional, cognitive, decision-making and well-functioning status in the workplace as a whole. In a slightly forced terminology, we could say that many employees have concealed their depression, for fear of social stigma or the idea that for a training in “psy” it is unthinkable to give in.

Resilience is a key concept in any crisis situation. It implies adaptability, flexibility of one's own concepts and defence mechanisms, relaxation of the Superego. From this point of view, resident doctors were the most exposed, not being backed up by the necessary resilience, nor having gone through other moments of crisis in the health system. The feeling of too much professional effort in relation to the satisfaction obtained, the effort to insist by phone up to 12 hours per event to find an available place in a life-support service, the overflow even of COSU (Operational Centre for Emergency Management) resources, the delegation by the consultant of too many responsibilities for the resident doctors (some of them in their first year) or for the nurses, increased at first their anxiety, then, quite quickly, their professional burn-out. A second important finding of the research is the result that resilience and intrinsic motivation are psychological aspects that differ among medical employees between the group of those who worked with Covid positive patients and the group of those who worked with Covid negative patients. Nursing staff in the non-covid ward were under less stress, caring for an issue and pathology they were used to [6].

We also concluded that there are significant differences in the level of stress experienced by employees of a psychiatric ward, depending on their status on the ward (physician, resident doctor, nurse, clinical psychologist, hospital attendant). Psychologists have had to quickly reshape their position (while most of them were performing clinical psychology examinations, now they had to be at the patient's bedside and provide counseling and psychological support) [14].

DİSCUSSİON

Moderation analyses indicate the importance of the professional status an employee has in a medical ward in the relationships between aspects of their own and others' (employees or patients) intrapsychic lives. The fact that the intensity of motivation at work increases on the basis of resilience only for the categories: attending physicians, resident doctors and clinical psychologists can be explained by the dedication to the profession, aspirations for status, dedication to others, the need for affirmation and the need to increase their professional status. The analysis of the relationship between anxiety and burn-out, with a strong increase among all employees in the medical department, except psychologists, maybe due to exactly the aspects previously presented [7].

But, the order of increase in burn-out levels (resident doctors have the highest level of burn-out related to anxiety, then nurses, then hospital attendants, and, last, attending physicians) is also important in order to rethink responsibilities in the medical hierarchy – operationalized not "ad hoc" in crisis situations, but through well-standardized internal procedures and protocols as mentioned by Chen too [4].

Finally, the result expressing how healthcare experience positively shapes the relationship between amotivation and fear of covid brings about important insights into the need for relevant medical training at the bedside, and clinical aspects that could bring diagnostic benefits being brought to the forefront [9].

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ORIGINAL ARTICLES

Quality of life impairments and stress coping strategies during the Covid-19 pandemic isolation and quarantine – A Web-based survey

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Abstract: *Isolation and quarantine during the Covid-19 pandemic affected the lifestyle and daily functioning of the population around the world, leading to social, psychological, and economic changes which further multiplied the stress related to the threat of coronavirus contagion by adding financial, relational, academic, professional and mental health vulnerabilities. To assess the impact of isolation and quarantine over the quality of life in the Romanian population, we conducted a Web-based survey focused on the evaluation of stress level, perception of lifestyle changes, communication patterns, mental health, major concerns, perception of one's future, but also on the preferred coping strategies that people have used to deal with the isolation stress. The answers were collected during one month and the results for the first 2 weeks of quarantine/isolation were compared with the results after one month of such regimen. Several recommendations based on the survey results analysis were formulated regarding possible strategies for decreasing the impact of stress factors over the general population and specific, vulnerable groups.*

Keywords: *Covid-19, pandemic, quality of life, stress coping strategies, functional impairment, depressive disorders, anxiety disorders, behavioral addictions*

QUALITY OF LIFE AND COPING STRATEGIES DURING QUARANTINE AND ISOLATION IN THE CONTEXT OF COVID-19 PANDEMIC

Quarantine is defined as a separation of people potentially exposed to contagious disease from other members of the society until the results of their analyses turn negative or until further medical interventions are needed [1]. Regarding the Covid-19 pandemic, this procedure has been considered necessary for people who traveled in the so-called ”red zones”, where high rates of coronavirus disease have been reported, and they were screened for infection initially and after two weeks of quarantine [2]. Also, the quarantine regimen involved special places for monitoring these people who were considered at risk for developing Covid-19.

Isolation is conceptualized as a separation of people who have been in contact with infected others, but who do not have any symptoms yet or have only mild symptoms, depending on national healthcare services' operational procedures [2]. This concept involves the isolation of people in their own homes for two weeks and active monitoring from their GP or Public Healthcare Services [2]. Self-isolation or lockdown is defined as a method to maintain social distancing by reducing the time spent out of the house for each asymptomatic person, and it was enforced during the Covid-19 pandemics by the law. Self-isolation is a broader

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concept but it is fundamentally a population-large method of prevention, applied in cases of contagious diseases, with a duration defined by national laws during the state of emergency. It does not involve relocation to a special quarantine-designed institution, each self-isolated person remained at his or her home for a duration specified by the law, and maintains the right to leave his/her home for a pre-defined set of specific purposes.

According to a recent review (n=24 papers), the main psychological negative effects of quarantine were post-traumatic stress symptoms, confusion, and anger, with the most important stressors being long quarantine duration, fear of contamination, frustration, boredom, lack of sufficient supplies, inadequate information, financial loss, and stigma [1]. During a SARS outbreak in Singapore, the contagious infection-related psychiatric and posttraumatic morbidity rates reached 22.9% and 25.8%, respectively [3]. According to this research, the most significant factors associated with psychiatric disorders, in general, were being seen at fever stations, younger age, increased self-blame, less substance use, while posttraumatic disorder was associated with the increased use of denial and planning, as coping mechanisms [3]. Another Web-based survey that examined the psychological impact in a quarantined Canadian population during a SARS outbreak (n=129) revealed a high prevalence of psychological distress, with symptoms of posttraumatic stress disorder and depression observed in 28.9% and 31.2% of responders [4]. A longer duration of quarantine was associated in this paper also with an increased prevalence of posttraumatic stress disorder, and acquaintance with or direct exposure to someone with a SARS diagnosis was also a risk factor for this pathology [4].

In the context of the Covid-19 pandemic complete social isolation during many consecutive weeks was considered in Italy similar to a large-scale, absolutely new social experiment [5]. Therefore, activation of healthy coping strategies could be of major importance in the prevention of psychiatric disorders onset in this population. Separation, isolation, boredom, loneliness, feelings of uncertainty are challenges for many quarantined or isolated people [6]. Healthcare workers suffered from quarantine as well, as they accused exhaustion, alienation, anxiety, irritability, insomnia, indecisiveness, decreasing work performance, etc [6, 7]. Also, healthcare personnel had more severe symptoms of posttraumatic stress disorder than the quarantined general population, experienced greater stigma, more avoidance after quarantine, were more likely to believe they had been contaminated and were more preoccupied with the risk of infecting others [7, 8].

Treatment adherence may be negatively affected by denial, anxiety, depression, feelings of despair, and also the risk of suicide or aggression should be taken into account by physicians who take care of patients diagnosed with Covid-19 [9]. Obsessive-compulsive symptoms may appear as a consequence of repeated washing and temperature checking [8]. Financial losses, societal rejection, disappointment, and discrimination are other factors that should be addressed by targeted interventions [1, 10].

Regarding the main keypoints for the psychological crisis intervention in Covid-19- diagnosed patients in China, a group of authors suggested understanding of the mental health status in different categories of population, identifying people who are at high risk of suicide and aggression, and targeted interventions for those in need [9]. Chinese researchers have established four levels of populations to design specific interventions for each one: level 1 population includes the most vulnerable people to mental health problems, e.g. hospitalized patients with severe organic diseases, frontline health care professionals, level 2 includes isolated patients with minimal symptoms and patients at fever clinics, level 3 includes people with close contacts, family members, colleagues, friends, etc, and level 4 people who are affected by the epidemic preventative measures, susceptible people and general population [9].

OBJECTIVES AND METHODOLOGY

Although stress is considered a normal response to the coronavirus pandemic, quarantine and isolation are periods of high risk for the development of stress-related disorders [11]. This risk is high especially in vulnerable populations, e.g. people with a known history of mental disorders, currently remitted or controlled by treatment, and professionals who are on the frontlines of the coronavirus pandemic (physicians, police officers, military personnel, etc), but since the lockdown was enforced by the law to the general population as a preventative measure against spreading the Covid-19, many other populations were exposed to the risk of developing stress-related disorders (e.g., elderly people, patients relying on the support of others due to physical or mental impairments, patients immobilized in their own homes, people with chronic treatments which involved regular visits to the hospital, people who were unable to work from home and who have lost their financial support, owners of small and medium enterprises that could not run their businesses during the state of emergency, etc).

The main objective of this research was to evaluate the impact of the isolation stress over the quality of life in people from Romania during the first period of the Covid-19 pandemic. The secondary objective was to find out how people have been coping with isolation-induced stress and related factors during the same period.

Data on the effect of isolation and quarantine in terms of quality of life and coping strategies were collected through an online survey that could be completed by the respondents between the 21st of March and 20th of April 2020. During one month, 941 respondents answered the online survey, and their answers were analyzed after the first two weeks (n=103) and at the end (n=941) of the before-mentioned period. A comparative analysis of the responses from the two stages was also performed.

The survey was entitled "The psychological impact of the Covid-19 pandemic in the population subjected to self-isolation/quarantine" and consisted of 34 questions (Q1-Q34), with a completion time of 10-15 minutes. The answers of the people who completed 100% of the survey questions were analyzed (n=941).

QUALITY OF LIFE IMPAIRMENTS AND COPING STRATEGIES TO STRESS DURING THE COVID-19 PANDEMIC

Respondents were adults (81.5%), elderly (17.5%) and adolescents (1%), coming from urban (86%) or rural (14%) environment, both citizens from Romania (97.3%) and Romanian citizens residing in other countries (Great Britain, Germany, Austria, Canada, the Republic of Moldova, Tunisia, Belgium, the Czech Republic, Spain, Cyprus, Portugal, Italy and the USA, 2.7%). The respondents were female (81%) or male (19%), and they were married 54.7%, unmarried 23.7%, widowed 8.6%, or divorced 13%.

People who completed the questionnaire were living mostly in self-isolation for prophylactic purposes (68%), in quarantine (6.5%) or isolation (symptomatic) (3%), for one week (11, 5%), two weeks (8.5%) or more than 14 days (80%) (Figures 1 and 2).

The respondents were professionals from the medical staff (10.5%), police officers and auxiliary personnel (1.2%), workers in trade business with direct contact with potentially-infected people (5.3%), or were professionals involved in other high-risk categories of contracting Covid-19 (12.3%). Answers provided by respondents who did not fall in any of the previously mentioned professional categories represented 70.7%.

The educational background of the respondents was university (43.4%), postgraduate (24.6%), or high school (19.2%).

Survey respondents lived in the same home with children up to 7 years old (9%), children aged 8-14 (6.7%), adolescents (11%), elderly (over 65 years old) (12.7%), people with disabilities or other severe illnesses (2%), people undergoing treatment for various organic diseases (5.6%), people diagnosed with mental disorders and currently on specialized treatment (1.1%), or people who took care of other vulnerable individuals (3%). Among the respondents, 3% had mental disorders and were currently receiving treatment, while 13% had somatic disorders.

Figure 1: In which situation are you now? (Q2)

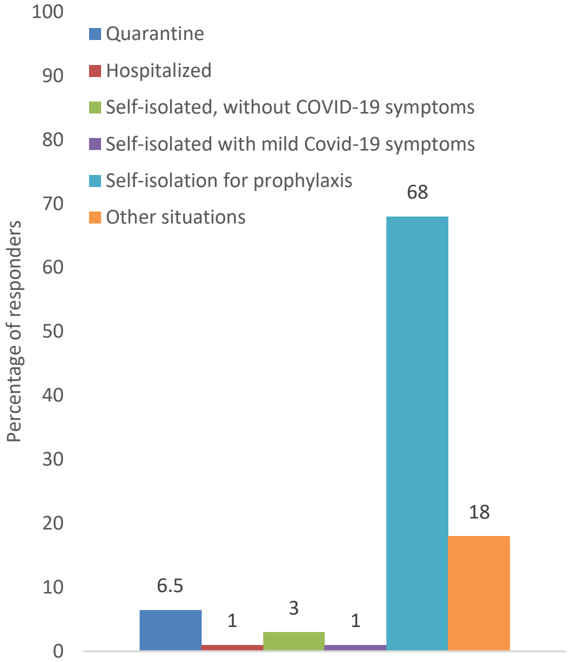

Figure 2: How long have you been in isolation or quarantine? (Q3)

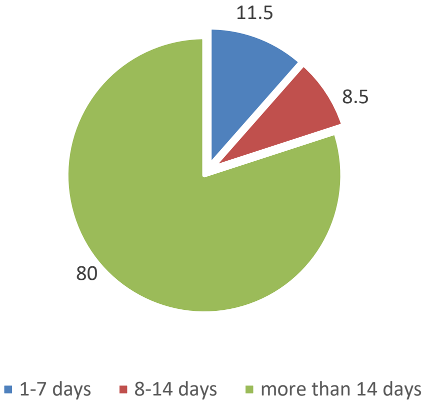

QUALITY OF LIFE IMPAIRMENTS DURING COVID-19 PANDEMIC- ASSOCIATED QUARANTINE AND ISOLATION

The level of stress induced by isolation or quarantine was felt differently by respondents, ranging from absent (10.6%) to extreme (7%) (Figure 3). Most of the respondents considered the level of stress associated with the quarantine or self-isolation regimen to be moderate (35.6%).

Communication is an important aspect of the quality of life in isolated populations, and changes in the communication patterns may have significant repercussions over people's social and psychological health. Our respondents continued to communicate with their relatives and friends by telephone calls (44.5%), through social networks using their smartphones (43.5%) or other electronic devices (laptop/ tablet/desktop) (10.1%) (Figure 4).

Figure 3: How do you evaluate the severity of the quarantine/ isolation-induced stress, from 1(absent) to 10 (extreme)? (Q11)

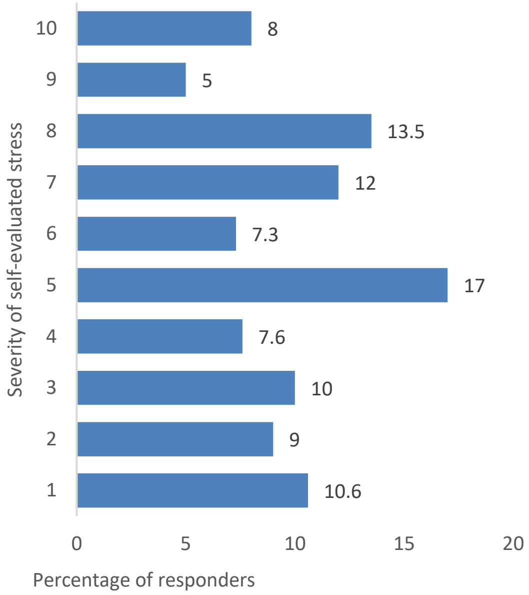

Inadequate information may negatively impact both mental health and quality of life, by raising the level of stress and fostering cognitive distortions. Most of the respondents collected data about the Covid-19 pandemic from the Internet (official sites) (57.5%), television (31.3%), Internet blogs and unofficial sites (5.3%), online or printed press (3.8%), and other sources (1.1%) (Figure 5). The majority of respondents stated they spent a maximum of one hour daily to find out news about the Covid-19 pandemic (71%), while 25% spent between one and 5 hours each day for the same purpose.

Regarding the most important concerns during the lockdown/quarantine that may influence the quality of life, our respondents mentioned primarily the concern about the health of those close to them (43.7%), then the concern related to their health (15.8%), followed by worries triggered by the family's financial security (12%), her or his financial future (7%), and his or her professional perspectives (4%). Other concerns reported by respondents were: the difficulties in running their own business, the current socio-political situation, the obligation to be vaccinated if a vaccine is to be discovered, the inability to continue daily routines they had before the pandemic, the restrictions applied to the freedom of movement, the restrictions applied to the access to religious services within the church, limitations to the professional/school activities, the collective mental health, the global economic crisis, the loneliness, the possible shortcomings in food production. About 13% of respondents did not mention any significant current concerns related to the Covid-19 pandemic that may relate to their quality of life.

Figure 4: How did you continue to communicate with your family, friends, neighbors during the isolation/quarantine? (Q13)

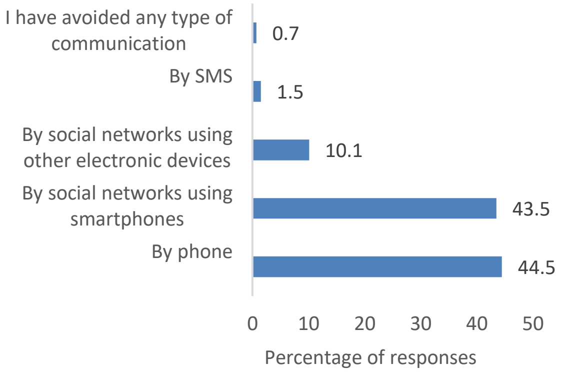

Figure 5: Where did you get your information about the CoViD-19 pandemic? (Q14)

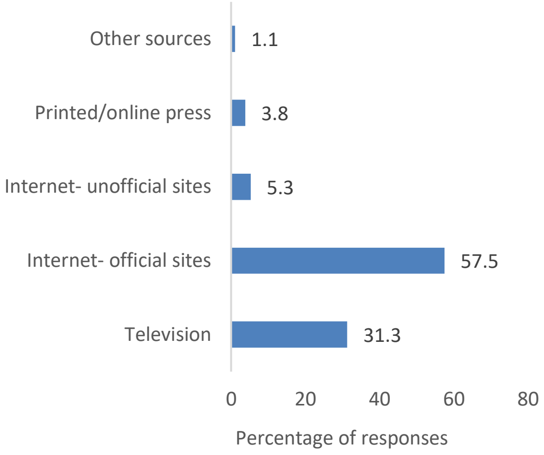

Financial aspects of quarantine and isolation have a significant potential to trigger changes in one's quality of life. During the period of self-isolation, 38.2% of people stated that they could work from home, without financial differences (20.8%), or with financial losses (17.4%), while

11% were in technical unemployment. It should be mentioned that 15.1% of the people stated they cannot work from home or receive financial support for this period, and a significant percentage of the respondents cannot work from home at all, because they have professions that cannot be done from distance.

Regarding the degree of optimism, respondents admitted a low (43%) or very low (35%) level, with only 20% recognizing a moderate level and 22% a high level of confidence in the possibility of solving the epidemiological crisis in the short-term (2-4 weeks) (Figure 6).

Figure 6: How do you rate your level of optimism regarding the possibility of solving the problem of the pandemic in a short time? (Q23)

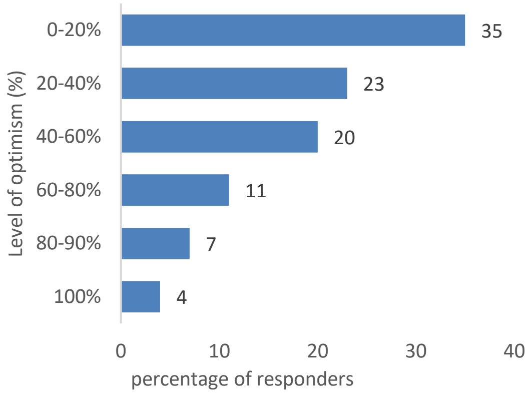

The way people look at their future is important for evaluating the quality of life in any population. Asked how they estimate their life will change as a consequence of the Covid-19-related (self)isolation/quarantine, the survey respondents said they expect to emerge stronger from this experience (47.7%), or that they will not suffer any medium or long-term negative impact of isolation (29.3%) (Figure 7). Only 10.1% of respondents consider that this experience will have a significant negative and permanent impact on their lives.

Regarding the appearance of psychopathological symptoms with functional impact during isolation, respondents mentioned insomnia (11.5%), nervousness/irritability (9%), unmotivated physical/mental fatigue (6.4%), panic (6%), catastrophic expectations related to pandemic (6%), increased appetite (4.5%), depressed mood (4%), decreased appetite (2%), crying spells (2.5%), ideas of uselessness or lack of self-worth (2.5%), concentration problems (2.2%), anhedonia (2.2%), feelings that life is meaningless (1.7%), drowsiness (1.7%), numbness in the hands or feet (1.6%), palpitations (1.6%), de-realization (1.3%), physical pain without an organic cause (1%), and many respondents admitted the presence of at least two of the listed symptoms. About 30% of respondents stated they did not experience any symptoms from the list presented.

Figure 7: What do you think that will change in your life as a result of isolation/quarantine? (Q29)

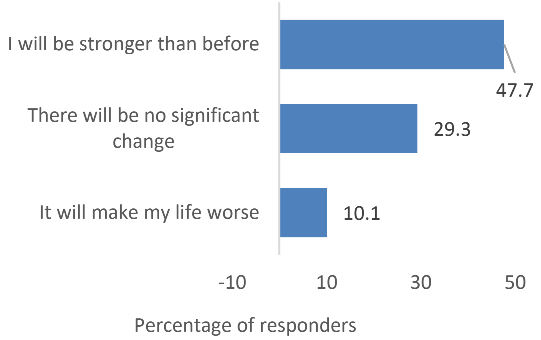

The communication with health services is another variable that may be correlated with the quality of life, especially during a world-scale epidemiological crisis. It should be noted that only 3.8% of the respondents sought the help of a mental health specialist during lockdown and quarantine. Of the people who went to the psychiatrist, 1% were already in treatment and adjustments of the current therapy were recommended, 1.2% were prescribed treatment for the first time, 0.5% did not receive any psychotropic treatment, being scheduled for a reassessment after the period of self-isolation/quarantine, 2.7% received a recommendation for online counseling/psychotherapy, and 40.1% did not receive a recommendation for any drug treatment or psychotherapy.

Of the patients who had medication recommended for any condition before self-isolation/quarantine, most of them stated that they were able to obtain the prescribed medications, but some people reported difficulties in procuring their usual medications (for hypothyroidism and diabetes, for example). Many respondents mentioned that they have appreciated the newly-launched system of electronic prescriptions sent by the physicians, without having them to travel to the doctors' office.

In terms of harmful behaviors with onset during the Covid-19-related self-isolation/quarantine, 23% of respondents ate more than before, 16% increased cigarette daily consumption, 9.6% resorted to excessive online shopping, which they later regretted, 5% took by themselves medication for sleep, and 4% increased alcohol use compared to their reference level. Other significant changes were abusive use of the Internet and changing in the sleep-wake rhythm, by advancing bedtime and waking up later than usual, and increased consumption of sweets. Changes in the physician-prescribed diet have also been reported, because people with dietary restrictions found harder on the market certain foods during this period. However, some respondents mentioned the appearance of certain favorable changes, stating that "I appreciate life more now", "I appreciate much more everything beautiful around me", "I treasure my health more", "I appreciate the freedom more”, “I have managed to quit smoking”, “I realized that I can adapt to more difficult situations without repercussions on my psychological status”.

COPING STRATEGIES DURING COVID-19- RELATED QUARANTINE AND ISOLATION

Interaction with other family members in self-isolation was the preferred method of coping with the situation (17%), followed in descending order by use of the Internet - social networks (13.4%), television – entertainment programs (12%), reading (11%), gardening (9%), Internet – entertainment (6%), Internet – online games (3.8%), television – news editions (3.6%), smoking (3.5%), sportive activities at home (3%), religious activities performed at home (3%), crosswords/sudoku/puzzles (2.6%), DIY (2.3%), Internet – educational programs (2%), board games with other self-isolated family members (1.6 %), Internet – shopping other than those of vital necessity (1%), television – cultural programs (1%) and alcohol consumption (0.75%).

When asked what they would advise other people placed in isolation to do while they are inside their homes, respondents said the best coping methods would be: structuring the daily program, in which to include activities as diverse as possible (e.g., "to arrange for the next day at least two activities that he/she likes"); to exploit the available time they have for doing things they have not been able to do so far because of the procrastination; to be patient, to keep calm and to follow the rules, to think about the fact that it is only a period that will pass; to do gymnastics or any kind of sport, in the house or outside; to adopt a pet; to get involved in religious activities that can be carried out at home; to avoid continuous contact with news programs (e.g., "to filter the information they receive very well", "to be informed about the current situation from the TV or other sources only if necessary"; "to watch the news only once daily”); to listen to cultural programs on the radio; to continue their professional activity at home, if possible; to discover new hobbies ("to discover something they will enjoy", "to do something creative"); gardening (e.g., "those who can move to a house with a garden should do it", "even if you are living in a flat you can take care of flowers, because flowerpots can be placed on the balcony"); to take advantage of free time and spend it with loved ones, to communicate with the family by phone/social networks (e.g., “those who have children should enjoy these moments, going to work usually robs us a lot of time that we should spent with our children”, “to communicate daily with the children and with the family by phone/Internet”); to reflect on the self (e.g., "to explore the self as much as possible using all the means at their disposal"), meditation, yoga; to clean the house in an organized way; to watch new movies with his/her family, but also to watch old movies, which he/she likes; crosswords, sudoku; Internet- games, social networks, entertainment (e.g., shows broadcasted online), personal development trainings, parenting, etc.; sewing, tapestry, embroidery, crocheting, knitting, tailoring; DIY; cooking (e.g., "to try to prepare new things in the kitchen, to explore..."); drawing, painting; music (e.g., "to listen to music daily, especially relaxation music"); reading (e.g., "to read some of the books we have long wanted to read"); development of skills that could be useful after the end of the pandemic- language courses, acquisition of professional qualifications or overspecializations, computer skills trainings (e.g., "to look for information related to a specialization in a new field"); to study what they wanted to study but failed due to lack of time; board games with other family members in the house; to keep a diary of this period; to avoid food excesses and to focus on a healthy diet; to build plans for the future (e.g., “to plan their activities in the post-isolation period, to take into account what they want to do, who they want to see”).

To the question "What do you not recommend to other self-isolated people to do?" the most common responses were: to avoid prolonged lying in bed during the day; to avoid binge-watching TV (e.g., "do not watch news programs for more than an hour daily") and to avoid data collected from unauthorized sources; avoid alcohol use and excessive smoking; do not do excessive shopping; do not isolate themselves emotionally; do not fall prey to monotony; do not consume excessively sweets and rich-calories foods in general; do not take medication without a doctor's recommendation; to avoid leaving the house if it is not necessary; to avoid excessive discussions on social networks about coronavirus; to avoid self-victimization and panic; do not abuse the Internet; to avoid online gambling; to avoid alcoholic drinks and to not increase the number of daily cigarettes.

Communication with people living in the same home was not significantly affected, according to the majority of respondents placed in isolation/quarantine. They stated that they enjoyed the time spent together, this time is an opportunity for mutual discovery or closeness. Such responses were, for example, “This isolation strengthened the connection between us, divergences faded, we mobilized to face a general threat together”, “We found, after a long time, the pleasure of time spent together; we have different stages of life but we have re-discovered common interests/opinions”, “I think this period has approached us”, “We had enough time to discuss everything we had not discussed so far”,“ Self-isolation facilitated communication between me and other family members, we had more time to spend together, a time we would not have allocated to ourselves if it were not for these extreme circumstances", "If it were not for the crisis, everyone would have had other things to do and we would have inadvertently avoided this approach".

There were however signals about monotony or increased nervousness - "Stress level is quite high, not everyone understands self-isolation measures", "This period increased our stress and made us feel trapped", "We became more irritable towards each other, because we spent too much time together”, "Because of the stress and anxiety there were more and more discussions and sometimes quarrels". There have also been situations when ignoring others has been reported as a method of coping with interpersonal stress.

The following reactions were reported during the communication with people (family, friends) outside the respondents' home: frustration due to lack of intimacy (“Emotionally, lack of contact with loved ones generates frustration”, “It is difficult not to have meetings, we can't enjoy the outdoors together", "I'm sorry I can't physically express my love for them", "I’m afraid we will become estranged"), worries about changing one's self and one’s relationships ("We became more lonely, more suspicious, more indifferent to each other”), concerns about the impact of one’s professional status over the family members (“I have to go to the hospital every day, being a doctor, and the family is worried about my health”), concerns about lower income that can affect family members, lack of food supplies on the markets, lack of enough anti-Covid-19 protective materials), reassessment of previous social experiences (“I miss my work colleagues, even those I did not value enough”). Some people tried to focus on the positive aspects ("We have more time to discuss, to talk about anything... For the first time I talked to my child for an hour without arguing", "It's hard not to see each other, but I know that's how we protect ourselves and others”,“ We communicate better”, “I think now I have time to talk to more people than before”).

Religion is seen as an important coping method by 47% of those in isolation or quarantine, even if participation in religious rituals was not allowed within the churches during the emergency state.

For people living with children in self-isolation/quarantine, the most commonly used methods of organizing children's time were encouraging online communication with classmates (7%), continuing school training (6.2%), board games (3.9%), encouraging participation in physical exercises (3.1%), unrestricted use of the Internet (“let them stay on the Internet as long as they want”) (2.8%), watching TV programs (“we do not limit access to watching TV anymore to a certain amount of time”) (0.8%).

Regarding the communication with children about the Covid-19 pandemic data, 32% of parents responded that they tell them what is happening and maintain constant communication with them about the pandemic, while 16% prefer to distract their attention from what happens through the use of games or other means. Almost 7% of the parents prefer to tell them that nothing bad is happening and that it is just a form of holiday, and only 2% said they forbid their children to come into contact with information sources about pandemic or do not answer children’s questions about the pandemic openly.

DYNAMIC INTERPRETATION OF THE QUALITY OF LIFE AND COPING STRATEGIES DURING THE QUARANTINE OR ISOLATION

Between March 21 and April 7, 2020, 103 respondents completed the online survey, and between April 8 and 20, 2020, the number of respondents reached 838. The interpretation of the answers must take into account the significant change in the demographic distribution of the participants, respectively an increase of the percentage of elderly people from 2% to 17.5%, with the corresponding decrease in the number of adults aged between 18 and 64 (95% vs. 81.5%), while the number of adolescents remained relatively constant (1% vs. 3%). Also, the marital status of the respondents was different, with an increase of the proportion of married (40% vs. 54.7%) and divorced people (3% vs. 13%), in parallel with a decrease in the number of unmarried people (55% vs. 23.7%). No major differences were observed in the geographical distribution of respondents (urban vs. rural, people located in Romania vs. Romanian citizens located in other countries). From the point of view of the educational background, a higher number of people with high school answered the questionnaire in the second stage of the survey, so that their proportion increased to 19.2% vs. to 9% in the first stage. Respondents who were not in those categories involving a professional risk of contracting Covid-19 increased from 56.5% to 70.7%, a difference that may be correlated with the change in the age of the respondents.

The people who completed the questionnaire were mostly self-isolating for prophylactic purposes both in the first half of the month and at the end of the one month, but the duration of the isolation regimen increased, as expected - the proportion of those who stayed in the house for more than 14 days was at the end 80% vs.11.7% at the half of the evaluation duration. The proportion of those who cared for children, adolescents, or the elderly was not significantly different in the two situations, as was the proportion of those who had mental or organic disorders in treatment.

The level of stress induced by isolation/quarantine did not change much over time, the proportion of those who consider this parameter to be moderate was 33% at two weeks and 35.6% after 4 weeks, those who considered it not significant were more numerous (6% vs. 10.6%), and the percentage of those who considered it unbearable also increased slightly (5% vs. 7%).

Interaction with other family members in self-isolation was the preferred method of dealing with the situation after two weeks and remained so at week 4 (23.5% and 17%, respectively).

Several coping strategies have increased their frequency of use – for example, use of the social networks (12% vs. 13.4%), watching entertainment programs, music, movies, television documentaries (10% vs. 13%), interest in gardening (4% vs. 9%), DIY (1% vs. 2.3%), smoking (3% vs. 3.5%), home sports/gymnastics (less than 1% vs. 3%), crosswords/sudoku (1% vs. 2.6%) and religious activities at home (less than 1% vs. 3%).

From the category of methods that have remained at the same level throughout the 4 weeks of the survey was the interest in reading (10% vs. 11%), sportive activities at home (8%), TV news (3% vs. 3.6%), Internet- educational programs (2%) and Internet- online shopping (1%). There was a slight decrease in the interest in Internet-entertainment and online games (13.5% vs. 9.8%), inboard games with other family members (3% vs. 1.6%), but also in the interest for alcoholic drinks (2% vs. 0.75%).

Respondents continued to communicate with those close to them by phone, through social networks on mobile phones or other electronic devices (laptop/tablet/desktop) in similar proportions in the two stages. Most respondents informed themselves about the Covid-19 pandemic from the Internet (official sites) (69.6% vs. 57.5%), but also unofficial sites or blogs (less than 1% vs. 5.3%). More respondents preferred to inform themselves from television at week 4 (22.5% vs. 31.3%), while the press remained at a constant level of interest (5% vs. 3.8%). Most of the respondents stated they have spent a maximum of one hour daily to find out data about the Covid-19 pandemic in a slightly increasing percentage (64.7% vs. 71%), while the number of those who spend between one and 5 hours each day for the same purpose decreased slightly (30.6% vs. 25%).

Asked what they would advise other people situated in isolation to do while they are inside their homes, the respondents offered the same categories of answers, namely those focused on the accuracy of information (using only official sources), spending more time with hobby activities, structuring of the daily time (including a list with various daily activities), caring for other family members with whom they live, communicating with friends/relatives outside the home by electronic devices, building plans for the future, developing new skills, adhering to the principles of healthy eating, practicing activities they have postponed due to lack of time, rediscovering themselves through specific activities (online personal development workshops, meditation, yoga). New recommendations have emerged regarding religious activities practiced at home, in the context of the Easter holidays.

To the question "What do you not recommend to other people in self-isolation to do?" the most common responses were similar in the two-time intervals assessed, namely avoiding long-term exposure to news programs, avoiding excessive eating, avoiding lack of activities during the day, avoiding leaving the house if it was not necessary, avoiding excessive discussions on social networks about coronavirus, avoid self-victimization, panic, and monotony, avoid alcohol use and excessive smoking. There were also recommendations to avoid prolonged bedtime during the day, excessive shopping, abuse of sweets, and high saturated fat foods, online gambling, and abuse of the Internet.

Communication with people situated in the same house was not significantly affected, according to the majority of respondents in self-isolation after two and 4 weeks, with a predominance of people who showed that they enjoyed the time spent together, this interval being considered an opportunity for mutual discovery or closeness.

During the communication with other people (family, friends) outside the respondents' homes, the following reactions were reported: frustration due to lack of privacy, concerns about the impact of professional status on the family’s general wellbeing, concerns about the lack of finance that may affect family members, reframing of the previous social experiences, but also equilibrium or even focusing on the positive aspects of the situation. Besides, concerns were raised about changing one's person and one’s relationships, which could be long-lasting, according to the respondents.

Regarding the most important concerns of this period, respondents mentioned especially the anxiety related to the potential health issues of the loved ones (67% vs. 43.1%, decreasing trend), then those related to their health (11.6% vs. 15.8%, increasing trend), followed by those fears triggered by financial uncertainty, professional or school prospects and the global political context.

During the period of self-isolation, 54% of people stated that they can work from home without financial differences (35% vs. 38.2%, slight increase), or with financial losses (14.5% vs. 17.4%, slight increase), while technical unemployment affected an increasing proportion of people (4% vs. 11%). It should be noted that 12% (after two weeks) and 15.1% (after 4 weeks) stated that they could not work from home or receive financial support for this period, and a significant percentage of respondents were not able to work from home at all due to the specifics of their jobs.

Regarding the level of optimism, respondents admitted a reduced level after 4 weeks comparative to the 2-week level (26% vs. 43%), only 20% admitted a moderate level, and 9% vs. 22% (significant increase) a high level of confidence in the short-term resolution (2-4 weeks) of the epidemiological crisis.

Religion was seen as an important resource for a growing proportion of people (33% vs. 47%) in self-isolation/ quarantine after 4 weeks comparative to the 2-week level.

For people living with children in self-isolation/quarantine, the most commonly used methods of organizing children's time were online communication with colleagues/teachers, board games, continuing school training program, then using the Internet, physical exercises, and watching TV. Regarding communication with children about Covid-19 pandemic data, a similar percentage of parents responded that they tell them what is happening and maintain constant communication with them about the pandemic (35% vs. 32%), while 17% vs. 16% prefer to distract them from what is happening outside by using games or other means.

Asked how they estimate they will be affected by self-isolation/quarantine, respondents said they expect to emerge stronger from this experience (55% vs. 47.7%, decreasing trend), or that they will not suffer any medium- or long-term negative impact of isolation (31% vs. 29.3%, relatively similar percentage).

Regarding the onset of psychopathological symptoms with functional impact during isolation, respondents mentioned insomnia (10% and 11%, respectively), followed by nervousness/irritability (10% vs. 9%) and anxiety/panic (5% vs. 6%, increasing trend), catastrophic expectations related to the pandemic (6%, increasing trend), unmotivated physical or mental fatigue (7% vs. 6.4%). Other reported symptoms were depressive mood (8% vs. 4%, decreasing trend), increased appetite (7% vs. 4.5%, decreasing trend), concentration problems (7% vs. 2.2%, decreasing trend), and several people have reported clusters of symptoms.

However, only 4.8% of the respondents sought the help of a mental health specialist during isolation/quarantine after two weeks and 3.8% after 4 weeks. Of the patients who had medication recommended for any condition before isolation/quarantine, most of them said they were able to purchase their prescribed medications.

During isolation/quarantine, harmful behaviors such as excessive online shopping decreased in time (13% vs. 9.6%), alcohol consumption compared to the previous level also decreased (7% vs. 4%), excessive smoking was increasingly reported (13.5% vs. 16%), and self-administered sedatives significantly increased (1% vs. 5%). Other important changes were the more intense use of the Internet and change in the sleep-wake rhythm by advancing the bedtime and waking up later than usual, and also an increase in the sweets consumption. After 4 weeks, changes in the structure of the diet were reported, people who had a diet recommended by the doctor stating that they can no longer buy those specific foods. After 4 weeks, some favorable changes were mentioned, such as raising awareness of the importance of life, health, and loved ones.

CONCLUSIONS AND RECOMMENDATIONS

The dynamic self-assessment of the stress level induced by isolation or quarantine in the context of the Covid-19 pandemic did not show significant changes between the analysis performed after two weeks and the one performed after 4 weeks. This stress level was very different from one respondent to another, being quite evenly distributed from absent to extreme, most often being chosen the average values. It should be noted that almost 50% of the respondents were caregivers for minors, elderly people, patients with organic or mental illnesses so that they were confronted with multiple stressors.

The most commonly reported coping methods used to deal with the stress of self-isolation were (1) direct communication with other family members with whom the respondents live, or by phone/Internet with loved ones who live outside their current place of isolation; (2) online entertainment (network games, movies, documentaries) or TV (movies, entertainment programs, sports); (3) reading,

(4) sports activities; (5) other – gardening, board games with other family members in isolation, TV news programs and educational programs (either TV or online); (6) keeping a diary, or planning daily activities for efficient time management; (7) personal development (online courses, meditation, yoga); (8) development of any professional skills that may offer an advantage on the labor market in the post-pandemic period (online language courses, computer programming workshops, overspecializations in one's field of activity, etc.).

It should be noted that the frequency of excessive smoking has increased after more than two weeks of isolation or quarantine, and this can be a dysfunctional coping method to deal with the isolation stress. Adequate measures of psychoeducation could be useful at the populational level for the prevention of excessive smoking, as well as dissemination of recommendations for adequate time management so that smoking can be replaced by relaxation methods free of harmful effects. Other dysfunctional coping methods were reported during self-isolation, namely, excessive online shopping, which was later regretted by the people concerned; compulsive eating, especially of sweets or other high-calorie foods; self-medication for sleep disorders; Internet abuse. In this regard, it should be noted that lockdown is a period during which behavioral addictions can develop unhindered if they are not actively fighting against - for example, the diet should be balanced to avoid the abusive use of sweets, between meals high-calorie foods are prohibited, prolonged sitting in front of the computer or TV should be considered harmful by people in isolation, and it is recommended to introduce active relaxation breaks between sessions of Internet or TV use; abusive online shopping or online gambling must be perceived as dangers and campaigned against; sleep medication should be taken only on prescriptions from a physician, strictly for the recommended duration.

The use of social networks and phone callings were used by respondents in relatively equal proportions to keep in touch with those close to them. The Internet was preferred as a source of information over television, but this is most likely the result of how respondents were selected (online platforms); the time spent daily informing about the Covid-19 pandemic has been reduced (over 70% estimated this time to be one hour), which indicates a realistic approach and avoidance of intoxication with data about the pandemic.

The main recommendations offered by the respondents for other people in isolation or quarantine were: (1) sports and relaxation activities (Pilates, yoga, gymnastics); (2) hobby activities (cooking, gardening, tapestry, drawing, dancing); (3) communication with loved ones; (4) caring for loved ones; (5) information only from official sources; (6) reframing of the current situation („you may consider that it is a holiday, a period of self-evaluation, of rediscovering your partner, of making plans for the future"); (7) developing new professional skills that may be useful in the future; (8) religious activities performed at home.

Contraindications that respondents considered important during self-isolation: (1) prolonged exposure to news programs; (2) self-blame and panic (it must be taken into account that the situation is limited in time anyway); (3) avoid excessive eating, smoking, and alcohol abuse; (4) avoid monotony by diversifying the daily program; (5) avoid unnecessarily leaving of one's own house; (6) avoiding lying in bed during the entire day; (7) avoid excessive consumption of sweets, online gambling and excessive use of the Internet.

Most of the people stated that there were no changes in communication with their loved ones who lived in the same house. However, from the category of those who felt such changes, most of them showed that they managed to enjoy the time spent together, using this period constructively and finding common interests and activities with their partner/children/other relatives in the house. The appearance of nervousness installed on the background of isolation-induced monotony was also reported, which leads to the need of implementing coping strategies based on the detection of common interests, on the practice of relaxation exercises, etc; ignoring one's partner or spouse was also signaled as a dysfunctional coping strategy, and in this case, we consider as optimal strategies the resignification of the situation, finding hobbies that several people from the same house may share, establish common plans for the future.

Communication with those outside the home was marked by (1) the feeling of lack of privacy which is commonly associated with physical, direct communication; (2) concerns about how the economic situation (technical unemployment, working from home with declining income, or even lack financial support) and social situation (separation from the group of colleagues, neighbors, friends) will affect oneself and his/her family; (3) emotional balance (acceptance of the current situation and associated changes of the communication paradigm); (4) capitalizing on current resources (using the time constructively, communication targeting the reconnection with old friends and relatives with whom they have not spoken for a long time, etc).

The respondents' main concerns were (1) worries related to the health of people close to them and their health; (2) financial security; (3) professional or academic future. Therefore, the officials should communicate more with the population about these issues and offer them adequate information about the pandemic-imposed crisis changes in the economy and academic activities.

The level of optimism regarding the resolution of the situation within a maximum of one month was reduced to 43% of the respondents, which shows a high level of awareness of the importance of the epidemiological situation.

Parents who live with their children at home have found as a means of organizing the children's time using board games within the home, the continuation of the school training program from home, then using the Internet for entertainment, online communication with colleagues and teachers, physical exercises and watching TV. The communication with the children regarding the Covid-19 pandemic data was good, in the sense that it was based on respecting the facts, with the concepts being formulated inappropriate terms.

The respondents did not expect self-isolation to change their long-term lifestyle, or even had favorable expectations, respectively to come out stronger from this experience, which shows a high level of confidence in their resources to cope and an effort to integrate this experience into the continuum of their life.

Psychopathological manifestations have been reported during self-isolation, in particular insomnia, nervousness/ irritability, anxiety/panic, unmotivated fatigue, depressed mood, appetite changes, concentration problems, and many people have shown symptomatic clusters, not just isolated symptoms. These elements had a recent onset and they can be largely attributed to the stress-induced by isolation or quarantine, given that only 3% of the survey respondents admitted to pre-existing mental disorders.

There was reported a reduced addressability to psychological or psychiatric services, while the use of one's methods of coping with symptoms increased in time (self-medication for sleep, coffee for drowsiness, alcohol for anxiety). Therefore, less than 4% of the respondents used the help of a mental health specialist during their isolation/ quarantine. These phenomena show not only that there is a risk of developing mental disorders in this period, especially anxiety and depressive disorders, which are to be expected in this period of isolation/quarantine, but also the risk that these disorders worsen rapidly in the absence of mental health-focused interventions. In this context, the possibility of accessing online medical platforms and psychological helplines dedicated to people going through difficult times should be disseminated into the population as an important means to prevent the worsening of stress-related disorders. Pharmacological treatment and psychotherapy should be used for anxiety, stress-related, and depressive-spectrum disorders, whenever needed [12]. Vulnerable populations to the isolation or quarantine-associated stress, represented by elderly patients, people with a psychiatric history or physical impairments should benefit from special attention from the mental health specialists, and special programs designed for the awareness of anxiety and depressive disorders should reach them through the media, general practitioners, or any official vector of information possible [13]. Depression is an important factor for lowering the quality of life and harms life expectancy so that it should be treated actively whenever detected [14, 15].

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VARIA

Redo procedure following Zenker`s diverticulum treatment – a clinical study

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Abstract: *Introduction: The prognosis of patients with Zenker`s diverticulum is dependent mainly on the type of procedure performed, either surgical or rigid/flexible endoscopy.*

Purpose - evaluation of the redo procedure by reviewing correlations in patients with iterative procedures.

Material and Methods: We included in the study 36 patients with cervicomediastinal Zenker diverticula treated from 2010 to 2017 at two university hospitals: Clinic of General and Esophageal Surgery. Mary Clinical Hospital Bucharest and Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca. For the patients included in the study we studied correlations of patients with symptoms persistence and to those with reintervention with epidemiological, clinical and the treatment criteria.

Results: Postprocedural symptoms we observed in 5 patients of the 29 treated endoscopically and 6 of the seven treated surgically. Statistical correlations were noted in patients with persistent symptoms after surgical treatment ($p < 0.01$) with patients with diverticulectomy (54.5%) ($p < 0.01$), grade III diverticula (36.4%) ($p < 0.01$) statistical association was noticed in patients who have required reoperation and those with persistent symptoms (63.6%) ($p < 0.01$) and with remaining dysphagia (100%) ($p < 0.01$) and those with grade 2 dysphagia persistence (71.4%) ($p < 0.01$), grade 3 diverticula (42.9%) ($p < 0.03$) and the high number of comorbidities. ($p < 0.04$)

Conclusion: Undoubtedly, endoscopic treatment is being used more and more even for those cases that require further treatment. Reoperation has been necessary for patients with persistent dysphagia with voluminous diverticula and a high number of comorbidities.

Keywords: *diverticulum Zenker, endoscopic diverticulostomy, diverticulectomy, iterative procedure*

INTRODUCTION

Zenker`s diverticulum is a cause of morbidity in elderly patients. According to van Overbeek's theory, the predominant role in diverticulum formation has an anatomical predisposition represented by Killian dehiscence weakness. [1] The externally surgical approach has long been the conventional treatment with satisfactory clinical success rates ranging from 80-100%. [2]

The prognosis for these patients depends on the surgical procedure performed. Some authors stated that diverticulectomy patients have a long-term symptom recurrence rate of 2% to 33%. [3] Studies have been published since 1981, which noted that surgical resection or suspension of the pouch performed without a concomitant

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myotomy fails to improve dysphagia and fail to prevent complications or recurrence of the diverticulum, a fundamental consideration of the endoscopic techniques developed later. [4]

Some authors found that patients with endoscopic stapler technique, have a long-term symptom recurrence rate ranging from 0 to 47%. Most authors consider that the reasons for symptomatology after the endosurgical approach is the incompatibility of the dimensions of the septum with the length of the stapler (3 cm). A small diverticulum limits access to the tip of the stapler and it may not be possible to perform a proper myotomy. [5]

Data from the literature indicate a recurrence rate for laser endoscopic diverticulostomy of about 5-25%. [6, 7]

Flexible endoscopic therapy is a relatively new approach compared to rigid endoscopic and surgical techniques. Data from the literature on flexible endoscopic therapy show that symptomatic improvement was observed at 85%, with a symptom recurrence rate of 18-20%. [8-11]

The higher recurrence rate after flexible endoscopic treatment could be caused by incomplete septal dissection. Some authors have shown that the success of the treatment correlates with the length of the septotomy. The risk of perforation by over dividing muscular fibers and difficult identification of the last fibers of the cricopharyngeal muscle could make the endoscopist to leave a residual part of the septum to avoid perforation into the mediastinum. [12]

MATERIAL AND METHODS

We included in the study group 36 patients with Zenker diverticula treated during 2010/2017 in two university hospitals: Clinic of General and Esophageal Surgery Sfanta Maria Bucuresti and Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca.

Inclusion criteria: patients with Zenker diverticulum admitted and treated endoscopically or by open surgery in the hospital during 2010/2017, over 18 years of age, investigated with barium swallow and upper GI endoscopy, patients with a follow-up period of at least 12 months post-procedure.

Exclusion criteria: immunocompromised patients, patients with associated malignant disease, patients lost from post-procedure follow-up.

All patients in the group were investigated by clinical examination, imaging with a barium swallow, upper endoscopy and functional tests with esophageal manometry when this exploration was possible.

Dysphagia was a constant symptom at presentation to the hospital, as well as the main symptom followed in the evaluation of the treatment efficiency. For the assessment of this symptom we used Mellow-Pinkas classification of dysphagia applied to the type of food that cannot be swallowed - grade 0 - No dysphagia, grade 1 - Dysphagia for solids, grade 2 - Dysphagia for semisolid, grade 3 - Dysphagia for solids and liquids grade 4 - Total dysphagia (inability to swallow saliva).

The sizes of the diverticula were appreciated both in centimeters and in degrees according to the Overbeeck classification by corroborating data obtained from the endoscopic exploration and the appearance of the barium contrast imaging. 1 – the small sac that has less than 1 vertebral body in length; 2 –intermediate sac 1-3 vertebral bodies in length; 3 – large sac greater than 3 vertebral bodies in length.

For all the patients surgically treated by cervicotomy, the resection of the diverticulum was performed with myotomy and manual esophageal suture, either single or double layer, or mechanically using a linear stapler. For the patients treated endoscopically, myotomy was performed with creating a wide communication of the diverticulum with the esophagus without resection of the diverticular pouch. A soft, flexible overtube (Cook Medical, Indiana, USA) was used to expose the septum, which was placed over the endoscope, with the short blade placed in the diverticulum and the long blade in the esophagus. After the septum was exposed, it was divided on the midline, from the esophageal lumen to the diverticular pouch, on an average length of 1.5 cm using a Dual knife (Olympus Co., Japan) followed by the closure of the mucosa with clips (2 -5 clips). All patients were clinically and imaged monitored at 3-month intervals during the first post-procedural year.

Study design

For the patients included in the study we studied correlations of patients with symptoms persistence, and to those with reintervention with epidemiological, clinical and the treatment criteria.

Statistical analysis

In comparison with the quantitative data, by the normality of the data, Student's t-test (for two groups with normally distributed data) and Mann-Whitney and Kruskal-Wallis (for data without normal distribution) were used. For the categorical data (synthesized as frequencies and percentages), Fisher exact tests (binary data) and Pearson

Chi-square were used as well as Likelihood Ratio (if 20% of expected frequencies were less than 5). The probability of error of less than 5% ($p < 0.05$) was considered as a significance threshold. The paper also included graphic representations, such as stratified bar type for data synthesized as frequencies and percentages.

RESULTS

Patients included were 29 patients treated endoscopically by myotomy in the Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca and 7 patients surgically treated by resection of the diverticulum associated with myotomy. In the Clinic of General and Esophageal Surgery of St. Mary's Hospital Bucharest. The intraprocedural complications were laminar hemorrhage in three patients during the endoscopic procedure, and one surgically treated patient developed a postoperative hematoma that required drainage. Post-procedural mortality was 0%, and morbidity was 13.88% - five patients (3 patients after endoscopic treatment and 2 patients after surgical treatment).

Persistent postinterventional symptomatology was reported by 11 patients and consisted of the persistence of dysphagia of a lower degree associated or not with the persistence of other symptoms: sialorrhea, regurgitation, nausea, and cough.

We did not observe associations regarding the correlation of the persistence of post-procedural symptomatology with the patients' sex, initial symptomatology or with diverticulitis. (Table 1)

Table 1: Correlations of post-intervention symptomatology - statistical processing of data

	Post opSymptoms = Yes (N=11)	**Post opSymptoms= No (N=25)**	**P_value (test)**
Sex=M	7/11 (63.6%)	14/25 (56.0%)	0.728773 (Fisher's Exact Test)
Grade			
1	0/11(0.0%)	2/25 (8.0%)	**0.063384** (Likelihood Ratio)
2	5/11 (45.5%)	15/25 (60.0%)	
3	2/11 (18.2%)	7/25 (28.0%)	
4	4/11 (36.4%)	1/25 (4.0%)	
No Symptoms			
1	3/11 (27.3%)	8/25 (32.0%)	0.266965 (Likelihood Ratio)
2	1/11 (9.1%)	4/25 (16.0%)	
3	4/11 (36.4%)	11/25 (44.0%)	
4	1/11 (9.1%)	2/25 (8.0%)	
5	2/11 (18.2%)	0/25 (0.0%)	
Proceduresurgicalendoscopic			
	6/11 (54.5%)	**1/25 (4.0%)**	**0.001423** (Fisher's Exact Test)
	5/11 (45.5%)	**24/25 (96.0%)**	
Pouch resection			
Yes	**6/11 (54.5%)**	**1/25 (4.0%)**	**0.001423** (Fisher's Exact Test)
Div Dimensions			
1	2/11 (18.2%)	12/25 (48.0%)	**0.003085** (Likelihood Ratio)
2	5/11 (45.5%)	13/25 (52.3%)	
3	**4/11 (36.4%)**	**0/25 (0.0%)**	
Dysphagia post op			
Yes	**10/11 (90.9%)**	**1/25 (4.0%)**	**0.000000** (Fisher's Exact Test)
Dysphagia Grade			
0	**1/11 (9.1%)**	24/25 (96.0%)	**0.000000** (Likelihood Ratio)
1	**4/11 (36.4%)**	1/25 (4.0%)	
2	6/11 (54.5%)	0/25 (0.0%)	
Hospitalisation			
	7.55±5.5563	2.56±1.5832	**0.005955** (Mann-Whitney Test)
	8.00 [2.00, 11.00]	**2.00 [2.00, 3.00]**	

Post-procedural symptomatology we observed only in five patients out of the 29 endoscopically treated and in six out of the seven surgically treated. The statistical association was observed for the type of intervention practiced – endoscopic or surgical treatment (p <0.01) and the persistence of postintervention symptomatology (endoscopic treatment = 45.5%) compared to those without postinterventional symptomatology (endoscopic treatment = 96.0%) – 96% of patients who did not report persistent symptoms had endoscopic treatment. (Table 1) (Figure 1)

Figure 1: Distribution of patients according to the presence of the post-intervention symptomatology to the surgical or endoscopic procedure type

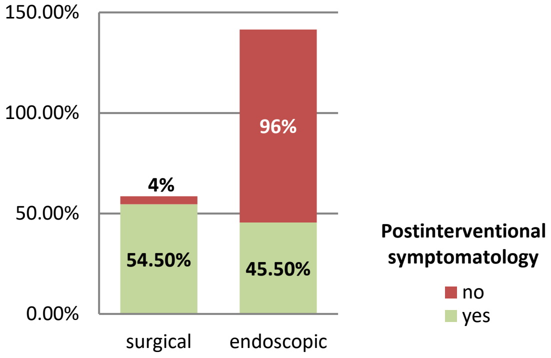

Resistant post-procedural symptoms may be influenced by the type of technique performed: resection of the diverticular pouch or just opening, and association of the myotomy or not. For the patients in the group, the resection of the diverticulum was performed only in the patients treated with open surgery.

The statistical association we observed comparing the percentage of resected Zenker's in patients with persistent postinterventional symptomatology (54.5%) compared to those without postinterventional symptomatology (4.0%) (p <0.01). (Table 1)

Of the 11 patients with postinterventional symptomatology, four patients had grade three diverticula. The correlation of the grade of the diverticulum with the persistence of the post-procedural symptomatology determined a statistical association to the dimension appreciated in degrees according to the Overbeeck classification.

The statistical association was found by correlating the persistence of postintervention symptomatology in patients with grade III diverticula (36.4%) compared with those without postinterventional symptomatology (0.0%) (p <0.01) (Table 1) (Figure 2)

Figure 2: Distribution of patients with post-intervention symptomatology to the dimensional degree of the diverticulum.

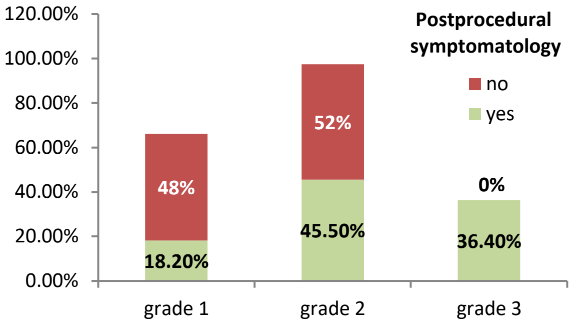

In the post-procedural symptomatology dysphagia was the dominant symptom of 10/11 patients – 4 patients had dysphagia grade 1 and 6 patients dysphagia grade 2. The statistical association was observed between the percentage of patients with delayed dysphagia (p <0.01) and those with postinterventional symptomatology (90.9 %) compared to those without postinterventional symptomatology (4.0%). (Table 1) Persistent postinterventional symptomatology was mainly dysphagia.

Post-procedural persistent dysphagia was reported by 11 patients – 5 patients after surgical treatment and 6 after endoscopic treatment, 10 patients had a lower degree of dysphagia than at the initial presentation. Of the 5 patients surgically treated for 3 dysphagia persisted in the first postoperative month and did not require treatment, for the 2 patients with grade 2 dysphagia, one required re-intervention for a hematoma, and another one with esophageal fistula required adequate surgical treatment. We observed a statistical association between persistent dysphagia and surgical treatment (p <0.03), probably due to the small number of patients treated surgically. (Table 2) (Figure 3)

Figure 3: Distribution of patients according to the persistence of postintervention dysphagia to the surgical or endoscopic procedure type.

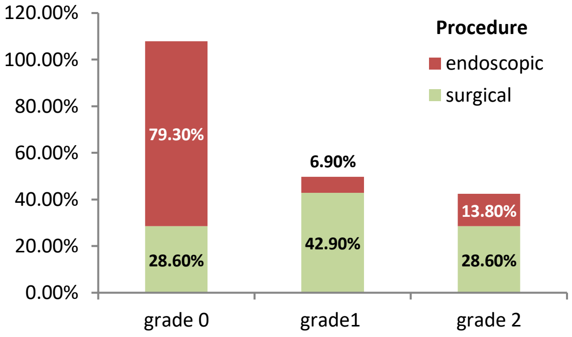

Tabel 2: Statistical correlation for redo procedure - statistical data analysis.

	Redo=Yes (N=7)	**Redo=No (N=29)**	**P_value (test)**
Sex=M	4/7 (57.1%)	17/29 (58.6%)	1.000000 (Fisher's Exact Test)
Grade			
1	0/7 (0.0%)	2/29 (6.9%)	0.328714 (Likelihood Ratio)
2	5/7 (71.4%)	15/29 (51.7%)	
3	2/7 (28.6%)	7/29 (24.1%)	
4	0/7 (0.0%)	5/29 (17.2%)	
No Symptoms			
1	3/7 (42.9%)	8/29 (27.6%)	0.633852 (Likelihood Ratio)
2	1/7 (14.3%)	4/29 (13.8%)	
3	3/7 (42.9%)	12/29 (41.4%)	
4	0/7 (0.0%)	3/29 (10.3%)	
5	0/7 (0.0%)	2/29 (6.9%)	
Hospitalisation			
	6.29±6.7506 **3.00 [2.00, 14.00]**	3.55±2.9469 **2.00 [2.00, 3.50]**	0.360412 (Mann-Whitney Test)
Persistent dysphagia			
yes	**7/7 (100%)**	**4/29 (13.8%)**	**0.000040** (Fisher's Exact Test)
Persistent Dysphagia grade			
0	**0/7 (0.0%)**	**25/29 (86.2%)**	**0.000009** (Likelihood Ratio)
1	2/7 (28.6%)	3/29 (10.3%)	
2	**5/7 (71.4%)**	**1/29 (3.4%)**	
Sialorrhea			
Yes	2/7 (28.6%)	2/29 (6.9%)	0.162567 (Fisher's Exact Test)
Size			
	3.86±1.2149 4.00 [3.00, 5.00]	**2.93±1.2516** 3.00 [2.00, 3.50]	**0.086359** (Student T)
Size classif.			
1	1/7 (14.3%)	13/29 (44.8%)	**0.023010** (Likelihood Ratio)
2	3/7 (42.9%)	15/29 (51.7%)	
3	**3/7 (42.9%)**	**1/29 (3.4%)**	
Procedure			
surgical	2/7 (28.6%)	5/29 (17.2%)	0.601669 (Fisher's Exact Test)
endoscopic	5/7 (71.4%)	24/29 (82.8%)	
Miotomy			
Yes	7/7 (100%)	28/29 (96.6%)	1.000000 (Fisher's Exact Test)
Pouch resection			
Yes	2/7 (28.6%)	5/29 (17.2%)	0.601669 (Fisher's Exact Test)
Intra op morbidity			
	1/7 (14.3%)	2/29 (6.9%)	0.488235 (Fisher's Exact Test)
Comorbidity			
yes/no	7/7 (100%)	21/29 (72.4%)	0.309298 (Fisher's Exact Test)
Comorbidities – No			
0	0/7 (0.0%)	8/29 (27.6%)	**0.033327** (Likelihood Ratio)
1	3/7 (42.9%)	5/29 (17.2%)	
2	4/7 (57.1%)	7/29 (24.1%)	
3	0/7 (0.0%)	8/29 (27.6%)	
4	0/7 (0.0%)	1/29 (3.4%)	

HTN	4/7 (57.1%)	15/29 (51.7%)	1.000000 (Fisher's Exact Test)
DM	0/7 (0.0%)	5/29 (17.2%)	0.558991 (Fisher's Exact Test)
Dyslipidemia	2/7 (28.6%)	12/29 (41.4%)	0.681030 (Fisher's Exact Test)
Other diverticula	1/7 (14.3%)	3/29 (10.3%)	1.000000 (Fisher's Exact Test)

Post-procedural sialorrhea was reported by four patients. It was not present as a unique symptom but it was always associated with persistent dysphagia. Post-procedural sialorrhea persisted in four patients.

Reintervention was necessary when the persistence of post-procedural symptoms was recorded for 7/11 patients. For the 5 patients treated endoscopically, who required reintervention for the persistence of the symptomatology, an average interval of 22.8 months was observed, while the reinterventions for the 2 patients treated by open surgery happened during the postoperative period. The statistical association was observed in patients who needed reintervention and those with postinterventional symptomatology (63.6%) ($p < 0.01$) (Table 1)

DISCUSSIONS

When comparing the technical and clinical results from the literature on the treatment of Zenker diverticulum, it should be critically emphasized that a direct comparison between studies and results may be inadequate, as the data are neither homogeneous nor standardized in regards of multiple variables. Noteworthy in this regard: symptom collection (dysphagia, dysphagia plus regurgitation, cumulative symptoms), symptom evaluation (objective scores for dysphagia, subjective grading of symptom relief and satisfaction), choice of one or another technique (sequentially, size of the diverticulum, clinical conditions, local policy), the definition of clinical success (total disappearance of symptoms, disappearance with improvement), success and extent of recurrences (after one or more sessions) and variable duration of follow-up (the shortest follow-up programs being reported after endoscopy) flexible, compared to the more recent series of patients treated transorally and with a historical cohort of patients treated surgically).

Patient follow-up included history taking, clinical examination, imaging investigations. Clinical criteria followed were persistence or recurrence of dysphagia and sialorrhea as with other similar studies. [13]

In patients in our group, we found the efficiency of endoscopic treatment of 96% and the whole group of 80%. Most cases published since 1995 demonstrate the efficacy and safety of flexible endoscopy, with clinical success rates between 56% to 100%. [14-15]

The success rate lower than 56% reported in a single series [10] is most likely because the clinical remission was evaluated according to the presence or absence of a group of symptoms and not only dysphagia.

The rate of recurrent symptoms was significantly higher in the surgical group than in the endoscopic group, results different from those reported in other studies where a 39% recurrence rate was found after endoscopic treatment and 0% recurrence in the patients who suffered surgery, probably due to the small number of patients treated surgically. [13]

In the studied group, the persistent postinterventional symptomatology was mainly represented by dysphagia, which is why we consider that in other studies, the efficacy of the treatment should be reported according to the improvement of this symptom. Data from the literature report clinical success increases to 84-100% when the unique series in which success is defined according to dysphagia is taken into account. [9, 11, 15]

As in our study and some other studies, treatment efficacy was reported after a single treatment session, [10, 16] while in other series the success rate was reported after several treatment sessions. [9, 17]

Unlike rigid staple endoscopy, the flexible septotomy technique for the Zenker diverticulum is neither univocal nor standardized. As already mentioned, different cutting techniques can be variably combined with different accessories. The optimal dividing technique is unknown because prospective randomized studies are lacking, and the choice is mainly based on the endoscopist's personal experience and preferences.

In evaluating the success rate of surgery, the most important factor is the subjective evaluation of the swallowing function performed by the patient. Both the primary and revision indications are guided by the patient's symptoms. [18]

Following rigid endoscopic or surgical treatment, persistent septum or residual pouch may still be present on barium contrast examination. Persistent symptoms may be due to other swallowing abnormalities and/or inappropriate myotomy, without correlating the size of the residual pouch and the recurrence of the symptoms. There is a consensus in the literature that the evaluation of treatment efficacy and the need for further treatment must be determined by the patient's symptoms [19, 20] - which we agree with.

Some authors concluded that the presence of a residual diverticulum on an early postoperative esophagogram does not warrant early surgical revision, even when dysphagia persists. In many cases, there may be a slow process of gradual fibrosis of residual dysfunctional cricopharyngeal muscles or atrophy of the remaining diverticular sac over time. In addition, a normal postoperative esophagogram probably cannot predict a good and persistent outcome.

Some authors have reported that only 12.5% of patients with remaining diverticula on the postoperative contrast check develop a recurrence of Zenker's diverticulum. [18]

The approach of redo interventions has changed over time. If in older studies, there is a high percentage of recurrent surgical treatment, [21, 22] we are currently attending an endoscopic revision intervention, and in our study was noted that on a case of redo was necessary to use an endoscopic method after open resection of the Zenker diverticulum for the treatment of dysphagia in case of postoperative stenosis.

Other authors point out the need for classical intervention to resect the residual sac, which, even after a complete myotomy, can be a reason for the persistence of food retention mechanism. This may be the cause of symptoms in patients with prolonged retention of the contrast substance in the pharyngeal pouch and difficult passage into the esophagus, even after complete cricopharyngeal myotomy. [2, 3]

Other authors also suggest that an incomplete cricopharyngeal myotomy may result in higher recurrence rates associated with flexible endoscopy. Ideally, the diverticulum should be reduced to <1 cm. [24]

Incomplete cricopharyngeal myotomy may be visible on radiographs as a cricopharyngeal impression. Hypopharyngeal dysfunction during swallowing of the contrast substance may indirectly suggest incomplete myotomy or recurrent laryngeal nerve injury. On radiographs with contrast substance, pharyngeal dysfunction is suspected if we see contrast substance in the larynx or the tracheobronchial tree. Some authors consider that the persistence of the septum rather characterizes the endoscopic technique and does not predict the failure of the treatment, especially during the immediate postoperative period. [25]

Other authors have tried to evaluate the efficiency of the procedure through manometric studies. Ozgursoy and Salassa reported that a large diverticulum can cause obstruction of the esophageal passage and can lead to high pressure, even if the tone of the upper esophageal sphincter remains normal at manometry. Such a theory leads to the hypothesis that the weak wall of the hypopharynx may play an important role in the development of the diverticulum and leaving the weak adynamic segment of the esophagus creates a favorable environment for recurrence. In addition, the creation of a large cavity after diverticulostomy favors endoscopic techniques over open diverticulectomy on manometric evaluation, because, in a large cavity, small pressures are difficult to record, even if part of the septum remains and this causes dysphagia. [26, 27]

Some studies have shown that post-procedural soluble contrast studies do not correlate with symptoms following endoscopic therapy or relapses and that the second radiological examination, three months after surgery, presents the final functional results, with which we also agree. [8, 25] We also carried out new post-procedural or post-operative radiological studies to exclude a fistula on the seventh postoperative day and in the post-procedural monitoring of patients. Maybe a more accurate assessment of the contrast substance study could be done 3 months after the procedure.

For the patients in the group, we found that the duration of hospitalization in patients with postinterventional symptomatology and those without postinterventional symptomatology was significantly different. The average duration of hospitalization was 2 days for patients without post-procedural morbidity and 7 days for those with post-procedural morbidity comparable to the data in the literature. In our study, the duration of hospitalization of patients with open diverticulectomy was comparable with the data in the literature. [28-30]

The persistence of dysphagia in the 11 patients included in the group usually required reintervention; most of the patients with persistent symptomatology required a reintervention of the same type. Similar studies have also been reported in the literature, most patients who failed the procedure also underwent endoscopic reintervention. [31]

In the patients in the group with bulky diverticula of degree III or larger than 4 cm, the most frequent post-procedural reinterventions were required, 3 out of 4 patients, aspects encountered in other studies. Partial septotomy in the diverticula greater than 5 cm has been proposed by some surgeons, even if the myotomy fails to completely remove the septum and may require more endoscopic interventions. [16, 32, 33]

According to recent literature, some authors recommend the endoscopic procedure for revision surgery as well. The great advantage compared to the open approach is that the endoscopic revision treatment is technically feasible and relatively easy, not more difficult than the primary endoscopic procedure, without an increase in risk. Furthermore, patient satisfaction was higher in those who underwent endoscopic revision surgery compared to those who underwent open surgery revision. [6, 18, 34]

Repeated procedures, in case of therapeutic failure or symptomatic relapse, can be easily and successfully performed by flexible or rigid endoscopy. An additional advantage is that repeated procedures for persistent or recurrent symptoms can be successfully achieved through a transoral (rigid or flexible) approach, while an open approach can be a major technical challenge. [5]

The data from the literature show recurrence rates of about 6% (range 0-22%), with the average recurrence rate of 5% compared to the classical approach, and according to data obtained in our study. [35]

The comorbidities of patients with diverticula included in the group also played a role in the emergence of the situations that required reintervention. [36]

Some authors consider that endoscopic findings tend to decrease over time and that incomplete septotomy is a significant factor for failure rate [37-40], an aspect we have also found.

CONCLUSIONS

Most of the time redo procedures were of the same type, the endoscopic iterative treatment being much easier than the surgical iterative treatment. Undoubtedly, endoscopic treatment has gained more and more confidence even in the field of revision interventions, considering that persistence or recurrence of dysphagia has as substrate an incomplete division of the septum requiring the same type of procedure. Redo procedures were necessary for patients with persistent dysphagia, bulky diverticula, and patients with a high number of comorbidities.

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ORIGINAL ARTICLES

Regression model for predicting the severity of acute poisoning cases by estimating PSS in the Emergency Department

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Abstract: *Acute poisonings represent an emergency pathology that associates an increased risk of unfavorable outcomes or death. The mortality rate can be improved by the fast recognition of its severity, which in turn allows a prompt intervention of the medical team. In the practical approach of the case, a standardized measure of predicting the patient's evolution would be necessary, which could be applied quickly to the patient's bed and easy to calculate and apply irrespective of the evaluator. Currently, for acute poisoning cases, Poisoning Severity Score (PSS) is used, a complex and dependable tool that accurately stages the severity of the case but has the disadvantage of being quantified retrospectively. This study presents the development and validation of a linear regression model that can be applied right in the emergency department (ED) and predicts the severity of the case by estimating PSS with an accuracy of 75%. The proposed model uses ten objective and quantifiable variables representing anamnestic, clinical, and biological parameters evaluated in the early stages of the poisoning. The regression was developed in the study group consisted of 62 pediatric patients diagnosed with severe acute poisoning with cardiotoxic agents complicated by cardiogenic shock.*

Keywords: *PSS, regression model, acute poisoning, cardiotoxicity*

INTRODUCTION

Acute poisoning is a global health problem, so preventing mortality is essential in intentional and unintentional exposures. Therefore, predicting the evolution of acutely poisoned patients is of clinical importance because it allows a prompt and adequate therapeutic intervention [1,2].

Like other medical specialties, Toxicology also benefits from classifying the severity of its characteristic pathology, for acute poisoning being used Poisoning Severity Score (PSS). It can be applied to both adults and children, regardless of the type and number of etiological agents involved. For classification, it is necessary to know the complete evolution of the case, considering the most severe clinical and/or paraclinical features recorded during hospitalization. For this reason, the score is quantified retrospectively after the patient's discharge or death. The classification only considers the directly observed signs and symptoms without estimating risks based on parameters such as the ingested dose or the serum/plasma level of the toxicant [2,3].

Predictive scores specific to toxicologic pathology that can be applied in an emergency case are rarely developed, even less so in pediatric patients. Emergency physicians typically use standard scores such as APACHE II/IV (Acute Physiology and Chronic Health Evaluation), SAPS II (Simplified Acute Physiology Score) or SOFA (Sepsis-Related Organ Failure Related). However, these scores do not evaluate the particularities of the clinical examination in a patient with acute poisoning or the characteristics of the toxicant [4].

The exhaustive evaluation that PSS performs using 12 categories of criteria, depending on the systems affected by

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the poisoning, makes it valuable and reliable. However, some studies signal some of its disadvantages [2]. Many variables analyzed, some of which have subjective evaluations such as "mild hypotension" or "rare/frequent seizures", make it difficult to quantify, time-consuming, and not consistently reproducible from one evaluator to another [5].

At admission, the examination of the case with acute poisoning may be within normal limits or discreetly modified, yet it may degrade in evolution to the point of requiring advanced therapeutic measures. Therefore it becomes necessary to identify criteria that raise the suspicion of the risk of unfavorable evolution since admission so that the medical team can make the best decisions in handling the case [6].

The importance of PSS for research is undeniable. However, in terms of clinical efficacy, a severity score should quickly assess the prognosis, establish the need for hospitalization and the department's rank where to be cared for, and especially be quickly calculated at the patient's bedside [7].

Considering that PSS is a tool for retrospective assessment of case severity, the premise of this study was to identify those features evaluated from the patient's initial examination that correlates with its subsequent evolution. The study aimed to develop a statistical model that predicts the severity of the case by quantifying these indicators from the emergency department.

METHODS

Study design and selection of study patients

This study is a retrospective analysis of a sample of patients recruited from the Department of Toxicology of the "Grigore Alexandrescu" Emergency Children's Hospital Bucharest. There were analyzed cases of acute poisoning hospitalized between 2014 - 2020, amounting to 7666 patients. Have been researched clinical observation files of the poisoned patients and identified those where the etiologic agent was cardiotoxic, so with a potential evolution to cardiogenic shock, obtaining a group of 1396 patients. After a thorough analysis of the patients at risk, have been selected those that have shown from admission or in evolution signs of cardiogenic shock, obtaining a batch of 62 severe cases.

Each of the 62 pediatric patients enrolled in the study group presented at least one of the following diagnostic criteria for cardiogenic shock: weakly or absent pulse in the major arteries, capillary recoloration time over 2 seconds, low blood pressure, cold, cyanotic or marbled extremities, decrease of diuresis below 1 ml/kg/hour or altered mental status [8,9].

Data analysis protocol

Base data for this research has been collected from the clinical observation files and included the baseline characteristics of patients with poisoning: age and sex; poisoning-related variables, such as the intent of poisoning, type of substance, route of exposure; time-related factors, such as time-lapsed between exposure and examination; and initial vital signs at the ED like heart rate (HR), systolic blood pressure (SBP), body temperature (BT), respiration rate (RR); and AVPU (A-alert, V-verbal response, P-pain response, U-unresponsive) scale of mental status (10,16,17). The collected data also contained admission medical tests and outcome-related variables, such as mortality at ED or after admission.

The work plan included:

(1) Assessment of statistically significant associations between variables: anamnestic, clinical and paraclinical indices, etiology, gender, length of hospitalization, development of complications.

(2) Verifying if the chosen variables can develop a regression model.

(3) Determining the coefficients of the regression model and its validation.

Statistical analysis

The data obtained from the analysis of the clinical cases in the study group were evaluated using the IBM SPSS v20 program, and the results were represented as graphs and tables to identify regression coefficients. The conditions for carrying out a linear regression model were assessed by verifying the existence of gross errors between the recorded values, also if the chosen variables are independent (Durbin-Watson statistical test) or correlated (Pearson test), respectively if there is a statistically significant link between input and output variables (ANOVA test). After all conditions were met, a linear regression model was developed to quantify the relationship between the independent and dependent variables.

RESULTS AND DISCUSSION

PSS is a standardized measure of the acute poisoning severity and is the main objective of the regression model. The usefulness of this model is that it estimates this score at admission, distributing patients into five categories according to the PSS classification.

Of all the variables recorded in the patients' database, ten were selected for having the highest potential to influence the prediction of the PSS [11]. These are intentional poisoning, ECG changes, systolic blood pressure (SBP), bradycardia (according to ages groups), coma, time-elapsed between ingestion and ED examination, transfer from another hospital, etiology represented by pesticides, etiology represented by a combination of beta-blocker with calcium channel blocker, the value of base excess. The values of all the selected variables are recorded in the emergency department after the initial examination [11,12].

In order to verify the model, the batch of 62 selected patients was divided into two samples. One, the derivation group, will be used to make the model, and the other, the validation group, to test the developed model. The number of patients in each group was determined based on Yamane's relationship:

$$n = \frac{N}{1 + N \cdot e^2}$$

where N - the number of patients in the group (N = 62), and e - the chosen marginal error (e = 5%) [13].

Based on the above formula, the group used to develop the model comprises 54 patients, and the rest will constitute the validation group. The eight patients in the test group were chosen at random and had the identification number: 28, 41, 43, 47, 49, 52, 54 and 62.

The development of the regression model was performed in two distinct phases. In the first phase, it is checked if the chosen variables can constitute a linear regression model, while in the second one, the values of the model coefficients are determined.

The following hypotheses were further verified [13]:

If the chosen variables contain gross errors

For this, the linear residues (errors) were determined and checked if their distribution was normal. As can be seen from Table 1, Standard Residuals fall in the range [-3, 3], so it can be concluded that the values used are not affected by gross errors.

Table 1: Determination of linear errors to identify gross errors (Residuals Statistics)

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	2.11	3.46	2.71	0.356	38
Residual	-0.880	1.072	0.000	0.439	38
Std. Predicted Value	-1.686	2.119	0.000	1.000	38
Std. Residual	-1.713	2.085	0.000	0.854	38

[1] If the variables are independent.

This hypothesis was verified based on the Durbin-Watson statistical test (Table 2), which imposes a maximum limit of 3 for independent variables. In our case, the value of the Durbin-Watson coefficient is 2.011.

Table 2: Calculation of the Durbin - Watson coefficient for the selected variables (Model Summary)

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.730	0.797	0.173	0.514	2.011

(2) If the variables are correlated

For this, the Pearson coefficients between the independent and the dependent variable were calculated, and because the value of these coefficients does not exceed the threshold of 0.59, it was concluded that there is no individual statistical correlation.

(3) If there is a statistical link between the independent and the dependent variables

This hypothesis was verified based on the ANOVA test, which produced a coefficient p <0.05 (Table 3), meaning that the statistical significance of the proposed regression model is significant. Therefore there is a statistically significant link between the independent and the dependent variables.

Table 3: ANOVA test applied for the chosen variables

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	4.685	10	0.469	1.774	0.015
Residual	7.131	27	0.264		
Total	11.816	37			

Because all the hypotheses were fulfilled, a linear regression model was made in the second phase, and the coefficients related to this model were determined (Table 4). In this case, all the coefficients are statistically significant for the model (the value of p <0.05) and have the values from the table.

The regression model developed is represented by equation below:

$$\text{PSS \_estimated} = \text{IP} \cdot 0.402 + \text{ECG} \cdot 0.097 - \text{SBP} \cdot 0.004 + \text{Coma} \cdot 0.495 + \text{Brad} \cdot 0.146 + \text{Texp} \cdot 0.001 - \text{Referral} \cdot 0.086 + \text{BE} \cdot 0.019 + \text{Pes} \cdot 0.499 + \text{BBCC} \cdot 0.565 + 2.407 \quad (1)$$

where: IP – intentional poisoning (0/1), ECG – ECG changes at presentation (0/1), SBS – systolic blood pressure at presentation (mmHg value), coma – coma at presentation (0/1), Brad – Bradycardia (0/1), Texp – time between exposure and examination (minutes), Referral (0/1), BE –

base excess at presentation (value mmol / l), Pes – Pesticides (0/1), BBBC – beta-blocker and Ca channel blocker (0/1), constant = 2.407.

Table 4: Determination of the coefficients related to the linear regression model

Variable	Linear regression model coefficients		Standardized coefficients	t	Sig.
	B	Standard Error	Beta		
Constant	2.407	0.414		5.809	0.000
Intentional poisoning	0.402	0.237	0.335	1.699	0.001
ECG modified	0.097	0.245	0.083	0.396	0.006
SBP (mmHg)	-0.004	0.005	-0.196	-0.777	0.044
Coma	0.495	0.276	0.344	1.791	0.035
Bradycardia	0.146	0.200	0.128	0.732	0.041
Time-lapse between exposure and examination (min)	0.001	0.000	0.234	1.433	0.013
Referred patient	-0.086	0.206	-0.076	-0.420	0.048
Pesticide	0.499	0.296	0.302	1.683	0.004
Base excess	0.019	0.032	0.118	0.590	0.050
Beta-blocker and Ca channel blocker combination	0.565	0.304	0.342	1.857	0.047

In order to avoid an additional phase of interpreting the results and obtain a quick estimate of the prognosis, in the development of the model, it was considered that the value range of the estimated PSS variable should be identical to that of the standard PSS variable. This fact led to the appearance of subunit coefficients with three decimals and, therefore, a complex function. We consider that this compromise is acceptable to keep the easy interpretation of the data. The practical calculation can be performed with the help of the Excel program.

Figure 1: Visual representation of the regression model

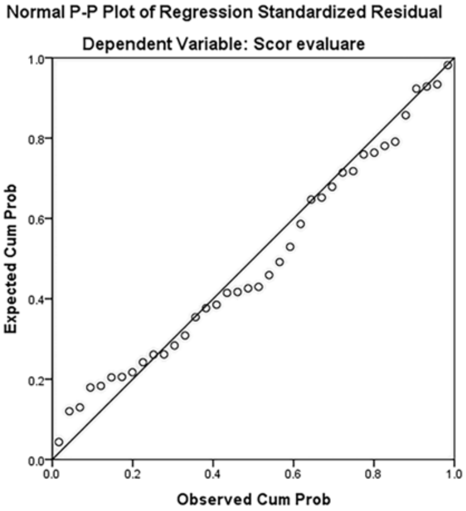

°: *PSS registered at the patient's discharge or death (training data)*

—: *PSS estimated using the proposed regression model*

Their similar appearance can be noticed by overlapping the estimated PSS graph and the registered PSS graph (Figure 1).

The validation of the model was done starting from the degree of correlation between the independent and dependent variables. Thus, based on the values in Table 5 below, we conclude:

- there is a high degree of correlation between the independent and dependent variables R = 0.730.
- 79.7% (R2 = 0.797) from the variation of the dependent variable is generated by the independent variables chosen in the model. The remaining 20.3% is influenced by other variables that could not be identified in the present study.

Table 5: Checking the correlation between the independent and the dependent variable

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.730	0.797	0.173	0.514	2.011

Further, to have a quantitative evaluation of the developed model, equation 1 was applied to the sample of eight patients in the test group. Their input data are summarized in Table 6.

Following the application of the regression model developed, in 6 of the 8 cases tested, PSS was accurately predicted (Table 7). This results in a success rate of 75%. It can be concluded that the developed model successfully predicts PSS, impacting choosing the optimal therapy for each patient [14].

Table 6: Input data of the 8 patients in the test group

ID	IP	ECG	SBP	Coma	Bradycardia	T_{exp}	Referral	Pesticide	BE	BBBC
28	0	0	74	0	0	40	1	0	-4.6	0
41	0	1	95	0	0	120	1	0	1	0
43	1	1	120	0	1	30	1	0	-2	0
47	0	0	90	0	0	300	1	1	-10	0
49	1	1	115	0	1	1440	0	0	1.4	0
52	0	0	100	0	0	60	0	0	-2	1
54	0	1	100	1	0	180	1	0	-1.1	0
62	1	1	147	0	0	120	0	0	11	0

Table 7: The results obtained in the test group following the application of the regression model

Estimated PSS	Rounded PSS	Recorded PSS	PSS differences
1.9416	2	2	0
2.069	2	2	0
2.451	3	3	0
2.3	2	4	2
2.7626	3	2	1
2.54	3	3	0
2.5101	3	3	0
2.539	3	3	0

Due to the subunit coefficients of the identified variables, the application of the model could be time-consuming and prone to errors. The alternative is represented by a multicriteria analysis derived from the proposed regression model, which would reduce the number of variables used and create a faster calculation template for estimating the PSS. However, its accuracy would decrease, being a compromise between the complexity of the regression model and precision [15].

There are numerous studies in the literature, but most of them performed on the adult population, which propose various scores to predict the severity or mortality of cases of acute poisoning. A Korean team (Han et al.) published a recent article proposing a new score for predicting poisoned patients mortality in the ED [10]. The developed template uses a hybrid approach where the model coefficients are divided into categories, and their value is standardized to facilitate the total calculation. However, the model requires an additional phase of interpreting the results and, in addition, only estimates the risk of mortality without making assessments about the cases' evolution.

CONCLUSIONS

The present study aimed and succeeded in developing a linear regression model that uses both toxicant-related characteristics and patients' physiological features in the emergency department, in order to predict Poisoning Severity Score with an accuracy of 75%, in the early stages of acute poisoning with cardiotoxic agents complicated by cardiogenic shock.

The proposed regression model can be the basis of multicriteria analysis, much easier and faster to apply by the examining physician, and which should be a valuable tool for predicting the patient's outcome in the emergency department.

Although the present study was carried on a small sample of patients, the applied methodology has the advantage of a smooth transition to a larger group. The accuracy of the model can be further improved by the increase of the training lot.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

All authors had an equal contribution to the conception and design of the study and also to the acquisition, analysis and interpretation of data.

Ethics approval and consent to participate

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital, Grigore Alexandrescu Children's Emergency Hospital Bucharest. The clinical observation sheets were analyzed following the medical legislation in force.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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ORIGINAL ARTICLES

Relationship between pregnancy and sense of taste

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Abstract: *Background: The change in taste is common for pregnant women. This study aimed to assess the sense of taste in pregnant women in comparison with non-pregnant women.*

Methods: A cohort study was conducted on 120 healthy women. The test group (n=60) included pregnant women presenting to a hospital for pregnancy care in their first, second and third trimesters. Control group (n=60) included healthy non-pregnant women presenting to the same center. The groups were matched in terms of age, marital status and oral hygiene. Assessment of sense of taste was done for two groups using sucrose, sodium chloride and citric acid solutions for perception of sweetness, saltiness and sourness. Serial dilutions of sucrose and sodium chloride solutions were prepared in 1.0, 0.1 and 0.01mM concentrations. Serial dilutions of citric acid were prepared in 3.2, 0.32 and 0.032M concentrations. Data were analyzed using SPSS version 17 and chi square test.

Results: Forty-four non-pregnant (73.3%) and 26 pregnant (43.3%) women gave wrong answer to 0.01mM sucrose solution and this difference wasn't statistically significant (p=0.07). One individual in each group (1.7%) gave wrong answer to 0.1 and 1mM concentration of sucrose. This difference wasn't statistically significant (P=0.9). Wrong answers in the two groups and among the three trimesters weren't significantly different for sodium chloride and citric acid (P=0.9). The difference among pregnant women in the three trimesters wasn't significant for any concentration or tested solution (P=0.9).

Conclusion: The sweet taste threshold decreases during pregnancy but the perception threshold for saltiness and sourness remains unchanged.

Keywords: *pregnancy; taste; taste threshold*

INTRODUCTION

Change in the sense of taste is a common complaint of pregnant women and can result in alterations in their nutritional regimen [1]. Nutrition of mother during pregnancy is a critical factor in health of mother and fetus. Poor quality of nutrition during pregnancy can lead to nutritional deficiencies, weight gain, and increased risk of pregnancy diabetes, high blood pressure and problems during delivery [1]. The pregnant women in their second trimester have higher sugar cravings [2]. The changes in sense of taste have a relatively high prevalence in pregnant women (26%) and most pregnant women in their study showed increased sensitivity to bitterness and decreased sensitivity to saltiness [3]. The sense of taste changes during pregnancy especially in the first trimester [4]. Change in sense of taste can serve as a protective mechanism since pregnant women have less craving for bitter foods, which often contain toxic agents and by doing so, the fetus is protected against toxic nutrients [5].

The mechanism of change in sense of taste during pregnancy has yet to be fully understood. However, hormonal alterations as the cause of changes in taste during pregnancy [6]. Changes in nervous system are also a suggested mechanism for change in sense of taste during pregnancy [7].

The studies on changes in sense of taste during pregnancy have yielded controversial results and discussed that this controversy might be attributed to different methodologies

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of studies and methods of assessment [8]. Also, change in sense of taste may be variable in different trimesters and it has been shown that alternations in sense of taste have the highest prevalence in the first trimester and decrease later in the course of pregnancy. The sense of taste returns to normal after delivery [3]. This study aimed to compare the sense of taste in pregnant women in different trimesters in comparison with non-pregnant women in the Mirzakoochak Khan Hospital, Tehran, Iran.

METHODS

Research design

This historical cohort study was conducted at the Gynecology and Obstetrician Department of Mirzakoochak Khan Hospital Tehran, Iran.

Research subjects

A total of 120 pregnant and non-pregnant women (60 pregnant and 60 non-pregnant women) were included into the study. The sample size was calculated based on a pilot study conducted on 10 pregnant and 10 non-pregnant women; the difference in percentage of wrong answers between the two groups was 20%. Considering the confidence interval of 90% and study power of 80%, sample size was calculated to be 60 in each group (a total of 120 in the two groups) [9].

Pregnant women comprised the test group and were selected among those presenting to Gynecology and Obstetrician Department of Mirzakoochak Khan Hospital for routine pregnancy care. Pregnancy of these patients had been confirmed by urine and blood tests.

The test group (n=60) included pregnant women presenting to a hospital for pregnancy care in their first, second and third trimesters.

The control group included non-pregnant women who were selected among those presenting to the same hospital for routine checkup and matched the test group in terms of age, marital status, oral hygiene and use of prosthetic or orthodontic appliances. The inclusion criteria were absence of systemic diseases, no history of sensory disturbances in the oral cavity, no smoking, no drug intake (except for vitamin supplements), no history of radiotherapy or chemotherapy, absence of mouth dryness [10] and absence of nausea and vomiting of pregnancy.

Oral hygiene measurements

To assess oral hygiene of participants, the Turesky-Gilmore-Glickman modification of Quigley Hein index was used [11]. Disclosing tablets were used to quantify plaque accumulated on buccal and lingual surfaces of teeth. Calculated plaque index ≤2 indicated good oral hygiene and plaque index >2 indicated poor oral hygiene.

Taste perception test

The sense of taste in participants was assessed according to the method described by Saluja et al. [12]. Three serial dilutions (1/2 log concentration) of sucrose, sodium chloride and citric acid solutions were prepared to assess the perception of sweetness, saltiness and sourness. Sodium chloride and sucrose solutions were serially diluted to obtain 1.0, 0.1 and 0.01mM concentrations. Citric acid was serially diluted to obtain 3.2, 0.32 and 0.032M concentrations. The solutions were diluted using distilled water. Participants were requested to refrain from eating and drinking for one hour before testing. They were asked to rinse their mouth 10 seconds prior to testing. Next, they were requested to taste 5mL of each solution for 10 seconds and then spit it out (starting from the lowest concentration). Participants were asked to rinse their mouth in-between tastings. Each test took 5-10 minutes in each individual and a minimum interval of 30 seconds was allowed between tests. Minimum concentration of each solution perceived by each participant was recorded. Participants were classified for their taste perception into two groups of correct and incorrect taste perception [12].

Assessment of taste perception was carried out between 9-11 a.m. every morning in a relaxed position under the same conditions for all patients.

Statistical analysis

Data were analyzed using SPSS version 17 (SPSS Inc., IL, USA) and the data were compared between the test and control groups using chi square test. A $p<0.05$ was considered statistically significant. Because the study was a qualitative research based answer of pregnant women, the Chi-square should be used for statistically significantly results.

Ethical clearance

Both test and control group subjects were thoroughly informed about the study and signed informed consent forms prior to participation in the study. Participants were ensured about the confidentiality of their information. The study protocol was approved in the Ethics Committee Of Dental Branch, Islamic Azad University (code: IR.IAU.DENTAL.REC.1395, 22).

RESULTS

A total of 120 participants including 60 pregnant and 60 non-pregnant women were evaluated. The mean age was

25.1±2.6 years in non-pregnant and 26.4±3.3 years in pregnant women. The two groups were not significantly different in terms of age (P>0.05). In terms of oral hygiene, in the group of non-pregnant women, 48 had well and 12 had poor oral hygiene. In pregnant women, 50 had well and 10 had poor oral hygiene. The difference in this regard between the two groups was not significant (P>0.05).

Table 1 shows the frequency of correct and incorrect taste perceptions in the two groups based on the concentration of sucrose solution tested. As seen in Table 1, in the group of non-pregnant women, 44 (73.3%) had wrong taste perception for 0.01mM concentration of sucrose; this rate was 43.3% (n=26) in pregnant women. According to chi square test, this difference between the two groups was statistically significant (P<0.05). One participant in each group (1.7%) had wrong taste perception for 0.1mM concentration of sucrose and the two groups were not significantly different in this respect (P=0.9). One participant in each group (1.7%) had wrong taste perception for 1.0 mM concentration of sucrose and the two groups were not significantly different in this respect (P=0.9).

Table 1: The frequency distribution of taste perception of participants in the two groups based on the tested concentration of sucrose

Group/Sucrose concentration (mM)	0.01		0.1		1	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
Non-pregnant	16 (26.7%)	44 (73.3%)	59 (93.3%)	1 (1.7%)	59 (93.3%)	1 (1.7%)
Pregnant	34 (56.7%)	26 (43.3%)	59 (93.3%)	1 (1.7%)	59 (93.3%)	1 (1.7%)
P value	P<0.05		P=0.9		P=0.9	

Table 2 shows the frequency distribution of participants in terms of taste perception of different concentrations of sodium chloride. As seen in Table 2, taste perception of different concentrations of sodium chloride was not significantly different between the two groups of pregnant and non-pregnant women (P=0.9).

Table 2: The frequency distribution of taste perception of participants in the two groups based on the tested concentration of sodium chloride

Group/Sodium chloride concentration (mM)	0.01		0.1		1	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
Non-pregnant	14 (23.3%)	46 (76.7%)	59 (93.3%)	1 (1.7%)	59 (93.3%)	1 (1.7%)
Pregnant	15 (25%)	45 (75%)	58 (96.6%)	2 (3.4%)	58 (96.6%)	2 (3.4%)
P value	P=0.9		P=0.9		P=0.9	

Table 3 shows the frequency distribution of participants in terms of taste perception of different concentrations of citric acid. As demonstrated in Table 3, taste perception of different concentrations of citric acid was not significantly different between the two groups of pregnant and non-pregnant women (P=0.9).

Table 3: The frequency distribution of taste perception of participants in the two groups based on the tested concentration of citric acid

Group/Citric acid concentration (mM)	0.01		0.1		1	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
Non-pregnant	1 (1.7%)	59 (93.3%)	59 (93.3%)	1 (1.7%)	60 (100%)	0
Pregnant	0	60 (100%)	59 (93.3%)	1 (1.7%)	59 (93.3%)	1 (1.7%)
P value	P=0.9		P=0.9		P=0.9	

Comparison of taste perception of women in the three trimesters for citric acid, sucrose, and sodium chloride showed no significant differences (P>0.05, Table 4).

DISCUSSION

The change in taste is common for pregnant women. This study aimed to assess the sense of taste in pregnant women in comparison with non-pregnant women. Forty-four non-pregnant (73.3%) and 26 pregnant (43.3%) women gave wrong answer to 0.01mM sucrose solution and this difference wasn't statistically significant (p=0.07). One individual in each group (1.7%) gave wrong answer to 0.1 and 1mM concentration of sucrose. This difference wasn't statistically significant (P=0.9). Wrong answers in the two groups and among the three trimesters weren't significantly different for sodium chloride and citric acid (P=0.9). The difference among pregnant women in the three trimesters wasn't significant for any concentration or tested solution

(P=0.9).

Table 4: The frequency distribution of pregnant women with correct taste perception of sucrose, sodium chloride and citric acid in the three trimester

Trimester/Solution	Sucrose			Sodium chloride			Citric acid		
	1	2	3	1	2	3	1	2	3
First trimester (n=20)	9	11	0	5	15	0	0	20	0
Second trimester (n=20)	13	6	1	6	13	1	0	19	1
Third trimester (n=20)	12	8	0	4	15	1	0	20	0
P value	P=0.9								

Proper and healthy nutrition plays a key role in a successful pregnancy [12]. Increased appetite is a common finding during pregnancy [14,15]. However, change in sense of taste and nutritional problems have been commonly reported by pregnant women [16]. This study compared the sense of taste of pregnant and non-pregnant women using different concentrations of sucrose, sodium chloride and citric acid solutions. These solutions were chosen for this study since they have no adverse effect on the mother and fetus.

Electrogustometric evidence shows that taste threshold increases with age. In other words, aging decreases the sense of taste [17]. These changes often commence at the age of 60 years in areas innervated by the chorda tympani and glossopharyngeal nerves and at the age of 70 years in areas innervated by the greater petrosal nerve [17]. Since the current study was conducted on 20 to 30 year-old women, the effect of age on sense of taste was not significant.

The current results revealed that the taste threshold of sucrose (sweetness) in pregnant women was lower than that in non-pregnant women. Similarly, the reduction in taste threshold of all tastes including sweetness in pregnant women [18]. Saluja et al, [12] also reported deceased taste threshold for sucrose in pregnant women. This change in taste has been attributed to hormonal changes during pregnancy. The changes in taste threshold of women during menstruation cycle and showed hyper-sensitivity to taste prior to ovulation and decreased sensitivity to taste after ovulation [19]. Since the sense of taste was found to be constant in males, such alternations in sense of taste especially for sweetness in women were attributed to female sex hormones and their alterations. They explained that increased sensitivity to taste prior to ovulation is probably attributed to high level of estrogen and decreased sensitivity to taste after ovulation is probably attributed to a combined function of estrogen and progesterone.

Our study only found a significant difference in taste of sweetness between pregnant and non-pregnant women while Kolbe et al. [20] reported higher taste threshold for all four main tastes in pregnant women. Also, Kuga et al. [4] reported increased taste threshold in pregnant women particularly in the first trimester. Difference between their results and ours may be explained by racial and ethnic differences between populations and different methodologies of the studies since Kuga et al. [4] did not perform an actual testing on participants and only questioned the participants verbally. The accuracy and reliability of this method (self-report) is somehow questionable. The method used by Kolbe et al. [20] was different from that in our study. Also, use of different concentrations of solutions can yield different results.

We did not find any significant difference between pregnant and non-pregnant women in terms of tasting saltiness, which was in line with the results of Saluja et al [12]. They reported that pregnancy does not affect on taste perception of saltiness. However, our results in this regard were in contrast to those of Brown and Toma [21], Hayes et al, [6] and Ochsenbein-Kölbleet al [18] since the afore-mentioned studies reported decreased taste threshold or higher craving for saltiness during pregnancy. These differences in the results of studies may be attributed to the use of different solutions with variable concentrations.

In our study, the difference between the two groups in terms of perception of sourness was not significant, which was in agreement with the results of Saluja et al. [12] who also reported that taste perception for sourness does not change during pregnancy. However, the some studies indicated that craving for sourness was different between non-pregnant women and those in the first, second and third trimesters of pregnancy. Also, Kolbe et al. [20] and Kuga et al. [4] indicated a change in perception of sourness in pregnant women. Racial, ethnic and geographical differences can all affect the nutritional habits of individuals and their perception of tastes such as sourness. Also, individual and genetic differences (variations in chemical receptors) can affect the sense of taste [22, 23]. It appears that each individual has their own gustation perception, and nutritional habits of individuals can also affect their taste threshold.

In general, in a healthy state, gestation of pregnant women is altered in a such a way to protect the health of mother and fetus. Changes in sense of smell of pregnant women also protect them against harmful chemicals. Changes in sense of taste during pregnancy enable consumption of a wider range of electrolytes and nutrients [20]. These changes include increased sensitivity to bitterness in the first trimester as a protective mechanism against toxins and increased craving for salty, sweet and sour foods in the second and third trimesters in order for the mother to consume a wide variety of nutrients required for the health of fetus [6]. Several physiological, psychological and social factors control nutritional habits of individuals. Psychological, physiological and behavioral factors can also affect the nutritional choice and consumption of food by pregnant women.

Several factors including oral hygiene, smoking, alcohol use, drug intake, systemic diseases and history of chemotherapy and radiotherapy may affect the sense of taste of individuals. Thus, in this study, the two groups were matched in terms of oral hygiene, and the other afore-mentioned factors were set as our exclusion criteria in order to eliminate their confounding effect on the results. This was strength of our study and has not been done in previous studies. One limitation of our study was that taste of bitterness was not evaluated. Quinine hydrochloride is often used for assessment of taste perception of bitterness. However, due to possible adverse effects of this agent on the fetus, we did not use it in our study. Future studies with larger sample sizes are required to confirm the results of this study.

CONCLUSION

The results of this study showed that the taste threshold of sweetness decreased during pregnancy but no difference was detected in taste threshold of saltiness or sourness between pregnant and non-pregnant women.

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ORIGINAL ARTICLES

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Renal angiomyolipoma – a short review of the literature

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Abstract: *Renal angiomyolipomas (AML) are the most common benign renal tumors. By containing an important amount of fat tissue they are easily identifiable on CT and MRI images, thus no biopsy should be needed for a diagnosis. However there is a small percentage of AMLs that have very little fat tissue and are very difficult to differentiate from renal cell carcinoma. Along fat tissue, they also contain smooth muscle and blood vessels. Renal AMLs can occur as a sporadic finding, or more frequently in association with tuberous sclerosis complex (TSC) or pulmonary lymphangioleiomyomatosis (LAM). These tumors are usually an incidental finding on imaging tools, but sometimes a serious presentation is rupture and hemorrhage, which can easily evolve to shock. Therapeutical management vary from active surveillance to surgical removal, and it needs to be individualized for every patient and according to the clinical presentation.*

Keywords: *renal angiomyolipoma, tuberous sclerosis, therapeutical management*

INTRODUCTION

Renal AMLs are the most common benign renal tumors, having a reported prevalence of 0.2-0.6%, with a female to male ratio in favor of females. Because of the increase use of imaging tools and the advances in imaging methods the frequency of detection in the general population is increasing. AMLs are more frequently associated with patients with tuberous sclerosis complex (TSC), having been reported in 75-85% of cases with renal lesions and in 49-60% in TSC patients overall [1-2]. In cases of lymphangioleiomyomatosis (LAM), aproximately 45-60% of patients tend to have renal AMLs, usually being multiple and bilateral [3-4]. Even though the prevalence of AMLs is low among patients who do not have TSC or LAM, combined with the fact that the prevalance of sporadic AML exceeds the estimated birth incidence of TSC or LAM, it makes it more common [5]. AMLs are a member of a group of tumors called neoplasms with perivascular epithelioid differentiation, arising by clonal proliferation of epithelioid cells distributed around blood vessels [6, 7].

Histological findings

Renal AMLs are made of smooth-muscle-like cells, adipocyte-like cells, and epithelioid cells, with the three types of cells appearing to be derived from pericytes, fact suggested by the fact that these cells express perycyte markers [8].

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AMLs are classified in two major histological groups. The most common histological variant is the classic one, having abnormally thick-walled vessels that lack a well-developed internal elastic lamina and varying amounts of spindle smooth-muscle-like cells and adipose tissue. However, some tumors consist solely of one component, while the other elements are present in very small amounts. In the classic variant epitheliod cells are rare, making up less than 10% of cells in this type of AML [9]. Classic AMLs are bening but, there are cases with local invasion into the perirenal fat, and in very rare ocasions into the collecting system, renal vein, or inferior vena cava and right atrium. The epithelioid variant, unlike the classic one can undergo malignant transformation. There is no consensus on the percentage of epithelioid cells that is required to make a diagnosis of the epithelioid variant, but studies sugest that a percent higher than 70% of epithelioid cells, a tumor size >7 cm, vascular invasion, ≥2 mitotic figures per 10 high-power fields, atypical mitotic figures and necrosis suggest an increased risk for malignant transformation [10].

Epithelioid cells are differentiated by their abundant eosinophilic and granular cytoplasm.

Epithelioid AML has less fat tissue than the classic variant, making it more difficult to diagnose by imaging, frequently resembling and be misdiagnosed as renal cell carcinoma.

In addition to these two major histological groups, there is a rare variant, characterized by solid and cystic areas, called angiomyolipoma with epithelial cysts. It is a benign lesion with no evidence of metastasis or recurrence following surgical excision [11]. The three variants are differentiated from each other only after biopsy.

DIAGNOSIS

Clinical presentation

Commonly patients are asymptomatic at the time of diagnosis, AMLs being an incidental finding. This is explained by the increasing use of imaging tools and the advances in imaging technology [12].

Among symptomatic patients, the most common sign is flank pain, followed by a palpable mass, haematuria, anaemia, urinary tract infection, or an impaired renal function. In a study conducted by Koo et al on 129 patients, 75% of them were asymptomatic [13]. Regarding symptomatic patients, Bissler et al found that out of the 32 sypmtomatic patients included into their study 20 had flank pain, 7 had hematuria and 2 had spontaneous rupture [14].

A serious form of presentation, seen in less than 15% of cases, is spontaneous retroperitoneal haemorrhage, which can lead in one third of patients to shock. Therefore, risk of a life-threatening bleeding is the main clinical concern in a patient diagnosed with AML [12].

The epithelioid variant of AMLs can undego malignant transformation. Though rare, it is characterized by local aggressivness, lymphadenopathy, and distal metastases. The larger the tumour, the more likely it is to spread, acting like a veritable retroperitoneal tumor [15-19] and can produce secondary hydronephrosis that must be drained with a double J stent [20, 21].

AMLs tend to be more aggressive in patients with TSC or LAM, thus symptomatic patients are more commonly found in these cases. It is important to screen patients diagnosed with AML for TSC and LAM. Patients with multiple, bilateral, or larger (≥4 cm) are at higher risk for TSC and the tumors are usually diagnosed at an early age.

Imaging features

By containing fat tissue AMLs are very easily diagnosed using imaging tools. However, in cases of hemorrhage, calcification, necrosis or low fat content the diagnosis becomes a challenge. A recent article classified renal AMLs as fat-rich, fat-poor, or fat-invisible, based on the amount of fat detected on imaging studies [22].

A hyperechoic lesion, resulting from a combination of fat, blood vessel, and muscle contents, with a posterior acoustic shadow, due to the multiple tissue interfaces is the classical appearance of an AML rich in fat tissue on ultrasound [23]. The echogenity of the tumor depends on the percentage of fat tissue in the AML, usually being the same as or greater than that of the renal sinus, but as the amount of fat gets lower, the echogenity decreases.

AML low in fat content are usually a mixture of hyperechoic and isoechoic lesions compared with the renal parenchyma, while a fat-invisible AML is homogenously isoechoic with it. In these cases it is hard to differentiate renal cell carcinoma from an AML on ultrasound [22, 24].

Computed tomography (CT) scan is recommended when hyperechoic lesion is found by ultrasound, having excellent sensitivity, specificity, and reproducibility regarding renal masses. Computed tomography can identify macroscopic fat in AML as low-density area of −10 HU or lower. Fat-poor AMLs are heterogeneously isoattenuating or hyper-attenuating, but on the other hand, fat-invisible AMLs appear homogenously hyperattenuating. Contrast-enhanced CT is not necessary for diagnosing fat-rich AML but shoud be performed where there is a risk for tumor bleeding, to identify the tortuous vessels, before therapeutic embolization can be scheduled [22, 25].

Kidney AML can be very similar with renal cell carcinomas and hemorrhagic cysts on the MRI images, thus fat suppression techniques may be helpful in differentiate them from each other [23]. As in computed tomography imaging, contrast enhanced MRI has a limited role in the diagnosis of AML.

In some cases, especially in fat-invisible AMLs, when both CT and MRI images are inconclusive and we cannot differentiate AMLs from renal cell carcinomas on imaging alone, percutaneous renal biopsy is recommended to avoid unnecessary treatment.

TREATMENT

AMLs being an incidental finding on imaging tools in asymptomatic patients, implies that a small percentage of cases requires active treatment.

The current guidelines regarding AMLs recommend active treatment in well-selected cases, such as: symptomatic tumours, the presence in women of childbearing age, poor access to follow-up or emergency care and large masses, but a size threshold remains controversial (historically a size greater than 4 cm was considered an indication for surgery).

Active surveillance

If the indication for active treatment are not present, all patients with AML should undergo routine monitoring by imaging to assess stability [26]. Patients with tumors less than 2 cm should do renal ultrasound every 3 to 4 years and those with masses measuring 2 to 4 cm should undergo ultrasound monitorisation every year. Patients with masses larger than 4 cm in diameter, who do not undergo surgery should be assesed, by ultrasound or CT every 6 months, and if stable, anually. The same with patients with epithelioid variants. Patients who have had surgical removal of an epithelioid AML, at high-risk for malignant transformation, should have whole-body CT six months after surgery and yearly thereafter for at least five years [27].

Embolization

Selective embolization of the renal artery is the preferred treatment for patients with life-threatening retroperitoneal hemorrhage. Embolization can be performed preoperatively to reduce the difficulty and complications of the surgical removal. It has a relatively high percentage of side effects including: fever, flank pain, leucocytosis, nausea, and vomiting within the first three days after the procedure, but nothing that cannot be managed conservatively. Other rare complications include non-target embolization of normal parenchyma or renal infarction [28,29]. Altough it is a generally well-tolerated procedure, it has a risk of regrowth and repeated haemorrhage after embolization [30].

Surgical excision

Partial or radical nephrectomy can be used to treat renal AMLs, being the only treatment that completely removes the renal mass. Current guidliness recommend that nephron-sparing surgery should always be performed whenever possible, being even more important in patients with TSC or LAM, because of the multifocality of the disease in this group of patients and the higher risk of recurrence. Radical nephrectomy should be used only in selected cases, with large masses and a high risk of malignant transformation, or when other treatment options are not viable. Sometimes, in an emergency setting, a radical nephrectomy can be a life-saver [18].

Minimal invasion techniques

Cryoablation and percutaneous radiofrequency ablation can be used as an alternative to embolization or surgery. Different studies show good efficacy with minimal complications, very few therapy sessions and no recurrences, but these minimal invasion techniques should be used on small (1-3 cm in diameter) and asymptomatic lesions [31].

Medical treatment

mTOR inhibitors, such as Sirolimus or Everolimus, decreases the risk of tumor progression and reduces the size of the tumor, especially in patients with TSC or LAM., but the use of these drugs in sporadic LAMs is still debatable. Recent guidelines recommend the use of mTOR inhibitors in patients with progressive renal LAMs greater than 3 cm in asymptomatic patients with TSC or LAM as the first line of treatment. Other treatments are required in case of secondary complications, such as cardiovascular diseases [32-36] or infections [37, 38].

DISCUSSIONS

A Japanese study, using a population-based ultrasound screening, included 18,000 healthy adults, and found that 0.1 percent of men and 0.2 percent of women had renal AML [39]. Rule et al stated that renal AMLs are more common seen in middle aged women, compared to men, findings supported by Koo and his team in their research on a group of 117 patients with sporadic AML, that found that 92% of patients were women with a mean age of 52 years old [16, 40].

Ewalt et al in their longitudinal study on 60 children with TSC found that renal AMLs were present in 48% of boys and 60% of girls at a mean age of 7 years old and in 76% of boys and 83% of girls at a follow-up age of 10 years old, suggesting that the prevalence of renal AMLs in patients with TSC is increasing with age [41].

In their paper on diagnosis and management of renal angiomyolipoma, Seyam and his colleagues included 60 patients with renal AMLs with a median age of 45 years old, from which 43 were females. Half the patients presented with flank pain and only 13 had hematuria. They screened patients for TSC and found this disease in 14 patients. More than half the patients were followed up expectantly, the mean size of the tumor being 4 cm in diameter, suggesting that the majority of patients with AMLs should undergo routine monitoring to assess stability. 29 patients needed active treatment to control bleeding and relieve pain or because of the risk of malignant transformation. Tumors that are larger in size have a higher risk of bleeding, the mean size of masses in which retroperitoneal hemorrhage was discovered was approximately 11 cm. Patients were followed up for a mean of 4 years and the lesions grew an average of 5 cm for TSC patients and less than 1 cm for the sporadic group, suggesting that patients with TSC are at a higher risk of progression. Furthermore, patients whom had TSC compared with the sporadic group were younger at presentation (26 versus 49 years old), had bigger renal masses (19 cm compared with a mean of 4 cm) and had multiple or bilateral lesions [42]. Similar findings were stated in a smaller group study conducted by Nelson et al (336 patients with 19% having TSC). The patients within the sporadic renal AMLs group were older (mean 52 years old compared with 30 years old), had smaller AMLs (mean 5 compared 9 cm), and were much less likely to have both multiple lesions (13% compared with 97%) and acute hemorrhage (14% compared with 44%) [43].

Some authors recommend that renal AMLs measuring 4 to 8 cm in diameter should be observed, if asymptomatic and close follow-up can be achieved [44, 45].

Bissler and his colleagues in their paper on mTOR inhibitors included 118 patients with AMLs, 5 of which were sporadic, the rest being in correlation with TSC. The target of at least 50 percent reduction in the total volume of the mass was achieved in 42% of the patients treated with everolimus compared with none of the patients treated with placebo, with a median response time of 3 months. Progression of AMLs was significantly more common in the placebo group (21%

compared with 4% in the mTOR inhibitors treated group) [46].

mTOR inhibitors treatment can be used to reduce the volume of tumors so the patients can benefit from a nephron-sparing surgery, fact stated in Staehler's paper on „Nephron-sparing resection of angiomyolipoma after sirolimus pretreatment in patients with tuberous sclerosis". Patients treated with Sirolimus had a 38 to 95% reduction in AML volume [47].

The efficacy of selective renal artery embolization was demonstrated in a study conducted on 30 patients with large renal AMLs (mean size of 8 cm). In 83% of AMLs that were embolized, the procedure was complete after a single sesion. The median reduction volume was 43% at a follow-up of 6 months and 81% at 12 months. They found that the response was poor for fat-rich AMLs compared with fat-poor tumors [48].

De Luca stated that sporadic AMLs smaller than 1.5 cm showed no growth over 5 year follow-up in 92% of cases [49]. Furthermore, Oesterling et al said that AMLs greater than 4 cm in diameter grow more rapidly and other authors found that younger patients compared with older individuals had a higher risk of progression [50, 51]. On the other hand, epithelioid variants of AMLs have a poorer prognosis. In one study conducted on 40 patients with renal epithelioid AMLs and atypia detected on histologic examination, local recurrence or distant metastases were present in 26% of cases [52]. Nese et al had the same results in their study on 41 patients with pure epithelioid AML, out of which 36% had a metastatic disease at the time of presentation, and 16% developed metastatic disease during follow-up [53].

CONCLUSION

AMLs are a benign group of renal tumors, commonly associated with TSC and LAM, but can also appear sporadically in the population. The detection of AMLs is increasing because of an increase in the use of imaging and advances in imaging technology. The diagnosis of AML is generally made by imaging in asymptomatic patients, although a biopsy may be required to diagnose fat-poor lesions. In terms of therapeutical management, several treatment option are available, though a small percentage of patients require it and the treatment should be individualized for every patient. Most of the AMLs can be managed by active surveillance. Pure epithelioid variants of AMLs have a poor prognosis comapared with the clasic variant.

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ORIGINAL ARTICLE

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Renal involvement in primary retroperitoneal tumors

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Abstract: *Retroperitoneal tumors grow quietly and are generally large in size, more than half of them being larger than 20 cm at the time of diagnosis. They usually present several therapeutic challenges because of their rarity, relative late presentation and anatomical location, often in close relationship with several important structures in the retroperitoneal space. Due to intimate relationships with vital organs in retroperitoneum, extensive surgery with en bloc resection of the involved organ is often required, most frequently the kidneys, followed by colon, pancreas and spleen. Malignant tumors have a poor prognosis, the most significant factors regarding survival rates being delayed diagnosis, high histological grade, inoperability due to invasion into vital organs, and a positive surgical border.*

Keywords: *primary retroperitoneal tumors, renal involvement, therapeutic management*

INTRODUCTION

The retroperitoneum is one of the largest spaces in the body, located behind the abdominal cavity, between the posterior leaf of the parietal peritoneum and the lumbar muscles fascia. It is delimited superiorly by the diaphragm and extending inferiorly to the pelvic floor, reaching the external borders of the lumbar muscles laterally [1]. It contains connective tissue, kidneys, adrenals, ureter, aorta with its branches, inferior vena cava with its tributaries, a portion of the duoden and pancreas, a part of the descending and ascending colon and lymph nodes. At the kidneys level, the retroperitoneum is divided into three spaces by the anterior and posterior renal fascia, as follows: anterior pararenal space, perirenal space and posterior pararenal space [2]. Primary retroperitoneal tumors represent a rare, but interesting group of neoplasms. Even though they are located in the same space, tumors of the kidneys, adrenals and pancreas, tumors originating from the organs situated partially in the retroperitoneum and retroperitoneum metastasis do not fall into this category. Primary retroperitoneal tumors do not originate from any retroperitoneal organ (parenchymatous or not), but develop from the actual retroperitoneal tissue (lymphatic, nervous, vascular, support muscle, connective, or fibroareolar tissue) or from embryonic rests of the urogenital ridge (wolffian or müllerian ducts, germ cells, primitive notochord) [3-6].

The retroperitoneum can host a wide spectrum of pathologies. Primary retroperitoneal tumors are a rare entity, having a reported incidence of 0.3-3.0%. More than half of the retroperitoneal masses are malignant (70-85%), leaving a percentage of 15-25% of the tumors to be benign. The majority of the tumors are of mesenchymal origin (75%), followed by the neural origin (24%), and last from embryonic

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rests (less than 1%) [7].

The retroperitoneum represents the second most common site of origin of malignant mesenchymal tumours after the lower extremities [7, 8]. Liposarcomas (70%) and leiomyosarcomas (15%) are the two most frequent histological subtypes of sarcomas. These tumors present as hard abdominal masses with an irregular surface; they are surrounded by a capsule that is rapidly outgrown by tumor growth and infiltrate the posterior parietal peritoneum and the intra-abdominal viscera attached to it, thus becoming intraperitoneal by direct, and not metastatic, invasion.

Benign tumors are often an incidental finding during an investigation for unrelated symptoms. The most common primary benign pathologies encountered in the retroperitoneum include benign neurogenic tumours (schwannomas, neurofibromas), paragongliomas (functional or non-functional), fibromatosis and retroperitoneal lipomas [9, 10].

Due to the inaccessibility of the region and since these tumours often give none or non-specific symptoms until they have reached a substantial size, they are usually large at presentation [11]. They grow silent and aggressively and usually invade the surrounding structures. In primary retroperitoneal tumors lymphadenopathy and distant metastases are a rare entity. More than half of the retroperitoneal tumors are larger than 20 cm in diameter at the time of diagnosis, the retroperitoneum space being “adaptable”.

The urologist has a crucial role in diagnosing and treating these tumors, because they are usually found in the anatomical region commonly used for surgical access in many major urological surgeries. However, in certain cases, a multidisciplinary approach should not be avoided.

Retroperitoneal tumors occurs frequently in the 5th and 6th decade, being found more often in female than in male patients [12, 13]. Certain histological types, such as embryonal rhabdomyosarcoma, teratoma and neuroblastoma, are commonly found in younger patients. Patients with primary retroperitoneal masses usually present with abdominal swelling and pain, early satiety, abdominal discomfort, most having a palpable abdominal mass. Many benign tumors are discovered as an incidental finding during imaging for unrelated symptoms [14, 15]. Pain can vary in intensity and severity and can also be located in the lumbar, inguinal or gluteal region. General symptoms such as asthenia, anorexia, weight loss or prolonged fever may be present. A study found out that out of the total number of patients, 44% had on admission gastro-intestinal symptoms such as: vomiting, abdo-minal distention, bloating, constipation, dyspepsia, gastrointestinal bleeding or intestinal blockage. Neurological symptoms (lower limb weakness, sensory loss of the obturator, femoral cutaneous or sciatic nerve, radiculitis, sciatica and sphincter dysfunctions) were also found in 3 out of the total 27 patients, and 10% showed signs of venous compression (edema, varicocele, ascites and genital swelling).

Retroperitoneal masses are usually diagnosed by clinical examination associated with blood tests and imaging studies (CT, MRI, abdominal ultrasonography). The imaging test of choice in the evaluation and staging of primary retroperitoneal masses is contrast-enhanced computed tomography of the thorax, abdominal and pelvic regions. It can distinguish between different densities which suggest the nature of the mass, defines the shape and size of the tumor, can discover enlarged lymph nodes and can assess the involvement of the neighboring organs [16, 17]. It can also be used to perform CT-guided biopsies. It can detect the presence or absence of pulmonary, bone, hepatic or peritoneal metastases and local or regional recurrence in patients who have already been treated [18].

It is important to confirm that the tumor is located within the retroperitoneal space and to exclude the possibility that the tumor originates from a retroperitoneal organ. Anterior displacement of the retroperitoneal organs is a strong indicator that the mass has a retroperitoneal origin. The migration of the large vessels can be helpful as well [19, 20].

Ultrasound is a fast, easy to use, repeatable method. It can reveal whether the mass is cystic or not, it can determine its volume, topography and can assess the condition of the inferior vena cava. The main disadvantage is that it is a subjective method, dependent on the doctor's experience [19].

If after the results of the imaging studies the diagnosis remains uncertain, an exploratory laparotomy or laparoscopy remains a last diagnostic resource.

THERAPEUTIC MANAGEMENT

Complete surgical resection is the treatment of choice for primary retroperitoneal tumors, but local recurrence occurs in the majority of the patients, being responsible for more than 75% of sarcoma-related deaths. Complete surgical ablation is not always possible, because of its invasion in vital structures. Despite the possibility of performing large organ resections, the possibility of a residual tumor is very high.

Macroscopic excision, tumour grade, multifocality and histological subtype are the main prognostic factors that dictate local recurrence and overall survival rates [21-23]. Local recurrence rates are higher and occurs at an earlier interval in high-grade tumors compared with low-grade ones. The possibility of obtaining negative margins is remarkably lower at the time of local recurrence and each successive operation is more difficult than the last. In the presence of a positive surgical border following the resection of a malignant retroperitoneal tumor, the 5-year survival rate decreases to 28%.

The most significant factors regarding survival rates are delayed diagnosis, high histological grade, inoperability due to invasion into vital organs, and a positive surgical border. For patients with high-grade tumors, the mean life expectancy is approximately 20 months, increasing to 80 months in patients with low-grade retroperitoneal masses.

A study found that tumors larger than 10 cm generally had distant metastasis at the time of diagnosis [24]. The risk of local recurrence is more important than the risk of metastasis regarding the progression of malignant primitive retroperitoneal tumors [25].

Due to the fact that a radical surgery is needed and that the tumor is usually large in size, an access route that allows a wide exposure for procedures is required. Midline laparotomy is the acces route of choice, allowing simultaneous examination of the abdominal and pelvic viscera, enabling management of the large vascular trunks, additional visceral surgery and the incision can be extended for access in the thoracic cavity. Chevron incision is usually used to remove large tumors with the invasion of inferior vena cava and lombotomy should be used to remove small retroperitoneal masses [26].

Because of the high risk of local recurrence, a strict follow-up with CT or MRI testing is necessary every six months for 2 or 3 years [27, 28]. Since primary retropritoneal tumors have such high rates of local recurrence, adjuvant therapy is recommended.

The use of high-dose therapy, in an attempt to lower the rate of local recurrence, is limited because these tumors are often adjacent to radiosensitive structures with low radiation tolerance (such as kidneys and intestines). The doze usually used is about 60 Gy, studies found that therate of local recurrence is 67% with doses below 50 Gy but only 17% at doses equal to or higher than 50 Gy [27]. Lymphomas, rhabdomyosarcomas, neuroblastomas and undifferentiated sarcomas seem to have good results with radiotherapy.

The use of chemotherapy in retroperitoneal tumors is controversial, having a similar response index with other therapeutic regimen and equally poor in single drug therapy or in combination [18]. The most commonly used drug, both in single drug therapy or in combination (adriamycin – dacarbazine, or cyclophosphamide – vincristine – adriamycin – dacarbazine), is Adriamycin. This drug is also used for metastatic disease, as a palliative form of treatment [28, 29].

If a tumor is considered unresectable or the patient has distant metastases, a core needle biopsy may be indicated to confirm the diagnosis and to enable consideration of alternative therapy.

Palliative surgery (incomplete resection for unresectable tumor) should be considered for symptom control and may improve quality of life [28].

DISCUSSION

Due to the fact that kidneys and ureteres are retroperitoneal organs, they are usually involved by retroperitoneal masses, either by extrinsec compression or direct invasion. If the tumor is left unchecked, it will grow to cause secondary hidronephrosis and renal parenchymal destruction that will lead to chronic kidney disease with severe hydro-electrolytic imbalance and serious consequences. Resistant hypertension [30] with dyselectrolytemia [31, 32], secondary heart failure [33] and cardiac arrhythmias [34] may be the result of compromised renal arteries by extrinsec compression caused by the retroperitoneal masses [9].

Urological manifestations, caused by possible urinary tract involvement (displacement, compression and invasion) at any level (kidneys, ureter, and bladder) were not as frequent, only 25% of the patients presented chronic lower back pain, or acute renal and ureteral colic. Hematuria, polakiuria, dysuria or urinary retention usually occur in tumors located in the pelvic region, near the bladder [13]. Hematuria may also appear if the retroperitoneal mass invades the ureters or the pyelocaliceal system [35].

In terms of treatment, the complete surgical resection of the tumor en bloc with the involved organs is the gold standard. It can vary from segmental ureterectomy, followed by a surgical repair of the defect, to radical nephrectomy. In cases with extrinsec compression of the ureter and secondary hidronephrosis, with no signs of invasion, an internal (ureteral stent) or external (percutaneous nephrostomy) renal drainage method can be used, until the tumor can be surgically removed. More than 83% of the patients require resection of neighboring organs, most frequently the kidneys, followed by colon, pancreas and spleen, for achieving a negative margin [27].

If the ureteral defect after extensive surgical removal of the retroperitoneal mass is proximal to the ureterovesical junction and it is not large in size, a ureteroneocystostomy or a primary anastomosis of the ureter (ureteroureterostomy) is usually preferred. When ureteroureterostomy or ureteroneocystostomy cannot be performed, the psoas hitch ureteral reimplantation is the best approach. For defects in the middle third of the ureter after partial ureterectomy, the optimal approach is ureteroureterostomy, but if it cannot provide a tension free anastomosis, a Boari flap is an optimal, but complex approach. The optimal procedure for repair of an upper ureteral injury is a ureteroureterostomy, if it can be performed without tension. A nephropexy with fixation to the psoas tendon can provide a tension-free anastomosis. When a tension-free anastomosis cannot be achieved, even after kidney mobilization, autotransplantation, ileal or appendiceal interposition graft, nephrostomy or a cutaneous ureterostomy can be performed. The ureteral anastomosis following any type of primary repair is typically stented.

In advanced cases, with inoperable tumor and secondary ureterohydronephrosis, an urgent drainage of the obstructed kidney should be used [36]. This can be achieved either by internal drainage using a double J ureteral stent [17] or by using a percutaneous nephrostomy. A nephrostomy deteriorates the patient's quality of life and leads to frequent infections, blockage of tubes or bleeding. Ureteral stenting, on the other hand, can be associated with irritative bladder symptoms, blockage, sepsis and a high failure rate, which ultimately leads to a nephrostomy tube [28].

Wrona AJ et al wanted to offer a better solution than ureteral stenting and nephrostomy for kidney drainage. In their study, they presented a subcutaneous pyelovesical bypass as a solution for ureteric obstruction (detour bypass). A detour bypass is made of two coaxial tubes, a 27F porous polytetrafluoro-ethylene (PTFE) outer tubeand an inner 17F silicone tube extending beyond the PTFE, with perforations on both ends and a radio-opaque ring on the proximal end (for an accurate placement of the proximal end of the stent). The detour is placed subcutaneously, using a tunneling device, and the distal end of the tube is placed into the dome of the bladder by a small incison. They concluded that this method is a safe, minimally invasive and highly effective alternative in patients with ureteral obstruction caused by both malignant and benign diseases and that the potential complications (infection, encrustation) may be easily and efficiently managed [37].

Andrea Montenegro et al presented a case of a 65 year-old female with a large retroperitoneal liposarcoma, in whom, due to the fact that it was very adherent to the left kidney, a complete resection of the tumor with concomitant left nephrectomy was practiced.

In a case report by Mariko Tanaka et al, they introduced the case of a 60 year old female who presented with abdominal distension. The CT revealed a large retroperitoneal tumor which proved to be a dedifferentiated liposarcoma at biopsy. Due to the fact that the tumor invaded the duodenum, head of the pancreas, right kidney, inferior vena cava and the aorta below the left renal artery, an extensive surgical removal of the tumor was performed, with en bloc resection of the right kidney, duodenum, head of the pancreas, a portion of the inferior vena cava and abdominal aorta, with concomitant vascular reconstruction [38].

In their study, Rodriguez et al found out that sarcomas, mainly liposarcomas, followed closely by leiomyosarcomas, were the most common type of primary retroperitoneal tumors. More than half of the patients included in the study presented at admission pain of varying severity and location (mainly abdominal and lumbar) and general symptoms such as asthenia, anorexia, weight loss or prolonged fever. Gastrointestinal manifestations (vomiting, abdominal distention, bloating, constipation, dyspepsia or intestinal blockage), due to compression/invasion of the gastrointestinal tract, were present in 44% of cases. Renal involvement was not as frequent, only one quarter of patients had urological symptoms, such as lower back pain, but also dysuria, polakiuria and urge [13].

Pereira et al reported the case of a 48-year-old male who presented for pain and right abdominal swelling. Clinical investigation revealed a right varicocele and imaging studies found a solitary hydronephrotic right kidney (personal history of left renal agenesis). Contrast enhanced computed tomography showed a retroperitoneal mass and a right hydronephrotic kidney. The particularity of this case was the presence of a solitary right kidney with left renal agenesis, with the possibility that the patient will become anephric after extensive surgery. Ultrasonography-guided biopsy was performed that confirmed a well-differentiated liposarcoma. Surgical excision of the retroperitoneal sarcoma was performed, sparring the right kidney, but the patient unfortunately died during chemotherapy [39-42].

Another case reported by the previous team presented with hypertension, anorexia, and weight loss (10 kg in 2 months). This patient had a solitary right kidney following a left radical nephrectomy subsequent to renal cell carcinoma. A right retroperitoneal mass was found during the study of his hypertension, which had a compressing effect on the right renal artery. Surgical excision of the mass was performed, but the patient died of local recurrence.

MRI of an 84-year-old male, who presented with complaints of pain and a palpable right lumbar mass, revealed a large retroperitoneal tumor invading the right kidney. Extensive surgical excision was made, with concomitant right nephrectomy.

Kazim et al stated that the location of intra-abdominal organs can shift in the presence of retroperitoneal tumors, due to the mass effect, with the kidneys being the most common viscera with an altered intra-abdominal location. In their series, they reported one retroperitoneal tumor, measuring 30 cm in diameter that pushed the left kidney to the right anterolateral direction. Since the left kidney was completely engulfed by the mass, the patient underwent extensive surgical excision of the mass and a left nephrectomy [43].

CONCLUSIONS

The retroperitoneum can host a wide variety of pathologies, including benign and malignant tumors. Primary retroperitoneal tumors are rare, almost all of them being malignant. Retroperitoneal masses grow silently, presenting late, usually invading neighboring structures at time of diagnosis. Contrast-enhanced computed tomography is the best way to evaluate this kind of tumors. Complete surgical resection is the only potential curative treatment modality for retroperitoneal masses, but the characteristics of retroperitoneal tumors (size, proximity to abdominal organs and structures, proximity to large retroperitoneal vessels) make surgical treatment a real challenge for surgeons. The efficiency of pre-operative and post-operative radiotherapy and chemotherapy is still a controversial issue, complete surgical excision with negative margins remains the gold standard procedure.

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Research for the development of logistics planning information support in health protection against biological agents

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Abstract: *Making medical countermeasures requires documentation to assess vulnerabilities, threats and operational risks and bioterrorism through the collection and processing of specific multidisciplinary data in human medicine, veterinary, phytosanitary, the environment, defense and national security, etc. Based on retrospective data and late information (medical intelligence), we can estimate the prospective risk to public health, respectively for troops and civilian population in a given area, the area of operations.*

The biological military or bioterrorist attack, whether overtly or masqueraded as human or animal epidemics can cause a major biological crisis for troops and the civilian population, for domestic and wild animals, for crops or wild plants or for the environment, which remains contaminated.

Therefore, it is important to constantly monitor the situation of infectious diseases in the area of responsibility (national territory, operations theaters) to assess vulnerability, threat and bioterrorist risk but also for making medical anti-bioterrorist countermeasures, which can only be effective by genuine cooperation between all specialized structures of the Ministry of Defense, other departments and the civil society.

Keywords: *biological attack, bioterrorism, medical countermeasures, logistics support, medical treatment*

INTRODUCTION

Medical protection logistics planning against biological warfare agents (ABR) is based on interdisciplinary studies and specific calculations.

The complexity of this approach involves processing a database of medical, pharmaceutical and logistics that can only be operated in real time by using a suitable IT support. It consists in hardware (IBM compatible standard configuration computer), software (Windows based NATO specific program), specialized personnel (NATO computer operators, specialists in military medicine, logistics, CBRN defense, etc.) and properly equipped and secure space.

Scenarios that simulate hypothetical situations reported in the territory, will be tested by constructive simulation as functional experimental models of "war games ".[1]

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EXPERIMENTAL MODELS

Making the experimental model of the information system for constructive simulation for medical protection against biological weapons and bio-terrorism and testing it as a military medical exercise demonstrated and validated the scientific data, using modeling and constructive simulation, together with UNAp/CISM, the current Center for War Games and doctrinal Experiments (CJRED) of the General Staff. [2] It was awarded a large database that can be exploited in different complementary approaches. Of all bacteria, Bacillus anthracis has been chosen for being used in the constructive simulation experiment, it is known as the biologically bacterial agent most used for biological attacks. [3]

Medical countermeasures must be known and individualized for each situation and appropriately applied, with appropriate forces and means. This requires an adequate and efficient logistic support because the scale of medical needs (medicines, sanitary materials, reagents, accommodation, transport, etc.) exponentially increases during an epidemic. As a result, at any level, health-pharmaceutical materials are rapidly becoming inadequate. The unintended consequence is that we cannot prevent illnesses or treat patients, so the affected communities are no longer capable of fighting and working and there is a risk of over mortality. Therefore, the medical protection against biological weapons requires a proper logistic planning that is also based on statistical analysis of some relevant information for this purpose. The exploitation of such data requires a dedicated computer system, with suitable software, trained personnel, quick and secure media and communications, etc. Because the computer system for the surveillance of infectious diseases caused by combat biological agents and by bioterrorism to be reliable, it must be implemented in stages, with feedback in the form of custom scenarios for biological attack or epidemic outbreak.

Achieving this pilot experiment in the Romanian Army, demonstrated the usefulness of extending simulation tests on other categories of warfare and on specific logistics for biological crisis. The system used was JCATS – Joint Conflict and Tactical Simulation. The information was delivered in the form of screen-shots that required image processing, computer analysis and interpretation. [5]

The data was processed and a computer media has been made for logistics planning of medical protection against biological warfare and bioterrorism agents through statistical analysis of the resulting data bank. To estimate the number of contaminants analysis were carried out on the three simulated hypothesis: without sensors, with military sensors and alarm on short (in barracks) and with military sensors and alarm on short distance (in barracks) together with a sensors system of the local Inspectorate for Emergency Situations (ISU) and alarm in the city. We have obtained information that was collated for each simulation, in numerical tables and graphs illustrating the impact such an undesirable event can have. [6]

The obtained data allowed the dynamic evaluation (an estimate every 5 minutes) on the effects of a biological attack with anthrax on cities with different number of inhabitants and military forces.

The post exercise analysis showed that in terms of exposed population, where a relatively small number of people, the presence of the sensor is useful, the number of people being affected (civilian and military) decreased after they triggered the alarm.

In a similar situation, in the case of larger cities it has been observed that activation of military sensors does not cause diminishing the number of affected civilians, but decreases the number of infected soldiers.

The comparative analysis of the post-biological attack, for the soldiers of the sea shows that there are a huge number of people affected in the absence of any alarm sensor (over 50%) as their protection or travel ability is restricted to the soldiers on land. Also, the percentage of affected soldiers who do not benefit from the ship protection is higher (70%), if they go out on the deck.

The major differences recorded between the high percentage of affected civilians and that of the military, leading to the idea that it is useful and necessary for the civilian population to be at least more informed about how they should act when triggering an alarm of any kind. [7]

Following statistical analysis of the database resulting from constructive simulation system experimentation resulted in an acquisition, storage, retrieval and information-processing algorithm as an interactive database. [8]

SANITARY LOGISTICS

The statistical analysis conduct to information somehow surprising by the extremely high number of people affected, at least from the civilian population, which is not trained to respond to any alarm. Regarding the military, which is a segment of the relatively "trained" population compared to civilians, we found that the number of those affected drastically decreases, especially when special sensors for detection exist. The analysis of relevant simulation for the purpose of the experiment showed that a possible attack with anthrax spores, even if it is not repeatable, it could result in a significant number of contaminated, many of which will develop the disease, and some will die. The evolution of the epidemic will depend on the capacity of the health system (military and civilian) to promptly and adequately act with the necessary forces and means and with the appropriate logistic support, depending on the epidemiological situation of the troops and the civilian population. [8]

This means that all exposed persons should be medically supervised: rapid laboratory analyzes for different areas/neighborhoods, nasal/throat exuding for identifying B. anthracis.

The contaminated must be prophylactic treated, also with antibiotics according to the therapeutic scheme Guide for 60 days and under medical supervision. Patients should be isolated and treated for 60 days. For those with severe forms (pulmonary anthrax) intensive oxygen therapy must be also applied, etc. [9, 10, 11]

The deceased must be disinfected and handed over to and Funeral Services (the civilians) or the Logistics department (military) for transport and burial, in accordance with epidemic provisions. Incineration is contraindicated due to the large number of corpses. Repatriation is not allowed because of the contagion risk. [12]

Overall, the medical forces and means are overburdened and there is an urgent need for specific additional procurement for a 7 week period.

For such need, specific measures are necessary because sanitary-pharmaceutical materials are rapidly becoming inadequate and the only way to overcome the situation involves cooperation with all own and allied military forces as well as civil-military cooperation (CIMIC).

CONCLUSION

- Medical countermeasures against biological or bioterrorist attack are complex and require specialized forces and means, in very large quantities and in real time.
- By medical intelligence the real situation must be known and the short, medium and long term development must be assessed in order to correctly estimate the forces and means necessary for the medical countermeasures.
- By appropriate application of the logistics assurance principles and CIMIC cooperation the specific logistical support can be achieved, necessary to carry out the medical countermeasures for the prevention of diseases and treating patients.
- The logistical support for medical countermeasures against biological or bioterrorist attack must be coherent, efficient and fast.

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REVIEW

Cardiotoxicity of Chemotherapy in Lynch Syndrome – A Literature Review

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Abstract: *Chemotherapy is an important treatment in oncological disease, with a vast number of side effects. The cardiotoxicity of several chemotherapeutic agents and appropriate risk stratification and patient follow-up must be ensured by a multidisciplinary team which must include an oncologist and a cardiologist. Lynch syndrome is associated with younger-onset malignant tumors of various localizations, requiring aggressive chemotherapy. FOLFOX chemotherapy which is frequently used in Lynch syndrome-associated colorectal cancer has several cardiotoxic effects with mechanisms ranging from increased reactive oxidative species to Krebs cycle blockade or coronary vasospasm. These complex effects on the cardiovascular system have varied clinical effects, such as heart failure, arrhythmias, or acute ischemic events***.**

Keywords: *heart failure, bradycardia, cardiotoxicity, chemotherapy, Lynch syndrome, FOLFOX, colon cancer, cardio-oncology*

INTRODUCTION

Cancer therapy is life-saving for patients requiring such medication. However, a wide array of side effects have been described, depending on the treatment used and the history and risk factors of the patient. Cardiotoxicity has been described as a side effect of many chemotherapic drugs.

In young patients with oncological disease, the diagnosis is usually determined at a later stage, requiring more aggressive chemotherapy. Moreover, younger-onset cancers might be associated with hereditary diseases, such as Lynch syndrome. This syndrome is associated with early-onset cancers of varied localizations, from colorectal cancer to endometrial cancer.

We present Lynch syndrome chemotherapy cardiovascular side-effects for the most commonly associated cancers.

DISCUSSION

Cancer is no longer a pathology reserved for the elderly. Several studies reveal an alarming increase in the incidence of colorectal cancers for young patients. Colorectal cancers at a younger age are more often localized on the left colon, are less differentiated, more advanced at diagnosis, and have a higher prevalence of signet cells. 30% of early-onset patients have hereditary cancer-predisposing syndrome mutations and 20% have familial colorectal cancer [1,2]. In clinical practice, still, in a young patient, cancer is often a diagnostic of exclusion, rarely taken into consideration due to its low incidence and nonspecific signs and symptoms. There are several distinct characteristics of cancer in the young population. These people often have no other diseases requiring frequent medical visits which might lead to a delayed diagnosis, with no potential surgical treatment. Moreover, the chemotherapy regimen used in advanced cancers is more aggressive, both in dosage and in the agents required, leading to various potential side effects and drug interactions that have to be managed by the medical team. The cardiotoxicity of these compounds is variable and has to be taken into consideration and monitored accordingly [3].

To assess the cardiac toxicity of various chemotherapy agents, several tests can be used – ECG, biomarkers (troponin, natriuretic peptides), and imaging. Cardiac echography is the most used imagistic test. Advanced echocardiographic methods using myocardial deformation analysis are important, as they can detect early alterations of cardiac function. Using Color Doppler tissue imaging, strain, and strain rate data can be collected and plotted to assess global and regional cardiac systolic function. Diastolic strain and strain rate data can be obtained, but it is not widely used. Global longitudinal strain reduction has been used as a parameter of global cardiotoxicity because it is modified earlier than LVEF in patients undergoing chemotherapy. However, there is significant inter-vendor variability in GLS cut-offs. Moreover, some agents such as anthracyclines may cause peak strain rate reduction even before inducing diastolic dysfunction, which suggests that some chemotherapic agents may cause initial systolic dysfunction without diastolic dysfunction [4].

An important cause of cancer in young patients with no other comorbidities is Lynch syndrome, which is the most common cause of inherited colorectal cancer (hereditary nonpolyposis colorectal cancer). It is an autosomal dominant disorder caused by mutations in various DNA mismatch repair genes, such as MLH1 (3p22.2), MSH2 (2p21-16), MSH6 (2p16.3), PMS2 (7p22.1), leading to early carcinogenesis. Mutations in the MSH2 gene are the most frequent, followed by MLH1 mutations [5], [6]. Loss of MLH1 expression is exemplified in Figure 1.

Figure 1: Loss of MLH1 expression in the tumoral cell mass

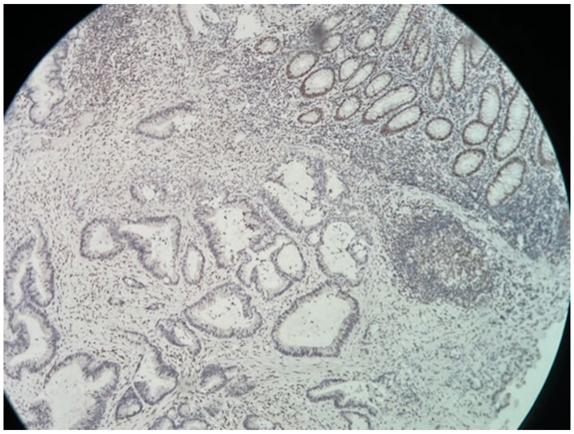

There are several cancers associated with Lynch syndrome, such as colorectal, endometrial, gastric, ovarian, renal/ ureteral, prostatic or skin cancers. The incidence of each type of cancer varies with the mutation involved in the etiology of Lynch syndrome. The classical aspect of Lynch syndrome associated with colon cancer can be seen in Figure 2. The prognostic of the affected individuals and the personalized screening program varies according to several variables. These variables include the pathogenic variant involved, the personal and familial history of cancer, and age at the first oncologic diagnostic. The most frequent malignancy is colon cancer, which is most frequently right-sided and can be associated with synchronous or metachronous tumors of different localization. Furthermore, the adenoma-carcinoma sequence in Lynch syndrome is accelerated and the cancer can develop directly from mucosal crypts in the colon [7].

Figure 2: HE, 10X, Mucinous colon adenocarcinoma with lymphocytic infiltration

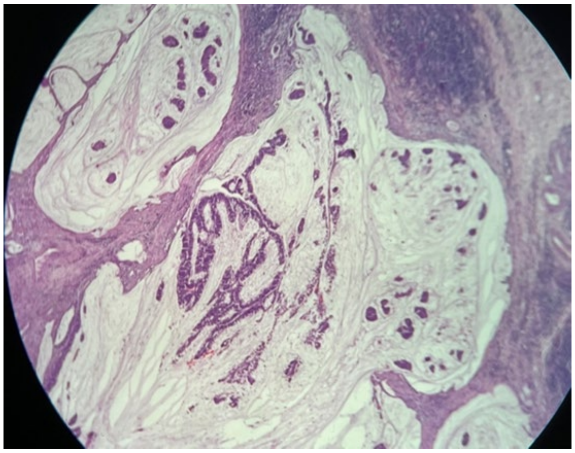

For about 50% of females that carry one of the LS mutations, the first malignant lesion is an endometrial carcinoma. Women with Lynch syndrome have a lifetime cumulative risk is 40-60%. This risk is similar to or exceeds their risk of colorectal cancer [8].

In young individuals with malignant tumors, genetic testing of the patient and their family members is paramount. All patients with colorectal cancer must be evaluated for DNA mismatch repair gene deficits by immunohistochemistry or microsatellite instability (MSI) by PCR [9]. Revised Bethesda Guidelines can be used to assess the need for oncogenetic screening.

According to the aforementioned guidelines, oncogenetic screening should be performed to [10]:

1. Patients with a diagnosed colorectal cancer before 50 years of age;

2. Patients with synchronous or metachronous colorectal tumors or with any other HNPCC-associated tumors, regardless of the patient's age;

3. Patients with colorectal cancer with MSI-H histology before 60 years of age;

4. Patients with colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one cancer being diagnosed before 50 years of age;

5. Patients with colorectal cancer are diagnosed in at least two first or second-degree relatives with HNPCC-related tumors,

regardless of age.

Amsterdam criteria have been modified since their original proposal, however the 3-2-1 rule remains useful in clinical practice, due to its' simple applicability: at least three relatives should have histologically confirmed CRC, spanning at least two successive generations and with at least one colorectal cancer being diagnosed before 50 years of age [11]. However, in current practice, the guidelines recommend the use of the Revised Amsterdam criteria. These criteria diagnose Lynch syndrome in patients with at least three relatives with HNPCC-associated cancer (colorectal cancer, endometrial cancer, enteral cancer, ureteral cancer, or renal pelvis cancer) associated with [12]:

1. One relative should be a first-degree relative to the other two;

2. At least two successive generations should be affected;

3. At least one patient should be diagnosed before 50 years of age;

4. Familial adenomatous polyposis should be excluded;

5. Tumors should be confirmed by pathological examination.

Cardiotoxicity of chemotherapy agents has been classified into type 1 and type 2 agents. Class 1 agents have dose-related cardiotoxic effects, that are mostly irreversible. They are cytotoxic and cause necrosis and apoptosis of cardiac cells, increasing troponin levels. Class 1 agents have a variable incidence of LV dysfunction, its' incidence increasing in the following order: antimicrotubule agents (docetaxel/paclitaxel) <alkylating agents (cyclophosphamide, 5-FU) <anthracyclines (doxorubicin, idarubicin, etc.). Cyclo-fosfamide may cause hypertension, myocardial infarction, Raynaud phenomenon, or hepatic vein occlusion [13]. Class 2 agents have variable cardiotoxicity even in the same category. Class 2 agents have a more complex pathophysiology, but their effects are generally reversible. In this class, anti-HER2 agents, VEGF inhibitors, and TK inhibitors are to be mentioned. They cause cellular dysfunction, leading to transient LV dysfunction [14–18].

One of the most important chemotherapy regimens in colon cancer is the FOLFOX regimen, which includes 5-fluorouracil, Folinic acid, and Oxaliplatin. The addition of oxaliplatin reduces relapse in stage III colorectal cancer compared to a regimen of 5-Fluorouracil and folinic acid [19]. To prevent cardiotoxicity development in cancer patients, the new guidelines for Cardio-oncology recommend risk stratification tools, such as the HFA-ICOS risk stratification [20]. In our presented case, VEGFi therapy cardiotoxic risk stratification could be guided by this score. Risk factors and their stratification in VEGFi therapy are exemplified in Table 1.

Table 1: Risk stratification for VEGFi

M1 risk factors	M2 risk factors	H risk factors	VH risk factors
Elevated baseline cTn or NP	Arrhythmia	DVT/PE	HF/Cardiomyopathy/CTRCD
Radiotherapy to left chest/mediastinum	$450 \le QTc < 480$ ms (men) $460 \le QTc < 480$ ms (women)	$QTc \ge 480$ ms	MI/PCI/CABG/Stable angina
Age 65-74	LVEF 50-54%	LVEF < 50%	Arterial vascular disease
CKD, Proteinuria, DM or hyperlipidemia		Age > 75 years	
Smoker/smoking history Obesity (BMI>30 kg/m^2)		HTN	

cTn, cardiac troponin; NP, natriuretic peptides; CKD, chronic kidney disease; DM, diabetes mellitus, BMI, body mass index, QTc, corrected QT; LVEF, left ventricle ejection fraction; DVT, deep venous thrombosis; PE, pulmonary embolism; HTN, arterial hypertension; HF, heart failure; CTRCD, cancer therapy-related cardiac dysfunction; MI, myocardial infarction; PCI, percutaneous intervention; CABG, coronary artery bypass graft;
Risk level: low risk (no risk factors or one M1 risk factor), moderate risk (moderate risk factors with a total of 2-4 points; M1 = 1 point, M2 = 2 points), high risk (moderate risk factors with a total of at least 5 points or any high-risk factor), very-high risk (any very high-risk factor)

Fluoropyrimidines are a class of chemotherapy drugs frequently used in digestive neoplasia and breast cancer. This class includes drugs such as 5-Fluorouracil (administered IV), its' metabolic precursor capecitabine (administered po), and others, such as Tegafur, or TAS-102. They act by inhibiting thymidylate synthase, blocking the synthesis of nucleic acids, and causing the insertion of 5-FU metabolites in cells' nucleic acid. Not only do these effects occur in cancer cells, but also in normal cells of various localizations, causing cellular dysfunction in many tissues [21]. The most frequent cause of fluoropyrimidine toxicity is caused by a deficiency of dihydropyrimidine dehydrogenase, an important enzyme in the metabolism of fluorouracil, which is the first-rate limiting step in its catabolic pathway. The deficiency of this enzyme's activity leads to the accumulation of fluoropyrimidine in the body, increasing the risk of dose-related chemotoxicity. This enzyme is encoded by the DPYD gene, which has important interindividual variability [22]. In clinical practice, dose adjustment must be taken into consideration by testing for DPYD polymorphisms or by testing the patient's pharmacokinetics of the drug.

Moreover, the duration of 5-FU infusion is also important to prevent toxic accumulation of 5-FU. Elastomeric chemotherapy pumps are very important to ensure a slow release of the chemotherapic agent, with an infusion duration of 46 hours. The medical engineering of newer, more accurate elastomeric pumps may lead to a lower incidence of over- or under-dosing. Furthermore, different methods can be implemented to detect and reduce pump dysfunction, such as pump weighing by the patient [23]. Differences between 5-FU and capecitabine-related cardiotoxicity have been reported, with studies reporting a higher incidence of cardiotoxicity in the capecitabine group [24]. However, whereas exposure to capecitabine was more often associated with chronic heart failure compared to 5-FU, exposure to the latter was associated with cardiovascular diseases [25]. However, in the literature, there have been cases of severe reversible LVEF reduction described, from 65% to 15%, after the first dose of 5-FU containing chemotherapy [26].

Mechanisms of 5-FU-related cardiotoxicity have not been completely determined. Various mechanisms have been suggested, such as coronary vasospasm, direct myocardial injury, vascular endothelium dysfunction with associated thrombosis, or a-fluoro-b-alanine accumulation which causes a Krebs cycle blockade due to fluorocitrate accumulation [27–30]. Focal coronary vasospasm was documented in the early 1990s by Luwaert and co after 2 boluses of 5-FU. Moreover, in both animal and human models, endo-thelium-independent vasoconstriction has been associated with the administration of progressively higher doses of 5-FU [31]. Moreover, fluoropyrimidine-induced diffuse coronary spasm is caused by endothelial cytotoxicity. This has been demonstrated by cell edema, cytolysis, and vessel wall and cell contraction, associated with platelet adhesion, aggregation, and fibrin formation. A proposed mechanism is via endothelial nitric oxide synthase (eNOS), which is an important factor of vascular tonus. Furthermore, patients with 5-FU cardiotoxicity have increased levels of free radicals and endothelin-1, which is an important vasoconstrictor [32,33]. Dihydropyrimidine dehydrogenase, an enzyme involved in the 5-FU catabolism, also plays an important role in the myocardial impairment associated with fluoropyrimidine therapy [34].

3 platinum-based drugs are frequently used in the treatment of cancer: cisplatin, carboplatin, and oxaliplatin. Platinum-based drugs cause crosslinking of DNA, inhibiting nucleic acid replication and transcription. Moreover, it inhibits DNA repair and synthesis, leading to cell apoptosis. There are various side effects reported for this class of drugs, such as nephrotoxicity (AKI, renal tubular acidosis, hyperuricemia, hypomagnesemia), ototoxicity, neurotoxicity (peripheral sensory neuropathy), and cardiotoxicity. Various cardiological side effects of platinum-based drugs have been reported in the last decade, such as arrhythmias (ranging from sinus bradycardia to tachyarrhythmias or complete heart block), vasospasm (which might result in angina, acute coronary syndrome, or stroke), heart failure or thromboembolic events, hypertension, thrombosis (including cerebrovascular events). More data suggests that vascular toxicity is very important in platinum-based chemotherapy. Persisting circulating platinum causes endothelial dysfunction, increasing endothelial injury markers such as von Willebrand factor, tissue-type plasminogen activator, and plasminogen activator inhibitor-1. Moreover, an increased carotid-intima index has been reported [35–40].

Folinic acid (leucovorin) is added to 5-FU because it potentiates the effects of the latter [41]. Being used as an enhancer for 5-FU, the cardiotoxic effects of folinic acid alone have not yet been described.

Vascular endothelial growth factor signaling pathways are involved in both normal and pathological angiogenesis and ensure appropriate vascular permeability. VEGF binds to tyrosine kinase receptors, having different effects in different cells depending on the type of VEGF receptor [42]. Vascular endothelial growth factor inhibitors such as bevacizumab are used in the treatment of numerous cancer types with various localizations, ranging from colon to thyroid. However, VEGF inhibitors are associated with cardiovascular morbidity. The most frequently reported cardiovascular side effect is arterial hypertension, which is dose-dependent and reversible upon drug discontinuation. The mechanism is related to the effect of anti-VEGF drugs on cancer cells, by inhibiting VEGFR2, leading to reduced NO production in vessels, increasing ROS, and determining vasoconstriction [43]. Several mechanisms involved in VEGF inhibitors-induced hypertension were also described: vascular remodeling along with a decrease in microcapillary density (vascular rarefaction) that subsequently leads to an increased systemic vascular resistance, increased endothelin-1 levels, or autonomic nervous system toxicity [44].

Other side effects are heart failure, acute vascular events (arterial events such as aortic dissection, stroke, vasospasm, arterial and venous thrombosis, and thromboembolism), and significant QTc prolongation [42,45–49].

Even if VEGFi associated FOLFOX regimen has been efficient in HNPCC-associated colorectal cancer, this is not a specific treatment. Lynch syndrome is associated with microsatellite instability and deficient mismatch repair, and specific immunotherapy with immune checkpoint blockers is highly efficient. Molecules such as Pembrolizumab (anti-PD-1 antibody, FDA approved in 2017) and Nivolumab [50] are available in the US and might come into current practice and national guidelines in Romania in the following years. In animal studies, PD-1 deletion causes autoimmune myocarditis in murine models, which was confirmed in some human trials. Anti-PD-1 treatments promote myocardial infiltration with both CD4+ and CD8+ T cells, with an increased activation level of the latter (which are also called cytotoxic lymphocytes). This increases the risk of autoimmune reactions in the myocardium and a dysregulated cellular metabolism, with proteomal alteration [51]. More PD-1 inhibitor-associated cardiovascular side effects are arrhythmias, cardiomyopathy (such as dilatative cardiomyopathy or Tako-Tsubo syndrome), pericarditis, or myocardial/cerebral ischemia. The proarrhythmic mechanism is considered to be autoimmune when myocarditis causes intracardiac conduction system alterations. This can lead to atrioventricular blocks. Another proarrhythmic mechanism is indirect. Immune checkpoint inhibitors can cause autoimmune thyroiditis which is associated with atrial fibrillation. The monitoring of cardiac side effects is very important, as CV events have a significant mortality. Tropoponin is a sensitive test for ICI-associated myocarditis, however non-specific, particularly in late-presentation patients [52,53]. Higher cardiovascular toxicity has been reported in dual ICI-therapy-treated patients (ipilimumab and nivolumab) [54–57]. Another important side effect that has been documented in immune checkpoint inhibitor anticancer therapy has been vasculitis, the most frequent one being temporal arteritis [58].

Endometrial cancer is also a frequent Lynch syndrome-associated neoplasia, with an incidence similar to CRC in women with Lynch syndrome. The chemotherapy regimens required to treat these malignancies are extremely potent, however, the patients develop significant side effects. The classes used are more varied than in CRC. Alongside already discussed alkylating agents, VEGFi, and platinum salts, we mention:

- Anthracyclines (such as Doxorubicin);

- Immune checkpoint inhibitors (Pembrolizumab, Dostarlimab);

- Taxanes (Paclitaxel, Docetaxel);

- HER2-inhibitors (Trastuzumab);

- TKI (Pazopanib, Lenvatinib).

These classes are not used at the same time, however, the arsenal of agents that can be used is more vast and the cardiovascular side effects must be taken into consideration. NCCN guidelines recommend cisplatin + radiotherapy + carboplatin/paclitaxel in chemoradiation therapy and carboplatin + paclitaxel + another agent (pembrolizumab or dostarlimab or trastuzumab depending on the stage of the tumor or its' HER-2 status).

Anthracyclines are frequently associated with left ventricular dysfunction and act by altering topoisomerase enzymes. These enzymes are responsible for DNA unwinding to allow nucleic acid replication. When topoisomerases are inhibited, tissue growth is impaired, causing oxidative stress pathways to become more active (such as MAPKs or SAPKs), which are important in cell survival, leading to programmed cell death in many tissues, such as the myocardium. [59] There are several other mechanisms proposed for anthracycline cardiotoxicity, such as impaired mitochondrial function, calcium homeostasis dysfunction, and autophagy. Moreover, it downregulates DNA-methyltransferase 1 (DNMT1), leading to hypomethylation and mitochondrial gene dysfunction (peroxisome proliferator-activated receptor γ coactivator-1-α/PGC-1a, nuclear respiratory factor 1/NRF-1, mitochondrial transcription factor A/TFAM). In cardiac cells, oxidative stress associated with doxorubicin damages the CKMB isoenzyme, generating peroxynitrite free radicals. Furthermore, cardiomyocyte apoptosis is stimulated by increasing heat shock factor 1 (HSF-1) which induces heat shock protein 25 (HSP-25). HSP-25 induction stabilizes the p53 protein, generating proapoptotic factors such as FasL, Fas, and c-Myc [60,61].

Taxanes can cause edema, bradyarrhythmias, or hypotension. They frequently potentiate anthracycline toxicity by increasing plasma levels of the latter, but may directly cause cellular lesions in cardiac cells by microtubule alterations, leading to an ineffective cytoskeleton required for cell regulation and proliferation [62]. In animal models, Paclitaxel increased TNF-α and Jun N-terminal kinase JNK expression, leading to cardiomyocyte apoptosis [63].

HER-2 inhibitor Trastuzumab (which is the prototype of class 2 chemotherapy drugs regarding cardiovascular toxicity) is associated with left ventricular dysfunction or heart failure. This is very important in anthracycline-treated patients, as they cause increased expression of HER2-HER4 survival pathways which are targeted by Trastuzumab, leading to cardiac dysfunction by ineffective HER2-HER4 pathways [64]. One suggested mechanism that has been observed in animal models is ferroptosis, which is a form of iron-induced cell death. Herceptin causes increased cellular in mitochondrial ROS and altered protein expression of glutathione peroxidase and recombinant solute carrier family 7 member 11 (SLC7A11) which were reversed by ferroptosis inhibitor ferrostatin-1 [65]. Another observed mechanism of HER cardiotoxicity has been by trastuzumab phosphorylation of HER1 and HER2 which triggers the autophagy-associated inhibitory Erk pathway in human cardiomyocytes, leading to cellular toxins accumulation in these cells [66]. However, the most likely mechanism of Herceptin cardiotoxicity is binding to an extracellular domain of HER2, inhibiting HER2-ErbB4 dimerization, which downregulates HER2 expression and triggers signaling of the PI3K-AKT-mTOR pathway. This pathway is involved in cell growth and proliferation. Furthermore, in anthracycline-exposed patients, myocardial cells are already undergoing apoptotic or necrotic cellular modifications, being more susceptible to trastuzumab cardiotoxicity and myocardiocyte death during HER-2 inhibitor treatment [67,68].

TKI has a wide range of side effects, ranging from hypertension or ischemia to QT prolongation, arrhythmias, or thromboembolism (venous or arterial) [69]. These effects can be explained by the complex intracellular mechanisms that can be altered in tyrosine kinase inhibitor treatment. One of these mechanisms is the increased endoplasmic reticulum stress pathways activation, inducing cellular change and increased reactive oxygen species and lipid peroxidation [70].

Endometrial cancer survivors have an overall higher cardiovascular risk, even after finishing chemotherapy. These patients have a higher incidence of arterial hypertension and vascular disease. Moreover, endometrial cancer survivors had a 47% higher risk of heart disease compared with patients with no endometrial cancer history [71]. This higher risk can be caused by early menopause induced by hormonal treatment (which is associated with increased cardiovascular risk) and surgical treatment by hysterectomy with bilateral adnexectomy (except for stage IA G1 endometrial cancer, according to the ESMO clinical practice guidelines) [72]. The cessation of estrogen cardiovascular protection leads to vascular dysfunction, arterial hypertension, and redistribution of body fat [73]. Thus, patients with Lynch syndrome-associated endometrial cancer are at significant risk of cardiovascular disease because of various factors – early menopause – surgical hormonal, and chemotherapy.

To prevent cardiotoxicity development in cancer patients, the new guidelines for Cardio-oncology recommend risk stratification tools, such as the HFA-ICOS risk stratification [20]. Risk factors and their stratification in VEGFi therapy are exemplified in Table 1.

CONCLUSION

Lynch syndrome-associated cancers are aggressive and require significant chemotherapy regimens. Some effects are transient; however, some may be permanent. This is an important reason for the necessity of introducing a consistent interdisciplinary team that includes a cardiologist and an oncologist in the management of oncological patients, especially young ones, such as patients with Lynch syndrome.

Conflicts of interest and sources of funding

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Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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REVIEW

Emotional Regulation Strategies in Lynch Syndrome Diagnosis Psychological Adjustment - A Review

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Abstract: *The purpose of this article is to investigate the role of emotional regulation (ER) as a process underlying a variety of symptoms associated with Lynch syndrome (depression, anxiety, fear of cancer recurrence (FCR), insomnia, fatigue, pain, and subjective cognitive difficulties in patients with Lynch syndrome. Firstly, we provided an overview of the main psychopathological symptoms found in cancer patients and their impact, and then an overview of emotional regulation strategies and discussed the main impact ER strategies have on cancer patients, especially emotional suppression and experiential avoidance, as found in evidence-based studies. Recent research has shown that two ER strategies generally considered maladaptive, emotional suppression and experiential avoidance were significantly associated with higher levels of anxiety, depression, FCR, fatigue, and cognitive difficulties. We propose that experiential avoidance and emotional suppression may be common transdiagnostic mechanisms for a common set of psychological symptoms in Lynch syndrome patients, a hypothesis to be tested in a future study***.**

Keywords: *emotional regulation; experiential avoidance; emotional suppression; Lynch syndrome*

INTRODUCTION

With genetic diagnosis being available for a high number of hereditary syndromes the number of families with knowledge about hereditary cancer is also increasing. Lynch Syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is a multi-cancer syndrome caused by inherited DNA mismatch-repair gene mutations that pose particularly high risks for colorectal, gynecological, stomach, small bowel, pancreas, hepatobiliary tree, brain and urinary tract cancers.

Genetic counseling provides information about traits, possibilities, and recommendations for cancer-preventive programs.

Particularly, in Lynch Syndrome, the diagnosis is requested by most members of a family and it is beneficial since, for example, colonoscopy reduces morbidity and mortality in colorectal cancer [1-5].

Family members with hereditary cancer often experience death from cancer from close relatives and it can be assumed this may represent a burden for them with studies addressing psychological well-being in those individuals with increased risk of developing cancer. Studies are primarily focusing on genetic counseling time framework and found increased levels of distress and anxiety with the return to baseline 6-12 months after the diagnosis.

10% maintained distress related to the diagnosis after the same period [6-8]. Meanwhile, there are few studies concerning the psychological impact of Lynch Syndrome diagnosis.

Thus, our research questions focused on highlighting the main psychological symptoms that are associated with cancer diagnosis and specifically, Lynch Syndrome patients. At the same time, we wanted to assess the literature concerning the main emotional regulation strategies used by patients with cancer and/or Lynch Syndrome patients.

After assessing the main variables described above our main research question concerned the possible relationship between specific emotional regulation strategies and psychopathological symptoms in cancer patients and, specifically, in Lynch Syndrome patients.

MATERIALS AND METHODS

Studies included in the review consisted of published English-language peer-reviewed articles focused on assessing correlations between various emotional regulation strategies and mental health outcomes in patients diagnosed with Lynch Syndrome. We utilized the following databases: Google Scholar, Elicit, PsycINFO, and conducted electronic searches from June 2023 to August 2023, examining articles published at any time prior to June 15, 2023. Searches were conducted with terms related to Emotional Regulation strategies (i.e., “emotional regulation,” “suppression”, „experiential avoidance” and Lynch Syndrome patients (i.e., “Lynch Syndrome,” “cancer genetic testing” “Lynch Syndrome mental health”).

The concepts of „emotional regulation strategies” and „Lynch Syndrome diagnosis” were scarcely mentioned throughout the literature thus we included broader concepts like „cancer patients”, „emotional regulation in cancer patients” and „mental health in cancer patients” to attain a broader perspective on our primary objectives. All published articles found using the above search terms and deemed to be related to the topic of focus were included based on their relevance and familiarity with the hypothesis asked.

EMOTIONAL REGULATION (ER) AND LYNCH SYNDROME

ER may play a significant role in the mental adjustment of individuals diagnosed with Lynch syndrome. It may represent a key factor in mental adaptation to this diagnosis in various contexts as follows: a) coping strategies, where ER helps individuals with Lynch syndrome cope with the stressors within the disease.

Communal coping, which involves perceiving stressors as a shared problem and mobilizing adaptive resources together, can be an effective strategy for families affected by Lynch syndrome [9]; b) psychopathology and well-being where ER strategies can be adaptive or maladaptive depending on their function, context, and timing. Effective ER can contribute to better mental well-being and reduce psychopathological symptoms in individuals with mental disorders [10,11].

Also, ER can help individuals manage the psychological impact of the condition and maintain a better quality of life; c) social support and emotional expression, where ER is closely linked to situations, for example, when individuals perceive their social network as less responsive, they may suppress their emotions and not express them openly. This emotional suppression can contribute to distress in individuals facing an increased genetic risk of cancer [12]; d) trauma and emotion regulation. Previous research suggests that trauma, including early life stress, can affect the acquisition of appropriate ER skills. Individuals who have experienced trauma may have difficulties understanding and regulating their emotions. This can have implications for individuals with Lynch syndrome who may have experienced traumatic events related to their condition [13,14].

Overall, emotional regulation may play a crucial role in the mental adaptation of individuals with Lynch syndrome. It may help them cope with stress, maintain mental well-being, navigate social support, and manage the psychological impact of their condition. Developing effective emotional regulation skills can contribute to better mental health outcomes for individuals with this diagnosis.

MENTAL HEALTH AND LYNCH SYNDROME

Lynch syndrome can have a significant impact on the mental health of affected individuals and their families. Individuals, whether they have had cancer, may experience an increase in depression and anxiety scores post-disclosure [15]. A study found that 10-12% of Lynch syndrome carriers displayed depressive symptoms [16].

Long-term psychosocial and behavioral adjustment in individuals receiving genetic test results in Lynch syndrome showed that participants who underwent genetic testing for Lynch Syndrome (LS) had higher levels of anxiety and depression compared to those who did not undergo testing [17]. Also, individuals with Lynch syndrome may experience long-term psychosocial and behavioral adjustment issues [17]. This can include difficulties with coping, emotional regulation, and social support.

An important part of the mental health impact factors is represented by the structure of emotional support networks in families, which can be affected. Communal coping, which involves perceiving stressors as a shared problem and mobilizing adaptive resources together, can be an effective strategy for families affected by LS [17,18]. However, if individuals perceive their social network as less responsive, they may suppress their emotions and not express them openly. This emotional suppression can contribute to distress in individuals facing an increased genetic risk of cancer, such as LS [19]. Regarding a study found that individuals with LS may have a lower sense of coherence (SOC) and self-concept. Low SOC has been found to be associated with anxiety and depression after genetic counseling [20].

In conclusion, LS can have a significant impact on the mental health of affected individuals and their families. It can lead to depression, anxiety, difficulties with psychosocial and behavioral adjustment, social support, and self-concept.

PSYCHOPATHOLOGICAL SYMPTOMS ASSOCIATED WITH CANCER AND CANCER TREATMENTS

Anxiety and depression

In the oncological context, psychological suffering is defined as a multifactorial and unpleasant experience of a psychological nature (either cognitive, affective, or behavioral), social or spiritual, which may affect the ability to cope effectively with cancer, physical symptoms, and its treatments [21].

Psychological suffering occurs along a continuum that varies from feelings of vulnerability, sadness, and concern about the disease, which are normal responses to cancer, to problematic and disabling symptoms such as depression, anxiety, and adaptive disorders [21].

Symptoms of a depressive disorder include a depressed mood and/or decreased interest in or pleasure in activities, accompanied by fluctuations in weight or appetite, sleep disturbances, psychomotor agitation or slowness, fatigue or loss of energy, feelings of worthlessness or excessive or inappropriate guilt, concentration difficulties, and recurring thoughts of death or suicidal [22].

Anxiety disorders, on the other hand, manifest themselves in the form of cognitive disorders (e.g., attentional bias toward threatening stimuli), affective (e.g., anxiety, nervousness), behavioral (e.g., avoidance), and physical (e.g., muscle tension). Symptoms of depression and anxiety are often experienced on a transient basis, which is part of the normal process of adapting to cancer. However, the simultaneous occurrence of several symptoms (5 of 9, including one of the first two in the case of major depression), accompanied by clinically significant distress or marked impairment of the person's social, occupational, or other functioning, indicates the presence of a psychological disorder [22].

When a person experiences excessive psychological suffering in relation to what is considered normal or which significantly affects normal functioning, within three months of exposure to an identifiable stressor, such as LS, an adaptation disorder may be diagnosed. Adaptation disorder may be accompanied by depressive and/or anxiety symptoms (but may not meet the criteria for a depressive disorder and/or anxiety or other emotional and behavioral disorders [22].

Prevalence

According to a meta-analysis of 70 studies of 10,071 patients with different types of cancer, 38% of patients suffered from depression, an anxiety disorder, or an adjustment disorder [23]. In comparison, the annual prevalence of major depressive disorder in the general population is 4% [24].

Fear of recurrence/Fear of cancer recurrence (FCR)

It is a more specific form of anxiety or concern about the idea that the disease/cancer returns or progresses [25]. In the original formulation [26] FCR is not an exclusively emotional phenomenon. It is rather a multidimensional construct in which the emotion of fear can result from negative interpretations or cognitions about cancer, which in turn would be triggered by certain internal causes (for example, physical symptoms) or external (for example, a cancer television program [26,27,28]. FCR can lead to maladaptive reactions (for example, anxiety concerns, avoidance, self-examination, and the search for excessive reassurance), which in turn are likely to increase the extent of this FRC [26]. Most patients consider this symptom to be a major concern and declare that they have not received enough help to manage it [29].

Prevalence

Due to the lack of consensus on the definition and measurement of FRC, the prevalence rates of this symptom vary considerably in the literature, ranging from 24% to 70% in breast cancer patients [30,31]. FRC is reported to be higher in younger patients, who have more physical symptoms related to cancer or its treatment, or who have a less favorable prognosis (head or neck cancer, cancer recurrence, more advanced stage of cancer) [32,33].

Fatigue

Several authors identify this symptom as being the most reported one in relation to cancer and its treatments, regardless of the location or treatment received [34]. Fatigue is present during the entire course of the disease [35]. The most commonly used definition of cancer-related fatigue is that provided by the National Cancer Comprehensive Network (NCCN), which describes fatigue as a subjective and persistent feeling of exhaustion associated with cancer or oncological treatments that is disproportionate to activity level (the level of daily activity) and that does not subside with rest and interferes with usual daily activities [35,36]. Fatigue affects between 38% and 84% of breast cancer (female) patients during cancer treatment and up to 40% after treatment ends [37,38].

Insomnia (I)

This is the most reported sleep disturbance in cancer patients [39]. It is characterized by dissatisfaction related to sleep or sleep quality, which may include difficulty falling asleep, maintaining sleep through the night, or waking in the morning with an inability to fall asleep again [22]. When symptoms of insomnia are severe (minimum 30 minutes to fall asleep or nighttime awakenings) occurring at least 3 nights per week and more than 3 months and recurring despite adequate opportunities for sleep and eliciting marked distress or significant impairment in functioning, then a sleep disorder may be assumed [22]. Insomnia can occur independently (primary insomnia) or in comorbidity with another sleep disorder, with another psychopathological disorder (e.g. depression), or can be related to the effects of a substance (e.g. caffeine) or to a disease such as cancer (comorbid insomnia).

Prevalence in the overall population, 30% of people have insomnia symptoms, while 10-15% have insomnia syndrome [40,41]. In breast cancer patients, these rates range from 16% to 58% for insomnia symptoms and 26% to 46% for insomnia syndrome [42,43].

Pain (P)

Pain is a multifaceted phenomenon resulting from a complex interaction between different physiological (e.g. tissue damage), psychological (e.g. psychological stress related to pain), cognitive (e.g. the meaning attributed to pain), and social factors. Pain can be caused by the tumor itself, by its metastases (e.g., bone), but also due to oncological treatments [44,45].

Prevalence

Between 44% and 64% of breast cancer patients report pain during or after their oncological treatment [46,47]. Pain is thought to increase with disease progression, affecting up to 64% of patients with advanced or terminal cancer. More than a third of patients who experience cancer-associated pain describe its intensity as moderate or severe [47].

Cognitive difficulties (CD)

Cognitive functioning includes a range of functions such as verbal and non-verbal memory, language, attention and concentration, psychomotor functioning, reading, and writing. These include reasoning, problem-solving, mental flexibility, and working memory [48]. These functions are assessed either objectively using standardized neuropsychological tests or subjectively using self-assessment questionnaires. Cognitive difficulties may be related to cancer either directly (e.g. tumor or metastases located in the central nervous system) or indirectly (e.g. metabolic or endocrine abnormalities, infections, and oncological treatments such as chemotherapy; [49]. The main cognitive difficulties reported by patients following cancer treatments include difficulties in learning added information, memory problems, and decreased attention and concentration [50,51,52]. It should be noted that, in general, both objective and subjective measures of cognitive functioning in cancer patients are poorly associated with each other [53]. Moreover, subjective complaints of cognitive impairment, rather than the results of objective neuropsychological tests, are more closely correlated with depression, anxiety, and fatigue. Therefore, it seems that cognitive performance and perception of one's cognitive abilities are two different phenomena. In this study, only subjective cognitive difficulties will be evaluated.

Prevalence

Between 21% and 90% of breast cancer patients report subjective cognitive difficulties [52]. Considering all types of cancer combined, the highest proportions of cognitive difficulties are observed during oncological treatment [54]. While some results indicate that cognitive functioning returns to normal 6 to 12 months after the end of treatment [55], others suggest that cognitive difficulties may persist for up to 10 or even 20 years after the end of treatment [56].

THE IMPACT OF PSYCHOPATHOLOGICAL SYMPTOMS ASSOCIATED WITH CANCER AND CANCER TREATMENTS

Psychopathological symptoms associated with cancer and oncology therapy have consequences for patients. Depression, anxiety, fear of cancer recurrence, fatigue, insomnia, pain, and cognitive difficulties are all associated with poorer quality of life and impaired daily functioning [57,58]. The presence of certain symptoms, such as depression and fatigue, is also related to the increased use of healthcare [57]. In addition, depressive symptoms, hopelessness, fatigue, and poorly controlled pain are risk factors for early death wishes and /or suicidal thoughts and actions in cancer patients [59,60].

Emotional Regulation (ER)- concept definition and strategies

Emotional regulation (ER), which has been the subject of increasing interest in the field of clinical psychology in recent years, maybe a psychological mechanism that could contribute to the etiology of psychological symptoms associated with cancer. In fact, problems related to emotions or emotional regulation characterize about 75% of the mental disorders listed in the Diagnostic and Statistical Manual of Mental Disorders [61]. An increasing number of studies suggest that difficulties with ER play a central role in developing and maintaining psychopathology [62,63,64].

Some psychopathologies such as anxiety and mood disorders are diagnosed primarily based on emotional disorders, including a predominance of negative affect [65]. ER also influences perception and adaptation to acute and chronic pain, fibromyalgia, and chronic fatigue [66-69].

Emotional problems are also considered to be associated with insomnia. On the one hand, emotions that have not been adequately regulated during the day tend to reappear when it is time to go to bed, fueling concern and anxiety and also affecting sleep and creating hyperactivation that is harmful to sleep [70]. On the other hand, sleep problems could be harmful to the emotional regulation of the next day by creating emotional hyperactivation [71]. This suggests that emotional regulation problems could underpin several symptoms associated with cancer, a hypothesis that has not yet been studied [61].

Emotional problems are also thought to be associated with insomnia. On the one hand, emotions that have not been adequately regulated during the day tend to resurface when it is time to go to sleep, fueling concern and anxiety affecting sleep, and creating hyperarousal that is detrimental to sleep [70,71]. On the other hand, sleep problems could be detrimental to daytime emotional regulation by creating affective hyperactivity [70]. This suggests that problems with emotional regulation may underlie more symptoms associated with cancer, a hypothesis that has not yet been studied [61].

To fully understand ER, it is important to first define the concept of emotion. The definition varies greatly in the literature, but different authors seem to agree on three general points. First, emotions involve changes in the fields of subjective experience, behavior, and physiology. Second, emotions are dynamic phenomena that occur over a period of time that varies from a few seconds to a few minutes [72].

Third, the same emotion can be beneficial or harmful, depending on the context [72]. Fear, which leads to the avoidance of a potentially serious threat, and joy, which strengthens social relationships, are examples of beneficial emotions that adapt to their context [72].

Emotions are harmful when their intensity, duration, frequency or type are inadequate to the situation and when they give rise to maladaptive behaviors or cognitions.

The modal pattern of emotions [72] schematically illustrates how the situation, attention, evaluation appraisal and response follow one another. First, an emotion is directed towards a specific object: it has a trigger, or external situational antecedents (e.g. watching a sad movie) or internal antecedents (e.g. anticipation of an exam). Second, the trigger must receive the person's attention for there to be an emotional response. Third, the trigger is assessed according to the person's priorities (e.g. passing an exam, pleasing the other person). Fourth, several physiological, behavioral, and experiential systems coordinate to produce an emotional response. Fifth, emotion is flexible, meaning that it can be overridden (nullified) by other imperatives or responses to the situation. This flexibility is what allows emotion to be regulated [61].

ER refers to the strategies by which individuals influence the nature of the emotions they experience, as well as when and how they experience these emotions [61,72]. ER strategies can be automatic or controlled, conscious or unconscious, and can occur at any point in the sequence of the production of the emotional response illustrated in the modal pattern of emotion [61]. ER can be used to reduce, maintain, or even increase the intensity of an emotion [61].

ER has some similarities to coping strategies. Adaptation is defined as the cognitive and behavioral efforts that are undertaken to cope with external or internal demands deemed to exceed the individual's resources. Both ER and coping are processes for regulating affective processes [72]. However, coping is generally defined as a global reaction that occurs in response to a stress factor and includes a set of reactions aimed at both changing a stressful situation, as well as stress regulation related to the stressor. ER, on the other hand, incorporates reactions to specific emotions, both positive and negative, that occur in a variety of contexts, including stressful situations [72]. Both ER and coping are dynamic processes that change over time [73]. Nevertheless, compared to ER, which occurs more limited in time, coping is described as a process that can take place over a long period of time (for example, coping with mourning over several months). As mentioned above, ER may include both automatic and conscious processes, while coping is considered to primarily reflect conscious processes [73]. In addition, while ER strategies may occur just before an emotion is generated, (for example, by avoiding exposure to a situation), coping occurs after exposure to stress [72,73]. ER and coping would therefore be separate constructions that are partially juxtaposed, their main common ground is that they involve efforts to regulate emotions in response to stressful events or circumstances [73]. ER strategies can target different components of the emotional response, namely the experiential (subjective experience of the emotion), behavioral (e.g. facial expression specific to an emotion such as disgust, fear, or joy), and physiological (e.g. activation of the sympathetic nervous system [72]. ER strategies were classified into five groups, based on the point in the emotional response sequence at which they occur. Situation selection strategies refer to the decision whether or not to expose oneself to a situation that might elicit an emotion, (i.e.) which is the choice to avoid or go to certain places, people, or activities. Subsequently, coping strategies make it possible to act on the situation to change its emotional impact.

Attentional deployment strategies involve directing attention to a specific aspect of the situation. Cognitive reappraisal strategies involve changing the meaning of the situation that the person attributes to it in order to change the associated emotions. These four types of strategies are initiated before the emotional response is fully deployed (e.g., behavioral change or physiological activation), as opposed to the fifth type of strategy, response modulation strategies, which aim to modify the emotional response already produced.

Each type of strategy can be used adaptively or maladaptively, depending on the context in which it is used [72]. The later ER occurs in the sequence of producing the emotional response, the more effort it requires [61]. Intervention as early as possible in the sequence of producing the emotional response would make it possible to adjust the emotion more efficiently [74]. ER is maladaptive when it does not alter the emotional response in the desired way (e.g., does not reduce negative affect), when the long-term costs of a strategy (e.g., disruption of functioning, interpersonal relationships) exceed the benefits associated with short-term emotional changes, or when the strategy used is not contextually appropriate [61].

ER can be evaluated either subjectively, using self-assessment questionnaires, or objectively, using physiological indicators, such as heart rate variability. The ER strategies that have been most studied in both the general population and cancer patients are cognitive reappraisal, suppression of emotional expression, and experiential avoidance [61].

Cognitive reappraisal

Cognitive reappraisal involves changing the appreciation of a situation to reduce or increase its emotional intensity [61]. In Gross and Thompson's model, this strategy occurs before the emotional response is fully unfolded [75].

In the general population, the use of reappraisal is associated with higher levels of effectivity, lower levels of negative affect, fewer depressive symptoms, higher self-esteem, greater satisfaction with life, better interpersonal functioning, and increased well-being [61]. In patients suffering from depression, less frequent use of cognitive reappraisal has been associated with higher levels of depressive symptoms [76]. Other results indicate that this strategy moderates the relationship between stress and depressive symptoms, in that, in high-stress situations, the use of cognitive reappraisal makes it possible to regulate negative emotional states likely to lead to depressive symptoms [77]. In addition, reappraisal has a low impact on physiological activation and cognitive resources [61]. It is also associated with lower levels of inflammation and reduced risk of cardiovascular disease [78]. Therefore, cognitive reappraisal is considered a generally appropriate CR strategy [79]. Learning this strategy is also a key element of several psychotherapies, including cognitive-behavioral therapy [80]. Although several clinical trials have demonstrated the effectiveness of CBT in reducing anxiety, depression, pain, insomnia, and fatigue in cancer patients [81], the specific impact of cognitive reappraisal on a range of psychological symptoms associated with cancer has been poorly studied. Only one study investigated the association between this strategy (Emotion regulation questionnaire; ERQ) and anxiety and depression in 144 patients with different cancers [82]. Lower levels of cognitive reappraisal were cross linked to more anxiety and depression three months after cancer diagnosis. Other studies have evaluated the effect of a subtype of cognitive reappraisal, positive reassessment, which involves changing one's evaluation of a situation so as to give it a positive meaning [83].

In a prospective study of a sample of 509 women with breast cancer, the use of cognitive reappraisal was evaluated using the Cognitive Emotion Regulation Questionnaire, a questionnaire that assesses various cognitive ER strategies under stressful conditions (including positive reappraisal). Positive reappraisal and depressive symptoms were assessed five to seven days after oncological surgery (T1), and then depressive symptoms were re-evaluated one month later (T2). Higher levels of positive reappraisal at T1 were associated with lower levels of depressive symptoms at T2. In a cross-sectional study it was analyzed the effect of certain social strategies (avoidance of talking to loved ones about cancer concerns and unsupportive behaviors from loved ones) and cognitive strategies (positive reappraisal, self-esteem, and confidence in one's ability to cope with cancer; COPE inventory) on CRP and depressive symptoms in 150 women diagnosed with gynecological cancer. Self-esteem has significantly mediated the relationship between CRP and depressive symptoms. A higher level of positive reappraisal has been associated with lower depressive symptoms, but this strategy was not significantly associated with CRP [84].

Results from another cross-sectional study that included 353 patients diagnosed with breast cancer showed a weak correlation between positive reappraisal (Mini-Mental Adjustment to Cancer Scale) and cancer-related fatigue [85]. Nevertheless, this association was significant only in a multivariate analysis model that also included age, depression, and grief: the unique relationship between positive reappraisal and fatigue was not significant. Finally, in another study, positive reappraisal (COPE Inventory), positive mood (Vigor subscale of the Profile of Mood States) and psychological distress (Anger, Depression, Tension, Fatigue and Confusion subscales of the Profile of Mood States Scale) were measured in 92 breast cancer patients at three-time points, namely 20 weeks after the end of oncological treatment (T1), at 3 months (T2) and 12 months (T3). The higher levels of positive reappraisal in T1 significantly predicted an improvement in the positive mood at subsequent measurement time points in participants who had low or medium levels of positive mood at T1. Positive reappraisal did not predict psychological distress.

In conclusion, existing studies suggest that cognitive reappraisal is an adaptive and cognitively somewhat expensive ER strategy. It is associated with lower levels of psychopathology (depression and anxiety) in the general population. In cancer patients, only five studies have evaluated the possible impact of this strategy. The results are contradictory and the literature has its limitations. First, only one study specifically investigated cognitive reappraisal in this population, while the others focused on a subtype of cognitive reappraisal, namely positive reappraisal. Thus, little is known about the impact of cognitive reassessment in general on adaptation to cancer. Results in the general population suggest that this strategy may be associated with better adaptation to cancer, but more studies are needed on this question. In addition, the existing literature is limited to depression, anxiety, CRF, and fatigue. The relationship between cognitive reappraisal and other isolated symptoms (pain, insomnia, cognitive difficulties) remains unknown, as does its association with symptom clusters.

Emotional suppression (ES)

Emotional suppression is defined as an individual's effort to reduce an unwanted emotional experience. Specifically, suppression consists of inhibiting behaviors related to emotional expressions, such as facial expression (e.g., maintaining a neutral face, suppressing crying or laughing) or verbal expression (e.g., avoiding mentioning the experienced emotion) in order not to communicate to others information about the emotional states of the person [86]. This strategy also includes suppressing thoughts associated with emotional reactions [87]. Suppression is not a highly effective RE strategy and involves various short- and long-term costs. Although this strategy may reduce the subjective experience of positive emotions, it has no effect on the subjective experience of negative emotions [72]. In many cases, suppression has the paradoxical effect of increasing the negative emotions that the individual is trying to suppress [88]. Therefore, suppression is associated with important levels of negative affect, reduced levels of positive affect, poorer social functioning, and a diminished sense of personal well-being [72]. It can also lead to cognitive overload, reduced cognitive performance, and impaired memory and attention [87,88]. Suppression would be less effective and require more effort than cognitive reassessment [72]. In addition, it appears that suppression is used more frequently in people with a mood or anxiety disorder than in those without a psychological disorder [89]. Several studies also associate suppression with poorer adjustment to pain and chronic fatigue syndrome, increased risk of insomnia, higher levels of inflammation, and an increased risk of cardiovascular disease [90]. Suppression may also be associated with an increased risk of death (cancer, cardiovascular mortality, and all-cause mortality), although more studies are needed to confirm and explain this association [91]. Conversely, a good ability to flexibly regulate (i.e., suppress and enhance) the expression of joy and sadness has been associated with less interference with physical symptoms (e.g., headaches, heart problems). Although the individual using suppression seeks to reduce the intensity of the emotions felt, this strategy is often associated instead with activation of the sympathetic nervous system, manifested by changes in skin conductance and temperature as well as the pulse amplitude at the finger [72]. For example, 180 participants were asked to watch either emotionally charged or uncharged movies (sad, neutral, and funny) in the usual way or using suppression. Suppression was not associated with any physiological effect for the neutral movie, but for the amusing one, it was associated with an increase in sympathetic activity as measured by digital pulse amplitude, finger pulse transmission time, and digital temperature, and a reduction in heart rate. For the sad film, the suppression was associated with an increase in skin conductance and sympathetic and respiratory activation [92].

In cancer patients, the results of the few studies available indicate that suppression is generally associated with higher psychological distress. In a cross-sectional study previously described, higher suppression levels were associated with higher levels of anxiety and depression [82]. A study evaluated the relationship between suppression (measured with the Courtauld Emotional Control Scale), anxiety (Manifest anxiety scale), and emotional distress (Mood profile scale) in 93 women who completed questionnaires before and after being diagnosed with cancer or benign tumor to the breast. High levels of anxiety and suppression measured before cancer diagnosis have been associated with significantly higher scores of emotional distress before and after diagnosis. Of the 21 participants diagnosed with cancer, those with higher levels of suppression before diagnosis reported more psychological suffering after diagnosis. The same researchers then evaluated the effects of suppression on the negative emotions of 14 breast cancer patients on their first visit to the outpatient clinic, immediately after being diagnosed with cancer, after surgery, and at a follow-up visit three months later [93]. Participants with higher levels of suppression measured before diagnosis reported more general psychological distress, anxiety, depression, and anger at all measurement times except immediately after surgery. In addition, higher levels of suppression before diagnosis were associated with more fatigue after diagnosis. In another prospective study involving 31 breast cancer patients, suppression (Courtauld Emotional Control Scale) and psychological distress (Mood Profile Scale) were measured before surgery and between one and six months after surgery. A higher level of suppression measured before surgery was associated with more psychological distress after surgery (higher scores on stress-anxiety, depression, anger-hostility, fatigue-inertia, confusion, and total scores subscales) [94]. A similar cross-sectional association between suppression (Courtauld Emotional Control Scale) and psychological distress (all subscales of the Profile of Mood States Scale except the anger subscale) was obtained in 27 breast cancer patients who had completed their oncological treatments. High levels of suppression have also been associated with stress symptoms (for example, muscle tension, and sympathetic activation), assessed by the Calgary Stress Symptoms Inventory (C-SOSI) [95]. The results of another cross-sectional study in 139 patients in remission from various cancers for at least five years showed an association between suppression (Control Courtauld scale emotional) and higher levels of stress (Perceived stress scale), anxiety, and depression (Hospital anxiety and depression scale; HADS) and poorer adaptation to cancer (Mini-Mental scale for cancer adaptation) [96]. Suppression predicted the level of perceived stress, even after controlling depressive symptoms. However, contradictory data were also obtained. In a study that included 241 patients with different types of cancer, suppression (Courtauld Emotional Control Scale) has been associated with less self-reported psychological suffering on Distress Thermometer and HADS (total scale and depression subscale, but not on the anxiety sub-scale) [97]. To explain this unexpected result, the authors suggest that the reluctance to report psychological suffering is consistent with the use of suppression because completing a questionnaire about psychological symptoms can be perceived as an emotional manifestation. For the time being, these results have not been replicated and this hypothesis has not received empirical support.

In summary, the literature about the general population shows that suppression is associated with increased symptoms of psychological distress, pain, fatigue and insomnia, cognitive difficulties, and activation of the sympathetic nervous system. In the oncological context, several studies have investigated the relationship between suppression and psychological suffering, stress, anxiety, depression, and fatigue in a transversal and prospective manner. Therefore, these results suggest that suppression could be involved in the development of psychological and psychophysiological symptoms associated with cancer. However, the number of studies that have investigated the role of suppression in the development of cancer-associated symptoms is limited, and these studies are restricted to symptoms of depression, anxiety, and fatigue. The relationship between suppression and other symptoms that are highly prevalent in people with cancer, such as pain, insomnia, CRP, and cognitive difficulties, remains unknown. Finally, existing studies have assessed the relationship between suppression and isolated symptoms: the relationship between suppression and symptom clusters does not appear to have been studied up to the present time.

Experiential avoidance

Experiential avoidance is defined as a tendency to avoid or flee from certain psychological experiences (e.g., emotions, but also thoughts, physiological sensations, memories, impulses, and cognitions) by attempting to alter their form, frequency, or intensity, even when these attempts are futile or interfere with the individual's goals or values [98]. Experiential avoidance incorporates strategies like those used in emotional suppression. However, while emotional suppression is clearly an emotional response modulation strategy, experiential avoidance can be used at any point in the emotion production sequence [98]. In this respect, experiential avoidance is a broader concept that involves the use of strategies relevant to the five groups identified [72], namely situation selection (e.g., avoiding starting an apprehensive project), situational modification (e.g., using reassurance from a friend or family member), and situation modulation (e.g., use of a moderator to modulate the emotional response), use of reassurance by an anxious person to tolerate an uncomfortable situation), attention deployment (e.g., avoiding paying attention to thoughts, sensations or memories likely to provoke unpleasant emotions), cognitive reappraisal (e.g., attempting to rephrase a thought associated with an emotion): (e.g., "This university interview is a test of my value as a student and as a person "and replace it with "This interview is an opportunity for me to learn more about this university and see if it is really right for me") and response modulation (e.g., attempts to control verbal, behavioral, and physiological expression of emotions, including emotion suppression) [98]. In the general population, experiential avoidance is associated with poorer quality of life, poorer perceived physical health, and higher levels of general psychopathology, depression, anxiety, and substance abuse [99]. This strategy is even considered by several authors to be a fundamental mechanism in the development and maintenance of psychopathology [88]. A study evaluated the relationship between experiential avoidance (acceptance and action questionnaire), cognitive suppression and reevaluation (Emotion regulation questionnaire), and several indicators of psychological well-being and psychopathology [88]. Results suggest that experiential avoidance is a better predictor of psychological distress and anxiety symptoms than suppression and cognitive reappraisal. In addition, experiential avoidance mediated the relationship between suppression and reappraisal, on the one hand, and positive and negative daily experiences and functioning, life satisfaction and social anxiety, on the other. It is also interesting to note that the results from clinical trials suggest that reducing experiential avoidance is a significant mediator of the effectiveness of mindfulness-based therapies and acceptance and commitment therapy for several mental (e.g., stress) and physical (smoking cessation) health problems [98].

Some studies already assessed the possible influence of experiential avoidance on cancer-related symptoms [100,101] In a clinical study aimed to measure the effect of a group psychological intervention targeting anxiety, depression, and stress, 54 breast cancer patients completed the Acceptance and Action Questionnaire (AAQ-II), which measures experiential avoidance before and after the surgical intervention. A smaller decrease in experiential avoidance between the period before and after treatment predicted a weaker improvement in depressive symptoms and anxiety. Other papers have focused on acceptance, an ER strategy that is thought to be the opposite of experiential avoidance [102].

CONCLUSIONS

Although there is limited information on LS emotional regulation interventions. some studies have explored the structure of emotional support networks in families affected by Lynch syndrome and others have mapped psychosocial interventions in familial colorectal cancer. One study suggested that practitioners may assess cancer-risk-related distress before genetic testing and offer distressed individuals interventions focused on changing emotion regulation [105-107]. Another systematic review of studies involving families with BRCA1, BRCA2, and LS found that proband-mediated interventions, such as genetic counseling with additional telephone or in-person follow-up, letters, videos, and decision aids, have the potential to increase disclosure of genetic risk in families with a BRCA or LS condition [108]. However, none of these studies specifically focused on emotional regulation interventions for LS. It is worth noting that some studies have explored emotion regulation in the context of cancer survivorship more broadly. For example, researchers have called for the study of emotion regulation in breast cancer survivorship [109]. The Difficulties in Emotion Regulation Scale (DERS) and the Emotion Regulation Questionnaire (ERQ) are two tools that have been used to assess emotion regulation strategies [109]. However, these studies are not specific to LS.

Future perspectives

Cancer patients suffer from a range of psychological symptoms (depression, anxiety, CRF, insomnia, fatigue, pain, and subjective cognitive difficulties). Several authors suggest that a common psychological mechanism may contribute to the development and maintenance of symptoms, but no study has investigated this hypothesis so far. One possible underlying mechanism is ER, which has been the subject of increasing interest in clinical psychology in recent years. One possible underlying mechanism is RE, which has been the subject of increasing interest in clinical psychology in recent years. Previous work has shown that two RE strategies, emotional suppression (inhibition of emotion-related thoughts and behaviors, such as facial expression of emotions) and experiential avoidance (avoidance of feelings, physical sensations, and emotion-related thoughts), have been associated with anxiety, depression, and fatigue in patients treated for cancer. In contrast, cognitive reappraisal, i.e. changing the meaning associated with a situation to alter its emotional load, appears to be associated with better adaptation to cancer, in particular with fewer depressive and anxiety symptoms. Therefore, studies are needed to determine the role of ER as a common psychological mechanism for a broader set of cancer-associated symptoms, both cross-sectionally and prospectively. The study of psychological mechanisms that may underlie clusters of symptoms associated with cancer is relevant both theoretically and clinically. From a theoretical point of view, it is necessary to better understand why and how these symptoms develop. Clinically, several authors have emphasized that it is vital to understand the processes underlying the emergence of symptom clusters in order to provide more effective treatments [110]. For example, transdiagnostic psychological treatments for psychopathologies (for example, anxiety and depressive disorders) are innovative interventions that are of growing interest to psychologists and clinicians. These treatments make it possible to treat multiple comorbid psychological disorders simultaneously because they target the affective, cognitive, and behavioral processes underlying the disorders (e.g., avoidance, rumination, and suppression, which are found in a variety of anxiety and mood disorders), rather than targeting each disorder individually [111]. The effectiveness of several transdiagnostic treatment protocols developed for mood and anxiety disorders has received a great deal of empirical support [112]. However, these protocols cannot be applied directly to cancer patients, as they do not include certain symptoms common to this population (e.g., CRF, fatigue, pain, insomnia, and cognitive difficulties).

Identifying a mechanism underlying psychological disorders in LS would be the first step in adding real help to classical treatments.

Author Contributions

ML and CGI contributed to the conception, and structure of the paper, and contributed to the analysis, and interpretation of available literature. FG and CGI contributed to the development initial draft. ML and FG reviewed and critiqued

the output for important intellectual content. All the authors contributed to the article and approved the submitted version.

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ORIGINAL ARTICLE

Overview of the Genetic Aspects of Lynch Syndrome

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Abstract: *Lynch syndrome (LS) is one of the most common inherited cancer predisposition syndromes and the most important cause of hereditary colorectal and endometrial cancers. It is inherited in an autosomal dominant pattern, caused by a pathogenic germline variant in one of the mismatch repair (MMR) genes (MLH1, MSH2, MSH6, or PMS2), which encode proteins responsible for maintaining genome stability. A defective MMR system confers an elevated risk of developing certain types of cancer, at a younger age than in the general population and at a high rate of multiple primary neoplasia. Lynch syndrome is not just one disease, but a collection of different subtypes of underlying molecular aspects with specific clinical implications. A better understanding of these subgroup profiles could offer better care for each patient, leading to more personalized risk-reduction and surveillance strategies. Lynch syndrome is a frequent but yet underdiagnosed condition that needs multidisciplinary increased awareness for correct diagnosis and management. The aim of this review is to provide a summary of available literature data on the genetic aspects of Lynch syndrome together with geno-type-phenotype correlations and its clinical implications in the detection, genetic counseling, genetic testing, risk stratification, and management of this condition*.

Keywords: *Lynch syndrome; cancer predisposition; hereditary cancer; mismatch repair deficiency*

INTRODUCTION

Cancer is a disease of the genome that originates from complex interactions between various genetic, epigenetic, and environmental factors. Even though most of the genetic alterations in cancer are acquired somatic events leading to sporadic cancers, common and rare germline variants have important effects on tumor development [1,2].

About 5-10% of all cancers are hereditary. In those cases, a major inherited genetic risk factor could be identified through molecular genetic testing [3,4].

Colorectal cancer is the second most common type of cancer (after breast cancer) and the second cause of cancer death (after lung cancer) [5]. Approximately 20% to 30% of colorectal cancer cases could be associated with genetic factors and hereditary predisposition syndromes constitute 3% to 5% of all cases [3].

Lynch syndrome is the most frequent cause of hereditary colorectal cancer that accounts for 1%–3% of all colorectal cancer diagnoses [6].

According to Globocan estimated data, the burden of all types of cancer will increase in our country as well as in the entire Europe by 2040, including colorectal cancer [7]. Therefore, even if there seems to be a small proportion of hereditary causes, given the high incidence of cancer, a significant number of selected patients should benefit from hereditary cancer risk assessment, genetic counseling, and genetic testing for a correct diagnosis and management.

LS affects 1 in 279 individuals predisposing to colorectal, endometrial, ovarian, stomach cancers, and other malignancies at an earlier onset age than in the general population and at a high rate of multiple primary cancers [8,9]. It still remains an underdiagnosed condition despite all the recommendations for the identification of LS cases [10,11].

MOLECULAR GENETICS OF LS

Microsatellites, or short tandem repeats (STRs) are structures consisted of one to six nucleotides structures dispersed all over the genome, with a highly variable number of repetitions [12]. At this level, the DNA polymerase enzyme is prone to errors during DNA replication. MMR proteins are important in the recognition and correction of mispaired nucleotides, small insertions, or deletions generated by slippage of DNA polymerase [12].

A compromised or defective MMR system leads to microsatellite instability (MSI) and prone DNA to the accumulation of somatic mutations that often promote cancer development [9]. It has been demonstrated that the presence of MSI increases the mutation rate to 100-fold [13,14].

Immunohistochemical (IHC) staining of the MMR proteins and molecular testing of microsatellite instability by polymerase chain reaction (PCR) constitute the basis for the identification of MMR deficiency [15]. MSI and/or immunohistochemical loss of expression of MMR proteins could be identified frequently in colorectal and endometrial cancers and represents hallmarks for Lynch syndrome [16]. Alterations of MMR genes result in LS-associated tumors lacking the IHC expression of the corresponding MMR protein [6].

Since 2009, The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group recommended testing all colorectal cancer cases for MSI or MMR deficiency through immunohistochemistry as a screening method for LS [17]. Furthermore, the National Comprehensive Cancer Network (NCCN) now recommends universal testing of endometrial carcinomas for MMR proteins/MSI [18,19]. Although being important red flags for Lynch syndrome, IHC loss of expression of MMR proteins and/or microsatellite instability could not differentiate between germline and somatic origin of the defects [9].

Germline and somatic defects of the MMR genes

Mismatch repair deficient tumors may develop from acquired, somatic events in sporadic cancers or in the context of a cancer predisposition syndrome that necessitates a genetic defect of a germline origin, present from birth in all the cells of an organism [20].

LS is caused by a pathogenic heterozygous germline variant in one of the mismatch repair (MMR) genes (MLH1, MSH2, MSH6, or PMS2), which encode proteins responsible for maintaining genome stability [8].

A deficient MMR system determined by a pathogenic constitutional variant of one allele requires a second somatic hit in the wild-type (normal) allele in order to confer the cell a mutator phenotype and MSI [13,15]. This second somatic defect could be acquired through a two-hit inactivation process (based on Knudson’s two-hit hypothesis for tumor suppressor genes) or by other mechanisms such as epigenetic inactivation that may confer haploinsufficiency [21].

Despite presenting as a recessive condition at the cellular level, Lynch syndrome displays an autosomal dominant pattern of inheritance [9].

Most of the MMR variants causing LS are inherited from one parent [22,23]. De novo occurrence of MMR mutations was described but appears to be a rare presentation [24]. One study focused on determining the frequency of these cases reported that 2.3 percent of LS diagnoses occurred due to de novo germline variants [24].

Nevertheless, MSI/MMR deficiency may be attributable to somatic events/biallelic somatic inactivation [20]. For example, 15% of MSI-high/MMRd colorectal tumors are determined by hypermethylation of MLH1 promoter on both alleles (homozygous), associated with somatic BRAF V600E mutated status [16,25]. Therefore, MLH1 promoter hypermethylation analysis and/or BRAF V600E variant testing should be performed in tumors lacking MLH1 protein expression prior to germline testing to assess for sporadic origin of the MSI [23,26].

A unique somatic positive methylation result without BRAF testing in colorectal cancer could not exclude a predisposition syndrome caused by germline hypermethylation of MLH1 [23]. Although rare, this mechanism has been described and some authors recommend MLH1 constitutional epimutation testing using normal tissue in cases with methylated MLH1 that fulfil the revised Bethesda guidelines [23,27]. Similarly, just a BRAF V600E negative result could not differentiate between constitutional epimutations and germline mutations of MLH1 [23,27].

In endometrial tumors, the usefulness of BRAF testing is unclear [28]. MLH1 promoter methylation analysis is important in discriminating between somatic and inherited cases. Nevertheless, cases of coexistence of both MLH1 promoter hypermethylation and MLH1 germline mutations have been described to such an extent that Lynch syndrome could not be completely excluded in the case of positive somatic methylation of MLH1 [28].

On the other hand, the presence of two germline (homozygous or compound heterozygous) MMR defects give rise to a distinct phenotype, constitutional mismatch repair deficiency syndrome (CMMRD) a very rare presentation, with an autosomal recessive inheritance and severe clinical implications. In these cases, MMR deficiency could be encountered in any of the normal tissues of the patients, not just in the tumor cells [21].

Germline genetic testing and genetic spectrum of LS defects

Genetic germline testing in patients or healthy individuals at a high risk of carrying a pathogenic variant in one of the MMR genes is a mandatory step in the diagnosis of Lynch syndrome.

Approximately 50% of Lynch syndrome cases are determined by germline pathogenic variants in MLH1 and 40% are caused by MSH2 defects. MSH6 gene is responsible for 7-10% of LS cases and PMS2 accounts for fewer than 5% of cases [23]. In a small proportion of cases (1-3%), LS is caused by a deletion in the EPCAM (epithelial cell adhesion molecule) gene that confers loss of expression of the MSH2 gene due to hypermethylation of its promoter [23,29]. Other rare mechanisms that could be involved in the pathogenesis of LS are germline or constitutional epigenetic silencing of one of the MMR genes present in normal tissues [9,30,31].

Most of the germline defects could be identified through sequencing of the DNA or deletion/duplication assays. In contrast, epimutations are a particular type of genetic alterations that do not alter the DNA sequence but determine the transcriptional silencing of active genes [32].

Germline epimutation occurs in the germ cells, is maintained in fertilization and embryonic development, and is present in all the cells of an individual. In contrast, constitutional epimutations arise in the early stages of embryonic development, inducing a mosaic of affected and unaffected somatic cells [31]. This separation interferes with the inheritance of epimutations. Germline epimutations could be reversible between generations depending on the gender of the affected parent [33]. Studies have shown that epimutations could be inherited from the mother because there is unlikely to be lost in oogenesis whilst they disappear during spermatogenesis [31]. On the contrary, constitutional epimutations are not inherited [31]. Epigenetic silencing could also be inherited when determined by a secondary germline defect in another gene within their vicinity [30,33,34].

Germline or constitutional MLH1 and MSH2 epimutations have been described in some cases although negative for germline defects in the MMR genes [30,33–36]. The methylation of MHS6 and PMS2 genes has never been described in the literature [37].

The pathogenic spectrum of MMR variants in Lynch syndrome includes missense, nonsense, frameshift, or splice site mutations, although copy-number variants and structural rearrangements have also been described [6,23]. Therefore, to cover the most frequent alterations responsible for LS, genetic germline testing should include DNA sequencing and copy-number analysis of MMR genes as well as copy-number variations (CNVs) detection of the EPCAM gene. This approach can detect up to 99% of LS causes [23]. Testing for germline or constitutional epimutations requires different types of genetic analyses that are not routinely included in the diagnostic approach of Lynch syndrome [30]. Molecular testing of PMS2 is technically challenging considering the presence of multiple highly homologous PMS2 pseudogenes as well as balanced large rearrangements [23,38,39]. Additional analyses may be required to cover all of the genetic alterations in this gene [38,40].

Over the last few years, next-generation sequencing (NGS) testing costs and turnaround time significantly decreased. [41]. Available multigene panels comprising the MMR and EPCAM genes represent a comprehensive and more efficient testing approach than single-gene testing [19,23]. Different laboratories now offer a lot of commercial NGS panels for patients suspected of Lynch syndrome or even larger panels for hereditary colorectal cancer predisposition. These assays may present small differences in terms of performance characteristics and limitations [41].

Interpretation of genetic results

Next-generation sequencing does not provide a simple positive or negative type of results. High-throughput sequencing, also known as NGS opens up a large amount of data. That comes up with challenges in interpretation and understanding the clinical significance of the identified sequence variants [42,43]. More than 15,000 different variants were reported in the MMR genes [23].

The American College of Medical Genetics and Genomics (ACMG) guidelines provide a classification of genetic variants into five categories of interpretation: Pathogenic (P), Likely pathogenic (LP), Uncertain significance (VUS), Likely benign (LB), and Benign (B) depending on a series of criteria [43,44]. In addition, laboratories should use a uniform reporting nomenclature as a clear, common language regardless of language or country of origin [43].

Benign and likely benign variants are considered harmless and do not require additional intervention. Pathogenic and likely pathogenic variants conversely, entail specific recommendations for the prevention and surveillance of Lynch syndrome-associated malignancies and enable cascade-testing of at-risk family members and family planning strategies [22].

Besides its recognized clinical utility, very often NGS brings to light variants of uncertain significance (VUS), a challenging, grey area of results with a high potential of misunderstanding [45,46]. A VUS result means that at the time of reporting, there is insufficient populational-based or functional evidence to determine the pathogenicity of the variant or there is conflicting data in the databases or the literature about the variant's role in the disease [45,46].

As the databases and the literature are enriched with relevant data, a VUS could be reclassified into pathogenic or benign. In addition, family segregation analysis (genotyping the family members to evaluate if they have the same variant) and assessing for de novo occurrence could aid in the reclassification process [43]. Furthermore, in the process of assessing the pathogenicity of a variant, a detailed patient's phenotype is very important to be provided by the ordering clinician [43,44].

ACMG stated that "a variant of uncertain significance (VUS) should not be used in clinical decision-making" and efforts should be made to reclassify the variant into pathogenic or benign [43]. A large retrospective study that included 1.45 million individuals tested for hereditary cancer risk reported that 7.7% of unique variants of uncertain significance were reclassified [47]. Among these, 91.2% were downgraded to benign or likely benign variants 8.7% were upgraded to pathogenic or likely pathogenic variants and the median time for reclassification was less than 2 years [47].

CLINICAL SPECTRUM OF LS-ASSOCIATED MALIGNANCIES AND GENOTYPE-PHENOTYPE CORRELATIONS

Lynch syndrome is associated with an increased lifetime risk for colorectal and endometrial cancer, as well as for other extracolonic malignancies (gastric, small bowel, pancreatic, hepato-biliary, prostate, ovarian, skin, central nervous system or upper urinary tract neoplasia) [10,21].

The risk of developing breast cancer is highly debated. There are conflicting results in the literature, still, some studies point out that pathogenic variants in MSH2/PMS2 may confer a 2 to 3-fold increased breast cancer occurrence [48–50].

Lynch syndrome is a highly heterogeneous disease with important variability within the molecular germline defects, somatic profile of the tumors, as well as the onset age of cancer diagnosis and penetrance for different types of cancer [6,15].

Genotype-phenotype correlations provide individualized surveillance protocols according to subsequent genetic alteration.

Mutation carriers of MSH2 and MLH1 have a significantly higher lifetime risk of cancer compared to MSH6 or PMS2 carriers. The lifetime cancer risk by age 70 for MSH2 is 57-80%, followed by MLH1 with 59-65%, 25% for MSH6, and 25-30% for PMS2 [21]. Moreover, MSH6 and PMS2 mutation carriers display higher ages of onset for different types of LS-associated cancers [21]. Therefore, MSH6 and PMS2 confer a milder phenotype and reduced penetrance of cancer compared with MLH1 and MSH2 carriers. This fact has important implications in surveillance strategies according to each gene's corresponding risk. Better tolerability of MSH6 and PMS2 variants compared to MLH1 or MSH2 could also explain their frequent presentation in a biallelic state in CMMRD syndrome [22]. In addition, variability regarding age at first cancer diagnosis is observed between families with the same affected gene as well as within the same family affected by the same variant [51]. A variety of environmental and genetic factors contributing to the different onset ages of cancer in Lynch syndrome families have been proposed [52].

Genetic anticipation, a phenomenon characterized by progressively earlier age at the onset of a hereditary disorder and/or increased severity in subsequent generations, has also been described in Lynch syndrome [51–53]. It was also observed that patients with de novo mutations have a younger age of onset than those with an inherited variant [24].

LS patients also manifest a variable expressivity depending on MMR underlying the affected gene. Particularly, mutation carriers of MLH1 display the highest risk of developing colorectal cancer among all patients diagnosed with LS, and MSH2 carriers have the most elevated risk for extracolonic malignancies [10,21]. A large study based on data from the prospective Lynch syndrome database (PLSD) reported that the cumulative risks for colorectal cancer at 70 years of age are 46% for MLH1 carriers, 35% for MSH2, 20% for MSH6 and 10% for PMS2. The corresponding risks for developing endometrial cancer were 34%, 51%, 49% and 24% [54].

Muir-Torre syndrome is a phenotypic variant of LS most frequently determined by MSH2 germline defects. It is characterized by a high incidence of cutaneous sebaceous tumors (sebaceous adenomas/carcinomas and keratoacanthomas) [9].

Turcot syndrome, another phenotypic variant of LS, describes patients presenting with colorectal cancers and central nervous system tumors (glioblastomas) [6,9]. However, Turcot syndrome could also be part of the familial adenomatous polyposis (FAP) syndrome caused by an APC germline defect when colorectal cancer is associated with medulloblastoma [6,9,21].

Besides these larger associated risks for any of the MMR genes, it was observed that even for the same type of cancer, different mutational patterns were described in the same organ partly depending on the germline MMR-affected gene [15].

According to the "three-pathway model of carcinogenesis", LS colorectal cancers could develop from MMR-proficient adenomas, MMR-deficient adenomas, or MMR-deficient crypts [15]. NGS analyses of colorectal tumors in LS have shown highly heterogenous molecular somatic defects that could be associated with different affected MMR genes [2,15] For example, colorectal cancers due to MLH1 defects have been associated frequently with CTNNB1 mutations that could explain nonpolyposis immediate invasive growth through MMR-deficient carcinoma pathway resulting in interval carcinoma presentation [15]. On the contrary, PMS2-associated colorectal tumors do not harbor CTNNB1 defects and do not show interval colorectal cancers [15].

This variability shows that LS is not just one disease with a unique profile. It could be divided into distinct subgroups, with different associated risks and surveillance strategies depending on the germline MMR-affected gene [15] A better understanding of these subgroup profiles could offer better care for each patient, leading to more personalized surveillance strategies.

STRATEGIES FOR DIAGNOSIS OF LYNCH SYNDROME

The evaluation of individuals at high risk of carrying one LS pathogenic variant could encompass the following scenario:

- traditional testing strategy based on clinicopathological criteria (Amsterdam I/II criteria and Revised Bethesda guidelines)
- personal history of MMR-deficient tumor;
- increased risk for LS based on clinical prediction models;
- presence of confirmed LS pathogenic variant in the family [9,10,42].

In the 20th century, before the discovery of the genetic background of LS, family history of cancer represented the main tool in identifying familial aggregates of malignancies [9].

Amsterdam criteria (I and II) and Bethesda Guidelines, based on family and personal history of cancer and age of cancer onset were used to identify individuals eligible for molecular analysis [3,10]. Requiring three or more family members diagnosed with cancers from the LS spectrum at an early age, Amsterdam criteria were very stringent [3,22]. This method of screening targeted families with highly penetrant and dominant cancers, thus being less sensitive in detecting LS cases determined by pathogenic MSH6 or PMS2 variants [55,56]. Subsequently, less stringent Bethesda Guidelines (based on patient and/or family history of LS cancers and MSI-high pathologic features of colorectal tumors) were formulated to provide wider clinical criteria for screening of LS [3,9,22,57].

Despite these family-history-based prediction tools, just some selected patients were offered testing for LS [55,58]. Studies have shown that families meeting one of the clinical criteria have 82% sensitivity and 77% specificity in detecting LS and that Bethesda guidelines may miss up to 25% of LS cases [9,59].

Later on, because of the low sensitivity of family-history-based clinical criteria, “universal tumor screening” of all newly diagnosed colorectal cancer cases for MMR-deficiency by immunohistochemistry testing or for MSI was proposed as an alternative strategy to guide Lynch syndrome evaluation [3,10]. This approach demonstrated improved sensitivity compared to revised Bethesda guidelines [3]. Afterward, universal tumor testing was expanded to include recommendations for screening all endometrial cancers for LS if not limited by resources to maximize the sensitivity of LS detection [3,58,60].

Computational prediction models such as PREMM5, MMRPro, and MMRPredict represent effective tools in guiding germline testing, especially in the absence of available tumor tissue [8,57–59]. These models incorporate information like family history of cancer in order to estimate the likelihood of carrying MMR pathogenic variants [57,58]. For example, PREMM5 provides comprehensive risk estimation for all MMR genes [61]. Referral for genetic evaluation and germline genetic testing is recommended for individuals with overall predicted probability scores ≥2.5% [61].

If a pathogenic family variant is known, genetic testing for both affected and at-risk family members should be performed targeting that particular mutation [9,42]

Individuals meeting any of these scenarios should be referred for genetic counseling and genetic testing [42].

GENETIC COUNSELING IN LS FAMILIES

Patients following any germline genetic testing should receive appropriate pre-test and post-test genetic counseling [42].

Collecting detailed personal and family medical history (with at least three-generation pedigree on each side of the family) is an important step in understanding hereditary risk in a family [42,57]. Comprehensive cancer risk assessment should be focused on the type of diagnosed cancers in the family members (colorectal cancer, endometrial cancer, and other extracolonic LS-associated malignancies) as well as subtypes, pathology details, age of diagnosis, age of death, and causes of death, details about the patient or family’s country of origin or ancestry, and any known possible consanguinity [42]. Still, a negative family history of LS cancers should not exclude a possible LS diagnosis. De novo occurrence of a germline variant, constitutional epimutations, reduced penetrance, cancers not yet manifested in carriers, or early death of family members by other causes could represent sources of negative family history in LS cases [24,31,62]. Therefore, overreliance only on family history of LS-associated cancers to guide the selection of families for LS testing could contribute to general underdiagnosis of this condition [22,24].

Universal testing of colorectal and endometrial tumors could efficiently identify symptomatic patients at risk of Lynch syndrome. Genetic counseling is not required prior to testing of the tumor tissue for MMR proteins/MSI, but if a positive screening test result is obtained, patients should receive specialist counseling [42,58].

Pre-test genetic counseling is focused on providing essential information about the possible outcomes of germline testing, including positive or negative results as well as variants of uncertain significance (VUS), unexpected incidental findings, and limitations of the test [42,57].

Education of the patient or asymptomatic at-risk individuals on possible management options depending on the genetic test result, inheritance patterns, heterogeneity of the disease, penetrance, and variable expressivity helps in the informed, voluntary decision-making process [9,42,57].

Genetic testing should be performed after obtaining informed consent in individuals at high risk where a positive result will impact the medical management of the case and/or their family members [4,42,58].

After disclosure of results, post-test genetic counseling is highly recommended in order to explain genetic results and their implications and to discuss possible management options.

Patients diagnosed with Lynch syndrome should be offered intensive screening and follow-up according to genetic test results, family history, and personal history of cancer [4,6,60]. Because of the increased lifetime risk of colorectal, endometrial, and other extra-colonic cancers, LS patients and their families require lifelong coordinated multidisciplinary care [55].

Preventive measures (i.e. risk-reducing chemo-preventive agents and risk-reducing surgery) and surveillance protocols such as colonoscopy for early detection of cancers are proven to have a positive impact on patient outcomes and facilitate early diagnosis in individuals with confirmed LS diagnosis [6,42,55].

Genetic counseling and cascade testing of family members at risk of carrying LS-causing variants is highly recommended. Patients should be advised to inform their relatives about the risk of inheriting the identified P/LP variant [42]. In case of a VUS result, family testing could be recommended just for segregation studies to help in variant reclassification, but not for risk estimation or any clinical purposes [43].

Lynch syndrome is typically inherited in an autosomal dominant manner. Each child of an individual diagnosed with LS has a 50% (1/2) risk of inheriting the same P/LP variant [62]. Usually, germline testing for hereditary cancer predisposition of pre-symptomatic individuals is not recommended under 18 years old unless the risk of developing cancer is very high at younger ages and results would impact medical care in childhood [42,62]. Constitutional MMR deficiency (CMMRD) syndrome is one of the exceptions in which testing prior to age 18 is recommended to guide medical care [42].

Prenatal diagnosis or preimplantation genetic testing with selective implantation of unaffected zygotes is technically feasible if a pathogenic variant is known in the family [57,62-64].

CONCLUSIONS

Lynch syndrome is the most common hereditary colorectal cancer syndrome, characterized by high genetic and clinical heterogeneity. Being associated with the early onset of colorectal cancer and other extracolonic malignancies, individuals diagnosed with LS and their families require multidisciplinary care during their entire lifetime. Comprehensive genetic counseling educates patients and at-risk family members about their risks, genetic testing advantages and limitations, and implications of diagnosis. Specific genotype-phenotype correlations were observed and germline molecular testing helps individualize risk-reduction and surveillance strategies according to underlying genetic defect.

Conflicts of interest and sources of funding

The author declares no conflict of interest.

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REVIEW

Lynch Syndrome: Approach from the Gastroenterologist's Point of View

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Abstract: *Colorectal cancer is one of the main causes of death by cancer in the European Union and in Romania. Lynch Syndrome is an autosomal dominant inherited disease that affects around 3% of all colorectal cancer patients, defined by mutations in the mismatch repair genes (MLH1, MSH2, MSH6, PMS2). Aside from the high risk for colorectal cancer (CRC), they exhibit a high risk for endometrial cancer, ovarian cancer, gastric cancer, pancreatic cancer, brain cancer, small bowel cancer, and sebaceous gland cancer. Diagnosis is often made by the gastroenterologist, usually when he meets a young patient with symptoms suggesting the presence of a colonic tumor and a family history of CRC. The role of the gastroenterologist is to investigate the best practices in order to have an early diagnosis and to prevent interval cancers according to the newest guidelines or other gastrointestinal tract tumors associated with Lynch Syndrome. Registries play an important role in the optimal surveillance of these particular categories of patients and should be put into place in every country. They will allow clinicians to have a better understanding of the disease and a more standardized quality of care for them*.

Keywords: *Lynch Syndrome, colonoscopic surveillance, patient registry*

INTRODUCTION

Colorectal cancer (CRC) represents the second leading cause of death by cancer after lung cancer in the European Union and the second one by frequency of occurrence, making it an important problem of public health in many countries [1]. In Romania, the National Institute of Public Health estimates an incidence rate of 34.6‰$_{00}$ people in 2020, representing 12.7 % of the newly diagnosed malignancies in the oncologist's office. Analyzing past data, we can observe a marked increase in the incidence of new cases from 31.0‰$_{00}$ in 2012 to 34.6‰$_{00}$ in 2020 which medical professionals should take into account [2].

It is estimated that 3-5% of CRC diagnoses are associated with a genetic syndrome, but what is really concerning, is that 35% of CRC have a hereditary predisposition. Lynch Syndrome (LS) is the most common hereditary CRC syndrome and it's the cause of approximately 3 % of all CRC, with an estimate of 1/279 (95% CI, 192–403) of the population carrying a mutation in the mismatch repair genes [3].

LS is an autosomal dominant inherited syndrome genetically diagnosed by a mutation in one of the mismatch repair (MMR) genes (MLH1, MSH2/EPCAM, MSH6, PMS2) [4].

It is characterized by an overall increased risk of CRC, manifested by a predominance of diagnosed right-sided tumors, and a high incidence of metachronous or synchronous tumors [5]. Patients who carry a pathogenic variant of the MMR genes are also at high risk of developing endometrial cancer and some other cancers based on the respective gene defect [6].

Recent guidelines recommend that all newly discovered CRC regardless of age of diagnosis, should undergo testing for mismatch deficiency by immunohistochemistry (IHC) staining for MMR proteins, or microsatellite instability (MSI) by polymerase chain reaction (PCR). If the tumor is either MMR deficient or presents high instability, the patient should automatically be tested for LS. Ideally, genetic diagnosis should happen before chemotherapy is started, considering that, immunotherapy is associated with an improved prognosis in this category of patients compared with the ones having an MMR-proficient tumor. In cases of early-onset CRC, all patients should undergo genetic counseling and be tested for germline genetic mutations regardless of the results of IHC/PCR testing [6].

When faced with a young individual, the patient’s history is very important. The clinician should try to find out all the family history details, making a comprehensive review and ideally also a family pedigree. All causes of death in the family should be noted in order to understand how many of them were caused by the underlying condition and to observe the penetrance of the gene in the respective case [7].

MATERIALS AND METHODS

The studies that we included in this review, are peer-reviewed articles, published in English focused on gastroenterological surveillance in Lynch Syndrome. We used the PubMed database, Springer Link, and Google Scholar and we selected articles published before the 1st of August 2023. We use terms like "Lynch Syndrome" combined with "Colonoscopic surveillance", "Endoscopic Surveillance", "colon cancer", "Pancreatic cancer", and "non-invasive surveillance" and selected relevant articles to the subject. We didn’t include articles that presented small lots of patients and had insufficient data presented. The articles that complied with the search and contained relevant information for our study were included and presented in our study. We also researched the newest guidelines on hereditary colorectal cancer such as the NCNN latest guideline version, the ESMO guidelines but also the Mallorca consensus, and the new Delphi initiative on early-onset colorectal cancer.

RESULTS

As gastroenterologists, we are concerned about the early detection of Lynch-associated tumors and how to best prevent premalignant lesions. New studies discussed the pathways that lead to CRC cancer and what gastroenterologists can do to prevent new malignant lesions. Also, concerning the rarer GI tract cancers associated with Lynch Syndrome, there are new surveillance recommendations published very often and gastroenterologists need to stay up to date in order to offer the best care.

We are going to focus on the best practices in gastrointestinal surveillance, therefore we are going to discuss the early detection of patients, surveillance, and better ways to improve patients’ adherence to a lifetime of screenings.

Detection of patients

When talking about the gastroenterologist's perspective, it is usually concerning a CRC case at diagnosis. The most frequent motives for a doctor's appointment are hematochezia (46%), iron deficiency anemia (13%), and weight loss (10%) [8-11]. Physicians have a tool by applying the Amsterdam II Criteria or the revised Bethesda Guidelines when assessing a patient’s risk for Lynch Syndrome [12-13] (Table 1).

Table 1: Amsterdam II Criteria and Revised Bethesda Guidelines

Amsterdam II criteria
At least three relatives must have cancer associated with LS (colorectal, endometrial, small bowel, ureter, or renal pelvis); all of the following criteria should be present:
One must be a first-degree relative of the other two
At least two successive generations must be affected
At least one relative with cancer associated with LS should be diagnosed before age 50
Familial adenomatous polyposis should be excluded in the CRC cases (if any)
Tumors should be verified whenever possible
Revised Bethesda Guidelines
Tumors from individuals should be tested for MSI in the following situations
CRC diagnosed in a patient who is younger than age 50 years
Presence of synchronous or metachronous colorectal or other LS-related tumors regardless of age
CRC with MSI-high histology diagnosed in a patient who is younger than age 60 years
CRC diagnosed in a patient with one or more first-degree relatives with LS-related cancer, with one of the cancers being diagnosed below age 50
CRC diagnosed in a patient with two or more first or second-degree relationships with LS-related cancer regardless of age

Although throughout the years they proved themselves to be useful tools in the office, the more recently recommended approach is to define the MMR status via IHC staining or MSI using the polymerase chain reaction. Furthermore, the molecular alternative plays an important part in the aftermath of the diagnosis by being a useful indicator of a positive response to the use of immune checkpoint inhibitors [14].

In particular situations, where no tumor tissue is available, like patients with a high suspicion of Lynch Syndrome that don’t have a cancer diagnosis, clinical prediction models have been developed, in order to assess the indication for genetical testing.

The PREMM model ((http://premm.dfci.harvard.edu/), is a useful tool for clinicians, and easy to incorporate in day-to-day work. Recently Harvard developed the new PREMM5 model with an increased chance of predicting whether a patient might carry a germline variant of the genes involved in LS. If the prediction is above the 2.5% limit in the case of the PREMM5 model or more than 5% in older versions, the patient should be referred for genetic testing and further investigations in a geneticist's office [15].

From a gastroenterologist's point of view, the interview is based on onset symptoms, previous lab results, family history, and personal risk factors such as smoking status, gender, and age at diagnosis. Afterward, depending on the situation, whether there is a patient from a Lynch family or a newly identified patient, there are different ways to approach the screening. Patients may be referred to a geneticist in order to receive genetic counseling and to be explained familial risk. A study was conducted in Denmark on the manner in which individuals were informed about their cancer risk. There is a Lynch Syndrome registry implemented since 1991 with continuous improvements, where there is approval from the authorities to send unsolicited letters to patients at risk of familial cancer in order to include them in a surveillance program. The reactions to these letters were studied, and while the results were good, there still was a category of patients upset or angry to be informed of their genetic heritage. Even though the standard recommendation is that family members inform one another, depending on familial situations, and personal preferences, it was decided that the unsolicited letters were helpful in reaching as many members as possible. Potential individuals at risk were invited to genetic counseling and genetic testing in order to assess their further need for surveillance [16].

Colonoscopic surveillance

When talking about colonoscopic surveillance in LS there are several issues to be discussed. First, there are multiple recommendations regarding the screening both concerning the onset age to begin colonoscopic surveillance and also the interval between the procedures. The consensus is that the interval between colonoscopies should not be longer than 3 years as it was demonstrated in some studies that a 3-year window was associated with metachronous cancer [17]. A study conducted by Ahadova et al opened the window to new challenges regarding colonoscopy surveillance. The study presents a new theory that states the fact that carcinogenesis in Lynch Syndrome may occur via more pathways opposite to the previous theory stipulating that it's an accelerated road from adenoma to adenocarcinoma (Table 2). While the classic pathway with the adenoma to adenocarcinoma is still present, they show that adenocarcinomas can develop on macroscopically normal tissue, concluding that a schedule with more frequent colonoscopies won't necessarily prevent the development of new tumors [18]. Even though endoscopists will perform polyp resections when deemed necessary, they can't analyze the normal tissue. In summary, colonoscopy screening with polypectomies performed when necessary did not guarantee that the patient would be risk-free and tumor could appear on apparently normal mucosa. This new information makes colonoscopy surveillance even more important, and respecting the recommended intervals with no more than 2 years interval between exams is crucial in detecting tumors early.

Table 2: Pathways to Carcinoma in Lynch syndrome as stated by Ahadova et al. [18]

Pathway 1	Normal mucosa to Adenoma MMR proficient to MMR deficient Adenoma to Carcinoma
Pathway 2	Normal mucosa to MMR deficient crypt foci to MMR deficient Adenoma to Carcinoma
Pathway 3	Normal mucosa to MMR deficient crypt foci to Carcinoma

Seeing as new information appears every year, new guidelines were published with different recommendations regarding the timing of colonoscopy surveillance, and also the interval between the examinations. Recent data concerning LS patients shows that the on-set age should be different depending on the mutation, with the latest NCCN recommendations stating that in the case of MLH 1 or MSH 2, colonoscopy should start at age 20-25 years or 2-5 years before the earliest CRC diagnosis if it was diagnosed before 25 years of age, with an interval of 1-2 years between examinations. When talking about MSH 6 and PMS2, the guidelines are more relaxed, recommending starting at 30-35 years of age or 2-5 years before the earliest CRC diagnosed before the age of 30 years with an accepted interval of 1-3 years between examinations. If adenocarcinoma is detected in one of the examinations, colonoscopy on the remaining part of the colon should be made every 1-2 years, the same indication going in cases of adenomas that were successfully resected endoscopically. If there are situations when adenomas aren't suitable for endoscopic resection, surgery may be indicated depending on the particular situation [19].

The British Society of Gastroenterology published a new guideline in 2020 on the management of hereditary colorectal cancer making similar recommendations, the difference being in intervals between examinations. It is recommended a 2-year interval between colonoscopies and they also state that screening should end at age 75 years [20]. The ESMO 2019 guidelines are in consensus with the previous ones, making recommendations based on the known gene mutation with a

1-2 years interval between examinations [21]. The ACG guidelines state a slightly different approach to screening with a recommended age at onset between 20-25 years at least every 2 years with a suggestion to perform it annually in confirmed mutation carriers [22] (Table 3).

Table 3: Summary of guideline recommendations for colonoscopic surveillance

Guideline	Age	Interval
NCCN	MLH1/MSH2: 20-25 years or 2-5 years before earliest CRC diagnosis if prior to 25 years of age MSH6/PMS 2: 30-35 years or 2-5 years before earliest CRC diagnosis if prior to 30 years of age	1-2 years 1-3 years
ESMO	MLH1/MSH2: 25 years MSH6/PMS2: 35 years	1-2 years
BSG	MLH1/MSH2: 25 years MSH6/PMS2: 35 years	2 yearly until age 75 years
ACG	Age 20-25 years	1-2 years

One study conducted by the Dutch in 2022 [23] on colonoscopic surveillance of diagnosed LS patients revealed some difficulties encountered on the way regarding adherence to follow-up by the patients. One important difference that was acknowledged was the call for an appointment. Patients who were called by the hospital in order to make the appointment were more likely to undergo colonoscopic surveillance than the ones who were supposed to initiate the call themselves. Another issue at hand was the burden of bowel preparation. Patients were more open to smaller volume preparations than larger ones. Another issue that should be taken into account is the psychological burden. Out of the 197 patients included in the study, 41 answered that they considered quitting the colonoscopic surveillance. Out of them, 6 complained that it reminded them of having LS, 8 said that it did not fit into their schedule, and for 17 of them, the colonoscopy was burdensome. This was mostly found in populations under 40 years of age. The findings suggested that individuals would be more likely to undergo screening colonoscopy when the hospital is the one initiating the discussion in the context of a centralized cancer registry like the one already in place in the Netherlands because it was revealed that many of the patients didn't know which was the right schedule to appoint a new examination. They concluded by recommending new approaches to surveillance in Lynch Syndrome and exploring less invasive methods like noninvasive biomarkers, that would make patients more likely to adhere to a surveillance program.

Another study from the Prospective Lynch Syndrome Database shows interesting results that somehow contradict the before-known recommendations in the matter of interval between examinations. They compared results from different countries that have different examination schedules from yearly in Melbourne to every 3 years in Finland. They concluded that irrespective of the time since the last colonoscopy was performed, the tumor stage distribution was similar. They proposed another theory, one that exists in breast cancer from before, the overdiagnosis [24]. There are cases described in the literature that formulate the hypothesis that the host's immune system could detect neoantigens present on the surface of MSI cells and destroy them. So, performing colonoscopies too often could result in additional unnecessary surgical procedures that could be handled by the immune system [25].

Noninvasive surveillance

Over the last few years, there have been several studies conducted in order to try to find a suitable noninvasive screening method to be implemented in order to avoid performing colonoscopy yearly or every 2 years depending on the country of origin. There have been small studies concerning imaging methods, blood biomarkers, or fecal biomarkers that researchers hoped would help patients avoid the burden of undergoing colonoscopy.

In Australia, Lim et al conducted a small prospective study to compare Magnetic resonance colonography with colonoscopy results, in patients undergoing both procedures on the same day. The MR colonography images were reviewed by two radiologists. They managed to detect three lesions in three patients that were large, 70, 36, and 17 mm respectively, but failed to discover one small-sized adenocarcinoma sized 3 mm, two tubulo-villous adenomas, and one sessile serrated adenoma. They concluded that the examination still had a poor sensitivity in detecting small-sized polyps even though they detected the larger ones. Comparing the methods by patient comfort, even though other studies confirmed that patients admit a lower burden in performing MR colonography than colonoscopy, the burden of bowel preparation remains [26]. There have been other studies made on small sets of patients regarding other noninvasive methods such as stool tests (fecal immunochemical test or microbiota) or blood tests but at the moment none of them have proven to be able to replace screening colonoscopy [27-29].

Upper gastrointestinal (GI) tract surveillance

While most of the gastroenterologist's efforts are driven towards a high quality lower gastrointestinal tract surveillance, considering the high risk of colorectal cancer, gastric cancer, and small bowel cancer are two entities that should be taken into account when talking about Lynch Syndrome surveillance. There is no consensus on onset, interval, or even recommendation to perform upper GI endoscopy up until present. Recent guidelines recommend an individualized approach to gastric cancer screening depending on personal risk; that includes, family members with a diagnosis of gastric cancer, male sex, or increasing age. However, there were cases where risk factors weren't present, which made this a clinician's decision [30]. There is one common point that most guidelines agree upon, universal Helicobacter Pylori testing should be performed, the infection eradicated when discovered, and ulterior testing to confirm the eradication to be done [31]. Recent studies advocate for an early first Upper GI Endoscopy, at 30 years of age or 2-5 years earlier than the first upper GI tract cancer diagnosed in the family, with multiple biopsies taken in order to assess the presence of atrophic gastritis or a presumable Upper GI infection. If symptoms appear, an earlier examination should be performed. While there is no consensus on the best interval between exams, a 2-3 years interval could be a good option if there are no other risk factors to be taken into consideration. Also, the best way to ensure patient adherence to screening is to perform both colonoscopy and upper endoscopy in the same session [32].

Pancreas surveillance

While rarer than the other pathologies mentioned above, pancreatic cancer presents a higher risk in LS patients than in the general population (3.7% vs. 1.5%) [33]. With MLH 1 and MSH 2 mutations being most frequently involved. Another difference between LS patients and the general population is the age at diagnosis, with a mean age of 70 years, in this particular situation it may be diagnosed at a younger age. [34] Unfortunately, there is no standardized screening method for this pathology with the most useful tools at the moment being endoscopic ultrasound and magnetic resonance imaging, both of them being more accurate than a CT scan [35].

DISCUSSION

Lynch Syndrome is a diagnosis with a high impact burden on the patient, that became more common in the past few years with the implementation of standard IHC in all colorectal tumors that are surgically resected. Unfortunately, over the years many patients were missed because of the high cost of the investigations in underdeveloped countries, and physicians made everything possible to try not to miss any patients that may have been at risk for any hereditary cancer syndrome. While there are programs for some genetic mutations to be tested for free, germline mutation for Lynch Syndrome is at the moment available only if patients support the full cost, this situation making many patients refuse it for the moment being. Romania is a country undergoing medical developments with new projects to create national registries set into motion. Seeing as in other countries registries helped medical professionals follow certain categories of patients and improve their outcome, all countries are trying to achieve the same results.

The gastroenterologist is an important part of the diagnostic equation in this syndrome, alongside the gynecologist is usually the first medical professional that identify potential LS patients. That being considered, it is very important that all gastroenterologists should know the clinical features of LS in order to correctly identify patients at risk and send them to a geneticist. Colonoscopic surveillance, but also upper GI surveillance should not be performed at any center but only in the highly specialized ones by trained endoscopists with experience in screening colonoscopies for particular populations. A high-quality colonoscopy should be performed, ideally with the help of chromoendoscopy and of high definition white light endoscopy. Trained specialists know how to assess lesions properly when discovered and to decide which one should be excised endoscopically and which should go to surgery. It is a field in continuous development where new information appears every year. The existence of patient registries is very important and should be made a priority in every country, to allow patients to have high-quality surveillance in specialized centers and receive the best care possible to improve their lifetime expectancy and quality of life.

CONCLUSIONS

Surveillance of Lynch Syndrome patients is very challenging, with new information emerging every day, making the physician's job even more interesting. That is why it is very important to have more gastroenterologists specialized in the pathology associated with LS to ensure an appropriate standard of care for this category of patients.

Conflicts of interest and sources of funding

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Authors' contribution

L.S.D. – writing, original draft preparation, case research, C.G. – review and editing, supervision.

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ORIGINAL ARTICLE

An Endocrine Perspective on Lynch Syndrome

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Abstract: *Background: A germline mutation of the MisMatch Repairgene that causes a malfunctioning DNA system is the defining feature of the hereditary illness known as Lynch syndrome. This review will discuss the endocrine aspects of LS and highlight current advancements in the area. Methods: We searched the available literature of the last 10 years for terms such as endocrine tumors and LS. Our goal is to provide a summary of the most recent information available on the endocrine perspective in Lynch syndrome. Results: The hormonal chemoprevention methods cited are the use of combined oral contraceptives, the use of progestogen-releasing intrauterine devices, and the use of progesterone-only drugs. In addition, after surgical exclusion of the uterus and ovaries, a method frequently adopted for LS patients, it is necessary to start hormonal menopausal therapy, taking into account certain age-specific features. In addition, numerous LS-associated endocrine tumor types have been described. Conclusions: Many hormonal variants are available that are useful in the chemoprevention involved in the treatment of LS. Menopausal hormone therapy is imperative for LS patients who need it. Clinicians need to be aware of the possible association of certain types of aggressive endocrine cancers associated with LS.*

Keywords: *Lynch syndrome, endocrine tumors, menopausal hormone therapy*

INTRODUCTION

The most prevalent hereditary colorectal cancers are those caused by Lynch syndrome (LS), also known as hereditary non-polyposis colon cancer. This autosomal-dominant disorder causes colorectal cancers to develop on average 10 years sooner than sporadic colorectal neoplasia [1].

Lynch syndrome is caused by inherited mutations or pathogenic variations of the genes of the MisMatch Repair (MMR) system involved in the DNA repair system: in the mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6) and PMS1 homolog 2 (PMS2) genes. In addition, epithelial cell adhesion molecule (EpCAM) deletions of the 3' end may result in the epigenetic silencing or inactivation of MSH2. More rarely, germline hypermethylation of the MLH1 promoter area might result in hereditary inactivation of the MMR system. Failure of the MMR system causes a loss of expression of one or two MMR proteins with a consecutive increase in the number of MMR proteins and leads to an increase in errors during DNA replication. In addition, tumors that develop in the spectrum of LS also exhibit microsatellite instability and loss of expression of one or two MMR proteins [1,2]. The future of LS screening may involve using next-generation sequencing to check for microsatellite instability and find mutations in the MMR genes [3].

This syndrome is associated with a significantly increased risk of colorectal cancers along with carcinomas of the endometrium, upper urinary tract (mainly renal pelvis and ureter), ovary and small intestine, stomach, sebaceous tumors (adenomas and sebaceous neoplasms, keratoacanthomas), brain tumors (gliomas), pancreatic and bile duct tumors [4]. Furthermore, there have been observed genotype-phenotype connections, such as an increased incidence of extracolonic tumors or multiple malignancies in individuals with MSH2 mutations [3].

From the endocrinologist's perspective, patients with Lynch syndrome may require clinical and therapeutic management of surgically induced primary menopause with all its associated risks, as well as screening and possible treatment of other associated endocrine cancers.

Additionally, it's critical to accurately and promptly diagnose LS using medical genetic techniques because it affects both the patient and their family in terms of the moment and type of surgery, long-term monitoring, and screening tests for various tumor types.

MATERIAL AND METHODS

We searched PubMed for English and French full-length publications, aiming to compile a narrative mini-review, through a search starting from the terms ''lynch syndrome'', and associated "endocrine tumors" searching for "thyroid tumors", "adrenal tumors", "pituitary tumors", "testicular tumors". We used as inclusion criteria original publications, regardless of the level of statistical significance, full-length articles, and the French and English language, for the last 10 years. Exclusion criteria were non-English papers, non-French articles, and non-endocrine disorders associated with LS, usually not referred to the endocrinologist. We followed the main endocrine tumors associated with LS, as well as the importance of hormonal treatment in women with LS. Searching for the term "thyroid tumors" associated with Lynch syndrome, we found 17 publications, including 4 case reports and 3 cohort studies. In addition, searching for the term "adrenal tumors" associated with Lynch syndrome in the last years, we found 21 articles, 5 of which were case reports. Similarly, when searching for the term pituitary tumors, the search resulted in 6 publications of which 3 case reports. Searching for the term "testicular tumors" associated with Lynch syndrome in the last 5 years, we found 7 articles, 2 of which were case reports. In addition, searching for other endocrine tumors described with Lynch syndrome we found a case report with a parathyroid carcinoma and one clinical case of thymic carcinoma.

RESULTS

From the endocrinologist's perspective, of interest are how to optimally treat and monitor premature menopause associated with LS treatment and the clinical approach to endocrine tumors developed within this syndrome, especially thyroid, adrenal, but also testicular, pituitary, parathyroid, and thymic tumors.

Premature menopause may develop in patients with LS either induced by prophylactic or curative surgery or secondary to chemo and radiotherapy procedures that may be part of the therapy. The impact on patients, often young women under the age of 45 years, has numerous implications both endocrinologically, on fertility, as well as metabolically, cardiovascular, cognitive, bone, and not least psychologically.

The clinical management of premature menopause is carried out as a team with the gynecologist who is responsible for the diagnosis of ovarian and endometrial pathology associated with the LS. Our article will not focus on the diagnosis and treatment of gynecological pathology, but only on the hormonal and non-hormonal treatment of endocrine consequences, i.e. the treatment of premature menopause and the possible hormonal approach to LS patients. After scanning the specialized literature we will systematize the information found according to endocrine disorders.

Thus, we will first discuss the endocrine consequences of the clinical management of ovarian or endometrial cancer and the possible hormonal and non-hormonal treatments of premature menopause associated with LS. Then, we will detail other endocrine tumor types associated with this syndrome.

1. Ovarian and endometrial cancer

The foremost conditions associated with gynecologic cancers include LS and hereditary breast and ovarian cancer. About 2-3% of all endometrial cancers are considered to be associated with LS [5].

LS genes vary in clinical penetrance, with a lifetime risk of developing endometrial cancer of up to 60% and a risk of up to 24% for developing ovarian cancer, especially in the carriers of MSH2 [6]. On the other hand, PMS2 carriers, in particular, have a lower lifetime risk of cancer than other LS genes[7].

In terms of clinical approaches, we will discuss in the following both general methods of lifestyle optimization and methods of chemoprevention, as well as surgical options to reduce the risk of gynecological cancer.

Regarding lifestyle suggestions, patient education includes methods to lower cancer risk factors generally, such as quitting smoking, getting regular physical exercise (150-300 minutes of moderate-intensity weekly exercise), maintaining a healthy weight, eating a healthy diet (rich in vegetables and fruits and less red meat), limiting or eliminating alcohol, guarding against sexually transmitted diseases, avoiding sun exposure, and getting adequate cancer screenings [8]. However, no particular research has been done on how this broad advice may affect LS patients.

Chemoprevention

The first drug therapy used as chemoprevention in LS is the use of aspirin, as it can reduce the incidence of colorectal cancer. Studies indicate starting with a dose of 325 mg of aspirin per day, with consecutive increases to a dose of 650 mg per day after several months of treatment if aspirin is tolerated without side effects. In patients who have side effects following aspirin treatment or those with a personal and family history suggesting a lower risk of cancer, a lower dose of aspirin may be used [9]. In addition, taking aspirin diminishes the risk of developing all neoplasia in LS gene carriers. Aspirin appears to reduce the risk of endometrial neoplasia in obese women with LS compared to non-obese women [10].

In addition, in terms of hormonal chemoprevention, combined oral contraceptive pills (COC), progestin-only pills, or progesterone-containing intrauterine devices (IUD), are an alternative form of risk-reduction for those with LS, before to completion of the childbearing period and prior to risk-reducing surgery.

Moreover, data on the prevention of endometrial cancer with hormonal therapies in women with LS are limited and most of the evidence is extrapolated from studies in the general population.

Regarding COC, their use is known to decrease the lifetime risk of endometrial and ovarian neoplasia by 50%. Importantly, the protective effects of using COC become stronger the longer the use [11]. A retrospective cohort study showed that patients with LS who received hormonal contraceptives for more than 1 year had a reduced risk of endometrial cancer [12]. However, COC use raises the risk of breast cancer by up to 38% in the general population, if used for more than 10 years [13].

In a retrospective analysis, women with LS who used hormonal contraception for at least a year — in the form of a COC progestin-only pill, or an implant, had a 61% lower risk of developing endometrial cancer [14].

Table 1: Non-surgical prevention methods in LS *(adapted after Lynch syndrome for the gynecologist, Neil AJ Ryan et al)*

	Combined oral contraceptives	**Menopausal hormone therapy**	**Levonogestrel Intrauterine Device**	**Depo Provera Medroxyprogester one Acetate**	**Lifestyle measures***	**Aspirin**
Timing	Before surgical prophylactic surgery, 2 years after menarche	After surgical excision until the normal age of menopause	Method of chemoprevention, 2 years after menarche	Method of chemoprevention, over 18 years old	Any time	Method of chemoprevention, From 18 years old
Mechanism of action	Prevention of ovulation decreased endometrial proliferation	Anti-inflammatory impact decreased endometrial proliferation	Decreased endometrial proliferation	Decreased endometrial proliferation	Decrease of cancer risk factors	Not fully described
Contraindications	Hypertension, breast cancer, ischemic heart disease, migraines with auras, endometrial cancer, cirrhosis, hepatocellular adenoma, malignant hepatoma, history of venous thromboembolism	History of estrogen-dependent or breast cancer, active arterial thromboembolic disease, undiagnosed vaginal bleeding, thrombophilia disorder, history of venous thromboembolism	Sexually transmitted infection, congenital uterine abnormality, pelvic inflammatory disease, Confirmed or suspicion of uterine or cervical malignancy, abnormal uterine bleeding	Undiagnosed vaginal bleeding, active thrombophlebitis or past history of thromboembolic disorders or cerebral vascular disease, gallbladder disease, history of diabetes, coronary artery disease, or a current tobacco smoker age ≥ 35	Those who have health issues that would prevent them from doing too much physical activity	Severe heart failure, hemophilia, peptic ulcer disease, active abuse of alcohol
Adverse effects	Breakthrough bleeding, nausea, headaches, abdominal cramping, breast tenderness, decreased libido	Dysuria, skin reactions, mood alterations	Headache, acne, breast tenderness, weight gain, cramps or pain during menstruation, depression	Headache, acne, hirsutism, weight gain, depression	-	Dyspepsia, digestive haemorrhage

** reduce weight, increase physical exercise, healthy diet, smoking cessation*

Moreover, the use of depo-medroxyprogesterone acetate (depo MPA) or COC was specifically evaluated in LS patients, showing evidence of a marked decrease in endometrial proliferation [15]. However further investigation of these hormonal agents in this high-risk population is needed.

The levonorgestrel intrauterine device (IUD) releases progestin directly into the endometrium, attenuating the stimulatory effects of estrogen and it is frequently used to treat endometrial intraepithelial neoplasia and low-grade endometrial cancers. Studies have shown that all levonorgestrel IUD users have a reduced risk of endometrial and ovarian cancer in the general population, without an increase in the risk of breast cancer and this decreased risk persists for 5 years following discontinuation [14,16]. Unfortunately, POET, a randomized trial that aimed to ascertain whether the levonorgestrel IUD was effective at preventing endometrial neoplasia in women with LS, was prematurely stopped due to a lack of enrollment [8].

Thus, data on general lifestyle measures and chemoprevention methods used in LS have been systematized in Table 1.

Risk-reducing surgery

Regarding the option of preventive surgery, the Manchester Consensus Group recommends that total hysterectomy with bilateral salpingo-oophorectomy should be offered to patients no earlier than 35-40 years after cessation of the childbearing period in carriers of proven pathogenic MLH1, MSH2, and MSH6 genes. Furthermore, for pathogenic PMS2 heterozygotes, the usefulness of preventive surgery is not proven. It is also recommended to consider a woman's personal risk in order to provide individualized advice on the optimal timing of the surgical procedure [2].

Moreover, women with LS, especially when they are premenopausal and/or women over 40 who have chosen not to undergo total hysterectomy with bilateral salpingo-oophorectomy and who opt for periodic surveillance should have an annual follow-up with a gynecological specialist with knowledge of the syndrome [8]. In addition, a study reported that the risk is higher in the case of MSH6 gene mutation compared to MLH1 and MSH2 gene mutations [17].

The largest study comparing patients with LS concluded that prophylactic hysterectomy with bilateral salpingo-oophorectomy is an effective strategy for preventing endometrial and ovarian cancer in women with LS [18].

Currently, there is no recommended screening strategy for gynecologic cancers with demonstrated efficacy in the setting of LS. The annual association of the clinical examination and the endovaginal ultrasound is proposed [8]. In addition, some studies indicate the performance of the annual endometrial biopsy, is correlated with the monitoring of the CA-125 marker levels [2]. In any case, women should be warned that the appearance of metrorrhagia should lead to a specialist consultation as soon as possible and a diagnostic examination. Most endometrial cancers are diagnosed at an early stage, with a favorable prognosis similar to sporadic ones. In particular, the distribution of uterine tumor localization seems to be different in patients with LS [19]. It is worth noting that while in the case of endometrial cancer patients without LS, the tumor develops in the uterine body, for patients with this syndrome there is a greater risk of tumors of the lower segment of the uterus, which can be misdiagnosed as cervical adenocarcinomas [5].

These risk-reducing operations might lead to early menopause, infertility, and surgical complications, which would have a major negative impact on general health and quality of life. Patients need to be informed about the advantages and disadvantages of this gynecological surgery in terms of the morbidity of the operation and especially the medium and long-term consequences of surgically induced menopause.

The surgical complications rate varies by 1.6-9%, including events such as bowel and urinary tract injuries, bleeding, and infections [18]. Patients should be informed about the symptoms of surgically induced premature menopause, including vasomotor symptoms, sleep disturbances, decreased libido, symptoms of vaginal atrophy but also long-term systemic implications such as osteoporosis, the risk of cerebrovascular disease, cardiovascular disease, and accelerated neurodegenerative aging [20] .

However, studies continue to advise risk-reducing surgery for LS patients who have finished the childbearing period rather than cancer surveillance and chemoprevention [8].

Impact on Fertility

In addition, patients with LS should be referred shortly after diagnosis to clinicians with expertise in female reproductive endocrinology and gynecologic oncology for counseling on gynecologic cancer surveillance. In addition, patients need counseling on fertility, fertility preservation options, use of assisted human reproduction techniques (ART) as well as other options regarding the desire to have children, such as adoption or surrogacy. Additionally, in the case of ART, preimplantation genetic testing to prevent implantation of an affected embryo or prenatal diagnosis to determine if the fetus is affected is recommended in LS [21].

Consideration of fertility preservation options is an important component of the management of patients with LS. Early referral of patients known to carry pathogenic gene variants to reproductive endocrinology specialists at age 20-30 is recommended so that they can be informed about their own follicular reserve, fertility preservation, and potential for preimplantation genetic testing. This allows early assessment of baseline ovarian reserve and enables interested women to achieve spontaneous pregnancies at younger ages or to access ART when the methods are most likely to be successful. Through ART, patients have the option of cryopreserving and storing oocytes, embryos, or both [22].

Generally, during a cycle of controlled ovarian hyperstimulation, the ovaries are stimulated with exogenous gonadotropins, followed by ultrasound-guided transvaginal needle aspiration of the oocytes. However, there is some concern in patients carrying gene mutations about the effect of high levels of oestradiol generated during ovarian hyperstimulation on the risk of developing gynecological cancer. Fortunately, data from the literature indicate that the use of fertility drugs does not appear to increase the risk of breast cancer in patients with prior breast cancer or BRCA gene mutation carriers, having no impact on survival rates, at least in the short term. However, in these patients, it is recommended to minimize the increase in oestradiol levels by using an aromatase inhibitor (letrozole) in addition to using the gonadotropin stimulation protocol, which leads to lower, physiologically similar oestradiol levels. In the case of a patient who has cryopreserved oocytes or embryos prior to bilateral adnexectomy, pregnancy can still be achieved by hormonal preparation of the preimplantation uterus and embryo transfer. However, if a woman does not have her own oocytes or embryos available, she may consider using donor oocytes, or donor embryos, or turning to adoption [21].

For a woman of reproductive age who has opted for total hysterectomy either for treatment or as a preventive method to reduce the risk of cancer, who wants a child from her own gametes, ART can be used to obtain her own oocytes, which are then used in a gestational carrier. Medical, legal, and psychological counseling should be considered for both the prospective parents and the gestational carrier. Uterine transplantation is also a procedure that has recently resulted in live births, but its role in preserving fertility in patients at high risk of hereditary gynecological cancer has not yet been determined [5].

For patients in whom testing reveals a low ovarian reserve, we would recommend that pregnancy is unlikely and that options for becoming parents include adoption or the use of donor eggs or embryos.

Concerns regarding the long-term safety of fertility drugs and procedures have been raised due to the usage of reproductive treatments that may lead to repeated ovulation and changes in endogenous hormone levels, especially in hereditary gynecologic cancers, such as LS [23]. Although certain clinical research has revealed a connection between fertility medications and the risk of cancer, it can be challenging to evaluate the findings of these investigations. Based on the available data, the risk of invasive ovarian cancer is not significantly increased after the use of fertility drugs in infertile women [21].

Offering choices for prenatal testing, including pre-implantation genetic diagnosis, and carrier testing to persons of reproductive age is crucial. About 50% of a parent's kids can be affected by the autosomal dominant condition LS, which can be passed on from either parent. Couples should also be informed of the risks and potential outcomes of conceiving a child with constitutional MMR deficiency syndrome if both spouses have pathogenic mutations in the same MMR gene. To find out if the spouse carries pathogenic mutations in the same MMR gene, carrier testing might be made available. This is especially important since MSH6 and PMS2 mutations are widespread in the community and may not be strongly correlated with a substantial family history of cancer, even though they may be linked to a more moderate penetrance phenotype [24].

Menopausal hormone treatment

In terms of treatment decisions, endocrinologists generally form a clinical management team with gynecologists.

Menopausal hormone treatment (MHT), is a general term that refers to both unopposed estrogen usage for women who have had hysterectomies and also the combination estrogen-progestin therapy for women who still have their uteruses intact but require a progestin treatment to avoid estrogen-associated endometrial hyperplasia. MHT is sometimes referred to as "hormone therapy", although in the past it was known as "hormone replacement therapy". MHT can prevent and relieve most of the symptoms associated with surgically induced premature menopause. Non-hormonal treatment strategies are also available for women who have contraindications to MHT for the treatment of vasomotor symptoms and for the alleviation of genitourinary syndrome symptoms, including selective serotonin reuptake inhibitors (SSRIs), alpha 2 adrenergic agonists, and alternative medicine approaches such as the use of black cohosh, soy, flaxseed, vitamin E , yoga exercise and meditation [25].

Decisions about whether or not to use MHT should be individualized, taking into account the potential risk-benefit balance, severity of estrogen deprivation symptoms, personal and family history of cancer, and therapeutic goals [25].

In addition, recommendations in the Endocrine Society's 2015 Clinical Practice Guidelines suggest calculating cardiovascular and breast cancer risks before initiating MHT for any patient [26].

Although it has not been specifically researched in a Lynch population, MHT is generally regarded as safe for the treatment of surgical menopause after prophylactic surgery in premenopausal women. Women also benefit from requiring only estrogen replacement therapy, which is also advised after hysterectomy for endometrial cancer risk reduction in LS. In particular, for women with LS who undergo premenopausal surgery, experts advise considering MHT [8,14]. It's interesting to note that there is evidence that MHT protects against the development of colorectal cancer in the general population, which may be beneficial for women with LS, regardless of the type of oral or cutaneous MHT product, although further studies in this specific population would be needed [27]. Furthermore, researchers stated that MHT is advantageous in survivors of colorectal cancer [23].

Thus, in the case of young LS patients who opt for preventive surgery with full knowledge of the consequences, MHT should be started as soon as possible. In the absence of contraindications, MHT is recommended for clinical management of relief symptoms as well as for bone, cardiac, and cerebral health.

In addition, the guidelines recommend initiating MHT immediately after the diagnosis of premature menopause and prescribing higher doses of estrogens to mimic endogenous estrogen levels in younger, menstruating women compared to women who are menopausal at a normal age in their 50s. In addition, it states that MHT should be continued until the age of natural menopause, at which point the appropriateness of extending MHT should be reassessed [20].

In the absence of contraindications, MHT is recommended for relief of clinical symptoms as well as for cardiac, cerebral, and bone health and benefits [23].

It is recommended to start treatment with transdermal oestradiol which is biochemically similar to endogenous oestradiol. In addition, the use of the transdermal route avoids hepatic metabolism, with less metabolic impact. Alternatively, oral estrogens or a vaginal ring through which systemic doses of estrogen are administered can be used. Depending on the patient's preferences and whether she has a uterus, endometrial protection can be provided in several ways. If the patient wishes to become pregnant, it is recommended to use micronized progesterone rather than a synthetic progestogen for 12 to 14 days per month[28]. The absence of bleeding at the end of therapy or near the end of cyclic progestin therapy is an indication of a pregnancy test. If conception has occurred, MHT should be discontinued. For women without a uterus, established evidence does not justify the concomitant use of progesterone because endometrial protection is not necessary [5,20,26].

Women who underwent bilateral salpingo-oophorectomy before the age of 45 and were treated with estrogens until at least 45 years of age had a decreased risk of osteoporosis [29].

However, following basic guidelines for preserving bone health, bone mineral density should be assessed as soon as the start of premature menopause [20].

In addition, in a study of the Women's Health Initiative, women assigned to receive estrogen therapy who underwent bilateral salpingo-oophorectomy before the age of 45 years had a reduction in long-term all-cause mortality [30].

Guidelines for the prevention of bone and cardiovascular disease also recommend the adoption of general healthy lifestyle measures: weight regulation, exercise, restful sleep, smoking cessation, moderation of alcohol consumption, adequate calcium and vitamin D intake, and management of blood pressure, lipid and glucose levels [20].

Thus, it should be noted that the use of estrogen comes with a number of warnings and precautions, including a higher risk of gallbladder disease; if severe hypercalcemia, eyesight loss, or cholestatic jaundice develops, estrogen usage should be stopped. In addition, women getting thyroid replacement treatment should have their thyroid function checked regularly [5].

2. Endocrine tumors associated with LS

Adrenocortical carcinoma

Adrenocortical carcinoma (ACC) is a rare, aggressive cancer that starts in the adrenal gland's outer layer. ACC was associated with LS, even though it is known that it belongs to the Li Fraumeni syndrome tumor spectrum due to a TP53 mutation [31]. In a recent study, 3 patients out of a total of 634 patients carrying a germline mutation in the MSH2 gene were diagnosed with non-secretory ACC, corresponding to an incidence of 0.47% of all patients and 1.7% of all patients with MSH2 mutation, similar to other less common neoplasms considered to be part of the LS spectrum.

The association of ACC and LS was initially described in 2013 in a study that reported that 3.2% of LS patients had ACC [32]. Moreover, in 2016, the first case of familial ACC in association with an MSH2 pathogenic mutation was reported [33].

However, at this time, there is no need to advocate routine ACC screening for individuals with LS; nevertheless, physicians should have ACC on their minds for LS patients who have adrenal masses or other clinical signs of overactive adrenal glands [34].

Thyroid cancer

The first case described in the literature was an LS patient with a germline MSH2 mutation who developed undifferentiated thyroid cancer as an index case. Another case described was a thyroid cancer case in a patient with LS with anaplastic thyroid cancer as index cancer.

Another case reported was a synchronous association of papillary thyroid carcinoma and metachronous breast cancer with progression to metastasis [35]. In addition, a cohort study of 383 participants showed co-occurrence of familial non-medullary thyroid cancer (FNMTC) with LS, showing a heterozygous MSH2 gene modification exclusively in the FNMTC-LS family. FNMTC was more prevalent in females in 68.3% and was characterized by a mean tumor size of 1 cm, having multifocal growth in 44% of cases and extrathyroidal extension in 11.3% of cases [36].

Pituitary tumors

Additionally, a patient with LS who had an MSH2 mutation and had significant clinical Cushing disease was documented. He had corticotroph pituitary carcinoma. Furthermore, an invasive nonsecreting macroadenoma and a microprolactinoma, two clinically significant pituitary tumors, were described in a cohort study of 910 patients with LS from Sweden [37].

Testicular tumors

Moreover, a histopathological case report also described a clinical case that led to the broadening of the clinical tumor spectrum of LS by a clinical case of testicular seminoma associated with loss of MSH2 function [38]. Although there have been reports of MSI and MMR deficiency in testicular cancers that are resistant to chemotherapy and have a shorter time to recurrence, testicular cancer is not considered a member of the LS tumor spectrum. Despite the conflicting data reports, it is probably still desirable to investigate individuals with chemoresistant or recurrent testicular cancer for a potential link with LS [34].

Parathyroid carcinoma

Even while parathyroid carcinoma is extremely uncommon — it only makes up 0.005% of all malignancies — and isn't typically seen of being a member of the group of cancers that are connected with LS, one case report was recorded. Thus, a patient with severe hypercalcemia resistant to zoledronic acid accompanied by hyperparathyroidism and MSH2 and MSH6 deficiency was noted, demasking metastatic parathyroid carcinoma [39].

Thymoma and thymic cancer

Thymoma in LS patients has also been documented, as well as a unique occurrence of thymic cancer in a patient who carries the MLH1 heterozygous mutation [40].

CONCLUSIONS

Endometrial and ovarian cancer are more common in people with LS. Hormonal therapies, including combined oral contraceptive pills, only progestin treatment, or progesterone-containing intrauterine devices, are an alternative form of chemoprevention for those with LS. The risk of passing on hereditary pathogenic gene variants to their offspring, future fertility options, and the potential use of hormone replacement therapy after risk-reducing surgery should all be discussed with women who have LS.

Bone mineral density should be measured as early as the onset of premature menopause, following general recommendations for maintaining bone health. In addition, cardiovascular risks should be assessed and managed, and the patient should be encouraged to follow healthy lifestyle measures.

Further studies are needed on the role played by drugs involved in the chemoprevention of endometrial and ovarian cancers, to decide the appropriate time for prophylactic surgery and the ideal time for fertility, in order to enable physicians to prevent and identify Lynch-associated cancers earlier, thus reducing morbidity and mortality caused by this syndrome.

Other endocrine tumors have been described such as adrenal carcinoma, thyroid cancer, aggressive pituitary tumors, parathyroid, testicular, and thymic tumors.

Conflicts of interest and sources of funding

The author declares no conflict of interest.

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SYSTEMATIC REVIEW

Lynch Syndrome – An Overview of the Worldwide Management of This Silent Killer Within Our Genes

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Abstract: *Lynch syndrome, also known as hereditary non-polyposis cancer syndrome, represents the most common autosomal dominant genetic predisposition for the early onset development of several malignancies. Nearly three decades ago, the discovery of microsatellite instability, a distinctive feature of pathogenic variants within genes encoding mismatch repair proteins, marked a significant leap forward in understanding cancer biology and the underlying spectrum of cancers triggered by these mutations – typical of Lynch syndrome. In recent years, a new treatment paradigm, using immune checkpoint inhibitors, as well as preventive measures has drastically improved the survival rates. Identifying individuals with an inherent predisposition to cancer, through diagnostic protocols followed by personalized screening and treatment algorithms, holds the potential to mitigate premature cancer-related fatalities as well as preventable mortality. It is estimated that only a limited number of patients have been diagnosed, underscoring the importance of implementing specific screening programs for early detection of malignancies to which these patients are susceptible. This article aims to underline the importance of a national protocol tailored to guide a Western-inspired practice for Lynch syndrome patient management, the main aim of the Romanian Society for Lynch Syndrome, by providing an overview of similar initiatives throughout the world.*

Keywords: *Lynch syndrome, national registry; international consortium*

INTRODUCTION

In this article, we provide an overview of the key historical milestones in the management of Lynch's syndrome. Our review spans global geography, with the aim of highlighting the imperative for a national Romanian registry of Lynch syndrome, alongside tailored management initiatives. Additionally, we aim to identify the most straightforward and expeditious approaches to implementing global guidelines within the Romanian healthcare system.

Hereditary non-polyposis cancer syndrome was first studied by American pathologist A. S. Warthin, in 1895, in the family of a seamstress, with a generous background of cancers. [1] Warthin didn't manage to convince the scientific community that cancer prevalence within this family was not a coincidence, despite having published the oncological background of the seamstress's German family, known as "Family G", that had a history of very specific cancers, mostly digestive and gynecological. As such, the topic was left dormant for years. [2] In 1971, Henry T. Lynch, MD revisited "Family G", gathering data on 929 descendants. The clear presence of an autosomal dominant cancer predisposition pattern prompted the initiation of molecular investigations. Subsequent analysis revealed microsatellite instability as a distinguishing feature of these tumors. The underlying reason was attributed to a deficiency in the Mismatch Repair (MMR) system, with the responsible genes duly identified, reporting on the specific mutation in the MSH2 gene for the "G Family". [3,4] Henry T. Lynch formulated the fundamental principles of oncogenetic syndromes (heritable cancers): an early age of cancer onset, a distinct pattern of primary cancers, and a Mendelian inheritance model observed in families worldwide. He emphasized that identifying the genetic predisposition to develop cancers holds both prognostic and therapeutic implications. [4]

The acknowledgment of a genetic predisposition that substantially increases the risk of cancer, predictably affecting specific organs, has prompted global efforts to identify susceptible patients; this is driven by an understanding that closely monitoring organs prone to such risks – the digestive tract, reproductive and urinary systems, as well as the skin – represents the optimal approach for bolstering survival rates. To aid in the identification of potential patients, the Amsterdam Criteria were introduced in 1990. [4]. Understanding the biology of deficient MMR tumors, their accelerated growth rate [5], and the tumor immunogenicity carrying therapeutic implications, through the widespread employment of immunotherapy [6] or prophylactic measures like vaccines which are already in advanced stages of clinical studies [7,8] form the foundation for early detection and standardized management of these patients.

MATERIAL AND METHODS

We conducted a comprehensive search on PubMed for English full-length publications with the intention of creating a concise narrative review. Our query used the keyword “Lynch syndrome management”, aiming to encompass information from several continents. Our inclusion criteria featured original publications from the past 10 years, provided they were in full-length format and written in the English language. We excluded non-English papers, articles focusing solely on isolated tumors associated with Lynch syndrome, and those lacking a systemic impact. Throughout our research, we adhered to prominent international guidelines and consensus related to the diagnosis, screening, and treatment of Lynch syndrome. We found 872 relevant publications for the search term “Lynch syndrome management”. Furthermore, when exploring the intersection of "consortium" and Lynch syndrome over the recent years, we identified 84 articles. Similarly, our query for “Lynch syndrome management guidelines” yielded a total of 182 relevant publications, narrowed down to 95 when focusing on the last 5 years. When we searched for “Henry T Lynch” as an author, we found 294 publications, from which 82 regarded Lynch syndrome pathogenesis, manifestations spectrum, or management options.

RESULTS

The prodigious scientific activity of Henry Lynch is explicitly synthetized in almost 100 articles in which he aimed to find an optimal methodology for diagnosis, being aware that the new paradigm in oncology will impact in a significant way the survival of these patients. Describing the biological particularities of tumors attributed to Lynch Syndrome, he was a visionary regarding tailored screening programs, novel therapeutic options, and chemoprevention. Several international consortiums were coagulated aiming to find families and to study their particularities.

The Birth of Diagnostic Criteria

The Netherlands took the first step by identifying 22 families with a genetic predisposition to develop cancers. In 1983, a registry was set up in The Netherlands, overseen by a committee comprising multiple hospitals. The foundation’s objectives include enhancing monitoring within families with predispositions to hereditary tumors, ensuring consistent periodic assessment, and providing guidance to both general practitioners and medical specialists on screening approaches. [9] Dutch researcher Hans Vasen, MD was one of the leading minds behind the inception of the “International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer” (ICG-HNCPCC). This initiative was conceived during a CRC conference in Jerusalem in 1989 and held its inaugural gathering in Amsterdam in 1990. It was during this meeting that Vasen introduced the initial Amsterdam Criteria, also known as the 3 2 1 rule. Following this, the ICG-HNPCC would meet every year until 2001. [4]

The inception of a prominent new organization, InSiGHT (International Society for Gastrointestinal Hereditary Tumors), stemmed from the merger of ICG-HNPCC with the Leeds Castle Polyposis Group (LCPG), carried out over several meetings, in 1999 in Lorne, Australia, in 2000 in Tiberias, Israel, and in 2001 in Venice, Italy, respectively. [10,11]

These guidelines played a crucial role in pinpointing familial groups that ultimately unveiled the connection between the HNPCC syndrome and inherited mismatch repair gene mutations (MMR) [12-14]. However, the guidelines do not account for extracolonic cancers or apply to smaller family groups.

Both sets of criteria were later revised, as their original variants presented insufficient diagnostic sensibilities and specificity, leading to the omission of numerous patients with Lynch syndrome.

The Revised Amsterdam Criteria (Amsterdam II)

The identification of at least three relatives with HNPCC-associated cancer (cancer of the colon or rectum, endometrium, small bowel, ureter, or renal pelvis) and:
I. One should be a first-degree relative to the other two;
II. At least two successive generations should be affected;

III. At least one should be diagnosed before age 50;
IV. Familial adenomatous polyposis should be excluded;
V. Tumors should be verified by pathological examination. [14]

The Revised Bethesda Guidelines
I. Individual with CRC diagnosed by age 50;
II. Individual with synchronous or metachronous CRC or other HNPCC-associated tumors, regardless of age;
III. Individual with CRC and MSI-H histology diagnosed by age 60;
IV. Individual with CRC and more than one FDR with an HNPCC-associated tumor, with one cancer diagnosed by age 50;
V. Individuals with CRC and more than two FDRs or SDRs with an HNPCC-associated tumor, regardless of age. [15,16]

In November 1996, the Early Detection Branch of the U.S. National Cancer Institute held a global workshop titled "The Intersection of Pathology and Genetics in the Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Syndrome", in Bethesda, Maryland. The workshop sought to elucidate the genetic influence on HNPCC pathology, discussing topics like genomic instability, multistep carcinogenesis, mismatch repair gene involvement, HNPCC histopathology, potential molecular correlations, cell proliferation markers, their relevance to early diagnosis and prognosis, and potential clinicopathologic criteria for identifying more HNPCC patients. [17]

The diagnostic landscape of Lynch syndrome has seen significant advancements through the formulation of the Amsterdam and Bethesda criteria (and their revised versions). As we delve into this realm, it becomes relevant to also consider the contributions of the Ontario Familial Colon Cancer Registry (OFCCR). The organization is an innovative database gathering family histories, epidemiological records, blood samples, and tumor specimens from a relevant sample of colorectal cancer patients and their families. Achieving substantial response rates in genetic family investigations is particularly difficult, due to time demands and confidentiality concerns. Notably, the inaugural year of the registry saw a 61% response rate, with 1,395 probands taking part. [18]

The main goal of the Colon Cancer Family Registry (CCFR), an international consortium comprising 7 institutions from the USA, Canada, and Australasia. [18]

This database allowed for the differentiation between sporadic and familial forms of colorectal cancer in the subsequent year. These distinctions formed the basis for preventive, early diagnostic, and therapeutic strategies for colorectal cancers. [19]

Scientific research into the distinction between sporadic and hereditary cancers within these databases has yielded numerous conclusions that now play a crucial role in primary and secondary prophylaxis, optimal screening methods, and treatment approaches:

- Assessing the therapeutic effectiveness of extensive colectomies [20];
- Creating a model for distinguishing hereditary colorectal cancers from sporadic ones that exhibit microsatellite instability based on hyper-methylation, as well as morphological and clinical criteria [21];
- Comparing the diagnostic values obtained through IHC (Immunochemistry) tests against PCR (Polymerase Chain Reaction) [22];
- Factors that influence the adherence to screening programs [23]
- Smoking as a risk factor and NSAIDs emerging as a protective factor [24];
- The potential reduction of colorectal cancer risk through phytoestrogen intake [25];
- Estrogen-progesterone therapy [26];
- BMI, as underweight and obese postmenopausal women with colon cancer face an elevated risk of death, with comorbidities potentially contributing to this increased risk [27];
- Multiparity and pregnancy decrease the risk of colorectal cancer; studies showed an approximately 30% – 50% lower risk of gravidi-ty-associated colon cancer when considering parity. A rise in gravidity and parity appeared to show a declining trend in rectal cancer risk (p for trend = 0.07). In comparison to women with an equal number of pregnancies and live births, those who were nulligravid and nulliparous faced a 40% - 60% increased risk of colon cancer. Additionally, having one more pregnancy than live birth hinted at a potential decrease in both colon cancer and rectal cancer risk. Furthermore, there was a suggestion of an increased risk of MSI-high tumors in connection with nulligravidity and nulliparity. [28]
- Smoking increases the risk of rectal cancers in patients with Lynch syndrome [29];
- Physical activity decreases the risk of colon cancer [30].

In 2007, the registry of familial colorectal cancers became an international resource for the epidemiological study of colorectal cancers, employing two distinct recruitment strategies: population-based (tracking patients with colorectal cancer and identifying the proband through MSI testing) and clinic-based (identifying families with a genealogical history of cancers). This resource supports an ongoing, comprehensive research initiative that spans various fields and disciplines. The extensive and well-defined resource is open to the wider scientific community for studies on colorectal cancer. [31]

Western Countries Which Have Adopted Best Practice Guidelines

United States of America

In the USA, the implementation of screening recommendations for Lynch syndrome involved the collaboration of the CDC in 2009, resulting in the "Evaluation of Genomic Applications in Practice and Prevention (EGAPP) recommendations for Lynch syndrome. [32]

Figure 1: xxx

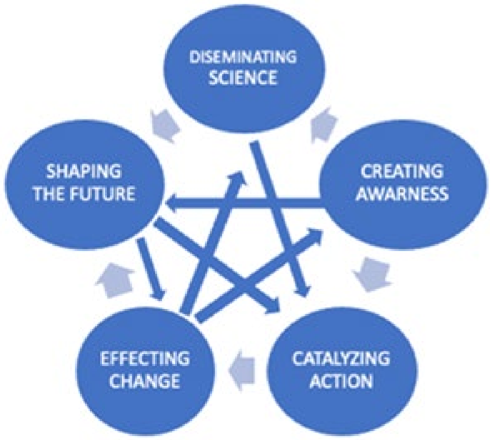

Health outcomes are the ultimate goal, driven by the 5 domains of influence. Disseminating science can include publication of findings in peer-reviewed journals, presentation at conferences, or through other media channels. Creating awareness involves receiving recognition and may include awards, general awareness, or acceptance of a concept or findings by the scientific community or policymakers, generating new discussion. Catalyzing action may include partnerships and collaborations, technology creation, congressional hearings or bills, or introduction in practice. Effecting change may include building public health capacity, legal or policy change, cultural, social, or behavioral change, or economic change. Shaping the future may include new hypotheses or strategies, implementation of new programs/ initiatives, or quality improvement." [33,34]

The genetic testing standard for hereditary colorectal cancers and polyps, set by the American College of Medical Genetics and Genomics, underwent its latest revision in 2021. It mandates universal testing for colorectal and endometrial cancers, irrespective of age at diagnosis, using techniques such as immunohistochemistry (dMMR testing) or molecular biology (microsatellite instability testing). Both methods are considered interchangeable alternatives.

Simultaneously, The International Mismatch Repair Consortium (IMRC) operates as a global collaboration comprising over 115 researchers from 59 centers/clinics across Africa, Asia, Australasia, Europe, North, and South America. These researchers are engaged in clinical care and/or research related to Lynch syndrome families. Professors Jenkins, Macrae, and Haile have established this consortium, harnessing the expertise of leading groups worldwide, facilitated by the International Society for Gastrointestinal Hereditary Tumors (InSiGHT) and the Collaborative Group of the Americas on Inherited Colorectal Cancer (CGA). Cumulatively, the IMRC possesses pedigree data for more than 8,800 Lynch syndrome families, encompassing over 20,000 confirmed carriers of germline mutations in mismatch repair genes. [34]

Canada

Canada initiated the registry for hereditary colorectal cancers in 2011 following a multidisciplinary consensus involving experts in genetics, gastroenterology, surgery, oncology, pathology, and healthcare systems. This collaboration resulted in the establishment of five consensus statements:

- Consensus Statement #1: Hereditary colorectal cancer registries will improve the identification of individuals with an elevated risk of hereditary colorectal cancer.

- Consensus Statement #2: Hereditary colorectal cancer registries will increase access to suitable clinical and genetic screening for individuals at increased risk of colorectal cancer.

- Consensus Statement #3: Increased access to clinical and genetic screening will contribute to reducing the occurrence of colorectal cancer and enhancing survival rates among relatives who are carriers at risk.

- Consensus Statement #4: Every province's population should have access to a provincial hereditary colorectal cancer registry.

- Consensus Statement #5: A network of hereditary colorectal cancer registries in Canada would facilitate both clinical care and collaborative research. [35]

The latest recommendation derived from the Canadian hereditary colorectal cancer registry is that incorporating universal screening within a publicly funded system will enhance the effectiveness of identifying Lynch syndrome patients.

Given potential limitations in genetic testing and counseling resources within public systems, the integration of secondary screening involving BRAF and MLH1 promoter hypermethylation testing is crucial for further enhancing efficiency. [36,37,38]

United Kingdom

In February 2017, the National Institute for Health and Care Excellence (NICE) issued updated diagnostic guidance: Pathology departments at 14 hospital trusts in the Yorkshire and Humber region of the United Kingdom were requested to submit material from patients aged 50 years or older with newly diagnosed colorectal cancer between April 1, 2017, and March 31, 2019, for Lynch syndrome screening. The testing protocol included immunohistochemistry for MLH1, PMS2, MSH2, and MSH6, followed by BRAF mutation analysis with or without MLH1 promoter methylation testing if MLH1 loss was identified. A total of 3,141 individual specimens were submitted from 12 departments, comprising 3,061 distinct tumors and 2,791 prospectively collected CRC patients. dMMR was observed in 15% of cases. Among cases with MLH1 loss, 76% exhibited a detectable BRAF mutation, while 77% of the remaining cases showed MLH1 promoter hypermethylation. Among patients included in the final analysis, 81 (2.9%) had indications for germline testing.

The implementation of LS screening through the NICE diagnostics guidance pathway can be effectively executed on a large scale, identifying a substantial cohort of patients with dMMR. This data is used to refer patients to regional clinical genetics services and also plays a crucial role in shaping treatment routes, including decisions regarding adjuvant/ neoadjuvant chemotherapy and immunotherapy. [39]

Australia

The first case of Lynch syndrome was identified by the Genetic Services Western Australia in 1994, shortly after pathogenic mutations in MMR genes were established as the underlying cause of the condition previously referred to as HNPCC. Over the following 11 years until 2004, there were 1 to 4 new cases identified annually. This observation was deemed lower than anticipated, given that the annual incidence of CRC in WA was approximately 1,000 cases during this period and considering the assumption that LS contributes to 1–3% of all CRC cases.

Australia has a single registry for familial cancers, which, starting in 1994, compiled data accompanied by Lynch syndrome screening results using routine IHC testing of MMR or MSI through PCR for cancers in patients under 60 years old. This approach quadrupled the annual number of identified LS cases. [40]

The Netherlands

The Dutch Hereditary Cancer Registry was established in 1985 with the support of the Ministry of Health (VWS). The main goals of the registry are:

I. Facilitating the identification of families with hereditary cancer;

II. Encouraging high-risk individuals' participation in surveillance programs;

III. Ensuring the continuity of lifelong surveillance examinations;

IV. Promoting research, especially for enhancing surveillance protocols.

Currently in the Netherlands, the diagnosis of Lynch Syndrome relies on a nationwide guideline for MSI analysis, introduced in January 2008 by the Netherlands Comprehensive Cancer Organization. Leveraging the Netherlands Cancer Registry [41], this guideline facilitates dynamic enhancements in Lynch Syndrome Management. This guideline closely resembles the MIPA (Multitesting Indicated by a Pathologist) criteria. MSI analysis (along with IHC of MMR proteins) is requested by pathologists for patients recently diagnosed with CRC or endometrial carcinoma under the age of 50, or those with two LS-associated tumors (including synchronous and metachronous CRCs) before 70. Cases with multiple LS-associated cancers are documented in PALGA, the nationwide network and registry of histopathology and cytopathology in the Netherlands [42]. For MSI analysis prompted by a positive family history, referral to a clinical geneticist is advised. In these instances, MSI analysis is generally conducted when the (revised) Bethesda or Amsterdam Criteria are met, contingent upon the availability of archival paraffin-embedded tumor tissue. [43,44]

Sweden

The National Swedish Registry (now known as the SCRCR) was founded in 1995, with a focus on rectal cancers, and later expanded to include colon cancers in 2007. The use of a unique personal ID number by the SCRCR enables the tracking of patient data over time. The study indicated that a minority of young CRC patients received genetic counseling, which goes against clinical guidelines. Consequently, hereditary CRC is likely to be underdiagnosed, even within the younger population. [45,46]

In Sweden, families suspected of having Lynch syndrome are directed to the regional department of clinical genetics in Umeå, Uppsala, Stockholm, Linköping, Gothenburg, or Lund for counseling and genetic testing.

Switzerland

The present approach to Lynch syndrome screening in Switzerland incorporates two tumor-based assessments (IHC and BRAF V600), followed by DNA sequencing. Germline DNA screening exhibits almost 100% accuracy, thus identifying all instances of Lynch syndrome and eradicating false negative and false positive diagnoses. This strategy also averts the possibility of CRC patients being lost to follow-up and prevents deaths stemming from undetected LS cases. Additionally, it identifies a greater number of cancer-free relatives and new CRC cases at early stages through colonoscopy screening. Despite the notable expenses linked to providing DNA testing for all CRC patients, universal LS screening enhances the overall effectiveness of genetic testing at a reasonable cost. [46]

Iceland

Iceland introduced universal screening for Lynch syndrome by conducting immunohistochemistry for MMR proteins on resected colorectal carcinomas and endometrial carcinomas in 2017. The primary challenge involves establishing a robust implementation of the screening algorithm by pathologists and ensuring accurate referrals for genetic counseling. One suggested solution is the automatic application of IHC staining on all CRC and EC cases, with any abnormal staining outcomes promptly reported to designated pathologists overseeing the ordering and interpretation of additional molecular tests. When genetic counseling is recommended, effective communication with the surgeon in charge, and ideally, the genetic counselor, is of paramount importance, preferably through verbal means [47].

Austria

The Austrian AGO (the workgroup for oncological gynecology) recommends IHC tissue screening for both type-I and type-II endometrial cancers for patients below the age of 70, and for endometrioid and clear-cell ovarian cancers, irrespective of patient age. If necessary, IHC can be supplemented with MLH1 promoter hypermethylation testing and/or microsatellite instability analysis on tissue samples. To confirm a Lynch syndrome diagnosis, identifying a germline mutation through molecular genetic testing of the mismatch repair genes using the patient's blood is required. This genetic examination should be conducted directly without prior tissue screening if the family history of LS-associated cancer patients aligns with either the Amsterdam II or the revised Bethesda criteria.

There are two distinct tumor tissue-based screening processes that significantly raise suspicion of Lynch syndrome:

- IHC identification of the absence of any of the four relevant MMR proteins: MLH1, MSH2, MSH6, or PMS2 in tumor tissue.
- Detection of MSI by examining five specific microsatellites in tumor DNA using RT-PCR; the presence of two or more positive markers indicates a strong suspicion of Lynch syndrome.

However, for individuals with a confirmed LS-associated carcinoma and a positive family history in accordance with either Amsterdam II or the revised Bethesda criteria, the aforementioned screening protocol should be bypassed, and a germline analysis should be promptly provided.

In alignment with the 2014 National Comprehensive Cancer Network guidelines, AGO Austria advises the testing of tumor tissue for Lynch syndrome in all women with endometrial carcinoma (both types I and II) who are under the age of 70. [48]

Furthermore, AGO Austria also advocates for the performance of these tests regardless of the age at diagnosis for endometrioid and clear-cell ovarian cancers. Even in cases where all screening outcomes yield negative results, if the family history still strongly indicates the possibility of Lynch syndrome (i.e., Amsterdam II or revised Bethesda criteria are fulfilled), comprehensive molecular genetic testing of all identified LS-associated genes should be contemplated.

Japan

Until the early 2000s, Lynch syndrome screening predominantly targeted cancer patients with high-risk attributes as per the Amsterdam II criteria or the revised Bethesda guidelines. However, in subsequent years, the practice of universal screening, involving MMR IHC and/or MSI analysis (with or without age restrictions), gradually took precedence over these population-based selections. Consequently, the approach to identifying Lynch syndrome has transitioned towards widespread universal screening [49].

Today, the advent of cancer genomic-based precision medicine has ushered in the use of Next-Generation Sequencing (NGS) for comprehensive somatic and germline MMR sequencing, spanning selected-gene or genome-wide assessments.

However, the main challenge of universally applying sequencing for germline MMR analysis pertains to the assessment of pathogenicity, which relies on the public genome database. This arises due to the substantial presence of Variants of Uncertain Significance (VUSs), and the eventual transition to a pathogenic level over time.

High rates of gastric cancer were observed in Japanese individuals affected by Lynch syndrome, and this occurrence was linked to Helicobacter pylori infection. The deficiency of MMR proteins was found to contribute to the formation of malignant tumors in LS patients. [50]

Advancing Lynch Syndrome Management

Results of Universal Screening

Presently, there are differing approaches to tumor testing methods across hospitals, resulting in a range of 0 - 19% of patients having incomplete mismatch repair deficiency test results. Due to the existing gaps in Lynch syndrome testing and referrals, additional efforts are required to establish effective approaches for seamlessly integrating LS testing into clinical practice; this could serve as a blueprint not only for hereditary cancers but also for broader genomic medicine. The implementation of standardized reporting may assist clinicians in comprehending tumor test outcomes and initiating appropriate subsequent steps.

Extending Testing to Other Organs

In 2020, Stanford University proposed the implementation of MMR testing for all digestive tract cancers, not solely limited to colon cancers. This recommendation stemmed from a study involving 1,425 consecutive malignant neoplasms that underwent MMR testing post-surgery. Surprisingly, 7.2% of these cases exhibited dMMR. The highest prevalence rates were observed for gastric cancers at 10%, followed by colorectal cancers at 9.1%, pancreatic cancers at 5.3%, and gastroesophageal cancers at 4.5%. [51]

Improving the Implementation of Diagnostic Guidelines

Based on these findings, and starting in 2008, routine MSI and/or IHC testing was recommended for all CRC patients under 60 years of age in Western Australia, regardless of their family cancer history. However, the systematic implementation of these tests at a population level for LS identification has proven to be challenging due to the need for collaboration and effective communication among various disciplines, including gastroenterology, pathology, surgery, oncology, and medical genetics. By the end of 2012, GSWA had identified 115 unrelated LS families with MMR gene mutations, comprising 40 MLH1, 51 MSH2, 11 MSH6, and 9 PMS2 germline mutations.

A significant number of Lynch syndrome patients detected through screening did not have a family history of cancer meeting the Amsterdam or Bethesda criteria, highlighting the importance of laboratory screening tests in their identification. Findings strongly suggest that the routine use of MSI and IHC screening for CRC has substantially improved LS detection in Western Australia.

Drawing from past experience with population-based screening in Western Australia, three crucial components take shape that are essential for the successful implementation of LS screening in other states or regions:

I. Reflex IHC testing should be conducted through accredited pathology services with consistent quality control systems in place.

II. The establishment of a centralized reference laboratory at the state or regional level is essential for MSI testing, serving to validate any abnormal or uncertain IHC results initially detected by pathology service providers. This is a crucial mechanism in preventing unnecessary referrals of individuals with false-positive IHC results to familial cancer clinics. In previous observations, the MSI test displayed reduced susceptibility to interpretational errors and greater potential for standardization, compared to IHC.

III. Thirdly, a pivotal aspect is the presence of state or regional-wide LS coordinators, ensuring that all red flag cases discovered by laboratory testing are promptly referred to familial cancer clinics before patients' health deteriorates significantly or life-threatening situations arise. [51]

Defining the Phenomenon of Genetic Anticipation

Genetic anticipation is a phenomenon where symptoms of a hereditary disease appear at an earlier age and/or are more severe in successive generations. [52]

A 2009 study examined the evolution of 125 families, encompassing 824 individuals (with 427 being female). This study yielded a quantified inference about anticipation. It revealed that the cancer appeared 3 years earlier from generation to generation. [53]

Since Alfred Whartin's initial description of Lynch syndrome in 1925, there has been documentation of a gradual reduction in the age of CRC diagnoses across successive generations. Over the years, a multitude of studies have examined the possibility of anticipation in this syndrome, showing variances from 3 to 13 years. [54]

Cost-Effectiveness Analysis for Universal Screening

A cost-effectiveness analysis conducted in Switzerland examined universal screening for Lynch syndrome in individuals diagnosed with colorectal cancer, followed by cascade genetic testing of their relatives. The Evaluation of Genomic Applications in Practice and Prevention working group has endorsed the practice of screening for LS among all newly diagnosed cases of CRC, regardless of age and family history, followed by the sequential testing of blood relatives. [55] The study assesses the economic viability of the alternative approach to identifying LS cases, considering the perspective of the Swiss healthcare system.

An analytical framework was formulated, merging decision trees with Markov modeling to calculate the cost-effectiveness of universal LS screening for individuals newly diagnosed with CRC, followed by cascade testing. Markov models encompassed four states: well-being, CRC, metachronous CRC (mCRC), and death. When diminishing the CRC risk in LS-diagnosed relatives to 25%, the program's cost rose to CHF

103,385 per QALY, surpassing the cost-effectiveness threshold. Universal LS screening combined with the sequential testing of relatives is cost-effective in around 80% of cases. The number of averted deaths is linked to yearly shifts from a state of health to CRC, and from CRC to death, which are significantly lower in populations subjected to testing and screening. [56]

Contemporary Challenges

Among path_MMR carriers undergoing colonoscopy surveillance, deaths resulting from non-CRC LS surpassed those from CRC. Addressing mortality associated with non-CRC presents a significant contemporary medical challenge in managing Lynch syndrome. [57]

International consortia

Genturis

The European Reference Network (ERN) is a network that connects healthcare providers and specialized healthcare centers, aiming to enhance access to diagnosis, treatment, and the delivery of exceptional healthcare services to patients diagnosed with rare diseases, regardless of their location in Europe. The governance of ERN also involves the participation of patient representatives. ERN employs international consensus guidelines for patient recruitment:

- Endorsed guidelines for Lynch, and polyposis, and hereditary CRC. The Manchester International Consensus Group recommendations for the management of gynecological cancers in Lynch syndrome

- Endorsed guidelines for Lynch, and polyposis, and hereditary CRC. Endoscopic management of Lynch syndrome and of familial risk of colorectal cancer – the European Society of Gastrointestinal Endoscopy (ESGE) Guideline.

- Endorsed guidelines for Lynch, and polyposis, and hereditary CRC. European guidelines from the EHTG and ESCP for Lynch syndrome – an updated third edition of the Mallorca guidelines based on gene and gender.

Middle East Network on Hereditary Colorectal Cancer

An association of medical professionals from Middle Eastern and North African (ME/NA) countries was established to enhance the identification and care of LS families. The organization conducted a survey in order to assess the existing healthcare services for families with LS in this region.

The acknowledgment and management of Lynch syndrome within ME/NA countries are suboptimal, leading to the majority of LS families in the area remaining unidentified. Future initiatives should focus on raising awareness about Lynch syndrome among both the public and medical practitioners and on improving the infrastructure in these countries. [58]

Future initiatives should concentrate on raising LS awareness among the public and medical professionals and enhancing the infrastructure within these nations.

Moreover, it's crucial to establish registries for families with hereditary cancer. This step aims to motivate participation in prevention programs and ensure the seamless continuation of lifelong surveillance initiatives. [59]

Countries With Limited Adoption of Western Best Practice Guidelines

Numerous countries continue to exclusively employ clinical diagnostic criteria, namely Amsterdam and Bethesda. The majority are in Asia and Africa, although most of these countries lead the diagnosis until genetic confirmation.

In Taiwan only a small number of medical centers offer Lynch syndrome diagnostics [60]; For a long time, China relied solely on the clinical diagnostic criteria. [61] In 2021, knowledge about LS-related EEC among the Chinese population remained scarce. The most extensive Chinese multi-center population study was undertaken in 2021, involving an analysis of 95 cases of dMMR. [62]; Based on the Singapore Polyposis Registry, an analysis was conducted on 59 unrelated families meeting the clinical diagnostic criteria, specifically Amsterdam II, and revised Bethesda. All identified genetic abnormalities involved MLH1 and MSH2, leading to the detection of LS in 17 families. [63] In a study conducted at a tertiary referral center focused on the rising cases of early-onset colorectal cancer, histopathologically processed tumor specimens revealed a potential increase in Lynch Syndrome incidence among CRC patients in Indonesia. They identified 32 likely cases of LS. [64]; The 2011-founded Russian Society for Clinical Oncology lacks specific recommendations for hereditary colon cancer. Lynch syndrome treatment seems to lean towards immunotherapy, with no clear genetic counseling recommendations. [65]

In South Korea, the largest study, which analyzed colo-rectal cancers recorded over a span of 10 years involving 5,520 patients, applied the Amsterdam II criteria to identify patients with LS. Only 1.3% of participants met these criteria, and their tumors were tested for microsatellite instability. Out of the latter group, only 25 patients, representing 6.9%, tested positive for MSI. Genetic tests were conducted on half of this latter group, yielding positive results for only 11 patients. [66]

Qatar, Saudi Arabia, and Pakistan conducted studies that represent foreplay for genetic testing, but no standardization is available for the moment [67, 68, 69].

African countries

South Africa consists of 9 provinces, with an approximate mid-year population of 58.78 million.

Genetic services are primarily extended to recently diagnosed LS patients and their family members within these areas through academic institutions situated in the Western Cape. Additional initiatives reach out to smaller neighboring towns and rural regions. [70-73]

Colonoscopic surveillance for at-risk family members is arduous and costly in resource-limited settings. Nevertheless, previous LS studies in the Western Cape region of South Africa have demonstrated that identifying the causative mutation significantly streamlines and reduces surveillance expenses. [74]

Morocco lacks a national protocol, the largest study involved 385 patients; for 214, MMR IHC testing was conducted; 159 underwent BRAF mutation testing as well. Among those diagnosed with LS, 35.6% were under 50 years old. These findings underscore the significant prevalence of CRC in young patients and the frequent occurrence of rectal localizations within the Moroccan population. [75]. In Tunisia, diagnosis is primarily based on clinical criteria. [76]

Identification of Lynch Syndrome Risk Variants in the Romanian Population

So far, two important research initiatives have been conducted in Romania.

After sequencing 61 CRC patients, 11 uncommon coding variants were identified in CRC genes: six in MLH1, one in MSH6, one in PMS2, and three in APC. ClinVar19's literature and clinical trial submission data for pathogenicity assessment were cross-referenced. Among the 11 variants, six were previously documented in ClinVar, each with a frequency below 1% in the ExAC database. [77]

Paget's Disease of the Bone and Lynch Syndrome: An Exceptional Finding

The patient's medical record included a diagnosis of well-differentiated rectal adenocarcinoma at the age of 34, which was treated with chemotherapy and radiotherapy, resulting in secondary amenorrhea, as well as the removal of an oesophageal papilloma through endoscopy. Furthermore, she had undergone surgery for three lumbar herniated discs and dealt with autoimmune chronic thyroiditis while maintaining normal thyroid function.

Concerning her family's medical history, the patient's mother had been diagnosed with polyostotic PDB, impacting her skull, femur, and spine at 44. Additionally, her father passed away from colorectal cancer at the age of 47. As of now, no targeted genetic testing has been conducted.

Although the simultaneous occurrence of PDB and LS has not been documented previously, instances of two distinct autosomal dominant conditions co-occurring, albeit rare, have been previously reported. [78].

DISCUSSION

The Romanian Society for Lynch Syndrome is working, through the national Lynch syndrome network, to align Romania with the countries practicing optimized management of LS patients.

CONCLUSIONS

Globally, colorectal cancer ranks as the first or second most common cancer in both sexes. Lynch syndrome, the most prevalent autosomal dominant genetic syndrome, predisposes to the development of numerous cancers, primarily driven by a genetic defect in MMR proteins. The colon is most frequently affected, with up to an 80% elevated risk of neoplasia compared to the general population. Multiple organs can be involved, including the endometrium, ovaries, and other parts of the digestive tract, identified since the syndrome's inception. Early diagnosis, both for the proband and their family, is pivotal for primary prevention and tailored screening programs. As of 2018, distinct therapeutic approaches have been introduced, including immunotherapy [79, 80].

Currently, there are numerous guideline recommendations and countries that have implemented them. However, widespread adoption of these recommendations has the potential to enhance cancer-related epidemiological indices (incidence, prevalence, morbidity, mortality), as well as economic profiles. Additionally, the significance of enriched databases enriches our understanding of the disease's biology, which still harbors many unknowns – prognostic factors for cancer onset, immuno-logical or genetic means (vaccines) of prevention, and more.

Conflicts of interest and sources of funding
The author declares no conflict of interest.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed following the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at: http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent before their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects from being recognized (or an eye bar should be used).

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

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– Identifies trials with a unique number; and

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These are invited by the Editor-in-Chief or their delegated editor and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure, and cite up to 15 references, including the source article. This should be cited as Rom. J. Mil. Med. (year); (vol): [issue].

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Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Acknowledgments

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EDITORIAL

Benchmarks of the evolution and revolution of military medicine in the XXI century – Tradition, Trust, Professionalism

Florentina Ioniță Radu

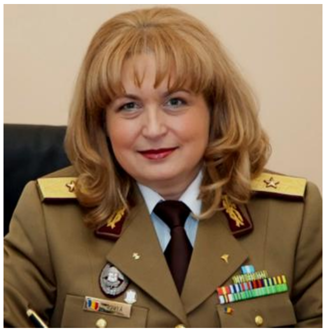

Brig. Gen. FLORENTINA IONIȚĂ RADU

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Associate Professor, Titu Maiorescu University, Bucharest

There is no secret to anyone that we live in a society marked by uncertainty and unpredictability, a society in which information flows through all communication channels, both in cyberspace and in mass media. Therefore, it is increasingly difficult for the „modern man” to decide upon the veracity and value of the informational content so that, eventually, based on the latter, he can upgrade his knowledge in any area of interest.

In such a context, it is difficult for society to control its evolution and to identify valuable markers that would ensure a valid growth in time and space. Lacking history, hierarchy, elite, rules and order, one can talk neither about the development of an organization nor about the development of society as a whole.

I think we have all passed, in recent years, through a "period of resistance" to the wave of non-values that invaded us and created an organic need to protect and preserve the values our parents and grandparents taught us. Despite the appearance of resignation, the accumulation of such energy has made us stronger, more aware of our role and this made us persistent and gave us the power and the chance to change our future and our children’s future.

Returning to the reason that brought us together, by means of this page, you – the reader and me – the author, I can say without any doubt that every cell of my being has reacted with emotion at the course and unrest of the last years of military medicine. It was only when I became the Commander (General Manager) of the "Dr. Carol Davila" Central Clinical Emergency Military Hospital (SUUMC), that I fully understood the huge potential of this noble profession – military doctor – potential generated by the excitement of the trust and respect offered each day by the public opinion in general and especially by citizens.

I have always considered that medicine is a field in which evolution ignores borders, bureaucratic rules, customs and paradigms, because trying to rescue somenone’s life is such a pure and noble approach that no sacrifice for this purpose can ever be considered too big.

This year the "Dr. Carol Davila" Central Clinical Emergency Military Hospital, a symbol for the history of Romanian society, celebrates the honorable age of 185 years of tradition, trust and professionalism, dedicated to research, development and innovation in medicine. 185 years in the service of life, without any deviation from the fulfillment of the two oaths: the military oath and Hipocrate's oath.

We should always keep, in our minds and hearts, the memory of those who, on the battlefield of the two World Wars, nursed their fellows and some of them even lost their lives trying to rescue the latter, the persistence of the struggle with their own limits taking care of the victims of the earthquake that shook Bucharest in March 1977; the emotion lived when faced with the young soldiers, victims of the construction of Transfăgărăşan and of the Danube – Black Sea Canal. Recently, the victims of the tragic fire at Club Colectiv were a challenge for the doctors and nurses in our hospital. All the departments of the hospital worked as a real efficient and dedicated team having only one purpose: to save the lives of the victims who were our patients.

During 185 years, together with the Romanian Army, we have been in the service of the country and of the population, doing our best in our duty with modesty, professionalism, ethics and team spirit. We did not pose as mentors, although here, in this area of health, the foundations of medicine, pharmacy and education in this field in Romania were laid. Medical and surgical specialties were also born here, specialties that today are spoken of as a fact, not as a gift from our ancestors, military doctors – thoracic surgery, urology, epidemiology etc.

The only thing military medicine was deprived of until now, is "a single voice of professionals" in this field, segment considered by most of our civil colleagues relatively narrow as area of specialty and therefore lacking the capital injection necessary to ensure its technical and cutting-edge technology, which apparently decreased our competitiveness in the market of medical services provided. Not true! In the absence of facilities, the human resource of this system accepted the professional challenge and approached a sphere of increasingly complex pathology, and on the other hand, the interdisciplinary collaboration has strengthened human links and increased our performance as professionals. It is true that until three years ago, we may not have had the latest surgical technologies but we beat cancer through the physician's sharp spirit, the surgeon's, the pathologist's and oncologist's experience.

Three years do not mean anything for a hospital, but for someone diagnosed with a relentless disease they mean everything... This was one of my thoughts that motivated me to develop centers of excellence – assigned and equipped to the highest standards – capable of providing prevention, treatment and monitoring of disease in the oncologic pathology, strokes, cardiovascular diseases and digestive diseases; lung transplant – a medical and surgical activity of high human and professional performance, which also involves a great responsibility assumed by SUUMC, on medium and long term, for patient's life quality, service which is not available at this time, in any other public hospital in Romania; neurological and neuromotor rehabilitation center for the veterans from the operation theaters.

Romania's geopolitical and geostrategic situation, as a state located at the EU and NATO border, obliges me, at the same time, to analyze the development of the operational medicine component in the Central Military Clinical Emergency Hospital "Dr. Carol Davila", so that, in wartime, we can be prepared to face the specific challenges related to the pathology and casuistry of a military confrontation.

Both as a doctor, and especially as the manager of the Central Military University Emergency Hospital "Dr. Carol Davila", I understand that the expectations of the whole society towards military medicine are very high, whether we speak of peace or war. People expect us to be a performant regional hospital, which provides at safe and high standards, a medical act comparable to that granted by a specialty hospital from abroad, while in wartime the hospital should become an elite military medical facility, ROL 4, able to provide medical care to the standards required by our NATO allies.

With a background of 185 years, the Central Military Clinical Emergency Hospital "Dr. Carol Davila", returns in force, as an important player on the market of medical services in Romania and elsewhere, proving that to the three qualities that have pierced the veil of history – TRADITION, TRUST, PROFESSIONALISM –

we should add the key to success in order to ensure the evolution of this type of institution, within the criteria imposed by the information society, consisting of flexibility, predictability, resilience and responsiveness in the case of a crisis or disaster.

As arguments in support of the above, I would point out some key milestones of the work of the last three years, namely, that SUUMC is the only institution in the medical network of the Ministry of Defence running projects from European funds for research, development and innovation in the medical field. It is connected to military medical centers in the Balkans, by means of the collaboration platform of telemedicine type, IMIHO. The hospital has developed collaboration agreements with major centers in the field, in the country and abroad (Belgium, Austria, Turkey and Serbia) to conduct training programs for medical staff and it is a serious instruction and training institution for students and residents, as a consequence of the extension of academic and university partnerships.

At the beginning of a new stage of life, I hope that The Romanian Journal of Military Medicine, in this new format, supported by a high scientific editorial board, will fulfill its objective to disseminate knowledge, to bring added value to the market of specialized publications in this field, from Romania and from abroad, to support the research activity and to motivate young people who make their first steps on the road to a career dedicated to the most precious thing that we all receive: health.

As a team player who strongly believes in the power of military medicine elite, in the interdisciplinary dialogue of this discipline, I assure you that I will support this approach, as I have already done it so far, in order to increase its quality every year and to borrow from the brilliance of the historic celebration of 185 years from the signing of the birth certificate of the Army Hospital.

REVIEW ARTICLE

Article received on February 12, 2016 and accepted for publishing on March 1, 2016.

The use of low-intensity ultrasound system in Orthopedics for treatment of fractures

Alexandra Șopu[1]

INTRODUCTION

The treatment of non-union or delayed union of fractures includes a growing number of approaches and techniques, one of which is bone growth stimulation using low-intensity ultrasound (LIUS). The treatment is effective, painless and safe. Currently, Exogen is the only device marketed worldwide that uses LIUS to influence the fracture healing process.

THE HEALING PROCESS OF THE FRACTURED BONES

All the fractured bones have to go through a healing process. This is true in cases of surgery or injury. The process of bone healing has three stages. The first stage is inflammation. This starts when the bone has been fractured. Bleeding always takes place in the area of the fracture. This leads to clotting of blood and inflammation in the area. This provides the framework of a new bone. The second stage is bone production. With time, the clotted blood formed in the inflammation stage is replaced by cartilage and fibrous tissues. As the healing continues, the hard callus replaces the soft callus. This can be seen by x-rays some days after the fracture [1, p 20]. The last stage is bone remodeling. This stage goes on for months. In this stage, the bone continues to become compact and returns to its original shape and structure. The blood circulation in the area of the fracture improves. Once some bone healing has taken place, weight bearing encourages the bone remodeling stage [2, p 99].

THE EXOGEN ULTRASOUND BONE HEALING SYSTEM

This is an approved and clinically tested system for treatment of bone fractures. Exogen makes use of the painless ultrasound waves in order to activate the cells that are near the site of the fracture. This speeds up the natural repair process [3, p 1999].

Clinical studies have shown that the system accelerates the healing process of the fractured bones by 38 % in acute fractures [4, p 655] and 86% in non-union or delayed union. It has been shown that Exogen can improve the fracture healing process of older people, those who take tobacco and those who are obese, cases in which the healing process might be delayed. Exogen system can accelerate the process of healing as much as 50 % for the patients who smoke [6, p 254].

The picture below offers a basic explanation of how Exogen works at the cellular level.

To enhance bone healing, Exogen releases low intensity pulsed ultrasound waves at the point of the fractured bone. This jump-starts the natural healing process of the body. The waves move through the skin and the soft tissues to stimulate the critical cells involved in bone healing.

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Figure 1: The way Exogen Accelerates the Healing Process of a Fractured Bone [14]

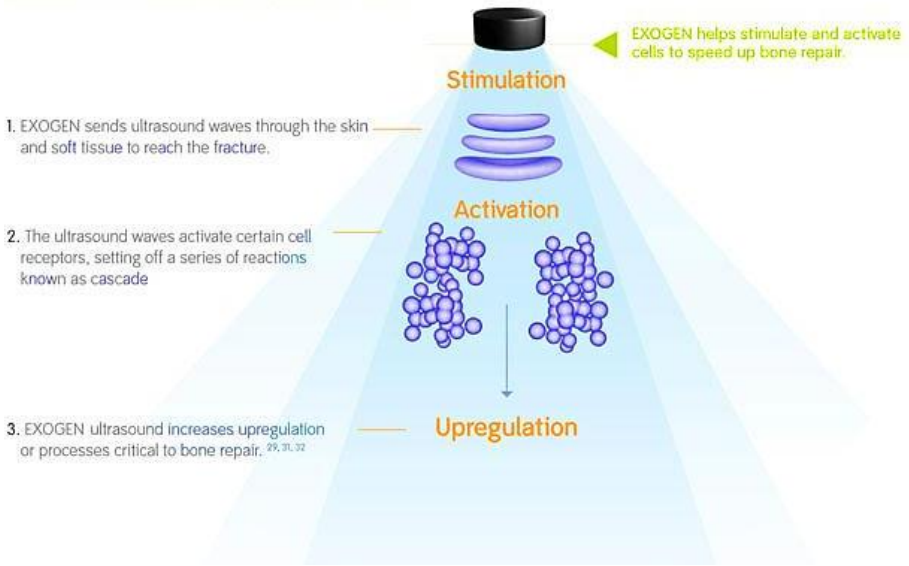
EXOGEN helps stimulate and activate cells to speed up bone repair.
Stimulation
1. EXOGEN sends ultrasound waves through the skin and soft tissue to reach the fracture.
Activation
2. The ultrasound waves activate certain cell receptors, setting off a series of reactions known as cascade
3. EXOGEN ultrasound increases upregulation or processes critical to bone repair. 29, 31, 32
Upregulation

In order to understand the role that the ultrasound plays in healing of the fractured bones, there is a need to understand what it is and how it works. It uses transcutaneous acoustic energy. The piezoelectric crystal produces sound waves which are transmitted through the various body tissues to make a number of changes which are implicated in the tissue healing [8, p 1098]. The density of the tissue is directly proportional to the sound waves that the tissue absorbs. The bone possess high density which makes the ultrasound waves to target the areas which may be affected or have bony abnormalities.

Low intensity pulse ultrasound (LIPUS) specifically works as a potential noninvasive therapeutic in healing of fractures. The waves which are produced by LIPUS introduce micromechanical stress in the area that has been fractured. This stimulates the cellular and molecular responses that are involved in the normal fracture healing. The osteogenic and angiogenic effects caused by the administration of LIPUS are non-thermal. The effects are mechanical in nature with a temperature variation of less than 10C [9, p 802].

The optimum parameters used to archive the maximum benefits of LIPUS are: a pulse width of 200us, an intensity of 30 Mw/cm2 and a 1.5 MHz frequency repeated at 1 kHz every day for 20 minutes [6, p 254].

HOW ULTRASOUND EXERTS MECHANICAL STRESS

There are two mechanisms by which LIPUS induces the mechanical stress in tissues. First it achieves it by displacement of the ends that are fractured and secondly by cavitation.

Research shows that displacement of the fractured ends occurs in at Nano metric scale [9, p 802]. This then stimulates the cellular and the molecular pathways which are involved in the bone healing process [10, p 1022]. It has been shown that LIPUS causes micro motion at the hard and soft tissues interface also. This action produces a mechanical stimulus that is more salient to the integrin mechanoreceptors which take part in osteogenic differentiation and cellular signaling. However it is not yet clear through the available research which displacement mechanism is dominant [11, p 703].

The second mechanism involves the acoustic streaming and cavitation. This mechanism promotes the idea that the sound waves that are emitted from LIPUS allow the accumulation of gas bubbles in the tissues and the cells [12, p 411]. This creates a cavity which gives room for the acoustic streaming. The streaming leads to turbulence or circular flow in the fluids from the tissues as the sound waves move around the bubble of gases. The acoustic streaming produced increased cell permeability. Once this has happened, the blood pressure rises in the area where the fracture has occurred. The increase in the blood pressure leads to the acceleration of healing by ensuring that there is sufficient gas exchange and the delivery of nutrients to the site. When the cavitation is not stable, the bubbles burst and the resultant energy stimulates the surrounding tissues [13, p 2642].

THE EFFECT OF LIPUS ON CELLS AND MOLECULES

There have been studies that have demonstrated the ability of LIPUS to promote fracture healing through the alteration of molecular and cellular mechanisms which are involved in the process of healing. Integrins are crucial in signaling modulation involved in fracture healing [14, p 345].

They act as mechanoreceptors and are also reactive to pressure changes and vibration caused by LIPUS in the cells environment. The mechanical stimuli increase the clusters of integrin on the fibroblasts. They also up regulate the mRNA expression of the integrin in osteoblasts [15, p 284]. The changes that occur enhance the sensitivity of the respective cells to motion and increase the cells intercellular signaling capacity. An important outcome associated with the intercellular signaling induced in the osteoblasts is the increased activation of ocyclooxygenase-2 enzyme.

This increases the production of prostaglandin which is critical in the mineralization during the process of endochondral ossification especially on the soft callus [16, p 660]. The endochondral ossification which is enhanced by LIPUS leads to formation of the bony callus by the process of augmented mineral deposition [17, p 77].

LIPUS also stimulates the differentiation of the cells which are involved in the process of fracture healing, chondroblasts, fibroblasts, mesenchymal and osteoblasts. Additionally, it increases the way aggrecan expresses itself [18, p 1957]. Aggrecan is a stimulator of chondrogenesis. Presence of aggrecan accelerates the differentiation of chondroblasts into chondrocytes. The production of chondroitin sulfate at the site of injury is accelerated by the presence of more chondrocytes.

Chondroitin sulfates are essential components in supporting bonny and cartilaginous structures. Because of the cavitation process aforementioned, LIPUS increases pressure at the site of injury due to the increase in the vascular permeability [19, p 727]. The increase in pressure has been associated with mesenchymal stem cells differentiation which enhances the development of fibro cartilaginous callous.

LIPUS increases the expression of the early osteogenic genes which include osteonectin, the insulin growth factor and osteopontin. These genes have an important role in making sure that proper osteoblast differentiation takes place [20, p 3190]. The osteoprogenitor cells from the bone marrow may also differentiate into the osteoblasts at a high rate when LIPUS is used. This will enhance the bone healing process and remodeling.

CONCLUSION

The use of low-intensity ultrasound system is a relatively new technology that has been proven to be safe and efficient in the treatment of acute fractures or in cases of non-union or delayed union. The effects at the cellular and molecular level demonstrate its efficacy.

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REVIEW ARTICLE

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A new global threat for the public safety: Zika virus

Simona Bicheru[1], Ion Ştefan[2], Marius Necşulescu[1], Diana Popescu[1], Lucia Ionescu[1], Gabriela Dumitrescu[1], Viorel Ordeanu[1,3]

Abstract: *Zika virus, the etiological agent of Zika fever, is transmitted by mosquitoes and has been affecting the South American continent starting with 2015. It was reported in several European countries, carried by the people who returned from Latin America, as reported by the health authorities in those countries. Today, according to the World Health Organization (WHO), the virus suspected to cause serious birth defects in the fetus has also been confirmed in 21 of the 55 countries of South America, but also in other states from Europe and North America. Zika virus is a single stranded positive sense RNA virus belonging to Flavivirus genus (family Flaviviridae) and was first identified in 1947 in Uganda rainforest Zika. The increased number of cases of microcephaly, in children from northern Brazil, suggested a connection with Zika virus, but it has not yet been proven. Also, the virus can be transmitted sexually and through blood or blood products. Diagnosis of the infection is made using Polymerase Chain Reaction (PCR). So far, there is no specific antiviral treatment or vaccine against the infection with Zika virus. The best form of prevention is to avoid mosquito bites. WHO has estimated that the spread of Zika virus, transmitted through mosquito bite, is "a global public health emergency". The priority is to protect pregnant women and to control the mosquitoes.*

Keywords: *Zika virus, Flaviviruses, microcephaly, Aedes aegypti, mosquitoes*

INTRODUCTION

Zika virus, the etiological agent of Zika fever, is transmitted by mosquitoes and has been affecting the South American continent starting 2015. It was reported in several European countries, carried by the people who returned from Latin America, as stated by the health authorities in those countries. Today, according to the World Health Organization (WHO), the virus suspected to cause serious birth defects in the fetus has also been confirmed in several countries from Europe: Great Britain, Italy, Netherlands, Portugal, Denmark and Switzerland.

Zika virus has been reported sporadically in Africa and Asia for several decades. Zika fever outbreaks were reported for the first time in 2007 (Yap island found in the Pacific Ocean), then in 2013 (French Polynesia). In 2015, outbreaks occurred in Africa (Cape Verde Islands) and South America (Brazil and Colombia). From here, Zika virus quickly spread to several countries in South and Central America and was finally reported in 21 countries of the region in early 2016. [1][2][3] On January 28, the World Health Organization announced that 3-4 million people in

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America could be infected with Zika virus in 2016. [4] On February 1, 2016, the WHO has estimated that the spread of Zika virus, transmitted through the bite of *Aedes aegypti* mosquito, is “a global public health emergency”.

THE ETIOLOGICAL AGENT

Zika virus is a single stranded positive sense RNA virus belonging to *Flavivirus* genus (family *Flaviviridae*) and was first identified in 1947 in Uganda rainforest Zika. It is the etiological agent of Zika fever, a viral infectious disease transmitted by infected mosquitoes. [1][2][3]

SYMPTOMATOLOGY

The incubation period is 3-12 days after an infected mosquito bite. Most infections caused by Zika virus are asymptomatic (60-80%). Symptoms are usually mild and disappear within 2-7 days without severe complications or death. The main symptoms are rash (macular or a papular exanthema that initially appears on the face and then spreads throughout the body), moderate fever, arthralgia, myalgia, headache, non-purulent conjunctivitis with conjunctival hyperemia. Vertical transmission can occur during labor (in the viremic stage) or transplacentar. The increased number of microcephaly cases in children from northern Brazil suggested a connection with Zika virus, but it has not yet been proven. Also, the virus can be transmitted sexually and through blood or blood products. [1][2][3]

TRANSMISSION

Zika virus is transmitted by mosquitoes and was isolated from species of the genus *Aedes*, such as *A. aegypti* (Figure1), *A. africanus*, *A. apico argenteus*, *A. furcifer*, *A. hensilli*, *A. luciocephalus* and *A. vitattus*. Studies showed that the incubation period in mosquitoes is approximately 10 days [22]. Zika virus can be transmitted through interpersonal sexual contact and can cross the placenta, affecting the unborn fetus. An infected mother (in the viremic stage) can transmit the virus to the newborn at the time of labor. [24][25][26]

The vertebrate hosts of the virus are the monkeys.

Figure 1: Aedes aegypti (http://www.cdc.gov)

Before the current pandemic, which began in 2007, Zika virus rarely generated collateral infections in humans, even in areas recognized as highly enzootic [7]. In 2009, Brian Foy, a biologist at Colorado State University, reported the sexual transmission of the Zika virus. [13][14]. In 2015, the Zika virus was detected in the amniotic fluid of two pregnant women, indicating that it is possible to cross the placenta and cause infections in newborns. [4][34][4][5][35][36]

DISEASE

Zika virus infection symptoms normally include mild headache, maculopapular rash, fever, malaise, conjunctivitis and arthralgia. [33] The first thoroughly documented cases of Zika virus infections were described in 1964, being characterized by mild headache, progressing through maculopapular rash, fever and back pain. Within two or three days, the initial symptoms remitted, but the rash persisted. Patients with Zika fever are advised to rest and are prescribed fluids and acetaminophen, while aspirin and nonsteroidal anti-inflammatory drugs should be used only after Denga fever was ruled out, in order to reduce the risk of bleeding. [38]

COMPLICATIONS

Microcephaly. In December 2015, it was suspected that an infection of the fetus with Zika virus, transmitted transplacental, may cause microcephaly and brain damage. [5][34] Consequently, the European Centre for Disease Prevention and Control issued a comprehensive update on the possible association of the Zika virus with congenital microcephaly. [35] (Figure 2)

According to CDC Atlanta (Centers for Disease Control and PreventionUSA), "were reported cases of microcephaly defects in children of mothers who were infected with the Zika virus during pregnancy."

Figure 2. Comparison between a child with microcephaly (left) and a normal child (http://www.cdc.gov)

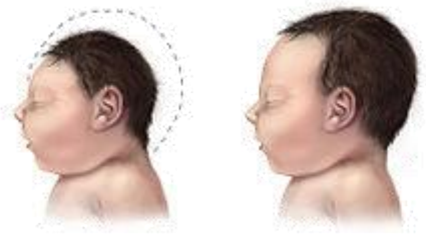

Zika viral infections were confirmed in several newborns with microcephaly; the number of microcephaly cases associated to Zika virus infection is unknown. [8][10][34]

On January 17, 2016 the Pan American Health Organization (PAHO), the Regional Office of the WHO, has recommended that its member states "establish and maintain the ability to detect and confirm cases of infection with Zika virus, given the increased number of congenital anomalies, Guillain-Barré syndrome and other neurological or autoimmune syndromes recorded in areas affected by Zika, to prepare healthcare facilities to respond to a possible increasing demand for specialized care for neurological syndromes and to strengthen prenatal care." [28][29]

DIAGNOSTIC

Zika virus is detected through PCR (gene amplification by Polymerase Chain Reaction). [15] [16] Since the viremic stage may be short, the WHO recommends that the RT-PCR testing should be done on the sera collected within 1 to 3 days after symptom onset or on the saliva or urine samples collected during the first 3 to 5 days after onset. [20][21][22][23]. Serological diagnosis can also be used by detection of specific IgM antibodies. Those appear towards the end of the first week of illness. [18]

Serological diagnosis can be difficult, given possible cross-reactions with other flaviviruses, for example the *Dengue* virus, *West Nile* virus or *Yellow Fever* virus. Zika commercial diagnostic tests are available at Euroimmun (www.euroimmun.com).

TREATMENT

Until present time, there is no specific antiviral treatment or vaccine available against Zika virus infection. The best form of prevention is to avoid mosquito bites.

Prophylaxis

The existence of mosquitoes and larval nests is an important risk factor for the infection with Zika virus. Disease prevention consists in decreasing the number of mosquitoes at source (elimination or modification of larval nests) and reducing contacts between these insects and humans. Those traveling to endemic areas should be informed on methods of protection against mosquito bites. These involve avoiding outdoor exposure at dusk and dawn, wearing clothes that cover as much exposed skin, using repellents, nets for beds impregnated with insecticide, accommodation in rooms with air conditioning and mosquito nets. It is also recommended emptying, cleaning and covering all containers that can collect water such as buckets, flower vases and tires in order to eliminate the places where mosquitoes can reproduce. During outbreak, health authorities can proceed to spray insecticide.

Vaccination

There are effective vaccines against several *Flaviviruses*. Vaccines against the *Yellow Fever* virus (antiamarilic), *Japanese encephalitis* and *Tick-Borneencephalitis* have been introduced from 1930, while the dengue fever vaccine just became available for use. [37]

According to Anthony Fauci, of the National Institute of Allergy and Infectious Diseases, attempts are made in order to obtain a Zika virus vaccine. [38] Researchers at the Research Center for Vaccines have extensive experience in obtaining vaccines against other viruses such as the Chikungunya and the Dengue fever virus. [38] Nikos Vasilakis, from the Center for Biodefense and Emerging Infectious Diseasesappreciated that getting the vaccine could take up to two years, but an effective vaccine for public use, approved by regulatory authorities, could be obtained in 10 – 12 years. [38][39][40][41]

EPIDEMIOLOGY

In 1947, scientists researching Yellow Fever revealed the onset of fever in a *Macaccus Rhesus* monkey located in a cage in the Zika Forest (Zika meaning "too high", in the Luganda language), near the Research Institute of Virology East Africa, in Entebbe, Uganda. The researchers have isolated from the monkey's serum a transmissible agent, which was first described in 1952 as the Zika virus [17] and was subsequently isolated in 1954 from a person in Nigeria. Since its discovery until 2007, rare cases of Zika virus infection were recorded in Africa and Southeast Asia. [40] In April 2007, the first outbreak outside Africa and Asia was declared on the island of Yap, in the Federated States of Micronesia; the cases were characterized by skin rash, conjunctivitis and pain, initially associated with Dengue, Chikungunya or Ross River disease. [41] However, the serum samples analyzed from patients in the acute phase of infection confirmed Zika virus infection. There have been 49 confirmed cases and 59 cases were disproved. There was no need for hospitalization and no deaths were reported. [42] Subsequently, outbreaks have appeared in Polynesia, Easter Island, the Cook Islands and New Caledonia. [5]

Starting April 2015, an epidemic broke out in Brazil with Zika virus (Figure 3) and has spread to South and Central America, the Caribbean, having a trend of global spread. In January 2016, CDC issued a level 2 travel alert for people traveling to affected areas (Table 1, Figure 4).

Figure 3: Zika virus epidemic map at 01.22.2016 (www.cdc.gov)

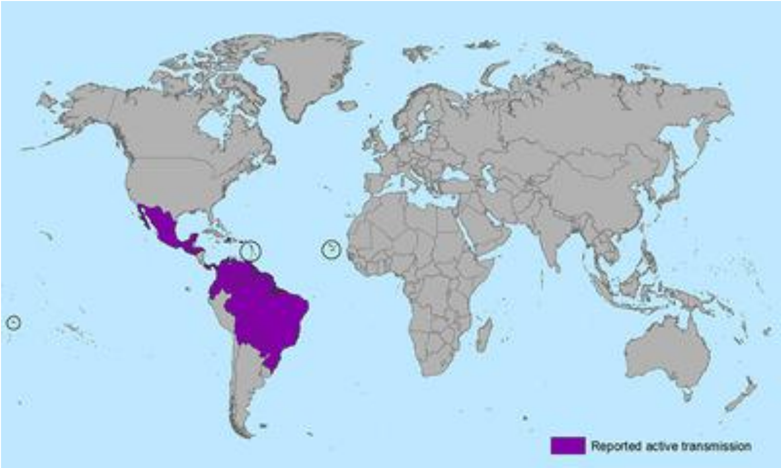

Figure 4: The geographical distribution of the Zika virus(www.cdc.gov)

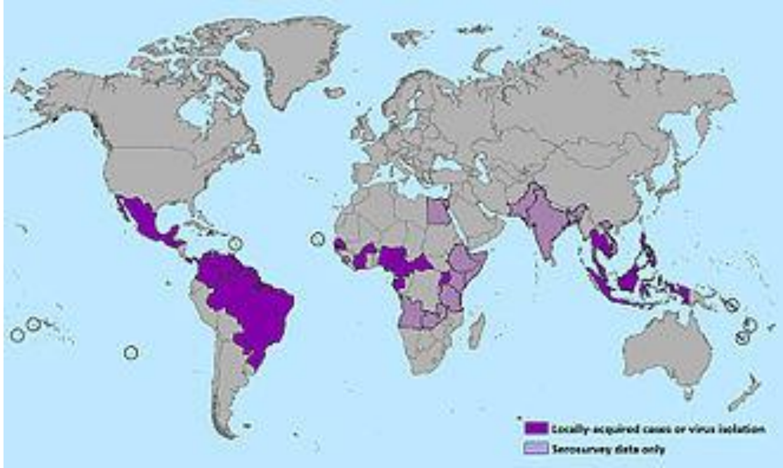

Table 1: Number of cases diagnosed with Zika virus (April 2015-present) (www.cdc.gov)

Country	Confirmed cases	Update
Australia	2	2 Feb 2016
Barbados	3	20 Jan 2016
Bolivia	1	20 Jan 2016
Brazilia	est. 1,5 mil	30 Jan 2016
Canada	4	30 Jan 2016
Chile	3	2 Feb 2016
Colombia	est. 20.000	30 Jan 2016
Curaçao	1	31 Jan 2016
Dominican Republic	8	27 Jan 2016
Ecuador	6	20 Jan 2016
El Salvador	est. 2.500	31 Jan 2016
French Guiana	2	8 Jan 2016
Guadeloupe	1	21 Jan 2016
Guatemala	1	27 Nov 2015
Guyana	1	21 Jan 2016
Haiti	5	21 Dec 2015
Honduras	3.649	1 Feb 2016
Jamaica	1	30 Jan 2016
Martinique	2	8 Jan 2016
Mexico]	18	31 Jan 2016
Nicaragua	3	31 Jan 2016
Panama]	50	28 Jan 2016
Paraguay	6	3 Dec 2015
Puerto Rico	22	26 Jan 2016
Saint Martin]	1	21 Jan 2016
Suriname	3	24 Jan 2016
United States	40	2 Feb 2016
Venezuela	3.700	28 Jan 2016
Total	**Est. 1.5 mil**	**up to 30.01.2016**

IMPLICATIONS FOR THE PUBLIC HEALTH

The WHO recommends that women planning to become pregnant should consult their physician before traveling to regions affected by the disease. [9][44] Governments and health agencies in the European Union, including UK and Ireland, as well as those in New Zealand, Canada, Colombia, Ecuador, El Salvador and Jamaica have issued similar travel warnings. Specific plans and measures were announced by authorities in Rio de Janeiro, Brazil, to prevent the spread of the Zika virus during the Olympic Games, taking place in this city, in 2016. [11][45][46]

According to CDC, Brazilian health authorities have reported more than 3,500 cases of microcephaly between October 2015 and January 2016. Some affected infants had a severe type of microcephaly, while others died.

These manifestations may be associated with Zika infection, occurred during pregnancy. Several studies are planned to assess the risks of the Zika virus infection during pregnancy. [45] In the worst affected regions in Brazil, about 1% of newborns are at risk for microcephaly. [46]

COMMENT

In humans, Zika virus causes an infectious disease called Zika fever, characterized by fever, headache, maculopapular rash, malaise, conjunctivitis, arthralgia etc.

The WHO has warned that the spread of Zika virus, transmitted by mosquitoes, is ''a global public health emergency'' and can be correlated with an increased number of newborns with congenital malformation recorded in Brazil last year.

WHO warns that Zika virus, transmitted mainly by mosquitoes, "explosively" spreads in South America, where in 2016 could be millions of cases. The priority is to protect pregnant women and to control the mosquito populations.

Medical experts are concerned about the very rapid transmission, over long distances of the virus with devastating consequences. From the confirmation of the presence of Zika virus, in May 2015, the virus has spread so far in 23 countries, including Brazil, Bolivia, Paraguay, Mexico, Venezuela and the epidemic continues to spread.

CONCLUSIONS

The World Health Organization has warned that the spread of Zika virus, transmitted by mosquitoes, is a

”global public health emergency”, which can be correlated with the increased number of newborns with congenital malformation recorded in Brazil last year and that the virus "explosively" spreads in South America, where in 2016 could be millions of cases.

Experts are concerned about the very rapid transmission, over long distances of the virus; the consequences can be devastating, and the priority is to protect pregnant women and to control the mosquito populations.

National health authorities are requested to take necessary measures for the prevention, diagnosis and treatment of Zika fever cases and to isolate import cases, in order to impede the spread of the disease.

Also, scientific research is needed in order to obtain an effective vaccine against Zika virus.

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SYSTEMATIC REVIEW

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Morphopatological and histochemical highlights in normal and varicose vein wall

Alina Condor[1], Caius Solovan[2], Liliana Vasile[2]

Abstract: *The nutrition of the venous wall appears to be an important factor in the vascular-fibrillar trophicity and in the dynamic of the extracellular matrix formation for the normal veins and, for the chronic venous ulcers of legs, on period of healing. Sequential biopsies were taken at various levels of venous wall of external and internal saphena in 16 cases presenting a chronic condition of legs venous system (35-58 years old patients, both sexes). 8 vein fragments with normal macroscopic appearance were also taken, in necropsy.*

These samples were analyzed using regular morphological methods and some histochemical reactions to reveal the glycogen, glycoproteins, and glycosaminoglycans substrates. There were been used the Gomori silver impregnation and orcein to expose some specific substrates like reticulin or elastin. Other staining methods, like Gomori trichrome, were used to differentiate the specific structures of the vein wall, were used to differentiate the specific structures of the vein wall.

A rich vascularization of normal and dilated vein wall could be remarked.Angiogenesis in vein wall and vasa vasorum changes as well as alcianophilic of vascular intima seem to be reactive and protective factors, depending on the applied therapeutic modalities. The veins are weak structures whose integrity depends on the thickness of the media and the support of neighboring structures.

INTRODUCTION

The emergence of varicosities it is supposed to be a consequence of defective development of venous wall and appears to be inherited. The proper nourishment of the venous walls appears to be an important factor in their trophicity. The incidence of varicose veins is about 1% of the adult population [5]. Both obesity and aging are involved as risk factors for emergence of defects in venous wall tissue support. The etiology and pathophysiology of varicose disease remains controversial. We emphasize the involvement of the following lesional links amid stasis hypoxia:

- Redistribution of microcirculation with arrangement in lobular aggregate of vessels in the superficial dermis;

- Areas of floride proliferation mimicking Kaposi's sarcoma (acro-angio-dermatitis), reticular dermis fibrosis and massive deposits of hemosiderin, degeneration of dermal fibroblasts (senescent fibroblasts converted by modulation into myofibroblasts having a role in periulceros tegument retraction) to extended tissue matrix lysis;

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-Outbreaks of fat necrosis concomitant with a proliferative-alternative response of adjacent epidermal, hyper parakeratosis and orthokeratosis and pseudoepitheliomatous hyperplasia.

The presence of lytic degradative tisular factors (metalloproteinase) and activation of monocito-macrofagic system induced degradation of the basal membrane and chronic venous ulcers.

Morpho-histochemical current study aims to deepen the importance of veins wall nutrition in normal and dilated veins. It also envisages the re-active participation of supra-adjacent dermo-epidermal tissue, the possible influence on vessel permeability and vasoprotective or anticoagulant medication used in selected cases in the study.

MATERIALS AND METHODS

16 biopsies were taken sequentially from the varicose saphenous in areas of dilated vein (12 cases) and in the vicinity of shank chronic venous ulcer (4 cases); Saphenous fragments from normal vein were collected at necropsy (8 cases). Cases have included both sexes. The selected cases were with at least 5 years of disease duration and the type of treatment was also considered.

Patients in the study were selected from the inmates in Department of Surgery of Military Emergency Hospital and University Clinic of Dermatology in Timisoara.

Processing of samples was performed at the Department of Histology of Medicine and Pharmacy University in Timisoara by usual histological techniques: all samples were fixed in 10% formalin, buffered formalin, AFA fixator (Alcohol - Formaline - Acetic acid), paraffin inclusionated, sectioned at 5 µm, then colored by hematoxylin-eosin. For histochemical study, there were been applied reactions PAS (blue Alcian (BA)) and salivary amylase-PAS (Alcian blue used at pH 2.8 and pH 0.5); blue toluidine (BT) at pH 5 and pH 2.8; it was used Gomori silver impregnation for reticulin and it was also used trichrome Gomori reaction.

Orcein staining method was used to expose elastin.

Figure 1: Lipodermatosclerosis (*toluidine blue, pH 2,8, ×200*)

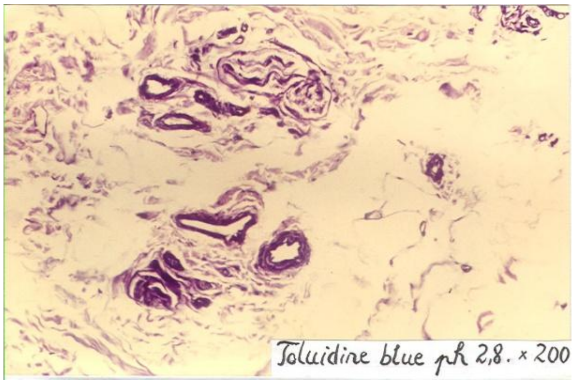

RESULTS

In all examined cases, the wall of external and internal saphenous veins was well vascularized, containing arterioles, venules, capillaries in musculo-fiber media. Constant, parietal capillaries from vasa vasorum were not flattened. In varicose veins adventitia, in small and medium vessels, were frequently found leukocytic marginations and acidophilic deposits and PAS positive, weak metachromatic fibrin (Figure 1).

Fibrin sleeves were also observed in the wall vessels of varicose veins in which angiogenesis is very marked. For AT, were remarked weak metachromatic perivascular sheaths, at pH 5 and chromophobe tissue matrix of dilated veins sheaths, at pH 2.8. Orthochromatic of nuclei allows observing the phenomenon of leukocytes margination (mostly lymphocytes) in the parietal small and medium vessels up next to intima, for both pH used (Figure 2).

Figure 2: Perivascular sheaths and parietal leucocytes margination in small and medium sized vessels, close to venous intima (*toluidine blue, pH 5, ×100*)

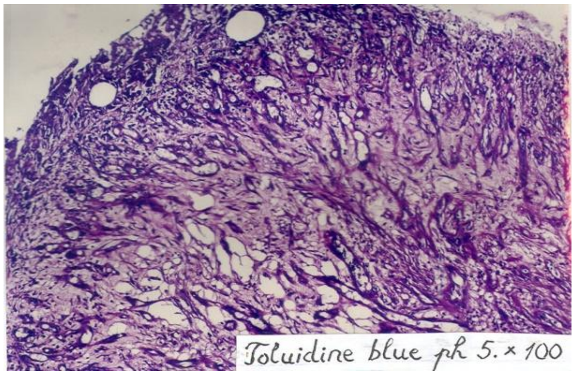

In all methods used in this study, the average media of varicose veins appears thickened through a rich smooth muscle proliferation (HE col., Gomori trichrome col.), with weak orceinic material fragmented through the wall layers (Orcein col.). There were observed fake elastic limitations, fragmented, disorganized (orcein, Gomori trichrome). In one case of all cases examined, using combined staining AA-PAS, which stands under anticoagulant therapy, an edemic intima and hyperplasia were observed, with fundamental alcianophilic substance (FS) and somewhere with deposits PAS positive as a rich interstitial material PAS positive all that pushing aside and cleaving muscle layers of the vascular media. Other intimal changes reported in our study are notches and extensive fibrosis, miointima hyperplasia of saphenous varicose veins.

Alcianophilic fibrillar material with reticular disposition is perivascular distinguished in the neo-capillaries that invade from adventitia media. Silver impregnation for reticulin (Figure 3) shows neo-formation basic membrane by budding vasa vasorum from adventitia to media to decline to extinction the reticulin network of media muscular interstice and vasa vasorum walls.

Figure 3. Reactive angiogenesis in variceal venous wall (*silver impregnation, ×200*)

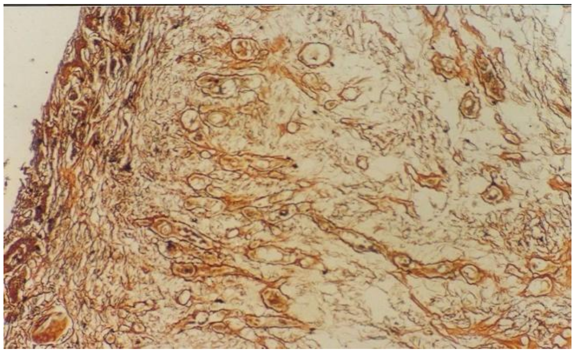

The phenomenon seems to affect 2/3 of external walls of the dilated veins. In neighboring dermal and epidermal tissue and in the perivascular support appear lesions like: lipodermatosclerosis, fibrosis and perivascular fibrin deposits and interstitial fragmentation of elastic fibers in the deep of dermis, tinctorial variations of epidermal cells cytoplasm for which an accumulation of PAS positive material could be noted and cemented SF was PAS reactive. For silver impregnation, the dermal epidermal limit appears fibrillary fine, thinner than the normal appearance.

DISCUSSIONS

The changes of vein wall tissue architectural pattern take place simultaneously with the progression of venous distension in varicose vein disease. It is noticed an important reaction of vessels in vasa vasorum which angiomatosic proliferate, invading media, almost to intima, leading to muscular media hypertrophy [5]. Vascular proliferation can be reactive to miointima hyperplasia and to varicose wall media. Chronic shank venous ulcer it is possible to occur, in time.

Thus, as a reaction to increased venous pressure in a vein much thickened, on account of media and intima and hence to the shank edema, fibrin sleeves occur, those may inhibit the angiogenesis [4]. However, it is created a protection against increased venous additional pressure with a possible role in causing tisular ischemia [6][8].

Fibrin sleeves were found around the capillaries of the dermis in lipodermatosclerosis. Massive deposits of fibrin are involved in elastin fragmentation of the deep dermis and in elastic limitations of vascular walls. Vasa vasorum angiomatosis proliferate in invading varicose vein media. Since 1992, "leukocyte sleeve" was reported as a response to chronic stasis. White blood cells can accumulate in the lumen of capillary and venular, increasing perivascular resistance.

Activated endothelium becomes thrombogenic surface (via tissue factor secreted by endotheliocyte: factor VIIa, factor X-Xa). Simultaneously, activated endotheliocyte was involved in angiogenic peptide synthesis, stimulating neovascularization of dermis and ectasiated venous walls. Tissue anoxia and ulceration is installed.

Consistent with the cited literature, we found also an increased number of capillaries in the skin of patients with venous hypertension in the lower limbs.

Wall vessels are the only ones bringing oxygenated blood, draining interstitial fluid and extravasated colloidal material [7][1][3]. This explains the rapid drainage and metastasis of tumor cells via veins and lymphatics with rich vascularity, having intraparietal capillaries opened.

Adventitial enriched positive PAS material, periadventitial and intraparietal, is represented by fibrin, and also by a macromolecular glycoprotein substrate containing laminin, fibronectin, tenascin detected by immunocytochemical investigations by some cited authors [2][5].

So we can correlate the "leukocytic margination" phenomenon in vasa vasorum and intraparietal vessels with depolymerization of SF tissue more evident in the 2/3 of external portion of the varicose venous wall.

The histochemical reactions used in our study, draw attention to the changes in the three fibrillar component. In varicose veins (dilated segments thereof) we found a quantitative increase of mature collagen fibers and reticulin, morpho-histochemical revealed, with a decrease and fragmentation of elastic fibers in the dilated walls. Together with the degradation of elastin, reticulin network growth can be an important factor in the parietal frailty in shank venous insufficiency evolution.

Marked alcianophilia of intima (AA pH 2.8) calls attention to presence at this level of complex carbohydrates with carboxyl groups and hydroxyl radicals in position of vic-glycol (sialic acid and hyaluronic acid) which act as intima hydrator, but may decrease macromolecular complexes stability of glycoproteins (with increase of sialic acid amount).

Sialic acid carboxyl groups and other substances not yet identified are responsible for basophilia and metacromazia of certain reticulin networks and basement membranes. The enriched PAS positive interstitial material in dilated vessels media is favorable to reticulin collagenization (gradual multiplication of preexistent collagen fibers in a glycoprotein matrix apparently unchanged).

Reticulin collagenization occurs when the hydroxyl group probably belonging to hydroxyproline and hydroxylysine achieve the appropriate concentration [9].

In the cases we investigated, collagenization is conducted in a glycoprotein reticulin dominant matrix (in the external 2/3 dilated vein wall), progressively decreasing toward intima, simultaneously decreasing nourishment intake by vessels of the vein wall fibrosing.

CONCLUSIONS

Morpho-histochemical methods draw attention to the complex of walls reshuffles of venous leg during chronic venous insufficiency. There are being considered the three coats of veins in that appear to be successive lesional patterns: mio-intimal hyperplasia, medial muscle hypertrophy, reactive angiogenesis, lipodermatosclerosis, collagenization of medial muscle.

The chronic venous stasis can be quantified by morphological parameters: mio-intimal hyperplasia, medial muscle hypertrophy, reactive angiogenesis, lipodermatosclerosis, collagenization of medial muscle. The prolonged venous hypertension modifies both the local microcirculation conditions and selecting of fibril-forming cells, triggering the evolution of chronic venous ulcers.

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ORIGINAL ARTICLES

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Expandable stents in digestive pathology – present use in an emergency hospital

Mădălina Ilie[1], Vasile Șandru[1], Cristian Nedelcu[1], Bogdan Popa[1], Gabriel Constantinescu[1]

Abstract: *Introduction: Self expandable metal stents (SEMS) are developed lately, as an effective and safe, less invasive alternative of surgery for the treatment of malignant intestinal/biliary obstruction. Recently, SEMS are also introduced in benign pathology.*

Aim: The aim of this presentation is to report a retrospective analysis of the total number of SEMS placed for esophageal, enteral, colorectal and biliary obstruction during the last 3 years in Clinical Emergency Hospital Bucharest, as well to review the literature published on this issue.

Methods: Between 2013-2015 in Clinical Emergency Hospital Bucharest, we have placed: 232 esophageal stents, 23 enteral stents, 5 colonic stents and 75 biliary stents under radiologic guidance. The main parameters followed were represented by: sex, age, grades of obstruction, stent diameter and type, immediate and late complications and survival rate.

Results: Regarding the esophageal stenting, most of the indications were malignant obstruction (155 cases of esophageal cancer and 30 cases of extrinsic compression), but also for esophageal fistula, peptic stenosis and even traumatic esophageal rupture. The majority of the enteral and colonic stents were inserted for malignant obstructions, having only 2 cases with benign obstructions. This is also the case for biliary stenting, were most of the indications were represented by pancreatic cancer. Technical and clinical success rates were approximately 92% and 80%, respectively. There were no major complications of perforation, bleeding, or death.

Conclusions: SEMS insertion can be performed safely, with minimal complications and hospitalization allowing the restart of oral feeding and improvement of nutritional status for the digestive obstruction or jaundice disappearance in case of biliary obstruction. It represents the first option for unresectable digestive/biliary malignant obstruction.

Keywords: *expandable stents, malignant obstruction, fistula, stenosis.*

INTRODUCTION

Over the past decades, the endoscopic approach for palliation of malignant obstruction has overcome the use of percutaneous approach. The options for endoscopic stenting are either self-expandable metal stents (SEMS) or plastic stents (PS). PSs are composed of polyethylene, polyurethane, or Teflon,whereas SEMSs are made of various metal alloys that are constructed to achieve adequate radial expandable force without sacrificing flexibility and conformability to the duct [1].SEMSs can be either uncovered or covered with material to prevent tumour ingrowths. Recently, uncovered self-expandable biodegradable stents were added to this portfolio [2].

The ideal stent have to be pliable, atraumatic but also forceful to maintain patency and position in the lumen. However, stents should also be easily removable and without any risk for benign hyperplastic or malignant tumor ingrowths or overgrowth.

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[1] Clinical Emergency Hospital Bucharest

The current guidelines from the American Society for Gastrointestinal Endoscopyfor distal malignant biliary obstruction recommend either SEMSs or PSs, with PSs preferred in cases of distant metastasis and short life expectancy. However, studies have shown that although PSs are less expensive, metal stents have better drainage and longer patency,with recent data showing they are more cost-effective [3][4].

METHODS

We have done a retrospective, non-randomized study between 2013-2015 in Clinical Emergency Hospital Bucharest. We have placed: 232 esophageal stents, 23 enteral stents, 5 colonic stents and 75 biliary stents under radiologic guidance. The main parameters followed were represented by: sex, age, grades of obstruction, stent diameter and type, immediate and late complications and survival rate. We had exclusion criteria: an INR more than 3 and an estimated survival time less than 3 weeks. Regarding the dysphagia we have used Mellow and Pinkas's scale.

0 = able to eat normal diet / no dysphagia.
1 = able to swallow some solid foods
2 = able to swallow only semi solid foods
3 = able to swallow liquids only
4 = unable to swallow anything / total dysphagia

We have used most the nitinol stents (alloy of nickel and titanium), because of their ability to conform to anatomical angulations and the latter for their removability.

RESULTS

More than 65% of the patients were male with age varying between 49 and 83 years old.

Regarding the esophageal stenting we have placed in Clinical Emergency Hospital Bucharest, 232 oesophageal stents for the following indications (Figure 1):

-155 cases of esophageal cancer
-30 cases of extrinsic compression
-43 cases of esophageal fistula
- 3 cases of benign stenosis
- 1 case of traumatic rupture of the esophageal wall

Figure 1: Graphic representation of esophageal stenting indications

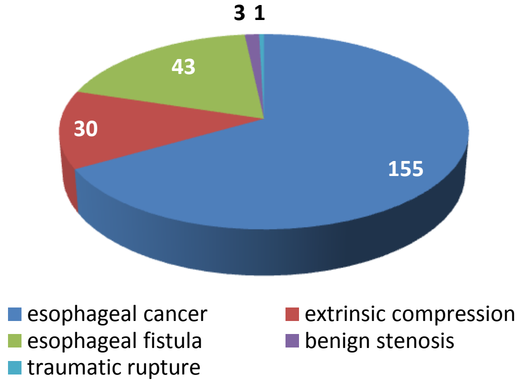

The majority of the cases (> 50 %) have unresectable disease at the time of diagnosis, either because of distant metastases or unsuitable candidates for surgical resection.

The aims of palliative therapy are to ameliorate symptoms of dysphagia, treat complications, maintain oral intake, minimize hospital stay, relieve pain and ultimately improve their quality of life.

We have used: totally covered SEMS or partially covered SEMS. Fully covered stents are more prone to stent migration; removable and theoretically may be more suitable for benign strictures/fistula whereas partially covered stents have a small portion of exposed bare metal in both proximal and distal ends to allow embedding into the oesophageal wall, which helps to decrease stent migration. The uncovered portion of the partially covered stents allows embedding and anchoring. No differences in survival rates were observed between these two types [5].

The techniques used for inserting the stent were: OTG – over the guidewire (most of the cases) or TTS (through the stent) both under radiologic view. At the beginning the lesion is visualized under endoscopy (Figure 2A – oesophageal fistula) and upper end is marked by injection of contrast agent into the submucosa (Figure 2B).

It is very important for the marking to be done internally and not to put external marks that may move during the procedure. The stents are mounted in a preloaded constrained position on a delivery catheter.

A guide wire is passed through the lumen of the catheter (Figure 2C), and when the wire has been advanced beyond the obstruction, the stent is passed over it and positioned across the stricture with the upper end at the position of the internal contrast mark (Figure 2 D, E, F).

Figure 2A: Esophageal fistula

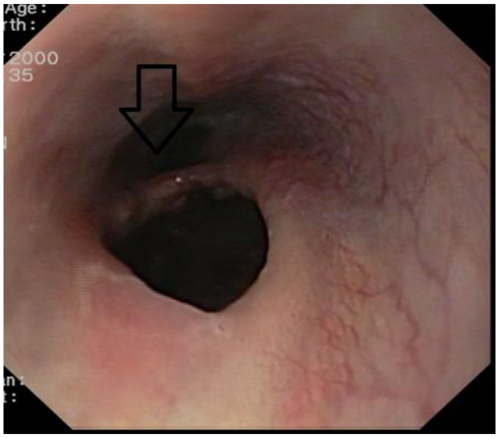

Figure 2B: Contrast injection

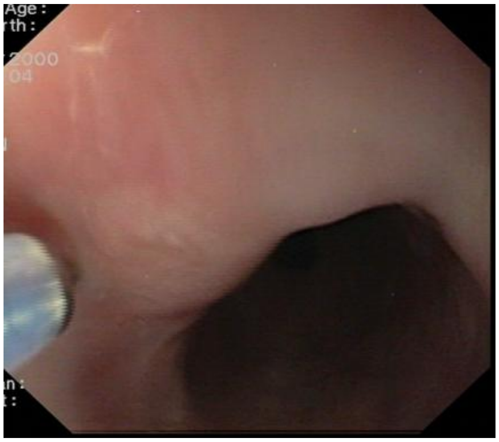

The immediate complications encountered were: retrosternal pain (60%), alleviated after antalgics, stent migration while as late complications we had: food impactation (7%),fibrous tissue invasion (4%), tumoral invasion (5%),uncovered fistula and upper GI bleeding.

Figure 2C: Guidewire insertion

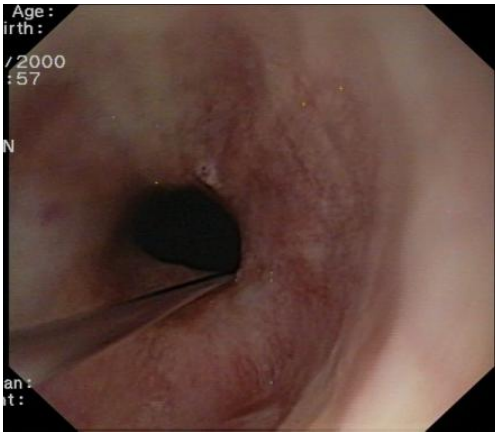

Figure 2D: Stent deployment

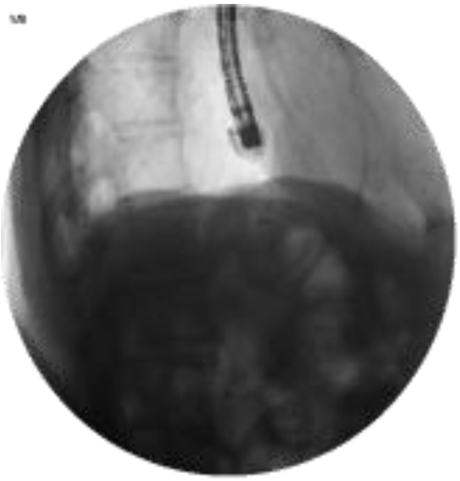

Figure 2E: Stent view on RX

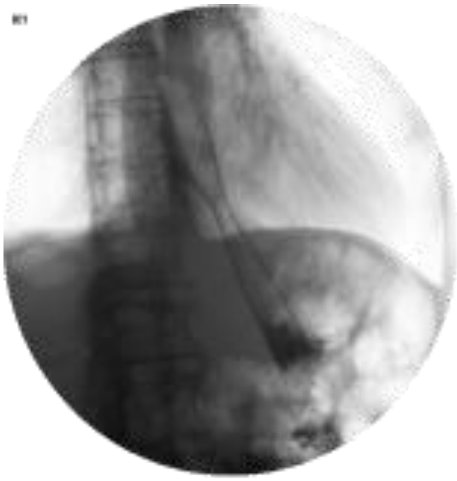

Regarding the enteral stenting we had 23 total cases: 21 for malignant extrinsic compression and 2 cases for benign stenosis. The technique used was TTS (through the scope) with low rate of complications.

Figure 2F: Stent under endoscopy view

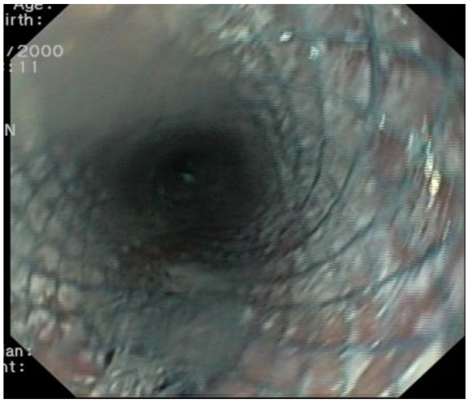

A specific situation was double stenting, both enteral and biliary (Figure 3 A, B), especially in pancreatic head malignancies with obstructive components.

Figure 3A: Double stenting (enteral+biliary)

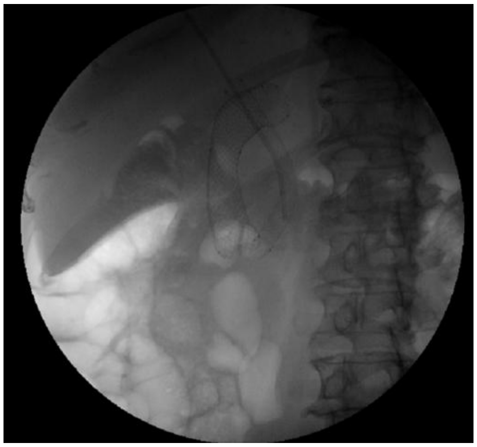

Figure 3B: Enteral stent-endoscopic view

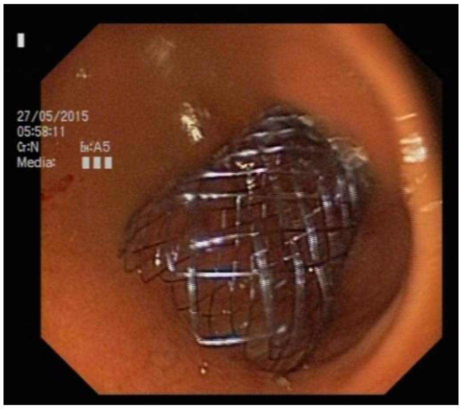

Colonic SEMS placement is recommended as the preferred treatment for palliation of malignant colonic obstruction, except in patients treated or considered for treatment with antiangiogenic drugs [6]. Stent insertion may be considered as an alternative to emergency surgery in those who have an increased risk of postoperative mortality. We have placed only 5 colonic stents (Figure 4), mainly because of ease of accessibility to the surgical service in our hospital.

Figure 4: Colonic stent

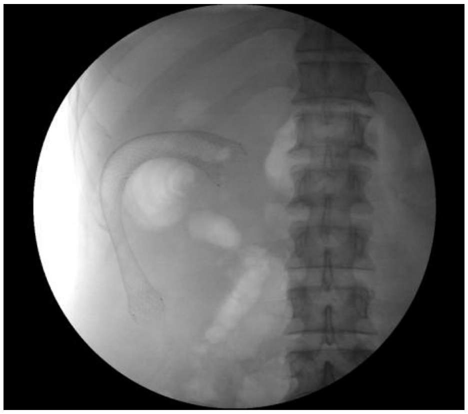

In case of biliary stenting we have inserted 73 stents for malignant obstruction (approx. 60% pancreatic cancer) and only 2 for benign obstruction (chronic pancreatitis). SEMSs range from 4 to 12 cm in length with diameters when expanded ranging from 6 to 10 mm.

Self-expanding metal stents (SEMSs) are built from different metal alloys, mainly nitinol, that are used to achieve adequate radial expansive force leading to increased patency duration and reduced recurrent obstruction.

SEMSs are preloaded in an outer sheath with a diameter of 8.5F or smaller, allowing use with a therapeutic duodenoscope. After placement in the duct, the outer sheath is withdrawn, allowing the stent to expand (Figures 5A, B, C).

SEMSs can be covered, partially covered, or uncovered.

Partially or totally covered SEMSs can be repositioned or fully removed, reduce the rate of stent occlusion but they have risk of cholecystitis because of involvement of the cystic duct orifice). Acute cholecystitis may occur in as many as 10% of patients with intact gallbladders after placement of a covered SEMS across the cystic duct. In opposition, uncovered SEMS migrate less but have risk of tumor ingrowths [3].

SEMS insertion at patients with malignant biliary obstruction palliate jaundice, anorexia and most important help the initiation of chemotherapy that otherwise would be contraindicated.

Initial insertion of a plastic stent is most cost-effective if patient life expectancy is shorter than 4 months; if it is longer than 4 months then initial insertion of a SEMS is more cost-effective.

Figure 5A: Distal biliary obstruction; stent insertion radiologic view

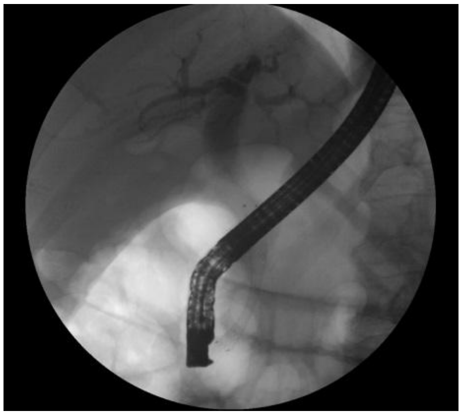

Figures 5B, 5C: Endoscopic views

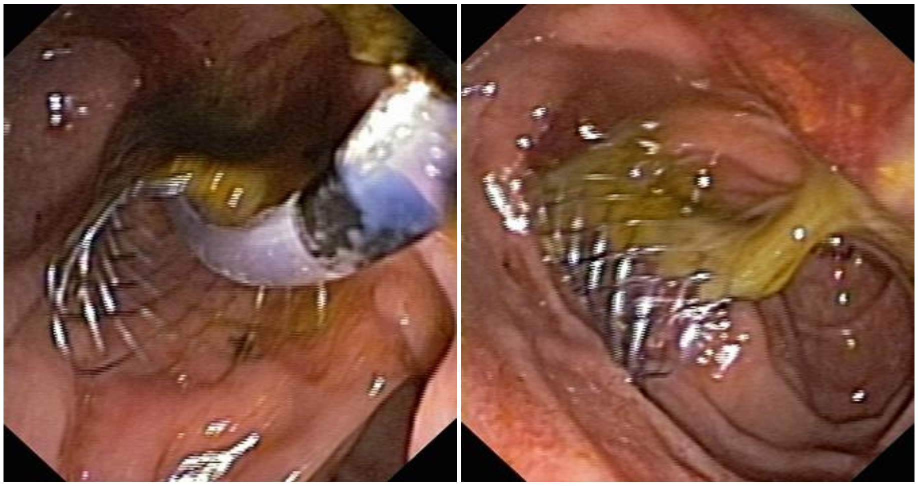

CONCLUSIONS

SEMS represents a very useful alterative, with minimal complications to classic surgery for palliation of unresectable digestive/biliary malignant obstruction. With the current development and applications of new materials and technologies, the future of stenting is evolving.

New trends include drug-eluting stents, biodegradable biliary/esophageal stent and the use of biodegradable polymers for local drug delivery, having also curative aim [7] . Radiation therapy, a cornerstone of treatment for malignant disease of the esophagus, may also be administered in conjunction with placement of esophageal endoprosthetics [8].

Regarding the biliary malignant obstruction, we recommend that SEMSs should be the first option because they have better patency, a lower occlusion rate, less need for reintervention, and fewer adverse events than plastic stents.

In conclusion, expandable metal stent placement is a very effective way of re-establishing luminal patency with negligible complications for an expert endoscopist.

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Hepatic hydatid cysts

Cătălina Diaconu[1], Mădălina Ilie[2,3], Alexandru L. Chiotoroiu[3], Laura Voicu[1], Daniel O. Costache[1], Raluca S. Costache[1,2]

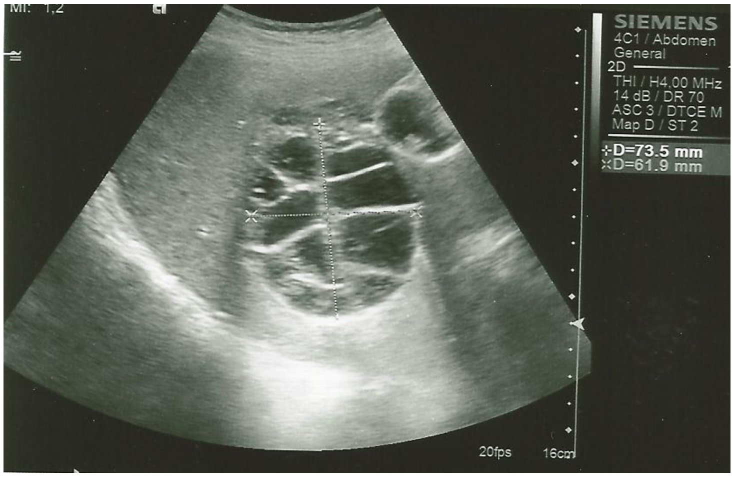

43 years old man presented with recently installed abdominal pain localized in the hypocondrium and epigastrium associated with jaundice and pruritus.

The patient's history included operated bilateral inguinal hernia. Physical examination yielded jaundice, normal aspect of postoperative scars in the inguinal fossae (left and right), pain in the hypocondrium and epigastrium, no guarding, rebound or bruits were found.

Laboratory findings reveal leukocytosis with eosinophilia, hepatic cytolisis (alanine aminotransferase = 219 U/L), hyperbilirubinemia with a higher conjugated bilirubin (total bilirubin=6.01mg/dL, conjugated bilirubin=4.7mg/dL).

Ultrasonography reveals a round transonic image of 7/6cm localized in the IV and VIII segment, with septa that suggests liver hydatid cyst, hyperechoic image of 9mm (hydatid cyst) and a 9mm gallbladder polyp. Computed tomography shows hepatomegaly with a cystic multiseptate image in the IVth segment of 8.5 cm.

Under treatment against pain, proton pump inhibitor, hydratation and regime during hospitalization the symptoms disappear. However the hepatic cytolysis worsens with the progressive growth of alanine aminotransferase to values of 916 U/L, hence antihelminthic treatment is delayed.

Enchinococcal infection caused by the larval form of *Echinococcus granulosus* remains an important health issue worldwide. Hepatic hydatid cyst is the most frequently encountered form (50-93% of the cases) and left untreated these grow and lead to: developing fistulae in adjacent organs, rupture in the peritoneal cavity, produce daughter cysts or dye (rarely)[1]. Clinical manifestations appear after the cyst is larger than 10cm in diameter and only a third of the patients experience symptoms.

Even though biological finding are nonspecific, elevated bilirubin and alkaline phosphatase may appear. Leukocytosis may appear due to infection of the cyst and eosinophilia is present only in a quarter

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of patients [2].

Despite the fact that ultrasonography remains the main pillar in the diagnosis of the disease, computed tomography and serology help improve the accuracy of diagnosing liver hydatidosis. WHO Ultrasound classification of echinococcal cysts helps establish the treatment: 1 (unilocular, anechoic cyst with double line sign), 2 (multiseptate honey comb cyst), 3a (cyst with detached membranes), 3b (cyst with daughter cysts in solid matrix), 4 (heterogenous contents and no daughter cysts) and 5 (solid plus calcified wall). CE4 and 5 are inactive.

On the other hand Gharbi classification also divides in 5 types: type I cysts consist of pure fluid; type II has a fluid collection with a split wall; type III cysts contain daughter cysts (with or without degenerated solid material); type IV has a heterogeneous echo pattern; and type V has a calcified wall. Therefore our case presents a hepatic hydatid cyst stage CE2 after WHO classification and type III after Gharbi classification.

Uncomplicated and small lesions (under 5cm) CE1,2 and 3 can be treated with oral albendazole (10-15mg/kg/day) and close monitoring. However, large CE1 and CE3 cysts need treatment with both albendazole and PAIR (percutaneous aspiration, introduction of scolicide and reaspiration), performed after initiation of albendazole.

Primary surgical treatment has been replaced with less invasive methods since the relapse rate can reach 20%. In patients with complicated cysts, surgery is the treatment of choice. CE4 and 5 only need to be monitored [1][3].

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CLINICAL PRACTICE

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More than simple hepatic cysts

Daniela Tabacelia[1], Mădălina Ilie[1], Alina Constantin[1], Anca Macovei Oprescu[2], Gabriel Constantinescu[1]

Abstract: *Caroli diseaseis a rare congenital disorder that classically causes saccular dilatation of the bile ducts. The complications of Caroli include choledochal cysts with recurrent cholangitis, abscess formation, septicaemia, intrahepatic lithiasis and amyloidosis.We report a rare case of a young female with Caroli disease pointing out the intrahepatic lithiasis as a rare complication of the disease.*

Learning points

- *Caroli disease is an uncommon condition that should be considered in the differential diagnosis of hepatic essential cysts.*
- *Clinically, it is characterized of recurrent episodes of fever and pain.*

The correct and early diagnostic is important because of the different complications and treatment unlike the essential hepatic cysts.

Keywords: *Caroli disease, intrahepatic lithiasis, endoscopic retrograde cholangiopancreatography*.

CASE REPORT

A 28 years old female presented to the emergency department with abdominal pain and fever after an ERCP procedure performed 2 weeks ago. From her disease history we retain an acute pancreatitis that was classified Balthazar B, one year ago, assumed to be caused by biliary main duct lithiasis; at that moment her initial biology showed ASAT 156U/L, ALAT 126U/L, lipase 2,398U/L, bilirubin 32 mmol/l and white cells 14,590/mmc; there was no sign of complication (acute cholangitis) that would have required a surgical treatment in emergency. Her family history was inconclusive for any specific digestive condition.

In our hospital, on physical examination her body temperature was reaching 38^0C, the heart rate 110 bpm, and blood pressure 110/70mmHg; on palpation she presented mild tenderness on epigastric and right quadrant; the usual blood tests showed white cell count with neutrophils raised, hepatic cytolysis and cholestasis until 5 times normal value. Next step was performing an abdominal ultrasound which revealed hepatic cysts (dilation of intrahepatic bile ducts) with liver hyperechoic images with acoustic shadow suggestive of intrahepatic lithiasis (Figures 1 A and B). A CT scan and a MRI were performed and revealed a normal pancreas without Wirsung dilatation, the biliary main duct with a 7 mm diameter without stones inside, and normal gall bladder without signs of cholecystitis but with beaded intrahepatic biliary duct dilatation until 18

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mm alternated with normal caliber ducts with numerous images of stones inside with a 12 mm maxim diameter. The dilated biliary canaliculi wall presented signs of cholangitis. All clinical and paraclinical data were typical for a Caroli disease with intrahepatic lithiasis and secondary cholangitis. The patient received treatment with urso-deoxy cholic acid (UDCA), antibiotics, anti-inflammatory drugs and pain medication followed by the resolution of the inflammatory episode.

Figure 1A

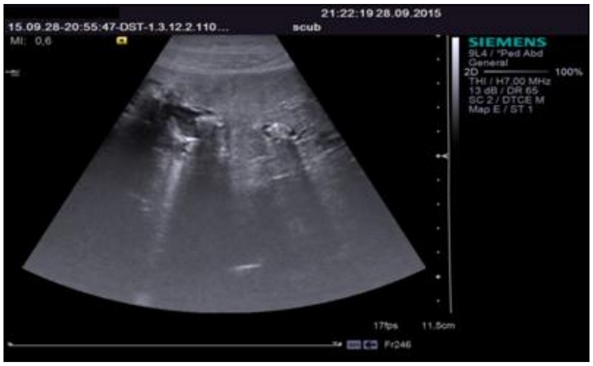

Figure 1B: Abdominal ultrasound revealing hepatic cysts with intrahepatic lithiasis

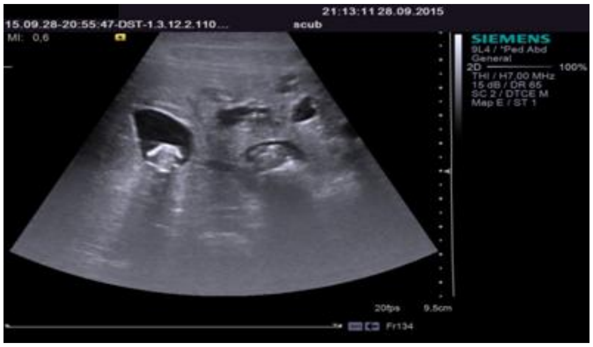

DISCUSSION

The hallmark of Caroli disease is intrahepatic duct dilatation [1]. Patients with Caroli disease are usually presenting to the hospital, when they develop complications as the result of biliary stasis, which leads to stone formation and infection. The stones are brown pigmented stones, composed of inspissated bile[1].

Caroli disease has two forms, one associated with congenital hepatic fibrosis and a simpler form occurring alone. The former, called Caroli's syndrome is associated with portal hypertension, and it's complications including splenomegaly, hematemesis and melena. Caroli disease is also associated with liver failure and polycystic kidney disease. The cause appears to be genetic; the simple form is an autosomal dominant trait while the complex form is an autosomal recessive trait.[2] Females are more prone to Caroli disease than males.[2] Family history may include kidney and liver disease due to the link between Caroli Disease and ARPKD (Autosomal recessive polycystic kidney disease). The symptoms include fever, intermittent abdominal pain, and hepatomegaly. Occasionally jaundice occurs.[3] Laboratory tests indicate cholestasis and hepatocytolysis associated with an inflammatory syndrome in the acute stages.

Caroli disease usually occurs in the presence of other diseases, such as autosomal recessive polycystic kidney disease, cholangitis, gallstones, biliary abscess, septicemia, liver cirrhosis, renal failure, and cholangiocarcinoma (7% affected).[2] People with Caroli disease are 100 times more at risk for cholangiocarcinoma than the general population.[3] Modern imaging techniques allow the diagnosis to be made more easily and without invasive imaging of the biliary tree.[4] Abdominal ultrasound can detect saccular or fusiform dilation of the bile ducts. Images taken by CT-scan or MRI will show enlarged intrahepatic (in the liver) bile ducts due to ectasia; cholangiography is the best approach to show the enlarged bile ducts as a result of Caroli disease.

The treatment of Caroli's disease depends on the clinical features and the location of the biliary abnormalities. Cholangitis is treated with appropriate antibiotics. In case of intrahepatic cholelithiasis litholytic therapy with urso-deoxy cholic acid (UDCA) is indicated[5]. When the ductal abnormalities are localized to one lobe, lobectomy relieves symptoms and appears to remove the risk of malignancy. In case of diffuse involvements of both lobes of liver, treatment options include conservative management, endoscopic therapy (sphincterotomy for clearance of intra-hepatic stone), internal biliary bypass procedures and in carefully selected cases liver transplantation[5].

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Nutritional approach in late gastric stenosis after gastric sleeve

Adina Mazilu[1]

INTRODUCTION

Laparoscopic gastric sleeve(LGS) is a very frequent used procedure nowadays in Romania, with more than 100,000 reported patients operated until now, the most part of patients being operated in private settings. It is a simple and efficient procedure, less expensive than gastric by-pass, with fewer complications, but can reduce obesity complications almost as well as gastric by-pass. Indications for this type of surgery are:

- Body Mass Index is greater than 40 kg/m^2 or between 35-40 kg/m^2 and patient has significant pathology that may benefit from weight loss-like diabetes, hypertension
- Severe co morbidities (cardiac, pulmonary, liver disease)
- Advanced age at time of operation
- Inflammatory bowel disease (Crohn's disease)
- Patient uses chronic medications (anti-inflammatory or immunosupressive)
- Need for continued surveillance of the stomach (that couldn't be evaluated after a gastric bypass)
- Severely enlarged liver found during the operation
- Severe adhesions or scarring to the bowel found during the operation
- Any combination of the above that significantly increases the patient's anesthetic or surgical risk

LGS may be used also combined with gastric by-pass done at 12-18 months after LGS, when anesthesia is less risky and liver has reduced diameters.

Acute complications are represented by hemorrhage reported in 1-6% of cases and gastric leaks in up to 5% in the immediate period after operation [1]. Gastric stenosis has a 0 to 4 % rate of appearance for this type of operation[4]. Most cases can be solved by endoscopic dilation with balloons;however some cases need to be converted to gastric by-pass.

In an article from 2014,in a study done on 565 patients, the incidence of complications was relatively low- the authors[2] reported in 7.79% of patients infarcts of the posterior pole of the spleen, 1.42% gastric fistulas in the His angle region, with 5 deaths among these patients – 3 due to septic complications in the course of fistula, 1 due to encephalopathy and 1 as a result of myocardial infarction.

Chronic complications include strictures, malnutrition and GERD. Chronic strictures usually require further intervention. Endoscopic dilatation is an useful tool used for short segment stenosis. Successive treatments in 4 to 6weeks interval are adequate to treat stricture and ameliorate patient symptoms. In contrast, long segment stenosis and failure of endoscopic management demands a surgical intervention – laparoscopic or open seromyotomy or conversion to Roux-en-Y gastric bypass. Parikh and colleagues reported an incidence of 3.5% of symptomatic stenosis following LGS in their series of

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230 patients; 2 patients required conversion to a Roux-en-Y gastric bypass owing to failure of endoscopic management.

In a recent study by Gehrer and colleagues[3], the prevalence of vitamin B12, vitamin D, folate, iron and zinc deficiency were reported to be 3%, 23%, 3%, 3% and 14%, respectively, after LGS In general, these investigators found micronutrient deficiencies to be less prevalent after LGS than Roux-en-Y gastric bypass; however, folate deficiency was slightly more common after LSG than Roux-en-Y gastric bypass (22% vs. 12%).

CLINICAL CASE

Our patient, a 25 years girl operated – LSG-1 year before, presented with severe malnutrition and dehydration, associating also hypokaliemia. She has been vomiting at least 4 times a day during the past 6 months and she lost almost 100 kg in 1 year (from 130 Kg, 167 cm to 37 Kg at admittance). She has done upper endoscopy 10 days ago and dilation of gastric stricture was not possible. She was told she needed surgical intervention after gaining 10 kg.

She was very pale, with brittle hair and dry skin, with Bichat's sign positive, hypotensive – 70/50 mmHg, tachycardic and refused admittance even if she was explained that she had a very high risk of death. She agreed to come and stay in hospital after solving the problems for her job. As she associated hypokaliemia we tried to correct it with 20 mEq of kalium in saline, but patient reported intense pain on peripheral i.v. infusion, so we used first 1,000mL of saline, and then Ringer and Kalium progressively.

Family reported repeated episodes of binge eating at home with ingestion of Coke and high amounts of food, after that unprovoked vomiting. Discussion with patient revealed also depression, this kind of food pattern was the patient's cry for help due to unresolved adolescence trauma, even if she had family and friends support now. We tried also to relieve the pressure from job.

Next day iv saline 500 ml, Ringer 1000 ml, Kabiven were introduced, with almost complete correction of hypokaliemia.As patient had urinary tract infection we added Gentamycin 320 mg/day and Ciprofloxacin 400 mg/day, for seven days, with good evolution.

Third day we introduced Fresubin – enteral formula – 500 ml/day, divided in 7-8 meals, no more than 100 ml/meal, associated with Osetron and Controloc on i.v. line. Patient stopped vomiting the third day and the next day we introduced small amounts of baby food – milk and egg, fruits puree, small amounts of soup with chopped meat.

By seventh day patient gained enough weight so we tried to reduce the amount of Fresubin and Kabiven and leave her on oral intake of mashed food, but this induced reappearance of vomiting. X-ray revealed no passage of barium due to complete gastric stenosis, so i.v. nutrition was resumed.

Laboratory 8 a.m. serum cortisol was more than 10 mcg/dl, so we excluded Addison disease.Patient had low TSH and low T3 levels – confirming sick euthyroid syndrome and hypotalamic amenorhhea with low Estradiol levels – 17 pg/ml.

In the last 3 days we introduced small amounts of rapid insulin – 10 U/day to improve anabolism.Patient reached 47 kg, with mild edema in the legs and was discharged in the 12th day of admittance.She still has mild hypoproteinemimia – albumin 3.2 mg/dl, total proteins 5,6 g/dl and mild hypocalcemia – 8.8 mg/dl.She was discharged with proton pump blocker and prokinetic treatment, associated with buvable ferrum sulfate.

She repeated the endoscopy and was converted to gastric by-pass after 3 days without more complications.

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CLINICAL PRACTICE

Fever and abdominal tumoral masses

Augustin C. Dima[1], Teodor Artenie[1], Daniel Florea[1], Florina Bold[1], Paul I. Oprea[1]

Abstract: *49 year-old man presented to our clinic for pain in the right hypochondrium, diarrhea, and fever. The clinical examination highlights a tumoral formation in the right side of the abdomen, with firm consistency, poorly defined margins, and present mobility in the deep structures. On biological exams, leukocytosis with neutrophilia, inflammatory syndrome, and hypoalbuminaemia were identified. The first computed tomography exam described parietal thickening of the ascending colon, with infiltrative aspect, and multiple local adenopathies, lomboaortic and interaortocave. Moreover, four nodular liver tumors, with hypodense image in native examination, were identified. The lab tests for infectious diseases were all inconclusives: three hemocultures, three stool samples, and three coproparasitological exams were all negatives.*

Interdisciplinary examinations, internal medicine and infectious diseases, sustained the diagnosis of colonic neoplasm with peritumoral abscess and liver pseudo-tumoral masses. The colonoscopy did not revealed any bowel lesions relevant for neoplasia. This result as well as the bio-clinical context imposed abstention from surgical intervention.

Wide spectrum antibiotics and symptomatic treatment were initiated. But, ten days after hospitalization, the second computed tomography exam showed reduction of the ascending colon wall thickness associated with significant increases of the liver tumors is so revealed. The investigations for other possible etiologies were so continued.

Keywords: *colon tumor, intestinal amoebiasis, liver abscesses.*

INTRODUCTION

In current clinical approach of abdominal pathology one must not forgot the rare disorders whose clinical picture and imaging could mime neoplasia, pathology so frequent today.

The current diagnosis of colon tumors is made especially using imaging diagnostic methods, whose accuracy is high, facilitating anaccurate stage diagnosis, very important in determining therapeutic tactics. But it must not be forgotten also the importance of classical clinical features that could bring essential elements for the certainty diagnosis.

CASE REPORT

We present the case of a 50-years old patient, smoker, diabetic, hypertensive, who presented for right upper hypochondrium pain, diarrhea, nausea, febrile syndrome, with the onset of symptoms about 48 hours prior to presentation.

Clinical examination revealed hypochondrium and right flank sensitive abdomen, tumor mass in the right flank, with firm consistency, imprecisely defined, relatively mobile on the deep plans, without signs of peritoneal irritation, fever (38.7˚C), shivering.

---

[1] Carol Davila Central Emergency Military Hospital, Bucharest

Biologically, neutrophilic leukocytosis, inflammatory syndrome, and hypoalbuminemia were noted. Plain chest and abdominal radiography were without pathological changes (Figure 1). Abdominal ultrasound identified hypoechogenic liver formations.

Figure 1: Biological evolution during diagnostic and after specific treatment

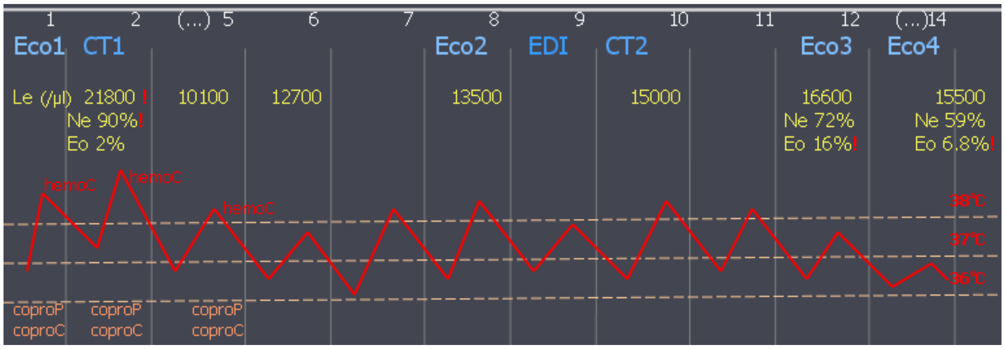

CT exam identifies hepatomegaly and four nodular liver formations: hypodense on native examination, irregularly shaped, with uneven structure, apparently separated from their dense walls (Figures2a, 3a, 4a). In addition, a parietal tumoral formation belonging to ascending colon, located toward liver angle, showing asymmetrical narrowing partially iodophile (with a maximum thickness of 2.1cm), infiltrating the cecum – ascendant digestive wall and the adjacent fatty abdominal tissue that adheres to the liver capsule. Perilesional, lumbar aortic and interaortocaval adenopathies, (with maximum dimensions of 1.3cm) (Figures5a, 6a, 7a) were also described.

Figure 2(a, b): Computer tomography exam: first two liver masses evolution under treatment

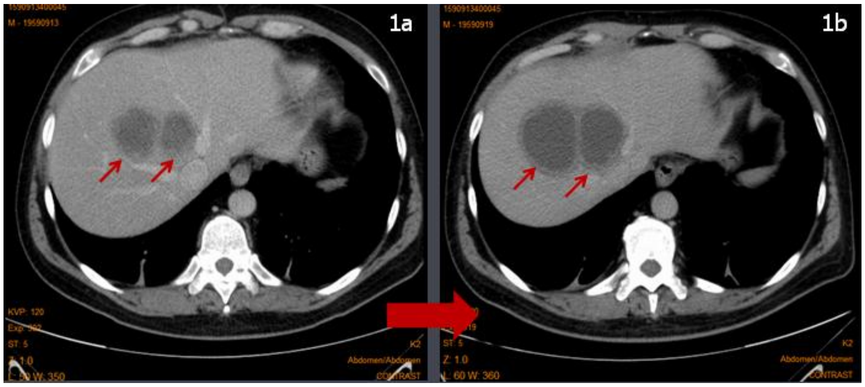

Figure 3(a, b): Computer tomography exam: liver masses evolution under treatment

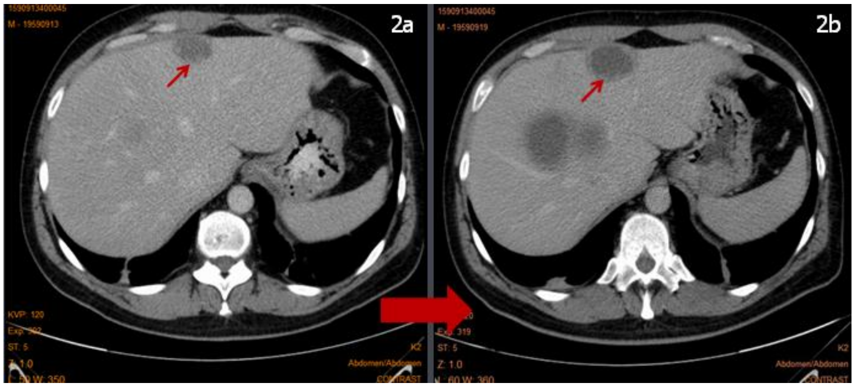

Figure 4(a, b): Computer tomography exam: liver masses evolution under treatment of the third tumoral mass

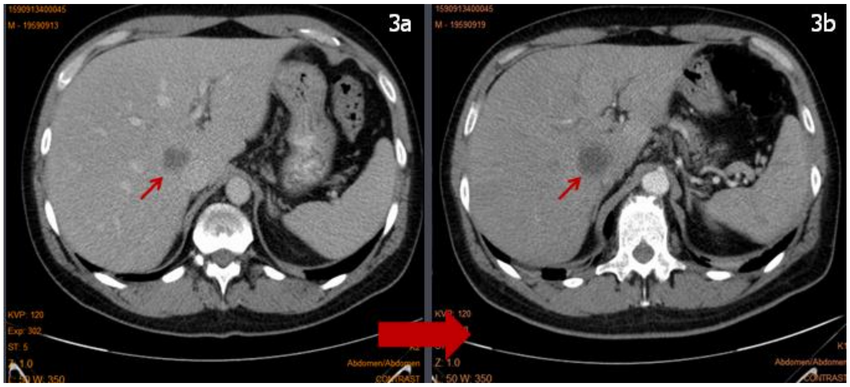

Figure 5(a, b): Computer tomography exam: colon tumor evolution under treatment

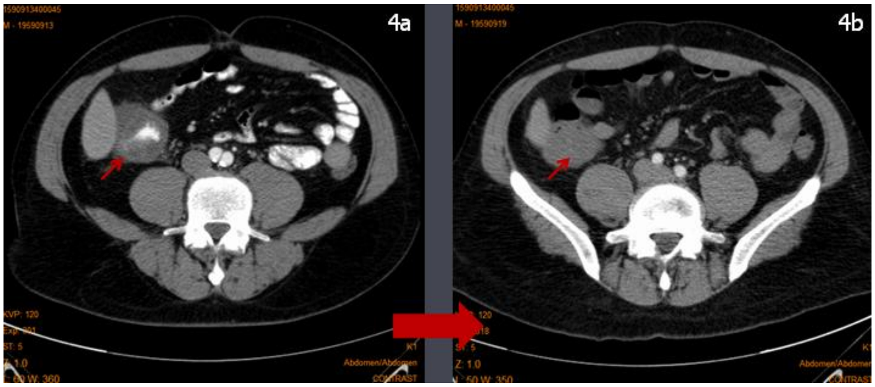

Figure 6(a, b): Computer tomography exam: colon tumor and local adenopathies evolution under treatment

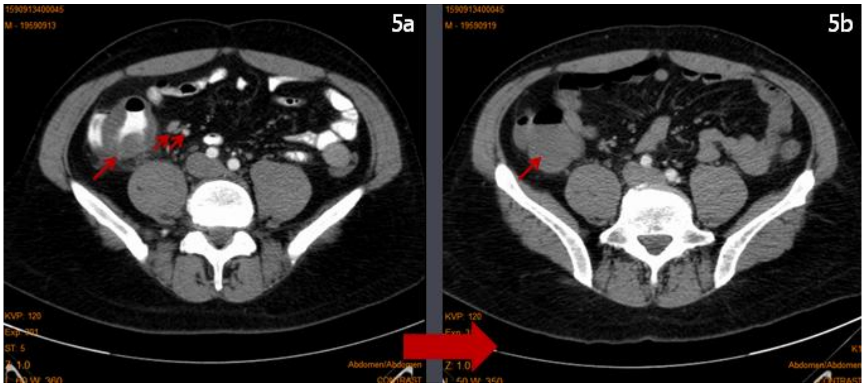

Figure 7(a, b): Computer tomography exam: colon tumor evolution

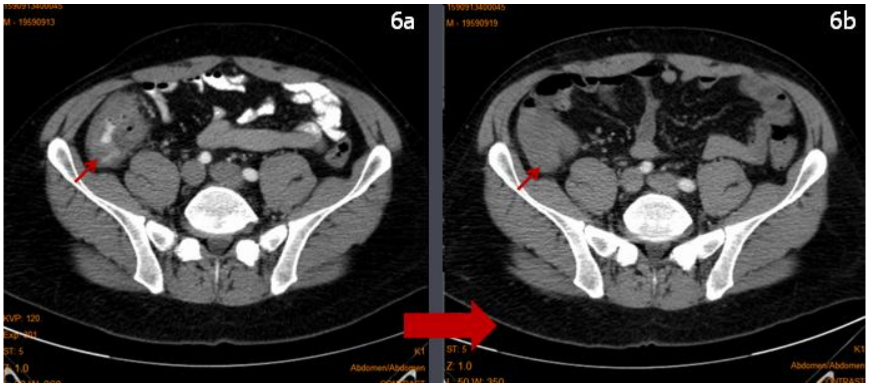

The paraclinical picture was completed with three negative blood cultures, three negative copro-parasitological exams, three negative coprocultures and tumor markers (carcinoembryonic antigen, CA 19-9, alpha-fetal protein) negative (Figure 1).

Internal medicine and infectious diseases examinations declines the eventuality of infectious or parasitic primary etiology of colony formation and supports the diagnosis of colon cancer with peritumoral abscess and septic liver determinations.

At this point, the imaging seems to support thediagnosis of ascending colon tumoral mass associated with secondary liver metastases or with local associated infection and liver abscesses.

Clinical arguments, however, given the relatively sudden symptoms onset, are pleading for diverticulitis or segmental colitis of the ascending colon with septic determinations in liver. Therefore, we consider it appropriate to postpone surgery and to make further investigations.

It was initiated treatment with broad antibiotic spectrum (imipenem – cilastatin 500 mg/ 500 mg, 4 times daily, linezolid 600 mg 2 times daily, both for 10 days and Moxifloxacin 400 mg daily for 5 days) associated with symptomatic treatment.

Lower digestive endoscopy describes in the rectum, at 4-5 cm from the anus, a 1 mm polyp covered by normal mucosa, at 10 cm from the anus a 2 mm sessile polyp, covered with congestive mucosal, biopsy was performed. In the sigmoid colon, to about 25 cm, a 10 mm semi pedunculated polyp, cylindrical, covered by the congestive mucosa, supple for biopsy. At the descending colon polyp plan, ovoid of 7-8 mm, covered with inflamed mucosa, supple for biopsy. Transverse and ascending colon without changes.

Caecum, opening of appendix and the ileocecal valve without modifications.

The morphopathologic diagnosis of colon polyps was of tubular adenoma. So, the endoscopic examination does not argue for tumor etiology.

The evolution under treatment was favorable: reducing the number of daily diarrheal stool (from 10 to 12 per day to 2-3 per day) and also fever – under vesperal fever and the overall condition has improved. Biologically, continuing with leukocytosis with neutrophilic, but at lower values and the increasing of cholestasis defining liver enzymes (Figure 1).

We repeat, on day 10 – from the admission – the CT examination and we see a significant increase in volume of liver formations, the largest with a diameter of about 7cm, with better separation than previously, clear evidence of the relatively thick walls and of clear fluid content (Figures2b, 3b, 4b). Also, the marked reduction in the ascending colon thickness and reduction in the size of abdominal adenopathy (Figures5b, 6b, 7b) were noted.

Diagnosis of infectious disease is gaining before a possible neoplastic etiology, se we ask forparasitology and infectious diseases check-up and serological determinations.

Positive diagnosis is supported by the presence of Ig G type anti-Entamoeba histolytica antibody (negative Entamoeba histolytica antigen, negative Echinococcus antibodies, negative Giardia duodenalis CoproAg and Cryprosporidium CoproAg) for which specific treatment is initiated.

Thus, the positive diagnosis was: liver amoeba disseminated abscesses in both lobes, in remission and intestinal amoebiasis affecting the ascending colon, submitted.

The patient’s evolution after the initiation of the etiological treatment was favorable with improvement of the general condition, normalization of blood counts at two weeks of antiparasitic ambulatory treatment and marked reduction of liverabscesses dimension with their disappearance in a year.

DISCUSSIONS

Entamoeba histolytica is a protozoan that is an important health problem in tropical and subtropical countries [1]. Infection is fecal-oral, 90% of those affected remain asymptomatic, being determined by ingestion of food contaminated with parasite cysts [2]. It affects particularly the gastrointestinal tract (more frequently the check and ascending colon) and liver [3].

The difficulty of diagnosis in this case was given by the initial CT imaging appearance of ascending colon tumor, accompanied by the negative coprocultures and coproparasitological exams.

Imaging appearance of colonic amoebiasis mimicking neoplastic pathology has been described in the literature, but this presentation as a colon tumor formation remains a rare occurrence[4][5]. Most frequently, the colonic amebiasis diagnosis is done by highlighting the parasite in the stool, the examination of three successive stools leading to a positive diagnosis in 70% of patients [6].

The correct diagnosis in the presented case was obtained only after achieving immunological determinations, serology for Entamoeba histolytica is positive in up to 90% of patients with amoebic colitis [7].

Also, another feature that hindered the diagnosis was the initial presence of neutrophilic leukocytosis, with no eosinophilia (Figure 1), which could be explained by possible plurimicrobial superinfection of the parasite gateway from ascending colon.

After starting antiparasitic treatment an increase in eosinophils in serum is observed, most likely secondary to parasitic destruction and thus the emergence of new antigenic elements.

Important in the proper management of this patient was refraining from any surgery and introduction of specific therapy (Metronidazol) despite the pressure brought by imaging and biological data. Surgery in Entamoeba histolytica infection is a must in cases of acute colitis with necrosis and perforation when it may be necessary even total colectomy [8][9] and could also be followed by many complications.

Another problem of therapeutic tactic was the discussion on the need for drainage of liver abscesses, their sizes being significant. The ultrasound tracking has shown that the effective antiparasitic treatment was enough, observing the gradual decrease of their sizes, with their disappearance one year after the initiation of antiparasitic treatment.

In general, the literature does not support the need of drainage for uncomplicated liver amoebian abscesses [10]. Could be drained big liver abscesses (>300 cm^3) or when there are questions about the etiology of liver abscess, like in the case of pyogenic liver abscess [11][12] when cultures from drainage samples could be informative on the etiology and further treatment.

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Romanian Journal of Military Medicine

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- *Benchmarks of the evolution and revolution of military medicine in the XXI century – Tradition, Trust, Professionalism*
- *Management of Helicobacter pylori infection – new insights*
- *The use of low-intensity ultrasound system in Orthopedics for treatment of fractures*
- *A new global threat for the public safety: Zika virus*
- *Morphological and histochemical highlights in normal and varicose veins wall*
- *Expandable stents in digestive pathology – present use in an emergency hospital*
- *Hepatic hydatid cyst*
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EDITORIAL

Benchmarks of the evolution and revolution of military medicine in the XXI century – Tradition, Trust, Professionalism

Florentina Ioniță Radu

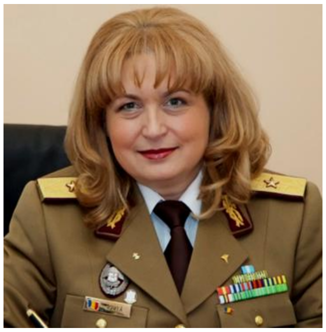

Brig. Gen. FLORENTINA IONIȚĂ RADU

Commander, Central Emergency Military Hospital, Ministry ofNational Defence, Romania
Associate Professor, Titu Maiorescu University, Bucharest

There is no secret to anyone that we live in a society marked by uncertainty and unpredictability, a society in which information flows through all communication channels, both in cyberspace and in mass media. Therefore, it is increasingly difficult for the „modern man” to decide upon the veracity and value of the informational content so that, eventually, based on the latter, he can upgrade his knowledge in any area of interest.

In such a context, it is difficult for society to control its evolution and to identify valuable markers that would ensure a valid growth in time and space. Lacking history, hierarchy, elite, rules and order, one can talk neither about the development of an organization nor about the development of society as a whole.

I think we have all passed, in recent years, through a "period of resistance" to the wave of non-values that invaded us and created an organic need to protect and preserve the values our parents and grandparents taught us. Despite the appearance of resignation, the accumulation of such energy has made us stronger, more aware of our role and this made us persistent and gave us the power and the chance to change our future and our children’s future.

Returning to the reason that brought us together, by means of this page, you – the reader and me – the author, I can say without any doubt that every cell of my being has reacted with emotion at the course and unrest of the last years of military medicine. It was only when I became the Commander (General Manager) of the "Dr. Carol Davila" Central Clinical Emergency Military Hospital (SUUMC), that I fully understood the huge potential of this noble profession – military doctor – potential generated by the excitement of the trust and respect offered each day by the public opinion in general and especially by citizens.

I have always considered that medicine is a field in which evolution ignores borders, bureaucratic rules, customs and paradigms, because trying to rescue somenone’s life is such a pure and noble approach that no sacrifice for this purpose can ever be considered too big.

This year the "Dr. Carol Davila" Central Clinical Emergency Military Hospital, a symbol for the history of Romanian society, celebrates the honorable age of 185 years of tradition, trust and professionalism, dedicated to research, development and innovation in medicine. 185 years in the service of life, without any deviation from the fulfillment of the two oaths: the military oath and Hipocrate's oath.

We should always keep, in our minds and hearts, the memory of those who, on the battlefield of the two World Wars, nursed their fellows and some of them even lost their lives trying to rescue the latter, the persistence of the struggle with their own limits taking care of the victims of the earthquake that shook Bucharest in March 1977; the emotion lived when faced with the young soldiers, victims of the construction of Transfăgărăşan and of the Danube – Black Sea Canal. Recently, the victims of the tragic fire at Club Colectiv were a challenge for the doctors and nurses in our hospital. All the departments of the hospital worked as a real efficient and dedicated team having only one purpose: to save the lives of the victims who were our patients.

During 185 years, together with the Romanian Army, we have been in the service of the country and of the population, doing our best in our duty with modesty, professionalism, ethics and team spirit. We did not pose as mentors, although here, in this area of health, the foundations of medicine, pharmacy and education in this field in Romania were laid. Medical and surgical specialties were also born here, specialties that today are spoken of as a fact, not as a gift from our ancestors, military doctors – thoracic surgery, urology, epidemiology etc.

The only thing military medicine was deprived of until now, is "a single voice of professionals" in this field, segment considered by most of our civil colleagues relatively narrow as area of specialty and therefore lacking the capital injection necessary to ensure its technical and cutting-edge technology, which apparently decreased our competitiveness in the market of medical services provided. Not true! In the absence of facilities, the human resource of this system accepted the professional challenge and approached a sphere of increasingly complex pathology, and on the other hand, the interdisciplinary collaboration has strengthened human links and increased our performance as professionals. It is true that until three years ago, we may not have had the latest surgical technologies but we beat cancer through the physician's sharp spirit, the surgeon's, the pathologist's and oncologist's experience.

Three years do not mean anything for a hospital, but for someone diagnosed with a relentless disease they mean everything... This was one of my thoughts that motivated me to develop centers of excellence – assigned and equipped to the highest standards – capable of providing prevention, treatment and monitoring of disease in the oncologic pathology, strokes, cardiovascular diseases and digestive diseases; lung transplant – a medical and surgical activity of high human and professional performance, which also involves a great responsibility assumed by SUUMC, on medium and long term, for patient's life quality, service which is not available at this time, in any other public hospital in Romania; neurological and neuromotor rehabilitation center for the veterans from the operation theaters.

Romania's geopolitical and geostrategic situation, as a state located at the EU and NATO border, obliges me, at the same time, to analyze the development of the operational medicine component in the Central Military Clinical Emergency Hospital "Dr. Carol Davila", so that, in wartime, we can be prepared to face the specific challenges related to the pathology and casuistry of a military confrontation.

Both as a doctor, and especially as the manager of the Central Military University Emergency Hospital "Dr. Carol Davila", I understand that the expectations of the whole society towards military medicine are very high, whether we speak of peace or war. People expect us to be a performant regional hospital, which provides at safe and high standards, a medical act comparable to that granted by a specialty hospital from abroad, while in wartime the hospital should become an elite military medical facility, ROL 4, able to provide medical care to the standards required by our NATO allies.

With a background of 185 years, the Central Military Clinical Emergency Hospital "Dr. Carol Davila", returns in force, as an important player on the market of medical services in Romania and elsewhere, proving that to the three qualities that have pierced the veil of history – TRADITION, TRUST, PROFESSIONALISM –

we should add the key to success in order to ensure the evolution of this type of institution, within the criteria imposed by the information society, consisting of flexibility, predictability, resilience and responsiveness in the case of a crisis or disaster.

As arguments in support of the above, I would point out some key milestones of the work of the last three years, namely, that SUUMC is the only institution in the medical network of the Ministry of Defence running projects from European funds for research, development and innovation in the medical field. It is connected to military medical centers in the Balkans, by means of the collaboration platform of telemedicine type, IMIHO. The hospital has developed collaboration agreements with major centers in the field, in the country and abroad (Belgium, Austria, Turkey and Serbia) to conduct training programs for medical staff and it is a serious instruction and training institution for students and residents, as a consequence of the extension of academic and university partnerships.

At the beginning of a new stage of life, I hope that The Romanian Journal of Military Medicine, in this new format, supported by a high scientific editorial board, will fulfill its objective to disseminate knowledge, to bring added value to the market of specialized publications in this field, from Romania and from abroad, to support the research activity and to motivate young people who make their first steps on the road to a career dedicated to the most precious thing that we all receive: health.

As a team player who strongly believes in the power of military medicine elite, in the interdisciplinary dialogue of this discipline, I assure you that I will support this approach, as I have already done it so far, in order to increase its quality every year and to borrow from the brilliance of the historic celebration of 185 years from the signing of the birth certificate of the Army Hospital.

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REVIEW ARTICLE

Management of Helicobacter pylori infection – new insights

Andrei Gavrilă[1],Andrada Popescu[1], Petruț Nuță[1], Raluca S. Costache[1,2], Mariana Jinga[1,2], Săndica Bucurică[1,2], Bogdan Macadon[1], Mihăiță Pătrășescu[1], Florentina Ioniță Radu[1,3]

Abstract: *Introduction: Our objective is to review current international guidelines for Helicobacter Pylori treatment and our department`s experience in this field.*

Materials and methods: Helicobacter pylori is a Gram-negative, microaerophilic bacterium that can be found mainly in the gastric mucus or on the inner surface of the gastric epithelium, infecting up to 50% of the population. Colonization with this bacterium is not a disease in itself, but can cause chronic gastritis, peptic ulcer, gastric cancer and MALToma. Because of this, infection with H. pylori continues to be a major healthcare burden, especially in less-developed countries.

A multitude of non-invasive tests are available for the diagnosis of Helicobacter pylori infection (blood antibody, stool antigen or urea breath test), but the most reliable method of diagnosis is histological examination from two sites after endoscopic biopsy, combined with either a microbial culture or rapid urease test.

Treatment of Helicobacter pylori infection is becoming a challenge, as eradication following standard triple therapy is decreasing worldwide due to increased bacterial resistance against antibiotics, which has led to the development of newer therapies such as the sequential treatment in which a PPI and amoxicillin is given for 5 days followed by a PPI, clarithromycin and metronidazole for another 5 days, or the quadruple therapy based on a PPI, bismuth subcitrate, metronidazole and tetracycline for 10 days.

Results and conclusion: H. pylori infection remains one of the most challenging infectious diseases, causing high morbidity and mortality, mainly because none of the actual antibiotic therapies can provide successful eradication.

Keywords: Helicobacter pylori, ulcer, adenocarcinoma, antibiotics, PPI

INTRODUCTION

Helicobacter pylori (Hp) is a helix shaped, Gram-negative, microaerophilic bacterium that can be found mainly in the gastric mucus or on the inner surface of the gastric epithelium[1]. Hp infection is the most common chronic infection in humans, but colonization with this bacterium is not a disease in itself. Although most infected individuals remain asymptomatic for life, Hp is responsible for a number of pathological manifestations including chronic gastritis, gastric or duodenal ulcer, adenocarcinoma

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of the stomach or gastric mucosa–associated lymphoid tissue (MALT) lymphoma[2].

THE DISCOVERY OF HELICOBACTER PYLORI

The Helicobacter pylori bacterium was discovered by two Australian researchers by the name of Barry Marshall and Robin Warren, who also deciphered its role in gastritis and peptic ulcer disease (PUD)[3]. Their discovery was published in 1982, at a time when it was a long-standing belief that stress and lifestyle factors were the major causes of PUD. The scientific community of the time met their findings with skepticism and a lot of criticism, refusing to belief that any life-form can survive in the acid medium of the stomach. This is why it took a very long time and effort for their discovery to become widely accepted[4].

Marshall`s 1985 “self-help” experiment rebutted the “stress and life-factors” dogma. He first underwent gastric biopsy to show the absence of the bacterium, then, to the horror of his assistant, ingested a turbid, foul-tasting solution of Hp. Soon he developed a number of symptoms including nausea and vomiting and an endoscopy with biopsy was performed, which revealed signs of gastritis and the presence of Helicobacter Pylori[3].

EPIDEMIOLOGY

Transmission of Hp is believed to be by gastro-oral or fecal-oral routes as the bacterium can be cultured from vomitus or diarrhea stools. Therefore, the overall prevalence of infection can be influenced by lack of proper sanitation and basic hygiene, of safe drinking water, as well as overcrowding, making it a public-health issue in developing countries[1].

One of the most important risk factors is childhood socioeconomic status, meaning that infection is acquired at an early age, especially in developing countries. It is common for spontaneous clearance to occur during early childhood, but the chance of reinfection is greater in developing countries compared to developed ones – where it is estimated to occur in less than 0.5% of cases per year[5].

Due to these factors, prevalence of infection can reach up to 80 % by the age of 20-30 in developing areas, whereas in developed countries prevalence is less than 20 % in individuals younger than 30 years, but can reach up to 40-50 % in those 60 of age or older[2].

PATHOPHYSIOLOGY

Clinical outcome of Hp infection is dependent on sophisticated interactions between the bacterial, host and environmental factors. In order to promote chronic infection, H. pylori first has to survive in the harsh acidic environment of the gastric medium[6]. One of the most important bacterium survival technique is the “acid acclimation mechanism” that adjusts periplasmic pH by regulating activity of urease. Another factor on which successful colonizationof the gastric epithelium depends is bacterial motility provided by the presence of 4 to 6 functional unipolar flagella. Recent studies show that peptidoglycan-degrading enzymes are necesary for the proper assembly ofthese flagella[7]. After colonization, adherence to gastric epithelial cells is necessary and this is done thanks to a variety of outer membrane proteins (OMPs), several of which can serve as adhesins, the most important being BabA and SabA. H. pylori employs genetic diversification to adapt to the host immune response and promote persistent infection[8].

This pathogen possesses various virulence factors known to be significant in the induction of disease during infection. One of the most studied effector molecule is cytotoxin-associated gene A (CagA) which is injected into the host cell upon contact via the cag pathogenicity island (cagPAI)-encoded typeIV secretion system. Once intracellular, CagA localizes on the inner surface of the celular membrane and is subjected to a tyrosine-phosphorylation process by Src family kinases. The phosphorylated CagA subsequently induces a signaling cascade, causing proinflammatory responses in epithelial cells[9]. While the literature is contradictory, some studies, including those of Kang et al. and Papadakos et al., sugest that inflamation is induced via the nuclear factor (NF)-kB signaling pathway and subsequent interleukin (IL)-8 secretion[7]. Taking this into consideration, and also the fact that CagA translocation and phosphorylation are mediated by cholesterol-rich microdomains of the plasma membrane, Lin et al. found that methylantcinate B, a triterpenoid extracted from the Antrodia camphorata mushroom, attenuates CagA translocation and phosphorylation and inhibits CagA functions, including NF-kB pathway activation and IL-8 secretion; but further in vitro research is needed to assess the possibility of a new, antibiotic-free regimen[9].

DIAGNOSIS

The diagnostic procedure of Hp infection can be difficult due to the fact that up to 80-85% of infected individuals are asymptomatic, while the rest develop a series of non-specific symptoms, such as nausea, vomiting, abdominal pain, heartburn, halitosis or diarrhea[1]. This is why specific indications for testing were stipulated. These include active or documented history of peptic-ulcer disease, early-stage gastric MALToma, early gastric cancer, or uninvestigated dyspepsia in high prevalence areas[4]. Testing for the primary prevention of gastric cancer can be performed in individuals with high risk, such as those with family history of gastric cancer, or first-generation immigrants from a region with high-incidence for this malignancy. Some physicians recommend testing prior to starting long-term non steroidal anti-inflammatory drugs or proton pump inhibitors (PPI)[10].

There is also clear evidence that Hp can be involved in the pathogenesis of some extra digestive diseases, with a clear indication to test for infection in individuals with unexplained iron-deficiency anaemia, idiopathic thrombocytopenic purpura (ITP) and vitamin B12 deficiency[11]. Other studies have tried to find a link between Hp infection and some cardiovascular, lung, hepatobiliary or neurological disorders, but failed to produce an unequivocal causative association thus far[12].

Several methods are currently available for detecting Hp infection. Unfortunately, there is no gold-standard diagnostic test due to the fact that every method has it`s limitations and disadvantages [13] (as seen in table 1).

Table 1: Diagnostic tests for Helicobacter pylori infection [14]

Endoscopic Testing	**Advantages**	**Disadvantages**
A) Histology	Excellent sensitivity and specificity	Expensive. Requires infrastructure and trained personnel
B) Rapid urease testing	Inexpensive. Provides rapid results. Excellent specificity and very good sensitivity.	Posttreatment sensitivity significantly reduced
C) Culture	Excellent specificity. Allows antibiotic sensitivities determination	Expensive. Difficult to perform. Not widely available.
D) Polymerase chain reaction (PCR)	Excellent sensitivity and specificity. Allows antibiotic sensitivities determination	Not standardized across laboratories. Not widely available
Nonendoscopic Testing	**Advantages**	**Disadvantages**
A) Antibody testing (quantitative and/or qualitative)	Inexpensive. Widely available Very good NPV	PPV dependent upon background Hp prevalence. Not recommended after Hp therapy
B) Urea breath tests (^{13}C and/or ^{14}C)	Identifies active Hp infection. Excellent PPV and NPV regardless of Hp prevalence. Useful before and after Hp therapy	Reimbursement and availability remain inconsistent
C) Fecal antigen test	Identifies active Hp infection. Excellent NPV and PPV, regardless of Hp prevalence. Useful before and after Hp therapy	Polyclonal test less well validated compared to the UBT in posttreatment settings. Monoclonal test appears reliable before and after Hp therapy.
The sensitivity of all tests (endoscopic and nonendoscopic) that identify active Hpi infection is reduced by the recent use of PPIs, bismuth, or antibiotics PPI = proton pump inhibitor; NPV = negative predictive value; PPV = positive predictive value; UBT = urea breath test.		

Table 2: Possible reasons for Helicobacter pylori treatment failure.

1	resistance acquired by mutations
2	the low gastric pH limits antibiotic efficacy
3	antibiotic concentration can be insufficient if there is a high bacterial load
4	existence of sanctuaries to which antibiotics do not diffuse
5	existence of viable bacteria in dormant forms not accessible to antibiotics
6	impaired host mucosal immunity
7	risk of re-infection
8	lack of patient compliance (due to adverse effects or poor understanding of complex antibiotic regimen)

TREATMENT

Historically, various combinations of antibiotics have been used to eradicate the infection, however, no optimal treatment has yet been defined, as there is not a single drug regimen that can eradicate it. There are many factors that have increasingly compromised the effectiveness of most commonly used therapies (as seen in table 2)[1].

These factors have reduced the eradication rates to unacceptable levels (< 80% in some geographic areas). As a response, new treatment strategies have been studied and recently been validated to replace the standard ones[15].

Eradication therapies should be guided ideally by individual susceptibility testing. As this is not cost-effective, local antibiotic resistance patterns should dictate the regimen. Another factor that influences the treatment strategy is drug availability in different countries[16]. These regimens can be divided into first-line treatment and second-line or rescue treatment and are summarized in table 3[1].

Table 3: Treatment regimens for Helicobacter pylori infection

First-line therapies		
Regimen name	*General characteristics*	*Duration*
"Standard triple treatment"	simultaneous PPI + clarithromycin + amoxicillin (each twice daily)	7 days
10 or 14 days triple	simultaneous PPI + clarithromycin + amoxicillin (each twice daily)	10 or 14 days
Sequential therapy	5 or 7 days simultaneous PPI + amoxicillin, followed by 5 or 7 days simultaneous PPI + clarithromycin + 5-nitroimidazole (each twice daily)	10 or 14 days
Quadruple therapy	Simultaneous PPI (twice daily) + bismuth subsalicylate, metronidazole and tetracycline (each 4 times daily)	10 or 14 days
Second-line and rescue therapies		
Regimen name	*General characteristics*	*Duration*
Concomitant therapy	Simultaneous PPI + 3 antibiotics (often amoxicillin, clarithromycin, and 5-nitroimidazole) (each twice daily)	7, 10 or 14 days
Levofloxacin therapies	Levofloxacin will replace clarithromycin in triple, sequential or concomitant regimens	7, 10, 14 days
Hybrid therapy	7 days simultaneous PPI + amoxicillin, followed by 7 days simultaneous PPI + amoxicillin + clarithromycin + 5-nitroimidazole (each twice daily)	14 days
Rifabutin triple therapy	PPI + amoxicillin + rifabutin (each twice daily)	14 days

PPIs are mandatory compounds in every regimen and are administered twice daily at standard doses: omeprazole 20 mg, esomeprazole 40 mg, lansoprazole 30 mg or pantoprazole 40 mg (with studies showing higher eradication rates for the more potent second-generation PPIs – namely esomeprazole). Standard antibiotic dosages are used in all treatment regimens: amoxicillin 1 g, clarithromycin 500 mg, metronidazole 500 mg, tinidazole 500 mg, bismuth subsalicylate 524 mg or bismuth subcitrate 420 mg, tetracycline 500 mg, levofloxacin 500 mg, and rifabutin 300 mg[17].

A recently published meta-analysis by Li et al. has compared many of the available regimens for efficacy and tolerance. In terms of efficacy, the standard triple therapy was outranked by all other regimens, with the best eradication rates for the sequential therapy amongst first-line treatments and for the concomitant therapy amongst rescue treatments. The same study has shown that increased treatment duration improves eradication rates, but at the same time enhances the likelihood of adverse effects, lowering patient compliance – almost all rescue regimens were poorly ranked in terms of tolerance[18]. Because of the fact that none of these regimens is ideal, eradication should always be assessed, preferably using a non-invasive test (eg: urea breath test or stool antigen test). Confirmation of eradication can also provide an early image on the pattern of antibiotic resistance in a specific population[15].

All this effort in treating Hp infection is justified by the many benefits of eradication for patience with PUD, but also some malignant conditions. For example a cured individual has better ulcer remission rates for both gastric and duodenal ulcer and does not need maintenance acid suppression therapy after eradication and ulcer healing[10]. It is also more cost-effective and superior compared to maintenance acid suppressive therapy in preventing duodenal ulcer[1]. As for malignant pathologies, early stage low-grade MALT lymphoma can be cured by Hp eradication up to 80% of cases and there is also the possibility of regression or decrease in progression of precancerous gastric lesions (atrophic gastritis and intestinal metaplasia) after eradication[15].

Other studies have shown inverse associations between Hp infection and the prevalence of certain diseases. A recent study published by Rubenstein et al. confirmed prior studies that showed a strong negative association between Hp infection (particularly the cagA strain) and erosive esophagitis, Barrett's esophagus or adenocarcinoma of the esophagus. However, contrary to the prevalent hypothesis explaining this association, they were unable to detect a negative association between Hp infection and gastroesophageal reflux disease symptoms[19]. Lebwohl et al. found a strong inverse relationship between Hp presence and celiac disease (CD), but failed to establish the mechanism by which Hp might be protective against CD[20]. Other studies have postulated that Hp is protective against some extradigestive conditions, especialy asthma and other allergic pathologies in children[21].

THE ROLE OF PROBIOTICS IN HP MANAGEMENT

Probiotics are defined by the World Health Organization (WHO) as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Most commonly used in clinical practice are lactic-acid-producing micro-organisms (Lactobacillus spp. or Bifidobacterium spp.). Lactic acid produced by these probiotics can have a direct antimicrobial effect by lowering the pH, but can also inhibit the Hp urease. There is also increasing evidence in animal models that some strains of probiotics can inhibit H. pylori growth by competing with different adhesion sites in the gastric mucosae. Another mechanism being stipulated is that some probiotics can increase the expression of the MUC2 and MUC3 genes, which can subsequently lead to an increase of mucus thickness in both antrum and corpus after long-term probiotic intake.

Many studies have been conducted in this field and by analyzing some of them Enzo et al. have reached the conclusion that the use of probiotics as an adjuvant to PPI–antibiotic treatment could improve eradication rates, but the main benefit of this association is the prevention of side-effects, leading to better patient compliance[22].

FUTURE THERAPIES

Efforts to develop a vaccine against H. pylori began in early 1990`s with initial attempts to promote a localized mucosal immune response in the stomach via oral vaccines. Recently, an intramuscularly trivalent vaccine (recombinant Cag-A, Vac-A, and neutrophil-activating protein) was developed, but failed to induce immunity, despite the fact that these antigens were recognized by the host's humoral and cellular immune systems. Although considerable progress has been made in understanding the innate and adaptive immune response against Hp infection, it is still uncertain how to promote the development of host immunity with the final goal of creating a successful vaccine[23].

DISCUSSIONS AND CONCLUSIONS

Despite the constant progress made in the management of Helicobacter pylori infection, it is still considered a major public health issue due to it`s high prevalence (especially in developing countries) and various conditions that can arise from it (even though 80-85% of infected individuals are asymptomatic). The main reasons for treatment failure is resistance acquired by mutations and lack of patient compliance and recent studies are showing that the standard triple therapy is out-performed in effectiveness by most other treatments. Probiotic adjuvant therapy appears to have a clear effect in reducing side effects, but insufficient data exists to conclude that their use improves eradication rates. Knowing that there is no "gold-standard" in Hp eradication therapy and falling short of the individual susceptibility testing goal, the regimen should be dictated by local antibiotic resistance patterns.

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REVIEW ARTICLE

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The use of low-intensity ultrasound system in Orthopedics for treatment of fractures

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INTRODUCTION

The treatment of non-union or delayed union of fractures includes a growing number of approaches and techniques, one of which is bone growth stimulation using low-intensity ultrasound (LIUS). The treatment is effective, painless and safe. Currently, Exogen is the only device marketed worldwide that uses LIUS to influence the fracture healing process.

THE HEALING PROCESS OF THE FRACTURED BONES

All the fractured bones have to go through a healing process. This is true in cases of surgery or injury. The process of bone healing has three stages. The first stage is inflammation. This starts when the bone has been fractured. Bleeding always takes place in the area of the fracture. This leads to clotting of blood and inflammation in the area. This provides the framework of a new bone. The second stage is bone production. With time, the clotted blood formed in the inflammation stage is replaced by cartilage and fibrous tissues. As the healing continues, the hard callus replaces the soft callus. This can be seen by x-rays some days after the fracture [1, p 20]. The last stage is bone remodeling. This stage goes on for months. In this stage, the bone continues to become compact and returns to its original shape and structure. The blood circulation in the area of the fracture improves. Once some bone healing has taken place, weight bearing encourages the bone remodeling stage [2, p 99].

THE EXOGEN ULTRASOUND BONE HEALING SYSTEM

This is an approved and clinically tested system for treatment of bone fractures. Exogen makes use of the painless ultrasound waves in order to activate the cells that are near the site of the fracture. This speeds up the natural repair process [3, p 1999].

Clinical studies have shown that the system accelerates the healing process of the fractured bones by 38 % in acute fractures [4, p 655] and 86% in non-union or delayed union. It has been shown that Exogen can improve the fracture healing process of older people, those who take tobacco and those who are obese, cases in which the healing process might be delayed. Exogen system can accelerate the process of healing as much as 50 % for the patients who smoke [6, p 254].

The picture below offers a basic explanation of how Exogen works at the cellular level.

To enhance bone healing, Exogen releases low intensity pulsed ultrasound waves at the point of the fractured bone. This jump-starts the natural healing process of the body. The waves move through the skin and the soft tissues to stimulate the critical cells

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involved in bone healing.

Figure 1: The way Exogen Accelerates the Healing Process of a Fractured Bone [14]

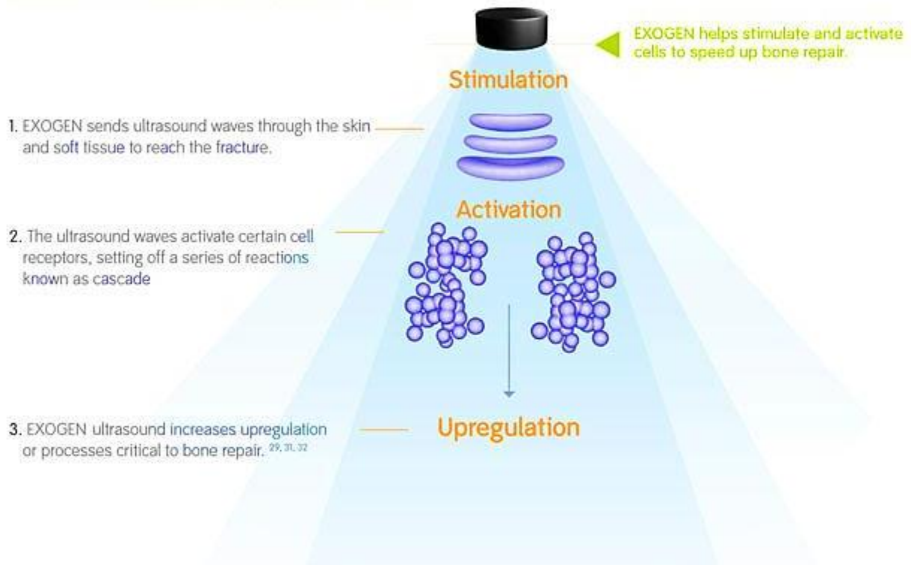
EXOGEN helps stimulate and activate cells to speed up bone repair.
Stimulation
1. EXOGEN sends ultrasound waves through the skin and soft tissue to reach the fracture.
Activation
2. The ultrasound waves activate certain cell receptors, setting off a series of reactions known as cascade
Upregulation
3. EXOGEN ultrasound increases upregulation or processes critical to bone repair. 29, 31, 32

In order to understand the role that the ultrasound plays in healing of the fractured bones, there is a need to understand what it is and how it works. It uses transcutaneous acoustic energy. The piezoelectric crystal produces sound waves which are transmitted through the various body tissues to make a number of changes which are implicated in the tissue healing [8, p 1098]. The density of the tissue is directly proportional to the sound waves that the tissue absorbs. The bone possess high density which makes the ultrasound waves to target the areas which may be affected or have bony abnormalities.

Low intensity pulse ultrasound (LIPUS) specifically works as a potential noninvasive therapeutic in healing of fractures. The waves which are produced by LIPUS introduce micromechanical stress in the area that has been fractured. This stimulates the cellular and molecular responses that are involved in the normal fracture healing. The osteogenic and angiogenic effects caused by the administration of LIPUS are non-thermal. The effects are mechanical in nature with a temperature variation of less than 10C [9, p 802].

The optimum parameters used to archive the maximum benefits of LIPUS are: a pulse width of 200us, an intensity of 30 Mw/cm2 and a 1.5 MHz frequency repeated at 1 kHz every day for 20 minutes [6, p 254].

HOW ULTRASOUND EXERTS MECHANICAL STRESS

There are two mechanisms by which LIPUS induces the mechanical stress in tissues. First it achieves it by displacement of the ends that are fractured and secondly by cavitation.

Research shows that displacement of the fractured ends occurs in at Nano metric scale [9, p 802]. This then stimulates the cellular and the molecular pathways which are involved in the bone healing process [10, p 1022]. It has been shown that LIPUS causes micro motion at the hard and soft tissues interface also. This action produces a mechanical stimulus that is more salient to the integrin mechanoreceptors which take part in osteogenic differentiation and cellular signaling. However it is not yet clear through the available research which displacement mechanism is dominant [11, p 703].

The second mechanism involves the acoustic streaming and cavitation. This mechanism promotes the idea that the sound waves that are emitted from LIPUS allow the accumulation of gas bubbles in the tissues and the cells [12, p 411]. This creates a cavity which gives room for the acoustic streaming. The streaming leads to turbulence or circular flow in the fluids from the tissues as the sound waves move around the bubble of gases. The acoustic streaming produced increased cell permeability. Once this has happened, the blood pressure rises in the area where the fracture has occurred. The increase in the blood pressure leads to the acceleration of healing by ensuring that there is sufficient gas exchange and the delivery of nutrients to the site. When the cavitation is not stable, the bubbles burst and the resultant energy stimulates the surrounding tissues [13, p 2642].

THE EFFECT OF LIPUS ON CELLS AND MOLECULES

There have been studies that have demonstrated the ability of LIPUS to promote fracture healing through the alteration of molecular and cellular mechanisms which are involved in the process of healing. Integrins are crucial in signaling modulation involved in fracture healing [14, p 345].

They act as mechanoreceptors and are also reactive to pressure changes and vibration caused by LIPUS in the cells environment. The mechanical stimuli increase the clusters of integrin on the fibroblasts. They also up regulate the mRNA expression of the integrin in osteoblasts [15, p 284]. The changes that occur enhance the sensitivity of the respective cells to motion and increase the cells intercellular signaling capacity. An important outcome associated with the intercellular signaling induced in the osteoblasts is the increased activation of ocyclooxygenase-2 enzyme.

This increases the production of prostaglandin which is critical in the mineralization during the process of endochondral ossification especially on the soft callus [16, p 660]. The endochondral ossification which is enhanced by LIPUS leads to formation of the bony callus by the process of augmented mineral deposition [17, p 77].

LIPUS also stimulates the differentiation of the cells which are involved in the process of fracture healing, chondroblasts, fibroblasts, mesenchymal and osteoblasts. Additionally, it increases the way aggrecan expresses itself [18, p 1957]. Aggrecan is a stimulator of chondrogenesis. Presence of aggrecan accelerates the differentiation of chondroblasts into chondrocytes. The production of chondroitin sulfate at the site of injury is accelerated by the presence of more chondrocytes.

Chondroitin sulfates are essential components in supporting bonny and cartilaginous structures. Because of the cavitation process aforementioned, LIPUS increases pressure at the site of injury due to the increase in the vascular permeability [19, p 727]. The increase in pressure has been associated with mesenchymal stem cells differentiation which enhances the development of fibro cartilaginous callous.

LIPUS increases the expression of the early osteogenic genes which include osteonectin, the insulin growth factor and osteopontin. These genes have an important role in making sure that proper osteoblast differentiation takes place [20, p 3190]. The osteoprogenitor cells from the bone marrow may also differentiate into the osteoblasts at a high rate when LIPUS is used. This will enhance the bone healing process and remodeling.

CONCLUSION

The use of low-intensity ultrasound system is a relatively new technology that has been proven to be safe and efficient in the treatment of acute fractures or in cases of non-union or delayed union. The effects at the cellular and molecular level demonstrate its efficacy.

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REVIEW ARTICLE

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A new global threat for the public safety: Zika virus

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Abstract: *Zika virus, the etiological agent of Zika fever, is transmitted by mosquitoes and has been affecting the South American continent starting with 2015. It was reported in several European countries, carried by the people who returned from Latin America, as reported by the health authorities in those countries. Today, according to the World Health Organization (WHO), the virus suspected to cause serious birth defects in the fetus has also been confirmed in 21 of the 55 countries of South America, but also in other states from Europe and North America. Zika virus is a single stranded positive sense RNA virus belonging to Flavivirus genus (family Flaviviridae) and was first identified in 1947 in Uganda rainforest Zika. The increased number of cases of microcephaly, in children from northern Brazil, suggested a connection with Zika virus, but it has not yet been proven. Also, the virus can be transmitted sexually and through blood or blood products. Diagnosis of the infection is made using Polymerase Chain Reaction (PCR). So far, there is no specific antiviral treatment or vaccine against the infection with Zika virus. The best form of prevention is to avoid mosquito bites. WHO has estimated that the spread of Zika virus, transmitted through mosquito bite, is "a global public health emergency". The priority is to protect pregnant women and to control the mosquitoes.*

Keywords: *Zika virus, Flaviviruses, microcephaly, Aedes aegypti, mosquitoes*

INTRODUCTION

Zika virus, the etiological agent of Zika fever, is transmitted by mosquitoes and has been affecting the South American continent starting 2015. It was reported in several European countries, carried by the people who returned from Latin America, as stated by the health authorities in those countries. Today, according to the World Health Organization (WHO), the virus suspected to cause serious birth defects in the fetus has also been confirmed in several countries from Europe: Great Britain, Italy, Netherlands, Portugal, Denmark and Switzerland.

Zika virus has been reported sporadically in Africa and Asia for several decades. Zika fever outbreaks were reported for the first time in 2007 (Yap island found in the Pacific Ocean), then in 2013 (French Polynesia). In 2015, outbreaks occurred in Africa (Cape Verde Islands) and South America (Brazil and Colombia). From here, Zika virus quickly spread to several countries in South and Central America and was finally reported in 21 countries of the region in early 2016. [1][2][3] On January 28, the World Health Organization announced that 3-4 million people in

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America could be infected with Zika virus in 2016. [4] On February 1, 2016, the WHO has estimated that the spread of Zika virus, transmitted through the bite of *Aedes aegypti* mosquito, is "a global public health emergency".

THE ETIOLOGICAL AGENT

Zika virus is a single stranded positive sense RNA virus belonging to *Flavivirus* genus (family *Flaviviridae*) and was first identified in 1947 in Uganda rainforest Zika. It is the etiological agent of Zika fever, a viral infectious disease transmitted by infected mosquitoes. [1][2][3]

SYMPTOMATOLOGY

The incubation period is 3-12 days after an infected mosquito bite. Most infections caused by Zika virus are asymptomatic (60-80%). Symptoms are usually mild and disappear within 2-7 days without severe complications or death. The main symptoms are rash (macular or a papular exanthema that initially appears on the face and then spreads throughout the body), moderate fever, arthralgia, myalgia, headache, non-purulent conjunctivitis with conjunctival hyperemia. Vertical transmission can occur during labor (in the viremic stage) or transplacentar. The increased number of microcephaly cases in children from northern Brazil suggested a connection with Zika virus, but it has not yet been proven. Also, the virus can be transmitted sexually and through blood or blood products. [1][2][3]

TRANSMISSION

Zika virus is transmitted by mosquitoes and was isolated from species of the genus *Aedes*, such as *A. aegypti* (Figure1), *A. africanus*, *A. apico argenteus*, *A. furcifer*, *A. hensilli*, *A. luciocephalus* and *A. vitattus*. Studies showed that the incubation period in mosquitoes is approximately 10 days [22]. Zika virus can be transmitted through interpersonal sexual contact and can cross the placenta, affecting the unborn fetus. An infected mother (in the viremic stage) can transmit the virus to the newborn at the time of labor. [24][25][26]

The vertebrate hosts of the virus are the monkeys.

Figure 1: Aedes aegypti (http://www.cdc.gov)

Before the current pandemic, which began in 2007, Zika virus rarely generated collateral infections in humans, even in areas recognized as highly enzootic [7]. In 2009, Brian Foy, a biologist at Colorado State University, reported the sexual transmission of the Zika virus. [13][14]. In 2015, the Zika virus was detected in the amniotic fluid of two pregnant women, indicating that it is possible to cross the placenta and cause infections in newborns. [4][34][4][5][35][36]

DISEASE

Zika virus infection symptoms normally include mild headache, maculopapular rash, fever, malaise, conjunctivitis and arthralgia. [33] The first thoroughly documented cases of Zika virus infections were described in 1964, being characterized by mild headache, progressing through maculopapular rash, fever and back pain. Within two or three days, the initial symptoms remitted, but the rash persisted. Patients with Zika fever are advised to rest and are prescribed fluids and acetaminophen, while aspirin and nonsteroidal anti-inflammatory drugs should be used only after Denga fever was ruled out, in order to reduce the risk of bleeding. [38]

COMPLICATIONS

Microcephaly. In December 2015, it was suspected that an infection of the fetus with Zika virus, transmitted transplacental, may cause microcephaly and brain damage. [5][34] Consequently, the European Centre for Disease Prevention and Control issued a comprehensive update on the possible association of the Zika virus with congenital microcephaly. [35] (Figure 2)

According to CDC Atlanta (Centers for Disease Control and PreventionUSA), "were reported cases of microcephaly defects in children of mothers who were infected with the Zika virus during pregnancy."

Figure 2. Comparison between a child with microcephaly (left) and a normal child (http://www.cdc.gov)

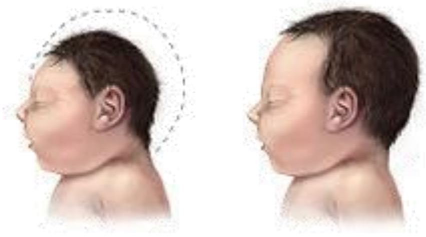

Zika viral infections were confirmed in several newborns with microcephaly; the number of microcephaly cases associated to Zika virus infection is unknown. [8][10][34]

On January 17, 2016 the Pan American Health Organization (PAHO), the Regional Office of the WHO, has recommended that its member states "establish and maintain the ability to detect and confirm cases of infection with Zika virus, given the increased number of congenital anomalies, Guillain-Barré syndrome and other neurological or autoimmune syndromes recorded in areas affected by Zika, to prepare healthcare facilities to respond to a possible increasing demand for specialized care for neurological syndromes and to strengthen prenatal care." [28][29]

DIAGNOSTIC

Zika virus is detected through PCR (gene amplification by Polymerase Chain Reaction). [15] [16] Since the viremic stage may be short, the WHO recommends that the RT-PCR testing should be done on the sera collected within 1 to 3 days after symptom onset or on the saliva or urine samples collected during the first 3 to 5 days after onset. [20][21][22][23]. Serological diagnosis can also be used by detection of specific IgM antibodies. Those appear towards the end of the first week of illness. [18]

Serological diagnosis can be difficult, given possible cross-reactions with other flaviviruses, for example the *Dengue* virus, *West Nile* virus or *Yellow Fever* virus. Zika commercial diagnostic tests are available at Euroimmun (www.euroimmun.com).

TREATMENT

Until present time, there is no specific antiviral treatment or vaccine available against Zika virus infection. The best form of prevention is to avoid mosquito bites.

Prophylaxis

The existence of mosquitoes and larval nests is an important risk factor for the infection with Zika virus. Disease prevention consists in decreasing the number of mosquitoes at source (elimination or modification of larval nests) and reducing contacts between these insects and humans. Those traveling to endemic areas should be informed on methods of protection against mosquito bites. These involve avoiding outdoor exposure at dusk and dawn, wearing clothes that cover as much exposed skin, using repellents, nets for beds impregnated with insecticide, accommodation in rooms with air conditioning and mosquito nets. It is also recommended emptying, cleaning and covering all containers that can collect water such as buckets, flower vases and tires in order to eliminate the places where mosquitoes can reproduce. During outbreak, health authorities can proceed to spray insecticide.

Vaccination

There are effective vaccines against several *Flaviviruses*. Vaccines against the *Yellow Fever* virus (antiamarilic), *Japanese encephalitis* and *Tick-Borneencephalitis* have been introduced from 1930, while the dengue fever vaccine just became available for use. [37]

According to Anthony Fauci, of the National Institute of Allergy and Infectious Diseases, attempts are made in order to obtain a Zika virus vaccine. [38] Researchers at the Research Center for Vaccines have extensive experience in obtaining vaccines against other viruses such as the Chikungunya and the Dengue fever virus. [38] Nikos Vasilakis, from the Center for Biodefense and Emerging Infectious Diseasesappreciated that getting the vaccine could take up to two years, but an effective vaccine for public use, approved by regulatory authorities, could be obtained in 10 – 12 years. [38][39][40][41]

EPIDEMIOLOGY

In 1947, scientists researching Yellow Fever revealed the onset of fever in a *Macaccus Rhesus* monkey located in a cage in the Zika Forest (Zika meaning "too high", in the Luganda language), near the Research Institute of Virology East Africa, in Entebbe, Uganda. The researchers have isolated from the monkey's serum a transmissible agent, which was first described in 1952 as the Zika virus [17] and was subsequently isolated in 1954 from a person in Nigeria. Since its discovery until 2007, rare cases of Zika virus infection were recorded in Africa and Southeast Asia. [40] In April 2007, the first outbreak outside Africa and Asia was declared on the island of Yap, in the Federated States of Micronesia; the cases were characterized by skin rash, conjunctivitis and pain, initially associated with Dengue, Chikungunya or Ross River disease. [41] However, the serum samples analyzed from patients in the acute phase of infection confirmed Zika virus infection. There have been 49 confirmed cases and 59 cases were disproved. There was no need for hospitalization and no deaths were reported. [42] Subsequently, outbreaks have appeared in Polynesia, Easter Island, the Cook Islands and New Caledonia. [5]

Starting April 2015, an epidemic broke out in Brazil with Zika virus (Figure 3) and has spread to South and Central America, the Caribbean, having a trend of global spread. In January 2016, CDC issued a level 2 travel alert for people traveling to affected areas (Table 1, Figure 4).

Figure 3: Zika virus epidemic map at 01.22.2016 (www.cdc.gov)

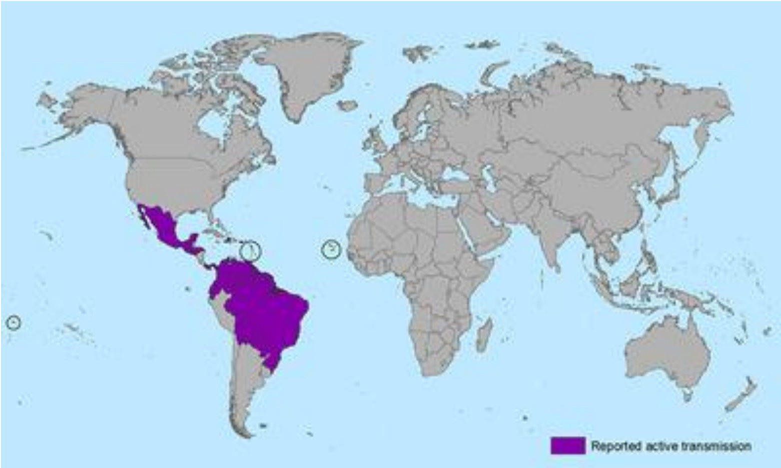

Figure 4: The geographical distribution of the Zika virus(www.cdc.gov)

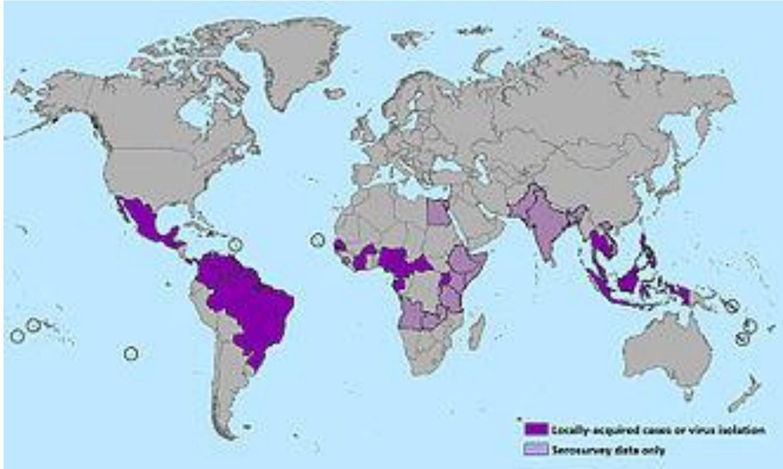

Table 1: Number of cases diagnosed with Zika virus (April 2015-present) (www.cdc.gov)

Country	Confirmed cases	Update
Australia	2	2 Feb 2016
Barbados	3	20 Jan 2016
Bolivia	1	20 Jan 2016
Brazilia	est. 1,5 mil	30 Jan 2016
Canada	4	30 Jan 2016
Chile	3	2 Feb 2016
Colombia	est. 20.000	30 Jan 2016
Curaçao	1	31 Jan 2016
Dominican Republic	8	27 Jan 2016
Ecuador	6	20 Jan 2016
El Salvador	est. 2.500	31 Jan 2016
French Guiana	2	8 Jan 2016
Guadeloupe	1	21 Jan 2016
Guatemala	1	27 Nov 2015
Guyana	1	21 Jan 2016
Haiti	5	21 Dec 2015
Honduras	3.649	1 Feb 2016
Jamaica	1	30 Jan 2016
Martinique	2	8 Jan 2016
Mexico]	18	31 Jan 2016
Nicaragua	3	31 Jan 2016
Panama]	50	28 Jan 2016
Paraguay	6	3 Dec 2015
Puerto Rico	22	26 Jan 2016
Saint Martin]	1	21 Jan 2016
Suriname	3	24 Jan 2016
United States	40	2 Feb 2016
Venezuela	3.700	28 Jan 2016
Total	**Est. 1.5 mil**	**up to 30.01.2016**

IMPLICATIONS FOR THE PUBLIC HEALTH

The WHO recommends that women planning to become pregnant should consult their physician before traveling to regions affected by the disease. [9][44] Governments and health agencies in the European Union, including UK and Ireland, as well as those in New Zealand, Canada, Colombia, Ecuador, El Salvador and Jamaica have issued similar travel warnings. Specific plans and measures were announced by authorities in Rio de Janeiro, Brazil, to prevent the spread of the Zika virus during the Olympic Games, taking place in this city, in 2016. [11][45][46]

According to CDC, Brazilian health authorities have reported more than 3,500 cases of microcephaly between October 2015 and January 2016. Some affected infants had a severe type of microcephaly, while others died.

These manifestations may be associated with Zika infection, occurred during pregnancy. Several studies are planned to assess the risks of the Zika virus infection during pregnancy. [45] In the worst affected regions in Brazil, about 1% of newborns are at risk for microcephaly. [46]

COMMENT

In humans, Zika virus causes an infectious disease called Zika fever, characterized by fever, headache, maculopapular rash, malaise, conjunctivitis, arthralgia etc.

The WHO has warned that the spread of Zika virus, transmitted by mosquitoes, is ''a global public health emergency'' and can be correlated with an increased number of newborns with congenital malformation recorded in Brazil last year.

WHO warns that Zika virus, transmitted mainly by mosquitoes, "explosively" spreads in South America, where in 2016 could be millions of cases. The priority is to protect pregnant women and to control the mosquito populations.

Medical experts are concerned about the very rapid transmission, over long distances of the virus with devastating consequences. From the confirmation of the presence of Zika virus, in May 2015, the virus has spread so far in 23 countries, including Brazil, Bolivia, Paraguay, Mexico, Venezuela and the epidemic continues to spread.

CONCLUSIONS

The World Health Organization has warned that the spread of Zika virus, transmitted by mosquitoes, is a

”global public health emergency”, which can be correlated with the increased number of newborns with congenital malformation recorded in Brazil last year and that the virus "explosively" spreads in South America, where in 2016 could be millions of cases.

Experts are concerned about the very rapid transmission, over long distances of the virus; the consequences can be devastating, and the priority is to protect pregnant women and to control the mosquito populations.

National health authorities are requested to take necessary measures for the prevention, diagnosis and treatment of Zika fever cases and to isolate import cases, in order to impede the spread of the disease.

Also, scientific research is needed in order to obtain an effective vaccine against Zika virus.

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SYSTEMATIC REVIEW

Article received on January 12, 2015 and accepted for publishing on January19 2015.

Morphopatological and histochemical highlights in normal and varicose vein wall

Alina Condor[1], Caius Solovan[2], Liliana Vasile[2]

Abstract: *The nutrition of the venous wall appears to be an important factor in the vascular-fibrillar trophicity and in the dynamic of the extracellular matrix formation for the normal veins and, for the chronic venous ulcers of legs, on period of healing. Sequential biopsies were taken at various levels of venous wall of external and internal saphena in 16 cases presenting a chronic condition of legs venous system (35-58 years old patients, both sexes). 8 vein fragments with normal macroscopic appearance were also taken, in necropsy.*

These samples were analyzed using regular morphological methods and some histochemical reactions to reveal the glycogen, glycoproteins, and glycosaminoglycans substrates. There were been used the Gomori silver impregnation and orcein to expose some specific substrates like reticulin or elastin. Other staining methods, like Gomori trichrome, were used to differentiate the specific structures of the vein wall, were used to differentiate the specific structures of the vein wall.

A rich vascularization of normal and dilated vein wall could be remarked.Angiogenesis in vein wall and vasa vasorum changes as well as alcianophilic of vascular intima seem to be reactive and protective factors, depending on the applied therapeutic modalities. The veins are weak structures whose integrity depends on the thickness of the media and the support of neighboring structures.

INTRODUCTION

The emergence of varicosities it is supposed to be a consequence of defective development of venous wall and appears to be inherited. The proper nourishment of the venous walls appears to be an important factor in their trophicity. The incidence of varicose veins is about 1% of the adult population [5]. Both obesity and aging are involved as risk factors for emergence of defects in venous wall tissue support. The etiology and pathophysiology of varicose disease remains controversial. We emphasize the involvement of the following lesional links amid stasis hypoxia:

- Redistribution of microcirculation with arrangement in lobular aggregate of vessels in the superficial dermis;

- Areas of floride proliferation mimicking Kaposi's sarcoma (acro-angio-dermatitis), reticular dermis fibrosis and massive deposits of hemosiderin, degeneration of dermal fibroblasts (senescent fibroblasts converted by modulation into myofibroblasts having a role in periulceros tegument retraction) to extended tissue matrix lysis;

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-Outbreaks of fat necrosis concomitant with a proliferative-alternative response of adjacent epidermal, hyper parakeratosis and orthokeratosis and pseudoepitheliomatous hyperplasia.

The presence of lytic degradative tisular factors (metalloproteinase) and activation of monocito-macrofagic system induced degradation of the basal membrane and chronic venous ulcers.

Morpho-histochemical current study aims to deepen the importance of veins wall nutrition in normal and dilated veins. It also envisages the re-active participation of supra-adjacent dermo-epidermal tissue, the possible influence on vessel permeability and vasoprotective or anticoagulant medication used in selected cases in the study.

MATERIALS AND METHODS

16 biopsies were taken sequentially from the varicose saphenous in areas of dilated vein (12 cases) and in the vicinity of shank chronic venous ulcer (4 cases); Saphenous fragments from normal vein were collected at necropsy (8 cases). Cases have included both sexes. The selected cases were with at least 5 years of disease duration and the type of treatment was also considered.

Patients in the study were selected from the inmates in Department of Surgery of Military Emergency Hospital and University Clinic of Dermatology in Timisoara.

Processing of samples was performed at the Department of Histology of Medicine and Pharmacy University in Timisoara by usual histological techniques: all samples were fixed in 10% formalin, buffered formalin, AFA fixator (Alcohol - Formaline - Acetic acid), paraffin inclusionated, sectioned at 5 µm, then colored by hematoxylin-eosin. For histochemical study, there were been applied reactions PAS (blue Alcian (BA)) and salivary amylase-PAS (Alcian blue used at pH 2.8 and pH 0.5); blue toluidine (BT) at pH 5 and pH 2.8; it was used Gomori silver impregnation for reticulin and it was also used trichrome Gomori reaction.

Orcein staining method was used to expose elastin.

Figure 1: Lipodermatosclerosis (*toluidine blue, pH 2,8, ×200*)

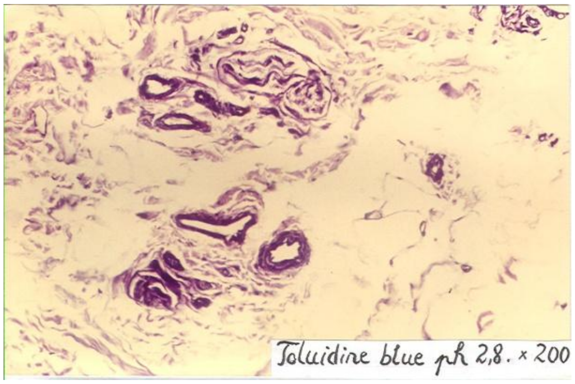

RESULTS

In all examined cases, the wall of external and internal saphenous veins was well vascularized, containing arterioles, venules, capillaries in musculo-fiber media. Constant, parietal capillaries from vasa vasorum were not flattened. In varicose veins adventitia, in small and medium vessels, were frequently found leukocytic marginations and acidophilic deposits and PAS positive, weak metachromatic fibrin (Figure 1).

Fibrin sleeves were also observed in the wall vessels of varicose veins in which angiogenesis is very marked. For AT, were remarked weak metachromatic perivascular sheaths, at pH 5 and chromophobe tissue matrix of dilated veins sheaths, at pH 2.8. Orthochromatic of nuclei allows observing the phenomenon of leukocytes margination (mostly lymphocytes) in the parietal small and medium vessels up next to intima, for both pH used (Figure 2).

Figure 2: Perivascular sheaths and parietal leucocytes margination in small and medium sized vessels, close to venous intima (*toluidine blue, pH 5, ×100*)

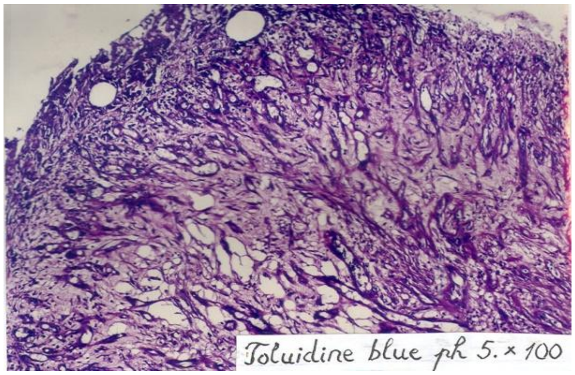

In all methods used in this study, the average media of varicose veins appears thickened through a rich smooth muscle proliferation (HE col., Gomori trichrome col.), with weak orceinic material fragmented through the wall layers (Orcein col.). There were observed fake elastic limitations, fragmented, disorganized (orcein, Gomori trichrome). In one case of all cases examined, using combined staining AA-PAS, which stands under anticoagulant therapy, an edemic intima and hyperplasia were observed, with fundamental alcianophilic substance (FS) and somewhere with deposits PAS positive as a rich interstitial material PAS positive all that pushing aside and cleaving muscle layers of the vascular media. Other intimal changes reported in our study are notches and extensive fibrosis, miointima hyperplasia of saphenous varicose veins.

Alcianophilic fibrillar material with reticular disposition is perivascular distinguished in the neo-capillaries that invade from adventitia media. Silver impregnation for reticulin (Figure 3) shows neo-formation basic membrane by budding vasa vasorum from adventitia to media to decline to extinction the reticulin network of media muscular interstice and vasa vasorum walls.

Figure 3. Reactive angiogenesis in variceal venous wall (*silver impregnation, ×200*)

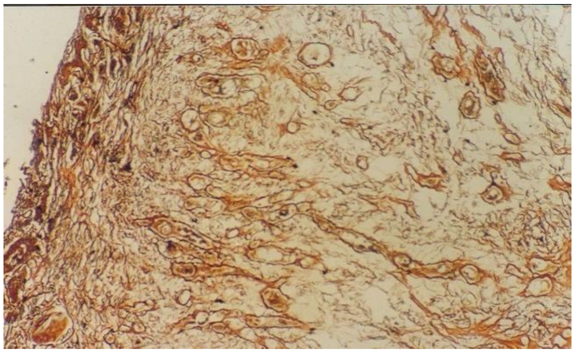

The phenomenon seems to affect 2/3 of external walls of the dilated veins. In neighboring dermal and epidermal tissue and in the perivascular support appear lesions like: lipodermatosclerosis, fibrosis and perivascular fibrin deposits and interstitial fragmentation of elastic fibers in the deep of dermis, tinctorial variations of epidermal cells cytoplasm for which an accumulation of PAS positive material could be noted and cemented SF was PAS reactive. For silver impregnation, the dermal epidermal limit appears fibrillary fine, thinner than the normal appearance.

DISCUSSIONS

The changes of vein wall tissue architectural pattern take place simultaneously with the progression of venous distension in varicose vein disease. It is noticed an important reaction of vessels in vasa vasorum which angiomatosic proliferate, invading media, almost to intima, leading to muscular media hypertrophy [5]. Vascular proliferation can be reactive to miointima hyperplasia and to varicose wall media. Chronic shank venous ulcer it is possible to occur, in time.

Thus, as a reaction to increased venous pressure in a vein much thickened, on account of media and intima and hence to the shank edema, fibrin sleeves occur, those may inhibit the angiogenesis [4]. However, it is created a protection against increased venous additional pressure with a possible role in causing tisular ischemia [6][8].

Fibrin sleeves were found around the capillaries of the dermis in lipodermatosclerosis. Massive deposits of fibrin are involved in elastin fragmentation of the deep dermis and in elastic limitations of vascular walls. Vasa vasorum angiomatosis proliferate in invading varicose vein media. Since 1992, "leukocyte sleeve" was reported as a response to chronic stasis. White blood cells can accumulate in the lumen of capillary and venular, increasing perivascular resistance.

Activated endothelium becomes thrombogenic surface (via tissue factor secreted by endotheliocyte: factor VIIa, factor X-Xa). Simultaneously, activated endotheliocyte was involved in angiogenic peptide synthesis, stimulating neovascularization of dermis and ectasiated venous walls. Tissue anoxia and ulceration is installed.

Consistent with the cited literature, we found also an increased number of capillaries in the skin of patients with venous hypertension in the lower limbs.

Wall vessels are the only ones bringing oxygenated blood, draining interstitial fluid and extravasated colloidal material [7][1][3]. This explains the rapid drainage and metastasis of tumor cells via veins and lymphatics with rich vascularity, having intraparietal capillaries opened.

Adventitial enriched positive PAS material, periadventitial and intraparietal, is represented by fibrin, and also by a macromolecular glycoprotein substrate containing laminin, fibronectin, tenascin detected by immunocytochemical investigations by some cited authors [2][5].

So we can correlate the "leukocytic margination" phenomenon in vasa vasorum and intraparietal vessels with depolymerization of SF tissue more evident in the 2/3 of external portion of the varicose venous wall.

The histochemical reactions used in our study, draw attention to the changes in the three fibrillar component. In varicose veins (dilated segments thereof) we found a quantitative increase of mature collagen fibers and reticulin, morpho-histochemical revealed, with a decrease and fragmentation of elastic fibers in the dilated walls. Together with the degradation of elastin, reticulin network growth can be an important factor in the parietal frailty in shank venous insufficiency evolution.

Marked alcianophilia of intima (AA pH 2.8) calls attention to presence at this level of complex carbohydrates with carboxyl groups and hydroxyl radicals in position of vic-glycol (sialic acid and hyaluronic acid) which act as intima hydrator, but may decrease macromolecular complexes stability of glycoproteins (with increase of sialic acid amount).

Sialic acid carboxyl groups and other substances not yet identified are responsible for basophilia and metacromazia of certain reticulin networks and basement membranes. The enriched PAS positive interstitial material in dilated vessels media is favorable to reticulin collagenization (gradual multiplication of preexistent collagen fibers in a glycoprotein matrix apparently unchanged).

Reticulin collagenization occurs when the hydroxyl group probably belonging to hydroxyproline and hydroxylysine achieve the appropriate concentration [9].

In the cases we investigated, collagenization is conducted in a glycoprotein reticulin dominant matrix (in the external 2/3 dilated vein wall), progressively decreasing toward intima, simultaneously decreasing nourishment intake by vessels of the vein wall fibrosing.

CONCLUSIONS

Morpho-histochemical methods draw attention to the complex of walls reshuffles of venous leg during chronic venous insufficiency. There are being considered the three coats of veins in that appear to be successive lesional patterns: mio-intimal hyperplasia, medial muscle hypertrophy, reactive angiogenesis, lipodermatosclerosis, collagenization of medial muscle.

The chronic venous stasis can be quantified by morphological parameters: mio-intimal hyperplasia, medial muscle hypertrophy, reactive angiogenesis, lipodermatosclerosis, collagenization of medial muscle. The prolonged venous hypertension modifies both the local microcirculation conditions and selecting of fibril-forming cells, triggering the evolution of chronic venous ulcers.

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ORIGINAL ARTICLES

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Expandable stents in digestive pathology – present use in an emergency hospital

Mădălina Ilie[1], Vasile Șandru[1], Cristian Nedelcu[1], Bogdan Popa[1], Gabriel Constantinescu[1]

Abstract: *Introduction: Self expandable metal stents (SEMS) are developed lately, as an effective and safe, less invasive alternative of surgery for the treatment of malignant intestinal/biliary obstruction. Recently, SEMS are also introduced in benign pathology.*
Aim: The aim of this presentation is to report a retrospective analysis of the total number of SEMS placed for esophageal, enteral, colorectal and biliary obstruction during the last 3 years in Clinical Emergency Hospital Bucharest, as well to review the literature published on this issue.
Methods: Between 2013-2015 in Clinical Emergency Hospital Bucharest, we have placed: 232 esophageal stents, 23 enteral stents, 5 colonic stents and 75 biliary stents under radiologic guidance. The main parameters followed were represented by: sex, age, grades of obstruction, stent diameter and type, immediate and late complications and survival rate.
Results: Regarding the esophageal stenting, most of the indications were malignant obstruction (155 cases of esophageal cancer and 30 cases of extrinsic compression), but also for esophageal fistula, peptic stenosis and even traumatic esophageal rupture. The majority of the enteral and colonic stents were inserted for malignant obstructions, having only 2 cases with benign obstructions. This is also the case for biliary stenting, were most of the indications were represented by pancreatic cancer. Technical and clinical success rates were approximately 92% and 80%, respectively. There were no major complications of perforation, bleeding, or death.
Conclusions: SEMS insertion can be performed safely, with minimal complications and hospitalization allowing the restart of oral feeding and improvement of nutritional status for the digestive obstruction or jaundice disappearance in case of biliary obstruction. It represents the first option for unresectable digestive/biliary malignant obstruction.

Keywords: *expandable stents, malignant obstruction, fistula, stenosis.*

INTRODUCTION

Over the past decades, the endoscopic approach for palliation of malignant obstruction has overcome the use of percutaneous approach. The options for endoscopic stenting are either self-expandable metal stents (SEMS) or plastic stents (PS). PSs are composed of polyethylene, polyurethane, or Teflon,whereas SEMSs are made of various metal alloys that are constructed to achieve adequate radial expandable force without sacrificing flexibility and conformability to the duct [1].SEMSs can be either uncovered or covered with material to prevent tumour ingrowths. Recently, uncovered self-expandable biodegradable stents were added to this portfolio [2].

The ideal stent have to be pliable, atraumatic but also forceful to maintain patency and position in the

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[1] Clinical Emergency Hospital Bucharest

lumen. However, stents should also be easily removable and without any risk for benign hyperplastic or malignant tumor ingrowths or overgrowth.

The current guidelines from the American Society for Gastrointestinal Endoscopyfor distal malignant biliary obstruction recommend either SEMSs or PSs, with PSs preferred in cases of distant metastasis and short life expectancy. However, studies have shown that although PSs are less expensive, metal stents have better drainage and longer patency,with recent data showing they are more cost-effective [3][4].

METHODS

We have done a retrospective, non-randomized study between 2013-2015 in Clinical Emergency Hospital Bucharest. We have placed: 232 esophageal stents, 23 enteral stents, 5 colonic stents and 75 biliary stents under radiologic guidance. The main parameters followed were represented by: sex, age, grades of obstruction, stent diameter and type, immediate and late complications and survival rate. We had exclusion criteria: an INR more than 3 and an estimated survival time less than 3 weeks. Regarding the dysphagia we have used Mellow and Pinkas's scale.

0 = able to eat normal diet / no dysphagia.
1 = able to swallow some solid foods
2 = able to swallow only semi solid foods
3 = able to swallow liquids only
4 = unable to swallow anything / total dysphagia

We have used most the nitinol stents (alloy of nickel and titanium), because of their ability to conform to anatomical angulations and the latter for their removability.

RESULTS

More than 65% of the patients were male with age varying between 49 and 83 years old.

Regarding the esophageal stenting we have placed in Clinical Emergency Hospital Bucharest, 232 oesophageal stents for the following indications (Figure 1):

-155 cases of esophageal cancer
-30 cases of extrinsic compression
-43 cases of esophageal fistula
- 3 cases of benign stenosis
- 1 case of traumatic rupture of the esophageal wall

Figure 1: Graphic representation of esophageal stenting indications

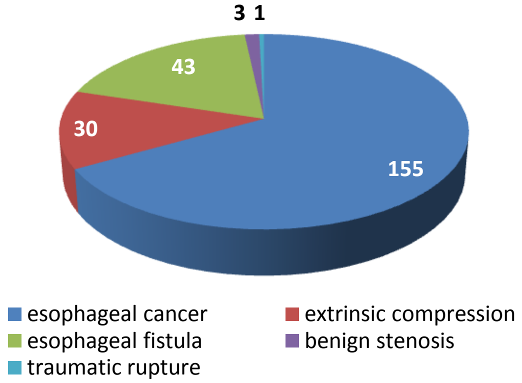

The majority of the cases (> 50 %) have unresectable disease at the time of diagnosis, either because of distant metastases or unsuitable candidates for surgical resection.

The aims of palliative therapy are to ameliorate symptoms of dysphagia, treat complications, maintain oral intake, minimize hospital stay, relieve pain and ultimately improve their quality of life.

We have used: totally covered SEMS or partially covered SEMS. Fully covered stents are more prone to stent migration; removable and theoretically may be more suitable for benign strictures/fistula whereas partially covered stents have a small portion of exposed bare metal in both proximal and distal ends to allow embedding into the oesophageal wall, which helps to decrease stent migration. The uncovered portion of the partially covered stents allows embedding and anchoring. No differences in survival rates were observed between these two types [5].

The techniques used for inserting the stent were: OTG – over the guidewire (most of the cases) or TTS (through the stent) both under radiologic view. At the beginning the lesion is visualized under endoscopy (Figure 2A – oesophageal fistula) and upper end is marked by injection of contrast agent into the submucosa (Figure 2B).

It is very important for the marking to be done internally and not to put external marks that may move during the procedure. The stents are mounted in a preloaded constrained position on a delivery catheter.

A guide wire is passed through the lumen of the catheter (Figure 2C), and when the wire has been advanced beyond the obstruction, the stent is passed over it and positioned across the stricture with the upper end at the position of the internal contrast mark (Figure 2 D, E, F).

Figure 2A: Esophageal fistula

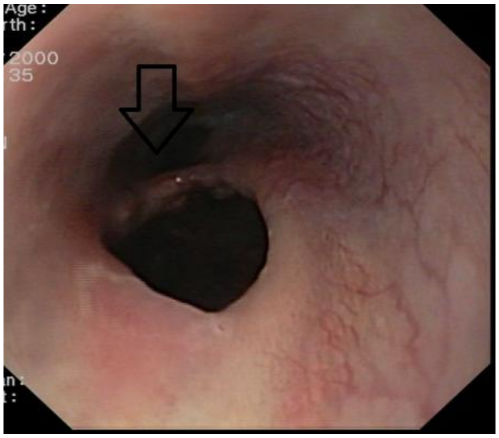

Figure 2B: Contrast injection

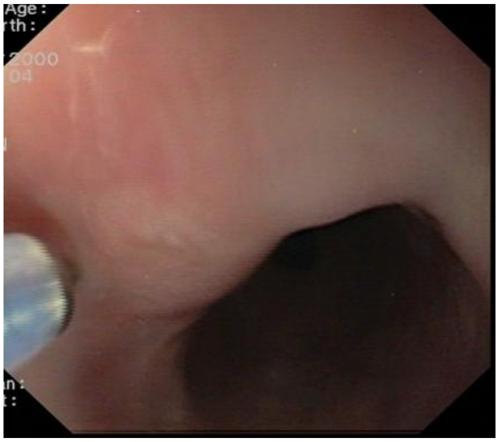

The immediate complications encountered were: retrosternal pain (60%), alleviated after antalgics, stent migration while as late complications we had: food impactation (7%),fibrous tissue invasion (4%), tumoral invasion (5%),uncovered fistula and upper GI bleeding.

Figure 2C: Guidewire insertion

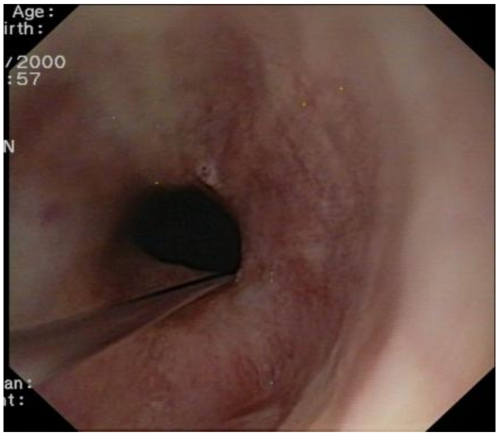

Figure 2D: Stent deployment

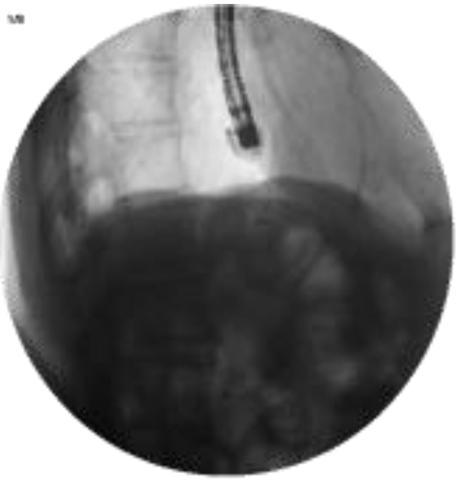

Figure 2E: Stent view on RX

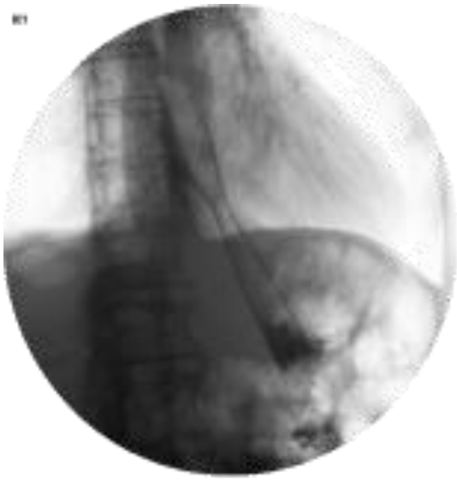

Regarding the enteral stenting we had 23 total cases: 21 for malignant extrinsic compression and 2 cases for benign stenosis. The technique used was TTS (through the scope) with low rate of complications.

Figure 2F: Stent under endoscopy view

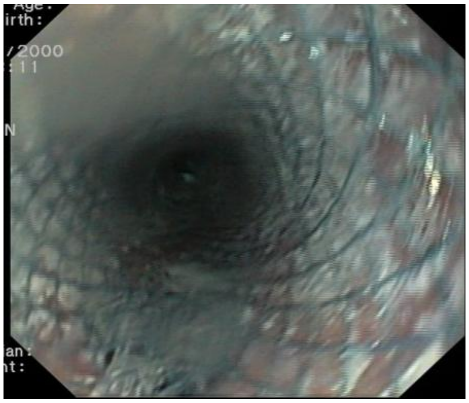

A specific situation was double stenting, both enteral and biliary (Figure 3 A, B), especially in pancreatic head malignancies with obstructive components.

Figure 3A: Double stenting (enteral+biliary)

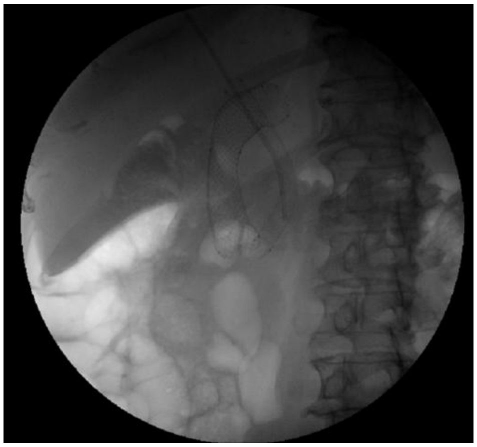

Figure 3B: Enteral stent-endoscopic view

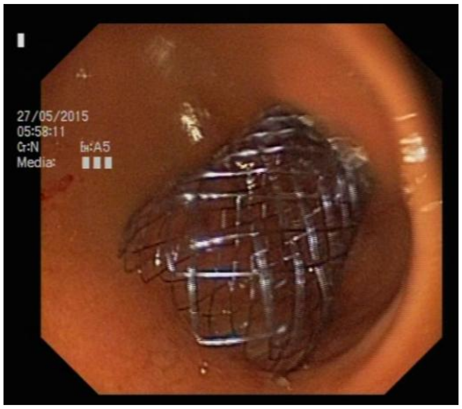

Colonic SEMS placement is recommended as the preferred treatment for palliation of malignant colonic obstruction, except in patients treated or considered for treatment with antiangiogenic drugs [6]. Stent insertion may be considered as an alternative to emergency surgery in those who have an increased risk of postoperative mortality. We have placed only 5 colonic stents (Figure 4), mainly because of ease of accessibility to the surgical service in our hospital.

Figure 4: Colonic stent

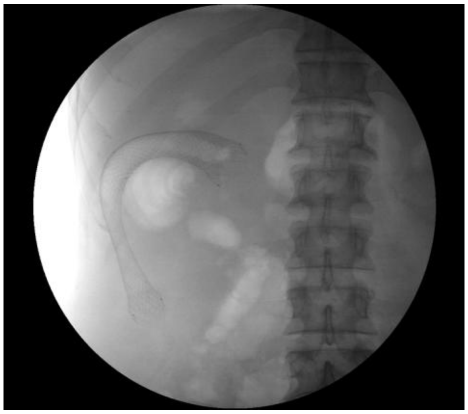

In case of biliary stenting we have inserted 73 stents for malignant obstruction (approx. 60% pancreatic cancer) and only 2 for benign obstruction (chronic pancreatitis). SEMSs range from 4 to 12 cm in length with diameters when expanded ranging from 6 to 10 mm.

Self-expanding metal stents (SEMSs) are built from different metal alloys, mainly nitinol, that are used to achieve adequate radial expansive force leading to increased patency duration and reduced recurrent obstruction.

SEMSs are preloaded in an outer sheath with a diameter of 8.5F or smaller, allowing use with a therapeutic duodenoscope. After placement in the duct, the outer sheath is withdrawn, allowing the stent to expand (Figures 5A, B, C).

SEMSs can be covered, partially covered, or uncovered.

Partially or totally covered SEMSs can be repositioned or fully removed, reduce the rate of stent occlusion but they have risk of cholecystitis because of involvement of the cystic duct orifice). Acute cholecystitis may occur in as many as 10% of patients with intact gallbladders after placement of a covered SEMS across the cystic duct. In opposition, uncovered SEMS migrate less but have risk of tumor ingrowths [3].

SEMS insertion at patients with malignant biliary obstruction palliate jaundice, anorexia and most important help the initiation of chemotherapy that otherwise would be contraindicated.

Initial insertion of a plastic stent is most cost-effective if patient life expectancy is shorter than 4 months; if it is longer than 4 months then initial insertion of a SEMS is more cost-effective.

Figure 5A: Distal biliary obstruction; stent insertion radiologic view

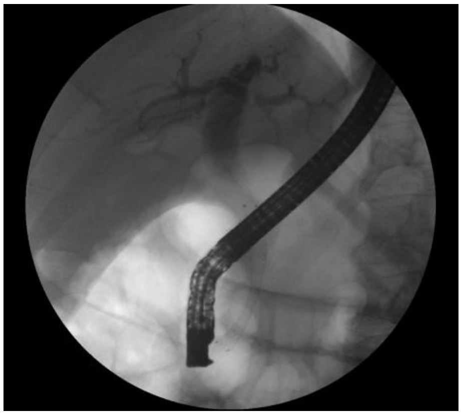

Figures 5B, 5C: Endoscopic views

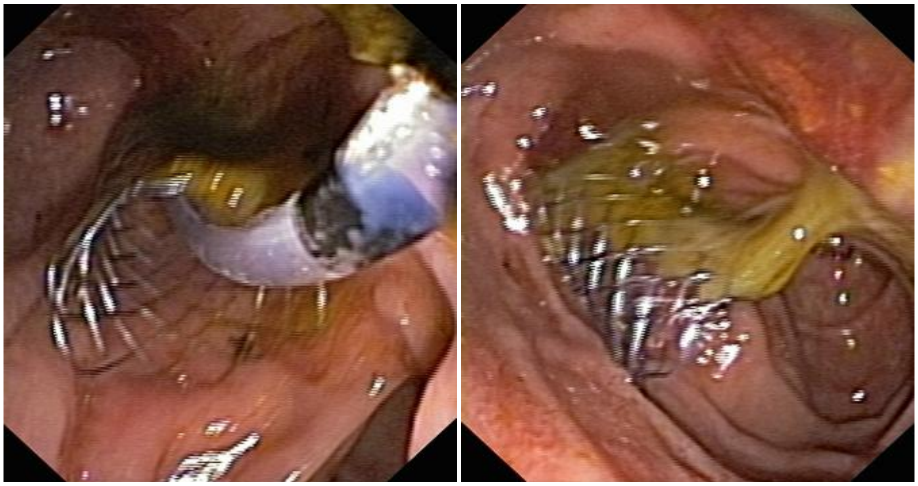

CONCLUSIONS

SEMS represents a very useful alterative, with minimal complications to classic surgery for palliation of unresectable digestive/biliary malignant obstruction. With the current development and applications of new materials and technologies, the future of stenting is evolving.

New trends include drug-eluting stents, biodegradable biliary/esophageal stent and the use of biodegradable polymers for local drug delivery, having also curative aim [7] . Radiation therapy, a cornerstone of treatment for malignant disease of the esophagus, may also be administered in conjunction with placement of esophageal endoprosthetics [8].

Regarding the biliary malignant obstruction, we recommend that SEMSs should be the first option because they have better patency, a lower occlusion rate, less need for reintervention, and fewer adverse events than plastic stents.

In conclusion, expandable metal stent placement is a very effective way of re-establishing luminal patency with negligible complications for an expert endoscopist.

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EDUCATION AND IMAGING

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Hepatic hydatid cysts

Cătălina Diaconu[1], Mădălina Ilie[2,3], Alexandru L. Chiotoroiu[3], Laura Voicu[1], Daniel O. Costache[1], Raluca S. Costache[1,2]

43 years old man presented with recently installed abdominal pain localized in the hypocondrium and epigastrium associated with jaundice and pruritus.

The patient's history included operated bilateral inguinal hernia. Physical examination yielded jaundice, normal aspect of postoperative scars in the inguinal fossae (left and right), pain in the hypocondrium and epigastrium, no guarding, rebound or bruits were found.

Laboratory findings reveal leukocytosis with eosinophilia, hepatic cytolisis (alanine aminotransferase = 219 U/L), hyperbilirubinemia with a higher conjugated bilirubin (total bilirubin=6.01mg/dL, conjugated bilirubin=4.7mg/dL).

Ultrasonography reveals a round transonic image of 7/6cm localized in the IV and VIII segment, with septa that suggests liver hydatid cyst, hyperechoic image of 9mm (hydatid cyst) and a 9mm gallbladder polyp. Computed tomography shows hepatomegaly with a cystic multiseptate image in the IVth segment of 8.5 cm.

Under treatment against pain, proton pump inhibitor, hydratation and regime during hospitalization the symptoms disappear. However the hepatic cytolysis worsens with the progressive growth of alanine aminotransferase to values of 916 U/L, hence anti-helminthic treatment is delayed.

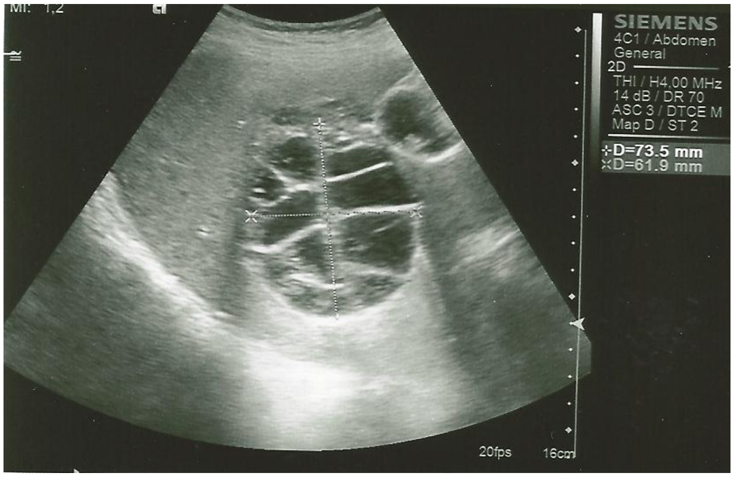

Enchinococcal infection caused by the larval form of *Echinococcus granulosus* remains an important health issue worldwide. Hepatic hydatid cyst is the most frequently encountered form (50-93% of the cases) and left untreated these grow and lead to: developing fistulae in adjacent organs, rupture in the peritoneal cavity, produce daughter cysts or dye (rarely)[1]. Clinical manifestations appear after the cyst is larger than 10cm in diameter and only a third of the patients experience symptoms.

Even though biological finding are nonspecific, elevated bilirubin and alkaline phosphatase may appear. Leukocytosis may appear due to infection of the cyst and eosinophilia is present only in a quarter of patients [2].

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Despite the fact that ultrasonography remains the main pillar in the diagnosis of the disease, computed tomography and serology help improve the accuracy of diagnosing liver hydatidosis. WHO Ultrasound classification of echinococcal cysts helps establish the treatment: 1 (unilocular, anechoic cyst with double line sign), 2 (multiseptate honey comb cyst), 3a (cyst with detached membranes), 3b (cyst with daughter cysts in solid matrix), 4 (heterogenous contents and no daughter cysts) and 5 (solid plus calcified wall). CE4 and 5 are inactive.

On the other hand Gharbi classification also divides in 5 types: type I cysts consist of pure fluid; type II has a fluid collection with a split wall; type III cysts contain daughter cysts (with or without degenerated solid material); type IV has a heterogeneous echo pattern; and type V has a calcified wall. Therefore our case presents a hepatic hydatid cyst stage CE2 after WHO classification and type III after Gharbi classification.

Uncomplicated and small lesions (under 5cm) CE1,2 and 3 can be treated with oral albendazole (10-15mg/kg/day) and close monitoring. However, large CE1 and CE3 cysts need treatment with both albendazole and PAIR (percutaneous aspiration, introduction of scolicide and reaspiration), performed after initiation of albendazole.

Primary surgical treatment has been replaced with less invasive methods since the relapse rate can reach 20%. In patients with complicated cysts, surgery is the treatment of choice. CE4 and 5 only need to be monitored [1][3].

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CLINICAL PRACTICE

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More than simple hepatic cysts

Daniela Tabacelia[1], Mădălina Ilie[1], Alina Constantin[1], Anca Macovei Oprescu[2], Gabriel Constantinescu[1]

Abstract: *Caroli diseaseis a rare congenital disorder that classically causes saccular dilatation of the bile ducts. The complications of Caroli include choledochal cysts with recurrent cholangitis, abscess formation, septicaemia, intrahepatic lithiasis and amyloidosis.We report a rare case of a young female with Caroli disease pointing out the intrahepatic lithiasis as a rare complication of the disease.*

Learning points

- *Caroli disease is an uncommon condition that should be considered in the differential diagnosis of hepatic essential cysts.*
- *Clinically, it is characterized of recurrent episodes of fever and pain.*

The correct and early diagnostic is important because of the different complications and treatment unlike the essential hepatic cysts.

Keywords: *Caroli disease, intrahepatic lithiasis, endoscopic retrograde cholangiopancreatography*.

CASE REPORT

A 28 years old female presented to the emergency department with abdominal pain and fever after an ERCP procedure performed 2 weeks ago. From her disease history we retain an acute pancreatitis that was classified Balthazar B, one year ago, assumed to be caused by biliary main duct lithiasis; at that moment her initial biology showed ASAT 156U/L, ALAT 126U/L, lipase 2,398U/L, bilirubin 32 mmol/l and white cells 14,590/mmc; there was no sign of complication (acute cholangitis) that would have required a surgical treatment in emergency. Her family history was inconclusive for any specific digestive condition.

In our hospital, on physical examination her body temperature was reaching 38^0C, the heart rate 110 bpm, and blood pressure 110/70mmHg; on palpation she presented mild tenderness on epigastric and right quadrant; the usual blood tests showed white cell count with neutrophils raised, hepatic cytolysis and cholestasis until 5 times normal value. Next step was performing an abdominal ultrasound which revealed hepatic cysts (dilation of intrahepatic bile ducts) with liver hyperechoic images with acoustic shadow suggestive of intrahepatic lithiasis (Figures 1 A and B). A CT scan and a MRI were performed and revealed a normal pancreas without Wirsung dilatation, the biliary main duct with a 7 mm diameter without stones inside, and normal gall bladder without signs of cholecystitis but with beaded intrahepatic biliary duct dilatation until 18 mm alternated with normal caliber ducts with numerous images of stones inside with a 12 mm maxim diameter. The dilated biliary canaliculi wall presented signs of cholangitis. All clinical and paraclinical data were typical for a Caroli disease with intrahepatic lithiasis and secondary cholangitis. The patient received treatment with urso-deoxy cholic acid (UDCA), antibiotics, anti-inflammatory drugs and pain medication followed by the resolution of the inflammatory episode.

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Figure 1A

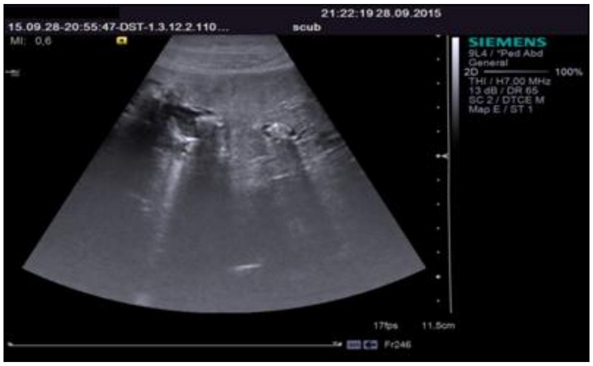

Figure 1B: Abdominal ultrasound revealing hepatic cysts with intrahepatic lithiasis

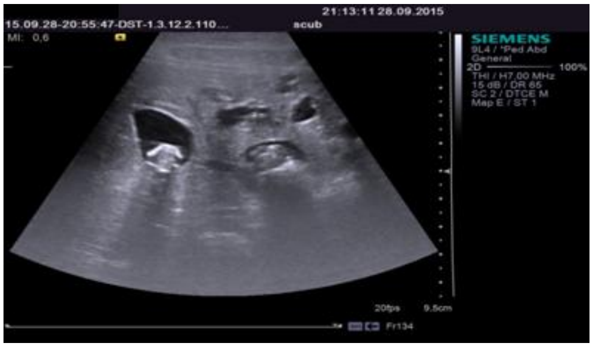

DISCUSSION

The hallmark of Caroli disease is intrahepatic duct dilatation [1]. Patients with Caroli disease are usually presenting to the hospital, when they develop complications as the result of biliary stasis, which leads to stone formation and infection. The stones are brown pigmented stones, composed of inspissated bile[1].

Caroli disease has two forms, one associated with congenital hepatic fibrosis and a simpler form occurring alone. The former, called Caroli's syndrome is associated with portal hypertension, and it's complications including splenomegaly, hematemesis and melena. Caroli disease is also associated with liver failure and polycystic kidney disease. The cause appears to be genetic; the simple form is an autosomal dominant trait while the complex form is an autosomal recessive trait.[2] Females are more prone to Caroli disease than males.[2] Family history may include kidney and liver disease due to the link between Caroli Disease and ARPKD (Autosomal recessive polycystic kidney disease). The symptoms include fever, intermittent abdominal pain, and hepatomegaly. Occasionally jaundice occurs.[3] Laboratory tests indicate cholestasis and hepatocytolysis associated with an inflammatory syndrome in the acute stages.

Caroli disease usually occurs in the presence of other diseases, such as autosomal recessive polycystic kidney disease, cholangitis, gallstones, biliary abscess, septicemia, liver cirrhosis, renal failure, and cholangiocarcinoma (7% affected).[2] People with Caroli disease are 100 times more at risk for cholangiocarcinoma than the general population.[3] Modern imaging techniques allow the diagnosis to be made more easily and without invasive imaging of the biliary tree.[4] Abdominal ultrasound can detect saccular or fusiform dilation of the bile ducts. Images taken by CT-scan or MRI will show enlarged intrahepatic (in the liver) bile ducts due to ectasia; cholangiography is the best approach to show the enlarged bile ducts as a result of Caroli disease.

The treatment of Caroli's disease depends on the clinical features and the location of the biliary abnormalities. Cholangitis is treated with appropriate antibiotics. In case of intrahepatic cholelithiasis litholytic therapy with urso-deoxy cholic acid (UDCA) is indicated[5]. When the ductal abnormalities are localized to one lobe, lobectomy relieves symptoms and appears to remove the risk of malignancy. In case of diffuse involvements of both lobes of liver, treatment options include conservative management, endoscopic therapy (sphincterotomy for clearance of intra-hepatic stone), internal biliary bypass procedures and in carefully selected cases liver transplantation[5].

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Nutritional approach in late gastric stenosis after gastric sleeve

Adina Mazilu[1]

INTRODUCTION

Laparoscopic gastric sleeve(LGS) is a very frequent used procedure nowadays in Romania, with more than 100,000 reported patients operated until now, the most part of patients being operated in private settings. It is a simple and efficient procedure, less expensive than gastric by-pass, with fewer complications, but can reduce obesity complications almost as well as gastric by-pass. Indications for this type of surgery are:

- Body Mass Index is greater than 40 kg/m^2 or between 35-40 kg/m^2 and patient has significant pathology that may benefit from weight loss-like diabetes, hypertension
- Severe co morbidities (cardiac, pulmonary, liver disease)
- Advanced age at time of operation
- Inflammatory bowel disease (Crohn's disease)
- Patient uses chronic medications (anti-inflammatory or immunosupressive)
- Need for continued surveillance of the stomach (that couldn't be evaluated after a gastric bypass)
- Severely enlarged liver found during the operation
- Severe adhesions or scarring to the bowel found during the operation
- Any combination of the above that significantly increases the patient's anesthetic or surgical risk

LGS may be used also combined with gastric by-pass done at 12-18 months after LGS, when anesthesia is less risky and liver has reduced diameters.

Acute complications are represented by hemorrhage reported in 1-6% of cases and gastric leaks in up to 5% in the immediate period after operation [1]. Gastric stenosis has a 0 to 4 % rate of appearance for this type of operation[4]. Most cases can be solved by endoscopic dilation with balloons;however some cases need to be converted to gastric by-pass.

In an article from 2014,in a study done on 565 patients, the incidence of complications was relatively low- the authors[2] reported in 7.79% of patients infarcts of the posterior pole of the spleen, 1.42% gastric fistulas in the His angle region, with 5 deaths among these patients – 3 due to septic complications in the course of fistula, 1 due to encephalopathy and 1 as a result of myocardial infarction.

Chronic complications include strictures, malnutrition and GERD. Chronic strictures usually require further intervention. Endoscopic dilatation is an useful tool used for short segment stenosis. Successive treatments in 4 to 6weeks interval are adequate to treat stricture and ameliorate patient symptoms. In contrast, long segment stenosis and failure of endoscopic management demands a surgical intervention – laparoscopic or open seromyotomy or conversion to Roux-en-Y gastric bypass. Parikh and colleagues reported an incidence of 3.5% of symptomatic stenosis following LGS in their series of

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230 patients; 2 patients required conversion to a Roux-en-Y gastric bypass owing to failure of endoscopic management.

In a recent study by Gehrer and colleagues[3], the prevalence of vitamin B12, vitamin D, folate, iron and zinc deficiency were reported to be 3%, 23%, 3%, 3% and 14%, respectively, after LGS In general, these investigators found micronutrient deficiencies to be less prevalent after LGS than Roux-en-Y gastric bypass; however, folate deficiency was slightly more common after LSG than Roux-en-Y gastric bypass (22% vs. 12%).

CLINICAL CASE

Our patient, a 25 years girl operated – LSG-1 year before, presented with severe malnutrition and dehydration, associating also hypokaliemia. She has been vomiting at least 4 times a day during the past 6 months and she lost almost 100 kg in 1 year (from 130 Kg, 167 cm to 37 Kg at admittance). She has done upper endoscopy 10 days ago and dilation of gastric stricture was not possible. She was told she needed surgical intervention after gaining 10 kg.

She was very pale, with brittle hair and dry skin, with Bichat's sign positive, hypotensive – 70/50 mmHg, tachycardic and refused admittance even if she was explained that she had a very high risk of death. She agreed to come and stay in hospital after solving the problems for her job. As she associated hypokaliemia we tried to correct it with 20 mEq of kalium in saline, but patient reported intense pain on peripheral i.v. infusion, so we used first 1,000mL of saline, and then Ringer and Kalium progressively.

Family reported repeated episodes of binge eating at home with ingestion of Coke and high amounts of food, after that unprovoked vomiting. Discussion with patient revealed also depression, this kind of food pattern was the patient's cry for help due to unresolved adolescence trauma, even if she had family and friends support now. We tried also to relieve the pressure from job.

Next day iv saline 500 ml, Ringer 1000 ml, Kabiven were introduced, with almost complete correction of hypokaliemia.As patient had urinary tract infection we added Gentamycin 320 mg/day and Ciprofloxacin 400 mg/day, for seven days, with good evolution.

Third day we introduced Fresubin – enteral formula – 500 ml/day, divided in 7-8 meals, no more than 100 ml/meal, associated with Osetron and Controloc on i.v. line. Patient stopped vomiting the third day and the next day we introduced small amounts of baby food – milk and egg, fruits puree, small amounts of soup with chopped meat.

By seventh day patient gained enough weight so we tried to reduce the amount of Fresubin and Kabiven and leave her on oral intake of mashed food, but this induced reappearance of vomiting. X-ray revealed no passage of barium due to complete gastric stenosis, so i.v. nutrition was resumed.

Laboratory 8 a.m. serum cortisol was more than 10 mcg/dl, so we excluded Addison disease.Patient had low TSH and low T3 levels – confirming sick euthyroid syndrome and hypotalamic amenorhhea with low Estradiol levels – 17 pg/ml.

In the last 3 days we introduced small amounts of rapid insulin – 10 U/day to improve anabolism.Patient reached 47 kg, with mild edema in the legs and was discharged in the 12th day of admittance.She still has mild hypoproteinemimia – albumin 3.2 mg/dl, total proteins 5,6 g/dl and mild hypocalcemia – 8.8 mg/dl.She was discharged with proton pump blocker and prokinetic treatment, associated with buvable ferrum sulfate.

She repeated the endoscopy and was converted to gastric by-pass after 3 days without more complications.

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CLINICAL PRACTICE

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Fever and abdominal tumoral masses

Augustin C. Dima[1], Teodor Artenie[1], Daniel Florea[1], Florina Bold[1], Paul I. Oprea[1]

Abstract: *49 year-old man presented to our clinic for pain in the right hypochondrium, diarrhea, and fever. The clinical examination highlights a tumoral formation in the right side of the abdomen, with firm consistency, poorly defined margins, and present mobility in the deep structures. On biological exams, leukocytosis with neutrophilia, inflammatory syndrome, and hypoalbuminaemia were identified. The first computed tomography exam described parietal thickening of the ascending colon, with infiltrative aspect, and multiple local adenopathies, lomboaortic and interaortocave. Moreover, four nodular liver tumors, with hypodense image in native examination, were identified. The lab tests for infectious diseases were all inconclusives: three hemocultures, three stool samples, and three coproparasitological exams were all negatives.*

Interdisciplinary examinations, internal medicine and infectious diseases, sustained the diagnosis of colonic neoplasm with peritumoral abscess and liver pseudo-tumoral masses. The colonoscopy did not revealed any bowel lesions relevant for neoplasia. This result as well as the bio-clinical context imposed abstention from surgical intervention.

Wide spectrum antibiotics and symptomatic treatment were initiated. But, ten days after hospitalization, the second computed tomography exam showed reduction of the ascending colon wall thickness associated with significant increases of the liver tumors is so revealed. The investigations for other possible etiologies were so continued.

Keywords: *colon tumor, intestinal amoebiasis, liver abscesses.*

INTRODUCTION

In current clinical approach of abdominal pathology one must not forgot the rare disorders whose clinical picture and imaging could mime neoplasia, pathology so frequent today.

The current diagnosis of colon tumors is made especially using imaging diagnostic methods, whose accuracy is high, facilitating anaccurate stage diagnosis, very important in determining therapeutic tactics. But it must not be forgotten also the importance of classical clinical features that could bring essential elements for the certainty diagnosis.

CASE REPORT

We present the case of a 50-years old patient, smoker, diabetic, hypertensive, who presented for right upper hypochondrium pain, diarrhea, nausea, febrile syndrome, with the onset of symptoms about 48 hours prior to presentation.

Clinical examination revealed hypochondrium and right flank sensitive abdomen, tumor mass in the right flank, with firm consistency, imprecisely defined, relatively mobile on the deep plans, without signs of peritoneal irritation, fever (38.7˚C), shivering.

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Biologically, neutrophilic leukocytosis, inflammatory syndrome, and hypoalbuminemia were noted. Plain chest and abdominal radiography were without pathological changes (Figure 1). Abdominal ultrasound identified hypoechogenic liver formations.

Figure 1: Biological evolution during diagnostic and after specific treatment

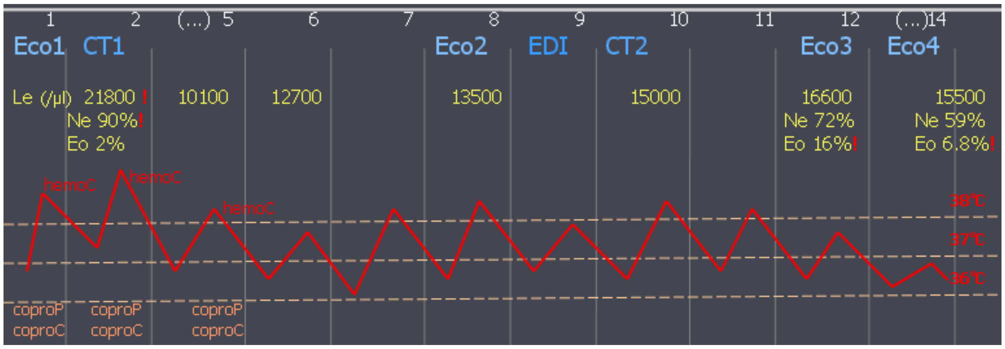

CT exam identifies hepatomegaly and four nodular liver formations: hypodense on native examination, irregularly shaped, with uneven structure, apparently separated from their dense walls (Figures2a, 3a, 4a). In addition, a parietal tumoral formation belonging to ascending colon, located toward liver angle, showing asymmetrical narrowing partially iodophile (with a maximum thickness of 2.1cm), infiltrating the cecum – ascendant digestive wall and the adjacent fatty abdominal tissue that adheres to the liver capsule. Perilesional, lumbar aortic and interaortocaval adenopathies, (with maximum dimensions of 1.3cm) (Figures5a, 6a, 7a) were also described.

Figure 2(a, b): Computer tomography exam: first two liver masses evolution under treatment

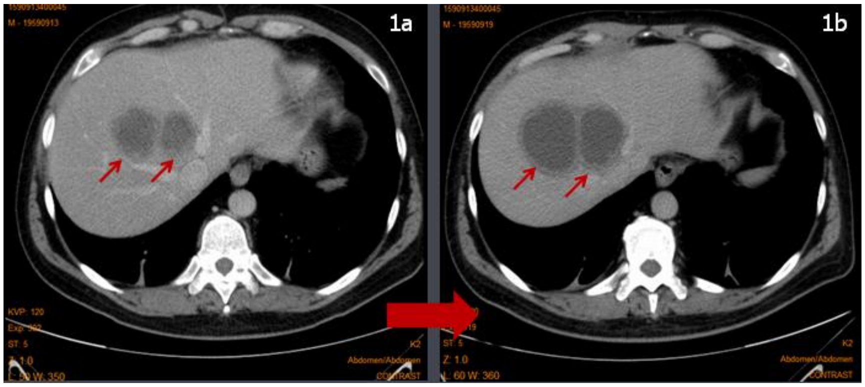

Figure 3(a, b): Computer tomography exam: liver masses evolution under treatment

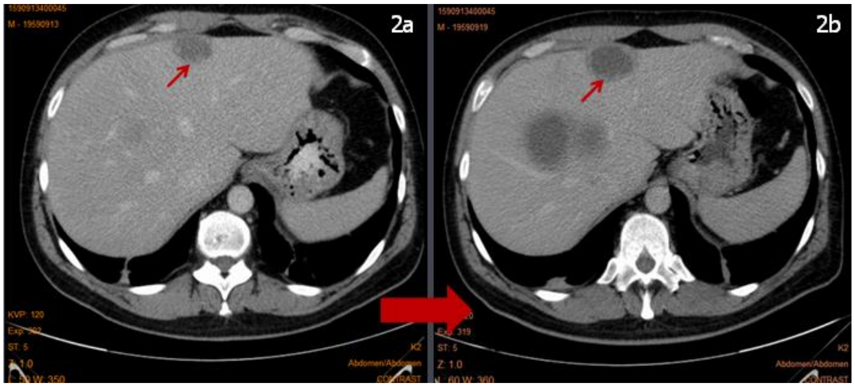

Figure 4(a, b): Computer tomography exam: liver masses evolution under treatment of the third tumoral mass

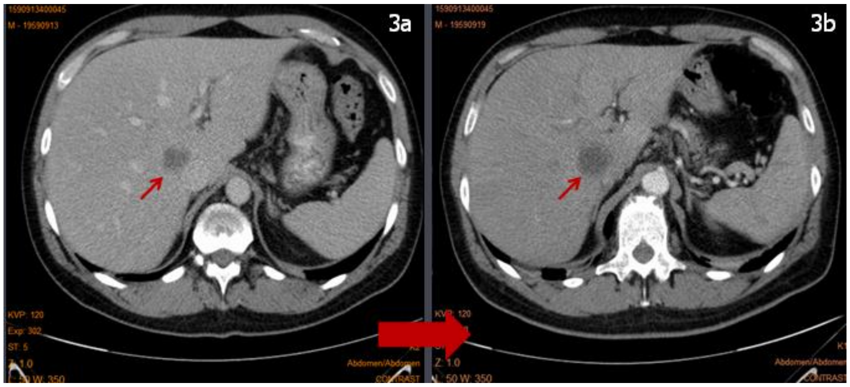

Figure 5(a, b): Computer tomography exam: colon tumor evolution under treatment

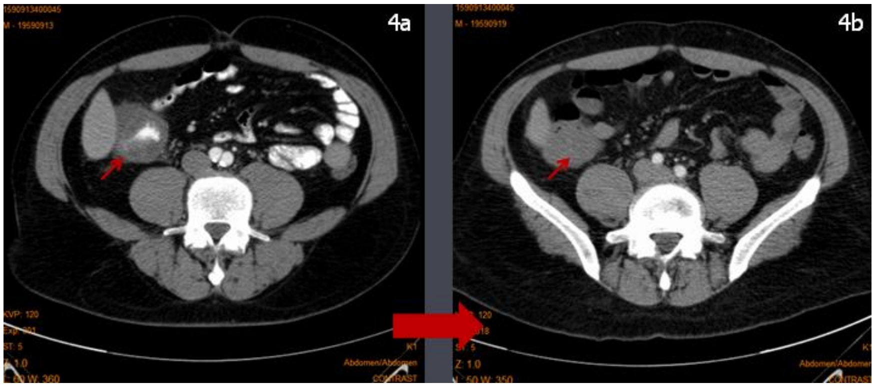

Figure 6(a, b): Computer tomography exam: colon tumor and local adenopathies evolution under treatment

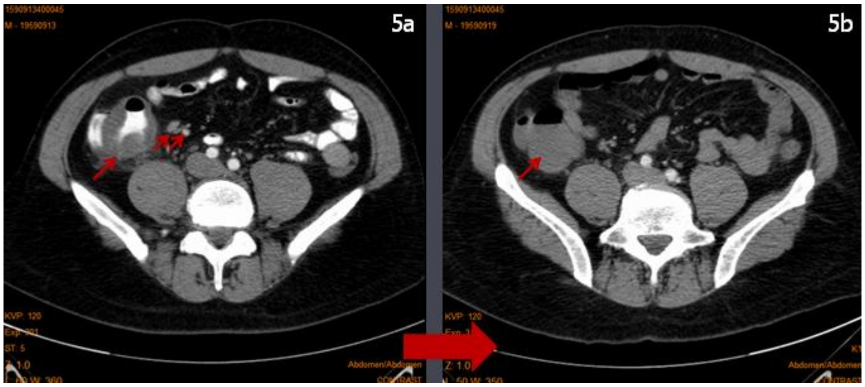

Figure 7(a, b): Computer tomography exam: colon tumor evolution

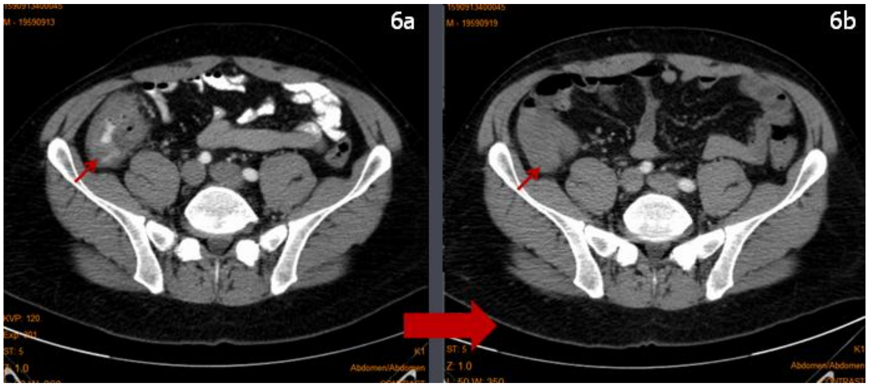

The paraclinical picture was completed with three negative blood cultures, three negative copro-parasitological exams, three negative coprocultures and tumor markers (carcinoembryonic antigen, CA 19-9, alpha-fetal protein) negative (Figure 1).

Internal medicine and infectious diseases examinations declines the eventuality of infectious or parasitic primary etiology of colony formation and supports the diagnosis of colon cancer with peritumoral abscess and septic liver determinations.

At this point, the imaging seems to support thediagnosis of ascending colon tumoral mass associated with secondary liver metastases or with local associated infection and liver abscesses.

Clinical arguments, however, given the relatively sudden symptoms onset, are pleading for diverticulitis or segmental colitis of the ascending colon with septic determinations in liver. Therefore, we consider it appropriate to postpone surgery and to make further investigations.

It was initiated treatment with broad antibiotic spectrum (imipenem – cilastatin 500 mg/ 500 mg, 4 times daily, linezolid 600 mg 2 times daily, both for 10 days and Moxifloxacin 400 mg daily for 5 days) associated with symptomatic treatment.

Lower digestive endoscopy describes in the rectum, at 4-5 cm from the anus, a 1 mm polyp covered by normal mucosa, at 10 cm from the anus a 2 mm sessile polyp, covered with congestive mucosal, biopsy was performed. In the sigmoid colon, to about 25 cm, a 10 mm semi pedunculated polyp, cylindrical, covered by the congestive mucosa, supple for biopsy. At the descending colon polyp plan, ovoid of 7-8 mm, covered with inflamed mucosa, supple for biopsy. Transverse and ascending colon without changes.

Caecum, opening of appendix and the ileocecal valve without modifications.

The morphopathologic diagnosis of colon polyps was of tubular adenoma. So, the endoscopic examination does not argue for tumor etiology.

The evolution under treatment was favorable: reducing the number of daily diarrheal stool (from 10 to 12 per day to 2-3 per day) and also fever – under vesperal fever and the overall condition has improved. Biologically, continuing with leukocytosis with neutrophilic, but at lower values and the increasing of cholestasis defining liver enzymes (Figure 1).

We repeat, on day 10 – from the admission – the CT examination and we see a significant increase in volume of liver formations, the largest with a diameter of about 7cm, with better separation than previously, clear evidence of the relatively thick walls and of clear fluid content (Figures2b, 3b, 4b). Also, the marked reduction in the ascending colon thickness and reduction in the size of abdominal adenopathy (Figures5b, 6b, 7b) were noted.

Diagnosis of infectious disease is gaining before a possible neoplastic etiology, se we ask forparasitology and infectious diseases check-up and serological determinations.

Positive diagnosis is supported by the presence of Ig G type anti-Entamoeba histolytica antibody (negative Entamoeba histolytica antigen, negative Echinococcus antibodies, negative Giardia duodenalis CoproAg and Cryprosporidium CoproAg) for which specific treatment is initiated.

Thus, the positive diagnosis was: liver amoeba disseminated abscesses in both lobes, in remission and intestinal amoebiasis affecting the ascending colon, submitted.

The patient’s evolution after the initiation of the etiological treatment was favorable with improvement of the general condition, normalization of blood counts at two weeks of antiparasitic ambulatory treatment and marked reduction of liverabscesses dimension with their disappearance in a year.

DISCUSSIONS

Entamoeba histolytica is a protozoan that is an important health problem in tropical and subtropical countries [1]. Infection is fecal-oral, 90% of those affected remain asymptomatic, being determined by ingestion of food contaminated with parasite cysts [2]. It affects particularly the gastrointestinal tract (more frequently the check and ascending colon) and liver [3].

The difficulty of diagnosis in this case was given by the initial CT imaging appearance of ascending colon tumor, accompanied by the negative coprocultures and coproparasitological exams.

Imaging appearance of colonic amoebiasis mimicking neoplastic pathology has been described in the literature, but this presentation as a colon tumor formation remains a rare occurrence[4][5]. Most frequently, the colonic amebiasis diagnosis is done by highlighting the parasite in the stool, the examination of three successive stools leading to a positive diagnosis in 70% of patients [6].

The correct diagnosis in the presented case was obtained only after achieving immunological determinations, serology for Entamoeba histolytica is positive in up to 90% of patients with amoebic colitis [7].

Also, another feature that hindered the diagnosis was the initial presence of neutrophilic leukocytosis, with no eosinophilia (Figure 1), which could be explained by possible plurimicrobial superinfection of the parasite gateway from ascending colon.

After starting antiparasitic treatment an increase in eosinophils in serum is observed, most likely secondary to parasitic destruction and thus the emergence of new antigenic elements.

Important in the proper management of this patient was refraining from any surgery and introduction of specific therapy (Metronidazol) despite the pressure brought by imaging and biological data. Surgery in Entamoeba histolytica infection is a must in cases of acute colitis with necrosis and perforation when it may be necessary even total colectomy [8][9] and could also be followed by many complications.

Another problem of therapeutic tactic was the discussion on the need for drainage of liver abscesses, their sizes being significant. The ultrasound tracking has shown that the effective antiparasitic treatment was enough, observing the gradual decrease of their sizes, with their disappearance one year after the initiation of antiparasitic treatment.

In general, the literature does not support the need of drainage for uncomplicated liver amoebian abscesses [10]. Could be drained big liver abscesses (>300 cm^3) or when there are questions about the etiology of liver abscess, like in the case of pyogenic liver abscess [11][12] when cultures from drainage samples could be informative on the etiology and further treatment.

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

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(2) searchable by standard, electronic (Internet-based) methods;

(3) open to all prospective registrants free of charge or at minimal cost;

(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

Review Articles

RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

Meta-Analyses or Systematic Reviews

RJMM particularly welcomes submission of Meta-Analyses and Systematic Reviews, which underpin evidence-based medicine. Guidelines for preparation of Meta-Analysis and Systematic Reviews are similar to other reviews, and articles are subject to the usual peer review process. Meta-Analyses and Systematic Reviews have a word limit of 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

Original Articles (including clinical trials)

RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

Education and Imaging

The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as ‘What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?’ Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

MANUSCRIPT PREPARATION

Style

Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors’ revised ‘Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication’, as presented athttp://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster’s Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader’s task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’

industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

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Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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Authors should use subheadings to divide the sections of theirmanuscript: *Introduction, Methods, Results, DiscussionAcknowledgments and References*.

References

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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If the above items are not included in the cover letter, manuscripts cannot be sent for review.

Please also note that the cover letter does not require a detailed or lengthy description of the content or structure of the manuscript itself.

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EDITORIAL

About clinical medicine: Is there still such a thing?

Dan Mischianu

Gral (R) Prof DAN MISCHIANU
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The other day, a distinguished and well known specialist radiologist from Bucharest shared with me two absolutely surprising things.

First: not long ago, an experienced internal medicine physician addressed expressly with the request to radiologicaly examines a patient with, in his opinion "acute pulmonary edema"! The radiologist told me that the “acute pulmonary edema” was actually a "spontaneous pneumothorax" probably due to rupture of emphysematous bubbles. The thoracic surgeon solved the so-called "acute pulmonary edema" actually "spontaneous pneumothorax" by specific means...

The second issue told by the radiologist: I can sit at home in front of my laptop and receive scanned images of patients from different locations (clinics, private hospitals) in the country (Bacau, Constanta, Baia Mare and so on), then I say my opinion and the fee is in my account!

I asked him slightly amazed: Yeah, well, but do you not feel the need to learn more about that patient? Did you forget what we learned in medical and surgical semiology that 80% of the diagnosis belongs to anamnesis? Do you not need to palpate, to auscultate or to listen to what the patient tells you...? Have you not learned anything from the mistake of the internist who "din not use a stethoscope" to find, in fact, that he had no vesicular murmur? My friend did not answer…

In fact what do I want to say in this editorial?

First I want to say that our guild lives in a perfect confusion: notions or concepts of "evidence-based medicine (EBM)", "alternative medicine", "noninvasive" medicine, "medicine x, y, z" and many others can only leave room for speculation. Medicine, as illustrated, has always been great at removing great speculations because of phantasmagoric individuals like Paracelsus or Mesmer...

Secondly, in Adevarul newspaper from 02/19/2016, Theodora Şopaltă, a journalist, was writing sententiously: "There are five types of doctors. Which one do you prefer?" The first type, in the opinion of the author is “The clever" (30% of respondents voted for him), then comes "The Digital doctor” (which probably specify diagnosis texting his phone! preferred by 8% of respondents), then comes “The Wise man" who do not talk to patients about their illness but gives them an accurate diagnosis. Go figure it! In fourth position lies "The Idealist", preferred by 24% of patients, said to be a younger version of "intelligence" and the ending is marked by "Only You", a "pragmatic option of alternative medicine."

Thirdly, I say that doctors may be labeled as "made and born" to practice this noble profession, for which are made after the Hippocratic precepts and not by strange and awkward ideas, good and honest doctors with knowledge of human nature, easily grasping which in this profession gives you a disadvantage and "good for nothing doctors" who easily realize that there is no place for them amongst us and change their job to managers, adventurers, free – thinkers and so on.

To end this Editorial I conclude by saying that a good and honest clinician is the one that accepts and applies the perennial teachings of Hippocrates of Cos and takes into account the lessons of the Great surgeon and Great clinician, Professor Dr. Ion Juvara:

"Whatever novelty and complexity of laboratory methods of investigation, clinical retains its entire value, observation of man by man offering truths that never grow old”

I believe and hope that there still is the so-called "Clinical medicine"!

REVIEW ARTICLE

Article received on March 2, 2016 and accepted for publishing on May 21, 2016.

MRI fusion biopsy vs transrectal ultrasound guided biopsy – A literature review

Constantin A. Rădulescu[1], Mihai F. Rădulescu[2], Gina A. Ciucă[1], Dan Mischianu[1,3]

Abstract: *Introduction: Current diagnosis of prostate cancer is based on transrectal ultrasound guided random biopsies. These procedures alongside with the discovery of PSA lead to a tremendous improvement of prostate cancer diagnosis rate in the last decades. Random ultrasound guided biopsy has its minuses and several attempts were made to improve the detection rate of the disease. MRI-US fusion targeted biopsies is one of them.*
Aim: This paper is a literature review of several important European studies and tries to draw a conclusion for the usefulness of MRI-US image fusion prostate biopsy in improving prostate cancer detection
Material and methods: We have analyzed 5 papers published since 2013 which compare random ultrasound biopsies with MRI-US fusion. The analyzed parameters were clinical significant cancer detected as described by the study and any cancer detected
Results: All 5 papers show superiority in cancer detection rates for both clinically significant and any cancer.
Conclusions: MRI-US is a useful tool for improving detection rate of clinically significant prostate cancer.

INTRODUCTION

Prostate cancer is a serious public health matter, being the first cancer in men in terms of incidence and the second cause of cancer death in the U.S. Cancer, in general is taught to surpass in the following year cardiovascular diseases as the leading cause of mortality. Prostate cancer, in particular is becoming more and more important due population aging. [1]

While throughout the history prostate cancer was poorly understood, with few cases recorded due to the low life expectancy, during the XXth century groundbreaking discoveries were made in understanding, diagnosing and treating the disease.

Early diagnosis methods included open transperineal biopsies, blind transperineal needle biopsy and finger guided transrectal biopsy. First attempts to use transrectal ultrasound for prostate imaging were made by Takahasi and Ouchi in 1963 but they obtained poor images [2]. Watanabe in 1968 performed first clinical useful TRUS. In 1989 Hodge and al. started the modern era of standard sextant

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transrectal ultrasound guided needle biopsy. Stamey developed a technique which consisted in performing a biopsy without knowing the tumor location within the prostate. Stamey's method was a major advance over older methods in which biopsy needles were guided only by the examining finger.(2) During the late 1990's and early 2000's standard protocol was modified introducing extended and saturation biopsies. Nowadays it is estimated that several million biopsies are performed each year around the globe.

Ultrasound guided transrectal biopsies have 2 major disadvantages: detection of focal carcinomas with little or no clinical significance on one side and the important percentage of falsely negative biopsy cores.

MRI was invented in 1971 by Paul C. Lauterbur for which he was granted Nobel Prize for Medicine in 2003. MRI evolved as being the most important tool for prostate cancer staging, especially if performed in a multiparametric manner.

Prostate biopsies can be performed directly under IRM guidance or images may fused either in a cognitive manner, a method dependent on operator's experience, or MRI-US image fusion may be used. When using US-MRI fusion, standard transrectal ultrasound is performed and software is utilized to fuse the ultrasound images, at the time of the examination, with IRM images that were obtained before.

3D images of the prostate are obtained [3]. Fusion devices use a variety of technologies to perform image fusion. There are devices which perform robotic tracking via a mechanical arm with built-in encoders with commercial products: Artemis and BioJet. Electromagnetic tracking is used by the following UroNav and Hi-RVS. A 3D US probe is used for tracking and fusing images by Urostation. [4]

MATERIALS AND METHODS

We have analyzed 5 major studies which were performed since 2012 in terms of number of subjects, medium PSA level, overall cancer detection and clinically significant cancer detection rates. All 5 studies analyzed were paired cohort studies which compared transrectal standard biopsy with MRI-US fusion biopsy. The population analyzed was heterogenous. The only thing that was common to all patients was high PSA levels. One study, (Sonn GA et al, 2014)[5] had only patients with prior negative biopsy while the other 4 studies had mixed patients with or without prior negative biopsies. The percentage between patient with and without negative biopsies varied.

The number of patients varied between 30 (Fiard et al, 2013) [6] and 347 (Kuru et al, 2013) [7] with a total of 677 patients. Medium PSA recorded in each study varied between 6.3 ng/ml (Fiard et al, 2013) and 9.85 ng/ml (Kuru et al, 2013).

Clinically significant prostate cancer was defined by each study. Sonn et al defined clinical significance as Cancer positive cores with length 4 mm or more or Gleason 3+4.

Kuru et al used the NCCN (National Comprehensive Cancer Network) criteria. Fiard et al and Rud[9] et al considered clinical significance when Gleason 7 (3+4) or higher was recorded on biopsy cores while Rastinehad et al[8] used Epstein criteria for clinical insignificant tumors: clinical stage < T1c; PSA density < 0.15 ng/ml; fewer than 3 positive cores and less than 50% cancer on positive cores.

Clinically significant disease, defined as above, was diagnosed by means of transrectal ultrasound guided biopsy in 4.8% (Rud et al,2012) to 38% cases (Kuru et al, 2013) with a mean value of 25.26% and by means of MRI-US fusion biopsy in 21.7% (Son et al, 2014) to 50% cases (Rud et al, 2012) with a mean value of 40,76%.

The difference in diagnosing clinically significant disease varied between 7% to 41.4%. MRI-US fusion biopsy was more accurate in diagnosing clinically significant prostate cancer compared to US transrectal biopsy with an overall advance of 17.36%.

When analyzing all types of cancer both clinically significant and insignificant 4 out 5 studies showed superiority of MRI-US fusion biopsy ranging from 0.2% to 53.2%. Sonn GA et al reported 27.5% for

TRUS biopsy vs 23.7 % for MRI-US fusion. This difference in favor of TRUS is probably linked to the study design as it is the only cited study that enrolled only patients that had undergone a prior negative TRUS biopsy.

Table1: Prostate cancer both clinically significant and any cancer

Paper	Clinically significant TRUS	Clinically significant MRI-US fusion	Any cancer TRUS	Any cancer MRI-US fusion
Sonn GA et al	14,7%	21,7%	27,5%	23,7%
Kuru et al	41,1%	50,4%	50,4%	50,6%
Fiard et al	33,3%	50%	43%	55%
Rastinehad et al	32,4%	44,8%	48,6%	50,5%
Rud et al	4,8%	46,2%	14,3%	67,5%
Medium values	25,26%	42,62%	36,76%	49,46%

DISCUSSIONS

All studies show superiority for detecting clinically significant disease with a smaller number of biopsy cores for MRI-US fusion biopsy and all but one showed overall superiority in detection of prostate cancer.

The disadvantages of this review are the fact that there isn’t a standardized definition of clinical significance, the lack of homogeneity of the analyzed population, the fact that some studies have enrolled both patients with prior biopsies and patients without biopsies, and the lack of standardized protocol for MRI-US fusion biopsy as there is a standard protocol for TRUS biopsy.

CONCLUSIONS

MRI-US fusion biopsy has proven to be more accurate in detecting clinically significant cancers but is still long way from becoming a standard procedure because of the complex technique required, the financial burden caused by the need of a prior MRI.

MRI is capable of accurately diagnosing prostate cancer, in assessing its extension, its aggressiveness. MRI is also a useful tool for following patients with prior diagnosis of prostate cancer and probably, in the future it will be the major tool for focal therapy of the tumor.

Transrectal ultrasonography still holds the crown for prostate cancer detection and will certainly remain the most used guidance technique for prostatic biopsies for long time with good results in the general population. Patients with negative biopsies and who experience a continuing rise in PSA levels, patients at risk when talking of hereditary antecedents of prostate cancer and some other risk categories may and will certainly benefit from the development of the MRI fusion technique.

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REVIEW ARTICLE

Article received on March 5, 2016 and accepted for publishing on May 16, 2016.

The role of contrast-enhanced ultrasound in risk assessment of carotid atheroma

Silviu Stanciu[1], Cristina Enciu[1], Ioana Răduţă[1], Dan Stoicescu[1], Adrian Anghel[1], Daniela Anghel[1], B. Olan[1], Lucian Ciobîcă[1]

Abstract: *Background and objective: Contrast-enhanced ultrasound, used to assess atherosclerotic carotid plaques, improves visualization of vessel wall irregularities and depicts intraplaque neovascularization. This article illustrates the use of contrast-enhanced ultrasound in the risk assessment of carotid atherosclerotic lesions, especially in challenging plaques evaluation.*

Materials and methods: For 23 patients with difficult duplex ultrasound examination due to carotid tortuosity or calcifications we assessed plaque morphology (contour, echogenicity and stenosis degree) using contrast substance (Sonovue, Braco) with dedicated vascular low mechanical index CPC software.

Conclusion: Contrast-enhanced ultrasound is a new, noninvasive, and safe procedure for imaging carotid atherosclerotic lesions. It is a valuable tool for evaluating the vulnerable plaque at risk for rupture and for the diagnostic of the development and severity of systemic atherosclerotic disease.

INTRODUCTION

Carotid atheromatous pathology is responsible for almost 1/3 of strokes through 3 basic mechanisms: atherothrombosis, distal embolism from plaque origin and hemodynamic effect in the case of significant, severe stenosis (1).

Carotid ultrasound technique remains the benchmark for the assessment of carotid-vertebral arterial axis, mainly regarding the presence of obstructive atherosclerotic plaques with a proper evaluation of morphology and stenosis degree, by combining the two methods of examination: Duplex mode-B mode and Doppler mode (Color and Spectral).

Numerous studies have shown the beneficial role of endarterectomy or stenting in stroke rate reduction, in symptomatic and asymptomatic patients with significant stenosis of over 70%, but there is no clear attitude regarding the therapeutic approach of asymptomatic patients with a lower degree of stenosis. In these patients, in whom stenosis has no cerebral hemodynamic effect, several studies emphasize on plaque morphology, by identifying unstable plaques which would play an important role in the development of symptoms (2).

Often, we encounter in daily clinical practice cases where carotid axe imaging becomes challenging due to intense calcifications in the carotid bulb and origin, due to the presence of carotid tortuosity or because of the impossibility to distinguish between

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preoclusive (over 90%) stenosis and complete occlusion, which would change the therapeutic approach (3,4).

To overcome these limitations and better characterize unstable plaques, by highlighting the intraplaque neoangiogenesis (5), recently a new imaging technique has been developed using contrast substance, which has the advantage of strictly vascular and plaque and wall vascularization distribution.

MATERIALS AND METHODS

During the last year, for 23 patients with difficult duplex ultrasound exams due to carotid tortuosity or calcifications we performed a contrast enhanced evaluation in order to assess plaque vascularization and stenosis degree.

In consequence, the protocol for plaque morphology description (contour, echogenicity and stenosis degree) consists in 1 ml bolus of contrast substance (Sonovue, Braco) administered through a catheter in the right arm, followed by 10 ml bolus of saline with the acquisition of 6 seconds clips during 4-5 minutes, maneuver which can be repeated to achieve the objectives of the study.

In our department we currently use dedicated vascular CPC software with low mechanical index, integrated into a SIEMENS Sequoia 512 CV system.

In each case we observed the contrast substance plaque penetration, carefully identifying and assessing intraplaque vascular pattern.

Detecting intraplaque angiogenesis

In the majority of patients (68%) with echogenic plaques and no significant stenosis (lower than 70%) intraplaque vascularization has been found, with outside to inside filling pattern, data similar to literature findings (see images).

The existence of this new angiogenesis vessels, mainly situated at the base of atheromatosic plaques, can be considered a risk zone for possible plaque rupture, resulting in major stroke.

Figure 1: Transverse cross section right after left CCA bifurcation, 30 seconds after contrast substance has been administered. Note the lack of opacification of left ICA due to the presence of atheromatous plaque responsible for a 60-70% stenosis.

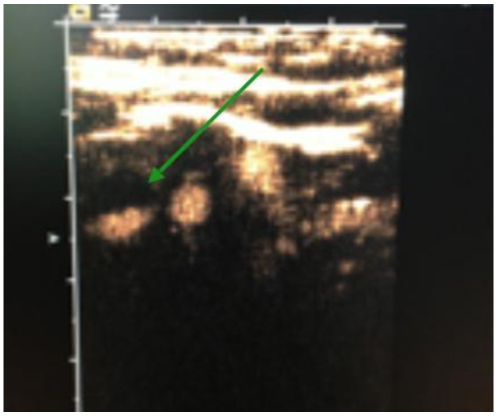

Figure 2: Longitudinal section 40 sec after the injection allowing plaque visualization at the origin of the left internal carotid artery with contrast enhancement at the base (arrow)

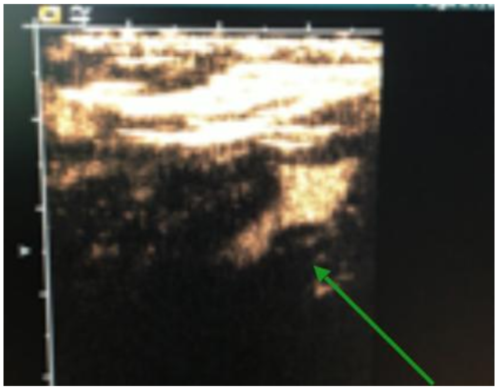

Figure 3: Same plaque from figure 2 at 60 seconds after injection

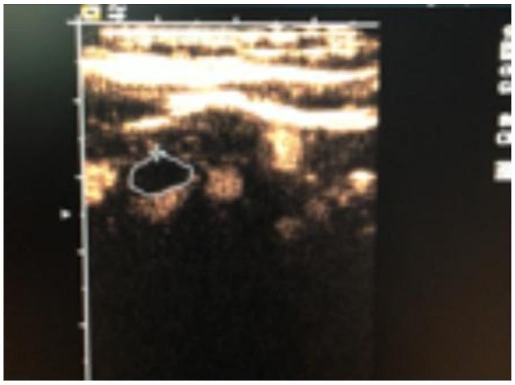

Figure 4: Contrast enhancements at 70 seconds with a filling patter from the outside to inside

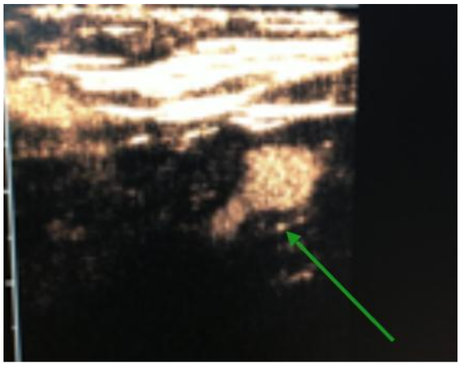

In consequence, identifying intraplaque neovascularization in patients with no significant carotid stenosis can be considered a noteworthy additional argument when considering an interventional procedure (stenting or endarterectomy) for stroke prevention.

DISCUSSION AND CONCLUSIONS

The concept of unstable or vulnerable plaque, similar to that seen in coronary pathology, is based upon identifying dangerous plaques with immediate stroke like complications, with acute inflammation and angiogenesis (which precede and cause plaque rupture or ulceration), that can't always be detected through conventional duplex ultrasound.

Carotid ultrasound remains the leading non-invasive exploration regarding pre-op/pre-interventional or post-op/post interventional functional and anatomic evaluation. Contrast-enhanced ultrasound studies, bring additional information regarding carotid stenosis quantification in difficult evaluation situations (calcified plaques or difficult to explore vascular tortuosity) but especially brings functional information regarding intraplaque vascularization, thus identifying unstable and high stroke risk plaques allowing an appropriate therapeutic approach.

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REVIEW ARTICLE

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The importance of life quality questionnaire in patients with prostate cancer, pre- and post-radical prostatectomy

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Abstract: *This study analyzes the satisfaction of the patients with radical prostatectomy according to the signs and symptoms included in the EORTC QLQ – C30 and EORTC QLQ – PR25 questionnaires, including their relation with the health state that influences the pre and postoperative life quality.*

Fifty patients with prostate cancer in a localized stage were studied and analyzed in a prospective manner and for a period of 12 months, before and 6 months after the radical prostatectomy. In choosing the patients, the common denominator was the use of the same postoperative plan and the lack of postoperative complications, as well as the lack of chronic diseases.

Erectile dysfunction, urine incontinence were quite frequent after radical prostatectomy, while the urinary obstruction and the weak urinary stream were less frequent. In this regard, people noticed a significant decrease of life quality.

Although the study addressed a small group of patients, the results are similar to those belonging to other clinical studies. Even if the aspect of life quality is more rarely taken into account, the questionnaires regarding the life quality are specific, useful and quite cheap in evaluating different therapies.

Keywords: *life quality, prostate cancer, questionnaire*

INTRODUCTION

Prostate cancer represents a disease with increased prevalence, being the second cause of death in U.S.A, on the first place being the lung cancer. However, the prostate cancer shows optimistic survival rates, the rate of the deaths caused by the prostate cancer decreased in the U.S.A, from the beginning of 2007 with 3.2%[4]. This fact led to the appearance of a model of patients with prostate cancer, patients for whom the quality of life is important, as well as the impact of the disease on this.[5].

For those who study the clinical problem, the aim of evaluating life at the patients with prostate cancer consists in evaluating the effects of the treatment on the symptoms and in identifying the negative impact of the disease on the life of the patient.

The quality of life is a concept that has started to be studied and taken into account in 1946 when in the

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Preamble of the Constitution of World Health Organization, health was defined as it follows: ,,*Health is the state of complete physical, mental and social wellbeing that does not reduce at the absence of a disease or of an infirmity"[1,2].*

Smith and his contributors consider the notions of life quality and the health as being two defined concepts, the life quality being related even more to the mental health, while the state of health hints at the physical function [3].

Meaning ,,a holistic auto evaluation of satisfactions regarding the individual", the life quality is a much – disputed concept, multidimensional that, though seems quite easy to understand it, is difficult to be defined.

In a more restrictive meaning is used the term of life quality regarding health ,,health related quality of life" (HRQL),to define the influence of health and nursing on the life quality, being influenced by numerous factors like: financial state, habitat, the work place, the spirituality, the social support and the health of the individual [2].

The evaluation of the aspect regarding the quality of life in patients with prostate cancer is done at a large scale in the clinical studies, but it must be underlined the importance of using these evaluation methods in clinical practice [1].

The questionnaire, a descriptive instrument, is the one through which the evaluation is done in a subjective manner a wide range of the disease effects that are, later, resumed into a final score.

The evaluating instruments of the quality of life come to complete the other methods of investigation of the patient with prostate cancer, for this very reason it would be opportune to facilitate the use of these by the people who study clinical problems, the subsequent therapeutic conduct being able to improve the quality of life for these patients.

The most used questionnaires in the clinical practice in evaluating the patients with prostate cancer are the following: EORTC QLQ-PR25 and EORTC C-30 questionnaires.

The quality of life as a medical concept explains the effects of diseases and those of the therapy on the stage of comfort for the patient that is the subject of evaluation with the help of the questionnaires.

The questionnaires can be completed by the medical personnel, the patient being asked the questions after having read and signed the informed consent or directly by the patient.

The first method has the advantage that the doctor can explain the patient the questions more accurately, diminishing the misunderstandings, but the result cannot have a maximum accuracy as not all the patients can be very honest while facing other persons.

MATERIAL AND METHOD

Fifty patients with prostate cancer in a localized stage were studied prospectively for 12 months, before and at 6 months after the radical prostatectomy between January 2015 – December 2015, at the Urology Clinic of the Carol Davila Central Military Hospital.

In choosing the patients, the common denominator was the use of the same postoperative plan and the lack of postoperative complications, as well as the lack of chronic diseases.

The average age of the patients included in study was of 65.

The patients were asked to fill in the EORTC C-30 and EORTC QLQ-PR25 in Romanian language, after having read and signed the informed consent.

The characteristics of the group of patients were recorded and processed in a SPSS database (version 22 – Chicago k, USA).

The EORTEC C – 30 questionnaire has 30 questions that shows us a pre and postoperative image of the patient, regarding the cognitive, emotional, social functions or regarding the signs and symptoms of the patients, using a scale with more items or singular ones.

The questionnaire, excepting the two last items, can be completed with values between 1 and 4, so,(A) the absolute amplitude of the variation of answer is 3. The scale of life quality is given by the answers at the questions 29 and 30 at which the patients can choose values from 1 to 7, the absolute amplitude being of 6.

Items	Number of items	Number of question
Physical state	5	1 - 5
Daily activities	2	6, 7
Emotional states	4	21 - 24
Cognitive functions	2	20, 25
Social and family relations	2	26, 27
Tiredness	3	10, 12, 18
Nausea and Vomiting	2	14, 15
Pains	2	9, 19
Dyspnoea	1	8
Insomnia	1	11
Anorexia	1	13
Constipation	1	16
Diarrhea	1	17
Financial problems	1	28
General health state	1	29
Life Quality	1	30

To analyze the questionnaires I used a different formula for functions, namely: Value _functions = 1 – ((Raw score – 1)/Absolute Amplitude)*100 comparing to Value _ symptoms = Raw score -1/Absolute Amplitude)*100.

As a result, I noticed that a higher value of the functions shows a higher rate of functionality and independency, while a higher value of the symptoms shows problems of dependency of the patient.

One can notice in the chart that, only emotionally, the patient feels better after 6 months after surgery. I must mention that the younger patients (<65 years old), consider that, physically and socially speaking, they were better during the preoperative period.

The indicator of the physical state is made up of 5 items: the resistance of hard activities, independency in dressing and eating, as well as the chronicle tiredness and the need of laying in bed. From the questionnaire, one can see that the physical resistance of the patient decreased from 94.11 to 90.23.

Figure 1: Score of life quality regarding functions

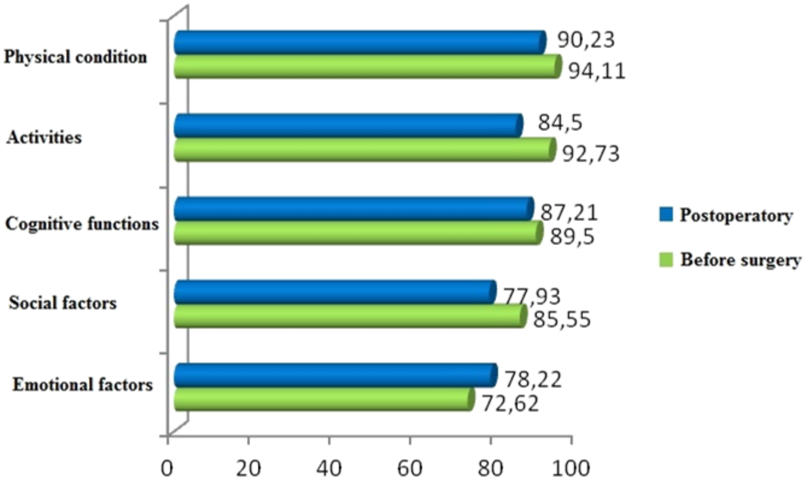

One can see an increase of tiredness after prostatectomy, from 11.1 to 16.2 and, also in the symptoms of nausea and vomiting.

The postoperative pain had a significant increase from 10.33 to 12.22.

The tiredness increases proportional with insomnia and the sensation of weakness, and the pain interferes with the daily activities especially at the patients of more than 65 years old.

Figure 2: Score of signs and symptoms

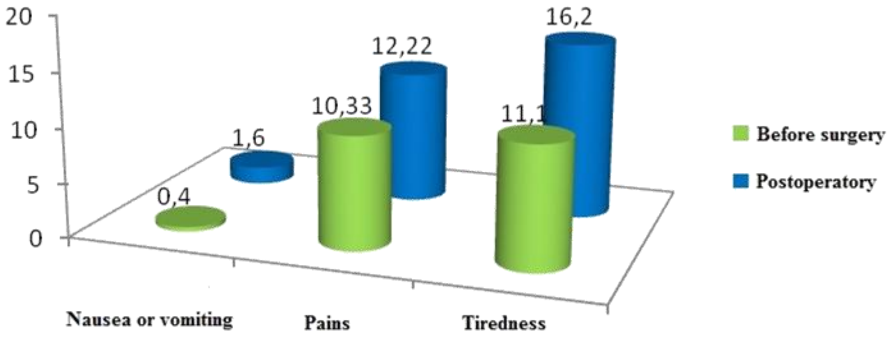

Figure 3: Signs and symptoms

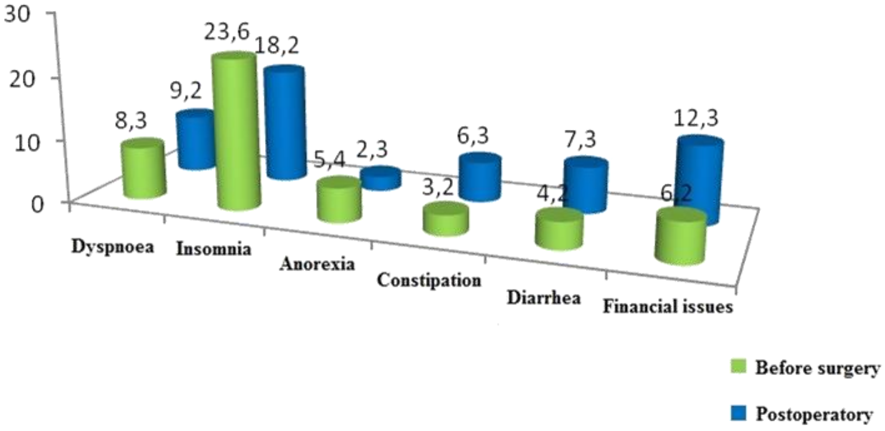

Figure 4: Quality of life

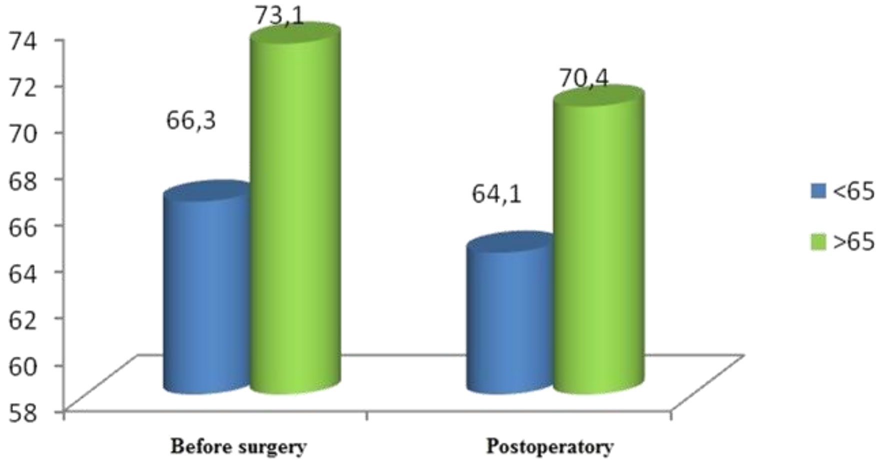

One can see an improvement of the quality of life of the postoperative patient regarding insomnia and anorexia. The digestive symptoms and the financial problems will accentuate postoperatively especially at the patients after 65 years old and the younger ones.

The last items are like a conclusion of the questionnaire permitting the patient to define the last week by the point of view of his health and the manner he sees the quality of his life at a certain time.

Analyzing the data, one can see the most unsatisfied patients on the ground of their physical state and quality of life are the patients younger than 65 years old. Totally, the difference between the quality of life appreciated by people younger than 65 years old pre and postoperative was of 2.2.

Younger patients appreciated the quality of life pre and postoperative lower than the patients of 65 years old.

The EORTC OLO – PR 25 questionnaire contains 25 questions divided into four evaluation functional scales.

Items	Number of items	Number of question
Urinary symptoms scale	9	31-39
Intestinal symptoms scale	4	40-43
Scale regarding the symptoms owing the treatment	6	44-49
Sexual functions scale	6	50-55

The significant differences were regarding the urinary symptoms scale, postoperative 13.86 comparing to preoperative of 7.88. In accordance to the age, there were differences regarding urinary symptoms scale, those of 65 years old had lower scores, at an average of 9.86.

Regarding the sexual functions scale, those younger than 65 had higher scores, at an average of 10.11 units.

The erectile dysfunction appears in a degree of 67% at different levels, while 34.9% shows no affliction of the erectile function.

In the postoperative group 23% presented with mild incontinence while severe and moderate incontinence was present in 4, 7% of the patients.

Postoperatively the frequency of the urinary incontinence was greater in patients older than 65 than those younger than 65.

CONCLUSIONS

To identify the type of treatment is vital for the success of the oncologic therapy of the prostate cancer, though the complications appeared after a radical prostatectomy influences the life quality of the patient. One of the objectives in carrying about patients is in helping at stabilization of the urinary continence and of sexual function, in improving the life quality of the patient. A more critical evaluation of the type of treatment is essential for developing an efficient strategy.

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SYSTEMATIC REVIEW

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Tumor markers in gastroenterology: useful or useless

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INTRODUCTION

Tumor markers are biomarkers that can be found in blood, urine or different body tissues, produced either by cancer cells or normal cells in response to cancer or in different noncancerous conditions (gastrointestinal stromal tumors). Their use varies from cancer screening on a population basis, to staging different tumors or to evaluate the efficiency of the treatment. Most frequently used in gastrointestinal malignancies are: carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9 and α-fetoprotein. However there are many markers that can be useful in dealing with gastrointestinal cancer such as: carbohydrate antigen 125, cyfra 21-1, chromogranin, tumor M2-PK, neuron-specific enolase, smooth muscle actin [1]. Future research show the utility of multiple tumor marker test, when by combining more tests in parallel the predictive value might be increased. For example: when talking about pancreatic or stomach cancer one might combine: CEA, CA19-9 and CA 72-4 [1,2].

ALPHA FETOPROTEIN

So far, alpha fetoprotein is the most studied serological marker in the surveillance of hepatocellular carcinoma, derived from the liver cells and yolk sac [3]. Its' use is debatable. Some studies show that a cut-off of 20 ng/mL carries a sensitivity of 41% to 65% and specificity between 80% and 94%, with a positive and negative predictive value of 25 and 98 percent. Even though The American Association for the Study of Liver Disease (AASLD) guidelines shows that cirrhosis is a possible cause of elevated AFP, its sensitivity (60%) is higher than that of ultrasound (58%). Therefore current data from the literature support the use of AFP combined with ultrasound as the surveillance tests of choice, taken every 6 months, leading to early detection of hepatocellular carcinoma (HCC) [4]. Despite the fact that more than 40% of the patients with HCC have normal AFP, serum levels higher than 500 mcg/L in a high-risk patient is diagnostic of HCC [3,5].

There is a demonstrated correlation between the tumor size and serologic AFP: higher levels with a sudden acceleration appear with HCC that measure more than 3 cm [6]. Moreover serum levels from 1,000 to 10,000 mcg/L are encountered in tumors past 5 cm in diameter. Date from the literature associate high levels of AFP with poorly-differentiated tumors, but further studies are required [7].

False positive values of AFP may be related to flares of activity in chronic viral hepatitis (B or C). Therefore the use of this serologic marker is very important in patients with hepatitis B or C during treatment with nucleotide analogues for hepatitis B or drug acting agents (interferon free) for hepatitis C, when we cannot encounter flares of transaminases [9].

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AFP remains an important prognostic marker in patients after resection or in those who are taken into consideration for transplant. High values over 1,000 mcg/L (believed a surrogate of vascular invasion) associates extremely high risks of recurrent disease after transplantation. Moreover some studies assign this value as an exclusion criterion for liver transplantation in patients with HCC that meet the Milan criteria, in order to improve posttransplant outcome [10]. An assay of serum AFT post-transplant is in order every 3 months for 2 years if initially elevated, then every 6 months. This serologic marker remains an independent prognostic predictor of outcome after ortotopic liver transplant for HCC [11].

Table 1: Causes of elevated serum alpha-fetoprotein [8]

Causes of elevated serum AFP

- Hepatocellular carcinoma
- Cirhossis due to hepatitits B/C
- Non-seminomatous germline tumours
- Ataxia-teleangiectasia
- Pregnancy with babies that carry neural tube defects or Down syndrome
- Other gastrointestinal tumours (gastric cancer, biliary tract cancer, pancreatic cancer)

CARCINOEMBRYONIC ANTIGEN

Discovered in 1965, CEA is a glycoprotein associated with colorectal cancer with a rather low sensitivity and specificity. Even though CEA is not used as a diagnostic test, levels over 5.0 ng/mL can predict a unfavorable prognosis, regardless the tumor stage [12].

A Cochrane review showed that a cut-off value of 2.5mcg/L for CEA carries a pooled sensitivity of 82 percent and specificity of 80 percent. For a cut-off of 10 mcg/L the sensitivity lowered to 68%, but the specificity reached 97 percent, due to fewer false positive values. CEA testing should be done once every 3 to 6 months. One must bear in mind the possible false positive values of CEA (most encountered between 5 and 10 ng/mL). One possible cause is due to the adjuvant therapy with fluorouracyl (therefore some recommend the end of adjuvant treatment before measuring CEA).

There are many arguments against using CEA as a screening test such as: almost half of the recurrences of CRC present normal values of CEA; is has no impact on improving survival and the quality of life and it is not cost-effective. One strong argument in favor of testing CEA is the detection of recurrences [13].

Even though CEA is prohibited in the mass screening and diagnostic pathway of colorectal carcinoma (CRC), it has value in the follow-up of patients with diagnosed CRS according to the American Society of Clinical Oncology guidelines: from surgical treatment planning to post-treatment follow-up and prognosis [14,15]. High serum levels of CEA after 4 weeks of the surgery (this being twice the plasma half-life of CEA) indicate the persistence of the disease, occult metastasis and the need to reevaluate the prognosis

[8]. In conclusion, CEA levels can be associated to the TNM classification of CRC, due not only to its prognostic value, but also the decision of adjuvant chemotherapy.

Table 2: Benign and malignant causes of high levels of seric CEA[8,13]

BENIGN	MALIGNANT
Gastritis	Colorectal cancer
Peptic ulcer disease	Breast cancer
Diverticulitis	Lung cancer
Pancreatitis	Gastric cancer
Liver disease	Pancreatic cancer
Chronic obstructive pulmonary disease	Cholangiocarcinoma
Diabetes	
Cigarette smokers	

Studies showed that high CEA even in node negative patients show a worse prognosis, a higher rate of recurrence, therefore adjuvant chemotherapy could be considered [16].

CEA can be used to monitor the response to treatment in metastatic disease. Whilst the decrease of seric levels of CEA shows the favorable response to the treatment, the rising level of CEA is incompatible with tumor regression [8].

Some studies show that bile CEA levels are elevated in cholangiocarcinoma, but not in benign diseases. Used as a single tumor marker, CEA is neither sensitive enough, nor specific enough to diagnose cholangiocarcinoma [8]. Different combinations of CEA and CA 19-9 are used to diagnose cholangiocarcinoma in patients with primary sclerosing cholangitis.

When using cut-off values of 5.2ng/mL for CEA and 180 U/mL for CA 19-9 the sensitivity was 100% and the specificity reached 78%. Despite this, other data from the literature show a sensitivity of 63% for detecting cholangiocarcinoma in patients with PSC, whilst the specificity when using a combined CA 19-9 and CEA only reaches 33%, albeit with higher specificity [13,17].

CARBOHYDRATE ANTIGEN 19-9

Most frequently used serological marker for cholangiocarcinoma, CA 19-9 has a wide variation in sensitivity and specificity. However, values over 1000 IU/mL are encountered in advanced disease with peritoneal dissemination [18].

CA 19-9 levels can be elevated in 45% of patients with non-malignant affliction since the tumor marker is produced by abnormal cyst epithelium. Benign diseases that can be associated with high values of CA 19-9 are: cholestasis, cirrhosis and cholestatic diseases [18,19].

In patients with primary biliary cholangitis CA 19-9 levels can be increased due to cholangitis or biliary obstruction. Literature states that a cut-off value of 100 IU has a 86% sensitivity and a 89% specificity for cholangiocarcinoma [4]. However CA 19-9 remains the most commonly used serological marker in cholangiocarcinoma with a sensitivity of 89% and a specificity of 86% for a cut-off over 100 IU/mL.

Moreover values over 1,000 IU/mL are associated with unresecability [17].

CA 19-9 has a higher specificity in the diagnosis of cholangiocarcinoma in patients with PBS. Therefore CA 19-9 combined with ultrasound or magnetic resonance cholangiopancreatography (MRCP) are recommended in the annual screening of PBS patients for cholangiocarcinoma [17,20].

Studies shows that the median preoperative CA 19-9 levels in pancreatic adenocarcinoma were 379 IU/mL in patients who would not benefit a curative resection, whilst in patients where it was resected it was 130 IU/mL.

Moreover, values over 130 U/mL postoperative demonstrate unresectability, the likelihood of R0 resection and long-term outcomes [4]. CA 19-9 has a low specificity; therefore studies show that it should not be used as a screening test for pancreatic cancer. However serial testing once every one to three months of this tumor serological marker in the follow-up of patients after curative surgery or in patients with advanced disease that receive chemotherapy was proven useful. Moreover, elevated values of CA 19-9 could precede the radiological images of recurrent disease (must be confirmed with imaging studies or biopsy) [21].

Table 3: Malignant causes of high values of CA 19-9 [18]

- Hepatocellular carcinoma
- Biliary cancer (gallbladder, cholangiocarcinoma, Vater ampullary cancer)
- Pancreatic exocrine or neuro-endocrine cancers
- Gastric, ovarian or colorectal cancer (less frequent)
- Lung, breast,uterine (rare)

Table 4: Cut-off values for CA 19-9 related to the presence of cholangitis (may associate fever, right upper quadrant pain and leukocytosis) or cholestasis (serum bilirubin larger than 3mg/dL) [18]

cut-off ≥ 37 IU/mL

- NO cholestasis
- NO cholangitis

cut-off ≥ 300 IU/mL

- cholestasis
- cholangitis

In patients that underwent curative surgery, the normalization of the values of CA 19-9 within 6 months is associated with twice the mean of survival than in patients that kept elevated CA 19-9 [17].

In individuals with negative Lewis phenotype (7% of the population), CA19-9 is unreliable since the tumor marker needs the expression of Lewis blood group antigen, leading to a false negative response [21].

Tumor markers could be used to differentiate chronic pancreatitis and pancreatic cancer. CA 19-9 was once used for this purpose, however due to its' low specificity and sensitivity this purpose was forgotten. Despite the fact that CA 19-9 is not used for surveillance in chronic pancreatitis, it can be helpful in families with hereditary pancreatitis [22].

OTHER TUMOR MARKERS

Cancer Antigen 125 (CA 125) is a glycoprotein most frequently used in the diagnosis, screening and prognosis of ovarian cancer, but with a very low sensitivity and specificity especially in early disease. It can have elevated values in non-gynecologic cancers such as: colon, liver, gallbladder cancer [4,8].

Carbohydrate Antigen 72.4 (CA 72.4) is a serological tumor marker used as a specific predictor for clinical recurrence in patients with gastric and colorectal cancer that undergo surgery and to follow up the treatment [23,24]. CA 72.4 with values higher than 7 IU/mL postoperative are associated with a worse prognosis, higher risk of recurrence and higher death risk. Studies show that in stage II colorectal cancer this tumor marker discriminates better than CEA the patients that will relapse or die from those with a favorable prognosis [25,26].

Chromogranin A is a protein contained by the neuroendocrine tumor cells. Elevated values can be encountered in a number of benign and malignant diseases. When referring to gastrointestinal disease we can find high values of chromogranin A in: carcinoid tumors of the gastrointestinal tract, pancreatic neuroendocrine tumors, colon cancer, hepatocellular carcinoma, pancreatic adenocarcinoma, liver cirrhosis, chronic atrophic gastritis, inflammatory bowel disease, and others. Chromogranin A has been associated with treatment response and might have prognostic value in neuroendocrine tumors [4,27].

CONCLUSION

Tumor markers remain a helpful tool in different afflictions. *AFP* is now considered the surveillance test of choice along with ultrasound in hepatocellular carcinoma. *CEA* is not used in the diagnosis of colorectal cancer, but in the prognosis and follow-up after curative surgery. *CA 19-9* is especially useful in the diagnosis pathway of cholangiocarcinoma in patients with PBS. Moreover, values over 1000 IU/ml are highly suggestive for cancer.

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ORIGINAL ARTICLES

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Air MEDEVAC in case of multiple casualties – The experience of civilian-military cooperation in RoAF

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Abstract: *Introduction: Starting September 2010 in Romania was created the Military Emergency Medical Service (SMMU) by the Ministry of National Defense, which has as main mission to provide first aid and save the lives of military personnel during military operations using special equipped MEDEAVC aircraft. Nationwide exist the national emergency system which operates thru 112- SMURD acting in support of the civilian population. In case of accidents with multiple victims the experience has shown the need for collaboration between the two systems, in order to save lives. In the last 5 years there has been an increasing Airlift missions (MEDEVAC) with multiple victims executed by joint civil-military medical teams using military aircraft.*

Material and methods. This paper provides a review of the most important aspects of particularities, advantages and disadvantages of this type of medical transport using the MEDEVAC missions based study carried out by the Air Force in recent years.

Results and conclusions. Performing these tasks presents challenges to mission planning, use of medical equipment and procedures, command-control system, exercise programs jointly joint medical teams and, of course, managing a large number of patients in flight.

The large number of patients transported safely and in the shortest time, regardless of weather conditions recommends this type of medical intervention.

Given the Romanian military presence in various theaters and that NATO strategic medical evacuation is a national responsibility, the capacity of air transport in case multiple casualties is a priority.

Keywords: *RoAF, flight surgeon, in flight medical care, multiple casualty, interoperability, cooperation, joint civil-military medical teams*

INTRODUCTION

Starting September 2010 in Romania the Military Emergency Medical Service (SMMU) was created by the Ministry of National Defense, which has as main mission to provide first aid and save the lives of military personnel during military operations using special equipped MEDEVAC aircraft. Nationwide exist the national emergency system which operates thru 112 emergency number – SMURD (Serviciul Mobil de Urgență, Reanimare și Descarcerare; Mobile Emergency Service for Resuscitation and Extrication) acting in support of the civilian population. In case of accidents with multiple victims the experience has shown the need for collaboration between the two systems, in order to save lives. In the last 5 years there has been an increasing Aeromedical evacuation missions (AME - MEDEVAC) with multiple victims

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Given the Romanian military presence in various theaters and that NATO strategic medical evacuation is a national responsibility, the capacity of air transport in case of multiple causality is a priority.

In this modern time beside the classical war conflict we are facing more and more new challenges like mass causality due to terrorist attacks, car accidents, fire and explosions in our civilian day by day life.

Due to financial and personnel restraints, neither one of the 2 system, civilian or military one, can properly face by itself this kind of incidents and it's just a question of time until the number of patients will overwhelm the capability and capacity of each system.

In Romania 2 systems coexist for Aeromedical Evacuation: the civilian SMURD (Mobile Emergency Service for Resuscitation and Extrication) using 8 EC135 helicopter and 1 Piper PA-42 Cheyenne III airplane and the military system under de MoD/RoAF command with 6 IAR 330 Puma helicopters and 1 C27J Spartan airplane. The drawback of the civilian system is that there aircraft don't fly at night and in severe meteorological condition. In the military one we don't have enough emergency medical personnel to cover 24/7. So this is the basis on which we start cooperation.

Due to the fact that national evacuation it's a civilian responsibility the military AME assets may be used to augment civil capability in 2 ways: Military aircraft assets with civilian medical crew and Military aircraft assets with mixed civilian-military medical crew.

Military aircraft used in this type of missions are:

● IAR 330 Puma MEDEVAC helicopter: fully equipped as an intensive care unit can carry up to 3 critically ill patient, in all-weather condition day or night with a range of 500 km;

● C130 Hercules has a maximum capacity of 74 stretchers with a range of 2000 km and have been use for years for CASEVAC missions from Iraq and Afghanistan;

● C27 Spartan: the newest and most modern aircraft from RoAF fleet can carry maximum 24 stretchers with autonomy of 6 hours and 2500 km. The characteristic of this aircraft is the possibility of carry 2 special ICU modules with dedicated monitor, ventilator and O2 capability.

Another characteristic is the MEDEVAC pressurization mode in which the aircraft will maintain the cabin pressure at ground level until flight level 130 (4000 m) in order decrease the hypobaric stress on the patient.

The last 6 years statistics show that from a total of 53 MEDEVAC missions with RoAF aircraft 15 (28.31%) where missions with multiple patients with a total of 100 patients (72.46%). The vast majority of this missions were made with C27J Spartan (12 – 80%), 2 with C130 Hercules (13.33%) and only 1 using helicopters (6.66%). That can be explained by the fact that the IAR 330 Puma helicopters can carry only 3 stretcher patient but at this load the space is severely limited. Another factor was the meteorological condition. This type of mission with multiple patients is used on longer distances (more than 300 km) and this is why the fix wing aircraft are used more (speed and distance advantages).

Messaging and alerts: because the civilian medical system can't put on alert the RoAF assets the messaging system goes like this: when somewhere in Romania an accident is happening the initial receiving medical facility contact the secondary or tertiary medical center if the complexity of the injury require a higher level of medical treatment. If the patients or patients are accepted for transfer then the medical coordinator of SMURD send an aeromedical evacuation request to CNMC (National Command Military Centre) who pass it to RoAF COA (Air Operation Component). At this level the Aeromedical Evacuation Officer have an up-to date image of the position, availability and the response time of each aircraft. The decision is made about which type of aircraft (airplane or helicopter) shall be used and the alert message is scrambled.

The particularity of multiple air MEDEVAC mission is that because the number of patients and the diversity of pathology the necessary medical equipment and the component of the medical team on board must be carefully planned on very short notice.

The military medical crew consists normally in 1 or 2 flight surgeons (medical officer, permanent working in an air base, having 1 year course in emergency medicine, 6 month training in aviation medicine, fit to fly according with the medical standards of the National Institute of Aviation Medicine) and 2 to 4 flight nurses (3 years degree in nursing after graduating Nurse College, 6 month training in emergency medicine, fit to fly) but in case of multiple cases this crew can or must be updated with ICU specialist or neonatologist accordingly to the medical need (in June 2012 MEDEVAC mission from Bucharest, ROU to Hanover, DEU with 3 children from 7 day to 3 years old with cardiac malformations in with the crew was upgraded with 1 neonatologist and 2 nurses beside the flight surgeon and the flight nurse from RoAF).

In term of medical equipment due to the national standardization there are no compatibility problems in term of coupling power tension or O_2 pressure. The only real challenge is how to be able to get all the necessary equipment in time and in the right quantity (in July 2008 after a explosion in a Gheorghieni local wood factory we accomplished a MEDEVAC mission for 10 severely burn patients, 2nd 3nd and 4th degree burns, all respiratory assisted and mechanically ventilated so the problem was how to have 10 ventilators and 10 multifunction monitors in that plane in less than 2 hours.)

AEROMEDICAL ASPECTS

Hypoxia is the greatest threat to anyone flying. Physiological effects of hypoxemia can be detected in healthy individuals at lower altitudes of 3000 m but more rapidly in certain patients. It occurs due to changes in ambient pressure. Cabin pressurization reduce this problem especially airplanes, but patients with respiratory failure may be at risk for hypoxemia current cabin altitude achieved. Adjusting the fraction of inspired oxygen to maintain the partial pressure of inspired oxygen constant throughout the mission is a technique useful clinically for prevention of hypoxemia. The wide availability of pulseoximeter decreased incidence of hypoxemia in the MEDEVAC missions with early recognition.

Accelerations: Acceleration or deceleration is a vector quantity, having both magnitude and direction. For this reason, the correct positioning of the patient in the idea to limit acceleration-induced forces sustained should be carried out. Acceleration forces that are experienced in helicopters or planes during routine operations (due to flight techniques and procedures ensuring low range of acceleration forces during MEDEVAC missions) tend to be small in size or as great as those observed in terrestrial transport vehicles.

Volume of gas: ambient pressure decreases with increasing altitude. Pressure changes with altitude and the change can affect a number of medical devices and patients. Contrary to common belief, cabin pressurization does not eliminate the problem. Pressurization allows a comfortable flight altitude manned which could not otherwise be achieved, but in general, not altitude from sea level, and thus, the equipment and the patient will be exposed to a pressure change. Any gas enclosed in a structure therefore becomes a concern. Air trapped in the sinuses, for example, can expand and cause discomfort and equipment (air-filled cuffs) can be damaged or can injure patient with the change altitudes.

Humidity: Humidity is a particular concern in fixed-wing missions because cabin air is taken from the rarefied atmosphere outside, and even in pressurized aircraft and when heated may contain very little moisture. This can lead to dryness and discomfort of the patient secretions during flight.

Noise: Modern aircraft produce quite a lot of noise. In most aircraft cabins is pretty quiet for conversation and patient evaluation but cabins of helicopters are so loud that exclude pulmonary auscultation. Soundproofed helmets and intercom systems are needed.

Vibration: Vibration is a form of repetitive alternative motion. The two major sources of vibration during MEDEVAC missions are powerplant and turbulence of the air moving aircraft. The vibrations cause discomfort and fatigue to patients and also to medical team, and are transmitted to medical equipment in flight and can be the source of errors and monitoring failures.

CONCLUSION

The Multiple victim accidents are a REALITY and the transport of those patients to an adequate level of care facility is a NECESSITY. Air medical transport put additional stress on patients (fear of flying, physical discomfort), on medical staff (unconventional and insufficient workspace with great limitation regarding medical equipment and medication and the impossibility to receive advice and expertise) and on medical equipment (operating in a vibration, pressure and temperature differential environment). Influence appears to be greater for the transport helicopters and in case of multiple patients.

Air medical evacuation missions with multiple victims require: rigorous planning, exercises (based on realistic scenarios) and resources (time, money, personnel, equipment).

Romanian Air Force aircraft equipped MEDEVAC are on alert 24/7 and the training of medical staff allow prompt intervention in case of accidents with multiple victims;

Civil-military intervention can be a solution for those situations, on the condition of legislation harmonization in order to provide fast and efficient mobilization of resources and cooperation between military and civilian institutions.

ORIGINAL ARTICLES

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Finite element analysis of a modified short hip endoprosthesis

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Abstract: *A finite element simulation of the mechanical static features for a modified short hip endoprosthesis was performed. The corkscrew-like femoral stem was modified introducing more turns of the thread. By such an approach it is expected that for some cases the mechanical fixation of the prosthesis to the bone will be improved or the use of the cement for bonding is not necessary. Our scenario was estimated for titanium and stainless steel, and both materials show good safety factors. Mechanical stress is expected to be distributed more uniform in the bone for the new design with more turns of thread.*

Keywords: *modified short hip endoprosthesis; design; finite element analysis; titanium; stainless steel*

INTRODUCTION

The increase in the average life expectancy rose the age at which different surgical interventions are performed. For example, the total replacement of the hip is made on a typical patient of 60-80 years old, and often patients are older, in some cases even over 100 years old. The primary hip intervention can be followed by several revision surgeries [1,2,3]. This is a reason for designing modular endoprosthesis, where damaged parts can be easily replaced.

A short femoral stem is recommended for patients where the femoral bone allows a minimal resection, and the bone structure has enough mechanical resistance to support the stress induced by implant [4]. A positive aspect of our design consists in screwing the implant into the bone, avoiding its hammering [5,6] that has a high risk of femoral neck fracturing. The prosthesis has a cylindrical metallic body, with an exterior thread, continued with a truncated sleeve, and ended with a junction neck (Figure 1). Inside of the implant there is a hexagonal hole for the insertion of a torque screwdriver (not shown). The distribution holes are built-in and they are intended to be used to spread the cement for fixation after the stem is screwed in the bone.

In this work a modified design is proposed. Namely, more turns (7 instead of 3) [7] of the screw-component of the endoprosthesis are considered. Expectations are to obtain an improved implant-bone fixation and a more uniform distribution of the forces that will allow a longer lifetime with less revision surgeries: it is well known that after about 10-15 years the prosthesis loosens and a replacement may

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be required [8]. For certain cases it is thought that our improved design may also provide a cement-free fixation. Finite-element analysis is used to estimate mechanical features of the prosthesis with the modified design. Two materials, titanium and stainless steel are considered.

MATERIALS AND METHODS

1. CAD design and materials properties

The endoprosthesis was designed [9] in Inventor Professional 2016, and the materials properties were assigned from the software database.

The proposed materials for the endoprosthesis are titanium and stainless steel (Table 1). Materials are considered isotropic and linearly elastic.

2. Mesh settings

All the settings are detailed in [6]. The total number of the resulted elements was 1,694, and the total number of the nodes was 3,287.

Table 1. Mechanical properties of materials used in simulation.

Material	Density [g/cm^3]	Young's Modulus [GPa]	Poisson's ratio	Shear Modulus [GPa]	Yield strength [MPa]	Tensile strength [MPa]
Titanium	4.51	102.81	0.36	44	275.6	344.5
Stainless Steel	8.00	193.00	0.30	86	250.0	540.0

3. Boundary and loading conditions

The endoprosthesis, either the initial or the modified design, is considered fixed on its stem, thread, and under the supporting disk for the scenario when using cement (Figure 1.a, blue region). For the cementless approach only the thread and the part under supporting disk are considered fixed. (Figure 1.b, blue region).

A load of 6000 N magnitude is analyzed, as the most unfavorable case [7]. Both scenarios are studied without the implant/cement/bone interactions.

A static compression load is distributed on the upper surface of the disk (Figure 1.c, blue region denoted with an arrow). The safety factor is based on the yield strength.

RESULTS AND DISCUSSION

The simulation results are shown in Table 2 and Figures 3, 4 and 5. The differences between the initial and modified designs are not significant, for selected materials, titanium and stainless steel.

A longer thread suits better for a cementless fixation (Figure 1.b), due to the larger contact area. For the stainless steel, the analysis is shown in Figure 5. The static finite element analysis shows no significant differences when compared to the "cemented" scenario.

Figure 1. Fixed constrained areas of the modified short femoral endoprosthesis: (a) for "cemented", and (b) "cementless" scenarios. (c) Selected area for force distribution.

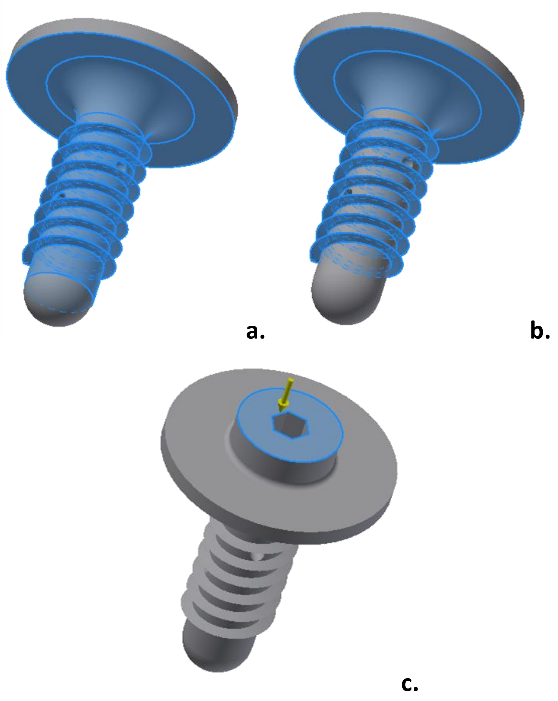

This approach confirms that the new design does not affect the mechanical behavior of the endoprosthesis. A longer thread is also expected to provide a better stress distribution in the femoral neck and further studies are required.

Table 2. Maximum displacements, maximum Von Mises Stress, and the minimum safety factor for a 6000 N load and different threads.

Materials	Displacement [max, µm]		Von Mises Stress [max, MPa]		Safety factor [min, ul]	
	initial	modified	initial	modified	initial	modified
Titanium	2.407	2.42	58.55	59.51	4.71	4.63
Stainless Steel	1.319	1.326	59.68	59.86	4.19	4.18

Figure 3. Displacement magnitude (a, b), Von Mises Stress distribution (c, d), and overall safety factor (e, f) for *titanium* endoprosthesis, “cemented” scenario, for the initial (a, c, e) and modified (b, d, f) design.

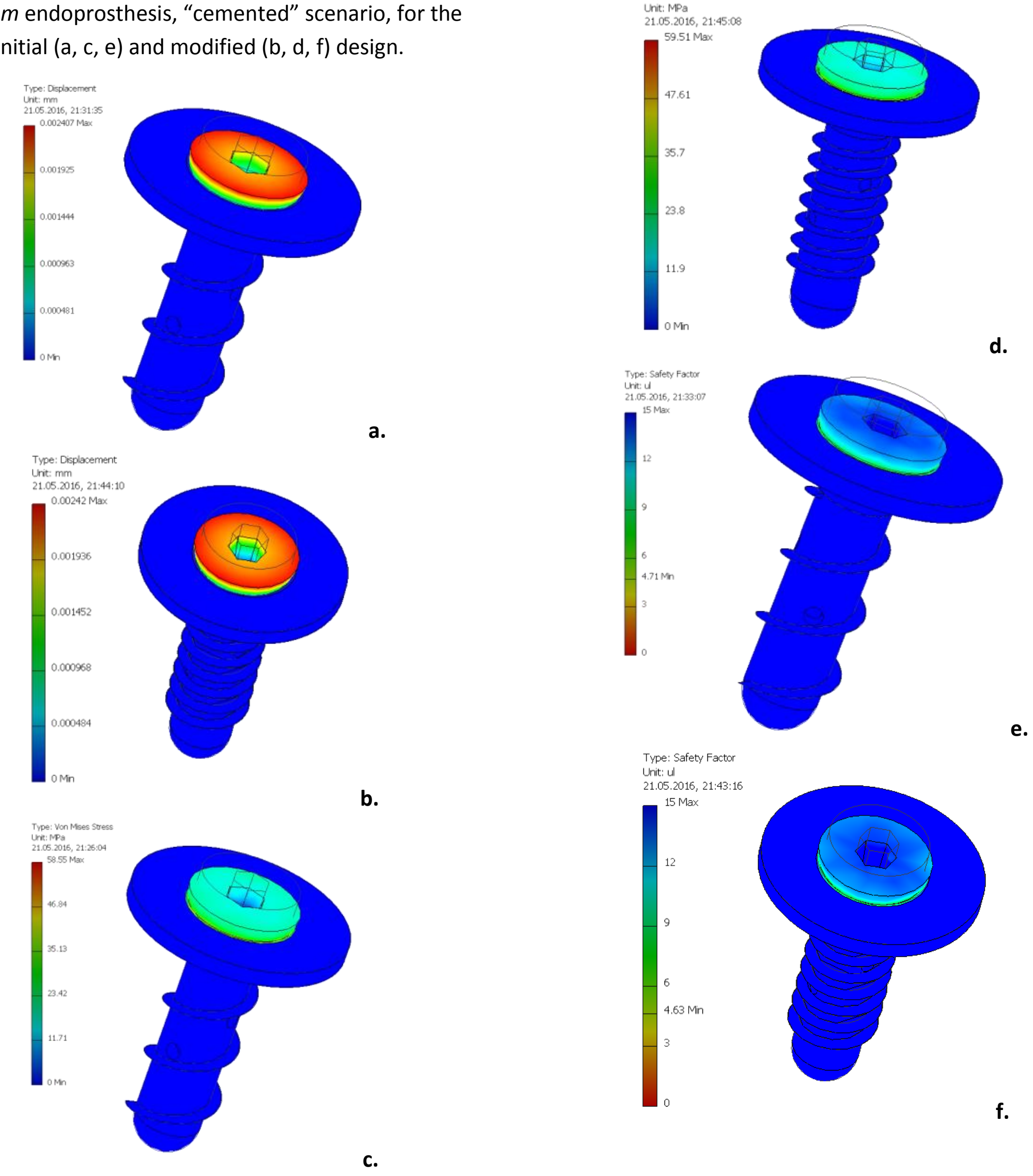
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Unit: mm
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0.001925
0.001444
0.000963
0.000481
0 Min
a.
Type: Displacement
Unit: mm
21.05.2016, 21:44:10
0.00242 Max
0.001936
0.001452
0.000968
0.000484
0 Min
b.
Type: Von Mises Stress
Unit: MPa
21.05.2016, 21:26:04
58.55 Max
46.84
35.13
23.42
11.71
0 Min
c.
Type: Von Mises Stress
Unit: MPa
21.05.2016, 21:45:08
59.51 Max
47.61
35.7
23.8
11.9
0 Min
d.
Type: Safety Factor
Unit: ul
21.05.2016, 21:33:07
15 Max
12
9
6
4.71 Min
3
0
e.
Type: Safety Factor
Unit: ul
21.05.2016, 21:43:16
15 Max
12
9
6
4.63 Min
3
0
f.

Figure 4. Displacement magnitude (a, b), Von Mises Stress distribution (c, d), and overall safety factor (e, f) for *stainless steel* endoprosthesis, “cemented” scenario, for the initial (a, c, e) and modified (b, d, f) design.

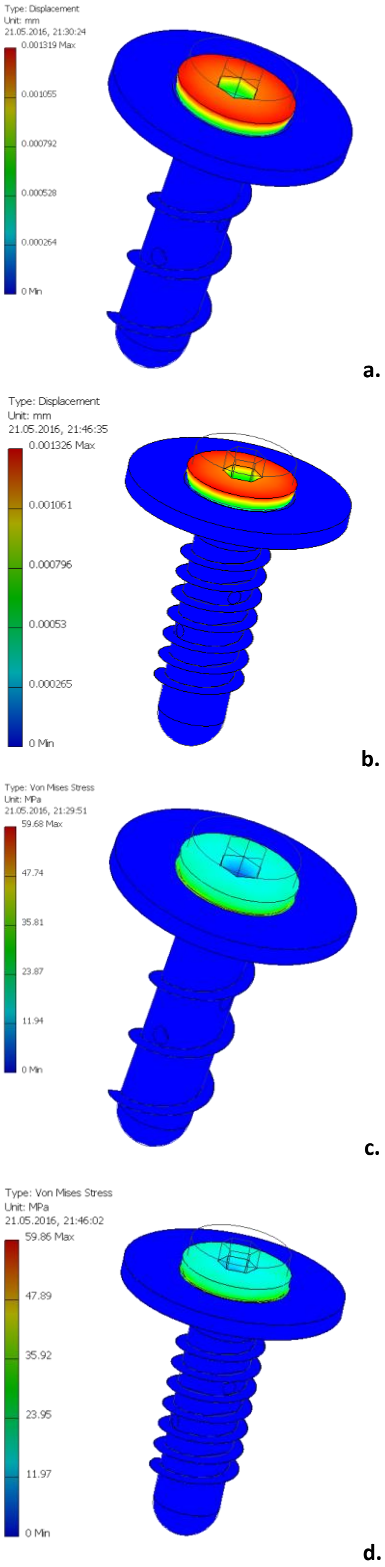
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a.
Type: Displacement
Unit: mm
21.05.2016, 21:46:35
0.001326 Max
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0.00053
0.000265
0 Min
b.
Type: Von Mises Stress
Unit: MPa
21.05.2016, 21:29:51
59.68 Max
47.74
35.81
23.87
11.94
0 Min
c.
Type: Von Mises Stress
Unit: MPa
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35.92
23.95
11.97
0 Min
d.

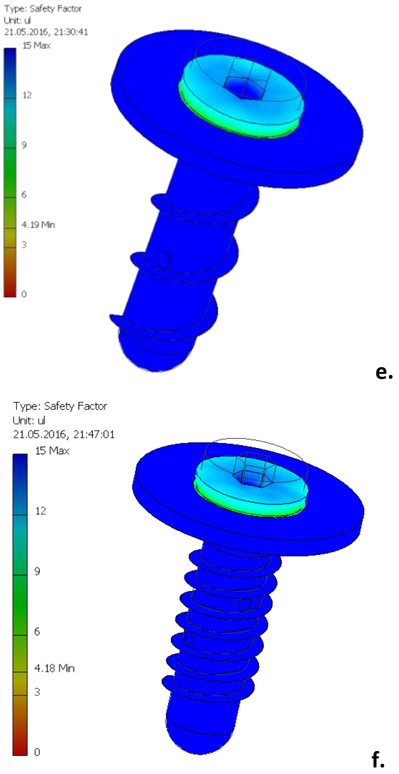
Type: Safety Factor
Unit: ul
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15 Max
12
9
6
4.19 Min
3
0
e.
Type: Safety Factor
Unit: ul
21.05.2016, 21:47:01
15 Max
12
9
6
4.18 Min
3
0
f.

Figure 5. Displacement magnitude (a), Von Mises Stress distribution (b), and overall safety factor (c) for the *stainless steel* modified endoprosthesis. A “cementless” scenario (6000 N load) is considered.

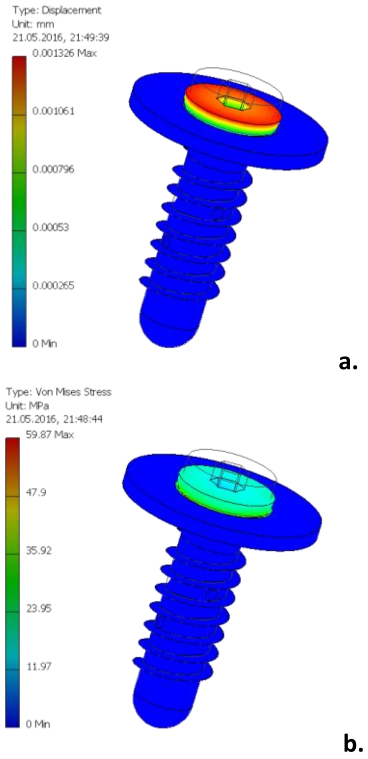
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0.000265
0 Min
a.
Type: Von Mises Stress
Unit: MPa
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59.87 Max
47.9
35.92
23.95
11.97
0 Min
b.

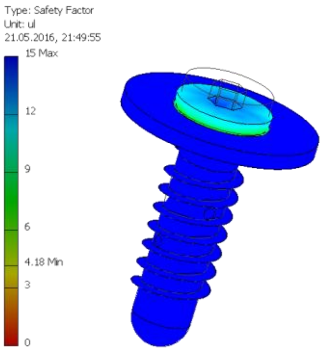

c.

CONCLUSIONS

A modified short hip endoprosthesis design is proposed as a better solution than the previous one, where a fixation using cement is considered. The new simple design consists in a longer thread that increases the friction force between endoprosthesis and bone by increasing the contact surface. The finite element analysis shows that the modified design does not affect the mechanical behavior. This design may allow, in some cases, a cementless fixation. Further tests are necessary to confirm our results.

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Optical “biopsy” in laryngeal cancer detection

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INTRODUCTION

Squamous cell carcinomas of the larynx is by far the most frequent malignant tumor of the head and neck region, representing 1-2% of all malignancies diagnosed world-wide. Treatment results of early cancer are not unfavorable: for the reported 5-year-survival rate of Tis, T1, T2 laryngeal cancer patients ranges from 80 to 90% [1]. At present, indirect laryngoscopy is widely used for the evaluation of laryngeal cancer and precancerous lesion, while direct laryngoscopy under general anesthesia with biopsy remains to be the gold standard.

The expression optical “biopsy” has entered into usual speech among researchers in the domain of biomedical optics. In fact this joining of terms is a paradox, because “biopsy” refers exactly to the removal of tissue, while the signification of “optical” is that tissue is not removed. The real meaning of this expression consists in amount of optical measurements mediated by optical fibers which allow the physician to perform an instant tissue diagnosis in vivo, in situ, noninvasively and in real time, previously possible only by using histological analysis. The main reason of this is to avoid the surgical removal of biopsy tissue samples.

These refined optical techniques go beyond standard endoscopic methods by offering improved image resolution, contrast, tissue penetration and delivering biochemical and molecular knowledge about mucosal disorders. For some endoscopic application, for which “random” biopsies are repeatedly taken to discover premalignant or early malignant lesions, optical measurement could enable “guided biopsy”, with increased probability for sampling a diseased site, reducing the number of tissue samples. Thus, additional purpose is certified by the potential for reduced health-care costs by eliminating unnecessary histology. Besides, the instant diagnostic information can reduce the emotional trauma to the patient awaiting an answer [2].

Recent technological advances in fiber optics, light sources and detectors have stimulated the tremendous development of many optical methods that promise to notably improve capability to visualize and evaluate human epithelium in vivo [3,4,5]. Some of these techniques are used for detection of laryngeal cancer: fluorescence spectroscopy, optical coherence tomography, narrow-band imaging, contact endoscopy and confocal endomicroscopy. This review describes the basic biophysics of light-tissue interactions, evaluates the strengths and weaknesses of each technique and explores clinical and preclinical evidence for each approach.

OVERVIEW

Fundamental parameters that characterize tissue

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optics in terms of radiative transfer are: absorption coefficient, scattering coefficient and anisotropy and measuring those constitutes a central issue in biomedical optics [6].

a. The absorption coefficient (μa) determines the probability of absorption events for a photon that propagates in tissue. Absorption occurs due to coupling of photons energy at a particular frequency with electronic or vibrational transitions energy. Depending on the spectral field, absorption is caused by:
- Cellular DNA and protein (amino acids and nucleic acids) in ultraviolet;
- Specific chromophores (hemoglobin, melanin, etc.) in visible;
- Water, OH and amine infrared.

b. Scattering coefficient (μs) determines the probability of a photon to be scattered during propagation. Scattering is caused by fluctuations of the refractive index of the environment in microscopic scale (ultra-structural heterogeneity) such as water-lipid interfaces membranes inside the cell and surrounding cells and fibers of collagen in the extracellular matrix. We can say that the absorption properties of tissue are tied to tissue biochemistry, while the scattering ones are related to its morphology.

c. Anisotropy (g) is the average cosine scattering angle and weighted the scattering coefficient effectiveness.

d. Alongside the three basic parameters, in medical practice, are also used: attenuation coefficients (total and effective); reflection; the refractive index; reflectance.

Cheong et al [7] realized (1990) a comprehensive compilation of optical tissue properties (absorption coefficients, scattering, total and effective mitigation and anisotropy for different biological tissues at a variety of wavelengths). They concluded that for a specific tissue, large areas of these parameters are frequent, indicating the sensitivity and vulnerability of such measurements to sample variation, detection equipment, boundary conditions and adopted light propagation model. Confidence in the obtained values can be compromised by any of these factors. After 1990, numerous studies have improved this knowledge, creating optical exploration methods that will be presented below.

1. Spectroscopy

1.1. Autofluorescence endoscopy (AFE)

Autofluorescence was first observed by Policard [8] in 1924, but Alfano [9] demonstrated in 1984 differences in spectral profiles of cancerous and normal tissues. In the last decades, this technique was used for screening in early detection of cancers of upper- and lower-GI tract and also lower-airway, and recently it was used in identifying precancerous and cancerous lesion [10].

Tissue autofluorescence is based on the presence of endogenous fluorophores, which emit visible green light on appropriate excitation. This involve structural proteins (collagen, elastin) mainly located in the submucosa, but also keratin, porphyrin and enzymes of the respiratory chain such as nicotinamide adenine dinucleotide and flavin adenine dinucleotide within the epithelium [11].

Figure 1: Squamous cell carcinoma of the right vocal fold: White light endoscopy (a); Autofluorescence endoscopy (b) [13]

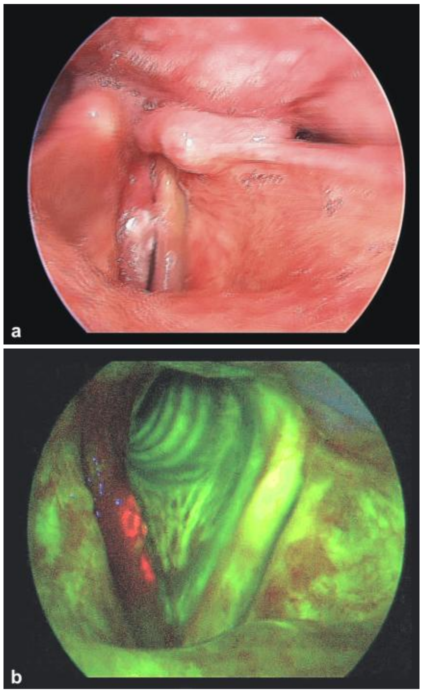

Because of an altered metabolism in tumor cells, premalignant and malignant tissues show a decreased quantity of fluorophores. Moreover, epithelial thickening prevents the excitatory blue light from reaching the submucosa. Both mechanism results in a loss of autofluorescence, with a color change from green to red-violet [12].

In AFE, healthy mucosa and benign lesion covered by normal epithelium (polyps, nodules, cysts, Reinke's edemas) present green color (no loss of autofluorescence). Scar tissue following surgery or radiotherapy shows a violet color; the same aspect is found in hyperplasia and chronic inflammation.

AFE can be used in early detection of premalignant and malignant lesions, in guided biopsy and oncological follow-up. This technique can only detect changes on epithelium, but not in the deeper structures [14].

Many studies have found that AFE has a better sensitivity and specificity in detecting laryngeal malignant changes than the classic white light endoscopy (WLE) [15,16,17]; a meta-analysis made by Kraft and co. in 2010 concluded: in identifying precancerous and cancerous lesions of the larynx, sensitivity (91% vs. 73%), specificity (84% vs. 79%) and accuracy (88% vs. 77%) of AFE were superior to WLE alone[18]; false-positive diagnoses result from chronic laryngitis, teleangiectatic polyps, granulomas[19] and papillomas; false-negative diagnoses are found in extreme hyperkeratosis, necrosis and inflammation [20,21].

There is a variant of this method involves the study of autofluorescence laryngeal mucosa after topical application of aminolevulinic acid, preferentially captured by the cancer cells, that became colored in orange-red (because protoporphyrin IX), while normal mucosa appear green [22].

Figure 2: Normalized mean and difference spectra for squamous cell carcinoma and non malignant tissue in larynx [26].

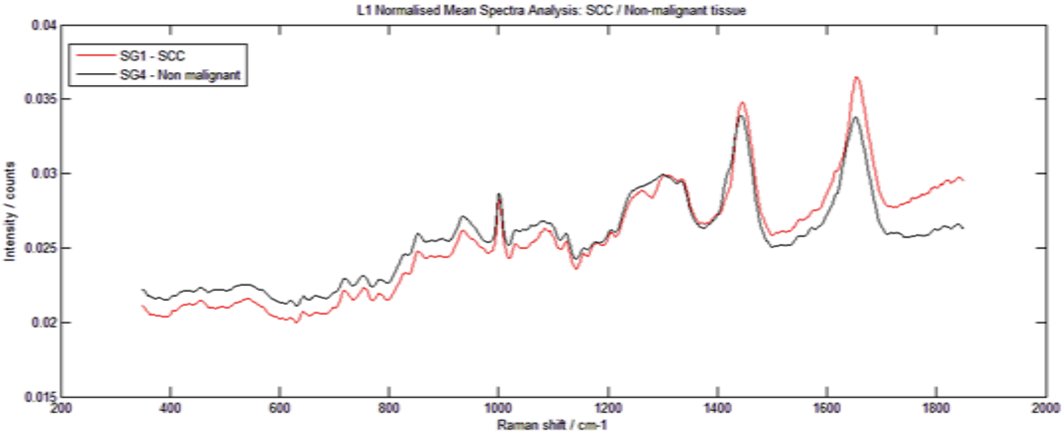

1.2. Raman spectroscopy

This method involves an effect whereby a small portion of tissue scattered light has a different frequency from that of the incident beam, with an equal amount of vibration frequencies of the molecules in the tissue; the effect is very weak, but with laser, it is possible to study the movements of rotation and vibration of molecules [23]. From the received signal, the percentage of biological molecules can be extracted in samples, such as collagen, elastin, beta-carotene, cholesterol (fig.2).

Introduced in medical practice in 2000, Raman spectroscopy is used to differentiate normal laryngeal tissue of precancerous and cancerous lesions [24]. Some authors publishing studies that indicate sensitivity between 70-90% and specificity in differentiating between normal tissues/malignant between 85-95 % [25].

2. Optical coherence tomography (OCT)

OCT is a modern method of investigation, in high resolution, mainly used in ophthalmology. It is derived from low coherence optical interferometry and uses non-ionizing optical radiation in the near infrared field.

OCT allows measurements in real time, in situ, with resolution up to 10 times higher than conventional radiology (CT, MRI), making it possible to identify microscopic tissue structures (villi, glands, crypts, blood vessels). Despite this exceptional resolutions (1-2 µm), the method is limited by the very low degree of depth (penetration of tissue) of 1-3 mm [27]. Articles published in the past decade shows that it can be used to detect an epithelial membrane rupture and overcoming of the laryngeal carcinoma or epithelial thickness measurement (normally between 98-185 µm, about 500 µm in hyperkeratotic lesions) [28].

Figure 3: T2 squamous cell carcinoma of the left vocal fold; (A) Endoscopic photograph. (B) Optical coherence tomography image demonstrating normal epithelium with an intact basement membrane on the right of the image, and invasive cancer with increased backscattering and loss of the basement membrane on the left. A transition zone is seen between these two areas. Note the area of motion artifact on the left of the image (bm-basement membrane; ca-invasive cancer; e-epithelium; tz-transition zone; SLP-superficial lamina propria) [29]

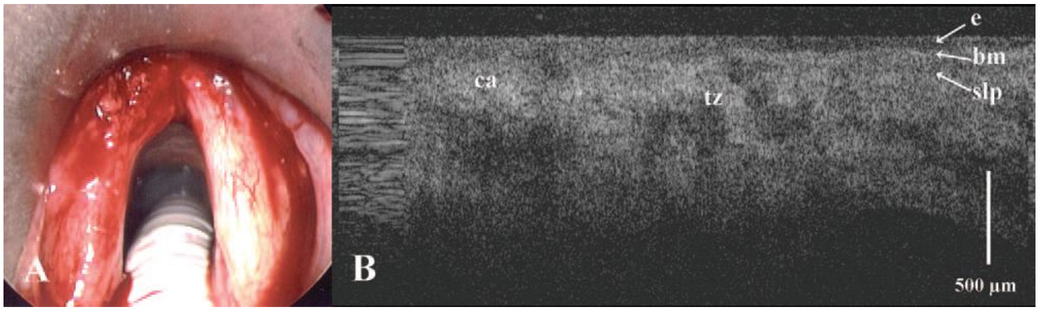

3. Narrow band imaging (NBI)

It is a modern endoscopic technique for investigating the vocal cord that uses certain wavelengths of light to improve the viewing of epithelial and sub epithelial microvascular pattern. They are using wavelengths of 415 nm (blue) and 540 nm (green), which are absorbed by hemoglobin, causing epithelial microvasculature to be colored in brown and become visible. The obtained images can be improved and recorded by using a HD (High-Definition) video system [30,31].

Neoplasia suspicion rises when on a brownish area, well-marked, is observed thick dark spots and/or tortuous course of the microvasculature, or more, a hypertrophic blood vessel that branches in small vascular loops [32,33]. There are studies that demonstrate that this optical method can even make the difference between neo-vessels specific to carcinoma and the ones altered by post-radiation inflammation [34].

The method was originally used for early detection and monitoring post-surgery esophageal [36], and tracheobronchial [37] neoplasm lesions, but recent studies show that is gradually earning supporters using it for the same goals at the larynx [38,39] (good results in subglottis injuries). Thus, Japanese authors demonstrate that narrow-band imaging has a sensitivity of 91.3% and a specificity of 91.6% [40], in laryngeal cancer lesions, while a team from Italy (Brescia) reports 90% specificity and 98% sensitivity [41].

Another recent study [42], multicenter, prospective, randomized, which included patients with esophageal cancer and pharyngeal and laryngeal cancer, showed that narrow-band imaging compared with conventional endoscopy detected a higher number of superficial carcinomas synchronous in both locations, making them the authors to conclude that this method could be the standard examination for early detection of superficial cancer in those localizations.

But the interpretation of the images must be made by an ENT specialist with a good experience, to avoid false negative or false positive. Also false negative may occur on lesions where the keratin layer has a greater thickness.

Figure 4: A. Conventional white-light image of the larynx. The left subglottic region is displayed slightly reddish. B. NBI image of the same site as a. Demarcated brownish area is seen in the left subglottic region surrounded by green area. C. Close white-light view of a. D. Close NBI view of b. There are scattered brown spots in the lesion. E. Histopathologic evaluation of the biopsy specimen revealed moderately differentiated squamous cell carcinoma of the larynx [35].

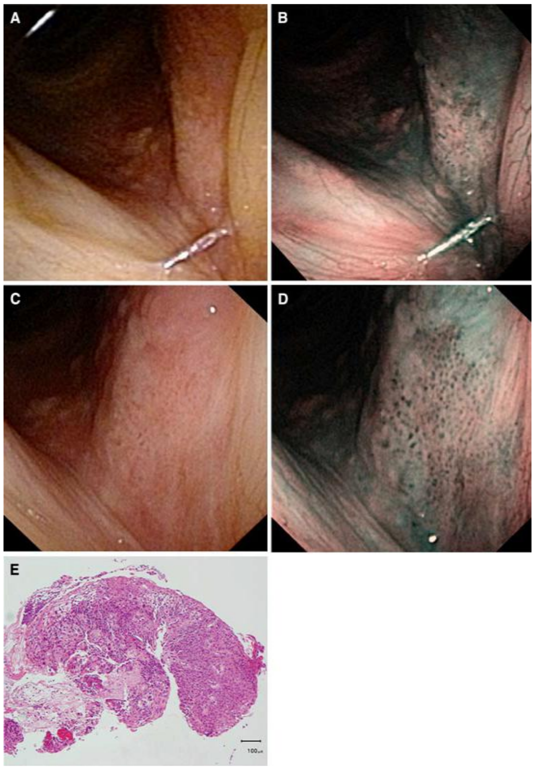

4. Confocal endomicroscopy

This technique is introduced into medical practice in 2004 by R. Kiesslich for in vivo diagnosis of colorectal cancer[43]. The principle of the method consists in illuminating the examined tissue with a laser wave of 488 nm which allows viewing epithelium in depth up to 250 µm. For a better image quality is given intravenously a contrast substance (fluorescein). The examination is made simultaneously with both endoscopic and endomicroscopic, acquiring images to identify the characteristics of epithelial cells (size, shape, appearance of the nucleus) near the basal membrane [44].

The limitation of this method is shallow penetration (250 µm), knowing that the cancer epithelial thickness can reach 500-1000 µm, being unable to view the membrane. Also it requires a pathologist with experience.

Introduced recently in Otolaryngology, there are still no studies on a large number of cases, but those in the gastrointestinal field [45,46] give high hopes for the future of this method.

There is a pilot study [47] clearly demonstrates the possibility to detect dysplastic cells near to the basal cell layer and within the subepithelial space in lesions with small leukoplakia (thin keratin layer). These results can have an impact on microlaryngoscopy to improve the precision for biopsy and on microlaryngoscopic laser surgery of the larynx to identify the margins of the pre-malignant lesion.

Figure 5: Confocal endomicroscopy image of the human supraglottis captured in vivo following topical application of 0.05% acriflavine hydrochloride [48]

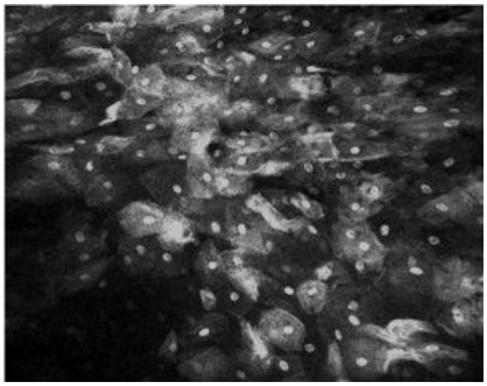

5. Contact endoscopy

Contact endoscopy is another novel noninvasive optical diagnostic imaging method that allows in vivo and in situ examination of the cellular architecture of the superficial layers of the mucosal epithelium. Magnified images are obtained using Hopkins' rod-lens endoscope placed on the surface of the dye stained mucosal tissue. This method allows evaluation of precancerous and cancerous lesions in vivo and has significant potential in the histopathologic diagnosis of many suspicious head and neck mucosal lesions without tissue biopsy. The first reported use of CE in otolaryngology head and neck surgery was by Andrea et al. as a diagnostic tool in the evaluation of various pathologies in the larynx in the 1990s [49]. They were able to visualize and diagnose laryngeal mucosal pathology from the magnification of vocal fold epithelium and microvasculature during microlaryngoscopy after staining the vocal cords with methylene blue dye.

Few members of our group [50] have systematically drawing vascular changes of the vocal fold, with clinical and pharmacological applications. They demonstrated the changes of microvascularity, occurring since stage of hypertrophic chronic laryngitis (hypervascularity with dilated vessels, but parallel to the free edge of the vocal fold) till the carcinomatous lessions (with hypervascularity and areas of keratin, changes of the archaeological and cytological parameters: **U**niformity of the cellular-field, **R**atio (nucleus/cytoplasm), **S**ize and **S**hape of the epithelial cells [51].

There are more advantages of contact endoscopy. Most significantly, it offers a noninvasive, quick, and repeatable in vivo evaluation of the cytological architecture while avoiding the need for an invasive biopsy and its associated risks. CE provides immediate results, with the possibility of examining many mucosal areas in a short time. CE can also assess a wider surface mucosal area, providing more information than a selected histological section taken by biopsy [52]. It also avoids tissue damage and alteration of cellular architecture which may occur in the biopsy and histological preparation. Subsequently, this results in a dramatic improvement of the diagnostic yield of the biopsy. Other potential roles of CE include the rapid diagnosis of benign and malignant mucosal lesions in an outpatient or operating room setting, surveillance, guided biopsies, and intraoperative evaluation of tumor resection margins. Despite its advantages, CE does have its limitations. Most notably, CE can only evaluate the most superficial cell layer of the mucosal epithelium. This is most likely due to a number of factors including (i) poor penetration of methylene blue which only stains a few superficial layers, (ii) short focal distance of the scope (i.e., CE can only assess to a depth of 80 um at 60x magnification and 30 um at 150x magnification), and (iii) optical artifact at high magnification due to glare from light reflected from cells not in focus. The lack of depth of penetration prevents the evaluation of important histological information especially when vertical extent of dysplasia is crucial in distinguishing the different grades of dysplasia from carcinoma in situ and invasive carcinoma. As a result, these factors could affect the sensitivity of CE, thus accounting for some of the false negative diagnostic results noted by authors. The potential impact of CE missing a malignant lesion needs to be taken into consideration if this technology is to one day substitute histopathology. Future investigation into better penetrating dyes, advances in digital optics, and image enhancements will eventually allow better vertical staining and increased resolution of the deeper cell layers which would translate CE in becoming a much more sensitive and accurate diagnostic tool.

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ORIGINAL ARTICLES

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Detection of epidermoid squamous-cell carcinoma by laser induced autofluorescence – preliminary results

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Abstract: *Epidermoid squamous-cell carcinoma is by far the most common malignant tumor of larynx (95-96%), representing 1.6-2% of cancers in men and 0.2-0.4% of malignancies in females, with a world growing incidence and a slight dominance in urban areas. By exposing cells and tissues to UV light, the excitation of naturally occurring chromophores occurs in part by non-radiative deactivations, in part via fluorescence emission. Using laser induced autofluorescence (LIAF) in natural tissues not impregnated with photosensitizers as a noninvasive autofluorescence technique for both diagnosis and intraoperative assessments of laryngeal cancer we can improve the tumor malign identification in vivo. A total of three laryngeal biopsies (i.e. three pairs of tissues, each pair containing a healthy and a tumor tissue sample extracted from the same patient) were considered in this study. The samples were collected from patients previously diagnosed with stage T3 laryngeal carcinoma. Immediately after the surgery fragments of normal tissue and neoplastic tissue were collected, fragments of which later, after freezing, were sectioned in 25-30 µm thickness slices and stretched to quartz slides. All samples were subjected to controlled laser irradiation using a pulsed diode laser (λ=375nm, pulse width=87ps, frequency 31MHz) and the autofluorescence and its lifetime were collected using two optical fibers (inner diameter 400µm and 1500µm, respectively) positioned in a 45° geometry. The signals were recorded using a spectrograph and a photo-sensor module, the output of which was fed to a digital oscilloscope.*

We assessed the impact of laser induced autofluorescence and autofluorescence lifetime measurements in order to identify the differences between healthy and tumoral laryngeal tissue and outlining them, in terms of differences between the laser autoinduced fluorescence averaged intensity. The results determined the usefulness of laser induced spectroscopy in the diagnosis of laryngeal squamous cell carcinoma, discriminating between the malignant and normal tissue by analyzing the differences in spectral autofluorescence intensity and autofluorescence lifetime.

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Keywords: *Laser induced autofluorescence, autofluorescence lifetime, laryngeal squamos cell carcinoma*

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INTRODUCTION

Cancer is a growing dilemma in the developed countries. Laryngeal carcinoma is the most common cancer of the head and neck. Epidermoid squamous-cell carcinoma is by far the most common malignant tumor of larynx (95-96%), representing 1.6-2% of cancers in men and 0.2-0.4% of malignancies in females, with a world growing incidence and a slight dominance in urban areas [1].The main risk factors are smoking, alcohol consumption, harmful dietary habits, exposure to the irritant chemical substances [2].

Early identification and pre-operative evaluation of laryngeal carcinoma and its precursor lesions are essential for function-preserving surgical treatment. Accurate determination of the site and extent of carcinomas is very important for planning therapy and establishing prognosis [3]. Attempts to optimize diagnostic methods to obtain more sensitive detection and precise description of laryngeal pathology remain a challenge for the otolaryngologist [4].

Microlaryngoscopy with biopsy is the standard diagnostic procedure for the detection of laryngeal carcinoma. Anyway, the microscopic view added to the palpation of the larynx is not always enough to the evaluation and precise description of precursor lesions. Some new techniques, like videostroboscopy, contact endoscopy, confocal endomicroscopy, optical coherence tomography, narrow-band imaging and fluorescence endoscopy have been developed to come up with the detailed examination and the histopatological information about mucosal lesions [5,6].

Fluorescence is a optical phenomenon of the light emission by some molecules after their excitation by the light at a particular wavelength. These fluorescent molecules are called fluorophores. Every fluorophore exhibits its characteristic absorption and emission bands whose shapes and positions of their maxima on the excitation and emission light wavelength axes depend on the physical and chemical properties of its nearest environments. The interest in the use of fluorescence in medical diagnostics has grown during the last decades. Fluorescence spectroscopy provides information on the biochemical and morphological aspects of different tissues [7].

The procedures using the fluorescence spectroscopy for the differential diagnosis of malignant lesions are based upon the differences in the autofluorescence emission due to the pathological changes of the tissue. These changes include the modifications of tissue architecture (tissue layer loss or thickening), light absorption and scattering by neovasculature, and the alterations of metabolic status of the tissue such as changes of the concentration and distribution of fluorophores [8]. As a result, recorded fluorescence emission spectra of cancerous lesions reveal differences in fluorescence intensity and fluorescence band shapes, when compared to healthy tissue.

MATERIALS AND METHODS

The spectroscopic measurements were investigated in three larynxes from laryngectomized cancer patients treated in ENT Clinic, Carol Davila Central University Emergency Military Hospital, Bucharest. Histological analysis of the tumor tissues revealed laryngeal epidermoid squamous-cell carcinoma (SCC) in both cases, stage T3 (Figure 1). Immediately after the surgery (total laryngectomy), the larynx was carefully washed in saline solution to remove any blood contamination.

After that, fragments of normal tissue and neoplastic tissue were collected, fragments of which later, after freezing, were sectioned in 25-30 µm thickness slices and stretched to glass slides (reconfirmed histological) and stored at -80ºC for further examination. All participants provided written consent to participate in the experiment. The study was approved by the local ethics committee.

Experimental setup (Figure 2) was performed using laser beam exposure of the samples a picosecond pulsed laser diode (Alphals, type PicoPower LD-375-50) emitting at 375 nm with 31MHz pulse repetition rate, 87 ps full time width at half-maximum laser pulses, and the average power of about 0.5 mW. A laser beam of 1.6x1.8 mm (elliptical) diameter was sent to the sample in double-pass vertical irradiation geometry through a 355 nm dichroic mirror and a totally reflecting mirror.

Two types of measurements on tissue samples were performed: laser induced autofluorescence (LIAF) and autofluorescence life-time. The autofluorescence signal was collected using two optical fibers positioned each, at 45° in the same plane with respect to the pumping laser beam.

Figure 1. Larynx after surgical ablation for SCC (T3)

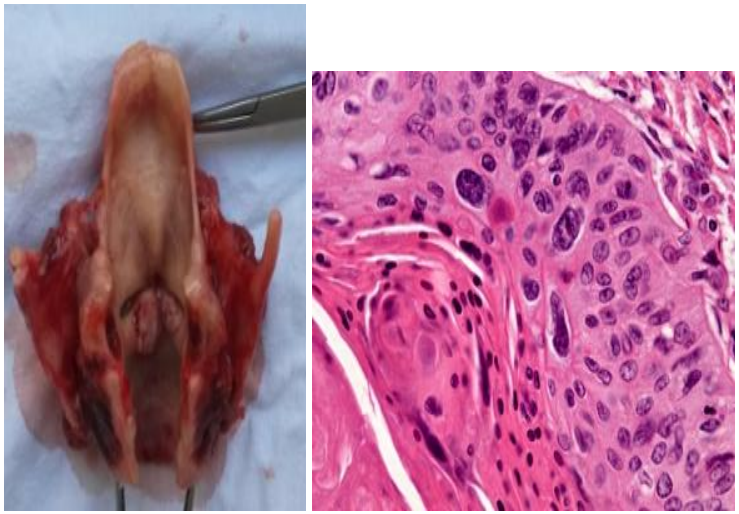

Figure 2: Experimental setup

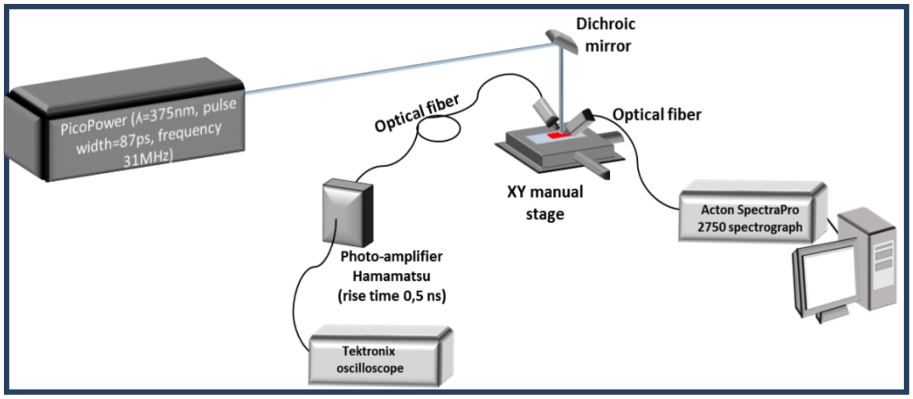
Dichroic mirror
PicoPower (Λ=375nm, pulse width=87ps, frequency 31MHz)
Optical fiber
Optical fiber
XY manual stage
Acton SpectraPro 2750 spectrograph
Photo-amplifier Hamamatsu (rise time 0,5 ns)
Tektronix oscilloscope

The optical fiber with the core diameter of 400 µm collects the signal for LIAF spectrum analysis; another fiber of 1,500 µm collects the remitted signal for autofluorescence life-time analyses. The LIAF signals were recorded using a spectrograph (Princeton Instruments, type Acton SpectraPro 2750) with an optical resolution of 0.08 nm and; a photosensor module (Hamamatsu, type H6780-20) having 0.78 ns rise time, was used for LIAF life-time measurements and its output was fed to a digital oscilloscope (Tektronix, type DPO7254). The signal acquisition by spectrograph and photosensor module was synchronized with the laser pulse through a pulse delay generator (SRS, DG 535). During the LIAF measurements, the probe (sample and substrate/slide) was placed on a XY manual stage with a displacement resolution of 5 µm. Each type of measurement was performed collecting the LIAF signal from three different spots for each sample. For each healthy and tumoral tissue two slides with the same type of samples were prepared.

RESULTS

The LIAF spectra of the healthy and tumoral tissues are presented in Figures 3-5 and are compared in terms of peak intensity (*I*), Area (*A*) under fluorescence spectral distribution curve and ratio between the intensity and the corresponding full width at half maximum (*I/FWHM*) signal.

In case I, two healthy tissues of different thicknesses, 20 µm and 30 µm, were compared. Figures 3A and 3B presents the LIAF spectra of the three spots from the duplicate samples and Figure 3C shows the corresponding averaged spectra. The peaks of LIAF spectra are placed between 456 – 462 nm for the 30-µm tissue thickness and between 460-463 nm for the 20-µm tissue thickness. In Figures 3A and 3B are observed variations in peak intensity, area and I/FWHM and approximately the same values for FWHM. These variations are, dominantly and most probably, due to different concentrations of fluorophores in the samples. The comparison between the averaged spectra of 20 µm and 30 µm shows 11.4% autofluorescence intensity, a 9.5 % area and a 14.4% I/FWHM increase in 30 µm with respect to 20 µm case.

Figure 3. Case I. LIAF intensity for healthy tissue for different thickness: A) 20 µm and B) 30 µm; collected in three spots of the duplicate samples and the averaged spectrum; C) LIAF intensity of the averaged spectra from the 20 µm and 30 µm thickness samples.

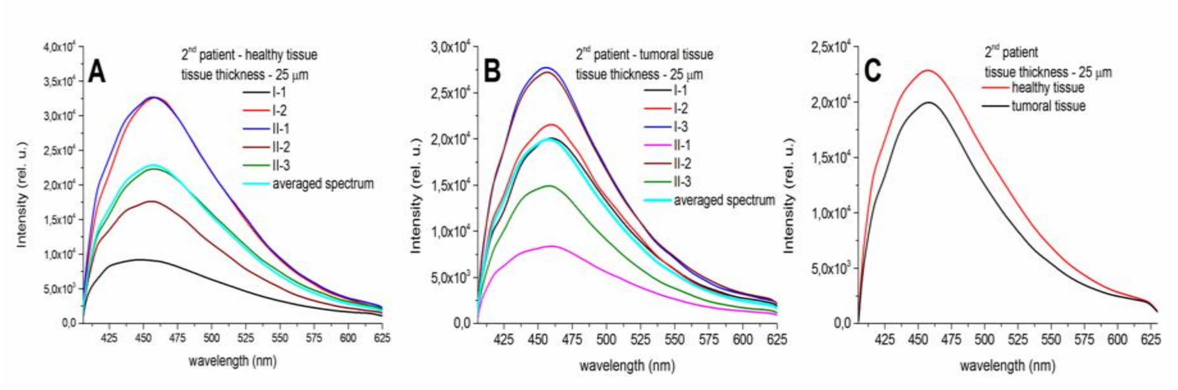

Figure 4. Case II. LIAF intensity for 25 µm tissue thickness: A) healthy and B) tumoral; collected in three spots of the duplicate samples and the averaged spectrum; C) LIAF intensity of the averaged spectra from the healthy and tumoral samples.

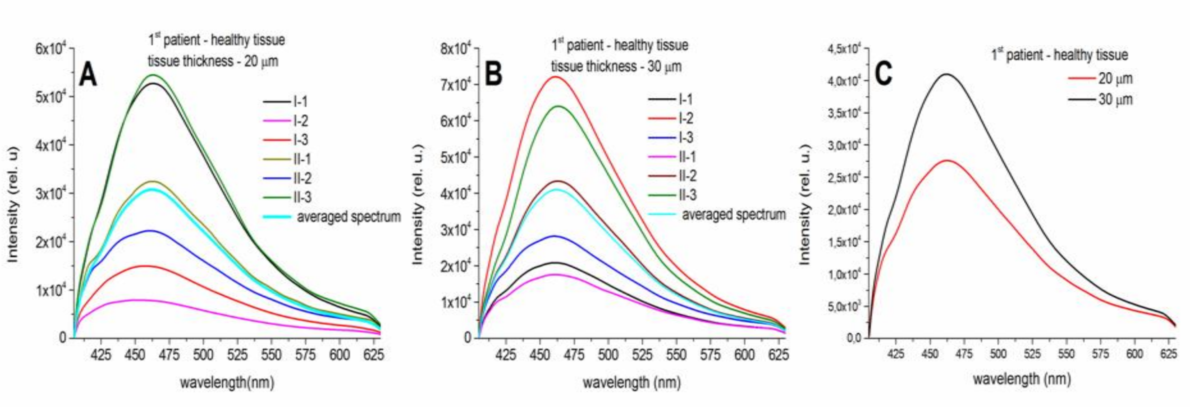

In case II, one healthy and one tumoral tissue of 25-µm thickness were analyzed. Figures 4A and 4B present the LIAF spectra of the three spots from the duplicate samples and the corresponding averaged spectrum (Figure 4C). The peaks of LIAF spectra are placed between 445 – 462 nm for healthy tissue and between 456 – 459 nm for tumoral tissue. In Figures 4A and 4B, are observed variations in peak intensity, area and I/FWHM and approximately the same values for FWHM.

In general, the maximum value of LIAF's intensity is smaller for the cancerous tissue (with 16.7%) than the healthy one. The area and the I/FWHM ratio of LIAF for cancerous tissue is also smaller than for healthy tissue (with 17.1% and 4.7%).

In case III, one healthy and one tumoral tissue of 30-μm thickness were analyzed. Figures 5A and 5B present the LIAF spectra of the three spots from the duplicate samples and the corresponding averaged spectrum (Figure 5C). The peaks of LIAF spectra are located between 457 – 458 nm for healthy tissue and between 456 – 459 nm for tumoral tissue. In Fig. 5A and 5B, are observed variations in peak intensity, area and I/FWHM, but smaller than those from case II, and approximately the same values for FWHM.

In Figure 5C, the maximum value of LIAF's intensity is smaller for the cancerous tissue (with 71%) than the healthy one. The area of LIAF for cancerous tissue is also smaller than healthy tissue (with 66.7%). At the same time, maximum spectrum for tumor sample is shifted to blue with nine nm (449-458 nm) than healthy tissue and the I/FWHM ratio decreases with 74.2% when comparing the healthy tissue with the tumoral one.

Figure 5. Case III. LIAF intensity for 30 μm tissue thickness: A) healthy and B) tumoral; collected in three spots of the duplicate samples and the averaged spectrum; C) LIAF intensity of the averaged spectra from the healthy and tumoral samples

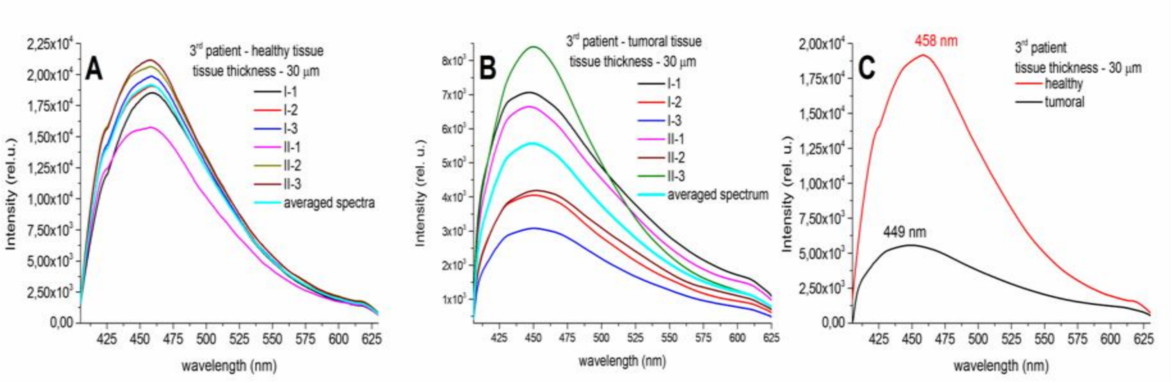

The computed parameters based on the experimental data extracted from LIAF spectra for the 2nd and 3rd patient healthy and tumoral tissues are shown in Table 1.

The results indicate that values of LIAF's parameters (wavelength, intensity, area, I/FWHM) are helpful and complementary diagnostic tools in detection of laryngeal carcinoma. These variations are due to the different concentrations and distribution of fluorophores in the samples.

Table 1. Parameters extracted from LIAF spectra for the 2nd and 3rd patient healthy and tumoral tissues (rel. u. are different from one column to the other; the same values of rel.u. are used for different patients)

Patient	Tissue	λ_{peak} (nm)	I_{max} (rel. u.)	FWHM (nm)	Area (nm x rel.u.)	I/ FWHM
2nd	Healthy	457	2.29x10^4	107.48	2.58x10^6	212.6
	Tumoral	457	2x10^4	98.5	2.13x10^6	202.5
3rd	Healthy	458	1.92x10^4	101.69	2.07x10^6	188.5
	Tumoral	449	5.56x10^4	114.6	6.87x10^6	48.56

Regarding LIAF's life-time (Figure 6), performed measurements show that the life-time of the healthy tissue decreases with thickness increasing, both for first patient case (Figure 6A) and for the second and third patients (Figures 6B and C, respectively). In Figure 6A, the life-time value decreases from 4.06 ns for the tissue of 20 µm thickness to 3.6 ns for that of 30 µm thickness, both values being obtained by averaging 4 samplings.

Figure 6. LIAF's life-time. A) 1st patient, healthy tissue at two thickness, 20 and 30 µm; B) 2nd patient, healthy and tumoral tissues at 25 µm tissue thickness; C) 3rd patient, healthy and tumoral tissues at 30 µm tissue thickness; blue dash line indicates the exponential decay fit function whose time constant τ represents life-time of the analyzed process.

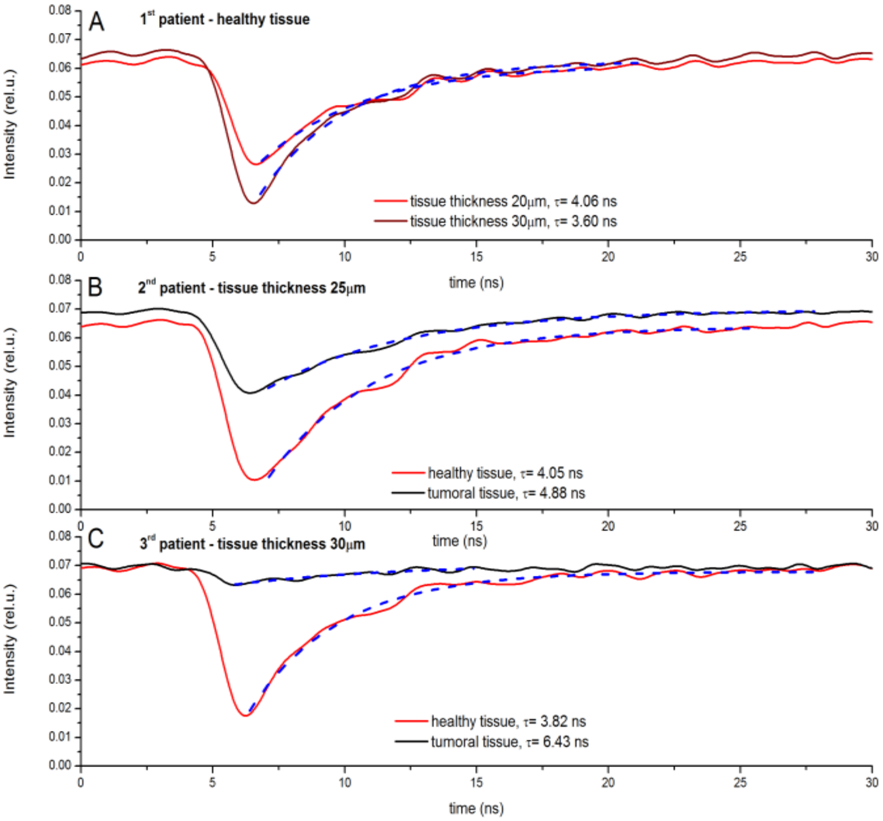

It is to mention that the life-time values of healthy tissue for second and third patients are similar with those for the first patient considered for the same tissue thickness. For both patients exhibiting developed malignant tumor, the life-time values were higher for the cancerous tissues than the healthy samples (4.88 vs 4.05 ns for second patient; 6.43 vs 3.82 ns for third patient) as we see in Figures 6B and C.

DISCUSSION

The prognosis for patients with laryngeal cancer is significantly better with early detection of malignant lesions. Therefore, otolaryngologists look for supplementary techniques, as laser-induced autofluorescence, which may improve accuracy of real time cancer detection.

Malzahn et al. [9] have demonstrated high sensitivity of autofluorescence endoscopy in the diagnosis of early laryngeal cancer compared with light endoscopy alone. In the study of Zargi et al. [10] the sensitivity and specificity of described method were 86.9% and 82.8%.

The aim of the presented study was to analyze the applicability of laser-induced autofluorescence measurements to the differentiation of the changes in the cancerous and healthy tissue of the larynx. For the measurements, it was used a laser diode emitting pulses of picosecond duration and λ = 375 nm. The chosen wavelength was found to excite the endogenous fluorophores: tyrosine (max. emission at 310 nm), tryptophan (max. emission at 340 nm), oxidized form of nicotinamide adenine dinucleotide (NAD+) (max. emission at 460 nm), the collagen cross-links (max. emission at 420–460 nm), the reduced form of nicotinamide adenine dinucleotide (NADH) (max. emission at 460nm), and the oxidized form of flavin adenine dinucleotide (FAD) (max. emission at 520 nm) [11]. The levels of the mentioned fluorophores, especially NADH and FAD, provide a strong emission signal and are significantly altered in cancerous cells, enabling the differentiation of the healthy and neoplastic tissues by autofluorescence measurements [12, 13, 14].

In both cases of the study, the LIAF's intensities of the cancerous tissue samples are lower in comparison with the adjacent healthy tissue samples. These results are in accordance with other studies where the decrease of intensity may be a consequence of reduced concentration of oxidized flavoproteins, the breakdown of collagen cross-links, the thickening of the cancerous tissue or the blood absorption (neovascularisation, inflammation)[15,16]. Neoplastic cells infiltrating submucosa express matrix metalloproteinases activity, which participate in breaking collagen cross-links [17].

We also observed in both cases that the area of LIAF for cancerous tissue is smaller than healthy tissue, the value of the ratio Intensity/ Full width at half maximum (I/FWHM) decreases in cancerous tissue sample and maximum spectrum for tumor sample is shifted towards blue. As for LIAF's life-time, in both cases the values were higher for cancerous tissues than healthy samples. Measurement of these parameters gives to our study originality, as well as the experimental setup.

Our results indicate that LIAF's parameters values (intensity, area, I/FWHM, life-time) are potentially a very helpful, complementary, diagnostic tool in detection of laryngeal carcinoma. The clinical prospective research on a great number of patients should be performed to establish utility of these measurements in everyday practice.

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Mucous herpes zoster in elderly

Daniel O. Costache[1], Ionel Ţoropoc[1], Ciprian Jurcuţ[1], Cătălina Diaconu[1], Laura Voicu[1], Raluca S. Costache[1,2]

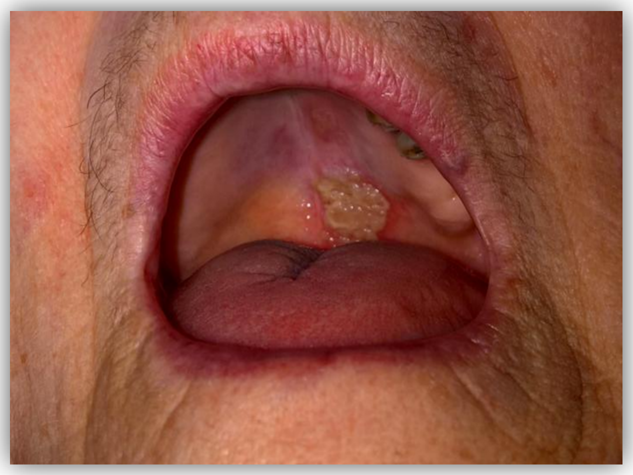

78 years old female is admitted in hospital for low back pain with moderate dysfunction and headache, secondary associated with vesicular/ pustular and ulcerative lesions in the mouth (image).

The patient has a cardiovascular medical history with severe arterial hypertension, grade 1 atrio-ventricular block, paroxistic atrial fibrillation, mitral and tricuspidian regurgitation, congestive heart failure NYHA II and chronic renal disease stage IIIB.

Biologic findings shows slight normochrome anemia, WBC was within normal range and no signs of inflammation. Ag Hbs, Ac anti HVC, HIV were negative. Beta haemolitic streptococcus and staphylococcus aureus were absent.

The imagistic standard evaluation (ultrasound) has normal findings.

Patient developed an erythematous patch on the soft palate, with pustules and ulcerations, slightly painful, and some vesicles on the left side of the tongue. No lymphadenopathy was present. It was interpreted as mucous herpes zoster and Acyclovir treatment, 4 grams daily for 7 days was initiated. From the 5th day the subjective symptoms disappeared and local manifestations tend to diminish.

Herpes zoster is the result of VZV reactivation and its spread from a single ganglion to the neural tissue of the affected segment and the corresponding cutaneous dermatome. When the virus is located and multiplies in the geniculate ganglion, facial, aural, and oral lesions of herpes zoster may emerge. Reactivation in the geniculate ganglion can lead to facial nerve (VII) paralysis (because sensory and motor nerves conjoin in nerve VII). VZV and HSV account for the majority of cases of Bell palsy (idiopathic facial paralysis). Our case was not one of those.

Under treatment, evolution was favorable, and 1 month after stopping treatment there were no residual symptoms.

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CLINICAL PRACTICE

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Syrian war shrapnel injury: cubital nerve defect grafting during humanitarian surgical mission. Clinical case presentation

Argentina Vidrașcu[1], Paul Polihovici[2]

Abstract: *Background and aim of this clinical case presentation is to reveal the importance of early nerve injury diagnosis and surgical treatment in war wounded patients.*
Methods. The author treated patients in Amman Charity Hospital were among different plastic surgery cases where limb nerve injuries with nerve grafting indication. The presented case was treated with autologus sural nerve graft.
Results were evaluated at 3 months after the surgery and revealed detectable nerve conductibility at the Electromiography test.
In conclusion, in cases with delayed nerve repair surgical treatment in war wounded patients, the vascularised nerve graft can be a better solution for nerve defect surgical treatment.

Keywords: *NERVE injury, axonotmesis, war wounded patient, sural nerve graft, microsurgery, ulnar claw*.

CASE REPORT

Acute and chronic war wounded Syrian refugees from Zaatari camp and the Syrian battlefields are directly admitted in Amman Al Maqqased Charity Hospital every day.

Among patients treated by the author during November 2015 Humanitarian Mission was a left cubital nerve defect due to a bomb explosion injury.

Peripheral Nerve injury described by Sunderland classification can be: neuropraxia as the lowest degree of nerve injury in which is affected only the nerve conduction and no disruption is done. The second degree is axonotmesis where the axon is damaged but the surrounding tissue remains healthy (1). The most severe form of nerve lesion is neurotmesis where both the surrounding tissue and the axones are disrupted (2).

Patient's surgical and medical history

Female patient, 42 years injured 8 month prior to surgery. Immediately after the explosion the patient received first aid surgical assistance: wound cleaning and direct closure.

The patient felt on the first postoperative day that she completely lost the tactile sense on 4th and 5th fingers on her injured left hand.

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[2] Iuliu Hatieganu University of Medicine and Pharmacy Cluj Napoca

During the healing process, the wound infection and sutures opening occurred and the patient was treated with antibiotics and wound dressings until the wound has completely closed.

Two months after the injury she was examined and diagnosed with post traumatic cubital nerve injury.

Clinical and paraclinical information

The patient presents left cubital nerve chronic palsy with "ulnar claw" appearance and extensive (12 cm) longitudinal scar on the palmar aspect of the forearm (Figure 1). The metacarpophalangeal joints of the 4th and 5th fingers are extended and the interphalangeal joints of the same fingers are fixed in extension.

Figure 1: Left hand war injury with antebrahial wound and hypothenar region muscular atrophy.

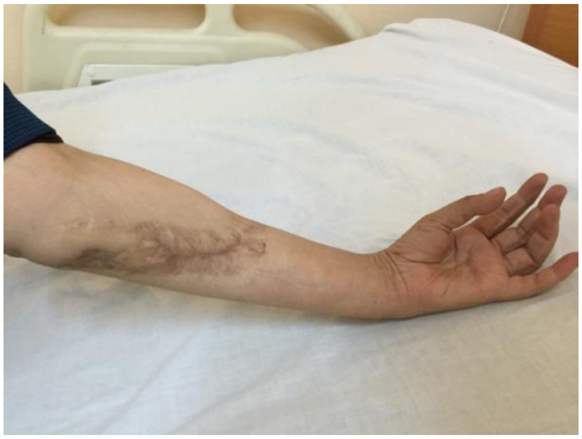

Figure 2: Ulnar claw hand aspect with insufficient flexion of the 4th and 5th finger's interfalangial joints.

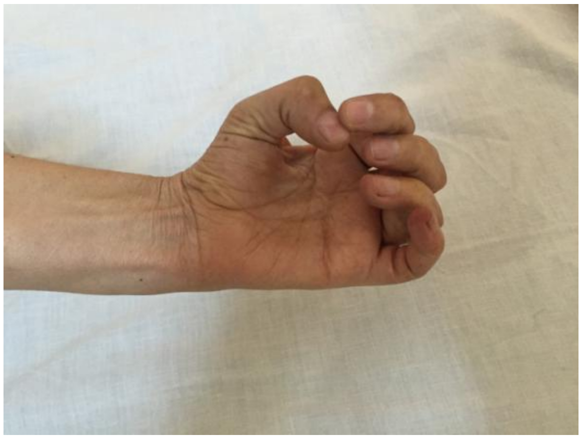

The claw is most obvious when the patient is asked to flex all fingers: the 1st and 3rd digits are flexing due to intact medial nerve innervation and the 4th and 5th ones are remaining extended because of high cubital nerve injury (Figure 2).

The final diagnosis is clarified by dorsal and hypothenar regions denervation and atrophy and by Electromiography testing which reveals the left ulnar transmission is completely disrupted at the scar site (Figure 3).

Figure 3: The dorsal aspect at the cubital denervated hand with muscle atrophy.

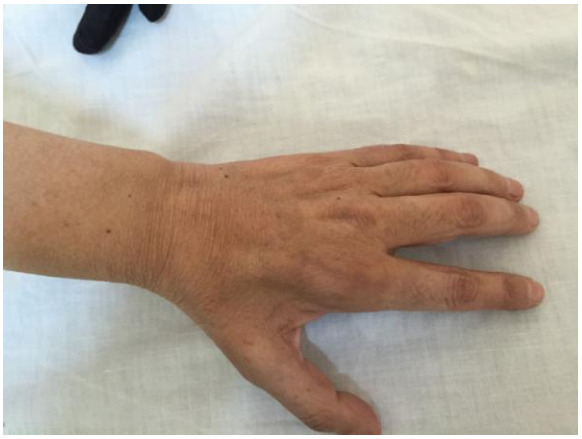

The nerve injury is grade 5 according to Sunderland's system.

Surgical method

Clinical practice and research on nerve injury treatment indicates that the primary surgical repair during the first week is required. If the nerve is disrupted on more than 2 cm length the sural nerve graft must be done.

The surgery was performed under general anesthesia using an interfascicular microsurgical nerve suture technique.

The partial scar excision was done and fibrosis was devided.
Cubital nerve was found being disrupted on 10 cm length at the medial 1/3 of the forearm. After debridement and neurolisis due to myeline degenerative process, the gap real size was 14 cm long (Figure 4).

The autogenous sural nerve grafting was decided to be done.

The right 18 cm long sural nerve graft was harvested and a cable graft designed to bridge the gap (Figure 5).

Figure 4: Cubital proximal and distal nerve stump after surgical debridement.

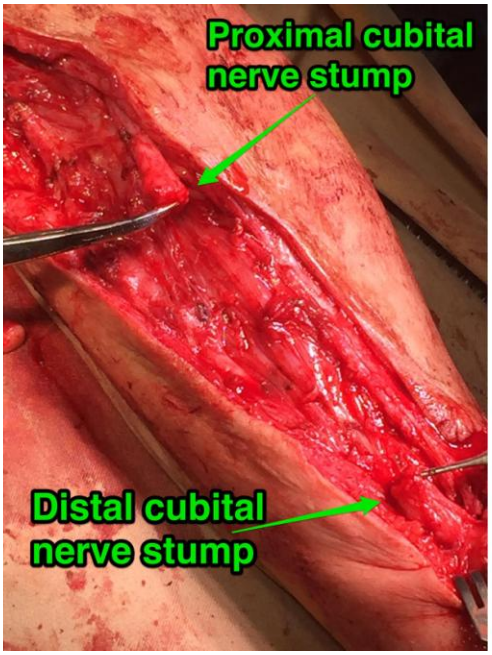

Figure 5: Right Sural nerve graft harvested.

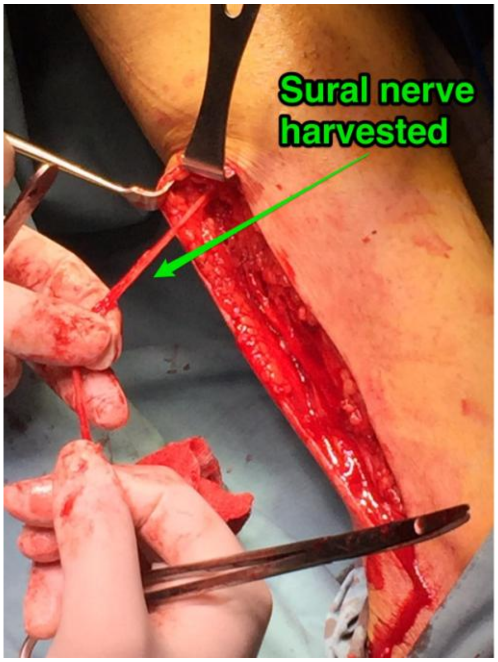

The microsurgical sutures were done at the both nerve ends to achieve nerve connections with separate nonabsorbable 7-0 sutures (Figure 6).

Skin suture and hand splinting was done.

The patient's evolution was favorable under antibiotic therapy for 7 days with no wound closure complications.

Results

After 3 months of kinetotherapy and electrotherapy the nerve conductibility was evaluated as being detectable at the lowest level by Electromiography test. No sensitivity or lightning in the 4th and 5th fingers was detected.

Figure 6: Sural nerve graft sutured on site.

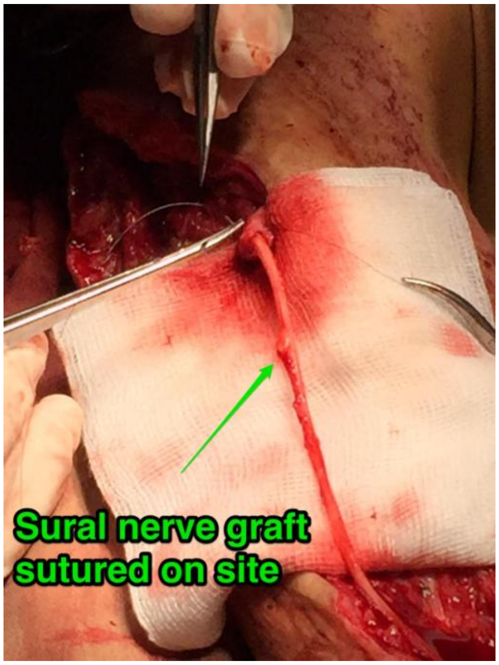

Continued hand therapy rehabilitation is indicated and, 6 and 12 months postoperative evaluation by Electromiography and two point discrimination tests are mandatory at each following checkup.

DISCUSSION AND CONCLUSION

The nerve injury repair should undergo in most of the cases on the emergency surgical treatment basis.

In neurotmesis cases the immediate surgical repair attitude (one week) is the preferred approach for nerve defect treatment in order to obtain a high sensitive and motor functions recovery rate (3).

In chronic palsy (lasting > 3-4 months) associated with muscle weakness, atrophy, surgical outcome is less certain. The duration of palsy and muscle weakness are key factors influencing the prognosis (4).

No conservative treatment can be done in such cases just bridging segmental nerve defect with autogenous nerve graft or decellularized nerve allograft and nerve tubulisation in repairing defects less than 5 cm (5).

In present case the grafting was done 8 months after surgery – this may lead to a less motor recovery but sensitive recovery still can be a good one 6 months after continuous hand rehabilitation(6-8). This demonstrates that 3 months after surgery the cubital nerve conduct was detected even at the lowest level.

In war areas where patients cannot seek specialized medical attention immediate after the injury and their nerve palsy is diagnosed later on, a vascularized nerve grafting should be considered as a clinical alternative for nerve reconstruction if sensitive sense it is not improved after nerve grafting.

Further research comparing late nerve grafting versus vascularized nerve grafting must be done.

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The not so good and the not so bad

Silviu V. Dumitrescu[1]

Today's question: How good is the good cholesterol?

Since before med school we knew that is a bad cholesterol and a good one. And like old days vigilantes we fight the bad one and support the good. But, like the characters in the novels, the bad one seems to have a bright side and the good one has dark secrets.

First let's talk about the LDL. It's involvement in atherosclerosis it's a fact and it's a silent killer. The cardiologists are fighting it since 1987 when the introduction of lovastatin in ischemic cardiac disease was a real breakthrough. But what the neurologists have to say? After the initial enthusiasm was tempered they started to ask: our patients have a LDL cholesterol level way below 70 mg/dl. They are safe. But they look dumber. Why? The statin lead to a mental decline or the low LDL level is to blame? In my opinion is the LDL level. I noticed that in several of my patients with very low LDL levels: they develop very fast dementia symptoms that regress dramatically when their LDL levels rise above 70 mg/ml. Is it a coincidence? Maybe. But what if the bad has good side?

Figure 1: The good and the bad cholesterol [5].

Now let's talk about the sheriff, HDL cholesterol. The one that protect us in the front of vascular aggressors. We know that the bigger is the better and we are very sad when our patient have low levels of it. But 500 years ago we knew that the earth is flat, so what is the truth?

Recently 4 Big Pharma firms developed a new class of lowering cholesterol drugs – Cholesteryl Ester Transfer Protein Inhibitors (CETPi) that were announced to be the new milestone in the treatment of dyslipidemia. Preliminary studies showed a spectacular HDL levels rise.

First to go first to fall – torcetrapib – shortly the mortality increased from an unknown cause.

Next dalcetrapib and evacetrapib – HDL levels reached the top. Well tolerated, few side effects, no increased mortality.

Then the bomb dropped: the studies were shutdown prematurely. Why? Headmaster of the late ACCELERATE study, Steve Nissen declared for Forbes magazine that “the drug didn’t work". I don't think that was the accurate response. In my opinion the drug worked because patients achieved incredible high HDL levels. The problem was that despite these very high HDL levels the cardiovascular events were not different from the placebo group. So I would say that drug proved to be effective in what it was made to do but high HDL levels were useless.

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Here comes the question: is the HDL as protective as we used to believe or just handsome good-for-nothing cholesterol as suggested by those studies?

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) accessible to the public at no charge;

(2) searchable by standard, electronic (Internet-based) methods;

(3) open to all prospective registrants free of charge or at minimal cost;

(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

Review Articles

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication', as presented at http://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

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Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

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industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

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- *About clinical medicine: Is there still such a thing?*
- *MRI fusion biopsy vs transrectal ultrasound guided prostate biopsy – A literature review*
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EDITORIAL

About clinical medicine: Is there still such a thing?

Dan Mischianu

Gral (R) Prof DAN MISCHIANU
Chief of Urology Clinic, Carol Davila Central Emergency Military Hospital
Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

The other day, a distinguished and well known specialist radiologist from Bucharest shared with me two absolutely surprising things.

First: not long ago, an experienced internal medicine physician addressed expressly with the request to radiologicaly examines a patient with, in his opinion "acute pulmonary edema"! The radiologist told me that the “acute pulmonary edema” was actually a "spontaneous pneumothorax" probably due to rupture of emphysematous bubbles. The thoracic surgeon solved the so-called "acute pulmonary edema" actually "spontaneous pneumothorax" by specific means...

The second issue told by the radiologist: I can sit at home in front of my laptop and receive scanned images of patients from different locations (clinics, private hospitals) in the country (Bacau, Constanta, Baia Mare and so on), then I say my opinion and the fee is in my account!

I asked him slightly amazed: Yeah, well, but do you not feel the need to learn more about that patient? Did you forget what we learned in medical and surgical semiology that 80% of the diagnosis belongs to anamnesis? Do you not need to palpate, to auscultate or to listen to what the patient tells you...? Have you not learned anything from the mistake of the internist who "din not use a stethoscope" to find, in fact, that he had no vesicular murmur? My friend did not answer…

In fact what do I want to say in this editorial?

First I want to say that our guild lives in a perfect confusion: notions or concepts of "evidence-based medicine (EBM)", "alternative medicine", "noninvasive" medicine, "medicine x, y, z" and many others can only leave room for speculation. Medicine, as illustrated, has always been great at removing great speculations because of phantasmagoric individuals like Paracelsus or Mesmer...

Secondly, in Adevarul newspaper from 02/19/2016, Theodora Şopaltă, a journalist, was writing sententiously: "There are five types of doctors. Which one do you prefer?" The first type, in the opinion of the author is “The clever" (30% of respondents voted for him), then comes "The Digital doctor” (which probably specify diagnosis texting his phone! preferred by 8% of respondents), then comes “The Wise man" who do not talk to patients about their illness but gives them an accurate diagnosis. Go figure it! In fourth position lies "The Idealist", preferred by 24% of patients, said to be a younger version of "intelligence" and the ending is marked by "Only You", a "pragmatic option of alternative medicine."

Thirdly, I say that doctors may be labeled as "made and born" to practice this noble profession, for which are made after the Hippocratic precepts and not by strange and awkward ideas, good and honest doctors with knowledge of human nature, easily grasping which in this profession gives you a disadvantage and "good for nothing doctors" who easily realize that there is no place for them amongst us and change their job to managers, adventurers, free – thinkers and so on.

To end this Editorial I conclude by saying that a good and honest clinician is the one that accepts and applies the perennial teachings of Hippocrates of Cos and takes into account the lessons of the Great surgeon and Great clinician, Professor Dr. Ion Juvara:

"Whatever novelty and complexity of laboratory methods of investigation, clinical retains its entire value, observation of man by man offering truths that never grow old”

I believe and hope that there still is the so-called "Clinical medicine"!

Article received on March 2, 2016 and accepted for publishing on May 21, 2016.

REVIEW ARTICLE

MRI fusion biopsy vs transrectal ultrasound guided biopsy – A literature review

Constantin A. Rădulescu[1], Mihai F. Rădulescu[2], Gina A. Ciucă[1], Dan Mischianu[1,3]

Abstract: *Introduction: Current diagnosis of prostate cancer is based on transrectal ultrasound guided random biopsies. These procedures alongside with the discovery of PSA lead to a tremendous improvement of prostate cancer diagnosis rate in the last decades. Random ultrasound guided biopsy has its minuses and several attempts were made to improve the detection rate of the disease. MRI-US fusion targeted biopsies is one of them.*

Aim: This paper is a literature review of several important European studies and tries to draw a conclusion for the usefulness of MRI-US image fusion prostate biopsy in improving prostate cancer detection

Material and methods: We have analyzed 5 papers published since 2013 which compare random ultrasound biopsies with MRI-US fusion. The analyzed parameters were clinical significant cancer detected as described by the study and any cancer detected

Results: All 5 papers show superiority in cancer detection rates for both clinically significant and any cancer.

Conclusions: MRI-US is a useful tool for improving detection rate of clinically significant prostate cancer.

INTRODUCTION

Prostate cancer is a serious public health matter, being the first cancer in men in terms of incidence and the second cause of cancer death in the U.S. Cancer, in general is taught to surpass in the following year cardiovascular diseases as the leading cause of mortality. Prostate cancer, in particular is becoming more and more important due population aging. [1]

While throughout the history prostate cancer was poorly understood, with few cases recorded due to the low life expectancy, during the XXth century groundbreaking discoveries were made in understanding, diagnosing and treating the disease.

Early diagnosis methods included open transperineal biopsies, blind transperineal needle biopsy and finger guided transrectal biopsy. First attempts to use transrectal ultrasound for prostate imaging were made by Takahasi and Ouchi in 1963 but they obtained poor images [2]. Watanabe in 1968 performed first clinical useful TRUS. In 1989 Hodge and al. started the modern era of standard sextant

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transrectal ultrasound guided needle biopsy. Stamey developed a technique which consisted in performing a biopsy without knowing the tumor location within the prostate. Stamey's method was a major advance over older methods in which biopsy needles were guided only by the examining finger.(2) During the late 1990's and early 2000's standard protocol was modified introducing extended and saturation biopsies. Nowadays it is estimated that several million biopsies are performed each year around the globe.

Ultrasound guided transrectal biopsies have 2 major disadvantages: detection of focal carcinomas with little or no clinical significance on one side and the important percentage of falsely negative biopsy cores.

MRI was invented in 1971 by Paul C. Lauterbur for which he was granted Nobel Prize for Medicine in 2003. MRI evolved as being the most important tool for prostate cancer staging, especially if performed in a multiparametric manner.

Prostate biopsies can be performed directly under IRM guidance or images may fused either in a cognitive manner, a method dependent on operator's experience, or MRI-US image fusion may be used. When using US-MRI fusion, standard transrectal ultrasound is performed and software is utilized to fuse the ultrasound images, at the time of the examination, with IRM images that were obtained before.

3D images of the prostate are obtained [3]. Fusion devices use a variety of technologies to perform image fusion. There are devices which perform robotic tracking via a mechanical arm with built-in encoders with commercial products: Artemis and BioJet. Electromagnetic tracking is used by the following UroNav and Hi-RVS. A 3D US probe is used for tracking and fusing images by Urostation. [4]

MATERIALS AND METHODS

We have analyzed 5 major studies which were performed since 2012 in terms of number of subjects, medium PSA level, overall cancer detection and clinically significant cancer detection rates. All 5 studies analyzed were paired cohort studies which compared transrectal standard biopsy with MRI-US fusion biopsy. The population analyzed was heterogenous. The only thing that was common to all patients was high PSA levels. One study, (Sonn GA et al, 2014)[5] had only patients with prior negative biopsy while the other 4 studies had mixed patients with or without prior negative biopsies. The percentage between patient with and without negative biopsies varied.

The number of patients varied between 30 (Fiard et al, 2013) [6] and 347 (Kuru et al, 2013) [7] with a total of 677 patients. Medium PSA recorded in each study varied between 6.3 ng/ml (Fiard et al, 2013) and 9.85 ng/ml (Kuru et al, 2013).

Clinically significant prostate cancer was defined by each study. Sonn et al defined clinical significance as Cancer positive cores with length 4 mm or more or Gleason 3+4.

Kuru et al used the NCCN (National Comprehensive Cancer Network) criteria. Fiard et al and Rud[9] et al considered clinical significance when Gleason 7 (3+4) or higher was recorded on biopsy cores while Rastinehad et al[8] used Epstein criteria for clinical insignificant tumors: clinical stage < T1c; PSA density < 0.15 ng/ml; fewer than 3 positive cores and less than 50% cancer on positive cores.

Clinically significant disease, defined as above, was diagnosed by means of transrectal ultrasound guided biopsy in 4.8% (Rud et al,2012) to 38% cases (Kuru et al, 2013) with a mean value of 25.26% and by means of MRI-US fusion biopsy in 21.7% (Son et al, 2014) to 50% cases (Rud et al, 2012) with a mean value of 40,76%.

The difference in diagnosing clinically significant disease varied between 7% to 41.4%. MRI-US fusion biopsy was more accurate in diagnosing clinically significant prostate cancer compared to US transrectal biopsy with an overall advance of 17.36%.

When analyzing all types of cancer both clinically significant and insignificant 4 out 5 studies showed superiority of MRI-US fusion biopsy ranging from 0.2% to 53.2%. Sonn GA et al reported 27.5% for

TRUS biopsy vs 23.7 % for MRI-US fusion. This difference in favor of TRUS is probably linked to the study design as it is the only cited study that enrolled only patients that had undergone a prior negative TRUS biopsy.

Table1: Prostate cancer both clinically significant and any cancer

Paper	Clinically significant TRUS	Clinically significant MRI-US fusion	Any cancer TRUS	Any cancer MRI-US fusion
Sonn GA et al	14,7%	21,7%	27,5%	23,7%
Kuru et al	41,1%	50,4%	50,4%	50,6%
Fiard et al	33,3%	50%	43%	55%
Rastinehad et al	32,4%	44,8%	48,6%	50,5%
Rud et al	4,8%	46,2%	14,3%	67,5%
Medium values	25,26%	42,62%	36,76%	49,46%

DISCUSSIONS

All studies show superiority for detecting clinically significant disease with a smaller number of biopsy cores for MRI-US fusion biopsy and all but one showed overall superiority in detection of prostate cancer.

The disadvantages of this review are the fact that there isn’t a standardized definition of clinical significance, the lack of homogeneity of the analyzed population, the fact that some studies have enrolled both patients with prior biopsies and patients without biopsies, and the lack of standardized protocol for MRI-US fusion biopsy as there is a standard protocol for TRUS biopsy.

CONCLUSIONS

MRI-US fusion biopsy has proven to be more accurate in detecting clinically significant cancers but is still long way from becoming a standard procedure because of the complex technique required, the financial burden caused by the need of a prior MRI.

MRI is capable of accurately diagnosing prostate cancer, in assessing its extension, its aggressiveness. MRI is also a useful tool for following patients with prior diagnosis of prostate cancer and probably, in the future it will be the major tool for focal therapy of the tumor.

Transrectal ultrasonography still holds the crown for prostate cancer detection and will certainly remain the most used guidance technique for prostatic biopsies for long time with good results in the general population. Patients with negative biopsies and who experience a continuing rise in PSA levels, patients at risk when talking of hereditary antecedents of prostate cancer and some other risk categories may and will certainly benefit from the development of the MRI fusion technique.

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REVIEW ARTICLE

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The role of contrast-enhanced ultrasound in risk assessment of carotid atheroma

Silviu Stanciu[1], Cristina Enciu[1], Ioana Răduţă[1], Dan Stoicescu[1], Adrian Anghel[1], Daniela Anghel[1], B. Olan[1], Lucian Ciobîcă[1]

Abstract: *Background and objective: Contrast-enhanced ultrasound, used to assess atherosclerotic carotid plaques, improves visualization of vessel wall irregularities and depicts intraplaque neovascularization. This article illustrates the use of contrast-enhanced ultrasound in the risk assessment of carotid atherosclerotic lesions, especially in challenging plaques evaluation.*

Materials and methods: For 23 patients with difficult duplex ultrasound examination due to carotid tortuosity or calcifications we assessed plaque morphology (contour, echogenicity and stenosis degree) using contrast substance (Sonovue, Braco) with dedicated vascular low mechanical index CPC software.

Conclusion: Contrast-enhanced ultrasound is a new, noninvasive, and safe procedure for imaging carotid atherosclerotic lesions. It is a valuable tool for evaluating the vulnerable plaque at risk for rupture and for the diagnostic of the development and severity of systemic atherosclerotic disease.

INTRODUCTION

Carotid atheromatous pathology is responsible for almost 1/3 of strokes through 3 basic mechanisms: atherothrombosis, distal embolism from plaque origin and hemodynamic effect in the case of significant, severe stenosis (1).

Carotid ultrasound technique remains the benchmark for the assessment of carotid-vertebral arterial axis, mainly regarding the presence of obstructive atherosclerotic plaques with a proper evaluation of morphology and stenosis degree, by combining the two methods of examination: Duplex mode-B mode and Doppler mode (Color and Spectral).

Numerous studies have shown the beneficial role of endarterectomy or stenting in stroke rate reduction, in symptomatic and asymptomatic patients with significant stenosis of over 70%, but there is no clear attitude regarding the therapeutic approach of asymptomatic patients with a lower degree of stenosis. In these patients, in whom stenosis has no cerebral hemodynamic effect, several studies emphasize on plaque morphology, by identifying unstable plaques which would play an important role in the development of symptoms (2).

Often, we encounter in daily clinical practice cases where carotid axe imaging becomes challenging due to intense calcifications in the carotid bulb and origin, due to the presence of carotid tortuosity or because of the impossibility to distinguish between

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preoclusive (over 90%) stenosis and complete occlusion, which would change the therapeutic approach (3,4).

To overcome these limitations and better characterize unstable plaques, by highlighting the intraplaque neoangiogenesis (5), recently a new imaging technique has been developed using contrast substance, which has the advantage of strictly vascular and plaque and wall vascularization distribution.

MATERIALS AND METHODS

During the last year, for 23 patients with difficult duplex ultrasound exams due to carotid tortuosity or calcifications we performed a contrast enhanced evaluation in order to assess plaque vascularization and stenosis degree.

In consequence, the protocol for plaque morphology description (contour, echogenicity and stenosis degree) consists in 1 ml bolus of contrast substance (Sonovue, Braco) administered through a catheter in the right arm, followed by 10 ml bolus of saline with the acquisition of 6 seconds clips during 4-5 minutes, maneuver which can be repeated to achieve the objectives of the study.

In our department we currently use dedicated vascular CPC software with low mechanical index, integrated into a SIEMENS Sequoia 512 CV system.

In each case we observed the contrast substance plaque penetration, carefully identifying and assessing intraplaque vascular pattern.

Detecting intraplaque angiogenesis

In the majority of patients (68%) with echogenic plaques and no significant stenosis (lower than 70%) intraplaque vascularization has been found, with outside to inside filling pattern, data similar to literature findings (see images).

The existence of this new angiogenesis vessels, mainly situated at the base of atheromatosic plaques, can be considered a risk zone for possible plaque rupture, resulting in major stroke.

Figure 1: Transverse cross section right after left CCA bifurcation, 30 seconds after contrast substance has been administered. Note the lack of opacification of left ICA due to the presence of atheromatous plaque responsible for a 60-70% stenosis.

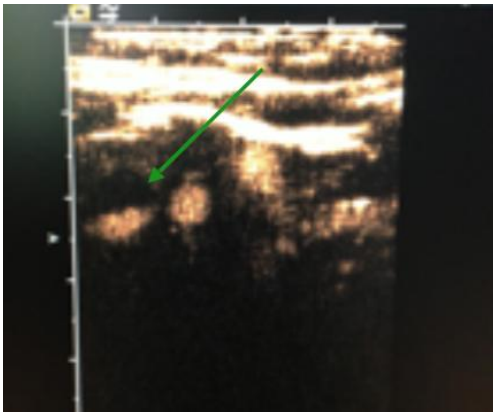

Figure 2: Longitudinal section 40 sec after the injection allowing plaque visualization at the origin of the left internal carotid artery with contrast enhancement at the base (arrow)

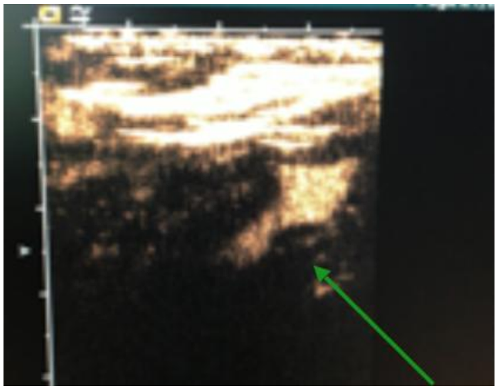

Figure 3: Same plaque from figure 2 at 60 seconds after injection

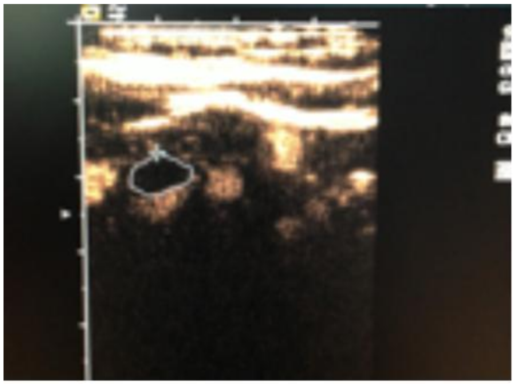

Figure 4: Contrast enhancements at 70 seconds with a filling patter from the outside to inside

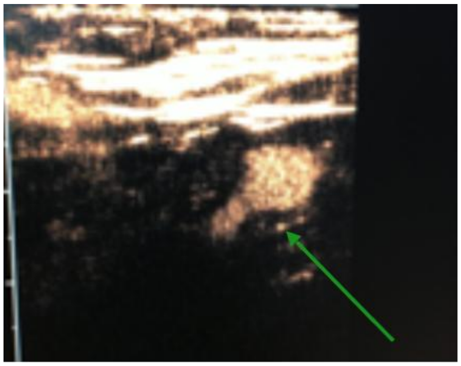

In consequence, identifying intraplaque neovascularization in patients with no significant carotid stenosis can be considered a noteworthy additional argument when considering an interventional procedure (stenting or endarterectomy) for stroke prevention.

DISCUSSION AND CONCLUSIONS

The concept of unstable or vulnerable plaque, similar to that seen in coronary pathology, is based upon identifying dangerous plaques with immediate stroke like complications, with acute inflammation and angiogenesis (which precede and cause plaque rupture or ulceration), that can't always be detected through conventional duplex ultrasound.

Carotid ultrasound remains the leading non-invasive exploration regarding pre-op/pre-interventional or post-op/post interventional functional and anatomic evaluation. Contrast-enhanced ultrasound studies, bring additional information regarding carotid stenosis quantification in difficult evaluation situations (calcified plaques or difficult to explore vascular tortuosity) but especially brings functional information regarding intraplaque vascularization, thus identifying unstable and high stroke risk plaques allowing an appropriate therapeutic approach.

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REVIEW ARTICLE

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The importance of life quality questionnaire in patients with prostate cancer, pre- and post-radical prostatectomy

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Abstract: *This study analyzes the satisfaction of the patients with radical prostatectomy according to the signs and symptoms included in the EORTC QLQ – C30 and EORTC QLQ – PR25 questionnaires, including their relation with the health state that influences the pre and postoperative life quality.*

Fifty patients with prostate cancer in a localized stage were studied and analyzed in a prospective manner and for a period of 12 months, before and 6 months after the radical prostatectomy. In choosing the patients, the common denominator was the use of the same postoperative plan and the lack of postoperative complications, as well as the lack of chronic diseases.

Erectile dysfunction, urine incontinence were quite frequent after radical prostatectomy, while the urinary obstruction and the weak urinary stream were less frequent. In this regard, people noticed a significant decrease of life quality.

Although the study addressed a small group of patients, the results are similar to those belonging to other clinical studies. Even if the aspect of life quality is more rarely taken into account, the questionnaires regarding the life quality are specific, useful and quite cheap in evaluating different therapies.

Keywords: *life quality, prostate cancer, questionnaire*

INTRODUCTION

Prostate cancer represents a disease with increased prevalence, being the second cause of death in U.S.A, on the first place being the lung cancer. However, the prostate cancer shows optimistic survival rates, the rate of the deaths caused by the prostate cancer decreased in the U.S.A, from the beginning of 2007 with 3.2%[4]. This fact led to the appearance of a model of patients with prostate cancer, patients for whom the quality of life is important, as well as the impact of the disease on this.[5].

For those who study the clinical problem, the aim of evaluating life at the patients with prostate cancer consists in evaluating the effects of the treatment on the symptoms and in identifying the negative impact of the disease on the life of the patient.

The quality of life is a concept that has started to be studied and taken into account in 1946 when in the

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Preamble of the Constitution of World Health Organization, health was defined as it follows: ,,*Health is the state of complete physical, mental and social wellbeing that does not reduce at the absence of a disease or of an infirmity"[1,2].*

Smith and his contributors consider the notions of life quality and the health as being two defined concepts, the life quality being related even more to the mental health, while the state of health hints at the physical function [3].

Meaning ,,a holistic auto evaluation of satisfactions regarding the individual", the life quality is a much – disputed concept, multidimensional that, though seems quite easy to understand it, is difficult to be defined.

In a more restrictive meaning is used the term of life quality regarding health ,,health related quality of life" (HRQL),to define the influence of health and nursing on the life quality, being influenced by numerous factors like: financial state, habitat, the work place, the spirituality, the social support and the health of the individual [2].

The evaluation of the aspect regarding the quality of life in patients with prostate cancer is done at a large scale in the clinical studies, but it must be underlined the importance of using these evaluation methods in clinical practice [1].

The questionnaire, a descriptive instrument, is the one through which the evaluation is done in a subjective manner a wide range of the disease effects that are, later, resumed into a final score.

The evaluating instruments of the quality of life come to complete the other methods of investigation of the patient with prostate cancer, for this very reason it would be opportune to facilitate the use of these by the people who study clinical problems, the subsequent therapeutic conduct being able to improve the quality of life for these patients.

The most used questionnaires in the clinical practice in evaluating the patients with prostate cancer are the following: EORTC QLQ-PR25 and EORTC C-30 questionnaires.

The quality of life as a medical concept explains the effects of diseases and those of the therapy on the stage of comfort for the patient that is the subject of evaluation with the help of the questionnaires.

The questionnaires can be completed by the medical personnel, the patient being asked the questions after having read and signed the informed consent or directly by the patient.

The first method has the advantage that the doctor can explain the patient the questions more accurately, diminishing the misunderstandings, but the result cannot have a maximum accuracy as not all the patients can be very honest while facing other persons.

MATERIAL AND METHOD

Fifty patients with prostate cancer in a localized stage were studied prospectively for 12 months, before and at 6 months after the radical prostatectomy between January 2015 – December 2015, at the Urology Clinic of the Carol Davila Central Military Hospital.

In choosing the patients, the common denominator was the use of the same postoperative plan and the lack of postoperative complications, as well as the lack of chronic diseases.

The average age of the patients included in study was of 65.

The patients were asked to fill in the EORTC C-30 and EORTC QLQ-PR25 in Romanian language, after having read and signed the informed consent.

The characteristics of the group of patients were recorded and processed in a SPSS database (version 22 – Chicago k, USA).

The EORTEC C – 30 questionnaire has 30 questions that shows us a pre and postoperative image of the patient, regarding the cognitive, emotional, social functions or regarding the signs and symptoms of the patients, using a scale with more items or singular ones.

The questionnaire, excepting the two last items, can be completed with values between 1 and 4, so,(A) the absolute amplitude of the variation of answer is 3. The scale of life quality is given by the answers at the questions 29 and 30 at which the patients can choose values from 1 to 7, the absolute amplitude being of 6.

Items	Number of items	Number of question
Physical state	5	1 - 5
Daily activities	2	6, 7
Emotional states	4	21 - 24
Cognitive functions	2	20, 25
Social and family relations	2	26, 27
Tiredness	3	10, 12, 18
Nausea and Vomiting	2	14, 15
Pains	2	9, 19
Dyspnoea	1	8
Insomnia	1	11
Anorexia	1	13
Constipation	1	16
Diarrhea	1	17
Financial problems	1	28
General health state	1	29
Life Quality	1	30

To analyze the questionnaires I used a different formula for functions, namely: Value _functions = 1 – ((Raw score – 1)/Absolute Amplitude)*100 comparing to Value _ symptoms = Raw score -1/Absolute Amplitude)*100.

As a result, I noticed that a higher value of the functions shows a higher rate of functionality and independency, while a higher value of the symptoms shows problems of dependency of the patient.

One can notice in the chart that, only emotionally, the patient feels better after 6 months after surgery. I must mention that the younger patients (<65 years old), consider that, physically and socially speaking, they were better during the preoperative period.

The indicator of the physical state is made up of 5 items: the resistance of hard activities, independency in dressing and eating, as well as the chronicle tiredness and the need of laying in bed. From the questionnaire, one can see that the physical resistance of the patient decreased from 94.11 to 90.23.

Figure 1: Score of life quality regarding functions

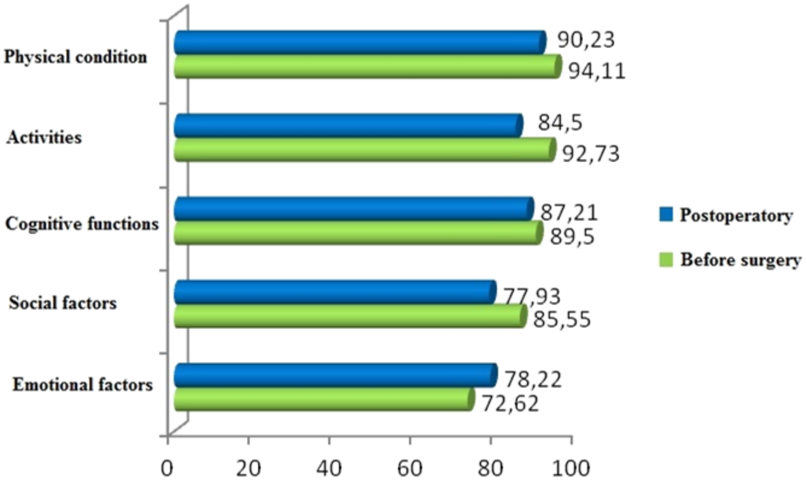

One can see an increase of tiredness after prostatectomy, from 11.1 to 16.2 and, also in the symptoms of nausea and vomiting.

The postoperative pain had a significant increase from 10.33 to 12.22.

The tiredness increases proportional with insomnia and the sensation of weakness, and the pain interferes with the daily activities especially at the patients of more than 65 years old.

Figure 2: Score of signs and symptoms

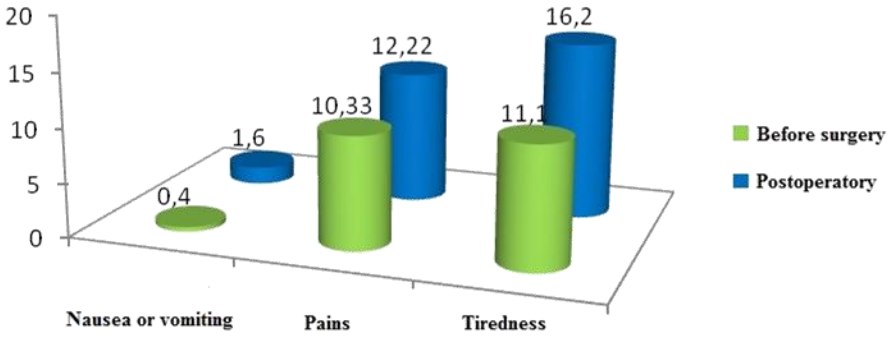

Figure 3: Signs and symptoms

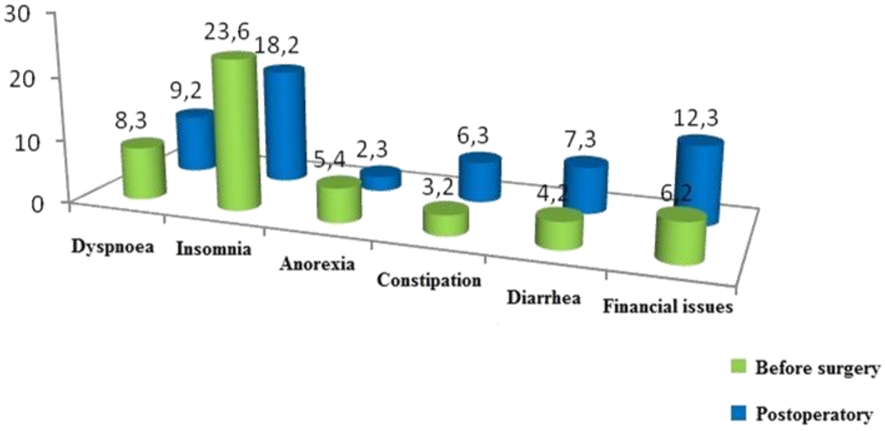

Figure 4: Quality of life

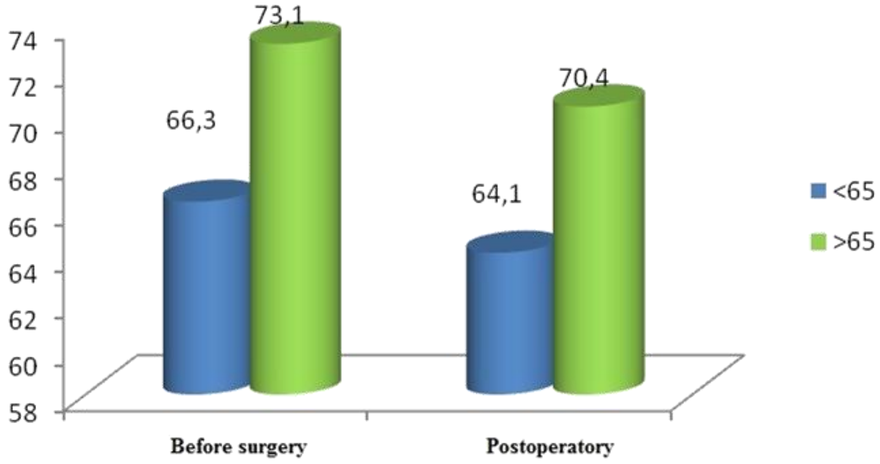

One can see an improvement of the quality of life of the postoperative patient regarding insomnia and anorexia. The digestive symptoms and the financial problems will accentuate postoperatively especially at the patients after 65 years old and the younger ones.

The last items are like a conclusion of the questionnaire permitting the patient to define the last week by the point of view of his health and the manner he sees the quality of his life at a certain time.

Analyzing the data, one can see the most unsatisfied patients on the ground of their physical state and quality of life are the patients younger than 65 years old. Totally, the difference between the quality of life appreciated by people younger than 65 years old pre and postoperative was of 2.2.

Younger patients appreciated the quality of life pre and postoperative lower than the patients of 65 years old.

The EORTC OLO – PR 25 questionnaire contains 25 questions divided into four evaluation functional scales.

Items	Number of items	Number of question
Urinary symptoms scale	9	31-39
Intestinal symptoms scale	4	40-43
Scale regarding the symptoms owing the treatment	6	44-49
Sexual functions scale	6	50-55

The significant differences were regarding the urinary symptoms scale, postoperative 13.86 comparing to preoperative of 7.88. In accordance to the age, there were differences regarding urinary symptoms scale, those of 65 years old had lower scores, at an average of 9.86.

Regarding the sexual functions scale, those younger than 65 had higher scores, at an average of 10.11 units.

The erectile dysfunction appears in a degree of 67% at different levels, while 34.9% shows no affliction of the erectile function.

In the postoperative group 23% presented with mild incontinence while severe and moderate incontinence was present in 4, 7% of the patients.

Postoperatively the frequency of the urinary incontinence was greater in patients older than 65 than those younger than 65.

CONCLUSIONS

To identify the type of treatment is vital for the success of the oncologic therapy of the prostate cancer, though the complications appeared after a radical prostatectomy influences the life quality of the patient. One of the objectives in carrying about patients is in helping at stabilization of the urinary continence and of sexual function, in improving the life quality of the patient. A more critical evaluation of the type of treatment is essential for developing an efficient strategy.

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SYSTEMATIC REVIEW

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Tumor markers in gastroenterology: useful or useless

Cătălina Diaconu[1], Mădălina Ilie[2], Gaudia Mănescu Avram[1], Laura Voicu[1], Daniel O. Costache[1], Raluca S. Costache[1,2]

INTRODUCTION

Tumor markers are biomarkers that can be found in blood, urine or different body tissues, produced either by cancer cells or normal cells in response to cancer or in different noncancerous conditions (gastrointestinal stromal tumors). Their use varies from cancer screening on a population basis, to staging different tumors or to evaluate the efficiency of the treatment. Most frequently used in gastrointestinal malignancies are: carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9 and α-fetoprotein. However there are many markers that can be useful in dealing with gastrointestinal cancer such as: carbohydrate antigen 125, cyfra 21-1, chromogranin, tumor M2-PK, neuron-specific enolase, smooth muscle actin [1]. Future research show the utility of multiple tumor marker test, when by combining more tests in parallel the predictive value might be increased. For example: when talking about pancreatic or stomach cancer one might combine: CEA, CA19-9 and CA 72-4 [1,2].

ALPHA FETOPROTEIN

So far, alpha fetoprotein is the most studied serological marker in the surveillance of hepatocellular carcinoma, derived from the liver cells and yolk sac [3]. Its' use is debatable. Some studies show that a cut-off of 20 ng/mL carries a sensitivity of 41% to 65% and specificity between 80% and 94%, with a positive and negative predictive value of 25 and 98 percent. Even though The American Association for the Study of Liver Disease (AASLD) guidelines shows that cirrhosis is a possible cause of elevated AFP, its sensitivity (60%) is higher than that of ultrasound (58%). Therefore current data from the literature support the use of AFP combined with ultrasound as the surveillance tests of choice, taken every 6 months, leading to early detection of hepatocellular carcinoma (HCC) [4]. Despite the fact that more than 40% of the patients with HCC have normal AFP, serum levels higher than 500 mcg/L in a high-risk patient is diagnostic of HCC [3,5].

There is a demonstrated correlation between the tumor size and serologic AFP: higher levels with a sudden acceleration appear with HCC that measure more than 3 cm [6]. Moreover serum levels from 1,000 to 10,000 mcg/L are encountered in tumors past 5 cm in diameter. Date from the literature associate high levels of AFP with poorly-differentiated tumors, but further studies are required [7].

False positive values of AFP may be related to flares of activity in chronic viral hepatitis (B or C). Therefore the use of this serologic marker is very important in patients with hepatitis B or C during treatment with nucleotide analogues for hepatitis B or drug acting

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agents (interferon free) for hepatitis C, when we cannot encounter flares of transaminases [9].

AFP remains an important prognostic marker in patients after resection or in those who are taken into consideration for transplant. High values over 1,000 mcg/L (believed a surrogate of vascular invasion) associates extremely high risks of recurrent disease after transplantation. Moreover some studies assign this value as an exclusion criterion for liver transplantation in patients with HCC that meet the Milan criteria, in order to improve posttransplant outcome [10]. An assay of serum AFT post-transplant is in order every 3 months for 2 years if initially elevated, then every 6 months. This serologic marker remains an independent prognostic predictor of outcome after ortotopic liver transplant for HCC [11].

Table 1: Causes of elevated serum alpha-fetoprotein [8]

Causes of elevated serum AFP

- Hepatocellular carcinoma
- Cirhossis due to hepatitits B/C
- Non-seminomatous germline tumours
- Ataxia-teleangiectasia
- Pregnancy with babies that carry neural tube defects or Down syndrome
- Other gastrointestinal tumours (gastric cancer, biliary tract cancer, pancreatic cancer)

CARCINOEMBRYONIC ANTIGEN

Discovered in 1965, CEA is a glycoprotein associated with colorectal cancer with a rather low sensitivity and specificity. Even though CEA is not used as a diagnostic test, levels over 5.0 ng/mL can predict a unfavorable prognosis, regardless the tumor stage [12].

A Cochrane review showed that a cut-off value of 2.5mcg/L for CEA carries a pooled sensitivity of 82 percent and specificity of 80 percent. For a cut-off of 10 mcg/L the sensitivity lowered to 68%, but the specificity reached 97 percent, due to fewer false positive values. CEA testing should be done once every 3 to 6 months. One must bear in mind the possible false positive values of CEA (most encountered between 5 and 10 ng/mL). One possible cause is due to the adjuvant therapy with fluorouracyl (therefore some recommend the end of adjuvant treatment before measuring CEA).

There are many arguments against using CEA as a screening test such as: almost half of the recurrences of CRC present normal values of CEA; is has no impact on improving survival and the quality of life and it is not cost-effective. One strong argument in favor of testing CEA is the detection of recurrences [13].

Even though CEA is prohibited in the mass screening and diagnostic pathway of colorectal carcinoma (CRC), it has value in the follow-up of patients with diagnosed CRS according to the American Society of Clinical Oncology guidelines: from surgical treatment planning to post-treatment follow-up and prognosis [14,15]. High serum levels of CEA after 4 weeks of the surgery (this being twice the plasma half-life of CEA) indicate the persistence of the disease, occult metastasis and the need to reevaluate the prognosis

[8]. In conclusion, CEA levels can be associated to the TNM classification of CRC, due not only to its prognostic value, but also the decision of adjuvant chemotherapy.

Table 2: Benign and malignant causes of high levels of seric CEA[8,13]

BENIGN	MALIGNANT
Gastritis	Colorectal cancer
Peptic ulcer disease	Breast cancer
Diverticulitis	Lung cancer
Pancreatitis	Gastric cancer
Liver disease	Pancreatic cancer
Chronic obstructive pulmonary disease	Cholangiocarcinoma
Diabetes	
Cigarette smokers	

Studies showed that high CEA even in node negative patients show a worse prognosis, a higher rate of recurrence, therefore adjuvant chemotherapy could be considered [16].

CEA can be used to monitor the response to treatment in metastatic disease. Whilst the decrease of seric levels of CEA shows the favorable response to the treatment, the rising level of CEA is incompatible with tumor regression [8].

Some studies show that bile CEA levels are elevated in cholangiocarcinoma, but not in benign diseases. Used as a single tumor marker, CEA is neither sensitive enough, nor specific enough to diagnose cholangiocarcinoma [8]. Different combinations of CEA and CA 19-9 are used to diagnose cholangiocarcinoma in patients with primary sclerosing cholangitis.

When using cut-off values of 5.2ng/mL for CEA and 180 U/mL for CA 19-9 the sensitivity was 100% and the specificity reached 78%. Despite this, other data from the literature show a sensitivity of 63% for detecting cholangiocarcinoma in patients with PSC, whilst the specificity when using a combined CA 19-9 and CEA only reaches 33%, albeit with higher specificity [13,17].

CARBOHYDRATE ANTIGEN 19-9

Most frequently used serological marker for cholangiocarcinoma, CA 19-9 has a wide variation in sensitivity and specificity. However, values over 1000 IU/mL are encountered in advanced disease with peritoneal dissemination [18].

CA 19-9 levels can be elevated in 45% of patients with non-malignant affliction since the tumor marker is produced by abnormal cyst epithelium. Benign diseases that can be associated with high values of CA 19-9 are: cholestasis, cirrhosis and cholestatic diseases [18,19].

In patients with primary biliary cholangitis CA 19-9 levels can be increased due to cholangitis or biliary obstruction. Literature states that a cut-off value of 100 IU has a 86% sensitivity and a 89% specificity for cholangiocarcinoma [4]. However CA 19-9 remains the most commonly used serological marker in cholangiocarcinoma with a sensitivity of 89% and a specificity of 86% for a cut-off over 100 IU/mL.

Moreover values over 1,000 IU/mL are associated with unresecability [17].

CA 19-9 has a higher specificity in the diagnosis of cholangiocarcinoma in patients with PBS. Therefore CA 19-9 combined with ultrasound or magnetic resonance cholangiopancreatography (MRCP) are recommended in the annual screening of PBS patients for cholangiocarcinoma [17,20].

Studies shows that the median preoperative CA 19-9 levels in pancreatic adenocarcinoma were 379 IU/mL in patients who would not benefit a curative resection, whilst in patients where it was resected it was 130 IU/mL.

Moreover, values over 130 U/mL postoperative demonstrate unresectability, the likelihood of R0 resection and long-term outcomes [4]. CA 19-9 has a low specificity; therefore studies show that it should not be used as a screening test for pancreatic cancer. However serial testing once every one to three months of this tumor serological marker in the follow-up of patients after curative surgery or in patients with advanced disease that receive chemotherapy was proven useful. Moreover, elevated values of CA 19-9 could precede the radiological images of recurrent disease (must be confirmed with imaging studies or biopsy) [21].

Table 3: Malignant causes of high values of CA 19-9 [18]

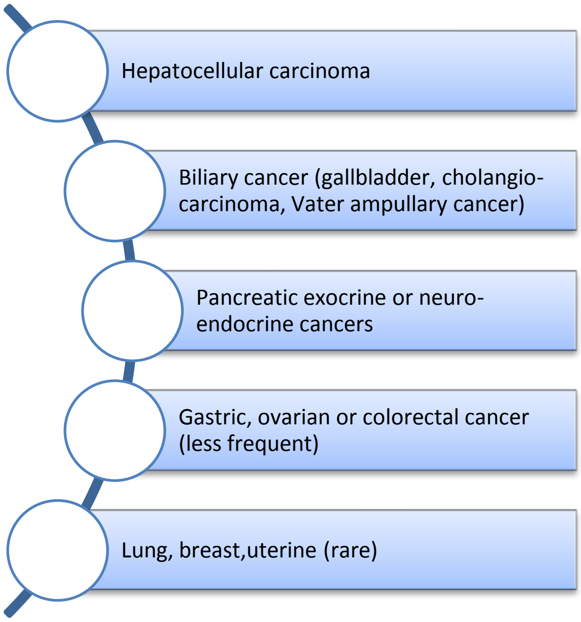
Hepatocellular carcinoma
Biliary cancer (gallbladder, cholangio-carcinoma, Vater ampullary cancer)
Pancreatic exocrine or neuro-endocrine cancers
Gastric, ovarian or colorectal cancer (less frequent)
Lung, breast,uterine (rare)

Table 4: Cut-off values for CA 19-9 related to the presence of cholangitis (may associate fever, right upper quadrant pain and leukocytosis) or cholestasis (serum bilirubin larger than 3mg/dL) [18]

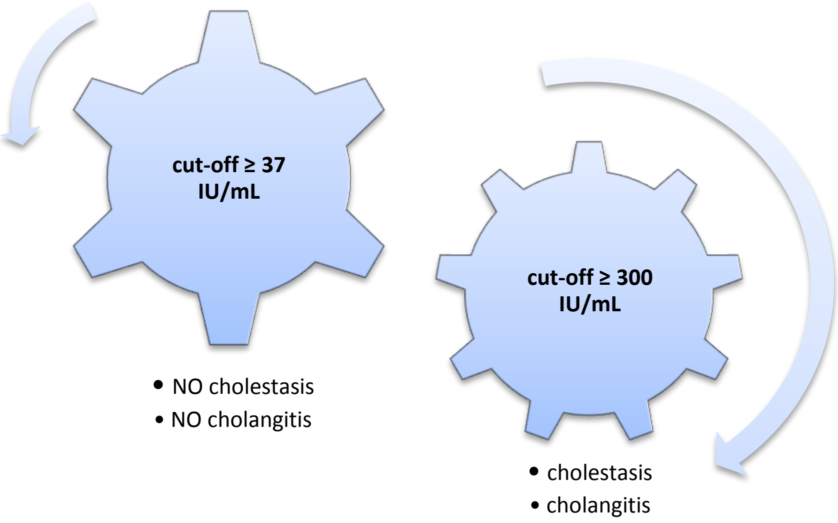
cut-off ≥ 37 IU/mL
• NO cholestasis
• NO cholangitis
cut-off ≥ 300 IU/mL
• cholestasis
• cholangitis

In patients that underwent curative surgery, the normalization of the values of CA 19-9 within 6 months is associated with twice the mean of survival than in patients that kept elevated CA 19-9 [17].

In individuals with negative Lewis phenotype (7% of the population), CA19-9 is unreliable since the tumor marker needs the expression of Lewis blood group antigen, leading to a false negative response [21].

Tumor markers could be used to differentiate chronic pancreatitis and pancreatic cancer. CA 19-9 was once used for this purpose, however due to its' low specificity and sensitivity this purpose was forgotten. Despite the fact that CA 19-9 is not used for surveillance in chronic pancreatitis, it can be helpful in families with hereditary pancreatitis [22].

OTHER TUMOR MARKERS

Cancer Antigen 125 (CA 125) is a glycoprotein most frequently used in the diagnosis, screening and prognosis of ovarian cancer, but with a very low sensitivity and specificity especially in early disease. It can have elevated values in non-gynecologic cancers such as: colon, liver, gallbladder cancer [4,8].

Carbohydrate Antigen 72.4 (CA 72.4) is a serological tumor marker used as a specific predictor for clinical recurrence in patients with gastric and colorectal cancer that undergo surgery and to follow up the treatment [23,24]. CA 72.4 with values higher than 7 IU/mL postoperative are associated with a worse prognosis, higher risk of recurrence and higher death risk. Studies show that in stage II colorectal cancer this tumor marker discriminates better than CEA the patients that will relapse or die from those with a favorable prognosis [25,26].

Chromogranin A is a protein contained by the neuroendocrine tumor cells. Elevated values can be encountered in a number of benign and malignant diseases. When referring to gastrointestinal disease we can find high values of chromogranin A in: carcinoid tumors of the gastrointestinal tract, pancreatic neuroendocrine tumors, colon cancer, hepatocellular carcinoma, pancreatic adenocarcinoma, liver cirrhosis, chronic atrophic gastritis, inflammatory bowel disease, and others. Chromogranin A has been associated with treatment response and might have prognostic value in neuroendocrine tumors [4,27].

CONCLUSION

Tumor markers remain a helpful tool in different afflictions. *AFP* is now considered the surveillance test of choice along with ultrasound in hepatocellular carcinoma. *CEA* is not used in the diagnosis of colorectal cancer, but in the prognosis and follow-up after curative surgery. *CA 19-9* is especially useful in the diagnosis pathway of cholangiocarcinoma in patients with PBS. Moreover, values over 1000 IU/ml are highly suggestive for cancer.

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ORIGINAL ARTICLES

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Air MEDEVAC in case of multiple casualties – The experience of civilian-military cooperation in RoAF

Dragoș C. Tudose[1], Daniel Nică[1]

Abstract: *Introduction: Starting September 2010 in Romania was created the Military Emergency Medical Service (SMMU) by the Ministry of National Defense, which has as main mission to provide first aid and save the lives of military personnel during military operations using special equipped MEDEAVC aircraft. Nationwide exist the national emergency system which operates thru 112- SMURD acting in support of the civilian population. In case of accidents with multiple victims the experience has shown the need for collaboration between the two systems, in order to save lives. In the last 5 years there has been an increasing Airlift missions (MEDEVAC) with multiple victims executed by joint civil-military medical teams using military aircraft.*

Material and methods. This paper provides a review of the most important aspects of particularities, advantages and disadvantages of this type of medical transport using the MEDEVAC missions based study carried out by the Air Force in recent years.

Results and conclusions. Performing these tasks presents challenges to mission planning, use of medical equipment and procedures, command-control system, exercise programs jointly joint medical teams and, of course, managing a large number of patients in flight.

The large number of patients transported safely and in the shortest time, regardless of weather conditions recommends this type of medical intervention.

Given the Romanian military presence in various theaters and that NATO strategic medical evacuation is a national responsibility, the capacity of air transport in case multiple casualties is a priority.

Keywords: *RoAF, flight surgeon, in flight medical care, multiple casualty, interoperability, cooperation, joint civil-military medical teams*

INTRODUCTION

Starting September 2010 in Romania the Military Emergency Medical Service (SMMU) was created by the Ministry of National Defense, which has as main mission to provide first aid and save the lives of military personnel during military operations using special equipped MEDEVAC aircraft. Nationwide exist the national emergency system which operates thru 112 emergency number – SMURD (Serviciul Mobil de Urgență, Reanimare și Descarcerare; Mobile Emergency Service for Resuscitation and Extrication) acting in support of the civilian population. In case of accidents with multiple victims the experience has shown the need for collaboration between the two systems, in order to save lives. In the last 5 years there has been an increasing Aeromedical evacuation missions (AME - MEDEVAC) with multiple victims executed by joint civil-military medical teams using military aircraft.

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[1] 90th Airlift Base Romanian, Romanian Air Forces

This paper provides a review of the most important aspects of particularities, advantages and disadvantages of this type of medical transport using the MEDEVAC missions based study carried out by the Romanian Air Force in recent years.

Performing these tasks presents challenges to mission planning, use of medical equipment and procedures, command-control system, exercise programs jointly joint medical teams and, of course, managing a large number of patients in flight.

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Given the Romanian military presence in various theaters and that NATO strategic medical evacuation is a national responsibility, the capacity of air transport in case of multiple causality is a priority.

In this modern time beside the classical war conflict we are facing more and more new challenges like mass causality due to terrorist attacks, car accidents, fire and explosions in our civilian day by day life.

Due to financial and personnel restraints, neither one of the 2 system, civilian or military one, can properly face by itself this kind of incidents and it's just a question of time until the number of patients will overwhelm the capability and capacity of each system.

In Romania 2 systems coexist for Aeromedical Evacuation: the civilian SMURD (Mobile Emergency Service for Resuscitation and Extrication) using 8 EC135 helicopter and 1 Piper PA-42 Cheyenne III airplane and the military system under de MoD/RoAF command with 6 IAR 330 Puma helicopters and 1 C27J Spartan airplane. The drawback of the civilian system is that there aircraft don't fly at night and in severe meteorological condition. In the military one we don't have enough emergency medical personnel to cover 24/7. So this is the basis on which we start cooperation.

Due to the fact that national evacuation it's a civilian responsibility the military AME assets may be used to augment civil capability in 2 ways: Military aircraft assets with civilian medical crew and Military aircraft assets with mixed civilian-military medical crew.

Military aircraft used in this type of missions are:

- IAR 330 Puma MEDEVAC helicopter: fully equipped as an intensive care unit can carry up to 3 critically ill patient, in all-weather condition day or night with a range of 500 km;

- C130 Hercules has a maximum capacity of 74 stretchers with a range of 2000 km and have been use for years for CASEVAC missions from Iraq and Afghanistan;

- C27 Spartan: the newest and most modern aircraft from RoAF fleet can carry maximum 24 stretchers with autonomy of 6 hours and 2500 km. The characteristic of this aircraft is the possibility of carry 2 special ICU modules with dedicated monitor, ventilator and O2 capability.

Another characteristic is the MEDEVAC pressurization mode in which the aircraft will maintain the cabin pressure at ground level until flight level 130 (4000 m) in order decrease the hypobaric stress on the patient.

The last 6 years statistics show that from a total of 53 MEDEVAC missions with RoAF aircraft 15 (28.31%) where missions with multiple patients with a total of 100 patients (72.46%). The vast majority of this missions were made with C27J Spartan (12 – 80%), 2 with C130 Hercules (13.33%) and only 1 using helicopters (6.66%). That can be explained by the fact that the IAR 330 Puma helicopters can carry only 3 stretcher patient but at this load the space is severely limited. Another factor was the meteorological condition. This type of mission with multiple patients is used on longer distances (more than 300 km) and this is why the fix wing aircraft are used more (speed and distance advantages).

Messaging and alerts: because the civilian medical system can't put on alert the RoAF assets the messaging system goes like this: when somewhere in Romania an accident is happening the initial receiving medical facility contact the secondary or tertiary medical center if the complexity of the injury require a higher level of medical treatment. If the patients or patients are accepted for transfer then the medical coordinator of SMURD send an aeromedical evacuation request to CNMC (National Command Military Centre) who pass it to RoAF COA (Air Operation Component). At this level the Aeromedical Evacuation Officer have an up-to date image of the position, availability and the response time of each aircraft. The decision is made about which type of aircraft (airplane or helicopter) shall be used and the alert message is scrambled.

The particularity of multiple air MEDEVAC mission is that because the number of patients and the diversity of pathology the necessary medical equipment and the component of the medical team on board must be carefully planned on very short notice.

The military medical crew consists normally in 1 or 2 flight surgeons (medical officer, permanent working in an air base, having 1 year course in emergency medicine, 6 month training in aviation medicine, fit to fly according with the medical standards of the National Institute of Aviation Medicine) and 2 to 4 flight nurses (3 years degree in nursing after graduating Nurse College, 6 month training in emergency medicine, fit to fly) but in case of multiple cases this crew can or must be updated with ICU specialist or neonatologist accordingly to the medical need (in June 2012 MEDEVAC mission from Bucharest, ROU to Hanover, DEU with 3 children from 7 day to 3 years old with cardiac malformations in with the crew was upgraded with 1 neonatologist and 2 nurses beside the flight surgeon and the flight nurse from RoAF).

In term of medical equipment due to the national standardization there are no compatibility problems in term of coupling power tension or O_2 pressure. The only real challenge is how to be able to get all the necessary equipment in time and in the right quantity (in July 2008 after a explosion in a Gheorghieni local wood factory we accomplished a MEDEVAC mission for 10 severely burn patients, 2nd 3nd and 4th degree burns, all respiratory assisted and mechanically ventilated so the problem was how to have 10 ventilators and 10 multifunction monitors in that plane in less than 2 hours.)

AEROMEDICAL ASPECTS

Hypoxia is the greatest threat to anyone flying. Physiological effects of hypoxemia can be detected in healthy individuals at lower altitudes of 3000 m but more rapidly in certain patients. It occurs due to changes in ambient pressure. Cabin pressurization reduce this problem especially airplanes, but patients with respiratory failure may be at risk for hypoxemia current cabin altitude achieved. Adjusting the fraction of inspired oxygen to maintain the partial pressure of inspired oxygen constant throughout the mission is a technique useful clinically for prevention of hypoxemia. The wide availability of pulseoximeter decreased incidence of hypoxemia in the MEDEVAC missions with early recognition.

Accelerations: Acceleration or deceleration is a vector quantity, having both magnitude and direction. For this reason, the correct positioning of the patient in the idea to limit acceleration-induced forces sustained should be carried out. Acceleration forces that are experienced in helicopters or planes during routine operations (due to flight techniques and procedures ensuring low range of acceleration forces during MEDEVAC missions) tend to be small in size or as great as those observed in terrestrial transport vehicles.

Volume of gas: ambient pressure decreases with increasing altitude. Pressure changes with altitude and the change can affect a number of medical devices and patients. Contrary to common belief, cabin pressurization does not eliminate the problem. Pressurization allows a comfortable flight altitude manned which could not otherwise be achieved, but in general, not altitude from sea level, and thus, the equipment and the patient will be exposed to a pressure change. Any gas enclosed in a structure therefore becomes a concern. Air trapped in the sinuses, for example, can expand and cause discomfort and equipment (air-filled cuffs) can be damaged or can injure patient with the change altitudes.

Humidity: Humidity is a particular concern in fixed-wing missions because cabin air is taken from the rarefied atmosphere outside, and even in pressurized aircraft and when heated may contain very little moisture. This can lead to dryness and discomfort of the patient secretions during flight.

Noise: Modern aircraft produce quite a lot of noise. In most aircraft cabins is pretty quiet for conversation and patient evaluation but cabins of helicopters are so loud that exclude pulmonary auscultation. Soundproofed helmets and intercom systems are needed.

Vibration: Vibration is a form of repetitive alternative motion. The two major sources of vibration during MEDEVAC missions are powerplant and turbulence of the air moving aircraft. The vibrations cause discomfort and fatigue to patients and also to medical team, and are transmitted to medical equipment in flight and can be the source of errors and monitoring failures.

CONCLUSION

The Multiple victim accidents are a REALITY and the transport of those patients to an adequate level of care facility is a NECESSITY. Air medical transport put additional stress on patients (fear of flying, physical discomfort), on medical staff (unconventional and insufficient workspace with great limitation regarding medical equipment and medication and the impossibility to receive advice and expertise) and on medical equipment (operating in a vibration, pressure and temperature differential environment). Influence appears to be greater for the transport helicopters and in case of multiple patients.

Air medical evacuation missions with multiple victims require: rigorous planning, exercises (based on realistic scenarios) and resources (time, money, personnel, equipment).

Romanian Air Force aircraft equipped MEDEVAC are on alert 24/7 and the training of medical staff allow prompt intervention in case of accidents with multiple victims;

Civil-military intervention can be a solution for those situations, on the condition of legislation harmonization in order to provide fast and efficient mobilization of resources and cooperation between military and civilian institutions.

ORIGINAL ARTICLES

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Finite element analysis of a modified short hip endoprosthesis

Augustin Semenescu[1], Florentina Ioniță Radu[2], Ileana M. Mateș[2], Petre Bădică[3], Nicolae D. Batalu[1]

Abstract: *A finite element simulation of the mechanical static features for a modified short hip endoprosthesis was performed. The corkscrew-like femoral stem was modified introducing more turns of the thread. By such an approach it is expected that for some cases the mechanical fixation of the prosthesis to the bone will be improved or the use of the cement for bonding is not necessary. Our scenario was estimated for titanium and stainless steel, and both materials show good safety factors. Mechanical stress is expected to be distributed more uniform in the bone for the new design with more turns of thread.*

Keywords: *modified short hip endoprosthesis; design; finite element analysis; titanium; stainless steel*

INTRODUCTION

The increase in the average life expectancy rose the age at which different surgical interventions are performed. For example, the total replacement of the hip is made on a typical patient of 60-80 years old, and often patients are older, in some cases even over 100 years old. The primary hip intervention can be followed by several revision surgeries [1,2,3]. This is a reason for designing modular endoprosthesis, where damaged parts can be easily replaced.

A short femoral stem is recommended for patients where the femoral bone allows a minimal resection, and the bone structure has enough mechanical resistance to support the stress induced by implant [4]. A positive aspect of our design consists in screwing the implant into the bone, avoiding its hammering [5,6] that has a high risk of femoral neck fracturing. The prosthesis has a cylindrical metallic body, with an exterior thread, continued with a truncated sleeve, and ended with a junction neck (Figure 1). Inside of the implant there is a hexagonal hole for the insertion of a torque screwdriver (not shown). The distribution holes are built-in and they are intended to be used to spread the cement for fixation after the stem is screwed in the bone.

In this work a modified design is proposed. Namely, more turns (7 instead of 3) [7] of the screw-component of the endoprosthesis are considered. Expectations are to obtain an improved implant-bone fixation and a more uniform distribution of the forces that will allow a longer lifetime with less revision surgeries: it is well known that after about 10-15 years the prosthesis loosens and a replacement may

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be required [8]. For certain cases it is thought that our improved design may also provide a cement-free fixation. Finite-element analysis is used to estimate mechanical features of the prosthesis with the modified design. Two materials, titanium and stainless steel are considered.

MATERIALS AND METHODS

1. CAD design and materials properties

The endoprosthesis was designed [9] in Inventor Professional 2016, and the materials properties were assigned from the software database.

The proposed materials for the endoprosthesis are titanium and stainless steel (Table 1). Materials are considered isotropic and linearly elastic.

2. Mesh settings

All the settings are detailed in [6]. The total number of the resulted elements was 1,694, and the total number of the nodes was 3,287.

Table 1. Mechanical properties of materials used in simulation.

Material	Density [g/cm^3]	Young's Modulus [GPa]	Poisson's ratio	Shear Modulus [GPa]	Yield strength [MPa]	Tensile strength [MPa]
Titanium	4.51	102.81	0.36	44	275.6	344.5
Stainless Steel	8.00	193.00	0.30	86	250.0	540.0

3. Boundary and loading conditions

The endoprosthesis, either the initial or the modified design, is considered fixed on its stem, thread, and under the supporting disk for the scenario when using cement (Figure 1.a, blue region). For the cementless approach only the thread and the part under supporting disk are considered fixed. (Figure 1.b, blue region).

A load of 6000 N magnitude is analyzed, as the most unfavorable case [7]. Both scenarios are studied without the implant/cement/bone interactions.

A static compression load is distributed on the upper surface of the disk (Figure 1.c, blue region denoted with an arrow). The safety factor is based on the yield strength.

RESULTS AND DISCUSSION

The simulation results are shown in Table 2 and Figures 3, 4 and 5. The differences between the initial and modified designs are not significant, for selected materials, titanium and stainless steel.

A longer thread suits better for a cementless fixation (Figure 1.b), due to the larger contact area. For the stainless steel, the analysis is shown in Figure 5. The static finite element analysis shows no significant differences when compared to the "cemented" scenario.

Figure 1. Fixed constrained areas of the modified short femoral endoprosthesis: (a) for "cemented", and (b) "cementless" scenarios. (c) Selected area for force distribution.

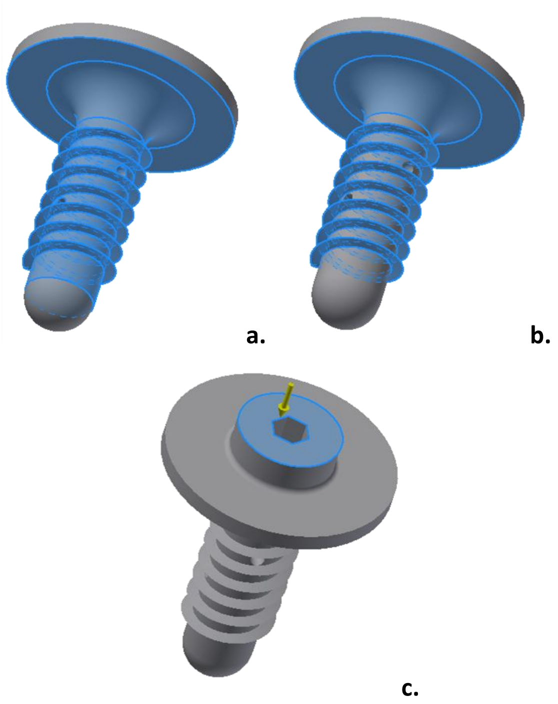

This approach confirms that the new design does not affect the mechanical behavior of the endoprosthesis. A longer thread is also expected to provide a better stress distribution in the femoral neck and further studies are required.

Table 2. Maximum displacements, maximum Von Mises Stress, and the minimum safety factor for a 6000 N load and different threads.

Materials	Displacement [max, µm]		Von Mises Stress [max, MPa]		Safety factor [min, ul]	
	initial	modified	initial	modified	initial	modified
Titanium	2.407	2.42	58.55	59.51	4.71	4.63
Stainless Steel	1.319	1.326	59.68	59.86	4.19	4.18

Figure 3. Displacement magnitude (a, b), Von Mises Stress distribution (c, d), and overall safety factor (e, f) for *titanium* endoprosthesis, “cemented” scenario, for the initial (a, c, e) and modified (b, d, f) design.

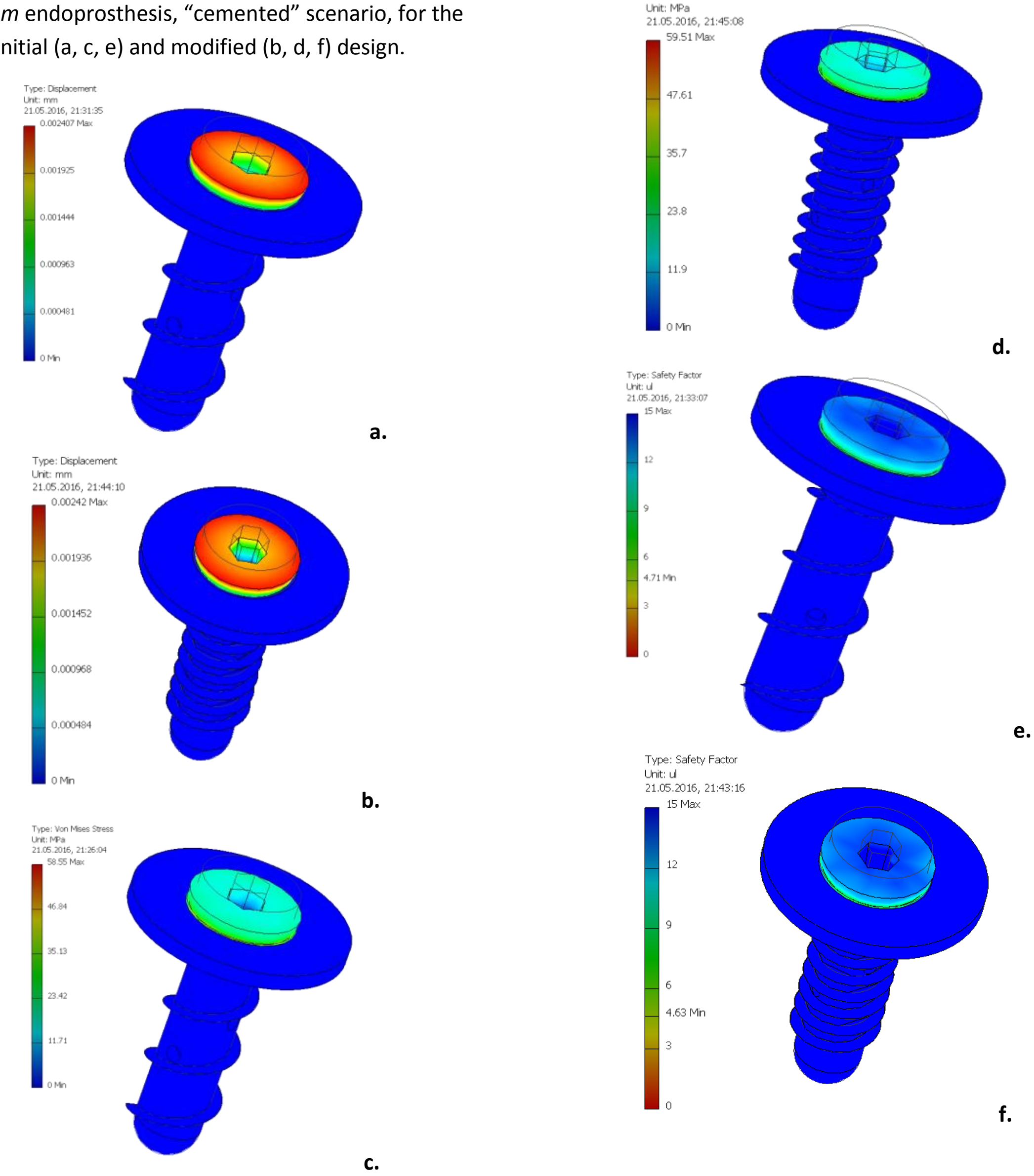
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0 Min
a.
Type: Displacement
Unit: mm
21.05.2016, 21:44:10
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0.001936
0.001452
0.000968
0.000484
0 Min
b.
Type: Von Mises Stress
Unit: MPa
21.05.2016, 21:26:04
58.55 Max
46.84
35.13
23.42
11.71
0 Min
c.
Type: Von Mises Stress
Unit: MPa
21.05.2016, 21:45:08
59.51 Max
47.61
35.7
23.8
11.9
0 Min
d.
Type: Safety Factor
Unit: ul
21.05.2016, 21:33:07
15 Max
12
9
6
4.71 Min
3
0
e.
Type: Safety Factor
Unit: ul
21.05.2016, 21:43:16
15 Max
12
9
6
4.63 Min
3
0
f.

Figure 4. Displacement magnitude (a, b), Von Mises Stress distribution (c, d), and overall safety factor (e, f) for *stainless steel* endoprosthesis, “cemented” scenario, for the initial (a, c, e) and modified (b, d, f) design.

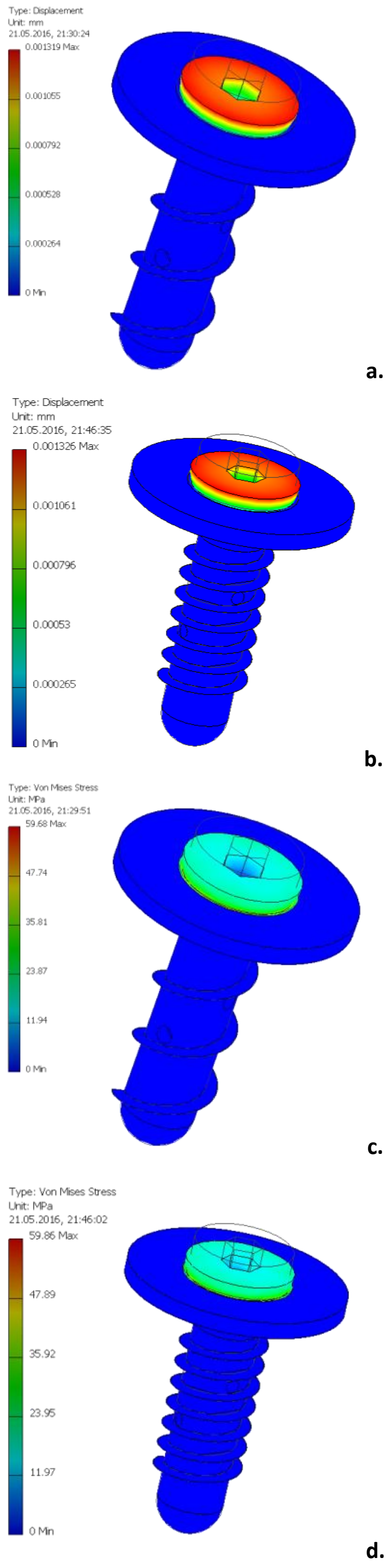
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0 Min
a.
Type: Displacement
Unit: mm
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0.00053
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0 Min
b.
Type: Von Mises Stress
Unit: MPa
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47.74
35.81
23.87
11.94
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c.
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Unit: MPa
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47.89
35.92
23.95
11.97
0 Min
d.

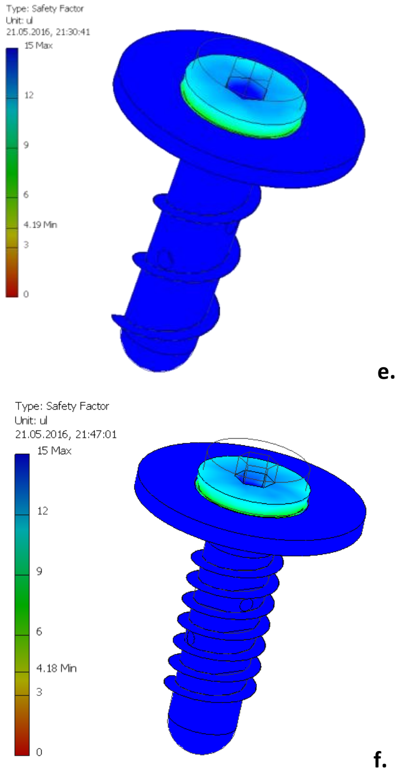
Type: Safety Factor
Unit: ul
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15 Max
12
9
6
4.19 Min
3
0
e.
Type: Safety Factor
Unit: ul
21.05.2016, 21:47:01
15 Max
12
9
6
4.18 Min
3
0
f.

Figure 5. Displacement magnitude (a), Von Mises Stress distribution (b), and overall safety factor (c) for the *stainless steel* modified endoprosthesis. A “cementless” scenario (6000 N load) is considered.

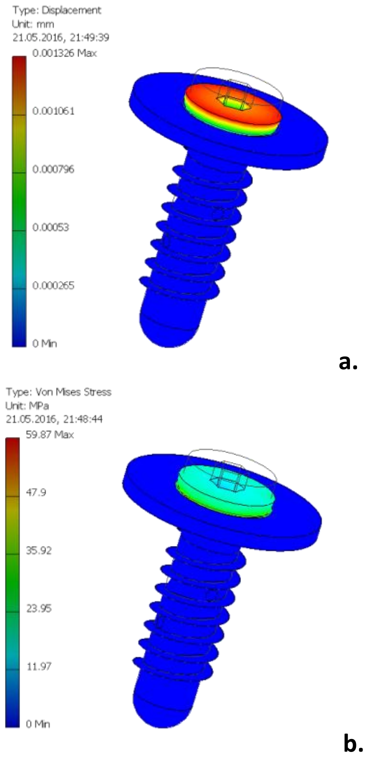
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a.
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59.87 Max
47.9
35.92
23.95
11.97
0 Min
b.

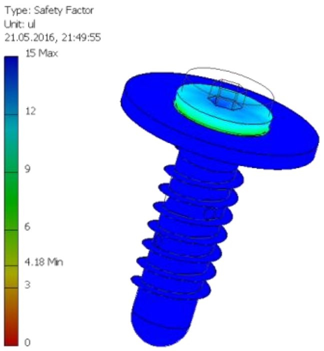

c.

CONCLUSIONS

A modified short hip endoprosthesis design is proposed as a better solution than the previous one, where a fixation using cement is considered. The new simple design consists in a longer thread that increases the friction force between endoprosthesis and bone by increasing the contact surface. The finite element analysis shows that the modified design does not affect the mechanical behavior. This design may allow, in some cases, a cementless fixation. Further tests are necessary to confirm our results.

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Optical "biopsy" in laryngeal cancer detection

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INTRODUCTION

Squamous cell carcinomas of the larynx is by far the most frequent malignant tumor of the head and neck region, representing 1-2% of all malignancies diagnosed world-wide. Treatment results of early cancer are not unfavorable: for the reported 5-year-survival rate of Tis, T1, T2 laryngeal cancer patients ranges from 80 to 90% [1]. At present, indirect laryngoscopy is widely used for the evaluation of laryngeal cancer and precancerous lesion, while direct laryngoscopy under general anesthesia with biopsy remains to be the gold standard.

The expression optical "biopsy" has entered into usual speech among researchers in the domain of biomedical optics. In fact this joining of terms is a paradox, because "biopsy" refers exactly to the removal of tissue, while the signification of "optical" is that tissue is not removed. The real meaning of this expression consists in amount of optical measurements mediated by optical fibers which allow the physician to perform an instant tissue diagnosis in vivo, in situ, noninvasively and in real time, previously possible only by using histological analysis. The main reason of this is to avoid the surgical removal of biopsy tissue samples.

These refined optical techniques go beyond standard endoscopic methods by offering improved image resolution, contrast, tissue penetration and delivering biochemical and molecular knowledge about mucosal disorders. For some endoscopic application, for which "random" biopsies are repeatedly taken to discover premalignant or early malignant lesions, optical measurement could enable "guided biopsy", with increased probability for sampling a diseased site, reducing the number of tissue samples. Thus, additional purpose is certified by the potential for reduced health-care costs by eliminating unnecessary histology. Besides, the instant diagnostic information can reduce the emotional trauma to the patient awaiting an answer [2].

Recent technological advances in fiber optics, light sources and detectors have stimulated the tremendous development of many optical methods that promise to notably improve capability to visualize and evaluate human epithelium in vivo [3,4,5]. Some of these techniques are used for detection of laryngeal cancer: fluorescence spectroscopy, optical coherence tomography, narrow-band imaging, contact endoscopy and confocal endomicroscopy. This review describes the basic biophysics of light-tissue interactions, evaluates the strengths and weaknesses of each technique and explores clinical and preclinical evidence for each approach.

OVERVIEW

Fundamental parameters that characterize tissue

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optics in terms of radiative transfer are: absorption coefficient, scattering coefficient and anisotropy and measuring those constitutes a central issue in biomedical optics [6].

a. The absorption coefficient (μa) determines the probability of absorption events for a photon that propagates in tissue. Absorption occurs due to coupling of photons energy at a particular frequency with electronic or vibrational transitions energy. Depending on the spectral field, absorption is caused by:
- Cellular DNA and protein (amino acids and nucleic acids) in ultraviolet;
- Specific chromophores (hemoglobin, melanin, etc.) in visible;
- Water, OH and amine infrared.

b. Scattering coefficient (μs) determines the probability of a photon to be scattered during propagation. Scattering is caused by fluctuations of the refractive index of the environment in microscopic scale (ultra-structural heterogeneity) such as water-lipid interfaces membranes inside the cell and surrounding cells and fibers of collagen in the extracellular matrix. We can say that the absorption properties of tissue are tied to tissue biochemistry, while the scattering ones are related to its morphology.

c. Anisotropy (g) is the average cosine scattering angle and weighted the scattering coefficient effectiveness.

d. Alongside the three basic parameters, in medical practice, are also used: attenuation coefficients (total and effective); reflection; the refractive index; reflectance.

Cheong et al [7] realized (1990) a comprehensive compilation of optical tissue properties (absorption coefficients, scattering, total and effective mitigation and anisotropy for different biological tissues at a variety of wavelengths). They concluded that for a specific tissue, large areas of these parameters are frequent, indicating the sensitivity and vulnerability of such measurements to sample variation, detection equipment, boundary conditions and adopted light propagation model. Confidence in the obtained values can be compromised by any of these factors. After 1990, numerous studies have improved this knowledge, creating optical exploration methods that will be presented below.

1. Spectroscopy

1.1. Autofluorescence endoscopy (AFE)

Autofluorescence was first observed by Policard [8] in 1924, but Alfano [9] demonstrated in 1984 differences in spectral profiles of cancerous and normal tissues. In the last decades, this technique was used for screening in early detection of cancers of upper- and lower-GI tract and also lower-airway, and recently it was used in identifying precancerous and cancerous lesion [10].

Tissue autofluorescence is based on the presence of endogenous fluorophores, which emit visible green light on appropriate excitation. This involve structural proteins (collagen, elastin) mainly located in the submucosa, but also keratin, porphyrin and enzymes of the respiratory chain such as nicotinamide adenine dinucleotide and flavin adenine dinucleotide within the epithelium [11].

Figure 1: Squamous cell carcinoma of the right vocal fold: White light endoscopy (a); Autofluorescence endoscopy (b) [13]

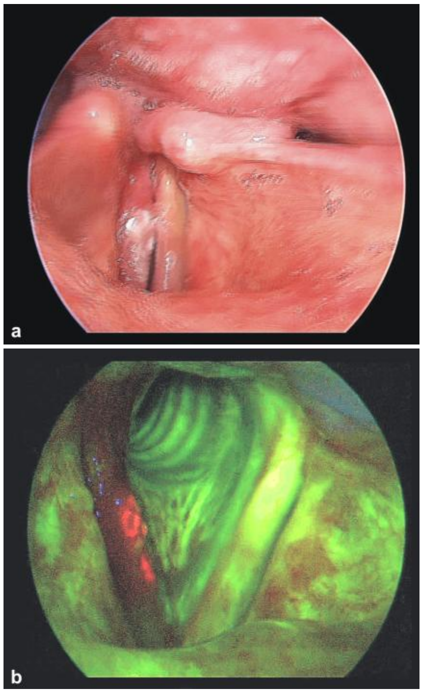

Because of an altered metabolism in tumor cells, premalignant and malignant tissues show a decreased quantity of fluorophores. Moreover, epithelial thickening prevents the excitatory blue light from reaching the submucosa. Both mechanism results in a loss of autofluorescence, with a color change from green to red-violet [12].

In AFE, healthy mucosa and benign lesion covered by normal epithelium (polyps, nodules, cysts, Reinke's edemas) present green color (no loss of autofluorescence). Scar tissue following surgery or radiotherapy shows a violet color; the same aspect is found in hyperplasia and chronic inflammation.

AFE can be used in early detection of premalignant and malignant lesions, in guided biopsy and oncological follow-up. This technique can only detect changes on epithelium, but not in the deeper structures [14].

Many studies have found that AFE has a better sensitivity and specificity in detecting laryngeal malignant changes than the classic white light endoscopy (WLE) [15,16,17]; a meta-analysis made by Kraft and co. in 2010 concluded: in identifying precancerous and cancerous lesions of the larynx, sensitivity (91% vs. 73%), specificity (84% vs. 79%) and accuracy (88% vs. 77%) of AFE were superior to WLE alone[18]; false-positive diagnoses result from chronic laryngitis, teleangiectatic polyps, granulomas[19] and papillomas; false-negative diagnoses are found in extreme hyperkeratosis, necrosis and inflammation [20,21].

There is a variant of this method involves the study of autofluorescence laryngeal mucosa after topical application of aminolevulinic acid, preferentially captured by the cancer cells, that became colored in orange-red (because protoporphyrin IX), while normal mucosa appear green [22].

Figure 2: Normalized mean and difference spectra for squamous cell carcinoma and non malignant tissue in larynx [26].

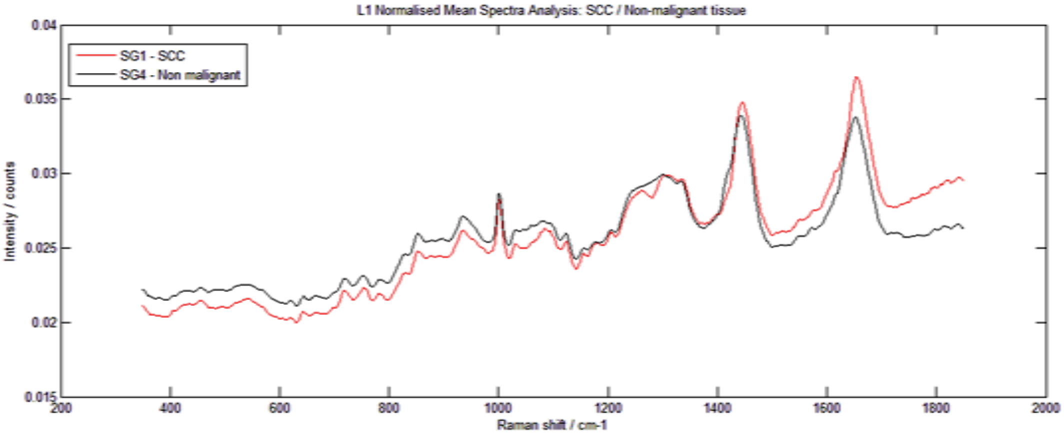

1.2. Raman spectroscopy

This method involves an effect whereby a small portion of tissue scattered light has a different frequency from that of the incident beam, with an equal amount of vibration frequencies of the molecules in the tissue; the effect is very weak, but with laser, it is possible to study the movements of rotation and vibration of molecules [23]. From the received signal, the percentage of biological molecules can be extracted in samples, such as collagen, elastin, beta-carotene, cholesterol (fig.2).

Introduced in medical practice in 2000, Raman spectroscopy is used to differentiate normal laryngeal tissue of precancerous and cancerous lesions [24]. Some authors publishing studies that indicate sensitivity between 70-90% and specificity in differentiating between normal tissues/malignant between 85-95 % [25].

2. Optical coherence tomography (OCT)

OCT is a modern method of investigation, in high resolution, mainly used in ophthalmology. It is derived from low coherence optical interferometry and uses non-ionizing optical radiation in the near infrared field.

OCT allows measurements in real time, in situ, with resolution up to 10 times higher than conventional radiology (CT, MRI), making it possible to identify microscopic tissue structures (villi, glands, crypts, blood vessels). Despite this exceptional resolutions (1-2 µm), the method is limited by the very low degree of depth (penetration of tissue) of 1-3 mm [27]. Articles published in the past decade shows that it can be used to detect an epithelial membrane rupture and overcoming of the laryngeal carcinoma or epithelial thickness measurement (normally between 98-185 µm, about 500 µm in hyperkeratotic lesions) [28].

Figure 3: T2 squamous cell carcinoma of the left vocal fold; (A) Endoscopic photograph. (B) Optical coherence tomography image demonstrating normal epithelium with an intact basement membrane on the right of the image, and invasive cancer with increased backscattering and loss of the basement membrane on the left. A transition zone is seen between these two areas. Note the area of motion artifact on the left of the image (bm-basement membrane; ca-invasive cancer; e-epithelium; tz-transition zone; SLP-superficial lamina propria) [29]

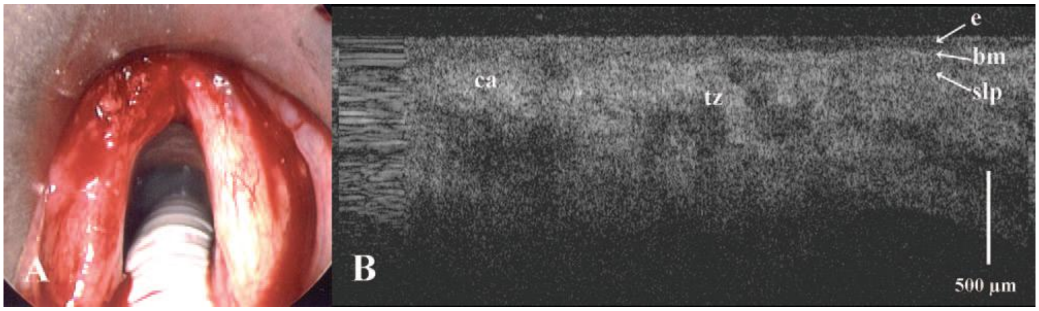

3. Narrow band imaging (NBI)

It is a modern endoscopic technique for investigating the vocal cord that uses certain wavelengths of light to improve the viewing of epithelial and sub epithelial microvascular pattern. They are using wavelengths of 415 nm (blue) and 540 nm (green), which are absorbed by hemoglobin, causing epithelial microvasculature to be colored in brown and become visible. The obtained images can be improved and recorded by using a HD (High-Definition) video system [30,31].

Neoplasia suspicion rises when on a brownish area, well-marked, is observed thick dark spots and/or tortuous course of the microvasculature, or more, a hypertrophic blood vessel that branches in small vascular loops [32,33]. There are studies that demonstrate that this optical method can even make the difference between neo-vessels specific to carcinoma and the ones altered by post-radiation inflammation [34].

The method was originally used for early detection and monitoring post-surgery esophageal [36], and tracheobronchial [37] neoplasm lesions, but recent studies show that is gradually earning supporters using it for the same goals at the larynx [38,39] (good results in subglottis injuries). Thus, Japanese authors demonstrate that narrow-band imaging has a sensitivity of 91.3% and a specificity of 91.6% [40], in laryngeal cancer lesions, while a team from Italy (Brescia) reports 90% specificity and 98% sensitivity [41].

Another recent study [42], multicenter, prospective, randomized, which included patients with esophageal cancer and pharyngeal and laryngeal cancer, showed that narrow-band imaging compared with conventional endoscopy detected a higher number of superficial carcinomas synchronous in both locations, making them the authors to conclude that this method could be the standard examination for early detection of superficial cancer in those localizations.

But the interpretation of the images must be made by an ENT specialist with a good experience, to avoid false negative or false positive. Also false negative may occur on lesions where the keratin layer has a greater thickness.

Figure 4: A. Conventional white-light image of the larynx. The left subglottic region is displayed slightly reddish. B. NBI image of the same site as a. Demarcated brownish area is seen in the left subglottic region surrounded by green area. C. Close white-light view of a. D. Close NBI view of b. There are scattered brown spots in the lesion. E. Histopathologic evaluation of the biopsy specimen revealed moderately differentiated squamous cell carcinoma of the larynx [35].

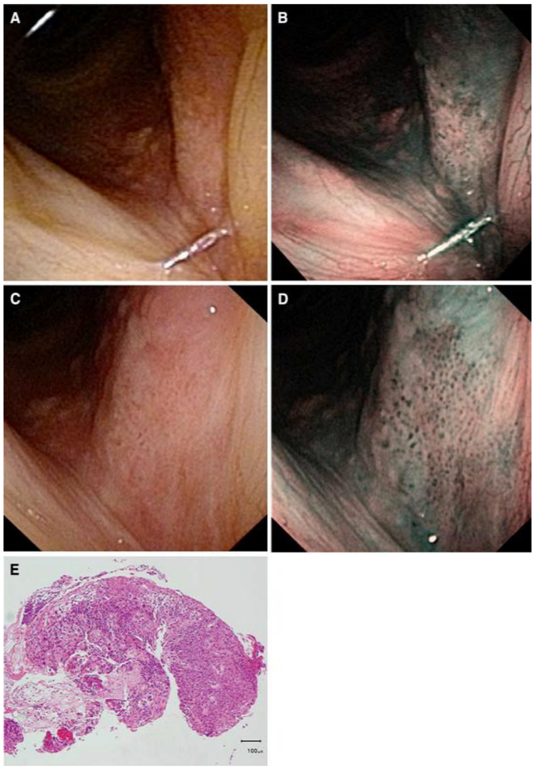

4. Confocal endomicroscopy

This technique is introduced into medical practice in 2004 by R. Kiesslich for in vivo diagnosis of colorectal cancer[43]. The principle of the method consists in illuminating the examined tissue with a laser wave of 488 nm which allows viewing epithelium in depth up to 250 µm. For a better image quality is given intravenously a contrast substance (fluorescein). The examination is made simultaneously with both endoscopic and endomicroscopic, acquiring images to identify the characteristics of epithelial cells (size, shape, appearance of the nucleus) near the basal membrane [44].

The limitation of this method is shallow penetration (250 µm), knowing that the cancer epithelial thickness can reach 500-1000 µm, being unable to view the membrane. Also it requires a pathologist with experience.

Introduced recently in Otolaryngology, there are still no studies on a large number of cases, but those in the gastrointestinal field [45,46] give high hopes for the future of this method.

There is a pilot study [47] clearly demonstrates the possibility to detect dysplastic cells near to the basal cell layer and within the subepithelial space in lesions with small leukoplakia (thin keratin layer). These results can have an impact on microlaryngoscopy to improve the precision for biopsy and on microlaryngoscopic laser surgery of the larynx to identify the margins of the pre-malignant lesion.

Figure 5: Confocal endomicroscopy image of the human supraglottis captured in vivo following topical application of 0.05% acriflavine hydrochloride [48]

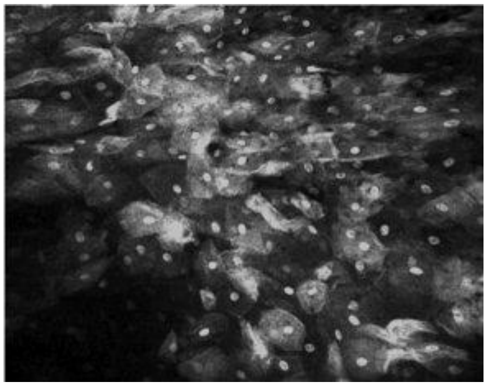

5. Contact endoscopy

Contact endoscopy is another novel noninvasive optical diagnostic imaging method that allows in vivo and in situ examination of the cellular architecture of the superficial layers of the mucosal epithelium. Magnified images are obtained using Hopkins' rod-lens endoscope placed on the surface of the dye stained mucosal tissue. This method allows evaluation of precancerous and cancerous lesions in vivo and has significant potential in the histopathologic diagnosis of many suspicious head and neck mucosal lesions without tissue biopsy. The first reported use of CE in otolaryngology head and neck surgery was by Andrea et al. as a diagnostic tool in the evaluation of various pathologies in the larynx in the 1990s [49]. They were able to visualize and diagnose laryngeal mucosal pathology from the magnification of vocal fold epithelium and microvasculature during microlaryngoscopy after staining the vocal cords with methylene blue dye.

Few members of our group [50] have systematically drawing vascular changes of the vocal fold, with clinical and pharmacological applications. They demonstrated the changes of microvascularity, occurring since stage of hypertrophic chronic laryngitis (hypervascularity with dilated vessels, but parallel to the free edge of the vocal fold) till the carcinomatous lessions (with hypervascularity and areas of keratin, changes of the archaeological and cytological parameters: **U**niformity of the cellular-field, **R**atio (nucleus/cytoplasm), **S**ize and **S**hape of the epithelial cells [51].

There are more advantages of contact endoscopy. Most significantly, it offers a noninvasive, quick, and repeatable in vivo evaluation of the cytological architecture while avoiding the need for an invasive biopsy and its associated risks. CE provides immediate results, with the possibility of examining many mucosal areas in a short time. CE can also assess a wider surface mucosal area, providing more information than a selected histological section taken by biopsy [52]. It also avoids tissue damage and alteration of cellular architecture which may occur in the biopsy and histological preparation. Subsequently, this results in a dramatic improvement of the diagnostic yield of the biopsy. Other potential roles of CE include the rapid diagnosis of benign and malignant mucosal lesions in an outpatient or operating room setting, surveillance, guided biopsies, and intraoperative evaluation of tumor resection margins. Despite its advantages, CE does have its limitations. Most notably, CE can only evaluate the most superficial cell layer of the mucosal epithelium. This is most likely due to a number of factors including (i) poor penetration of methylene blue which only stains a few superficial layers, (ii) short focal distance of the scope (i.e., CE can only assess to a depth of 80 um at 60x magnification and 30 um at 150x magnification), and (iii) optical artifact at high magnification due to glare from light reflected from cells not in focus. The lack of depth of penetration prevents the evaluation of important histological information especially when vertical extent of dysplasia is crucial in distinguishing the different grades of dysplasia from carcinoma in situ and invasive carcinoma. As a result, these factors could affect the sensitivity of CE, thus accounting for some of the false negative diagnostic results noted by authors. The potential impact of CE missing a malignant lesion needs to be taken into consideration if this technology is to one day substitute histopathology. Future investigation into better penetrating dyes, advances in digital optics, and image enhancements will eventually allow better vertical staining and increased resolution of the deeper cell layers which would translate CE in becoming a much more sensitive and accurate diagnostic tool.

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ORIGINAL ARTICLES

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Detection of epidermoid squamous-cell carcinoma by laser induced autofluorescence – preliminary results

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Abstract: *Epidermoid squamous-cell carcinoma is by far the most common malignant tumor of larynx (95-96%), representing 1.6-2% of cancers in men and 0.2-0.4% of malignancies in females, with a world growing incidence and a slight dominance in urban areas. By exposing cells and tissues to UV light, the excitation of naturally occurring chromophores occurs in part by non-radiative deactivations, in part via fluorescence emission. Using laser induced autofluorescence (LIAF) in natural tissues not impregnated with photosensitizers as a noninvasive autofluorescence technique for both diagnosis and intraoperative assessments of laryngeal cancer we can improve the tumor malign identification in vivo. A total of three laryngeal biopsies (i.e. three pairs of tissues, each pair containing a healthy and a tumor tissue sample extracted from the same patient) were considered in this study. The samples were collected from patients previously diagnosed with stage T3 laryngeal carcinoma. Immediately after the surgery fragments of normal tissue and neoplastic tissue were collected, fragments of which later, after freezing, were sectioned in 25-30 µm thickness slices and stretched to quartz slides. All samples were subjected to controlled laser irradiation using a pulsed diode laser (λ=375nm, pulse width=87ps, frequency 31MHz) and the autofluorescence and its lifetime were collected using two optical fibers (inner diameter 400µm and 1500µm, respectively) positioned in a 45° geometry. The signals were recorded using a spectrograph and a photo-sensor module, the output of which was fed to a digital oscilloscope.*

We assessed the impact of laser induced autofluorescence and autofluorescence lifetime measurements in order to identify the differences between healthy and tumoral laryngeal tissue and outlining them, in terms of differences between the laser autoinduced fluorescence averaged intensity. The results determined the usefulness of laser induced spectroscopy in the diagnosis of laryngeal squamous cell carcinoma, discriminating between the malignant and normal tissue by analyzing the differences in spectral autofluorescence intensity and autofluorescence lifetime.

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Keywords: *Laser induced autofluorescence, autofluorescence lifetime, laryngeal squamos cell carcinoma*

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INTRODUCTION

Cancer is a growing dilemma in the developed countries. Laryngeal carcinoma is the most common cancer of the head and neck. Epidermoid squamous-cell carcinoma is by far the most common malignant tumor of larynx (95-96%), representing 1.6-2% of cancers in men and 0.2-0.4% of malignancies in females, with a world growing incidence and a slight dominance in urban areas [1].The main risk factors are smoking, alcohol consumption, harmful dietary habits, exposure to the irritant chemical substances [2].

Early identification and pre-operative evaluation of laryngeal carcinoma and its precursor lesions are essential for function-preserving surgical treatment. Accurate determination of the site and extent of carcinomas is very important for planning therapy and establishing prognosis [3]. Attempts to optimize diagnostic methods to obtain more sensitive detection and precise description of laryngeal pathology remain a challenge for the otolaryngologist [4].

Microlaryngoscopy with biopsy is the standard diagnostic procedure for the detection of laryngeal carcinoma. Anyway, the microscopic view added to the palpation of the larynx is not always enough to the evaluation and precise description of precursor lesions. Some new techniques, like videostroboscopy, contact endoscopy, confocal endomicroscopy, optical coherence tomography, narrow-band imaging and fluorescence endoscopy have been developed to come up with the detailed examination and the histopatological information about mucosal lesions [5,6].

Fluorescence is a optical phenomenon of the light emission by some molecules after their excitation by the light at a particular wavelength. These fluorescent molecules are called fluorophores. Every fluorophore exhibits its characteristic absorption and emission bands whose shapes and positions of their maxima on the excitation and emission light wavelength axes depend on the physical and chemical properties of its nearest environments. The interest in the use of fluorescence in medical diagnostics has grown during the last decades. Fluorescence spectroscopy provides information on the biochemical and morphological aspects of different tissues [7].

The procedures using the fluorescence spectroscopy for the differential diagnosis of malignant lesions are based upon the differences in the autofluorescence emission due to the pathological changes of the tissue. These changes include the modifications of tissue architecture (tissue layer loss or thickening), light absorption and scattering by neovasculature, and the alterations of metabolic status of the tissue such as changes of the concentration and distribution of fluorophores [8]. As a result, recorded fluorescence emission spectra of cancerous lesions reveal differences in fluorescence intensity and fluorescence band shapes, when compared to healthy tissue.

MATERIALS AND METHODS

The spectroscopic measurements were investigated in three larynxes from laryngectomized cancer patients treated in ENT Clinic, Carol Davila Central University Emergency Military Hospital, Bucharest. Histological analysis of the tumor tissues revealed laryngeal epidermoid squamous-cell carcinoma (SCC) in both cases, stage T3 (Figure 1). Immediately after the surgery (total laryngectomy), the larynx was carefully washed in saline solution to remove any blood contamination.

After that, fragments of normal tissue and neoplastic tissue were collected, fragments of which later, after freezing, were sectioned in 25-30 µm thickness slices and stretched to glass slides (reconfirmed histological) and stored at -80ºC for further examination. All participants provided written consent to participate in the experiment. The study was approved by the local ethics committee.

Experimental setup (Figure 2) was performed using laser beam exposure of the samples a picosecond pulsed laser diode (Alphals, type PicoPower LD-375-50) emitting at 375 nm with 31MHz pulse repetition rate, 87 ps full time width at half-maximum laser pulses, and the average power of about 0.5 mW. A laser beam of 1.6x1.8 mm (elliptical) diameter was sent to the sample in double-pass vertical irradiation geometry through a 355 nm dichroic mirror and a totally reflecting mirror.

Two types of measurements on tissue samples were performed: laser induced autofluorescence (LIAF) and autofluorescence life-time. The autofluorescence signal was collected using two optical fibers positioned each, at 45° in the same plane with respect to the pumping laser beam.

Figure 1. Larynx after surgical ablation for SCC (T3)

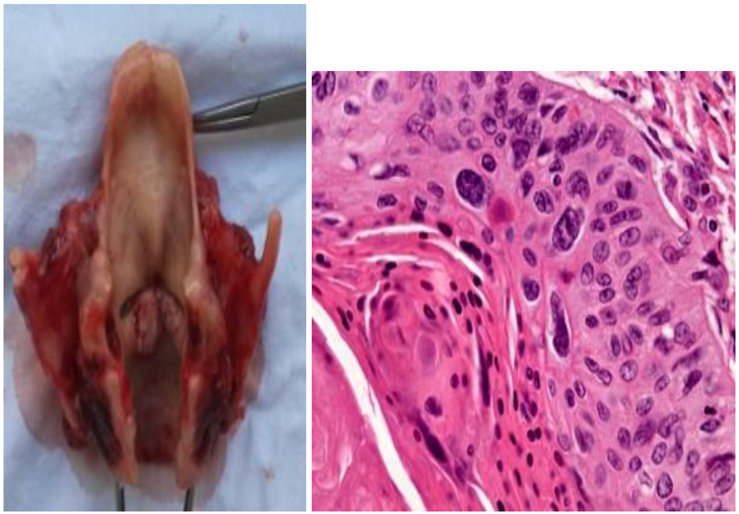

Figure 2: Experimental setup

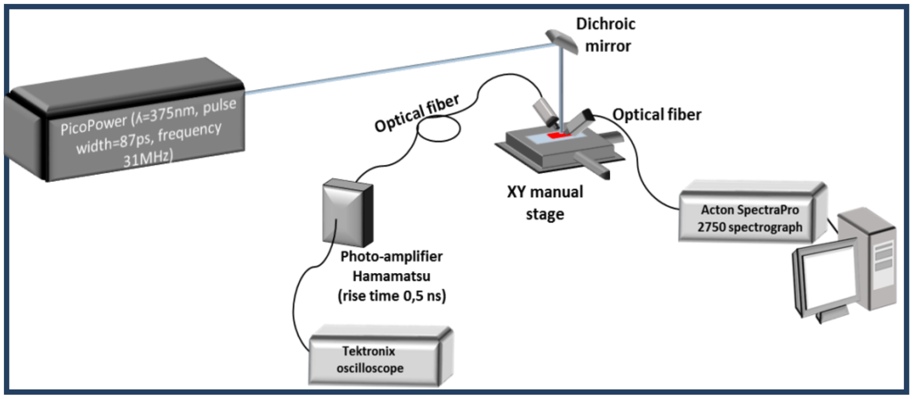
Dichroic mirror
PicoPower (λ=375nm, pulse width=87ps, frequency 31MHz)
Optical fiber
Optical fiber
XY manual stage
Acton SpectraPro 2750 spectrograph
Photo-amplifier Hamamatsu (rise time 0,5 ns)
Tektronix oscilloscope

The optical fiber with the core diameter of 400 µm collects the signal for LIAF spectrum analysis; another fiber of 1,500 µm collects the remitted signal for autofluorescence life-time analyses. The LIAF signals were recorded using a spectrograph (Princeton Instruments, type Acton SpectraPro 2750) with an optical resolution of 0.08 nm and; a photosensor module (Hamamatsu, type H6780-20) having 0.78 ns rise time, was used for LIAF life-time measurements and its output was fed to a digital oscilloscope (Tektronix, type DPO7254). The signal acquisition by spectrograph and photosensor module was synchronized with the laser pulse through a pulse delay generator (SRS, DG 535). During the LIAF measurements, the probe (sample and substrate/slide) was placed on a XY manual stage with a displacement resolution of 5 µm. Each type of measurement was performed collecting the LIAF signal from three different spots for each sample. For each healthy and tumoral tissue two slides with the same type of samples were prepared.

RESULTS

The LIAF spectra of the healthy and tumoral tissues are presented in Figures 3-5 and are compared in terms of peak intensity (*I*), Area (*A*) under fluorescence spectral distribution curve and ratio between the intensity and the corresponding full width at half maximum (*I/FWHM*) signal.

In case I, two healthy tissues of different thicknesses, 20 µm and 30 µm, were compared. Figures 3A and 3B presents the LIAF spectra of the three spots from the duplicate samples and Figure 3C shows the corresponding averaged spectra. The peaks of LIAF spectra are placed between 456 – 462 nm for the 30-µm tissue thickness and between 460-463 nm for the 20-µm tissue thickness. In Figures 3A and 3B are observed variations in peak intensity, area and I/FWHM and approximately the same values for FWHM. These variations are, dominantly and most probably, due to different concentrations of fluorophores in the samples. The comparison between the averaged spectra of 20 µm and 30 µm shows 11.4% autofluorescence intensity, a 9.5 % area and a 14.4% I/FWHM increase in 30 µm with respect to 20 µm case.

Figure 3. Case I. LIAF intensity for healthy tissue for different thickness: A) 20 µm and B) 30 µm; collected in three spots of the duplicate samples and the averaged spectrum; C) LIAF intensity of the averaged spectra from the 20 µm and 30 µm thickness samples.

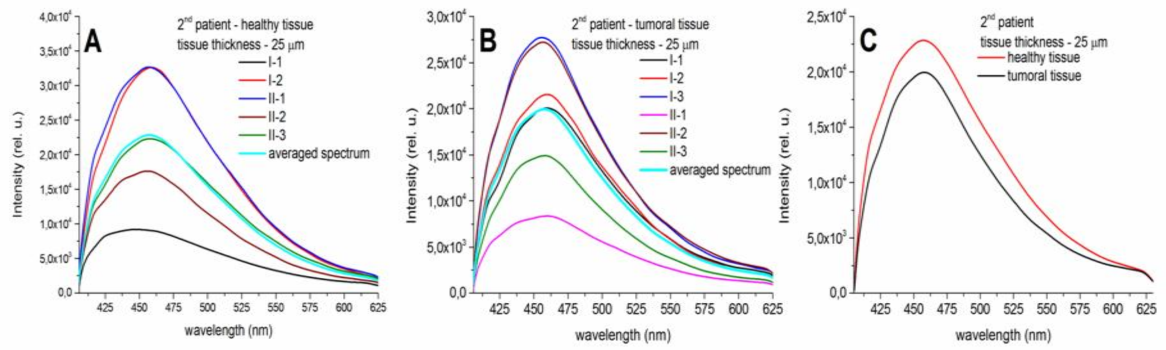

Figure 4. Case II. LIAF intensity for 25 µm tissue thickness: A) healthy and B) tumoral; collected in three spots of the duplicate samples and the averaged spectrum; C) LIAF intensity of the averaged spectra from the healthy and tumoral samples.

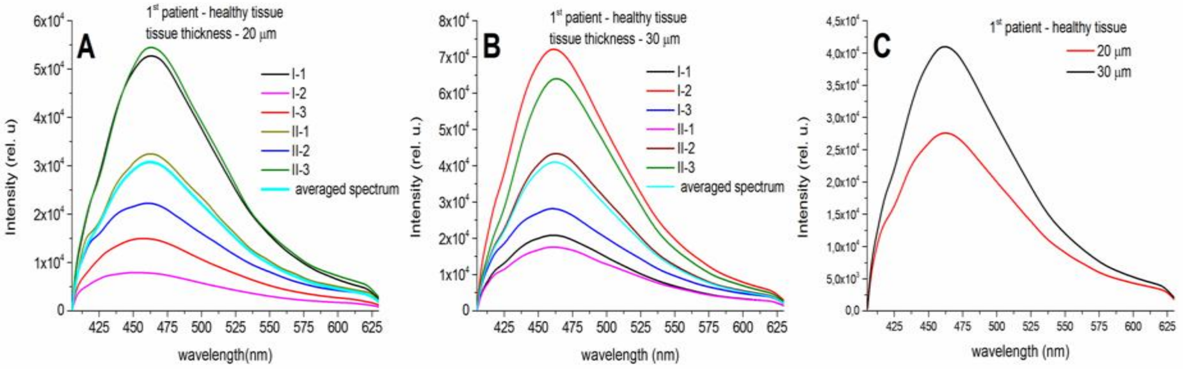

In case II, one healthy and one tumoral tissue of 25-µm thickness were analyzed. Figures 4A and 4B present the LIAF spectra of the three spots from the duplicate samples and the corresponding averaged spectrum (Figure 4C). The peaks of LIAF spectra are placed between 445 – 462 nm for healthy tissue and between 456 – 459 nm for tumoral tissue. In Figures 4A and 4B, are observed variations in peak intensity, area and I/FWHM and approximately the same values for FWHM.

In general, the maximum value of LIAF's intensity is smaller for the cancerous tissue (with 16.7%) than the healthy one. The area and the I/FWHM ratio of LIAF for cancerous tissue is also smaller than for healthy tissue (with 17.1% and 4.7%).

In case III, one healthy and one tumoral tissue of 30-μm thickness were analyzed. Figures 5A and 5B present the LIAF spectra of the three spots from the duplicate samples and the corresponding averaged spectrum (Figure 5C). The peaks of LIAF spectra are located between 457 – 458 nm for healthy tissue and between 456 – 459 nm for tumoral tissue. In Fig. 5A and 5B, are observed variations in peak intensity, area and I/FWHM, but smaller than those from case II, and approximately the same values for FWHM.

In Figure 5C, the maximum value of LIAF's intensity is smaller for the cancerous tissue (with 71%) than the healthy one. The area of LIAF for cancerous tissue is also smaller than healthy tissue (with 66.7%). At the same time, maximum spectrum for tumor sample is shifted to blue with nine nm (449-458 nm) than healthy tissue and the I/FWHM ratio decreases with 74.2% when comparing the healthy tissue with the tumoral one.

Figure 5. Case III. LIAF intensity for 30 μm tissue thickness: A) healthy and B) tumoral; collected in three spots of the duplicate samples and the averaged spectrum; C) LIAF intensity of the averaged spectra from the healthy and tumoral samples

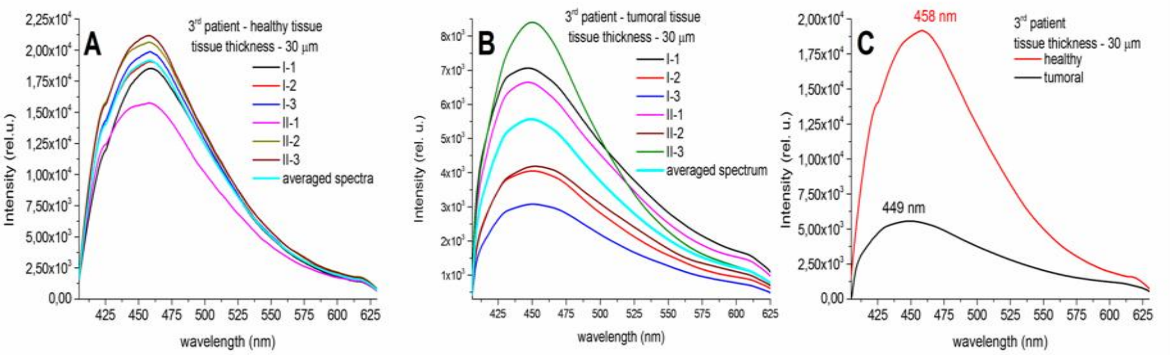

The computed parameters based on the experimental data extracted from LIAF spectra for the 2nd and 3rd patient healthy and tumoral tissues are shown in Table 1.

The results indicate that values of LIAF's parameters (wavelength, intensity, area, I/FWHM) are helpful and complementary diagnostic tools in detection of laryngeal carcinoma. These variations are due to the different concentrations and distribution of fluorophores in the samples.

Table 1. Parameters extracted from LIAF spectra for the 2nd and 3rd patient healthy and tumoral tissues (rel. u. are different from one column to the other; the same values of rel.u. are used for different patients)

Patient	Tissue	λ_{peak} (nm)	I_{max} (rel. u.)	FWHM (nm)	Area (nm x rel.u.)	I/ FWHM
2nd	Healthy	457	$2.29x10^4$	107.48	$2.58x10^6$	212.6
	Tumoral	457	$2x10^4$	98.5	$2.13x10^6$	202.5
3rd	Healthy	458	$1.92x10^4$	101.69	$2.07x10^6$	188.5
	Tumoral	449	$5.56x10^4$	114.6	$6.87x10^6$	48.56

Regarding LIAF's life-time (Figure 6), performed measurements show that the life-time of the healthy tissue decreases with thickness increasing, both for first patient case (Figure 6A) and for the second and third patients (Figures 6B and C, respectively). In Figure 6A, the life-time value decreases from 4.06 ns for the tissue of 20 µm thickness to 3.6 ns for that of 30 µm thickness, both values being obtained by averaging 4 samplings.

Figure 6. LIAF's life-time. A) 1st patient, healthy tissue at two thickness, 20 and 30 µm; B) 2nd patient, healthy and tumoral tissues at 25 µm tissue thickness; C) 3rd patient, healthy and tumoral tissues at 30 µm tissue thickness; blue dash line indicates the exponential decay fit function whose time constant τ represents life-time of the analyzed process.

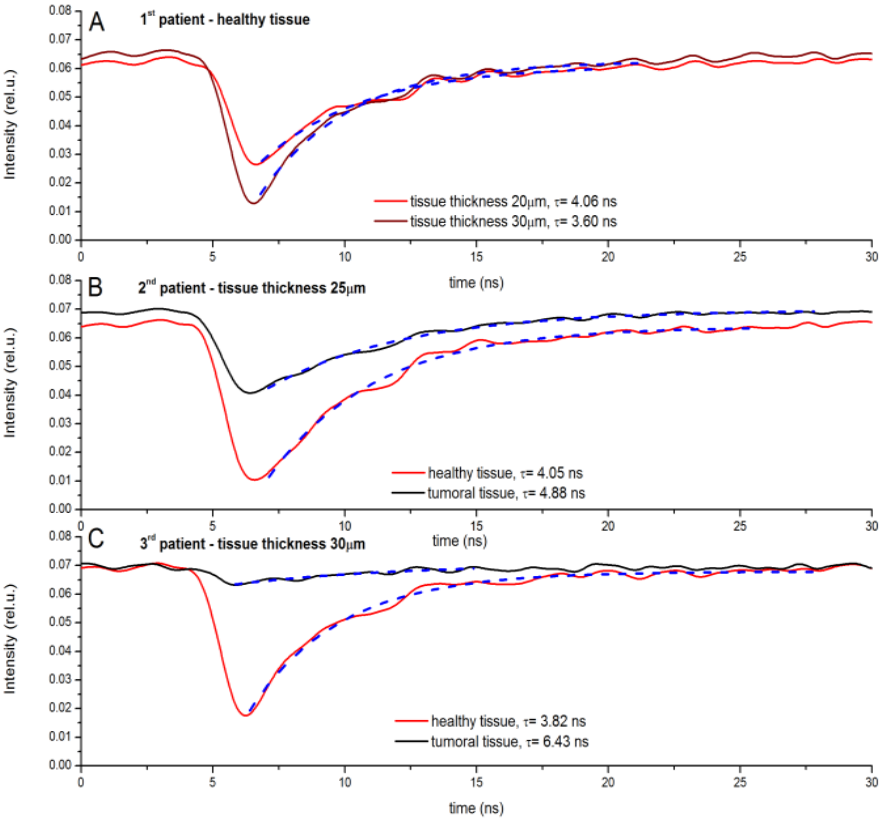

It is to mention that the life-time values of healthy tissue for second and third patients are similar with those for the first patient considered for the same tissue thickness. For both patients exhibiting developed malignant tumor, the life-time values were higher for the cancerous tissues than the healthy samples (4.88 vs 4.05 ns for second patient; 6.43 vs 3.82 ns for third patient) as we see in Figures 6B and C.

DISCUSSION

The prognosis for patients with laryngeal cancer is significantly better with early detection of malignant lesions. Therefore, otolaryngologists look for supplementary techniques, as laser-induced autofluorescence, which may improve accuracy of real time cancer detection.

Malzahn et al. [9] have demonstrated high sensitivity of autofluorescence endoscopy in the diagnosis of early laryngeal cancer compared with light endoscopy alone. In the study of Zargi et al. [10] the sensitivity and specificity of described method were 86.9% and 82.8%.

The aim of the presented study was to analyze the applicability of laser-induced autofluorescence measurements to the differentiation of the changes in the cancerous and healthy tissue of the larynx. For the measurements, it was used a laser diode emitting pulses of picosecond duration and λ = 375 nm. The chosen wavelength was found to excite the endogenous fluorophores: tyrosine (max. emission at 310 nm), tryptophan (max. emission at 340 nm), oxidized form of nicotinamide adenine dinucleotide (NAD+) (max. emission at 460 nm), the collagen cross-links (max. emission at 420–460 nm), the reduced form of nicotinamide adenine dinucleotide (NADH) (max. emission at 460nm), and the oxidized form of flavin adenine dinucleotide (FAD) (max. emission at 520 nm) [11]. The levels of the mentioned fluorophores, especially NADH and FAD, provide a strong emission signal and are significantly altered in cancerous cells, enabling the differentiation of the healthy and neoplastic tissues by autofluorescence measurements [12, 13, 14].

In both cases of the study, the LIAF's intensities of the cancerous tissue samples are lower in comparison with the adjacent healthy tissue samples. These results are in accordance with other studies where the decrease of intensity may be a consequence of reduced concentration of oxidized flavoproteins, the breakdown of collagen cross-links, the thickening of the cancerous tissue or the blood absorption (neovascularisation, inflammation)[15,16]. Neoplastic cells infiltrating submucosa express matrix metalloproteinases activity, which participate in breaking collagen cross-links [17].

We also observed in both cases that the area of LIAF for cancerous tissue is smaller than healthy tissue, the value of the ratio Intensity/ Full width at half maximum (I/FWHM) decreases in cancerous tissue sample and maximum spectrum for tumor sample is shifted towards blue. As for LIAF's life-time, in both cases the values were higher for cancerous tissues than healthy samples. Measurement of these parameters gives to our study originality, as well as the experimental setup.

Our results indicate that LIAF's parameters values (intensity, area, I/FWHM, life-time) are potentially a very helpful, complementary, diagnostic tool in detection of laryngeal carcinoma. The clinical prospective research on a great number of patients should be performed to establish utility of these measurements in everyday practice.

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Mucous herpes zoster in elderly

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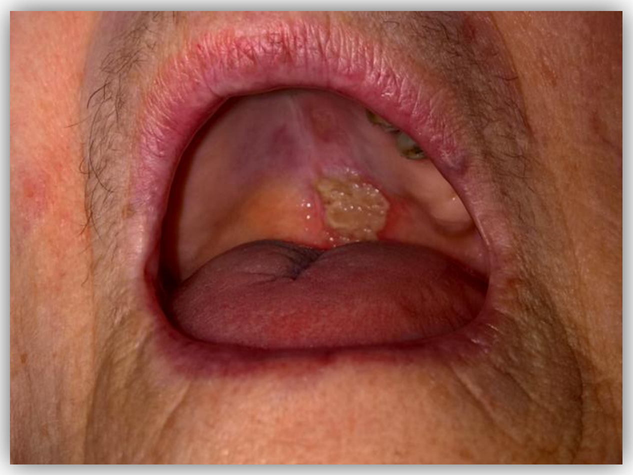

78 years old female is admitted in hospital for low back pain with moderate dysfunction and headache, secondary associated with vesicular/ pustular and ulcerative lesions in the mouth (image).

The patient has a cardiovascular medical history with severe arterial hypertension, grade 1 atrio-ventricular block, paroxistic atrial fibrillation, mitral and tricuspidian regurgitation, congestive heart failure NYHA II and chronic renal disease stage IIIB.

Biologic findings shows slight normochrome anemia, WBC was within normal range and no signs of inflammation. Ag Hbs, Ac anti HVC, HIV were negative. Beta haemolitic streptococcus and staphylococcus aureus were absent.

The imagistic standard evaluation (ultrasound) has normal findings.

Patient developed an erythematous patch on the soft palate, with pustules and ulcerations, slightly painful, and some vesicles on the left side of the tongue. No lymphadenopathy was present. It was interpreted as mucous herpes zoster and Acyclovir treatment, 4 grams daily for 7 days was initiated. From the 5th day the subjective symptoms disappeared and local manifestations tend to diminish.

Herpes zoster is the result of VZV reactivation and its spread from a single ganglion to the neural tissue of the affected segment and the corresponding cutaneous dermatome. When the virus is located and multiplies in the geniculate ganglion, facial, aural, and oral lesions of herpes zoster may emerge. Reactivation in the geniculate ganglion can lead to facial nerve (VII) paralysis (because sensory and motor nerves conjoin in nerve VII). VZV and HSV account for the majority of cases of Bell palsy (idiopathic facial paralysis). Our case was not one of those.

Under treatment, evolution was favorable, and 1 month after stopping treatment there were no residual symptoms.

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CLINICAL PRACTICE

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Syrian war shrapnel injury: cubital nerve defect grafting during humanitarian surgical mission. Clinical case presentation

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Abstract: *Background and aim of this clinical case presentation is to reveal the importance of early nerve injury diagnosis and surgical treatment in war wounded patients.*
Methods. The author treated patients in Amman Charity Hospital were among different plastic surgery cases where limb nerve injuries with nerve grafting indication. The presented case was treated with autologus sural nerve graft.
Results were evaluated at 3 months after the surgery and revealed detectable nerve conductibility at the Electromiography test.
In conclusion, in cases with delayed nerve repair surgical treatment in war wounded patients, the vascularised nerve graft can be a better solution for nerve defect surgical treatment.

Keywords: *NERVE injury, axonotmesis, war wounded patient, sural nerve graft, microsurgery, ulnar claw.*

CASE REPORT

Acute and chronic war wounded Syrian refugees from Zaatari camp and the Syrian battlefields are directly admitted in Amman Al Maqqased Charity Hospital every day.

Among patients treated by the author during November 2015 Humanitarian Mission was a left cubital nerve defect due to a bomb explosion injury.

Peripheral Nerve injury described by Sunderland classification can be: neuropraxia as the lowest degree of nerve injury in which is affected only the nerve conduction and no disruption is done. The second degree is axonotmesis where the axon is damaged but the surrounding tissue remains healthy (1). The most severe form of nerve lesion is neurotmesis where both the surrounding tissue and the axones are disrupted (2).

Patient's surgical and medical history

Female patient, 42 years injured 8 month prior to surgery. Immediately after the explosion the patient received first aid surgical assistance: wound cleaning and direct closure.

The patient felt on the first postoperative day that she completely lost the tactile sense on 4th and 5th fingers on her injured left hand.

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During the healing process, the wound infection and sutures opening occurred and the patient was treated with antibiotics and wound dressings until the wound has completely closed.

Two months after the injury she was examined and diagnosed with post traumatic cubital nerve injury.

Clinical and paraclinical information

The patient presents left cubital nerve chronic palsy with "ulnar claw" appearance and extensive (12 cm) longitudinal scar on the palmar aspect of the forearm (Figure 1). The metacarpophalangeal joints of the 4th and 5th fingers are extended and the interphalangeal joints of the same fingers are fixed in extension.

Figure 1: Left hand war injury with antebrahial wound and hypothenar region muscular atrophy.

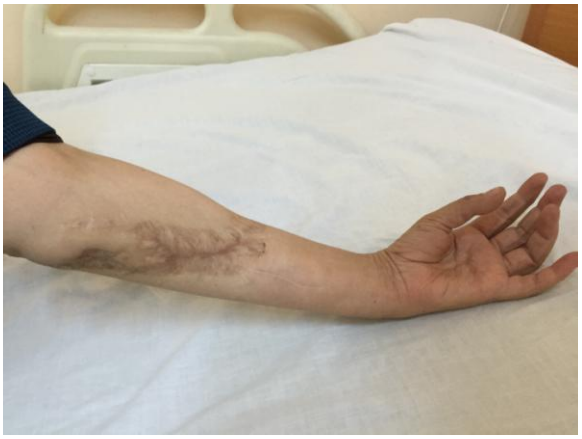

Figure 2: Ulnar claw hand aspect with insufficient flexion of the 4th and 5th finger's interfalangial joints.

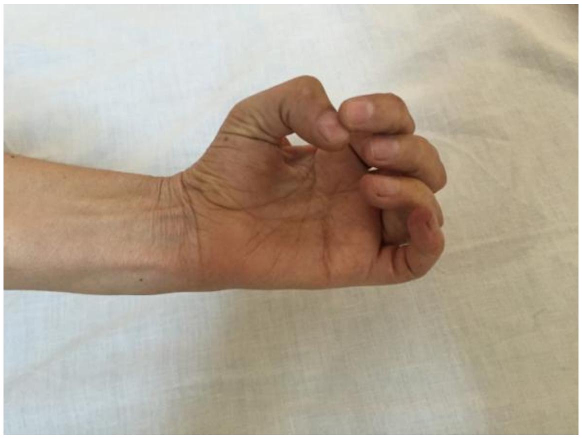

The claw is most obvious when the patient is asked to flex all fingers: the 1st and 3rd digits are flexing due to intact medial nerve innervation and the 4th and 5th ones are remaining extended because of high cubital nerve injury (Figure 2).

The final diagnosis is clarified by dorsal and hypothenar regions denervation and atrophy and by Electromiography testing which reveals the left ulnar transmission is completely disrupted at the scar site (Figure 3).

Figure 3: The dorsal aspect at the cubital denervated hand with muscle atrophy.

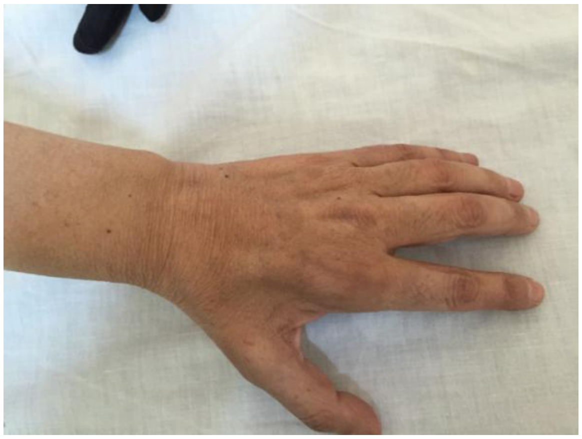

The nerve injury is grade 5 according to Sunderland's system.

Surgical method

Clinical practice and research on nerve injury treatment indicates that the primary surgical repair during the first week is required. If the nerve is disrupted on more than 2 cm length the sural nerve graft must be done.

The surgery was performed under general anesthesia using an interfascicular microsurgical nerve suture technique.

The partial scar excision was done and fibrosis was devided.

Cubital nerve was found being disrupted on 10 cm length at the medial 1/3 of the forearm. After debridement and neurolisis due to myeline degenerative process, the gap real size was 14 cm long (Figure 4).

The autogenous sural nerve grafting was decided to be done.

The right 18 cm long sural nerve graft was harvested and a cable graft designed to bridge the gap (Figure 5).

Figure 4: Cubital proximal and distal nerve stump after surgical debridement.

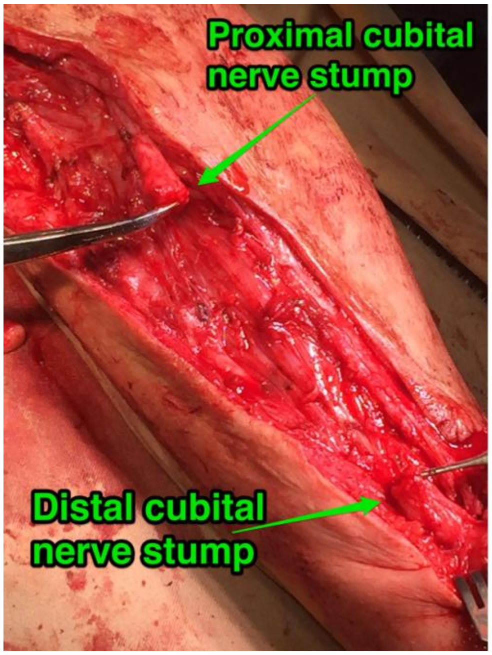

Figure 5: Right Sural nerve graft harvested.

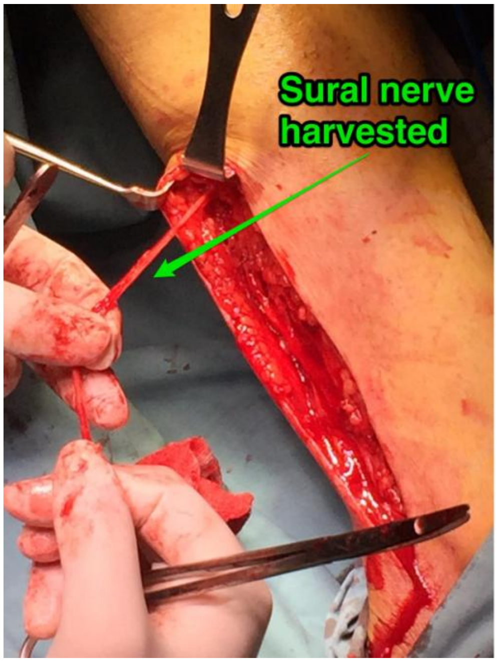

The microsurgical sutures were done at the both nerve ends to achieve nerve connections with separate nonabsorbable 7-0 sutures (Figure 6).

Skin suture and hand splinting was done.

The patient's evolution was favorable under antibiotic therapy for 7 days with no wound closure complications.

Results

After 3 months of kinetotherapy and electrotherapy the nerve conductibility was evaluated as being detectable at the lowest level by Electromiography test. No sensitivity or lightning in the 4th and 5th fingers was detected.

Figure 6: Sural nerve graft sutured on site.

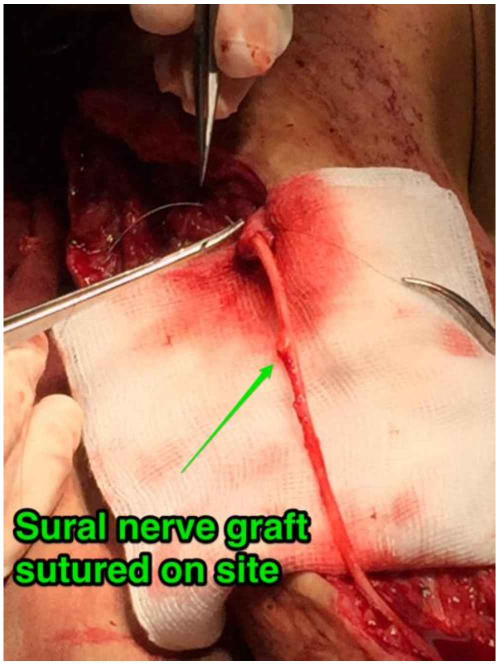

Continued hand therapy rehabilitation is indicated and, 6 and 12 months postoperative evaluation by Electromiography and two point discrimination tests are mandatory at each following checkup.

DISCUSSION AND CONCLUSION

The nerve injury repair should undergo in most of the cases on the emergency surgical treatment basis.

In neurotmesis cases the immediate surgical repair attitude (one week) is the preferred approach for nerve defect treatment in order to obtain a high sensitive and motor functions recovery rate (3).

In chronic palsy (lasting > 3-4 months) associated with muscle weakness, atrophy, surgical outcome is less certain. The duration of palsy and muscle weakness are key factors influencing the prognosis (4).

No conservative treatment can be done in such cases just bridging segmental nerve defect with autogenous nerve graft or decellularized nerve allograft and nerve tubulisation in repairing defects less than 5 cm (5).

In present case the grafting was done 8 months after surgery – this may lead to a less motor recovery but sensitive recovery still can be a good one 6 months after continuous hand rehabilitation(6-8). This demonstrates that 3 months after surgery the cubital nerve conduct was detected even at the lowest level.

In war areas where patients cannot seek specialized medical attention immediate after the injury and their nerve palsy is diagnosed later on, a vascularized nerve grafting should be considered as a clinical alternative for nerve reconstruction if sensitive sense it is not improved after nerve grafting.

Further research comparing late nerve grafting versus vascularized nerve grafting must be done.

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CLINICAL PRACTICE

Article received on March 9, 2016 and accepted for publishing on May 12, 2016.

The not so good and the not so bad

Silviu V. Dumitrescu[1]

Today's question: How good is the good cholesterol?

Since before med school we knew that is a bad cholesterol and a good one. And like old days vigilantes we fight the bad one and support the good. But, like the characters in the novels, the bad one seems to have a bright side and the good one has dark secrets.

First let's talk about the LDL. It's involvement in atherosclerosis it’s a fact and it's a silent killer. The cardiologists are fighting it since 1987 when the introduction of lovastatin in ischemic cardiac disease.

Figure 1: The good and the bad cholesterol [5].

Now let's talk about the sheriff, HDL cholesterol. The one that protect us in the front of vascular aggressors. We know that the bigger is the better and we are very sad when our patient have low levels of it. But 500 years ago we knew that the earth is flat, so what is the truth?

Recently 4 Big Pharma firms developed a new class of was a real breakthrough. But what the neurologists have to say? After the initial enthusiasm was tempered they started to ask: our patients have a LDL cholesterol level way below 70 mg/dl. They are safe. But they look dumber. Why? The statin lead to a mental decline or the low LDL level is to blame? In my opinion is the LDL level. I noticed that in several of my patients with very low LDL levels: they develop very fast dementia symptoms that regress dramatically when their LDL levels rise above 70 mg/ml. Is it a coincidence? Maybe. But what if the bad has good side?

lowering cholesterol drugs – Cholesteryl Ester Transfer Protein Inhibitors (CETPi) that were announced to be the new milestone in the treatment of dyslipidemia. Preliminary studies showed a spectacular HDL levels rise.

First to go first to fall – torcetrapib – shortly the mortality increased from an unknown cause.

Next dalcetrapib and evacetrapib – HDL levels reached the top. Well tolerated, few side effects, no increased mortality.

Then the bomb dropped: the studies were shutdown prematurely. Why? Headmaster of the late ACCELERATE study, Steve Nissen declared for Forbes magazine that “the drug didn’t work". I don't think that was the accurate response. In my opinion the drug worked because patients achieved incredible high HDL levels. The problem was that despite these very high HDL levels the cardiovascular events were not different from the placebo group. So I would say that drug proved to be effective in what it was made to do but high HDL levels were useless.

---

[1] Acad. St. Milcu Medical Diagnostic and Treatment Outpatient Centre, Bucharest

Here comes the question: is the HDL as protective as we used to believe or just handsome good-for-nothing cholesterol as suggested by those studies?

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ADMINISTRATIVE ISSUES

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We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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(4) validates registered information;

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RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related "mini-series" of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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EDITORIAL

About health, with hope

Dan Mischianu

Gral (R) Prof DAN MISCHIANU
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Health is an extremely complicated subject, but to talk about health is like howling in a cave: anyone can hear, no one understands! ...maybe only the echo?!

The World Health Organization (WHO) proposed in 1948 when it was established, the following definition: "Health is a fully favorable physically, mentally and socially status, not merely the absence of disease or infirmity." Then came an addition: 'ability to lead a productive life, socially and economically. "

I recently finished reading a bestseller also promised to the Romanian reader by Polirom publisher "Sapiens A Brief History Of Humankind" Yuval Noah Harari, Vintage Books, London, 2014. A strange creature this "homo sapiens" is, strange since all his derivatives propose other approaches and other perceptions on the concept or "health"! To primordial "sapiens" health had other connotations...

Perhaps they were cleverer, crueler, and that is why they prevailed and multiplied logarithmically. Then the diseases appeared: congenital, posttraumatic, neoplastic, infectious and so on...

Returning to our nation, Romanians produced with unmistakable grace many sayings, in which they truly believe: "Health is better than all!", "Health is the best wealth!"

Or, pessimistically "Until you're sick, you cannot be healthy!"

The traditional stories regarding health record something else: "Health, contentment and trust are your most valuable possessions and freedom is the greatest joy of yours."

I am absolutely convinced that we all want to reach or to "apply" (Anglo-Saxon term) to an entity which we believe is intangible: "Health comes first!"

Unfortunately, after we solve almost all our goals and desires, we find that health left at the end, it is "unsolvable"!...

Romanians deep and law abiding Christians, ancestral pessimistic have another saying "Cherish health when you lose it!"

Even I do not understand this allegory, why?

Because I work in a field that many pretend to understand, but few have deciphered the "anatomy and physiology" of its development, an area where financiers who led it wanting to make a profit, now keep their mouths shut nor can i forget that there were great men, deciding the destiny of many nations, who had a absolutely unfortunate history of "health": Emperor Napoleon III died in urosepsis under the supervision of the most famous urologist of its time, Emperor Wilhelm II, the first grandson of Queen Victoria of the United Kingdom, born with left brachial plexus palsy after a labor dystocia (1859), "irritable, angry, violent and passionate" started The First World War and Generalissim – Iosif Vissarionovich Stalin's apparently had the same malady that which made him as reckless and cruel.

They followed, and still follow many others, the bad, and the complexed!

It is these thoughts that make me not to forget Miron Costin's assertion: "There are times under the man, but the poor man under these times." ("Chronicle of Moldavia after Aron Voda").

For this editorial, unfortunately slightly skeptical about the future, it will appear in the new year 2017 I ask the reader of these lines to believe and hope that Romania will benefit from a new configuration of a credible, effective and with a tendency towards the many health system.

I further wish you a happy New Year full of accomplishments, or, as people say in our country, “A Happy New Year!"

REVIEW ARTICLE

Article received on March 7, 2016 and accepted for publishing on August 25, 2016.

Capabilities for identification and confirmation of bacterial biological agents

Diana M. Popescu[1], Marius Necșulescu[1], Lucia E. Ionescu[1], Simona N. Bicheru[1], Gabriela V. Dumitrescu[1], Viorel Ordeanu[1]

Abstract: *Military Medical Service is able for detection, identification and confirmation of biological agents; it is part of medical protection against CBRN weapons. We are specialized capabilities for in vitro tests, under construction, the maximum containment laboratory designed for work with Risk Group Microorganisms. An efficient primary containment system must be in place, consisting of one or a combination of the following: Class III safety cabinet laboratory, passage of two doors, suit laboratory, controlled access, controlled air system. Negative pressure in the facility, supply and exhaust air must be HEPA-filtered, decontamination of effluents, sterilization of waste and materials, airlock entry ports for specimens, materials and animals must be provided etc. Complementary is an Animal facility for in vivo tests. This is suitable for work with animals that are deliberately inoculated with microorganisms in Risk Group.*

Keywords: *biological security, military capability, biological attack, biological agents, CIMIC, medical protection*

CAPABILITIES FOR IDENTIFICATION AND CONFIRMATION OF BIOLOGICAL AGENTS

Military Medical Service is abilities for the detection, identification and confirmation of biological agents. It is part of the medical protection against CBRN weapons, with mobile and stationary capabilities. MMRC participate, specifically the medical protection against biological weapons and bioterrorism with a series of microstructures: Biological mobile intervention team with mobile platform; Biological analytical laboratory CBRN defense, integrated platform for scientific and medical-military research for biological agents, with: Laboratory of Microbiology high secure, Secured animal facility laboratory for confirmation of biological agents, laboratory of molecular biology and genetics, toxicology laboratory and Annex facility. [1, 2, 3, 4]

SPECIALIZED CAPABILITIES

Stationary

The maximum containment microbiological laboratory is designed for work with Risk Group Microorganisms, for in vitro diagnostic. Operational maximum containment laboratories should be under the control of national or other appropriate health authorities. [5,

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[1] Military Medical Research Center, Bucharest

6]

Laboratory design and facilities:

- Primary containment. An efficient primary containment system must be in place, consisting of one or a combination of the following; Class III cabinet laboratory. Passage through a minimum of two doors prior to entering the rooms containing the Class III biological safety cabinet is required; [5, 7] Suit laboratory. A protective suit laboratory with self-contained breathing apparatus differs significantly in design and facility requirements from a Biosafety Level 4 laboratory with Class III biological safety cabinets. [5]

- Controlled access. The maximum containment laboratory must be located in a separate building or in a clearly delineated zone within a secure building. Entry and exit of personnel and supplies must be through an airlock or pass-through system. [5, 8, 9, 10, 11]

- Controlled air system. Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. There are significant differences in the ventilating systems of the Class III cabinet laboratory and suit laboratory. [5]

- Decontamination of effluents. All effluents from the suit area, decontamination must be decontaminated before final discharge. Heat treatment is the preferred method. Effluents may also require correction to a neutral pH prior to discharge. Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment. [5, 8, 9, 10]

- Sterilization of waste and materials. A double-door, pass-through autoclave must be available in the laboratory area. Other methods of decontamination must be available for equipment and items that cannot withstand steam sterilization. [5, 6]

- Airlock entry ports for specimens, materials and animals must be provided. [5]

- Emergency power and dedicated power supply lines must be provided. [6]

- Containment drains must be installed. [5]

Because of the great complexity of the engineering, design and construction of Biosafety Level facilities, in cabinet or suit configuration, schematic representations of such facilities have not been included. [5] (Figure 1)

Figure 1: Microbiological laboratory for biological agents

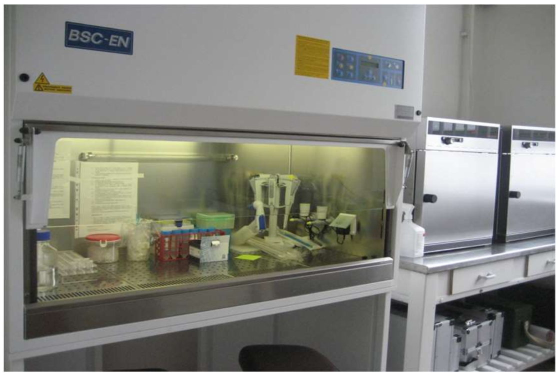

Animal facility

This is suitable for work with animals that are deliberately inoculated with microorganisms in the Risk Group, for in vivo diagnostic. [12, 13, 14] The following safety precautions apply:

- All the requirements for animal facilities – Biosafety Level must be met. [12, 13, 14, 15]

- Biohazard warning signs (see Figure 1) should be posted on doors and other appropriate places. [12, 14]

- The facility must be designed for easy cleaning and housekeeping. [12, 14]

- Doors must open inwards and be self-closing. [12, 14]

- Heating, ventilation and lighting must be adequate. [12, 14]

- If mechanical ventilation is provided, the airflow must be inwards. Exhaust air is discharged to the outside and should not be recirculated to any part of the building. [12, 14]

- Access must be restricted to authorized persons. [12, 14]

- No animals should be admitted other than those for experimental use. [12, 14]

- There should be an arthropod and rodent control program. [12, 14]

- Windows, if present, must be secure, resistant to breakage and, if able to be opened, must be fitted with arthropod-proof screens. [12, 14]
- After use, work surfaces must be decontaminated with effective disinfectants. [12, 14]
- Biological safety cabinets (Class II) or isolator cages with dedicated air supplies and HEPA-filtered exhaust air must be provided for work that may involve the generation of aerosols [12, 13, 14, 15, 16]
- An autoclave must be available on site or in appropriate proximity to the animal facility. [12, 13, 14, 15, 16]
- Animal bedding materials must be removed in a manner that minimizes the generation of aerosols and dust. [12, 13, 14]
- All waste materials and bedding must be decontaminated before disposal. [12, 13, 14]
- Use of sharp instruments should be restricted whenever possible. Sharps should always be collected in puncture-proof/-resistant containers fitted with covers and treated as infectious. [12, 13, 14]
- Material for autoclaving or incineration must be transported safely, in closed containers. [12, 13, 14]
- Animal cages must be decontaminated after use. [12, 13, 14]
- Animal carcasses should be incinerated. [12, 13, 14]
- Protective clothing and equipment must be worn in the facility, and removed on leaving. [12, 13, 14]
- Hand-washing facilities must be provided. Staff must wash their hands before leaving the animal facility. [12, 13, 14]
- All injuries, however minor, must be treated appropriately, reported and recorded. [12, 13, 14]
- Eating, drinking, smoking and application of cosmetics must be forbidden in the facility. [12, 13, 14]
- All personnel must receive appropriate training. [12, 13, 14]

This facility is complementary to the microbiological laboratory (Figures 2, 3).

Figure 2: Animal facility for microbiological diagnosis

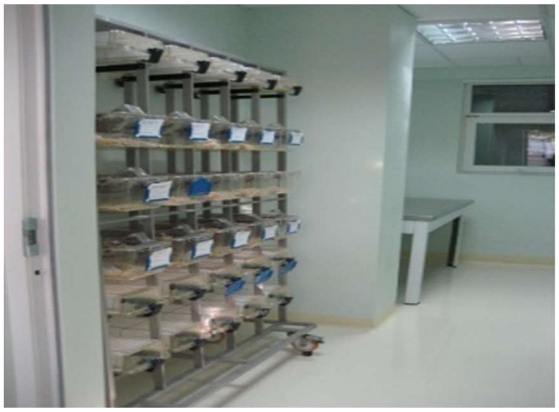

Figure 3: Secured animal facility laboratory

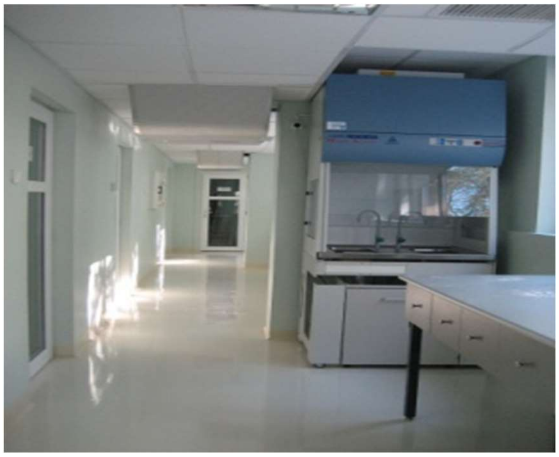

SPECIALIZED MOBILE CAPABILITIES FOR Bio-Det SYSTEM

Specialized Mobile capabilities for Bio det system:

- provides monitoring, sampling, detection and identification;
- detects long line releases and identifies up to 6000 species and sub-species (data base).
- up to 96 samples can run simultaneously, no time needed to re-deploy.
- deploy worldwide and conduct biological surveillance operations in order to provide identification of biological agent employment.
- provide information to other forces (rescue teams, medical, etc)

Bio-Det Systems

Configuration:

- Vehicle with 2 internal compartments, with power supply, specific furniture and safety cabinet class III.

For monitoring: Verotect – determines if a bio-mass is present within aerosol particles of a certain size range

For identification:

- MALDI TOF Microflex LT 20 with vehicle mount
- Water, soil and air sampling kits
- Sample preparation equipment (incubator, centrifuge, freezer, autoclave, handling tools, consumables and reagents)

Miscellaneous:

- Personal protection for the operators (suits, masks, oxygen tubes)
- Hardened laptop etc.

Mass Spectrometry

Those are complex instruments which record an ionic flow as a spectrum, in relation to both mass and the relative occurrence of the ions; Mass spectrometry is a confirmation analytical technique which allows identification of organic compounds, based on the mass/electrical charge ratio of the molecule fragments of the analyze.

The mass spectrometer consists of 4 elements: sample loading port, where a certain amount of sample is introduced at the required pressure in order to be exposed to the ion source and to be ionized at maximum efficiency. Ion source, where the neutral atoms and molecules are ionized and directed into the instrument as a monenergistic bundle.

Mass (energy) analyzer, which by electrical and/or magnetically deflections selects the ions according to the q/m values, their masses, concomitantly focusing them towards an outlet gap. Detector, comprising the system which transforms the ionic flow either into a photographic image or an electric current that can be recorded with an appropriate instrument.

MALDI TOF Microflex

Microflex LT20 is a MALDI TOF system equipped with Bioprofiler software and it is used for the identification and taxonomic appointment of the biological agents from air, water, soil and other surfaces, after a previous specific processing of the collected samples. The main element is a MALDI TOF spectrometer, with a mass domain from 2,000 to 20,000 Da. The instrument is controlled by means of a computer with Windows operating system and specialized software (Bioprofiler). The data processing system controls all acquisition, saving and storage processes of the spectral information, allowing later processing of the information or access to the data base.

Microflex LT 20 Bruker system

It has the ability to identify the biological agents from various samples (liquid, solid, gaseous). Identification is performed by comparing the obtained spectrum to the existing data base of memorized biological agents.

The software indicates the matching of the spectrum under test with spectra from the database, according to the obtained scores. Scores are generated by evaluation and comparison between spectra and are not percents. If a biological agent is not included in the data library, the agent with the closest structure is exhibited, but the score indicates that the agent under test is not included in the data base of the instrument (Figure 4).

Figure 4: Mass spectrometer for microbiological diagnosis (The Microbiology Laboratory MMRC, I. Cantacuzino)

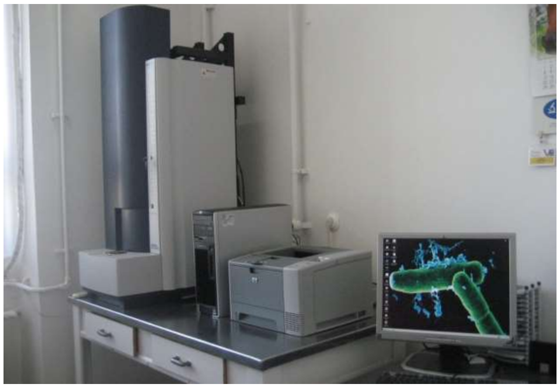

Operation steps Microflex

Preparation of samples for MALDI TOF analysis is done by extracting 16S ribosomal proteins that can be achieved by several techniques depending on the organism analyzed. [17]

The instrument can measure mass spectra from 2,000 up to 20,000 Da, i.e. a very wide range of organic molecules originating from any kind of biological agents (bacteria, spores, viruses, fungi, yeasts, toxins). The software and its data base are capable to identify biological agents of civil and medical-military interest.

The equipment is foreseen with a device for attachment to mobile laboratories, allowing its use in the field, either in campaigns or, in peace time, in case of biological events or crises.

The quality of the incorporated mass spectrometer as well as the structure of the soft that can be upgraded in time, strongly recommends the MALDI TOF – Bioprofiler MICROFLEX LT20 system for identifying biological agents, to be used for precise microbiologic diagnostic, species confirmation for the biological agent, medico-military research and medical defense against biological weapons or bioterrorist attacks.

Military Medical Service is able for detection, identification and confirmation of biological agents. It is part of medical protection against CBRN weapons, in civil-military cooperation (CIMIC). [17]

CONCLUSIONS

CCSMM has specific capabilities for scientific research for medical protection against CBRN WMD.

Anti-infective medical protection laboratory and Epidemiologic emergency laboratory have capability of performing detection, identification and confirmation of biological agents within the limits of available supplies.

The capacities and capabilities of health protection against biological weapons are interoperable with NATO.

Detection, identification and confirmation of the presence of bacterial biological agents are executed sequentially and modular, specialized capacities through cooperation, which must have the capability to perform these tasks.

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REVIEW ARTICLE

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The consequences of Chernobyl accident

Ion Chioșilă[1]

Abstract: *These days marks 30 years since the Chernobyl nuclear accident, followed by massive radioactive contamination of the environment and human in Belarus, Ukraine and Russia, and resulted in many deaths among people who intervened to decrease the effects of the nuclear disaster. The 26 April 1986 nuclear accident contaminated all European countries, but at a much lower level, without highlighted consequences on human health. In special laboratories, the main radionuclides (I-131, Cs-137, Cs-134 and Sr-90) were also analyzed in Romania from environmental samples, food, even human subjects. These radionuclides caused the population to receive a low dose of about 1 mSv in 1986 that is half of the dose of the natural background radiation (2.4 mSv per year). As in all European countries (excluding Ukraine, Belarus and Russia) this dose of about 1 mSv fell rapidly by 1990, reaching levels close to ones before the accident at the nuclear tests.*

Keywords: *nuclear accident, Chernobyl disaster, radionuclide, radioactive contamination, effective dose*

INTRODUCTION

2016 marks 30 years since the major accident in the history of nuclear power plants (active zone melting and thermal explosion) that took place on 26th April 1986 at 4th reactor of Chernobyl Nuclear Power Plant (Ukraine). Emissions of radio nuclides were particularly high and affected many countries in Europe, including our country. The 4 reactors in operation at the time run on natural uranium (U-238) with low-enriched U-235, a high producer of Pu-239 (strategic material for nuclear weapons).

Although this type of reactor was well known by the operating personnel, they conducted an experiment with the turbine running in the inertial mode and lowering reactor's power at 10%, but the return to full power (thousand MW) took only 2 seconds which led to a loss of control and the impossibility of an emergency shutdown of reactor no. 4. The rapid and uncontrolled increase in temperature of the reactor core produced two successive explosions seconds after each other that threw the 1000 tons concrete for protection, as well as large amounts of nuclear fuel with fission products and activation products, along with the burning graphite moderator [1].

Definitions of specific terms

1. Activity (radioactivity) of a radionuclide: the unit of work in the International System (S.I.), is one disintegration per second, or s-1, with the name Becquerel and symbol Bq. Curie - special name for the tolerated unit of the activity of a radionuclide, with the symbol Ci; is 3.7 x 1010 disintegrations per second

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[1] Romanian Society for Radiological Protection, Bucharest

(approximate activity of radon – 222 in equilibrium with one gram of radium – 226) and respectively 3.7 x 1010 Bq.

2. Half-life – the time after which the number of nuclei of a radionuclide is reduced to half by radioactive decay with symbol T1/2, or Tf is used.

The biological half-life – the time required for a biological system to remove half of the nuclei (stable or radioactive) by metabolic processes with symbol Tb. The effective half-life – the time needed to halve the number of radioactive nuclei (activity) by decay and metabolic processes with Tef symbol.

3. Effective dose – weighted sum of equivalent doses from external and internal exposure, performed on all organs (tissues) of the body (the absorbed dose multiplied by the weighting factor of radiation and weighting factor of tissue or organ). In the S.I. unit is J/kg with the name Sievert and symbol Sv.

The effective dose limit for occupationally exposed workers – effective dose limit for people working in environmental radiation is 20 mSv per year. The effective dose limit for the population – effective dose limit for an individual in the population is 1 mSv per year.

CONTAMINATION OF THE ENVIRONMENT

Fission products (Cs-137, Sr-90, Sr-89, I-131, etc.) and activation products (Cs-134, Co-60, Fe-55, Fe-59, etc.) were released in atmosphere and transported by air currents throughout the northern hemisphere. The most affected (radioactive contamination) were Belarus, Ukraine and European Russia; less contaminated air currents reached many other countries in NW Europe (mainly Norway, Sweden, Finland), and to S and S-V Europe, inclusively to Romania.

Rainfall and dry deposition made possible contamination of the environment (soil, surface and biosphere).

Cs-137 was the easiest to detect (beta-emitting and gamma-emitting radiation) and has a great half-life period (Tf or 1/2) of approximately 30 years, for which most of the estimations of environmental and human contamination reported to this radionuclide.

Table 1: Surfaces contaminated with Cs-137 in some countries affected by the Chernobyl nuclear accident [2]

Country*)	**37-185 (kBq/m^2)**	**185-1480 (kBq/m^2)**	**over 1480 (kBq/m^2)**
Russia	49800	7800	300
Belarus	29900	14400	2200
Ukraine	37200	4100	600
Sweden	12000	-	-
Finland	11500	-	-
Norway	5200	-	-
Austria	8600	-	-
Bulgaria	4800	-	-
Switzerland	1300	-	-
Greece	1200	-	-
Slovenia	300	-	-
Moldavia	60	-	-

*) In Romania, most of the surface was contaminated with Cs-137 with values below 2 kBq/m^2. There were large areas contaminated 2-10 kBq/ m^2 in Oltenia, Banat, N-E Moldova, central and northern Muntenia; here they were found locations with contamination exceeding 10 kBq/ m^2.

ASPECTS OF RADIATION PROTECTION IN ROMANIA

Monitoring environmental radioactivity and food radioactivity in Romania [1,3] was carried out by:

- 47 Environmental Radioactivity Monitoring Stations (belonging to the former National Council for Water, today 37 stations form the National Network of Environmental Radioactivity Monitoring and belongs to the Ministry of Environment)
- 22 Ionizing Radiation Hygiene Laboratories, of which 4 were from the Institute of Hygiene and Public Health in Bucharest, Iasi, Cluj and Timişoara (Ministry of Health)
- departamental laboratories (Institute of Atomic Physics – IFA Măgurele – Bucharest, Institute of Nuclear Energy Reactors – IRNE Pitești, Ministry of National Defense – MND, etc) (The Laboratory for radiochemistry of the Military Center for Medical Research also had tasks of human and environmental radioactivity monitoring in addition to the experimental research. There was also a laboratory to

measure the radioactive contamination of man (with whole body counter).

In the period immediately after the accident (the night of April 30, 1986 when contaminated air masses came first in the N-E) and in the years after the accident the following analyzes were performed:

- global beta measuring and gamma spectrometry of aerosol filters, total atmospheric deposition, surface and drinking water analysis, soil, wild vegetation and crops analysis, animal products and food menus from various canteens in Bucharest and in the country analysis
- direct measurement of radionuclides incorporated into man (I-131, Cs-137 and Cs-134) with MND and IFA whole body counter
- radiation dose exposure estimation for humans.

Among the measures taken for radiation protection of population in Romania, it is worth mentioning:
- consumer restrictions on certain foods (dairy products contaminated with I-131, Cs-137 and Cs-134) based on the values determined by measuring the radioactivity of food in the most contaminated counties
- forbidding sport events scheduled to take place outdoors on May 2
- administration of stable iodine for children, beginning with 3th May

Recommendations:
- extensive washing of vegetables before consumption
- reduced time spent in the open in the first week after the accident.

Negative aspects concerning the application of radiation protection measures:

- insufficient public information on the accident,
- KI administration started with a two days delay leading to reduced performance at about 50% and the administration was not performed at all children in areas contaminated with I-131.

ENVIRONMENTAL, FOOD AND HUMAN CONTAMINATION IN ROMANIA

After corroborating the results of radioactivity monitoring of the environmental factors, water and food, it was possible to create a map of radioactive fallout in our country in three stages: 1st to 2nd of May, 3rd - 4th of May and 5th to 6th of May respectively. So, the greatest contamination occurred due to the fallout of 3rd to 4th of May, which came from N-E and followed the Eastern and Southern Carpathians, with the southern area being the most contaminated (Figure 1) [4].

In atmospheric aerosols there were readings of a maximum of 103 Bq/m^3 for I-131, respectively 63 Bq/m^3 for Cs-137 at Toaca station in Ceahlău on 1st of May 1986; also readings of 17 Bq/m^3 I-131 and 14 Bq/m^3 for Cs-137 at Fundata station (Braşov county). These peaks declined rapidly in the second half of May and early June 1986, going as low as a 1000 times lower. Regarding the average concentration of Cs-137 in the aerosols, from about 106 Bq.s/m^3 in 1986 fell below 105 in 1987 and in 1991 it went back to the value from before the accident (about 200 Bq.s/m^3) [4,5].

Atmospheric radioactive depositions values were in 1986 over 3 orders of magnitude higher compared to before Chernobyl accident. As late as 1993 the average values of Cs-137 deposition reach 1 order of magnitude over that of 1985 because of wind carrying out the contaminated dust and returning in the form of radioactive fallout.

Average concentrations of Cs-137 in untilled soil samples were of 350 Bq/kg, with peaks in excess of 1000 Bq/kg in the northern county of Gorj. High levels of Cs-137 in untilled soil were recorded at Toaca-Ceahlău, Targu Mures, Gheorghieni and Parâng, with maximum levels of 2050 Bq/kg of dry soil.

In surface water, high values of Cs-137 were determined 2 or 3 days later than the atmospheric reading, measuring 50 Bq/m^3, with rapid drop below 10 Bq/m^3 in 1987 and 1988, returning to the values from 1985 as late as 1993.

The contamination of wild vegetation was carried out by fine radioactive particle deposition and measuring average values of Cs-137 as high as 1000 Bq/kg of dry vegetation, with values declining to before the accident in 1990. Contamination of crops by absorbing radionuclides in the soil was quite low, so radionuclide values decreased rapidly in the coming years [3].

The highest level of contamination with I-131 in food was found in ewe`s milk, with values of about 1300 Bq/L or higher, which led to temporary restriction in consumption imposed by the Ministry of Health, for dairy products. Cs-137 values in milk and dairy products were about 500 Bq/kg (Table 2). This radionuclide content in food has decreased since 1990, and since 2000 has reached the 1985 values.

Figure 1: Contamination Romanian territory since May 1, 1986 [4]

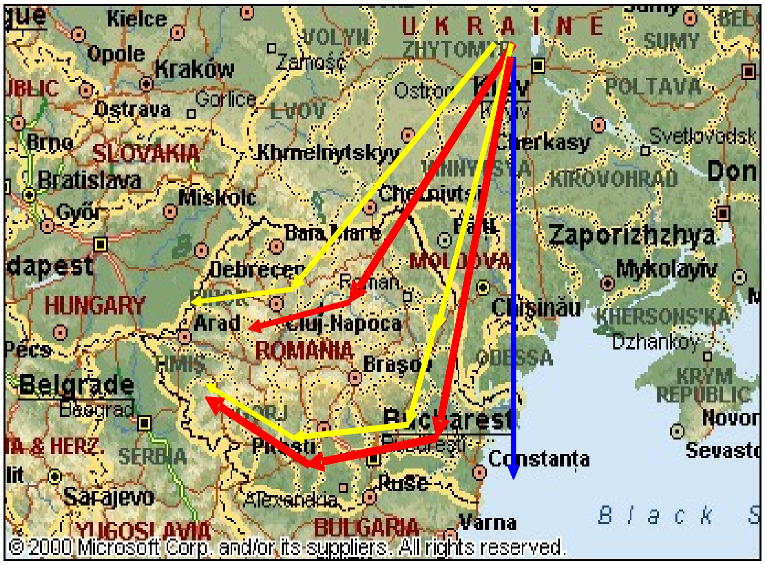
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Brno
SLOVAKIA
Khmelnytskyy
Cherkasy
Svetlovodsk
Bratislava
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KIROVOHRAD
Don
Győr
Chernivtsi
Baia Mare
Bălţi
Zaporizhzhya
Budapest
Debrecen
MOLDOVA
Mykolayiv
HUNGARY
Roman
Chişinău
KHERSONS'KA
Arad
Cluj-Napoca
Dzhankoy
Pécs
ROMANIA
Braşov
ODESSA
TIMIŞ
KRYM REPUBLIC
Belgrade
Beograd
Bucharest
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Constanta
Sevasto
Sarajevo
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Three stages of contamination in Romania: 🟥 1 – 2 🟨 3 – 4 🟦 5 – 6

Table 2: Content of Cs-137 (Bq/L or kg) in drinking water and foods [5, 6]

Food	1986	1987	1988 - 1991	2003
Water	-	0.05	0.002 – 0.009	0.002 – 0.009
Milk	10 - 200	27.92	1.02 – 5.13	0.01 – 0.137
Dairy products	10 - 500	61.98	5.07 – 16.61	-
Meet	50 - 300	47.50	1.31 – 39.25	0.014 – 10.28
Meet products	10 - 727	23.19	1.50 – 19.15	-
Grain products	50 - 100	42.16	1.01 – 12.15	0,251
Fruits and vegetables	7 - 411	46.43	1.89 – 23.39	0.037 – 0.265
Food menu/24 hours	-	23.69	1.50 – 4.49	-

If immediately after accident experts paid particular attention to radionuclides I-131 and Cs-137, easily determined by gamma spectrometry directly from samples, by the end of the year and in following years measurements were performed to determine the content of Sr-90 in water and food. Aerosols contamination levels with Sr-90 were less than 1/10 the levels of Cs-137 so the food contamination with Sr-90 was much lower, generally below 10 Bq/kg or L of product (Table 3).

Radionuclides Cs-137, Cs-134 and Sr-90, a little transferable from soil to plant, however they were detected many years after the accident; radioactive contents fell sharply in 1987 and then steadily, currently reaching in most foods well below 1 Bq/kg, hard to detect even with radiochemical analysis. There still are very low values for Cs-137 (units Bq/kg) in berries, mushrooms and wild venison.

Table 3: Content of Sr-90 (Bq/L or kg) in drinking water and foods [5, 6]

Food	1987	1988 - 1991	2003
Water	0.04	0.003 – 0.010	0.001 – 0.066
Milk	0.43	0.14 – 0.33	0.068 – 0.930
Dairy products	0.75	0.19 – 0.76	-
Meet	0.46	0.10 – 0.24	0.087 – 0.690
Meet products	0,62	0.09 – 0.20	-
Grain products	0.70	0.25 – 0.76	0.135 – 0.730
Fruits and vegetables	0.97	0.10 – 0.98	0.047 – 1.060
Food menu/24 hours	2.30	0.19 – 0.23	-

Assessment of human internal contamination with radionuclides I-131, Cs-137, Cs-134 and Sr-90 (Table 4) was performed both directly for the first three radionuclides (measuring radionuclides from humans) and by estimating the contamination based on the values of radioactive content determined in drinking water, food, and in real or hypothetical food menu (Table 2).

Table 4: Content of Cs-137, Cs-134 and Sr-90 in the human body (direct measurement/ estimation from ingestion of food) in Romania [6]

Radionuclide		1986	1987	1988	1989	1990	1991	1992	1993
Cs-137	measurement	1000 - 2000	2000 - 2500	800 - 1700	500 - 1100	300 - 500	< 300	< 300	< 300
	estimation	1538 - 20383	2335 - 12774	415 - 673	106 - 198	82 - 150	80 - 140	60 - 119	19 - 38
Cs-134	estimation	686 - 8703	729 - 4894	94 - 153	18 - 33	10 - 18	5 - 10	1 - 3	-
Sr-90	estimation	30 - 100	18 - 57	12 - 38	12 - 34	11 - 32	10 - 30	9 - 28	8 - 25

After about 80 days I-131 disappeared from humans and food through physical decay (if 10 Tf pass, it is considered that radionuclide decreases in values too difficult to determine; Tf for I-131 is 8.1 days). In terms of I-131 content found in thyroid, it presents very different values to a few thousand Bq only in May and June, depending on the level of contamination of the studied area and the food consumed. These rates were still much lower compared to human radioactivity/radiation dose received in the case of thyroid scintigraphy (tens - hundreds of kBq) [1,6].

For humans, Cs-137 + Cs-134, from about 2500 Bq in 1986 the values have gradually decreased to a point where after four years they went below 300 Bq. The loss of activity of the two radionuclides in humans was attributed to their reduction in food and to metabolic processes in the human body (Tb for the two radionuclides is 100-150 days) or by disintegration for Cs-134 (Tf = 2.05 years); so that after 1990 the Cs-137 values fell below the detection limit of the equipment used for the direct measurement. Sr-90 contamination for humans was much lower, estimated values below 100 Bq (radionuclide emits only beta radiation that can only be detected in small biological samples and the low contamination of foods did not allowed measuring this radionuclide in humans) [1,6].

ESTIMATION OF THE EXPOSURE DOSE FOR THE POPULATION OF ROMANIA

Immediately after the accident, Oncescu and Galeriu (IFA Bucharest – Măgurele) have calculated the exposure dose due to radioactive contamination of the population in our country which calculated to be about 1 mSv for the first year after the accident. Values are similar to those published by WHO (World's Health Organization) and IAEA (International Atomic Energy Agency) for other European countries (Finland: 0.44 mSv, Germany: 0.5 to 1.1 mSv, Italy: 0.61 mSv, Poland: 0.95 mSv, Switzerland: 1 3 mSv etc.) [1].

The subsequent calculation of the effective dose received by man from radionuclides determined in air, water and food for the most contaminated areas in Romania, except the contribution of I-131, resulted in average values close to 1 mSv in the first year after the accident, with a greater fall in 1988 (the greatest contribution to contamination was from Cs-137 and Cs-134 in food). Between 1990 and 2000 and now, the annual effective dose for humans fell below 50 µSv, values over 40 times lower than the dose received by humans from background radiation [4].

Figure 2: Exposure assessment of Romania's population – dose reconstruction by food based on Cs-137 concentration in atmospheric deposition [4, 5]. Minimum exposure dose of 0.2 mSv in Satu Mare County, maximum exposure dose of 2.1 mSv in Gorj County.

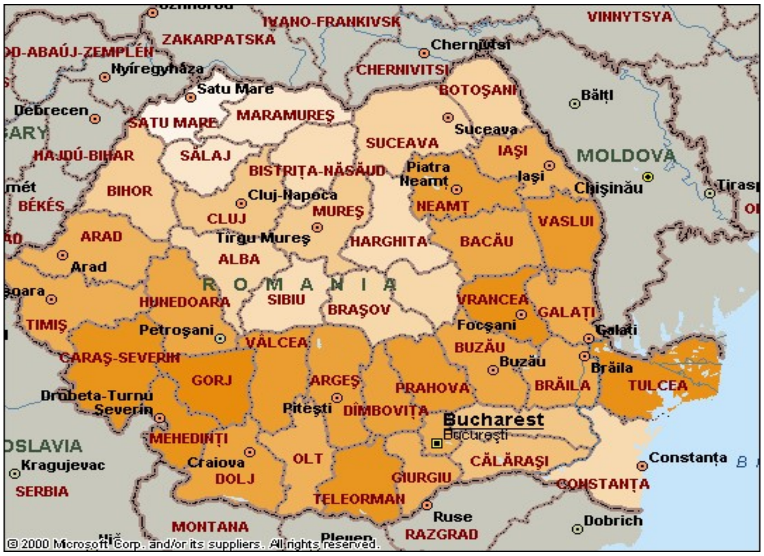
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Nyíregyháza
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BOTOŞANI
Bălţi
Debrecen
SATU MARE
MARAMUREŞ
Suceava
SUCEAVA
IAŞI
MOLDOVA
HAJDÚ-BIHAR
SĂLAJ
Piatra Neamţ
Iaşi
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BISTRIŢA-NĂSĂUD
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BIHOR
Cluj-Napoca
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BÉKÉS
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VASLUI
ARAD
Tîrgu Mureş
HARGHITA
BACĂU
Arad
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ROMANIA
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GALAŢI
BRAŞOV
TIMIŞ
Petroşani
Focşani
Galaţi
VÂLCEA
BUZĂU
CARAŞ-SEVERIN
Buzău
Brăila
GORJ
ARGEŞ
PRAHOVA
BRĂILA
TULCEA
Drobeta-Turnu Severin
Piteşti
DÎMBOVIŢA
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Kragujevac
Craiova
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Doses of radiation exposure of the population in Romania after the nuclear accident vary by an order of magnitude between 0.2 and 2.1 mSv in the first year (Figure 2), based on contaminated air masses movement (Figure 1) as well as wet and dry deposition in our country. Thus, there were areas (counties) with quite small exposure doses, less than 0.5 mSv such as Satu Mare, Salaj, Maramures, Sibiu, Brasov, Harghita, while in other counties exposure doses were even over 1 mSv such as: Caras-Severin, Gorj, Mehedinți, Tulcea, Neamt, Vrancea, Teleorman.

The average dose received by most of the European countries of about 1 mSv is incomparably lower than that of more than 10 mSv received by population in heavily radioactive contaminated areas of Belarus, Ukraine and Russia. This dose of about 1 mSv in the first year after the accident is below the natural background exposure (irradiation) had a low radiological significance on health.

Natural background radiation exposure (cosmic radiation, earth radiation given by natural radionuclides present in the environment, food and humans) is about 2.4 mSv per year for the entire planet, with the exception of areas with high natural background radiation (even more than 20 mSv per year in some areas of India, Brazil, Congo, Sweden) due to the massive presence of natural radionuclides [1].

Thus, the additional exposure dose received by most of the population of Europe in 1986 was about half of the natural background exposure, which is a quite low dose, closely to effective dose limit (of 1 mSv per year) supported by legislation for an individual of the population. To be mentioned that the effective dose limit allowed for workers exposed for professional reasons (who work with ionizing radiation in the environment) is 20 mSv per year.

Given the levels of radioactive contamination of the environment in Romania, much lower in comparison with Ukraine, Belarus and Russia, the exposure doses received by our population were relatively low (0.2 to 2.1 mSv in 1986) well below those that could produce significant biological effects on human health. Moreover, studies carried out by the hygiene institutes of Romania did not reveal an increased incidence of congenital malformations in children born between 01.10.1986 and 31.12.1987 compared with the reference group (those born before 1986) [3].

Exposure doses and biological effects post-Chernobyl [2]

Average exposure doses received by the affected people in Ukraine following the nuclear accident were distributed as follows:

- 100 mSv, about 240 000 people participating in decommissioning the damaged reactor
- 30 mSv, about 116 000 people evacuated,
- 10 mSv, tens of thousands of people that continued living in contaminated areas, in the first years after the accident.

It is estimated that the maximum exposure dose can be even with an order of magnitude higher in some areas with massive radioactive contamination from the three countries Belarus, Russia and Ukraine.

In other European countries, the maximum dose was about 1 mSv in the first year after the accident, with a progressive decrease in the coming years. The dose is well below the natural background exposure (irradiation) has a low radiological significance.

Another 200,000 workers who received doses of 0.01 to 0.5 Sv were at increased risk of developing late effects, including cancer that could put their lives in danger.

Of the 600 workers that participated directly in the decommissioning, 134 received doses of 0.7 and 13.4 Sv and develop radiation sickness (ARS – Acute radiation syndrome). Of these, 30 died shortly after the accident [2].

In areas heavily contaminated in the three countries, measures taken to limit radiation exposure have changed the lives of millions of people (returning to homeland, modification of eating habits, restrictions and job changing) to damage health conditions including psychiatric problems.

Thyroid cancer was given particular attention, about 1800 cases for exposed children, which greatly exceeds estimates in the 1986. The cases of leukemia (latency 2-10 years) weren't significantly higher in staff directly involved in decommissioning. The greatest increase in cases of thyroid cancer for children has been reported in the Gomel region of Belarus (near Chernobyl) [7].

In the most contaminated areas of Russia, Belarus and Ukraine there are still values of Cs-137 and Sr-90 easily detectable in water and food; thus, in the Bryansk region (Russia), Cs-137 had values of 90 kBq/kg in predatory fish meat, high value for humans too, up to 50 kBq, which led to estimation of doses ranging up to 1.8 mSv in 1996. These values are well above those detected in Romania immediately after the accident [2].

Ever since 1987, a special monitoring program of about 7 million people affected by the accident has begun: liquidators of consequences of the accident (1986 - 1987), evacuated population in 1986 and established in other areas of the former USSR, residents of areas heavily contaminated with Cs -137, Sr-90 and Pu-239, descendants of the irradiated persons (liquidators, people evacuated), people contaminated with I-131, aged 0 to 18 years during the accident [7].

Epidemiological studies have revealed: an increase in the number of people with psychological problems, number of suicides and overall violent deaths among liquidators and evacuated population due to insufficient information on the effects of ionizing radiation, social dislocation and diminishing quality of life. Only complete epidemiological studies will halt the trend of attribution of increasing incidence of all cancers exclusively to the effects of the nuclear disaster, especially in areas heavily radioactive contaminated [8].

CONCLUSIONS FOR CONSEQUENCES OF THE CHERNOBYL ACCIDENT IN ROMANIA

1. The level of radioactive contamination of the environment in Romania after the nuclear accident in 1986 was relatively similar to that of other central and eastern European states; 10 kBq/m^2 at ground level, with few areas over this value.

2. The main radionuclide contaminants, I-131, Cs-137, Cs-134, even Sr-90 from deposition and aerosols immediately after the accident passed quickly in plants and animals. I-131 showed values close to 1 kBq/kg, Cs-137 and Cs-134 values of several hundred Bq/kg, whereas the Sr-90 was below 10 Bq/kg.

3. Radionuclides Cs-137, Cs-134 and Sr-90 transfer difficult from soil to plants trough absorption, were still detectable in food many years after the accident; radioactive contents fell sharply in 1987 and then steadily, reaching below 1 Bq/kg between 1990 and 2003 in most foods. At the same time, there were somewhat higher values detected in berries, mushrooms and venison.

4. In humans, Cs-137 from 1000 to 2000 Bq gradually decreased, so in 1990 reached about 300 Bq. The annual effective dose decreased from about 1 mSv in the first year after the accident, to below 50 µSv between 1990 and 2000, respectively below 10 µSv between 2000 and 2010.

5. Besides the three heavily radioactive contaminated states (Belarus, Ukraine and eastern Russia), other contaminated European countries reported no biological effects on the population after the accident.

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REVIEW ARTICLE

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Using direct anticoagulants (DOACs) in cancer and other high-risk populations

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Abstract: *The major practical advantage of the direct oral anticoagulants (DOACs), comprising the thrombin inhibitor dabigatran and the factor Xa inhibitors apixaban, edoxaban, and rivaroxaban, over vitamin K antagonists is their fixed dosing without the need for laboratory monitoring. With the recent, rapid introduction of the DOACs for the treatment of acute venous thromboembolism (VTE), clinicians are now faced with various questions regarding the efficacy and safety of these compounds overall and in specific high-risk populations. The collective evidence from 6 large clinical trials involving 27,000 patients has demonstrated that DOACs are as effective as vitamin K antagonists (VKA) in preventing recurrent VTE while being associated with a significantly lower risk of major bleeding. These findings are consistent in subgroups of patients with pulmonary embolism, the elderly, and those patients with a high body weight or moderate renal insufficiency, making these agents suitable for a broad spectrum of patients with VTE. DOACs are also an attractive treatment option in patients with VTE and concomitant cancer, thrombotic antiphospholipid syndrome, or heparin-induced thrombocytopenia, but the currently available clinical data is insufficient to make evidence-based recommendations on the use of DOACs in these settings. Several studies evaluating the efficacy and the safety of DOACs in these high-risk populations are underway.*

BACKGROUND

Anticoagulant treatment of thrombotic conditions dates back to the 1930s and 1940s when unfractionated heparin (UFH) and vitamin K antagonists (VKA) were the first drugs to become available. They proved to be effective in preventing death in patients with venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), and remained the mainstay of anticoagulant therapy in the decades thereafter. The introduction of low-molecular-weight heparin (LMWH) in the 1980s marked the next breakthrough. These drugs could be injected subcutaneously in fixed doses, which simplified the initial management of acute VTE greatly and opened up the opportunity to treat these patients at home. From that time on, a treatment regimen of initial LMWH followed by long-term VKA for at least 3 months was considered standard of care for patients with VTE.

Recently, within a time span of nearly 4 years, the thrombin inhibitor dabigatran [1] and the factor Xa inhibitors apixaban, edoxaban, and rivaroxaban [2,3] collectively termed direct oral anticoagulants (DOACs), were compared with conventional therapy for

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treatment of acute VTE in several large clinical trials. Now that these agents have been approved by the respective regulatory agencies around the world, physicians yet have another treatment option for their patients with VTE. This contemporary DOAC revolution has led to a quickly changing landscape of antithrombotic treatment. Clinicians are faced with various clinical questions regarding the efficacy and safety of these drugs overall and in subgroups of high-risk patients. Therefore, in the present review, we briefly discuss the collective evidence from the phase 3 clinical trials evaluating DOACs for VTE treatment. In addition, we will assess the potential role of DOACs in patients with cancer, the antiphospholipid syndrome (APS), or heparin-induced thrombocytopenia (HIT).

METHODS OF DOAC TRIALS IN THE SETTING OF ACUTE VTE

More than 27,000 patients were enrolled in several randomized clinical trials that compared the efficacy and safety of either one of the DOACs with VKA therapy targeted at INR of 2-3 in the treatment of acute, symptomatic DVT or PE. Importantly, the definitions of recurrent VTE and bleeding were similar across all studies and fallowed the recommendations of the International Society on Thrombosis and Hemostasis (ISTH). Outcome events in all trials were adjudicated by the same independent committee who was blinded for treatment allocation, resulting in a similar objective judgment of recurrent VTE and bleeding. Noteworthy differences between the trials are the use of an initial LMWH lead-in period of 5 days in patients receiving dabigatran or edoxaban, whereas apixaban and rivaroxaban where given as oral monotherapy, and the open-label design of the studies evaluating rivaroxaban opposed to the double-blinding in the other studies.

Recently, we reviewed and summarized the evidence from these phase 3 trials in a meta-analysis [4]. Using these data, we will answer clinically relevant questions to aid the physician in choosing the optimal treatment for his or her patient with VTE. Specifically, we will discuss the overall efficacy and safety of DOACs compared with VKA, differences between dabigatran and the factor Xa inhibitors, and the efficacy and safety of DOACs in high-risk subgroups of patients with PE, elderly patients, obese patients, and patients with moderate renal impairment.

CLINICAL QUESTIONS REGARDING THE EFFICACY AND SAFETY OF DOACS FOR VTE TREATMENT OVERALL AND IN HIGH-RISK SUBGROUPS

Clinical question 1: what is the overall efficacy and safety of DOACs in the treatment of acute symptomatic VTE compared to VKAs?

The main finding of the aforementioned meta-analysis was that DOACs are as effective as VKA in preventing recurrent VTE. Because all trials were designed to demonstrate non-inferiority of DOACs, this should reassure the clinician of the overall efficacy of these agents. Most importantly, the comparable efficacy observed in DOAC recipients was paralleled by a 40% relative reduction in major bleeding compared with VKA. Consistent with this substantial reduction in major bleeding, DOACs were associated with a significant 60% relative reduction in the incidences of intracranial and fatal bleeding, the most feared components of major bleeding. In line with the reductions in major bleeding, patients on DOACs had a 25% lower relative risk of experiencing clinically relevant non-major (CRNM) bleeding. Of note, no significant difference in the incidence of major gastrointestinal bleeding between DOACs and VKA was observed, unlike earlier reports raising concerns on an increased risk of gastrointestinal bleeding in patients on DOACs. In support of this finding, a recent population-based study confirmed a similar risk of gastrointestinal bleeding associated with DOACs compared with VKA in non-atrial fibrillation patients [5].

Clinical question 2: are there any differences between the factor Xa inhibitors and thrombin inhibitors with respect to efficacy and safety?

Although the result of the studies evaluating oral direct thrombin and factor Xa inhibitors are frequently combined, in fact they are two distinct drug classes with important pharmacokinetic and pharmacodynamic differences. Compared to oral factor Xa inhibitors, dabigatran has a lower bioavailability requiring administration as a prodrug, has a longer half-life, and is more dependent on renal clearance. Dabigatran is not hepatic metabolized by the P450 cytochrome system and could therefore be a treatment option in patients with liver disease. In contrast, factor Xa inhibitors undergo significant CYP3A4 metabolism and thus are contraindicated in patients with significant liver impairment. Of note, also among the factor Xa inhibitors there are some differences in pharmacologic properties. Clinicians should take these drug characteristics into account when choosing the DOAC that best suits their individual patient, but they also want to know whether there are any important differential effects between these two classes of DOACs in terms of efficacy and safety. Overall, these are no significant differences in efficacy and safety between dabigatran and factor Xa inhibitors. Hence, the choice for a specific DOAC should primarily be based on drug characteristics (eg, renal clearance), treatment regimen (eg, once vs twice daily dosing), and preference for a LMWH lead-in rather than the efficacy or safety profile.

Clinical question 3: can I prescribe DOACs also to high-risk patients including those with PE, body weight>100 kg, the elderly (ie, age≥75 years), or a moderate renal insufficiency?

The overall favorable efficacy and safety of DOACs in the setting of VTE compared with VKA is reassuring and justifies their use in the general population. However, not all clinicians are confident in prescribing these drugs in specific subgroups of high-risk patients. They may have questions regarding the efficacy of DOACs in patients presenting with PE, have concerns that patients with a high body weight will not reach sufficient drug blood levels putting them in risk of recurrent VTE, and fear that the risk of DOAC-related major bleeding complications may be higher in elderly patients, as well as in those with moderate renal insufficiency given the partial renal clearance of DOACs. The similar efficacy of DOACs compared to VKA is consistent in patients presenting with PE, patients with a body weight >100 kg, patients aged 75 years or older, and patients with a creatinine clearance between 30 and 50 mL/min. Moreover, the overall significant reduction in major bleeding associated with DOACs is maintained in the subgroups of elderly patients and patients with moderate renal insufficiency. Based on these results, DOACs should be considered to be an appropriate treatment option for acute VTE in a broad spectrum of patients.

OTHER HIGH-RISK POPULATIONS WITH VTE

Clinical question 4: are DOACs suited for my cancer patients with VTE?

It is estimated that 20% of all VTE are related to cancer and anticoagulant treatment in these vulnerable patients is often challenging. Cancer patients with VTE are at increased risk of developing recurrent VTE compared to non-cancer patients, but also have a higher risk of major bleeding. LMWH is currently recommended over VKA for treatment of cancer-associated VTE due to its better efficacy in preventing recurrent VTE with a similar risk of bleeding compared to VKA.[6] However, LMWH therapy may be burdensome, particularly in cancer patients that often require indefinite anticoagulant treatment. Because DOACs offer a simple oral treatment regimen without the need for anticoagulant control, they could be an attractive alternative. However, at present, clinicians should refrain from providing these drugs to cancer patients since they have not been compared directly to LMWH. Other concerns are possible drug-drug interactions with antineoplastic agents, in particular P-glycoprotein inhibitors, and the gastrointestinal absorption of DOACs in vomiting cancer patients or those with chemotherapy-induced intestinal mucosal defects. Nevertheless, the subgroup analysis of the 1,500 cancer patients enrolled in the DOAC trials does provide guidance for those situations when LMWH is not an option, for instance when patients are unable or unwilling to take daily subcutaneous injections. Interestingly, in these patients, a possibly better efficacy of DOACs compared with VKAs without an increased risk of major bleeding was observed.[4] It should however be realized that cancer patients in the trials probably had a favorable cancer prognosis compared to the real-world cancer population. Patients with a limited life expectancy were excluded from the trials, as were patients in whom long-term VTE treatment with LMWH was anticipated.

Recently, the pooled results of the subgroup of cancer patients enrolled in the trials comparing dabigatran with VKA was reported providing information on the characteristics of these patients as well as on cancer-prognostic data.[7] Active cancer was defined as cancer within 5 years prior to the VTE diagnosis. Compared with non-cancer patients, the 221 patients were older and had a lower renal clearance. The time spent within the therapeutic INR range of 2-3 was comparable between VKA-treated cancer and non-cancer patients (54.5% and 58.3%, respectively) which is higher than in the CLOT17 (46%) and CATCH (47%) trials. The most prevalent tumor types were prostate, colorectal, and breast cancer and 13% had metastasis. During 6 months follow-up, no significant differences were observed between dabigatran and VKA with respect to recurrent VTE or major bleeding. Detailed results of the cancer subgroups from the studies evaluating apixaban and edoxaban are expected shortly.

Before DOACs become an accepted treatment option for cancer-associated VTE, they have to be evaluated in a head-to-head comparison with LMWH. To this end, an important study comparing the efficacy and safety of edoxaban to LMWH in the setting of cancer-associated VTE has recently been started. One thousand patients will be randomized and followed for the occurrence of one of the components of the combined primary outcome of recurrent VTE or major bleeding. Following observations of a similar risk of recurrent VTE in patients with active cancer and those with a history of cancer, patients with VTE will be allowed to enter the study wither with active cancer or cancer treated within 2 years prior to the VTE diagnosis. Moreover, incidentally detected lower extremity DVT or PE are allowed as an entry diagnosis because treatment and outcome is similar for this group of patients. A last innovative feature of the study is the follow-up time of 12 months, which will provide valuable data regarding the treatment of patients with VTE and cancer beyond 6 months.

In conclusion, DOACs may become an attractive treatment for cancer-associated VTE, but at present LMWH remains the preferred option. Although the results of the trial population with cancer are encouraging, further clinical studies are needed to evaluate the efficacy and safety of DOACs directly against LMWH in this population.

Clinical question 5: can I treat my patients with thrombosis and the antiphospholipid syndrome with a DOAC?

APS is an autoimmune disorder characterized by arterial or venous thrombosis or pregnancy complications in patients who repeatedly test positive for circulating antiphospholipid antibodies (aPLA), i.e. lupus anticoagulant, anti-β2-glycoprotein-1 antibodies, or anti-cardiolipin antibodies. It is estimated that 10% of all patients with VTE have circulating aPLA and these patients are at increased risk of recurrent VTE compared to VTE patients without aPLA. When patients test positive for all three types of aPLA, the risk of on-treatment recurrence is as high as 25% over 5 years. The treatment of thrombotic APS is similar to the treatment of VTE in the general population (VKA targeted at an INR of 2 to 3), but is often extended indefinitely in case of persisting aPLA.

Now that DOACs are increasingly used for treatment of VTE, the question arises whether drugs are effective and safe for VTE treatment in the setting of APS too. From a patient perspective, DOACs may be a significant amelioration in this usually young population for whom lifelong anticoagulation with VKAs and frequent INR monitoring is not attractive [8]. Given that aPLA are prevalent in patients with VTE, the aforementioned DOAC trials in the setting of VTE must have enrolled patients with APS because this was not an exclusion criterion. However, patients were not routinely tested for aPLA and outcomes for the patients with known aPLA have not been reported. Therefore, the current knowledge of the efficacy and safety of DOACs for thrombotic APS is exclusively derived from case series and retrospective cohort studies. These represent a select population of thrombotic APS patients that were switched to a DOAC often because of labile INRs, bleeding on VKA, or logistic problems with INR monitoring.

In a retrospective multicenter cohort study, Noel et al [9] enrolled 26 DOAC-treated patients who had thrombotic APS for a median duration of 3 years. Seven patients had tested positive for all 3 types of aPLA and another 12 patients had lupus anticoagulant. Six of 26 patients received rivaroxaban or dabigatran as first-line treatment, whereas the others had been treated previously with another anticoagulant for 1 to 23 years. During a median follow-up of 1.5 years, 1 patient (3.8%) developed recurrent VTE and in 2 patients (7.7%) a bleeding event occurred that led to discontinuation of DOAC therapy.

However, other authors raised concerns about the effectiveness of DOACs to prevent recurrent APS-related VTE. Win and Rodgers [10] presented 3 thrombotic APS patients developing recurrent thrombosis 6-12 months following initiation of second-line DOAC treatment. The cause of these observations of DOAC-failures is unknown. The recurrence rate in APS patients is high and these failures may have otherwise developed under VKA therapy. Moreover, compliance may have been a contributing factor in these patients who were often switched to a DOAC due to difficulties with their INR control. Alternative explanations may be mechanisms intrinsically related to DOACs or their dosing regimens. For instance, inhibition of a single clotting factor might be insufficient to completely block the thrombotic potential in severe APS patients, or the short-life of DOACs causes the procoagulant stimulus to overcome the anticoagulant effect at trough levels.

In summary, current anecdotal evidence on the use of DOAC in thrombotic APS patients is conflicting. As a consequence, physicians should be careful with prescribing DOACs in these patients until robust data become available.

Clinical question 6: are DOACs a treatment option for preventing or treating thrombosis in patients with heparin-induced thrombocytopenia?

HIT is a complication of treatment with UFH or, less commonly, LMWH and is characterized by formation of antibodies to heparin-platelet factor 4 (PF4) complexes. These antibodies may trigger platelet activation in turn resulting in venous or arterial thrombosis, so-called HIT associated with thrombosis (HTT). A decrease in platelet counts is usually observed 5-10 days after initiation of heparin exposure. In patients with confirmed HIT, cessation of heparin therapy alone is not sufficient to prevent HITT and treatment with an alternative anticoagulant is required. Traditionally, 2 classes of anticoagulants have been used for the initial treatment of HIT: parenteral direct thrombin inhibitors, including argatroban, bivalirudin, and desirudin, and nonheparin antithrombin-dependent anticoagulants, including fondaparinux and danaparoid. Although these drugs are widely used in the treatment of HIT, they have several disadvantages ranging from high costs, low availability, need for drug monitoring, dependence on renal clearance, and the absence of an antidote. DOACs could potentially overcome all of these issues. The biochemical structure of DOACs has no similarities with heparin; hence, interactions with PF4 or heparin-PF4 antibodies are not to be expected. This has been confirmed in preclinical laboratory studies that demonstrated that rivaroxaban, dabigatran, and apixaban [13] do not cross-react with HIT antibodies and do not induce platelet activation in the presence of HIT antibodies. Hence, on theoretical grounds, these drugs could be used to treat HIT. These observations suggest that DOAC are a safe alternative to traditional HIT treatment, but confirmatory studies are needed.

In summary, the clinical experience with DOACs for HIT is currently limited, but they have the potential to become the mainstay of anticoagulant treatment in this setting. They do not interact with HIT antibodies and do not cause platelet activation in the presence of HIT antibodies. Together with their favorable pharmacokinetics, high availability, and lower costs, they could be particularly suited for patients with HIT. The results of the ongoing clinical study have to be awaited before the evidence-based use of DOACs in patients with HIT can be recommended.

CONCLUSIONS

With the recent introductions of the DOACs, clinicians now have multiple therapeutic options for oral treatment of patients with VTE. DOACs are as effective as VKA in preventing recurrent VTE, but are associated with a significant reduction in the risk of major bleeding and are easier to use with no need for dose adaptations. These findings in the general trial population are consistent with those in patients with PE, the elderly patients, heavy patients, and patients with moderate renal impairment, making DOACs a suitable treatment option for a broad spectrum of patients. Whether DOACs can also safely be used in patients with cancer-associated VTE, thrombotic APS, or HIT is currently not proven. Studies are underway to evaluate DOACs in these high-risk populations.

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SYSTEMATIC REVIEW

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Risk factors and quality of life in late-life depressive disorders

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Abstract: *Late-life major depression is a high-incidence and difficult-to-treat affective disorder. Diagnosis of major depression in old age could also could be a challenge, due to aspects like (1) there is a higher vulnerability to the stigma of depression in this population, (2) hypochondriac ideation and somatic symptoms are the main symptoms, while depressive disposition or anhedonia are under-reported by such patients, (3) differential diagnosis includes various organic diseases, but also several psychiatric disorders, like neurocognitive disorders, organic affective disorders, drug induced affective disorders etc. Reduction of social relationships caused by retirement and loss of spouse and friends, as well as a decrease of personal income could precipitate or maintain depressive disorders during late-life. Quality of life in patients diagnosed with depressive major disorders is a rarely monitored parameter, although its importance for case management could not be overemphasized. A reduction of life quality correlates with a poorer functional prognosis, persistence of neglected residual symptoms, low adherence to treatment plan etc.*

Keywords: *late-life depression, geriatric depression, quality of life, risk factors, institutionalized population*

BACKGROUND

Late-life depressive disorders have a high impact over patients' quality of life, general well-being, risk of organic diseases recurrence, involvement in daily activities and life expectancy.

Although depression could be perceived as a part of normal process of ageing, this is only a relatively widespread misconception. Adjustment to changes in life style due to retirement, loss of the spouse, death of close friends, financial losses, decreasing of physical capacities etc, should not be confounded with major depressive disorder. Depression has been identified in 17-37% of elderly patients treated in primary care facilities, and 30% of these have a diagnosis of major depressive disorder [1].

Elderly patients are more sensitive to stigma associated with depression and this is a reason for them to declare less frequently than young adults that they have dispositional symptoms during epidemiological studies [2]. Therefore this kind of data should be regarded with caution, as an underestimation of the depression real incidence in late-life is very possible.

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Table 1: Risk factors for depressive disorders in late-life population

Risk factor	Overall impact
Bereavement after loss of spouse, a child or other close relatives	Bereavement after a recent loss correlates with depressive symptoms in elderly [5] Elderly bereaved patients seek frequently medical help for their emotional discomfort [6] Most of the depressive symptoms are reported by mothers who have lost a child after a prolonged suffering and in fathers who lost a child by suicide; it's possible that old adults have a lower risk of depression caused by the death of a child than younger adults, possible due to better coping mechanisms [7]
Retirement	People who retired recently have a higher risk for depression than those who continued to work, although self-esteem scores didn't change significantly, as reported by a prospective evaluation during 2 years [8] Depressive symptoms increase when the retirement is sudden and perceived by the subject as being too early or forced [9] There is a reciprocal relationship, as patients diagnosed with depression tend to retire with 1.5 years earlier than those without depression [10]
Income decrease	Women who have lost their spouse have as main mediator of depression financial burden, while widowed males have mainly problems with house holding activities [11]
Interpersonal relationships decreasing	Loneliness was the only factor which correlated with depression scores in 8% of people aged 60-98 who were institutionalized, thus suggesting that loneliness is an independent risk factor for major depression [5]
Lower physical capacity	Old adults with sedentary lifestyle and physical disabilities had a higher risk (OR=2.44) for depressive symptoms appearance than those with good mobility and that were physically active, according to a 8 years follow-up study; the risk maintained high (OR=1.99) in those with low mobility but active life-style, when compared to those who were both physically active and have an intact mobility [12]
Somatic symptoms, pain included	Presence of several organic diseases predicts late-life depression [5] Organic pathologies which associate important pain have a risk for developing more depressive symptoms and a worsening of pre-existing depressive manifestations [13] Quality of life is decreased more in old adults with symptoms like physical pain, because this is associated with limitations in functionality, fatigue, sleep disturbances, depressive mood [14]
Physical or psychological symptoms of patient's spouse	There is a high correlation between depressive symptoms in both spouses: if one has depressive manifestations, there is a high risk for the other to have the same type of symptoms, and this correlation increases in frequency with the patients' age; age difference between spouses has been associated with more depressive symptoms, and those with younger spouses reported more depressive symptoms in 65+ age than spouses with minor age differences [15]
Psychological symptoms, including decrease in cognitive function	Elderly patients (60+) with major depression who have persistent insomnia have a 1.8-3.5 times higher probability to maintain depressive symptoms than those who doesn't have insomnia [16] Depression appears frequently in almost every type of neurocognitive disorder, even in mild cognitive disorder (MCI), and the risk for depression to increase the risk of MCI conversion to dementia is investigated [17] Late-life depression leads to further functional and cognitive decline [18]
Decrease autonomic functioning	Mobility deficits predict a higher risk of mortality and loss of autonomy in elderly patients [14]
Relocation in other area (from city to rural areas, or from his/her own residence into a long-term care facility)	Institutionalization, lower frequency of visits from friends or relatives and less involvement in social activities have been associated with greater risk for late-life depression [5] In institutionalized patients, depression is more common in women who were widow/single, younger or much older than the mean group age, with lower educational background, poor social and economic status, who live alone [20].

Detecting vulnerable populations for depression disorders is an important process if we look at the risk of early death from natural and unnatural causes in patients diagnosed with depressive disorder (71.1 vs. 75.9 years old) and loss of potentially years of life (13.4 vs. 10.2 years) comparative to the non-depressed population [3].

Risk factors for development of depressive disorder in late-life are intricate, with genetic vulnerability, cognitive diathesis, age-associated neurobiological changes, and stressful events, while protective factors are higher education, high socioeconomic status, and involvement in desired activities, inclusive of religious and spiritual nature [4].

Clinical manifestations of major depression in elderly patients has several peculiarities, like denial or dissimulation of feeling sad or anhedonia, but with predominance of somatic complaints, irritability and anxiety, self-isolation, low level of energy and slow movements.

OBJECTIVE

To assess the specific risk factors for late-life depression and to search for impact of this pathology over the patients quality of life, by analyzing data from literature.

Table 2: Quality of life in late-life depressive disorder

Lin J-H, Huang M-W, Wang D-W et al. [21]	Depressed elderly inpatients have less satisfaction and quality of life; female gender, duration of hospital stay, and in-hospital rehabilitation correlated significantly with quality of life scores.
Doraiswamy PM, Khan ZM, Donahue RM, Richard NE [22]	Late-life depression associates significant quality of life impairments in 5 out of 8 baseline SF-36 items; women rated their quality of life as worse than men on physical functioning and role physical; subjects aged over 70 reported lower quality of life on the summary physical component and a trend for higher scores on the mental component of the SF-36
Dombrovsky AY, Lenze EJ, Dew MA et al. [23]	All domains of health-related quality of life except physical functioning improved with successful acute and continuation treatment using paroxetine and/or monthly interpersonal psychotherapy
Hunkeler EM, Katon W, Tang L et al [24]	Collaborative care intervention (including depression care manager, primary care doctor, psychiatrist offering education, behavioral activation, antidepressants, a brief behavior based psychotherapy, relapse prevention geared to personal needs and preferences) was superior to usual care for depression in elderly patients (60+) diagnosed with major depressive disorder regarding quality of life, self-efficacy, satisfaction with care, remission of depression after 12 months
Heiligenstein JH, Ware JE Jr, Beusterien KM et al [25]	Treatment with fluoxetine for 6 weeks in patients over age 60, diagnosed with major depressive disorder, had improvements vs. placebo in all dimensions of SF-36 health status survey; before treatment these patients had lower values in mental health, role limitations due to emotional problems, social functioning, vitality, role limitations due to physical problems, bodily pain- as dimensions of quality of life and health-related dimensions.

METHODS

Main electronic databases (PubMED, EMBASE, CINAHL, Cochrane) were searched using as keywords „old age depression”, „late life depression”, „geriatric depression”, combined with „clinical dimensions”, „quality of life”, „risk factors”, „prognosis”, „evolution”.

All articles were analyzed, including clinical trials, systematic reviews, meta-analyses, case reports, case series, guidelines and editorials.

We formulated the following inclusion criteria for the selected papers: population age over 60; diagnosis of major depressive disorder, organic depression or mixed anxiety-depressive disorder; institutionalized or community dwelling residents; specified statistical method for data processing; risk factors and/or quality of life as specified variables.

Exclusion criteria were: unspecified age of studied population; age under 60; diagnosis of drug induced depressive disorders, or bipolar disorder- major depressive episodes.

RESULTS

According to the analysis of the data from literature, main risk factors for depressive disorders in elderly are illustrated in table 1. Each factor has a different impact over the onset or evolution of depressive symptoms, but these factors appear seldom isolated, therefore it is hard to quantify each variable's impact.

Patients diagnosed with depressive disorders in late-life have various risk factors for this affective disorder, like social events (d.e. retirement, lack of social interaction), psychologic aspects (d.e. bereavement, cognitive deficits) and organic diseases (d.e. somatic pathologies, decreasing of physical mobility). Therefore, it's important to configure a management plan that includes all these factors and adequate monitoring methods, in order to improve depressive symptoms severity, prognosis and risk of relapse.

Regarding the quality of life in depressed elderly patients, we found several studies which are presented in table 2. All the cited trials are randomized and included quality of life and/or health-related quantitative measurements as main or secondary variables.

Although only a small number of trials dedicated to evaluation of life quality in depressed elderly patients was found, we can conclude from the available data that (1) a significant correlation between depression and quality of life decrease was found; (2) treatment, either psychotherapy or pharmacotherapy, could help in resolving both depressive symptoms and alterations in quality of life; (3) several risk factors like female gender, duration of hospital stay, and in-hospital rehabilitation correlated significantly with quality of life scores.

CONCLUSIONS

There are many risk factors that could be correlated with the onset of a new depressive disorder in old age, or with a relapse or recurrence of a previously diagnosed major depression. Some of these risk factors could be corrected and thus patient's prognosis could be improved (d.e. symptoms related to organic comorbidities, like pain, dizziness, or medical aids for visual or hearing deficits), while others could be only monitored and assisted through counseling (d.e. reduced immobility due to untreatable or only partially treatable conditions, lower physical capacity or bereavement).

Quality of life is an important predictor of prognosis in depressive disorders and monitoring the patients' well-being should be more frequently recommended during psychopharmacologic or psychotherapeutic treatment in elderly population. An increased attention should be placed in patients with depressive disorders and risk factors for lower quality of life, like female gender or long duration of hospitalization.

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ORIGINAL ARTICLES

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Modern medicine has a new technology: Therapeutic electroporation

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Abstract: *Electroporation is considered a new start-up in the treatment of various tumors; currently, researches are being conducted in order to develop this technology with medical applications. The technique consists in the significant increase in the electrical conductivity and permeability of the plasma membrane of cells resulting from the application of an external electric field. It is routinely used in molecular biology to transform bacteria, yeast, protoplasts and is performed using the electroporators. Currently, the process seems to be a real solution that enables a targeted drug to act with maximum efficiency on cells and tissues requiring treatment, resulting in obtaining a good therapeutic effect without major side effects. Therefore, pharmaceutical companies are trying to demonstrate through preclinical studies the potential efficacy of this technology, succeeding in recent years to achieve important steps in this direction.*

Keywords: *electroporation, therapy, tumor, medical application*

INTRODUCTION

Modern medicine is always looking for new technologies applicable to the diagnosis, prevention and treatment of diseases. Among these, the applications of molecular biology represent a direction of the future, particularly in the treatment of diseases currently considered incurable. Translational medicine applies the new discoveries, in molecular and genetic biology, as well as the techniques resulted from these, for therapeutics based on biology, which gradually replaces therapeutic based on chemistry. For example, by applying electric current at cell level, can be inserted into altered cells, the therapeutic molecules or transfection of genetic material can be achieved.

ELECTROPORATION

Electroporation is the electropermeabilization of cell membranes, which consists in increasing the electrical conductivity and permeability of the plasma membrane of cells, being caused by the application of an external electric field. This procedure is particularly useful in molecular biology in order to insert certain substances in a cell such as a molecular probe or a drug that can induce changes in cell functions or coding DNA [1].

Electroporation is a dynamic process that depends on local transmembrane voltage for each point at cell membrane level. For a certain type and duration of the

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pulse, there is a threshold of the transmembrane voltage in order to allow the expression of transmembrane electroporation phenomenon between 0.5 V and 1 V. This leads to defining threshold value of the magnitude of the electric field necessary to achieve the phenomenon of electroporation. Only the cells found in an electric field greater than the *threshold* value can be electroporated. If a second threshold is reached or exceeded, electroporation will compromise the viability of the cells, this phenomenon being called *irreversible electroporation* [2].

In molecular biology, the process of electroporation can be used to transform prokaryotic and eukaryotic cells. Bacteria that present a cell wall composed of peptidoglycans for protection, resistance and transport of substances are protected from environmental changes. Based on this characteristic, bringing the bacteria into contact with particular plasmids of interest may have the effect of transferring the plasmid to other bacterial cells, subsequent to an electroporation process by applying several hundred volts over a distance of a few millimeters. Subsequently, the cells must be handled carefully until they divide into new cells that contain new plasmids; this process is about ten times more efficient than chemical transformation [3].

This process is also highly efficient for the insertion of foreign genes in cell cultures and in particular in mammalian cell lines. For example, the process is used to produce *knock-out* mice (transgenic mice in which is inactivated a single target gene for the study of phenotypic characteristics encoded by this) as well as in tumor treatment, gene therapy, and cell-based therapy. The process for the insertion of foreign DNA molecules into eukaryotic cells is known as transfection. Electroporation is particularly effective for transfecting cells in suspension in a cultivation medium using an electroporation cuvette.

Electroporation has proven effective for use on *in vivo* tissues for *in utero* or *in ovo* transfection applications. The adherent cells to the substrate can also be transfected using electroporation, thereby providing an alternative to researchers for the trypsinization of the cells prior to transfection. Electroporation is performed using electroporators, purpose-built appliances which creates an electromagnetic field in the cell suspension, which is pipetted into a glass or plastic cuvette foreseen with two aluminum electrodes on its sides. For the electroporation of bacterial cells, a suspension of about 50 µl is used which, prior to electroporation, is brought into contact with the plasmid of interest. The mixture is pipetted in the cuvette, the voltage and the volume to be applied to the probe are set, and the cuvette is placed in the electroporator. Immediately after electroporation, 1 ml of liquid medium is added to the Eppendorf cuvette /tube and is incubated at a temperature which allows the multiplication of bacteria, to recover the cells and to express **antibiotic resistance**, followed by seeding on plates with nutrient agar.

For electroporation to be successful, the plasmid must be "*free of all salt*" because solutions with high concentration of sodium chloride can cause an electrical discharge (known as arcing), a phenomenon which often reduces cell viability.

For a more detailed investigation of the process, special attention should be given to the output impedance of the porator device and to the input impedance of the cell suspension (e.g. the salt content). Because the process requires direct electrical contact between the electrodes and the suspension, and it does not occur between isolated electrodes, it is obvious that the process requires the existence of electrolytic effects due to mild currents and electric fields [4].

Electroporators are special laboratory equipment that give the possibility to achieve electroporation for multiple samples at the same time, with sets of electrodes specific for cell cultures, such as X2HT Gemini system manufactured by BTX-Harvard. Electroporators can be set to different operating parameters, allowing researchers to optimize the electric field strength depending on the cell type and by the presence or absence of the cell wall [5].

Electroporators have been used on a wide range of cells, including *Escherichia coli* (for transformation) and mammalian cells (neurons, astrocytes, neuroglial, lymphocytes, monocytes, fibroblasts, epithelial and endothelial cells) from humans, mice, rats, and monkeys (for transfection [6].

Physical mechanisms of electroporation

Electroporation allows inserting into the cell DNA molecules, which do not have the ability to passively cross the cell membrane (the lipid hydrophobic bilayer). There are several ways in which changes can be caused to the electric field at the cell membrane level with very different mechanisms and effects. The electroporation advantage lies in the fact that lipid molecules are not altered in terms of chemical and the membrane has the ability to form a pore that will serve as a conductive path through the lipid bilayer [4].

Electroporation is a process with many stages and distinct phases [7]. Initially, a brief electrical pulse of 300-400 mV must be applied for less than 1 ms, which causes changes at membrane level related to the migration of ions in the surrounding solution. Once the critical stage is reached, a local rearrangement on the morphology of the lipids rapidly occurs and the resulting structure is considered to be a "pre-pore" because it is not a good conductor, but quickly leads to the formation of a conductive pore. Most evidence in support of this idea, come from the "flickering" process that occurs at the level of the pre-pores with the formation of hydrophobic regions of approximately 3Å [8].

If this theory is correct, the transition to the conductive pore may be explained by a rearrangement at the edge of the pores in which the lipid ends create a hydrophilic interface.

Finally, the conductive pores may suffer processes of reorganization, expansion or rupture, which can be influenced by various local factors such as the place and the moment in which the change occurs, the mechanical stress and the energy of the lipid bilayer existing at the time of trial [9].

MEDICAL APPLICATIONS

The optimization of electroporation is difficult to achieve, but it has been demonstrated that high voltage electroporation can almost irreversibly destroy the target cells, neighboring cells remaining unaffected. Thus, electroporation can form a new therapeutic approach in the treatment of cancer, cardiac disease and other pathological conditions which require the excision of tissue [10].

A recent technique called **non-thermal irreversible electroporation** has proved successful in the treatment of various types of tumors. This procedure involves the use of small electrodes (about 1 mm in diameter), placed inside or around the target tissue for applying short electrical pulses, repeating at a predetermined frequency and voltage, which increases resting transmembrane potential. As a result, when the applied electricity is higher than the threshold value for the target tissue, forming nanopores in the plasma membrane can become permanent and the cell repair mechanisms of “immortal” cancer cells become ineffective and the cells die.

The procedure is unique, due to the fact that compared to other tumor ablation techniques it does not affect the healthy tissue surrounding the tumor. In contrast, **reversible electroporation** occurs when electricity is applied through the electrodes below the threshold level of the electric field of the target tissue. Applying an electrical energy below the threshold allows the cells to rebuild the phospholipid bilayer and to resume normal operation. Reversible electroporation is carried out in the normal way for the administration of treatments which involve the insertion of some therapeutic molecules (drugs, genes, etc.) into cells. Since not all tissues have the same threshold of the electric field, precise calculations are needed before starting treatment, to ensure operating efficiency.

A major advantage of using non-thermal irreversible electroporation is that when done correctly, as a result of careful calculations made, it affects only the target tissue, while the proteins, the extracellular matrix and some critical structures such as blood vessels and nerves remain unaffected by this treatment. This allows a faster recovery and enables a faster replacement of the necrotic tumor cells with healthy cells. The first successful treatment of malignant skin tumors in mice was conducted in 2007 by a group of scientists (Garcia, Paulo A. & al.), who conducted the complete ablation of the tumor in 12 of 13 mice. They accomplished this by sending 80 pulses of 100 µsec to 0.3 Hz with an amplitude of the electric field of 2500 V / cm, to treat skin tumors. Prior to performing the procedure, the therapist must carefully calculate exactly what should be done and to customize the therapy to the patient needs using imaging (CT and MRI) to create an accurate three-dimensional image of the tumor. This information is very important because one can estimate the tumor volume and decide on the type of therapy, place and angle of insertion of electrodes and applied voltage, sometimes even during the procedure, especially when electroporation is used to treat brain tumors.

The whole procedure is quick (about 5 minutes); the success rate is high and therefore very promising for future treatment extrapolation to humans. A disadvantage would be that the released electricity can stimulate muscle cells causing their contraction, which in some cases can have serious or even fatal consequences. Therefore, a muscle relaxant agent (curare-like compounds) should be used while performing the procedure. The researches conducted so far have been successful, but the taken action must be subject to the existence of a risk when general anesthesia is being used. A recent technique called **irreversible high-frequency electroporation**, uses electrodes to apply bipolar electrical pulses at a high frequency, as opposed to low frequency monopolar electrical pulses. This type of procedure also leads to tumor ablation with the difference that it does not produce muscle contraction and therefore does not need a muscle relaxant agent [11].

Researchers have had success in treating the test animals with multiple tumor types. Even if you are not prepared to routinely apply this technique in the treatment of tumors in humans, non-thermal irreversible electroporation is used in practice for treating cutaneous and subcutaneous tumors. There have also been attempts to treat human cancer of the prostate, lung, kidney and liver. There are still many things to be set in place, on how to damage the animals and especially the man. Kenneth Thompson et al. tested the safety of applying this technique on humans who had tumors in their lungs, kidneys and liver. The treatment was found effective in 71% of cases and, respectively, in 49 patients out of the 69 cases of patients treated was achieved complete ablation of the tumor [12]. The highest rate of success was recorded in liver tumors. The result of this study thus provides encouraging evidence related to the future use of this technology as an alternative method of treating cancer in humans.

Electroporation can also be used for the insertion of drugs or genes into the target cell through the use of short, intense electrical pulses to transiently permeabilize the cell membrane. This procedure is referred to as **electrochemotherapy** when the molecules to be transported are represented by a chemotherapeutic agent or **gene electrotransfer** when the molecule to be transported is the genetic material (DNA) [4].

PHARMACEUTICAL APPLICATIONS

Some pharmaceutical companies have performed surveys on the development of electroporation procedures with the scope of providing a viable alternative to cellular uptake of drugs. This approach is based on the fact that human cells do not allow the free entry of foreign matter through the cell membrane, which results in delay in the operational efficiency of a drug. Therefore, electroporation can be a real solution to allow a drug to act directly, quickly and with maximum efficiency on cells and tissue requiring treatment, resulting in obtaining a good therapeutic effect without major side effects.

Therefore, Inovio and OncoSec tested the effectiveness of this technology and managed in 2013 to achieve important steps in this direction. The results allowed them to also achieve substantial gains: the company Inovio Pharmaceuticals (NYSEMKT: INO had gains of almost 500% and the company OncoSec Medical had gains of 150%. Inovio performs studies using synthetic vaccines, while OncoSec is testing therapeutic agents that are already used during cancer treatment. The company Inovio Pharmaceuticals has developed about 12 "*electroporation pipes*", for the treatment of cancer and infectious diseases. Thus, the tests performed on the H1N1 influenza vaccine developed by the company's specialists have highlighted the protective immune response comparable to conventional vaccines. Also, the SynCon DNA vaccine caused total protection against the infection with the viruses of Ebola and Marburg hemorrhagic fevers [13].

Preliminary studies for both types of vaccine, although in the initial stage, show that these exhibit a strong effect against the spread of these deadly viruses. On June 14th, 2014, Inovio announced that the H7N9 vaccine generated protective antibody HA with a percentage of 100% of the animals tested, and on July 10th, the results obtained from testing the HIV vaccine were published in the Journal of Infectious Diseases. Inovio found that the CELLECTRA system used for administering the Pennvax - B HIV vaccine improved the efficacy of the medicine in the first phase of testing. On July 18th, 2014 Inovio announced that electroporation significantly improves the outcomes of the DNA therapy in terms of stimulating the multiplication of blood elements. Administering with the CELLECTRA system the hTERT DNA vaccine used in cancer therapy and the anti-MERS vaccine has demonstrated that a good immune response has been generated during preclinical studies, improving the survival of patients [14]. OncoSec Medical conducted in preclinical testing centers studies for the treatment of metastatic melanoma, Merkel cell carcinoma (MCC) and cutaneous T cell lymphoma. In the study were included 15 patients with MCC, and so far the company's electroporation platform has been shown to be safe; in the last check, all patients showed an increase of IL-12 absorbing of at least 100 times, and in some cases up to 1,000 times.

This provides hope in regards to reducing the side effects that occur subsequent to immunotherapy. Also, studies have been conducted on a group of 25 melanoma patients. Were reported complete records for 21 of them and was observed that 38.1% had a favorable complete response lasting more than 6 months and in 61.1% of patients was observed a 30% regression of the tumor. All this led to an increase in the company's market share by 63%. It is considered that the near future will bring a series of novelties in this field, such as:

- the extension of the studies and obtaining positive results, for more than small populations of patients;

- reporting by Inovio of the data from the Phase 2 Study for VGX-3100 in the treatment of cervical dysplasia, which in Phase 1 has shown good immune response;

- reporting by OncoSec of the final data from the study of melanoma and for the Merkel cell carcinoma;

- presenting plans to launch a study for treating cutaneous lymphoma with T-cells, but also for the initiation of a new research for the treatment of solid tumors;

- OncoSec presenting the company's plans that expressly foresees combining the electroporation device with the anti-CTLA4, the anti-PD-1 and the anti-PD-L1. As is well known, the anti-PD-1s, particularly, was among the most frequently discussed cancer therapies last year, after Merck and Bristol-Myers have reported incredible data to the American Cancer Society (ASCO). All these led to the idea that electroporation increases the assimilation of some therapeutic agents and reduces side effects, thus making them even more efficient. Therefore, careful monitoring of therapy with anti-PD-1s and the OncoSec plans to expand the researches for the ImmunoPulse platform, finally pursue further researching for this therapeutic agent [15].

CONCLUSIONS

Therapeutic electroporation is a new treatment technology at cellular level. The method is based on the electroporation of the cell membrane which becomes selectively permeable to the therapeutic agents used. *In vitro* and *in silico* experiments are promising and have allowed the insertion of chemical or biological molecules in the target cells. The *in vivo* experiments on laboratory animals have given good therapeutic results, therefore, in the future may be applied to humans. Therapeutic electroporation can become in the near future, an important treatment for oncology, cardiac, infectious diseases etc., envisioning the predominant biological perspective of therapeutics in modern medicine.

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ORIGINAL ARTICLES

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The incidence of tracheoesophageal fistulas and it major determinant factors

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Abstract: *The tracheoesophageal fistula which occurred during oro-tracheal intubation of a patient in intensive care unit is a true challenge both in diagnostic and in therapeutic approach. The best treatment is prevention, by identifying risk factors but especially is important which the mechanism in the occurrence of tracheoesophageal fistula was. The occurrence of this complication in the evolution of hospitalized patients in ICU is accompanied by significant increase in mortality, contributing to negative prognostic. We have started a large multicentric study in April 2016 regarding all patients who required intubation longer than 7 days. The study will finish at the end of 2020. We are looking for a definite conclusion, in this moment we do not have enough data for a conclusion.[1, 2]*

INTRODUCTION

The tracheoesophageal fistula is an abnormal communication between the esophagus and trachea. It can be congenital or acquired. Most of them are congenital with a frequency of 1 to 3500/ 6000 births. In this case usually are associated, or appear within, with other birth defects (trisomy 13, 18 or 21, tetralogy of Fallot, other digestive problems or muscle skeletal VACTERL Syndrome (**V**ertebral anomalies, **A**nal atresia, **C**ardiac defects, **T**racheoesophageal fistula and/or **E**sophageal atresia, **R**enal & **R**adial anomalies and **L**imb defects.). Then, there are the malignant ones which appear in the posterior infiltrating tracheal wall or the anterior wall of the esophagus. The last ones are those acquired, non-malignant [1, 3, 4].

They occur particularly after a prolonged period of intubation with or without mechanical ventilation. Fistula can result from incidents of intubation maneuver, in the operating room but especially in ICU. The first fistula appeared after a prolonged period of intubation and was initially described in 1956 by d'Avignon [2, 4].

Several factors are involved in these serious complications during a prolonged period of intubation. The most important and most discussed about in recent years is represented by the cuff pressure of orotracheal tube. If the cuff is overinflated, it leads to compression on the rear wall membranous trachea, which in time leads to ischemia and necrosis, basically forming an abnormal communication between the trachea and esophagus. Other possible complication of a hyper-inflated cuff is tracheal stenosis or bleeding erosion of a significant blood vessel. Often, both situations can occur simultaneously. The

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tracheoesophageal fistulas were more common when the high cuff pressure tubes were used. Once with the use of tubes with high volume and low pressure cuff, the incidence of fistulas has dramatically dropped down. In the international literature the occurrence of these fistulae has a relatively low frequency, of approximately 1% [4, 5, 6].

The widely accepted indication is that the cuff pressure tube should not exceed 25 cm H_2O and the volume should ideally be of 6-8 ml air. More than 10 ml of air should raise the question about tracheal injury. There are data from multi-center studies which have shown dangerously high cuff pressure after patient mobilization. These recommendations are given after multiple multi-center, international researches, but the low pressure cuff has the same danger like the high-pressure ones. If the trachea is not fully secured, then the patient is prone to tracheobronchial aspiration, despite the good and high efficient nursing [7, 8].

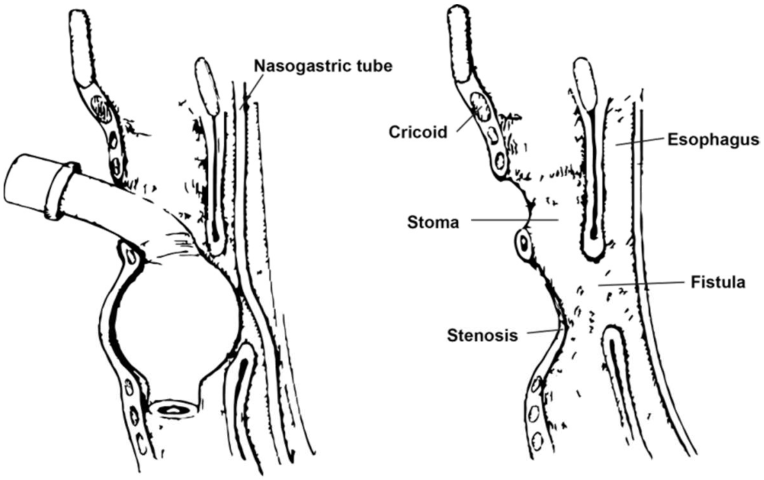

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The tracheostomy does not reduce the risk of tracheoesophageal fistula, the cuff of the cannula can injure the trachea in the same way. If the cannula's material is too rigid, the direct injury of tracheal wall may become possible through angulation [2].

The other factors involved in the occurrence of tracheoesophageal fistula are the presence of rigid nasogastric or orogastric tube, anemia, malnutrition, hypoxemia, anxiety, changing positions in bed, steroid treatment, advanced age, low perfusion from severe shocks [9, 10].

Tracheoesophageal fistula are located usually at the superior half of trachea, and in case of tracheostomy, 1-2 cm below the stoma [10].

The diagnosis of tracheoesophageal fistula is difficult. Suspicion of fistula is based on clinical signs: loss of air from breathing circuit, air on gastric tube, recurrent pneumonia, etc. We can do clinical test by administering blue methylene on the tube, but the diagnostic choice remains the bronchoscopy, images of computed tomography and upper gastrointestinal endoscopy [8, 11].

Treatment is usually surgical; less invasive procedures can be performed particularly for smaller defects, consisting in placing endoscopic stents [12].

PATIENTS AND METHODS

We have started a large prospective multicenter study in two intensive care units, the Central Military Hospital and University Emergency Hospital, Bucharest, Romania. The study started in April 2016 and will finish in the end of 2020.

All patients admitted to the intensive care unit which required orotracheal intubation more than 7 days, with or without mechanical ventilation, will be enrolled. Each observation paper will be attached in a special sheet and will include all the monitored parameters.

The parameters which will be monitored: counting days of orotracheal intubation, the pressure in the tube cuff, presence or absence of gastric tube, nutritional status of the patient, and hemoglobin during hospitalization, necessary of inotropic substances, presence or not of steroid treatment. Bronchoscopy will be performed regularly for early identification of changes of tracheal wall.

RESULTS

The occurrence of this complication in the intensive care unit of Military Central Hospital Bucharest during last five years was zero among the admitted patients (about 1500/year).

From 2,800 patients which were intubated, 1,100 needed tracheoesophageal intubation or tracheostomy more than 48 h. It is obvious that incidence of tracheoesophageal fistulas is inferior compared to actual literature information. So, we are looking forward the results of this study, in this moment the data we have aren't enough for a conclusion.

DISCUSSION

Our study aim is to highlight exactly the incidence of tracheoesophageal fistulas among endotracheal intubated patients in the ICU and their determinant factors.

Early identification of these complications with direct impact on morbidity and mortality of patients is extremely important. Secondary occurrence of pulmonary fistula and subsequent contamination of the lung may results in severe suppurative complication. Prompt treatment, either conservative or surgical, should be immediately applied.

In conclusion, the occurrence of tracheoesophageal fistulas can be lowered by aggressive nursing measures and eliminating/correcting determinants, including days of orotracheal intubation.

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CLINICAL PRACTICE

Ankylosing spondylitis and cardiovascular risk – Case report

Daniela Anghel[1], Lucian M. Ciobîcă[1], Silviu M. Stanciu[1], Ciprian V. Jurcuţ[1], Gabriel D. Stoicescu[1], Ioana A. Răduţă[1], Ancuţa Coca[1]

Abstract: *Introduction: Ankylosing spondylitis (AS) is a chronic progressive inflammatory disease of the axial skeleton and peripheral joints associated with HLA B27 antigen and with the predominance of the male gender (with an average between 20 and 30 years old).*

Case presentation

A 48 years old male patient was admitted to our clinic, having a long history regarding this disease since he was 16.

This patient has switched 3 therapies with anti TNF alpha agents until now, and we hope to obtain a good response for a long time.

During the treatment with Etanercept he presented an acute anterior uveitis which had a good response to therapy.

Conclusion: The ankylosing spondylitis management is complicated when we have the possibility to choose only three anti TNF alpha agents. If a patient does not respond to the first or second agent we are constrained to follow the last one. Therefore the principal problem regarding this special case is that the patient is non responder at the last agent. So the question that arises is witch will be the next therapy for this patient?

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic progressive inflammatory disease of the axial skeleton and peripheral joints – asymmetric.

Its major characteristic is the early damage of the sacroiliac joint, ascending to ankylosis. The incidence is increased for males between 20 and 30 years old, thus most of the patients are associating a HLA B27 antigen. The existence of cardiovascular risk due to inflammatory rheumatic diseases is known for decades but the real significance and therapy designed to control this risk has been recently evaluated.

Systemic inflammation because of inflammatory rheumatic diseases accelerates atherosclerosis and destabilizes plaque of atheroma, contributing to an increased frequency of fatal cardiovascular events such as myocardial heart attacks and strokes. The ankylosing spondylitis cardiovascular events include: aortic insufficiency, heart muscle damage (dilatation/hypertrophy), nerve impulse conduction disorders, pericarditis, and accelerated atherosclerosis.

Anti TNF-alpha therapy leads to a decrease of the disease activity score (BASDAI) and also the nonspecific inflammatory syndrome.

The anti TNF approved drugs which are currently in clinical practice are:

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1. Infliximab (*Remicade®*) – chimeric monoclonal antibody anti-TNF alpha;
2. Adalimumab (*Humira®*) – fully human monoclonal antibody anti –TNF alpha;
3. Etanercept (*Enbrel®*) – a fusion protein between the type 2 p75 receptor of TNF and an IgG1 Fc fragment.

CASE PRESENTATION

We present the case of a man, A.I., 48 years old, from urban medium, nonsmoker, having an insignificant family history, diagnosed since 1982 when he was 16 years old with juvenile arthritis.

He came for the last hospitalization, for clinical and biological reassessment, with ankles light arthralgias.

From his medical history we know that the disease has started when he was 16 years, with ankles and knees arthritics, at radiocarpal joints, at bilateral elbows, acute back pain and fever. The patient was diagnosed with juvenile arthritis and treated with Sulphasalazine 6 grams and nonsteroidal anti-inflammatory drugs. Under this treatment he had a slightly favorable evolution with partial improvement of symptoms. After 2 years of treatment disease flares have appeared, therefore the dose of Sulphasalazine at 8 grams/day was increased and a quantity of Methotrexate 7.5 mg/week was also added. After 1 year his clinical and biological evolution became favorable and the dose of Sulphasalazine was reduced to 4 grams/day, keeping Methotrexate and nonsteroidal anti-inflammatory drugs at the same dose. In 1998 he was diagnosed with ankylosing spondylitis according to the New York criteria and the association of HLA-B27 antigen. The patient gave up Methotrexate, the swelling and pain in large joints persisted so we increased the Sulphasalazine dose to 8 grams/day. The patient has annually done physio-kinesiotherapy. In 2009 it was decided to initiate biological therapy criteria (low back pain and morning stiffness, bilateral sacroiliitis grade 4, BASDAI 8.6) according to Guidelines for the treatment of ankylosing spondylitis.

In March 2009, the Infliximab (*Remicade®* 100 mg / fl) 5 mg/kg treatment was initiated. After a loading with Infliximab at 0, 6, 12 weeks, the patient had a clinically and biologically favorable development for 18 months.

Figure 1: Indications of biological therapies in ankylosing spondylitis by therapeutics protocols

Ankylosing spondylitis certain diagnostic according to the criteria of New York 1984:
• Low back pain and morning stiffness more than 3 months, being improved with effort and does not disappear at rest • Movement limitation of lumbar spine in sagital and frontal planes • Limitation of chest expansion • Unilateral or bilateral sacroiliitis grade 3-4 grade 2-4 (imaging criteria). • Diagnosis involves the imaging criteria presence (radiology, MRI) associated to at least one clinical criteria.
Active and severe disease
• BASDAI ≥ 6 for at least 4 weeks • Erythrocyte sedimentation rate > 28 mm/h • C reactive protein > 20 mg/L and 3 x VN (quantitated)
The failure of traditional therapies
•At least 2 nonsteroidal anti-inflammatory drugs continuously administrated for at least 3 months, at maximum recommended doses or tolerated for patients with axial forms • Patients with severe axial do not need a remitting (Sulphasalazine) before the biological therapy • Nonsteroidal anti-inflammatory drugs and Sulphasalazine in peripheral forms, at least 4 months of treatment at the maximum tolerated dose (3 g/d) • Ineffective response to at least one local administration of a corticosteroid injection in peripheral arthritis and/or active enthesopathies, if indicated.
The presence of hip joint damage and extra-articular manifestations are additional factors allowing anti-TNF therapy administration at a lower score of disease activity, with BASDAI ≥4

At the end of the year he suffered an acute infero-lateral myocardial infarction. The patient was hospitalized in the cardiology department, a coronary angiography was performed which revealed a 80-90% anterior descending artery stenosis, to the distal 50-60% left coronary artery stenosis, 80% stenosis in the origin of the AC, 90% oblique median artery stenosis.

The biological therapy was stopped and a surgical intervention was performed with a triple coronary artery bypass graft with autologous saphenous vein.

A carotid Doppler ultrasound was performed showing that carotid vascular system was without hemodynamically significant stenosis. Cardiac ultrasound revealed (left ventricular hypertrophy with anterolateral myocardial contractility low post, left ventricular ejection fraction 55%). Three months postoperatively the biological therapy with *Remicade®* was restarted but the second adverse reactions has occurred (generalized rash, severe hypotension). The patient received Sulphasalazine 6 grams/day, painkiller, intermittent nonsteroidal anti-inflammatory drugs and cardiological medication. Biological evaluation showed a mixed dyslipidemia, associating a grade 1 obesity.

Rheumatic symptoms were significantly exacerbated (peripheral arthritis and low back pain, intense predominantly nocturnal), thus it was decided to initiate therapy with another anti-TNF alpha (*switch*) respectively Adalimumab (*Humira*®). In February 2011 a first dose of Adalimumab – *Humira*® 40 mg/2 weeks sc was administrated.

After 6 months of biological treatment with Humira, the following peripheral arthritis, acute lombalgy and inflammatory syndrome have persisted, BASDAI score was 8.1 and anginous pain occurs during small efforts. The angiography was repeated after which angioplasty was performed. Cardiac ultrasound is performed together with control cardiac stress tests, highlighting a grade 2 mitral regurgitation, posterior mitral ring atheromatosis, hypokinesia in the left ventricle lower part. Our patient had high blood pressure values being diagnosed with a 2 grade essential hypertension, thus instituting a antihypertensive therapy treatment. Therapeutics commission (rheumatologist – cardiologist) has decided to interrupt the biological therapy, considering the patient as being non responder according to the guide treatment ankylosing spondylitis criteria. The patient has continued the background therapy with Sulphasalazine 8 grams/day, pain relievers and cardiological medication.

Persistent inflammatory syndrome associated with peripheral arthritis (*de novo*, costo-vertebral arthritis and intense bilateral talalgia), BASDAI score was 9.1, has imposed a board therapeutic meeting whose opinion was to switch on the last anti TNF alpha, which remained the only therapeutic option, Etanercept (*Enbrel®* 50 mg/week).

Before the Enbrel therapy initiation a whole-body bone scintigraphy was performed (osteogenic lumbar spine and right acetabulum reactions), CT abdominal-pelvic (grade I hydronephrosis left kidney, left kidney chronic pyelonephritis) and TB QuantiFERON test (negative) to exclude a subclinical tuberculosis infection.

Figure 2: Myocardial Perfusion Scintigraphy

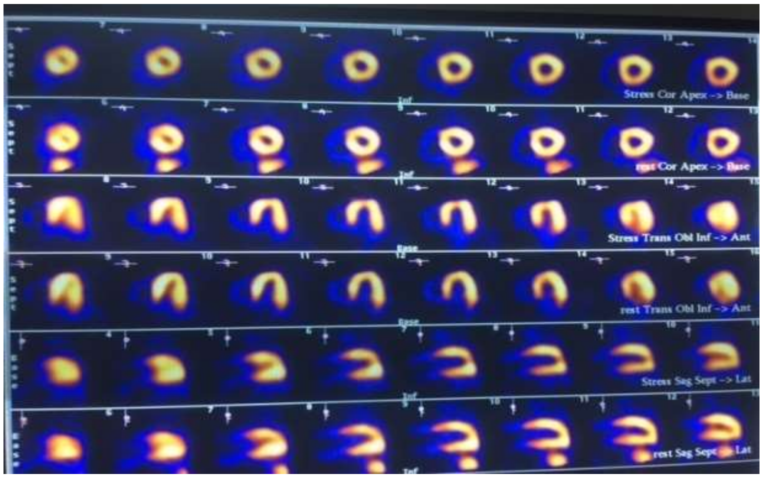

After 3 months the patient has presented a favorable evolution with a significant remission of the biological inflammatory syndrome, the peripheral joint tumefactions, persistence of a moderate intensity arthralgia and improvement of overall mobility. The patient was cardiologically reassessed, renal without the evidence of evolutionary elements. An angiocoronarography control was conducted (with 50% restenosis of the anterior descending artery stent), together with a myocardial treacherous tomoscintigraphy ECG synchronized (infero-septal dyskinesia level and decreased left ventricular ejection fraction regional effort – stress left ventricular ejection fraction 49%, resting left ventricular ejection fraction 60 %).

In 2014 the patient experienced an episode of acute anterior uveitis treated with corticosteroids and local mydriatic.

At the last hospitalization in February 2016 the clinical and biological evolution of the patient was favorable, the BASDAI score was 1.6. The treatment include *Enbrel®* the 50 mg/week, Sulphasalazine 1.5 grams/day, and nonsteroidal anti-inflammatory drugs intermittently. The patient is monitored at every 3 months by the rheumatologist and cardiologist and monthly by the family physician evidence the early possible complications.

Particularities of the case

1. Patient with juvenile arthritis, with ankylosing spondylitis evolution and complexes cardiovascular complications;

2. Lack of response to anti-TNF alpha 2 (*Remicade®*- side effects and *Humira®* - non responder);

3. Favorable answer and sustained to the 3rd anti TNF alpha (*Enbrel®*) after 3 years of treatment.

CONCLUSIONS

The introduction of TNF-alpha anti agents in treatment guidelines of ankylosing spondylitis, has revolutionized the therapy of patients with this disease, efficiency confirmed by numerous international clinical trials.

Anti TNF-alpha agents by reducing systemic inflammation creates a real cardiovascular protection for patients with ankylosing spondylitis, aspect highlighted for the case of our patient who has presented progressive cardiovascular events.

The complexity of the presented case is revealed by the associated comorbidities (cardiovascular, ocular, osteoarticular), which responded favorably after two switches of anti TNF-alpha concluding that switch between anti TNF alpha may be an effective therapeutic option in controlling the underlying disease and its complications.

Debut at the young age and association with HLA B 27 antigen are unfavorable prognostic factors for cardiovascular complications.

Interdisciplinary collaboration (rheumatologist, ophthalmologist, and cardiologist) is important for the management of patients with ankylosing spondylitis.

The evolution of this ankylosing spondylitis case is to be followed and the initiation in case of non-responder to *Enbrel®*, with a anti IL type 17 therapy, with sustained international studies having very good results in controlling this disease.

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CLINICAL PRACTICE

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Behçet disease – Case presentation

Lucian M. Ciobîcă[1], Sârbu Iolanda[1], Silviu M. Stanciu[1], Ancuţa Coca[1]

Abstract: *Behcet's disease is a rare and poorly understood condition with multiple systemic manifestations. The disease causes inflammation in blood vessels throughout the body which leads to numerous symptoms that may appear and disappear unpredictable.*

Case presentation

A 33 year old woman was admitted (interned) to our clinic. Her family medical history reveals – multiple strokes (father) and autoimmune thyroiditis (sister).

The onset of her symptomatology was in 2013 and it consisted of fever (39-40 Celsius degrees) sicca syndrome and persistent headaches, recurrent oral and genital ulcerations.

An important event is essential to be mentioned -the patients has suffered an episode of upper gastrointestinal bleeding (hemoglobin has dropped to 2.5g/dl which has led to cardiac arrest-resuscitated).

The lab tests showed: C3 hypocomplementemia, Anti-centromere antibodies (-), anti-b2gp1 antibody (+), lupus anticoagulant (+), U1RNP(-).

We started to administrate cyclophosphamide to the patient, thus his condition has improved after the 2nd dose.

At the 12-month evaluation we were able to see a significant clinical and biological improvement.

Conclusions

In order to be able to talk about "evidence based medicine" for the management of Bechet's disease, a large number of clinical trials is required to provide to the attending physicians the necessary data for diagnosis and treatment.

INTRODUCTION

Behcet disease is a rare condition with a sinusoidal evolution which can affect almost all the organs and human systems, determining multiform symptoms with a variable severity. The medical practitioner has at his disposition a diversity of therapeutic options (from symptomatic therapy to a major systemic immunosuppression); at this moment we can't discuss about fundamental therapeutics protocols and unanimously recognized.

Behcet disease is an inflammatory systemic condition with an uncertain etiology. In the disease pathogenesis both environmental and genetic factors are involved like for the majority autoimmune diseases. This disease has been worldwide described but the maximum prevalence is registered in Middle East, adjacent zones of Mediterranean Sea and Asia

Considering the potential muco-cutaneous, articular, ophthalmic, neurologically, etc. damages, the interdisciplinary collaboration becomes more important. Due to the lack of specific guidelines for the diagnosis and treatment, the therapeutic approach of

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individuals' cases still remains dependent on the doctor treating experience or on the clinics where these complex cases are monitored.

CASE PRESENTATION

A 33 year old woman was admitted to our clinic presenting severe muscle weakness and joint pain.

Her medical history has revealed that in the last years the patient was admitted several times in the Infectious Diseases department with persistent fever and severe headaches.

Her family medical history has revealed – multiple strokes (father) and autoimmune thyroiditis (sister).

The onset of her symptomatology was in 2013 and it consisted of fever (39-40 degrees Celsius) sicca syndrome and persistent headaches. As the disease was progressing, our patient presented oral ulcerations, odynophagia, muscle weakness and blindness of the left eye.

The onset of the decreased visual acuity started with the right eye. An ophthalmologic examination has revealed uveitis and chorioretinitis.

Considering an inflammatory demyelinating disease (multiple sclerosis), the neurological exam suggested a cranial magnetic resonance imaging (which has revealed optic nerve edema) and anti-aquaporin 4 antibodies screening (which was negative). The patient treatment has started on Corticosteroids (methylprednisolone in pulse therapy) resulting a slight improvement of visual acuity, persistent joint pains, xerophtalmia.

An important event is essential to be mentioned -the patients has suffered an episode of upper gastrointestinal bleeding (hemoglobin drops to 2.5 g/dl which leads to cardiac arrest – resuscitated).

When the patient was admitted to our clinic the symptoms become more aggressive; the patient present extreme fatigue (with orthostatic intolerance), recurrent oral and genital ulcerations, odynophagia (due to the pain caused by oral and esophageal ulcerations she was not able to solid food or liquids) and blindness of the left eye.

Figure1: Atrophy of retinal layer

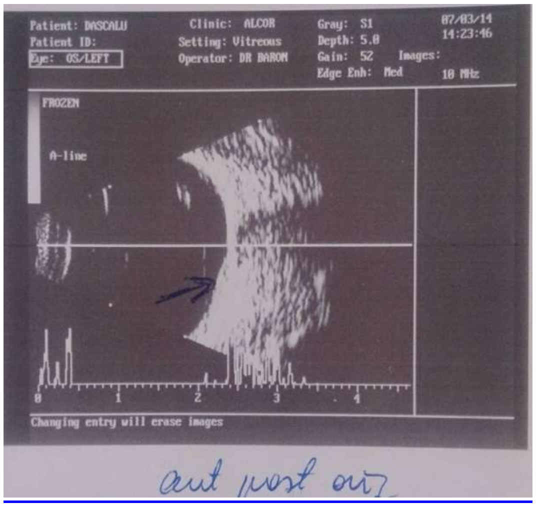

Figure 2: FOS – thicknesses of retinal layer

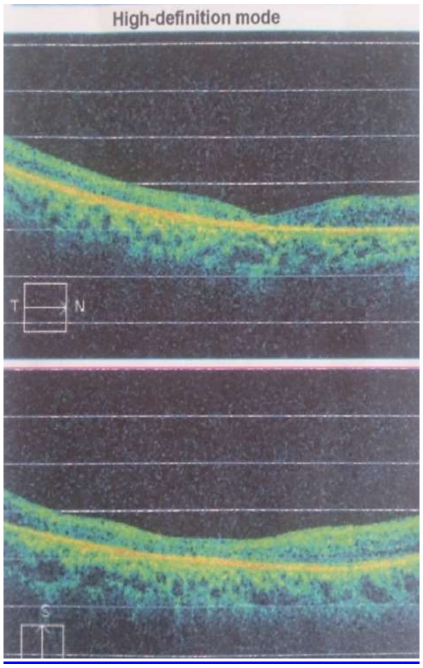

Despite the muscle weakness and severe fatigue that the patient suffered from throughout this whole period, muscle enzymes was never elevated.

Nevertheless we continued our investigations with an electromyogram. The EMG showed acute polymyositis aspect and sensory polyneuropathy lesion.

Then neurological exam showed spastic paraplegia, left brachial plegia and visual acuity loss. The brain MRI revealed a lacunar ischemic stroke in the vertebrobasilar territory.

The ophthalmological exam showed optic nerve atrophy.

We were facing with a patient, having a chronic, inflammatory multisystem condition of unknown etiology. The blood tests revealed nothing but a persistent inflammatory syndrome (ESR = 80-100 mm/h); the immunology tests didn't provide enough information either (C3 hypocomplementemia, Anti-centromere antibodies (-), anti-B2GP1 antibody (+) lupus anticoagulant (+), U1RNP (-), anti-aquaporin 4 antibodies (-) HLA B51 (-), Leiden V factor (+), ANA (+).

However we were in possession of his clinical examination highlights: chronic fatigue, recurrent mucosal ulceration, neurological symptoms, bilateral posterior uveitis, inflammatory arthritis various symptoms that we were able to identify as features of Behcet's disease.

By the time an accurate diagnostic could be established, the patient had already suffered some severe life threatening complications: upper GI bleeding with severe anemia that led to cardiac arrest, severe sepsis caused by infected decubitus injuries and medullar aplasia caused by high dose of immunosuppressive medication.

Behcet disease – generally accepted therapeutic objectives:

1. It's essential having a clear diagnostic and well founded as well as a clear setting of a lesional exact balance, with the progressive potential appreciation of the identified lesions;

2. The remission induction and maintenance;

3. The prevention of irreversible organs lesions (for example the ophthalmological one), which tends to appear early, especially in some problematic groups like young men;

4. The articular and muco-cutaneous exacerbations prevention can significantly improve the quality of life;

5. The multisystem involvement imposes for most cases interdisciplinary implication.

We want to underline the importance of having an accurate and precise diagnostic before starting a treatment, but in this particular case, a rapidly evolving disease worsened by the upper mentioned events, we decide that it was extremely necessary for the patient to start a remissive treatment. We initiated a moderate dose of cyclophosphamide (800 mg – 15 mg/kg bolus intravenous) and a low dose of oral corticosteroid (methylprednisolone 8 mg).

A significant improvement was observed after the second course of cyclophosphamide. The patient has regained the strength and muscular tonus, neurological symptoms has disappeared and she did no longer mucosal ulcerations.

Although the inflammatory ocular symptoms were suppressed, the significant loss of visual acuity persisted (it was caused by initial optical neuritis).

At the 12-month evaluation we were able to see a significant clinical and biological improvement. The patient is now able to do her daily activities therefore her lifestyle has improved.

The brain MRI shows normal brain parenchyma, normal vascular structures and normal blood flow.

Ophthalmological exam: minor restant vitrean abnormalities.

CONCLUSIONS

In the past decade we have witnessed a significant improvement in the therapeutic approach of Behcet's disease by optimal use of the old available drugs and also by the development and clinical use of new therapeutic solutions.

Behcet's disease still remains a multidisciplinary challenge for attending physicians.

Available data in the medical literature consists mainly in clinical cases and series of clinical cases with a limited statistic value.

In order to be able to talk about "evidence based medicine" for the management of Bechet's disease, a large number of clinical trials is required to provide for the attending physicians the necessary data for diagnosis and treatment algorithms.

Abbreviation

[1] U1RNP – Anti-Ribonucleoprotein

[2] EMG – Electroyography

[3] ESR – Erythrocyte Sedimentation Rate

[4] HLA B51 – Human leukocyte antigen B51

[5] ANA – Anti-nuclear antibody

[6] GI – Gastrointestinal

[7] MRI – Magnetic resonance imaging

[8] B2GP1 – Beta 2 Glycoprotein 1

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VARIA

In memoriam – Cristian Babalâc (1954-2006)

Dan Mischianu

I am absolutely convinced that you have never wondered what the etiology of the *ferpar* word is.

The Romanian *ferpar* has its origin linked to the French "fair-part", which, according to the dictionary means a letter "announcing birth, engagement, marriage or, most often someone's death". The above lines are not intended to be a late *ferpar.*

These lines are supposed to bring back the memory of an absolutely exceptional man, a thorough man and a fighter, a true military doctor - Col. Dr. Cristian Babalâc (1954-2006), my University colleague. I also hope that these lines will make you realize that time passes quickly destroying everything on its way.

And time, a decent gentleman for some, covering us with a veil of forgetfulness, time "has a bad habit of being irreversible", as we learned from Octavian Paler.

Our desire is to present the portrait of the man, the military doctor, the professional Cristian Babalâc, because Dr. Cristian Babalâc was – and "to be" in the past tense sounds so sad! – a devoted man and practitioner that always proved an absolute professional availability.

My group colleague and my friend, Dr. Cristian Babalâc was born in Bucharest, on 13 January 1954 on Teodosie Rudeanu street, near North Railway Station, a few hundred meters from the Institute of Military Medicine, the place or "origin" of our emergence, the ones from yesterday, today and future serving Romanian military medicine.

Graduated from High School "Ion Neculce" Bucharest (1973), Faculty of Medicine Bucharest (1979), Doctor of Medicine with the thesis "The value of laser in the surgical treatment of soft part tumors" (1997), specialist doctor in Orthopedics and Traumatology (1989), Senior researcher (1990), consultant in Orthopedics and Traumatology (1995).

He participated as a member or responsible at research contracts, guided numerous diplomas and internships, conducted/aided the full range of orthopedics – traumatology procedures, 15 patents and 7 innovation patents for different medical devices: gurney for the transport of traumatized, forearm electronic prosthesis controlled by sensors, bioelectronic prosthesis for superior limb, immobilizer and transportation device for polytrauma patients, external bipolar monoplane fixator, semicircular external fixator, circular external fixator, universal external fixator, grapples for osteotomy. He introduced "CO_2 surgical laser treatment in musculoskeletal limb tumors" for the first time in the country.

Published the treatise: "Laser in the treatment of musculoskeletal diseases" (co-author) Military Publishing House Bucharest (1999), "Course of emergency and disaster medicine" (co-author) MTM Publishing, Consult Publishing (2003), 147 (20 as first author) communicated papers at congresses, conferences, symposiums in the country and abroad, 28 articles (4 as first author) published in journals: "Journal of Military Medicine", "Journal of Orthopedics and Traumatology", "News Update", "Balkan Military Medical Journal", a member of SOROT – Romanian Society of Osteoporosis Treatment, Romanian Society of Laser in Medicine and Biology, Romanian Association of Military Physicians and Pharmacists.

I knew him as a student and we operated together as specialist surgeons. Dr. Cristian Babalâc was a hard worker by definition, an absolute perfectionist, continually dissatisfied with his achievements – although he managed to accomplish everything he undertook, a deep thinker, a cerebral man, a genuine family man.

He was particularly pleased with the silence at home, the achievements of his wife, the distinguished Head of Hospital Pharmacy of "Prof. Theodor Burghele" Hospital – Ms. Pharmacist Gabriela Babalâc, and the professional accomplishments of their son – Cătălin Babalâc.

Col. Dr. Cristian Babalâc was a "serious man", a professional of orthopedics and traumatology of the Central Military Hospital. Nevertheless, medicine is very often defeated by disease severity.

I confess that I had a sense of absolute helplessness when I was near the bedside of Dr. Cristian Babalâc!

Lack of health, the implacable decline, and the mortal character of all beings – but most of all those close to our soul – are almost unapproachable subjects. As I was writing these lines I was overwhelmed by bleak thoughts.

My intention was only one, to restore the memory of those who knew and valued him, a decade after his passing, the luminous figure of Dr. Cristian Babalâc, although "slightly frown as a result of the multitude of his thoughts".

Leaving us ten years ago after unimaginable suffering, I think Dr. Cristian Babalâc kept one hope in his noble and good soul: maybe better is yet to come!

May God bless his soul!

ADMINISTRATIVE ISSUES

Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine*. Please read the complete Author Guidelines carefully prior to submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

ETHICAL CONSIDERATIONS

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) accessible to the public at no charge;

(2) searchable by standard, electronic (Internet-based) methods;

(3) open to all prospective registrants free of charge or at minimal cost;

(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

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Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster’s Collegiate Dictionary.

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Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

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The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’

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Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

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Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/ mesh/meshhome.html).

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals, and cited in consecutive order in the text. Each figure should be supplied as a separate file, with the figure number incorporated in the file name.

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Military Medicine

REVISTA DE MEDICINĂ MILITARĂ

- *About health, with hope*
- *Capabilities for identification and confirmation of bacterial agents*
- *The consequences of Chernobyl accident*
- *Using direct anticoagulants (DOACs) in cancer and other high-risk populations*
- *Risk factors and quality of life in late-life depressive disorders*
- *Modern medicine has a new technology: therapeutic electroporation*
- *The incidence of tracheoesophageal fistulas and its major determinant factors*
- *Ankylosing spondylitis and cardiovascular risk*
- *Behçet disease – Case presentation*
- *In memoriam Cristian Babalâc*

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EDITORIAL

About health, with hope

Dan Mischianu

Gral (R) Prof DAN MISCHIANU
Chief of Urology Clinic, Carol Davila Central Emergency Military Hospital
Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Health is an extremely complicated subject, but to talk about health is like howling in a cave: anyone can hear, no one understands! ...maybe only the echo?!

The World Health Organization (WHO) proposed in 1948 when it was established, the following definition: "Health is a fully favorable physically, mentally and socially status, not merely the absence of disease or infirmity." Then came an addition: 'ability to lead a productive life, socially and economically. "

I recently finished reading a bestseller also promised to the Romanian reader by Polirom publisher "Sapiens A Brief History Of Humankind" Yuval Noah Harari, Vintage Books, London, 2014. A strange creature this "homo sapiens" is, strange since all his derivatives propose other approaches and other perceptions on the concept or "health"! To primordial "sapiens" health had other connotations...

Perhaps they were cleverer, crueler, and that is why they prevailed and multiplied logarithmically. Then the diseases appeared: congenital, posttraumatic, neoplastic, infectious and so on...

Returning to our nation, Romanians produced with unmistakable grace many sayings, in which they truly believe: "Health is better than all!", "Health is the best wealth!"

Or, pessimistically "Until you're sick, you cannot be healthy!"

The traditional stories regarding health record something else: "Health, contentment and trust are your most valuable possessions and freedom is the greatest joy of yours."

I am absolutely convinced that we all want to reach or to "apply" (Anglo-Saxon term) to an entity which we believe is intangible: "Health comes first!"

Unfortunately, after we solve almost all our goals and desires, we find that health left at the end, it is "unsolvable"!...

Romanians deep and law abiding Christians, ancestral pessimistic have another saying "Cherish health when you lose it!"

Even I do not understand this allegory, why?

Because I work in a field that many pretend to understand, but few have deciphered the "anatomy and physiology" of its development, an area where financiers who led it wanting to make a profit, now keep their mouths shut nor can i forget that there were great men, deciding the destiny of many nations, who had a absolutely unfortunate history of "health": Emperor Napoleon III died in urosepsis under the supervision of the most famous urologist of its time, Emperor Wilhelm II, the first grandson of Queen Victoria of the United Kingdom, born with left brachial plexus palsy after a labor dystocia (1859), "irritable, angry, violent and passionate" started The First World War and Generalissim – Iosif Vissarionovich Stalin's apparently had the same malady that which made him as reckless and cruel.

They followed, and still follow many others, the bad, and the complexed!

It is these thoughts that make me not to forget Miron Costin's assertion: "There are times under the man, but the poor man under these times." ("Chronicle of Moldavia after Aron Voda").

For this editorial, unfortunately slightly skeptical about the future, it will appear in the new year 2017 I ask the reader of these lines to believe and hope that Romania will benefit from a new configuration of a credible, effective and with a tendency towards the many health system.

I further wish you a happy New Year full of accomplishments, or, as people say in our country, “A Happy New Year!"

REVIEW ARTICLE

Article received on March 7, 2016 and accepted for publishing on August 25, 2016.

Capabilities for identification and confirmation of bacterial biological agents

Diana M. Popescu[1], Marius Necșulescu[1], Lucia E. Ionescu[1], Simona N. Bicheru[1], Gabriela V. Dumitrescu[1], Viorel Ordeanu[1]

Abstract: *Military Medical Service is able for detection, identification and confirmation of biological agents; it is part of medical protection against CBRN weapons. We are specialized capabilities for in vitro tests, under construction, the maximum containment laboratory designed for work with Risk Group Microorganisms. An efficient primary containment system must be in place, consisting of one or a combination of the following: Class III safety cabinet laboratory, passage of two doors, suit laboratory, controlled access, controlled air system. Negative pressure in the facility, supply and exhaust air must be HEPA-filtered, decontamination of effluents, sterilization of waste and materials, airlock entry ports for specimens, materials and animals must be provided etc. Complementary is an Animal facility for in vivo tests. This is suitable for work with animals that are deliberately inoculated with microorganisms in Risk Group.*

Keywords: *biological security, military capability, biological attack, biological agents, CIMIC, medical protection*

CAPABILITIES FOR IDENTIFICATION AND CONFIRMATION OF BIOLOGICAL AGENTS

Military Medical Service is abilities for the detection, identification and confirmation of biological agents. It is part of the medical protection against CBRN weapons, with mobile and stationary capabilities. MMRC participate, specifically the medical protection against biological weapons and bioterrorism with a series of microstructures: Biological mobile intervention team with mobile platform; Biological analytical laboratory CBRN defense, integrated platform for scientific and medical-military research for biological agents, with: Laboratory of Microbiology high secure, Secured animal facility laboratory for confirmation of biological agents, laboratory of molecular biology and genetics, toxicology laboratory and Annex facility. [1, 2, 3, 4]

SPECIALIZED CAPABILITIES

Stationary

The maximum containment microbiological laboratory is designed for work with Risk Group Microorganisms, for in vitro diagnostic. Operational maximum containment laboratories should be under the control of national or other appropriate health authorities. [5,

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[1] Military Medical Research Center, Bucharest

6]

Laboratory design and facilities:

- Primary containment. An efficient primary containment system must be in place, consisting of one or a combination of the following; Class III cabinet laboratory. Passage through a minimum of two doors prior to entering the rooms containing the Class III biological safety cabinet is required; [5, 7] Suit laboratory. A protective suit laboratory with self-contained breathing apparatus differs significantly in design and facility requirements from a Biosafety Level 4 laboratory with Class III biological safety cabinets. [5]

- Controlled access. The maximum containment laboratory must be located in a separate building or in a clearly delineated zone within a secure building. Entry and exit of personnel and supplies must be through an airlock or pass-through system. [5, 8, 9, 10, 11]

- Controlled air system. Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. There are significant differences in the ventilating systems of the Class III cabinet laboratory and suit laboratory. [5]

- Decontamination of effluents. All effluents from the suit area, decontamination must be decontaminated before final discharge. Heat treatment is the preferred method. Effluents may also require correction to a neutral pH prior to discharge. Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment. [5, 8, 9, 10]

- Sterilization of waste and materials. A double-door, pass-through autoclave must be available in the laboratory area. Other methods of decontamination must be available for equipment and items that cannot withstand steam sterilization. [5, 6]

- Airlock entry ports for specimens, materials and animals must be provided. [5]

- Emergency power and dedicated power supply lines must be provided. [6]

- Containment drains must be installed. [5]

Because of the great complexity of the engineering, design and construction of Biosafety Level facilities, in cabinet or suit configuration, schematic representations of such facilities have not been included. [5] (Figure 1)

Figure 1: Microbiological laboratory for biological agents

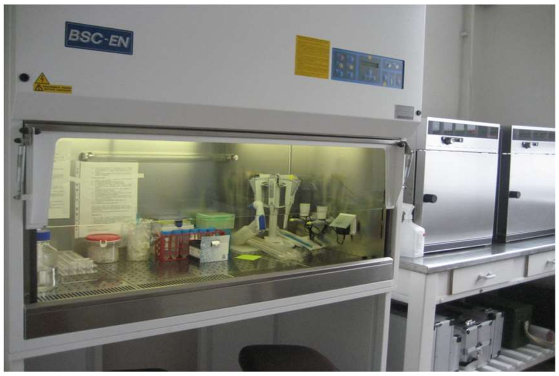

Animal facility

This is suitable for work with animals that are deliberately inoculated with microorganisms in the Risk Group, for in vivo diagnostic. [12, 13, 14] The following safety precautions apply:

- All the requirements for animal facilities – Biosafety Level must be met. [12, 13, 14, 15]
- Biohazard warning signs (see Figure 1) should be posted on doors and other appropriate places. [12, 14]
- The facility must be designed for easy cleaning and housekeeping. [12, 14]
- Doors must open inwards and be self-closing. [12, 14]
- Heating, ventilation and lighting must be adequate. [12, 14]
- If mechanical ventilation is provided, the airflow must be inwards. Exhaust air is discharged to the outside and should not be recirculated to any part of the building. [12, 14]
- Access must be restricted to authorized persons. [12, 14]
- No animals should be admitted other than those for experimental use. [12, 14]
- There should be an arthropod and rodent control program. [12, 14]

- Windows, if present, must be secure, resistant to breakage and, if able to be opened, must be fitted with arthropod-proof screens. [12, 14]
- After use, work surfaces must be decontaminated with effective disinfectants. [12, 14]
- Biological safety cabinets (Class II) or isolator cages with dedicated air supplies and HEPA-filtered exhaust air must be provided for work that may involve the generation of aerosols [12, 13, 14, 15, 16]
- An autoclave must be available on site or in appropriate proximity to the animal facility. [12, 13, 14, 15, 16]
- Animal bedding materials must be removed in a manner that minimizes the generation of aerosols and dust. [12, 13, 14]
- All waste materials and bedding must be decontaminated before disposal. [12, 13, 14]
- Use of sharp instruments should be restricted whenever possible. Sharps should always be collected in puncture-proof/-resistant containers fitted with covers and treated as infectious. [12, 13, 14]
- Material for autoclaving or incineration must be transported safely, in closed containers. [12, 13, 14]
- Animal cages must be decontaminated after use. [12, 13, 14]
- Animal carcasses should be incinerated. [12, 13, 14]
- Protective clothing and equipment must be worn in the facility, and removed on leaving. [12, 13, 14]
- Hand-washing facilities must be provided. Staff must wash their hands before leaving the animal facility. [12, 13, 14]
- All injuries, however minor, must be treated appropriately, reported and recorded. [12, 13, 14]
- Eating, drinking, smoking and application of cosmetics must be forbidden in the facility. [12, 13, 14]
- All personnel must receive appropriate training. [12, 13, 14]

This facility is complementary to the microbiological laboratory (Figures 2, 3).

Figure 2: Animal facility for microbiological diagnosis

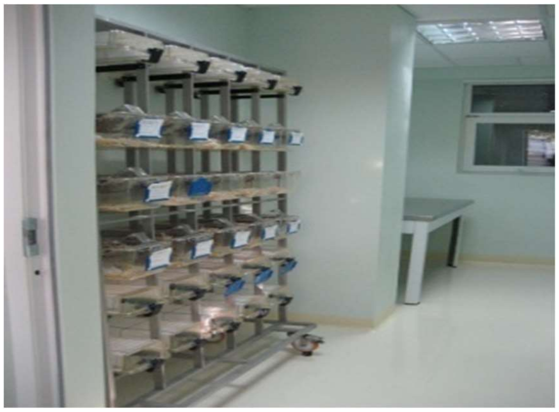

Figure 3: Secured animal facility laboratory

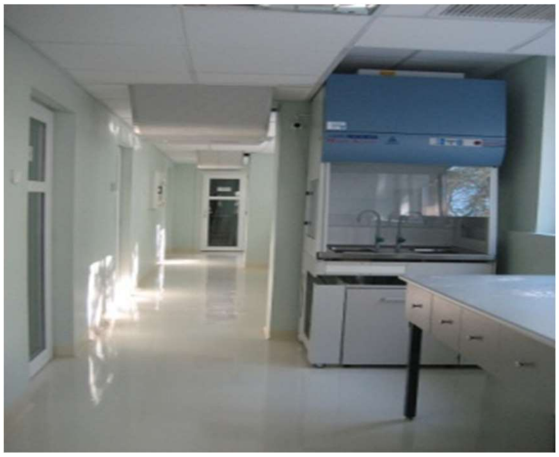

SPECIALIZED MOBILE CAPABILITIES FOR Bio-Det SYSTEM

Specialized Mobile capabilities for Bio det system:

- provides monitoring, sampling, detection and identification;
- detects long line releases and identifies up to 6000 species and sub-species (data base).
- up to 96 samples can run simultaneously, no time needed to re-deploy.
- deploy worldwide and conduct biological surveillance operations in order to provide identification of biological agent employment.
- provide information to other forces (rescue teams, medical, etc)

Bio-Det Systems

Configuration:

- Vehicle with 2 internal compartments, with power supply, specific furniture and safety cabinet class III.

For monitoring: Verotect – determines if a bio-mass is present within aerosol particles of a certain size range

For identification:

- MALDI TOF Microflex LT 20 with vehicle mount
- Water, soil and air sampling kits
- Sample preparation equipment (incubator, centrifuge, freezer, autoclave, handling tools, consumables and reagents)

Miscellaneous:

- Personal protection for the operators (suits, masks, oxygen tubes)
- Hardened laptop etc.

Mass Spectrometry

Those are complex instruments which record an ionic flow as a spectrum, in relation to both mass and the relative occurrence of the ions; Mass spectrometry is a confirmation analytical technique which allows identification of organic compounds, based on the mass/electrical charge ratio of the molecule fragments of the analyze.

The mass spectrometer consists of 4 elements: sample loading port, where a certain amount of sample is introduced at the required pressure in order to be exposed to the ion source and to be ionized at maximum efficiency. Ion source, where the neutral atoms and molecules are ionized and directed into the instrument as a monenergistic bundle.

Mass (energy) analyzer, which by electrical and/or magnetically deflections selects the ions according to the q/m values, their masses, concomitantly focusing them towards an outlet gap. Detector, comprising the system which transforms the ionic flow either into a photographic image or an electric current that can be recorded with an appropriate instrument.

MALDI TOF Microflex

Microflex LT20 is a MALDI TOF system equipped with Bioprofiler software and it is used for the identification and taxonomic appointment of the biological agents from air, water, soil and other surfaces, after a previous specific processing of the collected samples. The main element is a MALDI TOF spectrometer, with a mass domain from 2,000 to 20,000 Da. The instrument is controlled by means of a computer with Windows operating system and specialized software (Bioprofiler). The data processing system controls all acquisition, saving and storage processes of the spectral information, allowing later processing of the information or access to the data base.

Microflex LT 20 Bruker system

It has the ability to identify the biological agents from various samples (liquid, solid, gaseous). Identification is performed by comparing the obtained spectrum to the existing data base of memorized biological agents.

The software indicates the matching of the spectrum under test with spectra from the database, according to the obtained scores. Scores are generated by evaluation and comparison between spectra and are not percents. If a biological agent is not included in the data library, the agent with the closest structure is exhibited, but the score indicates that the agent under test is not included in the data base of the instrument (Figure 4).

Figure 4: Mass spectrometer for microbiological diagnosis (The Microbiology Laboratory MMRC, I. Cantacuzino)

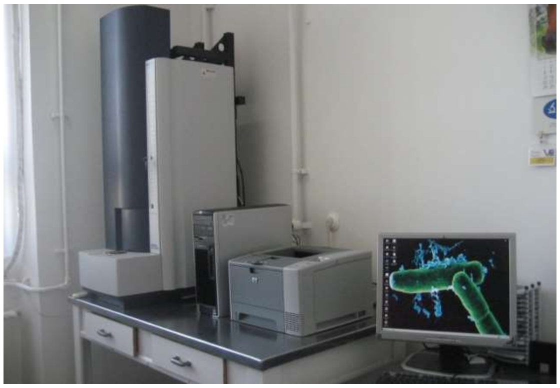

Operation steps Microflex

Preparation of samples for MALDI TOF analysis is done by extracting 16S ribosomal proteins that can be achieved by several techniques depending on the organism analyzed. [17]

The instrument can measure mass spectra from 2,000 up to 20,000 Da, i.e. a very wide range of organic molecules originating from any kind of biological agents (bacteria, spores, viruses, fungi, yeasts, toxins). The software and its data base are capable to identify biological agents of civil and medical-military interest.

The equipment is foreseen with a device for attachment to mobile laboratories, allowing its use in the field, either in campaigns or, in peace time, in case of biological events or crises.

The quality of the incorporated mass spectrometer as well as the structure of the soft that can be upgraded in time, strongly recommends the MALDI TOF – Bioprofiler MICROFLEX LT20 system for identifying biological agents, to be used for precise microbiologic diagnostic, species confirmation for the biological agent, medico-military research and medical defense against biological weapons or bioterrorist attacks.

Military Medical Service is able for detection, identification and confirmation of biological agents. It is part of medical protection against CBRN weapons, in civil-military cooperation (CIMIC). [17]

CONCLUSIONS

CCSMM has specific capabilities for scientific research for medical protection against CBRN WMD.

Anti-infective medical protection laboratory and Epidemiologic emergency laboratory have capability of performing detection, identification and confirmation of biological agents within the limits of available supplies.

The capacities and capabilities of health protection against biological weapons are interoperable with NATO.

Detection, identification and confirmation of the presence of bacterial biological agents are executed sequentially and modular, specialized capacities through cooperation, which must have the capability to perform these tasks.

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REVIEW ARTICLE

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The consequences of Chernobyl accident

Ion Chioșilă[1]

Abstract: *These days marks 30 years since the Chernobyl nuclear accident, followed by massive radioactive contamination of the environment and human in Belarus, Ukraine and Russia, and resulted in many deaths among people who intervened to decrease the effects of the nuclear disaster. The 26 April 1986 nuclear accident contaminated all European countries, but at a much lower level, without highlighted consequences on human health. In special laboratories, the main radionuclides (I-131, Cs-137, Cs-134 and Sr-90) were also analyzed in Romania from environmental samples, food, even human subjects. These radionuclides caused the population to receive a low dose of about 1 mSv in 1986 that is half of the dose of the natural background radiation (2.4 mSv per year). As in all European countries (excluding Ukraine, Belarus and Russia) this dose of about 1 mSv fell rapidly by 1990, reaching levels close to ones before the accident at the nuclear tests.*

Keywords: *nuclear accident, Chernobyl disaster, radionuclide, radioactive contamination, effective dose*

INTRODUCTION

2016 marks 30 years since the major accident in the history of nuclear power plants (active zone melting and thermal explosion) that took place on 26th April 1986 at 4th reactor of Chernobyl Nuclear Power Plant (Ukraine). Emissions of radio nuclides were particularly high and affected many countries in Europe, including our country. The 4 reactors in operation at the time run on natural uranium (U-238) with low-enriched U-235, a high producer of Pu-239 (strategic material for nuclear weapons).

Although this type of reactor was well known by the operating personnel, they conducted an experiment with the turbine running in the inertial mode and lowering reactor's power at 10%, but the return to full power (thousand MW) took only 2 seconds which led to a loss of control and the impossibility of an emergency shutdown of reactor no. 4. The rapid and uncontrolled increase in temperature of the reactor core produced two successive explosions seconds after each other that threw the 1000 tons concrete for protection, as well as large amounts of nuclear fuel with fission products and activation products, along with the burning graphite moderator [1].

Definitions of specific terms

1. Activity (radioactivity) of a radionuclide: the unit of work in the International System (S.I.), is one disintegration per second, or s-1, with the name Becquerel and symbol Bq. Curie - special name for the tolerated unit of the activity of a radionuclide, with the symbol Ci; is 3.7 x 1010 disintegrations per second

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[1] Romanian Society for Radiological Protection, Bucharest

(approximate activity of radon – 222 in equilibrium with one gram of radium – 226) and respectively 3.7 x 1010 Bq.

2. Half-life – the time after which the number of nuclei of a radionuclide is reduced to half by radioactive decay with symbol T1/2, or Tf is used.

The biological half-life – the time required for a biological system to remove half of the nuclei (stable or radioactive) by metabolic processes with symbol Tb. The effective half-life – the time needed to halve the number of radioactive nuclei (activity) by decay and metabolic processes with Tef symbol.

3. Effective dose – weighted sum of equivalent doses from external and internal exposure, performed on all organs (tissues) of the body (the absorbed dose multiplied by the weighting factor of radiation and weighting factor of tissue or organ). In the S.I. unit is J/kg with the name Sievert and symbol Sv.

The effective dose limit for occupationally exposed workers – effective dose limit for people working in environmental radiation is 20 mSv per year. The effective dose limit for the population – effective dose limit for an individual in the population is 1 mSv per year.

CONTAMINATION OF THE ENVIRONMENT

Fission products (Cs-137, Sr-90, Sr-89, I-131, etc.) and activation products (Cs-134, Co-60, Fe-55, Fe-59, etc.) were released in atmosphere and transported by air currents throughout the northern hemisphere. The most affected (radioactive contamination) were Belarus, Ukraine and European Russia; less contaminated air currents reached many other countries in NW Europe (mainly Norway, Sweden, Finland), and to S and S-V Europe, inclusively to Romania.

Rainfall and dry deposition made possible contamination of the environment (soil, surface and biosphere).

Cs-137 was the easiest to detect (beta-emitting and gamma-emitting radiation) and has a great half-life period (Tf or 1/2) of approximately 30 years, for which most of the estimations of environmental and human contamination reported to this radionuclide.

Table 1: Surfaces contaminated with Cs-137 in some countries affected by the Chernobyl nuclear accident [2]

Country*)	**37-185 (kBq/m²)**	**185-1480 (kBq/m²)**	**over 1480 (kBq/m²)**
Russia	49800	7800	300
Belarus	29900	14400	2200
Ukraine	37200	4100	600
Sweden	12000	-	-
Finland	11500	-	-
Norway	5200	-	-
Austria	8600	-	-
Bulgaria	4800	-	-
Switzerland	1300	-	-
Greece	1200	-	-
Slovenia	300	-	-
Moldavia	60	-	-

*) In Romania, most of the surface was contaminated with Cs-137 with values below 2 kBq/m². There were large areas contaminated 2-10 kBq/ m² in Oltenia, Banat, N-E Moldova, central and northern Muntenia; here they were found locations with contamination exceeding 10 kBq/ m².

ASPECTS OF RADIATION PROTECTION IN ROMANIA

Monitoring environmental radioactivity and food radioactivity in Romania [1,3] was carried out by:

- 47 Environmental Radioactivity Monitoring Stations (belonging to the former National Council for Water, today 37 stations form the National Network of Environmental Radioactivity Monitoring and belongs to the Ministry of Environment)
- 22 Ionizing Radiation Hygiene Laboratories, of which 4 were from the Institute of Hygiene and Public Health in Bucharest, Iasi, Cluj and Timişoara (Ministry of Health)
- departamental laboratories (Institute of Atomic Physics – IFA Măgurele – Bucharest, Institute of Nuclear Energy Reactors – IRNE Pitești, Ministry of National Defense – MND, etc) (The Laboratory for radiochemistry of the Military Center for Medical Research also had tasks of human and environmental radioactivity monitoring in addition to the experimental research. There was also a laboratory to

measure the radioactive contamination of man (with whole body counter).

In the period immediately after the accident (the night of April 30, 1986 when contaminated air masses came first in the N-E) and in the years after the accident the following analyzes were performed:

- global beta measuring and gamma spectrometry of aerosol filters, total atmospheric deposition, surface and drinking water analysis, soil, wild vegetation and crops analysis, animal products and food menus from various canteens in Bucharest and in the country analysis
- direct measurement of radionuclides incorporated into man (I-131, Cs-137 and Cs-134) with MND and IFA whole body counter
- radiation dose exposure estimation for humans.

Among the measures taken for radiation protection of population in Romania, it is worth mentioning:
- consumer restrictions on certain foods (dairy products contaminated with I-131, Cs-137 and Cs-134) based on the values determined by measuring the radioactivity of food in the most contaminated counties
- forbidding sport events scheduled to take place outdoors on May 2
- administration of stable iodine for children, beginning with 3th May

Recommendations:
- extensive washing of vegetables before consumption
- reduced time spent in the open in the first week after the accident.

Negative aspects concerning the application of radiation protection measures:

- insufficient public information on the accident,
- KI administration started with a two days delay leading to reduced performance at about 50% and the administration was not performed at all children in areas contaminated with I-131.

ENVIRONMENTAL, FOOD AND HUMAN CONTAMINATION IN ROMANIA

After corroborating the results of radioactivity monitoring of the environmental factors, water and food, it was possible to create a map of radioactive fallout in our country in three stages: 1st to 2nd of May, 3rd - 4th of May and 5th to 6th of May respectively. So, the greatest contamination occurred due to the fallout of 3rd to 4th of May, which came from N-E and followed the Eastern and Southern Carpathians, with the southern area being the most contaminated (Figure 1) [4].

In atmospheric aerosols there were readings of a maximum of 103 Bq/m^3 for I-131, respectively 63 Bq/m^3 for Cs-137 at Toaca station in Ceahlău on 1st of May 1986; also readings of 17 Bq/m^3 I-131 and 14 Bq/m^3 for Cs-137 at Fundata station (Braşov county). These peaks declined rapidly in the second half of May and early June 1986, going as low as a 1000 times lower. Regarding the average concentration of Cs-137 in the aerosols, from about 106 Bq.s/m^3 in 1986 fell below 105 in 1987 and in 1991 it went back to the value from before the accident (about 200 Bq.s/m^3) [4,5].

Atmospheric radioactive depositions values were in 1986 over 3 orders of magnitude higher compared to before Chernobyl accident. As late as 1993 the average values of Cs-137 deposition reach 1 order of magnitude over that of 1985 because of wind carrying out the contaminated dust and returning in the form of radioactive fallout.

Average concentrations of Cs-137 in untilled soil samples were of 350 Bq/kg, with peaks in excess of 1000 Bq/kg in the northern county of Gorj. High levels of Cs-137 in untilled soil were recorded at Toaca-Ceahlău, Targu Mures, Gheorghieni and Parâng, with maximum levels of 2050 Bq/kg of dry soil.

In surface water, high values of Cs-137 were determined 2 or 3 days later than the atmospheric reading, measuring 50 Bq/m^3, with rapid drop below 10 Bq/m^3 in 1987 and 1988, returning to the values from 1985 as late as 1993.

The contamination of wild vegetation was carried out by fine radioactive particle deposition and measuring average values of Cs-137 as high as 1000 Bq/kg of dry vegetation, with values declining to before the accident in 1990. Contamination of crops by absorbing radionuclides in the soil was quite low, so radionuclide values decreased rapidly in the coming years [3].

The highest level of contamination with I-131 in food was found in ewe`s milk, with values of about 1300 Bq/L or higher, which led to temporary restriction in consumption imposed by the Ministry of Health, for dairy products. Cs-137 values in milk and dairy products were about 500 Bq/kg (Table 2). This radionuclide content in food has decreased since 1990, and since 2000 has reached the 1985 values.

Figure 1: Contamination Romanian territory since May 1, 1986 [4]

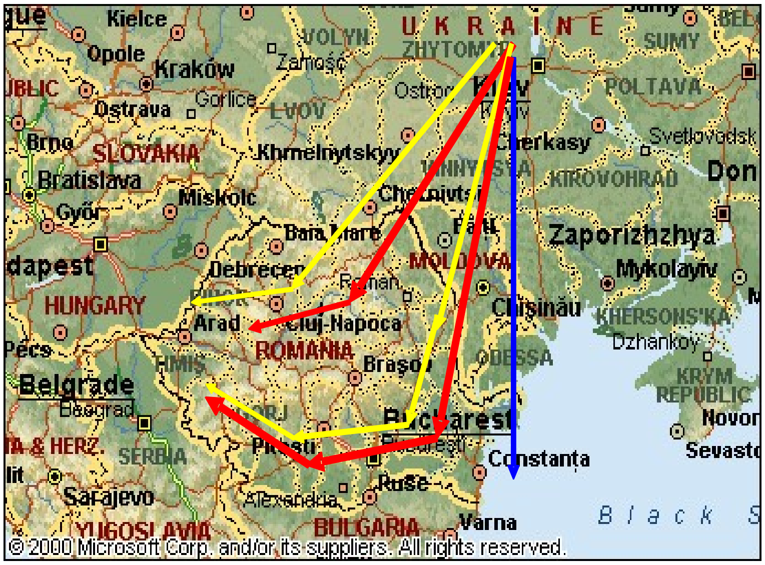
Kielce
Opole
Kraków
VOLYN
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SLOVAKIA
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Cherkasy
Svetlovodsk
Bratislava
Miskolc
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KIROVOHRAD
Don
Győr
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dapest
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Three stages of contamination in Romania: 🟥 1 – 2 🟨 3 – 4 🟦 5 – 6

Table 2: Content of Cs-137 (Bq/L or kg) in drinking water and foods [5, 6]

Food	**1986**	**1987**	**1988 - 1991**	2003
Water	-	0.05	0.002 – 0.009	0.002 – 0.009
Milk	10 - 200	27.92	1.02 – 5.13	0.01 – 0.137
Dairy products	10 - 500	61.98	5.07 – 16.61	-
Meet	50 - 300	47.50	1.31 – 39.25	0.014 – 10.28
Meet products	10 - 727	23.19	1.50 – 19.15	-
Grain products	50 - 100	42.16	1.01 – 12.15	0,251
Fruits and vegetables	7 - 411	46.43	1.89 – 23.39	0.037 – 0.265
Food menu/24 hours	-	23.69	1.50 – 4.49	-

If immediately after accident experts paid particular attention to radionuclides I-131 and Cs-137, easily determined by gamma spectrometry directly from samples, by the end of the year and in following years measurements were performed to determine the content of Sr-90 in water and food. Aerosols contamination levels with Sr-90 were less than 1/10 the levels of Cs-137 so the food contamination with Sr-90 was much lower, generally below 10 Bq/kg or L of product (Table 3).

Radionuclides Cs-137, Cs-134 and Sr-90, a little transferable from soil to plant, however they were detected many years after the accident; radioactive contents fell sharply in 1987 and then steadily, currently reaching in most foods well below 1 Bq/kg, hard to detect even with radiochemical analysis. There still are very low values for Cs-137 (units Bq/kg) in berries, mushrooms and wild venison.

Table 3: Content of Sr-90 (Bq/L or kg) in drinking water and foods [5, 6]

Food	1987	1988 - 1991	2003
Water	0.04	0.003 – 0.010	0.001 – 0.066
Milk	0.43	0.14 – 0.33	0.068 – 0.930
Dairy products	0.75	0.19 – 0.76	-
Meet	0.46	0.10 – 0.24	0.087 – 0.690
Meet products	0,62	0.09 – 0.20	-
Grain products	0.70	0.25 – 0.76	0.135 – 0.730
Fruits and vegetables	0.97	0.10 – 0.98	0.047 – 1.060
Food menu/24 hours	2.30	0.19 – 0.23	-

Assessment of human internal contamination with radionuclides I-131, Cs-137, Cs-134 and Sr-90 (Table 4) was performed both directly for the first three radionuclides (measuring radionuclides from humans) and by estimating the contamination based on the values of radioactive content determined in drinking water, food, and in real or hypothetical food menu (Table 2).

Table 4: Content of Cs-137, Cs-134 and Sr-90 in the human body (direct measurement/ estimation from ingestion of food) in Romania [6]

Radionuclide		1986	1987	1988	1989	1990	1991	1992	1993
Cs-137	measurement	1000 - 2000	2000 - 2500	800 - 1700	500 - 1100	300 - 500	< 300	< 300	< 300
	estimation	1538 - 20383	2335 - 12774	415 - 673	106 - 198	82 - 150	80 - 140	60 - 119	19 - 38
Cs-134	estimation	686 - 8703	729 - 4894	94 - 153	18 - 33	10 - 18	5 - 10	1 - 3	-
Sr-90	estimation	30 - 100	18 - 57	12 - 38	12 - 34	11 - 32	10 - 30	9 - 28	8 - 25

After about 80 days I-131 disappeared from humans and food through physical decay (if 10 Tf pass, it is considered that radionuclide decreases in values too difficult to determine; Tf for I-131 is 8.1 days). In terms of I-131 content found in thyroid, it presents very different values to a few thousand Bq only in May and June, depending on the level of contamination of the studied area and the food consumed. These rates were still much lower compared to human radioactivity/radiation dose received in the case of thyroid scintigraphy (tens - hundreds of kBq) [1,6].

For humans, Cs-137 + Cs-134, from about 2500 Bq in 1986 the values have gradually decreased to a point where after four years they went below 300 Bq. The loss of activity of the two radionuclides in humans was attributed to their reduction in food and to metabolic processes in the human body (Tb for the two radionuclides is 100-150 days) or by disintegration for Cs-134 (Tf = 2.05 years); so that after 1990 the Cs-137 values fell below the detection limit of the equipment used for the direct measurement. Sr-90 contamination for humans was much lower, estimated values below 100 Bq (radionuclide emits only beta radiation that can only be detected in small biological samples and the low contamination of foods did not allowed measuring this radionuclide in humans) [1,6].

ESTIMATION OF THE EXPOSURE DOSE FOR THE POPULATION OF ROMANIA

Immediately after the accident, Oncescu and Galeriu (IFA Bucharest – Măgurele) have calculated the exposure dose due to radioactive contamination of the population in our country which calculated to be about 1 mSv for the first year after the accident. Values are similar to those published by WHO (World's Health Organization) and IAEA (International Atomic Energy Agency) for other European countries (Finland: 0.44 mSv, Germany: 0.5 to 1.1 mSv, Italy: 0.61 mSv, Poland: 0.95 mSv, Switzerland: 1 3 mSv etc.) [1].

The subsequent calculation of the effective dose received by man from radionuclides determined in air, water and food for the most contaminated areas in Romania, except the contribution of I-131, resulted in average values close to 1 mSv in the first year after the accident, with a greater fall in 1988 (the greatest contribution to contamination was from Cs-137 and Cs-134 in food). Between 1990 and 2000 and now, the annual effective dose for humans fell below 50 µSv, values over 40 times lower than the dose received by humans from background radiation [4].

Figure 2: Exposure assessment of Romania's population – dose reconstruction by food based on Cs-137 concentration in atmospheric deposition [4, 5]. Minimum exposure dose of 0.2 mSv in Satu Mare County, maximum exposure dose of 2.1 mSv in Gorj County.

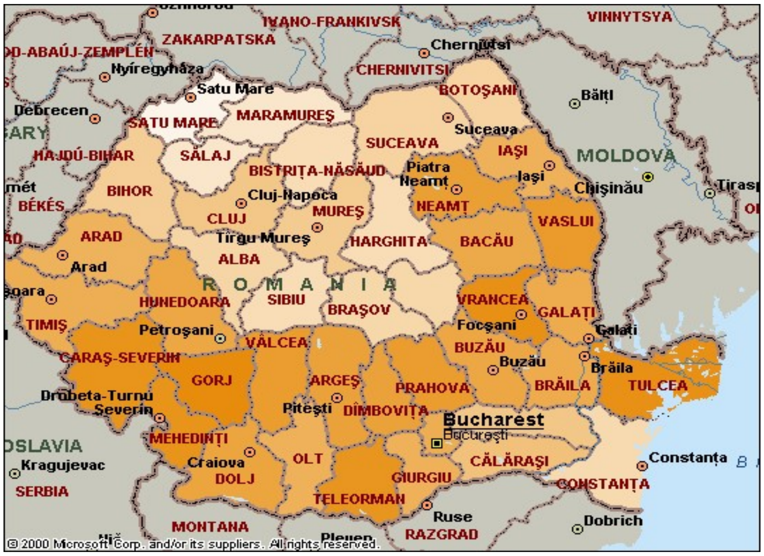
IVANO-FRANKIVSK
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D-ABAÚJ-ZEMPLÉN
Chernivtsi
Nyíregyháza
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Satu Mare
BOTOŞANI
Bălţi
Debrecen
SATU MARE
MARAMUREŞ
Suceava
SUCEAVA
HAJDÚ-BIHAR
SĂLAJ
IAŞI
MOLDOVA
BISTRIŢA-NĂSĂUD
Piatra Neamţ
Iaşi
Chişinău
Tiraspol
BIHOR
Cluj-Napoca
BÉKÉS
CLUJ
MUREŞ
NEAMŢ
VASLUI
ARAD
Tîrgu Mureş
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TIMIŞ
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Petroşani
Galati
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PRAHOVA
BRĂILA
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Drobeta-Turnu Severin
Piteşti
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SLAVIA
MEHEDINŢI
Kragujevac
Craiova
OLT
CĂLĂRAŞI
Constanţa
DOLJ
GIURGIU
CONSTANŢA
SERBIA
TELEORMAN
Ruse
Dobrich
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Pleven
RAZGRAD
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Doses of radiation exposure of the population in Romania after the nuclear accident vary by an order of magnitude between 0.2 and 2.1 mSv in the first year (Figure 2), based on contaminated air masses movement (Figure 1) as well as wet and dry deposition in our country. Thus, there were areas (counties) with quite small exposure doses, less than 0.5 mSv such as Satu Mare, Salaj, Maramures, Sibiu, Brasov, Harghita, while in other counties exposure doses were even over 1 mSv such as: Caras-Severin, Gorj, Mehedinți, Tulcea, Neamt, Vrancea, Teleorman.

The average dose received by most of the European countries of about 1 mSv is incomparably lower than that of more than 10 mSv received by population in heavily radioactive contaminated areas of Belarus, Ukraine and Russia. This dose of about 1 mSv in the first year after the accident is below the natural background exposure (irradiation) had a low radiological significance on health.

Natural background radiation exposure (cosmic radiation, earth radiation given by natural radionuclides present in the environment, food and humans) is about 2.4 mSv per year for the entire planet, with the exception of areas with high natural background radiation (even more than 20 mSv per year in some areas of India, Brazil, Congo, Sweden) due to the massive presence of natural radionuclides [1].

Thus, the additional exposure dose received by most of the population of Europe in 1986 was about half of the natural background exposure, which is a quite low dose, closely to effective dose limit (of 1 mSv per year) supported by legislation for an individual of the population. To be mentioned that the effective dose limit allowed for workers exposed for professional reasons (who work with ionizing radiation in the environment) is 20 mSv per year.

Given the levels of radioactive contamination of the environment in Romania, much lower in comparison with Ukraine, Belarus and Russia, the exposure doses received by our population were relatively low (0.2 to 2.1 mSv in 1986) well below those that could produce significant biological effects on human health. Moreover, studies carried out by the hygiene institutes of Romania did not reveal an increased incidence of congenital malformations in children born between 01.10.1986 and 31.12.1987 compared with the reference group (those born before 1986) [3].

Exposure doses and biological effects post-Chernobyl [2]

Average exposure doses received by the affected people in Ukraine following the nuclear accident were distributed as follows:
- 100 mSv, about 240 000 people participating in decommissioning the damaged reactor
- 30 mSv, about 116 000 people evacuated,
- 10 mSv, tens of thousands of people that continued living in contaminated areas, in the first years after the accident.

It is estimated that the maximum exposure dose can be even with an order of magnitude higher in some areas with massive radioactive contamination from the three countries Belarus, Russia and Ukraine.

In other European countries, the maximum dose was about 1 mSv in the first year after the accident, with a progressive decrease in the coming years. The dose is well below the natural background exposure (irradiation) has a low radiological significance.

Another 200,000 workers who received doses of 0.01 to 0.5 Sv were at increased risk of developing late effects, including cancer that could put their lives in danger.

Of the 600 workers that participated directly in the decommissioning, 134 received doses of 0.7 and 13.4 Sv and develop radiation sickness (ARS – Acute radiation syndrome). Of these, 30 died shortly after the accident [2].

In areas heavily contaminated in the three countries, measures taken to limit radiation exposure have changed the lives of millions of people (returning to homeland, modification of eating habits, restrictions and job changing) to damage health conditions including psychiatric problems.

Thyroid cancer was given particular attention, about 1800 cases for exposed children, which greatly exceeds estimates in the 1986. The cases of leukemia (latency 2-10 years) weren't significantly higher in staff directly involved in decommissioning. The greatest increase in cases of thyroid cancer for children has been reported in the Gomel region of Belarus (near Chernobyl) [7].

In the most contaminated areas of Russia, Belarus and Ukraine there are still values of Cs-137 and Sr-90 easily detectable in water and food; thus, in the Bryansk region (Russia), Cs-137 had values of 90 kBq/kg in predatory fish meat, high value for humans too, up to 50 kBq, which led to estimation of doses ranging up to 1.8 mSv in 1996. These values are well above those detected in Romania immediately after the accident [2].

Ever since 1987, a special monitoring program of about 7 million people affected by the accident has begun: liquidators of consequences of the accident (1986 - 1987), evacuated population in 1986 and established in other areas of the former USSR, residents of areas heavily contaminated with Cs -137, Sr-90 and Pu-239, descendants of the irradiated persons (liquidators, people evacuated), people contaminated with I-131, aged 0 to 18 years during the accident [7].

Epidemiological studies have revealed: an increase in the number of people with psychological problems, number of suicides and overall violent deaths among liquidators and evacuated population due to insufficient information on the effects of ionizing radiation, social dislocation and diminishing quality of life. Only complete epidemiological studies will halt the trend of attribution of increasing incidence of all cancers exclusively to the effects of the nuclear disaster, especially in areas heavily radioactive contaminated [8].

CONCLUSIONS FOR CONSEQUENCES OF THE CHERNOBYL ACCIDENT IN ROMANIA

1. The level of radioactive contamination of the environment in Romania after the nuclear accident in 1986 was relatively similar to that of other central and eastern European states; 10 kBq/m^2 at ground level, with few areas over this value.

2. The main radionuclide contaminants, I-131, Cs-137, Cs-134, even Sr-90 from deposition and aerosols immediately after the accident passed quickly in plants and animals. I-131 showed values close to 1 kBq/kg, Cs-137 and Cs-134 values of several hundred Bq/kg, whereas the Sr-90 was below 10 Bq/kg.

3. Radionuclides Cs-137, Cs-134 and Sr-90 transfer difficult from soil to plants trough absorption, were still detectable in food many years after the accident; radioactive contents fell sharply in 1987 and then steadily, reaching below 1 Bq/kg between 1990 and 2003 in most foods. At the same time, there were somewhat higher values detected in berries, mushrooms and venison.

4. In humans, Cs-137 from 1000 to 2000 Bq gradually decreased, so in 1990 reached about 300 Bq. The annual effective dose decreased from about 1 mSv in the first year after the accident, to below 50 µSv between 1990 and 2000, respectively below 10 µSv between 2000 and 2010.

5. Besides the three heavily radioactive contaminated states (Belarus, Ukraine and eastern Russia), other contaminated European countries reported no biological effects on the population after the accident.

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REVIEW ARTICLE

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Using direct anticoagulants (DOACs) in cancer and other high-risk populations

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Abstract: *The major practical advantage of the direct oral anticoagulants (DOACs), comprising the thrombin inhibitor dabigatran and the factor Xa inhibitors apixaban, edoxaban, and rivaroxaban, over vitamin K antagonists is their fixed dosing without the need for laboratory monitoring. With the recent, rapid introduction of the DOACs for the treatment of acute venous thromboembolism (VTE), clinicians are now faced with various questions regarding the efficacy and safety of these compounds overall and in specific high-risk populations. The collective evidence from 6 large clinical trials involving 27,000 patients has demonstrated that DOACs are as effective as vitamin K antagonists (VKA) in preventing recurrent VTE while being associated with a significantly lower risk of major bleeding. These findings are consistent in subgroups of patients with pulmonary embolism, the elderly, and those patients with a high body weight or moderate renal insufficiency, making these agents suitable for a broad spectrum of patients with VTE. DOACs are also an attractive treatment option in patients with VTE and concomitant cancer, thrombotic antiphospholipid syndrome, or heparin-induced thrombocytopenia, but the currently available clinical data is insufficient to make evidence-based recommendations on the use of DOACs in these settings. Several studies evaluating the efficacy and the safety of DOACs in these high-risk populations are underway.*

BACKGROUND

Anticoagulant treatment of thrombotic conditions dates back to the 1930s and 1940s when unfractionated heparin (UFH) and vitamin K antagonists (VKA) were the first drugs to become available. They proved to be effective in preventing death in patients with venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), and remained the mainstay of anticoagulant therapy in the decades thereafter. The introduction of low-molecular-weight heparin (LMWH) in the 1980s marked the next breakthrough. These drugs could be injected subcutaneously in fixed doses, which simplified the initial management of acute VTE greatly and opened up the opportunity to treat these patients at home. From that time on, a treatment regimen of initial LMWH followed by long-term VKA for at least 3 months was considered standard of care for patients with VTE.

Recently, within a time span of nearly 4 years, the thrombin inhibitor dabigatran [1] and the factor Xa inhibitors apixaban, edoxaban, and rivaroxaban [2,3] collectively termed direct oral anticoagulants (DOACs), were compared with conventional therapy for

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treatment of acute VTE in several large clinical trials. Now that these agents have been approved by the respective regulatory agencies around the world, physicians yet have another treatment option for their patients with VTE. This contemporary DOAC revolution has led to a quickly changing landscape of antithrombotic treatment. Clinicians are faced with various clinical questions regarding the efficacy and safety of these drugs overall and in subgroups of high-risk patients. Therefore, in the present review, we briefly discuss the collective evidence from the phase 3 clinical trials evaluating DOACs for VTE treatment. In addition, we will assess the potential role of DOACs in patients with cancer, the antiphospholipid syndrome (APS), or heparin-induced thrombocytopenia (HIT).

METHODS OF DOAC TRIALS IN THE SETTING OF ACUTE VTE

More than 27,000 patients were enrolled in several randomized clinical trials that compared the efficacy and safety of either one of the DOACs with VKA therapy targeted at INR of 2-3 in the treatment of acute, symptomatic DVT or PE. Importantly, the definitions of recurrent VTE and bleeding were similar across all studies and fallowed the recommendations of the International Society on Thrombosis and Hemostasis (ISTH). Outcome events in all trials were adjudicated by the same independent committee who was blinded for treatment allocation, resulting in a similar objective judgment of recurrent VTE and bleeding. Noteworthy differences between the trials are the use of an initial LMWH lead-in period of 5 days in patients receiving dabigatran or edoxaban, whereas apixaban and rivaroxaban where given as oral monotherapy, and the open-label design of the studies evaluating rivaroxaban opposed to the double-blinding in the other studies.

Recently, we reviewed and summarized the evidence from these phase 3 trials in a meta-analysis [4]. Using these data, we will answer clinically relevant questions to aid the physician in choosing the optimal treatment for his or her patient with VTE. Specifically, we will discuss the overall efficacy and safety of DOACs compared with VKA, differences between dabigatran and the factor Xa inhibitors, and the efficacy and safety of DOACs in high-risk subgroups of patients with PE, elderly patients, obese patients, and patients with moderate renal impairment.

CLINICAL QUESTIONS REGARDING THE EFFICACY AND SAFETY OF DOACS FOR VTE TREATMENT OVERALL AND IN HIGH-RISK SUBGROUPS

Clinical question 1: what is the overall efficacy and safety of DOACs in the treatment of acute symptomatic VTE compared to VKAs?

The main finding of the aforementioned meta-analysis was that DOACs are as effective as VKA in preventing recurrent VTE. Because all trials were designed to demonstrate non-inferiority of DOACs, this should reassure the clinician of the overall efficacy of these agents. Most importantly, the comparable efficacy observed in DOAC recipients was paralleled by a 40% relative reduction in major bleeding compared with VKA. Consistent with this substantial reduction in major bleeding, DOACs were associated with a significant 60% relative reduction in the incidences of intracranial and fatal bleeding, the most feared components of major bleeding. In line with the reductions in major bleeding, patients on DOACs had a 25% lower relative risk of experiencing clinically relevant non-major (CRNM) bleeding. Of note, no significant difference in the incidence of major gastrointestinal bleeding between DOACs and VKA was observed, unlike earlier reports raising concerns on an increased risk of gastrointestinal bleeding in patients on DOACs. In support of this finding, a recent population-based study confirmed a similar risk of gastrointestinal bleeding associated with DOACs compared with VKA in non-atrial fibrillation patients [5].

Clinical question 2: are there any differences between the factor Xa inhibitors and thrombin inhibitors with respect to efficacy and safety?

Although the result of the studies evaluating oral direct thrombin and factor Xa inhibitors are frequently combined, in fact they are two distinct drug classes with important pharmacokinetic and pharmacodynamic differences. Compared to oral factor Xa inhibitors, dabigatran has a lower bioavailability requiring administration as a prodrug, has a longer half-life, and is more dependent on renal clearance. Dabigatran is not hepatic metabolized by the P450 cytochrome system and could therefore be a treatment option in patients with liver disease. In contrast, factor Xa inhibitors undergo significant CYP3A4 metabolism and thus are contraindicated in patients with significant liver impairment. Of note, also among the factor Xa inhibitors there are some differences in pharmacologic properties. Clinicians should take these drug characteristics into account when choosing the DOAC that best suits their individual patient, but they also want to know whether there are any important differential effects between these two classes of DOACs in terms of efficacy and safety. Overall, these are no significant differences in efficacy and safety between dabigatran and factor Xa inhibitors. Hence, the choice for a specific DOAC should primarily be based on drug characteristics (eg, renal clearance), treatment regimen (eg, once vs twice daily dosing), and preference for a LMWH lead-in rather than the efficacy or safety profile.

Clinical question 3: can I prescribe DOACs also to high-risk patients including those with PE, body weight>100 kg, the elderly (ie, age≥75 years), or a moderate renal insufficiency?

The overall favorable efficacy and safety of DOACs in the setting of VTE compared with VKA is reassuring and justifies their use in the general population. However, not all clinicians are confident in prescribing these drugs in specific subgroups of high-risk patients. They may have questions regarding the efficacy of DOACs in patients presenting with PE, have concerns that patients with a high body weight will not reach sufficient drug blood levels putting them in risk of recurrent VTE, and fear that the risk of DOAC-related major bleeding complications may be higher in elderly patients, as well as in those with moderate renal insufficiency given the partial renal clearance of DOACs. The similar efficacy of DOACs compared to VKA is consistent in patients presenting with PE, patients with a body weight >100 kg, patients aged 75 years or older, and patients with a creatinine clearance between 30 and 50 mL/min. Moreover, the overall significant reduction in major bleeding associated with DOACs is maintained in the subgroups of elderly patients and patients with moderate renal insufficiency. Based on these results, DOACs should be considered to be an appropriate treatment option for acute VTE in a broad spectrum of patients.

OTHER HIGH-RISK POPULATIONS WITH VTE

Clinical question 4: are DOACs suited for my cancer patients with VTE?

It is estimated that 20% of all VTE are related to cancer and anticoagulant treatment in these vulnerable patients is often challenging. Cancer patients with VTE are at increased risk of developing recurrent VTE compared to non-cancer patients, but also have a higher risk of major bleeding. LMWH is currently recommended over VKA for treatment of cancer-associated VTE due to its better efficacy in preventing recurrent VTE with a similar risk of bleeding compared to VKA.[6] However, LMWH therapy may be burdensome, particularly in cancer patients that often require indefinite anticoagulant treatment. Because DOACs offer a simple oral treatment regimen without the need for anticoagulant control, they could be an attractive alternative. However, at present, clinicians should refrain from providing these drugs to cancer patients since they have not been compared directly to LMWH. Other concerns are possible drug-drug interactions with antineoplastic agents, in particular P-glycoprotein inhibitors, and the gastrointestinal absorption of DOACs in vomiting cancer patients or those with chemotherapy-induced intestinal mucosal defects. Nevertheless, the subgroup analysis of the 1,500 cancer patients enrolled in the DOAC trials does provide guidance for those situations when LMWH is not an option, for instance when patients are unable or unwilling to take daily subcutaneous injections. Interestingly, in these patients, a possibly better efficacy of DOACs compared with VKAs without an increased risk of major bleeding was observed.[4] It should however be realized that cancer patients in the trials probably had a favorable cancer prognosis compared to the real-world cancer population. Patients with a limited life expectancy were excluded from the trials, as were patients in whom long-term VTE treatment with LMWH was anticipated.

Recently, the pooled results of the subgroup of cancer patients enrolled in the trials comparing dabigatran with VKA was reported providing information on the characteristics of these patients as well as on cancer-prognostic data.[7] Active cancer was defined as cancer within 5 years prior to the VTE diagnosis. Compared with non-cancer patients, the 221 patients were older and had a lower renal clearance. The time spent within the therapeutic INR range of 2-3 was comparable between VKA-treated cancer and non-cancer patients (54.5% and 58.3%, respectively) which is higher than in the CLOT17 (46%) and CATCH (47%) trials. The most prevalent tumor types were prostate, colorectal, and breast cancer and 13% had metastasis. During 6 months follow-up, no significant differences were observed between dabigatran and VKA with respect to recurrent VTE or major bleeding. Detailed results of the cancer subgroups from the studies evaluating apixaban and edoxaban are expected shortly.

Before DOACs become an accepted treatment option for cancer-associated VTE, they have to be evaluated in a head-to-head comparison with LMWH. To this end, an important study comparing the efficacy and safety of edoxaban to LMWH in the setting of cancer-associated VTE has recently been started. One thousand patients will be randomized and followed for the occurrence of one of the components of the combined primary outcome of recurrent VTE or major bleeding. Following observations of a similar risk of recurrent VTE in patients with active cancer and those with a history of cancer, patients with VTE will be allowed to enter the study wither with active cancer or cancer treated within 2 years prior to the VTE diagnosis. Moreover, incidentally detected lower extremity DVT or PE are allowed as an entry diagnosis because treatment and outcome is similar for this group of patients. A last innovative feature of the study is the follow-up time of 12 months, which will provide valuable data regarding the treatment of patients with VTE and cancer beyond 6 months.

In conclusion, DOACs may become an attractive treatment for cancer-associated VTE, but at present LMWH remains the preferred option. Although the results of the trial population with cancer are encouraging, further clinical studies are needed to evaluate the efficacy and safety of DOACs directly against LMWH in this population.

Clinical question 5: can I treat my patients with thrombosis and the antiphospholipid syndrome with a DOAC?

APS is an autoimmune disorder characterized by arterial or venous thrombosis or pregnancy complications in patients who repeatedly test positive for circulating antiphospholipid antibodies (aPLA), i.e. lupus anticoagulant, anti-β2-glycoprotein-1 antibodies, or anti-cardiolipin antibodies. It is estimated that 10% of all patients with VTE have circulating aPLA and these patients are at increased risk of recurrent VTE compared to VTE patients without aPLA. When patients test positive for all three types of aPLA, the risk of on-treatment recurrence is as high as 25% over 5 years. The treatment of thrombotic APS is similar to the treatment of VTE in the general population (VKA targeted at an INR of 2 to 3), but is often extended indefinitely in case of persisting aPLA.

Now that DOACs are increasingly used for treatment of VTE, the question arises whether drugs are effective and safe for VTE treatment in the setting of APS too. From a patient perspective, DOACs may be a significant amelioration in this usually young population for whom lifelong anticoagulation with VKAs and frequent INR monitoring is not attractive [8]. Given that aPLA are prevalent in patients with VTE, the aforementioned DOAC trials in the setting of VTE must have enrolled patients with APS because this was not an exclusion criterion. However, patients were not routinely tested for aPLA and outcomes for the patients with known aPLA have not been reported. Therefore, the current knowledge of the efficacy and safety of DOACs for thrombotic APS is exclusively derived from case series and retrospective cohort studies. These represent a select population of thrombotic APS patients that were switched to a DOAC often because of labile INRs, bleeding on VKA, or logistic problems with INR monitoring.

In a retrospective multicenter cohort study, Noel et al [9] enrolled 26 DOAC-treated patients who had thrombotic APS for a median duration of 3 years. Seven patients had tested positive for all 3 types of aPLA and another 12 patients had lupus anticoagulant. Six of 26 patients received rivaroxaban or dabigatran as first-line treatment, whereas the others had been treated previously with another anticoagulant for 1 to 23 years. During a median follow-up of 1.5 years, 1 patient (3.8%) developed recurrent VTE and in 2 patients (7.7%) a bleeding event occurred that led to discontinuation of DOAC therapy.

However, other authors raised concerns about the effectiveness of DOACs to prevent recurrent APS-related VTE. Win and Rodgers [10] presented 3 thrombotic APS patients developing recurrent thrombosis 6-12 months following initiation of second-line DOAC treatment. The cause of these observations of DOAC-failures is unknown. The recurrence rate in APS patients is high and these failures may have otherwise developed under VKA therapy. Moreover, compliance may have been a contributing factor in these patients who were often switched to a DOAC due to difficulties with their INR control. Alternative explanations may be mechanisms intrinsically related to DOACs or their dosing regimens. For instance, inhibition of a single clotting factor might be insufficient to completely block the thrombotic potential in severe APS patients, or the short-life of DOACs causes the procoagulant stimulus to overcome the anticoagulant effect at trough levels.

In summary, current anecdotal evidence on the use of DOAC in thrombotic APS patients is conflicting. As a consequence, physicians should be careful with prescribing DOACs in these patients until robust data become available.

Clinical question 6: are DOACs a treatment option for preventing or treating thrombosis in patients with heparin-induced thrombocytopenia?

HIT is a complication of treatment with UFH or, less commonly, LMWH and is characterized by formation of antibodies to heparin-platelet factor 4 (PF4) complexes. These antibodies may trigger platelet activation in turn resulting in venous or arterial thrombosis, so-called HIT associated with thrombosis (HTT). A decrease in platelet counts is usually observed 5-10 days after initiation of heparin exposure. In patients with confirmed HIT, cessation of heparin therapy alone is not sufficient to prevent HITT and treatment with an alternative anticoagulant is required. Traditionally, 2 classes of anticoagulants have been used for the initial treatment of HIT: parenteral direct thrombin inhibitors, including argatroban, bivalirudin, and desirudin, and nonheparin antithrombin-dependent anticoagulants, including fondaparinux and danaparoid. Although these drugs are widely used in the treatment of HIT, they have several disadvantages ranging from high costs, low availability, need for drug monitoring, dependence on renal clearance, and the absence of an antidote. DOACs could potentially overcome all of these issues. The biochemical structure of DOACs has no similarities with heparin; hence, interactions with PF4 or heparin-PF4 antibodies are not to be expected. This has been confirmed in preclinical laboratory studies that demonstrated that rivaroxaban, dabigatran, and apixaban [13] do not cross-react with HIT antibodies and do not induce platelet activation in the presence of HIT antibodies. Hence, on theoretical grounds, these drugs could be used to treat HIT. These observations suggest that DOAC are a safe alternative to traditional HIT treatment, but confirmatory studies are needed.

In summary, the clinical experience with DOACs for HIT is currently limited, but they have the potential to become the mainstay of anticoagulant treatment in this setting. They do not interact with HIT antibodies and do not cause platelet activation in the presence of HIT antibodies. Together with their favorable pharmacokinetics, high availability, and lower costs, they could be particularly suited for patients with HIT. The results of the ongoing clinical study have to be awaited before the evidence-based use of DOACs in patients with HIT can be recommended.

CONCLUSIONS

With the recent introductions of the DOACs, clinicians now have multiple therapeutic options for oral treatment of patients with VTE. DOACs are as effective as VKA in preventing recurrent VTE, but are associated with a significant reduction in the risk of major bleeding and are easier to use with no need for dose adaptations. These findings in the general trial population are consistent with those in patients with PE, the elderly patients, heavy patients, and patients with moderate renal impairment, making DOACs a suitable treatment option for a broad spectrum of patients. Whether DOACs can also safely be used in patients with cancer-associated VTE, thrombotic APS, or HIT is currently not proven. Studies are underway to evaluate DOACs in these high-risk populations.

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SYSTEMATIC REVIEW

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Risk factors and quality of life in late-life depressive disorders

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Abstract: *Late-life major depression is a high-incidence and difficult-to-treat affective disorder. Diagnosis of major depression in old age could also could be a challenge, due to aspects like (1) there is a higher vulnerability to the stigma of depression in this population, (2) hypochondriac ideation and somatic symptoms are the main symptoms, while depressive disposition or anhedonia are under-reported by such patients, (3) differential diagnosis includes various organic diseases, but also several psychiatric disorders, like neurocognitive disorders, organic affective disorders, drug induced affective disorders etc. Reduction of social relationships caused by retirement and loss of spouse and friends, as well as a decrease of personal income could precipitate or maintain depressive disorders during late-life. Quality of life in patients diagnosed with depressive major disorders is a rarely monitored parameter, although its importance for case management could not be overemphasized. A reduction of life quality correlates with a poorer functional prognosis, persistence of neglected residual symptoms, low adherence to treatment plan etc.*

Keywords: *late-life depression, geriatric depression, quality of life, risk factors, institutionalized population*

BACKGROUND

Late-life depressive disorders have a high impact over patients' quality of life, general well-being, risk of organic diseases recurrence, involvement in daily activities and life expectancy.

Although depression could be perceived as a part of normal process of ageing, this is only a relatively widespread misconception. Adjustment to changes in life style due to retirement, loss of the spouse, death of close friends, financial losses, decreasing of physical capacities etc, should not be confounded with major depressive disorder. Depression has been identified in 17-37% of elderly patients treated in primary care facilities, and 30% of these have a diagnosis of major depressive disorder [1].

Elderly patients are more sensitive to stigma associated with depression and this is a reason for them to declare less frequently than young adults that they have dispositional symptoms during epidemiological studies [2]. Therefore this kind of data should be regarded with caution, as an underestimation of the depression real incidence in late-life is very possible.

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Table 1: Risk factors for depressive disorders in late-life population

Risk factor	Overall impact
Bereavement after loss of spouse, a child or other close relatives	Bereavement after a recent loss correlates with depressive symptoms in elderly [5] Elderly bereaved patients seek frequently medical help for their emotional discomfort [6] Most of the depressive symptoms are reported by mothers who have lost a child after a prolonged suffering and in fathers who lost a child by suicide; it's possible that old adults have a lower risk of depression caused by the death of a child than younger adults, possible due to better coping mechanisms [7]
Retirement	People who retired recently have a higher risk for depression than those who continued to work, although self-esteem scores didn't change significantly, as reported by a prospective evaluation during 2 years [8] Depressive symptoms increase when the retirement is sudden and perceived by the subject as being too early or forced [9] There is a reciprocal relationship, as patients diagnosed with depression tend to retire with 1.5 years earlier than those without depression [10]
Income decrease	Women who have lost their spouse have as main mediator of depression financial burden, while widowed males have mainly problems with house holding activities [11]
Interpersonal relationships decreasing	Loneliness was the only factor which correlated with depression scores in 8% of people aged 60-98 who were institutionalized, thus suggesting that loneliness is an independent risk factor for major depression [5]
Lower physical capacity	Old adults with sedentary lifestyle and physical disabilities had a higher risk (OR=2.44) for depressive symptoms appearance than those with good mobility and that were physically active, according to a 8 years follow-up study; the risk maintained high (OR=1.99) in those with low mobility but active life-style, when compared to those who were both physically active and have an intact mobility [12]
Somatic symptoms, pain included	Presence of several organic diseases predicts late-life depression [5] Organic pathologies which associate important pain have a risk for developing more depressive symptoms and a worsening of pre-existing depressive manifestations [13] Quality of life is decreased more in old adults with symptoms like physical pain, because this is associated with limitations in functionality, fatigue, sleep disturbances, depressive mood [14]
Physical or psychological symptoms of patient's spouse	There is a high correlation between depressive symptoms in both spouses: if one has depressive manifestations, there is a high risk for the other to have the same type of symptoms, and this correlation increases in frequency with the patients' age; age difference between spouses has been associated with more depressive symptoms, and those with younger spouses reported more depressive symptoms in 65+ age than spouses with minor age differences [15]
Psychological symptoms, including decrease in cognitive function	Elderly patients (60+) with major depression who have persistent insomnia have a 1.8-3.5 times higher probability to maintain depressive symptoms than those who doesn't have insomnia [16] Depression appears frequently in almost every type of neurocognitive disorder, even in mild cognitive disorder (MCI), and the risk for depression to increase the risk of MCI conversion to dementia is investigated [17] Late-life depression leads to further functional and cognitive decline [18]
Decrease autonomic functioning	Mobility deficits predict a higher risk of mortality and loss of autonomy in elderly patients [14]
Relocation in other area (from city to rural areas, or from his/her own residence into a long-term care facility)	Institutionalization, lower frequency of visits from friends or relatives and less involvement in social activities have been associated with greater risk for late-life depression [5] In institutionalized patients, depression is more common in women who were widow/single, younger or much older than the mean group age, with lower educational background, poor social and economic status, who live alone [20].

Detecting vulnerable populations for depression disorders is an important process if we look at the risk of early death from natural and unnatural causes in patients diagnosed with depressive disorder (71.1 vs. 75.9 years old) and loss of potentially years of life (13.4 vs. 10.2 years) comparative to the non-depressed population [3].

Risk factors for development of depressive disorder in late-life are intricate, with genetic vulnerability, cognitive diathesis, age-associated neurobiological changes, and stressful events, while protective factors are higher education, high socioeconomic status, and involvement in desired activities, inclusive of religious and spiritual nature [4].

Clinical manifestations of major depression in elderly patients has several peculiarities, like denial or dissimulation of feeling sad or anhedonia, but with predominance of somatic complaints, irritability and anxiety, self-isolation, low level of energy and slow movements.

OBJECTIVE

To assess the specific risk factors for late-life depression and to search for impact of this pathology over the patients quality of life, by analyzing data from literature.

Table 2: Quality of life in late-life depressive disorder

Lin J-H, Huang M-W, Wang D-W et al. [21]	Depressed elderly inpatients have less satisfaction and quality of life; female gender, duration of hospital stay, and in-hospital rehabilitation correlated significantly with quality of life scores.
Doraiswamy PM, Khan ZM, Donahue RM, Richard NE [22]	Late-life depression associates significant quality of life impairments in 5 out of 8 baseline SF-36 items; women rated their quality of life as worse than men on physical functioning and role physical; subjects aged over 70 reported lower quality of life on the summary physical component and a trend for higher scores on the mental component of the SF-36
Dombrovsky AY, Lenze EJ, Dew MA et al. [23]	All domains of health-related quality of life except physical functioning improved with successful acute and continuation treatment using paroxetine and/or monthly interpersonal psychotherapy
Hunkeler EM, Katon W, Tang L et al [24]	Collaborative care intervention (including depression care manager, primary care doctor, psychiatrist offering education, behavioral activation, antidepressants, a brief behavior based psychotherapy, relapse prevention geared to personal needs and preferences) was superior to usual care for depression in elderly patients (60+) diagnosed with major depressive disorder regarding quality of life, self-efficacy, satisfaction with care, remission of depression after 12 months
Heiligenstein JH, Ware JE Jr, Beusterien KM et al [25]	Treatment with fluoxetine for 6 weeks in patients over age 60, diagnosed with major depressive disorder, had improvements vs. placebo in all dimensions of SF-36 health status survey; before treatment these patients had lower values in mental health, role limitations due to emotional problems, social functioning, vitality, role limitations due to physical problems, bodily pain- as dimensions of quality of life and health-related dimensions.

METHODS

Main electronic databases (PubMED, EMBASE, CINAHL, Cochrane) were searched using as keywords „old age depression”, „late life depression”, „geriatric depression”, combined with „clinical dimensions”, „quality of life”, „risk factors”, „prognosis”, „evolution”.

All articles were analyzed, including clinical trials, systematic reviews, meta-analyses, case reports, case series, guidelines and editorials.

We formulated the following inclusion criteria for the selected papers: population age over 60; diagnosis of major depressive disorder, organic depression or mixed anxiety-depressive disorder; institutionalized or community dwelling residents; specified statistical method for data processing; risk factors and/or quality of life as specified variables.

Exclusion criteria were: unspecified age of studied population; age under 60; diagnosis of drug induced depressive disorders, or bipolar disorder- major depressive episodes.

RESULTS

According to the analysis of the data from literature, main risk factors for depressive disorders in elderly are illustrated in table 1. Each factor has a different impact over the onset or evolution of depressive symptoms, but these factors appear seldom isolated, therefore it is hard to quantify each variable's impact.

Patients diagnosed with depressive disorders in late-life have various risk factors for this affective disorder, like social events (d.e. retirement, lack of social interaction), psychologic aspects (d.e. bereavement, cognitive deficits) and organic diseases (d.e. somatic pathologies, decreasing of physical mobility). Therefore, it's important to configure a management plan that includes all these factors and adequate monitoring methods, in order to improve depressive symptoms severity, prognosis and risk of relapse.

Regarding the quality of life in depressed elderly patients, we found several studies which are presented in table 2. All the cited trials are randomized and included quality of life and/or health-related quantitative measurements as main or secondary variables.

Although only a small number of trials dedicated to evaluation of life quality in depressed elderly patients was found, we can conclude from the available data that (1) a significant correlation between depression and quality of life decrease was found; (2) treatment, either psychotherapy or pharmacotherapy, could help in resolving both depressive symptoms and alterations in quality of life; (3) several risk factors like female gender, duration of hospital stay, and in-hospital rehabilitation correlated significantly with quality of life scores.

CONCLUSIONS

There are many risk factors that could be correlated with the onset of a new depressive disorder in old age, or with a relapse or recurrence of a previously diagnosed major depression. Some of these risk factors could be corrected and thus patient's prognosis could be improved (d.e. symptoms related to organic comorbidities, like pain, dizziness, or medical aids for visual or hearing deficits), while others could be only monitored and assisted through counseling (d.e. reduced immobility due to untreatable or only partially treatable conditions, lower physical capacity or bereavement).

Quality of life is an important predictor of prognosis in depressive disorders and monitoring the patients' well-being should be more frequently recommended during psychopharmacologic or psychotherapeutic treatment in elderly population. An increased attention should be placed in patients with depressive disorders and risk factors for lower quality of life, like female gender or long duration of hospitalization.

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ORIGINAL ARTICLES

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Modern medicine has a new technology: Therapeutic electroporation

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Abstract: *Electroporation is considered a new start-up in the treatment of various tumors; currently, researches are being conducted in order to develop this technology with medical applications. The technique consists in the significant increase in the electrical conductivity and permeability of the plasma membrane of cells resulting from the application of an external electric field. It is routinely used in molecular biology to transform bacteria, yeast, protoplasts and is performed using the electroporators. Currently, the process seems to be a real solution that enables a targeted drug to act with maximum efficiency on cells and tissues requiring treatment, resulting in obtaining a good therapeutic effect without major side effects. Therefore, pharmaceutical companies are trying to demonstrate through preclinical studies the potential efficacy of this technology, succeeding in recent years to achieve important steps in this direction.*

Keywords: *electroporation, therapy, tumor, medical application*

INTRODUCTION

Modern medicine is always looking for new technologies applicable to the diagnosis, prevention and treatment of diseases. Among these, the applications of molecular biology represent a direction of the future, particularly in the treatment of diseases currently considered incurable. Translational medicine applies the new discoveries, in molecular and genetic biology, as well as the techniques resulted from these, for therapeutics based on biology, which gradually replaces therapeutic based on chemistry. For example, by applying electric current at cell level, can be inserted into altered cells, the therapeutic molecules or transfection of genetic material can be achieved.

ELECTROPORATION

Electroporation is the electropermeabilization of cell membranes, which consists in increasing the electrical conductivity and permeability of the plasma membrane of cells, being caused by the application of an external electric field. This procedure is particularly useful in molecular biology in order to insert certain substances in a cell such as a molecular probe or a drug that can induce changes in cell functions or coding DNA [1].

Electroporation is a dynamic process that depends on local transmembrane voltage for each point at cell membrane level. For a certain type and duration of the pulse, there is a threshold of the transmembrane voltage in order to allow the expression of transmembrane electroporation phenomenon between 0.5 V and 1 V. This leads to defining threshold value of the magnitude of the electric field necessary to achieve the phenomenon of electroporation. Only the cells found in an electric field greater than the *threshold* value can be electroporated. If a second threshold is reached or exceeded, electroporation will compromise the viability of the cells, this phenomenon being called *irreversible electroporation* [2].

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In molecular biology, the process of electroporation can be used to transform prokaryotic and eukaryotic cells. Bacteria that present a cell wall composed of peptidoglycans for protection, resistance and transport of substances are protected from environmental changes. Based on this characteristic, bringing the bacteria into contact with particular plasmids of interest may have the effect of transferring the plasmid to other bacterial cells, subsequent to an electroporation process by applying several hundred volts over a distance of a few millimeters. Subsequently, the cells must be handled carefully until they divide into new cells that contain new plasmids; this process is about ten times more efficient than chemical transformation [3].

This process is also highly efficient for the insertion of foreign genes in cell cultures and in particular in mammalian cell lines. For example, the process is used to produce *knock-out* mice (transgenic mice in which is inactivated a single target gene for the study of phenotypic characteristics encoded by this) as well as in tumor treatment, gene therapy, and cell-based therapy. The process for the insertion of foreign DNA molecules into eukaryotic cells is known as transfection. Electroporation is particularly effective for transfecting cells in suspension in a cultivation medium using an electroporation cuvette.

Electroporation has proven effective for use on *in vivo* tissues for *in utero* or *in ovo* transfection applications. The adherent cells to the substrate can also be transfected using electroporation, thereby providing an alternative to researchers for the trypsinization of the cells prior to transfection. Electroporation is performed using electroporators, purpose-built appliances which creates an electromagnetic field in the cell suspension, which is pipetted into a glass or plastic cuvette foreseen with two aluminum electrodes on its sides. For the electroporation of bacterial cells, a suspension of about 50 µl is used which, prior to electroporation, is brought into contact with the plasmid of interest. The mixture is pipetted in the cuvette, the voltage and the volume to be applied to the probe are set, and the cuvette is placed in the electroporator. Immediately after electroporation, 1 ml of liquid medium is added to the Eppendorf cuvette /tube and is incubated at a temperature which allows the multiplication of bacteria, to recover the cells and to express **antibiotic resistance**, followed by seeding on plates with nutrient agar.

For electroporation to be successful, the plasmid must be "*free of all salt*" because solutions with high concentration of sodium chloride can cause an electrical discharge (known as arcing), a phenomenon which often reduces cell viability.

For a more detailed investigation of the process, special attention should be given to the output impedance of the porator device and to the input impedance of the cell suspension (e.g. the salt content). Because the process requires direct electrical contact between the electrodes and the suspension, and it does not occur between isolated electrodes, it is obvious that the process requires the existence of electrolytic effects due to mild currents and electric fields [4].

Electroporators are special laboratory equipment that give the possibility to achieve electroporation for multiple samples at the same time, with sets of electrodes specific for cell cultures, such as X2HT Gemini system manufactured by BTX-Harvard. Electroporators can be set to different operating parameters, allowing researchers to optimize the electric field strength depending on the cell type and by the presence or absence of the cell wall [5].

Electroporators have been used on a wide range of cells, including *Escherichia coli* (for transformation) and mammalian cells (neurons, astrocytes, neuroglial, lymphocytes, monocytes, fibroblasts, epithelial and endothelial cells) from humans, mice, rats, and monkeys (for transfection [6].

Physical mechanisms of electroporation

Electroporation allows inserting into the cell DNA molecules, which do not have the ability to passively cross the cell membrane (the lipid hydrophobic bilayer). There are several ways in which changes can be caused to the electric field at the cell membrane level with very different mechanisms and effects. The electroporation advantage lies in the fact that lipid molecules are not altered in terms of chemical and the membrane has the ability to form a pore that will serve as a conductive path through the lipid bilayer [4].

Electroporation is a process with many stages and distinct phases [7]. Initially, a brief electrical pulse of 300-400 mV must be applied for less than 1 ms, which causes changes at membrane level related to the migration of ions in the surrounding solution. Once the critical stage is reached, a local rearrangement on the morphology of the lipids rapidly occurs and the resulting structure is considered to be a "pre-pore" because it is not a good conductor, but quickly leads to the formation of a conductive pore. Most evidence in support of this idea, come from the "flickering" process that occurs at the level of the pre-pores with the formation of hydrophobic regions of approximately 3Å [8].

If this theory is correct, the transition to the conductive pore may be explained by a rearrangement at the edge of the pores in which the lipid ends create a hydrophilic interface.

Finally, the conductive pores may suffer processes of reorganization, expansion or rupture, which can be influenced by various local factors such as the place and the moment in which the change occurs, the mechanical stress and the energy of the lipid bilayer existing at the time of trial [9].

MEDICAL APPLICATIONS

The optimization of electroporation is difficult to achieve, but it has been demonstrated that high voltage electroporation can almost irreversibly destroy the target cells, neighboring cells remaining unaffected. Thus, electroporation can form a new therapeutic approach in the treatment of cancer, cardiac disease and other pathological conditions which require the excision of tissue [10].

A recent technique called **non-thermal irreversible electroporation** has proved successful in the treatment of various types of tumors. This procedure involves the use of small electrodes (about 1 mm in diameter), placed inside or around the target tissue for applying short electrical pulses, repeating at a predetermined frequency and voltage, which increases resting transmembrane potential. As a result, when the applied electricity is higher than the threshold value for the target tissue, forming nanopores in the plasma membrane can become permanent and the cell repair mechanisms of "immortal" cancer cells become ineffective and the cells die.

The procedure is unique, due to the fact that compared to other tumor ablation techniques it does not affect the healthy tissue surrounding the tumor. In contrast, **reversible electroporation** occurs when electricity is applied through the electrodes below the threshold level of the electric field of the target tissue. Applying an electrical energy below the threshold allows the cells to rebuild the phospholipid bilayer and to resume normal operation. Reversible electroporation is carried out in the normal way for the administration of treatments which involve the insertion of some therapeutic molecules (drugs, genes, etc.) into cells. Since not all tissues have the same threshold of the electric field, precise calculations are needed before starting treatment, to ensure operating efficiency.

A major advantage of using non-thermal irreversible electroporation is that when done correctly, as a result of careful calculations made, it affects only the target tissue, while the proteins, the extracellular matrix and some critical structures such as blood vessels and nerves remain unaffected by this treatment. This allows a faster recovery and enables a faster replacement of the necrotic tumor cells with healthy cells. The first successful treatment of malignant skin tumors in mice was conducted in 2007 by a group of scientists (Garcia, Paulo A. & al.), who conducted the complete ablation of the tumor in 12 of 13 mice. They accomplished this by sending 80 pulses of 100 µsec to 0.3 Hz with an amplitude of the electric field of 2500 V / cm, to treat skin tumors. Prior to performing the procedure, the therapist must carefully calculate exactly what should be done and to customize the therapy to the patient needs using imaging (CT and MRI) to create an accurate three-dimensional image of the tumor. This information is very important because one can estimate the tumor volume and decide on the type of therapy, place and angle of insertion of electrodes and applied voltage, sometimes even during the procedure, especially when electroporation is used to treat brain tumors.

The whole procedure is quick (about 5 minutes); the success rate is high and therefore very promising for future treatment extrapolation to humans. A disadvantage would be that the released electricity can stimulate muscle cells causing their contraction, which in some cases can have serious or even fatal consequences. Therefore, a muscle relaxant agent (curare-like compounds) should be used while performing the procedure. The researches conducted so far have been successful, but the taken action must be subject to the existence of a risk when general anesthesia is being used. A recent technique called **irreversible high-frequency electroporation**, uses electrodes to apply bipolar electrical pulses at a high frequency, as opposed to low frequency monopolar electrical pulses. This type of procedure also leads to tumor ablation with the difference that it does not produce muscle contraction and therefore does not need a muscle relaxant agent [11].

Researchers have had success in treating the test animals with multiple tumor types. Even if you are not prepared to routinely apply this technique in the treatment of tumors in humans, non-thermal irreversible electroporation is used in practice for treating cutaneous and subcutaneous tumors. There have also been attempts to treat human cancer of the prostate, lung, kidney and liver. There are still many things to be set in place, on how to damage the animals and especially the man. Kenneth Thompson et al. tested the safety of applying this technique on humans who had tumors in their lungs, kidneys and liver. The treatment was found effective in 71% of cases and, respectively, in 49 patients out of the 69 cases of patients treated was achieved complete ablation of the tumor [12]. The highest rate of success was recorded in liver tumors. The result of this study thus provides encouraging evidence related to the future use of this technology as an alternative method of treating cancer in humans.

Electroporation can also be used for the insertion of drugs or genes into the target cell through the use of short, intense electrical pulses to transiently permeabilize the cell membrane. This procedure is referred to as **electrochemotherapy** when the molecules to be transported are represented by a chemotherapeutic agent or **gene electrotransfer** when the molecule to be transported is the genetic material (DNA) [4].

PHARMACEUTICAL APPLICATIONS

Some pharmaceutical companies have performed surveys on the development of electroporation procedures with the scope of providing a viable alternative to cellular uptake of drugs. This approach is based on the fact that human cells do not allow the free entry of foreign matter through the cell membrane, which results in delay in the operational efficiency of a drug. Therefore, electroporation can be a real solution to allow a drug to act directly, quickly and with maximum efficiency on cells and tissue requiring treatment, resulting in obtaining a good therapeutic effect without major side effects.

Therefore, Inovio and OncoSec tested the effectiveness of this technology and managed in 2013 to achieve important steps in this direction. The results allowed them to also achieve substantial gains: the company Inovio Pharmaceuticals (NYSEMKT: INO had gains of almost 500% and the company OncoSec Medical had gains of 150%. Inovio performs studies using synthetic vaccines, while OncoSec is testing therapeutic agents that are already used during cancer treatment. The company Inovio Pharmaceuticals has developed about 12 "*electroporation pipes*", for the treatment of cancer and infectious diseases. Thus, the tests performed on the H1N1 influenza vaccine developed by the company's specialists have highlighted the protective immune response comparable to conventional vaccines. Also, the SynCon DNA vaccine caused total protection against the infection with the viruses of Ebola and Marburg hemorrhagic fevers [13].

Preliminary studies for both types of vaccine, although in the initial stage, show that these exhibit a strong effect against the spread of these deadly viruses. On June 14th, 2014, Inovio announced that the H7N9 vaccine generated protective antibody HA with a percentage of 100% of the animals tested, and on July 10th, the results obtained from testing the HIV vaccine were published in the Journal of Infectious Diseases. Inovio found that the CELLECTRA system used for administering the Pennvax - B HIV vaccine improved the efficacy of the medicine in the first phase of testing. On July 18th, 2014 Inovio announced that electroporation significantly improves the outcomes of the DNA therapy in terms of stimulating the multiplication of blood elements. Administering with the CELLECTRA system the hTERT DNA vaccine used in cancer therapy and the anti-MERS vaccine has demonstrated that a good immune response has been generated during preclinical studies, improving the survival of patients [14]. OncoSec Medical conducted in preclinical testing centers studies for the treatment of metastatic melanoma, Merkel cell carcinoma (MCC) and cutaneous T cell lymphoma. In the study were included 15 patients with MCC, and so far the company’s electroporation platform has been shown to be safe; in the last check, all patients showed an increase of IL-12 absorbing of at least 100 times, and in some cases up to 1,000 times.

This provides hope in regards to reducing the side effects that occur subsequent to immunotherapy. Also, studies have been conducted on a group of 25 melanoma patients. Were reported complete records for 21 of them and was observed that 38.1% had a favorable complete response lasting more than 6 months and in 61.1% of patients was observed a 30% regression of the tumor. All this led to an increase in the company’s market share by 63%. It is considered that the near future will bring a series of novelties in this field, such as:

- the extension of the studies and obtaining positive results, for more than small populations of patients;

- reporting by Inovio of the data from the Phase 2 Study for VGX-3100 in the treatment of cervical dysplasia, which in Phase 1 has shown good immune response;

- reporting by OncoSec of the final data from the study of melanoma and for the Merkel cell carcinoma;

- presenting plans to launch a study for treating cutaneous lymphoma with T-cells, but also for the initiation of a new research for the treatment of solid tumors;

- OncoSec presenting the company’s plans that expressly foresees combining the electroporation device with the anti-CTLA4, the anti-PD-1 and the anti-PD-L1. As is well known, the anti-PD-1s, particularly, was among the most frequently discussed cancer therapies last year, after Merck and Bristol-Myers have reported incredible data to the American Cancer Society (ASCO). All these led to the idea that electroporation increases the assimilation of some therapeutic agents and reduces side effects, thus making them even more efficient. Therefore, careful monitoring of therapy with anti-PD-1s and the OncoSec plans to expand the researches for the ImmunoPulse platform, finally pursue further researching for this therapeutic agent [15].

CONCLUSIONS

Therapeutic electroporation is a new treatment technology at cellular level. The method is based on the electroporation of the cell membrane which becomes selectively permeable to the therapeutic agents used. *In vitro* and *in silico* experiments are promising and have allowed the insertion of chemical or biological molecules in the target cells. The *in vivo* experiments on laboratory animals have given good therapeutic results, therefore, in the future may be applied to humans. Therapeutic electroporation can become in the near future, an important treatment for oncology, cardiac, infectious diseases etc., envisioning the predominant biological perspective of therapeutics in modern medicine.

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ORIGINAL ARTICLES

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The incidence of tracheoesophageal fistulas and it major determinant factors

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Abstract: *The tracheoesophageal fistula which occurred during oro-tracheal intubation of a patient in intensive care unit is a true challenge both in diagnostic and in therapeutic approach. The best treatment is prevention, by identifying risk factors but especially is important which the mechanism in the occurrence of tracheoesophageal fistula was. The occurrence of this complication in the evolution of hospitalized patients in ICU is accompanied by significant increase in mortality, contributing to negative prognostic. We have started a large multicentric study in April 2016 regarding all patients who required intubation longer than 7 days. The study will finish at the end of 2020. We are looking for a definite conclusion, in this moment we do not have enough data for a conclusion.[1, 2]*

INTRODUCTION

The tracheoesophageal fistula is an abnormal communication between the esophagus and trachea. It can be congenital or acquired. Most of them are congenital with a frequency of 1 to 3500/ 6000 births. In this case usually are associated, or appear within, with other birth defects (trisomy 13, 18 or 21, tetralogy of Fallot, other digestive problems or muscle skeletal VACTERL Syndrome (**V**ertebral anomalies, **A**nal atresia, **C**ardiac defects, **T**racheoesophageal fistula and/or **E**sophageal atresia, **R**enal & **R**adial anomalies and **L**imb defects.). Then, there are the malignant ones which appear in the posterior infiltrating tracheal wall or the anterior wall of the esophagus. The last ones are those acquired, non-malignant [1, 3, 4].

They occur particularly after a prolonged period of intubation with or without mechanical ventilation. Fistula can result from incidents of intubation maneuver, in the operating room but especially in ICU. The first fistula appeared after a prolonged period of intubation and was initially described in 1956 by d'Avignon [2, 4].

Several factors are involved in these serious complications during a prolonged period of intubation. The most important and most discussed about in recent years is represented by the cuff pressure of orotracheal tube. If the cuff is overinflated, it leads to compression on the rear wall membranous trachea, which in time leads to ischemia and necrosis, basically forming an abnormal communication between the trachea and esophagus. Other possible complication of a hyper-inflated cuff is tracheal stenosis or bleeding erosion of a significant blood vessel. Often, both situations can occur simultaneously. The

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tracheoesophageal fistulas were more common when the high cuff pressure tubes were used. Once with the use of tubes with high volume and low pressure cuff, the incidence of fistulas has dramatically dropped down. In the international literature the occurrence of these fistulae has a relatively low frequency, of approximately 1% [4, 5, 6].

The widely accepted indication is that the cuff pressure tube should not exceed 25 cm H_2O and the volume should ideally be of 6-8 ml air. More than 10 ml of air should raise the question about tracheal injury. There are data from multi-center studies which have shown dangerously high cuff pressure after patient mobilization. These recommendations are given after multiple multi-center, international researches, but the low pressure cuff has the same danger like the high-pressure ones. If the trachea is not fully secured, then the patient is prone to tracheobronchial aspiration, despite the good and high efficient nursing [7, 8].

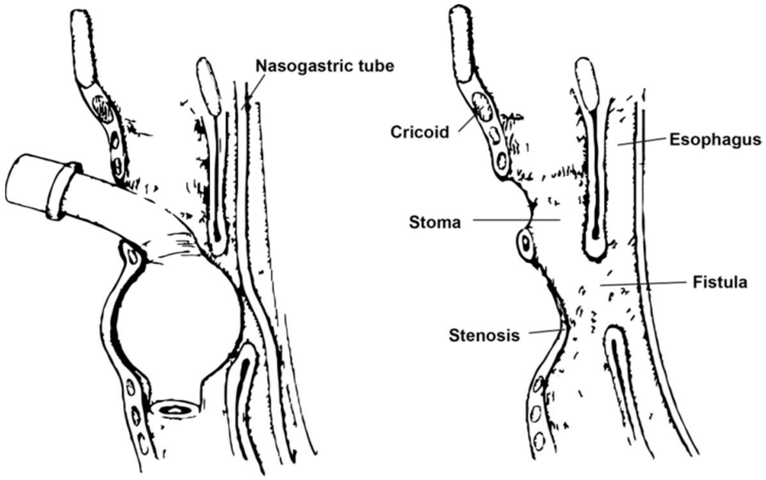

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The tracheostomy does not reduce the risk of tracheoesophageal fistula, the cuff of the cannula can injure the trachea in the same way. If the cannula's material is too rigid, the direct injury of tracheal wall may become possible through angulation [2].

The other factors involved in the occurrence of tracheoesophageal fistula are the presence of rigid nasogastric or orogastric tube, anemia, malnutrition, hypoxemia, anxiety, changing positions in bed, steroid treatment, advanced age, low perfusion from severe shocks [9, 10].

Tracheoesophageal fistula are located usually at the superior half of trachea, and in case of tracheostomy, 1-2 cm below the stoma [10].

The diagnosis of tracheoesophageal fistula is difficult. Suspicion of fistula is based on clinical signs: loss of air from breathing circuit, air on gastric tube, recurrent pneumonia, etc. We can do clinical test by administering blue methylene on the tube, but the diagnostic choice remains the bronchoscopy, images of computed tomography and upper gastrointestinal endoscopy [8, 11].

Treatment is usually surgical; less invasive procedures can be performed particularly for smaller defects, consisting in placing endoscopic stents [12].

PATIENTS AND METHODS

We have started a large prospective multicenter study in two intensive care units, the Central Military Hospital and University Emergency Hospital, Bucharest, Romania. The study started in April 2016 and will finish in the end of 2020.

All patients admitted to the intensive care unit which required orotracheal intubation more than 7 days, with or without mechanical ventilation, will be enrolled. Each observation paper will be attached in a special sheet and will include all the monitored parameters.

The parameters which will be monitored: counting days of orotracheal intubation, the pressure in the tube cuff, presence or absence of gastric tube, nutritional status of the patient, and hemoglobin during hospitalization, necessary of inotropic substances, presence or not of steroid treatment. Bronchoscopy will be performed regularly for early identification of changes of tracheal wall.

RESULTS

The occurrence of this complication in the intensive care unit of Military Central Hospital Bucharest during last five years was zero among the admitted patients (about 1500/year).

From 2,800 patients which were intubated, 1,100 needed tracheoesophageal intubation or tracheostomy more than 48 h. It is obvious that incidence of tracheoesophageal fistulas is inferior compared to actual literature information. So, we are looking forward the results of this study, in this moment the data we have aren't enough for a conclusion.

DISCUSSION

Our study aim is to highlight exactly the incidence of tracheoesophageal fistulas among endotracheal intubated patients in the ICU and their determinant factors.

Early identification of these complications with direct impact on morbidity and mortality of patients is extremely important. Secondary occurrence of pulmonary fistula and subsequent contamination of the lung may results in severe suppurative complication. Prompt treatment, either conservative or surgical, should be immediately applied.

In conclusion, the occurrence of tracheoesophageal fistulas can be lowered by aggressive nursing measures and eliminating/correcting determinants, including days of orotracheal intubation.

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CLINICAL PRACTICE

Ankylosing spondylitis and cardiovascular risk – Case report

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Abstract: *Introduction: Ankylosing spondylitis (AS) is a chronic progressive inflammatory disease of the axial skeleton and peripheral joints associated with HLA B27 antigen and with the predominance of the male gender (with an average between 20 and 30 years old).*

Case presentation

A 48 years old male patient was admitted to our clinic, having a long history regarding this disease since he was 16.

This patient has switched 3 therapies with anti TNF alpha agents until now, and we hope to obtain a good response for a long time.

During the treatment with Etanercept he presented an acute anterior uveitis which had a good response to therapy.

Conclusion: The ankylosing spondylitis management is complicated when we have the possibility to choose only three anti TNF alpha agents. If a patient does not respond to the first or second agent we are constrained to follow the last one. Therefore the principal problem regarding this special case is that the patient is non responder at the last agent. So the question that arises is witch will be the next therapy for this patient?

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic progressive inflammatory disease of the axial skeleton and peripheral joints – asymmetric.

Its major characteristic is the early damage of the sacroiliac joint, ascending to ankylosis. The incidence is increased for males between 20 and 30 years old, thus most of the patients are associating a HLA B27 antigen. The existence of cardiovascular risk due to inflammatory rheumatic diseases is known for decades but the real significance and therapy designed to control this risk has been recently evaluated.

Systemic inflammation because of inflammatory rheumatic diseases accelerates atherosclerosis and destabilizes plaque of atheroma, contributing to an increased frequency of fatal cardiovascular events such as myocardial heart attacks and strokes. The ankylosing spondylitis cardiovascular events include: aortic insufficiency, heart muscle damage (dilatation/hypertrophy), nerve impulse conduction disorders, pericarditis, and accelerated atherosclerosis.

Anti TNF-alpha therapy leads to a decrease of the disease activity score (BASDAI) and also the nonspecific inflammatory syndrome.

The anti TNF approved drugs which are currently in clinical practice are:

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1. Infliximab (*Remicade®*) – chimeric monoclonal antibody anti-TNF alpha;
2. Adalimumab (*Humira®*) – fully human monoclonal antibody anti –TNF alpha;
3. Etanercept (*Enbrel®*) – a fusion protein between the type 2 p75 receptor of TNF and an IgG1 Fc fragment.

CASE PRESENTATION

We present the case of a man, A.I., 48 years old, from urban medium, nonsmoker, having an insignificant family history, diagnosed since 1982 when he was 16 years old with juvenile arthritis.

He came for the last hospitalization, for clinical and biological reassessment, with ankles light arthralgias.

From his medical history we know that the disease has started when he was 16 years, with ankles and knees arthritics, at radiocarpal joints, at bilateral elbows, acute back pain and fever. The patient was diagnosed with juvenile arthritis and treated with Sulphasalazine 6 grams and nonsteroidal anti-inflammatory drugs. Under this treatment he had a slightly favorable evolution with partial improvement of symptoms. After 2 years of treatment disease flares have appeared, therefore the dose of Sulphasalazine at 8 grams/day was increased and a quantity of Methotrexate 7.5 mg/week was also added. After 1 year his clinical and biological evolution became favorable and the dose of Sulphasalazine was reduced to 4 grams/day, keeping Methotrexate and nonsteroidal anti-inflammatory drugs at the same dose. In 1998 he was diagnosed with ankylosing spondylitis according to the New York criteria and the association of HLA-B27 antigen. The patient gave up Methotrexate, the swelling and pain in large joints persisted so we increased the Sulphasalazine dose to 8 grams/day. The patient has annually done physio-kinesiotherapy. In 2009 it was decided to initiate biological therapy criteria (low back pain and morning stiffness, bilateral sacroiliitis grade 4, BASDAI 8.6) according to Guidelines for the treatment of ankylosing spondylitis.

In March 2009, the Infliximab (*Remicade®* 100 mg / fl) 5 mg/kg treatment was initiated. After a loading with Infliximab at 0, 6, 12 weeks, the patient had a clinically and biologically favorable development for 18 months.

Figure 1: Indications of biological therapies in ankylosing spondylitis by therapeutics protocols

Ankylosing spondylitis certain diagnostic according to the criteria of New York 1984:
• Low back pain and morning stiffness more than 3 months, being improved with effort and does not disappear at rest • Movement limitation of lumbar spine in sagital and frontal planes • Limitation of chest expansion • Unilateral or bilateral sacroiliitis grade 3-4 grade 2-4 (imaging criteria). • Diagnosis involves the imaging criteria presence (radiology, MRI) associated to at least one clinical criteria.
Active and severe disease
• BASDAI ≥ 6 for at least 4 weeks • Erythrocyte sedimentation rate > 28 mm/h • C reactive protein > 20 mg/L and 3 x VN (quantitated)
The failure of traditional therapies
•At least 2 nonsteroidal anti-inflammatory drugs continuously administrated for at least 3 months, at maximum recommended doses or tolerated for patients with axial forms • Patients with severe axial do not need a remitting (Sulphasalazine) before the biological therapy • Nonsteroidal anti-inflammatory drugs and Sulphasalazine in peripheral forms, at least 4 months of treatment at the maximum tolerated dose (3 g/d) • Ineffective response to at least one local administration of a corticosteroid injection in peripheral arthritis and/or active enthesopathies, if indicated.
The presence of hip joint damage and extra-articular manifestations are additional factors allowing anti-TNF therapy administration at a lower score of disease activity, with BASDAI ≥4

At the end of the year he suffered an acute infero-lateral myocardial infarction. The patient was hospitalized in the cardiology department, a coronary angiography was performed which revealed a 80-90% anterior descending artery stenosis, to the distal 50-60% left coronary artery stenosis, 80% stenosis in the origin of the AC, 90% oblique median artery stenosis.

The biological therapy was stopped and a surgical intervention was performed with a triple coronary artery bypass graft with autologous saphenous vein.

A carotid Doppler ultrasound was performed showing that carotid vascular system was without hemodynamically significant stenosis. Cardiac ultrasound revealed (left ventricular hypertrophy with anterolateral myocardial contractility low post, left ventricular ejection fraction 55%). Three months postoperatively the biological therapy with *Remicade®* was restarted but the second adverse reactions has occurred (generalized rash, severe hypotension). The patient received Sulphasalazine 6 grams/day, painkiller, intermittent nonsteroidal anti-inflammatory drugs and cardiological medication. Biological evaluation showed a mixed dyslipidemia, associating a grade 1 obesity.

Rheumatic symptoms were significantly exacerbated (peripheral arthritis and low back pain, intense predominantly nocturnal), thus it was decided to initiate therapy with another anti-TNF alpha (*switch*) respectively Adalimumab (*Humira*®). In February 2011 a first dose of Adalimumab – *Humira*® 40 mg/2 weeks sc was administrated.

After 6 months of biological treatment with Humira, the following peripheral arthritis, acute lombalgy and inflammatory syndrome have persisted, BASDAI score was 8.1 and anginous pain occurs during small efforts. The angiography was repeated after which angioplasty was performed. Cardiac ultrasound is performed together with control cardiac stress tests, highlighting a grade 2 mitral regurgitation, posterior mitral ring atheromatosis, hypokinesia in the left ventricle lower part. Our patient had high blood pressure values being diagnosed with a 2 grade essential hypertension, thus instituting a antihypertensive therapy treatment. Therapeutics commission (rheumatologist – cardiologist) has decided to interrupt the biological therapy, considering the patient as being non responder according to the guide treatment ankylosing spondylitis criteria. The patient has continued the background therapy with Sulphasalazine 8 grams/day, pain relievers and cardiological medication.

Persistent inflammatory syndrome associated with peripheral arthritis (*de novo*, costo-vertebral arthritis and intense bilateral talalgia), BASDAI score was 9.1, has imposed a board therapeutic meeting whose opinion was to switch on the last anti TNF alpha, which remained the only therapeutic option, Etanercept (*Enbrel®* 50 mg/week).

Before the Enbrel therapy initiation a whole-body bone scintigraphy was performed (osteogenic lumbar spine and right acetabulum reactions), CT abdominal-pelvic (grade I hydronephrosis left kidney, left kidney chronic pyelonephritis) and TB QuantiFERON test (negative) to exclude a subclinical tuberculosis infection.

Figure 2: Myocardial Perfusion Scintigraphy

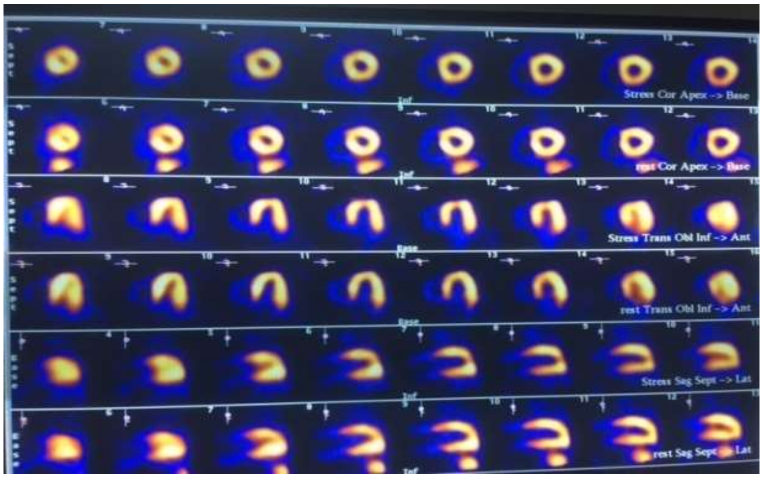

After 3 months the patient has presented a favorable evolution with a significant remission of the biological inflammatory syndrome, the peripheral joint tumefactions, persistence of a moderate intensity arthralgia and improvement of overall mobility. The patient was cardiologically reassessed, renal without the evidence of evolutionary elements. An angiocoronarography control was conducted (with 50% restenosis of the anterior descending artery stent), together with a myocardial treacherous tomoscintigraphy ECG synchronized (infero-septal dyskinesia level and decreased left ventricular ejection fraction regional effort – stress left ventricular ejection fraction 49%, resting left ventricular ejection fraction 60 %).

In 2014 the patient experienced an episode of acute anterior uveitis treated with corticosteroids and local mydriatic.

At the last hospitalization in February 2016 the clinical and biological evolution of the patient was favorable, the BASDAI score was 1.6. The treatment include *Enbrel®* the 50 mg/week, Sulphasalazine 1.5 grams/day, and nonsteroidal anti-inflammatory drugs intermittently. The patient is monitored at every 3 months by the rheumatologist and cardiologist and monthly by the family physician evidence the early possible complications.

Particularities of the case

1. Patient with juvenile arthritis, with ankylosing spondylitis evolution and complexes cardiovascular complications;

2. Lack of response to anti-TNF alpha 2 (*Remicade®*- side effects and *Humira®* - non responder);

3. Favorable answer and sustained to the 3rd anti TNF alpha (*Enbrel®*) after 3 years of treatment.

CONCLUSIONS

The introduction of TNF-alpha anti agents in treatment guidelines of ankylosing spondylitis, has revolutionized the therapy of patients with this disease, efficiency confirmed by numerous international clinical trials.

Anti TNF-alpha agents by reducing systemic inflammation creates a real cardiovascular protection for patients with ankylosing spondylitis, aspect highlighted for the case of our patient who has presented progressive cardiovascular events.

The complexity of the presented case is revealed by the associated comorbidities (cardiovascular, ocular, osteoarticular), which responded favorably after two switches of anti TNF-alpha concluding that switch between anti TNF alpha may be an effective therapeutic option in controlling the underlying disease and its complications.

Debut at the young age and association with HLA B 27 antigen are unfavorable prognostic factors for cardiovascular complications.

Interdisciplinary collaboration (rheumatologist, ophthalmologist, and cardiologist) is important for the management of patients with ankylosing spondylitis.

The evolution of this ankylosing spondylitis case is to be followed and the initiation in case of non-responder to *Enbrel®*, with a anti IL type 17 therapy, with sustained international studies having very good results in controlling this disease.

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CLINICAL PRACTICE

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Behçet disease – Case presentation

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Abstract: *Behcet's disease is a rare and poorly understood condition with multiple systemic manifestations. The disease causes inflammation in blood vessels throughout the body which leads to numerous symptoms that may appear and disappear unpredictable.*

Case presentation

A 33 year old woman was admitted (interned) to our clinic. Her family medical history reveals – multiple strokes (father) and autoimmune thyroiditis (sister).

The onset of her symptomatology was in 2013 and it consisted of fever (39-40 Celsius degrees) sicca syndrome and persistent headaches, recurrent oral and genital ulcerations.

An important event is essential to be mentioned -the patients has suffered an episode of upper gastrointestinal bleeding (hemoglobin has dropped to 2.5g/dl which has led to cardiac arrest-resuscitated).

The lab tests showed: C3 hypocomplementemia, Anti-centromere antibodies (-), anti-b2gp1 antibody (+), lupus anticoagulant (+), U1RNP(-).

We started to administrate cyclophosphamide to the patient, thus his condition has improved after the 2nd dose.

At the 12-month evaluation we were able to see a significant clinical and biological improvement.

Conclusions

In order to be able to talk about "evidence based medicine" for the management of Bechet's disease, a large number of clinical trials is required to provide to the attending physicians the necessary data for diagnosis and treatment.

INTRODUCTION

Behcet disease is a rare condition with a sinusoidal evolution which can affect almost all the organs and human systems, determining multiform symptoms with a variable severity. The medical practitioner has at his disposition a diversity of therapeutic options (from symptomatic therapy to a major systemic immunosuppression); at this moment we can't discuss about fundamental therapeutics protocols and unanimously recognized.

Behcet disease is an inflammatory systemic condition with an uncertain etiology. In the disease pathogenesis both environmental and genetic factors are involved like for the majority autoimmune diseases. This disease has been worldwide described but the maximum prevalence is registered in Middle East, adjacent zones of Mediterranean Sea and Asia

Considering the potential muco-cutaneous, articular, ophthalmic, neurologically, etc. damages, the interdisciplinary collaboration becomes more important. Due to the lack of specific guidelines for the diagnosis and treatment, the therapeutic approach of individuals' cases still remains dependent on the doctor treating experience or on the clinics where these complex cases are monitored.

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CASE PRESENTATION

A 33 year old woman was admitted to our clinic presenting severe muscle weakness and joint pain.

Her medical history has revealed that in the last years the patient was admitted several times in the Infectious Diseases department with persistent fever and severe headaches.

Her family medical history has revealed – multiple strokes (father) and autoimmune thyroiditis (sister).

The onset of her symptomatology was in 2013 and it consisted of fever (39-40 degrees Celsius) sicca syndrome and persistent headaches. As the disease was progressing, our patient presented oral ulcerations, odynophagia, muscle weakness and blindness of the left eye.

The onset of the decreased visual acuity started with the right eye. An ophthalmologic examination has revealed uveitis and chorioretinitis.

Considering an inflammatory demyelinating disease (multiple sclerosis), the neurological exam suggested a cranial magnetic resonance imaging (which has revealed optic nerve edema) and anti-aquaporin 4 antibodies screening (which was negative). The patient treatment has started on Corticosteroids (methylprednisolone in pulse therapy) resulting a slight improvement of visual acuity, persistent joint pains, xerophtalmia.

An important event is essential to be mentioned -the patients has suffered an episode of upper gastrointestinal bleeding (hemoglobin drops to 2.5 g/dl which leads to cardiac arrest – resuscitated).

When the patient was admitted to our clinic the symptoms become more aggressive; the patient present extreme fatigue (with orthostatic intolerance), recurrent oral and genital ulcerations, odynophagia (due to the pain caused by oral and esophageal ulcerations she was not able to solid food or liquids) and blindness of the left eye.

Figure1: Atrophy of retinal layer

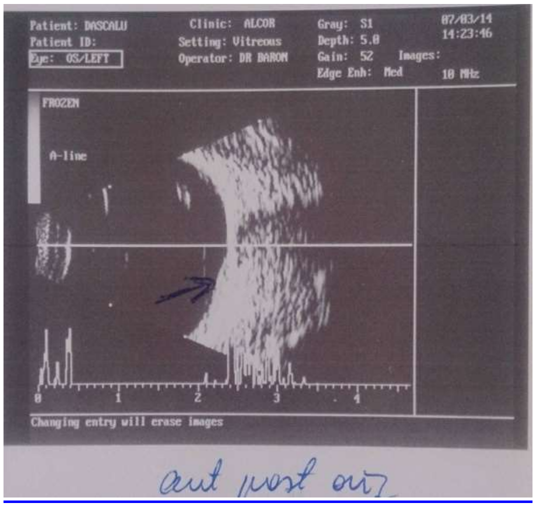

Figure 2: FOS – thicknesses of retinal layer

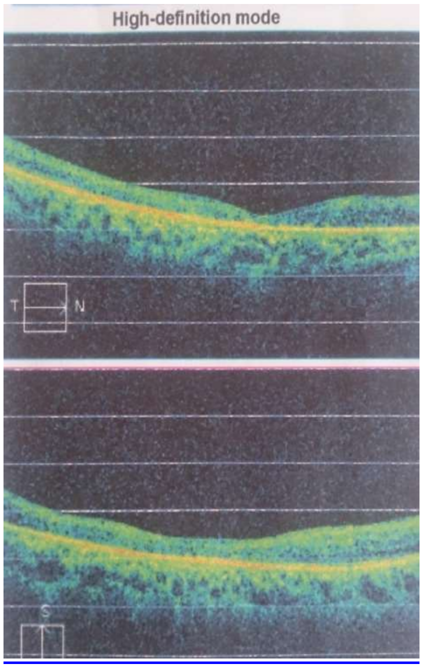

Despite the muscle weakness and severe fatigue that the patient suffered from throughout this whole period, muscle enzymes was never elevated.

Nevertheless we continued our investigations with an electromyogram. The EMG showed acute polymyositis aspect and sensory polyneuropathy lesion.

Then neurological exam showed spastic paraplegia, left brachial plegia and visual acuity loss. The brain MRI revealed a lacunar ischemic stroke in the vertebrobasilar territory.

The ophthalmological exam showed optic nerve atrophy.

We were facing with a patient, having a chronic, inflammatory multisystem condition of unknown etiology. The blood tests revealed nothing but a persistent inflammatory syndrome (ESR = 80-100 mm/h); the immunology tests didn't provide enough information either (C3 hypocomplementemia, Anti-centromere antibodies (-), anti-B2GP1 antibody (+) lupus anticoagulant (+), U1RNP (-), anti-aquaporin 4 antibodies (-) HLA B51 (-), Leiden V factor (+), ANA (+).

However we were in possession of his clinical examination highlights: chronic fatigue, recurrent mucosal ulceration, neurological symptoms, bilateral posterior uveitis, inflammatory arthritis various symptoms that we were able to identify as features of Behcet's disease.

By the time an accurate diagnostic could be established, the patient had already suffered some severe life threatening complications: upper GI bleeding with severe anemia that led to cardiac arrest, severe sepsis caused by infected decubitus injuries and medullar aplasia caused by high dose of immunosuppressive medication.

Behcet disease – generally accepted therapeutic objectives:

1. It's essential having a clear diagnostic and well founded as well as a clear setting of a lesional exact balance, with the progressive potential appreciation of the identified lesions;
2. The remission induction and maintenance;
3. The prevention of irreversible organs lesions (for example the ophthalmological one), which tends to appear early, especially in some problematic groups like young men;
4. The articular and muco-cutaneous exacerbations prevention can significantly improve the quality of life;
5. The multisystem involvement imposes for most cases interdisciplinary implication.

We want to underline the importance of having an accurate and precise diagnostic before starting a treatment, but in this particular case, a rapidly evolving disease worsened by the upper mentioned events, we decide that it was extremely necessary for the patient to start a remissive treatment. We initiated a moderate dose of cyclophosphamide (800 mg – 15 mg/kg bolus intravenous) and a low dose of oral corticosteroid (methylprednisolone 8 mg).

A significant improvement was observed after the second course of cyclophosphamide. The patient has regained the strength and muscular tonus, neurological symptoms has disappeared and she did no longer mucosal ulcerations.

Although the inflammatory ocular symptoms were suppressed, the significant loss of visual acuity persisted (it was caused by initial optical neuritis).

At the 12-month evaluation we were able to see a significant clinical and biological improvement. The patient is now able to do her daily activities therefore her lifestyle has improved.

The brain MRI shows normal brain parenchyma, normal vascular structures and normal blood flow.

Ophthalmological exam: minor restant vitrean abnormalities.

CONCLUSIONS

In the past decade we have witnessed a significant improvement in the therapeutic approach of Behcet's disease by optimal use of the old available drugs and also by the development and clinical use of new therapeutic solutions.

Behcet's disease still remains a multidisciplinary challenge for attending physicians.

Available data in the medical literature consists mainly in clinical cases and series of clinical cases with a limited statistic value.

In order to be able to talk about "evidence based medicine" for the management of Bechet's disease, a large number of clinical trials is required to provide for the attending physicians the necessary data for diagnosis and treatment algorithms.

Abbreviation

[1] U1RNP – Anti-Ribonucleoprotein

[2] EMG – Electroyography

[3] ESR – Erythrocyte Sedimentation Rate

[4] HLA B51 – Human leukocyte antigen B51

[5] ANA – Anti-nuclear antibody

[6] GI – Gastrointestinal

[7] MRI – Magnetic resonance imaging

[8] B2GP1 – Beta 2 Glycoprotein 1

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VARIA

In memoriam – Cristian Babalâc (1954-2006)

Dan Mischianu

I am absolutely convinced that you have never wondered what the etiology of the *ferpar* word is.

The Romanian *ferpar* has its origin linked to the French "fair-part", which, according to the dictionary means a letter "announcing birth, engagement, marriage or, most often someone's death". The above lines are not intended to be a late *ferpar.*

These lines are supposed to bring back the memory of an absolutely exceptional man, a thorough man and a fighter, a true military doctor - Col. Dr. Cristian Babalâc (1954-2006), my University colleague. I also hope that these lines will make you realize that time passes quickly destroying everything on its way.

And time, a decent gentleman for some, covering us with a veil of forgetfulness, time "has a bad habit of being irreversible", as we learned from Octavian Paler.

Our desire is to present the portrait of the man, the military doctor, the professional Cristian Babalâc, because Dr. Cristian Babalâc was – and "to be" in the past tense sounds so sad! – a devoted man and practitioner that always proved an absolute professional availability.

My group colleague and my friend, Dr. Cristian Babalâc was born in Bucharest, on 13 January 1954 on Teodosie Rudeanu street, near North Railway Station, a few hundred meters from the Institute of Military Medicine, the place or "origin" of our emergence, the ones from yesterday, today and future serving Romanian military medicine.

Graduated from High School "Ion Neculce" Bucharest (1973), Faculty of Medicine Bucharest (1979), Doctor of Medicine with the thesis "The value of laser in the surgical treatment of soft part tumors" (1997), specialist doctor in Orthopedics and Traumatology (1989), Senior researcher (1990), consultant in Orthopedics and Traumatology (1995).

He participated as a member or responsible at research contracts, guided numerous diplomas and internships, conducted/aided the full range of orthopedics – traumatology procedures, 15 patents and 7 innovation patents for different medical devices: gurney for the transport of traumatized, forearm electronic prosthesis controlled by sensors, bioelectronic prosthesis for superior limb, immobilizer and transportation device for polytrauma patients, external bipolar monoplane fixator, semicircular external fixator, circular external fixator, universal external fixator, grapples for osteotomy. He introduced "CO_2 surgical laser treatment in musculoskeletal limb tumors" for the first time in the country.

Published the treatise: "Laser in the treatment of musculoskeletal diseases" (co-author) Military Publishing House Bucharest (1999), "Course of emergency and disaster medicine" (co-author) MTM Publishing, Consult Publishing (2003), 147 (20 as first author) communicated papers at congresses, conferences, symposiums in the country and abroad, 28 articles (4 as first author) published in journals: "Journal of Military Medicine", "Journal of Orthopedics and Traumatology", "News Update", "Balkan Military Medical Journal", a member of SOROT – Romanian Society of Osteoporosis Treatment, Romanian Society of Laser in Medicine and Biology, Romanian Association of Military Physicians and Pharmacists.

I knew him as a student and we operated together as specialist surgeons. Dr. Cristian Babalâc was a hard worker by definition, an absolute perfectionist, continually dissatisfied with his achievements – although he managed to accomplish everything he undertook, a deep thinker, a cerebral man, a genuine family man.

He was particularly pleased with the silence at home, the achievements of his wife, the distinguished Head of Hospital Pharmacy of "Prof. Theodor Burghele" Hospital – Ms. Pharmacist Gabriela Babalâc, and the professional accomplishments of their son – Cătălin Babalâc.

Col. Dr. Cristian Babalâc was a "serious man", a professional of orthopedics and traumatology of the Central Military Hospital. Nevertheless, medicine is very often defeated by disease severity.

I confess that I had a sense of absolute helplessness when I was near the bedside of Dr. Cristian Babalâc!

Lack of health, the implacable decline, and the mortal character of all beings – but most of all those close to our soul – are almost unapproachable subjects. As I was writing these lines I was overwhelmed by bleak thoughts.

My intention was only one, to restore the memory of those who knew and valued him, a decade after his passing, the luminous figure of Dr. Cristian Babalâc, although "slightly frown as a result of the multitude of his thoughts".

Leaving us ten years ago after unimaginable suffering, I think Dr. Cristian Babalâc kept one hope in his noble and good soul: maybe better is yet to come!

May God bless his soul!

ADMINISTRATIVE ISSUES

Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine*. Please read the complete Author Guidelines carefully prior to submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

ETHICAL CONSIDERATIONS

Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

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(2) searchable by standard, electronic (Internet-based) methods;

(3) open to all prospective registrants free of charge or at minimal cost;

(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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All articles, with the exception of Editorials, must contain an abstract of no more than 250 words. Abstracts for original articles should be formatted into subheadings, as detailed below. Titles must not be longer than 120 characters (including spaces).

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These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

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RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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Original Articles (including clinical trials)

RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

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Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’

industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must

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4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

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WELCOME ADDRESS

Constantin Ștefani

Col. CONSTANTIN ȘTEFANI
Chief of the Medical Directorate
of Romanian Armed Forces

Dear Generals, Admirals, distinguished guests and colleagues,

As the Chief of the Medical Directorate of Romanian Armed Forces, as well as the Head of Romanian Delegation at the 21st Balkan Military Medical Committee Congress, it is a real honour, but also a pleasure to welcome and host you in Bucharest, the capital of Romania.

For us, people destined by history to live in Balkans, the Congress of BMMC, has already acquired a well deserved successful tradition, being an opportunity of approaching the many-sided military medical issues. Together with our colleagues from Albania, Bulgaria, Greece, Serbia and Turkey we have been contributing to the increase of the prestige of this reunion and of its scientific periodical. I am sure that Romanian branch of BMMC will succeed in continuing the best achievements of the previous edition.

In a constantly evolving international environment, we all have numerous issues and perspectives to share and coordinate within the medical community and many questions are raised for many nations but, among these, one is particularly relevant: how to maintain the high level of professionalism reached by military medical personnel. The main topic of this year edition "Balkan Military Medicine – Future challenges" is not only "Food for thought" but an opportunity to exchange ideas and knowledge on Military Medical topics, which is of great importance and value for all of us, considering the present geo-political circumstances in our area and in the world.

In the hope that the congress we are about to commence will be an outstanding example of true scientific, ethical and friendly spirit, I welcome you in Bucharest, a city with more than 800 years of history, once known as the "Little Paris". For those who are for the first time in Bucharest I do hope they will enjoy it and for those, who have been before, to experience new places the capital of our country is willing to offer.

Yours sincerely,

Col. Assist. Prof. Constantin ŞTEFANI, MD, PhD
Chief of the Medical Directorate of the Romanian Armed Forces
President of 21st BMMC Congress

ORAL PRESENTATIONS

OP session 1 – Military medicine

EXPLOITING STRATEGIES OF IMPROVING HUMAN PERFORMANCE FOR MILITARY OPERATIONS

Radu Hertzog

During military operations, the war fighters are exposed to several threats. CBRN agents, blast related events, extreme and austere environments, lack of family, stress and emotional shock jeopardize health, protection, performance and even human condition. Many medical approaches or technologies have been developed or proposed for human performance optimization (HPO), human systems integration (HIS) and human performance enhancement (HPE) in order to support sustainability and survivability as well as to improve combat readiness and effectiveness. The presentation covers few aspects of animal kingdom to understanding of biological underpinnings of high level performance. Nature is providing us an unlimited source to discover bio-tech solutions to counteract the threats that could affect the life of our soldiers.

MILITARY INFORMATION SUCH AS SPECIFIC IN NATURE AND USE INFORMATION

Chobanov N., K. Hristova, S. Kolev, K. Paskalev

PURPOSE

1. Defining the place of military medical information to the general flow of information that serves military science.

2. Determine the purpose and addressees of the information that is gaining SRLMMI.

3. Participation in the creation of a network for the exchange of military medical information.

MATERIALS AND METHODS

1. Materials – NATO standardization documents: Allied Joint Medical Doctrine (AJP - 4.10) and Allied Joint Medical Doctrine for Medical Intelligence (AJMedP - 03); Doctrine for medical insurance. National publication of the Armed Forces of the Republic of Bulgaria (NP-4.10); collections, manuals, lectures and publications of SRLMMI.

2. Methods – systematizing, analyzing, evaluating and interpreting information.

RESULTS

1. Promotion of the mission, strategic goals and objectives of SRLMMI in the short and long term.

2. Creating conditions for future sharing unclassified products conducted by military medical intelligence structures with similar aims and purposes.

CONCLUSIONS

1. Identify hazards to health and life of soldiers during the preparation, implementation and after implementation of missions outside the Republic of Bulgaria is important for successful implementation.

2. The dissemination and exchange of classified military medical products conducted by intelligence structures with similar aims and purposes increases the efficiency of their operations.

ANALYSIS OF THE COST OF MEDICINES AT THE MILITARY MEDICAL ACADEMY

Virsavia Vaseva, Ventsislav Mutafchiiski, Rumen Popov, Lyubomir Aleksiev, Nikolay Petrov

Introduction: Facing conditions of market economy and financial deficits each health manger is challenged to provide cost-effective functioning of its entrusted hospital. In the structure of the treating costs, medicines take leading position, according to leading authors, their share reaches 40% of the total cost. For this reason, introducing scientifically based and cost-effective strategy for analysis and control the costs of medicines proves extremely relevant.

Aim: To analyze the use of medicines at the Military Medical Academy and to propose a model for control.

Material and methods: We used data from Hospital Information System and Financial Department about received income from the National Health Insurance Fund and expenditure on medicinal products in MMA in 2014 and 2015. In 2015 the system for control of medicines spending was introduced. We compared the results of the 2 years. A comparative analysis was made on quantitative and qualitative criteria of medical services for 2014 and 2015. The share of revenue from the NHIF and the cost of drugs to all clinics was calculated. A relative ratio income/expense is calculated, as results are processed by comparative analysis.

Results: Our study shows that in 2015 revenues of NHIF exceed those of 2014 by 3% and the cost of medicines in 2015 decreased by 33% compared to 2014 as the quality of medical services retain their values. The share of the medicine cost to the price of the clinical pathways in 2015 decreased by 7% compared to 2014. For surgical clinics average rate of cost of drugs is 9% and 12% for therapeutic. Surgical clinics have better relative index income/expense than the therapeutic.

USE OF THE ELECTRONIC HEALTH RECORD FOR IMPROVEMENT DISEASES CONTROL IN BULGARIAN ARMED FORCES

Petkov Assen, Valchanova-Stoykova Lubomira, Alexandrov Alexandar, Aleksieva Ivanka, Popivanov Ivan

Based on oral interviews conducted with medical advisers of Logistics Directorate - MoD, Joint Forces Command, Land Forces, Air Force, Navy and doctors from major military units, the authors found the most common reasons for hiding information on changes in health status of military personnel and civilian employees in Bulgaria Armed Forces: concern of dismissal due to unfitness, ethical concerns, discomfort of commanders and colleagues, ineligibility for certain positions, restrictions for participation in missions abroad. The impact of those reasons on the individual and group security in the permanent garrisons and on missions is analyzed.

Based on published till now data from the Ministry of Health and information from the authors about the project “Electronic Health Record” (eHealth record), the authors explore the possibilities of access to health information by military doctors for each serviceman and civilian employee in Bulgaria Armed Forces. The aim is strict control of morbidity and prevention of threats to personal and collective security in both Armed Forces and society. Fundamental ethical and legal problems of access to the eHealth records are analyzed and well-grounded proposals for regulatory amendments are made.

INFORMATION SYSTEM FOR AUTOMATING AND MANAGING PROCESSES IN THE MILITARY MEDICAL ACADEMY

Anna Krasteva, Georgi Yanchev, Rumen Popov, Virsaviya Vaseva

The information system GAMMA HIS possess every module needed for automating and managing the processes at a medical facility.

Military Medical Academy has the experience of creating and implementing medical software solutions for achieving automation and optimization of the processes in outpatient, hospital and para-clinical activities related to the collection, processing and storage of medical statistics.

Main directions of our activity of the Military Medical Academy are:

- Development and implementation of application software in the field of health
- Development of information systems for the needs of hospital care
- Consulting and training of staff in hospitals in the structure of MMA
- Operational maintenance of embedded systems

The range of software products in GAMMA are integrated into a single system, but can function independently on their own. All statements and specifications of software products comply with the requirements of The National Health Insurance Fund (NHIF) and the Ministry of Health, as well as the existing regulations under the Accounting Law. The product GAMMA CODEMASTER is intended to register outpatient and inpatients in hospitals and outpatient medical care. The program keeps the most important data on enrollments, it allows economic analysis and preparation of statistical reports.

The purpose of the program GAMMA MULTILAB is the implementation of automated management of activities related to the process of registration and control of paraclinical services, to store the results and their connection to the electronic medical record of the patient. The product supports a barcode labeling of specimens. Related to system analysts will automatically transfer data and after validation are available for the recipients. The program meets the requirements of the NFC for software labs.

The software Gamma Store - "Hospital pharmacy and warehouse" is designed for automated maintenance of records in pharmaceutical and other warehouses of hospital units that are providing supply and dispensing of medicines and materials to the units of hospitals. The system allows the organization of the work of many warehouses in the health facility with sub warehouses for different types of materials: drugs, supplies, sanitation, food and more. Ordering of medicines and materials can be carried out electronically. The system supports restricting the visibility of availability and prices.

The software GAMMA PHARMA is designed for automated maintenance of records in hospital pharmacies and pharmaceutical warehouses ensuring supply and dispensing of drugs for bedridden patients in inpatient health establishments and other units of hospitals. The program has the ability for monitoring the supply of drugs in competitions and control correspondences between the contract and delivery prices, as well as set the order for delivery of drugs to a supplier.

MEDICAL INTELLIGENCE ORGANISATION IN ALBANIAN ARMY FORCES

Kukeli Edlira, Muhameti Rushan, Duraku Kujtime, Reso Elton

Introduction: Medical Intelligence is a main factor in medical support planning and Medical Force Protection. Medical Intelligence Doctrine should be adapted and implemented in multinational operations conducted in the NATO- lead operations. Main principals, MI cycle and its organisation in Albanian Army Forces play an inportant role in assesment of operational risk.

Objective: The assessment of troop’s healthy risks, preventive measures implementation, support medical services management in missions abroad.

Material and Methods: The descriptive study was conducted in the period of time 2010-2011.The study sample included military contigents redeployed back home from abroad missions. The data was collected from medical records of “Trauma University Hospital”.

Results: This study was conducted at a military contingent (of 120 solders) with mission in Chad. There were identified 10 cases deployd back home with infective disease (malaria). Medical Intelligence is neccessary in operational planning and provides medical information for geografic factrors (clima, flora and fauna) endemic and epidemic deseases, enviroment, toxic, industial risks and taken measures related with Weapons of Mass Destruction (CRBN). Albanian Medical Service Structures lead and cordinate the organisation, planning and medical controls for medical force protection. Albanian Preventive Medical System included Health Inspectoratet that cordinates the medical activity and Military Medical Unit as a specialized institution for health inssurance effectives.

Conclusion: Medical Intelligence has an important role for medical preventive measures, immunisations, healthy education and troop’s medical evaluation in mission.

THE ROLE OF THE BULGARIAN MILITARY MEDICAL TEAM IN THE EU TRAINING MISSION IN MALI

Kesov Georgi, Svetlin Nikolov, Alexiev Lybomir, Karakanov L., Petrov Nikolay, Mutafchyiski Ventsislav, Popivanov Georgi

Introduction: Since 2013 by decision of EU a training mission was established in Mali in order to increase the preparation of the army and to improve the anti-terroristic response. Since its establishment ten Bulgarian military medical teams with 40 medics (20 doctors) have been participated. The purpose of the work is to present the specific tasks of the mission, the most common morbidity, security issues, climate characteristics and the costs.

On the background of the increased Islamic activity in the region this mission has a growing responsibility and significance. It requires specific military medical training of the participants. The close collaboration between medical teams (Role 1 and Role 2) in the training bases is a prerequisite for successful work of the team.

CONCERNING CREATING A STRUCTURAL MODEL FOR DETERMINING THE CROSS-BORDER ENVIRONMENTAL SECURITY ALONG THE BULGARIAN-ROMANIAN BORDER

Hristova Krassimira

PURPOSE

1. Formulation of problems in cross-border environmental security.

2. Theoretical studies to create a structural model for determining the cross-border environmental security along the Bulgarian – Romanian border.

MATERIALS AND METHODS

1. Materials – Transnational program "Danube 2014 to 2020"; „Cross-border program between Romania and Bulgaria for the period 2014-2020”.

2. Methods – systematization, analysis, evaluation and interpretation of available information.

RESULTS

1. New structural model by which to reflect the time and phases of the development of cross-border risks and threats.

2. Formulation of future researches on cross-border risks and criticality.

CONCLUSIONS

1. Cross-border environmental hazards are appropriate to define hypotheses as to their substance.

2. Cross-border environmental threats are a combination of information about the quantity, quality and composite indicators of all the constituent system components.

A SPECIAL POPULATION: PREGNANT WOMEN IN ARMED CONFLICTS

Karasahin K. Emre, Fidan Ulas, Alanbay Ibrahim, Kinci Ferdi, Yenen Mufit Cemal

OBJECTIVE: To investigate the possible effects of war on pregnant women and to delineate the basic healthcare standards for caring pregnant women in armed conflicts.

MATERIAL AND METHOD: An internet research was made using the keywords of “pregnancy” “war” “armed conflict”, “refugee” and “conflict”. It is understood that there is either not much data on the topic or the conflict zones were too busy or deprived for a reliable data to be presented.

RESULTS: Obstetrics can present a large health problem in case of a war. Despite the difficulties of war, high-risk pregnant women could be properly identified and could be referred to a more sophisticated center if cooperation with other (either governmental or non-governmental) organizations could be provided. Resources can diminish fast in cases of mass casualties or high number of people fleeing armed conflict zones. Small and efficient obstetric teams could be set up with mobile ultrasound, or cardiotocographs may be helpful. Basic laboratory elements for urinalysis and hematocrit are desirable. Normal delivery with as little cesarean as possible should be aimed.

CONCLUSION: Although there are numerous guidelines on management of pregnant women in disastrous states, the war situation can be quite different. Medical personnel should be ready to adapt to rapidly changing conditions, such as the weather, advancing hostile forces, the triage priorities, availability of surgical approaches etc. The medical personnel should be protected by solid legal approaches in order to be immune to malpractice cases due to the unforeseen outcomes in obstetric management in war zones. Neonatal outcomes cannot be predicted, and the survival of mother and the neonate may supersede the long term outcomes. The medical personnel should also be psychologically prepared and supported to complete the mission successfully.

A WAR SURGEON: CAPABILITIES AND EDUCATION

Harlak Ali, Kaymak Sahin, Eryilmaz Mehmet, Coskun Ali Kagan, Lapsekili Emin, Ersoz Nail, Unlu Aytekin, Zeybek Nazif, Kozak Orhan

OBJECTIVE: This study aims to discuss the optimal capability of a war surgeon and his educational program. The main mission of military medicine is to threat wounded soldiers.

The War Surgery can be defined as advanced surgical management of war wounds. In daily surgical practice every surgeon works in his special area. For example general surgeons perform usually gastrointestinal operations. They don’t interest in a thoracic pathology. If necessary a thoracic surgeon can participate to the operation. But military trauma causalities have usually complex injuries and their initial treatments are made in a field hospital with limited conditions. A war surgeon must have basic skills for the stabilization of wounded soldiers.

MATERIAL AND METHOD: This study evaluates the wounded compartment of causalities from a military conflict and the role of doctors from different specialties. We have tried to make a conclusion about the basic capabilities of a war surgeon. We also compared our training program in war surgery with the clinical practice.

RESULTS: Duration of war surgeon training program in Gulhane Military Medical Academy is two year after general surgery residency. In this period a war surgery fellow works 13 months in general surgery, 3 months in thoracic surgery, 2 months in orthopedic surgery, 1 month in plastic and reconstructive surgery, 1 month in radiology department. In the latest military conflict, the distribution of wounded compartment and distribution of surgical specialist which participate to the treatment were as fallows: cranial 10 %, facial 14%, neck 4%, thoracic 8%, abdominal 7%, pelvic 6%, extremities 54%, vascular 28%.

CONCLUSION: The results show that a war surgeon must have basic knowledge about orthopedic, vascular, abdominal, thoracic, cranial surgery.

CAN BLUE OCEAN STRATEGY BE APPLICABLE IN MILITARY HEALTHCARE INSTITUTIONS?

Ugur Ugrak, Ugurluoglu Ozgur

OBJECTIVE: As we know, Blue Ocean Strategy can be defined as a new strategy without any competition. In this study, we aimed to analyze of applicability of “Blue Ocean Strategy” in healthcare sector including Military Healthcare Institutions.

MATERIAL AND METHOD: All publications reached in literature surveying with keywords “blue ocean strategy”, “healthcare”, “healthcare management”, “hospital”, “health” and various combination of all were analyzed in context of this study purpose.

RESULTS: Military Hospitals in Turkey have faced with competitive market after starting giving healthcare service to public. Competition is important, however, institutions focusing on competition strategy might skip more crucial strategic aspects. Blue ocean strategy, contrary to traditional rules, doesn’t use competition as a comparison point, instead, aims at reaching untapped markets with creating new value for both themselves and consumers. Blue ocean strategy claims that with tools such as “strategy canvas” and “four action framework”, it can make competition irrelevant and get reached markets in which new demands exist. According to literature review “Blue Ocean Strategy” could be applied successfully in various sector. Even, Malaysia accepted “Blue Ocean Strategy” as a national strategy. Successful example of Blue Ocean Strategy were also seen in healthcare sector.

CONCLUSION: For Military Hospitals, which have been faced with competitive markets, Blue Ocean Strategy is thought to provide useful tools to make competition irrelevant and has a potential to lead those institutions to untapped markets in which new demands exist. Additionally, it is thought that “Blue Ocean Strategy” might be useful to determine unnecessarily focused factors in Military Health Care System, which are complex and highly specialized.

OP session 1 – Microbiology, clinical research and studies

A NEW EXPERIMENTAL INVASIVE MODEL IN THE TREATMENT OF GASTROOSEPHAGEAL REFLUX: IMPLANTATION OF ENDOSCOPIC STEM CELL INTO THE LOWER ESOPHAGEAL SPHINCTER

Caliskan Bahadir, Kantarcioglu Murat, Gunal Armagan, Demirbag Suzi, Bagci Sait

INTRODUCTION: Gastroosephageal reflu disease has an incidence of 23% in Turkey. Endoscopic treatment modalities are used commonly. In this study we endoscopically implantated the stem cell derived from female endometrial tissue to rabbits low esophageal sphincter (LES), observe the differentiation to muscle cell in this area and the effect of this procedure to sphincteric pressure

MATERIAL METHOD: Stem cells 1x106 implantated to 10 rabbits (Group 1) and % 0.9 NaCl implantated to ten rabbits (Group 2). Rabbits LES pressure were studied before implantation and 3 weeks later. On day 21 lower esophageal sphincter dissected.

RESULTS: Histopathological examination of group 1 showed GFP expressing cells in the lower esophageal sphincter of the rabbits. It has been observed that these cells express MYO D protein that play a key role in muscle differentiation and factor MHY-2 which contributes to the formation of myocytes. When compared to controls, increase in LES pressure in the stem cells-implanted group was obtained.

DISCUSSION: Endoscopic human endometrial progenitor stem cell implantation to LES leads to the differentiation and an increase in sphincter pressure. Our findings suggest that, implantation of autologous stem cells, using the natural human cells in selected gastroesophageal reflux disease patients with LES failure can be a new invasive endoscopic therapeutic model.

MOLECULAR STUDIES REGARDING SOME TICK BORNE – PATHOGENS IN ROMANIA

Alexandru Vladimirescu, Victoria Gabriela Dumitrescu, Lucia E. Ionescu, Valentina Elena Moraru, Marius Necşulescu, Simona Bicheru, Diana M. Popescu, Doina Daneș, Stelian Bărăităreanu, Marius Ciocănău, Valeria Purcărea-Ciulacu, Gabriela Nicolescu

Our studies undertaken between 2006 – 2015 have shown that the most frequent species of ticks in Romania is Ixodes ricinus. Three Romanian counties were selected as ticks sampling sites (Sibiu, Tulcea and Giurgiu). Over 400 I. ricinus and Hyalomma sp. ticks were collected and analyzed by Real-Time PCR methods (included in the new TicKitqPCR detection concept) that give us results on Borrelia burgdorferi, Francisella tularensis, TBEv and CCHFv presence in the vectors.

Total RNA and DNA were and analized by in house real-time PCR reagents (included in the new TicKitqPCR detection concept) and 2 commercial kits for the BFTC detection in the I. ricinus and Hyalomma sp. pools. All reverse-transcriptions were made using random hexamers.

Specific DNAs from B. burgdorferi sl. were detected (Fla B gene target) in 20% of I. ricinus ticks and specific DNAs for F. tularensis and/or Francisella-like endosymbionts were detected (IS Ftu2 genomic isertion-like element) in 2% of the same vector species.

Specific RNAs from TBEv were detected (3’ UTR-genomic region) in < 1% of I. ricinus pools. No specific CCHFv RNAs were detected (S–genomic region) among the Hyalomma sp. pools.

The results strengthen the concern that already exists in Romania, for the enhancement the surveillance and the control measures for the tick populations but also for the means of active information of the human population about the danger of the diseases transmitted by ticks in some risk areas.

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NERVE AGENTS SIMULANTS ISOPROPYL P-NITROPENYL METHILPHOSPONATE AND ETYL P-NITROPHENYL METHILPHOSPONATE AND THEIRS APLICATION AS ACHETYLCOLINESTERASE INHIBITORS

Cristina Anca Secară, Bogdan Pătrinichi, Mihaela Ionescu, Lavinia Hinescu, Mihail-Silviu Tudosie

Background: Armies of NATO member countries are interested in the synthesis and improvment of new antidotes against chemical warfare agents.

The inherent toxicity and limited laboratory supply of CWAs has led to the development and study of safer compounds that simulate the chemistry and the reactivity of CWAs.

Objectives: Reducing the risk of exposure to chemical nerve staff who test experimental therapeutic efficacy using their simulants suitable laboratory work;

Material and method: Two nonvolatile compounds were GC-MS identified as CWA simulants, 90% purity: 4-nitro-phenyl ethyl methylphosphonate (NEMP, VX simulant), and 4-nitrophenyl isopropyl methylphosphonate (NIMP, sarin simulant). In vivo study of these compounds was performed in order to determine LD50 values, cholinesterase inhibition degree, and oxime assisted protective ratio.

Experimental data statistical analysis was perfotmed by using Student's T test.

Results: NIMP: DL50 0.45 ± 0,002 mg/kg i.p; AChE inhibition in the range 53 to 95%, antidote protective ratio: 2.5; NEMP: DL 50 0.51 ± 0,003 mg/kg i.p. AChE inhibition in the range 56 to 88%;

Conclusions: Comparative analysis of LD50 values for NIMP, NEMP and CWA correspondents showed lower intrinsic toxicity of the compounds synthesized. Inhibiting activity is sufficient to mimic exposure to CWa, being present all the clinical signs typical of poisoning.

Thus it can be concluded that the two compounds studied show similar pharmacodynamic effects and references due to lower intrinsic toxicity may be suitable as a surrogate for studies in the laboratory.

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THE IMPACT OF CYP2C19 POLYMORPHISMS, CLINICAL AND LABORATORY VARIABLES ON PLATELET RESPONSE TO ANTIPLATELET

Alina Mărginean, Claudia Bănescu, Valeriu Moldovan, Alina Scridon, Mihai Mărginean, Rodica Bălaşa, Smaranda Maier, Mariana Ţăruşi, Minodora Dobreanu

Introduction: Antiplatelet drugs are widely used in patients with acute coronary syndromes or stroke. Despite adequate antiplatelet therapy, some patients develop acute ischemic events. This is partly attributed to the fact that they have poor inhibition of platelet reactivity despite treatment.

This study aimed to assess the impact of clinical and laboratory variables on platelet response to clopidogrel and/or aspirin, evaluated using impedance aggregometry in a Romanian population.

Methods: The study included 189 patients with acute coronary syndromes and non-cardiogenic ischemic stroke. Platelet aggregation was evaluated by impedance aggregometry. CYP2C19 loss-of-function polymorphisms were detected using the Polymerase Chain Reaction - Restriction Fragment Length Polymorphism technique. Various clinical and laboratory data were also recorded.

Results: In our data set, 81% of the patients were responders and 19% non-responders to antiplatelet therapy. The distribution of CYP2C19 polymorphisms was as follows: 61.1% of patients were CYP2C19 wild-type homozygotes, 27.7% CYP2C19*2 heterozygotes, 1.1% CYP2C19*3 heterozygotes and 10% of patients CYP2C19*2 homozygotes. The highest level of association with clopidogrel response status was found for CYP2C19 polymorphisms, concomitant aspirin treatment, leukocyte and platelet count, history of myocardial infarction and arterial hypertension. Aspirin response status was associated with coadministration of beta-adrenergic blockers and with history of myocardial infarction.

Conclusion: The prevalence of antiplatelet resistance in our population was in line with that reported for Western populations. Clopidogrel response was significantly influenced by the presence of CYP2C19 polymorphisms. Interestingly, the concomitant use of aspirin had a significant impact on platelet response to clopidogrel, indicating a synergic interaction between these drugs.

IS ONE-SIZE-FITS-ALL STRATEGY ADEQUATE FOR PLATELET STORAGE?

Eker Ibrahim, Yilmaz Soner, Cetinkaya Riza Aytac, Unlu Aytekin, Pekel Aysel, Acikel Cengizhan, Sebahattin Yilmaz, Gursel Orhan, Avci Ismail Yasar

BACKGROUND: Preservation of platelets in room temperature increases their recovery and survival in vivo following transfusion, thus their use decreases the frequency of prophylactic platelet transfusions and the risk of alloimmunization in patients with hypoproliferative thrombocytopenia.

However, recent data also shows that the viability and hemostatic functions of platelets preserved at 4°C are higher than the platelets preserved at 22°C. We investigated and compared the viability and hemostatic functions of platelets stored at both 4°C and 22°C.

MATERIALS AND METHODS: Fresh apheresis-derivated (ADPs) and random platelet suspensions (RPSs) (n = 10 per group) were assigned to storage at 4°C or 22°C in agitators for 48 hours. Viability levels and hemostatic functions of all groups were assessed using flow cytometry and thrombin generation tests, respectively.

RESULTS: The mean platelet counts of ADPs and RPSs were 1195.2 ± 153.5 x 103 /μL and 1008 ± 304.9 x 103 /μL, respectively.

There were no statistically significant differences between the viability of platelets in ADPs stored either at 4 °C or 22 °C for 48 hours. Likewise, viability of platelets in RPSs stored either at 4 °C or 22 °C for 48 hours were not statistically significantly different. Although the ADPs and RPSs stored at 4 °C for 48 hours generated higher total thrombin levels, the differences were not statistically significant when compared to suspensions stored at 22 °C.

On the other hand, ADPs and RPSs at 4 °C for 48 hours groups generated statistically significantly higher peak thrombin levels. Moreover, 4°C/48 hours group ADPs' time to thrombin generation and time to peak thrombin generation were significantly shorter. (Table 1, 2)

CONCLUSION: The viability of platelets stored at 4°C was not different but their hemostatic activity was higher than the platelets at room temperature.

Given the superior safety profile of refrigerated platelets for bacterial contamination, creating storage conditions for specific transfusion targets may be a prudent approach, especially for improving the outcome of bleeding trauma casualties of military conflicts.

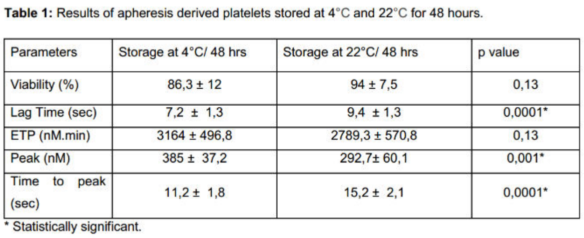

Table 1: Results of apheresis derived platelets stored at 4°C and 22°C for 48 hours.

Parameters	Storage at 4°C/ 48 hrs	Storage at 22°C/ 48 hrs	p value
Viability (%)	86,3 ± 12	94 ± 7,5	0,13
Lag Time (sec)	7,2 ± 1,3	9,4 ± 1,3	0,0001*
ETP (nM.min)	3164 ± 496,8	2789,3 ± 570,8	0,13
Peak (nM)	385 ± 37,2	292,7± 60,1	0,001*
Time to peak (sec)	11,2 ± 1,8	15,2 ± 2,1	0,0001*

* Statistically significant.

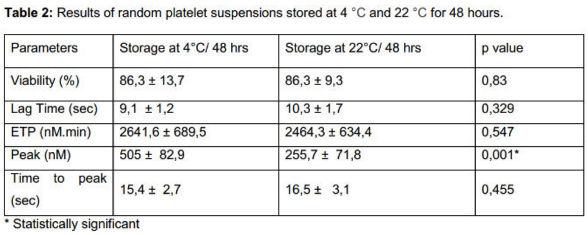

Table 2: Results of random platelet suspensions stored at 4 °C and 22 °C for 48 hours.

Parameters	Storage at 4°C/ 48 hrs	Storage at 22°C/ 48 hrs	p value
Viability (%)	86,3 ± 13,7	86,3 ± 9,3	0,83
Lag Time (sec)	9,1 ± 1,2	10,3 ± 1,7	0,329
ETP (nM.min)	2641,6 ± 689,5	2464,3 ± 634,4	0,547
Peak (nM)	505 ± 82,9	255,7 ± 71,8	0,001*
Time to peak (sec)	15,4 ± 2,7	16,5 ± 3,1	0,455

* Statistically significant

THE PREVALENCE OF PARASITES IN DONKEYS FROM DIFFERENT REGIONS OF GREECE

Starras Alexandros, Dimanopoulou Anastasia

Aim: The purpose of present study was to investigate the prevalence of donkey parasites in Greece.

Materials- Methods: The total number of the donkeys examined was 143. From every donkey included in the survey there were collected samples of faeces directly from the rectum and blood samples from the jugular vein. Furthermore, every animal was inspected in detail so as to find ectoparasites such as: a) lice especially in the body regions of neck, crest, withers, shoulders and back, b) ticks in all over the body, c) mange mites with the use of scraping test from parts of the skin appearing relative symptoms and d) fleas by using a specific comb to examine the presence of the parasites or their faeces.

Results: The endoparasites found in donkeys' faecal samples were strongyles (71.32%), Anoplocephala spp. (29.37%), Parascaris equorum (18.18%), Strongyloides westeri (6.29%), stomach spirurids (Habronema spp, Draschia spp.) (3.49%), Oxyuris equi (1.39%) and Dictiocaulus arnfieldi (1.39%). On the other hand, the only ectoparasites found in this survey were the lice species Damalinia equi (14.68%).

Conclusions: Donkeys, as reservoir host for equine parasites, can affect the health of other equids still used in many native armies. Moreover, nowadays donkeys are used for milk production. Hence, knowledge about their health status is also essential for public health.

SHOULD SANITARY DOOR OPENER BE USED TO PREVENT BACTERIA TRANSMISSION VIA THE HANDS OF KITCHEN WORKERS?

Celik Cagatay, Oz Baris, Topcu A.

BACKGROUND: WHO emphasizes the importance of preventing healthcare-associated infection (HCAI) by giving priority to the promotion of hand hygiene best practices in health care. poor hand hygiene has effects on Food Hygiene and Safety which may cause deadly foodborne illnesses all over the world. We aimed to determine bacterial flora on door handles as one of the main points of cross contamination in facility, and assess efficacy of "Sanitary Door Opener" as a troubleshooter.

METHODS: The study was carried out different 80 barracks in 3 months. Merck Swab Test Kit - Envirocheck® Contact C (Merck 1.02136) slides were used to obtain surface samples and bacteria counts. Samples were collected spontaneously from restrooms' door handles in facility used by dining facility and kitchen workers.

RESULTS: Eighty samples were collected and evaluated for Total Aerobic Bacterial Count and Coliform Bacteria Count. The results were obtained from plate count agar by counting colonies to obtain total aerobic bacterial count and from Chromocult coliform agar by counting red colonies to count coliforms. Overall, 81.25% and 81.25% of samples were positive for total aerobic bacteria and coliform bacteria, respectively.

CONCLUSION: Coliform microorganisms were present at significant levels, 81.25%, on restroom door handles in dining facilities and kitchens. At the handling level of food processing in kitchens, Coliform microorganisms are indicator bugs as in the other levels. Therefore, should take preventive measures to get rid of possibility of contamination from hand to hand by using restroom door handles. This study recommends use of ''Sanitary Door Opener'' more than the other options such as automatic folding door in terms of the cost effectiveness.

PERIPHERAL NERVE INJURY AND NEURON REGENERATION

Andreea Benga, Fatih Zor, Ahmet Korkmaz, Bogdan Marinescu

Peripheral nerve injuries can be most disabling, resulting in loss of sensitivity, motor function and autonomic control in the involved anatomical segment. Although capable of regeneration there is still no optimal solution for their reconstruction. Nerve regeneration has several vulnerable points that could be addressed to improve outcome: (1) neuron survival after trauma, (2) gap crossing and orientation of neurites, (3) neuroma formation, (4) target-organ trophicity.

Inflammation is an important sequence in nerve regeneration. Cytokines released by macrophages and fibroblasts have mitotic effect on Schwann cells. Fibroblasts are present in epineurium, perineurium and endoneurium. If they proliferate extensively, scar formation occurs that impedes nerve regeneration. Scar tissue contains repulsive proteoglycans (such as chondroitin sulfate) and semaphorins that inhibit neurites' elongation.

Nerve repair still faces two immense challenges: fascicules orientation that could be addressed by improving the surgical technique and the time race against fibrosis and end-organ atrophy amenable to pharmacological therapy.

The aim of this study is to review the literature for the most frequent chemicals used in experimental studies to address neuron survival, reduce scar formation and improve overall nerve regeneration.

SEASONAL INFLUENZA VACCINATION: FACILITATING OR INHIBITING FACTORS

Mellidis Christos, Sfikas Georgios, Katsimardou Alexandra, Iosifidis Georgios, Iosifidou Eleni, Koumaras Charalambos, Doumaki Eleni, Psomas Evangelos, Tapazidis Vasileios

Purpose: We investigated the percentage of influenza vaccinated patients among those who where clinically examined in the ER by an internal medicine specialist. Reasons for this percentage were sought.

Materials and methods: A total of 6528 patients with influenza symptoms visited the ER of 424 General Military Hospital during the influenza outbreak periods (November till March) from 2008 till 2014. During history taking, patients were given a relative questionnaire.

Results: High risk patients were found to be vaccinated 39% of the time, whereas 45% of the total patients examined were vaccinated. Reported reasons for the vaccination were: desire to avoid illness (61%), family doctor advice (35%) and desire to avoid illness spreading in family environment (4%). Reasons against the vaccination were: personal belief that one does not belong to a high risk group (37%), lack of family doctor guidance (48%), family doctor omitting vaccination (12%), vaccines shortage in the pharmacies (3%).

Conclusions: Total influenza vaccination coverage rate is low. Consulting high risk groups and general population about influenza and vaccination mainly via family doctors will aid in: increase of coverage rates among high risk groups, avoidance of vaccine shortage in the market.

OP session 1 – Students session

EFFECTS OF LPS AND IFN-γ COMBINATION ON MATURATION AND FUNCTION OF HUMAN MONOCYTE DERIVED DENDRITIC CELLS

Danilovic Milos, Isailovic Jelena, Stevanovic Jovana, Tomic Sergej, Mihajlovic Dusan, Colic Miodrag

OBJECTIVE: Dendritic cells (DCs) are potent antigen-presenting cells, which are increasingly being investigated in therapy of malignance. Their maturation is mostly induced by standard cocktail containing tumor necrosis factor-α, interleukin (IL)-1β, IL-6 and prostaglandin E2. Previous findings suggested that interferon (IFN)-γ stimulates the synthesis of IL-12, which mediate a strong anti-tumorous immune response via T-helper (Th1) cells. Additionally, lipopolysaccharide (LPS) is a known DC maturation agent acting via Toll-like receptor (TLR)-4. Therefore, we compared the effects of LPS/IFN-γ combination and standard cocktail on maturation, allostimulatory and Th polarization capability of human

monocyte-derived DCs.

MATERIAL & METHODS: DCs were generated by cultivating monocytes with granulocyte macrophage colony-stimulating factor and IL-4 for 6 days. Immature DCs were then stimulated with the cocktail or with LPS/IFN-γ combination. After 24h, phenotypic analysis and DCs functions were investigated.

RESULTS: The cocktail induced a higher expression of HLA-DR, CD54 and CD83, but a lower expression of CD40 by DCs. LPS/IFN-γ-stimulated DCs produced more IL-12, but less IL-23. In addition, the co-culture supernatants of LPS/IFN-γ-matured DCs and allogeneic CD4+T cells contained higher concentrations of IFN-γ and IL-17, compared to the co-cultures with the cocktail-treated DCs. In contrast, cocktail-matured DCs induced a stronger allogeneic CD4+T cell proliferation.

CONSLUSION: Although the maturation protocol with LPS/IFN-γ exhibits weaker phenotypic maturation potency for DCs, it induced much stronger Th1 and Th17 immune responses than the protocol with standard cocktail.

IMPACT AND INTERVENTIONS OF MUSIC ON HEALTH

Charitidis Nektarios, PagkratI Vasiliki

Purpose: Music is a universal feature of all human cultures and appreciated as such. The primary and specific aim of this study is to record the therapeutic effect of music as a healing tool for hospitalized patients and its' effect on a wide range of diseases. The secondary aim, is to investigate and document the positive contribution of music in all branches of general public health.

Material and Methods: Collected data came from a review of the international bibliography (research studies and articles found on Pub Med).

Results: From the comparison and screening of the collected data, most studies confirm and document the beneficial effects of music on separate and general fields of health. Regarding hospitalized patients, music helps and enhances the healing and recovery process of many diseases and unbalanced health conditions; it also generates a serene, stress-free and patient-friendly hospital environment for patients, within which, healthcare professionals can perform "lege artis”. In terms of general public health, research proves that music is a beneficial factor that improves productivity and stamina and also results in physical, social and mental well-being, by reducing stress, depression, grief, loss, sorrow and similar emotional states.

Conclusion: Music is a low-cost, universal and multicultural therapeutic tool that can be used as an alternative therapy in many cases, a fact well supported by search-based findings, but not yet accepted by the majority of medical community. Therefore, healthcare professionals should educated further in order to implement and evolve music in patient care, because of its effectiveness.

MULTIPLE CASUALTIES INCIDENTS – THE IMPORTANCE OF EARLY SPECIFIC TRAINING OF MILITARY MEDICAL PERSONNEL

T.C. Mures, Maria M. Ancuta, R. A. Zamfir, B. S. Zamfir

Introduction: MCI has always been a challenge even for well-developed health systems. The geo-climatic and socio-economic current conditions clearly predispose us to increased incidence and magnitude of such events. Moreover, the current trend of the evolution of armed conflict to asymmetric and unconventional confrontations causes increasingly frequent incidents which involves civilians in armed military conflicts, representing an additional risk factor. In this context, the contribution of military medicine at the management of civil MCI can be particularly valuable both in human terms and in terms of capabilities.

Material: The sudden and unexpected onset, the major destructive effects, unpredictable developments and major impact both physical and psychological on the intervention personnel are the main features of the MCI. These characteristics superimposed over a high level of current activity destabilize the health systems. To ensure an adequate emergency response, procedures are conducted after specific and complex operation plans that differ substantially from those used in the current work routine. The knowledge and application of these procedures accurately by the staff involved is essential to ensure actional interoperability between the structures participating in the intervention.

Conclusion: Acquiring, maintaining and improving knowledge and skills needed to perform, in conditions of extreme physical and mental stress, the complex activities provided in emergency response procedures require completion of a gradual and continuous program of specific training. Ideally, this program will begin as early as possible in the medical career.

CONSIDERATIONS REGARDING TACTICAL

EVACUATION CARE

Iulia-Madalina Staicu

Introduction: Lessons learned from more than a decade of war in Afghanistan showed that many potentially preventable deaths have occurred until casualties reached a medical treatment facility (MTF).

Materials and methods: Research work on the available medical literature by searching databases (Medline, Wiley, Science Direct), selected articles published since 2012 to 2015. Search terms included: medical treatment facility, tactical evacuation care, stress, aeromedical assets.

Results: Our paper deals with one important factor which could reduce these deaths – the third phase of tactical combat casualty care – tactical evacuation care (TEC). After a brief history regarding medical evacuation, we describe the concept of TEC with stress on some important factors which influence the mortality during this phase of care when are used aeromedical assets.

Conclusions: Almost 90% of all combat deaths occur before the casualty reaches a Medical Treatment Facility (MTF). One of the most important factors to reduce the mortality in tactical evacuation phase of care is to have well equipped evacuation assets, manned with well-prepared medical personnel.

RELATIONSHIP OF SOLITARY GLIOBLASTOMAS TO THE FUNCTIONAL REGIONS OF THE BRAIN

Bulatovic Jelena, Ljubenovic Nenad, Hadzija Helena, Despotović Dragana, Peric Predrag

OBJECTIVE: Maximal safe resection of glioblastoma (GB) depends on its size and anatomical localization (AL). Functional localization (FL) limits or disables tumor resectability.

AIM: Determination of AL and size of GB, and their relationship to the functional brain regions (FBRs).

MATERIAL & METHODS: We analyzed pre-operative magnetic resonance imaging of the brain in DICOM format of 19 patients with primary, solitary GBs of the brain. The size of GB was determined as maximal clinical diameter (MCD) of tumor hyperintensity signal on T2W/FLAIR sequence, and as maximal morphological diameter (MMD) of tumor contrast enhancement on T1W+C sequence. FL was defined as FBRs according to Sawaya and co-workers.

RESULTS: In analyzed patients, GB was frequently localized in temporal lobe and eloquent brain regions (52.6% and 42.1% respectively). In females, GB was significantly more frequent in eloquent regions (p = 0.039). Independently to the tumor FL, significant correlation between MMD and MCD (p = 0.022) was found, as well as highly significant difference between them.

DEVELOPMENT OF A POLICY TO INCREASE DETECTION RATES OF RARE DISEASES

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Introduction: The term “Orphan Diseases” refers to extremely rare medical conditions, rare being 1:2000 in the population according to the European definition and affecting less than 200.000 people worldwide according to the American definition. There are 6 to 8 thousand rare diseases on Earth. Countries use different strategies and policies to treat or cure these conditions. First in 1983 in America, then in 1999 in Europe, different political approaches have been adopted. Rare diseases constitute a global problem; thus, they require creation of shared databases and extensive studies. We don’t have an extensive study about rare diseases in our country yet.

Method: First, the website “orpha.net” was utilized to collect information about the 100 most common rare diseases. Then, our hospital’s data center was searched for records of the ICD-10 codes of these diseases between January 2011 - February 2015.

Results: We found 11.238 diagnoses of rare diseases among these admissions. We analyzed some clinical qualifications of these patients in SPSS.

Discussion: We aimed to acquire epidemiological data about these diseases, since there was not a related research in our country. If this research can be applied in other hospitals in the country, nationwide epidemiological results can be achieved, and a shared database for further studies will be possible.

PUBLIC AWARENESS IN CHRONIC DISEASES

Eda Demirtas, Bekir Dogan, M. Emin Karayigit, Yavuz S. Yalcin, Faruk Colak, Sevde B. Okuducu, Nurperi H. Acar, Aydın Bagdat, Okkes Yigit, Muhsin F. Yildirim, Abdulgani Bakar, Sumeyra Altun, Merve Arslan, Ugur Isik, Unal

Zengin, Ilker Yilmaz

Objective: By definition, a chronic disease is one lasting 3 months or more. Chronic diseases constitute 63% of all death causes. The most frequent causes of chronic disease are: 1. increased blood pressure (13%), 2. tobacco products (9%), 3. increased blood glucose (6%), 4. insufficient physical activity (6%), 5. overweight and obesity. We aimed to increase the awareness of people about hypertension, cigarette, diabetes and obesity risk factors.

Material- Method: Four different risk evaluation forms were developed to assess four major health problems of cigarette, diabetes, hypertension and obesity. Then, informative posters were prepared and communication booths were established in our hospital. Blood sugar levels, blood pressure, waist circumference, height and weight were recorded for every visitor. In light of the measurements, we filled the risk evaluation forms for each patient. We recommended patients whose conditions were found to be risky in terms of four health problems to visit related outpatient clinics. We also noted the contact information of the patients for further follow-up studies.

Results: In light of both our observations during the activity and results obtained from the risk evaluation forms, consciousness and awareness were significantly insufficient among the patients. Overall, our data suggest patients do not tend to quit cigarette unless they had had heart attack or malignancy. Also, patients do not develop healthy nutritional habits unless they are diagnosed with diabetes. There were some patients whose blood pressures were measured for the first time in their lives.

Conclusion: Public awareness in chronic diseases is lower than expected. Further studies aiming to inform the public about complications of chronic diseases are urgently needed.

EVALUATION OF ENVIRONMENTAL HEALTH AND CONDITIONS OF STUDENT LIVING AREAS IN A FACULTY OF MEDICINE

Zeynep Eslem Gokcek, Furkan Taha Birinci, Burak Cengeloglu, Fethi Dilber, Hulya Kapucu, Talha Kivanc, Muhammed Muaz Kusen, Ali Uygun, Burhan Poyraz, Yavuz Selim Yalcin, Recai Ogur

Objective: Environmental health is focused on the natural and built environments for the benefit of human health. In this study, we focused on 5 major factors that we considered are most influential on our campus life: microbiological air quality, electromagnetic radiation (EMR), noise, swab culture and illumination.

Methods: Microbiological analyses, EMR, noise measurements, swab culture and illumination measurements were carried out in the following places with respective specific devices: dormitories, study halls, bathrooms, public toilets, hallways and cafeterias.

Results: After the statistical evaluation of microbiological measurements, statistically significant differences were found between room population and bacterial pollution (p=0.020). For EMR, a statistically significant difference was found between room population and study rooms' measurement at 20.00 PM. Also, statistically significant differences were found between room population and EMR measurements at 01.00 AM (p=0.028, p=0.016; respectively). Also, there were statistically significant differences between noise measurements at count time and 01.00 AM (p<0.001) A significant statistical difference was found between gender and colonization in fridges according to colony counts of swab cultures (p=0.011). There were statistically significant differences between room types and illumination in all conditions of lighting (p<0.01).

Conclusion: Conditions and problems of our faculty were investigated using 5 major parameters.

OP session 1 – Nurses session

TURKISH NURSING STUDENTS' KNOWLEDGE OF AND ATTITUDE TOWARD HIV/AIDS AND THEIR

WILLINGNESS FOR CARE RELATED TO PATIENTS WITH HIV/AIDS

Kok Gulsah, Guvenc Gulten, Kaplan Zeliha

OBJECTIVE: Studies have shown that healthcare providers have negative attitudes toward and fear of people living with HIV/AIDS because of lack of knowledge and the stigma attached to the disease, and fear of the possibility of becoming infected, and hesitate to provide care to HIV/AIDS. The aim of this study was to determine Turkish nursing students' HIV/AIDS knowledge, attitudes, and willingness care related to patients with HIV/AIDS.

MATERIAL AND METHOD: Cross-sectional design was used in this descriptive study. Participants were nursing students from a School of Nursing in Ankara, Turkey during 2013–2014 academic years. A total of 325 nursing students volunteered to attend this study and all of them were female. Data collection form, HIV/AIDS Knowledge Questionnaire, and AIDS Attitude Scale were used for data collection. The data were analyzed using SPSS for Windows version 15.0.

RESULTS: The mean age of participants was 20.56±1.05. The mean score for AIDS attitude scale was 49.50±10.57. The mean subscale scores for contagion, negative emotions, and professional resistance were 20.88±4.24; 12.99±3.64; 15.61±6.10 respectively. The mean knowledge score was 13.76 ±2.88, and mean score for willingness to care was 4.30±1.76. There were significant differences in median scores of knowledge, AIDS attitude scale and subscales among nursing students.

CONCLUSION: The current results reveal that nursing students have moderate level of knowledge about HIV/AIDS and that their attitudes need to be improved. Inadequate knowledge, negative attitudes towards patients with HIV/AIDS, and consequent fear may result in less than optimal care for patients with HIV/AIDS.

FLIGHT NURSE SCHOOL IN THE HELLENIC AIRFORCE

Katsika Alexandra

Purpose: To inform about the operation of Flight Nurse School in the Hellenic Airforce, under the Aerospace Medicine Centre, in order to provide specialized nursing personnel for air evacuated patients, during peace and war time, as well as to advance the quality of nursing care and ensure their ability to respond in every emergency condition involving medevac. The structure and performance of school conducted in accordance to US/Canada FN Schools, corresponded to the abilities and needs of our country.

Material: Data were collected from Flight Nurse School records and the Operation's Regulation of Aerospace Medicine Centre.

Results: Since 1988 a total number of 284 Officer Nurses and 27 petty officers of the HAF, 11 Officer Nurses of the HN, 20 Officer Nurses of the HA and 24 civilian paramedics of the Hellenic National Emergency Aid Centre, have graduated from school Education at school includes:

- Theoretical part about stresses of flight, mission planning, emergency situations in flight, combat casualty care and triage.
- Practical part includes low pressure chamber, practice in sea survival and real flights.

Conclusions: FN School offers a unique and broad field to professional nursing, with an opportunity to serve patients, community and Armed Forces as a basic part of Aerospace Medicine. Greece is one of NATO members that can afford specialized Flight Nurses during Medevac, according to NATO STANAGS, while we are considering the possibility of training others' countries officers, as soon as specific difficulties will be overcome.

NURSE'S ROLE IN LASER ASSISTED REFRACTIVE SURGERY

Mihai Florentina, Grigore–Ghita Ana–Maria

Introduction: Refractive surgery is one of the most rapidly evolving fields in ophthalmology. It is used to correct refractive errors such as myopia, hyperopia, astigmatism and presbyopia.

This field of surgery evolved rapidly with the development of excimer laser assisted procedures, the most commonly used being LASIK.

Systematic review methodology: This study is a review on laser assisted refractive surgery, emphasizing the role of the nurse in patient's care.

Results: A critical part of this procedure is the perioperative care of the patient and, as such, the nurse. Nursing involves deep human interpersonal relationships between nurses and patients and it is an essential part of the patient's care.

Conclusions: The nurse is a critical part of the perioperative care of the patient.

If a patient decides, after careful consideration, to do this procedure, he must meet some criteria, carefully selected to limit the postoperative complications.

Even if patient’s satisfaction after this type of procedure is usually high, there are various complications that may occur, depending on the type of procedure used.

BREAST CANCER IN YOUNG WOMEN

Margareta Popa

Living with breast cancer can be a difficult proposition. In women who have had a mastectomy, altered body image can be a challenge. Young women are especially vulnerable because of their age.

Breast cancer at a young age though the age definition for young is very variable, has been reported to pursue a more aggressive clinical course and to be associated with a more unfavorable prognosis compared with the disease in older patients.

About 5-10% of all breast cancer is thought to be hereditary. When a women is diagnosed with breast cancer at a young age (i.e. under 40 years), we are concerned that she might have developed her cancer due to an inherited predisposition.

Young women who learn they carry a mutation in a gene conferring a high risk of breast cancer must make a plan for managing their cancer risk over time. Today, there are several option for women, but unfortunately, all involve significant trade-offs:

- Increased surveillance
- Prophylactic surgery
- Chemoprevention

Diagnosis of breast cancer presents the young woman with a whole range of challenges. In addition to the physical and existential threats, there are the prospects of disfiguring surgery and complex adjuvant treatment with its unpleasant side effects. She is also at risk of psychological, familial, occupational, financial and social problems.

Breast cancer at young age often occurs before reproductive plans have been completed and reproductive issues, including fertility, pregnancy, contraception and menopause, are therefore of great importance for this growing number of young breast cancer survivors.

Expectations of young women concerning psychological intervention are slightly different from the needs of the older patients. However, family support is the most important for them, there is strong expectation of psychological support from clinical professional. Availability of such help should be maintained much more intensely.

PRESENT AND FUTURE OF HEARING AIDS

Ileana Mariana Mateș

The hearing aids (Receiver-In-The-Ear, Behind-The-Ear, In-The-Ear, Canal Hearing Aids, Cochlear Implants, Bone Anchored Systems) have performed a vital process for people suffering of hearing problems for almost two centuries, but fortunately they have rapidly evolved after decovering the microprocessor in 1970s.

If we look to the future, scientists are working for developing new and exciting solutions in hearing aid design. Scientists from all over the world are developing future devices including

- laser-signalled hearing aids with a larger frequency range,
- permanent in-ear hearing aids which never have to be removed because they use induction charging rather than batteries.
- regeneration of damaged parts of the inner ear using stem cells

In our days, the major players in the hearing aids market are Cochlear Limited (Australia), GN Store Nord A/S (Denmark), MED-EL (Austria), SeboTek Hearing Systems, LLC (U.S.), Sivantos Pte. Ltd. (Singapore), Sonova (Switzerland), Starkey Hearing Technologies, Inc. (U.S.), Widex (Denmark), William Demant Holding A/S (Denmark), and Zounds Hearing, Inc. (U.S.).

The hearing aid devices segment is expected to account for the largest share of the global hearing aids products market in 2016, and the global hearing aids market is expected to reach USD 8,373.9 Million by 2020 from USD 6,183.3 Million in 2016.

OP session 2 – Aerospatial and diving medicine

THE INFORMATION SEEKING BEHAVIOR OF GREEK AIR FORCE MILITARY DOCTORS

Evangelos Persakis, Petros Kostagiolas, Eleni Lazaridou, Georgios Toloumis

Aim: This paper aims to study the information seeking behavior of Greek Air Force military doctors and thereafter inference on the role of information needs satisfaction at operational level. We call this interaction "INFORATION" combining the words "information and operation."

Material – Method: The research was based on empirical evidence from a survey informed by Wilson's macro model of information-seeking behavior. The data includes 101 military doctors of 251 Air Force General Hospital, in Athens, Greece.

Results: The method was successful in identifying operational information needs, information resources employed and the obstacles military doctors face when seeking information. The most important information needs of the physicians were related to diagnosis, treatment, research, updating of knowledge and instruction-educational issues. Online resources such as scientific databases, search engines and scientific journals were the most important information resources, while the major obstacle encountered by the doctors was the lack of access to online scientific databases from the workplace. Significant associations were identified and discussed.

Conclusions: The information seeking behavior and needs of Greek Air Force military doctors should be, at operational level, addressed as an integral part of the development of information systems and information services especially for that category of doctors.

NIGHT VISION DEVICES USE IN HELICOPTER AIRCREW - HUMAN FACTOR ASPECTS

Stefanescu Cristina, Stefanescu Cristian-Dragos

Introduction: Night Vision Devices (NVD) have become an essential component of modern military aviation. It offers enhanced performance qualities compared to unaided night vision, more useful during the execution of missions at night, but at the same time their use is tributary to some limitations that should be known and accepted (both technical, and human factor related).

Material and methods: We used an anonymous questionnaire (transversal observational study) applied to helicopter pilots using different types of NVD (military flight), totaling 46 respondents. Thus we could analyzed various aspects of using these devices in terms of human factors (assessment of their benefit in terms of morphoscopic acuity for different distances, stereoacuity and depth perception, visual field extend ,contrast sensitivity quality, benefits in different phases of flight and in various flight conditions), and problems faced by the user: the fatigue phenomenon (description, cause, duration), spatial disorientation and "black hole illusion", if the simulator can reproduce the situations faced by the pilot in real flight, and the importance of a basic professional course as well as continuous training.

Conclusions: The aria of concerns about flight safety must include the mandatory use of NVD. Most important for quality skills are proper and continuous training.

ROMANIAN MILITARY MEDICINE CONTRIBUTION IN AIRMEDEVAC CAPACITY BUILDING IN BOSNIA AND HERZEGOVINA

Pintilie Daniel-Iacob, Tudose Dragoş-Cristian, Nică Daniel-Florin

As of 2008, Bosnia and Herzegovina applied for NATO membership as Partnership for Peace member. Therefore, in order to achieve NATO standards, one of the objectives was to develop a structure to provide AirMEDEVAC capabilities for Bosnian and NATO forces as well. The project started in 2010 with Romanian Air Force Mobile Training Team as part of ALTHEA mission in BiH, and continued from 2012 with Romanian Air Force AirMEDEVAC Advisory Team. The Romanian Air Force developed an Air Medevac structure, evaluated by NATO through STARTASSES process.

Materials & methods: This paper provides a review of the most important aspects of the capacity building regarding AirMEDEVAC capability: available resources, project development, challenges, and further development. The project is to be evaluated by NATO during 2017.

Results: The long term mission and the tremendous logistic, medical and operational challenges resulted in building an updated functional structure in BiH Armed Forces dedicated to Air Force missions in general and medical evacuation in particular.

Conclusion: The recent experience of Romanian Air Force in developing an AirMEDEVAC structure is of help for surpassing the tremendous challenges encountered by BiH Armed Forces in the process of building their own AirMEDEVAC capacity.

DECOMPRESSION RISK AND INTENSIVE PHYSICAL EFFORT AFTER DIVING

Shopov Nikola, Vazharov Ivaylo

Introduction: The effect of post-dive exercise on bubble formation remains controversial. There are studies, including ours, showing that moderate physical activity before diving can reduce the risk of decompression. One 2006 study suggests that heavy post-dive exercise might be beneficial, although it was done on fit military. After the number of cases of decompression sickness (DCS) in divers involved in catching shells does not diminish, our hypothesis is that heavy exertion immediately after diving is probably inadvisable.

On the other hand the most likely factor initiating DCS is believed to be the formation of bubbles in the blood and the tissues. It is generally accepted that the incidence of DCS is lower when few or no bubbles are present in the circulation from hyperbaric exposures. Further, it is hypothesized that heavy anaerobic efforts immediately after the dive will significantly increase Doppler detected bubbles, which may be the reason for the increase in cases of DCS.

Purpose: The purpose of this study was to examine the impact of strenuous physical activity after a dive on decompression risk.

Materials and Methods: 22 non well-trained divers participate for this study (average age: 32 ± 8 years and Body Mass Index: 24 ± 4.2 kg m^{-2}). Each diver performed two type of dives (during 3 days) in a hyperbaric chamber in depth 18 m for 60 min with decompression stop on 3 m for 5 min according to the French Tables de plongee a l’air Marine Nationale (MN 90). One type dive is without performing physical effort after decompression and after the second dive was carried out intense exercise. Physical exercise consisted of two cycles of five minutes, with one minute rest, consistently performing 10 crunches, 5 push-ups and 10 squats with 20 kg weight. After each dive be performed Doppler monitoring by using a bi-directional Doppler (BIDOP ES 100-V3 – HADECO, Japan) with 2MHz transducer. Bubbles are detected in the precordial area with the usage of the Kisman-Masurel code. Monitoring was performed at 30, 60 and 90 min after surfacing and we observed for the maximum bubble grades - MBG (bubble peak). The bubble grade was subsequently used to calculate the Kisman integrated severity score (KISS). KISS was assumed to be a meaningful linearised measure of post-decompression intravascular bubble activity status that may be treated statistically.

Results: Anaerobic intensive workloads significantly increased Maximum Bubble Grades (Δ = 0.53; r = 0.718**; p < 0.01) and KISS (Δ = 6.72; r = 0.869**; p <0.01). Nobody demonstrated a decrease in venous bubble grade after dives with exercises.

Conclusion: These results suggest that post-dive strenuous exercises after a single dive significantly increased post-dive gas bubble formation in poorly trained divers and perhaps leading to increased of decompression risk.

COSMIC RADIATION AFFECT ON WOMEN WHO USE WARPLANES: A CUMULATIVE DOSE STUDY

Ozturk Mustafa

BACKGROUND During flights, pilots, flight personnel, and passengers are exposed to cosmic radiation originating from galactic rays and solar system. The term cosmic radiation is the conjunction of solar and galactic radiation that originates from the sun and other planets and stars, respectively. Cosmic radiation consists of neutrons, protons, electrons, and photons, and human skin is permeable to these particles, which can cause alterations within live cells on a molecular level. This article reviews the effects of cosmic radiation on women who use warplanes.

MATERIALS AND METHODS We calculated the cosmic radiation exposure of women who use warplanes. We implanted cosmic radiation calculators into pilots’ (F 16, CASA and helicopter) uniforms. Exposure to cosmic ionizing radiation was calculated by the Turkish Atomic Energy Authority after 1 month. All flights were calculated according to July datasets because the maximal radiation exposure effect is considered to be in the month of July.

RESULTS According to the reports of the ICRP in 2003 and 2007, doses below 10 rads (1 rad = 10 mSv) have no demonstrable influence on humans. For nonpregnant people, the cosmic radiation response dose has been established at 50 mSv per month. The allowed radiation doses are 0.5 mSv per month during pregnancy. Domestic flights do not exceed the recommended monthly limit for women.

CONCLUSION We evaluated the maximum cosmic radiation exposure effects on women using warplanes. The exposure for pilots was found to be below the risk level.

AEROMEDICAL EVACUATION OF ABDOMINAL GUN SHOT WOUNDED PATIENTS IN POSTOPERATIVE EARLY PHASE

Acar Yahya Ayhan, Uyguner Cem, Tezel Onur, Salman Necati, Dede Hakan

OBJECTIVE: There is still debate on optimal post-operative time for aeromedical evacuation of abdominal surgery patients. This study aims to evaluate the risk factors associated with aeromedical evacuation of patients with abdominal gunshot injury in early postoperative phase.

MATERIAL AND METHOD: This is a retrospective analysis of abdominal gunshot wounded patients which were transported by air ambulance system of Turkish Armed Forces. Data were collected retrospectively from the patient records between 1998 and 2015. Patients divided into two groups according to postoperative transport time; patients transported within postoperative three days (GROUP 1) and later than three days (GROUP 2). Besides demographic data, flight crew number, pre- and post-transport vital signs of the patient, airway status (spontaneous, mechanically ventilated), vasopressor and/or volume replacement therapies used in flight were recorded. SPSS 15.0 (SPSS Inc., Chicago, IL) was used for statistical analysis and $p<0.05$ considered as statistically significant.

RESULTS: A total of 217 patients were enrolled in the study. All of them were transported with a fixed wing aircraft (CN-235) air ambulance. No deaths occurred during transport. There was not any difference in age and gender between two groups ($p=0.385$ and 0.244, respectively). Mechanically ventilated patients and vasopressor use showed a higher rate in group 1 ($p=0.001$ and 0.011, respectively). There was not any difference in pre- and post-flight systolic blood pressure levels and flight crew number between two groups ($p=0.122$, 0.775,and 0.865, respectively).

CONCLUSION: Patients with abdominal gunshot injury can be transported safely by air ambulance in early postoperative phase.

THE ROLE OF MILITARY AIRCRAFTS IN TRANSPORT OF ROMANIAN CITIZENS INVOLVED IN MASS CASUALTY INCIDENTS

Leonard-Marin Lupu, Bogdan Teusdea

A mass casualty incident (MCI) is any incident in which emergency medical services resources, such as personnel and equipment, are overwhelmed by the number and severity of casualties.

A MCI can involve many and varied types of responders and agencies. Once an MCI has been declared, a definite and well coordinated flow of events will occur, using three separate phases: triage, treatment, and transportation. When a complex type of injures are present (for example severe burns or major trauma injuries), which need special medical facilities to be treated, then the situation may become critical considering the public impact of the incident.

A complex process must be implemented very quickly in order to identify domestic or foreign hospitals specialized in burn or trauma treatment, to make all the necessary arrangements, to transport the patients in a proper way and to sustain all this efforts.

The use of military medical aircrafts proved to be a valid option for long-distance evacuations. Military assets could play a great role in an MCI situation but without preplanning and scenario based exercises this missions may become difficult.

PATIENTS’ SATISFACTION LEVEL IN A GREEK MILITARY HOSPITAL

Trachanatzis Theodore

Aim: The last decades, the interest of health care professionals increasingly focuses on patients’ satisfaction level of the provided care. The cost of hospitalization coupled with general quality has been the subject of a heated debate. The main objective of this research is to estimate the patients’ satisfaction level in the 251 Air Force Hospital of Athens. Additional objectives are the investigation of the factors that define it.

Material-Methods: The study population consisted of patients just before their discharge from the hospital. 150 questionnaires (close-typed) were distributed within a six-month period (May-October 2014) in total to the orthopedic and cardiology clinics. Of these, 109 were completed correctly and used in the analysis. For the statistical evaluation, Cronbach's coefficient, and the SPSS program were used.

Results: 58.7% of the sample were men. The majority of the participants was above 61 years old (26.6%), married (47.7%), with an average monthly income of 1001-1500 euros. Overall satisfaction in terms of friendliness and courtesy among patient- therapist seemed to be in a high level (81.9% and 84% respectively for the 2 clinics). Furthermore, based on the regression analysis, the education of the patients affects mainly their perception about the quality of the services.

Conclusions: On the whole, useful conclusions have been drawn regarding the provision of care. It is a fact that the high educational level and general competence of the physicians and nurses are the key elements that reflect on the patients' high satisfaction score. Quality is undoubtedly a challenge, and also an inalienable right based on the principle of humanism, and the Hippocratic ideal!

THE PREVALENCE OF THYROID ABNORMALITIES ON ULTRASONOGRAPHY AND ASSOCIATED FACTORS PREDICTING RISK PROFILE IN AVIATORS

Okur Aktaş Gökcan, Karacaer Zehra, Karaduman Mehmet, Tirmik Uzeyir

OBJECTIVE: To evaluate the prevalence of thyroid abnormalities detected on ultrasonography and its relationship with metabolic parameters which are important to describe a risk profile among aviators.

MATERIAL AND METHOD: We retrospectively reviewed medical charts of 259 aviators who were admitted to the Military Aviation Medicine Center for the annual examination in year 2014. Thyroid ultrasonography findings, age, body mass index (BMI), presence of liver steatosis on ultrasonography, laboratory parameters (TSH, fasting blood glucose, BUN, creatinine, total cholesterol, LDL, HDL, VLDL and triglyceride, TC/HDL ratio) were obtained.

RESULTS: Mean age was 35.12±5.9 years. All subjects were male. 67 subjects (26%) had thyroid abnormalities on ultrasonography. According to BMI, 69 subjects (26%) were slightly overweight (BMI 25-30 kg/m2) and of them 19 subjects had thyroid abnormalities on ultrasound (28%). Liver steatosis was found in 67 subjects (26%) and 20 subjects with liver steatosis had thyroid abnormalities on ultrasonography at the same time (17 subjects were overweight and 3 subjects had normal BMI).

Older age, higher BMI were found closely related with the presence of thyroid nodules ($p<0.05$ for all). Presence of liver steatosis and laboratory parameters including TSH were identified as independent predictors for the existence of thyroid nodules.

CONCLUSION: This study showed a positive relationship between metabolic parameters and thyroid abnormalities in aviators. Our results support that increases in thyroid nodules is partly secondary to increase in obesity.

Metabolic and sonographic abnormalities of thyroid gland and liver might be clinical indicators for further evaluation of aviators whose clinical risk profile could be underestimated by classical risk factors and hence, annual examinations for aviators should be designed to include thyroid ultrasonography.

OP session 2 – Emergency medicine and trauma management

COLECTIVE MASS CASUALTY INCIDENT – OUR EXPERIENCE

Negoiţă DD, Teuşdea CB, Toma LM, Butoi A

On the 30th of October 2015 a fire occured in a nightclub where there were a few hundred people, causing tens of deaths and dozens of injuries. A few of them got by their own means to the ED of the University Emergency Central Military Hospital Bucharest. Several of them were brought by SMURD ambulances from 2 other Emergency Hospitals (Floreasca and Coltea) following the Emergency Red Intervention Plan. 11 patients were admitted to ICU during that night. One patient denied admission at his own risk (having minor injuries). We present the peculiarities of management of these burn patients in the ER.

ULTRASOUNDS CAN ESTIMATE CEREBRAL EDEMA IN MANY WAYS THAT CAN ALSO BE APPLIED TO PREHOSPITAL EMERGENCY CARE

Karellas Ioannis, Plakas Sotirios, Lafazanos Spiridon, Tsakalakis Christos, Liapis Georgios, Gourtzelidis Pavlos

Purpose: Traumatic Brain Injuries (TBIs) are a leading cause of morbidity and mortality in modern battlefield. Increased Intracranial Pressure (ICP) due to cerebral edema is a serious complication of TBIs. This study is investigating if Transcranial Doppler (TCD), Doppler of Internal Carotid Arteries (ICAs) and Ocular Nerve Sheath Diameter (OSND) can estimate increased ICP, very important for decision making and guidance of therapy.

Material and Methods: 24 TBI patients, presented with Glasgow Coma Score<8, were sedated, intubated, mechanically ventilated and admitted in ICU. In all patients intracranial catheters were applied for direct monitoring of ICP, Mean Arterial Pressure (MAP) was invasively monitored and ONSD was measured. When Cerebral Perfusion Pressure (CPP=MAP-ICP) decreased below 50 mmHg, in parallel to aggressive therapy, a TCD scan was held, the spectral analysis of flow in ICAs was obtained and OSND was measured. Blood flow velocities were measured in both Mean Cerebral Arteries, PI (PI=Peak systolic velocity-End diastolic velocity/Mean velocity) and CPP were calculated. The TCD spectral analysis was compared with that of ICAs.

Results: 365 TCD examinations were conducted and PI and CPP was calculated. There was a strong correlation between CPP and PI (Pearson’s Correlation Coefficient: -0.782, p<0.0001) and between ONSD and ICP (Spearmen: p<0.05). The ICAs Doppler spectral analysis resembled that of TCDs and lost its diastolic component when ICP>dBP.

Conclusion: There are many ways that ultrasounds can estimate of CPP in TBI patients. Compact devices make ultrasounds applicable to prehospital care most useful at frontline for the on site evaluation of casualties.

SEPSIS – AN UPDATE

Dogaru S, Teusdea CB, Popa Luminiţa Mariana

Sepsis is not something new. Neither is the increasing awareness toward associated morbidity, mortality and costs burden. Many organizations, including regulatory agencies and hospitals are focused on sepsis quality improvement programs.

In addition, greater understanding of underlying pathophysiologic mechanisms responsible for cellular dysfunction and the significant amount of data obtained through evidence based medicine led to constant updated definitions, biomarkers study, admission criteria and protocol based treatment (Surviving Sepsis Campaign 2008 and 2012).

Fast growing medical knowledge and increasing standardization require active concern toward the very essence of this subject. This is the reason why the Sepsis Definition Task Force recently (February 2016) updated the definitions for sepsis and septic shock of whose previous versions dated back in 1992 and 2003.

Although great progress had been made there much still to be done.

POINT-OF-CARE TESTING (POCT) DEVICES IN THE EMERGENCY DEPARTMENT – OUR EXPERIENCE

Teusdea CB, Toma LM, Dogaru S, Popa Luminiţa Mariana

Point-of-care testing (POCT), or bedside testing is defined as medical diagnostic testing at or near the point of medical care - that is, at the time and place of patient care. POCT is to bring the test conveniently and immediately to the patient, making the results quicker, which allows for immediate clinical management decisions to be made.

POCT includes: blood glucose testing, blood gas and electrolytes analysis, rapid coagulation testing, rapid cardiac and sepsis markers diagnostics, drugs of abuse screening, urine strips testing, pregnancy testing, infectious disease testing and cholesterol screening.

In this study, we made a comparison between the results obtained from blood tests on POCT instruments (blood count and biochemistry), and blood tests obtained in the Central Laboratory of the hospital to determine if these analyzers can be used routinely to the emergency room.

Our study showed little difference between the two laboratory tests but with no statistical significance. Thus, POCT type tests can be successfully used in evaluating patients in ED, representing a possibility to reduce the time to treatment and to other medical decisions.

TRAUMATIC RUPTURE OF THE DIAPHRAGM

S. N. Romedea, S. Luncă, R. Tiutiuca, B. S. Zamfir

Introduction: Diaphragmatic rupture remains a diagnostic challenge because of the lack of an accurate test demonstrating the injury. The aim of this paper was to analyze our experience with management of these injuries and to identify predictors of outcome.

Material and method: Sixty-five patients were treated for diaphragmatic rupture in “St John” Emergency Hospital Iasi and Emergency Military Hospital “Jacob Czihac” Iasi, between January 2003 and April 2012. They were 52 men (80 %) and 13 women (20 %), with a mean age of 39.34 ±6.35years. Blunt trauma accounted for 16 injuries (24.61%) and penetrating trauma for the rest of 49 injuries (75.38%).

Results: The preoperative diagnostic was established for 18 patients, 7 blunt and 11 penetrating. Thirty-one patients (47.69%) presented with hemorrhagic shock upon admission and 50 patients (76.92%) had associated injury with an average Injury Severity Score of 25. Sixty-one cases (93.84%) were operated within 12 hours from admission. Two blunt lesions were treated after 18, respectively 24 hours and two cases presented after 5 respectively 12 years from the trauma episode. Intraoperatively 16 right lesions (24.61%), 49 left (75.38%) were identified. There were 8 deaths (12.30%) and 17 patients (26.15%) developed complications. Hemorrhagic shock upon admission, Injury Severity Score, mechanism of the injury and age strongly influenced the outcome ($p<0.05$).

Conclusions: In emergency trauma settings the diagnostic for diaphragmatic injuries is mainly established by laparotomy. A high index of suspicion and a thorough examination of both diaphragms during laparotomy is mandatory in order to avoid missing traumatic diaphragmatic injuries.

MANAGEMENT OF MASS CAUSALITY INCIDENT IN ROMANIA

Teusdea CB, Toma M, Dogaru S, Popa Luminiţa Mariana, Negoiţă DD

Romania is a Central European country, which has a various relief and it can be affected by many hazards. Hazards can be classified in natural and technological hazards.

In this context the healthcare system in Romania has two parts: civil and military which are providing the medical assistance in our country in every situation. The assessment and the coordination of an emergency situation are realized by National System for Emergency Situation and his components.

The system is activated by any person who calls 112 – “the unique number for emergencies”, where a trained dispatcher assesses the emergency: type, type of hazard involved, location, number of victims, status of the victims and the need for help. The dispatcher from 112 Center/ Integrated Emergency Dispatch Center alerts the qualified first responder team – an ambulance for first aid from SMURD (Emergency Mobile Resuscitation and Extrication Service) or from the Ambulance Service which goes to the incident/accident site. If the dispatcher has information’s that is a Mass Casualty Incident (MCI)/disaster he alerts the coordinator (medical doctor from a Regional Hospital - Emergency Department) and also the Chief Inspector for Emergency Situation from Regional Inspectorate for Emergency Situation. The Chief Inspector announces the Prefect which declares active the Red Intervention Plan for prehospital activities and the White Plan for in-hospital activities. At the MCI site goes also the Police intervention team and a team from the Fire Department.

THE ENCOUNTER OF ASYMMETRIC WARFARE INVOLVES ADDITIONAL SUPPORT TO THE EMERGENCY NATIONAL HEALTH SYSTEMS

Karellas Ioannis, Daliakopoylos Stavros, Houliaras Eleutherios, Sourlas Sotirios, Arsenoglou Athanasios, Liapis Georgios

Purpose: Asymmetric warfare, bypassing armed forces, can cause mass casualties mainly civilians. Depending on the scale of the event, timely medical support can be demanding. This study is investigating the potential of National Health Systems to respond promptly to such a challenge and the readiness of Armed Forces to contribute, minimizing the time that proper medical help is provided to the site of incident, on the ground that the frequency of such incidents in large cities is increasing and that proper medical care on site is lifesaving.

Material and methods: At a 4000000 people city, we investigated how medical emergencies are managed. We interviewed the paramedics and recorded the patients that daily and yearly, emergently requested medical support. We acknowledged the patients that were properly evacuated. The readiness of Armed Forces to support National Medical Services in a mass casualty’s situation was examined.

Results: In a year, almost 240000 patients emergently request medical help. From those, 40000 (16%) choose own ways to access medical care. Anytime, 30-60 ambulances are on the move and yearly, respond to 200000 medical requests, performing 160000 emergency evacuations. Additionally, they perform 50000 scheduled medical evacuations. Great work burden and lack of psychological support the main complains of employed paramedics. Armed Forces use a significant number of ambulances but not specially trained paramedics.

Conclusion: National Emergency Health Systems manage to meet the emergency demands in peace time. Mass casualties can also follow asymmetric warfare. It would be expected, the additional support needed in such occasion, to come from the potential of Armed Forces.

THE MANAGEMENT OF THE MAJOR BURNT PACIENT WITH ASSOCIATED AIRWAY INJURY IN THE CLINICAL CONTEXT OF MULTIPLE CASUALTIES INCIDENT: CASE PRESENTATION – EXPERIENCE ACQUIRED

Marinescu B., Marinescu Penelopia, Ioana Tuhar, Benga Andreea, Popescu F., Oprea T

Purpose: The objective of this study is to present the outcomes of the correct management of the burned pacient, and to share the experience acquired secondary to this event.

Methods: The case sample included 12 patients from the same multiple casualties' incident, with the severity of the burn lesions varying between gr IIb-III and between 5-50% body surface, mainly affecting the upper half of the body (thorax, upper limbs, head and neck). As a common determinant all the cases presented respiratory tract lesions due to the nature of the event-fire in inclosed space followed by toxic fumes inahalation.

The surgical team involved was able to ensure in less than 10 hours the emergency surgical primary care for all of the 12 patients admitted.

Discussion: The distinctiveness of the presented cases consists in the great number of patients simultaneously admitted and treated.

Results: We can report a 100 % survivability, at 50 days all pacients being discharged only with minor granulating areas left to heal spontaneously under local treatment. Most of the studies that we have found and we could reffer to, on the matter of proper airway burn injuries and toxic fume inhalation did not statistically differ from our outcomes, even compared with consecrated burn centres from Western Europe.

Conclusions: The good overall results can be related to the interdisciplinary approach, right from the admission of the patients. Our hospital is a level I medico-military unit, concieved from the beginning to provide emergency medical assistance for the most complex cases.

OP session 2 – Neurology and toxicology

COULD ERYTHROPOIETIN BE USED AS A GOLD STANDARD NEUROPROTECTIVE AGENT IN PATIENTS WITH SEVERE CLOSED BRAIN INJURY?

Stavros Gourgiotis, Stavros Aloizos, Georgios Veloudis, Evangelos Falidas, Constantinos Villias

Aim: Our research was focused on the neuroprotective function of erythropoietin (Epo) in patients with severe closed traumatic brain injury (TBI).

Material - Methods: Our model examined the influence of the outcome and neurological recovery in 42 adults with TBI who were admitted to ICU within 6hrs of their injury and were recruited into a randomized controlled study of two groups; only the patients of the intervention group received 10,000 i.u. of Epo for 7 consecutive days. Prognostic model based on CRASH II injury model and outcome was measured by survival and Glasgow Outcome Scale-Extended version (GOS-E) score at 6 months post-injury.

Results: Six patients (18.7%) died during the first two weeks; 4 of the control group and 2 of the intervention group. A mortality rate of 22.2% and 8.3% for the control and intervention group respectively was observed. A lower rate of good outcome (GOS-E score >4) at 6 months was mentioned among patients of the control group.

Conclusions: The study provides evidence of lower mortality and better neurological outcome for the patients who received Epo increasing the possibility that Epo therapy could be used in clinical practice, limiting neuronal damage induced by TBI.

VALIDATION OF HIPPOCAMPAL VOLUMETRY USING 3-D MAGNETIC RESONANCE IMAGING ACQUISITION IN MULTIPLE SCLEROSIS PATIENTS

V. Petrova, K. Genov, M. Penkov

Introduction: Different Magnetic Resonance (MR) imaging methods have been used to investigate and follow the pathological changes in central nervous system and their evolution in multiple sclerosis (MS) patients. Measuring tissue loss (brain atrophy) is considered a global marker of the adverse outcome of the disease. Hippocampus is a structure critical for cognition. It is sensitive to inflammation. Hippocampal atrophy is detected early in the disease course. MR determination of hippocampal volume (HV) could be useful tool in evaluating correlation between neurodegeneration and cognitive impairment in MS.

Purpose: Validation of hippocampal volumes measured using 3-D acquisition in a PERP protocol.

Methods: MR imaging was performed on a 1.5-T unit. We got 3-D volumetric acquisitions of hippocampus for each subject in a plane perpendicular to the axis of hippocampal formation (PERP protocol). 10 healthy subjects were studied. The results were compared with data obtained from hippocampal volume determination in 10 relapsing-remitting multiple sclerosis patients (RRMS). Both groups were age matched (20-40 years).

Results: For the control group (right hippocampus) the mean hippocampal volume was 9.46 cm (standart deviation 0.98). For the RRMS group was registered mean HV of 8.16cm (standart deviation 0.54). The difference of the mean volume is statistically significant (P =0.003) between two groups. The correlation with age was not significant in both groups (considering the small number of subjects).

Conclusion: Brain volume acquisition provides a measure of axonal loss. Atrophy is a sensitive and reliable marker in evaluating the neurodegenerative process in multiple sclerosis. Hippocampal biometry, in particular, could be applied in estimating data obtained from nonconventional MR techniques like magnetic resonance spectroscopy.

NEUROLOGICAL AND NEUROMUSCULAR SYMPTOMS CAUSED BY WEST NILE VIRUS INFECTION

Diana M. Popescu, Viorel Ordeanu, Marius Necsulescu, Alexandru Filip Vladimirescu, Simona N. Bicheru, Lucia E. Ionescu, Gabriela V. Dumitrescu

West Nile virus (WNV), isolated first from the blood of a febrile woman in the West Nile district of Uganda in 1937, was then identified and re-isolated from sick people, birds and mosquitoes in Egypt (since 1950). The virus have been classified in the genus Flavivirus, family Flaviviridae and since 1999 its geographical distribution include in addition to Africa: Europe, South Asia, Middle East and U.S.A.

The Encephalitic flaviviruses, including WNV, usually crosses the blood-brain barrier and probably they have the ability to multiply in the vascular endothelium.

The clinical manifestations of West Nile fever are medium to severe, occurring after 2-15 days (average 1-6 days) of incubation. Most human infections are clinically inapparent.

About 1 in 150 infected people, presents severe illness. The most important risk factor for neurological damage is age. People over 50 years have a higher risk of developing neurological symptoms. The most common neurological manifestations are: meningitis, meningo-encephalitis and encephalitis.

During the epidemics in the US from 2002 to 2003 neuromuscular manifestations (severe muscle weakness) were recognized as the main causes that have led to increased morbidity and mortality.

Although the potential for contamination, infection and invasion of neurotropic viruses is very high, infections and illnesses of central nervous system are rare. This is explained by the functioning of the host defense mechanisms.

IS THERE A CORRELATION BETWEEN HAND PREFERENCE AND VERTEBRAL - SUBCLAVIAN ARTERY DIAMETER AND VERTEBRAL ARTERY VOLUME FLOW?

Karaman Bulent, Hamcan Salih, Abduramani Asaf, Tasar Mustafa

Introduction: Systemic mastocytosis, often termed systemic OBJECTIVE: During cerebral angiography of cerebral vascular structures it may be necessary to predict the dominant vertebral artery side. The reason of the right and left vertebral artery diameter asymmetry is not clear. We think that there may be a correlation with hand preference and vertebral artery dominance and subclavian artery diameter. In this article we aimed to present a study that investigates correlation between hand preference and vertebral-subclavian artery diameter and vertebral artery flow.

MATERIAL AND METHOD: A total 112 patient were included to the study. We examined fourteen left handed and 98 right handed individuals. The measurements were done with 7.5 MHz linear probe between the fifth and sixth cervical vertebrae at each side. Vertebral artery diameter and volume flow and subclavian artery diameter was measured by two different radiologist and average values were taken.

RESULTS: There were no significant difference in diameter and volume flow of right and left VA between right and left handed individuals ($p<0.05$). Beside this, any significant difference was found in diameter of both subclavian artery between right and left handed individual ($p=0.042$).

CONCLUSION: We found no correlation between differences in vertebral artery diameter and hand preference. Beside that there was no correlation between differences in right and left vertebral artery volume flow and hand preference either. We also found any correlation between both subclavian artery and hand dominance. New and comprehensive researches are needed for the reason and mechanism of vertebral artery diameter asymmetry.

METHOD FOR STUDY THE ELIMINATION ALUMINIUM TO PATIENTS WITH CHORONIC KIDNEY DISEASE

Caragea G., Tudosie M.T., Ştefani C., Forje M., Ardelean L., Bărcănescu S., Ionică M

Alluminium is a toxic microelement. Some toxicity can be traced to deposition in bone and the central nervous system. The toxicity is particularly increased in patients with reduced renal function. In patients with chronic renal disease, the trace elements are removed only during the dialysis procedure. For the determination of alluminium concentrations, a Varian graphite furnace – atomic absorption spectrometer (GF-AAS) was used. We have performed a comparative study concerning alluminium concentrations in blood, determined on subjects in a control group, versus blood alluminium concentrations in subjects belonging to a group of patients undergoing dialysis procedure. Average concentrations of aluminum in the blood of patients with chronic kidney disease, before and after the dialysis procedure is not significantly different statistically, but it is different for subjects with normal renal function. In order to emphasize the dynamic removal of the aluminum in the dialysate during the dialysis procedure, six samples were taken from the dialysis time: zero, 30 minutes, 1, 2, 3 and 4 hours. These values were interpolated using a polynomial Lagrange method. By integrating this equation, one can calculate the amount of aluminum removed during dialysis procedure or can appreciate the amount that could be eliminated if it increased during the dialysis procedure. The average amount of alluminium removed by dialysis procedure is about 5 times higher than the amount removed in 24 h by the patients with normal renal function. GF-AAS method is sensitive, reproducible and has a relatively low cost.

CURRENT DRUG RECIPES EVOLUTION

Forje M., Tudosie M.T., Ştefani C., Caragea G., Ionică M.

Drug recipes suffered an increasing evolution from heroin-carbamazepine mixtures and traditional abuse substances to new “psychotomimetics” with complex structure including ingredients with different functions as: psychotropes, addiction accelerators, nonspecific cholinesterase inhibitors, synthetic drug design LSD, LSA and synthetic cannabinoids. Despite restrictive policies, these products are still commercialized with other uses and benefits, apparently harmless, such as: traditional and medicinal plants, plant fertilizer, bath salts. Consumption of this association can result in very severe clinical symptoms that can be in some cases fatal.

The FPIA and GC-MS techniques highlighted the presence of opiates, antipsychotics, anticonvulsivants, benzodiazepines, and antidepressants of different generations, phencyclidines, cocaine, amphetamines, methamphetamines and LSD-like structures completing the formula of these “recreational” drugs. Because many of assayed compounds lack monoclonal antibodies and also mass spectra required for identification for the new drug design compounds it is necessary to continuously improve methods and techniques in extraction, detection, quantification and assay of biological samples.

The authors present a FPIA and GC-MS method to be used in the identification of psychotomimetic mixtures and special condition liquid-liquid extraction for LSD-like structures to be used in GC-MS method.

The urine samples have been collected from the patients presented in the ER and other similar medical facilities in Bucharest and have been analyzed on a FPIA-COBAS Integra 400 Plus and also on a GC-MS Saturn 2000 Varian. PMW, NIST, Wiley libraries were used for identification.

A CASE OF ACUTE VOLUNTARY INTOXICATION WITH INSECTICIDES CHOLINESTERASE INHIBITORY

Dumitraşcu M., Macovei R.A., Dănescu I., Tudosie M.T., Caragea G, Forje M., Ionică M.

In the Clinical Emergency Hospital Bucharest, cases of poisoning are frequent cholinesterase inhibitor compounds, representing a challenge for physicians in the therapeutic conduct. The use of these compounds widely in agriculture and households, leads to an increased number of accidental poisonings. At the same time, it remains a method used quite frequently suicidal purposes. Always laboratory tests guides physicians regarding treatment. Therefore, the development of toxicology lab was a priority. These compounds are quickly metabolized in the body, causing either laboratory compound itself, but more often, its metabolites. The method is the determination of the compounds and/or their metabolites is gas chromatography coupled with mass spectrometry (GC-MS). The clinical picture includes symptoms of associated diseases, the most important remains rapid determination of compound that produced intoxication, thus leading doctor right treatment.

The paper presents a case of voluntary intoxication with a mixture of insecticide widely used: cyfluthrin, transfluthrin, prallethrin, propoxur and chlorpyrifos, an old patient with cardiac and neurological pathology associated. Existing flaws could mislead the physician in the diagnosis, the patient is comatose, found by neighbors in the house, without being able to perform a history too broad. Cardiac and neurological symptoms could mask toxidrom cholinergic if some signs would not be determined doctor, who examined the patient, to recommend a toxicological analysis, which was done quickly and confirmed the suspicion of acute poisoning with cholinesterase inhibitors. Treatment was symptomatic management reactivated without cholinesterase, as in this case, they can produce serious neurological side effects.

ADMINISTRATION OF VITAMIN K IN PATIENTS WITH OVERDOSE OF ORAL ANTICOAGULANTS

Truţă E., Ştefani C., Tudosie M.T., Constantin L., Stoica A., Caragea G., Forje M., Ionică M

The relation between the anticoagulants and body´s ability to control homeostasis is complicated. Doses that are too law may prevent clots, while doses that are too high may cause severe bleeding. In order to obtain a clinical efficacy and limit the risk of either bleeding or clothing vitamin K antagonists require rigurous and continuous adjustment of INR (international normalized ratio). Vitamin K is mainly indicated as an antidote against hypoprohrombinemia due to excesive coumarin anticoagulation. Our aim was to assess if vitamin K is being used acording to the available clinical evidence, estimating the impact of unnecesary prescriptions. We included 40 oral anticoagulants prescription written during 2015 year. The patients handed their treatment on their own (100%) and took an average 5.5 different drugs a day. All patients had increased INR. The main reasons for increased INR were: neglect monitoring INR; did not meet the doses indicated by the doctor (10); were not able to travel for collection of biological samples (3); voluntary ingested (2); mistook drugs (2); overdose history (2), possible drug interactions (10). Lenght of hospitalization was between 2 - 19 days. Vitamin K used as an antidote was not required, 5 patients, or doses administered were not recommended by the literature, 20 patients. It is one of the medical staff´s responsability to make sure that the patients have a good understanding of their treatment and it´s monitoring.

OP session 3 – General surgery

DAY SURGERY DEVELOPMENT AND PRACTICE: KEY FACTORS FOR A SUCCESSFUL PATHWAY

Rasvan Hristea, Cristian Dragos Stefanescu

To understand history is to begin to understand the future. This paper explores the beginnings of modern day surgery and via its growth and advantages looks at how it can develop in the future. The advantages of day surgery are clear and well documented. But, as in the past, barriers exist to its expansion and these are examined. The reasons for this include medical conservatism and protectionism. However, in the longer term there is no doubt that no country, rich or poor, will be able to resist a move to day surgery because of the economic benefits combined with quality treatment that accrues from this approach. In the not too distant future, the question will not be 'Can this patient be treated on a day basis?' but 'Why cannot this patient be treated as a day case?' Day surgery rather than inpatient surgery will become the norm for elective surgery.

ANESTHESIA FOR DAY SURGERY

Teodora Serban, Simona Butoi, Rasvan Hristea, Cristian Dragos Stefanescu

As with all cases of surgery, the choice of a proper anaesthetic drug and technique for day surgery should be based on considerations of safety, quality and cost effectiveness, with the safety issue as the most important concern. As day surgery has some specific characteristics, the way of looking at these three main issues may be different from major inpatient surgery. Day surgery should be well planned, elective surgery done during daytime, usually of minor or intermediate invasiveness and involving stable patients of good or fairly good general health. This implies that the risk of severe problems arising from patient safety is less likely to occur. On the other hand, if problems do occur they will be much less tolerated and accepted by patients and society. The discussion on the ideal choice of anaesthetic technique should be based on demands for a close to zero risk of mortality and permanent disability, and then moving the focus into aspects of quality and cost efficacy. Further, the success of a chosen anaesthetic is highly dependent upon adjuvant issues, such as the skill and routines of the personnel involved, proper monitoring and dosing, and the use of non-anaesthetic adjuvant drugs and methods for pain and nausea protection.

SLEEP SURGERY IN ONE DAY IN ONE PLACE

Cristian Dragos Stefanescu, Cristina Stefanescu, Teodora Serban

INTRODUCTION. The appropriate level of postoperative monitoring for most patients undergoing surgery for obstructive sleep apnea (OSA) remains controversial. Our objective was to document the postoperative course of patients undergoing OSA surgery.

MATERIALS AND METHODS. Study Design: Prospective cohort study. All patients were evaluated (polisomnography included) at the somnology department of The National Institute of Aerospace Medicine and undergoing „on demand" to surgery for OSA at our day surgery center, from 2009 to 2012. Outcome measures were: 1) incidence of respiratory complications requiring nursing intervention, 2) level of postoperative blood oxygen saturation divided into three groups: mean oxygen saturation in recovery room, mean oxygen saturation in step-up unit and lowest oxygen saturation over the 24 hour period.

RESULTS. The overall incidence of nursing intervention in response to a respiratory complication was less than expected. Mean SpO_2 was above 90%.

CONCLUSIONS. In selected patients undergoing to planed and minimally invasive surgery for OSA routine ICU (intensive care unit) postoperative monitoring is not mandatory.

MINIMALLY INVASIVE vs OPEN GASTRIC CANCER RESECTIONS

Kyosev Vasil, Kotashev Georgi, Mutafchiyski Vencislav, Vasilev Krasimir, Ivanov Plamen, Grigorov Grigor, Hristova Vasilena, Popivanov Georgi, Petrov Hristo

Aim: Minimally invasive surgery of gastric cancer has emerged as a result of the technical advances, better understanding of gastric physiology, and more knowledge of the biologic behavior of gastric cancer. The aim of this study was to present our experience with minimally invasive radical resection for distal localize gastric tumors, to compare with open gastric resections, to evaluate the surgical outcomes and oncologic efficacy of minimally invasive approach.

Material and Methods: This study included 20 patients: eight (40%) underwent minimally invasive subtotal gastrectomy (MG), while twelve (60%) patients open subtotal gastrectomy (OG). The following variables were evaluated: age, sex, comorbid conditions, tumor size, and histological type, depth of wall invasion, and presence or absence of lymph node metastasis. The surgical variables investigated included operating time, blood loss, postoperative complications, and length of postoperative stay.

Results: The demographics, preoperative data, and characteristics of the tumor were similar. Median operative time for the minimally invasive approach was 258 min (range 150-385 min) compared with median of 136 min (range 85-205 min) in the open group (p < 0.01). Minimally invasive surgery resulted in less intraoperative blood loss compared with OG. All resected margins in two groups were free of tumor invasion. The mean number of resected lymph nodes was 31.6 ± 14.4 in the OG group and 28.0 ± 11.9 in the MG (P = not significant). MG-patients had an earlier return to normal bowel function and length of hospital stay was 6 days (range 4-14 days), compared with 8 days (range 6-19 days) in the open group (p = 0.01).

Conclusions: This study demonstrated that minimally invasive subtotal resection for gastric cancer had good result and were feasible and safe procedure. Minimally invasive approach can obtain adequate margins, follow oncologic principles and is an excellent alternative to the more traditional open approach.

THE IMPORTANCE OF BARIATRIC SURGERY IN ARMY FORCES – PRESENTATION OF OUR PRELIMINARY RESULTS

Tasis Nikolaos, Sotiropoulos Georgios, Barkolias Christos, Fradelos Aggelos, Barbalia Ioanna, Panagakis Georgios, Terzis Ioannis, Xanthopoulou Georgia, Georgopoulos Nikolaos

Aim: Aim of our study is to highlight the effect of bariatric surgery in improving quality of life and working efficacy of obese personnel.

Material – Method: Our department contacted a retrospective study, collecting data from 41 patients who underwent bariatric surgery in our hospital between February 2009 and November 2014. Patients were asked to fill a questionnaire collecting their demographic data and calculating their BMI before surgery, at its lowest point and at present time. Furthermore, the patients were asked to evaluate their changes in quality of life and working efficacy after surgery in a scale from 1 to 10, with 5 indicating stability, 10 maximum improvement and 1 maximum aggravation.

Results: Thirty eight patients completed our questionnaire. Five, of them, underwent gastric bypass surgery and thirty three, of them, laparoscopic sleeve gastrectomy. The average body mass index before the procedure was 45.99. The average body mass index after the procedure was 33.67. The average lowest body mass index after the procedure was 31.59. Mean evaluation of their working efficacy after surgery was 8.26. Mean evaluation of their quality of life after surgery was 8.31.

Conclusions: Significant improvement was recorded in both working efficacy and quality of life. Patients productiveness was increased, their working environment improved, as well as, their social life. Further data is collected by our department, at the moment, for future more detailed reports on this subject.

BARIATRIC SURGERY: TWO YEAR OF PRACTICE IN OUR CENTER

Diana Toma, Serban Vasile, Mircea Lica, Rely Manolescu, Paula Neicutescu

Introduction: Bariatric surgery is the most effective therapy available for morbid obesity and can result in improvement or complete resolution of obesity comorbidities.

The morbid obese patient have a diversity of clinical and occult obesity-related comorbidities that necessitates a multidisciplinary team approach in the preoperative evaluation - cardiac, pulmonary, psychiatric, and endocrine.

Laparoscopic sleeve gastrectomy (LSG), it’s a procedure that reduce the dimensions of the stomach to about 15-20% of its original size by removing of a large portion of it, following the greater curve.

We present our results after two years of constant practice of LSG in morbid obese patients in Medlife - Life Memorial Hospital.

Matherial and method: In our bariatric center, 82 patients with obesity, underwent to laparoscopic sleeve gastrectomy, between 2014 – 2015. Body Mass Index (BMI) at admission ranged from 32 to 53 (mean 42) and most of the patients were women (86 %). 39 patients (47 %) presented associated comorbidities – hypertension, diabetes mellitus, sleep apnea syndrome. Laparoscopic sleeve gastrectomy was performed in all patients; conversion rate was null.

Results: No major complication (leakage or active bleeding) was encountered. Readmision rate was 4.8 % (4 patients) mostly for postoperative nausea and vomiting. One year scheduled control from the beginning of the programme encoutered excess weight loss percentage (% EWL) of 68.4 %.

Conclusions: Bariatric surgery gained a major role in the treatment of severe and morbid obesity. Low complications rates and weight loss above 50% entitle us to consider that sleeve gastrectomy is a safe and effective method of treatment in severe and morbid obesity.

OUR FIVE YEARS EXPERIENCE IN SLEEVE GASTRECTOMY ON MILITARY PERSONNEL – OUR PRELIMINARY RESULTS

Barkolias Christos, Sotiropoulos Georgios, Tasis Nikolaos, Fradelos Aggelos, Panagakis Georgios, Barbalia Ioanna, Terzis Ioannis, Xanthopoulou Georgia, Georgopoulos Nikolaos

Aim: Aim of our study is to assess the long term results of laparoscopic sleeve gantrectomy regarding BMI reduction and patient satisfaction.

Material – Method: Our department landed a retrospective study, including 34 patients who underwent laparoscopic sleeve gastrectomy in our hospital between 2009 and 2014. Patients were divided in 3 groups. The first consisted of those who received surgery in 2014 (group A), the second of those who received surgery in 2013 (group B) and the third from patients who were subjected to surgery between 2009 and 2012 (Group C). Demographic and somatometric data was collected in order to calculate BMI score before surgery, at the lowest point after surgery and at present time. Satisfaction of patients from the surgical results was evaluated in a scale of 1 to 10, 5 indicating neutrality, 10 maximum satisfaction and 1 maximum dissatisfaction.

Results: Both groups A and B achieved similar maximum BMI reduction (32% and 34% respectively) and managed to maintain a steady BMI until today. Group C reached a similar maximum BMI reduction (33%), but it presented a partial relapse, as it now presents a 26% BMI reduction compared to its original BMI. Satisfaction remains high in all groups.

Conclusions: Our preliminary results indicate the promising role of laparoscopic sleeve gastrectomy in treating obese personnel. The partial relapse of group C might urge the need for choosing gastric bypass as treatment for selected patients. Further data is collected by our department for more conclusive results.

CYSTIC DUCT REMNANT CALCULUS AS A CAUSE OF POST-CHOLECYSTECTOMY SYNDROM: CASE REPORT

Atanaskovic Aleksandar, Kostic Zoran, Neskovic Branimir

AIM: There are several causes of post-cholecystectomy syndrome (PCS), and the presence of stones within cystic duct remnant is one of them, with estimated incidence <2.5%. The objective of this work is to emphasize cystic duct remnant calculi as potential cause of PCS, its diagnostics and treatment.

MATERIAL-METHOD: 67 year old patient who has undergone cholecystectomy 28 years ago, were evaluated for unspecific abdominal pain and dyspeptic symptoms, with no jaundice. The patient was diagnosed with stone in remnant cystic duct by MSCT and US of the abdomen, gastroscopy and endoscopic US of biliary tree, and then treated surgicaly.

RESULTS: PCS is defined as the recurrence of symptoms similar to those before the cholecystectomy. It usually takes the form of upper abdominal pain and dyspepsia, with or without jaundice. The sympthoms were unspecific and could have indicate to several conditions. Laboratory findings were in refered range. US of the upper abdomen indicated, and MSCT of the abdomen as well as endoscopic US confirmed diagnosis. Surgery has been performed trough right subcostal laparothomy, and excision of remnanat cyst duct with stone removal has done. Dilatated remnant cystic duct 4cm wide is found, with 2cm cholesterol calculus in it.

CONCLUSION: Leaving behind a cystic duct stump for too long predisposes stone formation as a cause of PCS, even many years after cholecystectomy. MRCP is the imaging modality of choice, and is not mandatory in all patients.

OPEN ABDOMEN - EARLY OR DELAYED FASCIAL CLOSURE?

V. Oprea, D. Leuca, D. Gheorghescu, F. Buia

Background: Open- abdomen is an effective treatment for abdominal catastrophes in traumatic and general surgery with a formidable task upon surgeon and patient. We analyse a retrospective series of patients with open abdomen in order to determine when the best moment for fascial closure is: early or delayed.

Methods: between January 2010 and December 2015 all medical records patients with open abdomen admitted in the Department of Surgery of the Military Hospital from Cluj-Napoca were reviewed. Demographics, co- morbidities, etiology of the open-abdomen and the time and methods of fascial closure were recorded. Patients were classified according to Bjork open abdomen grading system. The morbidity and mortality of the method and of the fascial closure were analysed.

Results: 27 patients (9 female) with a median age of were analysed. Average time for open abdomen therapy was 12 days (2-39 days). Etiology of open abdomen was a major leakage of visceral anastomoses in over 50% of cases. Other cause: visceral perforation, severe acute pancreatitis, abdominal compartment syndrome and mesenteric ischemia. Mean width of the abdominal defect - 21 cm (17-27 cm). The abdomen was open in 16 patients (59%) from which 6 died. Global mortality - 63%.

Conclusions: high mortality rate for both primary cause and method too.

MINIMALLY INVASIVE TREATMENT OF SPIEGEL'S HERNIA

A. Popentiu, D. Moga, I. Barb, C.G. Smarandache, V. Marcu, A. Sabau, D. Sabau

The purpose of this paper is to provide an overview of laparoscopic procedures used for the treatment of Spiegel's hernia. A number of methods using mesh prosthesis or just the herniorraphy were imagined in time, each presenting several advantages and disadvantages.

At present, minimally invasive treatment is becoming more and more the gold standard in terms of treating the abdominal wall defects, including the rare forms of hernia, such as Spiegel's. The laparoscopy offers several advantages, resulting in decreased length of hospital stay, reduced rate of complications and reccurences.

Spiegel's Hernia, as an uncommon clinical entity, benefits from the rapid evolution of laparoscopic techniques.

Technical execution variants must be chosen according to the surgeon's experience, technical cappabilities of the department and not at least, to the patient's will.

OP session 3 – Thoracic surgery and surgical oncology

CHEST WALL TUMORS – RECONSTRUCTION OPTIONS AFTER THORACIC PARIETAL TUMORECTOMY

C. Grozavu, M. Iliaş, D. Marin, D. Pantile

Introduction: Chest wall tumors are complex surgical conditions with a complex treatment which includes surgical thoracic teams with plastic surgery training. Authors propose surgical procedures and a classification for chest wall tumors based on histological type and solutions. We analyze parietal reconstruction methods used in our department.

Material and method: Patients with chest wall tumors undergoing resection during the past 10 years were reviewed. The analysis was made on a lot with chest wall tumors with various topographies. Diagnosis was established with: CT, MRI, bone scintigrafic scan. The surgical intervention includes: tumoral resection within oncological limits and chest wall reconstruction (soft tissues and bones). The target of reconstruction is chest wall stability and lung hernia prevention.

Results and conclusions: The results were very promising using our surgical techniques. "Spider web" technique and prosthetic materials (Thoratex reinforced mesh) and STRATOS osteosynthesis system we used in chest reconstruction give resistance and stability to the chest wall. We did not have any lung hernia in postoperative period. Rebuilding soft tissues was made with mammary gland and skin/muscle flaps from neighboring region. The outcome was normal.

MINIMALLY INVASIVE REPAIR FOR PECTUS EXCAVATUM – A SINGLE INSTITUTION EXPERIENCE

C. Grozavu, M. Iliaş, D. Marin, D. Pantile, T. Augustin

Introduction: Pectus excavatum is the most frequent anterior thoracic wall congenital malformation. This malformation becomes more evident with the aging process and has its peak incidence during teenage, when the clinical symptoms become more acute and psychological effects are real important. Over time many treatment techniques have been proposed, conservative or surgical. The minimally invasive repair of pectus excavatum, "Nuss technique", developed after 1987, is the most used technique worldwide.

Material and Method: This presentation analyzes 85 patients, admitted on University Emergency Military Hospital “Carol Davila” - Thoracic Surgery Department, over an 8-year timeframe (2007 – 2016), diagnosed, investigated and surgically treated according to Nuss procedure. Therapeutic and diagnostic protocols will be presented and analyzed: clinical and paraclinical evaluation, indications and contraindications of Nuss procedure, as well as possible intraoperative and postoperative complications.

Results: Nuss procedure’s esthetical benefits will be presented, as well as improvements of lung functional parameters. Patients’ degree of satisfaction is presented according to the 2-step Nuss questionnaire.

Conclusions: Nuss procedure has a series of advantages: minimally invasive surgical procedure reduced operative time, minimal blood loss and fast socio-professional reinstatement.

UNCOMMON APPROACH FOR A GIANT SOLITARY FIBROUS PLEURAL TUMOR

C. Nistor, A. Ciuche, A. Iordache, D. Pantile, D. Marin, Olivia Arhire, B. Petre, Roxana Brîncoveanu

Introduction: Primary pleural tumors are either localized (solitary mesothelioma) or diffuse pleural lesions. The etiology of solitary fibrous tumors of the pleura is unclear – most authors consider that these tumors arise from the mesenchymal cells of the mesothelium’s subjacent areolar tissue, rather than the pleural mesothelial layer. Besides, asbestos exposure, typical for pleural mesotheliomas, is uncommon in solitary fibrous pleural tumors.

Material and method: We discuss the case of a patient admitted in our Thoracic Surgery department for a giant thoracic tumor, occupying the entire left hemithroax. The patient accused fatigue, severe dyspnea, palpitations, and left thoracic heaviness. Chest X-Ray shows an opacity occupying the whole left hemithorax, with right tracheal and cardiac deviation. The CT scan identifies a 25 cm diameter tumor, well defined, without invasion into any adjacent structure, occupying the whole left hemithorax. We decided to perform surgery in order to resect the tumor. Left bi-thoracotomy is performed (intercostal 4th and 6th spaces) with the “en-bloc” excision of a giant pleural tumor (weighting approximately 5 kg).

Results: With a favorable postoperative outcome, the patient was discharged 6 days after surgery. The left lung was fully expanded, and the abnormal heart rhythm, as well as the dyspnea, disappeared.

Conclusions: We believe that the surgical intervention was the optimal choice for the patient, with all the perioperative risks that might have appeared. Postoperative the patient had a comprehensive socio-professional reintegration.

MULTIDISCIPLINARY APPROACH FOR A GIANT TORACO-LUMBAR TUMOR

C. Nistor, A. Ciuche, A. Iordache, D. Pantile, C. Năstase, V. Ștefănescu, B. Petre, Roxana Brîncoveanu

Introduction: Hereditary exostosant malady (Ombredanne malady) represents a congenital condition with autosomal dominant transmission, characterized by the onset of multiple skeletal osteocondromas.

Material and method: We discuss the case of a 19 y.o. male, admitted in our Thoracic Surgery Department for a giant tumor, located at the base of the left hemithorax and upper left lumbar area. The patient accuses fatigue and thoraco-lumbar heaviness caused by the tumor’s mass effect. The CT scan shows a tumor with its origin at the level of T12-L3 vertebral bodies, invading the left 12th rib and the left iliac crest.

We decide to perform the surgical intervention with a mixed team: thoracic surgeon, general surgeon and neurosurgeon. A 25 cm long incision is performed at the tumor’s level, from the spine to anterior of rectus abdominis muscle. Complete tumor resection is then performed, also resecting the 12 rib, performing T12-L3 laminectomy and T12-L3 foramenotomy.

Results: With a favorable postoperative outcome, the patient was discharged 6 days after surgery.

Conclusions: We consider this mixed approach the right choice of surgical treatment for this type of tumor. The favorable postoperative outcome sustains our decision for a mixed surgical team.

THORACOSCOPIC RESECTION OF A BRONCHOGENIC ESOPHAGEAL CYST

A. Ciuche, C. Nistor, D. Pantile, D. Marin, O. Arhire, A. Tudose, S. Bucurica, C. Betianu, R. Brîncoveanu

Introduction: Esophageal bronchogenic cyst is an uncommon entity and surgical experience of their removal by thoracoscopic surgery is limited.

Material and method: We present our patient outcome and surgical thoracoscopic technique in the treatment of an esophageal bronchogenic cyst.

The study included 1 patient, male, 52 years with important obesity. The only clinical manifestation was intermittent dysphagia. Some imagistic findings at CT scan and transesophageal echography suggest the presence of a solid intraparietal benign esophageal tumor.

The intervention was performed using 4 thoracoscopic entry points. Thoracoscopic removal of the cyst needed careful manipulation and subsequent dissection of the cyst sac from the structures to which it was attached (esophageal wall and mediastinal pleura). The intramural esophageal localization with local esophageal mucosa being intact and the particular aspect of the tumor (filled with mucoid material and contain cartilage fragments) were able to make the positive diagnosis (cystic intramural esophageal tumor) and eliminated the preoperative diagnosis (solid intramural esophageal tumor).

Results: The patient had a favorable outcome, without any complications and was discharged the 3rd day after surgery. The dysphagia was relieved immediately after the operation. The follow-up was well 6 months after the surgery.

Discussions and Conclusions: The preoperative computed tomography and endoscopic ultrasound had some limits in this case (distinguishing between cystic and solid tumor was difficult).

Standard surgical treatment is removal of the cyst by thoracotomy. While difficulties in removal and possible complications and recurrence are similar to those of classical posterolateral thoracotomy, video-assisted thoracoscopy offers clear postoperative advantages.

INTRALOBAR LUNG SEQUESTRATION-THORACOSCOPIC SURGICAL PROCEDURE

Adrian Ciuche, Claudiu Nistor, Dragos Marin, Daniel Pantile, Laura-Mariana Constantin, Roxana Brîncoveanu

Introduction. The authors present the case of a 73 y.o. male admitted for a left lower lung lobe tumor identified on a standard chest X-Ray.

Material and method. First we present the radiological findings and then intraoperative images. Using minimally invasive thoracic surgery we managed to diagnose a left lower lung lobe sequestration (highlighting the vascularization of the sequestered lung territory from the descendent aorta, right above the diaphragm), and to perform a thoracoscopic wedge resection of the lesion, clipping the aberrant vessel.

Results. With favorable surgical outcome, several aspects are being detailed: postoperative evaluation, as well as the radiological findings one-month postoperative.

Conclusion. Minimally invasive thoracic surgery has permitted a better exposure of this rare lung malformation, allowing at the same time a better diagnosis and treatment procedure (thoracoscopic wedge resection), with a fast and complete postoperative recovery of the patient.

UNINTENTIONAL PARATHYROIDECTOMY DURING THYROID SURGERY

Dimitrios K. Manatakis, Nikolaos Stamos, Dimitrios Balalis, Sotirios Gantzoulas, Vassilios Berdelis, Georgios Bouboulis, Dimitrios P. Korkolis, Nikoalos Kouris, Georgios Plataniotis, Emmanouil Gontikakis

Purpose: Incidental parathyroidectomy is a well recognised complication of thyroid surgery, occurring in 5-30% of patients, however controversy still remains concerning its clinical impact. The aim of our study was to evaluate the incidence of unintentional parathyroidectomy in our Department, its clinical and biochemical consequences and to identify potential patient-related risk factors.

Material and Methods: A retrospective study of prospectively collected data was conducted, on patients who underwent total or near-total thyroidectomy between January 2006 and December 2015. Demographic characteristics, preoperative and postoperative data were collected for each case. Pathology reports were reviewed to identify those patients who had an inadvertent parathyroidectomy. Calcium levels were calculated preoperatively, on the day of surgery and on postoperative days 1 and 2.

Results: 205 patients underwent total/near-total thyroidectomy between January 2006 and December 2015 (47 males, 158 females) with a median age of 55 years (range 17-85). Incidental parathyroidectomy was noticed in 56 (27%) cases, with 44.6% (25/56) found in an intrathyroidal location. Evidence of postoperative biochemical hypocalcemia was noticed in 66% of patients with inadvertent parathyroidectomy (37/56), compared with 30% (44/149) in the no-parathyroidectomy group (p<0.001). Symptomatic hypocalcemia was observed in 3.6% (2/56) and 3.4% (5/149) respectively (p>0.05,NSS). Thyroiditis was recognized as a potential risk factor (p<0.001), whereas age, sex and malignancy did not differ significantly between the two groups.

Conclusion: Incidental parathyroidectomy is a relatively common complication of total thyroidectomy and a significant percentage of those cases is due to the intrathyroidal location of parathyroid glands. Our study found an association of unintentional parathyroidectomy with postoperative biochemical hypocalcemia, but not with clinically symptomatic disease.

TOTAL THYROIDECTOMY IN THE ELDERLY

Dimitrios K. Manatakis, Nikolaos Stamos, Vassilios Berdelis, Sotirios Gantzoulas, Dimitrios Balalis, Konstantinos Damalas,Georgios Bouboulis, Dimitrios P. Korkolis, Nikolaos Kouris, Emmanouil Gontikakis, Georgios Plataniotis

Purpose: Prevalence of thyroid nodules increases with age and thyroid neoplasms in older patients tend to be more aggressive. Our study aimed to analyze surgical indications and perioperative outcomes of thyroid surgery in elderly patients (>65 years).

Material and Methods: Patients who underwent total thyroidectomy between January 2005 and December 2015 were retrospectively reviewed and data were collected on demographics, pathology and postoperative complications.

Results: In total, 234 patients were divided into group A (<65 years, n=172, mean age 48.1±11.1 years) and group B (>65 years, n=62, mean age 71.8±5.0 years). Male-to-female ratio and preoperative TSH levels did not differ significantly. Incidence of malignancy, incidental papillary micro-carcinomas and thyroiditis were also comparable. Graves disease, although rare in our sample, occurred only in the younger group (4/172). Malignant tumors were marginally larger in group B (1.95 vs 2.39cm, p=0.06). Perioperative mortality was nil. In group A, 3 patients suffered transient RLN palsy, while 2 patients required reoperation due to postoperative haematoma.

Unintentional parathyroidectomy rates were comparable (24.4 vs 32.3%, p>0.05), however evidence of biochemical hypocalcemia was significantly more frequent in younger patients (36.6 vs 11.6%, p<0.01). Symptoms of hypoparathyroidism were only noticed in 5/172 (2.9%) and 2/62 (3.2%) of patients respectively (p>0.05).

Conclusion: Total thyroidectomy is a safe procedure in the elderly and age should not be contraindication to surgery. Moreover our study did not find any differences in postoperative pathology reports, in terms of malignancy or thyroiditis.

RECURRENT HYPERTHYREOIDISM AFTER SUBTOTAL THYROIDECTOMY

Vesela MIHNEVA, Ventzislav Mutafchiyski, Tzvetelina Totomirova, Ivona Daskalova

Background: Subtotal thyroidectomy is a surgical procedure, in which the surgeon leaves a small thyroid remnant in situ to preserve thyroid function, thereby preventing lifelong thyroid hormone supplementation therapy.

Aim: To evaluate thyroid function after subtotal thyroidectomy for hyperthyroidism.

Subjects and Methods: We retrospectively reviewed the medical records of all patients (n = 36) who underwent subtotal thyroidectomy for recurrent hyperthyroidism between 2010 and 2014 in Military Medical Academy - Sofia. Thyroid function was defined according to plasma TSH and free T4 values.

Results: Median followup after operation was 6 months (range 4.1–7.9 months). Only 9 % of patients were euthyroid after surgery. The majority of patients (76 %) became hypothyroid, whereas 15% of patients had persistent or recurrent hyperthyroidism. Permanent recurrent laryngeal nerve palsy and permanent hypocalcaemia were noted in 1.2% and 2.8 % of patients, respectively.

Conclusion: In our series, subtotal thyroidectomy for hyperthyroidism was associated with a high risk of postoperative hypothyroidism and a smaller, but significant, risk of persistent hyperthyroidism. Our data suggest that subtotal thyroidectomy seems to provide very little advantage over total thyroidectomy in terms of postoperative thyroid function.

OP session 3 – Orthopedic surgery

TREATMENT PRINCIPLES FOR OSTEOCHONDRAL LESIONS IN THE FOOT AND ANKLE

Athanasios Badekas, Maria Takvorian, Erasmia Politi, Vasiliki Malkaki

Purpose: To assess the efficacy of surgical correction of stage II tibial tendon deficiency with medial translational calcaneus osteotomy and flexor digitorum longus tendon transfer to the navicular,

Material and methods: A medial translational osteotomy of the calcaneus and transfer of the flexor digitorum longus tendon into the navicular were done. The patients were examined, radiographs were obtained, and isokinetic evaluation of both feet was performed at a mean of 5.2 years postoperatively. The AOFAS Score and Short Form Health Surgery (SF-36) were used to evaluate patients postoperatively.

Results: The mean AOFAS score at follow-up was 79 points (range, 54-93). There were seven significant complications in six patients. Isokinetic inversion and plantarflexion power and strength were symmetric with the contralateral limb in 95 patients, mildly weak in 18 patients, and moderately weak in eight patients. Subtalar joint motion was normal in 56 (44%), slightly decreased in 66 (51%), and moderately decreased in seven patients (5%). Correction was significant ($p < 0.05$) in all four radiographic parameters evaluated. Patients were entirely satisfied (118 patients), partially satisfied (seven patients), or dissatisfied (four patients). Further, 125 (97%) experienced pain relief, 121 (94%) showed improvement of function, 112 (87%) experienced improvement in the arch of the foot, and 108 (84%) were able to wear shoes without shoe modifications or orthotic arch support.

Conclusions: The surgical correction of stage II posterior tibial tendon deficiency with medial translational calcaneus osteotomy and FDL tendon transfer to the navicular yielded excellent results with minimal complications and a high patient satisfaction rate.

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AUDIT OF ORTHOPAEDICS DEPARTMENT AT ATHENS NAVAL & VETERAN HOSPITAL - RECORDING THE OPERATION NOTES OF ARTHROSCOPIES ACCORDING TO THE RCS GUIDELINES

Tsouknidas Ioannis, Festas Georgios, Papoutsis Konstantinos, Tsioros Konstantinos

Purpose: Accurate record of surgical procedures are of crucial meaning nowadays, because of clinical, academical and medicolegal reasons. This essay is meant to examine the way that arthroscopies' operation notes were written comparing to the guidelines of Royal College of Surgeons of England.

Material and Methods: A list of shoulder and knee arthroscopies that took place during the period 01/01/2014 – 31/05/2015 were detected in the operation lists. Arthroscopies' operation notes were collected from the Electronic System "Medico" and the recorded files of the Hospital. A random sample of the arthroscopies operation

notes were assessed against the criteria of RCS guidelines. Other supplementary data, concerning the hand written/ electronic record, the legibility of hand written operation notes were recorded too. Findings were presented and discussed at the clinical meeting of Orthopaedics Department.

Results: The structure of 56 operation notes were compared to the 20 points of a right recorded operation note according to the RCS guidelines: Criteria such as the date of the operation, the names of surgeons and anaesthiosiologist and the surgical procedure were covered in more than 80% of the operation notes. However time of the operation, elective/emergency of the procedure, postoperative care instructions and some other points were poorly described or not refered at all.

Conclusion: New operation note templates regarding shoulder and knee arthroscopies were proposed and discussed at the clinical meeting. The new templates were modified to meet the standards of arthroscopies procedure. A second re-audit is meant to be done in 3 months time.

VAC IN ORTHOPEDIC AND SEPTIC SURGERY

Kovachev G, Mutafchiyski V, Stefanov D, Dimitrov D, Popivanov G, Petrov. N

The most frequent problems encountered in acute and chronic wounds are bacterial contamination or infection, tissue edema and wound secretion, which significantly hamper the wound healing process. Numerous reports in the last three decades have been shown that Negative Pressure Wound Therapy facilitates the wound healing and is superior to the conventional techniques. It allows for complete exudate evacuation, reduction of tissue swelling, stimulation of granulation tissue. Additionally it significantly improves the quality of life, alleviates the nursing care and decreases the hospital costs.

Here we present a part of our 10-year experience with severe combat injuries, open fractures, wounds with major tissue insufficiency and decubital ones.

CALCIUM PHOSPHATE CERAMICS IN NON-UNIONS: WHAT CAN WE EXPECT?

B. Zlatev, A. Iotov, L. Stokov, G. Kovachev, Tzachev, A. Nikolova

Introduction: Achieving bone regeneration in some cases still presents a substantial problem. The numerous current strategies for stimulation of bone healing do not guarantee a 100% success and an individual approach to each case is indicated.

Material and methods: The series include 8 males and 2 females at the age between 23 and 57. There were 3 infected non-unions and 7 aseptic. The non-unions are: 1 hypertrophic, 4 oligotrophic and 5 atrophic. All NU are after operative treatment of fresh fractures. Two fractures were open, GA II. In 2 cases there were 2 previous reoperations including autologous bone grafting in at least one of them. The non-union locations were following: tibial shaft-3, humeral shaft-3, distal tibial metadiaphysis – 1, radius - 2, femur – 1. The used bone substitutes included bone marrow aspirate and calcium deficient hydroxyapatite in 3 cases; nanocristaline hydroxyapatite in 4; β-TCP in 3. β-tricalcium phosphate is used in 2 atrophic nonunions /radius shaft, femur shaft/ and 1 oligotrophic/ tibial shaft/; bone marrow aspirate and calcium deficient hydroxyapatite is used in 3 infected nonunion /tibia – 2, radius - 1/ as a second stage procedure; nanocristaline hydroxyapatite in 1 hypertrophic NU /humeral shaft/ and 3 oligrotrophic ones/ humeral shaft 2, tibial shaft 1/. Three reosteosynthesis are performed.

Results: Bone healing is achieved in 8 of 10 patients. Two developed again atrophic NU/ β-TCP used/.

Conclusion: Synthetic CaP ceramics are not contraindicated for usage in non-unions. Their use requires a good assessment of the local biological environment and if needed, a combination with osteoinductive or osteogenic substitute.

CaP BONE SUBSTITUTES IN TRAUMA SURGERY: DO WE GET THE SAME?

Zlatev Borislav, Yotov Andrei, Stokov Lubomir, Kovachev Georgi, Nikolova Ana

Introduction: The management of bone defects caused by trauma requires consideration of different patients’ factors such as age, co-morbidities, nutritional status, and state of the soft tissues and the characteristics of the bone defect.

Material and method: For a period of 60 months, 33 patients with 33 closed fractures were operated and followed up. There were 17 women and 16 men at the age between 23 and 71 years old. In each case the bone defect was a result of acute fracture. The involved bones include proximal humerus, proximal tibia, calcaneus and metacarpals. The lost of substance was mainly cancellous and cortico-cancellous. The synthetic bone substitutes include - beta tricalcium phosphate in 13 cases, calcium deficient hydroxyapatite in 11 cases and hydroxyapatite in 9. Osteosynthesis was performed in 33 cases. The volume used is between 5and 15cc.

Results: The follow up period was between 12 and 60 months. The criteria of Irwin et al. for radiological assessment of graft incorporation were used for assessment of the osteointegration process. Bone healing was achieved in 33 patients. Resorption of the substitutes and the bone regeneration process were defined as two simultaneously going processes with equal speed. The biodegradion process was assessed as faster for TCP and calciumdeficient hydroxyapatite than synthetic hydroxyapatite. No case of fibrous encapsulation of the substitute or heterotopic ossification was observed.

Conclusion: Different biodegradation rate do not lead to clinical and functional differencies. The different mechanical strength did not led to secondary collaps in load bearing areas. The biodegradion rate depends on the recipient bone, area, ceramics' properties and the volume used.

HYPERBARIC OXYGEN THERAPY IN THE TREATMENT OF OSTEONECROSIS OF THE FEMORAL HEAD: A SYSTEMATIC REVIEW

Uzun Gunalp, Mutluoglu Mesut, Ersen Omer, Yildiz Senol

OBJECTIVE: To review the current literature on the use of hyperbaric oxygen therapy (HBO2) in the treatment of osteonecrosis of femoral head (ONFH).

MATERIAL AND METHOD: We searched PubMed, Directory of Open Access Journals (DOAJ), EMBASE, Web of Science, Academic Search Complete, CINAHL and MEDLINE through April 2015. We hand searched relevant textbooks, conference proceedings and the reference lists of review articles and clinical studies Randomized controlled trials (RCT) and observational studies (cohort study, case-control study, case series) that reported the outcome of patients that received HBO2 therapy for ONFH were included. Only English language articles were included. Study quality was not used as an exclusion criterion. Two authors independently assessed trials for inclusion, extracted data and presented to other authors. Disagreements were resolved by consensus.

RESULTS: We identified eight clinical studies; two RCTs; one historically controlled study; and five case series. The majority of the studies were small-scale, heterogeneous and methodologically weak. In four of the studies HBO2 therapy was combined with other treatment modalities, making it impossible to draw firm conclusions on the specific effects of HBO2 therapy. Hip survivorship in studies wherein HBO2 therapy was used alone was 95.5% in Steinberg Stage I lesions, 89% in Steinberg Stage II lesions and 100% in Ficat Stage II lesions.

CONCLUSION: There is a room for HBO2 therapy in the management ONFH. Further randomized controlled trials, however, are required to better elucidate the role of HBO2 therapy in the treatment of ONFH.

OP session 4 – Mental health and psychology

EXAMINATION OF THE RELATIONSHIP BETWEEN PERSONALITY CHARACTERISTICS AND EMPATHY OF TURKISH MILITARY PHYSICIANS

Uzuntarla Yasin, Teke Abdulkadir, Cihangiroglu Necmettin, Bakir Bilal

OBJECTIVE: Empathy, the essence of good relation between health workers and patients has been found to be related clinical competency and personal satisfaction. This study has been conducted to examine the relationship between personality characteristics and empathy tendencies of the military physicians at the biggest military hospital in Ankara.

MATERIAL AND METHOD: This was a cross sectional study which carried on 415 physicians working at all internal and surgical services of Gulhane Military Training Hospital between 9 to 16 April 2015. An inquiry form with three parts including The Big Five Inventory (BFI) and Jefferson Scale of Physician Empathy (JSPE) scales was used to collect data among physicians. Data was analyzed using SPSS package program version 22.0.

RESULTS: It was found the majority of physicians have openness personality and their empathy skills are higher than moderate level. While a negative relationship was found between Neuroticism and empathy scores, a positive relationship was determined between empathy and other personality characteristics namely extraversion, friendliness (Agreeableness), Conscientiousness and openness ($p<0.01$).

CONCLUSION: The empathy tendencies of physicians have varied depending on their personality characteristics. If physicians use more empathy skills, this will contribute the quality of health services and will improve communication with patients.

“BURN-OUT” SYNDROME, IN MILITARY HOSPITAL PERSONNEL

Vasha Dorela, Basha Entela, Reso Elton, Beja Ergina

Objective: “Burn-out” is a phenomenon characterized by fatigue and frustration, usually to professionals whose work is faced with high level of stress that comes as a result of dedication to a cause or a way of living that does not match expectations of the person. Although this appears to be associated with risk factors derived from professional environment, this problem can be present in every person. The aim is to evaluate the level of “burn-out” in the military hospital personnel.

Method: This is a cross-sectional study conducted through the burnout self administered test to the military medical personnel. The Maslach Burn-out Inventory was used as it is the most commonly used tool to self-assess whether you might be at risk of burn-out through three components: exhaustion, depersonalization and personal achievement. According to the level of burn-out it was made a comparison to the types of clinic (therapeutic, surgical, and emergency) and to the level of responsibility.

Results: The study shows that medical personnel of surgical and emergency clinics have a higher level of burn-out than those of the therapeutics clinics. The head of clinics and doctors have a higher level of burn-out than nurses.

Conclusions: There is a clear need for adaptation and expansion of a conceptual framework for a promising approach for designing interventions to help clinicians in mitigation, which cause anxiety and prevent build the flexibility they need to support themselves in clinical services.

AUDIO-VISUAL ENTRAINMENT: AN IMPORTANT TOOL IN MANAGING MALADAPTATIVE STRESS RESPONSE IN THE MILITARY

Ciumaşu-Rîmbu Mălina

Occupational stress in the military comes from a variety of sources both in the form of chronic, garisson stres through work overload, issuficient logistics, troubled work relationships, role conflicts etc and acute, combat stress in a challenging new environment with unknown biological, physical, chemical and psychological stressors. The ability of the military to cope with different stressors is critical for its own health and safety or its unit and the military organization. Thus lymbic system becomes a leading actor in this equation of coping and the ability to modulate it through medication or non-drug treatment method of therapy becomes a major target of the occupational stress management protocols in the military. Audio-visual entrainment (AVE) consists of constant, repetitive stimuli of the proper frequency and intensity to stimulate the thalamus and neocortex thus improving cognition and behavioral problems, and alleviating stress and pain. AVE achieves its effects through several mechanisms simultaneosly including altered EEG activity, dissociation, limbic stabilization, improved neurotransmitter production and altered cerebral blood flow. The aims are to present the possibilities of AVE in managing maladaptative stress response and even PTSD and the experiece of our lab with AVE technique. Material and methods: We used standardised protocols and the Romanian AVE device. Results: AVE produces a calming effect on limbic structures regulating exagerrated cardiovascular stress response. Conclusions: AVE can be considered a reliable tool part of the occupational stress management protocols in the military aiming limbic stabilisation.

THE IMPORTANCE OF THE 24-HOUR HELPLINE IN THE HELLENIC ARMED FORCES

Aikaterini Stasini, Miltiadis Soultanis, Foteini

Athanasiadoy, Nektarios Voydiklaris

AIM: To present the history, function, usefulness and the general impact of the Helpline, as well as to underline its important connection to the crisis intervention services, especially in the Armed Forces.

MATERIAL & METHODS: The official form –questionnaire was used in order to collect the data. The analysis of the data was conducted using the Queries Database SQL and the MS Excel.

RESULTS: The Helpline offers its services to both military personnel as well as civilians. Comparative results will be presented in relation to several requests of the callers. In addition, systemic, psychodynamic and group-analytic perspectives for the qualitative analysis of the statistical data will be illustrated.

CONCLUSIONS: The 24-hour psychological support Helpline promotes preventive, counseling and therapeutic mental health services for the Hellenic military personnel without excluding the civilians.

EVALUATING THE INTENSITY OF PAIN AND THE EMOTIONS OF PATIENTS WITH LOW BACK PAIN ACCORDING TO THEIR SOCIOECONOMIC STATUS. A CROSS-SECTIONAL STUDY

Michalea-Dimoulea Eleni

Aim: (1) To record the incidence of low back pain in pain clinics in Greece; (2) to determine whether patient's demographic and socioeconomic characteristics affect the intensity of pain and their emotional situation.

Material-Methods: A cross-sectional study of patients complaining about low back pain was conducted at 6 different pain clinics in Attica. Patients' demographic and socioeconomic characteristics, their pain intensity and their emotional situation had been evaluated. Multiple linear regression analysis in a stepwise method was used. Statistical significance was set at $p<0.05$ and analyses were conducted using SPSS statistical software.

Results: Most of the patients were women (69.7%), over 60 years old (64.2%) and had low income (76.2%). University alumni and patients with postgraduate studies having less intense pain than primary/ middle school and high school graduates ($\beta=0.09$; SE=0.03; $p=0.005$ and $\beta=0.07$; SE=0.03; $p=0.045$ respectively). Female sex ($\beta=0.30$; SE=0.14; $p=0.038$), painkillers use ($\beta=0.37$; SE=0.18; $p=0.036$) and increased pain score ($\beta=0.37$; SE=0.04; $p<0.001$) were associated with greater negative emotions. Age ($\beta=0.57$; SE=0.18; $p=0.003$ for patients 60-69 years old and $\beta=0.63$; SE=0.17; $p<0.001$ for patients over 70) and marital status ($\beta=0.44$; SE=0.15; $p=0.004$) were associated with more positive emotions.

Conclusions: Low back pain was the first non-malignant reason for someone to visit a pain clinic. Patients' demographic and socioeconomic characteristics seem to affect the intensity of their pain and their emotional situation.

PSYCHOLOGICAL SUPPORT GROUPS FOR THE STAFF AT THE NAVAL HOSPITAL OF CRETE

Asimina Eleftheria

Aim: Occupational burnout in the field of military hospitals is a reality. The testimonies of professionals prove that daily encounter with disease, pain and death affects them emotionally.

At the Naval Hospital of Crete, in an attempt to prevent occupational strain, psychological support to the staff was provided by the psychiatric department.

Material-Methods: The head nurse of the consultation-liaison psychiatry service organised and coordinated individual as well as group sessions from 2009 to 2015. There were 7 groups developed on a monthly basis and 561 individual sessions took place. These sessions were based on a group-analytic and socio-therapeutic approach and the principles of the therapeutic community.

The subjects discussed were professional relationships, managing confrontation and work-related stress, facing disease and death, interpersonal relationships, personal and family problems, financial and social crisis.

Results: The results achieved by these sessions are emotional discharge, an attempt to better understand one another, setting boundaries on both professional and personal relationships. An increased awareness of personal responsibility for improving working conditions and professional relationships is remarkable. This has minimized the attribution of responsibility and passive expectation of solutions by Administration.

Conclusions: In conclusion, we are confident that psychological support for the staff at a military hospital enables better cooperation and improves their psychological well-being without affecting the military structure or hierarchy. Fears and reluctance were expressed by both the personnel and the administration.

The participants' request and the administration's sensitivity to preventing occupational burnout was stronger than any doubts or difficulties.

RELATIONSHIP BETWEEN ANXIETY AND CARDIOVASCULAR DISEASE RISK IN HEALTHY HELLENIC ARMED FORCES

Athanasiadou Foteini, Koukia Eumorfia, Alevizopoulos George, Kallergis George

AIM: Coronary heart disease (CHD) is referred to as a psychosomatic disease.

Phenomena such as anxiety, depression, mental exhaustion and socioeconomic situation have not been well examined (Albus et al., 2004).

The aim of this study was to examine the relationship between anxiety and the risk of CHD appearance in healthy Greek Military Earth Force population.

MATERIAL-METHODS: Four sixty four (464) Earth Force officers (358 men and 106 women) aging from eighteen (18) till sixty (60) years old complete the 20-item State-Trait Anxiety Inventory (STAI) (Spielberg et al., 1970). Responses are recorded on a four-point scale. Two anxiety traits were measured. Pearson r correlations were conducted to reveal if any relationship between trait and/or state anxiety and the risk of CHD appearance exists.

RESULTS: Frequency results revealed that a total of 46.0% feel a little calmness, 28.6% feel a continuous agony for bad situations that might happen. Respectively, 31.0% was in an anxiety state, feeling nervous, 24.6%, and in continuous arousal, 27.7%. Pearson r resulted in a positive correlation between smoking habbit and State Anxiety, r=.238, p=.010, as well as with Trait Anxiety, r=.238, p=.010. Results showed that STAI statements directly connected with anxiety are positively correlated with either State Anxiety, r=.920, p=.010, and Trait Anxiety, r=.920, p=.010. Positive correlations were found between given STAI statements and blood measurement results (blood pressure, LDL).

CONCLUSIONS: Results confirm the initial hypothesis that anxiety as autonomous trait has a direct relationship with CHD appearance. This research applauses the influence of anxiety to sudden cardiac death.

THE EMPLOYEES’ PERCEPTION OF HUMAN RESOURCE PRACTICES AND THEIR RELATION TO THE ORGANIZATIONAL COMMITMENT AND JOB SATISFACTION IN A TERTIARY GREEK HOSPITAL

Stavros Gourgiotis, Evangelos Falidas, Georgios Veloudis, Constantinos Villias

Aim: Human resources management (HRM) is an important task in the field of healthcare. We tried to evaluate the HRM practices in the health sector through the practical investigation of employees’ perceptions about the HRM practices and the possible relation of these perceptions to the organizational commitment and job satisfaction.

Material - Methods: A structured questionnaire including social-demographic characteristics, HRM policies and practices scales, organizational commitment scale, and job satisfaction scale was distributed among the departments of a tertiary hospital. 206 questionnaires were properly filled out.

Results: Employees’ attitudes regarding the reward, the compensation, and the evaluation of job performance HRM practices were negative, while the commitment and the job satisfaction ranged in low levels. Gender, age, education, marital status and the years of experience did not influence the perceptions of employees’ about HRM practices in contrary with specialty. The doctors in training, the technology staff, and the nurses had more negative views than the specialized doctors and the administrative staff. The position of the respondents influenced the views of employees about the HRM practices were related to the possibility of involvement. There was also statistical positive relationship between employees’ perceptions about HRM practices and job satisfaction and organizational commitment.

Conclusions: The health sector regarding remuneration, evaluation, and reward needs significant improvement in the terms of HRM practices. Although practices such as selection, training, participation, and working conditions have moderately improved, organized strategies mainly regarding the lower hierarchical levels of the hospital are needed.

PROGNOSTIC SIGNIFICANCE OF IPDE DIMENSIONAL SCORES IN THE PREDICTION OF SUICIDE. A STUDY IN MILITARY, CLINICAL AND CONTROL GROUP

Tony Donchev, Krasimir Kostadinov

Introduction: We used our data from the validation study of the Bulgarian version of IPDE for a statistical analysis, seeking a correlation between dimensional scores of IPDE and suicide attempts in our population.

Methods: We studied 457 subjects between 18 and 40 years in three groups: 185 inpatients; 178 soldiers, control group of 94 students. They were evaluated with IPDE for ICD-10. Binary logistic regression was used to assess the relationship between the probability for a suicide attempt and dimensional personality traits measured by IPDE.

Results: Binary logistic regression model showed that dimensional traits that have a prognostic value are schizoid, histrionic, emotionally unstable - borderline and impulsive type and dissocial.

Conclusion: The study showed that personality dimensional scores carry prognostic significance in the assessment of the probability of a future suicide attempt. Our results support the view that personality disorders characterized with the impulsivity traits have higher suicide risk.

OP session 4 – General surgery, urology and gynaecology

SURGICAL TREATMENT METHODS OF COLORECTAL CANCER

Savulescu Florin, Cirlan Cristian

The evolution of science as well as the technological advances have allowed, over time, the development of several methods for surgical treatment of colorectal cancer. From all of these methods, we will consider only those therapeutic methods that have proven effective over time. We will not discuss a certain procedure, but we will refer to the following therapeutic categories as follows: classical surgery, laparoscopic surgery and robotic surgery.

Materials and methods: The information collected came from a retrospective study conducted over a period of 18 months starting with October 2014. In this study were included patients diagnosed with different colorectal cancers.

Conclusion: The findings of this study are presented as tables that have centralized the information obtained.

Discussions: In the conclusions of this presentation, based on the information and findings presented, calling upon the free will, are depicted some ideas regarding the three therapeutic methods, leaving the reader to determine the appropriateness of applying a certain method.

KEY POINTS IN FUNCTIONAL SURGICAL TREATMENT OF RECTAL CANCER

O. Albita, T. Rogin, O. Sima, D. Orosan, C. Dutu, C. Musat, R. Nica, N. Bajenaru, V. Varjoghe, F. Savulescu

Introduction: The colorectal cancer (CCR) represents the 3rd main cause of neoplasic disease and the 2nd cause of death worldwide. Modern complex treatment of CCR involves association of various paraclinic investigations with modern surgical techniques and postoperative chemotherapy, aiming to obtain best oncological outcomes and a good quality of patient’s life.

Materials and Method: This study compares Group 1(57 patients 2004-2014) with Group 2(61 patients 1998-2002) regarding postoperative genitourinary function, with or without pelvic autonom nerves preservation (PANP-Sugihara).

Results: Statistical study of results reveals reduction of urinary impairment from 67% to 43.75% and sexual disorders from 32.1% to 28.1%, emphasizing the leading role of PANP.

Conclusions: The Gold Standard of CCR’s surgical treatment is the total mesorectal excision (TME) with PANP and saving sphincter technique. Only adequate knowledge of pelvic autonom nerves anatomy facilitates identification and preservation during surgery with optimal postoperative outcome of genitourinary function.

DEEP SURGICAL SITE INFECTIONS WITH FASCIAL NECROSIS - CLINICAL ASSESSMENT AND COST ANALYSIS - PRELIMINARY RESULTS

Mutafchiyski V, Popivanov G, Petrov N., Savov E., Vaseva V, Kjossev K, Gergova I, Ivanov P, Petrov H, Penchev D, Tabakov M

Introduction: Deep surgical site infections are a significant source of morbidity and increase significantly the hospital costs. Although negative pressure wound therapy has been received worldwide recognition as an effective technique for temporary abdominal closure in several conditions, the results of treatment of fascial necrosis and dehiscence are rather unsatisfactory. We sought to perform clinical assessment and economic analysis of the deep SSIs and the effectiveness of NPWT in such cases.

Material and methods: The study includes only cases with neglected deep surgical site infections and partial or complete fascial dehiscence. All cases were treated by serial debridement and V.A.C® from one team in the period September 2013-February 2016. Open abdomen was applied in 13 cases with partial or complete fascial dehiscence. No major break in the surgical technique was noted. They were compared with 20 matched controls with uncomplicated postoperative course. The hospital stay and the cost of the treatment were considered as a primary outcome measure, whereas PFC rate, days with NPWT and mortality were set as secondary outcome measures.

Results: The mean age was 70 years and 76.5% (n=13) of them had at least two risk factors. The rate of SSI was 1% (17/1610). Microbiological confirmation of the infection was achieved in 11 cases (64.7%). Polyresistant hospital strains were isolated in 4 cases (23.5%). NPWT was applied for a mean of 19.5 (4-36) days with mean 7.8 dressing changes. The mean hospital length of stay and hospital costs per patient in complicated and uncomplicated cases was 30 vs 7.3 days and 86 vs 719 Euro, respectively.

Primary fascial closure was achieved in 6 of 13 patients (46%), whereas planned ventral hernia through skin closure only was created in 10 (59%). None of the patients develop enteroatmospheric fistula. Lethal outcome was noted in 1 case (5.9%).

Conclusion: Deep surgical site infections led to fascial necrosis and dehiscence are troublesome postoperative complication associated with a tremendous increase of the hospital costs. NPWT alone yields low PFC rate and the combination with dynamic fascial closure is highly recommendable.

LAPAROSCOPIC RETROPERITONEAL LYMPH NODE DISSECTION FOR RESIDUAL POST-CHEMOTHERAPY NON-SEMINOMATOUS TESTICULAR CANCER

Dimitrios K. Manatakis, Georgios Bouboulis, Ioannis Passas, Dimitrios Balalis, Konstantinos Damalas, Sotirios Gantzoulas, Vassilios Berdelis, Georgios Sakorafas, Georgios Plataniotis, Emmanouil Gontikakis, Dimitrios P. Korkolis

Purpose: The role of laparoscopic retroperitoneal lymph node dissection (RPLND) for residual post-chemotherapy retroperitoneal masses in non-seminomatous tumors remains controversial, largely due to its inherent technical difficulty and long learning curve. The aim of this retrospective study is to present our technique and initial perioperative results with the laparoscopic approach for modified template dissection in clinical stage II non-seminomatous testicular cancer.

Material and Methods: Between January 2014 and December 2015, 28 patients underwent post-chemotherapy therapeutic RPLND, of whom 10 selected patients with a mean age of 28.8±7.7 years were offered and consented to the laparoscopic approach. Six left-sided and 4 right-sided modified template dissections were performed, for residual masses of 2-5 cm (mean 3.5 cm).

Results: Mean operative time was 240 minutes (range 150-330) with an average estimated blood loss of 250 ml (range 100-1500). One patient (10%) was converted to the open approach, due to significant hemorrhage from injury of the ipsilateral renal vein. Mean length of hospital stay was 3.2 days (range 2-7). Antegrade ejaculation was maintained in all cases, while one patient (10%) developed chylous ascites postoperatively, due to intraoperative injury of the cisterna chyli and was managed conservatively. An average number of 12 lymph nodes were harvested (range 5-25) and harbored disease in 70% of patients (viable tumor cells 20%, mature teratoma 50% and fibrosis/necrosis 30%).

Conclusion: Laparoscopic RPLND is a technically challenging operation, however in experienced hands it is a feasible and safe option for residual post-chemotherapy retroperitoneal masses in non-seminomatous testicular cancer, with similar oncological results compared to open dissections. The laparoscopic approach offers all the advantages of minimally invasive surgery, in terms of shorter hospitalization, faster return to normal activity and better cosmesis.

CONSERVATIVE TREATMENT OF TUBAL PREGNANCY

Nicolae Niculescu, Ovidiu V. Nicodin, Bogdan Panaite, Costin Parvulescu, Ioana Niculescu

Objectives: Establishing the indications of conservative treatment in ectopic pregnancy.

Materials and Methods: Between 2012-2014, thirtheen patients with ectopic pregnancy have been treated with conservative surgical techniques.

Pre- and intraoperative exclusion criteria were: ectopic gestational sac size exceeding 6 cm, fetal heart activity, β-hCG serum levels > 20,000 mIU/ml, previous surgery on the affected fallopian tube, isthmic ovular implantation.

Thus were selected the following cases: abcence of the controlateral fallopian tube/functional compromised, gestational sac unrupted and ampulo-pavilionar implantation; we decided to apply laparoscopic linear salpingotomy, gestational sac extraction with bipolar hemostasis.

Results: We have obtained five intrauterine pregnancies and two ectopic pregnancies which required radical treatment (salpingectomy). Two pregnancies have been completed with gestation carried to term. There were no favorable results in patients who had other associated factors of infertility.

Conclusions: In selected cases laparoscopic surgery allows the anatomical and functional restauration of the fallopian tube affected by pregnancy and offer a chance in getting a desired pregnancy.

PROINFLAMMATORY CYTOKINES IN PATIENTS WITH BENIGN PROSTATE HYPERPLASIA

Florin Rusu, Ovidiu Pacu, Armand Iliescu, Andrei Paun, Marius Dinu, Viorel Jinga, Emilia Rusu, Dan Mischianu

AIM: Inflammation of the prostate may be a mechanism for hyperplastic changes that happens in the prostate. In this study it was evaluated the relationship between anthropometric, metabolic factors, and proinflammatory cytokines in patients with benign prostate hyperplasia (BPH).

METHODS: 312 men were included into prospective study for prostate cancer screening. 25 patients were diagnosed with prostate cancer and were excluded from this analysis. Others (287 patients) were divided into 2 groups – group A 180 patients without BPH and, group B – 107 patients with BPH. Body weight, waist circumference, hip circumference, blood pressure were determined. Body mass index (BMI) was calculated. Biochemical analyses including fasting plasma glucose (FPG), HbA1c, total cholesterol, triglycerides, high-density lipoprotein (HDL-C), fasting plasma insulin (FPI), adiponectin, leptin, TNF alpha, IL-6 and prostate-specific antigen (PSA) were performed. Insulin resistance (IR) was determined using Homeostasis model assessment (HOMA-IR). The prostate gland volume was measured using transrectal ultrasound.

RESULTS: Men with BPH were older (58.05±9.1 versus 57.91±7.58 years). Body weight, waist circumference, hip circumference, BMI, systolic and diastolic blood pressure don't differ into groups. Patients with BPH (group B) have fasting plasma glucose, HbA1c, total cholesterol and triglycerides, HOMA-IR significantly higher (all $p<0.05$). Leptin, TNF alpha, IL-6 were higher in patients with BPH (all $p <0.05$). PSA level was correlated positively with age ($r=0.69$, $p=0.005$), triglycerides ($r=0.45$, $p=0.03$), total cholesterol ($r=0.32$, $p=0.04$), fasting plasma insulin ($r=0.46$, $p=0.03$), and HOMA-IR ($r=0.49$, $p=0.02$) and negatively with BMI ($r=-0.22$, $p=0.03$).

CONCLUSIONS: PSA level was influenced by age and metabolic factors (BMI, triglycerides, HDL-C, fasting plasma insulin, insulin resistance). Obesity, elevated level of triglycerides, total cholesterol, fasting plasma insulin, HOMA-IR are associated with BPH. The results suggest that BPH can be a facet of metabolic syndrome. Chronic inflammation may lead to events that can cause proliferation within prostatic tissue through a variety of mechanisms which involves proinflammatory cytokines.

Funding: A part of this study was supported by the Romanian National Authority for Scientific Research as a part of the PNCDI 2 program ADENODIAG 41-085/2007.

OPEN SURGERY – STILL A VIABLE ALTERNATIVE TO THE MINIMALLY INVASIVE TREND TREATMENT OF STAGHORN STONES

Andrei Paun, Ovidiu Pacu, Cornel Stanescu, Florin Rusu, Armand Iliescu, Marius Dinu, Dan Mischianu

In literature there are described several methods of treatment of renal lithiasis:

- extracorporeal shock wave lithotripsy (ESWL)
- percutaneous nephrolithotomy
- retrograde or antegrade ureteroscopy
- open surgery

Considering that minimally invasive techniques can sometimes require adjacent procedures to resolve remaining fragments that can spread over long periods of time, in selected cases open surgery can be used.

We would like to bring to your attention the case of a 60-year-old male patient diagnosed with type A bilateral staghorn stones.

Blood tests - within normal limits.

KUB – bilateral type A staghorn stones

Urinalysis - Proteus mirabilis UTIs.

IVU – secretion/excretion present bilateral associated with bilateral grade 1 hydronephrosis.

Left pielolithotomy is performed with full removal of the staghorn stone. Postoperative evolution is favorable. The patient is discharged after 7 days.

One month after the first surgery patient returns to continue therapy management. Right pielolithotomy is performed. Postoperative evolution is simple. The patient is discharged after 6 days.

Radiological and ultrasound investigations performed after the two surgical procedures did not show any remaining sones. Urinalysis – sterile.

In certain selected cases of kidney stones open surgery can be performed to achieve the goal of "STONE FREE".

EXTRAGONADAL GERM CELL TUMOR

Cornel Stanescu, Florin Rusu, Ovidiu Pacu, Marius Dinu, Adrian Marincas, Armand Iliescu, Andrei Paun, Cristian Iatagan, Dan Mischianu

INTRODUCTION: Extragonadal germinal tumors have a low incidence, of 1-5% of all germinal cell tumors, and malignant transformation is due to the remaining germinal cells along the longitudinal axis of the body (from the pineal gland, through the mediastinum to the retroperitoneum and sacrococcygeal region) during their migration in early embryonic stages.

METHODS: We present a clinical case of an extragonadal retroperitoneal germinal cell tumor in a 44 years old patient.

RESULTS: A 44 year old male patient diagnosed using imaging with gigantic retroperitoneal lymph nodes, in correlation with a high seric level of beta-hCG is admitted for further investigation and appropriate treatment. The clinical exam and scrotal echography reveal solely a hypotrophic left testicle. Decision is made for surgery and biopsic orchidotomy is practiced under spinal anesthesia trough an inguinal approach: normal macroscopic semblance; extemporaneous HP exam shows no malignant lesions, the gland is conserved. Echographic percutaneous guided biopsy is performed on a giant laterocystic lymph node with a positive HP diagnostic of seminoma. The patient undergoes polychemotherapy. The follow-up PET-CT reveals the retroperitoneal lymph nodes to be residual and metabolically inactive.

CONCLUSIONS: The first line of treatment for large volume germinal seminal tumors (with lymph nodes > 5cm) is polychemotheraphy. For the exposed case we accomplished full metabolic response to the oncological treatment.

MESH SURGICAL TREATMENT FOLLOW-UP IN GENITAL PROLAPSED

Ovidiu Bratu, Dragoș Marcu, Dan Spânu, Marius Dinu, Dan Mischianu

Objective: Genital prolapse represents a frequent pathology in elderly women and it is the source of many genital and urinary complications.

The aim of this article is to evaluate the surgical and functional results of minimallly invasive procedures that we have used in this pathology.

Matherial and metods: We have analysed 50 women with genital prolapse. For anterior compartiment prolapse we have used a four arm mesh and for posterior prolapse a two arm mesh anchored to the sacrospinous ligament.

Results: We have encountered three relapses in the cases of patients with IVth grade prolapse. Occult urinary stress incontinence appeared in 7 out of 20 cases with cystocele. 7 women out of 16 who have undergone mesh procedures for posterior prolapse were sexually active and 5 patients out of these 7 have complained of pain during intercourse, postprocedure. No mesh rejection or septic complications have occurred.

Conclusions: Nowdays, mesh surgical treatment represents the standard treatment for genital prolapse. Most of the late complications are well accepted by patients. We don't recommend posterior implant in sexual active women.

LAPAROSCOPIC VERSUS OPEN PYELOPLASTY FOR URETEROPELVIC JUNCTION OBSTRUCTION

Mădan V, Aungurenci A, Rădulescu A, Spînu D, Iatagan C, Pacu O, Farcaş C, Botea V, Mischianu D, Dinu M

Introduction: Among the multitude of surgical techniques, the dismembered pyeloplasty (Heynes-Anderson) is considered the gold standard for ureteropelvic junction obstruction treatment. In the last two decades the laparoscopic approach started to replace open surgery. The aim of our study is to compare the surgical and functional outcomes between laparoscopic and open pyeloplasty.

Method and materials: We analyzed a series of 30 patients admitted in our clinic between January 2015 and March 2016. Twelve patients underwent laparoscopic pyeloplasty while the rest of 18 underwent open surgery. For the patients operated open the retroperitoneal approach was elective. The laparoscopic transperitoneal approach was performed in 10 cases, while the retroperitoneal was performed only in 2 cases. A ureteral stent was inserted to all operated patients.

Results: The surgical time for laparoscopic pyeloplasty ranged between 240 and 360 minutes while for open surgery ranged between 90 and 210 minutes. The number of hospitalization days for laparoscopy was between 5 and 12 days (mean ratio - 6.63), while for open pyeloplasty was between 7 and 21 days (mean ratio - 12.68). Postoperative complications appeared at 6 patients out of 18 operated openly, while only 3 out of 12 patients operated laparoscopic had complications. Functional outcomes were evaluated by mean of Uro-CT scan or renal scintigraphy at 3 months postoperative. Most patients (28) had improved postoperative results.

Conclusions: In our experience, both laparoscopic and open pyeloplasty had similar functional results, but laparoscopic technique is favourable because of reduced hospitalization time and lower postoperative complications.

OP session 4 – Oral and maxillofacial surgery, ophtalmology

IDENTIFICATION OF MYOEPITHELIAL CELLS AND THEIR ROLE IN DIAGNOSIS OF SALIVARY GLAND TUMORS

Gunhan Omer

OBJECTİVE. Myoepithelial cells are an important component of salivary gland tumors and are responsible from the diverse histology of them. This study is focused on the myoepithelial cell differentiation by using immune-histochemical stainings in a various types of salivary gland tumors. The relation of myoepithelial cells with stromal matrix and the associated epithelial cells were evaluated.

MATERIAL AND METHOD. Retrospective analysis of histologic slides of twenty benign and twenty malignant salivary gland tumors were examined for morphologic and immunohistochemical identification of different myo-epithelial cells such as; spindle-stellate, polygonal-epitheloid, plasmacytoid, basal and clear types. P63, S-100 protein, smooth muscle actin, cytokeratin 7 antibodies were used for identification of myoepithelial and epithelial cells.

RESULTS. The best examples of myoepithelial cells were detected by immunohistochemically and particularly in pleomorphic adenomas. Basal and clear cell types of myoepithelial cells closely resembled the epithelial cells and their identification was relatively difficult. More than one, different type antibodies are necessary for myoepithelial cells identification. Morphologic identification of myoepithelial cell types was easier when they were associated with stromal matrix material.

CONCLUSION. Myoepithelial cell components of various salivary gland tumors may be quite different and identification of myoepithelial cell types may pose difficulties. A confident identification of myoepithelial cells can be achieved with the use of combination of morphology and antibodies of P63, S-100 protein and smooth muscle actin. Correct identification of myoepithelial cells is one of the critical part of diagnosing salivary gland tumors.

FEATURES REGARDING VICIOUSLY CONSOLIDATED ORBITAL BLOWOUT FRACTURES

Moraru L., Dumitru C., Gabără A., Bădoiu C., Căruntu A.

Introduction: The isolated blowout orbital fractures occur when traumatic agents that are comparable to the size of its aperture frontally hit the orbit. The traumatic energy is transmitted to eyeball, which transmits it to orbital walls that fracture easily, thus sparing visual analyzer. The symptoms are influenced by the displacement of the fracture, ranging from periorbital bruise to diplopia. Since orbital walls cannot be directly examined, many cases go undiagnosed and heals in a vicious manner being accompanied by atrophy and scarring of soft tissue thus making surgery difficult.

Methods: We retrospectively reviewed cases with blowout orbital fractures viciously healed with secondary diplopia treated surgically in our unit over the last two years.

Results: Compared with recent fractures, treatment, though identical (reconstruction of orbital walls with preformed low profile titanium mesh) was more difficult and required more time in the operating room. The interventions resulted in remission of diplopia shortly after surgery without complications, but couldn’t equally correct enophtalmia.

Conclusions: Viciously consolidated orbital blowout fractures can be successfully treated in terms of eyeball functionality, but technically more difficult, with potentially higher perioperative risks and variable aesthetic results. Therefore proper diagnosis and early treatment are preferred.

PIEZOSURGERY WITH PLATELET-RICH PLASMA OR CONVENTIONAL ROTATORY INSTRUMENTS FOR INFERIOR THIRD MOLAR EXTRACTION

Cheshmedzhieva Atanaska, Mitov Stefan, Kolev Stanislav

PURPOSE: The surgical removal of mandibular third molars is frequently accompanied by significant postsurgical sequelae, and different protocols have been described to decrease such adverse events. The aim of this study was to investigate the performance of piezosurgery compared with traditional rotating instruments during mandibular third molar removal.

PATIENTS AND METHODS: Our research includes 40 patients who needed surgical removal of bilateral mandibular third molar teeth. They are served in Departement of Oral and Maxillofacial surgery in Military Medical Academy, Sofia. Each patient was treated, at the same appointment, using bur removal on 1 side of the mandible and a piezoelectric device on the contralateral side. On the side where we used ultrasonic surgery we put Platelet-Rich Plasma (PRP) in the socket of third lower molar after its extraction. Postoperative sequelae as oedema, trismus, pain, and the duration of surgery were analyzed.

RESULTS: The study consisted of 40 healthy patients, 20 male and 20 female. The pain evaluation reported by patients who underwent surgery with piezosurgery was significantly lower than that reported after bur (conventional) removal, reaching statistical difference 2 days ($P = .043$). The clinical value of orofacial swelling and trismus at day 3, was lower in the piezosurgery group ($P < .005$). The average surgical duration was significantly shorter in the cases with bur exstraction than in the piezosurgery cases combined with PRP. ($P < .05$).

CONCLUSION: The results of our survey showed that piezoelectric surgery and applying PRP significantly reduced the occurrence of postoperative sequelae -oedema, trismus, and pain compared to the conventional rotary instrument technique in lower third molarsurgery. The only disadvantage of the piezoelectric technique was the duration of the, which was increased by approximately 8 minutes; however, this effect was offset by reducing the morbidity and increasing patients’s post-op comfort.

OPERATIONAL TREATMENT OF A HEAVY FRACTURE OF AN EYE-SOCKET AND A CHEEK-BONE

Mitov St., Chesmedjieva At., Karjin E.

Major S M. was transported to the Military Medical Hospital in the beginning of May 2015. He had been injured during the safemaking of the detonated munitions store near the village of Iganovo. He had fallen from a height of about three metres in the course of the execution of his duties. As a consequence of this height trauma, he had a broken leg, three ribs on his left side, a left eye socket and a left cheek-bone, as well as a concussion of the brain.

S M. first entered intensive care unit for the treatment of this combined trauma. After a seven day treatment in the reanimation ward, his condition was stabilized and a traumatological operation on his broken left leg was performed. No operational treatment was necessary for his broken ribs as the fracture was without a displacement.

On the eighth day after his trauma, he was admitted to maxilla-facial surgical department for treatment. A new X-Ray was done and-a 3D reconstruction of the facial skeleton performed. It was established that due to the heavy eye-socket fracture, there is a collapse of the floor of the eye-socket in the direction of the upper- jaw sinus. The broken left-hand side cheek-bone was wedged into its underlying bone structures. This had led to a change in the position of the left eye and the corresponding sight disturbance-double vision. After pre-operative preparation, we proceeded to surgical treatment under general anaesthesia.

A horizontal slit was made in the area of the lower lid of the left eye and a vertical one of the side edge of the left eye-socket reaching up to the lower end of the left eyebrow. After the fracture lines with the help of the Limberg bone hook through a horizontal skin slit in the area of the left cheek were revealed, we reached the fracture line in the area of crysta zygomaticomaxillaris. In this way, the wedged eye-socket was moved and pulled outwards and upwards with the help of the Limberg bone hook. A good reposition of the fragments was achieved. A defect of the bone with dimensions of 2/1.5 cm was established in the area of the floor of the eye-socket. A titan net was set in the area of the defect which was fixed with a screw at about 1 cm below the infraorbital edge. A disk osteosynthesis was made in two places-infraorbital, and a second one in the area of the link of the cheek-bone with the frontal bone. A very good fixation of the fragments was achieved. The post-operational period proceeded well and the patient was discharged from the hospital seven days later. It was established during a check examination 30 days after the discharge that the patient had no visual disturbance. S M. returned to work three months after the trauma and now he performs his everyday military duties.

PROBLEMATIC AIRWAY IN MAXILLOFACIAL SURGERY - ANAESTHESIOLOGIC APRROACH

Krstic Lecic Ivana

Today it is well know that over one third of unfavourable anaesthesiologic outcomes are connected to inadequate ventilation, while almost one third of lethal outcomes due to anaesthesia direct consequence of impossibility establishment and maintenance of problematic airway.

Specificity of anaesthesiology work during maxillofacial surgical procedures is based on fact that the most of the patients because of the characteristic pathology belongs to category of those in whom difficult or impossible ventilation and complicated intubations is expected in advance. Protocols for management of difficult airway are pretty similar in most countries that have them, and all these protocols are renewed and reviewed in difficult periods of time. Numerous appliances and device for easier establishment and maintenance of problematic airway are available today, such as for example, various types of LMAs, laryngeal tubes, rigid fiberoptic guides, flexible bronchoscopes and sets for percutaneous tracheotomy. Each of these appliance and device are of a great help in cases of expectedly or unexpectedly problematic airway, but only in hands of skilled, educated and experienced staff. Of course, evaluation of the patient remains the most important factor that is going to have an impact on choice of strategy and evaluation of the airway itself, especially onto the site which is at the same time an operation field that has to be easily approached by surgeon, while monitoring a stable and safe patient.

COMPUTER BASED PROGRAMME FOR SCREENING OF MACULAR DISEASES AND AFTER EYE TRAUMA

Vidinova Christina, Voinov Latcezar

Aim: The aim of this survey is to describe our programme for early screening for macular changes in diabetic retinopathy, AMD and after eye trauma.

Material – Method: We present 32 patients who have been examined in our clinic after being screened for macular diseases with MACUTEST.

In all cases VA examination, computer perimetry tests, fundus photography and OCT have been performed. The OCT programmes include: HD line, Cross line and EMM5.

Results: For early screening of macular diseases we use - MACUTEST. An online internet based test, broadly accessible and easy to use.

From the 32 patients referred to us after screening 7 were with diabetic retinopathy with macular edema. The hard exudates and foveal changes have been detected on the fundus photographs and OCT.

In 5 patients traumatic maculopathy after eye injury has been diagnosed. Apart from edema and epiretinal gliosis, cases of post traumatic macular holes have been found.

In the majority of patients - 20 different stages of AMD have been found. Patients with macular atrophy, neovascularisation and RPE detachments have been detected.

Conclusions: Macular diseases are a large group of eye disorders whose early detection is of substantial importance for their successful treatment. The MACUTEST is a screening test, which can be done at home, easily and without specialized equipment. That makes it very useful in finding patients with macular disorders and enables their quick hospitalization and treatment.

REGENERATING AGENTS (RGTAs) – MATRIX THERAPY IN OPHTHALMOLOGY

Ştefan Cornel, Timaru Cristina Mihaela, Anghel Gheorghe, Burcea Marian, Şelaru Daniela, Muşat Ovidiu, Zemba Mihail, Manole Horaţiu, Macovei Laura, Pulbere Liliana, Armegioiu Monica

Introduction: The RGTA- based technology (“ReGeneraTing Agents”) is a new therapeutic approach also named “Matrix Therapy”, aiming to preserve and even restore the extracellular matrix (ECM) and providing encouraging results in ocular tissue regeneration. The results of this therapy are promising for the management of anterior segment diseases, especially corneal diseases – persistent epithelial defects and neurotrophic keratitis.

Material and method: Patients with persistent epithelial defects or corneal superficial punctuate keratitis of various etiologies,refractory to conventional treatments and patients who underwent refractive surgery or corneal transplant surgery, were included in a single-center uncontrolled, prospective study. The clinical examination included slit lamp biomicroscopy, florescein staining and anterior segment OCT.

Results and conclusions: The RGTAs appear as apromising new therapy in the context of persistent epithelial defects and neurotrophic keratitis. It is effective in improving subjective and objective symptoms, corneal healing and patient’s comfort, thus providing a solution for patient’s refractory to conventional treatment.

OCULAR TRAUMA WITH RETAINED FOREIGN OBJECT. SURGICAL PARTICULARITIES

Gh. Anghel, O. Musat, C. Stefan, D. Selaru, M. Zemba, H. Manole, M. Armegioiu, L. Pulbere, L. Macovei

This paper presents, by the use of videos, surgical solutions for penetrating ocular trauma with retained foreign object of hospitalized patients in the ophthalmology department of the Central Universitary Emergency Military Hospital.

In all cases the retained foreign object removal was followed by pars plana vitrectomy, laser treatment, tamponading agents such as expanding gases and silicone oils as required by each case.

VISION RELATED QUALITY OF LIFE IN SERBIAN PATIENTS

Kovac Bojan, Vukosavljevic Miroslav, Resan Mirko, Kontic Marko, Petrovic Nenad

PURPOSE: To asses vision related quality of life and health related quality of life in most common ophthalmic deseases and to measure effectivness of catarct surgery throu quality of life assessment.

METHODS: The Serbian version of NEI VFQ-25 was translated in accordance with standard methods that have been adopted internationally. Descriptive analyses and a generalized linear estimating equation analysis were undertaken to measure change in vision related quality of life after surgery.

RESULTS: The overall index score on the NEI VFQ-25 ranged from 65.3 to 67.8 with a mean of 67.4 ± 15.0. The average value of a composite score before surgery was equal to 73.1 (SD, 18.8). After cataract surgery on the worse eye, there was an increase in the value of the composite score by an average of 20.2 points on 93.3 (SD, 11.6).

CONCLUSION: Cataract surgery significantly improved vision related quality of life among bilateral cataract patients and this study has provided valuable information about change in vision related quality of life after surgery in Serbia. Due to the benefits that cataract surgery has for Vision-related quality of life, ensuring that the older population has access to regular eye examinations and timely treatment for cataract is paramount.

OP session 5 – Gastroenterology, diabetes and nutrition

INTESTINAL PERFORATION AS THE FIRST PRESENTATION OF CROHN’S DISEASE: A RARE CASE IN A YOUNG ADULT

Pachiadakis Ioannis, Sfikas Georgios, Lttzakri Dimitra, Psochias Polykarpos, Katsimardou Alexandra, Gatsiou Marina, Kafalis Nikolaos, Pehlivanidis Anthimos

Purpose: We report the case of a young male who presented with fever of a ten-day duration and intestinal perforation as first manifestations of Crohn’s disease with no other symptoms from the gastrointestinal tract.

Materials and methods: A male of 19 years with no medical record was admitted to our hospital because of fever over 39 C that persisted for ten days. The patient mentioned no other symptoms. The clinical examination revealed no findings and the laboratory evaluation showed elevated inflammation markers and thrombophilia. The tests for antibodies against a wide spectrum of viruses were proven negative. The patient underwent a CT scan of the thorax and upper and lower abdomen, which revealed a severe inflammation of the ileum and sigmoid, intestinal perforation and presence of contrast dye in the urinary tract. After ten days of conservative treatment, the patient underwent a new CT scan of the abdomen, which further revealed the formation of an intraabdominal abscess. The patient complained of haemorrhagic stool and his general condition deteriorated. He was submitted to surgical drainage of the abscess. He further underwent colonoscopy that revealed ileitis and colitis.

Results: The patient was treated with corticosteroids, immunosuppressive therapy and antibiotics and his clinical and laboratory status substantially improved. The pathologic findings were indicative of Crohn’s disease.

Conclusions: Crohn’s disease may rarely present as a mild feverish condition with no symptoms from the gastrointestinal tract which, despite the good clinical condition of the patient, may hide very severe complications.

THE EXPRESSION AND THE SURVIVAL EFFECT OF Β-CATENIN IN RELATION TO PATIENTS' OUTCOME IN COLORECTAL CANCER

Constantinos Villias, Georgios Veloudis, Dimitrios Keramidaris, Christos Liatsos, Stavros Gourgiotis

Aim: Aberrant activation of the Wnt signaling pathway has been implicated as a key regulator of colorectal cancer (CRC) tumorigenesis. β-catenin plays a key role in the signaling output of the Wnt cascade. There are many reports about the significance of β-catenin in CRC, yet correlations with prognosis remain highly variable and contradictory. The aim of this study was to investigate β-catenin expression and its relationship to clinicopathological parameters and prognosis in CRC.

Material - Methods: The study included 29 female and 28 male patients with a mean age of 67±10.4 years. Immunohistochemistry analyses were performed to characterize the expression of β-catenin in CRC tissues.

Results: Cytoplasmic and focal nuclear accumulation of β-catenin was observed in 88% (50/57) and 25% (14/57) of patients respectively. There was no significant association between β-catenin overexpression in the cytoplasm and the patients’ clinicopathological parameters. β-catenin overexpression in the nucleus was associated with advanced N stage ($p=0.04$). Kaplan-Meier survival curves demonstrated that patients with β-catenin overexpression in the nucleus presented a significantly unfavorable overall survival (OS) time ($p=0.04$). Multivariable COX regression analysis adjusted for age, gender, tumor location, TNM stage and grade of differentiation, showed that β-catenin overexpression in the nucleus is an independent prognostic factor for OS. There was no significant association between β-catenin overexpression in the cytoplasm and OS.

Conclusions: The protein expression level of nucleus, rather than cytoplasmic β-catenin predicts CRC patients with worse prognosis.

EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF E-CADHERIN IN PATIENTS WITH COLORECTAL ADENOCARCINOMA: RESULTS FROM A RETROSPECTIVE STUDY

Georgios Veloudis, Stavros Gourgiotis, Christos Liatsos, Dimitrios Keramidaris, Evangelos Falidas, Constantinos Villias

Aim: The epithelial-mesenchymal transition (EMT) represents a critical event in the progression and metastases of many epithelial tumors. E-cadherin is the best-characterized molecular marker of EMT and loss of expression has been linked to poorer prognosis in several cancers. However, in patients with colorectal cancer (CRC), the correlation between E-cadherin and patients' prognosis remains controversial. The aim of this study was to investigate the E-cadherin expression in CRC patients.

Material - Methods: E-cadherin mRNA/protein expression was investigated in a series of 57 patients and related with their clinicopathological features and prognosis. Quantitative PCR and immunohistochemistry analyses were performed to characterize the expression of E-cadherin in CRC tissues.

Results: The positive expression rates of E-cadherin mRNA and protein were 53% and 42%, respectively. E-cadherin immunostaining showed no correlation with the patients' clinicopathological features. There was a trend towards higher mRNA expression levels in patients with well in comparison with them with poor differentiated tumors (p=0.09). Kaplan-Meier survival curves demonstrated that patients with negative E-cadherin mRNA and protein expression, presented a significant and marginally significant unfavorable disease-free survival (DFS) time (p<0.001 and p=0.05, respectively). Patients with negative E-cadherin mRNA expression presented a significant unfavorable overall survival (OS) time (p=0.02). Multivariable COX regression analysis adjusted for age, gender, tumor location, TNM stage, and grade of differentiation, showed that E-cadherin mRNA expression was an independent prognostic factor for OS.

Conclusions: E-cadherin downregulation is an important component of disease progression in CRC patients. Gene expression levels are a strong predictor of OS and DFS.

3D REGIMEN FOR TREATMENT OF HCV INFECTION

Konaktchieva Marina, Simonova Marieta, Katzarov Krum

Hepatitis C virus (HCV) infection is one of the most common etiology of liver disease requiring liver transplantation (LT). HCV causes almost always a re-infection of the graft, often resulting in rapid progression to severe fibrosis or cirrhosis, and even graft failure. Cirrhotic patients and liver transplant recipients are difficult to treat with combination of pegylated interferon and ribavirin because of the limited efficacy and severe side effects.

The aim of this paper is to present the initial experience in treatment of HCV with the new direct-acting antiviral agents (DAAs) in our center.

Seven patients with various liver diseases, caused by HCV infection were included in the study. We evaluated the efficacy and safety of Viekirax (ombitasvir/paritaprevir/ritonavir), Exviera (dasabuvir) and weight based ribavirin given for 12 and 24 weeks.

In all patients HCV RNA was undetectable on week 4, measured by real-time PCR. Side effects like fatigue, headache and asthenia were mild in severity. Three patients had haemoglobin levels decreased to less than 9.0 g/dL and underwent a ribavirin dose reduction on week 2 and discontinued therapy with ribavirin on week 3. One patient experienced post-baseline serum ALT and bilirubin elevation on week 4. Dosage adjustment of tacrolimus was required in all patients (3/7) with HCV re-infection after liver transplantation. HCV RNA was undetectable by all patients on week 24 after end of treatment.

Our results suggest that the introduction of DAAs is a very promising option for safer and more effective treatment of patients with HCV infection, which are experiencing reduced quality of life and survival.

THE RELATIONSHIP BETWEEN ACUTE PANCREATITIS DISEASE ACTIVITY AND IL-6 AND PROCALCITONIN LEVELS

Gul Vahit Onur, Ahioglu Serkan, Destek Sebahattin

OBJECTIVE: Acute pancreatitis (AP) is the formation of autoactivation of pancreas tissue as a result of pancreatic enzymes become active inside in the pancreas. Various scoring systems are used to determine the prognosis and clinical severity of acute pancreatitis. This study aims that compare Ranson, APACHE II and Balthazar scoring with procalcitonin and IL-6 levels and investigate the relations between statistically.

MATERIAL AND METHODS: 49 patients with a diagnosis of acute pancreatitis in Edremit Military hospital between 2011-2015 (32 male, 17 female age: 29-75) were included in this study. Ranson and APACHEII scores with serum procalcitonin and Interleukin-6 (IL-6) levels were determined for all patients.Patients were classified as mild and severe AP according to Ranson and APACHE II scores. Abdominal CT were performed for all severe patients.

RESULTS: Biliary causes for 23 patients (%47), hyper-triglyceridemia for 5 patients (%10), chronic alcohol abuse for 3 patients (%6), and idiopathic causes for 18 patients (%37) were responsible in the etiology of acute pancreatitis. The patients were separated into two groups as mild and severe AP. Correlation of AP severity, according to APACHE II and Ranson scores between procalcitonin levels and interleukin-6 levels (P <0.001) were statistically significant. Abdominal CTs were performed to the severe AP patients and scored according to Balthazar classification. Balthazar classification scores also showed statistically significant correlation (P <0.001) with Procalcitonin and IL-6 levels.

CONCLUSION: Ranson, APACHE II and Balthazar scoring used to determine the acute pancreatitis severity and measurement of early inflammation markers IL-6 and procalcitonin levels were both statistically significant for disease severity.

STEP BY STEP TREATMENT OF DIABETIC FOOT - NEW TREATMENT POSSIBILITIES

Ciprian Constantin, Georgiana Constantin, Marinela Sirbu, Ana Spuderca

Introduction: Different drugs and therapies for the management of diabetic foot ulcers comprises: local therapies, vasodilatation drugs, antibiotics, neuropathic and neurotrofic drugs, wound dressings, skin substitutes, growth factors and inflammatory modulators.

Background: The majority of therapies target the treatment of diabetic foot ulcer, but a local intervention (surgical intervention) to address the local area and to remove ulcers and necrotic areas is necessary. However, do not apply only one treatment, but applies a lot of intervention and a serial procedures are necessary to be practice on this situation in tentative of a tratment for diabetic foot ulcers.

Material and method: This is a case presentation of a 68 years old patient who was hospitalized in our clinic in February of 2015. This patient with known type 2 diabetes mellitus is presenting wounds in the region of single leg, left side of the plant straight appearance of necrotic ischemic purple of finger 5 and lesions located in region between finger 4 and 5, swelling and redness in the middle plantar region, which progressively worsened.

Results: Despite unfavorable initial prognostic and despite the medical advice to amputate a large part of the limb, the patient was recovered with a minimal intervention. Actual stage of disease and actual risk factors expose this patient to a new amputation.

Conclusion: Some new therapies (as growths factors or stem cells) could be an inovative solution for this kind of patient, ideally before that this stage of disease is diagnosed. We need a lot of other markers to diagnose this kind of pathology before the stage when is necessary a large amputation.

COMBINED METHOD OF INTRA- AND POSTOPERATIVE ANALGESIA IN PATIENTS WITH DIABETIC FOOT BY CSEA AND TREATMENT WITH HBO – A CASE REPORT

Bozov H.

For diabetes is known to have many complications and one of the most anxiety is from diabetic foot ulcer, which often leads to amputations.

The aim of this study is to present a new own methodology for intra - and postoperative behavior of the anesthesiologist in patients with diabetic foot, who undergone amputation of the part of lower limb. Hyperbaric oxygenation therapy (HBO) is a good addition to the treatment.

Material and method:

51 - year old man with diabetic foot, treated with conventional therapy and HBO undergone surgery - debridement and amputation of the finger remains on the right foot. Anesthesia - combined spinal and epidural/CSEA/. A level of puncture for spinal anesthesia - L3-L4, 15 mg 0,75% Levobupivacaine was introduced, level of analgesia Th 10 - S 5. Epidural catheter was placed at the level L2-L3. Tip of the catheter at the level L1. Postoperative analgesia with a mixture of Levobupivacaine 0.5% 4 ml and 1% Lidocaine 4 ml bolus 2 times a day. In the postoperative period were conducted 6 HBO treatment sessions. 10 minutes before entering in the hyperbaric chamber in the Epidural catheter were injected 3 ml of 2% Lidocaine. Hemodynamic was monitored immediately before and after leaving the decompression chamber.

Results:

1. Blood pressure - drop by 5-10 mm Hg from baseline after application of Lidocaine and return to the original values after HBO treatment sessions.

2. Pulse rate - unchanged.

3. Touch block - change in temperature sensation after application of Lidocaine, Became back to the normal after 70-80 minutes.

4. Motor block - without dynamics.

After significant improvement in the local status patient was signed out from the hospital.

Conclusion: Although only one clinical case is presented, methodology shows good healing result.

CONTINUOUS GLUCOSE MONITORING SYSTEM IN TYPE 2 DIABETES - EXPECTATIONS AND PRACTICAL USE FOR THE DIABETOLOGIST

Brindusa Cofaru

Context: The overall assessment of glycaemic control in patients with type 2 diabetes should include the monitoring of three parameters that are usualy described as “glucose triad”: HbA1c, fasting plasma glucose and postprandial glucose excursions.

However, one additional marker, the so- called “glucose variability” might be as important as the three others since it has been demonstrated that both upward and downward glucose fluctuations are potent activators of oxidative stress.

Self monitoring of blood glucose (SMBG) can provide only intermittent snapshots of blood glucose levels, missing often hyperglycaemic or hypoglycaemic excursions.

In the modern diabetes monitoring, Continuous Glucose Monitoring (CGM) could be considered as an important pillard, since it provides information on day-to-day change of blood glucose levels and help achiving treatments targets without increasing the risk of hypoglycaemia.

Evidence Acquisition: The two major sources of data acquisition included medical literature and personal clinical experience of the author.

Conclusions: Glucose monitoring systems could be extended to current clinical care of type 2 diabetic patients especially for motivating them to change their lifestyle and to accept earlier insulin treatments in case of “oral antidiabetic drug secondary failure” and further for choosing the most appropriate insulin regimen in order to improve glycaemic control.

Short term retrospective CGM use may be beneficial in certain clinical situations such as to detect nocturnal hypoglycaemia, to assist in the management of hypoglycaemic unawareness and when significant therapeutic changes are performed.

COMPARISON OF THE DATA FROM CONTINUOUS GLUCOSE MONITORING AND CONTROL ASSESSMENT PARAMETERS IN PATIENTS WITH TYPE 1 AND TYPE 2 DIABETES

Tzvetelina Totomirova, Ivona Daskalova, Vesela Mihneva

Aim: Continuous Glucose Monitoring (CGM) is used to adjust therapy in diabetic patients on intensified insulin treatment regimen. Additionally, CGM helps to evaluate glucose control during observed days. The aim of our study is to compare the results from CGM and control assessment parameters in patients with diabetes.

Material and methods: We studied 106 patients (60 men, 46 women; mean age 58.23±10.81 years) with type 2 diabetes (31 - on oral therapy; 33 - on premixed insulin; 21 - on multiple insulin injections) and with type 1 diabetes (21). CGM was performed for seven days by using iProTM. Glycated hemoglobin (HbA1c) was measured. CGM-results were assessed in accordance of control parameters.

Results: In type 2 diabetes, HbA1c shows a good positive correlation (r=0.44-0.68) with mean blood glucose level, standard deviation, number of high excursions, AUC above the limits and percent of time above the limit, while there is a good negative correlation to percent of time within the limits (r=-0.65; -0.67; -0.52). There are no significant differences in type 2 groups. No such correlations are found in type 1 group (r=-0.1-0.07). Only in oral treated patients there is correlation between HbA1c and percent of time below the limit (r=-0.42)

Conclusion: Correlation between results derived from CGM and disease control parameters depend on type of diabetes. In patients with type 1 diabetes CGM should not be used as control assessment tool, while in patients with type 2, glucose monitoring could present overall disease control, not depending on treatment regimen.

DIETARY INTAKE AND NUTRITIONAL HABITS AMONG ALBANIAN SPECIAL OPERATION FORCES

Berdo Elva, Kukeli Edlira, Dervishi Genc

Introduction: Special Operation Forces regularly engage in physically demanding combat operation and field training exercises, resulting in high energy expenditure which increased energy requirements. Success requires the mustering of all strength and endurance both physical and mental. Appropriate nutritional habits and interventions under the most rigorous conditions, can enhance performance and preserve health.

Objective: To access and evaluate nutritional habits and dietary intake among Special Armed Forces.

Material and Method: A cross-sectional study conducted through a self-administrated questionnaire was used to collect data from a sample of 100 Special Operation Forces in the period of time October – December 2015.

Results: The median age was 21-22 years (at a range of 18-27 years).The majority of SOF reported taking meals regularly (92%) with 64% eating 5 times per day and 36 eating at least 3 times per day. SOF eat a nutritionally balanced breakfast and lunch at the unit. 4% of them don’t eat regularly the dinner. 36% smoke cigarettes and few subjects drink alcohol. 73% are aware of the concept of nutritionally balanced food but only 13% apply this concept when selected food from a menu. 37% consume at least once a week fast foods. 54 % know foods richer in protein, 48% vitamin, 38% carbohydrates, 47% saturated fat, 21 % unsaturated fat, 32% fiber.

Conclusion: Insufficient nutritional knowledge often leads to wrong food choices having not a maximal nutritional efficiency.

OP session 5 – Oncology and dermatology

DOES WNT SUPPRESSOR, AXIN-2, PROMOTE COLON ADENOCARCINOMA ONCOGENIC ACTIVITY?

Georgios Veloudis, Stavros Gourgiotis, Christos Liatsos, Dimitrios Keramidaris, Evangelos Falidas, Constantinos Villias

Aim: The Axin-2 protein plays an important role in the regulation of the stability of the Wnt signaling pathway. Although initially described as a tumor suppressor, recent findings support the presence of a pro-tumorigenic role. The aim of this study was to investigate the association of Axin-2 mRNA and protein expression with clinicopathological parameters and survival in colorectal cancer (CRC) patients.

Material - Methods: Fifty seven patients, who were diagnosed with adenocarcinoma of the colon and rectum and underwent surgical resection, were included in this study. Expression of Axin-2 was investigated using quantitative PCR and immunohistochemical staining.

Results: In most cases, Axin-2 immunolocalization was detectable in the cytoplasm as opposed to only one case where it was shown to be expressed in the nucleus.

The positive expression rates of Axin-2 mRNA and protein were 51% and 33%, respectively. Statistical analysis showed no association between Axin-2 mRNA/protein expression and patients' clinicopathological parameters and survival.

Conclusions: In the present study Axin-2 mRNA and protein expression failed to provide with significant prognostic information in CRC patients.

These findings may reflect the complex tumorigenic role of this marker in CRC.

ROSAI-DORFMAN DISEASE

Vîrlan Cristina-Georgiana, Ghiațău Anca, Șotcan Mihai, Chirita Doru, Dănăilă Eduard

Introduction: Histiocytosis defines a series of diseases of the reticulo-endothelial system - cells with the origin in promonocytes which the mononuclear phagocytic system. The proliferation of Langerhans cells (which have the role of defense in the skin) means histiocytosis. The symptoms are variable depending on the affected organs- the osteo-articular system is affected in 80% of cases, skin, lymphatic system are a frequent target. There are cases of localized histiocytosis (just skin/mono or poliostotic lesions) or an extensive form with visceral implication with or without organ dysfunction. The positive diagnosis is made on biopsy of the skin lesions or curettage of the bone lesions.

Material and method: We present the case of a young man -27 years who was admitted on Dermatology for a series of subcutaneous, erythematous skin nodules on the torso.

Results: A skin biopsy was performed by surgically removing a cutaneous flap which histologically and imuno-histochemically resembled Rosai Dorfman disease. We continued investigating the systemic implication by performing a cerebral and thoraco-abdomino- pelvic tomography which identified numerous peripheral and hepatic hilar lymphadenopathy thus contouring the diagnosis of multisystem histiocytosis.

Conclusion: Multisystem histiocytosis without lung, lymphatic system, cerebral parenchyma, liver involvement has low mortality and a good prognosis but left untreated can lead to disease extension. With parenteral treatment with Vinblastine and SoluMedrol the evolution was favorable – the skin lesions and lymphadenopathy disappeared.

MANAGEMENT OF SURRENAL METASTASES BY STEREOTACTIC ABLATIVE BODYRADIOTHERAPY (SABR)

Beyzadeoglu Murat, Gamsiz Hakan, Sager Omer, Demiral Selcuk, Dincoglan Ferrat, Uysal Bora

OBJECTIVE: Surrenal metastases constitute a frequent distant metastasis site for lung cancer. Stereotactic Ablative Body Radiotherapy (SABR) emerged as a viable therapeutic option in the treatment of surrenal metastases to replace adrenalectomy, however, there is paucity of data regarding its utility for this indication. In this aspect, we evaluated the use of SABR in the radiodebulking management of surrenal metastases from non-small cell lung cancer (NSCLC) and report our single center experience in this study.

MATERIAL AND METHOD: Fifteen NSCLC patients (9 male, 6 female) with 17 surrenal metastases referred to Department of Radiation Oncology, Gulhane Military Medical Academy were treated using Active Breathing Control 4D-SABR. 10 Gy per fraction dose in 3 fractions was delivered to a total dose of 30 Gy in 3 consecutive days for all patients. The mean adrenal gland Gross Tumor Volume (GTV) was 28.4 cc (range: 6.6-101.5 cc) and mean adrenal gland Planning Target Volume (PTV) was 57.4 cc (range: 16.5-143.8 cc).

RESULTS: At a median follow-up time of 16 months, local control was 86.7% and overall survival was 33.3%. Median disease-free survival was 10 months. Treatment response according to RECIST (Response Evaluation Criteria in Solid Tumors) was noted as complete response in 3 patients (20%), partial response in 5 patients (33.3%), stable disease in 5 patients (33.3%), and progressive disease in 2 patients (13.3%). Patients experienced minimal grade 1 nausea (n=7) and grade 1 fatigue (n=12). There were no cases of grade 4-5 (life threatening or fatal) acute or late toxicity.

CONCLUSION: SABR offers a safe and efficacious noninvasive management strategy for surrenal metastases from NSCLC by providing excellent local control with negligible treatment related toxicity replacing the burdensome surgical adrenalectomy.

PROSTATE CARCINOMA - CLINICAL AND HISTOPATHOLOGICAL COMPARASIONS

Drandaska Ivanka

Prostate cancer is the fourth most common neoplastic disease among men in Bulgaria. The incidence of prostate cancer increases rapidly in recent years.

Currently the prostate cancer is the most commonly diagnosed malignant tumor in men in many countries and it is the second after the lung cancer as a disease that leads to death.

Retrospectively / 2012-1014 / are studied 115 cases of prostate cancer in men diagnosed in MMA-Sofia. All cases of the prostate cancer are established on histological biopsies / tru-cut biopsies or prostatectomie/.

Prostate cancer was histological defined and staging through G and the Gleason grading system. Immunohistochemie was used in cases with ambiguous histology and data on suspected carcinoma. We analyzed age, anatomical location, differentiation and staging of prostate cancer. We compared the histological findings from both tru-cut biopsy and prostatectomy.

Today the main efforts of scientists and clinicians are focused on early diagnosis of prostate cancer and adequate treatment in order to preserve its radicalismand quality of life of patients.

ACUTE ERYTHROID LEUKEMIA OR MDS

Taulla Eriselda, Perolla Adela, Asqeri Tajar

OBJECTIVE: how to differentiate an AEL from MDS. AEL is a rare type of acute leukemia, it consistutes 5 % of all cases.

CASE REPORT: A 74 year old man was hospitalized with a two weeks history of severe weakness, anorexia, weight loss. He suffered of diabetes mellitus type II insulinodependent, and HTA. The physical examination showed cutaneous and conjuctival pallor, large ecchymosis in the low extremities and hepatosplenomegaly.

CBC showed: pancytopenia and macrocytosis, neutrofiles 60%, normoblasts 4:100 and reticulocyte count 3%. Serum B12 - 1225pg/ml and folates - 21ng/ml. DTC negative, ITC negative, LDH 1660 U/L, total bilirubin 1.1 mg/dl, total proteins 5.6 g/dl, glucose 388 mg/dl, and other parameters within the normal range.

The bone marrow aspiration revealed: mieloblasts 6%, promyelocytes 1%, myelocytes 6%, metamyelocytes 6%, bands 5%, neutrophils 4%, proerythroblasts 4%, basophilic normoblasts 4%, polychromatophilic normoblasts 4%, promegaloblasts 7%, basophilic megaloblasts 10%, polychromatophilic megaloblasts 36%, and orthochromatic megaloblasts 7%.

The rate myeloid/erythroid = 1/3, showing the predomination of the erythroid line which constitute 65%

of all cells. The proportion of proerythroblasts and basophilic erythroblasts was 38% of all erythroblastic cells. The proportion of megaloblastic forms was 80% of all erythroblasts. PAS staining of bone marrow smears showed typical cytoplasmatic positivity of pathologic erythroblasts: granular in more immature cells and diffuse in more mature ones, confirming the diagnosis of erythroleukemia.

We treat him with Cytarabine 100 mg/m² every 12h day 1-7. The patient had an extremely aggressive clinical deterioration and he died ten days after admission.

CONCLUSION: the diagnosis was Acute Erythroid Leukemia. The % of myeloblasts in bone marrow was >20% of non-erythroid cells

DERMATOLOGICAL QUALITY OF LIFE IN VITILIGO PATIENTS

Sinani Ardiana, Roshi Enver, Lico Rovena, Osmenllari Artan

Introduction: Vitiligo is an acquired dermatological pigmentation disorders of the skin. It causes loss of pigment on effected areas of the skin or mucosae and is characterised by milk white, nonscaley lesions with distinct margins.

Objective: Aim of this research study about Vitiligo is to investigate the impact of vitiligo on the life quality of affected patients.

Results: 1758 individuals who come in Military Hospital Tirane, Albania in Dermatolgy Service in period October 2014 - March 2015 was examined having or not vitiligo. 39 cases with Vitiligo were diagnose through the physical standard examination. In these 39 patients diagnosis with Vitiligo were applied DLQI questionnaire:

From 39 patient taken in consideration in this research study 22 (56.4%) were females and 17 (43.6%) were males, it was noticed that gender was influential in the degree of concern that brings Vitiligo (p=0.01,), and in the degree of embarrassed and anxiety due to this skin disease (p=0.01), where females were proven to be the most sensitive category. Resulted that age group was determinant in the skin disease (vitiligo) impact on social activities (p=0.007), in the skin disease impact for the selection of clothes (p=0.007), in problems with relatives as the result of skin disease states (p=0.007) and in problems level caused from the skin disease treatment (p=0.000). Categories which are more sensitive from skin disease (vitiligo) are the age groups 15-24 and 25-34, where subjects which belong to these age groups categories represent the highest percentage of the persons who have concerns as the result of the skin disaese states. Residence feature is determinant for the concerns level as the result of skin disease states (p=0.01), skin disease impact level on social activities (p=0.026), sexual difficulties level as the result of skin disease states (p=0.0047).

Conclusion: Vitiligo is not life-threatening skin diseases but have a significant impact on the quality of life of patients and cause considerable psychological distress.

PIGMENTATION DISORDERS, DISEASES WITH A NEGATIVE IMPACT ON THE SOCIAL LIFE OF INDIVIDUALS AFFECTED

Sinani Ardiana, Lico Rovena, Hoti Brunilda

Introduction: Peutz-Jeghers syndrome is a rare inherited disease characterized by the presence of 1. gastrointestinal polyps which have a very high risk of becoming malignant. 2. the presence of the macula, pigmented, mainly around the area of the mouth, lips, eyes, in the mucosa of the lips, mouth, hands, feet.

Clinical case: patient aged 24 years presented to the dermatologist doctor complained about the presence of dark brown spots around the mouth, which according to her, had been added since childhood, the patient will marry and seek to remove these stains by her face, she did not refer any other problem or concern, despite the fact that her sister had such problems too.

From differential diagnosis of pigmention disorders and clear clinical appearance of these characteristic spots around the mouth, thought for a lentiginous, from which, with the above features were consistent with Peutz-jeghers syndrome.

The patient was hospitalized in the dermatology clinic, where she was subjected to laboratory examination of the blood count and biochemical balance that resulted normal.

On fibro gastroscopic examination, saw numerous polyps in the stomach (hamartomatous polips). Final diagnosis Peutz-jeghers syndrome.

Discussion: More and more are added attention and noted that the skin is an early tumoral markers to diagnosis of malignant internal diseases. Changes in the skin may be the first signs of an internal problem, including malignant diseases. Cutaneous markers are divided into two groups. Paraneoplastic syndrome that occurs as a result of substance that produces underlying cancer.

Genetically determined syndrome with cutaneous component (genodermatoses) that predispose risk to develop the cancer. Example as Gardner syndrome, Gorlin syndrome, neurofibromatosis, Peutz-Jeghers Syndrome, etc., the truth is that never be neglected cutaneous signs of systemic disease and are the first dermatologist who make their assessment and helping to establish the diagnosis. Were small brown spots that helped in establishing a diagnosis of a rare inherited syndrome.

CHRONIC URTICARIA PATIENTS REFERRED TO A TERTIARY CARE CENTER

Kostic Kristina, Storebra Tanja, Zecevic Rados, Kandolf Sekulovic Lidija

BACKGROUND: Chronic idiopathic urticaria (CIU) is a common disease, demoralizing for patients and in its severe form, can be a therapeutic challenge to the treating physician. Chronic idiopathic urticaria (CIU) encompasses at least two subgroups. One of these is chronic autoimmune urticaria (CAU), due to autoantibodies against either the high-affinity IgE receptor FcεR1 or, less commonly, IgE. The autologous serum skin test (ASST) is a routine diagnostic test for differentiating these two subgroups.

OBJECTIVE: to compare two groups of patients with urticaria: those with chronic idiopathic urticaria (CIU) and those with probable chronic autoimmune urticaria (CAU).

METHODS: A total of 105 chronic urticaria in patients treated from 2009-2013, were identified from our hospital database. Patients were classified in two groups depending on ASST result: group of patients with positive ASST (CAU group) and those with negative ASST (CIU group). Demographic data (sex, age) and data about the duration of disease, presence of angioedema, associated diseases and treatment were collected and analyzed in these two groups.

RESULTS: ASST was performed in 85 patients (81%). ASST was positive in 29 patients (28%) - CAU group. In 56 patients ASST was negative (CIU group). The group of patients with positive ASST had prolonged duration of urticaria, more frequent presence of angioedema and associated thyroid disease. Significant number of patients with positive ASST (CAU group) was treated with cyclosporine A with good clinical response.

CONCLUSION: ASST is a routine clinical test considered a screening test for chronic autoimmune urticaria (CAU). Patients with chronic autoimmune urticaria have a more severe form of disease with increased need for immunotherapy for disease control.

SUCCESFUL TREATMENT OF GIANT BASAL CELL CARCINOMA WITH TOPICAL IMIQUIMOD 5% CREAM

Dinic Miroslav, Kostic Kristina

AIM: the use of the topical imiquimod 5% cream offers a noninvasive, nonsurgical, and an effective option for the treatment of primary small (5 cm) BCC with imiquimod are rare.

MATERIALS AND METHODS: we present our experience in the treatment of giant sBCC (6×5 cm^2) on the forehead of 97 years-old woman who refused surgical treatment, with imiquimod 5% cream, 5 days/week for 8 weeks, followed by 3 days/week application for the next 4 weeks.

RESULTS: tumor was cured with clinical evidence, leaving vitiligo-like scar.

CONCLUSION: Imiquimod 5% cream, a topical immune response modifier, is safe and effective modality for treatment of giant sBCC when surgical excision is not the option.

OP session 5 – Pneumology

THE EXPERIENCE OF DEPARTAMENT OF PNEUMOLOGY SUUMC IN ULTRASOUND GUIDED TRANSBRONCHIAL PUNCTION (EBUS - TBNA)

Stefanescu Ioana Ruxandra, Firoiu Elvis Florian, Oprea

Ioana, Vasilescu Florentina, Chim Aneta Serbescu

Introduction: Endobronchial ultrasound (EBUS) is a minimally invasive but highly effective procedure used to diagnose lung cancer and other diseases causing enlarged lymph nodes in the chest. Although the method appeared in the late 90s, emerged in Romania for several years, initially in Iasi, after that in Cluj-Napoca. The Military Hospital is the first hospital in Bucharest that brought this technique in December 2015.

Materials and methods: We conducted a descriptive observational study in the Department of Pneumology of SUUMC. 11 patients were investigated between December 2015 - March 2016 by EBUS-TBNA, with moderate sedation (Midazolam and Propofol).

Results: Most of the patients were imagistic diagnosed with pulmonary tumors and mediastinal lymph nodes. All patients with diagnostic imaging of lung tumors were confirmed by EBUS-TBNA both cytology and histopathology (cell block). The pacients with suspicion of Sarcoidosis where not confirmed.

Conclusions: EBUS-TBNA is a minimally invasive method, with small risk, highly effective in the diagnosis and staging of lung cancer. This method could replace in some cases mediastinoscopy as a method of diagnosis of lung cancer, especially in patients with severe comorbidities. The method is not very effective in the histopathological diagnosis of sarcoidosis.

SOLITARY PULMONARY NODULE - A DIAGNOSTIC CHALLENGE

Jascu Ionut, Stefanescu Ioana, Ionita Catalin, Firoiu Elvis, Popovici Claudia, Andras Gabriela

Introduction. A solitary pulmonary nodule (SPN) is an imagistic entity (radiological or tomographic), that has long challenged the clinician. A SPN is defined as a single pulmonary opacity, of 3 cm diameter or less, surrounded by normal lung tissue, that is not associated with adenopathy or atelectasis. The frequency with which SPN is identified on chest radiography is on the order of 1 to 2 per thousand chest radiographs. The diagnostic and the management of SPN are not yet very well standardized. A significant percentage of SPN are malignant.

Material and methods. In the Pulmonology Department of the "Dr. Carol Davila" Emergency Military Hospital Bucharest we investigated in 2015 a total of 30 patients with SPN, discovered during routine imaging evaluations. For all patients was applied a rigorous diagnostic management, including noninvasive (tomography) and low invasive (bronchoscopy) procedures and those with suspected malignancy were referred to the Thoracic Surgery Department. We conducted a thorough retrospective analysis of cases investigated, assessing the role of the clinician in diagnosing approach of SPN.

Results. Of all cases 30% had imagistic malignancy features and lung cancer was confirmed in about 20% of the cases we investigated.

Conclusions. The first objective of SPN management is identifying the nature of benign or malignant by noninvasive methods, which will guide the subsequent conduct of diagnosis and treatment, and the ultimate goal is to ensure an early and complete surgical treatment of lung cancer while avoiding thoracotomy in patients with benign nodules.

THE PREVALENCE OF BRONCHIECTASIS IN YOUNG TURKISH MALE PATIENTS ALONG WITH CLINICAL AND RADIOLOGIC FEATURES

Ozkara Bikemgul, Okur Aktas Gokcan

OBJECTIVE: The purpose of this study was to determine prevalence of bronchiectasis and highlight clinical significance with extension and severity on high-resolution computed tomography (HRCT) in young male patients.

MATERIAL AND METHOD: A medical chart review showed sixty-one male patients diagnosed with bronchiectasis among 6829 patients during a period between September 2014-April 2015. Retrospectively interpreted chest x-ray and HRCT images of 61 patients were recorded. Bronchiectasis features on HRCT images were classified according to localization, extension, and severity. Average clinical history, and previous identified etiology were recorded with laboratory test results.

RESULTS: The average age was 21. Lower lob involvement, mainly left lower lobe, was found the most favored localization [65%, n: 40]. Multilobar involvement was the most common finding. Moderate bronchial dilatation (varicose bronchiectasis) was found higher than mild dilatation. The most common additional finding was alveolar infiltration [34.4%, n: 21]. There was a weak correlation found between mediastinal lymphadenopathy and ground glass density (r=0.269, p=0.036). Similarly, decreased attenuation (air-trapping) and ground glass density (r=0.357, p=0.007) was shown weak correlation. Neutrophil cell and WBC increase was the most common finding at biochemical analyses (respectively, 19.7%, n: 12, 16.4%, n: 10).

CONCLUSION: Bronchiectasis is still considered an under and misdiagnosed condition. To determinate the extension and severity of lung damage can help to proper management in young patients. Isolated bronchiectasis cases can be treated by surgical resection of affected lobe. Radiologic interpretation of findings on HRCT is crucial and may help to clinicians in determining treatment and follow-up plan.

PHYSICAL EFFORT IMPACT ON RESPIRATORY PARAMETERS OF ALBANIAN SPECIAL OPERATION FORCES

Muhameti Rushan, Kukeli Edlira, Berdo Elva, Gjovreku Eva, Alizoti Xhemil

Introduction: Medical periodic controls of Albanian Special Operation Forces play a main role for respiratory parameters evaluation. Physical efforts are of a dynamic and static nature and require the special care for respiratory function assessment of ASOF.

Objective: Identification of links between static and dynamic respiratory parameters for determining of effective paramilitary contingents that would compete in Special Operation Forces.

Material and Method: The analytic study was performed with two contingents of Albanian Special Operation Forces. The individuals underwent measurements anthropometric and respiratory evidence testing. Analysis of data was conducted according to T student test.

Results: In this study were taken two groups, the trained and untrained group, and effective of Special Operation Forces. Total of individuals is 30 respectively 25 individuals for each group. Age of individuals is 18 -24 years. Each group underwent anthropometric measurements (there were taken in consideration average values and deviation standard for each parameter) and evidence of spirometry. For each individual was performed the measurement of CV, VEMS and TIFNO.

Conclusion: Static respiratory parameters (Vital Capacity) and dynamic respiratory parameters (VEMS) are significant indicatores in physical effort evaluation of Albanian Special Operation Forces. Respiratory parameters are important indicatores to assess funcional respiratory performance. Respiratory functional changes should be followed in dynamic during physical training of Albanian Special Operation Forces.

INFECTION BY LEGIONELLA PNEUMOPHILA IN A MALE PATIENT AS LOBULAR PNEUMONIA AND ACUTE RENAL FAILURE

Sfikas Georgios, Psallas Michail, Koumaras Charalambos, Roukas Ioannis, Doumaki Eleni, Giourtzis Theodoros, Kartas Anastasios , Minasidis Ilias, Tapazidis Vasileios

Purpose: We present the rare case of a patient with lobular pneumonia and oliguric acute renal failure due to infection by Legionella Pneumophila.

Materials and methods: We present the case of a 52-year old male who was admitted to our department because of fever (39 C) during the previous ten days with malaise, arthralgias-myalgias, hemoptysis, vomiting and oliguria. He had no significant medical record. He was a heavy smoker and alcohol user and he used to work as cloth manufacturer in air-conditioned environment. The laboratory tests revealed leukocytosis with neutrophilia, hyponatremia, elevated liver enzymes and impaired renal function. The chest X-ray showed an infection of the lower lobe of the left lung, which was confirmed by a thorax CT scan.

Results: The patient underwent haemodialysis during the first two days of hospitalization and treated with moxifloxacin and intravenous hydration. He presented a rapid improvement in his clinical condition and laboratory findings. The blood and urine samples revealed antibodies to Legionella Pneumophila, indicative of a recent infection.

Conclusion: Infection by Legionella Pneumophila may present as a severe polyorganic disease and therefore, the diagnosis requires the combination of medical history and laboratory findings with high clinical suspicion.

CLINICAL AND PATHOLOGICAL FEATURES IN MALIGNANT PLEURAL EFFUSIONS IN ALBANIAN PATIENTS

HILA Elona, XHEMALIAJ Daniela

Objective: An overview of malignant pleural effusions in hospitalized patients, in Respiratory Diseases Hospital

Tirane. The role of cytological examination of MPE, in further management of these patients'. Clinic correlation and main symptoms of malignant pleural effusions among Albanian patients.

Methods: a retrospective study for the period of time from January 2012 till December 2013.

We have examined all clinical files, of patients with pleural effusion. Used methods are: Pearson Chi-Square test, B variate correlation, and Likelihood Ratio. Consensus was taken from all the patients before doing the procedure of thoracocentesis. Total patient number 1291.

Results: malignant pleural effusions contains 10 % of all pleural effusions, (n=129).The rest was of inflammatory origin (Tb, pneumonia, pulmonary embolism, RA, etc.) (n=1162). Male to female ratio is: 52% male and 48% female. Age range was (18-91), with mean age is 63.2 years old with SD 15.1. Position: 53% of MPE were in the right lung, 38% in the left lung and only 9% were bilateral. Smoking history in only 46% of them. Personal history of neoplastic disease: 3 %, but in malignant pleural effusion was found in 37% of cases. Pleural effusion as first sign was found in 27% of patients. Familiar history of neoplastic disease: 14%. Other systematic diseases: High blood pressure 37%, Diabetes mellitus 3.4%, other diseases 8.6%. Main clinical symptoms were: hemoptysis 27% (n=348), cough 46 % (n=593), dyspnea 45% (n=590), and chest pain 22% (n=284). Hospitalization days: average is 9 days, and day range was (1-31) with SD 5.4. Main medical treatment: ceftriaxone 41% and broad spectrum antibiotics. Cytological pleural fluid examination was performed in all cases. Biopsy was performed in 48% of cases. Positive results of pleural biopsy in 97% of cases. Main histological types: Adenocarcinoma of lung (35%) mostly in Men, Ductal carcinoma of breast (18%), follow by Adenocarcinoma of lung (10%) mostly in in women. Performance status of all cases: 60.2% improved, 37.2% idem, 2.3% dead in hospital.

Conclusions: In developing countries like ours, where investigations tools and health care facilities are inadequate, pleural fluid analysis and cytology should to be performed as first-line investigation in all pleural effusion cases. It is a convenient, cost-effective and safe investigation. In combination with pleural biopsy can enhance its usefulness in diagnosing pleural malignant effusions.

OP session 6 – Cardiology

CORONARY EMERGENCIES IN THE MILITARY ENVIRONMENT THERAPEUTIC AND DIAGNOSTIC PECULIARITIE

Florea Mihail

OBJECTIVE: highlighting of the increasing incidence of the acute coronary syndromes in young and younger military groups and the peculiarities of cardiovascular risk factors with major impact on this kind of pathology, and pointing the steps and key objectives in diagnosis and efficient and modern treatment, emphasizing on the need of the interventional treatment.

MATERIAL AND METHOD: clinical and statistical analysis of all cases of the acute coronary syndromes encountered in military population in the last 10 years within the competence area of Military Hospital Cluj-Napoca and the presentation of the peculiarities of therapeutic solution regardless of the initial hospitalization site.

CONCLUSION: in our evidence acute coronary syndromes were represented both by unstable angina and acute myocardial infarction without ST segment elevation, which was usually complicated. Emergency coronary angiography was the key element in diagnosis and was most oftenly followed by percutaneous coronary angioplasty, with or without stent implantation.

SERUM BIOMARKERS IN EVALUATION OF CARDIOVASCULAR DISEASES

Costea Florea, Teusdea CB, Toma M, Costea Jaqueline Roberta, Arsene Anca

Biomarkers represent indicators of biological process that can be measured and evaluated for therapeutic interventions. The categories of serum biomarkers used in cardiovascular diseases are: inflammatory syndrome markers (CRP, hs-CRP), markers for plaque instability (MPO, MPMs), cardiac ischemia and necrosis markers (troponine I and T, CK-MB, myoglobine), and also markers for cardiac failure (proBNP, NT proBNP). In this paper we analyze the role of these markers in evaluation of cardiac pathologies in the Emergency Department.

PREDICTORS OF SUDDEN CARDIAC DEATH

Djuric Predrag, Jovic Zoran, Spasic Marijan, Mladenovic Zorica, Stajic Zoran, Gudelj Ognjen, Matunovic Radomir, Djuric Ivica

INTRODUCTION. Sudden cardiac death (SCD) is defined as the unexpected natural death from a cardiac cause within a short period of time from the onset of symptoms in a prior apparently healthy person. Previously asymptomatic patients are particularly jeopardise by acute coronary thrombosis due to a lack of conditioning. The most important risk factors for SCD are coronary artery disease (CAD), previous myocardial infarction, reduced left ventricular ejection fraction (LVEF), heart failure, previous sudden cardiac arrest (SCA) episode, prior ventricular tachycardia (VT), ventricular fibrillation (VF), hypertrophic cardiomyopathy, long QT syndrome, Brugada Syndrome.

MATERIALS & METHODS. In Military Medical Academy from 2013 year we have implanted 12 ICDs and 15 CRT Ds (cardiac resynchronization therapy with defibrillator). ICD therapy consists of pacing, cardioversion and defibrillation, and was indicated in patients who were survivors of SCA due to ventricular fibrillation or hemodynamically unstable sustained VT after excluded any reversible disorder. CRT D therapy was indicated in patients in NYHA III-IV class, who were symptomatic despite optimal medical treatment, with reduced LVEF <35% and QRS prolongation > 130 ms.

RESULTS. All the procedures were performed without complications. Prevention and treatment of SCA also include medical therapy, lifestyle modification, catheter ablation which can definitely isolate arrhythmogenic focus.

CONCLUSION. There are numerous environmental, lifestyle, clinical and hereditary factors that can trigger SCA. Early diagnosis and appropriate treatment, especially with ICD is the most effective in preventing SCD. Introducing of new screening method for the detection of patients at increased risk of SCD is of crucial importance.

ACUTE PULMONARY EMBOLISM IN A PATIENT UNDER TREATMENT WITH ANTICOAGULANT AGENTS: A RARE CASE PRESENTATION

Sfikas Georgios, Pehlivanidis Anthimos, Kartas Anastasios, Pasatas Ioannis, Kotsakis Dionysios, Iosifidis Georgios, Psallas Michail, Iosifidis Michail

Purpose: We present the case of a male patient who was admitted to our hospital with acute pulmonary embolism while on cumarinic oral anticoagulants and an INR value equal to 2.7.

Materials and methods: A 71-year-old male was transferred to the ER department due to gradually deteriorating dyspnea since three days, with no fever and a history of chronic obstructive pulmonary disease and chronic atrial fibrillation. The clinical examination revealed tachypnea (22 breaths/ min), tachycardia and anasarca with dilated jugular veins. The ECG showed atrial flutter (180 beats/min), while laboratory evaluation showed renal dysfunction, an INR value of 2.7 due to treatment with cumarinic oral anticoagulants and a high value of D-dimers. The echodiagram revealed severe right heart failure with dilated right cardiac cavities and pulmonary hypertension (90 mmHg). The clinical suspicion of multiple pulmonary embolisms was confirmed with CT angiography of the thorax and ventilation-perfusion scan. Ultrasonography of the lower extremities revealed the presence of multiple thrombi (deep vein thrombosis).

Results: The patient was treated with low molecular weight heparin, oral anticoagulants, diuretics, amiodarone and intravenous hydration with improvement of his clinical and laboratory status. A filter was inserted in the inferior vena cava to prevent future incidents of embolism. The thrombophilia tests were negative.

Results: Pulmonary embolism in a patient theoretically sufficiently treated with anticoagulants may evade diagnosis and further treatment is a matter of debate with very few literature references.

ORAL CONTRACEPTIVES AND VENOUS THROMBOEMBOLISM RISK - A SAFETY UPDATE AND ALGORITHM PROPOSAL

Aurelian-Emil Ranetti, Anca-Pati Cucu

Introduction: The daily combined oral contraceptive (COC) remains the most commonly used nonbarrier method of reversible contraception. Due to its non contraceptive effects it is also indicated in several reproductive tract/hormonal disorders. Active women of fertile age need an efficient risk-free method of contraception and treatment. Venous thrombembolism (VTE) induced by COC risk was first described in the 60s, having an increasing incidence in the past years.

Objectives: To prevent severe consequences of COC induced thrombosis

Material and method: All formulations more or less induce a prothrombotic phenotype with increased levels of coagulation activation markers, reduced levels of natural anticoagulants, and an acquired resistance to APC. The risk of VTE associated with hereditary thrombophilias is further increased with COC use.

Results: Current data shows strong epidemiological evidence that use of second generation COCs increased the risk of VTE approximately 2-fold as compared with non-users, whereas third-generation pills were associated with a 4-fold increase. Progestogen only pill is associated with no or only a marginally increased risk of VTE

Conclusion: About half of women use COCs correctly and consistently, still 37% of women reported that they discontinued their COC because of side effects. Based on current guidelines we propose an algorithm for the optimal choice of oral contraception, suitable for active women of reproductive age and extremely useful especially during deployment.

RELATIONSHIP OF CYSTATIN C WITH CORONARY ARTERY DISEASE AND ITS SEVERITY

Doganer Y. Cetin, Aydogan Umit, Aydogdu Aydogan, Aparci Mustafa, Akbulut Halil, Nerkiz Polat, Turker Turker, Cayci Tuncer, Barcin Cem, Saglam Kenan

OBJECTIVE: Cystatin C, which is an endogenous marker for renal function, is reported to be a novel marker for coronary atherosclerosis. In this study, we aimed to evaluate its role in determining the presence and also the severity of coronary atherosclerosis in patients with coronary artery disease (CAD).

MATERIAL AND METHOD: Eighty-eight patients who underwent elective coronary angiography were enrolled in the study. Patients with heart failure, renal failure, diabetes, and thyroid disease were excluded from the study. The study population was divided into three groups: individuals with normal coronary arteries, patients with critical CAD, and patients with noncritical CAD. We also analyzed the relationship of Cystatin C levels with the presence and the severity of CAD and the number of vessels involved.

RESULTS: The mean age of the study group was 51.73±9.21 years, and the majority were men (n= 71, 80.7%). Cystatin C levels were significantly lower in patients with CAD (1334.86±93.45 vs. 836.49±411.29, P <0.001). It was significantly lower in patients with critical CAD compared with those with noncritical CAD and normal individuals (656.60±346.35, 1016.38±396.54, and 1334.86±393.45, P < 0.001, respectively). Serum levels of Cystatin C according to the numbers of coronary vessels such as none, single-vessel, two-vessel, three-vessel, and four-vessel disease were as follows: 1334.86±393.45, 801.67±418.70, 993.90±457.34, 744.09±354.53, and 682.30±294.43, respectively (p<0.001).

CONCLUSION: Lower cystatin C levels may be associated with increased severity of CAD in clinically stable patients, whereas higher levels may indicate the presence of any vulnerable plaque. It may also guide the diagnostic and therapeutic options for the clinical scene on the presentation.

OP session 6 – Best papers presentation

SIGNIFICANT REDUCTION IN THE PREVALENCE OF HELICOBACTER PYLORI INFECTION IN ALBANIAN CHILDREN

Begaj Bajram, Resuli Bashkim, Hoxha Liri

Introduction: In developing countries, Helicobacter pylori infection is very common and begins in the very early childhood.

Objective: To determine the prevalence of H pylori infection in Albanian children aged 8-10 years comparing with the data of the study in 1994 and to identify factors associated with H pylori infection.

Methods: The prevalence of H pylori infection was studied in 308 asymptomatic children between of 8 and 10 years from mixed urban and rural areas. Of two different districts of Albania. H pylori status was evaluated by stool antigen test (SAT).

Urea-breath test were used in 1994 study. Demographic information, socio-economic feature, and living and hygiene condition, such as type of the house, number of person/children living in the house, practices related to water use and toilet facility were evaluated by a detailed questionnaire completed by the teachers with the cooperation of parent of each children.

Results: The overall prevalence of H pylori infection was 58%. Compared to the data of the 1994 study, there were a significant drop to 33%. There were no significant difference between males and female in both studies. The children who grew up in villages had a higher prevalence than those grew up in the city ($p<0.001$). There were no significant difference in H pylori infection prevalence between the three socio-economic classes. The prevalence were inversely associated with clean water index, crowding index and toilet facility, $p<0.001$ respectively.

Conclusions: This study confirms the nearly two-fold reduction in the prevalence of H pylori infection in Albanian children 8-10 years of age between 1994 and 2014, consequence of improvement in standard of living and hygiene practices. The childhood is a period of major risk for acquisition of H pylori.

A COMPARATIVE ANALYSIS OF THE CONTEMPORARY BLAST, GUNSHOT AND CONVENTIONAL TRAUMA

Popivanov G, Mutafchiyski V, Kjossev K, Petrov, N, Chipeva S, Ivanov P, Petrov H, Penchev D

Introduction: The unceasing terror directed toward civilian people significantly has challenge the current health care system. Several authors have warned against the insufficient preparedness of the civilian health care system to face the consequences of the contemporary terrorism. The aim of the present study is to analyze and compare these three trauma types in order to facilitate the optimal health care response.

Material and methods: Prospectively collected cohorts with 73 cases blast and 81 gunshot trauma cases were compared with a retrospective cohort with 416 conventional injuries.

Results: The mean ISS was 20, 13 and 11 in blast, gunshot and conventional trauma. ISS > 17 was noted in 51%, 27% and 13%, respectively. The rate of involved regions were following – head (31%, 5% and 11%), face (18%, 3%, 18%), neck (11%, 6%, 0%), chest (38%, 28%, 18%), abdominal (21%, 32%, 4%) and extremities (69%, 53%, 87%). The need for ICU treatment and 48 hour mortality rate were 44%, 20%, 13% and 10%, 4% and 4%, respectively. Missed injuries were noted in 3% in the blast group versus zero in the other two groups.

Conclusion: Blast and gunshot traumas are associated with significantly higher frequency of chest and abdominal involvement. Penetrating thoracoabdominal injuries and limb vessels injuries are more frequent in gunshot trauma. The rate of head and neck injuries, burns, multiple body regions involvement, limb amputations, the need for CT, DCS and ICU treatment, 48 hour mortality and missed injuries are higher in blast trauma.

TRANSCRANIAL DOPPLER ULTRASOUND IS IDEAL FOR THE IMMEDIATE EVALUATION OF PATIENTS WITH TRAUMATIC BRAIN INJURY AND PUPIL DILATATION

Karellas Ioannis, Stouraitis Georgios, Sourlas Sotirios, Arsenogloy Athanasios, Tsakalakis Christos, Plakas Sotirios, Liapis Georgios

Purpose: Pupillary size and light reactivity are constantly examined in ICU in patients in danger of developing cerebral edema. Pupil dilatation (mydriasis) in these patients is alarming and requires immediate evaluation. Transcranial Doppler Ultrasound (TCD) is a practical, bedside examination that directly provides information about cerebral blood flow and intracranial pressure. This study is aiming to demonstrate the effectiveness of TCD in the evaluation of the patient with severe traumatic brain injury (TBI) that abruptly develops pupil dilatation.

Naterials and Methods. 14 TBI patients with GCS<8, were sedated, intubated and mechanically ventilated. All patients were hemodynamically stable, blood pressure was invasively monitored and their pupils were examined in 15 minutes intervals. When pupil dilatation was observed, aggressive therapy was initiated and TCD and brain CT was held.

Results: In 8 patients TCD revealed compromised cerebral blood flow indicating severe brain edema which was also demonstrated in brain CT. Intracranial catheters were applied in all these patients for continuous ICP monitoring and decompressive craniectomy was performed in 5. In one patient TCD revealed cerebral circulatory arrest and CT scan revealed findings suggesting cerebral herniation. In the patients that TCD and brain CT was normal, pupil dilatation was attributed to nonconvulsive epileptic activity in 2 and to medications in 3.

Conclusion: Pupil dilatation in TBI patients requires immediate evaluation. TCD needs training, practical skills and cannot be used for continuous monitoring. Nevertheless, it is the examination of choice since it is reliable, affordable, and readily accessible and gives direct information about cerebral blood flow, guiding to the appropriate therapy.

EMERGENCY MANAGEMENT OF THE SEVERE BURNED PATIENT

Teusdea CB, Salceanu M

The burn patients, especially the severe ones and the cases with multiple victims (like the recent and dramatic "Colectiv" nightclub fire), represent a real emotional and professional challenge for all emergency team members. Also the initial management (both prehospital and in the emergency department) of burned patients has a critical impact in terms of morbidity and mortality. This calls for better understanding and implementation of emergency treatment algorithm for severe burned patient.

Burns treatment should be initiated by the first responders at the site of the accident and it continues throughout prehospital care as well as during transport to the burns centre or to the closest emergency department.

In order to do that it is mandatory to follow a number of steps: rescuer safety, primary and secondary survey and simultaneous treatment, including analgesia and burn wound management, assessment of burn severity (based on burned surface area, depth of the burn, burned body site, inhalation injury, additional trauma and patient history) and transfer to specialized burns treatment facility.

The main goal of this algorithm is to help physicians in the initial management of burn victims until they reach a specialized burns center.

IMPLANTABLE CARDIOVERTER DEFIBRILLATOR AND CATHETER ABLATION AS A GOLD STANDARD FOR TREATING PATIENT WITH SUSTAINED VENTRICULAR TACHYCARDIA

Djuric Predrag, Spasic Marijan, Gudelj Ognjen, Mladenovic Zorica, Jovic Zoran, Stajic Zoran, Matunovic Radomir

INTRODUCTION. Ventricular tachycardia (VT) in clinical practice, according to duration, can be classified as: non sustained (0-30 seconds) and sustained (>30 seconds), which can be potentially fatal. Myocardial infaction in numerious of cases can be complicated by forming of scar, which can represent potentially dangerous arrhythmogenic focus. Despite appropriate medical therapy for the management of VT, incidence of sudden cardiac death (SCD) is stil unacceptable high. Implantationem of implatable cardioverter defibrillator (ICD) is indicated for prevention of SCD, while catheter ablation can definitely isolate arrhythmogenic focus.

CASE REPORT. We have presented a case 78-years old male with post infarct dilated cardiomyopathy and sustained ventricular tachycardia, and previously implanted VVIR pace maker. Four years ago he had inferoposterior myocardial infarction. Coronary angiography revealed occluded right coronary artery (RCA) with heterocollateral from left anterior descending artery (LAD). A few months after ICD implantationem as primary prevention strategy, he was admitted to hospital due to fast ventricular tachycardia and lost of consciousness. Electophysiology study was performed and catheter ablation of arrhythmogenic focus in scar-related part of inferior myocardium was successufully done.

CONCLUSION. Implantable cardioverter defibrillator and catheter ablation as compatible procedures are gold standard for treating patient with scar-related heart disease presenting with VT.

TRANSLATIONAL STUDY OF COMPOSITE EYE AND PERIORBITAL ALLOTRANSPLANTATION FLAP: FROM RAT TO CADAVER MODEL

Zor Fatih, Kulahci Yalcin, Bozkurt Mehmet, Siemionow Maria

OBJECTIVE: Vascularized Composite Allotransplantation (VCA) is a novel alternative for reconstruction of severe periorbital defects with functional recovery, which can offer a new hope for restoring vision to these patients. The aim of this study is to describe a composite tissue allotransplantation model of eyeball and periorbital tissues on rats and fresh cadavers.

MATERIAL AND METHOD: In the rat model, we harvested a composite tissue flap composed of eyeball, optic nerve, periorbital fat, extraocular muscles and periorbital skin (Figure 1a, b). Vasculature of the demonstrated flap with angiography (Figure 1c). We performed heterotopic composite eyeball and periorbital transplantation between rats. We also performed a nerve coaptation between optic nerve of the donor and great auricular nerve of the recipient. As a continuation of the study, we performed a cadaver study. Borders of the composite eyeball and periorbital allotransplantation flap were infraorbital rim at inferior, nasal dorsum at medial, eyebrow at superior and lateral orbital rim at lateral (Figure 2a, b, c). The perfusion of the flap was confirmed with SPY Elite System.

RESULTS: Rat model showed that the eyeball and periorbital soft tissues were all viable and there were 35 % volumetric decrease in the eyeball (Figure 1d). In fresh cadaver study indocyanin injection identified perfusion zones and visualized arterial inflow and perfusion (Figure 2d).

CONCLUSION: Our rat and cadaver model showed that composite eye transplantation is technically possible, as perfusion of the rat and human flap was confirmed. We think that these studies are cornerstones of the future composite eyeball transplantation.

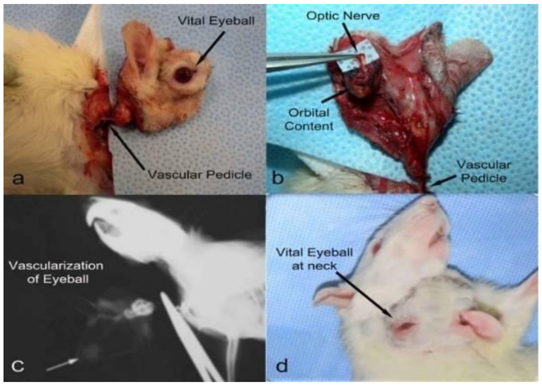

Figure-1: Composite eyeball transplantation in rat. (a) composite eyeball is harvested on vascular pedicle, (b) inner surface of flap showing the optic nerve, (c) angiography of the flap, (d) Vital eyeball on neck of the recipient rat after transplantation

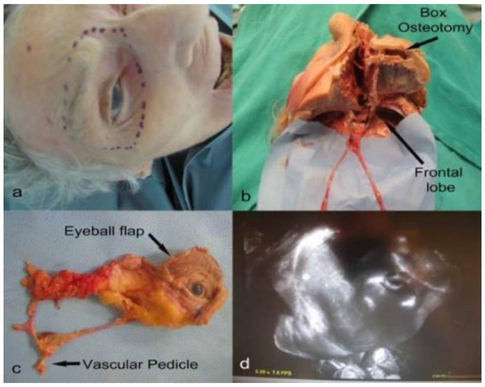

Figure-2: Composite eyeball transplantation in cadaver. (a) outline of the flap, (b) harvested flap after frontal craniotomy and box osteotomy, (c) appearance of the flap after harvest (d) SPY image of the flap showing perfusion of the indocyanine green injection.

OP session 6 – Varia

SHOULD SURGICAL MANAGEMENT OF EPILEPSY BE GIVEN MORE PRIORITY IN THE YOUNG ADULT PATIENTS?

Simsek Hakan, Zorlu Emre, Kendirli M.Tansel, Bakal Omer, Duz Bulent

OBJECTIVE: Epilepsy is featured by unprovoked and recurrent seizures that cause loss of working ability; so three cases are presented to discuss surgical timing in epilepsy of young patients.

MATERIAL AND METHODS: A 21-year-old young man in active military service presented with status epilepticus. After controlling his seizure with benzodiazepines, magnetic resonance (MR) images were obtained. A left mesial temporal hyperintensity and edema was detected. He had seizures for a year but did not have MR imaging study. He was operated and oligodendroglioma of the temporal lobe was diagnosed. Following surgery, he was seizure-free. Another 32-year-old man with intractable seizures despite multiple antiepileptic medications was evaluated with cerebral MR imaging study that did not reveal any lesion. EEG was also non-specific. We placed a vagal nerve stimulator on the left side and seizures were shorter and frequency decreased more than sixty percent in his sixth-month follow-up. A 38-year-old woman presented with mesial temporal sclerosis and a meningioma lying over the right Sylvian fissure and impaired EEG pointing the whole right temporal lobe underwent surgical removal of the tumor. We reserved mesial temporal lobectomy for later and she became seizure-free with one antiepileptic drug.

DISCUSSION AND CONCLUSION: Surgical intervention is considered as the last alternative if all other treatment methods have failed. In fact, surgery should be among the first options considered if early trials with anticonvulsant medications are ineffective. Thus active military service capacity of some of the young patients can be preserved.

CLINICAL PREDICTORS OF WOUND HEALING

Mutluoglu Mesut

OBJECTIVE: To determine the clinical predictors of wound healing.

MATERIAL AND METHODS: We performed a literature search using Pubmed, Embase and Google Scholar to identify studies related with wound healing. We used the following key words: “absolute change in area”, “percentage change area”, “healing rate”, and “area-ratio”.

RESULTS: The majority of studies were performed on either neuropathic diabetic foot ulcers (NDFU) or venous stasis ulcers (VSU). The association of wound size, wound duration and wound grade on healing were extensively studied. Additionally, several other surrogate endpoints for the treatment of NDFUs and VSUs were reported. Among these, percent change in wound are over a 4-week period was determined to be a robust predictor of complete healing at 12-week in both wound groups. In patients with a NDFU Patients with a reduction in ulcer area greater than 53% had a 12-week healing rate of 58%, whereas those with reduction in ulcer area less than 53% had a healing rate of only 9% ($P<0.001$). In VSUs using a percent change in area of 28.79 at four weeks as a cut point, complete healing of the primary ulcer by 24 weeks could be predicted with a sensitivity of 0.67, a specificity of 0.69, a positive predictive value of 0.80, and a negative predictive value of 0.52.

CONCLUSION: Among several surrogate endpoints for predicting eventual healing of wounds, percent change in wound are over a 4-week period seems to be the most reliable predictor of eventual healing.

CBRN DECONTAMINATION UNIT OF TURKISH MILITARY MEDICAL ACADEMY AS A ROLE MODEL FOR BALKAN COUNTRIES

Kenar Levent, Ortatatli Mesut

OBJECTIVE: The threat from unconventional warfare agents including chemical, biological, radiological and nuclear (CBRN) biological agents has recently been considered as both military and civilian issue. Decontamination which is one of the important steps in the first-aid and treatment of contaminated CBRN casualties is such a neutralization process for removal of CBRN agents to facilitate the further medical care.

MATERIAL AND METHOD: A main military hospital like Gulhane Military Medical Academy (GMMA), the top-level military medical organization of our country must possess the ability to immediately decontamination and treat CBRN so called Weapons of Mass Destruction (WMD) casualties. Therefore, GMMA decided to establish a decontamination site in a proper structure where the CBRN victims could be extensively taken under care prior receiving to the hospital interior. This site has its own specialized air-conditioning and waste disposal system.

RESULTS: GMMA Hospital Decontamination Center architecturally is integrated with Emergency Unit owing a lounge and recording section resembling hot zone, undressing and leaching station, and contamination control and dressing sections in order (Figure). A total of 420 ambulatory and 60 unconscious injured victims are able to be thoroughly decontaminated within an hour before dispatch to the concerned clinics.

CONCLUSION: It is obvious that all hospitals existing in both civilian and military health system should be prepared to keep CBRN casualties and contamination out of hospital building by an effective decontamination flowchart similar to GMMA decontamination site as a role model which will be explained in this presentation in details.

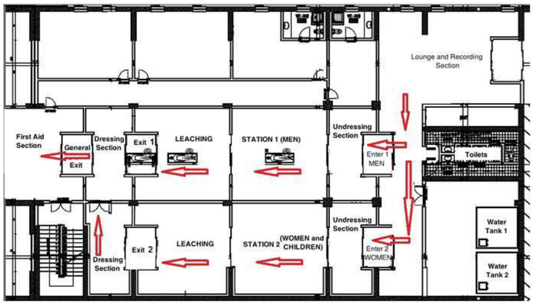

Figure-1: Architectural plan of GMMA Hospital Decontamination Centre.

A CASE OF BRUCELLA MELITENSIS INFECTION - CLINICAL COURSE AND EPIDEMIOLOGICAL CHARACTERISTICS

Popivanov Ivan, Doychinova Tzetza Md, Terzieva Kalina, Gancheva Galya, Shalamanov Dimitar

Background: The epidemic process of Brucellosis in Bulgaria after 1950 has been characterized with only sporadic cases of the disease, usually with 1-2 cases yearly. In two long periods (1973-1984 and 1986-1992) were not even registered any cases. In 2005-2008, in Southeastern Bulgaria (Haskovo Province) emerged group cases among humans – a total of 71 confirmed cases. In 2015 occurred 33 cases in Southwestern Bulgaria (Kyustendil Province) and for the first time two cases in Northern Bulgaria (Pleven Province).

Objectives: Clinical features, laboratory diagnosis, clinical course and epidemiological survey of patient, treated for Brucellosis in Clinic for Infectious disease, Medical University – Pleven, Bulgaria are presented. Discussion of the main challenges of epidemiological control of Brucellosis at this stage in Bulgaria.

Materials and methods: Diagnosis, clinical features and treatment of case of human brucellosis in Pleven region in 2015 are presented by using clinical, microbiological and laboratory methods. Data from the epidemiological survey of patient are presented and special features of the disease in this stage using methods of epidemiological analysis are discussed.

Results and discussion: A case of a 24-year-old male, admitted to hospital with nonspecific symptoms of general weakness, headache and low-grade fever. The diagnosis was confirmed by National Center of Infectious and Parasitic Diseases, Sofia by Brucella Capt test titer 1/5,120. Etiological treatment with Amikacin was carried out. The outcome of the disease was favorable. Goat cheese was established as the probable vehicle of infection. An upward trend in incidence rate was outlined with dissemination in new regions in the country.

Conclusion: For Bulgaria Brucellosis is re-emerging infectious disease. Bulgarian military physicians are inexperienced in early recognition and diagnosis of the disease. Thus and the possibility of disguised beginning with nonspecific symptoms, can lead to delays in diagnosis, therapy and epidemic measures. These are preconditions for protracted clinical course, long-term treatment and temporary or permanent loss of personnel (military or civilian) for the military institution. Furthermore, for military medicine the disease is a matter of interest because the causative agent is in the list of potential BW agents (Category B).

CHRONIC RENAL SEQUELAE OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN SERBIA

Obrencevic Katarina, Petrovic Marijana, Jovanovic Dragan, Maksic Djoko, Vavic Neven, Radojevic Milorad, Ignjatovic Ljiljana

INTRODUCTION/OBJECTIVE: The Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) which is characterized by fever, hemorrhage and acute renal failure. The long-term prognosis of HFRS has been considered favorable. There are, however, some reports about subsequent chronic renal failure after previous Hantavirus infection.

MATERIALS AND METHODS: 135 patients with HFRS have been treated in our unit during the period from 1989 to 2007. Diagnosis was confirmed by serologic tests. The aim of our study was to investigate long-term renal sequelae in HFRS patients and its association with clinical severity of the acute phase of the disease.

RESULTS: The three serotypes of Hantaviruses were serologically confirmed: Hantaan in 48%, Belgrade in 33% and Puumala in 19%. Half of our patients had long convalescent phase - over 3 months. After the convalescent phase we noted chronic renal failure (CRF) in 11 patients (8.15%) who had severe form of the disease. The cause of the disease in 5 patients was Belgrade, and in 6 Hantaan virus. In 4 patients infected with Belgrade we noted progression to terminal renal failure, in 2 of them kidney transplantation has been done, another two are on chronic dialysis program. Another 4 patients with CRF (1 infected with Belgrade and 3 with Hantaan) are not on chronic dialysis yet, and the last 3 were lost for follow up. CONCLUSION: HFRS can cause chronic renal dysfunction in patients who had severe form of illness, particulary in those infected with Belgrade and Hantaan viruses.

ACUTE PYELONEPHRITIS WITH ASSOCIATED LEUKOPENIA: A CASE REPORT

Pehlivanidis Anthimos, Sfikas Georgios, Konstantinou Vasileios, Psochias Polykarpos, Tzakri Dimitra, Kartas Anastasios, Karapiperis Dimitrios, Tapazidis Vasileios

Purpose: We present the rare case of a patient with acute pyelonephritis and leucopenia. Acute pyelonephritis is an infection of the upper urinary tract, which usually requires hospitalization. Classical clinical and laboratory findings of the disease include high fever, chills, nausea, tenderness on renal palpation and leukocytosis. Without early initiation of treatment it can lead to renal abscess, sepsis or chronic nephritis.

Materials and methods: We report a case of a 54-year old immunocompetent female who was hospitalized for uncomplicated acute pyelonephritis, accompanied by leukopenia. The symptoms of the patient in the emergency department were high fever, vomiting and left flank pain. She did not take any medication before admission. White blood cells were 3,820/μlt on admission and fell down to 2,410/μlt, during the third day of hospitalization. Urine cultures revealed E.coli infection. There was no clinical evidence of sepsis during the course of hospitalization.

Results: Cefuroxime and amikacin were administered to the patient with resolution of symptoms within 48 hours. The number of white blood cells was restored to normal levels one week later.

Conclusion: Acute pyelonephritis without complicated sepsis is rarely presented with leukopenia and it may be a misleading factor concerning the diagnosis. The most likely explanation is bone marrow suppression due to infection.

A WOMAN WITH SYSTEMIC AMYLOIDOSIS PRESENTED WITH ENLARGED TONGUE AND PLAQUES ON SKIN

Marineli Filitsa, Brouziotis Athanasios, Xatzidakis Ioannis, Gongaki Stavroula, Evagelia Zarogianni, Mostratou Eleni, Symeonidis Nikolaos

Purpose: A case of systemic amyloidosis presented with enlarged tongue and skin lesions from 5 years.

Materials and methods: A 71 year old female, was admitted because of dyspnea on exertion.

Results: On admission patient had enlarged tongue and waxy, rough plaques on hands and legs. An electrocardiogram showed low-voltage QRS complexes and chest radiographques i bilateral pleural infusions. Laboratory exams showed creatinine 1.6 mg/dl, elevated free lambda light chain levels (284 mg/l), b2 microglobulin 3.14 mg/l. 24h urine protein was negative. Urine immunofixation demonstrated monoclonal free lambda chain. The echocardiogram showed left ventricular hypertrophy, normal left systolic function and granular speckling of the ventricular wall, findings suggestive of infiltrative cardiomyopathy. A bone marrow biopsy was positive for plasma-cell myeloma. Abdominal fat-pad biopsy and tongue biopsy with Congo red staining revealed amyloid. Patient referred to hematologic clinic for further management.

Conclusion: Patients with amyloidosis are usually presented with cardiac and renal failure. AL amyloidosis (also called primary amyloidosis), is associated with organ damage, cardiac involvement presenting as restrictive cardiomyopathy, rapidly progressive heart failure and renal involvement (46%), with renal failure requiring dialysis in less than 5%. AL amyloidosis is always the result of a clonal plasma-cell neoplasm. This patient had involvement of soft tissue (tongue enlargement and skin lesions) which is rare (3%) and because of its atypical presentation, diagnosis of amyloidosis is generally made late in the disease process. Congo red staining of biopsies is necessary, as there is an apple-green birefringence under polarized light that is characteristic of amyloid.

DERMATOMYOSITIS IN A FEMALE PATIET WHO UNDERWENT GASTRIC PARTITION FOR TREATMENT OF OBESITY

Sfikas Georgios, Pehlivanidis Anthimos, Gatsiou Marina, Katsimardou Alexandra, Kostopoulos Georgios, Zisopoulos Dimitrios, Iosifidis Michail

Purpose: We present the case of a female patient who was admitted to our hospital with dermatomyositis after a gastric partition for treatment of obesity.

Materials and methods: A 48-year-old female was transferred to our clinic due to walking deterioration and a laboratory evaluation which showed normochromic,

normocytic anemia and elevated inflammation markers. The patient had a history of hypertension and diabetes mellitus. The neurological/neurophysiologic evaluation revealed lower limb polyneuropathy with bilateral peroneal nerve involvement. Extensive radiology studies were conducted, including brain − thorax − abdominal CT scan, brain MRI − thoracic−lumbar spine MRI, gastroscopy, colonoscopy and a complete immunologic profile, all without abnormal findings. Skin and muscle biopsy revealed dermatomyositis related findings.

Results: The patient was treated with azathioprine, corticosteroids and intensive kinesiotherapy with improvement of her clinical and laboratory status.

Conclusions: Dermatomyositis is an autoimmune disease, which has been associated with many causal factors. This case is interesting regarding its rare initiating cause and its non typical clinical and laboratory status.

POSTER PRESENTATIONS

PREVALENCE OF SMOKING IN ALBANIA

Tafa Holta, Hafizi Hasan, Bardhi Donika, Habazaj Altin, Duraku Kujtime

Introduction: Tobacco is a leading disease risk factor. Understanding national trends in prevalence and consumption is critical for prioritizing action and evaluating tobacco control progress.Smoking prevalence in Albania remains high. According to WHO we rank 13th among European countries, and the smoking daily prevalence is 24%. During the last decade female smoking rate is increased with 4 %. Burden of Obstructive Lung Disease (BOLD) study, the first study of its kind ever-implemented in Albania, assessed smoking prevalence alongside COPD prevalence.

Methods: A nation wide representative sample was drawn using a stratified, multi-stage randomized procedure. It included 2400 non-institutionalized individuals divided into 2 major age group categories: 20-39 and ≥40-80 years old, each of them including 600 men and 600 women.

Regarding smoking habits, the individuals were interviewed according to BOLD Smoking Questionnaire.

Results: The prevalence of current smoking for ≥40-80 years old age group was 21.6% in total, with a noticeable prevalence of smoking in males as compared to females: 35.8% versus 7.3%, respectively. The prevalence of current smoking for 20-39 years old age group was 35.1% in total; 50.5% for men and 20.3% for women, thus exhibiting a narrower male to female distribution pattern.

Conclusion: This study shows that the prevalence of smoking in Albania is high with a predominant male smoking rate. In younger age groups it is observed a statistically significant increased percentage of smoking among women. Higher smoking rate in young generations needs to be addressed more comprehensively.

NONOPERATIVE MANAGEMENT OF BLUNT SPLENIC INJURY

Asqeri Tajar, Ceka Sotir

Introduction: The spleen is one of the most injured organ in blunt abdominal trauma. Nonoperative management of blunt splenic trauma is the preferred option in hemodynamically stable patients, especially in children. In this study we intend to identify predictors for the successful nonoperative management of patients with blunt splenic trauma.

Methods: We conducted a retrospective review of patients with isolated blunt splenic injury, nonoperative managed. They were admitted in Military Hospital, Tirana within the period

2011- 2015.We analysed sex and age, mechanism of blunt injury, hemodynamic status, spleen injury grade in ultrasonography and computed tomography, presence and extent of extra-abdominal injury, blood analysis, hospital stay.

Results: A total of 77 patients with splenic trauma within the period 2011- 2015 were admitted, of whom 53 patient underwent splenectomy. 24 patients were nonoperative managed and they had all blunt mechanism of injury. Penetrating injuries to the spleen are most managed operatively, often because of concerns about associated intraperitoneal injuries. Analysis identified stable blood pressure during admission and observation, minimal and no-increased in time hemoperitoneum, insignificant drops of red blood cells and hematocrit within 3-4 hours after admission, grade I-II splenic injuries, and limited extra-abdominal injuries as predictors for the success of nonoperative management.

Conclusions: With improvement of intensive care, interventional radiology and trauma protocols, it is reported that blunt splenic injuries can now be managed conservatively with success. Computed tomography with intravenous contrast remains the gold standard in a stable patient.

PREVALENCE OF HELICOBACTER PYLORI INFECTION

Begaj Bajram, Cuko Liri, Resuli Bashkim, Saraci Sonja

Introduction: Helicobacter pylori infection is the most common bacterial infection in human beings.Probably 50 % of the earth's population in all age groups is infected with this infection. The infection is received at an earlier age and occurs more frequently in developing nations. The majority of cases are acquired in childhood. Prevalence is depending on socioeconomic status and living conditions in childhood. Other factors include age, housing density, race, overcrowding, sharing a bed, and lack of running water.

Objective: The aim of this study is to show the prevalence of Helicobacter pylori in Albanian children and to compare that with the prevalence funded 20 years ago.

Material and Methods: Children at age between 8-10 from several schools in Tirana city and villages (Kavaja, Ndroq, Gose, Golem, Zall-Herr) participated in the study. The total number of children was 308 from which 43% were males and 53% females. Demographic data were the same with those made 20 years ago. Confirmation of helicobacter pylori infection was made by using tests that provide direct evidence of active H. pylori infection. While 20 years ago was used the urea breath test, in this study was used the monoclonal stool antigen test with the same sensibility and specificity.

Results: Actual prevalence of helicobacter pylori infection in children between ages 8-10 is 43 %. After 20 years the prevalence of helicobacter pylori infection is more than two times less.

Conclusions: Descent percentage of helicobacter pylori infection from 91 % to 43 %, more than two times, is related with improvement of socioeconomic status of Albanian families and living conditions. One other reason is related to up-to-date treatment of infected persons.

NURSING CARE IN GASTRIC CANCER

Begaj Bajram, Misho Valbona, Kolleshi Manjola, Cakoni Suzana

Introduction: Incidence of Gastric cancer has been decline recently in developed country. This disease is more common in men than women and mortality is very high. In all over the word the nursing care is very important for getting a good quality of medical care. Patients with gastric cancer disease need very high capable nurse personnel and nursing care is very crucial.

Objective: The aim of this study is to provide epidemiological, clinical and endoscopicaly data for the patient with gastric cancer. Risk factor are related with incidence and prevalence of this disease.To evaluate the nursing care in those patients.

Methods: There is a retrospective study. There were includec in this study 70 patients with gastric cancer disease. Those patients have been admitted in military Hospital from 2007-2010.Data were collected from chart of the patients. A questinare was made to provide information about socioeconomic data, risk factors, clinic of the disease and endoscopic exam.Nursing care was evaluated by using data for history of the patient's disease and nursing examination.

Results: Data collected by the study shows that 70 % of the patients are men and 30 % are women. Rate betwen men and women 2.33:1. Risk factor are heredity, gender, age and H.pylori infection. Mean age of the patient was 65 years old (50-70). This disease is more common on men than in women. 7 patient or 10 % of them are without metastases and 63 patient or 90 % with metastases. The diagnosis of the patient's disease was made to late in advance stade with metastasis.

Conclusions: Nursing profession and nursing care is big challenge for 21 century. In patients with gastric cancer diseasse nursing care is very important and is realted with better care for the patient. Data collected from history of the patients and nursing exam should be as a guide for the nursing care.

INTENSIVE CARE UNIT-ACQUIRED URINARY TRACT INFECTION, DURING 2014-2015

Beqaj Akela, Hodaj Age, Dushaj Dritan

Introduction: Urinary Tract Infection is the most common hospital-acquired infection. About 80% of nosocomial UTIs are related to urethral catheterization and are acquired after 48-72 hours of hospital admission.

Objective: Identification of microorganisms isolated, causing a nosocomial urinary tract infection and evaluation of antibiotic resistence of these cultures, in Intensive Care

Unit, at the University Trauma Hospital, during 2014-2015.

Methods: Laboratory methods used, are: Cultures of urine samples on blood–agar, Mac Conkey and Sabauraud dextrose agar, Myller-Hinton agar, Biochemical test (with Entero-Pluri Test and TSI), Oxidase-test, antibiotics susceptibilities by Kirby-Bauer disc diffusion method.

Results: From a total of 375 samples, 90 (24%) isolates were positive cultures, out of this: 15 (16%) isolates of E.Coli; 9 (10%) isolates of Enterobacter spp.; 5 (5.5%) of isolates Proteus Mirabilis; 4 (4.4%) of isolates K.pneumoniae; 11 (12%) of isolates is Acinetobacter calcoaceticus; 10 (11%) of isolates is Enterococcus faecalis; 10 (11%) of isolates is P.aeruginosa; 5 (5.5%) of isolates is S.aureus; 21 (23.3%) of isolates is Candida albicans. Male: 70 (77%), female 20 (22.2%).

Conclusion: Patients with asymptomatic bacteriuria can generally be treated initially with catheter removal or catheter exchange, and do not necessarily need antimicrobial therapy. Symptomatic patients should receive antibiotic therapy. Resistance of urinary pathogens to common antibiotics is currently a topic of concern.

THE PREVALENCE OF ALCOHOLIC INTOXICATION AT “TRAUMA UNIVERSITY HOSPITAL OF TIRANA”

Berdo Elva, Kukeli Edlira, Muhameti Rushan, Fazlli Valbona

Introduction: Acute Alcohol intoxication is commonly encountered in Emergency Medicine. Excessive alcohol consumption (regular or binge drinking) produces physical and physiological alterations. The severity and clinic data vary widely depending upon the individual tolerance and amount o alcohol used. Complications may include respiratory arrest, coma and death.

Objective: The aim is to estimate the prevalence of acute alcohol intoxication at Emergency Department of “Trauma University Hospital”

Method: A cross-sectional study, data of which were collected from a retrospective review of hospital medical records. The cases are patients presented to the Emergency Department of “Trauma University Hospital”, with acute alcohol intoxications, during the period of time January2015 -December 2015.

Results: A total of 501 patients with alcohol intoxications treated to Emergency Department in one year period. 407(81%) were male, 94 (19%) were female. Significantly there were more males than females. The median age was 35-36 years (range= 15 to 79 years). 2.6% of the patients were intoxicated both from narcotics and alcohol. Most of the intoxication in the age group 15-30 years old happened during the weekends, fundamentally at night, while in the > 30 years, the weekly distribution is more homogeneous and there is a bigger number in the afternoons.

Conclusion: It’s alarming the increasing number of alcohol intoxicated patients in the young age. This is a major reason why early and intensive prevention strategies are required.

GENERALISED GRANULOMA ANNULARE AS THE FIRST MANIFESTATION OF DIABETES MELLITUS

Dajci Violeta, Sinani Ardiana, Osmenllari Artan, Qalliu Tushe

Introduction: GA is a dermatitis that occurs with papular elements like a ring, with elevated borders and boundaries clearly with healthy skin. Elements appear mainly in the extremities (the dorsum of them), and can be isolated and small alone, or generalized. Pathology is asymptomatic and is associated with the pathology of other systemic disease.

Material and methods: Tthe patient, women 53 years old presented with papular elements at her lower extremities, which cover almost all the surface of the dorsum pedis, the lesions were smaller, circular with the raised borders. History shfqajes a year. Case paekzaminuar and untreated. 2. D. V 57 year old. Patients underwent cutaneous biopsy, which confirmed the clinical diagnosis of Granuloma Annulare. Glucose hemoglobin was almost at twice the rate. Blood and chemical examinations other in normal limits.

Conclusions: Generalized Granuloma Annulare, at this case was the first sign of diabetes mellitus type II, as in the cases presented. The patient did not know that suffer from diabetes mellitus type II. She underwent therapy with insulin under control of endocrinologist and got local treatment with cortisone-based ointment. A month after the start of treatment of diabetes, the lesions in the skin were improved significantly.

AGE-HISTOPATHOLOGY RELATED PATTERNS OF PRIMARY CENTRAL NERVOUS SYSTEM (CNS) IN ALBANIA

Djamandi Pavllo, Brace Gramoz, Kaloshi Gentian, Basha Entela, Duraku Kujtime

Introduction: Central nervous system tumors are a heterogeneous group of neoplasms that include all forms of primary and secondary neoplasms developed within the cranial and vertebral cavity. In 0-84 years of age, the proportion of CNS tumors is around 8.2 -8. % per 100 000

Methods: Retrospective, observational study.

Results: A total of 1883 of malignant, benign and uncertain behavior brain tumors were registered from 1993-2013, 997 (49%) were interviewed.

Examining trends in high grade glioma incidence versus low-grade glioma were observed an increased incidence and convergence in the age group 15-44 years, while seen a “dramatic” divergence in the incidence of high grade tumors (increase) and low grade glioma (decrease) in the age groups above 45 years old. Although various factors may be implicated in some epidemiological studies but no factor risk was identify with absolute impact in these data.

Conclussion: Age remains the main factor in determining the incidence and prognosis of CNS tumors. The incidence of tumors seems to appear in a symbiotic relationship with the patient's age. This due to the fact that different tumors have a higher incidence in certain age groups.

EVALUATION OF ENVIRONMENTAL RISK FACTORS IN BRAIN TUMORS

Djamandi Pavllo, Brace Gramoz, Kaloshi Gentian, Basha Entela, Kopani Valbona

Introduction: Different occupations and exposure to certain substances are continuously charged as risks for brain tumors. Lower incidence of brain tumors makes it difficult to resolve the debate on the role of environmental triggers in their development

Methods: Retrospective observational study We used OR – exposure odds ratios and IC -95% confidence interval to determine if risk factors are considered with an increased risk for developing tumors brains,

Results: Among the environmental factors we could not find a link between the effects of domestic, electromagnetic field, but managed to confirm that residential exposure may be an important factor in inducing tumors especially in the age of childhood.

Our results showed that the risk occupations such as agricultural pesticides or other chemicals have a relatively increased risk for brain tumors, we have failed to confirm the use of mobile as risk factors.

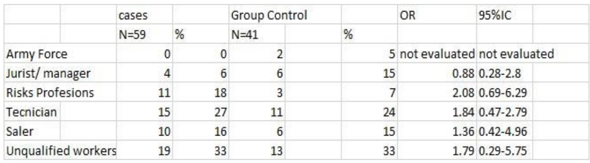

	cases		Group Control		OR	95%IC
	N=59	%	N=41	%		
Army Force	0	0	2	5	not evaluated	not evaluated
Jurist/ manager	4	6	6	15	0.88	0.28-2.8
Risks Profesions	11	18	3	7	2.08	0.69-6.29
Tecnician	15	27	11	24	1.84	0.47-2.79
Saler	10	16	6	15	1.36	0.42-4.96
Unqualified workers	19	33	13	33	1.79	0.29-5.75

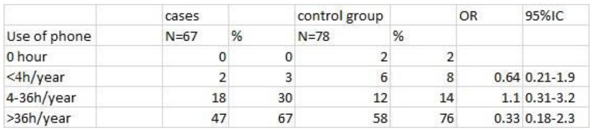

	cases		control group		OR	95%IC
Use of phone	N=67	%	N=78	%		
0 hour	0	0	2	2		
<4h/year	2	3	6	8	0.64	0.21-1.9
4-36h/year	18	30	12	14	1.1	0.31-3.2
>36h/year	47	67	58	76	0.33	0.18-2.3

Conclussion: The role of exposure to radiation, chemicals, radiofrequency is unclear, althout this is limited by the number of patients included in our study.

INCIDENCE PATTERNS OF PRIMARY CENTRAL NERVOUS SYSTEM TUMORS (CNS) IN ALBANIA

Djamandi Pavllo, Brace Gramoz, Kaloshi Gentian, Basha Entela, Duraku Kujtime

Introduction: Central nervous system tumors are a heterogeneous group of neoplasms that include all forms of primary and secondary neoplasms developed within the cranial and vertebral cavity.

Methods: Retrospective, observational study

Results: A total of 1883 cases with CNS tumors were registered during 1993 - 2013, of these 977 cases (49 %) were evaluated and interviewed during the period 2010 - 2013. The following table (see tab 1) shows increasing incidence of CNS tumors during years. Interpretation of this increase is complicated greatly from two peaks, in 1995-1996 and 2001-2002, the period corresponding to the addition of diagnostic procedures (CT scanner) or improving their (the introduction of MRI). Age and gender are important risk factors in development of brain tumors, from 55- 80 years old the incidence is higher (see tab 3), and we determined the exclusive predominance of women in meningeoma and a predominance of males in glioma (see tab 2).

Conclusion: The impact of improved diagnostic methods was seen particularly in the growth of incidence over the years. Age and gender are important risk factors in development of various brain tumors. Environmental and medical factors can be implicated in some studies, but we not noticed any factor risk with impact absolute.

MEDICAL RISK FACTORS IN THE DEVELOPMENT OF BRAIN TUMORS

Djamandi Pavllo, Kaloshi Gentian, Basha Entela, Duraku Kujtime

Introduction: Several diseases and medical treatments are discussed as risk factors for the development of brain tumors, including infections, allergy, alterations of immune system, cranial trauma, hormonal factors, epilepsy, cancer family history… etc.

Results: A total 1883 patients with brain tumors are registered from 1993 -2013, 977 (49%) of them were interviewed to review the analysis of risk factors, during the period December 2010-December 2013. We've failed to find a potential correlation between infections and brain tumors, only 2 cases was speculated or rather a coexistence between JC virus and cerebral tumors. Regarding allergies by comparing the data of 176 patients with glioma, 134 with meningeoma and 53 subjects of control, we confirmed that subjects with whatever allergies story (eczema, asthma, etc.) have less risk to be affected by glioma (OR = 0.6 , 96%, 0.5-0.8), but no change for meningeoma. We found in our study not a significant correlation between trauma and brain tumor, while family history was useful in detecting tumors with secondary or metastatic nature, this occurred in 84 patients in our study. Hormonal factors increase the risk for glioma in female, and we determine that the data between epilepsy and tumors are problematic

Conclussion: The role of medical factors in development of brain tumors is important for some of them, especially hormonal factors, cranial trauma, family history and is without effect especially in allergy, epilepsy, infections.

BUCCO-DENTAL CONSEQUENCES DUE TO HYPOPHOSPHATEMIC RICKETS CAUSED BY CHROMOSOMAL X DISORDER

Gjovreku Eva, Ivalle Bruno, Qamurani Sofika, Berdo Elva, Nikollari Luan

Introduction: Hypophosphatemic rickets caused by chromosomal X disorder is a hereditary disease which appear in a person from 20000 persons.

Objective: Recognition of dental signs by dentist staff and the effective treatment of this pathology.

Material and Methods: It is a descriptive study based in our experience in Dentistry Department of "Trauma University Hospital" Tirana.

Results: In our experience was treated a case (30years male) with above disorder. If the disease not treated in the effective way during the growth age of children seriously will impact in mineralization of dentin and will cause spontaneous pulpal necrosis as in temporary and permanent teeth. On the other hand we have indicated that a effective medical treatment in early age of combination of vitamin D hydroxyl shape and phosphorus limited consequences and disturbances of this disease. In each therapeutic medical situation effective medical reinforced bucco-dental treatment is recommended. The data clinic is always discoverable by imaging and histological examinations.

Conclusion: It's always necessary that dental staff to cooperate with specialist physician during the medication of this patients in a way that interventions (medical treatments, orthodontic, paradental, implants) do not have bones consequences.

PTERIGIUM SURGERY WITH CONJUCTIVAL AUTOGRAFT

Kambo Blerina, Halili Ismail

Objective: We intend to demonstrate the effectiveness and results of conjuctival autograft in Pterigium Surgery.

Methods: 44 patients were operated in Military Hospital and Laser Eye Clinic in Tirana with conjuctival autograft within the period 2014-2015. 20 of patients had bilateral primary pterigium. 11 of patients had recurrent pterigum. 13 patients had primary unilateral pterigium. Graft margins were secured to the recipient site.

Results: In 17 cases were used vicryl 10.0, in 15 patients were used neylon 10.0 and in 12 patients were used vicryl 9.0 sutures. There was no significant difference between women and men concerning the recurrence of the disease. After surgery we had no more recurrence. The average age in the group was 42.6 years old. In our study- period we did not identify any significant differences in application of sutures 10.0 neylon, 10.0 vicryl or 9.0 vicryl. There weren't differences between use of autograft in primary and recurrent pterygium. The visual quality was improved after pterigium surgery in 24 patients and without changes in 20.

Conclusions: Conjunctival autograft surgery appears to be an effective surgical technique in preventing pterygium recurrence and it can also help in improving the best corrected visual acuity.

PRESEPTAL CELLULITIS FROM THE FLEGMONA OF THE LACRIMAL SACCUS

Kasa Katerina, Qalliu Tushe

Introduction: Preseptal cellulitis is an infection of the subcutaneous tissues anterior to the orbital septum. It must be differentiated from the orbital cellulitis, in which it might evolve.This is an emergency diagnose.

Case Report: The case present a 32 years old woman, which has presented symptoms that persisted from 3 days. Her right eye presented painless periorbital oedema, erythema and hyperemia, crusts nasally in inferior eyelid. Visual acuity, pupillary reaction and ocular motility were unimpaired.

Swelling of the eyelid and adjacent preseptal soft tissues in contrast to the orbital cellulitis, without proptosis and chemosis. Differential diagnosis were made with orbital cellulitis and preseptal cellulitits. From anamnesy the initial localization of the infection seemed that came from the obstruction of the lacrimal saccus. Axial CT crani confirmed the diagnosis: It showed irregular formation (38 x 40 mm) directed to the medial part of the eye. An obliteration of the right frontal sinuses. Blood tests showed leucocytosis. Stage by Chandeler: Preseptal cellulitis. The therapy given were: topical oil antibiotics, intravenous cefalosporines, gentamycin, and metronidazole. After 5 days the patient undewent the intervent of dacrocistectomia to drain the flegmona.

Conclusion: Periorbital cellulitis, which frequently derives from the spread of the severe infection of the adjacent lacrimal saccus, is an emergency ocular diagnosis, which needs immediate medical and chirurgical therapy. The differential diagnosis is made first with orbital cellulitis, is made easier combining anamnesy with the CT crani examination.

MEDICAL TREATMENT OF THE ACUTE ABDOMINAL PAIN IN EMERGENCY DEPARTMENT OF “TRAUMA UNIVERSITY HOSPITAL”

Kukeli Edlira, Begaj Bajram, Hodaj Vrenos, Koceku Gerta, Berdo Elva, Fazlli Valbona

Introduction: In Emergency Department frequent case were presented with strong abdominal pain. Normally acute abdominal pain caused from intra-abdominal pathology therefore the medical treatment and management should be performed at appropriate level. The patients with abdominal colic needed immediately surgical intervention depending on diagnosis.

Objective: To present our experience in diagnosing and medical management of acute abdominal pain in Emergency Department of “Trauma University Hospital”.

Method and Material: The cross-sectional study was conducted in Emergency Department of Trauma University Hospital during the period of time 2014-2015. There were treated 1788 cases based (medical records, performed examinations and consultations with specialized physicians).

Results: Age of cases were 17-83 years. There were treated 540 (30%) colonopathy spastic 610 (34%) enterovirosis, 390 (22%) cholecystitis acute, 15 (1%) pancreatitis acute, 233 (13%) appendicitis acute. 27% of the identified cases were sent for immediately surgical intervention in “Mother Theresa” University Hospital Center.

Conclusion: The medical treatment of abdominal colic should take into consideration, the patient anamnesis, clinical data, lab examinations, imaging examinations and the service cost. Medical Staffs of mixed emergencies must perform an effective medical treatment of abdominal colic.

THE PREVALENCE OF HELICOBACTER PYLORI IN PATIENTS WITH GERD

Luka Merita, Begaj Bajram

Introduction: Gastro-esophageal reflux is a normal physiologic reflux experienced intermittently by most people, particularly after eating. Gastro-esophageal reflux disease occurs when the amount of gastric juice that refluxes into the esophagus that exceeds the normal limit, causing symptoms with or without associated esophageal mucosal injury. There are several independent factors that have an interesting role in causing GERD symptoms but also erosive esophagitis. One of them is the role of helicobacter pylori in GERD patients which is still very controversial. Still many studies suggest that there is an elevated incidence of reflux in ulcerous patients after H. pylori eradication and other studies suggest that this infection improves the efficacy of antisecretory treatment in healing esophagitis and maintenance of remission.

Objectives: To determine the prevalence of helicobacter pylori (H. pylori) infection in patients with gastroesophageal reflux disease (GERD) 2: to study the percentage of helicobacter pylori–positive in GERD patients according to different grades of esophagitis.

Material and methods: A total of 80 patients who presented at the department of Gastro-Enterology at the Military Hospital of Tirana with symptoms of GERD where included and interviewed according to validated GERD questionnaire. It included questions regarding GERD symptoms mostly heartburn and acid regurgitation. Every subject underwent an upper endoscopy for the evaluation of erosive esophagitis and in each case we made a classification of erosive esophagitis based on Los Angeles Classification.

Also H. pylori infection in GERD patients was determined by using an H. Pylori antigen stool test. Subsequently, the percentage of H. Pylori infection was analyzed in the different grades of esophagitis, according to the Loss Angeles Classification.

Results: In this study we determine that the prevalence of the helicobacter pylori in GERD patients is 40% (32/80) .The prevalence of H.Pylori in patients with esophagitis grade A is 58% (10/17), in patients with esophagitis grade B is 27% (6/22), in patients with esophagitis grade C is 22% (4/18) and in patients without esophagitis the prevalence of helicobacter pylori was 52% (12/23).

Conclusions: The prevalence of helicobacter pylori in GERD patients included in this study is 40% and its absence is associated with more severe grades of the disease. These results indicate that H. Pylori plays a protective role against GERD.

HIATAL HERNIA AND GASTRO-ESOPHAGEAL REFLUKS DISEASE

Luka Merita, Begaj Bajram, Cakoni Suzana

Introduction. Patients with substantial Hiatal Hernia (HH), regardless of whether or not they had a hypotensive low esophageal sphincter, have been shown to incur an increase number of acid reflux episodes.

Objective: The aim of the present study was to examine the prevalence of Gastro Esophageal Reflux Disease (GERD) in the patients with HH and the presumed role of HH in the severity of esophageal mucosal breaks.

Methods. The 480 patients with HH, 264 (55.1%) male and 216 (44.9%) female with an average age of 42±38 years, were drawn from 1439 consecutive persons referred for upper endoscopy due to heartburn and reflux symptoms. Esophageal mucosal lesions of 271 (56.4%) patients with GERD and HH, assessed by Los Angeles classification, were compared to the group of the patients (188) with GERD without HH.

Conclusions. The GERD was present in the 56.6% of the patients with HH. On the other hand, HH plays an important role in the severity of esophageal mucosal injure.

ENDOBRONCHIAL TUBERCULOSIS

Muhameti Rushan, Alizoti Xhemil, Argjiri Dhimitraq, Ibro Mustafa

Introduction: Endobronchial tuberculosis (EBTB) is defined as tubercular infection of the tracheobronchial tree with microbial and histopathological evidence

Objective: To present our experience related with medical treatment of endobhroncial tuberculosis in Trauma University Hospital.

Material and Method: The descriptive study was conducted during the period of time 2011-2013.

Results: It is seen in 10-40% of patients with active pulmonary tuberculosis. More than 90% of the patients with EBTB have some degree of bronchial stenosis. 10 to 20 percent have normal chest radiograph. Therefore a clear chest radiograph does not exclude the diagnosis of EBTB. Bronchoscopic sample has been the key to the diagnosis peoducing more than 90% yield on smera as well as on culture Bronchoscopy and computed tomography (CT) are the methods of choice for accurate diagnosis of bronchial involvement and assessment for the surgical intervention. Characteristic HRCT findings of EBTB are asymmetric centrilobular nodules and branching lines (tree-in-bud appearance). Early supervised antituberculosis therapy results in minimal structural and functional residual. Corticosteroid therapy may not influence the outcome of endobronchial turbeculosis.

Conclusion: Early diagnosis and medical treatment before the development of fibrosis is important to prevent complications of endobronchial turbeculosis, such as bronchostenosis.

ENDOBRONCHIAL TREATMENT OF BENIGN AND MALIGNANT PATHOLOGIES OF THE RESPIRATORY TRACT BY INTERVENTIONAL BRONCHOSCOPY

Muhameti Rushan, Kukeli Edlira, Ibro Mustafa, Alizoti Xhemil

Introduction: The patients with bronchial tract pathologies underwent procedures of the endobronchial treatment

Objective: Assessment of the endoscopic procedures role in the patient's life saving, life expectancy and quality of his life.

Material and Method: The retrospective study was performed for patients treated at the “Trauma University Hospital” and “Ikeda” Clinic. Target group: There were selected 48 patients with bronchial tract pathologies.

Results: All the patients who were submitted to the above mentioned procedures that have not been any contingent for postoperatory or intraoperatory major complications. Patients with benign pathology including ages of 13-70 years have won full rehabilitation, quality of life and have a completely normal life. The patients with malignant pathology which included age 31-72 years have received lifetime, better quality of life, thanks to the combination of endobronchial resectionschemotherapy and radiotherapy.

Conclusion: Interventional bronchoscopy is the greatest guarantee for the patient, due to major complications which might happen. For benign pathologies the patients get complete rehabilitation, while for those malignant the patient gets the quality of life. An important point is not only the experience of interventionist physician but also the anesthetist doctor who may apply intravenous or endotracheal anesthesia.

DYNAMIC STABILITY OF THE KNEE AFTER DAMAGE ON THE CRUCIAT ANTERIOR LIGAMENT

Saraci Manushaqe, Stanaj Luljeta, Duraku Kujtime, Lico Rovena

Introduction: Recovery of dynamic stability of the knee is not only a necessity but also an emergency for patients who suffer damage on the cruciat anterior ligament (LCA) especially to return to different activities from before the injury as amateur sports activities, competitive or even your career or normal social life.

Objective: To highlight the importance of rehabilitation on the dynamic stability of the knee during or after the intervention on LCA on patients with moderate sportive activities.

Material and method: The study includes January 2014-April 2015 period. They were rehabilitated according to (Post-Operator Recostruction Protocoll Wrightington Hospital) with the Hop-Test method which consists on: Jumping forward with one leg. Time of jump for 6 meters, successive jumps, Diagonal jump. The patients which studies were conducted on have been a subject to Arthroscopy for the LCA reconstruction not longer than 4 weeks after the injury. Fourth month results of the rehabilitation were compared with those of the fifth month

Studies were conducted on 87 patients of 18-40 years old. Average age of 26 ± 3 years old. Average weight of 65 ± 5 kg, M/F Ratio 63 males injured in football, 24 women (11 injured in basketball and 13 in volleyball)

Results: Hop-Test was used for the assessment of motor control of patients. To perform this test theres required a high level of dynamic stability of the knee which is represented by LSI (Limb Symetry Index), Jump distance with one leg for 88.23% of the patients resulted with LSI>85%

Distance of three successive jumps for 94.11% of the patients resulted with LSI>85%, Distance of diagonal jump for 94.11% of the patients resulted with LSI>_85%, Time of jump in a 6 meter distance for 94.11% of the patients resulted with LSI<85%.

Conclusions: 94.11% of patients with LSI>85% rehabilitated by this method have returned to their activities by the end of the fourth month.

HALLUX VALGUS, EVALUATION OF RESULTS ACHIEVED BY TREATMENT WITH SCARF OSTEOTOMY

Ruci Julian, Duni Artid, Shehu Kreshnik, Sejati Erion, Murati Lorenc

Introduction: Hallux valgus is one of the most common pathologies of the foot, mainly occurs on women. This pathology is characterized with the medial deviation of the first metatarsal bone and lateral deviation of proximal phalange of the big toe. Objective: The purpose of this study is to define an efficient surgical method for the treatment of Hallux Valgus. Also, the review of the patient treatment result that went under surgery between the periods 2009-2015 with the osteotomy based on the Scarf method, compared with the results of other surgical methods of the Orthopedic Trauma services.

This is a tranversal (kors-seksional) study on which 106 patients with the hallux valgus diagnose were taken in condiseration. From the total no. patients 91, 85.8% of them were female and 14.2% male. The average age of the participants was 49.2.

Results: Scarf Oteotomy resulted an efficent operating technique for the treatment of Hallux Valgus, mainly on moderate to severe forms. This tecnique is followed with the improvement of the angles HVA and IMA, respectively with 18.27° and 5.8°.

Conclusion: The results clearly show that the Scarf operating technique is superios in comparison with all the others operating techniques that have been taking in consideration during this study. This based on the better improvement of two parameters after the surgery: HVA angle and IMA angle.

TREATMENT OF IDIOPATHIC SCOLIOSIS WITH CORSET CHENEAU TYPE

Ruci Julian, Duni Artid, Shehu Kreshnik, Sejati Erion, Murati Lorenc

Introduction: Idiopathic scoliosis are a developmental deformity of the vertebral column, which influences the form and function of young individuals. Corsets and physiotherapy are conservative treatment methods of the column deformation, in light and moderated cases.

Objective: To present our experience in thoraco-thoraco scoliosis, lumbar and lumbar rigid, based on the use of Chaneau corset type. Studies have shown that keeping correctly the corsets has influenced on the natural progression of Scoliosis and possibly up to the elimination of surgical intervention.

Material & Methods: Our 100 patients are treated by our team, consisted of doctors, physiotherapist and ortoprothesist technicians for the preparation of orthotics. The duration of treatment, is dated from 2010 to 2014. In this group there are included only the patients who after being diagnosed, have agreed to be treated and to partially use the data related to them for study reasons.

Results: Improvement, a decrease of Cobb angle of 6 degrees or more. Stabilised, not more than 5 degrees Progression or improvement. Progressed with the Cobb angle lower than 50 degrees. Progressed with Cobb angle of more than 50 degrees, being considered promising candidates for surgery. Besides this classification, the particulars in relation to sex, age, angle and duration of treatment were taken into consideration in each case treated.

Conclussion: Cheneau corset treatment and physiotherapy of idiopathic scoliosis is successful in 45 percent of the cases. The results of this paper prove its role in limiting the incidence of surgical but natural progression in improving diagnosis.

XANTHOGRANULOMA JUVENILE A RARE TYPE OF NON-LANGERHANS CELL HISTOCYTOSIS IN 5 MONTH OLD BABY

Sinani Ardiana, Vasha Dorela, Lico Rovena, Nikollari Edlira

Introduction: Juvenile xanthogranuloma is a type of non - Langerhans cell histocytosis. It is usually benign and self-limiting. It occurs most often in the skin of the head, neck, and trunk but can also occur in the arms, legs, feet, and buttocks. Skin lesions of Juvenile xanthogranuloma are presents predominantly in infants and in young children, more often in males but 10% of cases are adults. However, most cases of childhood juvenile xanthogranuloma are self-limited and resolve without treatment but in adults tends to be more complicated and often are not resolve without treatment.

Case Report: 5-month-old child's parents came to the dermatology service, worried about their child. They had noticed two skin elements, one on the scalp and the other in left armpit of child. During objective examination of skin were seen two lesion, with yellowish appearance, in 0.5 cm diameter. This skin lesions were increased in size for two months, the child seemed healthy and the parents did not refer further concerns for their child. Clinical diagnosis of these lesions coincide with granulomatous formations similar to Juvenile Xanthogranuloma. Parents refused to make a cutaneous biopsy and the child was kept under surveillance every three months.

Discussion: Juvenile xanthogranuloma in most cases of childhood is a rare self-limited disease and the correct diagnosis is based in a cutaneous biopsy. The lesions are made of collections of histiocytes. Most of the nodules are 0.5-1 cm in diameter but giant nodules may be as large as 2 cm. They may be on any site of the body, but more frequently appear on the trunk and upper extremities. Occasionally they also appear in the eye or internal organs. There is no treatment for juvenile xanthogranulomas, they are harmless growths and disappear eventually over 2 to 3 years, usually without scarring. If they are giant nodules a surgical treatmet may be done.

HEALTH ASSESSMENT AND KNOWLEDGE OF MILITARY EFFECTIVES

Vasha Dorela, Beja Ergina, Reso Elton, Asqeri Denisa

Objective: The primary health care in the Albania Armed Forces provides: primary prevention and health promotion;

risk factor detection for different disease; disease management, support for self-help and self care; surveillance/reporting. The aim is to evaluate the health assessment and health knowledge of the effectives.

Method: This is a cross-sectional study conducted through the interviews of a 5 scale questionnaire made to the effectives of the Support Command that were presented to the medical center of the command for any medical visit for a 2 months period. Data like how easily they can assess and understand the health information, were collected from the interviews and has been processed by the SPSS program.

Results: The study shows that most of the effectives interviewed find it almost easily to almost difficult to assess health information, to understand it and follow the different instructions for preventing diseases or for their treatment.

Conclusions: There is a need for availability and implementation of health programs related to the most common diseases and more promoting seminars with the effectives.

RISK FACTORS FOR THE APPEARANCE OF DIABETES, HBP, IN THE ARMED FORCES

Vasha Dorela, Reso Elton, Beja Ergina, Asqeri Denisa

Objective: Obesity, family predisposition, smoking, life-style are some risk factors for many highly prevalent and potentially fatal chronic diseases, including diabetes, cardiovascular diseases, etc. The aim of this study is to evaluate some of the risk factors that influence in the appearance of these diseases in the ranks of the Armed Forces.

Method: This is a cross-sectional study conducted through the interviews made to the ranks of the Land Forces Command that were presented to the medical center of the command for any medical visit for a 2 months period. Data like blood pressure, glicemia, BMI, and questions around medical history, family predisposition, feeding habits, etc were collected from the interviews and has been processed by the SPSS program.

Results: The study shows that overweight and obesity is more common in the elderly males, due to increased total calories intake and decreased physical activity. Obesity and family predisposition for diabetes shows important factors for the appearance of type 2 diabetes. Smoking, family predisposition and changes in diet shows potentially risk factors for High Blood Pressure.

Conclusions: The ageing population, increased total calories intake and decreased physical activity, smoking, family predisposition, and obesity are some of the factors that influence in the appearance of diabetes, HBP and many other diseases.

TRAUMATIC TREATMENT OF TEMPORARY TEETH

Xhafaj Alba, Eni, Kociaj Ani

Introduction: Little kids tooth damage may cause serious and long life consequences. This damage can lead to, discoloration, malformation, or lose of teeth. Most damages of the first teeth happen during the age of 1 ½ and 2 ½. The structures included in fractures are as following: Enamel, Dentina, or pulp.

Objective: The aim is to determine pathological consequences of traumatized teeth.

The teeth are at the risk of the pulpes necrosis. They suffer from lack of collateral blood. The thin band of tissue vascular nervo, may get apart even by tiny strokes. The most risky damage of the permanent teeth is their effect on the nonerupted under development teeth. If this damage happens during development period of the permanent tooth, it causes hypoplazi e enamelit or hypocalcification.

Conclusion: Teeth fractures: the treatment of these fractures depends on the eventual fracture level. The best prognosis is when it has happened at the 2/3 of the root. Most of these teeth keep their vitality and the moving is minimal. They are clinicly obvious as the tooth is sensitive when it bites. The tooth treatment should be out of the occlusion. Hard moving is a usual reaction of the tooth to the traumas. As a treatment it is advisable that the kid does not chew with those teeth for about a month.

MEDICAL CONTRIBUTION OF ALBANIAN ARMED FORCES AT MULTINATIONAL MISSIONS

Zeka Valbona, Kopani Valbona

Introduction: In context of security environment transformation Albanian Armed Forces are engaged intensively at NATO’s operations for providing of stability and peace by integrated strategic orientation. Participation in NATO’s operations requires training improvement for continuity of medical personal in prevention of conflicts crisis management, peace support operations and humanitarian assistance.

Objective: Predeployment medical assessment of operational medical capacities of Albanian Armed Forces in multinational missions.

Method: The descriptive study was conducted during the period of time 2010-2015. The data were taken in retrospective way from medical documentation in Military Hospital related with participation in ISAF operation in Afganistan.

Results: There are 1070 (medical personnel) underwent the periodic medical assessment. Resulted 930 mission capable and 180 mission unable (80 AgHbS positive and 60 with visual difficulties.)

Conclusion: The medical personnel participating of Albanian Armed Forces should be demonstrated improving of operational medical capacities comparable with members of the alliance in multinational missions.

ANALYSIS OF TRENDS FOR CHANGE OF MEDICAL PERSONNEL IN BULGARIA FOR THE PERIOD 2010 - 2014 YEAR

A. Aleksandrov, A. Petkov, I. Aleksieva

Introduction: The observed quantitative and structural changes of the medical personnel in the last 20-25 were the subject of this study. These changes relate mainly to the reduction of the staff, which is a constant trend expressed in varying degrees for the different medical specialties. They can lead to serious problems in the health care system of the country in the medium term and therefore they were of interest to explore.

Material and Methods: An essential part of the analysis was the availability of a medical staff in 25 medical specialties. The study covers the period 2010 - 2014 year. The total number of doctors and dentists was investigated for the period 1990 – 2014. Statistical data for the periods of the study was used and processed. We analysed the change of medical personnel in different specialty and general changes and new trends in the availability of doctors in Bulgaria.

Results and discussion: Statistical surveys showed that the widespread opinion that doctors and dentists in the last 25 years have decreased is not true. There is some increase in the absolute number and the physicians and dentists.

Conclusions:

1. The number of doctors for the period 1990 - 2014 has not changed significantly - 28 497 doctors in 1990 and 28842 doctors in 2014. / 1.21% increase /

2. Dentists have increased in the same period from 6109 to 7013 / 14.80% /

3. The study of the change in the number of doctors in the last five years in different specialties shows different situation for each specialty.

CONDUCTING PSYCHOLOGICAL ASSESSMENT TO PATIENTS AT CLINIC OF NEUROLOGYCAL DISEASES - MMA

Krasen Daskalov, Krasimir Genov, Mirena Georgieva, Maria Dimitrova, Iglika Parashkevova

The paper presents psychological work done by specialists of Center for Mental Health and Prevention at the Clinic of Neurologycal Diseases of Military Medical Academy in Sofia. It discusses the types of tests and assesement on request by the physicians to support the process of diagnostics and providing psychological assistance to patients.

260 patients passed psychological assesement at the Clinic of Neurologycal Diseases for the last year. In 121 cases are tested cognitive skills, in 102 cases are tested personality potential, 37 cases are combined cognitive and personality testing. The cognitive psychological research is required for memory disorders, vascular dementia, Alzheimer's and Parkinson's disease. It is performed with neuropsychological battery including a measurement of concentration and stability of attention, memory, thinking, abstract thinking, constructive praxis, reasoning ability.

The assesement of personality parameters includes measuring the level of anxiety, self-esteem, self-protective mechanisms, adaptive abilities and coping strategies, patterns of behavior and involvement in social interactions. It clarifies the attitude of the patient about the illness process and support the development of coping strategies. Explore anxiety-depressive symptoms, presence of stressors affecting the optimal personality functioning, not worked traumas and conflicts that exacerbate the process of illness. For the assesement of personality were used personality questionnaires as MMPI, test of T. Leary, depression scale of Zung, projective methods.

In conclusion, psychological assesement of personalilty not inferior to the number of cognitive assesement associated with specific neurological symptoms. The need of measuring the personal abilitys and emotional state is directly responsible to the diversity of symptoms cared for at the department of neurology of MMA. The growing need for psychological interventions in neurological practice shows systematic approach to medical care for patients according to their individual needs and personality traits that directly affect the illness process.

CARDIAC OXIDATIVE STRESS AFTER ACUTE POISONING BY ORGANOPHOSPHOROUS COMPOUNDS

D. Dimov, K. Kanev, I. Samnaliev

INTRODUCTION: The main mechanism of action of the organophosphorus compounds, including nerve agents (Tabun, Sarin, Soman, and VX), is irreversible inhibition of acetylcholine esterase (AChE), resulting in accumulation of toxic levels of acetylcholine (ACh) at the synaptic junctions. However, our previous studies showed that organophosphorus compounds might have toxicological effects on the body by non-cholinergic mechanism.

AIM: The objective of the study was to analyze nitrotyrozine production as a marker oxidative stress in heart after acute Tabun poisoning.

MATERİALS and METHODS: We investigated by immune-histochemistry the expression of 3-nitrotyrozine, a marker of NO-induced oxidative stress, in three different groups of animals: (i) treated by 0.5 LD50 Tabun; (ii) vehicle and (iii) atropine and specific re-activator applied immediately after Tabun.

RESULTS: Heart nitrotyrozine expression 24 h after Tabun treatment was dramatically increased in comparison with its levels without treatment. More than 70% of cardiac cells were positive with a high intensity. After application of atropine and AChE re-activator, we observed decrease in nitrotyrozine levels of about 10% compared to Tabun treatment, but still far from the normal levels in non-treated heart

CONCLUSİON: Our study evidently demonstrated that nerve agent Tabun triggers oxidative-nitrosative stress in heart and these cellular effects should be protected by an additional anti-oxidant therapy, since atropine and AChE re-activator are not efficient in this manner.

MORPHOLOGICAL ASPECTS OF LUNG INJURIES CAUSED BY FIREARMS WEAPONS IN EXPERIMENT

Drandarska I., S. Nikolov, V. Mutafchiiski

The aim of this study is to examine the morphological features of lung injuries associated with experimental gunshot. We analyzed the lung injuries caused by firearms weapons with or without silencers in experiment with pigs.

A detailed description is given of changes in the lungs of experimental animals with various types of weapons. Histological tissue changes were diagnosed and compared in intensity depending on various factors of the shot.

The scientific research is conducted in accordance with the principles of ICLAS/FELASA and the respective Bulgarian legislation acts regarding the humane attitude and welfare of the experimental animals.

PATHOHISTOLOGICAL INJURIES IN ABDOMINAL CAVITY CAUSED BY FIREARMS WEAPONS IN EXPERIMENT

Drandarska I., S. Nikolov, V. Mutafchiiski

The aim of this study is to examine the macro and microscopical features in abdominal cavity associated with experimental gunshot wounds in pigs. We used the firearms weapons with or without silencers. The severity of injuries depends on many factors: bullet factors, firearms weapons factors and tissue factors.

We put all the changes occurring in the abdominal cavity due to the use of both types of weapon with and without the silencer. Histological we studied tissue materials from affected organs to clarify the degree of damage in different types of weapons.

The scientific research is conducted in accordance with the principles of ICLAS/FELASA and the respective Bulgarian legislation acts regarding the humane attitude and welfare of the experimental animals.

CHALLENGES WITHIN THE CASUALTY DECONTAMINATION AFTER CHEMICAL EMERGENCIES

Galabova A., Dimov D., Kanev K

INTRODUCTION: Industrial accidents or chemical terrorist attacks may lead to contamination of environment and people. The decontamination procedures are an integral part of the countermeasures against intoxications by reducing contamination, elimination and spread of the harmfull effects. In order to be effective, the evidence based research is of a vital importance.

The AIM of the present material is to outline the challenges, which should develop additional awareness during the planning process for chemical emergencies.

MATERIALS AND METHODS: Survey of the open literature on the current status and trends of the problem.

RESULTS: In chemical emergencies the timely reaction is of a vital importance, but the arrival and depolyment of the emergency teams may be of a great delay. Decontamination, triage, administration of antidotes and life saving medicines are supposed to happen in limited time conditions. In serious circumstances, as mass poisonong cases, it is expectes that casualties will use their own methods to reach medical facilities, thus spreading the contamination. It is accepted widely decontamination to be performed on site, but in this case medical personnel in hospitals will be of a great risk. Within hospitals patient flow should be controlled and decontamination plan should be tailored before entering the emergency medical departments.

CONCLUSION: The readiness for mass casualty chemical emergencies is a responsibility for the medical and non medical personnel. Ensuring that hospital have the resourses and are trained is essential.

CURRENT SOCIOMEDICAL PROBLEMS OF THE DEPARTMENT OF EMERGENCY CARE OF MILITARY MEDICAL ACADEMY - SOFIA

Grigorov Nedyalko, Manoilova Yuliya, Popivanov Ivan, Nedelcheva Svetla

Object: To specify the most important current sociomedical problems of the Department of emergency care in Military Medical Academy - Sofia determined via an analysis of three-month (October, November, December 2015) experience. To create an algorithm of engagement of the Social Services for more effective care.

Materials and Methods: We have used retrospective and statistical analysis. We have discussed the working methods of the Emergency department with homeless patients, aggressive patients, victims of violence, and patients without health insurance, the evaluation of their condition with different methods and a general check-up of these patients.

Results:

1. 1516 patients have been treated over a period of three months in the Emergency department. Almost every sixth patient is without health-insurance.

2. 39 784 patients have been treated over a period of one year (01.01.2015 – 31.12.2015) in the Emergency department. 742 (1.87 %) of them are victims of criminal acts (except for crashes), 15 % - suicide attempts, 10 % - homicide attempts, 10 % - accidents at work, 30 % - victims of domestic violence, 30 % - victims of robbery, 5 % - victims of sexual violence.

Conclusions:

1. The victims of domestic violence after hospital discharge should be placed in special centers by the Social Services.

2. If NHIF (National Health Insurance Fund) allocates more money to the ambulatory treatment, fewer patients will visit the Emergency department on the occasion of non-emergency conditions and routine check-up.

3. In the Emergency department often arrive aggressive patients who endanger the security of the medical workers.

4. We need to achieve a better coordination with caseworkers. There should be a non-stop telephone contact with the Social Services in purpose to find lodgings for the homeless patients without health problems.

5. The involvement of schools and media in health education will lead to early detection and treatment of the emergency cases as well as to their prevention.

6. If NHIF starts paying implants for emergency operations, all patients in the Emergency department will be able to receive advanced treatment.

BIODOSIMETRY IN THE HUMAN LYMPHOCYTES AFTER IN VITRO IRRADIATION

M. Hristozova, D. Denkova, V. Rangelov, R. Popov

INTRODUCTION: The cytogenetic method is used for assessing the radiation damage and to support triage, medical treatment decisions, and prognosis of radiation casualties. Its advantage is that it is relatively cheap method and easy to perform. Requiered laboratory backup along with time delay to acquire the first results of cytogenetic assessment make these methods unsuitable for military field conditions. These problems have been overcome with the new biodosimetric methods by including protein marker or serum protein analyses. Nevertheless, these assays are being developed and have not yet been tested in a real triage situation. Therefore, cytogenetic methods still remain a standart of biodosimetry.

PURPOSE: The aim of this study is to evaluate these parameters in human blood lymphocytes after in vitro irradiation and to assess its contribution to biodosimetry. The in vitro cytokinesis-block micronucleus (CBMN) assay is a cytogenetic method based on the assessment of micronuclei in nucleated cells that have completed only one nuclear division.

CONCLUSION: Our results suggest that micronuclei measured in binucleated cells are the best biomarker of ionising radiation evaluated by CBMN cytome assay and assessment of other parameters is possible but their use is limited due to insignificant differences and overlapping of the 95% confidence intervals between neighbouring doses. Values of micronuclei measured in binucleated cells were used to construct in vitro linear-quadratic dose-response calibration curve.

EFFECT OF IONIZING RADIATION ON MAMMALIAN CELL CULTURES

M. Hristozova, V. Rangelov, D. Denkova, P. Petrunov

Introduction: At the cellular level, ionizing radiation distort growth, division of cells, the structure of the nucleus, induced chromosomal aberrations, fragmentation and other. These disorders lead to cytolysis and cell death. Tracking radiation damage at the cellular level using biological markers representing quantitative changes that occur in biological systems under the radiation effect. Damage to the cell directly depends on the dose of ionizing radiation - in small doses amendments may have reversible character. At the high doses cell death occurs.

The aim of the study was to follow radiation effect (Co-60) on mammals’ cell cultures taking into consideration the overall impact of the gamma radiation on the cell. In the resulting survival curves was monitored dose-effect as in the tumor and in normal cell lines.

Used four cell lines: MDCK (kidney calf - epithelial cells), Fl (normal human amniotic tissue), A-549 (carcinoma of human lung), P (human embryonic lung).

Irradiation of samples was conducted in Gamma irradiation with 4 source cobalt (Co-60) to ensure homogeneity of the irradiated area. The dose rate is 22 sGy/min. Used a wide range of radiation doses from 1 to 17.5 Gy.

Observe for morphological characteristics of the cells, the degree of growth or degeneration, the condition of the monolayer and change of medium.

Determine the percent of cells viability and preparing survival curves.

Conclusion: Death rates of irradiated cell lines is a function of dose, and has an exponential dependence. This pattern was not demonstrated in all our attempts, which may be due to: asynchrony of the population, the presence of hypothermia, altered permeability of the cell membranes.

THE "GOLDEN HOUR" OF EMERGENCY TOXICOLOGY

Kanev Kamen, Paskalev Kuzman, Neykova - Vasileva Ludmila

The pattern of intoxications in humans in the modern world is changing with the development of pharmacy, chemistry and others branches of industry and agriculture.

This necessitated the development of new antidotes and treatments for the cases of poisoning. This requires the development of new antidotes and therapeutic regimens for the treatment of poisoning.

The PURPOSE of this work is to analyze the most important factors for rescue activities, limiting the harmful effects of toxic substance within the "golden hour".

MATERIALS AND METHODS: The current therapeutic regimen for the treatment of poisoning are logically analyzed.

RESULTS: The optimal treatment of a poisoning depends on the availability of antidotes administered on time in sufficient quantity based on the "benefit / risk" principle in the light of good medical practice. In case of accidents, toxicological emergency assistance increases its efficiency with the use of modern formulations.

CONCLUSION: Therefore, the adherence to the timeframe of the "golden hour" is a concept that should be guiding for the therapeutic behavior of the medical staff in emergency toxicological assistance.

This concept ensures a successful outcome by the inclusion of all the suitable methods and ignoring unacceptable for each particular case.

ACTINOMYCOSIS: A RARE CAUSE OF LARGE BOWEL OBSTRUCTION

Kirien Kjossev, Evgeni Belokonski, Petko Dimov, Georgi Popivanov

Aim: Abdominal actinomycosis is a chronic suppurative infection, accounting only 20% of cases caused by Actinomyces species. Recent investigation finds 481 reported cases with ab¬dominal wall and abdominal viscera involvement. The condition has been shown to mimic appendiceal tumor and abdominopelvic actinomycosis is associated with abdominal surgery and intestinal perforation or occlusion. Bowel obstruction due to actinomycosis is very rare, and few cases have been reported.

Method: We report two cases of large bowel actinomycosis leading to obstruction and preoperatively misdiagnosed as neoplastic disease. The first patient with pelvic form probably caused by a long-standing intrauterine device presented with signs of obstruction and colonoscopic data for malignant sigmoid occlusion. In second case ileocecal form of the disease is presented with CT signs of obstructed and perforated neoplastic disease of the right colon.

Results: Both cases were treated with wide surgical excision and long-standing administration of antibiotics without local recurrence at follow up.

Conclusion: Even though abdominal actinomycosis is very rare, it should be included in a list of differential diagnoses in large bowel obstruction. Accurate preoperative diagnosis and efficacy of antibiotic treatment may spare the patient from unnecessary surgery.

MEANING OF DELAYED FLAP COVERAGE OF OPEN EXTREMITY FRACTURES AFTER PREVIOUS VACUUM-ASSISTED CLOSURE (VAC) THERAPY

Dimitar Kostov, Nedelcho Tzachev

AIM: Dispute remains regarding timing in the management of complex traumatic lower extremity defects.Some originators recommend a definitive bony and soft tissue reconstruction within a critical period of 72 h, yet in many patients this may be impossible due to concomitant injuries or delayed referral. However, little data are available on the results of delayed flap reconstruction of complex traumatic extremity defects, especially using new technologies of wound coverage such as vacuum-assisted closure VAC therapy which may reduce the disadvantages of conventional open wound therapy prior to a subsequent flap reconstruction.

MATHERIAL AND METHODS: We retrospectively analysed the soft tissue reconstructions in 52 open extremity fractures during a 6-year period (between 2008 and 2014) with special regard to complications, overall flap loss and wound infection.

RESULTS: A total of 38 male and 14 female patients with 38 open fractures of the lower and 14 of the upper extremity were included. All patients had been referred from a trauma centre at a mean interval of 25 days (range 5-86days) after the trauma event with temporary VAC of their wounds after initial fracture fixation and initial debridement of necrotic tissue. Flap reconstruction was thus only possible later than 72 h and definitive wound closure was achieved at a mean time of 28 days (range 5-98 days).

CONCLUSION: According to this study, the flap reconstructions performed beyond the frequently quoted critical interval yielded similar results to those of immediate reconstruction within the first 3 days, as reported in the literature.

This strategy is in accordance with the principles of ‘Damage Control Orthopaedics (DCO)’ and may reduce the importance of emergency reconstructions, especially in poly-traumatised patients.

EVOLUTION OF PSYCHIATRIC SYNDROMS DURING PARTICIPATION IN MILITARY ACTIONS

Iliya Marinov, Silviya Dimitrova

Aim: The object of this publication is to explore the development of scientific concepts of psychiatric syndromes during military actions from the American Civil War to modern operations other than war.

Material and metods: Based on the review of various literatures (theoretical and applied research) we look at the evolution of the concepts associated with stress reactions and disorders in participation in military actions.

Results: At the beginning of the studies of this issue the focus was primarily on physical and physiological symptoms as a response to an unpleasant influence of the combat stressors. With the development of psychology and psychiatric sciences, mainly psychological and psychiatrical effects from the impact of the battle stressors are examined.

The survey results show that the concepts of war related psychiatric syndromes vary in historical aspects depending on the development of science and the assumed concepts at that stage.

Concusions: Publication of the findings of numerous research projects, articles, reports and monographs of clinicians, psychiatrists and psychologists, as well as the research of military doctors who participated in the conduct of military actions, have contributed to the formation and determination of the diagnostic criteria of mental disorders (battle stress, gross stress reaction, battle fatigue, post traumatic stress disorder, etc.) as distinct diagnostic units that are included in the DSM of the American psychiatric association and subsequently in the ICD (International Classification of Diseases).

CLOZAPINE INTOXICATION - CASE REPORT

Neykova-Vasileva Lyudmila, Kanev Kamen, Traykova Vera

INTRODUCTION: Clozapine (Leponex) is an atypical antipsychotic medicine. Induced for treat ment of schizophrenic patients, resistant to standard antipsychotics. Despite its limited application it could lead to heavy intoxications.

AIM: The objective of the material is to make a logical analysis of the therapeutic algorithm for the treatment of such poisoning.

METHOD AND MATERIAL: We introduce severe intoxication with Clozapine, in '60 in female patient with a primary diagnosis: paranoid schizophrenia. The toxic-chemical analysis proved lethal concentration of Clozapine in her blood.

DISCUSSION: Detoxicating measures aim to limit the harmful effects of xenobiotic on the patient. The depuratsionna complex therapy a pplied by us, according to the standard of "Clinical Toxicology" and according to modern international standards - of charcoal hemoperfusion (twice) and lipid therapy (twice), under constant laboratory control, master end the life-threatening intoxication. The patient was discharged without current acute toxic problem, but with exacerbat ion of her mental illness.

CONCLUSION: Clinging strictly to the procedures in "medical standard" in cases of severe and deteriorating intoxications may be ineffective. This requires the implementation of new modern depurative methods taking in account the nature of the toxic products and good the medical practice.

COMBINED TRAUMA – RECTAL LESIONS DUE TO OPEN PELVIC FRACTURES. TREATMENT STRATEGIES

Teodosiev I, Popivanov G, Mutafchiiski V, Kjossev K

Aim: Open pelvic fractures combined with recto-sigmoid lesions are associated with high morbidity and mortality. Treatment needs the involvement of a multidisciplinary team. This report presents our experience in the treatment of this kind of traumatism.

Methods: We present four cases with combined trauma due to different causes (two gunshot injuries, one road accident and one Crush injury). The common features are combined trauma - open pelvic fracture and lesion of the recto-sigmoid.

Results: All patients were males with mean age of 36 years. Median ISS was assessed as 35(20-55). The four patients passed through prolonged but successful curative period. There was no mortality in this short series. Loop colostomy and VAC wound therapy as a part of the treatment strategy are procedures defined as “golden standard” and resulted in remarkable efficiency and reduced rates of morbidity and mortality.

Conclusions: Open pelvic fractures combined with recto-sigmoid lesions present a challenge for managing and treatment with successful results. Multidisciplinary team has to be involved in the patients care. According to our initial experience and literature data early fecal diversion with loop colostomy and VAC wound therapy are given as procedures of choice leading to good result.

CLINICAL CASE OF COMBINED HAMARTOMA OF THE RETINA AND RPE IN YOUNG ADULT

Vidinova Christina, Voinov Latcezar

Purpose: The aim of our study is to report a clinical case of a young adult shown with the picture of combined hamartoma of the RPE and discuss the diagnostic strategies and follow up.

Material and methods: We report a case of a 26 year old adult who complained of progressive loss of vision of one eye in a period of one year. She was accepted in the ward and underwent a complete ophthalmologic examinations including OCT (RTVue OPTOVUE), fluorescein angiography and fundus autofluorescence.

Results: Ophthalmoscopically we found a partly pigmented, slightly elevated, peripapillary lesion with strong distortion of the retinal blood vessels. Fluorescein- angiography showed early filling of the dilated, large caliber, anomalous retinal vessels, which in the periphery made a specific network of vessels with immense glial proliferation and leakage of dye in the mid and late phases of the study. The OCT showed generalized edema in the retina and peripapillary zone and autofluorecence was typical for RPE proliferations.

Conclusion: Combined hamartomas of the retina and RPE (CHRPE) are rare occasions, usually congenital. What is interesting in our case is that it occurred in a later age of 26 years. The proper diagnosis of such cases is important because this benign lesion may be confused with more threatening conditions such as malignant melanoma or retinoblastoma. An appropriate diagnosis through clinical appearance and fluoresce in angiography is imperative to prevent unnecessary enucleation.

TELEMEDICINE - OPPORTUNITIES FOR SERVICING OF MISSIONS

Georgi Yanchev, Rumen Popov, Ventzislav Mutafchyski, Virsaviya Vaseva

The project to build a "Telemedicine" in Bulgaria is very old. The new things in it were driven by the need to cover different parts of the world in need of medical advice from qualified professionals. That will provide a connection between points, so that it can exchange information in electronic form (graphic, imaging, multimedia, internet, text, etc.).

Nothing is as important as the health care relationship between patient and doctor. Preservation and care are sometimes defined as the heart of medicine.

Today, telemedicine programs are focused on accessing inaccessible areas, field areas and missions where there is little or no specialists. "Telemedicine" has the potential to access necessary health services and medical expertise especially in the above mentioned areas.

Doctors practicing telemedicine using two core technologies:

1. live interactive (interactive live)
2. store-and-forward.

Specialists also communicate with the patient, using e-mail and the Web. The link "Telemedicine" is based on the deployment of Eutelsat satellites validated with telemedicine applications. The number of network interfaces can be expanded as necessary and standard interface can be modified. Thanks to these and other features of the system parameter and optimized real-time satellite monitoring and control, medical specialists- users can be confident that here is high quality servicing (maintenance), speed and reliability of information.

Feedback is very relevant to the use of each of these technologies. Between seeing patients live and interactive service conformity diagnosis is about 80%. This is the same percentage as if two specialists see the same patient in a medical room. This percentage increases to 90% if the specialists put different diagnoses rather than a single diagnosis.

BREAST RECONSTRUCTION AFTER MASTECTOMY

Yolanda Zayakova, Ivailo Vajarov, Nikolai Pashaliev, Anton Stanev

Aim: We present our surgical experience and analyze the results as well as the appropriateness of the applied techniques for breast reconstruction.

Material – Method: During the period between 2010-2015, we performed 39 reconstructive breast surgeries. The surgical technique involve 16 one stage and 23 delayed procedures.

The treatments included the use of expanders, implants and pedicled flaps.

Results: The mean follow – up is 24 months. Few complications were observed: seroma (n=2); necrosis of the skin (n=2), infection (1).

Conclusions: Based on our clinical data we can conclude that each of the used reconstructive techniques can lead to a good aesthetic outcome as long as the type of mastectomy and the individual anatomical features are taken into account.

SURGERY OF SCALP DEFECTS BASED ON LOCAL FLAPS AND EXPANSION

Yolanda Zayakova, Ivailo Vajarov, Nikolai Pashaliev, Anton Stanev

Aim: The purpose of this article is to present the use of local flaps and tissue expansion for covering simple and composite head defects.

Material – Method: Over the last five years we treated 25 patients aged 35-78, with scalp defects caused by burn, basal cell carcinoma, melanoma, and trauma. The wound size varied from 5x7 cm to 25x14cm. The surgeries were either one stage or two-stage depending on the need for tissue expansion. In the cases of composite defects we applied titanium mesh plate, while the simple ones required only flap coverage.

Results: The results were favorable with all patients. The flaps survived entirely and the recovery was comparatively quick.

Conclusions: The described techniques of surgical reconstruction are recommended for treating large scalp defects which may be either simple or composite. The methods provide healthy, stable, hair-bearing tissue and good aesthetic result.

ARMED FORCES IN MENTAL HEALTH MANAGEMENT OF TRAUMATIZED COMMUNITIES

Andreadaki Evangelia

Purpose: The aim of this paper is to provide with rough outline for managing psychological trauma in afflicted civil communities from the military point of view.

Material and Methods: An extensive literature review was conducted using the Medline database with the help of PubMed interface. The key words used for this purpose were: “mental trauma”, “army”, “community”, ”recovery”. Abstracts for conferences and papers written in a language other than English were excluded. A total of thirty six eligible articles were identified.

Results: Massive traumas are so disruptive that the forces of disintegration may overwhelm society’s restorative capacity transposing in the individual with display of a gamut of pathogenic mental manifestations. Military personnel, either as traumatized community members themselves either as response and mitigation forces, pose a key pillar in the healing process. Establishing a sense of security, deconditioning fear, catalyzing social solidarity, elaborating screening and outreach intervention programs, constructing a spectrum of specialized modalities and stratified therapeutic initiatives for psychiatric sequelae are only fragments of their integrated involvement after a calamity has taken place. The cadre of specialized mental health team approach emerges as a focal point to elicit a proactive seamless readiness-rehabilitation plan for all stakeholders.

Conclusions: In the therapeutic milieu after a massive trauma, no evidence based directives exist, only compilation of broad range norms to facilitate military actors involved, alleviate massive suffering. Army corps can undisputable undertake the ombudsmen role in reinstating sustainability, enhance psychological and physical societal serenity and provide with collaborative civil-army recovery matrices.

EVALUATING QUALITY OF LIFE AND JOB STRAIN IN HELLENIC AIR FORCE NURSE CORPS UNDER THE RESTRAINS OF CAPITAL CONTROL ENFORCEMENT

Andreadaki Evangelia

Purpose: The aim of this study is to assess Quality of Life (QOL), Occupational Stress (OS) and their association with organizational and financial variables in Hellenic Air Force (HAF) Nursing Personnel, in the unprecedented context of economic recession and capital control imposition.

Material and Methods: A total of 89 HAF Nurses participated in the study. The survey included the Greek versions of WHO QOL Questionnaire (WHOQOL-BREF) and Effort-Reward Imbalance (ERI) Questionnaire. Ceteris paribus, results were paired with the outcomes of a base line study conducted two years earlier, in the same population sample. Statistical analysis was performed using SPSS v.18.0.

Results: Degradation in psychological health, albeit the promotion of perceived job security, is documented. Health status remained highly sufficient, although one out of four participants face obstructive musculoskeletal problems or mood disorders. Receiving financial aid by a third party, shouldering bank loans, confronting health deficits and having offsprings independently predicted elevated OS along with impaired QOL attributes.

Conclusions: In face of the challenges posed by the current adverse socioeconomic framework, HAF Nurse Corps designate as a bastion against an imminent health crisis, serving their duty in high morale while providing first-line quality care for the armed forces and their beneficiaries. Policy makers and military leaders should take initiatives to engage in a transformational, more rewarding institutional climate as well as devise countermeasures to mitigate the escalation of nursing shortage. Evincing a more person focused leadership can reinforce resilience in military staff and leverage strong esprit de corps to full potential.

FEELINGS OF ANXIETY AND STRESS: WHICH IS THE RELATIONSHIP TO TIME?

Athanasiadou Foteini, Papastamatelou Julie, Unger Alexander, Giotakos Orestis

Aim: We tested the relationship between the dimensions of the Zimbardo Time Perspective Inventory (ZTPI- Zimbardo & Boyd, 1999) and anxiety disorder / perceived stress, while also taking into account the Deviation of the Balanced Time Perspective (DBTP).

Methods: In study 1 clinicians of the Mental Health Centre of the Armed Forces Athens diagnosed anxiety in a number of male participants of the overall sample (n= 204). In addition, participants completed a validated Greek translation of the ZTPI. In study 2 a sample of students (n= 71) of Panteion University Athens completed the Greek translation of theZTPI (Anagnostopoulos&Griva, 2012)and the Greek version (Andreou, Alexopoulos, Lionis, Varvogli, Gnardellis, Chrousos, &Darviri, 2011) of the Perceived Stress Scale (PSS 14-item scale; Cohen, Kamarck, &Mermelstein, 1983). We hypothesized that the Past Negative, the Present Fatalistic and the Future ZTPI dimensions would be significantly associated with anxiety disorder and perceived stress.

Results: Study 1: Independent samples t-tests showed that Future orientation was related to non-anxiety disorder (t(202) = 2.07, p = .040). The DBTP was higher in persons with an anxiety disorder (t(202) = 2.46, p= 0.15). This result was confirmed by a binary logistical regression. Study 2: Independent samples t-tests revealed that the Past Negative (t(69) = -4.68, p = < .00) and the Present Fatalistic (t(69) = -3.38, p = .001) orientations correlated with high-perceived stress. Pearson's correlations and a linear regression confirmed the results. Furthermore, high-perceived stress was associated with a higher DBTP (t(69) = -2.37, p = .021). A linear regression confirmed this result.

Conclusions: The negative correlation between Future time perspective and anxiety could be attributed to the positive bias displayed by the items of the ZTPI Future scale. Further investigation is needed in this respect.In addition, the non-significant relationship between Past Negative and anxiety, as well as between Present Fatalistic and anxiety could be a result of the measurement tool we have used to assess anxiety. The high predictability of DBTP on anxiety and perceived level of stress can inform therapeutic interventions whichhave the aim of altering problematic time perspectives.

RELATIONSHIP OF STRESS, CANNABIS USE AND FIRST PSYCHOTIC EPISODE

Selakovic Mirjana, Kakavos Vasilis, Athanasiadou Foteini, Mitropoulos Georgios, Dikeos Dimitris, Giotakos Orestis

Aim: To check if the interaction between cannabis use and stress induced by recruitment to the army is related to the emergence of first psychotic episode (FPE), we compared data on cannabis use between two groups of male patients, with FPE, a group of newly recruited soldiers (N=20) vs. a group of patients from public hospitals (N=20). All patients were of the same age (18-29 y.o.).

Methods: Cannabis use and its age of onset were assessed by taking of personal history. The use was considered as heavy frequent/when it occurred more than 50 times a year. Two groups were considered based on onset before or after 18 y.o.

Current status clinical examination provided information on basic clinical characteristics of psychotic episode including presence of any delusions, bizarre delusions, agitation/ aggression, withdrawal, suicidal ideation. Statistical analysis was based on chi-square tests; significance was set at 0.05.

Results: 9/20 newly recruited patients with FPE reported cannabis use (which for 6 was heavy/frequent) vs. 13/20 general hospital patients with FPE (which for all was heavy/frequent). The difference between the two groups was statistically significant (p=0,025) regarding use being more often heavy/ frequent among general hospital patients.

Delusions (p=0,025) and suicidal ideation (p=0,035) were more frequent among recruits than among general hospital patients.Agitation/aggression was associated with a history of cannabis use (p=0,018). Bizarre delusions seemed also to be more frequent among cannabis users (they were reported 5/22 cannabis users vs.1/18 non-users), but the difference was not statistically significant (p=0,130).

Conclusions: Cannabis use frequency does not differ between general hospital patients with FPE and army recruits with FPE; there is an indication that it is more frequent and heavy among the former. Our hypothesis, that the interaction of being recruited to the army and cannabis use has a more negative impact on the onset of FPE than each one by itself is not supported. Stresses due to recruitment and cannabis use do not seem to interact in producing a strongernegative impact on the onset of FPE than the impact that each condition has by itself.

Delusion and suicidal ideation are more frequent in the group of recruits vs. general hospital FPE patients, probably because of the inability of recruits to adapt quickly to a stress life situation, such as the service in army. Suicidal ideation is more frequent among army recruits who were substance users.

Cannabis use seems to influence the kind and frequency of FPE symptoms.

SOCIOECONOMIC FACTORS AND RELATED PARAMETERS IN HELLENIC ARMY STAFF

Athanasiadou Fotini, Voudiclaris Nectarios, Shmanthrakhs Dimitrios, Stasini Aikaterini, Stavridou Androniki, Giotakos Orestis

Introduction: Studies show that socio-economic factors such as economic recession, declining wages, debt or disruption of family life, which found in conditions of economic recession, seems to be related to the emergence of psychopathology (Economou et al., 2012).

Aim: The present study was designed to investigate the related parameters between socioeconomic factors and psychopathology occurrence in soldiers and military officers.

Material and Method: The study was conducted in the Military Hospital for Special Diseases 414 (SNEN). The survey included 663 army soldiers and officers, who visited the outpatient psychiatry and psychiatric clinic of 414 SNEN, 94.5% men and 5.5% women, with a higher percentage in the ages 18-44 years, during January and September 2015. The 633 participants were assessed through a completion of the Pre-Assessment Critical Indices Screening Questionnaire, PA-CISQ (Triantafylou & Giotakos), after having been informed that their participation was voluntary and anonymous.

Results: After analyzing the variance in terms of monthly income, revealed that low income affects significantly the appearance of disorder schizophrenic spectrum, panic disorder, agoraphobia symptoms, generalized anxiety disorder, obsessive compulsive disorder, social phobia, suicidal ideation, the hypomanic symptoms, depressive disorder, the enactment, substance use, physical problems and eating disorders ($p < .001$), while it seems not to affect the appearance of alcoholism. The Pearson correlation analysis revealed a high degree of positive correlation between stress and life events with the index depression and low positive correlation with outstanding loans and debts

Conclusions: It appears that confidence in a supportive context (family, friends, colleagues, health services) has a positive effect in reducing incidence of all psychopathological behaviors. It has been observed that there is a direct proportional relationship between stress and depression, so every time there is an increase between stress and debts, respectively increases depression. The linear regression analysis revealed that the loan default and life events are powerful predictors of developing depression and anxiety. They provide 32% of the variability of the total score.

SUTTON NEVUS: A RARE LESION MIMICKING MELANOMA OR LYMPHOMA

Avraam Konstantinos, Zacharis Evangelos, Almpanis Zannis, Chaleplidis Nikolaos, Tsantopoulos Margaritis

Aim: To report a case of a Sutton nevus (also known as "Leukoderma acquisitum centrifugum," "Perinevoid vitiligo,"and “Halo nevus”) and to emphasize the importance of its correct diagnosis.

Material/Methods: A 27 years-old female patient with a disease-free medical history underwent a biopsy of the back skin in order to excise a round-shaped brownish mass 0.4 cm in the anatomic area for the past ten years. The surgical specimen was fixed in formalin, the samples were embedded in paraffin blocks and histologic sections were taken and stain with hematoxylin- eosin.

Results: Histopathologic examination revealed a nevus composed of residual melanocytes with heavy infiltration by lymphocytes and histiocytes that destroy pigment containing cells. Fibrosis did not observed.

These findings suggested the diagnosis of a Sutton nevus.

Conclusion: Sutton nevus represents an unusual benign type of nevus surrounded by zone of hypopigmented skin usually located at the back or less common at the head due to regression caused by cell mediated immunity, or less commonly humoral immunity or granulomatous inflammation. It is an uncommon lesion concerning 1% of population before adulthood with no racial or gender predilection and may be asssociated to Turner syndrome. Pathologists should be diagnose this entity with great caution and try not to misdiagnose it with lymphoma or melanoma.

LANGERHANS CELL HISTIOCYTOSIS: AN UNUSUAL AND RARE PRESENTATION OF ORAL MANIFESTATIONS (2 CASE REPORTS)

Bompou Panagiota, Simopoulou Vicky, Tselkas Orestis

Introduction: Langerhans cell histiocytosis (LCH) is an uncommon disease due to the abnormal proliferation of immature dendritic cells (histiocytes). The pathogenesis is yet unknown, although trauma, viral or toxic agents have been implicated. Manifestation of the disease in the oral mucosa and mandible is extremely rare, diagnosed by its specific macroscopic and microscopic appearance: rapidly increasing lesions with hard limits.

Aim: To emphasize the importance of accurate diagnosis of LCH in the further treatment of these patients. To present two extremely unusual occurrences of LCH in the oral cavity of two adult patients who were admitted to the Maxillofacial Surgical Department of the Military Hospital of Athens.

Materials and methodology: Two patients presented to the Maxillofacial Clinic with pain in the left mandible and teeth mobility in the area. Clinical examination was inconclusive. Appropriate investigations were carried out with full blood count, radiological imaging biopsy and histological analysis.

Results: LCH was diagnosed in both patients and was managed successfully with surgical removal. Through the examinations, lesions in the lungs were identified in the chest radiographs in both patients, a life-threating situation that was immediately referred to the respiratory physician.

Conclusions: The diagnosis of Langerhans cells histiocytosis is performed by a combination of clinical and histopathological examinations. The presentation of the disease exclusively in the oral cavity is a unique and relatively recently localized entity with unclear etiology, pathogenesis and treatment. The importance of differential diagnosis and thorough examination can lead not only to the diagnosis of LCH, but also to other unknown life-threatening situations.

THE PREVALENCE OF KNEE OSTEOARTHRITIS IN 100 ATHLETICALLY ACTIVE MILITARY PERSONNEL (AGE 35-55)

Paxinos Odysseas, Karavasili Alexandra, Delimpasis Georgios, Stathi Afroditi

Aim: To sonographically investigate the prevalence of knee osteoarthritis in a group of athletically active male military personnel.

Material – Method: A group of 100 athletically active Greek military personnel 35 to 55 years old (mean age 49.60 SD± 5.9) were examined with ultrasound for knee OA and they were administered the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire.

Results: The prevalence of OA in this group was 33%. Femoral cartilage thickness and altered knee biomechanics were not related with the prevalence of OA (p=0.740). Only Pain Score was increased in the self-administered KOOS questionnaire without any significant relation to the sonographic findings.

Conclusions: Diagnostic ultrasound is a valuable tool in detecting early knee OA lesions even before they get really symptomatic.

PHYSIOTHERAPY CENTRE IN A GREEK ARMY MARINE CORPS' BRIGADE

Pippas Christos, Arvanitakis Chrysostomos, Kolokotronis Vasileios, Basagiannis Christos, Zigras Filippos, Giannoglou Dimitrios

Aim: Assessment of a pilot physiotherapy centre in a Greek Marine Corps military unit.

Material – Methods:

1) Operating period: Two (2) months (9/7/2015-11/9/2015)

2) Material: Treatment table, electrotherapy equipment, taping equipment, elastic bandages, massage oil, cold therapy packs, treatment gel

3) Treatment methods: Massage, kinesiotherapy, functional restoration, ballistic/continuous/hold-relax stretching, sport rehabilitation, electrotherapy, cryotherapy, mobilization and manipulation of central and peripheral joints, traction, friction technique, ischemic pressure.

Results: Overall, fifty-five patients were treated (one citizen, forty soldiers, ten NCOs and four officers). Three of them were not subjected to therapy while twenty-two were referred to the hospital.

Sessions were held in the physiotherapy centre of 32 Marine Corps Brigade of Volos and patients had either musculoskeletal or neurological problems.

Injuries	Shoulder	Elbow	Cervical	Lumbar	Hip	Tibia	Knee	Ankle
Cases	5	2	6	11	2	1	25	3

The outcome was very positive, as all of the patients were relieved from at least one of their symptoms. Some of the benefits of the treatment were: functional recovery, range of motion improvement, pain reduction or deletion, reduction of muscle spasm.

Conclusions: Our results indicate that physiotherapy centers in large military units are useful as they prove to be an effective and reliable solution for musculoskeletal and neurological injuries' restoration, and help to save money and time.

NONSYSTEMIC AND SYSTEMIC VASCULITIC NEUROPATHY: A CLINICOPATHOLOGICAL STUDY OF 72 CASES

Bougea A, Giatas K, Gerakoulis E Papadimas P, Papadopoulos K, Anagnostou E, Kararizou E

Aim: Vasculitis of the peripheral nervous system (PNS) occurs rarely either in the context of systemic vasculitis or isolated (non-systemic vasculitic neuropathy - NSVN). This is the first large prospective study which aims to investigate the clinical, and pathological features of both systemic and nonsystemic vasculitic neuropathy in order to establish the clinical manifestations and to promote the earlier diagnosis of the syndrome.

Material – Method: Biopsies were selected from over 855 sural nerve biopsies performed at the Section of Neuropathology, Neurological Clinic of Athens University Hospital between 1985 and 2005 and were followed up until 2014. The diagnosis of vasculitis was based on established clinicopathological criteria. Complete laboratory, clinical, electrophysiological, and pathological studies were performed in all cases.

Results: Nerve biopsies of 22 (2.5 %) patients were diagnosed as NSVN. Systemic vasculitis (5.8%) included: 15 rheumatoid arthritis, 9 Churg-Strauss syndrome, 7 cryoglobulinemic vasculitis, 7 Systemic lupus erythematosus, 5 Sjogren disease, 3 polyarteritis nodosa, 2 Behcet's disease, 1 Crest Ankylosing spondylitis. The pathological features were vasculitis and predominant axonal degeneration with a varying pattern of myelinated fiber loss. The vasculitic changes were found mainly in small epineural blood vessels. Mononeuritis multiplex and distal symmetrical sensorimotor neuropathy were equally frequent.

Conclusions: Although less common than systemic vasculitis NSVN should be suspected in a case of unexplained polyneuropathy without evidence of systemic involvement. Clinical and neurophysiological studies are essential for the detection of nerve involvement, but the specific diagnosis of NSVN may be missed unless a biopsy is performed.

THE IMPACT OF PROSTATE CANCER ON THE PATIENT'S QUALITY OF LIFE. A HOLISTIC APPRAISAL OF DATA EXTRACTED FROM A TERTIARY CENTER

Malioris Ap, Gkekas Ch., Kalyvas Vas., Papathanasiou Mich

Aim: The physical consequences of prostate cancer are adequately studied and published.It is a simple task of observing and reporting the symptoms. Our purpose is to study beyond the symptom itself and assess the impact of the disease in general.

Materials and methods: We used the Esper's questionnaire validated and translated into Greek. It consists of five block of questions which holistically assess the physical, psychological and social implications of prostate cancer. The questionnaire was addressed to a total of 120 patients. 70 patients were allocated in the radical prostatectomy group, 30 patients were in the active surveillance group and 20 in the radiotherapy group.

Results: The answers were cross-checked. 83% of the patients in the radical prostatectomy group could live up to their family role spending the least time bed rested. On the other hand, those in the radiotherapy group objectively experienced much less support from friends with 50% of them reporting a "weak" support and the rest 50% "no support at all". This can barely compare to the 88.6% of those in the radical prostatectomy group feeling "enough" or even "very much" supported. The overall 6.7% of the 120 patients who reported "a little" or "not at all" in the family support question were all married.

Conclusion: Our conclusions implicate that even in patients physically suitable for a specific treatment there are additional variables that need to be addressed. The most impaired patients were those on radiotherapy tending to be the least physically active, the most pessimistic and the least satisfied to the treatment. This is to an extent objectively perceived as such due to the physical botherness that radiotherapy induces.

POTABA INDUCED DRESS SYNDROME. A RARE MEDICAL ENTITY IN THE UROLOGICAL PRACTICE

Kalyvas Vas, Gkekas Ch., Malioris Ap., Papathanasiou Mich.

Aim: DRESS SYNDROME (DRUG INDUCED EOSINOPHILIA WITH SYSTEMIC SYMPTOMS) is a rare drug induced hypersensitivity reaction initially linked to antiepileptic drus, sulfonamides and antibiotics. So far only six cases have been reported linking POTABA to DRESS. We present a case of a 45 year old man with Dress aiming to add to the growing body of evidence related to the disease.

Materials and methods: A 45 year old male suffering from Peyronie's disease was prescribed POTABA 9 gr/day. His disease appeared to be of early stage with fluctuating shaft angulation, pain and no calcifications. After 8 weeks off administration he developed fever and a generalized, itching, morbilliform ruch which gradually went diffuse with signs off jaundice.

Results: The patient admitted to the ER and examination revealed a diffuse erythema covering the trunk, upper and lower extremities equaling to more than 50% of BSA, peripheral eosinophilia , jaundice and liver injury (10 fold increase of liver enzymes). Serologic examination for Hep A, B, C viruses, CMV and EBV was negative. The patient's past history was unremarkable and a diagnosis of drug induced hypersensitivity reaction with solitary visceral involvement was established in the absence of other pathology. 9 weeks after discontinuation of POTABA he completely recovered.

Conclusion: POTABA induced DRESS syndrome is a drug related complication which is not due to a direct cytotoxic effect of the drug, which is known to happen occasionally, but an antigenic property of the PABA moiety of the drug which triggers a systemic autoimmune response. This response could involve several solid organ and has up to 5 - 10% mortality.

SUBINGUINAL VARICOCELECTOMY WITH THE USE OF INTRAOPERATIVE MINI DOPPLER. A RELIABLE ALTERNATINE TO MICROSURGERY

Gkekas Ch., Malioris Ap., Kalyvas Vas., Papathanasiou Mich.

Aim: The gold standard for the operative treatment of varicocele is the microsurgical subinguinalvaricocelectomy which spares the testicular artery and lymph vessels. Nevertheless it requires a surgical microscope which is not always available. We present a feasible alternative with the use of a mini Doppler stylus device overcoming the drawback of increased cost.

Materials and methods: Between January and December 2014, 20 patients underwent subinguinalvaricocelectomy with the use of a 12MHz mini Doppler (HadecoMinidop). The spermatic cord was exposed through a 1.5-2cm subinguinal incision and the pampiniform plexus degloved from its fascial layer. With the use of the mini Doppler the testicular artery was initially recognized and spared. The small venous branches of the pampiniform plexus were individually ligated and after every step the testicular artery was checked for pulsation with the mini Doppler. In 9 patients local instillations of papaverine were additionaly applied. By combining the use of mini Doppler to the classic Marmarvaricocelectomy we enjoyed the confidence and safety that only microsurgery can offer. Upon completion of the operation the testicular artery was finally checked along with the ipsilateral testis for pulsations.

Results: Mean operative time was 48min+ 8 mins. No major perioperative complications occurred. The follow up period is 12 months and we had no long term complications, namely testicular atrophy. We had 2 cases of persistent – recurrent varicocele at 12 months and 1 case of hydrocele.

Conclusion: The intraoperative use of mini Doppler offers a significant advantage during subinguinalvaricocelectomy and equals in terms of safety the liability of microsurgery. It is a reasonable alternative in cases where the operative microscope is not available.

BRUCELLA SPECIES MISINDENTIFIED AS BERGEYELLA ZOOHELCUM

Karapsias Stergios, Sgourou Agathi

Aim: To report misidentification of Brucella species as Bergeyella zoohelcum by a common bacterial identification system.

Material and Methods: In 2013, three patients with symptoms of brucellosis (fatigue, fever >39oC, painful joints of hands, knees and spine) were admitted to pathological wards of 251 General Air Force Hospital, Athens, Greece. Three sets of blood culture samples were taken from each patient. All blood samples turned out to be positive within 48 hours of incubation (Bactec, Becton Dickinson) and then were inoculated onto Petri dishes. Microbe colonies were observed only onto blood and chocolate agar dishes. Gram stain, oxidase testing, brucella agglutinating methods and biochemical identification of bacterial strains followed.

Results: All isolates were oxidase-positive Gram negative bacteria and revealed positive reactions when tested with brucella agglutinating sera (Remel), both M (Brucella melitensis, title: 1/1280) and A (Brucella abortus, title: 1/40). Wright serum tests were positive in all three patients (positive titles: 1/640 up to 1/5120). Yet, all strains were biochemically identified as Bergeyella zoohelcum (MicroScan WalkAway, Siemens). Diagnosis of brucellosis (Brucella melitensis) was established upon clinical evidence and laboratory results, without taking into account Bergeyella zoohelcum identification. Patients were treated with brucellosis antibiotic therapy (rifampicin, tetracycline, gentamycin). Six months later, they fully recovered from illness.

Conclusions: Brucella species may be misidentified as Bergeyella zoohelcum using MicroScan WalkAway. Thus, diagnosis of brucellosis should be based upon clinical evidence and basic laboratory results such as Wright test, positive blood cultures and evaluation of colonies (Gram stain, oxidase test, brucella agglutinating sera).

INCIDENCE OF MULTIDRUG - RESISTANT RALSTONIA PICKETTII

Karapsias Stergios, Sgourou Agathi

Aim: To observe the occurrence of Ralstonia pickettii, a non-fermenting opportunistic Gram negative bacterium, from clinical samples.

Material and Methods: R. pickettii had been investigated at 251 General Air Force Hospital, Athens, Greece for three years (2012-2014). All strains, detected among specimens of hospitalized patients, were registered. Biochemical identification and susceptibility testing of isolates followed (MicroScan WalkAway, Siemens).

Results: Three R. pickettii stains were isolated from blood cultures within a three-month period (March to May, 2013). R.pickettii antibiotic susceptibility proved to be almost the same in all strains (Table1).

Table 1. Antibiotic susceptibility of R. pickettii strains.

Antibiotic agent	Strain 1	Strain 2	Strain 3	Susceptibility
Amikacin	R	R	R	100% R
Aztreonam	R	R	R	100% R
Ceftazidime	R	R	R	100% R
Ciprofloxacin	S	S	S	100% S
Colistin	R	R	R	100% R
Gentamycin	R	R	R	100% R
Imipenem	I	I	I	0% S
Levofloxacin	S	S	S	100% S
Meropenem	I	R	I	0% S
Piperacillin/ tazobactam	S	S	S	100% S
Ticarcillin	R	R	R	100% R
Tigecycline	S	S	S	100% S
Trimethoprim/ Sulfamethoxazole	S	S	S	100% S

S=Sensitive, I=Intermediate, R=Resistant.

Conclusions: Multidrug-resistance, including aminoglycosides, aztreonam, carbapenems and colistin, rises as a problem in therapeutics of R.pickettii. Thus, R.pickettii should be treated on the basis of susceptibility testing.

A META-ANALYSIS OF STAPHYLOCOCCUS RESISTANCE CONCERNING METHICILLIN, ERYTHROMYCIN AND CLINDAMYCIN

Karapsias Stergios, Sgourou Agathi

Aim: To evaluate the epidemiological profile of staphylococcal infections concerning methicillin, erythromycin and clindamycin resistance during a 13-year period.

Material and Methods: The study comprised all Staphylococcus strains derived among the inpatients of 251 General Air Force Hospital in Athens, Greece, from January 1995 to December 2007. Staphylococcus aureus and Coagulase Negative Staphylococci (CoNS) had been isolated from all clinical specimens. Oxacillin was used to confirm methicillin-resistance, which represents resistance against β-lactamic agents. Statistical process of data was executed using x2 criterion.

Results: A sum of 2570 strains was registered during 13 years. The majority of 1688 strains were identified as CoNS (65.68%) while the remainder of 882 strains was defined as S. aureus (34.32%). Mean annual resistance-percentages of S. aureus concerning methicillin, erythromycin and clindamycin were calculated as 37.15% (C.I. 95%, 30.65% - 43.65%, Methicillin Resistant S. aureus, MRSA), 36.15% (C.I. 95%, 30.57% - 41.74%) and 30.85% (C.I. 95%, 23.92% - 37.78%), respectively. CoNS mean annual resistance-percentages concerning methicillin, erythromycin and clindamycin were estimated as 65.69% (C.I. 95%, 61.20% - 70.18%, Methicillin Resistance CoNS, MR-CoNS), 72.62% (C.I. 95%, 67.16% - 78.07%) and 61.23% (C.I. 95%, 55.52% - 66.95%), respectively. Methicillin, erythromycin and clindamycin staphylococcal resistances were proved to be statistically associated with CoNS ($p<0.001$).

Conclusions: The study confirms recent reports suggesting CoNS to be a genetical tank responsible for the enrichment of S.aureus with pathogenetic abilities such as antibiotic resistance. Thus, S.aureus and CoNS may share parallel evolutionary pathways by exchanging resistance genes concerning β-lactamic agents (mecA), macrolides and lincosamides (iMLSB).

MRSA RESISTANCE CONCERNING VANCOMYCIN, TEICOPLANIN, DAPTOMYCIN AND LINEZOLID

Karapsias Stergios, Sgourou Agathi

Aim: To report methicillin-resistant Staphylococcus aureus (MRSA) resistance against vancomycin, teicoplanin, daptomycin and linezolid.

Material and Methods: The study took place at 251 General Air Force Hospital in Athens, Greece, where MRSA strains were registered within a three-year period (2011-2013). All clinical specimens had been under investigation. MRSA isolates were certified via biochemical identification and susceptibility testing (MicroScan WalkAway, Siemens). Statistical process of data was executed using x^2 criterion.

Results: A sum of 82 MRSA strains was isolated. Resistance levels of MRSA against selected antibiotics were estimated as: 0% teicoplanin, 0% vancomycin (7.32% intermediate), 0% daptomycin (8.54% intermediate) and 3.66% linezolid. MRSA susceptibility phenotypes are exhibited in Table 1.

Table 1. MRSA susceptibility phenotypes.

Vancomycin MIC (μg/ml)	Teicoplanin MIC (μg/ml)	Daptomycin MIC (μg/ml)	Linezolid MIC (μg/ml)	Sum of strains (%)
<1 S	≤1 S	≤1 S	≤2 S	34 (41.46)
<1 S	≤1 S	4 I	>4 R	1 (1.22)
2 S	≤1 S	≤1 S	≤2 S	37 (45.12)
2 S	≤1 S	4 I	≤2 S	3 (3.66)
2 S	≤1 S	4 I	>4 R	1 (1.22)
4 I	≤1 S	≤1 S	4 S	4 (4.88)
4 I	2 S	4 I	4 S	1 (1.22)
4 I	2 S	4 I	>4 R	1 (1.22)
S=Sensitive, I=Intermediate, R=Resistant.				82 (100)

Conclusions: Half MRSA strains (41/82) presented elevated MIC levels concerning vancomycin (2 μg/ml). No resistance to teicoplanin was revealed. Linezolid-resistance proved to be statistically associated with daptomycin-intermediate resistance (p<0.001).

PERIOPERATIVE HEALTH PROFESSIONALS' ATTITUDE TOWARDS MANAGEMENT IN OPERATING ROOM

Konstantinia Karathanasi, Panagiotis Prezerakos, Maria Malliarou, Ioannis Koutelekos, Paulos Sarafis

Aim: The aim of this study is to investigate perioperative health professionals' attitude towards surgical suite management.

Material – Method: The study was conducted on a convenience sample of health professionals working in OR environment. For the collection of data an anonymous self-completed questionnaire was developed in order to collect information about current operating room management as well as opinions and expectations about OR Management. Statistical analysis was carried out with SPSS 20 statistical package.

Results: 1615 questionnaires were issued, of which 982 were completed (r.r. = 60.8%) from 40 hospitals with minimum of 5 operating rooms (September-November 2014). 69.8% of the respondents believed that a master in management is an essential qualification for the OR Manager since 72% of his/her most important responsibilities is improving the quality of care. 78.4% also believed that OR Manager should have an authority over the perioperative healthcare professionals in order to successfully run the OR. Over 4/5 of the sample agreed that an OR Manager would definitely positively contribute to better organization (92.5%), efficacy (90.2%), functioning (85.4%) and also in better collaboration between OR stuff (88.4%). %). There was also a different approach between doctors and nurses about who should be responsible for the daily management while there was a positive correlation of OR Manager increased perception score to the reduced rating of the OR functioning (b=_0.05, p=003).

Conclusions: Successful management of the OR requires an OR Manager in an organizational structure, with effective and important role. The specialty or profession is of secondary importance.

EFFICACY AND SAFETY OF TOCILIZUMAB IN PATIENTS WITH RHEUMATOID ARTHRITIS

Panagopoulos Panagiotis, Katsifis Gkikas

Purpose: Tocilizumab (TCZ), a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R), has been approved for the treatment of rheumatoid arthritis (RA). The aim of this study was to evaluate the safety and efficacy of intravenous tocilizumab in every day clinical practice.

Methods: Patients were prospectively monitored for disease activity, physical function and adverse events (AEs). ACR 20, 50 and 70 responses, Disease Activity Score 28 (DAS28) and physical function (Health Assessment Questionnaire, HAQ) were evaluated at 24 months. Safety was assessed at each scheduled visit day.

Results: Twenty-three (26) patients with active RA received

TCZ 8 mg/kg every 4weeks. Baseline characteristics were: age: 56.5±2.4 years, disease duration:14.2±2.1 years, HAQ:1.8±0.1, DAS28:6.1± 0.5, CRP:17.5± 6.0, swollen joints:12.1±2.4 and tender joints:10.4±1.8. About 63% of patients had received biologic drug(s) prior to TCZ. Methotrexate (MTX) and corticosteroids were used concomitantly in 85.0% and 70.0% of patients, respectively. At 12 months ACR 20, ACR 50 and ACR 70 responses were 64%, 28% and 20%, respectively. 18% of the patients achieved DAS 28-defined remission (DAS28<2.6) and 65% clinically meaningful HAQ responses (reduction of ≥0.3 units). Tocilizumab was generally safe and well tolerated. The most common adverse events were infections of the respiratory and urogenital tract.

Conclusion: Tocilizumab induced clinically relevant improvement in disease activity and physical function and had acceptable safety over 24 months in patients with an inadequate response to DMARDs and/or anti-TNFα therapy. Treatment with tocilizumab is an effective option in clinical practice.

THE CURRENT TRENDS IN OPHTHALMIC OPERATIONS - A 2015 REVIEW

Klados Nektarios, Kourkoutas Dimitrios, Georgiou Iordanis, Xanthopoulou Paraskevi, Tsartsara Aikaterini, Lygeros Michail-Nikolaos

Aim: The purpose of this study was to observe trends in ophthalmic operations performed during the period of January to December 2015.

Material-Methods: Our Clinic’s special database was analysed.

Results: The vast majority of ophthalmic operations were phacoemulsification with PC-IOL implantation. Oculoplastic surgeries were the next most common group followed by glaucoma operations.

Conclusion: Phacoemulsification remains the most common ophthalmic operation in our Clinic.

EPIDEMIOLOGY OF AMETROPIA OF GREEK ARMY RECRUITS

Klados Nektarios, Kourkoutas Dimitrios, Georgiou Iordanis, Xanthopoulou Paraskevi, Lygeros Michail-Nikolaos

Aim: To determine the prevalence of low and mid-level refractive errors in Greek military conscripts with perfect vision.

Material - Methods: A retrospective review of ophthalmic examinations for entry to the military service was performed. The health examination data of the candidates that are saved in the Informatics Research Office of 401 General Army Hospital of Athens was used. The data of the candidates examined between 1 January 2015 and 31 December 2015 were evaluated. The total number of candidates was 2467. The ametropic recruits with perfect vision were 1572.

Results: The retrospective epidemiological evaluation revealed that myopia or myopic astigmatism lower than 4.50 DSE was the most frequent refractive error counted participants (71%). Ametropia between 4.50 and 8.00 DSE was the next most common diagnosis counted 396 participants (25%) whereas ametropia 8.00-12.00 DSE counted 64 participants (4%).

Conclusion: The vast majority of ametropic recruits with perfect vision are simple myopes or myopic astigmats.

ULTRASTRUCTURAL ALTERNATIONS IN THE FEMORAL NERVE IN WISTAR RATS AFTER ALENDRONATE ADMINISTRATION PER OS

Kymioni Vasiliki-Maria, Gogadis Aristeidis , Dietrich Eva-Maria, Papamitsoy Theodora, Sioga Antonia, Koimitzis Georgios, Neloum Esthelle

PURPOSE: The femoral nerve inflammation is frequently associated with an underlying malignancy or infections. Bisphosphonate (BP) uptake has been recently identified as a new etiology for the syndrome. The mechanism that lies behind this neuropathy and the involvement of BPs has not been elucidated, yet. We hypothesize that BPs, taken per os, are capable of producing degenerative changes to the femoral nerve.

MATERIALS: 15 female Wistar rats, 12-month old, weighing approximately 500 gr., were used in the experiment.

METHOD: The animals were randomly allocated into two groups: Group A, the experimental group that consisted of 10 animals that were given Alendronate per os and Group B, the control group that also consisted of 5 animals, which were given normal saline. The duration was 13 weeks. The samples were observed under a Transmission Electron Microscope.

RESULTS: Degenerative changes were defined as:

vacuolization of the myelin sheath, detachment of the axon, local thickening and/or disruption of the myelin sheath. Local myelin thickening and detachment of the axon was found in both groups. Restricted areas of disorganization of myelin with tendency to protrude in the inner layers were also noticed. Curves of sheath were also formed. Myelin also invade in the axons.

CONCLUSIONS: Possible pathophysiological mechanisms causatively related to the histological alterations are: 1. alterations to the blood vessels of the nerve, 2. toxicity effects on Schwann and neuronal cells, or 3. changes to the innate or acquired immunity. The last may, probably, result from the presence of microbial films that cause cytokine production. The hypothesis provides scientific evidence for the presence of degenerative changes after BPs administration and helps in understanding of possible pathophysiological mechanisms.

ANXIETY OF GREEK ARMED FORCES RETIRED PERSONNEL. A RESEARCH STUDY

Maria Malliarou, Kypraiou, Pavlos Sarafis, Evaggelia Kotrotsiou

Aim: Retirement is a turning point in human life, a loss with practical and symbolic dimensions. The aim of this study was to identify anxiety levels of Greek Armed Forces retired personnel.

Material – Method: Participants were 507 retired Greek Armed forces personnel (93.7% males and 5.3% females). Anxiety symptomatology was measured using Trait Anxiety Inventory while a section with demographics was also included. The State-Trait Anxiety Inventory (STAI) consists of 40 self-report items pertaining to anxiety affect and distinguishes between a person’s state and trait anxiety levels. The A-Trait and A-State scales comprise 20 items each, scored on a 4-point Likert-type response scale. Scores range from 20 to 80, with higher scores suggesting greater levels of anxiety. Low scores suggest mild anxiety, median scores suggest moderate anxiety, while high scores suggest severe anxiety.

Results: Male 94.6%, Female 5.4% with an average retirement age of 49.1 years old. Retirement led the 81.3% of respondents to a significant loss of family income which affected their quality of life of 79.3% of those. Retirement decision caused by unfriendly or stressful working environment associated with significantly more symptoms of anxiety. Career satisfaction, satisfactory lifestyle change and keeping fit are connected to lower scores on the scale of anxiety. Inclusion of new activities, support from the family environment and good physical and mental health were related to less both temporary and permanent stress. Systematic alcohol consumption, obesity and health problems were associated with more anxiety symptoms.

Conclusions: The stressful working conditions, family problems, diseases, high body mass index (BMI), frequent alcohol consumption, smoking and dissatisfaction related to lifestyle changes have statistically significant effect on symptoms of anxiety. Military retirees can benefit with higher retirement satisfaction and better adjustment to civilian life if a retirement planning took place.

DEPRESSION AND QUALITY OF LIFE OF GREEK ARMED FORCES RETIRED PERSONNEL

Maria Malliarou, Kypraiou, Sophia Zyga, Maria Tsironi, Pavlos Sarafis

Aim: The aim of this study was to measure quality of life of Greek Armed Forces retired personnel.

Material – Method: Participants were 507 retired Greek Armed forces personnel (93.7% males and 5.3% females). Sf-12 questionnaire was used to measure quality of life, CES-D scale was used to measure depressive symptomatology, while a section with demographics was also included. Sf-12 questionnaire uses just 12 questions that are summarized into two summary scores, the Mental Component Summary (MCS) and Physical Component Summary (PCS) scales. The two scores range between 0 and 100, with increasing values equating to better health. Participant with total score of 16 or higher in CES –D scale is considered depressed.

Results: Male 94.6%, Female 5.4% with an average retirement age of 49.1 years old. Half of the participants who smoked maintained smoking habit while 77.8% of participants who consumed alcohol regularly maintained habit after retirement. 64.0% of participants maintained at a same level using health services after retirement. 38.6% of veterans did nothing to prepare for retirement. Retirement led the 81.3% of respondents to a significant loss of family income which affected their quality of life of 79.3% of those. Means scores for the PCS and MCS scales have been found to be 54.43 (SD=7.35) and 50.50 (SD=9.54). Mean value of CES – D was found to be 8.54 (SD=9.09). There was a significant negative correlation between CES – D and quality of life dimensions. After multivariate linear regression analysis, having as dependent depression scores of participants and as independent demographic and working factors and the scores on dimensions of quality of life it was found that stressful working conditions as a reason for retirement, veteran's satisfaction by their lifestyle change and PCS and MCS scales were found to be independently associated with depression scores.

Conclusions: Better physical and mental health and supportive family environment can be associated with better quality of life and lower levels of depression.

GREEK MILITARY REGISTERED NURSES' CULTURAL COMPETENCE

Aikaterini Oikonomou, Stella Nika, Maria Malliarou

Aim: The purpose of this study is to investigate the cultural competence of the military nursing personnel in Greece.

Material-method: The study is a descriptive cross-sectional study with synchronical design. The sample of the study consisted of 127 military registered nurses (RR: 98%) who completed an anonymous questionnaire. The tool used in the study was «Transcultural Self-Efficacy Tool (TSET)» of Marianne R. Jeffreys (2006) and it validated in Greek culture by Sarafis et al. Transcultural Self-Efficacy Tool (TSET) was designed as a diagnostic tool to measure and evaluate nurses' transcultural self-efficacy perceptions for performing general transcultural nursing skills among diverse client populations. The statistical analysis was processed through the Statistical Package of Social Sciences, SPSS 17.0.

Results: Reliability analysis of cultural knowledge, understanding, skills/competences was found measuring Cronbach's a>0.98. 81.1% of military nurses were female and the 43.3% of the sample was 41-50 years old. 14.2% had participated in postgraduate programs of abroad and 18.1% had worked abroad. 32.3% of military RN's possess a Master degree. 7.1% reported "every day" contact with patients of different culture, while the 66.1% reported "sometimes per year". Those who had studied and worked abroad showed greater levels of cultural knowledge and skills respectively, while officer nurses who had been trained in transcultural nursing showed greater levels of understanding the need for taking in mind different cultural aspects in care. Those, who seem to be culturally sensitive and consider cultural competence important, seem to have more cultural skills.

Conclusions: Improving military nurses' cultural competence could improve quality of health care and patient's satisfaction and contribute to positive health outcomes.

PROS AND CONS OF USING DRG'S TO CUT HOSPITAL COSTS

Maria Malliarou, Eirini Tzourtzoukli, Konstantinia Karathanasi, Panagiotis Prezerakos

Aim: A modern compensation mechanism and control of hospital costs, last decades used in modern health systems with individual variations, is the funding under similar diagnostic groups (Diagnosis Related Groups, DRGs). The aim of this study was to show pros and cons of using DRGs in the process of trying to cut hospital costs.

Material – Method: Literature review was conducted on Pubmed Google Scholar and for articles (10 years old) about the theme using as key words: nursing, DRG's, cost.

Results: Diagnosis Related Groups (DRG) is a system classifying in-hospital patient cases into categories with similar resource use. The grouping is based on diagnoses, procedures performed, age, sex and status at discharge. In European countries using DRG's it is believed that DRGs have helped with homogenizing care procedures and have improved inpatient care organization however it is challenging to integrate in the payment system an implicit set of clinical guidelines defining how to treat a homogeneous group of patients. There is evidence that the cost containment pressure created by the introduction of DRG-based payment in USA could have an adverse impact on patient outcomes in terms of readmission and mortality rates. Different patient groups can also experience various impacts, depending on the price incentives provided by different DRGs.

Conclusions: DRGs tend to promote cost transparency, and make it easier to determine which patients are likely to use more or less healthcare resources; however the pressure for cost-containment created by the DRG-based payment system can also adversely affect care quality. The pressure for efficiency introduced by DRG-based payment systems might help to improve organization of care, accelerate the adoption of technology, and hence improve quality or if not Particular attention is paid to ensure that high-severity groups are adequately accounted for in the DRG system, quality of care will be affected for these patients.

THE ROLE OF OXIDATIVE STRESS ON AMYLOID-LIKE PROTEIN FORMATION AND AORTIC VALVE

CALCIFICATION

Mamarelis Ioannis, Koutoulakis Emmanouil, Kotoulas Christophoros, Mamareli Vasiliki, Dritsa Vasiliki, Anastassopoulou Jane

Background: Calcification of aortic valve is a common abnormality leading to heart valve replacement, but the mechanism of calcium salts and fibril formation is not clear. The purpose of the present research is to investigate the mechanism and the role of amyloid-like protein formation on aortic valve calcification and stenosis during oxidative stress.

Methodology: Fourier Transform Infrared (FT-IR) spectroscopy and Scanning Electron Microscopy (SEM) were used to study the molecular structure of 54 calcified aortic valves from patients of 38-83 years old, presented with severe aortic stenosis and underwent aortic valve replacement under cardiopulmonary bypass.

Results: The high intensity bands of FT-IR in the region 3000-2800 cm-1, arising from the stretching vibration bands of vCH3 and vCH2 groups of membranes' lipids and phospholipids are related with increasing of lipophilic environment due to amyloid-like protein formation. From the shifts to lower frequencies of the characteristic absorption bands of Amide I and Amide II, it is resulting that the proteins do change their secondary structure from α-helix to β-parallel and β-antiparallel sheets confirming the amyloid formation. SEM was used to evaluate the morphology and architecture of the sample surfaces. The formation of low biological hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and $CaHPO_4$ was supported by XRD analysis.

Conclusion: The data showed that oxidative stress is one of the pathways of amyloid-like protein formation leading to aortic valve calcification mainly on the disulfide (S-S) cross-linking or branched sites of proteins. Moreover, SEM-EDAX and XRD data show substitution of Ca^{2+}cations by Mg^{2+}cations is leading to amorphous hydroxyapatite formation, preventing, thus, the aortic valve stenosis. It also suggests that treatment with magnesium salts maybe could avoid the calcification of aortic valves.

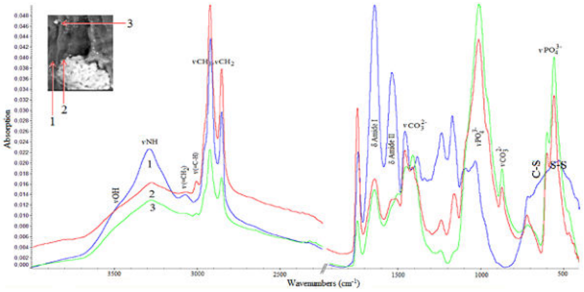

Figure 3. Representative Fourier transform infrared spectra of an aortic valve taken from the spots. 1, organic phase, 2 interface between organic and mineral phase and 3 individual mineral deposit.

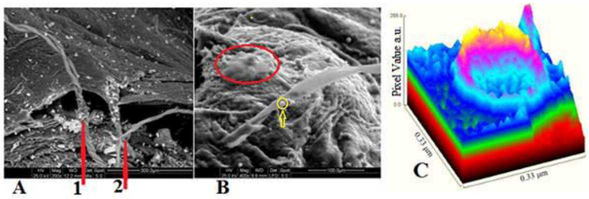

Figure 2. Scanning electron microscope images of morphology and architecture of calcified aortic valve. A; general morphology with minerals and fibrils. The arrows 1 and 2 show the fibril and protein branch-polymerization, respectively. (Scale 300 µm, M200x), B; Clear view of misfolding proteins and aggregates. The red circle outlines the membrane inflammation. (Scale 100 µm, M400x). C; ImagJ analysis of an aggregate (yellow circle in B), showing the barrel configuration

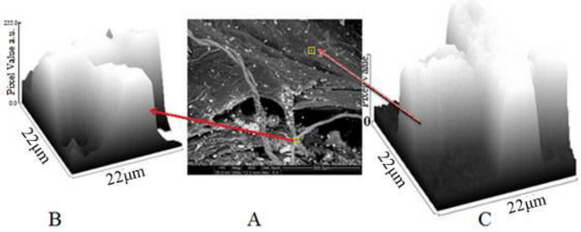

Figure 3. A; Scanning electron microscope morphology of calcified aortic valve. B; ImagJ analysis of branched proteins and C; ImagJ analysis of cross-linked proteins

DISEASES IN THE STAFF OF HELLENIC NAVY

Mastrokostopoulos Antonios, Roumpi Aikaterini, Bounas Pavlos, Sampani Anthi, Rigas Grigorios, Tsoukalos Grigorios, Kapiri Elpiniki, Emmanouil Georgios, Lampadakis Ioannis, Panagiotakos Demosthenes, Toutouzas Konstantinos, Kasotakis Emmanouil, Katsimagklis Georgios

Aim: To reduce the burden of cardiovascular disease in the staff of Hellenic Navy, to identify new prognostic factors for cardiovascular diseases, to improve the quality of life in the staff of Hellenic Navy.

Materials – Methods: Our study population consists of all the naval personnel enlisted from 2015. After completing a consent form the subjects are evaluated in our Hospital and the following tests are performed:

1. Clinical examination
2. Questionnaire concerning medical history
3. Questionnaire for dietary habits
4. Zung DRS and STAI Questionnaire
5. IPAQ questionnaire for physical activity
6. Blood sample for testing
7. Carotid IMT
8. Carotid Microwave Radiometry (MR)

It is an ongoing study. We will every year enroll the new enlisted personnel, and we will have a re-evaluation every 5 years for a period of 15 years.

Results: We are still processing our data and we are willing to publish our data during the congress.

Conclusions: It is the first study in the Hellenic Navy that addresses this topic and the first with the collaboration of the 1st Department of Cardiology Athens Medical School and the Dept of Nutrition and Dietetics Harokopio University.

CHANGE MANAGEMENT

Matthaios Marios-Thomas

Aim: Become familiar with some managerial theories, that may be useful to adapt smoothly to upcoming changes.

Material – Methods: Scientific literature. Content analysis.

Results: Those theories provide unique ways to understand and approach the implementation of innovations.

Conclusions: Any medical manager should be ready to deal with the upcoming changes and be able to communicate them with the other personnel.

Change can be an opportunity for some or a threat and loss for others. The last decade and especially through the financial crisis we can hardly identify an agency or an organization that has not been in the urgent need for major changes (quality improvements). Technological and medical advancements, new laws and obligations, the changing needs of patients, further reducing of government expenses and also the human inner need for change and evolution push or pull health organizations and people to change and modify practices continually.

The concept of managing the change is to:

- Understand the culture of an organization.
- Identify the kind of changes.
- Create a vision and set new goals.
- Develop strategy and tactics.
- Communicate the change.
- Inspire and motivate.
- Coach and mentor.
- Stakeholder Analysis.
- Decision making.
- Risk management.
- Complete and anchor the change.
- Feedback and evaluate.
- Study why most attempts to change fail and what should be avoided.

Change Management is a dynamic, complex and challenging process and a 'one-size-fits-all' approach is probably ineffective. Each change event is different and also every organization has its own structure, culture and needs. Despite the diversity of approaches there are some common factors for delivering effective change. A structured approach to manage change (John Kotter's, McKinsey's and Lewin's models) may help us to understand why, when and how we will manage to change.

“DEPRESSION AND FATIGUE IN MULTIPLE SCLEROSIS (MS)”

Papadopoulou Athina, Efstathiou Vasiliki, Papadopoulou Athanasia, Gerasimoy Charilaos, Christos Christodoulou

AIM: The literature review of depression and fatigue on patients with multiple sclerosis and the relationship between them.

MATERIAL AND METHODS: The search was performed in electronic databases (Medline, Scopus, PsycINFO) using the keywords: multiple sclerosis, depression, fatigue.

RESULTS: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, causing disability and other problems. It affects approximately 2.5 million people worldwide and is the most common cause of neurological disability in young and middle-aged people. Research has shown that almost one in two patients with multiple sclerosis will face depression in their lifetime. Psychological and psychiatric effects of the disease worsen the physical symptoms such as fatigue. Although fatigue is a very common symptom in patients with MS, its causes and mechanisms are poorly understood. Both depression and sleep problems of MS patients may worsen fatigue. Depression can also manifest with fatigue symptoms and thus many symptoms can be confused. Although fatigue can be attributed to depression and vice versa, their relationship has not been clarified completely. Several studies indicate the existence of fatigue on MS patients without depression or disability (EDSS), stressing the need to consider fatigue as a distinct MS symptom that is not explained by depression. However, when both are present, depression treatment appears to reduce fatigue.

CONCLUSIONS: Since both depression and fatigue coexist so often with MS, it is essential to further investigate their relationship in order to provide the appropriate treatment and help patients with MS to improve their quality of life.

“STRESS, COMBAT STRESS, PTSD: PREVENTION STRATEGIES IN THE MILITARY”

Papadopoulou Athina, Efstathiou Vasiliki, Gerasimoy Charilaos, Xygki Ioanna, Kalliora Maria, Efstathiou Elpida, Gaitanou Konstantina

AIM: The literature review of the prevention strategies for stress, battle stress and PTSD in the Armed Forces.

MATERIAL AND METHODS: The search was performed in electronic databases (Medline, Scopus, PsycINFO) using the keywords: stress, combat stress, prevention strategies, PTSD, military.

RESULTS: Relevant research has shown that the stressors associated with the military environment and the military missions are many, thus often leading the Armed Forces staff to face extreme stress and disorders associated with it. The preventive strategies that have been implemented are based on personnel information, "psycho-education", cognitive restructuring, addressing the symptoms and preventing possible relapses. Specifically, the primary prevention of Battle stress and traumatic disorder appears to include a careful selection of personnel, awareness programs as well as “psychoeducation” of those who will potentially be exposed to traumatic events. Secondary prevention includes psychological techniques such as Psychological Debriefing, immediately following a traumatic event.

Stress management is applied at three levels:

1st Level → self-help measures, help from friends and executives who belong to the same unit and are aware of the working and living conditions of the staff.

2nd Level → crisis management groups consisted of mental health professionals and military executives.

3rd Level→ psychotherapy techniques implemented by psychologists and psychiatrists.

CONCLUSIONS: Stress and related disorders is a field that has concerned the Armed Forces for many years. The implementation of primary and secondary prevention as well as of relevant research studies is necessary in order to design effective prevention and treatment programs.

“SUICIDE PREVENTION STRATEGIES IN THE MILITARY”

Papadopoulou Athina, Efstathiou Vasiliki, Gerasimoy Harilaos, Kalliora Maria, Xygki Ioanna, Efstathiou Elpida, Gaitanou Konstantina

AIM: The literature review of the suicide prevention strategies in the military.

MATERIAL AND METHODS: The search was performed in electronic databases (Medline, Scopus, PsycINFO), using the keywords: suicide prevention/preventive strategies, military.

RESULTS: Integrated interventions for suicide prevention aim at addressing the risk factors and strengthening the protective ones. The fact that the Armed Forces are a highly organized structure, with special bonds between their members, constitutes an important advantage in suicide prevention. A key component of prevention programs is to enhance knowledge and awareness of the Armed Forces staff regarding suicide (warning signs, myths and facts), suicide risk factors (e.g. substance abuse and mental health problems), as well as the appropriate actions in response to at-risk individuals before the intervention of a mental health professional. Moreover, determining a number of the Armed Forces staff as «Gatekeepers» (in each unit) and their further – graded – education about suicide in order to serve as sources of prevention, has proved to be an effective prevention strategy, as it enhances the early identification of individuals at risk and thus their early treatment. Meanwhile, the prevention programs aim to reduce the stigma of mental illness, provide information about available mental health services and remove barriers to seeking treatment. It is necessary that access to high-risk means for suicide (eg firearms) should be controlled, especially for individuals who suffer from high psychosocial stress.

CONCLUSIONS: A key factor of anthropocentric approach of the armed forces should be the development of structures and programs to prevent suicides.

ABBERANT RIGHT SUBCLAVIAN ARTERY WITH UNUSUAL LATE ONSET PRESENTATION: REPORT OF TWO CASES

George Sotiropoulos, Maria Chounti, Ioanna Barbalia, Sotirios Moraitis, Panagiotis Hountis

Purpose: Aberrant right subclavian arteries (ARSA) are congenital vascular anomalies that usually do not cause any symptoms. When symptomatic they are considered as a rare cause of dysphagia. This presentation is known as dysphagia lusoria and was first described by Kommerell. They are diagnosed by barium swallow or contrast-enhanced computed tomography, although it may be an incidental finding.

Material and Methods: We present here a late onset symptomatic finding of two patients with ARSA. Both patients were presented with chest pain without dysphagia and dysphagia mainly for liquids respectively, which are both considered as non-typical presentations of the anomaly. Dysphagia becoming worse with liquids may indicate a motility problem but not dysphagia lusoria.

Results: Motility studies such as barium swallow and manometry were performed as the patients were managed conservatively but they failed to attend for follow up. On the other hand, symptoms persisted for about a year which is typical in such cases. In both patients conservative management was selected with lifestyle changes and dietary modifications.

Conclusions: Management varies from life modifications to surgical intervention. In our cases both patients were considered as atypical late onset symptomatic presentation because of the presence of aberrant right subclavian artery withmain symptom chest pain without dysphagia. Due to age and medical comorbidities both patients where managed conservatively.

HYBRID SOLUTION FOR VISCERAL ISLAND ANEURYSM AFTER THORACOABDOMINAL AORTIC ANEURYSM REPAIR

George Sotiropoulos, Maria Chounti, Ioanna Barbalia, Sotirios Moraitis, Panagiotis Hountis

PURPOSE: Repair of a visceral artery island aneurysm after thoracoabdominal aortic aneurysm surgery poses significant technical challenges. We report a case with a visceral island aneurysm who had a one-stage repair using a hybrid approach.

METHODS: A 63 years old Caucasian male presented with severe continuous back pain. He underwent replacement of the entire aorta in stages over a 16-year period.

The celiac, superior mesenteric and right renal arteries were reimplanted in the thoracoabdominal graft. The left renal artery was reimplanted in the thoracoabdominal graft via an 8 mm Dacron graft. Serial CT angiograms demonstrated a progressive dilatation of the visceral island from 3.4 cm to 7 cm.

Via a midline abdominal incision, the celiac, superior mesenteric, right and left iliac arteries were exposed. After heparin was given, the 14 mm limb of a 14x8 mm bifurcated Dacron graft was anastomosed to the right common iliac artery. One limb of the 14x8 Dacron graft was anastomosed to the superior mesenteric artery and the other limb was antastomosed to the celiac artery in an end to side fashion.

Subsequently, a stent graft was deployed across the anastomosis between the thoracoabdominal graft and the graft to the visceral island, avoiding the origin of the graft to the left renal artery, via the left common iliac artery.

RESULTS: A completion aortogram showed complete exclusion of the aneurysm and excellent perfusion of the celiac, SMA and left renal arteries. The patient was discharged on the 8th postoperative day.

CONCLUSIONS: Hybrid techniques may facilitate repair of visceral island aneurysms after thoracoabdominal aortic aneurysm repair, by avoiding aortic cross-clamping and reentering the thoracoabdominal cavity.

POSTOPERATIVE NEUROLOGIC COMPLICATIONS AFTER DESCENDING AND THORACOABDOMINAL AORTIC ANEURYSM REPAIR

George Sotiropoulos, Maria Chounti, Ioanna Barbalia, Sotirios Moraitis, Konstadinos Plestis, Panagiotis Hountis

PURPOSE: Surgical repair of descending and thoracoabdominal aortic aneurysms started in the early 1950s, and have been traditionally associated with high mortality rate and a significant risk of paraplegia and multiple organ complications.

The aim of our study is to analyse postoperative neurologic complications on patients operated for Descending and Thoracoabdominal aortic aneurysms.

MATERIALS AND METHODS: We reviewed 94 consecutive cases of descending and thoracoabdominal aortic aneurysm repairs to determine the impact of modern adjuncts on postoperative neurologic deficit and mortality. Between December 1999 and March 2005, 24/94 (26%) patients were operated on for thoracoabdominal aortic aneurysm type I, seven (7%) for type II, 25/94 (27%) for type III or type IV, and 38/94 (40%) for descending thoracic aneurysms.

RESULTS: Twenty (21%) of the patients required hypothermic circulatory arrest for conduction of the operation. The postoperative rate of paraplegia was 3% (3/94). One patient developed temporary paraparesis. Overall operative mortality was 10% (9/94). This included 12/94 (13%) patients who underwent surgery emergently for ruptured or contained rupture of aortic aneurysm.

CONCLUSION: Surgical repair of descending and thoracoabdominal aortic aneurysms require a systematic approach to prevent devastating complications especially ischemic spinal cord injury. Newer operative strategies are key factors for spinal cord protection during these operations. Use of perioperative cerebrospinal fluid drainage, distal aortic perfusion and permissive hypothermia result in a low incidence of spinal cord injury and a low operative mortality.

SOMATOSENSORY EVOKED POTENTIAL MONITORING IN ANEURYSM REPAIR

George Sotiropoulos, Maria Chounti, Ioanna Barbalia, Sotirios Moraitis, Konstadinos Plestis, Panagiotis Hountis

PURPOSE: Descending thoracic (DTA) and thoracoabdominal aortic aneurysm (TAAA) repair have been complicated by paraplegia. In this study we compared postoperative neurologic complications in patients with DTAs and TAAAs operated with and without the use of continuous SSEP monitoring.

MATERIALS AND METHODS: Between June 1999 and November 2007, 165 patients underwent descending (n = 51) and TAAA (n = 114) aneurysm repair. SSEP monitoring was used in 74 patients (group A), while 91 patients did not have any spinal cord monitoring (group B). The median age was 64+14 years in group A and 63±13 in group. In group A, 28 patients (37.8%) had type I or II aneurysms. In group B, 31 patients34%) had type I or II TAAA. All patients had distal aortic perfusion, cerebrospinal fluid drainage, and mild hypothermia (32 -34 °C). Deep hypothermiccirculatory arrest (DHCA) was used in and 25 patients (33.7%) in group A, and in 17 patients (18.6%) in group B. In group A, 17 patients (22%) required reimpantation of intercostals arteries based on intraoperative SSEP changes, while in group B 40 patients (43.9%) had reimplantaiton of intercostals arteries.

RESULTS: In hospital mortality was 6.7% (n=5) in group A, and 6.5% (n=6) in group B (p>0.05). One patient (1.3%) sustained immediate and one (1.3%) delayed paraplegia in group A. In group B, 2 patients (2.1%) developed immediate and 2 (2.1%) delayed paraplegia. All patients had normal SSEPs at the completion, but one patient developed immediate paraplegia.

CONCLUSIONS: SSEP monitoring appears to be a reliable method of ruling out spinal cord ischemia injury during thoracic aortic aneurysm repair. In our study it lead to a significant decrease in the number of patients that required intercostal artery reimplantation.

THE IMPORTANCE OF EARLY DETECTION AND TREATMENT OF BURNOUT SYNDROME IN HELLENIC MILITARY POPULATION

STAVRIDOU A., ATHANASIADOU F., STASINI A., ZACHARIADIS A., SERAFEIM T., TSAMADOU H., GIOTAKOS O.

Aim: The present study was designed to investigate the incidence of burnout syndrome among executives of the Greek army, in relation to their position of responsibility and expertise.

Material – Method: The survey was conducted at the Military Psychiatric Clinic (414 SNEN), in Mental Health Joint Centre of Armed Forces, at 401 General Military Hospital but also in camps throughout Greece (11th

Infantry Regiment, 95th Military Hospital). The period of investigation was the second half of 2015. The 152 participants were assessed through the completion of the questionnaire of burnout of Maslach (MBI) and Readjustment Scale of Holmes and Rahe, after having been informed that their participation was voluntary and anonymous.

Results: About 40 % of participants have a sense of reduced personal achievements, 18 % high depersonalization prices while not experienced high emotional exhaustion prices. The high management position, the vast work experience and limited presence of traumatic events favor the emergence of personal achievements feeling.

Conclusions: Recognition of the value of each tier of government, to develop prevention skills of burnout and the strengthening of the role of psychological support of personnel of the Armed Forces could prevent a widespread occurrence of burnout in the near future.

A RARE CASE OF UNDIAGNOSED FAMILIAL DIGITAL CLUBBING IN A MILITARY CAMP AND THE ROLE OF THE GP ARMY DOCTOR

Tsilogianni Zoi, Grapatsas Konstantinos, Tsantilas Apostolos

Purpose: To present the rare case of undiagnosed hereditary digital clubbing and explain this clinical sing.

Material and methods: A 35-year old professional soldier presented in military camp's clinic with a common cold. During the clinical examination digital clubbing was observed in both hands and feet, for which the patient had no knowledge or symptoms and led us to more thorough examination and questions about his medical and family history.

Results: Digital clubbing is characterized by round enlargement of fingers' distal phalanx. The most common ways of recognizing this sign are measurement of Lovibond angle and index of nail curvature; also Schamroth's sign is observed. Digital clubbing is a sign of underlying disease; it is mainly asymptomatic and can be bilateral or not. The differential diagnosis includes mainly pulmonary (chronic lung infections, malignancy and chronic interstitial lung diseases), but also other conditions (cyanotic congenital heart disease, infective endocarditis, cirrhosis of the liver, and inflammatory bowel disease); rarely is due to idiopathic-primary cause. Our patient had no medical history that could explain this clinical sign and all his recent medical exams were at normal rates. However he informed us of the same sign at the family history with his father having the same type of fingers.

Conclusion: Digital clubbing is an important sign concerning multiple serious medical conditions. Idiopathic cause is rare. The doctor in primary medical care should recognize this clinical sign and proceed to more thorough examination of the patient.

ACL RUPTURE

Violakis-Georgiladakis Emmanouil, Panagoulis Evangelos, Loukovitis Eleftherios, Xenakis Andreas

Definition. The ruptures of the anterior cruciate are constantly increasing due to the high population participation in sports. Usually the rupture occurs after internal rotation and anterior displacement of the tibia.

Genetics. Researches associate rupture of the anterior cruciate ligament with genetic factors. More specifically, the genes expressing metalloproteinases show greater correlation with the risk of injury and rupture.

Epidemiology. Women predominate in number about four to eight times compared to men. The incidence of injuries of the anterior cruciate ligament is greater in people who participate in sports.

Presentation. Typical symptoms that the patient feels after injury of the anterior cruciate ligament is:

a) Pain and swelling, which occur immediately after injury

b) Stiffness of the knee, difficulty of effecting movements due to swelling

c) Difficulty in walking

d) Feeling of instability in the joint.

Diagnosis. For the diagnosis there are many clinical tests with the most important the Lachman test and the anterior drawer test. The MRI shows accurately the rupture of the ligament amounts up to 95 % which are then confirmed arthroscopically.

Treatment. It can be divided into conservative and surgical. Techniques applied are either the traditional open surgery or arthroscope. The rupture of the anterior cruciate ligament can't be restored by stapling back the ligaments.it is replaced with a piece of other grafts. Usually ligament graft is harvested from the patellar ligament.

"THE ASSOCIATION OF DEMOGRAPHIC AND SOCIOECONOMIC CHARACTERISTICS OF PROFESSIONAL MILITARY PERSONNEL WITH THE ONSET OF DEPRESSIVE SYMPTOMS IN GREEK ARMY"

Vlazakis Alexandros, Athanasiadou Foteini, Siagkri Vasiliki, Giotakos Orestis

Aim: The aim of the present study was to examine the association of socio-demographic data, financial data, major life events and self-perceived social support with the onset of depressive symptoms in professional military personnel of the Greek Army.

Material – Method: This is a quantitative relational study of secondary analysis.

As data collection instrument, a structured self-supplemented questionnaire of 56 questions was used, with an average completion time of six minutes. The questionnaire included questions on socio-demographic characteristics of participants and a custom selection of items from already used valid scales.

The analysis initially included the analysis of demographic characteristics and the examination of the internal consistency of the scales used. Then a series of stepwise analyses using the method of multivariate regression in SPSS v.22 were conducted.

Results:

As far as the demographic and socioeconomic characteristics are concerned,

1. The gender (b = 0.13, t = 2.87 p <0,004, DR2 = 0.04), as the women consistently showed higher levels of depressive symptoms,
2. The life events (b = 0.23, t = 5.11 p <0,001, DR2 = 0.07),
3. The inability of loan repayment (b = 0.22, t = 4.77 p <0,001, DR2 = 0.06) and
4. Social support (b = –0.21, t = –4.75, p <0.001, DR2 = 0.04) significantly predicted depression (F (4.403) = 27.35, p <0.001).

With regard to life events:

1) Problems with the supervisor (b = 0.24, t = 5.12 p <0,001, DR2 = 0.07),

2) Extreme changes in the working tasks (b = 0.19, t = 4.10 p <0,001, DR2 = 0.04),

3) Non-affordable mortgage or loan (b = 0.13, t = 2.71 p <0,007, DR2 = 0.02),

4) Divorce (b = 0.13, t = 2.81, p <0,005, DR2 = 0.02) and

5) The death of close friend / th (b = 0.10, t = 2.16, p <0.03, DR2 = 0.01) predicted significantly depression.

Conclusions: The results of this study highlighted the relationship of gender, loan repayment and significant loss events in the personal life and problems in working life of the professionals of the Greek Army with the onset of depressive symptoms and the role of social support as a protective factor with a negative correlation with the occurrence of depressive symptomatology. The need to emphasize, in the primary prevention of depressive symptoms through regular health examinations, providing further facilities and welfare measures in the permanent military personnel and the expansion of the network and of the provided psychosocial care services to members of the Armed Forces is also highlighted.

LUTEMBACHER SYNDROME IN A PATIENT WITH RHEUMATIC MULTIVALVULOPATHY AFTER PERCUTANEOUS MITRAL BALLOON VALVULOPLASTY

Votsis Stefanos, Nikolaidou Chrysovalantou, Hadjimiltiades Stavros, Karamitsos Theodoros, Culum Gordana, Karvounis Haralampos

PURPOSE: To report a rare clinical case of Lutembacher syndrome, which was developed in a patient with rheumatic multivalvulopathy who underwent percutaneous mitral balloon valvuloplasty with a rapidly deteriorating aortic valve stenosis also present.

MATERIAL AND METHODS: We present the case of a 67-year-old patient with rheumatic multivalvulopathy (moderate aortic stenosis and severe mitral stenosis) who, after being initially deferred for radical surgical treatment, underwent percutaneous mitral balloon valvuloplasty with an initial satisfactory operational, clinical and haemodynamic result. After the lapse of a 3-year period, the patient was diagnosed with Lutembacher syndrome, the cause of which was the deterioration of the aortic valve stenosis (from moderate to severe), which consequently not only prevented the progressive closure of the iatrogenic atrial shunt of the aforementioned percutaneous mitral balloon valvuloplasty, but actually caused its anatomical and haemodynamic deterioration, resulting also in newly diagnosed severe tricuspid regurgitation, mild right-ventricle failure and initial-stage pulmonary hypertension due to volume overload.

RESULTS: After a thorough imaging investigation to establish the cause and the anatomical and haemodynamic parameters of the current Lutembacher syndrome presentation (including TEE, cardiac catheterization and cardiac MRI), the patient finally underwent a) surgical replacement of the mitral valve b) surgical replacement of the aortic valve c) surgical repair of the tricuspid valve and d) surgical closure of the atrial septal defect, with satisfactory operational and clinical result.

CONCLUSIONS: Lutembacher syndrome is sometimes iatrogenic after atrial septum puncture, and can be caused in cases of aortic valve stenosis deterioration, even if the initial mitral valve stenosis is ameliorated.

"BODY IMAGE IN WOMEN WITH BREAST CANCER"

Xygki Ioanna, Kalliora Maria, Papadopoyloy Athina

Aim: A literature review surveying the alterations in women's body image due to the development of breast cancer.

Material – Methods: Relevant papers published from 2006 to 2014 were traced in electronic databases using the keywords: breast cancer, body image, body change stress, quality of life.

Results: Meta-analysis of the available literature suggests that approximately half of breast cancer patients experienced two or more body image problems some of the time, due to their exposure to marked changes in their physical appearance. A high investment in appearance, as well as the body image before the development of the disease, could function as vulnerability factors. The latter is a part of personality that is not easily modified after it has become established, not even by treatment for breast cancer. Negative body image of breast cancer patients has been also associated with the type of surgical treatment a patient receives. As a consequence, changes in body image can lead to a maladaptive coping of the illness, which can impede the treatment of breast cancer.

Conclusions: Health care professionals that work with cancer patients should carefully consider body image issues during the course of the disease and discuss surgical options with patients. Providers are urged to help women redefine beauty, sexuality and femininity in a way that does not automatically exclude any woman who has suffered from this debilitating illness.

HYPER-IgG4 SYNDROME (IgG4-RELATED DISEASE-IgG4 RD) IN A PATIENT WITH SUBMANDIBULAR GLAND ENLARGEMENT

Filio Marineli, Ioannis Lianos, Ioannis Xatzidakis, Stavroula Gongaki, Konstantinos Kounouklas, Eustathios Koutsostathis, Basilios Fasias, Evagelia Zarogianni, Petros Savourdos, Eirini Theodoropoulou, Eirini Zarvou, Dimitra Stathi, Panagiotis Petrikkos, Eleni Mostratou, Nikolaos Symeonidis

Purpose: Presentation of an IgG4-related disease case with left submandibular gland enlargement.

Materials and Methods: A 51 year old woman was referred to our Department from the ENT Division for investigation of a left submandibular gland enlargement started 6 months previously. An operation for (R) submandibular gland excision was performed 7 months previously. Results came up negative for malignancy and showed reactive etiology. Her past medical history included chronic rhinosinusitis and asthma. Physical exam revealed eyelids edema, painless jugular lymph node enlargement and a significant left submandibular lymph node enlargement with a diameter >2cm. Differential included lithiasis of salivary gland, salivary gland infection, Sjogren syndrome, sarcoidosis, salivary gland neoplasm, metastatic disease, Wegener disease, and IgG4-related disease.

Results: Lab tests revealed elevation of LFTs and elevated IgG4. Department was suspicious for a case of IgG4-releated disease. Paraffin block from the ENT division was obtained and sent for immunohistochemistry stain. Results came positive for IgG4 infiltration with a percentage of >50%. A PPD test was performed (negative), and patient started on 0.6 mg/kg dose of prednisone with significant clinical improvement. Patient is asymptomatic on a dose of 2.5 mg prednisone.

Conclusion: IgG4-related disease is a rare disease which must be included in the differential in cases of unexplained lymph node enlargement. IgG4 levels should be included in the lab work. Prednisone is treatment of choice. Duration and dose of treatment is under further discussion. More studies are needed.

PITUITARY APOPLEXY MIMICKING STROKE WITH OCULOMOTOR NERVE PARESIS

Filio Marineli, Ioannis Lianos, Ioannis Xatzidakis, Stavroula Gongaki, Konstantinos Kounouklas, Basilios Fasias, Evagelia Zarogianni, Eirini Zarvou, Eleni Mostratou, Nikolaos Symeonidis

Purpose: Presentation of a patient with pituitary apoplexy, with clinical findings mimicking stroke.

Materials and methods: A 69-old man was admitted for headache from seven days, with fatigue and low blood pressure, accompanied by right eye ptosis, unsteadiness and dysarthria. Personal history: glaucoma, psoriasis, alcohol abuse. Physical exam revealed skin hyperpigmentation and slight right eyelid ptosis. Differential diagnosis included glaucoma, stroke, temporal arteriitis, and adrenal insufficiency. Laboratory tests included cortisol levels, urine cortisol, aldosterone and corticotropin (ACTH).

Results: Computed tomography was perfomed twice upon admission 2 days later and came up negative. Magnetic resonance imaging (MRI) revealed pituitary enlargement. MRI of cella turcica revealed findings consistent with recent bleeding of pitiutary gland. Laboratory exams showed low levels of cortisol and ACTH, hyponatremia (Na 123 mEq/l) and elevated CRP: 47 mg/dl. Acute phase proteins were elevated. Patient started on hydrocortisone, with a significant neurologic improvement and discharged asymptomatic. He was advised to follow neurosurgical and endocrinological consultations.

Conclusion: Sudden hemorrhage into the pituitary gland is called pituitary apoplexy which is very serious and it often occurs into a pituitary adenoma. It can result in sudden, severe headache, diplopia due to pressure on the oculomotor nerve and hypopituitarism. Cortisol deficiency is the most serious complication, because it can cause life-threatening hypotension. Hydrocortisone is the medicine of choice. MRI may confirm clinical suspicion where as CT scan can miss diagnosis.

ASSOCIATION OF PRE-OPERATIVE NEUTROPHIL-TO-LYMPHOCYTE RATIO WITH PATHOLOGICAL CHARACTERISTICS OF PAPILLARY THYROID CARCINOMA

K. Manatakis, Nikolaos Stamos, Dimitrios Balalis, Sotirios Gantzoulas, Vassilios Berdelis, Konstantinos Damalas, Georgios Bouboulis, Dimitrios P. Korkolis, Nikolaos Kouris, Georgios Plataniotis, Emmanouil Gontikakis

Purpose: Neutrophil-to-lymphocyte ratio (NLR) has been used as a surrogate systemic inflammatory marker in a variety of tumors. Although thyroid cancer has a well-established connection with chronic inflammation, application of NLR in the pre- and postoperative evaluation of patients is limited and controversial. Our study aimed to identify potential associations of NLR with pathological characteristics of papillary thyroid carcinomas.

Materials and Methods: All cases of papillary thyroid carcinomas between January 2006 and December 2015 were retrospectively analyzed and pathology reports were reviewed for tumor size, multifocality, lymphovascular and capsular invasion. NLR was calculated as the absolute neutrophil count divided by the absolute lymphocyte count, based on the preoperative complete blood count.

Results: In total, 72 patients (21 men, 51 women) with papillary thyroid carcinomas were identified, with a mean age of 55 years. Mean tumor size was 1.16±1.13 cm, multifocality was observed in 27/72 cases (37.5%) and capsular/vascular invasion in 12/72 cases (16.7%). NLR was significantly higher in larger carcinomas (>3cm) compared to microcarcinomas (3.08 vs 2.13, $p=0.002$), but did not reach statistical significance compared to carcinomas of 1-3 cm (3.08 vs 2.38, $p>0.05$). NLR in multifocal carcinomas was higher compared to unifocal lesions, but the difference was not statistically significant (2.47 vs 2.17, $p=0.10$). On the contrary, invasive carcinomas showed significantly higher NLR than non-invasive neoplasms (2.80 vs 2.18, $p=0.02$).

Conclusion: High baseline NLR in patients with papillary thyroid cancer has been associated in the past with older age at presentation, larger tumor size and poorer prognosis. We observed a tendency towards higher NLR in more aggressive carcinomas, which reached statistical significance in tumors larger than 3cm and capsular/vascular invasion. As inflammatory marker, NLR is inexpensive and readily available and warrants further study in larger trials.

CASE REPORT: SURVIVAL IN SEVERE LACTIC ACIDOSIS IN ACUTE ORAL ANTI-DIABETIC COMPOUNDS POISONING

Ardeleanu Dida, Serban Cristina

Objective: Metformin is an oral anti-diabetic drug chosen for the treatment of thype 2 diabetes. The correct management of diagnosis and intensive care measures led to the survival of the patient with severe complications in acute poisoning with oral anti-diabetic compounds. The most common symptoms following overdose appear to include vomiting, diarrhea, abdominal pain, tachycardia, drowsiness. The major potentially life-threatening complication of biguanide overdose is lactic acidosis, which is due to lactate accumulation. Secondary to lactic acidosis, the patient may experience changes in the central nervous system, cardiovascular collapse, renal failure, and death.

Materials & methods: We describe: woman 60 year old, overweight, and transferred in our department 12 hours after a voluntary acute poisoning with metformin (avandamet). On admitance: drowsiness, tachypnea, tachyarrhythmia, vomiting, abdominal pain, arterial hypotension, severe lactic acidosis and acute respiratory failure. The treatment was complex: supportive, concomitent with hemodyalisis sessions.

Results: Treatment of metformin overdose is generally supportive as is no specific antidote. Lactic acidosis is initially treated with sodium bicarbonate, but severe lactic acidosis has not responded to sodium bicarbonate and was established hemodialysis. The clinical evolution of patient was favorable.

Conclusion: The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment. Acute oral anti-diabetic compounds poisoning has sometimes a severe clinic evolution with severe lactic acidosis and respiratory disease. The primary goals of therapy are restoration of acid-base status and removal of metformin, using hemodialysis and bicarbonate therapy. The intensive care measures can change the vital prognosis of the patient.

CASE REPORT: THE SEVERE EVOLUTION IN ACUTE COPPER SULPHATE POISONING

Ardeleanu Dida, Serban Cristina

Objective: Copper sulphate is commonly known as "Blue Vitriol" or "Blue Stone". It is used chiefly for agricultural purposes as a pesticide and in leather industry. It is consumed mainly with suicidal intentions. Accidental poisonings have been reported. The clinical course of the copper sulphate intoxicated patient is often complex involving intravascular hemolysis, jaundice and renal failure. The treatment is mainly supportive. In severe cases methemoglobinemia needs treatment. Mortality is quite high in severe cases. The copper-albumin complex represents the toxicological active portion of the serum copper.

Materials & methods: We describe here a fatal case of copper sulphate poisoning: a 24-year-old man who was admitted to the toxicology emergency department after accidental ingestion of blue vitriol sprinkled in garden used. Tox reveals elevated copper in blood and urine. Malaise, Reed coma, cyanosis, hemolytic anemia. During hospitalization: methemoglobin values begin to rise; acute renal failure, jaundice, hepatic cytolysis syndrome, vomiting, diarrhoea, hypotension. The treatment was supportive and methylene in symptomatic methemoglobinemia; hemodialysis.

Results: 12 days after admission the clinic parameters register a permanent instability and subsequently an irresuscitable cardiac arrest is installed followed by the decease of the patient.

Conclusions: Copper sulphate poisoning is associated with high mortality in severe cases due to methemoglobinemea, hepatotoxicity and renal failure. Mainstay of treatment is supportive, including careful fluid therapy and methylene in symptomatic methemoglobinemia. Chelation therapy though tried in many cases, their benefits are not established in controlled trials. The role of dialysis is limited to the management of associated renal failure.

PSYCHOLOGICAL AND PSYCHIATRIC ASPECTS OF STRESS MANAGEMENT IN THE MILITARY ORGANIZATIONS

Guiță-Alexandru Iuliana, Bulacu Ion

The events at the beginning of the XXI century are generating major changes in the geopolitical and security environment. By their geostrategic position, the countries of Southeast Europe must sustain their role of a balancing factor of stability and security in the region.

Military organizations have had to quickly adapt and to face new geostrategic contexts, with major technological changes as well as an increase in interconnection between civilians and military world. From the psychological point of view, this permanent effort of adaptation can produce an accumulation of tension, feelings of inadequacy and discomfort and could traumatically affect physical and mental health and generate psychological vulnerability. When stress factors exposure exceeds individual's ability to keep control, there appears a brain sensitivisation to specific neural networks. Human body's response to stress may generate pathological emotional or psychological reactions (irritability, disbelief, sadness, anxiety, apathy etc.), or somatic reactions (palpitations, insomnia, fatigue, headache etc.).

Stress pathological effects' managements could include both elements of psychological counseling and psychotherapy (with a broad recommendation for the use of cognitive-behavioral therapy in treating stress-related symptoms) and appropriate psychopharmacological interventions. A special consideration should be given to preventing stress consequences, which would mean much lower costs than treating them as job-related diseases.

In conclusion, to improve the satisfaction level of the military and to ameliorate occupational health, great importance should be given to continue psychological assistance and, where is needed, to early diagnosis and treatment of psychiatric disorders occurring as a consequence of chronic exposure to stress.

BIOSCAVENGER ENZYMES AS NEW STRATEGY FOR MILITARY PROTECTION AGAINST CHEMICAL WEAPON NERVE AGENTS EXPOSURE

Poştoarcă G. Angela, HINESCU G. Lavinia, Drăghici Constantin, Cosmescu Cristiana, Tudosie Mihail-Silviu, Ionescu Mihaela

Introduction: An efficient therapeutic strategy to combat against accidental or intentional exposure to organophosphate (OP) or other chemical warfare nerve agent is of high demand both in civilian and military sectors. Last decade experimental studies have defined two enzyme (butyrylcholinesterase - BuChE and paraoxonase - PON1) that can be used as bioscavenger of OP molecule.

Materials/Methods: Anion exchange chromatography (Q-Sepharose Fast Flow) was used in order to develop methods of purifying enzymes. The enzymatic fractions collection was carried out in an integrated Gradifrac Pharmacia Biotech. Continuous monitoring of parameters: flow (3-5mL/min), NaCl concentration in the elution buffer (Tris-HCl, pH 8.5), time of fraction collection (1.5min/fraction), absorbance (280nm), allowed the process optimization. The ability to reactivate acetycholinesterase and butyrylcholinesterase was tested by in vitro/ex vivo experiments on whole blood samples inhibited to 95% with Ethyl Paraoxon.

Results: From the three collected fractions, FII has had the best specific enzymatic activity - 0.82U/mg and total protein concentration - 0.94 mg/mL. The combination of the purified fraction with oxime (OX 1, OX 2) led to a reactivation of acetycholinesterase up to 40-48% compared to 60-69% for butyrylcholinesterase. Measurement of AChE and BuChE activity variation is considered as a biomarker of exposure, effect or susceptibility to OP intoxication of soldiers in neurotoxicology.

Conclusions: Due to the small volume of plasma processed through the Q Sepharose FF column, fractions with low specific activity were obtained. However, the yield of cholinesterase reactivation creates the foundation to obtain an enzyme pharmaceutical product usable subsequently for in vivo experiments.

3D CONFORMAL RADIOTHERAPY TREATMENT IMPLEMENTATION IN CENTRAL MILITARY EMERGENCY UNIVERSITY HOSPITAL" DR. CAROL DAVILA" BUCHAREST (SUUMC)

S. Vlad, G. Balasa, M. Matei, M. Dumitrache, A. Tănase

INTRODUCTION: Radiation therapy efficiency and making it safe require substantial capital investment in equipment and room arranging, but also require substantial investment in human resources. New advanced technology have made possible implementation of 3D conformal radiotherapy.

3D conformal term is used to describe the development of radiation therapy treatment planning, based on the data acquisition of 3D images obtained from computed tomography dedicated to radiotherapy, in order to treat the clinical target volume and avoid the possible organs at risk.

MATERIALS AND METHODS: Transition to 3D conformal radiotherapeutic treatments require complex resources in terms of technology, equipment, medical staff and skills. To enable a modern radiotherapeutic treatment for various oncological diseases, Department of Radiotherapy from SUUMC was equipped with a medical accelerator model UNIQUE Power Varian, a CT Simulator and a HDR brachytherapy unit.

CONCLUSIONS: With our modern radiotherapy equipments, during 2015, we have doubled our capacity, offering 3D radiotherapy treatment to approximately 1000 patients, with various cancer diseases like cervix, breast, lung, prostate and bone cancer. In near future we plan to implement advanced treatment techniques, mainly used for convex or concave planning target volume, located near organs at risk, like spinal cord, optic nerves, rectum, etc.

SYRIAN WAR SHRAPNEL INJURY: CUBITAL NERVE DEFECT GRAFTING DURING HUMANITARIAN SURGICAL MISSION. CLINICAL CASE PRESENTATION.

Argentina Vidrascu

Introduction: Acute and chronic war wounded Syrian refugees from Zaatari camp and the Syrian battlefields are directly admitted in Amman Al Maqqased Charity Hospital every day.

Among patients treated by the author during November 2015 Humanitarian Mission was a left cubital nerve defect due to a bomb explosion injury.

Aim of presentation: Background and aim of this clinical case presentation is to reveal the importance of early nerve injury diagnosis and surgical treatment in war wounded patients.

Methods: The author treated patients in Amman Charity Hospital were among different plastic surgery cases where limb nerve injuries whith nerve grafting indication. The presented case was treated with autologus sural nerve graft.

Cubital nerve was found being disrupted on 10 cm length at the medial 1/3 of the forearm. After debridement and neurolisis due to myeline degenerative process, the gap real size was 14 cm long.

The autogenous sural nerve grafting was decided to be done.

The right 18 cm long Sural nerve graft was harvested and and a cable graft designed to bridge the gap.

The microsurgical sutures were done at the both nerve ends to achieve nerve connections with separate nonabsorbale 7-0 sutures

Results: were evalueted at 3 months after the surgery and revealed detectable nerve conductibility at the Electromiography test.

In conclusion, in cases with delayed nerve repaire surgical treatment in war wounded patients, the vascularised nerve graft can be a better solution for nerve defect surgical treatment.

PREVALENCE OF OBSTRUCTIVE SLEEP APNEA IN THE ROMANIAN MILITARY POPULATION

Ciumaşu-Rîmbu Mălina, Lovin Sanziana

Sleep apnea syndrome is common in the Romanian civilian population, but little is known about it in members of the military. Obstructive sleep apnea (OSA) constitutes a potentially serious condition for military members, since excessive daytime sleepiness can impair performance, cognition,interfere with mental clarity and alertness, increase accidents in dangerous environments, such as during a deployment or while working with firearms. Long-term effects of OSA include associations with increased mortality and medical disorders like cardiovascular disease, obesity and diabetes. The objectives of this study were to describe the prevalence of obstructive sleep apnea in a sample of ministery of defence (MoD) employed personnel referred for occupational medicine routine evaluation and identify relationships between demographic characteristics, comorbid diagnoses, and OSA. Material and methods: We performed a retrospective analysis on the data from one year occupational medicine referred cohort (4444 MoD employees with 83.23% military employees). Results :We have found a small percentage (0.42%) of OSA,twice higher in the military employees compared with the civilian ones, with most patients having severe OSA (68.42%) and a significant statistical relationship between obesity, glycemic and hepatic impairment, dyslipidemia and cardiovascular disease. Conclusion: The small percentage of the found OSA it’s just the tip of the iceberg since 9.5%percent of the same cohort are obese. We need further detailed screening studies on the obese MoD population and on larger numbers of employees to confirm and/or evaluate the prevalence of OSA in the Romanian military population.

RISK FACTORS AND PROGNOSIS IN ENDOMETRIAL CARCINOMA

Bodac Aniela

Introduction: Endometrial carcinoma is the most common gynecologic malignancy in the developed countries and occurs mainly in the menopausal women (1). There are two types of endometrial cancer: Type I is mostly seen in younger women, estrogen-dependent and has better prognosis. Meanwhile, type II has a higher prevalence in menopausal women and it is diagnosed in advanced stages, with worse prognosis than type I carcinoma (2).

Material and Methods: We developed a retrospective study that included 40 women between 50-65 years old, admitted in our service from January to April with endometrial carcinoma. We applied to our study patients a questionnaire that included weight, number of births, miscarriages, family history, undergoing treatments, other related diseases, and the age the menopause occurred. Also, we collected the histopathology results in order to see the adverse prognostic factors such as FIGO staging, histological type, myometrium invasion, lymphovascular invasion and tumor expression for ER (estrogen receptor) and PR (progesterone receptor).

Results: In our study group the most prevalent endometrial carcinoma was type II. The most important risk factor for our patients was obesity, followed by hormonal therapy and late menopause.

Conclusions: As reaching menopause all women should be informed about the risks and symptoms of endometrial carcinoma in order to diagnose it in early stages. Also, women with high risk of developing this malignancy should be offered screening, tissue sampling of the endometrium.

VOLUMINOUS ADRENAL CYST - MINIMALLY INVASIVE SURGICAL TREATMENT. CASE REPORT

R. Nica, C. Muşat, F. Vasilescu, C. Pleşa, A. Mihai, D. Alexe, C. Cîrlan, F. Săvulescu

Introduction: The adrenal tumor formations are often discovered by chance during a review of imaging (abdominal ultrasound, computed tomography, magnetic resonance), for a problem that is not related to the adrenal gland, they do not usually show any signs or symptoms specific to an adrenal gland pathology.

Case presentation: The patient is hospitalized accusing mild abdominal pain, which started about 2 months before hospitalization, without no other signs or symptoms. The usual blood tests were normal. Abdominal ultrasound revealed a cystic tumor formation relatively large, about 8-10 cm with a possible liver etymology. CT examination could not exactly determine the origin of the tumor, describing it interhepatorenal. Due to the acute stage of patient pain, surgery is to be laparoscopic. Surgery is completed with abdominal cystic tumor ablation.

Results: The patient's postoperative evolution was favorable, with a completely remittance of the painful symptoms. Postoperative histopathologic exam establishes the diagnosis of adrenal endothelial benign cyst.

Conclusions: Laparoscopic surgery is useful for diagnosis in patients presenting atypical abdominal pain growing in intensity, especially when imaging tests cannot provide accurate data on the present pathology. Minimally invasive surgery is the current "gold standard" of the adrenal tumor excision.

IXODES RICINUS – VECTORS FOR TICK BORNE ENCEPHALITIS VIRUS IN ROMANIA

Ionescu E. Lucia, Dumitrescu V. Gabriela, Popescu M. Diana, Necşulescu Marius, Ordeanu Viorel, Bicheru N. Simona, Vladimirescu Alexandru

Ixodes ricinus is the most widespread tick in Romania and is the vector for many important pathogens for humans and domestic animals. In 1999 in Romania was reported an outbreak caused by tick borne encephalitis virus (TBEv). TBEv is a flavivirus transmitted to humans by the bite of infected tick or by the ingestion of unpasteurized milk from the animal host.

We tested Ixodes ricinus, collected from some localities from Transylvania for the presence of TBE virus.

Pools of adult ticks were tested using RT-PCR with specific primers for 3’UnTranslated Region (UTR) of the RNA genome.

We have determined the presence of TBEv in the Ixodes ricinus pools, mostly collected from Sibiu County, the positive results have demonstrated that Ixodes ricinus is implicated in the TBE virus transmission in Romania.

The research is part of the project TicKitqPCR, funded by MEN-UEFISCDI PN II “Partnerships in priority areas” program, Grant No. 295/2014.

MALDI TOF MASS SPECTROMETRY USED FOR RAPID IDENTIFICATION OF SPORULATED BIOLOGICAL AGENTS

Simona Nicoleta Bicheru, Diana Mihaela Popescu, Lucia Elena Ionescu, Gabriela Victoria Dumitrescu, V. Ordeanu, A. Vladimirescu, M. Necşulescu

Using MALDI TOF system (Matrix - Assisted Laser Desorption/Ionization Time - of - Flight Mass Spectrometry) for identification and characterization of pathogen germs, it is a fast and safe method, which measures molecular fingerprint unique to each microorganism by mass spectrometry. Bacteria are biological agents that can be easily multiplied, stored and conditioned like weapons and disseminated.

The cultures of Bacillus cereus were seeded via loop prick on the simple agar and blood agar, in Petri plates in order to obtain isolated colonies. Bacterial colonies obtained were processed for 16S ribosomal proteins extraction, by the "Microorganisms Profiling" extraction trifluoro acetic acid (TFA 80%) procedure and analyzed with Microflex® LT 20 equipment. The method is used for extraction of ribosomal proteins in vegetative form of bacteria, but lends itself particularly well for biological material sporulated.

Spectra analysis of the genus Bacillus cereus biological agents representing the polypeptide fragments of the 16S ribosome revealed a mass range between 800 and 12000 Daltons. The values of characteristic peaks strain of Bacillus cereus strain IC 11549 are in the range of 2500-11250 Daltons. The characteristic peak "protein species signature" of the species Bacillus cereus is set in the range of 7080 Daltons. All mass spectra obtained were identified as belonging to the tested bacterial species, with results which were confirmed by classical bacteriological techniques.

The Microflex LT 20 system allows quick and accurate identification of biological agents therefore being an important confirmation/validation mean for other microbiological diagnostic methods.

PERIPHERAL ARTERIAL DISEASE (PAD) AND TYPE 2 DIABETES MELLITUS (T2DM) PATIENTS – RAPID GUIDE CONSIDERING DIAGNOSIS AND TREATMENT

Ciprian Constantin, Ovidiu Cotea, Catalina Popa

Introduction: The assessment of PAD at T2DM patients is a must have of screening and treatment of this patient category. Methods of diagnostic and evolution of these methods imply a rapid and preclinical diagnostic in our days.

Objectives: To evaluate the value of old and new methods dedicated to diagnosis PAD at T2DM patients. A real good review will serve for residents as a valuable tool to create an image about the place and role of actual tools to diagnose and evaluate the treatment of PAD.

Materials and methods: Articles were selected using next key words: T2DM, PAD, tools and PAD, drugs and PAD, diagnostic of PAD. International guidelines and multicenter studies were main sources of this algorithm.

Results: The actual data suggest that is simple to evaluate and to predict the role of a diagnostic of PAD in the future evolution for a patient with T2DM. For this patient will be necessary to use a class of medication or an intervention for prevent amputation of inferior limbs. The control of risk factors that could contribute at PAD evolution will better controlled and better treated if actual tools will diagnostic at a preclinical stage this complication/comorbidity of T2DM patients. The role for a cardiovascular risk score (dyslipidemia, high blood pressure etc) could be evaluated here. Results from major clinical trials are presented.

Conclusion: The key contribution of this paper is an actual update of literature for a new or experimented medical doctor in a better understanding of PAD at T2DM patients.

ULTRASOUND POWER DOPPLER IN CLINICAL REMISSION IN PATIENTS WITH RHEUMATOID ARTHRITIS

Daniela Anghel, Mihai Lucian Ciobica, Nicolae Cristian Anghel, Ciprian Viorel Jurcut, Adrian Anghel, Ancuta Coca

Background: The goals of treatment for patients with rheumatoid arthritis are remission or decreased disease activity, stopping the rate of joint damage. The subclinical synovitis is associated, despite clinical remission, with progression of structural damage. Imaging technique such ultrasound is capable to provide a more accurate measure of disease activity.

Objectives: To assessed in patients with rheumatoid arthritis in clinical remission the presence of subclinical synovitis by ultrasound.

Methods: The study included 58 patients with RA (ACR/EULAR 2010 criteria) in clinical remission. Medium age is 45 (range 30-59) years. The patients was in clinical remission (DAS<2.6); 81% are female; 65% are positive for rheumatoid factor and 85% for ACCP (anticitrullinate peptide antibody). PDUS examination was performed using Esaote US machine equipped with linear probes (5-12MHZ). PDUS (power Doppler ultrasound signal) investigated metacarpophalangeal and proximal interphalangeal joints and wrist symmetrical. PDUS used 4 grade (semi quantitative score) from grade 0 to 3. PDUS was performed at baseline and after 6 months to beginning of treatment. Furthermore, DAS 28 and laboratory data (ESR, CRP) were obtained at baseline and after 6 months to beginning treatment and clinical remission.

Results: All patients were in clinical remission after 6 months of treatment. PDUS were used as a measure of active disease. Synovitis grade 0 has been found in 29 patients (remission) PDUS grade 1 has been found in 10 patients, PDUS grade 2 in 9 patients and no PD grade 3 has been found after 6 months. PDUS was more frequently observed in the wrists (45%), MCP 2 (26%), PIP 3 (9%).

Conclusion: The results of study confirm that clinical remission doesn't reflect an absence of synovial inflammation. PDUS is useful in assessing of patients considered to be in remission. PDUS detected subclinical synovitis in the small joints of hands.

The other parameters don't show an evident association with the presence or absence of PDUS.

COMPLEX RECONSTRUCTION FOR A THORACIC PARIETAL METASTASIS WITH PERICARDIAL INVASION – CASE PRESENTATION

Adrian Ciuche, Claudiu Nistor, Dragos Marin, Daniel Pantile, Laura-Mariana Constantin, Roxana Brîncoveanu

Introduction. The authors present the case of a 61 y.o. male with a left pneumonectomy, performed 2 years ago for left lung cancer, followed by chemotherapy and radiotherapy, admitted with an anterior thoracic wall metastasis invading the overlying skin and the pericardium.

Material and method. The surgical technique is being detailed – surgical resection of C2, C3, and C4 anterolateral rib arches, en-bloc with adjacent structures (skin and pericardium). After removal, a complex reconstruction of the thoracic wall has been performed (using a musculocutaneous flap including the latissimus dorsi muscle and the overlying skin with the preservation of the thoracodorsal vascular pedicle), as well as a pericardial reconstruction (with Mersilene® mesh).

Results. With favorable surgical outcome, several aspects are being detailed: postoperative respiratory function, aesthetic and socio-professional reintegration.

Conclusion. When dealing with thoracic wall metastasis invading surrounding anatomical structures, their complete resection requires complex reconstruction. In this case is being performed a musculocutaneous flap transposition preserving the vascular pedicle and pericardial reconstruction.

TRANEXAMIC ACID IN MAJOR TRAUMA AT HIGH RISK OF BLEEDING

Dijmarescu Alisa-Elena

OBJECTIVE: Tranexamic acid isan antifibrinolytic drug that was shown to increase survival in severely injured trauma patients who have or are at risk for severe hemorrhage.

Our objective in this paper was to perform a systematic review to evaluate the effectiveness and safety of tranexamic acid in reducing bleeding in trauma.

MATERIALS AND METHODS: We included 15 studies, case reports and review articles.Literature was searched inPubmed, Cochrane, SciELO between 2013-2016.

RESULTS: This systematic reviewshowed that the administration of tranexamic acid significantly reduced the risk of death due to bleeding by up to 25-30%, also reduced the need for blood transfusion, without apparentserious adverse effects, such as increased risk of deep vein thrombosis and pulmonary embolisms.

The protocols for the management of bleeding consider TXA may be administered to patients with traumatic injuries, who are hemorrhaging or at risk for hemorrhage. TXA is contraindicated in patients who have an isolated traumatic brain injury. Administration of tranexamic acid, ideally within 3 hours, can reduce mortality, regardless of baseline risk. The initial bolus dose of TXA is 1 g I.V infused over 10 minutes, followed by an infusion of 1 g over 8 hours.

After the CRASH-2 trial, TXA was endorsed by the World Health Organization in March 2011 as an essential medicine with sufficient evidence to support use in adult patients with trauma and significant risk of hemorrhage. The military are using tranexamic acid to treat combat casualties.

CONCLUSION: The tranexamic acid should be administered early as possible to the trauma patient who is bleeding or at risk of significant hemorrhage.

ATYPICAL AORTIC COARCTATION – A RARE CASE PRESENTATION

Ancuta Coca, Lucian Mihai Ciobica, Daniela Anghel, Ioana Raduta, Cristina Trofin, Silviu Marcel Stanciu

Coarctation of the aorta (CoA) is a congenital cardiac malformation with a reduced life expectancy for patients who have not undergone correction. Survival in case of elderly is rare meanwhile the mean survival rate is around 35 years. A typical CoA is a rare condition which affects 0.5 % to 2 % of the individuals with CoA.

We describe the case of a woman, firstly diagnosed with an atypical coarctation of aorta. A 25-year-young man presented to our department with chest discomfort, increasing fatigue, and exertional dyspnea. On physical examination, he was identified a grade 2/6 systolic murmur in the apex and left second intercostals space and the femoral pulse delay was palpable bilaterally. The systolic pressure gradient between upper and lower extremities is 25 mmHg. Aortic coarctation was suspected, and a further investigation was performed. The electrocardiogram (EKG) revealed left ventricular hypertrophy, while the transthoracic echocardiography (TTE) showed wall-motion abnormalities, a bicuspid aortic valve and the left ventricular ejection fraction was 40 %. The continuous wave Doppler revealed a peak flow velocity of -3.33 m/sec in the descending aorta and the peak pressure gradient was estimated at 44.3 mmHg. The patient then underwent a transesophageal echocardiogram (TEE) that confirmed the results. A thoracic CT scan was proposed and it showed a severe coarctation of aortic arch, between the left common carotid artery and the left subclavian artery, with an axial diameter of 12 mm and a post- stenotic dilatation of 29 mm.

Considering the severe coarctation, the patient have an obvious indication for cardiovascular surgery, which is one of the most challenging conditions for surgeons and inclusive for the patient.

COMPLICATIONS OF PRIMARY ANGIOPLASTY IN PATIENTS WITH STEMI

Alice Munteanu, Silviu Dumitrescu, Liviu Chiriac, Daniel Nita, Razvan Rosulescu, Nicoleta Avram, Irina Florescu, Cristina Calcan

The major limitations of balloon angioplasty have been represented by the acute obstruction and restenosis of blood vessels. Early studies of intracoronary stents have shown that these devices are very effective in treating or preventing acute blood vessel obstruction, thus avoiding emergency surgical bypass interventions. Two randomized trials – the Benestend and STRESS (Stent Restenosis Study) have shown that stenting lesions de novo on native coronary have reduced angiographic restenosis by about 30% compared to conventional balloon angioplasty. Implanting stents leads to a luminal diameter larger than balloon angioplasty, both right after the surgery and in the follow up, thus to a lower restenosis rate.

Indications for interventional myocardial revascularization are: STEMI, NON-STEMI, stable angina, angina equivalent – dyspnea, arrhythmias, syncope, asymptomatic patients or moderately symptomatic with a medium-large surface of viable myocardia, or moderately-severe myocardial ischemia on noninvasive tests, angiographic – hemodynamically significant lesions on myocardia arteries which irrigate a viable myocardia and have a diameter higher than 1.5 mm.

Relative contraindications for interventional myocardial revascularization are: relevant comorbidities, small diameter coronaries or venous grafts with diffuse stenosis, LAD stenosis in a patient with CABG recommendation (surgery remains the preferred therapy method for such cases), PCI unsuitable coronary anatomy.

Contraindications for interventional myocardial revascularization are: patients with contraindications for antiplatelet or anticoagulant therapy, coronary lesions which prevent fully inflating the balloon and properly implanting the stent.

Vasile Candea Emergency Clinical Centre for Cardiovascular Diseases is part of the national program RO-STEMI for 5 years. During this period 3453 patients were admitted with STEMI. 2952 patients received primary PCI.

The results regarding procedures performed were: 14.5% were not admitted to angiography, 4.4% received only PCI without stent, 52.6% of patients received one stent, 21.5% 2 stents, 6.4% 3 stents and 0.6% 5 stents. The vessel responsible for STMI was in 57% of cases LAD, in 30.2% was RCA, in 11% was CXA and in 1.7% was not specified. The rate of early complications after angiography was 9.3%: 3.5% of patients suffered acute stent thrombosis, 3.2% of patients had pseudoaneurysm, 1.7% of patients had procedural failure and 0.9% had coronary dissection. At discharge 71.5% of patients had not had simptoms of heart failure. 25% of patients showed signs of left heart failure and 3.5% of patient admitted in programme died.

THE TAMING OF THE FIRE BY SURGEONS: A HISTORY OF THE CAUTERY

A. Popentiu, D. Moga, I. Barb, C. G. Smarandache, V. Marcu, A. Sabau, D. Sabau

Since the dawn of human history, the fire was present amnog us. The healers, and especialy the surgeons, used it as a very important tool in their practice. From the raw flame, the fire evolved into the modern surgical devices that use the latest in space-age technology.

A brief history of the electrosurgical equipement and accessories, together with some basic notions about the physics aspects involved is presented in our work. The evolution of equipement and the latest advances in this field are also highlited.

MANAGEMENT OF STROKES INYOUNG ADULTS AND MILITARY ACTIVITY

Emilia Furdu Lungut, Carmen Adella Sirbu, Cristina Florentina Plesa, Iustinian Porfir Furdu

Stroke is not an uncommon event in young adults (ages 30 to 45 years) accounting for an estimated 3 percent of cerebral infarctions in many series.

We are followed many cases of young military adults, in stress conditions, privacing to sleep, period of adaption to particularly conditions (missions of NATO).

Nevertheless most of the strokes could be accounted for by three categories, more or less equal in size: atherosclerotic thrombotic infarction (usually with a recognized risk factor) cardiogenic embolism (particurlaly in the post association with rheumatic heart disease, bacterial and verrucous endocarditis, paradoxic embolism through patent foramen ovale and prosthetic heart valves; and one of several nonatherosclerotic vasculopathies (arterial trauma dissection of the carotid artery moyamoya, lupus erithematosus drug induces vasculitis).

In conclusion: it"s possible that young adults, with possibly risk factors and that are in particulary condition can have stoke mor or less serious.

INTRACARDIAC EXTENSION OF INTRAVENOUS LEIOMYOMATOSIS

Ioana Alina Raduta, Lucian Mihai Ciobica, Daniela Anghel, Silviu Marcel Stanciu, Gabriel Stoicescu, Adrian Anghel, Lutuc Raluca, Ancuta Coca

Intravenous leiomyomatosis (IVL) is a rare histologically benign tumor that is biologically malignant. Less than 200 IVL cases of which 113 were involving the heart, have been reported.

This case presents a rare leiomyoma in the inferior vena cava (IVC). A 66-old woman with a history of hypertension and an episode of pulmonary thrombembolism was admitted to our departement because of an acute dyspneea and enlargement of the abdomen. The laboratory data showed normal tumoral markers (CA 125, AFP, CEA, CA19-9). The transthoracic echocardiography (TTE) relieved a large aortic stenosis and a mass of moderate echogenicity inside the right atrium (RA). The transesophageal echocardiography (TEE) relieved an elongated multilobular mass inside the RA in the bicaval 110° view. A pelvic-abdominal CT scan showed us a voluminous abdominal pelvic tumor, a tumoral mass in the IVC witch expands to RA and 1 lesion expanded into the right pulmonary artery. Spiral CT scanning with multiplanner reformation (MPR) is in work.

The patient underwent surgical excision of the tumor (9 kg), and shortly thereafter a hysterectomy and salpingo-oophorectomy. The histological examination showed a benign leiomyomas arising from the tunica media of the intramyocardial vessels. IVL extension to inferior vena cava and to the heart is one of the most challenging conditions for surgical treatment.

The patient has to be subjected to an excision of the IVC tumor/thromb or a placement of an IVC filter.

DEFINING FACTORS THAT CONTRIBUTE TO WEAKENED DETERMINATION FOR QUITTING SMOKING

Bucan Vesna, Tepsic Ostojic Vesna, Anastasovska Zorica, Zivic Bratislav

BACKGROUND: Clinical research has proven that only 1-2% of those dependent on nicotine have the required determination to arbitrarily quit smoking. Earlier studies of addictions in our country showed that the lack of information and attitude both contribute to this prevalent addiction.

OBJECTIVE: Aside from noting sociodemographic aspects, the intent is to document and assess smoking dynamics, nicotine dependence level, corresponding compulsion, overall awareness and the level of commitment to quit smoking.

METHODS: A specifically adapted questionnaire was used with 254 participant smokers that are also employees serving in military medical clinic.

RESULTS: Out of all the respondents, 65.8% is in their prime years of professional service; 62.25% has secondary education; 67.3% have family members that smoke; 95.3% have friends that smoke. On dependence, 28.2% have high, while 61% have low dependence on nicotine. Furthermore, 58.3% is aware of accompanying health consequences; 89.9% believes their smoker appearance is not socially attractive. On quitting, 60.6% of respondents tried quitting; 63.4% is actively considering quitting; 35.8% believes that they could quit at any moment. Lastly, 50% never accepted any related help or advice.

CONCLUSION: Based on our findings, low nicotine dependence level here indicates that persistence in smoking can be contributed to habits and other sociocultural factors. Additionally, high percentage of respondents believed that they could quit smoking at any moment and without any professional help. This highlights a substantial lack of critical thinking as well as misapprehension about commitment and determination levels that are needed in the process of addiction withdrawal.

WAR WOUNDS OF THE HAND SURGICAL TREATMENT - OUR EXPERIENCE

Kozarski Jefta, Djordjevic Boban, Milicevic Sasa

The significance of war injuries of the hand is the direct consequence of the incidence of these wounds and the resulting disability. According to the World War II data, the incidence of hand injuries is approximately 7% of all war injuries. Inadequate management and surgical treatment produce severe disability. High speed projectiles (above 600 m/s) cause extensive tissue defects on their course through the tissue. Around 50% of these wounds are associated with tissue defect. War wounds caused by expolosive devices are almost always associated with the defect of tissue, tendons, nerves, bone and joint injuries, with irregular „pockets” filled with secondary bone projectiles. These injuries are most commonly multilocular and associated with blast injury, but can also be penetrating.

This paper aims at presenting surgical management of war injuries of the hand according to the principles of war surgery and to establish the place of plastic surgery in the management of war injuries of the hand.

Methods: Management of the wounds with tissue defects was done in accordance with the principles of war surgery doctrine. Since the wounds had been primarily contaminated with polymorphic bacteria, all the patients received antitetanus and antibacterial treatment. Surgical treatment of war wounds of the hand involve:

1. Primary surgical management. Primary surgical management of the injured hand is performed within 24 hours of wounding using «economical debridement» of devitalized tissue in accordance with war surgery principles all the way to clinically vital tissue. Dislocated osseous fragments are repositioned and periosteal fragments are removed. After debridement, the hand is immobilized in plaster of Paris in neutral position. In accordance with war surgery doctrine, osteosynthesis of the phalanges and metacarpal bones is not allowed, but we used Kurschner wires in some cases for internal immobilization.
2. Additional surgical management (if required). Subsequent tissue necrosis, after primary management of the wound, caused by pathophysiological mechanisms of wounding and found on «second look».
3. Delayed wound closure. For wound closure we used partial thickness skin grafts as primary or final reconstruction procedure. Different flap types were used: local skin flap, fasciocutaneous, fascial, muscle flap, arterial, reverse, regional, direct distant or free microvascular flaps.
4. Secondary reconstructive procedures on damaged deep structures. Reconstruction of deep hand structures with complex tissue defects (tendons, nerves, bones, joints) was performed two months or later after wound closure. For injured tendon reconstruction we commonly used tendon grafts, and for injured nerves additional neuroraphy was done or we used nerve grafts. For injured bones we used bone grafts and for joints we utilized capsulotomy or arthrodesis.

Results: At the Clinic for Plastic Surgery and Burns, Military Medical Academy, 37.6% of the wounded had war wounds with tissue defects caused by firearms. War wounds caused by explosion were recorded in 55.2%, while in 7.1% some thermical agent was the cause. More than half of the wounds treated at the Military Medical Academy were associated with defects of various tissues. War wounds with tissue defects were most common on the extremities (72.3%; upper, 35.8%), while in 16.6% the hand was affected. Phalanx injuries were detected in 12.44%, while metacarpal bones were injured in 10.97%. Direct suture was used to close soft tissue defects in 12.68% and skin grafts were used as either temporary or definitive reconstructive procedure in 40.86%. We made use of various flaps: local – cutaneous (fasciocutaneous, fascial, arterial, and reverse) in 9.89%, regional in 16.78%, distant direct in 9.89% or free microvascular ones in 2.37% of the wounded. Finger amputations or surgical management of the explosion-caused hand amputation were performed in 7.52% (5). Reconstructive surgical procedures on deeper structures in the treatment of war wounds with complex tissue defects (tendons, bones, joints) were performed two months after the treatment of soft tissue defects. Tendon graft was most commonly used in the reconstruction of injured tendons; additional neurorraphy or nerve graft was used in nerve reconstruction, while bone structures were reconstructed with skin grafts. Transpositional tendoplastics was utilized after tendon, bone and nerve reconstructions in order to improve hand function. Various prosthetic devices were used for amputated hands.

Conclusion: War wounds of the upper arm and the hand with tissue defects make up more than one third of all war wounds. Due to highly specialized anatomic structure characterized by a high ratio of skin surface and the volume of deeper tissue structures and complex functional mechanisms it possesses, the hand is an organ the injury of which requires specific diagnostic and surgical measures of several tissue structures (tendon, nerve, bone, joint). Since war injuries of the hand are mostly complex and associated with tissue defects and always cause a degree of permanent disability, it is essential that a surgeon has good command of the reconstruction methods in wound closure in the reparatory phase, as well as of appropriate atraumatic technique in the reconstruction of tendons, nerves, bones in the reconstruction phase. Plastic and reconstruction surgery has a very prominent role in the surgical management of war wounds of the hand, during primary and primary delayed surgical treatment, in wound closure later on, and in the phase of reconstruction of deep tissue defects.

MICROSURGERY IN HEAD AND NECK RECONSTRUCTION

Kozarski Jefta, Milicevic Sasa, Djordjevic Boban

The head and neck region’s defects presents a great challenge for reconstructive surgeons.We report our experience in the treatment of 54 patients with head and neck free flap reconstruction (15 tumor resection, 2 with burns, one with postburned neck’s contracture, and 36 wounded patients).

We applied six fibular, four scapular, three radial forearm, one dorsalis pedis and one latissimus dorsi free flaps. Fibular and radial forearm flaps were used for reconstruction of the mandibular bone, while scapular, dorsalis pedis and latissimus dorsi flaps were used for filling the defects of the mouth floor, orbital or maxilar region. We used scapular free flaps for covering the burn of the face, scapular and parascapular flaps for the covering of the neck and exposed mandibular bone and preexpanded scapular and parascapular flap for correction of the neck’s postburn contracture.

We treated extensive wartime tissue defects of the lower third of the face with composite free flaps: seven scapular, nine radial forearm and twenty fibular flaps. Length of the mandibular defects varied from 5-16 cm. The bones were fixed by wire, miniplates or external fixation. The skin parts of the flaps were used in reconstruction of lower lip, chin and/or cheek, respectively. Vascular pedicles of the flaps were microsutured to either the superior thyroid, facial or external maxillary and carotid vessels.

Our experience and results in microvascular reconstruction in the head and neck region support the use of this reconstructive method in peace-time pathology and in treatment of war wounds with complex tissue defect.

VACUUM-ASSISTED COMPRESSION THERAPY SETTINGS FOR APPLICATION IN PLASTIC SURGERY

Milicevic Sasa, Stepic Nenad, Kozarski Jefta, Djordjevic Boban

OBJECTIVE: Nowadays vacuum-assisted compression therapy plays an important role in plastic surgery, especially for preparing good basis for covering skin defects.

MATERIALS & METHODS: During the last four years, we treated 30 patients with different etiology skin defects, using different vacuum-assisted compression therapy settings. Depending on the location and depth of the wound, the presence of an infectious agent in the wound and the general condition of the body, vacuum-assisted compression therapy was administered at once or more times before skin transplantation.

RESULTS: The whole settings was corrected due to the granulation response. In 10 patients we were used vacuum-assisted compression therapy in intermitent mode and pressure of 125 mm Hg, in the next 10 patients we used it in continuous mode and in the rest of patients we used it in both intermitent and continuous mode. The healing time was significantly lower in the last mode.

CONCLUSION: Vacuum-assisted compression therapy plays an important role in the formation of granulation tissue, but using combination of intermitent and continuous mode gives better results than any single mode.

INCIDENCE OF PAPILLARY MICROCARCINOMA OF THYROID GLAND IN PATIENTS WITH SURGICALLY TREATED GRAVES' DISEASE – AN INSTITUTIONAL EXPERIENCE

Kovacevic Bozidar, Ignjatovic Mile, Petrovic Milan, Karajovic Jelena, Kuzmic Jankovic Snezana, Cerovic Snezana

Papillary carcinoma (PC) is the most common malignant tumor of thyroid gland with the constant increase of incidence in different parts of the world. In total number of diagnosed PC an important part belongs to its histological variant – papillary microcarcinoma (PMC). Papillary microcarcinoma is a tumor measuring less than 10 mm and usually represents an incidental finding at surgically removed thyroid glands for benign diseases including all types of hyperfunctional conditions. Association between thyroid carcinoma and Graves' disease is controversial with significant difference in reported incidence from 0.5% - 32%. The aim of our analysis is to determine the frequency of PMC in surgically treated patients with Graves' disease. Seven hundred eighty four thyreoidectomias were performed in two years period at Military Medical Academy. PC is detected in 357 patients from which 157 are PMC (44.6%). We analyzed an incidence of PMC in 109 surgically treated patients with Graves' disease and we detected microcarcinoma in 20/109 cases (18.34%) and 20/157 (12.73%) from total number of diagnosed PMC. Thyroid carcinomas in Graves' disease are not rare and most of them are papillary microcarcinomas. Our results show high prevalence of PMC (18.34%) at surgically treated patients with Graves'disease wich represents one of the highest prevalences in incidentally discovered carcinomas in this pathological condition.

RECANALIZATION RATE AND CLINICAL OUTCOMES AFTER THROMBOLYSIS IN PATIENTS WITH OCCLUSION OF MAJOR CEREBRAL ARTERIES IN STROKE UNIT, MMA

Pasovski Viktor, Grunauer Marija, Veljancic Dragana, Krsmanovic Zeljko, Labovic Boban, Lepic Toplica, Raicevic Ranko

INTRODUCTION: Intravenous administration of recombinant tissue plasminogen activator is still the most common therapy for the treatment of acute ischemic stroke (AIS) but recanalization rate of large cerebral arteries occlusion largely does not lead to the desired therapeutic outcome.

OBJECTIVE: Evaluation of results of intravenous thrombolysis in the patients with large vessel occlusion (anterior and posterior circulation stroke) comparing to those without it.

METHODS: From February 2013 until January 2016, we analyzed 71 patients treated with thrombolytic therapy at MMA in retrospective study design.We divided patient in two group, those with occlusion of anterior and posterior circulation present in major arterial branches (ACI, ACM (M1), AV (V4) and a. Basilaris) confirmed with CTA, with the other group of trombolysed patients.

RESULTS: We compared average age of the patients as well as percentage of males and females and average door- to needle time was estimated in minutes in both groups. Level of neurological dysfunction was being established with NIHSS score before and 24 hours after the applied thrombolytic therapy and thrombolytic effect of rtPA was being estimated by CTA that had been done before and 24 hours after the treatment, with estimation of success of recanalization. The frequency of cardioembolic stroke, atherosclerosis of large blood vessels as well as the existence of small blood vessels disease are shown as percentage in overall etiology of AIS. Patients with mRS 0-2 presented the group with favourable treatment results and patients with mRS 3-6 the group with unfavourable results. Complications of applied thrombolytic therapy – haemorrhagic transformation, symptomatic ICH and system haemorrhage are presented in percentage (final results of the study are in the final stage of stat! istical data processing).

CONCLUSION: Our results suggest limited recanalization potential of IVT in acute large vessel occlusive strokes and imposes the need for introduction of endovascular treatment in routine practice whose effectiveness is confirmed by recent published studies.

THE CORRELATION BETWEEN TICK BITES AND ECOLOGICAL INDEX OF LYME DISEASE RISK IN BELGRADE PARKS

Krstic Milena, Lazic Srdjan, Stajkovic Novica, Djordjevic Milutin, Radakovic Sonja, Jadranin Zeljko, Mladenovic Jovan

OBJECTIVE: The goal of this paper was to determine the value of ecological index on green areas in 9 parks in Belgrade and establish the correlation of this index with tick bites in humans.

MATERIALS & METHODS: Ticks were collected monthly by using the flag hour's method and the infection rate was determinated by dark field microscopy. Point values were assigned to certain parameters and ecological index was evaluated for each habitat. The data on tick bites from the surveyed habitats were obtained from the Protocol of patients bitten by ticks of the Institute of epidemiology, Military Medical Academy (Belgrade).

RESULTS: In Belgrade parks high potential risk of Borrelia burgdorferi transmission on average was determined, while the actual risk on most of habitats was categorised as the limited risk. Statistically, in terms of actual risk values, the 2 habitats, Hajd Park and Topčider, were significantly different.

RESEARCH OF MORPHOLOGICAL AND HISTOCHEMICAL CHARACTERISTICS OF FAMILIAL ADENOMATOUS POLYPOSIS

Cvetkovic Marija, Jovanovic Jelena, Veselinovic Marija

INTRODUCTION: FAP is a hereditary gastrointestinal polypose, complicated by malignant alteration in 100% cases. It is greatful for studing histogenesis of colorectal cancer, which there exist in thousants.

AIM: Project's aim is studing gradual carcinogenesis where are included lots of adenomatouse.

MATERIAL AND METHODS: Operating material, total colectomy, is surgically removed because of 100% malignant alteration of adenomatouse. After tissue treatment, material is processed and colored by clasical HE method, histochemical PAS method and combinated HID-AB, pH= 2,5 method. Histological and mucinous-histochemical characteristics of 20 adenomatouse are studed.

RESULTS: In surroundings of the present colorectal mucinous adenocarcinoma are found mucuinous secretion villous adenomatouse, that affirns the hypothesis of histogenesis mucinous adenocarcinoma from villous adenomatouse.

CONCLUSION: The conclusion is that malignant potencial of removed adenomatouse which were around cancer, is influenced by the number of adenomatouse, their histological type, as well the level of dysplasia.

A RARE TUMOUR IN ANORECTAL REGION: PRIMARY ANORECTAL MALIGNANT MELANOMA

Akgun Veysel, Ozen Alptug, Hamcan Salih, Battal Bilal

OBJECTIVE: Primary anorectal malignant melanoma (AMM) is very rare when compared with the adenocarcinoma of this region. It has imaging clues that can be important in treatment planning. The aim of this report is to represent magnetic resonance imaging (MRI) findings of a case with primary AMM.

MATERIAL AND METHOD: A 64-year-old male consulted to our service complaining for rectal bleeding lasting for 3 months. A pigmented tumour visualized in rectum during colonoscopy, biopsy was taken and the pathologic diagnosis was malignant melanoma. Pelvic MRI and PET-CT were scheduled to demonstrate the extent of the tumour.

RESULTS: A mass lesion, which was hyperintense in T1A and hypointense in T2A relatively to muscular tissue, involving a 10 cm segment of rectum was revealed. Adipose tissues between the mass and prostate and seminal vesicles were obscured. 8 lymph nodes were detected within mesorectal adipose tissue.

CONCLUSION: Primary AMM is seen in less than 4% of all anorectal cancers. It is more aggressive and seen more commonly in males. In contrast to adenocarcinoma, AMM is hyperintense in T1A and hypointense in T2A due to its melanin content. MRI imaging can be very useful for differential diagnosis of AMM from other malignant tumours due to distinct signalization specialties.

BILATERAL KÖHLER DISEASE

Balyemez Ugurcan, Verim Samet, Inan Kemal, Ors Fatih

OBJECTIVE: Köhler's disease is the self limiting avascular necrosis of navicular bone. The disease typically affects boys, but girls could also be affected. It's commonly seen at 4-5 years of age and is usually unilateral. The last ossifying tarsal bone is navicular bone and it could be compressed by already ossified talus and cuneiform bones. The compression causes an obstruction in navicular's blood supply. Thus, avascular necrosis occurs. We aimed to present a patient with bilateral avascular necrosis of navicular bones.

MATERIAL AND METHODS: 5-year old male patient, presented with pain in his both ankles. Patient was examined by bilateral anteroposterior and lateral ankle radiographs.

RESULTS: His physical examination revealed pes planus deformity and bilateral anteroposterior and lateral ankle radiographs were obtained which revealed bilateral significant volume loss, sclerosis and deformation of navicular bone and diagnosed as Köhler's disease.

CONCLUSION: Köhler disease is the avascular necrosis of navicular bone, effecting typically boys at 4-5 years of age. Although Köhler's disease is a self limiting entity, early diagnosis and treatment of the patient is important in limiting the symptomatic period of disease. It could be diagnosed in early stages by plain radiograophs and must be in differntial diagnosis in patients with supporting symptoms. Moreover, disease could rarely occur bilaterally as in our case.

INVASIVE DUCTAL CARCINOMA DERIVED FROM POSTOPERATIVE PARENCHYMAL DISTORTION AREA

Balyemez Ugurcan, Hamcan Salih, Uğurel M. Sahin, Tasar Mustafa

OBJECTIVE: In mammographic examinations architectural distortion areas are classified into two groups according to the presence of a mass density at the center of distortion. Lesions with mass density are called “white star", and others are called “black star". Generally, black star areas are used to define benign entities such as radial scars or postoperative paranchymal changes. We aimed to present the follow up mammographies of an invazive ductal carcinoma case derived from postoperative paranchymal distortion area.

MATHERIAL AND METHOD: A 45-year old woman with a history of excisional biopsi from breast and a positive family history in her mother examined with mammography, ultrasonography and excisional biopsy.

RESULTS: Pleomorphic microcalsifications were detected in screening mammography. Radioguided occult lesion localization (ROLL) was performed with mammography since sonographic examination revealed negative in microcalsification area. Excisional biopsy revealed as “fibrocystic changes”. Following that, follow up mammographies were reported as postoperative paranchymal distortion (black star) at the biopsy area for 4 years. The last mammography of the patient showed an increase in density and size of the black star area. Although there is fat necrosis type course calsifications, the increase in size and density were considered to be suspicious and a second excisional biopsy was performed. The pathological result of the biopsy revealed invazive ductal carsinoma.

CONCLUSION: Paranchymal distortion areas on mammograms are associated with benign entities such as radial scars, complex sclerosing lesions, postoperative changes or malignancies. Benign paranchymal distortions particularly postoperative paranchymal distortions as in our case, don’t have a central density, seen as a radiolusent distortion area and named “black star”. A decrease in density and size of the area is expected in postoperative paranchymal distortions. If an increase is detected in these parameters, radiologist should suspect of malignancy and perform a biopsy.

A RARE ANOMALY: DIVERTICULA IN THE RIGHT VENTRICLE

Balyemez Ugurcan, Hamcan Salih, Ors Fatih, Bozlar Ugur

OBJECTIVE: Right ventricular diverticules are extremely rare and generally associated with congenital anomalies. Aneurysms or pseudoaneurysms are in the differential diagnosis of the diverticules. Ventricular diverticules are differentiated from these entities by narrow neck and synchronized contraction with ventricle. We aimed to present CT findings of a case with right ventricular diverticula and concomitant pericarditis.

MATERIAL AND METHOD: A 22-year old male patient with a history of pericarditis and presented with chest pain. He was examined by cardiac CT angiography study with 320-slice CT.

RESULTS: Pericardial effusion measuring 9mm, pericardial thickening and pericardial contrast enhancing were detected. In addition to these findings, a 8x12mm sized diverticule was seen on apical section of the right ventricular wall.

CONCLUSION: Ventricular diverticules and particularly right ventricular diverticules are extremely rare anomalies. Cardiac CT is a useful method in detecting ventricular diverticules since it enables multiplanar and functional imaging.

ACTIVE BREATHING CONTROL (ABC) FOR RESPIRATORY MOTION MANAGEMENT IN RADIOTHERAPY OF THORACOABDOMINAL TUMORS

Beyzadeoglu Murat, Sager Omer, Gamsiz Hakan, Dincoglan Ferrat, Uysal Bora, Demiral Selcuk, Elcim Yelda, Gundem Esin, Onal Elif, Ekmen Ayca, Askin Semiha, Taspinar Kadir, Dirican Bahar

OBJECTIVE: Respiratory motion management in radiotherapy has gained utmost importance recently with tremendous recent progress in imaging technology and treatment delivery techniques. Active Breathing Control (ABC) system offers a viable strategy for motion management in radiotherapy of thoracoabdominal tumors. Herein, we aim to review the rationale, indications, and potential advantages of ABC system which is the primary method of managing respiratory motion in our department.

MATERIAL AND METHOD: ABC system has been developed for facilitating reproducible breath-holding for respiratory motion management. Moderate-deep inspiration breath-holding with the ABC system has widespread utilization for management of thoracoabdominal tumors. An education session is typically performed for patients appropriate for 4-D radiotherapeutic management. After acquisition of treatment planning images at the breath-holding phase, treatment planning is done and patients undergo 4-D treatment at moderate-deep inspiration breath-holding. Image guidance is typically used in conjunction with 4-D radiotherapy to achieve excellent treatment precision.

RESULTS: Practical applications of 4-D radiotherapy using the ABC system includes treatment of left-sided breast cancers, locally advanced lung cancers, early stage non-small cell lung cancers, pulmonary oligometastases, adrenal metastases, liver metastases, and locally recurrent pancreatic cancers. Primary benefits of using ABC system include minimization of breathing-induced motion thereby allowing the use of reduced internal margins, elimination of image artifacts, and minimizing heart exposure during irradiation of left-sided breast cancers.

CONCLUSION: Improved accuracy and precision with the ABC system may possibly translate into enhanced treatment efficacy and normal tissue sparing with great potential for optimizing the therapeutic ratio in radiotherapy of thoraco-abdominal tumors.

RUPTURE OF HYDATIC CYST OF LIVER AND LUNG IN CHILDREN

Caliskan Bahadir, Altan Bilal, Bulut E. Burak, Demirbag Suzi, Surer Ilhami

Introduction: Hydatid cyst disease is a parasitic infection commonly caused by Echinococcus granulosus. In hydatid cysts of the liver and lung, spontaneous or traumatic perforation can occur. Perforation may arise next to the bile ducts, bronchus, neighboring organs, or directly into the abdominal cavity. Treatment of ruptured hydatid cyst requires emergency surgical intervention.

Case Report: The children’s presenting complaints were abdominal pain, nausea, skin eruptions, vomiting, coughing and urticaria. Diagnosis was achieved through physical examination, blood tests, lung graphy, USG, and CT. All patients underwent surgery after presentation. Two cysts were located in the liver, and one was in the lung. Treatment involved cystectomy and drainage. The abdomen of two patients was washed with 3% NaCl, and the cyst pouch was irrigated with hypertonic saline (20%) followed by isotonic saline.

Discussion: Perforation of the hydatid cyst in the peritoneal cavity is a very rare event, even in areas where the disease is endemic. The cyst may be ruptured after a trauma, or spontaneously as a result of increased intracystic pressure. Ultrasonography and CT are the main diagnostic methods. Emergency surgery is the main treatment for intraperitoneal and intrabronchial rupture of hydatid cysts, and medical treatment should be given postoperatively.

SURGICAL EXCISION OF TAILGUT CSYT IN A CHILD DIAGNOSED WITH MAGNETIC RESONANCE

Caliskan Bahadir, Bulut E. Burak, Bayir Y. Burak, Guven Ahmet, Demirbag Suzi

Introduction: The Tailgut cyst (cystic hamartoma) is rare congenital anomalies that are believed to arise from the embryonic hindgut. The most important complications of these cysts are infection with secondary fistulization and malignant degeneration. MRI has evolved to be the investigation of choice for the evaluation of presacral tumors as it can provide excellent anatomic detail and soft tissue contrast

Case Report: 5 year old girl presented with abdominal pain. Clinical examination revealed no significant abnormalities. During laboratory and radiologic investigation a retrorectal cystic mass nearly 2 cm and an urolithiazis nearly 7 mm in the right kidney were found incidentally by abdominal US. Digital rectal examination revealed a soft cystic lesion on the right side of retrorectal area. Serum alphafetoprotein and beta hCG were normal. MRI lumbo-sacral spine showed well defined multicyistic lesion nearly 2 cm which is brightly hypertense on T2W1 and hypotense on T1W1 posterior to sacrum and coccyx with no evidence of connection to the thecal sac. Complete excision of the cyst was done. Histopathology report shows cyst wall partially lined with stratified squamous epithelium and cyst wall shows spaces lined by cuboidal epithelium and nerve bundles with no evidence of malignancy suggestive of tailgut cyst.

Discussion: Tailgut cysts are rare congenital anomalies derived from the remnants of the embryonic hindgut. MRI is a non-invasive useful imaging investigation with high diagnostic accuracy when a retrorectal cyst is suspected. Complete surgical excision is the treatment of choice as this provides a definite diagnosis and prevents possible complications such as infection, fistula formation and malignant degeneration.

VAGINAL ATRESIA, UTERUS DIDELPHYS AND HEMATOMETRACOLPOS: A RARE CAUSE OF COLIC ABDOMINAL PAIN IN A TEENAGE

Caliskan Bahadir, Fidanci Ulas, Bulut E. Burak, Bayir Y. Burak, Guven Ahmet, Demirbag Suzi

INTRODUCTION: Hypoparathyroidism, deafness and renal dysplasia (HDR) syndrome is an autosomal dominant genetic disorder characterized by hypoparathyroidism, sensori-neural deafness and renal dysplasia. Rarely, genital neoplasms, hematocolpos, hematometrocolpos, and renal anomalies are reported in association with didelphys uterus.

CASE REPORT: A 13-year-old girl was admitted to our clinic with a complaint of severe abdominal pain in the right lower quadrant of the abdomen for a duration of 2 days. Physical examination revealed a suprapubic mass in the abdomen. A gynecologic examination revealed vaginal atresia. The patient has the diagnosis of HDR syndrome and she has right atrophic kidney, left cohen operated kidney, deafnesss and low IQ levels. The Abdominal US examination showed a cystic lesion in the adnexial region and absence of the right kidney. An MRI revealed a uterine didelphys, bicollis, and lower atresia of duplicated hemivaginas. There was a collection of fluid that exhibited a high signal intensity on T1 and a low signal intensity on T2 sequences, both in the uterus, and in the atretic vagina referred to as hematometrocolpos. Ultrasound-guided hematometrocolpos aspiration was performed. One week later Hysterectomy and bilateral salphingectomy was performed because of the family's preference. The girl was discharged uneventfully on post operative day 5.

DISCUSSION: Complex congenital anomalies of the female reproductive tract are uncommon and occur most commonly as a result of Mullerian dysregulation during embryogenesis. Rarely, genital neoplasms and endometriosis are reported in association with cases of didelphys uterus. Detailed examination of urogenital system is important. The definitive treatment depends on the complexity of anomaly.

A RARE CARDIAC PATHOLOGY, ACQUIRED SINUS VALSALVA ANEURYSM AND RUPTURE: 320 DETECTOR ROW CT FINDINGS

Celikkanat Serhat, Hamcan Salih, Bozlar Ugur, Tasar Mustafa

OBJECTIVE: Sinus valsalva aneurysm (SVA) was first discovered in 1835 by Hope in an autopsy examination and it was first described in 1840 by John Thurnam. This rare pathology is usually congenital. The pathophysiology of this disorder is thinning and seperation between aortic media layer and annulus fibrosus that leads pathologic dilatation. Spontaneous rupture is usually occur in 3rd and 4th decades of life. If it is not operated in emergency conditions it may be fatal. We aimed to present imaging findings of a rare 52-year-old male with acquired SVA rupture.

MATERIAL AND METHOD: Coronary angiography was made due to sudden onset of chest pain.

RESULTS: Right coronary sinus was enlarged than the other sinuses. An 8 mm defect in the anteroinferior side of the right SV that was fistulized to right ventricle was determined.

CONCLUSION: Incidence of this rare disorder is 0.14-0.23%. Syphilis, bacterial endocarditis, trauma, atherosclerosis, media necrosis and previous operations are the acquired reasons of this commonly seen as congenital pathology. In our case SVA and rupture of it that are based on atherosclerotic disease were present. SVA ruptures are commonly develop in right coronary sinus and drain into the right ventricle. More rarely thrive in the non-coronary sinus and can be drained into the right atrium. Whether it fistulizes to pericardium severe cardiac tamponade may be thrive. 320 detector row CT is not only the choice of modality to diagnose this entity but also the other cardiac abnormalities.

A RARE IMITATOR OF MALIGNANCY, ISOLATED HEPATIC TUBERCULOSIS: MRI FINDINGS

Celikkanat Serhat, Akgun Veysel, Battal Bilal, Tasar Mustafa

OBJECTIVE: Hepatic tuberculosis (TB) can be seen in secondary to miliary TB and its incidence is 50-80% in disseminated TB cases. But, isolated hepatic TB is a very rare entity and less than 100 cases were reported in literature. In this article, we aimed to present an isolated hepatic TB that mimics liver malignancy.

MATERIAL AND METHOD: A 21-year-old male patient with high fever was referred to Radiology Department for abdominal ultrasound (US). Multiple hypoechoic lesions were determined in liver and for further evaluation dynamic liver magnetic resonance imaging (MRI) was performed.

RESULTS: Hepatomegaly and multiple T1 hypointense and T2 hyperintense lesions 0.5-1.5 cm in size were determined. These lesions were enhancing peripherally on post-contrast images.

CONCLUSION: There are 5 types of liver tuberculosis: miliary TB, pulmonary TB with hepatic involvement, focal tuberculoma or abscess, primary hepatic TB and TB cholangitis. Isolated hepatic TB is the rarest one among these subtypes (1%). Hepatic TB can mimic primary hepatocellular carcinoma, cholangiocarcinoma, liver abscess and metastasis. Therefore, the diagnosis of isolated hepatic TB can be challenging. In cases with multiple focal hepatic lesions that are unexplained even though it is rare but hepatic TB should be kept in mind. Definitive diagnosis can be made by demonstrating caseous granuloma histopathologically.

A RARE MALIGNANCY IMITATOR: PSEUDOMALIGN MYOSITIS OSSIFICANS

Celikkanat Serhat, Akgun Veysel, Battal Bilal, Tasar Mustafa

OBJECTIVE: Myositis ossificans (MO) is a benign pathology that is characterized by heterotopic ossification within muscles. In this article, we aimed to present magnetic resonance (MR) features of a case with MO and prostate cancer.

MATERIAL AND METHOD: An 83-year-old patient with pathologically proven prostate cancer had received radiotherapy and chemotherapy previously. Due to elevated prostate specific antigen (PSA) values, 99mTc-Methyl diphosphonate bone scintigraphy and dynamic prostate MRI were performed.

RESULTS: There were T2 hyperintense areas adjacent to pubic rami and callus formation in both pubic rami that were not calcified yet. In scintigraphy examination these areas showed mild uptake values suggestive of osteoblastic metastasis of prostate cancer. Nevertheless, another suspicious lesions with low signal intensity on T2 sequence were detected within the insertion of adductor and gracilis muscles.

CONCLUSION: MO is commonly seen in young and after trauma. There are four types of MO: myositis ossificans circumscripta, MO associated with neurologic disorders, fibrodysplasia ossificans progressiva and pseudomalign myositis ossificans (PMO). PMO is rarely seen and often not associated with trauma. PMO has various imaging features related to its clinical grade. Especially early and mid-term PMO can mimic metastatic lesions in patients with known malignancy and making the diagnosis difficult.

A RARE PELVIC MALIGNANT TUMOR, AGRESSIVE ANGIOMYXOMA: 320 SLICE CT FINDINGS

Celikkanat Serhat, Akgun Veysel, Battal Bilal, Tasar Mustafa

OBJECTIVE: Agressive angiomixoma (AAM) is a rare mesenchymal neoplasm that originates from pelvic and perineal soft tissues. It was first described by Steeper and Rosai in 1983 in the literature. This extremely rare entity is most commonly seen in female adults, shows agressive behaviour and invades adjacent structures. In this poster, we aimed to present CT findings of a 41-year-old female with AAM.

MATERIAL AND METHOD: A 41-year-old female patient was admitted to Gynecology Department with abdominal pain. Ultrasound examination (US) was performed and a pelvic mass was determined. For further evaluation the patient was referred to Radiology Department for the CT scan.

RESULTS: A homogenous enhancing mass that occupies right obturator region was seen in the CT images. The mass was 11x5x6 cm in size, invading adjacent structures and deplacing uterus and urinary bladder to left.

CONCLUSION: AAM has homogenous structure appears as hypodense according to muscle tissue in non-contrast-enhanced CT images and usually invades surrounding structures. It shows diffuse and homogenous contrast enhancement. In magnetic resonance imaging (MRI), it shows low signal intensity in T1 and high signal intensity in T2 sequences. Its radiological diagnosis may be challenging therefore it is important to understand the clinical features of AAM. In cases with pelvic malignancies that has agressive behaviour this rare malignancy should be considered.

EPIPERICARDIAL FAT NECROSIS, A RARE PATHOLOGY THAT MIMICKS PULMONARY EMBOLISM: 320 SLICE CT ANGIOGRAPHY FINDINGS

Celikkanat Serhat, Hamcan Salih, Bozlar Ugur, Tasar Mustafa

OBJECTIVE: Epipericardial fat necrosis is a rare pathology among the causes of chest pain. The major symptom of this entity is an acute pleuritic chest pain like a stabbing wound and it may mimic pulmonary embolism. Epipericardial fat necrosis is mostly located on the left side of cardiophrenic angle. We aimed to present CT angiography (CTA) findings of an epipericardial fat necrosis.

MATERIAL AND METHOD: A 22-year-old male patient was admitted to Emergency Department with a pleuritic chest pain. There was no history of trauma to explain the chest pain. Due to a suspicion of pulmonary embolism, the patient was referred to the Radiology Department for computed tomography angiography (CTA) imaging of pulmonary arteries. Follow up imaging is done a month later.

RESULTS: There was a mass in fat attenuation localized in the right side of anterior mediastinum adjacent to the right side of pericardium and pleura. The lesion was encapsulated, 3x2 cm in size and contained dense strandings which suggests inflammatory processes. A minimal pleural effusion was also detected in the right lung.

CONCLUSION: The majority of the cases have prior dyslipidemia or obesity. This rare pathology is usually detected incidentally and it may be misdiagnosed by emergency staff and/or overlooked by radiologists. Epipericardial fat necrosis should be kept in mind alongside with the other possible anterior mediastinal pathologies that cause chest pain. Multislice CT imaging is helpful to diagnose not only epipericardial fat necrosis but also other wide range of pathologies that occupy anterior mediastinum.

TWO CASES OF A RARE RENAL ANOMALY: SEGMENTAL MULTICYSTIC DYSPLASTIC KIDNEY: MRI FINDINGS

Celikkanat Serhat, Akgun Veysel, Battal Bilal, Tasar Mustafa

OBJECTIVE: Multicystic dysplastic kidney (MCDK) is a rare renal anomaly that is composed of multiple cysts separated by dysplastic renal parenchyma. The incidence of this entity that impairs renal function is 1:4300. Segmental MCDK is a rare form of this disorder and can be seen in 4% of all children with MCDK. Less than 50 cases were reported in pertinent literature. In this article, we aimed to present imaging findings of this rare entity.

MATERIAL AND METHOD: A 3-year-old male with recurrent fever and elevated acute phase reactants and a 39-year-old male with elevated urea and creatinine values were evaluated by abdominal ultrasound (US) and magnetic resonance imaging (MRI).

RESULTS: In the first case lobulated multiple cystic lesion with thin septations in the upper pole of right kidney was depicted. Mild enhancement was determined in these septations in post-contrast images. In the second case, multiple cysts separated by thin septations in the upper pole of right kidney was determined.

CONCLUSION: Segmental MCDK is usually unilateral and effects upper pole of kidney. Frequent and recurrent urinary infection, abdominal mass and renal function impairement may be seen in effected patients. This rare entity is common in pediatric group. Radiologic features of Segmental MCDK are: Multiple cysts, thin septations, segmental involvement, lack of renal function in this region and normal parenchyma in the rest of kidney.

A RARE AUDITOR NEUROPATHY, ISOLATED COCHLEAR NERVE ABSCENCE: 3T MRI FINDINGS

Celilov Ramil, Verim Samet, Battal Bilal, Akgun Veysel

OBJECTIVE: Cochlear nerve growth retardation or developmental absence is a condition that goes along with congenital hearing loss. The incidence of congenital hearing loss is reported about 1-3: 1000. Therefore, the existence of cochlear spiral ganglion cells and nerves to transmit signals to the brain have to be determined before cochlear implanting procedure. Also MRI for posterior fossa has significant role for preoperative detection of cochlear nerve aplasia, hypoplasia or agenesis. On this article we present magnetic resonance (MR) images of an 11-year-old pediatric patient with auditory neuropathy.

MATERIAL AND METHOD: Male pediatric patient with left hearing loss, was referred to our clinic for posterior fossa MR imaging.

RESULTS: On posterior fossa MR images, while intracanalicular and cisternal segments of 7th cranial nerve were found to be normal, the left cochlear nerve was not observed in at all. Left cochlear aperture was small and narrow. Middle and upper rotations of cochlea have dysplastic appearance.

CONCLUSION: Cochlear nerve absence can be isolated or associated with the undevelopment of cochlear structures. CT can show the absence of the bone channel at the cochlear turn and MRI helps to detect the absence of cohlear nerve. Auditor nerve absence is seen in 1% of cases with developmental deafness. This pathology is diagnosed by the absence of auditor nerve in internal auditor channel. Transverse size of the inner ear and size of the vestibulocochlear nerve thickness is essential for surgical applications.

A RARE INTRACRANIAL PATHOLOGY, RUPTURED DERMOID CYST: MRI FINDINGS

Celilov Ramil, Celikkanat Serhat, Hamcan Salih, Battal Bilal, Tasar Mustafa

OBJECTIVE: Intracranial dermoid cyst (IDC) is one of the rare developmental ectodermal cysts and can display the characteristic features. 0.5% of all primary intracranial tumors are IDC's. This pathology may not be symptomatic over years and can be detected incidentally. These cysts can cause complications if they rupture into subarachnoid or intraventricular spaces. We aimed to report MRI findings of a case with a rupture of an ICD.

MATERIAL AND METHOD: Because of the 3 months ongoing story of night shift a 27-year-old patient has been referred to our clinic to be evaluated by computed tomography (CT). After detection of multifocal intracerebral lesions with fat densities, MRI was performed.

RESULTS: Cystic soft tissue mass was seen in the anterior cranial fossa, mostly consisting of fat tissue, about 60x35x48 mm in size. Multifocal fat tissue formations secondary to rupture of a dermoid tumor were observed on both sides of serebral hemispheres, also in the Sylvian fissure and the basal cisterns.

CONCLUSION: Such as other epidermoid cysts the IDC's are surrounded by mature squamous epithelium. They are usually located in the midline. Usually seen in the younger people and showing demographic peak in the second and third decades. Cyst rupture usually cause seizures, although it may cause deadly chemical meningitis, coma, cerebral vasospasm and infarction. Depending on the fat content ICD's have hyperintense signal on T1-weighted images and mixed signal intensity on T2-weighted MRI images. While rupturing into the subarachnoid space and ventricular system, hyperintense lesions of fat-fluid level may be seen.

CALVARIAL OSSIFIED FIBROMA IN AN ATYPIC APPEARANCE: CT AND MRI FINDINGS

Celilov Ramil, Verim Samet, Battal Bilal, Akgun Veysel

OBJECTIVE: Fibroosseous lesions of the skull and facial bones are usually slow-growing and benign tumors but despite to these lesions can be clinically more aggressive. These lesions are locally destructive and can cause deformities in bones. It was reported in the literature that recurrent lesions can grow into sarcomas. We aimed to present 27 -year-old male patient's computed tomography (CT) and magnetic resonance (MR) images of calvarial fibroosseous lesions, which shows different characteristics beyond usual findings.

MATERIAL AND METHOD: A 24-year-old male patient with headaches for 2 months and falling of the left eyelid was admitted to the neurosurgery department and for further evaluation patient was referred to our clinic for CT and MRI.

RESULTS: On CT a soft tissue density lesion in the left frontal bone was determined. The lesion was 32x24x28 mm in size and caused expansion and cortical erosion of frontal bone. As a differential diagnosis primarily an epidermoid cyst was thought. On MRI, lesion has high signal intensity both on T1 and T2 weighted images. Differential diagnosis included hemorrhagic containing bone cysts, intraosseous epidermoid lesions and also frontal sinus mucocele. Intraoperative and postoperative histopathological examinations result as “fibroosseous lesion” (ossifying fibroma).

CONCLUSION: Benign, unilocular or multilocular fibroosseous lesions shows slow growth and complete surgical excision is sufficient to prevent recurrence. In rare cases, it can cause tumors to grow faster and reach cosmetic and functional problems. Due to the wide spectrum of imaging features of these lesions diagnosing by CT and MRI can be quite challenging for the radiologist.

DISASTER MEDICINE EDUCATION IN MEDICAL FACULTIES IN TURKEY

Demirtas Unal, Yildiran Nuri, Ozturk Gultekin, Unlu Caglar

OBJECTIVE: Disaster medicine has been defined as “the science for analysis and development of the methodology requested to handle situations where available resources are insufficient in relation to the immediate need of medical care”. Any doctor, nurse and ambulance crew can be involved in a major accident or disaster at any time. Therefore, it is self-evident that basic knowledge in disaster medicine should be included in their undergraduate curriculum. It is important for creating awareness in terms of disaster medicine education to the medical faculty student during their education. The aim of this study to investigate disaster medicine lessons delivered in medical faculties in Turkey.

METHOD: Academic program of medical faculty of 58 state and 25 private universities have been examined and tried to determine the name and period of lessons about disaster medicine.

RESULTS: When academic program of medical faculties analyzed, lessons about disaster medicine have been identified in 29 of the 58 state universities (44.8%) and five of 25 private universities (20%). 70% of the disaster medicine education (24 medical faculties) is situated in the 3rd grade academic program. The titles of the lessons are Disaster Medicine, Disaster Management, Disaster Health Services and Disaster Culture. Disaster Culture lessons have been continued during two academic term in only one medical faculty.

CONCLUSIONS: Disaster medicine education is very important in terms of creating awareness for doctors. We evaluated that these lessons should be compulsory lessons in all medical faculties and common education content should be determined.

CASE OF AN ABDUCTOR POLLICIS MUSCLE: MULTIPLE TENDONS

Develi Sedat, Yildirim Ahmet

OBJECTIVE: This study aims to discuss anatomic variations of supernumerary tendons of an abductor pollicis muscle.

MATERIAL AND METHOD: During routine dissection of a 73-year-old male cadaver for gross anatomy education, a variant abductor pollicis longus (APL) muscle was encountered on the right side. In this variant muscle, belly of the muscle was divided into four tendons and tendons were attached to os trapezium and 1st metacarpal. The muscle was innervated by a branch of posterior interosseous nerve. In normal anatomy, APL originates from middle third of the posterior surface of the antebrachium and inserts on the base of the 1st metacarpal. The main action of the muscle is to abduct the thumb at the carpometacarpal joint and to assist rotation of the thumb. Therefore this muscle is of importance in performing skills with the hands.

RESULTS: Due to abnormal attachment sites, supernumerary tendons may alter biomechanics of the thumb. Unbalanced contraction force may alter abduction the thumb. Also, supernumerary tendons may cause impingement in first extensor compartment which APL and extensor pollicis brevis pass through. Inflammation of tendons within this compartment may cause de Quervain tenosynovitis. On the other hand, surgeons should be aware of supernumerary tendons in surgical procedures of wrist. These accessory tendons may be useful as tendon grafts in tendon reconstructions.

CONCLUSION: Clinicians and surgeons should be aware of anatomic variations of APL. Because existence of accessory tendons of APL may cause de Quervain’s syndrome or supernumerary tendons may be used as tendon grafts.

FIBULARIS BREVIS MUSCLE: CASE REPORT OF VARIANT INSERTION

Develi Sedat, Yalcin Bulent

OBJECTIVE: This study aims to discuss abnormal insertion of a fibularis brevis muscle.

MATERIAL AND METHOD: During routine dissection of a male cadaver for gross anatomy education (unknown age), a variant insertion of fibularis brevis muscle (FB) was encountered on the right side. In this case, belly of the muscle was originating from distal lateral side of fibula, as usual. Its main tendon was inserting on the basis of fifth metatarsal bone. But from the upper side of the main tendon, a tendinous slip was originating. This accessory tendinous slip was inserting on the caput of the fifth metatarsal bone.

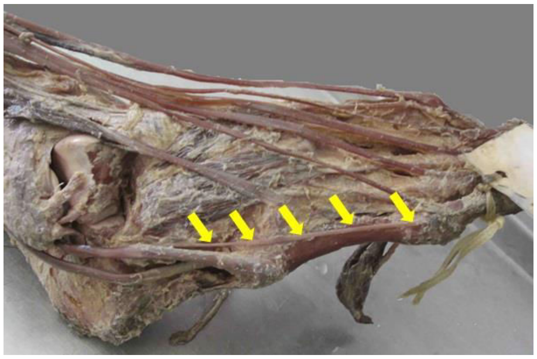

RESULTS: Accessory tendinous slips may alter biomechanics and stability of the joints. Abnormal attachment sites may affect the balanced distribution of the strength of the muscle. Also, accessory tendons may cause unexpected impingement or avulsion fractures at unexpected locations. Inflammation of these tendons may also cause tenosynovitis. Variant insertions of FB should be kept in mind by surgeons, in terms of surgical procedures of foot. These accessory tendons may be useful as tendon grafts in tendon reconstructions.

CONCLUSION: These accessory tendons may be used in tendon or peroneal retinaculum repair. The knowledge of these variations will help physicians in evaluating clinical conditions which will provide more accurate diagnosis and better image interpretation.

DOES THE TRAIT ANXIETY AFFECT THE DENTAL FEAR IN PRIMARY CARE PATIENTS?

Doganer Y. Cetin, Aydogan Umit, Yesil Hande U., Rohrer James E., Williams Mark D., Agerter David C.

OBJECTIVE: The aims of the present study were to evaluate possible associations between trait anxiety, dental fear and the predictors of these interactions including demographic characteristics and dental history of patients applied to dental care center.

MATERIAL AND METHOD: A sample of 607 enrollees responded to Turkish version of Modified Dental Fear Survey (MDFS), the State–Trait Anxiety Inventory (STAI-T) and a questionnaire regarding previous dental experience. Multiple logistic regression analysis was used to identify the association between dental fear and the independent variables including trait anxiety, age groups, education level, dental visit frequency, experience and the source of dental knowledge.

RESULTS: Table 1 demonstrates the demographic characteristics of the participant patients. There was a trend for increasing in trait anxiety scores with greater levels of dental fear in medium level of dental fear group (odds ratio; OR=1.055, 95% CI [1.025-1.086]; p<0.001) and in high level of dental fear group (OR=1.090, 95%CI [1.057-1.124]; p<0.001). Comparing to low level of dental fear group; participants of medium dental fear level intended more likely go to the dentist when they have a complaint instead of regularly going (OR=3.177, 95%CI [1.304-7.741]; p=0.011). Participants of high dental fear level tended to be less likely to have experienced no problem (OR=0.476, 95%CI [0.284-0.795]; p=0.005) than the low level of dental fear group (Table 2)(Table 3).

CONCLUSION: We strongly indicate that higher dental fear scores have a predisposition of having high trait anxiety scores. Unpleasant dental experiences increased the risk for high dental fear levels. Avoiding regular visits, patients with dental fear tended only visited dentists due to necessities.

Table 1. Descriptive features of the participants (n=607)

	Frequency (n)	Percent (%)
Age groups		
<20 years	121	19.9
20-25 years	454	74.8
>25 years	32	5.3
Gender (male)	588	96.9
Education levels		
<12 years	263	43.3
≥12 years	344	56.7
Dental visit frequency		
Never	104	17.1
Having a complaint	467	76.9
Regularly	36	5.9
Experienced a problem?*		
No	358	71.2
Yes	145	28.8
The source of dental knowledge		
Parents	138	22.7
Dentist	294	48.4
TV-internet	175	28.8

*: n=503 (patients who never have a dental visit were excluded).

Table 2. Dental fear and trait anxiety comparison in terms of independent variables (n=607)

	MDFS total score mean/median (sd)	P*	STAI-T Anxiety mean/median (sd)	P*
Age groups				
<20 years	25.02/22 (9.52)	0.547	40.37/40 (7.57)	0.017
20-25 years	25.56/22 (10.94)		42.35/43 (8.55)	
>25 years	27.81/25 (12.80)		43.91/44.5 (6.51)	
Education levels				
<12 years	26.61/23 (11.24)	0.056	44.36/46 (8.46)	<0.001
≥12 years	24.78/22 (10.36)		40.27/40 (7.74)	
Dental visit frequency				
Never	26.23/22.5 (11.78)		44.73/46 (8.05)	
Having a complaint	25.64/22 (10.50)	0.080	41.43/41 (8.18)	0.001
Regularly	22.75/18.5 (11.22)		42.22/42.5 (9.25)	
Experienced a problem? **				
No	24.22/21 (9.87)	<0.001	40.94/41 (8.18)	0.032
Yes	28.45/26 (11.61)		42.83/42 (8.31)	
The source of dental knowledge				
Parents	23.34/21.5 (8.21)		41.33/41 (8.77)	
Dentist	25.65/22 (11.10)	0.040	42.15/42 (8.28)	0.470
TV-internet	27.21/24 (11.72)		42.42/42 (7.97)	

* Dichotomized groups were analyzed with Mann-Whitney U test, trichotomized groups were analyzed with Kruskal-Wallis test

** n=503 (patients who never have a dental visit were excluded)

Gender differences were not shown regarding majority of participants (96.9%) were composed by males

MDFS: Modified Dental Fear Survey, STAI-T: State-Trait Anxiety Inventory - (Trait)

Table 3. Multivariate logistic regression analysis of MDFS [Medium (36.6%, n=184) or High dental fears (32.8%, n=165) vs Low dental fear (30.6%, n=154)]

			OR (95% CI)	P
Medium				
	Trait anxiety	STAI-T	1.055 (1.025-1.086)	< 0.001
		<20 years	1.100 (0.376-3.217)	0.862
	Age groups	20-25 years	1.039 (0.386-2.796)	0.939
		>25 years	Reference	.
	Education	<12 years	0.810 (0.506-1.298)	0.381
		≥12 years	Reference	.
	Dental visit frequency	Having a complaint	3.177 (1.304-7.741)	0.011
		Regularly	Reference	.
	Experienced a problem?	No	1.110 (0.655-1.881)	0.698
		Yes	Reference	.
	The source of dental knowledge	Parents	1.114 (0.617-2.010)	0.720
		Dentists	1.191 (0.694-2.044)	0.526
		TV-internet	Reference	.
High				
	Trait anxiety	STAI-T	1.090 (1.057-1.124)	< 0.001
		<20 years	1.184 (0.395-3.550)	0.763
	Age groups	20-25 years	0.970 (0.355-2.647)	0.952
		>25 years	Reference	.
	Education	<12 years	0.990 (0.605-1.620)	0.968
		≥12 years	Reference	.
	Dental visit frequency	Having a complaint	2.035 (0.855-4.843)	0.108
		Regularly	Reference	.
	Experienced a problem?	No	0.476 (0.284-0.795)	0.005
		Yes	Reference	.
	The source of dental knowledge	Parents	0.539 (0.286-1.014)	0.055
		Dentists	0.839 (0.484-1.455)	0.533
		TV-internet	Reference	.

*n=503 (patients who never have a dental visit were excluded in multivariate logistic regression analysis)

THE EFFECTS OF ULTRASOUND THERAPY ON WOUND HEALING PROCESS AFTER EXPERIMENTAL N. ISCHIADICUS LESIONS IN RATS

Gezer Ayhan Hilal, Aktas Mustafa, Yildiz Funda

OBJECTIVE: To assess the effects of ultrasound therapy on nerve healing process after experimental N. ischiadicus lesions in rats.

MATERIAL AND METHOD: The material of this study comprised of 30 female Wistor albino rats divided in two groups as the study and control groups. Crush lesions were inflicted on the ischiadicus nerves through a predefined surgical procedure. In the experimental group, ultrasound therapy was applied on the nerves at a dose of 1/10.3 MHz and 0.1w/cm2 for 5 minutes every 48 hours on the defective nerve portion, for two weeks. The control group was managed with only the traditional methods. Physiologic capacity changes were measured every 48 hours by comparing step length and finger space in each group. For this purpose, corridors of walking were laid onto white paper 10 x15cm in size. Glycerin and methylene blue dye were put under the feet of the rats and then the marches of rats were selected as target. The footprints of all rats were taken as an endpoint of study. The dimensions of the right foot of the rats were measured. Seven rats were sacrificed post-operatively on the 8th and 15th days in both groups. Histopathologic and macroscopic samples were studied.

RESULTS: The number of Schwann cells and mononuclear cells were significantly increased in the study group. The findings of this study implicate that ultrasound therapy

positively influences and accelerates the nerve healing process.

CONCLUSION: Ultrasound therapy can be a positive contribution to healing in frequently encountered N. ischiadicus lesions.

A LEFT VENTRICULAR DIVERTICULUM WITH MITRAL SUBVALVULAR LOCATION: CT ANGIOGRAPHIC FINDINGS

Hamcan Salih, Abduramani Asaf, Bozlar Ugur, Mustafa Tasar

OBJECTIVE: Congenital ventricular diverticulae are rare, left located outpouching ventricular anomalies. They are divided into: 1. fibrous type: non-contractile and generally originating from the left ventricular base. 2. muscular type: Contractile and generally originating from the apicoposterior and inferior left ventricular wall. This report is aimed to present the CT angiographic findings of the coincidentally found congenital left ventricular diverticulum with mitral subvalvular location.

MATERIAL AND METHOD: A 39-year-old male presented with exertional dyspnea. A prospective ECG-triggered cardiac CT angiography study was done with a 320-detector CT with injection of 80 cc contrast for 5cc/sec.

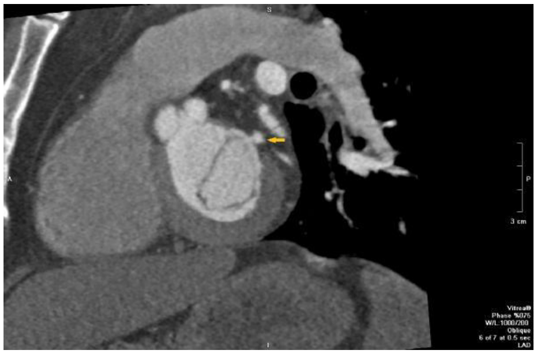

Left ventricular diverticulum in the sagittal oblique MPR images (arrow)

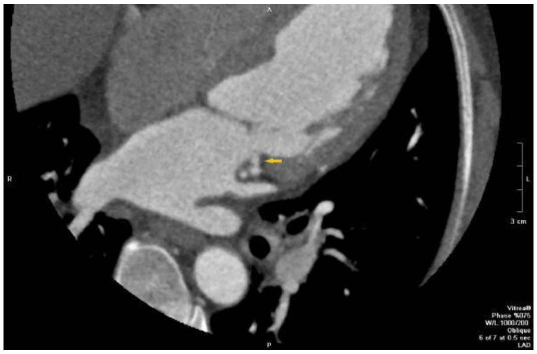

Transverse oblique MPR images of left ventricular diverticulum (arrow)

RESULTS: In CTA evaluation, a 5x6 mm diverticulum was observed just beneath the posterior valvule of the mitral valve and elongating into the atrioventricular sulcus. Differentiation of fibrous and muscular diverticulae can be made by CT and MRI. The contracting diverticulae are known as muscular diverticulae and vice versa for fibrous diverticulae. As evaluated retrospectively with the diastolic images, its location was compatible with a fibrous diverticulum.

CONCLUSION: Cardiac CT angiography is a successful imaging method in diagnosing the rare anomalies such as ventricular diverticulae and studying their detailed morphology.

ENDOVASCULAR EMBOLIZATION OF A GIANT HEMATOMA IN BLADDER DUE TO HEMORRHAGIC CYSTITIS BECAUSE OF CHEMOTHERAPY SIDE EFFECTS: CASE REPORT

Hamcan Salih, Arik Seref Barbaros, Karaman Bulent, Tasar Mustafa

PURPOSE: Hemorrhagic cystitis may develop as a complication of high-dose chemotherapy and hematopoietic stem cell transplantation. In this paper, we aimed to present endovascular embolization of a giant hematoma in bladder due to hemorrhagic cystitis, which cause severe hemodynamic instability that occurred after a chemotherapy cycle.

METERIALS and METHODS: CT images and ultrasonography showed a giant hematoma in bladder of a 13-year-old girl with hematuria after bone marrow transplantation (BMT). As there was no active bleeding focus in scintigraphy, possibility of intermittent bleeding was raised and the patient was referred to interventional radiology department for diagnosis and treatment.

RESULTS: When vesical arteries were studied superselectively, pseudoaneurysms and extravasations from distal branches were observed. Spherical embolization particles (Bead Block 300-500 µm and 500-700 µm Terumo) were injected superselectively to active bleeding distal branches in four sessions, one week apart. In control angiograms, pseudoaneurysms and extravasations were not observed.

CONCLUSION: Although there are widely accepted international guidelines for the diagnosis and management of some common complications related to high dose chemotherapy, which cause significant morbidity and mortality, there are no such standards for definition, severity, classification and optimum therapy of hemorrhagic cystitis. DSA is beneficial in the diagnosis and management of patients with severe hemorrhage and resistance to medical therapy and are preferable to surgical approaches, which cause high mortality and morbidity and cause permanent anatomic changes and genitourinary system malfunctions.

CAN THE ZIKA VIRUS BE CONSIDERED AS A MEDICAL THREAT?

Karaardic Levent, Demirtas Unal, Ozturk Gultekin

OBJECTIVE: The Zika virus was first isolated from a monkey in Uganda in 1947 through a monitoring network of sylvatic yellow fever. The first documented Zika outbreak occurred in the Pacific islands. Since 2003 cases and outbreaks of the disease have been reported from the Western Pacific, Americas and Africa. Cases have been reported in 27 different countries and territories including European countries in 2016. The aim of this study is to evaluate the Zika virus disease in the context of medical threat for military personnel, troop and humanitarian relief operations.

METHOD: We reviewed World Health Organization reports and briefs on Zika virus disease and evaluated this situation in the context of medical threat.

RESULTS: The incubation period is not clear. The symptoms are fever, skin rashes, conjunctivitis, muscle and joint pain, malaise and headache. These symptoms are usually mild and last for 2-7 days. The relationship between Zika virus and birth malformations and neurological syndromes has not been established yet. There is no specific treatment or vaccine available. The best prevention from Zika is preventing mosquito bites. Prevention and control relies on reducing mosquitos through source reduction and reducing contact between mosquitos and people.

CONCLUSIONS: Due to its potential effect on fetuses, especially female military personnel who plan to conceive should be careful in military operations and humanitarian relief operations in endemic countries. These outbreaks could affect the health services support and cause inefficiency and interruption of services. Also Zika virus could cause outbreaks in camps of refugees where socio-economic and environmental conditions are less favorable.

INCIDENTALLY DETECTED TRANSIENT INTUSSUSCEPTION IN A NAVY PILOT: CASE REPORT

Karademir Ibrahim, Cakmak Tolga, Akin Ahmet, Metin Suleyman

OBJECTIVE: Transient (non-obstructing) intussusception, a process in which a segment of intestine invaginates into the adjoining intestinal lumen with no bowel obstruction, is most frequently detected incidentally. We present the case of 29 year-old asymptomatic male navy helicopter pilot with transient intussusception who visited our center for aircrew periodical medical examination.

MATERIAL AND METHOD: The standard examination consists of physical exam, chest x-ray, ECG, transthoracic echocardiography, pulmonary function test, and abdominal sonography and biochemical tests.

RESULTS: All work-up were within normal limits except abdominal sonography. A pseudokidney image was seen at subgastric area on duodenal-jejenual junction. This image was confirmed with CT scan which revealed a 13 cm long intussusception. The characteristic presentation suggesting intussusception including cyclic abdominal pain, palpable mass, and jelly stool was absent. There was not any lead-point detected. Due to the risk of fatally complications, an intussusception with this length of size should be taken seriously. The pilot was referred to Gulhane Military Medical Academy for further examination. The following day, a second abdominal sonography conducted at the academy showed that the intussusception was spontaneously resolved. The pilot was assessed as fit to fly.

CONCLUSION: It cannot be foreseen whether the intussusception is temporary or will progress to a serious condition. This case would have needed an immediate operation, if he had atypical US findings or clinical deterioration with persistent intussusception. If not treated early, it may become a medical emergency during flight operations and can challenge the flight safety by causing a partial or total incapacitation.

BRAF MUTATION, WHICH WAS CONVERTED FROM NEGATIVE TO POSITIVE IN THE COURSE OF MALIGNANT MELANOMA

Nuri Karadurmuş, İbrahim Demirci, Birol Yıldız, Şükrü Özaydın, Mustafa Öztürk

Introduction: Malignant melanoma is the 6th most common type of cancer in adults and BRAF protein is the key component of RAS-RAF pathway, which is associated with normal cell proliferation and survival. BRAF V600E mutation is found between 40-45% of the patients with metastatic malignant melanoma and is very important on determining the therapeutic agent and prognosis.

Case: 38-year-old female patient was admitted to hospital in 2012 for an atypical nevi located on her right knee, and nodular-type malignant melanoma was diagnosed by biopsy. Breslow thickness was 1.2 mm. Sentinel lymph node involvement was found positive in dissection and no other pathological lymph node or focus detected in the body. Because of the node involvement, adjuvant interferon therapy administered to the patient who was negative for BRAF mutation. Patient, who did not come to follow-up visits regularly, admitted to hospital in February 2015 for the palpable swelling in the right breast and dyspnea. In the examinations, a 4 cm hemorrhagic mass with well-defined borders in her right breast, widespread metastatic lesions in bilateral lungs and liver, and pancytopenia were detected. Diagnostic biopsy was performed from the mass in her right breast and histologic analysis was consistent with malignant melanoma metastasis. But this time, BRAF mutation was detected positive and bone marrow biopsy and histologic analysis also revealed malignant melanoma infiltration. Vemurafenib treatment was started and the patient's hemogram returned to normal at the end of the first month, and partial response was obtained at the sixth month.

Conclusion: While the patients with early stage malignant melanoma can be followed without any treatment, metastatic disease might be extremely aggressive and mortal. Although the PDR-1 inhibitors is widely available and have highly successful outcomes, especially BRAF V600 mutation positive patients, BRAF inhibitors constitute the first step of treatment. In literature, it is shown that the BRAF mutation - negative cases can be detected as BRAF positive at 2nd analysis at a rate of 7-11%, and from another aspect, as seen in our case, the early stage BRAF-negative melanomas can be converted to BRAF-positive at advanced stages.

EFFİCACY OF PAZOPANİB THERAPY IN TREATMENT RESİSTANT SOFT TİSSUE SARCOMA: THREE CASES

Nuri Karadurmuş, B. Bahadır Başgöz, Birol Yıldız, Şükrü Özaydın, Mustafa Öztürk

INTRODUCTION: Soft tissue sarcomas are the fourth most common malignant and aggressive tumors seen in childhood and young adolescents after lymphoma, central nervous system tumors and neuroblastoma. In our study, we presented efficacy of Pazopanib treatment in three patients resistant to multidrug chemotherapy.

CASE -1: 21 years old female patient with diagnosis of clear cell sarcoma originated from left kidney received vincristine, doxorubicin, cyclophosphamide alternating with ifosfamide ,etoposide (VAC/IE alternating) as first line chemotherapy and received gemcitabine and taxotere combination as second line chemotherapy. Despite this therapy disseminated disease findings was continuing in the lungs, liver and the peritoneum. Ifosfamide, carboplatin and etoposide (ICE) regimen administered as third line chemothreapy and minimal response obtained after third course of chemotherapy but disease progressed in follow-up. After Pazopanib administration, pneumothorax occurred due to therapy but also partial response obtained. Currently, treatment with 800 mg/day dose is continued.

CASE-2: 57 years old female patient with paraspinal mass received ifosfamide, mesna, Adriamycin as first line chemotherapy after resection surgery. Patient received gemcitabine and taxotere combination as second line chemotherapy due to disseminated bone metastases and oral etoposide administered as third line chemotherapy due to progression of disease. In follow, pazopanib therapy was started to the patient who can not tolerate oral etoposide therapy and well tolerated except mild fatigue side effect. PET-CT scan performed in third month revealed findings in accordance with stable/minimal response in disease.

CASE-3: 40 years old male patient with diagnosis of soft tissue sarcoma in periprostatic localization received three courses of ifosfamide, mesna, Adriamycin as neoadjuvant chemotherapy and three courses as adjuvant chemotherapy after surgery. Patient received gemcitabine and taxotere combination as second line chemotherapy due to lung metastases and received ICE as third line chemothreapy. During all this therapy period, patient received two times palliative radiotherapy for painful bone metastases. Pazopanib administered as fourth line chemotherapy with 800 mg/day dose and minimal response obtained. Currently, treatment is continued with same dose.

CONCLUSION: In recent years, clinical usage of pazopanib, which is one of the targeted treatments with inhibitory effect on the VEGF receptors, is increased. Pazopanib is well tolerated, safe agent with minimal side effects and could be an alternative therapy for patients with multidrug chemotherapy resistant soft tissue sarcoma.

GASTRIC CANCER AND PRIMARY HYPERPARATHYROIDISM: HYPERCALCEMIA AND WIDESPREAD BONE LESIONS

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Introduction: Paraneoplastic syndromes are commonly encountered particularly in the lung cancers, whereas it can be seen in gastric and pancreatic cancers. While hypercalcemia is one of the most common paraneoplastic syndromes, each hypercalcemia associated with malignancy may not be a paraneoplastic syndrome.

Case: 35 years old female patient was admitted to hospital in February 2015 to investigate the cause of the weight loss and anemia. At first a mass detected at the lesser curvature of the stomach, and by further investigations gastric adenocarcinoma was diagnosed. In staging examinations, common area of lytic lesions in rib and vertebral bone tissues were observed. The patient's serum calcium level was 15.4 mg/dL and she was evaluated as stage 4 gastric cancer with bone metastases. No pathological findings revealed in the ECG of the patient who had a history of cardiac arrest during delivery and resuscitation by CPR. Although the serum magnesium and phosphorus levels were normal, serum PTH level was measured because of the prolonged constipation and abdominal pain complaints, and it was found as > 300 ng/ml. Parathyroid gland adenoma of 1.3 cm in diameter was detected in the scintigraphic examinations and primary hyperparathyroidism diagnosis was made; and depending on the diagnosis, bone findings were thought to be associated with hyperparathyroidism, instead of bone metastasis of gastric cancer. The patient underwent gastrectomy and adjuvant chemotherapy protocol including cisplatin and capecitabine was administered. The serum calcium and PTH levels of the patient who remain to receive adjuvant chemotherapy decreased to normal levels, bone lesions remained stable and gastrointestinal symptoms were resolved completely.

Discussion: Hypercalcemia is an electrolyte imbalance that can cause serious and life-threatening cardiac side effects. Treatment of patients with malignant hypercalcemia should be made without delay while the researching the etiology and determining the goal of treatment on the other hand.

PNEUMOTHORAX CASE OCCURED DUE TO PAZOPANIB TREATMENT OF A PATIENT WITH REFRACTORY SOFT TISSUE SARCOMA

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Introduction: Sarcomas can originate from bone or soft tissues. In the treatment of soft tissue sarcomas, especially in the cases refractory to the multi-line chemotherapy, good response rates can be achieved with targeted therapies. In this case report, we present a young patient who had pneumothorax during pazopanib treatment.

Case: 21-year-old female was admitted to hospital in July 2012, for abdominal pain and shortness of breath. She underwent an operation because of a mass surrounding her right kidney and malignant mesenchymal tumor (renal clear cell sarcoma) diagnosed by pathology. Following the diagnosis an alternating VAC-IE chemotherapy was given. Following 6th cure chemotherapy, liver metastases were detected and 6 cycles of gemcitabine and taxotere treatments were given to the patient. Due to the continuing progression of symptoms despite the treatment, 6 cycles of ICE chemotherapy (Ifosfamide, Mesna, Carboplatin, and Etoposide) were administered to the patient. This protocol also couldn’t help to stop progression of peritoneal involvement and liver metastases. Indication approval received and Pazopanib treatment of 800 mg/day was initiated. Except grade 1 fatigue, pazopanib treatment was well tolerated by the patient. In the 3rd month of the treatment the patient complained of chest pain and dyspnea. On the chest X-ray, partial pneumothorax and cavitation was detected in the left lung, and she was treated with VATS resection by chest tube. In the literature, the risk of pneumothorax associated with pazopanib use was found as 3%, and in some case reports about soft tissue sarcomas, it was found to be up to 14%. The development of necrosis, cavitation and therapeutic erosion secondary to pazopanib therapy were considered as possible mechanisms of the pneumothorax. She has been discharged from the hospital and still under treatment of pazopanib as outpatient, and partial response has been observed her follow-up visits.

Conclusion: Pazopanib, in general, is a well tolerated therapeutic agent by patients. Although secondary pneumothorax in metastatic lung lesions are extremely rare, it should be kept in mind that with the increasing use of targeted-therapies, side effects such as pneumothorax can be encountered and they can interfere with the progression of the primary clinical disease.

SKIN TUMOR MIMICKING THE SKIN INVOLVEMENT OF MANTLE CELL LYMPHOMA; PILOMATRICOMA

Nuri Karadurmuş, Birol Yıldız, Şükrü Özaydın, Mustafa Öztürk, İbrahim Demirci

Introduction: Mantle cell lymphoma is a heterogeneous disease ranging from indolent form to leukemia like aggressive form. In this case report, we present a benign skin tumor that mimics skin involvement in a patient who is taking bendamustine treatment for relapsed and refractory disease.

Case: The 58-year-old male patient was diagnosed as mantle cell lymphoma with the enlargement of cervical lymph nodes at first, in 2011. He was evaluated as Stage 4 disease because of extensive supra- and infra-diaphragmatic lymph node involvement and bone marrow metastases detected on PET-CT scans. 3 cycles of R-CHOP and 3 cycles of R-DHAP alternating regimen high-dose chemotherapy and autologous stem cell transplantation was performed. In the inspections performed at the 6th month following the transplantation, left cervical painless lymphadenopathy found in the left cervical region, and by excisional biopsy of the node, mantle cell lymphoma recurrence was diagnosed although he had achieved a complete response before transplantation. Following the diagnosis, R-Bendamustine salvage chemotherapy given and extremely well tolerated by the patient and very good partial response was achieved at the end of 4th cure. At the beginning of the 5th cure, a lesion was observed on left side of the nose, raised from the hyperemic ground and about 2 cm in diameter. Dermatology consultation and biopsy was performed and benign pilomatricoma was reported by pathology. In the literature review, some studies were found, referring that Pilomatricomas could be detected more frequently in acute leukemias with APC gene mutations and in aggressive lymphomas expressing Beta catenin gene. The patient is still under treatment and has lymphadenopathies of his primary disease, and his skin findings are followed periodically.

Discussion: Mantle cell lymphoma, which can be quite aggressive, is a disease that can be treated by autologous stem cell transplantation and for the refractory cases, by allogeneic stem cell transplantation. Both the clinical course and the treatment process is similar to leukemias rather than lymphomas, and also can present with cutaneous involvement like acute leukemias. As seen in our case applied with leukemia cutis like skin lesions, all the concomitant atypical skin lesions should be evaluated with diagnostic biopsy. Even if they are histologically benign, these findings may have the risk of malignant transformation in time.

ANALYSIS OF THE HEALTH CARE NEEDS WHICH PERCEIVED BUT UNMET AMONG RECRUITS: A STUDY IN A TRAINING CENTER IN ANKARA

Kir Tayfun, Fedai Turan, Turk Yusuf Ziya

Objective: While some of health care needs are perceived and demanded, important part of them are not demanded or not perceived even as needs. One of most important aims of health policies and plans is to make unperceived needs be perceived ones. One of good way to reach this aim is carrying out screening programs. In this study, we aimed finding out perceived and real health care needs of soldiers evaluating determined health problems of recruits during first medical examinations by military physicians while joining in a Training Center.

Material and Methods: This is a descriptive study. Without sampling all recruits, who trained at a Recruit Training Center for Armored Forces in Ankara, were included into the study. Recruiting System of Turkish Armed Forces delivers recruited soldiers randomly to training centers for basic military training, using computer program. So, our findings may reflect unmet needs of all recruits who delivered to armored forces.

So, we carried out the study among recruits of a Training Center as a convenient population. All recruits' first medical examination records in the year of 2014 were included to the study.

Results: Number of recruits assigned to Recruit Training Center for Armored Forces, Ankara was 16,825. After first medical examinations of the recruits, 3,227 transferred to military hospital and 814 transferred to psychological adviser office of the Center. By psychologist, 457 of 814 recruits sent to military hospital for psychiatrist consultation.

Conclusion: These are early results of the study. After we completed the evaluation of records (including hospital records of recruits who transferred hospital), determination of real and perceived health needs ensure four benefits for us: To ensure having information on family physicians' examinations during recruiting, to determine the needs for health problems which may affect military manpower in the future, to plan health facilities and personnel, and to make more effective the health evaluation procedures.

ARTERIAL BLOOD SUPPLY OF THE PANCREAS: AN ANATOMICAL AND SURGICAL STUDY

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There is a controversy in the literature regarding definition or interpretation of pancreatic arteries. This study is planned to study the results of studies, which were previously performed by analyzing wide radiological series, on cadavers.

All of the important pancreaticoduodenal arterial, venous and nervous arches are localized between the fusion fascies of Treitz and pancreatic parenchyma. The arterial supply of the head and collum of pancreas is from anterior, intermediate, posterior pancreaticoduodenal and prepancreatic arterial arches. Since the head and collum of the pancreas are reported in the literature to be supplied from minor resources in addition to pancreaticoduodenal arterial arches and there is still controversy among the authors, there is still a need for detailed examination of the vessels of the pancreas in anatomical and surgical aspects.

This study is performed on 60 formalin-fixed pancreas and surrounding tissues. Superior pancreaticoduodenal artery arising from gastroduodenal artery, inferior pancreatico-duodenal artery arising from superior mesenteric artery and dorsal pancreatic artery, splenic artery and their branches are tracked and measurements regarding pancreas morphology are performed. Variational situations observed other than classical arterial supply or coming from a different source are recorded. Acquired results are compared to the results in the literature.

This study may yield a new perspective to the arguments on the blood supply of the pancreas; acquired results may reveal the variational differences in the arteries that directly supply the pancreas and these data may contribute to surgery during pancreatic resections.

ANALYSIS OF MALE PATIENTS WHOSE GAMETE CELLS HAVE BEEN CRYOPRESERVED FOR FERTILITY PRESERVATION IN THE ASSISTED REPRODUCTIVE TECHNIQUES (ART) CENTER OF GULHANE MILITARY MEDICAL ACADEMY

Korkmaz Cem, Baykal Baris, Muslu Bal Esin, Seferbay Senem

OBJECTIVE: This study aims to analyse the properties of male patients, whose gamete cells have been cryopreserved in the ART Center of Gulhane Military Medical Academy.

MATERIAL AND METHOD: Gamete cell cryopreservation is being performed for male patients, who are indicated to undergo chemotherapy and radiotherapy because of various cancer diseases in the ART Center of Gulhane Military Medical Academy for almost 15 years. One of the side effects of chemotherapy and radiotherapy performed for the treatment of cancer diseases especially like genital tumors, leukemia and lymphoma is the disruption of spermatogenesis in the patients and as a result of this: azoospermy. This negative effect can be short term or permanent depending on the effectiveness and duration of the therapy and agents used for the therapy. For this reason, in order to preserve the fertility of the patients who would undergo cancer treatment, Polge et al. have suggested cryopreservation and performed it by using glycerol in 1949. The first live birth after cryopreservation of sperm sample with glycerol and storage in liquid nitrogen, has been reported in 1964. Recently, this method is being widely used to preserve fertility as a precaution before cancer treatments. Sperm cells given by the patients are being mixed with cryoprotectants in the laboratory and frozen and stored in liquid nitrogen at -196°C. If normal sperm production has not been detected to begin at controls after the completion of cancer treatments of the patients who want to have a baby, croprotected sperm samples are being successfully used in assisted reproductive techniques. The number and age distribution of sperm samples cryoprotected in the ART Center of Gulhane Military Medical Academy between the years 2006 and 2015 are presented in Table 1.

AGES	2006-2009	2010	2011	2012	2013	2014	2015
≤20	-	-	13	3	4	19	4
20-25	-	-	13	32	28	21	38
26-30	-	-	3	6	12	9	11
31-35	-	-	6	4	2	4	7
36-40	-	-	3	1	2	0	2
≥41	-	-	1	2	0	2	4
TOTAL	22	23	39	48	48	55	66

CONCLUSION: The increase in numbers within the past years can be due the increase in the awareness about this issue and also may be an indicator of the increase in the cancer incidence in the males at reproductive ages.

PHYGITAL INNOVATION IN HEALTH SERVICES: AN EXAMPLE OF MEDICAL DESIGN AND MANUFACTURING CENTER IN GULHANE MILITARY MEDICAL ACADEMY

Orhan Fatih, Altinel Ozcan, Varol Semsettin, Tuncer Selahattin, Oguz Erbil

Aim: Health system is a system, which includes different specializations. An important feature of this system is that changing and innovation are the most common. In this study, we aimed to reveal innovation combining physical and digital elements, which is called phygital innovation recently

Introduction: Phygital innovation in health system has wide range of topics from ethics to 3-D video calls/holograms with patient's relatives in ICU. Thanks to technologic facilities and digital technology, many benefits may be provided at the same time such as patient safety, patient rights, ethical and moral elements.

Results: Gulhane Military Medical Academy, Medical Design and Manufacturing Center (METU) are a unique center in terms of capacity and medical technologic opportunities in both Turkey and Europe. 3-D pictures of the patient and wounded area can be taken and then the information can be shared with other medical staffs far away from the scene. It may give an opportunity for physicians to simulate operation prior to real operation and may provide us with titanium models for prosthesis such as cranial injuries.

Conclusion: METU is a phygital innovation center which can combine physical with digital facilities for both the patient and physician. All the data can be collected through METU. Operation and prosthesis can be planned and made due to the data which can be collected previously. In this context, both Medical and engineering knowledges can be used for the same purpose.

PROLOTHERAPY FOR LATERAL EPICONDYLITIS (TENNIS ELBOW)

Orscelik Aydan, Seven Mehmet Murat, Turker Turker, Yildiz Yavuz

OBJECTIVE: Chronic lateral epicondylitis (CLE) is common, debilitating and often refractory. Prolotherapy (PrT) is an injection therapy for tendinopathy. In this retrospective study the efficacy of PrT for CLE was evaluated.

MATERIAL AND METHOD: Sixteen military patients between the ages of 35 to 51 (mean age: 44.6 years) with CLE for more than 3 months, refractory to conservative treatments, were enrolled. PrT solution was injected onto the lateral epicondyle. Then, up to 5 mL of the solution was "peppered" on bone along a short segment of the tendon and annular ligament at the areas of palpated tenderness. In multiple injections 3 weeks expected to next injection. All patients were prescribed with a home standard exercise program. The outcome measures Visual Analogue Scale (VAS) and DASH (The Disability of the Arm, Shoulder and Hand) score were assessed at baseline and 3 months after last injections.

RESULTS: PrT was performed for all 16 patient. 12 patients received a triple, 1 patient received a double and 3 patients received a single injection according to healing process. The VAS scale showed a significant improvement: the baseline score of 8.1±1.3 decreased to 1.2±1.2 at 3 months after last injections ($p<0.001$). The DASH score also showed a smilar positive trend: the baseline score of 78.6±11.4 decreased to 33.5±8.3 at 3 months after last injections ($p<0.001$).

CONCLUSION: PrT resulted in safe, significant improvement of elbow pain and function compared to baseline status. This pilot study suggests the need for a definitive trial.

VIEUSSENS ARCH ANEURYSM AND FISTULIZATION TO PULMONARY ARTERY: CT ANGIOGRAPHY FINDINGS

Ors Fatih, Ozen Alptug, Hamcan Salih, Bozlar Ugur

OBJECTIVE: Vieussens arch is a connection between conus branch of the right coronary artery and proximal right ventricular branch of the left anterior descending artery (LAD). Pathologies of this connection are really rare. The aim of this report is to represent CT angiography findings of aneursym on Vieussens Arch and fistulisation to pulmonar artery in a case.

MATERIAL AND METHOD: A 73 year-old female patient consulted for chest pain in the emergency service. A suspicious fistula to pulmonary artery was visualized in echocardiographic evaluation and coronary CT angiography was scheduled for further evaluation. The CT angiography was performed with 640-slice CT scanner with 75 cc non-ionic intravenous contrast agent. CT angiography images were evaluated in dedicated workstation.

RESULTS: Atherosclerotic plaques were detected proximal and middle segments of LAD causing mild luminal stenosis on CT angiography. A focal aneurysm in size of 4 x 5.5 mm was detected on the Vieussens Arch, which between right coronary artery, and LAD. Additionally, thin connections visualized in between Vieussens Arch and pulmonary artery. CONCLUSION: Fistulas and aneurysms of Vieussens arch are extremely rare. CT angiography is a very helpful and useful technique in evaluating coronary artery fistulas and aneurysms due to its non-invasivity, easily and rapid accessibility and capability to give detailed three-dimensional anatomical information.

A RARE COMPLICATION OF ACUTE PANCREATITIS: MRI FINDINGS OF WALLED OFF NECROSIS

Ozen Alptug, Akgun Veysel, Battal Bilal, Tasar Mustafa

OBJECTIVE: Necrotizing pancreatitis (NP) is a severe medical condition seen in 30% of acute pancreatitis cases. Walled off necrosis is a late-term complication of acute NP. The aim of this article is to represent magnetic resonance imaging (MRI) findings of acute complicated NP.

MATERIAL AND METHOD: A 59-year-old female consulted to emergency service with abdominal pain 48 hours after ERCP procedure. The patient was on steroid treatment for asthma. Her blood tests indicated leukocytosis and high levels of amylase. For further evaluation, contrast enhanced computed tomography (CT) performed and findings were compatible with acute NP. Dynamic upper abdominal MRI and right hip MRI were performed for atypical complaints.

RESULTS: Inflammation and necrosis in pancreatic tissue and peripancreatic area, well-limited collections in three different locations in peripancreatic area were depicted. In addition, there was a wide septated zone with heterogenous intensity including fat intensities, extending from right perirenal to retroperitoneal area. In hip MRI that was performed to exclude a possible aseptic necrosis of the femoral head, bone tissue was totally normal but there was a fluid collection adjacent to the right hip joint.

CONCLUSION: Walled off necrosis is a severe clinical condition seen in 2-9% of acute NP cases. Differential diagnosis with pseudocyst is crucial due to different treatment techniques. Although CT is the modality of choice for diagnosis of acute pancreatitis, MRI is a referred technique in complicated patients due to its capability of differentiating edematous and necrotizing pancreatitis more reliably and defining complex fluid collections more accurately.

A RARE COMPLICATION OF HCC FOLLOWING PERCUTANEOUS BIOPSY: SEEDING METASTASIS

Ozen Alptug, Akgun Veysel, Battal Bilal, Tasar Mustafa

OBJECTIVE: Hepatocellular carcinoma (HCC) is the 5th most diagnosed malignant cancer worldwide. Alfa-feto protein levels, imaging techniques and biopsy are main diagnostic methods. Seeding metastasis in biopsy tract is a rare but known complication. The aim of this report is to represent magnetic resonance imaging (MRI) findings of this rare complication.

MATERIAL AND METHOD: A 77 year-old male patient with a history of percutenous biopsy for his liver mass consulted to our service for right subcostal swelling and rush. Ultrasonography revealed nodular lesions in the skin, subcutaneus tissue and within intercostal muscular tissues. For further evaluation, dynamic upper abdominal MRI in 3T equipment was scheduled.

RESULTS: T2 hyperintense lesions that were showing contrast wash-out were compatible with multifocal HCC in 4A, 5 and 6 liver segments were revealed. Additionally, in skin, subcutaneus tissue and within intercostal muscular tissues adjacent to lesion in segment 6 were determined compatible with seeding metastases showing similar signal and contrast features.

CONCLUSION: Seeding metastasis of HCC is a rare complication that can prevent surgical treatment. Therefore, coaxial percutaneous biopsy should be preferred in cases suspicious for HCC. Radiologists should always keep seeding metastasis in mind in follow-up cases with HCC.

EMPHYSEMATOUS PYELONEPHRITIS: CT AND MRI FINDINGS

Ozen Alptug, Akgun Veysel, Battal Bilal, Tasar Mustafa

OBJECTIVE: Emphysematous pyelonephritis (EP) is a rare acute supurative necrotizing infection with gas formation in renal parenchyma, collecting system and perirenal tissue. Mortality rate of this urologic emergency is relatively high. This condition occurs in mostly diabetic patients (90-96%) and is diagnosed by imaging procedures. The aim of this report is to represent computed tomography (CT) and magnetic resonance imaging (MRI) findings of a case with EP.

MATERIAL AND METHOD: A 35-year-old female with poorly controlled diabetes consulted to E.R. in general poor-health condition. Leukocytosis and pyuria were detected in first examination. In ultrasonography, left kidney was enlarged and gas-indicating echogenities were detected in collecting system. Abdominopelvic CT and MRI were performed for further evaluation.

RESULTS: Left kidney size was increased and parenchyma was heterogenous in abdominopelvic contrast-enhanced CT. Parenchymal air densities and large retroperitoneal abscess formation were observed. In upper abdominal contrast-enhanced MRI, two abscesses in left kidney, which are hypointense in T1A, hyperintense in T2A, were detected and showed peripheral contrast enhancement. In addition, multiple air-bubbles without any signal were existed in perirenal area in all sequences. Imaging findings were indicating EP accompanying with abscess formations.

CONCLUSION: EP is a rare but life-threatening medical condition and early diagnosis is crucial. This condition is seen in female patient’s 6-times more than males and most of the patients have history of poorly controlled diabetes. There are no specific clinical symptoms and laboratory findings and thus, diagnosis depends on radiological examinations. MRI is very useful in diagnosis and follow-up of EP.

PATENT DUCTUS ARTERIOSUS ASSOCIATED WITH SUPRAAORTIC VASCULAR ANOMALIES: CT ANGIOGRAPHY FINDINGS

Ozen Alptug, Bozlar Ugur, Ors Fatih, Ugurel Mehmet Sahin

OBJECTIVE: It is crucial to identify supraaortic vascular abnormalities before surgical or endovascular treatments. The aim of this case report is to represent CT angiography findings.

MATERIAL AND METHOD: A 61 year-old male patient consulted complaining from right central facial paralysis and left hemiparesis and directed to our service for CT angiography of head and neck arteries. The CT angiography was performed with 640-slice CT scanner with 80 ml non-ionic intravenous contrast agent. CT angiography images were evaluated on dedicated workstation.

RESULTS: An 8.5 mm sized patent ductus arteriosus was detected between proximal decending aorta and left main pulmonary artery. Additionally, it was detected that right subclavian artery originating from arcus aorta as the last vessel and reaching to right subclavian region coursing at the posterior of trachea and eusophagus (aberrant right subclavian arter anomaly). Also, right vertebral artery was originated from right main carotid artery instead of right subclavian artery.

CONCLUSION: CT angiography is a very useful technique for the evaluation of aorta and supraaortic vascular structures due to its non- invasiveness, easy accessibility, feasibility, high spatial resolution and availability of multiplanar imaging.

COULD BE TREATED A YOUNG MAN WİTH HYPERTENSİON BY SURGERY: A CASE REPORT WİTH EXTRAADRENAL PARAGANGLİOMA

Ozer M. Tahir, Demirbas Seref, Ince Mehmet, Kahraman S. Deniz, Kozak Orhan

Paraganglioma arising from neural crest cell in the autonomic nervous system are rare tumors. The tumors are benign but often carries up to 10% of malignant features. They originated from chemoreceptor of the body.Clinical symptoms such as hypertension, headache, sweating and palpitations are secondary to increased catecholamine releasing, are nonspecific. Treatment is surgical excision. We shared a case diagnosed as extraadrenal paraganglioma.

21-year-old male was admitted with palpitation and he was diagnosed as secondary hypertension. Urine WMA, normetanephrine and norepinephrine levels were significantly higher. On the abdominal-CT revealed a soft tissue mass in the presacral region which was surrounding the abdominal aorta. The CT-angiography made in order to determine the relationship between blood vessels and mass. The hypervascular mass contained calcification, surrounds aortic wall was found at the level of distal abdominal aorta. Also, a similar natured mass was found at the level of L5 vertebra.

The mass has seen as described in the CT and it was to compresses to the vena cava on the exploration. Tumors were removed in coordination with the anesthesia. Postoperatively, the patient's blood pressure returned to normal values and structures that were reported in accordance with lesions histologically paraganglioma to be removed. The patient was discharged on the 10th postoperative day without any problems.

Functional paragangliomas can be detected in the early stages because of symptoms and they can be resected as surgically with curative treatment. The alpha and beta-adrenergic blockers should be given and patients should be closely monitored during the operation.

EVALUATION OF CURRENT CONCEPTS AND RECENT ADVANCES IN STEREOTACTIC RADIOSURGERY (SRS) OF GLOMUS JUGULARE TUMORS (GJT)

Sager Omer, Dincoglan Ferrat, Uysal Bora, Demiral Selcuk, Gamsiz Hakan, Beyzadeoglu Murat

OBJECTIVE: Stereotactic radiosurgery (SRS), a very advanced and modernized form of radiation therapy, allows the delivery of high fraction doses to well-defined targets in the brain. Herein, we review current concepts, recent advances, and future perspectives in SRS of GJT.

MATERIAL AND METHOD: SRS is a relatively new treatment concept for the management of glomus jugulare tumors, however, there is compiling data from studies with increasing follow-up durations which support the use of SRS as a safe and effective therapeutic option.

RESULTS: Recent improvements in SRS include advances in neuroimaging, radiation treatment techniques, equipment, treatment planning and delivery systems. Gamma knife technology has recently been capable of extended anatomical reach, which may have implications for treating some GJT below the skull base. Moreover, recent introduction of noninvasive, relocatable cranial immobilization systems allow for fractionated stereotactic treatments with the Gamma knife technology, which also enable accurate radiotherapeutic management of larger GJT with the high mechanical accuracy of Gamma knife radiosurgery systems. Despite limited experience with LINAC-based systems for GJT radiosurgery, comparable safety and efficacy has been demonstrated.

CONCLUSION: Compiling data from studies with increasing follow-up durations demonstrate excellent local control with a favorable toxicity profile for SRS of GJT. Recently, SRS is being more increasingly used both as the upfront management modality and as a complementary or salvage therapeutic option for GJT. As a safe and effective treatment strategy, SRS appears to play an increasing role in the management of most patients suffering from GJT in the near future.

STEREOTACTIC BODY RADIATION THERAPY (SBRT) FOR LOCALLY RECURRENT LUNG CANCER

Sager Omer, Gamsiz Hakan, Dincoglan Ferrat, Demiral Selcuk, Uysal Bora, Beyzadeoglu Murat

OBJECTIVE: Local recurrence is a predominant pattern of failure for lung cancers. Stereotactic Body Radiation Therapy (SBRT) emerged as a viable therapeutic option in judicious management of locally recurrent lung cancers. Herein, we review current concepts in radiotherapeutic management of recurrent lung cancers and report a case treated with highly sophisticated SBRT for local recurrence of lung cancer.

MATERIAL AND METHOD: A 62-year-old male patient was admitted to our clinic with primary diagnosis of locally advanced non-small cel lung cancer (NSCLC). After thorough evaluation by the multidisciplinary team of experts, he received 60 Gy of radiotherapy to the primary lung tumor and involved node regions in the primary disease setting. Periodical follow up examinations revealed treatment response, but he was found to have locally recurrent disease in 2016. Since the lesion was not deemed amenable to surgical resection, the patient was referred to Radiation Oncology Department for SBRT. After detailed analysis of critical organ doses, he received 30 Gy in 3 fractions using fractions of 10 Gy each on alternating days.

RESULTS: At the first follow up after SBRT in 2016, the patient has partial response with reduction in the size of locally recurrent lesion. He had no significant toxicity attributed to radiation therapy. The patient still attends periodical follow up examinations without any evidence of disease progression.

CONCLUSION: SBRT may offer a viable non-invasive management strategy for locally recurrent lung cancers.

MEDICAL AIR TRANSPORT OPERATIONS OF TURKISH ARMED FORCES DURING OPERATION ENDURING FREEDOM OF AFGHANISTAN

Salman Necati, Acar Yahya Ayhan, Tezel Onur

OBJECTIVE: We aimed to present our experiences and data about medical air transport operations of Turkish Armed Forces during Operation Enduring Freedom of Afghanistan.

MATERIAL AND METHOD: A retrospective observational study. We collected the written data of medical air transport cases between 2003 and 2014. Data includes date (month/ year), medical team (physician, nurse, anesthesiology technician) of flight; gender, age, Glasgow

coma scale, medical condition of patient (good/moderate/bad), life threatening condition (+/-), endotracheal intubation (+/-), spontaneous ventilation (+/-), medications used during flight, systolic blood pressure, diastolic blood pressure, pulse per minute, saturation of oxygen, trauma (+/-, type of injury), trauma site and surgical intervention data of patients.

RESULTS: The NATO-led International Security and Assistance Force (ISAF) conducted training, development, and humanitarian activities in addition to security operations during its 12 years in Afghanistan. During 12 year period, 18 patients were transported by Turkish medical team at 10 medical air transport operations from Kabul to Ankara. Five of patients (28%) were civilians and 13 (72%) of patients were soldiers. Physicians reported that 10 of patients (56%) had life threatening conditions and five (28%) of the patients were endotracheal intubated at the handover procedures.

CONCLUSION: Medical air transport facilities had an important role at both military medical activities and humanitarian care facilities in operation enduring freedom of Afghanistan. We can conclude that particularly the patients with life threatening conditions benefited from these operations.

NUTCRACKER SYNDROME: 320 DETECTOR ROW CT FINDINGS

Sari Sebahattin, Ersen Mehmet, Hamcan Salih, Celikkanat Serhat, Bozlar Ugur, Tasar Mustafa

OBJECTIVE: The nutcracker syndrome (NS), alias renal vein entrapment syndrome, is a rare entity that can be easily overlooked. Pathophysiological findings like pain, hematuria, and proteinuria, pelvic congestion in females or varicocele in males could be seen after renal vein narrowing which is caused by outer compressions. The narrowing of renal vein is most commonly seen in left renal vein between the abdominal aorta (AA) and superior mesenteric artery (SMA). In this report we aimed to show the CT angiography (CTA) findings of nutcracker syndrome.

MATERIAL AND METHOD: A 22 year-old-male patient with abdominal pain and hematuria had CT examination as the result of left renal vein expansion that is observed in US imaging.

RESULTS: Axial and coronal CTA images showed that left renal vein is narrowed between the SMA and AA also the proximal part of left renal vein is expanded.

CONCLUSION: NS is a clinical condition, which is related with outer compression of the aortomesenteric renal vein. Due to its rarity it might usually be misdiagnosed by clinicians and radiologists. It is first described by El Sadr and Mina in 1950, that the left renal vein is compressed between the abdominal aorta and superior mesenteric artery. Then, in 1972, Dr. Schepper named this disease as Nutcracker Syndrome. Recently it is understood that the patients with hematuria and proteinuria who previously treated as nephritis are having NS. Duplex Ultrasound should be used as the initial imaging instrument. In the other hand CTA provides superior information about vascular and non-vascular abnormalities.

NURSES’ EXPERIENCES, KNOWLEDGE AND EXPECTATIONS ABOUT GENETICS AND GENETIC COUNSELING

Tenekeci Elif Gokce, Kara Belguzar

OBJECTIVE: This study aimed to determine the experiences, knowledge and expectations of nurses regarding genetics and genetic counseling and their related factors.

MATERIAL AND METHOD: The sample of this cross-sectional study consisted of 113 nurses working in a military hospital in Turkey. An information form and the questionnaire on roles of nurses in genetic applications and the questionnaire on genetic consultancy, basic conceptual genetic knowledge and information sources of nurses in genetic applications were used for collect data. Descriptive statistics and Chi-square test were conducted.

RESULTS: The mean age of the nurses was 33.0±6.8 years and 64.6% had a baccalaureate degree in nursing. The majority of the nurses defined their roles in genetics as providing information to patients and their families about a genetic test (74.3%) and gathering family history (85.8%). Many nurses did not have any information about the genetic counseling centres (97.3%) and they wanted to participate in a course on genetic diseases and counseling (75.2%). Many nurses reported that their knowledge on genetic counseling (62.8%) and ethical aspects of human genetics (77.0%) was insufficient. Knowledge about genetic consultancy was higher among nurses with longer working duration ($\chi^2=13.05$, $p=0.01$). Knowledge on ethical aspects of human genetics was higher in nurses who had higher education levels ($\chi^2=6.81$, $p=0.03$).

CONCLUSION: The results indicated that nurses did not have adequate information on genetics and genetic counseling. Therefore, nurses should be given information about this topic in undergraduate education. The courses should be organized to meet training requirements for nurses.

PERSPECTIVES OF HOSPITALIZED OLDER PATIENTS TOWARDS PRACTICES OF INTERN NURSES

Tenekeci Elif Gokce, Kara Belguzar, Sari Elif Tugba, Bayraktar Serife

OBJECTIVE: This study aimed to determine the views of hospitalized older patients towards practices of intern nurses (fourth-grade nursing students) and their related factors.

MATERIAL AND METHOD: This cross-sectional study included 70 adults aged 65 years or older hospitalized for different reasons in a military hospital in Turkey. Data were collected by using a questionnaire form related to socio-demographic characteristics and the views about the intern nurses. Descriptive statistics and Chi-square test were performed.

RESULTS: The mean age of the older patients was 72.6±6.2 years. The majority of the older patients reported that the intern nurses got into communication with them and helped them to feel better (92.4%) and the intern nurses respected for their privacy (86.9%). Some patients reported that they would be worried if the intern nurses perform invasive interventions (35.7%). It was found that older patients would not feel uncomfortable to be provided with service by the intern nurses when they apply to the hospital next time (85.7%). The patients who perceived support for themselves and their caregivers from intern nurses were more likely to be aged 75 years and over ($\chi 2=20.01$, $p<0.001$).

CONCLUSION: The results indicated that intern nurses mostly communicated with their older patients, informed them concerning the applications to be performed and respected their privacy. In order decrease the concerns of the older patients caused by the invasive interventions of the intern nurses, it might be suggested to increase the applied training opportunities and to perform these applications under the supervision of clinic nurses.

THREE DIFFERENT CASES OF FOREIGN BODY ASPIRATION: CT FINDINGS (SYMPTOMS OR RESULTS)

Toktoraliev Joomart, Verim Samet, Inan Kemal, Ors Fatih

OBJECTIVE: Foreign body aspiration is any object getting stuck in the tracheobronchial system during the oral or nasal breathing. In the majority of cases more than 75% of foreign body aspiration affects children of 1-3 years old. It is more common and frequent in male babies. Unlike those in adults up to 15 years each in pediatric patients two main bronchi branch through similar terms. Therefore, foreign body aspiration in this patient group is close to the rate observed in both main bronchus. Up to 80% of aspirated foreign bodies are radiolucent. Foods most commonly and especially nut fragments, account for the majority of aspirated objects. Foreign body aspiration is most of seen in children with bronchial air trapping. The removal of foreign bodies’ whithin the four hours quickly improves the prognosis. The aim of this paper is to present the CT findings and three cases of foreign body aspiration.

MATERIALS AND METHODS: One year, three years and seven years old males’ patients was referred to our clinic at different times for evaluation of suspected foreign body aspiration. Before bronchoscopy to all patients was performed localization, CT examination for differential diagnosis and possible accompanying symptoms.

FINDINGS: At the chest radiography opacity of foreign bodies was seen only on seven years old male patient. During the CT exemination foreign bodies were observed in one patient (7 years old) in the right main bronchus and other patients (1 year and 3 years) in the left main bronchus. Year old male patient was monitored as in the left lower lobe consolidation and interpreted as aspiration pneumonia.

RESULT: Foreign body aspiration is frequently seen in patients of the pediatric age group and it should be always taken into consideration to pediatric patients with sudden onset of shortness of breath. Unlike adult patients, pediatric patients with suspected foreign body should not only focus on the right main bronchus, but both main bronchi must be examined carefully.

A PATIENT OF TESTICULAR CARCINOMA, ADMITTED FOR CONSTIPATION COMPLAINTS

Birol Yıldız, İbrahim Demirci, Nuri Karadurmuş, Şükrü Özaydın, Mustafa Öztürk

INTRODUCTION: In many of the studies, only 30% of the patients with testicular masses were admitted to the hospital for complaints that localized to testes, and the majority was with symptoms of other localizations, often as back pain. In our case, the patient was admitted to the hospital for constipation, which was a very rare complaint.

CASE: 30 year-old male patient was evaluated in healthcare institutions due to the constipation complaint which began on August 2014 and existing for the last 6 months. Retroperitoneal mass was detected in the patient, and orchiectomy was performed following scrotal examination and ultrasonographic evaluation. The patient was diagnosed and treated with testicular neoplasm after orchiectomy and he is still under medical follow-up.

DISCUSSION: Testicular carcinoma is the most common malignancy in male patients in between 15-35 years of age and presents frequently with low back pain. It should be kept in mind that there could be atypical presentations as seen in our case.

INFERIOR VENA CAVA SYNDROME: AN EXCEPTIONAL COMPLICATION OF HEPATOCELLULAR CARCINOMA

Birol Yıldız, Nuri Karadurmuş, Şükrü Özaydın, Mustafa Öztürk, Bülent Karaman, İbrahim Demirci

INTRODUCTION: Inferior Vena Cava (IVC) syndrome, which develops due to the compression, thrombosis, invasion or constriction of the vein, is a rarely encountered condition and presents with generalized edema in the lower extremities, scrotal edema and ascites. Although it's not as mortal as superior vena cava syndrome, IVC syndrome also worsens morbidity; and early diagnosis and treatment of syndrome is very important for patient comfort.

CASE: 66-year-old male patient admitted to hospital for dyspnea and abdominal distention on November 2015. In the examinations, a mass was detected in liver and biopsy was performed. By the hystological analysis, hepatocellular carcinoma was diagnosed. Because the lesion was not suitable to radioembolisation procedure, he underwent chemoembolization procedure in interventional radiology clinic. Following the procedure, sorafenib treatment was started. During his follow-up visits, diffuse abdominal distension, lower extremity edema and scrotal edema were observed, and his symptoms did not decrease despite the appropriate diuretic treatment and ascites drainage. In the Abdominal imaging, it was found that Inferior Vena Cava was obstructed by compression of a conglomerated lymph node. The patient was scheduled for placing a stent in interventional radiology clinic.

CONCLUSION: Vena cava inferior syndrome is a rare syndrome and often interferes with the lower extremity edema, scrotal swelling and abdominal ascites symptoms, which expected to develop in primary liver cancers or their metastases. Diagnosis of inferior vena cava syndrome should be considered, if symptomatic relief cannot be obtained in a patient presenting with these symptoms despite appropriate treatment.

METASTATIC MALIGNANT MESENCHYMAL TUMOR DEVELOPED ON THE GROUNDS OF PREEXISTING NEUROFIBROMATOSİS TYPE-I: A CASE REPORT

Birol Yıldız, İbrahim Demirci, Nuri Karadurmuş, Şükrü Özaydın, Mustafa Öztürk

Introduction: Neurofibromatosis type I (NF-1) is a multisystem genetic disorder that is caused by the mutation of a gene on long (q) arm of chromosome 17 and seen in one of the 3000-4000 newborn. Diagnose is based on clinical and radiological findings. Depending on the localization of fibroma, many different symptoms and clinical signs can be encountered. In neurofibromatosis skin, eye, brain and bone tissues are primarily involved, but sometimes benign and malignant neoplasms can occur in abdominal region. In our case, we present an aggressive type of sarcoma developed on the ground of NF-1.

Case: 27-year-old male patient, who was diagnosed as NF-1 in December 2012, was admitted to hospital for weight loss, abdominal pain and constipation complaints in October 2015. A diffuse abdominal mass was detected in the examinations, compatible with some peritoneal and liver metastases.

A biopsy taken from the mass, and following the histological, clinical and radiological assessments, the patient was diagnosed as malignant mesenchymal tumor and 1st cure chemotherapy administered. The first cure of chemotherapy was well tolerated the patient and a reduction was observed in the size of liver mass. The second cure chemotherapy couldn't be administered because of the patient's impaired health condition. He has been taken to a follow-up program with palliative care support.

Conclusion: The patients with Neurofibromatosis type 1 (NF-1) are susceptible to the development of malignant and benign tumors.

Regular follow-up visits starting from the time of diagnosis will improve the chance of early diagnosis and treatment of these patients.

SINONASAL UNDIFFERENTIATED CARCINOMA PRESENTED WITH BILATERAL VISUAL LOSS

Yildiz Birol, Basgoz B. Bahadir, Karadurmuş Nuri, Ozaydin Sukru, Ozturk Mustafa

INTRODUCTION: Sinonasal undifferentiated carcinoma is a very rare and aggressive tumor of parazal sinuses and nasal cavity. It's difficult to distinguish it from other sinonasal tumors.

Treatment of the disease is difficult and prognosis also is poor. Despite presenting with visual loss is common, bilateral visual loss is very rare in the literature.

CASE: 21 years old male patient applied to hospital with complaints of nasal congestion in January 2005, visual loss in left eye in October 2015 and bilateral visual loss in November 2015.

There was no pathology found in fundoscopic examination however loss of bilateral light perception was detected. A mass that compressing the optic nerve and completely filling paranasal sinuses and nasal cavity was founded in computed tomography (CT) scan of paranasal sinuses and immediate surgery was performed to reduce compression effect of mass ON optic nerve.

Pathology specimen was reported as sinonasal undifferentiated carcinoma and hepatic metastasis was founded in body scanning for staging.

Chemotherapy protocol was administered a chemo-radiotherapy was planned in follow-up.

No improvement was obtained in visual loss and the patient is still under treatment and follow-up.

CONCLUSION: Sinonasal undifferentiated carcinoma is very aggressive tumor and early diagnosis and treatment is vital for better morbidity and mortality.

THROMBUS IN LEFT VENTRICUL APEX IN A CASE OF LUNG MALIGNANCY WITH PRESERVED LEFT VENTRICULAR EJECTION FRACTION

Birol Yıldız, Erol Gürsoy, İbrahim Demirci, Nuri Karadurmuş, Uygar Çağdaş Yüksel, Şükrü Özaydın, Mustafa Öztürk

INTRODUCTION: Malignancy patients are prone to have thrombosis. In this group, thrombosis frequently occurs in the venous system and cause mortality through embolism in 20% of them. In our case, we presented a treatment of a thrombosis detected in left ventricular apex during the assessment of chest pain complaint, of a patient with preserved left ventricular ejection fraction.

CASE: 41 year old male patient with 20-25 pack/year smoking history and without any other known disease, was evaluated for shortness of breath, headache and trismus complaints that started 5 months ago. In the radiologic scan, infiltration in apicoposterior zone of the left lung and left pleural effusion was found. Bronchoscopic biopsy was performed and reported as malignant epithelial tumor, primarily compatible with adenocarcinoma, and pleural effusion cytology was also reported to be compatible with adenocarcinoma. For brain tissue involvement, Whole Brain Radiotherapy (RT) of 10 sessions was performed to the patient who was reported to have widespread metastases on the PET/CT imaging. Following the hospitalization in our clinic for chemotherapy, the patient complained crushing type chest pain and Electrocardiography (ECG) was performed. Although there were no ST segment changes in ECG, an echocardiography was performed because of ongoing sinus tachycardia and crushing type chest pain. In echocardiography, left ventricular apex was observed slightly hypokinetic but there were no severe valve problem. Left ventricular ejection fraction was measured as 58%. An organized 16x18 mm thrombosis seen at the apical side of left ventricle. With preliminary diagnosis of acute coronary syndrome, he was urgently taken to the hemodynamics laboratory and underwent emergency coronary angiography procedure. After coronary angiography showed normal coronary anatomy, patient was taken to the coronary care unit for surveillance. Following the confirmation of that his cardiac thrombus were not associated with acute coronary syndrome and after his chest pain relieved, the patient was transported to our clinic and low molecular weight heparin was administered. The patient is still under treatment and follow-up.

CONCLUSION: Since they are prone to having thrombosis and thromboembolism, patients with malignancy need prophylactic treatment, and diagnostic procedures are to be done quickly in case of having symptoms of thrombosis. It should be kept in mind that, in patients with malignancy, thrombosis can develop not only in the venous system but also in the arterial system, and morbidity-mortality rate can be reduced by early diagnosis and treatment.

ADMINISTRATIVE ISSUES

Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine.* Please read the complete Author Guidelines carefully prior to submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) accessible to the public at no charge;

(2) searchable by standard, electronic (Internet-based) methods;

(3) open to all prospective registrants free of charge or at minimal cost;

(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or

actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

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RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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RJMM particularly welcomes submission of Meta-Analyses and Systematic Reviews, which underpin evidence-based medicine. Guidelines for preparation of Meta-Analysis and Systematic Reviews are similar to other reviews, and articles are subject to the usual peer review process. Meta-Analyses and Systematic Reviews have a word limit of 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

Original Articles (including clinical trials)

RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

Education and Imaging

The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as ‘What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?’ Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

MANUSCRIPT PREPARATION

Style

Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors’ revised ‘Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication’, as presented at http://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster’s Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and

Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader's task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

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The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

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Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/ mesh/meshhome.html).

Text

Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

References

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals, and cited in consecutive order in the text. Each figure should be supplied as a separate file, with the figure number incorporated in the file name.

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2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
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Two Word-files need to be included upon submission: A title page file and a main text file that includes all parts of the text in the sequence indicated in the section 'Parts of the manuscript', including tables and figure legends but excluding figures which should be supplied separately.

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Each figure should be supplied as a separate file, with the figure number incorporated in the file name. For submission, low-resolution figures saved as .jpg or .bmp files should be uploaded, for ease of transmission during the review process. Upon acceptance of the article, high-resolution figures (at least 300 d.p.i.) saved as .eps or .tif files will be required.

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EDITORIAL

The 6th Edition of Carol Davila University Central Emergency Military Hospital Scientific Days

Florentina Ioniță Radu

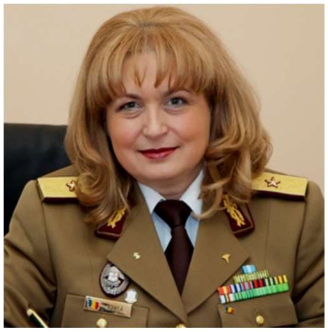

Brig. Gen. FLORENTINA IONIȚĂ RADU
Commander, Central Emergency Military Hospital, Ministry of National Defence, Romania
Associate Professor, Titu Maiorescu University, Bucharest

Prestigious and distinguished guests from home and abroad

On behalf of the Organizing Committee of the 6th Edition of the Carol Davila University Central Emergency Military Hospital Scientific Days, with the theme "185 Years of Excellence in Military Medicine," I have the great pleasure and honor to welcome you all at this scientific event which managed to enroll on the map – not so large – including manifestations that have specialized audience.

As an organizer, I can only be honored by the fact that the need for support and development of military medicine was identified as a theme in the goals and concerns of the decision structures, the event being under the patronage of the Minister of National Defence. We also have partners from the civil society, recognized – both in the country and abroad – for their work in the academic field, in the field of medical innovation, namely Carol Davila University of Medicine and Pharmacy and Titu Maiorescu University.

The anniversary, on September 13th, of 185 years since the decree for the set up of the Central Military Hospital was issued, by Alexandru Ghica, ought to be shared today with the whole modern Romanian society, mentioning all its implications – tradition, vision, determination, professionnalism and academic excellence.

Central Military Hospital was born with a predetermined destiny, that of being a landmark of Romanian society, a melting pot that mixes souls and crystallizes the purest form of science, loaded on a chosen structure, an alert and firm spirit, respecting the historical values of the Hippocratic Oath and especially the oath for the country.

During its 185 years of existence, the Central Military Hospital has gone through many difficult times, that is why I need to point out a few of these moments, because only by understanding the past you value more the present and the future and only by respecting you forebears, you have the power to face the unknown times that will come.

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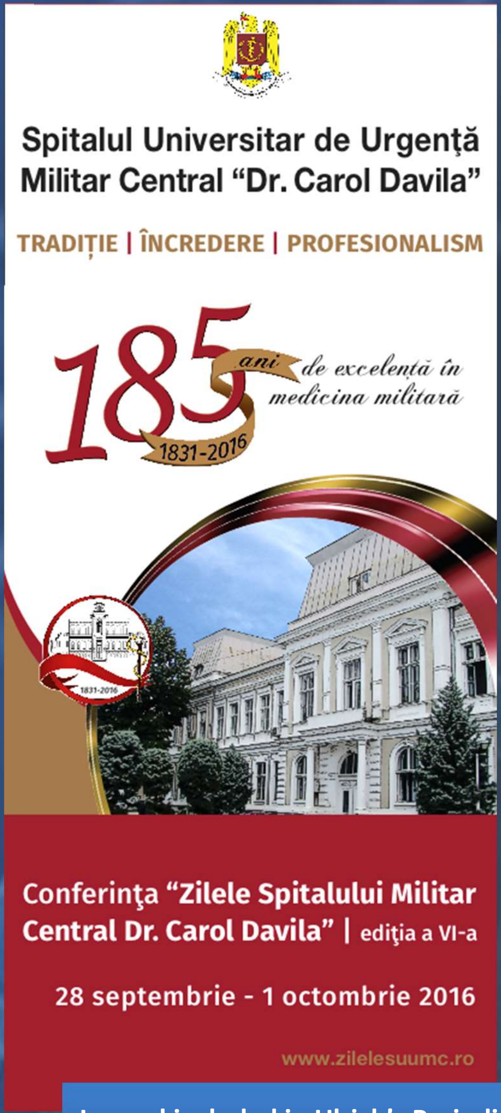

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EDITORIAL

The 6th Edition of Carol Davila University Central Emergency Military Hospital Scientific Days

Florentina Ioniță Radu

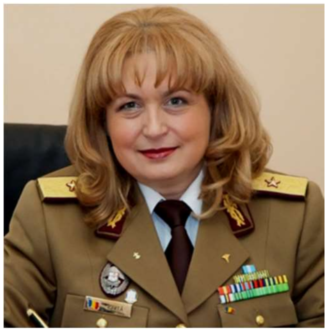

Brig. Gen. FLORENTINA IONIȚĂ RADU
Commander, Central Emergency Military Hospital, Ministry of National Defence, Romania
Associate Professor, Titu Maiorescu University, Bucharest

Prestigious and distinguished guests from home and abroad

On behalf of the Organizing Committee of the 6th Edition of the Carol Davila University Central Emergency Military Hospital Scientific Days, with the theme "185 Years of Excellence in Military Medicine," I have the great pleasure and honor to welcome you all at this scientific event which managed to enroll on the map – not so large – including manifestations that have specialized audience.

As an organizer, I can only be honored by the fact that the need for support and development of military medicine was identified as a theme in the goals and concerns of the decision structures, the event being under the patronage of the Minister of National Defence. We also have partners from the civil society, recognized – both in the country and abroad – for their work in the academic field, in the field of medical innovation, namely Carol Davila University of Medicine and Pharmacy and Titu Maiorescu University.

The anniversary, on September 13th, of 185 years since the decree for the set up of the Central Military Hospital was issued, by Alexandru Ghica, ought to be shared today with the whole modern Romanian society, mentioning all its implications – tradition, vision, determination, professionnalism and academic excellence.

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OP session MD1

Tuberculosis – a diagnostic challenge in Romania. Considerations on a clinical case

G. Stoicescu, L.M. Ciobîcă, Daniela Anghel, S. Stanciu, Iolanda Sârbu, Ancuța Coca

A 42 years old patient present cough, respectively a productive mucopurulent sputum, in a small quantity and asthenia. From her medical history we retain a diagnostic of uterine tumor, about seven years – therapeutic neglected and a recent asthma – 2 months ago, in a normal clinical exam context and a normal radiologic examination.

Clinic, without elements of bronchospasm, without bronchospasm seizures in the last 2 months and an abdominal pelvic tumor at the 1st palpation.

We performed a chest radiograph which highlighted several mild nodular opacities and a hydroaeric image of 4/2 cm in the right base. Respiratory functional tests were normal and the bronchoscopy with lavage, highlighted an intense mucosal congestion in basal segments. The cultures from the sputum and tracheobronchial aspirate, haven't evidentiate the BAAR presence. The hemoleucogram indicates a medium ferriprive anemia and martial treatment was followed. Detailed exam of the pulmonary CT bring in discussion, suspicion of a nodular and ulcerative pulmonary TBC – multiple and bilateral.

In this context, after pulmonology exam the patient is guided in a specialized department. The BK culture result at 2 months has come positive.

As a particularity of the case, the TBC pulmonary with a fast evolution toward a pulmonary abscess, in less than 2 months, after a pulmonary exam with normal pulmonary radiography. The patients were not in a clinic or biological context of immunosuppression.

http://www.zilelesuumc.ro/

Improving treatment adherence in schizophrenia diagnosed patients by using long acting injectable antipsychotics

O. Vasiliu, D. Vasile, A.G. Mangalagiu, B.M. Petrescu, C. Tudor, C. Cândea, R.E. Bazac-Bratu, F.T. Androne, A.F. Alboaie, M. Pătrașcu, E.A. Morariu

Treatment adherence in psychotic disorders is an important factor that correlates with disorder's prognosis, risk of complications, duration and frequency of hospitalizations, social and professional reinsertion.

Long acting injectable treatments are available and their administration could improve patients' adherence to antipsychotics. From the clinician's perspective, long acting injectable antipsychotics are preferred to oral medication due to a more accurate way of treatment monitoring and to the maintaining of stable active drug serum concentrations throughout the treatment duration, therefore avoiding high drug levels associated with adverse events, but also lower drug levels which could be responsible for relapses.

From the patients' perspective, fewer presentations to the physician could be perceived as a benefit. From the caregivers' point of view, a lower rate of relapse and fewer visits with the patient to the physician could reduce the disease burden.

Several options are currently available in clinical practice – risperidone microspheres could be administered every 2 weeks, aripiprazole monohydrate is recommended for administration every 4 weeks, olanzapine pamoate every 2 or 4 weeks, and paliperidone palmitate every 4 weeks. Other formulations are FDA approved but not yet marketed in Europe, like aripiprazole lauroxil (administered every 4 or 6 weeks) and paliperidone palmitate with a longer, 12 weeks, effect.

A trend to develop drug formulas with longer intervals between injections is observed and this is good news regarding the problem of treatment adherence in schizophrenia.

Osler-Weber-Rendu Disease – familial case report

Cristina Vîrlan, Florina Topliceanu, Lavinia Bârsan, M. Șotcan, E. Dănăilă

Introduction: Osler-Weber-Rendu disease (OWRD, also known as hereditary hemorrhagic telangiectasia) is a rare autosomal dominant disease, which manifests through mucous-cutaneous telangiectasia and arteriovenous malformations, a potential source of serious morbidity and mortality. Recurrent and severe epistaxis is the most common presentation, frequently leading to severe anemia that necessitates transfusion.

Material and methods: A 40 year-old woman presented to our clinic with recurrent epistaxis. Clinical examination revealed mucous-cutaneous telangiectasia. Imaging studies showed ectasias of portal and splenic veins. She confirmed that her mother had also had recurrent mucosal and oral bleeding, but she has not investigated them. The diagnosis of OWRD was established as all the criteria were met.

Results: As the OWRD is a hereditary disease, we investigated the first-line blood relatives of the patient. One of her two sons presented the same symptoms and was also diagnosed with OWRD. Currently, the patient and her son are periodically evaluated and treated for anemia. There was no need for surgical intervention, as the arteriovenous malformations were minimal and not life-threatening.

Discussion: Symptom onset may be delayed until the fourth decade of life (~90% of patients manifest by age 40 years) or later. Screening family members for signs of OWRD is reasonable and should include a complete history, physical examination, chest radiography, and arterial blood gas testing (with measurement of the shunt fraction).

Macroamylasemia – a source of confusion in clinical practice

V. Smedescu, Alexandra Gireadă, Corina Taubner, I. Copaci

Introduction: Macroamylasemia represents the presence of circulating complexes, consisting of amylase bound to immunoglobulins (IgA or IgG), not filtered by the kidney, generating increased amylase serum levels.

Materials and methods: We mention the case of a 44 year old woman who presented with a dyspeptic syndrome and whose laboratory tests indicated an increased amylase serum level with a normal lipase serum level. In the absence of another cause of hyperamylasemia (an intraabdominal or salivary condition or renal failure), macroamylasemia was suspected.

Results: The diagnosis was confirmed by the amylase creatinine clearance ratio (ACCR), less than 1%.

Discussions: Macroamylasemia is encountered in about 2.5% of hyperamylasemic patients and in 1% of healthy individuals. There are three types of macroamylasemia. In the first, most common type, the amylase activity is increased in serum and decreased in urine. In the second type, the ratio macroamylase/normal amylase in serum is lower than in the first type and the urinary amylase is increased. The third type is characterized by normal serum and urine amylase activity.

As a conclusion, in some cases, like ours, the diagnosis can be confirmed by the ACCR, but sometimes the detection of macroamylase in serum may be necessary.

Holistic approach of the bacterial resistance to antibiotics phenomenon

V. Ordeanu

The main objective of this work is how to investigate the microbial resistance and, in particular, the bacterial resistance to antibiotics (AB) as it is a worrying phenomenon for the public health, globally, in all WHO regions. The spread of this phenomenon leads to the risk of not being able to control infectious diseases, described as "the end of the antibiotics era" that would lead to "the end of modern medicine."

In order to prevent this public health’s major risk, we study the phenomenon scientifically and exhaustively, interdisciplinary and multidisciplinary, bio-medically and socio-economically, in order to identify critical points and propose concrete measures of action.

The "One health" innovative Concept of the WHO allows, for the first time, to bring together the bacterial and antibiotic resistance in human medicine, military medicine, veterinary medicine, zootechnics, pharmacy, environment etc., being appropriate for studying and fighting against this dangerous to public health phenomenon.

The innovative holistic approach to medical and paramedical aspects of the microbial resistance, a subject in which our team already has a long experience, will constitute a useful database for scientists, practitioners and healthcare policymakers. This project was proposed and accepted of Grand Challenge at Bill and Melinda Gates Foundation, USA, 2016.

Primary prevention in unipolar depression

A. Nistor, T. Hara, C. King, F. Blebea, A. Iftodi, Corina Tudor, O. Vasiliu, D. Vasile

The concept of primary prevention in psychiatry remains controversial, met with skepticism even today. Goldstein (1977) sees it as an idea that is ripe to become practice, and a possible achievement of modern psychiatry, Cummings (1972) sees more costs versus benefits in its implementation, Henderson (1975) judges it to be an illusion, Erlenmeyer-Kimling (1977) as something that belongs in the distant future, while those responsible for allocating funds for its realization, consider it a luxury. These are points of view still present when we try to explain the passive attitude of our communities regarding this field.

Nevertheless, medical progress in the field of psychiatry brings hope that primary prevention in mental health can prove to be effective, with unipolar major depression being the most researched topic, as depression is among the leading contributors to the global burden of illness-related disability, and is predicted to be the greatest contributor to the illness burden by 2030.

There is a paucity of primary preventive programs aimed at the general population. Therefore, it is a matter of public health to find ways to implement timely and effective strategies to prevent major depression. As primary prevention addresses the healthy individual, a collaborative multi-disciplinary approach focused on universal primary prevention (Shinn and Toohey-2001) that would target the communities in their entirety would be most successful.

Rare cause of a severe coagulopathy

Anca Manolache, V. Duțescu, Elena Busuioc, I. Copaci, V. Smedescu, V. Balaban

We report a case of a 40 year old man who presented himself in emergency department for multiple spontaneous ecchymoses and hematomas over the last 12 hours. He had been prescribed NSAID for renal colic 3 days before presentation. In the beginning, biological tests showed inflammatory syndrome without anemia.

Clinical exam revealed pale teguments and mucosa, ecchymosis and diffuse tenseness to the right forearm, left and right leg.

Laboratory results showed a prothrombin time of 21 msec and an activated partial thromboplastin time of 98 seconds. Platelets were normal and hemoglobin level was 7.2g/dl. The patient presented also hypoproteinemia, hypocalcemia and hypokalemia associated with severe inflammatory syndrome (fibrinogen 875 mg/dl, ESR 90 mm/1h).

Because the patient became unstable due to fast progression of anemia (Hb5g/dl) over 4 hours associated with the extension of hematomas and with a slow response of the treatment, we decided the admission in the intensive care unit.

We continued to investigate the coagulopathy and we excluded the acute promyelocytic leukemia. We excluded a possible organophosphate poisoning after the cholinesterase level came out normal. We took blood test for hemophilia as per indication of the hematologist.

The CT scan showed intestinal walls thickened due to possible hematomas at this level.

After we started treatment with FFP, vitamin K, the coagulation parameters became normal. Because hydroelectrolytic disturbance persisted and patient continued to present modification of intestinal transit (one/two soft stools per day), it rose the suspicion for celiac disease. We performed an esophagogastroduodenoscopy that revealed a typical pattern for celiac disease. Also the serology was positive with an increased level of antitransglutaminase IgA antibody (320 U/ml).

After we started the free gluten diet, the clinical and biological evolution of the patient was favorable.

Symptomatic coagulopathy represents a very rare manifestation of celiac disease, in this particular case revealed by the consumption of NSAIDs medication.

Noninvasive ventilation in obesity hypoventilation syndrome

I. Jascu, Ioana Ștefănescu, C. Ioniță, E. Firoiu, Claudia Popovici, Gabriela Andraș

Introduction: Obesity hypoventilation syndrome (OHS) is a condition in some obese people in which poor breathing leads to lower oxygen and higher carbon dioxide levels in the blood. This syndrome arises from a complex interaction between sleep-disordered breathing, diminished respiratory drive, and obesity-related respiratory impairment, and is associated with significant mortality and morbidity.

Material and methods: In the last year we investigated several obese patients for OHS presenting with acute to chronic respiratory failure. In most of the cases the diagnosis was confirmed and many patients were diagnosed or already had obstructive sleep apnea or COPD too. We initiated noninvasive ventilation (NIV) in daytime in three periods of two hours and six hours in the night. Few of them, who came with severe condition, were admitted in the Intensive Care Unit and needed tracheal intubation and mechanical ventilation. After improving, they were transferred in our department and continued NIV.

Results: The patients had a good and rapid recovery with carbon dioxide level lowered almost to the normal values and concomitantly with oxygen partial pressure growth. Severe patients could be mobilized after a few days and in 7-10 days were discharged. Most of them now use NIV only in the night and have a better quality of life.

Conclusions: Patients with OHS can be treated with NIV during an episode of acute hypercapnic respiratory failure. NIV introduction in our department practice determined a faster recovery of severe patients with shortening hospital admittance and costs lowering.

Malabsorption and hematuria – what is the connection

Anca Manolache, V. Duțescu, Elena Busuioc, I. Copaci, V. Smedescu, L. Eftimie, G. Becheanu

We present the case of a 60 years old woman which was sent from the Psychiatric Department to our establishment to investigate a malabsorption syndrome. For 4 months the patient presented symptoms of asthenia, fatigue and modified stools associated with weight loss of approximately 10 kg.

The clinical exam showed low weight (BMI 17 kg/m^2), pale teguments and mucosa, cutaneous hyperpigmentation, leg edema and absent bilateral vesicular murmur on the base of the lungs.

Since the beginning, the exams were showing severe feriprive anemia, hypocalcemia, hypoproteinemia with moderate inflammatory syndrome and also with negative serology for celiac disease. Urinary samples detected microscopic hematuria with 20% dysmorphic red blood cells, leucocyturia in the presence of positive urine cultures for Escherichia coli, without nephrotic or nefritic proteinuria.

We repeated the upper and lower endoscopy. After the esogastroduodenoscopy we've suspected the presence of intestinal lymphoma, subsequently denied by the histopathological exam. Ultrasonographic exam shows the presence of polyserositis.

The CT scan discovered in addition abdominal lymphadenopathies and bilateral renal cortical densification. After all the common causes were excluded, the persistence of hematuria and malabsorption lead us to investigate another rare cause like Whipple disease.

We repeated the upper gastro endoscopy with multiple biopsies samples for PAS coloration. The results were positive and confirmed by PCR exam for Tropheryma whippelli.

After we initiated the specific antibiotic treatment, the patient evolution was favorable with the remission of the malabsorption syndrome, hematuria and negativity of the PCR for T. whippelli.

The renal disorder due to Whipple disease it's very rare, a few cases were described in the literature of interstitial chronic nephritis and kidney failure due to renal amyloidosis.

A case of H1N1 flu complicated with bronchopneumonia, acute respiratory failure and BOOP

C. Ioniță, Ioana Ștefănescu, I. Jascu, E. Firoiu, Claudia Popovici

H1N1 is a flu virus. When it was first detected in 2009, it was called swine flu because the virus was similar to those found in pigs. The H1N1 virus is currently a seasonal flu virus found in humans, who spreads between people in the same way that seasonal flu viruses spread.

We present the case of a 49 years patient, non-smoker, with no medical history, presented in ER with: high fever, inspiratory dyspnea, productive cough (purulent sputum) and intense fatigue. Physical findings: bilateral intense crepitation, SaO_2 between 70% and 73% without oxygen, polypnea (34/minute). The chest X-ray revealed a mixt (alveolar and interstitial) pattern spread in both lungs, while the laboratory revealed leukopenia (3.700/mm^3) and increased values of Urea (67 mg/dl), Creatinine (1.75 mg/dl) and AST (92 UI/l), the patient being positive for H1N1 flu.

The patient was admitted in ICU, where he was intubated and mechanical ventilated (for three weeks), treated with large spectrum antibiotics and Tamiflu, with the persistence of respiratory failure (SaO_2 88% - 89% without O_2) and a mild improvement of the chest X-ray aspect. Due to the last aspect, a BAL (bronchoalveolar lavage) was performed, it's results being characteristic for BOOP (bronchiolitis obliterans organizing pneumonia), which determined the introduction of corticotherapy (Prednisone – 70 mg daily as initial dose, for about three weeks, followed by reduction with 5 mg every three weeks).

After four months of corticotherapy, despite the clinical and chest X-ray initial improvement, the prognosis remain reserved (the patient still presents shortness of breath, still uses O_2 from time to time, stationary aspect of the chest X-ray in the last 3 months).

OP session MD2

The value of Wells score in pulmonary embolism management in the ED

C. Florea, C.B. Teușdea, G. Ifrim, Alexandra David, Anca Arsene

The diagnosis of pulmonary embolism (PE) in the emergency department is challenging due to the wide range of non-specific symptoms, imperfect investigations and lack of clinical diagnostic criteria.

There are various scoring systems in an attempt to limit unnecessary investigations in those with low risk of PE. The Wells score for pulmonary embolism provides an estimated pre-test probability of pulmonary embolism and allows the clinician to exclude with some measure of accuracy those patients who are at very low risk of PE and for whom further testing and observation is not necessary.

The presenting symptoms are common and non-specific varying from shortness of breath chest pain, cough and hemoptysis, to syncope and cardiac arrest. There is a high false positive rate with non-invasive testing (D-dimmers) but relatively significant potential adverse events (radiation and contrast associated complications) with more invasive testing such as Computed Tomography Pulmonary Angiogram (CTPa).

While the original intent of this tool was to determine which patient was low risk enough to rule out testing with D-dimmers it is considered safer to use the three tier model which uses the score and d-dimmers testing as well as performing CTPa on all high risk patients regardless of D-dimmers results.

We analyzed the way our ER department currently manages pulmonary embolism cases and if our protocols could be further improved by introducing the Wells score. We aimed to increase documentation of pre-test probability and reduce inappropriate investigations.

http://www.zilelesuumc.ro/

Cardiac markers - ''odd'' laboratory results

Alexandra Gireadă, V. Smedescu, Corina Taubner, I. Copaci

Introduction: CK-MB is an essential cardiac marker used in the diagnosis of several acute cardiac syndromes, however there are several scenarios in clinical practice when it can create confusion.

Materials and methods: The presentation illustrates the case of a 78 year old man who was admitted for atypical chest pain and had a CK-MB level of 999 U/l and a total CK level of 764 U/l with normal troponin levels. An acute cardiac illness or rhabdomyolysis were excluded. The final diagnosis was urinary bladder neoplasm.

Results: The laboratory picture suggested the presence of macro CK in the serum of this patient.

Discussions: Usually, in cardiac patients, both CK and CK-MB levels are increased and the CK-MB/CK ratio is also modified (from its normal values of between 3% and 5% to 15-30% in certain clinical scenarios). When this ratio increases to values higher than 30%, other conditions must be considered. A laboratory result of > 100% is highly characteristic for the presence of macro-creatine kinase complexes (macro-CK), which are aggregates formed between CK isoenzymes, often involving immunoglobulins. They are usually generating false increases in the CK and CK-MB concentrations.

In conclusion, sometimes unusual laboratory findings can announce the presence of important pathological processes.

Difficulties of clinical classification in a case of aortitis and large vessels vasculitis

A. Ionescu, Roxana Diaconu, Denise-Ani Mardale, V. Smedescu, Oana Stancu, Magda Iriciuc, I. Copaci, C. Jurcuț

We present the case of a 22-year-old woman, diagnosed with Takayasu arteritis four years earlier, who recently started to complain of fatigue, claudication of the left arm and weight loss of about 7 kg in the last months. Her physical examination revealed signs of anemia, multiple bruits, asymmetrical arterial blood pressure with a colder left arm with no signs of acute ischemia, while her laboratory investigations showed moderate anemia with signs of systemic inflammation.

We performed a computed tomography angiography which described thickening of the wall of the entire aorta, with smoothly tapered luminal narrowing to a minimum of 9 mm, also involving most major branches of the aorta. At this point, the patient meets the classification criteria for Takayasu arteritis, established by the ACR in 1990. However, the laboratory work-up showed the positivity for specific systemic lupus erythematosus (SLE) antibodies. Thus, the possibility of aortitis in the context of SLE was suggested.

However, in the absence of direct histological examination, this differential diagnosis was not possible. No other causes for the patient's anemia, other than the chronic inflammatory state, were uncovered and a net improvement of her condition was observed after systemic glucocorticoids therapy (pulse therapy of methylprednisolone followed by oral prednisone) and IV iron supplement, the patient being stable thus far.

This case report emphasizes the difficulties of correct classification in patients with large vessels vasculitis in daily clinical practice.

Severe mitral regurgitation in young woman – when to search for a systemic cause

Denise Mardale, Roxana Diaconu, A. Ionescu, V. Smedescu, Oana Stancu, M. Șotcan, D. Cârpaciu, I. Copaci, C. Jurcuț

We report the case of a 29-year-old woman followed in the department of cardiology for severe mitral regurgitation. During the follow-up she developed deep vein thrombosis at the level of left upper limb (subclavian and jugular vein) associated with mild pulmonary hypertension, polyserositis, lymphadenopathies and anemia. The computed tomography confirmed the presence of deep vein thrombosis, bilateral pleural and pericardial effusion, the polyadenopathies and the hepatosplenomegaly. We performed the biopsy of an axillary adenopathy which was negative for any specific infectious, neoplastic and hematological diseases.

An extensive laboratory work-up was performed revealing the high level of anti-dsDNA antibodies associated with positivity for antiphospholipid antibodies and microcytic anemia. The other causes for anemia were carefully excluded. No other clinical signs (i.e. arthritis or skin lesions) of systemic lupus erythematosus (SLE) were observed. A diagnosis of SLE was made and a treatment with hydroxychloroquine, systemic glucocorticoids (prednisone) and oral anticoagulants was started with the remission of serositis, lymphadenopathies and anemia. Taking into account the severity of mitral regurgitation, the surgical valve replacement was planned.

This case emphasized the need for screening for systemic diseases (i.e. SLE) in young patients with cardiac valvular lesions without a clear etiology. Moreover, the antiphospholipid antibodies were reported to be associated with cardiac valvular lesions in patients with SLE and should be evaluated in these patients.

Pre-analytical variables in coagulation testing

Corina Taubner, Rodica Zemba, Irina Butte, Clara Neguţ

Introduction: The most commonly performed tests in the coagulation laboratory are the Prothrombin Time and the Activated Partial Thromboplastin Time. Coagulation tests results are important in the diagnosis and treatment of patients with bleeding or clotting disorders and also in monitoring the efficiency of anticoagulant therapy.

Materials and methods: The elements of the laboratory quality system, internal quality control and external quality assurance provide information on the analytical phase of the testing process by assessing the precision, sensitivity, accuracy of the testing method and by evaluating the long-term laboratory performance. Although significantly improved analyzers were developed and highly sensitive reagents are routinely used, samples for coagulation testing are particularly susceptible to pre-analytical errors.

Results: The pre-analytical variables such as specimen collection and handling account for up to 75% of errors within the diagnostic process and cannot be detected by current control quality control procedures. The activity of the coagulation laboratory was assessed regarding the quality and integrity of the coagulation samples affected by inappropriate specimen collection or handling. The identified errors include samples that are hemolysed, heparin contaminated, clotted or insufficient collected.

Discussion: Understanding the sources of the pre-analytical errors is a prerequisite for implementing measures in order to control the assay performance and lead to reliable test results that actually represent the clinical status of the patient and not just the status of a clinical sample received and tested.

OP session MD3

AVC recovery to youth

A. Iliuță, D. Nedelescu, Paulina Vintilă, Simona Ionescu

Stroke is an acute, serious neurological disorder resulting from blockage of blood supply to an area of brain or from cerebral hemorrhage.

Worldwide, stroke is the leading cause of morbidity and mortality because annual "kill" five million people and causes five million other severe disabilities.

Prospective studies show that this disease increases from year to year, both the incidence and prevalence, appreciating the World Health Organization experts, the stroke will become by 2030 the leading cause of mortality.

In Europe, the incidence of stroke varies from country to country, estimated between 100 and 200 strokes per 100,000 inhabitants annually in November.

Stroke is the main etiological factor of installing long-term disability in developed countries constituting the third leading cause of death after heart disease and neoplasms

Romania is in the top ten in the world in terms of the incidence of stroke by stroke. Mortality is three - four times higher in our country than in EU countries and six – seven times higher than the United States America. Maximum age on the incidence of strokes occur in 75% of cases after 65 years.

Patients who survive a stroke often show symptoms like persistent paralysis of motor function, sensory deficits, and deficits in perception, balance, aphasia, depression, dementia or other deterioration of cognitive function.

A neurologically ill health is a long process, continuously for life. Problems actual recovery will put just as long as there are signs of improvement and hopes for compensation or gain functional.

Particularities of diagnosis and treatment of cervical spine pain in medical rehabilitation

D. Nedelescu, A. Iliuță, Paulina Vintilă, Simona Ionescu

Cervical spine pain is a symptom that most people experienced in their lifetime.

Knowledge of spinal biomechanics and pathophysiology helps determine the most likely pain generators in each case. A variety of spinal structures can produce overlapping or obscure symptomatology. An accurate diagnosis provides the best opportunity for effective treatment. The purpose of this presentation is to show the particularities of diagnosis and treatment of major degenerative diseases of the cervical spine: 1. Chronic pain of the cervical spine; 2. Acute torticollis; 3. Cervical-cephalic pain syndrome; 4. Brachial neuralgia cervical; 5. Cervical spinal stenosis; 6. Syndrome Barre-Lieou. It is important to view the patient as a whole, and institute physical, pharmacologic, behavioral, and interventional treatments in the broad context of achieving what is best for the patient's physiologic and psychologic well-being.

Rehabilitation of patients with severe burns – our experience

Anca Sălceanu, G. Teodoru, Cristina Gorgonețu

The recent achievements in the multidisciplinary approach and starting intensive care treatment since the pre-hospital phase caused the increase in the number of survivors from fires involving large numbers of casualties how was the dramatic "Colectiv" nightclub fire almost a year ago. This approach brought great challenges in the rehabilitation of patients with severe functional deficits that in the past would not have survived until this phase.

In this paper we share our experience and results obtained in the treatment of patients with severe burns.

The medical rehabilitation principles is mandatory to be applied since the early postsurgical phase with functional positioning and avoiding complications of bed rest and should be continued for a long time until to complete the process of scar remodeling.

To obtain better outcome, patients should be regularly reassessed by all clinicians involved in their care and their treatment must be customized for each patient and each evolutionary stage.

Truth and myth in rehabilitation of patients with multiple sclerosis

Anca Sălceanu, G. Teodoru

Multiple sclerosis (MS), an inflammatory autoimmune disease is the most common demyelinating disease of the central nervous system. That is the second leading cause of disability of young adults after trauma.

The main problem of these patients is the inability to properly perform usual daily activities as a consequence of multiple neurological deficits.

For physical medicine and rehabilitation doctors the treatment of these complex patients represents a real challenge.

Management of them involves an interdisciplinary approach that requires close cooperation with neurologist, psychologist, physiotherapist, kineto-therapist, masseur, urologist and specialist in diet and nutrition.

For many years, patients with MS were advised to avoid exercise because of risk of increased neurological impairment, so for many years, and from our experience and in our days, many people with MS have limited their physical activity because of the fear of increased disability.

The goal of this paper is to shown, with several surveys, the benefits of physical training, with improvements in aerobic capacity, gait parameters and fatigue, and an influence on quality of life and less impact on fatigue.

Verrucous carcinoma arising in an area of necrobiosis lipoidica

Mihaela Georgescu, Viorica Marinescu, D.A. Chiriță, Florina Vasilescu

Introduction: Necrobiosis lipoidica is a granulomatous condition presenting as indolent atrophic plaques, often on the lower extremities, mostly in diabetic patients. It may occasionally be complicated by squamous cell carcinoma. This association is rare.

Case report: We present the case of a 69 years old female patient that was admitted with a verrucous, ulcerated tumor, of 4/3 cm, developed on a scleroderma like plaque, localized on the right ankle. The local examination showed the presence of large atrophic plaques with the center of porcelain color and erythematous-violet margins, with the diameter of 20/15 cm, on the 1/3 inferior part of the leg. The lesions were several years old.

The general physical examination was normal.

The primary clinical differential diagnosis was verrucous carcinoma developed on a lichen sclerosus lesion or a necrobiosis lipoidica lesion.

A biopsy was performed and the result was verrucous carcinoma (in situ squamous cell carcinoma) arising in an area of necrobiosis lipoidica.

Considering the high risk of metastasis of a squamous cell carcinoma, the tumor was completely excised. The necrobiosis lipoidica lesions were treated with topical corticosteroids and pentoxifylline 2 tb daily, with a mild favorable evolution.

Discussion: Only fifteen cases have been reported to date in the literature, regarding the development of a tumoral transformation on a classical necrobiosis lipoidica plaque.

Conclusion: Considering the association of verrucous carcinoma with necrobiosis lipoidica, clinicians should have a high index of suspicion when consulting a patient with a tumoral lesion arising within a plaque of necrobiosis lipoidica.

OP session MD4

Interferon-free treatment for chronic hepatitis C – efficacy in a small cohort

Florentina Ioniță Radu, Andrada L. Popescu, I.P. Nuță, Raluca S. Costache, Mariana Jinga, Săndica Bucurică, B. Macadon, M. Pătrășescu, Maria M. Chereja, A.I. Gavrilă

Introduction: The objective is to present our department`s experience in treating chronic hepatitis C patients with the new “interferon-free” regiment which became available in our country in the fall of 2015.

Materials and methods: Chronic hepatitis C affects approximately 3% of the world’s population and represents a significant global health issue especially due to its complications: cirrhosis and hepatocellular carcinoma. The hepatitis C virus (HCV) treatment landscape has greatly changed over the past years, with the development of direct-acting antiviral (DAA) drugs that target various steps in the HCV lifecycle.

Older interferon-based regimens were complex and full of side-effects; they required 6–12 months of therapy, with cure rates averaging around 45-50% for HCV genotype 1. DAA-based regimens have short durations, minimal side effects and efficacy approaching 90–100%.

In this small cohort study we aim to present our gastroenterology department`s experience in treating patients with advanced but compensated chronic hepatitis C – genotype 1b (Romanian population being very homogenous regarding hepatic C virus genotype infection), using an oral combination of ombitasvir, paritaprevir, ritonavir, dasabuvir and ribavirin; this being the only regiment that is available in our country at this time.

Results and conclusion: DAA-based regiment are a breakthrough in the long-term goal of eradicating hepatitis C, as more and more clinical studies confirm their very high efficacy, but the same clinical studies tell us that patients with sustained virologic response remain at risk for developing hepatocellular carcinoma, thus the need to further keep these patients under watch.

Rocky liver mass in HCV infected patient – a case report

Florentina Ioniță Radu, Andrada L. Popescu, I.P. Nuță, Mariana Jinga, Raluca S. Costache, Săndica Bucurică, B. Macadon, M. Pătrășescu, M. Șotcan, C. Bețianu, Maria M. Chereja, A.I. Gavrilă

Introduction: Hepatocelullar carcinoma is the fifth most common type of cancer and third most common cause for related cancer death with an increasing incidence worldwide.

Highly calcified hepatocellular carcinoma is considered a relatively rare condition, going to misleading diagnosis, since this is a common finding in granulomatous diseases or hydatic cysts.

Materials and methods: We present a unique case of extensive calcified liver mass. A 69-year old man with diabetes and HCV infection presented with nocturne hydrosis in the past three weeks.

Computed tomography showed a 13/11 cm highly calcified mass in the right hepatic lobe on a background of cirrhotic parenchyma, accompanied with multiple retroperitoneal masses.

On the contrast enhancement the mass didn’t have enhance in the arterial phase, nor portal venous and delayed phase washout, despite elevated alpha-fetoprotein level.

The liver mass had poorly defined margins, extensive psammomatous calcification, with dilatation of intrahepatic bile ducts and altered perfusion of the adjacent parenchyma.

Ultrasound guided liver biopsy of the mass was performed with difficulty due to massive calcification. Microscopically, the liver mass was composed of tumoral hepatoid large cells with granular eosinophilic cytoplasm disposed in trabeculae and cords.

The cells were diffuse immunoreactive to Glypican 3, to anti hepatocyte specific antigen antibody and focally positive for cytokeratin 7, but negative for cytokeratin 20 and antigen carbohydrate 19-9.

Results and conclusion: A calcified hepatocellular carcinoma is not such a rare condition, but it can be a challenge of diagnosis even in the presence of modern imagistic techniques.

A challenge in finding cause of extensive portal vein thrombosis with hepato-splenic infarction

R. Mateescu, Geanina Spulber, Florentina Ioniță Radu, M. Pătrășescu, B. Macadon, Andrada Popescu, Mariana Jinga, Raluca S. Costache, P. Nuță, Săndica Bucurică

Introduction: Portal vein thrombosis represents a blockage or narrowing of the portal vein by a blood clot.

In adults, cirrhosis is the major etiology. Neoplasms are another major cause, with hepatocellular carcinoma and pancreatic carcinoma, extrinsic compression or direct invasion of the portal vein and lead to thrombosis by inducing a hypercoagulable state.

Myeloproliferative disorders and inherited or acquired coagulation disorders are also incriminated, but in 8-15 % of cases have been reported to be idiopathic in the recent literature.

Case report: We report the case of a 52-year-old female, non-smoker, which denies completely alcohol consumption, with known hypertension, dyslipidemia, ischemic heart disease, stent on the coronary arteries, under treatment with beta blockers and antiplatelet, presented into the ER with a moderate to high intensity diffuse abdominal pain, nausea and vomiting, for at least 10 days.

The diagnosis was confirmed on a CT scan which showed thrombosis at all levels of portal venous system with LSH and spleen infarction.

We have started to investigate this patient for autoimmune, neoplastic, hematologic and hepatic diseases, in which all tests were resulting negative.

Conclusions: Portal vein thrombosis on a middle age woman without evident cause, as the etiology of the abdominal pain is very uncommon and raises a hard question: what are the etiologies and prevalence of this disease at non-cirrhotic patients?

Self-expandable metallic stents – an efficient palliative treatment for inoperable esophageal malignant tumors

M. Pătrășescu, P. Nuță, Raluca S. Costache, Săndica Bucurică, B. Macadon, Andrada Popescu, C. Bețianu, V. Balaban, A. Gavrilă, Roxana Călin, A. Lungu, C. Grozavu, Mariana Jinga, Florentina Ioniță Radu

Esophageal self-expandable metallic stents (SEMS) are frequently used in digestive oncology endoscopy for palliative treatment concerning malignant stenosis that are not suitable for surgical treatment. The most frequent symptom to be treated is severe dysphagia that hampers oral nutrition.

The complications associated with the use of SEMS may be early (within 2-3 weeks) or late. Early complications appear in 30% of patients and comprise of: migration of the stent (the most frequently encountered), prolonged retrosternal pain (12), severe hemorrhages (1%) and mediastinal perforation (rarely). The current paper reveals our 4 case experience of esophageal malignant stenosis.

The palliative treatment of dysphagia alleviates the degree of severity of this symptom from grade 4 (no esophageal passage of food) to grade 2 (passage of semisolids) (Nuehau classification). The deployment and the patency of the stents were performed under radiology and endoscopy control.

The most frequently encountered side effect was retrosternal pain, swiftly responsive to medical treatment with Metamizol.

A rare case of acute liver failure caused by hepatitis E virus infection in a neoplastic patient

Andrada Popescu, A.I. Gavrilă, I.P. Nuță, Raluca S. Costache, Mariana Jinga, Săndica Bucurică, B. Macadon, M. Pătrășescu, Mirela Chereja, Florentina Ioniță Radu

Introduction: We are presenting a case of acute liver failure, in a hospitalized patient with two neoplasia.

Case report: A 77-year-old male ex-smoker with lower lip squamous cell carcinoma diagnosed in 2014, came to our gastroenterology unit for jaundice, pruritus, fatigue and dysphagia, accompanied by acholic stools.

The patient had an acute hepatocytolytic syndrome (AST = 3010 UI/ml, ALT = 2559 UI/ml), a cholestatic syndrome and coagulopathy.

Viral markers were taken and send to laboratory for testing (IgM and IgG immunoglobulins for HAV, HEV, CMV, EBV, AgHBs and HCV antibodies), and supportive hepatic treatment was immediately conducted. We asked for the infection disease doctor opinion and took also the leptospira IgM antibodies.

After a few days the hepatocytolytic syndrome improved, we performed a gastroscopy and found a medium esophageal tumor. The thoracic-abdomen CT scan confirmed the esophageal tumor with lung metastases.

The laboratory tests showed rapid increase of cholestatic enzymes (TBil = 34 mg/dl, NCBil = 21.23 mg/dl), although the hepatocytolytic syndrome significantly improved (AST = 371 U/L, ALT = 449 U/L), and confirmed hepatitis E virus acute infection. Unfortunately the patient had signs of acute liver failure, encephalopathy and made also acute pancreatitis and IDC (he died in less than one month after the onset of symptoms despite the intensive hepatic treatment).

Results and conclusion: Acute viral hepatitis due to HEV is usually an acute, self-limiting illness, with bad evolution in pregnant women and chronic liver disease.

When complications occur – acute liver failure, the goals of treatment are to prevent further deterioration in liver function, reverse precipitating factors, and support failing organs.

Inflammatory bowel disease in neoplastic patients – a diagnosis and therapeutic challenge

Geanina Spulber, R. Mateescu, Florentina Ioniță Radu, Mariana Jinga, Raluca S. Costache, M. Pătrășescu, P. Nuță, Andrada Popescu, B. Macadon, Săndica Bucurică

Introduction: Inflammatory bowel diseases are a group of lifelong diseases arising from an interaction between genetic and environmental factors, and also they are complex disorders reflected by wide variation in clinical practice observed predominantly in the developed countries of the world.

Patients may live with a considerable symptom burden despite medical treatment in the hope that the etiology of ulcerative colitis will shortly be revealed and a cure emerge, and clinicians have to advise patients on the basis of information available today. Despite randomized trials there will always be many questions that can only be answered by the exercise of judgement and opinion. This leads to differences in practice between clinicians.

Case report: A 55 year-old male with a history of ulcerative colitis for 6 years in therapy with Salazopirine 4g/day, left inferior limb condrosarcoma in multiple sites for which he suffered a thigh amputation and chemotherapy diagnosed 3 years prior to presentation for flare of UC and corticodependent disease.

Discussion: There are a few studies addressing the overall cancer risk associated with thiopurine treatment in patients with IBD found no significantly increased risk but they have limited power, lack adequate control groups, present a limited degree of detail regarding drug exposure, or represent restricted populations.

Conclusion: Inflammatory bowel diseases represent a diagnostic challenge for gastroenterologist because of their pleomorphic manifestations which exposes a wide area for differential diagnosis and also they are a therapeutic challenge especially in neoplastic patients in which we have to estimate of the overall risk associated with the appropriate therapy we need to support clinical decision making in weighing the benefits against risks of therapy.

HCV infection - where do we stand?

Florentina Ioniță Radu, Andrada Popescu, Mirela Chereja, A.I. Gavrilă

Introduction: Our objective is to review current international data regarding hepatitis C virus infection, current incidence and prevalence, importance of early detection and treatment and global burden.

Materials and methods: Hepatitis C virus (HCV) is a leading cause of chronic liver disease, cirrhosis and hepatocellular carcinoma, with approximately 170 million (~3% of the global population) being affected.

Transmission of HCV is primarily through exposure to infected blood (transfusion before 1990`s, intravenous drug use, high-risk sexual activity, solid organ transplantation from an infected donor, occupational exposure, hemodialysis) and 70-85% of infected individuals develop chronic liver disease.

Taking into consideration that acute and chronic compensated hepatitis are asymptomatic in most patients, screening for infection using enzyme immunoassay to detect the HCV antibody is of great importance. Unfortunately, despite the fact that new direct antiviral drugs with success rates of over 90% are available, not all patients can have access to them via the national health system.

Our wish is that, in the near future, all chronic hepatitis cases can benefit from the best treatment available, as this is the best way to prevent natural evolution to cirrhosis and hepatocellular carcinoma, but also lower prevalence and ideally, some day, eradicate this disease.

Results and conclusion: Unfortunately, in the XXI`s century, despite the incredible scientific progress that allows easy diagnosis and next to ideal treatment of this viral infection, morbidity and mortality due to complications of chronic C hepatitis are still high, but the future seems bright.

Quality in colonoscopic screening

Cătălina Diaconu, Florentina Ioniță Radu, Mariana Jinga, P. Nuță, B. Macadon, Săndica Bucurică, M. Pătrășescu, Andrada Popescu, Gaudia Mănescu Avram, D.O. Costache, Raluca S. Costache

Introduction: For a long period of time the only method of screening in colorectal cancer was the guaiac fecal occult blood test. Colonoscopy has been taken into consideration more and more, having a higher specificity and sensitivity. Early detection of cancer increases the chance of successful treatment.

Studies show that screening colonoscopy reduces cancer incidence by 74% and right-sided cancer by 64%. There are plenty of risk factors in colorectal cancer, some that might be diagnosed endoscopically: polyps, prior colorectal cancer, familial adenomatous polyposis, inflammatory bowel disease or hereditary non-polyposis colon cancer. Combining the two diagnosis tests: fecal occult blood test and colonoscopy is a possible screening strategy, more effective than the tests conducted alone.

Patients and methods: We performed a retrospective study between January 5th and May 5th 2016 and included 171 patients diagnosed by colonoscopy with colon tumor (benign or malignant). Moreover, hystopathological examination was made on the masses taken through biopsy.

Results: The median age of our patients was 64.22 years, with a male prevalence of 72.51%. A total of 209 tumors were diagnosed, most of them in the descending colon, followed by rectal masses. Only 6.7% on the tumors were present in the cecum. Most of the tumors were under 1 cm (141). Most of the benign tumors (63%) were low grade dysplasia, 34% of the tumors were medium-grade dysplasia and only 7.3% are high-grade dysplasia. 29 out of 171 patients were diagnosed with adenocarcinoma.

Conclusion: Colonoscopy has become fundamental in the screening of colorectal cancer. There are many factors that influence the quality of colonoscopy: preparation, endoscopy unit and examiner. Determining the pathology of polyps has been the focus of many studies throughout the years.

The effectiveness and tolerability of DAA regimen (Viekirax+Exviera±Ribavirin) in patients

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Introduction: This study evaluates the effectiveness and tolerability of IFN-free therapy (Viekirax+Exviera±Ribavirin) in subjects with chronic HCV infection with genotype 1b and high degree of fibrosis (F3, F4).

Materials and methods: We included 30 patients (18 men + 12 women) with chronic HCV genotype 1b infection, aged 43 to 77 yo, with hepatic fibrosis F3/F4 (1 subject with F3 + 29 subjects with F4) and HCV RNA ranging from 20580 to 6500842 IU/ml, of whom 20 had been previous relapsers, 4 non-responders, and 6 naive.

Patients received Viekirax 12.5/75/50 mg + Exviera 250mg (subjects with F3) + Ribavirin 1/1.2g, according to the patient's weight (subjects with F4), daily for 12 weeks. Blood samples were collected at 4, 12, and 24 weeks.

We determined sustained virological response (SVR), treatment discontinuation rates and the occurrence of adverse events.

Results: Of 30 patients enrolled, SVR occurred in 28 (93.33%). The SVR rates among subgroups were: 100% in cases of cirrhosis, 100% in previous non-responders, 95% in previous relapsers, and 83.33% in naive patients.

Two patients discontinued treatment (6.66%) – the only patient having liver fibrosis F3, but serious comorbidities, died and another suffered a stroke because of uncontrolled hypertension.

Among the rest, the most frequent adverse events were fatigue (83.33%), pruritus (76.66%), nausea (56.66%) and 10 patients (33.33%) developed anemia.

Conclusions: IFN-free therapy has significantly improved SVR rates in patients with chronic HCV genotype 1b infection, reaching an almost unbelievable level of 96.66% in patients with fibrosis F4, with acceptable adverse events and tolerability for most subjects.

Gastric xanthomas – clinical and endoscopic characteristics in a large volume center

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Introduction: Gastric xanthomas are yellowish plaques characterized by the presence of lipid-laden histiocytes, located in the gastric mucosa or submucosa. Although their significance is not clearly known, some reports have associated them with mucosal injury and even premalignant conditions of the stomach. Our aim was to evaluate the clinical and endoscopic characteristics of gastric xanthomas in our endoscopy setting.

Methods: We retrospectively evaluated all upper gastrointestinal endoscopies performed in our high-volume center during the last three years (June 2013-June 2016). We selected patients in whom a xanthoma was described in the endoscopy report and confirmed by biopsy.

Results: Altogether 43 patients, 32.56% female, mean age 58 ± 11 years, had at least one xanthoma described in their endoscopy report. Among them, 11 were not biopsy-confirmed, and 6 were not biopsied. 26 patients were included in the final analysis – 11.5% had multiple xanthomas (range 2-5), while the others were single lesions. Regarding their distribution, 38.5% were located in the fundus, 19.2% in the body and 42.3% in the antrum. 20/26 (76.92%) of patients had associated gastritis – mostly erythematous-exudative (70%) and atrophic with/without metaplasia (15%). H. Pylori was tested in 14/26 patients and was positive in one third of them. Almost half of patients (46.15%) had associated dyslipidemia, mostly hyper-trygliceridemia. Only 7.69% had cholestasis.

Conclusions: In our cohort, gastric xanthomas were mostly solitary lesions, located in the fundus or antrum, associated with gastritis and dyslipidemia.

OP session MD5

Not all you see is a tumor

C. Sandu, Mihaela Gheorghiu, B. Teodorescu, M. Ștefănescu, F. Năftănăilă

Cerebellar abnormalities have a wide spectrum and can reveal us a surprising appearance and behavior, often becoming an interdisciplinary challenge.

The frequency and importance of the cerebral posterior fossa assessment have increased over the last years, having a close connection with the development of magnetic resonance technique.

The differentiation between tumor or non-tumor lesions requires a multimodal diagnostic approach, especially for the tumor-like forms of vascular, inflammatory and developmental abnormalities, that makes the definitive diagnosis difficult.

There are still problems to classify these potentially pathological features into certain groups. A good example could be dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease, part of COLD syndrome), a borderline disorder with slowly progressive tumor, hamartomatous and malformative elements, whose pathogenesis remains unknown. Recognition of this disease could help us to detect concomitant malignancies in these patients.

Posterior fossa lesions may influence prognosis and quality of life similar to supratentorial structures and we should always offer the same consideration to assessment.

Evaluation of 3D conformational techniques for breast cancer radiotherapy

Alina Tănase, M. Dumitrache, Ș. Vlad

Introduction: High incidence of breast cancer and anatomical variations from one case to other, led to different approaches for treatment planning, in order to fulfill QUANTEC dose constraints. Patients anatomy show a large variation in terms of geometry, and flexibility of a single technique is limited. In general, if we use photon tangential beams (internal – external) to get a homogeneous dose distribution, fluency must be modeled using filters or field in field technique. For patients with small breasts or chest wall with important concavity, classic technique is not always suitable due to the relatively high dose received by ipsilateral lung.

Materials and methods: For treatment planning evaluation we selected a case which has a relatively pronounced concave chest wall. A dose reduction was investigated in the ipsilateral lung through a different approach (3-4 fields) related to target volume. We analyzed the dose – volume histogram (DVH) obtained for the case under discussion, 3D dose distribution, homogeneity and conformity indices for the two different treatment planning methods.

Conclusion: In some cases the classical approach (tangential beams) is not appropriate, especially for pronounced concave geometry; the study shows that we can get a dose reduction to the ipsilateral lung using three fields technique, but with the disadvantage of a slight fall of dose homogeneity in the target volume. Meanwhile, according to conformity index (CI), this technique shows higher values of CI, compared to the classical approach.

Evaluation of lymph nodes using ultrasound

Carmen Tipar, Valerica Voicu, C. Mazilu, Raluca Mititelu

Introduction: Ultrasound evaluation of lymph nodes is an extension of the clinical examination. Detection and characterization of nodules is very important given the number of bodies who may be inflammatory or neoplastic diseases. Lymph nodes may be inflammatory, reactive, tumor or mixed.

Aim: Ultrasound’s contribution is to highlight the semiological characterization of lymph nodes and the role it has in differentiating benign from malignant lymph nodes.

Material and method: A retrospective study of patients who came to our department was performed to carry out an ultrasound ganglion (lateral-cervical, axillary, and inguinal). The study comprised a total of 15 patients.

Results: Of the total number of patients 6 showed with benign lymphadenopathy, while the rest had malignant lymph trait, their trait was later confirmed by pathological examination. Patients showed semiologic elements consistent with benign and malignant adenopathy.

Conclusion: Ultrasound imaging is useful exploring lymphadenopathy, and features standard ultrasound and color Doppler examination that are useful in their analysis. Ultrasound allows differentiation of metastatic lymph nodes reagents with a specificity of 83% and sensitivity of 95%.

Smoldering systemic mastocytosis

Florina Topliceanu, Cristina G. Vîrlan, M. Șotcan, E. Dănăilă

Introduction: Systemic mastocytosis, often termed systemic mast cell disease (SMCD), is a myeloproliferative neoplasm characterized by infiltration of clonally derived mast cells in different tissues, including bone marrow, skin, the gastrointestinal tract, the liver, and the spleen.

Manifestations of systemic mastocytosis may include the following: anemia, coagulopathy; GI symptom (abdominal pain); gastroesophageal reflux disease (GERD); pruritus, flushing, anaphylactoid reaction.

The major diagnostic criterion for systemic mastocytosis is the presence of dense infiltrates of mast cells in bone marrow or other extracutaneous tissues. Mast cells should be seen in aggregates of 15 or more.

Material and method: We present the case of a 68 years old men who was admitted on general surgery for abdominal pain in the left flank and left iliac fossa, weakness and weight loss.

Results: We started the investigation by performing a thoraco-abdomino-pelvic tomography which identified hepatic hilar and great omentum lymphadenopathy and hepatosplenomegaly. A lymph node biopsy was performed and histological and immuno-histochemical exams revealed systemic mastocytosis. A bone marrow biopsy was also performed which revealed mast cells infiltration of 45-50% thus confirming the diagnosis of systemic mastocytosis.

Discussions: Given the presence of B-findings (splenomegaly, lymphadenopathy) and none of the C-findings (organ failure) the patient is diagnosed with smoldering systemic mastocytosis. Under corticosteroid (Medrol) treatment the patient's condition has improved. The patient will always have on him an epinephrine pen (high risk of anaphylaxis).

Hodgkin lymphoma – prognosis and management

Cristina Vîrlan, Florina Topliceanu, E. Dănăilă, M. Șotcan

Introduction: Hodgkin lymphoma is a potentially curable lymphoma. Prognosis depends upon a large number of variables, including the stage of disease, presence or absence of B symptoms, age of patient and biological determinants, so the overall 5-years survival can go from 91% to 41%.

Material and methods: This is a case report of a 42 years-old man that presented to our clinic with high, prolonged fever, night sweats and weight loss. Together with the laboratory and imaging work-up, the diagnosis was strongly suggestive for Hodgkin Lymphoma, but the patient refused any further investigation.

Results: After 6 months from the initial presentation, the worsening state of the patient encouraged him to continue the management. The diagnosis of Hodgkin lymphoma was certified by the histopathological studies. Imaging studies showed general involvement of ganglia and dissemination to extranodal organs (liver and spleen). Chemotherapy was started. After 3 cycles, a CT-scan was performed that showed favorable evolution. An intermediate PET-CT evaluation presented complete remission.

Discussion: Even though Hodgkin lymphoma is a curable cancer, delay of treatment and spreading of the disease can bring a very low prognosis. Correct chemotherapy and supportive treatment may change the odds and result in complete remission.

Common errors in doctor-patient communication in oncology

Ș. Vlad, M. Matei, G. Bălașa

Objectives: Doctor-patient communication is essential in medicine. Communication aspect of the physician-patient relationship is very important in oncology, where diagnosis, prognosis and treatment decision are difficult tasks for oncologists. We try to identify, first the most frequent errors and the barriers in communication with patient in oncology, and second the interventions to enhance patient-centered communication and patient satisfaction and outcomes.

Methods: We identify the most frequent errors in communication with cancer patients: communicating in medical jargon, insufficient time for preparing communication of the cancer diagnosis or to discuss the prognosis, the use of internet in healthcare, not listening for patient’s emotions and fears, the symptom-focused nature of actual communication in oncology. We present the most efficient interventions to eliminate this errors and to enhance the doctor-patient relationship.

Conclusions: Having good communication skills, knowing the errors and barriers for efficient communication with cancer patients is essential to establish a good doctor-patient relationship. Efficient communication is important because it improves patient satisfaction, compliance with oncological treatment and patient’s emotional and functional status.

Retrospective analysis of patients with cancer of the cervix attending radiotherapy department – statistical analysis of DVH

Maria Vlăsceanu, Alina Tănase, M. Dumitrache, Ș. Vlad, G. Bălașa, M. Matei

Aim: It is well known that, the cervix cancer continues to be one of the most common gynecologic malignancy not only in Romania, but around the world. The aim of this study was to investigate the side effects of the small bowel in cervix cancer RT treatments, using 3D CRT technique, in accordance with our protocols.

Materials and methods: 177 selected patients were treated using box (four field) technique. Prescribed doses to PTV were between 45 Gy and 54 Gy with a fractionation of 1.8 - 2.0 Gy/session.

Treatment planning was done using Eclipse ver.11 with AAA algorithm and delivered by a UNIQUE medical accelerator with 80 leaves MLC.

3D reconstruction was based on a CT SIM SOMATOM Spirit from SIEMENS. For all patients we have used specific immobilization systems from Q-fix. Onboard imaging orthogonal technique was used for optimum positioning of every single case, starting with 3 consecutive verification and rechecked every two days along the whole treatment. Almost 95% of the patients in this study finished their RT treatment without any major complication.

Results and conclusion: The analysis was performed mainly on small bowel Dmax and V45. The delineated volume considered was the potential space within peritoneal cavity (OAR). In cases where radiotherapy was delivered concurrently with chemotherapy were observed side effects (e.g. nausea, vomiting, and diarrhea) for Dmax exceeding 48-50Gy. On the other hand, no statistical correlation between V45 and major side effects were observed for OAR volumes between 100-600 cc.

Ultrasonography importance in parotid lithiasis

Valerica Voicu, Carmen Tipar, Raluca Mititelu, C. Mazilu

Introduction: Sialolithiasis is caused by the obstruction of a salivary gland or its excretory duct by formation of calculi. It commonly involves the submandibular gland (80-95%), less frequently the parotid (5-20%) and the sublingual glands (1-2%). Parotid lithiasis usually appears on male patients between the age of 30 and 60. Calculi affecting the parotid gland are usually small, unilateral, and are located in the duct.

Material and methods: A 54 years old male came to the maxillofacial department of our hospital because he accused acute pain and unilateral inflammation in the left cheek region. The pain aggravated at mealtimes. There was no history of trauma at this level. Because ultrasound represents an excellent diagnostic technique, it was the first investigation for which he has opted.

Results: Ultrasound revealed a dilated duct with a relatively sinuous path along the entire length of 14 mm, with 8 mm diameter calculi.

Discussions and conclusion: The etiology and pathogenesis of salivary calculi is not known. Several hypotheses put forward to explain the etiology of these calculi include: mechanical, inflammatory, chemical, neurogenic, infections, strange bodies. Traditional theories suggest that the formation of sialoliths occur in two phases: formation of a central core and a layered periphery. Whereas parotid sialoliths are thought to be formed around a nidus of inflammatory cells or a foreign body.

Usually the parotid sialolithiasis are unilateral and they predominantly affect the salivary duct than the gland. Treatment depends on size and location of sialolith.

The incidence of tracheoesophageal fistulas and its major determinant factors

B. Petre, Denisa Ghinescu, Ioana Oprea, O. Bratu, N. Tănase, D. Corneci, Mirela Bidilică, L. Ene

A tracheoesophageal fistula is a congenital (frequency 1-5000) or acquired communication between the trachea and esophagus. This condition often lead to severe and fatal pulmonary complications.

In congenital condition diagnostics was made immediately following birth or during infancy.

Acquired tracheoesophageal fistula occur secondary to malignant infection, disease, trauma, and ruptured diverticula. In ICU characteristic is post-intubation tracheoesophageal fistula following prolonged mechanical ventilation with an endotracheal or tracheostomy tube.

If the tube cuff is overinflated, this will leads to compression of the rear wall membranous trachea, which in time goes to ischemia and necrosis, forming an abnormal communication between the trachea and esophagus. Another complication of the overinflated tube overinflated are tracheal stenosis or bleeding.

If cuff pressure is too low then air leaks can appear or microaspiration. This is the reason that cuff pressure must be kept within an optimal range (20-30mmHg) that ensures ventilation and prevents aspiration while maintaining tracheal perfusion.

Besides this major determinant factor – cuff pressure, are described many precipitating factors like age, nutritional status of the patient, presence or not of sepsis, hemoglobin, steroids, presence of gastric tube.

Appear of pulmonary fistula and subsequent contamination of the lung may lead in suppurative complication. This increase the use of antibiotics and prolong ICU stays.

The occurrence of this complication in the evolution of hospitalized patients in ICU have a negative prognostic and is associate with an increase in mortality, in spite of fast diagnostic and rapid therapeutic approach. Extremely important it is to prevent this complication by identifying and removing risk factors.

Daily cuff pressure measurement and reducing period of mechanical ventilation are the keys in preventing this complication.

A rare diseases in adult – pulmonary Langerhans cell histiocytosis, case report and literature review

Claudia Popovici, Gabriela Andraş, Ioana Ştefănescu, C. Ioniță, I. Jascu, E. Firoiu, Florina Vasilescu

Cigarette smoking has a clear epidemiological association with lung diseases, characterized by chronic inflammation of both the bronchiolar and interstitial lung compartments.

Adult pulmonary Langerhans’ cell histiocytosis (PLCH) is a rare disorder of unknown etiology that occurs predominantly in young smokers, with an incidence peak at 20–40 years of age.

High-resolution computed tomography (HRCT) of the chest is essential to the diagnosis. A high macrophage count in broncho-alveolar lavage (BAL) fluid is a common but nonspecific finding that merely reflects exposure to tobacco smoke.

BAL is useful for eliminating infections and the other infiltrating lung disorders that can be seen in young adults. Langerhans’ cells can be identified in BAL fluid, but, in contrast to what was initially hoped, this test shows a very low sensitivity and is rarely useful in the diagnosis of the disease.

The definite diagnosis of PLCH requires identification of Langerhans’ cell granulomas, which is usually achieved by surgical lung biopsy at a site selected by chest HRCT. In practice, however, lung biopsy is performed on a case-by-case basis.

No effective treatment is available to date, and improved understanding of the mechanisms involved in the pathogenesis of PLCH is urgently needed, and should help in the development of specific therapeutic strategies for patients with this orphan disease.

Smoking cessation may prove to be the most important and effective therapeutic option for patients with “smoking-related ILD,” and should be strongly encouraged.

OP session MD6

Random and systematic positioning error study in radiotherapy of cervix – Emergency Central Military Hospital experience

V. Bălăbăneanu, M. Dumitrache, Alina Tănase, Maria Vlăsceanu, Ş. Vlad, M.C. Matei, G. Bălașa

Introduction: The purpose of this paper is to analyze and recommend an appropriate method for taking into account geometric uncertainties during RT treatments of cervix cancer, based on verification using MV Portal Imager and to provide guidelines for the local clinical implementation at Emergency Central Military Hospital” Dr. Carol Davila”.

Materials and methods: During this study we identified 26 cases between 2014 and 2016, treated using 3D CRT box technique. Doses prescribed to PTV were between 46-50.4Gy (1.8-2.0 Gy/fraction). Treatment planning was performed using Eclipse v.11 and delivered by an UNIQUE medical accelerator. The patient positioning was based on EPID orthogonal images technique and offline reviewed in ARIA environment, where shifts for all 3 axis were determined for data study set. Specific immobilization tools from Q-fix were used in all cases.

Results and conclusions: It is well known that the overall mean set-up error is an indicator of any unwanted systematic component acting on all studied patients. Therefore, analyze is based on the individual mean set-up and random error calculated using shifts from offline review in order to determine the optimum PTV margins. By implementing the study results, our opinion is that it can be achieved better coverage for target volumes for cervical cancer in clinical daily practice.

Percutaneous core needle biopsy under CT-guidance

Alexandra Calu, M. Curea, L. Eftimie, Rodica Bulata, Liana Toma, Florina Vasilescu, C. Bețianu, M. Dumitrescu

Introduction: The treatment of cancer has significantly changed over recent years, with the advent of numerous targeted therapies, therefore a more complex approach of pathological diagnosis is being necessary.

Additional tests such as immunohistochemistry and molecular biology became almost indispensable and increased the need for adequate tumor samples. For this purpose, percutaneous core needle biopsy under image guidance has become widely accepted.

It is the baseline method when surgery is not possible nor indicated, offering a more adequate sample than fine needle aspiration.

Objectives: The aim of this report was to analyze the degree of concordance between the computed tomography and histopathological results in a cohort of patients undergoing a percutaneous needle biopsy under CT-guidance. We also wanted to provide information on our experience with CT-guided biopsy and to evaluate the accuracy and utility of this procedure.

Materials and methods: We retrospectively analyzed the results of CT-guided biopsies performed between January 2015 and July 2016 at the Central Military Emergency University Hospital "Dr. Carol Davila", collected from the hospital's database. Variables such as gender, age, CT findings, and histopathological features were assessed.

Conclusions: Percutaneous core needle biopsy under CT-guidance is a useful, minimally invasive and less expensive procedure, with a high rate of accuracy in the diagnosis of malignant lesions, providing adequate tissue specimens for the complete spectrum of the histopathological, immune-histochemical and molecular tests in different cancers in order to guarantee best patient care.

Cancer is not a matter of age... Case report and literature review

M. Curea, O.C. Voinea, Florina Vasilescu, L. Eftimie, Liana Toma, Rodica Bulata, M. Dumitrescu

Objectives: Metastases are a common cause of death in oncologic patients. When are found at first presentation for cancer diagnosis, life expectancy is very pour. This issue presents 3 problem: 1. the asymptomatic but rapid progression in some of the malignant proliferative lesions. 2. The attention by the general practitioner at the periodic exams; 3. The medical education of people

Methods: We present the case of a 29 years female, who died in the Neurology Department of our hospital. At presentation she came with signs of a stroke, but the medical papers revealed that the patient had been diagnosed a week before in another hospital with multiple tumors involving liver (for which she received emergency surgery), kidney, spleen, brain (cerebellum) and interventricular septum of the heart, without knowing the primary site. An autopsy was performed.

Results: The autopsy revealed the presence of tumors in the above mentioned organs and a tumor process involving the left ovary, adhering to the mesenteric and intestinal structures.

Conclusion: This case is a proof of the autopsy exam importance in explaining the thanatogenesis. Even postmortem, for medical knowledge and for the deceased's family, the histopathological diagnostic must be completed. It is also educational for general physicians to be aware of this issue. And it is important to keep in mind that some lesions may progress in a rapid but silent manner.

Is the tissue always necessary – correlations between cytological and histological changes in pathology

L. Eftimie, M. Curea, Rodica Bulata, Liana Toma, Florina Vasilescu, A. Calu, O.C. Voinea, M. Dumitrescu

Introduction: The clinical and imagistic diagnosis of malignancy must be confirmed in pathology departments. Histopathological diagnosis has evolved during years with the use of immunohistochemistry and molecular biology. However, histopathology and cytopathology are the main tools utilized in the diagnosis of cancer.

Objective: The aim of this study was to compare the accuracy between cytopathology and histopathology used in the diagnosis of cancer at the same patient, knowing that the costs for histological studies are higher than cytology.

Materials and methods: Medical records of patients requiring biopsy and cytology for diagnosis that were studied between August 2015 and July 2016, were retrospectively evaluated. Cytology samples were obtained by liquid aspirated from different parts of body and histopathologic samples was made by biopsy (less invasive, surgical techniques or at necropsy). Coloration methods was standard – Hematoxylin and Eosin (H&E) for histology and Papanicolau for cytology. On the cytology cases studied, we have noticed presence or absence of atypical neoplastic cells which we have correlated with biopsy diagnosis.

Conclusions: This study confirmed that cytological examination is a reliable and useful procedure only for rapid diagnostic and staging of malignant tumors (tumor invasivity) due to faster results, lower cost and lower invasiveness. However, cytology is not always sufficient without biopsy for a complex oncological treatment.

Role of pelvic MRI in establishing the clinical target volume in radiotherapy treatment of localized prostate cancer

M.C. Matei, Ş. Vlad, G. Bălaşa, Manuela Bârnă

Purpose: To compare the prostate volumes defined on magnetic resonance imaging (MRI) and non-contrast computerized tomographic (CT SIM SOMATOM Spirit - SIEMENS) scans used for three-dimensional (3D-CRT) treatment planning.

Methods and materials: Eight patients were simulated for treatment using standard immobilization devices from Q-fix. 3-D images were used to compare prostate volumes defined by MRI (4–6 mm thick slices) and CT images (3-5 mm thick slices). Segmentation ability of the following pelvic structures: prostatic apex (PA), prostate, rectum, bladder, and seminal vesicles (SV) were evaluated by four independent observers. Prostate volumes were calculated in cm^3 using Eclipse ver.11 3D planning system. MRI/CT images were merged using bony anatomy to define the regions of discrepancy on prostate volumes.

Results: The mean prostate volume was 16 % larger (range 5% to 29%) when defined by non-contrast CT compared to MRI. The areas of non-agreement tended to occur in three distinct regions of discrepancy: (a) the posterior portion of the prostate, (b) the posterior-inferior-apical portion of the prostate, and (c) the apex.

Conclusion: There is a tendency to overestimate the prostate volume by non-contrast CT compared to MRI. Awareness of this tendency should allow us to be more accurate in defining the prostate volume during 3-D treatment planning.

Use of lymphoscintigraphy in detection of sentinel nodes

C. Mazilu, Raluca Mititelu, Valerica Voicu, Carmen Tipar, B. Marinescu

Introduction: Regional lymph node involvement in oncologic patients is a very important prognostic factor, decreasing the 5-year survival rate. This has led to elective lymph node dissection in an effort to better classify patients for prognosis and treatment regimens. In complete lymphadenectomy, short-term complications include seroma, wound infection and breakdown, lymphedema, and paresthesia; long-term complications include lymphedema, paresthesia, and hernia formation.

Therefore, lymphoscintigraphy could help identify the lymph node at highest risk for metastasis, obviate radical lymph node, and possibly prolong survival in such patients.

Material: Lymphoscintigraphy for detection of sentinel nodes in patients with breast cancer or malignant melanoma was performed using intra-dermic peritumoral injection of Tc-coloidal agent (Nanocoll). We administered 2 mCi of radiotracer (4 sites x 0.5 mCi each). Dynamic and static acquisitions were performed using a gamma camera with double-header (PICKER AXIS). Sentinel node resection was performed after at least 6 hours following tracer administration.

Method: According to suspected pathology, planar and dynamic images were centered on injection site, with images of sentinel node - first lymph node which shows a communication with suspected/confirmed tumor location. Initial anterior and posterior images were completed with lateral and oblique images, obtained later. Sentinel node resection was performed in some cases in Plastic Surgery Department, using an intraoperatory gamma probe, followed by resection of the lymph node(s) with highest count activity.

Results: In most of the cases, sentinel node was detected in expected area – popliteal and inguinal region for foot/leg lesions, ipsilateral axilla for trunk lesions. Some patients revealed more than one sentinel nodes, located in the same/different lymph nodes stations. Due to guided surgical intervention and minimal post-surgery effects, lymphoscintigraphy is very useful in targeted lymph node resection in selected patients.

OP session MD7

A rare cause of rheumatoid syndrome

Oana Stancu, V. Smedescu, I. Copaci, M. Șotcan, C. Jurcuț

Establishing the etiology of a rheumatoid syndrome represents a challenge in daily clinical practice. Beside the common causes it is important to take into account the unusual etiology when we organize the imaging techniques and laboratory work-up.

We present the case of a 52-years-old, admitted in our department for diffuse bone and joint pain accompanied by important morning stiffness, involuntary weight loss and normochromic anemia of unknown etiology. Laboratory tests were negative for markers of autoimmune or neoplastic diseases. Moreover, upper and lower endoscopy were negative for any relevant disease. We performed a computed tomography which revealed: small lymph nodes <2cm and mixed bone lesions (appearance also revealed by bone scintigraphy). Serum protein electrophoresis and immune-fixation were normal. We performed flow cytometry examination of the peripheral blood that suggested the diagnosis of mantle cell lymphoma with minimal peripheral discharge. The next step was osteomedular biopsy with histopathology and immune-histochemical examination showing a hyper-cellular bone marrow with the presence of common focal infiltrates consisting of atypical mast cells. At immune-histochemical examination, aberrant presence of CD25 was showed. The final histopathological appearance was of systemic mastocytosis. Regarding the positive diagnosis of systemic mastocytosis we met the World Health Organization criteria. In patients with bone and joint symptoms without a very evident cause, the systemic mastocytosis might be taken into account.

What surprises can hide subacute infective endocarditis

Oana Stancu, C. Jurcuț, V. Duțescu, Anca Manolache, V. Smedescu, I. Copaci

Introduction: Acute mesenteric ischaemia (AMI) represents an important surgical emergency, which has a high morbidity and mortality. The most frequent causes of AMI are represented by arterial emboli, which are derived from cardiac sources, rarely in infective endocarditis (IE).

Material/methods: We present the case of a 67-year old man, smoker, known with arterial hypertension and COPD, who presented in our department for fever, marked fatigue, diffuse abdominal pain, and right upper limb paresthesia, started 5 days before admission. Three weeks before, he was admitted in another department for fever and cough, diagnosed as pneumonia and treated with antibiotics. During the hospitalization he presented acute abdominal pain, was diagnosed with acute mesenteric ischemia and an enterectomy with termino-terminal anastomosis was performed. Later, he showed transient clinical signs of acute right upper limb ischaemia. Transthoracic cardiac ultrasound was normal but transesophageal revealed a large vegetation on the aortic valve with no signs of regurgitation. Blood cultures were negative. After one week of afebrility fever reappears, despite antibiotic treatment, generated by an abdominal collection who was surgical treated and an ileostomy was performed. The evolution was favorable, but after 3 weeks of treatment he accused fever and chills, developing right jugular and subclavian veins thrombosis. We started anticoagulation therapy with NOAC with complete resolution.

Discussion/conclusions: IE is a complex disease, with various symptomatology, with many potential complications, very well illustrated by this case.

Ultrasound Power Doppler evaluation in patients with rheumatoid arthritis with anti TNF treatment

Dana Anghel, M.L. Ciobîcă, N.C. Anghel, G.D. Stoicescu, A. Anghel, Maria M. Negru, C.V. Jurcuț, Ancuța Coca

Background: The goals of treatment for patients with rheumatoid arthritis are remission or decreased disease activity, stopping the rate of joint damage. The subclinical synovitis is associated, despite clinical remission, with progression of structural damage. Imaging technique such ultrasound is capable to provide a more accurate measure of disease activity.

Objectives: To assessed in patients with rheumatoid arthritis in clinical remission the presence of subclinical synovitis by ultrasound.

Methods: The study included 60 patients with RA (ACR/EULAR 2010 criteria) in clinical remission. Medium age is 45 (range 30-59) years. The patients was in clinical remission (DAS<2.6); 81% are female; 65% are positive for rheumatoid factor and 85% for ACCP (anticitrullinate peptide antibody). PDUS examination was performed using Esaote US machine equipped with linear probes (5-12MHZ). PDUS (power Doppler ultrasound signal) investigated metacarpophalangeal and proximal interphalangeal joints and wrist symmetrical. PDUS used 4 grade (semi quantitative score) from grade 0 to 3. PDUS was performed at baseline and after 6 months to beginning of treatment. Furthermore, DAS 28 and laboratory data (ESR, CRP) were obtained at baseline and after 6 months to beginning treatment and clinical remission.

Results: All patients were in clinical remission after 6 months of treatment. PDUS were used as a measure of active disease. Synovitis grade 0 has been found in 28 patients (remission) PDUS grade 1 has been found in 13 patients, PDUS grade 2 in 9 patients and no PD grade 3 has been found after 6 months. PDUS was more frequently observed in the wrists (40%), MCP 2 (24%), PIP 3 (9%).

Conclusion:

1. The results of study confirm that clinical remission doesn't reflect an absence of synovial inflammation.

2. PDUS is useful in assessing of patients considered to be in remission. PDUS detected subclinical synovitis in the small joints of hands.

3. The other parameters don't show an evident association with the presence or absence of PDUS.

Mixed connective-tissue disease

L. Ciobîcă, I. Sîrbu, Ancuța Coca, F. Berghea, Alexandra David

Mixed connective-tissue disease (MCTD) has been more completely characterized in recent years and is now recognized to consist of the following core clinical and laboratory features: Raynaud phenomenon, swollen hands, arthritis/arthralgia, acrosclerosis, esophageal dysmotility, myositis, lung fibrosis, pulmonary hypertension, high level of anti–U1-RNP antibodies, antibodies against U1-70 kd small nuclear ribonucleoprotein.

We are presenting the case of a 63 years old man who presented in our department for acrosclerosis affecting his upper arms and mostly his hands. The symptoms began 2 years ago with mild Raynaud phenomenon and the patient ignored them. His medical history includes cardio-vascular diseases and strokes (2 transient and one ischemic) in the last 3 years.

On the clinical exam we found acrosclerosis on the upper and the lower limbs, clinical signs of pulmonary emphysema and normal blood pressure.

Biological: he had inflammatory syndrome with normal CBC.

We've continued our investigation with capillaroscopy and nuclear antibodies. An echocardiography and computer tomography of his chest were also performed.

After all the data were obtained the diagnosis of mixed connective tissue disease was sustained.

Sepsis – what we know by now

S. Dogaru, C.B. Teușdea, M. Toma

Sepsis in an increasing pathology on admission in emergency departments and intensive care units (about 20 %) being a major cause of mortality. Sepsis is also a major problem of public health (over 20 billion $- 5, 2% of admission funds in US). The heavy morbidity in growing old population let the sepsis survivors with long term physical, psychological and cognitive disabilities.

Increasing incidence and costs burden shifted the focus on algorithmic approach (the Surviving Sepsis Campaign guidelines), revised definitions and to early diagnosis.

Presepsin is used as one of the early inflammation markers. Increasing evidence suggest that presepsin compared to reactive C protein, procalcitonin, lactate or the newly introduced suPAR, sTREM1 or pre- adrenomedulin is at least a promising lead. Greater accuracy is obtained by combining sepsis with a score – MEDS, CURB65, or the newly introduced qSOFA. There are also problems to be solved – age and renal failure correlations.

OP session MD8

5 years in Romanian national program for interventional therapy in ST-elevation myocardial infarction

Alice Munteanu, S. Dumitrescu, L. Chiriac, D. Niță, R. Roșulescu, Nicoleta Avram, Irina Florescu

Cardiovascular diseases (CVDs) represent the main cause of death globally, being responsible for 1/3 of all deaths, more than cancer, chronic respiratory failure or diabetes mellitus. CVDs are a major cause of disability requiring high costs for monitoring, treatment and long-term care at home. Almost 17 million people die annually from CVDs. The total number of deaths from CVDs is estimated to reach 25 million by 2020. Every five seconds a death occurs as a result of a myocardial infarction.

Acute ST-elevation myocardial infarction (STEMI) is characterized by prolonged (15-20 minutes), severe chest pain nonresponsive to nitroglycerin administration. ECG shows ST-segment elevation ≥ 0.2 mV in V2-V3 and/or ≥ 0.1 mV in other derivations or new onset left bundle branch bloc. Biochemistry shows: increasing of cardiac enzymes (troponin, CK / CK-MB) showing myocardial necrosis.

A national program called RO-STEMI started in Romania in 2010 in 12 centers organized in a 24/7 system in five regional networks. The implementation of the program had significantly reduced the number of patients treated conservatively (32%). 63% of patients with STEMI were referred to myocardial primary angioplasty, facilitated or delayed, and only 5% received thrombolysis (RO-STEMI register). The number of patients with primary PCI was 10 times higher in 2011 compared to 2007 (SD Kristensen et al Eur Heart J 2014 August 1 35 29 1957-1970). AMI mortality rate decreased significantly, from 13% to 7.55% (RO-STEMI register). The mortality rate was approximately 4.4% in patients admitted for primary PCI, 8.3% in patients receiving thrombolysis and 17.1% in those treated conservatively. (Kristensen SD et al Eur Heart J 2014 August 1 35 29 1957-1970).

Vasile Candea Emergency Clinical Centre for Cardiovascular Diseases is part of the national program RO-STEMI for 5 years. During this period 3453 patients were admitted with STEMI. 2952 patients received primary PCI. 33.1% of the patients were brought to the ER directly by ambulance, 31.1% were transferred from other hospitals in Bucharest, 8.4% were transferred from hospitals at a distance <50 km, 10.2 % from a distance <100 km, 4.9% <150 km, 4.7% <200 km and 7.6% > 200 km. The average time from the onset of pain until calling to the emergency medical system was 3 hours, with a median of 2.5 ore. Only 21% of patients called the emergency medical system within 60min from the onset of chest pain. The time from first medical contact to balloon inflation was less than 90 min in 29% of patients.

The results regarding procedures performed were: 14.5% were not admitted to angiography, 4.4% received only PCI without stent, 52.6% of patients received one stent, 21.5% 2 stents, 6.4% 3 stents and 0.6% 5 stents. The vessel responsible for STMI was in 57% of cases LAD, in 30.2% was RCA, in 11% was CXA and in 1.7% was not specified. 43.6% of patients had single-vessel lesion, 38.1% had two-vessel lesions, 10.8% had three-vessel lesions/multivessel, 5.8% had non-obstructive coronary artery disease and 1.7% had permeable coronary arteries.

The rate of early complications after angiography was 9.3%: 3.5% of patients suffered acute stent thrombosis, 3.2% of patients had pseudoaneurysm, 1.7% of patients had procedural failure and 0.9% had coronary dissection. At discharge 71.5% of patients had not had symptoms of heart failure. 25% of patients showed signs of left heart failure and 3.5% of patient admitted in program died.

Acute myocardial infarction without chest pain

D. Negoiță, C.B. Teuşdea, L. Demiraş, Ana M. Demiraş

Acute myocardial infarction (AMI) can present itself without chest pain. This happens more often in the elderly, diabetics and women. We present the case of an elderly male patient who came to the ER with abdominal discomfort after an episode of faintishness. Careful history taking, physical examination, ECG (without STEMI criteria) and blood tests helped to establish the diagnosis. With this case we want to emphasize that a high index of suspicion for AMI is necessary in the elderly. Proper ECG interpretation of subtle ST changes is also mandatory.

Difficulties in diagnosis and treatment between STEMI and myocarditis

Alice Munteanu, L. Chiriac, R. Roșulescu, Irina Florescu, Cristina Calcan

A national program called RO-STEMI started in Romania in 2010 in 12 centers organized in a 24/7 system in five regional networks. Our hospital is part of this program for 6 years, under which we receive patients with criteria of STEMI for interventional myocardial revascularization.

We present a 59 years old female, hypertensive, obese (BMI 32,3kg/m^2), non-smoker, accusing the first episode of typical chest pain, started 2 hours before the presentation in ER. The diagnosis of STEMI was supported by electrical, biological and echocardiographic criteria. The drug therapy was initiated according to guidelines with double antiplatelet therapy, heparin and morphine. Emergency coronary angiography was performed distinguishing permeable epicardial coronary arteries.

We had to decide if it was STEMI with permeable coronary arteries or myocarditis. For a certain diagnosis she needed a cardiac MRI as soon as possible. In the meantime we had to decide the right treatment for this patient. Considering that we couldn`t exclude the diagnosis of STEMI we decided to continue the specific treatment for myocardial infarction – notice that this diagnosis has a worse prognosis vs. myocarditis.

After 3 months the patient was asymptomatic, ECG was normal, echocardiography showed normal systolic function, without parietal kinetic disorder. Cardiac MRI sustains the diagnosis of STEMI, so the patient continues the previous medication.

In conclusion, we had a patient with cardiovascular risk factors, with all criteria for STEMI but without coronary lesions. The differential diagnosis was challenging, but the decision of treating like a STEMI was right, this having the worst prognosis.

Cardiac investigation of the young patient with stroke

A. Anghel, S. Stanciu, Ioana Răduță, L. Ciobîcă, Lorena F. Davidescu

Stroke in the third cause of mortality and morbidity all over the world, but the number in the young patients is low but with major impact on the invalidity.

The strokes in young are ischemic and hemorrhagic

Most of the strokes in young patients are produced by cardiovascular pathologies, many of them unknown at the moment of the diagnosis.

Causes of strokes in young patients are:

1. Cardio embolic
   a. cardiac malformations (DSA, DSV)
   b. arrhythmias (atrial fibrillation, atrial flutter)
   c. endocarditis
   d. cardiac tumors
2. Atherosclerotic
   a. familiar dyslipidemia
   b. vasculitis
3. Hemorrhagic stroke
   a. hypertension (secondary?)
   b. vascular malformation

Cardiac investigation of the young patient with stroke must be complete including morphological exploration (trans-thoracic, trans-esophageal ultrasound, arterial or venous Doppler ultrasound), monitoring (ECG and TA monitoring) and, if necessary advanced imagistic methods (angio TC, angio MRI).

Because of the importance of the disease and the age of the patient the cardiac investigation is mandatory in the diagnosis but also in secondary and primary prevention.

Multiple etiology of deep venous thrombosis

L. Chiriac, R. Roșulescu, Alice Munteanu, S. Dumitrescu, Adriana Gârjău, Magda Gurzun

We would like to report a case of a male patient of 43 years old who was admitted at the emergency room of the Emergency Military Hospital in Bucharest accusing severe localized pains on the right tie which was swollen and presented all the acute inflammatory characteristics.

The clinical exam revealed severe unilateral edema in the right inferior limb associated with extreme pain and functional impairment. The cardiovascular and respiratory function of the patient were stable. Also hemodynamics wasn't affected.

The ECG was without anomalies.

The suspicioned diagnostic was profound venous thrombosis of the right inferior limb and confirmed by performing a venous compression ultrasonography and a Doppler exam.

To refine the etiology of the thrombosis the patient has performed a thoracic-abdominal CT scan native first which revealed no other morphological anomalies but a left retropsoic solid mass.

The thoracic-abdominal CT scan with intravenous and oral contrast revealed the absence of the inferior vena cava and identified the existence of a hypertrophic azygos and hemiazygos veins. The retropsoic solid mass was confirmed as a hypertrophy of the paravertebral venouse plexis anomalies which would indicate a compensatory morphological and functional adjustment to the congenital agenesia of the inferior vena cava.

The patient received anticoagulant therapy represented by high molecular mass heparin having an interval between 50 – 70 seconds as target APTT.

From this case is important to draw the conclusion that it may be important to think at the possibility of an anti-phospholipidic syndrome even in patients who have morphological congenital anomalies of the vascular system and proceed with the specific tests that will confirm or rule out this syndrome.

Is it a place for cardiac MRI in acute clinical settings

Maria Gurzun, Lavinia Florea, Raluca Popescu, G. Neagoe, Ana-Maria Cincă, R. Boiangiu, Ileana Hanțulie, Smărăndița Lacău, S. Dumitrescu

Multimodality imaging became mandatory in cardiac patient management nowadays. Among new imagining modalities cardiac MRI became very important during the last years. However, the long-time necessary for examination, the low availability and the high price make it a method less used in acute settings.

The purpose of this communication is to present a short case series for emphasis the utility of cardiac MRI in patient management during the acute phase.

The reported patients were admitted in cardiac care unit for chest pain, shortness of breath with EKG changes and elevated troponin levels. The two patients were angiographically explored and the coronary artery disease was excluded. The cardiac MRI performed during the first three days excluded the myocarditis for the first patient and confirm it for the second one. The second patient received conservative treatment, having a good recovery. During the hospitalization the first patient presented several hypertensive crises and pheochromocytoma was suspected and confirmed by bioumoral markers and abdominal computer tomography.

Therefore, cardiac MRI was essential in patients' management: in the first case excluded myocarditis and suggest another pathology involved, permitting the establishing of correct diagnosis; in the second case myocarditis was confirmed and the patient received the correct treatment.

To conclude cardiac MRI can be a useful tool for cardiac patient management even in acute clinical settings.

TILT table test

V. Ilieșe, R. Bolohan

Head-up tilt table test is used for the evaluation of the syncope, it starts to be used by Kenny and colleagues in 1986 like passive head-up posture.

The tilt testing is used for the evaluation of the reflex syncope and to discriminate between reflex, orthostatic hypotension syncope and jerking movement from epilepsy.

Preparation before the test, patients must be secured, should have a venous access, should fast 2-3 hours, they may continue their usual medication such as diuretics and antihypertensive. Beta-blockers and other medications for the treatment of syncope must be stopped at least 5 times half-life. The pre-test phase usually takes 5-20 minutes. After that the patient is put to 60-80 degrees; this is the passive phase of a minimum 20 minutes maximum 45 minutes, while the blood pressure and the heart rate is monitored from 5 to 5 minutes and from 1 min in case of symptomatology occurrence.

The pharmacologic provocation uses nitroglycerine and isoproterenol.

The response to tilt test is classified in type 1: mixed in which the heart rate and blood pressure decreases without asystole of <3 second; type 2 – cardio inhibitory 2A – without asystole an 2B – with asystole >3 seconds blood pressure decrease coincides with or occurs before the heart rate decrease; type 3: vasodepressor.

This year, in the first 6 months we performed 12 tests, out of which 3 were carotid sinus syndrome with asystole >3 seconds, 4 were with a mixed response, 5 were negative.

In 2014 we had a case of a 31 years old women, without any cardiovascular pathology, who followed medication for the epilepsy 1 month, she did not feel well, TILT test was positive, type 2B cardio inhibitory with a pause of 30 seconds, and as a consequence we implanted a permanent pacemaker.

Cardiac permanent pacing

V. Ilieșe, I. Țintoiu, R. Bolohan, D. Niță, D. Săvoiu, D. Cîrpaciu, L. Chiriac, Adriana Gîrjău, Andreea Teodorescu, Simona Almarichi, Alice Munteanu, L. Demiraș, Ana Demiraș, R. Roșulescu, G. Neagoe, S. Dumitrescu, Magdalena Gurzun, Ileana Hănțulie

Cardiac permanent pacing is an option for treatment of slow usually symptomatic rhythm. The recommendations for permanent cardiac pacing are well detailed in the ESC Guidelines on cardiac and cardiac resynchronization therapy 2013.

As of the beginning of 2016 we implanted 180 VVI; 56 DDD; we use the access from the left side usually horizontal cut. We always try to use the cephalic vein; other options can be the subclavian, axillary, jugular vein; the leads can be with active and passive fixation.

The leads are placed under radiologic guidance; for each lead we have to measure the threshold; wave amplitude; impedance; slew rate.

We use the active leads for the ventricular usually in the septum and the right appendage; the passive leads are used in old patient with low physical activity.

We choose the programming parameters according to patient pathology.

The ECG is the most useful method for the follow up.

The most common complication that we met is the local hematoma that usually doesn't need an intervention. For the lead dislodgment we had 4 cases.

Cardiac resynchronization therapy

I. Țintoiu, R. Bolohan, L. Chiriac, G. Neagoe, S. Dumitrescu, D. Niță, V. Ilieșe, Magdalena Gurzun, E. Pandea, L. Demiraș, D. Săvoiu, S. Cîrpaciu, Alice Munteanu, Andreea Teodorescu, R. Roșulescu, Adriana Gîrjeu, Simona Amarichi, Ana Demiraș, Ileana Hănțulie

Cardiac resynchronization therapy is an electrical therapy option for patients with heart failure with reduced ejection fraction (FE<35%) and a wide QRS duration (LBB>130ms & non-LBB >150ms), also in patients with reduced ejection fraction who need permanent right ventricular pacing (ESC Guideline: "Heart Failure 2016")

Response to cardiac resynchronization therapy is about to 60-70 % the non-responders are patient with ischemic cardiomyopathy and those with non-LBB:

Approach for the cardiac resynchronization: therapy can be done on the left or right side via subclavian incision. This is the most common procedure. For the venous axes we always use the subclavian vein with two punctures and one puncture we use for coronary sinus lead.

The most difficult part is usually the cannulation of the coronary sinus. There are two methods: the electrophysiological approach and the hemodynamic approach.

The electrophysiological approach uses the EP catheter suitable for the coronary sinus. The cannulation is done in the LAO 30° projection. Once the EP catheter is in the coronary sinus the guiding sheath is advanced in the coronary sinus and after that we do the venography of CS.

The hemodynamic approach can use the guiding sheath or the left Amplatz 2 catheter.

The CS lead can be unipolar, bipolar or quadripolar lead. The quadripolar lead allow us to do multisite pacing.

We have 8 cases of cardiac resynchronization: 4 were ischemic and 4 non ischemic; the patients also met the echocardiographic criteria for the atrio-ventricular, inter-intraventricular dys-synchrony; 1 was non responder he was an ischemic non–LBBB; 5 was responders with improvement of the clinical and echocardiographic parameters.

The principal tool that we use for programing CRT is the ECG. It can show you the site of pacing, the location of the lead and the fusion proportion.

For future we ca ensure more CRTs provided that we have the necessary equipment.

Inflammation, periodontal disease and subclinical vascular injury

S. Dumitrescu, L. Chiriac, Maria M. Gurzun, Ileana Hănțulie, R. Boingiu, S. Stanciu, D. Săvoiu, Raluca Popescu, H. Barbu

Introduction: There is a unanimously accepted connection between inflammation and periodontal disease (PD) and between hs-CRP levels and atherosclerosis, nevertheless there are few studies connecting all three.

Objective: We planned to investigate the relationship between inflammation, subclinical vascular disease and the periodontal disease (PD) status.

Methods: We evaluated 190 individuals in the course of a cardiovascular primary prevention program. Risk factors data were collected using an individual assessment sheet and through measurement of laboratory parameters (hs-CRP, lipid profile, blood glucose) in a period of +/- 3 days from the baseline clinical and echo evaluation. Vascular ultrasound data was collected on examinations made in the same day: brachial artery flow mediated dilatation (FMD) and carotid artery intima media thickness (IMT). Data on PD status were collected through a clinical exam performed in a period of +/- 7 days from the baseline evaluation and categorized the study group in a subgroup of normal periodontal status (NPS= 69 persons; 36,3%) and periodontal disease subgroup (PD= 121persons; 63,7%) which was further divided into a gingivitis subgroup (G=95 persons;

50%), mild to moderate periodontal disease subgroup (PM=19 persons; 10%) and severe periodontal disease subgroup (PS=7 persons; 3,7%).

Results: After data collection we compared the mean values for hs-CRP and vascular ultrasound measurements according to PD status. The persons with periodontal disease have an increased level of inflammation and vascular dysfunction (p<0,001). Accordingly, there is a significant correlation between hs-CRP level and both FMD (r=-0,785, p<0,001) and IMT (r=0,360, p<0,001). Subclinical vascular disease is widespread in subjects with abnormal PD status and inflammation (hs-CRP >1 mg/dl), 111 (92%) persons out of 121 having at least one type of subclinical injury. We calculated concordance coefficient for group classification based on inflammatory status (low risk: hs-CRP< 1mg/dl, moderate risk: hs-CRP =1 – 3 mg/dl and high risk: hs-CRP>3 mg/dl) compared with classification based on PD status and we found Kappa=0.715, (95%CI = 0.632-0.799; high concordance).

Conclusions: Our study confirms the connection between inflammation and periodontal disease and links both of them with evidence of subclinical vascular injury.

OP session MD9

Soft palate paralysis – a misleading onset of invasive mucormycosis involving nasopharynx and paranasal sinuses

V. Gheorghiță, R. Hainăroșie, R. Vasilescu, Eliza Grămadă, C. Socoliuc, Mona Popoiu, I. Ștefan, F.A. Căruntu

Introduction: Mucormycosis (MCM) is a life-threatening invasive fungal infection caused by fungi belonging to the Mucorales order. Despite aggressive therapy, the overall mortality rate remains unacceptably high (around 50%).

Material and methods: A 56-years-old diabetic male patient was referred to the "Matei Bals" National Institute for Infectious Diseases on August 7th, 2014 with suspicion of nasopharyngeal (N-Ph) MCM. The disease had been started 8 days before with severe odyno-dysphagia, regurgitation of liquids through the nose, right ear pains and headaches without fever. Laboratory analysis found hyperleukocytosis (21,651/mm^3), inflammatory biological syndrome (CRP 269 mg/L) and hyperglycemia (448 mg/dL). ENT check-up revealed an intense erythema of the posterior pharyngeal wall with a slight bleeding in the cavum and the right nasal fossa. The symptoms have improved under antibiotics (Ceftriaxone + Metronidazole + Levofloxacin), antifungals – Fluconazole and steroids treatment. In 6 days the initial lesions progress to the black necrotic eschar. The tissue biopsy was performed. Cerebral and facial CT scan showed maxillary, ethmoidal and sphenoidal right sinusitis, without brain lesions. We started treatment with combination of lipid formulation of amphotericin B (AMB 5 mg/kg/day) and posaconazole (POS-oral suspension, 800 mg/day) for 13 days, followed by POS monotherapy. Glycemic control was achieved with insulin therapy. Histopathological exam and culture confirmed MCM. Mucor was identified as Rhizopus oryzae by molecular methods. Resection of necrotic tissue was delayed until the 18th day of antifungal treatment.

Conclusion and discussions: In the high risk group of patients this clinical onset (with soft palate palsy) could be indicative for MCM. The early diagnosis was clearly associated with favorable outcome.

Thyroid dysfunction in sepsis in a prospective study

V. Gheorghiță, Alina E. Barbu, Monica L. Gheorghiu, I. Ștefan, A. Streinu-Cercel, Ruxandra Moroti, F.A. Căruntu

Introduction: The objective of our study was to evaluate the thyroid function during sepsis in order to identify a possible correlation with severity and final outcome of sepsis in a prospective on-going study.

Material and methods: A prospective, non-interventional cohort study was conducted in Matei Bals National Institute for Infectious Diseases between January and June 2015. Inclusion criteria included sepsis caused by bacterial infection in HIV negative adult patients who signed informed consent. Apart from demographics, clinical and microbiological data we measured serum thyroid hormones (TSH and fT4) and procalcitonin (PCT) at four different times: T0-admission, T1-24 hours, T2-72 hours and T3-7th day.

Results: 27 patients met the inclusion criteria. The median age was 67 years, (IQR, 57-78) and 55.5% (n=15) were male. 40.7% (n=11) of patients had severe sepsis and 18.5% (n=5) septic shock. The median value of PCT at diagnosis was 48.8 ng/mL, (IQR, 10.7-88.04). In 48.1% (n=13) of patients the etiology was established. The gram negative bacilli were identified in 84.6% (n=11) of cases. Thyroid dysfunction was present in 29.6% (n=8) of patients, 62.5% (n=5) having hypothyroidism. The median value of TSH in patients with hypothyroidism was 5.134 UI/mL, (IQR, 4.224-6.137). 75% (n=6) of patients with thyroid dysfunction had more severe disease at baseline. The mortality was 37.5% (n=3) in patients with thyroid dysfunction compared to 18.5% (n=5) in the whole cohort, p=0.32.

Conclusion: Thyroid dysfunction was diagnosed in about quarter of patients with sepsis and seems to correlate with the severity of sepsis and poor prognosis.

Psychiatry and terrorism

C. Cândea, D. Vasile, O. Vasiliu, B. Petrescu, A. Mangalagiu, Irina Cândea, A.M. Badic

Terrorism has dominated the domestic and international landscape since 9/11. Like other fields, psychiatry was not well prepared. It is time to consider what can be done to prepare before the next event. Much has been learned to provide knowledge and resources. The roles of psychiatrists are challenged by what is known of the causes of, consequences of, and responses to terrorism. Reflecting on knowledge from before and since 9/11 introduces concepts, how individuals become terrorists, how to evaluate the psychiatric and behavioral effects of terrorism, and how to expand treatments, behavioral health interventions, public policy initiatives, and other responses for its victims. New research, clinical approaches, and policy perspectives inform strategies to reduce fear and cope with the aftermath. This article identifies the psychiatric training, skills and services, and ethical considerations necessary to prevent or reduce terrorism and its tragic consequences and to enhance resilience.

Transcultural psychiatry

C. Cândea, D. Vasile, O. Vasiliu, B. Petrescu, A. Mangalagiu, Irina Cândea, A.M. Badic

This article deals with the main concepts of Transcultural Psychiatry and their applications to everyday psychiatric practice. Transcultural psychiatry has undergone a conceptual reformulation in the last two decades. Having started with a comparative approach, which focused on the diverse manifestations of mental disorders among different societies, it broadened its scope, aiming at present to incorporate social and cultural aspects of illness into the clinical framework. Therefore, transcultural psychiatry now focuses more on what is called the illness experience than on the disease process, the latter understood as illness as it is viewed by health practitioners. Western medicine, of which psychiatry is a part, is grounded in positivist epistemological principles that stress the biological processes of disease. The intention of the paper is to develop an interest in alternative but also complementary ways of thinking. Modern transcultural psychiatry interprets some epidemiological and clinical aspects of major mental disorders (such as schizophrenia and depression) in a different light. However, it also distances itself from the absolute relativism of antipsychiatry, centering on clinical facts and helping clinicians in their primary task of alleviating suffering. An important contribution in addressing this task is the formulation of a cultural axis within the DSM model of multiaxial evaluation. A clinical vignette of a cultural formulation applied to a clinical discussion of a case is described.

Expeditionary modular systems for sampling, detection and identification of CBRN agents

V. Ordeanu, I. Savu, A. Vladimirescu, Lucia E. Ionescu, Victoria G. Dumitrescu, Diana M. Popescu, Nicoleta S. Bicheru, M. Necșulescu, A.G. Corlan, M.S. Tudosie

The research, development and implementation of a modular expeditionary systems for sampling, detection and identification of CBRN agents, that is useful for providing information about the potential CBRN contamination, will ensure the samples necessary to perform the identification and confirmation testing's in specialized fixed and mobile laboratories with additional protection of the operators.

The novelty of the project proposal consists in designing and developing an integrated expeditionary systems, that can be transported by land, air, sea etc. and which will be provided with a remote drones for recognition of high risk areas and sampling.

Implementation of projects will follow defining the composition of the expeditionary modular system, depending on the missions of the mobile intervention teams, establish methods of sampling and integrating executed modules, making the decontamination devices of the remote drones and of the foldable mini-aerodrome, integration of the components and execution of an experimental model of the expeditionary modular systems, testing the modules under laboratory conditions in order to verify their correct functionality within the system, testing the experimental functional model on the site. The partners have experience in making kits and sampling kits for transport and testing of agents, explosives etc. The system does not exist in the manufacturing or marketing phase, in the proposed configuration.

The transfer for industry of the scientific and technological results is provided by industrial partner's capabilities, which has experience in the production of CBRN defense equipment, as a developer and supplier for beneficiaries of the national security system.

A rare case of hypokalemia – case report

V. Smedescu, Roxana Diaconu, Denise A. Mardale, Cristina Spiroiu, E. Firoiu, Anca Manolache, Mihaela Enache, I. Copaci, C. Jurcuț

We report the case of a 57-year-old woman, who is hospitalized for abdominal pain and jaundice with a one month onset.

The clinical exam further revealed hepatomegaly, dark urine and pale stools. Laboratory exams showed neutrophilic leukocytosis, hepatic cytolysis, cholestasis syndrome and severe hypokalemia.

The upper and lower gastrointestinal endoscopies were within normal parameters, while the chest and abdominal computed tomography showed a left lower lobe pulmonary tumor, multiple mediastinal and abdominal adenopathy and also liver and bone metastasis.

The pulmonary tumor was biopsied and small cell lung cancer was diagnosed. Despite treatment with oral and intravenous potassium salts the hypokalemia persisted, while the patient subsequently accused abdominal cramps and loose stools.

We did further tests which showed high serum levels for cortisol, chromogranin A and serotonin and also high urinary values for 5-HIIA, thus confirming the presence of two paraneoplastic syndromes: secondary Cushing syndrome and carcinoid syndrome.

The particularity of the case is represented by the rare association of these two paraneoplastic syndromes in this type of neoplasia and the discordance between the high hormonal values and the presence of minimal specific symptoms.

An unusual case of palpebral swelling and erythema

V. Smedescu, Oana Stancu, Anca Manolache, D. Chiriță, I. Copaci, C. Jurcuț

We report the case of a 47-year-old woman who presents with fever, left palpebral and zygomatic swelling and erythema, cervical adenopathy, hepatosplenomegaly and erythema nodosum on the right arm. The patient was treated with broad spectrum antibiotics without any improvement.

The laboratory exams showed an inflammatory syndrome, moderate anemia, thrombocytopenia, lymphopenia and mildly elevated aminotransferase levels. The tests for infectious diseases and tumoral markers were normal, while the screening for SLE, Sjogren syndrome and vasculitis was negative. The cerebral and cervical MRI revealed left lacrimal gland and periorbital subcutaneous fat inflammatory infiltrates. The thoracic and abdominal computed tomography showed hepatosplenomegaly and multiple mediastinal and intraabdominal adenopathy. The bone marrow examination was normal, while the pathological exams of an excised lymph node supported the diagnosis of reactive adenitis. A skin biopsy was performed which established the diagnosis of lupus erythematosus tumidus (LET). The symptoms were considerably improved after systemic course of glucocorticoids without recurrence at one year of follow-up. LET is a rare form of cutaneous lupus erythematosus, recently being redefined as an independent entity.

The particularity of this case is the association of LET with several systemic manifestations, however without enough criteria to support the diagnosis of systemic lupus erythematosus.

OP session MD10

Metformin effects on endometrial hyperplasia in women of reproductive age with PCOS

A. Ranetti, Anca Pati-Cucu

Background: Polycystic ovarian syndrome (PCOS) a metabolically determined gynecologic disorder with an incidence of 4 to 184% in women of reproductive age. PCOS diagnosis is set based on Rotterdam criteria, including hyper-androgenism, chronic anovulation and typical ultrasound appearance. Endometrial hyperplasia occurs in 1 to 48% of women with PCOS and can progress to endometrial cancer.

Material and method: 20 non-diabetic women between 25-35 years old, with PCOS untreated prior to presentation, with a BMI>30 kg/m^2, were included in a pilot study. Endometrial hyperplasia was defined as endometrial thickness more than 15 mm in the secretory phase. Endometrial biopsy in all women showed a simple hyperplasia without atypia. 10 women choose progestin-only treatment along with diet (non-Met-group) and 10 started a 6 months therapy with diet and metformin administered daily on a weekly increased dose up to a total of 2 g/day (Met-group). None of the patients were on a meat and dairy free diet. We also reviewed metformin influence on reproductive function with emphasis on endometrial effects.

Results: The Met-group had a significant weight reduction and a decreased endometrial thickness compared to non-Met and also ovulation occurred faster in Met-group.

Conclusions: Metformin, a biguanide used in treatment of type II diabetes mellitus, could be used single or in association with oral contraceptives or progestins in non-diabetic patients with EH. Still, further studies including larger cohorts of patients are needed.

The role of computed tomography (CT) in the evaluation of acute abdominal pain in patients without trauma

C. Sandu, Mihaela Gheorghiu, Cristina Sandu, Diana Soloman Năftănăilă, F. Năftănăilă

Non-traumatic abdominal pathology is one of the most common reasons for admission to the emergency department. Accurate and rapid diagnosis of these conditions helps in reducing related complications. Clinical assessment is often difficult due to availability of over-the-counter analgesics and self-medication practice, leading to less specific physical findings.

Laboratory and conventional radiographic findings are in many cases unhelpful. Thus, cross-sectional imaging plays a crucial role for initial evaluation and management of acute abdomen. Multidetector computed tomography is the primary imaging modality used for these cases due to fast image acquisition. There is no evidence of using one single strategy tool. The clinician may choose either routine ultrasound (US) evaluation complemented by CT in case the US is inconclusive or first-choice CT.

The usual causes of non-traumatic acute abdomen are bowel obstruction, acute pancreatitis, gastrointestinal perforation, diverticulitis, appendicitis, and cholecystitis. Less frequent, but also important causes are ruptured abdominal aneurysm, spontaneous abdominal bleeding due uncontrolled anticoagulant therapy, acute mesenteric ischemia or mesenteric venous thrombosis.

The role of ultrasound guided trans-bronchial puncture in diagnosis of lung cancer

Ioana Ştefănescu, E. Firoiu, Ioana Oprea, Florentina Vasilescu, Chim Aneta Şerbescu

Introduction: Endobronchial ultrasound (EBUS) is a minimally invasive but highly effective procedure used to diagnose lung cancer and other diseases causing enlarged lymph nodes in the chest. Although the method appeared in the late 90s, emerged in Romania for several years, initially in Iasi, after that in Cluj-Napoca. The Military Hospital is the first hospital in Bucharest that brought this technique in December 2015.

Materials and methods: We conducted a study in the Department of Pneumology of SUUMC on the patients with radiologic suspicion of pulmonary cancer, investigated between December 2015 - June 2016 by EBUS-TBNA, with moderate sedation (Midazolam and Propofol).

Results: All the patients were imagistic diagnosed with pulmonary tumors and mediastinal lymph nodes. The majority of patients with diagnostic imaging of lung tumors were confirmed by EBUS-TBNA both cytology and histopathology (cell block).

Conclusions: EBUS-TBNA is a minimally invasive method, with small risk, highly effective in the diagnosis and staging of lung cancer. This method could replace in some cases mediastinoscopy as a method of diagnosis of lung cancer, especially in patients with severe comorbidities. This technique can be improved by using thicker needles, although procedural risks may grow.

Multiple trauma diagnosis and treatment algorithm in ED

B. Teușdea, M. Toma, S. Dogaru

Trauma is in Romania a problem of public health, and it has the highest mortality in the population with ages between 15 and 44 years. Multiple trauma (MT) represents an acute severe status appeared after a mechanic, thermal, chemical or electrical impact, status characterized by multiple lesions at organ and body systems. Lesions that affect more than one organ are characteristic and are frequently fatal, in patients suffering from MT under different disasters /war, therefore this kind of patient needs a prioritization of the lesions, in order to treat them, and he represents a challenge for the trauma team.

The traumatized patient has an unpredictable evolution because it synthesizes multiple traumatic sites, with an immediate and delayed reaction, having a great potential for developing MSOF (syndrome of multiple organ failure). This type of patient develops shock of various origins with high severity. That's why a well-organized chain of medical aid increases the survival of patients with multiple trauma and decreases mortality and morbidity.

Emergency physicians play a vital role in the stabilization, diagnostic and initial treatment of MT patient at ED level. MT patients' management implies complex, time-dependent, decision–making, leadership capability and technical skill. Therefore at ED level we need instruments - algorithms that facilitate the diagnosis and treatment of MT patients. In this paper we present the algorithm for the diagnosis and treatment of MT patient because we consider that an algorithm, using ATLS principles, is necessary to guide management of this type of patients.

Our experience with point of care testing analysis in the Emergency Department

B. Teușdea, M. Toma, S. Dogaru, Luminița Popa

For emergency medicine, about 70% of the decisions, made by the physicians working in the ED and the physicians on duty, regarding admission, discharge and medication are based on lab results. In the last few years, Point of care testing (POCT) in the Emergency Department (ED) is becoming a lot more common.

POCT instruments are used every day in our ED for biomarkers determination, such as cardiac biomarkers (CK-MB, troponine I, NTproBNP etc.), sepsis biomarkers (presepsin), blood gas analysis (PaO2, PaCO2, pH etc.) and also for blood tests – blood cell count, biochemistry and urine tests (toxicology).

We made a comparison between the blood test results obtained on POCT instruments (biochemistry) and the blood tests obtained in the Central Laboratory of the hospital. The final result was a little difference between the 2 lab tests, but with no statistically significance.

The urosepsis

Ioana Oprea, Denisa Ghinescu, B. Petre, O. Bratu, D. Corneci, Mirela Bidilică, L. Ene

Sepsis is a life-threatening problem that leads to increase the mortality, morbidity and costs of healthcare services. In spite of huge efforts up to now, have no reached to safe therapy with reproducible effects systematically.

Within sepsis, interleukins, cytokines and other factors of inflammation reach to significant serum concentration and affect all organs and body systems, getting to acute dysfunctions and shortfall of these. According to Surviving Sepsis Campaign 2012, sepsis is defined as documented or suspected infection, accompanied by various laboratory or clinical variables. Due to major implications arose in the context of the diagnosis and complex therapeutic approach of affected patients, worldwide appear new ideas, concepts and assumptions with frequent updates in order to optimize the septic patient approach with or without associated organ dysfunction.

Medical approach must be multidisciplinary one, targeted both on the treatment of the cause (initial infection), but also to maintain internal homeostasis simultaneously with decreasing the damage of other organs and systems.

When the starting point of sepsis is an infection within the urinary tract, it is called urosepsis. This could be life-threatening, if emergency treatment is not carried out. Statistically, urosepsis accounts for approximately 25% of all cases of sepsis.

The urosepsis remains a challenge for the urologist, intensive care doctor, radiologist and microbiologist. It is very important to have a good cooperation between these specialties and teamwork.

Threat of Burnout Syndrome in the Emergency Department – our experience

B. Teușdea, M. Sălceanu

Burnout Syndrome is defined as a psychological syndrome that occurs among the employees who work in a stressful environment and as a result of an imbalance between expectations and resources. This syndrome is more than an extreme fatigue and involves emotional exhaustion, depersonalization, and a diminished sense of personal accomplishment with a secondary disengagement that occurred among different professionals who work with other people in challenging situations.

Through this paper we hope to raise awareness about this danger and we try to identify how this syndrome affects our staff in Emergency Department at Central Emergency Military University Hospital "Carol Davila" Bucharest.

For this purpose we use a new instrument called "The Oldenburg Burnout Inventory" (OLBI), applied to all our staff members, that includes positively and negatively statements to assess the two core dimensions of burnout: exhaustion and disengagement (from work). We found interesting data and correlations in matter of age, gender and level of qualifications.

Moreover we suggest methods of prevention and treatment in order to increase the quality of life and satisfaction both for employees, employer and the beneficiaries of the medical services that we offer.

Percutaneous tracheostomy – the experience in SUUMC ICU

Ș. Zahiu, L. Ene, Mirela Bidilică

Percutaneous dilatational tracheostomy (PDT) is a minimally invasive, rapid, bedside technique, elective procedure used in Critical Care services to create a stoma between skin and trachea. Its popularity has partly been due to the procedure being more readily available with less restraints from theatre availability, accessing surgeons, cost and time involved in coordinating patient transfer compared to a standard surgical tracheostomy (SST).

The main indications are: 1. Mechanical obstruction of the upper airways, 2. Protection of tracheobronchial tree in patients at risk of aspiration, 3. Respiratory failure, 4. Retention of bronchial secretions, 5. Elective tracheostomy.

Modern PDT was described in 1955 and in 1969. The dilating tracheostomy forceps were developed in 1989 and further by Griggs in 1990 who introduced the use of a guidewire. The most commonly used PDT kits are available from Cook Critical Care and Sims Portex.

In ICU/SUUMC we began the use of PDT in 2015 and up to August 2016 we performed 23 such procedures. The kits we used are Portex percutaneous tracheostomy with a special forceps that allows the passage of a guiding wire. Bedside bronchoscopy performed by pneumologists was mandatory pre-procedural during procedure and as a control after having performed the operation. There was one case when Ear-Nose-Throat specialist had to perform hemostasis 4 hours after tracheostomy.

PDT is a reliable procedure in ICU, performed by ICU physician, ICU nurse and pneumologist.

The post-streptococccal syndromes as border pathology – an infectious diseases practitioner point of view

B. Cîrciumaru

Background: The post-streptococcal syndromes are the sequelae of the streptococcal infections that frequently occur during childhood. They are borderline pathology within internal medicine specialties (including the infectious diseases) and a quite common condition for our country, thus organizing the clinical forms and therapeutically approaches becomes a necessity.

Materials and methods: I retrospectively studied, the post-streptococcal syndromes admitted into the Army`s Infectious Diseases Ward, within the last 10 years. Most of the patients were monitored twice yearly, and treated, sometimes, medical and surgical (e.g. for endocarditis). In order to have a consistent view of the problem, I had discussions with different specialists, including family doctors.

Results: I described the forms of the streptococcal infections (self-limiting) as well as the post-streptococcal syndromes (monophasic and multi-recurential) and I determined reasonable therapeutically conducts for the antibiotic treatment and the prophylaxis.

Conclusions: The antibiotic treatment is essential in order to eradicate the remnant infectious focuses: as using two doses of Benzathinpenicillin G every 5-7 days, and every 10-14 days for the secondary prophylaxis. The length of the secondary prophylaxis remains controversial, monitored by the clinical improvement, biological markers (e.g. the reduction of the inflammatory phenomena, the ASO dropping tendency) and other investigations. I presented my opinion regarding the monitoring and the antibiotic therapy, as part of multi-disciplinary approach. I support the inclusion of the conclusions within the Hospital Guidelines.

A complicated case report associated with rheumatoid arthritis

L. Ciobîcă, Daniela Anghel, S. Stanciu, Raluca Lutuc, I. Sârbu, Ancuța Coca

A 43 years old patient, diagnosed at age 16 with JIA and at 23 years with Hashimoto's autoimmune thyroiditis. Three years later she was diagnosed with seronegative RA, for which she followed the classical remission therapy without an effect. Beside the existing autoimmune pathologies, she added a type 1 insulin-necesitant diabetes, shortly complicated with retinopathy, mixed neuropathy and gastropathy.

Over the last 10 years appear recurrent episodes of iridocyclitis, the reason why we considered to initiate the biologic therapy with Etanercept, without final, due to a pulmonary tuberculosis diagnosed by tests according to the protocol. Subsequently the patient develop a secondary sicca syndrome, complicated with corneal ulcer paracentral wich became infected, for that we practiced the enucleation of OS (March 2015).

Consequently of the unbalanced diabetes (HgA1c = 17.6%), the patient developed an infected interdigital ulcer in the IV-V fingers foot, complicated with a shore perforating plantar right which became infected.

The patient showed a varied clinical picture, and the laboratory indicated a microcytic hypochromic anemia, a moderate inflammatory syndrome, a positive FR, ACPA = 103.3 IU/ML and not least a coproparazitologic exam - Positive for Clostridium difficile. At the ophthalmic reevaluations, the acute iridocyclitis was in a healing process, but with a complicated cataract and a proliferative diabetic retinopathy.

As a particularity of the case, a patient with mixed autoimmune pathologies, followed by a fulminant evolution which was therapeutic neglected. Recurrent episodes of iridocyclitis, infectious complicated, by an enucleation of the right eye.

OP session MD11

Sarcoidosis – challenging diagnosis

L. Ciobîcă, I. Sîrbu, Ancuța Coca, Alexandra David

Sarcoidosis affects people of all racial and ethnic groups and occurs at all ages, although it usually develops before the age of 50 years, with the incidence peaking at 20 to 39 years. The presentation in sarcoidosis varies with the extent and severity of organ involvement. Asymptomatic – incidentally detected on chest imaging ~ 5% of cases, systemic complaints: fever, anorexia ~ 45% of cases, pulmonary complaints: dyspnea on exertion, cough, chest pain ~ 50% of cases; Löfgren syndrome (fever, bilateral hilar lymphadenopathy, and polyarthralgias): common in Scandinavian patients, but uncommon in African-American and Japanese patients.

We are presenting 3 types of clinical presentation of sarcoidosis with rare onset of the diseases. They were in the age incidence peaking, one of them had unilateral ankle swelling, the other one had a single erythema nodosum and the last one had been diagnosed with pulmonary carcinomatosis.

After performing the investigations in our clinic all of them were diagnose with sarcoidosis.

Catheter sepsis in patient with rituximab treatment for non-Hodgkin lymphoma

C. Bănică, I. Ștefan, B. Cîrciumaru, S. Ionescu, O. Dunăreanu, I. Pandrea, O. Călina

Introduction: Catheter-related bloodstream infections accounts for 5 to 20% of hospital-acquired infection. Frequently, catheter-sepsis is caused by multidrug-resistant micro-organisms: gram-positive cocci like S. aureus, Enterococcus or gram-negative bacilli – Pseudomonas aeruginosa, Acinetobacter baumanii, Enterobacter. In immune-depressed patients fungal or polymicrobial infection have been reported. These patients are also at risk for severe evolution.

Presentation: We present the case of a 62 years-old female with non-Hodgkin lymphoma under treatment with Rituximab – the second therapeutic cycle. She got a port-a-cath device on her left subclavian vein. The patient was admitted in our department, after the Rituximab cycle, with high fever, chills, deterioration of the general status and she was diagnosed with catheter-related sepsis. Under large spectrum antibiotics the fever persisted. The patient recovered quickly only after the removal of the intravascular device. All the blood cultures were negative, although the catheter tip culture was positive for Enterococcus faecalis.

Conclusions: The use of central venous catheters is part of the modern practices in critically-ill patients. In neoplastic patients, with fragile peripheral veins, intravascular devices are useful for giving intravenous treatment, blood transfusions or taking blood tests, but they raise the risk for catheter-related infections. In these immune-depressed patients, multidrug-resistant bacteria or fungi can lead to severe sepsis and metastatic infectious complications.

Methods of evaluation for cardiovascular continuum state in type 2 diabetes mellitus patients

C. Constantin, Georgiana Constantin, A. Ranetti, C. Serafinceanu

Introduction: During life time, a patient with type 2 diabetes mellitus (T2DM) could develop complications and an adapted tool should be used for evaluate the cardiovascular risk. There are some score risks present in clinical practice and each of them are developed using some characteristics of patient. This presentment will develop tools for evaluation cardiovascular risk in different situation of T2DM patient.

Material and method: This presentation summarizes the most important evaluation tools for cardiovascular risk in T2DM patients. A real trained practioner use the appropriate score and short and long therapeutic attitude should be adapted using the best medical guidelines. The case management implications and also the vital risks will be appropriate evaluated and presented during an acute event (stroke, acute coronary syndrome, acute peripheral artery obstruction etc.) and could demonstrate the best predictive value for prediction of long-term mortality and morbidity in T2DM patients.

Results: There is not a single and a general available score to evaluate and to be used during lifetime of a T2DM patient. A real and integrated score risk is – in fact – a proper score applied at a proper moment.

Discussions: This short presentation will try to clearly describe these tools and the best moment when that tool should be applied. A lot of actual tools are present in cardiovascular risk market, but a clearly used algorithm for application of these scores should be implemented.

Fluid, gas, fibrosis, blood, calcifications and tumors in the retroperitoneal space. Imaging evaluation

D. Cuzino, Oana Baston, C. Blaj, F. Năftănăilă

Introduction: Retroperitoneal space is a challenging zone for imaging diagnosis. The lesions in this area can arise from the retroperitoneal organs but also from outside.

Material and method: We have revisited our cases in the last three years and we found diverse infectious, inflammatory, tumoral, traumatic and congenital lesions. All of these cases were explored first of all using CT. Some of these cases have been also explored using MRI for a better characterization of the lesion extension.

Conclusion: The complexity of the retroperitoneal lesions need anatomical and semiological analysis.

Imaging diagnosis also need to be interpreted in a strong correlation with the physio-pathological understanding of the region.

Infections in rheumatologic disorders

L. Ciobîcă, Daniela Opriș, Alexandra David

Arthritis tuberculosis has its origins in hematological spread due to activating sites, in the first stage of the disease. Extra-pulmonary infection with Mycobacterium tuberculosis has musculoskeletal involvement in up to 19% of cases. The most involved joints are: the spine, hip and knee. There is no specific clinical manifestation. The symptoms vary from night sweats, cough, and weight loss, with or without joint manifestation: rubor, calor, dolor and in some rare cases evacuation of caseum from a joint fistula.

We report a case of a 79 old female patient who presented in the emergency department for polyarthralgia with onset of symptoms in the last 3 weeks. The right shoulder joint was tender, swollen and red, no fever at the moment nor in the last half of the year. There was no recent history of trauma, of respiratory, infective, or joint disease; because the pain was not improving after usually non steroid drugs she was given for the last 10 days dexamethasone 8 mg/day. She was also known with splenic lymphoma, interstitial pulmonary disease and cardiovascular pathology.

Monitoring biological therapy – a continuous challenge

L. Ciobîcă, Daniela Anghel, D. Stoicescu, Iolanda Sîrbu, A. Dumitru, S. Stanciu, Ioana Răduță, Ancuța Coca

Biological therapy has revolutionized the treatment of rheumatic diseases in the past decade improving not only the heath of the patent but their quality of life.

However, monitoring the progressions of the disease and the possible side effects of the drugs can sometimes become a challenge.

We would like to bring to your attention the case of a 62 year old male with ankylosing spondylitis (HLAB 27+), type II diabetes and hypertension.

10 years after the onset of the disease he is started on etanercept and a few months later the patient presents with pain in the low back region.

The differential diagnosis in now important for excluding neoplastic lesions, tuberculous spondylodiscitis and bacterial spondylodiscitis and the Andersson lesion in ankylosing spondylitis.

The Andersson lesion is an inflammatory involvement of the intervertebral discs in spondyloarthritis. It is a non-infectious condition that occurs in about 8% of patients with ankylosing spondylitis.

OP session MD12

The Chicago classification and its contribution to high resolution esophageal manometry studying

Florentina Ioniță Radu, Andrada Popescu, Maria M. Chereja, A.I. Gavrilă

Introduction: This publication aims to summarize the state of our knowledge of the third Chicago classification criteria for esophageal motility disorders published by the International HRM Working Group, as well as the classification`s usefulness in clinical high resolution esophageal manometry studying.

Materials and methods: The primary objective of the Chicago classification (CC) is to categorize esophageal motility disorders in individuals with non-obstructive dysphagia and/or esophageal chest pain by applying standardized high resolution manometry (HRM) marks. The study is based on the standard HRM test in which the patient is asked to perform ten 5-ml swallows of water. On the color pressure topography plots that result, the software is able to define a set of parameters that are used in the classification and these are: contractile deceleration point (CDP), distal contractile integral (DCI), distal latency (DL) and integrated relaxation pressure (IRP).

The metrics obtained from the HRM study can be used to characterize individual test swallows (in terms of integrity of contraction, contraction pattern and intrabolus pressure pattern), but most important, they can define esophageal motility disorders and classify them in a) achalasia and EGJ (eso-gastric junction) outflow obstruction, b) major disorders of peristalsis and c) minor disorders of peristalsis.

Results and conclusion: The Chicago classification is a useful tool that can guide course of treatment in patients with esophageal motility disorders, despite the fact that metrics and criteria it uses can be hard to understand. It is an evolving process, with its third version incorporating recent advances in the understanding of esophageal motility pathology.

The impact of bariatric surgery on upper gastrointestinal symptoms in obese patients – the role of esophageal manometry

Andrada Popescu, A.I. Gavrilă, I.P. Nuță, Raluca S. Costache, Mariana Jinga, Săndica Bucurică, B. Macadon, M. Pătrășescu, Mirela Chereja, Florentina Ioniță Radu

Introduction: The role of this paper is to point out the importance of GI functional testing (especially esophageal manometry) before bariatric surgery, in order to reduce the prevalence of GERD symptoms, and the need for a medical protocol regarding this issue.

The literature data available reveals that the prevalence of GERD is higher in obese patients compared with normal weight controls, with an increased risk of 2.5 of developing symptoms and erosive esophagitis. This is most likely related to increased esophageal acid exposure. Several pathophysiological mechanisms may be involved: transient lower esophageal sphincter relaxation (TLESR), lower esophageal sphincter (LES) pressure, altered esophageal motility, presence of hiatal hernia and esophageal factors such as poor esophageal clearance, altered gastroesophageal pressure gradient and delayed gastric emptying. In morbidly obese patients, some of these mechanisms may occur increasing the risk of developing severe GERD, but they can be established with esophageal manometry.

We know from other studies that the Roux-en-Y gastric bypass is considered an effective method to alleviate symptoms of GERD, whereas laparoscopic sleeve gastrectomy appeared to increase the incidence of the disease. Adjustable gastric banding was seen to initially improve the symptoms of GERD; however, a subset of patients experienced a new onset of GERD symptoms during long-term follow-up. Thus performing esophageal manometry and pH-metry before bariatric surgery can give important information and help in choosing the right type of surgical intervention, reducing the prevalence of upper GI tract symptoms.

Conclusion: Careful medical assessment is mandatory before performing any type of bariatric surgery in obese patients, especially for those who already have GERD.

Update in the management of uncomplicated acute pancreatitis

Cătălina Diaconu, Gaudia Avram-Mănescu, Laura Voicu, Florentina Ioniță-Radu, Mariana Jinga, D.O. Costache, Raluca S. Costache

Acute pancreatitis represents the inflammation of the pancreas, inflammation that can be localized to the gland or it may extend to peripheral tissues or distant organ systems.

The treatment in acute pancreatitis is directly aimed at relief of symptoms. Fluid repletion, glucose repletion/nutrition and analgesia are the main pillars in managing acute pancreatitis. Studies show that early fluid resuscitation with 5-10 ml/kg/h is imperative in the first 12 to 24 hours. Moreover crystalloids are the first choice, rather than colloids. Ringer lactate was proven to have lower risk in developing systemic inflammatory response syndrome (SIRS) and might reduce the risk of metabolic acidosis and non-anion gap when compared to chloride-rich solutions (saline). The goals in fluid repletion are: to decrease of hematocrit and blood urea nitrogen and to maintain normal levels of creatinine. In patients with acute pancreatitis and cholangitis endoscopic retrograde cholangio-pancreatography (ERCP) is mandatory in the first 24 hours.

Antibiotherapy is not recommended as a routine treatment and should be reserved in infected pancreatic or extrapancreatic necrosis that fails to improve after 7-10 days of hospitalization and extrapancreatic infections (cholangitis, pneumonia, bacteriemia, cathether-acquired infection). In these cases carbapenems, quinolones and metronidazole are the treatment of choice. Antifungal agents are not recommended as prophylaxis.

Since severe abdominal pain is the main symptom, analgesia should be taken into consideration: opioids (fentanyl or hydromorphone). Enteral feeding is essential in acute pancreatitis, regardless the severity, once the symptoms subside. Even though most guidelines in the past recommended NPO (nothing by mouth) nowadays studies have shown that enteral nutrition is feasible and improves outcome and can be performed via nasogastric or nasojejunal tube and use low-fat semielemental formulas.

Hereditary hemochromatosis: pathogenesis, diagnosis and treatment

Gaudia V. Mănescu-Avram, Cătălina Diaconu, Laura Voicu, Florentina Ioniță-Radu, Mariana Jinga, D.O. Costache, Raluca S. Costache

Hereditary hemochromatosis is an autosomal recessive genetic disorder often determined by mutations in the HFE gene (typical patients have inherited a C282Y mutation in the HFE gene from each parent) and less commonly by mutations in the genes for hemojuvelin, hepcidin and feroportin which implies abnormal accumulation of iron in parenchymal organs, leading to organ toxicity.

The classic description of the disease also called ”bronze diabetes” implies cirrhosis, diabetes mellitus and cutaneous hyperpigmentation. A recent study of C282Y homozygotes in Australia suggested that 28% of men and only 1% of women have iron related symptoms, influenced by age, sex, dietary iron intake, alcohol, blood loss in menstruation and pregnancy. Symptoms usually appear after the fourth decade of life or when the stores of iron are 15-40 g. Hemochromatosis has multiple manifestations, but the major consequence remains the development of hepatocellular carcinoma (HCC) which appears in one third of patients with hemochromatosis and cirrhosis. An ultrasonography should be made every 6 months in these patients to screen for HCC. For the diagnosis of this affliction clinical, laboratory, genetic testing and pathology criteria are needed. Laboratory tests reveal increased serum transferrin saturation, which is often elevated in patients with HFE-linked hemochromatosis, with a sensitivity over 90% and increased serum ferritin level (but ferritin may also be elevated in various infections and inflammatory conditions). Liver biopsy is recommended in C282Y homozygotes patients or compound heterozygotes if liver enzymes are elevated or if ferritin is higher than 1000 µg/L. Response to phlebotomy may confirm the diagnosis and MRI can show moderate to severe iron overload.

It is recommended to initiate the treatment of hemochromatosis with phlebotomy (removal of 500 mL blood) before the development of cirrhosis and/or diabetes with an important reduction of morbidity and mortality. Phlebotomies should be done weekly/biweekly until serum ferritin reaches 50-100 µg/L, then 3-4 phlebotomies/year are recommended for maintenance.

Hematologic abnormalities in cirrhotic patients, a challenging diagnosis: case report

Florentina Ioniță Radu, M. Șotcan, Andrada Popescu, A.I. Gavrilă, Maria M. Chereja

Introduction: Cirrhosis is responsible for various hematological abnormalities. Different mechanisms are implied such as hypersplenism, hemolysis, altered synthesis of clotting factors and trombopoietin, nutritional deficiency of folate, B12, B6 and iron, variceal bleeding or drug induced bone marrow toxicity.

Identifying the real cause require a careful approach and must take into consideration other severe hematological diseases.

Materials and methods: We present the case of a 69 year old man with Child C cirrhosis secondary to VHC infection who developed progressive leucopenia and thrombocytopenia. Leucopenia with neutropenia were confirmed on peripheral blood count, without any pathological findings on blood smear. A moderate enlargement of the spleen was found on abdominal ultrasound. The leucopenia got worst two months later, associating severe megaloblastic anemia (6 g/dl). As the dosage of folate and B12 were normal and no gastroesophageal bleeding had occurred we continued further hematological investigations. Bone marrow aspiration showed hypercellularity, few blastic cells, mild erythroblastopenia, megakaryocytar hyperplasia and dysplasia of megakaryiocytes and erythroblastes. A marrow bone biopsy was performed. Blastic cells, found in a percent of 20-22%, were CD 34 positive (stem cell marker) and negative for CD 61 (megakaryocyte marker). The histopathological aspect was compatible with an acute myeloblastic leukemia secondary to a myelodysplastic syndrome.

Conclusion: A myelodysplastic syndrome can be hidden behind hematological abnormalities found in cirrhotic patients. A strong collaboration between hepatologist and hematologist is needed to identify as early as possible the correct cause and implement appropriate therapy.

Novel indicators for quality in endoscopy

D.V. Balaban, Iulia Enache, Alice Farcaş, Cătălina Diaconu, Andrada Popescu, M. Pătrăşescu, B. Macadon, Săndica Bucurică, Raluca S. Costache, P.I. Nuţă, Florentina Ioniţă Radu, Mariana Jinga

Quality standards of endoscopic procedures have been a constant concern among endoscopy services and regulation authorities in the last years. Although several quality indicators have been previously validated for colonoscopy, new ones have emerged recently and considerable attention has been paid to indicators for upper GI endoscopy also.

Along with the traditional quality metrics (cecal intubation rate, withdrawal time, adenoma detection rate, interval cancers), colonoscopy examinations are also being assessed by the number of polyps per patient; this new indicator is trying to differentiate endoscopists who are detecting one adenoma (and after identifying this one lesion continuing with less careful examination of the colon) from those who detect more than two adenomas/patient, thus being a good predictor of missing rates.

Regarding upper digestive endoscopy, several indicators have been proposed: inspection time, photo documentation of certain landmarks and lesions, use of mapping and scoring tools for premalignant conditions such as atrophic gastritis, gastric intestinal metaplasia or Barrett's esophagus and application of biopsy protocols for these conditions.

Development, validation and implementing of such indicators for endoscopic procedures is a good quality measure for endoscopic services, making them available for internal and external auditing and thus providing improved medical care for patients.

Relationship between helicobacter pylori infection and digestive oncological pathology

P. Nuță, Roxana Călin, A. Lungu

Helicobacter pylori infection is the most frequent infection, its high prevalence being higher in developing countries. H.

pylori colonizes the stomach and duodenum, and it is localized in the areas of gastric metaplasia. The inflammatory process associated with the presence of bacteria destroys acid secreting glands and lead to hypoacidity, favoring the emergence of gastric ulcers and gastric cancer.

Helicobacter pylori is a group I carcinogen (shown carcinogenic agent in humans), and is proven correlation between infection with the microorganism, in particular cagA + strain, and gastric adenocarcinoma. Helicobacter pylori infection increases the risk of gastric cancer for 8 times, except tumors located at cardial level. Also, Helicobacter pylori was detected in 75% of patients with MALT lymphoma (mucosa-associated lymphoid tissue), eradication of microorganism causing various degrees of tumor regression in 70-80% of patients. The role of Helicobacter pylori eradication therapy in reducing the risk of gastric cancer is undeniable.

Although Helicobacter pylori infection has been incriminated in the occurrence of gastric cancer, it seems that it has protective against esophageal malignancies. One explanation is the appearance of atrophic gastritis with achlorhydria and consequent reduction of acid reflux into the esophagus. Another hypothesis is the induction of apoptosis of atypical cells in patients with Barrett's esophagus associated esophageal reflux.

The link between Helicobacter pylori infection and other digestive cancers is controversial.

OP session SG1

Management of complications in laparoscopic pyeloplasty for ureteropelvic junction obstruction

A. Aungurenci, A. Rădulescu, D. Spînu, C. Iatagan, O. Pacu, C. Stănescu, S. Ioniță, A. Iliescu, F. Rusu, V. Botea, M. Dinu, V. Mădan, D. Mischianu

Introduction: In the last two decades the laparoscopic approach for ureteropelvic junction obstruction replaced the classical open surgery. But there are still specific complications that can jeopardize the final result of the operation. The aim of our study is to assess our main complications of laparoscopic pyeloplasty and their management.

Materials and method: We analyzed a series of 45 patients admitted in our clinic between January 2010 and December 2015 that underwent laparoscopic pyeloplasty. In all cases we used the transperitoneal approach. Heynes-Anderson pyeloplasty was performed in 43 cases while in the other 2 cases we performed Foley (Y-V) pyeloplasty. Postoperative complications were assessed according to Clavien-classification.

Results: The main postoperative complications encountered in our clinic were: urine leakage (6%) and recurrence of the pelviureteral obstruction (4.4%). Except for the patients with restenosis that was managed by open surgery, in all other cases were performed minimally invasive and endoscopic procedures. Conversion rate was approximately 9%, due to intraoperative complications that made impossible the laparoscopic approach, but this was recorded only in the first three years.

Conclusions: In our experience, the complications of laparoscopic pyeloplasty can be managed with minimal invasive techniques that ensured the same functional results as in uncomplicated cases. The restenosis of the ureteropelvic junction should be managed by open surgery.

Refractory priapism – case presentation

D. Marcu, D. Spînu, O. Bratu, A. Aungurenci, V. Mădan, I. Armand, A. Rădulescu, R. Popescu, D. Mischianu

Introduction: A rare health problem which untreated on time can have a devastating impact on the health status of an individual and, last but not least on the status of his private life.

Material and method: We present the therapeutic approach in the case of a patient who was admitted to the emergency clinic of urology of the Carol Davila SUUMC accusing penile pain and the presence of erection for more than 30 hours. Local examination, clinical and paraclinical confirmed the presence of venous priapism. Emergency surgical procedures consisting in Winter shunts and epinephrine injections were undergone for 3 times with a momentary therapeutic success. Open glandular shunts were the saving procedure in this particular case.

Results: Post-operative evolution was favorable, the patient presented at 2 and 4 four weeks without any local problems but also without any erectile function.

Conclusions: At 58 years old a man is still sexual active. Given the fact that no serious comorbidities affected this patient the cause remains unknown. What goes more than 6 hours of erectile function is in most cases irretrievable. Not only the sexual function could have been lost but his life could have been in danger.

Upper lumbar ureteral lithiasis – therapeutic approach

A. Păun, L. Chirilă, O. Pacu, F. Rusu, A. Iliescu, V. Botea, C. Stănescu, A. Aungurenci, V. Mădan, M. Dinu, D. Mischianu

Introduction: Urinary stones is a disease known since ancient times. This is an important health problem worldwide with a prevalence in the general population estimated at 2-3% and a recurrence rate during lifetime of 50 %. The apparent increase in incidence may be real, but also secondary to high rate detection through high performance investigations. The incidence of urinary stones is about 3 times higher in men.

Material and methods: The study included a total of 149 patients admitted in our clinic between January 2013 and December 2015 with a diagnosis of upper ureter lumbar lithiasis > 1cm. Surgical approach was open, laparoscopic, ureteroscopic or ESWL.

Results: Were made 82 ureteroscopy with lithotripsy laser "in situ" (± stent JJ), 11 laparoscopic ureterolithotomy, 19 open ureterolithotomy and in 20 cases ESWL was performed per primam (18 cases a stent JJ was placed first, then after normalize biological constants, ESWL was performed).

In patients with open or laparoscopic ureterolithotomy percentage of "stone free" was 100 %.

Of the 82 ureteroscopy, in 43 cases other secondary procedures were needed (URSR, double J stent placement, ESWL).

In patients in whom ESWL was first procedure, stone–free rate was 55 % after a single session.

Conclusion: Lumbar ureteric stones needs a multimodal treatment. In our study, the most effective methods for achieving the goal of "stone-free" were ureterolithotomy both the classical and laparoscopic approach.

It is important to notice that we have only analyzed the stone-free rate after the first procedure; hospitalization time and cost, postprocedural pain have not been analyzed.

Surgical challenges in kidney infections

D. Marcu, O. Bratu, D. Spânu, I. Oprea, M. Dinu, R. Popescu, A. Rădulescu, D. Mischianu

Introduction: Kidney infections should not be neglected, this pathology often leading to significant morpho-functional alterations and even to the loss of kidney function.

Material and method: We present the therapeutic approach that we have adopted in three patients with renal infectious pathology. The first patient presented for a renal cystic tumor, case in which the laboratory investigations lead to the diagnosis of renal hydatid cyst (increased titer of antiechinococcus granulosus antibody). In the second case we have practiced a lumbar nephrectomy for a lithiasic destroyed kidney, but the histopathological examination revealed that it was renal tuberculosis. The third patient was admitted for a gigantic renal cystic tumor for which we have practiced nephrectomy and the histopathological result was papillary carcinoma.

Results: Postoperative evolution was favorable in the first two patients, but in the case of the third patient secondary lung determinations were encountered at six months after the nephrectomy (CT scans).

Conclusions: In the absence of a correct diagnosis and a proper treatment, kidney infections may have a fatal trend, leading to the appearance of urosepsis, impaired renal function and even to the loss of the kidney.

The difficult vaginal hysterectomy

B. Panaite, N. Niculescu, I. Constantinescu, Ioana Niculescu, C. Pârvulescu, N. Alexe, Anca Popescu, Alexandra Năftănăilă, M. Nicodin, O. Nicodin

Our present work represents an overview of difficult surgical cases of vaginal hysterectomy that have been performed in our clinic.

The main criteria we have established when considering a vaginal hysterectomy to be a difficult one were: the increased volume of the uterus, the modified anatomy of the bony pelvis, previous caesarian section as well as other pelvic surgical interventions, obese patients and cases that have associated cervical elongation.

Our debate has focused on how a proper surgical technique that has accordingly adapted to each case previously mentioned together with the skills of a well-trained surgeon in the field of vaginal surgery can shift an absolute contraindication into a relative one when considering the vaginal approach of a hysterectomy.

Adiponectin blood level in patients with prostate cancer

F. Rusu, O. Pacu, A. Iliescu, A. Păun, M. Dinu, Lavinia Chirilă, V. Botea, V. Jinga, Emilia Rusu, Gabriela Radulian, D. Mischianu

Introduction: Recent data suggests that obesity can influence the incidence, progression and mortality of prostate cancer through hormonal changes, through insulin resistance, increasing production of inflammatory markers etc. Aside these factors, obese patients with PCa are more difficult to diagnose as they have mostly low serum PSA level and a higher prostate volume. Although, the effects of adiponectine on normal prostate cells are at the moment not understood, indirect evidence suggests an antiproliferative effect in prostate tissue. The present study assesses the level of adinopectine on the subjects with prostate cancer.

Material and method: The 414 selected patients were divided in 2 groups: group A: 381 patients with obesity, without PCa (O. PC(-)) and 33 patients with obesity and prostatic cancer (O. PC(+)). Specific anthropometric parameters were analyzed (height, weight, body mass index (BMI), waist circumference), blood tests (fasting blood glucose – (BPG), fasting insulinemia (FPI), HbA1, total cholesterol, triglycerides, HDL cholesterol, adiponectin, leptin, TNF alpha, IL -6, prostate specific antigen (PSA), free PSA (F-PSA), testosterone).

Results: The study results shows statistically significant differences in terms of age, BMI, PSA level, Free-PSA level, FPG, FPI, adiponectin, leptin, TNF-⍰, IL-6, testosterone and prostate volume in patients from the two groups (all values p <0.05). In terms of the abdominal circumference and diastolic blood pressure there were no differences in the two groups. Plasma adiponectin level was lower in patients with

PCa than the control group (p <0.001) and also in patients with those with aggressive disease (1.23 ng/ml in patients with Gleason score <7 vs 0.64 ng/ml for Gleason score 8-10) (the difference remained statistically significant after logarithmic transformation).

Conclusions: The results of the present study indicates that patients with prostate cancer have a lower plasmatic adiponectin level and are also associated with histological grade and disease stage. Further prospective studies are recommended to asses any causal relation between prostate cancer and plasmatic concentrations of adiponectin.

OP session SG2

Principles and techniques in cheek reconstruction – a review of cases

Monica Dărmănescu, V. Trifu, A.M. Țilea, Raluca I. Săftoiu

The cheek is the broadest subunit of the face, in close proximity to aesthetically important regions such as eyelids, nose and mouth. In most cases the extensive mobility of cheek skin provides for relatively simple closures following tumor extirpation, but in case of larger defects, those located near free margins, or those within areas of decreased skin laxity may require a more complicated approach.

We review the principles of cheek reconstruction, from the simplest option (primary closure) to more complex methods (grafts, flaps), with special considerations to specific reconstructive options. An essential principle of cheek reconstruction is to provide an accurate skin color and thickness match with locally recruited tissues, avoiding distortion of the facial free margins (lower eyelid, upper lip and oral commissure).

Each case must be approached with a unique plan for the defect and the patient, with a non-geometrical and three-dimensional design for every case in order to have very good aesthetic and functional outcomes.

Reconstruction of the cheek requires a thoughtful approach to the etiology of the defect, the functional status of the patient, and the best solution with the least morbidity. The dynamic function and contour of the cheek make it a challenging structure to repair.

Principles and techniques in forehead and scalp reconstruction – a review of cases

Monica Dărmănescu, V. Trifu, A.M. Țilea, Raluca I. Săftoiu

Reconstruction of scalp and forehead defects is a complex field; we have a broad variety of reconstructive options. A thorough understanding of the anatomy of anatomic structures of the scalp and forehead is paramount to optimal surgical result.

A case series of regional reconstruction of scalp and forehead area is presented, with a variety of reconstructive techniques. The anatomy of the region is reviewed; it is important to preserve the function of motor and sensory nerves, although aesthetic concerns are of great importance in scalp and forehead reconstruction. Respecting the normal position of facial landmarks such as the eyebrows and hairline can be challenging during reconstruction.

We review the principles of forehead and scalp reconstruction, from the simplest option (primary closure) to more complex methods (flaps).

Each case must be approached with a unique plan for the defect and patient, with a non- geometrical design, in order to achieve excellent aesthetic and functional outcomes.

Pigmented basal cell carcinoma – clinical and dermoscopical clues

M. Țilea, Mihaela Georgescu, Monica Dărmănescu, V. Trifu

Introduction: Pigmented basal cell carcinoma (pBCC) can pose a real dilemma even to the most skilled clinician. Clinical similarities with pigmented seborrheic keratosis (pSK) and pigmented cutaneous melanoma (PCM) can be misleading and may result in some unfortunate therapeutic approaches.

Case presentations: We present a series of cases of pigmented basal cell carcinomas that were diagnosed and treated in our clinic. While in some cases the diagnosis was established clinically, in other cases dermoscopy proved to be a valuable tool for reaching the correct diagnosis. All cases were confirmed with histopathology.

Discussion and conclusions: The clinical aspect of a pBCC is usually straightforward. The classical clinical signs of e.g., greyish translucent aspect (pearls in the periphery of a superficial lesion), the arborizing telangiectasias and the periods of clinical improvement alternating with periods of clinical deterioration (bleeding), can in most cases lead the clinician to the correct diagnosis. In our experience there is also another important clinical clue for the diagnosis of a pBCC – punctate pigmentation in the periphery of the lesion. The colour of the pigmentation can range from dark grey to brown to blue-black. The corresponding dermoscopical structures are: blue-grey ovoid nests and globules, leaf-like structures and spoke-wheel like structures. This clinical clue does not appear in pSK or in PCM.

A solid clinical diagnosis, aided by dermoscopy, can help a surgeon to better manage these tumors and as such to deliver a disease free, functional and aesthetical result.

A simple cataract procedure in a complex ophthalmic patient – case report

G. Anghel, O. Mușat, M. Zemba, C.H. Manole, Mioara L. Macovei, Liliana Pulbere, Monica M. Armegioiu, C.G. Ștefan, Daniela Șelaru, Camelia Constantin, Corina Cernat, D.C. Luncă

We report the case of a 65-year-old male, who is hospitalized for a slow progressive decrease in visual acuity over the past years. The clinical exam revealed: OU: primary open angle glaucoma (POAG) – (bilateral trabeculectomy in 1999) compensated under medical therapy, OD: pulsatile exophthalmy, exposure keratitis, paracentral vascularized leukoma, posterior chamber IOL, OS: complicated cataract, sectorial optic nerve atrophy, sequel of superior branch retinal vein occlusion. Past medical history: arterial hypertension, type 2 diabetes, gout, dyslipidemia. The visual acuity was OD: hand motion (HM), OS: counting fingers (CF) and the ocular pressures TOS=20 mm Hg NCT, TOD=similar digital tone as the left eye. The cranial computed tomography revealed the absence of the superior wall of the right orbit with direct communication between the cranial cavity and the right orbital content, after neurosurgery for right orbital cellulitis, in the early '90s. Given all these findings, the therapeutic intervention with the greatest benefit was considered to be the cataract procedure in the left eye.

The patient underwent uneventful phacoemulsification with posterior chamber intraocular lens implantation. The post-operative visual acuity was VOD=0.8 without correction.

The particularity of this case is represented by the association of multiple conditions that threaten sight and the pulsatile exophthalmia caused by the CSF pulsation transmitted through the bone defect of the right superior orbital wall.

Complex clinical association – Rhegmatogenous retinal detachment after perforant keratoplasty and psudofak

O. Mușat, G. Anghel, C. Ștefan, M. Zemba, H. Manole, Liliana Pulbere, Monica Armegioiu, Laura Macovei, Daniela Șelaru, Corina Cernat, Camelia Constantin, Uliana Ochinciuc

The paper reports the case of a 62 years old patient with a history of keratoplasty surgery after a concussional ocular trauma with central corneal leucoma, posttraumatic iris atrophy.

The patient underwent posttraumatic cataract surgery with artificial lens implant.

About 6 years later he developed rhegmatogenous retinal detachment, which was treated surgically: posterior vitrectomy, laser photocoagulation, silicon oil tamponade with no intraoperative complications and good final outcome.

This paper reports the particularities and difficulties of such a complex case.

Glaucoma after chemical burns

C. Ștefan, Cristina M. Timaru, G. Anghel, M. Burcea, Daniela Șelaru, O. Mușat, M. Zemba, H. Manole, Liliana Pulbere, Laura Macovei, Monica Armegioiu

Introduction: Glaucoma after chemical burns and radiation is a secondary, posttraumatic, open angle glaucoma. It is a frequently seen complication of chemical burns that can occur in the acute stage or as a late complication.

Material and method: This study is a presentation concerning glaucoma after chemical burns, coupled with a case study presentation of a female patient who suffered from a right eye chemical burn. The management of this case along with diagnosis and treatment are presented in this article.

Results and conclusions: Aldo it is not a frequently seen pathology, it is important to know how to diagnose and treat glaucoma after chemical burns. There are various options available for treatment, but choosing one is difficult because of the possible complications and scarring caused by the chemical substance.

Extensive corneoconjunctival tumor – a therapeutic challenge

M. Zemba, G. Anghel, O. Musat, H. Manole, Laura Macovei, Liliana Pulbere, C. Ștefan, Daniela Șelaru, Monica Armegioiu, M. Dumitrescu

Purpose: to show the surgical solution for a giant corneoconjunctival tumor.

Methods: the surgical technique is shown in a video film; we insisted on correct oncologic excision and ocular surface restoration.

Results: the visual acuity increased from light perception to 1 without correction.

Conclusions: a correct technique and suitable solutions for ocular surface restoration allow a fast and complete recovery for eyes apparently with very bad prognosis.

Peripheral nerve injury and neuron regeneration

Andreea Benga, F. Zor, A. Korkmaz, B.M. Marinescu

Peripheral nerve injuries can be most disabling, resulting in loss of sensitivity, motor function and autonomic control in the involved anatomical segment. Although capable of regeneration there is still no optimal solution for their reconstruction. Nerve regeneration has several vulnerable points that could be addressed to improve outcome: (1) neuron survival after trauma, (2) gap crossing and orientation of neurites, (3) neuroma formation, (4) target-organ trophicity.

Inflammation is an important sequence in nerve regeneration. Cytokines released by macrophages and fibroblasts have mitotic effect on Schwann cells. Fibroblasts are present in epineurium, perineurium and endoneurium. If they proliferate extensively, scar formation occurs that impedes nerve regeneration. Scar tissue contains repulsive proteoglycans (such as chondroitin sulfate) and semaphorins that inhibit neurites' elongation.

Nerve repair still faces two immense challenges: fascicules orientation that could be addressed by improving the surgical technique and the time race against fibrosis and end-organ atrophy amenable to pharmacological therapy.

The aim of this study is to review the literature for the most frequent chemicals used in experimental studies to address neuron survival, reduce scar formation and improve overall nerve regeneration.

Hydrosurgery – an effective method of wound debridement

B. Marinescu, Andreea Benga

Subject: The aim of the paper is to demonstrate the efficacy of using hydrosurgery in debridement of complex wounds.

Material and method: The method was used in 8 patients, treated in our clinic with wounds of different etiologies: burns, chronic, infected or post-surgical wounds. The hydrosurgical system permits simultaneously, an accurate ablation and aspiration of the nonviable tissue from wounds.

Results: In all of our cases the debridement was made rapidly and very precise. Direct closure of the wounds or skin grafting were performed in the same time.

Conclusions: Hydrosurgery represent an effective and selective method of debridement for difficult wounds. It minimize the tissue damage, preserve the healthy tissue and leave a clean and well vascularized wound bed.

Minim invasive solution for treatment of an intraocular lens subluxation

M. Zemba, G. Anghel, O. Mușat, H. Manole, Laura Macovei, Liliana Pulbere, C. Ștefan, Daniela Șelaru, Monica Armegioiu

Purpose: to show the surgical solution for a traumatic subluxation of a posterior chamber intraocular lens.

Methods: the surgical technique is shown in a video film.

Results: the visual acuity increased from 0.03 to 0.5 without correction.

Conclusions: a minim invasive technique may allow to obtain very good results, with fast recovery and low costs.

OP session SG3

Intralobar lung sequestration – thoracoscopic surgical procedure

A. Ciuche, C. Nistor, D. Marin, D. Pantile, Laura M. Constantin, Roxana Brîncoveanu

Introduction. The authors present the case of a 37 years old male admitted for a left lower lung lobe tumor identified on a standard chest X-Ray.

Material and method. First we present the radiological findings and then intraoperative images. Using minimally invasive thoracic surgery we managed to diagnose a left lower lung lobe sequestration (highlighting the vascularization of the sequestered lung territory from the descendent aorta, right above the diaphragm), and to perform a thoracoscopic wedge resection of the lesion, clipping the aberrant vessel.

Results. With favorable surgical outcome, several aspects are being detailed: postoperative evaluation, as well as the radiological findings one-month postoperative.

Conclusion. Minimally invasive thoracic surgery has permitted a better exposure of this rare lung malformation, allowing at the same time a better diagnosis and treatment procedure (thoracoscopic wedge resection), with a fast and complete postoperative recovery of the patient.

Thoracoscopic resection of a bronchogenic esophageal cyst

A Ciuche, C. Nistor, D. Pantile, D. Marin, Olivia Arhire, A. Tudose, Săndica Bucurică, C. Bețianu, Roxana Brîncoveanu

Introduction: Esophageal bronchogenic cyst is an uncommon entity and surgical experience of their removal by thoracoscopic surgery is limited.

Material and method: We present our patient outcome and surgical thoracoscopic technique in the treatment of an esophageal bronchogenic cyst.

The study included 1 patient, male, 52 years with important obesity. The only clinical manifestation was intermittent dysphagia. Some imagistic findings at CT scan and transesophageal echography suggest the presence of a solid intraparietal benign esophageal tumor.

The intervention was performed using 4 thoracoscopic entry points. Thoracoscopic removal of the cyst needed careful manipulation and subsequent dissection of the cyst sac from the structures to which it was attached (esophageal wall and mediastinal pleura). The intramural esophageal localization with local esophageal mucosa being intact and the particular aspect of the tumor (filled with mucoid material and contain cartilage fragments) were able to make the positive diagnosis (cystic intramural esophageal tumor) and eliminated the preoperative diagnosis (solid intramural esophageal tumor).

Results: The patient had a favorable outcome, without any complications and was discharged the 3rd day after surgery. The dysphagia was relieved immediately after the operation. The follow-up was well 6 months after the surgery.

Discussions and conclusions: The preoperative computed tomography and endoscopic ultrasound had some limits in this case (distinguishing between cystic and solid tumor was difficult).

Standard surgical treatment is removal of the cyst by thoracotomy. While difficulties in removal and possible complications and recurrence are similar to those of classical posterolateral thoracotomy, video-assisted thoracoscopy offers clear postoperative advantages.

Mediastinal paraganglioma – a surgical challenge

C. Grozavu, M. Iliaș, D. Pantile

Introduction: A paraganglioma is a rare neuroendocrine neoplasm that may develop at various body sites (including the head, neck, thorax and abdomen). Paragangliomas originate from paraganglia in chromaffin-negative glomus cells derived from the embryonic neural crest, functioning as part of the sympathetic nervous system.

Material and method: A 34-year-old woman came to our department with previous history of bilateral carotid glomic tumor. First, the right common carotid tumor has been surgically removed, followed one year later by the removal of the left common carotid tumor. Five years later the patient reported to the vascular surgery department, and after several investigations she was referred to our thoracic surgery department.

Results: On the basis of the symptoms and the previous surgical history we decided to operate on the patient.

We attempted and succeeded to dissect the tumor from the aorta, the left common carotid artery and the left subclavian artery and then resect the full tumor with acceptable blood loss.

Discussion: The treatment of choice for paragangliomas is, where possible, complete surgical resection.

Middle mediastinal paragangliomas are extremely rare, approximately 30 cases have been reported in the literature since 1959.

Conclusion: The surgical intervention was the appropriate approach for this case. When gently maneuvering the tumor, alterations of blood pressure can be avoided. Also, by performing a careful dissection and resection, the blood loss is acceptable and transfusion is not mandatory.

Laparoscopic hormonal suppression in breast cancer

N. Niculescu, O. Nicodin, B. Panaite, C. Pârvulescu, Ioana Niculescu

Objective: To compare the surgical laparoscopic hormonal suppression to the classical treatment, in the treatment of breast cancer.

Method: A retrospective study of 124 patients operated at the Gynecology Department of SCMC for breast cancer. From the patients who have had surgical intervention for hormonal suppression, 13% have had laparoscopic procedures.

Results: The average operative time for the classical approach was 40 minutes and the hospitalization time of 3-5 days. The laparoscopic approach for hormonal suppression took an average 14 minutes, and for bilateral laparoscopic oophorectomy followed by vaginal hysterectomy 63 minutes. The hospitalization time was 2 and 3 days respectively.

Conclusions: The surgical hormonal suppression is an effective complementary therapy in the complex management of breast cancer. The laparoscopic approach resulted in a reduced hospitalization time, a better cosmetic outcome and a quicker return to work for patients, therefore a better postoperative quality of life.

Right chylothorax – minimally invasive approach

C. Nistor, A. Ciuche, D. Pantile

Introduction: The authors present two cases, one of a 47 old woman, 3 years after mitral valvular replacement operation, presented with a postoperative pleural chylous effusion repetitive evacuated and recidivated, and another one of a man with an incidental radiological finding.

Materials and method: The authors presented a short review of the recent information about the pleural chylous effusions and the commonly therapeutically approach in these cases. They presented in details the thoracoscopic approach of the thoracic duct which was used in these cases.

Results: Postoperative evolution was favorable, with complete pulmonary expansion and complete resolution of the pleural chylous effusion.

Conclusions: The thoracoscopic approach, even if more difficult, allowed a good exposure of the thoracic duct, and also a fast and complete postoperative recovery.

“Refined” optical methods in pharyngolaryngeal tumor diagnosis

C. Romanițan, R. Costin, G. Ganea

The expression "optical biopsy" has entered everyday language for the last 2 decades, being imposed by biomedical researchers in the field of optics. It is a paradox, a word that joins two terms with meanings willed otherwise, "biopsy" meaning harvesting a tissue fragment, which does not happen in optical biopsy. This actually refers to a series of physical measurements using the optical properties of light and the interaction thereof with tissues, to allow the doctor the diagnosis of certainty in real time, in vivo, in situ and noninvasive (or minimally invasive).

It should be noted that it is widely accepted among ENT specialists that the "gold standard" in the diagnosis of malignant process is the histological examination by a pathologist made after harvesting the tumor tissue fragment. The biopsy itself may be simple, readily available to every ENT specialist in easily accessible areas (oropharynx, nasal passages) or difficult, sometimes requiring endoscopic technique under general anesthesia (cavum, hypopharynx, and larynx).

To overcome these drawbacks and anesthetic-surgical risks, to speed diagnosis of certainty and lower costs, we have developed new methods of exploration, that try to provide the clinician with new criteria to differentiate between healthy tissue/benign tumor/malignant tumor. New technical developments in the field of optical fibers, light sources, sensors, led to the unprecedented development of various optical methods which substantially improve visualization and diagnostic evaluation of the epithelium in vivo. These try to improve image resolution, contrast, and deep tissue penetration to standard endoscopic techniques, offering molecular and biochemical information about mucosal lesions.

Under the name "optical biopsy" there's actually: fluorescent spectroscopy, optical coherence tomography, narrow-band imaging and confocal endomicroscopy.

This paper describes bio-physical principles of interaction of light with tissues. It also compares the advantages and disadvantages of each optical methods used in ENT and brings statistical data from the literature.

Past and perspectives of local anesthesia in dental medicine and BMF surgery

A. Nistor, A. Diaconeasa Maier

Anesthetic substances plays an important role in pain management of various disease involving treatments in the oral-maxillofacial regions. Properly administered local or loco-regional anesthesia is the safest way to minimize the effects of various painful conditions or treatment regimens.

In this article there are evaluated the main types of anesthetic substances which are used most frequently in the current period. There are briefly set out the main characteristics of the major anesthetics used in dentistry and oral surgery regardless of technical management. Also, there is a highlighted focus of the advantages of using Articaine as a main anesthetic, adverse effects compared with other substances existing in current literature and personal considerations.

The main interventions of surgery and oral implantology, which were listed, are most indicated with the use of specific type of anesthesia but also come with some inconveniences and difficulties. Each anesthetic substances has an order of potential, toxicity and specific indications for use alone or in combination with Articaine.

Practical conclusions: Any dental surgery should not be accompanied by pain regardless of its intensity – the „pain management" is very important to be taken into account. Effectiveness of local anesthesia with Articaine, regardless of the concentration of vasoconstrictor is superior to all current anesthetics. The efficiency of Articaine allow it to be use in any dental intervention; Neurological disorders consecutive anesthesia with Articaine are statistically lower compared to the rest of dental anesthetics.

Surgical treatment of orbital blowout fractures

A. Gabără, Liliana Moraru, A. Căruntu

Introduction: The isolated blowout orbital fractures occur when traumatic agents that are comparable to the size of its aperture frontally hit the orbit. The traumatic energy is transmitted to eyeball, which transmits it to orbital walls that fracture easily, thus sparing visual analyzer. The symptoms are influenced by the displacement of the fracture, ranging from periorbital bruise to diplopia. Since orbital walls cannot be directly examined, many cases go undiagnosed and heals in a vicious manner being accompanied by atrophy and scarring of soft tissue thus making surgery difficult.

Methods: We retrospectively reviewed cases with blowout orbital fractures with secondary diplopia treated surgically in our unit over the last two years.

Results: Compared with recent fractures, treatment of viciously consolidated orbital blowout fractures though identical (reconstruction of orbital walls with preformed low profile titanium mesh), was more difficult and required more time in the operating room. The interventions resulted in remission of diplopia shortly after surgery without complications, but couldn't equally correct enophtalmia.

Conclusions: Viciously consolidated orbital blowout fractures can be successfully treated in terms of eyeball functionality, but technically more difficult, with potentially higher perioperative risks and variable aesthetic results. Therefore proper diagnosis and early treatment are preferred.

Discussions: The use of computer assisted intraoperative navigation as well as 3D printers before surgery for better surgical outcome.

The management of the major burnt patient with associated airway injury in the clinical context of multiple casualty's incident

B.M. Marinescu, Penelopia Marinescu, Ioana Tuhar, Andreea Benga, F. Popescu, T. Oprea

Purpose: The objective of this study is to present the outcomes of the correct management of the burned patient, and to share the experience acquired secondary to this event.

Methods: The case sample included 12 patients from the same multiple casualties' incident, with the severity of the burn lesions varying between IIb-III degrees and between 5-

50% body surface, mainly affecting the upper half of the body (thorax, upper limbs, head and neck). As a common determinant all the cases presented respiratory tract lesions due to the nature of the event-fire in enclosed space followed by toxic fumes inhalation.

The surgical team involved was able to ensure in less than 10 hours the emergency surgical primary care for all of the 12 patients admitted.

Discussion: The distinctiveness of the presented cases consists in the great number of patients simultaneously admitted and treated.

Results: We can report a 100% survivability, at 50 days all patients being discharged only with minor granulating areas left to heal spontaneously under local treatment.

Most of the studies that we have found and we could refer to, on the matter of proper airway burn injuries and toxic fume inhalation did not statistically differ from our outcomes, even compared with consecrated burn centers from Western Europe.

Conclusions: The good overall results can be related to the interdisciplinary approach, right from the admission of the patients.

Our hospital is a level I medico-military unit, conceived from the beginning to provide emergency medical assistance for the most complex cases.

Bisphosphonate related osteonecrosis of the jaw – role of surgery

A. Gabără, Liliana Moraru, C. Dumitru, A. Căruntu, C. Becheanu

Introduction: Recently described pathological entity, Bisphosphonate Related Osteonecrosis of the Jaw (BRONJ), although with low incidence in population needs, to be known and understood in health care system (especially among dental specialists), given the complexity of its treatment.

Methods: This article aims to review existing data in literature, along with the presentation of our conclusions from a retrospective analysis of patients treated in our department from 2003 till present day with and without use of PRF during surgical procedure

Results: All patients, except one, were treated for different malignancies with i.v. Zolendronic Acid. They have developed the disease about 1.5 years after the initiation of this therapy.

The mandible body was more affected than the upper jaw. At admission in our department, all patients presented advanced stages of disease. Surgical treatment consisted in limited bone debridement or sequestrectomy with watertight wound suture and since last year, local placement of PRF. In two cases, radical maxillary sinus surgery was performed. In one third of cases redo surgery was necessary before using PRF.

The condition was cured in all patients, except one. Biphosphonate therapy was stopped by prescribing physician for all patients.

Conclusions: Although prevention still remains the “best treatment” the use of PRF is a helpful tool in surgical management of BRONJ.

Discussions: The role of PRF in prevention of BRONJ.

OP session SG4

Lumbar spinal stenosis – surgical decompression

I. Cusciac, E. Popa, M. Mitrică

Object: Patient inclusion criteria were lumbar canal stenosis, no degenerative lumbar spondylolisthesis and no previous history of lumbar surgery.

Methods: 30 patients with LSS who have been operated between 2014 and 2015 were included in this analysis.

Results: At 25 of the patients the functional improvement was very good, at 4 patients was good and at one patient with polineuropathy there was no significant differences in the clinical outcome.

Conclusions: Surgical decompression is the intervention of choice for lumbar spinal stenosis when nonoperative treatment has failed.

Modern methods in the treatment of fractures of the spine

C. Năstase, M. Marinescu, M. Mitrică

Introduction: This study has the task of exposing modern methods in the treatment of fractures of the spine. The main neurosurgical treatment methods are: vertebroplasty technique and fusion.

Methods: Between January, 2014 and July 2016 a total of 35 patients received treatment with fusion, using titanium screws mounted transpedicular with different sizes and titanium rods, and "space-er" for the fusion between vertebral bodies (interbody fusion). In the same period, 15 patients experienced the beneficial effects of vertebroplasty treatment which consisted of injecting a cement with special properties under fluoroscopic control, this method is a minimally invasive method. Clinical aspects were: subjective (pain) and objective (clinical examination of the patient: static and dynamic examination of the spine, gait examination, neurological examination).

Results: After using fusion, we have notice the reduced misalignment, restoring physiological curves, fixing segmentation in multipoint attachment compression and restoration of vertebral body height. Regarding vertebroplasty technique except that it’s a safe and effective method, its benefit lies in improvement until pain accompanied by increasing mobility. There were no complications occurred. (Nerve compressions, thrombophlebitis, deep wound infections or local).

Conclusion: Vertebroplasty technique is used in elderly patients with vertebral compression due to osteoporosis. The treatment is effective in relieving pain and improving function of the spine, in such cases, without complications.

Surgical management of spondylolisthesis – case report

O. Sîrbu, A.V. Chirteș, M. Mitrică

Surgical management of spondylolisthesis has become increasingly common in spine surgery. A variety of pedicle screw systems have been described and new systems are being developed every day.

Spondylolisthesis represents an anterior subluxation of one vertebral body on another. Most commonly it occurs in lumbar region involving L5-S1 or L4-L5. Spondylolisthesis classification comprises five types: dysplastic, isthmic, degenerative, traumatic, and pathological and is divided into four levels depending on the percentage of subluxation. The clinical presentation may include back pain, radicular syndrome or cauda equine syndrome. Treatment is determined in relation to the presence of symptoms, extent and evolution of slipping.

In our neurosurgery department in the past 8 years we have gradually shifted from orthopedic – conservative treatment to the surgical approach. We will highlight through this case report the most important aspects of the surgical management of spondylolisthesis.

Magnetic resonance imaging evaluation of the cystic lesions of the knee

I. Codorean, I.B. Codorean, M. Ștefănescu, F. Năftănăilă

Objective: The objective of this study is to present and illustrate the spectrum of cystic lesions aspect, or "cyst like" revealed incidentally, in a group of patients investigated MRI for acute or chronic knee injury, suspected tumor lesions, inflammatory or infectious.

Material and method: This paper is a retrospective study conducted over a period of 8 years between - April 2007 - May 2015, and refers to a group of 2691 patients examined MRI. Age limits the patients were in the range of 14-77 years. The study group included 1851 (69%) men and 840 (31%) women.

Results and discussions: Of the 2691 patients were identified lesions in 197 patients with cystic appearance (7.32%). The shows and will illustrate the type of lesion identified, the location and appearance of articular MRI.

Conclusion: Magnetic resonance imaging is the method of choice in the study developed cystic lesions in the knee because it enables the detection, location, extent and relationship to adjacent structures.

Magnetic resonance imaging evaluation of the knee synovial pathology

I. Codorean, I.B. Codorean, M. Ștefănescu, F. Năftănăilă

Objective: The objective of this study is to present and illustrate aspects highlighted incidental pathological synovial knee in a group of patients investigated MRI for knee injuries acute or chronic, degenerative lesions, suspected tumor lesions, inflammatory or infectious.

Material and method: This paper is a retrospective study conducted over a period of 10 years within - April 2006 - December 2015, and refers to a group of 5776 patients examined the MRI. Age limits the patients were in the range of 14-87 years. The study group included 3638 (63%) men and 2138 (47%) women.

Results and discussions: Identified the following spectrum of synovial pathology: traumatic synovitis, infectious and inflammatory nonspecific synovitis, pigmented villonodular synovitis, localized and diffuse forms, condromatosis and osteocondromatosis as well as synovial tumors. In semiology terms, impaired MRI synovium, expressed, variable, in relation to disease stage, accumulation fluid articular in different quantities and locations, distension of the joint capsule, thickened synovium, synovial nodules, deposits of hemosiderin, bodies free joints.

Conclusion: Magnetic resonance imaging is the method of choice in the evaluation of native and contrast material of synovial pathology.

OP session SG5

Dunbar’s syndrome – case presentation

A. Dima, C. Mitru, D. Bădănoiu

A 33-year-old woman was investigated for persistent symptomatology as follows: post-prandial abdominal pain in the upper abdomen, constipation, and significant weight loss.

The computed tomography examination identified an almost complete stenosis of the celiac trunk by low arcuate median ligament insertion.

The imagistic aspect sustained the diagnosis of Dunbar’s syndrome (celiac artery compression syndrome or median arcuate ligament syndrome), a very rare pathology.

It was so performed the surgical management of the celiac trunk stenosis by laparoscopic arcuate median ligament release.

Post-operative follow-up was uneventful and patient was discharged at day 3 after surgery.

At 1, 2, and 3 months after the intervention controls, the patient was free of symptoms.

Myths among patients in laparoscopic cholecystectomy

C. Duțu, Andreea Gherghe, Andreea Rusu, Elena Stîrcu, F. Săvulescu

This presentation addresses a problem commonly encountered in the General Surgery ward, the one of patients’ myths related to the surgical treatment of gallstones. By listing the main misconceptions, the purposes of the paper are to identify them, to understand the causes of their occurrence and to find solutions in order to avoid their appearance in the future.

Two groups of patients with gallstones and their respective complications have been studied: 50 patients hospitalized at “Dr. Carol Davila” Central Military Emergency University Hospital, in the department of surgery II and 50 patients who have not received surgical treatment for this pathology. The patients were asked to answer a survey consisting of 9 questions to assess their knowledge regarding the treatment of their pathology.

There have been identified, among others, situations such as: confusion of the laparoscopic cholecystectomy with „the laser surgery”, distrust of the laparoscopic technique, aversion to surgery itself, a preference for dangerous and ineffective natural treatments, misunderstanding the term of cholecystectomy and confusing it with gallstones’ removal procedure (cholecystolithotomy). The ones who have consulted direct or online sources of general surgery and have received treatment present a lower risk of complications from therapeutic means set properly, while those who have sought information from nonsurgical sources are more prone to complications by delaying or refusing surgery.

Being part of the therapeutic arsenal for over 20 years and imposed as a "gold standard" in the treatment of gallstones, the laparoscopic cholecystectomy can still offer surprises in terms of its understanding and acceptance by patients. A correct information of the patients from authorized medical sources can reduce the rate of late-stage presentations and, consequently, morbidity and mortality.

Types of first artery approach in the head of pancreas cancers

A. Dima, T. Artenie, C. Mitru, F. Bold, D. Bădănoiu, I.P. Oprea

The „first artery approach" is the only currently recommended approach in cases of cancers of the head of pancreas. There are six types of first artery approach described in pancreatic head cancer, the choice and the combination of these techniques facilitates good surgical results.

All techniques are meant to primary assesse the integrity of the superior mesenteric artery in regard with the tumor in order to establish the resectability.

We present here our case series of patients in which this approach was performed, with good results comparable with those reported in literature.

In regard with the tumor localization, the combination of various types of artery first approach, it allows a safe radical pancreatic resection. The use of „first artery approach" requires a highly trained team in pancreatic surgery.

Complex treatment of advanced locally colorectal carcinoma

C. Drăgan

The colorectal carcinoma may require not only an appropriate colonic resection, but also a block resection of additional affected, invaded organs and tissues.

Objective: The objective of the present summary is to provide an overview on the surgical methodology and experience available at the Department of Surgery, University Military Emergency Hospital „Dr. C. Davila", concerning the treatment of locally colorectal carcinoma in advanced stages, their radicalism as well as immediate and long-term postoperative outcomes of surgical intervention.

Background: The carcinoma of the colon in its advanced stages is defined as a malignant neoplasia of the colon, which may appear locally or in a metastatic manner, invading other organs and tissue.

The local extension paths of the colon carcinoma are circular, as they follow the local lymphatic vessels, other paths of the carcinoma progress such as parallel of perpendicular path to the intestinal wall as well as perineural paths are rather seldom.

Methodology: Various cases with a diagnosis of local colorectal carcinoma in advanced stage, which were surgically treated at our Department, were assessed and discussed.

Results and conclusions: Our experience indicates that a (preventive) multi-organ resection performed in the cases of local colorectal carcinoma in advanced stage is the most reliable and safe methodology of surgical treatments. Because the palliative resection cannot predict or exactly determine the degree of tumoral metastasis, an ample tumoral resection is strongly indicated in order to improve the oncologic outcome, despite an increased risk of morbidity.

Particularities of myxopapillary ependymoma

R. Nica, C. Mușat, Alexandra Mihai, Florina Vasilescu, C. Pleșa, Diana Alexe, F. Săvulescu

The paper aims to highlight the particularities of tumor formations (lack of symptoms – even in the presence of tumor formation, at the location of the gluteal, but also the similarity in the clinical context with a pilonidal cyst), histologically diagnosed as myxopapillary ependymoma at a 22 years women.

Histopathological examination reveals tissue fragments with tubular and bulgy structures which reveals at Androgen receptor, S 100 immuno-histochemical tests – positive tumor cells.

The presentation contains images that shows the patient illness evolution from the beginning until the postoperatively status.

Radical antegrade modular pancreatosplenectomy

A. Dima, T. Artenie, C. Mitru, D. Bădănoiu, F. Bold, I.P. Oprea

The radical antegrade modular pancreatosplenectomy was for the first time proposed by Strasberg SM et al in 2003 in cases of pancreas body and tail malignant tumors.

The oncologic results of this technique are superior to other types of approach (i.e. posterior approach) especially due to a more efficient associated expended regional lymphadenectomy.

We present the results of a case series of six patients (ages 39 – 73 years) to whom this approach was applied, of which two laparoscopic interventions. There were registered no early or tardive complications.

The benefits of this method are represented by increased visibility in the dissection area, a better resectability of the ganglionar station N1, as well as the possibility of a posterior dissection with early vascular approach. Radical antegrade modular pancreatosplenectomy proved to be feasible and efficient in selected patients.

The therapeutically attitude in advanced abdominal cancer

T. Rogin, O. Albița, D. Orosan, C. Duțu, R. Nica, C. Mușat, F. Săvulescu

The purpose of this paper is to provide information about how pluri-visceral resection in intra-abdominal neoplasm, used together with oncological treatment, can improve survival rates in selected cases.

The paper presents the therapeutic attitude in abdominal neoplasms with pluri-visceral invasion, cases of multiple and serial resections in a number of patients with neoplasms in stages III and IV, with pursuing the cases over several years.

On our cases, we observed through imagistic methods surprisingly longer periods of time without recurrence of the disease in the abdominal region.

In conclusion, surgery remains the main approach for treating advanced intra-abdominal neoplasm and, together with complementary oncological treatment, can obtain greatly improved survival rates. We consider that maximal invasive surgery is justified.

Peritonitis localized through ileal perforation – case presentation

F. Săvulescu, C. Cârlan

In this paper we present the case of a woman, 50 years old, investigated in different hospital units, who consistently presented colicative pain localized at the level of the right iliac fossa. The particularities of the case are related to the fact that the patient showed a complex associated pathology that could justify the symptoms. Resorting to complex investigation methods, the diagnosis of ileal perforation by foreign body was established. This paper is accompanied by imagistic proof, images and videos that depict the diagnostic and therapeutic stages of the patient.

A giant recurrent liposarcoma of the retroperitoneal and peritoneal cavity

V. Ștefănescu, C. Mitru, F. Macău

Liposarcomas belong to the very seldom occurring group of malignant tumors of the retroperitoneal cavity. Usually they develop in adult patients. The pathogenic factors are still unknown. Growth of this type of tumor is slow and spreading. They can achieve very large sizes. The basic diagnostic examinations are ultra-sonography and tomography of the abdominal cavity. The basic treatment of liposarcoma is surgery, if possible radical resection of the whole tumor an organs involved, if possible. In this case, the tumor removed by our medical group weighed 11.5 kg in one part. It occupied not only the retroperitoneal cavity but also the whole abdominal cavity. Despite the removal of such a gigantic tumor, this kind of intervention is hard to consider as very radical. Literature data indicate that one of the essential risk factors of tumor recurrence is the size of the tumor. We would like to present the described case with regard to the rarity of occurrence: the enormous size, asymptomatic course of the disease, and recurrence 6 years after primary surgical treatment.

OP session SG6

A prospective multicentric randomized study comparing TAPP vs TEP

V. Ştefănescu, D. Moga, V. Oprea, C. Mitru, F. Macău

Background: Laparoscopic inguinal hernia repair has the advantage of lesser pain, early recovery and lesser wound morbidity.

There is no consensus on the best groin hernia repair.

There are two standard techniques of Laparoscopic Inguinal hernia repair – Total Extra Peritoneal repair (TEP) and Trans – Abdominal Pre-Peritoneal repair (TAPP).

There are not many trials available comparing the two techniques and questions remain about their relative merits and risks.

Method: Randomization plan was generated in November 2013; surgical interventions were performed in three military hospitals since January 2014 to august 2015 according randomization plan (49 TEP and 50 TAPP).

Results: Both TEP and TAPP mesh repair techniques were comparable in terms of operative time, intraoperative complications, and conversion to open, post-operative pain, time to resume normal activity and recurrence.

Conclusion: Laparoscopic repair of inguinal hernias is associated comparable results in both techniques, TAPP and TEPP and choice between TAPP and TEPP is a personal choice of concerned surgeon and/or surgical service.

The management of locally advanced gastric cancer

M. Tănase, I.P. Oprea

Worldwide, gastric cancer represents the fifth most common cancer and the third leading cause of cancer deaths. Although the overall 5-year survival for resectable disease was more than 80% in some countries due to the implementation of screening programs resulting in detection of disease at earlier stages, in Europe more than two thirds of gastric cancers are usually diagnosed in advanced stages with a survival rate of only 25%.

Surgical resection with extended lymph node dissection remains the only curative therapy for advanced gastric cancer, while adjuvant chemotherapies can improve the outcomes aimed at the reduction of recurrence and extension of survival.

In our surgical department’ statistics, the locally advanced gastric cancer represents 71% of gastric cancer cases.

The objective of the presentation is to develop an attitude in view of early diagnosis and extension of survival regarding locally advanced gastric cancer.

Vaginal hysterectomy on cicatricial uterus

B. Panaite, O. Nicodin

This presentation is based on a study developed in the Gynecology Clinic of Central Military Hospital- Bucharest „Carol Davila”- there have been analyzed 50 cases of vaginal hysterectomy which followed a caesarian delivery which 10 presented also a history of a vaginal delivery, 9 patients had a history of two or more cesarean sections. We have compared the percentage of bladder injuries to the simple vaginal hysterectomies (which had no history of cesarean section). There have been also analyzed: the operative time, the blood loss, the admission time, other complications.

Results: There haven’t been found significant differences between the two groups.

Incidenthaloma – Bartholin’s gland adenocarcinoma case report

Mădălina Prună, C. Mitru, N. Tănase, C.I. Beţianu, I.P. Oprea

Background: Malignant tumors of the Bartholin glands are very uncommon, with a reported incidence of 0.1 cases per 100,000 women.

Case: A 40 years old woman attended our clinic complaining of a painful, tender lump in the right side of her vulva. The immediate clinical suspicion was of a Bartholin's gland abscess. Incision, drainage and marsupialization were carried out, but after 4 months she returned with a recurrent abscess, treated with right Bartholin's gland excision. Pathological examination revealed a well-differentiated papillary adenocarcinoma originating in the Bartholin's gland. Our patient underwent wide local re-excision, bilateral pelvic and inguinal dissection. There was no metastases in the lymph nodes and the margins were free of tumor. According to the FIGO classification, the patient was diagnosed with stage I vulvar cancer. At 1 year after surgery there is no evidence of recurrence.

Discussion: In cases of recurrent vulvar abscess, even in young patients, complete excision should be done in order to prevent delay in diagnosis of a cancer. The therapy of this rare tumor has many controversial issues and there is no consensus regarding the optimal treatment.

Patient specific instruments for complex tumor resection – reconstruction surgery within the pelvis

E. Cernat, P.L. Docquier, L. Paul, X. Banse, I.B. Codorean

The pelvis bone resection-reconstruction surgery is one of the most challenging fields in orthopedics. Being applied for tumors, as for other complex reconstruction cases, this type of surgery needs careful planning and is time consuming, in order to obtain proper accuracy.

Unfortunately not all the time the expected accuracy is met, with consequences for the patients. PSI proved to provide good cutting accuracy during simulated tumor surgery within the pelvis. This article present a series of 4 patients operated in our department between June 2014 and Mars 2015 for tumors resection-reconstructions.

The patients were imaged using a CT and an MRI scan and the images were reconstructed in 3D. According to the bone bank stock, the most similar allograft was chosen and the stored CT scan was reconstructed in 3D.

Patient specific instruments (PSI) were designed and manufactured using rapid-prototyping technology for the resection of the native tissues as for the resection of the careful selected hemipelvic allografts. Allografts' fitting to the pelvis of the patients was excellent and allowed stable osteosynthesis.

Role of radical pelvic and paraaortic lymphadenectomy in utero-ovarian neoplasia

A.C. Dima, O. Florea, T. Artenie, C. Mitru, F. Bold, D. Bădănoiu, I.P. Oprea

The radical pelvic and paraaortic lymphadenectomy is indicated in endometrial, ovarian, and cervical cancer when diagnosed in advanced, but resectable stages.

This approach is laborious, with associated good results when the indications are respected and when it is correctly and completely realized.

There are presented here the data of a case series of 9 patients, ages between 42 and 71 years, with genital neoplasia to whom this approach was applied.

Radiotherapy, chemotherapy or radio-chemotherapy in neoadjuvant setting was done before surgery. In all cases, hysterectomy with bilateral salpingo-oophorectomy, combined with pelvic and paraaortic lymphadenectomy was performed.

In three cases, there was imposed a multi-organ resection was imposed. In two cases, an anterior pelvectomy was performed, and in one case a rectosigmoidectomy.

Even if these interventions were very laborious (with surgery time between 5 and 8 hours), the post-operative morbidity was highly acceptable.

Laparoscopic surgical technique in the conservative treatment of tubal pregnancy

N. Niculescu, O.V. Nicodin, B. Panaite, C. Pârvulescu, Ioana Niculescu

Objectives: Establishing the indications of conservative treatment in ectopic pregnancy.

Materials and methods: Between 2012 and 2014, thirteen patients with ectopic pregnancy have been treated with conservative surgical techniques.

Pre- and intraoperative exclusion criteria were: ectopic gestational sac size exceeding 6 cm, fetal heart activity, β-hCG serum levels > 20,000 mIU/ml, previous surgery on the affected fallopian tube, isthmic ovular implantation.

Thus were selected the following cases: absence of the controlateral fallopian tube/functional compromised, gestational sac un-ruptured and ampulo-pavilionar implantation; we decided to apply laparoscopic linear salpingotomy, gestational sac extraction with bipolar hemostasis.

Results: We have obtained five intrauterine pregnancies and two ectopic pregnancies which required radical treatment (salpingectomy). Two pregnancies have been completed with gestation carried to term. There were no favorable results in patients who had other associated factors of infertility.

Conclusions: In selected cases laparoscopic surgery allows the anatomical and functional restauration of the fallopian tube affected by pregnancy and offer a chance in getting a desired pregnancy.

OP session NS1

Extracorporeal circulation

Florentina Rizea, Marinela Bosincianu, Elena Iftode, Gabriela Batan, Andreea Teodorescu

A procedure in which blood is taken from a patient's circulation to have a process applied to it before it is returned to the circulation. All of the apparatus carrying the blood outside the body is termed the extracorporeal circuit.

The basic role of CPB is to maintain the systemic circulation at physiological parameters during the cardiac arrest and in the hipocontractillity period (reperfusion).

The adequate organ perfusion it's realized by maintaining the cardiac output and the perfusion pressure.

The blood flow through the arterial line, and therefore in the systemic vascular bed, is generated mechanically by a pump and it is not pulsatile. There are also technical devices that allow pulsatile flow generation. During CPB the organs vulnerable to hypoperfusion and hypotension should be especially protected (brain, kidneys).

CPB components: venous drainage; arterial cannula; venous reservoir; the actual pump; the oxigenator; heat exchanger; accessories; priming.

The perfusionist is the person in charge for the CPB functioning and who is endorsed on the type of the surgical intervention and patient's medical data. He is the one who calculates according to the patient's body surface, the priming necessary, the cardiac output (in order to avoid hemodillution) and selects the cannulae to be used.

The CPB starting is made on the surgeon's demand, who is maintaining a clear and bilateral communication with the perfusionist. The perfusionist repeats and confirms the surgeon's orders, informing him permanently on its activities.

The CPB exit (CPB-OFF)

It is initiated after the cardiac electric activity restore, after a reperfusion period, adequate to the surgical intervention's length. The perfusionist is gradually clamping the venous line and reduces the pump output, allowing to introduce the venous blood in the patient's circulation.

The blood pressure (BP) and heart filling (central venous pressure) are carefully monitored. If the Bp and heart filling are optimal, the CPB can be stopped.

The role of the professional nurse in the intraoperative care of the patient undergoing open heart surgery is beneficial for obtaining a positive outcome for the patient.

D vitamin or hormone

Florentina Dumitru, Mihaela Andreev

Through the ages, medical knowledge have evolved with the science and technology progress. A number of substances considered in a certain way then and there, turned out to fit into other classes of drugs nowadays.

Vitamins have diverse biochemical functions. Some, such as vitamin D, have hormone-like functions as regulators of mineral metabolism. Vitamin D promotes calcium absorption and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany.

Many scientific evidence confirm that "D-vitamins" act as a hormone in the body: modulation of cell growth, neuromuscular and immune function, and reduction of inflammation. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D.

These facts corroborate by the discovery of receptors of active form of vitamin D (1.25-dihydroxy-cholecalciferol) across cells in several types of tissues and organs, justify the new classification.

From the pharmaceutical point of view there are many different types of formulation and concentrations, each of them with its specificity and indications.

The new approach should be: What is more useful for us? A classification or the understanding and application of new medical knowledge in daily pursuits for everyone?

Cancer patients' rights

Elena Coman, L. Onel, Mădălina Ciobănescu

1. Right to early detection, diagnosis and treatment: All cancer patients, irrespective of age, sex, or various social conditions have rights to early disease detection, best methods of diagnosis and treatment available.

2. Quality of care: Quality of care should be the result of a comprehensive approach that responds to the particular needs of patients and their families, to promote improved quality of life and lead to more efficient use of resources.

3. Patient-health professional relationship: This relationship should develop in terms of mutual respect and trust and rely on well-defined rights and responsibilities.

4. Patient information: Access to information for patients and their families must be promoted to a better understanding of the disease and treatments, in order to decrease anxiety and to browse in a less stressful this stage of life.

5. Psychological support for patients and their caregivers: Psychological support is essential for the emotional needs of patients and their families at all stages of the disease, during and after treatment.

6. Socially and financially support

7. Rehabilitation and reintegration into society: Restoring physical and mental health and quality of life requires a complex, multidisciplinary approach (physical, social, occupational, psychological and spiritual).

8. Palliation: Patients' rights to medical care, which aim to improve the quality of life in advanced stages of malignant disease.

9. Social consultancy of patients: Concept established colloquially as "advocacy", which means all actions taken individually or through organizations, which aim primarily ever more active involvement of patients in medical decisions, but also in promoting health programs and policies.

Ebola

M. Leatham, K. Higgins

Following the United Nations declaration that the Ebola virus outbreak was a public health emergency in March 2014 the United Kingdom made commitment to provide support including providing care for the people in Sierra Leone. An international response involving WHO, US and British governments, the UK Department of Defense designed and oversight of the building of several Ebola Treatment Centers and Training Academy and provided logistic and planning support. In addition, approximately 800 Defense personnel were deployed to Sierra Leone including nurses, doctors and laboratory staff.

Based upon their current experience, skills and knowledge, two personnel from the British Army reservists were selected and deployed from 207 Manchester Field Hospital. Following further specialist training to ensure they could work in austere conditions treating people with this hemorrhagic viral disease that that claimed up to 11,325 lives of many civilians Lieutenant Colonel Marian Leatham and Major Kath Higgins work and lead a teams of nurses, combat medical technicians and healthcare workers to provide care and treatment for patients with confirmed or suspected Ebola.

This presentation gives an account of their experiences and the importance of team work and leadership from two different roles, one as a team leader the other as the Senior Nursing officer for the hospital in dealing with previously untested challenges in infection control and patient and personal safety during their time in Kerrytown Ebola Treatment Centre in Sierra Leone.

The nurse-patient relation

Florica Cristea

To practice medicine correctly, means to respond promptly, honestly, and with empathy, to the certain needs required by the medical act, always accompanied by a great sense of ethics.

The disease implies firstly a disturbance in the patient's inner equilibrium, which translates in physical or a mental disorder, a maladjustment to a new definitive or temporary situation. A person "enters in the disease" with a certain psychic, temperament, character and intelligence, with preconception and cultural horizon and that is why the patients assumes a certain attitudes towards the disease but also towards the medical team: trust, esteem, sympathy, but also possibly doubt, fear, hate and contempt.

The nurse has to treat all patients unconditionally. Also, the nurse-patient relation does not have to be with only about treatment but also connecting and understanding the patient in these difficult moments. Communication with the patient has to be in consonance with his sate, his has to be in consonance with his sate, his understanding and has to be associated with aiding elements of his ailment. The care of medical ethics consists of a member of more and professional traits regarding the nurse: honesty, altruism, a giving sense, respect, solicitude, comprehension.

This is way the medical profession has to be exercised with patience, generosity, passion, honesty, the central focus of all, being the patient who has to be understood and accepted as he is.

Laboratory investigations of autoimmune hemolytic anemia

Eva Dincă, Verginica Săvoiu, D. Săvoiu

Autoimmune hemolytic anemia is a condition in which effectors of the immune system erroneously attack own red blood cells, thus leading to lysis thereof.

Blood samples were collected from three patients suffering from immune hemolytic anemia. Vacutainers required for blood collection and Coombs kits, as well as microscope slides, thoroughly cleaned, degreased and dried have been used.

A complete blood count was performed, along with the leukocytic formula and a direct Coombs test.

Compared to normal values, all patients presented low values of the plasma concentration of HGb, Hct and of the erythrocytes count.

Elevated bilirubin, GOT, D-dimers and fibrinogen values were recorded.

A positive result of the Coombs Test confirms the autoimmune hemolytic anemia diagnosis.

Effect of Algisyl ventriculoplasty on functional status of patients with advanced heart failure

Daniela Nae, Magdalena Petrea, Iulia Dragomir, Iuliana Gheorghediac, Ileana Hănțulie, S.I. Dumitrescu

Study objective: Evaluation of functional status in the first six months after implantation of a new device, in first 10 patients of our center, randomized to the surgical arm of AUGMENT-HF clinical trial.

Methods: AUGMENT-HF clinical trial is a randomized, controlled study to evaluate the safety and cardiovascular effects of Algisyl-LVR™ as a method of LV augmentation in patients with chronic heart failure due to ischemic or non-ischemic dilated cardiomyopathy. Algisyl-LVR™ is a biopolymer classified as medical device by regulatory authorities that is implanted in free wall of the left ventricle aiming to alleviate LV dysfunction through a left thoracotomy followed by 10 - 19 biopolymer implants. Subsequently to device implantation the patients were evaluated by complex monitoring of clinical, laboratory and laboratory parameters comparing the surgical arm with the standard treatment arm.

Results: Assessment of clinical status at randomization and follow-up visits was done by NYHA class evaluation, 5-minute walk test and Kansas City cardiomyopathy Questionnaire.

	NYHA II/III/IV	6MWT	KCCQ
Randomization	0/7/3	271 ± 83	46 ± 21
3 months	2/6/2	302 ± 89	52 ± 24
6 months	3/5/1	311 ± 82	62 ± 27

Conclusions: The surgical procedure to implant the biopolymer alginate resulted in improved functional status in patients with advanced heart failure.

OP session NS2

Essential arterial hypertension

Elisabetha M. Ionescu, Petruța Zărnescu, Laurenția Leonte, V. Ilieșe

Introduction: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC).

Hypertension is defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg. The diagnosis of hypertension should be based on at least two BP measurements in the sitting position per visit on at least two visits.

Risk factors: Include age, male sex, smoking, dyslipidemia, glucose intolerance, obesity and family history of premature CVD. Asymptomatic organ damage mainly involves left ventricular hypertrophy, evidence of vascular damage and microalbuminuria.

Cardiovascular risk factors: Total, LDL and HDL cholesterol, and fasting triglycerides and glucose are considered routine tests in all hypertensive patients.

Treatment approach: Lifestyle changes. Appropriate lifestyle changes are the cornerstone for the prevention of hypertension and are also important for its treatment. The following lifestyle measures are recommended:

- Salt restriction to 5-6 g/day.
- Moderation of alcohol consumption (<20-30 g of ethanol per day in men and <10-20 g in women).
- Increased consumption of vegetables, fruits and low-fat dairy products.
- Reduction of weight to BMI of 25 kg/m
- Regular exercise (≥30 min of moderate dynamic exercise on 5-7 days per week)
- Smoking cessation
- Prompt initiation of antihypertensive drugs is recommended in patients at high or very high CV risk in elderly patients drug treatment is recommended when systolic BP is 160 mmHg, or ≥140 mmHg if younger than 80 years and treatment is well tolerated.

Conclusions: Diuretics, beta-blockers, calcium antagonists, ACE-inhibitors and angiotensin receptor blockers are all suitable for the initiation and maintenance of antihypertensive treatment, either as monotherapy or in combination therapy. Lifestyle changes. Appropriate lifestyle changes are the cornerstone for the prevention of hypertension and are also important for its treatment.

Blood investigations in Hodgkin lymphoma

Cătălina Mihăilescu, Matilda Marinache, Constantin Coman

Introduction: Lymphoma is a cancer that starts in lymphocytes of the immune system and appears as a solid tumor of lymphoid cells. These cells developed abnormal come from lymph nodes and spread outside the lymph system.

Thomas Hodgkin lymphoma published the first description in 1832, as called after him, Hodgkin lymphoma.

Material: Dynamics were followed in 2 patients diagnosed with Hodgkin lymphoma after about 10 years. Patients came for full blood regularly.

Explorations Blood: complete blood count with differential white blood count, erythrocyte sedimentation rate determination, and puncture and bone marrow biopsy performed iliac crest, ESR.

Biochemical explorations: liver function tests, electrophoresis, serum lactate dehydrogenase, serum calcium, urinary calcium, cupremia.

Biological sampling was done following bioethics standards, rules and norms aseptic SSM.

Method: Immunological abnormalities may occur. Such known cell-mediated immunity deficiency characterized by negative tuberculin skin test.

Results and discussions: Hemoglobin concentrations were slightly lower for patients investigated.

Leukocyte number was slightly increased.

The percentage of eosinophils was close to the maximum limit.

This Hodgkin's disease is suspected lymphadenopathy occurs when accompanied by fever, night sweats and weight loss.

To confirm the diagnosis of disease, it is necessary to carry out investigations where there Blood hemoglobin decreased, elevated ESR and fibrinogen for Epstein-Barr virus may increase the risk of lymphoma.

Diagnosis of the disease can be achieved after puncture ganglion; smear analysis reveals the presence of Reed-Sternberg cells, but for a diagnosis of certainty node biopsy is needed.

Advanced Parkinson's treatments and therapies

Olguța Preda-Cârșote, Violeta Gâlcă, Mioara Girea

Parkinson's disease is a chronic and progressive movement disorder, meaning that symptoms continue and worsen over time. The cause is unknown, and although there is presently no cure, there are treatments options such as medication and surgery to manage its symptoms. As Parkinson disease progresses, the amount of dopamine produced in the brain decreases, leaving a person unable to control movement normally. Medication is the main treatment used for the movement symptoms of Parkinson's The term 'advanced Parkinson's' usually refers to a time when symptoms are more complex and have more often an effect on patients' day-to-day life. In the later stages of Parkinson's, when non-motor symptoms begin to dominate quality of life, it may be appropriate for some drugs to be withdrawn.

There are few advanced therapies for patients with Parkinson disease when symptoms don't respond well to oral medication.

1) Apomorphine – an injection or infusion of dopamine agonist under the skin levodopa/carbidopa gel inserted into

your stomach by a tube. The dopamine agonist apomorphine provides relief from off periods when administrated as a subcutaneous injection. When applied continuously via a portable pump, oral medication can often be reduced considerably and dyskinesia improve in many patients. Continuous duodenal levodopa infusion it is used in treatment of advanced levodopa-responsive Parkinson's disease with severe motor fluctuations and hyper-/dyskinesia when available combinations of Parkinson medicinal products have not given satisfactory results. Duodopa is a gel for continuous intestinal administration.

2) Deep brain stimulation (DBS) is a surgical procedure used to treat a variety disabling neurological symptoms of Parkinson disease such as tremor, rigidity, stiffness, slowed movement and walking problems. At present, the procedure is used only for patients whose symptoms cannot be adequately controlled with medications. The majority of patients with Parkinson disease develop motor fluctuation and dyskinesia as their condition progress.

There are a number of therapies that can help people with Parkinson’s and offer advice on ways to help manage symptoms. Therapists and nurses are trained to provide advice and recommend exercises and treatments to keep them active and healthy. They are part of a team that can help patients manage their condition. The main therapies includes: occupational therapy, speech and language therapy, physiotherapy, seeing a dietitian and complementary therapies.

As a conclusion, Parkinson’s disease does not just affect the person living with it – it affects the entire family and an extended community of friends and loved ones.

Epilepsy (seizures disorders)

Cristina Lixandra, Oana Rada

Epilepsy is a syndrome of various etiologies caused by excessive neuronal discharges having a paroxysmal, transient, intermittent and interactive mechanism, characterized by sudden seizures of intermittent disorder of brain function often involved with loss of consciousness.

Antenatal causes: exogenous factors that deal with the mother during the pregnancy; abdominal trauma; maternal infections; toxic causes such as alcohol, professional noxae and so on.

Natal causes: difficult deliveries; fetal injuries during delivery or Rh incompatibility.

Postpartum causes: brain injuries; allergic factors; calcium and glucose metabolism disorders.

People with seizure would be classified according to the type of crisis, underlying cause, epileptic syndrome and episodes occurred during and around the crisis. Seizure types are classified depending on location or source of epileptic seizure (local seizures) or its distribution (grand mal seizures) in the brain. There are a number of factors contributing to the installation of the status epilepticus such as antiepileptic drug withdrawal; metabolism disorders; stroke or cerebral infarction; CNS infections, trauma or tumors.

Psychosocial effects of epilepsy include depression, anxiety disorders, migraines and attention deficit hyperactivity disorder. The symptoms vary according to the scale of seizure (partial seizures – affecting only one brain area or grand mal seizures – affecting large areas of the brain, located in both cerebral hemispheres).

They appear like small contraction localized in a small body segment or a series of abnormal perception of the environment, either visually or auditory perception. Clinical manifestations are very different because of the epileptogenic focus – “that small area of the brain where the seizures come from”.

Medication include anticonvulsant drug therapy and it is designed to reduce the frequency of epileptic seizures but it may also lead to their disappearance. The therapy for epileptic seizures is a specific one. Each patient, in terms of right chosen treatment and depending on the particular disease, about a third of them as an average, may not do the seizure or do them rarely.

So, they have the opportunity to conduct a normal life with a balanced diet and right behavior.

Penile tumor. Nursing for patients with penile tumor

A. Bozieru, Agnes Ciucă, D. Spînu, D. Mischianu, F. Rusu

Introduction: Penile tumors are a rare pathology that unfortunately remain untreated or treated late. A misunderstood sense of shame is often the biggest culprit for the late presentation to a doctor.

Material and method: We present the therapeutic approach adopted in the case of a patient with penile tumor, evolving for about ten years, hospitalized in the department of urology „Carol Davila” Hospital. The patient, male aged 78 years old, presented in our service complaining about diffuse left inguinal pain. Local exam confirmed the presence of a left groin palpable lymphadenopathy and a tumor involving the Glans with a minimal extension to the penian shaft. Biopsy confirmed low grade squamous cell carcinoma. Partial amputation of the penis and bilateral inguinal limfodisection was performed. Pathology result confirmed negative penian resection limits, but with left inguinal lymph nodes invaded (3/6).

Results: Postoperative evolution was favorable. Three months after surgery, a dilatation of the urinary meatus was necessary. He is now under oncological treatment.

Conclusions: The delay of the diagnosis due to a false sense of shame represents one of the largest mistakes. In the case of this patient, the neoplasia type has allowed and allows palliative treatment efficiency, unfortunately most often we come to cases of aggressive neoplasia that once metastatic dramatically reduces the life expectancy.

The role of nurses caring for patients in renal colic section of urology

Iulia Ene, Liliana Dogaru, Gabriela Pînzariu

Definition: Renal colic is a painful paroxysm with anteroinferior location for lumbar and iliac fossa irradiation, testis and scrotum in men and vulva in women, presenting an undulating, with maximum painful lull. It is a medical emergency and urological. Maximum intensity is usually located in costovertebral angle and lower right rib area XII, can have headquarters variable depending on the location of the obstacle.

The main cause of renal colic is represented by the sudden onset of a barrier to evacuate urine – kidney cavity, ureter, uretero-bladder junction which sharply increases the pressure in the pielo-uretero-caliceal system and renal receptors produce excitation preso-pain. Sudden obstacle that blocks urine is most often a stone, but may be just as likely, a clot (reno-ureteric bleeding) home suppuration material failure (renal TB). Other reasons: neighborhood processes (inflammation, tumors) extrinsic compression, causes iatrogenic following therapy (surgery, lithotripsy, radiotherapy) renal infarction.

Clinic: the patient shows paroxysmal pain, sudden onset, sometimes apparently in full health. The patient seeking a position analgesic that cannot find; useful element in the differential diagnosis with other abdominal pain syndromes that often immobilizes the patient.

Renal colic is accompanied by:

- Digestive symptomatology: nausea, vomiting, ileus, pseudoocluzive syndrome - retro-peritoneal irritation signs;

-Neuropsychiatric -signal: agitation, extreme anxiety;

- Cardiovascular symptomatology: usually shows no changes in BP and pulse.

- Fever association can quicken the pulse and BP lowering in this case suggests a UTI;

- Signal urination: Colic can be followed by hematuria which can be caused by a migrated stone, then became obstructive. Urine may be clear or cloudy excluding kidney affected by opposite kidney pain with hematuria and renal colic. The association with hematuria and histuria at the diabetic patient or consumer of analgesics can suggest papilar necrosis. The symptoms depends on the location of the obstacle: high obstacle will produce maximum pain, iliac fossa irradiation and genitals;

The role of nurses caring for patients in renal colic section of Urology:

Monitoring: assessment of vital signs (blood pressure, pulse, respiration, urine output, temperature) and physician reports the changes; assessment of pain characteristics and macroscopic appearance of urine; administration of treatment; follows the investigation indicated by the doctor; explain to the patient how to collect urine and urinate through cheesecloth (we could recover calculation for chemical analysis); obtaining blood and urine samples, prepare the patient for uiv ; treating the patient who shed; give moral support to the patient and family.

Transurethral bladder tumor resection

Adriana Ursu, Claudia Dumitru, Mihaela Marin, Angelica Mălăescu, Gina Popescu

Bladder cancer is a very common malignant affection which has a high rate of recurrence.

79% - 85% of the patients with bladder cancer are in an evolutionary stage of non-invasive bladder tumors, while the proportion for the recurrence is very significant.

On a global scale, the mortality caused by this disease is 2-10 men and 0.5-4 women out of 100.000 citizens per year. Bladder cancer is 3 to 4 times more frequent in men rather than in women.

In order to determine the right diagnosis, a transurethral tumoral resection has to be performed under spinal anesthetic agent.

Due to the risk of recurrence and progression, the patients with non-invasive bladder tumors have to be carefully monitored and the frequency and duration of the cystoscopy exams need to reflect the risks for every patient.

OP session NS3

The implementation of the protocol concerning the communication of bad news in the nurse's practice

B. Silveșan, Lucia E. Istrate

Bad news may be defined as "any information which adversely and seriously affects an individual's view of his or her future"

It is difficult and uncomfortable to deliver bad news in all medical areas especially in oncology even though we are referring to diagnosis, reoccurrences, disease progression or transition to palliations treatment. In any situation, this is a difficult and stressful task.

Studies showed that a significant part of the medical team experiment a strong emotion after communicating bad news, and the effect of stress lasts for hours and even days.

To realize an efficient communication there have been adopted a series of international protocols that can be used. One of them, named

S-P-I-K-E-S protocol, a recent but largely used strategy, that approaches the establishment of an appropriate context, checking the patient's perception; determines the amount of information known, desired and that will be transferred; the knowledge of medical realities before discussion; exploring the emotions that might appear during the interview and the establishment of a support strategy.

The role of palliative care for patients with severe chronic diseases

Ani Ivan, B. Silveșan

Palliative care is the active and total care of patients whose disease is not responsive to curative treatment. The control of pain and of other symptoms, the psychological, social and spiritual assistance are of major importance.

It is a benefit not just to those suffering from: cancer; HIV; progressive neurological disease; renal, cardiac, hepatic insufficiency or other diseases that cannot be cured, but also for their families considering that palliative care is very complex, it is a holistic care, is not only a medical work.

The goal of palliative care is to ensure the best possible quality of life for patients and their families.

Bone densitometry (DEXA, DXA)

Nicoleta Tudora, Cristina Popa, Carmen Hărdău

Bone Densitometry (DEXA = Dual-energy X-ray absorptiometry) is a paraclinic procedure which measure bone' density and osteoporosis detection, this phenomena appear especially in menopause women. Osteoporosis treatment prevents the risk factors, which improve the bones resistance.

DXA is recommended in 65 old women menopausal and with previous pathological fractures. Also in women with multiple risk factors (like familial vertebral fractures history) and in long periods of treatment with corticosteroids.

These examination is recommended for lumbar spine: 4 lumbar vertebrae L1 to L4, to coxofemoral joint (hips and pelvis) and forearm. For a complete test is necessary to exam 3 regions, but for a correct diagnostic 2 regions are enough.

DEXA is a simple, fast and noninvasive procedure. During the examination time, a very small dose of radiation is used. We mentioned that are no previous preparations for the patients and we can perform at any time of day. The patient can remain clothed during the examination, except them which contain metals things.

The patient should be lying on the table, between X ray and detector. The detector in moving in the part interested, without pain, bites or discomfort. Entire examination is finished in 5- 10 minute.

DEXA provides us 2 important results: T score and Z score. T score: provides the result of a comparison between the patient bone density and a maximum bone density (in a 30 years old man, young and healthy). A T score less than -1 or more is NORMAL. The values between -1 and -2.5 suggest the osteopenia and less than – 2.5 means OSTEOPOROSIS.

Z score: compare the patient bone density with a bone density in a person with the same age, this appreciate the damaged/lost grade of bone tissue. A negative Z score means that the patient is having fragile bones, and a positive score means that the patient is in normal limits.

Is snoring dangerous?

Ileana M. Mateș

Snoring is a sleep disorder which often precludes overnight rest, but it is especially important as a symptom of more serious respiratory disorders, such as the Syndrome of Sleep Apnea (SSA), the Obstructive Sleep Apnea (OSA) or the Syndrome of the Upper Airway Resistance (SUAR).

Snoring occurs when there is an obstruction to the flow of air into the area behind the nose and mouth, where the upper part of the pharynx and base of the tongue meet the soft palate and the uvula.

Severe snoring is often the symptom of serious health problems, with grave long-term effects, such as the obstructive sleep apnea (SAS).

In the sleep medicine a scale is commonly used to assess the degree of daytime sleepiness, called the Epworth Sleepiness Scale.

Hypertension and heart diseases are more common with people who snore, both men and women; moderate snoring involves a partial airway obstruction, and so an insufficient oxygenation during sleep – a fact which has serious consequences on the cardiovascular system.

In Western countries there is a wide range of products against snoring.

There are devices which prevent sleeping on the back or devices which change the position of the mandible.

If the snoring is very strong, an ENT doctor/ otorhinolaryngologist will examine the nose, mouth, soft palate and throat and will indicate if it is necessary to correct a nasal obstruction (deviated septum or polyps). An examination in the sleep lab may be necessary in order to determine how serious the snoring is and if it is coupled with sleep apnea thus affecting the patient health.

Meniere’s disease

Mihaela Arghir

Meniere’s disease is a disorder of the inner ear, whose causes are not fully known. It is characterized by the build-up of fluid in the inner ear, caused by either anxiety, high blood pressure or excessive salt consumption.

An acute episode of Meniere’s is characterized by the following symptoms: dizziness, nausea, vomiting, hearing loss, ringing in the ears, low tolerance for loud noises, headaches and a sensation of “full ears”, sometimes before the episode begins.

Diagnosing Meniere’s disease is managed by an otorhinolaryngologist, using techniques such as: tonal audiogram, electrocohleography, CT scans.

The treatment for Meniere’s usually starts by using drugs which lower the fluid quantity in the inner ear, and thus the pressure build up (diuretics).

The nurse – patient relationship

Georgeta Porojan, Alina Ispas

All medical care takes place in the framework of the nurse-patient relationship.

Communicating with the patient can bring together two people from different backgrounds, with different personalities, and different ways of seeing things. Therefore, the nurse-patient relationship is characterized through individuality and specificity.

The multiple times I’ve been the patient myself have given me the opportunity to better understand the point of view through which we, as medical professionals, are perceived. These experiences have also allowed me to ask myself about the ways we can better respond to the expectations a patient may have from the people who are in charge of their health care. This includes both the relationship between a single medical professional and a patient, and one between a team of health care professionals and a single patient. In spite of any differences, there’s always a basic way to communicate with the patient, which answers most of their expectations: empathy.

I have had experiences in which I first focused on the ‘’disease” before the patient. In order to avoid this situation, I considered six steps that I must always follow in order to see the patient as more than just a medical condition. These steps are: awareness, acceptance, responsibility, trust, purpose and integrity. Used in integrative psychotherapy, these six steps can provide an excellent framework for communication between medical professionals and patients.

Management of AAA – endovascular repair

Marinela Bosincianu, Elena Iftode, Mihaela Apostol, Roxana Constantin

Introduction: Abdominal aortic aneurysm is a multifactor disease that usually occurs in cardiovascular high risk patients and leads to death by rupture if not fixed on time. They are hemodynamic unstable, they need inotropic support and diagnostic is based on ultrasonography and computed tomography.

Objectives: Managing AAA needs pertinent screening and patient evaluation, in order to offer the best treatment, providing a safe and cost effective operation, with the best long term quality of life.

Method: Between 2015 and 2016 were selected a total of 6 patients with AAA who presented a high risk to the classic operation and the surgical treatment options for AAA was EVAR.

EVAR is performed by an interventional radiologist and a vascular surgeon, using x-rays to guide medical instruments inside the arteries. The benefits of EVAR over traditional surgery are: no large abdominal surgical incision, no sutures (stitches), or sutures only at the groin area, faster recovery and shorter time in the hospital, no general anesthesia (in most cases), less pain, reduced complications.

Results: Was analyzed the postoperative evolution, showing the special role of nurses in intensive care.

Conclusions: The results show that in the modern era of AAA treatment, EVAR is a safe and effective in patients with high-risk short and medium term.

Healthcare of patients with TAVI

Marinela Bosincianu, Elena Iftode, Roxana Tudor, Elena Anghel

Introduction: Aortic stenosis is a narrowing of the valve orifice that is responsible for forming a barrier against ventricular ejection during systole. Transcatheter aortic valve implantation (TAVI) involves inserting a new artificial heart valve inside the old tight valve using a balloon catheter.

The valve is made up of a metal frame (stent) and the outer lining (pericardium) of a cow’s heart.

Objectives: TAVI is a new technique with the potential for transforming the treatment of patients with aortic stenosis, who are at high risk for conventional open heart surgery or considered inoperable.

Working method: Case of Study-in period 2015-2016 in our clinic have conducted a number of 8 (3 transapical si 5 transfemoral)

Results: Stenotic aortic valve disease distribution by age and gender, low rate of hospitalization days.

Conclusion: Cardiovascular surgery is performed by more modern methods that greatly reduce trauma, postoperative pain and risk of infection.

During the interventions of cardiovascular surgery plays an important role. The intervening period is shorter, the patient's chances of recovery are higher. In this type of implant, shorter hospital stay and faster recovery.

The role of nurses in health postoperative cares of patients, supervision and monitoring with TAVI, and to educate patients and learn, to lead a healthy life it is very important.

OP session NS4

Transverse myelitis

Daniela I. Bradea, Mihaela Ivănică, Ioana Catalina, Oana Iliașii

Transverse myelitis is a neurological condition in which the spinal cord is inflamed. The inflammation damages nerve fibers, and causes them to lose their myelin coating leading to decreased electrical conductivity in the central nervous system. Transverse implies that the inflammation extends across the entire width of the spinal cord.

Although it is a very rare affliction, with an incidence rate of 1.34 in a million (according to Association for Transverse Myelitis), this syndrome can strike at any age in both women and men, but it`s mostly encountered between 10-19 years and after 40.

The patients who suffer from transverse myelitis recover differently depending on cause and severity of the illness. Almost 1/3 of these patients are completely recovered, for other 1/3 the results are average and for the last 1/3 the results are very low. The recovery is most efficient in the first 3-6 months after a transverse myelitis crisis and some of the patients need a few years for a total or partial recovery.

Benefits of the exploratory laparoscopy in acute surgical abdomen therapy

Claudia Buzatu, Daniela Mihai, Anca Pologea

With progress in methods of investigation and diagnosis, most of cases which goes to the hospital in emergency, receiving preoperative indication of their disease. There are some cases in which preoperative was set just it`s an acute surgical abdomen and that have operated. Here is where exploratory laparoscopy finds utility.

Also the exploratory laparoscopy contributes to complete partially elucidate preoperative diagnoses but with clear surgical indication.

In our general surgery department are operated in emergency between 250-300 patients with acute surgical abdomen. 15-20% of them receive benefits of exploratory laparoscopy.

This paper is a retrospective study of 684 patients operated in our service, in emergency conditions, in the last three years. 147 of them occurred through laparoscopic approach and laparoscopy was completed in 86 cases.

The paper is an according debate between pre and postoperative diagnosis, distribution by type of pathology and diseases in which laparoscopy had higher success rate.

Discussions aimed at length the advantages of exploratory laparoscopy in emergency conditions and diagnostic doubt.

The end of paper presents the conclusions that bring to the fore: shortening surgery; shortened hospitalization; rapid reintegration into family and society.

Gastroesophageal reflux disease

Alina Dinu, Anișoara Graure, Cristina Ioncescu

GERD has long been recognized as a significant public health concern in USA generating along the time, many discussion between gastroenterologists and surgeons. Once antireflux barrier was identified, and mechanism of reflux established, GERD can be defined as the failure of the antireflux barrier (represented especially by the lower esophageal sphincter, by gastric empty of gastric contents into the esophagus), allowing abnormal reflux of gastric contents into the esophagus. Positive diagnosis is setting by the presence of documented esophageal mucosal injury (esophagitis) or excessive reflux during 24 hours intra-endophageal pH monitoring. Medical treatment is efficient in acid suppression, but does not address the mechanical etiology, is too expansive and affect the quality of life of patients. Miniinvasive surgery was a boom in management of GERD, offering great satisfaction to patients, low costs and rapid social integration.

We present the role of surgery in GERD, therapy GERD which occur 85-93% control of reflux symptomatology, providing data from the literature on the techniques used, their advantages and limitations.

Ovarian cancer

Luciana Cristache, Costinela Lazăr, Ștefania Aldea, Claudia Rădulescu

Ovarian cancer represents one of the most common malignancies that affect women. It has a high death rate because the symptoms are nonspecific and is diagnosed in very advanced stages when the tumor has already evolved and metastasized.

The surgical treatment for ovarian neoplasm includes surgical intervention in early stages, laparoscopic exploration in order to staging and harvesting tissue for biopsy and procedures for tumoral mass reduction.

Surgery for breast cancer

Florina Aslan, Luminița Ștefan-Dragomir, Viorica Gogor

Through this study I want to make a presentation about the surgery and its indications for breast cancer.

Most women with breast cancer have some type of surgery as part of their treatment. Depending on the situation, surgery may be done for different reasons. For example, surgery may be done to: remove as much of the cancer as possible (breast-conserving surgery or mastectomy); find out whether the cancer has spread to the lymph nodes under the arm (sentinel lymph node biopsy or axillary lymph node dissection); restore the breast’s shape after the cancer is removed (breast reconstruction); relieve symptoms of advanced cancer.

There are two main types of surgery to remove breast cancer: breast-conserving surgery, mastectomy.

The study includes a short review about the breast cancer, describing the surgical procedure, its indications, surgical technique, incidents, complications, results, conclusions.

Bariatric surgery in the management of the cardiometabolic risk of extremely obese patients – case report

Tatiana Onofrei, Florentina Rogojină, Constanța Bandulea

Obesity has serious consequences on health and mortality. The general goals of every weight-loss program is to reduce and maintain a lower body weight, or prevent further weight gain in individuals who cannot lose weight. The treatment options are lifestyle modification (diet, exercise, and behavioral modification), pharmacotherapy and bariatric surgery. The three most commonly performed bariatric procedures are laparoscopic adjustable gastric banding, laparoscopic sleeve gastrectomy and the Roux-en-Y gastric bypass.

Objective: we present the case of a woman with extreme obesity, poor controlled diabetes mellitus – treated with insulin, dyslipidemia and coronary artery disease. She was submitted to bariatric surgery – laparoscopic sleeve gastrectomy. After surgery she lost more than 30 kilograms, with a good glycemic control on metformin only. Hypertension, dyslipidemia and her heart condition improved.

Conclusions: surgery is the most effective means of inducing significant weight loss in individuals with extreme obesity who are at greater cardiometabolic risk.

Longer the waist line, shorter the lifeline – obesity associated comorbidities

Nicoleta Mavriș, Aglaia Nedelcu, Florentina Rogojină

Overweight and obesity are associated with the development of a number of comorbidities and increased mortality. Hypertension, dyslipidemia, coronary artery disease, congestive heart failure, stroke, diabetes mellitus, non-alcoholic fatty liver, sleep apnea, gallstones, polycystic ovarian syndrome, osteoarthritis and cancers are more frequent in obese patients.

Objective: to evaluate the prevalence of comorbidities in obese patients admitted in our department during the last 5 years.

Results: diabetes mellitus, cardiovascular and cerebrovascular diseases are the most frequent obesity associated comorbidities. These diseases have a major impact on mortality.

Menopause – between physiology and pathology

Andreea Serafim, Ancuța Istrate, Carmen Miron

Menopause and menopausal transition are physiologic, but symptoms are frequent and often require medical attention. Approximately 60% to 80% of menopausal women experience hot flashes at some point during menopausal transition. They are more likely to develop anxiety symptoms at perimenopause and postmenopause and depressive symptoms or clinical depressive disorders in late perimenopause. Sleep disturbances are frequent.

Vaginal dryness and genital atrophy can cause sexual dysfunction. Lately, postmenopausal osteoporosis leads to increased morbidity and mortality as well as an important reduction of the quality of life as a consequence of fractures.

We present our experience with menopausal patients seeking medical attention in our department.

Macroprolactinoma in a diabetic patient with morbid obesity

Daniela Buzatu, Nicoleta Mavriș, Nicoleta Vale

Prolactinomas are prolactin secreting pituitary tumors that can occur between both sexes „the men’s unlike women’s macroprolatinomas predominates”. Clinical manifestations are given of prolactin hypersecretion „galactorrhea and gonadal dysfunctional”, related to tumor mass effect compression (neurologic and visual disturbances) and impairment of the other pituitary function.

We present a case of a 36 years old patient with macroprolactinoma and with 2 diabetes and morbid obesity, who has been presented for metabolic imbalance and visual disorder. The clinical exam was galactorrhea; disturbances of ocular motility and ptosis right eye, reduced visual acuity left eye; obesity (BMI = 42 kg/m^2), erysipelas right leg. Endocrine profile: prolactin, FSH, LH, TSH, serum cortisol, ACTH, testosterone, showed: hyperprolactinemia (5960.62pg/ml) and hypogonadotropic hypogonadism. CT and MRI brain showed a voluminous sellar mass by 42.5/39/32 mm, with invasion of right cavernous sinus; optic chiasma and right orbital apex compression. The patient started treatment with: dopamine agonist drug – carbegolin in high dose and testosterone, treatment hypogonadotropic hypogonadism, treatment for diabetes mellitus for erysipelas.

After 1 month has a good evolution with remission the visual acuity (left eye), MRI – showed a reduction of 1 cm pituitary tumor size, and prolactin decreased to 2950 pg/ml.

Nurse involvement in breast cancer prevention

Margareta Popa

In the last 20 years, breast cancer failed to be an incurable disease, being considered lately as a chronic disease, a curable disease and more important a disease which can be prevented.

Unlike other types of cancer, where a healthy style of life can prevent 70-80% of diseases, breast cancer has been always an exception.

There are many factors of risk coming into being breast cancer, factors we cannot control by our decisions: age over 50, blood relatives suffering from this disease, other breast previous diseases, excessive irradiation of mammary gland, precocious puberty (the first period before the age of 12), the late menopause (after 55), age of 30 for the first pregnancy, no birth, no nursing, contraceptive pills usage in the last 10 years, therapy for estrogen substitution, made more than 5 years in the last 10 years.

Nurse can have an important role in this, explaining all the risks the patients are exposed by ignoring the physician's advice regarding breast cancer prophylaxis.

Ignorance, fear of diagnosis, lack of sanitary education, lack of determined programs for prevention and screening, in our country, lead to discover the diagnosis in an advanced stage of the disease, when the treatment is useless. In this case the patient sufferance is enormous.

Breast cancer is a disease which can be activated no matter when, to whom and no matter social status or how rich you are. We cannot blame God, the people around us, or us for this disease. In our era, in 21st century, breast cancer is a curable disease, especially if it is traced in an early stage.

Physicians as well as nurses are involved in the fight against breath cancer, and our task, the nurse's task is to penetrate the science, as close as she can, to the patient heart and conscience, offering them the strength for fighting against the disease and beating death.

OP session NS5

Infections with human papilloma virus (HPV)

Cristina Râșnoveanu

Human papilloma virus (HPV) is a very common virus that is spread through sexual contact.

The majority of sexually active people (about 75%) will have a HPV infection sometime during their life.

Most HPV infections go away by themselves. However, some „ high risk" types of HPV might cause changes in cervical cells that might lead to cervical cancer.

With proper screening and early detection, cervical cancer has a high rate of cure. In order to diagnose early stages of cervical abnormalities we perform cervical smear (Pap-Test), HPV genotyping, colposcopy and guided biopsy.

Prevention and treatment of cervical cancer

Daniela Cuculea

Cervical cancer is a disease than can affect women of any age.

Cervical cancer is the second leading cause of cancer after breast cancer at women under age 45 years worldwide. Cervical cancer occurs more often at women who have started their sexual life earlier, living in an environment with poor intimate hygiene and those who have multiple sex partners.

Cervical cancer can be prevented by regular testing (PAP smear test) and by anti-HPV vaccination. Cervical cancer should be diagnosed intraepithelial carcinoma phase, thus reducing the prevalence of invasive cancer and mortality.

It easier to detect cervical cancer than to treat.

Decentralizing public health service

Gabriela Amoașei

Once the society started to develop it has emerged the notion of public service.

The notion of public service is legal conceptualized by Article 4, paragraph c, of Law no. 178/2010, according to the public service is '' all actions and activities which ensure the needs of general public interest or local collectivities."

The public service is organized and operates according to principles, namely: the principle of continuity; the principle of equality; the principle of adaptability.

Under these principles enters public health service, because this is vital for the community and must be provided continuously and permanently to every individual person regardless of nationality, age, gender, religious beliefs.

For a proper functioning of public services it has developed principles decentralization. Decentralization has occurred due to the fact that no matter how strong is the government, would not have a clear picture of all the problems facing the local community in each hand. Local communities, enjoying autonomy manage to identify best which are the needs of society and allocate the necessary resource where needed.

The legal provisions for decentralization of public service are found in the Article 195/2006, timber whom "Decentralization is the transfer of financial and administrative powers to the provision of public services from the central public authorities at the local government or the private sector.”

In 10th of May 2009, the Government adopted the Strategy on decentralization in the health system. Decentralization in health care law provides for the establishment of The Regional Institutes of Emergency under the Ministry of Health, which provides that certain emergency hospitals will go into the structure of these institutes. In exchange, the hospitals and the public health departments, converted in Directions of Nursing, will be transferred to the administration of the local authorities.

After decentralization, the county hospitals will pass administered by county councils, the municipal administration ones into the municipal councils, and town and village hospitals in the local council administration. Subordinated to Ministry of Health will remain national research institutes, clinical institutes, regional hospitals and some hospitals declared of national interest, where advanced medical acts performed.

Two of the principles underlying the strategy to achieve the objectives of health refers to placing the patient, and citizens in general, in the center of the health system, along with the responsibility for the decision makers before it. This implies a major change in the decision-making mechanisms and accountability, so that the decision can be taken as close to where they are provided and used health services. In this way ensures a better fit for the health needs of the population along with direct accountability to those who take decisions in the community.

Simultaneously central structures, particularly the Ministry of Health which is retreating from local management, can focus on strategic functions, i.e. development of sectoral policies, supervision and guidance of the entire system, including outside activities that impact on health and developing cross-sectoral cooperation mechanisms and structures. This requires increasing the institutional capacity of the Ministry of Health, to meet these new roles and develop new structures at central and local level, along with the modification status of health units.

In this context it provides a strategy of decentralization of healthcare that responds to the goals of the Government Program and conductive to achieve effective right to healthcare provided by the Constitution. In analyzing the decentralization of the health units, there is a greater interference of politics in the act reorganization, so we can appreciate the decentralization of hospital administration as a win for Romania.

In conclusion, decentralization is a major challenge for healthcare delivery in general. The active involvement of managers in developing reforms based on decentralization, standardization of financial allocations, international norms and standards and impact monitoring is essential to ensure equity and quality medical services and to improve efficiency.

Liver's tumors

Imola Grigore, Liliana Badea, Mihaela Moscaliuc, Carmen Florea

Definition : It's the most frequently form of primary hepatic tumors (80-90%); for children is on second place after hepatoblastoma; it's more frequently met on men than women; it can be developed either on a normal liver or a cirrhotic liver (B or C virus)

The liver's anatomy: the largest gland in human body; it is formed by two lobs (left and right) and about eight segments

Favorable factors: consumption of anabolic steroids, cirrhosis, B or C hepatitis, low immunity, obesity, smoking (especially to people who is infected to B or C hepatic virus), poisoning with arsenic.

Signs and symptoms: loss of weight, loss of appetite, strong pains in the upper abdomen, nausea and vomiting, weakness and general fatigue, grown liver, swollen abdomen, whitish feces

Etiology: most frequently met: chronic liver disease and cirrhosis (about 60-90%), chronic hepatitis with B virus, chronic hepatitis with C virus, chronic hepatitis with both viruses, chronic hepatitis with alcohol intake etiology, negative viral markers; most rarely met: hereditary hemochromatosis, porphyria, autoimmune hepatitis, primary biliary cirrhosis, Wilson disease, steroid oral contraception.

Diagnostic: blood analyses, imagistic tests (CT, MRI), and liver’s biopsy.

Treatment: surgical (partial hepatectomy), liver transplant, cryoablation and thermotherapy, chemoembolisation, radiotherapy, pharmaceutical treatment (with Soranfenibul).

Nurse's role: takes biological samples, prepares the patients for radiology test, abdominal punction, laparoscopy liver's biopsy and paracentesis, has to recognize and to watch for evolution and/or complication (hepatic coma or bleeding coma), and has to announce the doctor for any change or suspicions

Prevention: moderate alcohol consumption, to maintain the weight, vaccination against B/C viruses, personal hygiene measures.

Never use already used needles.

Communication in medical facilities

Nicoleta Voinea, Cristina Soare, Lucica Poenaru

Communication in medical facilities is a key component of public health and of the health care system. It comprises: patient-centered communication, communication between members of the same team, bedside manners (the ability of communicating with the patient), communication in emergency situation, communication in conflict situations.

We may consider that the three pillars of effective communication are: clear communication of the message, the patient’s perfect understanding of what he is told and the patient’s feeling that he is respected by the doctor.

Team communication is also very important, because members of a team working in a proper work environment can easily achieve their goals and have good results.

The best communication skills are: attention and sensitivity, empathy, respect, support and promoting partnership.

Emergency situation communication is critical and complex due to the dynamic and high level of stress atmosphere.

Conflict-situations communication is often difficult due to cultural differences and different perception of others and of the environment.

Communication in medical facilities is essential and leads to improvement of the population’s health state.

Osteoporosis

Elena I. Vasilăţeanu, Daniela Cernat, Florentina Socol

Osteoporosis is a progressive bone disease that is characterized by a decrease in bone mass and density which can lead to an increased risk of fracture. It is a major health problem and it affects especially older people.

Fractures are the most dangerous aspect of osteoporosis. The most common osteoporotic fractures are those of the wrist, spine, shoulder and hip.

The disease may be classified as primary type 1, primary type 2, or secondary. The form of osteoporosis most common in women after menopause is primary type 1 which is the osteoporosis of the spongious bone or post menopause osteoporosis. Primary type 2 osteoporosis or senile osteoporosis occurs when the patient is older than 75 years and is seen in both female and males are ratio of 2:1. Secondary osteoporosis may arise at any age and affects man and women equally. This form results from chronic predisposing medical problems or diseases or prolonged use of medication such us glucocorticoids.

There are many illnesses which can lead to osteoporosis:

- Endocrine disorders – hypothyroidism, type 1 diabetes;
- Hematologic disorders – multiple myeloma, leukemia;
- Gastrointestinal disorders – malabsorption, primary billiard cirrhosis;
- Medication – L thyroxin, Heparin, Methotrexate;
- Rheumatologic disorder – rheumatoid arthritis, ankylosing spondylitis;
- Smoking, excessive alcohol consumption.

The diagnosis of osteoporosis has few clinical signs but can be diagnose using: Conventional radiography, measuring bone mineral density. Dual-energy X- rays absorption – the gold standard for the diagnosis of osteoporosis.

Medication: drug for osteoporosis are useful in decreasing the risk of future fractures by increasing bone synthesis and decreasing reabsorption:

Fosamax – once a day at the beginning, and then once a week, and then once a month;

Bonviva – once a day;

Fosavance – contains fosamax and alfa D3;

Prolia – can be helpful for preventing osteoporotic fragility fracture and decreasing the risk of vertebral and non-vertebral fractures.

Nurse perception of bispectral index monitoring during general anesthesia

Mariana Ilie

Introduction: Bispectral index monitoring system (BIS) is a technology used to monitor depth of anesthesia.

Material: BIS monitoring is used to measure the effects of anesthetic and sedative drugs on the brain and to track changes in the patient's level of sedation and hypnosis. BIS monitoring provides information clinically relevant to the adjustment of dosages of sedating medication.

Method: The BIS monitor provides a number, which ranges from 0 (equivalent to EEG silence) to 100. A BIS value between 40 and 60 indicates an appropriate level for general anesthesia.

The essence of BIS monitoring is to take a complex signal (the EEG), analyze it, and process the result into a single number. When a patient is awake, the cerebral cortex is very active, and the EEG (electroencephalography) reflects vigorous activity. When asleep or under general anesthesia, the pattern of activity changes.

Results: The advantages of using BIS monitoring are numerous, including a decrease in time to extubation, use of hypnotic anesthetic drugs, incidence of nausea and vomiting, and occurrence of intraoperative awareness.

It can help the nursing personnel in preventing under- and over sedation during general anesthesia.

OP session NS6

Spinal injury nursing assistance

V. Rădăuceanu, Stela Platon

Spinal injury is a spinal cord or trauma accompanied by total or partial compromise of the affected spinal segment function. Such deficiencies are installed and vegetative, motor and sensory reflexes.

Medullary tissue destruction is caused by trauma or disease of spinal cord injury spinal (accidents, falls, blows to the spine) acting in tissue degenerative medullary (transverse myelitis, multiple sclerosis etc) compression by tumors, vascular disorders, thromboembolism.

Platelet rich plasma PRP

Maria Sandu

PRP is the acronym for Platelet Rich Plasma. It is a concentrated source of autologous platelets containing several different growth factors that can stimulate healing of bone and soft tissue.

PRP is made from patients own whole blood. The whole blood contains plasma, red blood cells, white blood cells and platelets. Platelets are small discoid cells that contain clotting and growth factors. Blood typically contains 6% platelets whereas PRP has a significantly increased platelet concentration. Although this level can vary depending on the method of extraction and equipment, studies have shown that clinical benefit can be obtained if the PRP used has an increased platelet concentration of 4 times greater than normal blood.

The product is obtained by collecting a small amount of whole blood from the patient which is afterwards centrifuged several times (depending on the method). The finite product (platelets enriched plasma) is then injected in the chosen area.

PRP is, in fact, an old therapy used in orthopedics, dentistry or sports medicine.

But only in the recent years has become well-known for the large public especially for its use in aesthetic medicine and surgery (the famous “vampire therapy”).

Inhaled medication

Adriana Chiru, Marinela Matei

The efficiency of a medication depends in equal measure, the preparation bioavailability and patient adherence to prescribed rules for its management.

If the oral preparations (solutions, tablets, etc.) the administration is relatively simple and well known, there are cases in which the patient must receive prior notice and sometimes even a practical demonstration to learn the correct administration of the product prescribed.

Inhaled pharmaceutical preparations, widely used for the treatment of acute or chronic respiratory diseases are a good example in this respect. Using the airway allows the administration of active substances with sympathomimetic bronchodilators such action mainly local or anticholinergic inflammatory or fixed combinations of those mentioned.

Inhaled medication has two major advantages: act very fast, very shortly after the drug reaches the bronchi; required much lower doses with fewer side effects, the same medication administered in tablets or injections.

There are several types of devices for administering drugs by inhalation such as sprays, dry powder inhalers, nebulizers.

Whatever type of device indicated for administration of inhaled medication, the patient must know exactly how to use it. Considering that the same active substance may be conditioned for various types of devices (e.g. Salbutamol - pressurized inhaler salbutamol – for nebulizer solution) to release the prescription the patient must receive all information regarding the administration technique. Careful reading of the instructions, followed by sequencing simulation management practice leads to learning the correct use of these devices and thus ensuring therapeutic efficiency act.

Nursing interventions in obstructive sleep apnea

Cristina Radu, Gabriela Drilea, Mariana Lazurcă

Sleep apnea is a breathing disorder and it occurs during sleeping. The condition is characterized by stoppage of breathing for ten seconds or longer and at least five times per hour. Sleep apnea is becoming a major health problem all over the world. Officially, about 800.000 adults in Romania have sleep apnea and in the same time many people may have the illness but they don't know it.

The nurses have an increased responsibility in the identification the symptoms, education and management of patients who are suffering from sleep apnea.

CPAP (Continuous Positive Airway Pressure) is the most effective form of treatment for sleep apnea but intolerance and incomplete compliance has made it difficult for efficacy.

The most common interventions are related to CPAP equipment and physical, psychological and social-economic side-effects. Some important aspects of nursing interventions are referred to change the patient’s lifestyle because many of them are obese, smokers.

Nurses' awareness and good knowledge help to increase the compliance to the treatment and minimize the side effects because can be a real risk that a patient with a negative experience may not persevere with the treatment plan for long-term period.

Sputum prelevation in tuberculosis diagnosis

Simona Bran, Mădălina Preda, Mihaela Mirea

Sputum represents all that is expelled secretions in the airway by coughing. Sputum represents the best product for finding Koch’s bacillus in the diagnosis of pulmonary tuberculosis.

Harvesting sputum in the morning, before eating or drug administration gives the highest rate of positivity in the diagnosis of pulmonary tuberculosis, even in patients who don’t cough and cough up a little. Getting positive diagnosis in Tuberculosis increases if 2-3 sputum samples, collected within 24-48 hours, are examined.

In people who do not cough, or swallow the sputum, special techniques are applied to produce and collect sputum: aerosol, larynx - tracheal lavage with sterile saline, gastric tubing using probes Nelaton or Einhorn, bronchial aspirate or broncho-alveolar lavage by fibrobronchoscopy.

A properly pathological material consists of a recently removed from the bronchial tree, with minimal amounts of oral or nasal secretions, and of satisfactory quality on the presence of a mucoid or mucopurulent material in an amount of at least 1.3 ml.

Transport to the laboratory must be immediately after harvest or maximum 24h after.

What are the potential complications of bronchoscopy?

Tatiana Lazăr, Mirela Barbu, Luminița Atudori

Bronchoscopy is a procedure during in which an examiner uses a viewing tube to evaluate a patient's lung and airways including the larynx, trachea, and many branches of bronchus.

Bronchoscopy is usually performed by a pulmonologist or a thoracic surgeon. Although a bronchoscope does not allow for direct viewing and inspection of the lung tissue itself, samples of the lung tissue can be biopsied through the bronchoscope for examination in the laboratory.

Bronchoscopy can be used for diagnosis or treatment.

Complications of bronchoscopy are relatively rare and most often minor. It is important to realize that all procedures may involve risk or complications from both known and unforeseen causes, because individual patients vary in their anatomy and response to medications.

Therefore, there is no guarantee that a procedure can be free of complications. The following is a list of potential complications: vocal cord injury, irregular heartbeats, lack of oxygen to the body's tissues, heart injury due to medications or lack of oxygen, bleeding from the site of biopsy, punctured lung (pneumothorax), and complications from pre-medications or general anesthesia.

Exacerbation of chronic obstructive bronchopneumopathy

Vasilica Ionescu, Mariana Tănăsescu, Doina Andrei

Obstructive chronicle bronchopneumopathy is an illness characterized by the limitation of the airways` airflow, incompletely reversible, limitation which is usually progressive and is accompanied by a systemic and pulmonary abnormal inflammatory answer regarding harmful particles or gases, especially the cigarette smoke.

COPD exacerbation is defined as an acute event in the natural evolution of the illness characterized by the modification of the dyspnea degree, of the cough and/or of the patients` expectorations, besides the daily variations which lead to the modification of the medication.

Exacerbations are important events during the evolution of the illness because it affects patients` life quality in a negative way, it requires weeks of recovery, accelerates he declining rate of the pulmonary functions, and are associated with a high rate of mortality, especially for those who need hospitalization which consequently implies high economic and social costs.

The treatment of exacerbation consists in oxygen therapy, inhalator bronchodilators administration with the help of a spacer or nebulization, oral corticosteroids or i.v. and antibiotics when there are signs of bacterial infection.

Patients` education represents an important method to improve their capacity of understanding this illness, to successfully make them give up smoking, to make them understand the aspects of their changing health in order to easily deal with exacerbations.

Minimally invasive repair for pectus excavatum

Emilia G. Raicu

Pectus excavatum is an abnormal development of the rib cage in which the breastbone (sternum) grows inward, which causes a sunken chest wall. Sometimes called "funnel chest," pectus excavatum is often present at birth (congenital) and can be mild or severe. If left untreated, pectus excavatum can sometimes cause compression (pressure) of the heart and lungs.

Pectus excavatum occurs in approximately 1 out of 400 children and is three to five times more common in males than females and it is diagnosed at birth or in the first year of life.

Genetic predisposition and stuffy sternum is sometimes associated with scoliosis, Marfan syndrome, Poland Syndrome, Noonan Syndrome, Turner Syndrome, Marfan syndrome.

The actual process of correction is minimally-invasive procedure, invented in 1987 by Dr. Donald Nuss for treating pectus excavatum.

After the Nuss procedure, children will return to school after two – three weeks and once fully recovered can return to daily activities.

Nuss procedure is an effective method with good aesthetic and functional results.

Preparing for digestive endoscopy

Aurica Avram, Mariana Moldoveanu, Monica Nicolescu, Daniela Tanasov

Preparing for digestive endoscopy includes general measures, necessary for all the endoscopic procedures and specific measures, related to the examined segment and the endoscopic procedures provided.

The first step in preparation is to establish the indication for the investigation.

Depending on the indication, the nurse is preparing the endoscopic accessories needed- biopsy forceps, polypectomy loops, variceal banding devices, etc. The discussion with the patient needs to address investigation purpose, description and estimated duration, the risks and benefits. An informed consent needs to be signed.

Before and during endoscopic procedures, medication may be used to diminish gastrointestinal motility, decrease the patient's anxiety or discomfort, and provide amnesia of the stressful intervention.

Before starting endoscopy, the medical personnel needs to be prepared to monitor vital signs (heart rate, oxygen saturation, blood pressure), especially in patients with cardiorespiratory pathology.

As a specific measure for the upper gastrointestinal endoscopy, patients shouldn’t ingest any solids for at least 6 hours before the procedure. For the colonoscopy, the colon needs to be cleansed of fecal material before the examination.

An oral purge with 2 to 4 liters of electrolyte lavage solution, after a short period of dietary restriction, and in some cases followed by enemas, results in adequately prepared colons.

Issues in caring for patients with gastrostomy

Iuliana Ghinea, Corina Marcovici, Petrica Gheorghe

Endoscopic gastrostomy is an intervention consisting in the creation of a direct link between the gastric cavity and the external environment through a catheter insertion.

Gastrostomy is a technique of nutrition "in- extremis", which in limited situation such tumors of the head and neck, radiation therapy of the head and neck region, head and neck trauma, nutritional difficulties after stroke, demyelination disease, dementia, allows life to continue, and leads to a relative normality feeding patient with fragmented, soluble food. The feeding tube can be temporary or permanent, depending on the reason of the intervention.

Innovation and continuity – attributes of gastrostomy – two words that apparently can't be places in the same sentence – are working together this time. The innovation in medicine through this technique supports life patient to continue his living and give back the person to society. The individual may conduct his existence in the familial environment and not laying on a hospital bed connected to infusion and nasogastric feeding tube, waiting for a lifesaving surgery or the end.

The endoscopic gastrostomy was first performed in 1980 by Gauderer and Ponski, and has the following advantages versus the surgical gastrostomy: short mounting time, elimination of general anesthesia, it lowers the risk of complication and has a good rapport cost/efficiency. It has a success rate of 95% of the cases.

The medicine comes again with solutions to the need of feeding and hydration the patient and the role of medical team is to ease the patient's situation with such problems for short or long term.

OP session NS7

Erysipelas – dermatologic emergency

Liliana Lăța, Dana Papa

Definition: Erysipelas is a localized skin infection caused by bacteria (β hemolytic group A Streptococcus, Staphylococci).

Pathogenesis: Skin infection can occur due to: a breach in the skin barrier (wounds, fungal infections, burns, venous leg ulcers); an infected nearby mucosa (pharyngitis, rhinopharyngitis, and suppurative otitis media); lymphatic dissemination (streptococcal lymphangitis); hematogenous dissemination – exceptionally (septicemia).

Symptoms: sudden onset with fever (39-40 degrees Celsius) and chills; erythematous plaque of erysipelas appears 6 to 12 hours from onset.

How should the nurse manage the case: immediately after recognizing the signs of the infection, let the doctor know in order to plan the therapeutic management; meanwhile, the nurse applies a protective wet dressing with antiseptics locally (Rivanol, 2% boric acid solution); the nurse should administer antipyretic medication and closely monitor the patient's temperature, pulse, blood pressure and the overall health state; explain to the patient what is going on, that the temperature and chills are part of the disease's clinical manifestations and what the possible future reactions are; educate the patient regarding the body's hygiene.

Sepsis – nurse's role in managing septic patient

Liliana Mocanu, M. Stanciu, E. Fărcășanu, C.B. Teușdea, S. Dogaru

Sepsis is a main presentation reason for reporting to the emergency department and hospital admission, being one of the major causes for death in non-cardiac intensive care units. Costs are increasing and reported incidence is higher than ever due to longer life span. Sepsis survivors have often long-term physical, psychological and cognitive comorbidities.

Time is essential in this equation and fast response to organ dysfunction is a key component for sepsis management. Septic patient is always an unpredictable one and nurse's role is essential in diagnosis and treatment optimization.

What you need to be an emergency nurse within the Emergency Department

Liliana Mocanu, M. Stanciu, M. Fleican, E. Fărcășanu, M. Toma, C.B. Teușdea

A question is always is rise: the real emergency nursing seems with a television program? Anyone can do this job?

My answer is NO. The emergency nursing is unpredictable, dramatic, challenging, and requires a team effort. As will present the emergency nursing is for some of us, and clearly not for everyone.

What you need to be an emergency nurse within the Emergency Department? Consider the following factors: 1. know yourself, 2. must have good assessment skills, 3. strong knowledge base in every area of nursing, 4. Critical thinking and sharp decision making, 5. Good communication skills.

As the health care system and technology advance, there will always be a need for emergency care and emergency nurses.

Current standards for non-invasive assessment of liver fibrosis

Magdalena Chiriac, Veronica Niculiţă, Liliana Bulat

Chronic liver diseases of various etiology (viral, alcoholic, and metabolic) constitute a significant proportion of pathology seen in a gastroenterology department. By complications that arise in their evolution, chronic liver diseases are an important cause of morbidity and mortality and carry a significant burden for health systems. According to the latest epidemiological data, in Romania there is a high prevalence of hepatitis B and C infections, 4.2% and 5.6% respectively, but much of the chronic liver diseases are caused by chronic alcohol intake and recently there has been an increase in the frequency of fatty liver disease (encompassing steatohepatitis, steatofibrosis and ultimately cirrhosis).

For staging of the disease, assessment of fibrosis severity and guiding treatment decisions, the gold standard until a few decades ago was represented by liver biopsy. The maneuver is invasive, carries risk of complications (bleeding, infection, damage to nearby organs), has come contraindications (coagulopathy, severe thrombocytopenia, ascites) and evaluates fibrosis in only one point in the liver parenchyma; however, considering the heterogeneous distribution of fibrosis in the liver, biopsy may underestimate the overall hepatic fibrosis.

In this setting a particular interest emerged to develop non-invasive methods for the quantification of liver fibrosis and there are now several diagnostic tools available: algorithms based on serum markers (Fibromax), transient elastography (Fibroscan, Echosens, Paris) ARFI elastography (Acoustic Radiation Force Impulse imaging), shear-wave elastography and not least magnetic resonance elastography.

Fibromax is a combination of 5 noninvasive tests (Fibrotest, ActiTest, SteatoTest, NashTest, AshTest) to assess fibrosis, steatosis and necro-inflammatory activity of the liver. The method involves the collection of blood and dosing of some surrogate markers of fibrosis, which are introduced into an algorithm with high diagnostic accuracy for fibrosis.

Fibroscan is a method of elastography which involves the transmission of pulses by means of a transducer to the hepatic parenchyma, and assesses fibrosis based on the reflection speed of these waves transmitted to the liver. The method is simple, quick, non-invasive, repeatable and painless. In addition, the add-on module CAP (Controlled Attenuation Parameter) can also give an estimation about the degree of hepatic steatosis.

These tests are available in our clinic and there is a rich experience with their use in patients with chronic viral hepatitis (B, C), as well as in those with alcoholic liver disease. As the literature has shown a good concordance of these non-invasive tests with liver biopsy, they can be used with confidence for fibrosis evaluation and monitoring of patients with chronic liver diseases.

Hemoptysis

Marcela Brighiu, Camelia Militaru

Introduction: Hemoptysis is the expectoration of varying amounts of blood from the airways. Hemoptysis is manifested by eliminating blood through the mouth (mixed with air, so "frothy with mucus") from the airways.

Material and method: This paper presents the classification, etiology, symptomatology, differential diagnosis, treatment indications and the treatment for hemoptysis.

Discussion: It is desirable for the treatment of hemoptysis to be applied at the place where hemoptysis occurred, avoiding the transportation (jerks, agitation), whatever the means of transport at hand. Best for the patient is to sit quietly and not to move, unless he receives emergency healthcare or is transported to a medical facility. Transportation will nevertheless be required if the doctor considers that a medical intervention cannot be performed without an emergency and surgery service, equipped with the appropriate equipment.

Conclusion: Hemoptysis is a medical emergency, even in small quantity. Its evolution may be serious or even critical.

Clostridium Difficile infection – a nurse`s point of view

Mariana Bogriceanu, Elena Zanfir, Stela Belea

Introduction: Our objective is to review current guidelines in managing Clostridium difficile colitis and our department`s experience in this field.

Materials and methods: Clostridium difficile is a Gram-positive spore-forming anaerobe that was identified, beginning with 1978 as the causative pathogen in the majority of antibiotic-associated diarrhea and colitis cases. It is believed that a small percent of healthy individuals have this bacterium in their colon, but it is more often found in the ground, water, animal or human feces, C. difficile being a growing cause for nosocomial disease.

The most common risk factor for C. difficile infection is the use of antibiotics, as they can disrupt the normal microbial balance in the colon.

This bacterium is dangerous because it can secret two toxins (A and B) that cause inflammation of the colonic mucosae, which can lead to moderate diarrhea, pseudomembranous colitis (most frequent form) or fulminant colitis. If the physician suspects a C. difficile infection, it is the nurse`s task to explain to the patient how to collect a stool sample and take it as fast as possible to the laboratory.

After infection is confirmed, it is up to the nurse to assure contact precautions: isolate the patient with single use gowns and gloves being available at room entrance; environmental disinfection; explaining the importance of hand hygiene and of course making sure that the patient takes his medication correctly, according to the physician`s recommendations.

Results and conclusion: C. difficile colitis has become the most frequent infectious cause of healthcare-associated diarrhea, causing high morbidity and mortality, mainly because of the use of broad-spectrum antibiotics.

The team working in the recovery of hemiplegic patients after stroke

Ecaterina Dane, Natașa Corpodeanu, Laura Iosif

Stroke is a brain infarction in ischemia or hemorrhage leading to discontinuation function.

Stroke is installed suddenly and is characterized by a neurological deficit, hemiparesis or hemiplegia known as half a body, depending on severity. 80% of strokes occur in the carotid arterial distribution and consist of muscle weakness of a body part (face, arm or leg, in any combination).

The incidence of stroke has declined in recent years, despite the aging of the population due to changing lifestyle, use of antiplatelet and proper treatment of heart disease. Yet the incidence of stroke increases with age. Risk factors for the disease include age, high blood pressure (hypertension), heart deficiency, history of stroke, transient ischemic attacks, diabetes.

Post-stroke recovery involves using compensatory techniques for mobility, the use of daily activities or ADL (activities of daily living) and promoting communication. AVC limited prognosis for recovery is given by the following elements: severe memory problems, the inability to understand commands, medical or surgical instability, previous stroke, sphincter incontinence, visual spatial deficits.

Routine diagnostic evaluation include: CT scan (computerized axial tomography) of the cephalic extremity, electroencephalogram (EEG), MRI, carotid Doppler studies, assessment of heart (echocardiogram, EKG), blood tests routinely. Patients post-stroke recovery starting in the acute phase and the recovery post-stroke uncomplicated, stable medically is done using passive and the active mobilization helped (assisted daily), tilting the bed and identifying communication deficits. Subsequently, the patient is sent to physiotherapy or occupational therapy wheelchair. Inpatient practicing transfer activities (in bed in a wheelchair and back), the preparatory activities for walking, daily activity or practicing self-care ADL and coating, communications training, following a therapy that addresses swallowing.

The effect of application interference current in scapulohumeral periarthritis

Georgeta Matei, Margareta Bărgăoanu, Laura Iosif

Periarthritis scapular humeral is a clinical syndrome characterized by pain in the shoulder and by stiffness caused by degenerative lesions and inflammation of the tendons and exchanges of the second joint of the shoulder, accompanied by limited arm movement, no signs of damage to the joint scapular humeral. After rheumatologist French Seze describing these clinical forms: simple shoulder pain, acute painful shoulder, shoulder blocked, pseudoparalitic shoulder joint and shoulder. Treatment aims to relieve the pain, combat inflammation and fibrosis trend, to improve joint mobility. It includes: segmental rest, anti-inflammatory and analgesic drugs, hydrotherapy, electrotherapy analgesic, massage, physical therapy.

Electrotherapy physiotherapy is part of the physical agent that uses artificial electric current applied to the body, either directly or indirectly.

Medium frequency currents are AC (sinusoidal) whose frequency is the thousands of hertz (1000-50000) and tens of thousands of Hz.

Interferential current effects: excitomotor – on striated muscle; muscle relaxant – obtained by average frequency (12-35 Hz) especially variable frequency between 0 - 100 Hz; analgesic effect; action on smooth muscles excitomotorie;

Contraindications: The application of medium frequency currents in the chest region; Neoplastic processes (electro therapy represents an excitation, so worse malignant process); in acute inflammatory processes in first states, where the decompensated heart disease and hypertension age; in scapulohumeral rheumatoid disease, the patient is applied on the painful shoulder type plate electrodes that are introduced in size synthetic coating texture previously dampened appropriate, may also apply vacuum or suction cup electrodes.

After treatment with interferential current combined with a program of analytical kinesiology, joint pain and inflammation disappears in all cases.

Nursing essentials for patients with lung cancer

Niculina Predescu

Treating patients with lung cancer can give a nurse one of the biggest professional satisfactions, in spite of the disastrous evolution of this disease.

Novel surgical techniques made possible an increase of these patients' survival rate.

This paper describes the most important aspects in the nursery of these patients: anatomy and physiology of the respiratory system; diagnosis of lung cancer; treatment and surgical techniques; nursing of patients with lung cancer

Despite a sustained work for over 25 years, lung cancer still represents a terrible killer. This is the reason why the nurses are actively involved in the nursing team, their actions being mandatory in order to have a long lasting recovery for these patients.

The nurse's role in cardiac rhythm disorders management in the ED

Tatiana Pană, Beatrice Ibănescu, Steluța Petrescu, Geanina Durlea

The nurse's role in early detection of cardiac rhythm disorders is extremely important considering the fact that in most cases the nurse is the first person who comes into contact with the patient and also the one who manages monitoring.

It is crucial for nurses to be educated and able to assess the gravity of the situation, especially in the ER department where time is of the essence. Education of course starts in nursing school but must be continued throughout the whole career.

The purpose of the present paper is to make a review of the most important cardiac rhythm disorders, the most common that present in the ED and the symptoms that are usually associated with them as well as taking a look at the types of patients that require cardiac monitoring and the methods that we use to ensure their proper care.

OP session NS8

Romanian medical team experience in field operations

Vasilica Mihis, Liliana Mocanu, M. Stanciu, M. Fleican, E. Fărcășanu, C.B. Teușdea

Since 1991 the Romanian army participated in missions under UN auspices to create a new architecture for peace, cooperation and stability in the world. That's why a Romanian field military hospital participated in mission in Iraq during the first Gulf War in 1991, but only for a month.

The first test took place after two years, when the field Romanian military hospital participated in the mission in Somalia (UNOSOM II) which was intended to treat military personnel involved in the peace mission, but treated and civilians (approx. 80 % of patients) victims of civil war.

Medical teams have performed medical triage, primary survey, resuscitation, secondary survey, surgical interventions and intensive treated patients who had or were brought to the ED of the field Romanian military hospital.

Kaposi sarcoma patients nursing – a challenge for quality care

Ioana Moisei, Andreea Mihai, Irina Vaciu

Definitions: Kaposi sarcoma is an endothelial cells neoplasia presenting with multiple vascular nodules, affecting both skin and other organs.

Clinical aspects: Kaposi sarcoma may be classified in four types: epidemic (HIV infection associated), immune-compromised, classical (sporadic) and endemic.

Kaposi sarcoma appears as red-purpuric macules or papules/nodules at any level of skin or mucous membranes. Initially, the lesions are small, painless, but may evolve with ulceration and became painful. Usually first lesions appear at legs, most of the times associating edema.

Treatment: nursing interventions are based mostly on: pain monitoring (pain killer drugs administration and comfortable body positions) and skin integrity monitoring. Digestive and respiratory functions also must be monitored. The nurse also monitors chemotherapy and radiotherapy side effects (nausea, anorexia, diarrhea and dizziness).

Kaposi sarcoma may have a favorable evolution. Yet, internal lesions can promote bleeding, sometimes massive, obstructions or organ perforations, amputations.

Kaposi sarcoma is an incurable disease but that may be controlled.

Actinic keratosis – real danger of today?

Irina Vaciu, Lucuța Teșa, F. Pieptea

Definition: Actinic keratosis (AK), also known as solar keratosis, is a precancerous lesion of the skin, usually appearing on long time sun exposed skin, mostly involving upper and lower limbs, face, nose and ears.

Clinical aspects: actinic lesions appear as keratotic plaques, yellowish-dark, 0.5 to 1.5 cm in diameter, either plane or slightly elevated.

Pathologic examination may show any of the following aspects: hypertrophy, akantolysis, atrophy, Bowenoid aspect or pigmentations. It may also define malignant transformation in spinocellular carcinoma.

Nursing roles are: prophylaxis, education, diagnostic (signs of infection, irritation), topical treatment correct application.

Complications may be present: bleeding secondary to electrocoagulation treatment, hard to cooperate with patients (self-medication such propolis, plants, tinctures etc, that may complicate post-operatory plagues), extended and hard to reach areas.

A history of anesthetic practice

Tudora Băraru

The presentation have assayed to review briefly the history of anesthesia specialty and to define the scope of anesthesiology, which even seems secure, but not yet mature and fully differentiated. Anesthesia is considered an American invention, although anyone who examines history will understand that innovation of such significance can hardly have arisen spontaneously.

In addition to the concept of standard of care, another kind of standards has grown in evidentiary importance. Organizations such as European Society of Anesthesia and the Romanian Society of Anesthesia as well as hospitals and departments, promulgate standards and procedural manuals or directive.

Operations had been performed over the centuries but always for simple affections such as fractures, amputation, cataract extraction, trephination of the scuff, or removal of bladder calculus. After 1960, in our country, valuable anesthetic practice developed and even improved due to the technological progress of medical and monitoring equipment along with medical substances.

Cardiovascular diseases

M. Lefter, V. Scarlat, N. Vasile

The term ”cardiovascular disease” (CVD) is attributed to a multiple diseases affecting the heart and blood vessels, and among them an ischemic cardiomyopathy (IC), cerebro-vascular disorder, (high blood pressure) or peripheral arterial disease (PAD).

Other cardiovascular disease are myocarditis (heart condition causes rheumatic fever) and congenital heart disease (heart malformations present at birth).

CVD is associated with heart attacks (myocardial infarction), angina and stroke.

Obstructive sleep apnea

M. Lefter, Mirela Barbu, M. Ene

Obstructive sleep apnea, is also known as apnea-hypopnea syndrome and obstructive sleep (SAS) – is a sleep disorder that involves substantial diminution or cessation of airflow inspired.

This is the most common type of sleep disorder and is characterized by recurrent episodes of airway blockage during sleep for 10 seconds more, leading to significant reductions in blood oxygen levels.

As a result, shallow sleep or waking phase comes total increases muscle tone in the throat and larynx to restore patency.

Status is followed by a series of deep breaths, usually with loud snoring. SAS patients have increased risk of developing heart diseases, hypertension, diabetes, disorders of potency.

Daytime sleepiness, memory and attention disorders can cause a car or work accident.

Tongue cancer

Nicoleta Ghica, Dorina Dumitru, Valentina Budai

Among all etiological factors potentially existent in the western world, tobacco and tobacco derivate use is primarily associated with the tongue cancer.

Tongue cancer represents the most frequent malignant oral form, having a more prognosis, especially in cases which are not discovered in earlier stages.

Tongue cancer requires specific client investigations.

Inner portion mandible abscess

Camelia Preda, Andreea Badea, Nicoleta Călin

Submaxillary area is situated in the inner horizontal arm of the mandible, having the mylohioidian, hypoglossal muscles and the mouth floor mucosa as the upper and medial limits.

Below and downwards is limited by the superficial cervical fascia.

Inner portion mandible abcedates as a result of septic processes developed in the inferior molars.

Clinical manifestations induced by teeth recesses are extremely variated and the treatment may be either very simple, either very difficult sometimes, requiring large surgical procedures, interdisciplinary assessment, specific medical treatment and a close patient care.

Biological treatments in R.A. (Abatacept)

Liliana I. Predescu, Aura Mandachi, Gina Pană

Rheumatoid arthritis is an autoimmune disease of the connective tissue of unknown etiology with a sampler of about 1% in the general population. Featuring through a symmetrical synovitis erosive, deforming and destructive arthropathy by systemic and extra-articular manifestations (rheumatoid nodules). The evolution of chronic, fluctuating disease, untreated can lead to progressive joint destruction, deformity accompanied by permanent joint with a motor deficits and reduction of life expectancy.

The advent of biological therapy within rheumatological was the beginning of a new era because the treatment had on hand until then it was limited.

Abatacept in combination with methotrexate is indicated for the treatment of active moderate/severe R.A. on adult patients who have insufficient response or intolerance to other disease-modifying anti-rheumatic drugs.

Abatacept (SC): each pre-filled syringe contains 125 mg of abatacept in 1 ml solution for injection. The content must be clear, colorless to pale yellow and is administered once a week.

Keep in the original packaging, protected from light, refrigerated (2-8° C), do not freeze. The liquid level must be above the line of control.

Preparing injection site: 30-60 minutes prior to administration remove the pre-filled syringe from refrigerator and leave at room temperature. Do not use heat sources to accelerate the heating of! Avoid region diameter of 5 cm around the navel. Do not inject into areas where the skin hypersensitivity, damage or crusting, swelling, tissue endured.

Choose a different place for the next administration injections (at least 3 cm from the previous).

Wipe in a circular motion the place chosen for injection with a pad soaked in rubbing alcohol and allow to dry.

Undesirable effects: headache, nausea and upper respiratory tract infections, fatigue, asthenia, erythema, pruritus, dyspnea.

OP session NS9

Schizophrenia

Lidia Dobrinescu, Cecilia Bizon

Schizophrenia involves a range of signs and symptoms including disturbances in sense of self and interpersonal communication. Deficits in functioning are observed in daily living family life, social interactions and employment.

Schizophrenia is an endogenous psychosis that most commonly occurs in late adolescence and early adulthood (15-40 years) affecting the self-identity, social interactions and interpersonal relationships.

People diagnosed with schizophrenia usually experience a combination of positive symptoms (hallucinations, delusions, transparency-influence syndrome), negative symptoms (low energy, back of emotions, poor or nonexistent social functioning) and cognitive symptoms (disorganized thoughts, speech and behaviors).

Continuous signs of the disturbance persist for at least 6 months. The course of schizophrenia is typically marked by alternating periods of remission and relapse. Episodes of relapse have significant implications both in terms of cost of health care as the personal implications of loss of functioning for the individual.

Schizophrenia is often described in terms of positive, negative and cognitive symptoms.

Positive symptoms include: transparency-influence syndrome, hallucinations, and delusions.

Negative symptoms include: apathy, depersonalization, ambivalence, social withdrawal, disorganized thinking and behaviors, deficits in attention, working memory, deficits in processing speed, verbal fluency, disorganized thinking: derailment or loose associations, abnormal social behavior; suicide attempt, homicide pyromania; theft.

The panic attack and panic disorder

Nicoleta Ene, Valentina Dinoiu, D. Vasilache

The panic attack is a sudden burst of fear or anxiety (an affective state which is characterized by psychomotor restlessness and unknown fear, without object) which causes patients alarming symptoms, but is not life threatening: accelerated heart rate, difficulty to breath, a feeling of losing control or imminent death. Usually, it lasts from 5 to 20 minutes, and can be caused by stressful situation or it can appear unexpectedly.

Panic disorder are diagnosed when a person has repeated panic attacks, is concerned about having a new one, and so avoids places that could trigger one. There is a possibility that a person could have panic attacks without it becoming a panic disorder, these attacks appear with anxiety disorder.

Causes: the exact cause of panic disorders is unknown. It is believed that is because of a chemical imbalance in the brain (neurotransmitters).

Risk factors: a history of panic disorders in the family, alcohol consumption, mitral valve prolapsed, previous sudden panic attacks.

Symptoms: the most important symptom of all is feeling of overwhelming fear and anxiety, along with other physical symptoms.

Pathophysiological mechanism: panic disorder can be diagnosed after some sudden panic attacks and continuous fear.

Treatment: initial treatment, maintenance treatment, ambulatory treatment.

Prophylaxis: panic disorders cannot be prevented. The bare avoidance of situation and places cannot guarantee that a panic attack will not happen under new circumstances.

The benefits of breast feeding

Ionela Brăiloiu

For most of us, breast feeding is one of the earliest events that helped determine our health for the rest of our lives. And while this is in the past for us and cannot be changed, it is yet to be determined for today's and tomorrow's babies. In this presentation, we look at the importance of breast feeding, the proper ways to breast feed including the correct positions for the mother and the baby and dietary tips for the mother.

We also discuss the medical and social benefits of breast feeding for the mother and for the baby, as well as pointing out problems that may arise during breast feeding, together with methods of avoiding or diminishing their impact. The first months of a baby's life is one of the most important periods for him or her, and also for the new mother – let's do it the right way.

Zika virus

Roxana Vasilescu

Zika virus is a mosquito-borne illness that is spread by the two Aedes species of mosquito – Aedes albopictus, known as the Asian Tiger mosquito, and the Aedes aegypti species. This species is mostly active during the day. These mosquitos can survive in both indoor and outdoor environments.

Zika virus cases typically occur in tropical climates such as Brazil, Colombia, Paraguay, Suriname, Venezuela and French Guiana. The World Health Organization (WHO) declared that the Zika virus outbreak constituted a Public Health Emergency of International Concern on 1 February 2016.

Signs and symptoms of Zika virus are vague and can last for up to a week. Diagnosis of the virus is typically confirmed with a blood test.

Zika infection can spread from a mother to a fetus during pregnancy. What is also of great concern is that the virus can be transmitted by sexual intercourse.

A growing concern that is currently under investigation is a possible link between maternal Zika virus infection and infant microcephaly. Brazil in particular has seen a surge in infants born with microcephaly.

At present, there is no special treatment for the virus and avoiding mosquito bites is a key aspect of prevention. There is currently no vaccine to protect against the disease

Romania's Health Ministry has received a confirmation of a three cases of Zika virus infection of a Romanian citizens outside the country: a young women coming from Martinica, a 10 year-old boy who lives with his family in French Guyana and a men return from Dominic Republic – people traveling to areas with Zika.

Global alert about Zika virus requires special measures in Romania. Institute of Public Health has asked all municipalities to take a combination of methods to prevent and control mosquitoes that spread viruses.

Doppler ultrasound in thyroid pathology

Daniela Zavragiu, Paula Sima, Cătălina Bejan Borangic

Ultrasonography or Ultrasound is a method of imaging examination that uses ultrasounds reflected by the human body as a vector of medical information.

The Doppler Effect is a physical effect described by Christian Andreas Doppler (1803-1853) in 1842.

This effect defines the wavelength behavior of the incoming wave when the wave source and the receiver wave are moving in relation to each other.

The Doppler effect is used both in radioastronomy to study celestial bodies moving with the aid of electromagnetic waves, and in industry and medicine where applications relate primarily to sound waves. In the human body the target being studied using the Doppler Effect is the blood flow.

The Doppler method in thyroid pathology allows for the assessment of the hipervascularity of the thyroid parenchyma, the absence or presence of arterial vascular signal at nodular level. The type of vascularization can also be used to guide treatment. Ultrasonography (ultrasound) opens new perspectives in evaluating the Doppler imaging of the thyroid.

Thyroid scintigraphy

Mariana Ciaușescu, Eugenia Rusu, Veronica Nicolau

Thyroid scintigraphy, nuclear medicine technique, is one of the oldest methods of imaging diagnosis, being in clinical practice from early 60’s. This technique is based on the thyroid property to extract from the circulatory system the administrated radiopharmaceutical and up taking it by an active transport mechanism, without involving it in the iodine intrathyroid cycle or up taking and processing it by the thyroid follicles.

By detecting at the surface the radiation emitted by the radiotracer can be assessed both the morphology (dimensions, localization, shape) and the thyroid gland function (overall and potential nodular structures). Thereby, the assessment of the morpho-functional variants of normal thyroid include: position (retrosternal, laterocervical or sublingual), the number of thyroid lobes (accesory lobe, lobe agenesis), the gland’s shape (oval, U-shaped or V-shaped) and possible radiopharmaceutical uptake asymmetry (preferential uptake in only one lobe).

Thyroid scintigraphy is usually performed with Tc99m-petechnetate, it implies the intravenous administration of a minimal quantity of radiotracer (with a minimum of radiation and after about 20 minutes from the administration, the acquisition of an thyroid gland image is performed (which takes about 5 minutes).

The thyroid scintigraphy’s indications are multiple:

The assessment of thyroid tissue function:

a) Diffuse or nodular goiter;

b) Function assessment of an singular nodule (cold, hot, fiery);

c) Indication of possible function autonomy of a fiery nodule;

d) The detection of ectopic thyroid tissue (lingual, substernal);

e) The assessment of the remaining thyroid tissue after a subtotal thyroidectomy, radioiodine treatment or cervical lodge irradiation.

Challenges in accurate positioning for head and neck cancer patients in 3D-conformal radiotherapy

A. Toma, N. Bocănială

Objectives: Radiotherapy is part of the multimodal treatment of head and neck cancer. Accurate positioning of the patient during CT simulation and treatment is essential, since a lot of critical organs surround the tumor target volumes. We try to identify the most frequent challenges in positioning our head and neck cancer patients during radiotherapy, as the setup can result in underdose the tumor or overdose the critical organs.

Methods: We start 3D-conformal radiotherapy in Radiotherapy Department, Carol Davila Central University Emergency Military Hospital in 2014. We use a Siemens CT Simulator for treatment planning and Unique Varian linear accelerator for treatment, and for positioning Aquaplast RT mask (Q-Fix, USA) custom-made by simulation. We analyze 12 patients with various head and neck cancers, treated in our department in 2015-2016 and in which cases we found image-guided setup errors during radiotherapy and need a second CT simulation.

Conclusions: The most frequent causes of inaccurate positioning during radiotherapy for head and neck cancers are: neck edema, tumor growth or important tumor regression, evolution of cervical adenopathy, patient cachexia.

OP session NS10

Pharmacotherapy in major depressive disorder

A. Meliaca, Stelica Fărcășanu Zoica

Depression is a major psychiatric disorder marked by sadness, loneliness, despair, low self esteem and self-reproach. Associated features psychomotor agitation or retardation, low social interactions and symptoms like insomnia, anorexia and pain.

Types of depression: depression with noradrenaline deficit; depression with serotonin deficit: anxious type, hostile type; depression with dopamine deficit; depression with GABA deficit and mixed depression: depression with serotonin/ noradrenalin deficit, depression whit serotonin and/or noradrenalin deficit associated with dopaminergic hyperactivity.

Severity scale: mild depression episode, moderate depression, severe depression without psychotic features, severe depression with psychotic features.

Minimal natural length for a depression episode is 3 to 9 months. The cours is quite variable: isolated depressive episode; from one to three depressive episodes in the course of life; multiple episodes.

Initiating tratement with antidepressants drugs associates specific risks for each antidepressant class, requiring a thoroughly selection based on medical criteria and potential risks evaluation. Throughout the treatment the patient will be strictly monitored to prevent the risk of serotoninergic syndrome cardiovascular changes (hypo and hypertension, QTc prolongation, dyscrazic blood changes and hepatic enzymes changes).

Recent studies signal the presence of metabolic syndrome in patients with depression undergoing antidepressant treatment. Taking this information to consideration, we recommend as precaution to inforce a monitoring algorithm similar to the one used for the antipsychotic treatment.

Psychiatric nursing strategies for alcohol consumption

Alina Popa, Elena Țolea, C. Gâlcă

It is very possible to have a drinking problem that is not defined or described as “Alcoholic”.

Although most people do not become addicted to alcohol on their first drink, a small proportion do. Many people who have experienced a strong liking of alcohol from their very first exposure then gone on to become addicted to it. At present it cannot be predicted who these people will be, so any exposure to alcohol has the risk of producing addiction in some users.

Many people use alcohol to cope with stress but do not realize that it exacerbates the problems in their lives. People drink to socialize, celebrate, and relax. There is drink of joy, sadness, out of habit, drink without reason, simply drink.

Alcohol enters the bloodstream from the first sip. Alcohol's immediate effects can appear within about 10 minutes. As you drink, you increase your blood alcohol concentration (BAC) level, which is the amount of alcohol present in your bloodstream.

Alcohol often has a strong effect on people – and throughout history we`ve struggled to understand and manage alcohol's power.

Alcoholism is a disease not a vice, it is a progressive disease, incurable, marked by the obsession to drink despite physical harm produced.

There are techniques and therapies available to help lessen the dependence on alcohol and rediscover balance in life.

Incompatibilities between drugs which are administered intravenous in the Intensive Care Unit

Mirela Bidilică, Gabriela Amoașei, N. Tănase, L. Ene

In ICU there are many drugs which, by their mixing in the same infusate solution, may determine incompatibility reactions, by chemical or physical mechanism. The incompatibility may appear as precipitation, chemical reactions, antagonism, and decomposition of biological molecules or the appearance of gaseous compounds.

Even though it is recommended the visual analysis of the admixture/carrier solution before its use in order to determine the precipitation, the color modification or the appearance of turbidity, not every incompatibility can be visually observed.

We will present the main mechanisms of intravenous drugs incompatibilities, their causes and consequences (sometimes severe|) involving the patient and also some preventive strategies.

In order to reduce the risk associated with the intravenous drugs which are used in the ICU we have implemented in our unit the using of a two dimensional chart which contains recommendations for dissolving and compatibilities between every drug commonly used in our department.

Nursing files

Gabriela Dediu

Considering that the healthcare system is undergoing a continuous transformation process, the quality of healthcare is often used a reference point for the evaluation of the medical act. Furthermore, it provides an accurate reflection of the quality of the patient care processes, while also bearing into consideration the latest technological and therapeutic developments.

The nurse's duties are to enact the doctor's recommendations, working towards improving the patient's health, while respecting safety regulations that have been certified, approved, understood and consented to, so that the quality of the medial act is ensured.

Evaluating the quality of the healthcare act implies assessing multiple aspects, such as: the efficaciousness, efficiency and the continuous nature of the healthcare procedures; patient safety; the competences of the medical team; patient satisfaction, as well as the satisfaction of the medical personnel.

In conclusion, it can be argued that the purpose of the standards guiding the activity of the medical personnel is to establish a set of criteria and optimal working conditions necessary for achieving the required quality in the healthcare system, while at the same time respecting the patient's life, dignity and individuality. These standards are fully documented in the nursing dossier.

The intraoperative nursing for surgical procedures longer than 3 h

Mariana Stoian, Voichița Militaru, Mihaela Mușat, Teodora Vasile, Adriana Oprea, Cornelia Nițu, B. Iordache, I. Cristea

The surgical procedures lasting longer than 3 h are highly difficult, regardless they are open-surgery or minimally–invasive (laparoscopic). They require special high-skilled nursing measures. We organized these special measures in an internal protocol adapted to the type of surgery.

The protocol involves measures dedicated to the patient:

- Invasive and non-invasive monitoring.
- Electrolitic acid lose, hematologic ecquilibrations.
- Preventing and treating hipothermia.
- Preventing thrombo-emboling complications.
- Preventing prolongead immobilisations

Measure for the preparation of the operating room:

- Aseptic and anti-septic measures
- The preparation of the medical devices, medications in sufficient quantities
- The registration of the medical activites according to internal protocols.

Our experience comprises approximatly 1000 longer then 3 hours procedures, due to the surgical complexity and the aplication of the internal protocol contributed to surgical success, led to the important reduction of the post-operating, complications and in reducing the operative time.

Course – Modern multidisciplinary approach on hepatic carcinoma

Hepatocellular carcinoma – a short review

Florentina Ioniță Radu, Andrada Popescu, A. Gavrilă, Maria M. Chereja

Introduction: Our objective is to review current international data regarding hepatocellular carcinoma.

Materials and methods: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer with an incidence ranging from 6.5 % in women to 7.9 % in men.

HCC has a high mortality rate being the third leading cause of cancer -related death worldwide, responsible for ~ 700,000 deaths annually. Regardless of the cause, cirrhosis is the most important risk factor for the development of HCC with an annual risk of 2-7%.

Biannual surveillance using both AFP and abdominal ultrasound remains the gold standard for HCC screening in cirrhotic patients even if they are not very sensible for early HCC detection. Other biomarkers, such as des γ carboxy-prothrombin (DCP), AFP-L3, and cross sectional imaging are of great interest, but there is insufficient evidence to consider using them for routine screening.

Radiological imaging is the primary tool used in HCC diagnosis, without a need for biopsy when typical appearance exists. Recent findings in genomics could provide new tools in HCC diagnosis.

Although there are many staging systems for HCC, the Barcelona Clinic Liver Cancer is the most widely accepted since it contains an evidence-based treatment algorithm.

There are multiple treatment options for HCC depending on patient performance status and liver dysfunction degree. There is a tendency in expanding the actual criteria for liver transplantation, but recent data show a higher posttransplant mortality.

Results and conclusion: Hepatocellular carcinoma is an aggressive solid tumor with a high mortality rate. Even if there are multiple treatment options, surveillance and detection of early HCC is the best option in increasing survival rate.

The hepatocelular carcinoma – what we know from guides

Gaudia Mănescu, Cătălina Diaconu, D.O. Costache, Raluca S. Costache

Hepatocellular carcinoma is a malignant tumor that is the fifth most common type of cancer, the third leading cause of cancer-related death globally (preceded only by the lung and the stomach cancers) and the the most common primary liver cancer. HCC was thought to be a special type of cancer prevalent only in the Southeast Asia and Africa, but it has become in a short time more common in other regions, especially in Europe and the United States, which has led to greater interest in the diagnosis and treatment of HCC worldwide.

90% of pacients with hepatocellular carcinoma associate cirrhosis. Chronic viral hepatitis (hepatitis B is an idependent predictor of HCC development), hemochromatosis and alcoholic cirrhosis are the most frequently associated with HCC.

The pathogenesis of HCC is not fully understood, but most probably are implicated the activation of proto-oncogenes, deactivation of tumor suppresor genes (e.g. p53 and pRb), changes in growth factor or growth factor signaling processes (e.g IGF, TGF), changes in telomeric length and activity or microsatellite instability.

Symptoms of HCC are commonly related to those of their chronic liver disease and include weakness or fatigue, weight loss, anorexia, pain in the upper abdomen usually in the right hypocondrium, bloating or jaundice.

Diagnosis is usually made by history, physical examination, laboratory studies (aminotransferase levels, alkaline phosphatase, serum bilirubin and optionally elevated serum AFP (> 400 ng/ml), because AFP is elevated in only 50-75% of cases), MRI or CT scan showing a liver mass consistent with HCC.

The treatment should be discussed and planned by a multidisciplinary team. The applicable treatment possibilities include surgical (liver resection, liver transplantation-OLT), ablative (percutaneous ethanol injection, transarterial chemoembolization, radio-frequency ablation) and systemic therapy (tyrosine-kinase inhibitor – Sorafenib).

The treatment is established using the Barcelona-Clinic Liver Cancer (BCLC) staging system which defines very early stage cancer (single nodule,<2 cm, Child-Pugh A) as stage 0, early stage cancer (1-3 nodules, <3 cm each, Child-Pugh A-B) as stage A, intermediate stage for multinodular HCC (stage B), advanced stage which involves vascular invasion or extrahepatic spread (stage C) and terminal stage at pacients with Child-pugh C cirrhosis. Resection, ablation and transplantation is recommended for stage 0 and A, transcatheter arterial chemoembolization for stage B, Sorafenib – stage C and best supportive care for terminal stage.

Surgical treatment of the hepatocellular carcinoma (HCC) – review

L. Mosoia

Hepatocellular carcinoma (HCC) is a rising cause of cancer related mortality and viral causes of cirrhosis appear to be a major cause. The data work stress out that surveillance helps to detect early stage disease and treatment options are determined by stage of presentation. Different classifications of HCC and specific treatment are presented.

Three potentially curative options are tumor resections, liver transplantation and radiofrequency ablation/cryotherapy.

Emerging therapies such as drug eluting beads – transarterial chemoembolizations, radioembolization or Sorafenib treatment will continue to advance options in HCC.

Early experience with laparoscopic bipolar radiofrequency device for liver resection in hepatocellular carcinoma

L. Mosoia, V. Ștefănescu, F. Măcău, C. Mitru, V. Dumitrașcu

Laparoscopic liver resection remains a surgical procedure of great challenge because of the risk of massive bleeding during liver transection and the complicated biliary and vascular anatomy in the liver. Various techniques and energy-based devices have been used to minimise the blood loss during transection of the liver parenchyma laparoscopically.

Methods.

The laparoscopic Habib™ 4X is a bipolar radiofrequency device consisting of a 2x2 array of needles arranged in a rectangle. It produces coagulative necrosis of the liver parenchyma sealing biliary radicals and blood vessels and enables bloodless transection of the liver parenchyma.

Two patients underwent laparoscopic liver resection in a period of 6 months. Indications for liver resection were marginal hepatocellular carcinoma (3.7/3.1cm and 8/7 cm) in 2 cirrhotic patients. Technical aspects were analysed.

Results. One patient underwent S6 resection, the other one underwent S2-S3 resection. Pringle manoeuvre was not used in any of the patients. Mean operative time was 180 minutes (range, 160–200 minutes). Bleeding control along the transection line was satisfactory. No conversion to laparotomy was required. Operative blood loss was minimal. No blood transfusion was recorded. The postoperative period was uneventful. Median postoperative hospital stay was 3 days. Histopathology revealed that the margins were disease free.

Conclusion. Laparoscopic liver resection can be safely performed with laparoscopic Habib™ 4X with a significantly low risk of intraoperative bleeding or postoperative complications.

Two stage surgical treatment for giant right hepatic hepatocellular carcinoma

L. Mosoia, T. Artenie, A. Dima, C. Mitru, V. Dumitrașcu, Florina Bold, Irina Zahiu, C. Bețianu

Hepatocellular carcinoma (HCC) is a primary tumor of the liver, usually associated with chronic liver disease, is less common in non-cirrhotic livers. In this report, we present a 75 yrs old patient who came to our attention one month after the initial ultrasound with palpable hepatomegaly.

The CT showed a hypervascularized solitary liver lesion measuring 22/18 cm originated from right hepatic lobe, with small for size left hemiliver at volumetry. Laboratory data showed normal liver function and hepatitis B and C serologies were negative. Serum tumor markers including CEA, CA 12.5, CA 19.9 were within normal range while AFP was elevated, > 500. Due to the insufficient future liver remnant, we performed right portal vein ligation (PVL) in the first instance, to convert the unresectable tumor to resectable for potential cure. We record the success of PVL with compensatory hypertrophy in the left liver, seen at seven weeks after PVL.

The patient underwent planned laparotomy and right hepatectomy with uneventfully postoperative follow-up and discharge in day 9.

The case presented, indicates that surgical resection for giant HCC is possible in selected patients, even if initially, the future liver remnant does not allowed surgical therapy.

Alternative treatment options for inoperable primary and secondary liver tumors

Săndica Bucurică

The treatment protocols proposed by EASL and AASLD are similar and it are made (based on Barcelona Clinic Liver Cancer staging) according to disease stage. As for early stages there are curative treatments for advanced stages (as inoperable, vascular invasion, extrahepatic spread, poor clinical and biological patient status).

It is considered that hepatic resection, liver transplantation (in carefully selected individuals), and radiofrequency ablation are considered curative.

Treatments that consider best supportive care for terminal-stage HCC, but not intend to be curative are transarterial chemoembolization, radioembolization, and systemic chemotherapy such as with sorafenib.

The goals of trying to treat inoperable hepatic tumors is desirable in order to obtain a longer time of progression, the extension of survival with a better life quality by minimizing the symptoms or to obtain regression of tumors sizes for ablation, resection or transplantation.

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We look forward to your submission.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

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(2) searchable by standard, electronic (Internet-based) methods;
(3) open to all prospective registrants free of charge or at minimal cost;
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(5) identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Acknowledgments

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
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4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

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OP session MD1

Tuberculosis – a diagnostic challenge in Romania. Considerations on a clinical case

G. Stoicescu, L.M. Ciobîcă, Daniela Anghel, S. Stanciu, Iolanda Sârbu, Ancuța Coca

A 42 years old patient present cough, respectively a productive mucopurulent sputum, in a small quantity and asthenia. From her medical history we retain a diagnostic of uterine tumor, about seven years – therapeutic neglected and a recent asthma – 2 months ago, in a normal clinical exam context and a normal radiologic examination.

Clinic, without elements of bronchospasm, without bronchospasm seizures in the last 2 months and an abdominal pelvic tumor at the 1st palpation.

We performed a chest radiograph which highlighted several mild nodular opacities and a hydroaeric image of 4/2 cm in the right base. Respiratory functional tests were normal and the bronchoscopy with lavage, highlighted an intense mucosal congestion in basal segments. The cultures from the sputum and tracheobronchial aspirate, haven’t evidentiate the BAAR presence. The hemoleucogram indicates a medium ferriprive anemia and martial treatment was followed. Detailed exam of the pulmonary CT bring in discussion, suspicion of a nodular and ulcerative pulmonary TBC – multiple and bilateral.

In this context, after pulmonology exam the patient is guided in a specialized department. The BK culture result at 2 months has come positive.

As a particularity of the case, the TBC pulmonary with a fast evolution toward a pulmonary abscess, in less than 2 months, after a pulmonary exam with normal pulmonary radiography. The patients were not in a clinic or biological context of immunosuppression.

http://www.zilelesuumc.ro/

Improving treatment adherence in schizophrenia diagnosed patients by using long acting injectable antipsychotics

O. Vasiliu, D. Vasile, A.G. Mangalagiu, B.M. Petrescu, C. Tudor, C. Cândea, R.E. Bazac-Bratu, F.T. Androne, A.F. Alboaie, M. Pătrașcu, E.A. Morariu

Treatment adherence in psychotic disorders is an important factor that correlates with disorder’s prognosis, risk of complications, duration and frequency of hospitalizations, social and professional reinsertion.

Long acting injectable treatments are available and their administration could improve patients’ adherence to antipsychotics. From the clinician’s perspective, long acting injectable antipsychotics are preferred to oral medication due to a more accurate way of treatment monitoring and to the maintaining of stable active drug serum concentrations throughout the treatment duration, therefore avoiding high drug levels associated with adverse events, but also lower drug levels which could be responsible for relapses.

From the patients’ perspective, fewer presentations to the physician could be perceived as a benefit. From the caregivers’ point of view, a lower rate of relapse and fewer visits with the patient to the physician could reduce the disease burden.

Several options are currently available in clinical practice – risperidone microspheres could be administered every 2 weeks, aripiprazole monohydrate is recommended for administration every 4 weeks, olanzapine pamoate every 2 or 4 weeks, and paliperidone palmitate every 4 weeks. Other formulations are FDA approved but not yet marketed in Europe, like aripiprazole lauroxil (administered every 4 or 6 weeks) and paliperidone palmitate with a longer, 12 weeks, effect.

A trend to develop drug formulas with longer intervals between injections is observed and this is good news regarding the problem of treatment adherence in schizophrenia.

Osler-Weber-Rendu Disease – familial case report

Cristina Vîrlan, Florina Topliceanu, Lavinia Bârsan, M. Șotcan, E. Dănăilă

Introduction: Osler-Weber-Rendu disease (OWRD, also known as hereditary hemorrhagic telangiectasia) is a rare autosomal dominant disease, which manifests through mucous-cutaneous telangiectasia and arteriovenous malformations, a potential source of serious morbidity and mortality. Recurrent and severe epistaxis is the most common presentation, frequently leading to severe anemia that necessitates transfusion.

Material and methods: A 40 year-old woman presented to our clinic with recurrent epistaxis. Clinical examination revealed mucous-cutaneous telangiectasia. Imaging studies showed ectasias of portal and splenic veins. She confirmed that her mother had also had recurrent mucosal and oral bleeding, but she has not investigated them. The diagnosis of OWRD was established as all the criteria were met.

Results: As the OWRD is a hereditary disease, we investigated the first-line blood relatives of the patient. One of her two sons presented the same symptoms and was also diagnosed with OWRD. Currently, the patient and her son are periodically evaluated and treated for anemia. There was no need for surgical intervention, as the arteriovenous malformations were minimal and not life-threatening.

Discussion: Symptom onset may be delayed until the fourth decade of life (~90% of patients manifest by age 40 years) or later. Screening family members for signs of OWRD is reasonable and should include a complete history, physical examination, chest radiography, and arterial blood gas testing (with measurement of the shunt fraction).

Macroamylasemia – a source of confusion in clinical practice

V. Smedescu, Alexandra Gireadă, Corina Taubner, I. Copaci

Introduction: Macroamylasemia represents the presence of circulating complexes, consisting of amylase bound to immunoglobulins (IgA or IgG), not filtered by the kidney, generating increased amylase serum levels.

Materials and methods: We mention the case of a 44 year old woman who presented with a dyspeptic syndrome and whose laboratory tests indicated an increased amylase serum level with a normal lipase serum level. In the absence of another cause of hyperamylasemia (an intraabdominal or salivary condition or renal failure), macroamylasemia was suspected.

Results: The diagnosis was confirmed by the amylase creatinine clearance ratio (ACCR), less than 1%.

Discussions: Macroamylasemia is encountered in about 2.5% of hyperamylasemic patients and in 1% of healthy individuals. There are three types of macroamylasemia. In the first, most common type, the amylase activity is increased in serum and decreased in urine. In the second type, the ratio macroamylase/normal amylase in serum is lower than in the first type and the urinary amylase is increased. The third type is characterized by normal serum and urine amylase activity.

As a conclusion, in some cases, like ours, the diagnosis can be confirmed by the ACCR, but sometimes the detection of macroamylase in serum may be necessary.

Holistic approach of the bacterial resistance to antibiotics phenomenon

V. Ordeanu

The main objective of this work is how to investigate the microbial resistance and, in particular, the bacterial resistance to antibiotics (AB) as it is a worrying phenomenon for the public health, globally, in all WHO regions. The spread of this phenomenon leads to the risk of not being able to control infectious diseases, described as "the end of the antibiotics era" that would lead to "the end of modern medicine."

In order to prevent this public health's major risk, we study the phenomenon scientifically and exhaustively, interdisciplinary and multidisciplinary, bio-medically and socio-economically, in order to identify critical points and propose concrete measures of action.

The "One health" innovative Concept of the WHO allows, for the first time, to bring together the bacterial and antibiotic resistance in human medicine, military medicine, veterinary medicine, zootechnics, pharmacy, environment etc., being appropriate for studying and fighting against this dangerous to public health phenomenon.

The innovative holistic approach to medical and paramedical aspects of the microbial resistance, a subject in which our team already has a long experience, will constitute a useful database for scientists, practitioners and healthcare policymakers. This project was proposed and accepted of Grand Challenge at Bill and Melinda Gates Foundation, USA, 2016.

Primary prevention in unipolar depression

A. Nistor, T. Hara, C. King, F. Blebea, A. Iftodi, Corina Tudor, O. Vasiliu, D. Vasile

The concept of primary prevention in psychiatry remains controversial, met with skepticism even today. Goldstein (1977) sees it as an idea that is ripe to become practice, and a possible achievement of modern psychiatry, Cummings (1972) sees more costs versus benefits in its implementation, Henderson (1975) judges it to be an illusion, Erlenmeyer-Kimling (1977) as something that belongs in the distant future, while those responsible for allocating funds for its realization, consider it a luxury. These are points of view still present when we try to explain the passive attitude of our communities regarding this field.

Nevertheless, medical progress in the field of psychiatry brings hope that primary prevention in mental health can prove to be effective, with unipolar major depression being the most researched topic, as depression is among the leading contributors to the global burden of illness-related disability, and is predicted to be the greatest contributor to the illness burden by 2030.

There is a paucity of primary preventive programs aimed at the general population. Therefore, it is a matter of public health to find ways to implement timely and effective strategies to prevent major depression. As primary prevention addresses the healthy individual, a collaborative multi-disciplinary approach focused on universal primary prevention (Shinn and Toohey-2001) that would target the communities in their entirety would be most successful.

Rare cause of a severe coagulopathy

Anca Manolache, V. Duțescu, Elena Busuioc, I. Copaci, V. Smedescu, V. Balaban

We report a case of a 40 year old man who presented himself in emergency department for multiple spontaneous ecchymoses and hematomas over the last 12 hours. He had been prescribed NSAID for renal colic 3 days before presentation. In the beginning, biological tests showed inflammatory syndrome without anemia.

Clinical exam revealed pale teguments and mucosa, ecchymosis and diffuse tenseness to the right forearm, left and right leg.

Laboratory results showed a prothrombin time of 21 msec and an activated partial thromboplastin time of 98 seconds. Platelets were normal and hemoglobin level was 7.2g/dl. The patient presented also hypoproteinemia, hypocalcemia and hypokalemia associated with severe inflammatory syndrome (fibrinogen 875 mg/dl, ESR 90 mm/1h).

Because the patient became unstable due to fast progression of anemia (Hb5g/dl) over 4 hours associated with the extension of hematomas and with a slow response of the treatment, we decided the admission in the intensive care unit.

We continued to investigate the coagulopathy and we excluded the acute promyelocytic leukemia. We excluded a possible organophosphate poisoning after the cholinesterase level came out normal. We took blood test for hemophilia as per indication of the hematologist.

The CT scan showed intestinal walls thickened due to possible hematomas at this level.

After we started treatment with FFP, vitamin K, the coagulation parameters became normal. Because hydroelectrolytic disturbance persisted and patient continued to present modification of intestinal transit (one/two soft stools per day), it rose the suspicion for celiac disease. We performed an esophagogastroduodenoscopy that revealed a typical pattern for celiac disease. Also the serology was positive with an increased level of antitransglutaminase IgA antibody (320 U/ml).

After we started the free gluten diet, the clinical and biological evolution of the patient was favorable.

Symptomatic coagulopathy represents a very rare manifestation of celiac disease, in this particular case revealed by the consumption of NSAIDs medication.

Noninvasive ventilation in obesity hypoventilation syndrome

I. Jascu, Ioana Ştefănescu, C. Ioniță, E. Firoiu, Claudia Popovici, Gabriela Andraș

Introduction: Obesity hypoventilation syndrome (OHS) is a condition in some obese people in which poor breathing leads to lower oxygen and higher carbon dioxide levels in the blood. This syndrome arises from a complex interaction between sleep-disordered breathing, diminished respiratory drive, and obesity-related respiratory impairment, and is associated with significant mortality and morbidity.

Material and methods: In the last year we investigated several obese patients for OHS presenting with acute to chronic respiratory failure. In most of the cases the diagnosis was confirmed and many patients were diagnosed or already had obstructive sleep apnea or COPD too. We initiated noninvasive ventilation (NIV) in daytime in three periods of two hours and six hours in the night. Few of them, who came with severe condition, were admitted in the Intensive Care Unit and needed tracheal intubation and mechanical ventilation. After improving, they were transferred in our department and continued NIV.

Results: The patients had a good and rapid recovery with carbon dioxide level lowered almost to the normal values and concomitantly with oxygen partial pressure growth. Severe patients could be mobilized after a few days and in 7-10 days were discharged. Most of them now use NIV only in the night and have a better quality of life.

Conclusions: Patients with OHS can be treated with NIV during an episode of acute hypercapnic respiratory failure. NIV introduction in our department practice determined a faster recovery of severe patients with shortening hospital admittance and costs lowering.

Malabsorption and hematuria – what is the connection

Anca Manolache, V. Duțescu, Elena Busuioc, I. Copaci, V. Smedescu, L. Eftimie, G. Becheanu

We present the case of a 60 years old woman which was sent from the Psychiatric Department to our establishment to investigate a malabsorption syndrome. For 4 months the patient presented symptoms of asthenia, fatigue and modified stools associated with weight loss of approximately 10 kg.

The clinical exam showed low weight (BMI 17 kg/m^2), pale teguments and mucosa, cutaneous hyperpigmentation, leg edema and absent bilateral vesicular murmur on the base of the lungs.

Since the beginning, the exams were showing severe feriprive anemia, hypocalcemia, hypoproteinemia with moderate inflammatory syndrome and also with negative serology for celiac disease. Urinary samples detected microscopic hematuria with 20% dysmorphic red blood cells, leucocyturia in the presence of positive urine cultures for Escherichia coli, without nephrotic or nefritic proteinuria.

We repeated the upper and lower endoscopy. After the esogastroduodenoscopy we've suspected the presence of intestinal lymphoma, subsequently denied by the histopathological exam. Ultrasonographic exam shows the presence of polyserositis.

The CT scan discovered in addition abdominal lymphadenopathies and bilateral renal cortical densification. After all the common causes were excluded, the persistence of hematuria and malabsorption lead us to investigate another rare cause like Whipple disease.

We repeated the upper gastro endoscopy with multiple biopsies samples for PAS coloration. The results were positive and confirmed by PCR exam for Tropheryma whippelli.

After we initiated the specific antibiotic treatment, the patient evolution was favorable with the remission of the malabsorption syndrome, hematuria and negativity of the PCR for T. whippelli.

The renal disorder due to Whipple disease it's very rare, a few cases were described in the literature of interstitial chronic nephritis and kidney failure due to renal amyloidosis.

A case of H1N1 flu complicated with bronchopneumonia, acute respiratory failure and BOOP

C. Ioniță, Ioana Ștefănescu, I. Jascu, E. Firoiu, Claudia Popovici

H1N1 is a flu virus. When it was first detected in 2009, it was called swine flu because the virus was similar to those found in pigs. The H1N1 virus is currently a seasonal flu virus found in humans, who spreads between people in the same way that seasonal flu viruses spread.

We present the case of a 49 years patient, non-smoker, with no medical history, presented in ER with: high fever, inspiratory dyspnea, productive cough (purulent sputum) and intense fatigue. Physical findings: bilateral intense crepitation, SaO_2 between 70% and 73% without oxygen, polypnea (34/minute). The chest X-ray revealed a mixt (alveolar and interstitial) pattern spread in both lungs, while the laboratory revealed leukopenia (3.700/mm^3) and increased values of Urea (67 mg/dl), Creatinine (1.75 mg/dl) and AST (92 UI/l), the patient being positive for H1N1 flu.

The patient was admitted in ICU, where he was intubated and mechanical ventilated (for three weeks), treated with large spectrum antibiotics and Tamiflu, with the persistence of respiratory failure (SaO_2 88% - 89% without O_2) and a mild improvement of the chest X-ray aspect. Due to the last aspect, a BAL (bronchoalveolar lavage) was performed, it's results being characteristic for BOOP (bronchiolitis obliterans organizing pneumonia), which determined the introduction of corticotherapy (Prednisone – 70 mg daily as initial dose, for about three weeks, followed by reduction with 5 mg every three weeks).

After four months of corticotherapy, despite the clinical and chest X-ray initial improvement, the prognosis remain reserved (the patient still presents shortness of breath, still uses O_2 from time to time, stationary aspect of the chest X-ray in the last 3 months).

OP session MD2

The value of Wells score in pulmonary embolism management in the ED

C. Florea, C.B. Teușdea, G. Ifrim, Alexandra David, Anca Arsene

The diagnosis of pulmonary embolism (PE) in the emergency department is challenging due to the wide range of non-specific symptoms, imperfect investigations and lack of clinical diagnostic criteria.

There are various scoring systems in an attempt to limit unnecessary investigations in those with low risk of PE. The Wells score for pulmonary embolism provides an estimated pre-test probability of pulmonary embolism and allows the clinician to exclude with some measure of accuracy those patients who are at very low risk of PE and for whom further testing and observation is not necessary.

The presenting symptoms are common and non-specific varying from shortness of breath chest pain, cough and hemoptysis, to syncope and cardiac arrest. There is a high false positive rate with non-invasive testing (D-dimmers) but relatively significant potential adverse events (radiation and contrast associated complications) with more invasive testing such as Computed Tomography Pulmonary Angiogram (CTPa).

While the original intent of this tool was to determine which patient was low risk enough to rule out testing with D-dimmers it is considered safer to use the three tier model which uses the score and d-dimmers testing as well as performing CTPa on all high risk patients regardless of D-dimmers results.

We analyzed the way our ER department currently manages pulmonary embolism cases and if our protocols could be further improved by introducing the Wells score. We aimed to increase documentation of pre-test probability and reduce inappropriate investigations.

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Cardiac markers - ‘’odd’’ laboratory results

Alexandra Gireadă, V. Smedescu, Corina Taubner, I. Copaci

Introduction: CK-MB is an essential cardiac marker used in the diagnosis of several acute cardiac syndromes, however there are several scenarios in clinical practice when it can create confusion.

Materials and methods: The presentation illustrates the case of a 78 year old man who was admitted for atypical chest pain and had a CK-MB level of 999 U/l and a total CK level of 764 U/l with normal troponin levels. An acute cardiac illness or rhabdomyolysis were excluded. The final diagnosis was urinary bladder neoplasm.

Results: The laboratory picture suggested the presence of macro CK in the serum of this patient.

Discussions: Usually, in cardiac patients, both CK and CK-MB levels are increased and the CK-MB/CK ratio is also modified (from its normal values of between 3% and 5% to 15-30% in certain clinical scenarios). When this ratio increases to values higher than 30%, other conditions must be considered. A laboratory result of > 100% is highly characteristic for the presence of macro-creatine kinase complexes (macro-CK), which are aggregates formed between CK isoenzymes, often involving immunoglobulins. They are usually generating false increases in the CK and CK-MB concentrations.

In conclusion, sometimes unusual laboratory findings can announce the presence of important pathological processes.

Difficulties of clinical classification in a case of aortitis and large vessels vasculitis

A. Ionescu, Roxana Diaconu, Denise-Ani Mardale, V. Smedescu, Oana Stancu, Magda Iriciuc, I. Copaci, C. Jurcuț

We present the case of a 22-year-old woman, diagnosed with Takayasu arteritis four years earlier, who recently started to complain of fatigue, claudication of the left arm and weight loss of about 7 kg in the last months. Her physical examination revealed signs of anemia, multiple bruits, asymmetrical arterial blood pressure with a colder left arm with no signs of acute ischemia, while her laboratory investigations showed moderate anemia with signs of systemic inflammation.

We performed a computed tomography angiography which described thickening of the wall of the entire aorta, with smoothly tapered luminal narrowing to a minimum of 9 mm, also involving most major branches of the aorta. At this point, the patient meets the classification criteria for Takayasu arteritis, established by the ACR in 1990. However, the laboratory work-up showed the positivity for specific systemic lupus erythematosus (SLE) antibodies. Thus, the possibility of aortitis in the context of SLE was suggested.

However, in the absence of direct histological examination, this differential diagnosis was not possible. No other causes for the patient's anemia, other than the chronic inflammatory state, were uncovered and a net improvement of her condition was observed after systemic glucocorticoids therapy (pulse therapy of methylprednisolone followed by oral prednisone) and IV iron supplement, the patient being stable thus far.

This case report emphasizes the difficulties of correct classification in patients with large vessels vasculitis in daily clinical practice.

Severe mitral regurgitation in young woman – when to search for a systemic cause

Denise Mardale, Roxana Diaconu, A. Ionescu, V. Smedescu, Oana Stancu, M. Șotcan, D. Cârpaciu, I. Copaci, C. Jurcuț

We report the case of a 29-year-old woman followed in the department of cardiology for severe mitral regurgitation. During the follow-up she developed deep vein thrombosis at the level of left upper limb (subclavian and jugular vein) associated with mild pulmonary hypertension, polyserositis, lymphadenopathies and anemia. The computed tomography confirmed the presence of deep vein thrombosis, bilateral pleural and pericardial effusion, the polyadenopathies and the hepatosplenomegaly. We performed the biopsy of an axillary adenopathy which was negative for any specific infectious, neoplastic and hematological diseases.

An extensive laboratory work-up was performed revealing the high level of anti-dsDNA antibodies associated with positivity for antiphospholipid antibodies and microcytic anemia. The other causes for anemia were carefully excluded. No other clinical signs (i.e. arthritis or skin lesions) of systemic lupus erythematosus (SLE) were observed. A diagnosis of SLE was made and a treatment with hydroxychloroquine, systemic glucocorticoids (prednisone) and oral anticoagulants was started with the remission of serositis, lymphadenopathies and anemia. Taking into account the severity of mitral regurgitation, the surgical valve replacement was planned.

This case emphasized the need for screening for systemic diseases (i.e. SLE) in young patients with cardiac valvular lesions without a clear etiology. Moreover, the antiphospholipid antibodies were reported to be associated with cardiac valvular lesions in patients with SLE and should be evaluated in these patients.

Pre-analytical variables in coagulation testing

Corina Taubner, Rodica Zemba, Irina Butte, Clara Neguţ

Introduction: The most commonly performed tests in the coagulation laboratory are the Prothrombin Time and the Activated Partial Thromboplastin Time. Coagulation tests results are important in the diagnosis and treatment of patients with bleeding or clotting disorders and also in monitoring the efficiency of anticoagulant therapy.

Materials and methods: The elements of the laboratory quality system, internal quality control and external quality assurance provide information on the analytical phase of the testing process by assessing the precision, sensitivity, accuracy of the testing method and by evaluating the long-term laboratory performance. Although significantly improved analyzers were developed and highly sensitive reagents are routinely used, samples for coagulation testing are particularly susceptible to pre-analytical errors.

Results: The pre-analytical variables such as specimen collection and handling account for up to 75% of errors within the diagnostic process and cannot be detected by current control quality control procedures. The activity of the coagulation laboratory was assessed regarding the quality and integrity of the coagulation samples affected by inappropriate specimen collection or handling. The identified errors include samples that are hemolysed, heparin contaminated, clotted or insufficient collected.

Discussion: Understanding the sources of the pre-analytical errors is a prerequisite for implementing measures in order to control the assay performance and lead to reliable test results that actually represent the clinical status of the patient and not just the status of a clinical sample received and tested.

OP session MD3

AVC recovery to youth

A. Iliuță, D. Nedelescu, Paulina Vintilă, Simona Ionescu

Stroke is an acute, serious neurological disorder resulting from blockage of blood supply to an area of brain or from cerebral hemorrhage.

Worldwide, stroke is the leading cause of morbidity and mortality because annual "kill" five million people and causes five million other severe disabilities.

Prospective studies show that this disease increases from year to year, both the incidence and prevalence, appreciating the World Health Organization experts, the stroke will become by 2030 the leading cause of mortality.

In Europe, the incidence of stroke varies from country to country, estimated between 100 and 200 strokes per 100,000 inhabitants annually in November.

Stroke is the main etiological factor of installing long-term disability in developed countries constituting the third leading cause of death after heart disease and neoplasms

Romania is in the top ten in the world in terms of the incidence of stroke by stroke. Mortality is three - four times higher in our country than in EU countries and six – seven times higher than the United States America. Maximum age on the incidence of strokes occur in 75% of cases after 65 years.

Patients who survive a stroke often show symptoms like persistent paralysis of motor function, sensory deficits, and deficits in perception, balance, aphasia, depression, dementia or other deterioration of cognitive function.

A neurologically ill health is a long process, continuously for life. Problems actual recovery will put just as long as there are signs of improvement and hopes for compensation or gain functional.

Particularities of diagnosis and treatment of cervical spine pain in medical rehabilitation

D. Nedelescu, A. Iliuță, Paulina Vintilă, Simona Ionescu

Cervical spine pain is a symptom that most people experienced in their lifetime.

Knowledge of spinal biomechanics and pathophysiology helps determine the most likely pain generators in each case. A variety of spinal structures can produce overlapping or obscure symptomatology. An accurate diagnosis provides the best opportunity for effective treatment. The purpose of this presentation is to show the particularities of diagnosis and treatment of major degenerative diseases of the cervical spine: 1. Chronic pain of the cervical spine; 2. Acute torticollis; 3. Cervical-cephalic pain syndrome; 4. Brachial neuralgia cervical; 5. Cervical spinal stenosis; 6. Syndrome Barre-Lieou. It is important to view the patient as a whole, and institute physical, pharmacologic, behavioral, and interventional treatments in the broad context of achieving what is best for the patient's physiologic and psychologic well-being.

Rehabilitation of patients with severe burns – our experience

Anca Sălceanu, G. Teodoru, Cristina Gorgonețu

The recent achievements in the multidisciplinary approach and starting intensive care treatment since the pre-hospital phase caused the increase in the number of survivors from fires involving large numbers of casualties how was the dramatic "Colectiv" nightclub fire almost a year ago. This approach brought great challenges in the rehabilitation of patients with severe functional deficits that in the past would not have survived until this phase.

In this paper we share our experience and results obtained in the treatment of patients with severe burns.

The medical rehabilitation principles is mandatory to be applied since the early postsurgical phase with functional positioning and avoiding complications of bed rest and should be continued for a long time until to complete the process of scar remodeling.

To obtain better outcome, patients should be regularly reassessed by all clinicians involved in their care and their treatment must be customized for each patient and each evolutionary stage.

Truth and myth in rehabilitation of patients with multiple sclerosis

Anca Sălceanu, G. Teodoru

Multiple sclerosis (MS), an inflammatory autoimmune disease is the most common demyelinating disease of the central nervous system. That is the second leading cause of disability of young adults after trauma.

The main problem of these patients is the inability to properly perform usual daily activities as a consequence of multiple neurological deficits.

For physical medicine and rehabilitation doctors the treatment of these complex patients represents a real challenge.

Management of them involves an interdisciplinary approach that requires close cooperation with neurologist, psychologist, physiotherapist, kineto-therapist, masseur, urologist and specialist in diet and nutrition.

For many years, patients with MS were advised to avoid exercise because of risk of increased neurological impairment, so for many years, and from our experience and in our days, many people with MS have limited their physical activity because of the fear of increased disability.

The goal of this paper is to shown, with several surveys, the benefits of physical training, with improvements in aerobic capacity, gait parameters and fatigue, and an influence on quality of life and less impact on fatigue.

Verrucous carcinoma arising in an area of necrobiosis lipoidica

Mihaela Georgescu, Viorica Marinescu, D.A. Chiriță, Florina Vasilescu

Introduction: Necrobiosis lipoidica is a granulomatous condition presenting as indolent atrophic plaques, often on the lower extremities, mostly in diabetic patients. It may occasionally be complicated by squamous cell carcinoma. This association is rare.

Case report: We present the case of a 69 years old female patient that was admitted with a verrucous, ulcerated tumor, of 4/3 cm, developed on a scleroderma like plaque, localized on the right ankle. The local examination showed the presence of large atrophic plaques with the center of porcelain color and erythematous-violet margins, with the diameter of 20/15 cm, on the 1/3 inferior part of the leg. The lesions were several years old.

The general physical examination was normal.

The primary clinical differential diagnosis was verrucous carcinoma developed on a lichen sclerosus lesion or a necrobiosis lipoidica lesion.

A biopsy was performed and the result was verrucous carcinoma (in situ squamous cell carcinoma) arising in an area of necrobiosis lipoidica.

Considering the high risk of metastasis of a squamous cell carcinoma, the tumor was completely excised. The necrobiosis lipoidica lesions were treated with topical corticosteroids and pentoxifylline 2 tb daily, with a mild favorable evolution.

Discussion: Only fifteen cases have been reported to date in the literature, regarding the development of a tumoral transformation on a classical necrobiosis lipoidica plaque.

Conclusion: Considering the association of verrucous carcinoma with necrobiosis lipoidica, clinicians should have a high index of suspicion when consulting a patient with a tumoral lesion arising within a plaque of necrobiosis lipoidica.

OP session MD4

Interferon-free treatment for chronic hepatitis C – efficacy in a small cohort

Florentina Ioniță Radu, Andrada L. Popescu, I.P. Nuță, Raluca S. Costache, Mariana Jinga, Săndica Bucurică, B. Macadon, M. Pătrășescu, Maria M. Chereja, A.I. Gavrilă

Introduction: The objective is to present our department`s experience in treating chronic hepatitis C patients with the new “interferon-free” regiment which became available in our country in the fall of 2015.

Materials and methods: Chronic hepatitis C affects approximately 3% of the world’s population and represents a significant global health issue especially due to its complications: cirrhosis and hepatocellular carcinoma. The hepatitis C virus (HCV) treatment landscape has greatly changed over the past years, with the development of direct-acting antiviral (DAA) drugs that target various steps in the HCV lifecycle.

Older interferon-based regimens were complex and full of side-effects; they required 6–12 months of therapy, with cure rates averaging around 45-50% for HCV genotype 1. DAA-based regimens have short durations, minimal side effects and efficacy approaching 90–100%.

In this small cohort study we aim to present our gastroenterology department`s experience in treating patients with advanced but compensated chronic hepatitis C – genotype 1b (Romanian population being very homogenous regarding hepatic C virus genotype infection), using an oral combination of ombitasvir, paritaprevir, ritonavir, dasabuvir and ribavirin; this being the only regiment that is available in our country at this time.

Results and conclusion: DAA-based regiment are a breakthrough in the long-term goal of eradicating hepatitis C, as more and more clinical studies confirm their very high efficacy, but the same clinical studies tell us that patients with sustained virologic response remain at risk for developing hepatocellular carcinoma, thus the need to further keep these patients under watch.

Rocky liver mass in HCV infected patient – a case report

Florentina Ioniță Radu, Andrada L. Popescu, I.P. Nuță, Mariana Jinga, Raluca S. Costache, Săndica Bucurică, B. Macadon, M. Pătrășescu, M. Șotcan, C. Bețianu, Maria M. Chereja, A.I. Gavrilă

Introduction: Hepatocelullar carcinoma is the fifth most common type of cancer and third most common cause for related cancer death with an increasing incidence worldwide.

Highly calcified hepatocellular carcinoma is considered a relatively rare condition, going to misleading diagnosis, since this is a common finding in granulomatous diseases or hydatic cysts.

Materials and methods: We present a unique case of extensive calcified liver mass. A 69-year old man with diabetes and HCV infection presented with nocturne hydrosis in the past three weeks.

Computed tomography showed a 13/11 cm highly calcified mass in the right hepatic lobe on a background of cirrhotic parenchyma, accompanied with multiple retroperitoneal masses.

On the contrast enhancement the mass didn’t have enhance in the arterial phase, nor portal venous and delayed phase washout, despite elevated alpha-fetoprotein level.

The liver mass had poorly defined margins, extensive psammomatous calcification, with dilatation of intrahepatic bile ducts and altered perfusion of the adjacent parenchyma.

Ultrasound guided liver biopsy of the mass was performed with difficulty due to massive calcification. Microscopically, the liver mass was composed of tumoral hepatoid large cells with granular eosinophilic cytoplasm disposed in trabeculae and cords.

The cells were diffuse immunoreactive to Glypican 3, to anti hepatocyte specific antigen antibody and focally positive for cytokeratin 7, but negative for cytokeratin 20 and antigen carbohydrate 19-9.

Results and conclusion: A calcified hepatocellular carcinoma is not such a rare condition, but it can be a challenge of diagnosis even in the presence of modern imagistic techniques.

A challenge in finding cause of extensive portal vein thrombosis with hepato-splenic infarction

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Introduction: Portal vein thrombosis represents a blockage or narrowing of the portal vein by a blood clot.

In adults, cirrhosis is the major etiology. Neoplasms are another major cause, with hepatocellular carcinoma and pancreatic carcinoma, extrinsic compression or direct invasion of the portal vein and lead to thrombosis by inducing a hypercoagulable state.

Myeloproliferative disorders and inherited or acquired coagulation disorders are also incriminated, but in 8-15 % of cases have been reported to be idiopathic in the recent literature.

Case report: We report the case of a 52-year-old female, non-smoker, which denies completely alcohol consumption, with known hypertension, dyslipidemia, ischemic heart disease, stent on the coronary arteries, under treatment with beta blockers and antiplatelet, presented into the ER with a moderate to high intensity diffuse abdominal pain, nausea and vomiting, for at least 10 days.

The diagnosis was confirmed on a CT scan which showed thrombosis at all levels of portal venous system with LSH and spleen infarction.

We have started to investigate this patient for autoimmune, neoplastic, hematologic and hepatic diseases, in which all tests were resulting negative.

Conclusions: Portal vein thrombosis on a middle age woman without evident cause, as the etiology of the abdominal pain is very uncommon and raises a hard question: what are the etiologies and prevalence of this disease at non-cirrhotic patients?

Self-expandable metallic stents – an efficient palliative treatment for inoperable esophageal malignant tumors

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Esophageal self-expandable metallic stents (SEMS) are frequently used in digestive oncology endoscopy for palliative treatment concerning malignant stenosis that are not suitable for surgical treatment. The most frequent symptom to be treated is severe dysphagia that hampers oral nutrition.

The complications associated with the use of SEMS may be early (within 2-3 weeks) or late. Early complications appear in 30% of patients and comprise of: migration of the stent (the most frequently encountered), prolonged retrosternal pain (12), severe hemorrhages (1%) and mediastinal perforation (rarely). The current paper reveals our 4 case experience of esophageal malignant stenosis.

The palliative treatment of dysphagia alleviates the degree of severity of this symptom from grade 4 (no esophageal passage of food) to grade 2 (passage of semisolids) (Nuehau classification). The deployment and the patency of the stents were performed under radiology and endoscopy control.

The most frequently encountered side effect was retrosternal pain, swiftly responsive to medical treatment with Metamizol.

A rare case of acute liver failure caused by hepatitis E virus infection in a neoplastic patient

Andrada Popescu, A.I. Gavrilă, I.P. Nuță, Raluca S. Costache, Mariana Jinga, Săndica Bucurică, B. Macadon, M. Pătrășescu, Mirela Chereja, Florentina Ioniță Radu

Introduction: We are presenting a case of acute liver failure, in a hospitalized patient with two neoplasia.

Case report: A 77-year-old male ex-smoker with lower lip squamous cell carcinoma diagnosed in 2014, came to our gastroenterology unit for jaundice, pruritus, fatigue and dysphagia, accompanied by acholic stools.

The patient had an acute hepatocytolytic syndrome (AST = 3010 UI/ml, ALT = 2559 UI/ml), a cholestatic syndrome and coagulopathy.

Viral markers were taken and send to laboratory for testing (IgM and IgG immunoglobulins for HAV, HEV, CMV, EBV, AgHBs and HCV antibodies), and supportive hepatic treatment was immediately conducted. We asked for the infection disease doctor opinion and took also the leptospira IgM antibodies.

After a few days the hepatocytolytic syndrome improved, we performed a gastroscopy and found a medium esophageal tumor. The thoracic-abdomen CT scan confirmed the esophageal tumor with lung metastases.

The laboratory tests showed rapid increase of cholestatic enzymes (TBil = 34 mg/dl, NCBil = 21.23 mg/dl), although the hepatocytolytic syndrome significantly improved (AST = 371 U/L, ALT = 449 U/L), and confirmed hepatitis E virus acute infection. Unfortunately the patient had signs of acute liver failure, encephalopathy and made also acute pancreatitis and IDC (he died in less than one month after the onset of symptoms despite the intensive hepatic treatment).

Results and conclusion: Acute viral hepatitis due to HEV is usually an acute, self-limiting illness, with bad evolution in pregnant women and chronic liver disease.

When complications occur – acute liver failure, the goals of treatment are to prevent further deterioration in liver function, reverse precipitating factors, and support failing organs.

Inflammatory bowel disease in neoplastic patients – a diagnosis and therapeutic challenge

Geanina Spulber, R. Mateescu, Florentina Ioniță Radu, Mariana Jinga, Raluca S. Costache, M. Pătrășescu, P. Nuță, Andrada Popescu, B. Macadon, Săndica Bucurică

Introduction: Inflammatory bowel diseases are a group of lifelong diseases arising from an interaction between genetic and environmental factors, and also they are complex disorders reflected by wide variation in clinical practice observed predominantly in the developed countries of the world.

Patients may live with a considerable symptom burden despite medical treatment in the hope that the etiology of ulcerative colitis will shortly be revealed and a cure emerge, and clinicians have to advise patients on the basis of information available today. Despite randomized trials there will always be many questions that can only be answered by the exercise of judgement and opinion. This leads to differences in practice between clinicians.

Case report: A 55 year-old male with a history of ulcerative colitis for 6 years in therapy with Salazopirine 4g/day, left inferior limb condrosarcoma in multiple sites for which he suffered a thigh amputation and chemotherapy diagnosed 3 years prior to presentation for flare of UC and corticodependent disease.

Discussion: There are a few studies addressing the overall cancer risk associated with thiopurine treatment in patients with IBD found no significantly increased risk but they have limited power, lack adequate control groups, present a limited degree of detail regarding drug exposure, or represent restricted populations.

Conclusion: Inflammatory bowel diseases represent a diagnostic challenge for gastroenterologist because of their pleomorphic manifestations which exposes a wide area for differential diagnosis and also they are a therapeutic challenge especially in neoplastic patients in which we have to estimate of the overall risk associated with the appropriate therapy we need to support clinical decision making in weighing the benefits against risks of therapy.

HCV infection - where do we stand?

Florentina Ioniță Radu, Andrada Popescu, Mirela Chereja, A.I. Gavrilă

Introduction: Our objective is to review current international data regarding hepatitis C virus infection, current incidence and prevalence, importance of early detection and treatment and global burden.

Materials and methods: Hepatitis C virus (HCV) is a leading cause of chronic liver disease, cirrhosis and hepatocellular carcinoma, with approximately 170 million (~3% of the global population) being affected.

Transmission of HCV is primarily through exposure to infected blood (transfusion before 1990`s, intravenous drug use, high-risk sexual activity, solid organ transplantation from an infected donor, occupational exposure, hemodialysis) and 70-85% of infected individuals develop chronic liver disease.

Taking into consideration that acute and chronic compensated hepatitis are asymptomatic in most patients, screening for infection using enzyme immunoassay to detect the HCV antibody is of great importance. Unfortunately, despite the fact that new direct antiviral drugs with success rates of over 90% are available, not all patients can have access to them via the national health system.

Our wish is that, in the near future, all chronic hepatitis cases can benefit from the best treatment available, as this is the best way to prevent natural evolution to cirrhosis and hepatocellular carcinoma, but also lower prevalence and ideally, some day, eradicate this disease.

Results and conclusion: Unfortunately, in the XXI`s century, despite the incredible scientific progress that allows easy diagnosis and next to ideal treatment of this viral infection, morbidity and mortality due to complications of chronic C hepatitis are still high, but the future seems bright.

Quality in colonoscopic screening

Cătălina Diaconu, Florentina Ioniță Radu, Mariana Jinga, P. Nuță, B. Macadon, Săndica Bucurică, M. Pătrășescu, Andrada Popescu, Gaudia Mănescu Avram, D.O. Costache, Raluca S. Costache

Introduction: For a long period of time the only method of screening in colorectal cancer was the guaiac fecal occult blood test. Colonoscopy has been taken into consideration more and more, having a higher specificity and sensitivity. Early detection of cancer increases the chance of successful treatment.

Studies show that screening colonoscopy reduces cancer incidence by 74% and right-sided cancer by 64%. There are plenty of risk factors in colorectal cancer, some that might be diagnosed endoscopically: polyps, prior colorectal cancer, familial adenomatous polyposis, inflammatory bowel disease or hereditary non-polyposis colon cancer. Combining the two diagnosis tests: fecal occult blood test and colonoscopy is a possible screening strategy, more effective than the tests conducted alone.

Patients and methods: We performed a retrospective study between January 5th and May 5th 2016 and included 171 patients diagnosed by colonoscopy with colon tumor (benign or malignant). Moreover, hystopathological examination was made on the masses taken through biopsy.

Results: The median age of our patients was 64.22 years, with a male prevalence of 72.51%. A total of 209 tumors were diagnosed, most of them in the descending colon, followed by rectal masses. Only 6.7% on the tumors were present in the cecum. Most of the tumors were under 1 cm (141). Most of the benign tumors (63%) were low grade dysplasia, 34% of the tumors were medium-grade dysplasia and only 7.3% are high-grade dysplasia. 29 out of 171 patients were diagnosed with adenocarcinoma.

Conclusion: Colonoscopy has become fundamental in the screening of colorectal cancer. There are many factors that influence the quality of colonoscopy: preparation, endoscopy unit and examiner. Determining the pathology of polyps has been the focus of many studies throughout the years.

The effectiveness and tolerability of DAA regimen (Viekirax+Exviera±Ribavirin) in patients

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Introduction: This study evaluates the effectiveness and tolerability of IFN-free therapy (Viekirax+Exviera±Ribavirin) in subjects with chronic HCV infection with genotype 1b and high degree of fibrosis (F3, F4).

Materials and methods: We included 30 patients (18 men + 12 women) with chronic HCV genotype 1b infection, aged 43 to 77 yo, with hepatic fibrosis F3/F4 (1 subject with F3 + 29 subjects with F4) and HCV RNA ranging from 20580 to 6500842 IU/ml, of whom 20 had been previous relapsers, 4 non-responders, and 6 naive.

Patients received Viekirax 12.5/75/50 mg + Exviera 250mg (subjects with F3) + Ribavirin 1/1.2g, according to the patient's weight (subjects with F4), daily for 12 weeks. Blood samples were collected at 4, 12, and 24 weeks.

We determined sustained virological response (SVR), treatment discontinuation rates and the occurrence of adverse events.

Results: Of 30 patients enrolled, SVR occurred in 28 (93.33%). The SVR rates among subgroups were: 100% in cases of cirrhosis, 100% in previous non-responders, 95% in previous relapsers, and 83.33% in naive patients.

Two patients discontinued treatment (6.66%) – the only patient having liver fibrosis F3, but serious comorbidities, died and another suffered a stroke because of uncontrolled hypertension.

Among the rest, the most frequent adverse events were fatigue (83.33%), pruritus (76.66%), nausea (56.66%) and 10 patients (33.33%) developed anemia.

Conclusions: IFN-free therapy has significantly improved SVR rates in patients with chronic HCV genotype 1b infection, reaching an almost unbelievable level of 96.66% in patients with fibrosis F4, with acceptable adverse events and tolerability for most subjects.

Gastric xanthomas – clinical and endoscopic characteristics in a large volume center

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Introduction: Gastric xanthomas are yellowish plaques characterized by the presence of lipid-laden histiocytes, located in the gastric mucosa or submucosa. Although their significance is not clearly known, some reports have associated them with mucosal injury and even premalignant conditions of the stomach. Our aim was to evaluate the clinical and endoscopic characteristics of gastric xanthomas in our endoscopy setting.

Methods: We retrospectively evaluated all upper gastrointestinal endoscopies performed in our high-volume center during the last three years (June 2013-June 2016). We selected patients in whom a xanthoma was described in the endoscopy report and confirmed by biopsy.

Results: Altogether 43 patients, 32.56% female, mean age 58 ± 11 years, had at least one xanthoma described in their endoscopy report. Among them, 11 were not biopsy-confirmed, and 6 were not biopsied. 26 patients were included in the final analysis – 11.5% had multiple xanthomas (range 2-5), while the others were single lesions. Regarding their distribution, 38.5% were located in the fundus, 19.2% in the body and 42.3% in the antrum. 20/26 (76.92%) of patients had associated gastritis – mostly erythematous-exudative (70%) and atrophic with/without metaplasia (15%). H. Pylori was tested in 14/26 patients and was positive in one third of them. Almost half of patients (46.15%) had associated dyslipidemia, mostly hyper-trygliceridemia. Only 7.69% had cholestasis.

Conclusions: In our cohort, gastric xanthomas were mostly solitary lesions, located in the fundus or antrum, associated with gastritis and dyslipidemia.

OP session MD5

Not all you see is a tumor

C. Sandu, Mihaela Gheorghiu, B. Teodorescu, M. Ștefănescu, F. Năftănăilă

Cerebellar abnormalities have a wide spectrum and can reveal us a surprising appearance and behavior, often becoming an interdisciplinary challenge.

The frequency and importance of the cerebral posterior fossa assessment have increased over the last years, having a close connection with the development of magnetic resonance technique.

The differentiation between tumor or non-tumor lesions requires a multimodal diagnostic approach, especially for the tumor-like forms of vascular, inflammatory and developmental abnormalities, that makes the definitive diagnosis difficult.

There are still problems to classify these potentially pathological features into certain groups. A good example could be dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease, part of COLD syndrome), a borderline disorder with slowly progressive tumor, hamartomatous and malformative elements, whose pathogenesis remains unknown. Recognition of this disease could help us to detect concomitant malignancies in these patients.

Posterior fossa lesions may influence prognosis and quality of life similar to supratentorial structures and we should always offer the same consideration to assessment.

Evaluation of 3D conformational techniques for breast cancer radiotherapy

Alina Tănase, M. Dumitrache, Ș. Vlad

Introduction: High incidence of breast cancer and anatomical variations from one case to other, led to different approaches for treatment planning, in order to fulfill QUANTEC dose constraints. Patients anatomy show a large variation in terms of geometry, and flexibility of a single technique is limited. In general, if we use photon tangential beams (internal – external) to get a homogeneous dose distribution, fluency must be modeled using filters or field in field technique. For patients with small breasts or chest wall with important concavity, classic technique is not always suitable due to the relatively high dose received by ipsilateral lung.

Materials and methods: For treatment planning evaluation we selected a case which has a relatively pronounced concave chest wall. A dose reduction was investigated in the ipsilateral lung through a different approach (3-4 fields) related to target volume. We analyzed the dose – volume histogram (DVH) obtained for the case under discussion, 3D dose distribution, homogeneity and conformity indices for the two different treatment planning methods.

Conclusion: In some cases the classical approach (tangential beams) is not appropriate, especially for pronounced concave geometry; the study shows that we can get a dose reduction to the ipsilateral lung using three fields technique, but with the disadvantage of a slight fall of dose homogeneity in the target volume. Meanwhile, according to conformity index (CI), this technique shows higher values of CI, compared to the classical approach.

Evaluation of lymph nodes using ultrasound

Carmen Tipar, Valerica Voicu, C. Mazilu, Raluca Mititelu

Introduction: Ultrasound evaluation of lymph nodes is an extension of the clinical examination. Detection and characterization of nodules is very important given the number of bodies who may be inflammatory or neoplastic diseases. Lymph nodes may be inflammatory, reactive, tumor or mixed.

Aim: Ultrasound's contribution is to highlight the semiological characterization of lymph nodes and the role it has in differentiating benign from malignant lymph nodes.

Material and method: A retrospective study of patients who came to our department was performed to carry out an ultrasound ganglion (lateral-cervical, axillary, and inguinal). The study comprised a total of 15 patients.

Results: Of the total number of patients 6 showed with benign lymphadenopathy, while the rest had malignant lymph trait, their trait was later confirmed by pathological examination. Patients showed semiologic elements consistent with benign and malignant adenopathy.

Conclusion: Ultrasound imaging is useful exploring lymphadenopathy, and features standard ultrasound and color Doppler examination that are useful in their analysis. Ultrasound allows differentiation of metastatic lymph nodes reagents with a specificity of 83% and sensitivity of 95%.

Smoldering systemic mastocytosis

Florina Topliceanu, Cristina G. Vîrlan, M. Șotcan, E. Dănăilă

Introduction: Systemic mastocytosis, often termed systemic mast cell disease (SMCD), is a myeloproliferative neoplasm characterized by infiltration of clonally derived mast cells in different tissues, including bone marrow, skin, the gastrointestinal tract, the liver, and the spleen.

Manifestations of systemic mastocytosis may include the following: anemia, coagulopathy; GI symptom (abdominal pain); gastroesophageal reflux disease (GERD); pruritus, flushing, anaphylactoid reaction.

The major diagnostic criterion for systemic mastocytosis is the presence of dense infiltrates of mast cells in bone marrow or other extracutaneous tissues. Mast cells should be seen in aggregates of 15 or more.

Material and method: We present the case of a 68 years old men who was admitted on general surgery for abdominal pain in the left flank and left iliac fossa, weakness and weight loss.

Results: We started the investigation by performing a thoraco-abdomino-pelvic tomography which identified hepatic hilar and great omentum lymphadenopathy and hepatosplenomegaly. A lymph node biopsy was performed and histological and immuno-histochemical exams revealed systemic mastocytosis. A bone marrow biopsy was also performed which revealed mast cells infiltration of 45-50% thus confirming the diagnosis of systemic mastocytosis.

Discussions: Given the presence of B-findings (splenomegaly, lymphadenopathy) and none of the C-findings (organ failure) the patient is diagnosed with smoldering systemic mastocytosis. Under corticosteroid (Medrol) treatment the patient's condition has improved. The patient will always have on him an epinephrine pen (high risk of anaphylaxis).

Hodgkin lymphoma – prognosis and management

Cristina Vîrlan, Florina Topliceanu, E. Dănăilă, M. Șotcan

Introduction: Hodgkin lymphoma is a potentially curable lymphoma. Prognosis depends upon a large number of variables, including the stage of disease, presence or absence of B symptoms, age of patient and biological determinants, so the overall 5-years survival can go from 91% to 41%.

Material and methods: This is a case report of a 42 years-old man that presented to our clinic with high, prolonged fever, night sweats and weight loss. Together with the laboratory and imaging work-up, the diagnosis was strongly suggestive for Hodgkin Lymphoma, but the patient refused any further investigation.

Results: After 6 months from the initial presentation, the worsening state of the patient encouraged him to continue the management. The diagnosis of Hodgkin lymphoma was certified by the histopathological studies. Imaging studies showed general involvement of ganglia and dissemination to extranodal organs (liver and spleen). Chemotherapy was started. After 3 cycles, a CT-scan was performed that showed favorable evolution. An intermediate PET-CT evaluation presented complete remission.

Discussion: Even though Hodgkin lymphoma is a curable cancer, delay of treatment and spreading of the disease can bring a very low prognosis. Correct chemotherapy and supportive treatment may change the odds and result in complete remission.

Common errors in doctor-patient communication in oncology

Ș. Vlad, M. Matei, G. Bălașa

Objectives: Doctor-patient communication is essential in medicine. Communication aspect of the physician-patient relationship is very important in oncology, where diagnosis, prognosis and treatment decision are difficult tasks for oncologists. We try to identify, first the most frequent errors and the barriers in communication with patient in oncology, and second the interventions to enhance patient-centered communication and patient satisfaction and outcomes.

Methods: We identify the most frequent errors in communication with cancer patients: communicating in medical jargon, insufficient time for preparing communication of the cancer diagnosis or to discuss the prognosis, the use of internet in healthcare, not listening for patient’s emotions and fears, the symptom-focused nature of actual communication in oncology. We present the most efficient interventions to eliminate this errors and to enhance the doctor-patient relationship.

Conclusions: Having good communication skills, knowing the errors and barriers for efficient communication with cancer patients is essential to establish a good doctor-patient relationship. Efficient communication is important because it improves patient satisfaction, compliance with oncological treatment and patient’s emotional and functional status.

Retrospective analysis of patients with cancer of the cervix attending radiotherapy department – statistical analysis of DVH

Maria Vlăsceanu, Alina Tănase, M. Dumitrache, Ș. Vlad, G. Bălașa, M. Matei

Aim: It is well known that, the cervix cancer continues to be one of the most common gynecologic malignancy not only in Romania, but around the world. The aim of this study was to investigate the side effects of the small bowel in cervix cancer RT treatments, using 3D CRT technique, in accordance with our protocols.

Materials and methods: 177 selected patients were treated using box (four field) technique. Prescribed doses to PTV were between 45 Gy and 54 Gy with a fractionation of 1.8 - 2.0 Gy/session.

Treatment planning was done using Eclipse ver.11 with AAA algorithm and delivered by a UNIQUE medical accelerator with 80 leaves MLC.

3D reconstruction was based on a CT SIM SOMATOM Spirit from SIEMENS. For all patients we have used specific immobilization systems from Q-fix. Onboard imaging orthogonal technique was used for optimum positioning of every single case, starting with 3 consecutive verification and rechecked every two days along the whole treatment. Almost 95% of the patients in this study finished their RT treatment without any major complication.

Results and conclusion: The analysis was performed mainly on small bowel Dmax and V45. The delineated volume considered was the potential space within peritoneal cavity (OAR). In cases where radiotherapy was delivered concurrently with chemotherapy were observed side effects (e.g. nausea, vomiting, and diarrhea) for Dmax exceeding 48-50Gy. On the other hand, no statistical correlation between V45 and major side effects were observed for OAR volumes between 100-600 cc.

Ultrasonography importance in parotid lithiasis

Valerica Voicu, Carmen Tipar, Raluca Mititelu, C. Mazilu

Introduction: Sialolithiasis is caused by the obstruction of a salivary gland or its excretory duct by formation of calculi. It commonly involves the submandibular gland (80-95%), less frequently the parotid (5-20%) and the sublingual glands (1-2%). Parotid lithiasis usually appears on male patients between the age of 30 and 60. Calculi affecting the parotid gland are usually small, unilateral, and are located in the duct.

Material and methods: A 54 years old male came to the maxillofacial department of our hospital because he accused acute pain and unilateral inflammation in the left cheek region. The pain aggravated at mealtimes. There was no history of trauma at this level. Because ultrasound represents an excellent diagnostic technique, it was the first investigation for which he has opted.

Results: Ultrasound revealed a dilated duct with a relatively sinuous path along the entire length of 14 mm, with 8 mm diameter calculi.

Discussions and conclusion: The etiology and pathogenesis of salivary calculi is not known. Several hypotheses put forward to explain the etiology of these calculi include: mechanical, inflammatory, chemical, neurogenic, infections, strange bodies. Traditional theories suggest that the formation of sialoliths occur in two phases: formation of a central core and a layered periphery. Whereas parotid sialoliths are thought to be formed around a nidus of inflammatory cells or a foreign body.

Usually the parotid sialolithiasis are unilateral and they predominantly affect the salivary duct than the gland. Treatment depends on size and location of sialolith.

The incidence of tracheoesophageal fistulas and its major determinant factors

B. Petre, Denisa Ghinescu, Ioana Oprea, O. Bratu, N. Tănase, D. Corneci, Mirela Bidilică, L. Ene

A tracheoesophageal fistula is a congenital (frequency 1-5000) or acquired communication between the trachea and esophagus. This condition often lead to severe and fatal pulmonary complications.

In congenital condition diagnostics was made immediately following birth or during infancy.

Acquired tracheoesophageal fistula occur secondary to malignant infection, disease, trauma, and ruptured diverticula. In ICU characteristic is post-intubation tracheoesophageal fistula following prolonged mechanical ventilation with an endotracheal or tracheostomy tube.

If the tube cuff is overinflated, this will leads to compression of the rear wall membranous trachea, which in time goes to ischemia and necrosis, forming an abnormal communication between the trachea and esophagus. Another complication of the overinflated tube overinflated are tracheal stenosis or bleeding.

If cuff pressure is too low then air leaks can appear or microaspiration. This is the reason that cuff pressure must be kept within an optimal range (20-30mmHg) that ensures ventilation and prevents aspiration while maintaining tracheal perfusion.

Besides this major determinant factor – cuff pressure, are described many precipitating factors like age, nutritional status of the patient, presence or not of sepsis, hemoglobin, steroids, presence of gastric tube.

Appear of pulmonary fistula and subsequent contamination of the lung may lead in suppurative complication. This increase the use of antibiotics and prolong ICU stays.

The occurrence of this complication in the evolution of hospitalized patients in ICU have a negative prognostic and is associate with an increase in mortality, in spite of fast diagnostic and rapid therapeutic approach. Extremely important it is to prevent this complication by identifying and removing risk factors.

Daily cuff pressure measurement and reducing period of mechanical ventilation are the keys in preventing this complication.

A rare diseases in adult – pulmonary Langerhans cell histiocytosis, case report and literature review

Claudia Popovici, Gabriela Andraş, Ioana Ştefănescu, C. Ioniță, I. Jascu, E. Firoiu, Florina Vasilescu

Cigarette smoking has a clear epidemiological association with lung diseases, characterized by chronic inflammation of both the bronchiolar and interstitial lung compartments.

Adult pulmonary Langerhans' cell histiocytosis (PLCH) is a rare disorder of unknown etiology that occurs predominantly in young smokers, with an incidence peak at 20–40 years of age.

High-resolution computed tomography (HRCT) of the chest is essential to the diagnosis. A high macrophage count in broncho-alveolar lavage (BAL) fluid is a common but nonspecific finding that merely reflects exposure to tobacco smoke.

BAL is useful for eliminating infections and the other infiltrating lung disorders that can be seen in young adults. Langerhans' cells can be identified in BAL fluid, but, in contrast to what was initially hoped, this test shows a very low sensitivity and is rarely useful in the diagnosis of the disease.

The definite diagnosis of PLCH requires identification of Langerhans' cell granulomas, which is usually achieved by surgical lung biopsy at a site selected by chest HRCT. In practice, however, lung biopsy is performed on a case-by-case basis.

No effective treatment is available to date, and improved understanding of the mechanisms involved in the pathogenesis of PLCH is urgently needed, and should help in the development of specific therapeutic strategies for patients with this orphan disease.

Smoking cessation may prove to be the most important and effective therapeutic option for patients with "smoking-related ILD," and should be strongly encouraged.

OP session MD6

Random and systematic positioning error study in radiotherapy of cervix – Emergency Central Military Hospital experience

V. Bălăbăneanu, M. Dumitrache, Alina Tănase, Maria Vlăsceanu, Ş. Vlad, M.C. Matei, G. Bălașa

Introduction: The purpose of this paper is to analyze and recommend an appropriate method for taking into account geometric uncertainties during RT treatments of cervix cancer, based on verification using MV Portal Imager and to provide guidelines for the local clinical implementation at Emergency Central Military Hospital" Dr. Carol Davila".

Materials and methods: During this study we identified 26 cases between 2014 and 2016, treated using 3D CRT box technique. Doses prescribed to PTV were between 46-50.4Gy (1.8-2.0 Gy/fraction). Treatment planning was performed using Eclipse v.11 and delivered by an UNIQUE medical accelerator. The patient positioning was based on EPID orthogonal images technique and offline reviewed in ARIA environment, where shifts for all 3 axis were determined for data study set. Specific immobilization tools from Q-fix were used in all cases.

Results and conclusions: It is well known that the overall mean set-up error is an indicator of any unwanted systematic component acting on all studied patients. Therefore, analyze is based on the individual mean set-up and random error calculated using shifts from offline review in order to determine the optimum PTV margins. By implementing the study results, our opinion is that it can be achieved better coverage for target volumes for cervical cancer in clinical daily practice.

Percutaneous core needle biopsy under CT-guidance

Alexandra Calu, M. Curea, L. Eftimie, Rodica Bulata, Liana Toma, Florina Vasilescu, C. Bețianu, M. Dumitrescu

Introduction: The treatment of cancer has significantly changed over recent years, with the advent of numerous targeted therapies, therefore a more complex approach of pathological diagnosis is being necessary.

Additional tests such as immunohistochemistry and molecular biology became almost indispensable and increased the need for adequate tumor samples. For this purpose, percutaneous core needle biopsy under image guidance has become widely accepted.

It is the baseline method when surgery is not possible nor indicated, offering a more adequate sample than fine needle aspiration.

Objectives: The aim of this report was to analyze the degree of concordance between the computed tomography and histopathological results in a cohort of patients undergoing a percutaneous needle biopsy under CT-guidance. We also wanted to provide information on our experience with CT-guided biopsy and to evaluate the accuracy and utility of this procedure.

Materials and methods: We retrospectively analyzed the results of CT-guided biopsies performed between January 2015 and July 2016 at the Central Military Emergency University Hospital “Dr. Carol Davila”, collected from the hospital’s database. Variables such as gender, age, CT findings, and histopathological features were assessed.

Conclusions: Percutaneous core needle biopsy under CT-guidance is a useful, minimally invasive and less expensive procedure, with a high rate of accuracy in the diagnosis of malignant lesions, providing adequate tissue specimens for the complete spectrum of the histopathological, immune-histochemical and molecular tests in different cancers in order to guarantee best patient care.

Cancer is not a matter of age... Case report and literature review

M. Curea, O.C. Voinea, Florina Vasilescu, L. Eftimie, Liana Toma, Rodica Bulata, M. Dumitrescu

Objectives: Metastases are a common cause of death in oncologic patients. When are found at first presentation for cancer diagnosis, life expectancy is very pour. This issue presents 3 problem: 1. the asymptomatic but rapid progression in some of the malignant proliferative lesions. 2. The attention by the general practitioner at the periodic exams; 3. The medical education of people

Methods: We present the case of a 29 years female, who died in the Neurology Department of our hospital. At presentation she came with signs of a stroke, but the medical papers revealed that the patient had been diagnosed a week before in another hospital with multiple tumors involving liver (for which she received emergency surgery), kidney, spleen, brain (cerebellum) and interventricular septum of the heart, without knowing the primary site. An autopsy was performed.

Results: The autopsy revealed the presence of tumors in the above mentioned organs and a tumor process involving the left ovary, adhering to the mesenteric and intestinal structures.

Conclusion: This case is a proof of the autopsy exam importance in explaining the thanatogenesis. Even postmortem, for medical knowledge and for the deceased’s family, the histopathological diagnostic must be completed. It is also educational for general physicians to be aware of this issue. And it is important to keep in mind that some lesions may progress in a rapid but silent manner.

Is the tissue always necessary – correlations between cytological and histological changes in pathology

L. Eftimie, M. Curea, Rodica Bulata, Liana Toma, Florina Vasilescu, A. Calu, O.C. Voinea, M. Dumitrescu

Introduction: The clinical and imagistic diagnosis of malignancy must be confirmed in pathology departments. Histopathological diagnosis has evolved during years with the use of immunohistochemistry and molecular biology. However, histopathology and cytopathology are the main tools utilized in the diagnosis of cancer.

Objective: The aim of this study was to compare the accuracy between cytopathology and histopathology used in the diagnosis of cancer at the same patient, knowing that the costs for histological studies are higher than cytology.

Materials and methods: Medical records of patients requiring biopsy and cytology for diagnosis that were studied between August 2015 and July 2016, were retrospectively evaluated. Cytology samples were obtained by liquid aspirated from different parts of body and histopathologic samples was made by biopsy (less invasive, surgical techniques or at necropsy). Coloration methods was standard – Hematoxylin and Eosin (H&E) for histology and Papanicolau for cytology. On the cytology cases studied, we have noticed presence or absence of atypical neoplastic cells which we have correlated with biopsy diagnosis.

Conclusions: This study confirmed that cytological examination is a reliable and useful procedure only for rapid diagnostic and staging of malignant tumors (tumor invasivity) due to faster results, lower cost and lower invasiveness. However, cytology is not always sufficient without biopsy for a complex oncological treatment.

Role of pelvic MRI in establishing the clinical target volume in radiotherapy treatment of localized prostate cancer

M.C. Matei, Ș. Vlad, G. Bălașa, Manuela Bârnă

Purpose: To compare the prostate volumes defined on magnetic resonance imaging (MRI) and non-contrast computerized tomographic (CT SIM SOMATOM Spirit - SIEMENS) scans used for three-dimensional (3D-CRT) treatment planning.

Methods and materials: Eight patients were simulated for treatment using standard immobilization devices from Q-fix. 3-D images were used to compare prostate volumes defined by MRI (4–6 mm thick slices) and CT images (3-5 mm thick slices). Segmentation ability of the following pelvic structures: prostatic apex (PA), prostate, rectum, bladder, and seminal vesicles (SV) were evaluated by four independent observers. Prostate volumes were calculated in cm³ using Eclipse ver.11 3D planning system. MRI/CT images were merged using bony anatomy to define the regions of discrepancy on prostate volumes.

Results: The mean prostate volume was 16 % larger (range 5% to 29%) when defined by non-contrast CT compared to MRI. The areas of non-agreement tended to occur in three distinct regions of discrepancy: (a) the posterior portion of the prostate, (b) the posterior-inferior-apical portion of the prostate, and (c) the apex.

Conclusion: There is a tendency to overestimate the prostate volume by non-contrast CT compared to MRI. Awareness of this tendency should allow us to be more accurate in defining the prostate volume during 3-D treatment planning.

Use of lymphoscintigraphy in detection of sentinel nodes

C. Mazilu, Raluca Mititelu, Valerica Voicu, Carmen Tipar, B. Marinescu

Introduction: Regional lymph node involvement in oncologic patients is a very important prognostic factor, decreasing the 5-year survival rate. This has led to elective lymph node dissection in an effort to better classify patients for prognosis and treatment regimens. In complete lymphadenectomy, short-term complications include seroma, wound infection and breakdown, lymphedema, and paresthesia; long-term complications include lymphedema, paresthesia, and hernia formation.

Therefore, lymphoscintigraphy could help identify the lymph node at highest risk for metastasis, obviate radical lymph node, and possibly prolong survival in such patients.

Material: Lymphoscintigraphy for detection of sentinel nodes in patients with breast cancer or malignant melanoma was performed using intra-dermic peritumoral injection of Tc-coloidal agent (Nanocoll). We administered 2 mCi of radiotracer (4 sites x 0.5 mCi each). Dynamic and static acquisitions were performed using a gamma camera with double-header (PICKER AXIS). Sentinel node resection was performed after at least 6 hours following tracer administration.

Method: According to suspected pathology, planar and dynamic images were centered on injection site, with images of sentinel node - first lymph node which shows a communication with suspected/confirmed tumor location. Initial anterior and posterior images were completed with lateral and oblique images, obtained later. Sentinel node resection was performed in some cases in Plastic Surgery Department, using an intraoperatory gamma probe, followed by resection of the lymph node(s) with highest count activity.

Results: In most of the cases, sentinel node was detected in expected area – popliteal and inguinal region for foot/leg lesions, ipsilateral axilla for trunk lesions. Some patients revealed more than one sentinel nodes, located in the same/different lymph nodes stations. Due to guided surgical intervention and minimal post-surgery effects, lymphoscintigraphy is very useful in targeted lymph node resection in selected patients.

OP session MD7

A rare cause of rheumatoid syndrome

Oana Stancu, V. Smedescu, I. Copaci, M. Șotcan, C. Jurcuț

Establishing the etiology of a rheumatoid syndrome represents a challenge in daily clinical practice. Beside the common causes it is important to take into account the unusual etiology when we organize the imaging techniques and laboratory work-up.

We present the case of a 52-years-old, admitted in our department for diffuse bone and joint pain accompanied by important morning stiffness, involuntary weight loss and normochromic anemia of unknown etiology. Laboratory tests were negative for markers of autoimmune or neoplastic diseases. Moreover, upper and lower endoscopy were negative for any relevant disease. We performed a computed tomography which revealed: small lymph nodes <2cm and mixed bone lesions (appearance also revealed by bone scintigraphy). Serum protein electrophoresis and immune-fixation were normal. We performed flow cytometry examination of the peripheral blood that suggested the diagnosis of mantle cell lymphoma with minimal peripheral discharge. The next step was osteomedular biopsy with histopathology and immune-histochemical examination showing a hyper-cellular bone marrow with the presence of common focal infiltrates consisting of atypical mast cells. At immune-histochemical examination, aberrant presence of CD25 was showed. The final histopathological appearance was of systemic mastocytosis. Regarding the positive diagnosis of systemic mastocytosis we met the World Health Organization criteria. In patients with bone and joint symptoms without a very evident cause, the systemic mastocytosis might be taken into account.

What surprises can hide subacute infective endocarditis

Oana Stancu, C. Jurcuț, V. Duțescu, Anca Manolache, V. Smedescu, I. Copaci

Introduction: Acute mesenteric ischaemia (AMI) represents an important surgical emergency, which has a high morbidity and mortality. The most frequent causes of AMI are represented by arterial emboli, which are derived from cardiac sources, rarely in infective endocarditis (IE).

Material/methods: We present the case of a 67-year old man, smoker, known with arterial hypertension and COPD, who presented in our department for fever, marked fatigue, diffuse abdominal pain, and right upper limb paresthesia, started 5 days before admission. Three weeks before, he was admitted in another department for fever and cough, diagnosed as pneumonia and treated with antibiotics. During the hospitalization he presented acute abdominal pain, was diagnosed with acute mesenteric ischemia and an enterectomy with termino-terminal anastomosis was performed. Later, he showed transient clinical signs of acute right upper limb ischaemia. Transthoracic cardiac ultrasound was normal but transesophageal revealed a large vegetation on the aortic valve with no signs of regurgitation. Blood cultures were negative. After one week of afebrility fever reappears, despite antibiotic treatment, generated by an abdominal collection who was surgical treated and an ileostomy was performed. The evolution was favorable, but after 3 weeks of treatment he accused fever and chills, developing right jugular and subclavian veins thrombosis. We started anticoagulation therapy with NOAC with complete resolution.

Discussion/conclusions: IE is a complex disease, with various symptomatology, with many potential complications, very well illustrated by this case.

Ultrasound Power Doppler evaluation in patients with rheumatoid arthritis with anti TNF treatment

Dana Anghel, M.L. Ciobîcă, N.C. Anghel, G.D. Stoicescu, A. Anghel, Maria M. Negru, C.V. Jurcuț, Ancuța Coca

Background: The goals of treatment for patients with rheumatoid arthritis are remission or decreased disease activity, stopping the rate of joint damage. The subclinical synovitis is associated, despite clinical remission, with progression of structural damage. Imaging technique such ultrasound is capable to provide a more accurate measure of disease activity.

Objectives: To assessed in patients with rheumatoid arthritis in clinical remission the presence of subclinical synovitis by ultrasound.

Methods: The study included 60 patients with RA (ACR/EULAR 2010 criteria) in clinical remission. Medium age is 45 (range 30-59) years. The patients was in clinical remission (DAS<2.6); 81% are female; 65% are positive for rheumatoid factor and 85% for ACCP (anticitrullinate peptide antibody). PDUS examination was performed using Esaote US machine equipped with linear probes (5-12MHZ). PDUS (power Doppler ultrasound signal) investigated metacarpophalangeal and proximal interphalangeal joints and wrist symmetrical. PDUS used 4 grade (semi quantitative score) from grade 0 to 3. PDUS was performed at baseline and after 6 months to beginning of treatment. Furthermore, DAS 28 and laboratory data (ESR, CRP) were obtained at baseline and after 6 months to beginning treatment and clinical remission.

Results: All patients were in clinical remission after 6 months of treatment. PDUS were used as a measure of active disease. Synovitis grade 0 has been found in 28 patients (remission) PDUS grade 1 has been found in 13 patients, PDUS grade 2 in 9 patients and no PD grade 3 has been found after 6 months. PDUS was more frequently observed in the wrists (40%), MCP 2 (24%), PIP 3 (9%).

Conclusion:

1. The results of study confirm that clinical remission doesn't reflect an absence of synovial inflammation.

2. PDUS is useful in assessing of patients considered to be in remission. PDUS detected subclinical synovitis in the small joints of hands.

3. The other parameters don't show an evident association with the presence or absence of PDUS.

Mixed connective-tissue disease

L. Ciobîcă, I. Sîrbu, Ancuța Coca, F. Berghea, Alexandra David

Mixed connective-tissue disease (MCTD) has been more completely characterized in recent years and is now recognized to consist of the following core clinical and laboratory features: Raynaud phenomenon, swollen hands, arthritis/arthralgia, acrosclerosis, esophageal dysmotility, myositis, lung fibrosis, pulmonary hypertension, high level of anti–U1-RNP antibodies, antibodies against U1-70 kd small nuclear ribonucleoprotein.

We are presenting the case of a 63 years old man who presented in our department for acrosclerosis affecting his upper arms and mostly his hands. The symptoms began 2 years ago with mild Raynaud phenomenon and the patient ignored them. His medical history includes cardio-vascular diseases and strokes (2 transient and one ischemic) in the last 3 years.

On the clinical exam we found acrosclerosis on the upper and the lower limbs, clinical signs of pulmonary emphysema and normal blood pressure.

Biological: he had inflammatory syndrome with normal CBC.

We've continued our investigation with capillaroscopy and nuclear antibodies. An echocardiography and computer tomography of his chest were also performed.

After all the data were obtained the diagnosis of mixed connective tissue disease was sustained.

Sepsis – what we know by now

S. Dogaru, C.B. Teușdea, M. Toma

Sepsis in an increasing pathology on admission in emergency departments and intensive care units (about 20 %) being a major cause of mortality. Sepsis is also a major problem of public health (over 20 billion $- 5, 2% of admission funds in US). The heavy morbidity in growing old population let the sepsis survivors with long term physical, psychological and cognitive disabilities.

Increasing incidence and costs burden shifted the focus on algorithmic approach (the Surviving Sepsis Campaign guidelines), revised definitions and to early diagnosis.

Presepsin is used as one of the early inflammation markers. Increasing evidence suggest that presepsin compared to reactive C protein, procalcitonin, lactate or the newly introduced suPAR, sTREM1 or pre- adrenomedulin is at least a promising lead. Greater accuracy is obtained by combining sepsis with a score – MEDS, CURB65, or the newly introduced qSOFA. There are also problems to be solved – age and renal failure correlations.

OP session MD8

5 years in Romanian national program for interventional therapy in ST-elevation myocardial infarction

Alice Munteanu, S. Dumitrescu, L. Chiriac, D. Niță, R. Roșulescu, Nicoleta Avram, Irina Florescu

Cardiovascular diseases (CVDs) represent the main cause of death globally, being responsible for 1/3 of all deaths, more than cancer, chronic respiratory failure or diabetes mellitus. CVDs are a major cause of disability requiring high costs for monitoring, treatment and long-term care at home. Almost 17 million people die annually from CVDs. The total number of deaths from CVDs is estimated to reach 25 million by 2020. Every five seconds a death occurs as a result of a myocardial infarction.

Acute ST-elevation myocardial infarction (STEMI) is characterized by prolonged (15-20 minutes), severe chest pain nonresponsive to nitroglycerin administration. ECG shows ST-segment elevation ≥ 0.2 mV in V2-V3 and/or ≥ 0.1 mV in other derivations or new onset left bundle branch bloc. Biochemistry shows: increasing of cardiac enzymes (troponin, CK / CK-MB) showing myocardial necrosis.

A national program called RO-STEMI started in Romania in 2010 in 12 centers organized in a 24/7 system in five regional networks. The implementation of the program had significantly reduced the number of patients treated conservatively (32%). 63% of patients with STEMI were referred to myocardial primary angioplasty, facilitated or delayed, and only 5% received thrombolysis (RO-STEMI register). The number of patients with primary PCI was 10 times higher in 2011 compared to 2007 (SD Kristensen et al Eur Heart J 2014 August 1 35 29 1957-1970). AMI mortality rate decreased significantly, from 13% to 7.55% (RO-STEMI register). The mortality rate was approximately 4.4% in patients admitted for primary PCI, 8.3% in patients receiving thrombolysis and 17.1% in those treated conservatively. (Kristensen SD et al Eur Heart J 2014 August 1 35 29 1957-1970).

Vasile Candea Emergency Clinical Centre for Cardiovascular Diseases is part of the national program RO-STEMI for 5 years. During this period 3453 patients were admitted with STEMI. 2952 patients received primary PCI. 33.1% of the patients were brought to the ER directly by ambulance, 31.1% were transferred from other hospitals in Bucharest, 8.4% were transferred from hospitals at a distance <50 km, 10.2 % from a distance <100 km, 4.9% <150 km, 4.7% <200 km and 7.6% > 200 km. The average time from the onset of pain until calling to the emergency medical system was 3 hours, with a median of 2.5 ore. Only 21% of patients called the emergency medical system within 60min from the onset of chest pain. The time from first medical contact to balloon inflation was less than 90 min in 29% of patients.

The results regarding procedures performed were: 14.5% were not admitted to angiography, 4.4% received only PCI without stent, 52.6% of patients received one stent, 21.5% 2 stents, 6.4% 3 stents and 0.6% 5 stents. The vessel responsible for STMI was in 57% of cases LAD, in 30.2% was RCA, in 11% was CXA and in 1.7% was not specified. 43.6% of patients had single-vessel lesion, 38.1% had two-vessel lesions, 10.8% had three-vessel lesions/multivessel, 5.8% had non-obstructive coronary artery disease and 1.7% had permeable coronary arteries.

The rate of early complications after angiography was 9.3%: 3.5% of patients suffered acute stent thrombosis, 3.2% of patients had pseudoaneurysm, 1.7% of patients had procedural failure and 0.9% had coronary dissection. At discharge 71.5% of patients had not had symptoms of heart failure. 25% of patients showed signs of left heart failure and 3.5% of patient admitted in program died.

Acute myocardial infarction without chest pain

D. Negoiță, C.B. Teuşdea, L. Demiraş, Ana M. Demiraş

Acute myocardial infarction (AMI) can present itself without chest pain. This happens more often in the elderly, diabetics and women. We present the case of an elderly male patient who came to the ER with abdominal discomfort after an episode of faintishness. Careful history taking, physical examination, ECG (without STEMI criteria) and blood tests helped to establish the diagnosis. With this case we want to emphasize that a high index of suspicion for AMI is necessary in the elderly. Proper ECG interpretation of subtle ST changes is also mandatory.

Difficulties in diagnosis and treatment between STEMI and myocarditis

Alice Munteanu, L. Chiriac, R. Roșulescu, Irina Florescu, Cristina Calcan

A national program called RO-STEMI started in Romania in 2010 in 12 centers organized in a 24/7 system in five regional networks. Our hospital is part of this program for 6 years, under which we receive patients with criteria of STEMI for interventional myocardial revascularization.

We present a 59 years old female, hypertensive, obese (BMI 32,3kg/m^2), non-smoker, accusing the first episode of typical chest pain, started 2 hours before the presentation in ER. The diagnosis of STEMI was supported by electrical, biological and echocardiographic criteria. The drug therapy was initiated according to guidelines with double antiplatelet therapy, heparin and morphine. Emergency coronary angiography was performed distinguishing permeable epicardial coronary arteries.

We had to decide if it was STEMI with permeable coronary arteries or myocarditis. For a certain diagnosis she needed a cardiac MRI as soon as possible. In the meantime we had to decide the right treatment for this patient. Considering that we couldn`t exclude the diagnosis of STEMI we decided to continue the specific treatment for myocardial infarction – notice that this diagnosis has a worse prognosis vs. myocarditis.

After 3 months the patient was asymptomatic, ECG was normal, echocardiography showed normal systolic function, without parietal kinetic disorder. Cardiac MRI sustains the diagnosis of STEMI, so the patient continues the previous medication.

In conclusion, we had a patient with cardiovascular risk factors, with all criteria for STEMI but without coronary lesions. The differential diagnosis was challenging, but the decision of treating like a STEMI was right, this having the worst prognosis.

Cardiac investigation of the young patient with stroke

A. Anghel, S. Stanciu, Ioana Răduță, L. Ciobîcă, Lorena F. Davidescu

Stroke in the third cause of mortality and morbidity all over the world, but the number in the young patients is low but with major impact on the invalidity.

The strokes in young are ischemic and hemorrhagic

Most of the strokes in young patients are produced by cardiovascular pathologies, many of them unknown at the moment of the diagnosis.

Causes of strokes in young patients are:

1. Cardio embolic
   a. cardiac malformations (DSA, DSV)
   b. arrhythmias (atrial fibrillation, atrial flutter)
   c. endocarditis
   d. cardiac tumors
2. Atherosclerotic
   a. familiar dyslipidemia
   b. vasculitis
3. Hemorrhagic stroke
   a. hypertension (secondary?)
   b. vascular malformation

Cardiac investigation of the young patient with stroke must be complete including morphological exploration (trans-thoracic, trans-esophageal ultrasound, arterial or venous Doppler ultrasound), monitoring (ECG and TA monitoring) and, if necessary advanced imagistic methods (angio TC, angio MRI).

Because of the importance of the disease and the age of the patient the cardiac investigation is mandatory in the diagnosis but also in secondary and primary prevention.

Multiple etiology of deep venous thrombosis

L. Chiriac, R. Roșulescu, Alice Munteanu, S. Dumitrescu, Adriana Gârjău, Magda Gurzun

We would like to report a case of a male patient of 43 years old who was admitted at the emergency room of the Emergency Military Hospital in Bucharest accusing severe localized pains on the right tie which was swollen and presented all the acute inflammatory characteristics.

The clinical exam revealed severe unilateral edema in the right inferior limb associated with extreme pain and functional impairment. The cardiovascular and respiratory function of the patient were stable. Also hemodynamics wasn’t affected.

The ECG was without anomalies.

The suspicioned diagnostic was profound venous thrombosis of the right inferior limb and confirmed by performing a venous compression ultrasonography and a Doppler exam.

To refine the etiology of the thrombosis the patient has performed a thoracic-abdominal CT scan native first which revealed no other morphological anomalies but a left retropsoic solid mass.

The thoracic-abdominal CT scan with intravenous and oral contrast revealed the absence of the inferior vena cava and identified the existence of a hypertrophic azygos and hemiazygos veins. The retropsoic solid mass was confirmed as a hypertrophy of the paravertebral venouse plexis anomalies which would indicate a compensatory morphological and functional adjustment to the congenital agenesia of the inferior vena cava.

The patient received anticoagulant therapy represented by high molecular mass heparin having an interval between 50 – 70 seconds as target APTT.

From this case is important to draw the conclusion that it may be important to think at the possibility of an anti-phospholipidic syndrome even in patients who have morphological congenital anomalies of the vascular system and proceed with the specific tests that will confirm or rule out this syndrome.

Is it a place for cardiac MRI in acute clinical settings

Maria Gurzun, Lavinia Florea, Raluca Popescu, G. Neagoe, Ana-Maria Cincă, R. Boiangiu, Ileana Hanțulie, Smărăndița Lacău, S. Dumitrescu

Multimodality imaging became mandatory in cardiac patient management nowadays. Among new imagining modalities cardiac MRI became very important during the last years. However, the long-time necessary for examination, the low availability and the high price make it a method less used in acute settings.

The purpose of this communication is to present a short case series for emphasis the utility of cardiac MRI in patient management during the acute phase.

The reported patients were admitted in cardiac care unit for chest pain, shortness of breath with EKG changes and elevated troponin levels. The two patients were angiographically explored and the coronary artery disease was excluded. The cardiac MRI performed during the first three days excluded the myocarditis for the first patient and confirm it for the second one. The second patient received conservative treatment, having a good recovery. During the hospitalization the first patient presented several hypertensive crises and pheochromocytoma was suspected and confirmed by bioumoral markers and abdominal computer tomography.

Therefore, cardiac MRI was essential in patients' management: in the first case excluded myocarditis and suggest another pathology involved, permitting the establishing of correct diagnosis; in the second case myocarditis was confirmed and the patient received the correct treatment.

To conclude cardiac MRI can be a useful tool for cardiac patient management even in acute clinical settings.

TILT table test

V. Ilieșe, R. Bolohan

Head-up tilt table test is used for the evaluation of the syncope, it starts to be used by Kenny and colleagues in 1986 like passive head-up posture.

The tilt testing is used for the evaluation of the reflex syncope and to discriminate between reflex, orthostatic hypotension syncope and jerking movement from epilepsy.

Preparation before the test, patients must be secured, should have a venous access, should fast 2-3 hours, they may continue their usual medication such as diuretics and antihypertensive. Beta-blockers and other medications for the treatment of syncope must be stopped at least 5 times half-life. The pre-test phase usually takes 5-20 minutes. After that the patient is put to 60-80 degrees; this is the passive phase of a minimum 20 minutes maximum 45 minutes, while the blood pressure and the heart rate is monitored from 5 to 5 minutes and from 1 min in case of symptomatology occurrence.

The pharmacologic provocation uses nitroglycerine and isoproterenol.

The response to tilt test is classified in type 1: mixed in which the heart rate and blood pressure decreases without asystole of <3 second; type 2 – cardio inhibitory 2A – without asystole an 2B – with asystole >3 seconds blood pressure decrease coincides with or occurs before the heart rate decrease; type 3: vasodepressor.

This year, in the first 6 months we performed 12 tests, out of which 3 were carotid sinus syndrome with asystole >3 seconds, 4 were with a mixed response, 5 were negative.

In 2014 we had a case of a 31 years old women, without any cardiovascular pathology, who followed medication for the epilepsy 1 month, she did not feel well, TILT test was positive, type 2B cardio inhibitory with a pause of 30 seconds, and as a consequence we implanted a permanent pacemaker.

Cardiac permanent pacing

V. Ilieșe, I. Țintoiu, R. Bolohan, D. Niță, D. Săvoiu, D. Cîrpaciu, L. Chiriac, Adriana Gîrjău, Andreea Teodorescu, Simona Almarichi, Alice Munteanu, L. Demiraș, Ana Demiraș, R. Roșulescu, G. Neagoe, S. Dumitrescu, Magdalena Gurzun, Ileana Hănțulie

Cardiac permanent pacing is an option for treatment of slow usually symptomatic rhythm. The recommendations for permanent cardiac pacing are well detailed in the ESC Guidelines on cardiac and cardiac resynchronization therapy 2013.

As of the beginning of 2016 we implanted 180 VVI; 56 DDD; we use the access from the left side usually horizontal cut. We always try to use the cephalic vein; other options can be the subclavian, axillary, jugular vein; the leads can be with active and passive fixation.

The leads are placed under radiologic guidance; for each lead we have to measure the threshold; wave amplitude; impedance; slew rate.

We use the active leads for the ventricular usually in the septum and the right appendage; the passive leads are used in old patient with low physical activity.

We choose the programming parameters according to patient pathology.

The ECG is the most useful method for the follow up.

The most common complication that we met is the local hematoma that usually doesn't need an intervention. For the lead dislodgment we had 4 cases.

Cardiac resynchronization therapy

I. Țintoiu, R. Bolohan, L. Chiriac, G. Neagoe, S. Dumitrescu, D. Niță, V. Ilieșe, Magdalena Gurzun, E. Pandea, L. Demiraș, D. Săvoiu, S. Cîrpaciu, Alice Munteanu, Andreea Teodorescu, R. Roșulescu, Adriana Gîrjeu, Simona Amarichi, Ana Demiraș, Ileana Hănțulie

Cardiac resynchronization therapy is an electrical therapy option for patients with heart failure with reduced ejection fraction (FE<35%) and a wide QRS duration (LBB>130ms & non-LBB >150ms), also in patients with reduced ejection fraction who need permanent right ventricular pacing (ESC Guideline: "Heart Failure 2016")

Response to cardiac resynchronization therapy is about to 60-70 % the non-responders are patient with ischemic cardiomyopathy and those with non-LBB:

Approach for the cardiac resynchronization: therapy can be done on the left or right side via subclavian incision. This is the most common procedure. For the venous axes we always use the subclavian vein with two punctures and one puncture we use for coronary sinus lead.

The most difficult part is usually the cannulation of the coronary sinus. There are two methods: the electrophysiological approach and the hemodynamic approach.

The electrophysiological approach uses the EP catheter suitable for the coronary sinus. The cannulation is done in the LAO 30° projection. Once the EP catheter is in the coronary sinus the guiding sheath is advanced in the coronary sinus and after that we do the venography of CS.

The hemodynamic approach can use the guiding sheath or the left Amplatz 2 catheter.

The CS lead can be unipolar, bipolar or quadripolar lead. The quadripolar lead allow us to do multisite pacing.

We have 8 cases of cardiac resynchronization: 4 were ischemic and 4 non ischemic; the patients also met the echocardiographic criteria for the atrio-ventricular, inter-intraventricular dys-synchrony; 1 was non responder he was an ischemic non–LBBB; 5 was responders with improvement of the clinical and echocardiographic parameters.

The principal tool that we use for programing CRT is the ECG. It can show you the site of pacing, the location of the lead and the fusion proportion.

For future we ca ensure more CRTs provided that we have the necessary equipment.

Inflammation, periodontal disease and subclinical vascular injury

S. Dumitrescu, L. Chiriac, Maria M. Gurzun, Ileana Hănțulie, R. Boingiu, S. Stanciu, D. Săvoiu, Raluca Popescu, H. Barbu

Introduction: There is a unanimously accepted connection between inflammation and periodontal disease (PD) and between hs-CRP levels and atherosclerosis, nevertheless there are few studies connecting all three.

Objective: We planned to investigate the relationship between inflammation, subclinical vascular disease and the periodontal disease (PD) status.

Methods: We evaluated 190 individuals in the course of a cardiovascular primary prevention program. Risk factors data were collected using an individual assessment sheet and through measurement of laboratory parameters (hs-CRP, lipid profile, blood glucose) in a period of +/- 3 days from the baseline clinical and echo evaluation. Vascular ultrasound data was collected on examinations made in the same day: brachial artery flow mediated dilatation (FMD) and carotid artery intima media thickness (IMT). Data on PD status were collected through a clinical exam performed in a period of +/- 7 days from the baseline evaluation and categorized the study group in a subgroup of normal periodontal status (NPS= 69 persons; 36,3%) and periodontal disease subgroup (PD= 121persons; 63,7%) which was further divided into a gingivitis subgroup (G=95 persons;

50%), mild to moderate periodontal disease subgroup (PM=19 persons; 10%) and severe periodontal disease subgroup (PS=7 persons; 3,7%).

Results: After data collection we compared the mean values for hs-CRP and vascular ultrasound measurements according to PD status. The persons with periodontal disease have an increased level of inflammation and vascular dysfunction (p<0,001). Accordingly, there is a significant correlation between hs-CRP level and both FMD (r=-0,785, p<0,001) and IMT (r=0,360, p<0,001). Subclinical vascular disease is widespread in subjects with abnormal PD status and inflammation (hs-CRP >1 mg/dl), 111 (92%) persons out of 121 having at least one type of subclinical injury. We calculated concordance coefficient for group classification based on inflammatory status (low risk: hs-CRP< 1mg/dl, moderate risk: hs-CRP =1 – 3 mg/dl and high risk: hs-CRP>3 mg/dl) compared with classification based on PD status and we found Kappa=0.715, (95%CI = 0.632-0.799; high concordance).

Conclusions: Our study confirms the connection between inflammation and periodontal disease and links both of them with evidence of subclinical vascular injury.

OP session MD9

Soft palate paralysis – a misleading onset of invasive mucormycosis involving nasopharynx and paranasal sinuses

V. Gheorghiță, R. Hainăroșie, R. Vasilescu, Eliza Grămadă, C. Socoliuc, Mona Popoiu, I. Ștefan, F.A. Căruntu

Introduction: Mucormycosis (MCM) is a life-threatening invasive fungal infection caused by fungi belonging to the Mucorales order. Despite aggressive therapy, the overall mortality rate remains unacceptably high (around 50%).

Material and methods: A 56-years-old diabetic male patient was referred to the "Matei Bals" National Institute for Infectious Diseases on August 7th, 2014 with suspicion of nasopharyngeal (N-Ph) MCM. The disease had been started 8 days before with severe odyno-dysphagia, regurgitation of liquids through the nose, right ear pains and headaches without fever. Laboratory analysis found hyperleukocytosis (21,651/mm^3), inflammatory biological syndrome (CRP 269 mg/L) and hyperglycemia (448 mg/dL). ENT check-up revealed an intense erythema of the posterior pharyngeal wall with a slight bleeding in the cavum and the right nasal fossa. The symptoms have improved under antibiotics (Ceftriaxone + Metronidazole + Levofloxacin), antifungals – Fluconazole and steroids treatment. In 6 days the initial lesions progress to the black necrotic eschar. The tissue biopsy was performed. Cerebral and facial CT scan showed maxillary, ethmoidal and sphenoidal right sinusitis, without brain lesions. We started treatment with combination of lipid formulation of amphotericin B (AMB 5 mg/kg/day) and posaconazole (POS-oral suspension, 800 mg/day) for 13 days, followed by POS monotherapy. Glycemic control was achieved with insulin therapy. Histopathological exam and culture confirmed MCM. Mucor was identified as Rhizopus oryzae by molecular methods. Resection of necrotic tissue was delayed until the 18th day of antifungal treatment.

Conclusion and discussions: In the high risk group of patients this clinical onset (with soft palate palsy) could be indicative for MCM. The early diagnosis was clearly associated with favorable outcome.

Thyroid dysfunction in sepsis in a prospective study

V. Gheorghiță, Alina E. Barbu, Monica L. Gheorghiu, I. Ștefan, A. Streinu-Cercel, Ruxandra Moroti, F.A. Căruntu

Introduction: The objective of our study was to evaluate the thyroid function during sepsis in order to identify a possible correlation with severity and final outcome of sepsis in a prospective on-going study.

Material and methods: A prospective, non-interventional cohort study was conducted in Matei Bals National Institute for Infectious Diseases between January and June 2015. Inclusion criteria included sepsis caused by bacterial infection in HIV negative adult patients who signed informed consent. Apart from demographics, clinical and microbiological data we measured serum thyroid hormones (TSH and fT4) and procalcitonin (PCT) at four different times: T0-admission, T1-24 hours, T2-72 hours and T3-7th day.

Results: 27 patients met the inclusion criteria. The median age was 67 years, (IQR, 57-78) and 55.5% (n=15) were male. 40.7% (n=11) of patients had severe sepsis and 18.5% (n=5) septic shock. The median value of PCT at diagnosis was 48.8 ng/mL, (IQR, 10.7-88.04). In 48.1% (n=13) of patients the etiology was established. The gram negative bacilli were identified in 84.6% (n=11) of cases. Thyroid dysfunction was present in 29.6% (n=8) of patients, 62.5% (n=5) having hypothyroidism. The median value of TSH in patients with hypothyroidism was 5.134 UI/mL, (IQR, 4.224-6.137). 75% (n=6) of patients with thyroid dysfunction had more severe disease at baseline. The mortality was 37.5% (n=3) in patients with thyroid dysfunction compared to 18.5% (n=5) in the whole cohort, p=0.32.

Conclusion: Thyroid dysfunction was diagnosed in about quarter of patients with sepsis and seems to correlate with the severity of sepsis and poor prognosis.

Psychiatry and terrorism

C. Cândea, D. Vasile, O. Vasiliu, B. Petrescu, A. Mangalagiu, Irina Cândea, A.M. Badic

Terrorism has dominated the domestic and international landscape since 9/11. Like other fields, psychiatry was not well prepared. It is time to consider what can be done to prepare before the next event. Much has been learned to provide knowledge and resources. The roles of psychiatrists are challenged by what is known of the causes of, consequences of, and responses to terrorism. Reflecting on knowledge from before and since 9/11 introduces concepts, how individuals become terrorists, how to evaluate the psychiatric and behavioral effects of terrorism, and how to expand treatments, behavioral health interventions, public policy initiatives, and other responses for its victims. New research, clinical approaches, and policy perspectives inform strategies to reduce fear and cope with the aftermath. This article identifies the psychiatric training, skills and services, and ethical considerations necessary to prevent or reduce terrorism and its tragic consequences and to enhance resilience.

Transcultural psychiatry

C. Cândea, D. Vasile, O. Vasiliu, B. Petrescu, A. Mangalagiu, Irina Cândea, A.M. Badic

This article deals with the main concepts of Transcultural Psychiatry and their applications to everyday psychiatric practice. Transcultural psychiatry has undergone a conceptual reformulation in the last two decades. Having started with a comparative approach, which focused on the diverse manifestations of mental disorders among different societies, it broadened its scope, aiming at present to incorporate social and cultural aspects of illness into the clinical framework. Therefore, transcultural psychiatry now focuses more on what is called the illness experience than on the disease process, the latter understood as illness as it is viewed by health practitioners. Western medicine, of which psychiatry is a part, is grounded in positivist epistemological principles that stress the biological processes of disease. The intention of the paper is to develop an interest in alternative but also complementary ways of thinking. Modern transcultural psychiatry interprets some epidemiological and clinical aspects of major mental disorders (such as schizophrenia and depression) in a different light. However, it also distances itself from the absolute relativism of antipsychiatry, centering on clinical facts and helping clinicians in their primary task of alleviating suffering. An important contribution in addressing this task is the formulation of a cultural axis within the DSM model of multiaxial evaluation. A clinical vignette of a cultural formulation applied to a clinical discussion of a case is described.

Expeditionary modular systems for sampling, detection and identification of CBRN agents

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The research, development and implementation of a modular expeditionary systems for sampling, detection and identification of CBRN agents, that is useful for providing information about the potential CBRN contamination, will ensure the samples necessary to perform the identification and confirmation testing’s in specialized fixed and mobile laboratories with additional protection of the operators.

The novelty of the project proposal consists in designing and developing an integrated expeditionary systems, that can be transported by land, air, sea etc. and which will be provided with a remote drones for recognition of high risk areas and sampling.

Implementation of projects will follow defining the composition of the expeditionary modular system, depending on the missions of the mobile intervention teams, establish methods of sampling and integrating executed modules, making the decontamination devices of the remote drones and of the foldable mini-aerodrome, integration of the components and execution of an experimental model of the expeditionary modular systems, testing the modules under laboratory conditions in order to verify their correct functionality within the system, testing the experimental functional model on the site. The partners have experience in making kits and sampling kits for transport and testing of agents, explosives etc. The system does not exist in the manufacturing or marketing phase, in the proposed configuration.

The transfer for industry of the scientific and technological results is provided by industrial partner's capabilities, which has experience in the production of CBRN defense equipment, as a developer and supplier for beneficiaries of the national security system.

A rare case of hypokalemia – case report

V. Smedescu, Roxana Diaconu, Denise A. Mardale, Cristina Spiroiu, E. Firoiu, Anca Manolache, Mihaela Enache, I. Copaci, C. Jurcuț

We report the case of a 57-year-old woman, who is hospitalized for abdominal pain and jaundice with a one month onset.

The clinical exam further revealed hepatomegaly, dark urine and pale stools. Laboratory exams showed neutrophilic leukocytosis, hepatic cytolysis, cholestasis syndrome and severe hypokalemia.

The upper and lower gastrointestinal endoscopies were within normal parameters, while the chest and abdominal computed tomography showed a left lower lobe pulmonary tumor, multiple mediastinal and abdominal adenopathy and also liver and bone metastasis.

The pulmonary tumor was biopsied and small cell lung cancer was diagnosed. Despite treatment with oral and intravenous potassium salts the hypokalemia persisted, while the patient subsequently accused abdominal cramps and loose stools.

We did further tests which showed high serum levels for cortisol, chromogranin A and serotonin and also high urinary values for 5-HIIA, thus confirming the presence of two paraneoplastic syndromes: secondary Cushing syndrome and carcinoid syndrome.

The particularity of the case is represented by the rare association of these two paraneoplastic syndromes in this type of neoplasia and the discordance between the high hormonal values and the presence of minimal specific symptoms.

An unusual case of palpebral swelling and erythema

V. Smedescu, Oana Stancu, Anca Manolache, D. Chiriță, I. Copaci, C. Jurcuț

We report the case of a 47-year-old woman who presents with fever, left palpebral and zygomatic swelling and erythema, cervical adenopathy, hepatosplenomegaly and erythema nodosum on the right arm. The patient was treated with broad spectrum antibiotics without any improvement.

The laboratory exams showed an inflammatory syndrome, moderate anemia, thrombocytopenia, lymphopenia and mildly elevated aminotransferase levels. The tests for infectious diseases and tumoral markers were normal, while the screening for SLE, Sjogren syndrome and vasculitis was negative. The cerebral and cervical MRI revealed left lacrimal gland and periorbital subcutaneous fat inflammatory infiltrates. The thoracic and abdominal computed tomography showed hepatosplenomegaly and multiple mediastinal and intraabdominal adenopathy. The bone marrow examination was normal, while the pathological exams of an excised lymph node supported the diagnosis of reactive adenitis. A skin biopsy was performed which established the diagnosis of lupus erythematosus tumidus (LET). The symptoms were considerably improved after systemic course of glucocorticoids without recurrence at one year of follow-up. LET is a rare form of cutaneous lupus erythematosus, recently being redefined as an independent entity.

The particularity of this case is the association of LET with several systemic manifestations, however without enough criteria to support the diagnosis of systemic lupus erythematosus.

OP session MD10

Metformin effects on endometrial hyperplasia in women of reproductive age with PCOS

A. Ranetti, Anca Pati-Cucu

Background: Polycystic ovarian syndrome (PCOS) a metabolically determined gynecologic disorder with an incidence of 4 to 184% in women of reproductive age. PCOS diagnosis is set based on Rotterdam criteria, including hyper-androgenism, chronic anovulation and typical ultrasound appearance. Endometrial hyperplasia occurs in 1 to 48% of women with PCOS and can progress to endometrial cancer.

Material and method: 20 non-diabetic women between 25-35 years old, with PCOS untreated prior to presentation, with a BMI>30 kg/m^2, were included in a pilot study. Endometrial hyperplasia was defined as endometrial thickness more than 15 mm in the secretory phase. Endometrial biopsy in all women showed a simple hyperplasia without atypia. 10 women choose progestin-only treatment along with diet (non-Met-group) and 10 started a 6 months therapy with diet and metformin administered daily on a weekly increased dose up to a total of 2 g/day (Met-group). None of the patients were on a meat and dairy free diet. We also reviewed metformin influence on reproductive function with emphasis on endometrial effects.

Results: The Met-group had a significant weight reduction and a decreased endometrial thickness compared to non-Met and also ovulation occurred faster in Met-group.

Conclusions: Metformin, a biguanide used in treatment of type II diabetes mellitus, could be used single or in association with oral contraceptives or progestins in non-diabetic patients with EH. Still, further studies including larger cohorts of patients are needed.

The role of computed tomography (CT) in the evaluation of acute abdominal pain in patients without trauma

C. Sandu, Mihaela Gheorghiu, Cristina Sandu, Diana Soloman Năftănăilă, F. Năftănăilă

Non-traumatic abdominal pathology is one of the most common reasons for admission to the emergency department. Accurate and rapid diagnosis of these conditions helps in reducing related complications. Clinical assessment is often difficult due to availability of over-the-counter analgesics and self-medication practice, leading to less specific physical findings.

Laboratory and conventional radiographic findings are in many cases unhelpful. Thus, cross-sectional imaging plays a crucial role for initial evaluation and management of acute abdomen. Multidetector computed tomography is the primary imaging modality used for these cases due to fast image acquisition. There is no evidence of using one single strategy tool. The clinician may choose either routine ultrasound (US) evaluation complemented by CT in case the US is inconclusive or first-choice CT.

The usual causes of non-traumatic acute abdomen are bowel obstruction, acute pancreatitis, gastrointestinal perforation, diverticulitis, appendicitis, and cholecystitis. Less frequent, but also important causes are ruptured abdominal aneurysm, spontaneous abdominal bleeding due uncontrolled anticoagulant therapy, acute mesenteric ischemia or mesenteric venous thrombosis.

The role of ultrasound guided trans-bronchial puncture in diagnosis of lung cancer

Ioana Ştefănescu, E. Firoiu, Ioana Oprea, Florentina Vasilescu, Chim Aneta Şerbescu

Introduction: Endobronchial ultrasound (EBUS) is a minimally invasive but highly effective procedure used to diagnose lung cancer and other diseases causing enlarged lymph nodes in the chest. Although the method appeared in the late 90s, emerged in Romania for several years, initially in Iasi, after that in Cluj-Napoca. The Military Hospital is the first hospital in Bucharest that brought this technique in December 2015.

Materials and methods: We conducted a study in the Department of Pneumology of SUUMC on the patients with radiologic suspicion of pulmonary cancer, investigated between December 2015 - June 2016 by EBUS-TBNA, with moderate sedation (Midazolam and Propofol).

Results: All the patients were imagistic diagnosed with pulmonary tumors and mediastinal lymph nodes. The majority of patients with diagnostic imaging of lung tumors were confirmed by EBUS-TBNA both cytology and histopathology (cell block).

Conclusions: EBUS-TBNA is a minimally invasive method, with small risk, highly effective in the diagnosis and staging of lung cancer. This method could replace in some cases mediastinoscopy as a method of diagnosis of lung cancer, especially in patients with severe comorbidities. This technique can be improved by using thicker needles, although procedural risks may grow.

Multiple trauma diagnosis and treatment algorithm in ED

B. Teușdea, M. Toma, S. Dogaru

Trauma is in Romania a problem of public health, and it has the highest mortality in the population with ages between 15 and 44 years. Multiple trauma (MT) represents an acute severe status appeared after a mechanic, thermal, chemical or electrical impact, status characterized by multiple lesions at organ and body systems. Lesions that affect more than one organ are characteristic and are frequently fatal, in patients suffering from MT under different disasters /war, therefore this kind of patient needs a prioritization of the lesions, in order to treat them, and he represents a challenge for the trauma team.

The traumatized patient has an unpredictable evolution because it synthesizes multiple traumatic sites, with an immediate and delayed reaction, having a great potential for developing MSOF (syndrome of multiple organ failure). This type of patient develops shock of various origins with high severity. That's why a well-organized chain of medical aid increases the survival of patients with multiple trauma and decreases mortality and morbidity.

Emergency physicians play a vital role in the stabilization, diagnostic and initial treatment of MT patient at ED level. MT patients' management implies complex, time-dependent, decision–making, leadership capability and technical skill. Therefore at ED level we need instruments - algorithms that facilitate the diagnosis and treatment of MT patients. In this paper we present the algorithm for the diagnosis and treatment of MT patient because we consider that an algorithm, using ATLS principles, is necessary to guide management of this type of patients.

Our experience with point of care testing analysis in the Emergency Department

B. Teușdea, M. Toma, S. Dogaru, Luminița Popa

For emergency medicine, about 70% of the decisions, made by the physicians working in the ED and the physicians on duty, regarding admission, discharge and medication are based on lab results. In the last few years, Point of care testing (POCT) in the Emergency Department (ED) is becoming a lot more common.

POCT instruments are used every day in our ED for biomarkers determination, such as cardiac biomarkers (CK-MB, troponine I, NTproBNP etc.), sepsis biomarkers (presepsin), blood gas analysis (PaO2, PaCO2, pH etc.) and also for blood tests – blood cell count, biochemistry and urine tests (toxicology).

We made a comparison between the blood test results obtained on POCT instruments (biochemistry) and the blood tests obtained in the Central Laboratory of the hospital. The final result was a little difference between the 2 lab tests, but with no statistically significance.

The urosepsis

Ioana Oprea, Denisa Ghinescu, B. Petre, O. Bratu, D. Corneci, Mirela Bidilică, L. Ene

Sepsis is a life-threatening problem that leads to increase the mortality, morbidity and costs of healthcare services. In spite of huge efforts up to now, have no reached to safe therapy with reproducible effects systematically.

Within sepsis, interleukins, cytokines and other factors of inflammation reach to significant serum concentration and affect all organs and body systems, getting to acute dysfunctions and shortfall of these. According to Surviving Sepsis Campaign 2012, sepsis is defined as documented or suspected infection, accompanied by various laboratory or clinical variables. Due to major implications arose in the context of the diagnosis and complex therapeutic approach of affected patients, worldwide appear new ideas, concepts and assumptions with frequent updates in order to optimize the septic patient approach with or without associated organ dysfunction.

Medical approach must be multidisciplinary one, targeted both on the treatment of the cause (initial infection), but also to maintain internal homeostasis simultaneously with decreasing the damage of other organs and systems.

When the starting point of sepsis is an infection within the urinary tract, it is called urosepsis. This could be life-threatening, if emergency treatment is not carried out. Statistically, urosepsis accounts for approximately 25% of all cases of sepsis.

The urosepsis remains a challenge for the urologist, intensive care doctor, radiologist and microbiologist. It is very important to have a good cooperation between these specialties and teamwork.

Threat of Burnout Syndrome in the Emergency Department – our experience

B. Teușdea, M. Sălceanu

Burnout Syndrome is defined as a psychological syndrome that occurs among the employees who work in a stressful environment and as a result of an imbalance between expectations and resources. This syndrome is more than an extreme fatigue and involves emotional exhaustion, depersonalization, and a diminished sense of personal accomplishment with a secondary disengagement that occurred among different professionals who work with other people in challenging situations.

Through this paper we hope to raise awareness about this danger and we try to identify how this syndrome affects our staff in Emergency Department at Central Emergency Military University Hospital "Carol Davila" Bucharest.

For this purpose we use a new instrument called "The Oldenburg Burnout Inventory" (OLBI), applied to all our staff members, that includes positively and negatively statements to assess the two core dimensions of burnout: exhaustion and disengagement (from work). We found interesting data and correlations in matter of age, gender and level of qualifications.

Moreover we suggest methods of prevention and treatment in order to increase the quality of life and satisfaction both for employees, employer and the beneficiaries of the medical services that we offer.

Percutaneous tracheostomy – the experience in SUUMC ICU

Ș. Zahiu, L. Ene, Mirela Bidilică

Percutaneous dilatational tracheostomy (PDT) is a minimally invasive, rapid, bedside technique, elective procedure used in Critical Care services to create a stoma between skin and trachea. Its popularity has partly been due to the procedure being more readily available with less restraints from theatre availability, accessing surgeons, cost and time involved in coordinating patient transfer compared to a standard surgical tracheostomy (SST).

The main indications are: 1. Mechanical obstruction of the upper airways, 2. Protection of tracheobronchial tree in patients at risk of aspiration, 3. Respiratory failure, 4. Retention of bronchial secretions, 5. Elective tracheostomy.

Modern PDT was described in 1955 and in 1969. The dilating tracheostomy forceps were developed in 1989 and further by Griggs in 1990 who introduced the use of a guidewire. The most commonly used PDT kits are available from Cook Critical Care and Sims Portex.

In ICU/SUUMC we began the use of PDT in 2015 and up to August 2016 we performed 23 such procedures. The kits we used are Portex percutaneous tracheostomy with a special forceps that allows the passage of a guiding wire. Bedside bronchoscopy performed by pneumologists was mandatory pre-procedural during procedure and as a control after having performed the operation. There was one case when Ear-Nose-Throat specialist had to perform hemostasis 4 hours after tracheostomy.

PDT is a reliable procedure in ICU, performed by ICU physician, ICU nurse and pneumologist.

The post-streptococccal syndromes as border pathology – an infectious diseases practitioner point of view

B. Cîrciumaru

Background: The post-streptococcal syndromes are the sequelae of the streptococcal infections that frequently occur during childhood. They are borderline pathology within internal medicine specialties (including the infectious diseases) and a quite common condition for our country, thus organizing the clinical forms and therapeutically approaches becomes a necessity.

Materials and methods: I retrospectively studied, the post-streptococcal syndromes admitted into the Army`s Infectious Diseases Ward, within the last 10 years. Most of the patients were monitored twice yearly, and treated, sometimes, medical and surgical (e.g. for endocarditis). In order to have a consistent view of the problem, I had discussions with different specialists, including family doctors.

Results: I described the forms of the streptococcal infections (self-limiting) as well as the post-streptococcal syndromes (monophasic and multi-recurential) and I determined reasonable therapeutically conducts for the antibiotic treatment and the prophylaxis.

Conclusions: The antibiotic treatment is essential in order to eradicate the remnant infectious focuses: as using two doses of Benzathinpenicillin G every 5-7 days, and every 10-14 days for the secondary prophylaxis. The length of the secondary prophylaxis remains controversial, monitored by the clinical improvement, biological markers (e.g. the reduction of the inflammatory phenomena, the ASO dropping tendency) and other investigations. I presented my opinion regarding the monitoring and the antibiotic therapy, as part of multi-disciplinary approach. I support the inclusion of the conclusions within the Hospital Guidelines.

A complicated case report associated with rheumatoid arthritis

L. Ciobîcă, Daniela Anghel, S. Stanciu, Raluca Lutuc, I. Sârbu, Ancuța Coca

A 43 years old patient, diagnosed at age 16 with JIA and at 23 years with Hashimoto's autoimmune thyroiditis. Three years later she was diagnosed with seronegative RA, for which she followed the classical remission therapy without an effect. Beside the existing autoimmune pathologies, she added a type 1 insulin-necesitant diabetes, shortly complicated with retinopathy, mixed neuropathy and gastropathy.

Over the last 10 years appear recurrent episodes of iridocyclitis, the reason why we considered to initiate the biologic therapy with Etanercept, without final, due to a pulmonary tuberculosis diagnosed by tests according to the protocol. Subsequently the patient develop a secondary sicca syndrome, complicated with corneal ulcer paracentral wich became infected, for that we practiced the enucleation of OS (March 2015).

Consequently of the unbalanced diabetes (HgA1c = 17.6%), the patient developed an infected interdigital ulcer in the IV-V fingers foot, complicated with a shore perforating plantar right which became infected.

The patient showed a varied clinical picture, and the laboratory indicated a microcytic hypochromic anemia, a moderate inflammatory syndrome, a positive FR, ACPA = 103.3 IU/ML and not least a coproparazitologic exam - Positive for Clostridium difficile. At the ophthalmic reevaluations, the acute iridocyclitis was in a healing process, but with a complicated cataract and a proliferative diabetic retinopathy.

As a particularity of the case, a patient with mixed autoimmune pathologies, followed by a fulminant evolution which was therapeutic neglected. Recurrent episodes of iridocyclitis, infectious complicated, by an enucleation of the right eye.

OP session MD11

Sarcoidosis – challenging diagnosis

L. Ciobîcă, I. Sîrbu, Ancuța Coca, Alexandra David

Sarcoidosis affects people of all racial and ethnic groups and occurs at all ages, although it usually develops before the age of 50 years, with the incidence peaking at 20 to 39 years. The presentation in sarcoidosis varies with the extent and severity of organ involvement. Asymptomatic – incidentally detected on chest imaging ~ 5% of cases, systemic complaints: fever, anorexia ~ 45% of cases, pulmonary complaints: dyspnea on exertion, cough, chest pain ~ 50% of cases; Löfgren syndrome (fever, bilateral hilar lymphadenopathy, and polyarthralgias): common in Scandinavian patients, but uncommon in African-American and Japanese patients.

We are presenting 3 types of clinical presentation of sarcoidosis with rare onset of the diseases. They were in the age incidence peaking, one of them had unilateral ankle swelling, the other one had a single erythema nodosum and the last one had been diagnosed with pulmonary carcinomatosis.

After performing the investigations in our clinic all of them were diagnose with sarcoidosis.

Catheter sepsis in patient with rituximab treatment for non-Hodgkin lymphoma

C. Bănică, I. Ștefan, B. Cîrciumaru, S. Ionescu, O. Dunăreanu, I. Pandrea, O. Călina

Introduction: Catheter-related bloodstream infections accounts for 5 to 20% of hospital-acquired infection. Frequently, catheter-sepsis is caused by multidrug-resistant micro-organisms: gram-positive cocci like S. aureus, Enterococcus or gram-negative bacilli – Pseudomonas aeruginosa, Acinetobacter baumanii, Enterobacter. In immune-depressed patients fungal or polymicrobial infection have been reported. These patients are also at risk for severe evolution.

Presentation: We present the case of a 62 years-old female with non-Hodgkin lymphoma under treatment with Rituximab – the second therapeutic cycle. She got a port-a-cath device on her left subclavian vein. The patient was admitted in our department, after the Rituximab cycle, with high fever, chills, deterioration of the general status and she was diagnosed with catheter-related sepsis. Under large spectrum antibiotics the fever persisted. The patient recovered quickly only after the removal of the intravascular device. All the blood cultures were negative, although the catheter tip culture was positive for Enterococcus faecalis.

Conclusions: The use of central venous catheters is part of the modern practices in critically-ill patients. In neoplastic patients, with fragile peripheral veins, intravascular devices are useful for giving intravenous treatment, blood transfusions or taking blood tests, but they raise the risk for catheter-related infections. In these immune-depressed patients, multidrug-resistant bacteria or fungi can lead to severe sepsis and metastatic infectious complications.

Methods of evaluation for cardiovascular continuum state in type 2 diabetes mellitus patients

C. Constantin, Georgiana Constantin, A. Ranetti, C. Serafinceanu

Introduction: During life time, a patient with type 2 diabetes mellitus (T2DM) could develop complications and an adapted tool should be used for evaluate the cardiovascular risk. There are some score risks present in clinical practice and each of them are developed using some characteristics of patient. This presentment will develop tools for evaluation cardiovascular risk in different situation of T2DM patient.

Material and method: This presentation summarizes the most important evaluation tools for cardiovascular risk in T2DM patients. A real trained practioner use the appropriate score and short and long therapeutic attitude should be adapted using the best medical guidelines. The case management implications and also the vital risks will be appropriate evaluated and presented during an acute event (stroke, acute coronary syndrome, acute peripheral artery obstruction etc.) and could demonstrate the best predictive value for prediction of long-term mortality and morbidity in T2DM patients.

Results: There is not a single and a general available score to evaluate and to be used during lifetime of a T2DM patient. A real and integrated score risk is – in fact – a proper score applied at a proper moment.

Discussions: This short presentation will try to clearly describe these tools and the best moment when that tool should be applied. A lot of actual tools are present in cardiovascular risk market, but a clearly used algorithm for application of these scores should be implemented.

Fluid, gas, fibrosis, blood, calcifications and tumors in the retroperitoneal space. Imaging evaluation

D. Cuzino, Oana Baston, C. Blaj, F. Năftănăilă

Introduction: Retroperitoneal space is a challenging zone for imaging diagnosis. The lesions in this area can arise from the retroperitoneal organs but also from outside.

Material and method: We have revisited our cases in the last three years and we found diverse infectious, inflammatory, tumoral, traumatic and congenital lesions. All of these cases were explored first of all using CT. Some of these cases have been also explored using MRI for a better characterization of the lesion extension.

Conclusion: The complexity of the retroperitoneal lesions need anatomical and semiological analysis.

Imaging diagnosis also need to be interpreted in a strong correlation with the physio-pathological understanding of the region.

Infections in rheumatologic disorders

L. Ciobîcă, Daniela Opriș, Alexandra David

Arthritis tuberculosis has its origins in hematological spread due to activating sites, in the first stage of the disease. Extra-pulmonary infection with Mycobacterium tuberculosis has musculoskeletal involvement in up to 19% of cases. The most involved joints are: the spine, hip and knee. There is no specific clinical manifestation. The symptoms vary from night sweats, cough, and weight loss, with or without joint manifestation: rubor, calor, dolor and in some rare cases evacuation of caseum from a joint fistula.

We report a case of a 79 old female patient who presented in the emergency department for polyarthralgia with onset of symptoms in the last 3 weeks. The right shoulder joint was tender, swollen and red, no fever at the moment nor in the last half of the year. There was no recent history of trauma, of respiratory, infective, or joint disease; because the pain was not improving after usually non steroid drugs she was given for the last 10 days dexamethasone 8 mg/day. She was also known with splenic lymphoma, interstitial pulmonary disease and cardiovascular pathology.

Monitoring biological therapy – a continuous challenge

L. Ciobîcă, Daniela Anghel, D. Stoicescu, Iolanda Sîrbu, A. Dumitru, S. Stanciu, Ioana Răduță, Ancuța Coca

Biological therapy has revolutionized the treatment of rheumatic diseases in the past decade improving not only the heath of the patent but their quality of life.

However, monitoring the progressions of the disease and the possible side effects of the drugs can sometimes become a challenge.

We would like to bring to your attention the case of a 62 year old male with ankylosing spondylitis (HLAB 27+), type II diabetes and hypertension.

10 years after the onset of the disease he is started on etanercept and a few months later the patient presents with pain in the low back region.

The differential diagnosis in now important for excluding neoplastic lesions, tuberculous spondylodiscitis and bacterial spondylodiscitis and the Andersson lesion in ankylosing spondylitis.

The Andersson lesion is an inflammatory involvement of the intervertebral discs in spondyloarthritis. It is a non-infectious condition that occurs in about 8% of patients with ankylosing spondylitis.

OP session MD12

The Chicago classification and its contribution to high resolution esophageal manometry studying

Florentina Ioniță Radu, Andrada Popescu, Maria M. Chereja, A.I. Gavrilă

Introduction: This publication aims to summarize the state of our knowledge of the third Chicago classification criteria for esophageal motility disorders published by the International HRM Working Group, as well as the classification`s usefulness in clinical high resolution esophageal manometry studying.

Materials and methods: The primary objective of the Chicago classification (CC) is to categorize esophageal motility disorders in individuals with non-obstructive dysphagia and/or esophageal chest pain by applying standardized high resolution manometry (HRM) marks. The study is based on the standard HRM test in which the patient is asked to perform ten 5-ml swallows of water. On the color pressure topography plots that result, the software is able to define a set of parameters that are used in the classification and these are: contractile deceleration point (CDP), distal contractile integral (DCI), distal latency (DL) and integrated relaxation pressure (IRP).

The metrics obtained from the HRM study can be used to characterize individual test swallows (in terms of integrity of contraction, contraction pattern and intrabolus pressure pattern), but most important, they can define esophageal motility disorders and classify them in a) achalasia and EGJ (eso-gastric junction) outflow obstruction, b) major disorders of peristalsis and c) minor disorders of peristalsis.

Results and conclusion: The Chicago classification is a useful tool that can guide course of treatment in patients with esophageal motility disorders, despite the fact that metrics and criteria it uses can be hard to understand. It is an evolving process, with its third version incorporating recent advances in the understanding of esophageal motility pathology.

The impact of bariatric surgery on upper gastrointestinal symptoms in obese patients – the role of esophageal manometry

Andrada Popescu, A.I. Gavrilă, I.P. Nuță, Raluca S. Costache, Mariana Jinga, Săndica Bucurică, B. Macadon, M. Pătrășescu, Mirela Chereja, Florentina Ioniță Radu

Introduction: The role of this paper is to point out the importance of GI functional testing (especially esophageal manometry) before bariatric surgery, in order to reduce the prevalence of GERD symptoms, and the need for a medical protocol regarding this issue.

The literature data available reveals that the prevalence of GERD is higher in obese patients compared with normal weight controls, with an increased risk of 2.5 of developing symptoms and erosive esophagitis. This is most likely related to increased esophageal acid exposure. Several pathophysiological mechanisms may be involved: transient lower esophageal sphincter relaxation (TLESR), lower esophageal sphincter (LES) pressure, altered esophageal motility, presence of hiatal hernia and esophageal factors such as poor esophageal clearance, altered gastroesophageal pressure gradient and delayed gastric emptying. In morbidly obese patients, some of these mechanisms may occur increasing the risk of developing severe GERD, but they can be established with esophageal manometry.

We know from other studies that the Roux-en-Y gastric bypass is considered an effective method to alleviate symptoms of GERD, whereas laparoscopic sleeve gastrectomy appeared to increase the incidence of the disease. Adjustable gastric banding was seen to initially improve the symptoms of GERD; however, a subset of patients experienced a new onset of GERD symptoms during long-term follow-up. Thus performing esophageal manometry and pH-metry before bariatric surgery can give important information and help in choosing the right type of surgical intervention, reducing the prevalence of upper GI tract symptoms.

Conclusion: Careful medical assessment is mandatory before performing any type of bariatric surgery in obese patients, especially for those who already have GERD.

Update in the management of uncomplicated acute pancreatitis

Cătălina Diaconu, Gaudia Avram-Mănescu, Laura Voicu, Florentina Ioniță-Radu, Mariana Jinga, D.O. Costache, Raluca S. Costache

Acute pancreatitis represents the inflammation of the pancreas, inflammation that can be localized to the gland or it may extend to peripheral tissues or distant organ systems.

The treatment in acute pancreatitis is directly aimed at relief of symptoms. Fluid repletion, glucose repletion/nutrition and analgesia are the main pillars in managing acute pancreatitis. Studies show that early fluid resuscitation with 5-10 ml/kg/h is imperative in the first 12 to 24 hours. Moreover crystalloids are the first choice, rather than colloids. Ringer lactate was proven to have lower risk in developing systemic inflammatory response syndrome (SIRS) and might reduce the risk of metabolic acidosis and non-anion gap when compared to chloride-rich solutions (saline). The goals in fluid repletion are: to decrease of hematocrit and blood urea nitrogen and to maintain normal levels of creatinine. In patients with acute pancreatitis and cholangitis endoscopic retrograde cholangio-pancreatography (ERCP) is mandatory in the first 24 hours.

Antibiotherapy is not recommended as a routine treatment and should be reserved in infected pancreatic or extrapancreatic necrosis that fails to improve after 7-10 days of hospitalization and extrapancreatic infections (cholangitis, pneumonia, bacteriemia, cathether-acquired infection). In these cases carbapenems, quinolones and metronidazole are the treatment of choice. Antifungal agents are not recommended as prophylaxis.

Since severe abdominal pain is the main symptom, analgesia should be taken into consideration: opioids (fentanyl or hydromorphone). Enteral feeding is essential in acute pancreatitis, regardless the severity, once the symptoms subside. Even though most guidelines in the past recommended NPO (nothing by mouth) nowadays studies have shown that enteral nutrition is feasible and improves outcome and can be performed via nasogastric or nasojejunal tube and use low-fat semielemental formulas.

Hereditary hemochromatosis: pathogenesis, diagnosis and treatment

Gaudia V. Mănescu-Avram, Cătălina Diaconu, Laura Voicu, Florentina Ioniță-Radu, Mariana Jinga, D.O. Costache, Raluca S. Costache

Hereditary hemochromatosis is an autosomal recessive genetic disorder often determined by mutations in the HFE gene (typical patients have inherited a C282Y mutation in the HFE gene from each parent) and less commonly by mutations in the genes for hemojuvelin, hepcidin and feroportin which implies abnormal accumulation of iron in parenchymal organs, leading to organ toxicity.

The classic description of the disease also called ”bronze diabetes” implies cirrhosis, diabetes mellitus and cutaneous hyperpigmentation. A recent study of C282Y homozygotes in Australia suggested that 28% of men and only 1% of women have iron related symptoms, influenced by age, sex, dietary iron intake, alcohol, blood loss in menstruation and pregnancy. Symptoms usually appear after the fourth decade of life or when the stores of iron are 15-40 g. Hemochromatosis has multiple manifestations, but the major consequence remains the development of hepatocellular carcinoma (HCC) which appears in one third of patients with hemochromatosis and cirrhosis. An ultrasonography should be made every 6 months in these patients to screen for HCC. For the diagnosis of this affliction clinical, laboratory, genetic testing and pathology criteria are needed. Laboratory tests reveal increased serum transferrin saturation, which is often elevated in patients with HFE-linked hemochromatosis, with a sensitivity over 90% and increased serum ferritin level (but ferritin may also be elevated in various infections and inflammatory conditions). Liver biopsy is recommended in C282Y homozygotes patients or compound heterozygotes if liver enzymes are elevated or if ferritin is higher than 1000 μg/L. Response to phlebotomy may confirm the diagnosis and MRI can show moderate to severe iron overload.

It is recommended to initiate the treatment of hemochromatosis with phlebotomy (removal of 500 mL blood) before the development of cirrhosis and/or diabetes with an important reduction of morbidity and mortality. Phlebotomies should be done weekly/biweekly until serum ferritin reaches 50-100 μg/L, then 3-4 phlebotomies/year are recommended for maintenance.

Hematologic abnormalities in cirrhotic patients, a challenging diagnosis: case report

Florentina Ioniță Radu, M. Șotcan, Andrada Popescu, A.I. Gavrilă, Maria M. Chereja

Introduction: Cirrhosis is responsible for various hematological abnormalities. Different mechanisms are implied such as hypersplenism, hemolysis, altered synthesis of clotting factors and trombopoietin, nutritional deficiency of folate, B12, B6 and iron, variceal bleeding or drug induced bone marrow toxicity.

Identifying the real cause require a careful approach and must take into consideration other severe hematological diseases.

Materials and methods: We present the case of a 69 year old man with Child C cirrhosis secondary to VHC infection who developed progressive leucopenia and thrombocytopenia. Leucopenia with neutropenia were confirmed on peripheral blood count, without any pathological findings on blood smear. A moderate enlargement of the spleen was found on abdominal ultrasound. The leucopenia got worst two months later, associating severe megaloblastic anemia (6 g/dl). As the dosage of folate and B12 were normal and no gastroesophageal bleeding had occurred we continued further hematological investigations. Bone marrow aspiration showed hypercellularity, few blastic cells, mild erythroblastopenia, megakaryocytar hyperplasia and dysplasia of megakaryiocytes and erythroblastes. A marrow bone biopsy was performed. Blastic cells, found in a percent of 20-22%, were CD 34 positive (stem cell marker) and negative for CD 61 (megakaryocyte marker). The histopathological aspect was compatible with an acute myeloblastic leukemia secondary to a myelodysplastic syndrome.

Conclusion: A myelodysplastic syndrome can be hidden behind hematological abnormalities found in cirrhotic patients. A strong collaboration between hepatologist and hematologist is needed to identify as early as possible the correct cause and implement appropriate therapy.

Novel indicators for quality in endoscopy

D.V. Balaban, Iulia Enache, Alice Farcaş, Cătălina Diaconu, Andrada Popescu, M. Pătrăşescu, B. Macadon, Săndica Bucurică, Raluca S. Costache, P.I. Nuţă, Florentina Ioniţă Radu, Mariana Jinga

Quality standards of endoscopic procedures have been a constant concern among endoscopy services and regulation authorities in the last years. Although several quality indicators have been previously validated for colonoscopy, new ones have emerged recently and considerable attention has been paid to indicators for upper GI endoscopy also.

Along with the traditional quality metrics (cecal intubation rate, withdrawal time, adenoma detection rate, interval cancers), colonoscopy examinations are also being assessed by the number of polyps per patient; this new indicator is trying to differentiate endoscopists who are detecting one adenoma (and after identifying this one lesion continuing with less careful examination of the colon) from those who detect more than two adenomas/patient, thus being a good predictor of missing rates.

Regarding upper digestive endoscopy, several indicators have been proposed: inspection time, photo documentation of certain landmarks and lesions, use of mapping and scoring tools for premalignant conditions such as atrophic gastritis, gastric intestinal metaplasia or Barrett's esophagus and application of biopsy protocols for these conditions.

Development, validation and implementing of such indicators for endoscopic procedures is a good quality measure for endoscopic services, making them available for internal and external auditing and thus providing improved medical care for patients.

Relationship between helicobacter pylori infection and digestive oncological pathology

P. Nuță, Roxana Călin, A. Lungu

Helicobacter pylori infection is the most frequent infection, its high prevalence being higher in developing countries. H.

pylori colonizes the stomach and duodenum, and it is localized in the areas of gastric metaplasia. The inflammatory process associated with the presence of bacteria destroys acid secreting glands and lead to hypoacidity, favoring the emergence of gastric ulcers and gastric cancer.

Helicobacter pylori is a group I carcinogen (shown carcinogenic agent in humans), and is proven correlation between infection with the microorganism, in particular cagA + strain, and gastric adenocarcinoma. Helicobacter pylori infection increases the risk of gastric cancer for 8 times, except tumors located at cardial level. Also, Helicobacter pylori was detected in 75% of patients with MALT lymphoma (mucosa-associated lymphoid tissue), eradication of microorganism causing various degrees of tumor regression in 70-80% of patients. The role of Helicobacter pylori eradication therapy in reducing the risk of gastric cancer is undeniable.

Although Helicobacter pylori infection has been incriminated in the occurrence of gastric cancer, it seems that it has protective against esophageal malignancies. One explanation is the appearance of atrophic gastritis with achlorhydria and consequent reduction of acid reflux into the esophagus. Another hypothesis is the induction of apoptosis of atypical cells in patients with Barrett's esophagus associated esophageal reflux.

The link between Helicobacter pylori infection and other digestive cancers is controversial.

OP session SG1

Management of complications in laparoscopic pyeloplasty for ureteropelvic junction obstruction

A. Aungurenci, A. Rădulescu, D. Spînu, C. Iatagan, O. Pacu, C. Stănescu, S. Ioniță, A. Iliescu, F. Rusu, V. Botea, M. Dinu, V. Mădan, D. Mischianu

Introduction: In the last two decades the laparoscopic approach for ureteropelvic junction obstruction replaced the classical open surgery. But there are still specific complications that can jeopardize the final result of the operation. The aim of our study is to assess our main complications of laparoscopic pyeloplasty and their management.

Materials and method: We analyzed a series of 45 patients admitted in our clinic between January 2010 and December 2015 that underwent laparoscopic pyeloplasty. In all cases we used the transperitoneal approach. Heynes-Anderson pyeloplasty was performed in 43 cases while in the other 2 cases we performed Foley (Y-V) pyeloplasty. Postoperative complications were assessed according to Clavien-classification.

Results: The main postoperative complications encountered in our clinic were: urine leakage (6%) and recurrence of the pelviureteral obstruction (4.4%). Except for the patients with restenosis that was managed by open surgery, in all other cases were performed minimally invasive and endoscopic procedures. Conversion rate was approximately 9%, due to intraoperative complications that made impossible the laparoscopic approach, but this was recorded only in the first three years.

Conclusions: In our experience, the complications of laparoscopic pyeloplasty can be managed with minimal invasive techniques that ensured the same functional results as in uncomplicated cases. The restenosis of the ureteropelvic junction should be managed by open surgery.

Refractory priapism – case presentation

D. Marcu, D. Spînu, O. Bratu, A. Aungurenci, V. Mădan, I. Armand, A. Rădulescu, R. Popescu, D. Mischianu

Introduction: A rare health problem which untreated on time can have a devastating impact on the health status of an individual and, last but not least on the status of his private life.

Material and method: We present the therapeutic approach in the case of a patient who was admitted to the emergency clinic of urology of the Carol Davila SUUMC accusing penile pain and the presence of erection for more than 30 hours. Local examination, clinical and paraclinical confirmed the presence of venous priapism. Emergency surgical procedures consisting in Winter shunts and epinephrine injections were undergone for 3 times with a momentary therapeutic success. Open glandular shunts were the saving procedure in this particular case.

Results: Post-operatory evolution was favorable, the patient presented at 2 and 4 four weeks without any local problems but also without any erectile function.

Conclusions: At 58 years old a man is still sexual active. Given the fact that no serious comorbidities affected this patient the cause remains unknown. What goes more than 6 hours of erectile function is in most cases irretrievable. Not only the sexual function could have been lost but his life could have been in danger.

Upper lumbar ureteral lithiasis – therapeutic approach

A. Păun, L. Chirilă, O. Pacu, F. Rusu, A. Iliescu, V. Botea, C. Stănescu, A. Aungurenci, V. Mădan, M. Dinu, D. Mischianu

Introduction: Urinary stones is a disease known since ancient times. This is an important health problem worldwide with a prevalence in the general population estimated at 2-3% and a recurrence rate during lifetime of 50 %. The apparent increase in incidence may be real, but also secondary to high rate detection through high performance investigations. The incidence of urinary stones is about 3 times higher in men.

Material and methods: The study included a total of 149 patients admitted in our clinic between January 2013 and December 2015 with a diagnosis of upper ureter lumbar lithiasis > 1cm. Surgical approach was open, laparoscopic, ureteroscopic or ESWL.

Results: Were made 82 ureteroscopy with lithotripsy laser "in situ" (± stent JJ), 11 laparoscopic ureterolithotomy, 19 open ureterolithotomy and in 20 cases ESWL was performed per primam (18 cases a stent JJ was placed first, then after normalize biological constants, ESWL was performed).

In patients with open or laparoscopic ureterolithotomy percentage of "stone free" was 100 %.

Of the 82 ureteroscopy, in 43 cases other secondary procedures were needed (URSR, double J stent placement, ESWL).

In patients in whom ESWL was first procedure, stone–free rate was 55 % after a single session.

Conclusion: Lumbar ureteric stones needs a multimodal treatment. In our study, the most effective methods for achieving the goal of "stone-free" were ureterolithotomy both the classical and laparoscopic approach.

It is important to notice that we have only analyzed the stone-free rate after the first procedure; hospitalization time and cost, postprocedural pain have not been analyzed.

Surgical challenges in kidney infections

D. Marcu, O. Bratu, D. Spânu, I. Oprea, M. Dinu, R. Popescu, A. Rădulescu, D. Mischianu

Introduction: Kidney infections should not be neglected, this pathology often leading to significant morpho-functional alterations and even to the loss of kidney function.

Material and method: We present the therapeutic approach that we have adopted in three patients with renal infectious pathology. The first patient presented for a renal cystic tumor, case in which the laboratory investigations lead to the diagnosis of renal hydatid cyst (increased titer of antiechinococcus granulosus antibody). In the second case we have practiced a lumbar nephrectomy for a lithiasic destroyed kidney, but the histopathological examination revealed that it was renal tuberculosis. The third patient was admitted for a gigantic renal cystic tumor for which we have practiced nephrectomy and the histopathological result was papillary carcinoma.

Results: Postoperative evolution was favorable in the first two patients, but in the case of the third patient secondary lung determinations were encountered at six months after the nephrectomy (CT scans).

Conclusions: In the absence of a correct diagnosis and a proper treatment, kidney infections may have a fatal trend, leading to the appearance of urosepsis, impaired renal function and even to the loss of the kidney.

The difficult vaginal hysterectomy

B. Panaite, N. Niculescu, I. Constantinescu, Ioana Niculescu, C. Pârvulescu, N. Alexe, Anca Popescu, Alexandra Năftănăilă, M. Nicodin, O. Nicodin

Our present work represents an overview of difficult surgical cases of vaginal hysterectomy that have been performed in our clinic.

The main criteria we have established when considering a vaginal hysterectomy to be a difficult one were: the increased volume of the uterus, the modified anatomy of the bony pelvis, previous caesarian section as well as other pelvic surgical interventions, obese patients and cases that have associated cervical elongation.

Our debate has focused on how a proper surgical technique that has accordingly adapted to each case previously mentioned together with the skills of a well-trained surgeon in the field of vaginal surgery can shift an absolute contraindication into a relative one when considering the vaginal approach of a hysterectomy.

Adiponectin blood level in patients with prostate cancer

F. Rusu, O. Pacu, A. Iliescu, A. Păun, M. Dinu, Lavinia Chirilă, V. Botea, V. Jinga, Emilia Rusu, Gabriela Radulian, D. Mischianu

Introduction: Recent data suggests that obesity can influence the incidence, progression and mortality of prostate cancer through hormonal changes, through insulin resistance, increasing production of inflammatory markers etc. Aside these factors, obese patients with PCa are more difficult to diagnose as they have mostly low serum PSA level and a higher prostate volume. Although, the effects of adiponectine on normal prostate cells are at the moment not understood, indirect evidence suggests an antiproliferative effect in prostate tissue. The present study assesses the level of adinopectine on the subjects with prostate cancer.

Material and method: The 414 selected patients were divided in 2 groups: group A: 381 patients with obesity, without PCa (O. PC(-)) and 33 patients with obesity and prostatic cancer (O. PC(+)). Specific anthropometric parameters were analyzed (height, weight, body mass index (BMI), waist circumference), blood tests (fasting blood glucose – (BPG), fasting insulinemia (FPI), HbA1, total cholesterol, triglycerides, HDL cholesterol, adiponectin, leptin, TNF alpha, IL -6, prostate specific antigen (PSA), free PSA (F-PSA), testosterone).

Results: The study results shows statistically significant differences in terms of age, BMI, PSA level, Free-PSA level, FPG, FPI, adiponectin, leptin, TNF-□, IL-6, testosterone and prostate volume in patients from the two groups (all values p <0.05). In terms of the abdominal circumference and diastolic blood pressure there were no differences in the two groups. Plasma adiponectin level was lower in patients with

PCa than the control group (p <0.001) and also in patients with those with aggressive disease (1.23 ng/ml in patients with Gleason score <7 vs 0.64 ng/ml for Gleason score 8-10) (the difference remained statistically significant after logarithmic transformation).

Conclusions: The results of the present study indicates that patients with prostate cancer have a lower plasmatic adiponectin level and are also associated with histological grade and disease stage. Further prospective studies are recommended to asses any causal relation between prostate cancer and plasmatic concentrations of adiponectin.

OP session SG2

Principles and techniques in cheek reconstruction – a review of cases

Monica Dărmănescu, V. Trifu, A.M. Țilea, Raluca I. Săftoiu

The cheek is the broadest subunit of the face, in close proximity to aesthetically important regions such as eyelids, nose and mouth. In most cases the extensive mobility of cheek skin provides for relatively simple closures following tumor extirpation, but in case of larger defects, those located near free margins, or those within areas of decreased skin laxity may require a more complicated approach.

We review the principles of cheek reconstruction, from the simplest option (primary closure) to more complex methods (grafts, flaps), with special considerations to specific reconstructive options. An essential principle of cheek reconstruction is to provide an accurate skin color and thickness match with locally recruited tissues, avoiding distortion of the facial free margins (lower eyelid, upper lip and oral commissure).

Each case must be approached with a unique plan for the defect and the patient, with a non-geometrical and three-dimensional design for every case in order to have very good aesthetic and functional outcomes.

Reconstruction of the cheek requires a thoughtful approach to the etiology of the defect, the functional status of the patient, and the best solution with the least morbidity. The dynamic function and contour of the cheek make it a challenging structure to repair.

Principles and techniques in forehead and scalp reconstruction – a review of cases

Monica Dărmănescu, V. Trifu, A.M. Țilea, Raluca I. Săftoiu

Reconstruction of scalp and forehead defects is a complex field; we have a broad variety of reconstructive options. A thorough understanding of the anatomy of anatomic structures of the scalp and forehead is paramount to optimal surgical result.

A case series of regional reconstruction of scalp and forehead area is presented, with a variety of reconstructive techniques. The anatomy of the region is reviewed; it is important to preserve the function of motor and sensory nerves, although aesthetic concerns are of great importance in scalp and forehead reconstruction. Respecting the normal position of facial landmarks such as the eyebrows and hairline can be challenging during reconstruction.

We review the principles of forehead and scalp reconstruction, from the simplest option (primary closure) to more complex methods (flaps).

Each case must be approached with a unique plan for the defect and patient, with a non- geometrical design, in order to achieve excellent aesthetic and functional outcomes.

Pigmented basal cell carcinoma – clinical and dermoscopical clues

M. Țilea, Mihaela Georgescu, Monica Dărmănescu, V. Trifu

Introduction: Pigmented basal cell carcinoma (pBCC) can pose a real dilemma even to the most skilled clinician. Clinical similarities with pigmented seborrheic keratosis (pSK) and pigmented cutaneous melanoma (PCM) can be misleading and may result in some unfortunate therapeutic approaches.

Case presentations: We present a series of cases of pigmented basal cell carcinomas that were diagnosed and treated in our clinic. While in some cases the diagnosis was established clinically, in other cases dermoscopy proved to be a valuable tool for reaching the correct diagnosis. All cases were confirmed with histopathology.

Discussion and conclusions: The clinical aspect of a pBCC is usually straightforward. The classical clinical signs of e.g., greyish translucent aspect (pearls in the periphery of a superficial lesion), the arborizing telangiectasias and the periods of clinical improvement alternating with periods of clinical deterioration (bleeding), can in most cases lead the clinician to the correct diagnosis. In our experience there is also another important clinical clue for the diagnosis of a pBCC – punctate pigmentation in the periphery of the lesion. The colour of the pigmentation can range from dark grey to brown to blue-black. The corresponding dermoscopical structures are: blue-grey ovoid nests and globules, leaf-like structures and spoke-wheel like structures. This clinical clue does not appear in pSK or in PCM.

A solid clinical diagnosis, aided by dermoscopy, can help a surgeon to better manage these tumors and as such to deliver a disease free, functional and aesthetical result.

A simple cataract procedure in a complex ophthalmic patient – case report

G. Anghel, O. Mușat, M. Zemba, C.H. Manole, Mioara L. Macovei, Liliana Pulbere, Monica M. Armegioiu, C.G. Ștefan, Daniela Șelaru, Camelia Constantin, Corina Cernat, D.C. Luncă

We report the case of a 65-year-old male, who is hospitalized for a slow progressive decrease in visual acuity over the past years. The clinical exam revealed: OU: primary open angle glaucoma (POAG) – (bilateral trabeculectomy in 1999) compensated under medical therapy, OD: pulsatile exophthalmy, exposure keratitis, paracentral vascularized leukoma, posterior chamber IOL, OS: complicated cataract, sectorial optic nerve atrophy, sequel of superior branch retinal vein occlusion. Past medical history: arterial hypertension, type 2 diabetes, gout, dyslipidemia. The visual acuity was OD: hand motion (HM), OS: counting fingers (CF) and the ocular pressures TOS=20 mm Hg NCT, TOD=similar digital tone as the left eye. The cranial computed tomography revealed the absence of the superior wall of the right orbit with direct communication between the cranial cavity and the right orbital content, after neurosurgery for right orbital cellulitis, in the early ‘90s. Given all these findings, the therapeutic intervention with the greatest benefit was considered to be the cataract procedure in the left eye.

The patient underwent uneventful phacoemulsification with posterior chamber intraocular lens implantation. The post-operative visual acuity was VOD=0.8 without correction.

The particularity of this case is represented by the association of multiple conditions that threaten sight and the pulsatile exophthalmia caused by the CSF pulsation transmitted through the bone defect of the right superior orbital wall.

Complex clinical association – Rhegmatogenous retinal detachment after perforant keratoplasty and psudofak

O. Mușat, G. Anghel, C. Ștefan, M. Zemba, H. Manole, Liliana Pulbere, Monica Armegioiu, Laura Macovei, Daniela Șelaru, Corina Cernat, Camelia Constantin, Uliana Ochinciuc

The paper reports the case of a 62 years old patient with a history of keratoplasty surgery after a concussional ocular trauma with central corneal leucoma, posttraumatic iris atrophy.

The patient underwent posttraumatic cataract surgery with artificial lens implant.

About 6 years later he developed rhegmatogenous retinal detachment, which was treated surgically: posterior vitrectomy, laser photocoagulation, silicon oil tamponade with no intraoperative complications and good final outcome.

This paper reports the particularities and difficulties of such a complex case.

Glaucoma after chemical burns

C. Ștefan, Cristina M. Timaru, G. Anghel, M. Burcea, Daniela Șelaru, O. Mușat, M. Zemba, H. Manole, Liliana Pulbere, Laura Macovei, Monica Armegioiu

Introduction: Glaucoma after chemical burns and radiation is a secondary, posttraumatic, open angle glaucoma. It is a frequently seen complication of chemical burns that can occur in the acute stage or as a late complication.

Material and method: This study is a presentation concerning glaucoma after chemical burns, coupled with a case study presentation of a female patient who suffered from a right eye chemical burn. The management of this case along with diagnosis and treatment are presented in this article.

Results and conclusions: Aldo it is not a frequently seen pathology, it is important to know how to diagnose and treat glaucoma after chemical burns. There are various options available for treatment, but choosing one is difficult because of the possible complications and scarring caused by the chemical substance.

Extensive corneoconjunctival tumor – a therapeutic challenge

M. Zemba, G. Anghel, O. Musat, H. Manole, Laura Macovei, Liliana Pulbere, C. Ștefan, Daniela Șelaru, Monica Armegioiu, M. Dumitrescu

Purpose: to show the surgical solution for a giant corneoconjunctival tumor.

Methods: the surgical technique is shown in a video film; we insisted on correct oncologic excision and ocular surface restoration.

Results: the visual acuity increased from light perception to 1 without correction.

Conclusions: a correct technique and suitable solutions for ocular surface restoration allow a fast and complete recovery for eyes apparently with very bad prognosis.

Peripheral nerve injury and neuron regeneration

Andreea Benga, F. Zor, A. Korkmaz, B.M. Marinescu

Peripheral nerve injuries can be most disabling, resulting in loss of sensitivity, motor function and autonomic control in the involved anatomical segment. Although capable of regeneration there is still no optimal solution for their reconstruction. Nerve regeneration has several vulnerable points that could be addressed to improve outcome: (1) neuron survival after trauma, (2) gap crossing and orientation of neurites, (3) neuroma formation, (4) target-organ trophicity.

Inflammation is an important sequence in nerve regeneration. Cytokines released by macrophages and fibroblasts have mitotic effect on Schwann cells. Fibroblasts are present in epineurium, perineurium and endoneurium. If they proliferate extensively, scar formation occurs that impedes nerve regeneration. Scar tissue contains repulsive proteoglycans (such as chondroitin sulfate) and semaphorins that inhibit neurites' elongation.

Nerve repair still faces two immense challenges: fascicules orientation that could be addressed by improving the surgical technique and the time race against fibrosis and end-organ atrophy amenable to pharmacological therapy.

The aim of this study is to review the literature for the most frequent chemicals used in experimental studies to address neuron survival, reduce scar formation and improve overall nerve regeneration.

Hydrosurgery – an effective method of wound debridement

B. Marinescu, Andreea Benga

Subject: The aim of the paper is to demonstrate the efficacy of using hydrosurgery in debridement of complex wounds.

Material and method: The method was used in 8 patients, treated in our clinic with wounds of different etiologies: burns, chronic, infected or post-surgical wounds. The hydrosurgical system permits simultaneously, an accurate ablation and aspiration of the nonviable tissue from wounds.

Results: In all of our cases the debridement was made rapidly and very precise. Direct closure of the wounds or skin grafting were performed in the same time.

Conclusions: Hydrosurgery represent an effective and selective method of debridement for difficult wounds. It minimize the tissue damage, preserve the healthy tissue and leave a clean and well vascularized wound bed.

Minim invasive solution for treatment of an intraocular lens subluxation

M. Zemba, G. Anghel, O. Mușat, H. Manole, Laura Macovei, Liliana Pulbere, C. Ștefan, Daniela Șelaru, Monica Armegioiu

Purpose: to show the surgical solution for a traumatic subluxation of a posterior chamber intraocular lens.

Methods: the surgical technique is shown in a video film.

Results: the visual acuity increased from 0.03 to 0.5 without correction.

Conclusions: a minim invasive technique may allow to obtain very good results, with fast recovery and low costs.

OP session SG3

Intralobar lung sequestration – thoracoscopic surgical procedure

A. Ciuche, C. Nistor, D. Marin, D. Pantile, Laura M. Constantin, Roxana Brîncoveanu

Introduction. The authors present the case of a 37 years old male admitted for a left lower lung lobe tumor identified on a standard chest X-Ray.

Material and method. First we present the radiological findings and then intraoperative images. Using minimally invasive thoracic surgery we managed to diagnose a left lower lung lobe sequestration (highlighting the vascularization of the sequestered lung territory from the descendent aorta, right above the diaphragm), and to perform a thoracoscopic wedge resection of the lesion, clipping the aberrant vessel.

Results. With favorable surgical outcome, several aspects are being detailed: postoperative evaluation, as well as the radiological findings one-month postoperative.

Conclusion. Minimally invasive thoracic surgery has permitted a better exposure of this rare lung malformation, allowing at the same time a better diagnosis and treatment procedure (thoracoscopic wedge resection), with a fast and complete postoperative recovery of the patient.

Thoracoscopic resection of a bronchogenic esophageal cyst

A Ciuche, C. Nistor, D. Pantile, D. Marin, Olivia Arhire, A. Tudose, Săndica Bucurică, C. Bețianu, Roxana Brîncoveanu

Introduction: Esophageal bronchogenic cyst is an uncommon entity and surgical experience of their removal by thoracoscopic surgery is limited.

Material and method: We present our patient outcome and surgical thoracoscopic technique in the treatment of an esophageal bronchogenic cyst.

The study included 1 patient, male, 52 years with important obesity. The only clinical manifestation was intermittent dysphagia. Some imagistic findings at CT scan and transesophageal echography suggest the presence of a solid intraparietal benign esophageal tumor.

The intervention was performed using 4 thoracoscopic entry points. Thoracoscopic removal of the cyst needed careful manipulation and subsequent dissection of the cyst sac from the structures to which it was attached (esophageal wall and mediastinal pleura). The intramural esophageal localization with local esophageal mucosa being intact and the particular aspect of the tumor (filled with mucoid material and contain cartilage fragments) were able to make the positive diagnosis (cystic intramural esophageal tumor) and eliminated the preoperative diagnosis (solid intramural esophageal tumor).

Results: The patient had a favorable outcome, without any complications and was discharged the 3rd day after surgery. The dysphagia was relieved immediately after the operation. The follow-up was well 6 months after the surgery.

Discussions and conclusions: The preoperative computed tomography and endoscopic ultrasound had some limits in this case (distinguishing between cystic and solid tumor was difficult).

Standard surgical treatment is removal of the cyst by thoracotomy. While difficulties in removal and possible complications and recurrence are similar to those of classical posterolateral thoracotomy, video-assisted thoracoscopy offers clear postoperative advantages.

Mediastinal paraganglioma – a surgical challenge

C. Grozavu, M. Iliaș, D. Pantile

Introduction: A paraganglioma is a rare neuroendocrine neoplasm that may develop at various body sites (including the head, neck, thorax and abdomen). Paragangliomas originate from paraganglia in chromaffin-negative glomus cells derived from the embryonic neural crest, functioning as part of the sympathetic nervous system.

Material and method: A 34-year-old woman came to our department with previous history of bilateral carotid glomic tumor. First, the right common carotid tumor has been surgically removed, followed one year later by the removal of the left common carotid tumor. Five years later the patient reported to the vascular surgery department, and after several investigations she was referred to our thoracic surgery department.

Results: On the basis of the symptoms and the previous surgical history we decided to operate on the patient.

We attempted and succeeded to dissect the tumor from the aorta, the left common carotid artery and the left subclavian artery and then resect the full tumor with acceptable blood loss.

Discussion: The treatment of choice for paragangliomas is, where possible, complete surgical resection.

Middle mediastinal paragangliomas are extremely rare, approximately 30 cases have been reported in the literature since 1959.

Conclusion: The surgical intervention was the appropriate approach for this case. When gently maneuvering the tumor, alterations of blood pressure can be avoided. Also, by performing a careful dissection and resection, the blood loss is acceptable and transfusion is not mandatory.

Laparoscopic hormonal suppression in breast cancer

N. Niculescu, O. Nicodin, B. Panaite, C. Pârvulescu, Ioana Niculescu

Objective: To compare the surgical laparoscopic hormonal suppression to the classical treatment, in the treatment of breast cancer.

Method: A retrospective study of 124 patients operated at the Gynecology Department of SCMC for breast cancer. From the patients who have had surgical intervention for hormonal suppression, 13% have had laparoscopic procedures.

Results: The average operative time for the classical approach was 40 minutes and the hospitalization time of 3-5 days. The laparoscopic approach for hormonal suppression took an average 14 minutes, and for bilateral laparoscopic oophorectomy followed by vaginal hysterectomy 63 minutes. The hospitalization time was 2 and 3 days respectively.

Conclusions: The surgical hormonal suppression is an effective complementary therapy in the complex management of breast cancer. The laparoscopic approach resulted in a reduced hospitalization time, a better cosmetic outcome and a quicker return to work for patients, therefore a better postoperative quality of life.

Right chylothorax – minimally invasive approach

C. Nistor, A. Ciuche, D. Pantile

Introduction: The authors present two cases, one of a 47 old woman, 3 years after mitral valvular replacement operation, presented with a postoperative pleural chylous effusion repetitive evacuated and recidivated, and another one of a man with an incidental radiological finding.

Materials and method: The authors presented a short review of the recent information about the pleural chylous effusions and the commonly therapeutically approach in these cases. They presented in details the thoracoscopic approach of the thoracic duct which was used in these cases.

Results: Postoperative evolution was favorable, with complete pulmonary expansion and complete resolution of the pleural chylous effusion.

Conclusions: The thoracoscopic approach, even if more difficult, allowed a good exposure of the thoracic duct, and also a fast and complete postoperative recovery.

“Refined” optical methods in pharyngolaryngeal tumor diagnosis

C. Romanițan, R. Costin, G. Ganea

The expression "optical biopsy" has entered everyday language for the last 2 decades, being imposed by biomedical researchers in the field of optics. It is a paradox, a word that joins two terms with meanings willed otherwise, "biopsy" meaning harvesting a tissue fragment, which does not happen in optical biopsy. This actually refers to a series of physical measurements using the optical properties of light and the interaction thereof with tissues, to allow the doctor the diagnosis of certainty in real time, in vivo, in situ and noninvasive (or minimally invasive).

It should be noted that it is widely accepted among ENT specialists that the "gold standard" in the diagnosis of malignant process is the histological examination by a pathologist made after harvesting the tumor tissue fragment. The biopsy itself may be simple, readily available to every ENT specialist in easily accessible areas (oropharynx, nasal passages) or difficult, sometimes requiring endoscopic technique under general anesthesia (cavum, hypopharynx, and larynx).

To overcome these drawbacks and anesthetic-surgical risks, to speed diagnosis of certainty and lower costs, we have developed new methods of exploration, that try to provide the clinician with new criteria to differentiate between healthy tissue/benign tumor/malignant tumor. New technical developments in the field of optical fibers, light sources, sensors, led to the unprecedented development of various optical methods which substantially improve visualization and diagnostic evaluation of the epithelium in vivo. These try to improve image resolution, contrast, and deep tissue penetration to standard endoscopic techniques, offering molecular and biochemical information about mucosal lesions.

Under the name "optical biopsy" there’s actually: fluorescent spectroscopy, optical coherence tomography, narrow-band imaging and confocal endomicroscopy.

This paper describes bio-physical principles of interaction of light with tissues. It also compares the advantages and disadvantages of each optical methods used in ENT and brings statistical data from the literature.

Past and perspectives of local anesthesia in dental medicine and BMF surgery

A. Nistor, A. Diaconeasa Maier

Anesthetic substances plays an important role in pain management of various disease involving treatments in the oral-maxillofacial regions. Properly administered local or loco-regional anesthesia is the safest way to minimize the effects of various painful conditions or treatment regimens.

In this article there are evaluated the main types of anesthetic substances which are used most frequently in the current period. There are briefly set out the main characteristics of the major anesthetics used in dentistry and oral surgery regardless of technical management. Also, there is a highlighted focus of the advantages of using Articaine as a main anesthetic, adverse effects compared with other substances existing in current literature and personal considerations.

The main interventions of surgery and oral implantology, which were listed, are most indicated with the use of specific type of anesthesia but also come with some inconveniences and difficulties. Each anesthetic substances has an order of potential, toxicity and specific indications for use alone or in combination with Articaine.

Practical conclusions: Any dental surgery should not be accompanied by pain regardless of its intensity – the „pain management” is very important to be taken into account. Effectiveness of local anesthesia with Articaine, regardless of the concentration of vasoconstrictor is superior to all current anesthetics. The efficiency of Articaine allow it to be use in any dental intervention; Neurological disorders consecutive anesthesia with Articaine are statistically lower compared to the rest of dental anesthetics.

Surgical treatment of orbital blowout fractures

A. Gabără, Liliana Moraru, A. Căruntu

Introduction: The isolated blowout orbital fractures occur when traumatic agents that are comparable to the size of its aperture frontally hit the orbit. The traumatic energy is transmitted to eyeball, which transmits it to orbital walls that fracture easily, thus sparing visual analyzer. The symptoms are influenced by the displacement of the fracture, ranging from periorbital bruise to diplopia. Since orbital walls cannot be directly examined, many cases go undiagnosed and heals in a vicious manner being accompanied by atrophy and scarring of soft tissue thus making surgery difficult.

Methods: We retrospectively reviewed cases with blowout orbital fractures with secondary diplopia treated surgically in our unit over the last two years.

Results: Compared with recent fractures, treatment of viciously consolidated orbital blowout fractures though identical (reconstruction of orbital walls with preformed low profile titanium mesh), was more difficult and required more time in the operating room. The interventions resulted in remission of diplopia shortly after surgery without complications, but couldn’t equally correct enophtalmia.

Conclusions: Viciously consolidated orbital blowout fractures can be successfully treated in terms of eyeball functionality, but technically more difficult, with potentially higher perioperative risks and variable aesthetic results. Therefore proper diagnosis and early treatment are preferred.

Discussions: The use of computer assisted intraoperative navigation as well as 3D printers before surgery for better surgical outcome.

The management of the major burnt patient with associated airway injury in the clinical context of multiple casualty’s incident

B.M. Marinescu, Penelopia Marinescu, Ioana Tuhar, Andreea Benga, F. Popescu, T. Oprea

Purpose: The objective of this study is to present the outcomes of the correct management of the burned patient, and to share the experience acquired secondary to this event.

Methods: The case sample included 12 patients from the same multiple casualties’ incident, with the severity of the burn lesions varying between IIb-III degrees and between 5-

50% body surface, mainly affecting the upper half of the body (thorax, upper limbs, head and neck). As a common determinant all the cases presented respiratory tract lesions due to the nature of the event-fire in enclosed space followed by toxic fumes inhalation.

The surgical team involved was able to ensure in less than 10 hours the emergency surgical primary care for all of the 12 patients admitted.

Discussion: The distinctiveness of the presented cases consists in the great number of patients simultaneously admitted and treated.

Results: We can report a 100% survivability, at 50 days all patients being discharged only with minor granulating areas left to heal spontaneously under local treatment.

Most of the studies that we have found and we could refer to, on the matter of proper airway burn injuries and toxic fume inhalation did not statistically differ from our outcomes, even compared with consecrated burn centers from Western Europe.

Conclusions: The good overall results can be related to the interdisciplinary approach, right from the admission of the patients.

Our hospital is a level I medico-military unit, conceived from the beginning to provide emergency medical assistance for the most complex cases.

Bisphosphonate related osteonecrosis of the jaw – role of surgery

A. Gabără, Liliana Moraru, C. Dumitru, A. Căruntu, C. Becheanu

Introduction: Recently described pathological entity, Bisphosphonate Related Osteonecrosis of the Jaw (BRONJ), although with low incidence in population needs, to be known and understood in health care system (especially among dental specialists), given the complexity of its treatment.

Methods: This article aims to review existing data in literature, along with the presentation of our conclusions from a retrospective analysis of patients treated in our department from 2003 till present day with and without use of PRF during surgical procedure

Results: All patients, except one, were treated for different malignancies with i.v. Zolendronic Acid. They have developed the disease about 1.5 years after the initiation of this therapy.

The mandible body was more affected than the upper jaw. At admission in our department, all patients presented advanced stages of disease. Surgical treatment consisted in limited bone debridement or sequestrectomy with watertight wound suture and since last year, local placement of PRF. In two cases, radical maxillary sinus surgery was performed. In one third of cases redo surgery was necessary before using PRF.

The condition was cured in all patients, except one. Biphosphonate therapy was stopped by prescribing physician for all patients.

Conclusions: Although prevention still remains the “best treatment” the use of PRF is a helpful tool in surgical management of BRONJ.

Discussions: The role of PRF in prevention of BRONJ.

OP session SG4

Lumbar spinal stenosis – surgical decompression

I. Cusciac, E. Popa, M. Mitrică

Object: Patient inclusion criteria were lumbar canal stenosis, no degenerative lumbar spondylolisthesis and no previous history of lumbar surgery.

Methods: 30 patients with LSS who have been operated between 2014 and 2015 were included in this analysis.

Results: At 25 of the patients the functional improvement was very good, at 4 patients was good and at one patient with polineuropathy there was no significant differences in the clinical outcome.

Conclusions: Surgical decompression is the intervention of choice for lumbar spinal stenosis when nonoperative treatment has failed.

Modern methods in the treatment of fractures of the spine

C. Năstase, M. Marinescu, M. Mitrică

Introduction: This study has the task of exposing modern methods in the treatment of fractures of the spine. The main neurosurgical treatment methods are: vertebroplasty technique and fusion.

Methods: Between January, 2014 and July 2016 a total of 35 patients received treatment with fusion, using titanium screws mounted transpedicular with different sizes and titanium rods, and "space-er" for the fusion between vertebral bodies (interbody fusion). In the same period, 15 patients experienced the beneficial effects of vertebroplasty treatment which consisted of injecting a cement with special properties under fluoroscopic control, this method is a minimally invasive method. Clinical aspects were: subjective (pain) and objective (clinical examination of the patient: static and dynamic examination of the spine, gait examination, neurological examination).

Results: After using fusion, we have notice the reduced misalignment, restoring physiological curves, fixing segmentation in multipoint attachment compression and restoration of vertebral body height. Regarding vertebroplasty technique except that it's a safe and effective method, its benefit lies in improvement until pain accompanied by increasing mobility. There were no complications occurred. (Nerve compressions, thrombophlebitis, deep wound infections or local).

Conclusion: Vertebroplasty technique is used in elderly patients with vertebral compression due to osteoporosis. The treatment is effective in relieving pain and improving function of the spine, in such cases, without complications.

Surgical management of spondylolisthesis – case report

O. Sîrbu, A.V. Chirteș, M. Mitrică

Surgical management of spondylolisthesis has become increasingly common in spine surgery. A variety of pedicle screw systems have been described and new systems are being developed every day.

Spondylolisthesis represents an anterior subluxation of one vertebral body on another. Most commonly it occurs in lumbar region involving L5-S1 or L4-L5. Spondylolisthesis classification comprises five types: dysplastic, isthmic, degenerative, traumatic, and pathological and is divided into four levels depending on the percentage of subluxation. The clinical presentation may include back pain, radicular syndrome or cauda equine syndrome. Treatment is determined in relation to the presence of symptoms, extent and evolution of slipping.

In our neurosurgery department in the past 8 years we have gradually shifted from orthopedic – conservative treatment to the surgical approach. We will highlight through this case report the most important aspects of the surgical management of spondylolisthesis.

Magnetic resonance imaging evaluation of the cystic lesions of the knee

I. Codorean, I.B. Codorean, M. Ștefănescu, F. Năftănăilă

Objective: The objective of this study is to present and illustrate the spectrum of cystic lesions aspect, or "cyst like" revealed incidentally, in a group of patients investigated MRI for acute or chronic knee injury, suspected tumor lesions, inflammatory or infectious.

Material and method: This paper is a retrospective study conducted over a period of 8 years between - April 2007 - May 2015, and refers to a group of 2691 patients examined MRI. Age limits the patients were in the range of 14-77 years. The study group included 1851 (69%) men and 840 (31%) women.

Results and discussions: Of the 2691 patients were identified lesions in 197 patients with cystic appearance (7.32%). The shows and will illustrate the type of lesion identified, the location and appearance of articular MRI.

Conclusion: Magnetic resonance imaging is the method of choice in the study developed cystic lesions in the knee because it enables the detection, location, extent and relationship to adjacent structures.

Magnetic resonance imaging evaluation of the knee synovial pathology

I. Codorean, I.B. Codorean, M. Ștefănescu, F. Năftănăilă

Objective: The objective of this study is to present and illustrate aspects highlighted incidental pathological synovial knee in a group of patients investigated MRI for knee injuries acute or chronic, degenerative lesions, suspected tumor lesions, inflammatory or infectious.

Material and method: This paper is a retrospective study conducted over a period of 10 years within - April 2006 - December 2015, and refers to a group of 5776 patients examined the MRI. Age limits the patients were in the range of 14-87 years. The study group included 3638 (63%) men and 2138 (47%) women.

Results and discussions: Identified the following spectrum of synovial pathology: traumatic synovitis, infectious and inflammatory nonspecific synovitis, pigmented villonodular synovitis, localized and diffuse forms, condromatosis and osteocondromatosis as well as synovial tumors. In semiology terms, impaired MRI synovium, expressed, variable, in relation to disease stage, accumulation fluid articular in different quantities and locations, distension of the joint capsule, thickened synovium, synovial nodules, deposits of hemosiderin, bodies free joints.

Conclusion: Magnetic resonance imaging is the method of choice in the evaluation of native and contrast material of synovial pathology.

OP session SG5

Dunbar’s syndrome – case presentation

A. Dima, C. Mitru, D. Bădănoiu

A 33-year-old woman was investigated for persistent symptomatology as follows: post-prandial abdominal pain in the upper abdomen, constipation, and significant weight loss.

The computed tomography examination identified an almost complete stenosis of the celiac trunk by low arcuate median ligament insertion.

The imagistic aspect sustained the diagnosis of Dunbar’s syndrome (celiac artery compression syndrome or median arcuate ligament syndrome), a very rare pathology.

It was so performed the surgical management of the celiac trunk stenosis by laparoscopic arcuate median ligament release.

Post-operative follow-up was uneventful and patient was discharged at day 3 after surgery.

At 1, 2, and 3 months after the intervention controls, the patient was free of symptoms.

Myths among patients in laparoscopic cholecystectomy

C. Duțu, Andreea Gherghe, Andreea Rusu, Elena Stîrcu, F. Săvulescu

This presentation addresses a problem commonly encountered in the General Surgery ward, the one of patients’ myths related to the surgical treatment of gallstones. By listing the main misconceptions, the purposes of the paper are to identify them, to understand the causes of their occurrence and to find solutions in order to avoid their appearance in the future.

Two groups of patients with gallstones and their respective complications have been studied: 50 patients hospitalized at “Dr. Carol Davila” Central Military Emergency University Hospital, in the department of surgery II and 50 patients who have not received surgical treatment for this pathology. The patients were asked to answer a survey consisting of 9 questions to assess their knowledge regarding the treatment of their pathology.

There have been identified, among others, situations such as: confusion of the laparoscopic cholecystectomy with „the laser surgery”, distrust of the laparoscopic technique, aversion to surgery itself, a preference for dangerous and ineffective natural treatments, misunderstanding the term of cholecystectomy and confusing it with gallstones’ removal procedure (cholecystolithotomy). The ones who have consulted direct or online sources of general surgery and have received treatment present a lower risk of complications from therapeutic means set properly, while those who have sought information from nonsurgical sources are more prone to complications by delaying or refusing surgery.

Being part of the therapeutic arsenal for over 20 years and imposed as a "gold standard" in the treatment of gallstones, the laparoscopic cholecystectomy can still offer surprises in terms of its understanding and acceptance by patients. A correct information of the patients from authorized medical sources can reduce the rate of late-stage presentations and, consequently, morbidity and mortality.

Types of first artery approach in the head of pancreas cancers

A. Dima, T. Artenie, C. Mitru, F. Bold, D. Bădănoiu, I.P. Oprea

The „first artery approach" is the only currently recommended approach in cases of cancers of the head of pancreas. There are six types of first artery approach described in pancreatic head cancer, the choice and the combination of these techniques facilitates good surgical results.

All techniques are meant to primary assesse the integrity of the superior mesenteric artery in regard with the tumor in order to establish the resectability.

We present here our case series of patients in which this approach was performed, with good results comparable with those reported in literature.

In regard with the tumor localization, the combination of various types of artery first approach, it allows a safe radical pancreatic resection. The use of „first artery approach" requires a highly trained team in pancreatic surgery.

Complex treatment of advanced locally colorectal carcinoma

C. Drăgan

The colorectal carcinoma may require not only an appropriate colonic resection, but also a block resection of additional affected, invaded organs and tissues.

Objective: The objective of the present summary is to provide an overview on the surgical methodology and experience available at the Department of Surgery, University Military Emergency Hospital „Dr. C. Davila", concerning the treatment of locally colorectal carcinoma in advanced stages, their radicalism as well as immediate and long-term postoperative outcomes of surgical intervention.

Background: The carcinoma of the colon in its advanced stages is defined as a malignant neoplasia of the colon, which may appear locally or in a metastatic manner, invading other organs and tissue.

The local extension paths of the colon carcinoma are circular, as they follow the local lymphatic vessels, other paths of the carcinoma progress such as parallel of perpendicular path to the intestinal wall as well as perineural paths are rather seldom.

Methodology: Various cases with a diagnosis of local colorectal carcinoma in advanced stage, which were surgically treated at our Department, were assessed and discussed.

Results and conclusions: Our experience indicates that a (preventive) multi-organ resection performed in the cases of local colorectal carcinoma in advanced stage is the most reliable and safe methodology of surgical treatments. Because the palliative resection cannot predict or exactly determine the degree of tumoral metastasis, an ample tumoral resection is strongly indicated in order to improve the oncologic outcome, despite an increased risk of morbidity.

Particularities of myxopapillary ependymoma

R. Nica, C. Mușat, Alexandra Mihai, Florina Vasilescu, C. Pleșa, Diana Alexe, F. Săvulescu

The paper aims to highlight the particularities of tumor formations (lack of symptoms – even in the presence of tumor formation, at the location of the gluteal, but also the similarity in the clinical context with a pilonidal cyst), histologically diagnosed as myxopapillary ependymoma at a 22 years women.

Histopathological examination reveals tissue fragments with tubular and bulgy structures which reveals at Androgen receptor, S 100 immuno-histochemical tests – positive tumor cells.

The presentation contains images that shows the patient illness evolution from the beginning until the postoperatively status.

Radical antegrade modular pancreatosplenectomy

A. Dima, T. Artenie, C. Mitru, D. Bădănoiu, F. Bold, I.P. Oprea

The radical antegrade modular pancreatosplenectomy was for the first time proposed by Strasberg SM et al in 2003 in cases of pancreas body and tail malignant tumors.

The oncologic results of this technique are superior to other types of approach (i.e. posterior approach) especially due to a more efficient associated expended regional lymphadenectomy.

We present the results of a case series of six patients (ages 39 – 73 years) to whom this approach was applied, of which two laparoscopic interventions. There were registered no early or tardive complications.

The benefits of this method are represented by increased visibility in the dissection area, a better resectability of the ganglionar station N1, as well as the possibility of a posterior dissection with early vascular approach. Radical antegrade modular pancreatosplenectomy proved to be feasible and efficient in selected patients.

The therapeutically attitude in advanced abdominal cancer

T. Rogin, O. Albița, D. Orosan, C. Duțu, R. Nica, C. Mușat, F. Săvulescu

The purpose of this paper is to provide information about how pluri-visceral resection in intra-abdominal neoplasm, used together with oncological treatment, can improve survival rates in selected cases.

The paper presents the therapeutic attitude in abdominal neoplasms with pluri-visceral invasion, cases of multiple and serial resections in a number of patients with neoplasms in stages III and IV, with pursuing the cases over several years.

On our cases, we observed through imagistic methods surprisingly longer periods of time without recurrence of the disease in the abdominal region.

In conclusion, surgery remains the main approach for treating advanced intra-abdominal neoplasm and, together with complementary oncological treatment, can obtain greatly improved survival rates. We consider that maximal invasive surgery is justified.

Peritonitis localized through ileal perforation – case presentation

F. Săvulescu, C. Cârlan

In this paper we present the case of a woman, 50 years old, investigated in different hospital units, who consistently presented colicative pain localized at the level of the right iliac fossa. The particularities of the case are related to the fact that the patient showed a complex associated pathology that could justify the symptoms. Resorting to complex investigation methods, the diagnosis of ileal perforation by foreign body was established. This paper is accompanied by imagistic proof, images and videos that depict the diagnostic and therapeutic stages of the patient.

A giant recurrent liposarcoma of the retroperitoneal and peritoneal cavity

V. Ștefănescu, C. Mitru, F. Macău

Liposarcomas belong to the very seldom occurring group of malignant tumors of the retroperitoneal cavity. Usually they develop in adult patients. The pathogenic factors are still unknown. Growth of this type of tumor is slow and spreading. They can achieve very large sizes. The basic diagnostic examinations are ultra-sonography and tomography of the abdominal cavity. The basic treatment of liposarcoma is surgery, if possible radical resection of the whole tumor an organs involved, if possible. In this case, the tumor removed by our medical group weighed 11.5 kg in one part. It occupied not only the retroperitoneal cavity but also the whole abdominal cavity. Despite the removal of such a gigantic tumor, this kind of intervention is hard to consider as very radical. Literature data indicate that one of the essential risk factors of tumor recurrence is the size of the tumor. We would like to present the described case with regard to the rarity of occurrence: the enormous size, asymptomatic course of the disease, and recurrence 6 years after primary surgical treatment.

OP session SG6

A prospective multicentric randomized study comparing TAPP vs TEP

V. Ştefănescu, D. Moga, V. Oprea, C. Mitru, F. Macău

Background: Laparoscopic inguinal hernia repair has the advantage of lesser pain, early recovery and lesser wound morbidity.

There is no consensus on the best groin hernia repair.

There are two standard techniques of Laparoscopic Inguinal hernia repair – Total Extra Peritoneal repair (TEP) and Trans – Abdominal Pre-Peritoneal repair (TAPP).

There are not many trials available comparing the two techniques and questions remain about their relative merits and risks.

Method: Randomization plan was generated in November 2013; surgical interventions were performed in three military hospitals since January 2014 to august 2015 according randomization plan (49 TEP and 50 TAPP).

Results: Both TEP and TAPP mesh repair techniques were comparable in terms of operative time, intraoperative complications, and conversion to open, post-operative pain, time to resume normal activity and recurrence.

Conclusion: Laparoscopic repair of inguinal hernias is associated comparable results in both techniques, TAPP and TEPP and choice between TAPP and TEPP is a personal choice of concerned surgeon and/or surgical service.

The management of locally advanced gastric cancer

M. Tănase, I.P. Oprea

Worldwide, gastric cancer represents the fifth most common cancer and the third leading cause of cancer deaths. Although the overall 5-year survival for resectable disease was more than 80% in some countries due to the implementation of screening programs resulting in detection of disease at earlier stages, in Europe more than two thirds of gastric cancers are usually diagnosed in advanced stages with a survival rate of only 25%.

Surgical resection with extended lymph node dissection remains the only curative therapy for advanced gastric cancer, while adjuvant chemotherapies can improve the outcomes aimed at the reduction of recurrence and extension of survival.

In our surgical department' statistics, the locally advanced gastric cancer represents 71% of gastric cancer cases.

The objective of the presentation is to develop an attitude in view of early diagnosis and extension of survival regarding locally advanced gastric cancer.

Vaginal hysterectomy on cicatricial uterus

B. Panaite, O. Nicodin

This presentation is based on a study developed in the Gynecology Clinic of Central Military Hospital- Bucharest „Carol Davila"- there have been analyzed 50 cases of vaginal hysterectomy which followed a caesarian delivery which 10 presented also a history of a vaginal delivery, 9 patients had a history of two or more cesarean sections. We have compared the percentage of bladder injuries to the simple vaginal hysterectomies (which had no history of cesarean section). There have been also analyzed: the operative time, the blood loss, the admission time, other complications.

Results: There haven't been found significant differences between the two groups.

Incidenthaloma – Bartholin's gland adenocarcinoma case report

Mădălina Prună, C. Mitru, N. Tănase, C.I. Beţianu, I.P. Oprea

Background: Malignant tumors of the Bartholin glands are very uncommon, with a reported incidence of 0.1 cases per 100,000 women.

Case: A 40 years old woman attended our clinic complaining of a painful, tender lump in the right side of her vulva. The immediate clinical suspicion was of a Bartholin's gland abscess. Incision, drainage and marsupialization were carried out, but after 4 months she returned with a recurrent abscess, treated with right Bartholin's gland excision. Pathological examination revealed a well-differentiated papillary adenocarcinoma originating in the Bartholin's gland. Our patient underwent wide local re-excision, bilateral pelvic and inguinal dissection. There was no metastases in the lymph nodes and the margins were free of tumor. According to the FIGO classification, the patient was diagnosed with stage I vulvar cancer. At 1 year after surgery there is no evidence of recurrence.

Discussion: In cases of recurrent vulvar abscess, even in young patients, complete excision should be done in order to prevent delay in diagnosis of a cancer. The therapy of this rare tumor has many controversial issues and there is no consensus regarding the optimal treatment.

Patient specific instruments for complex tumor resection – reconstruction surgery within the pelvis

E. Cernat, P.L. Docquier, L. Paul, X. Banse, I.B. Codorean

The pelvis bone resection-reconstruction surgery is one of the most challenging fields in orthopedics. Being applied for tumors, as for other complex reconstruction cases, this type of surgery needs careful planning and is time consuming, in order to obtain proper accuracy.

Unfortunately not all the time the expected accuracy is met, with consequences for the patients. PSI proved to provide good cutting accuracy during simulated tumor surgery within the pelvis. This article present a series of 4 patients operated in our department between June 2014 and Mars 2015 for tumors resection-reconstructions.

The patients were imaged using a CT and an MRI scan and the images were reconstructed in 3D. According to the bone bank stock, the most similar allograft was chosen and the stored CT scan was reconstructed in 3D.

Patient specific instruments (PSI) were designed and manufactured using rapid-prototyping technology for the resection of the native tissues as for the resection of the careful selected hemipelvic allografts. Allografts' fitting to the pelvis of the patients was excellent and allowed stable osteosynthesis.

Role of radical pelvic and paraaortic lymphadenectomy in utero-ovarian neoplasia

A.C. Dima, O. Florea, T. Artenie, C. Mitru, F. Bold, D. Bădănoiu, I.P. Oprea

The radical pelvic and paraaortic lymphadenectomy is indicated in endometrial, ovarian, and cervical cancer when diagnosed in advanced, but resectable stages.

This approach is laborious, with associated good results when the indications are respected and when it is correctly and completely realized.

There are presented here the data of a case series of 9 patients, ages between 42 and 71 years, with genital neoplasia to whom this approach was applied.

Radiotherapy, chemotherapy or radio-chemotherapy in neoadjuvant setting was done before surgery. In all cases, hysterectomy with bilateral salpingo-oophorectomy, combined with pelvic and paraaortic lymphadenectomy was performed.

In three cases, there was imposed a multi-organ resection was imposed. In two cases, an anterior pelvectomy was performed, and in one case a rectosigmoidectomy.

Even if these interventions were very laborious (with surgery time between 5 and 8 hours), the post-operative morbidity was highly acceptable.

Laparoscopic surgical technique in the conservative treatment of tubal pregnancy

N. Niculescu, O.V. Nicodin, B. Panaite, C. Pârvulescu, Ioana Niculescu

Objectives: Establishing the indications of conservative treatment in ectopic pregnancy.

Materials and methods: Between 2012 and 2014, thirteen patients with ectopic pregnancy have been treated with conservative surgical techniques.

Pre- and intraoperative exclusion criteria were: ectopic gestational sac size exceeding 6 cm, fetal heart activity, β-hCG serum levels > 20,000 mIU/ml, previous surgery on the affected fallopian tube, isthmic ovular implantation.

Thus were selected the following cases: absence of the controlateral fallopian tube/functional compromised, gestational sac un-ruptured and ampulo-pavilionar implantation; we decided to apply laparoscopic linear salpingotomy, gestational sac extraction with bipolar hemostasis.

Results: We have obtained five intrauterine pregnancies and two ectopic pregnancies which required radical treatment (salpingectomy). Two pregnancies have been completed with gestation carried to term. There were no favorable results in patients who had other associated factors of infertility.

Conclusions: In selected cases laparoscopic surgery allows the anatomical and functional restauration of the fallopian tube affected by pregnancy and offer a chance in getting a desired pregnancy.

OP session NS1

Extracorporeal circulation

Florentina Rizea, Marinela Bosincianu, Elena Iftode, Gabriela Batan, Andreea Teodorescu

A procedure in which blood is taken from a patient's circulation to have a process applied to it before it is returned to the circulation. All of the apparatus carrying the blood outside the body is termed the extracorporeal circuit.

The basic role of CPB is to maintain the systemic circulation at physiological parameters during the cardiac arrest and in the hipocontractillity period (reperfusion).

The adequate organ perfusion it's realized by maintaining the cardiac output and the perfusion pressure.

The blood flow through the arterial line, and therefore in the systemic vascular bed, is generated mechanically by a pump and it is not pulsatile. There are also technical devices that allow pulsatile flow generation. During CPB the organs vulnerable to hypoperfusion and hypotension should be especially protected (brain, kidneys).

CPB components: venous drainage; arterial cannula; venous reservoir; the actual pump; the oxigenator; heat exchanger; accessories; priming.

The perfusionist is the person in charge for the CPB functioning and who is endorsed on the type of the surgical intervention and patient's medical data. He is the one who calculates according to the patient's body surface, the priming necessary, the cardiac output (in order to avoid hemodillution) and selects the cannulae to be used.

The CPB starting is made on the surgeon's demand, who is maintaining a clear and bilateral communication with the perfusionist. The perfusionist repeats and confirms the surgeon's orders, informing him permanently on its activities.

The CPB exit (CPB-OFF)

It is initiated after the cardiac electric activity restore, after a reperfusion period, adequate to the surgical intervention's length. The perfusionist is gradually clamping the venous line and reduces the pump output, allowing to introduce the venous blood in the patient's circulation.

The blood pressure (BP) and heart filling (central venous pressure) are carefully monitored. If the Bp and heart filling are optimal, the CPB can be stopped.

The role of the professional nurse in the intraoperative care of the patient undergoing open heart surgery is beneficial for obtaining a positive outcome for the patient.

D vitamin or hormone

Florentina Dumitru, Mihaela Andreev

Through the ages, medical knowledge have evolved with the science and technology progress. A number of substances considered in a certain way then and there, turned out to fit into other classes of drugs nowadays.

Vitamins have diverse biochemical functions. Some, such as vitamin D, have hormone-like functions as regulators of mineral metabolism. Vitamin D promotes calcium absorption and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany.

Many scientific evidence confirm that "D-vitamins" act as a hormone in the body: modulation of cell growth, neuromuscular and immune function, and reduction of inflammation. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D.

These facts corroborate by the discovery of receptors of active form of vitamin D (1.25-dihydroxy-cholecalciferol) across cells in several types of tissues and organs, justify the new classification.

From the pharmaceutical point of view there are many different types of formulation and concentrations, each of them with its specificity and indications.

The new approach should be: What is more useful for us? A classification or the understanding and application of new medical knowledge in daily pursuits for everyone?

Cancer patients' rights

Elena Coman, L. Onel, Mădălina Ciobănescu

1. Right to early detection, diagnosis and treatment: All cancer patients, irrespective of age, sex, or various social conditions have rights to early disease detection, best methods of diagnosis and treatment available.

2. Quality of care: Quality of care should be the result of a comprehensive approach that responds to the particular needs of patients and their families, to promote improved quality of life and lead to more efficient use of resources.

3. Patient-health professional relationship: This relationship should develop in terms of mutual respect and trust and rely on well-defined rights and responsibilities.

4. Patient information: Access to information for patients and their families must be promoted to a better understanding of the disease and treatments, in order to decrease anxiety and to browse in a less stressful this stage of life.

5. Psychological support for patients and their caregivers: Psychological support is essential for the emotional needs of patients and their families at all stages of the disease, during and after treatment.

6. Socially and financially support

7. Rehabilitation and reintegration into society: Restoring physical and mental health and quality of life requires a complex, multidisciplinary approach (physical, social, occupational, psychological and spiritual).

8. Palliation: Patients' rights to medical care, which aim to improve the quality of life in advanced stages of malignant disease.

9. Social consultancy of patients: Concept established colloquially as "advocacy", which means all actions taken individually or through organizations, which aim primarily ever more active involvement of patients in medical decisions, but also in promoting health programs and policies.

Ebola

M. Leatham, K. Higgins

Following the United Nations declaration that the Ebola virus outbreak was a public health emergency in March 2014 the United Kingdom made commitment to provide support including providing care for the people in Sierra Leone. An international response involving WHO, US and British governments, the UK Department of Defense designed and oversight of the building of several Ebola Treatment Centers and Training Academy and provided logistic and planning support. In addition, approximately 800 Defense personnel were deployed to Sierra Leone including nurses, doctors and laboratory staff.

Based upon their current experience, skills and knowledge, two personnel from the British Army reservists were selected and deployed from 207 Manchester Field Hospital. Following further specialist training to ensure they could work in austere conditions treating people with this hemorrhagic viral disease that that claimed up to 11,325 lives of many civilians Lieutenant Colonel Marian Leatham and Major Kath Higgins work and lead a teams of nurses, combat medical technicians and healthcare workers to provide care and treatment for patients with confirmed or suspected Ebola.

This presentation gives an account of their experiences and the importance of team work and leadership from two different roles, one as a team leader the other as the Senior Nursing officer for the hospital in dealing with previously untested challenges in infection control and patient and personal safety during their time in Kerrytown Ebola Treatment Centre in Sierra Leone.

The nurse-patient relation

Florica Cristea

To practice medicine correctly, means to respond promptly, honestly, and with empathy, to the certain needs required by the medical act, always accompanied by a great sense of ethics.

The disease implies firstly a disturbance in the patient's inner equilibrium, which translates in physical or a mental disorder, a maladjustment to a new definitive or temporary situation. A person "enters in the disease" with a certain psychic, temperament, character and intelligence, with preconception and cultural horizon and that is why the patients assumes a certain attitudes towards the disease but also towards the medical team: trust, esteem, sympathy, but also possibly doubt, fear, hate and contempt.

The nurse has to treat all patients unconditionally. Also, the nurse-patient relation does not have to be with only about treatment but also connecting and understanding the patient in these difficult moments. Communication with the patient has to be in consonance with his sate, his has to be in consonance with his sate, his understanding and has to be associated with aiding elements of his ailment. The care of medical ethics consists of a member of more and professional traits regarding the nurse: honesty, altruism, a giving sense, respect, solicitude, comprehension.

This is way the medical profession has to be exercised with patience, generosity, passion, honesty, the central focus of all, being the patient who has to be understood and accepted as he is.

Laboratory investigations of autoimmune hemolytic anemia

Eva Dincă, Verginica Săvoiu, D. Săvoiu

Autoimmune hemolytic anemia is a condition in which effectors of the immune system erroneously attack own red blood cells, thus leading to lysis thereof.

Blood samples were collected from three patients suffering from immune hemolytic anemia. Vacutainers required for blood collection and Coombs kits, as well as microscope slides, thoroughly cleaned, degreased and dried have been used.

A complete blood count was performed, along with the leukocytic formula and a direct Coombs test.

Compared to normal values, all patients presented low values of the plasma concentration of HGb, Hct and of the erythrocytes count.

Elevated bilirubin, GOT, D-dimers and fibrinogen values were recorded.

A positive result of the Coombs Test confirms the autoimmune hemolytic anemia diagnosis.

Effect of Algisyl ventriculoplasty on functional status of patients with advanced heart failure

Daniela Nae, Magdalena Petrea, Iulia Dragomir, Iuliana Gheorghediac, Ileana Hănțulie, S.I. Dumitrescu

Study objective: Evaluation of functional status in the first six months after implantation of a new device, in first 10 patients of our center, randomized to the surgical arm of AUGMENT-HF clinical trial.

Methods: AUGMENT-HF clinical trial is a randomized, controlled study to evaluate the safety and cardiovascular effects of Algisyl-LVR™ as a method of LV augmentation in patients with chronic heart failure due to ischemic or non-ischemic dilated cardiomyopathy. Algisyl-LVR™ is a biopolymer classified as medical device by regulatory authorities that is implanted in free wall of the left ventricle aiming to alleviate LV dysfunction through a left thoracotomy followed by 10 - 19 biopolymer implants. Subsequently to device implantation the patients were evaluated by complex monitoring of clinical, laboratory and laboratory parameters comparing the surgical arm with the standard treatment arm.

Results: Assessment of clinical status at randomization and follow-up visits was done by NYHA class evaluation, 5-minute walk test and Kansas City cardiomyopathy Questionnaire.

	NYHA II/III/IV	6MWT	KCCQ
Randomization	0/7/3	271 ± 83	46 ± 21
3 months	2/6/2	302 ± 89	52 ± 24
6 months	3/5/1	311 ± 82	62 ± 27

Conclusions: The surgical procedure to implant the biopolymer alginate resulted in improved functional status in patients with advanced heart failure.

OP session NS2

Essential arterial hypertension

Elisabetha M. Ionescu, Petruța Zărnescu, Laurenția Leonte, V. Ilieșe

Introduction: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC).

Hypertension is defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg. The diagnosis of hypertension should be based on at least two BP measurements in the sitting position per visit on at least two visits.

Risk factors: Include age, male sex, smoking, dyslipidemia, glucose intolerance, obesity and family history of premature CVD. Asymptomatic organ damage mainly involves left ventricular hypertrophy, evidence of vascular damage and microalbuminuria.

Cardiovascular risk factors: Total, LDL and HDL cholesterol, and fasting triglycerides and glucose are considered routine tests in all hypertensive patients.

Treatment approach: Lifestyle changes. Appropriate lifestyle changes are the cornerstone for the prevention of hypertension and are also important for its treatment. The following lifestyle measures are recommended:

- Salt restriction to 5-6 g/day.
- Moderation of alcohol consumption (<20-30 g of ethanol per day in men and <10-20 g in women).
- Increased consumption of vegetables, fruits and low-fat dairy products.
- Reduction of weight to BMI of 25 kg/m
- Regular exercise (≥30 min of moderate dynamic exercise on 5-7 days per week)
- Smoking cessation
- Prompt initiation of antihypertensive drugs is recommended in patients at high or very high CV risk in elderly patients drug treatment is recommended when systolic BP is 160 mmHg, or ≥140 mmHg if younger than 80 years and treatment is well tolerated.

Conclusions: Diuretics, beta-blockers, calcium antagonists, ACE-inhibitors and angiotensin receptor blockers are all suitable for the initiation and maintenance of antihypertensive treatment, either as monotherapy or in combination therapy. Lifestyle changes. Appropriate lifestyle changes are the cornerstone for the prevention of hypertension and are also important for its treatment.

Blood investigations in Hodgkin lymphoma

Cătălina Mihăilescu, Matilda Marinache, Constantin Coman

Introduction: Lymphoma is a cancer that starts in lymphocytes of the immune system and appears as a solid tumor of lymphoid cells. These cells developed abnormal come from lymph nodes and spread outside the lymph system.

Thomas Hodgkin lymphoma published the first description in 1832, as called after him, Hodgkin lymphoma.

Material: Dynamics were followed in 2 patients diagnosed with Hodgkin lymphoma after about 10 years. Patients came for full blood regularly.

Explorations Blood: complete blood count with differential white blood count, erythrocyte sedimentation rate determination, and puncture and bone marrow biopsy performed iliac crest, ESR.

Biochemical explorations: liver function tests, electrophoresis, serum lactate dehydrogenase, serum calcium, urinary calcium, cupremia.

Biological sampling was done following bioethics standards, rules and norms aseptic SSM.

Method: Immunological abnormalities may occur. Such known cell-mediated immunity deficiency characterized by negative tuberculin skin test.

Results and discussions: Hemoglobin concentrations were slightly lower for patients investigated.

Leukocyte number was slightly increased.

The percentage of eosinophils was close to the maximum limit.

This Hodgkin's disease is suspected lymphadenopathy occurs when accompanied by fever, night sweats and weight loss.

To confirm the diagnosis of disease, it is necessary to carry out investigations where there Blood hemoglobin decreased, elevated ESR and fibrinogen for Epstein-Barr virus may increase the risk of lymphoma.

Diagnosis of the disease can be achieved after puncture ganglion; smear analysis reveals the presence of Reed-Sternberg cells, but for a diagnosis of certainty node biopsy is needed.

Advanced Parkinson’s treatments and therapies

Olguța Preda-Cârșote, Violeta Gâlcă, Mioara Girea

Parkinson's disease is a chronic and progressive movement disorder, meaning that symptoms continue and worsen over time. The cause is unknown, and although there is presently no cure, there are treatments options such as medication and surgery to manage its symptoms. As Parkinson disease progresses, the amount of dopamine produced in the brain decreases, leaving a person unable to control movement normally. Medication is the main treatment used for the movement symptoms of Parkinson's The term 'advanced Parkinson's' usually refers to a time when symptoms are more complex and have more often an effect on patients’ day-to-day life. In the later stages of Parkinson's, when non-motor symptoms begin to dominate quality of life, it may be appropriate for some drugs to be withdrawn.

There are few advanced therapies for patients with Parkinson disease when symptoms don't respond well to oral medication.

1) Apomorphine – an injection or infusion of dopamine agonist under the skin levodopa/carbidopa gel inserted into

your stomach by a tube. The dopamine agonist apomorphine provides relief from off periods when administrated as a subcutaneous injection. When applied continuously via a portable pump, oral medication can often be reduced considerably and dyskinesia improve in many patients. Continuous duodenal levodopa infusion it is used in treatment of advanced levodopa-responsive Parkinson's disease with severe motor fluctuations and hyper-/dyskinesia when available combinations of Parkinson medicinal products have not given satisfactory results. Duodopa is a gel for continuous intestinal administration.

2) Deep brain stimulation (DBS) is a surgical procedure used to treat a variety disabling neurological symptoms of Parkinson disease such as tremor, rigidity, stiffness, slowed movement and walking problems. At present, the procedure is used only for patients whose symptoms cannot be adequately controlled with medications. The majority of patients with Parkinson disease develop motor fluctuation and dyskinesia as their condition progress.

There are a number of therapies that can help people with Parkinson’s and offer advice on ways to help manage symptoms. Therapists and nurses are trained to provide advice and recommend exercises and treatments to keep them active and healthy. They are part of a team that can help patients manage their condition. The main therapies includes: occupational therapy, speech and language therapy, physiotherapy, seeing a dietitian and complementary therapies.

As a conclusion, Parkinson’s disease does not just affect the person living with it – it affects the entire family and an extended community of friends and loved ones.

Epilepsy (seizures disorders)

Cristina Lixandra, Oana Rada

Epilepsy is a syndrome of various etiologies caused by excessive neuronal discharges having a paroxysmal, transient, intermittent and interactive mechanism, characterized by sudden seizures of intermittent disorder of brain function often involved with loss of consciousness.

Antenatal causes: exogenous factors that deal with the mother during the pregnancy; abdominal trauma; maternal infections; toxic causes such as alcohol, professional noxae and so on.

Natal causes: difficult deliveries; fetal injuries during delivery or Rh incompatibility.

Postpartum causes: brain injuries; allergic factors; calcium and glucose metabolism disorders.

People with seizure would be classified according to the type of crisis, underlying cause, epileptic syndrome and episodes occurred during and around the crisis. Seizure types are classified depending on location or source of epileptic seizure (local seizures) or its distribution (grand mal seizures) in the brain. There are a number of factors contributing to the installation of the status epilepticus such as antiepileptic drug withdrawal; metabolism disorders; stroke or cerebral infarction; CNS infections, trauma or tumors.

Psychosocial effects of epilepsy include depression, anxiety disorders, migraines and attention deficit hyperactivity disorder. The symptoms vary according to the scale of seizure (partial seizures – affecting only one brain area or grand mal seizures – affecting large areas of the brain, located in both cerebral hemispheres).

They appear like small contraction localized in a small body segment or a series of abnormal perception of the environment, either visually or auditory perception. Clinical manifestations are very different because of the epileptogenic focus – “that small area of the brain where the seizures come from”.

Medication include anticonvulsant drug therapy and it is designed to reduce the frequency of epileptic seizures but it may also lead to their disappearance. The therapy for epileptic seizures is a specific one. Each patient, in terms of right chosen treatment and depending on the particular disease, about a third of them as an average, may not do the seizure or do them rarely.

So, they have the opportunity to conduct a normal life with a balanced diet and right behavior.

Penile tumor. Nursing for patients with penile tumor

A. Bozieru, Agnes Ciucă, D. Spînu, D. Mischianu, F. Rusu

Introduction: Penile tumors are a rare pathology that unfortunately remain untreated or treated late. A misunderstood sense of shame is often the biggest culprit for the late presentation to a doctor.

Material and method: We present the therapeutic approach adopted in the case of a patient with penile tumor, evolving for about ten years, hospitalized in the department of urology „Carol Davila” Hospital. The patient, male aged 78 years old, presented in our service complaining about diffuse left inguinal pain. Local exam confirmed the presence of a left groin palpable lymphadenopathy and a tumor involving the Glans with a minimal extension to the penian shaft. Biopsy confirmed low grade squamous cell carcinoma. Partial amputation of the penis and bilateral inguinal limfodisection was performed. Pathology result confirmed negative penian resection limits, but with left inguinal lymph nodes invaded (3/6).

Results: Postoperative evolution was favorable. Three months after surgery, a dilatation of the urinary meatus was necessary. He is now under oncological treatment.

Conclusions: The delay of the diagnosis due to a false sense of shame represents one of the largest mistakes. In the case of this patient, the neoplasia type has allowed and allows palliative treatment efficiency, unfortunately most often we come to cases of aggressive neoplasia that once metastatic dramatically reduces the life expectancy.

The role of nurses caring for patients in renal colic section of urology

Iulia Ene, Liliana Dogaru, Gabriela Pînzariu

Definition: Renal colic is a painful paroxysm with anteroinferior location for lumbar and iliac fossa irradiation, testis and scrotum in men and vulva in women, presenting an undulating, with maximum painful lull. It is a medical emergency and urological. Maximum intensity is usually located in costovertebral angle and lower right rib area XII, can have headquarters variable depending on the location of the obstacle.

The main cause of renal colic is represented by the sudden onset of a barrier to evacuate urine – kidney cavity, ureter, uretero-bladder junction which sharply increases the pressure in the pielo-uretero-caliceal system and renal receptors produce excitation preso-pain. Sudden obstacle that blocks urine is most often a stone, but may be just as likely, a clot (reno-ureteric bleeding) home suppuration material failure (renal TB). Other reasons: neighborhood processes (inflammation, tumors) extrinsic compression, causes iatrogenic following therapy (surgery, lithotripsy, radiotherapy) renal infarction.

Clinic: the patient shows paroxysmal pain, sudden onset, sometimes apparently in full health. The patient seeking a position analgesic that cannot find; useful element in the differential diagnosis with other abdominal pain syndromes that often immobilizes the patient.

Renal colic is accompanied by:

- Digestive symptomatology: nausea, vomiting, ileus, pseudoocluzive syndrome - retro-peritoneal irritation signs;

-Neuropsychiatric -signal: agitation, extreme anxiety;

- Cardiovascular symptomatology: usually shows no changes in BP and pulse.

- Fever association can quicken the pulse and BP lowering in this case suggests a UTI;

- Signal urination: Colic can be followed by hematuria which can be caused by a migrated stone, then became obstructive. Urine may be clear or cloudy excluding kidney affected by opposite kidney pain with hematuria and renal colic. The association with hematuria and histuria at the diabetic patient or consumer of analgesics can suggest papilar necrosis. The symptoms depends on the location of the obstacle: high obstacle will produce maximum pain, iliac fossa irradiation and genitals;

The role of nurses caring for patients in renal colic section of Urology:

Monitoring: assessment of vital signs (blood pressure, pulse, respiration, urine output, temperature) and physician reports the changes; assessment of pain characteristics and macroscopic appearance of urine; administration of treatment; follows the investigation indicated by the doctor; explain to the patient how to collect urine and urinate through cheesecloth (we could recover calculation for chemical analysis); obtaining blood and urine samples, prepare the patient for uiv ; treating the patient who shed; give moral support to the patient and family.

Transurethral bladder tumor resection

Adriana Ursu, Claudia Dumitru, Mihaela Marin, Angelica Mălăescu, Gina Popescu

Bladder cancer is a very common malignant affection which has a high rate of recurrence.

79% - 85% of the patients with bladder cancer are in an evolutionary stage of non-invasive bladder tumors, while the proportion for the recurrence is very significant.

On a global scale, the mortality caused by this disease is 2-10 men and 0.5-4 women out of 100.000 citizens per year. Bladder cancer is 3 to 4 times more frequent in men rather than in women.

In order to determine the right diagnosis, a transurethral tumoral resection has to be performed under spinal anesthetic agent.

Due to the risk of recurrence and progression, the patients with non-invasive bladder tumors have to be carefully monitored and the frequency and duration of the cystoscopy exams need to reflect the risks for every patient.

OP session NS3

The implementation of the protocol concerning the communication of bad news in the nurse's practice

B. Silveșan, Lucia E. Istrate

Bad news may be defined as "any information which adversely and seriously affects an individual's view of his or her future"

It is difficult and uncomfortable to deliver bad news in all medical areas especially in oncology even though we are referring to diagnosis, reoccurrences, disease progression or transition to palliations treatment. In any situation, this is a difficult and stressful task.

Studies showed that a significant part of the medical team experiment a strong emotion after communicating bad news, and the effect of stress lasts for hours and even days.

To realize an efficient communication there have been adopted a series of international protocols that can be used. One of them, named

S-P-I-K-E-S protocol, a recent but largely used strategy, that approaches the establishment of an appropriate context, checking the patient's perception; determines the amount of information known, desired and that will be transferred; the knowledge of medical realities before discussion; exploring the emotions that might appear during the interview and the establishment of a support strategy.

The role of palliative care for patients with severe chronic diseases

Ani Ivan, B. Silveșan

Palliative care is the active and total care of patients whose disease is not responsive to curative treatment. The control of pain and of other symptoms, the psychological, social and spiritual assistance are of major importance.

It is a benefit not just to those suffering from: cancer; HIV; progressive neurological disease; renal, cardiac, hepatic insufficiency or other diseases that cannot be cured, but also for their families considering that palliative care is very complex, it is a holistic care, is not only a medical work.

The goal of palliative care is to ensure the best possible quality of life for patients and their families.

Bone densitometry (DEXA, DXA)

Nicoleta Tudora, Cristina Popa, Carmen Hărdău

Bone Densitometry (DEXA = Dual-energy X-ray absorptiometry) is a paraclinic procedure which measure bone' density and osteoporosis detection, this phenomena appear especially in menopause women. Osteoporosis treatment prevents the risk factors, which improve the bones resistance.

DXA is recommended in 65 old women menopausal and with previous pathological fractures. Also in women with multiple risk factors (like familial vertebral fractures history) and in long periods of treatment with corticosteroids.

These examination is recommended for lumbar spine: 4 lumbar vertebrae L1 to L4, to coxofemoral joint (hips and pelvis) and forearm. For a complete test is necessary to exam 3 regions, but for a correct diagnostic 2 regions are enough.

DEXA is a simple, fast and noninvasive procedure. During the examination time, a very small dose of radiation is used. We mentioned that are no previous preparations for the patients and we can perform at any time of day. The patient can remain clothed during the examination, except them which contain metals things.

The patient should be lying on the table, between X ray and detector. The detector in moving in the part interested, without pain, bites or discomfort. Entire examination is finished in 5- 10 minute.

DEXA provides us 2 important results: T score and Z score. T score: provides the result of a comparison between the patient bone density and a maximum bone density (in a 30 years old man, young and healthy). A T score less than -1 or more is NORMAL. The values between -1 and -2.5 suggest the osteopenia and less than – 2.5 means OSTEOPOROSIS.

Z score: compare the patient bone density with a bone density in a person with the same age, this appreciate the damaged/lost grade of bone tissue. A negative Z score means that the patient is having fragile bones, and a positive score means that the patient is in normal limits.

Is snoring dangerous?

Ileana M. Mateș

Snoring is a sleep disorder which often precludes overnight rest, but it is especially important as a symptom of more serious respiratory disorders, such as the Syndrome of Sleep Apnea (SSA), the Obstructive Sleep Apnea (OSA) or the Syndrome of the Upper Airway Resistance (SUAR).

Snoring occurs when there is an obstruction to the flow of air into the area behind the nose and mouth, where the upper part of the pharynx and base of the tongue meet the soft palate and the uvula.

Severe snoring is often the symptom of serious health problems, with grave long-term effects, such as the obstructive sleep apnea (SAS).

In the sleep medicine a scale is commonly used to assess the degree of daytime sleepiness, called the Epworth Sleepiness Scale.

Hypertension and heart diseases are more common with people who snore, both men and women; moderate snoring involves a partial airway obstruction, and so an insufficient oxygenation during sleep – a fact which has serious consequences on the cardiovascular system.

In Western countries there is a wide range of products against snoring.

There are devices which prevent sleeping on the back or devices which change the position of the mandible.

If the snoring is very strong, an ENT doctor/ otorhinolaryngologist will examine the nose, mouth, soft palate and throat and will indicate if it is necessary to correct a nasal obstruction (deviated septum or polyps). An examination in the sleep lab may be necessary in order to determine how serious the snoring is and if it is coupled with sleep apnea thus affecting the patient health.

Meniere's disease

Mihaela Arghir

Meniere's disease is a disorder of the inner ear, whose causes are not fully known. It is characterized by the build-up of fluid in the inner ear, caused by either anxiety, high blood pressure or excessive salt consumption.

An acute episode of Meniere's is characterized by the following symptoms: dizziness, nausea, vomiting, hearing loss, ringing in the ears, low tolerance for loud noises, headaches and a sensation of "full ears", sometimes before the episode begins.

Diagnosing Meniere's disease is managed by an otorhinolaryngologist, using techniques such as: tonal audiogram, electrocohleography, CT scans.

The treatment for Meniere's usually starts by using drugs which lower the fluid quantity in the inner ear, and thus the pressure build up (diuretics).

The nurse – patient relationship

Georgeta Porojan, Alina Ispas

All medical care takes place in the framework of the nurse-patient relationship.

Communicating with the patient can bring together two people from different backgrounds, with different personalities, and different ways of seeing things. Therefore, the nurse-patient relationship is characterized through individuality and specificity.

The multiple times I've been the patient myself have given me the opportunity to better understand the point of view through which we, as medical professionals, are perceived. These experiences have also allowed me to ask myself about the ways we can better respond to the expectations a patient may have from the people who are in charge of their health care. This includes both the relationship between a single medical professional and a patient, and one between a team of health care professionals and a single patient. In spite of any differences, there's always a basic way to communicate with the patient, which answers most of their expectations: empathy.

I have had experiences in which I first focused on the ''disease" before the patient. In order to avoid this situation, I considered six steps that I must always follow in order to see the patient as more than just a medical condition. These steps are: awareness, acceptance, responsibility, trust, purpose and integrity. Used in integrative psychotherapy, these six steps can provide an excellent framework for communication between medical professionals and patients.

Management of AAA – endovascular repair

Marinela Bosincianu, Elena Iftode, Mihaela Apostol, Roxana Constantin

Introduction: Abdominal aortic aneurysm is a multifactor disease that usually occurs in cardiovascular high risk patients and leads to death by rupture if not fixed on time. They are hemodynamic unstable, they need inotropic support and diagnostic is based on ultrasonography and computed tomography.

Objectives: Managing AAA needs pertinent screening and patient evaluation, in order to offer the best treatment, providing a safe and cost effective operation, with the best long term quality of life.

Method: Between 2015 and 2016 were selected a total of 6 patients with AAA who presented a high risk to the classic operation and the surgical treatment options for AAA was EVAR.

EVAR is performed by an interventional radiologist and a vascular surgeon, using x-rays to guide medical instruments inside the arteries. The benefits of EVAR over traditional surgery are: no large abdominal surgical incision, no sutures (stitches), or sutures only at the groin area, faster recovery and shorter time in the hospital, no general anesthesia (in most cases), less pain, reduced complications.

Results: Was analyzed the postoperative evolution, showing the special role of nurses in intensive care.

Conclusions: The results show that in the modern era of AAA treatment, EVAR is a safe and effective in patients with high-risk short and medium term.

Healthcare of patients with TAVI

Marinela Bosincianu, Elena Iftode, Roxana Tudor, Elena Anghel

Introduction: Aortic stenosis is a narrowing of the valve orifice that is responsible for forming a barrier against ventricular ejection during systole. Transcatheter aortic valve implantation (TAVI) involves inserting a new artificial heart valve inside the old tight valve using a balloon catheter.

The valve is made up of a metal frame (stent) and the outer lining (pericardium) of a cow’s heart.

Objectives: TAVI is a new technique with the potential for transforming the treatment of patients with aortic stenosis, who are at high risk for conventional open heart surgery or considered inoperable.

Working method: Case of Study-in period 2015-2016 in our clinic have conducted a number of 8 (3 transapical si 5 transfemoral)

Results: Stenotic aortic valve disease distribution by age and gender, low rate of hospitalization days.

Conclusion: Cardiovascular surgery is performed by more modern methods that greatly reduce trauma, postoperative pain and risk of infection.

During the interventions of cardiovascular surgery plays an important role. The intervening period is shorter, the patient's chances of recovery are higher. In this type of implant, shorter hospital stay and faster recovery.

The role of nurses in health postoperative cares of patients, supervision and monitoring with TAVI, and to educate patients and learn, to lead a healthy life it is very important.

OP session NS4

Transverse myelitis

Daniela I. Bradea, Mihaela Ivănică, Ioana Catalina, Oana Iliașii

Transverse myelitis is a neurological condition in which the spinal cord is inflamed. The inflammation damages nerve fibers, and causes them to lose their myelin coating leading to decreased electrical conductivity in the central nervous system. Transverse implies that the inflammation extends across the entire width of the spinal cord.

Although it is a very rare affliction, with an incidence rate of 1.34 in a million (according to Association for Transverse Myelitis), this syndrome can strike at any age in both women and men, but it`s mostly encountered between 10-19 years and after 40.

The patients who suffer from transverse myelitis recover differently depending on cause and severity of the illness. Almost 1/3 of these patients are completely recovered, for other 1/3 the results are average and for the last 1/3 the results are very low. The recovery is most efficient in the first 3-6 months after a transverse myelitis crisis and some of the patients need a few years for a total or partial recovery.

Benefits of the exploratory laparoscopy in acute surgical abdomen therapy

Claudia Buzatu, Daniela Mihai, Anca Pologea

With progress in methods of investigation and diagnosis, most of cases which goes to the hospital in emergency, receiving preoperative indication of their disease. There are some cases in which preoperative was set just it`s an acute surgical abdomen and that have operated. Here is where exploratory laparoscopy finds utility.

Also the exploratory laparoscopy contributes to complete partially elucidate preoperative diagnoses but with clear surgical indication.

In our general surgery department are operated in emergency between 250-300 patients with acute surgical abdomen. 15-20% of them receive benefits of exploratory laparoscopy.

This paper is a retrospective study of 684 patients operated in our service, in emergency conditions, in the last three years. 147 of them occurred through laparoscopic approach and laparoscopy was completed in 86 cases.

The paper is an according debate between pre and postoperative diagnosis, distribution by type of pathology and diseases in which laparoscopy had higher success rate.

Discussions aimed at length the advantages of exploratory laparoscopy in emergency conditions and diagnostic doubt.

The end of paper presents the conclusions that bring to the fore: shortening surgery; shortened hospitalization; rapid reintegration into family and society.

Gastroesophageal reflux disease

Alina Dinu, Anișoara Graure, Cristina Ioncescu

GERD has long been recognized as a significant public health concern in USA generating along the time, many discussion between gastroenterologists and surgeons. Once antireflux barrier was identified, and mechanism of reflux established, GERD can be defined as the failure of the antireflux barrier (represented especially by the lower esophageal sphincter, by gastric empty of gastric contents into the esophagus), allowing abnormal reflux of gastric contents into the esophagus. Positive diagnosis is setting by the presence of documented esophageal mucosal injury (esophagitis) or excessive reflux during 24 hours intra-endophageal pH monitoring. Medical treatment is efficient in acid suppression, but does not address the mechanical etiology, is too expansive and affect the quality of life of patients. Miniinvasive surgery was a boom in management of GERD, offering great satisfaction to patients, low costs and rapid social integration.

We present the role of surgery in GERD, therapy GERD which occur 85-93% control of reflux symptomatology, providing data from the literature on the techniques used, their advantages and limitations.

Ovarian cancer

Luciana Cristache, Costinela Lazăr, Ștefania Aldea, Claudia Rădulescu

Ovarian cancer represents one of the most common malignancies that affect women. It has a high death rate because the symptoms are nonspecific and is diagnosed in very advanced stages when the tumor has already evolved and metastasized.

The surgical treatment for ovarian neoplasm includes surgical intervention in early stages, laparoscopic exploration in order to staging and harvesting tissue for biopsy and procedures for tumoral mass reduction.

Surgery for breast cancer

Florina Aslan, Luminița Ștefan-Dragomir, Viorica Gogor

Through this study I want to make a presentation about the surgery and its indications for breast cancer.

Most women with breast cancer have some type of surgery as part of their treatment. Depending on the situation, surgery may be done for different reasons. For example, surgery may be done to: remove as much of the cancer as possible (breast-conserving surgery or mastectomy); find out whether the cancer has spread to the lymph nodes under the arm (sentinel lymph node biopsy or axillary lymph node dissection); restore the breast’s shape after the cancer is removed (breast reconstruction); relieve symptoms of advanced cancer.

There are two main types of surgery to remove breast cancer: breast-conserving surgery, mastectomy.

The study includes a short review about the breast cancer, describing the surgical procedure, its indications, surgical technique, incidents, complications, results, conclusions.

Bariatric surgery in the management of the cardiometabolic risk of extremely obese patients – case report

Tatiana Onofrei, Florentina Rogojină, Constanța Bandulea

Obesity has serious consequences on health and mortality. The general goals of every weight-loss program is to reduce and maintain a lower body weight, or prevent further weight gain in individuals who cannot lose weight. The treatment options are lifestyle modification (diet, exercise, and behavioral modification), pharmacotherapy and bariatric surgery. The three most commonly performed bariatric procedures are laparoscopic adjustable gastric banding, laparoscopic sleeve gastrectomy and the Roux-en-Y gastric bypass.

Objective: we present the case of a woman with extreme obesity, poor controlled diabetes mellitus – treated with insulin, dyslipidemia and coronary artery disease. She was submitted to bariatric surgery – laparoscopic sleeve gastrectomy. After surgery she lost more than 30 kilograms, with a good glycemic control on metformin only. Hypertension, dyslipidemia and her heart condition improved.

Conclusions: surgery is the most effective means of inducing significant weight loss in individuals with extreme obesity who are at greater cardiometabolic risk.

Longer the waist line, shorter the lifeline – obesity associated comorbidities

Nicoleta Mavriș, Aglaia Nedelcu, Florentina Rogojină

Overweight and obesity are associated with the development of a number of comorbidities and increased mortality. Hypertension, dyslipidemia, coronary artery disease, congestive heart failure, stroke, diabetes mellitus, non-alcoholic fatty liver, sleep apnea, gallstones, polycystic ovarian syndrome, osteoarthritis and cancers are more frequent in obese patients.

Objective: to evaluate the prevalence of comorbidities in obese patients admitted in our department during the last 5 years.

Results: diabetes mellitus, cardiovascular and cerebro-vascular diseases are the most frequent obesity associated comorbidities. These diseases have a major impact on mortality.

Menopause – between physiology and pathology

Andreea Serafim, Ancuța Istrate, Carmen Miron

Menopause and menopausal transition are physiologic, but symptoms are frequent and often require medical attention. Approximately 60% to 80% of menopausal women experience hot flashes at some point during menopausal transition. They are more likely to develop anxiety symptoms at perimenopause and postmenopause and depressive symptoms or clinical depressive disorders in late perimenopause. Sleep disturbances are frequent.

Vaginal dryness and genital atrophy can cause sexual dysfunction. Lately, postmenopausal osteoporosis leads to increased morbidity and mortality as well as an important reduction of the quality of life as a consequence of fractures.

We present our experience with menopausal patients seeking medical attention in our department.

Macroprolactinoma in a diabetic patient with morbid obesity

Daniela Buzatu, Nicoleta Mavriș, Nicoleta Vale

Prolactinomas are prolactin secreting pituitary tumors that can occur between both sexes „the men's unlike women's macroprolatinomas predominates". Clinical manifestations are given of prolactin hypersecretion „galactorrhea and gonadal dysfunctional", related to tumor mass effect compression (neurologic and visual disturbances) and impairment of the other pituitary function.

We present a case of a 36 years old patient with macroprolactinoma and with 2 diabetes and morbid obesity, who has been presented for metabolic imbalance and visual disorder. The clinical exam was galactorrhea; disturbances of ocular motility and ptosis right eye, reduced visual acuity left eye; obesity (BMI = 42 kg/m^2), erysipelas right leg. Endocrine profile: prolactin, FSH, LH, TSH, serum cortisol, ACTH, testosterone, showed: hyperprolactinemia (5960.62pg/ml) and hypogonadotropic hypogonadism. CT and MRI brain showed a voluminous sellar mass by 42.5/39/32 mm, with invasion of right cavernous sinus; optic chiasma and right orbital apex compression. The patient started treatment with: dopamine agonist drug – carbegolin in high dose and testosterone, treatment hypogonadotropic hypogonadism, treatment for diabetes mellitus for erysipelas.

After 1 month has a good evolution with remission the visual acuity (left eye), MRI – showed a reduction of 1 cm pituitary tumor size, and prolactin decreased to 2950 pg/ml.

Nurse involvement in breast cancer prevention

Margareta Popa

In the last 20 years, breast cancer failed to be an incurable disease, being considered lately as a chronic disease, a curable disease and more important a disease which can be prevented.

Unlike other types of cancer, where a healthy style of life can prevent 70-80% of diseases, breast cancer has been always an exception.

There are many factors of risk coming into being breast cancer, factors we cannot control by our decisions: age over 50, blood relatives suffering from this disease, other breast previous diseases, excessive irradiation of mammary gland, precocious puberty (the first period before the age of 12), the late menopause (after 55), age of 30 for the first pregnancy, no birth, no nursing, contraceptive pills usage in the last 10 years, therapy for estrogen substitution, made more than 5 years in the last 10 years.

Nurse can have an important role in this, explaining all the risks the patients are exposed by ignoring the physician's advice regarding breast cancer prophylaxis.

Ignorance, fear of diagnosis, lack of sanitary education, lack of determined programs for prevention and screening, in our country, lead to discover the diagnosis in an advanced stage of the disease, when the treatment is useless. In this case the patient sufferance is enormous.

Breast cancer is a disease which can be activated no matter when, to whom and no matter social status or how rich you are. We cannot blame God, the people around us, or us for this disease. In our era, in 21st century, breast cancer is a curable disease, especially if it is traced in an early stage.

Physicians as well as nurses are involved in the fight against breath cancer, and our task, the nurse's task is to penetrate the science, as close as she can, to the patient heart and conscience, offering them the strength for fighting against the disease and beating death.

OP session NS5

Infections with human papilloma virus (HPV)

Cristina Râșnoveanu

Human papilloma virus (HPV) is a very common virus that is spread through sexual contact.

The majority of sexually active people (about 75%) will have a HPV infection sometime during their life.

Most HPV infections go away by themselves. However, some „ high risk" types of HPV might cause changes in cervical cells that might lead to cervical cancer.

With proper screening and early detection, cervical cancer has a high rate of cure. In order to diagnose early stages of cervical abnormalities we perform cervical smear (Pap-Test), HPV genotyping, colposcopy and guided biopsy.

Prevention and treatment of cervical cancer

Daniela Cuculea

Cervical cancer is a disease than can affect women of any age.

Cervical cancer is the second leading cause of cancer after breast cancer at women under age 45 years worldwide. Cervical cancer occurs more often at women who have started their sexual life earlier, living in an environment with poor intimate hygiene and those who have multiple sex partners.

Cervical cancer can be prevented by regular testing (PAP smear test) and by anti-HPV vaccination. Cervical cancer should be diagnosed intraepithelial carcinoma phase, thus reducing the prevalence of invasive cancer and mortality.

It easier to detect cervical cancer than to treat.

Decentralizing public health service

Gabriela Amoașei

Once the society started to develop it has emerged the notion of public service.

The notion of public service is legal conceptualized by Article 4, paragraph c, of Law no. 178/2010, according to the public service is '' all actions and activities which ensure the needs of general public interest or local collectivities."

The public service is organized and operates according to principles, namely: the principle of continuity; the principle of equality; the principle of adaptability.

Under these principles enters public health service, because this is vital for the community and must be provided continuously and permanently to every individual person regardless of nationality, age, gender, religious beliefs.

For a proper functioning of public services it has developed principles decentralization. Decentralization has occurred due to the fact that no matter how strong is the government, would not have a clear picture of all the problems facing the local community in each hand. Local communities, enjoying autonomy manage to identify best which are the needs of society and allocate the necessary resource where needed.

The legal provisions for decentralization of public service are found in the Article 195/2006, timber whom "Decentralization is the transfer of financial and administrative powers to the provision of public services from the central public authorities at the local government or the private sector."

In 10th of May 2009, the Government adopted the Strategy on decentralization in the health system. Decentralization in health care law provides for the establishment of The Regional Institutes of Emergency under the Ministry of Health, which provides that certain emergency hospitals will go into the structure of these institutes. In exchange, the hospitals and the public health departments, converted in Directions of Nursing, will be transferred to the administration of the local authorities.

After decentralization, the county hospitals will pass administered by county councils, the municipal administration ones into the municipal councils, and town and village hospitals in the local council administration. Subordinated to Ministry of Health will remain national research institutes, clinical institutes, regional hospitals and some hospitals declared of national interest, where advanced medical acts performed.

Two of the principles underlying the strategy to achieve the objectives of health refers to placing the patient, and citizens in general, in the center of the health system, along with the responsibility for the decision makers before it. This implies a major change in the decision-making mechanisms and accountability, so that the decision can be taken as close to where they are provided and used health services. In this way ensures a better fit for the health needs of the population along with direct accountability to those who take decisions in the community.

Simultaneously central structures, particularly the Ministry of Health which is retreating from local management, can focus on strategic functions, i.e. development of sectoral policies, supervision and guidance of the entire system, including outside activities that impact on health and developing cross-sectoral cooperation mechanisms and structures. This requires increasing the institutional capacity of the Ministry of Health, to meet these new roles and develop new structures at central and local level, along with the modification status of health units.

In this context it provides a strategy of decentralization of healthcare that responds to the goals of the Government Program and conductive to achieve effective right to healthcare provided by the Constitution. In analyzing the decentralization of the health units, there is a greater interference of politics in the act reorganization, so we can appreciate the decentralization of hospital administration as a win for Romania.

In conclusion, decentralization is a major challenge for healthcare delivery in general. The active involvement of managers in developing reforms based on decentralization, standardization of financial allocations, international norms and standards and impact monitoring is essential to ensure equity and quality medical services and to improve efficiency.

Liver's tumors

Imola Grigore, Liliana Badea, Mihaela Moscaliuc, Carmen Florea

Definition : It's the most frequently form of primary hepatic tumors (80-90%); for children is on second place after hepatoblastoma; it's more frequently met on men than women; it can be developed either on a normal liver or a cirrhotic liver (B or C virus)

The liver's anatomy: the largest gland in human body; it is formed by two lobs (left and right) and about eight segments

Favorable factors: consumption of anabolic steroids, cirrhosis, B or C hepatitis, low immunity, obesity, smoking (especially to people who is infected to B or C hepatic virus), poisoning with arsenic.

Signs and symptoms: loss of weight, loss of appetite, strong pains in the upper abdomen, nausea and vomiting, weakness and general fatigue, grown liver, swollen abdomen, whitish feces

Etiology: most frequently met: chronic liver disease and cirrhosis (about 60-90%), chronic hepatitis with B virus, chronic hepatitis with C virus, chronic hepatitis with both viruses, chronic hepatitis with alcohol intake etiology, negative viral markers; most rarely met: hereditary hemochromatosis, porphyria, autoimmune hepatitis, primary biliary cirrhosis, Wilson disease, steroid oral contraception.

Diagnostic: blood analyses, imagistic tests (CT, MRI), and liver's biopsy.

Treatment: surgical (partial hepatectomy), liver transplant, cryoablation and thermotherapy, chemoembolisation, radiotherapy, pharmaceutical treatment (with Soranfenibul).

Nurse's role: takes biological samples, prepares the patients for radiology test, abdominal punction, laparoscopy liver's biopsy and paracentesis, has to recognize and to watch for evolution and/or complication (hepatic coma or bleeding coma), and has to announce the doctor for any change or suspicions

Prevention: moderate alcohol consumption, to maintain the weight, vaccination against B/C viruses, personal hygiene measures.

Never use already used needles.

Communication in medical facilities

Nicoleta Voinea, Cristina Soare, Lucica Poenaru

Communication in medical facilities is a key component of public health and of the health care system. It comprises: patient-centered communication, communication between members of the same team, bedside manners (the ability of communicating with the patient), communication in emergency situation, communication in conflict situations.

We may consider that the three pillars of effective communication are: clear communication of the message, the patient’s perfect understanding of what he is told and the patient’s feeling that he is respected by the doctor.

Team communication is also very important, because members of a team working in a proper work environment can easily achieve their goals and have good results.

The best communication skills are: attention and sensitivity, empathy, respect, support and promoting partnership.

Emergency situation communication is critical and complex due to the dynamic and high level of stress atmosphere.

Conflict-situations communication is often difficult due to cultural differences and different perception of others and of the environment.

Communication in medical facilities is essential and leads to improvement of the population’s health state.

Osteoporosis

Elena I. Vasilățeanu, Daniela Cernat, Florentina Socol

Osteoporosis is a progressive bone disease that is characterized by a decrease in bone mass and density which can lead to an increased risk of fracture. It is a major health problem and it affects especially older people.

Fractures are the most dangerous aspect of osteoporosis. The most common osteoporotic fractures are those of the wrist, spine, shoulder and hip.

The disease may be classified as primary type 1, primary type 2, or secondary. The form of osteoporosis most common in women after menopause is primary type 1 which is the osteoporosis of the spongious bone or post menopause osteoporosis. Primary type 2 osteoporosis or senile osteoporosis occurs when the patient is older than 75 years and is seen in both female and males are ratio of 2:1. Secondary osteoporosis may arise at any age and affects man and women equally. This form results from chronic predisposing medical problems or diseases or prolonged use of medication such us glucocorticoids.

There are many illnesses which can lead to osteoporosis:

- Endocrine disorders – hypothyroidism, type 1 diabetes;
- Hematologic disorders – multiple myeloma, leukemia;
- Gastrointestinal disorders – malabsorption, primary billiard cirrhosis;
- Medication – L thyroxin, Heparin, Methotrexate;
- Rheumatologic disorder – rheumatoid arthritis, ankylosing spondylitis;
- Smoking, excessive alcohol consumption.

The diagnosis of osteoporosis has few clinical signs but can be diagnose using: Conventional radiography, measuring bone mineral density. Dual-energy X- rays absorption – the gold standard for the diagnosis of osteoporosis.

Medication: drug for osteoporosis are useful in decreasing the risk of future fractures by increasing bone synthesis and decreasing reabsorption:

Fosamax – once a day at the beginning, and then once a week, and then once a month;

Bonviva – once a day;

Fosavance – contains fosamax and alfa D3;

Prolia – can be helpful for preventing osteoporotic fragility fracture and decreasing the risk of vertebral and non-vertebral fractures.

Nurse perception of bispectral index monitoring during general anesthesia

Mariana Ilie

Introduction: Bispectral index monitoring system (BIS) is a technology used to monitor depth of anesthesia.

Material: BIS monitoring is used to measure the effects of anesthetic and sedative drugs on the brain and to track changes in the patient's level of sedation and hypnosis. BIS monitoring provides information clinically relevant to the adjustment of dosages of sedating medication.

Method: The BIS monitor provides a number, which ranges from 0 (equivalent to EEG silence) to 100. A BIS value between 40 and 60 indicates an appropriate level for general anesthesia.

The essence of BIS monitoring is to take a complex signal (the EEG), analyze it, and process the result into a single number. When a patient is awake, the cerebral cortex is very active, and the EEG (electroencephalography) reflects vigorous activity. When asleep or under general anesthesia, the pattern of activity changes.

Results: The advantages of using BIS monitoring are numerous, including a decrease in time to extubation, use of hypnotic anesthetic drugs, incidence of nausea and vomiting, and occurrence of intraoperative awareness.

It can help the nursing personnel in preventing under- and over sedation during general anesthesia.

OP session NS6

Spinal injury nursing assistance

V. Rădăuceanu, Stela Platon

Spinal injury is a spinal cord or trauma accompanied by total or partial compromise of the affected spinal segment function. Such deficiencies are installed and vegetative, motor and sensory reflexes.

Medullary tissue destruction is caused by trauma or disease of spinal cord injury spinal (accidents, falls, blows to the spine) acting in tissue degenerative medullary (transverse myelitis, multiple sclerosis etc) compression by tumors, vascular disorders, thromboembolism.

Platelet rich plasma PRP

Maria Sandu

PRP is the acronym for Platelet Rich Plasma. It is a concentrated source of autologous platelets containing several different growth factors that can stimulate healing of bone and soft tissue.

PRP is made from patients own whole blood. The whole blood contains plasma, red blood cells, white blood cells and platelets. Platelets are small discoid cells that contain clotting and growth factors. Blood typically contains 6% platelets whereas PRP has a significantly increased platelet concentration. Although this level can vary depending on the method of extraction and equipment, studies have shown that clinical benefit can be obtained if the PRP used has an increased platelet concentration of 4 times greater than normal blood.

The product is obtained by collecting a small amount of whole blood from the patient which is afterwards centrifuged several times (depending on the method). The finite product (platelets enriched plasma) is then injected in the chosen area.

PRP is, in fact, an old therapy used in orthopedics, dentistry or sports medicine.

But only in the recent years has become well-known for the large public especially for its use in aesthetic medicine and surgery (the famous “vampire therapy”).

Inhaled medication

Adriana Chiru, Marinela Matei

The efficiency of a medication depends in equal measure, the preparation bioavailability and patient adherence to prescribed rules for its management.

If the oral preparations (solutions, tablets, etc.) the administration is relatively simple and well known, there are cases in which the patient must receive prior notice and sometimes even a practical demonstration to learn the correct administration of the product prescribed.

Inhaled pharmaceutical preparations, widely used for the treatment of acute or chronic respiratory diseases are a good example in this respect. Using the airway allows the administration of active substances with sympathomimetic bronchodilators such action mainly local or anticholinergic inflammatory or fixed combinations of those mentioned.

Inhaled medication has two major advantages: act very fast, very shortly after the drug reaches the bronchi; required much lower doses with fewer side effects, the same medication administered in tablets or injections.

There are several types of devices for administering drugs by inhalation such as sprays, dry powder inhalers, nebulizers.

Whatever type of device indicated for administration of inhaled medication, the patient must know exactly how to use it. Considering that the same active substance may be conditioned for various types of devices (e.g. Salbutamol - pressurized inhaler salbutamol – for nebulizer solution) to release the prescription the patient must receive all information regarding the administration technique. Careful reading of the instructions, followed by sequencing simulation management practice leads to learning the correct use of these devices and thus ensuring therapeutic efficiency act.

Nursing interventions in obstructive sleep apnea

Cristina Radu, Gabriela Drilea, Mariana Lazurcă

Sleep apnea is a breathing disorder and it occurs during sleeping. The condition is characterized by stoppage of breathing for ten seconds or longer and at least five times per hour. Sleep apnea is becoming a major health problem all over the world. Officially, about 800.000 adults in Romania have sleep apnea and in the same time many people may have the illness but they don't know it.

The nurses have an increased responsibility in the identification the symptoms, education and management of patients who are suffering from sleep apnea.

CPAP (Continuous Positive Airway Pressure) is the most effective form of treatment for sleep apnea but intolerance and incomplete compliance has made it difficult for efficacy.

The most common interventions are related to CPAP equipment and physical, psychological and social-economic side-effects. Some important aspects of nursing interventions are referred to change the patient’s lifestyle because many of them are obese, smokers.

Nurses' awareness and good knowledge help to increase the compliance to the treatment and minimize the side effects because can be a real risk that a patient with a negative experience may not persevere with the treatment plan for long-term period.

Sputum prelevation in tuberculosis diagnosis

Simona Bran, Mădălina Preda, Mihaela Mirea

Sputum represents all that is expelled secretions in the airway by coughing. Sputum represents the best product for finding Koch’s bacillus in the diagnosis of pulmonary tuberculosis.

Harvesting sputum in the morning, before eating or drug administration gives the highest rate of positivity in the diagnosis of pulmonary tuberculosis, even in patients who don’t cough and cough up a little. Getting positive diagnosis in Tuberculosis increases if 2-3 sputum samples, collected within 24-48 hours, are examined.

In people who do not cough, or swallow the sputum, special techniques are applied to produce and collect sputum: aerosol, larynx - tracheal lavage with sterile saline, gastric tubing using probes Nelaton or Einhorn, bronchial aspirate or broncho-alveolar lavage by fibrobronchoscopy.

A properly pathological material consists of a recently removed from the bronchial tree, with minimal amounts of oral or nasal secretions, and of satisfactory quality on the presence of a mucoid or mucopurulent material in an amount of at least 1.3 ml.

Transport to the laboratory must be immediately after harvest or maximum 24h after.

What are the potential complications of bronchoscopy?

Tatiana Lazăr, Mirela Barbu, Luminița Atudori

Bronchoscopy is a procedure during in which an examiner uses a viewing tube to evaluate a patient's lung and airways including the larynx, trachea, and many branches of bronchus.

Bronchoscopy is usually performed by a pulmonologist or a thoracic surgeon. Although a bronchoscope does not allow for direct viewing and inspection of the lung tissue itself, samples of the lung tissue can be biopsied through the bronchoscope for examination in the laboratory.

Bronchoscopy can be used for diagnosis or treatment.

Complications of bronchoscopy are relatively rare and most often minor. It is important to realize that all procedures may involve risk or complications from both known and unforeseen causes, because individual patients vary in their anatomy and response to medications.

Therefore, there is no guarantee that a procedure can be free of complications. The following is a list of potential complications: vocal cord injury, irregular heartbeats, lack of oxygen to the body's tissues, heart injury due to medications or lack of oxygen, bleeding from the site of biopsy, punctured lung (pneumothorax), and complications from pre-medications or general anesthesia.

Exacerbation of chronic obstructive bronchopneumopathy

Vasilica Ionescu, Mariana Tănăsescu, Doina Andrei

Obstructive chronicle bronchopneumopathy is an illness characterized by the limitation of the airways` airflow, incompletely reversible, limitation which is usually progressive and is accompanied by a systemic and pulmonary abnormal inflammatory answer regarding harmful particles or gases, especially the cigarette smoke.

COPD exacerbation is defined as an acute event in the natural evolution of the illness characterized by the modification of the dyspnea degree, of the cough and/or of the patients` expectorations, besides the daily variations which lead to the modification of the medication.

Exacerbations are important events during the evolution of the illness because it affects patients` life quality in a negative way, it requires weeks of recovery, accelerates he declining rate of the pulmonary functions, and are associated with a high rate of mortality, especially for those who need hospitalization which consequently implies high economic and social costs.

The treatment of exacerbation consists in oxygen therapy, inhalator bronchodilators administration with the help of a spacer or nebulization, oral corticosteroids or i.v. and antibiotics when there are signs of bacterial infection.

Patients` education represents an important method to improve their capacity of understanding this illness, to successfully make them give up smoking, to make them understand the aspects of their changing health in order to easily deal with exacerbations.

Minimally invasive repair for pectus excavatum

Emilia G. Raicu

Pectus excavatum is an abnormal development of the rib cage in which the breastbone (sternum) grows inward, which causes a sunken chest wall. Sometimes called "funnel chest," pectus excavatum is often present at birth (congenital) and can be mild or severe. If left untreated, pectus excavatum can sometimes cause compression (pressure) of the heart and lungs.

Pectus excavatum occurs in approximately 1 out of 400 children and is three to five times more common in males than females and it is diagnosed at birth or in the first year of life.

Genetic predisposition and stuffy sternum is sometimes associated with scoliosis, Marfan syndrome, Poland Syndrome, Noonan Syndrome, Turner Syndrome, Marfan syndrome.

The actual process of correction is minimally-invasive procedure, invented in 1987 by Dr. Donald Nuss for treating pectus excavatum.

After the Nuss procedure, children will return to school after two – three weeks and once fully recovered can return to daily activities.

Nuss procedure is an effective method with good aesthetic and functional results.

Preparing for digestive endoscopy

Aurica Avram, Mariana Moldoveanu, Monica Nicolescu, Daniela Tanasov

Preparing for digestive endoscopy includes general measures, necessary for all the endoscopic procedures and specific measures, related to the examined segment and the endoscopic procedures provided.

The first step in preparation is to establish the indication for the investigation.

Depending on the indication, the nurse is preparing the endoscopic accessories needed- biopsy forceps, polypectomy loops, variceal banding devices, etc. The discussion with the patient needs to address investigation purpose, description and estimated duration, the risks and benefits. An informed consent needs to be signed.

Before and during endoscopic procedures, medication may be used to diminish gastrointestinal motility, decrease the patient's anxiety or discomfort, and provide amnesia of the stressful intervention.

Before starting endoscopy, the medical personnel needs to be prepared to monitor vital signs (heart rate, oxygen saturation, blood pressure), especially in patients with cardiorespiratory pathology.

As a specific measure for the upper gastrointestinal endoscopy, patients shouldn’t ingest any solids for at least 6 hours before the procedure. For the colonoscopy, the colon needs to be cleansed of fecal material before the examination.

An oral purge with 2 to 4 liters of electrolyte lavage solution, after a short period of dietary restriction, and in some cases followed by enemas, results in adequately prepared colons.

Issues in caring for patients with gastrostomy

Iuliana Ghinea, Corina Marcovici, Petrica Gheorghe

Endoscopic gastrostomy is an intervention consisting in the creation of a direct link between the gastric cavity and the external environment through a catheter insertion.

Gastrostomy is a technique of nutrition "in- extremis", which in limited situation such tumors of the head and neck, radiation therapy of the head and neck region, head and neck trauma, nutritional difficulties after stroke, demyelination disease, dementia, allows life to continue, and leads to a relative normality feeding patient with fragmented, soluble food. The feeding tube can be temporary or permanent, depending on the reason of the intervention.

Innovation and continuity – attributes of gastrostomy – two words that apparently can't be places in the same sentence – are working together this time. The innovation in medicine through this technique supports life patient to continue his living and give back the person to society. The individual may conduct his existence in the familial environment and not laying on a hospital bed connected to infusion and nasogastric feeding tube, waiting for a lifesaving surgery or the end.

The endoscopic gastrostomy was first performed in 1980 by Gauderer and Ponski, and has the following advantages versus the surgical gastrostomy: short mounting time, elimination of general anesthesia, it lowers the risk of complication and has a good rapport cost/efficiency. It has a success rate of 95% of the cases.

The medicine comes again with solutions to the need of feeding and hydration the patient and the role of medical team is to ease the patient's situation with such problems for short or long term.

OP session NS7

Erysipelas – dermatologic emergency

Liliana Lăța, Dana Papa

Definition: Erysipelas is a localized skin infection caused by bacteria (β hemolytic group A Streptococcus, Staphylococci).

Pathogenesis: Skin infection can occur due to: a breach in the skin barrier (wounds, fungal infections, burns, venous leg ulcers); an infected nearby mucosa (pharyngitis, rhinopharyngitis, and suppurative otitis media); lymphatic dissemination (streptococcal lymphangitis); hematogenous dissemination – exceptionally (septicemia).

Symptoms: sudden onset with fever (39-40 degrees Celsius) and chills; erythematous plaque of erysipelas appears 6 to 12 hours from onset.

How should the nurse manage the case: immediately after recognizing the signs of the infection, let the doctor know in order to plan the therapeutic management; meanwhile, the nurse applies a protective wet dressing with antiseptics locally (Rivanol, 2% boric acid solution); the nurse should administer antipyretic medication and closely monitor the patient's temperature, pulse, blood pressure and the overall health state; explain to the patient what is going on, that the temperature and chills are part of the disease's clinical manifestations and what the possible future reactions are; educate the patient regarding the body's hygiene.

Sepsis – nurse's role in managing septic patient

Liliana Mocanu, M. Stanciu, E. Fărcășanu, C.B. Teușdea, S. Dogaru

Sepsis is a main presentation reason for reporting to the emergency department and hospital admission, being one of the major causes for death in non-cardiac intensive care units. Costs are increasing and reported incidence is higher than ever due to longer life span. Sepsis survivors have often long-term physical, psychological and cognitive comorbidities.

Time is essential in this equation and fast response to organ dysfunction is a key component for sepsis management. Septic patient is always an unpredictable one and nurse's role is essential in diagnosis and treatment optimization.

What you need to be an emergency nurse within the Emergency Department

Liliana Mocanu, M. Stanciu, M. Fleican, E. Fărcășanu, M. Toma, C.B. Teușdea

A question is always is rise: the real emergency nursing seems with a television program? Anyone can do this job?

My answer is NO. The emergency nursing is unpredictable, dramatic, challenging, and requires a team effort. As will present the emergency nursing is for some of us, and clearly not for everyone.

What you need to be an emergency nurse within the Emergency Department? Consider the following factors: 1. know yourself, 2. must have good assessment skills, 3. strong knowledge base in every area of nursing, 4. Critical thinking and sharp decision making, 5. Good communication skills.

As the health care system and technology advance, there will always be a need for emergency care and emergency nurses.

Current standards for non-invasive assessment of liver fibrosis

Magdalena Chiriac, Veronica Niculiţă, Liliana Bulat

Chronic liver diseases of various etiology (viral, alcoholic, and metabolic) constitute a significant proportion of pathology seen in a gastroenterology department. By complications that arise in their evolution, chronic liver diseases are an important cause of morbidity and mortality and carry a significant burden for health systems. According to the latest epidemiological data, in Romania there is a high prevalence of hepatitis B and C infections, 4.2% and 5.6% respectively, but much of the chronic liver diseases are caused by chronic alcohol intake and recently there has been an increase in the frequency of fatty liver disease (encompassing steatohepatitis, steatofibrosis and ultimately cirrhosis).

For staging of the disease, assessment of fibrosis severity and guiding treatment decisions, the gold standard until a few decades ago was represented by liver biopsy. The maneuver is invasive, carries risk of complications (bleeding, infection, damage to nearby organs), has come contraindications (coagulopathy, severe thrombocytopenia, ascites) and evaluates fibrosis in only one point in the liver parenchyma; however, considering the heterogeneous distribution of fibrosis in the liver, biopsy may underestimate the overall hepatic fibrosis.

In this setting a particular interest emerged to develop non-invasive methods for the quantification of liver fibrosis and there are now several diagnostic tools available: algorithms based on serum markers (Fibromax), transient elastography (Fibroscan, Echosens, Paris) ARFI elastography (Acoustic Radiation Force Impulse imaging), shear-wave elastography and not least magnetic resonance elastography.

Fibromax is a combination of 5 noninvasive tests (Fibrotest, ActiTest, SteatoTest, NashTest, AshTest) to assess fibrosis, steatosis and necro-inflammatory activity of the liver. The method involves the collection of blood and dosing of some surrogate markers of fibrosis, which are introduced into an algorithm with high diagnostic accuracy for fibrosis.

Fibroscan is a method of elastography which involves the transmission of pulses by means of a transducer to the hepatic parenchyma, and assesses fibrosis based on the reflection speed of these waves transmitted to the liver. The method is simple, quick, non-invasive, repeatable and painless. In addition, the add-on module CAP (Controlled Attenuation Parameter) can also give an estimation about the degree of hepatic steatosis.

These tests are available in our clinic and there is a rich experience with their use in patients with chronic viral hepatitis (B, C), as well as in those with alcoholic liver disease. As the literature has shown a good concordance of these non-invasive tests with liver biopsy, they can be used with confidence for fibrosis evaluation and monitoring of patients with chronic liver diseases.

Hemoptysis

Marcela Brighiu, Camelia Militaru

Introduction: Hemoptysis is the expectoration of varying amounts of blood from the airways. Hemoptysis is manifested by eliminating blood through the mouth (mixed with air, so "frothy with mucus") from the airways.

Material and method: This paper presents the classification, etiology, symptomatology, differential diagnosis, treatment indications and the treatment for hemoptysis.

Discussion: It is desirable for the treatment of hemoptysis to be applied at the place where hemoptysis occurred, avoiding the transportation (jerks, agitation), whatever the means of transport at hand. Best for the patient is to sit quietly and not to move, unless he receives emergency healthcare or is transported to a medical facility. Transportation will nevertheless be required if the doctor considers that a medical intervention cannot be performed without an emergency and surgery service, equipped with the appropriate equipment.

Conclusion: Hemoptysis is a medical emergency, even in small quantity. Its evolution may be serious or even critical.

Clostridium Difficile infection – a nurse`s point of view

Mariana Bogriceanu, Elena Zanfir, Stela Belea

Introduction: Our objective is to review current guidelines in managing Clostridium difficile colitis and our department`s experience in this field.

Materials and methods: Clostridium difficile is a Gram-positive spore-forming anaerobe that was identified, beginning with 1978 as the causative pathogen in the majority of antibiotic-associated diarrhea and colitis cases. It is believed that a small percent of healthy individuals have this bacterium in their colon, but it is more often found in the ground, water, animal or human feces, C. difficile being a growing cause for nosocomial disease.

The most common risk factor for C. difficile infection is the use of antibiotics, as they can disrupt the normal microbial balance in the colon.

This bacterium is dangerous because it can secret two toxins (A and B) that cause inflammation of the colonic mucosae, which can lead to moderate diarrhea, pseudomembranous colitis (most frequent form) or fulminant colitis. If the physician suspects a C. difficile infection, it is the nurse`s task to explain to the patient how to collect a stool sample and take it as fast as possible to the laboratory.

After infection is confirmed, it is up to the nurse to assure contact precautions: isolate the patient with single use gowns and gloves being available at room entrance; environmental disinfection; explaining the importance of hand hygiene and of course making sure that the patient takes his medication correctly, according to the physician`s recommendations.

Results and conclusion: C. difficile colitis has become the most frequent infectious cause of healthcare-associated diarrhea, causing high morbidity and mortality, mainly because of the use of broad-spectrum antibiotics.

The team working in the recovery of hemiplegic patients after stroke

Ecaterina Dane, Natașa Corpodeanu, Laura Iosif

Stroke is a brain infarction in ischemia or hemorrhage leading to discontinuation function.

Stroke is installed suddenly and is characterized by a neurological deficit, hemiparesis or hemiplegia known as half a body, depending on severity. 80% of strokes occur in the carotid arterial distribution and consist of muscle weakness of a body part (face, arm or leg, in any combination).

The incidence of stroke has declined in recent years, despite the aging of the population due to changing lifestyle, use of antiplatelet and proper treatment of heart disease. Yet the incidence of stroke increases with age. Risk factors for the disease include age, high blood pressure (hypertension), heart deficiency, history of stroke, transient ischemic attacks, diabetes.

Post-stroke recovery involves using compensatory techniques for mobility, the use of daily activities or ADL (activities of daily living) and promoting communication. AVC limited prognosis for recovery is given by the following elements: severe memory problems, the inability to understand commands, medical or surgical instability, previous stroke, sphincter incontinence, visual spatial deficits.

Routine diagnostic evaluation include: CT scan (computerized axial tomography) of the cephalic extremity, electroencephalogram (EEG), MRI, carotid Doppler studies, assessment of heart (echocardiogram, EKG), blood tests routinely. Patients post-stroke recovery starting in the acute phase and the recovery post-stroke uncomplicated, stable medically is done using passive and the active mobilization helped (assisted daily), tilting the bed and identifying communication deficits. Subsequently, the patient is sent to physiotherapy or occupational therapy wheelchair. Inpatient practicing transfer activities (in bed in a wheelchair and back), the preparatory activities for walking, daily activity or practicing self-care ADL and coating, communications training, following a therapy that addresses swallowing.

The effect of application interference current in scapulohumeral periarthritis

Georgeta Matei, Margareta Bărgăoanu, Laura Iosif

Periarthritis scapular humeral is a clinical syndrome characterized by pain in the shoulder and by stiffness caused by degenerative lesions and inflammation of the tendons and exchanges of the second joint of the shoulder, accompanied by limited arm movement, no signs of damage to the joint scapular humeral. After rheumatologist French Seze describing these clinical forms: simple shoulder pain, acute painful shoulder, shoulder blocked, pseudoparalitic shoulder joint and shoulder. Treatment aims to relieve the pain, combat inflammation and fibrosis trend, to improve joint mobility. It includes: segmental rest, anti-inflammatory and analgesic drugs, hydrotherapy, electrotherapy analgesic, massage, physical therapy.

Electrotherapy physiotherapy is part of the physical agent that uses artificial electric current applied to the body, either directly or indirectly.

Medium frequency currents are AC (sinusoidal) whose frequency is the thousands of hertz (1000-50000) and tens of thousands of Hz.

Interferential current effects: excitomotor – on striated muscle; muscle relaxant – obtained by average frequency (12-35 Hz) especially variable frequency between 0 - 100 Hz; analgesic effect; action on smooth muscles excitomotorie;

Contraindications: The application of medium frequency currents in the chest region; Neoplastic processes (electro therapy represents an excitation, so worse malignant process); in acute inflammatory processes in first states, where the decompensated heart disease and hypertension age; in scapulohumeral rheumatoid disease, the patient is applied on the painful shoulder type plate electrodes that are introduced in size synthetic coating texture previously dampened appropriate, may also apply vacuum or suction cup electrodes.

After treatment with interferential current combined with a program of analytical kinesiology, joint pain and inflammation disappears in all cases.

Nursing essentials for patients with lung cancer

Niculina Predescu

Treating patients with lung cancer can give a nurse one of the biggest professional satisfactions, in spite of the disastrous evolution of this disease.

Novel surgical techniques made possible an increase of these patients' survival rate.

This paper describes the most important aspects in the nursery of these patients: anatomy and physiology of the respiratory system; diagnosis of lung cancer; treatment and surgical techniques; nursing of patients with lung cancer

Despite a sustained work for over 25 years, lung cancer still represents a terrible killer. This is the reason why the nurses are actively involved in the nursing team, their actions being mandatory in order to have a long lasting recovery for these patients.

The nurse's role in cardiac rhythm disorders management in the ED

Tatiana Pană, Beatrice Ibănescu, Steluța Petrescu, Geanina Durlea

The nurse's role in early detection of cardiac rhythm disorders is extremely important considering the fact that in most cases the nurse is the first person who comes into contact with the patient and also the one who manages monitoring.

It is crucial for nurses to be educated and able to assess the gravity of the situation, especially in the ER department where time is of the essence. Education of course starts in nursing school but must be continued throughout the whole career.

The purpose of the present paper is to make a review of the most important aspects of the most important cardiac rhythm disorders, the most common that present in the ED and the symptoms that are usually associated with them as well as taking a look at the types of patients that require cardiac monitoring and the methods that we use to ensure their proper care.

OP session NS8

Romanian medical team experience in field operations

Vasilica Mihis, Liliana Mocanu, M. Stanciu, M. Fleican, E. Fărcășanu, C.B. Teușdea

Since 1991 the Romanian army participated in missions under UN auspices to create a new architecture for peace, cooperation and stability in the world. That's why a Romanian field military hospital participated in mission in Iraq during the first Gulf War in 1991, but only for a month.

The first test took place after two years, when the field Romanian military hospital participated in the mission in Somalia (UNOSOM II) which was intended to treat military personnel involved in the peace mission, but treated and civilians (approx. 80 % of patients) victims of civil war.

Medical teams have performed medical triage, primary survey, resuscitation, secondary survey, surgical interventions and intensive treated patients who had or were brought to the ED of the field Romanian military hospital.

Kaposi sarcoma patients nursing – a challenge for quality care

Ioana Moisei, Andreea Mihai, Irina Vaciu

Definitions: Kaposi sarcoma is an endothelial cells neoplasia presenting with multiple vascular nodules, affecting both skin and other organs.

Clinical aspects: Kaposi sarcoma may be classified in four types: epidemic (HIV infection associated), immune-compromised, classical (sporadic) and endemic.

Kaposi sarcoma appears as red-purpuric macules or papules/nodules at any level of skin or mucous membranes. Initially, the lesions are small, painless, but may evolve with ulceration and became painful. Usually first lesions appear at legs, most of the times associating edema.

Treatment: nursing interventions are based mostly on: pain monitoring (pain killer drugs administration and comfortable body positions) and skin integrity monitoring. Digestive and respiratory functions also must be monitored. The nurse also monitors chemotherapy and radiotherapy side effects (nausea, anorexia, diarrhea and dizziness).

Kaposi sarcoma may have a favorable evolution. Yet, internal lesions can promote bleeding, sometimes massive, obstructions or organ perforations, amputations.

Kaposi sarcoma is an incurable disease but that may be controlled.

Actinic keratosis – real danger of today?

Irina Vaciu, Lucuța Teșa, F. Pieptea

Definition: Actinic keratosis (AK), also known as solar keratosis, is a precancerous lesion of the skin, usually appearing on long time sun exposed skin, mostly involving upper and lower limbs, face, nose and ears.

Clinical aspects: actinic lesions appear as keratotic plaques, yellowish-dark, 0.5 to 1.5 cm in diameter, either plane or slightly elevated.

Pathologic examination may show any of the following aspects: hypertrophy, akantolysis, atrophy, Bowenoid aspect or pigmentations. It may also define malignant transformation in spinocellular carcinoma.

Nursing roles are: prophylaxis, education, diagnostic (signs of infection, irritation), topical treatment correct application.

Complications may be present: bleeding secondary to electrocoagulation treatment, hard to cooperate with patients (self-medication such propolis, plants, tinctures etc, that may complicate post-operatory plagues), extended and hard to reach areas.

A history of anesthetic practice

Tudora Băraru

The presentation have assayed to review briefly the history of anesthesia specialty and to define the scope of anesthesiology, which even seems secure, but not yet mature and fully differentiated. Anesthesia is considered an American invention, although anyone who examines history will understand that innovation of such significance can hardly have arisen spontaneously.

In addition to the concept of standard of care, another kind of standards has grown in evidentiary importance. Organizations such as European Society of Anesthesia and the Romanian Society of Anesthesia as well as hospitals and departments, promulgate standards and procedural manuals or directive.

Operations had been performed over the centuries but always for simple affections such as fractures, amputation, cataract extraction, trephination of the scuff, or removal of bladder calculus. After 1960, in our country, valuable anesthetic practice developed and even improved due to the technological progress of medical and monitoring equipment along with medical substances.

Cardiovascular diseases

M. Lefter, V. Scarlat, N. Vasile

The term ”cardiovascular disease” (CVD) is attributed to a multiple diseases affecting the heart and blood vessels, and among them an ischemic cardiomyopathy (IC), cerebro-vascular disorder, (high blood pressure) or peripheral arterial disease (PAD).

Other cardiovascular disease are myocarditis (heart condition causes rheumatic fever) and congenital heart disease (heart malformations present at birth).

CVD is associated with heart attacks (myocardial infarction), angina and stroke.

Obstructive sleep apnea

M. Lefter, Mirela Barbu, M. Ene

Obstructive sleep apnea, is also known as apnea-hypopnea syndrome and obstructive sleep (SAS) – is a sleep disorder that involves substantial diminution or cessation of airflow inspired.

This is the most common type of sleep disorder and is characterized by recurrent episodes of airway blockage during sleep for 10 seconds more, leading to significant reductions in blood oxygen levels.

As a result, shallow sleep or waking phase comes total increases muscle tone in the throat and larynx to restore patency.

Status is followed by a series of deep breaths, usually with loud snoring. SAS patients have increased risk of developing heart diseases, hypertension, diabetes, disorders of potency.

Daytime sleepiness, memory and attention disorders can cause a car or work accident.

Tongue cancer

Nicoleta Ghica, Dorina Dumitru, Valentina Budai

Among all etiological factors potentially existent in the western world, tobacco and tobacco derivate use is primarily associated with the tongue cancer.

Tongue cancer represents the most frequent malignant oral form, having a more prognosis, especially in cases which are not discovered in earlier stages.

Tongue cancer requires specific client investigations.

Inner portion mandible abscess

Camelia Preda, Andreea Badea, Nicoleta Călin

Submaxillary area is situated in the inner horizontal arm of the mandible, having the mylohioidian, hypoglossal muscles and the mouth floor mucosa as the upper and medial limits.

Below and downwards is limited by the superficial cervical fascia.

Inner portion mandible abcedates as a result of septic processes developed in the inferior molars.

Clinical manifestations induced by teeth recesses are extremely variated and the treatment may be either very simple, either very difficult sometimes, requiring large surgical procedures, interdisciplinary assessment, specific medical treatment and a close patient care.

Biological treatments in R.A. (Abatacept)

Liliana I. Predescu, Aura Mandachi, Gina Pană

Rheumatoid arthritis is an autoimmune disease of the connective tissue of unknown etiology with a sampler of about 1% in the general population. Featuring through a symmetrical synovitis erosive, deforming and destructive arthropathy by systemic and extra-articular manifestations (rheumatoid nodules). The evolution of chronic, fluctuating disease, untreated can lead to progressive joint destruction, deformity accompanied by permanent joint with a motor deficits and reduction of life expectancy.

The advent of biological therapy within rheumatological was the beginning of a new era because the treatment had on hand until then it was limited.

Abatacept in combination with methotrexate is indicated for the treatment of active moderate/severe R.A. on adult patients who have insufficient response or intolerance to other disease-modifying anti-rheumatic drugs.

Abatacept (SC): each pre-filled syringe contains 125 mg of abatacept in 1 ml solution for injection. The content must be clear, colorless to pale yellow and is administered once a week.

Keep in the original packaging, protected from light, refrigerated (2-8° C), do not freeze. The liquid level must be above the line of control.

Preparing injection site: 30-60 minutes prior to administration remove the pre-filled syringe from refrigerator and leave at room temperature. Do not use heat sources to accelerate the heating of! Avoid region diameter of 5 cm around the navel. Do not inject into areas where the skin hypersensitivity, damage or crusting, swelling, tissue endured.

Choose a different place for the next administration injections (at least 3 cm from the previous).

Wipe in a circular motion the place chosen for injection with a pad soaked in rubbing alcohol and allow to dry.

Undesirable effects: headache, nausea and upper respiratory tract infections, fatigue, asthenia, erythema, pruritus, dyspnea.

OP session NS9

Schizophrenia

Lidia Dobrinescu, Cecilia Bizon

Schizophrenia involves a range of signs and symptoms including disturbances in sense of self and interpersonal communication. Deficits in functioning are observed in daily living family life, social interactions and employment.

Schizophrenia is an endogenous psychosis that most commonly occurs in late adolescence and early adulthood (15-40 years) affecting the self-identity, social interactions and interpersonal relationships.

People diagnosed with schizophrenia usually experience a combination of positive symptoms (hallucinations, delusions, transparency-influence syndrome), negative symptoms (low energy, back of emotions, poor or nonexistent social functioning) and cognitive symptoms (disorganized thoughts, speech and behaviors).

Continuous signs of the disturbance persist for at least 6 months. The course of schizophrenia is typically marked by alternating periods of remission and relapse. Episodes of relapse have significant implications both in terms of cost of health care as the personal implications of loss of functioning for the individual.

Schizophrenia is often described in terms of positive, negative and cognitive symptoms.

Positive symptoms include: transparency-influence syndrome, hallucinations, and delusions.

Negative symptoms include: apathy, depersonalization, ambivalence, social withdrawal, disorganized thinking and behaviors, deficits in attention, working memory, deficits in processing speed, verbal fluency, disorganized thinking: derailment or loose associations, abnormal social behavior; suicide attempt, homicide pyromania; theft.

The panic attack and panic disorder

Nicoleta Ene, Valentina Dinoiu, D. Vasilache

The panic attack is a sudden burst of fear or anxiety (an affective state which is characterized by psychomotor restlessness and unknown fear, without object) which causes patients alarming symptoms, but is not life threatening: accelerated heart rate, difficulty to breath, a feeling of losing control or imminent death. Usually, it lasts from 5 to 20 minutes, and can be caused by stressful situation or it can appear unexpectedly.

Panic disorder are diagnosed when a person has repeated panic attacks, is concerned about having a new one, and so avoids places that could trigger one. There is a possibility that a person could have panic attacks without it becoming a panic disorder, these attacks appear with anxiety disorder.

Causes: the exact cause of panic disorders is unknown. It is believed that is because of a chemical imbalance in the brain (neurotransmitters).

Risk factors: a history of panic disorders in the family, alcohol consumption, mitral valve prolapsed, previous sudden panic attacks.

Symptoms: the most important symptom of all is feeling of overwhelming fear and anxiety, along with other physical symptoms.

Pathophysiological mechanism: panic disorder can be diagnosed after some sudden panic attacks and continuous fear.

Treatment: initial treatment, maintenance treatment, ambulatory treatment.

Prophylaxis: panic disorders cannot be prevented. The bare avoidance of situation and places cannot guarantee that a panic attack will not happen under new circumstances.

The benefits of breast feeding

Ionela Brăiloiu

For most of us, breast feeding is one of the earliest events that helped determine our health for the rest of our lives. And while this is in the past for us and cannot be changed, it is yet to be determined for today's and tomorrow's babies. In this presentation, we look at the importance of breast feeding, the proper ways to breast feed including the correct positions for the mother and the baby and dietary tips for the mother.

We also discuss the medical and social benefits of breast feeding for the mother and for the baby, as well as pointing out problems that may arise during breast feeding, together with methods of avoiding or diminishing their impact. The first months of a baby's life is one of the most important periods for him or her, and also for the new mother – let's do it the right way.

Zika virus

Roxana Vasilescu

Zika virus is a mosquito-borne illness that is spread by the two Aedes species of mosquito – Aedes albopictus, known as the Asian Tiger mosquito, and the Aedes aegypti species. This species is mostly active during the day. These mosquitos can survive in both indoor and outdoor environments.

Zika virus cases typically occur in tropical climates such as Brazil, Colombia, Paraguay, Suriname, Venezuela and French Guiana. The World Health Organization (WHO) declared that the Zika virus outbreak constituted a Public Health Emergency of International Concern on 1 February 2016.

Signs and symptoms of Zika virus are vague and can last for up to a week. Diagnosis of the virus is typically confirmed with a blood test.

Zika infection can spread from a mother to a fetus during pregnancy. What is also of great concern is that the virus can be transmitted by sexual intercourse.

A growing concern that is currently under investigation is a possible link between maternal Zika virus infection and infant microcephaly. Brazil in particular has seen a surge in infants born with microcephaly.

At present, there is no special treatment for the virus and avoiding mosquito bites is a key aspect of prevention. There is currently no vaccine to protect against the disease

Romania's Health Ministry has received a confirmation of a three cases of Zika virus infection of a Romanian citizens outside the country: a young women coming from Martinica, a 10 year-old boy who lives with his family in French Guyana and a men return from Dominic Republic – people traveling to areas with Zika.

Global alert about Zika virus requires special measures in Romania. Institute of Public Health has asked all municipalities to take a combination of methods to prevent and control mosquitoes that spread viruses.

Doppler ultrasound in thyroid pathology

Daniela Zavragiu, Paula Sima, Cătălina Bejan Borangic

Ultrasonography or Ultrasound is a method of imaging examination that uses ultrasounds reflected by the human body as a vector of medical information.

The Doppler Effect is a physical effect described by Christian Andreas Doppler (1803-1853) in 1842.

This effect defines the wavelength behavior of the incoming wave when the wave source and the receiver wave are moving in relation to each other.

The Doppler effect is used both in radioastronomy to study celestial bodies moving with the aid of electromagnetic waves, and in industry and medicine where applications relate primarily to sound waves. In the human body the target being studied using the Doppler Effect is the blood flow.

The Doppler method in thyroid pathology allows for the assessment of the hipervascularity of the thyroid parenchyma, the absence or presence of arterial vascular signal at nodular level. The type of vascularization can also be used to guide treatment. Ultrasonography (ultrasound) opens new perspectives in evaluating the Doppler imaging of the thyroid.

Thyroid scintigraphy

Mariana Ciaușescu, Eugenia Rusu, Veronica Nicolau

Thyroid scintigraphy, nuclear medicine technique, is one of the oldest methods of imaging diagnosis, being in clinical practice from early 60’s. This technique is based on the thyroid property to extract from the circulatory system the administrated radiopharmaceutical and up taking it by an active transport mechanism, without involving it in the iodine intrathyroid cycle or up taking and processing it by the thyroid follicles.

By detecting at the surface the radiation emitted by the radiotracer can be assessed both the morphology (dimensions, localization, shape) and the thyroid gland function (overall and potential nodular structures). Thereby, the assessment of the morpho-functional variants of normal thyroid include: position (retrosternal, laterocervical or sublingual), the number of thyroid lobes (accesory lobe, lobe agenesis), the gland’s shape (oval, U-shaped or V-shaped) and possible radiopharmaceutical uptake asymmetry (preferential uptake in only one lobe).

Thyroid scintigraphy is usually performed with Tc99m-petechnetate, it implies the intravenous administration of a minimal quantity of radiotracer (with a minimum of radiation and after about 20 minutes from the administration, the acquisition of an thyroid gland image is performed (which takes about 5 minutes).

The thyroid scintigraphy’s indications are multiple:

The assessment of thyroid tissue function:

a) Diffuse or nodular goiter;

b) Function assessment of an singular nodule (cold, hot, fiery);

c) Indication of possible function autonomy of a fiery nodule;

d) The detection of ectopic thyroid tissue (lingual, substernal);

e) The assessment of the remaining thyroid tissue after a subtotal thyroidectomy, radioiodine treatment or cervical lodge irradiation.

Challenges in accurate positioning for head and neck cancer patients in 3D-conformal radiotherapy

A. Toma, N. Bocănială

Objectives: Radiotherapy is part of the multimodal treatment of head and neck cancer. Accurate positioning of the patient during CT simulation and treatment is essential, since a lot of critical organs surround the tumor target volumes. We try to identify the most frequent challenges in positioning our head and neck cancer patients during radiotherapy, as the setup can result in underdose the tumor or overdose the critical organs.

Methods: We start 3D-conformal radiotherapy in Radiotherapy Department, Carol Davila Central University Emergency Military Hospital in 2014. We use a Siemens CT Simulator for treatment planning and Unique Varian linear accelerator for treatment, and for positioning Aquaplast RT mask (Q-Fix, USA) custom-made by simulation. We analyze 12 patients with various head and neck cancers, treated in our department in 2015-2016 and in which cases we found image-guided setup errors during radiotherapy and need a second CT simulation.

Conclusions: The most frequent causes of inaccurate positioning during radiotherapy for head and neck cancers are: neck edema, tumor growth or important tumor regression, evolution of cervical adenopathy, patient cachexia.

OP session NS10

Pharmacotherapy in major depressive disorder

A. Meliaca, Stelica Fărcășanu Zoica

Depression is a major psychiatric disorder marked by sadness, loneliness, despair, low self esteem and self-reproach. Associated features psychomotor agitation or retardation, low social interactions and symptoms like insomnia, anorexia and pain.

Types of depression: depression with noradrenaline deficit; depression with serotonin deficit: anxious type, hostile type; depression with dopamine deficit; depression with GABA deficit and mixed depression: depression with serotonin/ noradrenalin deficit, depression whit serotonin and/or noradrenalin deficit associated with dopaminergic hyperactivity.

Severity scale: mild depression episode, moderate depression, severe depression without psychotic features, severe depression with psychotic features.

Minimal natural length for a depression episode is 3 to 9 months. The cours is quite variable: isolated depressive episode; from one to three depressive episodes in the course of life; multiple episodes.

Initiating tratement with antidepressants drugs associates specific risks for each antidepressant class, requiring a thoroughly selection based on medical criteria and potential risks evaluation. Throughout the treatment the patient will be strictly monitored to prevent the risk of serotoninergic syndrome cardiovascular changes (hypo and hypertension, QTc prolongation, dyscrazic blood changes and hepatic enzymes changes).

Recent studies signal the presence of metabolic syndrome in patients with depression undergoing antidepressant treatment. Taking this information to consideration, we recommend as precaution to inforce a monitoring algorithm similar to the one used for the antipsychotic treatment.

Psychiatric nursing strategies for alcohol consumption

Alina Popa, Elena Țolea, C. Gâlcă

It is very possible to have a drinking problem that is not defined or described as “Alcoholic”.

Although most people do not become addicted to alcohol on their first drink, a small proportion do. Many people who have experienced a strong liking of alcohol from their very first exposure then gone on to become addicted to it. At present it cannot be predicted who these people will be, so any exposure to alcohol has the risk of producing addiction in some users.

Many people use alcohol to cope with stress but do not realize that it exacerbates the problems in their lives. People drink to socialize, celebrate, and relax. There is drink of joy, sadness, out of habit, drink without reason, simply drink.

Alcohol enters the bloodstream from the first sip. Alcohol's immediate effects can appear within about 10 minutes. As you drink, you increase your blood alcohol concentration (BAC) level, which is the amount of alcohol present in your bloodstream.

Alcohol often has a strong effect on people – and throughout history we`ve struggled to understand and manage alcohol's power.

Alcoholism is a disease not a vice, it is a progressive disease, incurable, marked by the obsession to drink despite physical harm produced.

There are techniques and therapies available to help lessen the dependence on alcohol and rediscover balance in life.

Incompatibilities between drugs which are administered intravenous in the Intensive Care Unit

Mirela Bidilică, Gabriela Amoașei, N. Tănase, L. Ene

In ICU there are many drugs which, by their mixing in the same infusate solution, may determine incompatibility reactions, by chemical or physical mechanism. The incompatibility may appear as precipitation, chemical reactions, antagonism, and decomposition of biological molecules or the appearance of gaseous compounds.

Even though it is recommended the visual analysis of the admixture/carrier solution before its use in order to determine the precipitation, the color modification or the appearance of turbidity, not every incompatibility can be visually observed.

We will present the main mechanisms of intravenous drugs incompatibilities, their causes and consequences (sometimes severe|) involving the patient and also some preventive strategies.

In order to reduce the risk associated with the intravenous drugs which are used in the ICU we have implemented in our unit the using of a two dimensional chart which contains recommendations for dissolving and compatibilities between every drug commonly used in our department.

Nursing files

Gabriela Dediu

Considering that the healthcare system is undergoing a continuous transformation process, the quality of healthcare is often used a reference point for the evaluation of the medical act. Furthermore, it provides an accurate reflection of the quality of the patient care processes, while also bearing into consideration the latest technological and therapeutic developments.

The nurse's duties are to enact the doctor's recommendations, working towards improving the patient's health, while respecting safety regulations that have been certified, approved, understood and consented to, so that the quality of the medial act is ensured.

Evaluating the quality of the healthcare act implies assessing multiple aspects, such as: the efficaciousness, efficiency and the continuous nature of the healthcare procedures; patient safety; the competences of the medical team; patient satisfaction, as well as the satisfaction of the medical personnel.

In conclusion, it can be argued that the purpose of the standards guiding the activity of the medical personnel is to establish a set of criteria and optimal working conditions necessary for achieving the required quality in the healthcare system, while at the same time respecting the patient's life, dignity and individuality. These standards are fully documented in the nursing dossier.

The intraoperative nursing for surgical procedures longer than 3 h

Mariana Stoian, Voichița Militaru, Mihaela Mușat, Teodora Vasile, Adriana Oprea, Cornelia Nițu, B. Iordache, I. Cristea

The surgical procedures lasting longer than 3 h are highly difficult, regardless they are open-surgery or minimally–invasive (laparoscopic). They require special high-skilled nursing measures. We organized these special measures in an internal protocol adapted to the type of surgery.

The protocol involves measures dedicated to the patient:

- Invasive and non-invasive monitoring.
- Electrolitic acid lose, hematologic equilibrations.
- Preventing and treating hipothermia.
- Preventing thrombo-emboling complications.
- Preventing prolongead immobilisations

Measure for the preparation of the operating room:

- Aseptic and anti-septic measures
- The preparation of the medical devices, medications in sufficient quantities
- The registration of the medical activites according to internal protocols.

Our experience comprises approximatly 1000 longer then 3 hours procedures, due to the surgical complexity and the aplication of the internal protocol contributed to surgical success, led to the important reduction of the post-operating, complications and in reducing the operative time.

Course – Modern multidisciplinary approach on hepatic carcinoma

Hepatocellular carcinoma – a short review

Florentina Ioniță Radu, Andrada Popescu, A. Gavrilă, Maria M. Chereja

Introduction: Our objective is to review current international data regarding hepatocellular carcinoma.

Materials and methods: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer with an incidence ranging from 6.5 % in women to 7.9 % in men.

HCC has a high mortality rate being the third leading cause of cancer -related death worldwide, responsible for ~ 700,000 deaths annually. Regardless of the cause, cirrhosis is the most important risk factor for the development of HCC with an annual risk of 2-7%.

Biannual surveillance using both AFP and abdominal ultrasound remains the gold standard for HCC screening in cirrhotic patients even if they are not very sensible for early HCC detection. Other biomarkers, such as des γ carboxy-prothrombin (DCP), AFP-L3, and cross sectional imaging are of great interest, but there is insufficient evidence to consider using them for routine screening.

Radiological imaging is the primary tool used in HCC diagnosis, without a need for biopsy when typical appearance exists. Recent findings in genomics could provide new tools in HCC diagnosis.

Although there are many staging systems for HCC, the Barcelona Clinic Liver Cancer is the most widely accepted since it contains an evidence-based treatment algorithm.

There are multiple treatment options for HCC depending on patient performance status and liver dysfunction degree. There is a tendency in expanding the actual criteria for liver transplantation, but recent data show a higher posttransplant mortality.

Results and conclusion: Hepatocellular carcinoma is an aggressive solid tumor with a high mortality rate. Even if there are multiple treatment options, surveillance and detection of early HCC is the best option in increasing survival rate.

The hepatocelular carcinoma – what we know from guides

Gaudia Mănescu, Cătălina Diaconu, D.O. Costache, Raluca S. Costache

Hepatocellular carcinoma is a malignant tumor that is the fifth most common type of cancer, the third leading cause of cancer-related death globally (preceded only by the lung and the stomach cancers) and the the most common primary liver cancer. HCC was thought to be a special type of cancer prevalent only in the Southeast Asia and Africa, but it has become in a short time more common in other regions, especially in Europe and the United States, which has led to greater interest in the diagnosis and treatment of HCC worldwide.

90% of pacients with hepatocellular carcinoma associate cirrhosis. Chronic viral hepatitis (hepatitis B is an idependent predictor of HCC development), hemochromatosis and alcoholic cirrhosis are the most frequently associated with HCC.

The pathogenesis of HCC is not fully understood, but most probably are implicated the activation of proto-oncogenes, deactivation of tumor suppresor genes (e.g. p53 and pRb), changes in growth factor or growth factor signaling processes (e.g IGF, TGF), changes in telomeric length and activity or microsatellite instability.

Symptoms of HCC are commonly related to those of their chronic liver disease and include weakness or fatigue, weight loss, anorexia, pain in the upper abdomen usually in the right hypocondrium, bloating or jaundice.

Diagnosis is usually made by history, physical examination, laboratory studies (aminotransferase levels, alkaline phosphatase, serum bilirubin and optionally elevated serum AFP (> 400 ng/ml), because AFP is elevated in only 50-75% of cases), MRI or CT scan showing a liver mass consistent with HCC.

The treatment should be discussed and planned by a multidisciplinary team. The applicable treatment possibilities include surgical (liver resection, liver transplantation-OLT), ablative (percutaneous ethanol injection, transarterial chemoembolization, radio-frequency ablation) and systemic therapy (tyrosine-kinase inhibitor – Sorafenib).

The treatment is established using the Barcelona-Clinic Liver Cancer (BCLC) staging system which defines very early stage cancer (single nodule,<2 cm, Child-Pugh A) as stage 0, early stage cancer (1-3 nodules, <3 cm each, Child-Pugh A-B) as stage A, intermediate stage for multinodular HCC (stage B), advanced stage which involves vascular invasion or extrahepatic spread (stage C) and terminal stage at pacients with Child-pugh C cirrhosis. Resection, ablation and transplantation is recommended for stage 0 and A, transcatheter arterial chemoembolization for stage B, Sorafenib – stage C and best supportive care for terminal stage.

Surgical treatment of the hepatocellular carcinoma (HCC) – review

L. Mosoia

Hepatocellular carcinoma (HCC) is a rising cause of cancer related mortality and viral causes of cirrhosis appear to be a major cause. The data work stress out that surveillance helps to detect early stage disease and treatment options are determined by stage of presentation. Different classifications of HCC and specific treatment are presented.

Three potentially curative options are tumor resections, liver transplantation and radiofrequency ablation/cryotherapy.

Emerging therapies such as drug eluting beads – transarterial chemoembolizations, radioembolization or Sorafenib treatment will continue to advance options in HCC.

Early experience with laparoscopic bipolar radiofrequency device for liver resection in hepatocellular carcinoma

L. Mosoia, V. Ștefănescu, F. Măcău, C. Mitru, V. Dumitrașcu

Laparoscopic liver resection remains a surgical procedure of great challenge because of the risk of massive bleeding during liver transection and the complicated biliary and vascular anatomy in the liver. Various techniques and energy-based devices have been used to minimise the blood loss during transection of the liver parenchyma laparoscopically.

Methods.

The laparoscopic Habib™ 4X is a bipolar radiofrequency device consisting of a 2x2 array of needles arranged in a rectangle. It produces coagulative necrosis of the liver parenchyma sealing biliary radicals and blood vessels and enables bloodless transection of the liver parenchyma.

Two patients underwent laparoscopic liver resection in a period of 6 months. Indications for liver resection were marginal hepatocellular carcinoma (3.7/3.1cm and 8/7 cm) in 2 cirrhotic patients. Technical aspects were analysed.

Results. One patient underwent S6 resection, the other one underwent S2-S3 resection. Pringle manoeuvre was not used in any of the patients. Mean operative time was 180 minutes (range, 160–200 minutes). Bleeding control along the transection line was satisfactory. No conversion to laparotomy was required. Operative blood loss was minimal. No blood transfusion was recorded. The postoperative period was uneventful. Median postoperative hospital stay was 3 days. Histopathology revealed that the margins were disease free.

Conclusion. Laparoscopic liver resection can be safely performed with laparoscopic Habib™ 4X with a significantly low risk of intraoperative bleeding or postoperative complications.

Two stage surgical treatment for giant right hepatic hepatocellular carcinoma

L. Mosoia, T. Artenie, A. Dima, C. Mitru, V. Dumitrașcu, Florina Bold, Irina Zahiu, C. Bețianu

Hepatocellular carcinoma (HCC) is a primary tumor of the liver, usually associated with chronic liver disease, is less common in non-cirrhotic livers. In this report, we present a 75 yrs old patient who came to our attention one month after the initial ultrasound with palpable hepatomegaly.

The CT showed a hypervascularized solitary liver lesion measuring 22/18 cm originated from right hepatic lobe, with small for size left hemiliver at volumetry. Laboratory data showed normal liver function and hepatitis B and C serologies were negative. Serum tumor markers including CEA, CA 12.5, CA 19.9 were within normal range while AFP was elevated, > 500. Due to the insufficient future liver remnant, we performed right portal vein ligation (PVL) in the first instance, to convert the unresectable tumor to resectable for potential cure. We record the success of PVL with compensatory hypertrophy in the left liver, seen at seven weeks after PVL.

The patient underwent planned laparotomy and right hepatectomy with uneventfully postoperative follow-up and discharge in day 9.

The case presented, indicates that surgical resection for giant HCC is possible in selected patients, even if initially, the future liver remnant does not allowed surgical therapy.

Alternative treatment options for inoperable primary and secondary liver tumors

Săndica Bucurică

The treatment protocols proposed by EASL and AASLD are similar and it are made (based on Barcelona Clinic Liver Cancer staging) according to disease stage. As for early stages there are curative treatments for advanced stages (as inoperable, vascular invasion, extrahepatic spread, poor clinical and biological patient status).

It is considered that hepatic resection, liver transplantation (in carefully selected individuals), and radiofrequency ablation are considered curative.

Treatments that consider best supportive care for terminal-stage HCC, but not intend to be curative are transarterial chemoembolization, radioembolization, and systemic chemotherapy such as with sorafenib.

The goals of trying to treat inoperable hepatic tumors is desirable in order to obtain a longer time of progression, the extension of survival with a better life quality by minimizing the symptoms or to obtain regression of tumors sizes for ablation, resection or transplantation.

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- *Nonoperative management of high-grade splenic injury*
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- *Managing radial nerve injuries associated with humeral fracture*
- *Intraoperative parathyroid hormone assay in patients with primary hyperparathyroidism*
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- *Clinical and dermatoscopical aspects of pigmented basal cell carcinoma – Case series and literature review*
- *Compliance of school doctors' practice with medical legislation*
- *In memoriam – General (Ret.) Academician Prof. Vasile Cândea MD, PhD*

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SYSTEMATIC REVIEW

Efficacy and tolerability of antibiotic augmentation in schizophrenia spectrum disorders – A systematic literature review

Octavian Vasiliu[1], Daniel Vasile[2], Victor A. Voicu[2,3]

Abstract: *Background: A large number of augmentation agents have been tried in patients with schizophrenia spectrum disorders, because negative and cognitive symptoms are difficult-to-treat with current pharmacological agents, and because the high percentage of treatment-resistant cases requires new therapeutic solutions. Inflammation is one of the supposed pathophysiological mechanisms in schizophrenia; therefore, antibiotics have been explored as add-on agents. No systematic review or meta-analysis about the efficacy of antibiotics in psychotic disorders has yet been conducted. Objectives: To assess if the use of antibiotics as add-on to current antipsychotic treatment may improve core symptoms of schizophrenia spectrum disorders, global clinical status, overall functionality, and if this augmentation strategy is well tolerated. Methods: A systematic literature review was conducted, including papers published between 1980 and 2018, which have been found in the main electronic databases (PubMed/MEDLINE, CINAHL, NCBI, Embase, Thomson Reuters/Web of Science). The keyword search strategy was formulated using the following paradigm: „schizophrenia spectrum disorders"/ „schizophrenia"/ „schizo-affective disorder"/ „schizophreniform disorder", AND „antibiotics"/ all the names of currently marketed antibiotics classes. This review was registered to PROSPERO database with the protocol number CRD42019119152. Results: Based on reviewing the selected trials regarding the efficacy of antibiotics over core symptoms of schizophrenia, only two agents were detected – minocycline and D-cycloserine. Minocycline as add-on to antipsychotic treatment was associated with mixed results, while regarding the efficacy of d-cycloserine either no significant difference, or superiority to placebo over negative and cognitive symptoms was reported. Minocycline had an overall effect over the clinical impression that was not distinguishable from placebo, although there was one trial supporting the efficacy of this antibiotic. The impact of minocycline over the global functioning when added to antipsychotic in patients with schizophrenia was mixed, with one positive and one negative trial (moderate to high quality designed trials). D-cycloserine had no significant impact over global clinical status or patients' overall functionality. Conclusion: From all the data reviewed and hierarchized resulted that larger trials are needed in order to confirm the efficacy of antibiotics as add-on to antipsychotics over negative and cognitive symptoms of schizophrenia spectrum disorders.*

Keywords: *antibiotics, augmentation strategy, negative symptoms, cognitive symptoms, tolerability, schizophrenia spectrum disorders*

BACKGROUND

Negative and cognitive symptoms are extremely challenging for clinicians who are treating patients with schizophrenia, and these clinical phenomena are important to approach because they are related to functional prognosis, real-world performance and quality of life. If currently marketed antipsychotics can control positive, behavioral and sometimes affective symptoms of schizophrenia, when negative and cognitive symptoms are analysed

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longitudinally, the results are still disappointing [1]. The prevalence of treatment-resistant schizophrenia is estimated to be approximately 30% (range 10-45%) [2] and an important number of patients have „ultra-resistant" schizophrenia, defined as resistance to clozapine [3]. In all these cases, a significant number of psychotic symptoms persist, although several trials of antipsychotics have been conducted for enough time in adequate doses [3]. Treatment-resistant cases require additional therapeutic agents, and a large number of solutions have been formulated without reaching a consensus (except for clozapine, but as previously mentioned, there are cases resistant even to this second-line atypical antipsychotic).

A significant number of symptoms detected in treatment-resistant patients are based on cognitive deficits [4], and the N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis attempts explaining why antipsychotic treatment may attenuate the striatal dopaminergic dysfunction without having an influence over the prefrontal cortex in treatment-resistant cases [5]. Negative symptoms have been demonstrated to explain the highest part of variation in disease severity in treatment-resistant schizophrenia, and these symptoms mediated the effects of verbal fluency dysfunctions and high-level neurological soft signs on disease's severity [6].

Many treatments from a large area of pharmacological, psychotherapeutical and other somatic approaches (e.g., deep brain stimulation) have been investigated for the management of negative and cognitive symptoms of schizophrenia, but there is no first-line therapy yet established for these cases. Atypical antipsychotics used at the lowest dose necessary to control positive symptoms, antidepressants as add-on agents, and cognitive-behavioral therapy have been suggested with limited efficacy in schizophrenia with prominent negative symptoms [7]. Many different pharmacological targets have been suggested for addressing cognitive symptoms in schizophrenia, from D1-agonists to glycine site agonists, and from type A gamma-amynobutyric acid (GABA-A) receptors agonists to alpha7-nicotinic receptors partial agonists, but without significant effects [8-10].

Antibiotics have been explored in clinical practice not only for their anti-microbial properties, but also for their neuroprotective and antiinflammatory effects. As schizophrenia has been associated with increased levels of circulating pro-inflammatory markers and microglial activation in both animal and human studies, researchers have become increasingly interested in the therapeutic potential of antibiotics [11]. Many β-lactams antibiotics have been associated with increased glutamate type 1 transporter (GLT1) expression in animal models, which can lead to a prevention of toxic neuroexcitation by excessive glutamate transmission [12]. Macrolides may confer neuroprotection against ischemic damage following cerebral ischemia without cerebral blood flow impairments [13]. Doxycycline was proven to prevent amyloid-β toxicity both in vivo and in vitro in a Drosophila melanogaster model of Alzheimer disease [14]. Minocycline is a second generation, semi-synthetic tetracycline that has been associated with neuroprotective, anti-inflammatory and anti-apoptotic properties, which also can inhibit proteolysis, angiogenesis and tumour metastatis [15-17]. D-cycloserine is not only an antituberculosis agent, but also a glycine site partial agonist studied for its properties to enhance neuroplasticity in schizophrenia [18].

While many data on the effects of antibiotics over neuroplasticity are derived from animal models, information on the clinical impact of these pharmacological agents in patients diagnosed with schizophrenia spectrum disoders are contradictory [18-22].

The objectives of this systematic literature review are (1) the evaluation of antibiotics efficacy as add-ons in the management of the core symptoms of schizophrenia spectrum disorders, global clinical status, overall functionality, and (2) to verify if this augmentation strategy is well tolerated. These objectives are considered of clinical interest for psychiatrists, but they also may be useful for researchers oriented on finding new, non-dopaminergic-based treatments for psychotic disorders.

METHODS

This systematic review is based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Table 1) [23]. The protocol of this review was registered in the PROSPERO with the protocol number CRD42019119152 [24].

2.1. Outcomes

The primary outcome for this review was the efficacy of any antibiotic in decreasing symptoms severity in schizophrenia spectrum disorders. We considered the impact of these agents over positive, negative, cognitive, behavioral, and/or affective dimensions of psychosis, as determined by validated psychometric scales for this specific pathology.

The secondary outcomes were (a) tolerability of antibiotics, assessed by either subjective reports or validated instruments, (b) general functionality of patients, and (c) global clinical status, assessed by structured instruments.

2.2. Search strategy

A keyword search strategy was formulated using the following paradigm: „schizophrenia spectrum disorders”/„schizophrenia”/„schizo-affective disorder”/„schizophreniform disorder”, AND „antibiotics”/all the names of currently marketed antibiotics classes. Electronic databases searched were Medline (PubMed), Embase, Cumulative Index to Nursing and Allied Health Literature (CINAHL), National Center for Biotechnology Information (NCBI), and Thomson Reuters/Web of Science. Papers included in the references of the analyzed articles were also reviewed, and their results were included if the quality of research corresponded to the pre-defined inclusion and exclusion criteria. We selected clinical trials that were published in any language, between January 1980 and January 2019.

Table 1: PRISMA-P 2015 Checklist [25]

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		ADMINISTRATIVE INFORMATION			
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☒	☐	92-94
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☐	Not applicable
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	94
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	Title page
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	138-150
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☐	Not applicable
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	146-148
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☐	Not applicable
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☐	Not applicable
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	45-89
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☒	☐	135-136, Table 1
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☒	☐	124-133, Table 1
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	124-133
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	104-111
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	138-150

Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	138-150
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	138-150
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	138-150, Table 1
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☒	☐	96-102
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☒	☐	143-147
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☐	☒	
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☐	☒	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☐	☒	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☒	☐	156-164
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☐	☒	
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	52-57

This checklist has been adapted for use with protocol submissions to Systematic Reviews from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic Reviews 2015 4:1

Figure 1: Results of the PRISMA-based search paradigm

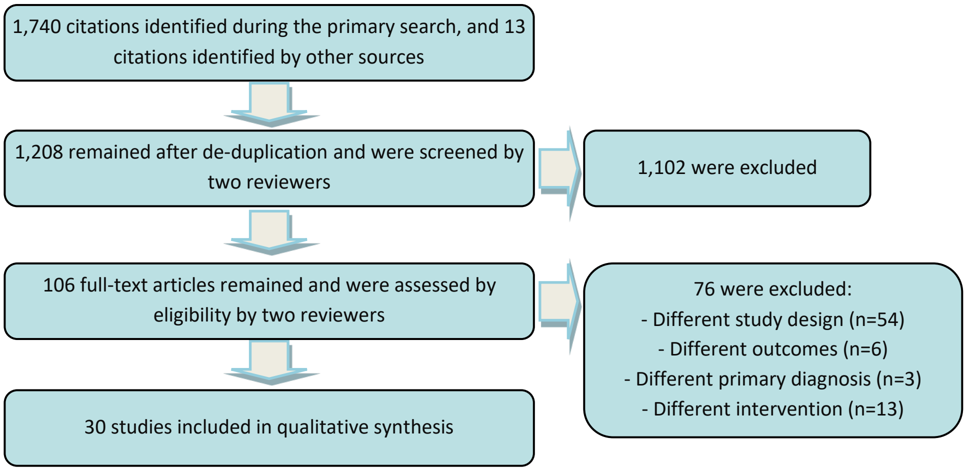
1,740 citations identified during the primary search, and 13 citations identified by other sources
1,208 remained after de-duplication and were screened by two reviewers
1,102 were excluded
106 full-text articles remained and were assessed by eligibility by two reviewers
76 were excluded:
- Different study design (n=54)
- Different outcomes (n=6)
- Different primary diagnosis (n=3)
- Different intervention (n=13)
30 studies included in qualitative synthesis

2.3. Inclusion and exclusion criteria

Studies were eligible for inclusion if they were randomized/non-randomized, open label, single blind or double blind, with or without active comparator, prospective/retrospective trials. These studies could be controlled with placebo, any other active drug, or usual care, and they must have specified diagnosis criteria for the explored clinical entity/entities. All patients included in these trials were over 18 years old at the initial visit, and no superior limit of age was established. Validated instruments for evaluation of the efficacy of the investigational product should be used at baseline and at least at end-point, e.g. Positive and Negative Syndrome Scale or Brief Psychiatric Rating Scale.

Only full-text articles were eligible for analysis, but if abstracts with outcomes corresponding to those defined by us were found, authors of the respective works were contacted to provide full-text of their papers. No limitation about the duration of the follow-up was included as a criterion of trials selection. A more detailed presentation of the inclusion and exclusion criteria is emphasized in Table 2.

Table 2: Inclusion and exclusion criteria

Operational criteria	Inclusion criteria	Exclusion criteria
Population	Minimum age of inclusion in the selected trial was specified as being 18, according to the study protocol. No superior age limit was specified. The main diagnosis was schizophrenia, schizo-affective disorder, schizophreniform disorder, or any combination of these entities. Diagnosis based on standardized criteria (DSM, ICD, or other compatible psychiatric classifications). Patients with resistant forms of schizophrenia were included (defined by resistance to at least 2 antipsychotic agents, each administered for a minimum duration and dosage, currently presenting symptoms of a minimum duration and severity determined by a standardized rating scale, and moderate or worse functional impairment) [15].	Patients with age under 18, except for cases where statistical procedures allowed for a separate evaluation of the adult vs. child population. The presence of severe somatic or psychiatric co-morbidities with significant impact over cognition, disposition, behavior, and overall functionality. The presence of infectious diseases, which require antibiotic therapy, as co-morbid or main diagnoses. Any co-morbidities which can create confusions in the domain of antibiotics efficacy over psychotic symptoms, either organic diseases (e.g., infections), or psychiatric (e.g., disorders induced or related to drugs or general medical conditions). Neurocognitive co-morbities were not allowed.
Intervention	Antibiotics used as add-on to any antipsychotic agent, either typical or atypical. Antibiotics from any class that is currently marketed, with no limitations related to the dosing regimen, adverse events profile, or any pharmacokinetic/pharmacodynamic property. The effects of the intervention over targeted symptoms of schizophrenia spectrum disorders were quantified using at least one validated instrument.	Trials controlled by psychotherapy only. Studies controlled by non-pharmacologic and non-psychotherapy interventions, e.g., deep brain stimulation, electroconvulsive therapy, transcranian magnetic stimulation. Over-the-counter products or dietary supplements as add-on or active comparators. Investigational pharmacologic agents used in phase II or III clinical trials. Administration of more than one antibiotic at a time.
Environment	Hospitals, day care, special care, permanent care, or intermediate care units. Both in-patient and out-patient regimen. A caregiver who is able to report on the patient's evolution under treatment and therapeutic adherence was considered optional.	Correctional environments. Unspecified environment.
Primary and secondary variables	Changes in psychotic symptoms (positive, negative, cognitive, behavioral, and/or dispositional) were evaluated by trained clinicians. Evaluation of the drugs tolerability, and patients overall functionality and global clinical status was also monitored. Studies were allowed to entered the analysis if they were focused on efficacy, tolerability, or both of these outcomes.	All trials using unspecified psychometric instruments, or only clinical observation were excluded.
Study design	Intervention was studied in randomized/non-randomized, open label, single blind, or double blind, with or without active comparator/placebo, prospective/retrospective trials. No limitation about the duration of the treatment was established. Only peer-reviewed studies were included.	Studies with unspecified or insufficiently specified design. Lack of methodologic specifications regarding statistic processing of the primary/secondary variables related to the efficacy. Case reports, case series. Animal model studies. Systematic literature reviews, meta-analyses.
Language	English, French, Spanish, Russian	Any other language, except for those mentioned in the „inclusion criteria”

2.4. Data extraction and analysis

Screening was performed by two independent reviewers, and the resulted material was filtered using inclusion and exclusion criteria mentioned above. Data regarding the main outcomes, the secondary outcomes, and other relevant information about the studies were extracted into the table 3. Data extraction and quality assessment of the studies were done independently by two reviewers (OV and either VAV or DV), and if any disagreement between the two appeared and could not be resolved by consensus, the third reviewer has been consulted.

Consensus was obtained regarding the methodological quality for the included studies after each study was independently rated using the Cochrane Handbook for Systematic Reviews of Interventions - Assessing risk of bias in included studies [26]. Each study was rated as having low, moderate or high risk of bias, based on the (1) randomization procedure, (2) blinding and treatment allocation, (3) use of placebo or an active comparator, (4) methods used for evaluation of outcomes, and (5) dimension of the study group.

All included trials were reviewed critically by authors of the current article, with consideration to the bias risk. A meta-analysis could not be performed because of the heterogeneity of the methods used within the selected trials.

RESULTS

A number of 1753 citations returned after the primary search, and only 1208 remained after de-duplication. After filtering-out articles non-compatible with our predefined inclusion/exclusion criteria, and safter excluding papers for which full text could not be found or provided by authors, a number of 30 trials resulted (Figure1).

3.1. Methodological quality assessment

Among the 30 articles included in this review, a majority were of moderate quality (n=15), while the high and low quality were almost equally represented (7 and 8, respectively). Most of these trials were randomized, placebo-controlled (n=28), one open-label, and one trial had a bi-phasic design, with a single-blind, followed by a double-blind period of treatment. Only 4 trials had a sample size over 100, while 19 trials included below 50 subjects, which reduced the power of the analysis for the poor-populated trials.

Frequently encountered problems were lack of information about concealment of the randomization, and about the intent-to-treat (ITT) vs. per protocol analysis. The mean percentage of agreement between the two reviewers for each trial was 90.7% ($p<0.0001$) for the validity criteria.

3.2. Evaluation of primary outcome: the efficacy of antibiotics in schizophrenia spectrum disorders

The only active interventions detected by our search were D-cycloserine (n=18 trials, most of them of moderate quality) and minocycline (n=10 trials, most of them of moderate and low quality).

The efficacy of minocycline as add-on to antipsychotic treatment was investigated in two large double-blind, placebo-controlled trials (n=200 and 207, respectively) and the results were negative [27, 28]. No improvement of negative (on medium and long term), cognitive and overall psychotic symptoms (on medium term) was detected by these two studies. A small trial (n=73 subjects) evaluated the impact of minocycline as add-on to clozapine over avolition and concluded that although higher plasma levels of clozapine were detected after minocycline initiation, there was no evidence that improvement in negative symptoms can be correlated with higher level of plasma antipsychotic [29].

Another small trial (n=52) concluded that minocycline did not improve significantly psychosis factor and overall BPRS (Brief Psychiatric Rating Scale) score, neither had it a significant impact over cognitive function, except for working memory [31]. The same trial reported a significant improvement in the BPRS anxiety/depression factor in the minocycline-treated patients [31].

Regarding the positive effects of minocycline, a small trial (n=24) detected significant improvements in patients treated with this drug over the positive and negative symptoms, compared to placebo, and a protective effect of this antibiotic over the gray matter loss in fronto-temporal areas involved in the pathophysiology of schizophrenia during 12-month of active treatment [32].

Another short-term trial (8 weeks, n=43 subjects) found that SANS (Scale for the Assessment of Negative Symptoms) total score decreased significantly compared to placebo, and the negative sub-scale of PANSS (Positive and Negative Syndrome Scale) also decreased, but not significantly compared to placebo [33].

A study that evaluated 92 patients with schizophrenia treated with risperidone who received minocycline as add-on for 16 weeks reported greater improvements on SANS total scores and PANSS negative subscale versus placebo [34]. This study observed no difference between groups in cognitive performances, except for the attentional domain [34].

Table 3: Qualitative analysis of the trials selected

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
Minocycline	Weiser M, Levi L, Burshtein S, et al. [27]	Efficacy of minocycline over positive, negative and cognitive symptoms of schizophrenia	16-week double-blind, placebo-controlled RCT; minocycline (200 mg/ day) /pramipexole /acetyl- salicilic acid/ placebo as add-on to antipsychotic treatment	N=200 patients with schizophrenia or schizo-affective disorder	Primary outcome measurements - PANSS total score at the end of the trial. Secondary outcomes- positive, negative and general psychopathology scored by PANSS; CGI-S, CGI-I; BACS; rates of drop-outs	No significant differences between minocycline and placebo for PANSS total score, PANSS subscales, CGI, and BACS were detected. No significant difference between groups in the frequency of adverse events.	High
Minocycline	Deakin B, Suckling J, Barnes TRE, et al. [28]	Efficacy of minocycline over negative symptoms in schizophrenia with recent onset	12-month double-blind, placebo-controlled RCT; minocycline (200 mg/ day for 2 weeks, then 300 mg/day for the remainder of 12 months) vs. placebo plus continuing treatment	N=207 patients with schizophrenia spectrum disorders that begun in the last 5 years	Negative symptom subscale score of the PANSS; prefrontal grey matter volume, dorsolateral prefrontal cortex activation during working memory task, and plasma concentration of IL6	No effect of minocycline was observed over ratings of negative symptoms. Biomarker outcomes did not change over time, and were not affected by minocycline. No difference in the appearence of serious adverse events was detected between groups.	High
Minocycline	Liu F, Xie L, Zhang B, et al. [30]	Effects of adjunctive minocycline over body metabolism in risperidone- treated patients with schizophrenia	16-week placebo-controlled, double-blind crossover RCT	N=63 patients diagnosed with schizophrenia,treated with stable dose of risperidone for ≥4 weeks	Body weight, BMI, waist circumference, fasting insulin, glucose and lipids serum levels	No difference between groups was detected (P's>0.3)	Moderate
Minocycline	Wehring HJ, Elsobky T, McEvoy JP, et al. [29]	Pharmacokinetic impact of minocycline over clozapine and correlation between modified levels of clozapine /norclozapine over negative symptoms in schizophrenia	10-week placebo-controlled RCT, minocycline vs. placebo as add-on to clozapine	N=73, DSM IV-TR-based diagnoses of schizophenia or schizo-affective disorder, currently treated with clozapine ≥200 mg/day and blood level of clozapine ≥350 ng/ml.	Serial plasma clozapine, norclozapine and total clozapine levels, SANS	Clozapine plasma levels are increased after the initiation of minocycline (21% increment to baseline) compared to placebo. However, smoking patterns were not analysed in relation to this plasmatic difference in concentration. Avolition improvement on SANS was not explained by changes in clozapine blood levels. There is no evidence that improvement in any symptom domain is due to higher clozapine levels.	Moderate
Minocycline	Kelly DL, Sullivan KM, McEvoy JP, et al. [31]	Efficacy of minocycline as adjunctive treatment to clozapine in chronic psychoses	10-week double-blind, placebo-controlled RCT; 100 mg p.o BID minocycline vs. placebo.	N=52 patients with schizophrenia and schizo-affective disorder with persistent positive symptoms	Positive and cognitive symptoms were primary outcomes. Avolition, anxiety/depression, and negative symptoms were secondary outcomes.	BPRS- psychosis factor and BPRS- total score were not changed significantly during treatment. Still, a change in total BPRS score of more than or equal to 30% was observed more frequently in minocycline treated patients (p=0.044). Global cognitive function (MATRICS) did not differ between groups, but significant	Moderate

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
						improvement in working memory favored minocycline. SANS total score did not differ, but minocycline improved significantly working memory (p=0.023). Significant improvement in the BPRS anxiety/depression factor was observed with minocycline (p=0.028). The overall tolerability of minocycline was good.	
Minocycline	Chaves C, Marque CR, Maia-de-Oliveira JP, et al. [32]	Effects of minocycline on brain morphology and cerebral perfusion in patients with recent-onset schizophrenia	12-month double-blind, placebo-controlled RCT of minocycline as add-on treatment (200 mg/day minocycline vs. placebo)	N=24 outpatients with recent-onset schizophrenia	SPECT and MRI were performed after 12 months of treatment for detection of morphologic changes.	Minocycline may have protective effect against gray matter loss and modulate fronto-temporal areas involved in the pathophysiology of schizophrenia. Significant lower gray matter volumes were observed in the midposterior cingulate cortex and in the precentral gyrus in placebo group, in comparison with minocycline group. Positive and negative symptoms were significantly decreased versus placebo.	Moderate
Minocycline	Ghanizadeh A, Dehbozorgi S, Omrani Sigaroodi M, Rezaei Z [33]	Effectiveness of add-on minocycline in schizophrenia	8-week placebo-controlled RCT; minocycline 200 mg/day + risperidone vs. placebo + risperidone.	N=43 patients diagnosed with DSM IV schizophrenia	Schizophrenia negative symptoms severity as primary outcome	SANS total score decreased significantly in the minocycline group at week 8, compared with placebo group. The decline of PANSS Negative score was more in the active treatment group than in the placebo group (not statistically signifi-cant). Tolerability of minocycline was good.	Moderate
Minocycline	Liu F, Guo X, Wu R, et al. [34]	Efficacy and safety of minocycline for the treatment of negative symptoms and cognitive impairments in patients with schizophrenia	16-week double blind, placebo-controlled RCT; minocycline 200 mg/day vs. placebo.	N=92 with early stage schizophrenia treated with risperidone	The primary outcome was represented by negative symptoms of schizophrenia, evaluated by SANS scores. Secondary outcomes were scores of response rate on SANS, PANSS, CGI, cognitive tests	Patients receiving minocycline had greater improvements on SANS total scores and PANSS negative subscale scores vs. placebo. Rates of treatment response in the minocycline group were significantly higher than those in the placebo group after 16 weeks of treatment. Cognitive performances were not improved significantly by minocycline, except for the attentional domain.	High
Minocycline	Khodaie-Ardakani MR, Mirshafiee O, Farokhnia M, et al. [35]	Efficacy and tolerability of minocycline add-on to risperidone in treatment of negative	8-week double-blind, placebo-controlled RCT; minocycline (titrated up to 200 mg/day) vs. placebo as add-on to	N=40 outpatients with chronic schizophrenia stabilized on risperidone for at least 8 weeks prior to baseline	Primary outcomes- negative symptoms evaluated by PANSS	Significant difference between groups on negative scale of PANSS favoured minocycline at the end of the trial (p<0.001). PANSS total score, positive score and general psychopathology score were also improved by minocycline compared to placebo.	Moderate

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
		symptoms in chronic schizophrenia	risperidone (maximum 6 mg/day)			No difference between groups regarding the severity of adverse effects was observed.	
Minocycline	Chaundhry IB, Hallak J, Husain N, et al. [36]	Efficacy of minocycline as add-on to treatment as usual over negative symptoms in early psychosis.	Placebo-controlled RCT, one year duration, minocycline (titrated up to 200 mg/day) vs. placebo.	N=144 patients with early psychosis	Primary outcomes- negative and positive symptoms evaluated by PANSS. Secondary outcomes- GAF, CGI, AIMS, neuropsychological outcome measures.	Minocycline improved negative symptoms ($p<0.001$) in the ITT population. No other outcomes were influenced significantly by minocycline. AIMS scores tend to improve more in the placebo group.	High
Minocycline	Levkovitz Y, Mendlovich S, Riwkes S, et al. [37]	Efficacy of minocycline as add-on treatment for alleviating negative and cognitive symptoms in early-phase schizophrenia	Double-blind, placebo-controlled RCT, 6 months duration, minocycline 200 mg/day vs. placebo + atypical antipsychotic (200-600 mg/day chlorpromazine-equivalent doses).	N=54 early-phase schizophrenia patients (DSM IV) - age 18-35 years	Primary outcome- negative symptoms severity evaluated by SANS. Cognitive measures evaluated by CANTAB. Functional measures and clinical evaluations by GAF, SOFAS, MCAS, CGI.	A significant time effect for SANS total scores was detected in the minocycline group, negative symptoms improved since week 14. CGI improved better with minocycline, as did cognitive functioning, mainly executive functions (working memory, cognitive shifting, and cognitive planning). GAF and SOFAS showed a better improvement with minocycline. Also, minocycline had a favourable effect in preventing weight gain during treatment.	Moderate
Minocycline	Miyaoka T, Yasukawa R, Yasuda H, et al. [38]	Efficacy and tolerability of minocycline as adjunct to antipsychotic medication in schizophrenia	Open-label, pilot study, minocycline 150 mg/day 4 weeks as adjunct to current antipsychotic medication	N=22 patients with schizophrenia	Psychotic symptoms severity determined by PANSS	All scales of PANSS improved during minocycline treatment. All patients tolerated the full dose during the study.	Low
D-cycloserine	Takiguchi K, Uezato A, Itasaka M, et al. [39]	Effects of d-cycloserine over positive, negative and cognitive symptoms of schizophrenia. To investigate if white matter integrity influences response to d-cycloserine.	6-week placebo-controlled, double-blind, cross-over design RCT; d-cycloserine (50 mg) vs. placebo as add-on to their current antipsychotic treatment	N=41 patients, in- and out- patients with DSM IV diagnostic criteria for schizophrenia	PANSS and SANS scores, BACS, EQS- interpersonal, intrapersonal and situational domains; correlations between d-cycloserine treatment and MR-DTI white matter integrity.	No improvement of positive, negative, or cognitive symptoms of schizophrenia. The better treatment effect of d-cycloserine on BACS was observed when fractional anisotropy was higher in sagittal striatum, cingulum, fornix stria terminalis, genu of corpus callosum, and external capsule, and the better treatment effect on PANSS- general psychopathology score was observed when fractional anisotropy of the splenium of corpus callosum was higher. The better treatment effect on PANSS- general psychopathology score and SANS-IV was observed when fractional anisotropy was lower in the posterior thalamic radiation.	Moderate

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
D-cycloserine	Forsyth JK, Bachman P, Mathalon DH, et al. [40]	Exploration of NMDA-receptor signaling on working memory and experience-dependent plasticity	Double-blind, placebo-controlled, between-group design RCT, 100 mg d-cycloserine vs. placebo; subjects were tested at one day after d-cycloserine or placebo administration	N=45 patients diagnosed with schizophrenia	Neuroplasticity changes evaluated by cognitive tests and EEG with high-frequency visual stimulation	D-cycloserine did not affect plasticity (similar neural potentiation in both active and placebo group), similar information integration and weather prediction tasks performances. Still, patients who received active drug and who were actively involved in memory task had superior 2-back performace compared to placebo-receiving patients. NMDA-receptor-signaling was not translated into synaptic plasticity change in schizophrenia.	Moderate
D-cycloserine	Cain CK, McCue M, Bello I, et al. [41]	Efficacy of d-cycloserine + cognitive remediation (CR) program in improving memory of patients with schizophrenia	8-week placebo-controlled RCT; 3-5 times per week CR program + once-weekly adjunctive treatment with 50 mg d-cycloserine or placebo administered before the first session within each week.	N=36 outpatients, stable medicated adults	Primary outcomes were performances on an auditory discrimination task, MATRICS battery composite scores, SANS total scores	Performance on the practiced auditory discrimination task significantly improved in the d-cycloserine group compared to placebo. Active treatment was associated with significantly greater negative symptom improvement for subjects symptomatic at baseline. Improvement on MATRICS was observed only in the placebo group.	Moderate
D-cycloserine	Gottlieb JD, Cather C, Shanahan M, et al. [42]	Efficacy of d-cycloserine as add-on to CBT for the management of delusions in schizophrenia	Double-blind, cross-over, placebo controlled RCT, single oral dose of d-cycloserine 50 mg vs. placebo, one hour post-drug administration patients received 2 CBT sessions focused on challenging patients' paranoid appraisals, with 7-day and 14-day re-evaluations	N=21 outpatients diagnosed with schizophrenia or schizo-affective disorder and moderately severe delusions	Delusional severity was assessed using SAPS; alternative explanations generated on the ABA; PSYRATS score	No significant effect of d-cycloserine on delusional distress or severity has been reported.	Moderate
D-cycloserine	Goff DC, Cather C, Gottlieb JD, et al. [43]	Investigation of d-cycloserine over negative symptoms and cognition in schizophrenia	8-week double-blind, parallel-group RCT; add-on of d-cycloserine 50 mg or placebo administered once-weekly.	N=50 adult outpatients treated with any antipsychotic except clozapine	Primary outcomes were change from baseline to week 8 on SANS total score and on a composite cognitive score.	D-cycloserine significantly improved SANS total scores compared to placebo at week 8. Cognitive performance did not improve with d-cycloserine at week 8. Delayed thematic recall was significantly improved with the first dose of d-cycloserine vs. placebo, but not immediate thematic recall and item recall.	High

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
D-cycloserine	Buchanan RW, Javitt DC, Marder SR, et al. [44]	Efficacy of glycine or d-cycloserine in patients with schizophrenia with moderate to severe negative symptoms and cognitive impairments.	16-week double-blind, double-dummy, parallel-group RCT of adjunctive glycine, D-cycloserine, or placebo	N=157 inpatients and outpatients diagnosed with DSM-IV schizophrenia or schizo-affective disorder and retrospective and prospective criteria for moderate to severe negative symptoms without marked positive, depressive, or extra-pyramidal symptoms	The primary outcome-average rate of change of SANS total scores and change in the average cognitive domain z scores	No significant differences in change of the SANS total score between glycine and placebo, or d-cycloserine and placebo. No significant differences between glycine and placebo or d-cycloserine and placebo were recorded on the average cognition z score.	High
D-cycloserine	Yurgelun-Todd DA, Coyle JT, Gruber SA, et al. [45]	Examination of patterns of cortical activation underlying d-cycloserine's therapeutic efficacy in patients with schizophrenia	2-week single-blinded, placebo lead-in phase RCT; after placebo phase, patients received 50 mg/day d-cycloserine or placebo in a double-blind manner for 8 weeks.	N=12 out- and in-patients with DSM-IV criteria for schizophrenia on stable dose of antipsychotic for at least 4 months and presenting prominent negative symptoms	fMRI patterns of cortical activation; SANS for severity of negative symptoms	Patients receiving d-cycloserine had a significant increase in temporal lobe activation, associated with reduction in negative symptoms.	Low
D-cycloserine	Goff DC, Herz L, Posever T, et al. [46]	Efficacy of d-cycloserine as augmenting agent for conventional antipsychotics in decreasing negative and cognitive symptoms of schizophrenia	Double-blind, parallel-blind RCT, 50 mg/day d-cycloserine or placebo for 6 months	N=55 patients with schizophrenia with prominent negative symptoms	Primary outcomes- negative symptoms and cognitive impairments evaluated by PANSS, SANS, cognitive battery	No difference between groups was detected on any primary outcome at 8 or 24 weeks (PANSS, SANS, cognitive tests).	High
D-cycloserine	Duncan EJ, Szilagyi S, Schwartz MP, et al. [47]	Efficacy of d-cycloserine over negative and cognitive symptoms of schizophrenia	Double-blind, parallel-group RCT, 50 mg QD d-cycloserine or placebo for 4 weeks	N=22 male patients with schizophrenia displaying prominent negative symptoms stabilized on typical neuroleptics	Symptoms severity (SANS, BPRS, Abrams and Taylor rating scale). Cognition was assessed by the Sternberg Memory Test and the Continuous Performance Test.	No significant differences between the d-cycloserine and placebo group on any symptom rating	Low
D-cycloserine	Evins AE, Amico E, Posever TA, et al. [48]	Effects of d-cycloserine when added to risperidone	Placebo-controlled RCT, 2 weeks of placebo and 4 doses of d-cycloserine	N=10 patients with schizophrenia treated with risperidone	Negative symptoms severity	D-cycloserine (50 mg/day) significantly reduced negative symptoms.	Low

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
		on negative symptoms of schizophrenia				Ratings of depression, extrapyramidal side effects, and cognitive function were unchanged.	
D-cycloserine	Heresco-Levy U, Ermilov M, Shimoni J, et al. [49]	To investigate the clinical effects of d-cycloserine when added to conventional antipsychotics, olanzapine, or risperidone for treatment-resistant schizophrenia	Double-blind, placebo-controlled, crossover study, 2 random-order 6-week treatment arms (d-cycloserine, 50 mg/day vs. placebo), separated by 2-week adjuvant treatment washout.	N=24 patients diagnosed with treatment-resistant schizophrenia, and they were on stable doses of medication for at least 3 months before study entry	Psychotic symptoms evaluated by PANSS, depression severity evaluated by HAMD, and adverse events quantified by SAS and AIMS	Significant reduction in negative symptoms in patients treated with d-cycloserine. No difference between patients treated with conventional neuroleptics or atypical antipsychotics was observed.	Moderate
D-cycloserine	Heresco-Levy U, Javitt DC, Ermilov M, et al. [50]	Efficacy of d-cycloserine over negative symptoms in schizophrenia	16-week, double-blind, placebo-controlled, adjuvant treatment RCT with 50 mg/day d-cycloserine	N=9 treatment-resistant chronic schizophrenia-diagnosed patients	Psychotic symptoms evaluated by PANSS, depression severity evaluated by HAMD, and adverse events quantified by SAS and SAS and AIMS	Symptoms changes between groups were not significant, except for a significant reduction in negative symptoms which was registered during treatment with d-cycloserine but not placebo. Greater reductions were registered in patients with lower baseline serum glycine levels. No side effects were registered.	Low
D-cycloserine	van Berckel BN, Evenblij CN, van Loon BJ, et al. [51]	Effects of d-cycloserine as add-on to typical antipsychotics in reducing negative symptoms in schizophrenia	Double-blind, parallel group, placebo-controlled RCT; 50 mg d-cycloserine BID or placebo for 8 weeks.	N=26 patients with DSM-IV schizophrenia	PANSS, CGI, ESRS	D-cycloserine slightly worsened positive symptoms and general psychopathology compared to placebo. No significant change in negative symptoms was detected or extrapyramidal symptoms. Tolerability of d-cycloserine was good.	Moderate
D-cycloserine	Goff DC, Henderson DC, Evins AE, Amico E [52]	Effects of d-cycloserine over negative symptoms in schizophrenia	6-week RCT, d-cycloserine 50 mg/day or placebo, crossover design separated by 1 week placebo washout for a total of 13 weeks	N=17 outpatients diagnosed with schizophrenia (DSM-IV criteria) treated with clozapine	PANSS, SANS, GAF, HAMD, AIMS, SAS, BAS	D-cycloserine significantly worsened negative symptoms compared to placebo (increased SANS scores and negative subscale of PANSS)	Moderate
D-cycloserine	Goff DC, Tsai G, Levitt J, et al. [53]	Efficacy of d-cycloserine over negative symptoms and cognitive function when added to conventional neuroleptics	8-week double-blind RCT, d-cycloserine 50 mg/day vs. placebo, added to their conventional neuroleptic	N=47 patients with schizophrenia meeting criteria for deficit syndrome	SANS total score	The mean reduction in negative symptoms with d-cycloserine was significantly greater than with placebo (SANS scores). No differences were found in performance on any cognitive test between groups or in changes in any other clinical measure.	Moderate

Intervention	Authors	Objectives	Design	Population	Outcomes	Results	Quality score
D-cycloserine	Goff DC, Tsai G, Manoach DS, et al. [54]	Efficacy of d-cycloserine as add-on over negative symptoms in patients treated with clozapine	2-week placebo-controlled RCT; 5, 15, 20, 50 and 250 mg/day d-cycloserine	N=10 outpatients with schizophrenia receiving clozapine who presented criteria for primary deficit syndrome	SANS	Significant dose effect of d-cycloserine on SANS scores, 50 mg/day produced a mean increase of 21% in SANS score. No improvement was detected on clozapine-treated patients with negative symptoms.	Low
D-cycloserine	Rosse RB, Fay-McCarthy M, Kendrick K, et al. [55]	Efficacy of d-cycloserine as add-on to molindone in schizophrenia	4-week placebo-controlled, double-blind, parallel-group RCT, 10 or 30 mg/day d-cycloserine as adjuvant to molindone (150 mg QD)	N=13 patients diagnosed with schizophrenia	BPRS, SANS, CGI	Neither dose of d-cycloserine had adjuvant therapeutic efficacy, although the tolerability of the d-cycloserine was good.	Low
D-cycloserine	Goff DC, Tsai G, Manoach DS, Coyle JT [56]	Efficacy of d-cycloserine over negative symptoms of schizophrenia	2-week trials of placebo and four doses of d-cycloserine; 5, 15, 50, 250 mg/day d-cycloserine	N=9 outpatients with schizophrenia (DSM III R criteria) treated with stable doses of conventional antipsychotics for at least 4 months prior to inclusion	SANS, Negative symptoms subscale of BPRS, SAS, AIMS, GAF, Sternberg's Item Recognition Paradigm	D-cycloserine at 50 mg/day produced significant reduction in negative symptoms and significantly improved reaction time (as a measure of executive dysfunction)	Low

RCT = randomized controlled trial; MR-DTI = Magnetic resonance - diffusion tensor imaging; PANSS = Positive and Negative Syndrome Scale; SANS = Scale for the Assessment of Negative Symptoms; EQS = Emotional Intelligence Scale; BMI = Body Mass Index; BPRS = Brief Psychiatric Rating Scale; ITT = Intent to treat analysis; GAF = Global Assessment of Functionality Scale; CBT = Cognitive Behavioral Therapy; SAPS = Scale for the Assessment of Positive Symptoms; ABA = Alternative Beliefs Assessment; PSYRATS = Psychotic Symptom Rating Scale; CANTAB = Cambridge Neuropsychological Test Automated Battery; SOFAS = Social and Occupational Functioning Assessment Scale; MCAS = Multinomah Community Ability Scale; HAMD = Hamilton Depression Rating Scale; SAS = Simpson-Angus Rating Scale; AIMS = Abnormal Involuntary Movement Scale; ESRS = Extrapyramidal Symptoms Rating Scale; BAS = Barnes Akathisia Scale; CGI-S = Clinical Global Impressions- Severity; CGI-I = Clinical Global Impressions- Improvements; BACS = Brief Assessment of Cognition in Schizophrenia

Another 8-week trial (n=40) confirmed significant differences between groups in favour of minocycline regarding the negative symptoms evaluated by PANSS, while positive and general psychopathology scores were non-significantly improved [35].

A large, long-term trial (n=144, 12 months) reported that minocycline improved negative symptoms in the ITT population (p<0.001) [36]. The efficacy of minocycline over negative symptoms was supported also by a study (n=54) which included early-phase schizophrenia patients, with difference between groups appearing at week 14 [37]. An open-label study confirmed a positive effect of minocycline over all scales of PANSS after 4 weeks [38].

D-cycloserine was associated with lack of efficacy over positive, negative and cognitive psychotic symptoms, in a small trial (n=41), but some interesting correlations were reported between MR-DTI (Magnetic resonance - diffusion tensor imaging) and score evolution of general and cognitive symptoms, i.e. fractional anisotropy in certain thalamic regions (where assymetric alterations in the white matter integrity were demonstrated) could be improved by this antibiotic and it may be a mediator of the therapeutic response [39].

No significant effects were observed in patients treated with d-cycloserine over neuroplasticity and cognitive performances, evaluated by cognitive tests and EEG with high-frequency visual stimulation, in a small trial (n=45) [40]. In a crossover design study, no significant effect of d-cycloserine on delusional distress or severity has been reported [42].

Data from a large trial (n=157) found no difference between d-cycloserine and placebo over the severity of cognitive and negative symptoms [44]. No significant differences were found between d-cycloserine and placebo in two trials, which included patients with prominent negative symptoms in the domains of negative and cognitive manifestations [46, 47]. D-cycloserine slightly worsened positive symptoms and general psychopathology compared to placebo, and it did not significantly change negative symptoms in an 8-week placebo-controlled trial (n=26) [51]. D-cycloserine significantly worsened negative symptoms compared to placebo in a 13-week trial (n=17) [52]. When added to clozapine, d-cycloserine worsened negative symptoms dose-proportionally in a small study (n=10) [54]. D-cycloserine added to molindone did not resulted in improvements of general psychopathology or negative symptoms (n=13) [55].

Regarding the positive results of d-cycloserine, this drug was significantly superior to placebo on reducing negative symptoms compared to placebo (n=36, 8-week trial) [41]. D-cycloserine significantly improved SANS total scores after 8 weeks in small trial with outpatients, but no effect was observed over cognitive functions (n=50) [43]. A small trial (n=12) found that d-cycloserine increased significantly temporal lobe activation, a phenomenon correlated with negative symptoms [45].

Table 4: Efficacy of antibiotics over symptom severity in schizophrenia spectrum disorder

Treatment	Clinical trials	GRADE Recommendations
Minocycline	[27-29,31-38]	Positive symptoms: ? Negative symptoms: D+ Cognitive symptoms: D+ Behavioral symptoms: ? Affective symptoms: D Overall symptoms: D+
D-cycloserine	[39-45,48,50-56]	Positive symptoms: ? Negative symptoms: D+ (but not as add-on to clozapine) Cognitive symptoms: ? Behavioral symptoms: ? Affective symptoms: ? Overall symptoms:?

GRADE recommendations for the level of evidence [57]: A=high quality= future research are very unlikely to change the validity of these recommendations; B= moderate quality= future research is likely to have an important impact on the appreciation of the recommendations; C=low quality= it is very likely that future research will have an important impact over the confidence in the estimate of effect and is likely to change the recommendations; D= very low quality= any estimate of the effect is very uncertain. Intermediate steps (+ or -) represent nuances of the recomedantions based on the quality of trials and tolerability of antidepressant agents, as resulted from these trials.

Another small trial (n=10) signaled reduced negative symptoms with d-cycloserine, but no effect of the same drug over cognitive function [48]. In a trial with treatment-resistant schizophrenia-diagnosed patients, adding d-cycloserine to current antipsychotic agent has lead to significant reduction in negative symptoms, without differences between patients treated with atypical or typical antipsychotics [49].

A small trial (n=9) reported significant improvements in negative symptoms versus placebo, especially in patients with lower baseline serum glycine levels [50]. A significant reduction in negative scores (evaluated by SANS) was observed in an 8-week double blind RCT (n=47), but no effect was detected over cognitive symptoms [53]. When added to conventional antipsychotics, D-cycloserine 50 mg/day produced significant reduction in negative symptoms and improved executive dysfunction in patients with schizophrenia (n=9) [56].

Table 5: Tolerability of antibiotics in patients diagnosed with schizophrenia spectrum disorders

Treatment	Clinical trials	GRADE Recommendations
Minocycline	[27, 28, 31-38]	Overall adverse events: B Extrapyramidal symptoms: B Body weight, waist circumference, lipid and glucose metabolism: B-
D-cycloserine	[44, 48-52, 55, 56]	Overall adverse events: B- Extrapyramidal symptoms: B- Body weight, waist circumference, lipid and glucose metabolism: ?

Table 6: Effect of antibiotics over global clinical status in patients diagnosed with schizophrenia spectrum disorders

Treatment	Clinical trials	GRADE Recommendations
Minocycline	[27, 34, 36, 37]	D-
D-cycloserine	[51, 52, 55, 56]	D-

Table 7: Effect of antibiotics over global functionality in patients diagnosed with schizophrenia spectrum disorders

Treatment	Clinical trials	GRADE Recommendations
Minocycline	[36, 37]	D
D-cycloserine	[51, 52, 55, 56]	D-

3.3. Evaluation of antibiotics tolerability in the studied population

Tolerability of minocycline was evaluated in a number of nine trials of moderate quality, most of them being double blind, placebo-controlled, that included 825 patients, and had variable duration (between 4 weeks and 12 months). Most of these trials included small groups, but there were also 3 large-group (over 140 patients each) studies [27, 28, 36].

No significant differences in the rate of adverse events between minocycline- and placebo-treated patients were reported [27, 28, 31, 32, 35, 36, 38].

No difference between minocycline and placebo was detected in a study focused on tolerability in the domain of body weight, waist circumference, fasting insulin, glucose and lipid serum levels [30]. Minocycline had a favourable effect in preventing weight gain during treatment in a trial with early-phase schizophrenia patients (n=54) [37].

Tolerability of d-cycloserine was evaluated systematically in 6 trials, of low-to-moderate quality, that included 99 patients, with a duration between 2 and 16 weeks [44, 48-51, 55]. All these trials reported a good tolerability of d-cycloserine, and the main adverse events monitored were extrapyramidal symptoms. However, none of the trials, which included scales for extrapyramidal symptoms, reported any significant changes during d-cycloserine administration [49-52, 56].

3.4. Evaluation of general functionality and global clinical status

Minocycline improved the global clinical impression in only one study (n=54) [37], while in other three studies (with a total of 436 subjects) it did not distinguished itself from placebo [27, 34, 36]. Trials included in this analysis were of high quality.

The global functioning was improved by minocycline more than by placebo in one trial (n=54) [37], but did not differ from placebo in another trial (n=144) [36]. The quality of these trials was high to moderate.

D-cycloserine had no significant impact over global clinical status or overall functionality, but data were derived from only 2 trials for each outcome, and they included low number of participants (39 and 26, respectively) and were of moderate to low quality [51, 52, 55, 56].

CONCLUSIONS

Based on reviewing the selected trials regarding the efficacy of antibiotics as add-on to antipsychotic treatment over core symptoms of schizophrenia, minocycline (n=10 trials, N=950 participants) was associated with mixed results regarding its efficacy, both positive [32-38] and negative [27-29, 31]. The large majority of these studies were of moderate and low quality, which further complicates the evaluation of their conclusions. Most of the data derived from these trials did not support a significant efficacy of minocycline over negative, cognitive, and overall psychotic symptoms, when it was added to various antipsychotic agents, clozapine included [27-29]. Other trials reported isolated efficacy of minocycline superior to placebo over anxiety/depression factor of BPRS, working memory, or significant improvement in negative and/or positive symptoms [31-38]. Non-significant superior efficacy of minocycline over placebo was also reported over positive and general psychopathology [35]. A definite conclusion about the efficacy of minocycline can not be formulated based on these data, and the GRADE recommendation could not be over the D+ level regarding the positive, negative, and overall psychotic symptoms, as well as D level for affective symptoms of schizophrenia (table 4).

D-cycloserine was investigated in moderate quality trials (n=18, N=626 participants) focused on its efficacy over symptoms of schizophrenia, when used as add-on to antipsychotics. No significant difference between d-cycloserine and placebo was observed in trials focused on negative and cognitive symptoms of schizophrenia [39-56]. A trend to the worsening of positive symptoms by d-cycloserine was also reported, as well as a trend to aggravation of negative symptoms, especially when added to clozapine [51, 52, 54]. D-cycloserine was also proven superior to placebo over negative symptoms [41, 43, 45, 48-50, 53, 56] and cognitive function [56], without difference between atypical and typical antipsychotics as main treatment [49]. Based on these contradictory data, d-cycloserine could be rated as D+ recommendation for the treatment of negative symptoms of schizophrenia, when added to typical or atypical antipsychotics, but not to clozapine. Thorough monitorisation of patients undergoing d-cycloserine augmentation should be initiated because there is a risk of worsening positive symptoms or negative symptoms (the last case has been reported in clozapine-treated patients).

After reviewing the trials focused on tolerability of antibiotics in patients diagnosed with schizophrenia as add-on to antipsychotics (n=9, N=825 participants), the data collected was considered as being of moderate quality (table 5). Almost all of them reported no difference in the rate of adverse events between minocycline and placebo-treated patients, and two studies showed a favorable effect of this antibiotic over metabolic status of the patients undergoing antipsychotic treatment [27, 28, 30-32, 35-38]. These data support a level B- for the tolerability of minocycline as add-on to antipsychotic regarding the metabolic profile, body weight and waist circumference, and level B for the overall adverse events (extrapyramidal symptoms included).

Tolerability of d-cycloserine was also evaluated as good in moderate to low quality trials (n=6 studies, N=99 participants), and the main adverse events were extrapyramidal symptoms [44, 48-51, 55]. These data support a level B- for the tolerability of d-cycloserine as add-on to antipsychotic regarding the overall adverse events (extrapyramidal symptoms included).

Minocycline had an effect over the clinical global impression that was not disguinshible from placebo [27, 34, 36], although there was one exception [37]. Data was derived from high quality studies (n=4, N=490 participants), and the recommendation for improving this outcome with minocycline as add-on is D- (table 6). The impact of minocycline over the global functioning when added to antipsychotic in patients with schizophrenia was mixed, with one positive and one negative trial (n=2, N=198 participants, moderate to high quality designed trials) [36, 37]. Based on these data a D level is formulated in this case.

D-cycloserine had no significant impact over global clinical status (n=2, N=39 participants) or overall functionality (n=2, N=26 participants) [51, 52, 55, 56]. These trials were of moderate to low quality and there was formulated a D-recommendation for each outcome based on the before-mentioned results (Tables 6 and 7).

From all the data reviewed and hierarchized it is evident that larger trials are needed in order to confirm the efficacy of minocycline and d-cycloserine over negative and cognitive symptoms of schizophrenia. The tolerability of these two antibiotics was good in almost all trials that were reviewed, which is encouraging in the perspective of designing new trials with these agents.

Limitations of this review are related to the heterogeneity of design in trials that were analysed, and the impossibility of contructing a meta-analysis. The use of different scales for monitoring the same cluster of symptoms made difficult the comparison of the results. In addition, most of the trials included in the current review were conduceted on small groups, which make the generalizability of data very difficult.

Disclaimer

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REVIEW ARTICLE

Nonoperative management of high-grade splenic injury. A review of the literature and case report

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Abstract: *Splenic injuries causedby traumatic events are common in the emergency departments, mostly because the spleen is frequently affected in trauma. The lesions of the spleen can be diagnosed using a contrast enhanced computed tomography and based on the results, the injury of the spleen is graded. The management of the patient can be operative or nonoperative. The choice of treatment is decided by the hemodynamic stability of the patient.*

INTRODUCTION

Blunt abdominal trauma mostly due to sports activities is common in the emergency departments in Europe and 30% of the polytrauma patients have abdominal organs injuries [1]. The most frequently injured organ in blunt abdominal trauma is the spleen, which is involved in about 32% of abdominal injuries [2, 3].

The splenic injuries could be treated surgically or by a nonoperative management. In cases of hemodynamically stable patients, nonoperative management of the blunt splenic injuries is preferred and the recognition of the overwhelming post splenectomy infections also sustains the nonoperative management [2, 4].

The splenic injury in trauma is diagnosed based on computed tomography (CT) and it can also show other injuries associated with trauma [5]. The sensitivity of the CT examination in detecting splenic injuries has been reported to be as high as 95% [6].

On trauma patients should be performed FAST to find the evidence of intraabdominal hemorrhage. The patients with positive FAST and unstable hemodynamically, with or without cardiovascular associated disorders [7-9], are rushed to the operating room and the stable ones are planned for contrast-enhanced computed tomography [10].

Based on the CT results, various grading systems were introduced to help physicians to take the best decision regarding the management of the patient [6].

The best known and used splenic and hepatic grading system is the one established by the American Associationfor the Surgery of Trauma (AAST) [11] (Figure 1).

Vascular injuries in splenic trauma with active bleeding could be detected on a contrast-enhanced helical CT scan as perisplenic material extravasation [6, 12].

The selection of the treatment, operative or nonoperative, is difficult, but there are some factors such as hemodynamic status, age, grade of splenic injury, quantity of the

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hemoperitoneum and associated injuries useful to decide the optimal standard of care for the trauma patient [11].

The rate of success in the nonoperative management of splenic injuries is very high in many published series, between 80-94% in the cases of adult trauma patients[2, 11].

There is some evidence that older patients might have a worse prognosis with nonoperative management than younger patients, and several series have reported thatpatients older than 55 years are less likely to have a successful nonoperativemanagement [11, 13, 14].

Other option of treatment in splenic lesions is the splenic artery embolization, which is classified as nonoperative treatment. Splenic artery embolization is used to reduce the incidence of reccurent bleeding, factor that would lead to hemodynamic instability [15-17]. Studies confirm a high rates of splenic salvage after the combined treatments such as splenic artery embolization and nonoperative management [3].

Table 1: The splenic organ in Jury Scaling System of the American Association for the Surgery of Trauma, 1994 revision

Grade*	Injury description	
I	Hematoma	Subcapsular, <10% surface area
	Laceration	Capsular tear, <1 cm parenchymal depth
II	Hematoma	Subcapsular, 10–50% surface area, <5 cm in diameter
	Laceration	1–3 cm parenchymal depth that does not involve a trabecular vessel
III	Hematoma	Subcapsular, >50% surface area or expanding; ruptured subcapsular or parenchymal hematoma; intraparenchymal hematoma >5 cm or expanding
	Laceration	>3 cm parenchymal depth or involving trabecular vessels
IV	Laceration	Laceration involving segmental or hilar vessels producing major devascularization (>25% of spleen)
	Laceration	Completely shattered spleen
V	Vascular	Hilar vascular injury that devascularizes spleen

**Advance one grade for multiple injuries up to grade III*

The operative treatment is preferred in all cases of instable hemodynamically patients with/without dyselectrolytemia [18, 19]. There are two surgical options that the operator can take after examining the injury: splenectomy or spleen preserving surgery [20, 21].

There are some types of spleen preserving surgery including spleen packing, partial splenectomy or splenorrhaphy [11].

Spleen packing is used in selected cases with isolated splenic injuries and stable patients.

If the patient is unstable with a grade IV or V spleen injury splenectomy should be performed. Another surgical technique that can be performed is the partial splenectomy and this is possible because of the segmental nature of the splenic blood supply [20].

One of the most dangerous complications that could appear after a splenectomy performed in trauma is OPSI (overwhelming postsplenectomy infection). OPSI is an emergent condition which at first may develop as a mild infection and then rapidly progress to sepsis [22, 23] that has a 50-70% risk of mortality with or without treatment [24-28].

Vaccination against S. pneumoniae, N. meningitidis, and HIB are recommended in addition to a yearly influenza vaccine to avoid OPSI, but the administration of these vaccines is recommended after the postoperative day 14 to increase the immune response in patients who have undergone splenectomy due to trauma [29-31].

MATERIAL AND METHOD

We highlight the key aspects of the nonoperative management of blunt trauma splenic injuries by doing a review of the literature.

We researched the PubMed and Cochrane datebases for studies involving nonoperative management of the splenic traumatic injuries. The studies we analyzed were published between 2017 and March 2019.

CASE REPORT

We present the case of a 25-year-old woman, who came into the emergency room after a motocycle accident. The clinical examination showed tenderness in the left upper quadrant and the right arm was painful, the patient was stable hemodynamically. An X-ray of the right arm showed fracture of the radius and cubitus that were immobilized in a cast [32].

Point of care abdominal ultrasound was performed and it suggested a splenic lesion – non-homogeneous echostructure and hypoechogenic areas (Figure 1), and free fluid in Morrison and Douglas pouches (Figure 2).

Figure 1: Ultrasound finding of splenic lesion

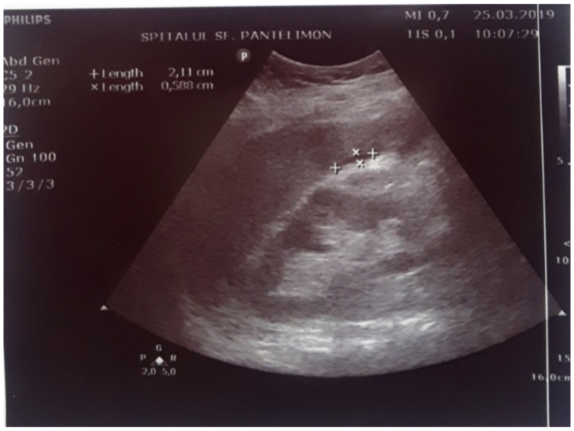
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×Length 0,588 cm

Figure 2: Hemoperitoneum: hypoechoic, nonhomogeneous paravesical image

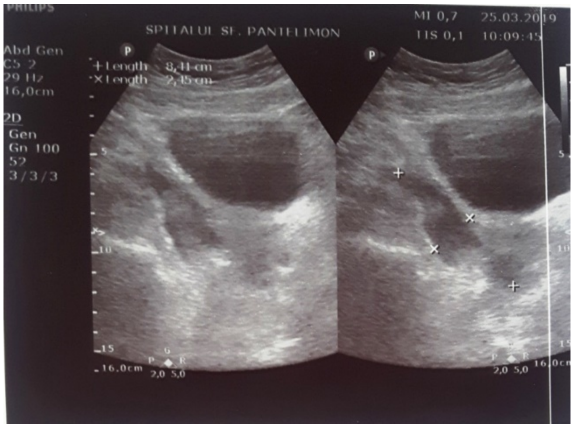
PHILIPS
SPITALUL SF. PANTELIMON
MI 0,7
TIS 0,1
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+Length 8,41 cm
×Length 2,45 cm

Figure 3: Native CT image showing grade III splenic lesion (hypodense zone – densities between 34 and 41 Hounsfield Units – from hilum to capsule)

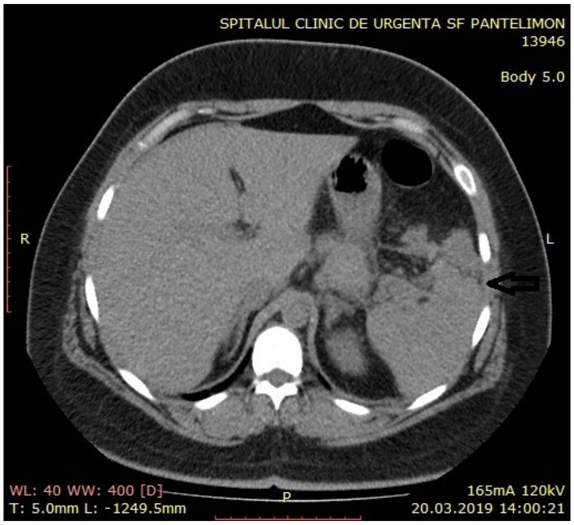
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13946
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Figure 4: Ultrasound finding of splenic lesion

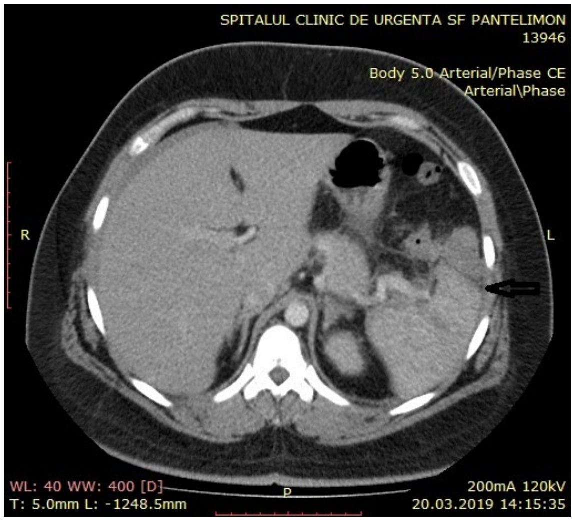
SPITALUL CLINIC DE URGENTA SF PANTELIMON
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Body 5.0 Arterial/Phase CE
Arterial\Phase
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WL: 40 WW: 400 [D]
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Figure 5: Contrast-enhanced CT scan - spleen hilum involvement

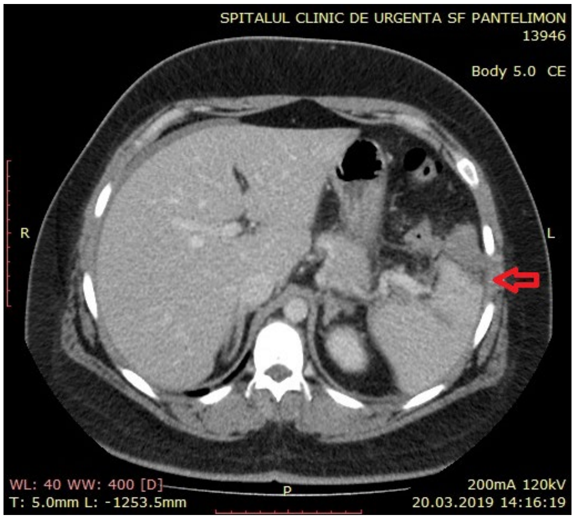
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WL: 40 WW: 400 [D]
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Figure 6: Blood accumulation in Douglas' pouch

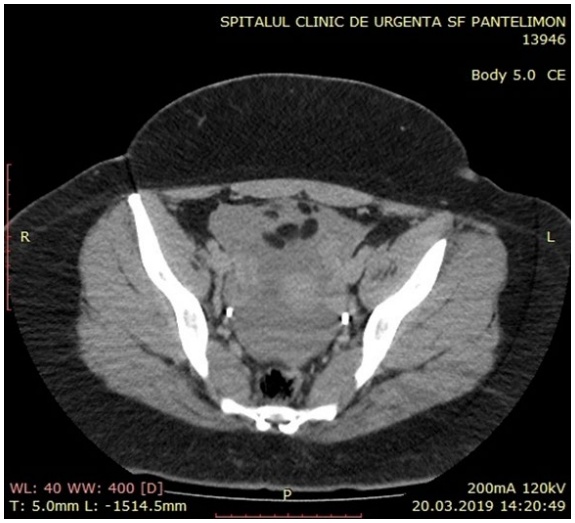
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The blood tests revealed anemia (hemoglobin 9.30 g/dL) and leukocytosis (14.510/uL).

Contrast-enhanced CT scan of the abdomen and pelvis revealed a grade III splenic injury (5.6 cm hematoma and 2 cm laceration) (Figures 3, 5), fluid surrounding the liver, in the Morrison pouch, in the right and left paracolic gutters (Figure 6). No contrast extravasation at level of the spleen was detected, no other traumatic injuries (Figure 4).

Hypodense zones of splenic tissue should be differentiated from cystic lesions of spleen, even hydatid cyst that, in case of rupture, could mimic acute abdomen [33].

We decided to treat the patient conservatory, to monitor her clinically every 6 hours, the blood pressure and pulse were measured also every 6 hours and she was monitored ultrasonographic daily. There were no modifications during the admission into the hospital and the patient was discharged in the fifth day. She was informed about the signs of hemorrhage (abdominal pain, light-headedness, syncope, difficulty breathing and chest pain) and she was instructed to call immediately a surgical emergency center if any of these symptoms appear.

One week after the discharge the patient came to follow up, she was examined clinically, ultrasonographically and biologically and everything was normal.

At this moment, there is no information about the nonoperative management of the splenic trauma regarding how long should the patient be admitted, how long should it be between the discharge and the follow up, how frequently should the level of hemoglobin and hematocrit be measured and how frequent should the patient be examined ultrasonographically [34, 35].

CONCLUSION

Splenic injuries are common and the patients stable hemodynamically who have grade III splenic lesions due to trauma could be managed nonoperatively. There are no protocols about the nonoperative management of the splenic injuries and we think their existance could influence the morbidity and mortality of these patients. Prospective trials could be performed to establish a protocol of treatment and follow up.

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REVIEW ARTICLE

Atherosclerosis in rheumatoid arthritis – the importance of imaging tests

Oana P. Ionescu[1], Silviu M. Stanciu[1], Mihai L. Ciobîcă[1]

Abstract: *As reported by literature, rheumatoid arthritis is a rheumatic disease with an increased risk of developing cardiovascular complications. Early detection of atherosclerosis by non-invasive techniques represent a current challenge of patient management. Current EULAR guidelines recommend careful monitoring of cardiovascular risk factors by stratification of patients and strict control of rheumatic disease activity to reduce it. The classic evaluation of intima-medium thickness (IMT) was completed by quantifying the degree of inflammation of the carotid plate using positron emission tomography or the degree of coronary calcifications by calculating Agatston score using Coronary Computer Tomography. These modern investigation techniques allow identification of several subclinical stages of atherosclerosis, the interest in their early detection resulting from the fact that, in this stage, through specific measures, the cardiovascular complications can be prevented.*

Keywords: *rheumatoid arthritis, atherosclerosis, cardiovascular risk, imaging tests*

INTRODUCTION

Rheumatoid arthritis is a rheumatic disease with an increased risk of cardiovascular events due to advanced atherosclerosis [1]. The development of atheromatous plaque is based on chronic inflammation found in both rheumatoid arthritis and atherosclerosis[2]. Recent studies have shown a higher prevalence of plaque in patients with rheumatoid arthritis, which explains their negative predictive role[3,4]. The higher frequency of traditional risk factors in patients with rheumatoid arthritis (hypertension, type II diabetes, smoking, high cholesterol and obesity) is another factor contributing to the development of cardiovascular complications. Its impact is different from that of the population not suffering of this pathology [5,6]. Current EULAR guidelines recommend careful monitoring of cardiovascular risk factors by stratification of patients and strict control of rheumatic disease activity to reduce it [6]. The early detection of atherosclerotic changes by non-invasive techniques is a current concern of patient management. Collaboration between rheumatologist and cardiologist and prospects of a new specialty - cardio-oncology will be encouraged in future in order to improve the prognosis of patients affected by rheumatoid arthritis and atherosclerosis.

NONINVASIVE IMAGING OF INTRACRANIAL VESSELS

Atherosclerotic plaque is the trigger for cardiovascular events, its vulnerability being due to plaque composition, inflammatory cell content, age, and not necessarily plaque size [7, 8]. In rheumatoid arthritis, advanced inflammation causes a particular phenotype of the plaque, thus presenting an increased risk of myocardial infarction or stroke [9, 10]. Transcranial Doppler examination is a method of measuring cerebral blood flow in the main intracranial arteries. This method uses 2 MHz ultrasound probes to dynamically detect intracranial arteries through thinner areas of the skull called

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acoustic windows [11, 12]. This study has proven inexpensive, reproducible and provides information on the prognosis of cardiovascular events [13, 14, 15, 16]. Measurement of average mean cerebral arterial flow rate, pulsatility index, and calculation of the relationship between these two indices revealed prognostic factors of recurrent cardiovascular events [17, 18] In patients with rheumatoid arthritis, there was an increase in the prevalence of distal occlusions of the middle cerebral artery and basilar artery detected by transcranial Doppler [11].

Another investigation commonly used in the clinic is the Doppler ultrasound of the carotid arteries. This is a good way to identify patients with rheumatoid arthritis at high cardiovascular risk who can benefit from active treatment.

Proper stratification of cardiovascular risk is a key element in the management of patients with rheumatoid arthritis. It is well known the EULAR's adaptation of cardiovascular risk assessment based on coronary risk assessment (SCORE) multiplied by 1.5 in patients with rheumatoid arthritis that meet two of the following three criteria: disease> 10 years, rheumatoid risk factor (RF) or anti-cyclic cytoplankin peptide (anti-CCP) antibody and presence of serious extra-articular manifestations [19]. However, the authors recommend performing a carotid ultrasound scan when SCORE (Figure 1)[20] does not report high cardiovascular risk, considering studies in which patients with rheumatoid arthritis were not considered at high risk for cardiovascular events by calculating SCORE risk, but who had significant cardiovascular events: myocardial infarction and stroke [21].

Figure 1: Cardiovascular risk estimation using SCORE

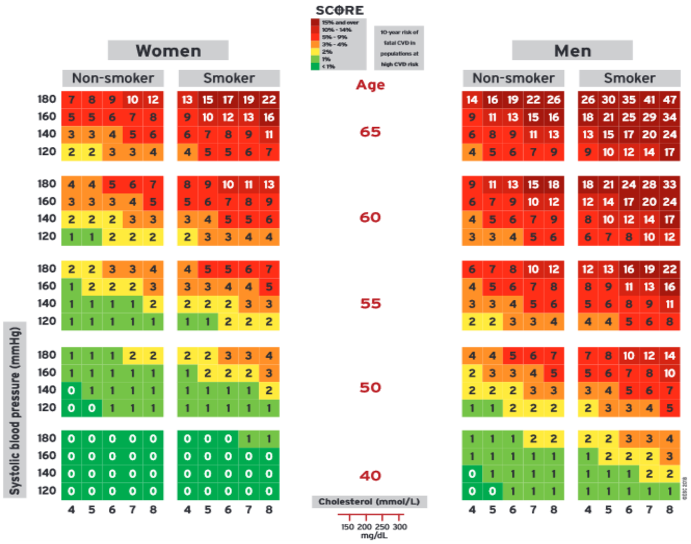
SCORE
15% and over
10% - 14%
5% - 9%
3% - 4%
2%
1%
< 1%
10-year risk of fatal CVD in populations at high CVD risk
Systolic blood pressure (mmHg)
Cholesterol (mmol/L)
150 200 250 300
mg/dL
© 2018 ESC

Age	Systolic blood pressure (mmHg)	Women Non-smoker 4	5	6	7	8	Women Smoker 4	5	6	7	8	Men Non-smoker 4	5	6	7	8	Men Smoker 4	5	6	7	8
65	180	7	8	9	10	12	13	15	17	19	22	14	16	19	22	26	26	30	35	41	47
	160	5	5	6	7	8	9	10	12	13	16	9	11	13	15	16	18	21	25	29	34
	140	3	3	4	5	6	6	7	8	9	11	6	8	9	11	13	13	15	17	20	24
	120	2	2	3	3	4	4	5	5	6	7	4	5	6	7	9	9	10	12	14	17
60	180	4	4	5	6	7	8	9	10	11	13	9	11	13	15	18	18	21	24	28	33
	160	3	3	3	4	5	5	6	7	8	9	6	7	9	10	12	12	14	17	20	24
	140	2	2	2	3	3	3	4	5	5	6	4	5	6	7	9	8	10	12	14	17
	120	1	1	2	2	2	2	3	3	4	4	3	3	4	5	6	6	7	8	10	12
55	180	2	2	3	3	4	4	5	5	6	7	6	7	8	10	12	12	13	16	19	22
	160	1	2	2	2	3	3	3	4	4	5	4	5	6	7	8	8	9	11	13	16
	140	1	1	1	1	2	2	2	2	3	3	3	3	4	5	6	5	6	8	9	11
	120	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4	4	5	6	8
50	180	1	1	1	2	2	2	2	3	3	4	4	4	5	6	7	7	8	10	12	14
	160	1	1	1	1	1	1	2	2	2	3	2	3	3	4	5	5	6	7	8	10
	140	0	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	4	5	6	7
	120	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	4	5
40	180	0	0	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2	3	3	4
	160	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2	2	2	3
	140	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	2	2
	120	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1

(Source: European Guidelines on CVD Prevention in Clinical Practice 2016 Eur J Prev Cardiol. 2016 Jul;23(11):NP1-NP96. doi: 10.1177/2047487316653709)

Carotid Doppler ultrasound evaluates intima-medium thickness (IMT) and highlights atherosclerotic plaques, making it an affordable, easy-to-use, reproducible, low cost study. A meta-analysis of 1384 patients with rheumatoid arthritis concluded that a carotid IMT increased from the control group population – 1147 patients (normal IMT <0.9 mm) [22]. Olah C. et al [11] also published in 2017 a study on the assessment of intracranial vessels in combination with carotid atherosclerosis and stroke in patients with rheumatoid arthritis in which more patients with rheumatic disease had at least a plaque in the right carotid artery, and its score was higher than that of the control group patients. Thus, we can say that carotid ultrasound remains an easy-to-use tool in practice, but different protocols for measuring the carotid plaque and operator-dependent, makes this investigation more and more replaced by carotid magnetic resonance.

Carotid magnetic resonance has the advantage of capturing the entire circumference of the carotid wall, including the adventitia and the outer layer, as opposed to carotid ultrasound, which only visualizes the intima of the carotid artery. In the multiethnic study of atherosclerosis, conducted in six US research centers, 698 patients were included and examined by carotid ultrasound and MRI. The results of the study showed that MRI could be a better predictor of cardiovascular events than carotid ultrasound, as the combination of wall thickness measured by magnetic resonance was stronger than that of ultrasound-measured IMT [23]. In addition, the arterial history revealed by MRI seems to be a better predictor of cardiovascular complications in a study that showed an increase in inflammatory cytokine expression in aortic adventitia in patients with rheumatoid arthritis who suffered from coronary artery bypasses compared to control patients [24, 25]. Another advantage of this method of non-invasive diagnosis is the strong correlation with the histological phenotype of atherosclerotic plaque, the dynamic contrast-enhanced MRI (DCE-MRI) allowing to evaluate the neovascularization of the atherosclerotic plaque, its vulnerability by the degree of inflammation and the degree of hemorrhage in plaque, and the effectiveness of therapeutic interventions [26, 27].

Another more modern technique capable of quantifying the degree of inflammation of the carotid plate is positron emission tomography. This exploration uses 18-F-fludeoxy-glucose, a glucose analogue, which has the ability to accumulate in inflamed atherosclerotic plaque, where exists a greater amount of marked pozitrons [28]. Thus, CT or magnetic resonance imaging is possible to map the affected area, the absorption of fludeoxoxiglucose being associated with the identification of lesions at high risk of embolization [29]. Another radioactive agent used in the detection of atherosclerosis is sodium fluoride (18-F-NaF), which is capable of binding to calcified unstable arterial zones. During the course of atherosclerotic disease, two types of vascular damage can occur: macrocalcification – conferring stability to plaque atheroma and microcalcification – associated with an increased risk of plaque vulnerability. Sodium fluoride PET-CT differentiates the two types of lesions presented above, the new technique being able to revolutionize the treatment of atherosclerosis, increasing the chances of preventing cardiovascular complications [30].

NONINVASIVE CARDIAC IMAGING

Advanced atherosclerosis in rheumatoid arthritis is also evident in the heart, manifesting itself in various forms: coronary ischemia - micro and macrovascular, inflammation or myocardial fibrosis (myocarditis) [31]. Rheumatoid arthritis should therefore be closely monitored using non-invasive techniques to identify patients at high risk of developing cardiovascular complications. A recent study performed by a Danish team, which included the entire population over 18 years old diagnosed with rheumatoid arthritis between 1978 and 2008, found an increased risk of heart failure in patients with rheumatoid arthritis, justifying the need for cardiovascular screening [32].

Coronary arteries can be assessed non-invasively by transthoracic or transesophageal ultrasound and magnetic resonance imaging computed tomography or computed tomography after endothelial-dependent vasodilation [33].

Transthoracic echocardiography is a classic method of evaluating cardiac function that, by its characteristics, can detect patients with a high cardiovascular risk, such as those with rheumatoid arthritis. Recent studies have demonstrated subclinical impairment of right and left ventricular function in patients with rheumatoid arthritis versus control, as demonstrated by the comparison of early ventricular (E-wave) filling, late ventricular (A-wave) filling, and the E/A ratio of the two lots [34]. A modern clinical application of transthoracic echocardiography is transthoracic stress echography with dipiridamol, able to evaluate the left coronary reserve. This represents the ratio of maximal coronary flow during stress to basal rate, which is a very sensitive diagnostic marker (> 90%) for coronary heart disease [35, 36]. In the literature, it has been reported that a value of coronary reserve flow <2 is associated with the presence of coronary stenosis [37]. In the absence of evidence of epicardial coronary stenosis, an abnormal coronary flow reserve may indicate a pathology of coronary microcirculation in patients with reperfused myocardial infarction, hypertension, diabetes mellitus, hyper-cholesterolemia, X syndrome or hypertrophic cardiomyopathy. Recent studies have also demonstrated a negative prognostic value of coronary reserve flow in patients with or suspected of coronary artery disease [38].

Transesophageal cardiac ultrasound is an investigation that can successfully complete transthoracic cardiac ultrasound, which has the disadvantage of visualizing the posterior structures of the heart, or which has the disadvantage of a difficult window of exploration in obese patients or affected by lung disease. Transesophageal cardiac ultrasound was used in a case-control study conducted on a group of 30 patients diagnosed with rheumatoid arthritis, which concluded that there was a high frequency of regurgitation of the mitral and aortic valves and obvious signs of cardiomyopathy in most patients [39].

Computer tomography is another method of assessing atherosclerosis present in the patient diagnosed with rheumatoid arthritis by completing the classical techniques mentioned above. A recent study aimed at examining the impact of rheumatoid arthritis on the prevalence and severity of coronary artery disease, concluded that the presence of coronary calcifications is common in patients with serum-positive rheumatoid arthritis [40]. This result was based on measurements of calcium score, an indicator of the degree of coronary artery calcification, by means of computer tomography. Figure 2 presents intense calcifications in the coronary artery walls, more evident on the LAD 2 (60-70%). The examination is non-invasive, without the use of a contrast substance, guided by the ECG; it does not require a long examination time and shows minimal irradiation.

Figure 2: Intense calcifications present in the coronary artery walls, more evident on the LAD 2 (60-70%). Normal detachment of coronary arteries.

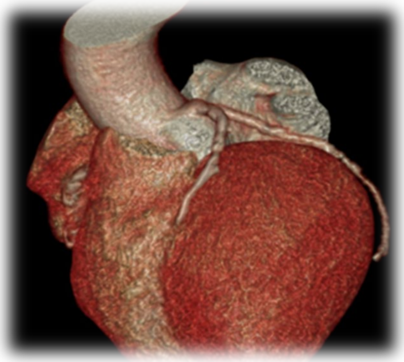

The patient is lying down during the exam, and the calcium score is measured in deep inspiration. Coronary CT scan provides information on cardiovascular risk by calculating Agatston score: a small risk is equivalent to a score below 100 Agatston units, an intermediate risk of 100 to 300 Agatston units, and an increased risk of more than 300 Agatston units (Figure 3). The presence of an elevated risk score (over 300 Agatson units) requires a myocardial scintigraphy and the presence of a score of over 600 Agatson Units to perform invasive coronarography.

Still in the field of noninvasive imaging techniques, cardiac nuclear magnetic resonance is increasingly used, which is the gold standard for the evaluation of myocardial viability.

This modern technique was used in a study comparing the structure and function of myocardial patients with rheumatoid arthritis compared to a control group included in the multi-ethnic Baltimore study on atherosclerosis, unaffected by rheumatic pathology.

Figure 3: Cardiac CT: Calcium Scoring 529.74 Agaston units (LAD 329 UA, LCX 70.62 UA, RCA 129.86 UA)

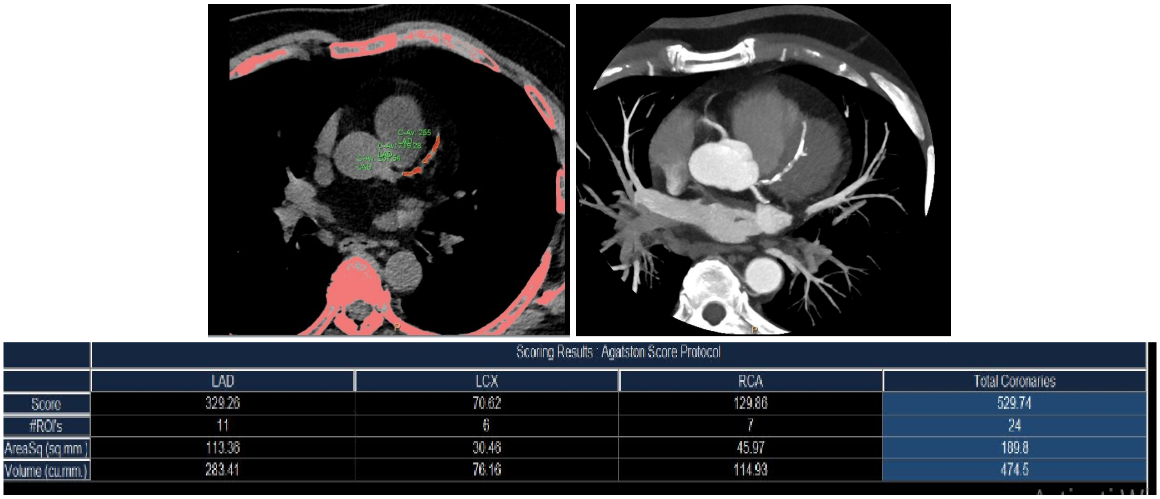

	Scoring Results : Agatston Score Protocol			
	LAD	LCX	RCA	Total Coronaries
Score	329.26	70.62	129.86	529.74
#ROI's	11	6	7	24
AreaSq (sq.mm.)	113.38	30.46	45.97	189.8
Volume (cu.mm.)	283.41	76.16	114.93	474.5

The findings showed that there was no statistically significant difference between the two volumes in the left ventricular and telediastolic volumes, but that the left ventricular ejection fraction and the cardiac output were lower in patients with rheumatic arthritis, indicating a progression of heart failure [41]. Cardiac Magnetic Resonance has also been applied to a group of rheumatoid arthritis patients with no history of cardiovascular disease (pulmonary hypertension, coronary artery disease, valvulopathy, atrial fibrillation or diabetes), and the results were detection of subendocardial infusion defects in correlation with disease activity, respectively with markers of inflammation – PCR and VSH [42]. These results confirm the major involvement of inflammation in worsening cardiovascular status in patients with rheumatoid arthritis.

NON-INVASIVE IMAGING OF PERIPHERAL CIRCULATION

Peripheral circulation can be investigated non-invasively by a variety of methods from the classical ankle-arm index, or measuring post-occlusive reactive hyperemia with a plethysmograph up to brachial artery mediated vasodilatation, measured ultrasonographically or by magnetic resonance. These tests are useful in assessing endothelial function and may have considerable potential in the future in assessing rheumatic patients.

CONCLUSIONS

In conclusion, we can state that the modern investigation techniques allow identification of several subclinical stages of atherosclerosis, the interest in their early detection resulting from the observation that in this stage, through specific measures, the cardiovascular complications can be prevented.

Systemic chronic inflammation in atherosclerosis and rheumatic diseases brings to the forefront the need for a multibiomarker approach in cardiovascular risk assessment. Biological markers correlated with imaging tests may be important prognostic elements capable of delaying the progression of atherosclerosis, providing the opportunity for early cardiovascular treatment.

Until now, atherosclerosis imaging has been more centered on the anatomical aspect, with current trends focusing on a more functional approach using multi-modal images. The ultimate goal would be to fuse between anatomical and functional data and to achieve a more faithful image of the atherosclerotic plaque. In the future, an exploration of molecular and cellular mechanisms of cardiovascular disease is expected through molecular imaging that is able to overcome various aspects of inflammation involved in atherosclerosis. Slowing or stopping the progression of the inflammation process will be the future target of patient management by improving their prognosis and decrease the rate of their cardiovascular complications.

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ORIGINAL ARTICLES

Managing radial nerve injuries associated with humeral fracture

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Abstract: *: The overall prevalence of radial nerve injury after humeral shaft fractures is up to 18% representing the most common peripheral nerve injury associated with long bone fractures.Spiral or oblique fractures of the junction between the middle and distal third of the humeral shaft are at greater risk for radial nerve injury. Iatrogenic damage to the radial nerve may also occur during manipulations of closed reduction.*

Material and methods: A prospective study between 2017-2019 following 5 patients (all male, with ages between 30 and 56 years old), who were treated at our clinic for complete radial nerve palsy after a high-energy fracture of the humerus

Results: All the patients were followed-up for a mean of 6 months (range 4 - 15 months) post op. The first 3 cases showed the initial signs of recovery 4 weeks after the operation. In the last 2 cases, a palliative radial intervention was required.

Conclusions: The consequences of the radial nerve palsy associated with humeral fractures is strongly related to trauma mechanism. In high-energy fractures, severe contusion or transection of the nerve must be expected. In this case, nerve recovery is unlikely and the patients should be informed of the poor prognosis and the need of tendon transfers.

Keywords: *humeral fracture, compression, upper limb, radial nerve, callus*

INTRODUCTION

Fractures of the humerus are often complicated with radial nerve palsy. The overall prevalence of radial nerve injury after humeral shaft fractures is up to 18% representing the most common peripheral nerve injury associated with long bone fractures.[1-5] This is caused by the relatively fixed position of the radial nerve and the direct contact with the periosteum of the humerus as the nerve courses through the middle and distal thirds of the upper arm and by the nerve's diminished mobility where it pierces the lateral intermuscular septum. As such, at this level the bone ends can easily entrap, contuse, or even lacerate the radial nerve, varying from neurapraxias to complete neurotmesis.

Spiral or oblique fractures of the junction between the middle and distal third of the *humeral shaft are at greater risk for radial nerve injury.[6] Iatrogenic damage to the radial nerve may also occur during manipulations of closed reduction or at the time of surgical intervention, during internal fixation with a intramedullary nailing or compression plate.[7, 8, 9]

Treatment is often expectant (non-operative), though surgery is also sometimes necessary by a variety of often imaginative procedures. Because radial nerve injuries are the least debilitating of the upper limb nerve injuries, results are usually satisfactory.[10]

PATIENTS AND METHODS

A prospective study between 2017-2019 following five patients (all male, with ages between 30 and 56 years old), who were treated at our institution for complete radial nerve palsy after a high-energy fracture of the humerus. In all cases, the fracture was stabilized and no other complications except the radial palsy. Baseline electrophysiological studies (i.e. nerve conduction velocity studies and electromyography) conducted before admittance showed a complete motor paralysis of the nerve and all had an absent Tinel's sign at each clinical examination.

Operative Procedure

Case 1: A 41-year-old male, with a 4 months old high-energy transverse diaphyseal fracture of the left humerus and faulty soft tissues following a car accident was treated with plating.

The same anterolateral approach used for plating was used during our surgical exploration where we found contusion of the radial nerve and perineurial adhesions of a small segment at the level of the humeral fracture (Figure 1).

Figure 1: Perineural adhesion of the radial nerve to the humerus

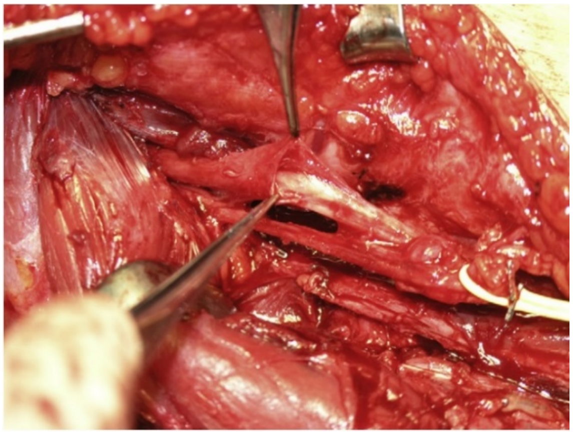

External neurolysis was practiced (Figure 2).

Figure 2: Neurolysis of the radial nerve

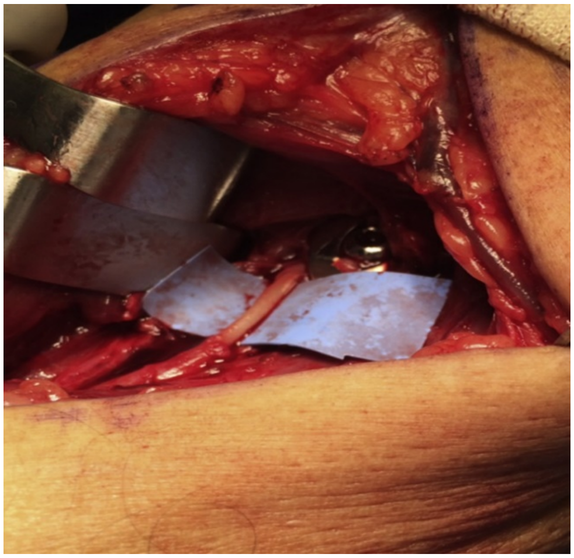

Case 2: A 38-year-old male, with 3 months old closed fracture of the right humeral shaft following a recreational arm wrestling match was treated with two non-staggered plates.

Upon surgical exploration via dorsal approach, a partially lacerated nerve draped over a loose compression plate.

Treatment associated the refixation of the plate and embedment of the nerve in the triceps muscle.

Case 3: A 40-year-old male, with a 3-month-old fracture of the humeral shaft following a car crush was treated with external fixator.

During surgical exploration, the distal pin of the external fixator was found to run through the brachioradial muscle into the body of the radial nerve destroying 15% of its circumference (Figures 3 and 4).

Figure 3: Distal pin winded up in the radial nerve

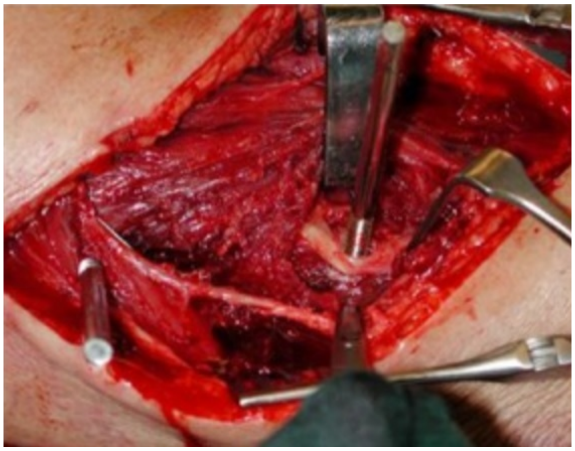

Figure 4: Radial nerve buried in the fracture callus

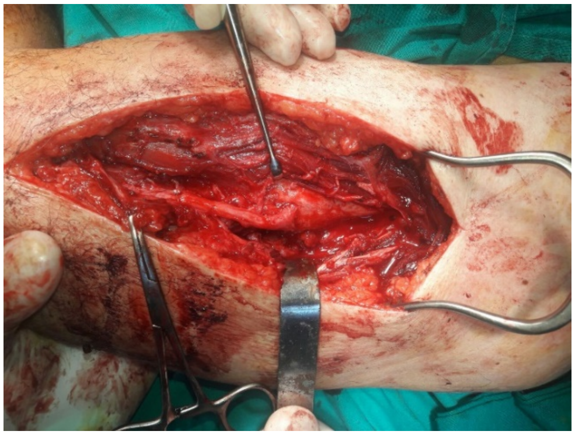

The pin was removed and reattached after releasing the nerve and embedding it in the triceps muscle.

Case 4: A 56-year-old male with a six-month-old fracture of the left humeral shaft was treated with internal fixation after crush in the conveyor belt.

Surgical exploration had a posterior approach through a lateral para-tricipital window revealing the nerve enveloped in the fracture callus more than 8 cm with the impossibility of finding the proximal end (Figure 5).

Figure 5: Presenting the donor tendons. From left to right: palmaris longus, flexor superficialis middle finger and flexor ulnaris carpi

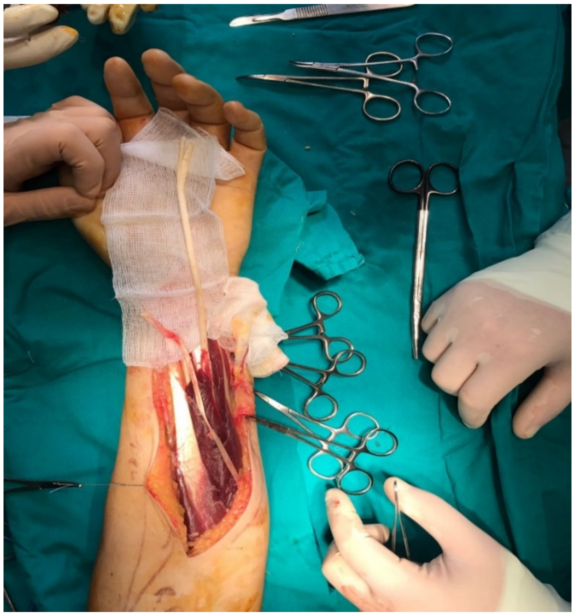

A follow-up tendon transfer procedure was carried out flexor ulnaris carpi to extensor ulnaris carpi, flexor superficialis of middle finger to extensor digitorum communis, palmaris longus to extensor pollici longus (Figures 6 and 7).

Figure 6: Tenoplasty of the extensor digitorum communis and extensor pollici longus by Pulvertaft technique

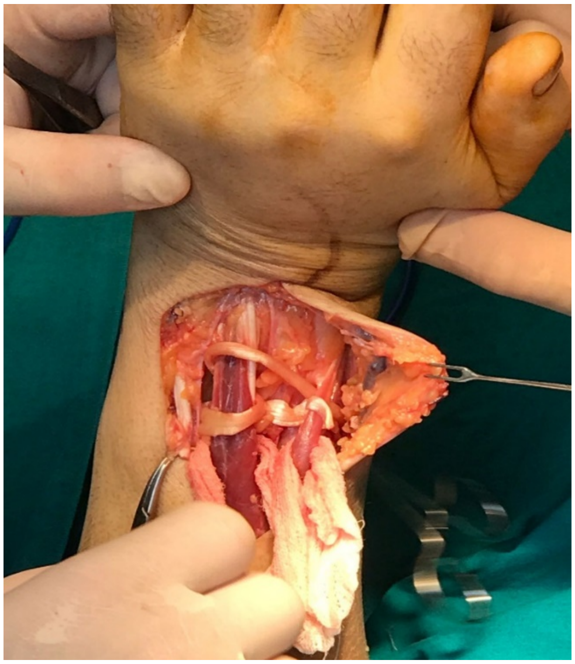

Figure 7: Active extension of the wrist, thumb and fingers 6 weeks post op

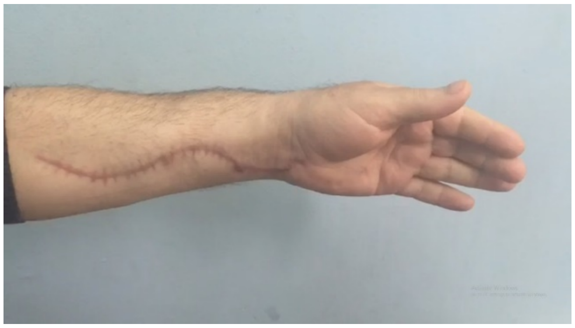

Case 5: A 30-year-old male, with a 12-month-old chainsaw trauma, which required open reduction and fixation of the humerus, anastomoses of the brachial artery with venous grafting and neurorrhaphy of median nerve and ulnar nerve, with the impossibility of finding the radial nerve ends, presented for second intervention in our clinic.

A palliative radial intervention practicing tendon transfer was used.

We transferred the pronator teres tendon to extensor carpi radialis tendon, flexor carpi radialis tendon to extensor digitorum communis and plamaris longus to extensor pollicis longus (Figures 8 and 9).

Figure 8: Presenting the donor tendons. From left to right: pronator teres, flexor radialis carpi and palmaris longus

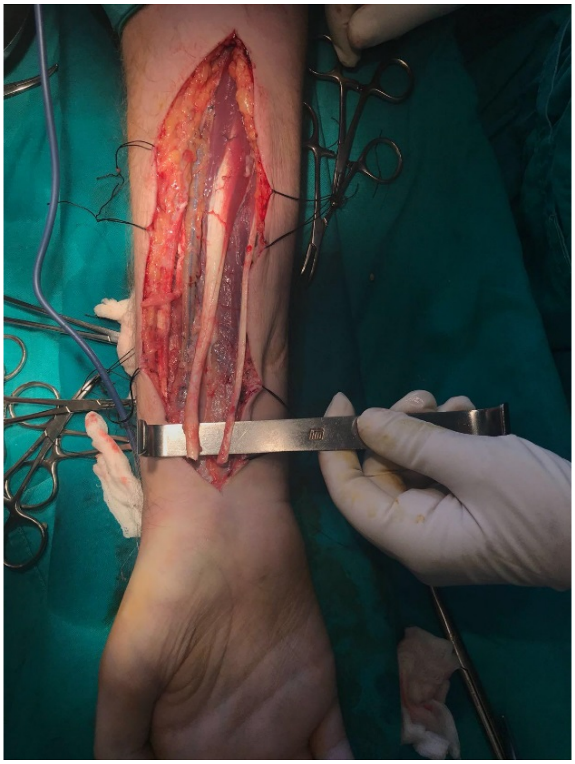

Figure 9: Tenoplasty of the extensor carpi radialis (over the scissors tip), extensor digitorum communis (forceps) by Pulvertaft technique

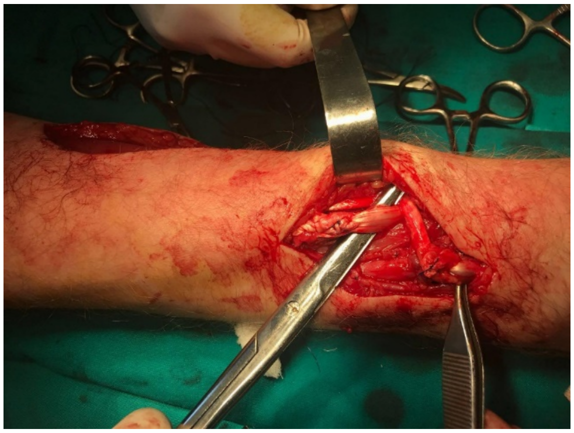

Postoperative follow-up examination included radiographic evaluation of the fracture, physical examination (i.e. Tinel's sign), and monthly electrophysiological studies for motor and sensory nerve recovery.

RESULTS

All the patients were followed-up for a mean of 6 months (range 4-15 months) post op. The first three cases showed the initial signs of recovery 4 weeks after the operation. Full nerve recovery was achieved in a mean of 19 weeks (range 16-22 weeks) with a slight sensory discomfort in the third case. An advancing Tinel's sign was a good predictor for nerve recovery.

In the last two cases where palliative radial intervention practicing tendon transfer was used, the imobilization was removed after approximately 4 weeks and a volar custom splint made by a hand therapist was applied with extension slings for the MP joint of the fingers. Active range-of-motion exercises of the fingers were begun at 3 to 4 weeks, and the wrist at 6 weeks.

Splinting as protection yielded excellent results and was done for 8 to 12 weeks postoperatively.

DISCUSSIONS

These cases highlights the necessity of a postreduction examination of the fracture. Nerve function should always be thoroughly assessed before and after reduction, with complete documentation in the medical record. To ensure accuracy, any patient who requires conscious sedation for reduction must return to full consciousness and alertness before obtaining the postreduction exam.

Radial nerve injury associated with a humeral shaft fracture is a very important injury pattern among trauma patients with an incidence up to 18%. [3] These injuries can be either primary or secondary depending on the timing of the palsy presentation. Early surgical exploration of the nerve is generally indicated for those cases, in which there was an open fracture, fractures that cannot achieve an adequate closed reduction requiring open reduction, fractures associating a vascular injury or a severe soft tissue injury [11].

Patients who demonstrate a primary radial nerve palsy have a reported spontaneous recovery rate of over 70% [12]. In the management of nerve palsy, nerve conduction examination with electrophysiological studies could be obtained at three months post injury, as motor potential should be recovered by this point, and these nerve conduction data will enable the clinician to determine if the injury should require surgical intervention. Early exploration revealing a nerve injury that can be repaired can lead to a quicker return to function and better nerve recovery. Degeneration of the motor endplate and irreversible muscle atrophy occurs if sufficient reinnervation is not present within 12–18 months after injury. [13] Poor functional recovery has been demonstrated in experimental studies when nerve repair is delayed for 6 months due to decreased motor neuron regeneration capacity. [14, 15]

Tendon transfer remains an essential technique in irreparable or long-standing radial nerve palsies for hand function restoration. Because the first signs of nerve recovery can be quite delayed, patience is merited before considering tendon transfers. A waiting period of 12 months is usually sufficient to see any evidence of nerve regeneration. Following a radial nerve injury, the extrinsic extensor function of the hand and wrist is lost, resulting in an inability to extend the fingers and wrist. It affects grip strength and has a large influence on thumb usage. Tendon transfer surgery must restore wrist, thumb and finger function without inflicting other motor deficits on the hand. [15] It is important that the joints have achieved good passive mobility in order to achieve optimal function post-tendon transposition. The donor muscles are tested for strength before surgery, and must come from a region unaffected by the pathology. [16]

Modern medicine sheds light on biological therapies and nerve regeneration. Research is being conducted with pluripotent stem cells and nerve conduits with promising developments using human amniotic membranes loaded with human umbilical cord mesenchymal stem cells in radial nerve repair. [17]

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ORIGINAL ARTICLES

Intraoperative parathyroid hormone assay in patients with primary hyperparathyroidism

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Abstract: *Introduction: Currently, parathyroidectomy represents the elective surgical treatment and the only one curative that relieves the patient of metabolic complications and improves the quality of life of the patient. Intraoperative confirmation of PTH is required to reflect the fact that the correct parathyroid gland has been extracted. Material and method: The study is retrospective. The period on which the data was gathered over a period of 2 years. The values of the PTH assay were obtained from blood drawn from the ipsilateral cephalic vein of the hyperproductive parathyroid gland: 1. at the moment of anesthesia induction; 2. at identification; 3. at 10 minutes after the extraction; 4. at 20 minutes after extraction. Results: We identified a number of 25 patients operated for primary hyperparathyroidism. Preoperative values of PTH were 380pg/mL with a maximum value of 821 pg/mL. PTH values decreased by 55% at 5 min, 68% at 10 minutes and 74% at 15 minutes. A marked reduction in PTH values occurred after the first 5 minutes while the interval between 10-15minutes associated a variation of only 8-10% decrease.*

Conclusion: Intraoperative PTH assay is a useful technique to predict the success of the intervention. The technique is standard although some issues have been raised and are still under discussion.

Keywords: *PTH, hyperparathyroidism*

INTRODUCTION

The first reported intraoperative dosage of the parathormone or PTH was reported in 1988 by Nassaum et al. [1] The level and the extension of the cervical dissection in patients with primary hyperparathyroidism is still under discussion. In the absence of any preoperative imagistic or laboratory investigations and using only the intraoperative exploration of the parathyroid glands Satava et al. confirmed a rate of succes up to 96% in identifying and extracting the correct parathyroid gland responsible of primary hyperparathyroidism [2].

However, this technique has its limitations which can lead to surgical failure if one encounters a case of multiglandular hyperplasia, multiple parathyroid adenomatous nodules or hyperfunctional parathyroid ectopic tissue.

Primary hyperparathyroidism is an endocrinological disorder commonly found to be produced in 87% of cases by a single adenoma [3, 4]. Hyperparathyroidism associated with the presence of two hyperfunctional adenomas associates an incidence of up to 15% [3, 4].

Currently, parathyroidectomy represents the elective surgical treatment and the only one curative that relieves the patient of metabolic complications and increases the quality of life. To achieve this goal, a minimally invasive

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surgical procedure, aimed at a single parathyroid gland that houses the hyperfunctional nodule, is now successfully used, thus replacing bilateral exploratory cervical dissections. This procedure was quickly adopted due to the effectiveness of paraclinical investigations in the field of scintigraphy that allow the exact identification of the parathyroid adenoma during the preoperative evaluation [5].

However, an intraoperative confirmation is required to reflect the fact that the correct parathyroid gland has been extracted. In this regard, the intraoperative PTH dosage is used which due to the reduced half-life of 2-5 minutes coupled with a 50% reduction of the circulating intraoperative values compared to the preoperative values confirms the correctness of the procedure or the opens up the possibility to a cervical exploration to identify other parathyroid glands or parathyroid ectopic tissue [6].

The current protocol involves 4 dosages of PTH: at the time of induction of anesthesia (which will be used as a threshold value) at the moment of identification, at 10 and 15 minutes after the gland has been extracted. Success rates when this technique is used can reach 94% [7].

However, literature has begun to bring into discussion the efficacy of PTH evaluation when multinodular hyperplasia is encountered and the cost-effectiveness of the technique [8].

The study represents a retrospective evaluation of the effectiveness of this technique incorrectly identifying the hyperfunctional parathyroid gland in patients operated in our clinic for primary hyperparathyroidism by a minimally invasive technique.

MATERIAL AND METHODS

The study is retrospective. The period on which the data was gathered over a period of 2 years (01.01.2017-01.01.2019). Data were obtained from the clinic of general surgery of the Clinical Emergency Hospital “Prof. Dr. Agrippa Ionescu,” Bucharest, Romania. The following variables were obtained: age, sex, preoperative values of PTH, intraoperative PTH values, postoperative complications, preoperative scintigraphy, surgery time, and length of hospital stay.

The patients were operated using the standard technique of minimally invasive parathyroidectomy.

PTH values were obtained from blood drawn from the ipsilateral cephalic vein of the hyperproductive parathyroid gland: 1. at the moment of anesthesia induction; 2. at identification; 3. at 10 minutes after the extraction; 4. at 20 minutes after extraction.

Laboratory PTH values were obtained using the Radioimmunoassay method.

Inclusion Criteria: Diagnosis of primary hyperparathyroidism and surgery for primary hyperparathyroidism.

Ethics: The retrospective analysis of the data has been approved by an Ethics Committee of the institution.

RESULTS

We identified 25 patients, which were operated for primary hyperparathyroidism with a minimally invasive technique on which intraoperative PTH hormone assay was done (Figure 1).

Figure 1: Right parathyroid adenoma operated with a minimally invasive technique – the incision had a length of 5 cm and was focused on the parathyroid gland. The adenoma is clearly visible.

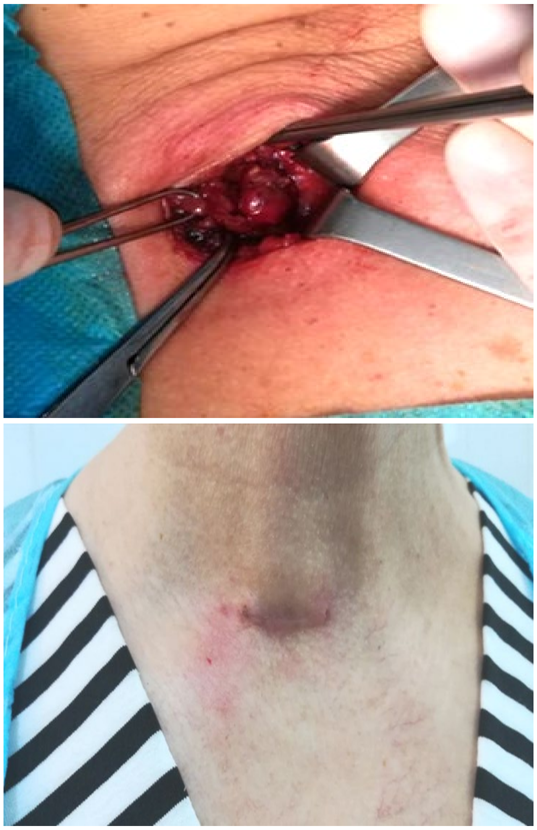

The average age at diagnosis was 54 years with a range of 32-68 years.

The study group was composed of 16 women and 9 men.

A number of 20 patients were diagnosed with singular parathyroid adenoma while multiple glandular adenomas were identified in five patients.

There were no mortalities reported in the studied group. There were no recurrent nerve lesions.

All of the patients were investigated before surgery with Tc 99m Scintigraphy to identify the location of the hyperproductive parathyroid gland. Only three cases did not correctly associate the preoperative scintigraphy with intraoperative exploration.

The average hospital stay was 2.3 days while a number of 8 patients were discharged on day 1 after surgery.

All of the patients were operated under general anesthesia.

Average preoperative values of PTH were 380pg/mL with a maximum value of 821 pg/mL.

The mean value (SD) for LVEDV was 144.37 (69.2) ml by 2DE and 143.6 (67.2) ml by 3DE, p=0.8, Spearman's coefficient 0.86. For LVESV the mean value (SD) was 106.26 (59.53) ml by 2DE, 103.24 (55.02) ml by 3DE, p=0.051, Spearman's coefficient 0.85 (Figure 2).

Figure 2: Decrease of intraoperative PTH values after parathyroidectomy at 5 minutes, 10 minutes and 15 minutes

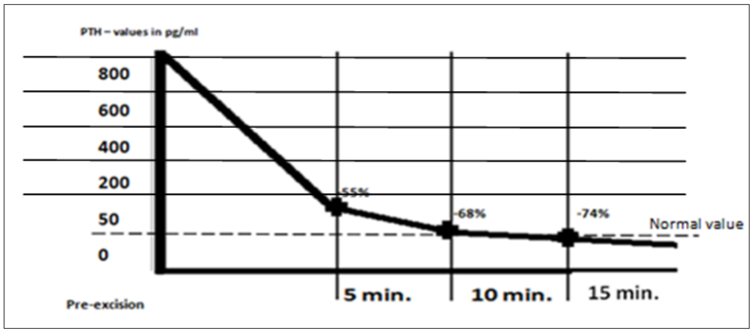

Average PTH values decreased by 55% at 5 min, 68% at 10 minutes and 74% at 15 minutes.

From the above graph, we can observe that a marked reduction in PTH values occurred after the first 5 minutes while the interval between 10-15 minutes associated a variation of only 8-10% decrease of PTH values.

There were no extended lateral cervical dissections done.

Two cases required local explorations by lifting and exploring the posterior region of the thyroid gland. In these cases, the PTH values decreased by 10% in one case at 20 minutes and the other they did not change.

DISCUSSIONS

The methods/techniques one uses to locate the parathyroid gland responsible for hyperparathyroidism has long been a topic of debate. Some surgeons advocate for preoperative investigations such as scintigraphy or cervical ultrasonography while others have fought for bilateral cervical exploration as a standard technique.

Today in order to identify the parathyroid gland, the scintigraphic examination is recognized as the gold standard, while other imaging solutions such as cervical ultrasound allow for a complete examination. Computer tomography and Magnetic Resonance Imaging are widely used to explore the suprarenal gland [9].

Ultrasound and scintigraphy are synergistic. When used together, they achieve a 95% sensitivity in identifying the hyperproductive parathyroid gland [10].

All of these investigations allow for a minimal-invasive targeted approach that translates into reduced hospitalization time, offer the possibility to use local anesthesia and ambulatory surgery, reduced operative time and low failure rates of only 1.5% [11, 12]. All of these advantages have led to a global reduction in costs [11, 12].

Surgical extraction of the parathyroid gland responsible for primary hyperparathyroidism produces a decrease in circulating PTH values thus confirming in real time the success of surgery or indicates towards a rapid change of tactics with the patient still on the operating table and under anesthesia. Failure of primary hyperparathyroidism surgery is largely related to correctly identify the parathyroid gland as it can be confused with lymphatic tissue or thyroid tissue [13].

Another cause of failure is multinodular hyperparathyroidism, which, in the absence of advanced diagnostic techniques, will force the operator to do a cervical exploration – to identify at least four thyroid glands, extraction of the macroscopically affected glands and to wait for the extemporaneous investigation. This technique associates increased comorbidities and a significant increase in operative time. In literature, so-called false-negative results in intraoperative PTH assay were reported which were due to increased PTH values after extraction of the gland or a delay of up to 30 minutes until the values were reduced. These are mainly due to manipulation of the parathyroid tissue at dissection [14].

No such cases have been encountered in the studied group.

In these situations, the extemporaneous examination of the resected parathyroid gland is of real use. Good communication with the anatomopathological and the laboratory physician is mandatory in order to establish the optimal therapeutic course.

Intraoperative PTH assay has seen its share of discussions. We used this technique with great success and it has proven to be effective in the majority of our surgical interventions. The reproaches are of economical nature, cost-effectiveness and the half-life of PTH, which seems to differ in cases of a single parathyroid adenoma and multiglandular parathyroid hyperplasia. Other issues raised are related to the technique: intraoperative time of PTH harvesting and the PTH value set as the baseline to which a reduction of more than 50% after extraction of the gland is considered effective [15].

Therefore, some authors recommend as the baseline the PTH values from the preoperative day while other surgeons use the PTH value as baseline obtained at anesthesia induction. On the studied group, we used as the baseline the PTH values obtained before induction although some authors consider this value to be 60% higher than the previous day dosing. Subsequent PTH assays after gland extraction showed a 30-40% drop in values and a reduction in of more than 50% at 15 minutes in the cases of correct parathyroid removal.

In cases of multinodular hyperparathyroidism, the extraction of the parathyroid gland can lead to so-called false-positive. In these cases, one can observe a more than 50% reduction in PTH values from baseline although there is incomplete removal of all the hyperproductive parathyroid tissue [16]. To avoid these situations when there is a suspicion of multiglandular hyperparathyroidism, it has been proposed that one should pursue a more than 70% reduction in PTH values from the baseline [17]. A reduction of over 70% of intraoperative PTH values to 10 minutes is associated with healing with a sensitivity of 85% and specificity of 100% (18). When multiglandular hyperparathyroidism exists, preoperative investigations imaging investigations in particular scintigraphy allow for a complete evaluation.

As we observed in our studied group, of great importance is the place where the blood is obtained for the PTH assay. It should be obtained from the ipsilateral cephalic vein of the hyperproductive parathyroid gland. Contralateral blood harvesting delays the decrease in PTH values to more than 10 minutes due to the circulatory time of the hormone. Such contradictory values can raise false doubts as to the fairness of the procedure. In order to increase the sensitivity of the PTH assay, one can harvest blood the upper, middle or inferior thyroid veins [19].

Study limits:

However, the study has limits. It was difficult to appreciate the evolution of the disease until surgery. We could not follow-up, in the long run, the effectiveness of the surgical procedure.

In conclusion, although medical treatment in primary hyperparathyroidism has evolved in time, surgery has remained one of the pillars of treatment. Direct PTH evaluation is a useful real-time technique and can predict utility of the surgical procedure. The technique is standard although some issues have been raised and are still under discussion. As demonstrated in our study a reduction of more than 50% of PTH values when compared to baseline at anesthesia induction can predict the success of the intervention in agreement with the literature. Preoperative scintigraphy plays a key role if one decides to use this technique as it can decrease surgery time and hospital stay.

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ORIGINAL ARTICLES

Metformin – old treatment for diabetes, new treatment for psoriasis

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Abstract: *Metformin was a revelation in the treatment of patients with type 2 diabetes, being widely used, with remarkable outcomes. Lately, research has greatly expanded the indications of metformin treatment, which can be used not only in diabetes but also in many other conditions. Although still debatable, several studies have shown the beneficial effects of metformin treatment in patients with psoriasis. This article is a review of the latest data of scientific literature about the effects of metformin administration in patients with diabetes and psoriasis, but also in nondiabetic patients with psoriasis.*

Keywords: *metformin, diabetes, psoriasis*

INTRODUCTION

Psoriasis is a chronic inflammatory disorder characterized by hyperproliferation and inflammation of keratinocytes. Psoriasis is a complex disorder, both from the standpoint of the disease and from the many associated comorbidities. Obesity, diabetes, metabolic syndrome, arthritis and cardiovascular disease are the most common comorbidities in patients with psoriasis [1, 2, 25]. Pathophysiologically, patients with psoriasis present changes of the tumor necrosis factor alpha and interleukins [6, 8, 12, 17, 19 and 23], highlighting the pathophysiological link with the metabolic syndrome [3]. There is a two-way link between psoriasis and diabetes, patients with psoriasis are at risk of developing diabetes, while patients with diabetes are at risk of developing psoriasis [4, 5].

Numerous therapies are available for the treatment of psoriasis, from topical to biological. Many of the current therapies for psoriasis are expensive and/or may cause toxicity; new and more accessible therapies with reduced adverse effects are needed.

Metformin, a herbal remedy, was first synthesized in 1922, and subsequently Jean Sterne highlighted the properties of the metformin as Glucophage compound (glucose eater) (6). Metformin is largely used in the treatment of diabetes, being accessible, easy to administer, with few side effects and with potential to decrease the body weight in obese patients. Since 2009, metformin has been recommended by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) in patients with type 2 diabetes as first-line oral therapy [7]. Many studies focused on the potential benefits of metformin administration in patients with diabetes and other conditions.

Although generally well tolerated, metformin has a number of contraindications to administration (Figure 1).

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Figure 1: Contraindications to metformin administration

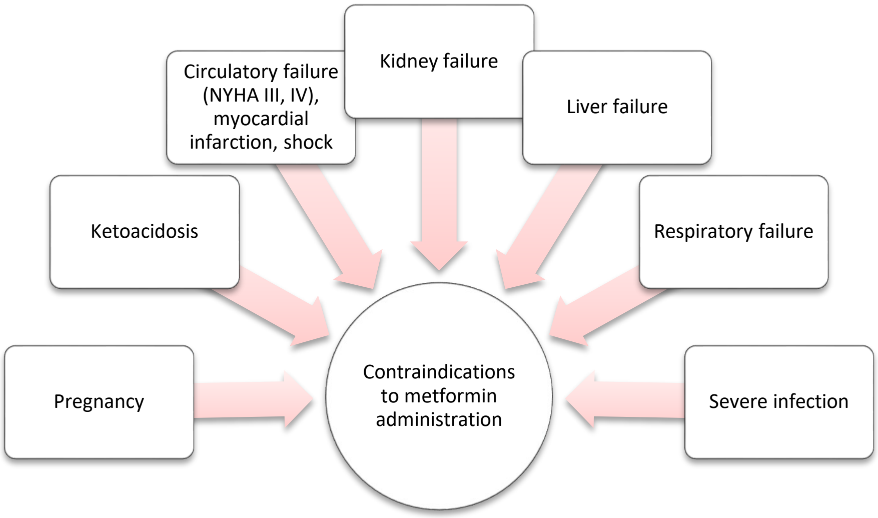
Circulatory failure (NYHA III, IV), myocardial infarction, shock
Kidney failure
Liver failure
Ketoacidosis
Respiratory failure
Pregnancy
Contraindications to metformin administration
Severe infection

METFORMIN IN THE TREATMENT OF DIABETES

Metformin is widely used for the treatment of type 2 diabetes. It is a first-line treatment as monotherapy or can be used as a combination therapy when monotherapy is no longer effective; insulin (regardless of the number of insulin injections/day) can be combined with a sulphonylurea, incretin or flozin [8]. In addition to the potential of lowering high blood glucose, metformin has a favorable effect on body weight, a very important aspect in diabetic patients.

Metformin does not cause hypoglycemia, because it does not stimulate the insulin secretion by the beta cells of the pancreas, the effect of lowering the serum glucose is due to inhibition of hepatic glucose production and sensitization of peripheral tissue to the effect of insulin.

Kirpichnikov et al emphasized the ability of metformin to reduce hemoglobin A1c by 1.3% - 2%, and may have a higher degree of reduction in patients with higher blood glucose levels during pre-treatment [9]. Although lifestyle changes seem to be more effective in the prevention of type 2 diabetes in people at risk, metformin can reduce the progression of diabetes and can be administered from the age of 10 years [10].

Following the administration of metformin in diabetics, in addition to the glucose-lowering effects, an improvement not only in the cardiovascular morbidity and mortality has been noticed, but also in the general health [11]. From the cardiovascular point of view, in patients at risk, the benefit of metformin administration is observed by the reduction of the body stress consequently to lower glucose levels, reducing the risk of myocardial infarction or arrhythmias.

In addition to the administration of metformin in type 2 diabetes, recent studies focused on its administration in type 1 diabetes. Nadeau et al, following the administration of metformin in patients with type 1 diabetes, observed a decrease in insulin dose, an improvement in body mass index and a decrease in waist circumference, especially in women [12]. Libman et al, in their study, have found that HbA1c was not significantly decreased following metformin administration, although the decrease in insulin dose was noted, and the patients complained of gastrointestinal distress [13]. Other observations following metformin administration in type 1 diabetes were improved outcomes of heart failure (regardless of decreased hemoglobin A1c) and decreased androgen levels in women with type 1 diabetes and hyperandrogenism [14, 15].

The benefits of using metformin in pre-diabetes were highlighted by the study of Capitanio et al, who have noticed a reduced risk of type 2 diabetes (by 31%) and a reduction of the body weight by average 2 kg in those who used metformin [16]. People with prediabetes should reduce their body weight and increase their daily physical activity; in addition, metformin can be considered for the prevention of type 2 diabetes in people at risk.

METFORMIN IN THE TREATMENT OF PSORIASIS

Starting from the pathophysiological mechanisms of psoriasis, with hyperproliferation and abnormal differentiation of epidermal keratinocytes, the main method of treatment of psoriasis focuses on inhibiting the excessive proliferation of keratinocytes [17}. HaCaT are cell lines derived from keratinocytes in normal adult skin, and excessive proliferation of these keratinocytes results in psoriatic lesions, with HaCaT cells being used as an in vitro model for the study of anti-psoriasis agents [18].

He et al investigated the effects of metformin on the proliferation of human immortalized keratinocytes. Metformin treatment regulated the level of p-AMPK and p-ERK1/2 in HaCaT cells and significantly inhibited the proliferation of HaCaT cells in vitro, by a mechanism associated with activation of the protein signaling pathway, mitogen-activated kinase [19].

Xuan et al have found differences in the quality of life of patients with psoriasis treated with metformin plus methotrexate, compared with patients treated with methotrexate alone. Combined metformin + methotrexate therapy contributes to a significant improvement in the quality of life of patients with psoriasis [20].

Because many psoriasis patients have also metabolic syndrome, Singh and Bhansali studied the effects of metformin (1000 mg/day) on the metabolic syndrome in psoriatic patients. These authors showed a marked improvement of the metabolic syndrome, following metformin administration, at 52.4%, compared with placebo (metformin versus placebo – OR (95% CI), 5.2 (1.3–20.7), P value 0.019) [21}. The proven efficacy of metformin on the metabolic syndrome is a potential axis for improving psoriasis. Because of metformin administration, 14.3% of patients compared with placebo (10%) had no changes or increases in IL-6 and TNF-α, followed by recurrence of psoriasis within the next 6 months [21].

Brauchli et al estimated a low risk of developing psoriasis (as a new diagnosis) using metformin compared to controls [OR = 0.77 (95% CI - 0.62-0.096)] [22].

Regarding the safety of metformin administration in patients with psoriasis and diabetes, it did not cause increased mortality, did not increase the severity of psoriasis or the rate of hospitalization for any other reason, metformin being safe to administer to patients with psoriasis [23].

Wu et al looked at the risk of developing psoriasis in patients with diabetes depending on the antidiabetic medication; they observed an increased risk of developing psoriasis in people with severe diabetes under insulin treatment, while the risk of developing psoriasis was not associated with metformin [5].

Starting from the cases of lichenoid rash and erythema multiforme that appeared following the administration of oral antihyperglycaemic drugs, we have found in the scientific literature only a case published in 2003 (possibly the first of this type reported), who suggests the occurrence of psoriatic rash following the administration of metformin, which eruption recovered after stopping the drug and reappeared with the reintroduction of the drug [24].

In addition to the proven beneficial effects of metformin on psoriasis, it seems that it has other applications in dermatology. Among the dermatological disorders studied from the point of view of metformin administration are acanthosis nigricans, acne, disorders of increased pigmentation, eruptive xanthomas, suppurative hidradenitis, hirsutism, skin cancer, squamous cell carcinoma, and melanoma.

CONCLUSION

The more extensive the metformin research areas, the more therapeutic applications appear. From the point of view of psoriasis, the use of metformin is safe and beneficial. Further studies are needed to determine the optimal dose of metformin recommended in patients with psoriasis, depending on the associated comorbidities.

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All the authors have equal contribution.

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CLINICAL PRACTICE

Improving the outcomes for pregnancies in the context of kidney disease

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Abstract: *Renal disease in pregnancy is a rare pathology, but it raises several problems in terms of normal pregnancy evolution. Among pregnant women with renal injury, the rate of complications such as spontaneous abortion, preeclampsia, hypertension, intrauterine growth restriction, premature birth, fetal and neonatal mortality are much higher.*

For a good understanding of the pathology and a correct management of pregnant women with renal disease, it is necessary to know the normal pregnancy changes and the differentiation of chronic kidney disease from the acute injuries occurring during pregnancy.

It is useful to ensure counseling prior to conception and to perform all prophylactic measures in order to prevent pregnancy complications and subsequent decline in renal function in all cases of pregnant women with chronic renal insufficiency.

Treatment of acute renal failure in pregnancy involves therapy of the underlying condition that caused renal injury, prevention of irreversible kidney injury and pre-vention of maternal and fetal secondary complications. Monitoring carefully this cate-gory of pregnant women, although additional risks strike, often results in favorabl pregnancy evolution.

Keywords: *pregnancy, chronic kidney disease, acute renal failure, complications, preeclampsia*

INTRODUCTION

Chronic kidney disease (CKD) affects 3% of pregnant women and represents an isolated risk factor for the occurrence of complications such as preeclampsia, arterial hypertension, premature birth and intrauterine growth restriction [1]. Such complications are not the only challenges that women with kidney disorders face during pregnancy, adding the potential teratogenicity of medications and the risk for disease progression. The management of chronic kidney disease in the context of pregnancy has lately become increasingly relevant to the practicing obstetrician, women becoming pregnant at more advanced age. Challenges brought by the presence of an underlying disease in pregnancy, such as autoimmune disease or diabetes mellitus, raise the necessity of interdisciplinary medical team to ensure satisfying maternal and fetal outcomes. The presence of acute kidney injury in pregnancy is generally declining worldwide, but still remains a significant health concern in developing countries, where pregnancy might be the first time to diagnose the kidney disease or the hypertension. Acute kidney injury affects a very small number of pregnant women and in this case the renal function decreases rapidly. The etiologies of acute kidney failure are diverse and require specific treatment [2-4]. It is critical to understand and evaluate normal physiologic changes in pregnancy in order to establish the proper diagnosis. The most common causes of chronic kidney disease are diabetes mellitus and hypertension [5]. Preconception counseling is required for women known with renal insufficiency, so patients would

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understand the potential risks of pregnancy and make various changes of the treatment for kidney disease, so the therapeutic agents won't affect the pregnancy's normal course [2, 5]. There are cases in which, through serum and urinalysis investigations carried out at the beginning of the pregnancy, patients are diagnosed for the first time with chronic kidney disease [5, 6].

PHYSIOLOGICAL CHANGES OF THE RENAL FUNCTION DURING PREGNANCY

The pregnancy changes in the renal and urinary tract are both anatomical and functional [2, 7]. Anatomically, the renal volume increases up to 30 %, attributed to the expansion of kidney vascular and interstitial volume, by approximately 1-1.5 cm, predominantly at the level of the collector system. A certain degree of ureteral and renal pelvis dilation occurs, mainly secondary to progesterone-induced relaxation of the muscular layers and due to mechanical compression on the ureters themselves [2, 8]. In almost 86% of pregnant women is a right-sided preponderance of hydronephrosis, attributed to the right ureter peculiar anatomy, crossing over the iliac and ovarian vessels at an angle before entering the pelvis, while the left ureter passes at less acute angle and travels in parallel with the ovarian vein [9]. As a result, these changes should not be interpreted as hydronephrosis during an ultrasound [2, 10].

From a functional point of view, during pregnancy, blood volume increases by up to 50%, systemic vascular resistance decreases, cardiac rate increases by up to 30%, blood pressure decreases in the first two trimesters, with a compensatory increase in heart rate, and returns to the values before the pregnancy in the third trimester. [5, 11, 12]. These cardiovascular changes lead to increased renal blood flow by up to 50%, increased filtration rate up to 30%, and serum creatinine levels decrease up to 20%.

PREGNANCY AND CHRONIC KIDNEY DISEASE

Chronic kidney disease is a pathophysiological process that ultimately results in end-stage renal disease through a progressive loss of nephron number and function that persist 3 or more months [5]. It affects up to 6% of women of childbearing age and is estimated to be associated in 3% of pregnancies. Although the etiology does not significantly influence the evolution of pregnancy, we underline the most common causes: diabetic nephropathy, chronic focal glomerulonephritis, systemic lupus erythematosus, scleroderma and nodulous periarteritis, chronic pyelonephritis, reflux nephropathy, polycystic kidney disease, chronic nephrolithiasis, post-nephrectomy status and congenital unique kidney [13-17] Women with chronic kidney disease are frequently impaired to make the renal adaptations needed for a normal course of preg-nancy. These patients often show the inability to increase levels of renal hormones leading to reduced expansion of plasma volume, normochromic normocytic anaemia and vitamin D deficiency [16]. Advanced renal dysfunction with poorly controlled underlying primary renal disease, associating proteinuria, frequently causing hypertension, are all significant risks for adverse maternal, fetal and postpartum renal outcomes. These are the main reasons to plan the pregnancies, whenever is possible, thus achieving the best outcomes, allowing the opportunity to counsel the future parents in advance and to optimize these risks.

Chronic kidney disease in pregnancy is classified according to serum creatinine levels in three categories:
- Mild-serum creatinine level < 1.5 mg/dl
- Moderate – serum creatinine level 1.5-2.5 mg/dl
- Severe – Serum creatinine level > 2.5 mg/dl [18].

Another important marker of renal function in pregnancy is proteinuria/24 h, also being a diagnostic factor for preeclampsia [18].

The pregnancies in patients with mild or moderate renal disease, normotensive, generally evolve favorably, although these patients' prone higher risks for complications compared to pregnant women with normal renal function. Pregnant women with severe chronic kidney disease are more exposed to complications such as intrauterine growth restriction or premature birth, but these cases are extremely rare, the fertility rate at this stage of the disease being low [1]. Although of great importance, the kidney function is not the only predictive factor for the pregnancy outcome, changes in endothelial function and active systemic disease are more likely to be involved.

It is considered that the pregnancy accelerates more rapidly the renal dysfunction due to proteinuria and hypertension, but there are insufficient data in this matter [1], moreover, several small single centre studies suggested that the rate of progression of renal disease to end stage is unaffected by gestation, despite the known fact that many women experience a temporary decline in renal function during pregnancy, given the pathophysiological effects of hyperfiltration [19].

MATERNAL AND FETAL RISKS

Preeclampsia

The risk of developing preeclampsia for pregnant women with renal disease is significantly higher compared to the general population. In mild renal dysfunction, the risk is 20%, and in the severe one increases up to 60-80%, compared to the 5% risk in the general population [5]. The main fetal risks generated by preeclampsia are the restriction of intrauterine growth and premature birth [14]. A reduction of the risk for preeclampsia by 25% was demonstrated by daily administration during pregnancy of 150 mg of aspirin [5, 20].

Renal function

The decline in renal function rarely occurs in women with mild disease, but the risk for moderate illness is 25% in terms of definitive loss of 25% of renal function [5].

Hypertension

Once placental development is fully achieved, by 16 weeks' gestation, a 50% rise in plasma volume occurs, and in the vast majority of women blood pressure falls, due to the vasodilator response accompanied by an elevation of the cardiac output, arterial underfilling, subsequent ADH release and the stimulation of the renin-angiotensinogen system. Relaxin may also play a role in this chain of changes, because it has been linked to ADH secretion and water drinking, while levels are increased during pregnancy. In the third trimester, blood pressure starts to rise, reaching almost the pre-pregnancy values.

Among patients with renal disease, preexisting pregnancy hypertension is common, but during pregnancy it becomes much harder to control, on the one hand, due to the adaptive changes in pregnancy in the second and third trimester [5,10] and on the other hand, by the inability to use therapeutic agents of election, as inhibitors of the conversion enzyme and receptor blockers for angiotensin [5].

To reduce maternal and fetal complications it is necessary to maintain blood pressure below 140/90 mmHg, but an important decrease below 120/70 mmHg was correlated with increased risk of fetal demise [21]. Therapeutic agents that can be used in pregnancy are Methyldopa, Labetalol, Nifedipine, and Hydralazine (Table 1) [1, 5, 10, 22-24].

Table 1: Drugs used in pregnancy for women with kidney diseases, diabetes or kidney transplant

Drug	FDA	Indication	Fetal/neonatal effects	Use in pregnancy	Teratogenicity
ACE inhibitors/ARB	D	Hypertension	Cardiovascular, central nervous system defects in first trimester, FGR and neonatal anuria in second and third trimesters, fetal renal tubular dysplasia, oligohydramnios, perinatal acute renal failure, and other major congenital anomalies	Stop at conception	Yes
Beta-blockers	C	Hypertension	FGR in first trimester Atenolol may cause fetal bradycardia in the first trimester	Labetolol often used first line Atenolol can be used safely later in pregnancy	No
Ca channel antagonists – nifedipine, amlodipine	C	Hypertension	Nimodipine is prohibited	Used second line with methyldopa or labetolol	No
Hydralazine	C	Hypertension		Used for more severe hypertension Used in combination with methyldopa or beta-blockers	No
Methyl-Dopa	B	Hypertension		Often used first line	No
Biguanides – Metformin	C	Diabetes		Administered from first trimester	No
Second generation oral sulfonylurea hypoglycemic agent – Glyburide	B	Diabetes	Neonatal hypoglycemia higher when compared to insulin	Administered from first trimester Does not cross the placenta	No
Isophane (NHP) insulin	C	Diabetes	Can cause fetal macrosomia, and due to increased oxygen requirements is a risk factor for fetal death	First choice long-acting insulin The most natural agent for hyperglicemia	No
Rapid-acting insulin analogues – Aspart, Lispro	C	Diabetes		May be preferable to start pre-pregnancy	No
Longer acting insulin anlogues: Detemir,Glargine	C	Diabetes		Few studies, but encouraging results	No

Prednisolone/IV methylprednisolone	C	Kidney transplantation	Rare – except at large doses (cataract, adrenal insufficiency and infection) Increased frequency of premature rupture of membranes	maternal side effects: glucose intolerance, hypertesion, weight gain, hirsutism, acne, cushingoid features, impaired wound healing, osteoporosis, mood changes	Possible increase in oral clefts and mental retardation
Azathioprine – Imuran	D	Kidney transplantation		Maternal increased risk of infection and neoplasia.	Crosses the placenta as an inactive form. No congenital abnormalities have been observed.
Calineurin Inhibitors	C	Kidney transplantation	Transient immune alterations in the neonate (Cyclosporine) Hyperkalaemia and renal impairment (Tacrolimus)	Increasingly used - they have decreased rejection episodes and improved survival in transplant recipients.	No
Mycophenolate mofetil	D	Kidney transplantation	Increased risk of miscarriage and congenital abnormalities. Congenital malformations of the external and middle ear, cleft lip and cleft palate, and other major structural malformations involving the eye, hands and fingers, cardiac, bowel and skeletal systems.	Should be discontinued before pregnancy	Yes
Sirolimus (Rapamune)	C	Kidney transplantation	Small-for-gestational-age infants and delayed bone ossification	Immunosuppressant that could be used during pregnancy	Has not been implicated in specific fetal malformations. Further investigations are required

Proteinuria

The gold standard to diagnose proteinuria of pregnant women is the 24-hour urine collection and quantification of urine proteins level. Even so, errors in timing and urine retention in the dilated systems make urine collections incomplete, because of the large volumes contained at one time in the upper urinary tract.

The increase of the excretory level of urinary proteins is associated with important risk for intrauterine growth restriction and increased risk for renal dysfunction. [18, 20, 21].

In patients developing nephrotic syndrome during pregnancy (proteinuria > 3g/day, albuminuria > 30 mg/L and edema) there is a high risk for thromboembolic accidents and maternal death, even if blood pressure is maintained at satisfactory levels. In this category of patients, anticoagulant prophylaxis with low molecular weight heparins is recommended, up to 6 weeks postpartum. [5, 25].

Preconception Counseling

As general rule, the conception should not be discouraged as long as possible risks are understood and careful monitoring of the pregnancy is available. [1, 14, 20]. It is necessary to discontinue and substitute therapeutic agents with teratogenic potential (Table 1).

Patients with active vasculitis or lupus should be advised to wait for the episode to end prior to the conception. [5, 26].

RENAL TRANSPLANTATION AND PREGNANCY

Maternal status and fetal development in posttransplant renal patients is favorable, significantly better when compared to pregnant women with advanced renal disease requiring dialysis, especially if the following conditions are fulfilled [1, 8]:

- Renal function is stable
- Minimum one year after renal transplantation; latest recommendations ex-tend the waiting period at 2 years, thus reducing the risk for allograft failure
- Proteinuria maintained under 1g/day
- Controlled hypertension
- Minimal doses of immunosuppressants required (prednisone, tacrolimus, azathioprine, ciclosporin) (Table 1)

- No prior or pending rejection of renal transplantation [5].

Thus, women with diagnosed advanced kidney disease are often encouraged to wait until after successful kidney transplantation to obtain a pregnancy. The reason for this recommendation is that fertility is improved after transplantation and the risks for pregnancy complications, such as preterm birth and hypertension, are much lower. Pregnancy is considered a sensitizing event, which can result in the formation of anti-HLA antibodies that may impend finding a future suitable donor.

The incidence of premature childbirth, intrauterine growth restriction and preeclampsia is higher compared to the general population and 30% of pregnancies will be affected by these complications.

Vaginal childbirth is not contraindicated in case of mothers with renal transplantation. When Caesarian section is preferred, the approach of the lower segment may be difficult due to the trajectory of the transplanted ureter. [1, 5].

DIABETIC NEPHROPATHY AND PREGNANCY

Lately, as women do tend to procreate later in life, diabetic nephropathy is probably becoming the most common CKD seen in pregnancy. Pregnancy in women with diabetes type 1 or type 2 is associated with a two- to four-time increased risk of pre-term delivery, preeclampsia and perinatal mortality compared with the general population [27]. Pregnancies in women with CKD associate poor placentation and fetal growth restriction, with an incidence of small for gestational age newborns of 31% [19]. It is rather unclear if growth restriction is based on poor placentation or on fetal hyperinsulinamia, but women with more severe renal impairment are exposed to a greater risk for pregnancies with poorly grown infants, despite maternal hyperglycaemia, thus antenatal documentation of fetal growth restriction has been linked with worse fetal outcomes than macrosomia.

The first clinical sign of kidney malfunction is microalbuminuria, and urinary albumin excretion of 30–300 mg/24 h, corresponding to a spot urine albumin/creatinine ratio of 30–300 μg/mg. The progression towards diabetic nephropathy is seen in all cases left untreated, when microalbuminuria becomes persistent proteinuria, associating hypertension and an unavoidably decline in GFR [28].

Diabetic nephropathy adversely affects the course of pregnancy by the following three mechanisms: development of severe hypertension superimposed on the deterioration of kidney function in the mother, preterm delivery due to high maternal blood pressure and preeclampsia, and fetal intrauterine growth restriction and fetal distress caused by placental insufficiency.

The prepregnancy preparations in women with diabetic nephropathy or microalbuminuria would comprise intensive glycaemic control, low-dose aspirin, and intensive antihypertensive treatment. The treatment with ACE inhibitors followed by strict metabolic control for at least 6 months, usually results in low levels of albumin excretion and it has been found to be associated with a high rate of successful pregnancy outcome [27]. On the onset of pregnancy, early in the first trimester, ACE inhibitors are linked to increased risk of congenital malformations [29], as for the last part of the pregnancy, are associated with abnormal fetal renal development and neonatal renal failure [30] therefore treatment with ACE inhibitors or AngII antagonists should be stopped before conception. In the event of an unplanned pregnancy, women with diabetic nephropathy which necessitates continuous treatment with blockers of the RAS (renin angiotensin system), can benefit from a shift to other antihypertensive drugs successfully in early pregnancy. Severe congenital malformations have been linked to poor glycemic control before pregnancy, therefore the goal is strict glycemic control and HbA1c as close to normal as possible, at least <7% is recommended [31, 32].

In all women with high risk for pre-eclampsia, treatment with low-dose aspirin is proven to have some preventive effect, thus, at least theoretically, the same treatment could be of benefit in women with diabetic nephropathy [20] and is recommended in American and British guidelines.

The goal for CKD and diabetic pregnacy should be BP <135/85 mmHg and urinary albumin excretion <300 mg/24 h, obtained with antihypertensive treatment with pregnancy-friendly drugs, strict glycemic control (HbA1c <6.0%) and low-dose aspirin.

For many years, a pregnancy in women with diabetic nephropathy was related to a decline in maternal kidney function and important perinatal mortality, but recent improvements in pregnancy management substantially reduced those risks.

Cesarean delivery should be considered for every case where fetal weight is estimated for more than 4500 g. Vaginal birth at 39 weeks gestation is safe whenever blood glucose levels are well controlled and kidney function is normal. In those cases with vascular complications and poorly controlled blood glucose, delivery should be considered at 36 0/7 to 38 6/7 weeks or even earlier, depending on maternal and fetal status [33].

ACUTE RENAL INSUFFICIENCY IN PREGNANCY

Acute renal insufficiency is defined as the sudden degradation of renal function, ranging from the slight decline in renal function to the necessity of renal function replacement therapy [3, 34].

In the first part of the pregnancy, infectious pathology and hypovolemia are the most common causes of renal injury, and in the second part of the pregnancy, the most frequent etiological factors are preeclampsia, placental abruption or severe anemia.

The best management of this pathology is possible using the classification of the acute renal insufficiency by the cause, as pre-renal, renal and post-renal. Pre-renal injury occurs predominantly secondary to hypovolemia, hemorrhage, congestive heart failure, sepsis. Renal insufficiency has as main etiologies acute tubular necrosis, renal cortical necrosis, thrombotic microangiopathy, preeclampsia, acute interstitial nephritis, and glomerulonephritis. Post-renal insufficiency occurs due to ureteropelvic block, but rarely causes bilateral renal injury [3, 5].

Clinically, pregnant women with acute renal insufficiency present oliguria, de-fined as a urine production < 0.5 ml/kg/h. [3, 35]. Another marker of renal injury is the increased level of serum creatinine, which is difficult to be appreciated due to its low level in normal pregnancy. Secondary to renal injury, as a compensatory mechanism for renal hypo-filtration, an increase in systemic blood pressure and intravascular volume appears and changes of urinary electrolytic values due to the action of the system renin-angiotensin-aldosterone [36-38].

Treatment of acute kidney failure involves treating the underlying condition that caused renal injury and focuses on the restoration of urine production, avoiding secondary injury and increasing renal infusion. The effects of this pathology on the fetus are diverse, specific to each etiology, and may require delivery, as in the case of preeclamsia or placental abruption. [39, 40].

CONCLUSIONS

Although the risk of complications in pregnancy in patients with chronic kidney disease is higher than in the general population, in most cases, with the correct management of pregnancy and kidney disease, the pregnancy has a favorable evolution. There are insufficient data to certify the pregnancy as a potential factor in the decline of the renal function.

Intensive pre-pregnancy antihypertensive treatment in patients with diabetic nephropathy and good glycaemic control results in preservation of kidney function and favorable maternal and fetal outcomes.

Acute renal insufficiency, occurring during pregnancy, may benefit from specific etiological treatment, but may also require emergency delivery.

Close obstetric surveillance and screening for kidney function is important to improve pregnancy outcomes in these high-risk pregnancies.

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CLINICAL PRACTICE

Differential diagnosis in case of overdose of antiepileptic treatment. Clinical case

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Abstract: *Acute drug-related acute intoxications or suicidal ideation following overdose with anticonvulsants may cause major morbidity, in many cases requiring intensive care and prolonged periods of hospitalization.*

The case of a 35-year-old patient known for a history of epilepsy with phenobarbital and carbamazepine treatment is brought to the Emergency Unit by transfer from another hospital in a comatose state with insufficient respiratory performance, bilateral active mydriasis. From the family members' claims, the patient discontinued treatment with phenobarbital two weeks ago. Following interdisciplinary examination, hospitalization in the Neurology Department with the diagnosis of epileptic status is decided. Due to the progressive aggravation of acute respiratory insufficiency, the ventilator support is constituted. Since the biological samples related to probability diagnosis and cerebral imaging were not conclusive, toxicological samples were collected. The GC/MS analytical toxicology screening in urine reveals the presence of carbamazepine and phenobarbital and their plasma levels (5.92 and 92.3 mg/l) as determined by the FPIA method indicate an overdose of phenobarbital therapy. The patient is taken over by the Department of Clinical Toxicology where specific intensive care measures are applied. Clinical evolution is favorable. The psychiatric examination highlights the depressive idea, suicidal ideas, and uselessness. The suicide attempt is confirmed. It also presents a review of the main information about acute poisoning with anticonvulsants, but also about the analytical laboratory methods that bring precious information in establishing the diagnosis of certainty.

Keywords: *phenobarbital, carbamazepine, GC/MS, urine, acute poisoning*

INTRODUCTION

Acute drug-related acute intoxications or suicidal ideation following overdose with anticonvulsants may cause major morbidity, in many cases requiring intensive care and prolonged periods of hospitalization. The evaluation and stabilization of the poisoned patient is similar to all cases presented at the Emergency Unit and aims at detecting vital risk disruptions and correcting them in the shortest possible time [1].

Phenobarbital ($C_{12}H_{12}N_2O_3$) is a drug of the barbiturates group used since 1912. It is generally administered to epileptic patients at doses of 60-200 mg/day, commonly in combination with other anticonvulsants [2]. In the chronic administration of 200 mg / day in epileptic patients, the concentrations had average values of 29 mg/l and 16-48 mg/l [3]. Plasma concentrations of 10-30 mg/l are generally considered appropriate for patients receiving phenobarbital as an anticonvulsant and may require 14-21 days to reach the level of equilibrium.

The compound had a plasma half-life of about 4 days [4]. Toxic reactions to phenobarbital at chronic doses occur at plasma concentrations exceeding 40 mg/l. Patients develop

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coma with reflexes present at plasma concentrations of 65-117 mg/l and reflexes loss occurs at concentrations of 100-134 mg/l [5].

The mechanism of action of barbiturates and implicitly of phenobarbital is related to their affinity for the GABAA receptor, which is fixed in distinct sites with both benzodiazepines and GABA (the main CNS inhibitor neurotransmitter), potentiating its effect. Barbiturates also block a glutamate receptor - the major excitatory neurotransmitter in the CNS [6].

In acute intoxication with barbiturates, because of the depression of central respiratory control mechanisms, following a general depression and anxiety, death may occur in the absence of artificial ventilation and vital functions support measures. In short-duration barbiturates intoxication, the cardiopulmonary arrest may be the cause of premature death and late: circulatory insufficiency, cerebral edema, acute pulmonary edema. Central nervous effects are related to the severity of intoxication. Because there is no specific antidote, the goal of therapy remains to increase the elimination of toxic and support vital functions. In determining the establishment of measures to increase the elimination of anticonvulsants (hemodialysis, hemoperfusion, plasmapheresis), it is also important to measure the plasma levels of these substances in relation to the clinical condition of the patient.

Carbamazepine is a classic anticonvulsant, largely used, with an iminostilbene nucleus, similar to that of tricyclic antidepressants, with a prolonged digestive absorbtion time. Its mechanism of action is partially known, acting on the neuronal membrane towards stabilizing its hyperexcitability, inhibiting repetitive neuronal discharges and reducing excitatory synaptic transmission by blocking voltage-dependent sodium channels. The reduction is glutamate discharge and the stabilization of the neuronal membrane contribute only supplementary to the antiepiletic effect [7]. Its main metabolite is 10,11-carbamazepine epoxide, an essential metabolite which possesses the same toxic effects as the basic drug. In pharmacological doses, the distribution volume of carbamazepine is of 1.4 l/Kg, and the half-life is of 15 hours.

The absorbtion of carbamazepine occurs slowly, 65-85% of it binds to plasma proteins and it is quickly distributed to all tissues. The maximum serum levels are reached within 6 to 8 hours following ingestion and steady-state plasma concentrations, considered to be therapeutic levels of carbamazepine, considerably vary between individuals; for most patients, the therapeutic level of carbamazepine is between 4 and 12 ml//l and the concentration of the active metabolite (10,11 epoxide-carbamazepine) is 30% of the plasma levels of carbamazepine [7]. In epilepsy, carbamazepine is administered in doses of 0.8 to 1.2 g / day, 2-3 times a day for an adult and 0.1-1 g / day (10-25 mg / kg / day) for children of ages from 1 to 15. Therapeutic doses can be optimized through therapeutic monitoring (TDM) due to a good correlation between plasma levels and the therapeutic effect [8]. As it is an enzymatic inducer, carbamazepine can induce its own metabolism, this phenomenon being responsible for the reduction of its half-life over repeated administrations.

In case of carbamazepine overdose, many of the observed effects can be tied to secondary mechanisms of its tricyclic structure. The symptomatology of an acute intoxication is characterized by coma, pyramidal signs, nausea/vomiting, hypotension, hypothermia, anticholinergic symptomatology (tachycardia, mydriasis, redness, urinary retention etc.) tremor, convulsions, respiratory depression, cyanosis, sinusal bradycardia or atrio-ventricular conduction disorders [9]. Plasma levvels of carbamazepine between 18-70 µg/ml have been measured in carbamazepine overdoses, while levels higher than 40 µg/ml have been associated with an increased risk of complications such as coma, convulsions, respiratory insufficiency, and cardiac conduction disorders [10].

CASE PRESENTATION

A.G, female gender, 35 years old, admitted for being comatose, with acute respiratory insufficiency and non-reactive bilateral mydriasis.

Personal medical history – known to have had previous comitial crises, treated with phenobarbital and carbamazepine

History – Comitial patient, brough from the Emergency Admission Unit in a coma, transferred from another hospital. From her family’s affirmations, the patient had stopped taking phenobarbital 2 weeks before.

Interdisciplinary consults performed in the Major Emergency Department:

- neurosurgical consult: coma GCS = 5 points, no neurological signs;

- internal diseases consult: PA = 100 mmHg, AV = 76 b/min, ECG sinusal rhythm with no pathological alterations.

CT scan: no heterodense lesions are present at brain level, ventricular system situated on median line.

The patient is taken over by the Neurology Department with a diagnosis of epilepsy.

Objective examination upon admission: second degree coma; non-reactive; oro-tracheal tube, spontaneously breathes through the tube; TA = 120/70 mmHg, AV = 85 b/min; gastric statis = 200 ml; diuresis = 400 ml; positive bilateral Babinski bilateral; fixed mydriasis, pupil OD>OS, fixed eyeballs; no meningeal signs; spontaneously and discreetely mobilizes right superior limb. Due to the progressively aggraveted acute respiratory insufficiency, mechanical ventilation is applied (BIPAP, PEEP = 5 mbar, FiO2 = 40%, Pmax = 19 mbar);

In order to confirm the diagnosis, lumbar punction was performed and probes of cerebrospinal fluid were sent for biochemstry, bacteriology and cellularity.

A central subclavicular catheter was also applied.

The pulmonary radiography does not show evolving pleuro-pulmonary changes.

As the diagnosis could not be confirmed by the biological samples, toxicological samples were taken. The GC/MS examination of the urine shows the presence of carbamazepine and phenobarbital.

Biological table: biochemistry, hematology, coagulation within normal limits.

The patient is taken over by the Clinical Toxicology Department.

Intensive therapy measures: ventilation support; ensuring permeability of respiratory pathways – aspiration of tracheo-bronchial secretions through the IOT probe; naso-gastric drainage tube; hydro-electrolytic rebalancing; plasmatic and urinary alkalinization under paraclinical control (EAB).

Support therapy: monitoring of vital functions; ventilatory support; hemodynamic monitoring; diuresis monitoring; analytical control of urine.

Clinical evolution is favorable – the patient becomes conscious, reacts to stimuli; shows spontaneous breathing signs; is detubed and restarts spontaneously breathing. Physical recovery measures continue.

Psychiatric exam reveals depressive ideation, suicidal ideas and ideas of uselessness. A suicide attempt is confirmed.

She is tranferred to the specialized psychiatry clinic.

Analytical diagnosis of certainty in case of acute drug intoxication

One of the most used methods in identifying the substances incriminated in acute drug intoxications is gas chromatography - GC coupled with mass spectrometry - MS. Thus, by gas chromatograph injection of a completely unknown matrix, if the working conditions are well chosen, at the exit of the GC they will be placed in turn at the detector's disposal. If the detector is of the MS type and the injection matrix contains organic substances whose molecular weight falls within the working range of the mass filter, it will determine the mass spectrum for each substrate. In our case, the urine sample collected from the patient is follows to the liquid-liquid analytical procedure and injected into the GC/MS system, with ion trape detection, electronic ionisation 70eV. Midazolam is used as an internal standard. The method chosen for the separation and qualitative identification of analytes by GC / MS is called medicamente.mth, with a 45-minute acquisition time. Upon completion of the acquisition program, read the total recorded chromatogram. Apply the background correction and then proceed to the identification of each chromatographic peak. It establishes the mass spectrum that corresponds to it and these spectra are automatically compared with the existing spectra in the laboratory database. The database includes specialized spectral libraries (Nist98, PMW, Wiley6).

The temperature program used was:

- temperature injector: 280 ° C,

- for column oven: Start at 1400C, 2 minute range, increase temperature by 5° C / min to 290° C, 13 minutes (total time 45 minutes).

Following these investigations, the result is established. In our case, we separated the chromatographic column and identified the following substances: Phenobarbital and carbamazepine at the retention times shown in Table 1, based on the mass spectrum obtained by EI.

Table 1: Data on identified compounds (molecular weight, retention times and characteristic spectral lines m/z)

Compound	MW (g/mol)	Retention time (min)	CAS. No.	Spectral lines (m/z)
Phenobarbital	232	16.001	50-06-6	117, 204, 231
Carbamazepine	236	16,944	298-46-4	193, 236
Midazolam (SI)	326	26,951	59467-70-8	310, 327

In case of acute drug intoxications, measuring plasma levels is important both to establish the seriousness of the intoxication and to monitor the efficiency of the medical treatment.

Therapeutic drug monitoring is performed based on the principle of fluorescent light polarization on a Cobas Integra 400/800 system for serum samples taken from patients. It is a quantitative method which uses the the fluorescence of substances and is based on immunological properties (antigen- antibody) of the analyzed substances. The principle of this method is based on exciting the sample with a fixed wavelength and measuring the quantity of light emitted by a substance called a luminophore, on another wavelength. The specific antigens, present in the tracer, bind to the antibodies present in the sample [11]. Thus, the quantity of light emitted by the sample will be inversely proportionate to the quantity of searched substance.

Figure 1: Phenobarbital mass spectrum (top) and full scan chromatogram obtained from SD urine extract

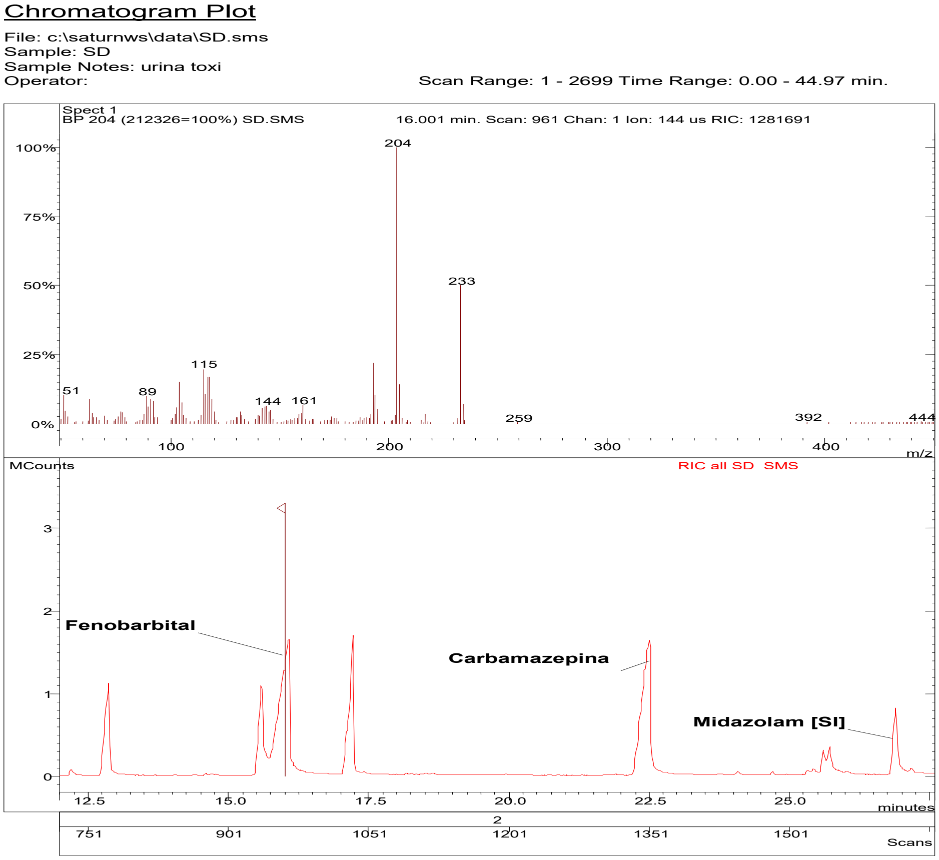

Figure 2: Calibration curve (in 6 points) for carbamazepine on the Cobas Integra 400+ System

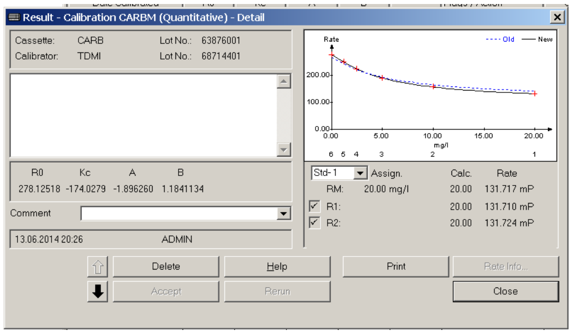

Cassette: CARB Lot No.: 63876001
Calibrator: TDMI Lot No.: 68714401

R0	Kc	A	B
278.12518	-174.0279	-1.896260	1.1841134

13.06.2014 20:26 ADMIN

Std-1	Assign.	Calc.	Rate
RM:	20.00 mg/l	20.00	131.717 mP
☑ R1:		20.00	131.710 mP
☑ R2:		20.00	131.724 mP

In this case, determinations of the plasma levels of carbamazepine and phenobarbital in the samples were performed using the FPIA method, which implies determinations on a calibration curve in 6 points. The quality control is performed for three known concentrations.

The plasma concentration of carbamazepine on the first and second day of hospitalization was 5.92 and 2.90 mg/L, respectively. Because this value falls within the therapeutic limit, no measurements have been made during hospitalization. In contrast, the plasma level of phenorbarbital was increased and its variation in the first 7 days of hospitalization is shown in Figure 3.

CONCLUSIONS

Antiepileptic treatment based on selective anticonvulsant medication (carbamazepine and phenobarbital) follows the avoidance and treatment of epilepsy seizures, and at therapeutic doses these crises stop. Unfortunately, cases of overdose of treatment may occur either accidentally or for a suicidal purpose.

In the case presented, the crisis appeared could be interpreted in a double context, in the absence of correct anamnestic data, either in the case of baseline, in the absence of anticonvulsant treatment or in the case of overdosing of the medication.

Table 2: The therapeutic and toxic plasma levels for phenobarbital and carbamazepine (The Cobas Integra 400+ System)

Compound	Therapeutic levels (mg/L)	Toxic levels (mg/L)
Phenobarbital	10-30	>40
Carbamazepine	4-8 (associated with other anticonvulsants) 6-12 (unique treatment)	>8 >12

Compound	*Plasma levels (mg/L)*						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Phenobarbital	94.5	121.8	117.9	93.7	87.4	57.6	35.9
Carbamazepine	5.92	2.90					

Figure 3: Plasma levels of phenobarbital during the first 7 days of hospitalization

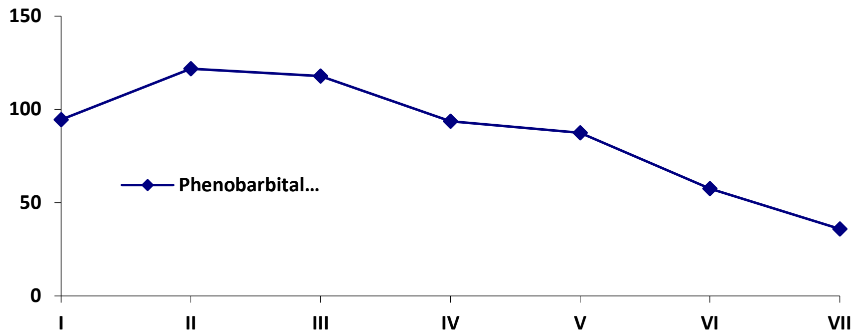

In establishing the differential diagnosis of certainty, toxicological screening of biological products was of great importance, both by identifying drug substances in the urine (by the GC/MS method), especially by measuring their plasma levels by the FPIA (Fluorescence Polarization Immunoassay). Measuring plasma levels of some drugs facilitates the optimization of therapeutic behavior but also reduces the toxic effects of these drugs in case of overdose

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CLINICAL PRACTICE

Cross-leg limb salvage solution: A case report regarding the management of a major defect in a patient with type IIIB open distal tibial fracture

M. Turbatu, C. Condrea, A. Oproiu, A. Cursaru, A. Lupu

Abstract: *Tissue coverage of lower limb defects after tibial fractures remain one of the hardest areas for a surgeon to deal with. The definitive management of Gustillo type IIIB fractures depends on a series of factors, which have a high impact in the multidisciplinary management of this severe trauma.*

Although free flaps have been used succesfully in the last two decades, there remains some cases where easier plasties using the contralateral limb tissue reserved could be indicated.

We describe our experience with a cross-leg flap in a severe-smoking patient for the reconstruction of a difficult leg defect.

Keywords: *Cross-leg plasty, Type IIIB fractures, External fixators*

INTRODUCTION

The tibia is reported to be the most commonly fractured long bone in the human body. Gustilo-Anderson IIIB injuries are a major trauma requiring aggressive excision, adequate fixation, and good coverage of tissue defects. They are open tibial fractures more difficult to manage than other I, II, and III A forms.

Stable skeletal fixation with immediate soft tissue cover has been the key to the optimal results in treating open tibial fractures, in particular, type IIIB fractures. The prognosis of these fractures depends on the degree of contamination of the wound, the devitalization of the soft tissue and area of the defect.

Pedicled cross-extremity flaps for calves wound coverage have been replaced by free tissue transfer in the last two decades. However, there are certain difficult situations where the free flap cannot be employed and alternative methods are needed.

University Emergency Hospital Bucharest, Plastic surgery and Orthopaedics, Bucharest, Romania

We describe our experience with a cross-leg gastrocnemius flap in a heavy-smoker patient with little tissue reserve necessary for the coverage of a defect in the middle to lower third anterior calf.

CASE PRESENTATION

We present the case of 58 years old patient who was admitted in the Orthopedics and Traumatology Clinic of the Emergency University Hospital of Bucharest with an injury after a direct trauma by axe and had pain in the calf, functional impotence of the inferior member and an open wound of about 10 cm on the inner side in the 1/3 middle anterior calf.

Following wound debridement (including post-debridement measurement) and skeletal fixation, the anterior defect was assessed [1].

The following days the patient underwent antibiotherapy and careful daily wound dressing until a proper defect coverage plan was decided.

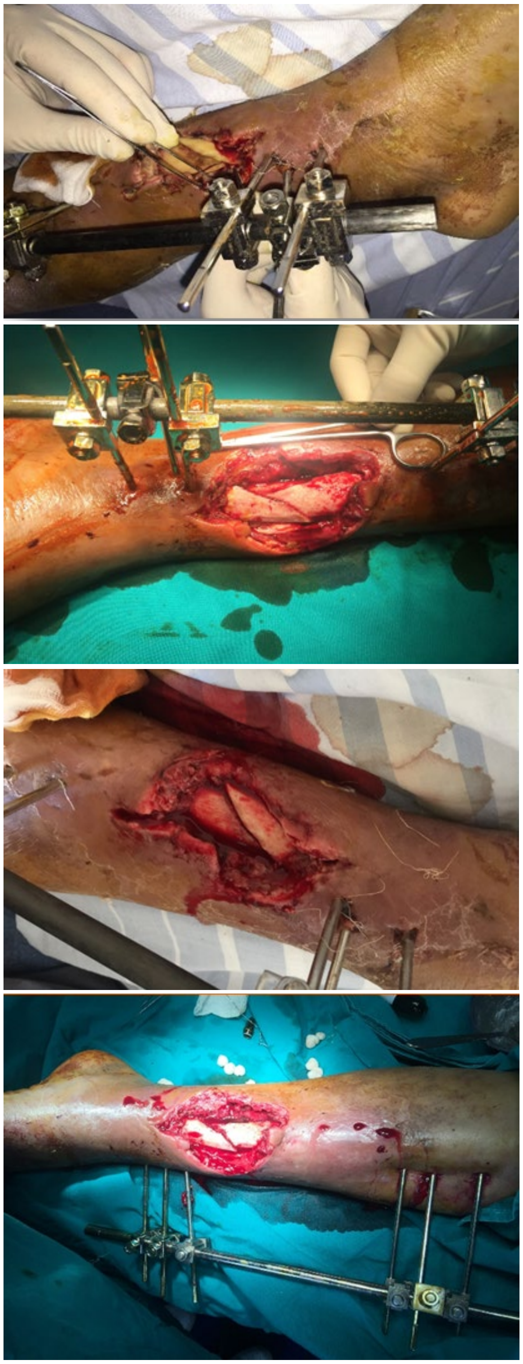

Free flaps are usually the first choice for soft tissue coverage in the distal leg. There continue to be, however, some clinical situations in which local fasciocutaneous and myocutaneous flaps are often not available [2].

Occasionally, a free flap may also have failed because of technical errors or damaged vasculature. In these situations, a cross-leg flap is the best choice.

In the end we decided to perform a pedicled cross-leg gasctrocnemius flap, with the utilization of an external fixator to keep both legs immobilized after the surgery.

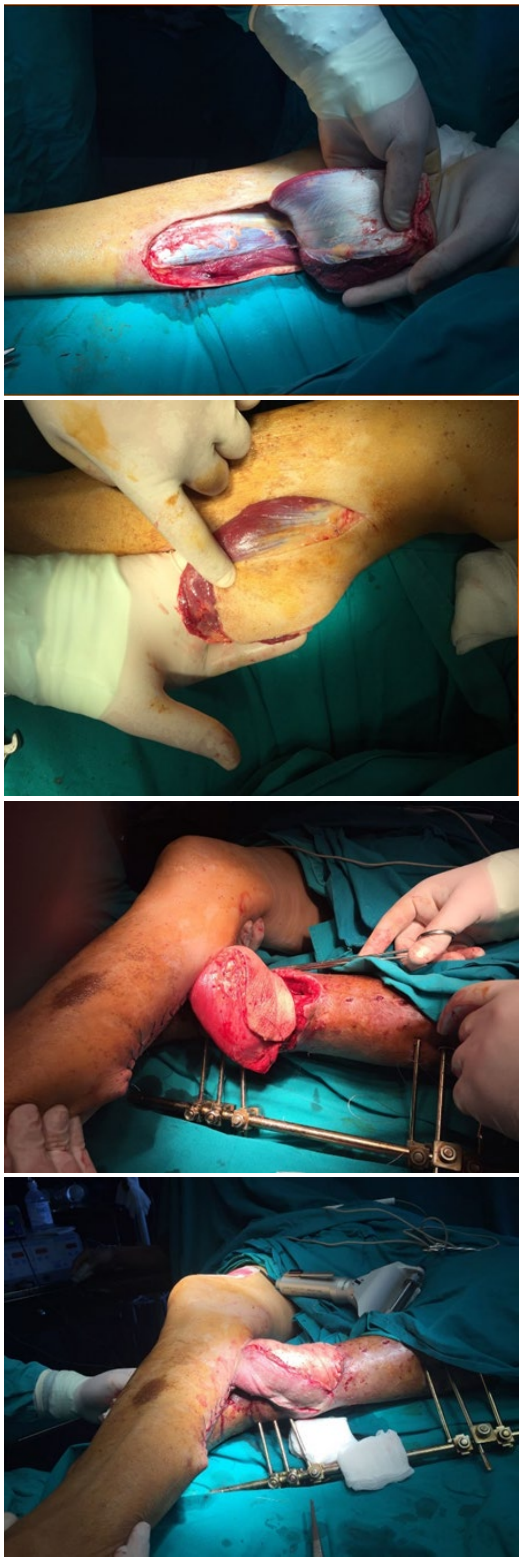

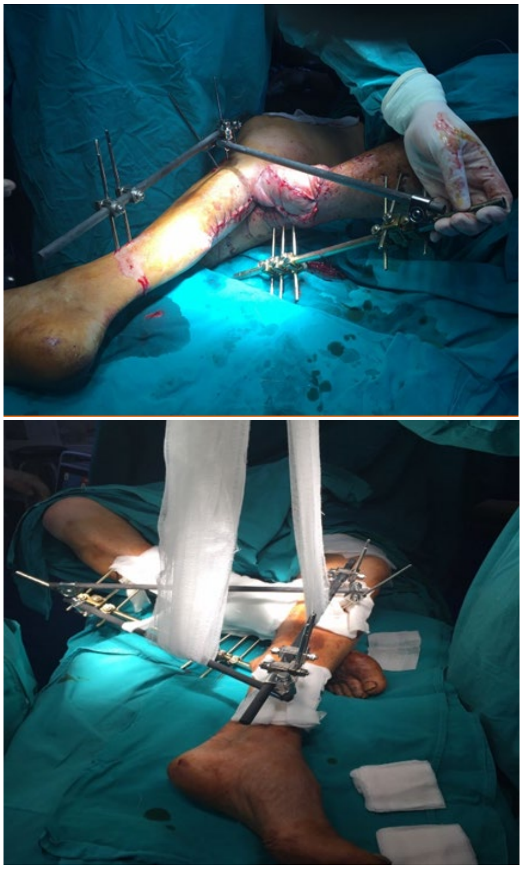

After proper preoperative markings, the flap was raised including the fascia. The inclusion of fascia in the flap makes the length-to-breadth ratio 3: 1 perfectly safe [3]. This allows much greater area of skin to be transferred with much more freedom of leg position. The donor site was covered using direct sutures and a split skin graft was also used to cover the donor muscle flap [4].

OUTCOME AND FOLLOW-UP

The release of the flap was performed at 21 days and the unaffected contralateral member was removed from fixation [5]. The patient continued treatment by monoplane external fixation for 8 weeks with progressive mobilization with the support of the unaffected limb. The patient evolution was satisfactory also making regular X-rays checks for the consolidation of the fracture.

At 4 months after the trauma, the ablation of the external fixation [6] was decided and the patient resumed progressive mobilization of both lower limbs. With the presence of the consolidated fracture, at 4 months postoperatively the patient successfully achieved a score of 62 according to Clinical rating system for the ankle and hindfoot [7].

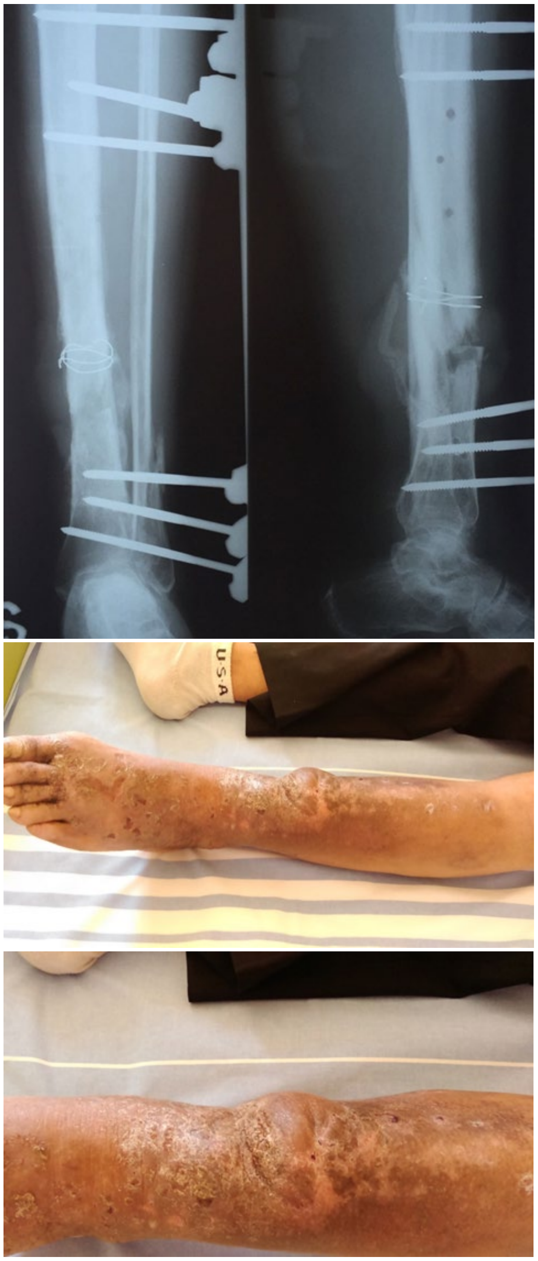

CONCLUSION

The cross-leg Filatov flap is still considered a safer and more reliable solution to free tissue transfer in certain situations of lower-limb trauma, especially open distal tibial fractures in which rotation flaps are not a viable solution.

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CLINICAL PRACTICE

Clinical and dermatoscopical aspects of pigmented basal cell carcinoma – Case series and literature review

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Abstract: *The diagnosis of pigmented basal cell carcinoma can bring significant distress to patients and physicians alike. However, astute clinicians that can employ correctly the clinical and dermatoscopical clues can reach a correct preoperative diagnosis, and as such can avoid unnecessary worry for the patient, additional work-up, or overtreatment. We present a case series of pigmented basal cell carcinomas and a comparative case with pigmented cutaneous melanoma, together with a literature review, that will highlight these aspects of the management of pigmented tumours of the skin.*

Keywords: *dermoscopy, basal cell carcinoma*

INTRODUCTION

Pigmented tumours of the skin bring significant distress to patients and to physicians alike. While pigmented basal cell carcinoma is not a benign tumour, its prognosis is highly favourable if it is diagnosed and treated early. The dermatologist is also in the position to tell the patient that even if the tumour to be excised is a malignant tumour, the treatment, i.e the excision of the tumour, will result in the complete healing of the lesion, with a low probability of local recurrence.

We present a case series of pigmented basal cell carcinomas and a review of the literature in an effort to show that this particular pigmented tumour of the skin can be easily identified by the experienced dermatologist with the aid of clinical and dermatoscopical clues.

REVIEW OF LITERATURE

Background

One of the oldest references [1] to pigmented basal cell carcinoma (pBCC) in PubMed involves a differential diagnosis with cutaneous melanoma. Indeed, the spectrum of pigmented cutaneous melanoma (PCM) can plague the management of a pigmented lesion of the skin leading to overtreatment, if a wide excision of the supposed PCM is intended, and/or to unnecessary worry of the patient and extended additional work-up, e.g. lymph-node ultrasonography, lung radioscopy etc.

Goldenberg et al [15] describe the age-adjusted incidence of BCC in American population to be 226 per 100000 persons and the age-adjusted prevalence of BCC to be 342 per 100000 persons.

Maloney et al [13] and Betti et al [14] describe the histopathology epidemiology of pBCC to be of around 6.7% and 8.5% respectively of the total cases of BCC taken into consideration.

Altamura et al [17] found that actually the dermatoscopically

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non-pigmented BCCs can be as low as 15.1%, and the rest of the BCCs have some degree of pigmentation, from low to heavy. They [17] have not used the diagnosis of pBCC according to the histopathology, but according to the dematoscopical features pigmentation, albeit all lesions were confirmed with histopathology to be BCC.

Both studies, of Maloney et al [13] and Betti et al [14] found that pBCC has a better prognosis with clear excision margins than the other types of BCC. A similar conclusion is reached also by Ito T et al [6] by saying that narrow-margins excision of 3mm may be enough to obtain clear margins for pBCC, as opposed to 4mm margins required for classic non-pigmented BCC.

Clinical aspect

The clinical aspect of BCC should not be disregarded as we consider that an astute clinician can observe significant clinical clues that can lead him to an appropriate preoperative diagnosis.

Bolognia et al [11] describe the clinical aspect of a nodular BCC as “shiny, pearly papule with a smooth surface and the presence of arborizing telangiectasias”. They [11] also describe a “thin rolled border” in superficial BCC.

Wu et al [12] describe the clinical aspect of BCC to have a “pearly or translucent quality” with telangiectasias, the lesions having a “periphery more raised than the middle” – a rolled border. In addition, they [12] say that “a shiny quality” can be observed when a superficial BCC is illuminated.

Huang et al [10] used translucency as a clinical indicator to learn computer aided systems to detect BCC and achieved a 93% accuracy.

Dermatoscopy

Dermatoscopy, if correctly employed, can be an invaluable tool in diagnosing BCC and especially pBCC.

A very thorough article on the dermatoscopy of pBCC is the one of Menzies et al [5]. They established that there are several highly specific dermatoscopical patterns, e.g. “maple leaflike areas” and “spoke wheel areas” that have a specificity of 100%, albeit a low sensitivity of 17% and respectively 10% [5, 16]. Other dermatoscopical criteria with high specificity were “large grey-blue ovoid nests”, “multiple grey-blue globules” and “arborizing (treelike) telangiectasia” [5 , 16].

Marghoob et al [7] also describe several specific dermatoscopy aspects for pBCC: large blue-grey ovoid nests, multiple blue-grey globules, leaf-like structures, spoke-wheel-like structures, arborizing telangiectasias and ulceration.

Kittler et al [8] describe the dermatoscopic patterns of pBCC as “radial lines, dots, clods and structureless zones” and they say that a common pattern is blue clods of different shapes and sizes. In addition, other structures may appear, such as brown clods, grey, blue and brown dots, white structureless zones and radial lines. They [8] also describe patterns that are very strong indicators against BCC, e.g. non-radial lines (reticular, branched, curved and parallel), pseudopods and circles. Also, the description of the typical vessels found in BCC would be: “branched serpentine vessels that originate from thick stem” – usually found in nodular BCC, while in superficial BCC a polymorphous pattern of vessel can appear, e.g. thin, serpentine and coiled vessels. The pattern of dot vessels is a strong indicator against BCC [8], unless found within ulceration.

Cabo et al [9] considers the dermatoscopical pigmented structures, e.g. maple leaflike areas, spoke-wheel area, multiple blue-grey dots or small globules, blue-grey ovoid nests to be characteristic for pBCC, while “white shiny blotches and strands” are an additional criterion only seen with polarized dermatoscopy.

Felder et al [18] and Turkmen et al [19] describe a dermatoscopical blue-white variant of pBCC. This type of pBCC is included in the class of heavily pigmented BCC as described by Altamura et al [17]

Lallas et al [21] describe dermatoscopic features to differentiate between superficial BCC (e.g. maple-leaf like areas and short, fine telangiectasias) and nodular BCC (e.g. blue-grey ovoid nests, arborizing vessels, ulceration). Wozniak - Rito et al [20] agree with these findings.

Additional work-up

Usually additional work-up is not necessary for the diagnosis of pBCC. However, cytology i.e fine needle biopsy can be used [3] in certain cases that the clinical and dermoscopical aspects are not enough to establish a certain diagnosis. Usually this happens when the lesions are pure nodular, without a superficial part.

CASE SERIES

Case 1

A 59 years old female patient presented to our dermatology department for a 2/2 cm, ulcerated, pigmented lesion in the left axilla. The lesion had a history of about 8 years. The patient also had a personal history with another BCC lesion operated 10 years prior to this admission.

The clinical aspect of the lesion (Figure 1):
- central ulceration with serohematic crust on the surface,
- the non-ulcerated part of the lesion has a translucent greyish feature with superimposed dark-brown, blue, black punctate pigmentation
- the periphery of the lesion has a pearly pigmented aspect
- the lesion has a smooth-shiny surface that does not continue the adjacent normal skin architecture

Figure 1: The clinical aspect of Case 1 has a very specific pearly-pigmented aspect of the periphery of the lesion along with a translucent quality

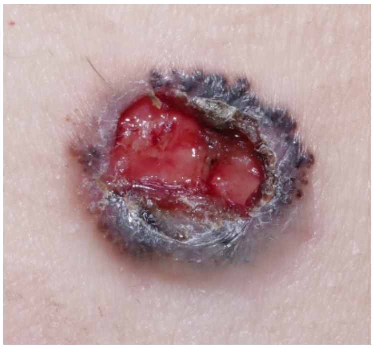

The dermatoscopical aspect of the lesion Figure 2:
- brown and blue-black-grey ovoid nests
- leaf-like areas (high specificity for pBCC)
- fine telangiectasias
- ulceration

Figure 2: The dermatoscopical aspect of Case 1 has leaf-like areas and brown and blue-black-grey ovoid nests

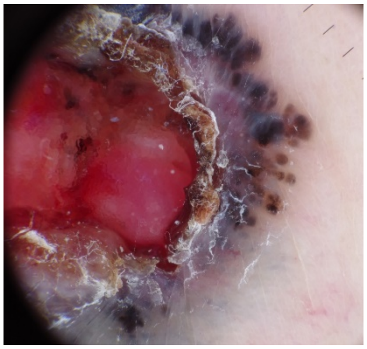

The lesion was excised with 4 mm margins and the histopathological examination established the diagnosis of pBCC, (Figure 3) with islands of basaloid cells with peripheral palisading, clefts between the islands and the stroma, and focal deposits of melanin within the basaloid islands.

Case 2

A 63 years old male patient presented to our dermatology department for a 1.5/2cm pigmented lesion on the posterior thorax. The lesion had a history of about 1 year. The patient had no significant medical history.

Figure 3: The histopathology of Case 1 established the diagnosis of pigmented basal cell carcinoma

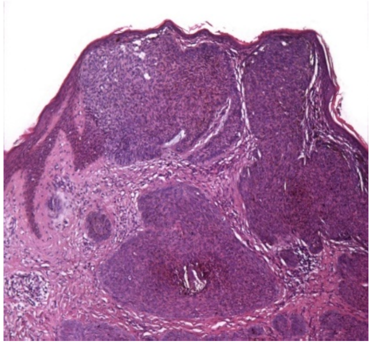

The clinical aspect of the lesion (Figure 4):
- small central ulceration
- translucent feature of almost the entire lesion with pigmentation – large pearly structures pigmented bluish-black
- shiny smooth surface that does not continue the architecture of the adjacent normal skin

Figure 4: The clinical aspect of Case 2 has an overall pigmented pearly and translucent feature with shiny smooth surface

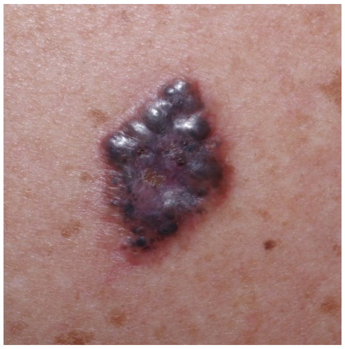

The dermatoscopical aspect of the lesion (Figure 5):
- blue-grey ovoid nests and globules
- arborizing telangiectasias
- the polarized dermatoscopy image showed white lines and blotches

The lesion was excised with 4mm margins and the histopathological diagnosis confirmed it was a pBCC.

Case 3

An 82 years old female patient, with no significant medical history, presented to our dermatology department for a 0.7/0.7 cm pigmented lesion on the left thigh. The lesion had a history of about 6 months.

Figure 5: The dermatoscopical aspect (polarized) of Case 2 has blue-grey ovoid nests, arborizing telangiectasias and white lines and blotches

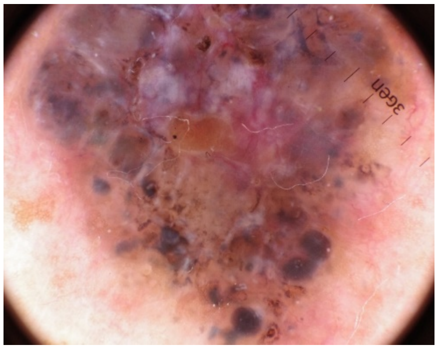

The clinical aspect of the lesion (Figure 6:

- central ulceration with serohematic crust on the surface
- translucent aspect the lesion with a general violaceous – bluish aspect and some brown pigmentation on the periphery of the lesion
- shiny smooth surface that disrupts the normal adjacent skin architecture (Figure 7)

Figure 6: The clinical aspect of Case 3 has a translucent violaceous-bluish aspect and a shiny smooth surface

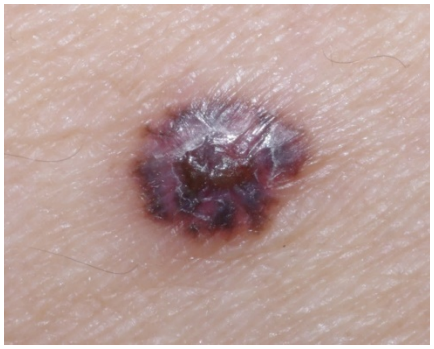

Figure 7: The particular aspect of shiny smooth surface that disrupts the normal adjacent skin architecture

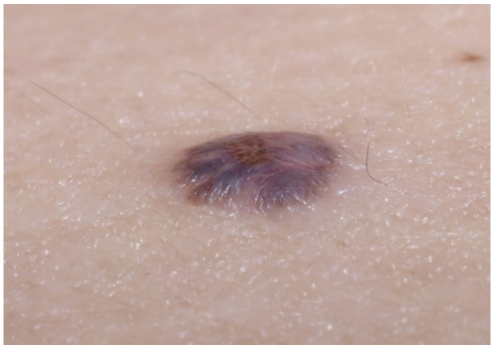

The dermatoscopical aspect of the lesion (Figure 8):

- blue-grey ovoid nests
- arborizing telangiectasias
- leaf-like structures (within the brownish periphery pigmentation)
- ulceration
- white lines and blotches (observed with polarized dermatoscopy)

Figure 8: The dermatoscopical aspect (polarized) of Case 3 has leaf-like areas and blue-black ovoid nests, arborizing telangiectasias and white lines and blotches

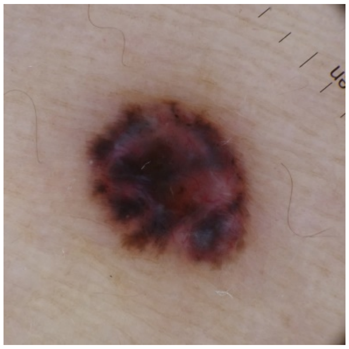

The histopathology of this lesion (Figure 9) confirmed the clinical and dermatoscopical diagnosis of pBCC, observing islands of basaloid cells with peripheral palisading, clefts between the islands and the stroma as well as deposits of melanin within the basaloid islands.

Figure 9: The histopathology of Case 3 observed islands of basaloid cells with peripheral palisading, clefts between the islands and the stroma, deposits of melanin within the basaloid islands reaching the diagnosis of pigmented basal cell carcinoma

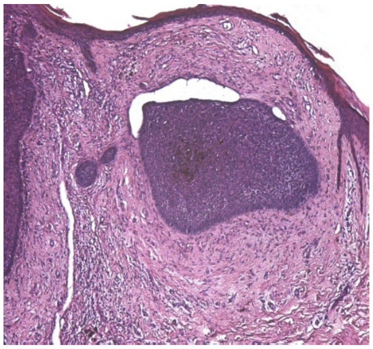

Case 4 – Comparative case

As our fourth case we present a lesion that highly resembles the clinical appearance of the Case 3 lesion (Figure 6), however the dermatoscopical aspect of this lesion has a rather conspicuous lack of clues for any specific diagnosis.

A 66 years old male patient, with no significant medical history, presented to our dermatology department for a 0.7/1.4 cm pigmented lesion on the abdomen. The lesion had a history of about 6 months.

The clinical aspect of the lesion (Figure 10):
- ulceration with serohematic crust
- pigmentation – pink – red – violaceous – black
- textured surface – tends to keep the architecture of adjacent normal skin, even if somewhat disorganized (the surface is not smooth - shiny)
- ovoid/rather rectangular shape

Figure 10: The clinical aspect of Case 4 observed rather similar type of pigmentation with Case 3, i.e. pink – red – violaceous – black, however with a textured surface (as opposed to Case 3, here the surface is not shiny)

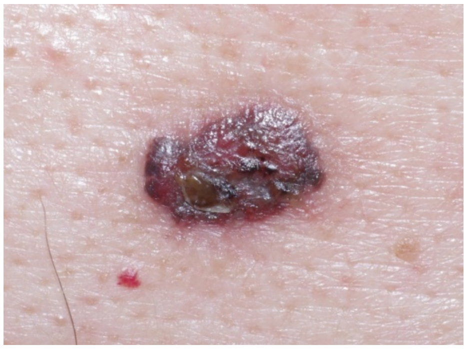

The dermatoscopical aspect of the lesion (Figure 11):
- ulceration
- pink – red – violaceous colour
- white lines and blotches on the periphery of the lesion (polarized dermatoscopy)
- lack of specific clues for pBCC (blue –grey ovoid nests, leaf-like structures, arborizing telangiectasias)

Figue 11: The dermatoscopical aspect of Case 4 has pink – red violaceous colour with lack of specific clues for pBCC

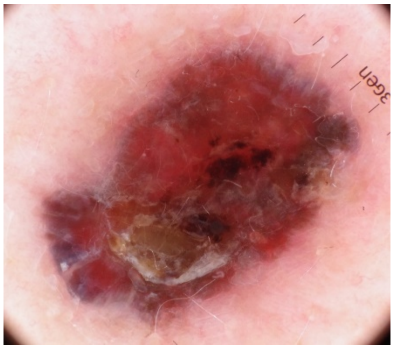

The histopathology of this lesion (Figure 12) reached the diagnosis of pigmented cutaneous melanoma (PCM), with a Breslow thickness of 0.8 mm.

As a discussion of Case 3 vs Case 4, Figure 13 presents a comparative view of both the clinical and dermatoscopical aspects of both lesions. On the clinical part, both lesions are ulcerated and have somewhat the same type of pigmentation.

Figure 12: The histopathology of Case 4 established the diagnosis of pigmented cutaneous melanoma, Breslow thickness of 0.8 mm, Clark IV

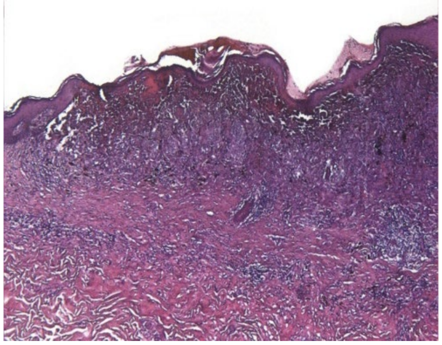

However the clinical clue of smooth – shiny surface that disrupts the normal adjacent skin architecture can be found in Case 3 and not in Case 4 where we find a textured surface of the lesion that tends to keep the normal adjacent skin architecture, even if somewhat disorganized. On the dermatoscopical part, both lesions present ulceration and similar type of pigmentation. However, while the Case 3 dermatoscopy image has conspicuous clues for pBCC, Case 4 dermatoscopy has a discomforting lack of clues for pBCC or for other pigmented lesion of the skin. Even though the polarized dermatoscopy clue of white lines and blotches are present in both Case 3 and Case 4, this comparative case shows that this particular clue cannot be used to differentiate between pBCC and PCM.

Figure 13: Comparative view of clinical and dermatoscopical aspects of Case 3 and Case 4

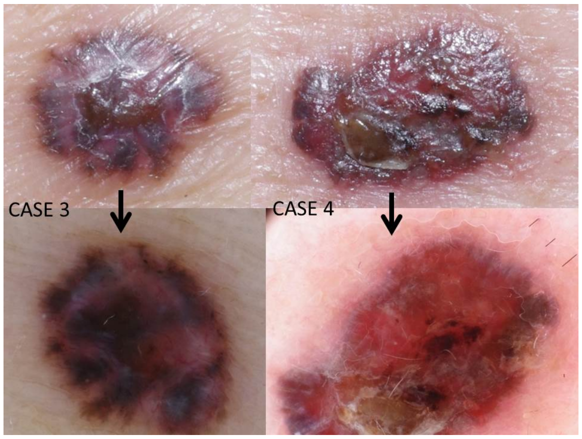

DISCUSSIONS

Regarding the epidemiology of pBCC we think that if the clinical and dermatoscopical aspect of pigmented structures within a BCC lesion are taken into consideration when establishing the pBCC diagnosis, the percentage may be significantly higher than what the studies of Maloney et al [13] and Betti et al [14] are suggesting, 6.7% to 8.5% . This may be because even in clinically non-pigmented BCC, the dermoscopy may reveal some pigmentation [17, 22], e.g. leaf-like structures, blue-grey ovoid nests or globules, and these structures may not appear in a given histopathology slide. To prove our point, Altamura et al [17] found that some degree of dermatoscopical pigmentation could be found in almost 85% of the BCC lesions. Therefore, the quantitative feature of the pigmentation may have an important role to play in reaching a histopathological diagnosis of pBCC.

To our knowledge, the clinical clue of disruption of the adjacent normal skin architecture within the lesion of a superficial pBCC has not been clearly described so far. There are papers that describe the “shiny quality” [12] or “shiny… smooth surface” [11] in superficial BCC and we think it refers to the same clinical quality. We consider this clinical clue important because superficial spreading cutaneous melanoma tends to conserve adjacent normal skin architecture within the lesion, as opposed to pBCC.

The clinical clue of pigmentation, even though is the most worrisome in the differential diagnosis of pBCC with PCM, should be observed within the overall aspect of the lesion, e.g. pearly translucent feature, rolled border.

If the clinical aspect of a suspected pBCC lesion is not enough to reach a correct diagnosis, usually dermatoscopy comes with supplementary clues. Moreover, some clues are highly specific for pBCC, e.g. leaf-like structures and spoke-wheel like structures [5] [7] [9]. Other clues that are indicative for pBCC are blue-grey ovoid nests, globules and dots, arborizing telangiectasias [5] [7] [9] (brown clods, grey, blue and brown dots, branched serpentine vessels that originate from thick stem [8]).

Even though the clinical and dermoscopical aspects of the pBCC are usually enough to diagnose a pBCC, there are papers [2 -4] that do not use dermoscopy as an aid.

On the other hand, papers focusing on dermatoscopy tend to overlook the clinical aspect of the lesion. As such, Turkmen et al [19], even though they present the clinical aspect of the lesions, the pictures are small and cannot be evaluated properly. In their article [19] the Case 3 and Case 6 seem to have a rolled border that may be a strong indicator for BCC, considering the fact that the dermatoscopical aspect, with blue-white veil cannot differentiate between pBCC and pigmented cutaneous melanoma (PCM).

While we consider the dermatoscopical aspect of pBCC of paramount importance, considering the highly specific clues that can simply establish the diagnosis of pBCC, we also consider the clinical clues very important, especially when the dermatoscopical clues are lacking, as we showed in our case series.

Considering the data regarding the clearing of margins in the pBCC management [6] [13] [14] we think that a careful evaluation, clinical and dermatoscopical, of a pigmented lesion can avoid unnecessary worry for the patient, additional work-up and overtreatment.

CONCLUSION

We consider that the clinical and dermatoscopical aspects, as well as the histopathological diagnosis should have a synergy within the management of pBCC.

Therefore, the clinical aspect of pearly border, translucency, and shiny surface – disruption of the adjacent normal skin architecture should be combined with the dermatoscopical aspect of “leaflike areas”, “spokewheel areas”, blue-grey ovoid nests and globules as well as arborizing telangiectasias and the diagnosis of pBCC can be reached in a swift manner. By doing so one can avoid unnecessary patient worry and additional work-up as well as one can adapt the treatment according to the diagnosis. Nevertheless, a biopsy should always confirm the clinical/dermatoscopical diagnosis.

Author Contributions:

The individual contributions of the authors is as follow: conceptualization, A.M.T., M.A.B.; methodology, A.M.T., B.D.; validation, G.S.T, V.T; formal analysis, A.M.T.; writing—original draft preparation, A.M.T.; writing—review and editing, D.O.C., R.S.C.; supervision, G.S.T.

Conflicts of Interest:

The authors declare no conflict of interest.

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VARIA

Compliance of school doctors' practice with medical legislation

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Abstract: *School doctors' practice comprises specific aspects governed by legal rules. Knowledge and compliance with the applicable legislation are necessary in order to ensure respect of the patient's rights, to avoid claims of medical malpractice and to assure medical act's quality.*

The present study investigated compliance of school doctors' practice with the applicable legal requirements.

All respondent doctors violate the applicable legal requirements. Future quantitative research is necessary to measure the amplitude of the identified vulnerabilities.

INTRODUCTION

School doctors' assistance implies ensuring special conditions for children with chronic diseases, coordinating the optimal response during medical emergencies and managing school environment's health and safety.

Sports competitions for students represent another special feature in this field, given the school doctor's task of issuing a favourable notice for competition to students who participate in such competitions following a brief clinical examination, without always having the student's personal background checks or the possibility to send the student to a specialist for control, without having an appropriate emergency kit and under improper conditions for a medical act which can all generate problems related to malpractice.

Specific aspects of their activity expose them in case of ignorance and non-compliance of the applicable legal regulations.

RESEARCH OBJECTIVE

The assumption under which our research was initiated was that school doctors from Bucharest are not fully aware and do not fully comply with the legislation in force.

The research objective was to collect information regarding the compliance of school doctors' practice with the applicable legal requirements.

MATERIAL AND METHOD

The present research aimed to identity the existence of a phenomenon (the issue regarding compliance of school medical activities with the applicable legal provisions) and to establish the framework for possible future quantitative research addressed to the newly developed area of interest.

The target group included 25 general practitioners activating as school doctors in Bucharest. Sample was selected taking into account the qualitative objects of the research, in the sense that we aimed to identify possible specific tendencies among school doctors. The investigative techniques were represented by interviews, which led to structuring of a questionnaire comprising five closed questions destined to evaluate the risk of malpractice in the school doctors'

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activity. Developed models because of previous research in the field of knowledge and compliance with medical legislation by doctors were used when preparing the questionnaire.

The questionnaires were then circulated and filled in anonymously during a workshop organized by the Administration of Hospitals and Medical Services Bucharest (Administrația Spitalelor și Serviciilor Medicale București - ASSMB) and which took place in Bucharest, on 8.12.2017.

Participants were asked to answer the questions based on their daily practice and not on theoretical knowledge or personal opinions regarding the respective matters.

Moreover, respondents were informed that their answers would be used exclusively for scientific purposes, for performing an objective valuation of the malpractice risk resulting from non-compliance with the legal framework applicable to school doctors' practice.

Definition of the valuation criteria for answers to the questionnaire's inquiries

Thus, the interviews aimed to investigate if medical practice of respondent doctors respects the applicable legislation.

The doctors' answers were marked as "correct" if they indicated the fact that the practice of the respondent doctor respects the legal requirements involved by the question or "wrong" when the answer demonstrated non-compliance of the law by the respondent doctor during his daily medical activity.

In addition, the alternative answers selected by respondent doctors were analysed in detail with the purpose of identifying some models of practice and their justifications. Programme IBMSPSS Statistic, version 20 was used for processing the obtained data.

RESULTS AND DISCUSSION

General results

None of the respondent doctors chose the "correct" answer to all the questions. Therefore, we can consider that, in their current activity, respondent doctors do not fully respect the legal framework applicable to school doctors.

Specific results

Based on received answers we identified some domains of medical practice in which specific legal requirements are not fully respected: informed consent, confidentiality of medical data, patients' access to personal medical information, filming/photographing of patients and limiting the doctor's activity to one's own medical specialty.

Question no. 1

"How do you proceed when a patient requires health care for an associated disorder beyond the scope of competence of your specialty?"

Proposed alternatives to answer:
a) I do not provide health care, regardless the patient's status.
b) I provide health care after consulting a specialized doctor in the field of the respective disorder.
c) I provide health care in emergencies.

The correct answer (to be chosen between the proposed alternatives) is: c)

The general representation of answers to Question no. 1 is included in Table 1 and Chart 1.

Chart 1: Representation of answers to Question no. 1, according to valuation: correct/wrong.

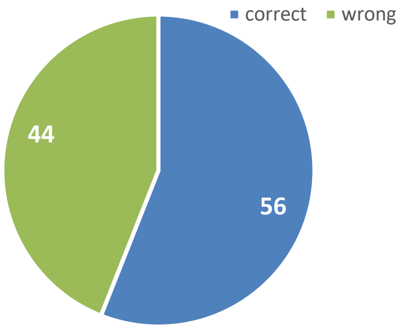

Table 1: Answers to Question no. 1

		Frequency	Percent	Valid percent	Cumulative percent
Valid	a	1	4.00	4.00	4.00
	b	4	16.00	16.00	20.00
	b, c	5	24.00	24.00	44.00
	c	14	56.00	56.00	100.00
	Total	25	100.00	100.00	

It may be noticed that 44% of answers were wrong. In order to identify the respondent doctors' attitude concerning limiting the medical practice to its own specialty we performed a detailed analysis of the received answers to each question, the results being included in Table 1 and Chart 1.

The correct action for the situation described by this question from the questionnaire is prescribed by legal texts: the medical practitioners are civilly liable for the damages caused during the exercise of their profession and when they exceed the limits of their competence. In case of some associated disorders', each of these conditions will have to be supervised/diagnosed/treated by a doctor specialized in the respective area of expertise/competence. Therefore, the legislation includes an interdiction for doctors to perform medical acts that are not covered by their specialty.

There is an exception to this rule in case of emergencies when there is an imminent danger/irreversible deterioration of patient's health state and when a medical practitioner with the necessary competence is not available. [3]

It may be noticed that 24% from respondents included the correct alternative c), but they also added alternative b) (which is wrong), while 16% from respondents mentioned only alternative b) as being correct.

The attitude of respondent doctors is to provide health care beyond their specialty in every situation after they ask a medical practitioner having the necessary area of expertise. These attitudes do not comply with the abovementioned legal requirements.

In situation when all 3 conditions are cumulatively met (urgency, imminent danger for patient's life/irreversible deterioration of patient's health state, lack of specialized medical practitioners) medical activities beyond one's area of expertise can be performed without the need to consult another medical practitioner.

Question no. 2

"*Can information regarding the treatment prescribed to a patient be communicated to third parties?*"

Proposed answers:
a) Yes, to non-governmental organizations which support the patients.
b) Yes, to patient's family members.
c) No.

The correct answer (to be chosen from the proposed alternatives) is: c).

According to the law, the doctor cannot disclose to any other person than the patient data regarding its health state. Information concerning patient's status, results of medical investigations, diagnosis, prognosis, treatment and personal data are confidential, even after the patient's death.

General representation of answers to Question no. 2 is included in Table 2 and Chart 2.

Wrong answers were generated by the doctors' attitude to communicate medical data to patient's family members. It may be supposed that doctors chose this answer given the fact that patients' majority (with the exception of students) are minors.

Chart 2: Representation of answers to Question no. 2, according to valuation: correct/wrong

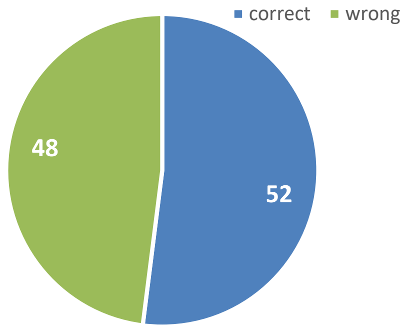

Table 2: Answers to Question no. 2

		Frequency	Percent	Valid percent	Cumulative percent
Valid	b	12	48.00	48.00	48.00
	c	13	52.00	52.00	52.00
	Total	25	100.00	100.00	

However, the question did not refer to minor patients. Even if we referred to minor patients, not every family member is a legal representative (there are various circumstances prescribed by law in which parents, relatives, other people can be legal representatives for a minor patient). Therefore, not even under this scenario, the alternative b) would have been correct. This represents more a vulnerability of respondent doctors' practice with respect to compliance of medical practice with the abovementioned legal requirements.

Question no. 3

"*The informed consent of the patient may be obtained*:"

Proposed answers were the following:
a) From the patient attaining the age of 18.
b) From a minor patient under some circumstances.
c) From one of the minor patient's parents.

Correct answer to this question: a), b), c).

According to law, the statutory age for giving an informed consent is 18.

Minor patients may give their consent in absence of parents or legal representative, in medical situations related to the diagnosis and/or treatment of sexual and reproductive problems, when specifically requested by a minor patient who is over 16 years old.

The important amount of wrong answers (96%) requires an in-depth analysis of answers to this question.

Chart 3: Representation of answers to Question no. 3, according to valuation correct/wrong

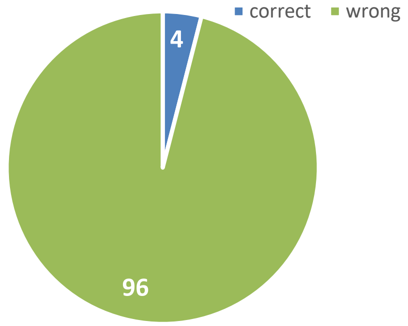

Table 3: Answers to Question no. 3

		Frequency	Percent	Valid percent	Cumulative percent
Valid		1	4.00	4.00	4.00
	a	3	12.00	12.00	16.00
	a, b, c	1	4.00	4.00	20.00
	a, c	20	80.00	80.00	100.00
	Total	25	100.00	100.00	

80% from respondent doctors only selected two of the three correct answers. Therefore, the legal prescription, which allows obtaining an informed consent from minor patients, which are at least 16 years old for sexual and reproductive problems, is not known and respected. Surprisingly, 12% of respondents did not identify the answers as being applicable to medical practice given the fact that these people consider that it is mandatory to obtain the informed consent from both parents of a minor patient.

Question no. 4

"*Should medical data concerning undertaken investigations, established diagnosis and recommended treatment be made available to the patient?*"

The proposed alternatives to answer:
a) It is not necessary; when discharging the patient from hospital, the treatment scheme and the necessity to come back for control should be explained to the patient.
b) Yes, always, in a complete manner.
c) Information related to diagnosis and established treatment should be made available to the patient"

The correct answer (to be chosen from the proposed alternatives) is b).

According to law no. 46/2003, the patient has unlimited access to all personal medical information and the doctor has to make it available to the patient and, in writing, if the patient requests it.

Almost half of respondent school doctors (44%) do not ensure unlimited access to personal medical information for patients/legal representatives.

Chart 4: Representation of answers to Question no. 4, according to valuation correct/wrong

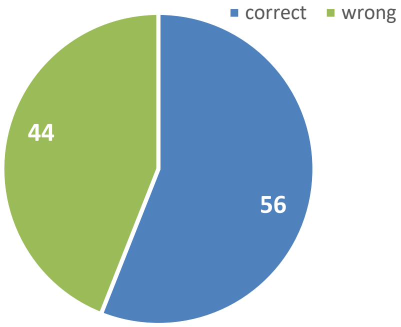

Table 4: Answers to Question no. 4

		Frequency	Percent	Valid percent	Cumulative percent
Valid		1	4.00	4.00	4.00
	b	15	60.00	60.00	64.00
	b, c	3	12.00	12.00	76.00
	c	6	24.00	24.00	100.00
	Total	25	100.00	100.00	

The analysis of chosen answers demonstrates that 24% from doctors consider that only information regarding diagnosis and treatment must be made available to the patient/legal representative.

This attitude may also be caused by an excess of legislation, which generates confusion. Thus, law no. 46/2003 was amended in 2016, the following article being added: "Article 12: the patient or the person expressly designated by the patient, according to articles 9 and 10, has the right to receive, when discharged from hospital, a written summary of investigations, diagnosis, treatment, medical care provided during hospitalisation and, upon request and once, a copy of high performance investigations' records.".

It may be generated the impression that information mentioned in this article could be accessed only by patients, but this interpretation would contradict article 9 of the norms for application with respect to Law no. 46/2003 regarding patient's rights which prescribes the following: "the hospital units must ensure unlimited access to personal medical data for patients ".

We believe that medical practice should take into account the most restrictive (unlimited access) legal requirement and this represents a correct attitude for a school doctor,

Question no. 5

"*May the medical personnel film the patient in the medical unit?*"

The proposed answers were the following>
a) After obtaining the patient's written consent.
b) By protecting the patient's identity.
c) For teaching and research purposes exclusively.

The correct answer (to be chosen from the proposed alternatives) is b).

The patient cannot be photographed or filmed in a medical unit without his consent, with the exception of the situation when the images are necessary for diagnosis and treatment and for avoiding the suspicion of medical malpractice.

Therefore, the medical personnel may film/photograph without the patient's or the legal representative's consent if the confidentiality of data which is obtained throughout this means is protected.

Chart 5: Representation of answers to Question no. 5, according to valuation correct/wrong

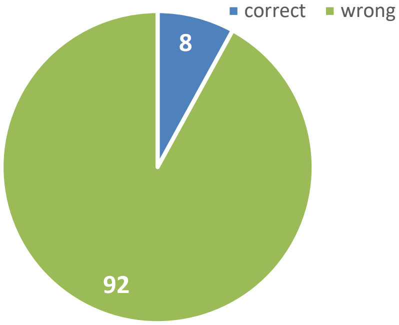

Table 5: Answers to Question no. 5

		Frequency	Percent	Valid percent	Cumulative percent
Valid		1	4.00	4.00	4.00
	a	11	44.00	44.00	48.00
	a, b	2	8.00	8.00	56.00
	a, b, c	6	24.00	24.00	80.00
	a, c	1	4.00	4.00	84.00
	b	2	8.00	8.00	92.00
	b, c	1	4.00	4.00	96.00
	c	1	4.00	4.00	100.00
	Total	25	100.00	100.00	

Most of wrong answers are generated by respondent doctors' conviction that they need patient's consent for filming/photographing him/her. It may be noticed that there are many alternatives of answers selected for this question that suggests the ignorance of the specific legal framework.

CONCLUSIONS

School doctors' practice does not respect entirely the applicable legal framework.

The consequences are that civil liability may be incurred against doctors and the protections of malpractice assurance ceases. Moreover, respecting patients' rights represents an element to measure medical act's quality, as defined by the National Authority of Quality Management in Health (Autoritatea Naţională de Management al Calităţii în Sănătate – ANMCS) throughout specific accreditation standards for medical units. Extensive further studies are necessary in order to determine this phenomenon's amplitude and configuring some education projects destined to cover the identified educational need is necessary.

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In memoriam – General (Ret.) Academician Prof. Vasile Cândea MD, PhD

A FLAME WENT OUT, A LIFE DEDICATED TO SCIENCE

On the morning of January 14, 2020, people working is the "Dr. Carol Davila” University Central Emergency Military Hospital (SUUMC) found out, with great regret and pain, about the passing away of one of Romania's greatest scientific personalities, a great physician, a visionary who put his whole life, intelligence and work power in the service of people and for the promotion of science, General (in retirement) Academician Professor Vasile CÂNDEA Ph.D., one of the parents of contemporary Romanian medicine, President of the Romanian Academy of Scientists and founder of the Army Emergency Clinical Center of Cardiovascular Diseases.

For us, it was a privilege to be contemporary with a scientist, a scholar, a Man like Vasile Cândea, with the Professor, the General, the Doctor, the Surgeon, the President, the Founder Vasile CÂNDEA, a Man who discovered the vocation of his existence in saving lives, working, studying, researching, in order to help and cure people.

Unfortunately, there are and were few such personalities, who managed to mix, at a higher level, professional, creative, scientific, managerial, relational and psycho-moral qualities, as Professor Cândea did. With a broad scientific and cultural horizon, with a comprehensive and profound understanding of man and his problems as a biological and social being, he made his voice heard in a constructive and creative sense as a great authority, not only in his professional activity - medicine, respectively, cardiology and cardio-vascular surgery, but also in the management positions of high responsibility that he exercised, without interruption, for more than four decades (1976-2017), in different national and international forums and institutions.

There would be much to say and even more to write about the strong impact of the personality of General Academician Prof. Vasile Cândea on society and especially on the destiny of Romanian medicine, but for editorial economy reasons, we will mention only a few historical landmarks.

Doctor Vasile Cândea, as he used to characterize himself and liked to be called, making proof of diplomacy and elegance, humbleness and love towards those suffering and especially of the beauty, fairness and respect inscribed in the DNA of the Romanian village of Bărăgan spirit as it was described by the great author Marin Sorescu, in the novel “Morometţii”, and additional to these, having an intact reputation, managed to cope both with the communist desert and with the difficult period of transition to democracy.

Professor Cândea started his activity in 1957 as a general practitioner and from April 1959 until October 1995 he worked in the Ministry of National Defense. At first he was a unit military physician from 1959-1962 (Timisoara, Constanta, Bucharest - Border Guard Command). Then he passed the clinical secondary exam - General Surgery 1962-1964 - Central Military Hospital of Bucharest, with the 3-month internship, Bucharest Emergency Clinic, Fundeni Urology Clinic in Bucharest, the Thoracic Surgery Clinic, then primary physician general surgery 1964-1972 - Surgery I Department from the Central Military Hospital, primary physician cardiovascular surgery (second specialty) - 1976, doctor in medical sciences in 1975 - confirmed in 1976, Head of Cardiovascular Surgery Department in the Central Military Hospital - 1976-1990, Manager of the Army Cardiovascular Diseases Center - May 1990-1995, Manager of the Institute of Cardiovascular Diseases “Prof. Dr. C.C. Iliescu ", Fundeni Bucharest, December 1995-2002, November 1996 - December 2000, Deputy in the Romanian Parliament, founding member, University Professor and Vice-Rector at" Titu Maiorescu" University of Bucharest, 1990-2003, Honorary Professor at" Ovidius "University Constanta , Faculty of Medicine and Pharmacy, April 2000, Consultant Professor, 2003-2008, "Titu Maiorescu" University in Bucharest, Consultant Professor, 2005-2008, Carol Davila University of Medicine and Pharmacy Bucharest.

Specializing in France, Austria, Israel, USA, he became the father of military cardiac surgery in Romania, he received the academic recognition of several scientific and academic, national and international forums out of which we mention: Academy of Scientists in Romania (President of Honor); Academy of Medical Sciences (Head of the Surgical Specialties Section); Romanian Society of Cardiovascular Surgery (ex-president, founding member); Balkan Medical Union (President of the Romanian National Section, 1992-1993, international secretary general - 1993 - until 2020); Mediterranean Medical Antanta (International Secretary General from 1993-2020); National Academy of Ecological Sciences of the Republic of Moldova (1999); Russian Academy of Natural Sciences (since 2007); Balkan Society of Angiology and Vascular Surgery (vice president; founding member); Romanian Phlebology Society (founding member); Romanian Society of Vascular Surgery and Angiology (founding member); European Society of Cardiovascular Surgery; International Society of Cardiovascular Surgery "Michael E. Debakey" (since 1976, New York); International Society of Angiology; Society of Military Doctors and Pharmacists.

Throughout his prodigious career, Academician Vasile Cândea received the Award of the Romanian Academy “Dr. Gh. Marinescu ”, 1975, the distinction“ The Man of the Year 2000 ”, offered by the American Biographical Institute, the National Order the Romanian Star – Knight degree, December 2002, the distinction of the Honorary Committee of the Diplomats of the Superior School of War in Brazil and of the Embassy of Brazil, September 7, 2009, Order "Military Merit", classes 3, 2, 1 and multiple diplomas of excellence, distinctions, medals and plaques, offered by scientific research institutes, universities, official state institutions (Ministry of Defense, Ministry of Internal Affairs, Ministry of Health, Bucharest City Hall etc.)

In 2007, he has been awarded the title of "Citizen of honor" of the village where he was born - Viişoara in Teleorman county. In 2011 he received the title of Citizen of honor of the city of Mioveni – Argeş County.

His contribution and involvement in the academic world earned him the title of Doctor "Honoris Causa" of several universities, such as: University of Medicine and Pharmacy Timisoara; "Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova; Ovidius University Constanta; Faculty of Medicine and Pharmacy; University of Oradea / Faculty of Medicine; "BIOTERRA" University; University of Medicine and Pharmacy Cluj Napoca; "Valahia" University Târgovişte; "Andrei Şaguna" University; National Defense University; "Apollonia" University, Iasi.

In the field of medicine, its name is linked to remarkable original contributions, with both national and international resonance, in the study and clinical-surgical treatment of cardiovascular diseases, lung, liver, pancreas and of the immune system.

Academician Professor Vasile CÂNDEA Ph.D. was a man with an exceptional moral standing and intellectual background, a true friend and a caring man who impressed many personalities and became the savior of thousands of lives. With each life saved he increased the nobility of the soul, he came closer to God, he became closer to his fellow human beings and, paradoxically, he became more humble and modest although, his personality is impressive and almost impossible to be described in a few words. He dedicated himself to others and gathered people around him, in the name of a common ideal: to dedicate his life to the rescue of other people lives. He set up and led research groups and surgical teams in difficult interventions which took hours, under the pressure of time. It happened more than often for him to be called in the middle of the night from home, because only him, the doctor Vasile CANDEA, was the guarantee and the hope that life will continue for the patient. He saved so many lives. So many people are still alive because the Good God has given this man, the grace and power to sacrifice himself for his fellow human beings.

More than two decades ago, Academician Professo Vasile CÂNDEA Ph.D. was a visionary of military medicine and not only of the latter, when he implemented, for the first time worldwide, an integrative solution for the problem of cardiovascular diseases, based on the idea of solving it in the format of a multidisciplinary team: clinical cardiology, interventional cardiology and cardiovascular surgery, since he was a promoter of teamwork.

Professor Cândea was the model and benchmark that we, those who had the chance to work and complete our career beside his impressive personality, as well as the new generations that come, still need.

We, all those who have had the privilege to be touched, even tangentially, by the light of the personality of the General Academician Professor Dr. Vasile CÂNDEA, cannot and do not want to tell him "Goodbye", because the spirit and values he has promoted throughout his career, will continue to be a natural and motivating presence in our medico-military and scientific world, as they have been up to present, and his name will continue to protect our destinies, being written on the frontispiece of the Cardiovascular Diseases Center of the Central Military Emergency University Hospital "Dr. Carol Davila"(SUUMC), all the necessary steps have already been taken for that.

In a world riddled with the avalanche of information for which there is no time for verification and validation, in which forms without essence are promoted, we, those left behind, promise you doctor that we will not get lost along the difficult way to performance, that we will stay united to defend and to preserve your professional values and standards, so that the legacy you leave us, will be handed to future generations, with pious gratitude while remembering you.

We have the honor to greet you, General Academician Professor Vasile CÂNDEA! May our tears and grief illuminate your path to angels, where you belong!

Condolences to the bereaved family! God rest his soul!

ADMINISTRATIVE ISSUES

Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine.* Please read the complete Author Guidelines carefully prior to submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review.

Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

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(3) Open to all prospective registrants free of charge or at minimal cost;
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(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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dorind a răsplăti meritele

redacției pubilcației „Revista de Medicină Militară",

în semn de apreciere a contribuţiei avute la creşterea prestigiului structurilor medicale din Armata României, cu ocazia aniversării a 120 de ani de la înfiinţare,

conferă ***Emblema de Onoare a Armatei României***
-cu însemn de pace-

Acordat în Bucureşti, 15 Noiembrie 2017

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Mihai-Viorel Fifor

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ORIGINAL ARTICLES

Diagnostic challenges in gastrointestinal infections

Nicoleta Negrut[1], Shamim A. Khan[2], Simona Bungau[3], Dana C. Zaha[4], Corb A.R. Anca[4], Ovidiu Bratu[5], Camelia C. Diaconu[6], Florentina Ionita-Radu[7]

Abstract: *Gastrointestinal infections are among the most common infectious diseases found all over the world, varying depending on the etiological agent. Symptoms usually include diarrhea, vomiting, and abdominal pain. Water and electrolyte imbalance is the main consequence of gastrointestinal infections. Most of them are cured or self-limited in few days, but at the same time, for a specific population such as immunocompromised, elderly patients or new-borns, these infections are potentially severe. In this context, it is very important to identify the etiological agents of acute diarrhea for the appropriate treatment and infection control measures. While routine laboratory diagnosis of parasitic diarrhea still depends largely on microscopic examination of fecal samples, immunological and molecular methods are becoming increasingly commercially available and, in well-resourced settings, will ultimately displace traditional methods. The present paper presents some of the most common and well-known pathologies of this type, being a brief presentation of the variety of gastrointestinal diseases, each with characteristic clinical manifestations and diagnosis.*

Keywords: *gastrointestinal infections, Rotavirus, Salmonella, Shigella, Helicobacter pylori*

INTRODUCTION

Worldwide, acute gastrointestinal infections are the second most common infectious diseases after respiratory tract infections and a major cause of morbidity and mortality among infants and children [1]. Gastrointestinal infections can be caused by a large number of microorganisms present in the water, food, or hospital environment, that cause inflammation of different segments of the gastrointestinal tract. The prevalence of the different microorganisms causing gastroenteritis depends on several factors, such as age (children, adults), community or nosocomial acquisition, country (developing or developed), patients who have traveled abroad and immune status [2].

The common clinical picture usually looks like acute gastroenteritis, with a sudden or insidious onset, immediately after or after a while from returning to the country of origin. At the first presentation, it is important to identify the patient's journey, with detailed information on particular aspects of the trip. Failure in identification of the epidemiological link may lead to delayed diagnosis and treatment, with increased risks of complications. Empirical intervention is often important when there is a strong possibility of a certain condition and confirmatory lab tests

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are delayed or unavailable, especially for infections that are rapidly progressive or with prolonged testing duration [3].

Traveling for business and pleasure is a small proportion of the total movement of people. Modern transport ways allow more goods and people to travel around the world at a faster pace; they also open up the airways to the transcontinental movement of vectors for infectious diseases. While traveling, people carry their genetic profile, immunological sequelae from previous illnesses, cultural preferences, traditions, and patterns of behavior. They are also accompanied by microbes, animals and other biological lives [4]. The range of infectious diseases present at a certain time and in a certain region has increased, with the increasing travel possibilities from one country to another and between continents. Transiting through different geographical areas, in a short time (as with long-distance flights), may increase the risk of contamination with foreign etiological agents. Cities are becoming important centers for the transmission of infectious diseases due to international travel and migration, as shown by recent pandemics. Several factors contribute to infectious disease emergence. Others commonly listed include microbial adaptation and change, demographics and behaviour, changes in the environment, technology and economic development, deterioration of public health and surveillance programs, and international travel and trade [5].

Many tropical diseases havea polymorphic clinical feature, making the diagnosis difficult, especially for clinicians from countries outside tropical areas. In developing and developed countries, doctors in urban settings need to be aware of developments in infectious diseases. Upon travel, an ill traveler's history must analyze exposure specifics, including visited destinations, activities, ingestion of infected food or drink, contact with insects, livestock, freshwater or blood and body fluids and other possible exposures. Information on the geographic dissemination of infectious disease is significant in creating the differential diagnosis and testing [6].

The purpose of this paper is to make a brief review of the most common gastrointestinal infections, with their relevant clinical features and diagnosis.

GASTROINTESTINAL INFECTIONS

In the geographical area of Romania, southeastern Europe, Rotavirus, Salmonella species, Shigella species, Clostridium difficile, Escherichia coli and Giardia lamblia are the most common etiological agents of gastrointestinal infections [7-9]. Infections with Helicobacter pylori, Campylobacter spp., and other viruses are also frequent. Several tropical infectious diseases, such as amebiasis, malaria, leishmaniasis, dracunculiasis, schistosomiasis, brucellosis, leptospirosis, dengue fever, yellow fever and viral haemorrhagic fever, can have a digestive onset, requiring increased attention from the clinician. Experimental animal studies and clinical research have shown that the virulence of infectious microbial agents can have variable severity, sometimes being involved even in deaths because of sepsis [10, 11].

Viral gastrointestinal infections

The most important etiological viral agents of diarrhea are rotavirus, norovirus, adenovirus, astrovirus, sapovirus and enterovirus. Gastroenteritis caused by norovirus, asingle-stranded RNA (ssRNA) viruses belonging to the family of Caliciviridae, may manifest with severe nausea, vomiting and diarrhea. The genogroup I (GI), II (GII) and IV (GIV) of Rotaviruses (double stranded RNA) are the main cause of diarrhea in children under 5 yo and responsible for the death of around 200,000 children/year. Rotaviruses belong to the Reoviridae family [12]. Major symptoms are usually abdominal pain, vomiting and waterydiarrhea (AWD). Rotaviruses serogroups A, B, and C are responsible for human infections.

Adenoviruses cause respiratory diseases, bladder infections, conjunctivitis. Adenoviruses belong to Adenoviriadae family of double-stranded (dsDNA) viruses. Taking into consideration 51 serotypes of human adenoviruses (classified into six groups, from A to F), gastroenteritis is mainly caused by Adenoviruses serotype 40 and 41.

Astroviruses discovered in 1975 are single-stranded (ssDNA) viruses and belong to the family of Astroviridae; the incidence of these infections is constantly increasing [13]. Clinical signs include vomiting and severe diarrhea, with moderate dehydration. In developed countries, the Astrovirus infection incidence is between 2-9%, affecting mainly children [14]. To date, serotypes 1 - 5 are the most relevant. Serological studies indicate that most children infected with Astroviruses develop antibodies to the virus early in life, which provide protection against future infections.

Sapoviruses are part of the family of Caliciviridae and are major causative agents of gastroenteritis worldwide, causing disease in humans of all ages. The highest incidence was described for children under 5 year-old, but infections are diagnosed in adults as well, with diarrhea, vomiting, and fever (similar clinical symptoms to those of norovirus infections). However, self-limited sapovirus infections lead to less severe gastroenteritis compared to norovirus-induced gastroenteritis [15].

There is a variety of different subtypes of human enteroviruses, such as human enterovirus 70/71, poliovirus, Coxsackie virus A and B, echovirus, with different degrees of tissue tropism and virulence, although retaining a basic antigenic identity. Infections are usually asymptomatic or present with mild cold-like symptoms. Severe enterovirus infections are poliomyelitis, meningitis and myocarditis. Coxsackie viruses are present worldwide and can cause the so-called “summer diarrhoea”. Other severe infections with Coxsackie virus or human enterovirus 70/71 can lead to conjunctivitis and myocarditis, whereas echovirus infections can result in aseptic meningitis, with echovirus 30 being the most common meningitis-causing serotype in Europe, America and Asia [16, 17].

Viral hemorrhagic fever (VHF) is characterized by severe multiple organ dysfunction syndrome. Etiologically, four particular RNA viral families (Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae) lead to VHF. The viruses that cause VHFs are spread across most of the globe. Haemorrhagic viruses are considered as potential biological warfare agents. Initially, clinical symptoms include fever, cephalgia, myalgia, arthralgia and chills, which can lead to massive haemorrhages, shock or even acute multiple organ dysfunction. Paraclinical parameters show an increase in liver enzymes and a decrease in albumin levels. The coagulation panel shows an increase in prothrombin time and a decrease in fibrinogen. These results of the paraclinical tests may be similar to diseases like disseminated intravascular coagulation, malaria, haemolytic uremic syndrome. Although the confirmation of the diagnosis requires detection of viral RNA, viral antigens, or specific antibodies, the clinician must raise the suspicion quickly, before laboratory results, because these diseases require strict isolation measures and biosafety level 4 laboratory facilities [18].

Dengue fever is a global arboviral infection spread by the mosquito genus Aedes (A) (primarily A. aegypti, but also A. albopictus, and A. polynesiensis), a mosquito found in the tropical and subtropical regions of the world, also involved in spreading Zika virus disease, yellow fever, and Chikungunya. About 40% of the world population, almost 3 billion people live in regions with a dengue threat [19]. In more than 100 countries, people are affected by dengue fever resulting in up to 22,000 deaths per annum globally [20]. In 2018, Romania reported 4 cases of dengue fever, all travel-associated [21]. With incubation of 3-14 days, 50-80% of patients are asymptomatic during the period of illness [22]. Classic dengue fever (break-bone fever) is distinguished by high fever, severe cephalgia, myalgia, arthralgia, retro-orbital pain, abdominal pain or tenderness with persistent vomiting, and hepatomegaly. The diagnosis is supported by the combination of thrombocytopenia, leukopenia, modifications of the liver function tests and serological tests for dengue virus.

Yellow fever virus etiologically belongs to Flaviviridae family, which causes acute viral disease transmitted by A. aegypti, and Haemagogus mosquitoes. The name's "yellow" refers to jaundice that affects most patients. In tropical regions of Africa and Central and South America, the virus is endemic. In 2018, one case of yellow fever was reported by Romania [21]. Severe cases can have a mortality rate of up to 50% [23, 24]. With an incubation period of 3-6 days, clinical manifestations in the second phase, which generally affect liver and kidney, consist in fever, vomiting, abdominal pain, jaundice, dark urine, hemorrhage, and even shock. Yellow fever, especially during early stages, is difficult to diagnose, it can be mistaken for diseases like leptospirosis, fulminant forms of viral hepatitis, or even severe malaria. Confirmation of the diagnosis requires the detection of the virus by identifying viral ribonucleic acid (RNA), specific antigen or immunoglobulins M.

Digestive infections caused by protozoans and parasites

Parasitic infections causing diarrhea are particularly important in young children, immunocompromised patients, and those living in conditions of poor sanitation and hygiene, with unsafe food and water supplies. Travelers from developed countries are another group at risk, particularly susceptible to parasitic pathogens, because they lack protective immunity acquired from previous exposure. Several of these organisms are of zoonotic origin, and there may be occupational or recreational exposure risks [25].

Giardia lamblia, Cryptosporidium spp., Entamoeba histolytica and Dientamoeba fragilis are the most important diarrhoea-causing protozoans. Helminths are less important as agents of diarrhea, but they may produce loose or even dysenteric stools in acute or heavy infections (for example, intestinal schistosomiases). One important exception is the nematode Strongyloides stercoralis, which has the unusual capacity to maintain subclinical infections for years or decades, being discovered only when the patient is immunosuppressed for various reasons, when severe or potentially fatal disseminated infection may occur [26].

Giardia lamblia infections (synonym G. Intestinalis, G. duodenales) in developed countries occur in 6-8% of children and 2% of adults, and around 33% of the population is infected with protozoans [27, 28]. In the US for example, CDC estimated 77,000 cases/year of Giardiasis [29].

One of the several species of the genus Cryptosporidium is

Cryptosporidium parvum, which, together with C. hominis, are the most common cause's ofcryptosporidiosis in humans [29]. Similar clinical symptoms could reveal infections by other Cryptosporidium spp. such as C. felis, C. meleagridis, C. canis, and C. Muris.Around 748,000 cryptosporidiosis cases/year occurred in the US [30, 31].

The causative agent of amoebiasis is Entamoeba histolytica - the only human pathogenic species of the genus Entamoeba. Amebiasis has a worldwide distribution, prevalent in Central and South America, Africa, and Asia, being produced by trophozoites of Entamoeba histolytica.

Clinical manifestations can appear up to 4 months from the contact with contaminated food or objects. In about 90% of cases, the disease may evolve asymptomatic, self-limiting, with recurrences. The rest of the patients may have different severity clinical forms, such as acute colitis (bloody diarrhea, fever, abdominal pain), severe colitis (fulminant, abscesses), and chronic form (ameboma, and inflammatory bowel disease). The etiological diagnosis is based on the microscopic identification of cysts and trophozoites in the stool of the patients, specific antibodies and antigens detection. Around 50 million people worldwide suffer from amoebiasis each year, resulting in 100,000 deaths each year [32].

One of the most underestimated diarrhea-causing protozoansis Dientamoeba fragilis and recent studies demonstrated the direct implication as a common cause of gastrointestinal disease and, in the meantime, his pathogenic potential.The prevalence of Dientamoeba fragilis varies from 0.3% to 52% and often exceeds that of Giardia lamblia [33].

Malaria is produced by infection with Plasmodium species, by the bite of Anopheles mosquito, being recognized as the second cause of death by infections in Africa. According to the European Centre for Disease Prevention and Control (ECDC), Romania confirmed and reported 18 cases of malariain 2018 [21]. The disease is endemic in sub-Saharan Africa and South Asia and has an incubation period between 8-25 days, with a clinical picture dominated by cyclical fever. The following manifestations: insomnia, weakness, arthralgias, myalgias, diarrhoea, abdominal pain, headache, and hepatosplenomegaly can be associated with the fever in case of acute malaria. Classical clinical triad present in malaria consists of fever, splenomegaly, and anaemia. The microscopic examination of thin or thick blood smears, the detection of the parasite nucleic acid by real-time polymerase chain reaction assay, the identification of the plasmodium antigen by immunochromatography are specific tests used to confirm the diagnosis of malaria [34].

Leishmaniasis is a neglected tropical disease, protozoan vector-borne infection, whose transmission is through the bite of an infected female sand fly (Phlebotominespecies). It is present in environments ranging from tropical forests in South and Central America to desert areas in West Asia, including North of Africa with cutaneous leishmaniasis, which is the most common form, mucocutaneous, and visceral leishmaniasis, the last one being the most severe form of the disease. The period of incubation varies widely, from a few weeks to several months after the initial bite, depending on the species. The wide spectrum of clinical manifestations in visceral leishmaniasis includes fever, anorexia, abdominal pain, emesis, diarrhea, hepato-splenomegaly. Diagnostic findings are based on skin biopsy, tissue culture, and presence of antibodies [35].

Dracunculiasis, also known as Guinea worm disease, can be found in rural areas of South Sudan, Chad, Ethiopia, and Mali, which relies on contaminated water supplies. Clinical symptoms can take up to a year to appear, and may include painful blister centered on the worm, usually localized in the lower extremity, fever, nausea, vomiting, and local lymphadenopathy [36].

Schistosomiasis is a disease caused by parasitic worms (Genus Schistosoma), also known as bilharzia, which is endemic in tropical and subtropical areas and considered as one of the neglected tropical diseases. With an incubation period of about 2-8 weeks, the acute clinical picture presents a flu-like syndrome, nausea, vomiting, abdominal pain, bloody diarrhoea, hepatosplenomegaly, and lymph-adenopathy. Laboratory findings include the direct detection of eggs by the microscopic examination or of the parasite's nucleic acid in patients'stool and urine, and serological tests for identifying the specific antibodies in patients returned from endemic areas, but without eggs demonstrated in faecal or urine samples [37].

Digestive infections caused by bacteria

One of the most common bacterial causes of gastroenteritis worldwide is Campylobacter spp. frequent especially in children under 2 year-old, causing diarrhoea, abdominal cramps, vomiting, fever and even bleeding. It is usually generated by the consumption of raw or undercooked meat (frequent/especially poultry) or contaminated milk.

Up to 25% of cases of antibiotic-associated diarrhea in healthcare institutions and hospitals are generated by Clostridium difficile. Elderly and immunocompromised patients have a higher risk. The most recent emergence of highly toxigenic and resistant C. difficile strains has led to more severe outbreaks, increasing mortality and morbidity [38].

In the developing countries, especially among children, Escherichia coli is a major cause of diarrheal disease and the leading cause of travellers'diarrhoea. The infections are produced by drinking water contaminated with human or animal feces. A special strain of this genus is Escherichia coli O157:H7, a Shiga toxin producing. These bacteria generate gastrointestinal infections with symptoms including vomiting and sometimes even bloody diarrhoea. Although it is usually cured after a few days, a percentage of 5-10% of infections lead to haemolytic uremic syndrome and its consequences (destruction of blood platelets, anaemia, kidney failure) [39].

Helicobacter pyloriis the main cause of gastritis and is associated with the development of gastric and duodenal ulcers or even stomach cancers. H. Pylori is the strongest etiological factor in both of these conditions, with the World Health Organization classifying it as a class I carcinogen. According to the CDC, approximately two-thirds of the population is infected with H. pylori, but less than 20% have a risk of developing ulcers or stomach cancer [40]. On the other hand, infection with H. pylori may protect against some cancers, such as oesophageal adenocarcinoma and gastroesophageal reflux disease [41]. It can cause stomach pain or nausea, but in many cases, there are no symptoms.

Salmonella and Shigella are food-borne GI illnesses. Salmonella is common and is found in raw meats, poultry, seafood and eggs, as well as milk and dairy products. Acute symptoms include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. Shigella is frequently found in water polluted with human feces. Symptoms of shigellosis or bacillary dysentery include abdominal pain, cramps, diarrhoea, fever, vomiting, and blood, pus, or mucus in stool.

Yersinia enterocolitica is a cause of diarrhea and abdominal pain relatively frequent after eating contaminated food, especially raw or undercooked pork products, milk or ice cream. Common symptoms are fever, abdominal pain and diarrhea, which is often bloody.

The most common cause of food intoxication, characterized by violent onset, severe nausea, cramps, vomiting, and diarrhea is Staphylococcus aureus. It is also associated with a large range of food, and could be found on humans (infected cuts, skin, throats and noses).

Brucellosis is a zoonosis caused by four different species of Brucella. It is considered as a major infectious disease in the Caribbean, the Middle East, Eastern Europe, Asia, and Africa. According to ECDC, one case of brucellosis was reported by Romania, in 2018 [21].The incubation period is between 2 weeks to 5 months. General brucellosis signs are usually vague and flu-like, with gastrointestinal symptoms such as dyspepsia, diarrhoea, constipation, vomiting, abdominal pain, hepatic abscesses. However, the clinical picture is dominated by fever, with undetected aetiology through routine laboratory investigations. The diagnosis of brucellosis, although easy to confirm based on serological tests, often requires a period of up to one year, due to the presence of a polymorphic, sometimes oligo-symptomatic clinical picture.

Leptospirosis is a global zoonotic infection with a high prevalence in Caribbean and Pacific islands, Central and South America, Southeast Asia, predominantly during the rainy period, caused by pathogenic spirochetes of genus Leptospira. In 2018, 51 cases of leptospirosis were confirmed in Romania [21]. Usually the incubation period is 1-2 weeks. It presents with a wide range of clinical symptoms, which could be easily mistaken for other diseases, including flu-like syndrome, nausea, vomiting, diarrhoea, rash, dry cough, jaundice, hepatomegaly and splenomegaly, oliguria/anuria, haemorrhages, with the involvement of multiple organs. Suspicion occurs in the case of association between digestive, liver, kidney manifestations with fever, and myalgia. Confirmation requires the determination of specific antibodies.

DIAGNOSIS

In the presence of symptoms of a possible gastrointestinal infection, the diagnosis could be confirmed by different old and modern laboratory tests using stool specimens first and others such as serum for specific antibodies (serological tests). The standard culture to isolate bacteria remains the gold standard for bacterial pathogens, even if it has a relatively low sensitivity and it is time-consuming (more than 24 h), but it may be followed by antibiotic susceptibility testing. In this way, the therapeutic options may be evaluated, but also the assessment of microbial resistance to antibiotic therapy [42]. Ova or parasite examination are ordered in case of suspicion of parasites. For this purpose, a direct examination by microscopy or a concentration method is used, but it has a low sensitivity and it is, once again, time consuming and requiring specific training of the laboratory staff. In addition, there are antigen tests used to detect Clostridium difficile (GDH, toxins), Giardia lamblia, Cryptosporidium spp., Helicobacter pylori, or Entamoeba histolytica. However, antigen tests can only detect a few specific parasites and therefore cannot replace ova and parasite examination. Antigen tests are useful to rapidly detect viruses causing gastroenteritis, such as rotavirus and adenovirus; however, these tests are not available for all entero-pathogens and show a variable sensitivity.

Taking into account the wide range of pathogens that can cause diarrhea and the similarity of the clinical manifestations produced by different pathogens; the molecular techniques seem to be the best tools for a rapid diagnosis. In the last years, some tools based on polymerase chain reaction (PCR) techniques have been developed to detect specific microorganisms or a panel of different entero-pathogens directly from stool samples [43-45]. Real-time PCR allows the simultaneous detection and differentiation of viral, bacterial and parasitic gastrointestinal pathogens. This assay also showed a high sensitivity for most pathogens included in the panel and efficient detection of coinfections. The main advantages of the molecular tools for the diagnosis of gastroenteritis are: high sensitivity, increased diagnostic spectrum, improved workflow (processing and diagnosis time is between 1 and 5 h), favorable impact on infection control and costs. Some disadvantages of molecular methods are the inability to distinguish between viable and nonviable microorganisms; they do not offer antibacterial susceptibility or epidemiological data; high cost; detection of the asymptomatic carriage could be difficult to distinguish from symptomatic/asymptomatic infections. Therefore, the application of these tools should be performed in patients selected based on clinical condition and/or the severity of gastroenteritis. Particularly in hospital settings, the rapid diagnosis provides important information for implementing infection control measures [46-48].

The clinical symptoms of all these diseases are nearly identical. Thus, it is difficult and almost impossible to determine the pathogenic cause based on clinical features alone. Rapid diagnosis, appropriate treatment, and infection control are important measures in these contexts. The early medical challenge is to include the possibility of tropical infectious diseases that are not common in the area, in the differential diagnosis. Not only the characterization of the symptoms but also the detection of infectious diseases based on a detailed history of the patient and a quick assessment can enormously help the clinicians in establishing a correct diagnosis and treatment.

Emerging evidence suggests that many gastrointestinal diseases are associated/caused by alterations in the gut microbiome. Specific microbiome signatures have been reported for irritable bowel syndrome and inflammatory bowel disease, and these have been associated with pro and anti-inflammatory effects, contributing to disruption or maintenance of gut homeostasis. Colorectal cancer is the third most common malignancy diagnosed worldwide, one of the leading causes for cancer-related mortality in both men and women. Different hereditary, environmental and inflammatory factors have been shown to increase the risk of colorectal tumorigenesis, but recently, bacteria and viruses have been described to be involved in colorectal carcinogenesis through direct modulation of epithelial cells, leading to genomic or chromosomal instability. Observational studies have found positive associations between gastric ulcers, periodontal disease, bacterial infections, and the risk of pancreatic cancer, the most rapid lethal cancer, with over 70% of patients dying within one year after diagnosis.

CONCLUSIONS

Concerning human mobility, several specific aspects must be considered, such as undiagnosed, atypical and subclinical types of diseases. These diseases are mistakenly treated as intercurrent diseases, and serious latent infections may develop. In the last few years, molecular methods have become readily available for many clinical laboratories and their utilization has resulted in a more rapid diagnosis and increased pathogens detection. As travel around the world continues, microbes will continue to evolve, adapting to new environmental conditions. The emergence of new infections will continue, and the distribution, severity and frequency of the identified infections will change, considering the new conditions. Travel or migration of people will continue to be an important factor in the occurrence and development of diseases. The analysis and monitoring of pathogens will focus the specialized actions and researches on the global movement and evolution of microbes. Integration of medical knowledge and multidisciplinary skills – social, biological and physical sciences – are therefore required.

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ORIGINAL ARTICLES

Diagnostic value of ultrasound in comparison with the pathologic results of fine-needle aspiration of thyroid nodules for rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital

Hojjatollah Khajehpour[1], Mohammad Ebrahimifar[2], Efat Naeimi[1], Mortaza Movahed[1]

Abstract: *Background and Aim: Various studies have reported that 4-7 percent of adults with palpable thyroid nodules that 50-70 percent of it's diagnosed with high-accuracy sonography. Although most thyroid nodules are non-cancerous and do not require treatment, a small percentage of them are associated with a potential risk of malignancy, which is an important early diagnosis by ultrasound or pathology. The present study aimed to compare the ultrasound with the pathologic results of fine-needle aspiration (FNA) of thyroid nodules for the rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital.*

Methods: The study population consisted of 100 (80 female, 20 male) patients with thyroid nodules in the age range of 19-82 years referred to the radiology ward of Baqiyatallah Hospital who were studied by the cross-sectional method. All patients were first scoring by ultrasound, then fine needle aspiration with thyroid nodule ultrasound was performed, and the specimens were referred to the pathology department for further evaluation.

Results: The pathologic results from fine-needle aspiration showed that only 8 of the 100 patients studied had malignant nodules that, by ultrasound scoring, 7 patients had 4 scores and 1 patient had 3 scores. However, the other 92 patients with benign nodules 57.60% of patients had score 1, 30.43% of patients had score 2 and 11.97% of patients had score 3.

Conclusion: According to the results of this study, it seems that ultrasound scoring is useful in the rapid diagnosis of thyroid nodules and prevent unnecessary thyroid nodule FNA.

Keywords: *fine needle aspiration, sonography, thyroid nodule, malignancy*

INTRODUCTION

The thyroid gland is one of the largest endocrine glands in the butterfly-shaped transverse section of the larynx. The units that make up the thyroid gland are a sinus or follicles, which are present in the central part of colloidal proteins as a source of thyroid hormone storage [1]. The thyroid gland has several key functions, such as iodine uptake and transfer, thyroglobulin secretion, iodine binding to thyroglobulin to make thyroid hormones, facilitating thyroid hormone secretion into the bloodstream, and interfering with body metabolism [2].

Thyroid hormones

Thyroglobulin, a large iodized glycoprotein, has been shown by researchers to be the first essential step in initiating the synthesis of thyroid hormones through iodine absorption through the food. Transmission of iodine to the thyroid gland occurs by binding to serum proteins, especially albumin, and

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unbound iodine is often excreted in the urine [3]. TSH, which is secreted by the anterior pituitary cells, plays a central role in the control and evaluation of thyroid function, which is regulated by the TRH hypothalamic hormone [4]. Various studies have shown that there is an inverse relationship between thyroid hormone levels and basal TSH production, indicating the role of thyroid hormones as a major regulator of TSH production [5].

Common disorders of the thyroid gland include hypothyroidism, hyperthyroidism, goiter or enlargement of the thyroid gland, thyroid cyst, thyroiditis (Hashimoto's) and thyroid nodules. According to the latest studies, 7-8% of the world population has one of the thyroid disorders [6].

The term thyroid nodule refers to a group of thyroid cells that grow abnormally and fall into a mass, nodule, or node, which are divided into two groups of benign nodules and malignant nodules [7]. Various studies have shown that most thyroid nodules have non-cancerous and benign cells and various reports have estimated 1 in 10 patients as malignant nodules [8].

The types of thyroid nodules are divided into 3 groups of benign (colloidal), malignant (papillary) and suspicious nodules based on pathological findings. Among the types of nodules mentioned, only malignant nodules require further surgery and care.

Symptoms of patients with thyroid nodules include neck, jaw or ear pain, difficulty swallowing, itching or tingling in the throat, shortness of breath, sore throat, hoarseness, and most studies have reported that most patients Thyroid nodules are without symptoms and are often discovered during examination for other causes [9].

Researchers have identified chronic follicular thyroid nodule (Hashimoto's), congenital malformations such as failure to form a lobe, thyroid abscess, simple cyst, and non-thyroid diseases such as lymphoma and teratoma [10].

Tests for the diagnosis of thyroid nodules include measurement of thyroid hormone and thyroid-stimulating hormone, ultrasound, scanning, fine needle aspiration, and pathological examination (cellular aggregation) [12, 11].

Since a rapid diagnosis of malignancy facilitates the process of thyroid nodule healing, therefore, finding the fastest method of diagnosis is important for initiating therapeutic measures.

The present study aimed to compare the ultrasound report only with the pathologic results of fine-needle aspiration of thyroid nodules with the aim of rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital.

MATERIAL AND METHOD

Following the approval of the research plan by the University Research Council, informed written consent was obtained from patients' participation and distributed to those who referred to the thyroid nodule interventional ultrasound department and the indication of fine-needle aspiration. No entry and exit criteria were considered for the study.

Finally, 100 patients (80 females, 20 males) from thyroid nodules in the age range of 19-82 years referred to the radiology ward of Baqiyatallah Hospital and the statistical population were selected by census method and studied by the cross-sectional method. After obtaining biographies and demographic information by the student, patients were referred to a skilled sonographer for ultrasound (using Philips Affinity fog) and fine-needle aspiration (with ultrasound guidance) and the specimens were referred to the pathology department for cytological examination.

In thyroid ultrasound, tissue is examined for ultrasound by echogenicity and rate and extent of calcification. 6 modes of Hyper echo nodule (white nodule in ultrasound), Hypo echo nodule (gray and pale nodule in ultrasound), cystic nodule (absolutely Echoless), solid nodule (uniform echo), microcalcification (calcification less than 2 mm) and macro calcification (calcification greater than 2 mm) was determined by scanning ultrasound for scoring from 1-4. The four nodule states were hypo echo, solid, microcalcification, and a 1 cm-high scoring criteria of 1-4. Each of the above was given a score of 1 and the nodule with all four attributes scored 4. In fine needle aspiration, the thyroid tissue is sampled using a fine needle for the microscopic examination of existing cells.

Among the characteristics of malignant nodules can be severe porcelain, cell size and shape variation, dense and double or multilayered cell masses, etc. These features were reported by a skilled pathologist.

Data were analyzed by spss23 software.

RESULTS

The mean age of patients was 48.26±08/13 and the results of this study showed that malignant thyroid nodules were more prevalent in 40-49 years old patients and women in our study ($p<0.05$).

Of the 100 patients with thyroid nodules, 8 (8%) had malignant nodules, indicating that a thyroid nodule may be about 12 times more likely to be benign.

Ultrasound reports showed that of the 92 benign nodules, 58 were hyper echogenic, 22 were hypo echogenic, and 12 were cystic nodular, whereas of the 8 malignant nodules, 5 were hypo and 3 were iso- echogenic.

It was observed that the ratio of solid in malignant nodules was more than benign. The calcification rate in benign nodules was 17% for microcalcification and 2% for macro calcification, which was 40% and 20% for malignant nodules, respectively.

Pathologic reports showed 53 patients with nodular goiter, 28 with adenomatous goiter, 1 with a follicular lesion, 8 with papillary carcinoma, 8 with thyroiditis, and two remained unknown due to insufficient sample that both nodules were cystic.

Of the 8 patients with malignant nodules reported by the pathologist from the side of ultrasound, 7 (87.5%) cases had score 4 and one patient (12.5%) had score 3. While 57.60% of benign nodules had score 1, 30.43% had score 2 and 11.97% had score 3.

From the side of ultrasound scoring, 53 cases received score 1, 28 cases received score 2, 7 cases received score 3 and 12 cases received score 4. In terms of thyroid nodule count were 47 single nodules and 53 multinodular nodules.

From hormonal tests, 4 cases were hypothyroid, 5 were hyperthyroid and 91 cases were normal, 3 cases were hypothyroid or hyperthyroid, and 5 cases were normal. Eleven of the patients had a family history of thyroid disorders, which was found to be 5 out of 11 people and 3 in people with no history of malignancy.

DISCUSSIONS

This study aimed to compare ultrasound only report with pathologic results of fine-needle aspiration of thyroid nodules with the aim of rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital. The pathologic results of fine-needle aspiration in the present study showed that only 8 of the 100 patients studied had malignant nodules which, by ultrasound scoring, 7 cases had score 4 and 1 case had score 3. However, the other 92 patients with benign nodules 57.60% had score 1, 30.43% had score 2 and 11.97% had score 3. In other words, by taking into account the pathologic results in this study, it can be concluded that ultrasound results can be used as a quick tool in predicting thyroid nodule malignancy. The reason for the high success of sampling accuracy in this study was the use of ultrasound guidance during biopsy and slide preparation by the pathologist.

In 2015, Siyadati et al investigated the diagnostic value of fine-needle aspiration compared to pathologic results in the diagnosis of thyroid nodules in Babol Shahid Beheshti Hospital, northern Iran, for 22 years. The researchers said that fine-needle aspiration of the thyroid is a selective method of examining thyroid nodules that have recently been questioned in the management of nodules. This cross-sectional study was performed on cytology specimens of 225 patients who underwent thyroidectomy in Shahid Beheshti hospital of Babol for 22 years (1989-91). Age, sex, physician performing FNA, type, and surgical site, FNA and pathology results were recorded. Pathologic FNA results were classified into four groups of inadequate, benign, malignant and suspicious and pathologic results were also divided into two benign and malignant and compared. The results showed that in the diagnosis of malignant thyroid nodules, sensitivity was 60%, specificity 96%, positive predictive value 65%, negative predictive value 95% and diagnostic accuracy of 81.8%. Of the 225 cytological specimens, 127 were benign (56.4%), 47 suspicious (20.9%), 27 malignant (12%), and 24 inadequate (10.7%). Final pathology was also benign in 150 patients (66.7%) and malignant in 75 patients (33.3%). According to the results of this study, fine needle aspiration can be used to diagnose thyroid nodules [13].

Rosario et al., 2013 in Brazil, designed a study to predict malignancies in thyroid nodules with non-diagnostic cytology and to evaluate the value of ultrasound. The results of this study showed that in patients with thyroid nodules and repeatedly diagnosed cytology findings, ultrasound findings are an excellent parameter for choosing to continue their treatment, which would be useful for periodic ultrasound [14].

In a 2005 study in Taiwan, Lin et al., in the study of thyroid nodules by ultrasound and fine-needle aspiration, concluded that ultrasound combined with fine-needle aspiration detected 10 years earlier than other methods of malignancy [15].

In 2006, in Italy, Baratolata et al. designed a study entitled Quantitative and Qualitative Evaluation of Thyroid Nodule with Ultrasound Contrast. These researchers concluded that quantitative data corroborated the subjective findings and contrast ultrasound of the thyroid gland was a feasible procedure but found other random events during ultrasound may interfere and are one of the reasons limiting the potential of this method for the detection of thyroid nodules [16].

In 2009, Tramaloni et al. evaluated ultrasound and Doppler ultrasound and fine-needle aspiration in thyroid nodules. In this study, microcalcification, hypoechogenic nodules, and intra-nodular vascularization were introduced as criteria for malignancy [17]. In our study, as the grayscale ultrasound was used, therefore, vascularization was not used in malignant scoring.

Rago et al. 2008 evaluated ultrasound and Doppler ultrasound and fine-needle aspiration for malignant patterns in thyroid nodules. In this study, microcalcification, hypoechogenic nodules, and intravascular nodular vasculature were introduced as criteria for malignancy [18]. In our study, as the grayscale ultrasound was used, therefore, vascularization was not used in malignant scoring.

CONCLUSIONS

According to the results of the present study, it seems that ultrasound scanning alone is useful in the rapid diagnosis of thyroid nodules and to suggest a more accurate result, the present study should be designed in a larger statistical population. More accurate and desirable results can be performed on specific types of lesions. Thyroid nodules with scoring 3 and 4 should also be subjected to the fine-needle aspiration to facilitate faster and more accurate diagnosis and to prevent the progression of malignancy. Among the properties of the nodule used in scoring, nodule size alone may not be a good criterion for nodule malignancy, whereas solidity, hypo echogenic, and microcalcification favor the malignancy of the nodule, and can exclude nodules that score 1 or 2 can be tracked by ultrasound and does not necessarily need to be sampled.

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Conflicts of interest: The authors state that there are no conflicts of interest in the present study.

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ORIGINAL ARTICLES

Overview of bilirubin as criterion for higher military education admission

ViorelM. Oniciuc[1]

Abstract: *This article was written because of the necessity of clarifying the recent biomedical selection criterion for the medical exam of the students who are applying for higher military education. In order to screen for or monitor liver disorders or hemolytic anemia the bilirubin is measured. The increased values of the bilirubin can be caused by multiple factors and they are presented in the following chapters.*

Keywords: *Bilirubin, liver, gall, alimentation, genetic diseases*

WHAT IS BILIRUBIN?

Bilirubin is a linear liposoluble tetrapyrrole derived from the enzymatic catabolism of the hem fraction from various hemoproteins in the body. Understanding changes in plasma levels of bilirubin under pathological conditions and differentiation of icteric syndromes involves a prior knowledge of the mechanism of formation, agglutination and elimination of this compound.

The normal values observed in the laboratory analysis are:
- Total bilirubin = 0.3 - 1.2 mg / dl, (or 0.1 - 1.2 mg / dl)
- Direct bilirubin = 0.1 - 0.35 mg / dl
- Indirect bilirubin = difference

Linear tetrapyrrole is the result of the enzymatic mechanism that catalysis the oxidative breakdown of the tetrapirolic (porphyrinic) ring of the hemoglobin heme molecule, primarily of the aged erythrocytes.

The process leads to hemoglobin formation in at least two stages. Bilirubin is mainly formed in the liver (about 80-85% up to 90% according to some authors), the rest having extrahepatic origin, in part reticuloendothelial (spleen, bone marrow).

Involvement of the liver in bilirubin production

The main degradation product of porphyrins is bilirubin, the red-orange bile pigment, which still possesses the four pyrrole rings. In the plasma, the pigment circulates as total bilirubin, consisting of two components: bilirubin bound to unconjugated albumin, indirectly referred to as free glucuronide bilirubin (mono- and diglucuronate), related to the diazotation reaction, Hijmans Van Den Bergh. Total bilirubin level serum concentration is 0.1-1.2 mg/dL or 17 µmol/l. Under normal circumstances, bilirubin accounts for 85% of the erythrocyte hemoglobin degradation and the remaining 15% originates in the degradation of other hemproteins such as myoglobin, cytochromes and other hemic enzymes (catalases, peroxidases, cytochrom-oxidases).

Direct bilirubin (agglutinated with glucuronic acid) circulates freely in the blood. Indirect bilirubin (primary, unconjugated) is transported by binding to albumin, partially in the form of the albumin-phosphatide complex and in small amounts bound to both metals, amino acids, peptides or other small molecules. In the gall there are bilirubin esters with sulfuric

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acid and other acids. By binding the bilirubin to the elastic fibers of the skin and the conjunctiva the symptoms of jaundice appear (yellowing of the skin and conjunctiva).

Bilirubin production is 35 mg per gram of hemoglobin, yielding 250-300 mg daily bilirubin. Hemoglobin degradation in bilirubin is performed in Kupffer stellate cells in the hepatic reticulo-histocithic system (RHS), which is the primary location of the process, the spleen and bone marrow being organs involved in this activity.

The secretion of the gall

One of the most important functions of the liver is the production of its external secretion, the gall, which reaches the duodenum through the biliary tract. The gall fulfills two major functions:

• elimination of endogenous substances – end products of metabolism and foreign substances such as bromsulfonaphthalein (BSP) drugs and dyes;

•intervention in lipid digestion and reabsorption – the liver secreted by hepatocytes, the hepatic ball, differs in appearance and concentration of vesicular bile. The first one is clear, yellow-gold and almost isotonic with blood, and the second is of brownish green (oxidized bilirubin), cloudy and filthy. Vesicular vesicle is more concentrated than the liver due to water reabsorption through the bladder wall.

INCREASE IN BILIRUBIN VALUES

Without entering into anatomical and physiological details and the claim of accurately discovering the causes that cause the increase of bilirubin values, applying filters such as the elimination of neo-natal casuistry, serious diseases (cancerous disease, cirrhosis etc), which have a very low incidence at the age of the students, we can focus on two main directions that could underpin the rise of increased bilirubin at our students:
1. Physiological-behavioural and social.
2. Inherited, triggering some hereditary disease syndromes.

Prior to the two directions, two further specifications are required:
- The triggering of the studied phenomenon belongs mainly to the age of adolescent-premature.
- In specialty work, the possibility of recording normal values occurs once in 3-5 laboratory tests, at intervals of at least three weeks, repeated bilirubin tests can be associated with other investigations, to determine normal hepatic functionality.

1. On the liver tract, intrahepatic and extrahepatic, gall bladder, biliary bladder, we narrowed down the multitude of possibilities with pathological manifestation (even anicteric, cholestasis mild hepatitis, confirmed by Dr. Anton).

However, it is worth mentioning that hyperbilirubinemia has been reported in a different institution, namely the "Football Academy" (according to A. Constantinescu – Fundeni University Clinic)

We noticed an increase of bilirubin in the cases of:
- Automedication (we advise that medication is administered and supervised by the medic):
  - Paracetamol, even a slightly increased dose,
  - Oral contraceptives,
  - Consumption of food supliments, protein for muscle mass.
  - Energy drinks, sweet juices, fast food
- Alimentation (canteen, home or other sources):
  - Lack of food diversity
  - Lack of lipids in the diet

The most important aspects of nutrition belong to food adjuvants mainly preservatives:
- Sodium metabisulphite (E223) in biscuits, cakes, fast food, juices, pickles, medications.
- Monosodium glutamate (called "good taste") is less directly incriminating, but it induces fatigue, headaches, sometimes aggravating breathing, causing stress. It is omnipresent in soups, sausages, vegetable preserves, flavors etc. This heat-treated (E621) adjuvant gives the browning reaction resulting in very toxic compounds.
- Sodium benzoate, preservative (E211).
- Carbon tetrachloride, hepatotoxic.

An important factor is "pernicious anemia" - ineffective erythropoiesis, lack of vitamin B12 due to lack of food, or more severe, due to the lack of intrinsic Castle's factor in gastric juice, necessary for absorption. B12 or perhaps stress-related somatostatin secretion that inhibits the secretion of the Castle's factor.

Sure, the theme is vast. Surveillance over time and complementing with specific analyzes, not just laboratory (CT, eco) will explain the causes.

2. On the track from the liver, intra-, extracapsal, bile duct to gall bladder several hereditary transmission syndromes can be manifested with precise localization and with a slightly variable pathological manifestation. They are triggered at adolescent age, according to the algorithm for the interpretation of the functional - liver test results (according to Henry J.B.). The most common genetic diseases are:

- *The Gilbert syndrome*

It is characterized by chronic, benign, intermittent, familial hyperbilirubinemia found in a routine control. It is caused by a defect in the transport and conjugation of unconjugated bilirubin. The jaundice is accentuated in feverish states, physical exertion or consumption of oral contraceptives.

- *The Crigler-Najjar syndrome*

Type I may be a homozygous of Gilbert's disease. Type II is characterized by partial glucuronide-transferase deficiency that bilirubin conjugates to the liver cell. Usually at adolescents, complications are rare and occur in the case of poor eating habits or by excluding lipids from the diet.

- *The Dubin- Johnson syndrome*

It is characterized by the inability to transport bilirubinuriuronide from hepatic cells to the tubule while the conjugation is normal. The stress periods are a factor. The jaundice is harmless and reversible and occurs after eating estrogen and contraceptives. It can be confused with mild viral hepatitis.

- *The Rotor Syndrome*

It is asymptomatic, benign; withdrawal and storage of conjugated bilirubin defect, and possibly related to transfer from liver to bile. Usually it is reported in adolescents (medication, exercise-physical stress). Other liver functions are normal.

- *Epstein-Barr virus infections (human herpesvirus 4)*

It induces bilirubin increases in about 30% of the patients, but if liver function abnormalities are not found, another diagnosis should be sought.

CONCLUSIONS

After analyzing the studied material together with the medical laboratory specialists (Chem. Stanciulescu C., Synevo, Biochem. Apircioaei O., former SMC), the two physicians mentioned above, Dr. Stanciulescu P., internal specialist and I tried to highlight some aspects of hyperbilirubinemia:

- Self-medication - forbidden.
- Nutrition - the main recommendations:
- Regular fresh fruits and vegetables consumption
- Reduced fast food and unhealthy drinks consumption.
- Reducing fast at the first deviations from the normal functioning of the body, especially during the body-growing period.
- Elimination, at least limitation of stress, in all forms of manifestation: physical, food, intellectual, psychosocial.
- Avoid fatigue of any kind (not enough sleep at night)

These landmarks must be all considered, taking care of only one recommendation cannot bring immediate positive results.

As for hereditary transmission diseases, unfortunately, the possibilities of reaction are very limited in Gilbert and Crigler - Najjar syndromes, phenobarbital brings a certain decrease (Jacques Wallach, p. 341, confirmed by Dr. Anton) only under the supervision of the doctor. The most important factor is however a varied diet, avoiding starvation and tiredness.

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ORIGINAL ARTICLES

Antibiotherapy: latest current affairs – state of the art 2019

Viorel Ordeanu[1,2], Lucia E. Ionescu[1], Victoria G. Dumitrescu[1], Roxana G. Pricop[1], Răzvan Neagu[1], Diana M. Popescu[1]

Abstract: *Given the continuous growth of the bacterial resistance phenomenon, the appropriate choice of antibiotherapy represents an important step in the treatment and prophylaxis of infectious diseases. Depending on the symptomatology, the dose required for the effective treatment of an infection may vary. Inadequate dosing not only reduces the effectiveness of the antibiotic, but also promotes the emergence of resistant bacterial strains. Also, too high of a dose increases the toxic side effects of antibiotics. Therefore, a personalised antibiotherapy according to: the patient's clinical status, body mass, associated chronic diseases, etc. represents a promising approach in the treatment of infectious diseases.*

The purpose of this study is to present the most important current affairs regarding the emergence of new antibiotics, the maximum doses recommended by the latest version of the "European Committee on Antimicrobial Susceptibility Testing" (EUCAST - version 9/2019) and providing an indication on whether these antibiotics are used in Romania.

Keywords: *antibiotics, antibiotherapy, maximum recommended dose, antimicrobial chemotherapics*

INTRODUCTION

Antibacterial chemotherapics (antibiotics) have become the emblematic drugs of the 20th century starting with the first antibacterial chemotherapic, Salvarsan (invented by Paul Ehrlich, of German origin, in 1909) and the beta-lactamase Penicillin (discovered by the American Alexander Fleming, in 1929). Currently, there are hundreds of antibacterial antibiotics (AB), of which 269 have been approved for antibiotherapy (ABT). It was believed that bacterial diseases could be eradicated, but the practice has shown since the middle of the last century that the phenomenon of bacterial resistance to antibiotics emerges, that much higher doses are now needed, with modified conditioning and dosage, targeted use, in phases and combined or associated, even the use of new antibiotics, as far as they can be produced [1-7].

ANTIBIOTHERAPY

Over time, the efficacy of ABT has been reduced, despite advances in medicine in general and pharmacology in particular. Resistant, multi-resistant and all-resistant bacteria create the most serious problems in medical practice and more and more patients die from infections or over-infections that do not respond to treatment. This worrying phenomenon has been thoroughly investigated, starting with the application manner of ABT. Thus, the website www.longitudeprize.org published in 2014 an international medical statistic [6] having as results the inappropriate use and prescription of ABs:

- 90% of the physicians prescribed AB under the pressure of the patient who wanted a certain AB (namely, "Customers is King?!");
- 70% of the physicians prescribed AB without being sure

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that the etiology of the disease is bacterial or viral;

• 49% of the physicians prescribed AB for a week or more, without a prolonged treatment being needed;

•44% of the physicians prescribed AB only preventively, after discharge from surgery.

Following the highlight of the multiple factors that lead to the irrational use of ABs, medical and especially non-medical, in 2017 UN/WHO launched a new conception on prophylaxis and treatment – One health – and officially formulated recommendations for the Member States, with the goal of maintaining the efficiency of ABT worldwide. Based on these recommendations, each country sets its own research directions for medical and pharmaceutical scientific research. In this respect, a prize was established worldwide, Longitude Prize (2014). It has a prize pool of £ 10m to reward a competitor who will develop a test targeted on the rapid diagnosis of the diseases that require ABT. Through the rational use of ABs, the test would lead to the conservation of efficiency and shall revolutionise healthcare worldwide. The test should be accurate, fast, inexpensive, easy-to-use and accessible to anyone, anywhere in the world. By this test, shall be identified which ABs are needed and which shall be used. But these requirements seem utopian in the current stage of development of science and technology, it being unlikely to discover something that corresponds at least partially. Therefore, the prize has not been awarded so far.

Inventing new ABs is costly and difficult. These must meet divergent conditions: high toxicity for microorganisms (thus, antimicrobial efficacy), but little or no toxicity for the treated macroorganism; also, it must not have significant side effects; it must be produced industrially with reasonable costs; it must be stored and managed conveniently etc.

For the in vitro evaluation of the antibacterial effect of the AB, the Minimum Inhibitory Concentration (MIC) testing is performed on the respective bacterium (Breakpoint by determination in microdilutions in liquid culture medium) and the Kirby-Bauer diffusimetric antibiogram (ABG) with the measurement of the diameter of the Inhibition Zone (IZ) on the solid culture medium [2, 7]. One can choose from theoretically efficient ABs [1, 3, 4, 5] those that inhibit isolated and identified bacteria from the patient, by interpreting in the SIR system (Susceptible, Intermediate and Resistant) the diameter measured in millimeters, compared to EUCAST tables [2]. Because often the result obtained in vitro was between susceptible and resistant, this is noted as intermediate and for clinicians it was equivalent to resistant, because it would not have the desired therapeutic effect. In the current conditions of spread of the bacterial resistance phenomenon towards antibiotics, it is recommended to increase the dose of ABs, where the pharmacological properties allow, so that the bacteria pass from the intermediate to the susceptible area. By this approach of bacterial susceptibility, the intermediate bacteria pass from the resistant group to the susceptible group, and the SIR system gains another significance. The paraclinical findings become a practical indicator for the guidance of the clinician:
S = susceptible to standard dosing regimen (usual dosage)
I = intermediate, susceptible to high exposure (high doses, where possible)
R = resistant (therefore, useless for use).

As a result, the most handy method of combating bacterial resistance is the justified increase of the consumption of ABs, by increasing the single dose, daily or per cure, the frequency of administration or the duration of treatment, where the pharmacological characteristics allow (for example, in order not reach the toxic dose). The maximum recommended doses can be per dose, per day or per cure, and the cure can be short (1-5 days), medium (5-10 days), long (10-15 days) or very long (15-60 days). The doses are approximated depending on the conditioning of the drug (single antibiotic package or fixed combination), as well as the body mass of the patient. Any drug is theoretically dosed for an average patient, with a net body weight of 70 kg; so for a patient over 140 kg it should be double dose and for one below 35 kg it should be half dose. Some drugs, including ABs, are given by calculating in mg/kg body weight, especially in paediatrics, where children may have very different weights. When choosing ABs and in determining the dose, one must take into account, in addition to the pharmacological criteria imposed by antibiotherapy (which are well known), also: the general and particular contraindications, the age, the general patient status, the associated diseases, possible allergies or intolerances, the need for combinations with anti-beta lactamases or combinations with ABs or other anti-infective or adjuvant drugs. Consideration should also be given to possible antagonisms, inactivation with drugs and food, unwanted adverse or secondary phenomena as well as possible Herxheimer-type reactions. All this leads to a bi-unequivocal personalized ABT: depending on the needs and characteristics of the patient as well as the susceptibility of the pathogenic bacterium [8-13].

THE CLASSIC RECOMMENDATIONS

Professor Balş, the professional owner of the hospital that bears his name formulated the basic principles of antibiotherapy (also called antibiotic therapy) regarding: indications, contraindications, errors, associations of ABs, which are still valid today. These principles have been applied in Romanian medical practice by Prof. Angelescu and his students, with undeniable successes. But at present, although all these principles have remained valid, their effectiveness in ABT practice is progressively diminished, due to the the spread of bacterial resistance to ABs, worldwide. As a result, we must complement these principles with new observations and discoveries in order to maintain the effectiveness of ABT. The most important news for ABT are not the new ABs, but the increased doses of those already known and their grouping by type of bacterial resistance.

MAXIMUM DOSES CURRENTLY RECOMMENDED

The new maximum doses are recommended by EUCAST 2019, p.96-99, systematized on AB groups, International Common Names (DCI) and with mentions for different clinical situations (2), and in the following we shall provide a summary, with the mention, „*" If used in Romania:

Penicillins: Benzylpenicillin* 1.2 g (2MU) x 4-6 iv, or 2.4 (4 MU) x 6 iv in meningitis and pneumonia; Ampicillin* 2 g x 4 iv, or 2 g x 6 iv in meningitis; Ampicillin+Sulbactam* 3 g x 4 iv; Amoxicllin* iv 2 g x 6 iv; Amoxicllin - oral 1 g x 3; Amoxicllin+clavulanic acid* iv 2.2 g x 3 iv; Amoxicllin+ clavulanic acid - oral 1 g x 3; Piperacillin* 4 g x 4 iv; Piperacillin+tazobactam* 4.5 x 4 iv; Ticarcillin* 3 g x 6 iv; Ticarcillin+clavulanic acid* 3.1 g x 6 iv; Phenoxymethyl-penicillin 2 g x 4 oral; Oxacillin* 1 g x 6 iv; Cloxacillin 1 g x 4 oral or 2 g x 6 iv; Dicloxacillin 2 g x 4 oral or 2 g x 6 iv; Flucloxacillin* 1 g x 4 oral or 2 g x 6 iv; Mecillinam 0.4 g x 3 oral.

Cephalosporins: Cefaclor* 0.25-1 g tidpo; Cefadroxil* 0.5-1 g bidpo; Cephalexin* 0.25-1 g bid-tid po; Cefazolin* 1 g tid-qid (or 2 g tid) iv; Cefepime* 2 g tid iv; Cefixime* 0.2- 0.4 g bid po, or 0.4 g po in gonorrhea; Cefotaxime* 2 g tid iv, or 2 g qid in meningitis; Cefpodoxime* 0.1-0.2 g bid po; Ceftaroline* 0.6 g tid iv (slow infusion – 2 hrs); Ceftazidime* 2 g tid iv or 1 g x 6 iv; Ceftazidime+avibactam* 2.5 g tidpev (2 hr infusion); Ceftibuten 0.4 g po; Ceftobiprole 0.5 g tid iv (slow infusion – 2 hrs); Ceftolozane+tazobactam* 1.5 g tid iv (slow infusion – 1 hr); Ceftriaxone* 2 g bid iv, or 4 g iv in meningitis; Cefuroxime* 1.5 g tid iv; Cefuroxime 0.5 g bid po.

Carbapenems: Ertapenem* 1 g x 1 iv; Imipenem* 1 g qid iv (30 mins perfusion); Meropenem* 2 g tid iv (3 hrs perfusion), or in meningitis 2 g tid iv; Meropenem+vaborbactam 4 g tid iv.

Monobactams: Aztreonam 2 g qid iv.

Fluoroquinolones: Ciprofloxacin* 0.75 g bid po, or 0.4 g tid iv; Levofloxacin* 0.5g bid po, or 0.5g bid iv; Moxifloxacin* 0.4g x 1 po, or 0.4g x 1 iv; Norfloxacin* 0.4g bid po; Ofloxacin* 0.4 g bid po, or 0.4 g bid iv.

Aminoglycosides: Amikacin* 30 mg/kg x 1 iv; Gentamicin* 7 mg/kg x 1 iv; Netilmycin* 7 mg/kg x 1 iv; Tobramycin* 7 mg/kg x 1 iv.

Glycopeptides and glycolipopeptides: Dalbavancin* 1 g x 1 iv; Oritavancin 1.2 g x 1 iv; Teicoplanin* 0.8 g x1 iv (single dose in slow infusion – 3 hrs); Telavancin 10 mg/kg x 1 (slow infusion – 1 hr); Vancomycin* 0.5 g qid iv, or 1 g bid iv, or 2 g x 1 in perfusion.

Macrolides, lincosamides and streptogramins: Azithromycin* 0.5 g x 1 po, or 0.5 g x 1 iv, or 2 g po in single dose; Clarithromycin* 0.5 g bid po; Erythromycin* 1 g qid, or 1 g qid iv; Roxithromycin 0.15 g bid po, Telithromycin 0.8 g x 1 oral; Clindamycin* 0.3 g qid po, or 0.9 g tid iv; Quinupristin+dalfopristin 7.5 mg/kg tid iv.

Tetracyclines: Doxycycline* 0.2 g x 1 po; Eravacycline 1 mg/kg bid iv; Minocycline* 0.1 g bid po; Tetracycline* 0.5 g qid po; Tigecycline*0.1 g followed by 50 mg bid iv.

Oxazolidinone: Linezolid* 0.6 g bid po, or 0.6 g bid iv; Tedizolid* 0.2 g x 1 po.

Other antibiotics: Chloramphenicol* 2 g qid po, or 2 g qid iv; Colistin* 4.5 MU bid iv; Daptomycin* 6 mg/kg x 1 iv; Fosfomycin* 8 g tid iv or 3 g x 1 po in a single dose; Fusidic acid* 0.5 g tid iv; Metronidazole* 0.5 g tid po, or 0.5 g tid iv; Nitrofurantoin* 50-100 mg tid-qid po; Nitroxoline* 0.25 g tid po; Rifampicin* 0.6 g bid po, or 0.6 g bid iv; Spectinomycin 2 g x 1 im; Trimethoprim 0.16 g bid po; Trimethoprim+ sulfamethoxazole* 1.44 g bid po, or 1.44 g bid iv.

CLINICAL GROUPING OF ABs

Changing the posology and increasing the doses of ABs, where necessary and possible, contributes to maintaining a reasonable susceptibility of pathogenic bacteria, maintaining the efficiency of ABT. Clinicians should rationally use ABs and shall apply, where appropriate, the new recommendations, which move the category of bacteria with intermediate sensitivity, from resistant to susceptible [2]. Consideration should also be given to the recent clinical classification of ABs in the three groups: for infections with common bacteria (Access group antibiotics), with resistant bacteria (Watch group antibiotics) and the reserve (Reserve group antibiotics), as well as special antibiotics (eg anti-tuberculosis, anti-leprosy), according to the WHO Model List Essential Medicines, no 20/2017 and 21/2019 [1]. The practical advantage for medicine is immense, but if in medical practice it is found that many clinicians still do not follow the rules of good practice of ABT, action will have to be taken. One of them could be, in extremis, that according to the recommendations of WHO 2017 and 2019, ABs from the therapeutic groups should be prescribed according to the competence of the doctor:

ABs group I, usual, by any doctor with the right to free practice,

ABs group II, for resistant or multidrug-resistant bacteria, by specialized clinicians,

ABs group III, reserved, by primary care clinicians,

Special ABs, by the clinicians, according to their specialty.

Obviously, "off-label prescriptions" could represent the exception, in justified cases; As an example, it has been found that the antiparasitic Metronidazole is also the most effective antianaerobic AB.

There are also some ABs, which, although authorized, are so little used in national medical practice that they could be considered as new ABs. It is interesting to see how many new ABs will be authorized in the coming years. It is very important to discover new anti-infective drugs, both with new molecules, and by highlighting antibacterial and/or antibiofilm properties in pharmaceuticals known for other pharmacological effects. This type of applied research involves smaller investments, and the results can be faster, going up to the patenting of new innovative anti-infective drugs, in the near future.

CONCLUSIONS

Antibiotherapy is constantly dynamic, in order to keep up with the adaptation of pathogenic bacteria to the selection of pressure exerted by ABs, We must be aware of these trends, apply new acquisitions in the field of drugs, dosage conditioning and dosage, to help maintain an effective ABT, and abandon, where appropriate, the old methods and habits. Unfortunately, it is possible that some healthcare workers may continue to prescribe ABs, not for treating the patient, but for their own peace of mind and as well as their patients' or for any other reasons.

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CLINICAL PRACTICE

Clinicopathological evaluation of Moyamoya disease. Case report and review of literature

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Abstract: *Moyamoya disease is a rare affliction, which progressively affects cerebral arteries, more frequently those in the circle of Willis and the internal carotid arteries. This condition has a genetic component, but the cause of the disease is unknown. It usually affects children but can affect adults as well, representing a health issue due to its mortality. Not in a few cases, Moyamoya disease has been difficult to recognize as a cause of ischemic or hemorrhagic strokes. In this article, we present the case of a young patient, with a medical history of cerebral hemorrhage, which was diagnosed with Moyamoya disease after clinical and cerebral angiographic corroboration in a specialized vascular center. The patient entered the Emergency Receiving Unit with a sudden comatose state. Paraclinical tests revealed massive intraparenchymal hemorrhage, with movement in the medial line and ventricular flooding.*

Keywords: *Moyamoya disease, stroke, cerebral death*

INTRODUCTION

Moyamoya disease is a rare progressive blood vessel disease characterized by progressive stenosis of cerebral arteries, more frequently those in the circle of Willis and the internal carotid arteries. A collateral blood flow is formed around the blocked arteries to compensate for the lack of blood flow, but those collateral vessels are small and fragile, thus increasing the risk of bleeding, aneurysm and thrombosis [1].

The name of Moyamoya disease comes from the Japanese language, where it means "cloud of smoke". This disease was discovered in 1957 as an internal carotid artery hypoplasia of the tunica intima [2].

This condition has a genetic component, but the cause of the disease is unknown. Patients may present chorea, headache, seizures, or cognitive impairment expression of transient ischemic attacks or ischemic stroke and a large proportion of patients, mostly adults presenting with intracerebral hemorrhage. Two age peaks are recognized: one during childhood and one in young adults [1, 3]. In most regions, women are more frequently affected than men [3].

Although Moyamoya is a vascular disease, it is associated with other medical conditions like autoimmune disease, Down syndrome, arteriosclerosis, neurofibromatosis type 1 or other multiple tumors developing in the spinal cord [4, 5, 6].

In this article we present the case of a young patient, with a medical history of cerebral hemorrhage, who was diagnosed with Moyamoya disease after clinical and cerebral angiographic corroboration in a specialized vascular center and we review the literature on this disease.

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CASE PRESENTATION

We present the case of a patient aged 39, from an urban environment, known with having a medical history of cerebral hemorrhage in the previous year (twice in 6 months). The patient was investigated in a specialized vascular center in western Romania, where she was diagnosed with Moyamoya disease and was recommended a course of treatment. She is brought into the Emergency Clinical County Hospital in Oradea, Romania, with a sudden comatose state which had come on several hours before. On physical examination, neurological testing reveals asymmetric mydriasis, bilateral flexion in the plantar cutaneous reflex, symmetrical dropping of the arms and feet on the bed and a Glasgow score of 3. Other examinations (skin, extremities, abdomen, musculoskeletal system) were within normal limits.

A cranial CT scan shows massive localized hemorrhage in the left temporoparietal region, of 5.6/4/1 cm, with ventricular flooding in the third and lateral ventricles and mass effect with movement in medial line of 8 cm (Figure 1).

Figure 1: Cranial CT image shows the hemorrhagic accumulation with fresh blood; intraparenchymatous temporo-parietal densities with 5.6/4.1 cm axial diameters; slight perilesional edema, ventricular flooding in the bilateral lateral and third ventricles, and mass effect, the median line is shifted to the right by approximately 8 cm.

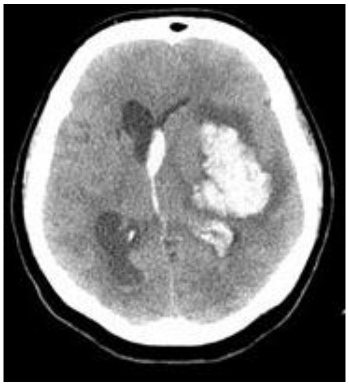

Laboratory results on admission showed hydro-mineral imbalance (hypokalemia and hypomagnesemia), which were corrected and remained within normal limits for the short duration of the patient's hospital visit. Complete blood cell count and examination of the peripheral smear showed normocytic anemia with normal leukocyte and platelet counts. Coagulation tests (D dimer, fibrinogen, partial thromboplastin time, prothrombin time, and thrombin time) were within normal limits.

The patient is then transferred to the Intensive Care Unit, where she receives orotracheal intubation and mechanical ventilation. Depletive and neurotrophic cerebral treatment is instituted, along with antibiotic therapy and acid-base and electrolytic balancing. The neurological state of the patient remains unchanged, with a Glasgow score of 3, fixed bilateral mydriasis and no cerebral reflexes. A control cranial CT scan is undertaken within another 48 hours, which shows the same collection of temporoparietal hemorrhage and no other modifications. The lateral ventricles have increased in size compared to the previous CT scan, with erased subarachnoid spaces (Figure 2). The suspicion of cerebral death is raised. After 72 hours, the suspicion of cerebral death was raised and the protocol in declaring cerebral death is followed, which is then confirmed by 2 EEG's, 2 apnea tests, ICU doctor, neurologist, neurosurgical assessment (Figure 3).

Figure 2: Cranial CT Image: the temporal-parietal intraparenchymal massive hemorrhagic left collection with ventricular intrusion in the right-side ventricle and ventricle 3 and relatively unchanged mass effect. The lateral ventricles have increased in dimension compared to the previous examination, bilaterally. Deleted subarachnoid spaces. Hypodensity of the cerebral parenchyma with deletion of the differentiation between the white subset and the newly developed gray substance which may suggest hypoxic ischaemia.

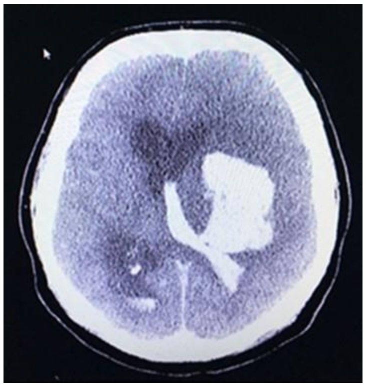

Figure 3: Evolution of patient

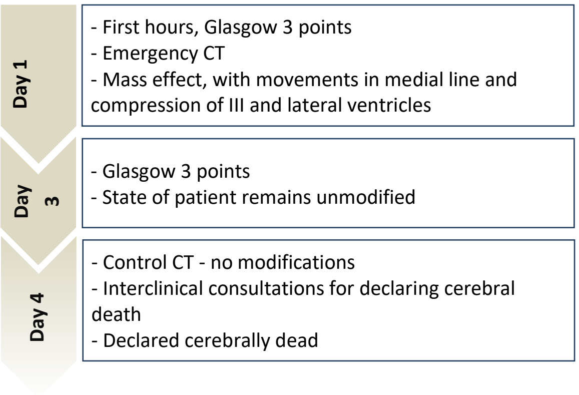

- First hours, Glasgow 3 points
- Emergency CT
- Mass effect, with movements in medial line and compression of III and lateral ventricles

- Glasgow 3 points
- State of patient remains unmodified

- Control CT - no modifications
- Interclinical consultations for declaring cerebral death
- Declared cerebrally dead

In the case we presented, the patient suffered a profound left cerebral vascular hemorrhagic accident, with three ventricular flooding and mass effect, as well as movement in the medial line by 8 cm, which made her comatose. Despite proper treatment, no signs of improvement were recorded. The family grants permission for transplant and investigations begin to facilitate organ transplantation. In the subsequent necrotic examination, a massive 7/6/3 intraparenchymal hemorrhage is observed, with erased channels and flattened circumvolutions, as well as reddish cerebrospinal fluid.

DISCUSSIONS

Moyamoya disease is a rare pathology, but its seriousness needs to make us aware of the risks that can appear. This disease is a medical problem from a variety of standpoints because it can appear in both children and adults, it progressively affects cerebral arteries through stenosis and occlusion and can lead to cerebral vascular accidents or repetitive transient ischemic attacks, which can kill the patient by themselves. Usually, children present symptoms attributed to slowly progressive cerebral hypoperfusion and repetitive ischemic symptoms, while adults have hemorrhagic or ischemic events [7-8].

The cause of Moyamoya disease is unknown, but it is believed that there is a genetic component involved [9]. According to some studies, this disease could be autosomal dominant, with incomplete penetration, depending on age and genomic factors [10]. Another study shows that the RNF213 gene is the main gene associated with Moyamoya disease [11]. In some studies, arterial stenosis was found to occur in not only the intracranial arteries but also renal, pulmonary, coronary, and pancreatic arteries. Renal artery stenosis is the most frequently reported associated with Moyamoya disease and the consequence is renovascular hypertension [12].

The walls of blood vessels have three layers in their structure: tunica intima, media and adventitia. From a morpho-pathological point of view, Moyamoya disease is characterized by the thickening of the tunica intima in internal carotid arteries bilaterally, which, at this level, can contain lipid deposits. The most frequently affected arteries are the anterior, middle and posterior cerebral arteries, which form the circle of Willis and can present with different degrees of stenosis and occlusion because of thickening in the tunica intima, curling in the internal elastic lamina and thinning of the tunica media.

The transversal sectioning of a basal artery in a patient with Moyamoya disease, with hematoxylin-eosin staining shows fibrosis and thickening of the tunica intima and proliferation of the smooth muscle cells (white arrows), as well as the folding and chronic contraction of the internal elastic lamina (blue arrows) and absence of inflammatory or atherosclerotic changes [13].

The incidence of the disease is greater in children around the age of five and adults around the age of 40. The condition is more frequent in women than in men, with a higher incidence in Asian population and a lower one in the European population. Women can present with transient ischemic attacks or cerebral hemorrhages in their medical history, or they can be asymptomatic. The recurrence in cerebral vascular accidents can be higher in these patients [14]. Clinically, patients with Moyamoya disease may present seizures, transient ischemic attacks, ischemic and hemorrhagic strokes. Headache, speech disturbances, visual deficits, intellectual deterioration, cranial nerve paralysis can also be seen in both children and adults. Mental retardation can be seen in children having Moyamoya disease.

Moyamoya disease is an important diagnosis to consider in children or young adults presenting stroke. Cerebrospinal fluid results of a lumbar puncture or electro-encephalography can be abnormal but are usually nonspecific. This pathology can be most frequently diagnosed in a quick fashion by using modern cerebral imaging methods such as an angio-CT, angio-MRI or 3D-DSA (rotational digital subtraction angiography), in a disease that is sometimes asymptomatic. Knowing all the radiological signs have a major influence on placing early and correct diagnosis, which can lead to prompt treatment and a possible increase in life expectancy in these patients.

Unfortunately, the therapeutic options are limited or inappropriate. Treatment in the acute phase is symptomatic for maintaining cerebro-vascular perfusion. Anticoagulant and antiplatelet agents, corticosteroids have shown no remarkable benefit. Surgical treatment procedures have been used to manage the hemorrhagic and ischemic consequences of Moyamoya disease to restore and maintain adequate cerebral perfusion, but there are serious adverse effects including cerebrovascular infarct or hemorrhage, motor or sensory dysfunction, cognitive decline, and death [15].

CONCLUSION

This pathology needs to be taken seriously because it presents a significant risk factor in creating cerebral vascular accidents, both ischemic and hemorrhagic, in young adults. Surgery can be beneficial, particularly if the diagnosis of moyamoya disease is made early. In patients with a history of cerebral accidents, angio-CT or angio-MRI and/or obtaining a protein RNF213 dosage would be recommendedto confirm or exclude Moyamoya disease in its early stages and institute proper specialized treatment.

Conflict of interests

None.

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CLINICAL PRACTICE

Elastofibroma – an uncommon cause of upper limbs pain

Natalia Dumitrescu[1], Bianca Sandu[2], Silviu V. Dumitrescu[2]

A female patient age 65 came to our clinic forcontinuous pain of the upper limbs (left more than right), increased when moving the scapulae. Patient declared that in the past few months the dorsal decubit became gradually painful and she cannot achive a good sleep. One year ago patient was diagnosed with bilateral suprascapular lipoma and a surgical removal was proposed.

At the visual examination, we found two well-circumscribed suprascapular tumor mobile with arms movements. At palpation, we noticed that each scapula was pushed from below from an underlying tumor with a very firm consistency.

Figure 1: Clinical aspects

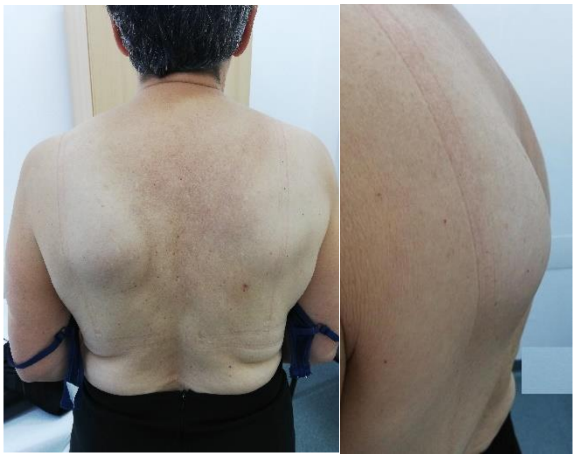

Chest radiography was normal.

A CT scan of torax was performed and revealed two subscapular unencapsulated, soft tissue masses with skeletal muscle attenuation interspersed with strands of fat attenuation [1, 2].

Figure 2: CT aspects

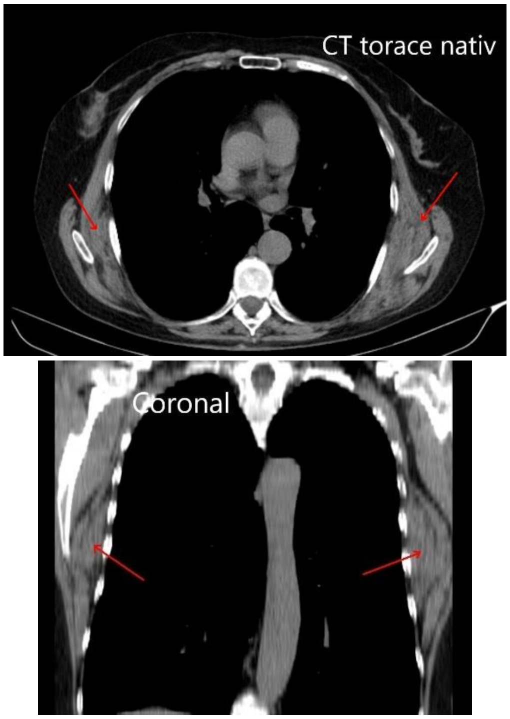

The patient was directed to thoracic surgery and the tumors were removed. The histological findings were consistent with the diagnosis of elastofibroma [3]. The recovery period was long, allmost two months passed before complete healing.

The long period needed for healing, usually burdened with local complications and the fact that the tumor is benign makes the surgery not recommendable for the patients with no discomfort or impared arms movement [4].

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CLINICAL PRACTICE

Crossed renal ectopia

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Abstract: *The case of 73 years - old male patient with inferior crossed renal ectopia diagnosed upon imaging workup using a GE LightSpeed 16 Slice CT, with urothelial carcinoma, at the level of the left kidney, nephrolithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery. The upper lobe of the right kidney fused with the lower lobe of the left kidney, on the left side of the midline. There were 6 renal arteries (4 on the left and 2 on the right) originating from the left side of the abdominal aorta, at the level of the termination of the abdominal aorta, the left common iliac artery and the right internal iliac artery. The patient presented with 6 renal veins (3 on the left and 3 on the right) that drained anteriorly on the left side of the inferior vena cava into the left common iliac vein and into the right internal iliac vein.*

Keywords: *crossed renal ectopia; arteries – veins*

INTRODUCTION

Crossed ectopic kidney (CFE) is an anomaly within the fused kidneys (FKs) group of malformations, consisting of the fusing of the upper pole of one kidney with the opposite lower pole of the other kidney traversing the midline, with a reversed L-shaped view. [1] describes 6 types of fused kidneys (FKs). [2], quoting Sousa Escandon and Aguilar Tubet, mentions that the first case of crossed renal ectopia was described by Pamarolus in 1654 and the classification of fused kidneys was done in 1957 by McDonalds and McClellan. According to [3], most frequently, the left kidney is the one fusing on the right part, aspect met in the case described by [1]. In our case, as well as in the first two cases described by [4] and in the case described by [5], it was the right kidney that traversed the midline in order to fuse with the left kidney. Numerical variation are frequently met in crossed fused ectopia of the renal veins, comporting 1 to 6 arteries originating from the aorta or the iliac veins (common and internal), and one or more veins that terminate into the inferior vena cava or in the iliac veins (common or internal). The ureters usually do not present variants or anomalies, being just one for each kidney. The renal hilum is anterior or anteromedial. Frequently, crossed fused ectopia is asymptomatic [2, 3, 5, 6] and incidentally discovered during a consult for different other medical reasons, although cases of crossed fused ectopia may present a pathology associated with congenital malformations. As associated pathologies, we have : hematuria, dysuria, urinary tract infections, renal failure, nephrolithiasis, obstruction of the ureteropyelic junction, hydronephrosis, urinary stasis, ectopic ureterocele, urothelial carcinoma [2, 3, 5, 6, 7]. As for congenital malformations, we may encounter the following: cardiac malformation, skeletal-muscle malformation, genital malformations, gastrointestinal malformation [3, 6, 7, 8, 9]. The case presented in our review was diagnosed with urothelial carcinoma of the left kidney, nephrolithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery.

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CASE STUDY

73-year old male patient, admitted at the Emergency Clinical County Hospital "Sf. Andrei" in Constanta, diagnosed with urothelial carcinoma of the left kidney, renal lithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery. After the CT scan that was performed using GE LightSpeed 16 Slice CT, the patient was diagnosed with crossed renal ectopia. The right kidney crossed the midline – its upper pole fused with the lower pole of the left kidney, forming a 129,4° angle, showing a reversed L-shape aspect. The diagnosis of inferior crossed renal ectopia – type D was made, according to the classification of [1].

The left kidney was located vertically in the upper part and presented with the upper pole laterally to the costal processes of the L2 vertebra, covering the inferior top of the 12th rib. Its inferior pole reached the pelvis, at the level of inferior half of the S1 vertebra, at the level of sacral ala and the upper medial of the left iliac fossa. The renal isthmus, slightly oblique-inferior to the right, was located above the superior margin of the L5 vertebra and at the level of the L5 – S1 intervertebral disk and at the level of the left lateral part of the S1 vertebral body, at the level of the sacral ala. The inferior pole of the left kidney was positioned anteriorly to the sacral ala and also anteriorly to the upper medial part of the left iliac fossa. The left renal hilum was positioned anteriorly. The right kidney was horizontal (slightly oblique-lateroinferior to the right) with its superior pole corresponding to the inferolateral part of the L5 vertebral body, to the right lateral part of the L5 – S1 intervertebral disk and to the left sacral media ala, and also corresponding to the anterolateral sides of the sacral S1 – S2 vertebras. Its inferior pole overpassed the midline of the sacrum corresponding to the right lateral side of the S3 vertebra, above the anterior third sacral hole (Figure 1). The right hilum was positioned anteroinferiorly.

Figure 1: Our case of CFE in relation with vertebral column

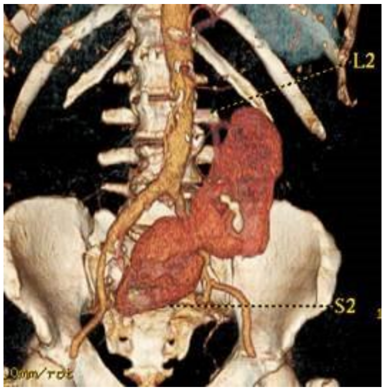

The complex of the 2 kidneys had a longitudinal diameter of 190.6mm, a transverse diameter of 113.8mm and with a volume of 295,788 cm^3. The left kidney (Figure 2) had a bipolar diameter of 122.9mm, a transverse diameter of 65.3mm and an anteroposterior diameter of 36.5mm, with a parenchymal thickness of 20mm and a volume of 160 cm^3.

Figure 2: Left kidney diameters

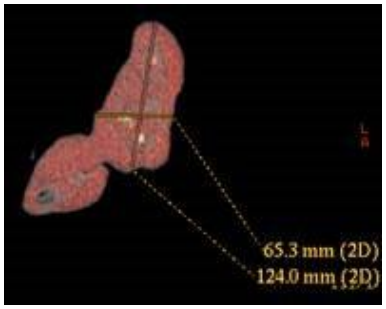

The renal isthmus (Figures 3, 4) had an anteroposterior diameter of 18.1mm, a length of 31 mm, located next to the L4 – L5 intervertebral disk, 30 mm laterally to the midline.

Figure 3: Isthmus antero-posterior diameter (18.1 mm)

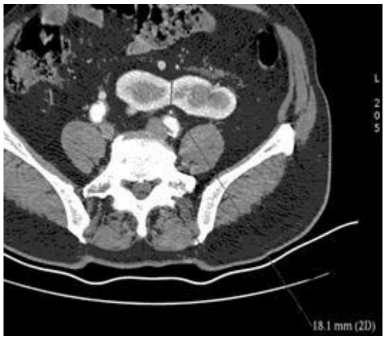

Figure 4: Isthmus lenght (30.6 mm)

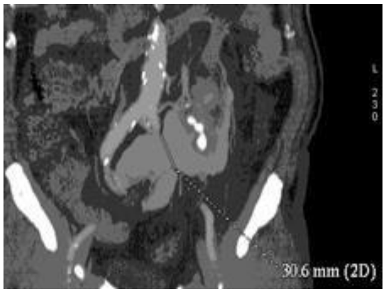

The right kidney (Figure 5) had a bipolar diameter of 105.7 mm, a transverse diameter of 58.8 mm, an anteroposterior diameter of 35.8 mm, with a parenchymal thickness of 20.3 mm and a volume of 136,295 cm^3.

The right ureter had a diameter of 6.0 mm and the left ureter presented with a diameter of 5.0 mm.

The 6 renal arteries of the cross fused ectopia (CFE) were positioned one under the other, originating at the level of the abdominal aorta and at the level of the internal and common iliac arteries, each artery with its own vascularization territory.

Figure 5: Right kidney diameters

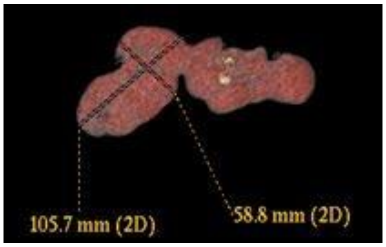

The first renal artery originated on the left side of the aorta, approximately 19mm inferiorly to the origin of the superior mesenteric artery, at the inferior 1/3 of the L1 vertebral body, with a diameter of 1.7 mm that penetrated the upper pole of the left kidney giving birth to a branch the descended anteriorly to the bassinet.

The second renal artery also originated from the left side of the aorta, approximately 55mm inferiorly to the first renal artery, next to the right upper part of the L3 vertebra, with a diameter of 4.3 mm penetrating the renal sinus from behind the bassinet of the left kidney.

The third renal artery originated at the level of the aortic termination, next to the L4 vertebral body, approximately 40 mm inferiorly to the second renal artery, with a diameter of 4.6 mm. It bifurcated into an anterior branch penetrating the renal sinus behind the bassinet and into a posterior branch penetrating the renal parenchyma of the inferior pole issuing a branch that penetrated the renal isthmus and branches for both renal poles.

The fourth artery originated in the left common iliac artery, approximately 60 mm from the bifurcation of the aorta, with a diameter of 2.6 mm. The artery bypasses the lower left pole of the kidney, penetrating the renal sinus anterior to the bassinet flowing into the inferior mesorenal.

The fifth renal artery originated in the right internal iliac artery, at the level of its origin, approximately 62mm from the bifurcation of the aorta, with a diameter of 6.8mm; it penetrated in the right renal sinus where it bifurcated into a prepyelic and retropyelic branches.

The sixth renal artery originated in the posterior trunk of the right internal iliac artery, approximately 62 mm inferiorly to the fifth renal artery, with a diameter of 2.2 mm, perforating the renal parenchyma next to the left inferior kidney.

The left testicular artery originated from the left side of the abdominal aorta, 28 mm under the first renal artery, next to the middle of the L2 vertebral body, with a diameter of 1.7mm and in its ascending route passing behind the left bassinet, issuing a branch for the tumor formation.

There were 6 renal veins (Figures 6, 7, 8), 3 veins for each kidney – for the left kidney we had V1, V2 and V4 and for the right kidney, we had V3, V5 and V6.

Figure 6: CFE 6 renal arteries (R1, R2, R3, R4, R5, R6) and vascular territories

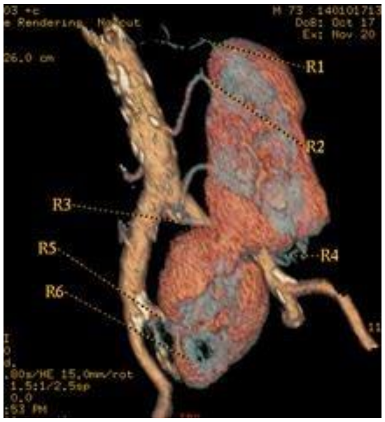

Figure 7: CFE 6 renal veins (V1, V2, V3, V4, V5, V6)

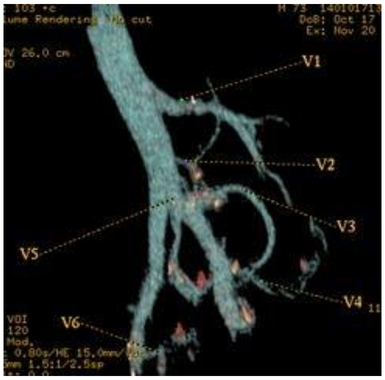

Figure 8: CFE veins and renal drainage territories

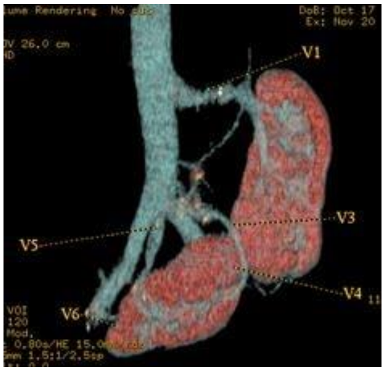

The first renal vein ended into the left side of the inferior vena cava, next to the L2 vertebra, it drained the left kidney, with a retropyelic and retroaortic trajectory and with a diameter of 9.3 mm.

The second renal vein formed by the fusing of an anastomotic branch with the first renal vein with a left lumbar branch, also ending into the left side of the inferior vena cava, next to the L3 vertebra, with a retroaortic trajectory, draining the left kidney and having a diameter of 4.8 mm.

The third renal vein originated from the right renal sinus anterior to the bassinet (also draining the upper pole), ending into the left common iliac vein, 19 mm from the formation of the inferior vena cava, with a diameter of 8.7 mm, draining the right kidney.

The fourth renal vein originated from the left renal sinus next to the inferior pole, accompaning the fourth renal artery, ending into the left common iliac vein, approximately 68 mm from the formation of the inferior vena cava, with a diameter of 5.4 mm.

The fifth renal vein originated from the renal sinus on the posterior side of the right bassinet along with the fifth renal artery was bypassing anteriorly the right common iliac artery, ending on the anterior side of the inferior vena cava, approximately 10 mm above the IVC origin and approximately 22 mm inferiorly to the second renal vein, with a diameter of 8.3 mm.

The sixth renal vein originated from the right inferior pole along with the sixth renal artery, ending in the right internal iliac vein at 33 mm inferiorly to the formation of the right common iliac vein, with a diameter of 5.2 mm.

DISCUSSION

Within the fused kidneys (FKs) group of malformations, the most frequently met is the horseshoe kidney (HK), the crossed fused ectopia (CFE) being the second anomaly in terms of frequency. The horseshoe kidney has a bigger rate of incidence: 1/400 cases according to Lawsley, Eisendrath, Bauer (quoted by 10), 1/600 according to Moore, or 1/1,200 according to Rubin (both quoted by [4]). According to [7], CFE is a rare anomaly of the kidneys, with a prevalence rate of 1/1000 – 1/7500 of cases (Wilmer, Abeshouse, quoted by 10), although a renal anomaly, according to [3] and Bauer (quoted by [7]) incidence rates of 1/300 – 1/7500 of cases. [10] considers the ratio between the frequency of CFE/HK is of 1/7.7. CFE is most frequent in male cases, i.e. double the cases in relation to female cases [9], Bauer (quoted by [7]), 3/1 Boyan (quoted by [5]) or 2.3/1 according to Muttarak (quoted by [5]). [2] describes 3 cases of CFE, 2 of which are female cases and 1 male case.

[4] describes 2 cases of CFE: in one case he finds 6 right renal arteries (2 arteries originating from the lateral side of the aorta and were destined to the left kidney; 1 artery originating from the posterior side of the aorta, vascularizing the isthmus and the left inferior kidney; 1 artery originating at the level of the bifurcation of the aorta that penetrated the left kidney on the posterior side and issued a voluminous branch for the right kidney; the right kidney was vascularized by 2 arteries – one originating on the anterolateral side of the aorta and the other originating from the right common iliac artery). In this case, he finds only 3 renal veins (2 right renal veins and 1 left renal vein). In the second case, he also finds 6 renal arteries: 2 left renal arteries originating on the lateral side of the aorta and 4 right renal arteries - 3 originating on the lateral side of the aorta and one artery originating at the level of the terminal bifurcation of the aorta. In this case, he finds only 2 renal veins – right and left.

[5] describes the case of a CFE that presented 4 renal arteries – 1 artery for the superior renal parenchyma (left kidney) and 3 renal arteries for the inferior renal parenchyma (right kidney). De venous drain was composed of 2 renal veins.

According to Papin, quoted by [7], fused renal anomalies may present with 2 renal arteries (in 20% of the cases), 3-5 renal arteries (in 66% of the cases) and more than 5 renal arteries (in 14% of the cases).

[3] quoting Bauer and bailey, considers that the total number of the arteries for a crossed fused ectopic kidney varies from 1 to most frequently met cases ranging from 2 to 4 arteries; he discovered 3 renal arteries for the left kidney and 1 renal artery for the right kidney.

[6] describes a case of CFE that presented 2 renal arteries originating in the common iliac arteries.

[9] describes one single case presented with only 2 renal arteries (originating on the sides of the aorta), the right renal artery having a precaval trajectory and 2 renal veins – the left renal vein with a retroaortic trajectory.

The origin of the CFE arteries varies, usually the main arteries or in the case where there are only 2 renal arteries originating in the abdominal aorta. [6] finds the origin of the 2 renal arteries in the common iliac arteries.

We found that out of the 6 renal arteries, 2 renal arteries (R1 and R2) originated on the left side of the abdominal aorta, one renal artery originating at the level of the terminal ramification of the aorta (R3) and into the left internal iliac artery (R4), the last 2 arteries originating in the right internal iliac artery – 1 at the level of the unique trunk of the artery (R5) and the other at the level of the posterior trunk of the right internal iliac artery (R5).

[34] finds that most frequently the origin of the CFE arteries is at the level of the abdominal aorta (lateral right side or left side, posterior side and aortic bifurcation), considering the origin of the artery at the level of the right common iliac artery.

In the case of [5], he finds the origin of the 4 renal arteries only at the level of the lateral sides of the aorta – on the right side, 1 artery and on the left side, 3 arteries.

According to [3] the origin of the fused ectopic kidney is at the level of the superior abdominal aorta (in 25% of the cases), whereas for the rest of the cases, he considers the origin at the level of the inferior aorta and the iliac arteries.

The 6 arteries penetrated the kidney at its superior pole (R1), at the level of the renal hilum (R2, R4 and R5), or at the level of the inferior pole of the kidney (R6). R3 terminated into a bifurcation that issued a hilar branch and another branch that inserted in the kidney through its inferior pole. R2 penetrated the hilum posteriorly to the bassinet whereas R4 anteriorly to the bassinet and R5 issued one prepyelic branch and another retropyelic branch.

In two of his cases, [4] finds the CFE arteries penetrating the kidneys about the same levels as ours and he also describes a renal artery for the left kidney that bifurcated into one hilar branch and into an isthmic branch, whereas for the right kidney he finds 2 renal arteries that penetrated the kidney through its anterior side, respectively at the level of its posterior side.

The most voluminous of the arteries was the fifth renal artery that vascularized the right kidney and the less voluminous artery was the sixth renal artery also destined to the right kidney.

There was no renal artery for the renal isthmus. It receives a branch through an artery destined to the inferior pole of the left kidney or through an artery destined to the right kidney.

Out of the 6 renal veins of the CFE, 3 of them terminated in the inferior vena cava, 2 in the left common iliac vein and 1 in the right internal iliac vein. The veins of the left kidney left the kidney through the upper pole (V1) and through the renal hilum (V2 and V4) whereas the veins of the right kidney left the kidney through the renal hilum (V3 and V5) and through the lower pole of the kidney (V6). Out of the veins of the right kidney, the first 2 renal veins had a posterior trajectory to the bassinet, presenting also with a retroaortic trajectory, and out of the veins of the right kidney, the fifth renal vein had a posterior trajectory to the bassinet. The veins of the left kidney terminated on the left side of the inferior vena cava (V1 and V2) and into the left common iliac vein, and the veins of the right kidney terminated into the anterior side of the inferior vena cava (V5) into the left common iliac vein (V4) and in the right internal iliac vein (V6). The first renal vein (V1) was the most voluminous one and belonged to the left kidney, and the less voluminous one was the second vein and originated also from the left kidney. There was no vein to serve exclusively the renal isthmus. Out of the 3 veins of the left kidney only the renal vein had a retroaortic trajectory.

In one of the cases [4] described, he met 3 renal veins: 2 right renal veins – one collecting the blood at the level of the left pole of the left kidney and from the level of the isthmus and flew into the left internal iliac vein, and the other collecting the blood of the upper part of the isthmus ending in the inferior vena cava, above its termination into the cava of the left renal vein. The left renal vein was normally positioned but it received an anastomosis from the left common iliac vein. The other case of CFE of [4] presented a normal left renal vein and 2 right renal veins: one originated at the level of the left pole of the kidney and terminated into the inferior vena cava, 2 cm under the termination of the left renal vein and the left pole of the right kidney with the isthmus presented a vena that terminated in the left iliac vein.

In the case described by [5] there were only 2 renal veins: the upper renal parenchyma was vascularized by a vein that terminated in the left side of the inferior vena cava and the lower renal parenchyma was drained by the second renal vein that terminated also in the left side of the inferior vena cava.

CONCLUSIONS

The case we presented is special due to its uniqueness, as we have not found a similar case of crossed renal ectopia presented with 6 renal arteries (4 left and 2 right) and 6 renal veins (3 left and 3 right) in the specialist literature.

There was no artery – vein correspondence in all 6 cases, such a correspondence was met only at the level of the pole vessels and there was only one case at the level of the hilum vessels. Regarding arteries, the most voluminous was a hilum artery, whereas the veins; the most voluminous, was a superior polar one.

We consider MDCT examination to be the best method of describing vascular and uretheral abnormalities in case of crossed fused ectopia, being able to indentify also, other associated malformation, aspect noted also by [3, 5, 7].

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VARIA

The synthesis and in vitro testing of symmetric bisquaternary salts with pyridin-isonitrosoacetanilide structure, active in the exposure to neurotoxic chemical agents

Cristina A. Secară[1], Bogdan Pătrânichi[1], Simona Bicheru[1], Mihail S. Tudosie[2]

Abstract: *Neurotoxic chemical agents induce complex toxicological effects with major adverse effects for those who are exposed to them. Medical countermeasures include administering atropine, a cholinesterase reactivating oxime and an anticonvulsant. The purpose of this reseach is the optimization of an antidote-based treatment in the intoxication with organophosphates through the synthesis and toxicological testing of new compounds with a pyridin-isonitrosoacetanilide structure and their comparison with obidoxime as reference.*

The objective of this study was the in vitro/in vivo assessment of the antidote properties and acute toxicity of synthethic oximes active in the exposure to neuroparalytic organophosphates.

The CT50 value for obidoxime was estimated, through calculus, to be 3.24 mg/ml and 9.33 ×10 -6 M respectively. The CT50 value for Bis1,3[2-hydroxyimino-N-(pyridyl)acetamide]oxapropandichloride (HIN 2) was estimated to be 2.72 mg/ml and 6.29 ×10 -6 M respectively.

The studied compounds showed values close to cellular viability at low and moderate equimolecular concentrations, administered in vitro to a cellular culture of fibroblasts. Bis1,3[2-hydroxyimino-N-(piridyl)acetamide]oxapropandichloride (HIN 2) showed an in vitro cellular viability value smalled than obidoxime at higher equimolecular concentrations, proving itself to be more toxic than obidoxime at high concentrations. Taking into account the close values of CT50, the newly synthethized compound, having this particular characterization from a toxicological standpoint, qualifies for further studies as an antidote.

Keywords: *paraoxon, acetylcholinesterase, obidoxime, N-acetyl-2-hydroxyiminomethylpyridinbromide (PYC8); 1,3(2-hydroxyimino-N-pyridin-3-propan bromide (HIN1); 1,3(2-hydroxyimino-N-pyridin-3-oxapropanchloride (HIN2)*

INTRODUCTION

High risk organophosphates (sarin, soman, tabun) are extremely toxic substances with a phosphoric acid esther structure. Due to their extremely high toxicity, they were registered on the list of substances that require destruction according to international regulations (Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction – Geneva). Their production, stockpiling and use are monitored by international organizations (Organization for prohibition of chemical weapons – OPCW). [4, 7, 23].

Neuroparalytic chemical agents have the property of irreversibly inhibiting erythrocyte acetylcholinesterase (AChE). The coupling reaction of the organophosphate at the level of the active site of the enzyme is shown in Figures 1, 2. [23]

The inhibition of AChE leads to the acummulation of acetylcholine (ACh) at the level of the synaptic cleft and the hyperstimulation of muscarinic and nicotinic receptors. The

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clinical signs of mild intoxication are pupil constriction, sweating, muscular fasciculation, and the decrease of cardiac frequency and of arterial tension.

Figure 1: The coupling mechanism of the organophosphate at the level of the active site of AchE

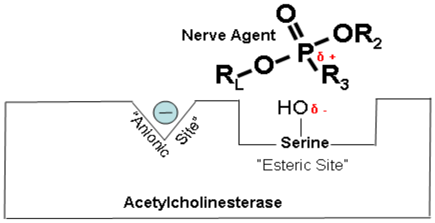

In high concentrations of the toxic compounds, convulsions occur and death takes place through respiratory insufficiency caused by the paralysis of respiratory centers. Cholinesterase-inhibiting neurotoxic organophospates possess, in their molecule, two organic groups, identical or different, and an active group that is inorganic or organic, bound to a pentavalent phosphorus atom. This atom can be bound to an oxygen or sulphur atom (phosphates, phosphonates, tiophosphates, tiophosphonates). [1, 4, 9, 23] In scientific literature, they were presented as potential simulants for each class or warfare chemical agents [4, 16, 22].

The laboratory use of neurotoxic chemical agents is restricted, as they are extremely toxic substances. Thus, antidote studies and those refering to their pharmacotoxicological properties are performed utilizing simulants of the paraoxon type. A simulant is defined as a compound possessing all relevant physical and chemical properties of the corresponding neurotoxic chemical agent, but with lower intrinsical toxicity; The utilization of simlants in research and testing activities contributes to the reduction of the stress and risk the personnel involved in the testing procedures is subjected to by handling chemical agents with high toxicity. [1, 23]

Figure 2: The interaction between AchE and the organophosphate

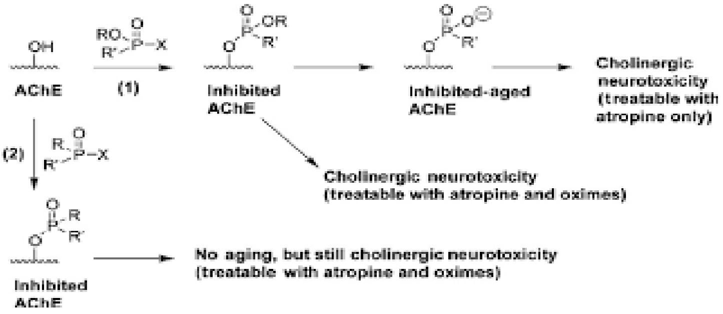
OH
AChE
RO
R'
P–X
(1)
OR
R'
Inhibited AChE
O
R'
Inhibited-aged AChE
Cholinergic neurotoxicity (treatable with atropine only)
Cholinergic neurotoxicity (treatable with atropine and oximes)
(2)
R
R'
P–X
Inhibited AChE
No aging, but still cholinergic neurotoxicity (treatable with atropine and oximes)

The general emergency measures necessary to stop the exposure are masks, decontamination and the evacuation of the individual from a contaminated environment. Medical countermeasures are the administration of atropine, of a cholinesterase reactivating oxime and of an anticonvulsant. The increase of antidote treatment efficiency in the intoxication with organophosphates is one of the main objectives of research activity at national and international level, taking into account both aspects of clinical toxicology of organophosphates used as pesticides and of experimental toxicological research of neuroparalytic war chemical agents. [4, 8, 12, 16, 19] Cholinesterase-reactivating compounds used in the UN member states (pralidoxime, obidoxime, HI6) possess therapeutic efficiency for different neuroparalytic chemical agents [1, 2, 3, 5, 11, 15, 17, 22]. Having quaternary and bisquaternary salts chemical structures with pyridinic rings, this substances are known and studied as efficient antidotes in the processes of activation-reactivation of acetylcholinesterase in the intoxication with organophosphates [6, 10, 22].

Bisquaternary compounds such as Hagedorn, HI-6, Obidoxime, are obtained through the quaternization of pyridine bases substituted (oximes, carboxamides) with reactive dihalogenated derivatives such as symmetric dichloromethylether (DCDMS) or 1,3-dibromopropane [6, 14, 22]. Symmetric quaternary salts obtained through the quaternization of pyridine substituted with oxime or amide groups in a 2:1 molar ratio to the dihalogenated derivate are intensely studied. The typical example is represented by obidoxime, the product of 4-pyridinaldoxime bisquaternization with exces symmetric dichlorodimethylether. This compound is easily obtained and has good stability. [6, 7, 10, 12, 14, 22].

Through the monoquaternization of a pyridine base or of another cyclic tertiary amine with DCDMS or 1,3-dibromopropane in excess, the monoquaternary salt is obtained, usually unisolable that, in the next step, can be quaternized with another different base, obtaining mixed products with an antidote efficiency that is higher and a reduced stability. In (bis)quaternization, tertiary bases that can be utilized are pyridines which are monosubstituted with aldoxime or carboxamide groups in all three positions, 1,3-N-methyl-2-imidazolilaldoxime or quinuclidinic bases: 3-quinuclidinonoxime, 3-quinuclidinyl-N,N-dimethylaminocarbamate etc, which, theoretically, through combination with dihalogenate derivatives cand lead to a large variety of antidotes [6, 14, 19, 20].

The present paper proposes the synthesis and characterization of new symmetric bisquaternization compounds with 3-pyridinylisonitroso-acetanilide as a precursor, the quaternization agents being symmetric dichlorodimethylether and 1,3-dibromopropane. These compounds are PAM-2 analoges, N-Methyl-2-hydroximinomethyl pyridine chloride being utilized for the first time as an antidote in the intoxications with neurotoxic organophosphates. [14, 19, 21]

MATERIAL AND METHOD

The reactivating potential of two oxime compounds with pyridin-isonitrosoacetanilide structure was tested in vitro: 1,3(2-hydroxyimino-N-pyridin-3-propane bromide (HIN1); 1,3(2-hydroxyimino-N-pyridin-3-oxapropanchloride (HIN2). The HIN 1 compound did not disolve in the culture medium. The acute toxicity of obidoxime and of HIN 2 was assessed through the determination of cellular viability the two produced on cell cultures from 3T3 fibroblast lines. The cells were incubated for 24 hours at concentrations of 1 mg/ml, 3 mg/ml, and 5 mg/ml and optical density was read at 490 nm.

For this purpose, two compounds with pyridinisonitroso-acetanilide structure were tested, compounds considered active in the intoxication with neuroparalytic chemical agents due to the presence in their molecule of the oxime group R=N-OH, as well as of the pyridine structure resembling obidoxime. (Figure 3)

Figure 3: Chemical structures of newly synthethized compounds

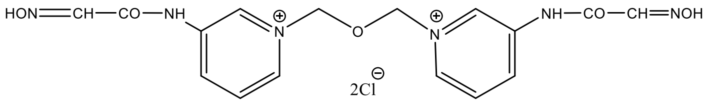

$C_{16}H_{18}Cl_2N_6O_5$
Masa Moleculara; 445,26

bis 1,3[2-hidroxiimino-N-(piridin-3-il)] oxapropandiclorura

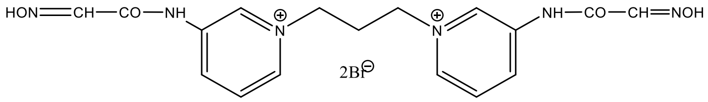

$C_{17}H_{20}Br_2N_6O_4$
Masa Moleculara: 532,19

bis 1,3[2-hidroximino-N(piridin-3-il)]propandiona

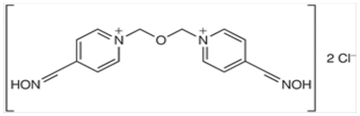

$C_{14}H_{16}N_4O_3Cl_2$
1,1-bis-metilen-bis-hidroxiiminometilpiridinium
Molecular mass 358,75

The synthesis of new symmetric pyridin-isonitroso-acetanilide bisquaternary salts with cholinesterase reactivation properties

The sythesis of the isonitrosoacetanilide of 3-aminopyridine named 2-(hydroxyimino)-N-(pyridin-3-yl) acetamide.

The isonitrosoacetanilide of 3-aminopyridine is obtained from 3-aminopyridine, chloral hydrate and hydroxylamine chlorohydrate in a saturated solution of sodium sulphate according to the following reaction in Figure4.

Figure 4: The synthesis of 2-(hydroxyimino)-N-(pyridin-3-yl) acetamide.

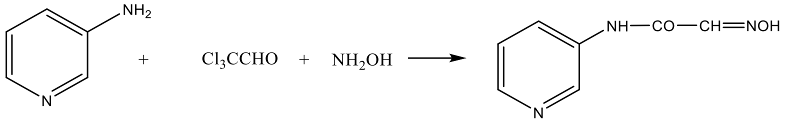

In a 2L balloon with efficient mechanical agitation, around 400ml of water are introduced. Under agitation, the sodium sulphate is brought in portions, and the chloral hydrate is added to the saturated solution within 2-5m. Seperately, the amine chlorhydrate is prepared in an aqueous solution of amine, 10ml HCl 35 and 20ml water. It is added under agitation to the previously prepared solution within 5m. Then, it is heated at 30-40^{0}C, and the hydroxylamine chlorhydrate is added in small portions after being dissolved in 50ml of water. The temperature is steadily increased to 80-90^{0}C within 1h, followed by boiling for another 15m. Then it is left to cool down at room temperature overnight and the sodium sulphate is filtered. The sodium sulphate is then washed twice with 50ml of water each time, and the filtered solution is added to the previous one. The reunited filtered solutions are neutralized with saturated ammonia solutions and are cooled down to 0-5 degrees. The precipitate is filtered, and the resulted solution undergoes extraction three times with 150ml methylen chloride each time. Through distillation, the solvent is removed, and the residue is recrystallized from methanol (through boiling with animal charcoal). The isonitrosoderivative is obtained with a yield of 65-70 %.

The compound was characterized through spectral RMN analyses, hydrogen and carbon spectrums which confirm the structure(Figure 5).

Figure 5

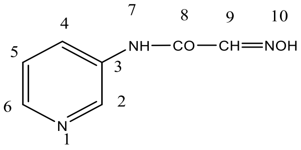

RMN characterization

1H-RMN – 1H-RMN (DMSO-d6, δ ppm, J, Hz) spectrum: 10.32(s, 1H, H-10); 8.85(dl, 2.6, 1H, H2); 8.29(dd, 4.4, 1.3, 1H, H-6); 8.12(dl, 4.3, 1H, H-4) (Figure 13).

Quaternization with symmetric dichlorodimethylether (DCMS) to bis1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamidî oxapropandichloride

The symmetric quaternization reaction of the isonitrosoacetanilide of aminopyridine with dichloroether in a 2:1 ratio is performed in anhydrous dimethylformamide at temperatures of 30-40^{0}C. The quaternization agent, symmetric dichlorodimethylether, is obtained from paraformaldehyde and thionyl chloride (Figure 6).

Figure 6: Isonitrosoacetanilide 3-aminopyridine –H-RMN spectrum

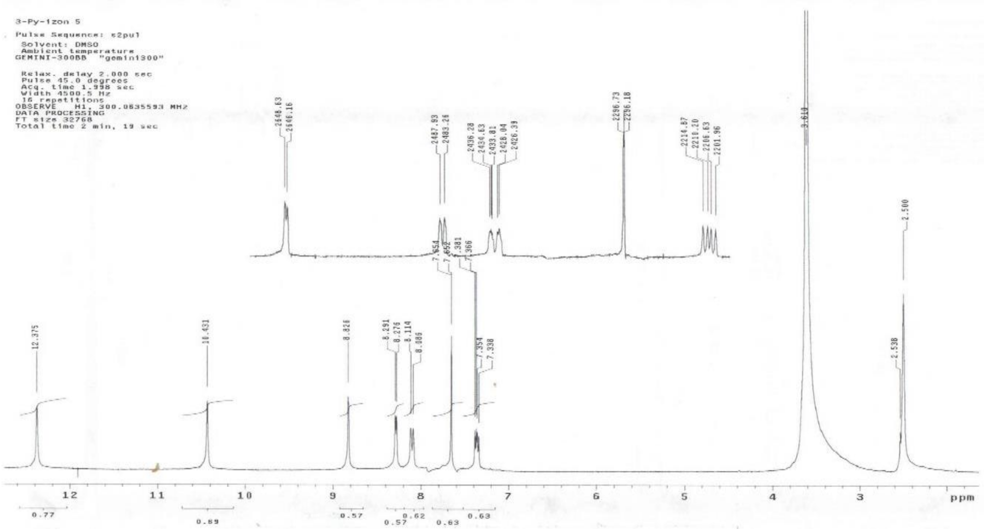

13C-RMN – 13C-RMN (DMSO-d6, δ ppm) spectrum: 160.91(C-8); 144.77(C-9); 143.80(C-6); 141.60(C-2); 135.22_C-3); 127.04(C-4); 123.69(C-5).

In an Erlenmayer, dimethylformamide and isonitroso-acetanilide are introduced. They are magnetically agitated to complete dissolution, and then dichlorodimethylether that was previously dissolved in 5ml of anhydrous dimethylformamide is added. The recipient is then sealed and agitated for 10h at room temperature and, finally, another 5h at 30-40^{0}C. It is cooled at 0-5^{0}C for 3h and is then filtered through vacuum filtering. The precipitate is repeteadly washed with anhydrous acetone and then dried in a vacuum, in the desiccator. 2.3 g of white product are obtained with m.p=145-150^{0}C, which corresponds to a yield of 52%. The synthethized compound, the bisquaternary salt, was characterized through spectral RMN and proton and carbon spectrum analyses which confirm its structure.

Figure 7: Isonitrosoacetanilide 3-aminopyridine - C- RMN spectrum

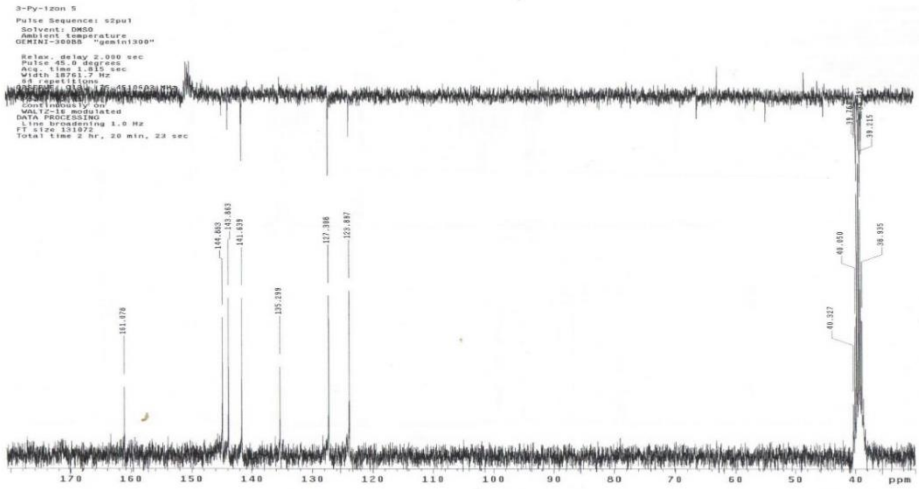

Figure 8: The synthesis of bis1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride

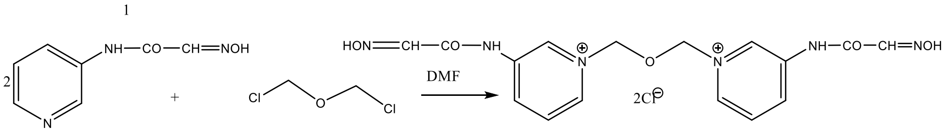

RMN characterization of the 1H-RMN hydrogen spectrum is shown in Figure 9 and the13C-RMN carbon spectrum is shown in Figure 10.

Figure 9: Bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride compounds - 1H-RMN spectrum

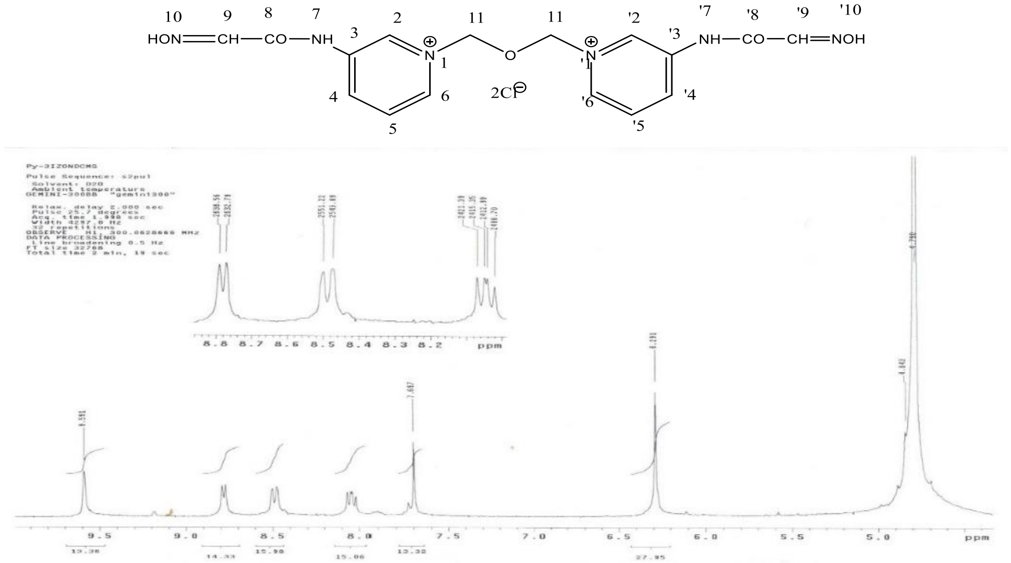

1H-RMN (D2O, δ ppm, J, Hz) hydrogen spectrum: 9.58(sl, 2H, H-2, H-2'), 8.77(dl, 6.0, 2H, H-6, H-6'), 8.48(dl, 9.7); 8.04(dd, 8.7, 6.3, 2H, H-6, H-6'), 6.28 (s, 4H, H-11, H-11')

Figure 10: Bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride compound - C-RMN spectrum

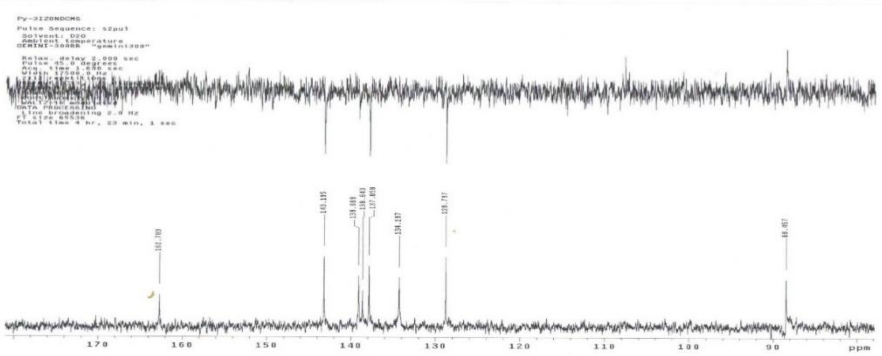

3C-RMN (D2O, δ ppm) carbon spectrum: 162.69(C-8, C-8'), 143.18(C-9, C-9'), 139.08(C-6, C-6'), 138.69(C-2, C-2'), 137.84 (C-4, C-4'); 134.30(C-3, C-3'); 128.79(C-5, C-5'); 88.48(C-11, C-11')

Quaternization with 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide.

The symmetric quaternization reaction of 3-pyridylisonitrosoacetanilide with 1,3-dibromopropane in a 2:1 ratio goes as described below and is also performed in anhydrous DMF DMF, at a temperature of 40-55^0 C (Figure 11).

Figure 11: The synthesis of bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propane dibromide

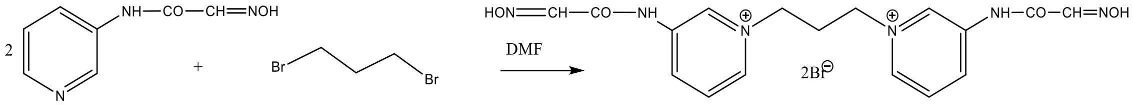

In an Erlenmayer glass, dimethylformamide and isonitrosoacetanilide are introduced. They are magnetically agitated to complete dissolution, then 1,3-dibromopropane that was previously dissolved in 5ml of anhydrous dimethylformamide is added. The recipient is sealed and agitated for 4h at room temperature and for another 10h at 40-60^{0}C. It is cooled down to 0-5^{0}C, and then vacuum filtered. The precipitate is repeteadly washed on the filter, with anhydrous acetone. It is dried in a desiccator in a vacuum overnight. 3.1 g of white crystal product are obtained with mp.180-182^{0}C and a yield of 59.0%.

The synthethized compound, the bisquaternary salt, was characterized through spectral RMN analyses, which confirm its chemical structure. (Figures 12, 13)

RMN characterization

The 1H-RMN hydrogen spectrum is shownin Figure 12and the 13C-RMN carbon spectrum is shownin Figure 18.

Figure 12: 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide - H-RMN spectrum

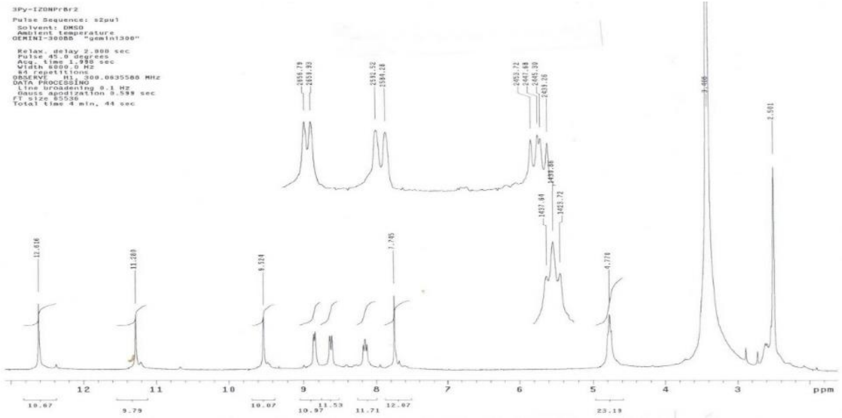

1H-RMN (DMSO-d6, δ ppm, J Hz) spectrum: 10.52 (s, 2H, H-7, H-7); 8.8 (dl, 2.4, 2H, H'-2, H-2); 8.3(dd, 5.0; 1.6, 2H, H-6, H-6); 8.1 (dl, 8.4, 2H, H-4,H-4)', 7.68(s, 2H, H-9, H-9'); 7.43(dd, 8.4, 4.7, 2H, H-5, H-5'), 4.71(t, 7.1, 4H, H-11, H-11'); 2.48(t, 7.1, 2H, H-12)

Figure 13: 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide - C-RMN spectrum

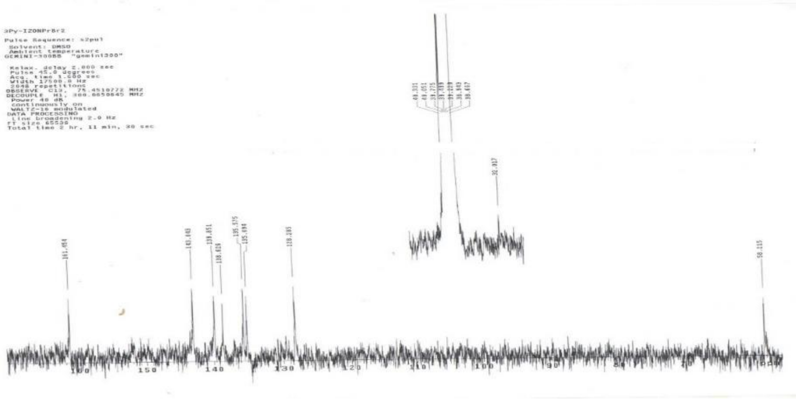

13C-RMN (DMSO-d6, δ ppm) carbon spectrum: 160.84 (C-8, C-8'); 143.97 (C-6, C-6'); 143.64 (C-2, C-2'); 140.70(C-9, C-9'), 135.36 (C-3, C-3'); 127.69(C-4, C-4'); 123.95(C-5, C-5'), 59.11(C-11, C-11'); 33.13(C12)

In vitro testing and toxicological characterization of symmetric bisquaternar compounds with a pyridin-isonitrosoacetanilide structure

The experiment was performed in sterile conditions in a microbiological niche.

In vitro testing incldued the two synthethized compounds: bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propane dibromide (coded under the name of HIN 1) and bis 1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2), utilizing obidoxime as reference. Each of these compounds was dissolved in a culture medium, and 3 solutions were obtained for each, with the following concentration: 5mg/ml; 3 mg/ml and 1 mg/ml. HIN 1 was insoluble in the culture medium at the above mentioned concentration.

On a 3T3 cellular line (fibroblasts) from Lonza from which a cellular subculture with a concentration of 1×10^5 cells /ml was obtained as follows:

- the cells were counted initially with the Burger Turck hemacytometer to establish the initial concentration of cells per ml;
- the dilution was performed so that a final concentration of 105 cells/ml would be obtained;
- the cells were cultivated with a proper medium (supplemented DMEM, Lonza reactive) for 24 hours at a temperature of 37 degree Celsius in a CO_2 atmosphere;
- 2 ml of tripsine solution are added (Sigma reactive);
- 5 minutes of centrifugation at 500 G at a temperature of 5-8 degrees Celsius;
- the supernatant is removed;
- resuspension in 5 ml of culture medium (DMEM);
- a volume of 200 microliters is placed in a plate with 96 wells;
- 6 h of incubation in CO_2 atmosphere for adherence;
- after 6 h of incubation for adherence, obidoxime and HIN2 are added after dissolution in the culture medium, equimolecularly, in 3 concentrations: 5 mg/ml, 3 mg/ml and 1 mg/ml;
- 24 h of incubation;
- after 24 h of incubation, 20 microliters of MTS reactive (Lonza) are added to each well;
- 4 h of incubation at 37 degrees Celsius in a CO_2 atmosphere.

The samples were read at 490 nm with a Biorad microplate reader and compared to the control sample represented by the culture medium.

RESULTS

The in vitro toxicity of HIN 2 and obidoxime was assessed through the values of their optical densities corresponding to different molar concentrations. The results are shown in Table 1 and in Figures 14 and 15.

The molar concentrations of studied compounds can be correlated with the cellular viability percentage.

The results are shown in Table 2 and in Figures 16 and 17.

In lower concentrations of 1 mg/ml, values that were close to the in vitro cellular viability were recorded (73.43% for obidoxime, and 82.42% for HIN 2).

For medium molar concentrations (3 mg/ml), cellular viability values were 67.75% for HIN 2 and 67.36% for obidoxime.

Table 1: The variations of optical density values of obidoxime and HIN 2

	Well1	Well2	Well3	Mean	Substraction blank
Untreated CTRL	1.068	1.112	1.67	1.283333±0.174	1.16083
Obi 5mg/ml	0.362	0.353	0.449	0.388±0.02	0.2655
Obi 1mg/ml	0.875	1.075		0.975±0.025	0.8525
Obi 3mg/ml	0.837	0.972		0.904 ±0.033	0.782
HIN 2 5mg/ml	0.141	0.149	0.152	0.147±0.012	0.0248
HIN2 1mg/ml	0.895	1.078	1.265	1.079±0.030	0.9568
HIN2 3mg/ml	0.849	0.969		0.9090±021	0.7865
CTRL BLANK	0.122	0.123		0.1225	0

Figure 14: Variation of optical density (DO) correlated with concentrations of obidoxime

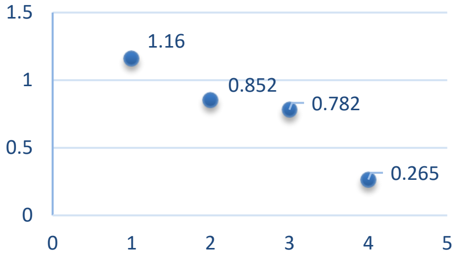

Figure 15: Variation of optical density (DO) correlated with concentrations of HIN 2

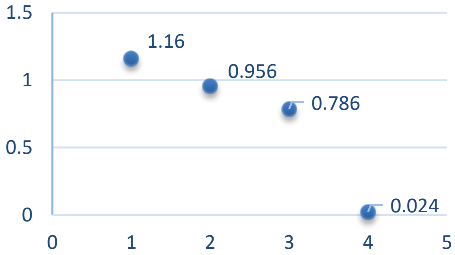

Table 2: Percetange variation of cellular viability for obidoxime and HIN 2

Dose mg/ml	DO Obidox	Cellular viability vs control (%)	DO HIN2	Cellular viability percentage vs control (%)
0	1.160833	100	1.160833	100
5	0.2655	22.8715	0.024833	2.139268
1	0.8525	73.43862	0.956833	82.42642
3	0.782	67.3654	0.7865	67.75305

For high doses, (5 mg/ml) HIN 2 showed a cellular viability value of 2.13%, much lower than that of obidoxime, and 22.87% respectively. Thus, HIN2 is more toxic than obidoxime.

Figure 16: Cellular viability percentage corresponding to different concentrations of obidoxime (y = -13.873 + 97.379)

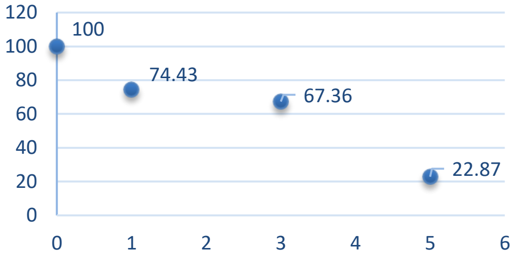

Figure 17: Cellular viability percentage corresponding to different concentrations of HIN 2 (y = -18.397 + 104.47)

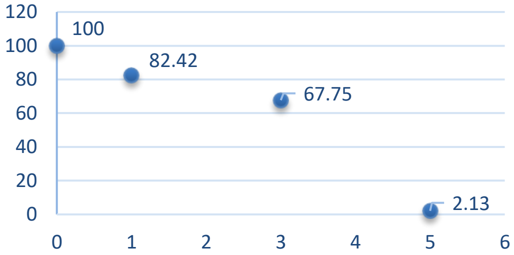

The assessment of CT50 was performed according to the working protocol described in the Romanian Pharmacopoeia.

In the regression line ecuations shown in Figures 14 and 15, the y value was replaced with the probit corresponding to the ideal mortality of 50 %. The probit is a statistical measure resulted from the transformation of the percentage of positive answer into a numeric value and is linearly dependent on the logarithm of the concentration. Thus, the x value in the ecuation of the regression line represents the logarithm of CT50. Through calculus, we could estimate CT50 for obidoxime at a value of 3.24 ± 0.005 mg/ml. The CT50 value for HIN 2 was estimated at 2.72 ± 0.009 mg/ml. The CT50 value being inversely proportional to the toxicity of the substance, it can be understood that the CT50 values are comparable (around the value of 3 mg/ml), HIN 2 being more toxic than obidoxime.

The statistical analysis of experimental results obtained showed statistically significant variations between the determined cellular viabilities of the two compounds in equimolecular concentrations only for high doses. The experimental results are represented in Tables 3-5.

Table 3: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 1 mg/ml (2.29×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	2.29×10^{-6} M;	1.160833333	0.276632	P ≥0,05 – mean values do not significantly differ
2	HIN 2	2.29×10^{-6} M;	0.956833333		

Table 4: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 3 mg/ml (6.94×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	6.94×10^{-6} M;	0.7820	0.4820	P ≥0,05 - mean values do not significantly differ
2	HIN 2	6.94×10^{-6} M;	0.7865		

Table 5: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 5 mg/ml (11.57×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	11.57×10^{-6} M;	0.2655	0.000723	P≤0,05 – mean values significantly differ
2	HIN 2	11.57×10^{-6} M;	0.0248		

CONCLUSIONS

The syntheses took into consideration the principle of obtaining a cholinesterase reactivating chemical compound that is stable, with a possible action at central nervous level, which could prove to be an alternative therapy to obidoxime, being usable in antidote studies.

Following solubility tests, only one compound, bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2) qualified for in vitro testing, the other being insoluble.

The CT50 value for obidoxime was estimated through calculus to be 3.24 ± 0.005 mg/ml, or 9.33×10^{-6} M;

The CT50 value for bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2) was estimated to be 2.72± 0.009 mg/ml or 6.29×10^{-6} M;

The in vitro tested compounds showed values close to cellular viability in low and moderate equimolecular concentrations in the fibroblast cellular culture (73.43% for obidoxime, and 82.42% for HIN 2).

The bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropan-dichloride compounds (HIN 2) showed a value of cellular viability in vitro lower than obidoxime at high equimolecular concentrations, proving to be more toxic. (2.13%, for HIN2, and 22.87% for obidoxime)

Taking into account the close CT50 values, the newly synthethized compound that is as such characterized from a toxicological standpoint can qualify for further studies as an antidote.

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VARIA

Orthodontic treatment and periodontal disease – between advantages and disadvantages

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Abstract: *Orthodontic treatment and periodontal disease can have an inter-conditional relationship, particularly when the accumulation of bacterial plaque, favoured by orthodontic appliances and poor oral hygiene can cause periodontal inflammation. The objective of the study is to highlight the influence of the risk factors' polymorphism on the therapeutic response in periodontal disease. 64 patients with mixed or definitive dentition and poor oral hygiene were included in the study, of whom 22 patients followed treatment with fixed orthodontic dispensers, 22 patients were treated with mobile orthodontic braces and 20 were non-attendants. From all patients gingival crevicular fluid was collected and assessed, before and after applying a chlorhexidine-based gel antiseptic agent.*

Keywords: *periodontal disease, orthodontic treatment, chlorhexidine gel, gingival fluid*

INTRODUCTION

Periodontal disease inflicts a wide range of oral changes, ranging from gingival tissue inflammation to tooth loss. During some orthodontic treatment protocols patients may not to compliant to keeping a good oral hygiene, possibly due to the difficulties that certain orthodontic appliances may impose on efficient teeth brushing [1]. Microbiological patterns involved in the genesis of periodontal disease are more common in orthodontic treatment patients, so the bacterial plaque is left undisturbed for a long period of time around the orthodontic appliances and can grow in bacteria number and pathogenic potential. Microbial periodontal pathogens, especially Gram-negative bacteria, are more frequently found around fixed/mobile orthodontic appliances [2]. Periodontal disease may have different clinical forms, with a wide range of manifestations, from gum tissue damage to periodontitis, which can even lead to tooth loss in some cases [3].

The clinical manifestations and evolution of periodontal disease may vary in accordance with the intensity of the bacterial challenge. In orthodontic treatment patients, the periodontal tissues are also challenged by the orthodontic forces which generate teeth movement. During teeth movement, all periodontal structures, including the gingival, the periodontal ligament and the alveolar bone are under constant mechanical stress [4]. This mechanical stress can cause a decrease of the tissue's defensive capabilities. Combined with the increased bacterial challenge, caused by poor oral hygiene, this creates very favourable conditions for the onset of periodontal disease [5]. Consequently, a good understanding of the periodontal disease's existing aetiology and risk factors is required for a correct diagnosis and

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treatment plan for a patient undergoing orthodontic therapy.

The objective of this study was to highlight the influence that certain risk factors have on the therapeutic response in periodontal disease in orthodontic patients and to assess the local impact that the use of an anti-plaque agent has in these patients.

MATERIAL AND METHOD

The study included a group of patients who were clinically examined and expressed similar symptoms during the first visit to the dental office. In order to collect the data obtained from the anamnesis, observation charts were prepared for each patient. The inclusion criteria used for the study were the following: (1) patients who were treated or not with orthodontic braces/mobile dispensers; (2) age 12-20 years; (3) the presence of mixed or definitive dentition; (4) the presence of gingivitis subjective and objective clinical signs (gingival bleeding, gingival oedema, halitosis). Following the clinical examination, a bacterial plaque index was calculated for the participating patients, its values being directly proportionate with the degree of gingival inflammation.

A total of 64 patients with mixed or definitive dentition and poor oral hygiene were selected, being divided into 3 study groups: (1) 22 patients carrying a mobile orthodontic device; (2) 22 patients with fixed orthodontic appliances and (3) 20 patients who did not require or have refused orthodontic treatment.

Before initial periodontal therapy (airflow cleaning and professional brushing) gingival crevicular fluid was sampled from the gingival sulcus of the participating patients. For sampling sterile paper, cones of 20 mm were kept for 30 seconds in the periodontal pocket/gingival sulcus, after the pre-removal of the supragingival bacterial plaque, in order to avoid contamination of the sample with supragingival bacteria. The collection and transport of the biological products and the stages of microbiological diagnosis were performed by the clinician and their assistant.

The results of microbiological investigations depend to a large extent on the quality of the sampling procedure, which needs to follow specific rules:
(a) the patient was informed about the aimed analysis and about the correct way of sampling procedure;
(b) the sampling instruments were sterile, disposable and adapted to the investigation;
(c) the pathological product was harvested prior to administration of any topical or general topical treatment; it is harvested from areas where it is most likely to find the bacterium that produced the infection;
(d) the outside of the container was disinfected if it has been contaminated during sampling procedure;
(e) the name of the patient and his provenance, the type of biological product, the date and time of collection was written on the container;
(f) each pathological product was accompanied by a bulletin containing information about the sample and the patient;
(g) transport was as quick as possible to the laboratory to prevent the degradation of the pathological product;
(h) transport media used ensured good survival of bacteria;
(i) for the oral cavity, the sampling procedure consisted in carefully cleaning the edge of the gum and tooth surface to remove saliva and food debris, afterwards a periodontal scalpel was used and the lesion secretion was removed;
(j) transport was carried out using an anaerobic transport system in less than 2 hours from sampling.

Microbiological tests were performed to detect aerobic and anaerobic gram-positive and gram-negative bacteria. After the initial periodontal treatment, the patients were randomly re-divided into two groups, as follows: (1) 12 patients with orthodontic devices (six fixed devices, six mobile devices) and (2) 12 appliance donors, who received applications of a chlorhexidine-based gel (Elugel 0.02%, Pierre-Fabre, France). The other 24 patients were only instructed for good oral hygiene and monitored. The ethical standards of medical research and GDPR (Global Data Protection) and the approval of the Ethics Committee of the Faculty of Dentistry of the “Titu Maiorescu” University in Bucharest were followed by the present study.

RESULTS

The distribution of the participating patients regarding orthodontic treatment type was similar between the patients with mobile and fixed orthodontic appliances (Figure 1).

Figure 1: Selection criteria: fixed/mobile orthodontic handlers/ carriers

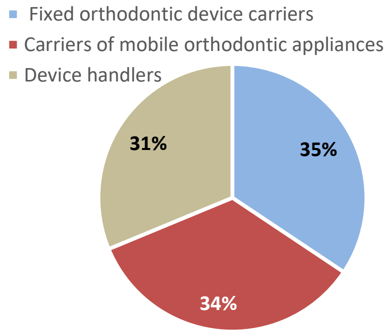

The microbiological assessment revealed bacteria of the violet periodontal complex: (Veillonella parvula, Actinomyces odontolyticus), yellow complex (Streptococcus spp), green complex (Eikenella corrodens, Capnocytophaga spp.) red complex (Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola) and of the orange complex (Prevotella intermedia/nigrescens, Micromonas micros, Campylobacter rectus, Fusobacterium nucleatum) (Figure 2). Compared to the microbial flora normally found in gingival tissue of healthy individuals with no periodontal problems, microbiological tests revealed the exaggerated growth of germs in the case of fixed carriers and a significant increase in the case of mobile carriers, as shown in Table 1.

Streptococcus spp, P. gingivalis, and F. nucleatum predominantly occured in fixed-bearer carriers, while Treponemma is constantly present whether or not the patient is treated with fixed or mobile devices. The Actinomyces spp were present within the saprophytic flora and does not develop during orthodontic treatment.

Figure 2: Bacteria type frequency in periodontal heath vs. gingival inflammation (%)

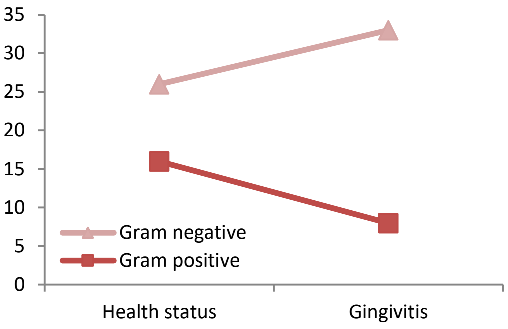

Table 1: Distribution of bacterial pathogens according to study groups

Pathogen	Health	Not carriers of orthodontic braces	Carriers of orthodontic braces	Carriers of mobile devices
Streptococcus	+++	+	+ ++	+ +
Actinomyces	+++	+	+	+
Veillonella	+	+	+	+
Aggregatibacter actinomycetemcomitans	-	++	+	?
Capnocytophaga	+/-	+/-	+/-	+/-
Treponeme orale	+/-	+++	+++	+++
Porphyromonas gingivalis	-	+	+++	++
Prevotella intermedia	+/-	+	+++	++
Tannerella forsythia	-	++	++	?
Fusobacterium nucleatum	+	++	+++	++

Figure 3: Patients treated with chlorhexidine-based gel antiseptic substance

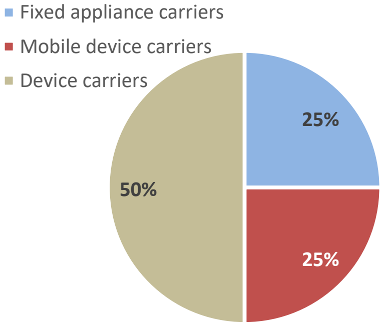

Following the application of the chlorhexidine-based gel antiseptic substance to orthodontic patients, it was found that after two weeks the results of the microbiological analysis of the gingival sulcus exudate revealed a reduction of the number of pathogenic bacteria in the orthodontic treated subjects and their disappearance in the non-attendant devices (Figure 3).

For the patients who did not benefit from the application of chlorhexidine-based gel substance, it was found that for the orthodontic handlers the microbial flora was still highly present. After 30 days, when the patients were reassessed, it was discovered that the pathogenic flora disappeared in all patients treated with chlorhexidine-based gel antiseptic substance, both with orthodontic treatment or not.

DISCUSSION

According to the literature, a study by Al-Bazi et al. from 2016 demonstrated that the formation of dental biofilm is a result of the increase in the number of microorganisms on the tooth surface, which was found to be 2-3 times higher in patients under orthodontic treatment than in young people without orthodontic treatment [6]. Previous studies have shown that regular dental brushing and the use of dental floss alone do not decrease levels of S. mutans in patients treated with fixed orthodontic appliances [7]. In our study, subjects treated with mobile orthodontic devices, compared to healthy individuals without periodontal problems, revealed an exacerbated growth of germs in the case of fixed-bearer carriers and a significant increase in the case of the mobile-bearer carriers. Some bacterial species (Streptococcus, Porphyromonas, Prevotella and Fusobacterium) are found mainly in fixed-bearer carriers, while Treponemma is constantly present whether or not the patient is a carrier of fixed or mobile devices and Tannerella forsytia is absent in patients' exudates.

Different protocols have shown the ability of CHX (Chlorhexidine) to inhibit the formation of dental biofilm and to reduce plaque development [8]. Randomized, controlled clinical trials demonstrated the efficacy of 0.50% and 0.75% CHX to control gingivitis and bleeding in orthodontic patients, concluding that lower concentrations of chlorhexidine may reduce the risk of tooth colouring, without compromising its effectiveness level against pathogenic bacteria [9]. In our study, applying the chlorhexidine-based gel antiseptic substance significantly reduced the number of bacterial colonies in all patients of the study. However, antiseptic treatment should not replace dental mechanical treatment, but be applied in a complementary manner [10]. Combining mechanical treatment with local application of antiseptics gives statistically better results than each of them taken separately does.

CONCLUSIONS

Given the results of the study, we can conclude that for the patients treated with orthodontic appliances, either fixed or mobile, it is impossible to completely clean all oral surfaces and to decrease the number of microbial colonisation without the application of local antiseptics during the treatment. The results of the study can be used to prevent periodontal disease in patients with orthodontic treatment.

Conflict of interest:
Nothing to declare.

Contribution Note:
All the authors equally contributed to the drawing up of the present paper.

Patient participation at the study respects ethical standards in medical research and GDPR.

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VARIA

Acute kidney injury incidence and models for mortality prediction in acute coronary syndromes

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Abstract: *Background and aim. The presence of acute kidney injury (AKI) in subjects diagnosed with acute coronary syndromes (ACS) represents a major predictor of a negative outcome.The purpose of this study is to reveal the incidence of AKI in ACS subjects and to illustrate the prognosis of these subjects, using special models for mortality prediction, based on the creatinine values and the presence of acute heart failure (AHF).*

Methods. Using multiple linear regression, several models were designed to predict the mortality including: comorbidities of the subjects, initial and highest value of the serum creatinine (sCr) during hospitalization, presence of theAHF and Killip class compared with sCr values, the last model illustrating the influence of all the previous variables.

Results. AKI had a significant incidence in the chronic kidney disease (CKD) group (53.11%). The mortality rate and the hospital readmission were higher in the CKD subjects (41.14%) versus the patients with normal kidney function (21.23%, p<0.01). The association of AKI and AHF, known as cardio-renal syndrome, was related to a negative outcome in the CKD group. The models for prediction of mortality emphasize that the age, dyslipidaemia, worst sCr, Killip class and AHF presence can be used to assess the mortality risk of patients with AKI and ACS. Previous pathological conditions such as diabetes, peripheral artery disease, coronary artery disease (CAD) and obesity, failed to achieve statistically significance for prediction of death.

Conclusions. AKI represents a serious pathological condition with a high prevalence in subjects with CKD; the association with AHF increases the risk of hospital readmission and fatal outcome.

Keywords: *acute coronary syndromes, acute kidney injury,acute heart failure, serum creatinine, predictors, mortality*

INTRODUCTION

AKI represents a pathological condition that resumes a complex interaction between various factors like subsequent renal failure, utilization of iodinated contrast substance during percutaneous coronary intervention (PCI), renal hypoperfusion caused by decreasing of the cardiac output during an ischemic event [1]. Manifestations of AKI may consist in a simple increase in sCr or severe renal failure with anuria requiring kidney replacement therapy [1]. There is a strong relationship between the renal function impairment and the CAD development, regardless the other common risk factor involvement. The prospective study realized by Jurkovitz et al. including 13,329 participants,

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without previous CAD, reflected that increased values of baseline sCr (defined as ≥1.2 mg/dL in women and ≥1.5 mg/dL in men) were associated with a high incidence of acute myocardial infarction (AMI) and death due to ischemic events, compared to normal values of the sCr [2]. The renal impairment has an important influence on the patient's outcome during cardiovascular events and a small decrease in glomerular filtration rate worsens the prognosis of these patients [3].

The complexity of the atherosclerosis process in CKD subjects is a result of the interaction between common and uncommon risk factors. Diabetes mellitus, hypertension, dyslipidaemia and smoking represent essential factors in atherosclerosis development by promoting chronic inflammation, oxidation of the low-density lipoproteins and reactive oxygen species formation [4]. Even secondary hypertension in young persons has an important role in atherosclerosis occurrence and the level of plasma renin in these subjects represents a clear indication [5, 6]. Systolic and diastolic values of the blood pressure are strongly related to alimentation in young persons and there are several actions to change the unhealthy eating habits, beginning with education regarding healthy diet from early age [7].

Various terms were attributed to AKI and that is the reason why the comparison of studies is difficult. There are more than 30 definitions of AKI and the most used are: the Risk, Injury, Failure, Loss and End-stage renal disease (RIFLE), criteria revised by the Acute Kidney Injury Network (AKIN) and the Kidney Disease: Improving Global Outcomes (KDIGO) AKI Work Group classification.

AKI definition (according to KDIGO) expresses an increase in sCr by 0.3 mg/dL (≥ 26.5 µmol/L) within 48 hours or increase in sCr 1.5 to 1.9 times baseline value, with known or presumed abrupt onset within the prior 7 days. There are 3 stages according to the sCr values and the urine output. The first stage is consistent with sCr variations mentioned in the definition and a urine output of less than 0.5 mL/kg/h for 6-12 h, whereas the second stage involves increase in sCr 2.0 to 2.9 times baseline and a urine output of 0.5 mL/kg/h for 12 h. The last stage represents an association between increase in sCr to ≥ 4.0 mg/dL (≥ 353.6 µmol/L) or ≥ 3.0 times baseline or usage of renal replacement therapy and a urine output of less than 0.3 mL/kg/h for ≥ 24 h or anuria for ≥12 hours [8, 9, 10].

Usually, AKI remains undetected due to the fact that the onset is earlier in ACS patients and the sCr from admission represents the reference value, without actually knowing the evolution of the creatinine before hospitalization. If the sCr does not have a remarkable increase after admission, there may be an error of AKI diagnosis. New biomarkers able to early detect the AKI are required in order to initiate the preventive treatment and minimalize the renal insult [11]. The beginning of AKI is usually in the early phase of the ACS, fact that determines the late detection of this condition.

ACS represents a challenging diagnosis in CKD subjects because most of them remain asymptomatic. The explanation is suggested by the fact that uremic milieu and associated diabetic neuropathy influence the perception of pain in these patients [12, 13].Inflammation and oxidative stress represent common elements for both CKD and ACS, and the association of these diseases increases the risk to develop AKI. High levels of inflammatory markers (such as C reactive protein (CRP), fibrinogen and procalcitonin (PCT)) were found in subjects who developed AKI after PCI. Kurtul et al. revealed that PCT represents an independent factor for the development of AKI in ACS patients undergoing PCI [14]. Subjects without AKI emphasize PCT values less than 0.1 µg/L15. Gao et al. analysed 7000 PCI and illustrated that CRP levels were higher in subjects with AKI16. Gu et al. illustrated that determination of both markers, CRP and PCT, has higher predictive value in identifying subjects at high risk of AKI development. Also, the AMI is an independent risk factor involved in AKI occurrence [17].The role of antioxidants in the cardiovascular field is well known and vegetables/fruits consumption has demonstrated its effect for people at risk of cardiovascular diseases, on immunologic, clinical and antioxidant markers [18].

The main purpose of the present research is to highlight the incidence of AKI in ACS patients, and to present prediction for these patients based on particular models for mortality prognosis taking into account the presence of AHF and creatinine values.

METHOD

This study designed a retrospective single-centre analysis, which enrolled 435 patients admitted with ACS diagnosis in the Cardiology department of the Clinical Emergency County Hospital of Oradea, January 2016 - January 2018. The study lot involved 209 patients with ACS and CKD; the control lot comprised 226 patients with ACS and normal kidney function. The exclusion criteria included allergy to contrast media, exposure to the contrast media 2 days before the coronary angiography procedure, malignant conditions.The AKI patients were detected using the KDIGO classification because it contains lower values of sCr than the AKIN criteria, so the underdiagnosis of the condition was avoided.

ACS was revealed according to the Fourth Definition of the

Myocardial Infarction using the association of thoracic pain, elevated cardiac necrosis enzymes, electrocardiographic changes and imaging evidence of new regional wall motion disturbances.ST-segment elevation myocardial infarction (STEMI) was defined due to the presence of persistent ST-segment elevation more than 1 mm in two contiguous leads with the exception of precordial leads that require an elevation of 1.5 mm in women, more than 2.5 mm in men under 40 years-old and less than 2 mm in men older than 40 years. Myocardial infarction in subjects with previous left bundle branch was revealed due to Sgarbossa's criteria. Non-ST-segment elevation myocardial infarction (N-STEMI) illustrates either a ST-segment depression $\geq$0.5 mm in two contiguous leads or deep inverted T wave more than 1 mm, along with elevated myocardial necrosis enzymes. Unstable angina (UA) expresses cardiac necrosis enzymes in normal limits associated with T negative wave, ST-segment depression or transitory ST-segment elevation [19]. Left ventricle ejection fraction (LVEF) was assessed using the Simpson's method. The normal value was considered between 50-72% in men and 52-74% in women [20].

Cardiac necrosis enzymes, cTnI (cardiac troponin I) and hscTnI (high-sensitive cardiac troponin I), were tested and abnormal values were defined as elevated when the result showed at least one value above the upper limit of the 99th percentile. Normal ranges were considered whenthe values were lower than 0.04 ng/mL for cTnI and 29 ng/L for hscTnI. Serial measurements were required to exclude thediagnosis of myocardial injury [21].

Killip Kimball classification was assessed in each patient with STEMI and the four classes. The first class illustrated subjects without signs of cardiac decompensation, the second class included patients with rales, third heart sound and increased jugular venous pressure. The third class was consistent with acute pulmonary oedema and the last stage revealed subjects with cardiogenic shock [22].

The statistical analysis was performed using Biostat software. Chi-square test was used when comparing prevalence in the two-groups, while Anova test was used to compare the mean values of certain parameters in the two groups. Univariate and multivariate regressions were done to assess the factors that correlate with the risk of death. A value of $p<0.0$ was considered statistically significant.

This research was performed respecting the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects), and it was approved by the Ethics Committee of the Clinical County Emergency Hospital of Oradea, Bihor County, Romania (decision no. 30088/December 10th, 2019); each patient included in the study signed the informed consent form.

RESULTS

The prevalence of the common risk factors and associated comorbidities was higher in the CKD lot. Conditions like hypertension, diabetes mellitus, dyslipidaemia and peripheral artery disease were present in an increased proportion in CKD subjects in comparison with patients without CKD (84.21% vs 63.27%, $p<0.01$; 58.85% vs 33.62%,$p<0.01$; 60.09% vs 49.95%, $p=0.02$; 20% vs 4.9%, $p<0.01$).The distribution of the ACS types was different between the two lots. STEMI was characteristic in subjects without CKD whereas the CKD lot revealed mostly N-STEMI cases, UA had a similar incidence in both CKD and non-CKD lots (Figure 1).

Figure 1: Categories of coronary syndrome according to the presence/absence of CKD

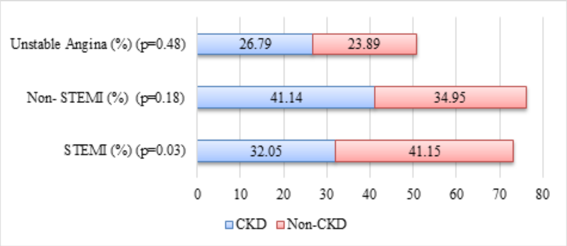

Myocardial interventional revascularization through PCI and stent implantation represented the therapeutic option for 53.59% of CKD subjects and 63.72% non-CKD patients ($p=0.03$). Coronary artery bypass surgery (CABS) was performed in 15.79% of CKD subjects, whereas the non-CKD lot had a proportion of surgical myocardial revascu1arization of 16.81% ($p=0.77$). The use of conservative treatment instead of PCI, known as renal nihilism, was objectified mostly in the CKD lot ($p<0.01$) (Figure 2).

Figure 2: Treatment of ACS according to the presence/absence of CKD

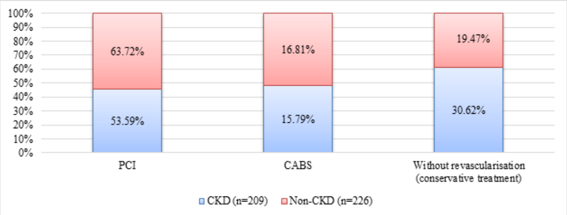

AKI has occurred in the CKD lot, revealing statistically significance ($p<0.01$). The association of two pathological conditions, such as AKI and AHF, had a major impact in subjects' outcome and was revealed in the CKD group ($p<0.01$). The uncomplicated evolution of the ACS cases was noticed in patients with normal kidney function (p<0.01) (Figure 3).

Figure 3: Presence of AKI or AHF according to the presence/ absence of CKD

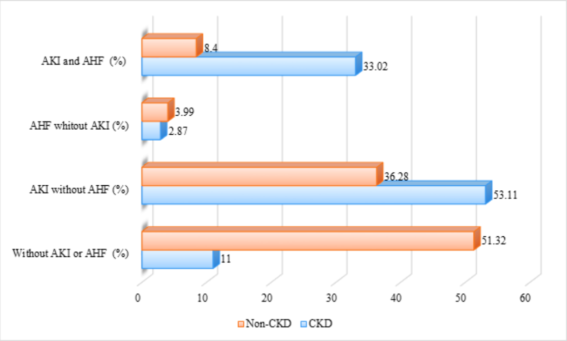

Deteriorated LVEF was noticed in a higher proportion in CKD lot, compared to patients without CKD (23.5% vs 20.17%, p=0.4). The average value of sCr was higher in the CKD lot (1.66 mg/dL vs 0.8, p<0.01), the standard deviation (Q1=1.01, Q2=0.20). Another important parameter was the worst value of the sCr which revealed a high level in the CKD lot with statistical significance (2.71 mg/dL vs 1.1 mg/dL, p<0.01), standard deviation (Q1=2.11, Q2=0.36).The analysis of the average sCr variation illustrated an increased value in the CKD group (1.03 mg/dL vs 0.3 mg/dL, p<0.01).The mortality rate was statistically significant in CKD subjects compared to patients with normal kidney function (13.39% vs 6.1%, p<0.05, SD CKD 8.39-17.61, SD non-CKD 4.39-7.69). Killip class assessment revealed that most of the subjects without CKD had first Killip class at admission (p<0.01), whereas the CKD patients were included in the third and fourth Killip classes (p<0.01).

The initial value of sCr illustrated importance in univariate analysis and predicted the development of AHF and AKI (p<0.01) (Figure 4, Figure 5, Table 1).

Figure 4: Correlation between worst value of sCr (mg/dL) and AKI development

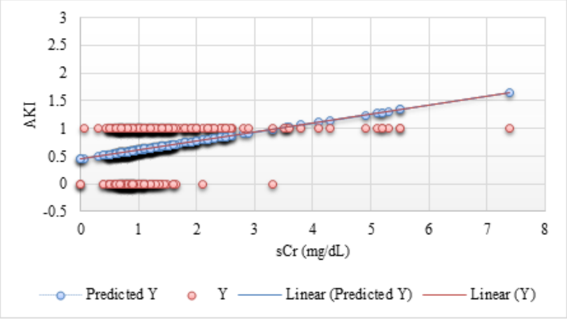

Models for mortality prediction were designed using multivariate analysis and including different factors to demonstrate the impact of AKI and other comorbidities or biomarkers regarding the outcome of patients with ACS (Table 2). The first model, based on the medical history and the associated cardiovascular risk factors of the subjects, illustrated that only the age and dyslipidaemia reached statistical importance to predict the mortality risk in ACS subjects with and without CKD (p=0.0404, p=0.0332).

Figure 5: Correlation between worst value of sCr (mg/dL) levels and AHF development

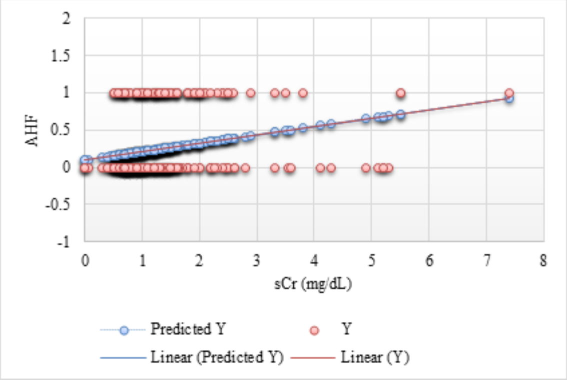

Table 1: Parameters of linear regression

	Coefficient	Standard error	LCL	UCL	t Stat	p value
Between worst value of sCr (mg/dL) and AKI						
Intercept	0.4489	0.0389	0.3724	0.5253	11.5427	*0.0000
Worst value of sCr (mg/dL)	0.1619	0.0263	0.1102	0.2136	6.1518	1.7451E-9
Between sCr (mg/dL) and AHF						
Intercept	0.1005	0.0352	0.0314	0.1696	2.8576	*0.0045
Worst value of sCr (mg/dL)	0.1120	0.0238	0.0652	0.1587	4.7053	3.4162E-6

**p<0.05, respectively p<0.001; LCL –low confidence limit; UCL–upper confidence limit; sCr – serum creatinine*

Diabetes, hypertension, smoking condition, history of peripheral artery disease, stroke did not predict the risk of death in the presented model. The second model included an analysis regarding the mortality risk of the study groups based on the presence of complications and biomarkers values. The presence of AHF and Killip class were statistically significant in anticipating the fatal outcome (p=0.0002, p<0.01). Other parameters like sCr value, age, troponin, LVEF failed to demonstrate their role in predicting the mortality. The third model evaluated the difference between baseline sCr and the worst value of sCr in identifying the patient's outcome. The worst value of sCr was the most important factor in mortality prediction in this model (p=0.0117). The fourth model represented an evaluation of all the variables in detecting the mortality risk. The Killip class and the AHF demonstrated statistical importance (p=0.0132, p= 0.0000).

Table 2: Prediction of mortality risk using multivariate analysis, based on 3 models

Parameter	Coefficient	Standard error	p value
Model 1. Associated cardiovascular risk factors and comorbidities			
Intercept	-0.0037	0.1521	0.9805
Age	0.0027	0.0013	*0.0404
Gender	-0.0004	0.0342	0.9918
BMI (kg/m^2)	-0.0030	0.0034	0.3700
Smoking	0.0137	0.0187	0.4656
Hypertension	-0.0342	0.0348	0.3270
Diabetes mellitus	-0.0111	0.0162	0.4945
CKD	0.0498	0.0327	0.1288
Anaemia	0.0496	0.0328	0.1318
Peripheral artery disease	0.0866	0.0469	0.0661
Chronic obstructive pulmonary disease	0.0131	0.0583	0.8229
Stroke	-0.0728	0.0491	0.1389
CAD	0.0020	0.0117	0.8651
Model 2. Complications and paraclinical results			
Intercept	-0.2572	0.0972	*0.0085
Age	0.0009	0.0010	0.3625
Initial value of sCr (mg/dL)	-0.0066	0.0328	0.8410
Worst value of sCr (mg/dL)	0.0144	0.0176	0.4138
AKI	-0.0175	0.0271	0.5200
AHF	-0.2284	0.0598	*0.0002
cTnI (ng/mL)	0.0057	0.0286	0.8426
Killip class	0.2300	0.0303	*<0.001
Decreased LVEF	0.0431	0.0322	0.1818
Model 3. Initial sCr and worst value of sCr			
Intercept	0.0176	0.0249	0.4791
Initial sCr (mg/dL)	-0.0027	0.0350	0.9375
Worst value of sCr (mg/dL)	0.0439	0.0173	*0.0117

**p<0.05, respectively p<0.001; BMI – body mass index; CKD – chronic kidney disease; CAD – coronary artery disease; AKI – acute kidney injury; AHF – acute heart failure; ctnI –cardiac troponin I; LVEF – left ventricular ejection fraction; sCr – serum creatinine.*

DISCUSSIONS

The present study outlines two significant ideas: (1) The serial determination of sCr is essential in identifying subjects with AKI and special attention should be paid to subjects with CKD undergoing the coronary procedure; (2) The presence of AKI in patients with ACS and CKD can be a fatal prognosis, especially when associated with AHF. Despite following accurate indications regarding coronary imaging, like the use of a low-osmolarity contrast agent (600-800 mOsm/kg) and the corresponding post-procedural hydration associated with diuresis stimulation, the incidence of AKI varies from 5% to 55% [1, 23, 24]. The variable incidence resides in variations of the nomenclature of this term [25].

As there is no safe and highly efficient prevention method for AKI, it is considered necessary to develop prediction scores to identify high-risk patients [26]. A first element that should be included is the Killip class, evaluated upon admission to the hospital, along with the presence of AHF. These elements should be identified particularly for CKD subjects, as multivariate analysis demonstrates the role of independent risk factors for the risk of death in these patients. The reduction of the number of functional nephrons, evidenced by the initial sCr analysis and the worst value of the sCr, together with an increased rate of reduced

LVEF are major predictors of AKI and AHF occurrence, an association known as cardio-renal syndrome [27].

Common cardiovascular risk factors have failed to outline the risk of mortality in multivariate analysis, except for dyslipidaemia, since in the case of CKD there are also factors specific to the uremic environment that significantly influence the prognosis of these patients. Early myocardial revascularization reduces the risk of AHF. A recent study confirms the importance of this therapeutic method, especially in subjects with N-STEMI and CKD, that associate increased values of cardiac biomarkers, such as the N-terminal fragment of the brain natriuretic peptide (N-terminal pro b-type natriuretic peptide, NT-proBNP, BNPT) or hscTnI, or a Killip class three or four [28]. For patients with ACS undergoing PCI, an independent predictor of developing AKI is the level of NT-proBNP at admission, being connected with high costs, extended hospitalization, high rates of terminal-stage renal failure, repeat revascularization, myocardial infarction and early and late mortality [29]. LVEF shows low values in subjects with ACS and CKD due to the increased incidence of multivariate CAD and especially to the impairment of left coronary artery trunk. Choosing the method of myocardial, interventional or surgical revascularization should consider the possibility of resolving all significant coronary lesions, the complexity of CAD and the surgical risk for the subjects [30].

The increased mortality evidenced in the group of subjects with CKD is explained by the increased incidence of AKI in these subjects. The mean hospitalization period was longer for patients with ACS and CKD who developed AKI, highlighting the severity of this complication (15.71 days vs. 13.15 days, $p <0.01$). For patients with STEMI, accentuated oxidative stress, sympathetic activation and pronounced inflammatory response were found to synergistically accelerate AKI [31]. The most powerful independent predictor of mortality within hospital in case of dangerous settings like for STEMI associated with cardiogenic shock was identified to be AKI [32].

A recent and further argument of the way AKI influences the long-term effect for patients suffering from STEMI subjected to primary PCI is presented by Narula et al. The 3-year follow-up revealed that the mortality rate for patients suffering from AKI was 16%, while for those without AKI it was 4%. As for the association of severe ischemic cardiac side effects and serious bleeding, the intensification of the side effects was more accentuated after three years, being of 40% for subjects with AKI and about 25% for patients without AKI. Corroborated, these data show that AKI evolution influences the complete prognosis of ACS patients [33]. Throughout primary PCI for STEMI, increased contrast volume is connected with increased levels of AKI and mortality, the contrast ratio and contrast volume determining AKI evolution [34]. A general complication in the case of patients suffering from shock and myocardial infarction is nephropathy, as recently revealed by a CULPRIT-SHOCK study that showed a high percentage of subjects (11.6-16.4%) who needed renal replacement therapy [35].

The decrease of renal mass is thought to be the main pathophysiology that is associated with CKD, AKI and the end stage renal disease. Considering these interesting findings and the fact that the common therapies do not influence the short-term prediction in AKI, supplementary preventive actions meant to ameliorate the health status for AKI survivors are extremely important. There is general evidence that most of the patients suffering from AKI are not regularly surveyed by nephrologists and the current clinical practice does rarely include follow up evaluation for AKI, dealing with this issue representing a considerable option for important change and better caring for this category of population [36]. Even patients with mild and moderate AKI, which in most cases is short lived and clinically reversible renal failure, had an elevated risk of death after hospitalization that did not dissipate over time. These conclusions arenovel and do not support the conventional teaching that AKI in its mild-form is inconsequential [37].

CONCLUSIONS

AKI negatively influences the short- and long-term prognosis of subjects diagnosed with ACS. CKD and AHF are other negative predictors of ACS evolution and their association with AKI has shown the highest mortality rate. Although it is not an accurate marker, the initial sCr and the worst value of sCr can be used in AKI detection to establish appropriate therapeutic measures. New predictive scores able to identify AKI in a timely manner are necessary, considering the fatal prognosis for patients with ACS and CKD.Early and complete myocardial revascularization in ACS is an optimal method, which decreases the risk of developing AHF and AKI, known as cardio-renal syndrome.

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VARIA

Bucharest mobile military hospital – response to the COVID-19 pandemic

Florentina Ionita Radu[1,2], Ileana Mariana Mates[1], Valeriu Gheorghita[1,3]

Abstract: *The idea of using mobile hospitals - a fully functional hospital is a system with a high degree of complexity - in case of a pandemic crisis, is not new, but shows how much they are needed.*

Solutions belonging to the category of mobile architecture are increasingly common, technical and logistic difficulties are solved in more perfect way. This applies mostly to objects with simple layouts and functional relationships, with relatively little technical equipment. In the case of healthcare facilities there are several factors increasing the difficulty: multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure.

The Romanian military and civilian specialists from Bucharest Central Military Emergency University Hospital (SUUMC) showed that is possible to build a hospital as a modular and mobile structure in order to fight COVID 19, but the strategic actions and the effort needed to organize and run mobile hospital far exceeds the expenditure, protocols and specialized M.D.s necessary to run the stationary hospital.

Keywords: *mobile hospitals, COVID-19, strategic actions*

INTRODUCTION

SARS-CoV-2, the causative agent of COVID-19, is a newly discovered coronavirus. It is transmitted through respiratory droplets and direct contact; other modes of transmission (i.e. airborne and digestive) have been proposed, but they are still under investigation. The average incubation period is estimated at 5 to 6 days, ranging from 1 to 14 days [1]. There is currently no approved specific treatment or vaccine against COVID-19.

Business-as-usual service delivery approaches are not sufficient to respond in the fight against COVID 19, once cluster of cases or widespread community transmission is registered and superior capacity will be needed.

While World Health Organization (WHO) advises that aggressive measures to find, isolate, test, treat and trace are the best ways to stop the spread of this virus, public health interventions to "flatten the curve" may slow transmission and mitigate peak capacity needs. Modelling studies point to the impact of nonpharmaceutical interventions (NPIs), such as physical distancing, school and university closures, banning of mass gatherings, and remaining indoors, on spreading the number of cases over a longer period to give health systems the opportunities they need to cope with caseloads [1, 2, 3, 4]

These models have helped to reinforce the message from WHO about the implementation of such measures, alongside widespread testing, to detect cases and break chains of transmission. In addition to these measures, phased plans for creating surge capacity in acute and intensive care will help respond to COVID-19 cases while maintaining essential health services.

Surge capacity is the ability of a health system to manage a sudden and unexpected influx of patients in a disaster or

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emergency situation.

Surge capacity can be created from intrinsic and extrinsic resources. The former covers all local resources that can be used for the response, while the latter involves leveraging resources from outside the affected area (across geography or across specialty).

As the COVID-19 pandemic necessitates both in tandem in all countries, this note covers both these aspects together. Creating surge capacity involves a comprehensive approach linking the 4 S's of surge capacity: space, staff, supplies and systems. [5]

RECOMMENDATIONS AND STRATEGIC ACTIONS

In the following, are presented the recommendations and strategic actions to activate surge capacity in line with the 4 S's drawing on past experiences in emergencies and on the emergent experiences of world countries in the COVID-19 response.[6]

Strategic action 1. **Create and activate a stepwise plan to expand physical space to care for COVID-19 patients respecting infection prevention and control protocols**.

This action requires the following steps, and is schematically presented in the Figure 1.

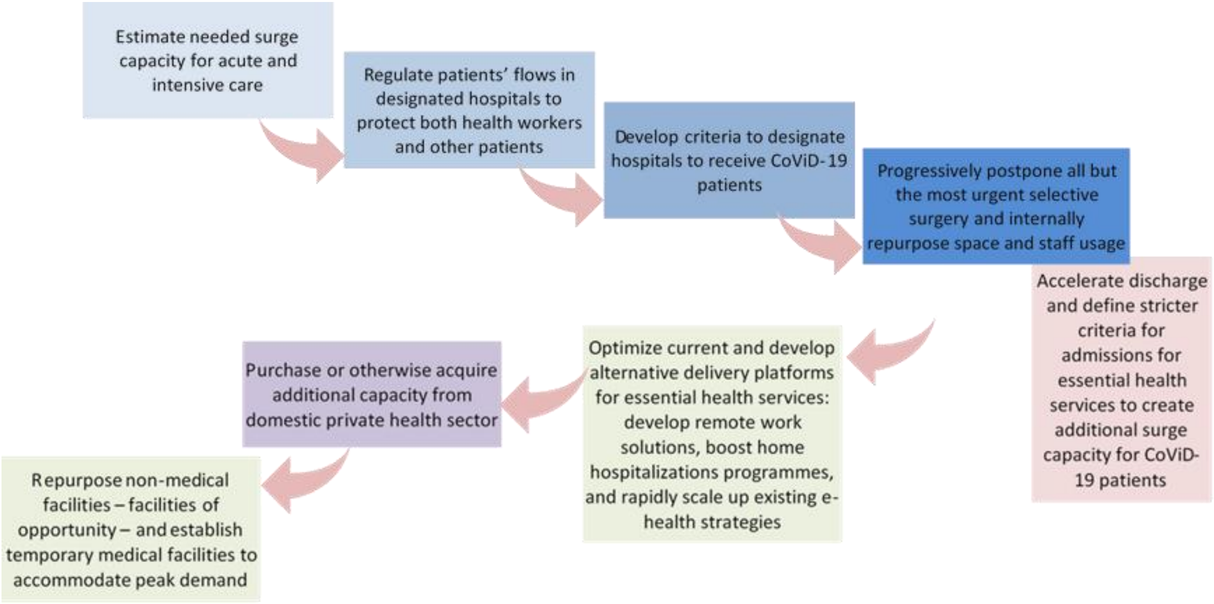
Estimate needed surge capacity for acute and intensive care
Regulate patients' flows in designated hospitals to protect both health workers and other patients
Develop criteria to designate hospitals to receive CoViD-19 patients
Progressively postpone all but the most urgent selective surgery and internally repurpose space and staff usage
Accelerate discharge and define stricter criteria for admissions for essential health services to create additional surge capacity for CoViD-19 patients
Optimize current and develop alternative delivery platforms for essential health services: develop remote work solutions, boost home hospitalizations programmes, and rapidly scale up existing e-health strategies
Purchase or otherwise acquire additional capacity from domestic private health sector
Repurpose non-medical facilities – facilities of opportunity – and establish temporary medical facilities to accommodate peak demand

Figure 1: Necessary steps for strategic action 1

Strategic action 2. Identify the health workforce available for surge capacity demands, and repurpose and upskill for rapid deployment to meet surge capacity needs

The steps necessary for this strategic action are depicted in Figure 2.

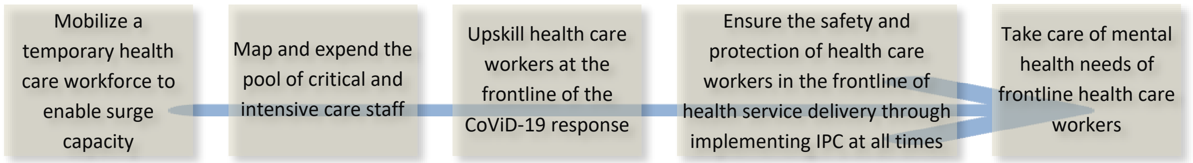
Mobilize a temporary health care workforce to enable surge capacity
Map and expend the pool of critical and intensive care staff
Upskill health care workers at the frontline of the CoViD-19 response
Ensure the safety and protection of health care workers in the frontline of health service delivery through implementing IPC at all times
Take care of mental health needs of frontline health care workers

Figure 2. Necessary steps for strategic action 2

Strategic action 3. Ensure adequate supplies to support surge in demand

The necessary steps for strategic action 3 are presented in Figure 3.

Strategic action 4. Establish systems to manage and align policies to meet surge in demand

The steps that are necessary for strategic action 4 are mentioned in Figure 4.

Figure 3: Necessary steps for strategic action 3

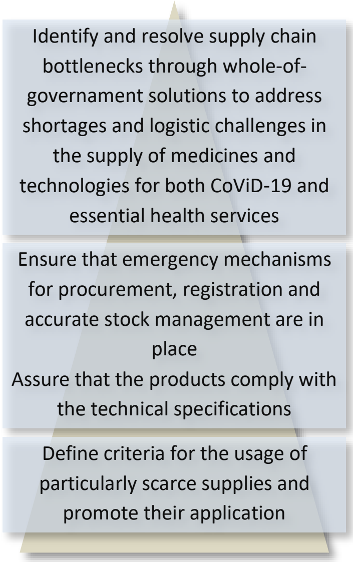
Identify and resolve supply chain bottlenecks through whole-of-government solutions to address shortages and logistic challenges in the supply of medicines and technologies for both CoViD-19 and essential health services
Ensure that emergency mechanisms for procurement, registration and accurate stock management are in place
Assure that the products comply with the technical specifications
Define criteria for the usage of particularly scarce supplies and promote their application

Figure 4: Necessary steps for strategic action 4

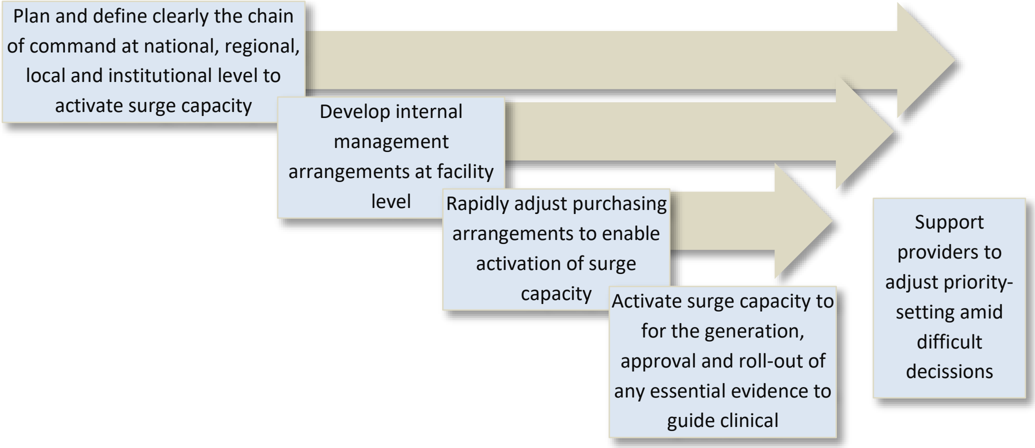
Plan and define clearly the chain of command at national, regional, local and institutional level to activate surge capacity
Develop internal management arrangements at facility level
Rapidly adjust purchasing arrangements to enable activation of surge capacity
Activate surge capacity to for the generation, approval and roll-out of any essential evidence to guide clinical
Support providers to adjust priority-setting amid difficult decissions

THE ROL 2 MILITARY HOSPITAL AT THE ANA ASLAN INSTITUTE IN OTOPENI, NEAR BUCHAREST – A CASE STUDY

The ROL 2 military hospital was installed at the Ana Aslan Institute in Otopeni city, near Bucharest, to support the Romanian government as a response to the Covid-19 pandemic. The ROL 2 was declared operational on Saturday, March 28, in the presence of President Klaus Iohannis and Prime Minister Ludovic Orban, the Ministry of Defense announced.

Once accredited by the Ministry of Health, the ROL 2 hospital will operate as an external facility of the Central Military Emergency University Hospital (SUUMC). The mild and medium cases of COVID-19 will be admitted and treated in this medical facility (Figure 5).

Figure 5: The ROL 2 first medical team

The ROL 2 mobile military hospital operate as a stand-alone entity (as in the American solutions), or in conjunction with the whole hospital – Bucharest Central Military Emergency University Hospital (but still autonomously, as in European solutions). These two variants are taking into account organisation of space or technological line flow. The difference between the two models comes down to one important aspect: in the European pattern patients' flow is unidirectional, almost linear, from the entrance of the patient to the segregation area (TRIAGE), through diagnostic and treatment facilities ending at the hospital bed wards). This is due to the principles of Emergency Department (DE) operation. ED is an integral part of the hospital and at the same time it is an emergency path of hospitalization. The American ED is more autonomous unit, operating often as an outpatient clinic compared to European standards: the TRIAGE and central nurse station become the most important areas of the ED. Other functions of the ED are grouped around the focal point of the nurse station. The flow of patients through the ED is less structured, without clearly separate movement paths, always through the nurse point.

In the case of building a mobile military hospital there are several factors increasing the difficulty: multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure, which includes both the military and civilian objectives.

The mobile military hospital is built as a reaction to disasters, both natural and those resulting from human factor, for "emergency" (acute and crisis situations) activities. An emergency medicine, no matter of the form of its organisation, is a set of medical actions, serving a life rescue.

The technical solutions are related to 2 terms: modularity and mobility. Modularity should provide a construction of possibly most numerous and diverse structures of various purposes. Mobility is to ensure a solution which enables the module (typical commodity container) to be loaded and transported by standard ways: truck or a ship, or even air-borne.

For this achievement, the management of the Bucharest Central Military Emergency University Hospital (SUUMC) conceived a procedure which has been developed to support hospital preparedness for the management of COVID-19 patients, based on European Centre for Disease Prevention and Control recommendations [7]. The procedure started with the procurement and stock management. A procurement procedure to acquire the necessary materials and supplies was activated on short notice and alternative suppliers have been identified. The acquisition team took into account that if main suppliers should run out of stock (especially for personal protective equipment (PPE)) a buffer stock of key supplies has been acquired (e.g. for hand and respiratory hygiene, PPE, isolation, ICU supplies, mechanical respirators). A procedure to monitor and regularly update the inventory and a stock inventory have been conducted. In order to avoid misuse or overuse, a plan was developed to keep track and custody of key supplies (e.g. PPE, ventilators, cleaning and disinfection material, alcohol solution etc.).

The maximum facility capacity, including the maximal number of ICU beds, mechanical ventilators, bins for infectious waste, and the required human resource capacities (M.D.s, healthcare workers and cleaning personnel) and supply capacity have been calculated.

Storage facilities which meet all demands with respect to temperature, humidity, cold-chain, logistics etc., for additional stock have been identified, also a proper amount of cleaning and disinfection products shown to be effective against coronaviruses is available. A procedure to ensure that equipment is in perfect working order and can be quickly replaced whenever necessary was developed.

All staff, healthcare and non-healthcare workers, have been informed and trained on the following topics: Case definitions, Hand and respiratory hygiene, How to use PPE's and know the documents outlining the procedures, Triage procedures, Placement and movement of patients in isolation, Internal and external communication lines and rules, Sick-leave policy and what to do if staff members show symptoms and Security plan.

Procedures for patient prioritization (e.g. triage, discharge criteria, triggers to postpone elective hospitalizations or interventions) are in place A patient' system before they arrive at the hospital is in place: phone/email/telemedicine services are in place for possible cases.

A procedure for the cleaning of the rooms on a regular basis and after a patient's discharge has been established.

Figure 6: The ROL 2 mobile military hospital – general view

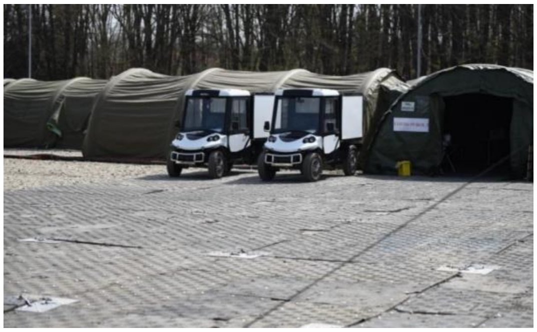

The ROL2 mobile military hospital, made up of medical tents and containers (Figure 6) is intended for the triage, diagnosis and hospitalization of patients confirmed with COVID-19. The structure of the hospital is composed of two parts: the medical and the administrative part. The medical part is structured in the following sections/laboratories/

compartments:
- Emergency Room Unit (ERU) with 20 beds
- Infectious Diseases Department (IDD) with 120 beds
- Intensive Care Unit (ICU) with 24 beds
- Imagistic Laboratory
- Medical Analysis Laboratory
- Pharmaceutical point

The hospital was equipped with medical equipment necessary for the diagnosis and treatment of patients with COVID-19, as follows: necessary RT-PCR for the identification of COVID-19, biochemistry analyzer, mobile radiology apparatus, ultrasound device, oxygen concentrators, pulse oximeters, laryngoscopes, intermittent mechanical compression devices, patient heating system, trolleys for the storage of sanitary materials and medicines, , air purification devices, wheel sprayers for car-trucks disinfection.

The Intensive Care Unit (ICU) was equipped with specific beds, secretion aspirators, oxygen pumps, automatic syringes with related stations, mechanical fans, and monitors for monitoring vital functions. Very important is the fact that telemedicine system has been installed with video cameras for the monitoring of patients, so the medical staff can keep them under observation, with minimal contact.

The Emergency Room Unit (ERU), adjacent to the patient triage area, is equipped with 8 beds and a stationary area featured with 6 compartments of 2 beds for patients waiting for lab results and for carrying out complementary investigations (interdisciplinary evaluations, imaging investigations). If the number of patients presented is greater than the capacity to take in the ERU, there are two waiting areas for patients featured with chairs located at a distance of at least 1 meter, in an adjacent tent (Figure 7).

The Infectious Diseases Department (IDD) is designated for the admission of patients confirmed with COVID-19. The section is provided with 120 beds equally distributed in 30 individual reserves with own sanitary group. Each reserve is provided with medical equipment and equipment for the permanent monitoring of patients, with the possibility of ensuring the oxygen requirement to patients who do not meet the criteria for admission to the intensive care unit. All IDD rooms are sterile environments; all personnel wear protective clothing (scrubs), shoe covers, masks, caps, eye shields and other coverings.

The inventory for 1 IDD reserve (Figure 8) includes: 4 beds, disposable linen, 1 TV set, 1 telephone and equipment for the medical supervision of patients (nurse-call system), 1 refrigerator, 4 stands of infusion, 1 acute care physiologic monitoring system (comprehensive patient monitoring systems that can be configured to continuously measure and display various parameters via electrodes and sensors connected to the patient), 4 pulse oximeters, 1 treatment table, 4 bedside tables. Lingerie is changed daily and whenever necessary.

Figure 7: The Emergency Reception Unit (ERU)

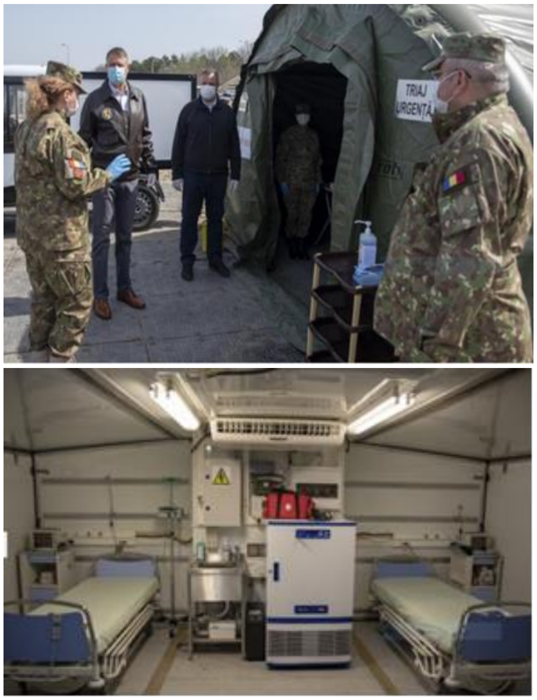

Upon admission to the IDD, each patient will receive a disposable kit containing: 1 disposable pajamas, 1 pair of disposable slippers, 1 toothbrush and toothpaste, 2 absorbent fields (towels) size 90/140 cm^2. These materials will be later distributed, whenever appropriate.

Figure 8: IDD reserve

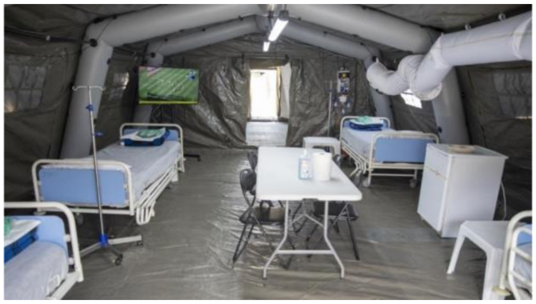

The medical part also comprises:

• **The Intensive Care Unit** (ICU) is undergoing an arrangement with 24 beds and it is located in the annex building of the ROL 2 hospital. Patients with severe respiratory disorders cannot be properly treated in tents, as they must benefit, for a certain period of time, of special care and connection to mechanical fans.

• **Two own laboratories** (Figure 9) where diagnostic tests (RT-PCR) and respectively hematological and biochemical investigations (complete blood count, CBC (hemoglobin), Erythrocyte Sedimentation Rate (ESR), coagulation test, myoglobin, D-Dimer, blood chemistry tests including Ferritin, troponin test, procalcitonin (PCT), presepsin (P-SEP), HBV, HCV, HIV Screening Tests, detection of SARS CoV 2 with RT-PCR) will be performed.

Figure 9: Laboratory for diagnostic tests (RT-PCR) and hematological - biochemical investigations

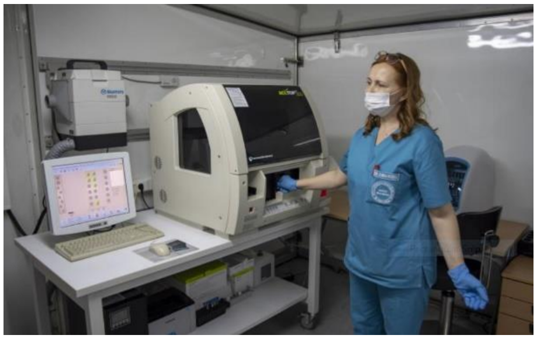

• **Imaging laboratory** provided with a mobile radiology device and Computer Tomography machine.

• **Pharmaceutical point** (Figure 10) where antiviral drugs necessary for the treatment of patients confirmed according to the national protocol adopted at the level of the Ministry of Health and taken over by this structure will be available.

Figure 10: Pharmaceutical point

At the ROL 2 hospital level, the functional circuits with the delimitation of the red and green areas, are ensured by providing separate flows for patients and medical staff, with rooms for equipping the medical and auxiliary staff and separate rooms with their own sanitary for undressing.

The sections and compartments of the ROL 2 hospital are computer interconnected between them and the command point, as well as with the computer network of S.U.U.M.C "Carol Davila".

The payroll comprises a total of approximately 270 positions distributed over each structure. For the operationalization of the ROL 2 Hospital, the personnel will be provided from the human resources of the S.U.U.M.C. and partly from the Medical Directorate.

The work program of the staff is structured on each separate compartment, in 12-hour shifts, over a 7-day cycle and is provided by working medical corps, made up of doctors, nurses, stretches. After this period of 7 days, the work team enters the period of self-isolation for a period of 7-14 days, a necessary measure for both the rest and the physical and mental recovery of the personnel and for the epidemiological supervision. The activity will be continued by other working medical corps, following the same structure.

At the entry and exit of the 7-day work cycle, the entire workforce will be clinically evaluated and the diagnostic test using RT-PCR for COVID-19 will be performed. Also, the staff will be tested as needed if it becomes symptomatic during the activity.

The work program is structured as follows:

- 7 consecutive days with 7 days rest for doctors and 14 days for nurses, nurses, stretches and medical administrators;
- Daily, there will be two shifts (day and evening) at 12 hours intervals; the ranges are from 08:00 – 20:00, respectively 20:00 – 08:00;
- Each 12-hour work program is provided by separate working medical corps.
- The structure and work schedule for a 7-day cycle of the IDD
- 2 specialist/consultant doctors (infectious diseases and pneumology or internal medicine);
- 6 residents (infectious diseases – 2 and internal medicine – 4);
- 12 nurses (also includes the head nurse);
- 4 orderlies;
- 2 stretches.

The procedure described may not be applicable to all military or civilian hospitals and may need to be adapted to the specific characteristics of the hospital, the individual national health system, legislation and community where the hospital is located.

“Today I visited the ROL 2 Military Hospital, a medical facility made in record time by the Army and the military medical personnel. Romania is prepared to cope as well as possible with this epidemic and I assure you that we will continue to

take all necessary measures to be even better prepared," Klaus Iohannis (Figure 11) said after visiting the hospital. [9]

Figure 11: The Romanian President Klaus Iohannis and Major General Assoc. Prof. Florentina Ioniță-Radu MD, PhD at the inauguration of the ROL 2 military hospital installed at the Ana Aslan Institute in Otopeni (Photo: Presidency.ro)

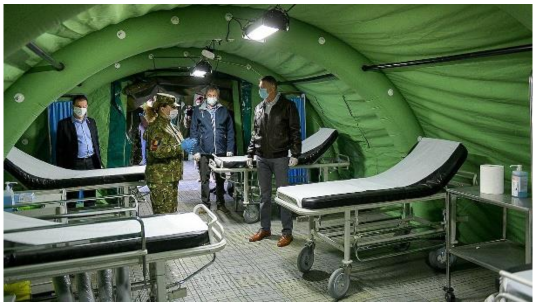

The same ROL 2 military hospital was deployed in 2015 when it was supposed to treat potential Ebola infection cases. It was also used in NATO military exercises in 2019, including Vigorous Warrior – the biggest medical military exercise ever organized by NATO, which was hosted by Romania.

"*I have entrusted the supreme ally commander of Europe with the task of coordinating all the means of supporting, coordinating and directing all the excesses at national level to the allies who need support at this particular moment, a first step taken by NATO, at this moment, is to provide the logistics of allies who need support in the fight against the coronavirus pandemic*." said Mircea Geoana, NATO Deputy Secretary General. [10]

CONCLUSIONS

The set up of the mobile military hospital installed at the Ana Aslan Institute in Otopeni, near Bucharest, to support the Romanian government as a response to the Covid-19 pandemic complies all the health requirements regulations defined by the National Authority of Quality Management in Health (Autoritatea Naţională de Management al Calităţii în Sănătate – ANMCS) throughout specific accreditation standards for medical units.

Further studies are necessary in order to determine the Covid-19 pandemic amplitude actions to end it, and future research projects destined to cover the need and development of these mobile hospitals, which involve a multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure is necessary.

Note

The ICU is arranged in the annex building of the ROL 2 hospital. Patients with severe respiratory disorders cannot be properly treated in tents, as they must benefit, for a certain period of time, of special care and connection to mechanical fans.

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Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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(5) Identifies trials with a unique number; and
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redacţiei publilcaţiei „Revista de Medicină Militară",

în semn de apreciere a contribuţiei avute la creşterea prestigiului structurilor medicale din Armata României, cu ocazia aniversării a 120 de ani de la înfiinţare,

conferă ***Emblema de Onoare a Armatei României***
-cu însemn de pace-

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REVISTA DE MEDICINĂ MILITARĂ

- *Evidence based medicine – lessons learned from the NATO Military Medical Center of Excellence*
- *The trend of bladder cancer among Iranian Military Community from 2007 to 2019*
- *Obesity and osteoporotic fractures*
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REVIEW ARTICLE

Evidence based medicine: lessons learned from the NATO Military Medical Center of Excellence

Tomáš Vašek[1], Jaroslav Žďára[1], Petr Král[2], Milan Růžička[1], Michal Potáč[1], Petr Smola[1], John Quinn[3]

Abstract: *Evidenced-based medicine drives best practices. The Lessons Learned (LL) process in clinical medicine for deployed and military support services drive the NATO Military Medical Center of Excellence (MILMED COE). This review article focuses on the Lessons Learned process and its use in NATO and partner countries medical services. Organizational learning drives development and progress; capturing lessons from mistakes, colleagues' interactions and institutional experience can be lost without an organized lesson learned process. Therefore, in 2014, NATO institutions and Centers of Excellence across disciplines began to focus on the quality management, information sharing and evidence-based practices to maximize outcomes. Since this inception, NATO has implemented the Lessons Learned process and expanded the impact across the alliance in order to save life and prevent illness. The Lessons Learned process and sharing of experience is also a way to improve the quality of care in the military medicine, from preventive, epidemiological, trauma related, casualty evacuation and forward surgical care, among many others, to the medical standardization and organization of military medical services. The Lessons Learned process is an undertaking and an instrument that can also be used to achieve better international and civil-military cooperation. Finally, we deal with the current situation and use of the information obtained from the areas of military health care.*

Keywords: *evidenced-based medicine, knowledge management, lessons learned, Military Medical Service, NATO*

INTRODUCTION – APPLICATION OF THEORY OF LEARNING

Each scientific discipline must be driven by data. One of the areas from which information can be drawn is Lessons Learned, through analysis and a constant cycle of learning and sharing data. This is most applicable in the military medicine and across the healthcare sector, where shared information can be used from various areas, rooted in preventive medicine, epidemiology, traumatology, and others adding to military medicine and healthcare organization and administration.

Institutions that promote health act as microcosms of organism that can evolve through adaptation. As Charles Darwin posited, it's not the strongest survivor, not even the most intelligent, but the one who can best adapt. Every living organism adapts best by learning from past mistakes. In a broader sense, it is about adapting to the environment, as well as the active role of the individual and the choice based on challenges. But learning is not mere adaptation, but it is an active process in which an individual acquires (changes, adapts, and repeats) something that is new. Learning outcomes are actively created personal hypotheses and meanings of reality [1].

There are many theories of learning, but the process we describe in this paper focuses on the theory of Classical Conditioning (I.P. Pavlov), Thorndike's theory of Instrumental Conditioning, Skinner's theory of Programmed Learning, and the theory of Learning through Imitation. Simply put, we

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[2] NATO Centre of Excellence for Military Medicine
[3] Prague Center for Global Health, Institute of Hygiene and Epidemiology, First Faculty of Medicine, Charles University

will do, repeat and develop what we have done and have some positive effect in a particular area. Conversely, we discuss what happened and had some negative consequences that should not be repeated. Based on applying these hypothesis listed above and core knowledge, every organization that wants to survive must behave to the basic strategy of learning from past mistakes.

THE WAY OF THE LESSONS LEARNED PROCESS TO MEDICAL SERVICES OF THE NATO ARMIES

In the early years of the 21st century, NATO understood the importance of transformation and has become a learning organization that has the ability to be constantly flexible and adaptive to remain the central core of European and North Atlantic Security structures in the promotion of safety and security across international communities. For this reason, NATO has issued guidelines outlining the process of learning and sharing experiences, with the clear aim of providing a standardized structure and process that enables this ability in all alliance command structures [2].

Moreover, as outlined within the Armed Forces Declaration, signed by the NATO Heads of State during the NATO Summit, Wales 2014, NATO was directed to enhance the sharing of best practices and lessons learned in support of its Armed Forces personnel and their families [3]. Through the Military Committee (MC), the Committee of the Chiefs of Military Medical Services in NATO (COMEDS) requested that the NATO Centre of Excellence for Military Medicine (MILMED COE) prepare a way ahead to be presented at the 42nd COMEDS Plenary. The proposal was approved and subsequently endorsed by the International Military Staff (IMS) and Military Committee (MC) [4].

LESSONS LEARNED PROCESS AS A PART OF KNOWLEDGE MANAGEMENT

Lessons Learned is defined as a major component of Knowledge Management (KM), not only in the military, but also in government and commercial organizations [5]. The priority objective of Knowledge Management is to achieve higher performance of the organization in fulfilling its tasks in a dynamic, asymmetric and unstable environment (i.e. across the domains of land, sea, air, space and cyber). Therefore, gaining knowledge and experience is a process whereby the Lessons Learned are explored in order to find the best way to deal with the situation and share the outcome to all stakeholders who can benefit from it, and preferably mitigate and reduce mortality and morbidity.

In addition, it is a process allows an organization to learn from its mistakes or use its achievements. Thus, Lessons Learned is an instrument for the continuous process of improving each organization based on evidence [6]. The essence of this process is that it reduces the risk of error repetition in the organization, and the likelihood of repetition of success increases. The introduction of this process within the military health service is important not only for updating the core doctrine that drives best practices, but also for the comprehensive preparation of units in both peacetime and deployed operations.

According to the NATO Bi-Strategic Command Directive 080-006 [7], which is a strategic document of the Lessons Learned process, the following components – the pillars through which the process is implemented – are needed (Figure 1):

- Processes – links between elements to implement the process, standardize the process to ensure its compatibility and universality both in the NATO structure and in collaboration with other partners.
- Structure – elements (bodies) that are responsible for individual subprocesses and which guarantee an efficient and smooth course.
- Tools – technology, selection and use of appropriate support tools and their optimization to support the Lessons Learned process and its subprocesses.
- Training and education – the main sponsor activities needed to understand the importance of this process.

Figure 1: Components of the Lessons Learned System (NATO, 2016, p. 9)

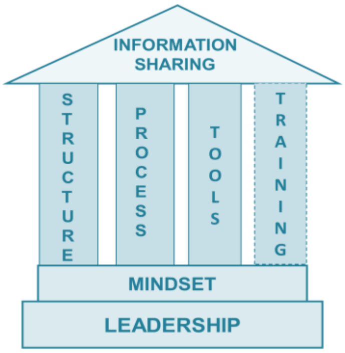

The ceiling of this process is the very important sharing of information that represents the culmination of the entire Lessons Learned process. However, the most important are the cornerstones of the whole process, without which this process could not work effectively. The base of the Lessons Learned process is made up of commander's support – leadership, with senior military management knowing and understanding the benefits of the process. Then it is the commitment and thought of all involved. It is therefore necessary to achieve a state where all the participants will understand the importance of this process and, in addition, the support of the commander will be clear to all [8].

The goal of the lessons learned proces

The general objective of the Lessons Learned process is to preserve the organization's knowledge, is to transform the individual's experience into the knowledge of the organization so that other members of the organization can use their experience in the absence of the expert [5].

Therefore, using lessons learned can positively influence other processes of the organization on a continuous cycle of improvement.

Scheme of lessons learned process

The NATO Bi-Strategic Command Directive 080-006 describes the individual parts and sequence of the Lessons Learned process, which is then illustrated in Figure 2.

Observation

This is an identified problem that requires action to be taken to improve the status or best practice for reuse. These are precisely described facts, events, claims, statements that are based on the evaluation of information that describes a certain activity of units, immediate causes, effects or effects.

Knowledge can be Raw Observation, which requires further study or analysis to understand the cause of the event and to supplement the information, or the Mature Observation, to which there is sufficient information, or the cause of the event is known and is not needed its further study and analysis [5, 6, 7].

Analysis

It is a study of the whole through careful examination of its individual parts and their interrelationships [5, 6, 7].

For the purposes of the Lessons Learned process, it is about describing a given phenomenon, its causes and consequences, including the initial design of measures to prevent repetition of inappropriate practices or to make repetition of the appropriate ones.

Lesson identified

Identified experience because of knowledge analysis. In addition to describing the event, the causes and the consequences, it includes a proposal for remedial measures, concrete steps and responsibilities for implementing these measures [5, 6, 7].

Lesson learned

It is the result of the implementation of measures that have increased the ability of a particular element [5, 6, 7].

Knowledge

Newly generated information, whether in the form of knowledge, identified or generalized experience has an individual value for the user corresponding to his/her knowledge (5). Interpreting such information in interaction with experience, facts, individual value, thought processes, and meanings can be understood as knowledge.

Share

One of the most important part of the Lessons Learned process, which is intertwined with the process, is sharing. If NATO really wants to work based on a learning organization, it is necessary for individual Member States to share knowledge with others.

Database of knowledge and experience

In addition, to centrally store, manage, share, and access the knowledge, national and international databases have been created to enable registered users to easily access the information they need.

IMPROVING THE QUALITY OF MEDICAL SERVICES ACCORDING TO THE NATO MEDICAL LESSONS LEARNED PROCESS

First, quality in healthcare can be elusive and very complex in the military medical paradigm. The World Health Organization (WHO) defines the quality of health care as the extent to which health services provided to individuals and patient populations improve health outcomes [9]. For this purpose, healthcare must meet these key properties for all. It must be safe, efficient, timely, effective, and fair and focused on people – all activities must be driven by data and evidence based clinical practice. Health care quality can also be explained as a continuous improvement in the quality of health services provided, the ultimate goal of which is to improve health, improve the quality of life and satisfaction of the people they care for [10].

Thus, the Health Care Quality System includes the entire process of creating procedures, collecting information, setting clinical standards and assessing health care, health services and most importantly healthcare outcomes. It is also worth noting that a quality system is an intrinsically interconnected concept of quality elements that should help health organizations meet the goals of their mission [11]. In general terms, this applies to both civilian and military healthcare that the objective of a particular quality system in healthcare is always to improve the quality of health and related care provided in accordance with individual needs and patient requirements and is rooted in evidence and best practice. In military medical operations, duty of care for warfighters and deployed personnel is the burden of the countries involved and the alliance; this is extended to the combatants and civilian population as outlined in the UN and Geneva Conventions and international norms. This includes the provision of healthcare to all at a standard of care as close to and in keeping with clinical standards provided at peacetime found in the participating countries [12]. This is an extremely difficult task for the provision of medicine for deployed personnel in the dynamic environment that is war, conflict and peacekeeping in remote, austere and resource challenged environment.

Figure 2: Scheme of Lessons Learned
(NATO, 2016, p. 11)

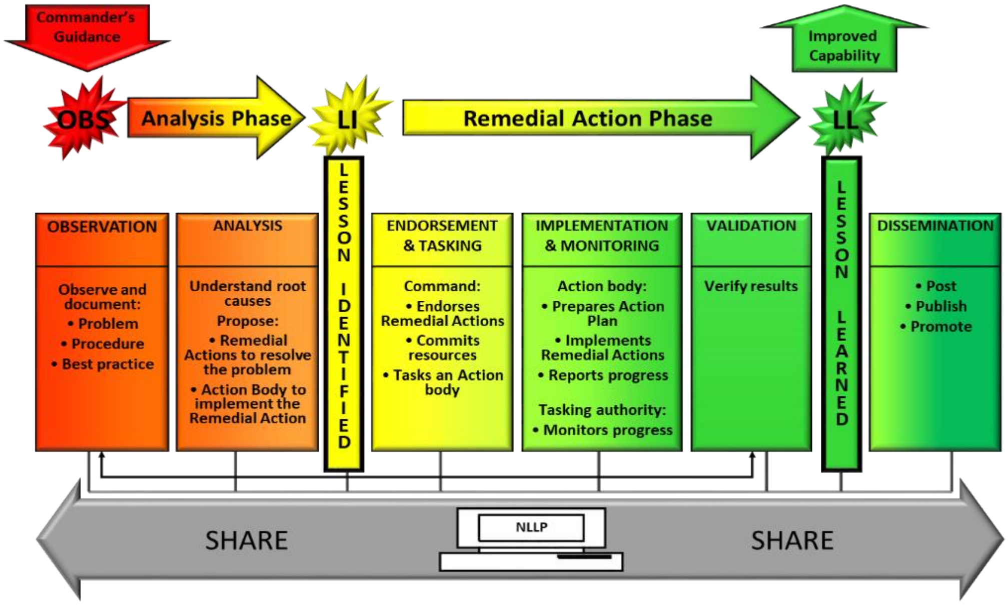
Commander's Guidance
Improved Capability
OBS
Analysis Phase
LI
Remedial Action Phase
LL
LESSON IDENTIFIED
LESSON LEARNED
OBSERVATION
Observe and document:
• Problem
• Procedure
• Best practice
ANALYSIS
Understand root causes
Propose:
• Remedial Actions to resolve the problem
• Action Body to implement the Remedial Action
ENDORSEMENT & TASKING
Command:
• Endorses Remedial Actions
• Commits resources
• Tasks an Action body
IMPLEMENTATION & MONITORING
Action body:
• Prepares Action Plan
• Implements Remedial Actions
• Reports progress
Tasking authority:
• Monitors progress
VALIDATION
Verify results
DISSEMINATION
• Post
• Publish
• Promote
SHARE
NLLP
SHARE

APPLICATION OF LESSONS LEARNED PROCESS IN NATO MILITARY MEDICAL SERVICES

In October 2014, the NATO Center of Excellence for Military Medicine (MILMED COE) hosted the International Security Assistance Force (ISAF), the NATO-led security mission in Afghanistan, Medical Lessons Learned Workshop. This was performed in order to preserve the knowledge gained in ISAF and discuss health care expertise in ISAF in Afghanistan. Examples of the use of appropriate combat application tourniquets during severe limb bleeding have clearly shown that collecting knowledge and sharing Best Practices saves lives and improves the clinical outcomes of those injured on the battlefield. Additionally, on the basis of Lessons Learned from Afghanistan, there was a demand for safe removal by a light armored medical vehicle for evacuation purposes. The MEDEVAC (Medical Evacuation) system was also created based loosely on this process, which can skip individual stages of health care and from point of injury for the warfighter or enemy combatant to ensure they are appropriately evacuated as soon as possible directly to a Medical Treatment Facility (MTF) at a ROLE 2E or ROLE 3 facility.

A number of shortcomings have also been identified in the quality system of medical support; these are of special interest when making the entire system more robust, more resilient and capable of fighting the next battle with potentially different weapon systems and challenges than the current.

The results were presented to the Committee of Military Health Service Chiefs (COMEDS) at their 42nd session. At this meeting, it was decided that NATO MILMED COE would become the main contact and coordination center for the Lessons Learned process within the NATO military health service. Lessons Learned Process for NATO Health Support Centers is illustrated in Figure 3 [13]. In effect, the MATO MILMED COE LL has become the clearinghouse and central crossroads for all thing's best practices in NATO Military Medicine across the alliance.

By positioning itself as the coordinator and collector of observations and lessons, the Lessons Learned Branch of the MILMED COE adds value to the NATO system, processing and sharing Lessons and best practices for all members of the Coalition across the alliance. The LL Branch are building a roadmap to the future of the delivery of Health Support

Services through best practices and rooted in clinical evidence. The members of the alliance have delivered amazing health care, and we need to collect and share the Best Practices across the organization to ensure that everything that has been learned can continue to save lives, financial resources and sparse clinical resources [14].

Currently, the NATO MILMED COE Lessons Learned Branch is developing an inclusive process to improve the collection, processing, analysis, storage and sharing of military health-related knowledge. All the information presented and analyzed is uploaded and stored in the Library of Medical Lessons Learned MILMED COE Information and Knowledge Management (IKM), which is located on the MILMED COE website (http://www.coemed.org/medlldb). An important achievement from the third Medical Lessons Learned Workshop, which took place in 2015, was the establishment of the Multinational Medical Lessons Learned Team (MMLLT), where the Czech Military Medical Service is represented and hosted the second meeting as well. Participating countries agreed to share national information and documents related to military health through the MILMED COE IKM portal. The MILMED COE KM Portal is a good starting point for sharing across the alliance. Each contributing nation should decide on a national level what kind of document they would share. COMEDS has requested to rename the MMLLT, because this name is too similar and thus be confused with Medical Lessons Learned Core Team. With these changes, the new name of the body is Medical Sharing Group.

Figure 3: Lessons Learned Process for NATO Health Support Centres (MILMED COE, 2015)

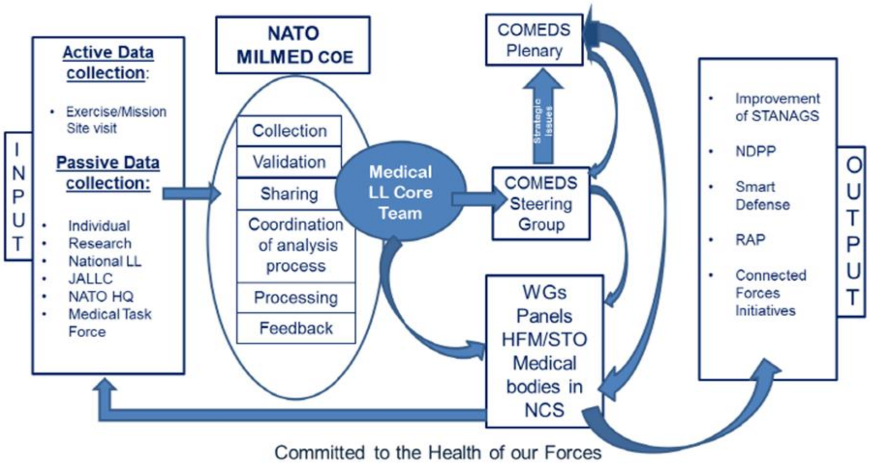
INPUT
Active Data collection:
Exercise/Mission Site visit
Passive Data collection:
Individual
Research
National LL
JALLC
NATO HQ
Medical Task Force
NATO MILMED COE
Collection
Validation
Sharing
Coordination of analysis process
Processing
Feedback
Medical LL Core Team
COMEDS Plenary
Strategic issues
COMEDS Steering Group
WGs Panels HFM/STO Medical bodies in NCS
Improvement of STANAGS
NDPP
Smart Defense
RAP
Connected Forces Initiatives
OUTPUT
Committed to the Health of our Forces

Moreover, the aim of the Medical Sharing Group is to establish the capability to share Lessons Learned information and knowledge across the Alliance [2]. Specifically, the Group members are responsible for reviewing Observations, Lessons Identified, and Lessons Learned information and knowledge within their national or organizational system and for forwarding any item to the NATO Medical Lessons Learned process that is considered beneficial to other Alliance Nations or to the Alliance as a whole. The intent is to empower the exchange of locally identified solutions or Best Practices with the goal of optimizing performance in current and future activities. The type and level of items to be shared will be established by the providing nation or organization and does not need to be the same for every participant. Additionally, the type and level of input provided by a participating entity does not have to remain the same over time and no formal process for change is required, enabling faster sharing of vital information [4]. The Medical Lessons Learned Process, an ongoing effort to improve process, is shown below in Figure 4.

Medical lessons learned conference on civilian – military interaction

A conference on civilian military cooperation has also been held several times. The main aim was to invite civilian and military organizations recently involved in Crisis Management, Humanitarian Assistance and relevant NATO, EU operations and share experiences, best practices and lessons learned. The last event was attended by 36 delegates from 12 nations and 13 organizations. A growing number of Crisis Management and Humanitarian Assistance missions/ events have been resulting in increasing demand for well-organized Civil-Military Interaction. Sharing individual perspectives on the situation and root causes of a crisis is the first step towards de-conflicting actions and complementing each other’s efforts, in effect to remove a duplication of effort. For the successful Civil-Military Interaction sharing of information is key importance to develop a common understanding about the situation and necessary measures.

Figure 4: Medical Lessons Learned Process
(MILMED COE, 2018)

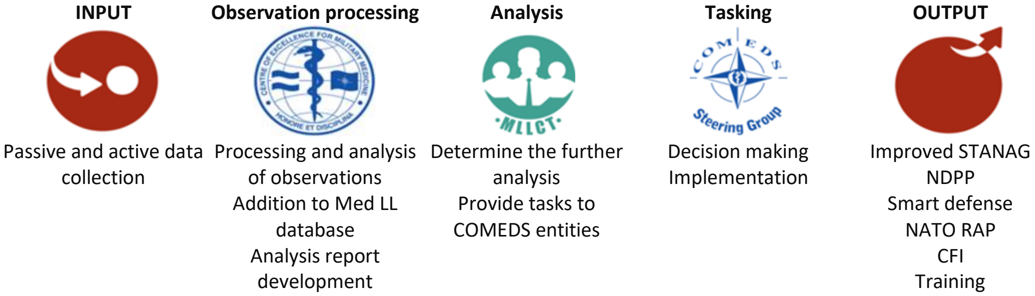

INPUT	Observation processing	Analysis	Tasking	OUTPUT
Passive and active data collection	Processing and analysis of observations Addition to Med LL database Analysis report development	Determine the further analysis Provide tasks to COMEDS entities	Decision making Implementation	Improved STANAG NDPP Smart defense NATO RAP CFI Training

Knowledge and tools of information collection

During the multinational medical exercise Vigorous Warrior 2017 a total of 391 pieces of observations were collected and analyzed. Five focus topics for further analysis were identified: Exercise Development, Interoperability, Patient Evacuation Coordination Cell, Bio-responsiveness and Experimentation [15]. Furthermore, a new tool for collecting and retrieving observations via smartphones was also introduced – the Observation, Discussion, Conclusion, Recommendation (ODCR) application, which was one of the tools for gaining knowledge (also those responsible for collecting knowledge - Office of Primary Responsibility (OPR) LL and paper and electronic forms). This application is fully functional in ANDROID and Apple´s iOS system (free download from Google play and AppStore). The previously mentioned Medical Knowledge Management Portal is a very important tool for preserving and sharing of observations and lessons. Today, there are over 1234 lessons from various areas of military and civilian health care (Exercises, Operations, UN, Ebola and others). Currently, the observations from the latest large-scale international medical exercise, Vigorous Warrior 2019, where 38 NATO and partner countries participated, are being analyzed within these new frameworks. The total number of primary findings is 464, of course, not all of the observations will be uploaded to the KM Portal avoiding repetition, and analysis will follow a standard approach.

DISCUSSION AND CONCLUSION

Evidenced-based medicine drives best practices. Knowledge sharing is an important prerequisite for the functioning and improvement of quality at all levels of healthcare within the deployment of medical units and medical teams across NATO operations. The quality of planning, which often takes place under time constraints and in a hostile environment, is improving and maximizes efforts across the NATO Alliance. The Lessons Learned (LL) process in clinical medicine for deployed and military support services drive the NATO Military Medical Center of Excellence (MILMED COE). This paper describes the Lessons Learned process and its use in NATO and partner countries medical services. Organizational learning drives development and progress and capturing lessons learned improves the quality of care in military medicine on a preventive, epidemiological, trauma, casualty evacuation and forward surgical care basis. The Lessons Learned process is an undertaking and an instrument that can also be used to achieve better international and civil-military cooperation. Lessons Learned process is one of the key elements of developing medical services in line with the changing operating environment.

Acknowledgment
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Conflict of interests
Authors state no conflict of interests.

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ORIGINAL ARTICLES

The trend of bladder cancer among Iranian military community from 2007 to 2019

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Abstract: *Purpose: Cancers are one of the main causes of mortality in the world. The incidence of cancers in the world and mainly in developing countries is increasing. Due to the importance of this issue, the purpose of the current study was to assess the trend of incidence and death of Bladder Cancer among Iranian Military Community (MC) from 2007 to 2019.*

Methods: This is a cross-sectional study using time series data. All registered bladder cancer in Iranian Military community from March 2007 to February 2017 entered in this study. The future trend predicted using ARIMA (p,d,q) model. All analyzes were performed using ITSM and Excel (2010) software.

Results: According to ARIMA (2, 2, 1) model, the bladder cancer among the Iranian military community had an increasing trend. The AR (12, 2, 0) model showed an increasing trend of this cancer among males but it seems the trend of bladder cancer among females will have a constant trend AR (12, 2, 1). The death due to bladder cancer was showed a decreasing trend using AR (14, 2, 1) model.

Conclusion: In spite of decreasing trend in death due to bladder cancer, the trend of incidence of the Bladder cancer among the Iranian Military Community was increasing. However, it is necessary to adjust the effect of many factors that may have an important role in this trend.

Keywords: *bladder cancer, military community, Iran, Time Series Analysis*

INTRODUCTION

Cancers are one of the main public health problems in the world with different pattern in developed and developing countries. Only 5-10% of cancers are related to human genetics and 90-95% of them are due to exposure to environmental factors and lifestyle [1]. Cancer tumors can affect different organs. One of the common organs involved in tumors is the bladder. Bladder cancer is one of the most prevalent cancers in the world. annually there are many people getting this cancer in the world [2]. Smoking and Infection with Schistosoma haematobium are the main risk factors for the disease [3, 4]. The highest and lowest incidence of the disease was observed in Northern Europe and Asia, respectively [5]. In Iran as a developing country in Persian Gulf Region (southwest Asia), an epidemiologic transition is ongoing from communicable to non-communicable diseases, bladder cancer is sixth most common cancer [6] with estimated age-standardized incidence rate of 10.92 and 2.80 in men and women,

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respectively [7]. In spite of a lower incidence of bladder cancer in the Asian region in comparing to western countries, in Iran, the bladder cancer is one of the most prevalent urological malignancy [8]. according to the results of research, some jobs such as Truck drivers, road construction workers, mechanics, petrochemical workers, plastic and metal industries, welding, and pipeline workers are most prone to getting bladder cancer [9]. To the best of our knowledge there is no study about bladder cancer morbidity and mortality trends among Iranian Military community (active, retired, family, veterans) = MC, so this study performed to time series analysis of bladder cancer in Iranian MC. The trend analysis and prediction of future trends can provide very beneficial information for health policymakers and managers to programing and designing prevention programs. Due to importance of problem the purpose of the current paper was to describe the incidence of registered cases of bladder cancer among Iranian MC during the March 2007 to February 2017 and Forecasting its incidence in the coming years using the Iranian Armed Forces Health Insurance database.

MATERIAL AND METHODS

Study Design

In the current cross-sectional study, the required data were extracted from the registered cases in the Iranian Armed Forces Health Insurance. This data was registered according to the time of diagnosed and registered cases.

Inclusion criteria

All registered bladder cancers in the Iranian MC during the March 2007 to February 2017 entered in this study.

Data analysis

Removing non-stationary in data

Due to the non-stationary nature of the used data set, the needed transformation applied to the used time series. To remove variance fluctuation the Box cox transformation was used. In addition, to remove non-stationary in the mean of data set the differencing was used.

Selection of models

The partial autocorrelation function (PACF) and auto-correlation function (ACF) graphs were used to selection of the prediction model. By using the mentioned graphs the parameters of ARIMA models that include Autoregressive (AR) (p) and Moving Average (MA) (q) were determined.to determine the best model of prediction the different parameters of P and q were tested and the model with minimum Akaike Information Criterion (AIC) statistic was selected to the prediction of the future trend.

Assessing the fitness of the selected models

To assess the fitness of selected model the tests of randomness on residuals that include Ljung - Box statistic, McLeod - Li statistic, Turning points, Diff sign points, Rank test statistic, Jarque-Bera test statistic (for normality) also the schematic checking of the residual graph were used.

Prediction

To prediction of future trends, the ARIMA (p, d, q) model with the minimum amount of AIC was used. By considering monthly time intervals, 120 months from 21 March 2007 to 20 February 2017 were entered into the time series model and the prediction was made from March 20, 2017, to 20 August 2019. All analysis was performed using the ITSM (Interactive Time Series Modeling) software and Excel 2010 by considering the α: 0.05 as a significant level.

RESULTS

The total registered cases of Bladder cancer during the March 2007 to February 2017 were 1,640 cases. The mean age of registered cases was 70.31±11.63 years. 1,386(84.51%) of total cases were males and 254(15.49%) of total cases were females. Most of cases have had been registered in March and June respectively (Figure 1).

An increasing trend was predicted by a time series Analysis with autoregressive integrated moving average (ARIMA) model with AR = 2, MA= 1, and Akaike information criterion statistics (AIC) = 263 were used (ARIMA [2, 2, 1]) for the coming years (Figure 2).

In addition, an increasing trend was predicted among males (autoregressive model (AR) model with AR = 12, MA= 0, and Akaike information criterion statistics (AIC) = 271 were used (AR [12, 2, 0]). While a constant trend was predicted among females, for the coming years (Figure 4). For the prediction of the trend of this cancer among females the autoregressive model (AR) model with AR = 12, MA= 1, and Akaike information criterion statistics (AIC) = 281 were used (AR [12, 2, 1]) (Table 1). Regarding the prediction of Bladder cancer Mortality among Iranian Military community, the results of time series analysis showed a decreasing trend for the coming years (Figure 5). For the prediction of the Mortality trend the autoregressive model (AR) model with AR = 14, MA= 1, and Akaike information criterion statistics (AIC) = 449 were used (AR [14, 2, 1]) (Table 1).

Figure 1: The registered cases of bladder cancer among Iranian military community in different month.

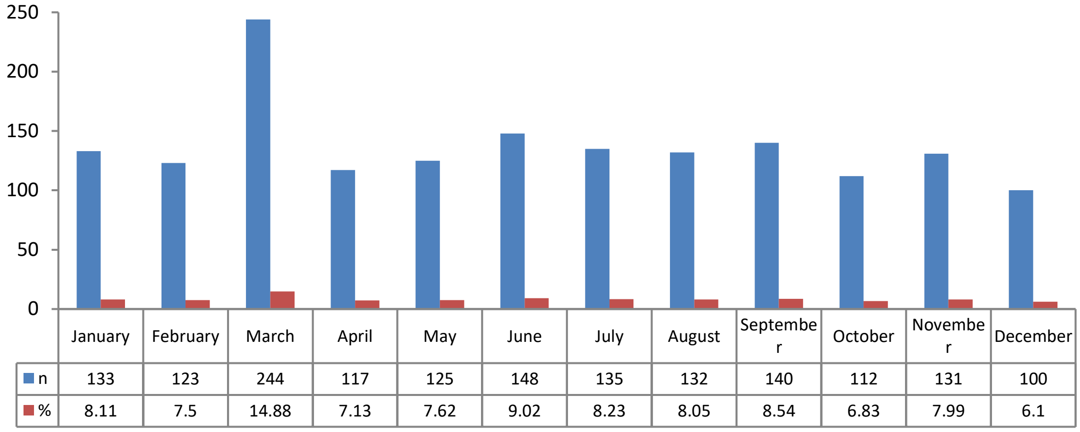

	January	February	March	April	May	June	July	August	September	October	November	December
🟦 n	133	123	244	117	125	148	135	132	140	112	131	100
🟥 %	8.11	7.5	14.88	7.13	7.62	9.02	8.23	8.05	8.54	6.83	7.99	6.1

Figure 2: The trend of bladder cancer and predicted value among Iranian military community during 2007-2019.

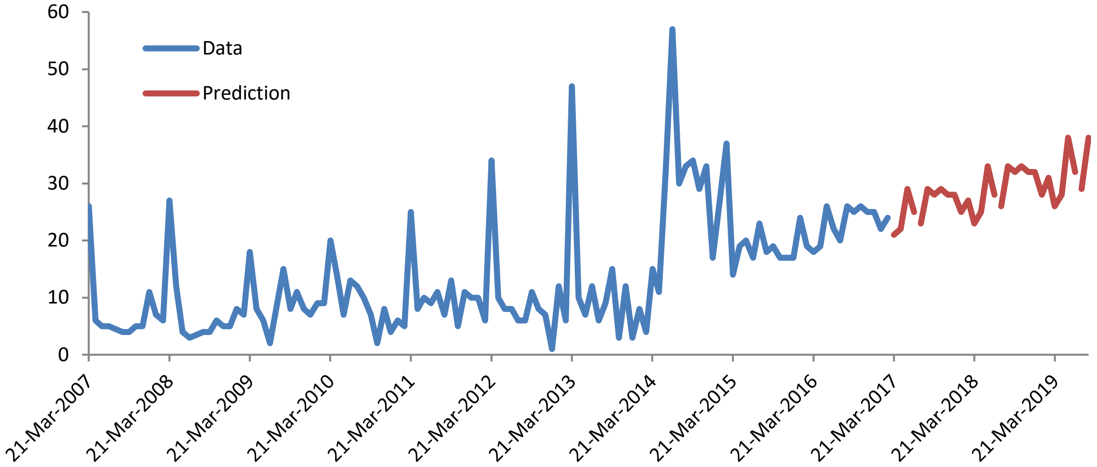

Figure 3: The trend of bladder cancer and predicted value among males of the Iranian military community during 2007-2019.

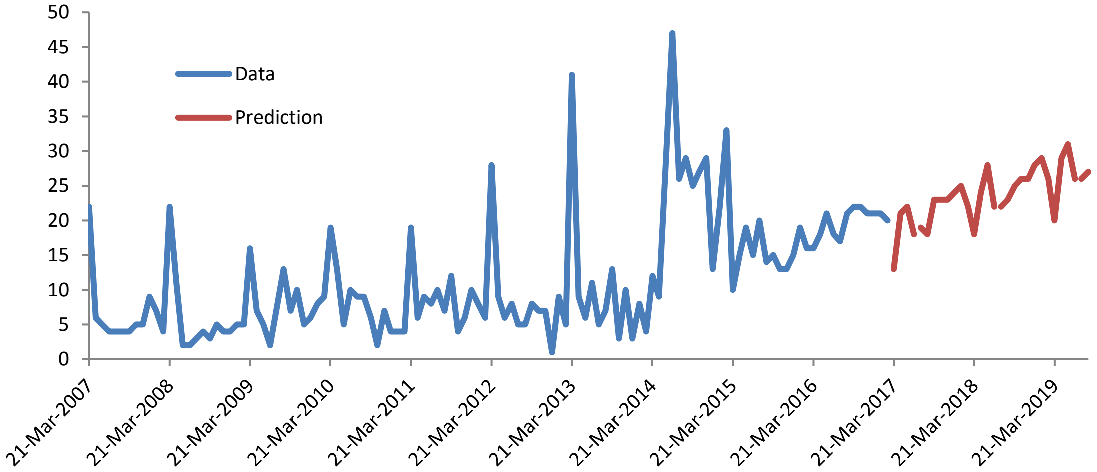

Figure 4: The trend of bladder cancer and predicted value among females of the Iranian military community during 2007-2019.

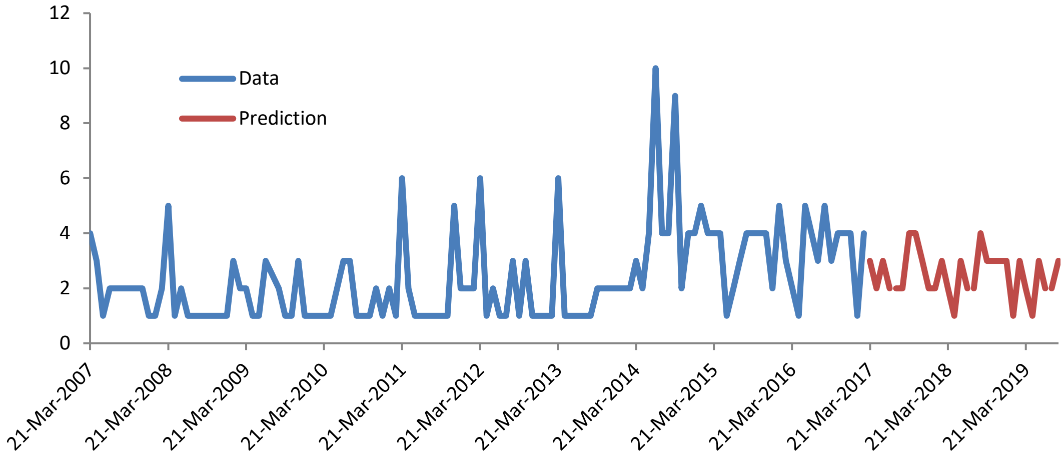

Figure 5: The trend of death due to bladder cancer and predicted value among females of the Iranian military community during 2007-2019.

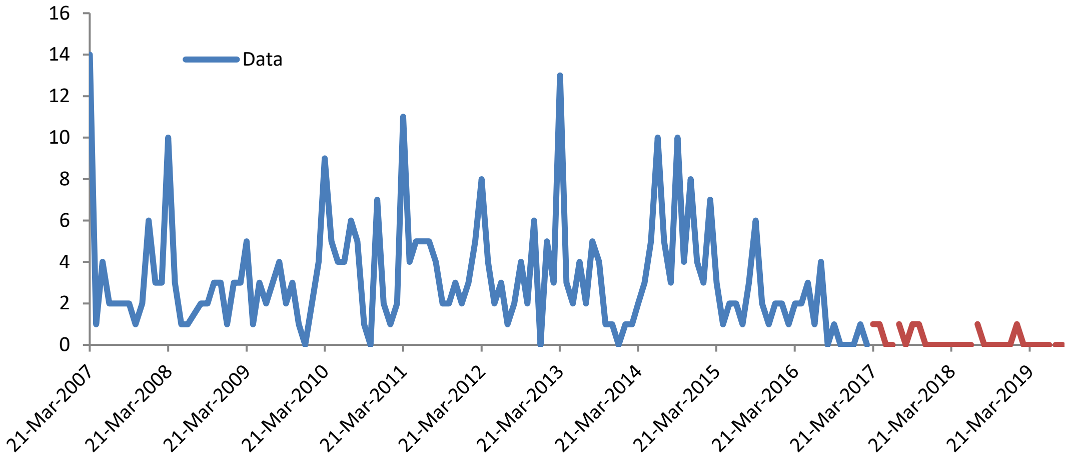

DISCUSSIONS

Cancer is one of the main causes of mortality in human populations. The high incidence of malignancies may be a consequence of some factors such as aging of the population, sedentary life style, lack of a healthy diet, specific occupational exposures, stress etc [10-12]. Age and gender are related to bladder cancer morbidity [13, 14]. The epidemiological pattern of diseases incidence in developing societies is not similar to that of industrialized nations. Therefore, the investigation and monitoring of overall patterns of malignancies is valuable and it could help public health authorities to trace the etiology and evaluate the effects of diagnostic and screening interventions [15]. Accordingly, the purpose of current study was to assess the trend of bladder cancer incidence and it's mortality among Iranian MC. To the best of our knowledge, no study has assessed both the incidence and mortality rates for Bladder cancer in Iranian MC. According to results of the current study, mean age of registered cases was 70.31±11.63 years. A research in Pakistan reported a mean age of 55.5 years [16] and a study conducted in Iran reported it as to 65.1±12.7 years [5], so it seems the risk of bladder cancer increases with increase in age. The global sex (male/female) ratio for the incidence of bladder cancer is 3.3:1.0. However, it has been reported as 1.1:1.0 in Africa and 5.1:1.0 in Europe [17]. In our sample, this ratio (5.5:1) was comparable to the figures reported in previous research in other studies [5, 16, 18]. More exposure to tobacco, opium, and occupational carcinogens in men may account for the difference in the incidence of bladder cancer between men and women [19]. The time series analysis results showed that the incident cases of bladder cancer among Iranian MC have an increasing trend. Maybe it is due to improving cancer registration system. In addition, changing some lifestyle factors, such as obesity and lack of physical activity should be regarded. Other studies also showed an increasing trend of bladder cancer in Iran [9, 20] and also in Egypt [21]. Several studies reported an increase in the overall incidence of bladder cancer [22, 23]. However, the trend of incidence may affect by some risk factors for bladder cancer, including tobacco, non-occupational and industrial carcinogens, and population aging [23]. In comparison future trend between males and females, the incidence of bladder cancer among males had increasing trend but the incidence of this cancer among females showed a constant trend. A study by Antoni et al. [24] has studied the global morbidity and mortality trends of bladder cancer. They reported that the incidence rates were diverging, with stabilizing or declining rates for men but increasing rates for females in many countries.

Table1: The related coefficients for prediction incidence of bladder cancer among Iranian military community

	Overall incidence	Incidence in males	Incidence in females	Total death
BOX COX	100	100	100	700
AR	2	12	12	14
MA	1	0	1	1
AIC	263643E+03	271944E+03	281468E+03	449345+03

Some studies showed that obesity and diabetes mellitus is associated with increased risk of bladder cancer [25-27]. However, the results of a recent systematic review and meta-analysis on Iranian military personnel indicate that the prevalence of overweight and obesity in Iranian military personnel is 41% (95% CI: 26.57%) and 13% (95% CI: 10.17%), respectively [28]. So probably, we will oppose to a higher incidence of bladder cancer in the future.

Our study has some limitations: Firstly, all analyses and predictions were performed based on registered data. Therefore, the quality of the registration system could affect the validity of the prediction. For example, to some extent, the increasing trend of incidence may be due to the improvement of the registration system and not related to the increase in the true incidence of cancer cases.

Second, as in most of epidemiological studies we were unable to distinguish between tumor stages because of the nature of the available data. This could reduce the amount of details that can be found from the analyses.

Third, sociodemographic and economic variances can affect the morbidity and mortality of bladder cancer [29]. So lack of investigation about these underlying factors, in the current study could be considered as a major limitation. For example, we don’t have any information regarding the size of the covered population by insurance organization of the Iranian military community so the increase in the size of that population may leads to an increase in incidence cases.

Finally, known risk factors, such as smoking habits, obesity, and occupational exposure may affect the interpretation of our results.

In addition, the impact of screening programs on registered cases of cancers should be considered. The improving and developing of such programs may help the diagnosis of cancer cases. Further studies are recommended in the future taking into account the mentioned points, in this regard.

CONCLUSIONS

Our study is the first report on trends of incidence and mortality of bladder Cancer in Iranian MC. Based on the results of this study the need for promoting screening programs is emphasized.

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ORIGINAL ARTICLES

Obesity and osteoporotic fractures

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Abstract: *Obesity correlates with a higher risk of fractures in some sites as vertebras, upper arm, and legs. This is a narrative mini-review focused on several key points that link obesity with osteoporosis. Waist circumference may a better predictor of fractures than body mass index (BMI) in obese females. Even obese menopausal women have an extra source of estrogens in fat tissue this actually is not enough to offer a complete protection against osteoporotic fractures. Low levels of testosterone in males induce a higher risk of fall dependent or independent of sarcopenia (which is more evident in elderly). High BMI is correlated with vitamin D deficiency. Obesity is a contributor to muscle damage and decline due to fat accumulation and inefficient fuel utilisation by the muscle, namely sarcopenic obesity, regardless the age. Associated type 2 diabetes mellitus involves bone damage and fracture risk due to glycated proteins of the matrix, the changes of adipokines, increased cortical porosity, high risk of fall because of blood pressure and glycaemia anomalies, visual disturbances, renal and peripheral nerves diabetes-associated conditions etc. The fracture risk is also augmented via fat derived cytokines and chronic inflammation. Even it seems logical that the weight correction though bariatric surgery reduces the fracture risk in obesity, actually data from longitudinal studies pointed that, despite the procedure is life saving, it does not actually protect against osteoporosis which is regarded as a potential long-term complication. A high fracture burden in obesity should increase the level of awareness for practitioners of different medical areas due to multiple levels of complications and to the epidemiological impact of obesity.*

Keywords: *obesity, osteoporosis, fracture*

INTRODUCTION

Obesity currently represents a pandemic issue and every medical and surgical speciality includes different complications of obesity from cardiovascular conditions, type 2 diabetes mellitus and insulin-resistance, some types of cancer to infections at any level including skin, lungs, urinary tract, gastrointestinal infections, respective infertility, sleep apnoea, non-alcoholic fatty liver disease, depression, and finally any of these or their combination may increase the overall mortality. [1, 2, 3, 4] The increasing epidemiological impact has brought a large area of concern worldwide. [1, 2, 3, 4] The endocrine frame of obesity perception is related to hypothyroidism, hypercortisolemia, male hypogonadism, female hyperandrogenemia, and lately to osteoporosis and fragility fractures, including hypovitaminois D. [5] A systematic review associated with a meta-analysis published in 2019 by van Hulsteijn et al. included 68 studies (meaning 19,996 obese subjects). [6] The study identified that 28% of the individuals had reduced thyroid function of different severities, while 0.9% of them had hypercortisolemia. [6] When it comes to males the prevalence of hypogonadism was between 32 and 42%

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depending on criteria. [6] Contrary to historical records that consider obese women protected against osteoporosis because of the estrogens excess at the level of adipose tissue, last two decades brought enough proves that actually this hypothesis is not entirely true. [7]

AIM

Our purpose is to introduce data related to the risk and prevalence of osteoporotic fractures in obese persons.

MATERIAL AND METHOD

This is a narrative mini-review of the literature based on a research focused on several practical key points that correlate obesity with osteoporosis. A number of 69 articles have been included in the selection.

RESULTS – GENERAL DATA

The influence of abdominal obesity versus body mass index

The link between obesity and fractures may be expressed as BMI (Body Mass Index), either as abdominal obesity. A cross-sectional study published in 2019 showed that among 669 elderly women 65 had fragility fractures of different sites, with an average age of 73.9 years. [8] BMI was not associated with fragility fractures but abdominal obesity suggested by a waist of more than 88 centimetres (cm) was, regardless the presence of different clinical risk factors as smoking or age etc. [8] Some authors suggested that actually the waist circumference is a better predictor of fractures than BMI in overweighed and obese females. [8,9] It seems that low body mass index as well as high content of body fat, respective increase BMI both are associated with high fracture risk, especially at vertebral site. [9] Generally the fat content of the body has been reported as an accurate predictor factor for several conditions including cardiovascular and metabolic morbidities. [10] On the other hand, the influence of BMI on osteoporotic fractures risk may include the fact that low and high BMIs are considered at risk for fractures despite Bone Mineral Density (BMD) values which are directly correlated with BMI and it might not reflect the real fracture risk in obese individuals. [11, 12]

Postmenopausal women

The female individuals in menopause suffer of estrogens deficiency related to menopause thus causing bone loss and low trauma fractures. [13, 14, 15] Even obese menopausal women have a second source of estrogens in fat tissue which is superior to normal weighted subjects this actually is not enough to offer a complete protection against osteoporotic fractures. [13, 14, 15] Some studies pointed a similar rate of fractures in menopausal women regardless they were obese or not and others showed a different distribution depending on site, for instance: a high risk is for vertebral fractures, humerus, upper arm, leg and ankle while a low risk has been described for hip and wrist. [13,14,15] This aspects are most probably related to the physics of fall depending on weight. [13, 14, 15]

Male hypogonadism

Even osteoporosis is less frequent in males, the prevalent fractures are found in obese men through associated hypogonadism independent of the BMI values. [16, 17, 18] Low levels of testosterone induce a higher risk of fall dependent or independent of sarcopenia (which is more evident in elderly). [16, 17, 18] Testosterone therapy may reduce the fracture risk. [16, 17, 18]

Hypercortisolemia

Glucocorticoid osteoporosis is a well known cause of obesity-related type of secondary osteoporosis. [19, 20, 21] The obese subjects without Cushing’s syndrome display chronic high values of basal cortisol which seems suppressible at dexamethasone inhibition test. [19,20,21] Chronic autonomous secretion of cortisol is identified in patients with adrenal incidentaloma with an age-dependent incidence and this has been reported to increase the risk of osteoporosis independent of age. [22, 23, 24]

Hypovitaminosis D

The interest in the area of hypovitaminosis D has been explosive during the last decades. A higher BMI is positively correlated with a high risk of vitamin D deficiency based on large epidemiological studies. [25, 26, 27[However, there is still a matter of debate regarding which is the cause and which is the effect. [28, 29, 30] The potential causes of vitamin D lack include: the relative immobility and decreased sun exposure, lower dietary intake, decreased intestinal absorption, high accumulation of vitamin D at the level of adipose tissue since it is a liposoluble vitamin thus a lower bioavailability of the vitamin D. [31, 32, 33] Decreased levels of 25-hydroxivitamin D are contributors to impair muscle resistance and to sarcopenia, thus increasing the risk of fall, and finally the risk of fracture. [34, 35, 36] Also, hypovitaminosis D is a contributor to metabolic disturbances as insulin resistance, hyperlipemia etc. [37, 38] The doses of vitamin D required for supplementation are usually two to three times higher in obese versus non-obese individuals due to fat accumulation. [39, 40]

Sarcopenic obesity

The loss of muscle mass and function is progressive since the age of 40 and accelerated after 60 years. [41, 42] Obesity is a contributor to muscle damage and decline due to fat accumulation and inefficient fuel utilisation by the muscle, namely sarcopenic obesity, regardless the age. [41, 42] The collateral contributors to this pathological status is represented by low 25-hydroxivitamin D levels and the presence of glucose metabolism anomalies. [43, 44] Overall, the weakness of muscles causes a higher risk of fall thus of fractures. [43, 44]

Type 2 diabetes mellitus

The metabolic anomalies are associated as seen in metabolic syndrome where obese individuals are also diabetics. [45, 46] The risk of fracture caused by type 2 diabetes mellitus is more evident in menopausal women. [45, 46] Type 2 diabetes is associates bone damage and high fragility fracture risk due to glycated proteins of the matrix, the changes of adipokines as leptin, increased cortical porosity, increased risk of fall due to blood pressure anomalies, visual disturbances, renal and peripheral nerves diabetes-associated conditions. [47, 48, 49, 50]

Chronic inflammation

The network between fat and bones includes anatomical aspects like high BMI associates high BMI or mechanical loading is protective for fracture, but also changes of the skeleton micro-architecture in obese subjects that differs with the skeleton site thus some types of osteoporotic fractures are more exposed. [51, 52] Moreover, the fracture risk is augmented via fat derived cytokines and chronic inflammation as seen in metabolic syndrome or abdominal adiposity. [51, 52]

Bariatric surgery

Even it seems logical that the weight correction though bariatric surgery might reduce the fracture risk in obese individuals, actually data from longitudinal studies pointed that, despite the procedure is life saving, it does not actually protect against osteoporosis. [53, 54] Why? Because of the changes involving the mechanical loading due to the weight decrease, of lower intestinal surface for nutrients absorption (including potential malabsorption), anomalies of gut-produced calcitropic hormones, and restrictive diets after surgical procedure etc. [55, 56] Even osteoporosis after bariatric surgery seems a small price to pay, the post-operatory fractures are currently regarded as a long term complication on patients of different ages. [57, 58]

Depression

A higher risk of depression has been reported in obesity. [59] This may further involve reduced physical activity, including decreased sun exposure with risk of hypovitaminosis D and other factors leading to higher risk of fall and fractures. [60] Some mediators anomalies (as well as antidepressants-related) including serotonin have been reported at risk of bone loss through peripheral changes of its concentration despite the fact that circulating 5-hydroxytriptamin has not been yet indicated as a useful bone turnover marker in daily practice and conditions with pathological increase of the molecule as neuroendocrine tumours are not conventionally on the list of secondary osteoporosis. [61, 62]

DISCUSSIONS

Fracture risk assessment

DXA (Dual-Energy X-Ray Absorptiometry) does not particularly reflect the fracture risk in obese individuals since high BMI is correlated with high BMD. [63] FRAX also uses as input BMI. [64] Bone turnover markers might be blunted in associated diabetes and dramatically increased after bariatric surgery. [65, 66] Also type 2 diabetic menopausal women may beneficiate from TBS (Trabecular Bone Score) analysis. [67] The assays of 25-hydroxivitamin D might also be useful. [68]

Current limits

Obese individuals are less likely to receive anti-osteoporotic drugs since they already have several non-osteoporotic complications requiring different therapies. [69, 70, 71] Also, if a specific advisable drug in these subjects should be used in order to treat osteoporosis and/or to prevent fractures is not clearly understood up to this moment which is that drug, and probably the therapy for osteoporosis should be done based on similar protocols as in normal weight subjects with osteoporosis. [69, 71] Moreover, higher doses of vitamin D are necessary in obesity. [69, 71] The importance of therapy in order to provide an adequate primary and secondary intervention regarding osteoporosis is essential since the complications of fractures may become more severe due to obesity co-morbidities. [69, 71, 72]

CONCLUSION

A high fracture burden in obesity should increase the level of awareness for practitioners of different medical areas due to multiple levels of complications and to the epidemiological impact of obesity.

Abbreviations:
BMI = Body Mass Index
BMD = Bone Mineral Density
cm = centimetres
DXA = Dual-Energy X-Ray Absorptiometrry
TBS = Trabecular Bone Scor

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ORIGINAL ARTICLES

Evaluation of the expression level of Sine oculis homeobox homolog 1 in cervical cancer tissue in comparison with healthy adjacent tissue

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Abstract: *Background and Aim: This study aimed to investigate the expression level of Sine oculis homeobox homolog 1(SIX1) in normal and cancerous cervical tissues. Methods: Real-time PCR was used to evaluate SIX1 expression level in fresh surgically obtained cervical biopsies. Also, cervical specimens were tested for HPV DNA detection by MY09/11, GP5+/6+ nested PCR method. Results: The expression level of SIX1 mRNA in Cervical cancer was markedly higher than in normal cervical tissue (p<0.0001). Moreover, HPV DNA was detected in 100% of cases and 21.05% of controls (P<0.0001). Conclusions: This finding indicates that increased SIX1 expression might be of relevance to the pathogenesis of cervical cancer. Perhaps, studies to find agents that specifically target SIX1, develop novel anti-cancer and anti-metastasis drugs. This idea has to be investigated in future clinical studies.*

Keywords: *cervical cancer, SIX1, molecular mechanism, pathogenesis, expression level*

INTRODUCTION

Cervical cancer (CC) is the fourth most common cancer in women with worldwide estimated 527,000 cases and 266,000 deaths in 2012 [1, 2]. Persistent infection with high-risk Human papillomaviruses (HPVs) known as the fundamental cause of CC; although, most of the infected women usually recover spontaneously and only a small proportion progress to CC [2, 3]. HPV E6 and E7 oncoproteins not only inactivate the tumor suppressors p53 and Rb respectively but also modulate a plethora of cellular proteins and signalling pathways related to DNA replication, cell survival, apoptosis, anoikis, epithelial to mesenchymal transition (EMT), anchorage-independent growth (AIG) and angiogenesis. Accordingly, alteration of these cellular cascades leads to cell immortalization, transformation and finally occurrence/development of CC and metastasis [4, 5]. Among these cellular factors, Sine oculis homeobox homolog 1 (SIX1) play a crucial role in CC onset and metastasis [6]. SIX1 is one of the important members of SIX family. This family, including a series of transcription factors, are homolog of the Drosophila sine oculis (so) gene and have a total of six members (SIX1 to SIX6) in vertebrates. Among them, SIX1 has attracted the attention of many researchers recently. SIX1 gene is located on human chromosome 14q23 and its structure composed of two conserved domains, the homeodomain (HD) and the SIX domains (SD). Many studies have shown that SIX1 is essential for the development of

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various human organs in physiological conditions and overexpressed in multiple human cancers, also associated with metastatic phenotype [7, 8]. It is indicated that SIX1 stimulates DNA replication, cell proliferation and maintains survival conditions in CC cells. In the other hand, HPV E7 oncoprotein can induce SIX1 overexpression. Consequently, HPV can obtain opportunity of effectively persistent infection in cervical cells and develop CC [9, 10]. Advanced stages of CC have not effective treatments and most of patients with metastatic CC die. Thus, the better understanding of HPV-induced CC molecular mechanism is indispensable and can assist to introduce more effective novel therapeutic approaches for HPV-induced CC prevention and treatment. In the present study, we evaluate the expression level of SIX1 mRNA by real-time PCR in CC biopsies in comparison with healthy adjacent tissues.

MATERIAL AND METHOD

2.1. Specimens

A total of 38 histopathologically confirmed fresh cervical biopsies consist of 19 cancer samples and 19 normal samples were surgically collected from the Department of gynecology at Imam Khomeini hospital in Tehran, Iran. Normal subjects were women referring to gynecology department of hospital for any cause (for example ovarian cancer), except for cervical involvement. No subjects received preoperative radiotherapy, chemotherapy, or other treatment. The study was approved by the medical ethics committee from Tehran University of Medical Sciences and written informed consent was obtained from all patients before enrollment (approval number 1395.1301).

2.2. DNA Extraction

DNA was extracted from biopsies by the High Pure Viral Nucleic Acid Kit (Roche, Mannheim, Germany) according to manufacturer's instructions. A Nanodrop spectrophotometer (Nanodrop Technologies) was used to determine DNA concentration and purity.

2.3. PCR Analysis

First, we confirmed the integrity of extracted DNA by amplification of a housekeeping gene β-globin (act as an internal control) using PCO3/PCO4 primers. The reaction conditions were as follows: 40 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min and a final extension at 72 °C for 7 min. Then, the presence of HPV DNA was examined by nested PCR assay in all of the positive specimens for the β-globin gene. For this purpose, we used MY09/11 outer followed by GP5+/6+ inner primers (are located within the L1 region of the HPV genome. The Sequences (5 to 3) of all PCR primers are listed in table 1. The temperature cycling program in the first round (MY09/11) and second round (GP5+/6+) PCR was identical as follows: 94°C for 4 min, 40 cycles of 94°C for 1 min, 42°C for 1 min, 72°C for 30 s and a final extension at 72 °C for 4 min [11].

2.4. RNA Extraction

The total RNA from tissue specimens was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted RNA was eluted in 50 µl Diethylpyrocarbonate (DEPC) water. The concentration and purity of RNA were determined by Nanodrop spectrophotometer (Nanodrop Technologies). In order to the prevention of genomic DNA contamination, RNA samples were treated with RNase-free DNase I (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions.

2.5. Real-Time PCR

One microgram of RNA samples was reverse transcribed to cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) following the kit’s instructions. Real-time PCR reactions were carried out in duplicate with Applied Biosystems 7500 Real-Time PCR system using SYBRs Premix ExTapTM II (Takara, Japan) and specific primers that listed in Table 1 [12]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was applied as an internal amplification control. The 2−ΔΔCT method was used for relative quantification of SIX1 expression levels and results were normalized to GAPDH, as a housekeeping gene.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Variables were compared between groups using the Fisher's exact test or student's t-test as appropriate. Numerical results are expressed as the mean ± SEM. The p-values of <0.05 were considered statistically significant.

RESULTS

3.1. Demographic characteristics

The mean age of the women in the CC group and control group was 61.3 ± 10.2 and 36.0 ± 8.3 years, respectively. Based on statistical analysis, the mean age in the CC subjects was significantly higher than the control group (p-value <0.0001).

Table 1: Sequences of primers used for real-time PCR reactions in current study

Primers	Sequence (5′–3′)
β-globin	PCO3 (Forward) ACACAACTGTGTTCACTAGC PCO4 (Reverse) CAACTTCATCCACGTTCACC
HPV	MY09 (Forward) CGTCC(A/C)A(A/G)(A/G)GGA(A/T)ACTGATC MY11 (Reverse) GC(A/C)CAGGG(A/T)CTATAA(C/T)AATGG GP5+ (Forward) TTTGTTACTGTGGTAGATACTAC GP6+ (Reverse) AAAAATAAACTGTAAATCATATTC
GAPDH	(Forward) GGAGCGAGATCCCTCCAAAAT (Reverse) GGCTGTTGTCATACTTCTCATGG
*SIX*1	(Forward) AAGGAGAAGTCGAGGGGTGT (Reverse) TGCTTGTTGGAGGAGGAGTT

HPV, Human papilloma virus; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; SIX1, Sine oculis homeobox homolog 1

3.2. Prevalence and type of HPV infection

HPV DNA was detected in 100% (19/19) of the cancer cases and 21.1% (4/19) of normal cervical specimens (Table 2). There was a significant difference between the two studied groups (p<0.0001). After sequencing analysis of HPV-positive specimens, we identified HPV-16 in all of the CC samples. Also genotyping of 4 HPV-positive specimens in normal group was HPV-16: 2 cases, HPV-66 and HPV-68: each one was one case (Table 2).

Table 2: Demographic and clinico-pathological characteristics in patients with cervical cancer and without cervical cancer

Variables	Cases (n = 19)	Controls (n = 19)	p-value
Age (mean years)	61.3 ± 10.2	36.0 ± 8.3	<0.0001, t-test
HPV positivity	19 (100%)	4 (21.1%)	<0.0001, Fisher'sisher's exact test
HPV type	HPV-16 = 19	HPV-16 = 2, HPV-66 = 1, HPV-68 = 1	

HPV, Human papilloma virus

3.3. SIX1 mRNA expression

Relative real-time PCR results showed that the mean SIX1 mRNA expression in the CC patients was 2.61 ± 0.09 and in the normal subjects was 1.12 ± 0.03 (Table 3).

Table 3: Expression level of SIX1 in patients with cervical cancer and without cervical cancer

Gene		Cases	Controls
*SIX*1	Mean ± SE	2.61 ± 0.09	1.12 ± 0.03
	CI	2.42 - 2.79	1.06 - 1.18
	Minimum expression	1.94	0.92
	Maximum expression	3.47	1.39
	p-value	<0.0001 t-test	

SIX1, Sine oculis homeobox homolog 1; SE, standard error; CI, confidence interval

Then, statistical analysis revealed that SIX1 mRNA levels were significantly (p<0.0001) higher in CC tissue compared to the normal cervical tissue (Figure 1).

Figure 1: The expression level of SIX1 in CC and normal cervical group. Data are presented as mean ± SEM and the fold increase of transcripts for SIX1 mRNA normalized by GAPDH housekeeping gene. ****p<0.0001.

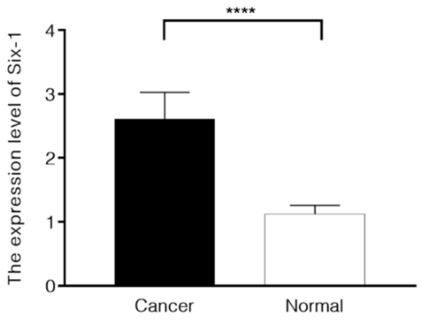

DISCUSSION

The sine oculis homeodomain (SIX) proteins are a family of evolutionarily conserved transcription factors that play a pivotal role in both embryogenesis and carcinogenesis. The homeoprotein transcription factor SIX1 is one of the members of this family that has attracted much attention from the scientific community in recent years [13]. Based on the previous studies, SIX1 upregulated in several human cancers such as breast, ovarian, cervical, liver, rhabdomyosarcoma and Wilms' tumor, also contribute to initiation and metastatic development of these malignancies. Researches have also observed the SIX1 overexpression strongly correlated with poor prognosis and shortened overall survival in cancer patients [14-16]. Consistent with previous studies, we demonstrated the SIX1 mRNA levels is significantly higher in CC than normal cervical tissues. The results of previous studies showed that SIX1 can drive cancer stem cell phenotype and EMT, also lymphangiogenesis, consequently metastasis via stimulation of the TGF-β signaling pathway and VEGF-C, respectively [17-19]. EYA2 act as a coactivator for SIX1, thus mediate its transcriptional activation. Previously it has been shown that inhibition of SIX1-EYA2 complex formation, may be a promising and more effective approach for treatment of cancer and tumor metastasis [20]. In CC cells, SIX1 act as principal regulator of DNA replication, as well as a key factor in metastasis process. Therefore, SIX1 upregulation can lead to malignant proliferation of cervical cells, also metastasis through stimulation of DNA replication-related genes and a5b1 integrin, respectively [10, 21]. Interestingly, high-risk HPV employ various mechanisms to promote metastasis in CC [4]. Anoikis, meaning homelessness, is a detachment-induced cell death. Anoikis resistance, EMT, AIG, and angiogenesis are important cellular events that closely associate with cancer metastasis [22]. Hr-HPV E7 oncoprotein can stimulate SIX1 overexpression in CC. SIX1 consider as an EMT-activating factor, thus its upregulation result in EMT through increasing of ZEB1 and decreasing of E-cadherin. Indeed, SIX1-induced EMT, increase cell survival in detached cancerous cells and drive anoikis overcoming and finally, metastatic dissemination [9]. In the present study, among the total of 38 fresh surgically obtained cervical biopsies, 100% (19/19) and 21.1% (4/19) were positive for HPV DNA, in CC and normal adjacent groups, respectively. Then, sequencing analysis revealed all of HPV-positive specimens in CC group were HPV type 16 that highlighted the indispensable role of HPV-16 in CC onset and progression. The previous studies demonstrated the high risk HPV-16 and HPV-18 account for about 70% of CCs [23]. In current study, four HPV-positive specimens in normal cervical group were: two samples of HPV-16 and one sample of HPV-66 and HPV-68. Based on international agency for research on cancer (IARC) classification, HPV-66 belongs to 2B category (probably carcinogenic). Also, HPV-68 belongs to 2A category (possibly carcinogenic) [24]. Our outcomes corroborated previous reports specifying that the SIX1 involved in the tumorigenesis of cervical cancer [13, 25]. The main limitation of our work was low sample size. In conclusion, SIX1 upregulated significantly in CC, which in turn induces TGF-β, VEGF-C and ZEB1 that collectively can have an association in the tumorigenesis and metastasis of CC.

CONCLUSIONS

We demonstrated SIX1 expression levels was frequently expressed in CC tissues. Also, HPV DNA was detected in 100% (19/19) of the cancer cases and 21.1% (4/19) of normal cervical specimens. HPV can upregulate SIX1 in CC. In addition, SIX1 can contribute to the development of CC and lymph node metastasis of cervical tumors. Nevertheless, the precise biologic role of SIX1 in CC is unclear yet and future works should study on the larger number of patients.

List of abreviation:

CC, Cervical cancer; HPV, Human papillomavirus; SIX1, Sine oculis homeobox homolog 1; EMT, Epithelial to mesenchymal transition; AIG, Anchorage independent growth; so, Drosophila sine oculis; HD, Homeodomain; SD, SIX domains; DEPC, Diethylpyrocarbonate; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; IARC, International agency for research on cancer.

Contribution Note:

EK, EF, MFP, TMA designed the study, EK, EF, MFP performed laboratory works, MFP, MF, MY statically analyzed the data, EK, EF, MFP, MF, MY, TMA wrote and revised the manuscript.

All authors read and approved the final manuscript.

Patient participation at the study respects ethical standards in medical research and GDPR.

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ORIGINAL ARTICLES

The strategic need for the implementation of a technological platform for the microproduction of antidotes for the CBRN medical protection

Viorel Ordeanu[1,2], Adrian A. Andrieș[2], Lucia E. Ionescu[1], Marius Necșulescu[1], Diana M. Popescu[1]

Abstract: *The "volatile" international context requires the strengthening of the national security measures, including in the field of CBRN and public health. The medical countermeasures for the defense are based ultimately on the prophylactic treatment (pre-exposure, intra-exposure and post-exposure) and on the curative treatment (of emergency, maintenance and recovery) of the contaminated and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is immediately applied. Although in times of peace this scenario is almost non-existent, during war or terrorist attack it can be a mass pathology, like an explosive epidemic, that requires specific therapeutic means.*

Military medicine had and has the conceptual capacity to make antidotes for CBRN medical protection; some have been patented and/or produced as experimental models, but it has no manufacturing capacity anymore.

Keywords: *medical protection, antidote, microproduction, technological platform, medical countermeasures, pharmaceutical technique, orphan drug production*

INTRODUCTION

The international context following the conclusion of the "Cold War" paradoxically requires the strengthening of national security measures, including in the field of CBRN and public health. The international context shows that although the risk of biological warfare has been reduced following the Geneva Convention (Biological and Toxins Wapons Convention – BTWC 1972), signed and ratified by about 90% of the world's countries, the risk of bioterrorism is real, as is the risk of pandemics, zooanthroponosis etc.

The World Health Organization (WHO), the European Union (EU) and the North Atlantic Treaty Organization (NATO) recommend concrete measures to reduce these risks and to increase the responsiveness of each country, of the alliance and of the international community. Therefore, in this context, it is absolutely necessary to implement cutting edge technologies for diagnostic and prophylactic treatment, taking into account the mechanism of action in infectious diseases, the therapeutic means of counteracting the effects of biological agents and the epidemiological surveillance.

The existing international concerns force us to align ourselves with the situation existing worldwide. Designing and equipping such platforms according to international recommendations, determines the level of performance of such infrastructure to contribute to the worldwide effort of medical protection against biological agents. The present study was performed at the request of the Medical Directorate, following the request of the Control Body of the National Ministry of Defence.

MEDICAL COUNTERMEASURES

The medical countermeasures for CBRN defense are based ultimately on the prophylactic treatment (pre-exposure,

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intra-exposure and post-exposure to the attack with pathogens) and on the curative treatment (emergency, maintenance and recovery) of the contaminated and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is applied immediately. In times of peace this case is almost non-existent; however, during war or terrorist attack it can be a mass pathology, like an explosive epidemic. that requires specific therapeutic means. Medical intervention requires the same strengths and the medical means all at the same time, so medical aid becomes inefficient in quantity. Moreover, the necessary drugs, in principle the specific antidotes, are orphan drugs, being little used because they are addressed to very rare diseases, and therefore they are manufactured in very small quantities and are not available when needed for urgent treatment for a large number of patients.

The medical protection against the effects of the weapons of mass destruction: chemical, biological, radiological and nuclear (CBRN WMD) is based on fundamental and applied scientific research (CDI) and later on its application in practice.

Critical situations caused by CBRN weapons/agencies are major medical emergencies and can be caused by CBRN WMD attacks, terrorism or criminal acts. The medical system must immediately have at its disposal the necessary force and means of special facilities and appropriate procedures, because "later it may be too late".

Antidotes are specific drugs for the emergency, prophylactic and curative treatment of diseases caused by physical, chemical or biological harmful factors, including CBRN agents, pollutants, etc. There is a risk of using a large variety of WMD agents: chemical warfare agents (CWAs), biological warfare agents (BWAs), radiological warfare agents (RWA), industrial toxicants, etc. which cause a wide range of diseases. As a result, many types of antidotes are needed, permanently available in stock. These must continuously exist, in appropriate forms and in reasonable quantities, for the emergency treatment of the contaminated, the suspected and the sick among the military and civilians, and possibly for the treatment of service animals, etc. In this category can be included decontaminants for internal and external use for humans and animals, in the form of solutions, powders, etc.

In medical practice, antidotes, in a broad sense, can be classified according to several criteria. WHO 20 list of essential medicines, 2017 edition, includes at chap. 4. Antidotes and orher substances used in poisonings, only 15 pharmaceuticals with antitoxic effect, but without taking into account the other CBRN agents. Antidotes can be classified pharmacologically, depending on the agent for which they are used, hence etiological. Antidotes can also be classified according to clinical use: preventive (eg protectors, vaccines), post-exposure prophylaxis (eg atropine, specific antibiotherapy, decontaminants for the skin, mucous membranes, eyes etc.), or therapeutic (e.g. decorporators, chelators, therapeutic sera). From a pharmaceutical point of view, there are antidotes for external use, including hygiene-cosmetics (solutions, lotions, pastes, ointments, powders, etc.), antidotes for internal use (buccal solutions, powders, granules, tablets, dredges, capsules etc.) used preventively, post-exposure or therapeutically, as the case may be. Injectable antidotes (specific vials and devices, self-injecting syringe, auto-injector) and infusions represent an important category in the medical-military arsenal of countermeasures against CBRN agents.

Like any other drug, antidotes can be prepared industrially, galenically or masterfully, from natural and/or artificial pharmaceuticals. These can be of mineral origin (eg sodium sulfate), vegetable (eg charcoal, atropine), animal (anti-venom), microorganisms (eg vaccines, immuno-modulators). Depending on the context, they can be used for prophylaxis (eg pyridostigmine bromide, vaccines), for post-exposure prophylaxis (eg decontaminants for skin and mucous membranes, atropine, anatoxins, antibiotics), curative (eg EDTA, therapeutic sera, antimicrobial chemotherapics).

Antidotes must meet a number of basic conditions required by the Medicines Law: proven therapeutic effect, undesirable side effects as less as possible, preparation in appropriate conditions, reasonable cost price, the possibility of being produced in the required quantities and to be stored for a certain time period, as well as being distributed in a timely manner for emergency treatment. However, an issue arises concerning the cost/efficiency: if within the antidote’s validity period there are no cases or there are very few (desirable situation), the remaining antidote must be discarded at the expiration of the term of validity. These stocks cannot be refreshed by "being put in use" in the sanitary units, because under normal conditions they are of no use except to a very small extent. As European Medicines Law no longer allows re-endorsement after expiry, at most, the antidotes that correspond to the pharmaceutical and pharmacological control may be used only for possible veterinary use, if applicable. In the event the numbers of cases require a certain antidote exceeding the existing stock, the product will have to be prepared quickly, according to a product portfolio located at the manufacturer. If there is no authorized manufacturer, a call can be made for emergency imports or international aid. In fact, the EU Charter also provides for a solidarity clause, whereby European countries are obliged to help each other in case of emergency, but it is obvious that this can be done only after covering their own needs.

In practice, it is difficult to find a pharmaceutical industry producer (one of the most profitable in the world) to manufacture the full range of antidotes, because the orders are small, the products are "niche", and the prices are monitored. This is because antidotes are orphan drugs, being used only for rare diseases, so the demand is so low that their manufacture would mean a lot of work for little money, and would not be cost effective. It turns out that a private producer could not be forced to manufacture them in normal circumstances.

The production of specific antidotes for CBRN medical protection can be achieved only in conjunction with:

1) the medical research's need to physically manufacture the newly designed products (medicines, diagnostic kits, kits and medical devices), at the level of experimental model (EM), functional experimental model (FEM), prototype and/or technological demonstrator, for endorsement, receipt and possibly patenting (as an invention or industrial model), with

2) the need of the industry to carry out the 0 series, the microproduction and the batch series production for clinical testing and for obtaining the marketing authorization (MA), so the principle of integration must apply: research, education and production.

This integrative principle promoting success in the development of specific antidotes, was introduced in Romanian medicine by Prof. Ioan Cantacuzino, since 1901, (Biography Cantacuzino, Ciuca) being applied at the Institute that bears his name, but was subsequently abandoned under unjustified circumstances. Obviously, nowadays, such a dedicated objective involves a pharmacological and pharmaceutical collaboration in the form of a technological platform for the production of antidotes for medical protection, whose components (facilities and utilities) include a Pharmaceutical Laboratory for CBRN antidotes and that must comply with the recommendations of the ANMDM (National Agency for Medicines and Medical Devices) and implicitly with those of the WHO.

State-of-the-art for the production of antidotes for CBRN medical protection is different from one country to another. Civil drug manufacturers (the pharmaceutical industry) generally avoid manufacturing antidotes, as these are "orphan drugs", meaning that they are rarely used under normal conditions, so it would be a difficult commodity to sell. State producers can be forced, through the Production Plan, to manufacture them, but in Romania there is no longer the case, as a result of privatization, and private producers risk financial losses or even bankruptcy, if they manufacture without and end profit.

The beneficiaries and the producers avoid ordering or storing antidotes, because they can remain unused until the expiration date and the prolongation of the duration is not allowed even if they maintain their therapeutic effect, so the medicines are canceled and financial problems arise. As a result, CBRN-specific antidotes do not appear as such in the List of Essential Medicines (WHO Model List of Essential Medicines, edition 21, WHO 2019).

The solution would consist in the specific pharmaceutical manufacture in military or militarized facilities, according to the needs of the national military forces, of the population exposed to specific risk and possibly for other allied armies or for the population in general.

PREVIOUS EXPERIENCE

In Romania there was the Pharmaceutical Production Laboratory of the Medical Directorate belonging to the Ministry of National Defence, which operated without interruption for almost a century, near the Sanitary Warehouse. It prepared specific usual medicines for the military staff, which were distributed free of charge to military hospitals and unit nurses. Different products were manufactured, depending on the needs, as solid forms (tablets, dragees, suppositories, spark plugs, powders), liquid forms (solutions, lotions), ointments, etc. But the injectable, infusable forms and the eye drops, which must be sterile, were prepared only in the pharmaceutical laboratories of the military hospitals. These drugs, along with others, manufactured in the country or imported, have been used to treat the military during peace and war: the Balkan Wars, the First World War, the Second World War, the Revolution, etc. After Romania's entry into NATO and the EU, this laboratory was closed by the pharmaceutical micro-production and moved to another headquarters because it did not comply with the new European norms for the pharmaceutical industry. At the entry into force of the Medicines Law, it was closed down and the machines, inventory objects and consumables (pharmaceuticals and chemicals) were redistributed, some of them ending up at CMMSR for scientific research. They no longer meet the conditions required for the pharmaceutical industry, can no longer be used for the initial purpose and cannot be exploited.

The laboratories of The Center for Military-Medical Scientific Research (CMMSR) have experimentally produced various specific drugs, including antidotes, some of which have been used for the troops in peace or war time. These include: Dostirav (Pyridostigmine) and other CBRN protective equipment provided, including in the Iraq war, 1991; the cyanide antidote that, although patented, is not manufactured by the pharmaceutical industry and the tragic consequences could be seen in the tragic accident which took place at “Colectiv” etc.

The applied research, through the module "Scientific and technological research on the production of antidotes and products with special purpose", has completed technological researches on the manufacture of protective and decomposing antidotes under conditions of peace and in critical conditions (campaign, natural and technological disasters) and, since 2002, ended by:

- establishing the technologies for industrial production and by micro-production under critical conditions for oral preventive antidotes (eg Dostirav/30 mg);

- establishing technologies for industrial production and by micro-production under critical conditions for decontaminants for skin and the mucous membranes (eg DCPM);

- establishing the structure and operating conditions of certain microproduction lines under critical conditions for antidotes;

- research on the synthesis of some organic derivatives of obidoxime with improved pharmacokinetic properties, etc.

These research projects were approved and accepted, the microproduction was performed at galenic level, and the financing of a Pharmaceutical Micro-production Laboratory for antidotes was approved. However, the necessary funds were not allocated and the objective was not achieved. This problem must be resumed in the current political-military context and updated according to the legislation in force. We present below, as an example, some products resulting from the CMMSR’s activity, such as Patents of invention/innovation and/or experimental/galenic models (Table 1). To these can be added numerous sets and diagnostic and treatment kits for medical intervention in CBRN events, some of which have been presented at military exhibitions: ACTTM Clinceni, EXPOMIL Bucharest, BSDA, Inventions fairs, etc. and have been used in applications and theaters of operations (Saudi Arabia, Somalia, Angola, Iraq, etc.).

Regarding orphan drugs, which are very rarely needed, the cost of making and placing a medicinal product on the market for the diagnosis, prevention or treatment of the disease, cannot be recovered through the expected sales of the medicinal product, and the pharmaceutical industry would be refractory to its realization, under normal market conditions. The antidotes for medical protection in case of CBRN attack, terrorism, catastrophe or industrial accident are also considered orphan drugs.

Another aspect of these medicines is represented by the expired drugs. The supplier guarantees the efficacy of the drug only within the deadline, so an orphan drug may expire before it is needed. This can create a financial problem that is difficult to solve. The US Army is currently studying the possibility of extending the shelf life of medicines from stocks, if they have retained their pharmacological properties (internet).

The operating costs differ depending on the chosen variant, the assortments and the quantities of antidotes manufactured, being able to reach the order of millions of euros, depending on the spaces, equipment, personnel and portfolio of pharmaceutical products.

Implementation Opportunities. Providing antidotes is mandatory in medical practice and especially in military medicine. Antidote insurance is nationally deficient and globally they are considered "orphan drugs".

A compromise formula, adapted to the current context in Romania, could be the strategic cooperation regarding fundamental, applied, developmental, innovation scientific research integrated with the production and education of the profile. Thus, the Ministry of National Defence, through virtual reorganization can achieve an Integrated Technological Platform, by sharing the scientific basis and launching the production of antidotes for public health needs and for CBRN crisis situations.

To this would contribute first of all the Military Medical Research Center (CCSMM), the “Cantacuzino” National Military-Medical Institute for Research Development (INCDMM Cantacuzino), the CBRN Defense and Ecology Scientific Research Center (CCSACBRNE), as well as other competent military entities and civilians, from the academic, university, industrial health field, etc. In order to launch the specific antidote micro-production or production, it may be necessary to build or adapt an appropriate facility (pharmaceutical laboratory or drug factory). As a back-up formula, in case of force majeure, a virtual "ad hoc" structure can be created, which will integrate different structures and microstructures existing in Ministry of National Defence, to achieve this common purpose.

Table 1: Examples of CBRN medical protection products resulting from the activity conducted by the Military Medical Research Center

No	Product	
1	Medicinal product with effective action against organophosphorous compounds	Patent State Office for Inventions and Trademarks 79914/1974 V. A. Voicu, C. Miu, M. Jiquidi, C. Furnica, I. Elian
2	Dixidextracaine 70, a drug intended for subarachnoid hyperbaric anestesia	Patent State Office for Inventions and Trademarks 81100/1975 I. Curelaru, C. Furnica, V. A. Voicu, E. Ionescu-Visan, C. Miu, P. Nitescu, L. Ivanescu, I. Manolache
3	Pharmaceutical combinations with antiseptic effects	Patent State Office for Inventions and Trademarks 65237/1977 Al. Bors, V.A.Voicu, C. Rizescu
4	Dynamic repolarization solution	Patent State Office for Inventions and Trademarks 65235/1977 Al. Bors, V .A. Voicu
5	Anti-arrhythmic compound	Patent State Office for Inventions and Trademarks 65236/1977 Al. Bors, V .A. Voicu
6	Equipement for objectifying drug interactions	Patent State Office for Inventions and Trademarks 74803/1980 Al. Ionescu-Stoian, V. A. Voicu, M. Jiquidi
7	Anti-arrhythmic compound and synthesis process	Patent State Office for Inventions and Trademarks 69027/1978 Al. Bors, V. A. Voicu, V. Aldea, E. Olteanu
8	Radioprotective compound	Patent State Office for Inventions and Trademarks 3198/1970 Al. Bors, V. A. Voicu, C. Paun, Gh. Dumitrescu
9	New anti-hypertensive compund	Patent State Office for Inventions and Trademarks 76911/1980 V. A. Voicu, St. Muresan, Gh. Radu, M. Jiquidi
10	New anti-hypertensive compund	Patent State Office for Inventions and Trademarks 76912/1980 V. A. Voicu, St. Muresan, Gh. Radu, M. Jiquidi
11	New antiseptic compound	Patent State Office for Inventions and Trademarks 80051/ 1979 Al. Bors, V. A. Voicu, A. Karassi
12	Anticholinergic compound	Patent State Office for Inventions and Trademarks 87010/1985 V. A. Voicu, Al. Ionescu-Stoian, L. Antoniu, M. Jiquidi
13	Herbal compound with anti-inflammatory, analgesic and vasoprotective action	Patent State Office for Inventions and Trademarks 87147/1985 Al. Ionescu-Stoian, L. Antoniu, V. A. Voicu, V. Florea
14	Complex antidote for the absorption, neutralization and inactivation of ingested toxins	Patent State Office for Inventions and Trademarks 99921/1989 V. Mihaila, V. A. Voicu
15	Medicine with anti-inflammtory, analgesic and decongestant effect	Patent State Office for Inventions and Trademarks 102954/1991 V. A. Voicu, C. Mircioiu, Al. Ionescu-Stoian, M. Jiquidi, E. Rolea
16	Collagen bandage for the treatment of burns	Patent State Office for Inventions and Trademarks 103550/1991 V. A. Voicu, V. Trandafir, T. Giurgiu, T. Safta, D. Burlacu, A. Nica, L. Dragoescu
17	Medicine with anti-inflammtory, analgesic and decongestant effect	Patent State Office for Inventions and Trademarks 106333/1996 V. A. Voicu, M. Jiquidi, C. Mircioiu
18	Method and compounds for the simultaneous determination of acetyl-cholinesterase and pseudo-colinesterase in blood	Patent 67856/19.10.1976
19	Versatile antidote against chemical agents, with effect on skin lesions and mucous membranes	Patent 7959/23.06.1978
20	Device for measuring the caloric response	
21	Protective mixture for impregnating fabrics	
22	Device for measuring contamination with radioactive isotopes of the human body	
23	Device for rapid desiccation of biological or chemo-pharmaceutical compounds	
24	Medicinal product with specific anti-stress, anti-wear synergistics, polyvalent, neuropsycholobiological revitalization effects	Invention Patent No. 87905/2005
25	Immuno-enzymatic test for the detection of microorganisms	Patent 91566/1984/09.01.1987
26	Enzyme conjugation process	Patent 90105/1984
27	Herbal compound with anti-inflammatory, analgesic and vascular-protective effect	Patent 9992/30.10.1989
28	Collagen bandage for skin lessions	Patent 103550/20.02.1989

Military medicine has had and has the conceptual capacity to make antidotes for CBRN medical protection, some of which have been patented, but it does not have the production capacity. It is possible to arrange or build a self-production capacity for specific antidotes, as orphan drugs, on constructive variants, depending on complexity. The total costs are important, but the objective can be amortized in a short time if the supplier has a regional opening and the social benefit is very high.

The problem of self-injection syringes

We consider that the most important and stringent problem is to equip the active and the non-commissioned military staff and the risk group population, with self-injection syringes (over 1 million people in Romania), according to NATO norms (STANAG 2871 CBRN MED, 3-6 syringes per person, plus reserves); therefore, a total of over 3-6 million self-injection syringes.

The issue of providing CBRN antidotes in Romania is not solved by NATO standards. For example, the RDG model self-injection syringes, made of stainless steel, that were in the Romanian Army's endowment, were withdrawn in the 1980s and have not been replaced until now. The Romanian model made of plastics (made by the Center for Military-Medical Scientific Research as an experimental model, a functional experimental model, a prototype and a zero series) was no longer manufactured in series due to the privatization of the manufacturer (the Plastics Factory Bucharest). At present, the Center for Military-Medical Scientific Research has developed a prototype of self-injecting syringe for antidotes, with the intention to carry out a technological transfer through projects funded by the Ministry of Research and innovation.

THE PHARMACEUTICAL LABORATORY

The pharmaceutical laboratory for the production of antidotes for CBRN medical protection must be a basic component of CBRN medical protection. It must be complex, must have functional autonomy (space, equipment, staff and specific procedures) and must operate in compliance with national and international norms in the field. Its main activity shall consist in the pharmaceutical research, manufacture and pharmaceutical control of CBRN medical protection medicines (antidotes and decontaminants), as well as the storage (in pre-determined quantities) and must be in charge of their distribution (in effect of an order) to the military or the civilian health network, nationally or internationally.

The Romanian Army had 17 facilities authorized for the microproduction of military-specific drugs, including antidotes: 12 in the pharmaceutical laboratories of the Military Hospitals, one in the pharmaceutical laboratory at the Bucharest Garrison Health Center (led by chief pharmacist colonels (eg Col. Pharm. Dr. Oiţa from Iasi who prepared and patented over 100 innovative drugs), a laboratory for blood and derivatives at the Hematology Center (led by Col. Pharm. Dr. Coreni), a galenic laboratory for antidotes at Military Medical Research Center (CCSMM) (led by Col. Pharm. Prof. Dr. Mircioiu), one at the Pharmacology and Drug Control Laboratory (led by Col. Vatasescu), a microproduction laboratory for specific drugs for the military staff, at the Sanitary Warehouse (led by Col.Pharm. Rosnovschi), and a chemical synthesis laboratory for antidotes (led by Col. Pharm. Miu) was also operating at CCSMM. These had incorporated a department for injectable and infusible medicines and were able to prepare any master or galenic drugs recommended by clinicians or researchers. However, all were abolished at the restructuring of the army from the beginning of this century, and the current legislation no longer allows them to be re-instated.

We recall that the Medical Directorate had a Pharmaceutical Microproduction Laboratory, with an old tradition, that provided specific sanitary-pharmaceutical materials, but it was abolished, after almost a century of existence, at the entry into force of the Medicines Law, because it did not comply with the requirements imposed by the European Union, although strictly speaking, they refer only to the “marketing authorization” of the products.

It can also be exemplified based on the experience of CCSMM, that had a Galenic Laboratory, which implemented the products created by the researchers from the laboratories of the Center (under direction of General Prof. Victor A. Voicu, MD, PhD, SR, member of Romanian Academy) but which was renamed and reorganized, because the new Medicines Law no longer provides the "galenic" category. This laboratory, with pharmacists and chemists, had the task of pharmaceutical, pharmacological research and prepared the reagents and drugs resulting from scientific research activities of the center, in quantities of up to 50 doses per lot, in order to remain in the field of microproduction. The products were used for physico-chemical tests, animal experiments or cell cultures and some were distributed to units located in Operations Theaters (for example in Iraq, Somalia, Angola), in applications (for example Campulung, Brasov, Tulcea), in specific missions (for example, anthrax, cholera, BDA epidemics), have been presented at different thematic exhibitions (Clinceni, Central Army House, Expomil, Inventions, BSDA) and many have been patented as inventions or innovations. However, at the present moment, the Romanian Army no longer has any facilities for drug production, so neither specific CBRN antidotes nor qualified personnel for pharmaceutical preparations.

REQUIREMENTS AND LEGISLATION IN FORCE FOR CERTIFICATION/ACCREDITATION/OPERATION

After designing, obtaining the necessary permits and financing, the platform can be built and the specific endowment can be started. From the moment it is put into operation, with the commissioning protocol, the sanitary-veterinary authorization of operation is requested from the Center for Preventive Medicine, then the RENAR and ANMDM approval for the manufacturing authorization. After completing these mandatory steps, it can be proceeded to micro-production, but for each particular product the marketing authorization must be obtained.

Corroborating the need of medical research to physically make the newly designed products (medicines, diagnostic kits, sets and medical devices), at the level of experimental model (EM), functional experimental model (FEM), prototype and/or technological demonstrator, in order to obtain the approval, commissioning protocol, authorisation and possibly patenting (as an invention or industrial model), with the need for the industry to carry out the microproduction and serial production of 0 series for clinical trials and obtaining the marketing authorization (MA), the integrative principle must apply: research, education and production.

The current legislative requirements can be found in: the Guide on good manufacturing practice for medicinal products for human use, the Guide on good practice in drug distribution, the Order 1807/2006 regarding the approval of the Norms for the application of some provisions of Regulation no. 141/2000/EC on orphan drugs, entered into force in 2007, GMP Regulations – Good Practice Regulations in production, consultancy-certification.ro, etc.

CONCLUSIONS

Medical countermeasures for CBRN defense are based on the prophylactic and curative treatment of the infected and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is immediately implemented. However, in time of peace, this case is almost non-existent, and during the war or during a terrorist attack it can be a mass pathology, like an explosive epidemic, in which the forces and means involved for medical counter-measures become insufficient.

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ORIGINAL ARTICLES

Non-melanoma skin cancer (NMSC): Extramammary Paget ’s disease

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Abstract: *Non-melanoma skin cancer (NMSC) comprises squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and severalrare skin tumors (i.e., Extramammary Paget’s disease), being the most common malignancy-affecting people worldwide. Itrepresents the majority of skin cancers and a significant percentage of all malignancies. Despite increasing public awarenessand scientific interest, the incidence of NMSC is constantly increasing. Even though most NMSC`s are associated with lessaggressive behavior, they can still be invasive locally and cause extensive damage to neighboring structures, inducingsignificant morbidity. In addition, different types and subtypes of NMSC tend to have frequent recurrences and may have significantmetastatic potential. As a direct consequence, NMSC has become an important issue for healthcare systems with a significantsocio-economic impact.*

Keywords: *Extramammary Paget’s disease; World Health Organization; keratinocyte stem cells; EMA*

INTRODUCTION & BACKGROUND

Non-melanoma skin cancer (NMSC) comprises squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and several rare skin tumors (i. e. Extramammary Paget’s disease), being the most common malignancy affecting people worldwide. It represents the majority of skin cancers and a significant percentage of all malignancies. Despite increasing public awareness and scientific interest, the incidence of NMSC is constantly increasing. Even though most NMSC`s are associated with less aggressive behavior, they can still be invasive locally and cause extensive damage to neighboring structures, inducing significant morbidity. Also, different types and subtypes of NMSC tend to have frequent recurrences and may have significant metastatic potential. As a direct consequence, NMSC has become an important issue for healthcare systems with a significant socio-economic impact.

EXTRAMAMMARY PAGET’S DISEASE (EMPD)

EMPD localized to the vulva is a rare disease that canmimic different types of dermatosis and only four cases havebeen reported in our practice in the last 5 years.

Definition

EMPD, according to World Health Organization, is a rare intraepithelial neoplasm characterized histopathologically by a prominent growth of large cells, with apocrine or eccrine glandular-like pattern, originating in the skin (primary EMPD) or representing intraepithelial spread of a hidden visceral carcinoma (secondary EMPD). This type of disease is represented by the International Classification of Diseases for Oncology with the following code: 8542/3. [1, 2]

EMPD is a marginated plaque that clinically and histologically

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resembles Paget's disease, but occurs in sites rich in apocrine glands, such as vulva, anogenital region and axilla. Currently, there is controversy as to how often this condition appears against on the background of an underlying carcinoma and how often it occurs primarily in the epidermis or apocrine ductal tissue in the affected area. This gave rise to the concept of primary and secondary extra-mammary Paget's disease [3, 4].

Epidemiology and Etiology

EMPD accounts for about 1 % of all ano-genital neoplasms. It develops more frequently in Caucasian women in the postmenopausal period [1] and usually begins in the fifth decade or later. Current opinion is that, in about 75% of cases, extracellular Paget's disease appears as a primary intra-epidermal neoplasm, possibly from ductal cells of the apocrine gland or keratinocyte stem cells. Another possible origin may be the Toker's cells [3, 5]. In the remaining 25% of cases, there is a hidden primary adenocarcinoma. These cases are referred as secondary Paget's disease.

Only a few cases of association between mammary and vulvar Paget's disease have been described. Under extraordinary circumstances, EMPD is a secondary event caused by extension of an adenocarcinoma either of the rectum to the perianal region, or of the cervix to the vulvar region. On the other hand, genital Paget's disease with a long evolution, can extend into the cervix and urinary tract [6].

Localization

The labia majora is the most widespread site, followed by the labia minora and clitoris. The perineum, perianal area, scrotum, penis, lower abdomen and inguinal folds can also be affected [1].

Based on origin, several classification schemes have been proposed [22] :
Type 1: primary vulvar cutaneous origin
Type 2: secondary to primary anal or rectal adenocarcinoma
Type 3: secondary to urothelial carcinoma

OR

Type I: Paget disease of primary cutaneous origin
Ia: as a primary intraepithelial neoplasm
Ib: as an intraepithelial neoplasm with invasion
Ic: as a manifestation of underlying adenocarcinoma of skin appendage or vulval glandular origin
Type II: Paget disease of noncutaneous origin
IIa: secondary to anorectal carcinoma
IIb: secondary to urothelial neoplasia
IIc: as a manifestation of another noncutaneous carcinoma: endocervical, endometrial or ovarian adenocarcinoma, etc.

Clinical features

The most common presentation is a relatively well-demarcated flat or slightly elevated, scaly, erythematous patch or plaque, accompanied by itching or burning, often simulating eczema. Unusual forms include lesions with hyper- or hypopigmentation and multi-centric bruising [1].

EMPD lesion has many features in common with Paget's disease of the nipple. The edges are sharp, rounded and slightly raised, and includes an area that is pink or red. The affected area may be tiled, and small, gray-colored crusts may cover erosion. Itching is an essential feature and there may be excoriations or lichenification. Areas of fluctuating hyperpigmentation may be present, causing confusion between extra-mammary Paget's disease and superficial spread melanoma. In some cases, leukoplakia may be present [3, 7, 8, 9].

The clinical presentation of the disease varies by site, the most frequent involved being vulva. The first symptom, especially in vulvar lesions, is itching and burning, which can be persistent and growing. It is often seen as dermatitis and may be disturbed by topical therapy. The surfaces of the lining of the labia are periodically redder than the skin when both areas are involved, and the change may proliferate towards the thighs, mons pubis and vaginal introitus. There will be a limited papillomatous area. Characteristic clinical features include fierce advancement, challenging all local applications, and the whetted margin. Eventually, one area may become thickened and ulcerated as evidence of downwards invasion. Lymph nodes involvement or distant metastases can appear. Although most cases of primary carcinoma result from a hidden adenocarcinoma of the sweat gland, it is essential to examine the patient for evidence of a later adenocarcinoma hidden in the cervix and rectum [3].

Pathogenesis

Histogenesis of primary EMPD is unknown [1].

Paget cells in the epidermis may appear as an in situ upward extension of an adenocarcinoma located in the deeper glands. Alternatively, EMPD may have a multifocal primary origin in the epidermis and skin appendages, and does not represent an epidermotropic spread or metastasis from an intrinsic carcinoma of the sweat gland. It can be shown that almost 25% of EMPD originates from primary adenocarcinoma of the secretory glands. Primary tumor and Paget cells are usually mucus secretory [10].

At one point, it was widely accepted that in primary EMPD, Paget cells in the epidermis are due to an in-situ upward extension of an in-situ adenocarcinoma of the sweat glands along the eccrine or apocrine ducts. This theory is comparable to the generally accepted view that secondary EMPD shows an extension of a cervical, rectal or urinary adenocarcinoma and that the progression of Paget breast disease is an extension of a lactiferous canalicular epidermotropic carcinoma. The fact that a hidden adenocarcinoma often could not be demonstrated, can be explained by the different anatomic characteristics between the breast, which has only 20 large, conspicuous lactiferous ducts, and the genital skin, which has thousands of small apocrine and eccrine glands, making the localization of this particular small gland invaded by carcinoma increasingly difficult [6].

Histopathology

EMPD disease has in common with Paget's disease the presence of varying numbers of Paget cells within the epidermis (Figure 1). In some cases, the Paget cells are found to be limited to the acanthotic epidermis, accompanied by hyperkeratosis, parakeratosis, erosion or ulceration. Frequently, Paget cells can be identified within the epithelium of hair follicles or eccrine sweat ducts. This types of in situ malignancy associated with EMPD has the same favorable prognosis as EMPD limited to the epidermis. However, the prognosis is much worse in cases in which the Paget cells have invaded the dermis from the epidermis or from an underlying sweat gland carcinoma. Glandular clusters with a central lumen, absent in mammary Paget's disease may be identified in the lower epidermis in extra-mammary Paget's disease [6].

Figure 1: EMPD. Paget cells in epidermis (photomicrograph taken from our cases), hematoxylin &eosin, magnification 200x

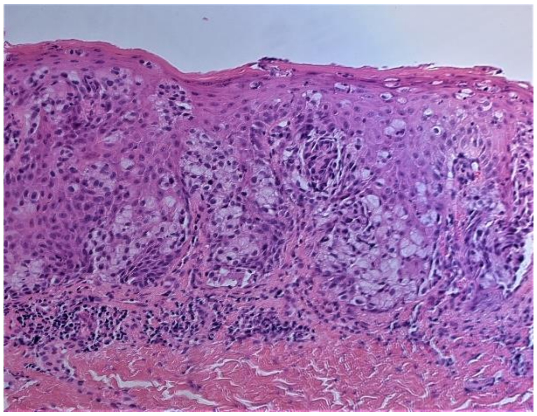

Serial sectioning of excised lesions of vulvar EMPD demonstrated that the lesions within the epidermis and its appendages have a multifocal origin. In the cases that have extensive epidermal lesions, the involvement of eccrine glands and ducts is occasionally, and the involvement of apocrine glands and ducts is absent, even if some Paget cells form apocrine glandular structures in the epidermis. Despite the existence of cohesion between the epidermal and the ductal foci, no one can definitive say that the extension is in an up or down direction. The dermal invasion in EMPD has its beginning point in the epidermis and not ductal or glandular structures.

The origin of the Paget cell may be the poral portion of an apocrine duct, or the pluripotential germinative cells within the epidermis [6]. Paget cells have an abundant basophilic to amphophilic cytoplasm and vesicular nuclei with pronounced nucleoli. They are generally distributed in a solitary fashion or may form gland-like structures or well-demarcated solid nests. Involvement of adnexa (hair follicles and eccrine ducts) is seen in most cases. Rarely, single cells, glands and solid nests can be found in the dermis; this is considered to be microinvasion. Dermal invasion is rare in vulvar EMPD, more common in perianal region. However, this type of microinvasion cannot be applied in cases that are associated with an underlying adenocarcinoma with a ductal in-situ component [1].

Immunohistochemistry

Immunohistochemical staining shows that Paget cells stain consistently positive for CK7 (cytokeratin 7) [1] (Figure 2) and EMA (Epithelial membrane antigen) – used by pathologists like a marker of Paget disease [11] (Figure 3).

Figure 2: Paget cells showed positivity for CK7 (cytokeratin 7also called as keratin 7, CK7, KRT 7, K7) (photomicrograph taken from our cases), magnification 200x

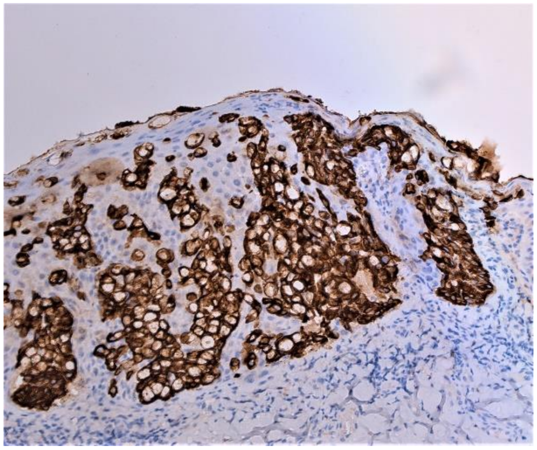

Paget cells often express GCDF-P15 (gross cystic disease fluid protein) (Figure 4), ERBB2 (HER2/neu) (Figure 5), CEA (carcynoembrionic antigen), CA125 (cancer antigen 125/ MUC16), and AR (androgen receptor) [1].

Figure 3: Paget cells showed positivity for EMA (epithelial membrane antigen also known CD227, MUC1 or episialin) (photomicrograph taken from our cases), magnification 200x

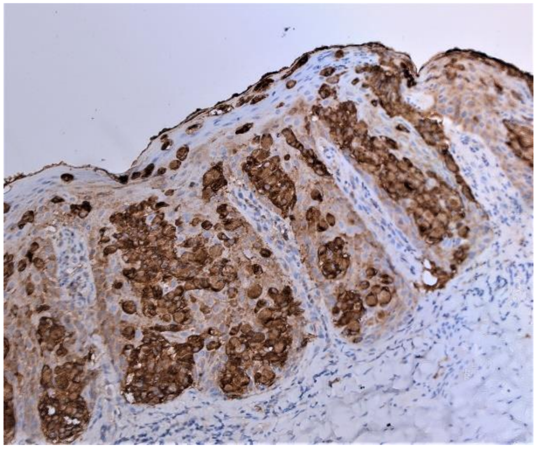

Figure 4: Paget cells showed positivity for GCDFP-15 (gross cystic disease fluid protein also known BRST2, PIP-prolactin induced protein) (a photomicrograph taken from our cases), magnification 200x

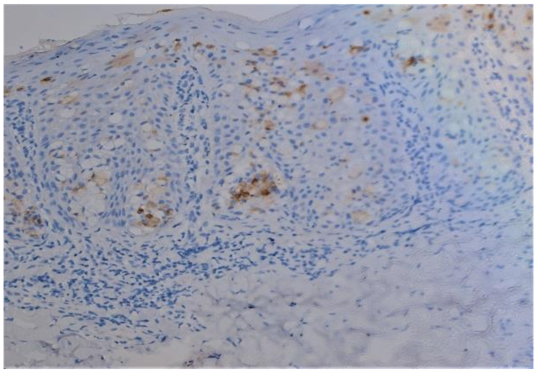

Figure 5: Paget cells showed positivity for ERBB2 (human epidermal growth factor receptor 2, also known HER2/neu, c-erbB2, CD340)(photomicrograph taken from our cases), magnification 200x

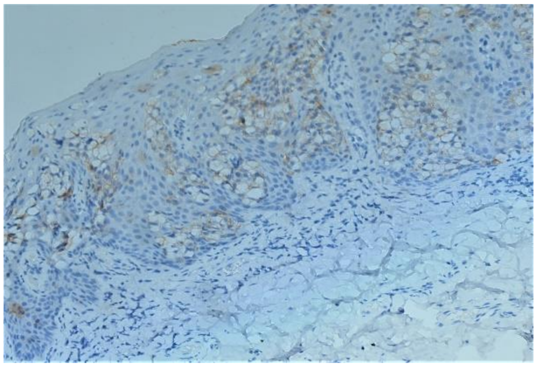

Differential diagnosis.

For correct diagnosis, immunohistochemistry and clinico-pathological correlation are essential [1].

The most pressing matter is distinction between primary and secondary EMPD (Table 1). Potential imitators of EMPD are Toker vulvar cells, vulvar and penile analogues of cervical stratified mucin-producing intraepithelial lesion, high-grade squamous intraepithelial lesion, melanoma, pagetoid dyskeratosis, and clear cell papulosis. [1]

Table 1: Differential diagnoses of Extra-mammary Paget's disease (Immunohistochemical Markers). [12]

Immuno-histochemical marker	Primary extra-mammary Paget's disease	Secondary extra-mammary Paget's disease	Bowen's disease	Superficial spreading melanoma
CK7	+	+	+ or -	-
EMA	+	+		
CEA	+	+	-	-
GCDFP-15	+	-	-	-
Melan-A	-	-	-	+
P63	-	-	+	
S100 protein	-	-	-	+
HMB45	-	-	-	+

For differential diagnosis with superficial spreading melanoma we currently use as immunohistochemical markers S100 protein and HMB45 (Figure 6), and we do not use Melan-A because it has often given false positive results (Figure 7).

Figure 6: Paget cells showed negativity for HMB45 (Human Melanoma Black) (photomicrograph taken from our cases), magnification 100x

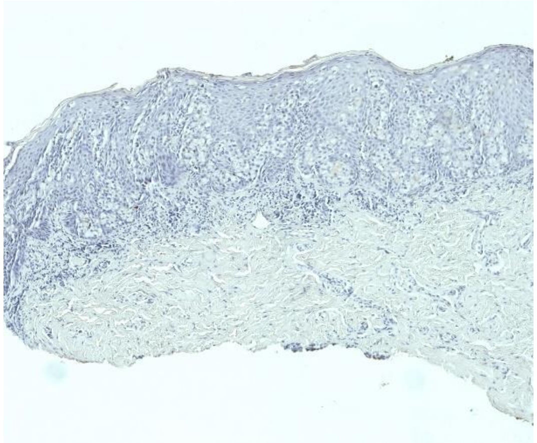

For proper localization of a large perineal lesion (vulvar or perianal) we usually use GCDFP-15 (positive for vulvar localization) and CK20 (positive for perianal localization) [13]

Prognosis and treatment

Primary EMPD’s prognosis generally is better than that of mammary Paget’s disease [6].

Local recurrence is frequent, even in cases with a wide surgical extraction, the recurrence rate being 12-58%. This may be related to positive surgical margins, skip areas, and multicentricity.

Figure 7: Paget cells showed false positivity for Melan-A (Melanoma Antigen, also called MART-1 – Melanoma Antigen Recognized by T cells 1)(photomicrograph taken from our cases), magnification 100x

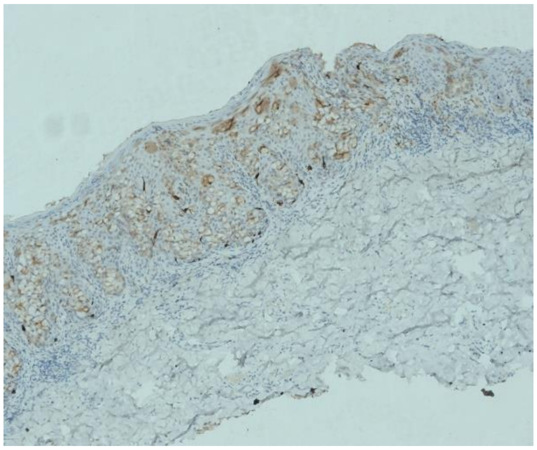

Adverse prognosis is associated with increased serum levels of CEA and the depth of invasion. Proper tissue sampling and other investigations are vital to determine if there is an associated underlying malignancy that requires surgical excision. If such a malignancy exists, it should be excised along with all clinically abnormal epithelium. If no underlying malignancy is clinically and surgically detected on careful examination, the entire affected area of skin should be excised.

In the case of primary EMPD associated with an invasive component, the prognosis is significantly worse, and it can undergo lymph node metastasis. Cases with gene mutation of PIK3CA and AKT1 are often invasive. [1]

An important method is “Mohs surgery” (microscopically controlled surgery) that minimizes the recurrence rate by carefully controlling excision margins of the tumor [14, 15, 16].

Photodynamic therapy is another important tool in the treatment of EMPD, but it requires larger series and longer periods of follow-up [17, 18].

Topical imiquimod has been used with good results in cases with limited disease [19].

Radiation therapy has been supported either as an adjuvant to surgical therapy [20] or as an alternative therapy for elderly patients in whom surgery may have a greater risk [21].

DISCUSSIONS

Because of the resemblance of the lesions in the EMPD with other dermatological conditions, it is important that the clinician always consider a possible diagnosis of EMPD, especially in the case of lesions in the genital and anal spheres.

Due to the false positive results that may appear at various markers used for the diagnosis of EMPD, the pathologist cannot always rely on immunohistochemical tests for differential diagnosis with other disorders. Therefore, a correlation between clinical, paraclinical and histopathological data is very important for a correct diagnosis.

CONCLUSION

EMPD is a very rare skin malignancy that needs to be diagnosed and treated accordingly [23]. Because it can mimic a lot of benign or malignant diseases, a correct differential diagnosis is crucial for the treatment and prognosis of the patient. Also, it is imperative to differentiate primary EMPD from a secondary type, in the latest the identification of the primary site of the tumor being vital for the patient.

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Conflicts of Interest: The authors declare no conflict of interest.

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ORIGINAL ARTICLES

Laparoscopic sleeve gastrectomy and esophageal motility

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Abstract: *Laparoscopic sleeve gastrectomy (LSG) is becoming popular between the bariatric procedures. There are few studies available in the literature about LSG impact on esophageal motility, with debatable results. Technically, it modifies the anatomy of the esophago-gastric junction; it can damage the phreno-esophageal ligament and decrease the lower esophageal sphincter pressure that will predispose to reflux. Gastroesophageal reflux disease (GERD) is already common in obese patients due to frequent hiatal hernia, high intraabdominal pressure, increased transient lower esophageal sphincter relaxations (TLESR), altered esophageal motility and poor esophageal clearance. LSG can worsen gastroesophageal reflux disease, but also the weight loss achieved after it and the decrease of acid production in the small gastric pouch will probably reduce the GERD symptoms. Dysphagia and odynophagia were reported after sleeve gastrectomy, but usually associated with anatomical narrowing of the gastric sleeve, caused by esophageal dismotility or secondary to diabetes mellitus, low tiamine level, the use of opioids and non-steroidal anti-inflammatory drugs, even hypothyroidism. In addition to upper GI tract endoscopy, high-resolution esophageal manometry (HRM) should be required for all patients planning to undergo a LSG, and in the follow-up period according to the contractility pattern.*

Keywords: *obese, sleeve gastrectomy, esophageal motility, esophageal clearance, reflux*

INTRODUCTION

According to World Health Organization (WHO), overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. If the Body Mass Index (BMI weight in kilograms divided by the height in square meters) is between 25-30 kg/m^2 a person is overweight, and if BMI > 30 kg/m^2 obese [1]. Unfortunately due to behavioral and nutritional changes more than 20% of the worldwide population is overweight and 10% obese, and in the next 20 years the estimation is that 2.16 billion people will be overweight and 1.12 billion obese [2].

Morbid obesity, defined by BMI greater than 40 kg/m^2 or between 35 and 40 kg/m^2 with any obesity-associated conditions such as diabetes, high blood pressure, cardiovascular disease, stroke, sleep apnea, osteoarthritis, is a serious invalidating health condition that can interfere with basic physical functions such as breathing or walking [3].

The first step to obtain weight loss is through behavioral and lifestyle changes, with low-calorie diet and exercise, but usually previous attempts of weight loss are followed by rapid weight regain. Many obese associate to diet and exercise pharmacological drug therapy, achieving maximum 10% weight loss [5]. Unfortunately, a lot of patients fail to achieve long-term weight loss through these methods, so they are good candidates for surgical treatment.

Bariatric surgery cause weight loss by restricting the amount

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of food in the stomach, or by a combination of gastric restriction and malabsorption[6]. The bariatric surgical procedures can be purely restrictive, such as laparoscopic adjustable gastric band (LAGB) or laparoscopic sleeve gastrectomy (LSG), or malabsorptive-restrictive: Roux-en-Y gastric bypass (RYGB) and biliopancreatic diversion (BPD)[3, 6]. All the bariatric surgical procedures may have local complications (stenosis at anastomotic sites, stomal ulcers, band erosion, fistulae) but they can be managed with proper follow-up and close monitoring.

Table 1: The known risk factors [4] for morbid obesity are:

1. Genetic factors (familyhistory of obesity)
2. Dietaryhabitsandsedentary lifestyle, **q**uittingsmoking, microbiom
3. Stressandanxiety (highlevel of cortisol, high-calorie food)
4. Sleepdisorders
5. Female (post pregnancyweightgain or menopause)
6. Aging (slowingdown metabolism)
7. Somemedications (antidepressants, steroidsand beta-blockers)
8. Cushing'ssyndrome, Prader-Willi syndrome

The laparoscopic adjustable gastric band (LAGB) involves an inflatable band that is placed around the upper portion of the stomach, creating a small gastric pouch and reducing the amount of food. Is a reversible procedure, with shorter hospital stay and low mortality rate, but the band can slip or create erosion into the stomach, requires a foreign device to remain in the body and strict adherence to the postoperative diet and follow-up visits. The weight loss achieved is slower than the other bariatric procedures, with low risk of vitamin/mineral deficiencies, but a high rate of re-operation [6].

The Roux-en-Y gastric bypass was considered the “gold standard” of weight loss surgery, restricting the amount of food consumed and modifying hormonal level of ghrelin, decreasing appetite an enhance satiety. But is technically more complex than LAGB or laparoscopic sleeve gastrectomy (LSG), has longer hospital stay with possible long-term vitamin/mineral deficiencies (vitamin B12, calcium, folate, iron). Nowadays, remains an effective method to alleviate symptoms of GERD and many bariatric procedures are finally converted to Roux-en-Y bypass [6].

Laparoscopic sleeve gastrectomy (LSG) consist of a vertical division on the larger curvature of the stomach, removing a large portion of the body and all of the fundus, the effect being both restrictive and endocrinologic – reducing ghrelin. LSG is very popular among the bariatric procedures, it induces rapid and sustainable weight loss of > 50% for 3-5 years, has relatively short hospital stay (48h), but is a non-reversable procedure with potential long-term vitamin deficiencies [6]. Also, transforming the stomach into a cylindrical structure and altering the anatomy of the esophago-gastric junction will probably influence the lower esophageal sphincter pressure and esophageal motility.

OBESITY AND ESOPHAGEAL HEALTH

Gastroesophageal reflux disease (GERD) is common in obese patients, and they can be even more sensitive to the presence of acid in the esophagus than a normal weight person. Also, hiatal hernia, a risk factor predisposing to GERD is more prevalent among the obese [7]. Obesity may trigger vagal abnormalities and a higher level of bile and pancreatic enzymes, worsening the effect on esophageal mucosa [8].

According to a meta-analysis published by Halmpel H. et al. in 2005 [9], obesity may increase the risk of GERD symptoms by 50%, and also cause a 2-fold increase in esophageal adenocarcinoma [9]. The pathophysiological mechanisms of increased GERD in obese are: high intraabdominal pressure, frequent hiatal hernia, increased transient lower esophageal sphincter relaxations (TLESR), lower esophageal sphincter (LES) pressure, altered esophageal motility with poor esophageal clearance [10, 11].

The esophageal transit time in obese has been in the researchers preoccupation from1985, when Mercer CD et. al [11] measured it by radionuclide scintigraphy using a swallowed technetium sulfur colloid bolus. The data obtained were compared from obese patients with GERD to normal weight with and without GERD. The esophageal transit time was significantly prolonged in the obese group compared with both lean groups (p less than 0.001). Also performing conventional esophageal manometry theyconfirmed that in obese patients gastroesophageal pressure gradient is elevated [12].

Furthermore, in a study published in Obese Surgery in 1999, Jaffin BW et al. revealed a high prevalence of asymptomatic esophageal motility disorders among morbidly obese patients. During a 3-year period, they evaluated manometric and completed a questionnaire of symptoms, medications, demographic data, other medical conditions associated, 111 obese patients for bariatric surgery. Abnormal manometric findings were observed in 61% patients: 14% hypertensive contractions of the esophageal body, 7% diffuse esophageal spasm (DES), 1% achalasia, 25% hypotensive lower esophageal sphincter defined by LES pressure < 10 mm Hg, and 14% were nonspecific esophageal motility disorders [13].

Similar results have been obtained by Koppman's team [14], after performing esophageal manometric evaluation in 116 obese patients selected for laparoscopic gastric banding in 2003. 41% of the patients had abnormal manometric findings: 23% nonspecific esophageal motility disorders, 11% hypertensive contractions, 1% diffuse esophageal spasm, 1% achalasia, 3% isolated hypertensive LES pressure (>35 mmHg), 3% isolated hypotensive LES pressure (<12 mmHg). Only one patient with abnormal esophageal motility reported noncardiac chest pain [14].

LAPAROSCOPIC SLEEVE GASTRECTOMY AND ESOPHAGEAL MOTILITY

In a review of the impact of laparoscopic sleeve gastrectomy on gastrointestinal motility, published in 2018 by Eleni Sioka et al. [15], after a search of the medical literature with two independent reviewers, they selected only 9 eligible studies that focused on esophageal motility [16]. The esophageal motility data were debatable, with differences regarding the follow-up timing (from 6 weeks to median 13 months), the study design, the surgical technique, the bougie size, and the distance from pylorus. To clarify this subject we need more data from future prospective studies on larger cohorts.

Del Genio G et al. [17] studied the impact of LSG on esophageal physiology, performing high-resolution impedance manometry (HRiM) combined 24-h pH and multichannel intraluminal impedance (MII-pH) before and after the procedure, in 25 patiens. After a median follow-up of 13 months, HRiM showed increased ineffective peristalsis and incomplete bolus transit, but normal LES function.

In a retrospective analysis of 53 cases of HRiM performed after LSG, Mion F. et al. [18] found in 77% of patients an increased intragastric pressure (IIGP), even if they were asymptomatic with no specific manometric or impedance changes, and this could be assimilated as a manometric marker of LSG.

An interesting study of Petersen WV et al. [19] published in 2012, proved that LSG increased lower esophageal pressure independent of weight loss achieved after the procedure, thus protecting obese patients from gastroesophageal reflux.

Dysphagia was reported after laparoscopic sleeve gastrectomy, but usually associated with narrowing of the gastric sleeve which means inability to pass a 9.6 mm gastroscope due to stenosis or a sharp angulation [19]. In a study published by A. Nath et al. in september 2016, from 352 patients included, 22.7 % had dysphagia, 9.3% narrow sleeve, 7.1 % sharp angulation, 2.3% stenosis, and they responded well to ballon dilatation (1-3 sessions). Other medical conditions were associated with post-operative dysphagia: esophageal reflux, diabetes, low tiamine level, opioids and non-steroidal anti-inflammatory drugs or hypothyroidism, so dysphagia is not a specific esophageal motility disorder sign [20].

PRACTICAL CONSIDERATIONS

We performed a prospective study in Carol Davila University Central Emergency Military Hospital to determine the manometric pattern of obese patients, the prevalence of esophageal motility disorders prior and after gastric sleeve and the upper GI complications post sleeve.

The aim was to do high-resolution esophageal manometry (HRM) to all obese patients proposed for LSG, before and 6 to 12 months after the procedure, plus upper GI tract endoscopy and GERD questionnaire (GERD-HRLQ). Starting from January 2017, we included in the study 61 morbid obese patients evaluated both endoscopically and manometric. The exclusion criteria were: previous gastric surgery, another bariatric procedure surgical or endoscopically, the use of medications that could affect esophageal contractility or lower esophageal sphincter pressure (prokinetics, calcium-blockers, nitrates). Half of the patients are now in the post-operative evaluation period, because of surgical intervention timing, and the final results will be later published.

Esophageal manometry was performed with a 36-channel High-Resolution Solid State Catheter from Sierra-Given, after 6 h of fasting and previous upper GI tract endoscopy evaluation. The manometric data were obtained through 10 single test swallows of 5 ml of water. The HRM results were then analyzed according to the Chicago Classification v3.0 of esophageal motility disorders, using the ManoView ESO software program (component of the ManoScan system), providing visual images and quantitative physiological parameters.

The principal manometric variables analyzed [21] were summarized in the Table 2.

Diffuse esophageal spasm (DES) causes chest pain and sometimes mixed dysphagia for both liquids and solids. The substernal chest pain may waken the patient from sleep or is associated with exercise and often confused with angina pectoris. Hot or cold liquids may aggravate the pain. From the current data available, it is possible that in time this disorder to evolve into achalasia with impaired esophageal peristalsis and lack of lower esophageal sphincter relaxation during swallowing. Usually barium swallow may show poor progression of bolus plus simultaneous contractions [22]. Fragmented peristalsis and ineffective motility are minor esophageal motility disorders according to Chicago Classification v3.0 which can be seen also in normal person.

A particular manometric pattern was found during our study, in a 42-year-old female with morbid obesity BMI = 44.81 kg/m^2 (W= 122 kg, H= 165 cm), obstructive sleep apnea and minor GERD symptoms prior to the surgical intervention. The upper gastrointestinal endoscopy revealed erosive esophagitis grade A, duodenitis with Helicobacter pylori infection both treated preoperative.

Table 2: Principal manometric variables analyzed

Lower esophageal sphincter(LES) resting pressure	Normal 13-43 mmHg
Deglutitive LES relaxation = Integrated Relaxation Pressure (IRP)	For Sierra-Given solid probe the normal value < 15 mmHg
Esophageal peristalsis = Distal Contractile Integral(DCI)	Normal 500 – 5000 mmHg-cm-sec; > 5000 Hypertensive > 50% contractions weak (DCI < 500) or failed (DCI < 100) = IEM Ineffective motility
Duration of contraction waves in seconds= Distal Latency (DL)	Normal value > 4.5 sec, if 2 contractions are premature < 4.5 sec the diagnose is Diffuse esophageal spasm(DES)
Peristaltic break > 5 cm	More than 50% of swallows = Fragmented peristalsis(FP)

Figure 1: The upper GI tract endoscopy image preoperative – erosive esophagitis grade A Los Angeles Classification

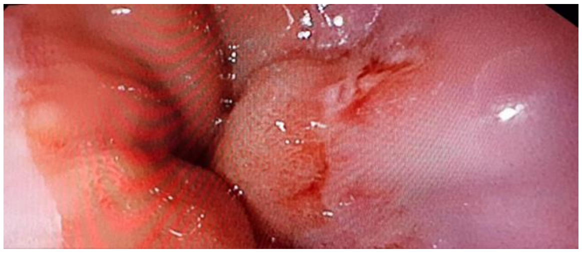

On the preoperative high-resolution esophageal manometry (HRM) she had normal lower esophageal sphincter (LES) basal pressure, LES length = 3 cm, normal deglutitive LES relaxation, with IRP median value of 6.6 mmHg, good esophageal contractility (DCI median= 956.1 mmHg-cm-sec and DCI maximum=1305.7 mmHg-cm-sec) but > 20% premature contraction (distal latency value < 4.5 seconds), aspect of diffuse esophageal spasm according to Chicago Classification v3.0.

Figure 2: Wet 5-ml test swallows recorded with HRM preoperatory – shorten distal latency

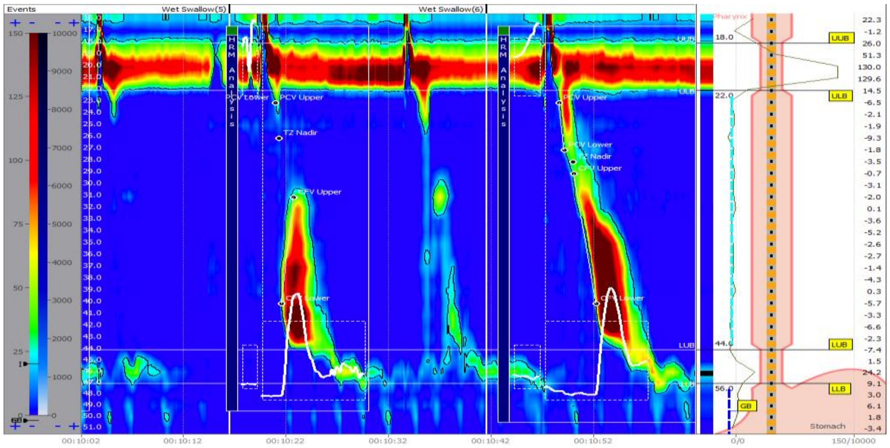

Considering the high cardio-vascular risk in a young patient, severe obstructive sleep apnea, the failure of diet and exercise and the need for sustainable weight loss, we didn't contraindicated the laparoscopic sleeve gastrectomy, but we've decided to closely follow the patient.

The laparoscopic surgical intervention went well, with good recovery after the procedure, good tolerance of food intake implementing the diet and exercise protocol. One year after LSG, the patient lost 40 kg, achieved a BMI of 30 kg/m^2 and had 82 kg weight. Clinically, now she had mild dysphagia, early satiety and fullness (that can be related to the low volume of the stomach), and less reflux symptoms compared to baseline.

The result of upper gastrointestinal endoscopy was encouraging: no evidence of erosive esophagitis, a normal gastric scar on the posterior wall of the stomach after LSG, as you can see in Figure 3.

Postoperative data from high-resolution esophageal manometry (HRM) examination revealed normal lower esophageal sphincter basal pressure (mean 20.2 mmHg, min -0.8 mmHg) with normal deglutitive relaxation of LES > 60%, mean IRP value 2.2 mmHg, the same LES length of 3 cm, but more than 50% of the esophageal contractions ineffective, with a DCI < 500 mmHg-cm-sec and normal distal latency, diagnosis of IEM ineffective motility according to Chicago Classification. Increased intragastric pressure (IIGP) after 5-ml water swallows was observed in 30% of the ten swallows examined.

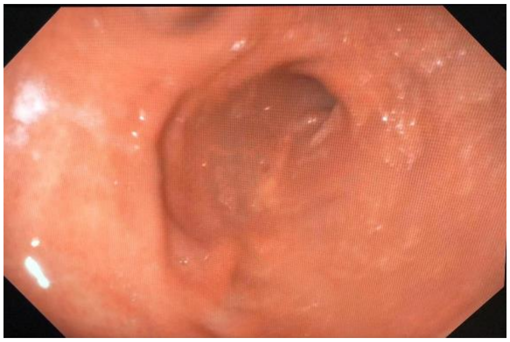

Figure 3: The longitudinal scar on the posterior wall of the stomach after LSG

Figure 4: Wet 5-ml test swallows recorded with HRM postoperatory – ineffective motility

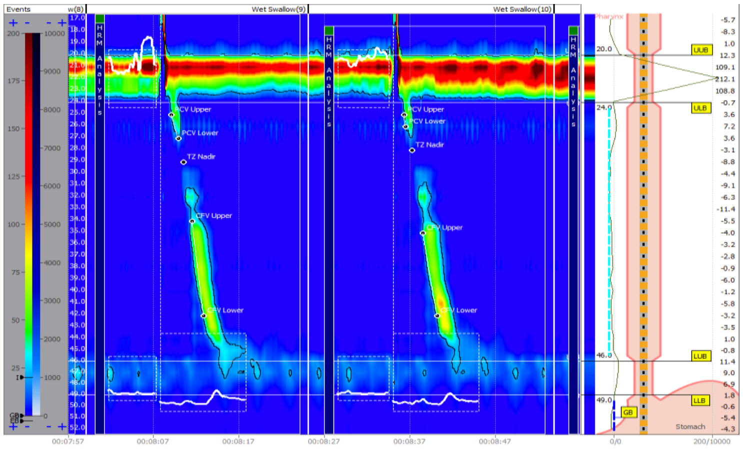

Table 3: The manometric data of LES 1-year after the LSG, no criteria of SED or achalasia as expected

Pressure meas. method	eSleeve	Normal
Basal (respiratory min.)(mmHg)	– 0.8	4.8-32.0
Basal (respiratory mean)(mmHg)	20.2	13-43
Residual IRP (mean)(mmHg)	2.4	<15.0
Residual (highest)(mmHg)	17.9	
Percent relaxation(%)	91	

Table 4: The manometric data of distal esophageal contractility 1-year after LSG (low mean value of DCI = hipocontractility)

High Resolution Parameters		Normal
Distal contractile integral(mean)(mmHg-cm-s)	444.4	500-5000
Distal contractile integral(highest)(mmHg-cm-s)	666.7	
Contractile front velocity(cm/s)	2.9	<9
Intrabolus pressure (@LESR)(mmHg)	– 0.5	<8.4
Intrabolus pressure (avg max)(mmHg)	3.4	<17

Table 5: Detail of all 10 wet test swallows recorded 1-year post LSG
(3 failed with DCI < 100 mmHg-cm-sec, 4 weak DCI < 500 mmHg-cm-sec).

	N	Med	1	2	3	4	5	6	7	8	9	10
DCI distal contractil integral (mmHg-cm-sec)	500-5000	444.4	666.7	N/A	422	182.6	N/A	375.7	557	515.2	391.3	N/A

Our patient experienced dysphagia after laparoscopic sleeve gastrectomy, but the GERD symptoms ameliorate. The HRM showed ineffective esophageal motility (IEM) and not achalasia as expected [16]. IEM is considered a minor motility disorder which can be seen also in asymptomatic patients according to Chicago Classification v3.0, but it was also cited by Del Genio G et al. after LSG [17]. We found 30% increased intragastric pressure (IIGP) after water swallows, corresponding to Mion F et al. data [18]. Further manometric follow-up is needed in this patient to see the contractility evolution pattern.

CONCLUSIONS

High resolution manometry (HRM) was already indicated for the preoperative assessment of peristaltic function in patients who needed antireflux surgery, but we consider that it is necessary to extend the HRM recommendation to bariatric surgery for better management of complications.

Studying the manometric changes in obese patients prior to laparoscopic sleeve gastrectomy is important, because it helps to select those patients who could develop postoperative esophageal motor complications and refractory reflux. In addition to upper GI tract endoscopy, high resolution esophageal manometry (HRM) should be required for all patients planning to undergo a LSG.

A proposed alternative to pharmacological treatment to relieve post laparoscopic sleeve gastrectomy GI symptoms is usually the Roux-en-Y gastric bypass, which is considered an effective method to alleviate symptoms of GERD, but it will not resolve the poor esophageal contractility. In conclusion, conversion to gastric bypass is not an universal method for managing GI complications, especially esophageal dysmotility after LSG, so we need to choose wisely the patient eligible for sleeve.

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CLINICAL PRACTICE

Bilateral pneumatization of middle and superior nasal turbinates

Mugurel C. Rusu[1], **C.J. Sava**[2], **Mihai D. Stoenescu**[3]

Abstract: *The pneumatization of all nasal turbinates is an extremely rare finding. Several previous reports have presented the bilateral superior, middle, and inferior conchæbullosæ. We report the bilateral pneumatization of middle and superior nasal turbinates. The variant was documented in cone beam computed tomography (CBCT) in all three dimensions. The inferior concha bullosa was a bilaterally moderate subtype of lamellar pneumatization that presented as a conchal recess of the maxillary sinus. The concha bullosa media was bilaterally of the extensive or true type and associated unilaterally with an uncinate bulla. The concha bullosa superior was asymmetrical bilaterally, narrower on the right side, and larger on the left where it was also septated and tri-cameral. The supreme conchæ were rudimentary, as were their pneumatizations. For an accurate anatomic diagnosis of conchæbullosæ in computed tomography, sagittal, coronal, and axial cuts, as well as three-dimensional reconstructions, should be used to ensure adequate planning of functional endoscopic sinus surgery.*

Keywords: *agger nasi; ethmoid bone; nasal fossa; nasal concha; nasal turbinate; anatomic variation*

INTRODUCTION

The lateral nasal wall attaches the nasal turbinates or conchæ. The inferior one is an independent bone. The middle, the superior, and the inconstant Santorini's supreme turbinates are the ethmoidal turbinates that are attached on the nasal side of the ethmoidal labyrinth [1].

A concha bullosa defines the pneumatization of a nasal turbinate. Although the term is commonly used to indicate the pneumatization of the middle turbinate, other nasal turbinates (inferior, superior, and supreme) have also been found to be pneumatized [2-15].

Sagit et al (2008) reported the first case of the pneumatization of six turbinates [16]. Fidan (2012) also assumed that the "pneumatization of all turbinates in a person has not been published yet" and suggested the term "panconcha bullosa" for such pneumatization of all six turbinates [17]. Ozturan et al (2013) failed to document these two previous reports and presented a case with "bilateral triple turbinate pneumatisations," noting, "Despite an extensive search of the medical literature, we could locate only one description of extensive turbinate pneumatisation involving anywhere near as many conchae as in our patient" [18].

Moreover, Bergman's Comprehensive Encyclopedia of Human Anatomic Variation [19] indicated only that the "pneumatization of several turbinates in the same patient has been reported," citing the reports of Sagit et al (2008), Ozturan et al (2013), and a third paper in which Pittore et al (2011) reported only a case of concha bullosa inferior [10], and not a panconcha bullosa. The reports of Fidan (2012) and Toplu et al (2013) were omitted [15, 19].

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We therefore report the fifth known case in the literature of the pneumatization of all nasal turbinates. To our knowledge, it is the first such case being documented in cone beam computed tomography (CBCT) and not on coronal CTs, as well as the first case in which findings were equally documented with bi-dimensional multiplanar reconstructions (MPRs) and three-dimensional volume renderizations (3DVRs). Nevertheless, we hereby report the pneumatization of eight, and not six, turbinates, as well as Santorini's first supreme turbinate was also found to be bullous.

ANATOMIC VARIATION

A retrospective CBCT study of the archived files of a 52 years old female patient was performed.

The subject had been scanned using a CBCT machine – iCat (Imaging Sciences International), the DICOM file being further documented with the Planmeca Romexis Viewer 3.5.0.R software, according to a previously detailed protocol of study [20-23]. We evaluated the nasal turbinates in all the three anatomical planes, as well as the three-dimensional volume renderizations. Relevant anatomical features were exported as image files. The patient has given written informed consent for all medical data to be used for research, provided they are anonymized.

In the case hereby reported, a Type 5 Mladina nasal septal deformation was found, with a horizontal septal spur sticking laterally and deeply in the right nasal fossa between the middle and inferior turbinates (Figure 1). The postero-superior part of the nasal septum accommodated an anterior (septal) recess of the right sphenoidal sinus. However, the septum did not appear deviated.

The inferior turbinates were bilaterally pneumatized by conchal recesses of the maxillary sinuses (Figures 1A–E, 2, 3F, 4, 6). The conchal pneumatization penetrated the horizontal segment of the lamellar part of each turbinate (Figure 6), with a 6.10 mm depth on the right side, and 3.02 mm depth on the left side (on coronal slices). These inferior conchæ bullosæ were located between the ethmoidal and maxillary processes of the inferior turbinate (Figure 6).

We also found bilateral pneumatizations of the middle turbinates (Figures 1A, 3, 4). These were both extensively pneumatized (true or extensive concha bullosa media) and were draining in the middle meatus on that side. An uncinate bulla was found unilaterally on the outer side of the right concha bullosa media (Figure 1A). It was pneumatized from an anterior adjacent lacrimal cell located, in turn, posterior to the agger nasi cell.

Figure 1: Anterior to posterior series (A–F) of coronal MPRs identifying the eight turbinates' pneumatizations. The beaked-shape pneumatization of each inferior turbinate is identified (A–E, white double-headed arrows). The bilateral concha bullosa of the middle turbinates (A, white arrows) is of extensive type, lamellar and bulbous. A right uncinate bulla is found (A, *). The superior turbinates are bilaterally pneumatized (B, white arrowheads). A horizontal septum divides the anterior part of the left superior concha bullosa in two chambers, superior (C, black arrowhead) and inferior (C, black arrow). Both drain into the upper nasal meatus (C, D). A third posterior chamber of the left superior concha bullosa (E, black double-headed arrow) appears as a medially evaginated posterior ethmoid air cell. On both sides of an anterior (septal) recess („X") of the right sphenoidal sinus the rudimentary supreme turbinates also appear pneumatized (F, black arrowheads).

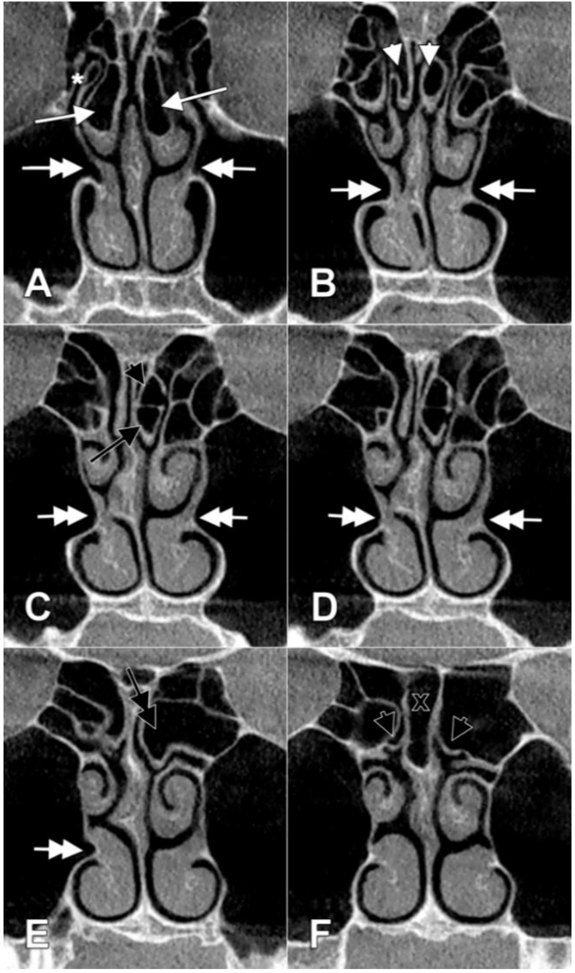

We also observed a bilateral concha bullosa superior (Figures 1B–E, 3-6). The conchal air space was narrow in the right side (1.75/4.01 mm on coronal slices). The left concha bullosa superior was larger (4.81/11.35 mm on coronal slices) and septated by an overturned "Y"-shaped septum (Figures 3A, 5).

Thus, it consisted of three chambers: antero-superior, antero-inferior, and posterior. The anterior chambers were draining in the left superior nasal meatus (Figures 1C, D).

Figure 2: Axial MPR demonstrating the bilateral inferior concha bullosa (double-headed arrows).

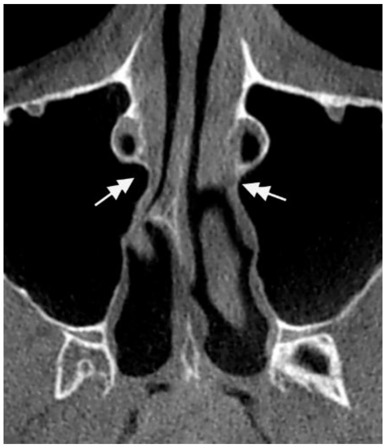

Figure 3: Lateral to medial series (A–F) of sagittal MPRs of the left lateral nasal wall. An agger nasi cell (A–F, *) is found anterior and superior to the anterior end of the middle nasal turbinate. Posterior to it lies the middle concha bullosa (A-–, white arrow). The inferior concha bullosa is indicated (F, white double-headed arrow). The superior concha bullosa is double-septated and consists of three chambers (A, B, white arrowheads): two anterior and one posterior. The posterior one is a conchal recess of a posterior ethmoid air cell (B–F, „X”), as is the supreme concha bullosa (D, black arrowhead). The superior (black arrow) and supreme (black double-headed arrow) turbinates are indicated (C–E).

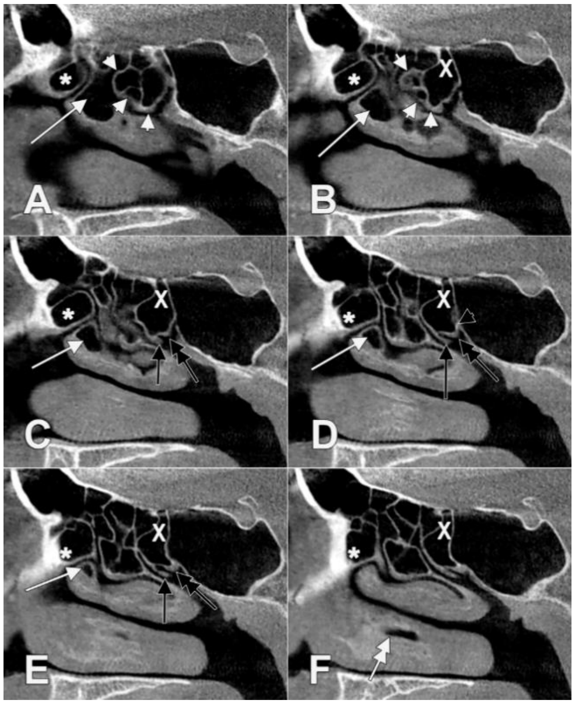

The posterior chamber appeared as the extension (conchal recess) of a posterior ethmoid air cell which, in turn, was also extending to form the left concha bullosa suprema (Figure 3).

As the concha bullosa suprema was also found on the right side (Figures 1, 3–5), we recorded this variation as bilateral. The rudimentary supreme conchæ bullosæ appeared as small diverticula of the respective posterior ethmoid air cells.

Figure 4: Sagittal MPR of the right lateral nasal wall crossing thorough all four conchal pneumatizations: inferior (double-headed arrow), middle (arrow), superior (white arrowhead), and supreme (black arrowhead).

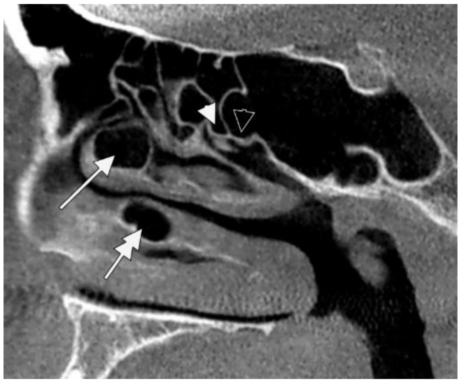

Figure 5: Three-dimensional sagittal volume renderization of the left lateral nasal wall. The superior concha bullosa is separated by septa in three chambers (diverging thin arrows). A posterior ethmoid air cell ("X") pneumatize the posterior chamber of the superior turbinate (white arrowhead) and the supreme turbinate (black arrowhead). The thick arrow indicates the middle concha bullosa and the double-headed arrow—the agger nasi cell. FS: frontal sinus; SS: sphenoidal sinus; IT: inferior turbinate; NS: nasal septum (partly).

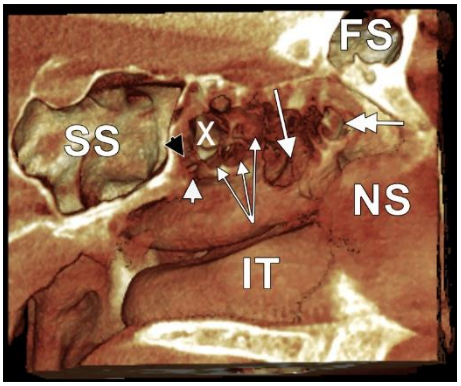

Agger nasi cells were found bilaterally. The right one was draining in the frontal recess and the left one in the ethmoidal infundibulum (Figure 7).

DISCUSSION

As was already discussed in the Introduction, reports of panconcha bullosa, such as this one, are rare. The nasal septum typically deviates away from an asymmetrically enlarged dominant concha bullosa [7].

Nasal septum deformities were classified by Mladina, the left-sided deformities being more prevalent than the right-sided ones [24]. According to these authors, type 5 indicates "an almost horizontal septal spur that sticks laterally and deeply into the nasal cavity," the opposite side of the septum being flat [24].

This corresponds to the septal morphology we found in the present case. The absence of an important septal deviation indicates that we encountered a case of bilateral pneumatizations balanced in size.

Figure 6: Three-dimensional coronal volume renderization of inferior turbinates (ITs). On each side, conchal recesses of the maxillary sinuses (MS) penetrate the turbinate roots (double-headed arrows) to form communicating lamellar inferior conchæ bullosæ. These pneumatizations are located on each side between the ethmoidal process of the ITs (1), which further articulates with the uncinate process (2), and the maxillary process of the ITs (3).

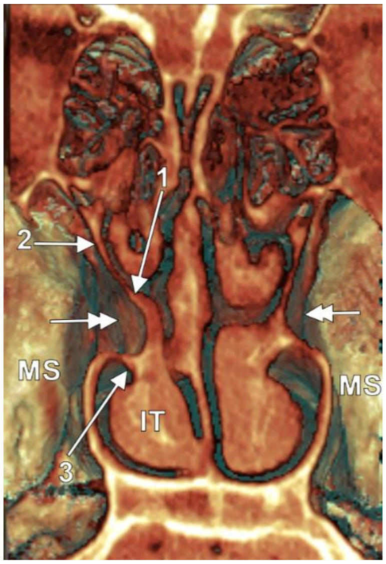

Figure 7: Sagittal MPRs demonstrate bilateral agger nasi cells (*). The right one communicates with the frontal recess (arrow), and the left one drains in the ethmoidal infundibulum (arrowhead).

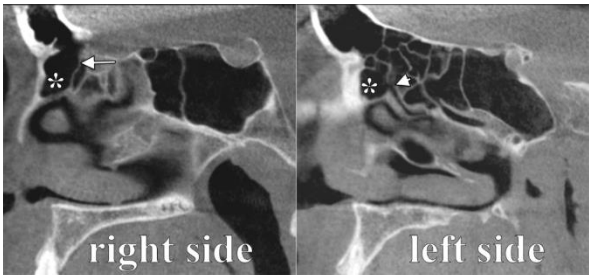

Figure 8: Reprinted from Hindawi – Turhan San, Selma San, Emre Gürkan, and Barış Erdoğan, "Bilateral Triple Concha Bullosa: A Very Rare Anatomical Variation of Intranasal Turbinates," Case Reports in Otolaryngology, vol. 2014, Article ID 851508, 2 pages, 2014. doi:10.1155/2014/851508, which is licensed under CC BY. We indicate (inset, digitally magnified) that the so-diagnosed left inferior conchal pneumatization is merely a strongly curved inferior turbinate, due to a septal spur that pushes the left inferior turbinate.

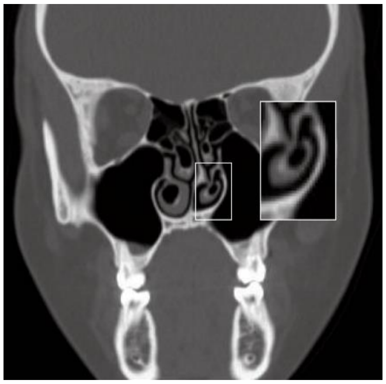

According to Kiroglu et al (2007), the pneumatization of the upper and middle nasal conchæ is commonly encountered, but a concha bullosa inferior is very rare [25]. Koo et al (2017) documented 1188 sides and found a unilateral concha bullosa media in 17.3%, a bilateral concha bullosa media in 36.4%, a unilateral concha bullosa superior in 11.3%, a bilateral concha bullosa superior in 27.4%, and concha bullosa inferior in only 1.0% [13]. A supreme concha bullosa was reported, to our knowledge, only once, by Măru et al (2015) [2].

The first report of an inferior concha bullosa as an anatomic possibility was that of Zinreich et al (1988), while the first paediatric case with a concha bullosa inferior was assumed by Kiroglu et al (2007).

Bolger et al (1991) evaluated in 202 patients the common anatomical variants of the middle nasal turbinate, such as its pneumatization and the paradoxical curvature [26]. In 53% of patients, the authors detected concha bullosa media [26]. They further classified the respective pneumatizations according to their location, within the lamellar part of the middle turbinate, or in its bulbous part, or in both these. It resulted in a classification of concha bullosa media, lamellar, bulbous, and extensive or true, respectively [26].

Further, Yang et al (2008) extended the classification of Bolger et al (1991) to the inferior turbinate [27]. Yang et al (2008) evaluated 59,238 sinonasal CTs and contributed 16 patients with concha bullosa inferior (pneumatized inferior turbinate) to the 28 cases they had already documented as reported at that time [5, 28-41]. They found that in relation with the maxillary sinus, a concha bullosa inferior can be either communicating or non-communicating [27]. This equally feeds the hypothesis of misinvaginated epithelium within the double chondral lamella of the developing inferior turbinate (the non-communicating type) and the hypothesis of maxillary sinus extension within the inferior turbinate (the communicating type) [27]. The communicating type of a concha bullosa inferior was found in 8/18 pneumatized inferior turbinates by Yang et al (2008), thus in 44% of the series. Yang further subdivided the communicating type of a concha bullosa inferior into three subtypes, according to the degree of communicative pneumatization (27). These are (a) a subtle type with a beaked shape of pneumatization, (b) a moderate type in which the pneumatization fills the superior part of the vertical plate of the inferior turbinate, and (c) a marked type, in which the pneumatization completely fills the vertical plate of the turbinate [27]. It is interesting to note in this context a study by Măru et al (2013) in which the authors investigated 100 cases documented by CBCT and found unilateral extensions of the maxillary sinus within the inferior turbinates in 6% and bilateral extensions in 16% , which they termed conchal recesses of the maxillary sinus. Although these last authors did not indicate these recesses as pneumatized turbinates, it is bona fide the same morphology previously described by Yang et al (2008) as pneumatized inferior turbinates. According to the Yang classification, in the case we report here, the inferior conchæ bullosæ we found are of the communicating lamellar type, i.e., the moderate subtype. They can be equally classified as conchal recesses of the maxillary sinuses, however. Such communicating conchal pneumatizations were also indicated as common cavities of the maxillary bone and inferior turbinates [42], thus antro-, or sino-conchal cavities.

One should keep in mind that, according to Yang et al (2008), pneumatized inferior turbinates could be either communicating with the maxillary sinus, or non-communicating. Therefore, a concha bullosa inferior that drains in the inferior nasal meatus is a false one. We support this comment with the report of a "bilateral triple concha bullosa" of San et al (2014), who documented such a concha bullosa inferior only on coronal CT in a four-reference paper in which the study of Yang et al (2008) was not quoted. This is a false concha bullosa inferior that communicates with the inferior nasal meatus, and not with the maxillary sinus; narrowing of the turbinate concavity is rather due to a septal spur on that side (Figure 8). The left nasal spur (Figure 8) corresponds to the apex of maximum left septal deviation and is higher than the right concha bullosa inferior. Therefore, the concha bullosa inferior was not bilateral, as is supported by Tomblinson et al (2016), who demonstrated that the apex of maximum nasal septal contralateral deviation is positioned more superiorly when a unilateral concha bullosa is present and that the severity of septal deviation increases in direct proportion to the concha bullosa's width [7]. Therefore, studies of conchal pneumatization should be evaluated in three dimensions and carefully documented.

In this study, we documented a septated concha bullosa superior that was not, to our knowledge, previously reported. There were, however, a few reports of septated concha bullosa media. The first septated concha bullosa media was found unilaterally and reported by Yanagisawa et al (2008) in a patient elected for functional endoscopic sinus surgery [43]. The second such case was reported by Perić et al (2009), who found a unilateral tri-cameral concha bullosa media [44]. A tri-cameral concha bullosa media was also reported later, by San et al (2013), and was also unilateral [45]. Septation of the middle turbinate is not significantly related to sinonasal pathologies [46]. As supported by our results, the septation of turbinates is not a specific feature of the middle one. Further studies should carefully observe whether the observed conchæ bullosæ contain septa that determine multiple drainage pathways of such pneumatizations.

CONCLUSION

In conclusion, the pneumatization of all nasal turbinates appears as a rare anatomic variation that sums the individual possibilities of the pneumatization of nasal turbinates. Conchæbullosæ are better documented in CBCT and in all three dimensions and, in turn, are of better use for surgeons dealing with functional endoscopic sinus surgery.

Conflict of interests

The authors declare that they have no conflict of interest.

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CLINICAL PRACTICE

Study on the applications of digital smile design planning technique in complete dentures

Ana Maria C. Țâncu[1], Elena Preoteasa[1], Alexandra Totan[1], Ioana A. Guțu[2], Mihaela Pantea[1], Marina Imre[1]

Abstract: *One of the most obvious trends in current prosthetic dentistry is the introduction of digital techniques. Of these, the techniques that offer the possibility of aesthetic prefiguration of the final prosthetic result are of particular interest to the dentist.*

The aim of this paper was to verify the applicability of the Digital Smile Design application, which is the most commonly, used aesthetic prefiguration application, an application designed for the dentate patient, to full edentulism. Two groups of full edentulous patients were formed: the first group, complete denture carriers, dissatisfied with the aesthetic aspects, that they wanted to improve in the new denture, and, the second group, newly bimaxillary full edentulous patients with high aesthetic expectations. Thus, on the first group, the tests were performed on the existing denture, and the second one with the maxillary occlusion template. However, the view of the final result offered the patients the chance to have a realistic picture of their desires regarding the shape, color, size of the teeth, which were inadequate in relation to age and facial typology. The use of this application also improves communication with the dental laboratory. In full edentulism, the current DSD technique can be used with some limitations and the use of it in this field needs more complex studies on larger groups of patients.

Keywords: *digital techniques, smile design, complete denture*

INTRODUCTION

Nowadays, dental aesthetics is becoming more and more a subject of interest in dental practice for those patients who want to improve their smile appearance.

If, some time ago, the esthetic requirement was the prerogative of younger people, now, given the increased time of active life, with the increase of life expectancy, this requirement is frequently found among seniors [1], including the full edentulous ones or those in the imminent moment of full edentulism.

Ageing causes a series of major aesthetic disorders, such as altering the soft parts of the face, emphasizing the perioral lines, oral commissures, nasogenian and mentolabial folds, the loss of elasticity of the tissues and the oro-facial muscles [2]. These signs of physiological aging are associated with the pathological signs following the installation of full edentulism. Oral commissures are lowered, and the mouth slit becomes a line with superior convexity. The lower part of the face is diminished, the prominent menton, thus generating a concave profile [3]. To meet patients' requirements, in order to improve the attractiveness of smiles, practitioners must perform a complex facial assessment in an objective and standardized manner. The fundamental criteria for aesthetic analysis include facial, dental, and dento-gingival parameters.

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Nowadays, digital smile simulation programs have been implemented for both research and clinical practice. These are multifunctional tools that strengthen the way of analysis and diagnosis of the facial and dental parameters, improve communication between team members, allow the education and motivation of patients by visualizing the final results of the proposed treatment solutions, and also increase the degree regarding treatment acceptance, the most commonly used technique within current practice being Digital Smile Design DSD [4-6]. Another smile design digital application for dentate but also for the edentulous patient, which takes into consideration patient's personality is Visagismile. Based on visagisme concept (Philippe Halawell), this digital application helps the dentists in their prosthetic work in order to obtain the best esthetic result reflecting patients' personality [7,8]. Designing the smile based only on mechanical parameters such as shape and size of the face will give a monotonous and stereotype appearance whereas customizing it with the person's attitudes and behavior will make it more life-like [9].

However, it addresses fixed dentures retained on teeth or implants [10]. Of course, the implant based dentures, in particular maxillary overdentures retained on implants, a solution with higher esthetic degree by compensating for the horizontal resorption of the vestibular slope through the denture base, supporting the soft parts, remains the first treatment alternative [11] especially for patients who find themselves in the imminent moment of becoming total edentulous. However, this alternative is often not possible due to extensive surgery, poor health, or high costs, the only solution remaining the conventional complete dentures.

When analyzing specialized literature, we observed that no publications tested the applicability of these systems through conventional complete dentures therapy. Thus, the purpose of our study was to check the possibilities and limitations of digital smile simulation applications regarding the total edentulous patient with conventional complete denture.

MATERIAL AND METHOD

The study was conducted between September 2017 and September 2018 on a group of 12 patients, 8 women and 4 men, aged 40 to 80 years, full bimaxillary edentulous, with and without conventional complete dentures, being treated within the Complete Denture Clinic of "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

The inclusion criteria of patients in the study were represented by the increased aesthetic requirements and expectations, but also the desire to preview the result before the treatment itself. The exclusion criteria were represented by patients manifesting disinterest in aesthetics and by uncooperative ones.

All 12 patients who participated in the trial have expressed and signed an informed consent regarding their participation in the study, publication of personal data and photos.

The working protocol consisted in applying and adapting the digital smile planning workflow to the full edentulous patients using the Digital Smile Design application (DSD Free App by Christian Coachman).

The group of patients was divided in 2 categories in order to compare results: patients with existing dentures, on whom simulations were performed, and patients without dentures, being at the clinical stage of determination of intermaxillary occlusion relations, for whom the upper occlusion template was used.

Patients were clinically examined and evaluated for facial features and smile elements, following these aspects:

- Facial features: Shape and Face Size: length/ width, skin, hair, and eye color.

- Smile components: Labial line of smile, smile arc, upper lip curvature – mouth commissures located at the same level (yes/no), buccal corridor – visibility in mm and gingival smile visibility.

Following extraoral clinical examination, each patient received extra and intraoral photos as indicated by the DSD specific working protocol, which we briefly described: uploading photos, calibration of the frontal facial photo, identification and positioning of the incision line and the lines corresponding to the distal faces of the maxillary central incisors, identifying and marking the labial line of the smile, the smile arc and recording the dimensions of the maxillary central incisors, choosing the appropriate teeth template according to the patient's facial typology and preference, generating the smile frame according to the references set in the previous steps and starting the smile simulation, marking the upper lip contour, choosing the teeth color from a series of four pre-set application shades and individualizing it, applying the false gum and individualizing its color, visualization of the final result.

RESULTS AND DISCUSSIONS

DSD simulation on the existing denture and definition of aesthetic elements that corresponded to the patients' aesthetic expectations were performed for the first group.

Pacient I.E., female, 80 years old, disatisfied with the existing complete denture, due to the colour of the artificial teeth

(Figure 1).

Figure 1: Patient disatisfied with the existing complete denture due to color of artificial teeth

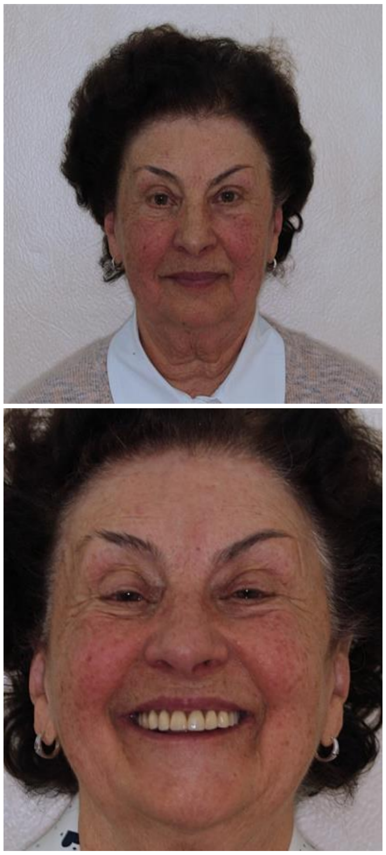

Facial features:
- Face shape: oval;
- Face size: 18 cm long, 16 cm wide;
- Skin color: dark;
- Hair color: brown;
- Eye color: brown.

Smile components:

1. Labial line of smile is at the level of the free gingival border and reveals the entire cervico-incisal height of the maxillary frontal teeth and the interdental papilla.

2. Smile arc is a curved line parallel to the upper border of the lower lip.

3. Upper lip curvature - mouth commissures are at the same level as the lower border of the upper lip and thus they do not have a curve, being straight.

4. Buccal corridor - visible on both sides - 3 mm.

5. The patient does not have a gingival smile.

We uploaded the frontal extraoral photos in smile and with apertures, while the patient had the maxillary denture applied to the prosthetic field, but in the smile simulation, we only used the frontal facial photograph. Calibration of the frontal facial photograph that captures the patient's smile with the maxillary denture was applied to the prosthetic field.

Then, the identification and positioning of the incision line and the lines corresponding to the distal faces of the maxillary central incisors was performed.

Afterwards, the identification and marking of the labial line of the smile, the smile arc, and the recording of the dimensions of the maxillary central incisors were performed.

We roughly established the width of the maxillary center incisor at 8 mm and its length at 10 mm and the application setting at an 84% ratio between width and length. These dimensions were going to be subsequently modified in the smile simulation process for a suitable adaptation of the teeth templates that were pre-set by the application to the elements of the facial assembly.

The next steps were the choice of the appropriate teeth template according to the patient's facial typology and preference, the generation of the smile frame according to the references set in the previous steps and the start of the smile simulation.

For patient I.E. we proposed three types of teeth templates: framing the templates in the smile frame, over the patient's pre-existing teeth, and altering their predefined shape, position, size, and color, depending on the patient's needs and preferences, as well as on the facial, dental, and gingival features. Then, we proceeded by marking the upper lip contour and choosing the teeth color from a series of four pre-set application shades and individualizing it. The last step consisted of applying the false gum and individualizing its color.

Final result

While smiling, the patient discovered the entire cervico-incisal height of the maxillary frontal teeth, thus allowing the proper application of the DSD working protocol, which required such a smile for a proper simulation of the smile and the achievement of aesthetic results. It was possible to keep the initial size of the teeth without distorting it, in order to allow the teeth to fit into the smile frame.

The teeth templates were selected in agreement with the patient, having a similar shape as the original ones. The patient was aesthetically pleased with the old denture, showing the desire to preserve the same aspect, but we tried to suggest other colors for the teeth, more suitable for the skin, eyes, and hair. During her first session, the patient had high aesthetic expectations and wanted a new denture with a teeth color that was much lighter than the initial one, very unaesthetic and inapropriate with her age. However, with the application, the patient being able to preview the final result, she understood the shortcomings of a lighter nuance for the teeth and finally opted for a color appropriate to her age and facial typology.

Figure3: Final result patient I.E.

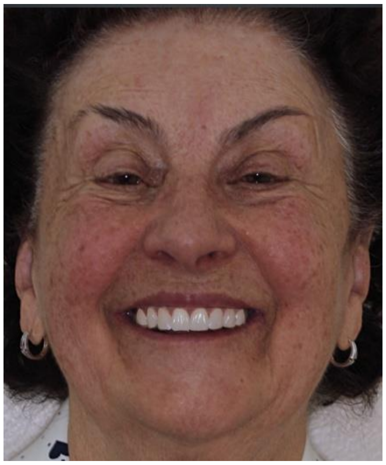

Patient Ș.M., male, 40 years old, bimaxillary full edentulous with great aesthetic expectations (Figure 3).

Figure3: Facial features

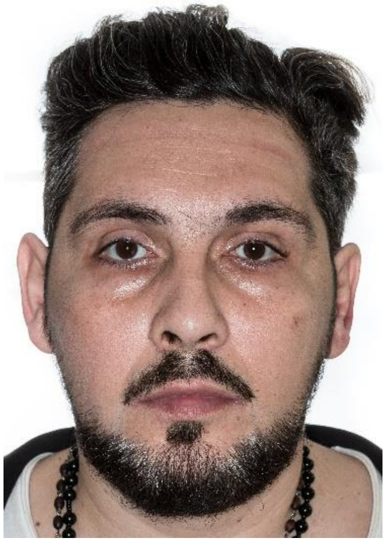

Facial features:
- Face shape: oval;
- Face size: 21 cm long, 18 cm wide;
- Skin color: light;
- Hair color: black;
- Eye color: black.

Smile components:

1. Labial line of smile: it is low and reveals less than 75% of the cervico-incisal height of the maxillary frontal teeth.

2. Smile arc is a curved line almost parallel to the upper border of the lower lip.

3. Upper lip curvature - the right mouth commissure is at the same level as the lower border of the upper lip, and the left one is located below, thus the upper lip has a downward curvature on the left side, while the right side is straight.

4. Buccal corridor – poorly visible on both sides – 2 mm.

5. The patient does not have a gingival smile.

DSD working protocol with example working on the maxillary template (second group).

Figure4: Frontal facial full smile

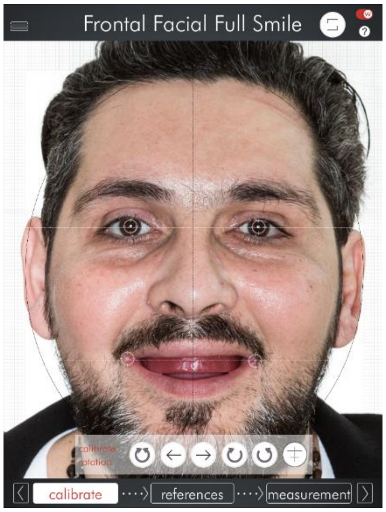
Frontal Facial Full Smile
calibrate
references
measurement

Figure5: Frontal facial full smile and smile design

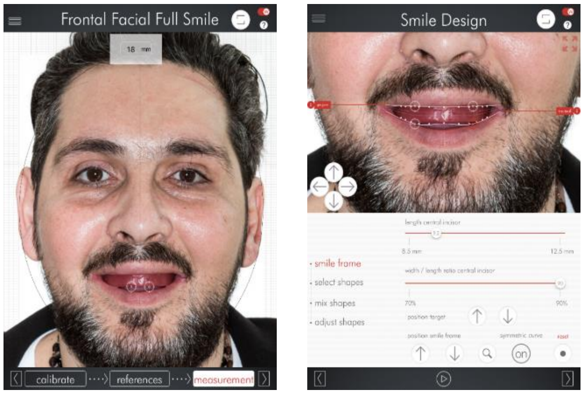
Frontal Facial Full Smile
18 mm
calibrate
references
measurement
Smile Design
length central incisor
8.5 mm
12.5 mm
smile frame
select shapes
mix shapes
adjust shapes
width / length ratio central incisor
70%
90%
position target
position smile frame
symmetric curve
reset
on

We roughly established the width of the maxillary center incisor at 9 mm and its length at 9.2 mm, the application setting a 90% ratio between width and length. These dimensions were going to be subsequently modified in the smile simulation process for a suitable adaptation of the teeth templates that were pre-set by the application to the elements of the facial assembly.

The next step consisted of choosing the appropriate teeth template, according to the patient's facial typology and preference, generating the smile frame according to the references set in the previous steps and starting the smile simulation itself (Figures4, 5).

For patient Ș.M., we proposed 3 types of teeth templates: framing the templates in the smile frame, over the patient's pre-existing teeth, and altering their predefined shape, position, size and color, depending on the patient's needs and preferences, as well as on the facial, dental and gingival features. Then we proceeded by marking the upper lip contour and choosing the teeth color from a series of pre-set application shades and individualizing it. The last step consisted of applying the false gum and individualizing its color.

Figure6: Final result patient Ș.M.

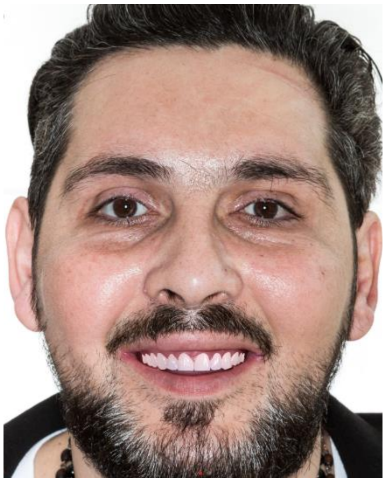

Taking a picture with the maxillary template allowed us to simulate a gingival smile because it had a very good hold on the prosthetic field.

The occlusion template gives total freedom in positioning the reerence points such as the incisal line, the lines corresponding to the distal faces of the maxillary central incisors, the labial line of the smile and the smile arc.

In addition, working on the template, we were able to establish several dimensions and shapes of the maxillary frontal teeth, not having to choose a certain size or shape close to the original one, which would cover the artificial teeth on the patient's old denture. In this case, the application guided us to choose rectangular teeth with well-defined angles for the new denture.

Digital Smile Design (DSD App by Christian Coachman) is a tool for digital smile planning used for total edentulous patients, facilitating the choice of shape, color, and size of the artificial teeth, and the possibility to preview the final result before the actual treatment. Moreover, it favors a faster integration of the denture by the patient through his active involvement in the whole process.

In case of full edentulous patients who cannot provide a gingival smile, either because of a poor maintenance of the maxillary denture that comes off the prosthetic field during the attempt to mimic a forced smile, or because of the patient's impossibility to expose the entire cervico-incisal height of the maxillary frontal teeth during the smile, the application has some limitations and requires a considerable reduction in the size of the teeth, in order to allow their optimal fitting within the smile frame, generating less aesthetic results.

Using a photograph with the maxillary template applied to the prosthetic field, instead of the old maxillary denture, within the smile simulation process, will provide the possibility to choose between several types of teeth templates without any limitations regarding the teeth shape close to the one on the old denture, which would generate similar results to the original design of the denture.

Another advantage of using the occlusion template instead of the denture is its good retention. This way, the patient can mimic a gingival smile without the risk of detaching the template from the field, the resulting photograph allowing the correct application of the DSD working protocol and the achievement of superior aesthetic results.

In terms of colors, the application offers a limited range of only four shades with the possibility of changing their features.

In specialized literature, there are still insufficient articles or studies illustrating the expansion regarding the use of digital smile planning applications in total edentulous patients, despite the fact that they can greatly facilitate the design concept of the complete denture, by being able to preview the final result and choose the shape, color, size and arrangement of the artificial teeth according to the facial typology and patient's wishes, thus the patient being involved in establishing the treatment plan.

CONCLUSIONS

Introducing digital techniques to preview the final result of the prosthetic treatment in current dentistry brings important benefits regarding the success of the treatment, communication with the patient and the dental laboratory.

DSD technique is a tool used in a relatively small percentage in current practice by dental practitioners, only to the dentate patient, which requires the use of digital photographs, templates, and digital applications.

In full edentulism, the current DSD technique can be used with some limitations related to: the dynamics of the lips in smile, execution of the original photographs for which the denture should have a good retention on the prosthetic field and the proposal of a limited number of templates that do not satisfy all facial typologies.

In full edentulism, the vestibular slope of the denture is an important part of its aesthetics, but the application cannot simulate it.

However, the preview of the end result offers the patient the chance to have a realistic picture of his desires, regarding the shape, color, size of teeth, which are inadequate in relation to the patient's age and facial typology.

In the future, studies that are more complex are needed on a larger group of patients, in order to make the application more efficient also in the field of full edentulism.

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VARIA

Determine the medical causes and diseases which led to early and permanent medical disqualification of military cadets

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Abstract: *Background and Aim: Efficient manpower is the most important of military capital, and many studies have shown the relationship between personal health and organizational performance. The preparation of military cadet volunteers to enter aeronautical training and the provision of optimal services in the form of well-trained pilots requires many material spiritual resources that disabilities, make them waste.*

Methods: This is a descriptive, cross-sectional, retrospective study with target population of Iranian air force cadets with early and permanent medical disqualification from 1986 to 2016.

Results: 80 cases were detected, including 25 kills and 55 early and permanent medical disqualification. The main causes were ear, nose and throat, psychiatry and cardiac, respectively. Common diseases include motion sickness, generalized anxiety disorder, adjustment disorder and epilepsy, respectively. The total number of lost service years was 1412 person years and the average of lost service years was 25.67 person years. Most of the lost service years were related to medical causes included ear, nose and throat, psychiatric and cardiac, and diseases included motion sickness, generalized anxiety disorder and adjustment disorder, respectively. Neurologic causes resulted in highest average of lost service years and high blood pressure was the major diseases, respectively.

Conclusions: Due to the lack of similar studies, the results of this study were compared with the data, which extracted from previous studies in the pilot's population. Because of occupational similarity between pilots and cadets, It was expected that the most common causes in pilots and cadets should be the same, but we found that the results are very different.

Keywords: *disabilities, medical disqualification, health, performance, prevention*

INTRODUCTION

Personal resources are the most important capital of armed forces, and many studies have shown the strong links between personal health and organizational performance. In order to increase productivity, organizations have anticipated numerous measures to evaluate pre-employment health and staff health during service. However, health risks remain the most important reducing factor for personal productivity. Disability is defined as the effect of a disorder on physical, mental, and social activity of a person and the work environment and family status are also effective elements. [1, 2, 3] Cadets are the future capital for professional aviation and the sufficient understanding of the medical causes and diseases leading to their early and permanent medical disqualification (EPMD), plays an important role in professional pilot training. According to statics, effective prevention of cardiovascular risk factors in aviation population has a significant role in improving the professional health index of pilots. The study of pilot's work environment and their lifestyle, active intervention, periodic

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regular examinations and screening test for early detection of diseases are among the basic measures. [9] Preparation of cadets to enter pilot's training courses and delivering desirable services in the form of expert military pilots requires 10 million dollars and extreme spiritual costs, and as a result disqualification, it will be a major loss of money and human budget for armed forces [21]. Thus, control of this dilemma has no way except of effective prevention. [1-5]

These days, the economic, mental and social problems caused by morbidity and mortality of armed forces are critical burdens. Regardless of the huge cost to run and maintain of powerful army, the financial and psychological burden of the victims can be devastating for any society. [20] In 1993, the United States army paid 500 million dollars to newly recruited armed forces. [6-7] In a study between 1980 and 1994 on the causes of disability in US air and sea forces, it has been shown that the disable armed forces has led to an excessive cost in about 1.5 million dollars for US army. [1] The federal aviation association(FAA) has conducted a great comprehensive study in 2014 on the causes of medical disqualification for pilots whose results indicate fifteen causes include pectoris angina, bipolar mood disorder, cardiac valve replacement, coronary artery diseases, diabetes mellitus, loss of consciousness with unknown reason, seizure, cardiac replacement, myocardial infarction, permanent pace maker, disabling personal disorder, psychosis, drug abuse and dependency and lack of neural control, respectively. [15] Cadets are the future maker of aviation and can become expert pilots in near future. Unfortunately, so far, there has not been a similar published study in the world, which makes it necessary to accomplish this research more effective and strategic.

The ultimate goal of this study was to determine the causes and diseases which led to EPMD of Iranian Air Force (IRIAF) cadets from 1986 to 2016. Other objectives include determining the exact numbers of cadet and aviation trainee kills in IRIAF, determining the lost service years (LSY) in IRIAF cadets with EPMD (with counting of seven years of aviation training course and a total service duration of 30 years in IRIAF), determination of the average LSY for IRIAF cadets with EPMD and the provision of scientific and practical solution to prevent the EPMD of IRIAF cadets.

METHODS

This study is a descriptive-cross sectional-retrospective research that its target population was the IRIAF cadets with EPMD from 1986 to 2016. IRIAF cadets with medical disqualification from 1986 to 2016, that their relevant official documents have been recorded in medical sessions and their medical profiles were included, but cadets with disqualification due to physical or mental illness and death in non-occupational accidents, non-medical reasons, personal requests or disciplinary punishments were excluded from study.

The ethical approval of this study was issued with registration No: 1016794 by the Ethics Committee of the Aerospace and diving medical faculty in Aja University of medical sciences. This study was conducted by the personal expense of the corresponding author and so, no governmental or military fund was not be used at all.

After obtaining administrative licenses (Allow access to information) and ethics approval (being ethical and maintaining the principle of patient privacy) in research ethics council of aerospace and diving medical faculty (which is subset of AJA University of medical sciences) and coordination with relevant authorities in IRIAF department of health, medical education and services for obtain permission of unlimited access to medical profiles and sessions, in order to maintain the medical confidentiality, the archivist assigned a secret and random code to each participant that was recorded on top of the electronic data forms. Required information including age, year of entry to and excite from service, main medical cause and disease which led to EPMD were inserted in an electronic form designed according to the study requirements. An individual archivist was used to enter data and in order to military and national information security, the total number of IRIAF cadets was not stated.

All of the IRIAF cadets were male and, in few cases, which there were more than one reason for EPMD, only the main cause was considered. Like other similar studies, all medical causes and diseases were classified based on the 10th version of international classification of diseases (ICD-10) and the causes in domain of nephrology and urology have been recorded as renal causes. However, possible differences in the statistics of similar studies may be related to their classification methods for causes and diseases. It should be noted that in any armed force, while training or real battle, casualties are inevitable and so killed cadets are not considered to be among the lost years of service. The data was collected and analyzed by the researcher using Microsoft office software (version 2016) and compared with the results of similar studies in pilot's population.

RESULTS

During the period from 1986 to 2016, 80 cases of early and permanent discharges were obtained from all the medical sessions and medical profiles, including 25(31.25%) kills and

55(67.75%) medical disqualification. The kills also included 17 cadets and 8 aviation trainees. This data are given in Table1.

Table1: Study Data

Occupation	Early and Permanent Discharge	Kills
Cadets	55	17
Aviation Trainees	0	8

Common causes of EPMD in IRIAF cadets included ear, nose and throat (ENT), psychiatric and cardiac, respectively. Their prevalence is depicted in Figure1. Common diseases resulted in EPMD of IRIAF cadets included motion sickness, adjustment disorder, epilepsy and, respectively and shown in Table2.

Figure 1: Causes lead to the EPMD of IRIAF cadets

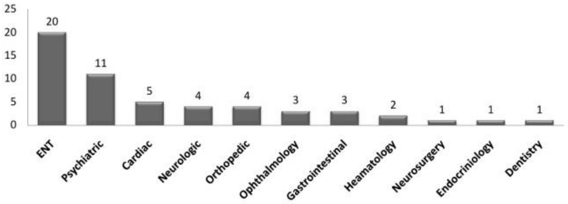

Table 2: Diseases lead to EPMD of IRIAF Cadets

Diseases	Motion Sickness	Generalized Anxiety Disorder	Adjustment Disorder	Epilepsy	High Blood Pressure	Others
N#	18	5	3	3	2	24

As 1412 LSY in IRIAF cadets, the most common causes include ENT, psychiatry and cardiac, respectively. More detail is on Figure2.Common diseases with most of LSY include motion sickness, generalized anxiety disorder, epilepsy, adjustment disorder, and high blood pressure, with detailed statistics in Table 3.

Figure 2: LSY of IRIAF cadets with EPMD according to causes

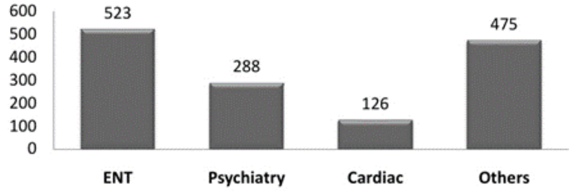

Table3: LSY of IRIAF Cadets with EPMD According to Diseases

Diseases	LSY
Motion Sickness	468
Generalized Anxiety Disorder	137
Epilepsy	82
Adjustment Disorder	74
High Blood Pressure	57
Others	594

The average of LSY in IRIAF cadets with EPMD was 25.67 person years (p/y), the frequency o which based on common causes, including neurology, psychology and ENT with comprehensive information shown in Figure3.

Common diseases resulted in the highest average of LSY in IRIAF cadets with EPMD including high blood pressure, occupational hearing loss, generalized anxiety disorder, epilepsy, and borderline personality disorder, as shown in Table4.

Figure 3: Average LSY of IRIAF cadets with EPMD according to causes

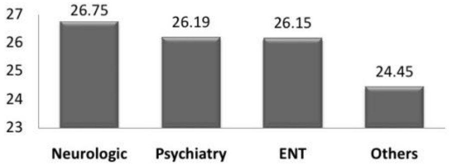

Table4: Average LSY of IRIAF Cadets with EPMD According to Diseases

Diseases	Average of LSY
High Blood Pressure	28.5
Occupational Hearing Loss	27.5
Generalized Anxiety Disorder	27.4
Epilepsy	27.33
Borderline Personality Disorder	26.5
Others	24

DISCUSSION

So far, there has been no investigation into the causes and diseases that lead to EPMD of military or civil cadets in Iran or the world. Therefore, in order to compare the results of this study, the studies in civil and military pilot's population were used, because cadets and pilots have similar occupational and social status. The main reasons for EPMD in IRIAF cadets during our study included ENT, psychiatric, and cardiac, respectively. In the study of Whitton-1984 on United State Air Force (USAF) pilots and navigators, common causes were cardiac and neurologic [16], while in the same study of Mc Crary-2002, the most common causes in 1995 to 1999, include cardiac, musculoskeletal, neurologic and endocrinology. [11] Also, in the study of Montazeri-2005, the most common causes of EPMD in IRIAF pilots included cardiac and musculoskeletal. [19] In the study of Ghazizade-2010 on the same population in 1992 to 2003, cardiac, nervous-skeletal and gastrointestinal were the most common causes. [18]

In the Dark study with the help of FAA, on medical disqualification of American airlines pilots in 1983 and 1984, the most commonly reported causes were cardiovascular and Neuropsychiatric events. [18] In the Nakanishi study on permanent disability of 260 Japanese crews, significant causes include malignancies, neurodegenerative, cardiovascular, gastrointestinal and musculoskeletal disorders. [8] In the review of trained pilots on Commonwealth countries from 1994 to 2004, the causes of cardiovascular were prevalent. [18] In the Arva study on 257 Norwegian civil pilots, the main causes were cardiovascular, neurologic, musculoskeletal and psychiatric disorders. [10] In the studies of Mitchell-2004 and Evan-2006 on sudden incapacitation of British civil pilots, 36 cases have been diagnosed and half of them were due to cardiac and cerebrovascular disorders and psychiatric disorders have been one of the leading causes. [12, 13] In the Nezami study on 200 civil pilots of Vnukovo airport in Moscow, cardiac, neurologic and digestive have been the main causes of medical disqualification. [9] Common diseases resulted in EPMD of IRIAF cadets, including motion sickness, generalized anxiety disorder, adjustment disorder, epilepsy and high blood pressure. In the Mc crary's review on USAF pilots and navigators in 1995 to 1999, more common diseases were ischemic heart diseases, high blood pressure, back pain, inter vertebral disc disorders and migraine. [11] In the study of Nezami on 200 civil pilots of Vnukovo airport in Moscow, ischemic heart diseases, myocardial infarction, high blood pressure, cerebrovascular sclerotic lesions and peptic ulcer have been common. [9]

The LSY was 1412 (p/y) and the highest was related to ENT, psychiatric and cardiovascular causes, respectively. Diseases with the highest LSY included motion sickness, generalized anxiety disorder, epilepsy, adjustment disorder and high blood pressure. The average of LSY was 25.67 (p/y) and the highest was related to neurologic, psychiatric and ENT causes, respectively. Diseases with the highest average of LSY included high blood pressure, occupational hearing loss, generalized anxiety disorder, epilepsy and borderline personality disorder. In the study of Montazeri-2005, the mean LSY of IRIAF retireds with EPMD was 6.5(p/y). [19] In the Ghazizade study on IRIAF medical disables from 1992 to 2003, the mean LSY was 6.14 (p/y) and the highest was 10 (p/y). [18]

This study, like other studies, has limitations that include: 1) the lack of a comprehensive electronic system for record of medical profile, 2) the possibility of oriented malingering in disorders which have not specific objective diagnostic methods and their detection are subjective, such as motion sickness, migraine, irritable bowel syndrome and etc, and 3) flying device factors(ergonomics and exhaustion), operational and airbase factors(work and rest schedule), Personal factors(life style, physical and psychological capabilities, socioeconomic state, use of legal and illicit drugs and habits), medical factors(medical and psychological advices and hygiene education) are important items which have effective influence on cadets health.

The present study has important benefits such as: 1) at yet, this is the first study on EPMD of military cadets in the world, 2) it has the longest cross-sectional period (35 years) that other longest studies has a period of 10 years, and 3) data presentation in order to kills of cadets and aviation trainees.

At the end, we are suggested that, while it is necessary to conduct similar studies in military and civil cadets and pilots (ever-in non-pilots' flight crew), this advises were recommended to prevent the recurrent medical dispensation of military cadets:

1) Facilitate the comprehensive electronic system for record military cadets' medical profile
2) Upgrading of on-the-job examinations of cadets based on the real requirements
3) Sufficient periodic in-service examination of cadets
4) Lifestyle modifications (habits, nutrition, smoking, use and abuse of drugs and substances, physical exercise etc)
5) Use of medical and psychological counseling (motivation, stress management, high-risk behaviors, risk aversion)
6) Hygiene and health education
7) Regular fitness-based training
8) Updating of flying systems, operational devices and air base facilities (ergonomics, work and rest schedule)
9) Effective and powerful preventive systems with regular updating
10) Improvement of services, which provide with occupational health centers.

List of Abbreviations

Early and Permanent Medical Disqualification (EPMD)
Permanent Medical Exchange in Service categories (PMES)
Iranian Air Force (IRIAF)
Lost Service Years (LSY)
Person Years (p/y)
Federal Aviation Association (FAA)
Myocardial Infarction (MI)

10th International Classification of Diseases (ICD-10)
Generalized Anxiety Disorder (GAD)
Post Traumatic Stress Disorder (PTSD)
Ear, Nose and Throat (ENT)
United State Air Force (USAF)

Declarations

Ethics approval and consent to participate:

The ethical approval of this study was issued with registration N# 1016794 by the Ethics Committee of the Aerospace and diving medical faculty.

Consent for publication:

I (Dr HamzeShahali as Corresponding Author), on behalf of the authors, expresses my satisfaction with the publication of this study in that valuable journal.

Availability of data and materials:

All the data of this study is available and could be accessible for publication by that valuable journal.

Competing interests:

I declare that there is no competing interest about the authors of this study, at all.

Funding:

This research was my special doctorate thesis and so, all the expenses were paid personally. I declare that I did not use any government funds to conduct this study, at all.

Authors' contributions:

Title Presentation, basic researching, approval the research subject, obtaining the necessary permits, collecting data and writing the paper was conducted by Dr HamzeShahali as Corresponding Author and Data analysis, editing and writing part of the article was done by Dr AzadeAmirabadi Farahani as Co-Author, too.

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VARIA

Fever of unknown origin

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Abstract: *Fever of unknown origin (FUO) is a diagnosis that describes a prolonged febrile illness without an established cause, despite investigations. From the first definition of the medical condition (given by Petersdorf and Beeson in 1961) the diagnostic criteria have changed over time, intending to improve the management and outcome of these patients. The main causes of FUO fall into 4 categories: infectious, non-infectious (inflammatory), neoplastic and diverse causes. The etiology of FUO has changed over time, due to ever-changing disease patterns and the development of diagnostic techniques. A significant percentage of patients with FUO remain without an etiologic diagnosis, despite advanced diagnostic tests. Gathering potentially diagnostic clues through history, physical examination and nonspecific key paraclinical abnormalities is the basis for the diagnosis of FUO.*

Keywords: *fever of unknown origin, infections, malignancies, systemic inflammatory diseases*

INTRODUCTION

Fever of unknown origin (FUO) was first defined in 1961 by Petersdorf and Beeson as a medical condition that has the following three characteristics [1]:

- Body temperature 38.3° C (101° F) at three or more successive measurements.
- A duration of at least 3 weeks.
- Uncertain diagnosis after one week of hospitalization.

In the following years, the definition of FUO changed. Immunosuppressed patients were excluded from this category because they have etiological peculiarities and require a different therapeutic approach. Thus, the current definition of FUO includes the following diagnostic criteria:

- a body temperature ≥38.3°C (101°F) at least 2 determinations;
- the duration of illness for ≥ 3 weeks or recurrent febrile episodes for ≥ 3 weeks;
- the absence of immunosuppression, defined as HIV infection or known hypogammaglobulinemia, treatment with prednisone 10 mg or equivalent for a duration ≥ 2 weeks in the previous 3 months before the onset of fever, and neutropenia for a period ≥1 week 3 months before the onset of fever;
- uncertain diagnosis, despite a rigorous case history and clinical examination and the following laboratory investigations: complete blood count, erythrocyte sedimentation rate, ferritin, three blood cultures, tuberculin skin test, C-reactive protein, electrolytes, total proteins,

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protein electrophoresis, creatinine, microscopic urinalysis, urine culture, creatine kinase, rheumatoid factor, antinuclear antibodies, alkaline phosphatase, lactate dehydrogenase, aminotransferases, chest X-ray, abdominal ultrasonography.

EPIDEMIOLOGY

Scientific and technological progress in the microbial diagnosis and classification of the pathogenesis of rheumatic diseases have led to a higher rate of etiological diagnosis among patients with fever. Thus, if in 1930, 75% of patients with fever had no identified causes, nowadays-true FUOs are uncommon [4, 5]. The causes of FUO vary greatly with age. Among children, the most common are viral infections, while among adults' multisystem diseases, such as rheumatic diseases or sarcoidosis, are more likely [5, 6]. Furthermore, geographical region and economic development have an important influence on the etiology of this disease. Thus, in developing countries, the most common causes of FUO are tuberculosis, typhoid fever, amebic liver abscesses and acquired immunodeficiency syndrome (AIDS). In developed countries, the travel of inhabitants to certain areas is a risk factor, resulting in the identification of rare diseases, such as malaria, filariasis, schistosomiasis, Q fever, relapsing fever, Zika virus, fever leash, etc. [5, 7]. Travelers may be infected with microorganisms to which local residents are not vulnerable, because of pre-existing immunity. The diseases contracted while abroad may have a very high incubation period, symptoms (such as fever) occurring after a period from the moment of return. This reduces the chance of an etiological diagnosis [5, 8].

A special category of patients is immunosuppressed patients, such as those with AIDS or known neutropenia. In the case of AIDS, the causes for fever are infections (79%) and malignancies (8%). In 9% of cases, the cause cannot be identified. Regarding the infectious agents, more than half of the cases were due to mycobacteria, especially atypical, the most common being the mycobacterium avium complex (MAC). Regarding malignancies among HIV-infected patients, lymphomas, particularly non-Hodgkin's lymphomas dominate [5]. Febrile episodes associated with neutropenia are most frequently caused by bacteremia, followed by the underlying disease, which has resulted in neutropenia, certain drugs, blood products, and in the case of transplanted patients, allograft rejection [5].

Usually, fever improves with an increase in neutrophils. When fever persists, although the neutrophil count has normalized, the diagnosis of hepato-splenic candidiasis can be considered [9].

ETIOLOGY

The etiology of FUO has changed over time, due to ever-changing disease patterns and the development of diagnostic techniques. The main causes of FUO fall into four categories: infectious, non-infectious (inflammatory), neoplastic and diverse causes [10]. There is also a significant percentage of cases when the etiology of fever remains unknown. A prospective multicenter study estimated the percentage of patients with FUO of 51% [11]. Over time, the frequency of malignancies and infectious diseases as causes of FUO has decreased in favor of noninfectious inflammatory diseases and cases without an etiological diagnosis [12]. From infectious diseases, the most common causes of FUO are tuberculosis and abscesses [13]. The most common malignancies presenting with fever are lymphoma, especially non-Hodgkin, leukemia, renal cell carcinoma, hepatocellular carcinoma, and liver metastasis [13]. Common noninfectious febrile inflammatory conditions correlate with patients' age. Thus, in young patients, systemic juvenile idiopathic arthritis and giant cell arteritis are frequent causes of fever [13]; in older patients, connective tissue diseases or granulomatous diseases are more frequently encountered. Table 1 presents the most common causes of FUO [10, 13, 14].

DIAGNOSIS

FUO remains a diagnostic challenge, considering that there are over 200 disorders that enter in the differential diagnosis. The diagnostic approach therefore should focus on a thorough history of the patient and finding hallmark clinical features that are characteristic of each disease, along with selective non-specific laboratory tests [13]. A detailed history of the patient can provide important diagnostic clues for FUO and should aim to orientate the paraclinical investigations necessary for the positive diagnosis [15]; it should include the history of present illness, data about recent travel, past medical history, drug history, social history and family history (Table 2) [16].

Every FUO category has clinical hallmarks the clinician should search during physical examination [15-18]. Searching potentially diagnostic clues (PDC) during the clinical exam is an important step in the diagnostic workup. PDCs are defined as "all localizing signs, symptoms, and abnormalities potentially pointing toward a diagnosis"[19]:

1. Infectious diseases:

- Spinal tenderness points: suggest spinal tuberculosis, subacute vertebral osteomyelitis;
- Hepatomegaly: Q fever, typhoid/enteric fever, rat bite fever, brucellosis;

- Splenomegaly: miliary tuberculosis, Epstein-Barr virus (EBV) infection, cytomegalovirus (CMV) infection, typhoid/enteric fever, subacute bacterial endocarditis, Q fever;
- Regional adenopathy: characteristic of cat-scratch disease;
- Epididymo-orchitis/epididymal nodule: EBV infection, renal tuberculosis, brucellosis;
- Cutaneous findings of endocarditis: Osler nodes, Janeway lesions, splinter hemorrhages;
- Heart murmurs: suggest bacterial endocarditis.

Table 1: The most common causes of FUO.

Infections	Malignancies	Systemic inflammatory diseases	Miscellaneous
• Bacterial (tuberculosis, occult abscess, complicated urinary tract infection, osteomyelitis, Q fever, rickettsia infections, enteric fever, brucellosis) • Viral (Epstein-Barr virus, cytomegalovirus, human immuno-deficiency virus) • Parasitic (malaria, toxoplasmosis) • Fungal (histoplasmosis)	• Hepatocellular carcinoma (or liver metastasis) • Leukaemia • Lymphoma, especially non-Hodgkin's • Myelodysplastic syndromes • Multiple myeloma • Renal cell carcinoma • Sarcoma • Colon cancer • Lung cancer • Atrial myxoma	• Giant cell arteritis • Polyarteritis nodosa • Adult Still's disease • Takayasu's arteritis • Granulomatosis with polyangiitis • Mixed cryoglobulinemia • Systemic lupus erythematous • Sarcoidosis • Granulomatous hepatitis • Antiphospholipid syndrome • Behçet's disease	• Drugs (e.g. sulfone-amides, penicillin, nitrofurantoin, vancomycin, antimalarials, H1- and H2-blocking antihistamines, barbiturates, phenytoin, nonsteroidal anti-inflammatory drugs, hydralazine, methyldopa, quinidine, procainamide, anti-thyroid drugs, etc.) • Environmental (metal and polymer fume fevers) • Factitious fever • Disordered heat homeostasis • Alcoholic hepatitis • Venous thrombosis and thromboembolism • Hematoma • Hyperthyroidism and subacute thyroiditis • Pheochromocytoma • Adrenal insufficiency • Familial Mediterranean fever • Tumour necrosis factor receptor-1–associated periodic syndrome (TRAPS) • Hyper-IgD syndrome • Muckle-Wells syndrome • Familial cold autoinflammatory syndrome

2. Malignant diseases:
- Cachexia;
- Diffusely enlarged lymph nodes: lymphoma;
- Erythema nodosum: lymphoma;
- Sternal tenderness points: bone marrow disorders: preleukemia, myeloproliferative disorders;
- Eye examination: Roth spots - lymphoma, atrial myxoma; cytoid bodies - atrial myxoma; retinal hemorrhages - preleukemia.

3. Systemic inflammatory disorders:
- Skin rash: may suggest systemic lupus erythematosus (SLE), adult Still's disease [14];
- Unequal pulse: Takayasu's arteritis;
- Lacrimal gland enlargement: late-onset rheumatoid arthritis (RA), sarcoidosis, SLE;
- Oral ulcers: Behçet's disease, SLE;
- Lymphadenopathy: SLE, sarcoidosis;
- Heart murmur with negative blood cultures suggests a possible diagnosis of Libman-Sacks endocarditis.

4. Miscellaneous diseases. Miscellaneous fevers of unknown origin are often diagnosed based on historical clues rather than clinical findings. Relative bradycardia may suggest drug fever. Cirrhosis is often overlooked as a possible cause of FUO [15, 16]. Splenomegaly may be another important clue that may suggest regional enteritis, cirrhosis or hyper-IgD syndrome.

Paraclinical investigations

Before initiation of any diagnostic tests, treatments, such as antibiotics or glucocorticoids, must be stopped, as they can mask different signs and symptoms and thus delay the diagnostic process.

In any patient with FUO, the following basic laboratory tests are necessary: erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), hemoglobin, platelet count, leukocyte count and differential, electrolytes, creatinine, total protein, serum protein electrophoresis, alkaline phosphatase, aminotransferases (AST, ALT), LDH, creatine kinase, antinuclear antibodies, rheumatoid factor, urinalysis, together with blood cultures, urine culture, a tuberculin skin test or Interferon-Gamma Release Assays (IGRA), and imaging studies - chest X-ray, abdominal ultrasonography [19].

Table 2: Key clues to FUO derived from history.

Infectious Diseases	Malignant disorders	Systemic inflammatory diseases	Miscellaneous Disorders
Prior/invasive procedures or surgeries (abscess)	Significant weight loss (2 lbs/week)	Arthralgias	Fever periodicity (cyclic neutropenia)
Dentition (apical abscesses, subacute bacterial endocarditis)	Anorexia	Myalgias	History of lymphadenopathy (Rosai-Dorfman, Kikuchi's disease)
Antecedent/concomitant infections	Post-hot bath pruritus	Dry cough (Giant cell arteritis)	Neck/jaw pain (subacute thyroiditis)
Animal/pet contact (Q fever, brucellosis, toxoplasmosis etc.)	History of adenopathy	Visual disturbances (Giant cell arteritis)	Inflammatory bowel disease (regional enteritis)
Mosquito/tick exposure (malaria, babesiosis)	History of malignancy	Acalculous cholecystitis (SLE, peri-arteritis nodosa)	Alcoholism (cirrhosis)
Rodent exposure (leptospirosis, rat bite fever)	Pel-Ebstein fever-week long high fevers with week-long remissions (Hodgkin disease)	Family history of systemic inflammatory diseases	Medications (drug fever)
Blood transfusions (CMV, HIV)	-	Jaw claudication (Giant cell arteritis)	Prolonged immobility, car trips, flights (thromboembolic disease)
Immuno-suppressive drugs	-	-	-
Nocturia (prostatitis)	-	-	-
High-risk sexual practices	-	-	-
Travel to an endemic area	-	-	-

Complete blood count contains often-overlooked clues, for example, leukopenia, monocytosis, lymphocytosis-relative lymphopenia, eosinophilia, basophilia, atypical/abnormal lymphocytes, thrombocytosis, and thrombocytopenia. In FUO, an isolated alkaline phosphatase elevation may be a clue for lymphoma [20, 21, 22]. Eosinophilia may suggest polyarteritis nodosa, drug fever, or visceral leishmaniasis. Low levels of hemoglobin or hematocrit may point toward occult hemorrhage or hematoma (often retroperitoneal).

After all potentially diagnostic clues retrieved from the patient's history, clinical examination and paraclinical investigations have been identified, the clinician should make a list of the most probable diagnoses; further specific investigations should be guided by these diagnoses.

If the diagnosis remains elusive, more advanced paraclinical investigations may be necessary. In patients with hepatomegaly or splenomegaly, abdominal computed tomography (CT) scan may be useful in detecting abnormalities such as adenopathy, abscesses or masses [23]. Echocardiography is important in case of suspicion of endocarditis or atrial myxoma. Positron emission tomography-computed tomography (PET-CT) scans help detect obscure infectious/neoplastic causes of FUO, for example, lymphomas, Q fever endocarditis or aortic graft infection [24]. Magnetic resonance imaging (MRI) may be useful in case of osteomyelitis. Digestive endoscopy may become necessary in case of suspicion of inflammatory bowel disease or sarcoidosis [25].

Invasive tests, such as lymph node biopsy, bone marrow biopsy, epididymal nodule biopsy, liver or ileal biopsy may have a high diagnostic yield [26-29]. However, these tests should be ordered based on narrowed diagnostic possibilities [30, 31].

TREATMENT

Treatment should be directed toward the underlying cause once a diagnosis is made. Because they may mask, delay or obscure the clinical manifestations, empirical therapeutic trials with antibiotics [32, 33], glucocorticoids, or antituberculous agents should be avoided in FUO, except in immunocompromised, neutropenic patients, in case of severe systemic illness or those with a rapid deterioration (patients with central nervous system or miliary tuberculosis or giant cell arteritis) [34, 35].

CONCLUSION

FUO remains one of the most difficult diagnostic challenges, as the differential diagnosis consists of over 200 malignant/ neoplastic, infectious, rheumatic/inflammatory, and miscellaneous disorders. It is essential for the clinician to obtain potentially diagnostic clues through a detailed history of the patient, complete physical examination and guided paraclinical investigations. Interdisciplinary consultations may be appropriate in a majority of patients with FUO.

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VARIA

Developing a conceptual model for healthcare services branding in the military hospital

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Abstract: *Introduction: Given fast grow and competition in the healthcare industry, managers and health activists in this sector need to adopt strategies for branding. The purpose of the study was to develop a conceptual model for branding in the healthcare industry to build brand advocacy and positive brand image.*

Methods: The study includes patients referred to a military hospital in May 2018. Data gathered by questionnaire, and 162 surveys were collected by simple random sampling method. To test the hypotheses, the conceptual model was analysed by structural equation modeling with SmartPLS® software.

Results: The affective commitment and brand trust could predict brand advocacy. CSR significantly impacts brand image and affective commitment, furthermore brand attitude and COB significantly impact brand trust. There was no significant relationship between brand trust and affective commitment with brand image.

Conclusion Organizations can improve brand image and establish brand support through investing resources on branding. This study contributes insights for marketing managers in developing successful branding strategies for health care organizations.

Keywords: *branding, health care industry, hospitals, SEM*

INTRODUCTION

Healthcare services are dynamic, competitive and full of turbulence. The number of hospitals and healthcare providers rising continuously and with this situation, the atmosphere becomes more and more competitive. Furthermore, in Iran, hospitals can't advertise themselves (It's a law) for gaining more patients and customers (Maleki, 2016). In the other view, measuring the efficiency of healthcare services is depending on lots of quantitative and qualitative criteria (like capable employees, behavioural and technical interactions, treatment matter, type of patient, public and private services) and because of that, it is hard to measure (Thantry et al., 2006).

Due to the hard situation for hardware and material distinction, organizations prefer to be distinct in software (Kotler & Keller, 2012). Huge different exists between services and product usage and because of that, considering people who use them, is different and need other conceptualization (Wang et al., 2009; Van Riel et al., 2001). Moreover, a brand in the service sector corresponds with "organization" whereas in the production sector correspond with "product" and according to this, conceptualizing in the service sector is essential (Wang et al., 2009; Chahal & Bala, 2012). When a high value of satisfaction and emotional commitment is issue, branding in hospitals that refer to customer needs is crucial (Kemp et al., 2014).

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Health care is one of the most critical services that a patient receives (Kemp et al., 2014). Like brand in product sector that has socio-psychological attributes, for service sector, brand is same and can be inspiring. This means there is a bond between brand and customers (Kemp et al.). The relationship between brand and customer is binary. Developing relationships between brand and customer can be a challenging procedure and in a different pattern. For example, it is possible that nexus among brand and customer is according to simple or emotional cognition (Park et al., 2009; Thomson et al., 2005; Brakus et al., 2009; Grisaffe & Nguyen, 2011). When there is a strong relationship between brand and customer, the organization has a significant advantage in competitive atmosphere and also can immune organization from rivals (Kemp et al.).

The progress of healthcare services and economic development are closely linked; reaching one without other is impossible (Khodadad & Behboudi, 2017). Branding in healthcare services is a newfound phenomenon, but managers are gaining intuition from its competitive advantage. Meanwhile, hospitals as an expensive part of the healthcare industry that assign a high portion of GDP, need more attention and considering (Nasiripour et al., 2010). Branding plays a vital role in service organizations through increasing customer trust, make a better condition to illustrate services, acts as a mean of competitive advantage and gives value to the customers (Chahal & Bala, 2012). Branding in healthcare services requires a strong and organized commitment to presenting unique services. For having successful branding, managers should have a strategy for maintaining and guiding values for making trust and also managing emotion (Kemp et al., 2014; Mangini, 2002).

The purpose of the study was to develop a conceptual model for branding in the healthcare industry to build brand advocacy and positive brand image.

METHODS

This descriptive study surveyed patients who went to a military hospital in 2018 may, using simple random sampling. To determine the minimum sample size before gathering data Kock and Hadaya (2018) suggest the use of the inverse square root method, which estimates 160 samples. After distributing the questionnaires, 162 samples were gathered. The questionnaire that distributed had two parts, first demographic questions and then 28 questions that evaluate survey variables in Likert-5 scale. The research constructs were developed based on prevalidated measures (Table 1). Instrument items were modified by experts in any aspects and rephrased.

Table 1: Validity and reliability

Construct	Cronbach Alpha (α)	Composite Reliability (CR)	Average Variance Extracted (AVE)	Source
Corporate Social Responsibility	0.856	0.920	0.700	Kemp et al, (7)
Brand Attitude	0.849	0.906	0.710	Kemp et al, (7)
Customer-oriented Behavior	0.9	0.929	0.768	Kemp et al, (7)
Affective Commitment	0.883	0.936	0.831	Kemp et al, (7)
Brand Trust	0.890	0.932	0.821	Kemp et al, (7)
Brand Image	0.809	0.872	0.583	Wu (67)
Brand Advocacy	0.739	0.869	0.623	Kemp et al, (7)

After data gathering, single variable and two variables measurement analyses with SPSS® version 25 software and analysis in structural equation modeling calculated with Smart-PLS® version 3.2.7 (Ringle et al., 2015). Cronbach alpha (recommended threshold ≥ .7) (Cronbach, 1951) and composite reliability (CR) (recommended threshold ≥ .7) (Hair et al., 2016) estimated for reliability and average variances extracted (AVE) (recommended threshold ≥ .5) (Fornell&Larcker, 1981) estimated for validity with Smart-PLS®. Results of Validity and Reliability analysis (Table 1) exemplified acceptable value as exceeded recommended threshold, which is an indication of a validated and reliable instrument.

RESULTS

Researchers can figure out data essence with descriptive results. Results of descriptive analysis like gender, age, education, insurance and hospital familiarity (or use duration) are in Table 2.

Table 2: Demographic statistics

Measure		Frequency	Percentage
Gender	Male	94	53.4
	Female	82	46.6
Age	Under 20	8	4.5
	21-30	54	30.7
	31-40	47	26.7
	41-50	33	18.8
	Above 50	34	19.3
Education	Under diploma	40	22.7
	Diploma	50	28.4
	Bachelor	56	31.8
	Master	24	13.6
	Doctoral	6	3.4
Having Insurance	Yes	170	96.6
	No	6	3.4
Time of being hospital customer or (familiarity)	Under 1 year	78	44.3
	1 year	30	17
	2 years	21	11.9
	3 years	10	5.7
	Above 3 years	37	21

Most participants were male (53.4%), 30.7% were 21-30 years old, 31.8% had bachelor degree, almost every participant has insurance (96.6%), and 44.3% of participants were being hospital customer under one year. Constructs averages and standard deviations are in Table 3.

Table 3: Average and Standard Deviation of research constructs

Construct	Average	Standard Deviation
Corporate Social Responsibility (CSR)	3.806	0.971
Brand Attitude (BA)	4.117	0.514
Customer-oriented Behavior (COB)	3.458	1.239
Affective Commitment (AF)	3.448	1.266
Brand Trust (BT)	4.152	1.06
Brand Image (BI)	4.190	0.793
Brand Advocacy (BA)	4.024	0.743

In two variable analyses, researchers used Spearman coefficient correlation. Results of this analysis are in Table 4.

Results of assessing hypotheses using structural model in smart-PLS® (Ringle et al., 2015) (standard and T-values, values in parenthesis in the model) are in Figure 1 which shows H1a, H1b, H2a, H2b, H3 and H4 confirmed and H2c, H5 and H6 rejected. Results are in Table 5.

Figure 1

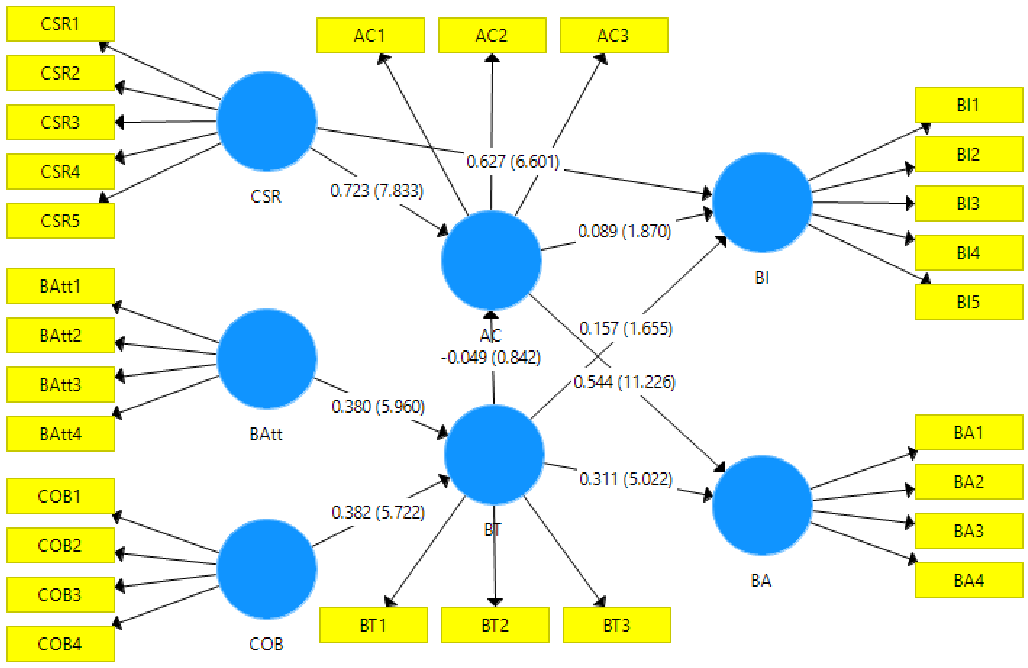
CSR1
CSR2
CSR3
CSR4
CSR5
CSR
AC1
AC2
AC3
AC
BI1
BI2
BI3
BI4
BI5
BI
BAtt1
BAtt2
BAtt3
BAtt4
BAtt
COB1
COB2
COB3
COB4
COB
BT1
BT2
BT3
BT
BA1
BA2
BA3
BA4
BA
0.627 (6.601)
0.723 (7.833)
0.089 (1.870)
0.157 (1.655)
-0.049 (0.842)
0.544 (11.226)
0.380 (5.960)
0.311 (5.022)
0.382 (5.722)

Structural model assessment: For assessing quality of structural model, R2 considered as criteria for assessing model (Hair et al., 2016) and results show that all variables are more than average (0.33 is the mid number). Table 6 shows the values.

Table 4: Spearman Correlation coefficient, all the numbers are significant at 0.01 level

Construct	CSR	BA	COB	AF	BT	BI	BA
Corporate Social Responsibility	1						
Brand Attitude	0.602	1					
Customer-oriented Behavior	0.501	0.262	1				
Affective Commitment	0.598	0.436	0.334	1			
Brand Trust	0.775	0.467	0.500	0.485	1		
Brand Image	0.712	0.477	0.463	0.481	0.637	1	
Brand Advocacy	0.666	0.445	0.379	0.701	0.627	0.571	1

Table 5

Hypothesis	Independent variable	Dependent variable	Path coefficient (β)	T-value	P-value	result
H1a	Corporate Social Responsibility	Brand Image	0.627	6.601	<0.01	Confirmed*
H1b	Corporate Social Responsibility	Affective commitment	0.732	7.833	<0.01	Confirmed*
H2a	Brand Attitude	Brand Trust	0.380	5.960	<0.01	Confirmed*
H2b	Customer-oriented behavior	Brand Trust	0.382	5.722	<0.01	Confirmed*
H2c	Brand Trust	Affective Commitment	-0.049	0.842	0.420	Rejected
H3	Affective Commitment	Brand Advocacy	0.544	11.226	<0.01	Confirmed*
H4	Brand Trust	Brand Advocacy	0.311	5.022	<0.01	Confirmed*
H5	Affective Commitment	Brand Image	0.089	1.870	0.059	Rejected
H6	Brand Trust	Brand Image	0.157	1.655	0.109	Rejected

* Significant at 99%

Table 6: Model assessment results

Construct	Average
Affective Commitment	0.446
Brand Trust	0.346
Brand Image	0.657
Brand Advocacy	0.553

Finally, according to Tenenhaus, the goodness of fit measured and according to formula

$$\mathrm{GOF} = \sqrt{\mathrm{Communality} \times \mathrm{R}^2} = 0.492$$

calculated that exhibits well goodness of fit (Tenenhaus et al., 2004).

DISCUSSIONS AND CONCLUSIONS

This research aimed to find a pattern for branding in the healthcare industry. Hence, variables like corporate social responsibility, brand attitude, customer-oriented behavior, affective commitment, brand trust, brand image, and brand advocacy recognized and the relationship between them through a conceptual model is investigated. Results showed corporate social responsibility has a significant effect on emotional commitment and brand image; these results are in accordance with recent researches (Kemp et al., 2014; Alrubaiee et al., 2017; Kolade&Ogunnaike, 2014). Brand image mostly previses corporate social responsibility. Therefore, by developing corporate social responsibility, brand image become better in customer mind, and marketing performance will improve. Besides, managers can determine policies and strategies in marketing more efficient due to this results and structures. Furthermore, it is crucial for hospitals to invest and help society, which can create an emotional bond and affective commitment.

According to the results, brand attitude and customer-oriented behavior predicted brand trust, and this is following Kemp et al. research (Kemp et al., 2014). Thus, creating a positive brand attitude and customer-oriented behavior from employees will improve brand trust. Educating employees for having better conduct and behavior with customers can improve customer-oriented behavior. Since the healthcare industry is a personal service and a close relationship exist between parties, trust is crucial due to personal information, mental and physical status exposure to healthcare providers (Kemp et al.).

Brand trust could not predict affective commitment that is in the opposite of previous surveys (Kemp et al., 2014; Patawayati et al., 2013; Feiz et al., 2016). However, it could predict brand support that is in accordance with previous researches (Kemp et al.; Moreira & Silva, 2015; Ansari &Mohammadi, 2016). Results showed with brand trust rise up; brand support will rise simultaneously. Hospitals should focus on high trust level in marketing (Kim et al., 2004). When a customer trust in healthcare service provider, it is possible that he/she continues to use that service provider and suggests it to others. Hospitals should use their sources to invest in developing more trust for creating more brand advocacy. Results showed affective commitment could predict brand advocacy and it is in accordance with previous surveys (Kemp et al.; Moreira & Silva; Feiz et al., Ansari &Mohammadi), so by developing an emotional relationship with customer, he/she becomes a brand advocator and finally, competitive advantage will be created.

This research demonstrates the advantages of branding and establishing a meaningful relationship with customers. Results of this research can help marketing managers and strategic planners. In the healthcare industry, vicinity with a hospital is a key factor for productivity (Beckham, 2000). Nevertheless, with increasing healthcare costs and more options for customers, marketers can act more strategic through creating competitive advantage, and distinguishing customers' needs (Brakus et al., 2009).

Healthcare organizations should effectively put their organization and its brand as a valuable factor in providing unique services. As proposed in this research, corporate social responsibility, affective commitment, customer-oriented behavior, customer engagement (like customer relationship management) and trust, are significant elements to reach this goal.

Although this study can help in creating and developing brands in healthcare services, future research and surveys in this field are imperative. Several limitations have existed in this study. First, as data gathered in a cross-sectional way, no cause and effect relationship can be established about research variables. Investing in a relationship between latent variables through longitudinal data is suggested. Future studies can investigate how the correlation between performance and actual satisfaction can create a connection between brands and consumers. Second, some discordance observed between the results of this research and literature, so the research results should expand cautiously. Third, respondents to questionnaires are from the same region so, gathering data from diverse regions is suggested. Forth, in this study, healthcare providers were limited to hospitals, more scopes like pharmacy, clinics, medical imaging, laboratories, and dental services should be investigated. Finally, respondents may somehow were not in their best physical and mental condition, so this maybe affects the data gathering and analysis.

At least once in a lifetime, people use healthcare services, and because of that, good experience and relationship with providers are crucial. As changing perspective in healthcare services in future years, research opportunity in strategical branding and marketing fields will be plentiful for healthcare service providers.

Conclusion Organizations can improve brand image and establish brand support through investing resources on branding. This study contributes insights for marketing managers in developing successful branding strategies for health care organizations.

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VARIA

Redo procedure following Zenker`s diverticulum treatment – a clinical study

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Abstract: *Introduction: The prognosis of patients with Zenker`s diverticulum is dependent mainly on the type of procedure performed, either surgical or rigid/flexible endoscopy.*

Purpose - evaluation of the redo procedure by reviewing correlations in patients with iterative procedures.

Material and Methods: We included in the study 36 patients with cervicomediastinal Zenker diverticula treated from 2010 to 2017 at two university hospitals: Clinic of General and Esophageal Surgery. Mary Clinical Hospital Bucharest and Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca. For the patients included in the study we studied correlations of patients with symptoms persistence and to those with reintervention with epidemiological, clinical and the treatment criteria.

Results: Postprocedural symptoms we observed in 5 patients of the 29 treated endoscopically and 6 of the seven treated surgically. Statistical correlations were noted in patients with persistent symptoms after surgical treatment ($p <0.01$) with patients with diverticulectomy (54.5%) ($p <0.01$), grade III diverticula (36.4%) ($p <0.01$) statistical association was noticed in patients who have required reoperation and those with persistent symptoms (63.6%) ($p <0.01$) and with remaining dysphagia (100%) ($p <0.01$) and those with grade 2 dysphagia persistence (71.4%) ($p <0.01$), grade 3 diverticula (42.9%) ($p <0.03$) and the high number of comorbidities. ($p <0.04$)

Conclusion: Undoubtedly, endoscopic treatment is being used more and more even for those cases that require further treatment. Reoperation has been necessary for patients with persistent dysphagia with voluminous diverticula and a high number of comorbidities.

Keywords: *diverticulum Zenker, endoscopic diverticulostomy, diverticulectomy, iterative procedure*

INTRODUCTION

Zenker`s diverticulum is a cause of morbidity in elderly patients. According to van Overbeek's theory, the predominant role in diverticulum formation has an anatomical predisposition represented by Killian dehiscence weakness. [1] The externally surgical approach has long been the conventional treatment with satisfactory clinical success rates ranging from 80-100%. [2]

The prognosis for these patients depends on the surgical procedure performed. Some authors stated that diverticulectomy patients have a long-term symptom recurrence rate of 2% to 33%. [3] Studies have been published since 1981, which noted that surgical resection or suspension of the pouch performed without a concomitant

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myotomy fails to improve dysphagia and fail to prevent complications or recurrence of the diverticulum, a fundamental consideration of the endoscopic techniques developed later. [4]

Some authors found that patients with endoscopic stapler technique, have a long-term symptom recurrence rate ranging from 0 to 47%. Most authors consider that the reasons for symptomatology after the endosurgical approach is the incompatibility of the dimensions of the septum with the length of the stapler (3 cm). A small diverticulum limits access to the tip of the stapler and it may not be possible to perform a proper myotomy. [5]

Data from the literature indicate a recurrence rate for laser endoscopic diverticulostomy of about 5-25%. [6, 7]

Flexible endoscopic therapy is a relatively new approach compared to rigid endoscopic and surgical techniques. Data from the literature on flexible endoscopic therapy show that symptomatic improvement was observed at 85%, with a symptom recurrence rate of 18-20%. [8-11]

The higher recurrence rate after flexible endoscopic treatment could be caused by incomplete septal dissection. Some authors have shown that the success of the treatment correlates with the length of the septotomy. The risk of perforation by over dividing muscular fibers and difficult identification of the last fibers of the cricopharyngeal muscle could make the endoscopist to leave a residual part of the septum to avoid perforation into the mediastinum. [12]

MATERIAL AND METHODS

We included in the study group 36 patients with Zenker diverticula treated during 2010/2017 in two university hospitals: Clinic of General and Esophageal Surgery Sfanta Maria Bucuresti and Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca.

Inclusion criteria: patients with Zenker diverticulum admitted and treated endoscopically or by open surgery in the hospital during 2010/2017, over 18 years of age, investigated with barium swallow and upper GI endoscopy, patients with a follow-up period of at least 12 months post-procedure.

Exclusion criteria: immunocompromised patients, patients with associated malignant disease, patients lost from post-procedure follow-up.

All patients in the group were investigated by clinical examination, imaging with a barium swallow, upper endoscopy and functional tests with esophageal manometry when this exploration was possible.

Dysphagia was a constant symptom at presentation to the hospital, as well as the main symptom followed in the evaluation of the treatment efficiency. For the assessment of this symptom we used Mellow-Pinkas classification of dysphagia applied to the type of food that cannot be swallowed - grade 0 - No dysphagia, grade 1 - Dysphagia for solids, grade 2 - Dysphagia for semisolid, grade 3 - Dysphagia for solids and liquids grade 4 - Total dysphagia (inability to swallow saliva).

The sizes of the diverticula were appreciated both in centimeters and in degrees according to the Overbeeck classification by corroborating data obtained from the endoscopic exploration and the appearance of the barium contrast imaging. 1 – the small sac that has less than 1 vertebral body in length; 2 –intermediate sac 1-3 vertebral bodies in length; 3 – large sac greater than 3 vertebral bodies in length.

For all the patients surgically treated by cervicotomy, the resection of the diverticulum was performed with myotomy and manual esophageal suture, either single or double layer, or mechanically using a linear stapler. For the patients treated endoscopically, myotomy was performed with creating a wide communication of the diverticulum with the esophagus without resection of the diverticular pouch. A soft, flexible overtube (Cook Medical, Indiana, USA) was used to expose the septum, which was placed over the endoscope, with the short blade placed in the diverticulum and the long blade in the esophagus. After the septum was exposed, it was divided on the midline, from the esophageal lumen to the diverticular pouch, on an average length of 1.5 cm using a Dual knife (Olympus Co., Japan) followed by the closure of the mucosa with clips (2 -5 clips). All patients were clinically and imaged monitored at 3-month intervals during the first post-procedural year.

Study design

For the patients included in the study we studied correlations of patients with symptoms persistence, and to those with reintervention with epidemiological, clinical and the treatment criteria.

Statistical analysis

In comparison with the quantitative data, by the normality of the data, Student's t-test (for two groups with normally distributed data) and Mann-Whitney and Kruskal-Wallis (for data without normal distribution) were used. For the categorical data (synthesized as frequencies and percentages), Fisher exact tests (binary data) and Pearson

Chi-square were used as well as Likelihood Ratio (if 20% of expected frequencies were less than 5). The probability of error of less than 5% (p <0.05) was considered as a significance threshold. The paper also included graphic representations, such as stratified bar type for data synthesized as frequencies and percentages.

RESULTS

Patients included were 29 patients treated endoscopically by myotomy in the Department of Diagnostic and Interventional Digestive Endoscopy of the Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor, Cluj-Napoca and 7 patients surgically treated by resection of the diverticulum associated with myotomy. In the Clinic of General and Esophageal Surgery of St. Mary's Hospital Bucharest. The intraprocedural complications were laminar hemorrhage in three patients during the endoscopic procedure, and one surgically treated patient developed a postoperative hematoma that required drainage. Post-procedural mortality was 0%, and morbidity was 13.88% - five patients (3 patients after endoscopic treatment and 2 patients after surgical treatment).

Persistent postinterventional symptomatology was reported by 11 patients and consisted of the persistence of dysphagia of a lower degree associated or not with the persistence of other symptoms: sialorrhea, regurgitation, nausea, and cough.

We did not observe associations regarding the correlation of the persistence of post-procedural symptomatology with the patients' sex, initial symptomatology or with diverticulitis. (Table 1)

Table 1: Correlations of post-intervention symptomatology - statistical processing of data

	Post opSymptoms = Yes (N=11)	Post opSymptoms= No (N=25)	P_value (test)
Sex=M	7/11 (63.6%)	14/25 (56.0%)	0.728773 (Fisher's Exact Test)
Grade			
1	0/11(0.0%)	2/25 (8.0%)	**0.063384** (Likelihood Ratio)
2	5/11 (45.5%)	15/25 (60.0%)	
3	2/11 (18.2%)	7/25 (28.0%)	
4	4/11 (36.4%)	1/25 (4.0%)	
No Symptoms			
1	3/11 (27.3%)	8/25 (32.0%)	0.266965 (Likelihood Ratio)
2	1/11 (9.1%)	4/25 (16.0%)	
3	4/11 (36.4%)	11/25 (44.0%)	
4	1/11 (9.1%)	2/25 (8.0%)	
5	2/11 (18.2%)	0/25 (0.0%)	
Proceduresurgicalendoscopic			
	6/11 (54.5%)	**1/25 (4.0%)**	**0.001423** (Fisher's Exact Test)
	5/11 (45.5%)	**24/25 (96.0%)**	
Pouch resection			
Yes	**6/11 (54.5%)**	**1/25 (4.0%)**	**0.001423** (Fisher's Exact Test)
Div Dimensions			
1	2/11 (18.2%)	12/25 (48.0%)	**0.003085** (Likelihood Ratio)
2	5/11 (45.5%)	13/25 (52.3%)	
3	**4/11 (36.4%)**	**0/25 (0.0%)**	
Dysphagia post op			
Yes	**10/11 (90.9%)**	**1/25 (4.0%)**	**0.000000** (Fisher's Exact Test)
Dysphagia Grade			
0	**1/11 (9.1%)**	24/25 (96.0%)	**0.000000** (Likelihood Ratio)
1	**4/11 (36.4%)**	1/25 (4.0%)	
2	6/11 (54.5%)	0/25 (0.0%)	
Hospitalisation			
	7.55±5.5563	2.56±1.5832	**0.005955** (Mann-Whitney Test)
	8.00 [2.00, 11.00]	**2.00 [2.00, 3.00]**	

Post-procedural symptomatology we observed only in five patients out of the 29 endoscopically treated and in six out of the seven surgically treated. The statistical association was observed for the type of intervention practiced – endoscopic or surgical treatment (p <0.01) and the persistence of postintervention symptomatology (endoscopic treatment = 45.5%) compared to those without postinterventional symptomatology (endoscopic treatment = 96.0%) – 96% of patients who did not report persistent symptoms had endoscopic treatment. (Table 1) (Figure 1)

Figure 1: Distribution of patients according to the presence of the post-intervention symptomatology to the surgical or endoscopic procedure type

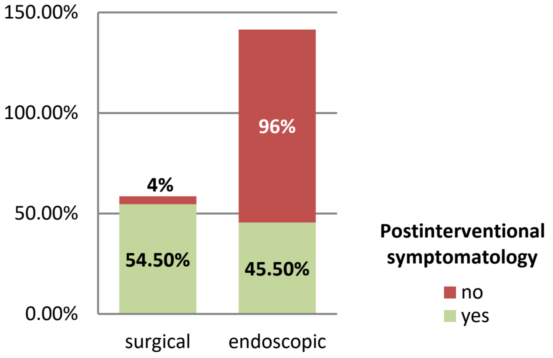

Resistant post-procedural symptoms may be influenced by the type of technique performed: resection of the diverticular pouch or just opening, and association of the myotomy or not. For the patients in the group, the resection of the diverticulum was performed only in the patients treated with open surgery.

The statistical association we observed comparing the percentage of resected Zenker's in patients with persistent postinterventional symptomatology (54.5%) compared to those without postinterventional symptomatology (4.0%) (p <0.01). (Table 1)

Of the 11 patients with postinterventional symptomatology, four patients had grade three diverticula. The correlation of the grade of the diverticulum with the persistence of the post-procedural symptomatology determined a statistical association to the dimension appreciated in degrees according to the Overbeeck classification.

The statistical association was found by correlating the persistence of postintervention symptomatology in patients with grade III diverticula (36.4%) compared with those without postinterventional symptomatology (0.0%) (p <0.01) (Table 1) (Figure 2)

Figure 2: Distribution of patients with post-intervention symptomatology to the dimensional degree of the diverticulum.

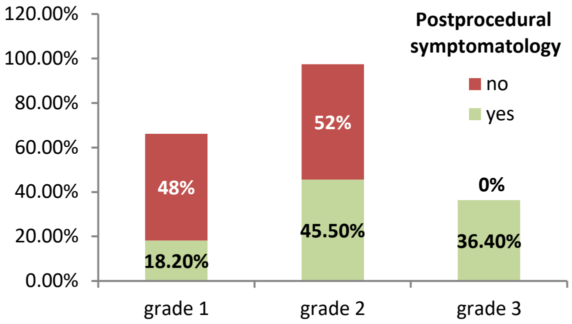

In the post-procedural symptomatology dysphagia was the dominant symptom of 10/11 patients – 4 patients had dysphagia grade 1 and 6 patients dysphagia grade 2. The statistical association was observed between the percentage of patients with delayed dysphagia (p <0.01) and those with postinterventional symptomatology (90.9 %) compared to those without postinterventional symptomatology (4.0%). (Table 1) Persistent postinterventional symptomatology was mainly dysphagia.

Post-procedural persistent dysphagia was reported by 11 patients – 5 patients after surgical treatment and 6 after endoscopic treatment, 10 patients had a lower degree of dysphagia than at the initial presentation. Of the 5 patients surgically treated for 3 dysphagia persisted in the first postoperative month and did not require treatment, for the 2 patients with grade 2 dysphagia, one required re-intervention for a hematoma, and another one with esophageal fistula required adequate surgical treatment. We observed a statistical association between persistent dysphagia and surgical treatment (p <0.03), probably due to the small number of patients treated surgically. (Table 2) (Figure 3)

Figure 3: Distribution of patients according to the persistence of postintervention dysphagia to the surgical or endoscopic procedure type.

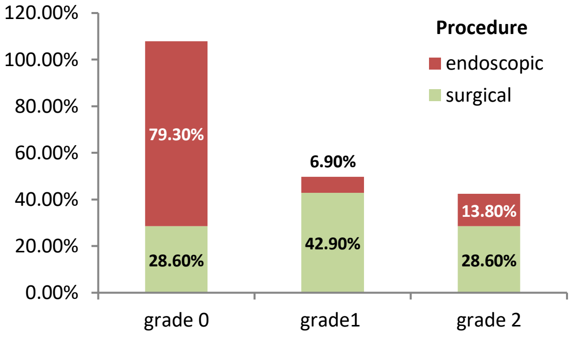

Tabel 2: Statistical correlation for redo procedure - statistical data analysis.

	Redo=Yes (N=7)	**Redo=No (N=29)**	**P_value (test)**
Sex=M	4/7 (57.1%)	17/29 (58.6%)	1.000000 (Fisher's Exact Test)
Grade			
1	0/7 (0.0%)	2/29 (6.9%)	0.328714 (Likelihood Ratio)
2	5/7 (71.4%)	15/29 (51.7%)	
3	2/7 (28.6%)	7/29 (24.1%)	
4	0/7 (0.0%)	5/29 (17.2%)	
No Symptoms			
1	3/7 (42.9%)	8/29 (27.6%)	0.633852 (Likelihood Ratio)
2	1/7 (14.3%)	4/29 (13.8%)	
3	3/7 (42.9%)	12/29 (41.4%)	
4	0/7 (0.0%)	3/29 (10.3%)	
5	0/7 (0.0%)	2/29 (6.9%)	
Hospitalisation			
	6.29±6.7506 **3.00 [2.00, 14.00]**	3.55±2.9469 **2.00 [2.00, 3.50]**	0.360412 (Mann-Whitney Test)
Persistent dysphagia			
yes	**7/7 (100%)**	**4/29 (13.8%)**	**0.000040** (Fisher's Exact Test)
Persistent Dysphagia grade			
0	**0/7 (0.0%)**	**25/29 (86.2%)**	**0.000009** (Likelihood Ratio)
1	2/7 (28.6%)	3/29 (10.3%)	
2	**5/7 (71.4%)**	**1/29 (3.4%)**	
Sialorrhea			
Yes	2/7 (28.6%)	2/29 (6.9%)	0.162567 (Fisher's Exact Test)
Size			
	3.86±1.2149 4.00 [3.00, 5.00]	**2.93±1.2516** 3.00 [2.00, 3.50]	**0.086359** (Student T)
Size classif.			
1	1/7 (14.3%)	13/29 (44.8%)	**0.023010** (Likelihood Ratio)
2	3/7 (42.9%)	15/29 (51.7%)	
3	**3/7 (42.9%)**	**1/29 (3.4%)**	
Procedure			
surgical	2/7 (28.6%)	5/29 (17.2%)	0.601669 (Fisher's Exact Test)
endoscopic	5/7 (71.4%)	24/29 (82.8%)	
Miotomy			
Yes	7/7 (100%)	28/29 (96.6%)	1.000000 (Fisher's Exact Test)
Pouch resection			
Yes	2/7 (28.6%)	5/29 (17.2%)	0.601669 (Fisher's Exact Test)
Intra op morbidity			
	1/7 (14.3%)	2/29 (6.9%)	0.488235 (Fisher's Exact Test)
Comorbidity			
yes/no	7/7 (100%)	21/29 (72.4%)	0.309298 (Fisher's Exact Test)
Comorbidities – No			
0	0/7 (0.0%)	8/29 (27.6%)	**0.033327** (Likelihood Ratio)
1	3/7 (42.9%)	5/29 (17.2%)	
2	4/7 (57.1%)	7/29 (24.1%)	
3	0/7 (0.0%)	8/29 (27.6%)	
4	0/7 (0.0%)	1/29 (3.4%)	

HTN	4/7 (57.1%)	15/29 (51.7%)	1.000000 (Fisher's Exact Test)
DM	0/7 (0.0%)	5/29 (17.2%)	0.558991 (Fisher's Exact Test)
Dyslipidemia	2/7 (28.6%)	12/29 (41.4%)	0.681030 (Fisher's Exact Test)
Other diverticula	1/7 (14.3%)	3/29 (10.3%)	1.000000 (Fisher's Exact Test)

Post-procedural sialorrhea was reported by four patients. It was not present as a unique symptom but it was always associated with persistent dysphagia. Post-procedural sialorrhea persisted in four patients.

Reintervention was necessary when the persistence of post-procedural symptoms was recorded for 7/11 patients. For the 5 patients treated endoscopically, who required reintervention for the persistence of the symptomatology, an average interval of 22.8 months was observed, while the reinterventions for the 2 patients treated by open surgery happened during the postoperative period. The statistical association was observed in patients who needed reintervention and those with postinterventional symptomatology (63.6%) ($p < 0.01$) (Table 1)

DISCUSSIONS

When comparing the technical and clinical results from the literature on the treatment of Zenker diverticulum, it should be critically emphasized that a direct comparison between studies and results may be inadequate, as the data are neither homogeneous nor standardized in regards of multiple variables. Noteworthy in this regard: symptom collection (dysphagia, dysphagia plus regurgitation, cumulative symptoms), symptom evaluation (objective scores for dysphagia, subjective grading of symptom relief and satisfaction), choice of one or another technique (sequentially, size of the diverticulum, clinical conditions, local policy), the definition of clinical success (total disappearance of symptoms, disappearance with improvement), success and extent of recurrences (after one or more sessions) and variable duration of follow-up (the shortest follow-up programs being reported after endoscopy) flexible, compared to the more recent series of patients treated transorally and with a historical cohort of patients treated surgically).

Patient follow-up included history taking, clinical examination, imaging investigations. Clinical criteria followed were persistence or recurrence of dysphagia and sialorrhea as with other similar studies. [13]

In patients in our group, we found the efficiency of endoscopic treatment of 96% and the whole group of 80%. Most cases published since 1995 demonstrate the efficacy and safety of flexible endoscopy, with clinical success rates between 56% to 100%. [14-15]

The success rate lower than 56% reported in a single series [10] is most likely because the clinical remission was evaluated according to the presence or absence of a group of symptoms and not only dysphagia.

The rate of recurrent symptoms was significantly higher in the surgical group than in the endoscopic group, results different from those reported in other studies where a 39% recurrence rate was found after endoscopic treatment and 0% recurrence in the patients who suffered surgery, probably due to the small number of patients treated surgically. [13]

In the studied group, the persistent postinterventional symptomatology was mainly represented by dysphagia, which is why we consider that in other studies, the efficacy of the treatment should be reported according to the improvement of this symptom. Data from the literature report clinical success increases to 84-100% when the unique series in which success is defined according to dysphagia is taken into account. [9, 11, 15]

As in our study and some other studies, treatment efficacy was reported after a single treatment session, [10, 16] while in other series the success rate was reported after several treatment sessions. [9, 17]

Unlike rigid staple endoscopy, the flexible septotomy technique for the Zenker diverticulum is neither univocal nor standardized. As already mentioned, different cutting techniques can be variably combined with different accessories. The optimal dividing technique is unknown because prospective randomized studies are lacking, and the choice is mainly based on the endoscopist's personal experience and preferences.

In evaluating the success rate of surgery, the most important factor is the subjective evaluation of the swallowing function performed by the patient. Both the primary and revision indications are guided by the patient's symptoms. [18]

Following rigid endoscopic or surgical treatment, persistent septum or residual pouch may still be present on barium contrast examination. Persistent symptoms may be due to other swallowing abnormalities and/or inappropriate myotomy, without correlating the size of the residual pouch and the recurrence of the symptoms. There is a consensus in the literature that the evaluation of treatment efficacy and the need for further treatment must be determined by the patient's symptoms [19, 20] - which we agree with.

Some authors concluded that the presence of a residual diverticulum on an early postoperative esophagogram does not warrant early surgical revision, even when dysphagia persists. In many cases, there may be a slow process of gradual fibrosis of residual dysfunctional cricopharyngeal muscles or atrophy of the remaining diverticular sac over time. In addition, a normal postoperative esophagogram probably cannot predict a good and persistent outcome.

Some authors have reported that only 12.5% of patients with remaining diverticula on the postoperative contrast check develop a recurrence of Zenker's diverticulum. [18]

The approach of redo interventions has changed over time. If in older studies, there is a high percentage of recurrent surgical treatment, [21, 22] we are currently attending an endoscopic revision intervention, and in our study was noted that on a case of redo was necessary to use an endoscopic method after open resection of the Zenker diverticulum for the treatment of dysphagia in case of postoperative stenosis.

Other authors point out the need for classical intervention to resect the residual sac, which, even after a complete myotomy, can be a reason for the persistence of food retention mechanism. This may be the cause of symptoms in patients with prolonged retention of the contrast substance in the pharyngeal pouch and difficult passage into the esophagus, even after complete cricopharyngeal myotomy. [2, 3]

Other authors also suggest that an incomplete cricopharyngeal myotomy may result in higher recurrence rates associated with flexible endoscopy. Ideally, the diverticulum should be reduced to <1 cm. [24]

Incomplete cricopharyngeal myotomy may be visible on radiographs as a cricopharyngeal impression. Hypo-pharyngeal dysfunction during swallowing of the contrast substance may indirectly suggest incomplete myotomy or recurrent laryngeal nerve injury. On radiographs with contrast substance, pharyngeal dysfunction is suspected if we see contrast substance in the larynx or the tracheobronchial tree. Some authors consider that the persistence of the septum rather characterizes the endoscopic technique and does not predict the failure of the treatment, especially during the immediate postoperative period. [25]

Other authors have tried to evaluate the efficiency of the procedure through manometric studies. Ozgursoy and Salassa reported that a large diverticulum can cause obstruction of the esophageal passage and can lead to high pressure, even if the tone of the upper esophageal sphincter remains normal at manometry. Such a theory leads to the hypothesis that the weak wall of the hypopharynx may play an important role in the development of the diverticulum and leaving the weak adynamic segment of the esophagus creates a favorable environment for recurrence. In addition, the creation of a large cavity after diverticulostomy favors endoscopic techniques over open diverticulectomy on manometric evaluation, because, in a large cavity, small pressures are difficult to record, even if part of the septum remains and this causes dysphagia. [26, 27]

Some studies have shown that post-procedural soluble contrast studies do not correlate with symptoms following endoscopic therapy or relapses and that the second radiological examination, three months after surgery, presents the final functional results, with which we also agree. [8, 25] We also carried out new post-procedural or post-operative radiological studies to exclude a fistula on the seventh postoperative day and in the post-procedural monitoring of patients. Maybe a more accurate assessment of the contrast substance study could be done 3 months after the procedure.

For the patients in the group, we found that the duration of hospitalization in patients with postinterventional symptomatology and those without postinterventional symptomatology was significantly different. The average duration of hospitalization was 2 days for patients without post-procedural morbidity and 7 days for those with post-procedural morbidity comparable to the data in the literature. In our study, the duration of hospitalization of patients with open diverticulectomy was comparable with the data in the literature. [28-30]

The persistence of dysphagia in the 11 patients included in the group usually required reintervention; most of the patients with persistent symptomatology required a reintervention of the same type. Similar studies have also been reported in the literature, most patients who failed the procedure also underwent endoscopic reintervention. [31]

In the patients in the group with bulky diverticula of degree III or larger than 4 cm, the most frequent post-procedural reinterventions were required, 3 out of 4 patients, aspects encountered in other studies. Partial septotomy in the diverticula greater than 5 cm has been proposed by some surgeons, even if the myotomy fails to completely remove the septum and may require more endoscopic interventions. [16, 32, 33]

According to recent literature, some authors recommend the endoscopic procedure for revision surgery as well. The great advantage compared to the open approach is that the endoscopic revision treatment is technically feasible and relatively easy, not more difficult than the primary endoscopic procedure, without an increase in risk. Furthermore, patient satisfaction was higher in those who underwent endoscopic revision surgery compared to those who underwent open surgery revision. [6, 18, 34]

Repeated procedures, in case of therapeutic failure or symptomatic relapse, can be easily and successfully performed by flexible or rigid endoscopy. An additional advantage is that repeated procedures for persistent or recurrent symptoms can be successfully achieved through a transoral (rigid or flexible) approach, while an open approach can be a major technical challenge. [5]

The data from the literature show recurrence rates of about 6% (range 0-22%), with the average recurrence rate of 5% compared to the classical approach, and according to data obtained in our study. [35]

The comorbidities of patients with diverticula included in the group also played a role in the emergence of the situations that required reintervention. [36]

Some authors consider that endoscopic findings tend to decrease over time and that incomplete septotomy is a significant factor for failure rate [37-40], an aspect we have also found.

CONCLUSIONS

Most of the time redo procedures were of the same type, the endoscopic iterative treatment being much easier than the surgical iterative treatment. Undoubtedly, endoscopic treatment has gained more and more confidence even in the field of revision interventions, considering that persistence or recurrence of dysphagia has as substrate an incomplete division of the septum requiring the same type of procedure. Redo procedures were necessary for patients with persistent dysphagia, bulky diverticula, and patients with a high number of comorbidities.

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REVIEW ARTICLE

Current treatment of posttraumatic stress disorder – A review of therapeutic guidelines and good practice recommendations

Octavian Vasiliu, Daniel Vasile, Andrei G. Mangalagiu, Bogdan M. Petrescu, Cristian A. Candea, Corina Tudor, Daniela Ungureanu, Madalina Miclos, Alina Draghici, Catalina Florescu, Roxana E. Bratu-Bazic

Abstract: *A unique approach to the treatment of posttraumatic stress disorder (PTSD) is difficult to support, because of the complexity and heterogeneity of this pathology. Multiple evidence-based guidelines for PTSD treatment exist, elaborated by prestigious institutions around the world, but a certain level of the inconsistency of their recommendations appears when these sources are compared with each other. Therefore, a review of current guidelines and extraction of the most supported by evidence recommendations is considered important for clinical practice, due to the need to approach such complex cases as PTSD-diagnosed patients in a stepwise manner. Structured monitoring of the clinical status during the treatment is also an important and rather neglected aspect of the case management in PTSD, and the necessity of follow-up visits for efficacy and tolerability should be taken into consideration. Psychotherapy (especially cognitive-behavioral oriented therapies and eye movement desensitization and reprocessing therapy) is supported by evidence as a first-line approach, as well as certain antidepressants, like the selective serotonin reuptake inhibitors and venlafaxine. Many second-line pharmacological agents and psychotherapies are also available, but there is an obvious need for more pragmatic trials with PTSD patients.*

Keywords: *post-traumatic stress disorder, psychotherapy, case management, antidepressants, therapeutic guidelines, good clinical practice*

BACKGROUND

While definitions of the diagnostic criteria for posttraumatic stress disorder (PTSD) are articulate and comprehensive in both the World Health Organization and American Psychiatric Association classifications of mental disorders [1-3], pragmatic recommendations for the treatment of this disorder are still lacking. Although there are several guidelines dedicated to this subject, they are based on randomized trials, and not on pragmatic, naturalistic studies, which leave some important questions unanswered. Even if the case management is based on these guidelines, there are several contradictions based on the different data they included. Therefore, we consider the first necessary step to be a critical analysis of these therapeutic guidelines, followed by the formulation of good practices and hierarchized recommendations, according to the GRADE level [4].

The diagnosis of PTSD could be formulated, according to ICD-11, if an extremely threatening or horrific event or series of events is followed by re-experiencing this trauma as intrusive memories, flashbacks, nightmares, accompanied

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by strong or overwhelming emotions and strong physical sensations; avoidance of thoughts and memories of the event(s), or avoidance of activities, situations, or people reminiscent of the events(s); persistent perceptions of heightened current threat, for example, indicated by hypervigilance or an enhanced startle reaction to stimuli such as unexpected noises [1]. These symptoms have to persist for at least several weeks and to cause significant impairment in personal, family, social, educational, occupational, or other important areas of functioning to support a PTSD diagnosis [1]. The ICD-10 mentions the capacity of the stressful event to cause pervasive distress in almost anyone who has been removed from the latest edition of this classification [1,3]. Also, differences in the structure of the symptoms list necessary to establish the PTSD criteria have been formulated between the two editions of ICD, from 13 in the tenth edition, to only 6, by removing the non-specific manifestations [1,3]. In the latest edition of the American Psychiatric Association classification of mental disorders (2013), there are 4 clusters of symptoms needed for the diagnosis: (1) intrusion symptoms associated with the traumatic event, (2) persistent avoidance of stimuli associated with the traumatic event, (3) negative alterations in cognitions and mood associated with the traumatic event, (4) marked alterations in arousal and reactivity associated with the traumatic event, and the duration of these manifestations are more than one month [2].

Complex PTSD may develop following exposure to an event or series of events of an extremely threatening or horrific nature, commonly prolonged or repetitive events from which escape is difficult or impossible [1]. Complex PTSD is characterized by severe and persistent problems in affect regulation, beliefs about oneself as diminished, defeated, or worthless, accompanied by feelings of shame, guilt, or failure related to the traumatic event; difficulties in sustaining relationships, and feeling close to others [1]. These symptoms cause significant impairment in personal, family, social, educational, occupational, or other important areas of functioning [1].

A high rate of comorbid major depressive disorder (up to 50%) has been reported in PTSD patients, a phenomenon which underlines the necessity for a thorough psychiatric evaluation, both initially and during the monitoring period [5,6]. Also, there has been described as an overlap between PTSD and psychosis that may raise important diagnosis challenges and treatment dilemmas [7, 8]. Substance-related disorders have also been described with relatively high incidence in this population, and specific psychotherapy algorithms have been developed for these patients with dual diagnosis, based on imaginal and in vivo exposure for PTSD, applied concomitantly with cognitive-behavioral techniques for substance use disorders [9]. Also, the pharmacological treatment should be adjusted according to the existing comorbidities, and the case manager should integrate all the data, taking into account the risk of pharmacologic interactions, contraindications, and side effects (e.g., avoidance of drugs with the risk of addiction, like benzodiazepines, is recommended in PTSD patients) [10].

ANALYSIS OF THE CURRENT THERAPEUTIC GUIDELINES FOR PTSD

Several prestigious institutions have elaborated evidence-based guidelines for the treatment of PTSD, therefore an important number of recommendations for approaching these patients to exist. While some therapeutic guidelines are focused on psychotherapy, others are based on pharmacological research, while still others encompass both types of treatment.

American Psychological Association Clinical Practice Guideline for the Treatment of PTSD (2017) strongly recommends as evidence-based psychotherapies in adults cognitive-behavioral therapy (CBT), cognitive processing therapy (CPT), cognitive therapy (CT), and prolonged exposure therapy (PE), and suggests the use of brief eclectic psychotherapy (BEP), eye movement desensitization and reprocessing therapy (EMDR), and narrative exposure therapy (NET) [11].

According to the **Australian Guidelines for the Treatment of Acute Stress Disorder and Posttraumatic Stress Disorder** (2013), individual-trauma focused CBT, including exposure and/or cognitive therapy, should be recommended if symptoms are consistent with acute stress disorder or posttraumatic stress disorder in the initial 4 weeks after a potentially traumatic event (level C evidence) [12]. Individuals with posttraumatic stress disorder benefit from trauma-focused CBT or EMDR (level A recommendation), and if the symptoms did not respond to these interventions, non-trauma focused approaches may be considered (e.g., stress inoculation technique) [12]. Also, there is some evidence that supports the efficacy of in vivo exposure therefore, by expert consensus, it is recommended to include this method in the treatment [12]. Group CBT (trauma-focused or non-trauma focused) may be offered as an adjunctive to trauma-focused individual therapy [12].

Medication should not be used for the treatment of acute stress reaction or posttraumatic stress disorder within the four weeks of symptom onset unless the severity of such symptoms- associated distress cannot be managed by psychological means alone [12]. The pharmacological approach may be continued while people are undertaking psychological treatments, but several agents, like benzodiazepines, may interfere with some effective psychological treatments [12]. SSRIs are considered the first line for the treatment of posttraumatic stress disorder [12].

According to the **Maudsley Prescribing Guidelines** (13th edition, 2018) patients diagnosed with posttraumatic stress disorder should be offered as the first line of treatment paroxetine, sertraline, fluoxetine (up to the maximum tolerated doses) or venlafaxine (37.5-300 mg/day) [13]. As second-line treatment the case manager could choose between antipsychotics (olanzapine 5-20 mg/day, risperidone 0.5-6 mg/day, quetiapine 50-800 mg/day), mirtazapine 15-45 mg/day, monoaminoxidase (MAO) inhibitors (phenelzine 15-75 mg/day), tricyclic antidepressants (amitriptyline 50-300 mg/day, imipramine 50-300 mg/day), and prazosin (2-15 mg nocte) for nightmare and sleep disturbances [13]. Possible choices if the first and second lines failed are duloxetine (60-120 mg/day), lamotrigine (up to 500 mg/day), phenytoin, valproate, or i.v. ketamine, and as non-drug therapies- eye-movement desensitization and reprocessing (EMDR) and trauma-focused CBT [13].

According to the **VA/DOD Clinical Practice Guideline for the Management of PTSD and ASD** (2017) pharmacotherapy in PTSD is classified as follows: sertraline, paroxetine, fluoxetine, venlafaxine as monotherapy (moderate evidence), nefazodone (low evidence), imipramine, phenelzine (very low evidence) [14]. The Work Group recommended against the use of atypical antipsychotics, benzodiazepines, and Divalproex as augmentation therapy due to low-quality evidence and potential adverse effects [14]. Also, prazosin is not supported by this guideline for the use in monotherapy or combination with other agents [14]. Individual trauma-focused psychotherapy for PTSD are Prolonged Exposure, Cognitive Processing Therapy, and EMDR, which are supported by the strongest evidence, but other therapies like specific cognitive therapies for PTSD, Brief Eclectic Psychotherapy, Narrative Exposure Therapy, written narrative exposure may be recommended [14]. If individual trauma-focused psychotherapy is not available, Stress Inoculation Therapy, Interpersonal Therapy, or Present-Centered Therapy may be recommended [14].

The **International Society for Traumatic Stress Studies new guidelines for the Prevention and Treatment of Posttraumatic Stress Disorder** (2019) has formulated the following recommendations according to their evidence-based status: (1) strong support for cognitive processing therapy, cognitive therapy, EMDR, individual CBT with a trauma focus, prolonged exposure; (2) standard recommendations for CBT without a trauma focus, guided Internet-based CBT with a trauma focus, narrative exposure therapy, present-centered therapy; (3) emerging evidence-couples CBT with a trauma focus, group and individual CBT with a trauma focus, reconsolidation of traumatic memories, single-session CBT, written exposure therapy, and virtual reality therapy [15].

Individual trauma-focused CBT intervention may be useful for adults who have acute stress disorder or clinically important symptoms of PTSD and have been exposed to 1 or more traumatic events within the last month- cognitive processing therapy, cognitive therapy, narrative exposure therapy, prolonged exposure therapy, according to the **NICE Guidelines** (2018) [16]. In adults with PTSD symptoms should be offered trauma-focused CBT, during 8-12 sessions, accompanied by psychoeducation about reactions to trauma, strategies for managing arousal and flashbacks, safety planning, or EMDR for patients with PTSD symptoms, if the patient has a preference for this method [16]. Trauma-focused computerized CBT for adults with a diagnosis of PTSD or clinically important symptoms of PTSD more than 3 months after a traumatic event, who do not want to be engaged in trauma-focused CBT or EMDR [16]. Symptom-specific CBT interventions for adults with a diagnosis of PTSD or clinically important symptoms of PTSD more than 3 months after traumatic events who are unable or unwilling to engage in a trauma-focused intervention that targets PTSD or have residual symptoms after a trauma-focused intervention [16].

Medication should not include benzodiazepines for the prevention of PTSD in adults, but venlafaxine or an SSRI may be chosen [16]. Antipsychotics may be offered if patients have disabling symptoms (psychotic) or if their symptoms have not responded to other drug or psychological treatments [16].

For patients with complex PTSD the case manager should help the patient control any symptoms (e.g. dissociation or emotional dysfunctions) that might interfere with engaging in trauma-focused therapies; ensure adequate time for the construction of the therapeutic relationship; to increase the number of trauma-focused therapy sessions according to the person's needs; plan any ongoing support the person needs and has implications in the therapeutic management [16].

DESCRIPTION OF THE MAIN PSYCHOTHERAPEUTIC METHODS IN PTSD

Cognitive-behavioral therapy (CBT) in PTSD is focused on the relationship among thoughts, feelings, and behaviors that are present-oriented and target current problems and symptoms [17]. CBT is based on emotional processing theory and social cognitive theory, therefore it claims that changing associations between reminders of the traumatic event and responses will lead to healthier behaviors while reframing the relation between trauma and existing beliefs about self, others and the world will also help decrease self-blame and improve the processing of the trauma [17].

Cognitive distortions in PTSD include perceiving the world as dangerous, seeing him/herself as powerless, and feeling guilty about surviving while other significant ones have died [18]. Cognitive therapy fosters the development of more realistic and adaptive cognitions, by successful processing of the traumatic event, with engaging and organization of the trauma narrative [18, 19].

Cognitive processing therapy (CPT) is an efficient method for treating PTSD as demonstrated by a recent meta-analysis (n=11 studies, N=1130 participants), outperforming inactive control conditions on PTSD outcome measures at posttreatment and follow-up, but also other active treatments at posttreatment but not at follow-up [20]. Effect sizes of CPT were superior to 89% of the inactive control conditions at posttreatment and 82% at follow-up, which indicates a large and sustained effect of the CPT [20].

Veterans who were diagnosed with both PTSD and alcohol use disorder (AUD) completed a 6-week CPT-based program had significantly better outcomes, including trauma-related cognitive distortions, PTSD symptoms, depressive symptoms, and trauma-cued substance craving [21]. In another study that included veterans (n=465), enrolled in CPT plus a written trauma account either group format or individual, the PTSD and depressive symptoms decreased significantly, with medium effects for the first option, and large effects for the second treatment [22].

Cognitive therapy (CT) has proven efficient in a group of 20 patients diagnosed with PTSD, as it decreased core PTSD features, but also depressive and anxiety symptoms [23]. Good treatment outcomes were related to more changes in dysfunctional cognitions related to the trauma [23]. Intensive CT was applied in 14 patients diagnosed with PTSD, in an 18-hour format during 5-7 working days, followed by one session a week later and up to 3 follow-up sessions; intensive CT led to similar outcomes as weekly-administered CT, but the intensive treatment improved the key PTSD symptoms over a shorter period and led to greater reductions in depression [24].

Prolonged exposure therapy (PE) is another trauma-focused CBT effective for improving PTSD, with response rates in 60-65% of trauma victims suffering from this disorder [25]. PE is based on two types of exposure techniques, imaginal exposure, and in vivo exposure, and it is used for enhancing emotional processing through systematic confrontation with trauma-related stimuli [26-28]. Intensive PE therapy (12 sessions, 90 minutes each, during 4 days) followed by four weekly 90-minute booster PE sessions in an open trial (n=73) patients diagnosed with PTSD who presented multiple interpersonal trauma had positive results after repeated treatment attempts [29]. Exposure therapy has an impact on the fear and contextualization circuits by facilitating communication between the ventromedial prefrontal cortex, hippocampus, and the amygdala [30].

Brief eclectic psychotherapy (BEP) for PTSD is a manualized technique that combines elements from psychodynamic, cognitive-behavioral, and directive psychotherapy [31]. Psychoeducation with the participation of the spouse, and exposure used to help the patient to access his or her feelings related to the traumatic event are also used during BEP [31]. A 16-session protocol of BEP in PTSD includes the following steps: psychoeducation for the development of self-control and motivation for therapy (the patient is invited to call up his memories and related emotions one last time); imaginal exposure or guidance (brief periods of muscle relaxation, relieving of the traumatic experience by imaginal exposure of 20-30 minutes); writing tasks and mementos (for uncovering the difficult feelings that may be related to the traumatic experience); meaning and integration (exploring and accepting a new view of the world and the self); farewell ritual (symbolic ritual created to encourage the expression of the sorrow the patient may still feel, and then to leave behind these feelings) [31, 32].

The first randomized clinical trial for BEP in patients with PTSD included 42 police officers who received 16 weekly sessions (60 minutes each) of psychotherapy [32]. At posttest and follow-up (at 3 months) BEP had produced significant improvement in PTSD symptoms, work functioning, and several comorbid conditions (e.g., agoraphobia) [32].

Another clinical trial included patients diagnosed with PTSD (n=24) randomly assigned to BEP or a waitlist group for 4 months, with superior efficacy on PTSD core symptoms and general anxiety symptoms in the active intervention group [33]. In this trial, BEP consisted of 16 weekly sessions (45-60 minutes each), and a high rate of the dropout was recorded (25% for the treatment group) [33].

Eye movement desensitization and reprocessing therapy (EMDR) is a type of psychotherapy developed by F.Shapiro for intervention in emotional trauma and other negative life experiences [34]. This psychotherapy consists of a protocol with 8 phases and bilateral stimulation targeting patient desensitization from the discomfort induced by traumatic memories [35]. The final objective of the EMDR is to achieve the reprocessing of these memories and to integrate them within the autobiographical memory [35].

According to a review of 24 randomized controlled trials, EMDR is an evidence-based approach for this kind of disorder, with 7 out of 10 studies reporting EMDR to be faster acting and/or more effective than trauma-focused CBT [34]. Two meta-analyses supported the efficacy of EMDR in treating PTSD, with results superior to various interventions and control conditions, and showed the efficacy of this psychotherapy over symptoms of depression, anxiety, and subjective distress [36,37]. A review of the trials published between 2014 and 2017 regarding the efficacy of EMDR in PTSD further supported this method, because it improved core symptoms over time, compared to relaxation therapy and a waiting-list control group [38].

Narrative exposure therapy (NET) is a psychotherapeutic intervention dedicated to the treatment of survivors of multiple and severe traumas, and 3-6 sessions may be sufficient to provide significant relief [39]. It is a short-term approach based on CBT and testimony therapy [40]. NET was evaluated in a group of 59 political detainees, 18 of which had full PTSD criteria, who were allocated in a randomized manner to either one session of psychoeducation or five sessions of NET [41]. After 6 months, NET but not psychoeducation produced a significant reduction in PTSD symptoms and depression scores, although four out of the nine patients receiving NET and eight out of the nine patients undergoing psychoeducation still had PTSD symptoms at 6 months [41]. In another trial, NET was compared to supportive counseling and psychoeducation (4, 4 and one sessions, respectively) in a group of Sudanese refugees (n=43), and the results after one year of treatment were superior for NET (29% vs. 79% vs. 80% still had PTSD criteria) [40].

Interpersonal therapy (IPT) is a time-limited focused on the patient's actual events and reframing of the social functioning as a way to tackle the symptoms and may be recommended where the patient does not want to be exposed to exposure-based approaches [42]. In a case study, IPT proved itself efficient comparative to the other two psychotherapies, after 14 weekly sessions [42]. In a 14-week randomized trial, IPT was compared to prolonged exposure and relaxation therapy in 110 unmedicated patients with chronic PTSD and the response rates were 63% for IPT, 47% for prolonged exposure, and 38% for relaxation therapy, without significant differences between groups [43]. IPT and exposure therapy improved the quality of life and social functioning more than relaxation therapy [43].

Group Interpersonal Therapy (G-IPT) was evaluated in 13 patients diagnosed with chronic PTSD, in an 8-week treatment program, with favorable results [44]. The improvement was significant in social functioning, general wellbeing and depressive symptoms, and a moderate reduction in the avoidant symptom cluster of PTSD [44]. These positive effects were stable at 3 months and associated with perceived intra-therapy progress in solving problematic domains identified during IPT [44].

Present-Centered Therapy (PCT) for PTSD is a manualized psychotherapy originally designed as a treatment comparator in studies focused on the effectiveness of trauma-oriented CBT [45]. A systematic review of the randomized trials (n=12, N=1837 participants) states that moderate-quality evidence supports a superior efficacy of PCT in reducing PTSD severity compared to control conditions, and PCT was associated with lower drop-out rates comparative to trauma-focused CBT [45].

Stress inoculation training (SIT) is derived from CBT and proved itself efficient in veterans with PTSD [46]. According to this model, PTSD symptoms are maintained by an imbalance between perceived situational demands and perceived resources to fulfill the environmental and personal demands [47, 48]. The purpose of SIT is to increase the patient's feeling of self-confidence by helping him or her to control anxiety and related symptoms and to inoculate patients against possible future episodes of severe anxiety [48].

In a population with PTSD and traumatic brain injury (n=65 veterans), SIT was applied for 18 months in an outpatient clinic and the results supported the efficacy of this psychotherapy because significant reductions in PTSD and depressive symptoms were detected, as well as increases in perceived stress tolerance and performance in multiple life domains [46]. Improvements in social and occupational functioning were also reported [46].

In another trial, 96 female assault victims with chronic PTSD were randomized on prolonged exposure, SIT, combined prolonged exposure-SIT, or wait-list control [49]. All active treatments (9 twice-weekly individual sessions) decreased PTSD and depression symptoms compared to wait-list but did not differ significantly from each other, and these results persisted at follow-ups (3, 6, and 12 months) [49].

GOOD CLINICAL PRACTICE RECOMMENDATIONS

Therapeutic guidelines offer various and sometimes contradictory recommendations, therefore a critical analysis of the literature is granted to find common management strategies. While several guidelines are more cautious when recommending pharmacotherapy [12] and support a psychotherapeutic-based approach especially in the first stage of the PTSD, others are more focused on drug therapy [13], and still, others consider the type of approach should be selected according to the individual variables, including patient's preference, availability, etc [14].

We formulated GRADE recommendations according to 4 levels: A= high quality, defined as the level to what future research are very unlikely to change the validity of these recommendations; B= moderate quality, meaning that future research is likely to have an important impact on the appreciation of the recommendations; C=low quality, which means that it is very likely that future research will have an important impact over the confidence in the estimate of effect and is likely to change the recommendations; D= very low quality, defined by the uncertainty of any estimate of the effect [4].

A wide range of psychotherapies have been studied for patients with PTSD, but CBT and related techniques (CPT, CT, PE), as well as EMDR, are the most evidence-based approaches [11-15]. We consider a GRADE level A for these therapies is granted, based on the existing evidence. Level B evidence exists for BEP, NET, IPT, PCT, and SIT [31-33, 39-49].

The same level of evidence exists for SSRIs and venlafaxine, as these are the most supported by evidence pharmacological agents in PTSD patients [12-15]. If the first-line agents fail, then second-line agents may be used-mirtazapine, MAO inhibitors or tricyclics, or add-ons like atypical antipsychotics (level B of evidence) [13].

A structured diagnostic approach is strongly encouraged and adequate instruments should be used. Structured interviews (e.g., Mini-International Neuropsychiatric Interview, Composite International Diagnostic Interview, or Schedules for Clinical Assessment in Neuropsychiatry) are considered useful because of the high comorbidity rate met in PTSD patients [50-52]. Because the evolution of PTSD is not linear, even under treatment, detection of new-onset psychiatric disorders is also important because of their high impact over the treatment plan. Therefore, using structured clinical interviews during the first 6-12 months of the treatment is also recommended.

Monitoring treatment efficacy in PTSD using standardized instruments is strongly encouraged. For this purpose, we recommend validated scales, inventories, or structured interviews, like the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5), PTSD Symptom Scale Interview (PSS-I), Mississippi Scale for Combat-related PTSD (MISS) [53-55]. This is considered a good clinical practice because it may increase the objectivity of the psychiatric evaluation, although it is not specifically recommended in all the reviewed therapeutic guidelines.

We consider good clinical practice in the PTSD treatment to actively monitor the tolerability of the pharmacological treatment, to detect early adverse events that may decrease therapeutic adherence [56]. For this purpose, the administration of the Treatment Satisfaction Questionnaire for Medication (TSQM) [57] or a simpler visual analogic scale for self-evaluation is considered useful.

CONCLUSIONS

A review of the existing therapeutic guidelines focused on PTSD management detected several common recommendations. These refer to the use of CBT and related therapies and EMDR as a first-line approach and SSRIs or venlafaxine as the main pharmacologic agents [1-5, 11]. Our team has added as good practice recommendations the necessity to use validated instruments for initial and follow-up diagnostic evaluations, as well as the monitoring of treatment tolerability and efficacy. Because of the high rate of comorbid disorders in PTSD patients, the case manager should be alert to new-onset psychiatric disorders that may appear as complications or just comorbidities in this population.

An integrated, psychological, and pharmacological approach should be considered for these patients, with frequent follow-up visits, and emergent disorders have to be integrated into the case management plan as soon as they are detected.

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ORIGINAL ARTICLES

A practical approach of the pediatric patient with obstetrical brachial plexus palsy starting from 28 cases – Single-center experience over 6 years

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Abstract: *Neonatal brachial plexus palsy is a complication that occurs during difficult vaginal delivery or due to unexplained causes, probably prenatal in which some or all brachial plexus branches are affected (avulsion, rupture, stretching). A cohort of 28 cases evaluated in our clinic in 6 years and 6 months (Jan 2007-July 2013) from the clinical and paraclinical point of view, treatment, and the outcome were analyzed. 17 (60.7%) of the patients were boys and 11 (39.2%) were girls. 14 (50%) had right brachial plexus palsy, 12 (42.8%) had left brachial plexus palsy and 2 (7.1%) had bilateral involvement. 5 (17.8%) of them had reconstructive surgery done. 11 (39.2%) had good to complete recovery. The purpose of this paper is to underline the importance of early presentation for neurological evaluation and the beginning of physical therapy.*

Keywords: *brachial plexus palsy, physical therapy, reconstructive surgery of brachial plexus*

INTRODUCTION

Neonatal brachial plexus palsy (NBPP) – is a functional disturbance due to brachial plexus injury, characterized by deficits in the areas served by the brachial plexus branches (C5 to T1). The following NBPP types have been described according to the lesion's level: total (C5 to T1) or partial – superior (C5 to C6), middle (C5 to C7), inferior (C8 to T1). The name of this dysfunction is controversial, some authors calling it obstetrical, because the trauma during a difficult delivery is the most common cause, while others name it congenital including obstetrical and unexplained cases due to a prenatal cause. In these circumstances, an umbrella term will be NBPP [2, 3, 6, 9]. Some or all the fibers of the brachial plexus branches suffer stretching, rupture or avulsion. The incidence is 0.1 - 0.4% of live births and has remained relatively constant over time [3, 6, 7, 9]. A multidisciplinary approach and consensus are needed for a better outcome.

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OBJECTIVES

This paper aims to analyze the cohort of patients with NBPP followed in the Pediatric Neurology Department, to highlight the variability of NBPP symptoms at presentation, to identify and correlate specific items related to clinical signs, investigations, and outcome, and to establish a protocol of diagnosis and follow-up for children with NBPP, according to the international recommendations adapted to the local needs. We propose a treatment algorithm, allowing optimum outcomes for these children.

MATERIALS & METHOD

The archive of the Pediatric Neurology Clinic was retrospectively analyzed over 6.5 years (January 2007 – July 2013), selecting the patients with NBPP. Approval from the Local Ethics Committee was obtained. Narakas's classification was used at the first admission to diagnose the NBPP type: superior, middle, inferior, and total [7]. The clinical status was registered at different times: at presentation, at 3, 6, 12 months follow-up. Active motor scale [1, 2, 4, 8, 9], Toronto score [1, 2, 4, 8, 9], and protocol for obstetrical brachial plexus palsy (Hospital for Sick Children Seattle) [8] were retrospectively applied, using registered neurological examinations, reports of physical therapy specialist, from files. Laboratory findings, EMG and nerve conduction velocities of the upper limb and brachial plexus MRI (performed if needed), treatment choices, and their results were also evaluated [10]. We designed a separate questionnaire for present status evaluation, including data about active movements, strength, trophic changes summarized in a score. The prognosis was quantified as follows: score 0-12 – poor recovery, score 13-24 – moderate recovery, 25-36 – good recovery, or complete remission. Statistical analysis used the SPSS17 program.

RESULTS

28 patients with the diagnosis of NBPP were identified between January 2007 – July 2013.

Evaluation schedule. All patients came at 3 months for neurological evaluation; 23 children (82.1%) returned for reevaluation at 6 months, and only 10 (35.7%) at 12 months.

History and clinical data. Among them, 17 were boys (60.7%) and 11 girls (39.3%), 4 days to 13 years old at admission. 14 (50%) cases had the right side, 12 (42,8%) had left side, and 2 (7.1%) had bilateral brachial plexus involvement. 6 (21.4%) of the cases have total NBPP and 22 (78.6%) – partial – superior (C5-C6) or extended superior (C5-C7) NBPP. All the children were born spontaneously, 13 (46.4%) of them had > 4000 g, another 8 (28.5%) had birth weight > 3900 g and eight (28.5%) patients had 2800 – 3900 g, but with difficult labor and delivery. Four patients (14.2%) had Claude Bernard Horner syndrome (CBH) and 3 (10.7%) had further associated pathology – global developmental delay, diplegia, microcephaly in evolution. 13 patients (46.4%) were first evaluated in our clinic in the first month of life, 12 (42.8%) between 1 and 3 months, 2 (7.1%) between and 1 (3.5%) after 6 months. 2 (7.1%) mothers had prenatal hypertension and hypertension during pregnancy, but that didn't affect the children.

Investigations. 6 patients (21.4%) had electrophysiological studies (EMG and nerve conduction velocities) performed in the first 2 months of life, in 3 cases (10.7%) repeated at 3 months and in 5 (17.8%) after 6 months. 6 patients were evaluated with MRI of the cervicobrachial region. 3 (10,7%) of them had avulsion (preganglionic lesions, usually associate pseudomeningoceles), 1(3.5%) had rupture (usually are postganglionic lesions, could associate pseudomeningoceles) and 2 (7.1%) had stretching/ elongation (postganglionic lesions, neuroma or hematoma or diffuse lesions). 4 (14.2%) of the children who had brachial plexus surgery had pre- and postganglionic lesions and 1 (3.5%) had postganglionic lesions.

Treatment. All patients received physical therapy, Vojta method (in our clinic); among them, two patients (7.1%) had already started physiotherapy through the Bobath method (in another Clinic). 12 patients (42.8%) were included in an intensive physical therapy program and 16 (57.1%) received inconstant or for a short period physical therapy. Good recovery was registered in 6 patients (21.4%) who performed intensive physical therapy and 5 patients (17.8%) among those with inconsistent therapy. Five patients (17.8%) had surgical reconstruction of the brachial plexus, 3 (10.7%) of them had total brachial plexus palsy and two (7.1%) had extended superior brachial plexus palsy.

Follow-up. Evolution at 3 month after physical therapy, evaluated with Toronto score (T score) showed score > 3.5 in five patients (17.8%) and values < 3.5 in 23 patients (82.1%). From the 23 patients (82.1%) with T score < 3.5, 6 (21.4%) were evaluated for surgical procedure and five (17.8%) were operated, in one (3.5%) case it was not necessary. The rest continued physical therapy. 18 patients (64.2%) with a T score <3.5 were not evaluated for surgery because they did not come for their scheduled evaluation/ follow-up.

Outcome. Eight (28.5%) patients had a poor recovery, 9 (32.1%) patients had a moderate recovery, 11 children (39.2%) had good recovery, among them 6 (21.4%) with complete remission. All of the children with good recovery to complete remission had superior or extended superior

NBPP.

DISCUSSIONS

Most of the children (18 – 64.2%) did not comply with the follow-up schedule. That is because they recovered with physiotherapy or spontaneously or because they were lost to follow-up. The last evaluation, consisting of a questionnaire applied on the phone showed that the majority had a good outcome, most probably because most of the cases were mild, superior type, and a good response to therapy (even those who have made physical therapy at home with their parents). Besides, we could not compare the types of reconstructive surgery, because the number of children undergoing surgery was too small (5). Among them, 4 children had reconstruction of the brachial plexus, and for one patient neurotomy was performed (the fibrous sleeve around the brachial plexus was removed).

Figure 1: Algorithm for diagnosis and treatment of NBPP

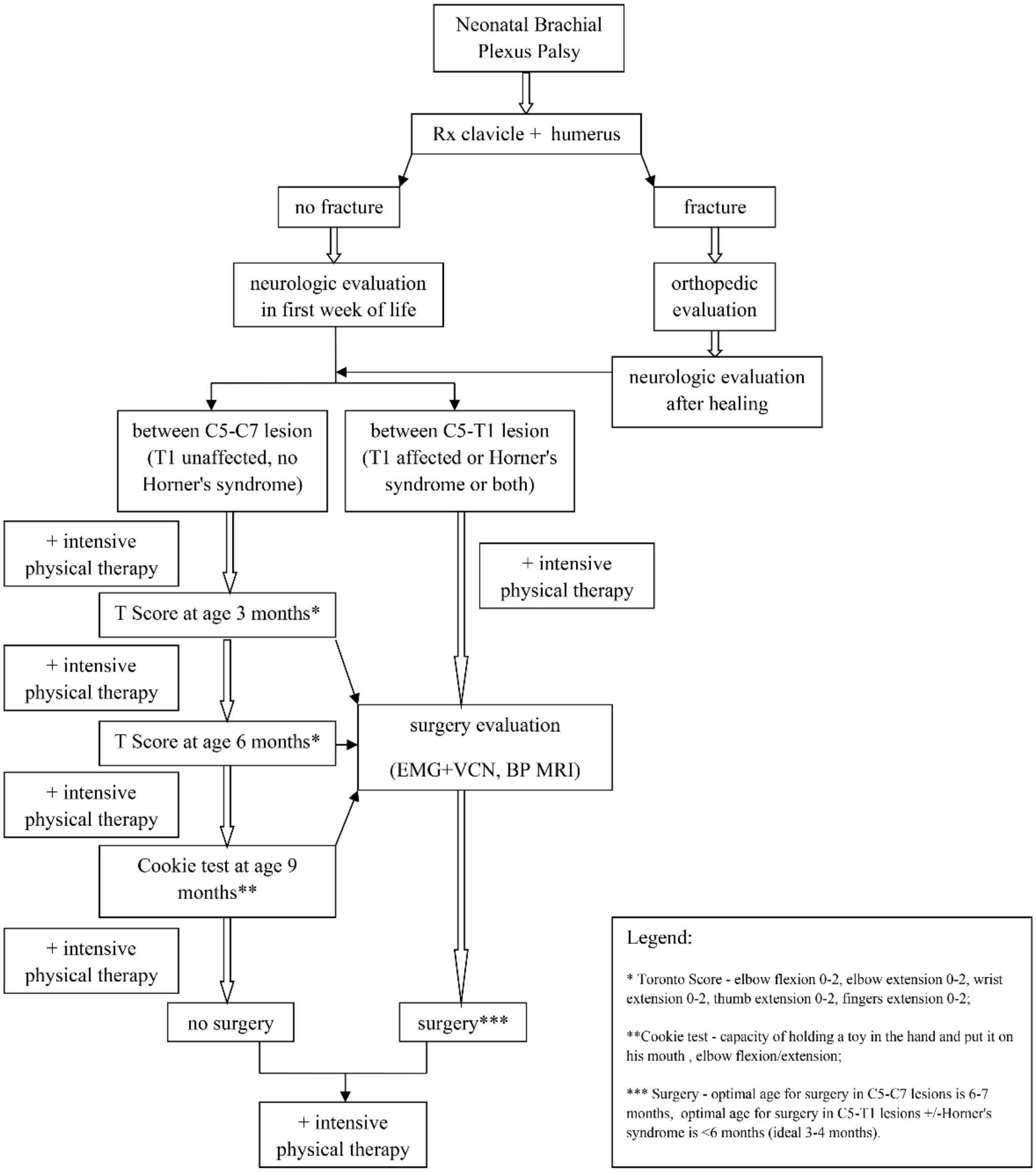
Neonatal Brachial Plexus Palsy
Rx clavicle + humerus
no fracture
fracture
neurologic evaluation in first week of life
orthopedic evaluation
neurologic evaluation after healing
between C5-C7 lesion (T1 unaffected, no Horner's syndrome)
between C5-T1 lesion (T1 affected or Horner's syndrome or both)
+ intensive physical therapy
T Score at age 3 months*
+ intensive physical therapy
T Score at age 6 months*
surgery evaluation
(EMG+VCN, BP MRI)
+ intensive physical therapy
Cookie test at age 9 months**
+ intensive physical therapy
no surgery
surgery***
+ intensive physical therapy

Legend:

* Toronto Score - elbow flexion 0-2, elbow extension 0-2, wrist extension 0-2, thumb extension 0-2, fingers extension 0-2;

**Cookie test - capacity of holding a toy in the hand and put it on his mouth , elbow flexion/extension;

*** Surgery - optimal age for surgery in C5-C7 lesions is 6-7 months, optimal age for surgery in C5-T1 lesions +/-Horner's syndrome is <6 months (ideal 3-4 months).

Age at first presentation in NBPP is of paramount importance for the subsequent treatment choice. It is important that the children with BPP to be early referred – even from birth for evaluation and treatment, in this way – T score at age 3 months will be the most valuable tool for an indication of early surgery if needed.

Cervico-brachial MRI was performed only in some of the patients with a T score <3.5. The importance of MRI is high for the treatment choice – in case of the complete avulsion, surgery is indicated.

Although some studies report 80-90% of the good recovery in unilateral, superior brachial plexus palsy, in our cohort we had only 11 (39.2%) patients with good recovery, probably due to inconsistent physical therapy and non-compliance to scheduled evaluations.

The results cannot be applied in the general population due to the small number of patients analyzed. A larger, prospective study is needed in the future to fill in the gaps. To be noted the good clinical practice of neonatology specialists, pediatricians, and general practitioners who evaluate the children in the first days of life and immediately take action, sending them immediately to pediatric neurology for evaluation and treatment.

– the age of first neurological evaluation and initiation of physical therapy decreased and the number of evaluated children with NBPP increased in the last few years in Romania although this is purely based on anecdotal data as no national registry exists for NBPP. That is why we strongly agree that the first protocol of diagnosis and treatment was needed in our country (Figure 1). Of course, that the experience of the next few years and the discoveries in the field will help us to permanently improve the protocol for better practice, according to the international rules.

CONCLUSIONS

The use of protocols for diagnosis and follow-up makes us „speak the same language", have the same criteria for diagnosis, evaluation, and treatment, with some particular situations due to the local needs.

Early diagnosis and treatment are important because the outcome is better – than raises the quality of life of these patients and the addressability for physical therapy. Vojta therapy has good results in NBPP. Better information of the patients concerning the steps of evaluation of NBPP and the importance of compliance with the protocol is critical for a better outcome and for avoiding a permanent deficit.

Reconstructive surgery must be done in selected cases for a better recovery.

This is a pilot study, with a proposal of protocol for diagnosis and treatment, according to the international protocols in force, but larger, prospective studies are mandatory to complete and refresh the existing data (Figure 1).

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ORIGINAL ARTICLES

Medical devices in current medicine

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Abstract: *The treatment of diseases is based on pharmacological and non-pharmacological means, but adequate devices are needed for their application. Medicine uses a wide range of medical devices. Currently, many medical devices are used, many of them being disposable. As recognition of the importance of medical devices, the national authority of the drug is the ANMDM (National Agency for Medicines and Medical Devices).*

Medical devices are indispensable for therapy, and their evolution has led to an extraordinary diversification of the field and the emergence of industry related to the pharmaceutical industry, with specific laws and regulations.

Keywords: *medical device, medicine, pharmacological action, non-pharmacological action, health legislation*

INTRODUCTION

The treatment of diseases is based on pharmacological and non-pharmacological means, but for whose application different devices are needed, suitable physical objects, without which treatment cannot be applied. Modern medicine uses a wide range of medical devices, some derived from classical forms, and others that are new and more complex, which revolutionize therapy. Although their use is very old, the term as such has been newly introduced in medical terminology, from the English terminology medical device. Another neologism, related to this, is disposable = available, which, together with a noun, also means "disposable". Currently, many medical devices are used, either small or large, simple or complex, many of them being disposable. As a recognition of the importance of medical devices for the medical and pharmaceutical field, as well as for the medical and pharmaceutical industry, the current national authority of the medicines (not a drug, apud WHO) has been renamed the ANMDM (National Agency for Medicines and Medical Devices) [1, 2].

GENERALITIES

Definition: "Medical device" means any instrument, apparatus, machine, appliance, or other item used alone or in combination, including the software necessary for its correct application, intended by the manufacturer to be used for human beings, and which does not achieve its primary intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its intended function by such means, for one or more of the specific medical purpose(s) of:

- diagnosis, prevention, monitoring, treatment or alleviation of the pain,
- diagnosis, monitoring, treatment, alleviation of or compensation for an injury,
- the investigation, replacement, modification, or support of

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the anatomy or a physiological process,
- control of conception (Law 176/2000).

Legislation

The specific legislation, including regarding medical devices, also appeared later on - in 1976, the Regulatory System for the MD - US. In 1993, the EU Regulatory System 93/42/EEC and Romania: Law 176/2000 on medical devices and Law 434/2004, with the Government Decision and Orders of the Ministry of Health. Example: HM Order 253/2010 regarding the reclassification of medical devices and 75/2010 for the Rules for Good Pharmaceutical Practice.

Within the HM, operate specific structures such as the Commission for Medical Devices and the National Agency for Medicines and Medical Devices. We mention that in the European Union, the pharmaceutical field was removed from the Health and included in the Industry, which caused some controversy in the medical world [3-23].

Authorization

Medical devices can only be marketed if they are effective, meet the intended purpose, and ensure patient safety. The certificate of conformity issued by the manufacturer guarantees the attestation of the product for being placed on the market and the classification in the corresponding risk class. The inscription must comply with the European Conformity Marking "CE" (conformitè europèene), which allows free movement in the EU space and should not be confused with "C E" (China Export), which has a space between letters. It turns out that a new medical device, being a product of the scientific research of development-innovation, will go through the following stages: Experimental model, Functional experimental model, Prototype (technological demonstrator), and zero series (at the manufacturer). The technical file of a medical device, which is subject to the approval of the NAMMD, contains information regarding the: device description, type of raw materials used for manufacturing, stability study, analysis bulletin, validation, description of the packaging, and instructions for use. The serial products must be manufactured with the technical assistance of those who have the patent for the product.

CLASSIFICATION

There are many types of medical devices and they can be classified according to several criteria:

1. Risk level
- Class I, Low risk: non-invasive medical devices (eg patches, bandages) and invasive (eg surgical gloves);
- Class II, Medium risk (use 1h-30 days) invasive (eg needles, catheters);
- Class II b, potentially high risk (use - more than 30 days) invasive (eg: probes, devices for repeated administration) and non-invasive (eg: modern dressings that come in contact with the damaged skin);
- Class III, High risk: surgically invasive, a tissue of animal origin, implantable or an integral part of a drug (eg dressings with antibiotics).

2. Access path
- Urinal,
- Digestive,
- Surgical,
- Respiratory,
- Parenteral.

3. Duration of use
- Transitional action, less than 1 hour,
- Short term, less than 1 month,
- Long term, over 1 month.

4. Frequency of use
- Single-use,
- Repeated,
- Permanent,
- When necessary.

5. Impact on the body
- Non-invasive,
- Invasive,
- Surgically-invasive,
- Implantable.

6. Method of manufacture
- Sterile (disposable or re-sterilizable),
- Nonsterile/aseptic.

7. Mode of operation
- With power source (eg electric battery),
- Without a power source.

8. Place of use
- Medical institution,
- Home,
- Portable.

After use in different activities:
Medical devices for:
- Diagnosis and monitoring,
- Administration of medicines,
- Parapharmaceutical (ex: dressings),
- Damaged tissue (orthopedics, surgery, burns),
- Oral hygiene,
- Sexual protection,
- Childcare,
- Medical imaging.

Categories of users:
- Manufacturing companies,
- Suppliers and distributors,
- Sanitary units,
- Medical technical workshops,
- Patients and clients.

QUALITY

The quality requirements are set out in the specific Reference Standards. Starting with 01.03.2019, ISO 13485: 2016 is mandatory. This standard is designed for organizations involved in the design, production, installation, and maintenance of medical devices and for risk management.

- Sterility

All devices and technical-medical products that come into direct contact with tissues and biological fluids are required to be sterile.

Sterilization by heat, gas, or radiation is chosen depending on the nature of the material to be sterilized and the efficiency of sterilization is checked according to FR X unless otherwise provided.

- Storage

The storage and transport of medical devices are done according to the general provisions of FR X unless otherwise provided. Some materials must be stored under special conditions: rubber, plastics, metal tools, dressing materials, and utensils.

LABELING

Markings

Medical devices must be labeled, on the product, on the packaging and the instructions for use with the information essential to the safety of the user:
- The product’s name,
- Identification of the manufacturer,
- Lot number and series, including the date,
- Storage conditions,
- Method of operation.

In some cases, additional data is required for special labeling:
- Sterile,
- Disposable,
- Exclusively for clinical investigations;

Different graphic symbols or warnings can be added.

RISK MONITORING

The safe handling of health products is called vigilance:
- Pharmacovigilance for pharmaceuticals and pharmaceutical raw materials,
- Materiovigilance for medical devices,
- Reacto-vigilance for in vitro diagnosis,
- Biovigilance for temporary-use cellular products,
- Hemovigilance for blood products.

Materiovigilance pursues the incidents arising from the use of the technical-medical products, to ensure the safety of the medical procedures, permanent monitoring of the incidents, and the risks of their occurrence. On the Materiovigilance Data Sheet are reported the essential information related to erroneous indications, omissions, or incomplete instructions. On their basis, studies and expertise are also carried out for the continuous evaluation of the risk level after being launched on the market.

RAW MATERIALS AND PACKAGING

The raw materials can be natural or artificial, single or in combinations: cellulose, cotton, metals, and alloys, natural and synthetic rubber, ceramic materials, glass, plastics, etc. or combinations thereof. All materials used must be compliant for medical use, from pharmaceuticals to the finished product, including packaging. As an example, medical devices for the application of innovative antibacterial and/or antibiofilm drugs fall within the general definition, according to the legislation in force. They must meet all the conditions for authorization, both as a package and/or applicator, and as a medicine administered for the treatment of the patient; they must come accompanied by a Technical File, Analysis Report, and a Conformity Certificate endorsed by the HM through the NAMMD. They fall into Class II b of Potentially High Risk, non-invasive as modern dressings, which are exposed to damaged skin or Class I Low risk, non-invasive. All components must be sterile or aseptic and must be stored and used properly. The labeling must be complete, accompanied by the appropriate markings and with the follow-up on the Materiovigilance. The pharmaceutical forms and the packages (applicators) in which they can be presented may differ, depending on the particularities of the treatment, being required several experimental models for choosing the prototype. Each of the components must come accompanied by a medical opinion for use, and if it does not, it must be requested from the NAMMD, which means time and money for taxes [3, 4, 9, 12].

As examples of medical devices for the application of pharmacologically active drugs may be listed the following:

1. Paper sachets, amylaceous, etc.
2. Capsules: soft or hard, gastro-soluble or gastro-resistant, with compartments or concentric (delayed), etc.
3. Pencils: liquid or powder dosage, etc.
4. Ampoules: glass or plastic, brittle, compartmentalized (lyophilized powder and solvent).
5. Bottles: glass or plastic, perforable, dropper, jet, sprayer (passive, active)
6. Bottle/dose atomizer under pressure (spray)
7. Patches: fast or delayed (for different areas) etc.
8. Implants: in an orifice, subcutaneous, submucosal, and visceral (eg: ovules, suppositories, spark plugs, cones, etc.)
9. Impregnated dressings: solutions, powders, solubles, ointments, absorbable or non-absorbable, hydrogel, etc.

COMMENTS

Currently, our research team is studying medical devices for the application of pharmacologically active drugs, for the substitute, adjuvant or complementary treatment to the anti-infective treatment, especially against resistant bacteria.

There are different medical devices, suitable for types of substances and dosages, authorized for medical use, from which we can choose the most suitable for the purpose; creating a new device, simple or complex, would require a complicated and costly procedure, but unnecessary in this case. The range of medical devices that can be filled with the appropriate pharmaceutical preparation is currently provided by the pharmaceutical industry upon request.

By combining the newly proposed drug with the chosen medical device, experimental model variants are achieved [24]. After experimenting with these under different conditions, the functional experimental model is obtained, as a masterful and/or galenic pharmaceutical preparation for the testing provided by the Law on medicinal products, and will subsequently enter under the pharmaceutical authorization procedures.

CONCLUSIONS

Medical devices are indispensable for therapy, pharmacological, or non-pharmacological. Their evolution has led to an extraordinary diversification of the field and the emergence of industry related to the pharmaceutical industry, with specific laws and regulations. Medical devices play a very important role in modern medicine.

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7. *** HG Nr. 55 din 29 ianuarie 2009 privind dispozitivele medicale implantabile active, cu modificările și completările ulterioare.

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12. *** Ordinul Ministrului Sănătății nr. 874/2015 pentru aprobarea formularelor de declare a activităților de sponsorizare în domeniul dispozitivelor medicale și al materialelor sanitare.

13. *** Ordinul Ministrului Sănătății nr. 308/2015 privind controlul prin verificare periodică a dispozitivelor medicale puse în funcțiune și aflate în utilizare.

14. *** Ordinul Ministrului Sănătății nr. 373 din 26.03.2014 privind aprobarea formularului cu regim special al procesului verbal de constatare și aplicare a sancțiunilor contravenționale privind nerespectarea dispozițiilor legale referitoare la dispozitivele medicale și activitățile conexe acestora.

15. *** Ordinul Mnistrului Sănătății nr. 1356/2013 privind aprobarea tarifelor aplicate de către ANMDM pentru activitățile desfășurate în domeniul dispozitivelor medicale.

16. *** Ordinului Ministrului Sănătății nr. 1032/2011 pentru aprobarea Normelor privind donațiile de medicamente, materiale sanitare, dispozitive medicale, vaccinuri, seruri și consumabilele aferente.

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ORIGINAL ARTICLES

Emergency surgery for complicated colorectal cancer – What we choose: a retrospective cohort study

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Abstract: *Introduction: Despite the progress that has been made regarding the diagnosis, a worrying percentage of patients are still admitted in an emergency, with complicated colorectal tumors, which often require interventions without a curative visa. The study aims to analyze the factors involved in choosing the type of surgical treatment.*

Patients and methods: We included in the study a group of 431 patients admitted and operated in an emergency for complicated colorectal cancer in the Surgery II Clinic of the Clinical Emergency County Hospital "Sf. Ap. Andrei" from Galați, between 2008-2017. We retrospectively analyzed the patients' data and we made statistical correlations between the type of emergency surgery and epidemiological, clinical, paraclinical, and intraoperative factors.

Results: Colostomy was mainly practiced in older patients (p<0.01), from rural areas (p<0.01), with associated cardiac diseases (p<0.01), with hydro electrolytic disorders (p<0.03), rectal tumors (p<0.01), peritoneal carcinomatosis (p<0.02), frozen pelvis (p<0.01). Hartmann's operations were associated with patients with: leukocytosis (p<0.01), intestinal perforation (p<0.01), sigmoid tumors (p<0.01). Internal derivations were associated with patients with: liver metastases (p<0.01), splenic angle tumors (p<0.01), tumors invading other organs (p<0.01), and stage IV of the disease (p<0.01). Resections with anastomosis were associated with transverse colon tumors (p<0.01), well-differentiated tumors (p<0.01).

Conclusions: The type of emergency surgery performed correlated well with the age of patients, the area, the comorbidities, the history of the disease, cachexia, anemia, oligoanuria, hydroelectrolyte disorders, tumor complication, and location of the tumor, hepatic metastases, and tumors' invasion in other organs, tumor grading and stage of the disease.

Keywords: *surgical treatment, colorectal cancer, emergency, correlations*

INTRODUCTION

In Romania, according to the GLOBOCAN 2012 analysis, colorectal cancer was the second [1]. According to the data provided by the GLOBOCAN 2018 statistics, colorectal cancer ranks third in incidence globally (after lung and breast

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cancer) and at the 2nd place as cancer mortality (after lung cancer) [2].

The incidence is increasing both in developed countries and in those where until a few decades ago, the colorectal cancer was not a major concern [1].

Both sexes are affected in approximately equal proportions and the most affected decades of age are the sixth and the seventh [1].

Despite the improvements that have been made regarding the diagnosis and the possibilities of screening, a worrying percentage of patients are still admitted in an emergency, with complicated colorectal tumors, which often require interventions without a curative visa [3].

Of all the complications of colorectal cancer, the intestinal occlusion is the most frequent and it occurs especially in the case of tumors located in the left colon and rectum [4].

There are no clearly established protocols regarding the choice of the type of emergency surgery for colorectal cancer. Various standardizations have been tried, but no consensus has yet been reached [5]. The most debated issue is the choice of the optimal intervention for occlusive left colon cancers [6].

The choice of the surgical treatment method in an emergency is made according to several factors, such as the biological status of the patients, the duration of the complication, comorbidities, age, local invasion of the tumors, the presence of metastases, the appearance of the affected colon. Whenever possible, it is ideal to choose a method that involves the tumor resection or allows it in a second time [7].

The most recent recommendations of the WSES Scientific Committee indicate in the case of occlusive or perforated colon tumors to practice a right hemicolectomy with a terminal ileostomy. The right hemicolectomy with ileocolic anastomosis can be performed if there is no significant increase in operative time and good bowel vascularization. For left colon occlusive tumors, the Hartmann operation is considered the procedure of choice. In patients with severe instability, a colostomy should be performed. For perforations on the left colon, Hartmann's procedure is recommended, with the mention of considering the open abdomen [8].

PATIENTS AND METHODS

We included in the study a group of 431 patients admitted and operated in an emergency for complicated colorectal cancer in the Surgery II Clinic of the Clinical Emergency County Hospital “Sf. Ap. Andrei” from Galați, between 2008-2017.

The patients’ data were collected from observation sheets, operating protocols, and pathological exams. The following epidemiological and clinical factors were analyzed: age, sex, provenance area, hereditary-collateral history, abdominal surgical history, comorbidities, preoperative diagnosis, and clinical status at admission (weight, height, diuresis).

We analyzed the following paraclinical factors: the values of hemoglobin, hematocrit, leukocytes, platelets, and ionograms at admission. Among the therapeutic factors, we analyzed the tumor location, the invasion in other organs, the intraoperative appearance of the frozen pelvis, the presence of hepatic metastases and peritoneal carcinomatosis, the type of surgery performed in an emergency, lymph node dissection, liver biopsy, stoma reversal after Hartmann procedure.

The inclusion criteria were patients with colorectal malignancies hospitalized and surgically treated in the Surgery II clinic of the Clinical Emergency County Hospital “Sf. Ap. Andrei" from Galați, between 2008-2017, patients over 18 years old.

The exclusion criteria were patients with benign colorectal pathology, patients with malignant colorectal tumors operated electively, patients whose observation sheets no pathological reports were attesting the type of tumor, grading, and classification in the pTNM system were not found.

Study design

Epidemiological, clinical, paraclinical, and therapeutic factors were retrospectively analyzed, to correlate them with the four types of surgery (external derivations, Hartmann procedure, internal derivations, and resections with anastomosis).

Statistical analysis

Using the SPSS version 23.0, statistical correlations were obtained, indicating the p-value with the Pearson, Chi-Square, and Likelihood Ratio tests. Statistical conclusions were formulated using a statistically significant difference threshold the value $p < 0.05$ for all calculations performed.

RESULTS

In each of the 10 years included in the observation period of this study, an average of 43 patients was hospitalized and operated in an emergency.

There have been practiced 179 (41.5%) external derivatives (of which 158 colostomies and 21 Pezzer cecostomies), 134 (31.1%) Hartmann operations, 29 internal derivatives (of which 16 ileo-transverse, 9 transverse-descending, 2 ileo-sigmoid, 1 ileo-descending, and 1 transverse-sigmoid anastomosis) and 89 (20.9%) resections with anastomosis (of which 47 right hemicolectomies, 13 left hemicolectomies, 26 transverse/sigmoid/descending segmental colectomies, 1 subtotal colectomy, 1 ileo-transverse anastomotic resection for a relapsed tumor and 1 rectal tumor resection with mechanical anastomosis).

The sex distribution of patients in the study reveals the predominance of men, with a ratio M/F = 256/175. Comparing the types of surgery performed (1 - colostomy/ cecostomy, 2 - Hartmann, 3 - internal derivations, 4 - resections with anastomosis), no association with the sex of the patients is obtained.

In the studied group, the patients from the urban area predominated. Patients in rural areas were associated with a colostomy ($p<0.01$) (Table 1).

Most patients (55.92%, n = 241) included were in the 5th and 6th decades of age. An association of the advanced age of the patients with the colostomy is noted ($p<0.01$) (Table 1).

43 (9.97%) of the patients had a significant hereditary-collateral history, as follows: 19 had a brother/sister with colorectal cancer, another 18 patients had a father diagnosed with colon or rectal cancer, and six patients had a son with colorectal cancer. Patients with a significant heredocolateral history for colorectal cancer were associated with colostomy and with Hartmann's procedure ($p<0.02$) (Table 1).

174 patients (40.37%) had a personal history of abdominal surgery, as follows: appendectomy - 72 patients, hysterectomy - 31 patients, cholecystectomy - 29, gastric resections (for gastroduodenal ulcer disease) – 12, caesarean section - 8, splenectomy (post-traumatic spleen rupture) -6, nephrectomy (renal lithiasis) - 6, segmental colectomy (descending colon cancers) - 4, right hemicolectomy - 2, adnexectomy - 2, hepatic hydatic cyst operated - 1 patient and colon esophagoplasty (for accidental ingestion of sodium hydroxide in adolescence) -1 patient. Comparing the patients with or without a surgical history, no statistical associations are found with the type of surgery.

Most patients (51.04%) included in the study had comorbidities. 148 of the patients (34.33%) had associated cardiac pathology, of which 77 (17.86%) with high blood pressure, 38 (8.81%) with atrial fibrillation with chronic anticoagulant treatment, 8(1.85%) with a history of myocardial infarction, 13 (3.01%) with varying degrees of heart failure, 12 (2.78%) with ischemic stroke; 42 (9.74%) patients were diabetic; 9 (2.08%) patients were diagnosed with liver cirrhosis, with thrombocytopenia; 9 other patients had colonoscopically diagnosed colon pathology, 5 of whom had colonic polyposis, 3 with Crohn's disease and one with diverticulosis. 2 of the patients had active pulmonary tuberculosis at the time of admission (transferred from another hospital). We also found statistical association by correlating the type of surgery with the presence of heart disease and atrial fibrillation, but not with liver cirrhosis. Thus, in patients with cardiac disease or atrial fibrillation, type 1 surgery (colostomy) was performed ($p<0.01$), respectively ($p<0.01$) (Table 1).

The majority of patients (87.94%, n = 379) had as main symptoms at admission the abdominal pain and the disorders of intestinal transit, and the rest (12.06%, n = 52) presented with rectal bleeding. Regarding the beginning of the symptomatology, the majority of patients (58%, n = 250) had symptoms (intestinal transit disorders and rectal bleeding) that they neglected. In patients with neglected symptomatology, it was performed a Hartmann procedure or colostomies ($p<0.01$). Patients with abdominal pain and intestinal transit disorders were associated with colostomy/ Hartmann ($p<0.01$) (Table 1).

Figure 1: Perforated sigmoid tumor (Surgery Clinic II of the County Hospital of Emergency Clinic „Sf. Ap. Andrei „Galati)

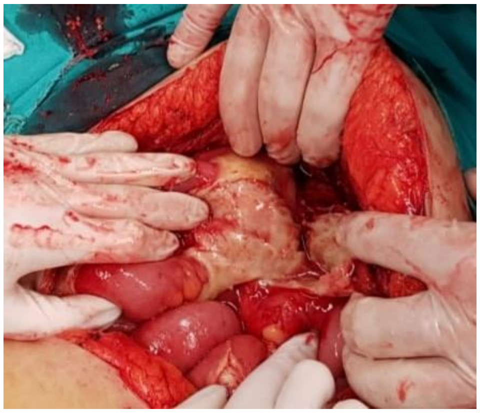

The preoperative diagnosis was intestinal obstruction in 332 patients (77.03%); 36 patients (8.35%) had intestinal perforation (tumor/diastatic) (Figure 1), 14 (3.25%) with imminent diastatic perforation (Figure 2) and 49 (11.37%) had rectal bleeding. Patients with hemorrhage or with imminent intestinal perforation (tumor/diastatic) at admission were associated with a colostomy; patients with intestinal occlusion were associated with internal derivations and resections with anastomosis. Patients with intestinal perforation were associated with the Hartmann procedure (p<0.01) (Table 1).

Table 1: Statistical correlations of the main types of surgery

	Type 1 (N=179 – 41.5%)	**Type 2 (N=134 – 31.1%)**	**Type 3 (N=29 – 6.7%)**	**Type 4 (N=89 – 20.9%)**	**p_value (test)**
Sex					
F	74/179	47/134	12/29	42/89	0.341089 (1)
M	105/179	87/134	17/29	47/89	
Age	72.35±12.5117	67.88±12.3811	62.72±10.2396	64.89±11.2426	0.000001 (2)
Area					
R	98/179	52/134	7/29	20/89	0.000001 (1)
U	81/179	82/134	22/29	69/89	
Number of days of hospitalization	10.0 [7.0, 15.0]	13.0 [10.0, 16.2]	14.0 [12.0, 16.5]	15.0 [12.0, 17.0]	0.000000 (3)
Abdominal pain=yes	135/179	133/134	29/29	83/89	0.000000 (1)
Intestinal transit disorders=yes	135/179	133/134	28/29	81/89	0.000000 (1)
HCA=yes	10/179	20/134	1/29	12/89	0.017821 (1)
Surgical abdominal antec.=yes	81/179	50/134	13/29	30/89	0.243466 (1)
History=yes	118/179	87/134	11/29	31/89	0.000001 (1)
Anemia=yes	134/179	76/134	22/29	67/89	0.002115 (1)
Anemia transfusions=yes	55/179	27/134	2/29	23/89	0.018961 (1)
Leukocytosis=yes	13/179	33/134	4/29	13/89	0.000342 (1)
Trombocytosis=yes	4/179	5/134	0/29	0/89	0.092185 (4)
Cachexia=yes	80/179	36/134	4/29	8/89	0.000000 (1)
HE disorders=yes	82/179	54/134	7/29	27/89	0.029122 (1)
Oligoanuria=yes	72/179	35/134	2/29	5/89	0.000000 (1)
Preop. diagnosis					
H	42/179	1/134	0/29	6/89	0.000000 (4)
I	11/179	3/134	0/29	0/89	
O	120/179	106/134	29/29	77/89	
P	6/179	24/134	0/29	6/89	
Tumor location					
Ascendant	1/179	0/134	14/29	45/89	0.000000 (4)
Descendant	6/179	23/134	12/29	16/89	
Rectum	149/179	0/134	0/29	2/89	
Sigmoid	19/179	102/134	0/29	12/89	
Transverse	4/179	9/134	3/29	14/89	
Tumor invasion=yes	69/179	26/134	22/29	9/89	0.000000 (1)
Synchronous tumors=yes	1/179	0/134	0/29	2/89	0.232949 (4)
Frozen pelvis=yes	38/179	5/134	0/29	2/89	0.000000 (1)
Hepatic MTS=yes	37/179	23/134	16/29	5/89	0.000000 (1)
Carcinomatosis=yes	9/179	2/134	2/29	0/89	0.019195 (4)
Cardiac diseases=yes	83/179	38/134	7/29	20/89	0.000133 (1)
FiA=yes	26/179	7/134	2/29	3/89	0.005035 (1)
HC=yes	4/179	5/134	0/29	0/89	0.092185 (4)
HP					
ADK	179/179	134/134	28/29	89/89	0.142874 (4)
GIST	0/179	0/134	1/29	0/89	

Grading					
1	60/179	26/134	1/29	28/89	0.002476 (1)
2	104/179	97/134	22/29	52/89	
3	15/179	11/134	6/29	9/89	
Mucoid=yes	6/179	3/134	1/29	6/89	0.411940 (4)
pTNM					
2	20/179	51/134	0/29	35/89	0.000000 (1)
3	120/179	59/134	12/29	50/89	
4	39/179	24/134	17/29	4/89	

Figure 2: Rectosigmoid tumor with necrosis and imminence of diastatic perforation (Surgery Clinic II of the County Hospital of Emergency Clinic „Sf. Ap. Andrei „Galati)

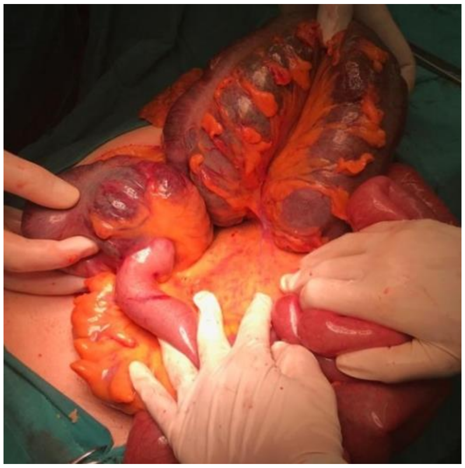

All patients benefited from preoperative imaging investigations, such as empty abdominal radiography and for those with a clinical diagnosis other than intestinal obstruction, abdominal ultrasonography was performed.

128 patients (29.69%) presented with cachexia when admitted (BMI <18.5). 114 patients (26.45%) had oliguria at admission (diuresis <= 500 ml). We found statistically significant differences in the cases of cachectic and oliguric patients at admission. In these patients, the colostomies were mainly practiced and only in very few cases the internal derivations/resections with anastomosis were chosen (p<0.01) (Table 1).

299 (69.37%) of all patients had anemia at admission. Of these, 107 required full blood transfusions or erythrocyte mass. According to the reference interval of our laboratory, we considered anemia values of hemoglobin less than 11g/dl and hematocrit less than 35%. Statistical analysis revealed the association of patients with anemia with a colostomy, and Hartmann's operations (p<0.01); as well as patients with anemia with blood transfusion with a colostomy, or internal derivations (p<0.02) (Table 1).

63 patients (14.61%) had leukocytosis at admission (a number of leukocytes> 10,000/µl). Thus, patients with leukocytosis were associated with Hartmann's interventions (p<0.01) (Table 1).

170 patients (39.44%) had hydro electrolytic disorders at admission (especially hypopotassemia). We considered that the patients with hydro electrolytic disorders were those whose ionograms on admission showed values of Na <136 mmol/l and potassium values <3.5 mmol/l. Patients with hydro electrolytic disorders were associated with a colostomy (p<0.03) (Table 1).

During the emergency surgery, in 13 cases (3.01%) the presence of peritoneal carcinomatosis was found (Figure 3). Peritoneal carcinomatosis has been associated with a colostomy (p<0.02). 81 (18.79%) patients had liver metastases, found either on preoperative ultrasound examination or the occasion of laparotomy (in most cases). Patients with liver metastases were associated with internal derivations (p<0.01) (Table 1).

Figure 3: Ascendant colon tumor, peritoneal carcinomatosis (II Surgery Clinic, Sf. Ap. Andrei Clinic Emergency County Hospital Galati)

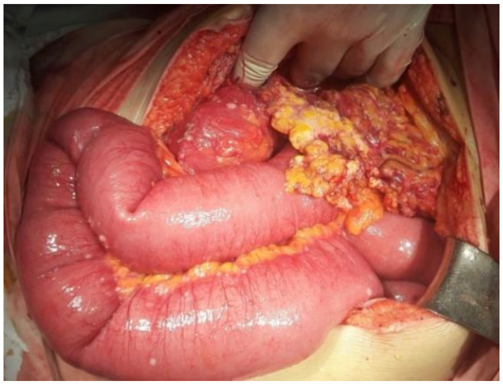

45 (10.44%) of the patients had "frozen pelvis" (tumor block comprising other pelvic organs, to which there is firm adherence, with very difficult or non-existent cleavage planes, to which small bowel loops adhere). Patients with frozen pelvis were associated with a colostomy (p<0.01) (Table 1).

Regarding the location of tumors, 60 (13.92%) were located at the level of the ascendant and hepatic angle of the colon, 30 (6.98%) at the level of the transverse colon, 57 (13.22%) at the level of the descending and splenic angle of the colon, 133 (30.85%) in the sigmoid colon and recto-sigmoid junction and 151 (35.03%) were rectal tumors.

For the tumors of the ascending colon and hepatic angle, in most cases, resections with anastomosis (right hemicolectomies) were practiced, followed by internal derivations and cecostomies ($p<0.01$), the patients with tumors located on the descending colon and the splenic angle mainly benefited from internal derivations or Hartmann's interventions ($p<0.01$), for patients with tumor location in the rectum, colostomies were practiced ($p<0.01$) and for the transverse colon locations, resections with anastomosis ($p<0.01$) were performed (Table 1).

In 126 cases (29.23%) we encountered tumors invading other organs: 27 invasive tumors in the prostate, 26 cases with tumors that invaded the small intestine, 18 invasive tumors in the gastrocolic ligament/large epiploon, 13 in the vagina, 11 in the abdominal wall, 10 in the sacrum bone, 6 tumors were invasive in the bladder, 4 in the duodenum (+/- head of the pancreas) and spleen, 3 invasive tumors in the right/left annexes, 2 in the left renal capsule and 2 tumors that invaded the pelvic ureter. The subset of patients with invasive tumors in other organs was associated with internal derivations ($p<0.01$) (Table 1).

Data from the histological reports revealed the predominance of adenocarcinomas (99.76% of cases) and only one GIST tumor. 16 (3.71%) of the patients had tumors with a mucoid component.

Regarding tumor grading, 115 (26.68%) were well-differentiated adenocarcinomas (grade 1), 275 (63.8%) medium differentiated tumors (grade 2) and 41 (9.52%) weak tumors differentiate (grade 3). Well, and medium differentiated tumors were associated with colostomies or with resections with anastomosis ($p<0.01$).

For postoperative staging, we used the classification recommended by The Union for International Cancer Control (UICC). Thus, according to pTNM staging, tumors in stages II and III predominated - 106 (24.59%), tumors in stage II, 241 (55.91%) in stage III, and 84 (19.5%) in stage IV. The patients in stage II were associated with resections with anastomosis or Hartmann operations ($p<0.01$), patients with tumors in stage III were associated with colostomies ($p<0.01$), and patients in stage IV were associated with internal derivations and colostomies ($p<0.01$) (Table 1).

DISCUSSIONS

According to literature data, up to 30% of colorectal cancer patients present with complicated tumors in emergency [9]. Despite many standardization attempts, the best surgical option for patients with complicated colorectal tumors is still under debate. [5, 10]

We have shown in this study that the type of surgery in patients with complicated colorectal cancer correlates with: age, provenance area, associated cardiac diseases, history of the disease, cachexia at admission, oliguria and hydro electrolytic disorders, diagnosis, presence of liver metastases, and frozen pelvis appearance, tumor location, tumor invasion in other organs and staging.

There is no consensus regarding the surgical treatment of the elderly affected by colorectal cancer, but it is generally accepted that patients over 80, who present in an emergency, compared to the younger ones, have a higher risk of postoperative complications and mortality, despite the progress made in preoperative care, surgical techniques, and intensive care.[11] Most authors reported significant correlations between the type of intervention and the age of the patients [6, 12], as we found in our study, but different from the data obtained by other authors.[13]

Several authors found significant correlations between the type of operation and the patients coming from the rural area [14], as we found in our study. Most authors agree that patients in rural areas have lower addressability due to more difficult access to medical services and therefore tend to show up later, with more advanced tumors.[15, 16]

The comorbidities, especially the cardiac ones, are associated in many studies with the practice of non-curative interventions in patients with complicated colorectal cancer.[17, 18] We also found this correlation in our study.

The pathophysiology of cachexia syndrome is characterized by negative energy and protein balance. In neoplastic patients, it is a major cause of morbidity and mortality [19-22]. A recent study, which included 235 patients with advanced colorectal cancer, reaffirms the negative impact of cachexia on the evolution of the patients with complicated colorectal cancer.

Oliguria is considered a specific and sensitive biomarker of renal dysfunction and a good predictor of mortality risk in critically ill patients, including complicated colorectal tumors.[24, 25] For patients with complicated colorectal cancer, with oligoanuria at admission, colostomies are performed, according to several reports [26, 27], as we found in our study.

Neoplastic patients frequently present with hydroelectrolyte balance disorders. These occur during tumor growth, because of inadequate absorption of electrolytes or because of the secretion of metabolically active substances by the tumor [28]. The hydroelectrolyte disorders present at admission are associated with the type of surgery performed in patients with complicated colorectal cancer [29], as we have shown in our study.

The liver is the most common location of secondary dissemination of primary colorectal tumors. Between 10% and 25% of colorectal cancer patients have liver metastases at the time of surgery [30]. Despite all the therapeutic progress made in recent years, the presence of liver metastases remains a negative prognostic factor.[31] Most of the time, these patients have advanced tumors and the only viable option in an emergency is a colostomy, which will allow them to survive for several months.[32] We made the same finding in our study.

The type of surgery is associated with the preoperative diagnosis, which, most commonly, is the intestinal obstruction. The therapeutic attitude in these cases depends on the location of the primary tumor, the stage, the general state of the patient, and the local changes (tumor resectability, colic distention, ischemic lesions of the colic wall, integrity of the ileocecal valve).[33, 34]

For perforated tumors, Hartmann's intervention is the recommended option in most published works [35-37], as we have shown in our study. For hemorrhagic tumors, the options are different depending on the location of the tumor.

In our study, most hemorrhagic tumors were rectal, and the emergency surgical option was most often a colostomy, after a local bleeding control. In the literature, there is also mentioned the practice of arteriographic embolization in these situations [38], a technical possibility that we did not have.

Patients with locally advanced colorectal tumors, with invasion in other organs, can be a therapeutic challenge [39-41].In most cases, in these situations, a terminal colostomy is practiced [42], which also happened in our study group. Other authors have considered the practice of plurivisceral resections in these situations.

The first such series was reported by Sugarbaker in 1946.[43] Croner et al. performed a study on 174 patients who underwent plurivisceral resections during the period 1978-2002 [44]. Most of the patients had sigmoid colon cancers and the most commonly invaded organs were the small intestine, the bladder, and the abdominal wall.

The type of emergency surgery performed for complicated colorectal cancer is significantly statistically correlated with the colorectal tumor location in all the analyzed studies. Most authors consider, as in our study, that single-phase procedures for left colon tumors are not indicated in emergency and that in these situations the Hartmann procedure is preferable.[45, 46] But some authors believe that, including for occlusive left colon tumors, the first option should be a resection with anastomosis [7, 13, 47, 48]. For the complicated tumors located on the right colon, there seems to be unanimity regarding the surgical option, in these cases the right hemicolectomy being the option.[8, 49]

The TNM staging proposed by the AJCC has remained a universal model since its first edition in 1977 [50]. Since then, the guides have been revised several times and the last update was proposed in 2018 [51]. In our study, stage II of disease correlated with Hartmann's operation and stage IV with a colostomy, as we found in other works.[52, 53]

CONCLUSIONS

1) The colostomy was associated with: older age, rural area, associated cardiac diseases, especially atrial fibrillation, neglected symptomatology, cachexia, oliguria, anemia, and hydro electrolytic disorders at admission, tumors complicated with hemorrhage or with imminent diastatic perforation, the intraoperative aspect of the frozen pelvis, rectal tumors, well or medium differentiated tumors, advanced stages of disease (III or IV pTNM).

2) Hartmann's operations were associated with the significant hereditary-collateral history, neglected symptomatology, intestinal transit disorders and abdominal pain upon admission, leukocytosis, intestinal perforation, sigmoid, and recto-sigmoid junction tumors, and stage II TNM.

3) Internal derivations were associated with the presence of intestinal occlusion at admission, liver metastases, and invasion in other organs, with tumors of splenic angle or descending colon and with stage IV disease.

4) Resections with anastomosis per primam were associated with the absence of comorbidities, tumors located at the level of the ascendant colon, hepatic angle, or transverse colon and well-differentiated tumors.

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ORIGINAL ARTICLES

Assessment of patient satisfaction with primary health care in a military setting: A cross-sectional study from Turkey

Alper Tunga Kokcu

Abstract: *Background and Aim: Information on patients' experiences with and evaluations of health care is considered an essential outcome indicator to assess the quality of health care. This study aims to evaluate patient satisfaction with primary health care in a military setting.*

Methods: This cross-sectional study was conducted at the primary health care center located in a military setting, in Ankara, Turkey. A total of 3080 military personnel who received primary care in the last 12 months formed the population of the study. Turkish version of the European task force on patient evaluations of general practice care (EUROPEP) questionnaire was administered to the participants.

Results: In the study, 2677 participants were reached with a response rate of 86.9%. The mean satisfaction rate (MSR) of clinical behaviour (69.3%) was higher than the MSR of the organization of care (58.4%). The MSR of the overall questionnaire was 67.1%. Significant differences were observed in the mean satisfaction scores of patients according to their descriptive characteristics including gender, age level, troop type, and the doctor providing the care.

Conclusions: This study highlights the low patient satisfaction levels, which may have resulted from the fact that the study population was young and relatively healthy, and that there was no possibility of choosing the general practitioner. The EUROPEP questionnaire, which is an internationally validated and standardized instrument, can also be used to improve primary health care in military settings.

Keywords: *doctor-patient relationship, military medicine, patient satisfaction, primary care, quality of care*

INTRODUCTION

The World Health Organisation's definition of quality of care is "the extent to which health care services provided to individuals and patient populations improve desired health outcomes. To achieve this, health care must be safe, effective, timely, efficient, equitable, and people-centered." [1]. There are various parameters or indicators to judge the quality of health care. The measurement of patient satisfaction is considered as an essential outcome indicator to evaluate health care quality. Questionnaires are the most widely used instruments to assess patient satisfaction [2,3]. Although there is a controversy about measuring patient satisfaction or patient experience of the quality of care, many authors hold that satisfaction is linked to patient expectations of ideal care and their real experience of care [4].

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In 1997, it was stated by the Council of Europe that the continuous improvement of quality of care is a key priority for all member states. According to this decision, information on the needs, priorities, and experiences of patients at all levels of care provision had to be gathered through appropriate methods ensuring the active participation of patients [5]. A valid and easy-to-use instrument to gather information on patients' experiences with and evaluations of general practice care was developed by the European working party on quality in general practice in 1999. The European task force on patient evaluations of general practice care (EUROPEP) instrument was designed to assess the quality of general practice and to provide relevant feedback to general practitioners, patients, and health care policymakers at different levels [6]. The EUROPEP questionnaire, which is an internationally validated and standardized instrument for patients' evaluations on general practice care, has been used in many European countries, including Turkey.

The objective of this study was to evaluate patient satisfaction with primary health care in a military setting by using the EUROPEP questionnaire. The impact of the differences in patient characteristics on their satisfaction was also investigated. Besides, comparisons were made between this study and other studies, especially those conducted with the EUROPEP questionnaire.

METHODS

This cross-sectional study was conducted at the primary health care center (PHCC) located in Gendarmerie and Coast Guard Academy, in Ankara, Turkey. A total of 3080 military personnel who received care from the PHCC in the last 12 months formed the population of the study. Since the whole population was included in the study, a sample was not chosen. The data were collected between August and October 2018.

Using a five-point answering scale with the extremes labeled as ‘poorest’ and ‘excellent,’ the EUROPEP questionnaire is a multidimensional instrument comprising 23 questions on evaluations by patients of specific aspects of general practice care. The questionnaire has two dimensions: clinical behavior (items 1-16), and organization of care (items 17-23). Through this questionnaire, patients are asked to rate the quality of care that they have received in the last 12 months from the general practitioner that they usually visit [6]. In 2002, Akturk et al adopted the English version of EUROPEP questionnaire into Turkish and demonstrated that the Turkish version of EUROPEP questionnaire is a valid and reliable instrument that can be used for the Turkish population [7]. Three items of organization of care (making an appointment and contacting PHCC or doctor by phone) not applicable in this study were removed from the EUROPEP questionnaire. In the study, the remaining 20 items of the Turkish EUROPEP questionnaire (items 1-16: clinical behavior; items 17-20: organization of care) were administered to the participants. Descriptive characteristics of the participants were also obtained through the questionnaire.

Participants who scored the two most positive answering categories (good and excellent) were considered as satisfied patients. The rate of satisfied patients was used for the description of patients’ views for each item. For the overall questionnaire and each dimension, the mean satisfaction rate (MSR) was used to assess patient satisfaction. Additionally, each item was scored on a five-point answering scale, ranging from one (poorest) to five (excellent). The mean satisfaction score (MSS) and the standard deviation of mean were used for each item. For the overall questionnaire and each dimension, MSS was used to assess patient satisfaction according to descriptive characteristics.

The internal consistency was assessed by using Cronbach’s alpha. The data analysis was performed using the SPSS 22.0 software package. As the dataset was neither distributed normally nor was homogeneous, non-parametric statistical tests were used in the analysis. Mann-Whitney U test and Kruskal-Wallis test were used to determine whether there was any difference in the answers of patients according to their descriptive characteristics. If the Kruskal–Wallis test was found to be statistically significant, Dunn-Bonferroni test was performed for pairwise comparisons. A value of $p<0.05$ was accepted as statistically significant. The descriptive tables including numbers, rates, and means are presented and the results are discussed at the end of the paper.

The necessary administrative permission was obtained from the command of the military setting before the study. The ethical approval for this study was provided by the relevant University Human Research Ethics Committee on 4 July 2018 (No:3304). The completion of the questionnaire was voluntary and anonymous. Consent forms were obtained from the participants before the administration of the questionnaire.

RESULTS

In the study, 2677 participants were reached with a response rate of 86.9%. In subgroups according to the descriptive characteristics of the participants, response rates ranged from 81.2% to 92.3%. All of the participants were military personnel of four different troops and received care from

PHCC at least one time over the last 12 months. General practice care was given by two doctors working in PHCC. Most of the patients were unmarried (n=2251; 84.1%) and male (n=2472; 92.3%). The mean age of patients was 24.8±2.5 and ranged from 18 to 40 years. The descriptive characteristics of the patients are summarized in Table 1.

In the study, all items of the questionnaire were aggregated into two dimensions: clinical behavior (items 1-16) and organization of care (items 17-20). The Cronbach's alpha ranged between 0.975 for "clinical behavior," and 0.883 for "organization of care." For the overall 20 items of the Turkish EUROPEP questionnaire, the Cronbach's alpha was 0.974.

Table 1: Descriptive characteristics of the patients (n=2677)

Characteristics	n	(%)
Gender		
Male	2472	(92.3)
Female	205	(7.7)
Marital status		
Unmarried	2251	(84.1)
Married	426	(15.9)
Age level		
18-20	158	(5.9)
21-25	1408	(52.6)
>25	1111	(41.5)
Troop type*		
A	1997	(74.6)
B	347	(13.0)
C	176	(6.6)
D	157	(5.8)
Visit number		
1-2	1663	(62.1)
3-4	637	(23.8)
>4	377	(14.1)
Dr. providing the care*		
Dr-A	1436	(53.6)
Dr-B	1241	(46.4)

**Names of the troops and doctors were encoded because of confidentiality*

For each item, the MSS and the rate of patients who scored the two most positive answering categories (good and excellent) are presented in Table 2. The highest satisfaction rate and MSS were observed for "keeping your records and data confidential" (81.2%; 4.15±0.86), "thoroughness" (75.9%; 4.06±0.97), and "listening to you" (75.9%; 4.04±0.96). The lowest satisfaction rate and MSS were found for "waiting time in the waiting room" (47.2%; 3.30±1.25), and "the helpfulness of the staff (other than the doctor)" (56.5%; 3.56±1.16). MSR of clinical behavior (69.3%) was higher than MSR of organization of care (58.4%). The MSR of the overall questionnaire was 67.1%. While the MSS of clinical behavior was 3.89±0.85, the MSS of organization of care was 3.60±1.00. For the overall general practice, the MSS was 3.84±0.84.

It was observed that there were significant differences in the MSSs of patients according to their descriptive characteristics including gender, age level, troop type, and the doctor providing the care. The MSS of female patients was lower than the MSS of male patients ($p<0.05$). The MSS of patients in the 18-20 age group was higher than those in the other age groups ($p<0.05$).

Regarding the overall questionnaire and each dimension, the patients from troop-A had lower MSS, compared to the patients from troop-C and troop-D ($p<0.05$). Regarding the dimension of clinical behaviour, the patients from troop-A also had lower MSS, compared to the patients from troop-B ($p<0.05$). Patients consulting Dr-A reported more satisfaction regarding the organization of care and overall general practice than the patients consulting Dr-B ($p<0.05$). However, there were no differences among other descriptive characteristics of patients such as marital status and the number of visits. The detailed results of comparisons of MSSs concerning the descriptive characteristics are given in Table 3 and Table 4.

DISCUSSION

It can be said that patient satisfaction is related to the physicians' adherence to the four principles of biomedical ethics including autonomy, beneficence, non-maleficence, and justice. Physicians' adherence to the principles of biomedical ethics, especially of autonomy, can be evaluated by using the instruments for assessing patient satisfaction. Most of the time, patients are not aware of the quality of health care delivered to them. Therefore, valid and reliable instruments are used to evaluate patients' experiences of health care.

In this study, the EUROPEP questionnaire was used for the assessment of patient satisfaction with primary health care in a military setting for the first time. Having this study conducted with the EUROPEP instrument allows us to make direct comparisons between the results of this study and the results of previous studies conducted with the same instrument. Similar to other studies, the current study revealed very high values for Cronbach's alpha for the EUROPEP questionnaire and its two dimensions [8-10]. For the overall questionnaire and each item, this study reported low patient satisfaction levels. Significant differences were observed in the MSSs of patients according to their descriptive characteristics including gender, age level, troop type, and the doctor providing the care. On the other hand, no differences were determined among other descriptive characteristics of patients, such as marital status and the number of visits.

Consistent with the previous EUROPEP studies, the results of this study showed that the patients were more satisfied with "clinical behavior" compared to the "organization of care." [8,9,11-14]. Similar to other studies, the highest and lowest satisfaction rates were for the items ''keeping your records and data confidential'' (81.2%), and "waiting time in the waiting room" (47.2%) [8-10,12-14].

Table 2: Descriptive statistics for 20 items of the questionnaire (n=2677)

Questions	MSS (Mean±SD)*	Good or excellent response [n (%)]**	
Clinical behaviour			
1. Making you feel you had time during consultations	3.80±1.02	1768	(66.0)
2. Interest in your personal situation	3.85±0.99	1802	(67.3)
3. Making it easy for you to tell him or her about your problems	3.97±0.95	1953	(73.0)
4. Involving you in decisions about your medical care	3.82±0.99	1763	(65.9)
5. Listening to you	4.04±0.96	2032	(75.9)
6. Keeping your records and data confidential	4.15±0.86	2173	(81.2)
7. Quick relief of your symptoms	3.85±1.00	1809	(67.6)
8. Helping you to feel well so that you can perform your normal daily activities	3.89±0.97	1867	(69.7)
9. Thoroughness	4.06±0.97	2033	(75.9)
10. Physical examination of you	4.00±0.99	1942	(72.5)
11. Offering your services for preventing diseases	3.89±0.98	1823	(68.1)
12. Explaining the purpose of tests and treatments	3.80±1.05	1744	(65.1)
13. Telling you what you wanted to know about your symptoms and/or illness	3.87±1.01	1833	(68.5)
14. Helping you deal with emotional problems related to your health status	3.72±1.05	1646	(61.5)
15. Helping you understand the importance of following his or her advice	3.86±0.99	1810	(67.6)
16. Knowing what he or she had done or told you during contacts	3.75±1.06	1688	(63.1)
MSS and MSR (items 1-16)	3.89±0.85		(69.3)
Organization of care			
17. Preparing you for what to expect from a specialist or hospital care	3.83±1.05	1784	(66.6)
18. The helpfulness of the staff (other than the doctor)	3.56±1.16	1512	(56.5)
19. Waiting time in the waiting room	3.30±1.25	1264	(47.2)
20. Providing quick services for urgent health problems	3.71±1.17	1690	(63.1)
MSS and MSR (items 17-20)	3.60±1.00		(58.4)

MSS: Mean satisfaction score; MSR: Mean satisfaction rate; SD: Standard deviation.

*Items were scored on a five-point answering scale, ranging from 1 (poorest) to 5 (excellent).

**Participants who scored the two most positive answering categories (good and excellent) were considered as satisfied patients.

By using the EUROPEP instrument, Petek et al. conducted a study in eight European countries in 2009. The study aimed to describe evaluations of general practice care by patients with a chronic illness. The MSRs ranged from 82.1% to 93.0% in the European countries [15]. It may be questionable that the study was conducted with a single group of chronic care patients. While a study reported that better health status was associated with a more positive evaluation of care, another study showed that patients reporting a chronic condition were more positive in their assessment of the general practice than patients without a chronic condition [16,17]. However, conducted by Rosemann et al., a previous study utilizing the EUROPEP instrument revealed that patient satisfaction was not determined by the disease-specific quality of life, at least not in osteoarthritis patients [18]. The Turkish Ministry of Health conducted a study in 2010 that surveyed 34472 patients across Turkey. The study aimed to measure patient satisfaction with PHCCs. The MSR was found to be 77.8% with the EUROPEP instrument [19]. The MSR of the current study (67.1%) is lower than that of the previous studies conducted in European countries and Turkey with similar methodology.

In contrast to some other studies, this study found that female patients' satisfaction levels were lower than those of the males [12, 16, 20]. On the other hand, the gender results of this study were consistent with the previous studies conducted in the military settings that found male patients to be more satisfied than female patients [21, 22].

Similar to the study conducted by Petek et al., no difference was found according to marital status in this study [15]. On the other hand, Baltaci et al. observed that the patient satisfaction level was higher for married patients than it was for the unmarried [20].

Table 3: Comparisons of mean satisfaction scores concerning the descriptive characteristics (n=2677)

Characteristics	n	(%)	MSSs (Mean±SD)[**] and p-values[***]					
			Clinical behaviour	p	Organization of care	p	Overall general practice	p
Gender								
Male	2472	(92.3)	3.91±0.84	0.000[a]	3.60±1.00	0.022[a]	3.85±0.84	0.000[a]
Female	205	(7.7)	3.68±0.87		3.49±0.83		3.64±0.83	
Marital status								
Unmarried	2251	(84.1)	3.89±0.84	0.679	3.60±0.99	0.308	3.83±0.84	0.897
Married	426	(15.9)	3.90±0.84		3.55±0.99		3.83±0.83	
Age level								
18-20	158	(5.9)	4.09±0.83	0.003[a]	3.81±0.99	0.008[a]	4.03±0.83	0.004[a]
21-25	1408	(52.6)	3.89±0.83		3.60±0.99		3.83±0.83	
>25	1111	(41.5)	3.85±0.85		3.56±0.99		3.80±0.84	
Troop type[*]								
A	1997	(74.6)	3.84±0.86	0.000[a]	3.55±1.00	0.000[a]	3.78±0.85	0.000[a]
B	347	(13.0)	3.98±0.79		3.66±0.97		3.91±0.79	
C	176	(6.6)	4.09±0.68		3.80±0.89		4.04±0.67	
D	157	(5.8)	4.07±0.85		3.77±1.00		4.01±0.84	
Visit number								
1-2	1663	(62.1)	3.89±0.79	0.149	3.63±0.95	0.254	3.84±0.79	0.441
3-4	637	(23.8)	3.87±0.89		3.53±1.05		3.80±0.88	
>4	377	(14.1)	3.92±0.96		3.55±1.07		3.84±0.94	
Dr. providing the care[*]								
Dr-A	1436	(53.6)	3.92±0.83	0.052	3.64±0.99	0.004[a]	3.87±0.83	0.035[a]
Dr-B	1241	(46.4)	3.85±0.86		3.54±0.99		3.79±0.85	

MSS: Mean satisfaction score; SD: Standard deviation.

[*] *Names of the troops and doctors were encoded because of confidentiality.*

[**] *Items were scored on a five-point answering scale, ranging from 1 (poorest) to 5 (excellent).*

[***] *Mann-Whitney U test and Kruskal-Wallis test were performed ([a] $p<0.05$).*

In contrast to other studies, this study found that younger age was associated with higher levels of patient satisfaction. [15, 16]. However, it is worth bearing in mind that the participants in this study were aged 40 or under.

A previous study conducted in a military setting revealed that waiting times were significantly correlated to overall patient satisfaction [23]. The current study found a difference in the satisfaction levels according to the troops the patients belonged to. In the military setting where the current study was conducted, according to the rules, the patient should visit PHCC with the other patients of his/her troop at the same time in the morning, except for emergencies. As the number of patients in the troop increases, the waiting time in the PHCC extends, which may be the underlying reason for the low patient satisfaction level in crowded troops like troop-A.

The current study couldn't confirm the results of previous studies that there is a positive association between the number of visits and the level of patient satisfaction [15, 16]. A majority of the participants of this study (85.9%) had a low number of visits (≤4), and the mean number of visits was 2.6. In Turkey, the total number of visits per person to a general practitioner in 2017 was 2.9 [24].

In this study, it was observed that there was a difference in the satisfaction levels depending on the doctor providing the care. While Dr-A is a military general practitioner, Dr-B is a civilian general practitioner, which may be one of the reasons for the low satisfaction levels of patients evaluating Dr-B.

A strength of this study is that it was conducted with a large population sample. On the other hand, there are relatively several limitations to the study. Because the study was conducted in a military setting, participants were younger than 40 and most of the visits to PHCC were due to acute health disorders. Due to the limited number of general practitioners working in PHCC where the study was conducted, the participants were able to evaluate two doctors in terms of general practice care.

Table 4: Pairwise comparisons of multiple groups whose comparisons were found to be statistically significant

Pairwise groups	p-values**		
	Clinical behavior	Organization of care	Overall general practice
Age level			
18-20 and 21-25	0.009[a]	0.019[a]	0.010[a]
18-20 and >25	0.002[a]	0.005[a]	0.003[a]
21-25 and >25	1.000	1.000	1.000
Troop type*			
A and B	0.034[a]	0.413	0.062
A and C	0.004[a]	0.010[a]	0.005[a]
A and D	0.003[a]	0.035[a]	0.005[a]
B and C	1.000	0.777	1.000
B and D	1.000	1.000	1.000
C and D	1.000	1.000	1.000

**Names of the troops were encoded because of confidentiality.*

***Dunn-Bonferroni posthoc test was performed ([a] $p<0.05$).*

This study highlights the low patient satisfaction levels, which may have resulted from the fact that the study population was young and relatively healthy, and that there was no possibility of choosing the general practitioner and PHCC. However, according to the results obtained through the questionnaire, taking necessary actions to promote patient satisfaction will improve the quality of primary health care in this military setting.

The EUROPEP questionnaire, which is an internationally validated and standardized instrument for patients' evaluations on general practice care, provides feedback to general practitioners and policymakers. The results of a study conducted with this instrument can be directly compared with other studies conducted with the same instrument. These comparisons can shed light on the similarities and differences between various PHCCs. This instrument can also be used to improve primary health care in military settings.

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Patient consent: Consent forms were obtained from the participants before the administration of the questionnaire.

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ORIGINAL ARTICLES

Total knee arthroplasty radiographic evaluation via a Bayesian belief network. A pilot study

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Abstract: *In an attempt to standardize the X-ray analysis and to create relevant correlations between it and the functional results, we present a pilot model of an artificial neural network based on a Bayesian belief network (BNN) designed for the automatic analysis of anteroposterior X-ray of the knee following total knee arthroplasty and more precisely the analysis of the tibial component.*

A prospective analysis was conducted in which 30 patients were analyzed and two groups resulted: the first was made up of 12 cases considered "normal" by the examiners (optimal positioning and cementation) and the second one, which was considered "pathological" (incorrect positioning or cementation) and included 16 cases.

Based on 22 points established on the tibial component several geometric sizes (calculated parameters) were computed. Several parameters were compared and the results were up to 75% accuracy, 81.25% sensitivity, and 66.7% specificity.

BBNs can be a solution for X-ray evaluation of total knee arthroplasty and can improve the understanding of its evolution. It needs confirmation in time, its accuracy rising with the number of patients introduced in the analysis algorithm.

Keywords: *TKA; Bayesian method; ANN; X-ray analysis*

INTRODUCTION

Artificial Neural Networking (ANN) are computer systems that create connections, similar to those existing in the biological neural network [1]. Such systems are programmed to learn based on examples, without being programmed in advance to identify specific tasks [2]. For example, artificial neural imaging systems can identify an area of osteolysis, the area that has been previously set as osteolysis using a yes/no algorithm on other images. They can perform this task without having the ability to understand what an osteolysis area means.

ANN is made up of a mass of nodes connected by artificial neurons just like the component of a biological brain. Artificial neurons are interconnected by synapses and can send information between them.

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The initial purpose of these artificial neural networks was to create a replica of the human brain and the latter to be able to make decisions similar to those made by a human. Following their application in different fields, the initial purpose was forgotten. They are currently used for verbal recognition, translation, social networks, but most importantly in the medical diagnosis, such as total knee arthroplasty (TKA) [3, 4, 5, 6].

Thus, for patients with diseased knee or hip joints, total knee or hip arthroplasty (TKA/THA) is considered as one of the most effective procedures for relieving pain and improving joint function [7]. Improvements in joint arthroplasty procedures will routinely witness rapid recovery, faster functional recovery, lower postoperative discomfort, and higher patient satisfaction, which is a win-win for patients and surgeons [8].

The importance of the restoration of the biomechanical axis of the pelvic limb is emphasized in current studies [9]. Proper prosthetic positioning, mobility restoration, and pain disappearance are factors predicting the long-term survival of the prosthesis as well as the postoperative patient satisfaction [10].

Figure 1: Postoperative TKA image used for Bayesian evaluation

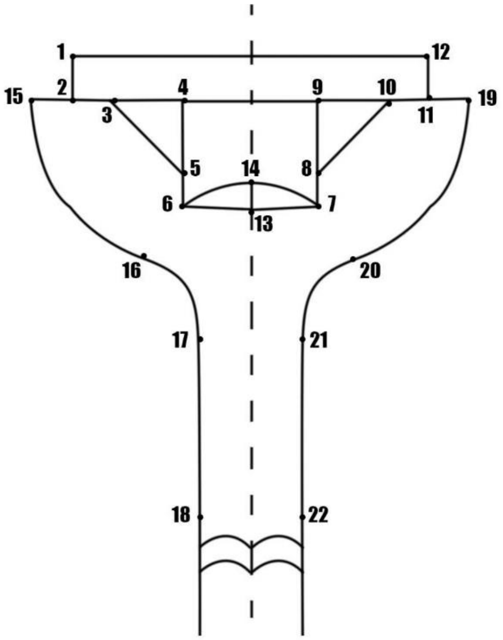

Radiological postoperative evaluation following total knee arthroplasty is essential to improve the quality of the surgical act. The standard procedure implies that two postoperative X-rays are performed, one anteroposterior, and one profile X-ray. They analyze the mechanical alignment of the knee, bone coverage by the prosthesis, femoral anterior and posterior offset, femoral flexion and extension, tibial slope (Figure 1).

Also, during the initial postoperative evaluation, any deficiencies in the cementation technique are identified. Ideally, it is sought to obtain a uniform cement coat for thickness and distribution.

In an attempt to standardize the X-ray analysis and to create relevant correlations between it and the functional results, we present a pilot model of an artificial neural network based on a Bayesian belief network (BNN) designed for the automatic analysis of anteroposterior X-ray of the knee following total knee arthroplasty and more precisely the analysis of the tibial component.

BNNs are frequently used in medicine, they provide the probability of an outcome based on creating intuitively relations between variables [11-15].

MATERIALS AND METHODS

A prospective study in which two lots of patients were used. In both, patients with gonarthrosis were included, who were subjected to total knee arthroplasty, which was performed in the same center by the same surgical team. The inclusion criteria were patients over 65 years old, who were subjected to TKA, with underlying gonarthrosis disease. Exclusion criteria included inflammatory arthritis, post-traumatic gonarthrosis with major deformities, unicompartmental arthroplasty.

The two lots of patients were conceived following the X-ray analysis on day 0 postoperatively by an independent team of orthopedists. Standing AP, profile, and full leg X-rays of 30 patients were analyzed and two groups resulted: the first was made up of 12 cases considered “normal” by the examiners (optimal positioning and cementation) and the second one, which was considered “pathological” (incorrect positioning or cementation) and included 16 cases. 2 patients were excluded following the appearance of postoperative complications. The same patient groups were followed up at 1 month, 6 months, and 1 year postoperatively. The same set of X-rays was performed at each visit.

Following the constitution of the two groups of patients, it was decided to start the computer analysis of the postoperative X-rays from 6 months and 1 year. To test the validity/relevance of the automatic analysis, we decided that the initial analysis should be performed at the tibia level, on front-loading X-ray. 22 control points were established at the level of the tibial component (Figure 2) that corresponds to the placement of the prosthesis and the tibial bone, as shown in the schematic picture, 14 points on the contour of the prosthesis and 8 points on the tibia.

Figure 2: 22 control points at the level of the tibial component

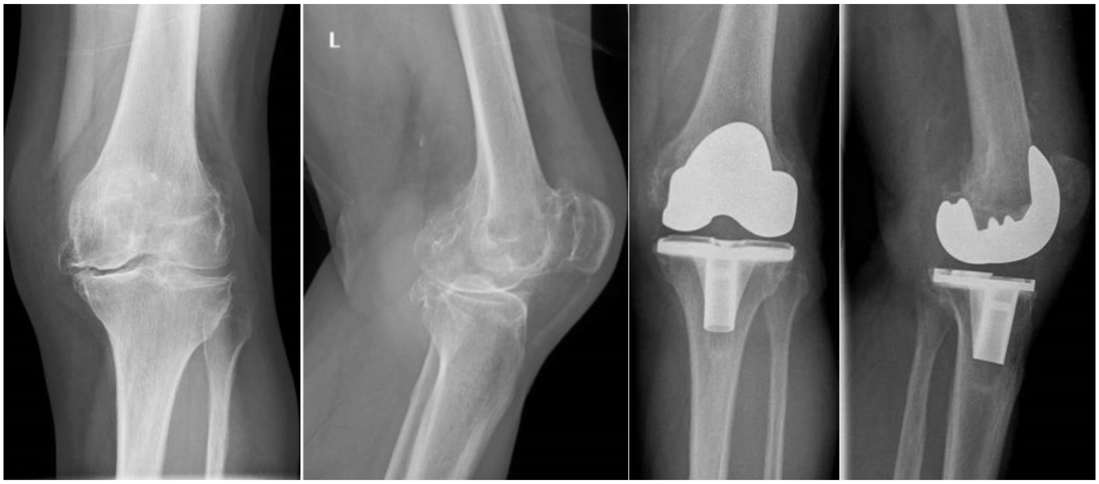

Using the identified points, several geometric sizes (calculated parameters) were computed:

1. The left-right rotation of the X-ray image, determined by the difference of the areas associated with the triangles determined by points 3 – 4 – 5 and 8 – 9 – 10 on the prosthesis.
2. Up-down rotation of the X-ray view, determined by the area associated with the ellipse determined by points 6 – 7 – 13 – 14 on the prosthesis.
3. The angle of vertical offset between the axis of the prosthesis (calculated as bisector of the angle formed by sides 4 – 5 – 6 and 7 – 8 – 9) and the axis of the bone, calculated as bisector of the angle formed by sides 17 – 18 and 21 – 22.
4. Horizontal offset angle between the base of the prosthesis (2 – 3 – 4 – 9 – 10 – 11) and the cut of the bone 15 – 19.
5. Percentage of coverage to the left of the prosthesis, determined by the part of the head bone not covered by the prosthesis at level 15 – 2.
6. Percentage of coverage to the right of the prosthesis, determined by the part of the head bone not covered by the prosthesis at level 11 – 19.
7. Variation of previously calculated parameters between postoperative images at 1 month and 6 months.

RESULTS

Following the schematic of the calculated parameters, a Bayes statistical model was applied.

DECISION 1: Normal-pathological differentiation tests at the level of individual X-rays

Of the many variants tested to make the decision, the best results were obtained based on measure 5 (percentage of coverage left of the prosthesis), with a threshold of 0.82 for differentiation (pathological > 0.82). Taking into account these conditions, the normal/ pathological decision correctly identified: 11 images from normal patients (out of the 24) and 26 images from pathological patients (out of the 32).

This led to the following sizes: 66.1% accuracy, 81.25% sensitivity, and 45.8% specificity.

DECISION 2: Normal-pathological differentiation tests at the level of the pair of individual postoperative X-rays at 1-6 months

Of the many variants tested to make the decision, the best results were obtained based on combined measures:

A. The decision according to a single parameter: the sum of parameter 1 (left-right rotation of the X-ray view) at 1 and 6 months, with a decision threshold of 0.47; pathological > 0.47). Taking into account these conditions, the normal/ pathological decision correctly identified: 9 pairs of images from normal patients (out of the 12) and 9 pairs of images from pathological patients (out of 16).

This led to the following sizes: 64.3% accuracy, 56.25% sensitivity, and 75% specificity.

B. The decision according to several parameters: the sum of parameter 1 (left-right rotation of the X-ray view) at 1 month and 6 months, with a decision threshold of 0.47, pathological > 0.47, or difference of parameter 3 (vertical offset angle between the axis of the prosthesis) at 1 month and 6 months, with a decision threshold of 0.75, pathological < 0.75 and difference of parameter 4 (horizontal offset angle between the axis of the prosthesis) at 1 month and 6 months, with a decision threshold of 1.8, pathological < 1.8.

Taking into account these conditions, the normal/ pathological decision correctly identified: 8 pairs of images from normal patients (out of 12) and 13 pairs of images from pathological patients (out of 16). This led to the following sizes: 75% accuracy, 81.25% sensitivity, and 66.7% specificity.

DISCUSSION

Artificial neural networks are increasingly present in classifications, group creation, pattern recognition, and prediction in many disciplines, medicine being among them. The huge potential is represented by speed, volume, and accuracy of data analysis.

The Bayesian system developed by us can recognize immediately and accurately any deficient positioning of the tibial prosthetic component. It can integrate this assessment into the clinical context and provide answers to a series of questions of patients with low satisfaction. It may be an optimal way of evaluating the series X-ray with results that are superimposed over those obtained by a CT scan.

Our model of X-ray analysis by BBN has several limitations. The number of patients is not large enough to obtain sufficiently conclusive results. X-ray analysis is performed only for the tibial component at present, a comprehensive X-ray analysis that also includes the femoral component is required. The registered data do not contain patient information: age, weight, degree of osteoarthritis, preoperative and postoperative mobility, pain scores, and so on.

CONCLUSIONS

BBNs can be a solution for X-ray evaluation of total knee arthroplasty and can improve the understanding of its evolution.

It needs confirmation in time, its accuracy rising with the number of patients introduced in the analysis algorithm.

It may provide an answer for low satisfaction rates of certain patients in whom the prosthetic components are optimally positioned and have no other postoperative complications.

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ORIGINAL ARTICLES

Prevalence of osteoarthritis disorders in Iranian chemical injured patients: A cross-sectional study using the COPCORD approaches

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Abstract: *Introduction: Osteoarthritis is considered as the most common joint disease. Sulfur mustard (SM) is a toxic compound, which was applied as a chemical warfare agent by Iraq against Iran veterans during 1980-1988. Chemical veterans suffer from chronic implications caused by sulfur mustard that not only adversely affect their quality of life, but also restrict their working abilities and their participation in social activities. This study aims to consider the prevalence of osteoarthritis disorders among Iranian SM-exposed veterans.*

Methods: In this cross-sectional study, 248 Iranian SM exposed patients were evaluated from 2013 to 2014. Veterans filled a special questionnaire for COPCORD studies and then general and specific rheumatology examinations were applied under the supervision of internists and rheumatologists. The obtained data from 245 filled questionnaires were analyzed by SPSS software and the results were reported as a percentage or mean ±SD.

Results: Bone and joint pain (72%), knee joint pain (52.5%), Joint stiffness (49.2%), and shoulder joint pain (45.8%) were the most common type of complications in these patients. Osteoarthritis in at least one joint was the most common type of osteoarthritis in veterans. 69.5% of veterans had osteoarthritis in at least one joint. Knee osteoarthritis was the second most common type of osteoarthritis, which has been found in 58.4% of all veterans. The prevalence of neck osteoarthritis in all veterans was 23.9%.

Conclusion: The total prevalence of musculoskeletal disorders among Iranian veterans was significantly higher than the average of society.

Keywords: *chemical warfare, osteoarthritis, prevalence, COPCORD*

INTRODUCTION

Osteoarthritis (OA) is considered as the most common joint disease and its main pathologic indication at the tissue level is joint cartilage breakdown. This disease is classified into two main groups: (1) primary or idiopathic osteoarthritis in

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which no underlying disease causes the disorder, and (2) secondary osteoarthritis in which an underlying predisposing factor imposes the disease locally or systemically [1]. However, another classification based on the number of involved joints is considered which includes generalized osteoarthritis in which more than 3 joints are involved and also localized osteoarthritis in which less than 3 joints are involved [2, 3]. Identification of predisposing factors gives better insights for the disease pathogenesis. There are various factors such as age, gender, race, genetic, bone density, sex hormones, nutritional status, and environmental factors that can affect the prevalence of OA. Age is the most important risk factor that is not controllable; as it increases, osteoarthritis prevalence rises [4, 5]. The type of joint involvement before 55 years is the same among both sexes; however, when age increases hand joint involvements more likely occur among females but hip joint involvements are more prevalent among men [6, 7]. The prevalence of OA can be increased along with aging and obesity. According to the United Nations, 130 million people will suffer from OA by 2050, of which 40 million will be severely disabled by the disease. The burden associated with OA includes costs for adaptive aids and devices, medicines, surgery, and time off at work. Osteoarthritis prevalence among black and white races is different [8]. The risk of osteoarthritis among individuals who have parents suffering from the disease is significantly higher [9]. Some studies have proposed a reverse relation between osteoporosis and osteoarthritis and some have suggested that bone density increscent is correlated to thigh and knee osteoarthritis but not hand osteoarthritis [10-12]. Endocrine or metabolic disorders such as diabetes, hyperparathyroidism, and acromegaly are in association with osteoarthritis [13]. Nutrition status and antioxidant materials in diets may prevent or postpone osteoarthritis. The damage caused by reactive oxygen species (ROS) is determined through the osteoarthritis trend [14]. Knee osteoarthritis progress among people suffering from vitamin C deficiency is significantly more than those consuming a high amount of this vitamin [15]. Obesity is one of the most important causes of osteoarthritis progress especially in the knee [16]. High pressure on joints due to sport or job, congenital disorders, and inflammatory or infectious diseases are the other affecting factors [16].

The chemical contamination from the wars is one of the main causes of various diseases in veterans. Sulfur mustard [17] is one of the warfare agents which has been utilized during various chemical wars and it was widely applied during the imposed war of Iraq against Iran (1980-88)[18]. The first report on the late implications of SM toxicity among 236 Iranian chemical veterans revealed 78% respiratory, 45% central nerve system, 41% skin, and 36% eye complications. These abnormalities were reported 2-28 months after SM exposure [19].

Chemical veterans are still suffering from the late effects of SM toxicity which negatively impacts their quality of life. More importantly, the progression of their disease restricts their working abilities and participation in social activities. There is little information about the prevalence of osteoarthritis or musculoskeletal disorders among chemical veterans. Since the status of nutrition, medicine, economy, mobility, and generally the lifestyle of the veterans are different from other parts of the society, low bone density or suffering from endocrine and metabolic disorders or chronic infectious disease is more likely to occur among this community [20]. Therefore, the purpose of this study was to evaluate the frequency of osteoarthritis disorders among Iranian chemical veterans who previously exposed to SM using the COPCORD approach.

METHODS

This cross-sectional study was conducted on Iranian chemical veterans in Tehran from 2013 to 2015. For sampling, the initial list of chemical veterans was provided from the lung clinic and medical commission of Baqiyatallah University of Medical Sciences, and individuals who fulfilled the inclusion criteria were entered into the study. Furthermore, according to the coordination of the Veterans and Martyrs Affair Foundation (VMAF), a list of patients who fulfilled the inclusion criteria were selected and invited to participate in the study. The Including criteria were as follows: having documented exposure to sulfur mustard (confirmed by related authorities), male gender, being over 40 years old, moderate and negligible involvement of lungs, and giving consent for participating in the study.

The special questionnaire for COPCORD studies was filled by veterans and the general and specific rheumatology examinations were applied under the supervision of internists and rheumatologists. This questionnaire consisted of demographic questions, complaints about osteoarthritis in the past 7 days, questions about the restrictions, accidents, damages, and external joint symptoms related to musculoskeletal diseases, and also questions in the scope of the treatment detailed examination by specialists and assessing medical histories elucidated the prevalence of different osteoarthritis complications for the mentioned population.

The final part of the questionnaire contained the recorded probable and final diagnosis of the patients who complained about at least one joint (pain, inflation, and stiffness) or external joint pain. If the patient needed more examinations such as radiology in the opinion of the physician, this information was obtained and attached to the questionnaire. Finally, the filled questionnaires were analyzed and the obtained data were revised and after excluding the defaced cases, the questionnaires data were entered into the SPSS software version .20 and the results were described as percentages and mean. The confidentiality of the information of the subjects studied by researchers and project implementers were considered as the ethical considerations of this study. The COPCORD questionnaire used in this study was provided by the Rheumatology Research Center at COPCORD website.

RESULTS

In this study, 245 subjects were evaluated in which 97 patients lived in Tehran and 148 subjects lived in Alborz Provinces. The mean age of the studied population was 51.1 years. Data on demographic information and smoking status can be seen in Tables 1 and 2, respectively.

Table 1: The frequency distribution of studied veterans based on demographic variable

Variable		Number (%)
Marriage status	Single	1 (0.4)
	Married	237 (98.8)
	Spouse dead	1 (0.4)
	Separated or divorced	1 (0.4)
Race	Fars	149 (62.1)
	Azeri	67 (27.9)
	Kurd	11 (4.6)
	Lor	7 (2.9)
	Other	6 (2.5)
Occupation status	Employee	76 (34.1)
	Worker	6 (2.7)
	Retired	76 (34.1)
	Self-employed	49 (22)
	Unemployed	15 (6.7)
	Disabled	1 (0.4)

Table 2: The smoking status of studied veterans

Smoking	Frequency	Percentage	Use average daily	Average of Time (year)
Never	178	75.4	--	--
I am smoking	23	9.7	10.9	19.9
I used to	35	14.8	12.7	6.8

The prevalence of bone and joint complaints in each age group is presented in Table 5. There was no significant difference in the percentage of bone and joint complaints between different age groups. Overall, bone and joint complain (78.4%), bone and joint pain (72%), knee joint pain (52.5%), Joint stiffness (49.2%), and shoulder joint pain (45.8%) were the most common types of bone and joint complains in studied patients.

The percentage of disease intensity and restriction following pain is summarized in Table 3. Most veterans have complained about the intensity of annoying and intolerable pain (73.9%). Furthermore, musculoskeletal pain is getting worse in 66.9% of veterans during activities. Approximately 68.8% of veterans wake up due to musculoskeletal pain.

Table 3: Prevalence of intensity and restriction following pain for complaining veterans

Variable	Percentage
Musculoskeletal pain going worse in the morning	54.1
Musculoskeletal pain going worse during activities	66.9
Waking up because of musculoskeletal pain	68.8
The intensity of annoying and intolerable pain	73.9
Restrictions due to the type or the number of activities in the present or past because of pain, sensitivity, etc.	55.7
Perfect adaptations with problems (pain, sensitivity, etc.)	55.2

Table 4: The relative prevalence of osteoarthritis based on different age groups

Type of the Disease	Age Group (year)			Total
	40-49	50-59	≥ 60	
osteoarthritis at least in one joint (%)	82(69.5%)	72(69.2%)	15(71.4%)	169(69.5%)
dorsal dick osteoarthritis	1(0.8%)	0(0%)	0(0%)	1(0.4%)
spinal osteoarthritis	2(1.7%)	2(1.9%)	0(0%)	4(1.6%)
shoulder osteoarthritis	1(0.8%)	1(0.8%)	0(0%)	2(0.8%)
wrist osteoarthritis	0(0%)	2(1.9%)	0(0%)	2(0.8%)
foot osteoarthritis	1(0.8%)	0(0%)	0(0%)	1(0.4%)
neck osteoarthritis	23(19.5%)	30(28.8%)	5(23.8%)	58(23.9%)
hand osteoarthritis	0(0%)	1(0.8%)	0(0%)	1(0.4%)
hip osteoarthritis	1(0.8%)	3(2.9%)	0(0%)	4(1.6%)
knee osteoarthritis	70(59.3%)	57(54.8%)	15(71.4%)	142(58.4%)

The relative prevalence of osteoarthritis based on different age groups is shown in Table 4. Osteoarthritis in at least one joint was the most common type of osteoarthritis in each age group. 69.5% of veterans had osteoarthritis in at least one joint. Knee osteoarthritis was the second most common type of osteoarthritis in each group. It has been found in 58.4% of all veterans. The prevalence of neck osteoarthritis in all veterans was 23.9%.

The prevalence of osteoarthritis is also considered in men with different age groups (Table 6). Similarly, osteoarthritis in at least one joint was the most common type of osteoarthritis in each age group. Knee osteoarthritis was the second most common type of osteoarthritis in each group.

Table 5: The prevalence of bone and joint complaints in the last 7 days based on the age group.

Type of implication	Percentage in age groups			Total
	40-49 years	50-59 years	≥60 years	
Bone and joint complain	77.1	81	72.2	78.4
Bone and joint pain	73.7	72	61.1	72
Joint inflation	28.8	32	27.8	30.1
Joint stiffness	47.5	50	55.6	49.2
Shoulder joint pain	46.6	47	33.3	45.8
Shoulder joint stiffness	21.2	26	16.7	22.9
Pain due to pressure in the shoulder	30.5	29	16.7	28.8
Elbow joint pain	26.3	22	22.2	24.2
Elbow joint inflation	6.8	8	5.6	7.2
Elbow joint stiffness	10.2	12	11.1	11
Pain due to pressure in Elbow	12.7	17	22.2	15.3
Wrist joint pain	24.6	27	27.8	25.8
Wrist joint inflation	7.6	10	11.1	8.9
Wrist joint stiffness	8.5	14	22.2	11.9
Pain due to pressure in the wrist	14.4	20	16.7	16.9
Pain in the hands and fingers joints	23.7	25	16.7	23.7
Inflation in the hands and fingers joints	12.7	6	11.1	9.7
Stiffness in the hands and fingers joints	9.3	14	11.1	11.4
Pain caused by pressure in the hands and fingers joints	13.6	17	16.7	15.3
Pelvic joint pain	33.1	28	16.7	29.7
Pelvic joint stiffness	11.9	19	11.1	14.8
Pain caused by pressure in the pelvic	19.5	28	22.2	23.3
Knee joint pain	50.8	55	50	52.5
Knee joint inflation	18.6	22	27.8	20.8
Knee joint stiffness	16.9	29	38.9	23.7
Pain due to pressure in the knee	28	34	33.3	30.9
Ankle joint pain	22.9	25	22.2	23.7
Ankle joint inflation	11.9	14	16.7	13.1
Ankle joint stiffness	10.2	18	22.2	14.4
Pain due to pressure in the ankle	15.3	22	16.7	18.2
Toes joint pain	16.9	21	16.7	18.6
Toes joint inflation	5.1	8	16.7	7.2
Toes joint stiffness	5.1	12	16.7	8.9
Pain due to pressure in the toes	12	18	27.8	15.7
Neck joint pain	37.3	39	16.7	36.4
Neck joint stiffness	18.6	21	16.7	19.5
Pain due to pressure in the neck	20.3	29	16.7	23.7
Spine joint pain	47.5	46	27.8	45.3
Spine joint stiffness	22	21	16.7	21.2
Pain due to pressure in the spine	29.7	32	16.7	29.7

DISCUSSION

Musculoskeletal implications negatively affect individuals' health, sanitary, and treatment systems [21]. In a previous study by Joshi and Chopra (2009), they considered the prevalence of osteoarthritis types in 8145 adult subjects in the Pune area located in India. Their results revealed that musculoskeletal pain was the most prevalent type of osteoarthritis in these individuals [22]. The results of the current study demonstrated that the prevalence of musculoskeletal complaints during the past 7 days among veterans is significantly high (78.4%). The most prevalent complaints from the veterans were related to Bone and joint pain, Knee, shoulder joint pains, and joint stiffness. The results of previous studies from different parts of Iran showed that the prevalence of joint complaints in the past 7 days is 54.13%, 41.9%, 42.8%, and 44.7% [23-26]. The comparison of the approached global studies indicated that the prevalence of musculoskeletal complaints in Iran is generally higher than in other Asian countries [27].

Table 6: Prevalence of osteoarthritis among men (Tehran COPCORD results)

Type of the Disease	Age Group (Year)		
	40-49	50-59	≥ 60
osteoarthritis at least in one joint (%)	39%	27%	16.6%
neck osteoarthritis	3.35%	2.41%	1.14%
hand osteoarthritis	7.14%	1.39%	0.5%
hip osteoarthritis	1.99%	0%	0.14%
knee osteoarthritis	36.32%	24.46%	14.49%

Based on the obtained results and final diagnosis of the following study, osteoarthritis, back pain, and pre-arthritis (69.9%, 30.5%, and 27.2%) had the highest frequency of the musculoskeletal diseases among the studied population. This finding confirmed the results of Tehran's study [27]. The results of a previous study on the rural population in Iran showed that approximately 20% of individuals suffered from osteoarthritis in at least one joint. The comparison of COPCPRD results in 17 countries revealed that Iran is second place in regards to osteoarthritis complications (with a frequency of 16.9%) [26[.

The results of this study demonstrated that the most prevalent type of osteoarthritis among veterans is knee (58.4%) and neck osteoarthritis (23.9%), respectively. Interestingly, knee osteoarthritis has been reported as one of the most prevalent rheumatic disorders among the Asian-Oceanic population [23]. The prevalence of knee osteoarthritis was previously calculated to be 5.8% in the rural areas of India, 22-28% in the urban areas of India, 25% in the rural populations in the north of Pakistan, 7.5, 9.2 and 10.6 in the rural, urban slumps and developed urban areas of Bangladesh, and 7.5% in China respectively [28-31]. The results of other studies on the Iranian population demonstrated that the prevalence of knee osteoarthritis in the southeast areas, rural areas, Sanandaj, and the whole country is 18.66%, 19.34%, 26.3%, and 15.5%, respectively [24-26, 28]. Recent studies have indicated that Iran is r ranked third for knee pain among 17 investigated countries [25]. According to previously accomplished data and results of the current research, the incidence of knee pain in veterans is significantly higher than that in the general population of Iran.

In this research, the prevalence of Gout arthritis in veterans was 0.4%. In previous studies, the prevalence of gout arthritis among individuals who lived in Tehran and Sanandaj was 0.13% and 0.12%, respectively [27, 32]. Dawatchi et al. indicated that Iran is ranked 11 among 17 countries regarding the prevalence of rheumatic diseases [25]. Further statistical analysis revealed that Indonesia (1.6%) and Australia (1.5%) had the highest frequency for this disease [25].

CONCLUSIONS

The results of this study described the frequency of musculoskeletal disorders and associated complaints among Iranian chemical veterans. Our data have revealed that the total prevalence of musculoskeletal disorders among Iranian veterans is significantly higher than the average of society. Due to the restrictions of these individuals and specific treatment needs, periodic screening tests, effective and proper nutrition, athletic and therapeutic interventions are strongly suggested to improve musculoskeletal disorders among chemical veterans.

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Declaration of interest

No competing interest was declared by any of the authors.

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ORIGINAL ARTICLES

CoViD-19 disease, Romanian health system response to outbreak and economic impact

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Abstract: *The pandemic called COVID-19 disease has an exponential course since the first cases were reported showing in 9th of May, 2020, more than 4 million cases of COVID-19. United States and European Council (EC), together with other European Union institutions, were closely monitoring the situation in order to take quick action.*

Objective: The aim of this article is to analyze the response to COVID 19 pandemic outbreak in term of strategy and money flow of Romanian health system in order to identify the strength and weakness and prepare for other similar provocative challenges.

Global and national strategy: United States Government is delivering a comprehensive package of services to support international partners around the world in combatting this disease adding American expertise for global benefit, saving lives by improving international partners abilities to respond to the COVID-19, and reducing secondary impacts. Meanwhile European Union is focused on 6 major coordinates limit the spread of the virus, ensure and provide medical supplies, decrease negative economic impact, support jobs, promoting research and help European citizens to repatriate. Despite all negative elements a proper and relatively quick response of Romanian Public Health System was noticed and the taken measures were harmonized and synchronized with the European coordinates, prioritize initiatives, build an action plan, establish a communication plan, workforce optimization and management strategy.

Conclusion: Global shock needs global response, with little or no government interventions, economic costs will be huge.

Keywords: *COVID-19, SARS-CoV-2, Coronavirus, pandemic financial impact, hospitalization cost*

BACKGROUND

COVID-19, coronavirus disease, is caused by a new type of coronavirus (SARS-CoV-2) and it was first identified in China in December 2019 when an outbreak in the municipality of Wuhan was described as quickly spreading to other regions of China and worldwide [1, 2]. By 7 of January 2020, isolated cases had appeared in some European Union (EU) member states. At the end of February 2020, Italy reported a significant increase of coronavirus disease (COVID-19) cases concentrated in two of the northern regions of the country [3]. By March 2020, all European Union (EU) member states had reported an exponential increase of COVID-19 cases. European Council (EC), together with other EU institutions, is closely monitoring the situation in order to take quick action [3]. This includes approving of relevant European

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Union legislation and ongoing coordination with member states to share information, assess needs and ensure a coherent European Union wide response under direct coordination of The World Health Organization (WHO) [3]. On 11 March 2020, the World Health Organization (WHO) declare COVID-19 as global pandemic with an important issue: in this declaration they were talking about geographic spread, and not about the severity of the disease [4].

The pandemic called COVID-19 disease has an exponential course since the first cases were reported showing in 9th of May, 2020, more than 4 million cases. Disease caused by the new coronavirus that was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, COVID-19, have been reported globally with more than 286,000 deaths in 180 countries, including all United States with 1,4 million persons recovered [3, 5].

As epidemiological pattern, all groups of ages are at risk for infection including here severe form of disease, even death. However, seems that the probability of fatal disease is highest in people aged more than 65 years, those with comorbidity and those living in a nursing home or long-term care facility [6, 7, 8]. Of underlying condition, especially when those are not controlled is appropriate to mention as major risk factor for severe or critical COVID-19 disease [3-7]: Hypertension, Obesity, Chronic respiratory disease, Cardiovascular disease, Cancer, Renal disease, Diabetes [9]. As for diagnose WHO recommends as gold standard nasopharynx samples to be used in order to detect SARS-CoV-2. Nasal swabs or oropharyngeal swabs may be acceptable alternatives [9]. Lower respiratory tract samples have a demonstrated higher sensibility than upper tract samples, but WHO recommend not to obtain because of concerns about aerosolization of virus during collection procedures. Regarding treatment, no drug has been proven to be safe and effective for treating COVID-19. There are no Food and Drug Administration (FDA) or EMEA -approved drugs specifically to treat patients with COVID-19. In the medical literature, reports have appeared claiming or promoting successful treatment of patients with COVID-19 using a variety of agents [6, 8, 9]. A single conclusion has emerging: definitive clinical trial data are needed to identify optimal treatments for this disease. General clinical management of patients with COVID-19 includes infection prevention, control measures and supportive care, including supplemental oxygen and mechanical ventilatory when indicated. In is also compulsory to mention that as in the management of any disease, treatment decisions ultimately reside with the patient and their health care provider [6, 7, 9, 10, 11, 12].

Behind the medical challenge generated by COVID-19 pandemic and all concerns, analyst anticipate an economic crisis. That is why worldwide governments are involved in generate measures in order to decrease negative economic impact.

OBJECTIVE

The aim of this article is to analyze the response to COVID 19 pandemic outbreak in term of strategy and money flow of Romanian health system in order to identify the strength and weakness and prepare for other similar provocative challenges.

GLOBAL ANALYSIS AND DISCUSSION

If we have to define the concept of support, probably in the last 50 years the United States (U.S.) will fit perfectly since they have been the largest contributor to global health security [4]. US built the foundation upon which the global health system is based, contributing more than $140 billion in global health assistance only in the last century and nowadays demonstrate leadership in fight to COVID-19 pandemic. From the beginning of the outbreak of COVID-19, the United States government provided more than $500 million in assistance specifically aimed to support anti-pandemic measures [3, 4, 13, 14].

But this is not all, US Government is delivering a comprehensive package of services to support international partners around the world in combatting this disease. The program should add American expertise for global benefit, saving lives by improving international partners abilities to respond to the COVID-19, and reducing secondary impacts [13, 14].

The strategy mentioned is focus to: Save lives by increasing international partners ability to respond to the pandemic; Reduce secondary impacts of the disease; Promote U.S. leadership for global benefit [13].

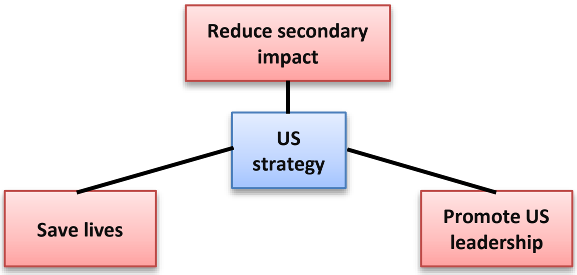

Figure 1: US Strategy for COVID-19 [13, 14]

The United States (US) was delivering a SAFE and more secure world from COVID-19 program as is state in the following table [13,14].

The European Union's (EU) response to COVID-19 outbreak focuses on 6 major coordinates as they are mentioned above [15,16].

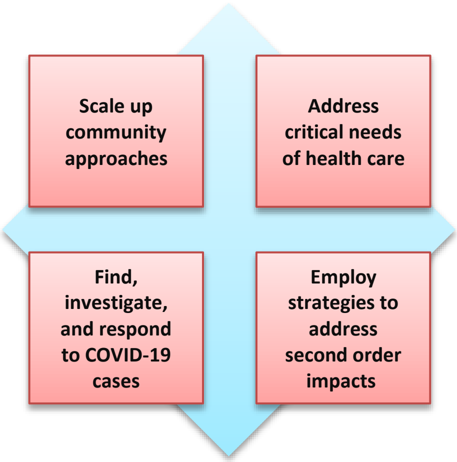
Scale up community approaches
Address critical needs of health care
Find, investigate, and respond to COVID-19 cases
Employ strategies to address second order impacts

Figure 2: US program SAFE for a safer and more secure world from COVID -19

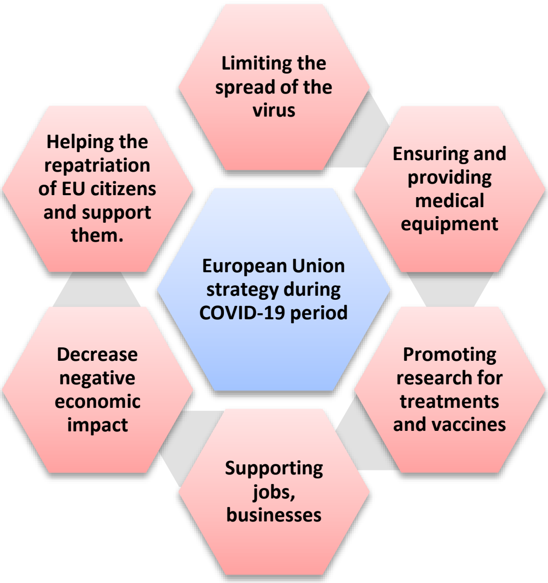
Limiting the spread of the virus
Ensuring and providing medical equipment
Promoting research for treatments and vaccines
Supporting jobs, businesses
Decrease negative economic impact
Helping the repatriation of EU citizens and support them.
European Union strategy during COVID-19 period

Figure 3: European Union strategy for COVID-19 disease [15, 16]

In order to slow down the transmission of the virus, European Union (EU) leaders agreed on a coordinated temporary restriction of non-essential travel to the EU for 30 days. The travel of European citizens outside the EU acquires negative recommendation. To coordinate the EU response to the COVID-19 outbreak the following measures were taken [15, 16, 17]:

- The Council activated the EU an integrated political crisis response (IPCR) with mandatory weekly roundtables gathering EU institutions, EU agencies experts and representatives of member states
- European Commission and the Council are gathering contact and coordination between relevant national ministries
- The European Centre for Disease Control (ECDC) quantify daily risk assessments and epidemiological updates for the EU population.

By using the union civil protection mechanism, the EU has: coordinated the deployment of medical teams to most affected areas, facilitated the sourcing for additional protective equipment, especially medical masks, activated the Emergency Response Coordination Centre, to coordinate support 24/7, created a new common European reserve of emergency medical equipment including also ventilators and lab supplies. More than €300 million was allocated for research and support initiatives in medical field and a European COVID-19 data portal was launched to ensure the rapid collection and sharing of available research data [15, 16, 18, 19].

The EU and its member states are taking action to minimize the negative impact of outbreak on the economy. On 9 April 2020, the Eurogroup generated, three immediate safety nets, worth €540 billion for: protect and generate new jobs and businesses in member states. The EU is putting forward temporary support to mitigate unemployment risks in an emergency (SURE) to help people keep their job during the crisis. The scheme consists of loans to member states of up to €100 billion to cover the costs related to the creation or extension of national short-time work schemes. European Investment Bank (EIB) Group to create a pan-European guarantee fund providing that way loans up to €200 billion for companies with a focus on small and medium-sized enterprises (SMEs) throughout the EU [15, 17, 18, 19].

European Stability Mechanism set up Pandemic Crisis Support based on an existing credit line, which will include loans available to all euro area member states up to 2% of their internal product estimated at €240 billion. The EU also has increased its budget for 2020, with €3.1 billion as response to the COVID-19 crisis [18, 19]. This additional fund will be used to purchase and distribute medical supplies, including protective gear and ventilators, boost the production of testing kits, build mobile hospitals, transfer patients for treatment in between member states, repatriate EU citizens stranded abroad [19].

The EU has also adopted measures to ensure additional flexibility in the use of structural funds. On behalf of

Coronavirus Response Investment Initiative Plus member states can transfer money between different capital lines in order to meet their needs, resources can be redirected to the most affected regions and the opportunity request up to 100% financing from the EU budget between 1 July 2020 and 30 June 2021 for programs involving measures with impact in the COVID-19 pandemic [15, 16]. The EU and its member states were working to repatriate more than 650.000 EU citizens stranded abroad. EU countries and EU delegations provide consular assistance to help EU citizens return home, providing 75% of the costs of the return flights and add measures in order to help them to cross EU borders [16].

Although Li at all [10] estimate that 86% of all infections were undocumented prior to 23 January 2020, when travel restrictions were beginning per person, the transmission rate of undocumented infections was 55% from all of documented cases. Due to their increasing factor, undocumented patients were the source for 79% of documented cases [12]. They conclude that two favorable condition are rising: existing estimates of case-fatality rates might be over-estimate and a grade of immunity is present in the system [12]. But they underline also that probably when interventions started in Europe and US the virus was already widely spread and simulation regarding on how measures of suppression will flatten the curve in the short period may be over-optimistic [12].

NATIONAL STRATEGY AND HEALTH ECONOMIC IMPACT

Geopolitical context

Romania is one of the countries that has to face the infection of the population with the new coronavirus. The most recent statistics shows that Romania has around 4.7 mil people that lives outside the country and they are spread all over the world [3, 19, 20].

According to statistics reported by the Ministry of Foreign Affairs from the total number of the Romanians that lives outside the borders in Italy lives 1.206.938 people which means that around 25% are located in the country that has the largest outbreak of the pandemic, and around 19% lives and works in Spain. This is the reason why Spain has become the second deadliest hotspot for COVID-19 after Italy with 27.967 cases and its death toll reached 24.824 on 1st of May 2020 [19, 20].

Having Italy as a “model “of how fast the virus spread among the citizens, medical stuff and hospitals and that no real actions were made until the spread was out of control, Romania introduced lockdown measures at an early stage in the outbreak: cancelled almost all flights in and out of the country, starting with the ones from the red zone, authorities were also quick to close bars, restaurants, cinemas and schools. Police were urging people to stay at home [19, 20].

The representatives of the Intersectoral Committee for the Prevention and Limitation of Diseases with the New Coronavirus (COVID-19), together with the representatives of the authorities responsible for this field, held a teleconference on the evening of February 22, 2020, taking into account the evolution of cases in Italy areas where many Romanian citizens are or traveling from [19].

After this point the public health authorities included persons arriving in Romania from the affected localities in the Veneto region and the province of Lodi / Lombardy or who have traveled to these localities in the last 14 days, in the category of those who must stay in quarantine for 14 days after entering the country. These measures were similar to those applied by the Italian authorities. At the same time, upon arrival in Romania, all asymptomatic passengers from the affected areas, respectively Hubei (China) or from 11 regions of Italy will be sent to quarantine for a period of 14 days [1, 2, 19].

New measures were sent by the Ministry of Health to the health units and family doctors, through the county and Bucharest public health directorates which included [21]:

- measures to control and prevent infection with the new coronavirus at land, sea / river and air border crossings. The Ministry of Health provided the necessary medical staff at the border crossing points, with specialists from the public health directorates, including by secondment from other counties and health units;
- all persons, who fall within the definition of a suspicious case, should be reported immediately by all health units where they appear (emergency units, hospitals, family doctors) to the public health departments and the Monitoring Service within Emergency Situation Department (DSU);
- Ministry of Health completed the legislative framework with necessary measures for quarantine and for the management of suspected and confirmed cases of coronavirus infection (COVID-19);
- public information campaign on the new coronavirus infection;
- individual and collective prevention measures were taken and extended with a daily information bulletin on the evolution of the situation at international level and any additional measures taken by the Romanian authorities;

Also, on March 10, 2020 the Ministry of Health operationalized the Operational Coordination Center, a unit that establishes in operational time the connection with the public health directorates and health units under the Ministry of Health subordination, to identify in real time the risk situations associated with COVID 19 infection and to establish measures that are required [20, 21].

The Operational Center had to provide up-to-date information and all the useful and vital information to establish the correct measures for the epidemic phases, real time stocks of medicines, medical equipment and disinfectants existing at national level in hospital units, the number of medical staff involved in the actions in order to limit the spread of infection, the number of free beds in infectious disease departments and Intesive Care Units [21].

Other important measure was to limit the scheduled hospitalizations in the hospitals coordinated by the Ministry of Health, in relation to the degree of bed occupancy (including ICU beds) and the local epidemiological evolution corroborated with daily report the situation of bed occupancy of the hospitals in the centralized electronic system of the Coordination Center. This measure was necessary both to ensure the capacity of hospitals to take over cases and to manage material and human resources correctly and efficiently [21].

Adequate public communication was ensured so that, during this period, the public could turn to hospitals only for emergencies. The aim was to avoid congestion in the sanitary units [21]. At that point was established a support network for infectious disease hospitals depending on the contagiousness of non-coronavirus cases. These hospitals are going to be support hospitals, in which patients will be transferred from the Infectious Diseases Hospital. In a first phase, was recommended that the support hospitals not be represented by the important emergency hospitals in the area, which can provide medical assistance for the other medical-surgical emergencies [21].

The Ministry of Health Plan also provides measures in case of extend of the crisis and in case of capacity exceed of Infectious Diseases Hospitals, as well regulations on the use of protective equipment [21].

On March 11, 2020 at the proposal of the Ministry of Health, the National Committee for Special Emergency Situations issued an order for including the temporary suspension, for a period of 6 months, of distribution, outside Romania, of medicines, medical devices and sanitary materials essential in prevention and treatment conditions associated with COVID-19 infection [21].

This measure is part of the action plan of the Ministry of Health which aims to provide adequate and continuous stocks of medicines, medical devices and essential medical materials in this situation. The list was going to be established together with the experts from the specialized commissions of the ministry, taking into account in particular, medicines such as: antivirals, anti-inflammatory, antibiotics, antipyretics, antifungals, anesthetics, sedatives, as well as other medicines considered necessary in the treatment of cases of COVID-19 and their complications [21].

The list also included medical supplies and medical devices such as overalls, gloves, syringes, masks, goggles, disinfectant solutions, as well as other medical supplies and devices identified as necessary for the management of COVID-19 cases [21].

The Centre for Supervision and Control of Transmissible Diseases issued recommendations which were published on 12 March 2020 [21].

- The preventive measures considered as most effective include: frequent hygiene of the hands with hydro-alcoholic substances through friction if hands are not visibly dirty, or with water and soap if the hands are dirty; avoid touching eyes, nose and mouth; practice respiratory hygiene by coughing or sneezing in the elbow, or use napkin/tissues and throw it away immediately; wearing a mask if the person has respiratory symptoms and ensuring in such case the hygiene of the hands after removing the mask; maintain the social distance of minimum 1 meter from the persons with respiratory diseases.

- Protective Personal Equipment (PPE) must be used based on exposure risk and based on the dynamic of transmitting the pathogen agent. The overall use of PPE will have a supplementary impact on the lack of supplies of PPE.

- The state office mentions that for the people with no symptoms, it is not recommended to use masks of any type. The wearing of the masks when it is not recommended can cause useless costs, a burden on purchases and can create a false feeling of security that can lead to the neglect of other essential prevention measures.

Threats and weaknesses

In order to analyze the response to COVID-19 outbreak, we identify a panel of weaknesses which conduct in early stage to quick spread of the virus and generate uncontrolled local outbreaks:

A panel of negative elements should be considered when we analyze the Romanian Health System response to outbreak.

- Low infrastructure in term of hospitals, circuits, Intensive Care Units and number of available PPE for health workers

- Administrative human resources with lack of management skills, unable to generate procedures and coordinates the Public Health Departments

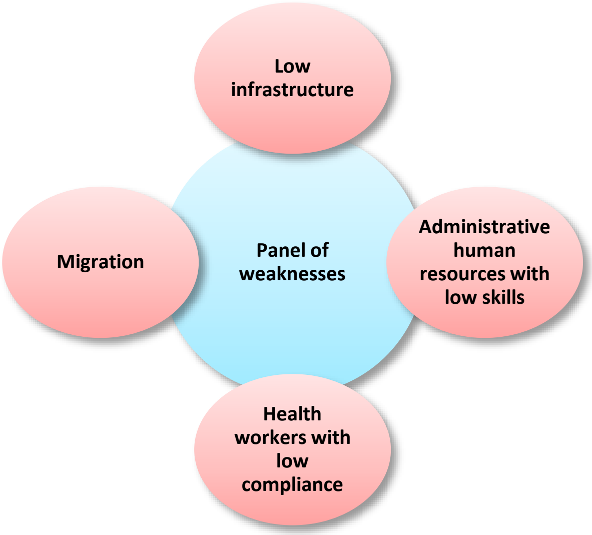
Low infrastructure
Migration
Panel of weaknesses
Administrative human resources with low skills
Health workers with low compliance

Figure 5: Panel of weaknesses regarding response to COVID-19 outbreak

- Health workers with low compliance at medical information and epidemiologic resources, unwilling in many situations to respect the new statement measures and legislation provided by Ministry of Health and National Health Institute

- Not as a last item, migration, the return of Romanian citizens from pandemic areas of Europe crossing the borders using in many situations false document of false declaration and being extremely low compliant of rules and measures, regarding quarantine or in self isolation

Ministry of Health strategy

Despite those negative elements a proper and relatively quick response of Public Health System and it could say that the taken measures were harmonize and synchronize with the European coordinates. Romanian Government through Ministry of Health established 5 directions as a strategy which enhanced the national response to COVID-19 disease.

When “everything is vital and important“, Ministry of Health and the other public institution involved in fighting the pandemic had to prioritize the initiatives. In order to do that a Plan of measures for the preparation of hospitals in the context of the COVID-19 Coronavirus epidemic was approved. This plan was the base of the strategy framework regarding the hospitals that can treat patients infected with COVID19. In collaboration with the local public administration authorities, based on the local analysis of patient care capacities, was generated and approved the List of support hospitals for COVID - 19 positive patients requiring treatment for specific pathologies (eg heart disease, orthopedics, etc.)

Thus, a number of 57 hospitals/outpatient departments/ buildings, 44 maternity hospitals, 60 hospitals and centers for performing dialysis procedures in all counties of the country and Bucharest were identified and designated as support units.

Supportive cases of COVID 19 that require medical care for other pathologies are also treated in support hospitals.

As a first step, designated support hospitals are non-emergency medical units that can provide medical care for other medical and surgical emergencies. Included in the network of support hospitals, municipal/city units and hospitals in other counties mainly those with Infectious Diseases departments, as well as hospitals in other networks than the Ministry of Health (Ministry of Transport, Ministry of National Defense, private health units).

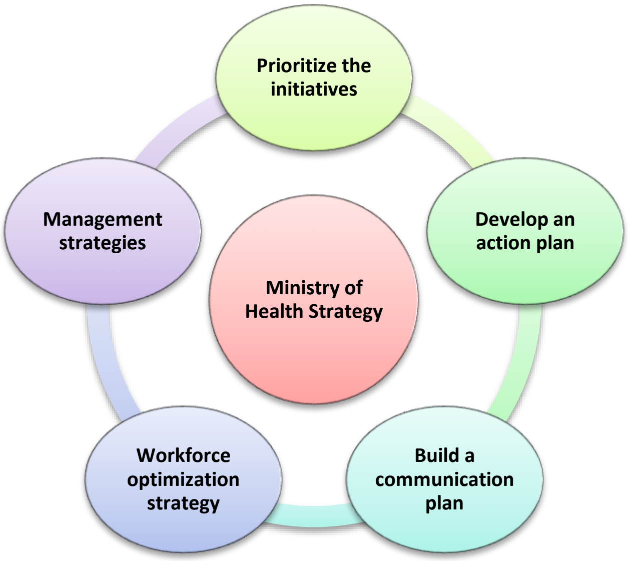
Prioritize the initiatives
Management strategies
Ministry of Health Strategy
Develop an action plan
Workforce optimization strategy
Build a communication plan

Figure 6: Romanian Ministry of Health direction strategy

“The plan of measures for the preparation of the hospitals was elaborated by the specialists within the specialized commissions ICU, infectious diseases and specialists within the Ministry of Health, based on the recommendations of the WHO and EC. I asked the managers of the health units, but also the public health departments, the ambulance services to inform the medical and auxiliary staff, repeatedly, the procedures to be followed in this crisis situation, all the existing measures in the plan of measures, especially the regulations. regarding the prevention measures and the correct use, depending on the exposure, of the medical staff, but also of the auxiliary" [21].

Also, all health units from public and private system have the obligation to ensure the provision of medical care to all patients, in accordance with the disposition of the approved plan of measures. Any refusal to ensure the provision of medical care according to the specific attributions it's going to be sanctioned according to the legal disposals [21].

Develop an action plan

In order to be able to control and establish the right measures Ministry of Health with the help of the Operational Center were able to collect in real time the stocks of medicines, medical equipment and disinfectants existing at national level from the hospitals, the number of medical staff involved and the number of free beds in infectious disease departments and Intensive Care Units.

As we could see the plan is to deliver the equipment or medication where is most needed, or to purchase the equipment or medication where is most needed.

In order to simplify the Purchases of materials, medicines and equipment necessary to combat the COVID-19 pandemic, during the state of emergency, are made directly by the central and local public authorities, the legal entities in which the state is the majority shareholder, the public health directorates, the health units, as well as the National Administration of Penitentiaries and the units subordinated based on the obligation to draw up a note justifying the necessity of the acquisition.

Regulations have been approved on the use of personal protective equipment (PPE) at the level of the health unit, rationally and correctly, depending on the department, staff and type of activity. Thus, in the context of COVID-19, personal protective equipment should be used based on the risk of exposure (eg type of activity) and the transmission dynamics of the pathogen (eg contact, drops or aerosols).

The type of PPE used when caring for patients with COVID-19 will vary depending on the situation, healthcare staff and work carried out. Medical personnel involved in the direct care of patients should use the following PPE: gowns, gloves, mask and eye protection (goggles or face shield).

Specifically, for aerosol-generating procedures for patients with COVID-19 (eg: intubation, noninvasive ventilation, tracheostomy, cardiopulmonary resuscitation, manual ventilation before intubation, bronchoscopy, gastroscopy, and COVID PCR testing) medical personnel should use as protection, gloves, gowns, masks FFP2 and FFP3; waterproof aprons will also be used if overalls/gowns are not waterproof.

Build a communication plan

Creating a communication plan to collect key information from stakeholders regarding the planned initiatives and the capacity building was an important step in order to manage media coverage of the topics related to the new coronavirus infection.

Therefore, by the Decision of the National Committee for Special Emergency Situations no. 2 from February 24 2020 The Strategic Communication Group was established and it consists experts from several institutions like Ministry of Health, Ministry of Defense, Emergency Situation Department, National Institute of Public Health, General Inspectorate for Emergency Situations.

In order to support the population a Free Direct Line (Telverde) has become operational for coronavirus information in coordination of National Institute of Public Health and people can request information on how the virus manifests itself, how to prevent the disease and other details related to preventive behavior. However, the experts informed the populations that if they have any health problems or suspect that you may be infected, to continue to call the emergency number.

Workforce optimizations strategies

The evolution of the epidemiological situation determined by the spread of coronavirus on the Romanian territory, as well as the declaration of the pandemic by the World Health Organization determined the public health risk assessment for the next period as a result of the increased number of active surveillance, early detection , isolation and treatment of positive COVID 19 cases, follow-up of contacts and prevention of the continuous spread of the infection which imposes the need for additional staff involved in these activities [3, 20].

Therefore, at the proposal of the Ministry of Health, the Government of Romania approved the supplementation with 2000 positions within the county public health directorates and county ambulance services for a determined period of 6 months with 2000 positions [21].

In the current situation, the operative crews of the ambulance services are involved both in the transport of patients confirmed with COVID-19, and in the collection of samples from people in quarantine or home isolation.

Also, the staff of the public health directorates ensures the epidemiological triage from the border crossing points, monitors the self-isolated persons at home, monitors the quarantined persons, collects field samples from the persons corresponding to the case definition for testing for COVID-19.

Currently, the number of posts allocated and fully distributed by ministerial order for the county and Bucharest public health departments is 3.862, and for the county ambulance services and the Bucharest-Ilfov Ambulance Service is 11.733 posts [21].

In order to prepare the health units and the health system and the emergency system to cope in case of an exponential increase of the cases with COVID and in case of signaling a personnel deficit in some areas it was necessary to detach the health personnel to cover the needs in the area. Therefore, specialized medical staff, primary care physicians, specialists or residents, medical staff, health auxiliaries and medical staff with higher education (chemists, biologists, biochemists, physicists, pharmacists and balneo-physiotherapists) employees of public health units are about to be seconded or delegated to other public health units that have a major staff shortage.

Management strategies

The socks which make up the pandemic cause a sharp drop in consumption and investment all over the world. With the previous experiences of pandemics, governments across the world have exercised a stronger caution towards outbreak by taking early measures, such as straitening the health infrastructure to prevent the outbreak.

With the measures taken by MoH regarding the fact that many hospitals were force to close outpatient departments and postponing or canceling elective visits and procedures, it's most likely that the financial viability to be threatened, especially for those with preexisting financial challenges and those heavily reliant on revenue from outpatient and elective services.

A 2018 report from the Agency for Healthcare Research and Quality suggested that elective admissions accounted for more than 30% of total inpatient hospital revenue [22]. Elective procedures, especially orthopedic and cardiac surgical procedures, are among the most profitable services for hospitals[23]. By one estimate, hospitals earn $700 more for elective admissions than for admissions through the emergency department [24]. Furthermore, for many hospitals, outpatient revenue now equals inpatient revenue, elective or otherwise [25].

Also in Romania though reduced outpatient and elective revenue may be partially offset by higher hospital and intensive care unit occupancy during the COVID-19 pandemic, and by an increase in services after the pandemic ends, this may not mitigate losses (particularly given the need for surge personnel and resources) or be evenly distributed across hospitals.

The COVID-19 pandemic represents an unprecedented medical and economic challenge for the Romanian health care system. In the absence of robust and sustained governmental support, almost all hospitals will experience financial difficulties.

But hospitals that are smaller, independent, rural, and have critical access status are particularly at risk. Policymakers provided dedicated support to these hospitals to access funds and allocated additional funding to them during the COVID-19 pandemic.

Table 1: Global Healthcare Systems Data

Country	Australia	New Zealand	Germany	UK	US	Romania
Healthcare expenditures per capita	AU $7845 ($5,612)	NZ $5,892 ($4,015)	€4,544 ($4,924)	£2,989 ($2332)	$10,612	€1,029 ($1,115)
Medical practitioners per 1,000 people	3,94	3,35	4,3	2,8	2,6	2,8
Beds per 1,000 people	3,9	2,6	6	2,5	2,8	6,8
Population aged +60 (%)	21,4	21,3	28,2	23,8	17	22,6

Source: https://insse.ro/cms/files/publicatii/Romania_in_cifre_breviar_statistic_2018.pdf, https://www.ibisworld.com/industry-insider/coronavirus-insights/effects-of-covid-19-on-global-healthcare-systems/ [26]

According to the Medical Group Management Association, 97% of medical group practices have experienced a negative financial impact directly or indirectly related to COVID-19. On average, these practices report a 55% decrease in revenue and a 60% decrease in patient volume since the start of the COVID-19 public health emergency [20, 24, 25].

In order to evaluate if Romania can face the expenditures due to COVID-19 outbreak we tried to compare data from different global healthcare system in their heroic and unprecedented ways to meet the challenges of COVID-19.

Hospitals and health systems face catastrophic financial challenges in light of the COVID-19 pandemic. We undertook four analyses to better understand and quantify these financial challenges.

Including:

- the effect of COVID-19 hospitalizations on hospital costs;
- the effect of cancelled and forgone services, caused by COVID-19, on hospital revenue;
- the additional costs associated with purchasing needed personal protective equipment (PPE); and
- the costs of the additional support some hospitals are providing to their workers.

a) Net Financial Impact of COVID-19 Hospitalizations

At this time there are no official estimates of the net financial impact of COVID-19 hospitalizations but for the over 6,800 patients tested positive until end of April, an expense of over 77 million lei (approximate 15 million euros) is estimated by the Ministry of Health [21, 26].

Table 2: Expenditure on protective equipment against COVID-19

Equipment	LEI	Euro
Protective coveralls	50,5 mil	10,4 mil
Disposable gown	15,2 mil	3,1 mil
Disposable caps	876,000	180,000
Disposable boots	3,1 mil	653,000
Disposable sort	732,000	150,000
Disposable gloves	7,7 mil	1,6 mil
Protective eyewear	2,2 mil	453,000
Protective visors	3,1 mil	640,000
FFP2 Mask	19,4 mil	4,1 mil
FFP3 Mask	2,4 mil	510,000
Surgical Mask	36,9 mil	7,6 mil
Disinfectant	21,8 mil	3,1 mil
Total	**153,9 mil**	**32,5 mil**

Source: Ministry of Health data collection from territory 30 April, 2020[27]

b) Total Revenue Losses from Cancelled Surgeries and Other Services

As a result of cancelled hospital services due to the COVID-19 pandemic, hospitals stand to lose approximately $200 million lei (approximate 40 million euros) in revenue over a period of four months, from March to end of April 2020. This includes cancelled surgeries, various levels of cancelled non-elective surgeries and outpatient treatment, and reduced emergency department services [26].

c) Additional Costs Associated with Purchasing Needed PPE

In order to estimate future expenditures and budget allocation Ministry of Health is collecting regularly data from territory regarding the consumption and demand for equipment and supplies, such as PPE, which considerably increased as a result of the COVID-19 pandemic. Hospitals have incurred additional costs as they struggle to acquire additional supplies to meet the needs of their patients and staff. Moreover, current guidelines require all hospital workers to wear some PPE, regardless of whether they are in direct contact with COVID-19 patients. These guidelines increase the need and expense for PPE relative to normal operations.

d) Costs of Additional Support Some Hospitals are Providing to their Front-line Workers

The govern could not yet estimate the cost of support for front-line hospital workers in COVID-19 hotspots for costs of providing child care, housing, transportation, and medical screening and treatment for COVID-19 for front-line workers but measures were taken to provide such services for them [27].

This report attempts to quantify these effects over the short-term, which are limited to the impacts over a two-month period from March 1, 2020 to April 30, 2020.

Although the government moved quickly to provide relief and the budget expenditures was increase by 2,5 billion euros in order to fight the financial impact, more help is needed [26, 27].

This includes:

- 120 million euros were added in staff costs, and this includes the payment of the risk incentive of 500 euros gross per month for doctors, medical staff, paramedical staff, including auxiliary staff directly involved during the emergency.
- Social security expenditures from National House increased by over 1,4 billion euros (technical unemployment and other health expenditures).
- Expenditures on the reserve fund increase by 3 billion lei.
- The Ministry of Labor also receives 1,6 billion euros and the Ministry of Health 785 million euros.

Following the immediate measures and reallocations of the government, the Ministry of European Funds obtained following the negotiations with the European Commission, a package worth 1.16 billion euros, European funds that will be injected urgently into the Romanian medical system to contribute to the socio-economic measures taken by the Government during this period [15, 16, 26, 27].

The allocation is going to cover expenditures as following [15, 16, 26, 27]:

- 100 million euros reimbursement of the risk incentive of 500 euros gross per month for doctors, medical staff, paramedical staff, including auxiliary staff directly involved during the emergency;

- 350 million euros for reimbursements of the purchases of medical equipment (ventilators, injectors, COVID-19 tests, etc.) and protective equipment (masks, gloves, coveralls, etc.), with the possibility of increasing the allocation to 500 million.

- 682 million euros for the purchase of medical equipment and equipment for the coordination system of the national emergency system, by financing an IGSU project.

The World Bank has allocated more than $1.4 billion to support the efforts of European and Central Asian countries to combat the effects of the COVID-19 pandemic. As of April 2, 2020, it has been approved to allocate approximately $ 490 million to be distributed for new emergency response programs [15,16]. In addition, it was decided to reallocate approximately $ 925 million, which will be redistributed from existing projects and loans, as well as additional funding, to support countries working to combat the effects of the COVID-19 pandemic [15]. Ministry of Health has purchased equipment for medical protection and treatment to fight against COVID worth 29 million euros from projects financed by the World Bank in Romania [27].

CONCLUSIONS

Global shock needs global response, with little or no government interventions, economic costs will be huge. For decrease negative impact, Government spending should be as large as the predicted economic costs, focusing directly on cash disbursement to firms and households. Central banks should support financial the government, not just through their own reserves but also by printing money if necessary. It is clear state that no country has fiscal capacity to stand alone [28].

Government priority should be on health expenditure but as the specialist anticipated is mandatory to generate a strategy to flatten the contagion curve that may rise back in the end of 2020. The tasks of test ‘at random’ representative samples of the population to identify the most contagious groups and isolate immediately the more vulnerable (e.g. the old) should be in front of strategy. A global expand of intensive care capacity (both beds and equipment) by building new units or convert available other type of buildings (e.g. hotel, barracks, etc.) is needed. Another imperative measures is to relocate patient who need intensive care to non COVID hospitals in order to increase capacity of that one placed in concentrated aria of contagion in terms of human resources and infrastructure.

Romania has harmonized with WHO and European Union requirements regarding preventive measures and legislation for COVID-19 disease and show a major and continuous concern in increasing testing capacity although the number of experts in that field was decreasing year by year. Despite the major weaknesses Ministry of Health adopted the best measures in order to combat the negative impact of pandemic. A perfect management strategy was setup in local outbreaks areas, introducing military management in hospitals in which wrong management decision were taken in early stage of COVID-19 disease.

Ministry of Health registered an extremely rapid reaction in support healthcare workers, purchase medical equipment and ventilators challenging the other health systems that faced with the same health problem forcing in a positive manner Public Health System for a simultaneous high inflow of SARS-CoV-2 positive patient in terms of human resources and infrastructure (mobile hospitals for patient with no and mild symptomatology, intensive care units etc.).

As part of European Nation, Romanian Government realized the harmonization of institution using emergency state providing this way an outstanding panel of experts in critical domains which initiate the best decision in a proper time, find the measures and optimal solutions for access to European available funds, cover the huge economic cost, helping Health System to fight against a complete new disease and providing the support for return of citizens in respect of European laws and Human Rights Declaration.

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All mentioned authors equally contributed.

JEL Classification: H51, I15, I18, I28

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CLINICAL PRACTICE

Therapeutic and diagnostic considerations in multiple eruptive clear cell acanthoma

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Abstract: *Clear cell acanthoma represent a benign solitary epidermal tumor of the clear glycogen containing epithelial cells, which is quite common in dermatologic practice. The etiology of this tumor formation is not known exactly at the moment. Clinically, the lesion appears as a single reddish papule or papule-nodule and the characteristic of this lesion is represented by a peripheral scaling collarette. Although solitary lesions represent the most common form of presentation of the acanthoma with clear cells, less than thirtieth cases of multiple clear cell acanthoma, from two up to four hundred lesions, have been described in the medical literature to date. The diagnosis based on clinical features frequently needs to be supported using a dermoscopy exam and, in most cases, a histopathology exam is required. The treatment consists is complete removal (standard surgical excision, cryotherapy, electrofulguration alone, carbon dioxide laser or shave removal or curettage followed by electrofulguration). For the cases with multiple lesions, cryotherapy, or topical 5-fluorouracil are preferred.*

We present the case of a 62-year-old male patient, presented to our Dermatology Department for multiple asymptomatic rounded papules and multiple exophytic nodular tumor formations (approximate fifteen), on the lower extremities, which had occurred approximately two years previously and gradually increased in number.

Keywords: *multiple clear cell acanthoma, glycogen containing epithelial cells, surgical excision, cryotherapy*

INTRODUCTION

Clear cell acanthoma represents a benign solitary epidermal tumor of the clear glycogen containing epithelial cells, which is quite common in dermatologic practice. The clear cell acanthoma was first described in 1962 by Dr. Degos et al. [1].

The etiology of this tumor formation is not known exactly at the moment. Clinically, the most frequent part of the body at which level the tumor can appear is represented by the lower limbs, although other portions of the body at which this lesion can develop are represented by the inguinal region or scrotum, scalp, face, vermilion mucosa, far palm, nipple, buttock, trunk, forearm, head and toe [2, 3]. The period with the highest incidence of this condition is represented by the middle-age, with a peak incidence

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between 50 and 60 years, and does not appear to occur in children. The studies did not show any sex predominance and there is no racial predilection.

Clinically, the lesion appears as a single reddish papule or papule-nodule and the characteristic of this lesion is represented by a peripheral scaling collarette, but this sign is not always present [4]. Some of the nodules are covered with a thin crust rather than a keratinous layer and quite often they are exuding slight moisture [1,3]. Other features that can be representative of clear cell acanthoma include vascular blush and stuck-on appearance [5]. The lesions usually reach a diameter between 1mm and 4 mm until a few centimeters, but a giant tumor has been described by Duperrat et al. [1].

The lesions with this pattern, located at the level of an extremity, the unequivocal differentiation from Bowen's disease can be really difficult if is based only on clinical and dermoscopic features alone and is required a biopsy for the histological exam. The reflectance confocal microscopy has been used for the examination of the non-melanocytic neoplasms. The exam is usually showing microscopic findings similar to the optical histology.

CASE REPORT

We present the case of a 62-year-old male patient, from the urban area, non-smoking, presented to our Dermatology Department for multiple asymptomatic rounded papules and multiple exophytic nodular tumor formations (approximate fifteen), on the lower extremities, which had occurred approximately two years previously and gradually increased in number. On dermatological examination, the exophytic nodular tumor formations were well delimited on palpation with the diameter between 3mm and 15mm, some of them with exudative surface and reddish-brown nipples (Figure 2), while the other of the tumor formations had a dry surface, with a squamous and hyperkeratotic consistency (Figure 1 and Figure 3).

Figure 1: Nodular tumor formations

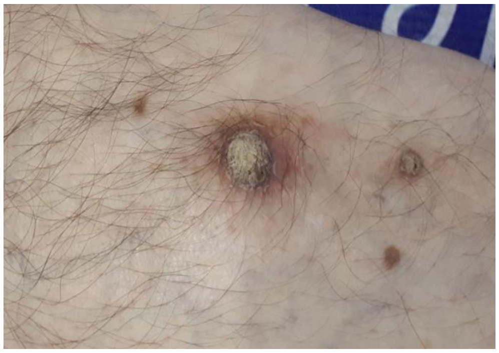

From his medical history, we found out that our patient is suffering from an HTA II degree and chronic venous insufficiency CEAP III; no history of drug ingestion was suspected. No other abnormal physical signs were found. Routine blood examination was in normal limits.

Figure 2: Nodular tumor formations

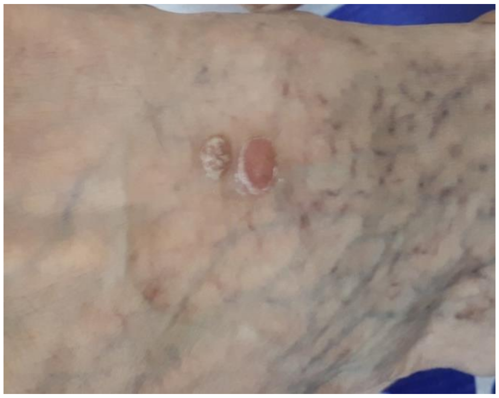

Figure 3: Nodular tumor formations with a dry surface and squamous and hyperkeratotic consistency.

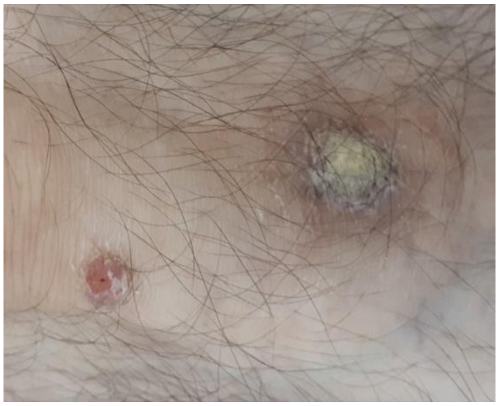

Dermoscopy showed glomerular and punctiform vessels with a “pearl necklace” distribution. This image is highly characteristic of clear cell acanthoma (Figures 4 and 5).

Figure 4: Dermoscopy

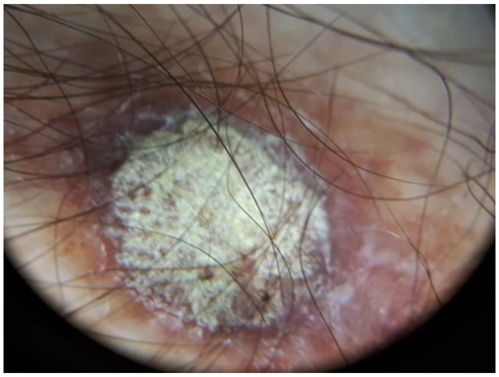

Figure 5: Dermoscopy

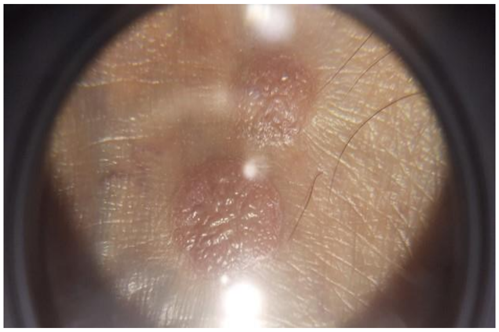

An excisional biopsy of one lesion followed by histopathological examination was performed. The histopathological examination showed psoriasiform elongation of plump and interconnected rete ridges, parchetosis and vascular ectasia in the papillary dermis (Figure 6), acanthosis, agranulosis, parakeratosis, and increased number of vessels in the papillary dermis (Figure 7); a periodic acid-Schiff reaction (PAS stain) demonstrated clear keratinocyte with pale cytoplasm, containing a large amount of glycogen (Figure 8).

Figure 6: Histopathological exam – psoriasiform elongation of plump and interconnected rete ridges, parchetosis, and vascular ectasia in the papillary dermis, HE stain x40.

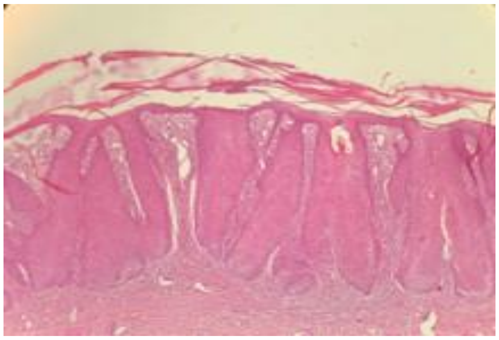

Figure 7: Histopathological exam – acanthosis, agranulosis, parakeratosis, an increased number of vessels in the papillary dermis, HE stainx100.

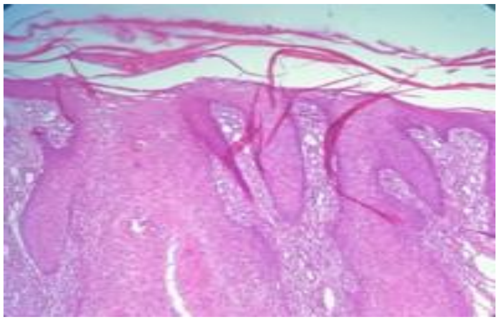

After establishing the diagnosis using the histopathological examination, the surgical excision of the larger tumor formations was decided, under local anesthetics with Xilina 1%, with the suture per-primam with non-absorbable suture threads and local dressing with Baneocin powder, and the others smaller lesions were treated by cryotherapy, using liquid nitrogen and a cotton wool swab, applied for fifteen seconds and repeated after one minute. All lesions were resolved with minimal residual scarring with no relapse at five months of dermatologic follow-up.

Figure 8: Histopathological exam – Clear keratinocyte with pale cytoplasm, containing a large amount of glycogen, demonstrated with a periodic acid-Schiff reaction, PAS stainx200.

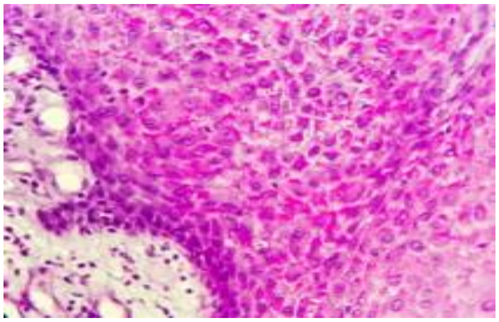

DISCUSSION

Although solitary lesions represent the most common form of presentation of the acanthoma with clear cells, less than thirtieth cases of multiple clear cell acanthoma, from two up to four hundred lesions, have been described in the medical literature to date [6]. The diagnosis based on clinical features frequently needs to be supported using a dermoscopy exam and, in most cases, a histopathology exam is required. Dermoscopy can show dotted or globular vessels, similar to those that can be seen in Bowen disease and psoriasis [7,8]. Also, research has shown an association between clear cell acanthomas and some other conditions including arthropod bites, viral infections, seborrheic keratosis, xerosis, hyttiosis, stasis dermatitis, and varicose veins [9].

The etiology of clear cell acanthoma is not well understood and is no evidence that trauma toxic, drugs, or substances lead to the onset of tumors. In clear cell acanthoma, exist a metabolic enzyme defect, an enzyme that has an important role in the synthesis of keratin [6]. Some authors consider that is an inter-relation with the output of melanocytes and the interaction between melanocyte and keratinocyte. Some of the authors consider that lesions are benign epithelial neoplasm, while others stand up the hypothesis that the origin of this tumor formation is an inflammatory one [7]. Some authors that clear cell acanthoma represent a variant of seborrheic keratosis with the keratinocytes containing abundant amounts of glycogen [10]. In the medical literature, have been reported neither spontaneous regression nor malignant transformation to data.

The most characteristic histopathologic features of clear cell acanthoma are the clear cells with acanthotic epidermis composed of a high composition of glycogen that can be highlighted by positive PAS-Periodic acid-Schiff-staining and the removal of the staining after the diastase digestion. The epidermis is in most cases infiltrated with an abundant number of neutrophils, which may lead to micro-abscesses in the corneum parakeratotic stratum. The Malpighian layer includes slight spongiosis or several pyknotic neutrophils in the intercellular spaces. The papillary dermal layer includes mixed cellular infiltrate and ectasia. A well definite line of demarcation limits the tumor. This line is produced by epidermal cells, which are increased in size, except those in the basal layer. The histochemical examination shows the absence of phosphorylase - a constitutive enzyme, able to demote glycogen [11]. The electron microscopy examinations have revealed glycogen granules in the dermal layer [12]. Video-dermatoscopy has been introduced among the modern methods, for the diagnosis of clear cell acanthoma, highlighting homogeneous pinpoint-like vascular lesions, that have a bush-like aspect if is set a higher magnification [13].

Multiple clear cell acanthoma – from two up to four hundred lesions – have been described in the medical literature until the moment date. The first multiple clear cell acanthoma has been first described by Delacretaz in 1964 [14].

Differential diagnoses include vulgar warts, pyogenic granuloma, Kaposi disease, amelanotic melanoma, dermatofibromas, traumatized hemangioma, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, irritated seborrheic keratosis, and psoriasis [15-21].

The treatment consists is complete removal (standard surgical excision, cryotherapy, electrofulguration alone, carbon dioxide laser or shave removal or curettage followed by electrofulguration) [22]. For the cases with multiple lesions, cryotherapy, or topical 5-fluorouracil are preferred [23, 24]. In our case, even if the lesions were multiple, the surgical approach supplemented with cryotherapy was found to be a good choice. Therefore, we opted for standard surgical excision, cheap and painless (because we used local anesthesia with Xiline) for larger tumor formations and the other smaller lesions were treated by cryotherapy, using liquid nitrogen. After two sessions, lesions were completely healed without relapse and with a good aesthetic result. In conclusion, we can say that cryotherapy is still an optimal method for treating forms of multiple eruptive clear cell acanthoma, but if there are lesions with a larger diameter among the tumors, classical surgical excision may also be combined.

CONCLUSIONS

Clear cell acanthoma is a rare benign epithelial tumor that presents typically as a solitary tumor, but there are cases with multiple lesions. The tumor is red/red-brown, dome-shaped papule, or nodule. A peripheral wafer-like scale collarette represents the classical presentation in majority lesions, but this sign may not always be present. The surface may have a crusted appearance or a moist appearance and may bleed with minor trauma. The tumor slowly enlarges over several years.

Dermoscopy reveals a highly characteristic pattern, a sign that can be utilized to aid in diagnosing a clear cell acanthoma - the the "pearl necklace" appearance (glomerular/pinpoint blood vessels present in a curvilinear and reticular pattern), but a skin biopsy is usually performed to confirm the diagnosis. The preferred treatment of clear cell acanthomas is complete removal (standard surgical excision, cryotherapy, electrofulguration alone, carbon dioxide laser or shave removal or curettage followed by electrofulguration). In our case, even if the lesions were multiple, the surgical approach (for larger tumor formations) supplemented with cryotherapy (for the smaller lesions) was found to be a good choice. In conclusion, we can say that cryotherapy is still an optimal method for treating forms of multiple eruptive clear cell acanthoma, but if there are lesions with a larger diameter among the tumors, classical surgical excision may also be used.

Although solitary lesions represent the most common form of presentation, less than thirtieth cases of multiple clear cell acanthoma have been described in the medical literature to date. Therefore, our case with approximate fifteen lesions could be classified as multiple eruptive clear cell acanthoma.

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VARIA

The effect of implementing collaborative care model on diet compliance in hemodialysis patients

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Abstract: *Non-compliance to diet and increased fluid intake can lead to unpleasant side effects and premature mortality in hemodialysis patients. Intervention strategies in educational and cognitive-behavioral domains can be used to improve diet compliance and restrict fluid intake in hemodialysis patients. The present study aimed to implement a participatory care model on diet compliance in patients undergoing hemodialysis referred to the Urmia Hemodialysis Center of Urmia in 1397.*

The present study is an experimental-randomized study that was performed on 60 patients undergoing diet without hemodialysis referring to Urmia Hemodialysis Center of Urmia. From 175 patients undergoing hemodialysis based on laboratory parameters of non-compliance patients and 60 patients were selected based on simple randomization and were randomly divided into intervention and control groups. After intervention steps of participatory care, diet compliance of the two groups was compared using the results of the comparable laboratory parameters. Analysis of the results of the present study showed that the collaborative care model significantly improved the results of laboratory parameters related to diet compliance in the intervention group compared to the results of the laboratory parameters before the intervention and the control group (p <0.05). Based on the results of the present study, it seems that participatory care intervention can be useful in improving diet compliance and fluid restriction in hemodialysis patients. The aim is to improve the quality of life of hemodialysis patients.

Keywords: *chronic kidney failure, collaborative care model, diet compliance, hemodialysis*

INTRODUCTION

Chronic renal failure is a progressive and irreversible disorder that causes the formation and increase of urea and other toxins in the blood [1]. In other words, chronic renal failure is defined as the progressive and irreversible loss of renal function, often leading to end-stage renal disease (ESRD) [2]. Hemodialysis is the main end-stage treatment for kidney disease (ESRD). Researchers have not considered the growing number of patients with ESRD as a growing problem in Iran and have estimated it to be a worldwide problem. Unfortunately, in Iran, due to a lack of control over diseases such as diabetes and blood pressure, there has not been significant success in preventing chronic kidney disease [3]. This failure to control the underlying diseases has resulted in an increase of 12% per year in the number of patients requiring hemodialysis nationwide (compared to the 6%

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average increase in hemodialysis patients worldwide) and thus the rate of renal disease growth. It is much higher than the world average [4-6]. During the hemodialysis process, extra fluids and wastes are extracted from the blood of patients and this improves the patient's life and prolongs their life, but without proper diet, fluid restriction, and appropriate drug therapy, hemodialysis is not effective [7]. Nutrition has a major impact on the treatment and fate of patients undergoing hemodialysis, and diet compliance is associated with mortality in this disease, such that compliance to a low-potassium diet leads to muscle weakness, arrhythmia, and stasis. Cardiac and death and elevated serum phosphate levels lead to hyperparathyroidism and coronary artery disease, as well as fluid compliance in these patients leading to weight gain between two dialysis sessions and complications such as muscle cramps, shortness of breath, Vertigo, anxiety, pulmonary edema, heart failure, and severe hypertension and threaten the patient's lifestyle [8, 9]. Low levels of blood albumin decrease as 1–91 g of the amino acid are lost in each hemodialysis session, increasing the need for hemodialysis patients to have more protein than normal. Decreased blood albumin levels are strongly correlated with hospitalization and mortality rates and are a determinant of mortality in these patients [10]. Therefore, due to the effect of renal failure on different parts of the body, proper nutrition, prevention or correction of malnutrition and dietary restriction are essential, so patients need a special diet to continue living. It includes restrictions on the consumption of food and liquids [11]. Patient compliance to diet is influenced by factors such as personal beliefs, cultural and social characteristics. Most studies on hemodialysis patients do not follow the proper diet regimen [12]. Although patient-centered dietary supplementation is complementary to hemodialysis and is recommended for the effective control of signs and symptoms of uremic syndrome along with the prevention of long-term complications, many patients do not follow the nutritional and fluid restriction plan. This will lead patients to dialysis inadequacy; therefore, to improve dialysis adequacy, educate, justify, and modify patients' attitudes about diet and the need to monitor the complexity of human behaviors in health decision-making and health decisions. Moreover, behavioral health promotion patterns the benefits of dialysis and timely referral are important [13]. The collaborative care model is one of the localized teaching methods that has been used in recent years in the Iranian nursing field. The model of participatory care is the use of participation in the care process. The purpose of this model is to establish and maintain an effective, balanced, and continuous relationship between the members and the therapeutic team and the patient, to increase collaboration, motivation, and collaboration, and to take responsibility for the treatment team in the treatment and care process [14]. Work-based on a nursing model helps to evaluate patients' status, establish meaningful useful communication between patients and nurses, set goals of care, improve quality of care, guide, and clarify care [15]. Therefore, the concept of "participation" in the care process can be used to improve and maintain an effective care relationship, which then creates an effective care relationship between the three main pillars of the care process: the patient, the nurse, and the physician. Collaborative care is a spectrum from 100% self-care to 100% professional care in which families or health providers, including health personnel, physicians, nurses, social workers, and pharmacists are partnered [16]. Collaborative care encompasses part of the spectrum of care, can be a large or small component of self-care, and can ultimately lead to improved health. It will be necessary to strengthen the caregiver's abilities as a patient with constant stress and anxiety [17]. Also, the use of new participatory teaching methods increases confidence, understanding, and learning, and improves communication skills among members [18]. The purpose of this study was to investigate the effect of the collaborative care model on diet compliance in patients undergoing hemodialysis referred to Urmia Hemodialysis Center of Urmia in 1397.

METHODS

The present study is an experimental study with code IRCT20180730040641N1 in which diet non-compliance is considered as a risk factor for hemodialysis patients. The statistical population in this study was all patients undergoing hemodialysis referred to Urmia Hemodialysis Center of Urmia who was enrolled in the study according to inclusion criteria. Inclusion criteria included no mental illness, cognitive impairment, known hearing, and visual impairment, no use of drugs and psychotropic substances, at least 6 months history of hemodialysis, and ability to stand on weights to control weight between two sessions. Then, of the 175 patients undergoing hemodialysis at the center based on the mean laboratory, sodium, potassium, phosphorus, and albumin parameters in the past 6 months, 60 patients were randomly selected as the study population (patients who did not adhere to their diet). They started studying. After informing the patients about the process of completing the study and completing the informed consent form in these patients, the collaborative care model was administered to the patients in the intervention group for 7 sessions and routine care was performed for the control group patients. The intervention was performed by implementing a collaborative care model. To achieve the objectives of the model, the following steps were presented in the form of a systematic structure that was followed by a logical and evolutionary set of interconnected and dynamic sequences, consisting of four steps: 1- Motivation: 2- Preparation; 3- Involvement; 4- Evaluation that was anticipated for each stage of the specific action plans.

Motivation Stage: To motivate patients by communicating with them appropriately, familiarizing them with the ward and facilities available, raising awareness of patients with chronic renal failure, its consequences, and dangerous consequences were summarized.

Educational need assessment was also done by reviewing and identifying patients' problems by taking biographies and interviews, which was a 2-hour session after filling out the demographic questionnaire during the hemodialysis process.

Preparation Stage: Model objectives and timing of participatory training programs and follow-up were identified (one-hour session).

Involvement phase: Training sessions and follow-up sessions were held at a stage (each session for 30 minutes) that included:

1) The first participatory training session

2) Second educational participatory meeting

3) Participatory follow-up meeting first (one-month interval between each session half an hour)

4) Second follow-up collaborative meeting (one-month interval between each session half an hour)

Evaluation Stage: Achievement of the goals was evaluated in the implementation stages of the model. The evaluation was performed in stages at the beginning and end of each training session as a question and answer session. The training steps were simple and lecture-based.

Implementation of the training program in 4 sessions in the patient's bedside with the nephrologist in the Hemodialysis ward of the Urmia Hemodialysis Center in Urmia for 30 minutes individually and individually for patients.

In the first session, the patient was provided with the necessary information about the nature of the disease, diagnostic methods, and treatment, in each training session after completing the training during the last 20 minutes, in the last 10 minutes, summarizing information, and asking patients questions about problems. The answer was raised. The second session provided information and skills on the importance and how to maintain a proper diet concerning the disease, and the third session on the final evaluation of education, with emphasis on follow-up and participation rates.

Educational information was also provided in the form of educational pamphlets and pamphlets.

Laboratory parameters were measured and reported in two stages, once at the end of the intervention and the second time at one month interval and compared with pre-study values. Also, educational content was collected from accredited hemodialysis and nurse nursing textbooks and approved patient education guidelines, physicians, and nursing professors.

Training sessions were also conducted by the researcher and supervised by the project executives. Data were analyzed using a t-test for independent samples and chi-square and repeated measures in SPSS 23 software.

RESULTS

The statistical population of this study consisted of 60 patients undergoing hemodialysis with laboratory parameters indicating diet compliance who were randomly divided into two equal groups of intervention and control.

The mean age of the patients in the intervention and control groups were (53.60 13 13.27) and (48.63 14 14.88) years, respectively, in terms of the mean duration of dialysis in both groups (intervention and control). It was equal to 31.02 20 20.45 and 33.13 18 18.54 months, respectively.

There was no significant difference between the two groups in terms of age and duration of dialysis. The t-test was homogeneous in terms of mean age (p = 0.17), duration of dialysis (p = 0.84) and patient weight (p = 0.16) (Table 1).

Table 1: Comparison of quantitative demographic characteristics of the studied samples in the two intervention and control groups

Variable	Intervention group		Control group		Test statistics Independent t-test	P-value
	Mean	SD	Mean	SD		
Age of patients	53.60	13.27	48.63	14.88	t=1.36 df=58	0.17
Duration of dialysis	31.02	20.45	33.13	18.54	t=-0.19 df=58	0.84
Weight of patients	68.83	11.59	72.98	10.98	t=-1.43 df=58	0.16

SD – standard deviation

Regarding the qualitative demographic characteristics that could influence the outcome of the intervention, based on the results of the Chi-square test in Table 2, no significant difference was observed between the two groups (intervention and control). In other words, two groups were gender (P = 0.43), occupational status (P = 0.46), educational status (P = 0.60), marital status (P = 0.71) and income (P = 0.51) were homogeneous.

Table 2: Comparison of qualitative demographic characteristics of the samples in the intervention and control groups

Variable		Intervention group		Control group		Test result
		Number	Percent	Number	Percent	
gender	female	17	56.7	14	46.7	X^2=0.60 Df=1 P=0.43
	man	13	43.3	16	53.3	
job	retired	11	36.7	14	46.7	X^2=1.55 Df=2 P=0.46
	housewife	9	30	5	16.7	
	does not have	10	33.3	11	36.7	
education status	illiterate	10	33.3	10	37	X^2=1/84 Df=3 p=0/60
	high school	15	50	12	44.4	
	diploma	1	3.3	3	11.1	
	above the diploma	4	13.3	2	7.4	
marital status	married	26	86.7	25	83.3	X^2=0.13 p=0.71
	single	4	13/3	5	16.7	
income	more than spending	4	13.3	3	10	X^2=1.34 Df=2
	spending equal expense	7	23.3	4	13.3	
	less than spending	19	63.3	23	76.7	

Table 3: Comparison of mean scores of laboratory parameters between the intervention and control groups in hemodialysis patients

Variable	Group	Intervention group	Control group	P-value
		Mean ± SD	Mean ± SD	
potassium	Before intervention	5.65±0.68	5.63±0.43	0.86
	After the intervention	5.11±0.52	5.41±0.42	0.017
	After one month of intervention	4.10±0.26	5.25±0.40	<0.001
	P-value	<0.001	<0.054	
Phosphorus	Before intervention	5.42±0.98	5.45±0.55	0.87
	After the intervention	5.19±0.7	5.34±0.54	0.31
	After one month of intervention	4.22±0.59	5.31±0.27	0.001
	P-value	<0.001	0.15	
Sodium	Before intervention	139.74±3.24	138.63±0.77	0.15
	After the intervention	138.07±3.25	137.90±2.33	0.82
	After one month of intervention	136.97±2.64	138.30±2.50	0.050
	P-value	<0.001	0.34	
Albumin (protein)	Before intervention	4.07±0.54	3.93±0.44	0.28
	After the intervention	4.24±0.46	3.78±0.41	<0.001
	After one month of intervention	4.54±0.42	3.59±0.52	<0.001
	P-value	<0.001	0.058	

SD – standard deviation

The results of the t-test of two independent groups concerning Table 3 show that before the intervention, the hypothesis of equality of mean laboratory parameters between the two groups at a 5% error level was not significant (P>0.05). There was no significant difference between the two groups in terms of mean laboratory parameters and after the intervention; there was a significant difference between the intervention and control groups (P<0.05). The results of the repeated measures test showed that in the intervention group there was a significant difference between before and after the intervention and after one month of intervention (P<0.05). Therefore, it can be said that the intervention was effective but in the control group was significantly higher than 0.05 indicating no significant difference in the control group before and after the intervention and after one month of intervention.

DISCUSSION AND CONCLUSSION

Various studies have shown that changes in the lives of individuals following the onset of chronic kidney disease require serious support and support to improve and tolerate behavior change [19]. Non-compliance to treatment regimens is common in chronic kidney patients and is associated with an increased rate of disease progression and mortality in these patients and is directly related [20]. Compliance with a diet is the result of a range of behaviors that are in line with the recommendations made in compliance with the prescribed dietary regimen, including medication, diet, and so on. Compliance with a diet as part of the treatment regimen can be effective in controlling many diseases [21]. Symptoms and symptoms include fluid and electrolyte imbalances, and accumulation of these substances in the body, hyperkalemia, metabolic acidosis, hyper-phosphatemia, anemia, hyperparathyroidism. Malnutrition, etc. are occurring and intensifying over time [22]. Protein-energy nutrition also occurs in 40% of patients with chronic kidney failure. One of the reasons for all these problems is the lack of diet; one of the needs of the patient at this stage is the need to obtain information about nutrition and nutrition. Nutrition in these patients suggests appropriate approaches to reduce the adverse effects of the disease. Increased mortality and morbidity due to toxins in the cassette may also be caused by the increased awareness of these patients in the diet [23]. Today, trying to adhere to a nutritional diet because of its cheapness compared to other health interventions and fewer side effects is considered an important treatment option in the early treatment of patients, and patient education is a valuable method. Compliance with the diet of the patients is considered. The purpose of this study was to evaluate the effect of the participatory care model on diet compliance in patients undergoing hemodialysis referred to Urmia Hemodialysis Center of Urmia in 1397. In the present study, two intervention and control groups, including hemodialysis patients who did not adhere to the diet, were compared after the intervention. The intervention group underwent an interventional process of collaborative care according to the standard model presented in the study method. The control group continued the normal hemodialysis procedure until the end of the study. At first, both groups were compared in terms of demographic characteristics. The results showed that there was no significant difference between the intervention and control groups in terms of demographic variables such as gender, age, marital status, education, patient occupation, monthly income, which could affect the results of the study. In other words, both groups were homogeneous. After successive steps of intervention, both groups were compared in terms of laboratory parameters related to diet compliance. The results showed that there was a significant relationship between the experimental and control groups in terms of laboratory parameters of phosphorus, potassium, sodium, and albumin. Participatory care reduces the toxicological parameters of patients with chronic kidney disease and reduces the weight difference due to the accumulation of toxic substances in the intervention group. In other words, participatory care has a significant direct relationship with compliance to the diet. Parvinian Nassab et al. 2014 A study "Determining the impact of collaborative care model on the anxiety of adolescents with beta-thalassemia major" by selecting 21 mothers available and randomly sampling in two control and intervention groups at Shahid Hospital Coliseum. Dastgheib Shiraz was designed for 3 months. The results of the study showed that the mean score of worry before intervention in the control and intervention groups was not statistically significant, but there was a significant difference between the mean worry in the intervention group before and after the implementation of the collaborative care model [24].

In 2015, Mamne et al. designed a study to investigate the impact of collaborative care model training on the nutritional behavior of type 2 diabetic patients. In this study, simple random sampling (random numbers) was performed from 411 cases to 42 cases in two experimental (control) and control (21 patients) groups at Tabriz Diabetes Training Center with two samples. The results showed that there was no significant difference in age, sex, educational level, occupation, marital status, and economic status between the two groups. Experimental group after training intervention in the areas of fast food consumption, fruit and vegetable consumption, canned, pastry, solid vegetable oil, sausage and sausage, the usual method of cooking and eating heavy and late dinner better performance than the control group. [25].

A quasi-experimental clinical trial study conducted by Khoshab et al. 2012 aimed to determine the impact of the collaborative care model on depression and anxiety in patients with heart failure in 11 patients with heart failure from CCU, PCCU, and internal heart wards. Afzalipour and Shafa hospitals of Kerman were randomly divided into control and experimental groups. The results of the study showed that the mean scores of anxiety in the intervention group decreased after implementing the collaborative care model [26].

The Lashkari et al. Study in 2015 aimed to investigate the effect of the collaborative care model on the fatigue of 12 conservative hemodialysis patients by purposeful sampling in two experimental and control groups randomly for 3 months in Golestan and Imam Ahwaz hospitals. According to the results of the study after intervention in the intervention group, the level of fatigue at a mild level increased (from 23.9% to 42.2%) and the level of fatigue at a severe level decreased significantly (from 42.3% to 7%). Severe fatigue in the control group increased from 42.2% before the intervention to 34.2% after the intervention. Results also showed that after the intervention, the mean score of total fatigue severity in the intervention group was reduced and it was statistically significant [27]. Our study was similar in terms of the implementation of the collaborative care model. The results of applying the collaborative care model to the crisis created in these studies were also in line with our study.

Based on the results of this study, it can be concluded that the participatory care model has been successful in explaining dietary compliance behavior. Therefore, it is suggested to implement a participatory care model of patients undergoing hemodialysis in planning to change dietary behaviors. Although the findings of this study provide an accurate description of the relationship between the incubation care model and diet compliance, it also has some limitations, including the ability to accurately and sequentially monitor laboratory parameters by a laboratory apparatus and an expert operator who can be useful to achieve better results. It is also recommended to consider the role of other standardized behavioral models in identifying the effective aspects of behavioral or behavioral changes in treatment planning and to consider effective educational methods to increase compliance with dietary restrictions. To improve both the quality of treatment and to reduce the cost of treatment for the consequences of noncompliance.

CONCLUSION

The findings of this study in general indicate the usefulness and effectiveness of the participatory care model in the process of compliance to diet in patients with hemodialysis. Therefore, participatory care intervention seems to play a key role in improving diet compliance and fluid restriction in hemodialysis patients, requiring comprehensive plans focusing on participatory model steps by health care professionals and hemodialysis patients. The effectiveness of the model as well as its simplicity is one of the strengths and weaknesses of this model compared to other models.

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Conflicts of Interest

No conflict of interest is declared by the authors.

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VARIA

Are there only records of success in urology, or are there errors, mistakes, and risks as well?

Viorel Tode

The history of urology dates back to the antiquity, before Christ. The panels decorated in low relief of Saqqara, located at the Institute for the History of Medicine in London, depict the oldest surgical procedure in the world – the circumcision – dating from 2200 BC.

Hippocrates knew about genito-urinary disorders and treated them with medication, leaving bladder stones to be treated by lithotomists.

Cystolithectomy is also known to be dating back to ancient times, having been performed in Egypt, the Middle East, and Ancient Greece.

Urology has always been and still is, an exceptional profession, requiring both intelligence and effort in scheduling and executing the necessary techniques, in assuming risks, and in accomplishing spectacular results.

I will only mention here several of the events that marked the exceptional evolution of urology: defeating pain using anesthesia, progresses of pathological anatomy, the development of the cystoscope by Nitze and the beginnings of endoscopic explorations, the use of Roentgen rays in medicine, Pasteur's contribution to the study of microbes and Lister's in asepsis and antisepsis, the renal transplantation – using which, the urologist gives another lease of life to the patient – the tremendous achievement of Mathias, Jaboulay and Alexis Carrel, percutaneous, laparoscopic and robotic urology as well as other exceptional accomplishments.

The title of this article is addressed as a question: are there only records of success in urology, or are there errors mistakes, and risks to take as well?

However, the word urology can be replaced by any other specialty: surgery, gynecology, ENT, ophthalmology, and why not even internal medicine, neurology, psychiatry, endocrinology, family medicine, and so on.

Hippocrates said: "In medicine, the one who has to be praised is the one who makes the fewer mistakes".

Reflecting upon my professional life, recalling now at senescence, I concluded that I remember less about my many successes in healing patients, but have more vivid memories of some intra or postoperative complications that I have lived intensely.

One can hardly forget, for instance, bleedings that were hard to contain, the slip of a ligature on a vascular pedicle, a vena cava or iliac tear, a large posttraumatic retroperitoneal hematoma, a complex kidney rupture, a pelvic fracture that affects the urethra, urinary bladder or rectum, a postoperative urinary fistula and so on.

In 1971, in medical literature, the second edition of a valuable work by Academy Professor Th. Burghele was published in French – "Erreurs, fautes et risqué en Urologie", from which I will cite only the title of the first chapter – The complexity of urology, factors of error and faults (La complexite de l'urologie, factor d'erreurs et de fautes) and the title of the last chapter – The ontological aspects of faults and errors in urological practice (Les aspects deontologiques des fautes et des erreurs de la practique urologique).

Specialty medical journals are rich in articles presenting, and rightfully so, the successes accomplished by urologists, however, very few publish the occasional failures: errors, mistakes, and risks met or faced during surgery. Professor Th. Burghele once introduced a chapter in the journal "Surgery" – deaths on the surgical table – but after a few case studies, no one published anything related anymore.

It would be difficult for me to believe that in medical practice, one could fully believe one never made a mistake. Medical mistakes in general, and surgical-urological ones in particular, given the human element on which the work is being done, can sometimes generate severe consequences and are thus not admitted.

Momentous errors punishable by law notwithstanding, some mistakes often look insignificant, that occasionally pass undetected by some and that only a urologist in the know would notice. Those are the kinds of mistakes that sometimes would genuinely shake the morality of the urologist tougher than any legal sanction.

Technical progress, modern means of investigation, and new therapeutic options have made urology more benign, with lesser risks and fewer possibilities for errors. The extension of urology to new areas, some not attempted before, exposed other possibilities for complications and postoperative incidents. However, with all the developments of urology and medicine in general, what we still only partially fathom are the reactions of the organism.

Despite technical or analytical errors, it is sometimes possible for the organism, utilizing its reactions to avert or at least limit important consequences that might follow. Other times, through a lack of response, paradoxical or unanticipated reactions, complications and mistakes might be aggravated, thus requiring re-intervention to ensure positive results.

No one in the world, in any profession, can be above mistake: the urologist has the moral duty to do whatever he can to minimize errors.

All active people, and especially those who try new things in the name of growth made mistakes. Progress in all fields, in medicine and particularly in surgery, meant repeated attempts with a mistake, repetition, effort, and sacrifice, and people often paid dearly for surgical advance. But often, from mistake came more knowledge than from success.

To admit errors and to look for their source is the essential condition for a better tomorrow.

Marshal Foch, the highest commander of allied armed forces, victorious in World War I said: “of course I have made many mistakes, but if I hadn’t made them, I wouldn’t have accomplished everything I did in 1918”.

But to misstep as little as possible is no simple or easy task. For the urologist, few mistakes mean having thorough professional training that never ceases to perfect.

For the urologist, making few mistakes means having the chance to stay young at heart, to have the will to constantly improve, and at the same time to keep an unshaken belief in the promise of a better future. It also means understanding and cherishing this ingrate but unimaginably beautiful profession which is urology. And to assimilate it well, the urologist is bound to forfeit his youth, sacrifice his freedom, and surrender all thoughts.

In my personal opinion, a resident in urology should only train in clinics that alongside highly qualified faculty, have the means of instructing residents in all areas of the specialty: open surgery, low and high endoscopic urology, ESWL, abundant imaging possibilities, laparoscopy, robotic surgery and so on.

Once a specialist, the urologist has to constantly oversee his professional progress and keep up with all modern attainments in our specialty.

To obtain the title of the primary physician, there should be a return to a competitive exam form factor, with a limited number of positions, exams not attestations, held before a commission with valued members of the faculty from the country. The primary physician title is a higher degree within the specialty, one that should be aspired to, and only received by the valuable urologists.

Competitive exams should be set to obtain the position of head of the unit, on a period of no more than 4 or 5 years, same as the presidency of the country. The head of the unit should be the best trained professional, highly competent, and capable of solving the most complex cases, known through his profession and case studies held at conferences, symposiums, or published in specialty publications.

There was once even an institute for specializing and perfecting doctors in Romania, housed within Fundeni Hospital. Bygone…

The urologist, like any other person, can slip, and without a doubt will make errors the more he works and the more intricate, tough problems he attempts. But what portrays a proper urologist is not that he is never mistaken, but the fact that he recognizes the mistake and has the conscience and power to fix it.

Celsius, the great anatomist and surgeon of the middle ages said from the beginnings of surgery:” recognizing mistakes is only fit for the intelligent man”.

Diagnosing and identifying complications that necessitate re-intervention is far from being a simple, easy feat.

The numerous paraclinical investigations available have particular importance in appreciating postoperative evolution and occurrences, with imaging being one of the most important means of investigating in diagnosing and indicating re-intervention.

However, all those, without minimizing their importance, cannot replace the clinical assessment, repeated examinations, careful and continuous observation of the patient, and especially the evaluation of the sometimes subtle signs of aggravation.

The character Sherlock Holmes, by Sir Arthur Conan Doyle, said: ”You know my method. It is founded upon the observation of trifles”.

Joseph Okinczy wrote the famous book that will forever remain in the history of surgery ”The small rules of perfect surgery”.

Due to the rapid progress of the last years in the area of biomedical sciences, and in cellular and molecular biology, genetics, and medical imaging, in particular, some clinicians developed the tendency to put less of an emphasis on the anamnesis and clinical examination, relying solely on "adequate" paraclinical examinations, which often prove to be inadequate. This leads frequently to not only unnecessary spending but to diagnosing faults which reflect on the patient's well-being.

The problem of re-intervening reflects upon the character of the urologist and his professional condition in the most profound dimensions of the specialty and subtlest forms of knowledge.

Whenever complications occur, the experience, expertise, and professional value, as well as the temper of the urologist are often put to the test.

Urology was not always as we see it currently. To achieve perfection and the shine we see today, every civilization contributed in spirit, every century laid its rocks, every people brought their input. In its constant evolution, it was only natural that the passive attitude towards incidents and complications had to change.

Currently, such accidents can undergo surgery, a large number of patients can be healed. A detailed study has shown how to best avoid those incidents.

Sure enough, several re-interventions have nothing to do with the primary procedure, nor any urological error. But when in doubt and more importantly when errors exist and the following complications require surgical remedy, re-intervention, no matter how severe, is the only act which allows the urologist to fix, often successfully, that which was wrong.

Within our need and tendency to better ourselves, we must not only see the mistakes of others. It would be despicable to comment in bad faith any errors of our colleagues while justifying our own.

The constant growth of urology offers indisputable potential for great success, but also occasions to fail.

As far as I am concerned, whenever I made a mistake, I prayed to the providence that it be the last one.

But I never forgot that the urologist, as long as he is working, cannot say that he has made his final mistake.

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Artificial Intelligence in cardiovascular medical imaging

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Abstract: *Artificial Intelligence (AI) has become an important tool for computer-aided diagnosis of medical imaging. This review aims to provide an overview for clinicians, explaining the relevant aspects of artificial intelligence and machine learning (ML) and presenting up-to-date applications of AI and ML techniques to medical imaging methods such as angiography, magnetic resonance, and echocardiography.*

For each imaging method, we present the acquisition process, the types of diagnostic test interpretation, and the challenges related to them, as well as how AI/ML techniques, have improved the process of decision making.

A summary of selected works applying AI/ML techniques to medical imaging is organized into a table, which highlights the scope of the study, the dataset used, the details of each approach as well as the measured results, including objectives and criteria. The overall benefits of AI in medical imaging are extracted based on the diverse applications and high evaluation scores. In the end, cardiologists should have an advanced understanding of using AI to integrate clinical data and making the final decision in diagnosis.

Keywords: *machine learning, cardiology, angiography, echography*

INTRODUCTION

The application of AI algorithms to a data set has the usual initial steps of developing the algorithm's ability to "learn" based on the training data and to apply the knowledge gained in making predictions. The "learning phase" of an algorithm consists of an incremental optimization of the model parameters with which it is configured, so that performance on a given training objective (e.g. classification of the most likely diagnosis, regression of numerical value of a health parameter) increases.

Machine Learning is a methodology used to recognize such patterns; the applicability of this can be extended very well in the context of medical imaging. Usually, this technique starts with the use of an ML-based system that determines those characteristics of the images that are considered to be important in making the prediction or diagnosis. As a result, the system identifies the best combination of these characteristics for the image classification and computes statistical data for the different regions identified.

The last decade demonstrates the huge potential of machine learning techniques in many domains along with the development of the graphical processing units (CPU/GPU),

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the availability of big data, and the growth of the learning algorithm efficiency [1].

Figure 1 shows the principal categorization of ML algorithms according to their main functionality. One can distinguish between Supervised Machine Learning (i.e. having an expert provide the so-called ground truth - the correct class or value - for each example in a dataset) and Unsupervised Machine Learning, where the objective is to find interesting patterns and frequent associations between examples in a dataset, without any human supervision (e.g. finding an automatic grouping of diseases by the similarity of symptoms).

Medical imaging applications mostly belong to the supervised ML category, because the correct interpretation (e.g. diagnosis, the classification of severity of a disease) of the result is provided by the medical experts who have analyzed it.

In the past decades, there was substantial growth in the healthcare applications one example is the deep learning algorithm that proved to have an outstanding accuracy in diabetic retinopathy identification [2].

Figure 1: Grouping the different types of machine learning algorithms according to the type of learning [3]

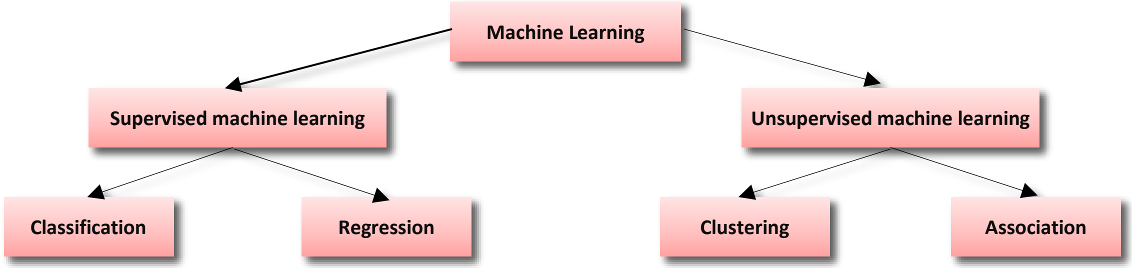

Yang et al [4] demonstrated the importance of AI about big data and parallel programming in medicine, especially in the field of cardiac imaging.

These last decades have also demonstrated the importance of medical imaging in the detection, diagnosis, and treatment of various conditions. In clinical applications, medical imaging is largely performed by a group of experts, such as cardiologists, radiologists, endocrinologists, and so on [5]. In this regard, the computer-assisted analysis enables a better interpretation of the medical image database available. Regarding the decoding of the parameters highlighted by the medical images, it is worth noticing the recent advances in deep learning techniques, which represent a considerable leap in the identification, classification, and quantification of medical conditions and/or health parameters based on input from different medical imaging techniques [6].

ARTIFICIAL INTELLIGENCE IN ECHOCARDIOGRAPHY

In the world of multimodal imaging in cardiology, echocardiography remains crucial for the diagnosis and management of patients with cardiovascular disease [7]. The advantages of the ultrasound of the heart compared to other imaging tools are low price, portability, the possibility of being repeated, short examination time, and the fact that the administration of contrast medium is unnecessary. However, errors in echocardiography quantification and conflict in the interpretation of echo images are frequent in everyday clinical practice.

Although it remains the main imagining modality used in cardiology, echocardiography has several shortcomings that can be improved. The echo machines have developed intensively during the last year (leading to accurate 2D and Doppler imaging and new technologies like tissue Doppler, speckle tracking, or 3D echocardiography). However, one of the main limitations of this method is high operator dependence. The learning curve in echocardiography is very steep and the knowledge, the experience, and sometimes even the mood of the echocardiographer are very important for the exam interpretation. For example, left ventricle ejection fraction is a crucial parameter for patient management but has a high inter- and intraobserver variability. Therefore, the result given by one observer can be different compared to another one or, even more, the same observer can give different interpretations when the reading is repeated. The purpose of AI is to overcome these issues.

Another important role of AI in this field is to solve the problem of a mismatch between the high number of echocardiography required in clinical practice and the number of cardiologists/echocardiographers available to interpret the studies. Moreover, a large volume of already acquired data is under-utilized during a routine echocardiogram and AI offers the possibility to interpret multiple datasets in a very short time [8].

The purpose of artificial intelligence in echocardiography is to detect the disease based on some specific echocardiographic criteria [9, 10]. AI can be used in the image-acquisition process as well and helps people with limited echocardiography training to obtain standard views by anatomic guidance.

Although the implication of AI in echocardiography is still less important compared to other imaging modalities, there are many examples of applications already being used.

Software algorithms are capable of correct identification of the ultrasound modality (2D, pulse wave Doppler or continuous wave Doppler) or the view (four-, two- or three-chamber view) [11]. Moreover, the identification of structures and even the quantification of some related parameters based on border detection are also possible. One of the first applications of AI in echocardiography was the assessment of left ventricular volumes and function initially based on 2D and subsequently on 3D [12]. Based on 3D echocardiography, the correct identification of the heart chamber borders offers results comparable to those measured by cardiac magnetic resonance (left ventricle volumes and function [13], right ventricle volumes, and function [14]. The new software can compute the chamber volumes not only at a maximum or minimum level but also dynamically through the cardiac cycle [15]. Some models for the automated diagnosis of regional wall motion abnormalities and myocardial infarction using deep learning algorithms are still developing [16].

AI is already aiding in valvular heart disease; there is automated software that offers the assessment of mitral [17] or aortic valve.

Some applications can already differentiate, with high sensitivity, between some pathological conditions that need an experienced human examination: left ventricular hypertrophy in athletic population vs. in hypertrophic cardiomyopathy [18], constrictive pericarditis vs. restrictive cardiomyopathy [19].

AI can change the way we read echocardiograms. Normally the interpretation of an echocardiogram is based on consecutive views analysis. For example, in aortic stenosis grading, the observer has to integrate information from multiple non-consecutive views (annulus diameter from the parasternal long axis, continuous wave and pulse wave Doppler data from apical 5 chamber or right parasternal). AI allows the classification of these images and the analysis of the ones related to aortic stenosis grading although they have not been acquired consecutively. Therefore, in the future, the reading of an echocardiogram will be based on the chamber or valve analysis in multiple views and not on the interpretation of consecutively acquired views.

A very important aspect is that using artificial intelligence in echocardiography can modify our understanding regarding the echocardiographic data. Our knowledge of echo interpretation is based on clinical trials that have included a target population. However, this population may not always be representative of the entire population. The large amount of data obtained by including artificial intelligence and machine learning in echocardiography can change our classifications, cut-offs, and interpretation of these data. For example, a recent study based on the NEDA registry (Australian New Zealand Clinical Trials registry) showed that patients with moderate aortic stenosis have similarly high rates of mortality compared to patients with severe aortic stenosis and, likely, we'll need to reevaluate our current data regarding the indications for intervention in aortic stenosis [20].

Therefore, in echocardiography, AI offers the possibility to interpret data from the real world and not from selected patients included in clinical trials and probably a better understanding of the diseases.

Recently, machine learning and deep learning have become the main direction of development in echocardiography, which is proved by the number of publications in PubMed [21]. The possible impact of artificial intelligence on echocardiography is impressive but for now, the human mind is still far too complex compared to AI [22].

Although we all dream of an automated program for echocardiogram interpretation, there is still a long journey ahead. Thus, cardiologists should have advanced knowledge and the capability to use artificial intelligence as extended intelligence.

ARTIFICIAL INTELLIGENCE IN CARDIAC MAGNETIC RESONANCE IMAGING

There is a concern among radiologists and medical students that AI will replace the radiologist. This is not likely to be the natural evolution, but radiologists who use artificial intelligence will replace those who do not or do not think they can use it.

The modern principles of medical imaging are based on the most efficient use of the techniques that bring the maximum amount of information in the shortest time possible, starting from the moment of clinical suspicion. This aspect makes it necessary for the clinician to know the value and the limits of each type of sectional imaging exploration, to optimize the indications of the examinations. Cardiac MRI has a very wide range of clinical applications and has been included in the guidelines for the assessment of congenital heart disease, heart tumors, pericardial disease, cardiac ventricular dysplasia, or myocardial ischemia - hibernating myocardium [23]. Other indications such as the evaluation of myocardial perfusion, ventricular or valvular function can be accurately evaluated by MRI, but compete with other imaging modalities, such as single-photon emission computed tomography (SPECT) and echocardiography, more commonly used in outpatient settings.

The major advantages of MRI are the absence of ionizing radiation and the very strong contrast and temporal resolutions (as opposed to CT, where the spatial resolution is very high [27]. Cardiac MRI provides a wealth of morphological and functional information about the heart. In some practices, the evaluation of the heart MRI requires a long global time, so the tendency is to standardize and reduce the time required for the exploration, but also for the analysis of the images obtained, by using the FAST MRI type techniques. To optimize these protocols for analyzing the data obtained from the scanning of the heart, AI techniques have been developed and used in clinical practice.

They are based on automatic machine learning techniques to enhance the ability to analyze images and data obtained by MRI scanning. The results are remarkable both in the optimization of scanning techniques and protocols, as well as in the analysis and integration of data and results. In an impressive achievement, the introduction of AI and the optimization through the machine learning algorithm can increase the speed of interpretation of the images obtained by cardiac MRI from an average of 13 minutes to about 4 seconds [24].

Cardiac MRI can establish the most appropriate plan for cardiac surgical planning, implantation of cardiac defibrillators, and assessment of cardiac toxicity in patients with oncological pathology. These decisions are based on the accuracy measurements made by MRI.

Multiple studies performed on significantly large numbers of patients have used the information to develop machine learning protocols [25]. These are based on neural network learning. For the time being, it is still necessary to validate the superiority of the efficient application of these AI-type protocols over the aspect of the presently considered examinations. At this moment, the cardiac MRI examinations intend to use a large database which, after a standardized examination and by using a minimum of subsequent maneuvers, the results will be offered in the form of measurements made automatically by the computer capable of filling an MRI result with multiple preformed fields. Also included in this result with preformed fields, the neural network algorithm also offers the diagnostic suspicions based on which differential diagnoses can be issued. The vision of those who work and create this advanced analysis and diagnostic software is to increase the speed of examination without sacrificing the accuracy and precision of interpretation.

The use of artificial intelligence is used to solve the following problems and challenges - the small number of radiologists, the long time required for each examination, the insufficient use of radiological equipment without corresponding efficiency [26].

Traditionally, CT and ultrasound are the most frequent techniques used for cardiac imaging examinations. However, MRI can occupy a very important place due to the very important contrast of tissue structures without ionizing radiation.

One of the major disadvantages of cardiac MRI is that the examination lasts for about an hour. For example, an existing software solution, called Heat Vista (available in the US), reduces the examination for suspected cardiac ischemia from 90 minutes to 15 minutes, and the solution is applied to existing equipment. The software recommends the protocol for about 10 seconds. All these technological advances also eliminate the need to examine through sequences with blocked breathing. This progress is achieved through sequences that recognize and detect artifacts and alert the technician if the sequence needs to be redone [28].

Another challenge is MRI examinations for patients with cardiac and vascular implants of various types of devices. It is necessary to adapt these protocols to the particular aspects of each patient and this aspect can be efficiently optimized by AI protocols depending on the type of implant and the manufacturer that produced them. Considering all the above, cardiac imaging and especially cardiac MRI will benefit from the integration of various AI solutions.

ARTIFICIAL INTELLIGENCE IN CARDIAC ANGIOGRAPHY

X-ray angiography is a medical imaging technique dedicated to the diagnosis and even treatment of vascular diseases. An angiography is obtained using an X-ray source and a detector located on both ends of a C-arm gantry. The imaging apparatus provides temporal images series for several seconds.

Table 1: Artificial Intelligence in cardiovascular medical imaging

Imaging Technique	**Scope**	**Dataset**	**Approach Details**	**Measured Results**
Cardiac MRI	Ventricle Segmentation [29]; Estimating Left Ventricle and Right Ventricle functioning	Cardiac MRI datasets, MICCAI 2012 RV segmentation challenge; 48 patients (16 training, 32 test)	Region of Interest (ROI) extraction using 960 cardiac MRI images with manually labeled RV and LV bounding boxes of size 150 x 150 px. Train CNNs for endocardium and epicardium semantic segmentation of RV on extracted bounding boxes.	Objective: contour overlap of segmented RV with ground truth Criteria: DM, HD, R, ME for EDV, ESV, and EF.
	Heart chamber segmentation [30]; Analyzing abnormality of wall motions	Cardiac MRI datasets, MICCAI 2012 RV segmentation challenge	Heart chamber ROI extraction using a deep learning approach for semantic segmentation. The segmentation approach was initialized with input from a prior edge detection algorithm.	Objective: contour overlap of segmented RV with ground truth Criteria: DM, HD, R, ME for EDV, ESV and EF.
Echocardiography	Regression task: left ventricular volume and ejection fraction computation; Decision making in valvular heart disease, heart failure and post-infarct care [31]	255 usable 2-dimensional echocardiography (2DE) studies	Local Ejection Fraction (EF) estimation. Comparative evaluation of automated EF measurements against manual inspection.	Objective: automated EF estimation Criteria: Intraclass correlation coefficients Bland-Altman analyses
	Regression task: left ventricular volume and Ejection Fraction estimation; [32]	218 patients including 165 with abnormal left ventricular (LV) function. 10,000 apical 4- and 2-chamber view images, from patients with cardiomyopathies, wall motion abnormalities, and dyssynchrony. Cardiac MRI for 21 patients	Identification of the Left Ventricle cavity. Manual tracing of the endocardial border. Left Ventricle volume estimation using modified Simpson's rule for Ejection Fraction computation.	Objective: automated EF estimation Criteria: 2-tailed Student t-test Bland-Altman analysis
Myocardial Perfusion Imaging (MPI) + Coronary angiography	Automatically predicting obstructive disease likelihood from myocardial perfusion imaging (MPI) [33]	1,638 patients without known coronary artery disease, undergoing stress 9mTc-sestamibi or tetrofosmin MPI in 9 different sites Coronary angiography performed within 6 months of MPI	Semantic segmentation of myocardium using a neural network method trained on raw and quantitative polar maps. Input for the network obtained using manual annotation of images obtained from nuclear cardiology software tools.	Objectives: obstructive disease prediction Criteria: area under the receiver-operating characteristic curve (AUC), sensitivity (SS)
SPECT+ computed tomography angiography	Estimating the likelihood of abnormality of coronary arteries in patients with ischemia [34]	1001 patients Coronary angiography within 60 days of myocardial perfusion SPECT for 59 patients with stable coronary artery disease	Semantic segmentation of artery zones with perfusion disorders. Use of segmentation to extract features such as shape, extent, location, count, perfusion homogeneity, regional motion, wall thickening, and sex	Objectives: obstructive disease prediction Criteria: SS, specificity, accuracy, AUC

DM – Dice metric, HD – Hausdorff distance, R – correlation coefficient, ME for EDV – mean errors for end-diastole volumes, ESV – end-systole volumes, EF – ejection fraction, MRI – magnetic resonance imaging

There are three types of angiograms: (i) monoplane angiography, which is the most common method and utilizes the single view principle, (ii) biplane angiography, which uses two C-arm systems for simultaneous acquiring of two different projections, and finally, (iii) rotational angiography, which can give the most accurate three-dimensional scenes by making a complete rotation around the patient.

In [33], the angiography and myocardial perfusion imaging are acquired from the same patient and used as training data on an artificial neural network (ANN) for an automatic prediction of obstructive disease. In [34], the coronary angiography is acquired within 60 days of myocardial perfusion SPECT for patients with stable coronary artery disease from a Japanese hospital. The purpose of ANN was to detect the coronary arteries responsible for the patients' cardiac ischemia. The algorithm accuracy to diagnose the culprit artery was similar to that of a medical expert.

Opportunities for AI applications at the peri-intervention stage are presented in [35] where diverse clinical investigations such as angiography, EKG, biomarkers are reunited for diagnosing STEMI patients. Some applications of machine learning techniques in angiography and other cardiac imaging methods can be found in Table 1.

CONCLUSIONS

The next challenge will be moving from focusing on technique and image analysis to tasks dedicated to physicians in the context of automation of other tasks. This may increase the time efficiency of the medical procedures, job satisfaction, better communication between patient-physician, and improvement of healthcare equality. AI-based solutions will contribute to fighting modern days' challenges, such as the decreasing number of skilled radiologists, leak of medical equipment used, and time-consuming diagnoses.

The final decision after integrating clinical data belongs to cardiologists. The role of artificial intelligence is not to replace humans but to make their work more accurate, fast, and efficient.

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VARIA

The role of career anchors in human resource strategy (Case study: Students of Management Faculty of Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University)

Mir M. Peidaie, Mohammad Belal

Abstract: *Maintenance of efficient, highly specialized, and valuable forces in the present age is one of the most important concerns of organizations, especially organizations with advanced technologies. The importance of human resources planning and management in these organizations has greatly increased, and in the various areas of human resource management, the approach to occupational anchor planning has been very much considered. The purpose of this study was to investigate the role of the job path anchors in human resource strategy among the target community. Participants were selected randomly from among 3500 students at the Faculty of Management of Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University in Tehran Province, Iran. The sample size was 346 by the Cochran method and the questionnaire was collected by electronic means. At the time of the study, 346 questionnaires were completed and the analysis was performed based on 346 questionnaires. To collect data, standard job anchor questionnaires, and Human Resources Strategic HR Questionnaire was used. The study population included 346 people, of whom 203 were male and 143 were female. The age of the people below the age of 20 was 38, from 20 to 25 years old, 94, between the ages of 26 and 30, 60, people aged 31 to 35, and 76 people over the age of 35 years. The reliability of the work anchors was 91% and the human resources strategy questionnaire was 80%. Investigations have shown that, on the anchors of the career path, the factors of creativity - entrepreneurship, autonomy-independence, pure challenges and public management with a committed strategy and public management, creativity-entrepreneurship, security-stability in the strategy of contracting businesses and creativity - entrepreneurship, Pure challenges, security with secondary jobs, and creativity-entrepreneurship, public management, pure challenges in parenting jobs. According to the results of this study, it seems that the emphasis on reducing organizational structures, empowerment, and participatory decision making can determine the necessary grounds for determining the type of human resources strategy in an organization.*

Keywords: *job path anchors, HR strategies, management, human resources, strategy*

INTRODUCTION

In today's world, the power, economic power, and prosperity of any country depend on the optimal use of its facilities, industries, and especially its human resources. Qualified and skilled human resources are the endless value and capital contributions to the growth and development of

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organizations and countries, and the greatest asset of a country is its growth factor. If labor, capital, and land were the main factors of production in the past, today technological change, manpower, and increased productivity is considered to be the drivers of growth [1]. In today's world, organizations are in constant competition. The intensity of competition increases every year and all organizations are constantly in need of improved organizational performance for their survival and growth. Therefore, through an efficient and effective human resources management system, individuals can be considered as strategic assets [2]. The term human resource strategy as a process is a specific set of human resource requirements developed by an organization to achieve its goals. Price, in defining a definition of the human resources strategy, stated that the human resources strategy encompasses all activities related to managing people in the organization in the form of coherent plans to achieve the strategic goals of the organization. Some writers have stated in the concept of strategy as a consequence that "human resource strategy is a model of decisions related to human resource system policies and actions" [3]. Creating a job in a world with diminished job security, changing technology, and increased personal responsibility for the training of certain skills, employment, and lifelong learning are key to meeting the challenges of today's world. Researchers have found that the complexity of the burgeoning context today has a significant impact on people's work attitudes and experiences in their working lives [4]. The concept of a career path, as a link between the organizations and the individuals they work for, is explored from both an internal and an external point of view. Slow and defined as organizational indicators of a career path, while the internal career path is the tendency for employees to move to or from the job anchor. It consists of three main categories: 1. Talents and abilities Self-perception; 2. Core values; 3. Evolved feelings about the motivations and needs that drive the career path [5].

Edgar Shine Model Career Anchors, with 8 Anchors:

1. Technical-Functional Competence: In this anchor, one is primarily motivated by automated content and tends to be employed in technical and specialized positions and jobs.

2. Managing Competency: This personal anchor is motivated by the opportunity to study complex conceptual issues under uncertain circumstances and incomplete information and tend to managerial positions and jobs in large organizations.

3. Security - Stability: In this personal anchor, it is promoted to a reputable organization through job security and long-term attachment.

4. Creativity - Entrepreneurship: In this anchor, one is motivated by the need to build or create projects that belong to him/her.

5. Autonomy - Independence: In this anchor, a person is essentially seeking job opportunities that enjoy maximum freedom in terms of organizational boundaries and are thus stimulated.

6. Serving and Sacrifice and Sacrifice: In this anchor, one is motivated to serve and pursue a sacred cause and to meet the needs of others.

7. Pure Challenges: In this anchor, the individual is challenged to overcome major obstacles, solve difficult problems, or win over competitors.

8. Lifestyle: People with this anchor tend to balance their career paths with their families [6].

Human Resources Strategy

In the early years of 1980, strategic human resource management was the focus of a large number of influential management consultants and researchers in practicing management practices. These authors found that effective human resource management can guarantee quality and be effective in terms of competitive advantage. Not surprisingly, in the mid-1980s, many HR researchers favored the view that more HR units should play a greater role. The first models of human resource strategy formulation were introduced in the middle of this decade. These models, with a strong emphasis on human resource strategy adherence to organizational strategy, presented a rational and logical approach to unifying strategic issues for managing the overall human resources of organizations [7]. But the first models that could offer different strategies for different organizational conditions were single-dimensional models based on the labor market type or control type factor in 1987. These models did not necessarily coincide with the strategy of the organization, thus preventing the possible errors in the process of formulating the organization's strategy into a strategy of human resources. Combined, by introducing the "one-piece model", they proposed the use of strategic reference points (SRPs) to formulate HR strategies (8). The importance of this model is that, in addition to the logic of attraction, for the reason that it is compatible with the fundamentals of management philosophy, it can be formulated and implemented without the need for organization strategy, while maintaining its alignment with the organization's strategy. On the other hand, the HR strategy formulation using this model does not rely on the examination and modeling of the status quo. Using two main dimensions of control (efficiency or process control) and labor market (concerning internal or external market), they have formulated four HR strategies [9].

Secondary strategy

This strategy is suitable for simple, duplicate, and standardized jobs that have a sufficient supply of labor outside the company and there is no need to nurture and retain them. In other words, they can be easily disconnected if they are not needed or unsatisfied with each other and can hire serious employees with the least training and cost if needed.

Paternal strategy

This strategy is also applicable to simple, repetitive, and standardized jobs, with the exception that corporate management tends to retain and enhance existing employees and align them with corporate culture. Therefore, vacancies can be filled by upgrading existing staff.

Contracting Strategy

This strategy is suitable for those complex and specialized jobs that require constant and formal hiring of relevant experts for the company, as the company needs these jobs in short and temporary terms. Therefore, these experts are usually highly compensated, solely for consulting or part of an invitation plan, and then return to the job market after the project is completed to provide similar services. Of course, some organizations (especially virtual organizations) also eliminate the constant need for their expert staff by using these experts' temporary or unnecessary services because the management of these organizations has been able to take full control of the organization's specific knowledge. Also, apply evaluation and promotion tools [10].

Committed strategy

This strategy is also applied to complex and specialized jobs, except that the experts in these jobs are constantly in need of the company and because they in many cases master the complex production network and specific knowledge required by the company. They are not easy to replace. Therefore, it is necessary to have an effective approach to the labor market within the organization, that is, to develop and train company experts and to strengthen their commitment and loyalty to the organization in such a way that these experts are exposed to higher market rights and benefits in the face of a secure and dynamic career future. Ignore work outside the organization. In the strategic reference point’s theory, a three-dimensional matrix is used to determine the reference points that includes the internal conditions of the organization, the external conditions of the organization, and time [11].

METHOD

The purpose of this study is cross-sectional applied in terms of purpose and type of research method and the research population is comprised of management students of Islamic Azad University, Yadegar Imam Branch - Shahre Ray. To ensure that the sample selected represents the real community, the sample was selected using correct sampling methods. Random sampling was also used to select the sample members. Morgan table was used to determine sample size in the present study. Since the target population in this study was 4 people, the sample size in this study was 9 people. Data were collected using the standard Anchor Job Inventory and HR strategy questionnaire as measured in previous studies. Surveying is one of the most common methods in quantitative social science research. Since quantitative research is not possible to study the behavior of all individuals in the community, the study is conducted through sampling. In this regard, a sample is selected and the result is generalized to the whole community [12]. After completing the questionnaire and collecting and removing the defective questionnaires, the data were analyzed with SPSS 23 software.

DISCUSSION

This section presents all the analyzes based on the assumptions of the present study. The analysis of the results will greatly help the scientific and purposeful discussion of the research topic.

The demographic variables of this study are as follows: The total number of participants was 346 persons, 203 men, and 143 women. The age group of people under the age of 20 is 38, 20 to 25, 94, between 26 and 30 are 60, people between 31 and 35 are 76, and people over 35 are 76. In terms of marital status, 192 were married and 154 were single; 118 were married, 36 were married, and 85 were single and 107 were single. The level of education in the associates is 50, 142 bachelors, and 154 masters. In terms of job status, 84 students were self-employed, 106 were employed in private companies, 109 were employed in public offices and 47 were employed in semi-public offices. Of these, 53 were freelance male, 78 were male, 78 were male, and 25 were semi-public, with a total population of 203.

Hypothesis 1: What are the career path anchors in committed business executives and what are their priorities?

To respond to this hypothesis, the relationship between the two factors of job anchors and commitment factors of the

HR strategy questionnaire was measured. Regression analysis can be used to determine the correlation between occupational anchor factors and the occupational commitment factor. Regression is a method for estimating and modeling the relationship of several independent variables to one dependent variable.

According to Table 1 and statistical significance at the level of 0.8, the regression model is significant. On the other hand, model estimation data show that independent variables account for approximately 5% of the total variance of the response variable. In other words, the 5 factor of job anchors is the ability to predict the factor of the committed jobs. Based on this, it is possible to determine appropriate modeling to determine the simultaneous relationship between occupational anchor factors and the occupational factor.

Table 1: Regression model and model evaluation indices

<table>
<tr><th colspan="2">Estimation error</th><th colspan="2">The adjusted coefficient of determination</th><th colspan="2">The coefficient of determination</th><th rowspan="2">Model evaluation</th></tr>
<tr><td colspan="2">5.103</td><td colspan="2">0.268</td><td colspan="2">0.285</td></tr>
<tr><td>P</td><td>F statistics</td><td>The average sum of square</td><td>Degrees of freedom</td><td>The sum of the regression squares</td><td></td><td></td></tr>
<tr><td rowspan="3">0.000</td><td rowspan="3">16.822</td><td>438.076</td><td>8</td><td>3504.610</td><td>regression</td><td rowspan="3">Significance of the model</td></tr>
<tr><td>26.041</td><td>337</td><td>8775.834</td><td>Error</td></tr>
<tr><td></td><td>345</td><td>12280.474</td><td>Total</td></tr>
</table>

According to the results of Table 2, variables A1, A4, A6, and A8 are not significant. In other words, in assessing the simultaneous relevance of job anchor factors, only the five key factors of public management, autonomy-autonomy, creativity-entrepreneurship, and sheer challenges are effective on the commitment factor. In other words, measuring and scoring these 5 factors can determine the type of person's job.

Table 2: Model coefficients and their significance

Model coefficients	Coefficients	Regression coefficient	Governor error	Standardized coefficient	T statistics	P
	Constant coefficient	9.224	1.921	-	4.801	0.000
	Technical-Applied Orientation (A1)	-0.087	0.112	-0.052	-0.773	0.440
	Public Management Tendency (A2)	0.452	0.092	0.299	4.892	0.000
	Autonomy - Independence (A3)	0.358	0.125	0.215	2.863	0.004
	Security-stability orientation (A4)	-0.001	0.083	-0.001	-0.013	0.989
	Creativity-Entrepreneurship (A5)	-0.258	0.085	-0.175	-3.012	0.003
	Serving and Sacrifice and Sacrifice (A6)	0.041	0.125	0.024	0.328	0.743
	Pure Challenges (A7)	0.451	0.097	0.300	4.636	0.000
	Lifestyle (A8)	-0.198	0.122	-0.117	-1.617	0.107

So the mathematical model would be as follows:

Committed Jobs = *A7 0.451+ *A5 0.258 - *A3 0.358 + *A2 0.452 + 9.224

This mathematical model implies that for each unit score increase in the A2, A3, A5, and A7 factors the commitment factor score will increase by 10.227 points.

Hypothesis 2: What are the career anchors of contractors and what are their priorities?

To answer this hypothesis, as in the first hypothesis, one can determine the relationship between job anchor factors and contractor job factors using a statistical regression method.

According to Table 3 and statistical significance at the level of 0.9, the regression model is significant. On the other hand, model estimation data show that independent variables account for approximately 5% of the total variance of the response variable. In other words, the 5 factor of job anchors is the ability to predict the factor of contracting jobs. Based on this, it is possible to determine appropriate modeling to determine the concurrent relationship between occupational anchor factors and contractor occupation factors.

Table 3: Regression model and model evaluation indices

Estimation error		**The adjusted coefficient of determination**		**The coefficient of determination**		**Model evaluation**
3.732		0.127		0.147		
P	F statistics	The average sum of square	Degrees of freedom	The sum of the regression squares		
0.000	7.271	101.275	8	810.203	regression	Significance of the model
		13.928	337	4393.799	Error	
			345	5504.003	Total	

According to the results of Table 4, variables A1, A3, A6, A7, and A8 are not significant. In other words, in evaluating the simultaneous relevance of job anchor factors, only the five key factors of public management, security-stability, and creativity-entrepreneurship are effective contractors. In other words, measuring and scoring these 5 factors can determine the type of person's job.

Table 4: Model coefficients and their significance

Model coefficients	Coefficients	Regression coefficient	Governor error	Standardized coefficient	T statistics	P
	Constant coefficient	5.508	1.405	-	3.920	0.000
	Technical-Applied Orientation (A1)	0.145	0.082	0.130	1.767	0.078
	Public Management Tendency (A2)	0.161	0.068	0.159	2.377	0.018
	Autonomy - Independence (A3)	-0.170	0.092	-0.152	-1.855	0.064
	Security-stability orientation (A4)	0.183	0.061	0.183	3.010	0.003
	Creativity-Entrepreneurship (A5)	0.175	0.063	0.178	2.804	0.005
	Serving and Sacrifice and Sacrifice (A6)	0.024	0.091	0.021	0.259	0.796
	Pure Challenges (A7)	-0.028	0.071	-0.028	-0.391	0.696
	Lifestyle (A8)	0.019	0.090	0.017	0.212	0.832

So the mathematical model would be as follows:

Contracting Jobs = *A5 0.175 +*A4 0.183+*A2 0.161 +5.508

The concept of this mathematical model is that for each unit score increase in the A2, A4, and A5 factors the contractor job factor score will increase by 6.0۲۷.

Hypothesis 3: What are the job anchors in secondary jobholders and what are their priorities?

To answer this hypothesis, as with the previous hypotheses, one can determine the relationship between job anchor factors and secondary job factors by the statistical regression method.

According to Table 5 and statistical significance at the level of 0.05, the regression model is significant. On the other hand, model estimation data show that independent variables account for approximately 23% of the total variance of the response variable. In other words, the 5 job anchor factors can predict the secondary job factor. Accordingly, it is possible to determine the appropriate modeling to determine the correlation between the job anchor factors and the secondary job factor.

Table 5: Regression model and model evaluation indices

Estimation error		The adjusted coefficient of determination		The coefficient of determination		Model evaluation
4.576		0.226		0.244		
P	F statistics	The average sum of square	Degrees of freedom	The sum of the regression squares		
0.000	13.565	284.084	8	2272.671	regression	Significance of the model
		20.943	337	7057.760	Error	
			345	9330.431	Total	

According to the results of Table 5 the variables A1, A2, A3, A6, and A8 are not significant. In other words, in assessing the concurrent relevance of job anchor factors, only the three key factors of security-stability, creativity-entrepreneurship, and sheer challenges to effective secondary jobs are effective. In other words, measuring and scoring these 3 factors can determine the type of person's job.

So the mathematical model would be as follows:

Secondary occupations = *A7 0.336- *A5 0.237 + *A4 0.384 + 6.340

The concept of this mathematical model is that for each unit score increase in A4, A5, and A7 the secondary job factor score will increase by 6.625 points.

Hypothesis 4: What are career path anchors in parenting and what are their priorities?

To answer this hypothesis, as with the previous hypotheses, one can determine the relationship between job anchor factors and secondary job factors by the statistical regression method.

According to Table 6 and statistical significance at the level of 0.05, the regression model is significant. On the other hand, model estimation data show that independent variables account for approximately 23% of the total variance of the response variable.

In other words, the 8 job anchor factors can predict the parent job factor. Based on this, appropriate modeling can be determined to determine the correlation between the job anchor factors and the parent job factor.

According to the results of the table below, variables A1, A3, A4, A6, and A8 are not significant. In other words, in evaluating the simultaneous relevance of job anchor factors, only the five key factors of public management, creativity-entrepreneurship, and sheer challenges are effective on the factor of hospitable jobs.

In other words, measuring and scoring these 3 factors can determine the type of person's job.

Table 6: Regression model and model evaluation indices

Estimation error		The adjusted coefficient of determination		The coefficient of determination		Model evaluation
5.734		0.227		0.245		
P	F statistics	The average sum of square	Degrees of freedom	The sum of the regression squares		
0.000	13.680	449.745	8	3597.964	regression	Significance of the model
		32.875	337	11071.929	Error	
			345	14676.893	Total	

So the mathematical model would be as follows:

Paternal Occupation = *A7 0.807+ *A5 0.261-A2 0.281+ 7.154

The concept of this mathematical model is that for each unit score increase in the A2, A5, and A7 factors, the secondary job factor score will increase by 7.981 points.

Table 7: Model coefficients and their significance

Model coefficients	Coefficients	Regression coefficient	Governor error	Standardized coefficient	T statistics	P
	Constant coefficient	7.154	2.159	-	3.314	0.001
	Technical-Applied Orientation (A1)	-0.169	0.126	-0.093	-1.342	0.181
	Public Management Tendency (A2)	0.281	0.104	0.17	2.704	0.007
	Autonomy - Independence (A3)	0.155	0.141	0.085	1.104	0.27
	Security-stability orientation (A4)	-0.024	0.093	-0.014	-0.254	0.800
	Creativity-Entrepreneurship (A5)	-0.261	0.096	-0.163	-2.718	0.007
	Serving and Sacrifice and Sacrifice (A6)	-0.135	0.14	-0.073	-0.965	0.335
	Pure Challenges (A7)	0.807	0.109	0.492	7.395	0.001
	Lifestyle (A8)	0.02	0.138	0.011	0.142	0.887

Explain the first hypothesis: Anchors of managerial competence, sheer challenges, and autonomy – independence and creativity – entrepreneurship are effective in committed jobs. Given that these jobs are complex and specialized and require specific knowledge, replacing them in these jobs is simply not possible and the specific characteristics of this group, which is autonomy and independence, the manager of the organization should have sufficient authority to act. It created these people. At the same time, people need to have adequate training in problem-solving to make the organization more effective. Management can use these people in managerial positions to advance organizational goals.

Explain the second hypothesis: In this hypothesis, job anchors include security – stability, creativity – entrepreneurship, and managerial competence. These contractors are highly specialized in the organization and have a high cost to the corporations, so hired individuals must possess the qualities of managerial competence, creativity, and entrepreneurship. In the meantime, they tend to do things that are more stable and secure. Besides, given the creativity of the staff, the organization can create the potential for individuals to emerge [13].

Hypothesis 3: Job Anchors are the most effective - security, stability, sheer challenges, and creativity - entrepreneurship. Given that, people in the workforce are simple, repeatable, and standardized, and there are enough people in the job market, so people are looking for job stability that they need to be creative and entrepreneurial to solve the challenge. Establish their sustainability organization [14].

Hypothesis 4: Job Anchors include pure challenges, managerial competence, and creativity-entrepreneurship. These jobs are very simple, repetitive, and standard. In these jobs, people have longer-term contracts, and the organization tends to stay in the organization and benefit from it when needed. In the strategy of committed and contracted jobs, due to the specialization of these jobs and jobs in this strategy, anchors of managerial competence and creativity - entrepreneurship are common and the subject of sheer challenges in commitment and security - stability in the contractor is different. The results show that managerial competence and creativity-entrepreneurship are an important issue in these two strategies. The results show that there are three factors of managerial competence, creativity-entrepreneurship, and pure challenges between committed and paternal strategies. The distinction between these strategies in autonomy is that they are required for extraordinarily specialized work (committed strategy) [15].

The results of the present study show that there is a significant and positive relationship between the components of public management competency, creativity-entrepreneurship, and pure challenges with HR strategies.

In the study of Amirtash et al. (2011), the comparison of job anchors and organizational commitment between physical education and non-physical education faculty members of Islamic Azad University of Iran was used by Edgar Shine Job Anchor Questionnaire. We have used [16].

Khayef Elahi et al. (2015).on the impact of anchor dimensions concerning job factors on organizational outcomes, in line with the results of the research that considers job path anchors as effective [17] is in line with the results of this study [17].

Gholamzadeh et al. (2012) used a bumper questionnaire in a study on the impact of HR strategy based on the theory of strategic bumper reference points and on the performance of managers and staff, which we also used [18].

CONCLUSION

The present study is the anchor of autonomy and independence as a common component in all four strategies, which emphasizes the importance of reducing organizational structures and participatory decision-making. It also emphasizes that it empowers employees and provides the necessary framework for staff decision-making. There is also a plan to help employees, such as solving personal problems of employees that impede their effective performance path. Where employees have the knowledge and skills and therefore the issue of managerial competencies is important. In management and specialist careers, the choice must be very careful. Managers are advised to pay attention to the provision and promotion of workforce development and support, as well as training and development of staff, and staff promotion based on their career path and merit. In the four strategies available, anchors of technical and functional competence, self-sacrifice, and sacrifice, as well as lifestyle, are missing. These anchors focus on the topics and personal lives of individuals and draw the attention of executives to motivate their employees to create a path that leads the individual forward with idealism and self-control. This raises the challenge that managers by integrating individual and organizational goals can play a special role in promoting the individual and the organization and enhancing the organization and the individual.

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VARIA

Overview of telemedicine use aspects in natural and technical disaster and combat situation

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Abstract: *Background: Preparation and hospital development estimated an essential part of the policies and strategic purposes of healthcare in each government. Today, applications of management to reduce the burden of natural and technical disasters appear are very necessary.*

Objectives: The study aimed to review the major relevant articles in the case of the application of telemedicine for trauma injuries in disaster and military situations.

Methods: Original clinical trials investigating the effects of telemedicine on trauma injuries published before April 2018 were aggregated, and used in this study. We search the scientific sources such as PubMed, MEDLINE, Wiley, EMBASE, ISI, and Scopus. The keywords that applied to identify these articles combined: Telemedicine, Mobile Health, Health, Mobile, mHealth, Telehealth, eHealth, Trauma, War, military, medicine, Wounds and Injuries, injuries.

Results: The text of the articles and abstracts were examined from various aspects, the 21 articles were evaluated for quality, and then 10 papers entered in a systematic review. We found that the use of telemedicine in trauma injury, rural and the military is very helpful for high-quality trauma care; also the telemedicine and telephone consultations can be were demonstrated reducing the risk of physician-related medicine mistakes. The economic evaluation of telemedicine was demonstrated cost-saving and cost-effective of telemedicine consultations. We also realized the importance and practicable of telemedicine for improving rural trauma care.

Conclusions: Ultimately, the telemedicine can help patients and health government organizations economically, the telemedicine consultations due to cost-saving and cost-effective. Further studies to evaluate the running of telemedicine need to abating the practical and high-efficiency method, that can be done everywhere and it's free from mistakes and defects.

Keywords: *telemedicine, trauma, disaster*

BACKGROUND

Preparation and hospital development estimated an essential part of the policies and strategic purposes of healthcare in each government. Today, applications of management to reduce the burden of natural and technical

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disasters appear are very necessary. [1, 2]. Telemedical technology is increasingly moving into the medical system. Teleconsultation between crisis positions with a lack of specialists' physician and a trauma center in surgical and trauma patients could save interventions [3-5]. In recent years, Telemedicine is growing more prevalent in intensive care medicine, but its impacts and the excellent organizational pattern is also ambiguous [6-8]. Particularly in situations that there is a shortage of extremely educated healthcare workers on-scene such as rural EMS, disaster, and military conditions, conditions teleconsultation could improvements in patient care via consultation of a specialist [3-7].

Telemedicine is a valuable method of implementing remote diagnoses such as ophthalmology, dermatology, and radiology [8]. Telemedicine represents the use of medical knowledge transferred from one position to another by electronic connections to enhance patients' health situation and care [2-5]. Crisis management in natural and technical disasters should decrease the harmful impacts of trauma events, mortality, and morbidity applying a designed program of development and mobilization. Notwithstanding, there are a lot of experiences in crisis management, it has limited application of telemedicine in disaster and military situations [2-6].

Application of the telemedicine in disaster and military situations management is the principal subject to maintain the health of accident patients [7]. Regarding the destruction created in the health services infrastructure, infrastructure has become a significant issue, the existence, and utilization of telemedicine technology and according to continuous development in electronic technology, communication to deliver health services in remote regions [6-8].

We try to review the major relevant articles in case of application of telemedicine for trauma injuries in disaster and military situations to help clinicians and patients to have a better understanding of these technologies and choosing the better therapeutic option. A highly standardized evidence-based review can be one of the best references for clinicians to decide about their treatment plan.

MATERIALS AND METHODS

Original clinical trials investigating the effects of telemedicine on trauma injuries published before April 2018 were aggregated, and used in this study. We search the scientific sources such as PubMed, MEDLINE, Wiley, EMBASE, ISI Web of Knowledge, and Scopus.

The keywords that applied to identify these articles combined: Telemedicine, Mobile Health, Health, Mobile, mHealth, Telehealth, eHealth, Trauma, War, military, medicine, Wounds and Injuries, injuries. Two authors have independently assessed the titles and abstracts of all papers obtained by the searching method outlined above.

The studies with the following criteria were excluded: did not evaluate the performance of Telemedicine; were not English-language study, and were review studies and letters to editors or conference abstracts. The two reviewers who carried out the literature search also individually made decisions and selected studies considering inclusion criteria. The following details were extracted from included studies independently by two reviewers, study location, year of publication, the first author's surname, study design, and outcomes.

Figure 1: Flow chart the inclusion studies

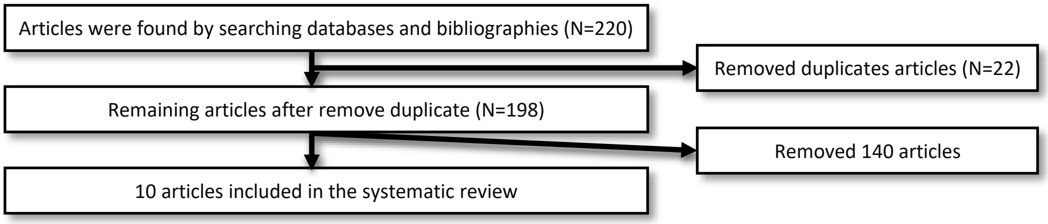
Articles were found by searching databases and bibliographies (N=220)
Removed duplicates articles (N=22)
Remaining articles after remove duplicate (N=198)
Removed 140 articles
10 articles included in the systematic review

RESULTS

Ten studies matched the criteria for analysis and discussion.

Daniel et al. (2013) in a randomized trial study examined emergency care between two groups. Doctor-staffed EMS teams compare to paramedic teams that were established telemedical by an EMS doctor. Overall 16 teams that each team contained one EMS physician and two paramedics randomly divided into the control and telemedicine group. Telemedical functionalities included 2 methods of audio communication, transportation of vital information, and video streaming from the situation place to the remotely placed EMS doctor. There were no significant differences in the case-specific items and, no potentially hazardous mistreatments were reported. They showed telemedical supported paramedic care as possible and at least not poor respect to conventional EMS teams with a doctor on-scene in these situations [9].

Table 1: Author's name, type of study and summaries of outcomes in the studies

Author's name	Type of study	Outcomes
Daniel et al. (2013) [9]	Randomized trial study	They showed telemedical supported paramedic care as possible and at least not poor respect to conventional EMS teams with a doctor on-scene in these situations
Brennan et al. (1998) [10]	Randomized trial study	They concluded that telemedicine was observed to be a satisfying system for pre-chosen emergency department patients and was observed by the doctors as an admissible way of complementary application.
Bowman et al. 2003 [11]	Cross-Sectional	Their results showed Telemedicine was a precise, reliable, and effective way of diagnosing and controlling these patients, particularly if slit-lamp images were applied.
Yang et al. 2015 [12]	Cross-Sectional	They concluded that a healthcare payer aspect, telemedicine to health care workers of severely sick and pediatric patients performing to rural EDs are cost-saving or cost-effective regarding the telephone.
Mohr et al. 2016 [13]	Observational cohort study	The telemedicine consultation was requested for severely injured rural traumatic patients (burns, penetrating trauma, and those with abnormal vital signs) in the Emergency Department, but Telemedicine consultation was not decreased interhospital transfer.
Oeveren et al. 2017 [14]	Cross-Sectional	They demonstrated a success rate of 96% of telemedicine during emergency intubation. Telemedicine-assisted intubation can be performed in rural hospitals practically.
Gilmore et al. 2014 [15]	Cross-Sectional	HBT can provide mental health services in a preferable procedure of location in military sexual trauma with PTSD patients and help to them for reducing mental pains and increasing readiness.
Dayal et al. 2016 [16]	Retrospective cohort study	The performance of a telemedicine program is designed to assist in the seriously ill children at admission to the PICU. They provided evidence for improving clinical outcomes in pediatric critical care with telemedicine programs.
Shah et al. 2016 [17]	Prospective cohort study	High-intensity telemedicine can be alternative to traditional severe disease care and it must be included in population health programs.
Dharmar et al. 2018 [18]	Retrospective chart reviews	They were demonstrated reducing the risk of physician-related medicine mistakes among critically sick and traumatic children of 8 rural EDs from academic children's hospital.

Brennan et al. (1998) conducted a randomized clinical study in the emergency department. Overall 104 patients were included. Fifty-four participated in the telemedicine group and 50 to the control group. There are no differences between the groups for an incident of 72 hours emergency department return visits; the requirement for further care; doctor communication; nurse communication; satisfaction. The mean patient throughput period for the telemedicine group was 106 minutes; the mean for the control group was 117 minutes. They concluded that telemedicine was observed to be a satisfying system for pre-chosen emergency department patients and was observed by the doctors as an admissible way of complementary application [10].

Bowman et al. (2003) assessed the precision and effectiveness of telemedicine in diagnosing and treating eye injuries. The methods were a clinical trial with a face-to-face group (40 patients) and a telemedicine group (40 patients). In the face-to-face group, consultations were in person; in the telemedicine group, researcher one handled video conferencing technology at 384 kbit/s, and observer two observed the patient face to face. In this study, 80 patients were included in the department. Principal result measures were agreement levels between the two observers for every phase, time of consultation, and several unnecessary recalls. There were more important conflicts applying telemedicine, in per sample the telemedicine diagnosis and management failed on the side of safety; consequently, no patient would have experienced via the improper administration because of the consultation using telemedicine. Their results showed Telemedicine was a precise, reliable, and effective way of diagnosing and controlling these patients, particularly if slit-lamp images were applied [11].

Yang et al. (2015) carried a financial assessment to determine the price, effect, and return on investment of telemedicine consultations presented to health care workers of severely sick and pediatric patients in rural EDs confronted with phone consultations from a healthcare payer prospect. The cost-effectiveness investigation was according to program effectiveness, obtained from change choices after telemedicine and telephone consultations. They concluded that a healthcare payer aspect, telemedicine to health care workers of severely sick and pediatric patients performing to rural EDs are cost-saving or cost-effective regarding telephone [12].

Nicholas M. Mohr et al. evaluated factors associated with telemedicine and interhospital transfer as a cohort study of all adult treated traumatic patients between 2008 and 2014 in North Dakota critical-access hospital (CAH). They demonstrated telemedicine consultation was requested for severely injured rural traumatic patients (burns, penetrating trauma, and those with abnormal vital signs) in the Emergency Department, but Telemedicine consultation was not decreased interhospital transfer [13].

Lucas Van Oeveren et al. evaluated intubations which done by telemedicine in rural EDs with ED telemedicine network. They were collected probable data of all patients with video telemedicine-assisted intubation from May 1, 2014, to April 30, 2015, and telemedicine involvement/intervention was evaluated using statistical analysis. Lucas Van Oeveren et al have involved 206 intubations with respiratory failure as most common. They demonstrated a success rate of 96% of telemedicine during emergency intubation. Telemedicine-assisted intubation can be performed in rural hospitals practically. This telemedicine-assisted intubation was done at first in emergency tracheal intubations via a live telemedicine link [14].

Amanda K. Gilmore et al. was described a randomized controlled trial comparing Prolonged Exposure (PE) delivered face-to-face and delivered via Home-based telemedicine (HBT) in female veterans, they focused on female veterans with military sexual trauma with PTSD. Their hypothesize was more be effective in improving the quality of life in PE delivered via HBT than SD at the post, 3-, and 6-month follow-up. Ultimately, Amanda K. Gilmore et al concluded HBT can provide mental health services in a preferable procedure of location in military sexual trauma with PTSD patients and help to them for reducing mental pains and increasing readiness. Generally, Home-based telemedicine can reduce systemic barriers for treatment [15].

Parul Dayal et al have compared the effects of access to a telemedicine program (pediatric critical care) on the intensity of disease and results for referral children to children's hospital PICU in the cohort study. The 582 patients with telemedicine that were younger and less sick than 524 patients from without telemedicine were compared during the study period. Parul Dayal et al was indicated the performance of a telemedicine plan for the care of seriously ill children. The performance of a telemedicine program is designed to assist in the seriously ill children at admission to the PICU. They provided evidence for improving clinical outcomes in pediatric critical care with telemedicine programs [16].

Manish N. Shah et al have evaluated the effect High-intensity telemedicine programs on emergency department use for the care of Six senior living community (SLC) residents in a cohort study over 3.5 years. They were indicated reducing emergency department use in High-intensity telemedicine care of SLC residents. Manish N. Shah et al was presented High-intensity telemedicine can be alternative to traditional severe disease care and it must be included in population health programs [17].

Madan Dharmar et al was evaluated the rate of physician-related medicine mistake on seriously 234 patients receiving 85 telephone consultations, 73 telemedicine consultation, and 76 no consultations in rural emergency departments, then outcomes of the study were compared. They were performed retrospective reviews critically sick and traumatic children of 8 rural EDs from academic children's hospital. Ultimately, Madan Dharmar et al was demonstrated reducing the risk of physician-related medicine mistakes among critically sick and traumatic children of 8 rural EDs from academic children's hospitals [18].

DISCUSSION

Traumatic injury is a common cause of death for those under the age of 44 years. On the other hand, accessing sufficient and adequate high-quality trauma care is a secondary problem after the trauma, and one of the main mechanisms associated with bad results in trauma patients. Generally, the improvement of trauma results is under the influence of triage of seriously traumatic patients. Telemedicine is a strategy to improve trauma care, with the aim of given tertiary level services locally. Using telemedicine is particularly increasing and improving the care of trauma patients, it can be valuable when the time-sensitivity is palpable in trauma interventions [19-30].

This study reviews several strengths aspects of telemedicine such as generally, treatment in a high-risk (population, condition, and location) and removal of barriers to care of traumatic patients.

In this systematic review, the literature searched in important databases for telemedicine in various fields such as trauma injury, war, rural, and military. The text of the articles and abstracts were examined from various aspects, the 21 articles were evaluated for quality, and then 10 papers entered in a systematic review. This manuscript is in the first study in various fields of telemedicine.

We found that the use of telemedicine in trauma injury, rural and the military is very helpful for high-quality trauma care; also the telemedicine and telephone consultations can be were demonstrated reducing the risk of physician-related medicine mistakes. Also, telemedicine can useful, practical, and preferable procedure of location for care military sexual trauma patients with PTSD and help to them for reducing mental pains and increasing readiness. The economic evaluation of telemedicine was demonstrated cost-saving and cost-effective of telemedicine consultations. We also realized the importance and practicable of telemedicine for improving rural trauma care.

Duchesne et al. [31] were reported that the performance of a telemedicine plan decreases trauma transfers to 89%. Many rural EDs have not board-certified physicians in emergency medicine [32] despite, the increasing of traumatic injury and trauma-related mortality in rural populations.

In a study that uses tele-emergency services, they were indicated to improve 95% quality of care by using tele-emergency services [33]. The clinical care is improved by using of telemedicine because access to specialized physicians [33]. in the other study, they found the utilization of expert physicians via telemedicine helped stabilize trauma patients during early care [34]. This study demonstrated a limit of 8% of patients have received care that may survive through using telemedicine consultation [34]. On the other hand, the levels of task-based communication of care services can be increased through telemedicine care [35]. Lucas Van Oeveren et al results were illustrated telemedicine improves care and enhances accessibility to treatment.

MST-related PTSD as one of the subjects for telemedicine was investigated by Gilmore et al. in particular; HBT increases treatment access women and even men with their MST histories which suffering from other physical or mental difficulties.

Although quality of care is fairly suitable in most EDs to children, it may behave rates of poor events, such as medication errors in non-children's hospitals for seriously ill children [36–38], nevertheless, the telemedicine can provide access to pediatric expertise for a decrease of physician mistakes [39].

The telemedicine programs significantly increase the quality of care and reduce mortality in traumatic injuries, also the telemedicine is the importance and practicable for improving rural trauma care. This producer can be a suitable alternative to traditional care, in other words, this method causes access to skilled and experienced physicians in far-off areas, subsequently; telemedicine can decrease the risk of physician-related ED medication errors particularly in a pediatric case. Besides, telemedicine can useful and practical producer for PTSD patients; and home-based telemedicine access for these patients in their home.

CONCLUSIONS

Ultimately, the telemedicine can help patients and health government organizations economically, the telemedicine consultations due to cost-saving and cost-effective. Further studies to evaluate the running of telemedicine need to abating the practical and high-efficiency method, that can be done everywhere and it's free from mistakes and defects.

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VARIA

Effect of pharmacist's patient education on patient compliance in the southeast of Iran: A quasi-experimental study

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Abstract: *Background: As an essential part of the treatment chain and also the link between physicians and patients, pharmacists play a major role in optimizing drug administration. Compliance with a therapeutic regimen is necessary for therapeutic success, while non-adherence can lead to disease progression and eventually treatment failure. This study aimed to evaluate the impact of pharmacist's patient education on different parameters of patient compliance.*

Results: The results showed the patients receiving the intervention compared to the control group, were significantly more adherence to the correct drug use dose (p = 0.016), time of use (p = 0.018), treatment duration (p =0.001), and frequency (p = 0.049).

Conclusions: This study indicates that detailed patient counseling at pharmacies can positively influence different parameters of patients' adherence.

Keywords: *pharmacists; monitoring drug therapy; disease management*

BACKGROUND

Medication adherence knowledge plays a crucial role in treatment success. Pharmacists' scientific skills to teach required information about drug administration, interactions, side effects storage is crucial to improve patient adherence and so treatment success [1, 2]. Patient education by pharmacists can decrease drug therapy problems arising from inappropriate drug administration or prescriptions and decrease overall health care costs [3-5]. Over the past 30 years, the scope of medication therapy management, as well as pharmaceutical care, focused on more specialized and patient-centered care through a more effective pharmacist-patient relationship [4]. Pharmacists also are one of the most important parts of rational drug use based on the World Health Organization (WHO) definition [6] and decreasing its burden [5, 7, 8]. On the other hand, inappropriate administration of antimicrobials may lead to drug resistance and treatment failure that is one of the most challenging problems today [7, 8]. Studies show that one of the main causes of irrational drug use is considering pharmacists only as drug distributors and ignoring their potential role as consultants and educators [9, 10]. In 2003,

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the WHO identified poor patient adherence to achieve all therapeutic goals as a major cause of treatment failure [4]. Although the impact of medication counseling by pharmacists on patient treatment outcome, has not been investigated truly, it has been shown that community pharmacists have a great impact on improving patient adherence especially in patients suffering from chronic diseases as well as improving overall community health [1, 11, 12]. Thus, growing an effective patient-pharmacist relationship is a key factor to optimize medication use and it seems necessary to focus on pharmacists consulting skills and supportive laws [13]. In the present study, considering the undeniable role of pharmacists in patient education and improving their compliance with therapeutic regimen, we evaluated the impact of medication consultation on the appropriate use of medication by patients referring to community pharmacies in Zabol city, southeast of Iran at 2019.

METHODS

Design and participants

This quasi-experimental study was conducted on 56 newly diagnosed patients in one governmental hospital (xxxx) affiliated to XXXX University of Medical Science in the southeast of XXXX from March 10, 2019, to August 30, 2019. Participants were selected using a simple random method and distributed to intervention and control groups. Patients were defined new to therapy if they had no evidence of filling prescriptions with their current medications during the past 6 months. Considering the mean scores for treatment belief for two control (5.04 ± 3.4) and intervention (2.52 ± 2.64) groups in pilot research, the type I error of 0.05, the test power of 0.80 as well as 20% sample loss, the required sample size was estimated as of 23 people in each group.

Intervention

The intervention group received their medications with written instructions on drug packages as well as face-to-face education by pharmacists mainly about dosage, regimen, and length of treatment of each drug in their prescriptions. In the control group received their medications only with routine written instructions on each drug package. Each patient in the control group who had a question that needs further education was excluded from the study.

Instruments

The patient's demographic information was included in data collection forms. Two separate questionnaires designed based on literature review and pilot tested. Patients or family caregiver received the first questionnaire, before collecting their prescriptions and the second one 14 days after. The first questionnaire consisted of four questions about their risk of non – adherence [14] as well as eight questions about their belief about prescribed medicines [15]. The second one consisted of five questions about the patient's practice (correct dose, time, frequency, and therapeutic course) about each of their prescribed medications. The investigator who reviewed patient medications after 14 days and filled out the second questionnaire did not know whether patients are in sample or control groups. The face and content validity of the questionnaire was determined by collecting the six experts' opinions. The questionnaires were then distributed to 20 people in a pilot form. The final questionnaires were designed after identifying the bugs and fixing them. To assess the reliability of the questionnaire. Belief about prescribed medication score and total performance score was obtained from the sum of scores of the relative questionnaires.

Statistical analysis

Statistical analysis of the present data was carried out using an independent t-test and chi-square test using SPSS software (version 21). P-value < 0.05 was considered as significant.

Ethical consideration

The study protocol was approved by the XXXX University of Medical Sciences Ethics Committee (code: XXXXXX) and patients provided written informed consent before participating in the study.

RESULTS

After considering the exclusion criteria, 56 patients were eligible to enroll in this study, 28 patients in both the intervention and control group. Our participants consisted of 45 (80.4%) male and 11 (19.6%) female 7.1% of whom were uneducated. Their ages ranged between 15 - 75 years with the mean age of 33.46 (14.20) years. The mean scores of the patient's primary risk of non-adherence and belief about prescribed medicines were 5.27 (0.92) and 23.89 (3.08) respectively.

There was no statistically significant difference between intervention and control groups in terms of age (p = 0.640), gender (p = 0.313), level of education l (p = 0.968), belief about prescribed medicines (p=0.180) and risk of non-adherence (p = 0.679) (Table 1).

Table 1: Demographic characteristics of sample and control groups

		Control	Sample	P-value
Age		30.03 (18.09)	24.43 (15.75)	0.640
Sex	Male	21 (75%)	24 (85.7%)	0.313
	Female	7 (25%)	4 (14.3)	
Level of education	Uneducated	2 (7.1%)	2 (7.1%)	0.968
	Under high school diploma	9 (32.2%)	7 (25%)	
	High school diploma	13 (46.4%)	14 (50%)	
	College degree	1 (3.6%)	3 (10.7%)	
	Bachelor degree	3 (10.7%)	2 (7.1%)	
Belief about prescribed medicines		24.11 (2.92)	23.68 (3.27)	0.679
Risk of non-adherence		5.11 (0.91)	5.43 (0.92)	0.180

Our results showed that patients receiving face-to-face education were significantly more adherent to the correct dose (p = 0.016), exact time (p=0.018), frequency of drug administration (0.049), and completing the therapeutic course (p <0.001), compared to the control group (Table 2).

Table 2: Comparison of patient’s adherence to correct dose, time, frequency and completing the therapeutic course in control and sample groups

	Control No (%)	Sample No (%)	P-value
Correct dose	16 (57.1%)	25 (89.3%)	0.016
Correct Time	7 (25%)	17 (60.7%)	0.018
Correct frequency	14 (50%)	22 (78.6%)	0.049
Completing therapeutic course	3 (10.7%)	19 (67.9%)	<0.001

Finally, 100 % of the patients in the intervention group and 17.9% of the control group were satisfied with the pharmacist's services (p < 0.001) (Table 3).

Table 3: Comparison of participants' satisfaction in control and sample groups.

Satisfaction		Control	Sample	P-value
Agree	No (%)	5 (17.9)	28 (100)	<0.001
No Idea	No (%)	7 (25)	0 (0)	
Disagree	No (%)	16 (57.1)	0 (0)	
Total	No (%)	28 (100)	28 (100)	

DISCUSSION

Pharmacists as an essential part of the treatment chain and also the link between physicians and the patients play major roles in health care systems [16]. Studies have shown that pharmacists can improve patient adherence and the rate of treatment success. Noncompliance can lead to irrational drug use, expansion of drug therapy costs, the prolongation of therapeutic courses, and adverse drug reactions. In this study, we evaluated the impact of patient education by pharmacists in different aspects of patient compliance and concluded its positive impacts. Our study population is divided into two intervention and control groups. The intervention group received medication counseling especially about dose, time, and frequency of drug administration and duration of therapy besides routine written instructions on drug packages that were consistent in both groups. We observed that although sample intervention groups were not significantly different in terms of demographic data, risk of non-adherence, and belief about their prescribed medication but their final behavior about their prescribed medications were completely different. Adherence to the correct dosage, timing, frequency, and therapeutic course was significantly higher in patients receiving drug education by pharmacists. They were also more satisfied with the pharmacist's services. These results were in agreement with the previous results of previous studies [17-21].

They proved positive impacts of patient counseling at the pharmacies on accomplishing therapeutic goals, reducing adverse drug reactions, and administration drug errors in their researches. Our findings showed that a relatively high number of patients who did not receive counseling did not properly follow their treatment course, which can lead to therapeutic failure and its related complications. Especially, when patients are on antibiotics, non-adherence can lead to drug resistance and finally affect both patients and society. The small sample size was one of our limitations in this study, besides if we could monitor a patient's performance more often, we would be able to provide more accurate results and lower possible bias. One of the strengths of this study was that patients with a history of the same disease were excluded from the study so that their previous experiences did not affect the final results.

CONCLUSIONS

It can be concluded that detailed patient counseling by pharmacists, can positively influence patients' adherence. In this regard, it seems necessary to focus on communicating and counseling skills of pharmacists and even motivational tools as counseling fees.

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VARIA

Clinical and therapeutic particularities of acute gallstones disease during COVID-19 pandemic

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Abstract: *Gallstones disease (GD) is a common pathology, with an incidence of approx. 20% in developed countries, a third of these cases being symptomatic. The paper aims to identify the diagnostic and therapeutic features of acute manifestations of gallstones during the COVID-19 pandemic.*

Materials and method: A retrospective, comparative study was performed of the cases presented to the emergency room (ER) and hospitalized for acute manifestations of gallstones in April-June 2020 vs similar period of 2019. The data regarding clinical form, associated pathology, therapeutic management were analyzed from the ER presentation register, hospitalization sheets and surgical protocols.

Results: During the COVID-19 pandemic, the number of hospitalized cases for acute cholecystitis and other acute manifestations of GD was significantly lower than in the same period last year (21 vs 49 patients). In both study groups the main form of presentation was acute catarrhal cholecystitis (52.4% vs 63.2%). During the SARS-Cov-2 pandemic, we report an increased incidence of complicated forms, mainly hydrops (23.8% vs. 4.1%, $p < 0.05$) and angiocholitis (19% vs. 4.1%, $p < 0.05$). Conservative treatment was chosen more frequently during the pandemic (48% vs. 29%). For operated patients, laparoscopic cholecystectomy was the main option, performed in 85.7% in both groups.

Conclusions: We maintain that laparoscopic cholecystectomy remains the gold standard for acute cholecystitis, even in the COVID-19 pandemic, as it guarantees the best outcomes for the patients. Conservative management may be an option for mild cases, but it carries the risk of recurrence of symptoms and increased incidence of severe complications.

Keywords: *laparoscopic cholecystectomy, gallstones disease, COVID-19 pandemic, acute cholecystitis*

INTRODUCTION

Gallstones disease (GD) is a common pathology, with an incidence of approx. 20% in developed countries, a third of these cases being symptomatic [1].

Laparoscopic cholecystectomy is the gold standard of treatment for this condition. Acute forms are of clinically variable severity, from possible regressive under conservative treatment to life-threatening, namely: biliary colic, acute cholecystitis, cholecysto-pancreatitis and acute angiocholitis. Early surgical treatment was recommended as

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standard in acute cholecystitis to prevent subsequent recurrent episodes, as well as migratory and infectious complications related to the displacement of stones in the main bile duct [2, 3].

The global spread of SARS-CoV-2 pandemic, with the declaration of a state of emergency in our country on the 16th of March 2020, for 60 days, followed by the state of alert since 15th of May, still in force today, had a profound impact on the surgical practice. The initial recommendations of the International and National Surgical Societies were to delay elective surgery, and to attempt first-hand a conservative management of mild and moderate forms presented to the emergency room. Another aspect was that of the aerosolization potential in laparoscopic interventions associated with the induction and dessuflation of the pneumoperitoneum, trocar leakage and laparoscopic smoke. Since the time of SARS-CoV, in 2003, there has been convincing evidence that aerosol-generating procedures (AGP) could potentially result in a wider human-to-human coronavirus transmission radius [4]. Bioaerosols range in size from 0.3 to 100 µm, and particles up to 5 µm remain airborne and can travel distances of more than 100 m, which may be a transmission path for SARS-CoV-2 [5]. Safety guidelines have been developed in laparoscopic surgery to prevent infection of medical personnel in patients suspected or infected with SARS-Cov2, which focuses on preoperative testing of patients, wearing appropriate PPE, consisting of FFP2 masks, goggles or faceshields, ultrafiltration of pneumoperitoneum and preventing accidental leakage of CO2 and plume during laparoscopic surgery [6-13]. Also, the addressability of the population and the desire for surgical treatment was lower for this pathology, partly explained by the fear of a possible contamination in the emergency room or hospital.

Aim

The paper aims to identify the diagnostic and therapeutic features of acute manifestations of gallstones during the COVID-19 pandemic.

MATERIALS AND METHODS

A retrospective, comparative study was performed of the cases presented to the guard room and hospitalized in emergency for acute manifestations of gallstones in April-June 2020 vs similar period of 2019. The data from the presentation register at the guard, hospitalization sheets and surgical protocols, on the particularities related to the clinical form at hospitalization, associated pathology, therapeutic management of patients with acute gallbladder disease, in the absence and during the SARS-CoV-2 pandemic. Changes in the surgical practice of patients with acute manifestations of gallstone disease imposed by international and national guidelines were also analyzed, as well as organizational changes in institutional circuits to prevent the spread of SARS-CoV-2 infection.

RESULTS

Establishing separate circuits within the department for the prevention of SARS-CoV-2 infection

During the pandemic COVID-19, our hospital, an emergency university center with many specializations, made sustained efforts to remain COVID-free, to be able to ensure the safe treatment of patients, and to prevent nosocomial contamination with SARS-Cov2.

In this regard, a strict protocol was implemented which involves testing by RT-PCR at the time of admission of all patients in the emergency room, their isolation respecting separate circuits until the result is obtained, simultaneously with the beginning of the appropriate treatment. If surgery is required immediately, it was performed by treating the patient as SARS-CoV-2 suspect, with all the precautions required by this: proper equipment of PPE in the operating room, avoidance as much as possible of aerosol-generating methods, including laparoscopic surgery, according to the recommendations of the national endoscopic surgery company ARCE in force during the analyzed period. When it was possible to delay the intervention for 24 hours until the result was obtained, in case of negative, the patient was operated laparoscopically, with standard PPE. If the test confirms the infection, the patient was transferred to one of the hospitals designated as a COVID-support hospital and further treated according to the guidelines in force.

Study group analysis

During April-June 2020, during the COVID-19 pandemic, the number of hospitalized cases for acute cholecystitis and other acute manifestations of GD was significantly lower than in the same period last year (21 vs 49 patients). Of the total presentations at the emergency room during the COVID-19 pandemic, only a percentage of 27.6.% agreed with the hospitalization (vs 48.5% in 2019), the rest expressing their request for conservative treatment.

The sex ratio was similar in the 2 groups (F: M = 2: 1), but the incidence of patients treated with rural origin was lower, this could be correlated with the travel difficulties imposed by the legislation during the state of expedite. The average age was higher in 2020, but statistically insignificant (64 vs 56 years).

Regarding the clinical forms, in both study groups the main form of presentation was acute catarrhal cholecystitis (52.4% vs 63.2%). We note, during the SARS-Cov-2 pandemic, the absence of mild forms of biliary colic, with increasing incidence of complicated forms, mainly hydrops (23.8% vs. 4.1%, p <0.05) and angiocholitis (19% vs. 4.1%, p <0.05).

From the analysis of associated comorbidities, we notice the increase of the share of cardiovascular diseases and diabetes in patients hospitalized during the pandemic, these diseases increasing the potential for aggravation of the general condition in patients with acute cholecystitis, making them unsuitable for conservative management.

Table 1: Comparative analysis of patients with acute manifestations of GD during the COVID-19 pandemic compared to the same period last year: demographic data, clinical forms at hospitalization, comorbidities

	April-June 2020	April-June 2019
Number	21	49
Male	33.3%	36.7%
Female	66.7%	63.3%
Urban/Rural	6:1	4:1
Age	64+/-3.2 years	56+/-2.7 years
Clinical form		
Biliary colic	0	8 (16.3%)
Acute cholecystitis		
- Cataral	11 (52.4%)	31 (63.2%)
- Phlegmonous	1 (4.7%)	2 (4.1%)
- Gangrenous	0	1 (2%)
Hydrops	5 (23.8%)	2 (4.1%)
Pio-cholecystitis	0	1 (2%)
Cholecistopancreatitis	1 (4.7%)	2 (4.1%)
Angiocholitis	4 (19%)	2 (4.1%)
Associated pathology:		
Obesity	6 (28.5%)	10 (20.4%)
Adherential Syndrome	5 (23.8%)	16 (32.6%)
Cardiac pathology	6 (28.5%)	6 (12.2%)
High Blood pressure	6 (28.5%)	16 (32.6%)
Diabetes	5 (23.8%)	3 (6.1%)
Gastritis/ Dyspeptic syndrome/Ulcer	3 (14.3%)	5 (10.2%)
Bronchopulmonary chronic obstructive disease	2 (9.5%)	0
Recent cerebrovascular accident	1 (4.7%)	0
Hepato-splenomegaly. Port vein thrombosis	1 (4.7%)	0
Hiatal hernia	0	1 (2%)
Neuropsychiatric disorders (epilepsy, drug addiction)	0	2 (4%)

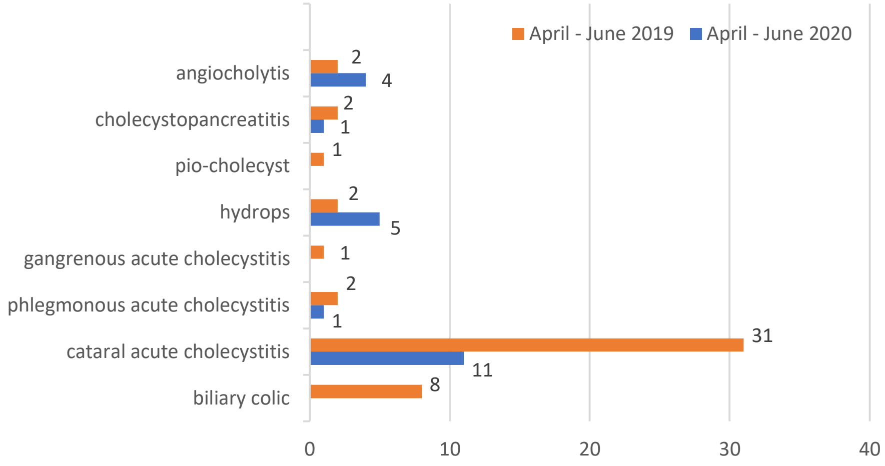

Figure 1: Comparative data for clinical forms during Covid-19 pandemic vs non-COVID period

After admission, all patients in the pandemic group were tested for Sars-CoV-2 infection and were hospitalized in a COVID-19 suspect area, with separate circuits from the rest of the department. Medical therapy was initiated, depending on the clinical form, with spasmolytics, analgesics, antibiotics and hydro-electrolytic rebalancing. In 20 of 21 cases, surgery could be delayed for up to 24 hours until the absence of COVID infection was documented. In one case, a piocholecyst with imminent of perforation was operated on in an immediate emergency, by open approach, and a cholecystectomy and subhepatic drainage were performed, with a slowly favorable evolution. In a case diagnosed with angiocholitis, SARS-CoV-2 positive, the patient was transferred for ERCP to a surgery department at a COVID-19 support hospital, evolving favorably under minimally invasive treatment.

Table 2: Therapeutic management and postoperative outcome in the COVID-19 pandemic group vs control group.

	April-June 2020	April-June 2019
% surgery	11 cases (52%)	35 cases (71%)
Type of surgery:		
LC	18 (85.7%)	30 (85.7%)
Conversion	0	2 (5.7%)
OC	1 (4.7%)	0
ERCP	1 (4.7%)	3 (8.6%)
ERCP+LC	1 (4.7%)	0
Hospital days	6.62	5.6
Postoperative complications:		
Deaths	2 (comorbidities)	0
Bleeding	1 (conservatory)	1
Infection of the wound	0	0

LC: laparoscopic cholecystectomy; OC: open cholecystectomy; ERCP: Endoscopic retrograde cholangiopancreatography

Regarding therapeutic management, there is an increase in cases that benefited from conservative treatment during the pandemic (48% vs. 29%). For operated patients, laparoscopic cholecystectomy was the main option, performed in 85.7% in both groups, except that during the pandemic, the intervention was delayed 24-48 hours to obtain confirmation of the absence of SARS-CoV-2 infection. There were no conversions for the pandemic group, a possible explanation being the better documentation of cases and the favorable effect of early medical therapy in cases with an increased risk of conversion due to local inflammatory anatomical changes.

The average hospital stay was slightly increased, but statistically insignificant, a possible explanation being the timing related to RT-PCR, but also the higher incidence of comorbidities and more severe forms of treated patients. The latter aspect also explains the higher rate of postoperative deaths (9.5% vs 0%), being related to cardiovascular and metabolic decompensation through the associated countries, in elderly patients.

DISCUSSION

The COVID-19 pandemic had a major impact on the management of acute gallstones. During this period, the proportion of cases treated conservatively increased, either outpatient, in the case of patients who refused hospitalization, or in hospitalized patients. This is communicated by similar studies conducted in Spain. Italy, UK or USA, also countries with a significant number of SARS-CoV-2 infections nationwide [1, 14-19].

One of the causes is the reluctance of patients to go to the emergency room or to consent to hospitalization for fear of infection. Harnett et al. estimates in this regard a decrease of up to 50% in the presentation of patients in the emergency room in the US during the pandemic [20]. On the other hand, given the increased pressure on health systems, but also of studies that show a bad outcome in surgeries performed on COVID + patients during the incubation period, international practice guidelines recommend conservative management and avoidance of surgery, if safe for the patient [6, 7, 12, 22-25].

Another important aspect is the reluctance to laparoscopic surgery, due to the possibility of aerosolization of surgical plume and CO2 at pneumoperitoneum desufflation [4]. Preoperative testing of patients is encouraged, as far as resources allow, however studies show a 30% chance of false-negative results, which can have unfavorable consequences for both the patient and the medical staff [26]. Considering possible presence of the infectious agent in blood, feces and intestinal wall and to avoid leakage near the trocar orifices, the use of low pneumoperitoneum pressure and low energy levels during electrocautery.

Regarding gallstone disease, laparoscopic cholecystectomy has established itself worldwide as a gold-standard, due to its indisputable advantages related to the reduction of operative complications, early recovery and reduced hospitalization time. Even during the COVID-19 pandemic, when the open approach was encouraged due to avoiding aerosolization, laparoscopic cholecystectomy remained the preferred technique in surgical practice in our department, being practiced in 87.5% of cases. Similar results are reported by Manzia, Narvaez and Kabir [1, 17, 27]. Moreover, Flemming and Kabir show that SARS-CoV-2 is not present in bile, gallbladder samples and peritoneal fluid [27, 28]. These preliminary findings suggest that the potential infectious risk for operating theater staff and surgeons during open and laparoscopic operations in SARS-CoV -2 patients is lower than initially expected.

Another observation found in the present study is the increase in the severity of cases hospitalized and operated during the SARS-Cov-2 pandemic compared to the same period last year. Gallstones disease, even if not an emergency, should receive surgical treatment when symptomatic. Neglecting this extremely common pathology can increase the incidence of severe, life-threatening complications such as cholangiopancreatitis or angiocolitis [28-33].

Additionally, a more advanced disease at the time of surgery may result in increasingly morbid operations, which are associated with prolonged hospital stay and higher costs. Since the global healthcare community has never faced a dramatic scenario such as the COVID-19 pandemic, it is difficult to forecast the effects of untreated GD and the development of its related complications during and after COVID-19 [1].

CONCLUSIONS

In light of recent findings, but changes to the surgical protocol are required to increased safety for medical personnel.

We maintain that laparoscopic cholecystectomy remains the gold standard for acute cholecystitis, even in the COVID-19 pandemic, as it guarantees the best outcomes for the patients.

Conservative management may be an option for mild cases, but it carries the risk of recurrence of symptoms and an increased incidence of severe complications.

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VARIA

Osteoporosis and the fracture risk in systemic lupus erythematosus

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Abstract: *Osteoporosis is a systemic skeletal condition, characterized by a microarchitectural degradation of bone density and quality, giving low resistance and an increased risk of fracture. Osteoporosis is an important public health problem, with considerable medical, social and economic impact. Data accumulated in recent years demonstrate a significant incidence of osteoporosis in the group of patients with major collagen diseases; of these, Systemic Lupus Erythematosus (SLE) is the prototype of autoimmune disease in which osteoporosis and associated complications have a significant clinical impact.*

Although additional studies are needed to deepen the relationship between SLE - osteoporosis - associated fractures, we believe that these patients should be managed from the early stages of the disease.

Keywords: *systemic lupus erythematosus, osteoporosis, risk factors, fragility fracture, therapeutic principles*

INTRODUCTION

Osteoporosis is a systemic skeletal condition, characterized by a microarchitectural degradation of bone density and quality, conferring low resistance and an increased risk of fracture [1, 4]. Osteoporosis is a significant public health problem, with considerable medical, social and economic impact, through the associated costs. The prevalence of osteoporosis in Romania in the population over 50 years of age is 20.5% in women and 6.2% in men [1].

Osteoporosis is an asymptomatic disease. The main clinical manifestation and at the same time complication of osteoporosis is the fragility fracture. Worldwide, it causes over 9 million fractures annually, approximately one osteoporotic fracture every three seconds [2].

Classification of osteoporosis [1, 3, 4]:

- Primary osteoporosis: type I (postclimax) and type II (elderly).
- Secondary osteoporosis: occurs in association with other conditions or with the use of certain drugs.

Causes of secondary osteoporosis [3]:

- Endocrinological: thyrotoxicosis, hyperparathyroidism, hypogonadism, Cushing's syndrome, insulin-requiring diabetes, Addison's disease, sarcoidosis.
- Gastrointestinal: severe liver damage, primary biliary cirrhosis, gastrectomy, malabsorption syndromes.
- Metabolic and nutritional: hypophosphatemia, hypercalciuria, hemochromatosis, amyloidosis, parenteral

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nutrition.

- Hematological disorders and neoplasms: hemophilia, thalassemia, myelomatosis, lymphoma and leukemia, tumors secreting PTH-like peptides.
- Inflammatory diseases: rheumatoid arthritis, systemic lupus erythematosus (SLE), etc.
- Others: chronic kidney disease, cardiovascular, neurological diseases, etc. [4].
- Medications: glucocorticoids (over 5 mg/day prednisone or equivalent, more than three months), anticonvulsants, proton pump inhibitors, heparin, antidepressants, etc.

This classification in primary and secondary is not delimited. Sometimes the causes of secondary osteoporosis can coexist with post-menopausal status or with aging.

Classical risk factors for osteoporosis

Modifiable risk factors:

- Nutritional deficiencies: Diet influences bone mass and risk of fracture. Nutrients with an important role in bone metabolism are calcium, vitamin D and, last but not least, proteins. Low protein intake is an essential determinant of bone mass. Malnutrition can increase the risk of falling both by affecting coordination and reducing the muscle strength [5-9].
- Physical inactivity: A significant cause of bone loss. It has been observed that forced immobilization in healthy volunteers or in patients with neurological disorders such as paraplegia, leads to decreased bone mineral mass. The negative effect of immobilization is much greater than the potential benefit of exercise on an already outpatient subject [10].
- Weight: A low body mass index correlates with a low bone mineral density (BMD), probably due to decreased peripheral estrogen production by adipose tissue and a lower mechanical load on the skeleton that metabolically influences bone composition [3].
- Smoking: After menopause, bone mineral density in women who smoke decreases by two percent/decade compared to non-smokers [12]. A much more significant loss of bone mass was observed in smoking men, especially in the femoral head. The risk decreases with smoking cessation but becomes significant only after ten years of withdrawal [13].
- Excessive alcohol consumption: Indirectly, influences the bone metabolism by decreasing protein and calcium intake, impaired gonadal function, mobility (increases the risk of fracture by falling), and directly, through the toxic effect on the osteoblast [3].

Non-modifiable risk factors:

- History of falls: Falls are the most common cause of osteoporotic fractures. The annual risk of falling increases by about 20% in women between 35 and 49 years and is over 30% in eldery men [14]. The risk of falling is directly correlated with the number of comorbidities (e.g., neurological disorders, cardiovascular diseases, urinary incontinence, depression, etc.). Also, medications such as hypnotics, sedatives, or antidepressants increase the risk of falling [14-17].
- Age over 65 or under 65 who associate several risk factors.
- Women: a higher risk of osteoporosis. Bone loss is 0.86-1.21% and increases after menopause, unlike men, where the loss is about 0.04-0.90% [18]. Despite this, secondary osteoporosis appears to be more common in men [19, 20].
- Fragility fractures in personal history: the presence of an osteoporotic fracture increases the risk of subsequent fractures. Thus, in both men and women who have suffered a distal fracture of the forearm, the risk of subsequent fractures of other skeletal regions is approximately doubled [21-24].
- Genetic predisposition: about half of the total bone mass, as well as bone architecture and geometry (elements relevant for bone strength), can be genetically determined [25, 26].

Preliminary data studied reveal a significant incidence of osteoporosis in patients diagnosed with collagen diseases. Of these, SLE is the prototype of autoimmune disease that predominantly (but not exclusively) affects young women, but also men. For this group of patients, osteoporosis is a frequent long-term complication.

With the progress of therapeutic strategies and the introduction of new effective treatments in SLE, organ complications are much better controlled, which has led to a significant increase in life expectancy. In this context, osteoporosis and associated complications have a greater clinical impact and the therapeutic attitude must focus on this aspect, from the early stages of the disease.

Regarding the method of objectification and osteoporosis diagnosis, there are several techniques for measuring bone mineral density, but the most common is using dual X-ray absorption (DXA). Bone densitometry provides two results: the T score, which provides the result by comparing the patient's bone density to a person with maximum bone density – a healthy 30-year-old, and the Z score, which compares the patient's bone density with that of a person of the same age to assess the degree of bone loss.

PARTICULARITIES OF OSTEOPOROSIS IN SLE

Osteopenia, defined as a T score between 1.0 and −2.5 in the spine and femoral head, has been reported in 25–74% of patients with SLE, while osteoporosis, defined as a T score less than −2.5, in 1.4–68% of patients with SLE in cohort studies [27].

Despite the large variability of epidemiological data, a significantly increased prevalence of low bone mineral density is recognized in SLE patients versus the general population.

Particular risk factors and mechanisms of occurrence

The etiology of bone density reduction and fractures is multifactorial, involving classical, disease-independent risk factors and particular risk factors. Among them, we mention:

- Inflammatory factors: Systemic inflammation contributes to bone loss by influencing the balance between bone resorption and osteosynthesis, causing an increase of osteoclasts activity. In addition, in the active forms of the disease, an increased level of tumor necrosis factor (TNF) and oxidized low-density lipoprotein (LDL) was observed, the latter interfering with the maturation of osteoblasts and activating T lymphocytes [27, 57, 58]. T lymphocytes involved in the synthesis of TNF and nuclear factor kappa B ligand receptor activator (RANKL) stimulate osteoclast differentiation [58]. A low complement C4 level (an indicator of disease activity) was considered a predictor of decreased bone density in the lumbar spine (Hopkins Lupus Cohort) [28]. A 5-year study showed a correlation between the frequency of disease outbreaks and the impact on bone density, confirming the hypothesis that systemic inflammation contributes to bone loss in SLE. In addition, more than half of diagnosed patients develop inflammatory nephritis during the disease, which can progress to renal failure [30]. In severe forms of renal impairment, the appearance of secondary hyperparathyroidism causes an increase in osteoclastic activity and bone resorption [29, 30]. Another mechanism may be a low level of 1.25 dihydroxy vitamin D by decreasing alpha1 hydroxylase activity in the kidney [59].

- Serological factors: Although autoantibodies role in influencing bone metabolism is unclear, anti-Ro may increase the osteoporosis risk [27, 31]. Up to date, no correlation between the presence of anti-dsDNA antibodies and bone mass has been demonstrated. The anti-Sm could have a protective role [31].

- Metabolic factors: Vitamin D deficiency in SLE can be determined by several factors such as photosensitivity, involving avoidance of sun exposure and use of SPF protection screen, kidney failure, disease activity, prolonged glucocorticoid therapy, the presence of anti-vitamin D antibodies (approx. 4 % of patients) [59].

- Hormonal factors: Unlike the general population at risk for osteoporosis, which has an estrogen deficiency (especially women over 50 years), in SLE, there is a predilection for females in the 15-45 age group, with high levels of estrogen. In those patients, low levels of androgen hormones appear to have a stronger influence on bone metabolism than relatively high levels of estrogen [32].

- Genetic factors: The density of bone structures could be influenced by the genetic polymorphisms of the vitamin D receptor - FOK I (FF, Ff, ff). However, a prospective study conducted during 5.3 years did not show a significant difference between the three genotypic groups [33].

The impact of medication on osteoporosis in SLE

Corticosteroid therapy influences in a dual way the bone mineral density in patients with SLE. On the one hand, its risk of inducing osteoporosis is well known, but it may also play a beneficial role by reducing systemic inflammation's effects on the bone. Two extensive prospective studies [29, 34] have shown that bone loss in SLE occurs predominantly in patients treated with at least 7.5 mg prednisone per day.

Hydroxychloroquine. The contribution of this treatment in osteoporosis prevention is not yet well defined. Despite this, the control of systemic inflammation with immunosuppressive drugs is important both by reducing inflammatory outbreaks and also by reducing the dose and duration of corticosteroid therapy. In women with SLE, HCQ use was associated with higher BMD in the lumbar spine [31, 61, 62] and hip [62]. However, more extensive studies are needed in patients with SLE and other rheumatic diseases in which hydroxychloroquine is used.

The risk of fracture in SLE

Fractures can be an important complication in the course of patients with SLE. The data accumulated in recent years demonstrates an increase of 1.2 - 4.7 times the risk of symptomatic fractures than the general population (Echblorn 2013). In the case of vertebral fractures, which can frequently be clinically inapparent, morphometric analysis is important. Morphometric identification of vertebral deformities is also significant for the attending physician because it is associated with significant impairment of quality of life and an increased risk of symptomatic fractures (e.g., hip) [35]. Current studies showed the presence of at least one vertebral fracture in 3.7-50% of patients with SLE [35, 36, 37-42].

The etiology of fractures is multifactorial in patients with SLE, including bone-dependent factors and factors associated with the risk of falling [36].

The main factors associated with the increased risk of symptomatic fractures in SLE are:

- Age;
- Menopause;
- Smoking, alcohol consumption;
- Duration of the disease;
- Kidney damage;
- Decreased BMD;
- Duration of treatment and cumulative dose of glucocorticoids.

In addition, patients with SLE have an increased risk of falling and associated conditions: physical asthenia, joint damage, neurological complications, impaired visual acuity or decreased muscle strength (which may be associated with both inactivity and corticosteroids or vitamin D deficiency) [8, 17].

A particular aspect of SLE patients is the relationship between bone mineral density and fracture risk. The idea of reduced BMD in patients with SLE is generally accepted, but not all studies showed a clear correlation with an increased risk of fracture. In addition, about 1/3 of patients with SLE and vertebral fractures have normal BMD [43, 44]. These data demonstrate the limited value of BMD in estimating fracture risk and the multifactorial etiology of SLE fractures.

PREVENTION AND TREATMENT OF OSTEOPOROSIS ASSOCIATED WITH SLE

SLE is a chronic condition that predominantly affects young women. Therapeutic regimens used to control the disease generally involve extensive and long-term use of glucocorticoids. Thus, decreased bone mineral density and increased fracture risk appear as natural problems in managing this condition. Lifestyle measures and control of risk factors are general principles in the management of osteoporosis and fracture risk. The current recommendations are based mainly on adopting a healthy lifestyle and preventing falls (as a central risk factor in fragility fractures).

Important aspects are: avoiding smoking, alcohol consumption, maintaining an optimal weight and regular physical activity. From a nutritional point of view, the measures are based on optimizing the intake of calcium, proteins and vitamin D, essential for bone mineralization and maintaining an adequate neuromuscular function. Data from the literature recommend a daily calcium intake of at least 1000 mg (1200-1500 mg in patients with corticosteroids), the dosage of serum level and supplementation with 800-1000 IU vitamin D per day and a protein intake of 1 g/kg per day [45].

Pharmacological therapy in SLE is addressed primarily to osteoporosis patients with a history of fragility fracture and glucocorticoid treatment.

The 2010 American College of Rheumatology (ACR) recommendations for preventing and treatment of glucocorticoid-induced osteoporosis include bisphosphonates as the first-line medication for postmenopausal women and corticosteroid patients [45]. This therapeutic class presents consistent evidence of efficacy in increasing BMD and reducing the risk of fracture. The antiresorptive agents inhibit bone resorption by attaching to the active remodeling sites causing apoptosis of osteoclast cells. The main representatives of the class currently used are the second generation drugs (e.g., alendronate, ibandronate, pamidronate) as well as those of the third generation, with much higher potency (e.g., risedronate and zoledronic acid). They are recommended for patients with normal renal function [45] to prevent and treat glucocorticoid-induced osteoporosis. Limited data on the teratogenic effects of bisphosphonates require their administration only to postmenopausal women [46]. In addition, patients should be informed of the risk of complications such as osteonecrosis of the jaw or atypical femoral shaft fractures, especially in patients on corticosteroid therapy [47-49].

Studies have identified new therapeutic targets in osteoporosis, such as cathepsin K, sclerostin, or the RANKL system. Of these, denosumab, an anti-RANKL monoclonal antibody, has entered and prevailed in clinical practice being an option for premenopausal women with glucocorticoid-induced osteoporosis, as well as for patients with SLE and renal impairment [27].

The risk of osteonecrosis of the jaw and atypical fractures remains in this category of therapeutic agents. Potential complications are the rebound fractures phenomenon and the greater tendency towards hypocalcemia [60].

Estrogen replacement therapy to prevent osteoporosis in patients with SLE is not recommended, as it is associated with an increased rate of inflammatory flares and thromboembolic events [50]. Raloxifene, a selective modulator of estrogen receptors, is an option for postmenopausal women with inactive lupus and without a history of thrombotic events or antiphospholipid antibodies [51].

Another treatment, the recombinant human PTH analogs (e.g., teriparatide), may be an option for men and postmenopausal women who have not responded to bisphosphonate therapy and are at increased risk of fracture [53]. The experience in the young adult population, including premenopausal women, is limited, so this treatment should be initiated only when the benefits outweigh the risks. Premenopausal women with glucocorticoid-induced osteoporosis should be considered at high risk of fracture if they have a pre-existing fracture or a combination of risk factors such as low bone mineral density, intensive treatment with glucocorticoids in high doses (≥7.5 mg/day) for at least six months or background disease in acute onset [52]. In patients with glucocorticoid-induced osteoporosis, this drug was associated with an increased BMD and a significant reduction of vertebral and non-vertebral fractures, but not of hip fractures, compared to the group treated with bisphosphonates [53, 52]. The administration must be made with caution in patients with moderate renal impairment [54].

SCREENING AND MONITORING OF TREATMENT

Patients should be monitored during the therapy by periodic reassessments, both biologically (alkaline phosphatase, blood calcium levels, phosphatemia, urine calcium levels, etc.) and imaging (bone densitometry, skeletal radiographs to document fractures). The inadequate response, low adherence, or adverse reactions to treatment, call into question the decision to discontinue or change it [55, 56].

Regarding the direct assessment of fracture risk, we mention that there is no current algorithm accepted and adapted to patients with SLE. The most widely used in practice is the Fracture Risk Assessment (FRAX), proposed by the World Health Organization [55]. FRAX has important limitations in SLE: it was developed for the postmenopausal period (and thus does not cover a significant subgroup of patients), does not take into account the cumulative dose of glucocorticoids (but only their use or not), and does not include fall risk assessment and the presence of vertebral deformities [27].

CONCLUSIONS

The last decades have made significant progress in the evaluation and treatment of patients with SLE, allowing current therapeutic strategies to control and stabilize affected organs. In this context, associated osteoporosis and fracture risk have become important in the medium and long term management of the disease.

At the moment, the practitioner has consistent data on the epidemiology, pathogenic mechanisms, and clinical impact of osteoporosis and fractures in patients with SLE. Current recommendations include: optimizing calcium and vitamin D intake, lifestyle adjustments and drug treatment. Special attention is directed to the group of women at fertile age, for whom treatment options are still limited.

Additional data on screening methodology and algorithms, morphometric assessment and fall risk are expected. In spite of this, there are currently several arguments for rheumatologists in order to turn their attention to the management of these complications from the early stages of the disease.

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Serum vaspin and chemerin changes in response to combat physical fitness test in military forces

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Abstract: *The study aimed to investigate serum vaspin and chemerin changes in response to combat physical fitness tests in military forces. In a semi-experimental design with pretest and posttest, twenty volunteers were engaged to participate in the study. Combat Physical Fitness Test consisted: (1) 25-yard(yd) sprint to J-hook, to 25-yd crawl, and 25-yd run through cones to 75-yd line; split (2) 75-yd casualty drag; split (3) ammo can run to grenade toss (75-yd); (4) ammo can run to end (75-yd); and (5) 650-yard endurance run. Pre-test and the post-test blood sample was gained. The data were analyzed by SPSS version 24. Serum chemerin did not change significantly compared to the pre-test whereas Serum vaspin was significantly higher after the test (p = 0.018). Afterward, a direct correlation was observed between serum chemerin and serum vaspin (p = 0.034, r=0.335). It is suggested that serum vaspin is responsive to short-term maximal power output activity required in the combat fitness test and positively correlates with chemerin. However, it appears that 3 to 4 minutes of high-intensity exercise induce comparatively moderate post-exercise serum vaspin increment, which appears to be inadequate to stimulate chemerin secretion.*

Keywords: *combat readiness test, vaspin, chemerin, military, adipokines*

INTRODUCTION

Adipokines are new bioactive adipose tissue secreted factors that induce a variety of local and systemic titled role in the body as systemic metabolic regulators, immunity, and endocrine function. Several adipokines including chemerin, vaspin, omentin-1, and apelin affect the whole-body homeostasis by influencing numerous biological, physiological, and pathophysiological processes [1, 2].

Chemerin is a16 kDa chemoattractant protein, an agonist of the orphan G-protein coupled receptor chemokine-like receptor 1 (CMKLR1, ChemR23) adipokine that play role in innate and adaptive immunity that is expressed highly by innate immune system cells [3, 4], liver, and white adipose tissue (WAT), and expressed moderately in lungs and brown adipose tissue, and weakly expressed in the heart, ovaries, and kidneys and as an adipokine exerts effects on many biological processes, including adipogenesis, angiogenesis, inflammation, immune responses, and food intake [4, 5]. Pathologically, several effector cells of the innate immune system are influenced by chemerin, including dendrite cells, monocytes, macrophages, and NK cells, and might influence the crosstalk between different cell types responsible for controlling the initiation and progression of inflammation [6]. Recent studies suggested either a proinflammatory or

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anti-inflammatory role for chemerin and emphasized it's as treatment strategies for obesity [7]. On the other hand, Vaspin(visceral adipose tissue-derived serine protease inhibitor) is a recently discovered 45 kDa adipocytokine predominantly secreted from visceral adipose tissue [1, 8]. Vaspin mRNA was reported to be expressed in subcutaneous and visceral adipose tissue which might play a role in inflammation expansion [9]. Multiple lines of evidence suggest that vaspin protects cells from inflammation and apoptosis. It has been demonstrated that vaspin inhibition of TNF-α induces the expression of adhesion molecules and subsequently decreased lymphocyte adhesion by a reducing ROS generation [10].

Several kinds of research studied the response of adipokines such as chemerin and vaspin to high-intensity exercise and reported contradicted results such as elevation [11, 12], no change [13, 14] or reduction [15, 16]. The most prior studies were focused on obese or metabolically patient subjects while to our knowledge there is no research to investigate acute chemerin and vaspin responses after MANUF. Also, the notable issue is that in previously performed protocols, the movement pattern/type was limited to one of the aerobic/resistance/power modality of exercise such as cycling or running [17-19].

Combat physical fitness is a key factor in armed forces [20]. The maneuver under attack fire (MANUF) is a relevant, high-intensity, and integrated test that its stages are executed by maximal power output and included combat physical items similar to war conditions [21]. Previously it was reported that high-intensity exercise stimulated free radical production in the skeletal muscle and myocardium. Also, high-intensity exercise (HIE) causes cell damage due to mechanical tension imposed on muscle cells that may stimulate the immune system and expand inflammation. In this regard, chemerin and vaspin may play the opposite role in response to high-intensity exercise. However, MANUF is a complete combination of war simulated tasks such as sprint, crawling, tossing a grenade, carrying casualties, or ammo boxes [21]. Hence, it's unclear whether utilizing different movement patterns or muscle groups throughout MANUF makes any difference in serum chemerin and vaspin responses or not. Therefore due to a lack of sufficient information about the metabolic response of the MANUF test, our first aim is to measure and compare serum chemerin and vaspin in pre and post-test. It is reported that serum chemerin and vaspin concentration are elevated after high-intensity exercise. A high-intensity exercise (severe muscle contraction) causes cell damage due to mechanical tension imposed on muscle cells that may stimulate the immune system and expand inflammation. Chemerin regulates activation of dendritic cells and macrophages by some intracellular factors such as the G protein-coupled receptors CMKLR1 (ChemR23), GPR1, and CCRL2. Based on Japanese cohort studies its reported a close association between serum vaspin levels and physical fitness in men also physical activity in women independent of the subject's body composition [22]. So secondly we postulate there is a proposed relationship between serum chemerin and vaspin.

MATERIALS AND METHOD

Statistical sample

In a quasi-experiment study design, fifties cadets volunteers were recruited. At the first visit to familiarize seasons information regards research procedures, its benefits, and potential risks were provided to participants. Written informed consent forms, and the health and physical fitness questionnaire were completed. Height and weight measurements and familiarization with the test were performed the day before the test. In the end, 20 subjects with an average age of 26.95 years, and, the weight of 73.3±7.34 kg were selected as the study sample. Inclusion criteria were healthy male military personnel or personnel with more than one year of fields’ military history. The participant exclusion factors were the history of fracture, cardiovascular disease, smoking, and consuming drugs.

The maneuver under attack fire (MANUF) test

The MANUF contains combat-related tasks. The MANUF that aims to assess the combat-related tasks consisted of movement types of actions that are made potentially in combat.

The sort of the items into a single timed event is both a combination of energy systems demonstration, strength/ anaerobic plus aerobic, and a measurement of military combat capability and physical fitness. The test included 300-yard field MANUF tasks such as performing sprint (25 yards), J-hook turn, low and high crawl (25 yards), casualty drag (75 yards); ammo can sweep (30 lb each, 1 in each hand) carries shuttle run (two 75 yards), a grenade toss and at the end, 650 yards running [21]. In the casualty drag item, there is a 10-yard drag, followed by a 65-yard fireman carry. Subjects encourage doing their maximal effort to achieving peak performance from start-up to course end.

Dietary and physical activity control

Subjects were requested to avoid intensive physical activity, supplement consumption such as caffeine and have their habitual diet in the preceding 24 hours of the test. Additionally, participants completed a 24-hour dietary recall before the combat test.

Enzyme-linked immunosorbent assays

Blood samples were collected into K3 EDTA tubes and were isolated by centrifugation (3,000 x G for 10 min). The obtained serum was aliquoted and stored at -80°C until analysis.

Serum chemerin was assessed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Biovendor-Laboratorni medicina a. s., Karasek, Czech Republic). Serum vaspin levels were assayed using a sandwich ELISA kit Vaspin (AdipoGen, Seoul, Korea) according to the manufacturer's instructions.

Statistical analysis

Statistical data analyses were done with descriptive statistic mean and standard deviation. A paired sample student t-test was used to analyze within groups comparison. The possible correlations between chemerin and vaspin serum levels were tested with Bivariate Pearson correlation coefficients. Statistically significant differences were set at P<0.05.

The SPSS (IBM Version 24, 2016) was used for data statistical analysis. Written consent form was completed by participants before taking part in the study.

All study protocol was performed according to the criteria set by the declaration of the Helsinki 1975, as revised in 2008, and were approved by Baqiyatallah University of Medical Sciences ethical committee (IR.BMSU.REC.1397.303).

RESULTS

Demographics

Twenty participants were engaged to participate; and completed all trials and are covered in the analysis. Participants' characteristics are shown in Table 1. All individuals had at least one combat deployment before the research with an average history of 43.1 ± 18 months of active duty service recruited.

Table 1: Subjects demographic and physiological characteristics

Variable	Mean± SD
Age (year)	26.95 ± 3.80
Weight (kg)	73.3±7.34
Height (cm)	176.65 ± 6.36
BMI (kg/m^2)	22.13 ±2.78
Rest Heart rate	78.63±12.5

BMI: body mass index, SD: standard deviation

The results of the pretest-posttest comparisons are represented in Table 2. The result of a paired sample t-test demonstrated that chemerin serum levels did not change significantly compared to pretest, While Serum vaspin levels increased significantly after the combat fitness test (p = 0.018, Table 2). Additionally, Pearson correlation analysis revealed a positive correlation between chemerin and vaspin serum levels changes in response to combat physical fitness test (p=0. 34, Figure 1).

Table 2: A within group's comparisons of serum chemerin and vaspin levels

	Pre-test (Mean±SD)	Post-test (Mean±SD)	t	P-value
Serum chemerin (pg/ml)	82.89 ± 19.56	87.38 ± 24.20	-0.68	0.34
Serum vaspin (pg/ml)	0.67 ±0.34	0.90 ±0.41	-2.58	0.02*

** Significant differences at p≤ 0.05*

DISCUSSIONS

The main finding of the present study was that vaspin significantly increased after combat fitness tests and there is a positive correlation between vaspin and chemerin increment although there was no dramatic change in chemerin levels comparing to pre-test level.

To the best of our knowledge, this is the first research that examines the effect of maneuver under attack on circulating serum chemerin and vaspin. Absence of dramatic change may be due to the low volume (frequency/duration) of applied exercise activity and following previous results of Zarei et al. 2018 and conflict with findings of Saremi et al.2010, Chakaroun et al.2012, Aghapour et al. 2013, Venojarvi et al. 2013, Malin et al. 2014, Kim et al 2014, Faramarzi et al. 2016 [23-29]. The present research failed to distinguish any dramatic alteration in serum chemerin levels after MANUF that is predominantly a high-intensity exercise in nature. Lack of such a change in our data series may be due to differences in the intensity, type, duration, and frequency of training compared to the noted studies.

Faramarzi et al. 2016 demonstrated a significant reduction in serum chemerin and vaspin levels in forty healthy women following 12 weeks of combined rhythmic aerobic and core stability exercise training [29]. Lioyd et al. 2016 reported that acute bouts of one hour of moderate-intensity walking (60-

65%Vo2peak) drop chemerin levels in 11 non-insulin resistant obese adults at one and two hours follow up time courses [30]. Liu et al. (2018) studied the effects of four weeks of low to moderate intensity of aerobic exercise in fifty obese adolescent female (14 years old) on chemerin serum levels and showed that whereas slice dieting did not have health benefits, 4 weeks of exercise plus dieting induces a significant decrease in chemerin and body fat [31]. Similar to our results Zarei et al. (2018) indicated nonsignificant changes in chemerin levels after 8 weeks of high-intensity interval training in comparison with control groups [13]. Based on these results, its proposed intensity-dependent changes in chemerin serum levels, in the form of its reduction in response to moderate exercise and non-changes or elevation following high-intensity exercise.

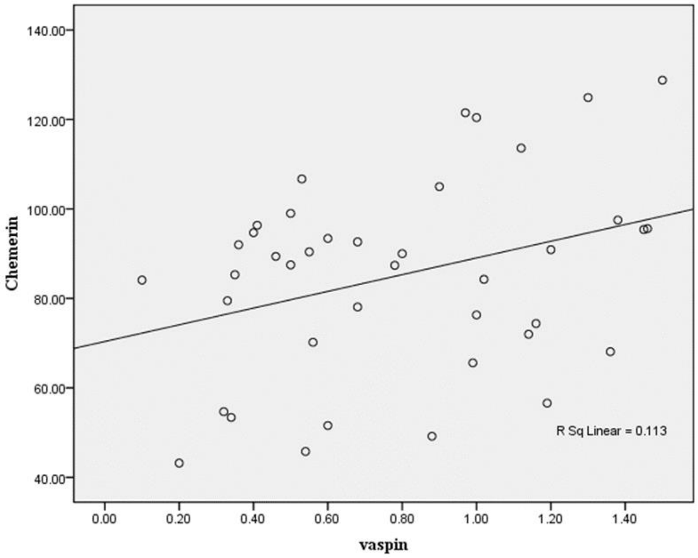

Figure1: The correlation between serum chemerin and vaspin

Furthermore, the characteristics of subjects also could be considered as counting factor. This study included a healthy young male army force without risk factors of cardiometabolic disease, while most other researches were carried out among middle/advanced-aged, overweight/ obese or diabetic [13, 30, 31] subjects who are exposed to high cardiometabolic risk. Further researches are necessary to clarify the impact of training type, frequency, and duration, and participant's health and fitness status on chemerin levels [6]. The mechanical tension leads to cell rupture and bioactive chemokine production to help macrophages for phagocytosis in the initial stage of inflammation [7]. As we observed a weak positive correlation between serum levels of chemerin and vaspin changes in response to one bout of acute exercise activity executed in MANUF test, it seems that there was not deleterious enough alteration in vaspin to induces a significant increase in chemerin. As it found a positive association between chemerin levels with obesity and T2DM and negative association of these diseases with vaspin levels, and low levels of vaspin reported in different stages of cardiovascular disease (CVD) [1], so optimal exercise intervention could be defined when it induced serum vaspin elevation while chemerin did not. In contrast to our results, Motawi et al. (2018) reported a strong negative relationship between chemerin and vaspin in grade I, II CVD that could be related to baseline variance levels of this adipokine in compare with the present study healthy arm force subjects. Subsequently, increased vaspin levels with non-changes in chemerin levels could be ideal regards the protective effects of exercise in CVD that needed to be clarified in further studies.

The result of the present study shown that vaspin levels increased at the end of the combat fitness exercise test. The recorded times to the completion of combat physical fitness tests, which were between 3 to 4 minutes that seems to be adequate to enhance vaspin secretion and augmenting its circulating levels. Base on previously available researches, the data about the response of vaspin after an acute bout of exercise particularly the maneuver under attack fire is still scarce and it is difficult to compare results towards reach a conclusion. The elevated serum level of vaspin seems to be related to the intensity of training. In this regard, Youn et al. 2008 reported that 4 weeks of high-intensity training causes an increase in serum vaspin levels along with weight loss in obese or type 2 diabetics subjects [32]. On the other hand, Oberbach et al [33] observed a significant decrease in serum vaspin concentration after 4 weeks of high-intensity resistance training in healthy young men. Also in a study conducted by Lee et al [34], vaspin levels in obese children significantly decrease after seven days of intensive lifestyle modification. Elevation in vaspin levels in the present study could be suggested as an optimal anti-obesity strategy as previously reported increase in serum vaspin levels depending on the degree of obesity and reduction in its levels with weight reduction programs [22]. Therefore, it seems that high-intensity aerobic exercise/training intervention induces serum however, it needs to be revealed in future studies.

CONCLUSIONS

These data specified that vaspin serum levels are responsive to short-term maximal power output exercise required in a combat fitness test and positively correlates with chemerin levels changes. However, it appears acute high-intensity exercise induce comparatively moderate post-exercise serum vaspin increment, which appears to be inadequate to stimulate chemerin elevation. It appears that there may be higher metabolic stress thresholds that are required for the activation of the compensatory and positive systemic anti-inflammatory responses and an extended exercise duration is expected to be a crucial element in achieving this, however further studies need to elucidate these.

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Authors contribution:
These authors also contributed equally in study conception and design, material preparation. Data collection and analysis were performed by A. Delpasan and B. Bazgir. The first draft of the manuscript was written by A. Delpasand and all authors read and approved the final manuscript.

Conflict of Interest:
The authors declare that there is no conflict of interest regarding the publication of this article.

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VARIA

Normal pressure hydrocephalus – Diagnosis and therapeutic challenges

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Abstract: *Normal pressure hydrocephalus (NPH) is a rare pathological condition that generally affects older people (>65 years old), and can take one of two forms: idiopathic or secondary. It is a reversible cause of dementia, and it manifests itself through a very specific triad: gait impairment, urinary incontinence, and dementia. The clinical diagnosis of NPH is particularly difficult to establish due to the association between these symptoms and old age, as well as due to the absence of specific paraclinical markers. Nevertheless, a holistic application of clinical, imagistic, paraclinical criteria, as well as of the various developed scales may, in time, lead to a diagnosis. Moreover, the evolution of magnetic resonance imaging has certainly made easier to establish the condition. The sole therapeutic method proven to be efficient is shunting surgery which has good results in the majority of the cases, showing clear signs of improvement from the very first-day post-surgery.*

Keywords: *normal pressure hydrocephalus, ventriculoperitoneal shunt, aqueductal stenosis, cerebral ventriculomegaly, communicating hydrocephalus, obstructive hydrocephalus*

INTRODUCTION

Normal pressure hydrocephalus (NPH) was firstly recognized by Adams et al. in 1965, by identifying a number of patients suffering from hydrocephalus, but having a normal pressure in the cerebrospinal fluid (CSF) at the lumbar puncture (LP) [1]. This represented a potentially reversible syndrome. The pathology is most often present in older people, and it manifests itself through a gait disturbance, urinary incontinence, and cognitive deficit. It has also been determined that gait impairment, along with one other characteristic element is essential for diagnosis. The clinical assessment demands further examinations, such as brain imagery or draining the CSF, to properly confirm the condition [2].

Traditionally, hydrocephalus was divided into two types: communicating hydrocephalus or obstructive hydrocephalus (non-communicating). The former type is characterized by an increase in the volume of CSF without any stenotic lesions in the drainage. In this case, the classification of NPH is still the one being used since 1965: idiopathic NPH (iNPH) – in roughly 50% of all cases, and secondary NPH (sNPH), which comes as a result of either subarachnoid hemorrhage (SAH), intracerebral hemorrhage (ICH), meningitis, intracranial tumors or trauma [1]. While iNPH is generally noticed at adults, sNPH may be present at any age, with both genders being affected equally [3]. Apart from the ventriculomegaly, there are no other observable radiological signs.

Obstructive or non-communicating hydrocephalus comes after the stenosis of the drainage between ventricles and the subarachnoid space, either congenital or acquired. The stenosis of the cerebral aqueduct is a common cause in young adults, although the symptoms are not visible until

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adulthood [4].

In the largest study ever conducted on NPH, in Sweden, the results estimated a 0.2% prevalence in the 70-79 age group, and 5.9% in those aged over 80 [5]. In Japan, the results estimated a 0.01% prevalence in the general population and 0.03% in those aged over 60 [6]. In another study on a Finnish cohort, the incidence was 1.58 for every 100.000 persons [7].

The comorbidities associated with NPH are: Alzheimer disease (15%), chronic high blood pressure (50%), hyperlipidemia (14%), and diabetes mellitus (20%) [6, 7]. The latter two factors increase the risk of developing NPH by doubling it, as compared to a normal patient [8]. Similarly, obesity and psychological stress, heart diseases, and strokes have also been named as contributory to the development of this pathology [8, 9].

Unfortunately, the criteria for NPH diagnosis remain uncertain. While some specialists consider it the most common form of hydrocephalus in adults, others contest its very existence [3, 10, 11].

NPH is difficult to diagnose due to its broad and general symptomology, commonly associated with old age. Thus, in individuals aged over 75, urinary incontinence is present in 20 to 30% of them, whereas 20% develop some kind of dementia and 20% show signs of ventriculomegaly [4]. Implicitly, the management of NPH is equally difficult, due to the complications associated with shunting. As such, the morbidity and mortality rates are 20% and 0.2%, respectively [12].

PHYSIOPATHOLOGY

The CSF is mainly secreted by the cells of the choroid plexus, other regions playing mainly a rather insignificant role. CSF's volume is between 125 and 150 ml, and its secretion varies between 400 and 600 ml daily, renewing itself 4 to 5 times every 24 hours in a healthy adult [13]. Its role is vital, namely, the protection and nutrition of the parenchyma [14]. Furthermore, its dynamic is highly dependent on the rate of production and absorption, as well as on the blood-related pulsatile flow through both the subarachnoid and Virchow-Robin spaces to the cerebral parenchyma [15, 16]. According to Adams et al. original theory, NPH develops when the level of CSF's reabsorption diminishes, thus resulting in intracranial hypertension, leading to ventricular dilation as a compensating mechanism [1]. Hence, a larger volume of CSF is redirected to the Virchow-Robin spaces, giving birth to a compression of the parenchyma and ischemia of the white matter.

The specific pathophysiology of iNPH is unclear, yet various mechanisms have been put forward as possibly involved in developing the pathology, such as [2]:

- Reduced compliance of the subarachnoid space
- Reduced reabsorption of CSF in the venous system
- Inadequate expression of TNF-alpha in CSF
- The hyperdynamic flux of CSF in the cerebral aqueduct
- The higher pressure of the CSF
- The reabsorption of CSF through abnormal mechanisms

CLINICAL PRESENTATION

iNPH must be suspected in older patients suffering from abnormal walking, sometimes combined with dementia or urinary incontinence [3].

Symptomatology can vary massively between patients. They may show signs with the classic triad in percentages of 30 to 50 (gait disturbance, urinary incontinence, and cognitive deficit), or only a combination of these: only gait impairment (5%), only dementia (2%), both combined (28%), gait abnormality together with urinary incontinence (4%) and dementia accompanied by urinary incontinence (1%) [6][17]. Approximately half of the patients with iNPH only show signs of a gait disorder, the other two characteristic symptoms being absent [6].

DIAGNOSIS

According to international guides, the imagistic diagnosis is based on the following elements [18]:

- Ventricular dilation with an Evans index > 0.3 [19]
- The absence of a macroscopic obstruction of the dynamics of the CSF
- At least one of the following [20]:
    - Callosal angle exceeding 40° [21]
    - Temporary dilated temporal horns of the lateral ventricles, but not fully due to the atrophy of the hippocampus
    - Changes in the CT and MRI signal caused by the alteration of the content of cerebral water, not fully owed to the microvascular ischemic modifications or demyelination.
    - Signs of a "flow void" in the MRI in the Sylvian aqueduct

The following imagistic methods can be engaged as well: MR elastography (MRE), glymphatic MRI, or the Silver index [22, 23, 24].

Regarding the Evans index, it is established based on a CT scan in the axial incidence. An index exceeding 0.3 indicates a pathological condition of the ventricles (sensibility of 80%) [19]. The threshold for indicating a pathological component varies by age, namely: between 65 and 69 years – 0.34; between 70 and 74 years – 0.36; between 75 and 79 years – 0.37; between 80 and 84 years – 0.37 [19].

Callosal angles are measured based on a CT or MRI scan, at the median point of the corpus callosum [21]. Identifying this point necessitates using the sagittal plane, placed parallel to the floor of the fourth ventricle [21]. The limit was 105.4° (42% sensibility, 87% specificity) [21]. It is deemed that, with the decrease of every degree in callosal angles, a patient has a 4% greater chance of benefitting from a shunting surgery [21].

The Japanese guidelines for diagnosing NPH do not regard periventricular changes as relevant. However, they do include two new criteria of diagnosis, i.e. Sylvian fissures and enlarged basal cisterns, as well as the narrowing down of the subarachnoid space and the cerebral sulci at the median level of the brain [25]. In 2017, the efficiency of the two guides was debated, since there are significant discrepancies from the international medical practice, the former being substantially more specific, demanding the presence of at least two symptoms of the classic triad to confidently identify the diagnosis [26]. Furthermore, the study has underlined the necessity of a common system for diagnosis, more objective than the current one [26].

Clinically, several procedures could be executed to diagnose NPH. Firstly, "tap test" or CSF's evacuation test consists in removing 30-50 mL of CSF through a lumbar puncture, to improve the symptomology, the association of the gait function with the frontal assessment battery score, the improvement of verbal fluency and gait [25, 27, 28, 29]. Secondly, different weights can be given to the various markers from CSF, such as the expression of certain isoforms of transferrin, of the tyrosine phosphatase Q-type receptor, or has-miR-4274. Similarly, the correlation between slow vasogenic waves (SWV) and intracranial pressure must be investigated [30].

DIFFERENTIAL DIAGNOSIS

The differential diagnosis must be performed with Alzheimer disease (AD), frontotemporal dementia, dementia with Lewy body, the one from Parkinson disease (PD), the one associated with AIDS, dementia caused through corticobasal degeneration and any other type.

Generally, the level of proteins in the CSF is measured, considered alongside biomarkers of the AD. In this particular case, the level of p-tau and t-tau are high, whereas the level of a β-amyloid peptide (Aβ) is low [4]. In the particular case of NPH, all these parameters are high, going back to normal values after shunting [31].

Apart from the AD, the PD, as well as other parkinsonian syndromes cause gait impairment [4]. Numerous investigations can be carried out, such as SPECT (Single Photon Emission Computed Tomography), this being limited through detecting an exhaustive set of neurodegenerative diseases, or FDG-PET (Fluorodeoxyglucose – Positron Emission Tomography), which can identify caudate hypometabolism, a potential marker of NPH [4].

TREATMENT

The clinical presentation of NPH is insufficient to recommend ventriculoperitoneal shunting surgery (VPS), because either one of the symptoms of NPH may have multiple potential etiologies. The diagnosis must be established clearly through the abovementioned methods in order to recommend surgical treatment.

VPS is the only efficient method of treatment for iNPH, by implanting a ventriculoperitoneal shunt. The international and Japanese guides, as well as AAN (American Academy of Neurology), confirm VPS as the only efficient method of treating iNPH, as opposed to the rather supposedly inefficient endoscopic approach of the third ventricle [25, 32, 33].

The purpose of a shunt is to drain CSF towards space where it may be reabsorbed. A shunt has three components: a proximal catheter, inserted at the level of the right lateral ventricle, a distal catheter in the peritoneal cavity, and a valve of shunting which contains a mechanism opening the valve up when the difference in pressure between the two cavities reaches a pre-established value [3]. Once the valve is open, CSF is drained in the peritoneum. Another approach is represented by lumboperitoneal shunt (LPS), which has the proximal end in the lumbar zone. In a study from Japan, it has been stated that LPS is used in 55% of the cases, whereas VPS is used in 43% of the surgical cases of NPH [6]. Another option of VPS is placing the distal end of the shunt between the two sheets of the greater omentum, the technique is considered as having good results and a low rate of post-surgery complications [34].

Concerning the efficiency of the shunting surgical treatment, in the first three months, in most cases, the headache is gone, and the gait deficit has improved in 85% of the cases, urinary incontinence in 70%, and cognitive functions in 65% [35].

In a study on the efficiency of LPS, it has been concluded that the method is comparable with VPS for patients showing signs of NPH. Nevertheless, the revision of the shunt took place more frequently in patients with LPS than in those with VPS [36].

Regarding the prognostic, 60% of the patients have benefitted from an improvement in symptomatology after the shunting surgery, and 40% have shown signs of significant improvement. These were measured using the mRS scale [9]. Both pre-surgery and post-surgery, patients must be evaluated using the following systems: Japanese NPH scale, Berg scale, DGI (Dynamic Gait Index), FIM (Functional Independence Measure), MMSE (Mini-Mental Status Examination), and TUG (Timed Up and Go) [2].

A series of post-surgery factors can suggest a good prognostic, such as: an aqueduct flux exceeding 42 µl, the absence of lesions of the white matter on MRI, resistance to the CSF debit exceeding 18 mmHg [2]. Antagonistic factors are severe dementia, dementia as a symptom of presentation and MRI abnormalities such as cerebral atrophy or lesions of the white matter [2].

Complications that may arise include:

- Surgical complications of the shunt: the failure of the shunt (3%), under- or over-drainage, subdural hematoma (3-4%), infections (1%) [37];
- Surgical complications unrelated to the shunt: seizures and intracerebral hemorrhage (under 5%) [2];
- Schizophrenia – 3 to 4 times more likely in patients with iNPH than in the healthy population [38];
- Hypertension and diabetes type 2 are frequent in iNPH patients, the latter risk factor causing increased mortality in affected patients [7].

CONCLUSIONS

NPH is a rare, yet treatable disease, and the diagnosis may be difficult to establish due to the rather broad symptoms. Both iNPH and sNPH cause similar features.

The difference between the idiopathic and secondary hydrocephalus has led to a difference in approach in a similar pathology, the latter being labeled as a surgical emergency. Both the concept of NPH, as well as the therapeutic approach retain a status of controversy. The diagnosis of iNPH must be considered when a patient shows signs either of the classic triad, or of some elements of it, or in the case of suggestive imagistic criteria.

Following this evaluation, treatment must be sought only when the risks and benefits of the shunt are carefully balanced. Recent innovations in the field of imaging have greatly improved the identification of patients who may benefit from shunting surgery. Furthermore, it should be also keep in mind that patients suffering from iNPH also present associated comorbidities, such as PD, AD, or other neurodegenerative diseases. Hence, the diagnosis must include tests that draw a clear distinction between NPH and these potentially present diseases. For the same reason, the patient's response to the drainage of CSF can be unpredictable.

NPH must be seen as a reversible cause of dementia. In the future, ample studies must be made to demonstrate the efficiency of shunting surgery.

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VARIA

The seasonal incidence of intracranial hemorrhages

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Abstract: *Objective: Cerebrovascular Accidents (CVAs) represent the second most prominent cause of death globally, surpassed only by ischemic heart diseases, thus constituting a significant economic burden for healthcare systems. Despite this, little is known about the correlation between the different times of the year and intracerebral hemorrhages. This review investigates said link, as well as seek to identify whether vascular risk factors (i.e. diabetes and high blood pressure) vary through seasons.*

Method: This paper undertakes the study of 97 patients, all of whom have suffered a hemorrhagic CVA and were hospitalized and treated within the Central Military Emergency University Hospital's Department of Neurology in Bucharest, in the period between 29th of June, 2017 and 29th of July, 2019. The scope of this study is to optimize prophylaxis within the groups most susceptible to the imminent danger of an intracranial hemorrhage during a certain season.

Results: Our study has shown that the season with the highest values of morbidity and mortality is autumn, followed closely by spring.

Conclusion: Given the set of yielded data, which is mirrored in the case of risk factors, we conclude that the results vary slightly from those put forward in the literature. Therefore, whilst hemorrhaging CVAs have a consequential impact upon society, both through the disastrous effects of the incident per se, and its disabling nature in the long-term, a positive percentage of them could be avoided by changing one's behavior during the high-risk periods.

Keywords: *intracranial hemorrhages, subarachnoid hemorrhage, subdural hematoma, hypertension, diabetes mellitus*

INTRODUCTION

Intracranial hemorrhage (ICH) is caused either by tearing apart several small penetrating vessels or as a result of some vascular anomalies. Risk factors such as high blood pressure (HBP) or diabetes mellitus (DM) are frequently associated with ICHs [1, 2]. While in developed countries, the incidence of this pathological condition has reduced due to better control of HBP, in developing countries, it has remained alarmingly constant for the past decades [3].

According to the WHO (World Health Organization), approximately 6 million deaths were attributed globally to ICH in 2016 [4]. In the context of its economic impact, in the US alone, in 2012, the total costs of stroke patients reached USD 71.5 billion, according to the AHA/ASA (American Heart

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Association/American Stroke Association). By 2030, these costs are estimated to reach USD 240 billion [5].

Intracerebral hemorrhages account for approximately 10 to 20% of all strokes [3]. The main cause of developing ICH is the alteration of the structure of the blood vessel caliber as a result of an untreated or inadequately treated chronic high blood pressure (HBP). Other incriminating causes include amyloid angiopathy and vascular malformations. It has been observed that the frequency of ICHs in developing countries is almost double that of developed countries, progressively growing with aging. Ergo, it has been determined that for the 35-54 age group, the incidence is of 6 cases for every 100,000 people, for the 54-74 age group, it increases to 37 for every 100,000 people, and, for the 75-94 age group, it peaks at 176 for every 100,000 people.

For young patients, the main cause of hemorrhages is represented by vascular malformations. In adults, this is represented by chronic HBP, whereas in the elderly, it is amyloid angiopathy. Moreover, it has been shown that, on average, regardless of age, the incidence of ICH is higher in men than in women [6].

Concerning chronic HBP, this leads, eventually, to hypertensive angiopathy which, in turn, causes degenerative modifications within the caliber of the small and medium penetrating vessels, modifications known as lipohyalinosis. Regarding chronic hypertension, it has been ascertained that degeneration takes place at the muscular layer, together with the fibrinoid necrosis of the subendothelial layer, accompanied by micro-aneurysms.

Amyloid angiopathy is characterized by deposits of β-amyloid peptide (βA) in the capillaries, arterioles, and, generally, in all small and medium vessels in the cerebral cortex, leptomeninges, and cerebellum [6]. Secondly, amyloid angiopathy is portrayed through deposits of intravascular βA in the leptomeningeal collaterals and small intracortical vessels. Although the mechanism of depositing and accumulating βA is unknown, its consequence is the degeneration of the blood vessel caliber through, more specifically, losing smooth muscle fibers, thinning the caliber, narrowing the lumen, and leading to aneurisms, the latter further giving birth to diffuse micro-hemorrhages [7].

Furthermore, apart from HBP, other causes include the dysfunction of self-regulating with excessive cerebral blood flow, arteriovenous malformations, ruptured aneurysms, cerebral angiopathy, hemorrhagic necrosis, cerebral venous thrombosis, and modified hemostasis.

The overflow of the resulting blood from the bursting of the caliber leads to a sudden increase of intracranial pressure, which brings about diffuse vasospasm, followed by the acute ischemia of the parenchyma – a mechanism which is frequently present in the potential vasospasm after subarachnoid hemorrhage [8, 9]. The BBB permeability heightens, thus aggravating the metabolic disorders of the brain, giving birth to the cerebral edema [10]. Despite the advancements in medicine, cerebral bleedings have retained a status of high morbidity and incremental mortality, unaddressed properly by the modern therapeutic methods developed hitherto. This is partly due to the complex, irreversible pathophysiology processes which occur the moment blood makes contact with the cerebral parenchyma. Understanding this phenomenon and ICH's evolution would allow identification of the potential therapeutic targets.

METHODS

A retrospective study has been conducted which includes patients who have had non-traumatic hemorrhaging vascular accidents, hospitalized and treated in the period between June 2017 and July 2019 within the "Carol Davila" Central Military Emergency University Hospital's Department of Neurology.

The selected patients have been diagnosed with non-traumatic intracerebral hemorrhage by a neurologist, subsequently confirmed through a CT scan. The patients' age varied between 23 and 97 years, with provenance from both countryside and urban environment, with diverse professional occupations. We have investigated the three main types of non-traumatic bleedings, namely, intraparenchymal hemorrhage (IPH), subarachnoid hemorrhage (SAH), and subdural hematoma (SDH).

RESULTS

Out of the 97 patients, 67% were male and 33% female. Out of these, as many as 77% have presented non-traumatic IPH, 13% SAH, and 10% SDH. 17% of the patients have deceased during hospitalization. The average age was 67 years old, 72% of those placed in medical care being older than 60 years old (Figure 1). The average period of hospitalization was 21 days, with a minimum of 2 days and a maximum of 587.

Seasons were defined in the same way as in the rest of the studies, to have a consistency between these, for the sake of comparing them and display our research as suitable, i.e. summer: 1st of June – 31st of August, autumn: 1st of September – 30th of November, winter: 1st of December – 28/29th of February, spring: 1st of March – 31st of May.

We have identified an incremental occurrence of the cases of cerebral hemorrhages in transitional seasons, namely, autumn and spring. Similar results have been produced in most of the past studies, regardless of whether, in some situations, these have shown a larger incidence in winter (based on the geographical areas where these have been carried out) due to the physiological differences of organisms at low temperatures [11, 12, 13].

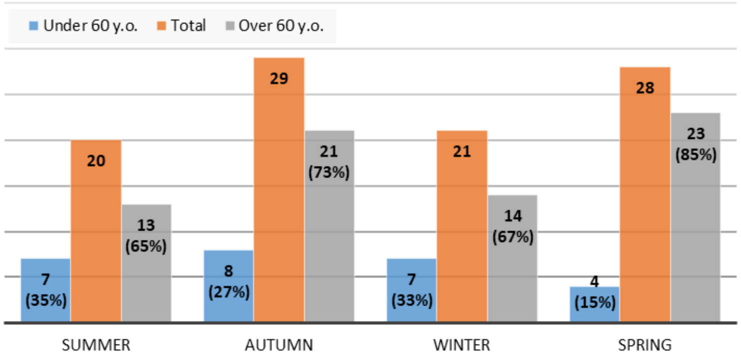

Legend: blue – the incidence of intracerebral hemorrhage (ICH) in the under 60 age group; orange – total incidence of both age groups; grey – the incidence of ICH in the over 60 age group

Figure 1: Number of cases (percentage) of ICH's seasonal incidence

Concerning the seasonal incidence of each type of hemorrhage (Figure 2), we observe an increase in IPH in autumn and spring, a constant incidence in SAH in summer, autumn, and winter, with a lower number of cases in spring, and a relatively constant number for SDH, with a maximum and a minimum number of cases in winter and autumn, respectively.

It is widely accepted that the main risk factor of ICH is represented by chronic HBP, corroborated with a hypertensive leap exceeding the limit of cerebral vascular resistance. The risk of hemorrhage in hypertensive patients is 1.5 times for BP ~ 140 mmHG and three times for BP > 180 mmHg than it is for normotensive patients [14].

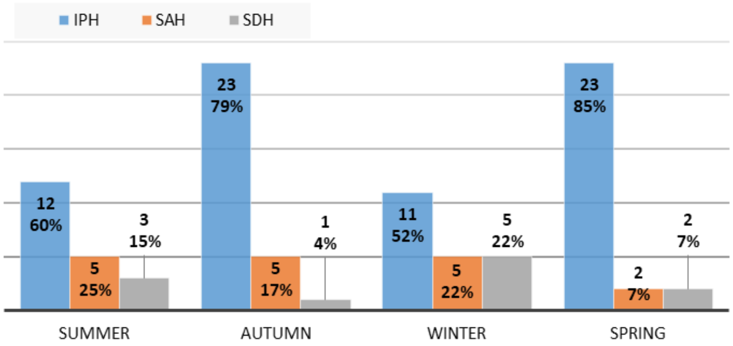

Legend: blue – frequency of intraparenchymal hemorrhage (IPH), orange – the incidence of non-traumatic subarachnoid hemorrhage (SAH); grey – the incidence of subdural hematoma (SDH)

Figure 2: Seasonal incidence of hemorrhages

Out of all diagnosed with IPH, 27% out of the 73 patients have had no associated diabetes mellitus (DM) or chronic HBP, 70% suffered from chronic HBP, 20% from diabetes, and 18% from both. In the context of SAH, 61% had no associated comorbidities, 31% had chronic HBP, 15% DM, and 7% both chronic HBP and DM. Out of those who have been hospitalized from non-traumatic SDH, 36% have not shown any of the abovementioned risk factors, 54% have shown HBP, 27% DM and 18% have shown both. In the analyzed lot, 61 patients suffered from HBP in different degrees, this number representing 62% of the hospitalized patients. The highest rate of cerebral bleedings associated with chronic HBP was registered in autumn, followed by spring (Figure 3). Besides this, we have also evaluated the association between DM and cerebral hemorrhage, as DM may determine an unpropitious prognostic in the case of ICH. Persons with diabetes have a 20% increased risk of suffering from cerebral bleeding, as opposed to a person not afflicted, as well as over a 50% chance of dying within 30 days after the hemorrhage [15]. In our study, we have identified a prevalence of diabetes of 29% among hospitalized patients.

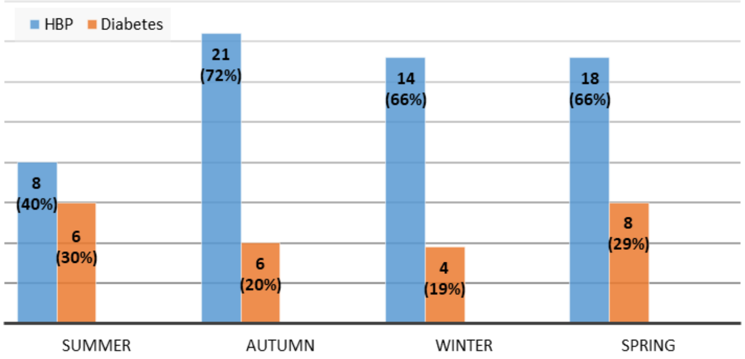

Legend: blue – the prevalence of high blood pressure (HBP) at ICH patients; orange – the prevalence of diabetes mellitus (DM) at ICH patients.

Figure 3: HBP and DM

According to this data, we observe that, indeed, diabetes does not seem to play an important role in contributing to ICH, yet there is high morbidity during summer and winter, seasons with extreme temperatures, and low morbidity during the transitional seasons, spring and autumn. Moreover, there is a higher risk of cerebral hemorrhaging during winter and summer, for those who have diabetes as an associated pathological condition. Apart from a heightened incidence of ICH in spring, we emphasize the more severe nature of these through lengthy hospitalizations periods, hence the increased economic burden on healthcare systems (Figure 4). Considering the number of days of hospitalization as an indicator of the gravity of the hemorrhage, as well as its occurrence in critical areas or through associated complications, we must submit a longer period of recovery for these patients and a lower degree of autonomy and social reintegration. Concerning deaths, we observe a relatively constant rate throughout the entire year, thus barring us from claiming any one risk factor in particular increases ICH's mortality. Apart from the increased morbidity, spring also registers the highest rates of mortality.

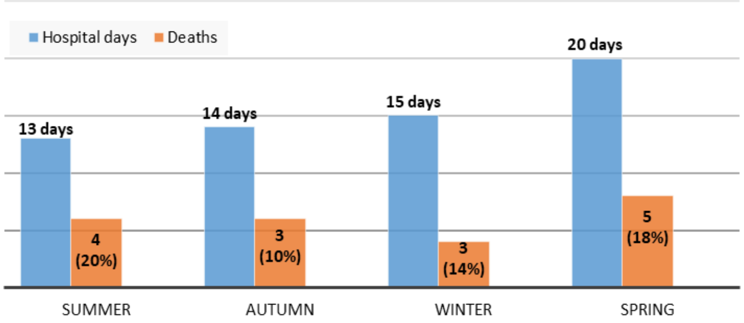

Legend: blue – the average number of hospital days; orange – number of deaths

Figure 4: Hospitalization and deaths

DISCUSSIONS

The results of this study show a variety of the incidence of hospitalization, in-hospital mortality, duration of hospitalization, and patients' prognostic throughout the year, in Bucharest, a region with a temperate continental climate. The data is new for this geographic zone, as, hitherto, there have been no recorded statistical studies (at least recent) to quantify the seasonal incidence of ICH in this area. Consequently, this research tackles on a novel theme, representing a pilot study. For the patients included in this study, the highest incidence was observed during the transitional seasons, especially during autumn, whereas the lowest was during summer. The yielded results contradict the larger part of studies available in the specialized literature, which have either failed to find a variety of incidence or claimed a heightened incidence in other seasons, particularly in winter and spring [12, 13, 16, 17]. Nonetheless, there are also similar results [11].

Regarding the seasonal distribution on age groups, the current study has registered a constant tendency of ICH debuting at patients under 60 throughout the year, apart from spring, when it has decreased, contrary to the constant incidence in the year evaluated by other studies [11, 12]. Conversely, regarding patients over 60, the seasons corresponding to high risk are spring and autumn, with two minimums registered during winter and summer, data which is in line with the results of other comparative studies [11, 12].

When the assessment also includes comorbidities, data is similar to that obtained in other research papers. Diabetes does not seem to play a decisive role in how a hemorrhage comes to be, but it may exacerbate the prognostic [15]. On the other hand, concerning HBP, this is seen as being the most important risk factor for hemorrhage and it has implications both for the prognostic and survival [18]. The mechanisms underlying these seasonal varying conditions are not clear, but it is probable for the latter to be associated with seasonal variations in biological factors, such as the level of serum lipids, some components of the blood, and a hypercoagulable state [19]. Concerning the number of deaths, we have not observed a significant variation in the year, thus being relatively constant, similar data having been obtained by other authors as well [12, 13].

The reason why the risk of ICH is modified throughout the year is not entirely known. The dysfunction of the environmentally adaptive physiological processes may be the underlying cause of hemorrhages. Variations in temperature and atmospheric humidity are considered the main catalyst for hemorrhaging events [20]. Low temperatures and low atmospheric humidity can increase evaporation, leading to an increased demand for salt, thus constituting HBP [20].

CONCLUSIONS

ICH is one of the most important neurological pathological conditions. Even though incidence is lower than it is in the case of ischemic strokes, morbidity and mortality are higher. There is still an ongoing need for thorough studies regarding the seasonal incidence of cerebral hemorrhages, as there is a subset of modifiable factors related to behavior and lifestyle which can potentially contribute to avoiding a hemorrhagic episode in either one of the seasons.

In this study, we have noticed a heightened tendency to cerebral bleeding during autumn and spring, as compared to other geographic zones or climates. Moreover, we have seasonally classified the type of ICH patients in this study were subject to, observing the predominant IPH throughout the entire year. We have also determined the average number of necessary hospital days for each season, emphasizing that spring is associated with the highest morbidity. Furthermore, in the context of this study, we have also found a significant correlation between chronic HBP and the incidence of cerebral hemorrhages, confirmed prior data that DM is a risk factor for triggering a hemorrhagic stroke, but with no real influence on mortality, as well as defined seasonal risk of ICH groups based on age and comorbidities.

In the future, it is paramount that we understand better the physiopathological process underlying cerebral hemorrhages to develop efficient methods with therapeutic potential. Although this may seem ideal, reducing seasonal incidence in risk groups through educating patients is particularly difficult to achieve. Until then, developing methods of treatment that focus on slowing down or completely barring the inflammation's progress and, implicitly, that of neurological deficits, is a short-term objective.

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REVISTA DE MEDICINĂ MILITARĂ

- *Academician Eugeniu Gh. Proca, founder of modern urology school in Romania. A memory*
- *Primary retroperitoneal tumours – a brief classification and differential diagnosis*
- *SGLT2 inhibition in patients with type 2 diabetes and cardiovascular diseases: which are the benefits*
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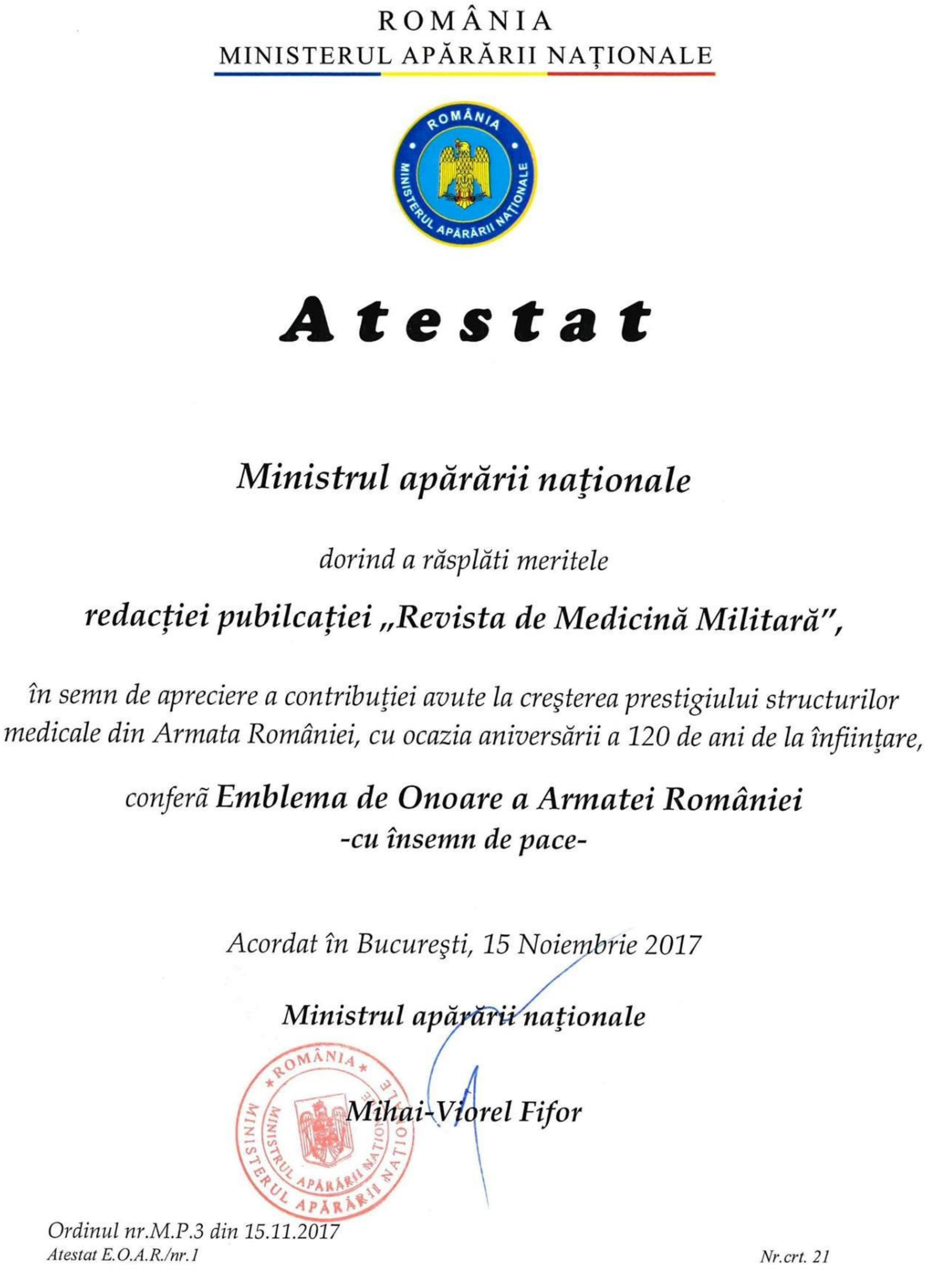

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EDITORIAL

Academician Eugeniu Gh. Proca, founder of modern urology school in Romania. A memory

Dan Mischianu

Gral (R) Prof DAN MISCHIANU

Chief of Urology Clinic, Carol Davila University Central Emergency Military Hospital Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Motto: “*You only live once – but if you work it right, once is enough*.” Joe E. Lewis

The history of Romanian Surgery will record, I am absolutely convinced, the name of Academician Eugeniu Gh. Proca (January 12, 1927 Godeni Muscel - 07.03.2004 Bucharest). As he testified in a televised interview, he wanted to become a general surgeon, he did not like urology at first, but in the end, as it happens in life, "you love who you live with." [1]

Image 1: Academician Eugen Proca at the age of great responsabilities and professional fulfilments

He didn’t regret at all the fact that he became a Romanian urologist, transforming the Romanian urology to which he added new development opportunities; therefore the title of this article is perfectly true. Moreover, after many years, I can see that his first love – general surgery –seems to disappear as a stand-alone specialty, slowly but surely…

Urological surgery is a noble, beautiful, difficult and extremely consuming profession. As one of my colleagues, a surgeon from Constanta – Professor Vasile Sârbu – wrote: "The Romanian surgery amazed through two miracles. One of them happened at the end of the nineteenth century when, in two to three decades, starting from almost nothing, it came to the level of the brightest surgical schools in the world. The second miracle took place in the twentieth century and it refers to the ability to improvise, to do great surgery in all areas, under dramatic conditions, with instrumental, material and technology shortcomings. "[2] Perhaps the chance of Romanian surgeons was the fact that in the twentieth century the three Ministers of Health, who were surgeons, proved an exceptional organizational spirit in their mandates, (Acad. N. Hortolomei 1939-1940, Prof. Dr. Voinea Marinescu 1954 -1966 and Acad. Eugen Proca 1978-1985). I do not mention the name of Prof. Constantin I. Angelescu, a surgeon and a professor at Brâncovenesc Hospital and then at Filantropia Hospital because his ministerial mandate – excelent in substance! – took place at the Ministry of Public Works 1914-1916 and at the Public

Instruction.

Image 2. At the Hospital Prof. Dr. Th. Burghele (previously called Panduri) near his colleagues: primary urologists and secondary urologists

Having all the available means of information from the present, in which we all become when we want and especially when it is not the case, curious "researchers" in the biographies and in the personal identification numbers of everyone from everywhere, I do not want to present the commonly known things regarding the life and work of an overwhelming man, such as Acad. Eugen Proca, I only want to reveal some rather special information.

The professor was born on January 12, 1927, in Godeni, from the former county Muscel. He was a "trueborn inhabitant of Muscel", the only son of Gheorghe C. Proca and Maria Proca. The people from Muscel, who lived close to the mountains, are known to be fiery and combative spirits (see "partisans from Muscel"). Brilliant graduate of "Dinicu Golescu" High School in Câmpulung Muscel, he becomes a student of the Faculty of Medicine in Bucharest in 1945 and at the same time, a student of the Medical-Military Institute. This educational ambivalence has almost always been a solution for many skilled children coming from families with modest material conditions.

Image 3. Standing beside Acad. L. Gherasim and Gl. Prof. Dr. Doc. Iuliu Şuteu

I will tell you something that I saw with my eyes and I heard with my ears a long time ago. A very long time ago...

I was a student of the Medical-Pharmaceutical Institute (currently University of Medicine and Pharmacy Carol Davila, Bucharest) and a Corporal student at the Medical-Military Institute. By the kindness of the Party Secretary of the Medical-Military Institute, as I was a student with honorable academic achievements, in the summer session 1974-1975, I was allowed to prepare the exams to come, late at night, in a room which was more protected against the ubiquitous noise in the area of the North Railway Station. It was then that I discovered the catalogues of former graduates, in the room called "IMM Museum". There were large, thick-tatted books in a dusty cabinet. This is how I browsed, with curiosity, the catalogues of promotions of future military doctors, in which the high school photo was attached to each student. The promotions of 1940 and 1942 showed me the young and confident faces of the future generals and surgeon professors – Iuliu Suteu and Traian Oancea, once emblematic people for the Central Military Hospital. Looking at the pages which turned yellow over time, I was contradicted to find out at some point (it was midnight), the name Eugene Gh. Proca, Godeni, Muscel 1927 January, who entered the Medical-Military Institute in 1945 and below, across the whole sheet of paper, it was written with a red, thick pencil, I have such a vivid recollection of the word: "EXPELLED!".

Image 4. Minister Eugen Proca beside some of his collaborators

Years passed incredibly fast for all of us and while I was a secondary physician, studying my second specialty – urology, during a short break between two surgeries, I told the Professor what I had discovered 20 years before. We were living in difficult times, quite similar to the present ones, but I saw his unblinking blue-grey eyes shining and he even told me the story which was also mentioned by Dr. M. Mihailide in the footnotes of one of his books [3]. He said he had been expelled from the institute because he had lit the fire in a stove using the newspaper published by the party – “Scanteia” newspaper. He also mentioned the way in which, on a Sunday from April 1961, being on duty at the hospital, he took care with full empathy and unaltered human kindness of his former faculty colleague, who needed dialysis. He was uremic and only dialysis prolonged his life.

His faculty colleague was the one who had informed the State authorities about the fire lit in the stove!

Academician Eugen Proca was a Christian orthodox who believed in life after death and who, I am sure, is looking at us with empathy from up there. Academician Eugen Proca is not only the creator of the modern urology school from our country. He is and will always be an emblematic figure for Romanian surgery.

For as long as I was around him, in my perception he was a true "Imperator", intelligent to extreme, self-exigent, caustic with cheaters, tenacious, with a huge culture, always eager to self-improve and aware, at all times, that a surgeon's job does not have a program.

Anyone using a computer can easily find out about his scientific and clinical achievements: performing the first hemodialysis – April 7, 1959, World Health Day – at the hospital raised by the Professor or Academician Th. Burghele, performing the first kidney transplant in Romania in February 1980 (the operation for which he had been mentally and spiritually prepared for a very long time) and about his paideic model. What almost nobody knows is that when he was the second Minister of Health as term of office (7 years), he used to work 24/7. At six o'clock in the morning he was examining patients. Then he went in the surgery room. At 11.00 a.m. – 12.00 p.m., he went to his office from the Ministry where he tried to solve many problems of that time: the birth issue (which is now of almost no interest to anyone), the introduction of medical promotion contests and many more, and in the evening, at 8.00 p.m. – 9.00 p.m., he returned at the hospital to see his patients...

Apparently, Ceausescu's wife was very proud of Minister Prof. Dr.Proca saying: "He is our only Minister who actually works."

The fact that the name of Academician Professor Dr. Eugeniu Proca, 15 years after passing into a better and fairer world, is not found in the memory of some of our institutions, is an additional proof that the Latin parable "Nemo propheta in patria sua " (Luke's Book, Chapter IV, verse 24) or in English translation: "No one is a prophet in his country" remains forever and, unfortunately, perfectly true!...

It seems that Oscar Wilde also enlightened our understanding with the following aphorism: "the truth is rarely pure and never simple…"

I dared to reveal something from my own truth about the work and life of this Man.

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REVIEW ARTICLE

Article received on September 30, 2018 and accepted for publishing on February 1, 2019.

Primary retroperitoneal tumors – A brief classification and differential diagnosis

Alexandru Cherciu[1], Lucian Iorga[1], Radu Anghel[1], Dragos Marcu[1,2], Raluca S. Costache[1,2], Ioana Bratu[1], Maria Manea[1]

Abstract: *According to literature the primary retroperitoneal malignant pathology is rare, representing less than 0.5% of all malignancies. In spite of its low incidence, this pathology often proves challenging in terms of diagnosis and treatment. The most com¬mon retroperitoneal malignancy is represented by lymphoma that accounts for approximately 33 % of the retroperitoneal malignancies. Other frequent retroperitoneal tumors are sarcomas: liposarcomas, leiomyosarcomas and malignant fibrous histiocytoma (in order of incidence). We have analyzed the existing data regarding the most common primary retroperitoneal tumors in order to realize a brief classification and their differential diagnosis considering their clinical, imagistic, histopathological and molecular characteristics.*

The technological developments that have been made over the years in terms of imaging investigations, as well as in biomelecular and cytogenetic studies have offered new possibilities for of assessing a retroperitoneal mass in order achieve more precise informations that can guide physicians to better distinguish between different types of retroperitoneal tumors and therefore their therapeutical protocol.

Keywords: *retroperitoneal tumors, diagnosis, imaging investigations*

INTRODUCTION

According to literature, approximately 70% – 80% of the primary retroperitoneal neoplasms it is estimated that are malignant, these tumors accounting for 0.1%–0.2% of all malignancies [1, 2]. These retroperitoneal masses can be divided into solid and cystic tumoral masses [3-5]. Because the treatment options vary, it is useful to be able to distinguish these masses and to diagnose them clearly in order to choose the correct therapeutical protocol. Imaging investigations and biopsy play a crucial role in establishing the tumors characteristics in terms of tumour content, size, shape, wall thickness, the presence of intratumoral septa, calcification, fat, necrosis, detecting the extension of the disease in nearby structures and also distant metastases, as well as in predicting the patients prognosis [1, 6, 7].

SOLID RETROPERITONEAL TUMORS

Solid retroperitoneal neoplasms can be split into four groups: 1. mesodermal neo-

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plasms; 2. neurogenic tumors; 3. germ cell, sex cord, and stromal tumors; 4. lymphoid and hematologic neoplasms [8].

Mesodermal Neoplasms

Retroperitoneal neoplasms are predominantly of mesodermal origin, the most frequent tumors located in the retroperitoneum being liposarcomas, leiomyosarcomas and malignant fibrous histiocytoma.

Retroperitoneal sarcomas represent approximately 0.2% of all malignancies, being more frequently discovered during the 6th decade of life. Quite often these retroperitoneal masses are found incidentally during imaging investigations, especially in early disease stages when the patient has no alarming sings, nor symptoms that could alert the patient and raise a question mark. Otherwise, a great percentage of retroperitoneal tumors are detected in advanced stages, when the tumor has important dimensions, often being associated with symptoms related to the nearby organ compression or invasion and therefore being less possible to achieve a complete and oncological surgical resection. The rate of relapse in such cases is high, often these patients will develop distant metastases, more frequently in the lungs, liver, bones and brain [1, 2].

Liposarcoma

Liposarcoma is the most common soft tissue sarcoma encountered in adult patients.This fatty cell tumor usually develops in middle-aged individuals as a mass of submucosal tissue that grows easily without pain and ulceration, yet some lesions can grow rapidly and become ulcerated. The most recent classification of sarcomas identifies five categories of liposarcomas: well differentiated, with adipocytic, sclerosing, and inflammatory subtype, dedifferentiated, myxoid, round cell, pleomorphic.

The histological type appears to be closely related to the anatomical distribution, so the well-differentiated liposarcoma appears more frequently in the soft tissues of both limbs and retroperitoneum, whereas the mixoid/round-cell liposarcomas and the pleomorphic liposarcomas have a striking predilection for limbs.

The tumor usually grows slowly over time, leading to progressive and diffuse abdominal distention and to other symptoms that appear to be secondary to the adjacent organ compression. Physical examination can prove itself to be inconclusive (especially in early stages when the tumor is small and without secondary signs nor symptoms), but in advanced stages (when the tumor has important dimensions) it may prove to be a useful tool in terms of raising the suspicion of a retroperitoneal mass.

Laboratory studies have demonstrated that cytogenetics have an important role in diagnosing lipomatous tumors based on the differences of chromosomal abnormalities between tumor types. The lipoblastoma usually exhibits modifications of bands 8q11-13 and the gene PLAG1 has been involved as the target of chromosomal changes [9].

Another important method used for evaluating these types of tumors is represented by imaging investigations. For example, computer tomography (CT) scanning has proven to be superior to magnetic resonance imaging (MRI) when we are investigating a bone erosion and demineralization, whereas MRI provides information's about the limbs characteristics, describing the fatty nature of the tumor. Liposarcomas usually have well-defined and mostly lobulated margins. The well-differentiated liposarcomas are formed of mainly fat tissue with septa or nodules. Myxoid liposarcomas are described as having a pseudocapsule with a homogeneous or heterogenous aspect. Pleomorphic tumors have a markedly heterogeneous structure. Another imagistic technique that can be used for the diagnosis process is angiography. This invesigation can demonstrate tumor malignancy on the basis of a prominent vascular component, therefore having an important value in planning the therapeutical protocol. The diagnostic procedure for liposarcoma is biopsy. For large (>3 cm) and deep tumors, the diagnosis implies open incisional biopsy accompanied by definitive resection. Fine-needle aspiration (FNA) should be followed by immunohistochemical and histological examination [10, 11].

Immunohistochemical examination is used for excluding other types of sarcoma. Lipid staining may be helpful, although oil red O stains or Sudan black are insufficient for diagnosis. Helpful stains include the following [12]:

- S-100 – Positive results in fat cells and lipoblasts;
- Alpha-1-antitrypsin – Positive results in malignant fibrous histiocytomas;
- Desmin – Positive results in leiomyosarcomas;
- Myoglobin – Positive results in rhabdomyosarcomas;
- Adipophilin stain to document lipid content in the cytoplasm of the neoplastic cells using formalin-fixed paraffin-embedded issue.

Histologically the identification of lipoblasts is decisive for the diagnosis of liposarcoma. A lipoblast is capable to produce and accumulate non–membrane-bound lipid within its cytoplasm. The most important morphologic features in diagnosing the liposarcoma are the demarcated cytoplasmic lipid that shifts the indentations in an irregular hyperchromatic nucleus and the characteristic scalloping of the nuclear membrane.

Well-differentiated liposarcomas usually contain a predominance of mature fat cells with relatively few, widely scattered lipoblasts. In the sclerosing subtype of a well-differentiated liposarcoma, collagen fibrils that encircle fat cells and lipoblasts make up a prominent part of the matrix.

Myxoid liposarcoma, the most common type, is diagnosed by the observation of a delicate plexiform capillary network that is associated with both primitive mesenchyme-like cells and a variable number of lipoblasts. In the round-cell type, lipoblasts are interspersed among sheets of poorly differentiated round cells.

Poorly differentiated pleomorphic liposarcoma is recognized by a mixture of bizarre, often multivacuolated lipoblasts and atypical stromal cells, many of which contain highly abnormal mitotic figures. Hemorrhagic and necrotic areas are common. Lipoblasts are present [12].

Leyomiosarcoma

Leiomyosarcoma derives from smooth muscle tissue, Wolffian duct remnants or blood vessels, being the second most frequently primary retroperitoneal tumor. This type of sarcoma can grow and reachenormous sizes (>10 cm), being more frequently encountered in female patients, usually in the 5th or 6th decade of life. Histopathologically, it is characterized by large areas of cystic degeneration and necrosis, but calcification is uncommon. Immunohistochemistry (IHC) experimental tests have showed that the tumor cells were significantly stained by desmin and actin antibodies, with some variation in staining intensity in different tumor cells. Relatively frail vimentin staining has been observed, especially if it is compared with the intense vimentin - specific decoration of endothelial cells and fibroblasts. Leiomyosarcoma can manifest itself predominantly as extravascular (62%), intravascular (5%) in the retroperitoneum or it can have a combination of extra- and intravascular components (33%). At CT examination, small tumors are usually homogeneously solid, although large tumors have areas of necrosis and occasional hemorrhage.

Extremely rare, leiomyosarcoma can appear as a cystic tumor. The MRI highlights these tumors to have intermediate to low signal intensity on T1-weighted images and intermediate to high signal intensity on T2-weighted images, depending on the amount of necrosis. So in conclusion, the existence of necrosis in a retroperitoneal tumor, with contiguous involvement of a vessel, is suggestive for leiomyosarcoma. Distant metastases usually occur late in the course of the disease, the most common locations being the liver, the lungs and/or lymph nodes [1, 13].

Approximately 6% of these retroperitoneal tumors arise from the inferior vena cava (IVC) [1]. These tumors have a large extravascular component that makes it difficult to distinguish them from a secondary involvement of the IVC. The most commonly affected location is the segment between the diaphragm and the renal veins. Tumors involving the superior segment of the IVC (above the hepatic veins) may manifest with Budd-Chiari syndrome, those of the middle segment (between the hepatic and renal veins) manifest with right upper quadrant pain, discomfort, nephrotic syndrome, or arterial hypertension [14-17] and the tumors of the inferior segment of the IVC (below the renal veins) can manifest with pain and lower extremity edema [13]. The differential diagnosis includes benign thrombus, angiosarcoma and a tumor extending to the IVC from adjacent organs [13, 18]. Tumors that develop from the middle segment of the IVC seem to have a better prognosis than those that arise from other IVC segments [18].

Malignant Fibrous Histiocytoma

This type of tumor is the third most common retroperitoneal sarcoma, but overall is the most frequently sarcoma in the body [19]. Malignant histiocytoma derives from primitive mesenchymal elements and it is estimated that approximately 15% of these tumors appear in the retroperitoneum [1]. In terms of gender distribution is it reported that male patients have higher risks for developing this type of tumor (75% male patients versus 25% female patients), being discovered especially in the fifth decade of life. Imaging techniques are nonspecific and usually describe a large, intrusive and heterogeneously enhancing mass with large areas of necrosis and hemorrhage, with invasion of the nearby organs, like other pathologies look alike [20]. Different patterns of calcification can be seen in the peripheral areas of these tumors. A cystic variant with peripheral calcification has been reported. The presence of calcification may help to distinguish leiomyosarcoma from malignant fibrous histiocytoma [1].

Germ cell, sex cord, and stromal cell tumors

Primary extragonadal Germ Cell Tumors

Even though germ cell tumors are present commonly in the testes or ovaries, a slight percent of germ cell tumors arise from an extragonadal location. These tumors are supposed to appear from abnormal primordial germ cell remnants that are cause of: germ cells distributed physiologically to liver, bone marrow, and brain or faulty migration of germ cells from the yolk sac or endoderm to the urogenital ridge [21]. Before an analysis of primary extragonadal germ cell tumor is made, a metastasis should be excluded. Sometimes the primary gonadal tumor could not be detectable because tumor relapse has left only a scar, with asynchronous or synchronous metastasis. This type of tumors is more frequently found in men. From the histopathological point of view, these tumors are classified in nonseminomatous germ cell tumors or seminomas, which involve teratoma, yolk sac tumor, choriocarcinoma, mixed germ cell tumors, and embryonal carcinoma. Increased levels of the beta subunit of human chorionic gonadotropin (choriocarcinoma) and of alpha-fetoprotein (yolk sac tumors, embryonal carcinoma) may be found with laboratory tests [21].

Extragonadal germ cell tumors are frequently seen near the midline, especially between the T6 and S2 vertebrae, the retroperitoneum being the second most common site after the mediastinum, a midline mass being more evocative for a primary extragonadal germ cell tumor than of metastasis. At imaging, the data collected are nonspecific for primary extragonadal germ cell tumors. The imaging characteristics are similar to those of gonadal germ cell tumors. Seminoma is occasionally located in the retroperitoneum and it can be recognized as a lobulated, large, well-defined homogeneous solid mass with fibrous septa and ring-like or speckled calcification. Non-seminomatous germ cell tumors are illustrated as heterogeneous tumors with areas of necrosis, hemorrhage and heterogeneous enhancement. The tumors in the extragonadal sites have a worse prognosis than that of their gonadal counterparts [22].

Lymphoid and Hematologic Neoplasms

The most common retroperitoneal malignancy is represented by lymphoma that accounts for approximately 33% of the retroperitoneal malignancies [1].

Lymphoma can be generally categorized into non-Hodgkin lymphoma and Hodgkin lymphoma. Hodgkin lymphoma usually occurs in patients during their second and sixth decade of life, having a bimodal age of distribution, often involving the spleen and mediastinum, manifesting itself with limited disease. Non-Hodgkin lymphoma is manifested in the forth-seventh decade of life, often associating extranodal disease in the spleen, bowel or liver. It is estimated that paraaortic lymph nodes are affected in 55% of the patients with non-Hodgkin lymphoma, as well as in 25% of the patients with Hodgkin lymphoma. A retroperitoneal mass can be discovered in approximately 15% of the subjects with non-Hodgkin lymphoma. Mesenteric lymph nodes are also frequently involved in non-Hodgkin lymphoma [1, 2].

At CT, lymphoma is identified as a circumscribed homogeneous mass, with moderate homogeneous contrast enhancement, that disseminates between normal structures without compressing them. The IVC and aorta can be anteriorly modified, producing the imagistic sign called “floating aorta”. Necrosis and calcifications are exceptional before therapy [23]. According to literature approximately 23% of the non-Hodgkin lymphomas are heterogeneous and they cannot be differentiated from other primary retroperitoneal tumors on the basis of their enhancement characteristics alone.

Ureterohydronephrosis, with secondary urinary tract infections [24, 25] and IVC obstruction may be found [1].

Image-guided needle biopsy is a first-line investigation when facing such cases, being useful in the diagnosis of lymphoma present in the retroperitoneum, especially if we consider its simplicity, as well as its noninvasiveness, often sparing the patients from radical procedures. Lymphoma comprises a heterogeneous group of lymphoproliferative malignancies, with varying morphologic features depending on the specific subtype [26, 27]. The abnormal lymphocytes in the lymph node, bone marrow, or in extranodal sites can be small, cleaved or noncleaved, intermediate or large cells and they can have a follicular or diffuse pattern. In contrast to reactive follicular hyperplasia, lymphomas usually alter the lymph nodes architecture, the capsule being usually involved [26, 28].

However, needle biopsy encounters several limitations; in few cases, core needle biopsy offers an amount of normal tissue or tissue that has lost its normal architecture, making diagnosis impossible [28, 29].

Immunophenotypic analysis aids to determine neoplastic from reactive lymphoid infiltrates, nonlymphoid from lymphoid malignancies, and specific lymphoid neoplasms. Although bcl-2 expression differentiates reactive follicular hyperplasia from follicular lymphoma, bcl-1 expression strongly supports a diagnosis of mantle cell lymphoma. CD30 expression is also important for the identification of anaplastic large cell lymphoma and it can also be found in the majority of Hodgkin lymphomas. Lymphoma is treated with chemotherapy or radiation therapy [30]. Soft-tissue masses can persist after treatment due to fibrosis and cannot be dif¬ferentiated from viable tumors [31].

Cystic Neoplastic Masses

Retroperitoneal cysts are infrequent, these tumors rarely producing symptoms, usually these symptoms being related to the cysts location and size, as well as to their complications. Differential diagnosis should be made between retroperitoneal cysts and omental, mesenteric, splenic and enteric duplication cysts.

Serous Cystadenocarcinoma

Serous cystadenocarcinoma is a rare primary epithelial retroperitoneal tumor that develops through mucinous or serous metaplasia of retroperitoneal coelomic mesothelium, which is located along ovarian descents [32]. Other approach of expansion includes a supernumerary ovary, an enterogenous cyst and a teratoma. Serous cystadenocarcinoma is discovered in women with normal ovaries. Histopathologically, the mass is multicystic and encapsulated, full with serous fluid and extruding papillary nodules. Imaging techniques are nonspecific and display a defined cystic mass with solid intramural nodules. Increase of the wall and solid components may be found. Other important diagnostic characteristics include: an elevated carcinoembryonic antigen level in the serum andglandular epithelial cells at biopsy [32]. This type of neoplasia needs surgical excision with wide margins [33]. Other rarely primary retroperitoneal epithelial neoplasms are mucinous cystadenocarcinoma, borderline mucinous tumor and borderline serous tumor [32].

CONCLUSIONS

Retroperitoneal masses can appear from different tissues and are a heterogeneous group, including some rare neoplasms.

Histopathology, immunohistochemistry, imaging techniques and other diagnostic exams are valuable in the evaluation of the retroperitoneal masses, particularly in staging as well as in assessing the nearby invasion. Although for now a specific diagnosis may be difficult to determine due to a lack of diagnostic information, the identification of certain characteristic features, along with clinical and demographic information, may help in narrowing the differential diagnosis.

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REVIEW ARTICLE

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SGLT2 inhibition in patients with type 2 diabetes and cardiovascular diseases: which are the benefits?

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Abstract: *Two major trials, EMPA-REG OUTCOME trial (with Empagliflozin) and CANVAS trial (with Canagliflozin) have demonstrated the beneficial effects of SGLT2 inhibitors in patients with type 2 diabetes at risk for heart failure. SGLT2 inhibitors possess a unique pharmacological profile and have shown a significant reduction of hospitalization for heart failure in diabetic patients, immediately after treatment commencement. It seems that their effects are beneficial both in heart failure with reduced ejection fraction and heart failure with preserved ejection fraction. SGLT2 inhibitors reduce cardiovascular endpoints in patients with diabetes and high cardiovascular risk, most likely through a reduction of heart failure-related events. Empagliflozin treatment exerts beneficial effects on vascular function and central haemodynamics. Various mechanisms seem to contribute to the beneficial effects of SGLT2 inhibitors in heart failure. Taking into consideration their cardiovascular benefits and the number of ongoing trials with these drugs, there is a possibility to expand their indication to nondiabetic patients with heart failure. This review focuses on the newest insights into the beneficial effects of SGLT2 inhibitors on the cardiovascular system.*

Keywords: *SGLT2 inhibitors, type 2 diabetes, empagliflozin, canagliflozin, heart failure*

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular disease (CVD) and, moreover, their association determines a higher mortality rate [1, 2]. On the other hand, heart failure represents an independent risk factor for developing T2DM [3], and a percentage varying from 20% to 40% of the patients with heart failure have also T2DM [4].

Pharmacological treatment of patients with T2DM is complex and has been continuously improved over the years. The treatment may be with oral antidiabetic agents (OAD) and/or insulin. The main oral antidiabetic agents are:

- Sulfonylureas.
- Alpha-glucosidase inhibitors.
- Thiazolidinediones.
- Glinides.
- Biguanides.
- Dipeptidyl peptidase-4 (DPP-4) inhibitors.
- GLP-1 agonists.

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- Sodium-glucose cotransporter (SGLT2) inhibitors.

ORAL ANTIDIABETIC TREATMENT

The first agent used as OAD in the treatment of T2DM was sulfonylurea that has hypoglycemic effects by stimulating the pancreatic β-cells to secrete insulin, primarily by closing the ATP-sensitive K channels from the β-cells plasma membrane inducing a series of events finalized with insulin release [5].

Alpha-glucosidase inhibitors are a class of OAD which were developed from bacteria. They are inhibiting the action of thealpha-amylase from the intestinal brush border. Their activity is independent of the pancreas and consists of delaying carbohydrate absorption in the gastrointestinal tract, obtaining the control of postprandial hyperglycaemia and, also, cardiovascular benefits [6].

Thiazolidinediones are a class of OAD formed by selective agonists of PPARγ from adipocytes, where they stimulate the fatty acid uptake and adipogenesis, especially in peripheral visceral fat (except the visceral fat). Lowering the concentration of circulating fatty acids and available lipids in the liver and muscle results in improving the patient's sensitivity to insulin. Also, they decrease the concentrations of hormones secreted by adipocytes, particularly adiponectin. Overall, they have mixed effects on circulating lipids and increase total body fat [7].

Glinides are OAD with a rapid effect onset, but short-acting duration [8], which stimulate the pancreatic release of insulin, thus lowering postprandial glycaemia.

Biguanides are OAD, metformin being the most important representative of this class. They are antihyperglycaemic agents, mainly through inhibition of hepatic glucose release and, also, in a lesser extent, through delaying the enteric absorption of glucose and stimulating the uptake of glucose at different levels – skeletal muscle, enterocytes and adipocytes [9].

Recently, new OAD classes, such as DPP-4 inhibitors, GLP-1 agonists or SGLT2 inhibitors, have been developed.

DPP-4 inhibitors act by preventing the inactivation of glucagon-like peptide 1 (GLP-1)[10] and glucose-dependent insulinotropic peptide (GIP) [11], resulting in an increased level of active GLP-1. The overall effect is the lowering of glucose levels through increased insulin secretion and reduced glucagon secretion [10].

GLP-1 agonists are grouped in long-acting and short-acting, after their effect duration. They stimulate insulin secretion and suppress glucagon secretion [12].

SGLT2 inhibitors act on the channels from the proximal convoluted tubules, where they stimulate glucose elimination through urine, by preventing its reabsorption. There is no risk of hypoglycaemia, because they act on blood glucose level and are independent of insulin's action, thus not affecting the pancreatic β-cells. Their limitation is represented by the impairment of the renal function [13].

SGLT2 inhibitors reduce glucose reabsorption in the early proximal tubule, leading to urinary glucose excretion and osmotic diuresis [14]. This class of medication has two main representatives-empagliflozin and canagliflozin, which demonstrated their beneficial effects in patients with T2DM at risk for heart failure in two major trials – EMPA-REG OUTCOME (with Empagliflozin) and CANVAS (with Canagliflozin). SGLT2 inhibitors have hypoglycaemic effects through increasing glycosuria and inhibiting renal glucose reabsorption [15]. They have also several additional beneficial effects, such as improving blood pressure [2, 13, 16–19], intrarenal haemodynamics [2] and albuminuria, reducing body weight [2].

For the management of cardiovascular risk in patients with T2DM, glycaemic control is very important, because it reduces the frequency of diabetic complications – especially microvascular ones [20] and has significant benefits in end-stage renal disease [21, 22]. Weight loss improves cardiovascular risk factors in T2DM on the short-term [23], but not on the long-term [24]. A 10 mm Hg reduction in systolic blood pressure reduces all-cause mortality, macrovascular and microvascular outcomes [25], but even a small blood pressure reduction can diminish cardiovascularrisk [26]. In consequence, it is very important to have a multifactorial approach in the management of T2DM [27].

SGLT2 inhibitors have several potential mechanisms that could explain their cardiovascular benefits, such as decreasing cardiac inflammation [16,28], decreasing cardiac oxidative stress [16,28], decreasing cardiac apoptosis [16,28], attenuating mitochondrial dysfunction [28], improving cardiac calcium and sodium ionic homeostasis [28] and hemodynamic effects, such as reduction in blood pressure [2,16,17] and intravascular volume [16,17] via osmotic diuresis [16] and natriuresis [17].

SGLT2 inhibitors posology is in tablets with rapid oral absorption [29], reaching peak plasma concentrationin 1-2 h [29, 30], and with a half–life of 10.2-13.1 hours [29,30], resulting in only one administration per day. The molecule metabolism is primarily through glucuronidation in the liver, followed by drug excretion through the urinary and faecal route. It is important to emphasize that SGLT2 inhibitors reduce the renal filtration rate (with up to 80 g per day), resulting in the necessity, for patients with an impaired renal function, of a more attentive follow-up and adjustment of doses [30]. The usual doses are canagliflozin – initially 100 mg, with a maximum of 300 mg per day, dapagliflozin – initially 5 mg, with a maximum of 10 mg per day, and empagliflozin – initially 10 mg, with a maximum of 25 mg per day [30].

EMPA-REG OUTCOME was a randomized, double-blind, placebo-controlled trial, which included 7028 patients with T2DM and established cardiovascular disease [1], who received empagliflozin once a day (10 mg or 25 mg dosage) versus placebo, compared to the usual standard of care. The study was multicentric, with 590 sites in 42 countries, and lasted until a minimum of 691 patients developed an adjudicated primary outcome - time to the first occurrence of any of the following adjudicated components of the primary composite endpoint (major adverse cardiovascular events [MACE] – CV death [including fatal stroke and fatal MI], non-fatal MI [excluding silent MI], non-fatal stroke). The trial lasted from September 2010 to April 2013 [1]. More than 99% of the included patients had established cardiovascular disease. The median observation time was 3.1 years, with a mean treatment duration of 2.6 years. As a cardiovascular outcome, a lower percentage of the primary outcome took place in the empagliflozin group (10.5%) than in the placebo group (12.1%) (HR for empagliflozin group, 0.86; 95.02%, CI, 0.74 to 0.99; $p<0.001$ for non inferiority and $p = 0.04$ for superiority); a significantly lower risk of death from cardiovascular causes (HR 0.62; 95% CI, 0.49 to 0.77; $p<0.001$), death from any cause (HR 0.68; 95% CI 0.57 to 0.82, $p<0.001$) and hospitalization for heart failure (HR 0.65; 95% CI 0.50 to 0.85; $p = 0.002$); the occurrence of myocardial infarction or stroke had no significant differences between groups [1]. Glycaemic control, estimated through glycated haemoglobin, was improved by empagliflozin at 12 weeks by −0.54 percentage points (95% CI, −0.58 to −0.49) in the 10 mg group, and by −0.60 percentage points (95% CI: −0.64 to −0.55) in the 25 mg group; at 94 weeks, the differences between empagliflozin group and the placebo group were by −0.42 percentage points (95% CI,−0.48 to −0.36) and by −0.47 percentage points (95% CI, −0.54 to −0.41), respectively; at 206 weeks, the differences between empagliflozin and placebo were by −0.24 percentage points (95% CI, −0.40 to −0.08) and by −0.36 percentage points (95% CI, −0.51 to −0.20), respectively [1]. Cardiovascular risk factors were slightly improved in the empagliflozin group: weight, waist circumference, uric acid level, systolic and diastolic blood pressure, but with small increases in both LDL and HDL cholesterol, with no increase in heart rate [1].

CANVAS consisted of two sister, multicentric, randomized, double-blinded, placebo-controlled trials, which included a total of 10,142 patients with T2DM and high cardiovascular risk [2]. Patients were administered canagliflozin versus placebo in 667 centres from 30 countries until a minimum of 688 cardiovascular events took place. CANVAS trial lasted from 2009 until February 2017 [2]. The inclusion criteria were: patients with T2DM, age ≥ 30 years, with a history of symptomatic atherosclerotic vascular disease, or ≥ 50 years old with 2 or more risk factors for cardiovascular disease. The primary end-point was represented by time to the first occurrence of non-fatal myocardial infarction, non-fatal stroke or cardiovascular-related death. Its results consisted of fewer primary outcome events in the canagliflozin group versus the placebo group (26.9 vs 31.5 participants, with an event per 1000 patient-years (HR 0.86; 95% CI of 0.75 to 0.97; p<0.001 for noninferiority; p = 0.02 for superiority). The renal outcome was a lesser progression of albuminuria in the canagliflozin group versus placebo group (89.4 vs. 128.7 participants, with an event per 1000 patient-years), with HR 0.73 (95% CI, 0.67 to 0.79); with a regression of albuminuria in canagliflozin group versus placebo group (293.4 vs. 187.5 participants per 1000 patient-years; HR 1.70; 95% CI, 1.51 to 1.91). An important outcome was the composite one -the 40% sustained reduction in eGFR, the need for renal replacement therapy, or death from renal causes, which was observed with a smaller frequency in the canagliflozin group versus the placebogroup (5.5 vs. 9.0 participants per 1000 patient-years HR 0.60; 95% CI, 0.47 to 0.77).

The empagliflozin patients had a mean age of 63 years, with a duration of diabetes of 10 years (57%), a mean HbA1c of 8.1%; cardiovascular risk was present in 99% of them through established cardiovascular disease, 10% of them had a history of heart failure, under treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in 81%, statins in 77% and acetylsalicylic acid in 83% of cases [1].

The canagliflozin patients had a mean age of 63 years, with a duration of diabetes >13.5 years, with a mean HbA1c 8.2%; cardiovascular risk was present, 65% had established cardiovascular disease and 35% multiple risk factors. 14.4% had a history of heart failure and were prescribed treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in 80%, statins in 75% and acetylsalicylic acid in 74% of cases [2].

SGLT2 inhibitors were evaluated through several trials. CANVAS-R, a prospective, randomized, double-blinded, placebo-controlled clinical trial, included patients with T2DM with a history or high risk of cardiovascular events [31]. The primary objective of this trial was limiting kidney disease progression and the results were fewer cases of albuminuria progression 430/2615 cases in the active group versus 581/2615 in the placebo group (HR 0.74), decreased cardiovascular mortality or hospitalized heart failure (61/2906 patients in active group versus 93/2905 patients in placebo group) (HR 0.66) and decreased cardiovascular death (40/2906 patients in the active group versus 60/2905 patients in the placebo group) (HR 0.68)[31]. CREDENCE trial was a randomized, double-blinded, placebo-controlled, parallel-group, multicentre study, with 4,402 participants, in whom the effect of canagliflozin on renal and cardiovascular primary composite endpoint was evaluated: end-stage kidney disease, doubling of serum creatinine, renal or cardiovascular death in subjects with T2DM and diabetic nephropathy [32, 33]. DECLARE-TIMI is a multi-national, randomized, double-blinded, placebo-controlled trial with 17,190 participants with T2DM that aimed to determine whether dapagliflozin versus placebo reduces cardiovascular events, such as cardiovascular death, myocardial infarction or ischaemic stroke [34]. Ertugliflozin CVOT is a randomized, double-blinded, placebo-controlled study, with the primary outcome the first occurrence of MACE after treatment with ertugliflozin in 8,000 patients with T2DM and established cardiovascular disease [35].

20% of diabetes mellitus patients over 65 years-old have heart failure [18]. There are very few OAD for patients with both heart failure and T2DM [18]. SGLT2 inhibitors, in this type of patients, are potentially OAD that can reduce the risk of cardiovascular mortality and heart failure [19]. They reduce all-cause mortality significantly when compared to placebo. Also, they reduce major adverse cardiovascular events when compared to placebo (OR 0.83; 95% CI 0.76–0.92; p<0.001), with the same efficacy for subgroups such as canagliflozin (OR 0.85; 95% CI 0.75–0.97; p=0.02) and empagliflozin (OR 0.82; 95% CI 0.71–0.94; p=0.006)[19]. Significant reductions were observed, when compared to placebo, for non-fatal myocardial infarction (OR 0.85; 95% CI 0.73–0.98; p=0.03) and for heart failure or hospitalisation due to heart failure (OR 0.67; 95% CI 0.59–0.76; p<0.001)(19). Insignificant reductions were observed, when compared with placebo, for non-fatal stroke (OR 1.02; 95% CI 0.85–1.21; p=0.87), atrial fibrillation (OR 0.61; 95% CI 0.31–1.19; p=0.15) and for the occurrence of unstable angina or unstable angina requiring hospitalisation (OR 0.95; 95% CI 0.73–1.25; p=0.73)[19].

CONCLUSIONS

SGLT2 inhibitors, such as empagliflozin, are very important, also, in treating patients with T2DM and heart failure, because they do not only reduce hyperglycaemia [2,13,18,19], but also lead to osmotic diuresis [18], reduce body weight [2,18,19] and blood pressure [2,13,18,19], without increases in heart rate, with favourable effects on markers of arterial stiffness and vascular resistance [18], albuminuria [18,19], and serum uric acid [13,18]. Another benefit of SGLT2 inhibitors is the reduction in the primary composite outcome that consists of cardiovascular death, non-fatal myocardial infarction or non-fatal stroke [18]. Treating heart failure in patients with T2DM is a real challenge, because of the lack of evidence-based guidelines for the management of such patients [18]. Important treatment goals are improving survival rates and preventing hospital admission [18].

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REVIEW ARTICLE

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Efficacy and safety of Ombitasvir/Paritaprevir/Ritonavir+ Dasabuvir and Ribavirin in patients with compensated HCV cirrhosis

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Abstract: *Background and aim: Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir in association with ribavirin was the only interferon-free regimen available in 2015 in Romania for compensated HCV cirrhosis. The aim of our study is to reveal our experience with interferon-free therapy in compensated HCV cirrhosis.*

Materials and methods: We conducted a multicenter prospective study including 90 patients with compensated (Child-Pugh A) HCV cirrhosis treated with Ombitasvir/Paritaprevir/Ritonavir + Dasabuvir and Ribavirin regimen for twelve weeks. The HCV infection was diagnosed using clinical, biological, and FibroMax tests. Each patient had follow-up visits at four, eight, twelve and twenty-four weeks after the initiation of the treatment.

Results: 44 (49% of) patients were female and 46 (51 % of) were male. The mean age of patients in the study was 61 years old. All the patients had HCV genotype 1b infection. 22 patients were naive and 68 had previous antiviral therapies based on pegylated IFN-α and ribavirin. At the initiation of treatment 64 (71%) of patients had cytolysis, 38 (42%) had thrombocytopenia, 19 (21%) patients had hyperbilirubinemia and 11 (12%) patients had anemia. It was noticed a decreasing of cytolysis, only 10% of 64 patients still having cytolysis after four weeks of treatment. One patient (1%) died after four weeks of treatment by complications of cirrhosis. 89 (99%) of patients had a virologic response (VR) at the end of treatment (EOT) and sustained virologic response (SVR) twelve weeks after the last dose administration.

Conclusion: The most common side effect among the patients in the study group was anemia (47% cases). Cytolysis disappeared after four weeks of treatment in 61% of patients. The interferon-free combination treatment was safe and highly effective in compensated HCV cirrhosis.

Keywords: *interferon-free regimen, HCV cirrhosis, anemia, Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir*

INTRODUCTION

The World Health Organizations estimates that there are 150 million people infected with hepatitis C virus [1].

Hepatitis C virus (HCV) affects individuals worldwide with a high degree of morbidity and mortality. Chronic HCV can lead to cirrhosis, hepatocellular carcinoma, the need for transplantation and death. The annual incidence of hepatocarcinoma in patients with HCV

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cirrhosis is between 1% to 7% [2, 3, 4, 5]. Genotype -1 is the most common HCV genotype from all the 7 genotypes [6]. Genotype-1b is the most prevalent HCV subtype in Europe, Central America, the Middle, and Asia [7].

Therapy with pegylated interferon and ribavirin for 12 weeks to 72 weeks was associated with low SVR (only 60% of them achieved sustained virologic response) and with adverse events [8, 9]. It can be a challenge for patients with cirrhosis to achieve SVR.

Sustained virologic response is defined as undetectable HCV-RNA at 12 weeks, after the end of treatment (EOT) and it is associated with improved survival and reduced risk of HCC development in patients with cirrhosis [10,11,12].

New IFN-free direct- acting antiviral (DAA) therapies have greatly improved SVR rates in patients with cirrhosis [13, 14, 15, 16, 17]. In most regimens for maximizing the SVR rates in patients with cirrhosis, it was required the addition of ribavirin [18, 19].

The available DAA are classified according to the viral target protein as NS3/4A protease inhibitor, NS5B polymerase inhibitors (including non-nucleoside and nucleoside inhibitors) and NS5A inhibitors [20].

Direct-acting antiviral agents were approved for the first time in 2011 for the treatment of hepatitis C. Although the addition of the NS3/4A protease inhibitors boceprevir, telaprevir or simeprevir to pegylated interferon and ribavirin regimens increased SVR rates in patients with genotype-1 infection efficacy rates have remained low in patients with cirrhosis [21, 22, 23, 24, 25].

The regimen containing ombitasvir/paritaprevir/ritonavir and dasabuvir with or without ribavirin was approved by the European Medicine Agency and/or the Federal Drug Administration for the treatment of patients without and with cirrhosis Child-Pugh-Turcotte class A.

Ombitasvir (OBV) is an NS5A inhibitor co-formulated with NS3/4A protease inhibitor paritaprevir (PTV), and the pharmacokinetic enhancer ritonavir which increases peak through drug allowing for once daily PTV dosing exposure [26,27]. Dasabuvir is a non-nucleoside NS5B polymerase inhibitor. This 3-DAA regimen with or without ribavirin (RBV) is approved in many countries to treat HCV genotype-1 infection.

Most of prospective phase II and III trials included only a few patients with cirrhosis.

The regimen Ombitasvir/paritaprevir/ritonavir and dasabuvir were evaluated in two studies in patients with compensated HCV cirrhosis. One study-TURQUOISE II trial, an open-label phase III trial evaluated the 3D regimen with ribavirin for 12 weeks vs 24 weeks in patients with compensated HCV cirrhosis. The other study – TURQUOISE III phase III b, open-label study evidenced if it could be achieved a good SVR without ribavirin at 3D regimen in 60 patients with compensated HCV cirrhosis. Ombitasvir/paritaprevir/ritonavir + dasabuvir with ribavirin became available in Romania for compensated HCV cirrhosis from November 2015 to November 2017 as the only interferon-free regimen.

We reported the results of a prospective study designed to asses the efficacy and safety of the regimen ombitasvir/paritaprevir/ritonavir+dasabuvir with ribavirin in patients with compensated HCV cirrhosis genotype-1b, with or without experienced pegIFN/RBV.[28, 29, 30].

PATIENTS AND METHODS

Study Patients

We conducted a multicenter prospective study including 90 patients with compensated (Child-Pugh A) HCV cirrhosis treated with Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir and Ribavirin regimen for 12 weeks. Patients were diagnosed by clinical, biological, and FibroMax tests.

Patients 18 years or older were eligible for the therapy if they had HCV genotype-1b infection for at least 6 months prior to screening, an HCV RNA > 15 UI/ml. Cirrhosis had to be documented by liver biopsy (Metavir score >3) or FibroMax (available within 3 months before therapy) and a Child-Pugh Turcotte score 5 or 6.

We included in the treatment-naive patients or those with prior exposure to PegIFN/RBV based therapy.

Decompensated cirrhosis and hepatocarcinoma were exclusion criteria.

Each patient had follow-up visits at four, eight, twelve and 24 weeks after treatment initiation. Biological tests included: hemoglobin, ALT, AST, platelets, total bilirubin, serum albumin, INR. We defined anemia as Hb<12 g/dl in women and Hb< 13 g/dl in men. Cytolysis was defined as values of ALT or AST > at least 2 times upper limit of normal. Hyperbilirubinemia was defined as a level of bilirubin > than 1.2 mg/dl. HCV RNA level in blood was evaluated at baseline, end of treatment (EOT) and 12 weeks after EOT. Undetectable HCV RNA was defined as a viral load <15 UI/ml, assessment by PCR. Virological response (VR) was defined as undetectable HCV RNA at EOT and sustained virologic response (SRV) as undetectable HCV RNA at 12 weeks after EOT.

Study design

All patients received the therapy for 12 weeks. Co-formulated OBV/PTV/r was administered once daily (25/150/100 mg) and DSV 250 mg twice daily. Patients were followed up at four, eight, twelve, and twenty-four weeks after treatment initiation.

Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Efficacy, safety and pharmacokinetic assessments

Plasma samples were collected at screening and at each study visit and HCV RNA levels were determined processed by real-time polymerase chain reaction using the COBAS TaqMan HCV version 2.0 (Roche Molecular Diagnostics). Efficacy was assessed by the percentage of patients with SVR12, defined as an HCV RNA below the lower limit of quantitation of 25 UI/ml and a lower limit of detection of 15 UI/ml after the last dose of study drug.

The primary efficacy endpoint was SVR12 (HCV RNA level <25 UI/ml 12 weeks after the last dose of study drug. The rates of SVR 4 weeks after the end of treatment (SVR4) and rapid virologic response (RVR; HCV RNA level <25 UI/ml) were also determined.

Treatment adverse events were collected from the start of study drug administration until 30 days after the last dose. Laboratory tests and clinical parameters were assessed during the study. The severity of the adverse events and their relationship with treatment were evaluated by the investigator.

Statistical Analysis

Number and percentage of patients in different categories are reported. Categorical variables included in the analysis were: sex, the previous treatment regimen. Continuous variables included were: age, cytolysis, thrombocytopenia, anemia, hyperbilirubinemia.

Results

Baseline Patient Demographics and Characteristics.

We screened patients from November 2015 to August 2016 at five sites in Bucharest. 90 patients were enrolled and received the study drug. The majority of patients had prior treatment experience with PegIFN/RBV. 22 patients were treatment-naive and 68 patients had experienced Peg/IFN/RBV.

Demographics and clinical characteristics are presented in Table 1 and Table 2.

Table 1. Baseline demographic characteristics

Parameter	**Patients** (n=90) n/%
Age (years)	65
Female gender	44 (49)
Male gender	46 (51)
Treatment naïve	22 (24)
Treatment experienced	58 (76)

Table 2. Baseline clinical characteristics

Parameter	**Patients** (n=90) n/%
Cytolysis	64 (71)
Anemia	11 (12)
Hyperbilirubinemia	19 (21)
Thrombocytopenia	38 (42)

Efficacy Outcomes

89-99% of patients completed 12 weeks of treatment with ombitasvir/paritaprevir/ritonavir, dasabuvir and ribavirin. After 12 weeks of treatment, 99% of patients achieved SVR 12 (89/90, 95% CI, 80%-99.9%) (Table 3). One patient died after four weeks of treatment.

Another patient (female, 60 years old, known with diabetes mellitus) gave up ribavirin after four weeks of treatment because of severe anemia (level of hemoglobin was 6 g/dl). We hadn't any virologic failure.

Table 3. Efficacy and safety of DAA regimen

Parameter	**Patients** (n=90) n/%
SVR	89 (99)
Anemia	42 (47)
Death	1 (1)

Improvement in laboratory assessments

At baseline 71 % (64) of patients had cytolysis and 42 % (38) had thrombocytopenia (Table 4).

Only 10% (9) of patients had cytolysis at the end of treatment. Thrombocytopenia was improved at the end of treatment, thus it was seen at only 26% of patients.

Table 4. Characteristics of patients at the end of treatment

Parameter	**Patients** (n=90) n/%
Cytolysis	9 (10)
Anemia	2 (47)
Hyperbilirubinemia	6 (40)
Thrombocytopenia	3 (26)

Safety outcomes

Most of the patients had adverse events and one patient died after four weeks of treatment. Anemia was the most frequent adverse event. At baseline only 12% of patients had anemia, but at the end of treatment the number of patients who developed anemia was increasing up to 47%. Another laboratory abnormality was hyperbilirubinemia. At the beginning of the treatment, only 21% of patients presented a high level of bilirubin. At the end of treatment, hyperbilirubinemia was seen in 40% of patients. One patient developed severe anemia (hemoglobin level was 6 g/dl) after four weeks of treatment and thus ribavirin was stopped.

DISCUSSION

Patients with HCV infection and cirrhosis are at the highest risk for hepatocarcinoma, decompensation and, mortality. In this study treatment with OBV/PTV/r, DSV and RBV had the SVR 12 rate of 99% in HCV genotype-1b infected patients with compensated cirrhosis. Treatment was well tolerated with a low rate of severe AE.

The most side effect among the patients in the study group was anemia in 47% cases. Cytolysis disappeared after four weeks of treatment at 61 % of patients. 89 of 99 patients (99%) had sustained virologic response at twelve weeks. One patient died after four weeks of treatment (1%). The treatment was safe and effective in compensated HCV cirrhosis.

In conclusion the 12 week 3-DAA regimen of OBV/PTV/r, DSV and RBV achieved the SVR 12 rate of 99% experienced and naive treatment patients with compensated cirrhosis HCV genotype-1b.

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ORIGINAL ARTICLE

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Renal involvement in primary retroperitoneal tumors

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Abstract: *Retroperitoneal tumors grow quietly and are generally large in size, more than half of them being larger than 20 cm at the time of diagnosis. They usually present several therapeutic challenges because of their rarity, relative late presentation and anatomical location, often in close relationship with several important structures in the retroperitoneal space. Due to intimate relationships with vital organs in retroperitoneum, extensive surgery with en bloc resection of the involved organ is often required, most frequently the kidneys, followed by colon, pancreas and spleen. Malignant tumors have a poor prognosis, the most significant factors regarding survival rates being delayed diagnosis, high histological grade, inoperability due to invasion into vital organs, and a positive surgical border.*

Keywords: *primary retroperitoneal tumors, renal involvement, therapeutic management*

INTRODUCTION

The retroperitoneum is one of the largest spaces in the body, located behind the abdominal cavity, between the posterior leaf of the parietal peritoneum and the lumbar muscles fascia. It is delimited superiorly by the diaphragm and extending inferiorly to the pelvic floor, reaching the external borders of the lumbar muscles laterally [1]. It contains connective tissue, kidneys, adrenals, ureter, aorta with its branches, inferior vena cava with its tributaries, a portion of the duoden and pancreas, a part of the descending and ascending colon and lymph nodes. At the kidneys level, the retroperitoneum is divided into three spaces by the anterior and posterior renal fascia, as follows: anterior pararenal space, perirenal space and posterior pararenal space [2]. Primary retroperitoneal tumors represent a rare, but interesting group of neoplasms. Even though they are located in the same space, tumors of the kidneys, adrenals and pancreas, tumors originating from the organs situated partially in the retroperitoneum and retroperitoneum metastasis do not fall into this category. Primary retroperitoneal tumors do not originate from any retroperitoneal organ (parenchymatous or not), but develop from the actual retroperitoneal tissue (lymphatic, nervous, vascular, support muscle, connective, or fibroareolar tissue) or from embryonic rests of the urogenital ridge (wolffian or müllerian ducts, germ cells, primitive notochord) [3-6].

The retroperitoneum can host a wide spectrum of pathologies. Primary retroperitoneal tumors are a rare entity, having a reported incidence of 0.3-3.0%. More than half of the retroperitoneal masses are malignant (70-85%), leaving a percentage of 15-25% of the tumors to be benign. The majority of the tumors are of mesenchymal origin (75%), followed by the neural origin (24%), and last from embryonic

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rests (less than 1%) [7].

The retroperitoneum represents the second most common site of origin of malignant mesenchymal tumours after the lower extremities [7, 8]. Liposarcomas (70%) and leiomyosarcomas (15%) are the two most frequent histological subtypes of sarcomas. These tumors present as hard abdominal masses with an irregular surface; they are surrounded by a capsule that is rapidly outgrown by tumor growth and infiltrate the posterior parietal peritoneum and the intra-abdominal viscera attached to it, thus becoming intraperitoneal by direct, and not metastatic, invasion.

Benign tumors are often an incidental finding during an investigation for unrelated symptoms. The most common primary benign pathologies encountered in the retroperitoneum include benign neurogenic tumours (schwannomas, neurofibromas), paragongliomas (functional or non-functional), fibromatosis and retroperitoneal lipomas [9, 10].

Due to the inaccessibility of the region and since these tumours often give none or non-specific symptoms until they have reached a substantial size, they are usually large at presentation [11]. They grow silent and aggressively and usually invade the surrounding structures. In primary retroperitoneal tumors lymphadenopathy and distant metastases are a rare entity. More than half of the retroperitoneal tumors are larger than 20 cm in diameter at the time of diagnosis, the retroperitoneum space being “adaptable”.

The urologist has a crucial role in diagnosing and treating these tumors, because they are usually found in the anatomical region commonly used for surgical access in many major urological surgeries. However, in certain cases, a multidisciplinary approach should not be avoided.

Retroperitoneal tumors occurs frequently in the 5th and 6th decade, being found more often in female than in male patients [12, 13]. Certain histological types, such as embryonal rhabdomyosarcoma, teratoma and neuroblastoma, are commonly found in younger patients. Patients with primary retroperitoneal masses usually present with abdominal swelling and pain, early satiety, abdominal discomfort, most having a palpable abdominal mass. Many benign tumors are discovered as an incidental finding during imaging for unrelated symptoms [14, 15]. Pain can vary in intensity and severity and can also be located in the lumbar, inguinal or gluteal region. General symptoms such as asthenia, anorexia, weight loss or prolonged fever may be present. A study found out that out of the total number of patients, 44% had on admission gastro-intestinal symptoms such as: vomiting, abdo-minal distention, bloating, constipation, dyspepsia, gastrointestinal bleeding or intestinal blockage. Neurological symptoms (lower limb weakness, sensory loss of the obturator, femoral cutaneous or sciatic nerve, radiculitis, sciatica and sphincter dysfunctions) were also found in 3 out of the total 27 patients, and 10% showed signs of venous compression (edema, varicocele, ascites and genital swelling).

Retroperitoneal masses are usually diagnosed by clinical examination associated with blood tests and imaging studies (CT, MRI, abdominal ultrasonography). The imaging test of choice in the evaluation and staging of primary retroperitoneal masses is contrast-enhanced computed tomography of the thorax, abdominal and pelvic regions. It can distinguish between different densities which suggest the nature of the mass, defines the shape and size of the tumor, can discover enlarged lymph nodes and can assess the involvement of the neighboring organs [16, 17]. It can also be used to perform CT-guided biopsies. It can detect the presence or absence of pulmonary, bone, hepatic or peritoneal metastases and local or regional recurrence in patients who have already been treated [18].

It is important to confirm that the tumor is located within the retroperitoneal space and to exclude the possibility that the tumor originates from a retroperitoneal organ. Anterior displacement of the retroperitoneal organs is a strong indicator that the mass has a retroperitoneal origin. The migration of the large vessels can be helpful as well [19, 20].

Ultrasound is a fast, easy to use, repeatable method. It can reveal whether the mass is cystic or not, it can determine its volume, topography and can assess the condition of the inferior vena cava. The main disadvantage is that it is a subjective method, dependent on the doctor's experience [19].

If after the results of the imaging studies the diagnosis remains uncertain, an exploratory laparotomy or laparoscopy remains a last diagnostic resource.

THERAPEUTIC MANAGEMENT

Complete surgical resection is the treatment of choice for primary retroperitoneal tumors, but local recurrence occurs in the majority of the patients, being responsible for more than 75% of sarcoma-related deaths. Complete surgical ablation is not always possible, because of its invasion in vital structures. Despite the possibility of performing large organ resections, the possibility of a residual tumor is very high.

Macroscopic excision, tumour grade, multifocality and histological subtype are the main prognostic factors that dictate local recurrence and overall survival rates [21-23]. Local recurrence rates are higher and occurs at an earlier interval in high-grade tumors compared with low-grade ones. The possibility of obtaining negative margins is remarkably lower at the time of local recurrence and each successive operation is more difficult than the last. In the presence of a positive surgical border following the resection of a malignant retroperitoneal tumor, the 5-year survival rate decreases to 28%.

The most significant factors regarding survival rates are delayed diagnosis, high histological grade, inoperability due to invasion into vital organs, and a positive surgical border. For patients with high-grade tumors, the mean life expectancy is approximately 20 months, increasing to 80 months in patients with low-grade retroperitoneal masses.

A study found that tumors larger than 10 cm generally had distant metastasis at the time of diagnosis [24]. The risk of local recurrence is more important than the risk of metastasis regarding the progression of malignant primitive retroperitoneal tumors [25].

Due to the fact that a radical surgery is needed and that the tumor is usually large in size, an access route that allows a wide exposure for procedures is required. Midline laparotomy is the acces route of choice, allowing simultaneous examination of the abdominal and pelvic viscera, enabling management of the large vascular trunks, additional visceral surgery and the incision can be extended for access in the thoracic cavity. Chevron incision is usually used to remove large tumors with the invasion of inferior vena cava and lombotomy should be used to remove small retroperitoneal masses [26].

Because of the high risk of local recurrence, a strict follow-up with CT or MRI testing is necessary every six months for 2 or 3 years [27, 28]. Since primary retropritoneal tumors have such high rates of local recurrence, adjuvant therapy is recommended.

The use of high-dose therapy, in an attempt to lower the rate of local recurrence, is limited because these tumors are often adjacent to radiosensitive structures with low radiation tolerance (such as kidneys and intestines). The doze usually used is about 60 Gy, studies found that therate of local recurrence is 67% with doses below 50 Gy but only 17% at doses equal to or higher than 50 Gy [27]. Lymphomas, rhabdomyosarcomas, neuroblastomas and undifferentiated sarcomas seem to have good results with radiotherapy.

The use of chemotherapy in retroperitoneal tumors is controversial, having a similar response index with other therapeutic regimen and equally poor in single drug therapy or in combination [18]. The most commonly used drug, both in single drug therapy or in combination (adriamycin – dacarbazine, or cyclophosphamide – vincristine – adriamycin – dacarbazine), is Adriamycin. This drug is also used for metastatic disease, as a palliative form of treatment [28, 29].

If a tumor is considered unresectable or the patient has distant metastases, a core needle biopsy may be indicated to confirm the diagnosis and to enable consideration of alternative therapy.

Palliative surgery (incomplete resection for unresectable tumor) should be considered for symptom control and may improve quality of life [28].

DISCUSSION

Due to the fact that kidneys and ureteres are retroperitoneal organs, they are usually involved by retroperitoneal masses, either by extrinsec compression or direct invasion. If the tumor is left unchecked, it will grow to cause secondary hidronephrosis and renal parenchymal destruction that will lead to chronic kidney disease with severe hydroelectrolytic imbalance and serious consequences. Resistant hypertension [30] with dyselectrolytemia [31, 32], secondary heart failure [33] and cardiac arrhythmias [34] may be the result of compromised renal arteries by extrinsec compression caused by the retroperitoneal masses [9].

Urological manifestations, caused by possible urinary tract involvement (displacement, compression and invasion) at any level (kidneys, ureter, and bladder) were not as frequent, only 25% of the patients presented chronic lower back pain, or acute renal and ureteral colic. Hematuria, polakiuria, dysuria or urinary retention usually occur in tumors located in the pelvic region, near the bladder [13]. Hematuria may also appear if the retroperitoneal mass invades the ureters or the pyelocaliceal system [35].

In terms of treatment, the complete surgical resection of the tumor en bloc with the involved organs is the gold standard. It can vary from segmental ureterectomy, followed by a surgical repair of the defect, to radical nephrectomy. In cases with extrinsec compression of the ureter and secondary hidronephrosis, with no signs of invasion, an internal (ureteral stent) or external (percutaneous nephrostomy) renal drainage method can be used, until the tumor can be surgically removed. More than 83% of the patients require resection of neighboring organs, most frequently the kidneys, followed by colon, pancreas and spleen, for achieving a negative margin [27].

If the ureteral defect after extensive surgical removal of the retroperitoneal mass is proximal to the ureterovesical junction and it is not large in size, a ureteroneocystostomy or a primary anastomosis of the ureter (ureteroureterostomy) is usually preferred. When ureteroureterostomy or ureteroneocystostomy cannot be performed, the psoas hitch ureteral reimplantation is the best approach. For defects in the middle third of the ureter after partial ureterectomy, the optimal approach is ureteroureterostomy, but if it cannot provide a tension free anastomosis, a Boari flap is an optimal, but complex approach. The optimal procedure for repair of an upper ureteral injury is a ureteroureterostomy, if it can be performed without tension. A nephropexy with fixation to the psoas tendon can provide a tension-free anastomosis. When a tension-free anastomosis cannot be achieved, even after kidney mobilization, autotransplantation, ileal or appendiceal interposition graft, nephrostomy or a cutaneous ureterostomy can be performed. The ureteral anastomosis following any type of primary repair is typically stented.

In advanced cases, with inoperable tumor and secondary ureterohydronephrosis, an urgent drainage of the obstructed kidney should be used [36]. This can be achieved either by internal drainage using a double J ureteral stent [17] or by using a percutaneous nephrostomy. A nephrostomy deteriorates the patient's quality of life and leads to frequent infections, blockage of tubes or bleeding. Ureteral stenting, on the other hand, can be associated with irritative bladder symptoms, blockage, sepsis and a high failure rate, which ultimately leads to a nephrostomy tube [28].

Wrona AJ et al wanted to offer a better solution than ureteral stenting and nephrostomy for kidney drainage. In their study, they presented a subcutaneous pyelovesical bypass as a solution for ureteric obstruction (detour bypass). A detour bypass is made of two coaxial tubes, a 27F porous polytetrafluoro-ethylene (PTFE) outer tubeand an inner 17F silicone tube extending beyond the PTFE, with perforations on both ends and a radio-opaque ring on the proximal end (for an accurate placement of the proximal end of the stent). The detour is placed subcutaneously, using a tunneling device, and the distal end of the tube is placed into the dome of the bladder by a small incison. They concluded that this method is a safe, minimally invasive and highly effective alternative in patients with ureteral obstruction caused by both malignant and benign diseases and that the potential complications (infection, encrustation) may be easily and efficiently managed [37].

Andrea Montenegro et al presented a case of a 65 year-old female with a large retroperitoneal liposarcoma, in whom, due to the fact that it was very adherent to the left kidney, a complete resection of the tumor with concomitant left nephrectomy was practiced.

In a case report by Mariko Tanaka et al, they introduced the case of a 60 year old female who presented with abdominal distension. The CT revealed a large retroperitoneal tumor which proved to be a dedifferentiated liposarcoma at biopsy. Due to the fact that the tumor invaded the duodenum, head of the pancreas, right kidney, inferior vena cava and the aorta below the left renal artery, an extensive surgical removal of the tumor was performed, with en bloc resection of the right kidney, duodenum, head of the pancreas, a portion of the inferior vena cava and abdominal aorta, with concomitant vascular reconstruction [38].

In their study, Rodriguez et al found out that sarcomas, mainly liposarcomas, followed closely by leiomyosarcomas, were the most common type of primary retroperitoneal tumors. More than half of the patients included in the study presented at admission pain of varying severity and location (mainly abdominal and lumbar) and general symptoms such as asthenia, anorexia, weight loss or prolonged fever. Gastrointestinal manifestations (vomiting, abdominal distention, bloating, constipation, dyspepsia or intestinal blockage), due to compression/invasion of the gastrointestinal tract, were present in 44% of cases. Renal involvement was not as frequent, only one quarter of patients had urological symptoms, such as lower back pain, but also dysuria, polakiuria and urge [13].

Pereira et al reported the case of a 48-year-old male who presented for pain and right abdominal swelling. Clinical investigation revealed a right varicocele and imaging studies found a solitary hydronephrotic right kidney (personal history of left renal agenesis). Contrast enhanced computed tomography showed a retroperitoneal mass and a right hydronephrotic kidney. The particularity of this case was the presence of a solitary right kidney with left renal agenesis, with the possibility that the patient will become anephric after extensive surgery. Ultrasonography-guided biopsy was performed that confirmed a well-differentiated liposarcoma. Surgical excision of the retroperitoneal sarcoma was performed, sparring the right kidney, but the patient unfortunately died during chemotherapy [39-42].

Another case reported by the previous team presented with hypertension, anorexia, and weight loss (10 kg in 2 months). This patient had a solitary right kidney following a left radical nephrectomy subsequent to renal cell carcinoma. A right retroperitoneal mass was found during the study of his hypertension, which had a compressing effect on the right renal artery. Surgical excision of the mass was performed, but the patient died of local recurrence.

MRI of an 84-year-old male, who presented with complaints of pain and a palpable right lumbar mass, revealed a large retroperitoneal tumor invading the right kidney. Extensive surgical excision was made, with concomitant right nephrectomy.

Kazim et al stated that the location of intra-abdominal organs can shift in the presence of retroperitoneal tumors, due to the mass effect, with the kidneys being the most common viscera with an altered intra-abdominal location. In their series, they reported one retroperitoneal tumor, measuring 30 cm in diameter that pushed the left kidney to the right anterolateral direction. Since the left kidney was completely engulfed by the mass, the patient underwent extensive surgical excision of the mass and a left nephrectomy [43].

CONCLUSIONS

The retroperitoneum can host a wide variety of pathologies, including benign and malignant tumors. Primary retroperitoneal tumors are rare, almost all of them being malignant. Retroperitoneal masses grow silently, presenting late, usually invading neighboring structures at time of diagnosis. Contrast-enhanced computed tomography is the best way to evaluate this kind of tumors. Complete surgical resection is the only potential curative treatment modality for retroperitoneal masses, but the characteristics of retroperitoneal tumors (size, proximity to abdominal organs and structures, proximity to large retroperitoneal vessels) make surgical treatment a real challenge for surgeons. The efficiency of pre-operative and post-operative radiotherapy and chemotherapy is still a controversial issue, complete surgical excision with negative margins remains the gold standard procedure.

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Digital techniques for anterior teeth form selection in complete edentulism

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Abstract: *The aim of this study was to compare two computerized digital esthetic design methods (DSD and Visagismile) in order to properly choose the appropriate form of the maxillary frontal teeth for complete bimaxillary edentulous patients and to validate the use of these applications in complete edentulism. An online questionnaire was submitted to a group of 100 persons (dental doctors, dental medicine students and persons activating in other fields); the respective persons were requested to choose the form of maxillary frontal teeth for a group of four complete bimaxillary edentulous patients, whose esthetic design had been performed by using the two computerized progams. Following the respondents' analysis of the esthetic previews elaborated for the four patients 60% of the total answers were in favour of DSD while the remaining 40% were in favour of Visagismile; these percentages are identical with the ones obtained on the basis of the answers given by dental doctors and dental medicine students. In case of the respondents activating in other fields the percentages were 66% in favour of DSD and 34% in favour of Visagismile. Based on the results of the study the two computerized methods were validated also for cases of complete edentulism but further studies to confirm this validation are still required. These two methods improve the dental doctor/patient communication and certainly provide a higher predictability of the esthetic results of prosthetic restorations not only for dentate patients but also in cases of complete edentulism.*

Keywords: *esthetic preview, complete edentulism, DSD, Visagismile*

INTRODUCTION

Choosing the most appropriate form for maxillary frontal teeth has always represented a challenge in the prosthetic restorations of complete edentulism as doctors had available a limited number of parameters (facial profile, sex, age, personality), which were often subjective methods of analysis and, as a consequence, the esthetic results had a low degree of predictability. [1].

Modern dental medicine uses digital technologies also in the field of dental esthetics, thus ensuring that the esthetic results of the prosthetic restorations meet as many requirements as possible having, at the same time, a high degree of predictability, given the possibility to preview the results.

A considerable number of such digital applications for smile simulation have been developed, among which: DSD App, VisagiSmile, Photoshop CS6, Keynote, Planmeca Romexis Smile Design, Cerec SW 4.2, Aesthetic Digital Smile Design, Smile Designer Pro [2].

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Smile planning applications adjust the digital technology to the smile design process. They can be used as multi-use conceptual tools, diagnosis and treatment plan preview instruments and can facilitate the communication with the patient and the dental technician, enhancing the predictability of the final results and allowing a thorough analysis of the facial and dental features otherwise superficially evaluated through clinical diagnosis, photographic assessment or by using models [3]. Among the esthetic software programs used at present in dentistry Visagismile and DSD are considered to be the most powerful tools in assessing and modifying the design of deficient smiles. [4].

Apart from the well-known DSD (Digital Smile Design) concept a new one, Visagismile, has been developed in the last years starting from the Hippocratic four types of temperament (sanguine, choleric, phlegmatic and melancholy) and the Jungian archetypes and symbols (basic geometric shapes and primary and secondary colours) [5]. The artist Philipp Halawell explained and developed the Visagism concept, which implies the creation of an image expressing a person's (patient's) type of personality, concept that has been integrated in the dental medicine, thus obtaining a teeth form that ensures a correlation between dental-facial harmony and the personality type [3].

The Visagism concept analyzes the relation between the facial features, temperament and patient's harmonious teeth configuration. The concept has been developed in the last years and validated for a large number of clinical cases (over 800). The software program is user-friendly, the doctor uploading one standard photography of the patient on the application, where it will be calibrated and the points composing the facial map of the patient will be selected. Based on the facial map and one brief psychological questionnaire (named "personality interview") filled in by the patient the application will establish the patient's temperament and appropriate teeth design. Afterwards the doctor can edit the dental configuration and superpose it on the patient's teeth in the photography so that the patient can preview the esthetic aspect to be obtained at the end of the treatment. All details related to the future teeth will be sent to the laboratory directly from the application in order to obtain the final prosthetic restoration [6].

We identified a large number of studies regarding the application of the esthetic software programs on dentate patients while the number of studies regarding the application of these programs on edentulous patients is quite limited. Starting from this finding we applied the two digital methods for choosing the form of the maxillary frontal teeth to complete edentulous patients.

The purpose of our work is to establish which of the two computerized methods used for choosing the form of the maxillary frontal teeth is more appropriate in case of complet edentulism.

MATERIALS AND METHODS

We performed a comparative study on a group of four patients of the Complete Denture Department of the Faculty of Dental Medicine within the University of Medicine and Pharmacy, "Carol Davila", Bucharest, Romania.

We used the following criteria for including the patients in the studied group: complete bimaxillary edentulous patients (both with and without dentures) and patients who gave their consent for participating in the studied group and for being photographed. The criteria for excluding the patients from the studied group were: dentate patients and non-cooperating patients.

For each patient a full set of photographs was taken, according to the protocols of the two software programs and all the data and photographs thus obtained were loaded and processed using Visagismile, DSD Keynote and Microsoft Excel programs. Previews of the form of the future artificial frontal teeth were obtained for each patient using both software programs; in achiving these previews, different steps were followed, as described in Table 1.

The ideal digital form of the future artificial maxillary frontal teeth was achieved for each of the four patients of the studied group by using the two computerized programs.

Table 1. Comparison between the steps for obtaining the previews corresponding to DSD Keynote and Visagismile programs

Keynote DSD	Visagismile
- Load the photograph on the software program and position the photograph in accordance to the calibrating lines - Draw the smile line and the mesio-distal limits of the future frontal teeth - Choose the forms of standard teeth that are preset by the program - Selection of the best fitting teeth-form by the doctor together with the patient - classical criteria for selection are taken into consideration (reversed face, age, sex, personality etc.)	- Load the photograph on the software program - ”Auto face reading” command performs the automatic calibration - The facial map results automatically by joining 27 facial points and a “facial type” is obtained, according to the four types of temperament (choleric, sanguine, melancholy, flegmatic) - The patient is subjected to the psychological test (a short interview consisting of four questions) - The software automatically ranks the facial type in percentages depending on the statistical parameters of the facial map and the results of the interview - Patient’s preferences as well as design related information can be inserted in the program in order to be submitted to the laboratory - Based on all the information inserted so far the program proposes a teeth design

In order to comparatively evaluate the two computerized methods used for choosing the form of maxillary frontal teeth with the aim of establishing which one is more appropriate for patients with complete edentulism, we submitted a questionnaire to a number of 100 persons. Out of 100 submitted questionnaires, 2 were considered invalid and 98 valid; the 98 persons were represented by 12 dental doctors, 53 students at the Faculty of Dental Medicine and 33 persons activating outside the medical field. This self-administered questionnaire (SAQ) included several questions and the two previews generated by the software programs (Figure 1), allowing us to receive feedback regarding the results obtained by using the two computerized methods from persons both activating in the dental medical field and outside the medical field. The questionnaire was filled in under the anonymity protection as the persons included in the study were not requested to provide personal information such as surname, name or personal identification number.

Figure 1. The self-administered questionnaire submitted online

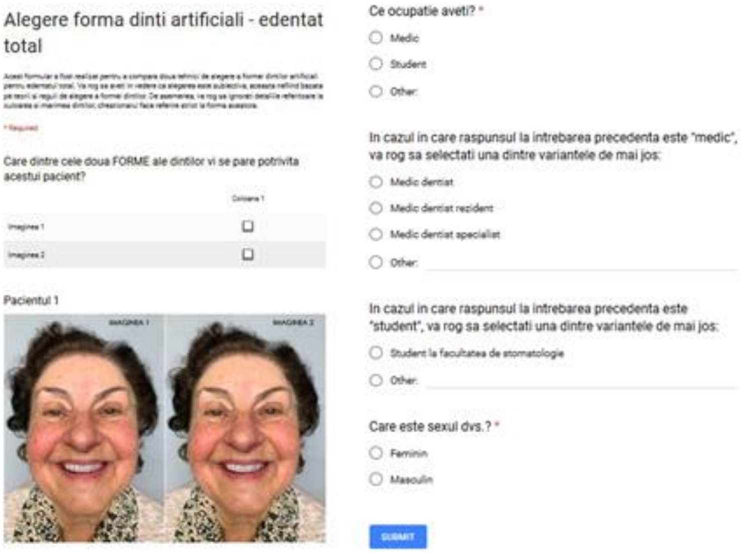

Alegere forma dinti artificiali - edentat total

Care dintre cele doua FORME ale dintilor vi se pare potrivita acestui pacient?

	Coloana 1
Imaginea 1	☐
Imaginea 2	☐

Pacientul 1

Ce ocupatie aveti? *

○ Medic
○ Student
○ Other:

In cazul in care raspunsul la intrebarea precedenta este "medic", va rog sa selectati una dintre variantele de mai jos:

○ Medic dentist
○ Medic dentist rezident
○ Medic dentist specialist
○ Other:

In cazul in care raspunsul la intrebarea precedenta este "student", va rog sa selectati una dintre variantele de mai jos:

○ Student la facultatea de stomatologie
○ Other:

Care este sexul dvs.? *

○ Feminin
○ Masculin

The questionnaire consisted of eight questions, of which the first four (closed-ended questions) regarded directly the four patients of the studied group while the remaining four were open-ended questions, referring to general information. The questionnaire, which was elaborated using Google Forms, was filled in online and it took sixteen days to receive all the answers. The information resulting from the answers to the questionnaires was further used, by employing Microsoft Office Excell 2018, to create statistical bases.

RESULTS

The results obtained after the questionnaire was filled in by the three categories of respondents are presented below:

- Dental doctors opted for DSD in proportion of 60% while 40% were in favour of Visagismile (Figure 2);

Figure 2. Options expressed by dentists

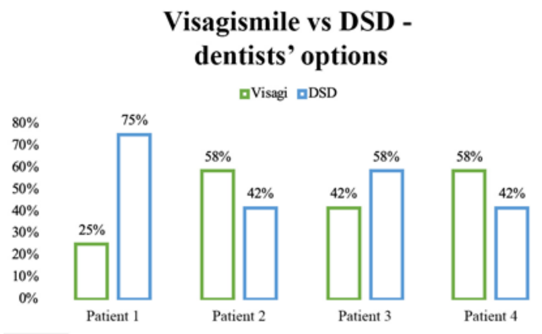

- Dental students were in favour of DSD in proportion of 60% while 40% chose Visagismile (Figure 3);

Figure 3. Options expressed by dental students

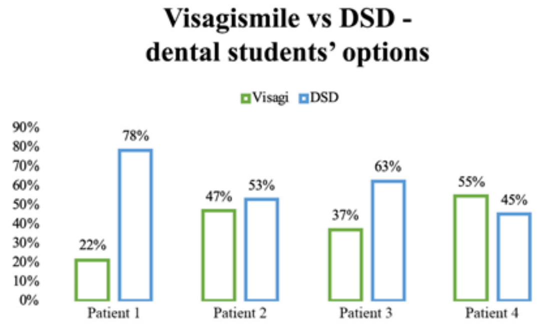

- The persons activating in non-medical fields were in favour of DSD in proportion of 66% while 34% opted for Visagismile (Figure 4).

Figure 4. Options expressed by persons from non medical field

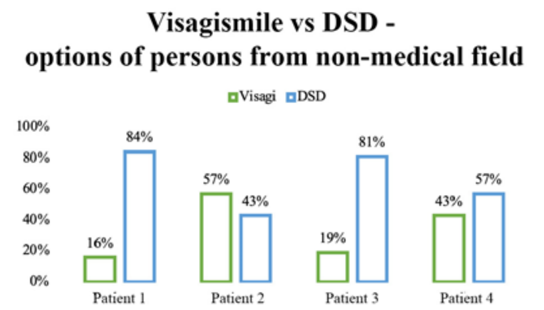

At the level of the whole group of respondents included in the study 60% opted for DSD while 40% were in favour of Visagismile (Figure 5).

Figure 5. General options

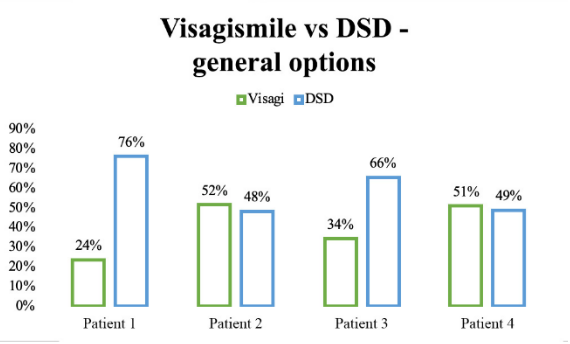

DISCUSSIONS

There are still insufficient articles and studies illustrating the use of the smile planning digital applications to patients with complete edentulism. The modern smile design techniques are more effectively controlled through data collection and different digital smile design tools, contributing to all treatment steps up to the fabrication of the definitive restorations, either fixed or removable. Utilizing these digital tools together with classical treatment-planning rules and principles facilitates a controlled and predictable therapy when redesigning a smile [8] both for dentate patients and for complete edentulism cases.

Visagismile concept supports both dentists and patients in deciding about the aesthetics of the final prosthetic restoration and it is mostly applied for dentate patients. Using a specific protocol, a smile design that corresponds to individual facial features, temperament and personal preferences of the patient can be achieved [7, 8]. This application can also facilitate to a great extent the conception of the complete denture design by allowing the preview of the final result and the choice of the form, colour, size and arrangement of artificial teeth depending on the facial typology and the desire of the patient, who is directly involved in elaborating the treatment plan.

In modern dentistry DSD programs are also successfully used as tools for diagnosis, treatment plan visualization and communication with the patient and the technician; all these elements can improve treatment outcome predictability. As regards the characteristics of the 3D functions, CAD/CAM connectivity and practicality, the DSD application proves to be even more performant than Visagismile. However, not all the DSD programs available today provide the same competency for the comprehensive analysis of the dentofacial esthetic parameters [8]. As in the case of complete edentulism, the actual DSD techniques can be used with certain limitations related to the following facts: dynamics of the lips when smiling; shooting of the initial photographs, which requires a good stability of the dentures; the wax occlusion rims that do not fit all the facial typologies. At the same time, the preview of the final prothestic result obtained by applying DSD offers the complete edentulous patient the possibility of having a realistic view on certain aspects related to teeth form, colour and size, which are not correlated with his/her age and facial typology.

On the other hand, the ability of the digital planning programs to perform an appropriate esthetic analysis is evaluated also depending on the number of the dental-facial esthetic parameters used. Thus, if Visagismile has the advantage of using additional elements related to the patient temperament, DSD uses more dental-facial esthetic parameters. [2] This could be the reason why the majority (between 60% and 66%) of the respondents to our questionnaire expressed their option for the DSD method.

CONCLUSIONS

Dental esthetics is a topical though subjective field and the techniques used for choosing the maxillary frontal teeth, either classical or modern (digital), have particular elements and, as a consequence, our study is a subjective one, based on the opinions expressed by the three categories of respondents.

DSD and Visagismile programs have proved their usefulness for dentate patients and our study could contribute to demonstrating their effectiveness also in case of complete bimaxillary edentulous patients. However, future studies of higher complexity and performed on larger groups of patients are necessary in order to validate the application of such programs in cases of complete edentulism.

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ORIGINAL ARTICLES

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Renal angiomyolipoma – a short review of the literature

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Abstract: *Renal angiomyolipomas (AML) are the most common benign renal tumors. By containing an important amount of fat tissue they are easily identifiable on CT and MRI images, thus no biopsy should be needed for a diagnosis. However there is a small percentage of AMLs that have very little fat tissue and are very difficult to differentiate from renal cell carcinoma. Along fat tissue, they also contain smooth muscle and blood vessels. Renal AMLs can occur as a sporadic finding, or more frequently in association with tuberous sclerosis complex (TSC) or pulmonary lymphangioleiomyomatosis (LAM). These tumors are usually an incidental finding on imaging tools, but sometimes a serious presentation is rupture and hemorrhage, which can easily evolve to shock. Therapeutical management vary from active surveillance to surgical removal, and it needs to be individualized for every patient and according to the clinical presentation.*

Keywords: *renal angiomyolipoma, tuberous sclerosis, therapeutical management*

INTRODUCTION

Renal AMLs are the most common benign renal tumors, having a reported prevalence of 0.2-0.6%, with a female to male ratio in favor of females. Because of the increase use of imaging tools and the advances in imaging methods the frequency of detection in the general population is increasing. AMLs are more frequently associated with patients with tuberous sclerosis complex (TSC), having been reported in 75-85% of cases with renal lesions and in 49-60% in TSC patients overall [1-2]. In cases of lymphangioleiomyomatosis (LAM), aproximately 45-60% of patients tend to have renal AMLs, usually being multiple and bilateral [3-4]. Even though the prevalence of AMLs is low among patients who do not have TSC or LAM, combined with the fact that the prevalance of sporadic AML exceeds the estimated birth incidence of TSC or LAM, it makes it more common [5]. AMLs are a member of a group of tumors called neoplasms with perivascular epithelioid differentiation, arising by clonal proliferation of epithelioid cells distributed around blood vessels [6, 7].

Histological findings

Renal AMLs are made of smooth-muscle-like cells, adipocyte-like cells, and epithelioid cells, with the three types of cells appearing to be derived from pericytes, fact suggested by the fact that these cells express perycite markers [8].

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AMLs are classified in two major histological groups. The most common histological variant is the classic one, having abnormally thick-walled vessels that lack a well-developed internal elastic lamina and varying amounts of spindle smooth-muscle-like cells and adipose tissue. However, some tumors consist solely of one component, while the other elements are present in very small amounts. In the classic variant epitheliod cells are rare, making up less than 10% of cells in this type of AML [9]. Classic AMLs are bening but, there are cases with local invasion into the perirenal fat, and in very rare ocasions into the collecting system, renal vein, or inferior vena cava and right atrium. The epithelioid variant, unlike the classic one can undergo malignant transformation. There is no consensus on the percentage of epithelioid cells that is required to make a diagnosis of the epithelioid variant, but studies sugest that a percent higher than 70% of epithelioid cells, a tumor size >7 cm, vascular invasion, ≥2 mitotic figures per 10 high-power fields, atypical mitotic figures and necrosis suggest an increased risk for malignant transformation [10].

Epithelioid cells are differentiated by their abundant eosinophilic and granular cytoplasm.

Epithelioid AML has less fat tissue than the classic variant, making it more difficult to diagnose by imaging, frequently resembling and be misdiagnosed as renal cell carcinoma.

In addition to these two major histological groups, there is a rare variant, characterized by solid and cystic areas, called angiomyolipoma with epithelial cysts. It is a benign lesion with no evidence of metastasis or recurrence following surgical excision [11]. The three variants are differentiated from each other only after biopsy.

DIAGNOSIS

Clinical presentation

Commonly patients are asymptomatic at the time of diagnosis, AMLs being an incidental finding. This is explained by the increasing use of imaging tools and the advances in imaging technology [12].

Among symptomatic patients, the most common sign is flank pain, followed by a palpable mass, haematuria, anaemia, urinary tract infection, or an impaired renal function. In a study conducted by Koo et al on 129 patients, 75% of them were asymptomatic [13]. Regarding symptomatic patients, Bissler et al found that out of the 32 sypmtomatic patients included into their study 20 had flank pain, 7 had hematuria and 2 had spontaneous rupture [14].

A serious form of presentation, seen in less than 15% of cases, is spontaneous retroperitoneal haemorrhage, which can lead in one third of patients to shock. Therefore, risk of a life-threatening bleeding is the main clinical concern in a patient diagnosed with AML [12].

The epithelioid variant of AMLs can undego malignant transformation. Though rare, it is characterized by local aggressivness, lymphadenopathy, and distal metastases. The larger the tumour, the more likely it is to spread, acting like a veritable retroperitoneal tumor [15-19] and can produce secondary hydronephrosis that must be drained with a double J stent [20, 21].

AMLs tend to be more aggressive in patients with TSC or LAM, thus symptomatic patients are more commonly found in these cases. It is important to screen patients diagnosed with AML for TSC and LAM. Patients with multiple, bilateral, or larger (≥4 cm) are at higher risk for TSC and the tumors are usually diagnosed at an early age.

Imaging features

By containing fat tissue AMLs are very easily diagnosed using imaging tools. However, in cases of hemorrhage, calcification, necrosis or low fat content the diagnosis becomes a challenge. A recent article classified renal AMLs as fat-rich, fat-poor, or fat-invisible, based on the amount of fat detected on imaging studies [22].

A hyperechoic lesion, resulting from a combination of fat, blood vessel, and muscle contents, with a posterior acoustic shadow, due to the multiple tissue interfaces is the classical appearance of an AML rich in fat tissue on ultrasound [23]. The echogenity of the tumor depends on the percentage of fat tissue in the AML, usually being the same as or greater than that of the renal sinus, but as the amount of fat gets lower, the echogenity decreases.

AML low in fat content are usually a mixture of hyperechoic and isoechoic lesions compared with the renal parenchyma, while a fat-invisible AML is homogenously isoechoic with it. In these cases it is hard to differentiate renal cell carcinoma from an AML on ultrasound [22, 24].

Computed tomography (CT) scan is recommended when hyperechoic lesion is found by ultrasound, having excellent sensitivity, specificity, and reproducibility regarding renal masses. Computed tomography can identify macroscopic fat in AML as low-density area of −10 HU or lower. Fat-poor AMLs are heterogeneously isoattenuating or hyper-attenuating, but on the other hand, fat-invisible AMLs appear homogenously hyperattenuating. Contrast-enhanced CT is not necessary for diagnosing fat-rich AML but shoud be performed where there is a risk for tumor bleeding, to identify the tortuous vessels, before therapeutic embolization can be scheduled [22, 25].

Kidney AML can be very similar with renal cell carcinomas and hemorrhagic cysts on the MRI images, thus fat suppression techniques may be helpful in differentiate them from each other [23]. As in computed tomography imaging, contrast enhanced MRI has a limited role in the diagnosis of AML.

In some cases, especially in fat-invisible AMLs, when both CT and MRI images are inconclusive and we cannot differentiate AMLs from renal cell carcinomas on imaging alone, percutaneous renal biopsy is recommended to avoid unnecessary treatment.

TREATMENT

AMLs being an incidental finding on imaging tools in asymptomatic patients, implies that a small percentage of cases requires active treatment.

The current guidelines regarding AMLs recommend active treatment in well-selected cases, such as: symptomatic tumours, the presence in women of childbearing age, poor access to follow-up or emergency care and large masses, but a size threshold remains controversial (historically a size greater than 4 cm was considered an indication for surgery).

Active surveillance

If the indication for active treatment are not present, all patients with AML should undergo routine monitoring by imaging to assess stability [26]. Patients with tumors less than 2 cm should do renal ultrasound every 3 to 4 years and those with masses measuring 2 to 4 cm should undergo ultrasound monitorisation every year. Patients with masses larger than 4 cm in diameter, who do not undergo surgery should be assesed, by ultrasound or CT every 6 months, and if stable, anually. The same with patients with epithelioid variants. Patients who have had surgical removal of an epithelioid AML, at high-risk for malignant transformation, should have whole-body CT six months after surgery and yearly thereafter for at least five years [27].

Embolization

Selective embolization of the renal artery is the preferred treatment for patients with life-threatening retroperitoneal hemorrhage. Embolization can be performed preoperatively to reduce the difficulty and complications of the surgical removal. It has a relatively high percentage of side effects including: fever, flank pain, leucocytosis, nausea, and vomiting within the first three days after the procedure, but nothing that cannot be managed conservatively. Other rare complications include non-target embolization of normal parenchyma or renal infarction [28,29]. Altough it is a generally well-tolerated procedure, it has a risk of regrowth and repeated haemorrhage after embolization [30].

Surgical excision

Partial or radical nephrectomy can be used to treat renal AMLs, being the only treatment that completely removes the renal mass. Current guidliness recommend that nephron-sparing surgery should always be performed whenever possible, being even more important in patients with TSC or LAM, because of the multifocality of the disease in this group of patients and the higher risk of recurrence. Radical nephrectomy should be used only in selected cases, with large masses and a high risk of malignant transformation, or when other treatment options are not viable. Sometimes, in an emergency setting, a radical nephrectomy can be a life-saver [18].

Minimal invasion techniques

Cryoablation and percutaneous radiofrequency ablation can be used as an alternative to embolization or surgery. Different studies show good efficacy with minimal complications, very few therapy sessions and no recurrences, but these minimal invasion techniques should be used on small (1-3 cm in diameter) and asymptomatic lesions [31].

Medical treatment

mTOR inhibitors, such as Sirolimus or Everolimus, decreases the risk of tumor progression and reduces the size of the tumor, especially in patients with TSC or LAM., but the use of these drugs in sporadic LAMs is still debatable. Recent guidelines recommend the use of mTOR inhibitors in patients with progressive renal LAMs greater than 3 cm in asymptomatic patients with TSC or LAM as the first line of treatment. Other treatments are required in case of secondary complications, such as cardiovascular diseases [32-36] or infections [37, 38].

DISCUSSIONS

A Japanese study, using a population-based ultrasound screening, included 18,000 healthy adults, and found that 0.1 percent of men and 0.2 percent of women had renal AML [39]. Rule et al stated that renal AMLs are more common seen in middle aged women, compared to men, findings supported by Koo and his team in their research on a group of 117 patients with sporadic AML, that found that 92% of patients were women with a mean age of 52 years old [16, 40].

Ewalt et al in their longitudinal study on 60 children with TSC found that renal AMLs were present in 48% of boys and 60% of girls at a mean age of 7 years old and in 76% of boys and 83% of girls at a follow-up age of 10 years old, suggesting that the prevalence of renal AMLs in patients with TSC is increasing with age [41].

In their paper on diagnosis and management of renal angiomyolipoma, Seyam and his colleagues included 60 patients with renal AMLs with a median age of 45 years old, from which 43 were females. Half the patients presented with flank pain and only 13 had hematuria. They screened patients for TSC and found this disease in 14 patients. More than half the patients were followed up expectantly, the mean size of the tumor being 4 cm in diameter, suggesting that the majority of patients with AMLs should undergo routine monitoring to assess stability. 29 patients needed active treatment to control bleeding and relieve pain or because of the risk of malignant transformation. Tumors that are larger in size have a higher risk of bleeding, the mean size of masses in which retroperitoneal hemorrhage was discovered was approximately 11 cm. Patients were followed up for a mean of 4 years and the lesions grew an average of 5 cm for TSC patients and less than 1 cm for the sporadic group, suggesting that patients with TSC are at a higher risk of progression. Furthermore, patients whom had TSC compared with the sporadic group were younger at presentation (26 versus 49 years old), had bigger renal masses (19 cm compared with a mean of 4 cm) and had multiple or bilateral lesions [42]. Similar findings were stated in a smaller group study conducted by Nelson et al (336 patients with 19% having TSC). The patients within the sporadic renal AMLs group were older (mean 52 years old compared with 30 years old), had smaller AMLs (mean 5 compared 9 cm), and were much less likely to have both multiple lesions (13% compared with 97%) and acute hemorrhage (14% compared with 44%) [43].

Some authors recommend that renal AMLs measuring 4 to 8 cm in diameter should be observed, if asymptomatic and close follow-up can be achieved [44, 45].

Bissler and his colleagues in their paper on mTOR inhibitors included 118 patients with AMLs, 5 of which were sporadic, the rest being in correlation with TSC. The target of at least 50 percent reduction in the total volume of the mass was achieved in 42% of the patients treated with everolimus compared with none of the patients treated with placebo, with a median response time of 3 months. Progression of AMLs was significantly more common in the placebo group (21%

compared with 4% in the mTOR inhibitors treated group) [46].

mTOR inhibitors treatment can be used to reduce the volume of tumors so the patients can benefit from a nephron-sparing surgery, fact stated in Staehler's paper on „Nephron-sparing resection of angiomyolipoma after sirolimus pretreatment in patients with tuberous sclerosis". Patients treated with Sirolimus had a 38 to 95% reduction in AML volume [47].

The efficacy of selective renal artery embolization was demonstrated in a study conducted on 30 patients with large renal AMLs (mean size of 8 cm). In 83% of AMLs that were embolized, the procedure was complete after a single sesion. The median reduction volume was 43% at a follow-up of 6 months and 81% at 12 months. They found that the response was poor for fat-rich AMLs compared with fat-poor tumors [48].

De Luca stated that sporadic AMLs smaller than 1.5 cm showed no growth over 5 year follow-up in 92% of cases [49]. Furthermore, Oesterling et al said that AMLs greater than 4 cm in diameter grow more rapidly and other authors found that younger patients compared with older individuals had a higher risk of progression [50, 51]. On the other hand, epithelioid variants of AMLs have a poorer prognosis. In one study conducted on 40 patients with renal epithelioid AMLs and atypia detected on histologic examination, local recurrence or distant metastases were present in 26% of cases [52]. Nese et al had the same results in their study on 41 patients with pure epithelioid AML, out of which 36% had a metastatic disease at the time of presentation, and 16% developed metastatic disease during follow-up [53].

CONCLUSION

AMLs are a benign group of renal tumors, commonly associated with TSC and LAM, but can also appear sporadically in the population. The detection of AMLs is increasing because of an increase in the use of imaging and advances in imaging technology. The diagnosis of AML is generally made by imaging in asymptomatic patients, although a biopsy may be required to diagnose fat-poor lesions. In terms of therapeutical management, several treatment option are available, though a small percentage of patients require it and the treatment should be individualized for every patient. Most of the AMLs can be managed by active surveillance. Pure epithelioid variants of AMLs have a poor prognosis comapared with the clasic variant.

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ORIGINAL ARTICLES

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Management of infectious complications in diabetes mellitus mellitus patients

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Abstract: *Diabetes mellitus is a serious condition, causing multiple health problems due to the disease itself, but also to the complications that these patients are predisposed to. Being a current health problem, many patients tend to disregard the doctor's recommendations concerning the necessary measures to maintain adequate and continuous control of the glycaemia levels. This, in turn, may cause complications, which include neuropathy, microangiopathy and a predisposition to infections due to weak local defence mechanisms.*

In otorhinolaryngology, the infectious pathology is one of the most often causes for presentation to the physician. Ranging from common otitis, acute pharyngitis or laryngitis and sinusitis, these different entities will cause significant discomfort for the patient, prompting the need for medical care. In patients with diabetes, the natural course of the disease is altered, with more severe symptoms, a more extended period of recovery and the predisposition for aggravated cases with the tendency to complications. Such complications may be life-threatening if we take into account the complications associated with acute sinusitis for example, which include orbital or cerebral abscesses.

Our paper aims to present the authors experience on the complete approach of patients with infectious pathology concerning the sinuses associating diabetes mellitus.

Always, we must keep in mind that patients with diabetes represent a particular class of patients that require a personalized approach. They are predisposed to a vicious circle, in which the infection causes diabetes imbalance, which in turn decreases the defense mechanism and predisposes to complications. The correct management must always include a proper control of diabetes, a complete evaluation that will determine if complications are already present and aggressive management of the infection, with regular check-ups to determine the evolution of the patient.

Keywords: *infections, diabetes mellitus, local complications*

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INTRODUCTION

Due to the high prevalence of diabetes mellitus in the general population, this group of patients has always benefited from practical guides concerning the proper management of the disease and the therapy protocol in case of imbalances or complications of the disease. The same principle applies for the complete approach of any secondary problems, such as electrolites imbalances in the normal body functions or associated infections [1, 2].

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It is accepted that patients with diabetes are predisposed to infectious complications due to the depressed local defense mechanisms, the body's incapacity to limit the infection and the high risk of unbalanced glycaemia levels [3-5]. However, studies that show the specific cellular dysfunctions that cause this problem are still limited. Some even stated that the link between diabetes and infections is difficult to establish [6], while other studies reinforce this concept [7]. However, the number of patients included remains limited and the conclusions scarce.

One fact remains unanimously accepted, and that is that the management of a patient with diabetes that associates an active infection is different and must be more thorough than that of a non-diabetic patient with the same illness. The guides clearly state that the infection must be limited, while the diabetes is kept under control with normal glycaemia levels, even if the use of insulin becomes necessary. In this manner we can avoid further complications that can prove to be life-threatening.

The mechanisms by which diabetes mellitus affects the normal immune capacity of the body are still unclear. Studies so far have demonstrated an association between the presence of diabetes mellitus and a decreased function of polymorphonuclear cells and macrophages [8, 9]. These cells involved in the natural processes of chemotaxis and phagocytosis present with a decreased response, thus lowering the host's capacity of self-defence. This was demonstrated when comparing the function of these cells in patients with diabetes compared to non-diabetic subjects [10].

Another mechanism that was described in order to explain the predisposition to infectious complications of diabetic patients is represented by disturbances in the humoral immunity, such as a low response of cytokines following stimulation or low levels of complement factor 4 [11].

In order to provide accurate and efficient management of these patients, it is essential to establish which mechanisms play the most significant role. Studies so far suggest that humoral adaptative immunity remains efficient also in patients with diabetes mellitus, as proven by their capacity to respond well to vaccines [12, 13]. As far as the cellular immunity is concerned, dysfunctions at this level have already been demonstrated, although at a lower level than in cases of HIV infections or other immunosuppressant conditions. Of this, one of the most important aspects is represented by lymphocytes dysfunctions in diabetes patients and delayed hypersensitivity reactions [14].

Diabetes mellitus may influence the onset and evolution of infectious diseases by other means as well. High levels of blood glucose create an environment in which a microorganism is more virulent and presents an increased adherence to the cells. This is especially true in patients with long-term unbalanced diabetes that present constant high levels of blood glucose.

Another problem that may occur in patients with diabetes and infections is the risk of diabetic ketoacidosis [3]. This complication may prove to be fatal in a large number of patients when it occurs, and it requires intensive care and aggressive management [15].

Due to all these factors, the management of the diabetic patient with an infection must be a multidisciplinary one. The team must include diabetes and nutrition specialist, an infectious diseases specialist, an intensive care unit doctor if the condition of the patients requires it, and specialist that treats the affected organ. Only with such a complex approach can these patients be balanced and healed in safe conditions.

Infections in diabetic patients can appear at any level and can involve primarily any organ. Starting from common cutaneous infections and up to severe ones, such as meningitis or myocarditis, these infections can appear alone or as part of a more severe condition and can lead to sepsis. The source of the primarly infection must be identified, and proper care measures must be ensured as soon as possible. These include broad-spectrum antibiotics as a first measure, followed by an antibiotic treatment according to an antibiogram after samples have been sent for testing. Other measures include anti-inflammatory drugs and the surgical drainage of any collection

MATERIALS AND METHODS

This paper aims to describe the proper management protocol used in our clinic for diabetic patients presenting with infections in the ENT field, more precisely the management of rhinosinusitis in diabetic patients.

Taking into account the septic state of ENT cavities, we must keep in mind that patients with diabetes are more predisposed than the general population to develop infectious conditions. These may include otic infections, laryngeal of pharyngeal problems, but one of the most common conditions is represented by sinusitis.

Patients diagnosed with sinusitis usually associate one or more of the following complaints: purulent nasal discharge, headache, cranio-facial pressure and vertigo. In acute cases, high fever may occur. The causes of sinusitis are divided into nasal and odontogenic. The last one may represent a severe complication in patients with diabetes due to their predisposition for dental problems.

In such patients, the primary protocol is similar to that of non-diabetic patients. Antibiotic treatment associated with anti-inflammatory drugs, antihistaminics and proper hydration are the most common measures and must always associate nasal decongestants.

Complete blood work must be performed in order to asses the toll this episode inflicts on the organism. Efficient monitoring of the patient is mandatory, especially in diabetic patients, due to the risk of rapid-onset complications.

Figure 1: CT images of a patient with diabetes and sinonasal mucormycosis.

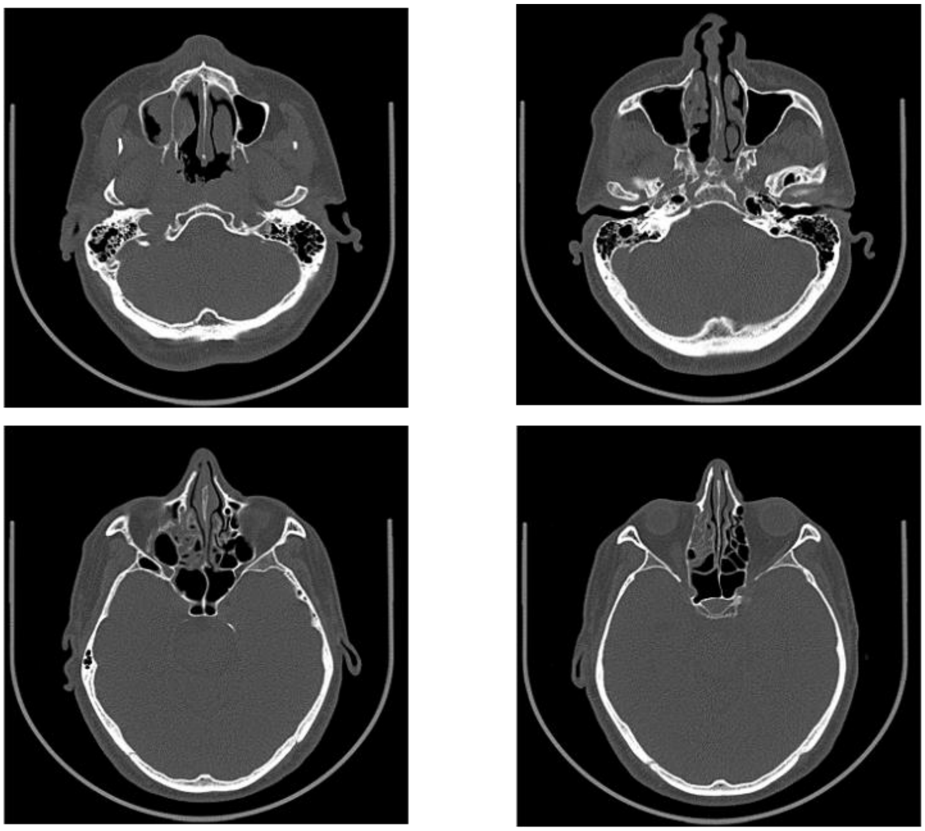

The complete diagnosis of these patients should include a computed tomography, which reveals the specific extension of any sinusitis. This is not routinely performed in the acute period, except for the cases that raise the suspicion of complications, such as an orbital abscess or meningitis. If the patients associate a positive response to therapy, the imagistic examination should be performed at a period of time following the acute infectious episode.

Taking into account the agent that causes the infection, sinusitis can be either bacterial or fungal. Each can occur at diabetic patients, but they are at risk of developing a more significant number of fungal infections than the general population. This may be caused by both low local defense mechanisms, but also to the high incidence of dental problems in these patients.

Figure 2: Mucormycosis lesion of the mucosa of the rhinopharynx in a diabetic patient – intraoperative view.

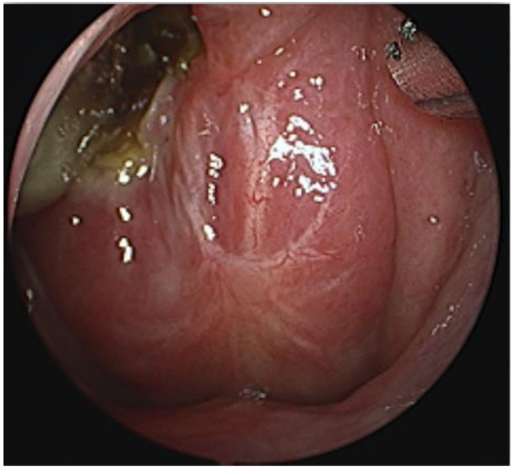

Figure 3: Intraoperative view of the same patient following an extensive debridation up to healthy tissue.

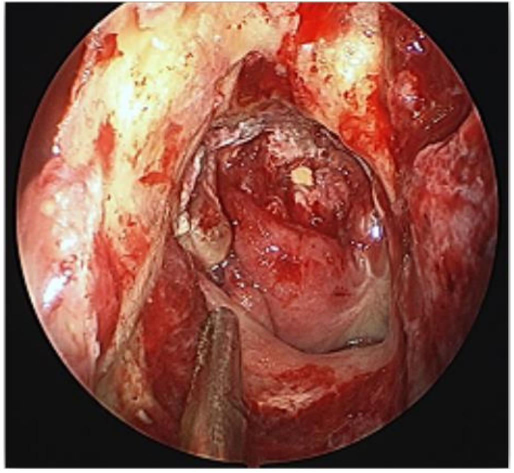

A significant aspect of the management of patients with sinusitis is the necessity to drain any purulent collection that prevents complete healing. The principle must be applied for all patients, but in diabetic patients, it is extremely important. Oral medication is more often inadequate in completely controlling the course of the disease. The surgical drainage may prove to be one of the simplest gestures that can be performed, with low risks. This principle applies to all patients but can be the only viable option in particular cases, such as Mucormycosis infections. In such cases, the surgical debridation of the affected area up to healthy tissue may be the only way to control the spread of the fungi.

The need for a multidisciplinary team remains, especially in patients with unbalanced diabetes that may require an aggressive approach [16]. Without adequate control of glycaemia levels, the healing process will be longer and less efficient. Also, an acute infection associates the risk of fluctuant glycaemia levels, with secondary complications. These patients have a higher risk of sepsis due to the decreased power of the body to contain the infection.

The surgical approach must be thorough and ensure adequate drainage of the infected sinuses. The same principle applies to bacterial or fungal sinusitis. Only the complete removal of the infectious reservoir will allow for a complete recovery.

RESULTS AND DISCUSSIONS

Patients with diabetes, especially if poorly controlled, represent a surgical challenge. Although the intervention itself follows the same principles as in non-diabetic patients, the surgeon must be even more thorough during the intervention and ensure that all infected sinuses have been drained and, in cases of fungal sinusitis, all affected tissue has been removed. The weak local defense mechanisms are not reliable in such cases, and any infectious reservoir remaining can cause a relapse in the disease [17].

These patients represent a challenge from the point of view of the anesthetist as well [18, 19]. The glycaemia levels are more difficult to control during the infectious process, they associate a higher risk of complications, including sepsis, and the response to usual therapy may be altered.

In our experience, the surgical gesture of draining the infected sinuses can be salvatory. Antibiotic or antifungal treatment is mandatory, depending on the type of sinusitis. However, therapy alone can be insufficient and unable to limit the infection. This is even truer in diabetes patients, due to their body's low defense mechanisms. In cases of sinusitis, after a complete diagnosis that must in some cases include an imagistic evaluation, the surgical intervention is advisable if the response to treatment in the first days is insufficient or if any complications appear. The imagistic examination of choice is represented by a CT scan, which can be completed with an MRI if complications, such as orbital or cerebral extension, are suspected.

The functional endoscopic sinus surgery is a minimally invasive one and ensures a good visualization of the affected area. It allows the surgeon to control the patency of every sinus and verify if all sources of infection have been removed.

The postoperative care is crucial, being a key element in the complete recovery of the patient. Especially in a diabetic patient, maintaining appropriate glycaemia levels in the postoperative period will increase the patient's chances of a full and uneventful recovery.

CONCLUSIONS

Diabetes remains a challenging disease, despite the advancements that are continuously made concerning the therapy. The diabetic patient remains a particular one, requiring a more thorough approach, especially when an infectious process is present. People with diabetes are at higher risk to develop complications and more susceptible to inadequate and incomplete healing. Another frequent problem is caused by the multiple problems caused by diabetes, even concerning the local defense mechanisms. In such patients, a vicious circle may occur, based on the relationship between infection and unbalanced diabetes, where diabetes favors the infection. This, in turn, causes difficulties in maintaining normal glycaemia levels, which paves the way for infectious complications.

The authors recommend a multidisciplinary approach of patients with ENT infections and diabetes. This applies even more for patients with sinusitis, which is a common disease but can develop even life-threatening complications if incompletely treated. If the disease does not respond well to medical therapy, it is advisable to ensure the surgical drainage of the affected sinuses and the ENT surgeon must do so before complications. For patients with diabetes, we must also bear in mind that the onset of complications may be more rapid and dramatic.

Once the infection has been drained, the healing process will have a normal course only if the patient is well balanced, with normal glycaemia levels. Insulin may be necessary, even in patients that did not require this prior to the event. Only the proper management of diabetes allows a normal recovery. Even so, these patients must be carefully observed to ensure no secondary complications or relapses occur, and if such cases occur, the necessary measures must be quickly taken, including revision surgery if needed.

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Maintenance pharmacologic therapies for opioid use disorders: beyond opioid agonists

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Abstract: *Opioid use disorder is a worldwide challenge for an increasing number of patients, and still the therapeutic options presented in clinical guidelines for this condition are very few. The first line pharmacological therapies for opioid use disorder continue to be opioid agonists as substitution treatment, but the need to find new treatments without the effect of prolonging opioid dependence become more and more obvious. This systematic review presents the most supported by evidence pharmacological agents that can be used as monotherapy for opioid use disorder patients (i.e., naltrexone, extended release/implant and oral formulation), or as adjuvants to substitution therapy (e.g. olanzapine for aggressive behaviours, memantine or dextromethorphan for reducing inflammatory processes related to opioid use). More good quality data derived from larger, well designed trials are needed in order to find solid recommendations for non-opioid agonists in the treatment of opioid use disorder.*

Keywords*: opioid use disorder, naltrexone, memantine, dextromethorphan, olanzapine, maintenance treatment*

INTRODUCTION

The importance of opioid dependence could not be overemphasized, and during the last decade a continuously increasing number of alerts have appeared both in public media and scientific environment regarding the negative functional impact, increasing societal costs and mortality risk associated with this pathology.

Opioid dependence is a significant contributor to the global disease burden, as between 24 and 35 million people globally used an illicite drug from this category in 2010 [1,2]. North America, Eastern Europe and Southern sub-Saharan Africa are the most strongly affected by the premature mortality related to opioid use disorder [1]. United Nations Office on Drugs and Crime 2017 Report shows an overall increase of the heroin and synthetic opioids use (oxycodone, hydrocodone, tramadol, and fentanyl) since 2009 and the trend to more diversification of the opioid market [3]. Disability-adjusted life years (DALY) attributable to opioid dependence in 2015 reached the value of 12 million, or 70% of the global burden of disease attributable to drug use disorder [4]. Relapse rate after detoxification is very high, ranging from 72% to 88% after 12-36 months [5].

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Given consideration to the importance of opioid use disorder both at individual and societal level, worldwide efforts have been constantly made in order to find treatments able to prevent opioid use relapse and to maintain patients in remission as long as possible. The most validated treatments for the maintenance phase are methadone and buprenorphine, both with agonist actions at µ-opioid receptors (the first being a partial agonist, while the second is a full agonist). Unfortunately, due to their mechanism of action, both these drugs have abuse potential (less in case of buprenorphine) and associate withdrawal symptoms (although milder than in case of heroin or morphine). Therefore, the need to find non-addictive agents which can be used during the maintenance phase is considered urgent.

OBJECTIVE AND METHODS

This systematic literature review has as primary objective finding non-opioid agonist treatments for the maintenance phase in opioid use disorder patients, based on good quality data. The secondary objective is to formulate evidence-based recommendations for this indication, starting from existing data in the literature.

Table 1. Inclusion/exclusion criteria.

Operational criteria	Inclusion criteria	Exclusion criteria
Population	Minimum age for inclusion was 18 years, no superior age limit was established. The main diagnosis was opioid use disorder. Secondary diagnoses were allowed, if the study design permitted statistical analysis for at least one outcome regarding opioid use disorder. Diagnosis was established according to DSM-IV, IV TR, 5 or ICD-10 criteria.	Patients under age of 18. Presence of severe somatic or psychiatric co-morbidities that may have impact over patients' outcomes, or due to their treatment may lead to confusion about the outcome by pharmacokinetic or pharmacodynamic interactions.
Intervention	Any pharmacological agent without agonism (either partial or complete) over opioid receptors, either as add-on or as monotherapy. Combined pharmacological and psychological treatment. Treatment duration over 4 weeks.	Psychotherapy or psychological counselling as only type of intervention. Non-pharmacological and non-psychotherapeutical interventions, OTC, investigational products used in phase I, II or III clinical trials.
Environment	Inpatients, outpatients. Hospitals, day care, special care, intermediate care units. Carceral environment.	Unspecified environment.
Primary and secondary outcomes	Changes in opioid use- related parameters, e.g. days of abstinence, number of toxicology tests negative, rate of rehospitalisation for opioid consumption. At least one outcome is related to the opioid use.	All trials with outcomes not related to the severity of opioid use disorder.
Study design	Prospective or retrospective clinical trials. Randomized, open label, single blind, or double blind. Case control studies. Epidemiological studies. Observational trials.	Trials with unspecified or insufficiently specified design. Variables referring to opioid use disorder were not included in statistical analysis, or not presented in the published paper. Systematic review, narrative reviews, meta-analyses, case reports, case series.
Language	English, French, German, Russian	Any other language, except for those already specified.

Methods were based on the qualitative review of data found in the main electronic databases- PubMed, Complete MEDLINE, EMBASE, Cumulative Index to Nursing and Allied Health Literature (CINAHL), National Library of Medicine and the National Institute of Health (NCBI), PsychInfo and Thomson Reuters/Web of Science. All clinical trials and epidemiological studies published between 1998 and 2018 in English, French, German, and Russian were included in the primary review. The search paradigm used was „opioid dependence", „opiate dependence", „opioid/opiate use disorder", combined with „non-opioid agonists" or „non-opioid pharmacological treatment". This literature review is based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P) principles of research [6].

Inclusion and exclusion criteria were applied to all papers found during primary search (table 1).

Therapeutic intervention was focused on drugs with non-opioid agonistic properties. If any psycho-therapeutic intervention was administered, than it was only as an add-on to the pharmacologic management, and not the main method of treatment. Because this review was focused on maintenance therapy, brief interventions were not included. The outcomes of therapeutic intervention had to be periodically evaluated, and statistically analysed. Only trial which produced inferential, statistically-based data were included in the final step of the review.

During the secondary analysis, papers were reviewed and their results were analyzed. Only papers to which full text was found were included in the final analysis.

All studies were ranked according to their level of significance (LOS). This level was determined based on the quality of the study design: (5) double-blind randomized controlled trial, with/without an active comparator/placebo; (4) single-blind trials, with/ without an active comparator/placebo; (3) open-label trials with/without an active comparator; (2) retrospective studies, observational trials; (1) any other study design.

GRADE recommendations for the level of evidence [7] were formulated based on the evidence found in the secondary analysis. GRADE recommendations included 5 levels: A= high quality= future research are very unlikely to change the validity of these recommendations; B= moderate quality= future research is likely to have an important impact on the appreciation of the recommendations; C=low quality= it is very likely that future research will have an important impact over the confidence in the estimate of effect and is likely to change the recommendations; D= very low quality= any estimate of the effect is very uncertain. Intermediate steps (+ or -) represent nuances of the recommendations based on the quality of trials and tolerability of intervention, as resulted from these trials [7].

RESULTS

After the primary search 2560 papers surfaced, but only 65 remained when the inclusion and exclusion criteria were applied (figure 1). A number of papers were excluded because full text was not found, so that 29 studies remained to be reviewed in the final step. All trials included in the secondary review are presented in table 2.

These 29 trials included in the secondary analysis had a total number number of participants of 3,159, and a mean LOS of 3.58. A number of 13 trials were found to be high quality designed trials (LOS=5), meaning double-blind, either placebo-controlled or with an active comparator, randomized clinical trials.

Analysis of interventions corresponding to the inclusion/exclusion criteria previously mentioned showed that non-opioid agonist drugs used in the maintenance phase in patients with opioid use disorder who have been previously detoxified were: (1) naltrexone implant; (2) oral naltrexone; (3) topiramate; (4) memantine; (5) dextromethorphan; (6) dronabinol; (7) olanzapine; (8) baclofen; (9) ibudilast; (10) buspirone; (11) sodium valproate; (12) fluoxetine; (13) paroxetine; (14) clonazepam; (15) gabapentin. The last five drugs were used only in combination with another active medication or as active comparators, so they were not evaluated separately in this review.

Figure 1: Stepwise filtration of titles found during electronic databases search

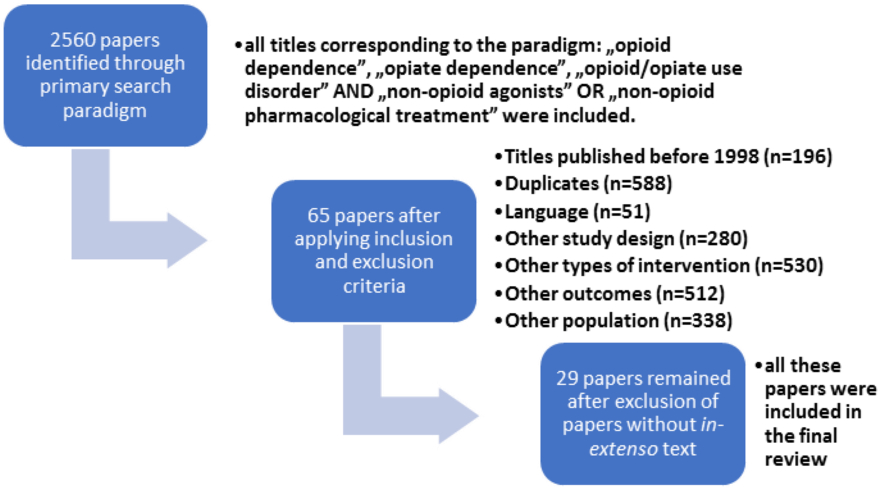
2560 papers identified through primary search paradigm
•all titles corresponding to the paradigm: „opioid dependence", „opiate dependence", „opioid/opiate use disorder" AND „non-opioid agonists" OR „non-opioid pharmacological treatment" were included.
65 papers after applying inclusion and exclusion criteria
•Titles published before 1998 (n=196)
•Duplicates (n=588)
•Language (n=51)
•Other study design (n=280)
•Other types of intervention (n=530)
•Other outcomes (n=512)
•Other population (n=338)
29 papers remained after exclusion of papers without *in-extenso* text
•all these papers were included in the final review

Naltrexone was the most investigated non-opioid agonist medication for the maintenance therapy in opioid use disorder, and 15 relevant trials focus on this drug's efficacy have been found during the secondary search. Naltrexone is an opioid antagonist, and both the parent drug and its active metabolite (6β-naltrexol) are competitive antagonists of the μ-, and to a lesser extend to κ- and δ-opioid receptor (10-25 times lower affinity for κ- and δ-receptors compared to the μ-receptors) [8]. Because oral naltrexone is extensively metabolized during first-pass metabolism, and because therapeutic adherence of opioid users is known to be very low, various implant formulations of this pharmacological agent have been developed.

Oral formulation of naltrexone has been investigated in comparison with buprenorphine/naloxone in the post-detox phase [9], methadone in stabilized patients [10], buprenorphine and placebo in detoxified patients [11], methadone in opioid dependent patients who were stablized on methadone maintenance treatment for controlling depressive and anxiety symptoms [12]. Oral naltrexone as monotherapy was compared with naltrexone plus gabapentin (1200 mg/day) for the management of muscle pain and craving [13], and naltrexone plus placebo was compared with naltrexone plus sertraline for controlling depression severity and opioid craving [14]. Another trial compared naltrexone (50 mg/day) + fluoxetine (20 mg/day) with naltrexone + placebo, fluoxetine + placebo, or placebo for 6 months plus psychotherapy for all groups for the monitoring of remission rate during 6 months [15]. The mean LOS of these studies was 3.85, and an overall number of 657 patients were included.

These trials supported a moderate efficacy and tolerability of oral naltrexone: buprenorphine/naloxone was superior to naloxone in the reduction of opioid-positive urine test and preserving patients participation in the treatment [6], naltrexone induced similar weight gain during 6 months of treatment when compared to methadone [16], naltrexone may improve depression in patients with opioid use disorder at 6 months [12], and naltrexone was inferior to buprenorphine (not statistically significant) in several outcomes regarding the rate of relapse in detoxified heroin dependent patients [11].

Naltrexone plus fluoxetine and individual psychotherapy has been proven a useful combination especially in women with heroin dependence, because it increased the remission rate and maintenance of remission in this population [15]. Naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine [13]. Naltrexone plus sertraline was a well-tolerated combination and increased on short-term treatment retention of recently non-depressed opioid abstinent patients, but the difference between this combo and naltrexone alone disappeared in time [14].

Naltrexone extended-release has been investigated in comparison with buprenorphine/naloxone [16], treatment as usual [5], oral naltrexone plus psychosocial therapy [17], and methadone [18]. Naltrexone implant was investigated also in several trials as single pharmacological agent, without an active comparator [19-22]. The mean LOS of these trials was 2.8, and the global number of participants was 1982.

The overall efficacy of naltrexone extended-release was good, although data are derived from poor and medium-quality designed trials. Its efficacy and tolerability were similar to buprenorphine/naltrexone in patients who wanted to switch from this combination of drugs to naltrexone implant [16], and it induced a lower rate of relapse when compared to treatment as usual, at least on short-term [23]. Heroin dependent patients who received extended release formulation of naltrexone had better outcomes than those treated with oral formulation of the same drug [17], and naltrexone implants but not methadone were associated with long-term benefits in decreasing opioid-related hospital morbidity [18].

In trials without an active comparator, naltrexone extended-release was efficient in preventing opioid overdose [22], improved depression severity at 4 weeks post-baseline, but it may also be responsible of worsening late insomnia [21], improved quality of life, rate of retention, opioid-negative urines, opioid craving, and rate of re-employment [20]. Also, naltrexone plus psychological counselling improved the acceptance of opioid dependence pharmacotherapy and prolonged the duration of abstinence to opioids, when compared to placebo plus counselling [19].

Topiramate is an anticonvulsant with GABA-enhancing properties and AMPA and kainate-inhibitory actions, involved in the modulation of extracellular dopamine release in the mesocortical and limbic system dopaminergic system, properties which recommended it as a potential treatment for cocaine dependence [24]. Patients with opioid use disorder who presented also cocaine dependence did not benefit from the addition of topiramate to their methadone maintenance therapy, because no difference to placebo could be found after 12 weeks [25].

Ibudilast is an anti-inflammatory drug with phosphodiesterase inhibition properties (especially PDE4) which has been associated in animal studies with the capacity of alterating several opioid-mediated effects [26, 27]. When compared with placebo in a 6-week RCT in patients detoxified from morphine, ibudilast treated patients presented lower rates of drug liking (oxycodone), lower craving to heroin/ tobacco/cocaine and lower subjective ratings of pain vs. placebo [26].

Memantine is a noncompetitive NMDA-receptor antagonist that blocks the effects of excessive concentrations of glutamate, currently used for Alzheimer Disease- related neurocognitive disorder and researched intensively for other dementias, ADHD, and as an agent able to reverse tolerance to opioids [28]. Memantine was administered in patients who were on buprenorphine/naloxone maintenance treatment, and compared to placebo [29], or in patients who were on methadone-maintenance-therapy, compared to placebo plus methadone-maintenance therapy [30, 31]. Memantine was also investigated as maintenance treatment in detoxified non-treatment seeker heroin dependent patients during 8 weeks of in-patient regimen [32].

These trials investigating the efficacy of mematine had a mean LOS of 4.25, and an overall number of 350 participants. It can be observed that the quality of trials focused on memantine efficacy was higher than previously mentioned research for other investigational products.

Memantine has been proven as a useful adjunctive medication for patients treated with buprenorphine/naloxone and it may provide an easier transition to buprenorphine-free therapeutic regimen [29]. Low dose memantine attenuated chronic opioid-dependence-induced cognitive impairment, decreased the daily dose of methadone, and had positive effect over probably NMDA-mediated inflammatory processes [30, 31]. Memantine decreased the subjective, but not the reinforcing effects of heroin in non-treatment seeker heroin-dependent patients [32].

Dronabinol is a synthetic form of 9-tetrahydro-cannabinol, with similar affinity for cannabinoid 1 and 2 receptors, currently approved for use in anorexia associated with weight loss in AIDS and for the treatment of nausea associated with chemotherapy resistant to other anti-nausea treatments [33]. A double-blind, placebo-controlled RCT investigated the effects of dronabinol in patients with naltrexone extended release and a significant effect of this drug in decreasing the severity of opiate withdrawal has been proven [34].

Dextromethorphan is an antitussive drug with N-methyl-D-aspartate (NMDA) receptor antagonist properties, involved also in protecting monoaminergic neurons against inflammation-mediated degeneration [35, 36]. Chronic opioid administration produces systemic inflammation, and for this reason the use of a drug with anti-inflammatory properties seems to be granted [37]. Based on its neuroprotective and anti-inflammatory properties, dextromethorphan was investigated as adjunctive agent in patients with opioid use disorder. Dextromethorphan 60 mg/day and 120 mg/day were compared with placebo as adjunctive to methadone treatment in opioid-dependent patients [37, 38], and in another trial dextromethorphan 240 mg/day was compared with placebo in methadone-stabilized patients [39].

These trials with dextromethorphan had a mean LOS of 5, and a total of 268 patients.

Dextromethorphan was useful in low doses as adjuvant treatment in methadone-treated patients both at inflammatory level and clinical level [37, 38], but in another trial the difference versus placebo was not significant in the otcome defined as mean methadone consumption [39]. The quality of life was superior in patients who reeived dextromethorphan as adjuvant to methadone versus placebo plus methadone [39].

Olanzapine is an atypical antipsychotic investigated in patients with opioid dependence who were on substitution therapy, as a drug targeting the aggresive behavior [40, 41]. The mean LOS of these trials was 3.5, and the total number of participants was 268.

In the first trial, olanzapine (2.5-15 mg) was compared with sodium valproate (600-1000 mg) in patients stabilized on methadone, and olanzapine was slightly superior in controlling aggressivity but did not differed from valproate in reducing the number of positive urine samples [40]. In the second, smaller-sample size, olanzapine was compared to fluoxetine/paroxetine and clonazepam and reduced aggressive/hostile behavior in heroin addicted patients undergoing substitution therapy [41]. In this second trial, olanzapine was superior to its comparators in the direct aggressiveness, overall hostility, verbal aggressiveness, and aggressive incidents; also, 70% of the completers achieved full remission, without significant differences between groups [41].

Buspirone is a non-benzodiazepine anxiolytic which possesses 5HT1A agonistic properties, acting as full agonist at 5HT1A presynaptic receptors and partial agonist at postsynaptic 5HT1A receptors [42]. A single trial that compared buspirone with placebo in patients with anxiety undergoing methadone therapy has been identified during the literature review [43]. Buspirone had no significant efficacy over anxiety symptoms in these patients, but it decreased depression severity and slowed down the return to substance abuse [43].

Baclofen is a GABA-B receptor agonist that may be helpful in promoting abstinence from a variety of drugs [44]. A double-blind, placebo-controlled, 12-week RCT with a LOS of 3, which included 40 patients with opioid dependence, recently detoxified, found a significantly better treatment retention in the baclofen-treated patients (60 mg/day) [45]. This trial had a small sample size and a high attrition rate, which make difficult to generalize its results.

Table 2. Trials focused on non-opioid agonist therapies during the post-detoxification phase in patients with opioid use disorder

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Pirnia B, Soleimani AA, Malekanmehr P, et al. [25]	Placebo-controlled RCT; topiramate (25-300 mg/day), 12 weeks, escalating doses; patients were treated also with methadone 20-140 mg/day and brief behavioral compliance enhancement treatment for cocaine dependence.	n=50 patients with dual cocaine and opiates dependence	Weekly urine drug screens.	Topiramate was no better than placebo in reducing cocaine use, but was superior to placebo in reducing cocaine craving.	The efficacy of topiramate in cocaine use reduction in patients with dual cocaine and opiates dependence was not supported.	5
Solli KK, Latif ZE, Opheim A, et al. [16]	Prospective cohort study, naltrexone-extended release vs. buprenorphine-naloxone 3 month + participants' choice of study medication for an additional 9 months; the active drug was administered every 4 weeks (380 mg).	n=54 opioid-dependent adults continuing naltrexone-extended release and n=63 opioid dependents inducted on the same treatment	Use of heroin and other illicit substances, opioid craving, treatment satisfaction, treatment retention, adverse events, addiction-related problems	Follow-up after 9 months showed completion rates of 51.9% among participants continuing naltrexone-extended release in the follow-up and 47.6% among those inducted on the active treatment, with opioid abstinence rates of 53.7 and 44.4%, respectively.	Opioid-dependent individuals who elect to switch from buprenorphine-naltrexone after 3 months to extended release naltrexone 9 months were experiencing similar results (treatment completion and abstinence rates) and similar rate of adverse events to those who had been on extended-release naltrexone from the start.	4
Metz VE, Jones JD, Manubay J, et al. [26]	6-week RCT focused on ibudilast (a nonselective phosphodiesterase inhibitor) effects over craving, reinforcing, analgesic effects of oxycodone in human volunteers diagnosed with opioid dependence; patients received maintenance treatment with ibudilast (50 mg bid) or placebo	n=11 patients detoxified from morphine addiction	VAS for measurement of subjective effects of oxycodone.	Patients receiving active ibudilast presented decreased rates of drug liking after 15 mg of oxycodone; heroin/ tobacco/cocaine craving was reduced under active ibudilast vs. placebo; mean subjective ratings of pain were lower in the active ibudilast condition.	Ibudilast may be useful in treating opioid use disorder and may enhance the analgesic effects of oxycodone.	3

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Earley PH, Zummo J, Memisoglu A, et al. [20]	Single-arm, multisite, open-label study; extended release naltrexone injections for up to 24 months, combined with counselling via intensive outpatient substance abuse treatment programs.	n=38 enrolled opioid-dependent healthcare professionals who had been detoxified for at least 2 weeks	Opioid drug tests monthly, measurements for quality of life, opioid craving, and treatment satisfaction	At 24 months, mean opioid craving fell by 45.2% and quality of life (SF-36) -mental component score improved by 31.1% from baseline; of the 22 unemployed subjects at baseline, 45.5% improved employment status at 24 months.	Long-term extended release naltrexone was associated with better quality of life, rates of retention, opioid-negative urines, opioid-craving reduction, re-employment	3
Lee JD, Friedmann PD, Kinlock TW, et al. [23]	Open-label RCT, 24-week extended-release naltrexone vs. usual treatment (brief counseling and referrals for community treatment programs);	n=308 criminal justice offenders with history of opioid dependence, abstinent from opioids at the time of randomization	Time to an opioid-relapse event (defined as 10 or more days of opioid use in a 28-day period).	Participants assigned to extended-release naltrexone had a longer time to relapse than did those who received usual treatment, lower rate of relapse, and higher rate of opioid-negative urine samples. After one year, the rates of opioid-negative urine samples were equal. During one year, there were no overdose events in the extended-release naltrexone treated participants, while in the usual-treatment group there were 7 cases.	Extended-release naltrexone was associated with a rate of opioid relapse lower than that with usual treatment, but after one year the protective effects waned.	3
Mokri A, Chwarski MC, Taherinakhost H, Schottenfeld RS [9]	Single-site, two-parallel groups, double-blind RCT, 12-week; oral naltrexone vs. buprenorphine/ naloxone.	n=102 participants with opioid use disorder in the post-detox phase	Initial duration of opioid abstinence (urine toxicology tests).	Mean number of days of initial duration of verified abstinence was higher with buprenorphine/naloxone (p=0.205); mean number of opioid-negative urine tests was higher in the buprenorphine/ naloxone-treated subjects (p=0.049); the mean number in treatment was also greater in buprenorphine/naloxone group (p=0.013); the rate of sustained, 12-week opioid abstinence was double in the buprenorphine/ naloxone group.	Sublingual buprenorphine/ naloxone was associated with a greater number of opioid-negative urine tests and treatment retention, but not significantly greater initial abstinence duration or proportions with sustained abstinence.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Gonzalez G, DiGirolamo G, Romero-Gonzalez M, et al. [29]	Double-blind placebo-controlled RCT, 13 weeks; patients already received treatment with buprenorphine/naloxone 16/4 mg/day and they were randomized to memantine 15 mg or 30 mg vs. placebo.	n=80 young adults with opioid dependence	Change in the weekly mean proportion of opioid use, and cumulative abstinence rates after rapid buprenorphine discontinuation at week 9	Memantine 30 mg/day significantly improved short-term treatment with buprenorphine/ naloxone by reducing relapse and opioid use after buprenorphine discontinuation, during the last 2 weeks of study participation.	Memantine could be an useful adjunctive treatment at 30 mg daily for patients treated with buprenorphine/ naloxone and helpful in the transition to buprenorphine-free therapeutic regimen.	5
Bisaga A, Sullivan MA, Glass A, et al. [34]	Double-blind RCT, dronabinol 30 mg/day (n=40) vs. placebo (n=20); patient underwent inpatient detoxification for opioid dependence and extended-release naltrexone induction; dronabinol was administered while inpatient and 5 weeks afterwards.	n=60 patients recently de-toxified from opioids	Severity of opioid withdrawal and retention in treatment.	The severity of opioid withdrawal during inpatient phase was lower in the dronabinol group vs. placebo; rates of successful induction onto extended release naltrexone and completion of treatment were not significantly superior for dronabinol vs. placebo.	Dronabinol reduced the severity of opiate witdrawal but had no effect on rates of extended-release naltrexone treatment induction and retention.	5
Chang YH, Chen SL, Lee SY, et al. [30]	RCT, memantine plus methadone-maintenance-therapy (n_1=57) vs. placebo plus methadone-maintenance therapy (n_2=77); healthy controls were matched with methadone-treated group; 12 weeks duration of monitoring.	n=134 patients stabilized on methadone	Cognitive performances	Both treatment groups showed improvements in their cognitive performances. Memantine add-on was superior to placebo in the post-treatment cognitive improvement.	Low-dose memantine (5 mg/day) may attenuate chronic opioid-dependence-induced cognitive decline.	5
Lee SY, Chen SL, Chang YH, et al. [37,38]	12-week RCT, dextrometorphan 60 mg/day (n_1=65), 120 mg/day (n_2=65) vs. placebo (n_3=66) as adjunctive to methadone treatment in opioid-dependent patients.	n=196 opioid dependents	Methadone dose required, plasma morphine level, retention in treatment.	Patients who received 60 mg/day dextromethorphan had significantly longer treatment retention and lower plasma morphine levels than did the placebo group. Plasma TNF-alpha was significantly decreased in the 60 mg dextromethorphan group compared to placebo.	Low dose dextromethorphan is useful as concomitant therapy in methadone-treated patients both at the pathogenic-inflammatory level, and at the clinical level.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Lee SY, Chen SL, Chang YH, et al. [31]	Double-blind RCT, placebo-controlled, 12-week duration; memantine (5 mg/day) (n_1=53) vs. placebo (n_2=75); patients with opioid dependence undergoing methadone maintenance therapy.	n=128 patients stabilized on methadone	Inflammatory bio-markers, overall dose of methadone	Memantine-treated patients required a somewhat lower methadone dose than did placebo-group (p=0.039), and they also had significantly lower plasma TNF-α and higher TGF-β1 levels.	Low-dose memantine may help patients during methadone substitution treatment because of the overall methadone dose decrease and positive impact over inflammatory process possible related to the NMDA-receptors.	5
Krupitsky E, Nunes EV, Ling W, et al. [19]	Double-blind, placebo-controlled multicentric RCT, 24 weeks; 380 mg extended-release naltrexone vs. placebo; counselling sessions were provided for both groups.	n=250 patients with opioid dependence, who underwent detoxification	Confirmed abstinence (urine drug tests and self-report).	Extended-release naltrexone-treated patients had significantly longer periods of confirmed abstinence (p=0.0002), more opioid-free days (p=0.0004), less craving (p<0.0001), more treatment-retention (p=0.0042).	Extended-release naltrexone in conjunction with psychosocial treatment improve the acceptance of opioid dependence pharmacotherapy and prolongs the duration of abstinence to opioids.	5
Krupitskii EM, Zvartau EE, Tsoi-Podosenin MV, et al. [15]	RCT, patients were randomized on naltrexone (50 mg/day) + fluoxetine (20 mg/day), naltrexone + placebo, fluoxetine + placebo, or placebo for 6 months; individual psychotherapy for the maintenance of remission was applied for all gropus (one session).	n=280 patients with heroin addiction	Remission rate, maintenance of remission	Naltrexone + fluoxetine was more effective than fluoxetine + placebo (p<0.01) and placebo (p<0.001) in the remission percentage. Naltrexone was superior to placebo and fluoxetine in the efficacy of maintenance of remission and preventing relapse in patients with heroin addiction. The combination of naltrexone and fluoxetine was more effective compared to naltrexone monotherapy only in women.	Naltrexone and fluoxetine could be useful in patients with heroin dependence, especially in women.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Zarghami M, Sheikhmoonesi F, Ala S, et al. [40]	12-week RCT, olanzapine (2.5-15 mg) vs. sodium valproate (600-1000 mg).	n=201 patients on methadone maintenance treatment	Aggressive behavior, urine toxicology.	Olanzapine and sodium valproate reduced the overt aggression and subscales of irritability, aggression, and suicidality. Olanzapine-treated patients presented more improvement in aggressivity. The percentage of positive urine samples (morphine, cannabis, methamphetamine) were not significantly different between the study groups.	Olanzapine and sodium valproate may be useful as adjunctive agent in decreasing aggressive behavior in heroin dependent individuals during methadone substitution treatment, with a slightly superior effect of olanzapine.	4
Salehi M, Zargar A, Ramezani MA [39]	Double-blind RCT, dextromethorphan vs. placebo; 6-month follow-up.	n=72 opiate abusers with methadone maintenance therapy	Methadone consumption dosage, quality of life, withdrawal symptoms.	The mean methadone consumption in the dextromethorphan-treated patients vs. control group were 62.7 mg/day vs. 70.4 mg/day. Total mean score of the quality of life was superior in the intervention group ($p>0.05$).	Dextromethorphan might be useful for opioid dependence treatment, but the differences vs. placebo were not significant.	5
Brooks AC, Comer SD, Sullivan MA, et al. [17]	Quasy-experimental comparison, long-acting injectable naltrexone ($n_1=42$) vs. oral naltrexone ($n_2=69$). Psychosocial therapy was also initiated for these patients.	n=101 patients on naltrexone	Days retained in treatment, severity of drug use	Long-acting injectable naltrexone had been associated with better outcome than oral naltrexone on days retained in treatment and other measurements for opiate use, while still other measurements for opiate use were not significantly different; heroin users with more severe baseline use showed better retention with oral naltrexone maintenance therapy, while less severe heroin users evidenced better outcomes when treated with long-acting injectable naltrexone.	Superior outcomes for heroin-dependents who received long-acting injectable naltrexone compared to oral naltrexone.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Mysels DJ, Cheng WY, Nunes EV, Sullivan MA [21]	Prospective evaluation using Hamilton Depression Rating Scale in patients treated with naltrexone depot maintenance and relapse prevention therapy; 2 and 4 -week post-baseline evaluations.	n=34 patients on naltrexone	Depression severity	Significant improvement of depression scores after 4 weeks post-baseline ($p<0.0004$), somatic and cognitive-affective subscales scores significantly decline; late insomnia increased significantly at 2 weeks post-baseline.	Naltrexone induction and maintenance does not worsen depression, but it may cause sleep impairments	2
Myesels DJ, Vosburg SK, Benga I, et al. [10]	Retrospective chart review; patients were maintained on methadone (n=16) or naltrexone (n=20);	n=36 opiate dependent patients stabilized on pharmacological treatment	Change in body weight from baseline to 3 months and 6 months into treatment.	No difference in weight was detected at baseline, 3 or 6 months between the two treatment groups.	No statistically difference over the weight between methadone and naltrexone maintenance treatment.	2
Schottenfeld RS, Chawarski MC, Mazlan M [11]	Double-blind RCT, 24 weeks; oral naltrexone vs. buprenorphine vs. placebo	n=126 detoxified heroin-dependent patients	Days to first heroin use (urine drug tests), days to heroin relapse, maximum consecutive days of heroin abstinence, reductions in HIV risk behaviours over 6 months.	Days to first heroin use, days to heroin relapse (3 consecutive positive tests), and maximum consecutive days abstinent had all supported the superiority of buprenorphine (significant to placebo). Differences between buprenorphine and naltrexone were not significant for days to heroin relapse and maximum consecutive days abstinent. Differences between naltrexone and placebo were not significant for any outcomes. HIV risk behaviours were significantly reduced by all three treatments.	Buprenorphine was superior to naltrexone, although for the most variables monitored the differences were not statistically significant.	5
Ngo HT, Tait RJ, Hulse GK [18]	Retrospective longitudinal follow-up; methadone ($n_1=522$) or naltrexone implant ($n_2=314$) as maintenance treatment for opioid- dependent patients. Evaluations at 6 months and 3.5 years post-treatment.	n=836 patients treated for opioid dependence	Hospital admissions rates, adjusted changes in risks and rates of overdose-related and non-overdose-related hospital morbidity associated with opioid vs. nonopioid drugs.	Naltrexone implant decreased risk for overdose and non-overdose-related events at 3.5 years, but not methadone; 5 drug-related deaths after methadone and 1 after naltrexone implantation.	Naltrexone implants, but not methadone, has been associated with long-term benefits in decreasing opioid-related hospital morbidity.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Gerra G, Di Petta G, D'Amore A, et al. [41]	12-week prospective, observational trial; open-label study; olanzapine vs. fluoxetine/ paroxetine+ clonazepam for controlling aggressiveness.	n=67 patients with heroin dependence undergoing substitution therapy	Hostility scores, incidence rates of aggressive incidents and attacks.	Direct aggressiveness, overall hostility scores, verbal aggressiveness scores, aggressive incidents rates were significantly lower compared to baseline in patients treated with olanzapine who comple-ted the treatment. Among completers, almost 70% achieved full substance abuse remission, 30% partial substance abuse remi-ssion, without signifycant differences between groups.	Olanzapine may be useful as adjunctive treatment in reducing aggressive/hostile behavior in heroin addicted patients who undergo maintenance substitution therapy.	3
Dean AJ, Saunders JB, Jones RT, et al. [12]	Open-label RCT, naltrexone 50 mg/day (n_1=42) vs. methadone (n_2=38) in opioid dependent patients who were stablized on methadone maintenance treatment; 6-month duration.	n=80 patients stabilized on methadone	Depressive symptoms severity, anxiety symptoms severity.	Patients who received naltrexone did not exhibit worsening of depressive symptoms; in patients attending follow-up assessments, those who received naltrexone presented an improvement in depression over time, compared to the control group; adherence to naltrexone correlated with lower depression severity scores.	Naltrexone has a positive impact over depressive symptoms during maintenance treatment in opioid dependent patients.	3
Hulse GK, Tait RJ, Comer SD, et al. [22]	Cohort study, pre-post design, data were prospectively collected; patients were evaluated before and after treatment with a sustained release naltrexone implant; a subgroup (n=146) had previously received treatment with oral naltrexone.	n=361 heroin dependent persons	Primary outcome was overdose events.	21 overdoses involving 20 persons in the 6 months pre-treatment; none was observed in the 6 months post-treatment; blood naltrexone levels at or above 3 ng/ml for approximately 6 months after injecttion; reduced number of opioid overdoses were also observed 7-12 months after implant; for those previously treated with oral naltrexone, more opioid overdoses occured in both 6-month prior to and after oral compared to the 6-month post-implant treatment.	Sustained-release naltrexone implant is efficient in preventing opioid overdose.	2

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Comer SD, Sullivan MA [32]	Detoxified, non-treatment-seeker-heroin dependent patients; 8 weeks; 0, 30, 60 mg/day memantine as maintenance treatment; intranasal heroin (0, 12.5, 50 mg); inpatient regimen.	n=8 patients with heroin dependence	Subjective ratings of drug quality, liking, willingness to pay for drugs, craving.	Memantine produced modest reductions in subjective ratings of drug quality, liking, willingness to pay for the drug, and craving for heroin. It had little influence over the reinforcing heroin effects.	Memantine could be a useful agent for decreasing the subjective but not the reinforcing effects of heroin.	2
McRae AL, Sonne SC, Brady KT, et al. [43]	RCT, 12-week, placebo-controlled study; patients on methadone who presented also anxiety symptoms; buspirone vs. placebo.	n=36 patients receiving methadone maintenance treatment	Anxiety symptoms	Buspirone did not significantly decrease anxiety symptoms in opioid-dependent patients.	Buspirone had no significant efficacy over anxiety symptoms in subjects undergoing methadone maintenance treatment, but it was associated with reduction in depression scale scores and slower return to substance use.	5
Assadi SM, Radgoodarzi R, Ahmadi-Abhari SA [45]	Double-blind, placebo-controlled, 12-week RCT; patients received fixed-schedule baclofen 60 mg/day or placebo.	n=40 patients with opioid dependence, recently detoxified	The primary outcome was retention in treatment.	Treatment retention was significantly higher in the baclofen group (p=0.01); 30% of patients treated with baclofen completed the trial vs. 10% patients on placebo. The rates of opioid-positive urine samples did not differ significantly between the two groups. Baclofen was superior to placebo in terms of opiate withdrawal syndrome and depressive symptoms. Intensity of opioid craving and self-reported opioid and alcohol use were more favourably influnced by baclofen, although not statistically significant. The drug side effects of the two groups were not significantly different.	Baclofen could be useful in the maintenance treatment of opioid dependence, but the sample size was small, and the attrition rates were high.	5

Authors	Study design, description of therapeutic intervention	Patients	Primary outcomes	Results	Conclusion	LOS
Kumar P, Jain MK [13]	Open label trial; patients received clonidine (for detox) and later naltrexone p.o. (50 mg BD) as maintenance therapy vs. naltrexone plus gabapentin (1200 mg/day) for 3 weeks.	n=20 opioid dependent patients (pentazocine)	Muscle pain and craving (rated on visual-analogue scale), psychological dependence severity.	Patients receiving naltrexone + gabapentin scored significantly lower on both parameters- pain score and craving score (p<0.001), and psychological dependence was significantly lower in this group (p<0.01).	Gabapentin is efficient in controlling muscle aching, and no tolerance effect was observed after 3 weeks. Gabapentin may reduce pain in opioid dependent patients when added to naltrexone, signaling a possible synergistic effect. Gabapentin may help in controlling the opiate craving and potentiated the effect of naltrexone in this regard.	3
Farren CK, O'Malley S [14]	Double-blind, placebo-controlled trial, naltrexone 50 mg qd plus placebo vs. naltrexone 50 mg plus sertraline 50 mg qd; 12-week monitoring duration.	n=13 recently non-depressed abstinent opiate addicts	Depression severity, opiate craving, adverse events	Similar side effect profile in both groups, and similar retention rates. Significant negative correlation between Beck Depression Inventory scores and duration in treatment in the combined therapy group. Opiate craving decreased over time in both groups.	Naltrexone plus sertraline was well tolerated and initially successful in increasing treatment retention, but the difference to the naltrexone alone diminished in time.	4

Based on these results a number of recommendations can be made in patients who need maintenance treatment for opioid use disorder, if a drug without opioid agonistic properties is preferred (table 3). These recommendations have been hierarchized according to the GRADE system [35].

Regarding the limitations of current review it can be mentioned the overall low quality design of the clinical research, only 13 articles included double-blind, controlled, randomized trials. Another limitation is related to the low number of drugs investigated, and except for naltrexone (oral and extended-release), data regarding the efficacy or tolerability of other pharmacological agents were derived from few good quality trials. Only one reviewer was involved in the processing of data collected, so a bias related to this aspect can not be excluded. More research is needed, and larger, well designed trials focused on a more diverse range of drugs are also expected to be launched in order to provide more accurate recommendation in a continuously extending pathology, like the opioid use disorder.

Table 3. Recommendation based on the literature review for patients with opioid use disorder with non-opioid agonist pharmacological agents

Treatment	Clinical trials	Advantages/disadvantages	GRADE recommendations
Naltrexone- oral administration	[9-15]	(+) naltrexone improve depressive symptoms in patients with opioid use disorder at 6 months (+) naltrexone plus fluoxetine and individual psychotherapy has been proven useful in women with heroin dependence (+) naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine (+) naltrexone plus sertraline increased short-term treatment retention of recently non-depressed opioid abstinent patients (-) buprenorphine/naloxone was superior to naloxone in the reduction of opioid-positive urine test and preserving patients participation in the treatment (-) similar weight gain during 6 months of treatment when compared to methadone (-) naltrexone was inferior to buprenorphine (not statistically significant) in several outcomes regarding the rate of relapse in detoxified heroin dependent patients	B
Naltrexone- extended release	[16-23]	(+) efficacy and tolerability were similar to buprenorphine/naltrexone (+) lower rate of relapse when compared to treatment as usual, at least on short-term (+) better outcomes than those treated with oral formulation of the same drug (+) naltrexone implants but not methadone were associated with long-term benefits in decreasing opioid-related hospital morbidity (+) efficient in preventing opioid overdose (+) improved depression severity at 4 weeks post-baseline (+) improved quality of life, rate of retention, opioid-negative urines, opioid craving, rate of re-employment (+) prolonged the duration of abstinence to opioids, in combination with counselling (-) worsened late insomnia (-) requires implant of the active drug (usually subcutaneous)	B+
Topiramate	[25]	(-) negative results in patients with opioid and cocaine use disorder	D-
Ibudilast	[26]	(+) induced lower rates of drug liking (oxycodone), lower craving to heroin/tobacco/cocaine and lower subjective ratings of pain vs. placebo	C
Memantine	[29-32]	(+) may provide an easier transition to buprenorphine-free therapeutic regimen (+) induced attenuation of chronic opioid-dependence-related cognitive impairment (+) decreased the daily dose of methadone (+) it had positive effect over probably NMDA-mediated inflammatory processes in opioid users (+) it decreased the subjective effects of heroin in chronic users (-) it did not decreased the reinforcing effects of heroin in non-treatment seeker heroin-dependent patients	C+

Treatment	Clinical trials	Advantages/disadvantages	GRADE recommendations
Dronabinol	[34]	(+) it decreased the severity of opioid withdrawal (-) no effect over rates of naltrexone-treatment induction or retention	D
Dextro-methorphan	[37-39]	(+) it was useful in low doses as adjuvant treatment in methadone-treated patients both at inflammatory level and clinical level (+) the quality of life was superior in patients who received dextromethorphan as adjuvant to methadone versus placebo plus methadone (-) the difference versus placebo was not significant in the otcome defined as mean methadone consumption	C
Olanzapine	[40,41]	(+) it reduced direct aggressiveness, overall hostility, verbal aggressiveness, and aggressive incidents (+) improved the rate of treatment retention	C
Buspirone	[43]	(+) positive effect over depressive symptoms associated to methadone maintenance treatment (-) no effect over anxiety	C-
Baclofen	[45]	(+) significantly better treatment retention	C-

From the data collected in this review only extended release naltrexone seems to achieve a B+ level of recommendation, based on 8 trials, and the benefits of using extended-release naltrexone tend to overcome its potential disadvantages in patients with opioid use disorder who underwent successful disintoxication. This naltrexone formulation had positive impact over a large number of outcomes related to opioid use disorder, including rate of relapse, quality of life, opioid-related hospital morbidity, and depressive severity [16-23]. As an alternative, oral naltrexone may be tried, although negative trials exist – level B recommendation, based on 7 trials [9-15]. Naltrexone plus fluoxetine and individual psychotherapy has been proven a useful combination especially in women with heroin dependence [15]. Naltrexone plus gabapentin improved muscle pain and psychological dependence severity in patients dependent on pentazocine [13]. Naltrexone plus sertraline were well-tolerated and increased on short-term treatment retention of recently non-depressed opioid abstinent patients [14].

Memantine has a level C+ recommendation for its use as adjuvant treatment in opioid use disorder because there more data to support its efficacy are needed. Low dose memantine attenuated chronic opioid-dependence-induced cognitive impairment and decreased the daily dose of methadone [30, 31]. However, information have been gathered about its positive effect over cognition, inflammatory processes, and decrease of methadone daily dose [29-32].

Olanzapine may be recommended for controlling aggressivity in patients with opioid use disorder, as adjuvant treatment (level C) [40, 41].

Dextromethorphan was associated with some positive results, although it did not decrease the necessary daily dose of methadone when used as adjuvant agent [37-39]. This drug was however useful in improving both clinical and inflammatory outcomes, and improved patients' quality of life [37-39].

Dronabinol did not prove itself efficient in the maintenance phase of treatment, although it may be of some help in alleviating withdrawal symptoms in opioid users [34].

Data for ibudilast, baclofen, and buspirone are insufficient to allow for stronger recommendations in the targeted population, but further research may change this perspective.

CONCLUSIONS

Data showing a continued increase of illegal opioids use are overwhelming, and it became obvious that clinicians must find new solutions for treating a continuously growing population affected by opioid use disorder. Opioid agonists like buprenorphine and methadone are considered now the first line for treatment of opioid dependent patients, but non-opioid agonist drugs should be investigated more, either as adjuvant agents or as monotherapy. These non-opioid agonist agents can lead to the administration of lower doses of opioid agonists (in case of adjuvant treatment), or even to their complete replacement, in both cases diminishing the risk for a new dependence.

From the data reviewed, extended-release naltrexone is the most supported by evidence recommendation, followed by oral naltrexone, in patients who were successfully detoxified from opioids. Naltrexone had positive results not only on the opioid use-related outcomes, but also over associated symptoms, like depression.

As adjuvant agents, olanzapine may be efficient for aggressive behaviours in opioid dependent patients, memantine could improve cognitive function and decrease the inflammatory processes associated with substance use disorders, and dextromethorphan may also help decreasing the inflammatory processes. Further research is needed in order to verify the efficacy and tolerability of olanzapine, memantine and dextromethorphan as adjuvant agents.

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CLINICAL PRACTICE

Survival following repetitive ventricular fibrillation caused by monoamine oxidase inhibitor overdosing (MAOI).

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Abstract: *One of the great dreams of humanity has been the improvement of its behavioural, affective and emotional status. Depression represents the suffering of beings over their existential course, as a point of passage between the human condition and its destiny. The attempts of psychiatry to introduce efficient and increasingly performing antidepressants represent one of medicine's great achievements. At the same time, antidepressant overdosing may lead to cardiotoxic manifestations presenting lethal risks as consequences [1]. We hereby present the case of a patient that was poisoned with tianeptine, ingested for suicidal purposes, who survived repetitive episodes of ventricular fibrillation, electrically converted to sinusal rhythm. The GC/MS analytical diagnosis played an essential part in detecting tianeptine in the biological samples. The rapid and very competent therapeutic intervention allowed for the patient's survival, despite further complications. A review of main tianeptine information is also presented, as this type of intoxication may have deadly consequences.*

Keywords: *tianeptine, ventricular fibrillation, acute intoxication*

INTRODUCTION

Tianeptine (fig. 1) was discovered and patented by the French Society for Medical Research in the 1960s. It is an atypical antidepressant, used mostly to treat major depressive disorder, but also to treat anxiety, asthma and irritable bowel syndrome. Regarding its chemical structure, tianeptine has the structure of a tricyclic antidepressant (TCA), but has a pharmacological profile that is very different from other TCAs and is grouped separately from those due to its unique mechanism of action [1]. As a modulator of the serotoninergic synapse, it has good efficiency in mild/moderate forms of depression associated or not with anxiety; it does not interfere with the metabolism of other drugs at the level of P45 cytochrome. Tianeptine does not have an affinity for muscarinic, alfa1-adrenergic or type 1 histaminergic receptors, which leads to less adverse events compared to TCAs.

Initial studies have shown that following acute and repeated administrations, the presence of tianeptine led to a decrease in extracellular levels of serotonin in rats' brains without a decrease in the release of serotonine, which is why it was called a "(selective) inhibitor of serotonine reuptake".

As opposed to TCAs and selective serotonine reuptake inhibitors (SSRI), tianeptine improves the synaptic uptake function of serotonine; despite all these, its efficiency against depressive episodes is comparable with that of TCAs and fluoxetine [2, 3]. Recently (2014), it has been demonstrated

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that tianeptine is an agonist of opioid receptors (MOR). Thus, as opposed to its therapeutic effects, MOR activation is probably the one responsible for tianeptine's toxicity in high doses, those that far surpass the normal therapeutic interval [1]. The therapeutic levels for tianeptine in whole blood, according to available data, are 0.3–0.7 mg/ml.

The absorption takes place almost entirely (99% bioavailability) at GI level, it is completely metabolized by the liver and it is not affected by the first pass effect [4]. Its distribution in the organism takes place quickly, 95% of it binds to plasma proteins (especially albumine) and the mean plasmatic half-life through distribution is approximately 0.7 hours.

Table 1. Adverse events for tianeptine – classified by frequency.

Type of adverse events	Adverse events
Common (frequency >1%)	Headaches (up to 18%) Dizziness (up to 10%) Insomnia/nightmares (up to 20%) Sleepiness (up to 10%) Dry mouth (up to 20%) Constipation (up to 15%) Nausea Abdominal pain Weight gain (~ 3%) Agitation Anxiety/irritability
Less frequent (frequency 0.1-1%)	Bitter taste Flatulence Epigastralgia Blurred vision Muscular pain Premature ventricular contractions Micturition disorders Palpitations Orthostatic hypotension Tremor
Rare (frequency <0.1%)	Hepatitis Euphoria ECG modifications Pruritus-like cutaneous reactions/alergy Muscular pain

Metabolizing in the liver takes place especially through beta-oxidation and, in a lower proportion, through N-demethylation, the main metabolites being propanoic acid (inactive metabolite) and pentanoic acid (active metabolite) [5]. Tianeptine is eliminated both renally and through extrarenal ways, mostly through urine (only 8 % is eliminated in unmetabolized form), fecces (15%), bile and has a half-life of 2-3 hours. [5].

Compared to other TCAs, tianeptine can lead to significantly less cardiovascular, anticholinergic (such as constipation or dry mouth), sedative and appetite stimulating effects [6, 7]. Recent studies have shown that it is amongst the most hepatotoxic (hepatic lesions) antidepressants, although proof supporting this concern is limited [8]. Also, it has been shown that TCAs can lead to cardiac arrhythmia, even though the data for tianeptine is limited [9].

Based on adverse event frequency, they are classified according to table no. 1 [5, 6, 10].

The certitude diagnosis is established by identifying tianeptine in biological fluids.

Figure 1. Tianeptine 2D Structure form

https://pubchem.ncbi.nlm.nih.gov/compound/Tianeptine#section=Top

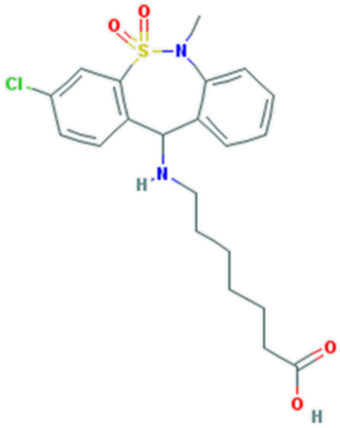

For this purpose, the GC/MS method (gas-chromatography coupled with mass spectrometry) is used. The GC/MS method, applied in the Analytical Toxicology Laboratory of the Military-Medicine Research Center, is performed by a GC/MS Varian SATURN 2000 computerized system: Varian gas-chromatograph STAR 3800 CX model and Varian mass spectrometer with an ion trap detector. The urine samples are analyzed using liquid-liquid extraction with organic solvents. For the extraction control, midazolam was used as an internal standard, which is a benzodiazepine whose retention time is situated in a different range from that of tianeptine. Mass spectra libraries were used, namely Pfleger–Maurer–Weber, NIST 98 and Wiley 6.

Figure 2: Total ion chromatogram and the mass spectra obtrained for lidocaine (peak no. 1), for tianeptine (peak no. 2) and for midazolam (peak no. 3).

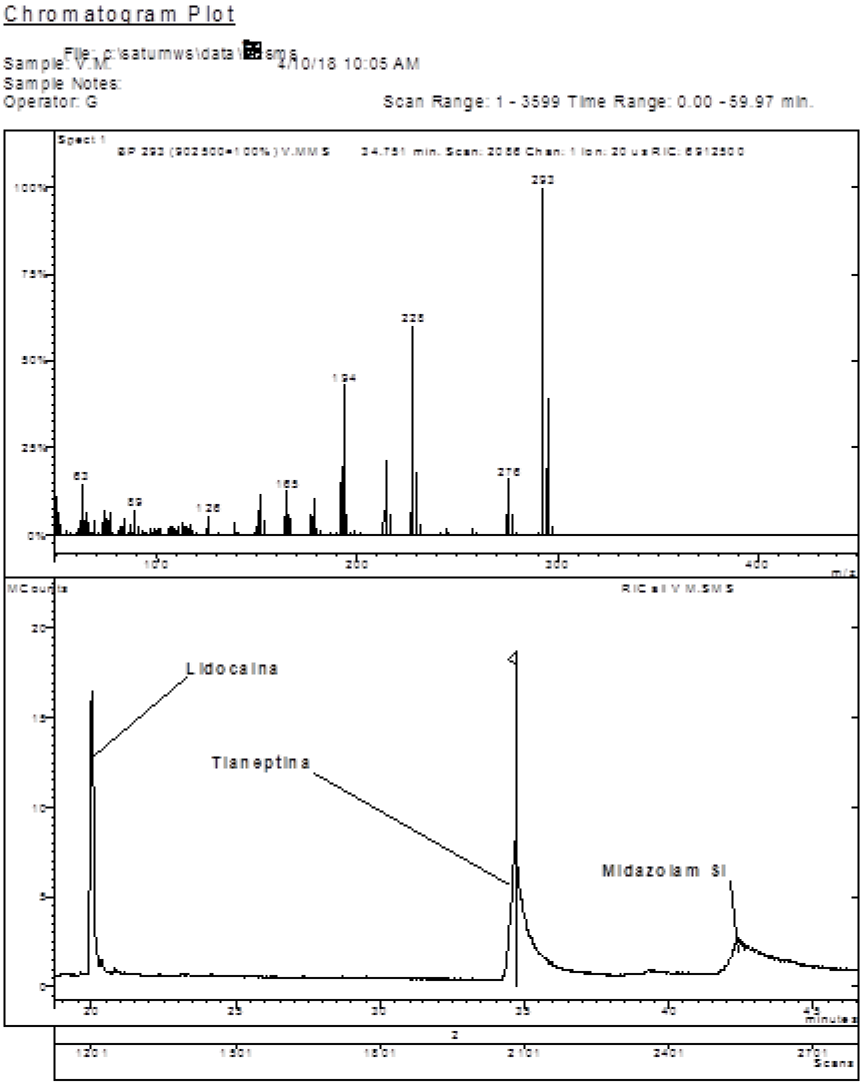

Table 2. The type of compound that was identified, its chemical formula and characteristic spectral lines

Identified compound	Chemical formula	Molecular mass	Spectral lines (m/z)
Lidocaine	$C_{14}H_{22}N_2O$	234	86, 234
Tianeptine	$C_{21}H_{25}ClN_2O_4S$	437	194, 214, 228, 276, 293
Midazolam (SI)	$C_{18}H_{13}ClFN_3$	327	310, 327

Following the analysis of the urine extract through the GC/MS method, the separation and identification of the following compounds were achieved: Lidocaine, Tianeptine and Midazolam (internal standard), presented in figure 2 and table 2.

CASE REPORT

Patient, aged 56 years old, with no pathologic history was brough by the medical ambulance to the Bagdasar hospital after being found at home following drug intake for suicidal purposes (declarated Nobixal –

Tianeptine) with agonistic respiration.

At home, the patient suffered two episodes of ventricular fibrillation, remitted after external electrical shocks (EES) applied by the ambulance medical staff. In the emergency care unit, the patient suffered another episode of ventricular fibrillation which responded to EES, with hemodynamic instability still persisting. Due to acute respiratory insufficiency, orotracheal intubation was necessarily performed.

Cardiological evaluation: arterial pressure 90/60 mmHg, AV 100 bpm, no murmurs, no rales, no edemas; ECG: regular sinusal rhythm, Q wave in DII, DIII, aVF.

The ECG shows a moderate/severe dystolic dysfunction of the left ventricle, most probably in the context of intoxication, followed by an EES, applied for rhythm disorders. The patient is sent to Emergency Clinical Hospital Bucharest, Intensive Care Unit II Toxicology.

Upon admission into Toxicology, the patient is in a serious condition sedated, orotracheal intubation with spontaneus respiration, presence of bilateral pulmonary vesicular murmur, hemodynamic instability on vasopressor support, arterial pressure = 100/58 mmHg, AV = 96/bpm, regular rhythm, normal abdominal movement, normal diuresis.

Biological parameters upon admission: increased values of glycemia, creatinine, liver enzymes (ALT and AST), LDH but also leukocytosis; coagulation tests being in normal ranges.

Table 3. Biological parameters

Sample	Result	
	Measured values	Normal values
Glycemia (*mg/dl*)	138	75-110
Urea (*mg/dl*)	25	19-43
Creatinine (*mg/dl*)	1.46	0.7-1.4
AST (*U/l*)	81	21-72
ALT (*U/l*)	107	14-50
LDH (*U/l*)	481	150-450
Sodium (*mmoles/l*)	144	137-145
Potassium (*mmoles/l*)	4.51	3.50-5.00
Leucocytes ($x\ 10^3/mm^3$)	16,8	4-9
Hemoglobine (*g/dl*)	13.10	12.6-17.2
Trombocytes ($x\ 10^3/mm^3$)	228	150-350
APTT (*sec*)	25.30	24-36.5
PT (*sec*)	13.1	11-14.5
INR	1.08	0.81-1.16

The toxicological urine analysis through the GC/MS method shows the presence of Tianeptine and Lidocaine.

Gastric lavage was applied and activated charcoal was administered through the naso-gastric tube; it were applied volemic, electrolitical and acido-basic repletion and complex clinical – biological monitoring. After 24 hours from admission in ICU II Toxicology, the ECG monitoring registers a new ventriculary fibrillation episode, followed by cardiac arrest. The resuscitation protocol was applied, the maneuvers were efficient. Intensive care measures being were continued, ventilatory support, vasopressor support – with dopamine in the continuous infusion, antiarrhythmic drug – Amiodarone continuous infusion, Lidocaine 1% in infusion. After 96 hours from admission, the patient is hemodynamically stable and the vasopressor support is suppressed; also the patient is weaned from ventilator support. The therapy continued with hepatotrofic and neurotropic drugs, with gastric protectors and general nursing. The evolution was favorable.

Psychiatric evaluation reveals a persistent suicidal idea, depressive facies and a marked depressive disposition, the patient was reffered to a psychiatric unit.

At cardiological reevaluation, 9 days after admission: the patient is hemodinamically stable, arterial pressure 108/77 mmHg, cardiac frequency 77 bpm. Regular rhythm, no murmurs, presence of bilateral pulmonary vesicular murmur, no stasis rales; ECG: sinusal rhythm, cardiac frequency 70 bpm, no ST segment modifications. Cardiac echography: left ventricle not dilated, VSTD 48 mm, not hypertrophied, SIV=PP= 10 mm, no segment kinetics disorders, with conserved ejection fraction 55%, no valve disorders, no pulmonary hypertension; pericardial liquid of 3 mm in diastole, laterally from right ventricle, no hemodynamic impact; diastolic dysfunction – delayed relaxation type.

The patient leaves the hospital toxicologically healed, conscious, cooperative, hemodynamically and respiratoriy balanced; stable from a cardiological point of view under antiarrhytmic treatment with Amiodarone.

Figures 3, 4: Cardiac rhythm abnormalities (episodes of polymorph ventricular tachycardia in the context of long QT syndrome) in the absence of diselectrolithemia, acidosis or hypoxemia after tianeptine overdose.

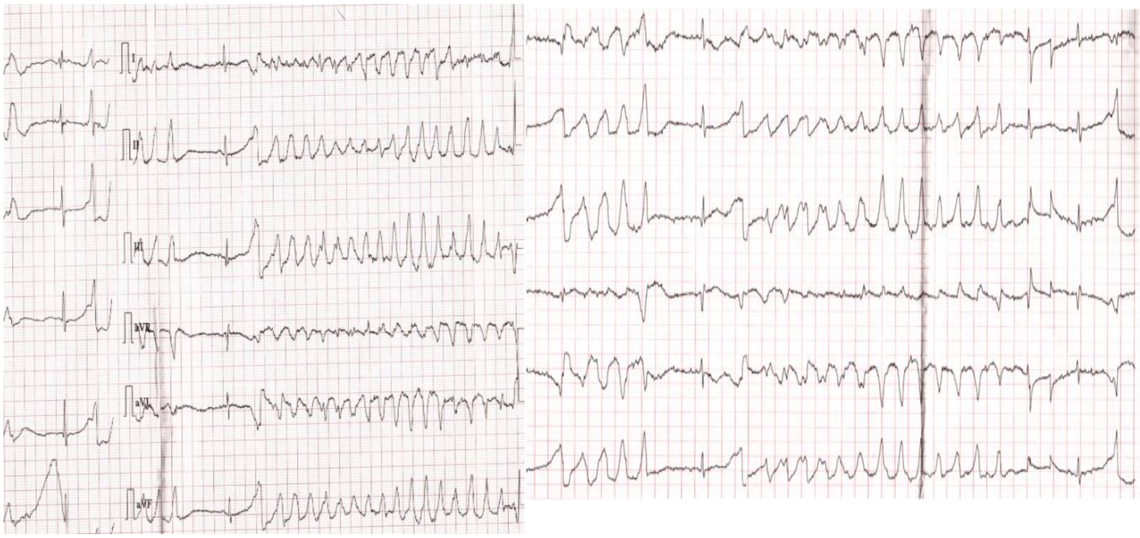

CONCLUSIONS

Although compared to TCAs, which have been proven scientifically to cause cardiac arrhythmia, the data for tianeptine is limited, the above case reiterates the possibility, even low, but with a deadly risk associated, of malignant arrhythmias being caused by it.

Although proof supporting the hepatotoxic potential of MAOI is limited, overdosing on the aforementioned generated phenomena of hepatic cytolysis, which is indicated by increased enzyme levels.

A correct management of acute poisoning with unclear substances, whether accidentaly or voluntary, involves a precise assessment and promptly initiated therapy.

GC/MS analytical method is the confirmatory method for urinary toxicological screening for a diagnosis of certainty in the case of acute drugs poisoning and is important in order to establish an effective therapeutic course.

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Principles of antimicrobial therapy in urosepsis: an approach from the perspective of the infectious diseases physician

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Abstract*: Background: This topic is aimed to review the treatment approach in adult patients diagnosed with urosespsis, in order to decrease mortality and to maximize the outcome of the antibiotic stewardship efforts.*

Methods: We present an approach of antibiotic therapy in urosepsis from an infectious diseases physician perspective, based on the recently published data integrating the epidemiology of the urinary tract infections in a multidisciplinary hospital from Romania.

Results: The principles of therapy in urosepsis should have five goals, but not limited to rapid diagnosis, specific intensive care unit treatment, obtaining urine and blood cultures, anti-infective treatment and identifying and correction of underlying risk factors. Due to the higher prognostic value, the antimicrobial therapy in urosepsis should consist in "two steps" regimen: initial antimicrobial therapy, based on "empirical" approach for the first 48 - 72 hours and directed or "definitive" antimicrobial therapy adapted to the susceptibility profile of the isolated pathogens, the penetration into the urinary tract and the patient clinical response. Clinicians should be aware of the different antibiotic susceptibility profile of the bacteria from the hospital compared to the community. Thus, the initial choice of antimicrobial agents depends on several criteria like the severity of illness and the risk factors for multidrug resistant pathogens: the immune status of the patient (age, debilitating chronic illnesses, immunosuppressive treatment or uncontrolled malignancies), broad-spectrum antimicrobial use and health care environment exposures in the previous 3 to 6 months, susceptibility of prior urinary isolates, and local community resistance prevalence of bacteria.

Conclusions: Our important messages highlighted the need of a holistic approach of urosepsis, in a multidisciplinary team, with integration of risk factors for AMR, focusing on the first phase of antibiotic treatment as the main driven of the survival rate.

Keywords: *urosepsis, antimicrobial therapy, infectious diseases, antibiotic resistance*

INTRODUCTION

In about 30% of the sepsis cases, the underlying condition is related to the urinary tract infections (UTIs). The main risk factors are obstructive uropathies and most frequently, urosepsis develops in the context of health care–associated (HCA) UTIs [1]. The prognosis of urosepsis is more favorable compared to that of sepsis in general, with a mortality rate about 20–40% for severe cases. Unlike etiology in all-cause sepsis, dominated by gram-positive bacteria (GPB), the most common pathogens isolated

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in urinary sepsis are gram-negative bacteria (GNB) [2]. In March 2018, the Centers for Disease Control and Prevention released the “Hospital Toolkit for Adult Sepsis Surveillance”, a guidance useful for hospitals to identify sepsis outcomes and evaluate the effectiveness of sepsis prevention efforts [3].

In our perspective, one of the main causes of failure to antibiotic treatment in sepsis is related to the antimicrobial resistance (AMR) and inadequate spectrum of the prescribed antibiotics at the beginning. Therefore, this topic aims to review the treatment approach in adult septic patients with acute complicated UTIs, in order to decrease mortality and to follow the antibiotic stewardship recommendations.

ETIOLOGY AND SUSCEPTIBILITY OF ISOLATED STRAINS

Worldwide, the etiology of urosepsis is dominated by enterobacteriaceae, with Escherichia coli (E. coli) accounting for about 52% of the cases, followed by Klebsiella species (spp), Pseudomonas aeruginosa, Enterobacter spp and GPB, such as enterococci (5%) [4]. In 2017, among the strains isolated from the urology department of our Romanian tertiary hospital, E. coli was encountered in 59.2% of all cases of UTIs, followed by other GNB like Klebsiella pneumoniae (K. pneumoniae) [unpublished data].

It has been reported that the resistance rates of pathogens in urosepsis are higher than 10% for almost all antibiotics. The European Antimicrobial Resistance Surveillance Network for 2016 showed that more than half (58.6%) of the E. coli isolates were resistant to at least one of the antimicrobial groups under regular surveillance (aminopenicillins, fluoroquinolones, third-generation cephalosporins, aminoglycosides and carbapenems) [5].

According to the European Centre for Disease Prevention and Control, the level of E. coli resistance to carbapenems remained rare in 2016 (< 0.1%), with a national percentage ranging from 0% to 1%, meanwhile for K. pneumoniae, data showed large differences, ranging from 0% to 66.9% [6]. Carbapenem-resistant enterobacteriaceae (CRE) represents a significant threat to patients and healthcare systems in all European countries, being associated with high mortality, especially due to the delay in administration of effective treatment and limited treatment options [6]. Despite the fact that in 2016 the E. coli carbapenem resistance level in Romania has dropped from 1.9% to 1%, the reported rates of AMR are very high and rising in comparison to other European countries [7].

MANAGEMENT

In the management of septic patients, we must consider that sepsis is a life threatening condition with a directly dependent prognosis on the timeliness and accuracy of therapeutic intervention. In the latest update of the Surviving Sepsis Campaign (SSC) bundle from 2018, the most important change of the 2016 guidelines was that the 3-h and 6-h bundles have been combined into a single “hour-1 bundle” [8]. Clinicians should initiate antibiotic treatment immediately, especially in patients with hypotension, rather than waiting and extending resuscitation measures. Other measures such as obtaining blood samples, urine and blood cultures, administration of fluids and antibiotics, should all begin immediately [8].

The principles of therapy in urosepsis should follow five rules [9]. First of all, we have to establish a rapid diagnosis based on clinical and paraclinical tests: classical symptoms (dysuria, frequent urges to urinate, flank pain and/or costovertebral angle tenderness, fever, chills, pyuria), quick Sepsis Related Organ Failure Assessment score (qSOFA), biomarkers of sepsis and imaging findings [9].

In addition, a specific intensive care unit (ICU) treatment should be start with fluid replacement, supportive and adjunctive therapy as needed [9]. Obtaining urine and blood cultures prior to antibiotic administration represent another important rule [9].

On the other hand, initiation of antibiotic treatment must be adapted to the patient profile (usually broad spectrum antibiotics) after the quick assessment by the clinician and/or infectious diseases specialist [9]. The last but not least rule is addressed to identification and correction of risk factors (calculus, obstruction, renal or perinephric abscess) by the urologist [9].

ANTIBIOTIC TREATMENT

Broad-spectrum antibiotic therapy aiming to cover all likely pathogens should be started immediately, ideally in the first hour. Data from the literature have shown an association between the rapid administration of antibiotics and improved survival in patients with sepsis. Initiation of empiric antibiotic treatment within the first hour of hypotension is associated with a survival rate of about 80% [10]. Each additional hour of delay was found to decrease the survival rate by 7.6% [10].

The initial choice of antimicrobial agents depends on several criteria like the severity of illness and the risk factors for multidrug resistant pathogens: the immune status of the patient (age, debilitating chronic illnesses, immunosuppressive treatment or uncontrolled malignancies), broad-spectrum antimicrobial use and health care environment exposures in the previous 3 to 6 months, susceptibility of prior urinary isolates, and local community resistance prevalence of bacteria. Clinicians should be aware of the different antibiotic susceptibility profile of the bacteria from the hospital compared to the community. Therefore, an accurate classification of the UTIs according to the place of acquisition of the infection is mandatory. There have been defined two major categories of infections comprising of community-acquired (CA) UTIs or health-care associated (HCA) UTIs with the following scenarios: community onset HCA UTIs or hospital onset HCA UTIs. In CA-UTIs, the possible pathogens are limited usually to susceptible enterobacteriaceae, ampicillin-susceptible Enteroccous faecalis or Staphylococcus saprophyticus. In HCA UTIs, in general we are dealing with resistant strains of bacteria such as extended spectrum beta-lactamases (ESBL)-producing enterobacteriaceae or CRE. It has been shown that inappropriate antibiotic treatment in critically ill patients was associated with decreasing in survival rate of at least 5 times in septic shock [11].

Table 1. Choice of antibiotic therapy in urosepsis

“Empirical” phase of antimicrobial therapy *(combination therapy (ß-lactam antibiotics plus the second antimicrobial agent) is preferred especially in critically ill patients with high risk of death*		
Community-acquired UTIs# *# in some regions the risk of ESBL enterobacteriaceae has to be considered also in community-acquired UTIs*	**Health-care associated UTIs**	
	without risk of non-fermenting GNB or hemodynamically stable patient	*risk of non-fermenting GNB or hemodynamically unstable patient*
Aminopenicillins +/- beta lactamase inhibitor (BLI) (Amoxicilin/ clavulanate) 3rd generation cephalosporins (e.g. Ceftriaxone) Fluoroquinolones (e.g. Ciprofloxacin, Levofloxacin) Aminoglycosides (e.g. gentamicin)	Carbapenems (e.g. Ertapenem) Ureidopenicillin plus BLI (e.g. Piperacillin/tazobactam) Aminoglycosides (e.g. Gentamicin, Amikacin, Netilmicin, Tobramycin) Glycopeptides (e.g. Vancomycin)	Carbapenems (e.g. Imipenem, Meropenem, Doripenem) Carbapenems plus BLI (e.g. Meropenem/vaborbactam) Ceftolozane/Tazobactam Ceftazidime/Avibactam Monobactams (Aztreonam) Colistin Glycopeptides (e.g. Vancomycin) Aminoglycosides (e.g. Gentamicin, Amikacin, Netilmicin, Tobramycin)
“Directed” phase of antimicrobial therapy±,¥		

±according to the antibiotic susceptibility profile of the detected pathogens

¥monotherapy is preferred (exception of critically ill patients with high risk of death and infections with CRE)

The antimicrobial therapy in urosepsis consists in “two steps” regimen:

a) Initial antimicrobial therapy, based on “empirical” approach, should starts ideally, after obtaining blood and urine cultures, during the first hour of the patient presentation in the emergency room or after the diagnosis of urosepis in case of hospitalized patients. Due to the prognostic value of this treatment phase, as a general rule, the broad-spectrum antibiotics with good penetration into the urinary tract are preferred for the first 48-72 h [8];

b) Directed or “definitive” antimicrobial therapy represent an adaptation of the initial regimen (e.g. de-escalation) according to the antibiotic susceptibility profile of the isolated pathogen, taking into account the clinical evolution of the patient [8]. The duration of definitive therapy should have a minimum duration with maximum efficiency, usually between 10 to 14 days [2];

Based on our clinical experience and the evidence base medicine, we present a personal approach, not validated in clinical trials, regarding the choice of antibiotic therapy in urosepsis (Table 1).

In addition to antimicrobial therapy, an underlying condition such as urinary obstruction, anatomical or functional urinary tract abnormalities (neurogenic bladder, bladder catheters, nephrostomy tubes, and ureteral stents), should be rapidly identified and managed [12]. One of the important issues is related to the appropriate collection of the urine sample in case of urinary tract obstruction. In case of kidney drainage (e.g. percutaneous nephrostomy) we must collect also the urine from the inserted tube in addition to the usual urine sample.

ASSESSMENT OF TREATMENT RESPONSE

Usually, symptoms improve rapidly when antimicrobial treatment is effective, but the fever can persist at least 3 days despite the appropriate antibiotic treatment. The response to treatment is assessed by the following criteria: clinical (fever remission), biological (decreasing levels of inflammatory markers, sepsis biomarkers, alleviating organ dysfunction) and bacteriological (negative urine and blood cultures). In the PRORATA trial, a multicentre randomized controlled trial, it has been evaluated the use of procalcitonin (PCT) to reduce the patient's exposure to antibiotics in ICU. It has been shown that a strategy consisting in measurement of PCT concentration on a daily base allowed for a reduction of antibiotic consumption in ICU [13]. Also, other studies have been proposed a PCT-based algorithm for starting, continuing or stopping antibiotics in patients with sepsis in the ICU [14]. A Swiss randomized controlled trial showed that an algorithm based on PCT concentration and quantitative pyuria measurements, significantly reduces antibiotic exposure, with about 30%, without apparent negative effects on clinical outcomes [15]. In case of persistent or worsening symptoms after 72 hours of appropriate antimicrobial therapy, an additional evaluation is required in order to identify potential factors that might interfere with the favorable clinical outcome. Dosing reevaluation, especially for aminoglycosides and glycopeptides, surgical drainage of the new abscesses, assessment of the inserted tube position and permeability, repeating urine and blood cultures and readjusting treatment to the antibiotic susceptibility profile of other isolated pathogens, should be considered.

CONCLUSIONS

Our important messages highlighted the need of a holistic approach in urosepsis, provided by a multidisciplinary team, with integration of risk factors for AMR, focusing on the first phase of antibiotic treatment as the main driven of the survival rate. The new sepsis "hour-1 bundle" and the principles of antimicrobial therapy should be followed in all emergency departments as a clinical tool intended to improve the general prognosis in urosepsis.

Author Contributions

Concept: VG, AMR; Design: VG, IS, AMR; Supervision: VG, IS, AMR; Materials: CG, FA, AMR; Data collection and/or processing: CG, FA, AMR; Analysis and/or Interpretation: CG, FA, IS; Literature Search: CG, FA, AMR; Writing Manuscript: VG, AMR; Critical Review: VG, IS;

Disclaimer

The authors have no conflict of interest to declare.

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Primary thyroid lymphoma – case report

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Abstract*: Introduction. Even though, primary thyroid lymphoma (PTL) is a rare thyroid malignancy and extranodal non-Hodgkin lymphoma, this type of disease should not be disgraced in order to achieve an early diagnosis and multimodal treatment.*

Materials and method. We selected for discussions a primary thyroid lymphoma patient with absent constitutional (B-type) symptoms, who undergoes a multidisciplinary approach of the disease, chemotherapy and radiotherapy. In this clinical case report a 84 yo male patient with stage IE diffuse large B-cell histological type (Ann Arbor staging) was treated using R-CHOP chemotherapy, followed by involved field radiotherapy, with an excellent treatment response and disease control.

Results and Discussion. Usually this type of extranodal non-Hodgkin lymphoma is present in the seventh decade of life, with males being affected earlier than females. One of the most common presentations of thyroid lymphoma is an enlarging neck mass and a history of Hashimoto's thyroiditis, which is the major risk factor. The most common PTL subtypes are diffuse large B-cell lymphoma (the most aggressive histological subtype, representing 70% of all PTLs) and MALT lymphoma (30% of the PTLs). Advances in both diagnosis and treatment in the last years have improved the early diagnosis and shortened the time for treatment implementation.

Keywords: *primary thyroid lymphoma; non-Hodgkin lymphoma; thyroid cancer; radiotherapy*

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INTRODUCTION

Even though lymphoma is one of the most common hematological malignancies, primary thyroid lymphoma (PTL) is very rare and accounts for 0.5-5% of all thyroid malignancies and about 2% of extranodal lymphomas [1]. Chronic Hashimoto's thyroiditis it is one of the main risk factors known to increase incidence of this type of malignancies [2]. PTL is a rare extranodal lymphoma which is defined as lymphoma that involves just the thyroid or the thyroid and the regional lymph nodes, without metastasis to other areas at the time of diagnosis [3].

Treatment and prognosis varies according to history and clinical stage. Surgery, chemotherapy, radio-therapy or their combination are the treatment of choice in this particular type of Non-Hodgkin Lymphoma (LNH). Such a case, with PTL, is presented in this article [4].

CASE REPORT

An 84 years old man, known with Hashimoto's autoimmune thyroiditis, with no other relevant personal or family history, visits the oncology department for recent-onset gross neck mass, throat discomfort, dysphagia and hoarseness (noticed about 1 month before presentation). The patient had no fever, night sweats or weight loss. The physical examination reveals firm, immobile and painless mass descending in the anterior chest wall. Thyroid hormones at presentation revealed high antithyreoglobulin antibodies, an increased thyrotropin level and normal thyroxin. The ultrasound showed a macronodular goiter and chronic lymphocytic cells. On the CT examination of the head and neck region, showed an enlarged thyroid gland, with a nodular structure, starting from the hyoid cartilage and ending in the upper mediastinum (Figure 1). The endotracheal biopsy and immune histological analyses led to the diagnosis of a Non-Hodkin diffuse large B-cell lymphoma (DLBCL), CD20+, Ki-67>90%. A bone biopsy was requested for disease staging, which showed no evidence of neoplazic cells on the examined tissue. Also, the CT scan of thorax, abdomen and pelvis showed no other signs of evolution.

Figure 1 (explication in text)

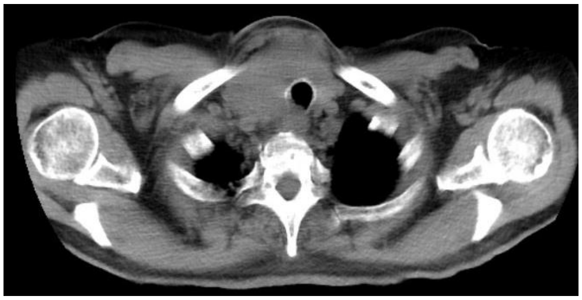

Regarding the fact that only the thyroid was involved (which is an extranodal site, on the same side of the diaphragm) and the fact that the patient had no B symptoms (fever, night sweats or weight loss), the stage of the disease, at the time of the diagnosis, was stage I AE.

After the diagnosis, the patient underwent 4 cycles of chemotherapy with R-CNOP chemotherapeutic protocol, i.e. rituximab (anti CD-20 antibody) 1400 mg, mitoxantrone 14 mg, cyclophosphamide 1350 mg, vincristine 2 mg and dexamethasone 16 mg. The treatment, after 4 cycles of chemotherapy, was stopped because of low tolerance. At the PET-CT scan, following chemotherapy was highlighted high metabolic activity in the right thyroid lobe.

Radiation therapy (RT) was recommended after chemotherapy. A total dose of 36 Gy (18 fractions, 2Gy/fraction) was administrated, using involved field technique to delineate the clinical target volume (CTV). For treatment preparation a fusion between PET-CT and planning CT-scan had to be used for accurate CTV delineation. Three months after radiotherapy, another PET-CT scan was made, to evaluate treatment response, showing low metabolic activity in the right thyroid lobe. The follow-up at 9 months post-chemoradiation was done using clinical examination and a CT scan, which showed a marked regression of the tumor mass (Figure 2).

Figure 2 (explication in text)

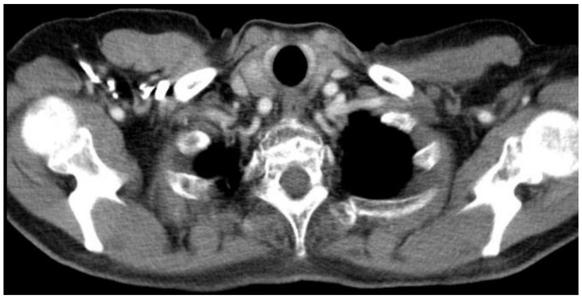

DISCUSSIONS

In general population this type of disease is rare, with an annual incidence rate of 2.1 persons per milion [6]. Also, it is known that PTL affects mainly women (5x times more common in women than men) between 50 and 80 years of age, whereas its incidence is low in individuals younger than 40 years of age [7].

We presented a patient with chronic lymphocytic thyroiditis (Hashimoto's thyroiditis), high levels of antithyroglobulin antibodies in different blood tests. These types of patients, with Hashimoto's disease, have a much higher risk of PTL than the general population. It has been presumed that abnormalities in immunity due to an approximately 20-year history of Hashimoto's disease increase the risk of developing PTL [8].

Clinical findings in our patient on presentation, made us consider an anaplastic carcinoma of the thyroid gland, because of the overlapping symptoms. After tumor and bone biopsy, the initial assumption was changed into primary thyroid B-cell lymphoma. The bone biopsy was made to exclude systemic involvement [9].

The current standard of care is chemotherapy followed by radiotherapy. The role of surgery has been diminished, because an aggressive debulking surgery would not have a more successful response over chemo-radiotherapy [10]. The recommended treatment was 6 cycles of R-CHOP followed by adjuvant RT [5]. In our case it was used R-CNOP chemotherapy because of mitoxantrones lower cardiotoxicity than doxorubicin, but with the same therapeutic outcome as the R-CHOP scheme [11]. RT treatment plan was realized by applying involved field radiotherapy technique, which included in the CTV the whole thyroid gland. A total dose of 36 Gy (2 Gy/fraction, 18 fractions) was administered at the end [7, 12].

An excellent prognosis for early stage PTL (stage IE and stage IIE) is achieved with proper modern treatment management and a multidisciplinary approach. A 5-year survival rate of 87.5% has been observed in patients with DLBCL type. Using modern treatment (combination of chemotherapy plus rituximab agent and radiotherapy), a good response is observed in this type of patients [7].

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The complex implications of dermatoses specifically associated with pregnancy

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Abstract*: Pregnancy-specific dermatoses are a heterogeneous group of inflammatory dermatoses associated exclusively with pregnancy. Although these dermatoses are rare, it raises problems in medical practice because of their appearance, being difficult to diagnose, treat and the possibility of the fetus being affected. Pemphigoid Gestationis (PG) is a rare, autoimmune, strongly pruritic, vesiculobullous dermatosis with a strong impact on the patient. Polymorphic Eruption of Pregnancy (PEP) is a benign, self-limited inflammatory skin disorder that usually affects first pregnancy in the third trimester or immediately in the postpartum period. Other dermatoses associated with pregnancy include: Intrahepatic Cholestasis of Pregnancy (ICP), Atopic Eruption of Pregnancy (AEP) and Pruritic Folliculitis of Pregnancy (PFP). We will extensively present PG and PEP, to draw attention to the importance of correct diagnosis and the implications of treatment during pregnancy.*

Keywords: *specific dermatoses in pregnancy, pemphigoid gestationis, polymorphic eruption of pregnancy*

INTRODUCTION

During pregnancy endogenous, immunological, metabolic and vascular complex changes can result in skin damage. The skin changes can be classified into: physiological skin changes, alteration of preexisting cutaneous conditions and specific dermatoses in pregnancy.

Pregnancy-specific dermatoses are a heterogeneous group of inflammatory dermatoses associated exclusively with pregnancy and/ or immediately after birth. Some are considered a significant risk to the fetus.

These relatively rare conditions, often with variable clinical appearance, and the lack of definitive diagnostic tests have led to confusion in terms of proper diagnosis and management of pregnancy-specific dermatoses.

While many physicians are familiar with normal physiological changes during pregnancy, the knowledge of dermatoses in pregnancy, which is rarely encountered, is often minimal. This review is designed to highlight pregnancy-related

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dermatoses for easier diagnosis and management.

PEMPHIGOID GESTATIONIS (PG)

Pemphigoid gestationis (PG) is a rare, autoimmune, strongly pruritic, vesiculobullous dermatosis, with an incidence of 1: 50,000 to 60,000 pregnancies in USA [1]. The results of European studies suggest a global incidence of 0.5 cases per million people per year [2]. It occurs more frequently in the white race, which may reflect the association with specific HLA haplotypes. Most often, the disease occurs in the second or third trimester of pregnancy, but cases have also been reported in the first trimester and postpartum period [3].

The etiology of the disease is unknown. Nearly half of the cases develop during the first pregnancy. Women with a history of PG are considered to have a higher prevalence of autoimmune diseases, including Graves’s disease, Hashimoto's thyroiditis, vitiligo, autoimmune thrombocytopenia, and pernicious anemia [4].

PG is associated with the following antigens: HLA-B8, HLA-DR3 and HLA-DR4 or combinations of HLA-DR3 and HLA-DR4 [5]. The presence of HLA-DR3/DR4 was highlighted in 45% of patients with PG compared with 3% of the general population, and all patients with a history of PG have anti-HLA antibodies [6].

The main antigen of PG is collagen XVII, present both in the skin and placenta, which is exposed to the maternal immune system through an abnormal expression of classical class II histocompatibility molecules in the placenta [7]. PG is exclusively a disease of pregnancy, so potential causes are focused on immunogenicity and potential cross reactivity between maternal-fetal transfer and placental tissue and skin [8]. PG onset in the first or second trimester may lead to negative outcomes, including premature delivery [9].

The diagnosis of PG relies on the clinical evaluation, histological findings, and direct immunofluorescence. Bullous changes are characterized by the formation of subepidermic blister, the bullae contain mostly eosinophils. Eosinophil infiltration is the most common histopathological finding [10].

The inflammatory cellular response of the upper dermis has numerous lymphoid cells, some neutrophils and eosinophilic leukocytes. Microscopic studies have confirmed that IgG is deposited along the basal membrane area, mainly in lucid lamina and located on the proximal anchor filament [11]. IgG class antibodies - the so-called herpes gestation factor (HG) - have an important effect on the genesis of this bullous dermatosis. These antibodies can be transmitted from mother to child, which can cause premature birth, low birth weight, bullous and papular lesions in the newborn; IgG antibodies can be detected by ELISA [12].

Laboratory tests are usually within normal limits. Peripheral eosinophilia may correlate with the severity of the disease, and changes in the level of immunoglobulins, erythrocyte sedimentation rates, acute phase reactants and antithyroid antibodies may occur [3].

A patient who suffered from PG at the previous pregnancy will also suffer from this disease in future pregnancies and the onset of PG will be earlier than in the previous pregnancy. Also, a patient with PG onset in the second trimester may experience remission, followed by exacerbation in the last 6 weeks of pregnancy. The mean duration of the disease at the end of the pregnancy is 4 weeks for bullous rashes and 14 weeks for the urticaria component. Neonatal PG is caused by the transfer of antibodies through the placenta, skin changes in these infants resolve spontaneously within a few weeks and rarely require treatment [13].

The clinical picture is characterized by pruritus, pain, disfigurement, papulovesicular polymorphic eruptions on the abdominal and distal extremities, including the palms, soles, chest and back. The lesions range from erythematous papules, edema papules, confluent vesicles and urticaria plaques through large and tight bullets [14].

Differential diagnosis should be made with: scarring pemphigoid, pruritic urticarial papules and pregnancy plaques, linear IgA dermatitis, multiform erythema, dermatitis herpetiforma, acute urticaria, allergic contact dermatitis, drug-induced bullous disorders, pruritic pregnancy folliculitis, papular dermatitis of pregnancy, scab [15].

As for treatment, consideration should be given to the fact that PG disappears after birth, but treatment is necessary to minimize pruritus and to prevent vesicles and bullae rash. In mild cases of PG, oral antihistamines and topical corticosteroids will be used, while systemic corticosteroids (prednisone 0.5-1.0 mg/kg/day) will be used in severe cases. Due to prolonged corticosteroid therapy, both the mother and the newborn should be followed to rule out adrenal insufficiency [16]. Alternatives to corticosteroids include azathioprine, dapsone, intravenous immunoglobulin, pyridoxine, cyclosporine, plasmapheresis, minocycline/ nicotinamide, methotrexate and cyclophosphamide. Immunoapheresis is a useful therapeutic option in patients who do not respond to conventional therapy [6, 17].

The clinical course of PG can be altered by changes in estrogen and progesterone levels. Although PG normally recurs in subsequent pregnancies, 8% of cases may not have PG in subsequent pregnancies. 25% of patients may experience relapse when using oral contraceptives or during menstruation. Abortion and premature birth were frequently reported [18].

POLYMORPHIC ERUPTION OF PREGNANCY (PEP)

Polymorphic eruption of pregnancy (PEP), a specific dermatosis of pregnancy also known as pruritic urticarial papules and plaques of pregnancy (PUPPP), is a benign, self-limited inflammatory skin disorder that usually affects first pregnancy in the third trimester or immediately in the postpartum period [19]. The incidence of PPE was estimated to 1 in 160 pregnancies, with the mean age being 29 years [15, 20]. PEP is considered to be the most common dermatosis of the pregnancy [21].

PEP is predominantly present either at the first pregnancy in 70% of cases [15] or at pregnancies that were not performed earlier (for example, spontaneous abortion). The increased incidence of multiple pregnancies in women with PEP has been reported [22]. A specific multiple gestation study in PEP showed that 2.9% of 8000 twin pregnancies had a PEP diagnosis and 14.3% of the triple pregnancies were associated with PEP [23]. It has been shown in several studies that there is a predilection of pregnant women who have PEP to give birth to male children.

Pathogenesis is still unknown, the theory of distension suggests that overstressing of the abdominal wall causes subsequent damage to the connective tissue, triggering an inflammatory response [20, 24].

Infants born from mothers diagnosed with PEP usually do not experience complications or skin manifestations. Premature birth was reported before 37 weeks in 18.4% of PEP patients, compared with 5.8% of patients in the control group [25]. A possible increase in the incidence of cesarean section and premature labor in PEP has been suggested, but there is no clear evidence that it would occur in later pregnancies.

In over 79% of cases, onset occurs in the third trimester of pregnancy, with remission of symptoms after birth, between 1 and 4 weeks [26].

PEP shows papules and pruritic urticarial plaques during pregnancy. Clinical findings were categorized into three PEP subgroups: type I, mainly characterized by papules and urticarial plaques; type II, which included erythematous patches and nonurticarial papules (1-2 mm) or vesicles; and type III, a combination of the other two types. All three types often also have some obstructions with surrounding blanched halos [26]. In terms of distribution, 91% of patients were first found to be the abdominal area, the proximal thighs, and occasionally both [25, 27].

PEP atypical lesions, including vesicles, complicate the diagnosis and have been reported in up to 40% of cases. A retrospective study showed that initially classic clinical manifestations are present in 98% of cases, but 51% of them develop changes in the form of vesicles, target lesions and several eczematous plaques [24]. In less than 2% of PEP patients, it can affect palms and soles, with papulous or vesicular lesions similar to dyshidrotic eczema [25, 28].

PEP is a disorder with a broad clinical spectrum and diverse clinical features, but with absence of pathognomonic identifying features. To exclude other conditions, laboratory and histopathological investigations will be performed, such as: complete blood cell counts, liver function testing, renal function testing, thyroid stimulating hormone level, urine level, and bile acid level. The differential diagnosis is made with: pemphigoid gestationis, contact dermatitis, toxic drug rash, viral sprain, pitiriasis rosea and urticaria.

Histopathologically, a superficial or deep perivascular dermatitis is found with lymphocytic vasculitis, eosinophils are often present, in some cases, acanthosis, hyperkeratosis and intraepidermal vesicles [29]. If the diagnosis is not conclusive, two biopsies should be performed, one from lesional skin for hematoxylin and eosin staining and one from perilesional skin for DIF. The histopathological examination confirms an inflammatory process underlying PEP and skin stretching has recently been suggested as a precipitation factor for "koebnerization" seen in vitiligo, psoriasis and lichen planus.

It was observed in PEP patients an increase in body weight of more than 15 kg and the infant weight at birth was on average 3.6 kg compared to 3.3 kg in the control group [30]. The prevalence of multiple pregnancies in PEP patients is 10 times higher than in the general population [31]. The skin from PEP lesions showed progesterone receptor immunoreactivity in the cytoplasmic keratinocyte suprabas, while non-lesional skin did not show [32].

Due to the fact that PEP is an autoimmune condition without significant maternal or fetal risk and without recurrence in future pregnancies, patients should not suffer from anxiety due to this condition. Interdisciplinary communication between dermatologist, obstetrician and neonatologist is essential in the management of severe cases of PPE.

Topical corticosteroids are the primary means of treatment for polymorphic eruption of pregnancy (PEP) [33]. The relief of symptoms may require high potency (Class I or II) topical steroids, such as fluocinonide (cream 0.05% / 0.1%, ointment 0.05%) or even systemic steroids (prednisolone 40-60 mg/day). Oral antihistamines can be effective. Symptomatic treatment therapy consists of cooling baths, frequent application of emollients, topical medications containing refrigerated menthol, lightweight cotton, first-generation sedative antihistamines (chlorpheniramine and diphenhydramine) and medically active topical corticosteroids [19, 34].

OTHER SPECIFIC DERMATOSES IN PREGNANCY

Intrahepatic Cholestasis of Pregnancy (ICP) also known as pruritus gravidarum, is a liver disorder characterized by severe pruritus and secondary skin lesions in the third trimester of pregnancy (25–30 weeks gestation). Pruritus typically starts on the palms and soles and later becomes generalized. Secondary lesions such as excoriations, scratch marks, and prurigo nodules might develop as a result of scratching [35]. The symptoms usually disappear 1-2 days after delivery, but is a high risk of recurrence of ICP in subsequent pregnancies (50–70%). ICP is correlated with fetal risks, the most common being premature birth (20–60%), intrapartal fetal distress (20–30%) and stillbirth (1-2%) [20, 36].

Atopic Eruption of Pregnancy (AEP) is a benign pruritic disease with eczematous or papular lesions occurring during pregnancy in patients with a predisposition or history of atopic dermatitis. Specific immunological changes in pregnancy are incriminated in the development of late onset [37]. Clinical manifestations include pruritus, prurigo lesions/ excoriations and eczematous skin lesions, the diagnosis being based on clinical features. Treatment is based on class III or IV topical corticosteroids; in severe cases, systemic or antihistamine corticosteroids may be administered [38, 39]. AEP is not associated with fetal risk factors, except for the child's uncertain risk of developing atopic dermatitis.

Pruritic Folliculitis of Pregnancy (PFP) manifests as small (3–5 mm), multiple, disseminated, erythematous, excoriated, follicular papules and sterile pustules [20]. PFP usually occurs in the 2nd or 3rd trimester of pregnancy (between the 4th and 9th gestation months). Etiology is unclear, has a good maternal prognosis, without recurrence in future pregnancies and without significant fetal harm. Differential diagnosis should be done with acne, bacterial folliculitis, or polymorphic eruption of pregnancy. Treatment includes topical low-potency corticosteroids, topical benzoyl peroxide 10 %, and ultraviolet B phototherapy [40].

CONCLUSIONS

Most skin manifestations that occur during pregnancy are mild and resolve after birth. A special category is pregnancy-specific dermatoses, which, although rare, can pose great problems in terms of diagnosis, treatment and fetal harm. Good knowledge of these dermatoses is necessary for both gynecologist, dermatologist and family doctor. Proper diagnosis and management are essential for symptom relief and, in the case of fetal risk, adequate monitoring during pregnancy and monitoring of newborns after birth are of major importance. Increased attention should be paid to pregnant patients to understand the risks, adverse effects, and drug interactions of their medications in the treatment of pregnancy-specific dermatoses.

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CLINICAL PRACTICE

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Salivary matrix metalloproteinases activity in periodontal disease

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Abstract*: Introduction: Matrix metalloproteinases (MMPs) are key enzymes involved in degradation of extracellular colagen matrix during periodontal disease progression. The aim of our work was to study the levels of MMP-1, MMP-2, MMP-7, MMP-9 and MMP-10 in saliva of patients with chronic periodontitis.*

Materials and methods: The study included 22 patients with chronic periodontal disease and 15 controls. We collect unstimulated whole saliva from all volunteers included in the study. Salivary levels of mentioned MMPs were analyzed using magnetic bead-based multiplex assays and Luminex technology.

Results: MMP-1, MMP-7, MMP-9 and MMP-10 present statistically increased salivary levels at patients with periodontal disease versus the control group ($p<0.05$). Salivary level of MMP-2 at patients with chronic periodontitis was increased but statistically insignificant compared with healthy subjects ($p=0.1$).

Conclusions. The increased level of these enzymes in patients diagnosed with periodontal disease reflects their implication in periodontal tissue degradation. Saliva is an excellent fluid useful in monitoring the severity of this oral disease that affects about half of the adult population around the world.

Keywords: *saliva, inflammation, proteases, periodontal tissue*

INTRODUCTION

Periodontal disease is one of the most common oral disease and affects the adult population around the world. Periodontal disease is a chronic inflammatory disorder that affects the supporting tissues of the tooth, leading to the progressive destruction of conjunctival tissue and alveolar bone.

The role of microorganisms in the etiology of periodontitis has been very well studied, from 600 types of bacteria that are able to colonize oral cavity about 10% plays an important role in the initiation of periodontitis. Porphyromonas gingivalis, Tanerella forsythensis and Troponema denticola, are directly associated with chronic periodontitis. Actinobacillus actinomycetemcomitans, was observed in early stages of periodontal disease and aggressive periodontitis. These

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microorganisms cause periodontal tissue damage either by their degradation products (toxins, enzymes, and lipopolysaccharide) or by activating of the host´s immune system, which leads to the activation of inflammatory cells, cytokine generation, proteolytic enzyme release, and osteoclast activation [1-4].

This inflammatory process is characterized by the increased levels of IL-1α, IL-1β, IL-6, and TNF-α and release of enzymes such as MMPs. MMPs-1, 2, 3, 8 and 9 are key proteases found in biopsy specimens of human inflammatory periodontal tissue [5].

The many advantages of the saliva, namely the rapid, inconvenient collection, anywhere and anytime, the lack of contamination has led many researchers to use it as a new diagnostic fluid for both systemic and oral disorders. Saliva is secreted by major and minor salivary glands, contains 99.5% water, 0.2% inorganic substances and 0.3% organic substances. Salivary proteins include proteins synthesized by salivary glands (mucoproteins and enzymes) and proteins of blood origin, with over 20 protein fractions. Plasma proteins may exceed 20% of salivary proteins and include: albumin, globulin (IgA, IgM, and IgG), α- and β-globulins. The total concentration of γ-globulins is less than 0.5% of the plasma concentration and is generally not affected by the salivary flow. Saliva also contains and non-protein organic compounds such as glucose, lipids, hormones, uric acid, vitamins [6-8].

The main aim of the present research is to detect salivary levels of MMP-1, 2, 7, 9 and 10 in unstimulated whole saliva of patients diagnosed with chronic marginal periodontitis using Luminex technology and Magnetic Bead-Based Multiplex assays.

MATERIAL AND METHODS

Patient selection: We included in our study 22 patients with chronic periodontitis and 15 healthy subjects who agreed to participate voluntary and signed and informed consent. The present research was approved by the Ethics Committee of Science Research from „Carol Davila University of Medicine and Pharmacy". Twenty-two subjects with chronic periodontal disease were recruited from a dental private office in Bucharest. We excluded from our study subjects under 18 years old or with systemic diseases, such as: diabetes mellitus, osteoporosis, chronic hepatitis, liver cirrhosis, HIV/AIDS, autoimmune diseases. All volunteers were diagnosed by the same experienced medical professional.

Clinical data: Demographic data, including age, sex, medical history, medication used and smoking habits (smoker/nonsmoker), were collected. The periodontal parameters performed were: for bleeding index (BI), probing depth (PD), plague index (PI). Chronic periodontitis was diagnosed according to the clinical case definitions proposed by the Center for Disease Control and Prevention.

Samples collection

For all participants in the study, saliva samples were collected after clinical examination. The collection method followed a well-established protocol and took place as follows: 2-4 mL of unstimulated whole saliva was given by each participant in the morning around 9 AM. The participants were asked not eating, drinking or smoking prior to saliva collection. All subjects rinsed with 5 mL of distilled water before collection. Immediately after the collection, the samples were kept on ice and then centrifuged at 5000 rpm for 10 minutes. Samples were aliquoted and stored at -80 ºC until further determinations.

Matrix metalloproteinases detection

All MMPs were detected using Luminex technology and Magnetic Bead-Based Multiplex assays. This method enables simultaneous detection of multiple salivary human biomarkers. Thus salivary levels of MMP-1, -2, -7, -9, -10 were assessed using MILLIPLEX MAP Human MMP Magnetic Bead Panel 2 – Immunology Multiplex Assay. All samples and controls were processed following the manufacturer's specifications. The assay required 25 µL of saliva diluted at 1:20; each MMP had its own detection interval as follows: MMP-1: 27 – 20,000 pg/mL; MMP-2: 68 -50,000pg/mL; MMP-7: 548 – 400,000pg/mL; MMP-9: 14 – 10,000pg/mL; MMP-10: 27 – 20,000pg/mL. The assay involved a 2 hour multistep procedure involving preparation of reagents, samples and controls, preparation of the 96 well-plate and analysis using a Luminex® 200™ and the xPONENT® software for data acquisition and analysis.

Statistical analysis: The statistical analysis was performed using SPSS software. The characteristics were expressed as median (quartile 1; 3). Statistically significant correlations were found using the Mann-Whitney test (a two-sided p-value less than 0.05 was noted as statistically significant).

Results:

Clinical data: Results for demographic data and clinical examination are present in Table 1. No significant differences were detected for gender and age between the periodontal patients and the control group. Table 2 present the MMPs values obtained in the two groups of patients included in the study.

Table 1. Clinical date of patients included in the study

Variables	Patients	Controls	p-values
Age (*years*)	44.88±12.81	20.67±2	-
Gender (*female/male*)	10/12	10/5	-
Smoking status	0	0	-
BI (*%*)	45±0.11	17±0.20	<0.05
GI (*%*)	59.7±0.24	12.36±0.12	<0.05
PD (*%*)	4.80±0.23	0	<0.05

Table 2. Salivary levels for MMP-1,-2,-7,-9 and -10

Variables	Patients	Controls	p-values
MMP-1 (*pg/mL*)	348.375±144.6	190.125±144.6	<0.05
MMP-2 (*pg/mL*)	116.37±230.12	9.56±230.12	=0.1
MMP-7 (*pg/mL*)	886.68±79.81	527.59±79.81	<0.05
MMP-9 (*pg/mL*)	6885.75±2800.08	5224.911±2800.08	<0.05
MMP-10 (*pg/mL*)	1569.56±168.04	598±168.044	<0.05

DISCUSSIONS

Salivary biomarkers are used in the diagnosis and monitoring of autoimmune disorders (cystic fibrosis, Sjrogen's syndrome), cardiovascular disease, diabetes, HIV, oral cancer, oral lichen planus and periodontal disease [9-13].

MMPs are metal-dependent proteolytic enzymes involved in both physiological (apoptosis, bone remodeling, angiogenesis) and pathological processes such as cardiovascular disease, cancer and periodontal disease [14].

In macrophages and other types of cells from periodontal tissue, lipopolysaccharide from Porphyromonas gingivalis, binds to Toll-like receptor 4 (TLR4) and activates an inflammatory cascade with the release of inflammatory cytokines, chemokines and prostaglandins. This inflammatory response actives inflammatory cells to release MMPs mainly from polymorphonucleate leukocytes (PMN) which will degrade extracellular collagen matrix [5, 15].

Because the extracellular periodontal matrix contains especially type 1 collagen, special attention has been given to MMPs capable of degrading this type of collagen such as MMP-2 MMP-8, MMP-9, MMP-13 [16, 17].

MMPs-1, -2, -8, -9 and -13, had been detected so far in patients with periodontal diseases, mostly from gingival crevicular fluid (GCF) and less from unstimulated whole saliva. The results of the studies reflects increased levels of all mentioned MMPs and their implication in periodontal tissue degradation [18, 19].

MMP-1 or collagenase-1 is the main collagenase found at patients with aggressive periodontitis. GCF and salivary levels of MMP-1 were increased during periodontal disease and decreased levels were observed after periodontal treatment [20-22].

MMP-1 present increased levels in inflammation, autoimmune and cardiovascular diseases [14, 23].

Our results revealed statistically increased salivary levels of MMP-1 in patients with chronic periodontitis compared with controls.

MMP-2 and MMP-9 are gelatinases that can be released by various type of cells from oral cavity such as gingival fibroblast and gingival keratinocytes [24].

In the present study, we obtained increased salivary levels of MMP-2 but statistically insignificant in patients with chronic periodontal disease versus the healthy group.

The salivary level of MMP-9 was statistically increased in our study at patients diagnosed with periodontal disease versus the healthy group.

Increased levels for MMP-2 and MMP-9 were observed in GCF at patients with periodontitis, versus patients' gingivitis and healthy subjects [25].

MMP-9 in involved in cell apoptosis, tissue remodeling, being associated with several systemic diseases such as systemic sclerosis and osteoarthritis. Meanwhile MMP-2 is involved in angiogenesis and pathological processes such as cardiovascular disease [14, 23].

MMP-7 or matrilysin-1 is produced in non-injured exocrine and mucosal epithelia. Levels of MMP-7 in GCF of patients with periodontal disease are the same with healthy subjects [26].

MMP-7 is associated with inflammation, cardiovascular and lung diseases and to physiological cell apoptosis [14, 23]. Salivary levels of MMP-7 at chronic periodontitis patients involved in our study reflect increased statistically levels compared with controls.

MMP-10 or stromelysin -2 acts on types III, IV and V of collagen and has been linked to cell apoptosis, liver and lung diseases, tissue remodeling [14, 19, 23].

Still now, MMP-10 has not been detected in saliva or GCF in patients with periodontal disease. Our study revealed increased statistically levels at periodontitis patients versus healthy volunteers.

CONCLUSIONS

In conclusion, increased levels of MMP-1, -2, -7, 9, and 10 are directly associated with the severity and progression of periodontal disease, especially through involvement in alveolar bone resorption and periodontal tissue breakdown. Salivary detection of this MMPs is a modern alternative to monitoring the activity of these enzymes.

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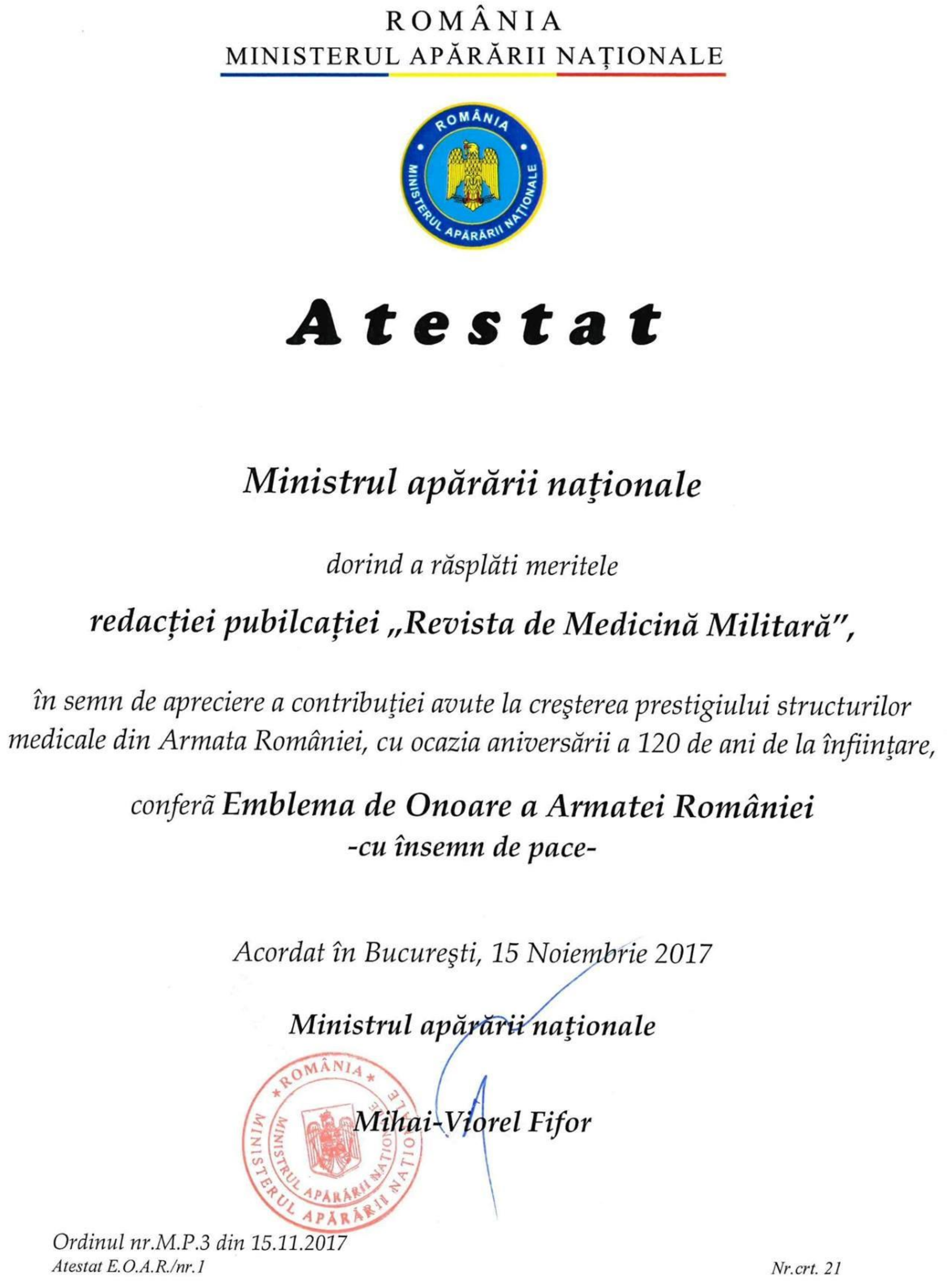

ROMÂNIA
MINISTERUL APĂRĂRII NAȚIONALE

Atestat

Ministrul apărării naționale

dorind a răsplăti meritele

redacției pubilcației „Revista de Medicină Militară",

în semn de apreciere a contribuției avute la creșterea prestigiului structurilor medicale din Armata României, cu ocazia aniversării a 120 de ani de la înființare,

conferă ***Emblema de Onoare a Armatei României***
-cu însemn de pace-

Acordat în București, 15 Noiembrie 2017

Ministrul apărării naționale

Mihai-Viorel Fifor

Ordinul nr.M.P.3 din 15.11.2017
Atestat E.O.A.R./nr.1

Nr.crt. 21

EDITORIAL

Academician General Victor Voicu at the anniversary

An anniversary is rather meant to reflect on the past, present and future, being at the same time, and a reason for celebration.

On Wednesday, June 26, 2019, the book of Academician Victor Voicu – "Under the sign of Hippocrates" was released in the Auditorium of the Romanian Academy – a book recently published by the prestigious European Idea Publishing House. It was a moment of great and wonderful celebration.

We will only remind you the essential things about Academician General Victor Voicu – a distinguished and emblematic personality of civil and military medicine (in the context in which the assertion of civilian physician – military physician is increasingly obsolete) from our country.

Born June 29, 1939 in the village of Bolovani, Dambovita county, student of "St. Sava" National College in Bucharest, transferred in the last year to "Ştefan cel Mare" Military Highschool in Iaşi (currently in Câmpulung Moldovenesc) – the moment when he put on the military coat, selected from the best for the Medical-Military Institute, the Faculty of Medicine – Medical Pharmace-utical Institute in Bucharest, which he graduated in 1962, apparently we would not have too much to say... and yet!

He chooses pharmacology and clinical pharmacology and the intuition of his master, Alfred Teitel, imposes the future creator of the School of Pharma-cology, Toxicology and Psychopharmacology from our country.

University Assistant, starting from 1966 Scientific Researcher at the Radiobiology and Molecular Biology Center, since 1972 Associate Professor and Head of the Pharmacology Department of the recently established Faculty of Medicine from Craiova, since 1990 Professor of Pharmacology, Toxicology and Psychopharmacology at the University of Medicine and Pharmacy "Carol Davila", Head of the Medical Department of the Ministry of National Defense between 1990-1995, Commander of the Medical-Military Scientific Research Center for 26 years (1987-2013), Corresponding Member (1991) and Full member (2001) of the Romanian Academy, Secretary-General in two legislatures and Vice-President of this High Authority from April 2018.

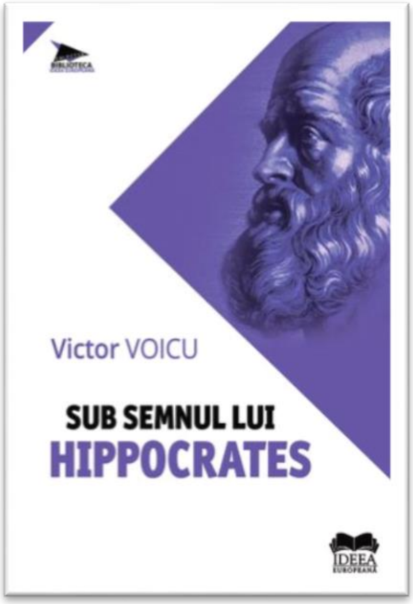

The life, work, efforts and achievements of a MAN cannot be reduced only to the previous sentence. Those who want to learn more about the frankness and the visionary spirit of Academician Victor Voicu, can do that by making use of "search engines", as they say nowadays.

Academician Nicolae Breban enlightened the thoughts of the audience when he pronounced the word "energetic", adding it to the joy felt by those present at the release of the book I previously mentioned.

General Academician Victor Voicu, a descendent in time of our common ancestor - General physician and pharmacist Academician p.m. (2003) Carol Davila is not only an "energetic" man, but also an "energetic" spirit for those around him, for all of us, when through his vision, calm and analytical spirit, he manages to clarify things and "indicate" the right direction to follow.

The release of the book, "Under the Sign of Hippocrates," was for all participants, as well as for future readers, a celebration of the spirit.

The editorial staff of the
Romanian Journal of Military Medicine
Wishes
Happy Birthday!
To

Academician Victor Voicu

Honorary Editor

Article received on February 21, 2019 and accepted for publishing on June 11, 2019.

SYSTEMATIC REVIEW

Effect of the selective serotonin reuptake inhibitors over coagulation in patients with depressive disorders – a systematic review and retrospective analysis

Octavian Vasiliu[1]

Abstract: *Several problems related to coagulation dysfunctions induced by selective serotonin reuptake inhibitors (SSRIs) were reported in literature, as serotonin is widely distributed in the human organism and it has significant contribution in various vascular and hematologic regulatory mechanisms. First, a systematic review has integrated main results from the field of SSRIs and anticoagulants pharmacologic interactions. Secondly, a retrospective analysis was performed, based on the medical charts of hospitalized patients diagnosed with SSRI-treated depressive disorders, who also received concomitant anticoagulant treatment with acenocoumarol. Platelet count, prothrombin time, activated partial thromboplastin time, and bleeding time were monitored during hospitalization and their values before and after SSRI initiation treatment were compared.*

Keywords: *major depressive disorder, antidepressants, anticoagulants, serotonin*

CURRENT STAGE OF RESEARCH IN THE FIELD OF SSRIs-ANTICOAGULANTS INTERACTIONS AND SSRIs-INDUCED COAGULATION DYSFUNCTIONS

Data in the literature regarding selective serotonin reuptake inhibitors (SSRI) treatment in patients diagnosed with depressive disorders and cardiovascular pathology for which they receive anticoagulants contain contradictions regarding the magnitude of antidepressants' effect over coagulation parameters.

Currently available informations suggest the existence of a decrease in platelets serotonin due to serotoninergic antidepressants action, and this phenomenon appears to be explained by a pharmacogenetic mechanism, through serotonin transporter promotor gene (5-HTTLPR) polymorphism [1, 2]. Also, it has been observed that serotoninnergic antidepressants could mitigate the depression- and anxiety-related procoagulant action [3], while yet another studies reveal the lack of significant effect of sertraline over coagulation in patients after an acute myocardial infarction [4].

An independent analysis of clinical trials published in the main electronic databases (PubMed, Cochrane, Medscape, and EMBASE), using keywords "anti-depressant", "serotonin selective reuptake inhibitors", "major depressive disorder (MDD)", "coagulation", "anticoagulants", "paroxetine", "sertraline", "escitalopram", "citalopram", "fluoxetine", "fluvoxamine", "warfarine", and "acenocoumarol" was performed. Only articles published between 1996 and 2018 have been selected.

Specific inclusion and exclusion criteria had been formulated according to Table 1.

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Table 1. Selection criteria for literature research

Operationalized criteria	Inclusion criteria	Exclusion criteria
Population	Inferior age limit is 18, no superior limit had been established. Diagnoses of MDD, dysthymia, adjustment disorder with depressive features, bipolar depression, mixed depressive and anxious disorder. Diagnoses according to DSM, ICD criteria, or compatible with these classifications. Lack of substance related comorbidities.	Children and adolescents. Heterogenous populations, where statistical procedures didn't allow a separate conclusion for patients aged over 18. Other disorders than those with a significant depressive component
Intervention	Any agent from the SSRI class. Monitored anticoagulant treatment.	Other, non-SSRIs-antidepressants. SSRIs in combination with other antidepressants. Concomitant use of CYP450 iso-enzymes inductors or inhibitors. Supra-therapeutic doses of SSRI agents.
Environment	In-patient or out-patient. In vitro or in vivo analysis of coagulation variables.	Unspecified environment.
Primary and secondary variables	Any coagulation-related indicator, like platelet count, bleeding time, prothrombin time etc. Discontinuation of treatment due to severe adverse events from the hematological domain, like gastro-intestinal bleeding, hemorrhagic stroke etc.	Any trial without coagulation related parameters as primary variables was excluded
Design	Randomized clinical trials, open-label, single-blind or double-blind, placebo-controlled or not, prospective or retrospective trials, case control studies, case reports or case series.	Unspecified design. Lack of a specific method for coagulation monitoring
Language	English, French, German, Romanian	Other language except for those mentioned

A number of 9 trials corresponded to these criteria and were included in analysis. A synthetic overview of the selected studies is presented in Table 2.

Table 2. Synthetic indicators for selected trials

Overall number of subjects	Mean duration of interaction	Age at inclusion
n=391 43.4 SD=70.9 Minimum=1 Maximum=225	n=2 7.5 weeks SD=6.3 Minimum=3 Maximum=12	n=3 68 years old SD=19.9 Minimum=45 Maximum=80

Due to the fact that not all of the papers published contain complete data, synthetic indicators were calculated using only those studies which included specified variables, like the number of participants, mean duration of treatment or the patients' age at inclusion.

The most relevant research data are presented in Table 3.

SSRIs as a pharmacologic class induced lower serotonin levels in the patients' platelets [1] and had a significant effect over several indicators of coagulation [3].

Fluoxetine increased significantly the bleeding time, although not above the normal values [5]. Another trial showed no significant influence of fluoxetine over mean prothrombine time during warfarin treatment [6].

Escitalopram had no effect on coagulation according to one trial [5].

Fluvoxamine could induced an over-anticoagulation status during acenocoumarol maintenance treatment, unlike other SSRIs used as controls [7]. Fluvoxamine effect over coagulation is supported also by two cases treated with warfarine [8, 9].

Sertraline is relatively safe in patients after acute MI, as it induced no changes in bleeding time [4]. Sertraline could interfere with warfarine at circulating binding proteins and displaced the anticoagulant, increasing its free fraction [10].

In conclusion, escitalopram, sertraline and fluoxetine seem to be quite safe during anticoagulant treatment, while fluvoxamine has a tendency for inducing an over-anticoagulation status. Not enough data have been found to formulate a conclusion regarding paroxetine and citalopram, but these agents seem safer than fluvoxamine, in trials who compared various serotoninergic agents. SSRIs, as a pharmacological class, doesn't induce significant effects over coagulation from a clinical point of view.

Table 3. Studies included in the systematic review

AUTHORS	DESIGN	RESULTS AND OBSERVATIONS
Reikvam AG, Hustad S, Reikvam H, et al., 2012 [1]	Case-control pilot study. In vitro measurements of platelet function. N=18. Blood donors using SSRIs vs. blood donors without treatment	Donors with SSRIs had significantly lower serotonin in their thrombocytes. Coagulation could be altered in patients using SSRIs based on lower serotonin platelet concentration.
Siddiqui R, Gawande S, Shende T, et al., 2011 [5]	Prospective, open-label, diagnosis of MDD. Bleeding time, clotting time, platelet count, prothrombin time, partial thromboplastin kaolin time – all were monitored for 12 weeks. N=40. Treatment with fluoxetine or escitalopram	At week 12 a significant increase in bleeding time was detected for fluoxetine, while escitalopram had no effect on coagulation variables. Fluoxetine is more powerful inhibitor of SERT than escitalopram. Fluoxetine increased significantly the bleeding time, but not beyond the normal values
Geiser F, Conrad R, Imbierowicz K, et al., 2011 [3]	Case-control study. Anxiety and comorbid depression. APTT, fibrinogen, factor VII, factor VIII, von Willebrand factor, von Willebrand ristocetin cofactor activity, prothrombin fragment 1 and 2, thrombin-antithrombin complex, d-dimer, alpha2-antiplasmin, PAP, tissue plasminogen activator and plasminogen activator inhibitor. N=62.	Fibrinogen, plasminogen activator inhibitor, PAP differentiated SSRIs treated patients from their matched controls. After controlling for smoke status and BMI, differences between groups were significant for PAP, von Willebrand ristocetin cofactor activity and APTT. Several coagulation indicators may be affected at significant levels ($p<0.05$) in anxious-depressive patients treated with SSRIs
Teichert M, Visser LE, Uitterlinden AG, et al., 2011 [7]	Prospective, populational-based cohort study. INR≥6 was the event monitored in patients treated with SSRIs during acenocumarol maintenance treatment. N=225 Age ≥ 45	The risk for over-anticoagulation during acenocoumarol maintenance treatment was increased in patients treated with fluvoxamine, but not with other SSRIs. Prothrombine time in users of acenocoumarol was increased by fluvoxamine above a critical value associated with bleeding risk. Number of exposed patients to other SSRIs except for fluvoxamine was low.
Limke KK, Shelton AR, Elliott ES, 2002 [8]	Case report. Warfarin+fluvoxamine 79 year old woman	Increased values of INR that persisted for 7 days. Fluvoxamine inhibits CYP1A2, 2C9, 2C19 and 3A4, while the metabolism of warfarine involves the same isoenzimes of CYP450.
Yap KB, Low ST, 1999 [9]	Case report. Warfarin + fluvoxamine. 80 year old woman	The interaction between fluvoxamine and warfarin could persist for up to 2 weeks after stopping the antidepressant
Shapiro PA, Lesperance F, Frasure-Smith N et al., 1999 [4]	Multicenter, open-label, pilot study. MDD patients identified 5 to 30 days after admission for acute MI. Serial bleeding time determinations. N=26	Bleeding time increased in 12 patients, decreased in 4 patients, was unchanged in 2 patients, 3 patients withdraw prematurely. No significant changes in coagulation measures. Sertraline seems to be relatively safe in patients after acute MI.
Ford MA, Anderson ML, Rindone JP, Jaskar DW, 1997 [6]	Open label. Stable dose of warfarin + fluoxetine. Prothrombine time was measured during the 22 days of the trial. N=6	No significant differences in mean prothrombine time before and during fluoxetine administration were detected. Fluoxetine at 20 mg/day doesn't influence the hypoprothrombinemic response of warfarin.
Apseloff G, Wilner KD, Gerber N, Tremaine LM, 1997 [10]	Non-blinded, randomised, placebo-controlled. Healthy male volunteer. Warfarin+sertraline or placebo. N=12	Increased prothrombine time significantly during sertraline versus placebo ($p=0.02$). After 22 days a significant increase ($p=0.02$) in unbound warfarine was observed in sertraline vs. placebo group. Differences between groups were statistically, but not clinically significant. Sertraline has minimal effect on the CYP2C9/10 isoenzyme, while warfarin is principally mediated by this enzyme.

SERT = serotonin transporter, BMI = body mass index, APTT = activated partial thromboplastin time, PAP = plasmin-alpha2-antiplasmin complex, INR = International Normalised Ratio, MI = myocardial infarction

Nevertheless, pharmacokinetic interactions between SSRIs and anticoagulants are possible, at the plasma protein binding level and at the CYP450 isoenzymes level, therefore monitoring of the main coagulation parameters is recommended in patients who undergo serotoninergic antidepressant and anticoagulant treatment.

RETROSPECTIVE ANALYSIS IN PATIENTS USING SSRIs AND ANTICOAGULANTS

Patients diagnosed with depressive disorders admitted in the hospital who received an SSRI agent, and who also were diagnosed with various cardio-vascular or hematologic diseases for which they received anticoagulant treatment, were analysed from coagulation parameters perspective.

Objective

To detect if significant statistical and/or clinical variation of coagulation variables are detected during combined, SSRIs and anticoagulant treatment.

Methods

We analyzed retrospectively charts of all patients evaluated for depressive disorders during one year in our department (01 January 2017 – 31 December 2017), which had anti-coagulant treatment with acenocoumarol and also received treatment with escitalopram, citalopram, fluoxetine, paroxetine, fluvoxamine or sertraline. Platelet count, prothrombin time as reflected in the INR values, APTT, and bleeding time were analyzed in all cases which were monitored during hospitalization and their values before and after SSRI initiation treatment were compared.

All included patients were over 18 years old, without previous treatment with an SSRI agent and were stabilized on acenocoumarol at time of the admission. Depressive disorders diagnoses were formulated according to ICD-10 criteria and included MDD, either first episode or recurrent, bipolar depression, dysthymia, adjustment disorder with depressive manifestations, mixed anxious-depressive disorder.

Patients diagnosed with comorbid substance related disorders were excluded from this analysis, and also those with mentioned history of non-adherence to their anticoagulation treatment.

Results

A number of 42 patients, mean age 56.6, 30 female and 12 male, diagnosed with MDD (n=23), mixed anxious-depressive disorder (n=10), bipolar depression (n=4), dysthymia and MDD (n=2) and adjustment disorder with depressive manifestations (n=3) were included in the statistical analysis. These patients had at least two determinations of INR and platelet count in their charts and most of them (62%) had at least 3 coagulation indicators recorded.

Figure 1: Treatment used during anticoagulation therapy

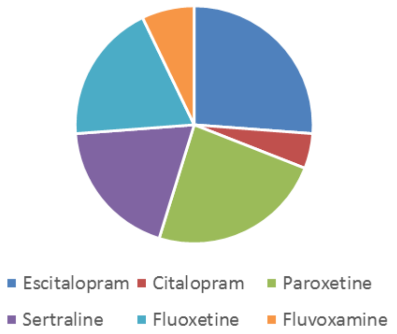

Patients received escitalopram (n=11), citalopram (n=2), paroxetine (n=10), sertraline (n=8), fluoxetine (n=8) or fluvoxamine (n=3).

Figure 2: SSRIs treatment influence over coagulation indicators (% of score variation)

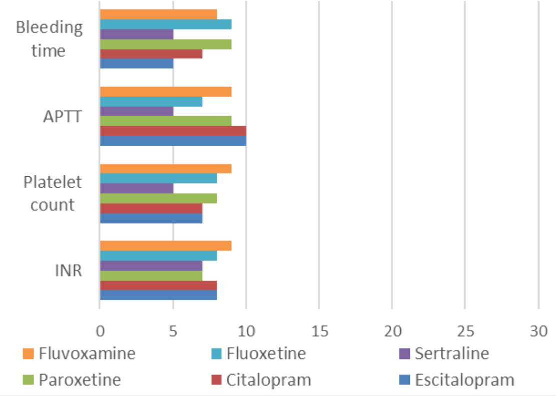

No significant differences (at p<0.05) were detected in any coagulation indicator for SSRIs as a pharmacologic class, as reflected by t test for dependent samples, when platelet count (p=0.166), INR (p=0.098), APTT (p=0.110), and bleeding time (p=0.102) were analyzed pre-SSRIs administration and after at least 7 days (mean duration of SSRIs treatment before hospital discharge was 8.4 days). When translated in percentage of absolute values variation, all SSRIs changes ranged from 5 to 10% in all the monitored parameters.

ANOVA univariate test resulted in F values ranging between 1.67 and 1.28, lower than Fcrit, at p<0.05, and η2 varied between 0.025 and 0.069, which signifies an influence of 2.5-6.9% of the SSRIs agent over INR, APTT, bleeding time, and platelet count variation during acenocoumarol treatment. A post-hoc analysis showed a superior effect over bleeding time for fluvoxamine over escitalopram and sertraline (p=0.043 and 0.46), although the clinical impact of this difference wasn't relevant.

No significant adverse event in the hematologic area was recorded during hospitalization period and mixed (SSRI and anticoagulant) treatment.

Two cases (one treated with fluvoxamine 150 mg/day and one who received paroxetine 40 mg/day) registered values of INR above therapeutic value (5.2 and 6.1, respectively) after 8 days of treatment, and acenocumarol doses were adjusted.

CONCLUSIONS

SSRIs had a minor influence over main coagulation parameters (INR, APTT, platelet count, and bleeding time) during acenocoumarol treatment in patients with depressive disorder and comorbid cardio-vascular or hematologic diseases.

Fluvoxamine has been associated statistically with a lesser safe profile than escitalopram and sertraline, although differences in the clinical domain are not detectable.

Disclaimer

Octavian Vasiliu was speaker for Servier and Bristol-Myers, and participated in clinical trials funded by Janssen Cilag, Astra Zeneca, Otsuka Pharmaceuticals, Sanofi-Aventis, Sunovion Pharmaceuticals.

Ethical considerations

All the analyzed medical charts included patients' informed consent for processing of their personal data for research and educational purposes.

List of abbreviations

5-HTTLPR= Serotonin-transporter-linked polymorphic region
APTT= Activated partial thromboplastin time
APTT= activated partial thromboplastin time,
BMI= body mass index
DSM= Diagnostic and Statistical Manual of mental Disorders
ICD= International Classification of Diseases
INR= International normalized ratio
MDD= major depressive disorder
MI= myocardial infarction
PAP= plasmin-alpha2-antiplasmin complex
SERT= serotonin transporter
SSRI= Selective serotonin reuptake inhibitor

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REVIEW ARTICLE

Improved methodology of using simulators develops better practical skills in laparoscopy of future residents

Cristian V. Toma[1,2], Cristian S. Sima[1,2], Daniel G. Radavoi[1,2], Traian Constantin[1,2], Daniel L. Bădescu[1,2], Viorel Jinga[1,2]

Abstract: *Background and aim: Minimally invasive surgery represents the actual tendency in many medical domains including urology. Gaining practical skills for these procedures becomes essential when preparing the future resident physicians in urology. The purpose of this study is to demonstrate that using medical simulation as an education tool improves the practical skills of the urology house officers in laparoscopy by using an accessible tool such as a box trainer.*

Methods: The study includes the objective and subjective evaluation of the practical skills of 54 students with no experience in laparoscopy. Each participant was evaluated before doing the practical activity on the simulator both in a subjective manner by filling a self-evaluation form and also objectively by timing the duration of doing laparoscopic basic tasks, knot tying and realizing a continuous suture. The participants were divided in two groups (A-28 participants, B-26 participants). Each group executed the same procedures with the same instruments, but with a different teaching technique.

Results and conclusions: Medical education with the help of a laparoscopic box trainer simulator is a useful tool for improving practical abilities and the time of execution of general procedures.

Keywords: *medical education, laparoscopy, box trainer simulator, students, methodology*

INTRODUCTION

On a continuous way to become an overall gold standard laparascopy and minimally invasive surgery in general shows better results in terms of patient recovery, hospitalization period, and fewer complications such as blood loss or side effects due to less required analgesia when compared to large open incisions [1]. Minimally invasive surgery also gives the advantage of smaller wound infection rates [2], fewer dehiscence rate and incisional hernia [1]. Key whole surgery requires more time, energy and financial resources. For example, the learning curve for laparoscopic radical prostatectomy was slower than the previously reported learning curves for open surgery [3]. All of these are due to less tactile real feel, loss of depth understanding, fulcrum effect and also due to manipulation: hand-eye coordination. Young urologists, especially residents, should be better prepared before performing their first interventions and this can be done in a safe, repeatable environment with the help of medical simulation.

Basic and essential skills such as camera manipulation, moving objects, manipulating intracorporeal materials (i.e. gauzes, tissue), knotting and suturing, tissue manipulation can be trained using medical simulation. In laparoscopy medical simulation can be trained with the help of basic pelvic trainers, virtual simulators which can also help improve procedural steps and live surgery on animal tissue such as porcine models.

The most reproducible, accessible and economic way of

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teaching laparoscopy to beginners is with pelvic trainers/box trainers. Unlike open surgery where one can encounter more learning possibilities, laparoscopic training for young surgeons is a challenge in their formation.

Training with the help of medical simulation has the purpose to transfer the acquired skills in the operating room [4]. In order to establish a minimum standard of training the European Society of Urology established a model called E-BLUS European Basic Laparoscopic Urologic Skills.

This model has a set of validated exercises which are applicable in urologic laparoscopic procedures. In addition to repeating the video showed skills one can improve the learning curves by identifying possible factors without taking into account the native ability of the surgeon or his previous surgical experience. [5] “And therefore I find that being a surgeon is a vocational profession, such as the athletes and airplane pilots” [6] thus meaning that the surgeon should also exercise before passing to real life scenarios, exactly how pilots train on advanced simulators in order to perfect their skills.

OBJECTIVE

To assess the better methodology of learning basic urologic laparoscopic skills by medical students using a box trainer.

METHODS

A study was realized with 54 medical students from all years of study from the University of Medicine and Pharmacy “Carol Davila” from Bucharest through from January 2017 to November 2018. The participants signed an informed consent and completed forms requiring basic demographic data such as age, year of study, gender, will to pursuit surgical careers and dominant hand.

The participants were divided in two groups. Group A had 28 students who were trained within 3 separate workshops (10, 9, 9) following one simple metho-dology; group B contained 26 participants who were divided in 3 separate workshops (10, 8, 8) and followed a more explicit and didactic technique.

All of the participants had to do the E-BLUS exercises. The first time they realized each exercise they were timed and after 15 minutes of practice they repeated the same measurement. The selected time was 15 minutes in order to have enough time, energy and concentration for the students to perform the tasks.

The instructors were represented by either urologists or general surgeons with laparoscopic experience. Pedagogical skills of the instructors were a criteria for inclusion in the study. All of the instructors who were part of the project had to train other senior students or residents with the aim of forming bench side instructors who would facilitate the communication between participants and main instructor and also give technical and logistic aid to the participants.

Tasks

The European training in Basic Laparoscopic Urological Skills consists of an online theoretical course which is followed by realizing 4 tasks in a specific amount of time. The tasks and their demo version can be found on the uroweb.org website. [7]

The tasks that were shown to GROUP A were similar to the ones from the E-BLUS. Peg transfer and circle cutting were identical. Needle guidance was observed in previous workshops to discourage students due to its difficulty and high energy consumption so we decided to take a thicker thread/suture without a needle so it would be easier to manipulate, but maintain the same rotation, piston like and hand eye coordination requirement. The laparoscopic suturing exercise was divided in two sub-tasks. The first one was to make a laparoscopic intracorporeal surgical knot, while the second one required for the volunteers to realize a surgical knot, make 3 sutures and then close with a second knot on a silicon model [8].

The methodology for group A was the following: instructor delivered two 15 minutes presentations on laparoscopy in general and laparoscopic instruments, after these all of the procedures were shown one by one by the main instructor at the demo pelvic trainer and immediately after the participants repeated each procedure with measuring the initial time and the after 15 minutes training timing.

Group B on the other hand repeated the same exercises, received the same live demonstration and video support. In addition to these, each of the exercise except circle cutting had a 3 minute training session with different exercises. Peg transfer exercise was preceded by lifting 6 pin board pins one by one, passing them from one instrument to the other and placing them in a plastic recipient. The thread passing exercise was preceded by a 3 minutes exercise which required passing of an articulated metal piece through a fixed orifice.

The breakthrough of this project besides improving the laparoscopic skills of all students was the significant improvement in time for the knot and suturing part of the workshop for Group B. The participants from Group B were made to repeat the intra-corporeal knot with the laparoscopic instruments under direct vision after the initial timing was measured.

This was done by disconnecting the monitor and changing the “abdominal wall” from the pelvic trainer with a transparent plastic sheet. In this way the movements were maintained restrained by the trocars, but the conversion from 2D to 3D natural view was present.

As a result the students at a cognitive level saw the procedure under 3D circumstances and when conversion to conventional 2D laparoscopic conditions was made the overall final improvement was significant as it will be shown in the results section.

RESULTS

The average year of study was 3.58, the male-female ratio was 1:1.4, and none of them had previous laparscopic surgery experience. All of the presented times are median scores over the same sector. All of the 28 scores of Peg Transfer Before section were summed and then divided by 28. The same principle was applied to all sections. The Time Difference column shows the performance in time before the Group B and Group A.

Figure 1: Comparison between groups

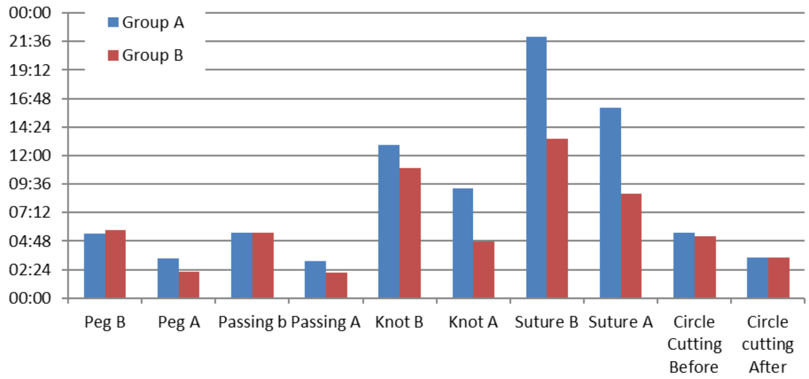

As one can see in Table 1 there is no significant difference in the before timing of the majority of exercises besides knotting and suturing which was enhanced in Group B probably due to the technique of showing the tasks under natural view and than reproduce the gestures and steps combining 3D cognitive perception with 2D laparoscopic vision.

Table 1: Overall timing in both groups A and B

Task		Group A	Group B	Time Diference	Significant[3]
1.	Peg Transfer Before	5:24	5:45	-0:21	No
	Peg Transfer After	3:22	2:11	1:11	Yes
2.	Thread Passing B[1]	5:31	5:32	-0:01	No
	Thread Passing A[2]	3:07	2:07	1:00	Yes
3.	Knot B	12:55	10:58	1:57	Yes
	Knot A	9:14	4:47	4:27	Yes
4	Suture B	21:59	13:26	8:33	Yes
	Suture A	16:03	8:49	7:14	Yes
5	Circle Cutting B	5:28	5:14	0:14	No
	Circle Cutting A	3:26	3:24	0:02	No

[1]B – before [2]A – after [3]Significant – more than 59 seconds difference

There was no significant difference in the Circle cutting group due to the fact that no extra exercise or technique was used in the Group B in order to enhance the performance.

In group B there was significant difference even in the “before” timing of knots and sutures probably due to the enhanced abilities given by the two extra exercises.

The overall extension of the Group B training time given by the three extra exercises of three minutes each was of nine minutes in practical extra activity and 21 minutes required to change the materials within the pelvic trainer.

Figure 2: Comparison – linear interpretation

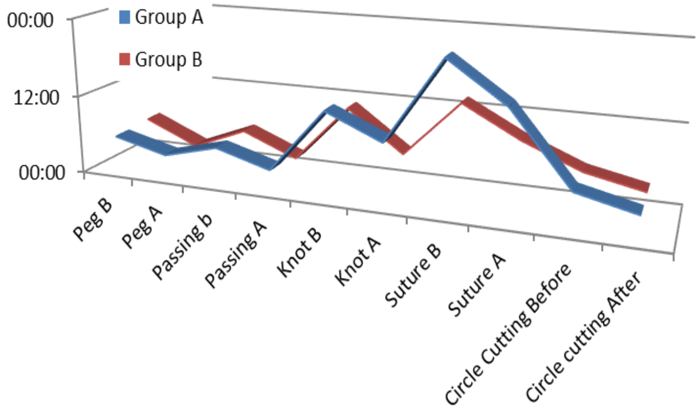

DISCUSSION

The timings could have been improved if the participants would have had more ergonomic instruments. All of the instruments had finger rings – Mayo Hegar laparoscopic needle holder.

Due to technical possibilities the table on which we placed the pelvic trainers had the same height which was inappropriate for short or tall students. Step stools were given to short students, but not offering them the best conditions. Tall students had the same problem.

The three speakers who took part in this project had two presences- one for group A and one for Group B. The sub-instructors were the same along the entire project.

CONCLUSION

It is well known that medical simulation in laparoscopy has its role in reducing the necessary time to acquire basic urologic skills in a safe and repeatable environment without the stress and pressure felt in operating room. This study demonstrates that even simple methodology can enhance the beginner's basic laparoscopic skills. In addition, due to experience, medical professional who teach these type of procedures can accelerate the process with tips and tricks which precede the proposed measuring tools. In this way the time from transferring the skills from laboratory to the operating room can be shortened and overall confidence of the surgeon can be enhanced.

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REVIEW ARTICLE

Current review of surgical management options for rotational alignment of the femoral and tibial component in total knee replacement

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Abstract: *Rotational malalignment complication following TKA, is common but can be avoided with proper surgical technique. This paper reviews the literature regarding rotational alignment during TKA, femoral and tibial rotation, and highlights the techniques prior in obtaining proper rotational positioning, nevertheless correct positioning in all three planes is important.*

Proper femoral component positioning in the axial plane is done using as landmarks the posterior condylar line (PCL), surgical transepicondylar axis (sTEA), anatomical transepicondylar axis and the trochlear anteroposterior (AP) axis. The paper describes the angular relationships between these landmarks and the distal femur. Axial tibial positioning is done when using intraarticular landmarks, the combination of more than one landmark could be a solution for solving this problem.

The consensus is that femoral component should be positioned according to TEA but the interobserver variability of this land mark is very high. The rotation of the tibial component remains an open subject, most studies suggesting a point between half of the distance of patellar ligament and 1/3 of the internal tuberosity as optimal landmark.

Keywords: *total knee arthroplasty; femoral component rotation; tibial component rotation*

INTRODUCTION

Total knee arthroplasty (TKA) is an intervention whose efficacy in the treatment of gonarthrosis is well known and documented [1]. The results of this intervention depend on the correct positioning of the prosthetic components in all three planes: frontal, sagittal, and axial [2]. The axial plane within TKA is represented by the alignment of the prosthetic components in the rotational plane. In 1979, Mochizuki and Schurman, who have shown that a lateral force produces the sprain of the patella when the tibial component is positioned according to the posterior plateau, described the importance of rotational positioning. They recommended that the tibial component should be aligned with the tibial tuberosity and the femoral component in a relative external rotation to the tibia at the time of a complete extension [3].

Femoral component positioning errors in the axial plane lead to complications in the femuropatellar joint, ligament instability and changes in normal kinematics [4]. The positioning of the tibial component from a rotational point of view has not been as studied, but its importance is equally large. The malposition of the tibial component is indirectly responsible for the femuropatellar complications that are often the cause of postoperative knee pain, reduced mobility and early revision [5]. Although current instrumentation methods have greatly reduced the malposition rate of

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components, the subject of rotation is not a closed one, and there is no consensus for the perfect positioning.

In this paper, we will try to present a review of recent literature on the correct rotational positioning of the femoral and tibial component, to describe the different axes guiding the rotational alignment, and to compare the known techniques.

ROTATION OF FEMORAL COMPONENT

The correct positioning of the femoral component from a rotational point of view is an important step for the success of TKA. The positioning of the femoral component in axial plane has repercussions on stability in flexion, knee kinematics, flexion alignment and femuropatellar joint [6, 7]. Berger demonstrated the relationship between the internal rotation of the femoral component and the patellar maltracking [8]. He was the first to use the CT to evaluate the rotation of prosthetic components. He showed that a low internal rotation, between 1 and 4o would lead to patellar maltracking and tilting, an average internal rotation, between 5 and 8o would lead to subluxation and a severe internal rotation of 7-17o, would lead to the sprain of the patella. For the analysis of both femoral and tibial rotation, Berger used the transepicondylar axis (TEA) and tibial tuberosity as anatomical landmark.

The external rotation of the femoral component also has negative repercussions on the TKA. Olcott et al. have shown that with the increase of external rotation of the femoral component, the medial flexion space increases, the result being a flexion instability symptomatology [9]. Another study led by Hanada et al. has shown that the excessive external rotation of the femur leads to varus flexion, the result being an excessive loading of the external compartment [10].

The consequences of external femoral rotation are well known; the best landmark for a good rotation is a topic yet debated. Many landmarks and axes have been described at the level of distal femur: posterior condylar line (PCL), transepicondylar axis (TEA), surgical transepicondylar axis (sTEA), anteroposterior axis (AP) or Whiteside line anterior femoral axis [11, 12].

“Tension gap” technique

The purpose of this technique is that after creating the gaps, the flexion space should be rectangular and equal to the extension space [13]. The soft parts of the knee are first balanced in complete extension by ligament rebalancing and subsequently at 90o flexion, either manually or with a laminar spreader. The aim is to obtain equal ligament tension both internally and externally and, after creating the posterior femoral cuts, the posterior femur should be parallel to the tibial surface after proximal tibial resection so that this space is rectangular. To support this theory, Laskin compared two groups of patients, in one group, the size of posterior femoral cuts was equal, and in the second group, the resection plane of posterior condyles was externally rotated to obtain a rectangular space. He noted that for this, it is necessary that the posterior medial femoral cut is larger than the lateral one, with a relative increase of this difference in the valgus knees due to lateral femoral condylar hypoplasia. The average external rotation for flexion space rectangulation was 3.20 to the posterior condylar line [14].

Correct valgus or varus extension positioning is a problem solved by current instrumentation systems but varus or valgus flexion alignment is often forgotten. This problem was investigated in a cadaver study, in which they compared the “tension gap” technique with the anteroposterior axis (Whiteside’s Line). Using the “tension gap” technique, they noted that they had good stability in extension and flexion, but the knees had varus deviations at flexion with an average of 8.2⍰, increasing pressure in the internal compartment. This phenomenon is largely due to the lateral collateral ligament, which in a normal knee is slightly laxer than the medial, this allowing the tibia to be pushed into varus (allowing the internal rotation of the femur against the tibia) when using the tensioning methods of equalizing the tensions between the two ligaments [15]. In the group in which the antero-posterior axis was used, the results were better in terms of stability and alignment.

Transepicondylar axis (TEA)

It is defined as a line crossing the two epicondyles, medial and lateral. This approximates the flexion-extension axis of the knee [16]. The positioning of the femoral component in the axial plane according to this line leads to an optimal patellar tracking, decreases the shearing forces at the beginning of flexion, and decreases the use of polyethylene insert. Internal or external rotations to this axis will result in changes in the patellar tracking [17]. The defect of this axis is that it cannot be located accurately intraoperatively. Berger et al. examined the knees of 75 cadavers and came to the following conclusions: surgical TEA is the axis between the lateral epicondyle and the medial sulcus, a channel below the medial epicondyle [18]. They concluded that the medial sulcus was an easily identifiable anatomical landmark and measured the difference between surgical TEA and the posterior condylar line as being 3.5° in males and 0.3° in females. After studying 32 cadavers, Yoshioka et al. concluded that there is a “condylar twist angle” of 5° in males and of 6° in females [19]. Although TEA is an axis difficult to highlight during surgery, especially in cases of significant destructive changes or in revisions, it could be the most accurate landmark for determining the rotation of the femoral component.

Posterior condylar line (PCL)

Using the posterior condylar line as a landmark for determining the femoral rotation is a well-known and generally accepted technique. PCL is on average 3-5° in internal rotation than TEA [20]. Most of the TKA instruments kits have additional pieces for external rotation attached to the instrument that palpates the posterior condyles, which try to position the two pins of the femoral cutting block parallel to TEA. In examination, PCL is identified by digitization and the desired external rotation is added to it.

Different studies highlighted that optimal patellar tracking was obtained with external rotation of the femoral component. Patients with the femoral component in external rotation do not need a rebalance of the extensor retinaculum and have better postoperative patellar tracking. This classic technique of positioning the femoral component in external rotation is easy to use but has the disadvantage that posterior condyles have to be perfectly palpated. This is not always possible due to the size differences of posterior condyles especially in cases of genu valgus with lateral femoral condylar hypoplasia in which there is a risk of internal rotation of the femoral component.

Trochlear anteroposterior (AP) axis

Also called the Whiteside line, the trochlear anteroposterior (AP) axis was originally described as a landmark in unicompartmental arthroplasty [21]. It was later used as a landmark for the positioning of the femoral component in axial plane in valgus deviation knees. It is defined as a line that joins the deepest point of femoral trochlea with the center of the deep lateral femoral notch. The perpendicular line on the AP axis is about 4° of external rotation compared to PCL. TEA has an average of 4.4° of external rotation compared with AP axis. The AP axis has the advantage of being easy to use and it can be identified during basic TKA.

TIBIAL COMPONENT ROTATION

The tibial component rotation is at least as important as the rotation of femoral component. The positioning of the tibial component in axial plane is responsible for optimal patellar tracking. An internal rotation will lead to a lateralization of tibial tuberosity and an increase of Q angle that will predispose to the subluxation of the patella [22]. The optimal tibial component position is not known exactly but we know that higher revision rates occur, and the clinical results are weaker at the time of malposition of the tibial component [23]. Many intra- and extra-articular landmarks are known. Intra-articular landmarks are tibial tuberosity, patellar ligament, and posterior tibial axis. There is also an option of alignment with the femoral component implanted according to TEA in extension [24]. Another option is to place the tibial component so that we have maximum tibial coverage [25]. The extra-articular landmarks are the transmalleolar axis of the ankle and the metatarsal II axis. The decision regarding what type of landmark to use for the axial alignment of the tibia is a difficult one and is influenced by other intraoperative factors. The external rotation of the plateau increases when an external parapatellar approach is used and an incomplete tibial plateau exposure resulting in internal rotation of the plateau [26].

Finding the optimal rotation of the tibial plateau requires a deep understanding of knee kinematics. In a normal knee, the tibia makes an internal rotation motion at the time the knee is in flexion and an external rotation motion at the end of the extension. This mechanism of external rotation at the end of the extension is called “screw home mechanism”, due to the geometry of the joint surfaces combined with the cruciate ligaments [27].

This physiological movement occurs between 0 and 15° of flexion. Often, this mechanism sometimes disappears, is low or paradoxically appears after TKA [28]. To reduce polyethylene use, we need to obtain a better axing of the two components in axial plane after TKA. The aim is to have a neutral rotation between the two components during the entire flexion, the rotational gap leading to femorotibial subluxation and early destruction of the polyethylene insertion.

Extra-articular landmarks

The most known extra-articular anatomic landmarks for rotation positioning of the tibial component are the transmalleolar axis of the ankle and the metatarsal II axis. Both axes have a significant variability in their knee orientation in different individuals [29]. In conclusion, these landmarks should not be the only ones used to determine the rotation. Extra-articular deformities secondary to trauma or arthrosis may result in alteration of these landmarks relative to the knee [30].

Intra-articular landmarks

Anterior tibial tuberosity is the most commonly used anatomical landmark for the proper determination of tibial plateau rotation. It is a landmark easy to identify and is not modified by gonarthrosis. This anatomical structure was split to increase the efficiency of its identification and its use. To determine the rotation, the internal edge of the tuberosity and 1/3 of the internal tuberosity are used [30].

In a study using the CT, the medial edge of the tuberosity was used in a lot of patients and in the second lot of patients, 1/3 of the internal tuberosity was used for the positioning of the tibial plateau in axial plane. The femoral component in all patients was positioned according to TEA. In the lot of patients in which 1/3 of the internal tuberosity was used as landmark, the femorotibial gap was lower, 67.5% of the patients between ±5°, 85% between ±10° and 97.5% between ±20°, compared to the lot of patients in which the medial edge of tuberosity was used, 3.8% of the patients between ±5°, 15% between ±10° and 68.8% between ±20°. In conclusion, the authors support the use of 1/3 of the internal tuberosity as landmark for the positioning of the tibial plateau [31].

Another study evaluated the relationship between TEA, the patellar ligament, and the posterior tibial axis in 30 healthy patients by MRI [32]. They found that the perpendicular line on TEA intersects the middle of the patellar ligament, the intersection being at about 41% of the width of the tendon measured from its internal edge. In order to have a smaller gap between the femur and the tibia, the anteroposterior axis of the tibial component must intersect the patellar ligament and the femoral component aligned with the TEA. This technique will finally result in a lack of coverage of the posteromedial tibia of about 5 mm with a variation between 2 and 10 mm. The patellar ligament is not modified by gonarthrosis, but its width is variable depending on the surgical approach. When the tibial plateau is positioned depending on the posterior cortex, it will enlarge the tibia coverage, but will also greatly raise the rate of the internal rotation of the tibial plateau. The authors of the study concluded that the optimal landmark is an immediate point located medial to the patellar ligament. The normal anteroposterior diameter of the tibial plateaus is different, and this will lead to the highlighting of the posteromedial bone at the time the tibial plateau is positioned correctly.

“Self-seeking” method

This method involves introducing trial components and performing full flexion and complete extension. The trial tibial plateau will be rotationally positioned according to the femoral component. The center of the trial tibial component is marked with the electrocautery and the final implantation is made using this point as landmark. This technique depends on the correct positioning of the femoral component, in femoral malrotation, the tibial component will also be affected. There is a risk that, when the femoral component is positioned in internal rotation and the tibial plateau is internally rotated, major problems of patellar tracking occur together with an increased instability. In order to overcome this problem and at the same time to use it, it is recommended to use a mobile plateau that will rebalance the tibiofemoral gap.

The mobile tibial plateau or “mobile-bearing” was built so that the polyethylene insertion follows the femoral component in rotation and the tibial component in flexion-extension. “Mobile-bearing” TKA reduces the need for tibial component placing in a perfect rotation and reduces the gap between the tibia and the femur. The disadvantage is that a second joint surface is created between the insertion and the tibial plateau and the destruction of polyethylene can occur at both interfaces.

CONCLUSIONS

TKA function and survival depend on an optimal rotation of the femoral component but also of the tibial component. The positioning of the femoral and tibial component is well known in the sagittal and frontal plane, but it is not very clear in the axial plane. There is a consensus that the femur should be positioned according to TEA but the methods this would be possible are not optimal. The combination of different techniques and the use of many landmarks could be a solution. The rotation of the tibial component remains an open subject, most studies suggesting a point between half of the distance of patellar ligament and 1/3 of the internal tuberosity as optimal landmark.

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ORIGINAL ARTICLES

The risk of bioterrorist and biocrime attack in the contemporary world

Ioana A. Gal[1], Teodora B. Eremia[1], Mihail S. Tudosie[1,2], Viorel Ordeanu[1,3,4]

Abstract: *The biological attack is the artificial spread by various means of pathogens that can cause serious infectious and contagious diseases as well as the spread of germ toxins that can be used by an aggressor as a means of fighting in order to reduce the troops' fighting force, by causing serious disease outbreaks or by killing people, animals and/or plants.*

Biological agents are microorganisms and/or microbial, animal or plant toxins, used as specific ammunition for biological weapons or used by terrorists in "bio-chem" attacks.

The risks of bioterrorism and biocrime attack in the contemporary world are real, and the history of the 20th century and the beginning of the 21st century confirms this.

It is necessary for preventive measures to be implemented on the unlawful use of biological agents. Early medical and non-medical countermeasures must be prepared for the prophylaxis, treatment and cessation of the consequences of any biological attack.

Keywords: *biological attack, bioterrorism, biocrime, medical protection*

INTRODUCTION

The biological attack is the artificial spread by various means of pathogens that can cause serious infectious and contagious diseases as well as the spread of germ toxins that can be used by an aggressor as a means of fighting in order to reduce the troops' fighting force, by causing serious disease outbreaks or by killing people, animals and/or plants.

Biological agents are microorganisms and/or microbial, animal or plant toxins, used as specific ammunition for biological weapons or used by terrorists in "bio-chem" attacks.

Terrorism, according to the League of Nations (1937), can be defined as the totality of criminal acts directed against a state or made or planned in order to create a state of terror in the minds of certain individuals, a group of people or the general public.

The objectives of terrorism can be grouped as follows:
a) Achieving political goals.

b) Attracting the attention of domestic and international public opinion to the "noble goal" pursued.

c) Undermining the authority of political regimes in some countries by creating a state of inner strain, insecurity and uncertainty, economic and social chaos.

d) Forcing authorities to meet certain requests.

g) Revenge on some officials.

h) Achieving military goals: management disruption at a strategic or operational level, diminishing or partially destroying the opponent's military potential, the disruption of the logistics system, paralyzing communications and telecommunication systems.

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i) Religious fanaticism.

Bioterrorism consists in using or threatening to use the weapon of mass destruction for claims, with the declared intent of affecting the public health and/or the environment.

Currently, the following means are known for dispersing biological agents:
- Using saboteurs;
- Using vectors of microorganisms such as insects, mites, rodents or other infected animals;
- Incorporation of biological agents into explosive ammunition;
- Aerosolization – through aerosols released from the ground, air or sea – is considered the most effective means of contaminating large areas of territory within the shortest feasible time interval.

With regard to the production of biological weapons, both ordinary and resistant biological agents can be used, the latter being preferred, because they cause a form of the disease that can not be treated with the usual drugs, causing a disease outbreak of greater magnitude and severity.

In general, biological attacks are masked by natural epidemics, epizooties and epiphytosis, the latter two being generally considered to be economic attacks, with the aim of decreasing trade, implicitly, causing the decline of the standard of living in the affected area.

Biological attacks can also be manifested in the form of zooanthroponoses, affecting both animals and humans alike. [1, 2].

BRIEF HISTORY

"Read the history and, thus, discover what will be" Nicolae Iorga rightly said. The first "chemical weapons" - "toxic smoke" were used around 424 BC in the Peloponnesian War. The first users of the "biological weapon" (around 400 BC) can be considered the Scythians, who used arrows soaked in decomposing bodies or mixed with manure. The Spartans used to cast sieves of sulfur-moistened wood on sieged fortresses. Greek, Persian, and Roman literatures describe how dead animals were used for fountain-contaminate.

The Mongols, in the siege of the Kaffa fortress in Crimea, during the war waged between 1346 and 1347, used the corpses of those killed by the plague in order to infect or create a breach among the fortress defenders.

In the fifteenth century, the Spanish infested the French wine with blood from those infected with the pests. Also, at that time, Pizzaro distributed to the Americans in South America garments contaminated with smallpox. In the sixteenth century, the same virus was used by the English to impregnate the blankets distributed to the ameridian tribes that helped the French. In the war against Sweden in 1710, the Russian troops used the same method as the Tartar army did 400 years prior – namely, the use of the corpses of those killed by the plague.

Table 1. Cases of illicit activity with biological agents around the world, confirmed by the judicial system, 20th century (W.S. Carus)

No	Objectives	Category			TOTAL cases	Comments
		Terrorist	Criminal	Others/not specified		
1	Killing	4	17	0	21	
2	Terror	6	9	22	37	
3	Extortion	0	13	3	16	
4	Losses	0	5	0	5	
5	Anti-animal or cultures	1	2	0	3	
6	Mass murder	4	0	0	4	
7	Revenge	0	3	0	3	
8	Disability	2	0	0	2	
9	Political	1	0	0	1	The fewest
10	Unknown	9	7	72	88	The most
TOTAL		27	56	97	180	
Comments		The fewest		The most		

Table 2. The objectives of using biological agents in the 20th century (W. S. Carus) [α]

No	Activity	Category			TOTAL cases	Comments
		Terrorist	Criminal	Others/not specified		
1	Acquisition and use	5	16	0	21	
2	Acquisition (possession)	3	7	2	12	
3	Interest (attempt)	6	4	0	10	The fewest
4	Threat or false attack	13	29	95	137	The most
TOTAL		27	56	97	180	
Comments		The fewest		The most		

During the Vietnam War, Americans used defoliant, desiccant and sterilizing herbicides. These substances did not fall under the Geneva Protocol of 1925 nor in the Convention on the Prohibition of Chemical Weapons. In the 1970s, ricin was used to assassinate Bulgarian dissidents Gheorghe Markov and Vladimir Kostov.

In 1976, the mayors of several US cities were the recipients of letters that were delivered in envelopes on which had been used an adhesive impregnated with lethal germs.

In 1979, among people working in a military microbiology unit, was recorded an epidemic of lung anthrax, resulting in over 60 deaths.

In 1984, in the US, members of the Bhagwan Shree Rajneesh sect have contaminated the food in Oregon restaurants with Salmonella Tiphi, causing 750 cases of major disease outbreaks.

In 1988, Iraq was spreading toxic and biological substances in Halabja, causing victims among the civilian population, predominantly children and women. Also in 1995, Iraq admitted to having in its posession Bacillus anthracis, botulinum toxin and aflatoxins, declaring to be ready to use them. All this demonstrates that, although the Convention on the Prohibition of Chemical Weapons was signed in 1972, the biological weapons research continued.

Since the end of 1992, there have been many more complex epidemics in the US, Milwaukee area, with wide-ranging effects: 400,000 cases of crypto-sporidium outbreaks from water consumption; in the spring of 1993, a respiratory epidemic with unidentified causes; during the fall of 1994, 250,000 cases of salmonella food poisoning.

On October 5th, 2001, in the US, Florida area, after having inhaled the anthrax bacillus, a tabloid's editor passed away. On October 20th, 2001, three letters containing anthrax spores were delivered to Washington DC, USA, having been received by Tom Daschle, a member of the Senate, and by Tom Brokaw from NBC News, as well as by the newspaper "The New York Post ". On November 2nd, 2001, a letter containing anthrax spores was found in Karachi, Pakistan, which was addressed to the newspaper "Daily Jang". In Santiago, a similar postal mailing resulted in disease outbreaks of thirteen people.

Figure 1. Frequency of the illicit use of biological agents in the 20th century, on decades (W. S. Carus) [α]

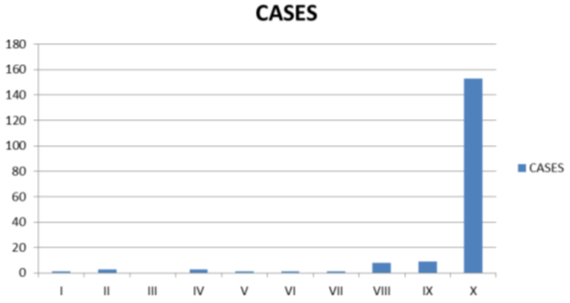

After September 11, 2001, the danger of massive terrorist attacks on the population of any part of the world is no longer just a working hypothesis. Chemical, biological or nuclear terrorism is, at present, one of the most serious threats to all states. Given the trend of interethnic and religious violence, as well as the number of cases of human rights violations in certain "high-risk" areas around the globe, analysts have warned against accentuating the risk of using these types of weapons in terrorist actions, underlining the imperative nature of urgently establishing non-proliferation measures.

Table 3. Type of biological agent used (W.S. Carus) [α]

1	Living agents	136 cases
2	Toxins	26 cases
3	Unknown	6 cases
TOTAL		168 cases

COUNTERMEASURES

Combating bioterrorist attacks involves, first of all, the intervention of the secret services, the police and the judicial system. Also, the intervention of the National Health System, the National Defense System, local communities and non-governmental organizations is crucial in the fight against these attacks. These non-medical contermeasures have an active, prophylactic role, preparing the population in the event of an attack. [3, 4, 5]

Table 4. Dissemination technique (W. S. Carus) [α]

No	Dissemination	Category			TOTAL cases	Comments
		Terrorist	Criminal	Others/not specified		
1	Aerosolization	2	0	0	2	The fewest
2	Injection/topic	6	10	0	16	
3	Food	1	20	1	22	
4	Water	4	0	2	6	
5	Natural vectors	0	1	1	2	The fewest
6	Not specified	10	13	79	102	The most
7	Unknown	5	10	2	17	
TOTAL		28	54	85	167	
Comments		The fewest		The most		

The responsibility for coordinating actions in these situations lies with the government of the country holding the EU Presidency, with the government of the country under attack and/or with the European Center for Infectious Disease Control.

The specific capabilities of the Medical Department of the Ministry of Defense are important for the medical protection against weapons of mass destruction and, in particular, for the fight against biological attacks. The Center for Military Medical Scientific Research has a Laboratory of Anti-infective Medical Protection and Epidemiological Emergencies, which functions as a specialized medical protection unit against biological weapons. It conducts military medical scientific research to protect troops and the civilian population against biological weapons and/or toxins, for biological warfare, bioterrorist attacks, or biological accidents.

Table 5. The mortality rate in the attack with biological agents (W.S. Carus) [α]

	Purpose	Cases	Deaths
1	Bioterrorism	751	0
2	Biocrime	130	10
TOTAL		881	10

Source: Carus W. S. „Bioterrorism and Biocrimes. The illicit use of biological agents since 1990", Center for Counterproliferation Research, National Defense University, Washington DC, 2001. [6]

If we make a retrospective of the 20th century, the most warrior of all, it is noticed that the practical use of weapons/biological agents was a reality. [6]

It is noted that in the last decade of the 20th century there were more cases than in the rest of the century.

OBSERVATION

An important component in the fight against biological attacks is represented by medical espionage, a constantly updated data base of new agents and existing diseases, thus, becoming the key to finding the most effective treatment.

With regard to the measures required for the imminence of a biological attack, individual protection means are used such as the gas mask - which is not 100% effective, but it decreases the amount of inhaled substance, having a protective role, because for most substances in order for harmfull effects to hapen it is necessary for large quantities to be inhaled.

Chemotherapics present much wider benefits in regards to the protection against biological weapons, acting switftly, having a broad spectrum and being easily administered in large communities.

Also, a very impressive step after a biological attack is the decontamination by appropriate physical and chemical means for the equipment, rooms, clothes, water sources, soil, etc. [7, 8, 9, 10]

CONCLUSIONS

The risks of bioterrorism and biocrime attacks in the contemporary world are real, and the history of the 20th century and the beginning of the 21st century confirms this. It is necessary to implement preventive measures on the unlawful use of biological agents. Early medical and non-medical countermeasures must be prepared for the prophylaxis, treatment and cessation of the consequences of any biological attack.

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ORIGINAL ARTICLES

The concept of biological warfare and real biological attacks

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Abstract: *In the current military-political context, with the Cold War having ended, we find ourselves in full anti-terror war, in which Romania is a direct participant, as a member of N.A.T.O. and the E.U., and the issue of biological warfare and bioterrorism is again highly topical but bearing other valences.*

There is information that there are still laboratories and plants specializing in the research and manufacture of biological weapons. Most of the results of such research are not intended to be published; however, a number of research guidelines that testify to the trends in the improvement of biological weapons and their means of use can be deduced from the data published by researchers from several research institutes.

We believe that the threats posed by bioterrorism are real and that it is mandatory to be prepared at any time to prevent, combat and liquidate the consequences of "bio-chem" attacks, respectively the management of the consequences.

Keywords: *biological attack, biological warfare, biological agents, international legislation, medical protection*

INTRODUCTION

In the current military-political context, with the Cold War having ended (1947-1990), we find ourselves in full anti-terror war (since 2001), in which Romania is a direct participant, as a member of NATO and the EU, and the issue of biological warfare and bioterrorism is again highly topical but bearing other valences.

DEFINITIONS

War is a short or lasting conflict (whether military or not, declared or not), between two or more groups, social categories or countries, aiming to achieve financial, ethnic, territorial, economic and political interests.

The biological warfare is the artificial spread by various means of pathogens that can cause serious infectious and contagious diseases as well as the spread of germ toxins that can be used by an aggressor as a means of fighting in order to reduce the troops' fighting force, by causing serious disease outbreaks or by killing people, animals and/or plants.

Weapons of mass destruction (WMD) are those weapons, which, used by the aggressor, cause extensive material damage (destruction of buildings, constructions, machinery, installations, means of transport, etc.) as well as a large number of victims among employees and unprotected animals.

Chemical, biological, radiological and nuclear defence (CBRN defence) represents the protection measures taken during times of war or terrorist attacks, in the event of a chemical, biological or nuclear attack.

The biological weapon is a system of unconventional weapons of mass destruction, whose ammunition carries biological agents and contaminates the enemy in order to cause disease outbreaks for the latter.

Biological agents are microorganisms and/or microbial,

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animal or plant toxins, used as specific ammunition for biological weapons or used by terrorists in "bio-chem" attacks.

Total war is a large-scale conflict in which policy makers mobilize all available resources to ensure the destruction of the rivals' ability to defend itself. During times of total war, there are no actual non-combatants, meaning that all people in a particular country - civilians and military alike – are considered targets.

The hybrid war represents undeclared wars by those participating states, where the military component is not explicitly assumed and is by no means singular (they are executed in concert as part of a flexible strategy with long-term goals).

THE BIOLOGICAL ATTACK

Under the conditions of modern warfare, the effects of using the biological weapon can be amplified by successive or concurrent use with other WMDs. In a concrete battlefield, the biological attack can occur with:

a) The enemy on the offensive – can use the biological weapon to weaken the opponent's defence power. The most likely objectives to be attacked with biological agents would be:

1. Concentration of troops, resistance points
2. Reserve and support units
3. Air and sea troops found at bridge ends, traffic routes clogs, operational reserves, etc.

In the tactical and operative space, pathogens of diseases of short and virulent incubation, but non-transmissible from human to human, will be used. Infected animals or insects will not be used, as they would be dangerous for own troops.

In the strategic space, the aggressor will spread biological agents that cause many types of diseases to disrupt the battlefield, cause panic and aggravate material insurance.

b) The enemy on defence – could use the biological weapon against opposing troop concentrations to reduce their force of attack. Can be attacked with biological agents, the units in the offensive, those in the second line or the back-ups, the foreign military bases, etc.

In the operative space, infected insects and animals may also be used to target troop concentrations; the passes, roads, and crossing points may be contaminated to hinder the pursuit.

Living organisms are the sole targets of these attacks, thus buildings (for e.g.) being left intact, these being able to be decontaminated and further used. The biological agent is massively released into the area by the enemy and can contaminate a large number of humans and/or animals (anthroposoonosis) turning them into secondary sources of infection by releasing in their turn pathogens in the external environment. Except for those destined exclusively for humans and for those common to both humans and animals, there are also those biological agents destined exclusively for the destruction of domestic animals and cultivated plants.

THE PROVISIONS OF INTERNATIONAL LAW

From the point of view of International Law, biological weapons are expressely prohibited because they fall under the mass destruction weapons category, and their effect cannot be limited in time and space, with medium-term effects not always predictable. Moreover, the military use of biological weapons is currently considered to be tactically and operationally inefficient, being tactically difficult and risky from a medical point of view, because an outbreak once released can get out of hand.

Exceptions on using WMD

An important issue in controlling the Convention's implementation is the existence of certain exceptions because the Convention cannot completely ban the use of living agents and their toxins. So far, no convention banning the use of a certain type of weapon against the enemy has explicitly prohibited its use on its own population.

First of all, biological agents and some toxins can be used in vaccine manufacturing, for diagnostic and treatment purposes. For example, biological agents may be used in the treatment of inflammatory bowel diseases such as Crohn's disease inflammatory form with moderate or severe activity that have not responded to conventional immune-suppressive therapy or where immunosuppressant therapy is contraindicated. They may also be used in patients with moderate/severe RCUH who have not responded to corticosteroid and immunosuppressive therapy or severe/ fulminant colitis. These patients are required to undergo treatment with drugs such as Infliximab or Adalimumab containing a chimeric anti-TNFα agent consisting of IgG1 monoclonal antibodies, 25% murine and 75% human, respectively a fully humanized anti-TNFα agent, IgG1 type.

At the same time, the emergence of vaccines has greatly revolutionized medical science since they carry an important prophylactic role.

The vaccine is a biological product containing suspensions (antigens) of attenuated, inactivated (killed) viruses or living bacteria, or fractions thereof, which are administered in order to induce a specific immune response. Depending on the type of vaccine, fragments of pathogen agents were used differently:

1. Viral vaccines – corpuscular vaccines with living attenuated viruses (oral polio vaccine, rotavirus vaccine, measles, rubella, varicella, amaryllis) or inactivated viruses (inactivated polio vaccine, rabies, Hep A) and antigenic fragments or viral subunits (Hep B, influenza vaccine)

2. Bacterial-corpuscular vaccines with living attenua-ted germs (BCG) or killed (germs), (cholera vaccine, pertussis), polysaccharide subunits (meningococcal, pneumococcal, Hib, typhoid vaccine) or purified (pertussis acelular)

3. Anatoxin – diphtheria, tetanus

Thus, the BTWC allows all signatory countries to possess and use reasonably small (grams) quantities of BWA for defensive scientific research to obtain new means of diagnosis, prophylaxis and treatment. But a real problem is that these living biological agents can be multiplied on demand and in any quantity or can synthesize toxins as specific ammunition for biological weapons.

Secondly, one of the BTWC articles states that in case of force majeure, when a state itself is threatened, it may notify the UN three months before resuming the production of biological weapons to deter the enemy. This also explains why Israel, "an island in a sea of hostile Arabs", has not signed and ratified the convention.

And Romania, which joined since 1972, ratified it only in 1979, in order to have an extra advantage in the face of a possible assault of the USSR in the context after 1968. It should be underlined that in fact Romania has not researched, manufactured, stored, and has never used biological or toxin weapons, nor did it have such an intention, but it was indeed, the target of covert biological attacks in the 20th century and, we must continue to take preventive measures.

In this complex situation, effective legal measures for the application of the BTWC need to be implemented worldwide, but concrete actions are difficult to apply in practice. The UN is in a position, as an international forum, to impose and control compliance with the Convention, being helped by the Security Council, the World Health Organization as a specialized body.

STANAG are NATO standards for different areas that set technical or operational standards in certain situations, including in the field of biological warfare, and which need to be adapted and implemented by Allied countries for interoperability.

PREVENTION OF BIOTERRORISM ATTACKS

Preventing bioterrorist attacks is mainly a political and social problem, which is primarily achieved through the intervention of the secret services, police and the justice system. Combating bioterrorist attacks also involves the intervention of the National Health System, the National Defence System, etc. The specific capacities of the Medical Department of the Ministry of Defence are important for the medical protection against the weapons of mass destruction and especially for fighting against biological attacks, permanently having the forces and means necessary for the medical protection against the C.B.R.N agents.

The Center for Military Medicine Scientific Research (CCSMM) has a Laboratory of Anti-infective Medical Protection and Epidemiological Emergencies, which functions as a specialized medical protection unit against biological weapons. It conducts military medical scientific research to protect troops and the civilian population against biological weapons and/or toxins, for biological warfare events, bioterrorist attacks, or biological accidents targeting primarily: bacteria, viruses, fungi, etc. and their toxins, to establish medical protection procedures, through cooperation between different medical specialties: microbiology, epidemiology, etc.

A first stage subsequent to the occurrence of a biological attack is the identification of the causal agent, being performed solely in a microbiology laboratory, starting from correctly harvested samples (from the air, soil, water, pathological products from the sick, etc.). The identification operation must be quick and specific.

In the event of an immediate biological attack, the problem at hand is the immediate decontamination by appropriate physical and chemical means of the staff’s equipment, of the rooms, the water sources and the food, etc. The appropriate anti-epidemic measures to limit the spread of the infection are, along with the above, another important stage in the post-attack protocol, in which we will: isolate the sick and the suspects, take care of individual and collective hygiene, qarantine the D.D.D. actions and so on. If the agent has been identified, we can perform emergency immunoprophylaxis by using vaccines, by administering prophylactic or therapeutic antibiotics, etc.

The protection against biological attacks depends on the moment when the attack is detected, thus, when the media shall signal the imminence of an attack, the possibility to benefit from individual protection (ex-gas mask) and collective protective means (shelters) should be available. The biological means of protection and the emergency prophylaxis with appropriate substances (serums, vaccines, etc.) can be of good efficacy. Immunostimulators and chemo-therapeutic agents (wide-spectrum antimicrobials) can also be associated with these.

THE BIOLOGICAL WARFARE – A HISTORICAL REVIEW

There are very few data from the Prehistory and Antiquity that testify to the use of biological weapons during warfare. However, the latest research attests to the fact that the Hittites were the pioneers of bioterrorism. The Hittite people lived on a territory that today belongs mostly to Turkey. Three thousand years ago, they used infected sheep aiming at conquering enemy people. According to the study performed by the Italian microbiologist Siro Trevisanato, the bacterium considered the first weapon of mass destruction in history was Francisella Tularensis, responsible for tularemia, an infectious and contagious rodent disease, caused by a bacterium and transmitted to domestic animals and humans, manifesting itself by fever, chills, vomiting, etc. The disease still exists nowadays and, if not treated properly, can lead to death. Studying ancient papers, researchers have proven that tularemia first appeared in a Phoenician city (located on the border between Lebanon and Syria today) in the Middle East in the 14th century BC. The Hittites plundered this city in 1325, bringing with them infected animals, which may have spread the disease – also referred to as the "Hittite Plague" - throughout its territory, as explained by the researcher. Trevisanato points out that there are several papers attesting the epidemic. At the time of tularemia’s utter virulence, the Hittites came to the attention of a neighboring population of the city of Arzawa (Western Anatolia), who took advantage of their weakness to invade their territory.

During the Middle Age as a Mongol saw his comrades die of plague in the three years of the siege of Caffa's Black Sea city, founded by the Genovese, he had the brilliant idea of throwing corpses over the city walls. The Genoese left the city, but the plague spread across Europe.

In the Modern Age – the First World War brought a more sophisticated biological warfare, with the development of microbiology, many pathogens causing diseases could be identified that could then be cultivated in the laboratory. There are suspicions about the use of plague against Russian troops at St. Petersburg. Petersburg in 1915, about horses being infected in US ports for British and French armies.

In the Contemporary Age – extraordinary intelligence has been invested in the development and modernization of all categories of weapons. Each country classified biological programs at the highest level of secrecy; there is little evidence today about the details of the programs being carried out, with only little evidence on the existence of such programs in the current media. Specific anti-human, anti-animal and anti-plant programs have been developed, agents have been standardized for use as weapons, and insects have been studied for use as natural dispersion vectors.

PERSPECTIVES

The range of pathogens used as biological weapons is very wide, which gives rise to particular difficulties in the elaboration of the protection and treatment measures.
Types of agents:
1. Bacteria: anthrax, plague, cholera etc;
2. Viruses: yellow fever, tick-borne encephalitis (TBE), smallpox, etc;
3. Rickettsia: Q fever, exanthema of typhus etc;
4. Pathogenic fungi: coccidioidoreo etiological agents, etc;
5. Toxins: bacterial (Botulinum, staphylococcal, etc.), fungal, of animals or plants (ricinotoxin).

These are only the pathogens found in nature. But new genetic techniques now allow us to obtain germs with new qualities, not naturally occurring (modified or hybridized) and which can be considered more dangerous by their pathogenic effect and increased resistance in the external environment.

Some agents have a lethal effect, while others have an incapacitating effect, leaving man out for a period of time ranging from a few hours to a few weeks.

They can be joined by immunosuppressive substances, which disrupt the mechanisms of the body's anti-infectious resistance, exposing it to current infections or to those deliberately disseminated.

In the case of a biological attack, it should be stressed that the possibility of using two or more biological agents simultaneously, whose effects combine or potentiate, or can be used with other weapons of mass destruction, is estimated.

We can also mention the existence of biological agents destined exclusively for destroying domestic animals and cultivated plants (e.g. avian influenza, barberry rust for plants) that can lead to large losses or can be used to destroy the economy of a competing country etc.

CONCLUSIONS

There is information that there are still laboratories and plants specializing in the research and manufacture of biological weapons. Most of the results of such research are not intended to be published; however, a number of research guidelines that testify to the trends in the improvement of biological weapons and their means of use can be deduced from the data published by researchers from several research institutes (e.g. the study of some of the most dangerous communicable diseases in humans and animals that currently have a very narrow spreading area). Another evidence of concern in the field of biological weapons is that the military personnel of some states are regularly exposed to prophylactic vaccines against some infectious diseases that are not circulating in the respective country.

We believe that the threats posed by bioterrorism are real and that it is mandatory to be prepared at any time to prevent, combat and liquidate the consequences of "bio-chem" attacks, respectively the management of the consequences.

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ORIGINAL ARTICLES

Morphological characteristics of the celiac-mesenteric trunk

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Abstract: *The present paper describes the morphological characteristics of 12 celiac-mesenteric trunk cases highlighted by angiography-computed tomography (CT), characteristics met only in male cases (1.82% of the cases). In relation to the vertebral column, the origin of the trunk was found in the the upper half of the L1 vertebra – intervertebral disk between L1 and L2 vertebrae. At the level of its origin from the aorta, the celiac-mesenteric trunk had an external diameter with values ranging from 8.7-13.4 mm, the aortic ostium of the celiac-mesenteric had a vertical diameter ranging from 8.8 to 13.1mm, the horizontal diameter values ranging from 8.8 to 11.2mm. At the level of its origin, the celiac-mesenteric trunk and the aortic wall had an angle with values ranging from 30.0o to 90.2o. The length of the celiac-mesenteric trunk had values ranging from 21.8 to 42.5 mm, most frequently met values were ranging from 30.7 to 33.5mm. At the level of its bifurcation, the celiac-mesenteric trunk had an angle with values ranging from 82.2 to 120,7, most frequently met values were over 90o. The celiac trunk resulted from the celiac-mesenteric bifurcation had an exterior diameter of 6.2 – 10.2 mm, values that in relation to exterior diameter the celiac-mesenteric originated, it represented 65.57 – 92.47% of its external diameter. The celiac trunk up to the end of its ramification had a length with values ranging from 6.3 to 16.8 mm. In all cases being a hepatosplenic trunk, the left gastric aorta originated in the abdominal aorta in 10 cases (83,3% of the cases) and in the other 2 cases, the left gastric aorta originated in one case under the end bifurcation of the celiac trunk whereas the other case in the celiac-mesenteric trunk, before its end bifurcation. The superior mesenteric artery resulting from the ramification of the celiac-mesenteric trunk had an external diameter with values ranging from 4.4 to 8.5 mm that represented 44.44 – 85.06% of the external diameter of the celiac-mesenteric trunk.*

Keywords: *celiac-mesenteric trunk – morphological characteristics*

INTRODUCTION

The celiac trunk and the superior mesenteric aorta are collateral branches of the abdominal aorta that are originating independently from its trunk, assuring the vascularization of the biggest part of the digestive tube and its annexed glands as well as the vascularization of the spleen. There have been cases where both arteries originated from the aorta through a common trunk, the celiac-mesenteric trunk, with frequencies of [1] and 3% [2, 3] – most authors pointing a percentage between 1.0 and 2.5% of the cases. According to [4], during the embryonal development, out of the 4 roots of the omphalomesenteric artery, roots 2 and 3 will disappear and the persistent root will unite by longitudinal anastomosis resulting into left gastric artery. The persistent root will give birth to the celiac trunk and the other one will issue the superior mesenteric artery. In the case where the first and the fourth root disappear, then the celiac-mesenteric trunk will originate.

MATERIAL AND METHODS

The material for this study comprised of 16 angiography-computed tomography (CT), performed with GE LightSpeed VCT high image resolution 64-slice CT system and GE LightSpeed 16- slice CT system, both systems within the

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premises of the Clinical Emergency County Hospital "Sf. Andrei" in Constanta. The above-mentioned 12 cases were studied out of 2220 patients that presented for a specialist consult for various medical reasons, during the period between July 2012 and June 2016, in Dobrogea area of the country, south - eastern part of Romania. From the total number of patients, 830 were females (37.39%) and 1390 were males (62.61%). The study was conducted with respect to the following parameters: the level of origin of the celiac-mesenteric trunk from the aorta in relation to the vertebral column, the trajectory and the angle formed from the origin of the celiac-mesenteric trunk with the aortic wall, the exterior diameter as well as the endovascular diameter of the celiac-mesenteric trunk at the level of its aortic origin, its length up to its bifurcation, the angle formed between its two terminal branches as well as the diameter and the trajectory.

RESULTS

In the present study, we met 12 cases with celiac-mesenteric trunk (0.54% of the total cases), all male cases (0.86% of the male cases). In relation to the vertebral column, the origin of the trunk was located in the upper half of the L1 vertebra – intervertebral disk between L1 and L2 vertebrae. In 7 cases (58.33% of the cases) the origin was located at the level of the upper half of the L1 vertebra, most frequently near the upper margin of the vertebral body. In 3 cases (25% of the cases), the celiac-mesenteric trunk originated in the lower half of the L1 vertebra, whereas in 2 cases (16.67% of the cases) it originated at the level of the intervertebral disk L1 – L2.

The celiac- mesenteric trunks originating from the aorta was located higher than the origin of the renal arteries, at a distance of 8.9 – 20 mm from the right renal artery, respectively 9.2 – 22.8 mm from the left renal artery. This indicates that the right renal artery originates most frequently from the aorta than the left renal artery.

The celiac-mesenteric trunk originating from the aorta had an external diameter with values ranging between 8.7 to 13.4 mm, in 8 cases (66.67% of the cases) with a diameter ranging between 11.4 and 13.4 mm. By comparing the external diameter of the celiac-mesenteric trunk with the external diameter of the abdominal aorta at the level of its origin, we have discovered differences in values, ranging from 9.3 to 13.8 mm between the two diameters, representing 40.09 – 58.01% of the external aortic diameter.

The aortic ostium of the celiac-mesenteric trunk had a vertical diameter with values ranging from 8.8 to 13.1 mm – the horizontal diameter ranged between 8.4 to 11.2 mm. In all cases the aortic ostium had an oval shape. In 8 cases (66.67% of the cases) the long axis was horizontal and in 4 cases (33.33% of the cases), the axis was vertical. By comparing the vertical diameter of the aorta, the ostium trunk had a vertical diameter with values ranging between 34.92 to 55.26% of the aortic one, whereas in the case of the horizontal diameter it presented with values ranging between 41.79 to 54.37% of the horizontal aortic diameter.

At the level of its origin, the celiac-mesenteric trunk created and angle with the aortin wall, with values ranging between 30° to 90.2° – extreme values had been found in one for each value, most frequent value for the angle being of 60°. This explains the trajectory of the celiac-mesenteric trunk, most frequently position being transverse anterolateral to the right, an aspect met in 10 cases (83.33% of the cases) and only 2 cases having a horizontal trajectory (transversal) presenting with an angle of 90,2° respectively 90,3°.

The length of the celiac-mesenteric trunk had values ranging between 21.8 to 42.5 mm, most frequently met values ranging from 30.7 to 33.5 mm.

The bifurcation of the celiac-mesenteric trunk into the two arteries had an angle with values ranging from 82.2° to 120.7°, most frequently met values being over 90° (83.33% of the cases). In the case that, after the bifurcation, the superior mesenteric artery had a transverse anterolateral to the right trajectory, the celiac trunk would always be ascending to the right, sometimes close to a vertical position.

The celiac trunk resulting from the celiac-mesenteric bifurcation had an external diameter with values ranging from 6.2 to 10.2 mm, extreme values have been met in only one case for each value; the rest of the cases with values ranging between 8.0 to 9.2 mm. In relation to the external diameter of the celiac-mesenteric trunk from which it originated, the celiac trunk had an external diameter with smaller values by 0.7 to 4.2 mm, representing 65.57 – 92.47% of its external diameter.

The length of the celiac trunk up to its ending ramification had values ranging between 6.3 to 16.8 mm – extreme measures were found in only one for each case. All cases presented with a hepatosplenic trunk with the left gastric artery originating from the abdominal aorta in 10 cases (83.33% of the cases), at a distance of 15.1 – 18 mm above the origin of the celiac-mesenteric trunk. In one case, the left gastric aorta originated from the terminal bifurcation of the celiac trunk whereas in the other case it originated from the celiac-mesenteric trunk before its ending ramification.

The endovascular ostium of the celiac trunk had a vertical diameter with values ranging from 7.1 to 8.6 mm, with a horizontal diameter of 6.6 to 8.6 mm. In 11 cases (91.67% of the cases) the horizontal diameter was bigger than the vertical diameter by 0.5 – 1.3 mm, thus having an oval-shaped horizontal long axis. In only one case (8.33% of the cases) the diameter of the ostium was bigger vertically than horizontally, with a difference of 0.5 mm thus having an oval shape with a vertical long axis.

The superior mesenteric artery resulted by the ramification of the celiac-mesenteric trunk had an external diameter with values ranging between 4.4 to 8.5 mm, smaller by 1.3 – 5.9 mm, representing 44.44 – 85.06% of the external diameter of the celiac-mesenteric trunk.

Table: Percentage frequency of the celiac-mesenteric trunk

Author	CMT frequency	Differences
Adachi	2.4 %	- 1.86 %
Babu	2.79 %	- 2.25 %
Chen	0.7 %	- 0.16 %
Eaton	0.54 %	0
Ferrari	1.7 %	- 1.16 %
Jones	1.7 %	- 1.16 %
Kornafel	1.5 %	- 0.96 %
Lippert	3 %	- 2.46 %
Lipshutz	2.4 %	- 1.86 %
Matusz	0.68 %	- 0.14 %
Michels	2.5 %	- 1.96 %
Mu CG	0.98 %	- 0.44 %
Natsume	0.60 %	- 0.06 %
Nelson	2.0 %	- 1.46 %
Panagouli	0.76 %	- 0.22 %
Piquand	2.0 %	- 1.46 %
Rio Branco	3.0 %	- 2.46 %
Song	1.06 %	- 0.52 %
Yadov	2.5 %	- 1.96 %
Our study	0.54 % of the total cases	0.86 % male cases

The endovascular ostium of the superior mesenteric artery had a vertical diameter with values ranging from 6.8 to 8.5 mm, with a horizontal diameter of 6.2 to 8.5 mm. In 11 cases (91.67% of the cases) the horizontal diameter was bigger than the vertical diameter by 0.3 – 1.1 mm, having an oval shape with a horizontal long axis. In only one case (8.33% of the cases) the diameters of the ostium were equal, thus having a round shape.

By comparing the external diameters of the celiac trunk and the superior mesenteric aorta, we have discovered that in 11 cases, the superior mesenteric aorta had a smaller diameter than the celiac trunk, representing 47.83 – 83.33% of the diameter of the celiac trunk and only in one case the diameter of the celiac trunk was smaller than the one of the superior mesenteric artery, representing 83.73% of its diameter.

DISCUSSIONS

Analyzing the results, we had and comparing them to the data in the existing literature referring to the morphological characteristics of the celiac-mesenteric trunk, we have discovered there is no thorough description of these, most authors mentioning only the frequency as a vascular variation.

By comparing the results of the study with the data existing in the literature we had the possibility to consult, we discovered that our results were the same with the results of [1], whereas in the rest of the cases we found differences with values ranging from 0.06 to 2.46%. Compared with the statistics of [5, 6, 7, 8, 9, 10] our results were smaller with values ranging between 0.06% [5] and 0.52% [10]. Compared with [11] the results we had were smaller by 0.96% and values ranging from 1 to 2.25% we most frequently met [Adachi, cited by 8, 12, 13, 14, 15, 16, 17, 18, 19]. Babu [19] is the only author mentioning the frequency of the celiac-mesenteric trunk in both genders – our results on male cases being smaller by 0.46%. Unlike our study where the presence of the celiac-mesenteric trunk was met only in male cases, Babu [19] discovers the presence of celiac-mesenteric trunk having a wider spread within female cases (1.47% of the cases). In relation to the statistics of [2, 3], the results are lower, the difference being by 2.46%. Lippert [3] discovers the presence of the gastro-hepato-spleno-mesenteric trunk in 2% of the cases, a difference of 1.95% from our study, and the hepato-spleno-mesenteric trunk with the origin of the left gastric aorta from the aorta in 1% of the cases, a difference of 0.55% from our study. Babu [19] finds that in 3 cases (15.79% of the met celiac-mesenteric trunks), the origin of the left gastric aorta was in the celiac trunk resulted from the celiac-mesenteric bifurcation at the level of the abdominal aorta. In a case described by Anupama [20], from the celiac trunk resulted the hepatic artery, the splenic artery, the left gastric artery and a branch that participated in formation of the gastro-duodenal artery. In the specialist literature, we have not met celiac-mesenteric trunk cases where the left gastric artery originated anteriorly to the ending ramification of the celiac-mesenteric trunk, closer to its origin.

The length of the celiac-mesenteric trunk given by Anupama [20] is of 13.00 mm, Tugrul [21] specifics a length of 13.42 mm, both values being smaller than the ones we found in our study by 8.80mm, respectively 8.38 mm smaller than the minimum length and 29.5 mm, respectively 29.08 mm smaller than the length we have measured in our study.

With regards to the diameter of the celiac-mesenteric trunk, Tugrul [21] finds an external diameter of 13.98 mm, a diameter bigger by 0.58 mm than what we have measured for the maximum value and with 5.28mm bugger than the minimum diameter. Compared to our results Tugrul [21] finds the celiac trunk resulting from the celiac-mesenteric bifurcation with a diameter (7.09 mm) bigger by 0.89 mm from the minimum diameter and smaller by 3.11 mm from the maximum value of the diameter we have measured. At the level of the superior mesenteric artery Tugrul [21] finds a diameter of 5.25 mm, bigger by 0.86 mm from the minimum diameter and smaller by 3.25 mm than the values of the maximum diameter we have measured in the study. Hemanth [22] finds an external diameter of 8.7 mm for the superior mesenteric artery, bigger by 4.3 mm than the minimum diameter, respectively by 0.2mm than the maximum value within our study.

CONCLUSION

There is a relatively low frequency of celiac-mesenteric trunk cases described in the literature, our case having the lowest frequency. The differences between different authors is related to the number of cases they have studied and due to the fact that cases where the hepatic artery was the only one originating from the trunk weren't taken into consideration as well as the methods used for the research. There is a difference between the classical methods (dissections, intramuscular injections) in relation to the modern methods, especially MDCD that has better results than the conventional angiography [17, 23]. With modern exploration methods, it has been noted that a greater number of vascular variants and malformations have been observed than those reported in the specialist literature involving other methods. We could add as a cause to the differences and the probable existence of some peculiarities related to the geographical area, which exist between authors who carried out their research on different meridians of the world.

We consider the name of vascular malformation used by some authors when talking about the celiac trunk is not the correct name, whereas vascular variant seems a more appropriate name since all vascular branches of the two arteries are present, having the same morphological characteristics as in the case where they originated independently from the aorta, also because they don't affect the vascular territories they serve.

The present study is interesting not only for a morphologist but also, knowing the different types of anatomical variants is fundamental for the planning of abdominal surgical procedure and is important to the radiologist as well, as specified by [10, 23].

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ORIGINAL ARTICLES

Local treatment options for management of loco-regional esophageal squamous cell carcinoma

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Abstract: *Aim: Surgical treatment is the main treatment method for esophageal cancer. The prognosis is poor due to high local recurrence and distant metastasis rates. Study aims to evaluate the most effective local treatment modality esophageal squamous cell carcinoma (SCC) according to real life data.*

Method: 136 patients were studied retrospectively. All patients were middle or lower esophageal cancer and had the SCC histology. Patients were divided into the surgical resection, definitive CRT (dCRT), and multimodal treatment groups according to curative local treatment they received.

Result: 32.4% were in the surgical, 36% were in the dCRT, and 31.6% were in the multimodal group. Median disease-free survival was 21 months (95% CI 14-27) in the surgical group, 8 months (95% CI 4-11) in the dCRT group, and 18 months (95% CI 0-39) in the multimodal group (p=0.059). The median overall survival was found to be 40 months (95% CI 0-92) in the surgical group, 19 months (95% CI 15-22) in the dCRT group, and 54 months (95% CI 11-96) in the multimodal group (p=0.012). In multimodal group, the number of patients receiving preoperative CRT was 25, and postoperative CRT was 18. Median OS was 47 months (95% CI 0-99) in the preoperative CRT group, and 64 months (NA) in the postoperative CRT group (p=0.302).

Conclusion: DFS and OS contributions of multimodal treatment in esophageal SCC have been shown in the present study. The addition of CRT to surgery in the preoperative or postoperative period has a contribution independently of the treatment sequence.

Keywords: *multimodal treatment, surgery, definitive chemoradiotherapy, squamous cell, esophagus cancer*

INTRODUCTION

Esophageal cancer is the seventh most common cause of cancer-related deaths worldwide. In 2012, 455,800 people were diagnosed with, and 400,200 people died from esophageal cancer [1].

Surgical resection has traditionally been the primary treatment for esophageal cancer in order to maintain local disease control and prolong life expectancy. The median survival is 15-18 months, and the 5-year survival rates are 20-25% after esophagectomy [2].

Failure in the treatment is generally due to recurrence and metastases. Developments in standard therapy are ongoing. Radiotherapy can provide locoregional control in esophageal cancer, while chemotherapy has both local and systemic antineoplastic effects. With the development of multidisciplinary treatment approaches, the use of

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chemoradiotherapy (CRT) in addition to surgery has become the most frequently used treatment method [3].

In esophageal cancer treatment, the results obtained from surgical treatment alone are not satisfactory in terms of locoregional recurrence or distant metastasis rates. This has paved the way for multimodal treatment approaches. Together with the multimodal treatment, chemoradiotherapy (CRT) added to surgery in the preoperative or postoperative period, aims to provide disease-free survival and an increase in life expectancy.

Preoperative chemoradiotherapy has begun to be used to increase the R0 resection rates by reducing the tumor stage before surgery, thereby contributing to survival [4-10]. The application of surgery after preoperative chemoradiotherapy is now a well understood and effective treatment [11, 12]. In the ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study (CROSS), a pilot study related to preoperative chemoradiotherapy, the preoperative chemoradiotherapy was reported to increase the median OS approximately 2-fold when compared to surgical treatment alone (49.4 vs 24.0 months) [4].

In the literature, there are data about the postoperative (adjuvant) activity of chemo-radiotherapy in addition to its preoperative activity. In the past, the major problem in applying chemoradiotherapy in the postoperative period was the inability of patients to complete the scheduled treatment due to poor performance of the patients after surgery, but currently developing surgical and radiotherapy techniques have partly removed this problem. Studies by Rice et al. [13] and Bedard et al. [14] have demonstrated the survival advantage of postoperative CRT. In the retrospective study by Rice, the median survival was stated to be 28 months with surgical postoperative CRT, and 15 months with surgery alone ($p<0.05$). In the study by Bedard, the median overall survival was reported to be 47.5 months with postoperative CRT and 14.1 months with surgery alone ($p=0.001$) [14].

Following the studies showing the benefits of preoperative and postoperative chemoradiotherapy, studies about treatment sequence emerged. Current data on the prognostic effect of local treatment sequence in esophageal cancer remain controversial [15, 16]. In a study based on the SEER data, it was determined that preoperative CRT ensured better survival compared to postoperative CRT [15], while a prospective study reported no difference in survival between taking the CRT in the preoperative or postoperative period [16].

In the light of this information, the prognostic implications of optimal multimodal treatment, surgery and chemoradiotherapy sequence in the treatment of esophageal cancer are still controversial. No standard guideline has been created in this particular. The main aim of this study was to evaluate the most effective local treatment modality in non-metastatic, middle and lower esophageal squamous cell carcinoma (SCC) according to real-life data.

METHODS

A total of 136 patients diagnosed between the years 2005-2016 in 3 different oncology clinics were included in the study. The patient data were reviewed retrospectively. Patients aged 18-70 years, without distant metastasis, treated for curative purposes, with ECOG performance status 0-2, with appropriate liver/renal reserve, middle and lower esophagus involvement, and with SCC histology were included in the study. Patients with adenocarcinoma histopathology, with cervical esophagus involvement, and those who only received RT as a local treatment were not included in the study.

All the patients were applied with thorax and entire abdomen computed tomography (CT) or positron emission tomography (PET-CT) or endoscopic ultrasonography (EUS) examinations for the purpose of staging prior to local treatment. The patients were divided into 2 groups as local and local advanced disease according to the radiological results at the time of diagnosis. Radiologically, patients with T4 tumors or lymph node positivity were considered to have locally advanced disease, while non-T4 patients with lymph node-negativity were considered to have local disease.

The patients were divided into 3 groups as those who underwent only surgical resection, only definitive CRT (dCRT), and multimodal treatment (preoperative CRT or postoperative CRT) according to the curative local treatments they received and were evaluated comparatively. Platinum-based combination therapies (fluorouracil or taxane) were administered concomitantly with the chemotherapy regimen in patients receiving chemoradiotherapy.

In CRT planning, a 3 mm interslice distance CT scan was performed on all patients in the supine position. In patients undergoing definitive and preoperative chemoradiotherapy, the gross tumor volume (GTV) was determined for primary mass and involved lymph nodes using thoraco-abdominal CT, PET-CT, endoscopy and EUS information, if available. The clinical target volume (CTV) was established by giving a margin of 4 cm in the superior-inferior direction and 1 cm in the radial direction to the primary mass. The planning volume was obtained by allowing 1 cm extra margin to this. The spinal cord, lungs, and organs at risk, such as the heart, remaining within the area were contoured. In postoperative therapy, bilateral supraclavicular or celiac lymph nodes were included in the area in the location of all mediastinum and primary masses.

The gastroesophageal intersection and proximal stomach were included in the area in distal localized tumors.

The radiotherapy (RT) plans were made by applying the 3-dimensional conformal technique. Patients undergoing definitive CRT (dCRT) received a total of 50.4 Gy radiotherapy, and those undergoing pre-operative or postoperative CRT received a total of 45 Gy radiotherapy, which was applied as 1.8 Gy daily for 5 days a week.

All patient records were reviewed clinically, pathologically and in terms of treatment characteristics. The long-term survival data of patients were obtained from the patient files and from the data of the Turkish civil registry office.

Approval for the study was granted by the Ethics committee of Sağlık Bilimleri University Dışkapı Yıldırım Beyazıt Training and Research Hospital.

The data were analyzed using SPSS 18.0 for Windows software. Descriptive statistics were expressed as mean ± standard deviation or median (minimum-maximum) for non-continuous numerical variables, and as number (n) and percentage (%) for categorical variables.

The progression and overall survival rates of all cases were investigated using Kaplan-Meier survival analysis. The 2-year and 3-year cumulative progression and overall survival rates together with mean lifespan and 95% confidence intervals for these terms were calculated. The demographic characteristics of the patients were assessed using descriptive statistical methods. Parameters that were significant in univariate analysis were assessed using Cox regression multivariate analysis. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

A total of 136 patients were evaluated comprising 32.4% (n=44) in the surgical group, 36% (n=49) in the definitive CRT (dCRT) group, and 31.6% (n=43) in the multimodal treatment group. Of the total patients, 52.2% were female and 47.8% were male.

The demographic characteristics of the patients are shown in Table 1.

Table 1: Demographic characteristics of the patients

		Surgery (n=44)	**dCRT** (n=49)	**Multimodal Treatment** (n=43)	***p* Value**
Age (years)	Median (min-max)	52 (32-76)	58 (25-77)	50 (32-70)	0.001
Gender	Female	26 (59.1%)	21 (42.9%)	24 (55.8%)	0.249
	Male	18 (40.9%)	28 (57.1%)	19 (44.2%)	
Smoking	Smokers	20 (45.5%)	26 (53.1%)	12 (39.5%)	0.426
	Non-smokers	24 (54.5%)	23 (46.9%)	26 (60.5%)	
Alcohol Use	Users	15 (34.1%)	14 (28.6%)	19 (44.2%)	0.288
	Non-users	29 (65.9%)	35 (71.4%)	24 (55.8%)	
Comorbid disease	Present	15 (34.1%)	16 (32.7%)	8 (18.6%)	0.208
	Absent	29 (65.9%)	33 (67.3%)	35 (81.4%)	
Cancer history in family	Present	9 (20.5%)	14 (28.6%)	9 (20.9%)	0.581
	Absent	35 (79.5%)	35 (71.4%)	34 (79.1%)	
Tumor Grade	Well	2 (4.5%)	5 (10.2%)	4 (9.3%)	0.240
	Moderate	8 (18.2%)	6 (12.2%)	5 (11.6%)	
	Poor	3 (6.8%)	12 (24.5%)	4 (9.3%)	
Location of tumor	Mid-esophagus	18 (40.9%)	25 (51.0%)	25 (58.1%)	0.271
	Lower esophagus	26 (59.1%)	24 (49%)	18 (41.9%)	
Pre-clinical stage	Early	26 (59.1%)	11 (22.4%)	20 (46.5%)	0.001
	Locally advanced	18 (40.9%)	38 (77.6%)	23 (53.5%)	

When the postoperative stages of 44 patients treated with surgery alone were evaluated, it was seen that 15.9% (n=7) of these patients were stage 1, 40.9% (n=18) were stage 2, and 43.2% (n=19) were stage 3. Adjuvant chemotherapy with cisplatin/fluorouracil combination was administered to 27.3% (n=12) of the patients in this group.

One patient died in the intensive care unit due to surgical complications in the postoperative period.

In the group of patients receiving definitive CRT, 1 patient died due to complications during treatment.

The number of patients receiving preoperative CRT was 25 and the number of patients receiving postoperative CRT was 18 in the multimodal treatment group. In the multimodal treatment group, a pathological complete response was determined in 64% (n=16) preoperative CRT group. In this group, 4 patients died in the postoperative period due to complication. In postoperative CRT group, 11.1% (n=2) of the patients were stage 1, 16.7% (n=3) were stage 2, and 72.2% (n=13) were stage 3 after surgery.

The median follow-up time was 31 months (2-142) in the surgical group, 11 months (2-106) in the definitive CRT group, and 20 months (4-140) in the multimodal treatment group.

The median disease-free survival was 21 months (95% CI 14-27) in the surgical group, 8 months (95% CI 4-11) in the definitive CRT group and 18 months (95% CI 0-39) in the multimodal treatment group (p=0.059). DFS curve shown in Figure 1.

Figure 1: Disease Free Survival

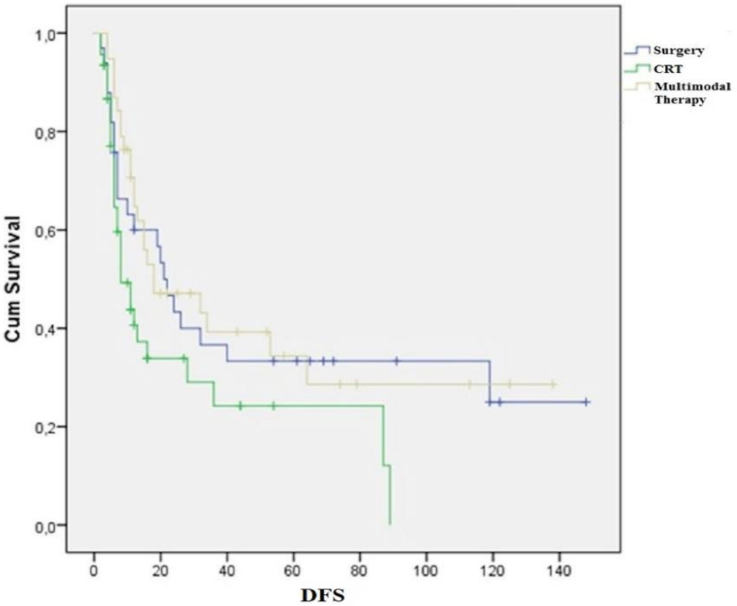

The 2-year disease-free survival (DFS) was 43% in the surgical group, 33% in the definitive CRT group, and 47% in the multimodal treatment group (p=0.05).

The median overall survival (OS) was 40 months (95% CI 0-92) in the surgical group, 19 months (95% CI 15-22) in the definitive CRT group and 54 months (95% CI 11-96) in the multimodal treatment group. The difference between the groups was statistically significant (p=0.012). Cumulative OS curve shown in Figure 2.

The 2-year OS was 68% in the surgical group, 40% in the definitive CRT group and 59% in the multimodal treatment group (p=0.01).

In the multimodal treatment group, the median OS was found to be 47 months (95% CI 0-99) in the preoperative CRT group, and 64 months (NA) in the postoperative CRT group. The difference between the groups was not statistically significant (p=0.302). OS curve when the multimodal group was divided into two as preoperative and postoperative CRT shown in Figure 3.

DISCUSSION

Surgical resection is the basic method that is conventionally effective in providing local disease control in non-metastatic esophageal cancer treatment. However, due to high locoregional recurrence and distant metastasis rates, surgical resection alone is insufficient. The median overall survival after esophagectomy is 15-18 months and the 5-year survival rate is 20-25% [2].

Figure 2: Overall Survival

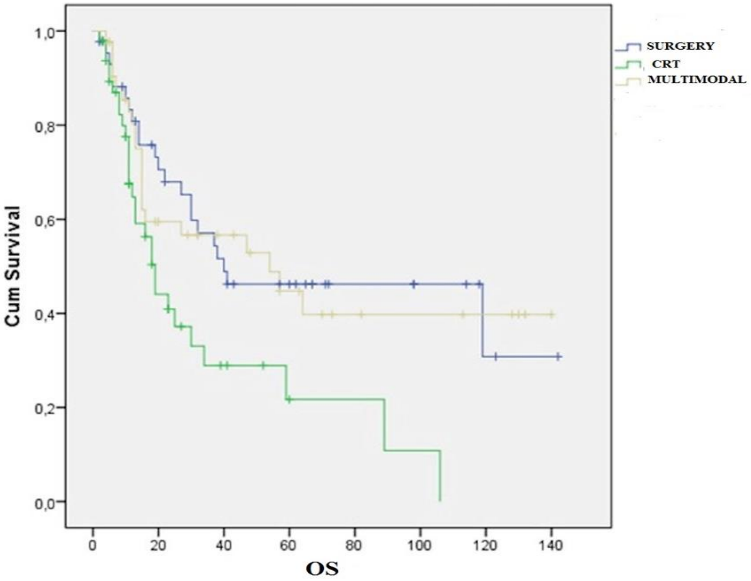

Figure 3: Overall survival in definitive chemoradiotherapy group

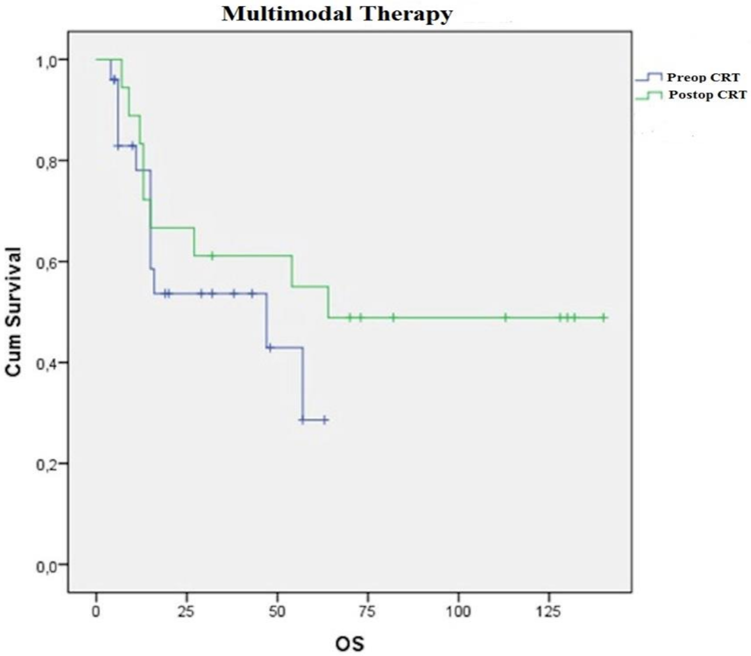

In a study by Orringer et al. conducted on 800 patients, the 5-year survival rates after esophagectomy were reported 23% [17]. In another study in which the surgery was compared with surgery after preoperative CRT, and patients were evaluated in the early stage, the median OS was reported as 18.6 months in the patients who underwent surgery [9].

In the present study, the disease-free survival was 21 months and the overall survival was 40 months in the group of patients who underwent surgery alone. In the surgical group, 27.3% of the patients (n=12) received adjuvant chemotherapy with Cisplatin/Fluorouracil combination. Compared to general literature data, the patients included in the current study had longer DFS and OS. Possible reasons for this may be the exclusion of upper esophageal tumors, which are known to have a worse prognosis, and the fact that approximately 25% of the patients who underwent surgery were administered adjuvant chemotherapy.

Although surgical resection is still the basic standard approach in non-metastatic esophageal tumors, definitive chemoradiotherapy is still an effective local treatment alternative for patients not suitable for curative surgery [18]. In one of the largest studies in which a direct comparison was made between definitive chemoradiotherapy and surgery, the two-year survival rates were 45.4% in the definitive CRT group and 56.2% in the surgical group [19]. This study supports the idea that all patients who cannot undergo surgery due to high risk may be recommended definitive chemoradiotherapy independent of the histology.

In the present study, the definitive CRT group showed a disease-free survival of 8 months, an overall survival of 19 months, and a 2-year survival rate of 43%. These results are consistent with the literature.

Although surgical resection is the curative treatment approach for esophageal cancer, the poor long-term survival outcomes have encouraged multimodal treatment approaches in this patient group. With preoperative chemoradiotherapy as one of the multimodal treatment methods, the treatment of micro metastases and regression of the tumor stage contribute positively to the overall survival rates of patients by increasing the rate of curative resection [4-10].

In the CALGB 9781 study, the median survival was 4.4 years vs. 1.7 years (p=0.002) and 5-year survival rates were reported to be 39% vs 16% in favor of multimodal treatment in esophagectomy after preoperative CRT, where the cisplatin/fluorouracil combination was used compared with esophagectomy only [10]. In the CROSS trial, patients who underwent surgery after preoperative chemoradiotherapy and patients who underwent surgery only were compared. The concomitant paclitaxel-carboplatin combination was used in this study. It was reported that the preoperative CRT improved the survival times compared to the surgery group (OS 49.4 months vs 24.0 months, HR for survival, 0.657; 95% CI, 0.495-0.871; p=0.003) [4]. The long-term results of the CROSS trial also showed that the contribution to overall survival continued [12].

Another multimodal treatment approach is postoperative adjuvant CRT. Many studies have shown the contribution to survival of postoperative chemoradiotherapy in patients who have undergone esophagectomy [13, 14, 20, 21, 22, 23]. In a study by Hwang et al., the 3-year OS rates in squamous cell esophageal cancer were 44.9% in patients who underwent postoperative CRT after esophagectomy, and 28.1% in patients who underwent surgery alone [20].

Following studies showing the contribution of preoperative and postoperative CRT in esophageal cancer, questions were asked about the prognostic contribution of surgery and the sequence of chemoradiotherapy and what should be the optimal treatment sequence. In a prospective study by Lv et al., where preoperative CRT, postoperative CRT, and surgery were compared, both 5-year OS (43.5% vs 42.3%) and 5-year DFS (37.5% vs 37.2%) were found to be similar between the preoperative CRT and postoperative CRT groups (p>0.05) [16]. Chen et al. emphasized that CRT together with surgery extended the overall survival independently of the treatment sequence [24].

In a retrospective study in which Hsu et al. evaluated the optimal treatment sequence for locally advanced esophageal cancer in 2017, the preoperative CRT and postoperative CRT groups were found to have similar median OS (26 vs 23 months, p=0.31) [25]. A statistically insignificant but clinically significant difference was found in disease-free survival (16.7 months vs 10.4 months, p=0.061). However, in that study, the rate of stage 3 patients was higher in the group receiving postoperative CRT.

Based on this information, in the present study, the multimodal treatment group was found to be superior to patients who underwent surgery only or definitive CRT in terms of both overall survival and disease-free survival. The treatment sequence in the multimodal group was not found to affect the overall survival and disease-free survival and these results are consistent with the current literature.

Since the present study was retrospective, it has some limitations. As patients from three different oncology clinics were included, it was not possible to obtain information on which criteria the patients were directed to different treatment groups when the initial treatment plan was made. No information could be obtained about why the patients with local disease could not be operated on. The fact that patients undergoing surgery were operated on by different surgical clinics was another limitation. Not all toxicity data could be obtained due to the retrospective nature of the study.

In conclusion, it was determined that the multimodal treatment of squamous cell medium and lower esophageal tumors increased the disease-free survival and overall survival, but the treatment sequence had no effect in this particular. However, there are questions that still need to be answered for operable esophageal tumors, such as what the ideal local treatment modalities and combinations are, whether it is preferable to apply the chemotherapy scheme synchronously with CRT, and the location of postoperative adjuvant chemotherapy. There is a need for further randomized phase studies on these subjects.

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ORIGINAL ARTICLES

Methods of assessing stable coronary artery disease by non-invasive imaging techniques

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Abstract: *Diagnosis of stable coronary artery disease is vital for prognosis, classification and early treatment. Current guidelines show that patients with stable angina who are suspected of CAD need to undergo a certain protocol for classification and further analysis. This review's aim is to present the most used non-invasive techniques for identification of CAD and to underline the current development of imaging technology and the possible reduction of invasive measures due to non-invasive techniques.*

Currently, non-invasive techniques used to diagnose stable coronary artery disease have a very high accuracy and newer methods seem to be comparable to the gold-standard. The majority of the methods discussed have an optimal performance for patients with PTP between 15-85%, and the future of diagnosis for these patients seem to involve less invasive measures and less radiation by improving the current devices and by usage of machine-learning algorithms.

Keywords: *coronary artery disease, PTP, stress echocardiography, SPECT, CMR, CCT*

INTRODUCTION

Diagnosis of stable coronary artery disease is vital for prognosis, classification and early treatment. Failure in doing so, might determine secondary events such as: disease progress, myocardial infarction or even death.

Current guidelines show that patients with stable angina who are suspected of CAD need to undergo a certain protocol for classification and further analysis.

This review's aim is to present the most used non-invasive techniques for identification of CAD and to underline the current development of imaging technology and the possible reduction of invasive measures due to non-invasive techniques.

METHODS

A PubMed review was performed, analyzing all publications from 1968 to 2019 concerning the topic "stable coronary artery disease" (keywords: coronary artery disease, PTP, stress echocardiography, SPECT, CMR, CCT). Human studies and meta-analyses, published in English, were cited in this review.

RESULTS AND DISCUSSION

Diagnosis and management

According to current guidelines, patients with suspected SCAD will follow a step-by-step approach for confirmation.

After the clinical examination and basic tests applied (ECG, bio-chemistry indicators, resting echocardiography), if the cause of chest pain is none other than CAD and the left ventricle ejection fraction is higher than 50%, the evaluation of suspected SCAD commences (patients with LVEF<50% have a high risk for cardiovascular events and they should have ICA without further testing). [1]

First step is to determine the pre-test probability of SCAD

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(PTP). This indicator is evaluated by the age, gender and the nature of symptoms for the suspected patient, and evaluates the probability of having a coronary artery disease before realizing any tests.

Non-invasive imaging techniques that assess coronary artery disease usually have sensitivities and specificities around the value of 85% (coronary computed tomography angiography – coronary CTA has the highest sensitivity (95%) [2] while stress echocardiography has the highest specificity (94.6%) [3]). This is why for 15% of the patients, most imaging techniques will be false positive and therefore, for these patients (with PTP < 15%) performing no non-invasive imaging tests will bring less incorrect results. Therefore:

- patients with PTP < 15% will not do any further investigations;
- patients with PTP between 15-65% are recommended to have an exercise ECG as the initial test and afterwards if necessary, perform imaging tests;
- patients with PTP between 66-85% must have a non-invasive imaging test for confirmation;
- patients with PTP over 85% are assumed to have SCAD, so they don't need further investigations. [1]

As such, patients with PTP between 15%-85% are required to be assessed with non-invasive techniques for risk stratification and selection for invasive measures (revascularization or ICA). [4]

Electrocardiogram exercise testing

As described above, for patients with PTP within 15-65% for suspected SCAD, an exercise electrocardiogram will be the first and best option.

According to the Bruce protocol, this test involves using a treadmill or an exercise bicycle in which patients are monitored using a continuous 12-lead ECG and their blood pressure measured, when they are exercising. The level of difficulty increases incrementally every 3-minutes until a certain target is reached (the predicted heart rate for the age of the patient) or the patient cannot continue the test. [5]

If ST-depression is present with a value of ≥ 0.1mV or 1 mm, in one or more ECG leads, which persist at least 0.06-0.08 seconds after the J-point, the test detects a possible CAD. [1]

Data shows that the usefulness of the test might come for its higher specificity, one study shows a sensitivity of 45%, and specificity of 85% [6] while another study shows higher values for men (sensitivity – 40%, specificity – 96%) than for women (sensitivity – 33%, specificity – 89%). [7]

Also, in a meta-analysis which compared the efficacy of exercise stress testing between 34 studies, results also showed that for men a positive exercise test results in a 89% probability of CAD, and for women results in a 69% probability of CAD. Whereas, a negative exercise test leads for men to a 37% probability of CAD and for women to an 18%. These values show that a large share of patients may be undetected after performing an exercise stress test. [8]

This method is useful only for patients that don't have ECG abnormalities at baseline. For example, patients with Wolff-Parkinson-White syndrome, paced ventricular rhythm, LBBB, use of digitalis, atrial fibrillation, left ventricular hypertrophy with repolarization changes or digoxin use, have a higher likelihood that the observed ST-segment depressions would happen because of the baseline pathology and not the CAD. Therefore, in these patients the ECG results aren't interpretable and exercise ECG testing should not be performed. [9]

Despite all the results, for low-risk patients, for example women, the standard ECG exercise treadmill test should be the first diagnostic strategy, because of its availability and lower costs of usage. Additional imaging techniques, like myocardial perfusion imaging (MPI) does not add any substantial benefit to the exercise treadmill test, but increases diagnostic costs significantly [10].

Also, it is safe to say that during exercise testing, the risk of having myocardial infarction because of the increase in the pro-coagulant activity, doesn't increase for patients with CAD (objectified angiographically) in comparison to those who don't have CAD, meaning that the method is in general safe. [11]

Although this method is accessible for diagnosis of CAD, in the near future it will probably be replaced with other more accessible, non-invasive imaging techniques. Cardio-goniometry (3D-ECG) was demonstrated to have a higher performance in diagnosis (sensitivity 75% vs sensitivity 68.1% - exercise ECG and specificity 74.4% vs specificity 38.1% - exercise ECG) of CAD in women, and being able to have this efficacy without having to undergo stress-testing and being practically free-of-risk, surely proves an improvement in easiness and performance in the diagnosis of CAD for the near future. [12]

Stress echocardiography

Another imaging technique for the assessment of stable CAD is stress echocardiography. This method usually involves a mechanical stressor (exercise performed on a treadmill or a bicycle) or a pharmacological agent.

The principle of stress echocardiography is the identification of functionality for all cardiac walls and further classification into normal, ischaemic, viable or necrotic myocardium:

- normal myocardium responds with normokinetic function at rest and during stress it may have a normokinetic or hyperkinetic function;
- ischaemic myocardium may have a normal function at rest, but during stress its response worsens, having a hypokinetic/akinetic/dyskinetic function;
- viable myocardium testing in dysfunctional myocardium at rest shows a improvement during stress response because it would indicate a region of hibernating myocardium that improves its function after revascularization;
- necrotic myocardium doesn't improve its function during stress, a region of dysfunctional myocardium at rest has its function fixed. [13]

Stress echocardiography using exercise stress is preferable because it provides additional data like exercise time, workload, heart rate and blood pressure variability. Pharmacological stress using dobutamine can be used for patients who are unable to exercise. [1]

Also using dobutamine bring the advantage of assessment of viability more accurately than exercise stress echocardiography. Ischaemic but viable myocardium at low doses of dobutamine (10-20 µg/kg/min) improves its function, but at high doses (40 µg/kg/min) its function worsens. This effect is due to the initial inotropic function of dobutamine at low doses, whereas in high doses its function changes to chronotropic and especially vasodilatory. [14]

Using additional contrast to the method (by using microbubbles of encapsulated gas) can bring enhancement of the images viewed, by improving image quality, accuracy of detection of CAD and reader confidence, especially when certain segments cannot be visualized at rest. [15]. Newer data show data coronary microvascular dysfunction (a potential cause for chest pain) can be observed using myocardial contrast echocardiography, a marker for possible CAD, patients with this issue having lower hyperemic perfusion and microvascular flux rate than patients with normal microvascular function. [16]

Diagnosis of CAD using stress echocardiography is quite efficient. Exercise stress echo, dobutamine and dipyridamole stress echo show sensitivities of 85%/80%/78% and specificities of 77%/86%/91% for detection of CAD, which are significantly better than electrocardiogram exercise testing, and similar to SPECT [17]

Also, using strain-rate imaging and tissue Dopple imaging can improve the quality of detection for CAD. Because the interpretation of images in most echocardiographies are subjective and prone to errors, measuring strain-rate can determine an objective view on the diagnosis. Voigt et al showed that ischemic segments had significantly higher peak systolic strain-rates and strains during ejection time during dobutamine stress than nonischemic segments [18] and Gupta et al., recently showed in a meta-analysis similar results, analysis of longitudinal strain imaging having higher AUC-ROC prediction of CAD (0.92) than assessment of wall motion (0.83). [19]

The newest advancement in echocardiography for detecting CAD markers is the 3D-speckle tracking echocardiography (3D-STE). One of the most used scoring systems to objectify CAD severity is the Gensini score. In one study, using 3D-STE to measure 4 parameters of CAD (global longitudinal strain – GLS, global circumferential strain – GCS, global radial strain – GRS and global area strain – GAS) it had been found that patients found with critical CAD after coronary angiography had significantly worse values of GLS,GCS,GRS,GAS than patients with noncritical CAD. Also the Gensini score had a significant positive linear correlation with GLS and GAD, meaning that 3D-STE was a very good method do identify the grade of severity of CAD. [20]

SPECT (Single photon emission computed tomography) – Myocardial perfusion scintigraphy

One of the most used imaging techniques, uses similar principles like the previously described methods. It uses either exercise stress or pharmacological stress (e.g. adenosine, dipyridamole, dobutamine, regadenoson) and measures the level of uptake of radioactive tracers at rest level and stress level from the myocardium. In ischemic tissue, due to the lower blood flow in stress response, the uptake of the tracer is reduced, meaning a positive test for diagnosis of CAD. [4]

Standard radiotracers are Technetium-99m (^{99m}Tc) or Thallium 201 (^{201}Tl), although the latter has a higher radiation dose. New developments in this technology (cadmium-zinc-telluride – CZT) show a significantly higher performance than conventional SPECT and a lower dose of radiation. [21] Also another radiotracer (for PET) that is frequently used is Rubidium 82 (^{82}Rb) that is associated with lower radiation exposure and high sensitivity (82%) and specificity (90%) for detecting ischaemia. [22]

The method also compares ischaemic areas of myocardium to normal areas. Some patients (with triple vessel disease) whom hearts have a widespread ischemia, will be detected false-negative for CAD because of the lack of inducing ischemia in the underperfused myocardium, proving a limitation of the method. [23]

Another limitation of the method would be diagnosis of CAD in patients with left bundle branch block (LBBB). In those patients, the intraventricular septum has a delay in contraction (being dyskinetic), and in the moment of image acquisition may have a lower thickness, indicating a LAD territory ischaemia, and thus a false-positive result. [24]

Also, for asthmatic patients that are undergoing SPECT, using adenosine is a contraindication because of the possible precipitation of bronchospasm due to activation of all adenosine receptors (especially A_1, A_{2B} and A_3). Regadenoson, a selective A_{2A} agonist (for producing hyperaemia) is currently used for these patients, because of its sufficient hyperaemic response, less side effects, and comparable efficacy to adenosine. [25]

The REASSESS study, in which in a prospective manner was assessed the diagnostic performance of coronary computed tomography angiography (CTA) vs. SPECT, showed that although many cases of stable CAD wouldn't have been diagnosed by SPECT if invasive measures (angiography) wouldn't have been taken, the overall performance of both of the methods (CTA and SPECT) was similar, in objectifying hemodynamically significant stenosis, having a similar accuracy (70% - CTA vs. 68% SPECT). [26]

The issue of false-negative results in SPECT, although present, is indicated to have a low prevalence and thus, a low impact. For example, in 133 patients with normal SPECT results, only 16% had anatomically and functionally significant stenoses, and from 180 vessels analyzed with normal SPECT results, only 8.33% had stenoses. As such, the prevalence of false-negative cases in normal SPECT remains low and a normal result stays important in diagnosis and follow-up. [27]

SPECT also predicts efficiently long-term outcomes. Patients with ischaemia burden, who had SPECT and also underwent early coronary revascularization had significantly lower all-cause mortality and cardiac mortality in comparison to patients who received only pharmacological therapy, indicating a significant diagnostic value for the severity of CAD by using SPECT. [28]

Usage of myocardial perfusion scintigraphy combined with positron emission tomography is more accurate in comparison to SPECT, having higher sensitivities (90% - PET, 85% - SPECT) and higher specificities (88% - PET, 85% - SPECT), also AUC-ROC prediction was higher for PET (0.95) in comparison to SPECT (0.90), according to Mc Ardle et al. [29]

A similar study showed better image quality and diagnostic accuracy of CAD for PET in comparison to SPECT (showing a percentage of 78%/79% excellent image quality of rest/stress PET scans vs. 62%/62% excellent image quality of rest/stress SPECT scans). [30]

Data also shows that using PET-MPI to determine myocardial blood flow is excellent and superior to the relative measurement of tracer uptake, being more efficient for the detection of CAD and eliminating false-negative patients with balanced ischaemia (triple vessel disease). [31]

New technology in PET-MPI for diagnosis of CAD show similar results as described above, for usage of flurpiridaz ^{18}F in comparison to SPECT, bringing more advantages for PET-MPI (higher quality images, diagnostic certainty of interpretation, and sensibility of detection of CAD). [32]

Cardiac magnetic resonance

One of the most advantageous methods of imaging techniques, because of the high-detail images, non-invasivity and lack of any ionizing radiation, cardiac magnetic resonance can identify CAD, even better than the previous methods discussed. Similar to all CAD detection methods, is uses a stress agent, exercise or pharmacological.

Stress cardiac magnetic resonance using both a bicycle [33] and a treadmill [34] seems feasible for detection of CAD. Recently, the EXACT study, which used stress CMR with a treadmill, showed a sensitivity of 79% and a specificity of 99% for detection of CAD after using a SPECT, also showed higher agreement of diagnosis with angiography (k=0.82) than SPECT (k=0.46), demonstrating as such excellent diagnostic value. [35]

Pharmacological stress uses a vasodilator (adenosine or regadenoson) or dobutamine. Added in the vasodilator method is the administration of i.v. gadolinium contrast, to enhance the perfusion defects that can be seen in ischaemic walls. Using dobutamine requires a similar method like in stress echocardiography, by viewing wall motion abnormalities in ischaemia response. [36]

In CMR the assessment of CAD can be either qualitative (by visual assessment of presence and degree of ischaemia) or quantitative by measuring absolute blood flow. A recent study showed that quantitative analysis had similar diagnostic accuracy (83%) as qualitative (80%) and observers considered a better approach to diagnose CAD using quantitative CMR. [37]

Recent data showed that CMR stress with perfusion in comparison with SPECT and PET showed good diagnostic accuracies, having a sensitivity of 89% (in comparison to SPECT – 88%, and PET – 84%) and a specificity of 76% (in comparison to SPECT – 61% and PET – 81%). Authors also considered that CMR could be a good alternative to PET, having a similar diagnostic accuracy. [38]

Nevertheless, current data shows that perfusion CMR has a better diagnostic performance value than SPECT, as shown in the MR-IMPACT trial [39]. Also, the CE-MARC study showed that CMR had better sensitivities than SPECT (88.7% for men vs. 50.9% for men – SPECT and 85.6% for women vs. 70.8% for women – SPECT) and similar specificities, and better diagnostic accuracies. [40]

The CE-MARC 2 study compared the efficacy of CMR, NICE guidelines and SPECT in reducing the percentage of unnecessary angiographies in patients with suspected angina. The results showed that the CMR strategy determined a lower probability of unnecessary angiographies within 12 months than the NICE guidelines strategy, CMR and SPECT reducing significantly the number of angiographies in comparison to NICE guidelines. [41]

The adverse effects for gadolinium-based contrast agents used in CMR have a low frequency (0.36%), the severe are even less (0.033%), and these adverse effects are more physiological than allergic (most frequently were dyspnea, hypersensitive reactions and emesis). [42].

Similar to SPECT, it had been shown that CMR can have long-term prediction values. Newer technology in CMR measures GLS (global longitudinal strain), this marker can be an independent predictor for long-term major adverse cardiac events (MACE): heart failure hospitalization, myocardial infarction, sustained ventricular tachycardia or death, in pacients with known or suspected coronary artery disease. That means CMR can identify patients with high risk of having dangerous adverse events in the near future because of CAD. [43]

Also, the newest data shows that CMR with quantitative myocardial perfusion mapping can detect coronary microvascular dysfunction separately from CAD, with high AUC-ROC prediction values (0.95 on vessels with MVD) with high sensitivity (90%) and specificity (89%), almost comparable with invasive procedures. [44]

More powerful magnets (3.0 Tesla), although not so widely available, used on CMR machines, have a net superiority over 1.5 Tesla CMR, having a far greater AUC-ROC prediction value (0.963 vs 0.645), sensitivity (90.5% vs. 61.9%) and specificity (100% vs. 61.9%). [45]

Computed tomography

Multidetector row CT systems used in cardiac CT are sufficient enough to visualize coronary anomalies, although this last method is preferable for measuring coronary anatomy and pathology occurring in this matter. There are two methods of using CT in the benefit of diagnosis for CAD: calcium scoring and CTCA (coronary computed tomography angiography).

Calcium scoring uses the principle of identifying coronary calcification, without use of contrast. Any pixel above 130 Hounsfield units is defined as coronary calcification and can be quantified using the Agatston score, which is dependent by the size of the plaque and the radiographic density. [46]

However, there can be identified obstructive coronary lesions which may be not calcified and vice-versa, critically calcified coronary arteries which aren't yet obstructed. Because of this matter, the preferable way to diagnose CAD is CTCA.

The North American Society for Cardiovascular Imaging (NASCI) agreed in their guidelines that patient selection is needed before usage of CTCA. As such:
- because of contrast usage, any patients with known history of severe anaphylactic reactions to contrast, have contraindication to this method;
- patients with history of acute myocardial infarction, severe hypotension, decompensated heart failure or renal impairment have also contraindication to this method;
- pregnant women or patients who are unable to cooperate to the breath-hold instructions are not able to have CTCA. [47]

The method involves usage of a bolus of contrast injected i.v., and after that realizing a coronary angiogram with complementary ECG gating. The ACCURACY trial showed that CTCA in comparison to ICA (invasive coronary angiography) had similar performances in measurements (CTCA sensitivity – 95% vs. 94%, CTCA specificity 83% vs. 83%, CTCA positive predictive value – 64% vs. 48% and CTCA negative predictive value 99% vs. 99%) for diagnosis of CAD with 50%/70% stenosis. [2]

Although the main issue in this non-invasive technique is usage of radiation and scan preparation, another problem is that presence of heavy calcification (objectively measured by an Agatston score > 400) in case of patients with severe CAD lowers the specificity, by creating beam hardening artefacts and excessive image noise. [48]

Analysis of outcome data, such as in the PROMISE trial, showed that patients with suspected CAD who did CTCA had in long-term observation, fewer catheterizations than patients who were functionally tested (using exercise electrocardiography, stress echocardiography or nuclear stress testing) although these patients hadn't any improvement in clinical outcome. [49], and also the SCOT-HEART trial showed that usage of CTCA reduced fatal and non-fatal myocardial infarction but not significantly in patients with suspected CAD. [50]

Improving the current performance in CTCA is possible. One method is measuring tthe fractional flow reserve (CTFFR). Although it is highly time-consuming and requires computer modeling, data shows that usage of CTFFR in CTCA is beneficial, increasing the diagnostic accuracy in comparison to standard CTCA. [51]

Additional usage of β-blockade and nitroglycerin before the exam seem to increase the diagnostic performance [52] and newer data show that CTFFR-CTCA is a safe alternative to ICA with a significantly lower rate of ICA in comparison to functional testing (61% cancelling rate), therefore lowering the clinical cost and having the same clinical outcomes. [53]

Machine-learning (based on a deep learning model) in CTFFR-CTCA seems to also make better the standard procedure, by reclassifying patients with nonsignificant stenosis and raising overall diagnostic accuracy (from 71% to 85%). [54]

CONCLUSION

Currently, non-invasive techniques used to diagnose stable coronary artery disease have a very high accuracy and newer methods seem to be comparable to the gold-standard. The majority of the methods discussed have an optimal performance for patients with PTP between 15-85%, and the future of diagnosis for these patients seem to involve less invasive measures and less radiation by improving the current devices and by usage of machine-learning algorithms.

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CLINICAL PRACTICE

Endoscopic eradication of nodular gastric vascular antral ectasia by using band ligation after argon plasma coagulation

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Abstract: *Gastric antral vascular ectasia (GAVE) is an important cause of gastro-intestinal bleeding. The most common clinical presentation of GAVE is chronic occult bleeding that leads to symptomatic iron deficiency anemia, but some cases could present with acute massive bleeding. Frequently, patients are dependent by iron suplimentation, or in severe cases even blood transfusions. Endoscopic therapy is frequently necessary in acute or chronic blood loss. Over the past several years, treatment for GAVE has continued to evolve as the number of available effective therapeutic interventions has increased. These included: YAG laser, argon plasma coagulation (APC), endoscopic band ligation, cryotherapy and surgical anterectomy. Argon plasma coagulation is the most commonly used technique, but has been associated with several complications like sepsis, post-APC bleeding, gastric outlet obstruction and increased incidence of hyperplastic polyps. Endoscopic band ligation (EBL), a mechanical procedure, has been reported in the past years as an effective salvage therapy for GAVE that is refractory to other approaches, or even as the first line treatment. We present a case of nodular GAVE treated succesfully with endoscopic band ligation after unsuccesufull sessions with argon plasma coagulation.*

Keywords: *gastric antral vascular ectasia, endoscopic band ligation, Argon plasma coagulation, gastro-intestinal bleeding*

INTRODUCTION

Gastric antral vascular ectasia (GAVE) or “watermelon stomach” is a relatively rare cause of gastrointestinal (GI) bleeding that mainly affects women aged 70 years and older. The incidence is estimated to be aproximately 4% of upper GI bleeding [1]. The term "watermelon stomach" is derived from the characteristic endoscopic appearance of longitudinal rows of flat, reddish stripes radiating from the pylorus into the antrum that resemble the stripes on a watermelon [2].

It usually presents as occult bleeding with chronic iron deficiency anemia. Even though GAVE was first diagnosed about six decades ago, its etiopathogenesis has not been fully established yet, with many hypotheses proposed such as mechanical stress, hormonal factors, and autoimmune factors. It is associated with liver cirrhosis, autoimmune disease, connective tissue disorders and collagen vascular disorders (eg, systemic sclerosis, Sjogren syndrome), chronic renal failure, bone marrow transplantation, acute myeloid leukemia and heart disease.

Frequently GAVE patients have chronic GI bleeding, but sometimes it can cause severe acute life-threatening bleeding especially in elderly with multiple chronic medical illnesses [2].

GAVE is typically located in the gastric antrum; however, it may be also found rarely in other areas of the GI tract, including cardia, duodenum, jejunum, and rectum. The

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involvement of the proximal part of the stomach is very rare and commonly located within a diaphragmatic hernia.

At endoscopy, GAVE may appear as 2 types. First type is the diffuse punctuate lesions in the antrum typically seen in male patients with cirrhosis and commonly accompanied by acute bleeding. Second one is the red lesions organized in stripes radially departing from the pylorus, known as “watermelon stomach”, mostly common in females with connective tissue diseases and usually present with occult bleeding [1].

Histopathologically, GAVE is characterized by vascular ectasia, spindle cell proliferation, and fibrohyalinosis. Immunohistochemical staining for CD61, a platelet marker, further confirms a diagnosis of GAVE [3].

Over the years, treatment for gastric antral vascular ectasia (GAVE) has continued to evolve and the number of available treatments has continued to increase, including surgical, medical and endoscopic therapies

Medical therapy has not clearly shown satisfactory results. Multiple drugs, such as hormonal (estrogen-progesterone) therapy, steroids, octreotide and tranexamic acid, have been tried to control GAVE-related bleeding. After all, no one has clearly shown satisfactory results in order to consider medical therapy as a valid alternative to an invasive approach.

Endoscopic therapies have rapidly become the first line therapy with argon plasma coagulation (APC) as the most common used method and more recently with radiofrequency ablation system (RFA) using Halo90 catheter and endoscopic band ligation (EBL). Both of them have been shown to be safe and effective for GAVE treatment. The latter two have been utilized in treatment of severe, diffuse, APC refractory GAVE. The clinical outcomes of ablative therapy such as APC treatment or HALO90 system were reported as 80%-100% success rate.

Other therapies include Nd:YAG (neodymium:yttrium-aluminum-garnet) laser coagulation, but with a higher risk of perforation given the deeper thermal effect. Endoscopic sclerotherapy, heater probe, cryotherapy have also been described in the literature. For unresponsive cases to endoscopic therapy, surgery with antrectomy can be considered but carries a high surgical risk, especially in the cirrhotic patients.

APC is most commonly used method, but has been associated with sepsis, post-APC bleeding, gastric outlet obstruction and increased incidence of hyperplastic polyps’ formation. It is needed multiple sessions of APC to reduce bleeding episodes and/or decrease transfusion dependence. APC has been found to be equally effective in the treatment of GAVE and is superior to ND:YAG laser in cost, convenience and complication rates(4).

The non-ablative treatment options is the endoscopic band ligation. EBL was firstly reported as the treatment for refractory GAVE in the patients who failed other treatment modalities such as APC or hormonal therapy by Sinha et al. [5]

Endoscopic band ligation has been reported as an alternative effective endoscopic therapy and the rate of complication is low in comparison with those reported for APC in retrospective studies [6].

A case series of 9 patients reported by Wells et al also showed superiority of EBL over endoscopic thermal therapy, which were APC and bipolar thermal probe therapy, for the rate of re-bleeding, duration of hospitalization and post procedure transfusion. The complications reported for this procedure were very small. Band ligation was prefered as the first line treatment regarding of the extensive involvement of the lesion in the patients. According to the result of the treatment mentioned above, endoscopic band ligation could be considered as first line treatment options for the GAVE patients especially for extensive area of involvement. [7]

A recent prospective study showed endoscopic improvement with the use of endoscopic band ligation in 91% of the patients, with a significant improvement in the hemoglobin and ferritin levels. Band ligation in GAVE has been associated with transient abdominal pain in a minority of the patients, but no major complications have been reported in the literature. [9]

Some studies suggest that the number of sessions required for GAVE eradication is inferior when using EBL compared to APC, resulting in inferior health care costs. However, randomized controlled trials are lacking to determine whether EBL is more cost effective than APC as the primary endoscopic therapy for GAVE. [9, 10]

Although initial reports of these endoscopic modalities are encouraging, well-performed, larger, prospective studies are needed before providing any definitive conclusion.

CASE PRESENTATION

Clinical data

We present a case of a 57-year-old female, diabetic with multiple microvascular complications – diabetic polineuropathy and chronic renal disease, hypertensive, with moderate hypochromic microcytic anaemia and a positive fecal occult blood test (FOBT), already in treatment with iron therapy who presented for abdominal pain, fatigability, phisical astenia and arthralgias. She denied no change in weight, bowel pattern, or stool color. The physical examination revealed paleness of the skin and mucosa and otherwise unremarkable.

Laboratory data

Labs indicates a moderate hypochromic microcytic anaemia (hemoglobine level – 9.4 g/dL, mean corpuscular volume – 70.2 fL, hematocrit – 31.1%, mean corpuscular hemoglobin – 21.2 picog and sideremia – 26 microg/L.

Endoscopy

Superior digestive endoscopy showed hypertrofic gastric antral vascular ectasia with mucosal eryhema, friability and visible small vessels extending radially in linear rows throughout the antrum and a duodeno-gastric reflux. She underwent serial argon plasma coagulation treatments and a second gastroscopy was performed next day which showed post argon plasma coagulation ulcers which were biopsied.

Histopathology data

Ulcer post argon plasma coagulation biopsy performed showed modifications of chronic inactive gastritis, but GAVE was not confirmed on biopsy.

Evolution

After two months and with iron therapy, the hemoglobin level was normalised (12.7 g/dL) with low mean corpuscular volume and mean corpuscular hemoglobin. At the endoscopic examination the macroscopic appearance persisted with multiple friable hypertrofic and nodular antral vascular ectasia and duodeno-gastric reflux.

At 3 months follow-up, hemoglobin level mantained in the normal interval (13.5 g/dL), but the endoscopic appearing was worst, with larger, friable, with edema and hypertrofic gastric atral vascular ectasia (Figure 1). We decided to apply 6 elastic bands on the biggest lesions with success (Figure 2). No complications of the procedure were reported, and the patient was discharged after 24h.

Figure 1: Endoscopic appearance from the index endoscopy, demonstrating multiple erythematous friable antral hypertrophic and nodular lesions, representing nodular gastric antral vascular ectasia (GAVE)

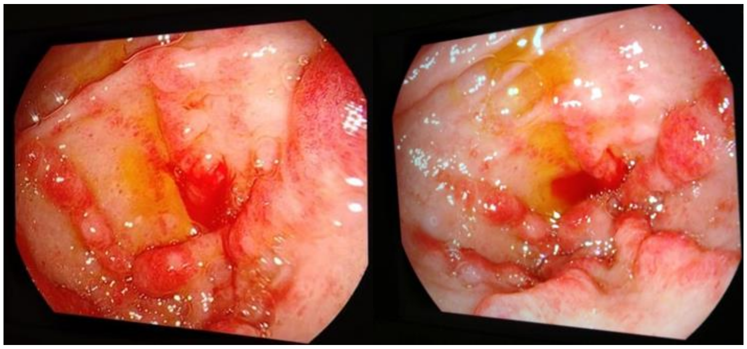

Figure 2: Endoscopic image from the band ligation of GAVE

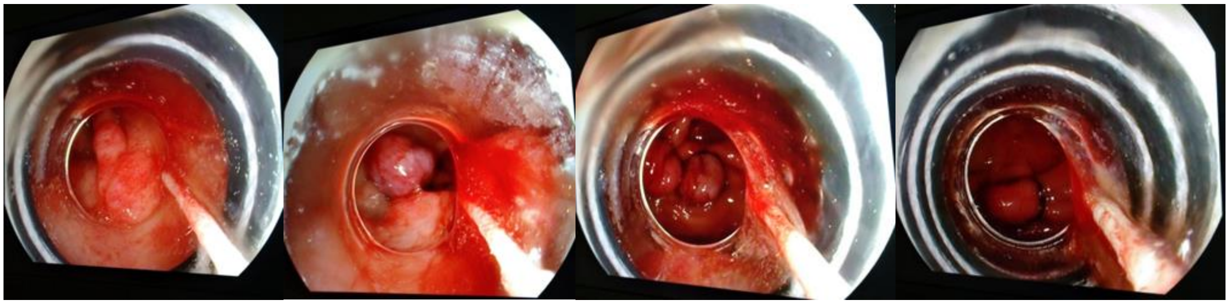

Two months later, hemoglobin level was stable (13.4g/dL). The upper endoscopy revelead small hyperemic antral circumferential disposed lesions with no signs of bleeding and no hypertrophy, with almost complete eradication of GAVE (Figure 3).

DISCUSSION

GAVE is a poorly understood entity, of unknown etiology, and an increasingly identifiable cause of chronic iron deficiency anemia. The pharmacological management of

GAVE had only poor results, so the mainstay of GAVE management remains endoscopic therapy.

Figure 3. Endoscopic appearance 2 months after endoscopic band ligation

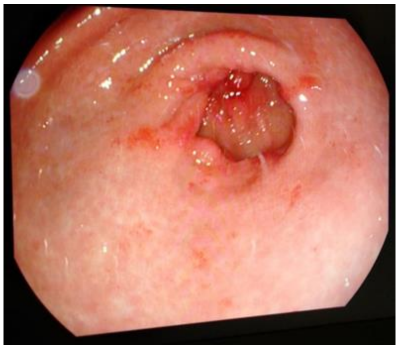

Figure 4. Suggested flow chart for treatment algorithm [14]. GAVE: Gastric antral vascular ectasia; APC: Argon plasma coagulation; RFA: Radiofrequency ablation; EBL: Endoscopic band ligation.

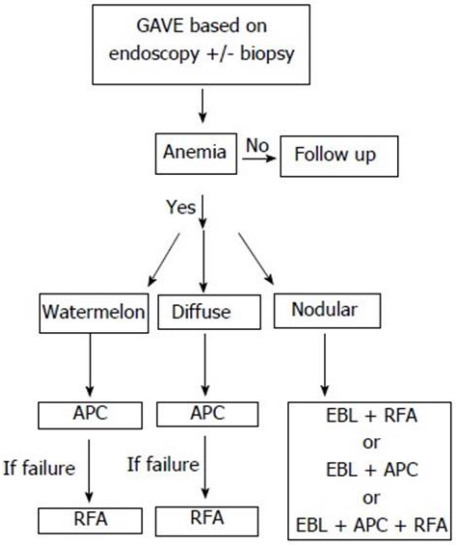

APC is a modality of non-contact electrocoagulation that applies high-frequency energy into tissue to cause thermal effects, which has been used successfully to treat GAVE with an effective transient response, but primary failure rates of therapy of up to 14% have been reported. Some authors suggest that this endoscopic method is insufficient in order to achieve medium and long-term treatment success, as it has been associated with a high recurrence rate (40–100%) [11].

In addition, APC might have complications, such as sepsis, pyloric stenosis and gastric outlet obstruction syndrome, in 20–33% of the patients [12].

EBL has been reported to be a relatively easy technique for GAVE therapy, has been shown to be safe and effective with lower complication rates in comparison with APC. Both Sato et al. [6] in 2012 and Prachayakul er al. [13] in 2013 conclude that EBL may be useful in the treatment of GAVE, to avoid the high recurrence rate after APC.

In this case, the patient was initially treated with APC after being diagnosed with GAVE as the cause of iron deficiency anemia, but this strategy was not successful. APC treatment might not be effective in some cases, especially in the case of hypertrofic and nodular type of GAVE, and this could be explained by the limited depth of thermal injury.

Since the histological changes are present in the mucosa and submucosal layer, EBL may be more effective for GAVE because of its ability to obliterate the submucosal vascular plexus like for esophageal varices. In this patient, the presence of extensive areas of the antrum affected with a high-density of both mucosal and submucosal vascular malformations is a likely explanation for the primary failure of the APC treatment.

It is becoming apparent that patients with severe, diffuse or refractory disease require multimodal therapy. Our case not only shows that, but also that patients specifically with nodular variant GAVE require and respond well to multimodal therapy.

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CLINICAL PRACTICE

Death due to a rare posttraumatic complication: fat embolism

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Abstract: *Fat embolism is a rare complication of high or medium intensity trauma. It is caused by the formation of fat particles in the territories of small terminal circulation, especially at pulmonary, tegumentary and cerebral levels. The mechanism underlying the fat embolism is described by several different theories in literature. In legal medicine, fat embolism raises many controversies upon the diagnostic certainty of the thanatogenerator mechanism leading to death. The occurrence of fat embolism syndrome followed by death must be properly explained so that the fatality can be adequately framed from a legal point of view. In this paper the authors present three cases in which victims of trauma died due to clinically undiagnosed fat embolism, the diagnosis being established only by postmortem histopathological examination. The authors underline the fact that the diagnosis of fat embolism syndrome, although it is a clinical one, it is often established only during autopsy.*

Keywords: *fat embolism, trauma, death, autopsy, diagnosis*

INTRODUCTION

Embolism represents the motion through circulatory blood flow of a material which is not present in blood in normal conditions and its subsequent lodging inside the blood vessels. In the case of fat embolism, the material carried through the blood is represented by fat particles that reach the level of microcirculation. Fat embolism syndrome represents the systemic manifestation of the fat particles being present inside the microcirculation.

Fat embolism was first described by Zenker in 1862 in the case of a railroad worker who died of crushing injuries. In 1873 Bergmann diagnosed fat embolism in a living patient who had suffered a fracture of the femoral bone [1, 2].

The causes of fat emboli formation are multiple, both traumatic and non-traumatic. The most frequent traumatic causes are: long bone fractures, orthopedic procedures, soft tissue trauma (fat tissue laceration), liposuction and mastectomy, neurosurgical interventions. Non-traumatic causes include: acute pancreatitis, extensive burns, diabetes, fatty liver due to diets rich in extrinsic fats and oils [2, 3]. Exceptionally, fat embolism has been observed in cases of hepatic steatosis (in alcoholics and diabetic patients with hepatic damage) due to the destruction of hepatocytes and subsequent release of fat particles [4, 5].

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Fat embolism occurs in 90% of patients who have suffered a trauma, but only 2-5% of them develop the fat embolism syndrome; particularly at risk are those with fractures of the long bones. It is difficult to estimate the duration of this syndrome because the symptoms are often subacute or masked by other symptoms of trauma [2, 3].

Post-mortem studies have shown a different but a very high incidence of fat embolism. Thus, a study by Behn C. et al. on 527 autopsies demonstrated the presence of fat embolism in 92 of these [6]. Another study conducted by Hiss et al. in 1996, on 53 victims of aggression followed by death, showed a high incidence of fat embolism. All the subjects studied were young, male, victims of aggressions, suffering severe trauma 24 hours before death. In thirty two cases the presence of fat embolism was observed in the main organs, with no other obvious cause of death. The authors of the study hypothesized that the source of fat embolism was the mechanical disintegration of the subcutaneous adipose tissue [7].

In cases of fat embolism there is a poor correlation between post-mortem and clinical findings. This discrepancy ultimately gave rise to the concept of "iceberg effect of fat embolism" [8- 12]. This justifies, as well as cases with a insufficiently outlined clinical picture for a particular pathology, or a clinical picture that cannot explain the occurrence of death, the importance of forensic autopsy and postmortem laboratory investigations (histopathological and toxicological) in order to establish the thanatogenerator mechanism and the causal link between trauma / illness and death, and ultimately the legal nature of the case, i.e. suicide, accident or homicide [13- 19].

MATERIALS AND METHODS

We present three cases of trauma in which the cause of death was the fat embolism syndrome. All the cases are characterized by a rapid clinical deterioration leading to death, so that a clinical diagnosis of fat embolism syndrome could not be established antemortem.

Case no. 1

Male, 72 years old, chronic alcohol consumer, with past medical history of left femoral fracture and right sided craniotomy, is admitted to hospital for head trauma. At the time of admission physical examination revealed multiple ecchymoses, pain at the pelvic level, lower limbs and abdomen. Clinical and paraclinical investigations established the following diagnosis: minor craniocerebral trauma, facial trauma, posttraumatic subarachnoid hemorrhage, dura mater hygroma, abdominal contusion, pelvic contusion. Surgical intervention was performed to evacuate the dura mater hygroma 6 days after the admission. Evolution was initially favorable. On the 4th postoperative day, the patient developed a febrile syndrome. Laboratory analysis highlighted: elevated leucocyte count (13.390 of which 11.270 neutrofils); elevated serum amylase (225 U/L); lowered erythrocyte count (3.83 milion); hematocrit levels as low as 40.08 %; low thrombocytes count (137.700); increased level of the liver enzyme AST to 48 U/L; low creatinine serum levels (0.69 mg/dl) and reduced glomerular filtration rate to 85 mil/min/1.73 m2. The next day the patient suffered cardiorespiratory arrest that did not respond to resuscitation maneuvers, followed by death. The forensic autopsy was performed. The external examination of the body showed: multiple injuries denoting violence such as ecchymoses and abrasions; signs of medical treatment at the level of the skull, i.e. recent surgical incision and an old whitish scar. The internal examination showed: on the head, 2 craniotomy areas, one of them being recent, subarachnoid hemorrhage, areas of flaccid cerebral structure, small petechial hemorrhagic areas disseminated throughout the brain. The examination of the lungs showed: pulmonary stasis and edema, and areas of pulmonary condensation. Fragments of brain and lungs were harvested during autopsy and subsequently examined by optic microscopy using the Sudan III staining. The microscopic examination revealed fat emboli in the brain and lungs. Following the postmortem macroscopic and microscopic examination we established that the cause of death was the fat embolism syndrome.

Case no. 2

Male, 54 years old, was admitted to the hospital with the diagnosis of: head trauma (subarachnoid hemorrhage, fracture of the left temporal bone with extension to the mastoid bone), face trauma (fracture of right jaw, comminuted fracture of the nose) and chest trauma (left pneumothorax, multiple rib fractures).

The patient was hospitalized for 35 days, during which multiple pleurotomies and bronchoscopies were performed. Laboratory examinations showed: increased leucocyte count (25.170 of which 21.100 neutrofils); low red blood cell count (2.612 milions); low hematocrit (21.240 %); low hemoglobin level (7.793 g/dl); normal thrombocyte count; increased level of AST (103 U/L); increased serum creatinine (4.28 mg/dl); increased GGT levels (93 U/L) and reduced glomerular filtration rate (10.8 mil/min/1.73 m2). Clinical evolution was initially favorable. On day 34 after admission the patient developed a feverish syndrome. The next day he suddenly suffered cardiorespiratory arrest, followed by death. A forensic autopsy was performed. The external examination of the cadaver revealed bruises and signs of recent medical treatment. The internal examination showed: linear head fracture, subdural hematoma under partial resorption, small petechial hemorrhagic areas in the brain, pulmonary edema, and areas of pulmonary condensation. Fragments of organs were harvested (brain, lung) and examined by optic microscopy using Sudan III staining. Following the macroscopic and microscopic examinations we concluded that the cause of death was the fat embolism syndrome.

Case no. 3

Male, 20 years old, victim of a traffic accident, was admitted to the hospital with the diagnosis of: polytrauma due to road traffic accident, major craniocerebral trauma with diffused cerebral edema, multiple rib fractures, fracture of the left iliac wing and left ischial bones, fracture of the left femoral neck, and type III A fracture of the femoral diafisis. Patient was admitted in the hospital for 7 days and underwent a surgical intervention for the femoral fracture. During the first days after the surgery the patient was hemodynamically stable. On day 4 the patient showed tachypnea, tachycardia, and fever and his clinical condition suddenly worsen. The laboratory examinations showed: low leucocyte count (1.56 of which 1.37 neutrofils); reduced numbers of erythrocytes (2.31 milions); low hematocrit (23.3%); low hemoglobin (6.77 g/dl); reduced number of thrombocytes (58.600/ mmc); increase in liver enzyme AST (272 U/L) and ALT (250 U/L); increased serum creatinine (2.43 mg/dl); increased serum urea (94.16 mg/dl) and reduction of glomerular filtration rate to 27.21 mil/min/1.73 m2. Death occurred on the same day. At autopsy, the external examination of the corpse revealed: multiple ecchymoses, abrasions, hematomas, signs of medical treatment. The internal examination showed: brain contusion; multiple rib fractures; pulmonary edema, pulmonary condensation areas, pulmonary infarction; fractures of the pelvis and of the left femor. The microscopic examination of the fragments of brain and lungs collected during the autopsy and stained with Sudan III showed fat embolism. Therefore, based on macroscopic autopsy findings and histo-pathological examination of the brain and lung fragments, we established that the cause of death was the fat embolism syndrome.

RESULTS

In all the three cases the victims suffered various trauma: in the first case the victim suffered soft tissues and head injuries, in the second case the victim suffered a polytrauma with multiple fractures and soft tissue injuries and in the third case the victim suffered craniocerebral trauma, soft tissue injuries and long bone fractures. Therefore, in all the cases, the etiological conditions for the fat embolism occurrence were met. However, in none of the cases the diagnosis was clinically established antemortem. The examination by optic microscopy of the fragments of brain and lungs collected during autopsy and stained with Sudan III showed in all the cases the fat microemboli in the form of orange globes disseminated throughout the fields examined microscopically (figures 1-4) and allowed the postmortem diagnosis of fat embolism. Therefore, in all three cases presented, the results of the histopathological examination of the fragments harvested from the brain and the lungs during the autopsy were essential for determining the cause of death.

Figure 1: Microscopic aspect of brain tissue
Sudan III stain x 40 (ice exam)

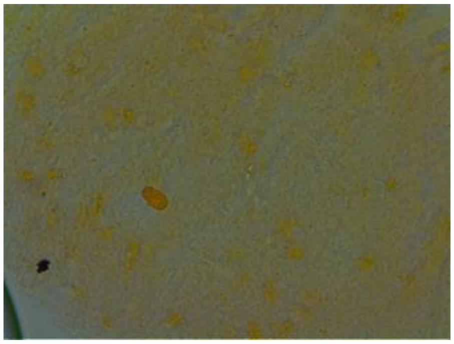

Figure 2: Microscopic aspect of lung tissue
Sudan III stain x 20 (ice exam)

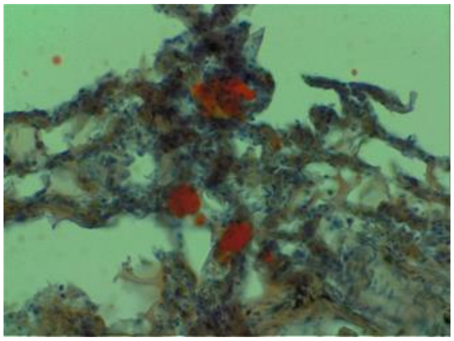

Figure 3: Microscopic aspect of lung tissue
Sudan III stain x 40 (ice exam)

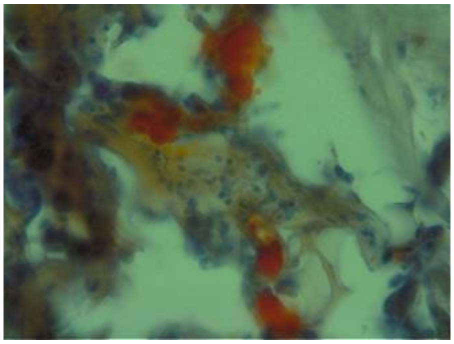

Figure 4: Microscopic aspect of lung tissue Sudan III stain x 10 (ice exam)

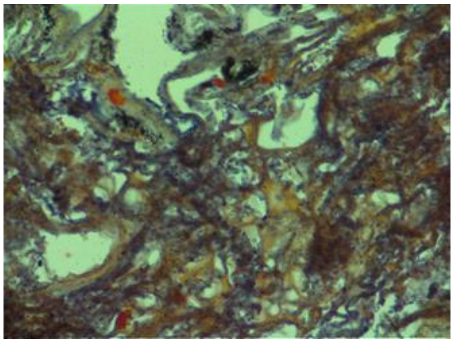

DISCUSSION

Fat embolism occurs due to the mobilization of fat cells or fat released by them, followed by their direct penetration into open blood vessels or lymphatic channels. Most emboli have a diameter of 15 to 40 μ; the fat emboli in the lungs are generally larger than those in the large arteries of the systemic circulation [11].

Several theories are mentioned in the literature to explain the pathogenesis of the fat embolism [20].

The mechanical theory shows that mobilization of fat particles occurs due to trauma to the bones or soft tissues. As a consequence, the fat particles released by the trauma enter the venous circulation from where they reach the lungs, where the majority remains, but some of them can also pass into the systemic circulation, reaching other organs.

The biochemical theory, also known as the emulsion instability theory, is mostly useful in explaining the non traumatic fat embolism.

The intravascular coagulation theory holds that in stressing conditions, substances that activate disseminated intra-vascular coagulation and aggregation of fat particles are released.

The toxic trauma theory claims that small blood vessels are affected by high concentrations of fatty acids in the plasma leading to increased vascular permeability.

The mechanical theory is based on the concept of the mobilization of fatty bone marrow from the long bones diaphysis. In the cases presented by us, only one patient suffered a recent fracture of the femur. In the other two cases we have to consider the trauma of soft tissues (hypodermic tissue) as the cause of the fat embolism, by noting that in both cases multiple ecchymoses were observed on the skin.

From a clinical point of view, Gurd and Wilson have developed the major and minor criteria which allow which allow for the diagnosis of fat embolism. The major criteria are: axillary or subconjunctival petechiae; hypoxaemia (PaO2 <60 mm Hg, FIO2 = 0); central nervous system depression disproportionate to hypoxaemia; pulmonary oedema; radiological signs and symptoms of respiratory insufficiency; cerebral changes unrelated to a cranial trauma or other illness; rash. The minor criteria are: tachycardia more than 110 bpm; pyrexia more than 38.5°C; fat globules present in urine; changes in renal function (reduced urine output); drop in haemoglobin level (more than 20% of the value upon admission); drop in haematocrit values; drop in platelet values (more than 50% of the value upon admission); increased erythrocyte sedimentation rate (greater than 71 mm per hour); fat globules present in the sputum; emboli present in the retina; tachicardia; fever; renal insufficiency; sudden thrombocytopenia. According to Gurd and Wilson the diagnosis of fat embolism can be established if either one major criterion and 4 minor criteria or 2 major criteria are met [21].

Lindeque suggested that the criteria of Gurd and Wilson may under-diagnose the syndrome, and proposed the following criteria based on respiratory parameters: Pao2 kPa of less than 8Fio2 0.21; Paco2 kPa or pH of less than 7.3 or more than 7.3; respiratory rate greater than 35 breaths/min−1 even after adequate sedation; increased breathing efforts showed by: dyspnea, use of accessory muscles, tachycardia and anxiety [22]. Any patient with a fractured femur and/or tibia, presenting one or more of these criteria, was diagnosed with fat embolism syndrome. These criteria led to the diagnosis of fat embolism syndrome in 29% of patients in a series of 55, which is higher than other series, especially since this study excludes patients with thoracic lesions, where some of Lindeque's clinical signs may appear in the absence of fat embolism [22].

Petechial eruption is considered a pathognomonic clinical sign for fat embolism syndrome. It may occur in about 60% of patients, usually on the conjunctiva, on the oral mucous membrane and on the skin of the neck and shoulders. This distribution can be explained by the drops of fat that accumulate in the aortic arch before the independent embolization at skin level through the subclavian and carotid vessels [20, 23-25]. The factors contributing to the petechial eruption are: stagnation of blood, loss of coagulation factors and platelets, and damage to the endothelial walls due to free fatty acids (FFA) leading to rupture of capillaries [25].

In the vast majority of cases, the fat embolism remains asymptomatic, and the fat emboli are dispersed into small cells and phagocyted by macrophages or embedded in hepatic cells [26, 27]. Thus, many cases remain undiagnosed before death.

In the three cases presented, the symptomatology before death was poor and unspecific (febrile syndrome, tachycardia, tachypnea), and remained undiagnosed. The diagnosis of fat embolism syndrome was established only after performing the autopsy, and the histopathological examination of the brain and lung fragments stained specifically with Sudan III. Therefore, for post mortem diagnosis of fat embolism syndrome, it is essential to perform a histopathological examination with special stains that highlight the presence of fat emboli in the tissues and organs.

In the cases presented in this paper, fat embolism at the pulmonary and cerebral level was observed. According to literature, brain and lung localizations are the most important, although in most cases pulmonary fat embolism remains clinically asymptomatic. In order to be able to certify fat embolism as the cause of death, it is necessary for histopathological examinations to find fat emboli on a large part of the examination fields. The gravity of the consequences of pulmonary embolism also depends on the size and amount of fat emboli, as well as whether or not they pass into the systemic circulation to the lung.

In the brain, emboli produce small petechial hemorrhages, areas of ischemic necrosis and hemorrhage or necrosis and demyelination. Cerebral embolism is considered in literature as a paradoxical event, because it requires the presence of cardiac atrial septal defects in order to be explained. In the presented cases, there was no autopsy finding that showed neither atrial or ventricular septal defects nor patent arterial canal that could explain the cerebral fat embolism. An explanation of the pathophysiological mechanism which determined the cerebral embolism could not be found in this study.

The cause of death in all the three cases presented in this paper was the fat embolism. The diagnosis was established with certainty only after autopsy, by a histopathological examination which revealed the presence of fat emboli in the lung and brain tissues. Of the cases presented only one suffered a recent fracture of the femur; another presented in addition to trauma paraclinical changes suggestive of acute pancreatitis as a contributing factor; none had cardiac malformations that could explain the paradoxical fat embolism in the brain; all of the cases showed multiple lesions of the subcutaneous tissue and death occurred shortly after the initial trauma or after surgical interventions.

Death due to fat embolism can fall into the category of violent or non-violent deaths. Regardless of the cause of fat embolism, from forensic point of view the fat embolism is considered a complication that leads to a secondary causal relationship; in other words between the trauma/ initial pathology and death an intermediate link is inserted represented by a complication (the fat embolism) which is connected to a greater or lesser extent to the initial trauma/ pathology [28-30].

CONCLUSION

From forensic point of view, the diagnostic certainty in posttraumatic lesions is a priority objective. From the diagnosis it is possible to reconstruct the thanatogenerator mechanism and finally it can be useful to respond to the legal aspects of the case, i.e. the causal relationship between trauma and death.

In the case of trauma with reduced thanatogenerator potential, which usually evolve without complications, the occurrence of fat embolism syndrome followed by death must be appropriately explained so that the case can be properly framed from a legal point of view. It is important to emphasize that the histopathological examination using specific stainings, such as Sudan III, has a fundamental and incontestable role in the diagnosis of fat embolism and in estimating its gravity, and therefore, its implication in the occurence of death.

Conflict of interest: *The authors declare that there is no conflict of interest.*

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Papillary thyroid carcinoma arising on a hypertrofic pyramidal lobe

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INTRODUCTION

Development of thyroid gland starts by the pharyngeal epithelium thickening floor which later forms a diverticulum which, in its development is pushed caudally. The descent path is usually anterior to hyoid bone but it can also be posterior or through the hyoid bone and ends on the anterior surface of the first few tracheal rings.

This primitive steam that connects primordium with pharyngeal floor becomes thyroglossal duct. Until the second month of gestation thyroglossal duct devolve, leaving at its place a small lump – the foramen cecum, at the unification of third medium with posterior third of the tongue, but portions of the duct associated with thyroid tissue can persist at any site between tongue and thyroid [1, 2].

Cells in the lowest portion of the thyroglossal duct which comprises normal thyroid tissue differentiates in forming pyramidal lobe of the thyroid gland. Pyramidal lobe often comes out from the thyroid isthmus, but can also come from the medial side of one or both thyroid lobes [1]. The pyramidal lobe is thought to be present in 15-75% of the general population [1, 3]. Thyroglossal duct fails to involute in approximately 7% of the population [4].

Many remnants of thyroglossal duct are never detected clinically (2) and malignant transformation is uncommon [5].

CASE REPORT

We present the case of a 50 years old female patient with chronic renal failure of unknown etiology (probably secondary to hyper blood pressure), with Graves Disease (in treatment with antithyroid drugs for about 1 year) who was admitted in our department after she has initially presented in an ENT department (Otolaryngology) for the investigation of a tumoral mass located in the midline upper neck. At that moment physical exam revealed a 2/3 cm mass between the hyoid bone and the thyroid cartilage, mobile on swallowing, painless spontaneously and on palpation, covered by normal skin. IRM exam revealed a bilobate lesion 2.9/1.1 cm, very well encountered, in close relationship with the adjacent muscles and the hyoid bone, without any other pathological changes on the structures of the anterior neck: no enlarged lymph nodes. There were no reference about thyroid gland on the IRM report. The IRM conclusion was: expansive anterior cervical soft tissues tumoral mass. Surgical excision of the tumoral mass and simultaneous removal of the central portion of the hyoid bone (Sistrunk procedure) was performed and the pathology report showed papillary thyroid carcinoma arising from thyroglossal duct. In our department, three months later, thyroid ultrasound showed hypoechoic, inhomogeneous echotexture suggestive for chronic autoimmune process, hypoechoic nodule with discrete Doppler flow, without hypoechoic halo located at the connection of the right thyroid lobe with isthmus and two micro lymph nodes with intense Doppler flow, without hilum of 0.5/0.4 cm and 0.7/0.4 cm located anterior of the larynx. Tumoral mass previously removed in the ENT department was reviewed and the pathology report showed tumoral multilobulated pyramidal thyroid lobe with pattern

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of solid and sclerosant variant of papillary carcinoma, infiltrative into fibro conjunctive, adipose and muscular adjacent tissue (Figure 1 and Figure 2) and areas of micro angioinvasion of the capsule (Figure 3).

Figure 1: Papillary carcinoma infiltrative into fibroconjunctive, adipos and muscular adjacent tissue (x20 HE)

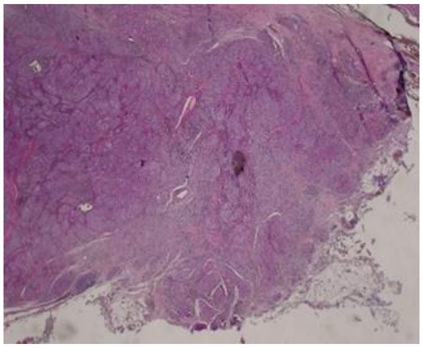

Figure 2: Papillary carcinoma infiltrative into fibroconjunctive adipose and muscular adjacent tissue (x40 HE)

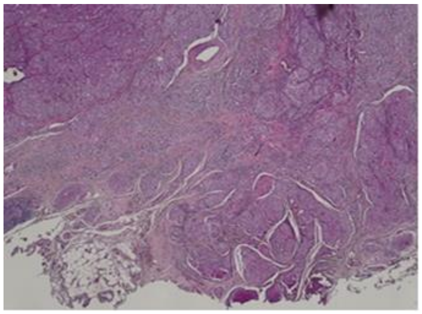

Figure 3: Micro invasion of the capsule (x200 HE)

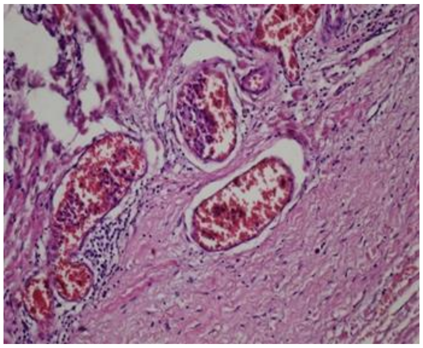

In this context, we decided total thyroidectomy (also as a treatment for Graves Disease) with dissection of central lymph nodes compartment. Histopathology report revealed embedded thyroid isthmus focus of papillary thyroid carcinoma 0.7/0.6 cm with tumoral pattern similar to that of tumoral pyramidal lobe previously removed (Figure 4), with areas of marginal invasion of the capsule (Figure 5) and micro angioinvasion of the capsule (Figure 6). The pathology report also showed reactive lymphadenopathy and post surgery staging was pT3N0.

Figure 4: Embedded thyroid isthmus focus of papillary thyroid carcinoma (x20 HE)

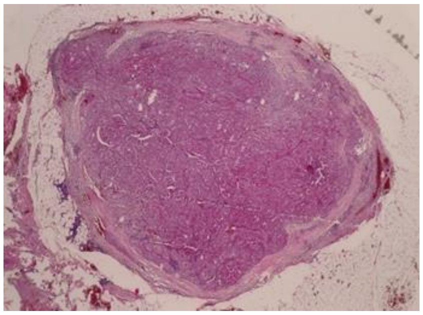

Figure 5: Marginal invasion of the capsule (x100 HE)

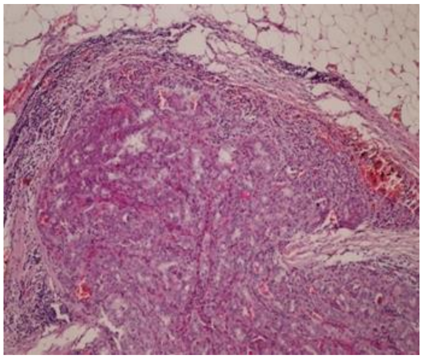

Figure 6: Microangioinvasion of the capsule (x100 HE)

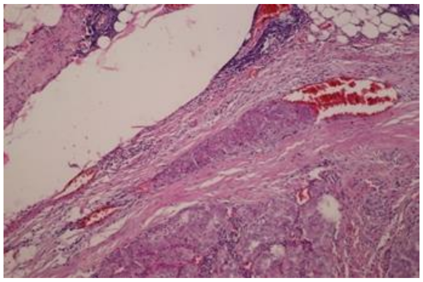

Radioactive iodine therapy was decided. Postsurgery, thyroglobulin level after Levothyroxine withdrawal is 0.2 ng/ml with unmeasurable thyroglobulin antibodies and 50 mCi 131I was administered. Post ablation thyroid scan showed no uptake of 131I in the thyroid bed or elsewhere in the body. Because of the association of BRAF mutation with papillary carcinoma of the pyramidal thyroid lobe, genetic testing of BRAF gene were performed and they were negative for somatic mutations in the 600 codon.

DISCUSSION

The detection of a pyramidal lobe or a thyroglossal duct in patients with hyperthyroidism is indicative of autoimmune hyperthyroidism. The vestiges of thyroglossal tract are more often seen in patients with Graves Disease compared with patients with autonomously functioning thyroid nodules and this is related with the presence of stimulating thyrotropin receptor antibodies [6, 7]. Pyramidal lobe can be the primary or secondary site of thyroid malignancy and during thyroidectomy, total excision of the pyramidal lobe is essential for patients with thyroid cancers who undergo radioactive iodine treatment (RAI) because the presence of pyramidal lobe prevents the increase in TSH and absorbs a large amount of the isotope and thus decrease the possibility to benefit from the treatment [8-10]. Residual pyramidal lobe can harbor cancer cells and from this point of view its total excision is indicated in thyroid cancers. In case of probable recurrence of malignant diseases due to pyramidal remnants, scintigraphy, ultrasound of the neck, computed tomography are indicated [11-13]

Thyroid pyramidal lobe can be the origin or recurrent site of papillary thyroid carcinoma. Malignant tumors arising from pyramidal lobe are rare and are associated with a high rate of concurrent thyroid cancer and it must be differentiated from thyroglossal papillary cancers because pyramidal cancer requires orthostatic thyroid surgery which is not always necessary in papillary thyroglossal cancers [14, 15]. Pyramidal lobe tumors are associated with poor prognostic factors such as: extra thyroidal extension, advanced T-stage, cervical lymph node metastasis, advanced AJCC stage (III, IV), BRAF mutation, multifocal thyroid cancer [16, 17]. In our patient there was one single embedded thyroid focus, no local lymph nodes metastasis and no distant metastasis. BRAF mutation was negative.

Malignant tumors arising from thyroglossal duct are also rare [18]. The majority are papillary carcinomas (about 94%) and less than 5% are squamous carcinoma [19]. The Sistrunk procedure that implies the simultaneous removal of the central portion of the hyoid bone to ensure the complete removal of the thyroglossal tract is enough for thyroglossal squamous carcinoma although they have a poor prognosis and a mortality rate of 30-40% [20]. On the other hand, papillary thyroglossal carcinomas are multicentric and multifocal and total thyroidectomy followed by 131I ablation and thyroid-stimulating hormone suppression is often required. There are still controversy regarding the need for total thyroidectomy, central or lateral compartment neck dissection and I131 ablation therapy in cases of papillary carcinoma of thyroglossal duct [21]. The decision depends on: tumor size (tumors larger than 1 cm require total thyroidectomy), abnormal findings of thyroid (multinodular goiter, cold nodule in a thyroid iodine uptake), histopathological findings, and the presence of enlarged lymph nodes or a history of neck irradiation [22].

In conclusion: in this report we have described a papillary thyroid carcinoma arising from a multilobulated pyramidal lobe including from a nodular peri-isthmic remnant of the pyramidal lobe (secondary to incompletely resected anterior tumor of the isthmus), in a patient with Graves Disease. The rest of the thyroid parenchyma is non tumoral. The complete resection of the thyroid is necessary because of tumor aggressiveness and the necessity of radioiodine ablation therapy.

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CLINICAL PRACTICE

Atypical Cogan syndrome; case report

Gabriela C. Musat[1,2], Roxana E. Decusara[2], Ovidiu Musat[3]

Abstract: *Cogan syndrome is a rare disease characterized by the concomitance of non-syphilitic interstitial keratitis with Meniere-like vestibulo-auditory symptoms. There are criteria for the diagnosis of both typical and atypical Cogan syndrome. We present the case of a 40 years old woman with sudden onset of hearing loss, tinnitus, intense vertigo, instability associated with kerato-conjunctivitis. The concomitance of the symptoms, the onset, and the evolution under treatment are consistent with the diagnosis of atypical Cogan syndrome.*

Keywords: *Cogan syndrome, atypical Cogan syndrome; vertigo; hearing loss*

INTRODUCTION

Cogan syndrome is a disorder characterized by the association between the Meniere-like vestibulo-auditory symptoms and ocular symptoms (interstitial keratitis). Although the first to describe a disorder associating ocular and inner ear symptoms were Morgan RF, Baumgartner in 1934 [1], the name of the disease comes from Dr. David Cogan who published in 1945 a series of 4 cases of patients with non syphilitic interstitial keratitis and vestibulo-auditory symptoms [2]. In 1980 Haynes et al proposed the enlargement of the criteria for the diagnosis, defining typical and atypical Cogan syndrome. They proposed that other ophthalmologic inflammatory manifestations such as episcleritis, uveitis, conjunctivitis, can be considered as disease criteria for atypical syndrome. [3]

For a disease described such a long time ago there is very little knowledge about the etiology of the disorder. Until now, approximately 250 cases have been published but we still don’t understand the etiopathogeny of the disease. It is considered an autoimmune disorder. This disease seems to affect young Caucasian adults with ages ranging between 25 to 35 years old, in most of the cases. [5]

CLINICAL FEATURES

Ocular manifestations: The main characteristic of the disease is the ocular involvement. Usually patients have red eye, eye pain and photophobia. The typical Cogan syndrome is defined by the presence of the non syphilitic interstitial keratitis. The examiner might notice granular and irregular infiltrate on the posterior part of the cornea. Neovascularization is also a possibility. Blindness and amaurisis can happen but usually the lesion regresses and the loss of visual acuity is moderate. In the majority of the cases both eyes are affected, the unilateral disease is infrequent [6]. In the atypical Cogan syndrome the vestibulo-cochlear manifestations can be associated with scleritis, episceritis, uveitis, optic neuritis, conjunctivitis or glaucoma. [7]

Vestibulo-cochlear symptoms: Cogan syndrome is classically characterized by sensory-neural hearing loss, vertigo and

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tinnitus in an association that resembles to Meniere's disease. The hearing loss might be profound, leading to cophosis in almost 52% of cases and usually it is bilateral. The auditory deficit is installed in days, months, too slow for a sudden nerosensorial hearing loss and too quick for a presbyacusys. The speech discrimination scores are poor. The hearing loss is associated with tinnitus. [4]

The vertigo can be important causing marked instability, ataxia, sometimes associated with nausea and vomiting. The nystagmus can be observed at the ocular examination. In most of the cases, complains are similar to Meniere's disease.

Other signs and symptoms

General manifestations of the disease are not rare, fever has been reported in many cases, weight loss and extreme asthenia can be found in patients with Cogan's.

Cardiac involvement, especially aortic insufficiency is present in as much as 15% of cases. Large vessels can also be affected causing heart murmur, abdominal pain, claudication of the members.

Musculo-scheletal involvement manifests as myalgia or arthritis (mono, oligo or polyarthritis).

Neurological signs appear in ¼ of cases [8], patients might have paresis, hemiplegia, aphasia, cerebellar syndrome, pyramidal syndrome, spinal cord disorders, epilepsy, and vigilance disorders. MRI can sometimes detect lesions of the white matter consistent with cerebral vasculitis.

Cutaneous lesions might appear during attacks taking the form of urticarial rash, vascular purpura, ulcerations or nodules.

Some patients might have gastro-intestinal or pulmonary symptoms.

Laboratory investigations

Biologic parameters can be modified in Cogan syndrome, especially during the attacks, but no laboratory test in pathognomonic for the disease. Leukocytosis, elevated ESR, anemia, hyperfibrinemia may appear. Several immune-logical modifications also can be noticed: rheumatoid factor, antinuclear antibodies, cryoglobulins, lupus anticoagulant but none of these are specific or relevant for the disease. [9]

In small series of patients, some authors determined the presence of specific antibodies for the inner ear or cornea but these studies were not relevant and could not be reproduced by other authors so cannot be used to support the diagnosis of Cogan.

Differential diagnosis

The first differential diagnosis one should bear in mind when facing a rapid onset hearing loss with vestibular symptoms and interstitial keratitis is syphilis. Another important differential diagnosis is Meniere's disease but in this case the ocular manifestations are absent.

Another diagnosis to be differentiated from Cogan is Susac syndrome, a retino-cochleo-cerebral vasculopathy involving the arterioles, manifested by central neurological disorders, visual acuity loss, and hearing loss.[10] Vogt- Koyanagi-Harada syndrome is characterized by uveitis, alopecia, vitiligo and audio-vestibular symptoms.[11] . Other systemic diseases such as Wegener granulomatosis, PAN, relapsing polychondritis, Behcet disease, and Sjogren syndrome can associate vestibulo-auditory symptoms with ocular involvement.

Evolution, prognosis

In some cases, the onset of the disease is preceded by upper respiratory tract viral infection.

The vestibular and auditory symptoms can be the first manifestations of the disease in 41% of cases. in 43 % of cases the Cogan syndrome debuts with the ocular symptoms. The involvement of the two organs is usually done in approximately 3 months [8]. In cases of atypical Cogan syndrome the complete symptomatology might be installed in a long period, even years.

Usually, after the first attack the disease enters a phase of remission without evident symptomatology. There is a possibility that there are recurrent episodes that repeat at variable intervals. Once installed the hearing loss is not remissible. The vestibular symptoms diminish as a result of the compensation mechanisms. The ocular symptoms have a variable evolution, but usually respond favorably to treatment.

Treatment

As the etiology and the pathogenic mechanisms are not known, there is not yet available a codified treatment for Cogan's syndrome.

Usually the first line of treatment is represented by corticosteroids. [12] In cases where the corticodependence is installed or in cases of corticoresistance there is the possibility of using other therapeutic agents such as immunosupressants (cyclophosphamide, azathioprine, and methotrexate). [13] The corticotherapy should be prescribed in high dosage (1-1.5 mg prednisone or equivalent) and interrupted in two weeks in cases where it is ineffective. Studies show that the vestibulo-cochlear symptoms respond to treatment only in one third of the cases (orphanet). Once the deafness is installed, it is usually non-reversible.

In the last years there were attempts to treat Cogan syndrome with TNF alfa blockers but there are not enough evidence based results [14, 15]

CASE REPORT

We present the case of a 40 years old woman with no remarkable medical history who presented at the emergency department of our hospital accusing sudden onset intense vertigo and dizziness, tinnitus and hearing loss in the right ear. The patient was admitted in the ENT department.

The physical examination of the patient yielded no relevant data, the ENT exam was within normal limits.

The vestibular examination pointed out to an important instability, the patient was unable to maintain orthostatic position or walk without support, no spontaneous nystagmus.

The cerebral IRM examination did not reveal any vascular or tumoral lesions. The neurologic examination did not discover any motor or sensorial deficit, no signs of localization.

The audiogram performed initially can be visualized in Figure 1. We diagnosed a profound sensoryneural hearing loss for the right ear and a medium sensory-neural hearing loss for the left ear.

Figure 1: Initial audiogram

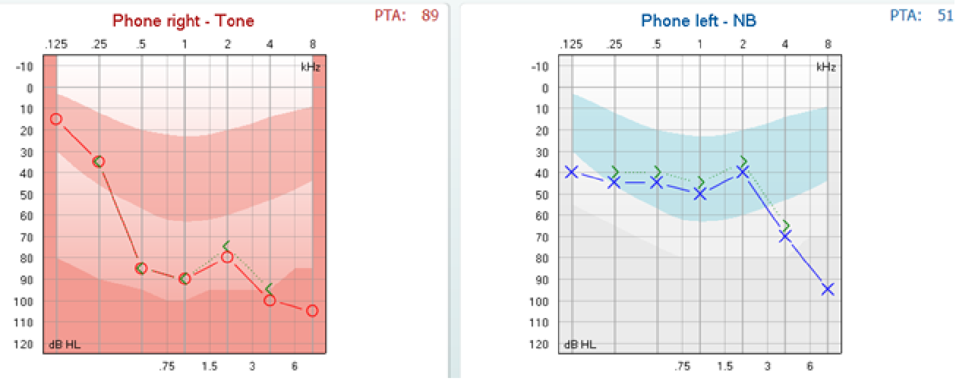

The rheumatologic examination revealed no remarkable findings: no arthritis, no cutaneous lesions, and no ocular symptoms.

The blood hematological and biochemical parameters were modified showing a slight leukocytosis with a white blood count of 11000/mm^3, the ESR also slightly elevated 25 mm/hour and the CRP had the value of 5. The modifications were interpreted as a reaction to an acute dental infection the patient had at that moment.

All the immunologic tests we performed were in normal limits, IgA, IgG, IgM, ANA, antibodies anti beta 2 glycoprotein, antibodies antiphospholipid were tested and the values were normal. Antibodies Ig M and Ig G for Epstein Bar virus, Toxoplasma, HIV, herpes virus, cytomegalovirus were all negative.

The ophthalmologic examination did not find any modifications of the anterior ocular pole at that initial moment.

The computerized posturography we performed at admission showed a severe vestibular deficiency pattern (Figure 2).

The videonystagmography with caloric testing evidenced a total right areflexia and an extremely important left hyporeflexia (Figure 3).

We started a treatment with high dose corticotherapy (solu-medrol), antiemetic (osetron), vestibular suppressant (diazepam), vasoactive agent (pentoxyphilin), vitamin (B1 and B6), plasma expander(dextran 40).

During the treatment, the patient presented a fluctuating evolution. The hearing level fluctuated especially on the left ear with PTA between 30 and 60 and on the right ear with PTA between 60 to 90.

In Figure 4 it can be noticed the aspect of two audiograms we performed during the treatment in which we could observe the fluctuant hypoaccusis in both ears.

The dizziness also fluctuated with episodes of severe vertigo. In these episodes, the direction of the nystagmus varied. We recorded horizontal rotatory nystagmus beating to the left but also to the right (Figure 5) alternating with periods of lack of nystagmus.

Figure 2: Computerized posturography

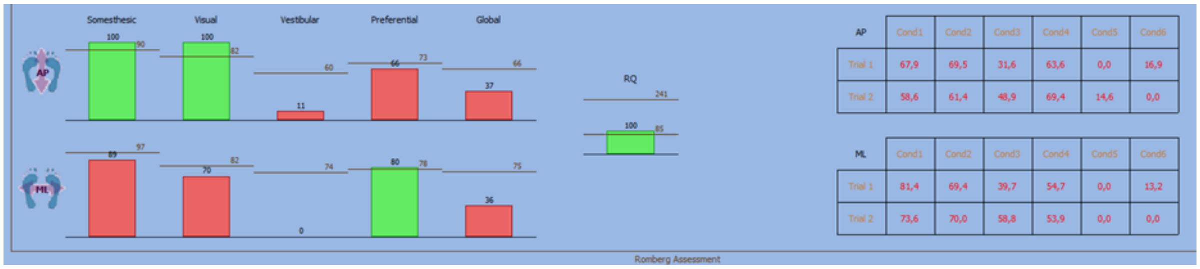
Somesthesic
Visual
Vestibular
Preferential
Global
AP
ML
100
90
100
82
60
11
66
73
66
37
89
97
70
82
74
0
80
78
75
36
RQ
241
100
85
Romberg Assessment

AP	Cond1	Cond2	Cond3	Cond4	Cond5	Cond6
Trial 1	67,9	69,5	31,6	63,6	0,0	16,9
Trial 2	58,6	61,4	48,9	69,4	14,6	0,0

ML	Cond1	Cond2	Cond3	Cond4	Cond5	Cond6
Trial 1	81,4	69,4	39,7	54,7	0,0	13,2
Trial 2	73,6	70,0	58,8	53,9	0,0	0,0

Figure 3: Videonystagmography

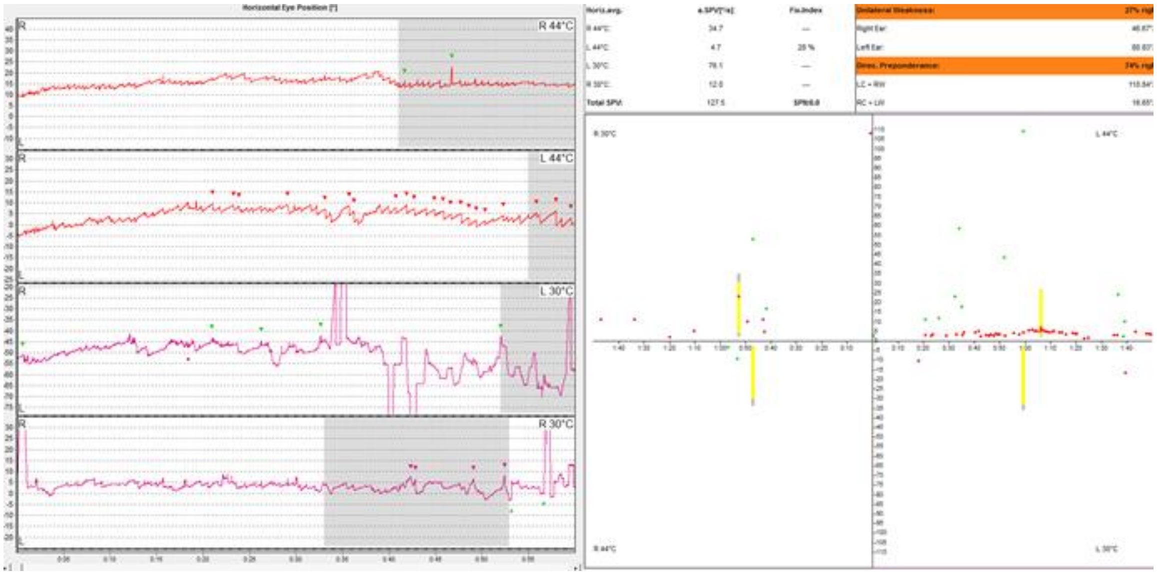

Horiz.avg.	a.SPV[°/s]:	Fix.Index
R 44°C:	34.7	—
L 44°C:	4.7	25 %
L 30°C:	78.1	—
R 30°C:	12.8	—
Total SPV:	127.5	SPN:6.0

Unilateral Weakness:	27% rig
Right Ear:	46.67
Left Ear:	80.67
Direc. Preponderance:	74% rig
LC + RW	115.84
RC + LW	16.65

Figure 4: Audiograms during the treatment

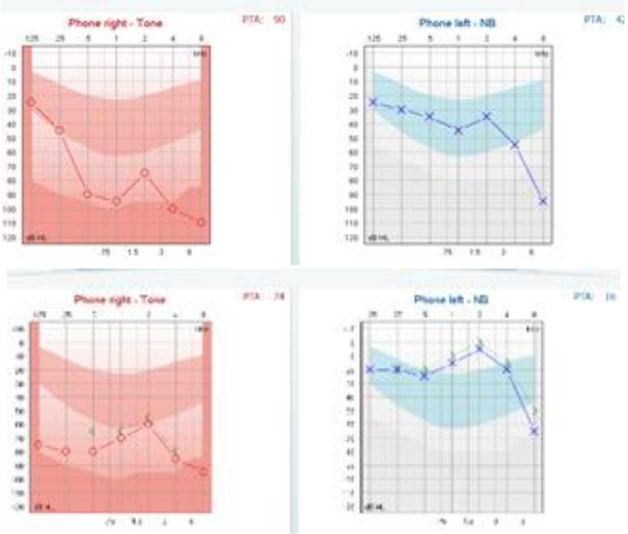

Figure 5: Horizontal rotatory nystagmus beating to the left but also to the right, alternating with periods of lack of nystagmus

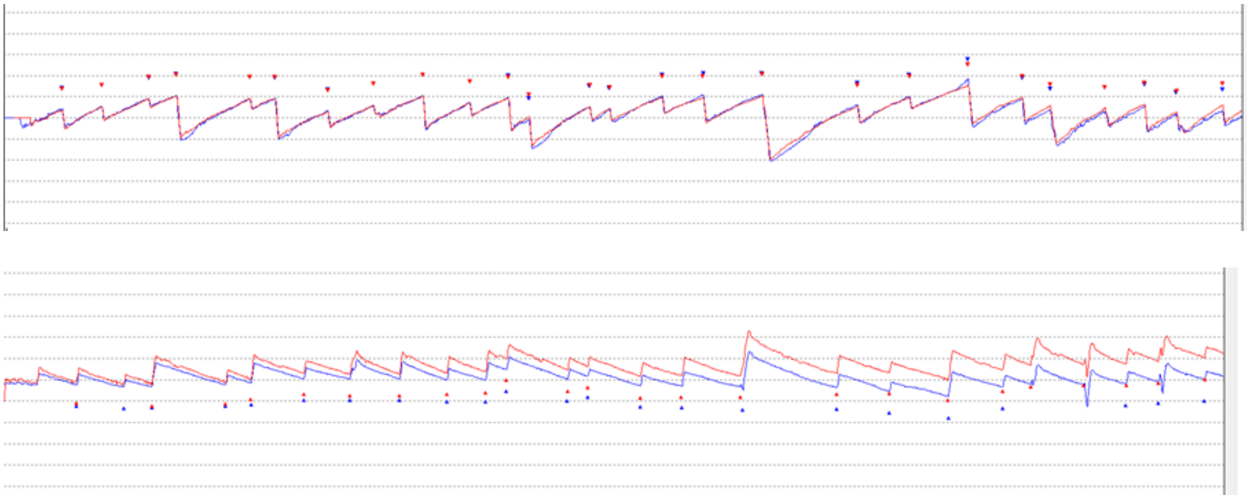

After 10 days of treatment in the hospital, the patient was discharged with an improvement of the hearing loss in the left ear, no vertigo and only a mild dizziness.

One month after this episode, the patient presented once again at the emergency department accusing intense vertigo, nausea, vomiting, tinnitus and aural fullness in the left ear. The audiogram evidenced a bilateral severe hearing loss (Figure 6).

Figure 6: Bilateral severe hearing loss

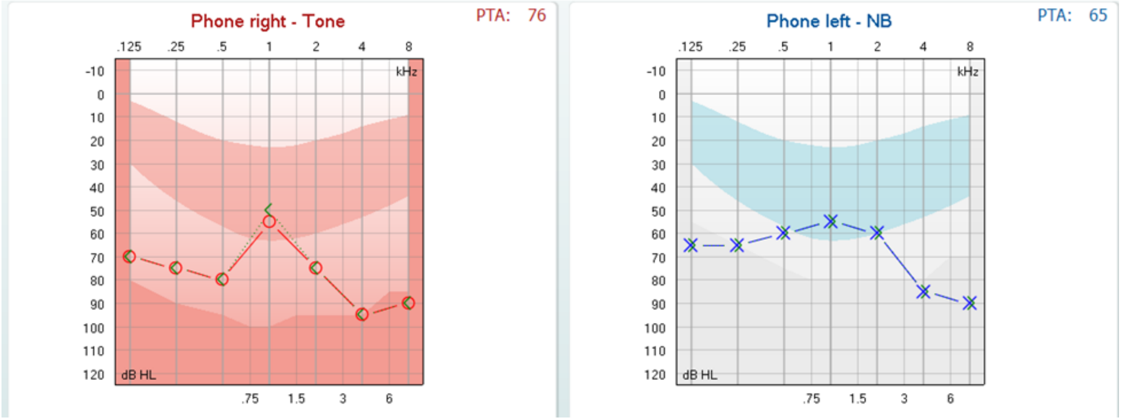

The rheumatologic examination did not reveal any modifications. The second day of hospitalization the patient suddenly presented eye pain with intense redness of the conjunctive. The ophthalmologic examination established the diagnosis of kerato-conjunctivitis (Figure 7).

In this moment, we were able to diagnose an atypical Cogan syndrome taking into account the association between the audio-vestibular symptoms with an inflammatory ocular disease.

We repeated the same treatment as in the first episode associating local eye topical corticosteroids and artificial tear solution with a major improvement of the hearing loss for the left ear PTA 10 (Figure 8).

The patient was discharged with a prescription of prednisone in low dose for a period of three months.

One year later, in the follow up, we noticed that the hearing in the right ear did not improve at all but the instability improved a lot so the patient was able to continue with everyday life. In this year she did not have any attack, no audiovestibular or ocular symptoms.

DISCUSSION

Haynes et al described atypical Cogan syndrome for the first time in 1980. The typical Cogan syndrome was described as an association between Meniere–like audio-vestibulary symptoms and non-syphilitic interstitial keratitis with an interval between the onsets of the symptoms of less than two years.

Figure 7: Kerato-conjunctivitis

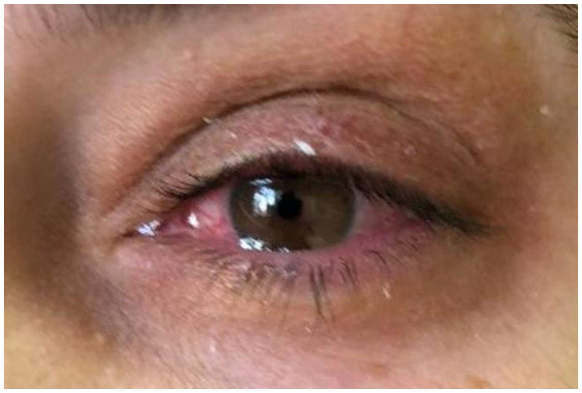

The atypical Cogan syndrome consists in an association of symptoms in which the disease criteria are grouped as follows:

- Inflammatory ocular manifestations with or without interstitial keratitis
- Typical ocular manifestations associated with audio-vestibular symptoms different from Meniere's
- A delay of more than 2 years between the onset of typical ocular and audio-vestibular manifestations.

The atypical Cogan syndrome with ocular manifestations other than interstitial keratitis tends to have a higher rate of systemic involvement with aortitis and has a worse prognosis. [3, 16]

It is the case of a 40-year-old woman with acute onset of intense audio-vestibulary symptoms with no remarkable medical history and no other general manifestations. At the first presentation the diagnosis of presumption was Meniere disease but there were characteristics of the evolution that did not entirely correspond. The disease was rapidly onset with bilateral and unequal involvement. The right ear had a profound hearing loss from the very beginning and practically did not respond to treatment. The left ear had a minor amelioration in the first episode and quite a good response in the second episode of the disease although the treatment was similar. During the admission we performed multiple audiograms evidencing the fact that the thresholds at both ears were in a continuous modification, not respecting the classical pattern of Meniere's. The videonystagmography with calorics showing bilateral vestibular lesion from the beginning of the disease, was also atypical for Meniere.

Figure 8: Major improvement of the hearing loss for the left ear

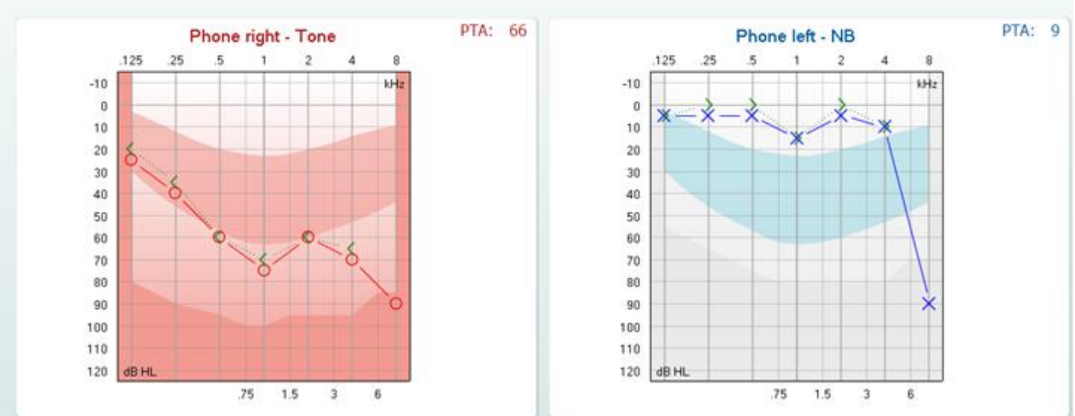

The onset and evolution of the disease made us believe that there is a autoimmune disorder of the inner ear but we did not have any disease criteria to classify. The laboratory tests were within normal limits, the minor leukocytosis and the slight elevated ESR were not noticeable. No other immunologic tests were modified or virus infections detected.

At the second episode of disease, the concomitance with the kerato-conjunctivitis was consistent with the diagnosis of atypical Cogan syndrome. We consider that this is the only disorder that can be taken into account for the diagnosis of this patient. Another remark is the fact that the patient is over the age of typical onset of the disease, but there are many reports of atypical Cogan with patients in the same age group. [17, 18]

The response to corticotherapy, was partial as the right ear did not recover, but the final result was considered satisfactory by the patient who could continue her daily activities.

CONCLUSION

Although Cogan syndrome is a rare disease, we must bear in mind that there is always a possibility of diagnosing it in the case of a patient with both vestibule-auditory symptoms and ocular manifestations.

Typical and atypical Cogan syndrome are diagnosed mainly based on clinical criteria as there are no laboratory tests able to evidentiate the disease.

The treatment consists mainly in corticotherapy. Immunosuppressants are an option in cases where corticotherapy is inefficient.

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VARIA

Patient-physician communication, an essential condition for an effective medical act

Carmen M. Voicu[1], Consuela M. Gheorghe[1]

An inconceivable reality for the medical world today is to consider the patient an active factor in his own healing process. Placement of the patient in the center of healthcare services aims at his loyalty, which means creating and maintaining correct and long-lasting relationships with him. In addition, in order to create such relationships with patients, both loyalty and stability, performance, and profitability strategies of the medical team must be thought on long-term.

Healthcare marketing situations differ, and medical units concerned with the essence of success must strive for a strategic alignment of relationships between all stakeholders (decision-makers, medical staff, patients, etc.) and the power of these relationships to improve their mutual value and even the relational context as a whole, turning patients into true brand promoters, thus enhancing marketing efforts [1].

The patient reaches to attitudes, judgements, and preferences about certain brands through a procedure of evaluating the features of these brands, developing a set of beliefs about the features that correspond to each brand [2].

Recognition of the strategic relationship that a patient desires is a medical marketing reality and involves greater commitment to accepting the challenge of creative thinking in often difficult situations, progressing through knowledge and understanding to mutual trust, converting emotions into balanced normality and the relationship thus created into an emotional-connected and loyal one for a certain period of time. This means concentrating efforts on patients, attention, and receptiveness to his wishes, but professionalism and rigor in such a way that his personal health expectations are not in vain. Moreover, it is very important to pay a close attention to communicating with him; it is even said that this type of communication is the broker of the relationship developed in a sensitive and dynamic environment like the healthcare field.

The complexity of providing healthcare services, on the one hand, and the wishes and expectations of patients, on the other hand, have as result special patient-physician communication valences and turn it into an essential condition. Permanent and effective communication with patients has become a condition of the existence of a qualitative medical act.

Given the continuous increase and diversification of health services, communication issues are becoming increasingly difficult and require much more laborious information efforts.

Due to the information explosion, patients tend to appreciate the quality of a product or service based on perceptions rather than on reality.

That is why efforts to optimize the technological and informational processes of communication between patients and medical staff, of exploring patients' behaviors regarding interpersonal communication in electronic environments in health services, of modeling negative emotions, of ways of transforming the healthcare infrastructure so that it offers an integrated insight of information through the optimized clinical and business processes, of welfare and health management, of patient-centered networks, of investigating the influence of virtual communities on the reputations of health organizations in Romania, has become a permanent necessity [3].

The relationship between the two sides, medical staff-patient, in the healthcare field, is much more complex, addressing social, psychological, and cultural aspects, which besides the base of the therapeutic level also implies a superior level of existential communication. The latter is found in medical communication because the medical act interferes with the destiny of the patient, linked in turn with elements of uncertainty and individual instability [4].

Interpersonal communication was the first spiritual instrument of the human being in the process of socializing, and is defined as the communication that occurs between two people in the context of their relationship and which, as it evolves, helps them negotiate and define the relationship [5].

Communication within the health organization takes place in a complex environment, where continuously modifying favorable and unfavorable factors coexist. Communication can take many forms and can be seen in different situations, but the most important of these is undoubtedly between patient and physician, which provide much of the data needed to establish the diagnosis and therapeutic attitude.

Interpersonal communication is formed by the combination of verbal forms (oral and written), nonverbal forms (gestures, mimics, posture, movement, aspect) and paraverbal forms (by voice attributes accompanying the word, such as intonation, rhythm, verbal flow), but, considering the importance of the information content of patient-physician communication (the diagnosis and treatment process), in the medical system, the emphasis is more on verbal communication. Non-verbal and paraverbal forms are important from the point of view of their emotional effect and the formation of sympathy.

In the health system, the relationship between the two sides, the medical staff-patient, is much more complex, involving a higher-level therapeutic type of existential communication. This type of communication [4] is involved in medical communication, because the medical act interferes with the destiny and evolution of the patient, being in turn linked to elements of uncertainty and individual instability.

On the other hand, the position of the two entities, namely the medical staff-patient, is different and unequal [6]. This relationship is established between members of two distinct social groups in terms of their prestige, power, and orientations. Thus, the physician has an extremely high status, given the level of information held and the specialized guidance through which he exercises his full authority.

The social role of the patient demonstrates his vulnerability, being forced to seek support whenever he needs. Thus, the patient is the most disadvantaged person, being influenced by physical and mental suffering, feeling the disease as a source of uncertainty and insecurity, while the physician is regarded as a person with multiple qualities, full of energy and sometimes with magical powers.

Gaining a high communicative competency needed by the specialist to create a real therapeutic alliance requires not only solid medical knowledge to diagnose and treat the disease but also the ability to obtain as much information as possible from the patient and interpersonal skills to respond to feelings and patient concerns and the ability to create and maintain the therapeutic relationship as a concrete offer of information and medical education [7].

Health communication is “the study and use of communication strategies to inform and influence the choices people make regarding their health”, and health information technology includes “digital instruments and services used to enhance self-care of patients, assisting patient-provider communication, informing about health behaviors, preventing health complications and promoting equity in health” [8].

A major concern of health services and primarily of hospitals has become communicating with patients, the quality of information provided to patients by physicians and the rest of the medical team. An essential component is the transmission of information, which has become increasingly important in medical deontology. This development is primarily driven by wishes of patients, more trained in health issues, and more cautious about the quality of the explanations they are given.

In the hospital environment, where the patient is subject to the attention of medical teams, the coherence of “what is said and what is not” becomes a permanent goal for patients and their relatives. Because, beyond anything “...there is an informational asymmetry between the physician and the patient: when they find themselves in the hospital (...), they feel ill, they do not feel what is going on with them, they do not know the possibilities of medical science, so they empower the physician with the freedom of choice” [9]. A good professional behavior is to state exactly how we are feeling and to write what we are saying. However, each physician is the supreme judge of how he or she does and can enrich his or her oral information by using information charts and, if considered necessary, by other documents or video support.

In healthcare services, access to online information has made patients more aware of their needs and desires so the result has materialized in a rigorous selection of service providers.

Therefore, healthcare managers and providers need to understand the stages a potential patient is going through in an online buying process or when expressing an intention to buy an online health product or service.

The winners will be those who build trust from the patient's perspective, with three progressive complex components in the relationship between the brand and him: credibility, care, and congruence. Certainly, what is very important is the amount of money spent by the patient for the recovery of health.

Nowadays, in health systems, the widened marketing mix enjoys considerable attention. It provides a simple organizational mechanism to understand the problems arising from the concerns of medical service providers. The widened mix has also developed as a partial solution to some of the most important problems faced by managers in terms of quality control of healthcare services provided and the relationship with patients in the process of providing these services. The widened mix explicitly recognizes the role of the staff and patients in the service delivery process, highlighting the role of marketing played by both sides.

The inclusion of Physical Evidence, Participants, and Process as distinct (contextual) elements underlines their importance and impact on marketing of health services. In addition, the key objectives are to increase patient satisfaction, quality of medical services offered and long-term patient gaining, which means that the widened marketing mix management is directly related to these objectives.

Due to the immaterial nature of healthcare service and the fact that, usually, they cannot be checked beforehand, patients are looking for tangible evidence of what they are about to experience in a particular immediate confrontation with the medical service. Even if a patient has a high experience with such a healthcare provider, variations, and contextual elements will affect patient's expectations related to the immediate confrontation with a medical service.

Elements of "Physical Evidence", such as noise level, smell, temperature, time, comfort, and even nutrition, will influence the perceptions of confronting with the healthcare service requested. Similarly, the attitudes and behavior of healthcare participants will influence the perceptions of current performance. Contextual elements can also influence satisfaction in the confrontation with the medical service, through their effects on attributions (of causes) for invalidation. Theory of attribution asserts that when results are not in line with expectations, people tend to look for reasons. The importance they give to "why", will influence the final evaluation of the outcome.

The communication process is very important within the medical unit; it is a process that affects the quality of the other processes. Through the positive impact on communication within the medical unit, the style oriented towards maintaining socio-emotional aspects and the leadership style oriented towards relationships is beneficial to the processes within the medical unit and to the emergence of positive socio-emotional states. This leadership style is associated with the existence of high quality communication relationships, which leads to the improvement of the quality of the other processes [10].

In the new context of health, development processes will need to focus not only on solving current problems, but also on anticipating future problems, not just on certain types of processes, but as many as possible, not just on technical resources, but more on human ones. Organizational-managerial excellence seeks not just the adaptation to circumstances, but also the becoming and the power to create the circumstances [11].

The depth of the process of change in health, its size, and its dynamics depend not only on the political will and on the subjective aspirations, but also on the existence and sufficiency of the necessary objective conditions, the managerial ones being the first.

In the context of today's healthcare problems, the special role of the manager is imposed by the need to create a general capacity for innovation, flexibility, stability, and ensuring success even in extreme situations. In this context, the theoretical but especially the methodological approach of the marketing of the medical system has special practical valences, requiring a change in the mental attitude of health managers towards an innovative strategic hospital marketing oriented towards and for the benefit of patients [12].

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VARIA

The tree we throw stones at

Mihail Mihailide

I stop to catch my breath on the crest of a hill in Predeluţ.

It's late autumn with a blue-diamond sky. Nevertheless, there is fresh snow fallen on the rugged tops of the mountains and on the hedges of dark fir trees at the foot of the Bucegi mountains – looking as if they were mounted lancers. The snow is glistening.

I feel the tall grass, dried by the season, under my feet. On the twisted and black branches of the old apple trees, red fruit swing in the scented wind. Around the roots of the some apple trees – there are spots of royal purple perennial irises. Although poisonous flowers, I can't resist their calling beauty and I lift a few threads.

Back on track, I turn my bicycle towards Bran, I descend by the Castel, pass by Vama Medievală, and ride along Turcu River to the place where it sweeps in Șimon stream. Then I turn left and head towards the Brandeberg guesthouse. Here I securely park my "Pegas". My destination is not the hotel but the building across the street, Parascheva Holy Church, on 340, Iancu Gonţea Street, whose frescoes were painted about three centuries ago by Nicolae Zugravu from Turcheş – Săcele. It is believed that he also put his talent at work at the Three Holy Hierarchs Monastery in Iași.

My objective as a traveller is not the church but the "cultural and medico-historical heritage" provided by the resting places around this holy edifice. Many important Romanian intellectuals – several of them physicians – were born in the village of Bran, today a tourist attraction, but also in the nearby villages; many sleep their eternal sleep in the cemetery of Şimon village, under crosses with their names engraved on them. I intend to leave the bunch of irises on Iancu Gonţea's grave… The surrounding mountains and the forests that reached the yard of my house when I was a child were like the lords of the land for me... It is at Şimon that I built a vault in order to make sure that I would not sleep my eternal sleep anywhere else. "Today, a commemorative plaque and the name of a street in Bran remind us of this son of the region", noted Emil Stoian, the brave chronicler of this beautiful mountain village, to whom the inhabitants owe a major contribution for preserving the image of those who really meant something for the Romanian people. Such people are numerous, in Bran: historians, philologists, lawyers, physicians, actors, officers, priests, professors and academicians; participants in the Great Union of 1918; heroes of the two World Wars. I have met some of the physicians…

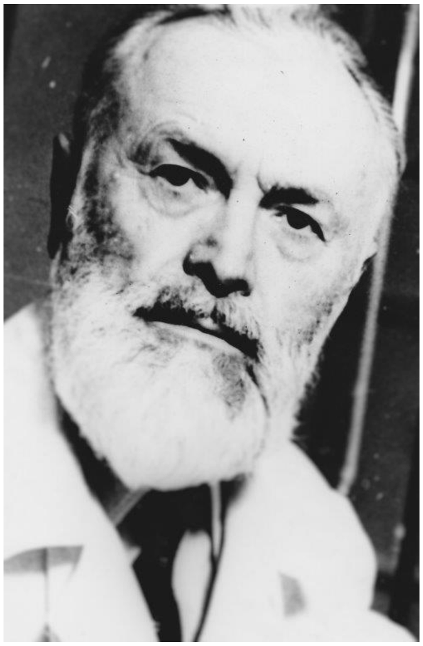

Found at the location, I feel disappointed: where is the tomb? I am told to go two kilometers uphill on the right side of the road. This time I decide to walk. Indeed, after passing the Primary School, on the yellowish wall of which a thin marble plaque reminds that the scholar attended his first classes "here", I pass by the house and the yard where he was born and spent his childhood, its gate always locked, and arrive at the church dedicated to St. Nicholas, standing a few meters retreated from the main street. Here, behind the building is a small cemetery. At its end, close to the hill, I finally find the vault: "Fam. Prof. Dr. Doc. Iancu Gonţea" with two rows of vertical crypts! It looks rather like a bunker. In the crypt by the side of the teacher lies his wife, Dr. Lucia L. Gonţea (1907-1998, born Popescu) buried 22 years after the professor.

From an oval photograph, embedded in the austere concrete wall, the professor gives me a harsh look. Where should I put the irises brought as a modest homage? It's difficult to find your way between the graves...

I have synthesized ergo-biographical data regarding Prof. Iancu Gonţea in a book [2], using sources coming mainly from the archives of the medical-historical documentation library of the National Public Health Institute of Bucharest, an institution whose enrichment with new information (manuscripts, photos, video tapes, books, magazines, etc.) and modern digitization was stopped for various reasons... Iancu Gonţea was one of my professors in my fourth year at the Bucharest Faculty of General Medicine. Decades have gone by. Before proceeding to "serious things", I ask the reader's permission to reproduce a few paragraphs, surely forgotten by the reader, perhaps funny, from the notes about the professor of the Food Hygiene Department, who was born in Şimon and returned there at the end of his life.

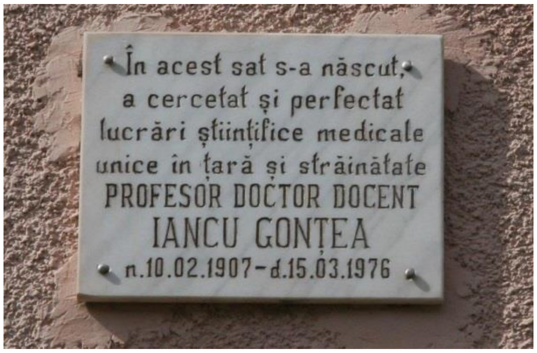
În acest sat s-a născut,
a cercetat și perfectat
lucrări științifice medicale
unice în țară și străinătate
PROFESOR DOCTOR DOCENT
IANCU GONȚEA
n.10.02.1907-d.15.03.1976

"We were two groups of fourth-year students of General Medicine, huddled in front of the laboratories of the Institute of Hygiene in Bucharest, where we were to take an oral exam in Food Hygiene. The professor – an unforgettable person... tall, slim, with penetrating eyes and a Hemingway-style beard, wearing an impeccable white gown – was sitting at the teacher’s desk, on a podium, with the assistant group on his right, and us, the wretched students (oh, how we cried out for mercy knowing his exigency!), one step down, in front of the examiners, at a tiled table full of Erlenmeyer balloons, pipettes, tripods, test tubes and dropping bottles. Usually, this type of final exam was the responsibility of the associate professors. It was only exceptionally that the professor would waste his time with us. Well, there we were, living... the exception! Each of us picked from a jar the ticket with the subjects written on it, thought of them for a few minutes and answered the three questions, in the order in which they were typed.

After ending our monologues (rather scanty, more often than not), followed a dialogue with the professor, Iancu Gonţea himself. At the end, he wrote down the grade that each of us deserved in our grade-books and we tiptoed out of the lab. In the hall, we were approached by our classmates who, browsing through their notebooks, were trying to learn everything they hadn’t learnt until then, before entering the lab room „to have their heads cut off".

– What subject did you pick? Does he also ask questions from «the practical experiments»? Is he harsh, is he kind? What grade did you get? Many of the questions were difficult to answer promptly... At a certain moment, one of the best students of our class came out of the lab, almost bursting into tears, obviously confused.

– What happened? What subject did you get?

– The third question, she explained us, as she started to feel better, was about the trophic content of the main foods. I explained how many proteins, lipids, fats and calories can be found in cow's milk, eggs, fish, chicken and I don't know what else I mentioned... I couldn't realize whether he was satisfied or whether I had gotten it all wrong. I finished what I had to say, but then he asked me additional questions, looking at me over his glasses:

– «What about ordinary vegetables, which of them have a higher vitamin content?» I gave the wrong answers, I stammered and then I said: carrots, spinach, tomatoes... Whatever!

– «What a-bout-par-sley?» he asked. I was as quiet as a fish. Total silence. He gave me the right answer and explained why his question was so important. Then he added:

– «Veronica, Veronica! You didn't really like Food Hygiene…» And then I saw him write 5 (five) in my grade-book and sign it. «You should come in the autumn to get a higher grade! he said, probably thinking that I would otherwise lose my scholarship…

The professor's question was not tricky, he had indeed told us in his lectures that parsley contains more vitamin C than lemon, orange or cabbage and, in addition to that, it also contains vitamins A, B1, B2, B6, K and E, as well as all the minerals useful to the body. Parsley is an antioxidant, antiallergic, antitumoral, which stimulates the gallbladder secretion, maintains elasticity of the vessels, and I can no longer remember now, more than half a century later, what other wonders we owe to this herb, commonly used for flavoring and adorning dishes!

Veronica had only remembered what the professor's assistant had told us at a seminar, smiling, about the qualities of this ancient vegetable: «It also ensures the good functioning of the genitals and even has a powerful aphrodisiac effect! »"[3]

Writing about himself in a curriculum vitae requested by the Academy of Medical Sciences (AMS), whose member he became, professor Iancu Gonţea believed that he had set a "new trend in the science he practiced, which was seen not only as food hygiene, studied separately from the man (in a narrow technical-sanitary concept), but as the science of biomedical, psycho-social, and economic relations between man and food"[4]. He has the merit of having contributed to determining the nutritional requirements in relation to one's occupational and environmental conditions, «for the first time demonstrating that physical activity increases protein and calcium needs, as well as vitamin C consumption, [and that] exposure to chemical noxae heightens the metabolism of thioamide acids and of ascorbic acid, increasing the needs of the body.»

In the same CV requested by the Academy of Medical Sciences, the professor listed the procedures he had devised for the biochemical control of vitamin nutrition status, as well as the methodology for studying the biological value of food, both highly appreciated by international scientific bodies.

The professor was to be awarded an important international prize (Maurel) as well as the silver medal in food science for his contribution regarding natural anti-nutritional substances in food and animal feed. I. Gonţea also demonstrated that no food is complete and set up an original classification of foods (by groups), embraced by experts of his time, in keeping with the benefits and the deficiencies of various food categories."[5]

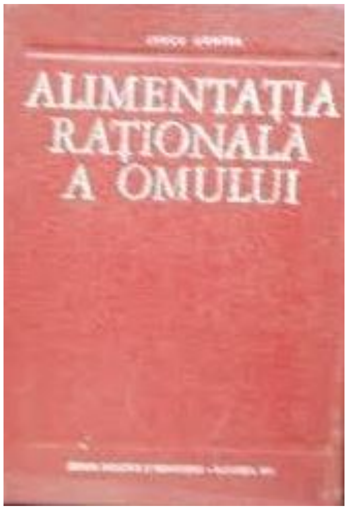

ALIMENTAȚIA
RAȚIONALĂ
A OMULUI

Given their originality and practical value, based on rigorous laboratory and field research, many of his writings have been translated into widely-circulated foreign languages. They cover the fields of effort physiology, pathophysiology, and medical clinic, but most of them include research on human nutritional needs under different physiological, occupational and environmental conditions: "Food Control" (1956), "Food Ration" (1956), "Rational Nutrition of Women during Maternity and Its Importance for Mother and Child Health" (1958) can be considered as major landmarks in medical literature, at least in the Romanian one. The last of the books mentioned were updated by the author, translated into German, and published by the prestigious publishing house

Gustav Fischer of Jena in 1965. Having received excellent reviews, this monograph was requested, from the German publishing house, by Dai-lichi Co. Ltd. Publishing House in Tokyo and became the first book by a Romanian scientist published in Japan, in 1974.

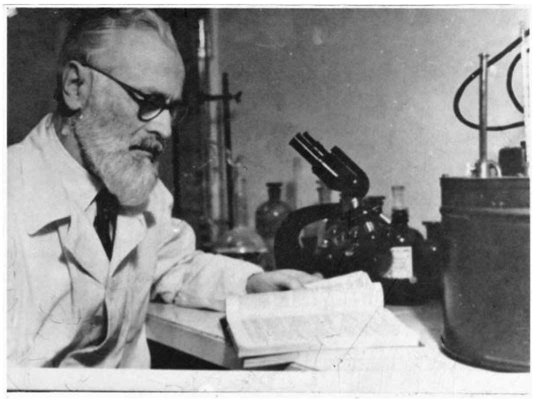

In 1963, the Medical Publishing House (Bucharest) released "The Bases of Nutrition". Three years later, another book was published: "Natural Anti-Nutritional Substances in Food and Animal Feed" (in association with Paraschiva Şuţescu, Ph.D.), subsequently translated into French and published by Vigot Frères Publishing House, in association with prof. Raymond Ferrando PhD (Paris, 1967), as well as into English, and published by Karger Publishers in Basel (Switzerland) [6] as well as in New York (1968). The Academy of the Socialist Republic of Romania awarded professor Gonţea the "Victor Babeş" prize for this book. Among the books that were published, almost every year, two titles still stand out, several decades later:" A Genetic Leap in the Nutritional Value of Corn – the Past and Present of this Cereal" (Ceres Publishing House) and "Alcoholism" (Medical Publishing House, 1976), an insight from the point of view of preventive medicine into the use and over consumption of alcoholic drinks – "considered to be a serious problem on a national level".

Professor Iancu Gonţea won high recognition in the scientific events he was invited to — international congresses and symposiums (Dresden, Moscow, where he accompanied the Romanian delegation as an expert in nutritional issues at C.A.E.R. meeting, 1969), at New Castle ("Women's Nutrition during the Reproductive Cycle", 1971), Mexico (where he presented a paper titled "Nutrition and Anti-Infectious Defense in Humans"), Austria ("The Effects of Sugar Abuse") etc. He received national and international awards and diplomas.

Under a Decree of the State Council of the Socialist Republic of Romania (153/1971), the professor was awarded the "Sanitary Merit", alongside other scientists rewarded "for the contribution to the Party's policy in the field of public health in our country". The chairman of the Council at the time was N. Ceauşescu, while the minister of health – Prof. Dr. Dan Enăchescu.

The fact that the romanian scholar did not also become a member of the Academy is rather amazing, since, forgive my insolence, many others were comfortably sitting in the "immortals"' seats of the Academy of the Socialist Republic for (scientific!) achievements, which, compared to his own, were a lot less significant...

*

Who would have thought that professor Iancu I. Gonţea – a former military physician (promoted major in 1948), hence a person whose past and family had been under the close scrutiny of the army's newly set-up personnel department and who had a "healthy" origin (being the son of a shepherd, born in the village of Şimon, on February 10, 1907), and therefore, seeming to be imbued with socialist "principles" and "ethics" – was to be placed under surveillance by the Securitate (being the subject of two files, "I. 498910")? [7]

He maintained correspondence with scientists from all over the world; often travelled to Western countries but also to the Soviet Union; he attended medical conferences; worked in the field of food hygiene, this having a great social impact. He represented the Socialist Republic of Romania in the World Health Organization. All this supposed that his loyalty to the "party and to the socialist homeland" were to be continuously tested by the "state bodies"...

The "vigilance" of the Securitate also translated into an internal decision issued on November 18, 1969, regarding the preservation of his "informative" file in the Operational Fund, under no. I. 715753, coming from the 3rd Directorate of the feared institution…

What was pursued Iancu Ion Gonţea accused of?

That "he was a member of the Iron Guard, working in the Sanitary Department of its Documentary Studies Center." That, "he was a major in the bourgeois army" (a member of the contingent of officers who took the military oath before the King). Later on that "he maintained correspondence with people from abroad, such as Prof. R. Ferrando (France) and foreign publishing houses." In an informative ("strictly secret") note obtained by major I. Ion, at agent "Gabriel" home and dated April 17, 1967, he declared that he met the person under surveillance during his studies at the Andrei Şaguna High School (1924-1927) and added that Iancu Gonţea (I.G.) "was a very good student, who graduated at the top of his class".

The note provided biographical data, generally consistent with that provided by Gonţea in his résumés. I.G. seems to been the son of poor peasants from Bran; he was not perform any legionary activities, although his uncle, a history teacher, dr. [Ioan] Moşoiu, "who was also the manager of the high school (the Andrei Șaguna Romanian Orthodox high school in Braşov) was known for his involvement in the legionary activity (he was the Commander of one of its local branches). After graduating from high school, I.G. joined the Medico-Military Institute (Bucharest) and "Gabriel" went to Prague. After 1948, they met again in Bucharest, on a daily basis, since I.G. taught at the Institute of Hygiene.

Despite being rather ill (suffering from diabetes), I.G. was very active, becoming a professor and the head of the Food Hygiene Department (Bucharest Institute of Hygiene). He was highly valued as a specialist, "Gabriel" convincingly using the communist jargon of the time: he "combines theory with practice and links laboratory research with practical activities, such as supervising in bakeries, canteens, etc." He had a vast journalistic activity, "Gabriel" citing in I.G.'s defense his contributions to Scânteia and Economic Life newspapers, ahead of the papers published in specialized magazines…

Next came a significant detail: although generally he did not have an active social life, I.G. celebrated his 60th birthday by organizing a "comradely lunch at the Faculty of Military Medicine". Among the guests featured professor [Nicolae] Nestorescu, a microbiologist and immunologist (who also was a former military physician) and lecturer Coman Petrescu, PhD (a pediatrician dealing with rational child nutrition). In "Gabriel"'s opinion, allegations that I.G. had been involved in the legionary movement activities as a student were simply "unfounded rumors."

However, in his Note, major I. Ion seemed keen to show his superiors how cautious he was: "Since I.G. is known by the Third Department (of the Securitate) as a former member of the Iron Guard, the agent will have to inform us of any data backing this assumption. In addition to that, the correspondence with foreigners will be kept under observation." Next came a list of names – French and German researchers and professors from (Paris) and (Heidelberg).

Here is what agent "Titi", who "kept him under observation during his teaching activities", wrote: "Always punctual, having a commanding attitude, he teaches the students notions of food hygiene during his two-hour lecture. During his lectures he seems to speak with passion, having a dignified attitude. From talking to other students, the source found out that professor Gonţea is very demanding during exams, asking the students to reproduce all the details, or better said the whole lecture, word by word. He does not hesitate to turn students out over the slightest mistake. His behavior gives the impression of a modest man, deeply involved in his profession."

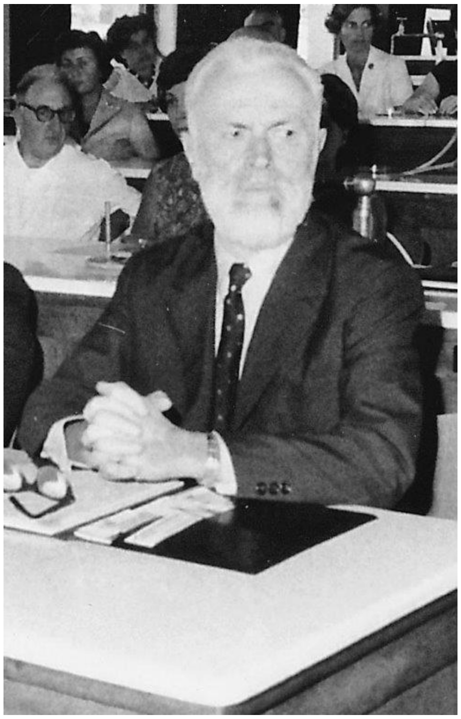

Elly – a professional informer

In Iancu Ion Gonţea's file, there is a note received by captainV. Ovidiu, on April 19, 1957, from agent "Elly", reeking of antipathy towards I.G., a text in which one can hardly tell the truth from "pure" lies. It is difficult, if not downright impossible, to check these allegations because the informer, hiding under the pseudonym given her by the Securitate, seems to be a professional of delation, citing people (giving their full names and the position held in the Institute) who could allegedly back her allegations, but who today, 60 years later, are most certainly dead...

"I inform you", wrote Elly, "that Dr. I.G., associate professor of nutrition at the Faculty of Medicine, living at 244, Dorobanti Street, phone 7.66.29, claims to be «untouchable» because he is backed by Acad. Ștefan Milcu and Acad. N. Lupu, who are «indebted» to him.

I.G. is the author of numerous fake public documents and characterizations (within the IMF – Institute of Medicine and Pharmacy), helped by former head of the personnel department At.). All these facts are known by the management. Dr. I. Per. from the Hygiene Institute, Laboratory of Food Chemistry, 1, Leonte Street, can provide references in this respect."

After this "introduction," "Elly" wrote that she knew I.G. since November 1954. "I know that he was a legionary, working in the cabinet of another Iron Guard physician, Dr. Ru, within the Legionary Ethics Committee – at the time, the steering committee of the Faculty of Medicine (?). At that time he worked wearing a green, Iron Guard shirt; at Iron Guard rallies he carried banners reading Nu merus nullus (sic!) and Nu merus claus (sic!). prof.dr. I.I.N., manager of the Institute of Physiology of the R.P.R. Academy, phone 4.20.59 (home) and Dr. Eug. D.), phone 7.31.16 (home) can give references about his activity at the time. Among the acts of hooliganism, I can mention the ones I heard about from Dr. Eug. D., namely that Assoc. Prof. Dr. I.G., under the legionary régime, tried to devastate the Cantacuzino Institute, kicking the doors when he entered the rooms, destroying laboratory equipment, etc. In the Military Sanitary Institute where he worked for some time, the whole institute knew him as a "passionate Iron Guard member" (Dr. Iac., Assistant at the physiology department, tel. 3.27. 90 ,home, can give references about this). In the bourgeois army, he was highly regarded because of the wealth he still holds, to a great extent:
- Mansion in Călimanesti
- Estate and mansion in Bran
- Houses in Bucharest
- Car etc.

Later on, Assoc. Prof. I.G. proved to be a hostile element, pleading in favor of Antonescu's criminal actions against the USSR. References: Dr. Eug. D. and Dr. P.

Thus, Assoc. Prof. Gonţea maintained ties with his former collaborators from the Legionary Nest, Assoc. Prof. Pr. Gh., from the Physiology Department, with Tudor G., also from the Physiology Department, with G. Maria from the same department, about whom I will tell you in detail further down. Assoc. Prof. I.G. has remained the same reactionary person, nurturing sympathy for the Iron Guard, and still indulging in hooliganism. Here are some facts:

1) He encourages the personnel not to apply for higher positions since exams are "based on the Russian model and have a temporary character". References can be given by C. Adrian, assistant at the Physics Faculty, phone 2. 68.09

2) Assoc .prof. I.G. sent two articles for publication to the Institute of Physiology of the Academy of the R.P.R. (People's Republic of Romania), registered with no. 36 / 6.III.1957 of the Nutrition Department:

- The bibliography of the first article includes 13 works, none of them Soviet.

- The bibliography of the second article includes ten works, all of them English and American.

- This amounts to hostile behavior towards Soviet science, and within the department, which contrary to the ideological orientation of the scientific research in the R.P.R.

3) He considers himself a "great patriot and a great Romanian". Under this mask, Assoc. Prof. I.G. hides his ferocious anti-semitism and hatred against elements dedicated to the working class. References: 1. Gabriela from Caritas, phone 2.90.40 who was an assistant at the Department of Nutrition, Dr. D. Eugen, phone 7.31.16; M. Maria. St. Constintin Street, No… Petre P. from the Cotroceni Hygiene Institute.

4) Assoc. Prof. Gonţea Iancu (hereafter names were blacked out by the CNSAS) – nurse G. Maria swore to me, in the autumn of 1955, on the health of her child, that, as head of the Nutrition Department, he had given her extra paid hours, the equivalent of a part-time job, [but] from the date of January 1, 1955 to September 1, 1955, he obliged her to give him 1/2 of her part-time wage. G. Maria told me this out of despair because (redacted by the CNSAS) his fabulous wealth helped him back the interests of the Iron Guard. [And] after she gave him this money, "which he literally grabbed from her and which were part of her due", as a reward, assoc.prof. Gonţea gave her the sack. The reason was another subordinate, dr. Şuţescu Paraschiva (anonymized words by the CNSAS) who [becoming a favorite] continued to work with I.G. G.Maria told me all this on the way between Ardealul Avenue and Plevnei Street, as she was going towards Progresului to the Rectorate of the Institute of Medicine and Pharmacy.

5) At the Nutrition Department, Assoc. Prof. Gonţea sells overpriced wool and veal meat from his estate in Bran to the staff, whom he obliges to buy, threatening to treat them badly if they don't. Assist. A. C. can give references, phone 3.11.10.

6) (Anonymised paragraph by the CNSAS) G. Maria was a notorious Iron Guard member, the daughter of the richest merchant in Constanța who lived in the countryside in Bulgaria until 1956, when she brought him back to Bucharest for good since "rich people are no longer oppressed as they used to be", according to her own words. G. Maria worked with the Legionnaires, being... (redacted). Thus she helped them set up camps in the V. Roaită resort and her father helped by providing food and transportation.

7) As far as his behavior towards students is concerned, I can mention that several of them have lodged complaints about his demagogic manifestations with the Dean’s Office of the

Faculty of Medicine, but to no avail. References can be given by student P. Aurel from the sixth year. His attitude towards the young employees of the Institute of Medicine and Pharmacy has remained fierily reactionary and he is doing his best to exclude those who had worked in ministries representing our régime. I am attaching the original statements by Prof. N., whom Assoc. Prof. Gonţea Iancu told that he would do his best so that Dr. Maria Mih. and her husband, C. Adrian, who had worked at the MAI [Ministry of Internal Affairs] would be sacked. C. Adrian was summoned to the C.C. of the P.M.R. for a mission, which Gonţea branded as a dangerous element for the reactionary clique he is a member of. (I should mention that the summons took place a few months after the appointment of assistant C. within the Medicine Faculty).

8) About Assoc. Prof. Gonţea Iancu's son, whose name is Gonţea Liviu, a fourth-year student at the Faculty of Medicine, I know that he was under investigation by the Ministry of Internal Affairs over hooliganism when he was a high-school student. I mention that Gonţea Iancu was connected to Lupaş Tudor, who provided him with articles translated from Hungarian, which had been broadcasted on the radio, which Gonţea Liviu handed to the students in his group. In a conversation with Tudor Geor., Lupas's uncle, who worked in the physiology laboratory, told me that «the guilty people are free while his nephew is in prison.»

When I asked him if Gonţea’s son had been involved, he did not give a straight answer, but made me understand that he had."

(signed) Elly

We find ourselves in 1957, a few months after the Hungarian revolution (called "counter-revolution" by the Communists), when the vigilance and coercive measures of the Securitate got a new "boost", stimulating the activity of informants. Moreover, on October 27, 1956, in university cities, students were out in the streets demanding an improvement in living conditions, the removal of the Russian language from the educational curricula, and the liberalization of social life, demands which were followed by reprisals and arrests. Students and professors thus becoming a target of the newly established Ministry of State Security, an institution distinct from the Ministry of Internal Affairs, at the time led by Alexandru Draghici.

A year later, in June, a plenary session of the C.C. took place, announcing a return to the struggle against bourgeois ideology, criticism of intellectuals accused of snobbery, cosmopolitanism, apoliticism, negativity etc. Thus, the departure of the Soviet troops who had been stationed on Romanian territory for 14 years, did not seem to be auspicious to acts of dissent, especially from those who had had certain political views in the past, even if they had let themselves be carried away; on the contrary, it meant increased vigilance8.

Elly´s denunciations were obviously not based on "proletarian intransigence" or "ethical" reasons, but rather on personal, vindictive motives, which, after so many years, cannot be properly weighed. It is possible, however, that the "witnesses" mentioned by the informer may have considered themselves, for various reasons, harmed by the teacher (possibly by the exigencies that he imposed) or "stopped" in their desire for rapid ascension in the professional field or on the social ladder. In that period, the "weapon" at hand chosen by an informant in ensuring his success was invoking an alleged Iron Guard activity, or at least sympathy, on the part of the "target".

One of the graduates of the Faculty of Cluj (whose name was blacked out), was "promoted", as early as his student years, to a party activist, to the "Central Committee structure”! Starting in 1949, he held important positions in the state hierarchy: adviser to the minister of Health, then Sanitary General Inspector. He became pro-rector of the Institute of Medicine and Pharmacy in Bucharest (IMF) then, based on this rapid ascension and, of course, "being a valuable person", was named associate professor. (This rapid fast-lane promotion took barely six years).Since 1955 he engaged in research as head of department at the Hygiene and Public Health Institute in Bucharest. How could "Elly” not cite his name and indicate him as a credible witness in order to assess professor Gonţea's activity and behavior?

And what about another rival, born in 1884? The latter hoped that, thanks to the protection of his administrative superiors and by "revealing" I.G.'s alleged "Legionnary activity" and backing Elly's allegations, he could indefinitely extend his teaching term, in no matter what department, if Gonţea could be forced to give up the position he held «based on merit».

Nevertheless, we should mention that Elly´s detailed statements found in this file were not backed by any "notes" or testimonies given by the witnesses she cited.

After August 23, 1944, the issue of the legionnaires had become a major concern for the new power. In 1945, former legionnaire’s members who were deemed as dangerous were arrested and sent to labour camps. Others were to be determined to become – and truly became – collaborators of the Communist Party, following Ana Pauker's intervention. On December 10, 1945, the Ministry of Internal Affairs issued an order "legalizing" some of the former Iron Guard members, who no longer posed a threat to the State and who had offered their services to the new régime. It was a bluff: the trio made up of Ana Pauker, Teohari Georgescu (minister of Internal Affairs) and KGB general Nicolski were later accused of having facilitated the infiltration of former Iron Guard members into the Communist Party. As a consequence, the three former Communist dignitaries were ousted.

At that time, however, the Legionnary Movement ghost had reappeared and informant "Elly" seized this opportunity to accuse Iancu Gonţea. It is true that many intellectuals, particularly younger ones, had sympathized with the Legionnary Movement in the 1930s, even though they later disavowed this affinity (see the notorious cases of Mircea Eliade, Eugen Ionesco, Emil Cioran.) Many intellectuals from the area of Braşov – Bran (eg, the great philologist Sextil Puşcariu), shared rightist, or even Iron Guard sympathies, an ideological confusion which they later regretted; among them there were also many physicians who had acquired some notoriety in their profession.

**

I once asked an older friend: why is there so much hatred towards a man who, through his knowledge and hard work, dedicated his life to medicine for the public welfare?

– "The he lazy man will throw stones only at the tree full of fruit, so that he can collect the fallen fruit effortlessly", my friend replied. "And then, we know from the Gospel that 'no good tree will bear bad fruit, nor will a bad tree bear good fruit.'"

References:

1. Stoian, Emil, „Portrete din Bran”, Dealul Medicilor Publishing House, Braşov, 2002

2. Mihailide, Mihail, „Insolitul ospăț al unui devorator de arhive”, Viaţa Medicală Românească Publishing House, 2017

3. Ibidem

4. Medicine History Documentary Library, National Institute for Public Health, Bucharest, Iancu Gonţea File.

5. Ibidem

6. Ib. id. Reper 2

7. National Council for the Study of Security Structure Archives (CNSAS), Central Archives Department, Operative file Gonţea Ion-Iancu, cote 498910, vol.I and vol.II * The files were studied by 10 other people between April 5, 1969 and March 3, 1976).

8. Collective, coordinator Valentina Bilcea, „Istoria românilor. Date Fapte. Oarneni”; Foreword: Professor Adrian Cioroianu Ph.D., Meronia Publishing House, Bucharest, 2018

ADMINISTRATIVE ISSUES

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To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review.

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We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) Accessible to the public at no charge;
(2) Searchable by standard, electronic (Internet-based) methods;
(3) Open to all prospective registrants free of charge or at minimal cost;
(4) Validates registered information;
(5) Identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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All articles, with the exception of Editorials, must contain an abstract of no more than 250 words. Abstracts for original articles should be formatted into subheadings, as detailed below. Titles must not be longer than 120 characters (including spaces).

Editorials

These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

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RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related "mini-series" of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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RJMM particularly welcomes submission of Meta-Analyses and Systematic Reviews, which underpin evidence-based medicine. Guidelines for preparation of Meta-Analysis and Systematic Reviews are similar to other reviews, and articles are subject to the usual peer review process. Meta-Analyses and Systematic Reviews have a word limit of 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication', as presented at http://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader's task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

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The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

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Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

Text

Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

References

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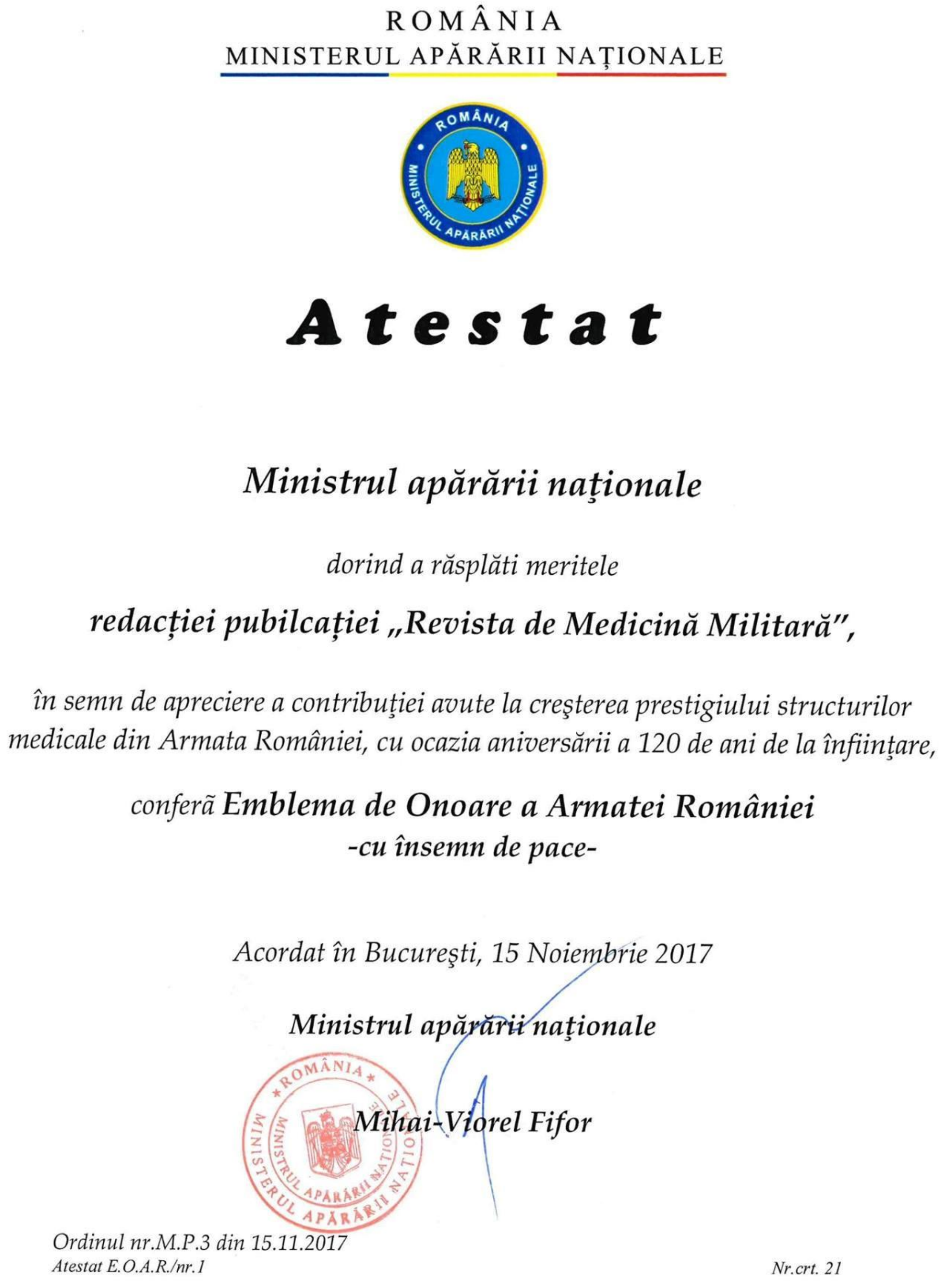

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redacţiei pubilcaţiei „Revista de Medicină Militară",

în semn de apreciere a contribuţiei avute la creşterea prestigiului structurilor medicale din Armata României, cu ocazia aniversării a 120 de ani de la înfiinţare,

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-cu însemn de pace-

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Nr.crt. 21

EDITORIAL

About malpraxis, with love… for your information

Dan Mischianu

The title of this article for the Romanian Journal of Military Medicine, is inspired from the title of an American movie from 1967, ''To Sir with love'', starring Sidney Poitier in the lead role. Maybe some of you still remember it!... It is said that once, when school did not oscillate between "variable concepts" and "palpable realities", between protocols and procedures, a hydrogeology professor, in a practical work with his students, asked them if they knew the significance of the walnuts planted near the houses of the Romanian peasants. All the students were amazed by the question and kept silence. Nobody made any gesture. The professor knew that this would be the reaction of the students - because this was the answer to the following question: "Why does the world need teachers?" The professor answered the questions and taught the students, as much as a professor can teach, that ''a walnut needs plenty of water to grow and the roots of the walnut are very deep, and that is why houses that have walnut trees next to them will not have problems with dampness...''

This parable, as understood by our mind, aims on one hand to draw the reader’s attention to the fact that "malpractice is defined as an improper or negligent treatment applied by a doctor to a patient, which causes the latter any kind of harm, in relation to the degree of damage of his physical and mental capacity", according to DEX, and on the other hand to state that the "WALNUT" – that is, the Teacher, in the way he teaches his students will keep the "house" (i.e. the workplace) safe against possible future malpractice charges.

And after all, malpractice can even be compared to dampness... for any professional organization. A superb allegory! I consider it a proper allegory and that is why I wanted to introduce it to you.

Gral (R) Prof DAN MISCHIANU

Chief of Urology Clinic, Carol Davila University Central Emergency Military Hospital
Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

I do not pretend to be an expert in the field of "medical malpractice" but being involved and exposed to it in my daily activities, I welcome some considerations on this topic, which I have found out from books, from the media, and also from journalistic practice.

This article also wishes to draw attention of the medical community and to make it more aware of the responsibilities that we have when we dare to practice this noble profession. All of us – physicians, nurses, stretcher-bearers, technical staff – have civil liability (and here is the context of a possible allegation of medical malpractice), disciplinary liability (in the relationships we have as full members of our professional organizations) and criminal liability (most often as defendants, sometimes as investigated parties or as witnesses).

I will present you several cases that have been the subject of disciplinary, civil and criminal investigations, all of them in the “urological filed”.

Case 1

A 55-year-old patient is admitted in the Surgery Department for urgent mumps and blurred urine. Diagnosis at admission: ''Urinary infection. Prostate adenoma. Hematuria.” Diagnosis after three days since admission: ''Medium periurethral adenoma with urethral fistula microabs. Piohematuria. Urinary Infection." Under antibiotic treatment, the symptomatology improves and an open surgery is performed – with the patient's consent – practicing transvesical adenomectomy with favorable post-surgery progress.

14 days after surgery, the urinary incontinence at effort is detected, which subsequently worsens with the occurrence of episodes of urinary tract infection recorded on a posterior urethral stricture. Urethrotomy and TUR-P for remaining adenomatous tissue are performed within urological university department. The histopathological result revealed prosthetic adenocarcinoma microfocus and chronic urethritis. Unfortunately, the functional diagnosis is probative: High overall deficiency, Invalidity degree II.

The patient's complaint was lodged against the surgery after which the patient was diagnosed with urinary incontinence that can only be cured by means of an artificial sphincter costing about 5,000 euros and retirement due to illness.

The Superior Disciplinary Commission of the Romanian College of Physicians considered that the moment when surgery was performed and the surgical approach were inappropriate, therefore it decided to sanction the physician. [2]

Case 2

The son of a patient lodges a complaint against his family physician for the unauthorized delay of the diagnosis of his father's renal carcinoma. The physician treated him for a year and six months for macroscopic hematuria and a so-called renal colic. After one year and 6 months, the patient is diagnosed with renal neoplasm, he goes through surgery and dies one year later.

The Superior Discipline Commission analyzes the facts and notes the following: Hematuria is a diagnostic urgency and therefore its etiology needs to be confirmed as soon as possible by an urologist, the diagnosis of renal carcinoma is not the responsibility of the family doctor, therefore the applied sanction has remained in force.

Case 3

This is a case distinct from the others due to a capital error. It involves a 74-year-old patient admitted in a Urology department and diagnosed with: prostate adenoma, complete urine retention, prostate litiation. Twenty-four hours after admission, the urologist intervenes and performs transvesic adenomyctomy.

Immediate post-surgery progression is favorable with the subsequent occurrence of a third degree urinary incontinence. Two years after the surgery, the patient lodges a complaint against the doctor.

The Territorial Discipline Committee ordered the action to be discontinued, but the patient filed an appeal with the Supreme Discipline Commission. The latter did not find any irregularities neither in terms of the technique used nor of the complication that has arisen, not even regarding the postsurgery treatment administered by the urologist, that is, Driptane. The lack of the informed consent from the patient's file was the only and actually the capital irregularity found, for which the physician was sanctioned.

The medical deontology code in Romania is very clear: "for any diagnostic or therapeutic medical intervention, the informed consent of the patient is necessary". Moreover, the consent is given only after informing the patient about his/her diagnosis, prognosis, therapeutic alternatives, along with their risks and benefits. [2]

Case 4

In the same spirit, there is also the case of a 39-year-old patient admitted in emergency with the diagnosis: right kidney colic. Two days after the admittance in hospital, surgery is performed: right pielolithotomy with internal drainage for calculus included in the right skin-ureteral junction and secondary uropionefrosis. The immediate post-operative progression was favorable up to the occurence of a cardio-respiratory stop during the first post-surgery night which was found to be irreversible. The cause of death was a massive pulmonary thromboembolism with probable causes either the lithotomy position, or more likely the presence of urinary sepsis.

The Superior Discipline Commission decided to sanction the physician for the lack of informed consent, lack of anticoagulation therapy and poor post-surgery follow-up [2, 3]

Case 5

The following is the case of a 54-year-old patient with a transient ischemic stroke, recently treated in a hospital from the country, where a Foley autostatic bladder ureter was mounted. From the patient’s personal history, we mention: type II diabetes, hypertension and ischemic heart disease. Approximately 8 (eight) days after urethro-bladder probe was mounted (according to the patient's report), physicians found the presence of an irreducible paraphimosis. Upon admission to the urology clinic, glandular necrosis, lack of local sensitivity, denuded penile body, penile scar tissue and necrosis, present at the base of the penis, free cutaneous mucous suture, skin remnants, cavernous body having an indurated appearance at palpation are found. The consultation of plastic and reparative surgery is entirely consistent with the urological clinical examination performed.

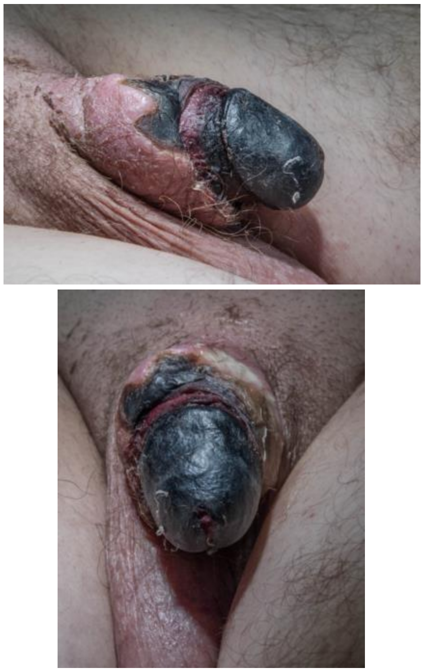

Figure 1: Preoperative aspect; the devitalization of the gland and the partially necrotic areas of the tegument can be seen

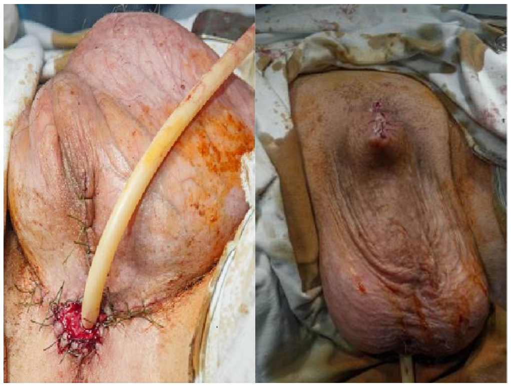

Figure 2: Postoperative final aspect; ureteral bladder and definitive perineal urethrostoma

A surgical intervention is performed, after the patient gave his written consent, consisting of the following: noncrectomy, lavage, double drainage both by the uretro - bladder probe and by suprapubic cistostomy.

After limiting the infectious process, a new surgical intervention is performed consisting of definitive perineal urethrostomy and closure of the penile arch. Post-operative evolution is surgically favorable [4]

The patient contacted the media, the criminal investigation organs, the territorial discipline commission, and is in charge of the Superior Discipline Commission.

CONCLUSIONS

We wanted to point out, against all inconsistencies, errors, mistakes and malpractice, which perhaps appear at first sight incriminating up to the contrary proof, that the presence during the professional training of a ''Teacher'' of a “Walnut'' absorbing all ''professional dampness'' is defining for the next years of professional maturity.

In relation to all the exposed cases, some of them not so relevant anymore, others still ''incendiary'' on which the public opinion and the indigenous media keep a close eye, I believe this journalistic warning should give us all something to think about.

The old Romanian saying teaches us: "The good deed speaks for itself", but here, in Romania, there is a new saying which has been quoted for about thirty years, in my opinion without any substantial background, which says: "No good deed remains unpunished!"

However, I have the absolute conviction that although you have performed 10,000 perfect surgeries, if you make a mistake on your last day of surgical practice, that mistake will not be forgiven!

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SYSTEMATIC REVIEW

Current therapeutic strategies for erectile function recovery after radical prostatectomy – literature review and meta-analysis

Cătălin Belinski[1], Alexandru Aungurenci[1], Dragoș Marcu[2], Dan Spînu[2], Ovidiu Bratu[2,3], Dan Mischianu[2,3]

INTRODUCTION

Radical prostatectomy is the most commonly performed treatment option for localised prostate cancer. In the last decades the surgical technique has been improved and modified in order to satisfy both oncological safety and postoperative functional results. Urinary incontinence and erectile dysfunction (ED) are the main postoperative functional impairments in patients that undergo radical prostatectomy (RP). [1]

According to the EAU Guidelines erectile dysfunction is defined as “the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance”. To this moment it is estimated that 25-75% of the patients who underwent RP develop ED with major implications in postoperative quality of life. [2] Various risk factors can be related to post-prostatectomy ED, among which the most important are the patient’s age, comorbidities, preoperative erectile function, but also surgical approach (robot-assisted, laparoscopic, open) and nerve-sparing technique (inter/intrafascial). [2, 3] Among physiopathological modifications that concur to ED, the nerve damage (neuropraxia) appears to be the main domino piece that triggers other alterations like structural changes of the smooth muscle, arterial damage and veno-oclusive dysfunction which lead to cavernosal oxygenation impairments. [4–6] The clinical results of physiopathological alterations are shortening of penile size, impairment of orgasm and ejaculation achievement which have tremendous impact on psychological and mental health. [5]

Various treatment options include oral medication with phosphodiesterase 5 inhibitors (PDE5i), intracavernosal injections or intraurethral applications of prostaglandin E1 (PGE1), vacuum or vibratory treatments. Though there are many options for ED treatment, to this date there is no definitive protocol for penile rehabilitation (PR) after prostatectomy. [5, 7] In this regard we have made a literature review and meta-analysis of the most important randomised trials published in the last 5 years in order to find the best strategies of penile rehabilitation after radical prostatectomy.

MATERIALS AND METHODS

We have systematically reviewed and collected data from randomised trials published in the last 5 years involving patients diagnosed with prostate cancer who experienced ED after radical prostatectomy. In order to find the necessary trials we have searched PubMed and ScienceDirect databases and used several key words like: erectile dysfunction, penile rehabilitation, phosphodiesterase 5 inhibitors, erectile functional recovery, prostate cancer, prostatectomy and nerve sparing – in various combinations. The first aspect for our meta-analysis inclusion criteria was to identify randomized (prospective or retrospective) placebo-controlled trials involving patients with localised prostate cancer that benefited of nerve sparing radical prostatectomy and developed postoperative ED. The key aspect of our research was the penile rehabilitation strategy which included the therapeutic means used for erectile

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function recovery and the treatment duration. Other aspects followed by us regarded the means of ED assessment, the surgical means and invasiveness and the rate of treatment success reported to ED severity.

In this regard we have conducted our research for each year between January 2014 and December 2018 and reviewed a total of 631 articles published on this theme collected from both search engines. For a time-efficient assessment we have read the title and abstract of each article and included only those articles that matched our criteria. We have excluded the reviews and meta-analyses and also the trials of ED that was not related to prostate cancer surgery. That left us 194 publications from which to select. We have also excluded the trials withdrawn from database for various reasons and articles that didn't followed the therapeutic means of ED recovery. Finally we have selected 15 articles matching our criteria for meta-analysis that were thoroughly full text reviewed. Seven out of the 15 were prospective randomised trials, 2 of them being double-blind, placebo-controlled. Another 2 were nonrandomised prospective trials and the rest of 6 articles were retrospective review. Although some studies included by us followed-up both the ED and postoperative incontinence problem, we have concerned strictly on erectile dysfunction issue and collected the necessary data.

For statistical analysis we have used ReviewManager 5.3 and Microsoft Excel software with data of treatment strategies and drug dosages collected from the studies. Although there was less heterogeneity between the study designs of the included articles we found important data about postoperative EF recovery. These data allowed us to include the studies in subgroups in order to analyse them.

Table 1. Distribution of included studies

Trial author	Design	Patient no.	Mean age	Cardiovascular comorbidities	Diabetes
Vickers et al. [8]	Randomised prospective	2162	64.2	NoS	NoS
Montorsi et al. [9]	Randomised double-blind placebo-control	423	58	NoS	NoS
Seo et al. [10]	Retrospective review	92	67.9	40 (43.5%)	14 (15.2%)
Nakano et al. [11]	Retrospective review	103	63.4	NoS	NoS
Fode et al. [12]	Randomised prospective control	68	62	NoS	NoS
Stolzenburg et al. [13]	Randomised double-blind	422	59	NoS	NoS
Canat et al. [14]	Randomised prospective	112	63	42 (37.5%)	18 (16.07%)
Yiou et al. [15]	Retrospective review	75	59.4	30 (40%)	6 (8%)
Haglind et al. [16]	Prospective controlled nonrandomised	2625	63	NoS	NoS
Kim D. et al. [17]	Randomised prospective placebo-control	97	54	NoS	NoS
Capogrosso et al. [18]	Retrospective review	2364	61	NoS	NoS
Sooriakumaran et al. [19]	Prospective non-randomised	2545	63.3	556 (35.2%)	99 (6.2%)

RESULTS

A total of 11,831 patients were enlisted in the 15 studies we have included for analysis. Mean age of all patients was 61.91 years with a range between 54 and 67.9 years. (Table 1) Although not all the studies specifically mentioned the most encountered comorbidity was the cardiovascular disease (37.5% - 100%) followed by diabetes mellitus (6.2% – 16.07%). For most ED patients the presence of cardiovascular comorbidities creates the premises of disease trigger, followed by diabetes and obesity. [] All patients were diagnosed (by means of PSA, prostate biopsy and pelvic MRI) with localised prostate cancer mainly T1c - T2c, with Gleason score ranging between 6 and 7 and PSA values below 10 ng/ml. (Table 2) These data were correlated with the postoperative pathology findings which in some cases changed the actual diagnostic stage according to d'Amico criteria. As it can be seen in Table 2 in some cases based on the degree of invasion the cancer stage was changed from localised to locally-advanced (T3b-T4) and based on Gleason score from low or intermediate risk to high risk (Gleason 8).

All patients were proposed for radical prostatectomy using various approaches: open surgery (n = 4,460), conventional laparoscopy (n = 1,371) or robot assisted laparoscopic prostatectomy (n = 4,929). (Table 2) Most patients benefited of bilateral nerve sparing (BNS) dissection of the nerve bundles (n = 6,852) by al known technical procedures (interfascial/intrafascial), which represented 63.68% of the operated patients. When case did not impose BNS dissection, unilateral nerve sparing technique was attempted (n = 3,209) which was performed in 29.82% of the operated patients. The rest of 6.5% of the patients either had not benefited of NS dissection either the information about the NS status was not mentioned. [16, 18, 19] The NS status at the end of the surgery was crucial because it ensured the premises for postoperative ED recovery although most of the patients experienced postoperative neuropraxia.

Table 2. PCa status and surgical approach for each study

Trial author	PCa clinical stage	Gleason Score	Surgical approach			Nerve sparing	
			OP	CLP	RALP	BNS	UNS
Vickers et al. [8]	T2a – T3	6 - 8	859 (40%)	546 (25%)	757 (35%)	657 (30%)	1498 (70%)
Montorsi et al. [9]	T1c – T2c	6 - 7	68 (48.9%)	29 (20.9%)	31 (22.3%)	423 (100%)	-
Seo et al. [10]	T2a – T2c	4 - 8	-	-	92 (100%)	57 (62%)	35 (38%)
Nakano et al. [11]	T2 – T3	6 - 7	NoS	NoS	NoS	24 (23.3%)	79 (76.7%)
Fode et al. [12]	T2 – T3	NoS	7 (10.3%)	-	61 (89.7%)	37 (54.41%)	31 (45.58%)
Stolzenburg et al. [13]	T2-T3	≤ 7	189 (44.78%)	88 (20.85%)	115 (27.25%)	422 (100%)	-
Canat et al. [14]	T2a-T2c	5 - 8	NoS	NoS	NoS	112	-
Yiou et al. [15]	T2 - T3	6 - 8	-	75 (100%)	-	75 (100%)	-
Haglind et al. [16]	T1 - T3	6 - 8	778	-	1847	1318	750
Kim D. et al. [17]	T1c – T3	6 - 8	58 (61.7%)	-	36 (38.3%)	96 (98.96%)	1 (1.04%)
Capogrosso et al. [18]	T2 - T4	NoS	614 (26%)	633 (27%)	1117 (47%)	2253 (95%)	78 (3%)
Sooriakumaran et al. [19]	T2 – T4	NoS	1792 (70.41%)	-	753 (29.58%)	1283 (50.41%)	737 (28.95%)
Kim S. et al. [20]	T2a – T2c	4 - 8	95 (100%)	-	-	95 (100%)	-
Kwon et al. [21]	NoS	NoS	NoS	NoS	NoS	NoS	NoS
Jo et al. [22]	T2a – T2c	5 - 7	-	-	120 (100%)	NoS	NoS

NoS – no specification; OS – open surgery; CLP – conventional laparoscopic prostatectomy; RALP – robot assisted laparoscopic prostatectomy; BNS – bilateral nerve-sparing; UNS – unilateral nerve-sparing.

Table 3. Preoperative EF assessment and postoperative EF recovery means and results

Trial author	Type of EF questionnaire	Preop. mean EF score	Means of penile recovery	Subgroups	Trial duration	Recovery rates
Vickers et al. [8]	IIEF-6	≥22 - 937 (59%)	PDE5i (NoS) PGE_1 ICI	-	2 years	36% (NoS)
Montorsi et al. [9]	IIEF-EF	≥22 - 423 (100%)	PDE5i	Tadalafil 5 mg – OaD – 139 (32.86%) Tadalafil 20 mg – PRN – 143 (33.8%) Placebo – 141 (33.3%)	9 months	Tadalafil 5 mg – 25.2% Tadalafil 20 mg – 19.7% Placebo – 14.2%
Seo et al. [10]	IIEF-5	≥22 - 92 (100%)	PDE5i	Tadalafil 5 mg – OaD Non-Tadalafil	1 year	Tadalafil 5 mg – 13.2% Non-Tadalafil – 7.7%
Nakano et al. [11]	IIEF-5	17.7	PDE5i	Vardenafil 10 mg – OD – 30 (29.12%) Vardenafil 20 mg – PRN – 5 (4.85%) No treat – 68 (66.01%)	1 year	Vardenafil 10 mg + Vardenafil 20 mg – 21 (60%) No treat – 26 (38.2%)
Fode et al. [12]	IIEF-5	25	PDE5i PVS	PDE5i + PVS - 30 PDE5i - 38	1 year	PDE5i + PVS – 16 (53%) PDE5i – 12 (32%)
Stolzenburg et al. [13]	IIEF-5	≥22 – 422 (100%)	PDE5i	Tadalafil 5 mg – OaD - 102 Tadalafil 20 mg – PRN - 112 Placebo - 106	9 months	Tadalafil 5 mg – OaD – 29 (28.43%) Tadalafil 20 mg – PRN – 24 (21.42%) Placebo – 27 (25.4%)
Canat et al. [14]	IIEF-6	≥ 22	PDE5i	Tadalafil 5 mg – OpW - 38 Tadalafil 20 mg – PRN - 40 Non-Tadalafil - 34	1 year	Tadalafil 5 mg – OpW – 19.89 (mean IIEF) Tadalafil 20 mg – PRN – 15.8 (mean IIEF) Non-Tadalafil – 13.47 (mean IIEF)
Yiou et al. [15]	IIEF-EF EHS	≥ 24 ≥ 2	PDE5i PGE_1 ICI	PDE5i + PGE_1 ICI – NoS No treatment - NoS	2 years	M12 – 19.6 (mean IIEF – treat.) M12 – 18.07 (mean IIEF – no treat.) M24 – 4.63 (mean IIEF – treat.) M24 – 4.92 (mean IIEF – no treat.)
Haglind et al. [16]	IIEF-5	≥ 21	-	RRP RALP	1 year	RRP – 124 (15.93%) IIEF-5 ≥ 21 RALP – 339 (18.35%)
Kim D. et al. [17]	IIEF-EF	≥ 21 (100%) (Mean 28.1)	PDE5i	Sildenafil 50 mg - OaD + Sildenafil 100 mg - PRN Placebo + Sildenafil 100 mg - PRN	13 months	Sildenafil 50 mg - OaD + Sildenafil 100 mg – PRN – 15 (32.4%) Placebo + Sildenafil 100 mg – PRN – 13 (29%)
Capogrosso et al. [18]	IIEF-6	≥ 24 (Mean 27)	PDE5i PGE_1 ICI	12 months - 1779 24 months - 1095	2 years	12 mo – 483 (27%) 24 mo – 377 (34%)
Sooriakumaran et al. [19]	Penile stiffness Morning erection	1702 (66.9%) PPP.	PGE_1 ICI PGE_1 IUr	RARP RRP	2 years	RARP – 377 (21%) RRP – 106 (14%)
Kim S. et al. [20]	IIEF-5	22.4	PDE5i	Tadalafil 5 mg – OaD – 2 years Tadalafil 5 mg – OaD – 1 year No Tadalafil	2 years	Tadalafil 5 mg OaD – 2 yr. – 16.1 (mean IIEF) Tadalafil 5 mg OaD – 1 yr. – 13.5 (mean IIEF) No Tadalafil – 9.4 (mean IIEF)

Kwon et al. [21]	Penile lenght SHIM	NoS	PDE5i (Sildenafil, Vardenafil, Tadalafil)	Complete recovery Incomplete recovery	1 year	CR – 318 (60.2%) IR – 210 (39.8%)
Jo et al. [22]	IIEF - 5	≥ 17	PDE5i (Sildenafil 100 mg)	Early treatment - 58 (3 wk. postop.) Delayed treatment - 62 (3 mo. postop.)	1 year	ET – 24 (41.4%) DT – 11 (17.7%)

PPP – preoperative potent patients; IIEF – International Index of Erectile Function; OaD – oance a day; PRN – pro re nata (on demand); ICI – intracavernosal injections; IUr – intrauretral;

For pre and postoperative EF assessment the IIEF questionnaire was preferred in most of the studies in different forms: the classical 15 questions IIEF-EF or the shorter IIEF-5/IIEF-6 questionnaires.

Most authors preferred the IIEF-5 questionnaire due to its reliability and rapid way of determining the EF status. (Table 3) In most of the studies that used the IIEF questionnaire a cut-of value of 22 points was established for preoperative potent men [8–10, 13, 14] while others established close cut-of values. [16, 20] Only two studies used other means of EF assessment. Sooriakumaran et al. used two questions about penile stiffness and morning erections, both having single choice answer from 5 possible variants. [19] In a particular case Kim et al. compared the penile length both pre and postoperative and additionally used the SHIM questionnaire for ED appraisal. [20]

The most frequently used treatment method for penile recovery was the administration of PDE5i which was tested with various ways of administration and dosages and compared with placebo or no treatment control groups. The most commonly used drug in the included trials was Tadalafil [9, 10, 13, 14, 20], followed by Sildenafil [17, 22] and Vardenafil. [11] Some trials studied the single use treatment of PDE5i compared with combinations of PDE5 with PGE1 ICI [8, 15, 18] or penile vibratory stimulation (PVS) devices. [12] There were also particular studies that did not compare the method of PDE5i administration but rather the rate of EF recovery [8, 21], the moment of treatment initiation [22] or the length of treatment administration. [18] Haglid et al. compared the EF recovery based only on the surgical approach type (open vs. RALP) and not interfering with any medication in this regard while Sooriakumaran et al. compared the same surgical approaches using PGE1 treatment setting. [16]

Figure 1: Multivariate comparison of PDE5i OaD and PRN treatment compared to control group

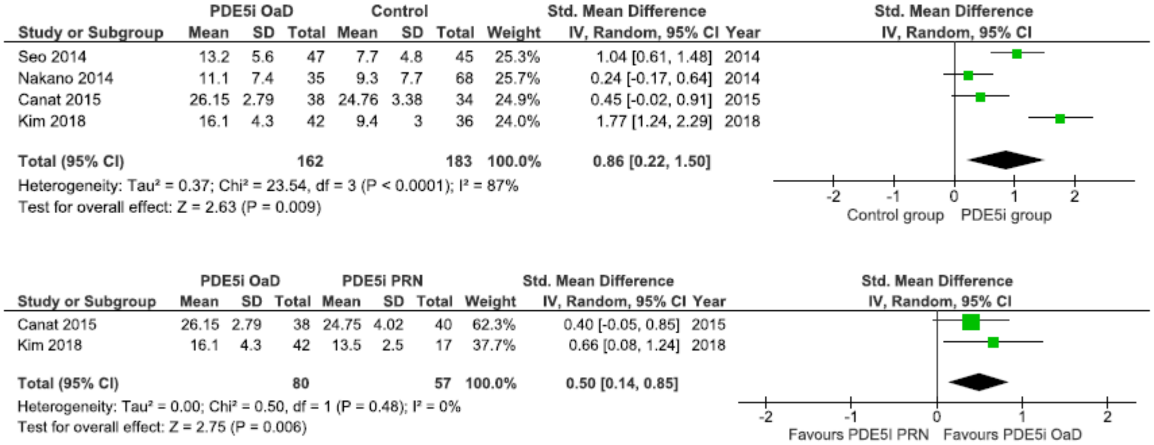

	PDE5i OaD			Control				Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	Year
Seo 2014	13.2	5.6	47	7.7	4.8	45	25.3%	1.04 [0.61, 1.48]	2014
Nakano 2014	11.1	7.4	35	9.3	7.7	68	25.7%	0.24 [-0.17, 0.64]	2014
Canat 2015	26.15	2.79	38	24.76	3.38	34	24.9%	0.45 [-0.02, 0.91]	2015
Kim 2018	16.1	4.3	42	9.4	3	36	24.0%	1.77 [1.24, 2.29]	2018
Total (95% CI)			**162**			**183**	**100.0%**	**0.86 [0.22, 1.50]**	

Heterogeneity: Tau² = 0.37; Chi² = 23.54, df = 3 (P < 0.0001); I² = 87%
Test for overall effect: Z = 2.63 (P = 0.009)

	PDE5i OaD			PDE5i PRN				Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	Year
Canat 2015	26.15	2.79	38	24.75	4.02	40	62.3%	0.40 [-0.05, 0.85]	2015
Kim 2018	16.1	4.3	42	13.5	2.5	17	37.7%	0.66 [0.08, 1.24]	2018
Total (95% CI)			**80**			**57**	**100.0%**	**0.50 [0.14, 0.85]**	

Heterogeneity: Tau² = 0.00; Chi² = 0.50, df = 1 (P = 0.48); I² = 0%
Test for overall effect: Z = 2.75 (P = 0.006)

Although the articles included in our meta-analysis had dichotomous criteria of study design we could find similarities between some of them, which allowed us to organise subgroups of trials. On multivariate analysis we have included trials in which PDE5i was administered as OaD and PRN treatment compared to a pacebo control group. The variables included in analysis were the mean IIEF scores recorded at the end of the trial for each group (OaD, PRN and control). As it can be observed in fig. 1 the higher rates of recovery are in favour of PDE5i OaD group compared to control group (CI 95%, P < 0.0001). On a lower scale, the same result could be observed when PDE5i OaD treatment was compared to PDE5i PRN although in this analysis only 2 studies had the necessary data to be included (CI 95%, P =

0.48).

In a univariate analysis we also compared the trials were only the percentage of recovered patients after PDE5i treatment was given. (Fig 2) As in previous assessment the PDE5i OaD group had higher recovery rates compared to control group (CI 95% P = 0.40). Also when we have compared PDE5i OaD with PRN group the better results were slightly higher in favour of OaD group thus resulting that the benefit of PDE5i OaD compared to PRN treatment was minor (CI 95%, P = 0.95).

Figure 2: Recovery rates in trials using PDE5i OaD and PRN treatment compared to control group

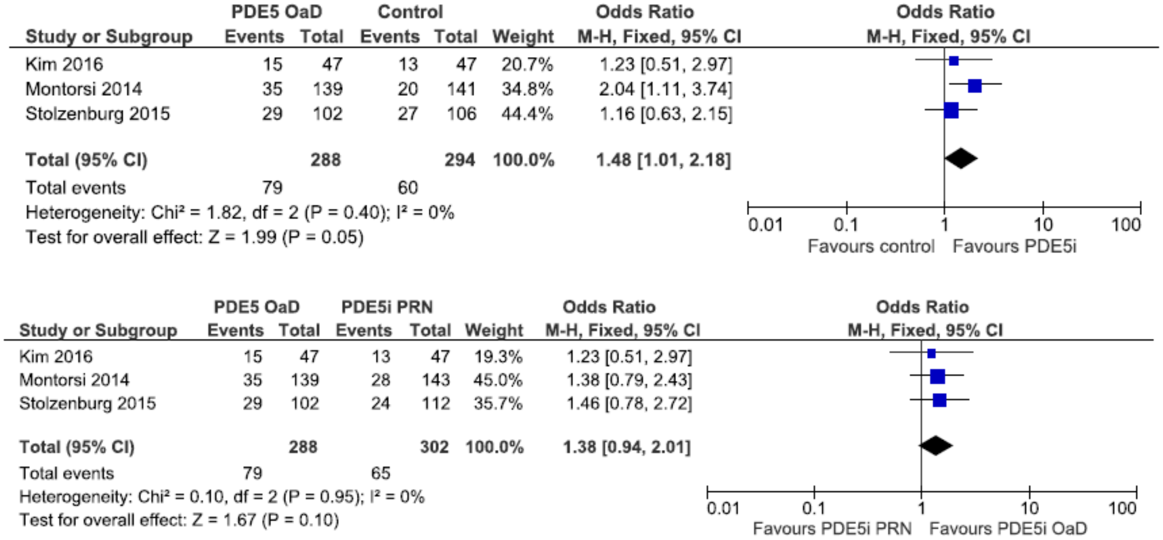

Study or Subgroup	PDE5 OaD Events	PDE5 OaD Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Kim 2016	15	47	13	47	20.7%	1.23 [0.51, 2.97]
Montorsi 2014	35	139	20	141	34.8%	2.04 [1.11, 3.74]
Stolzenburg 2015	29	102	27	106	44.4%	1.16 [0.63, 2.15]
Total (95% CI)		**288**		**294**	**100.0%**	**1.48 [1.01, 2.18]**
Total events	79		60			

Heterogeneity: Chi² = 1.82, df = 2 (P = 0.40); I² = 0%
Test for overall effect: Z = 1.99 (P = 0.05)

Study or Subgroup	PDE5 OaD Events	PDE5 OaD Total	PDE5i PRN Events	PDE5i PRN Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Kim 2016	15	47	13	47	19.3%	1.23 [0.51, 2.97]
Montorsi 2014	35	139	28	143	45.0%	1.38 [0.79, 2.43]
Stolzenburg 2015	29	102	24	112	35.7%	1.46 [0.78, 2.72]
Total (95% CI)		**288**		**302**	**100.0%**	**1.38 [0.94, 2.01]**
Total events	79		65			

Heterogeneity: Chi² = 0.10, df = 2 (P = 0.95); I² = 0%
Test for overall effect: Z = 1.67 (P = 0.10)

DISCUSSION

Erectile dysfunction is a major postoperative complication for men who undergo radical prostatectomy, alongside urinary incontinence. At this moment prostate cancer is the second most diagnosed neoplasia in men due to the systematic determination of PSA. [23] Radical prostatectomy remains the most important therapeutic procedure for localised PCa having a postoperative life-expectancy of 10 years. [2] Although the technical enhancements of prostatectomy reduced the rates of postoperative complication, ED still remains one of the most complex problem for PCa patient's quality of life.

Even if the nerve sparing dissection technique of the prostate bundles partially improved the ED rates, there are still problems regarding the postoperative penile rehabilitation. Neuropraxia is the main pathological alteration that leads to major structural changes of the penile tissue. This can also lead to veno-oclusive dysfunctions of blood drainage. Due to the constant state of hypo-oxygenation the penile smooth muscles suffers a process of apoptosis and fibrosis which in time are producing irreversible penile malformations such as penile shortening of 2-3 cm and Peyronie disease. [5, 24] In this regard it is advisable that urologists should discuss with their patients about the possibility of postoperative ED in various rates – from temporary to permanent. [25]

Two trials included in our meta-analysis studied the rate of ED occurrence and penile recovery degree after RALP compared to RRP in high volume centres from Sweden. In a 2015 prospective study, Haglid et al. found a slightly higher rate of penile recovery for RALP, but the differences were not significant for these two surgical approaches. [16] In a recently conducted prospective trial, Sooriakumaran et al. (2018) found earlier recovery rates of EF for RALP (21%) compared to RRP (14%) due to a more precise identification and preservation of nerve bundles during RALP. Also the rates of post-surgical positive margins were similar to open surgery which is an important aspect for oncological radicality. [19] Both studies were prospective non-randomised.

One key aspect of ED diagnosis is the use of pre and postoperative EF assessment questionnaires. As we have shown above the most frequently used questionnaire was IIEF-5 which is the short version of the IIEF-EF questionnaire. Other questionnaires like Sexual Health Inventory for Men (SHIM) or Expanded Prostate Cancer Index Composite (EHGS) have similar predictive results to IIEF and should be routinely used for postoperative ED diagnosis. [25, 26]

It is a known fact that older age, cardiovascular comorbidities and diabetes mellitus are the most important predictive factors for ED onset. [27, 28] On the other hand the younger age and the lower body mass index are demonstrated protective factors for ED recovery. [2] Even though not all the studies included in our meta-analysis reported the rate of cardiovascular disease and diabetes mellitus we could emphasize that in the post prostatectomy setting these two comorbidities could have significant impact on ED onset and the subsequent penile recovery. [10, 14, 15, 19, 20, 21]

Penile rehabilitation is the cumulus of therapeutically means which includes medication, medical devices or actions, simple or in various combination recommended for erectile function recovery. [5] PDE5i are the most frequently used drugs in clinical practice for penile rehabilitation and to this date is the most studied therapeutic method administered to ED patients. The main advantage of PDE5i therapy is that there are quick and easy to administer but it requires the integrity of at least 1 cavernosal nerve bundle. Also the treatment cost and side effects (headache, flushing and palpitations) are not negletable. [24]

In their common trial Montorsi et al. (2014) and Stolzenburg et al. (2015) studied the effect of Tadalafil administered once a day (OaD) and on demand (PRN) compared to a placebo-controlled group and found that the OaD administration of Tadalafil 5 mg has the best results for penile recovery after prostatectomy. [9, 13] Similar results for Tadalafil OaD administration were also found by Seo et al. (2014), Canat et al. (2015) and Kim S. et al. (2018) based on the mean IIEF score at the end of the trial. [14, 20] Nakano et al. (2014) and Kim D. et al. (2016) found similar results at the end of their trials although they have studied the effect of Vardenafil respectively Sildenafil. [11, 17] In our multivariate analysis the PDE5i OaD treatment has evident advantages over placebo and on demand PDE5i administration for postoperative penile recovery rate.

Regarding the choice of PDE5i treatment there are no specific trials that compare the efficiency of currently available substances in postoperative ED. In a 2005 open-label randomised trial Eardley et al. emphasized that Tadalafil is the preferred PDE5i used for ED treatment because of long-lasting effect in time compared to Sildenafil that allowed patients to have less concerns about spontaneity of erection. [29] Further more Hyndman et al. demonstrated in their randomised trial that Sildenafil could have major impact over urinary continence, thus impairing the overall recovery of PCa operated patients. [30]

Prostaglandin E1 intracavernosal injection (PGE1 ICI) was the first type of treatment for postoperative EF recovery, introduced and studied by Prof. Montorsi et al. [31] The most important advantage of PGE1 ICI is that is very effective in any type of surgical approach even if nerve sparing was not indicated, though the cost, the need to refrigerate the product, the invasiveness of the treatment and the side effects are a majos counterbalance for treatment initiation. [24] In our meta-analysis, Vickers et al., Yiou et al. and Capogrosso et al. have used PGE1 ICI in combination with PDE5i treatment for penile recovery. The results achieved by Yiou at 12 respectively 24 months of combined treatment were very similar in both drug-administered and control group. [15] On the other hand Capogrosso et al. found that at 24 months of combined PGE1 ICI and PDE5i treatment the rates of recovery were higher than at 12 months (34% vs. 27%). [18]

Vacuum and vibratory devices are non-invasive and very effective in EF recovery especially when combined with PDE5i treatment. Fode et al. demonstrated in their randomised prospective trial that EF recovery could be very effective when penile vibratory stimulation devices are used in conjunction with PDE5i (53% recovery rate) compared to PDE5i only group (32%). [12]

Regarding treatment initiation Jo et al. demonstrated that early penile rehabilitation commencement (at 3 weeks after surgery – immediately after urinary catheter removal) has better recovery results than delayed treatment initiation (at 3 months postoperative). [22]

CONCLUSIONS

The postoperative EF recovery of PCa patients can be achieved using a multitude of penile recovery strategies. For this purpose a preoperative assessment is very important, by using erectile function questionnaires. Patients should be informed about the postoperative ED risk and should be counselled in this area.

Nerve-sparing robot assisted laparoscopic prostatectomy is the most protective surgical approach for EF preservation. PDE5i is the main therapeutic approach for postoperative penile recovery.

Tadalafil 5 mg once a day was the most effective long term therapy for ED treatment both single use or in combination with PGE1 and other devices (vacuum or vibratory stimulation). Penile prosthesis implants should be regarded as last resort treatment option for cases when no penile recovery strategy has been successful.

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REVIEW ARTICLE

Toxoplasma Gondii infection and the signs of fetal affection during pregnancy as seen on ultrasound

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Abstract: *Toxoplasma Gondii is a protozoan parasite which causes multisystem affections and unusual clinical outcomes. Although primo- infection takes place frequently during childhood, there is a possibility for it to be found during pregnancy, when it could lead to congenital toxoplasmosis, with serious ocular and cerebral lesions (that can be identified during ultrasound). The positive diagnostic for the mother is made after a serologic test and for the fetus the parasite can be found in the amniotic fluid during amniocentesis.*

A prompt treatment can avoid the presence of cerebral lesions (hydrocephalus, ventriculomegaly, intracerebral calcifications).

Keywords: *protozoan, pregnancy, serology, amniocentesis, hydrocephalus, ventriculomegaly, intracerebral calcifications*

INTRODUCTION

Toxoplasma Gondii is a protozoan parasite that affects the human body in many ways; Infection generally occurs in infancy or adolescence, with declining prevalence in developed countries in the temperate climate [1, 2]. During an initial infection, an immunocompetent body will limit the spread of the parasite, but it remains cantonized in the nervous and muscular tissues, and cannot be completely eliminated [3].

When the infection occurs during pregnancy, there is a risk of transmission to the fetus, resulting in congenital toxoplasmosis that can be manifested by ocular and neurological damage. Proliferation of the parasite may continue, and cerebral tissue destruction may occur even after an important maternal immune response, including IgG production [1, 4].

The incidence of maternal infection during pregnancy varies between 1-8 ‰ [1, 5].

In the acute phase the infection is usually asymptomatic. If symptoms occur, these are nonspecific, such as fatigue, fever, headache, malaise, and myalgia [6].

Differential diagnosis is made by clinical appearance of acute Epstein-Barr infection, CMV, HIV, Treponema, sarcoidosis, Hodgkin's disease or lymphoma [1].

Positive diagnosis of maternal infection in pregnancy involves at least two blood test results with seroconversion from negative to positive for Toxoplasma-specific IgM or IgG. Note that the presence of only IgM in the blood does not automatically involve acute infection, with data showing that only 20% of patients with IgM positive had acute infection with Toxoplasma [7].

Screening for Toxoplasma is performed in some European countries on a monthly basis or at 3 months during

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pregnancy, with the major advantage of rapidly detecting such infection and increasing the effectiveness of therapy [8]. The US, Canada and UK do not routinely test pregnant women because the prevalence of primo-infection during pregnancy appear to be sufficiently low and the costs seem to outweigh the benefits [5,9]. On the other hand, repeated tests increase the number of false positive results, resulting in an unnecessary elevation in the number of prenatal invasive investigations and the number of treated patients. These drawbacks should be weighed against the potential benefit of early-onset therapy, which appears to be present only in the case of serious but rare cases of neurological sequelae of congenital toxoplasmosis [1,11].

IgM antibodies are positive in the first week of infection. Diagnosis of the primo-infection is much more precise when the initial testing shows IgM positive and negative IgG, and at the 14-day retest both of them become positive. This combination of results represents the safest diagnosis of Toxoplasma primo-infection two weeks ago and eliminates a possible false-positive result for IgM. If IgG retests remained negative, most likely positive IgM was only a false positive, and recent or older infection is excluded. An important problem is a positive IgG and IgM result in the first trimester because once IgG is present, IgG remains Positive for life [1].

Depending on the laboratory, the real risk of infection after conception is only 1-3% [12]. Since IgM is only positive after only two weeks but can remain positive for years and IgG reaches a maximum titer at 6-8 weeks and then decreases slowly over the next two years, the positive diagnosis of recent infection requires additional tests [13]. A very useful option is testing IgG avidity, which is very high in old infections, but may remain at a low level for some patients for some years [14]. IgG titration may be a variant, but is insufficiently evaluated and requires a reference laboratory for good quality of the results [1].

MATERIAL AND METHOD

Fetal infection occurs by transplacental transmission after maternal primo-infection. The risk of transmission to the fetus increases drastically with increasing gestational age [15]. In a meta-analysis of 1438 patients in 26 cohorts by individual patient data, it is estimated that the risk of maternal-fetal transmission is 15% when maternal seroconversion occurs at 13 weeks, 44% when seroconversion takes place at 26 weeks and 71% when seroconversion prevails at 36 weeks [15]. The study showed that early therapy seems to reduce the risk of developing congenital toxoplasmosis, but the strength of this assertion is low [15].

RESULTS

As mentioned earlier, the same meta-analysis has shown that with increasing gestational age to seroconversion, the risk of maternal-fetal transmission of T Gondii infection increases drastically, but the risk of cerebral fetal sequelae decreases and eye damage is not influenced by this factor [15].

Figure 1: A: Cerebral calcifications, B: Hydrocephaly, C: Vetriculomegaly

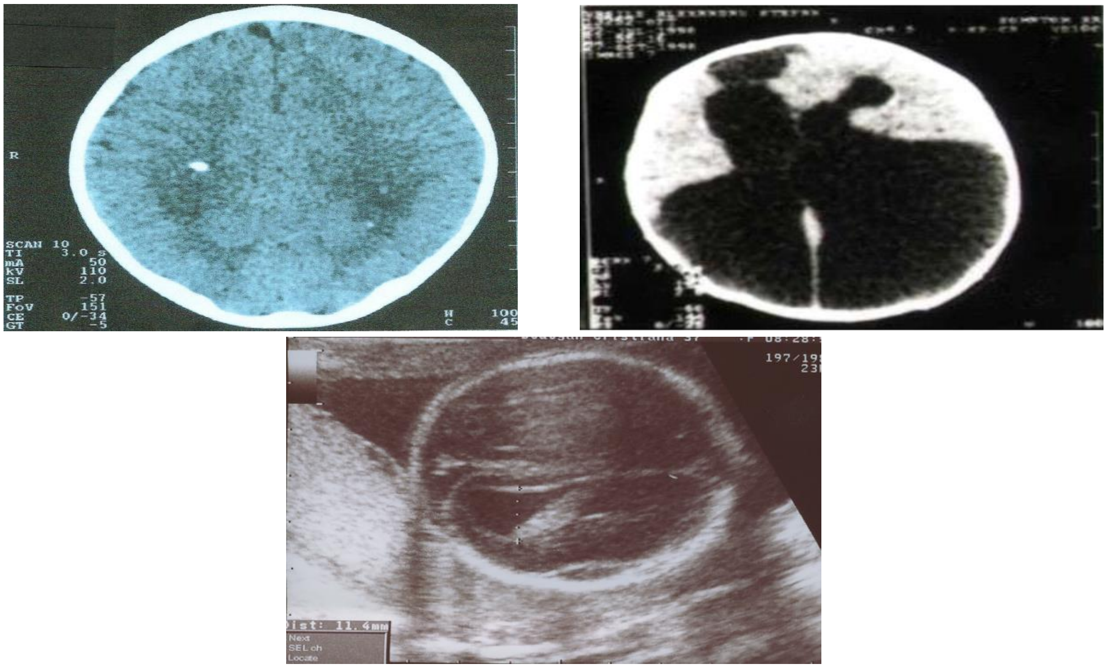

Ultrasound signs in Toxoplasma Gondii infection are generally nonspecific. The most common signs are intracerebral calcifications, intracerebral hyperecogenic seals and ventriculomegaly, usually medium or severe bilateral symmetry. The existence of brain pathological images usually means a gloomy prognosis with numerous possible impairments: corioretinitis, psychomotor retardation, blindness, epilepsy, postnatal hydrocephalus [16]. In fact, in a study of 8 confirmed children with congenital toxoplasmosis, only the absence of any pathological brain imaging or the existence of only a mild ventriculomegaly were associated with favorable postnatal prognosis [16]. Another study found that only 43% of fetuses with signs of impaired brain had a poor prognosis [17].

This study involving 32 fetuses with confirmed congenital toxoplasmosis showed that evolution intrauterine lesions is usually very fast, on the order of days [17]. However, the cranial pathological images were identified only after 21 weeks of pregnancy in all the studies quoted [16, 17]. Prognosis also appears to be more unfavorable in cases where seroconversion occurred earlier in pregnancy, or the patient did not receive treatment for this infection [16, 17].

Besides cerebral signs, fetal toxoplasmosis can be manifested along with other signs, but lacking specificity for this pathology. Thus intrahepatic calcifications, ascites, placental hyperecogenic images with thickened placenta, or rarely pleural and pericardial effusions [17, 18] can be discovered.

Restriction of intrauterine growth and microcephaly can be associated with this fetal infection, but they are not characteristic [16, 17].

Spontaneous fetal death of the fetus with the termination of pregnancy in evolution does not seem to be more common in affected fetus [17]. A slight tendency towards early childbirth appears to be influenced by obstetrical attitudes and not necessarily by fetal harm [17].

Prenatal diagnosis of this fetal infection can only be achieved by performing an amniocentesis with PCR analysis of the resulting amniotic fluid. Amniocentesis carries a risk of abortion or premature birth, a small but still present risk [15]. Patients should be informed of this risk and of the potential benefits of this diagnostic approach [15].

The main benefits of fetal infection confirmation with Toxoplasma Gondii refer to the subsequent therapeutic course in choosing the right drug combination. One problem, however, is that there are no randomized studies showing the benefit of a treatment regimen at the expense of another, most of the schemes being based on old, poor quality studies [15]. Another possible reason for carrying out amniocentesis is the exclusion of a possible fetal infection, which implies an easier to follow treatment by the patient with lower drug doses and reduced side effects. Last but not least, positive diagnosis can help the patient to make an informed decision about a possible discontinuation of pregnancy [15], knowing that a positive diagnosis accompanied by signs of fetal cerebral affection is associated with an unfavorable prognosis. On the other hand, a negative diagnosis of amniocentesis accompanied by a lack of any signs of fetal harm will limit the need for aggressive postnatal antibiotic treatment and will also have a beneficial effect on the mother's psyche.

CONCLUSION

T Gondii infection during pregnancy occurs in approximately 1-8 ‰ of the population and is usually asymptomatic. Diagnosis of this infection is done either by testing for nonspecific symptoms such as fatigue, fever, headache, malaise and myalgia, or through dedicated screening tests performed in pregnancy. There is a great variation in assessing the need for screening for this infection in the world, so only some countries offer such programs.

Diagnosis is performed by detecting specific IgM and IgG immunoglobulins in the serum, the proportion of which varies depending on the age of the infection. Generally, IgM is an acute phase control, and IgG indicates an old infection that will not cross the transplacental barrier. Testing IgG avidity against the parasite seems to help increase the sensitivity and specificity of acute infection detection.

Maternal infection with Toxoplasma Gondii during pregnancy may lead to fetal infection with this protozoan by transplacental transmission. Fetal infection does not necessarily imply an unfavorable prognosis, but the occurrence of suggestive echographic signs for this pathology is a serious factor. There are no signs of this kind of fetal infection, but the affected faces usually present some cerebral pathological echographic imaging, including cerebral calcifications, medium or severe symmetrical bilateral ventriculomegaly or hyperrecognized intracerebral seizures. There are other non-specific signs of fetal damage.

The positive diagnosis of fetal infection is done by testing amniotic fluid. This diagnosis will help in choosing the required treatment regimen, although there are no solid randomized studies indicating the use of a particular regimen in a given situation.

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ORIGINAL ARTICLE

High values of procalcitonin in non-septic patients with thermal and airway burns – an early severity predictor?

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Abstract: *Purpose – Combined airway burns with cutaneous burns is a further negative prognostic factor in burn patients. In this study we tried to identify the most relevant negative prognostic factors at admission in a relatively homogeneous population.*

Methods – In this study, ten young patients who suffered from skin and airway burns following a fire in a club, admitted to our ICU department, were included. At the intake, ABSI and APACHE II scores were calculated, and a series of biomarkers were also collected. The correlation and associations between gravity scores and biomarkers was then calculated using the Spearman correlation and Chi square tests.

Results – Based on data analysis, we found very high values of ABSI 8.9 ± 1.4 and APACHE II 25.4 ± 2.79 scores, procalcitonin (46.16 ± 68.18 ng/ml), white blood cells (41.26 ± 15.66 × 103/mm3), hematocrit (51.6 ± 7.31 %), and low corrected albumin values (22.08 ± 4.66 g/l). The statistical analysis revealed a good correlation between gravity scores and values of PCT and low albumin levels ($p< 0.05$). The mortality was also associated with the need for vasopressor support from the beginning ($p < 0.05$).

Conclusions – Adverse prognostic factors that we have identified in this group of young patients with skin burns and airway include: increased values of the severity scores and PCT, low values of corrected albumin and vasopressor support necessary at admission.

INTRODUCTION

Burns represent one of the most aggressive injuries of the human body known for high mortality, thus raising major public health issues such as increased length of hospitalization and recuperation, along with psychological and physiological traumas, all these leading to increased costs [1-4].

Burns-induced mortality rate is globally increased, and surprisingly high in the developing countries, including Europe's burn centers, where it ranges between 1.4% and 18% [5]. The high mortality is associated with a number of factors, including long-term severity of burn driven inflammatory response of the host, secondary human body frailty and abnormal response to further injuries like sepsis [6, 7]. The association of airways' and cutaneous burns induces a supplemental increase of the inflammatory response, difficult to quantify because of the inaccurate evaluation of the burns from the surface of the lungs and burn degree same time [8]. Consequently, in the case of mixed cutaneous-and-airway burns the mortality increases,

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despite the usage of advanced techniques to support lung function (Extracorporeal membrane oxygenation – ECMO) [9-12]. On the other hand, in the beginning, it is unclear whether a patient has both cutaneous and airway burns, as for the latter it might take up to 48 hours to observe any clinical manifestations. As such, the airways' burns assumption is made based on anamnesis and correlation between the high incidence of airways burns and the increased surface of cutaneous burns [11]. If one can clinically suppose an airways burn (in this case a bronchoscopy is usually performed for a certain diagnosis), there is no toll to quantify its' impact on the inflammatory response of human body. Currently, there is just an indirect estimation of their aggression on the human body using either dedicated markers or severity scores. Despite the wide availability of severity scores as Acute Physiology And Chronic Health Evaluation (APACHE II and APACHE III), Abbreviated Burn Severity Index (ABSI), and biomarkers (such as microalbuminuria, serum albumin, C-reactive protein, procalcitonin (PCT)), both used to estimate the severity of cutaneous burn injuries, they are not sufficiently standardized for the estimation of mortality risk in case of combined thermal and airway burns [13-16].

Considering the increased mortality and likeliness of combined burns complications, it is paramount to know the severity degree and the prognosis of the burn evolution from the beginning.

In the current study, we evaluated the absolute values of serological markers and severity scores upon admission to the Intensive Care Unit (ICU) in a group of young adult patients who presented both cutaneous and airway burns following the fire in a club in Bucharest, Romania and we studied their correlation with mortality.

MATERIAL AND METHOD

This study includes the victims of a fire at a club in Bucharest, in the night of 29-30 October 2015, all of them hospitalized that night in the Intensive Care Unit of the Emergency Hospital for Plastic Reconstructive Surgery and Burns, in Bucharest. All admitted patients underwent surgery (non-excisional debridement) and subsequently, they were connected to artificial breathing devices, central venous and urinary catheters being placed on them too.

Hydroelectrolytic rebalancing started from the operating room with a crystalloid solution (Ringer lactate), following the Parkland protocol (4 ml/kgc/Total Burn Surface Area-TBSA), while in patients who could not maintain a Mean Arterial Pressure (MAP) > 65 mmHg, epinephrine was administered in continuous perfusion from the beginning. Patients were admitted to ICU between 00:00 and 03:00 on 30 October, and the first set of blood tests was harvested at 6:00 on 30 October. To determine the quantitative procalcitonin (PCT) values, the testers arrived after about 48 hours from admission.

We estimated the severity of the patients' burns using ABSI and APACHE II scores.

In, order to estimate the appropriate level of serum albumin under hypovolemic conditions, we corrected it in accordance to the hematocrit (Ht) level, using the following formula:

$$corrected\ albumin\ level\ = measured\ albumin\ level\ \times \frac{normal\ Ht}{measured\ Ht}$$

where normal hematocrit (Ht) is 45% for men and 40% for women.

For the determination of the blood cells count, the Ac-T 5 diff CP (Beckman Coulter, Germany) was used. The biochemical markers were measured using the Konelab-20i/20XTI (Thermo Electron Corporation, Finland). Coagulation parameters were determined using the ACL-7000 instrument (Instruments Laboratory, Italy). PCT was assessed using the VIDAS, bio Merieux VITEK, France.

The conduct of this study was approved by the Ethics Committee of the Emergency Hospital for Plastic Reconstructive Surgery and Burns (number 5/10 July 2017).

Statistical analysis

The data is presented as the average ± standard deviation for quantitative variables, and absolute frequencies and relative frequencies, respectively for the qualitative variables. Batch variance analysis was performed with the Levene test. For the analysis of the difference between the environments the t-student test was used together with the calculation of the confidence intervals. For the correlation analysis Spearman correlation coefficient and chi-square nonparametric test were employed. In addition, for the correlation analysis we performed Percentile Bootstrap Correlation in order to calculate the Pearson parametric correlation coefficient. Confidence intervals for the value of the t test and the Pearson correlation coefficient were obtained through the bootstrap method with 10,000 and 1,000 replications, respectively. The results were considered statistically significant for a p value <0.05. For the statistical analysis IBM SPSS 18.0 and several R packages, such as wBoot, were used.

RESULTS

During the night of 29-30 October 2015, ten patients (seven male and three women) were admitted in our ICU. The deep cutaneous burn lesions (3rd degree) ranged between 5% and 50% of the body surface area and the age range of admitted patients was 18 to 40 years old. Their ABSI scores were between 7 and 11, associating a mortality between 10% and 60 - 80% and the APACHE II score ranged between 23 and 30, which is associated with a 46% to 70.3% mortality at admission. In Table 1 we report demographical data, several laboratory measures describing the admission conditions and, for some of them, the measures sampled 30 hours after admission.

Table 1. Characteristics of the patients included in the study

	Values
WBC (1000/ml)	41.26 ± 15.66
PLT (1000/ml)	142.5 ± 50
APTT (sec)	40.27 ± 10.76
INR	1.87 ± 0.45
pH	7.04 ± 0.11
Temperature (^{0}C)	35.95 ± 0.61
Albumin (g/l)	25.95 ± 4.52
Corrected Albumin (g/l)	22.08 ± 4.66
PCT (ng/dl)	46.16 ± 68.18
Creatinine (mg/dl)	1.39 ± 0.53
TGO (mg/dl)	70.8 ± 38.31
TGP (mg/dl)	54 ± 43.28
One month mortality (%)	80%
One year mortality (%)	90%
WBC (1000/ml)	41.26 ± 15.66
PLT (1000/ml)	142.5 ± 50
APTT (sec)	40.27 ± 10.76
INR	1.87 ± 0.45
pH	7.04 ± 0.11
Temperature (^{0}C)	35.95 ± 0.61
Albumin (g/l)	25.95 ± 4.52

Note: TBSA III = Total Burn Surface Area 3rd degree, WBC = white blood cells, PLT = platelets, APTT = activated partial thromboplastin time, INR = international normalized ratio, Corrected Albumin represents corrected values of albumin according to hematocrit, PCT = procalcitonin, TGO = glutamic oxaloacetic transaminase, TGP = glutamate-pyruvate transaminase.

For these variables, the values presented are mean ± standard deviation. Gender distribution and mortality after one month and one year are also provided in Table 1. Blood tests revealed: high levels of white blood cells (WBC) of 41.26 ± 15.66/mm3, elevated hematocrit (Ht) level51.6 ± 7.31%, albumin values of 25.95 ± 4.52 g/dl (uncorrected) and 22.08 ± 4.66 g/dl (corrected), creatinine level of 1.39 ± 0.53 mg/dl, glutamic oxaloacetic transaminase (TGO) values of 70.8 ± 38.31 mg/dl and glutamate-pyruvate transaminase (TGP) levels of 54 ± 43.28 mg/dl.

Forty-eight hour procalcitonin (PCT) values were extremely elevated: 49.85 ± 67.34 ng/ml (PCT was measured in eight patients). The coagulation parameters activated partial thromboplastin time (APTT), international normalized ratio (INR), and platelets (PLT) we report in Table 1 were measured at 30 hours from admission (the second set of analysis) because at the first set there were no changes (first set: APTT = 29.3 ± 3.01, INR = 1.15 ± 0.18, PLT = 306.2 ± 108.47). So we found: APTT = 40.27 ± 10.76 seconds, INR = 1.87 ± 0.45, PLT = 142.5 ± 50*103/ml. The pH values of 7.04 ± 0.11 presented in the table were obtained from central venous blood samples after the central venous catheters were placed. The temperature at admission had a mean value of 35.95 ± 0.61 Celsius degrees. Of the ten patients in ICU eight needed vasopressor support at admission and also during the next days.

Table 2 presents values of serological markers, Total Burn Surface Area 3rd degree in terms of mean ± standard deviation, and the number of patients who required vasopressor support with epinephrine (norepinephrine from the second day) from the admission, considering the total study group and the subgroup of those who did not survived.

Table 2. Values of serological markers, total burn surface area (TBSA) and norepinephrine status in the study group and subgroup

	Total	Non Survivors	p value
Albumin (g/l)	25.95 ± 4.52	24.63 ± 3.97	0.26
Corrected Albumin (g/l)	22.08 ± 4.66	21.25 ± 4.62	0.35
PCT (ng/ml)	46.16 ± 68.18	65.88 ± 71.52	0.35
Hematocrit (%)	51.6 ± 7.31	51.19 ± 5.07	0.43
WBC (1000/ml)	41.26 ± 15.66	44.00 ± 16.22	0.36
Norepinephrine	8 (10)	8	0.03
TBSA III (%)	25.7 ± 16.73	29.62 ± 16.27	0.30

Note: WBC = white blood cells, PCT = procalcitonin, Corrected Albumin represents corrected values of albumin according to hematocrit. TBSA III = Total Burn Surface Area 3rd degree. Data are presented as mean ± standard deviation.

In the case of norepinephrine requirements association chi-square test were performed.

Statistical significance is considered for a p value < 0.05. We performed parametric t-tests for the above variables in order to assess whether there are sizeable differences between the values recorded for all patients versus the values for those that died. Due to the existence of abnormal values for TBSA and PCT 48 hrs, we have eliminated TBSA values exceeding 8, and PCT 48H values under 200. Table 3 presents two versions of the t-tests: regular tests assuming unequal variances, and bootstrap tests with 10000 replications.

Table 3. T-tests and bootstrap confidence intervals

Parameter	T-test value	P-value	Bootstrap confidence interval limits			
			Lower 95%	Upper 95%	Lower 90%	Upper 90%
Corrected albumin	0.37421	0.7134	-2.90235	4.7619	-2.52655	4.1034
TBSA	-0.50211	0.6227	-17.32	9.86	-2.3798	4.0939
PCT	-0.38325	0.7093	-105.1441	8.8855	-100.0322	5.32
WCB (divided by 100)	-0.36157	0.7228	-16.404	8.845175	-13.9097	7.57075
TBSA>8	-0.28615	0.7793	-15.71875	11.5	-13.75	9.25
PCT48h <200	-0.4898	0.6367	-37.916	19.39814	-32.02486	14.458
Albumin	0.65438	0.5223	-2.195	4.57	-1.575	4.21
Hematocrit	0.16848	0.8683	-4.50495	5.85595	-3.6257	5.1709

All results show that differences between the two samples are non-significant at 90% confidence level. Further, we investigated the correlations between severity scores (ABSI and APACHE II), procalcitonin (PCT) and corrected albumin using the Spearman non-parametric (rank) correlation coefficient. Table 4 shows that these correlations are significant at 10% significance level. We pursued a more in-depth analysis of the correlations between the above variables calculating the Pearson parametric correlation coefficient using the Percentile Bootstrap Correlation Method available in the package wBoot of R. Table 5 presents the values of the correlation coefficient, the standard error as well as the 90% or 95% confidence intervals obtained after 1000 replications.

Table 4. Spearman correlations

Correlation	Correlation Coefficient	p value
ABSI - APACHE II	0.54	0.053
APACHE II - Corrected Albumin	-0.59	0.036
ABSI - PCT	0.73	0.019
APACHE II - PCT	0.73	0.019

Table 5. Pearson correlations: results from the Percentile Bootstrap Correlation Method, 1000 replications

Variables	Mean coefficient	Standard error	Confidence interval		
			Lower Limit	Upper Limit	Level
ABSI , Apache II	0.4904211	0.2582198	0.008991	0.8477	90%
ABSI , PCT	0.5594826	0.1655465	0.2769	0.8614	95%
Apache II, PCT	0.5487429	0.208531	0.1969	0.9677	95%
Apache II, Corrected Albumin	-0.5645653	0.2334818	-0.8677	-0.151	90%

Bronchoscopic aspects at 48 hours did not reveal severe changes, most of them related to mild or moderate impairment of the lungs (Grade 0-2), only one exception with severe lesions (Grade 3) being identified. Following the Berlin criteria for the classification of acute respiratory distress syndrome (ARDS), nine patients presented mild ARDS and only one moderate ARDS upon admission to ICU.

We have obtained a good correlation between the APACHE II score at admission and the procalcitonin values (at 48 hours), ABSI score and procalcitonin and also between the

APACHE II score and albumin (Table 3).

One month after admission, two of the ten patients survived and after one year, one patient survived.

DISCUSSION

Upon arrival, patients showed signs of increased burn severity including high values of PCT (at 48 hours from admission), WBC, APACHE II & ABSI scores, and Ht, as well as low levels of albumin. Eight of the ten admitted patients required vasopressor support from the beginning.

It is known that PCT, a molecule secreted by the thyroid, intestine, liver, kidney, muscle and adipocytes under the action of TNFα and IL-1β has elevated values especially in bacterial sepsis [17] and not in viral infections [18] and also has a rapid kinetic growth from the first few hours of injury to a maximum of 12 hours [19]. PCT is also increased in septic burned patients. Several studies were performed for identifying a cut-off value for sepsis in burned patients, the yielded values ranging from 0.5 ng/ml to 1.5 ng/ml [20-23]. The highest PCT values reported for non septic patients were 2.3 ± 3.7 ng/ml [20].

Nevertheless, PCT was also found to be increased in non-septic states by systemic inflammatory response syndrome-SIRS, which is logical considering that its secretion is stimulated by TNFα and IL-1β. Additionally, it has been noticed that in complex visceral liver/spleen plus thoracic trauma the PCT values go up to 9.37 ± 2.71 ng/ml [24], consequently elevated values were also found in other SIRS states [25, 26]. In burns it was found that PCT grows even in the absence of infection, so patients who associate PCT values > 2 ng/ml within the first 48 hours of admission have a mortality of 65% at 50 days [27]. According to the American Burns Association criteria (ABA) [28], we had just one patient who presented sepsis criteria in the first 48 hours, so the high PCT values observed in our patients (46.16 ± 68.18 ng/ml) were explained by an extremely aggressive inflammatory response to burn. Our opinion is that this reaction was induced not only by skin burns but also by pulmonary injury so that high PTC values on admission may also indicate an associated airway burn in patients who have such a suspicion.

Serum albumin is a marker of severity in critical patients and is associated with an increased mortality [29, 30]. Low levels of serum albumin and urinary albumin occur by increasing vascular permeability induced by pro-inflammatory mediators in SIRS or sepsis [31, 32]. Patients with burn injuries lose their albumin by increasing the capillary permeability induced by pro-inflammatory mediators and the serum albumin value correlates with the burned surface, so at a burned surface of 60%, serum albumin (at admission) is around 1.95 ± 0.02 g/l [33, 34, 35]. In a recent study, it was found that in patients with burn driven injuries an albumin value < 2 g/l is correlated with a mortality > 80% [36]. For our patients, the value of serum albumin on admission was 25.95 ± 4.52 g/dl and corrected albumin was 22.08 ± 4.66 g/dl. We consider it more useful to report corrected albumin values considering that patients have high hematocrit variations as most of them are hemoconcentrated at admission. In all of the studies we considered, the serum albumin values were presented without being adjusted to the hematocrit value.

The APACHE II score is known as a severity score that is used to estimate mortality in intensive care units [37]. Its correlation with mortality is applicable to burn patients [38, 39] and also to acute respiratory distress syndrome (ARDS) patients [40, 41]. In our group, the high APACHE II scores have been interpreted as being induced by an extreme inflammatory response probably induced by thermal and airway burns. The ABSI score is a good indicator of the severity of a thermal injury and seems to estimate quite well the burning severity in patients with airway burns [42, 43]. ABSI score has a similar prognostic value compared to APACHE II in estimating the severity of patients with both cutaneous and airway burns. ABSI and APACHE II predict a similar mortality and the correlation of their scores is at the p = 0.05 limit. In our patients, the WBC count at admission was extremely high 41.26 ± 15.66 *103/ml. These elevated values can be only partially explained by hemoconcentration because the mean value of the hematocrit was just slightly elevated. WBC count is also increased in other situations with a systemic inflammatory response but not to those values reported in our group [44, 45].

The mean serum creatinine value, at 6 hours after admission, was 1.39 mg/dl, which may be due to hypovolemia induced by renal hypoperfusion. On the other hand, elevated transaminases with predominantly TGO/TGP increased ratio may be explained by their release from the burn cutaneous area rather than by hepatic hypoperfusion. The triad: acidosis, hypothermia and coagulopathy has a poor prognosis in polytraumatized patients and in burn patients [46]. This triad is particularly presents in patients with cutaneous and airway burns. All the patients with skin and airway burns showed severe metabolic acidosis at admission. Coagulopathy did not immediately appear but emerged in the next 30 hours. Hypothermia (temperature under 35.5°C degrees) was present in just two patients at admission, and the mean value was about 35.95 Celsius degrees. The absence of hypothermia can be explained by the very short time until the patients were brought to the hospital, the fire being located in a club in Bucharest, relatively close to the hospital. Thus, out of the three parameters of the triad, in our patients, only 2 of them were present.

These changes of PCT, WBC and high APACHE II and ABSI scores can be explained by an extreme inflammatory reaction or septic shock. This inflammatory reaction could be caused by thermal cutaneous lesions and/or airway burns. Although we found a good correlation between the APACHE II score & PCT & corrected albumin, and also between ABSI score & PCT, we did not find a statistically significant correlation between the burn lesions surface or depth and the biological parameters. So we can state that the inflammatory reaction was determined by a mixed cause represented by the thermal cutaneous lesions and airway burns. For patients with airway burns, the etiology may be thermal or chemical. In most of our cases, bronchoscopy did not reveal significant structural changes in the upper airways, which led us to the idea that pulmonary injury was mixed, thermal and chemical. Although we did not obtain statistically significant changes, we found an increase in WBC, PCT and TBSA III values in the case of non-survivors compared to the mean values obtained in the entire group of patients. Yet, a statistically significant difference was obtained with regard to vasopressor support, so all eight patients requiring vasopressor support at the admission died in the first month.

The patients who survived one month from the incident, had PCT values at 48 hours of 3.14 ng/ml and 0.41 ng/ml, respectively, corrected albumin of 22.3 g/l and 28.45 g/l, respectively, while the APACHE II values were 24 and 23 respectively, and ABSI scores were 8 and 7 respectively. These were also the only patients who did not require vasopressor support at admission.

CONCLUSIONS

The association of airway burns to the cutaneous thermal lesions is linked to an increased mortality even in young patients. There is no dedicated marker or score for the assessment of the combined cutaneous and airways burns aggression on the human body but an association of markers and scores could improve the evaluation of the burn severity. The negative prognostic factors we identified in our group were high PCT values at 48 hours, low corrected albumin levels, vasopressor requirements and elevated APACHE II and ABSI scores. The WBC count and TBSA could be also considered in establishing the severity of the cases.

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ORIGINAL ARTICLES

Management of war-related vascular injuries: A civilian surgeon experience in the treatment of war casualties at a secondary care hospital

Bariş Akça[1]

Abstract: *Background: This study presents the management of patients with war-related injuries and early results of vascular injuries treated at a secondary care hospital neighboring to civil war.*

Methods: Between December 2013-December 2014 ninety-six war-related injury patients evaluated by a single vascular surgeon and participating disciplines according to components of injury in polytrauma patients. Thirty-six patients who underwent vascular surgery were retrospectively registered to the study. Demographics, treatment and early results of patients reviewed.

Results: Mean age was 26.69±11.80 (7-66) years and 91.66% (33/36) were males. Injuries were caused by shrapnel wound due to bombing 61.11% (22/36), stab wound 8.33% (3/36), gunshot wound 30.55 % (11/36). The number of patients with polytrauma was 29, seven patients had isolated vascular injury. Among 42 arterial and 31 venous injuries primary suture repair, autologous vein graft interposition and prosthetic graft interposition performed to 20.54% (15), 28.76% (21), 19.17% (14) respectively. Amputation and all-cause mortality were %8.3 (3/36), 13.8% (5/36) respectively.

Conclusions: Vascular injury with concomitant trauma is the main issue of war-related injuries. Surgeons should be cautious about patients who were initially deemed not to need surgery for vascular injury and then were found to need it. Repetitive physical examination is very important in case of any suspicion, especially in limited conditions for diagnostic tools like CT angiography. A rapid, careful multidisciplinary evaluation of experienced surgeons for the diagnosis and the treatment priorities in war-related injury patients may lead to increase life and limb salvage rates with a better quality of life.

Keywords: *vascular system injuries, war-related injuries, mortality, amputation, multiple trauma*

INTRODUCTION

Vascular injuries reported as varying rates of 2-3% of civilian trauma [1, 2], while about 7% of war-related trauma [3]. War-related vascular injuries frequently result in life-threatening hemorrhage or limb loss, and while the amputation rate was 60 % in the past, with ligation being the preferred technique for arterial injuries, today this rate has decreased to as low as 1 % through developments in early transport, damage control methods providing hemodynamic stability, diagnosis and complex treatment techniques [4, 5].

Early intervention is considered crucial for a successful outcome, although the management of vascular injuries is clinically challenging even for experienced surgeons when resources are limited, particularly in polytrauma patients, as imaging methods like computed tomography angiography (CTA) or angiography is necessary for prompt diagnosis, as well as for prompt and appropriate treatment. Ideally, war-related injuries should be treated by surgeons with military surgery experience, although civilian surgeons may find themselves in practicing military surgery without prior

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training or experience in this field [6].

As a result of ongoing civil war in Syria, an increasing number of patients with war-related polytrauma have been managed in a frontier secondary care hospital which neighboring the civil war, in Kilis, Turkey. This study presents the experiences of a single civilian surgeon with war-related injury management strategies and the early results of vascular injuries treated at a secondary care center with limited resources.

METHODS

Between December 2013 and December 2014, 96 patients with war-related injuries were evaluated for suspected vascular injury through a multidisciplinary approach involving a single vascular surgeon and other disciplines, who were involved depending on the components (cranial, chest, abdomen, bone fracture and soft tissue loss) of injury in polytrauma patients. Data of patients were recorded for retrospective analysis in real time. This retrospective study was conducted after obtaining the Ethical Committee approvals of Inonu University and where the work was carried out.

All patients were transferred to our hospital from the place where the injury occurred. In accordance with Advanced Trauma Life Support protocols and damage control principles [7, 8], tourniquets were routinely applied to all patients to achieve rapid hemorrhage control, and permissive hypotension (massive red blood cell, plasma, and minimal crystalloid infusion) was used to initiate a hemostatic resuscitation plan aimed at correcting metabolic imbalances. Subsequently, patients were evaluated for vascular and other injuries. In the absence of angiography and CTA, diagnosis of vascular injury was made almost entirely through physical examination findings, with a Doppler ultrasound (DUS) used when possible. If a patient presented with only hard signs of vascular injury, such as active pulsatile hemorrhage, absent distal arterial pulse, ischemia, expanding or pulsatile hematoma with no concomitant injury, immediately transported to the operating theater for vascular repair. After the multidisciplinary agreement about the benefit of vascular repair and concomitant surgical interventions, and an initial surgery decision according to clinical status at admission, patients with polytrauma were sent immediately to the operating theater. However, patients presenting with soft signs of vascular injury (non-pulsatile and non-expanding hematoma, palpable but diminished or unequal distal arterial pulses, bone fracture, peripheral nerve deficit and history of bleeding that subsided spontaneously) and with no immediate threat to life or limb, were preferred to be referred to a center where further investigations could be made. If there was no such opportunity, these patients were evaluated by DUS, if possible, or mostly through repetitive physical examinations, for clinical findings of ischemia, cyanosis, the absence of arterial pulse and hemorrhage. CTA or angiography was essential for diagnosis and the treatment options in 19 (19.79 %) polytrauma patients with uncertain physical examination findings, hence they referred to the relevant centers.

Among 96 patients evaluated for vascular injury, a total of 36 (37.5%) patients operated by vascular surgeon enrolled in the study. All patients received a tetanus vaccine and dual antibiotics prior to surgery. The initial surgical exploration was usually performed by the vascular surgeon, both for better bleeding control and arterial and venous repair, especially in injuries of the extremities, based on hemodynamic status and duration of ischemia. The exploration of injured vessels was performed via standard incisions if in the same course as the injury area; otherwise, proximal and distal control of the vessels was obtained directly from the injury area. In patients with extremity injuries (bone, nerve, and soft tissue damage, whether individually or together) vascular repair was performed immediately after proximal and distal control of the vessels to achieve better bleeding control and stable hemodynamic status in cases where extremity-threatening critical ischemia existed. Otherwise, vascular repairs were made after bone stabilization with external fixators.

Systemic Standard heparin was used intra-operatively, based on the level of bone and soft tissue damage/loss in non-coagulopathic patients. Thrombectomy was performed routinely with an embolectomy catheter after the routine assessment of proximal inflow and distal backflow. Also, the distal segment of the artery was cleansed with heparinized saline in patients for whom systemic standard heparin could not be used.

Vascular injuries were managed via simple repairs, end-to-end anastomoses, interpositions of the autologous saphenous vein graft or polytetrafluoroethylene (PTFE) graft and simple ligation methods. The vascular repair method was determined based on an intraoperative assessment of the injured vessels. The decision of ligation was based on life-threatening hemorrhage and distal arterial circulation with more than one artery (Ulnar/radial and one of the tibia arteries). Venous injuries of large veins, especially to the femoral, popliteal and axillary veins, were repaired via simple repair, vein graft or PTFE graft methods, when possible. Other minor venous injuries were ligated.

During the initial evaluation in the emergency room, soft tissue defects and necrotic tissues in patients with limb injuries were scrubbed with 2000–3000 cc of Ringer solution and cleansed from foreign bodies prior to the operation. Subsequently, the necrotic tissue debridement, foreign body removal and soft tissue reconstruction performed after forceful irrigation with Ringer solution in the operating theater.

Low molecular weight heparin was given to all patients postoperatively for anticoagulation, and a dual or triple antibiotic regimen was applied postoperatively, based on the contamination and severity of the injury.

Fasciotomy was performed by orthopedists intra operatively or during intensive care unit follow up, according to the follow up of patient and operative management of injury. Nerve injuries were repaired either in the same session or delayed for two to three weeks, by orthopedists or neurosurgeons.

The demographics, treatment and early results (in hospital period; up to postoperative seven days) of patients were reviewed, and the studied variables included age, gender, clinical signs and symptoms on admission, anatomical region of vascular injury, mechanism of injury, concomitant injuries in polytrauma patients, Mangled Extremity Severity Score (MESS) [9], type of vascular repair and clinical outcome.

The data were analyzed using PASW Statistics software (SPSS Inc, USA), and was presented as mean (SD), range or numbers and percentage (%), as appropriate.

RESULTS

The mean age was 26.69±11.80 (7-66) years and 33 (91.66%) were male. The mean MESS score was 8.47±2.64 (3-14), and extensive tissue defect/loss was observed in 12 (33.33%) patients. The cause of injuries were shrapnel wound due to the bombing, stab wounds and gunshot (mostly rifle) wounds in 22 (61.11%), 3 (8.33%) and 11 (30.55%) patients respectively.

The anatomical localization of injuries presented in Table 1.

Table 1. Anatomical localization and repair methods of injured 73 vessels in 36 patients

Repair method Vessel	n *(73)*	Primary repair *(15)*	Prosthetic graft *(14)*	Vein graft *(21)*	Ligation *(23)*
Carotid artery	1				1
Subclavian artery	1		1		
Brachial artery	4	1		3	
Radial artery	3			2	1
Ulnar artery	3	1			2
Common Femoral artery	5		2	3	
Superficial femoral artery	8	2	3	3	
Profunda femoral artery	3	1		1	1
Popliteal artery	5		3	2	
Anterior Tibial artery	5	1		3	1
Posterior Tibial artery	4			3	1
Internal jugular vein	1				1
Subclavian vein	1	1			
Common femoral vein	7	4		1	2
Profunda femoral vein	3	1			2
Superficial femoral vein	7	3	3		1
Popliteal vein	4		2		2
Anterior tibial vein	4				4
Posterior tibial vein	4				4
Total (%)		20.54	19.17	28.76	31.50

Of the patients, high-energy injuries were observed in 24 (66.6%), medium-energy injuries in 2 (5.55%), low energy injuries in 3 (8.33%) patients and 7 (19.44%) patients had crush injuries (rescued from beneath the wreckage of collapsed buildings after the bombing). The number of patients with polytrauma was 29 (80.55%) and concomitant injury distribution of patients presented in Table 2.

In the study, 7 (19.44%) patients had isolated vascular injury; 12 (33.33%) patients underwent vascular surgery with concomitant active bleeding; 17 (47.22%) patients presented with hypovolemic shock upon arrival at the emergency room; ischemia signs were observed in 24 (66.66%) patients, and the mean ischemia time was 5.45 ± 5.20 (1-24) h.

Table 2. Concomitant injury distribution of 36 patients enrolled in the study

	n	%
Cranial injury	3	8.3
Neck injury	1	2.7
Chest İnjury	5	13.8
Abdominal injury	7	19.4
Pelvic injury	4	11.1
Lower extremity injury	28	77.7
Upper extremity injury	11	30.5
Large tissue defect	12	33.3

Of the patients, 25 (69.44%) had single extremity injuries, 9 (25%) had double extremity injuries and one patient had three extremity injuries. In 20 (55.55%) patients, extremity injuries were accompanied by bone fractures, of which 17 (47.22%) were open fractures.

Initial physical examinations revealed motor loss in 20 (55.55%) patients. Intraoperative nerve injury was mostly determined as a full-thickness incision in 12 (33.33%) patients. Three (8.33%) patients with polytrauma had postponed surgery decision after a follow-up period for repetitive physical examinations due to uncertain initial physical examination findings.

A total of 42 arterial and 31 venous injuries were observed in the operating theater. The most common repair method was graft interposition with autologous saphenous vein and PTFE. Ligation was the second preferred method, especially for venous injuries, and 31.50% of total injuries were ligated. The details of repair methods presented in Table 1.

Postoperative complications are listed in Table 3.

Table 3. Postoperative complications of patients

	n	%
Graft Thrombosis	4	11.1
Amputation	3	8.3
Mortality	5	13.8
Reperfusion injury	1	2.7
ARDS	1	2.7
MODS	1	2.7

ARDS: Acute respiratory distress syndrome, MODS: Multiple organ dysfunction syndrome

Thrombectomy and/or embolectomy performed for all postoperative graft thrombosis patients. Three patients had autologous vein graft thrombosis and one had a prosthetic graft thrombosis. The amputation rate was 8.3% (3/36). Two (5.55%) patients had secondary amputation after vascular repair and one patient with mangled extremity underwent primary amputation via an intraoperative multidisciplinary decision in order to save life. All three patients who underwent amputation had injuries of the popliteal artery including trifurcation.

The mortality rate was 13.8% (5/36), four of them had high-energy injuries and one had medium-energy injury. Furthermore, two patients with concomitant abdomen injuries died from sepsis, one patient with concomitant cranial injury died due to intracranial hemorrhage. Two patients in hypovolemic shock with concomitant large soft tissue defect underwent surgery with preoperative early intubation and died from acute respiratory distress syndrome and multiple organ dysfunction syndrome. A fasciotomy was applied to three patients, of which two were closed and one was open.

DISCUSSION

As reported variously, the incidence of war-related vascular injuries has been increased gradually from World war II (0.2-4%) to the wars in Afghanistan and Iraq (6.8 %), also a recent study from the Lebanon war reported that the rate of vascular injuries increased to 7.6% of all casualties, and to 10.8% in the soldiers [4, 10-12]. This increase is possibly correlated with shortened evacuation time, improved immediate resuscitation, damage control by the progressive and effective use of tourniquets and temporary shunts. Furthermore, these developments over years have brought improved life and limb rescue rates in war-related injuries [13-15]. In this civilian study, however, the distance to the war zone (15 min. away by road to the hospital) was the most important factor affecting the increasing admittance of war-related trauma patients.

Recent experiences have triggered the management of such injuries, assessing especially the effect of explosives on injury patterns and treatment strategies [3]. Explosive weapons are designed to increase the number and energy of casing fragments, leading to multiple penetrating wounds [16]. In the present study, most common injuries were shrapnel wounds from bombing and rifle wounds, hence these kinds of high energy injuries includes both penetrating and blast effects on tissues as designed. Therefore, vascular injuries associated with multiple trauma often lead to high mortality rates, unless prompt and appropriate surgical management is made [17], hence war-related injury treatment should be provided by surgeons with military surgery experience [6].

Vascular injuries of extremities are the most common type of injury in war-related trauma patients [3, 12-14], as is the case in this study and it may lead to limb loss or mortality. Bleeding may lead to hypovolemic shock, as was the case with 47.2% of the patients in the present study at the admittance. Consequently, the primary aim is to avert exsanguination and death, and then to restore blood flow to prevent amputation.

The initial physical examination of patients who present with hypovolemic shock may be misleading about vascular injury due to diminished or absent distal arterial pulse and inadequate perfusion of extremities. In patients suffering from hypovolemic shock, recurrent physical examinations are important, as in patients with soft signs, and also should be carried out after appropriate resuscitation of hypovolemic shock. Three polytrauma patients of this study underwent surgery through recurrent physical examination findings.

If the initial evaluation of a patient revealed hard signs of vascular injury with a single entry and/or exit wound, and with no concomitant injury, there is consensus on the decision to make an immediate vascular exploration after resuscitation in the emergency room. However, there is a lack of consensus regarding the management of patients with only soft signs in polytrauma patients complicated with concomitant injury, multiple extremity injuries or single extremity multiple segment injuries with soft or no signs of vascular injury [18]. For this grey zone of patients with soft signs, additional diagnostic methods such as angiography or CTA are required to be applied prior to surgery in hemodynamically stable patients, both for diagnosis and choice of treatment [19-21]. Of these, angiography is the optimum diagnostic method, although CTA has gained increasing value in diagnosis and treatment planning, therefore some studies reported that CTA should be considered as the first line modality [19].

In this study, patients underwent immediate vascular surgery on the basis of physical examination findings and hard signs of arterial injury with the goal of shortening the ischemic period. As neither angiography nor CTA was present, hemodynamically stable patients with soft signs of arterial injury were referred to a center where these examinations could be made. In a very small number of patients DUS could be used. In the absence of contrast imaging, physical examination findings with hard signs of vascular injury seem to be an effective means of diagnosis, although the need for contrast imaging methods for patients with uncertain physical examination findings of vascular injury is undisputed.

It should not be forgotten that the possibility of arterial trauma still exists, particularly in the upper extremities, with palpable distal arterial pulses [18-22], also impaired perfusions with absent distal arterial pulses may not be noticed due to of collateral circulation.

In this study, ligation of vessels was considered as the final option especially in hemodynamically stable patients, although ligation is a good strategy in selected vessels that do not cause distal extremity ischemia, and in cases of life-threatening bleeding.

Similar to other studies [3, 23, 24], superficial femoral, common femoral, popliteal and brachial arteries were the most commonly injured arteries in this study. Vein injuries are mostly accompanying with the adjacent artery injuries. The most common repair method of this study was saphenous vein graft interposition, as recommended in most studies [3, 25]. Applying a prosthetic graft is still a controversial issue, increased risk of thrombosis and infection, thus poor outcome with a high secondary amputation rate [17, 23, 26]. In this study, contrast with the previous studies the absence of early infection despite the high ratio use of prosthetic grafts may be attributed to the factors like; a large amount of wound irrigation as an initial response in the emergency department and also in the operating room, careful and aggressive debridement of necrotic and contaminated tissues, additionally use of dual or triple antibiotic regimen for the follow up. In contrast with expectations, the number of autologous vein graft thrombosis was higher than prosthetic grafts. Revascularization of these polytrauma patients was carried out initially, followed by bone and soft tissue operations. Therefore, thrombosis developed due to inadequate length (short/long) of the graft. No temporary vascular shunting was used in this study.

The amputation ratio relies on many variables, including the severity of limb injury, mechanism of the injury, ischemia time, existence of accompanying injuries, and disaster conditions when dealing with mass injuries [13]. In this study, three patients with popliteal artery injuries underwent amputation, one pediatric patient who had an ischemia time of 2h with MESS score of 9 underwent primary amputation by an intraoperative multidisciplinary decision in order to save life. The ischemia times of these three patients were found to be 2h, 4h, and 8h. The MESS scores of patients who underwent amputation were 9, 10 and 11 while the mean MESS score of this study was 8.47±2.64. During the treatment of mixed skeletal, vascular and soft tissue injuries, limb ischemia specifies the treatment primacy, and also high limb loss rates related with popliteal artery involvement [19]. The amputation rate of this study is close to previous studies reported in varying rates (3-20%) [27, 28]. Mortality rate (13.8%) of this study was higher than the varying rates (1.5% - 6%) have been reported in previous studies [28, 29].

Retrospective design of the study, short follow up period of patients and relatively small number of patients are the basic limitations of this study.

CONCLUSIONS

When dealing with war-related injuries, especially in polytrauma patients, occult vascular injuries have been possibly missed due to uncertain initial physical examination findings of vascular injury and frequently presents later. Therefore, the definite diagnosis of vascular injury should be revealed by angiography or CTA. Following the diagnosis, some critical decisions arising such as: which patient should be operated first, among who applied at the same time and requiring emergency surgical intervention; which one of the injury components should be operated initially in patients with polytrauma; which type of injuries of polytrauma patients should be operated by two different disciplines at the same time, in the same session; and also which type of injury can be delayed. These are challenging situations for even most experienced surgeons in patients with complex polytrauma accompanying with systemic effects. Therefore, a multidisciplinary approach should be used when establishing treatment priorities.

Civilian surgeons unexpectedly finding themselves acting war surgery with no background, hence may tend to make the same traditional mistakes that military surgeons have already learned from previous wars, nevertheless a rapid, careful multidisciplinary evaluation of experienced surgeons for the diagnosis and treatment priorities in war-related injury patients may lead to increase life and limb salvage rates.

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ORIGINAL ARTICLES

3D echo in everyday life: Could it reset our threshold for interventions?

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Abstract: *Background Left ventricular ejection fraction (LVEF), the single most important metric in cardiology, is the cornerstone on which prognosis is estimated and costly decisions such as whether to implant an ICD are based. LVEF is most often assessed by 2D echocardiography (2DE), although 3D echocardiography (3DE) has been shown repeatedly to be more accurate.*

Aim of the study. We set out to assess whether using 3DE would reclassify the severity of LV impairment in patients with LVEF < 35% by 2DE.

Setting. Tertiary cardiac centre serving a population of one million, and performing approximately 100 ICD implants/year.

Methods. Successive patients in sinus rhythm, with good endocardial border definition and LVEF<35% by 2D Simpson's method, had scans according to the BSE protocol. 3D loops were acquired from the apical 4-chamber view and were analysed off-line using for regional wall motion abnormalities (RWMAs) and LVEF. The patients were classified in subgroups according to EF value: less than 20%, 20-25%, 25-30%, 35% and more than 35%. Moderate LV systolic was defined as LVEF between 35% and 45% and severe as LVEF ≤35%.

Results. We studied 100 patients (78 M, mean age (SD) 69.94 (13.54) years). 2DE had been requested for decision-making regarding ICD implantation in 86 patients (86%) and for LVEF measurement after acute coronary syndromes in 14 patients (14%). Regional wall motion abnormalities (RWMAs) were present in half of patients (55%, 51 pts – 56% in LAD territory, 31% in RCA/CX territory and 12% multiple territories).

3D LVEF regrouped 67% of patients: 10% to a lower EF and 57% to a higher EF subgroup. Twenty nine patients (29%) were reclassified from severe LV systolic dysfunction by to 2DE to moderate LV systolic dysfunction by 3DE. Patients with RWMAs were more often reclassified than patients without RWMAs (p=0.006). The LV dimensions were lower for the reclassified patients. The image quality had no effect on reclassification.

Conclusion Measuring LVEF by 3DE reclassifies the severity of LV systolic impairment in a substantial proportion of patients with 2D LVEF<35% and RWMAs, which may have important clinical and financial implications by resetting thresholds for costly interventions such as ICD implants.

Keywords: *biological attack, biological warfare, biological agents, international legislation, medical protection*

BACKGROUND

Left ventricular ejection fraction (LVEF), the single most important metric in cardiology, is the cornerstone on which prognosis is estimated and costly decisions (defibrillator implantation, resynchronisation therapy) [1] are based.

The clinical importance of echocardiography in ejection fraction measurement is undoubtedly, left ventricle systolic function evaluation being the most common reason for referring a patient for an echocardiogram [2]. In our department, from 117 patients scanned during one week, 60% were referred for LV systolic function evaluation. The main advantages of echocardiography comparing to more

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accurate methods (like MRI or CT (3)) are availability, inexpensive character, rapidity of performance, portability and radiation freedom.

Two-dimensional echocardiography (2DE) remains the most commonly used modality for EF estimation in clinical practice. The current guidelines recommend Simpson's discs method for LVEF calculation. However this method is subject to errors due to foreshortening and assumptions about LV shape (stacked disks with varying diameters).

Three-dimensional echocardiography (3DE) overcomes some of this limitation by capturing entire volumes, excluding foreshortening and geometrical assumption (very important in deformed ventricle). In addition, the endocardial position is measured at hundreds of points over the LV surface comparing to only two planes in conventional 2DE. Therefore, the calculation of LV volume is more accurate and reproducible.

OBJECTIVE

The primary aim of this study is to evaluate if LV EF assessment by 3DE in routine clinical practice can change the clinical decision making for patients with LV systolic dysfunction and if routine 3DE is useful and worthy versus largely used 2DE.

METHODS

We prospectively analyzed successive patients in sinus rhythm with good endocardial border referred to our echo department for left ventricle systolic function evaluation. The study was developed in a tertiary cardiac centre serving a population of one million, and performing approximately 100 ICD implants/year.

All patients had transthoracic 2D echocardiography obtained with harmonic imaging (Philips iE33, Philips Medical System, Holland). Left ventricle end-diastolic volume (LVEDV), left ventricle end-systolic volume (LVESV) and LVEF was measured on-line using Simpson's method of disks (using four and two chamber apical views). Patients with LVEF ≤35% were included in further analyse. The value of 35% was arbitrary selected because it is the cut-off limit for important and expensive treatments: ICD implant or cardiac resynchronisation therapy (1). LV systolic dysfunction was defined as severe for LVEF <35% and as moderate for LVEF 35-45%.

3D echocardiography was performed in the same time, using a matrix array ultrasonic transducer (X3-1 transducer, Philips iE33, Philips Medical System, Holland). An apical full volume image focused on LV, over four cardiac cycles was acquired. Measurement of 3DE LV volumes and ejection fraction was performed offline, using the 'mesh method' (Q- Lab 9, Philips Medical Systems) blinded to the 2D measurements. The semi-automated LV border detection was used for contour tracing, after first identifying the apex and the four mitral annular points (septal, lateral, anterior and inferior) (Figure 1A). A 3D endocardial shell was than produced and used for LV volumes and LVEF calculation (Figure 1B).

Figure 1. Full volume 3DE, 4 beats image of the left ventricle in a four chamber equivalent (apical probe position) and the corresponding end-diastolic four chamber (), two chamber () and short axis () view (A). The resulting end-diastolic volume (mesh), end-systolic volume (shell) and calculated 3DE after sequences analysis (B).

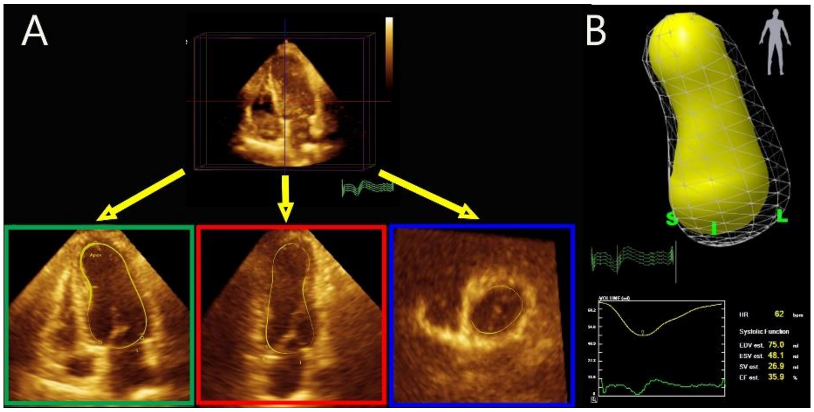

Statistical analysis: Agreement between 2DE and 3DE measurements was assessed by performing Bland-Altman analisys (MedCalc Software, version 12. 7.0, Acacialaan 22, B-8400 Ostend, Belgium). Means and standard deviations (SD) were calculated for continuous variables. Comparisons were performed using χ2 test for categorical data and Independent/Paired-Samples T test for continuous data with a two-tailed p value < 0.05 for statistical significance (IBM SPSS Statistics v.21, Armonk, NY).

RESULTS

During the enrollment period 106 patients were eligible. 6 patients were excluded due to inadequate quality of 3D acquisition (4 patients presented stitch artifacts and 2 patients had severely dilated LV, impossible to be included in 3D volume). The analyzed group was composed by 100 patients, 78 male, mean age (SD) 66.94 (13.54) years.

Transthoracic echocardiography had been requested for decision-making regarding ICD implantation in 86 patients (86%) and for LVEF measurement after acute coronary syndromes in 14 patients (14%). Regional wall motion abnormalities (RWMAs) were present in half of the cases: 51 patients (51%). The affected territory was corresponding to anterior descending artery in 29 patients (56%), to right coronary artery/circumflex artery in 16 patients (31%) ant to multiple vessels in 6 patients (12%). The QRS complex was narrow in 52% of patients and large in 48 patients (75% presented bundle branch block and 25% paced rhythm). Thirty two patients (32%) had left ventricle dilated (LVED volume >155ml).

Comparing 2DE and 3DE data

The mean value (SD) for LVEF calculated by 2DE (Simpson's method) was 27.36 (5.8%) and for LVEF calculated by 3DE was 30.53 (6.9%), p<0.001. The correlation between the two methods was good (Spearman's coefficient 0.79). On Bland-Altman analysis the bias was -3.2% and the agreement interval -4.1, -2.4 (Figure 2).

Figure 2. Bland-Altman plot representing the agreement between two methods for EF estimation: 2DE and 3DE.

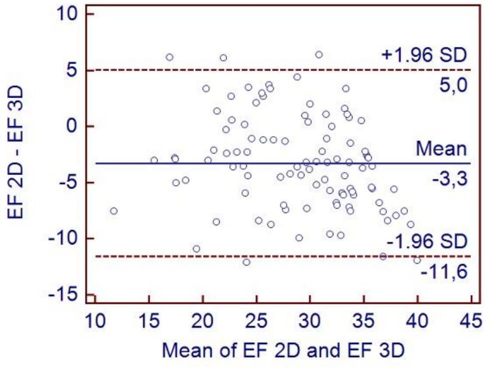

The mean value (SD) for LVEDV was 144.37 (69.2) ml by 2DE and 143.6 (67.2) ml by 3DE, p=0.8, Spearman's coefficient 0.86. For LVESV the mean value (SD) was 106.26 (59.53) ml by 2DE, 103.24 (55.02) ml by 3DE, p=0.051, Spearman's coefficient 0.85 (Figure 3).

Figure 3. Graphic representation of LVEDV and LVEDS distribution by 2DE vs 3DE.

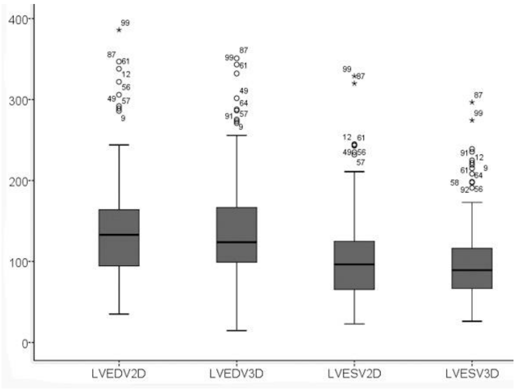

Reclassification by 3DE analysis

According to EF value, the patients were classified in four subgroups: <20%, ≥20% and <25%, ≥25% and <30%, ≥30% and ≤35%. Only for EF calculated by 3DE there was a new subgroup formed by patients with EF >35%. The 2DE and 3DE LVEF value for all patients is presented in Figure 4. The distribution of patients among subgroups comparing 2D to 3DE is illustrated in Figure 5. Considering the patients classification in these subgroups, 67% were reclassified by 3DE comparing to 2DE: 57% in a group with higher EF and 10% in a group with lower EF.

Twenty-nine patients (29%) were reclassified by 3DE from severe LV systolic dysfunction (EF ≤35%) to moderate LV systolic dysfunction (EF>35%). Eight patients reclassified from severe to moderate LV systolic dysfunction had 2DE EF 25-30% and 21 had EF 30-35% (Figure 4).

Figure 4. Graphic representation of 2DE EF (blue dots) vs 3DE EF (green dots) for all analyzed patients, grouped by 2DE in four subgroups (as previously mentioned).

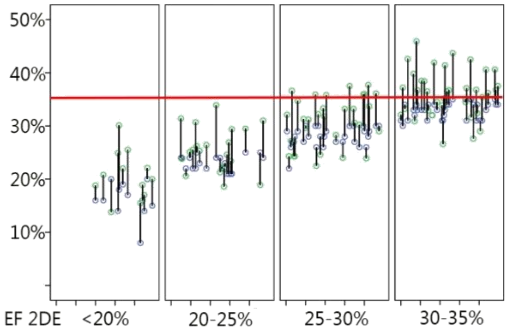

The difference between LVEF evaluated by 2DE and 3DE was more than 5% in 40 patients (40%). The mean (SD) difference EF was 3.2 (4.2) %. The difference between EF by 3DE and by 2DE was not significantly different among the 2DE defined four subgroups (mean value was 5.73% for EF <20%, 4.04% for EF 20-25%, 4.3% for EF 25-30% and 4.53% for EF 30-35%, p =0.4).

Characteristics of reclassified patients

a) Patients regrouped among subgroups by 3DE

The ejection fraction was not different between patients who were regrouped among the previously described subgroups and patients who remained in the same subgroup after 3DE. The end-diastolic and end-systolic LV volume was significantly lower for patients who were regrouped. The RWMAs and the QRS width did not influenced significantly the regrouping among subgroups (Table 1).

Figure 5. Graphic representation of number of patients from each subgroup by 2DE and 3DE.

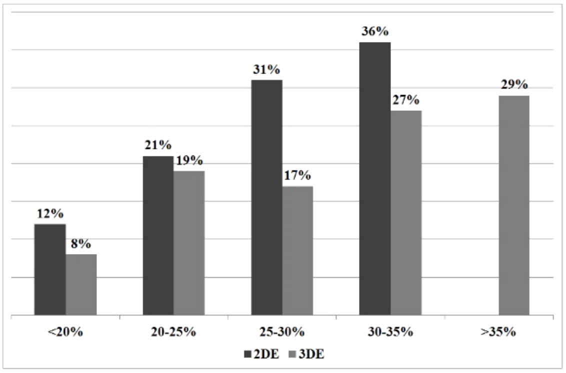

Table 1.

	Regrouped patients (subgroups)	Non-regrouped patients (subgroups)	p value
EF (SD)%	28.03 (5.09)	25.69 (7.0)	0.006
LVEDV (SD) ml	133.56 (57.58)	166.30 (85.22)	0.026
LVESV (SD) ml	97.25 (47.17)	127.60 (75.77)	0.016
RWMA Yes	72.5%	27.5%	0.02
RWMA No	61.2%	38.8%	
QRS narrow	65.4%	34.6%	0.7
QRS large	68.8%	31.3%	

Table 2.

	Reclassified patients (LVEF by 3DE>35%)	Non-reclassified patients (LVEF by 3DE≤35%)	p value
LVEDV (SD) ml	106.27 (37.39)	159.93 (73.34)	0.000
Dilated LV (LVEDV>155ml)	6.3%	93.8%	0.001
Normal LV(LVEDV <155ml)	39.7%	60.3%	
LVESV (SD) ml	71.14 (26.43)	122.02 (63.00)	0.000
Image quality average	30%	70%	0.82
Image quality good	28%	72%	
RWMA Yes	41.2%	58.8%	0.006
RWMA No	16.3%	83.7%	
QRS narrow	40.4%	59.6%	0.009
QRS large	16.7%	83.3%	

b) Patients reclassified from severe to moderate LV systolic dysfunction by 3DE

The end-diastolic and end-systolic LV volume was significantly lower in patients with severe LV systolic dysfunction who were reclassified as moderate systolic dysfunction after 3DE. The reclassified patients had more often regional wall motion abnormalities comparing to non-reclassified patients (Table 2). The number of reclassified patients was 16.3% form subjects with no regional wall motion abnormality, 48.3% for subjects with regional wall abnormality in LAD territory, 37.5% for RCA/Cx territory and 16.7% for multiple territory (p=0.018).

DISCUSSIONS

Can we trust 3DE? What do we know from cardiac magnetic resonance comparing studies?

A large number of studies during the last years have shown that 3DE is superior to 2DE for left ventricle quantification (LV volumes and EF) comparing to MRI. The superiority of the method is related to a better accuracy and a better reproducibility. The accuracy and the repeatability of 3DE are comparable with cardiac MRI for LV volumes and EF for unselected patients [4] or for patients with regional wall motion abnormalities [5, 6]. In a follow up study of patients with previous myocardial infarction the 3DE LV parameters changes were better correlated with MRI results comparing to 2DE [7]. Moreover, the 3DE estimation of LV volumes and EF was superior to 2DE measurement in predicting major cardiovascular events [8]. A recent meta-analysis showed that in studies comparing 2DE, 3DE and MRI LV measurements the 2DE/MRI pooled Bland Altman biases +/-SD were -48.2+/-55.9ml, -27.7+/-45.7ml and 0.1+/-13.9% for LVEDV, LVESV and EF. The 3DE/MRI pooled Bland Altman biases +/-SD were -15.7+/-31ml, -9.6+/-25.8ml and 0.0+/-9.2% for LVEDV, LVESV and EF. The differences in biases was not statistically significant for EF (p=0.42) [9]. However, most of the previous studies included only patients with good quality of echo pictures. In our study, we included all patients for which the EF calculation by Simpson's method was feasible, including patients with average quality of echo images.

Is 3DE evaluation a feasible method in clinical practice?

The feasibility of 3DE for LV parameters analysis was 89% in a large study, including unselected normal subjects [10]. In routine clinical practice 3DE was technically feasible in 83% of unselected patients [11]. The percentage of patients properly examined by 2DE that could not be analyzed by 3DE was not reported previously [12]. In our study, from 106 patients with adequate echo images quality for Simpson's evaluation, 6 patients (0.056%) could not be analyzed by 3DE.

Do we really need 3DE in clinical practice?

In nowadays clinical cardiology, when expensive decision makings are relied on ejection fraction value, its accurate determination becomes mandatory. The recent studies showed repeatedly that 3DE is superior to 2DE for LV quantification. However the EF estimation 2DE vs 3DE were not significantly different comparing to MRI in some studies [9]. Therefore the advantage of 3DE on 2DE in clinical echocardiography is uncertain.

Our data showed that routine 3DE use for LVEF estimation in every day practice can change the decision making in a significant number of patients. Twenty nine patients (29%) were reclassified from EF≤35% by 2DE to EF>35% by 3DE. Therefore, the clinical management of these patients (regarding ICD implant or CRT) should be reanalyzed in the view of the new data obtained by 3DE. The patients with 2DE severe LV systolic dysfunction and nondilated LV and RWMAs were often reclassified to 3DE moderate LV systolic dysfunction.

LIMITATIONS

The main limitation of the study is the lack of 'gold standard' measure. However, the superiority of 3DE over 2DE for LV assessment was previously demonstrated and the purpose of our study was to evaluate the possible clinical impact of 3DE in clinical practice.

CONCLUSIONS

Measuring LVEF by 3D echo reclassifies the severity of LV systolic impairment in a substantial proportion of patients with 2D LVEF<35% and RWMAs, which may have important clinical and financial implications by resetting thresholds for costly interventions such as ICD implants.

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ORIGINAL ARTICLES

Aptamer as a proper alternative instead of monoclonal antibody in diagnosis and neutralization of menacing biological agents

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Abstract: *Of the major threats to contemporary mankind, is the use of very dangerous and lethal biological agents as the biological weapons. The first step in confronting with this serious threat after prevention, is the accurate and rapid detection of this agents and neutralization of them. In this article, the role of molecules known as aptamer, has been studied in biological defense against these menacing biological agents. Traditional methods for detection of these agents are based primarily on immuno-affinity assays and the use of antibody molecules. While the modern methods, based on aptamer-affinity assays, are being replaced with traditional methods, due to the abundant advantages of them. The selection and preparation method of specific aptamer with high binding affinity to these biological agents is known as SELEX and the use of magnetic nanoparticles to perform this procedure (Mag-SELEX) is very common. The isolated aptamers with high specificity can also be used in neutralization and inhibition of menacing agents function, in addition to, quick and accurate diagnosis of these agents, utilizing them in nano-biosensors, based on aptamers (as the nano-aptasensors).*

Keywords: *Aptamer, SELEX, nano aptasensor, diagnosis and neutralization, menacing biological agents*

INTRODUCTION

Nowadays virulent and dangerous biological agents which can be used as biological weapons are becoming a considerable threat to us [1]. Some of very important agents used in bioterrorism are namely botulinum neurotoxins which are some of the strongest toxins known to men and produced by the anaerobic bacteria called clostridium botulinum [2]. Botulinum neurotoxins, especially three types of A, B and E, have been known as dangerous biologic weapons. Among these three serotypes, serotype A is more potentially virulent [3]. The first action against these very dangerous biological agents, after prevention is to identify and detect the agent accurately and rapidly and then to neutralize it [4]. Modern methods of identification and neutralization is based on molecules called aptamer [5]. In this article we will first introduce aptamer and chemical modifications possible to make on this molecule to approach certain purposes, and compare them to antibody molecules as a conventional tool in diagnoses and neutralization of the biological and viral agents or their exclusive products (such as toxins and etc.) and we will interpret concisely the works done around the world.

APTAMER, AS A NEW MOLECULAR DEVICE

Aptamer is generated from the Latin word of “Aptus” which means “to fit”. In fact, aptamers are oligopeptides or single stranded oligonucleotides (DNA or RNA) with high affinity for binding to target molecule (Figure 1). They create a bond with high specificity through their third structure to a target molecule [6]. In this article we discuss about oligonucleotide aptamers, especially DNA aptamers. By development of

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molecular sciences and setting up the SELEX technique, the specific DNA/RNA aptamers have been separated for binding to many molecules. These molecules are consisted of nucleic acids, amino acids, sugars and their polymers, and also mineral compounds, enzymes, growth factors, antibodies, the molecules binding to cells, chitins and even viral components [7].

Figure 1. A schematic representation of the binding of aptamer molecule to the target protein

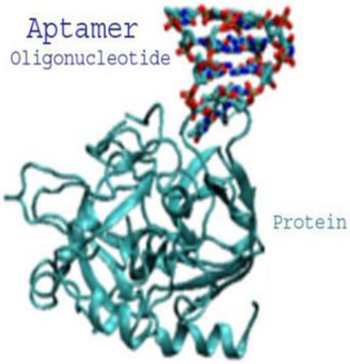

Natural aptamers in living systems

Naturally, aptamer is found in bacteria, archaea bacteria, fungi and plants, and acts as a part of riboswitch structures and often plays a role in regulation of expression of the genes involved in production of vitamins (co-enzymes) and in some cases is efficient in regulation of amino acids' production [8].

Evolution of aptamers in non-living systems

In 1990, Gold and Tuerk attained RNA fragments which were able to bind to T4 bacteriophage DNA polymerase. These oligonucleotide fragments were selected by a new combinatory technique called SELEX (systematic evolution of ligands by exponential enrichment). The meaning of this expression is that among a collection of aptamers with various sequences, it is possible to attain the specific sequence with high binding affinity to target molecule through some screening steps [9].

Aptamer library (AptLib)

The initial library for performing the SELEX process includes single stranded DNA (or RNA) oligonucleotides with a central core (approximately 40 bases) containing random and variable sequences (more than 1,015 different fragments), in which every oligonucleotide is consisted of certain flanking sites in both ends that are fixed positions for primer attachment [10]. In the middle of these constant regions (i.e. the central core), sequences of 15 to 75 (e.g. 40) nucleotides are randomly situated and every four base (A, G, C, T) may be situated in that position with the same probability [11]. These fragments are prepared by frequent synthetizations, with mixture of four types of nucleotides, coincidentally and by synthetization machine (will extendedly be discussed in results and discussion section) [12].

Aptamer library screening by SELEX cycles

In this stage, fragments with the highest affinity and specificity for target protein is selected from the aptamer library, through several SELEX cycles (usually 13-15 cycles). Repetition of these cycles is required for attaining the suitable affinity [13].

Using magnetic nano-particles for screening by SELEX cycles (Mag-SELEX)

Some of Mag-SELEX benefits include:
1) No requirement for special facilities or equipment,
2) Easiness of gathering and separation of aptamers by SELEX process only using a magnet or a simple centrifugation,
3) Possibility of using nano particles for two purposes: stabilization of target protein and also making aptamers single stranded in each cycle,
4) Possibility of performing the methods such as flow cytometry for evaluation of affinity,
5) Needing to little amount of protein in this technique (around 100 micrograms),
6) Possibility of coating nano particles with streptavidin for very strong interaction with biotin and thus prevention of wastage of stabilized protein during the washing process in which the conditions becomes tougher by rising cycle number of SELEX,
7) Expansion of protein contact surface with existent aptamers in the solution and rise in SELEX efficiency and abate of errors,
8) Easiness of stabilizing protein on these Nano particles.

It is worth mentioning that the main procedure of SELEX in different methods such as cell SELEX, CE SELEX, microfluidic SELEX (M-SELEX) and etc. are the same [14-16].

Corrections and chemical rearrangements of aptamer molecules

For increasing the stability and resistance of these molecules in clinical use, labeling or increasing the performance of these molecules, some modifications are made on these aptamer oligonucleotides.

The most important modifications include:

1) Corrections on carbon 2' of sugar including: a) Adding fluorine and amino group to pyrimidine bases. b) Adding hydroxyl group to purine bases. c) Adding o-methyl group to purine and pyrimidine bases. In clinical applications, 2'-O-

methyl oligomers are in priority, for they are less toxic than other corrections on carbon 2’ (these structures exist naturally in ribosomal RNAs) [17].

2) Capping of 3’-terminus of oligonucleotide including: a) Attachment of inverted thymidine (3’-idT) to 3’-end of oligonucleotide and creation of 3’-3’ connection. b) Attachment of biotin to the 3’-end [18]. 3) Capping of 5’-terminus of oligonucleotide including: a) Adding alkyl-amine group to 5’-end phosphate. b) Attachment of 5’-end of oligonucleotide to polyethylene glycol or on surface of liposome. c) Adding cholesterol, fatty acids and protein [19]. 4) Correction and chemical rearrangement of phosphate group which means replacement of sulfur group instead of the oxygen that doesn’t attend in phosphodiester bond (thio-aptamer). 5) Adding signal peptides to aptamers such as penetrating peptides including Tat and antennapedia and fusogenic peptides [20]. 6) Labeling aptamers with fluorescent molecules (for example derivatives of fluorescein such as FITC, FAM and etc.) for flow cytometry or with biotin molecules for stabilizing aptamer on solid surface coated with streptavidin (for performing ELISA or in biosensors design) [21].

It's rarely seen that the corrections and chemical rearrangements made for resistance improvement are effective on the main features, such as binding affinity and third structure. That's while in performance most of these modifications are possible in primary library before SELEX [22].

Comparison of aptamers with antibody molecules

1) Antibodies’ half-life is limited, while aptamers are very stable despite of the chemical rearrangements of their nucleotides. 2) Antibodies unlike aptamers are sensitive to environmental conditions such as temperature, pH and etc. 3) Antibodies have irreversible denaturation, that's while aptamers have denaturation and renaturation capacity because of their nucleic acid structure. 4) Antibodies unlike aptamers are immunogenic and this causes their rapid elimination in the body in clinical applications. 5) Size of antibodies unlike aptamers is massive (around 150 KDa) which causes long-term pause of them in blood and slow penetration in tissues and as a result, causes bone marrow toxicity in radioactive form [23]. 6) The procedure of antibody production is very difficult and for aptamers it is only difficult in the first time, during the panning steps of SELEX and then by sequencing of the aptamer selected from library, it is possible to synthesize the sequence attained easily. 7) Possibility of using aptamers in the chips that are based on DNA, so that in protein staining process aptamers do not get color. 8) Impossibility of antibody production for little non-immunogenic molecules; and possibility of aptamer production regarding to these molecules. 9) Factors like pH variation, salt concentration, heat, and chelating agents may help the process of aptamer reactivation and creation of functional structures in them. But for antibodies these agents would cause denaturation of them. 10) Stabilization of aptamers and labeling them are easier than antibodies.

These prominences have caused them to be widely used as molecular detectors in biosensors based on aptamer molecules (apta-sensors) and nanobiosensors based on aptamer molecules (nano-apta-sensors) [24].

AN OVERVIEW OF THE RESEARCH WORKS DONE UNTIL NOW, IN RELATION TO APTAMERS AND THEIR APPLICATIONS

Selection of aptamers for binding to antibodies, proteins and growth factors

- In 1996, Wiegand et al. succeeded in isolating aptamers) single stranded DNA and also RNA with 2’-amino chemical arrangement) against human IgE to inhibit the interaction of this molecule with “FcεRI” receptor on surface of basophilic leukemia cells of rat (as a new drug for the treatment of allergic diseases) [25].

- In 1995, Doudna et al. succeeded in selecting a RNA aptamer to detect the main epitope of insulin receptor antigen on surface of human cells [26].

- In 1995, Geling et al. selected RNA aptamers (with 2’-amino chemical arrangement) to inhibit the activity of fibroblast growth factor [27].

- In 2002, Lupold et al. isolated the RNA aptamer detecting prostate specific membrane antigen (PSMA) on surface of prostate cancer cells and labeled that aptamer by fluorescent molecules to observe its ligand-binding function in condition of “in vivo” [28].

Disruption of translation pathway signs of lymphocytes by Aptamar

- In 1997, Kubic et al. separated the binding and inhibiting RNA aptamer against the interferon gamma (INF-γ) which caused the inhibition of MHCI and ICAMI expression induction by INF-γ [29].

Selection of anti-viral aptamers

Some of these aptamers resulted in the elimination of virus cell cycle by interaction with the proteins necessary for virus proliferation and some of them were able to block surface binding proteins and consequently resulted in the prevention of attachment and entry of viruses into cells [30].

- In 1995, Pan et al. separated the detecting and neutralizing RNA aptamer against Rose Sarcoma virus to inhibit the binding of the virus [31].

Selection of specific aptamers for binding and inhibiting the function of enzymes

- In 1998, Brindonau et al. succeeded in isolating 2'-amino RNA aptamer by SELEX (as a new drug) for inhibition of phospholipase A2 enzymatic activity in patients [32].

- In 2011, Tzu-Wang Chang et al. succeeded in isolating the RNA aptamer specified for inhibition of enzymatic activity of botulinum neurotoxin type A [33].

Selection of aptamers for detection of small molecules

- In 1998 and some years later, one DNA aptamer for Sulforhodamine B, two RNA aptamers for sulforhodamine B and fluorescein and one RNA aptamer for malachite green were separated by Wilson and Szostak [34].

Aptamers for cell diagnosis

- In 1999, Bruno et al. detected Bacillus anthracis spore by a single stranded DNA (ssDNA) aptamer using magnetic beads [35].

- In 2005, Qin Pan et al. isolated the RNA aptamer detecting the strains of Solmonella enterica serovar Typhi containing type IVb pili [36].

- In 2007, Fan Chen et al. isolated the ssDNA aptamer detecting a virulent strain of Mycobacterium tuberclosis called H37Rv [37].

- In 2006, Vivekananda et al. isolated 25 sequences of the ssDNA aptamer identifying surface bacterial antigens of Francisella tularensis japonica [38].

- In 2007, Ikanovic et al. designed an optical biosensor based on aptamer for detection of Bacillus thuringiensis bacteria [39].

Aptamers as tools for drug delivery

- In 2006, Farrokhzad et al. prepared nano-capsules of poly lactic acid (PLA) containing drug and linked to them the special aptamer related to prostate cancer cells through the poly-ethylene glycol linkage (PEG) [40].

Using aptamers for designing biosensors (apta-sensors) and nano-biosensors (nano-aptasensors)

- In 2011, Lakshmi et al. designed a chemical-resistant nano-aptasensor, for detection of anthrax PA toxin based on single wall carbon nanotubes (SWNT) [41].

- In 2012, Jeffery Degrasse et al. isolated a selective DNA aptamer for detection of Staphylococcus aureus enterotoxin B (SEB) for designing nano-aptasensor on the basis of surface plasmon resonance (SPR) (Figure 2 – as seen in the figure, biotinylated apatamers have been stabilized on streptavidin-coated gold nanoparticles, and the above nanoparticles are also fixed onto gold discs and by use of corresponding device, signals from surface plasmon resonance (SPR) changes are measured after detecting the target ligand by aptamer.) [42].

Figure 2. A schematic representation of aptamer molecule application in the design of nano-aptasensor.

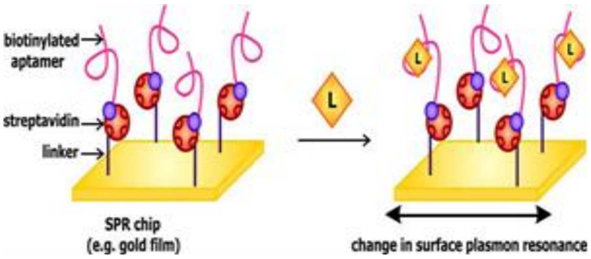
biotinylated aptamer
streptavidin
linker
L
SPR chip (e.g. gold film)
change in surface plasmon resonance

APTAMER AS A PROPER TOOL IN MODERN METHODS OF DIAGNOSIS

Nowadays traditional methods based on immune-affinity assays, in which antibody molecules are used, while transposing with new methods based on aptamers-affinity assays [43].

Functions and features of modern determination methods based on immune-affinity assays:

1. Aptamers are produced in vitro conditions and they do not require any living organisms, to which they allow us to use any molecules regardless of it being toxic for the organism and separate exclusive aptamers for determination.

2. After separation and selection of exclusive aptamers by SELEX, we could easily reproduce and mass-produce it in high purity of specified aptamers.

3. Mass production expenses are very low and they have proved to be cost effective.

4. Nucleic acids (including DNA-aptamers) by having some functional groups can more easily be adapted to their target exclusive ligand. Without any malfunctions or lessening in tendency for binding with exclusive ligand in the specified domain.

5. Aptamers are originally stable in a broader domain of conditions.

6. Aptamers have very high tendency to synthesize and dedicate compared to antibodies.

7. Aptamers with high affinity can be used for determination and detection of different sorts of ligands such as toxins, allergens, sugars (that are weak immunogens), haptens, nucleic acids, proteins and different kinds of little biological and non-biological molecules like morphine and etc. can be separated and used. It is while in previous methods there were many limitations regarding to this matter [44].

USING APTAMERS MOLECULES IN DETECTION AND NEUTRALIZATION (INHIBITION) OF BOTULINUM NEUROTOXINS

As latterly mentioned, until today there have been many researches around the globe on these oligonucleotides with detection and neutralization purposes specifically on botulinum neurotoxins (such as type A).

Reports have indicated that aptamers molecules separated from library are suitable and proper for this matter [45].

Diagnostic purposes

In diagnosis of botulism agent as the one of biologic threat factors, we can separate some aptamers with high binding tendency with SELEX method. Aptamers separated to interact with botulinum toxin and also with different neurotoxin regions including catalytic domain can be very effective in diagnosis purposes. But catalytic domains are more efficient and useful in diagnosis purposes for neurotoxin types specially type E [46].

Inhibitory goals

In inhibiting and neutralizing this extremely lethal factor we can get our hands on molecules with inhibiting and neutralizing effects on operational sites, by investigating inhibiting effects of separated aptamers for exclusive connections with high tendency to catalytic site [47].

These aptamers might hinder the activation of this operational site by connecting to de-activated catalytic site of the toxin’s body or by binding themselves to the freed catalytic site and cause the enzyme activity to stop [48]. In fact, inhibiting this position’s enzymatic function leads to restraining the toxin’s function. But antidotal aptamers can do the neutralizing process by connecting to other sites like connector sites and actually prevent the toxin from connecting to the cell and entering it [49].

Separating the aptamer against the catalytic domain of botulinum neurotoxin for recognizing and counteracting this toxin

Choosing the catalytic site from other operational sites of botulinum neurotoxin is important according to reasons mentioned below:

1. Results gained from conducted studies around the world show that this operational site is very suitable for getting the recognizing and counteracting goals.

- In 2011, Tzuu-Wang Chang et al. separated RNA-aptamer exclusively for inhibiting botulinum neurotoxin type A enzymatic activity [50].

- In 2009, Fang Wei et al. used separated aptamer for recognizing botulinum neurotoxin type A in designing electrochemical sensors [51].

- In 2009, Xinhul Lou et al. separated the special conjugative aptamers to catalytic domain of recombinant botulinum neurotoxin type A for recognizing this neurotoxin [52].

2. The main and operational part of this neurotoxin is the catalytic part and the quality of this neurotoxin is basically related to this part, in a way that said toxin will have no effect or special toxic function without this part [53]. So in conclusion the diagnosis and neutralizing value of this location will be much higher than other ones. And recognizing this location will lead to the certain recognition of infection to this type of neurotoxin, whereas recognizing other locations won’t necessarily mean that and it’s possible that other locations be used as recombinant with other factors [54].

3. The unique structure of botulinum neurotoxin type E and the importance of epitopes situated on this location and symmetric bond to the connector site, in a more general sense epitope makes the selection of this site for the toxin for this serotype exclusively more suitable (Figure 3 – the red represents the catalytic region, the green color indicates the translocating region and the yellow color indicates the binding region of the neurotoxin. As can be seen, both the catalytic and binding regions are located on one side of the translocating region, not on two sides as seen in the other types.) [55].

RESULTS AND DISCUSSION

Studies have shown that aptamers with central core of 75-100 base length are not suitable aptamers and on the other hand aptamers with the central core of lesser than 15-20 base also cannot be suitable aptamers [56]. If the length of aptamers fragments is longer than the above:

Figure 3. A schematic representation of second structures of neurotoxin botulinum type E and a symmetric relationship between catalytic and binding domains of toxin.

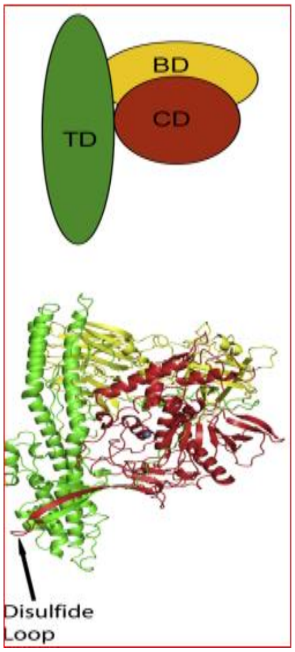

1. The possibility of creating similar sequences with primer in the central core is increased and with binding primer to this central parts and amplification, no particular fragments with shorter length will be the outcome.

2. On the other hand the possibility of connecting flanking sites of 2 sides (primer binding sites) with central sequences of various fragments, is increased and will cause an unhindered length increase and generation of fragments with larger sizes and PCR product of these two processes will be seen as smear.

3. Also the larger of these fragments be their particular operationally will also be decreased. Because after the formation of the 3rd structure there will be more binding sites to bind to target protein that each of which will have different affinity and can bind to our protein from different sites and positions and one of these position might have a high infinity to our target protein but no other position. So in conclusion the average affinity will drop and this means the decrement of specificity (affinity and specificity are two completely separate parameters) [57-59].

If the length of aptamers fragments is shorter than the above:

1. We'll witness a significant decrease in genetically variety of aptamer fragments and this will significantly limit our screening stages.

2. On the other hand the 3rd structures and conformation of aptamer which is the basis of aptamers binding to biomolecules will not form properly or won't form at all and this means the inefficiency of aptamer.

3. Another problem is the drawback in performing PCR and evaluating the target binding which distinguishing it from dimer primer is not easily achievable.

4. On the other side we'll face up to a huge problem in the purification of a production with such dimensions. Studies have shown that lengths about 40 base will be adequate and as we advance to larger or smaller numbers we will encounter the problems mentioned above more frequently although we are free to choose between 15-75 (20-100 according to some sources) (Figure 4) [60-62].

Figure 4. A schematic form of the single-stranded oligonucleotide structure of aptamer with a central core of 40 nucleotides. Scientifically and statistically, there is the probability of having a variety of 440 aptamer fragments (which is 1024 fragments) with a central core of 40 nucleotides, but optimistically virtually this variation is about 1015 aptamer fragments.

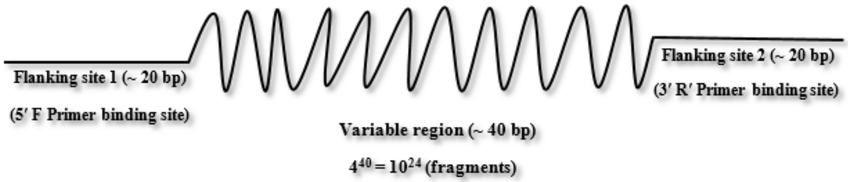
Flanking site 1 (~ 20 bp)
(5′ F Primer binding site)
Variable region (~ 40 bp)
$4^{40} = 10^{24}$ (fragments)
Flanking site 2 (~ 20 bp)
(3′ R′ Primer binding site)

In Figure 5 the schematic form of SELEX cycle on RNA aptamer library is shown. The primary library made of DNA is firstly turned into a RNA library during a transcription process and after being added to nano particles covered with target proteins and performing the stages of washing and reunifying by performing the process of "asymmetric RT-PCR", is transformed to c-DNA and after single threading and removing the non-original strand the next cycle is again continued by transacting on the product mentioned above and creation of a RNA aptamer library [63-65]. It is necessary to perform a stage of negative selection in the presence of similar proteins.

Figure 6 shows the increment of aptamers' affinity as with the progress of SELEX stages, which is evaluable using the

ELASA method (aptamer-based ELISA). Then we can perform a software based test on aptameric sequences using M-fold software.

Figure 5. A schematic representation of SELEX cycle on RNA aptamer library

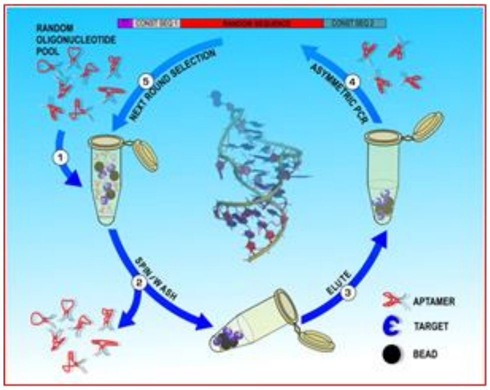
CONST SEQ 1
RANDOM SEQUENCE
CONST SEQ 2
RANDOM OLIGONUCLEOTIDE POOL
1
2 SPIN/WASH
3 ELUTE
4 ASYMMETRIC PCR
5 NEXT ROUND SELECTION
APTAMER
TARGET
BEAD

This investigation will give us the chance to understand how nucleotide changes in the final aptameric sequences causes a change in operational structure [66]. On the other hand, the software itself will predict different kinds of possible operational structures for that aptamer molecule and it will also suggest the best possible form. Sometimes the result of such investigations will show us the existence of a necessary operational site in the central core, which will have a key role in binding to the target protein [67].

Figure 6. Binding affinity of aptamers increases with the progress of the SELEX steps

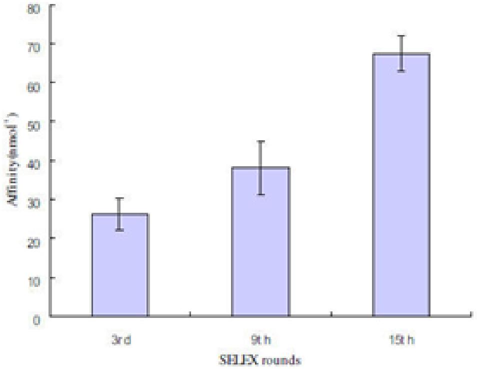

In a more accurate investigation for getting aptamers with higher affinities and higher specificity for binding to target protein we can put this specified operational site in the central core of a new aptamer library and perform the SELEX stages on that library again (Figure 7a and 7b) [68].

Figure 7. A schematic picture of two types of aptamers. 7a: An aptamer library with a central core containing a fixed region that is obtained by software evaluating by the M-Fold software. 7b: A simple aptamer library with a completely variable core

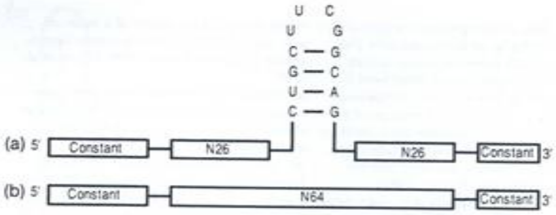
U C
U G
C — G
G — C
U — A
C — G
(a) 5'
Constant
N26
N26
Constant
3'
(b) 5'
Constant
N64
Constant
3'

In Figure 8 the pattern of aptamer nucleotides labeled with digoxigenin (DIG) recognizing the target molecule is shown, so in part A the flow cytometry is used for identifying the interaction between aptamer and target molecule and in part B the chemiluminescence is used. Part C shows the chemical formula of digoxigenin [69].

Figure 8. A) Digoxigenin labeled aptamer identifying the target molecule and measuring the binding by flow cytometry B) Digoxigenin labeled aptamer identifying the target molecule and measuring the binding with the ELISA method C) The chemical formula of digoxigenin.

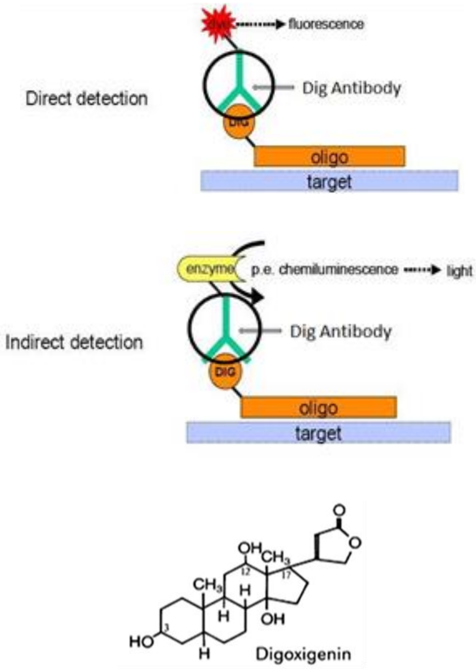
fluorescence
Direct detection
Dig Antibody
DIG
oligo
target
enzyme
p.e. chemiluminescence
light
Indirect detection
Dig Antibody
DIG
oligo
target
O
O
OH
CH3
12
17
CH3
H
H
OH
HO
3
H
Digoxigenin

In Figure 9 an image of the function of flow cytometer machine is shown. In a way that the sample containing the aptamers marked with fluorescent (marked aptamers bound to nanoparticles) is passed through a fluorescent sensor and after radiating laser light on that the number of marked nanoparticles are counted [70].

Figure 9. Flow cytometer device operation

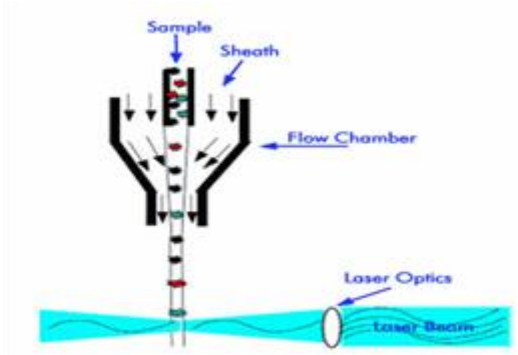

CONCLUSION

The most recent discoveries related to aptamer molecule shows the existence of particular properties and unique attributes in this biomolecule for the means of getting to the considered goal and the beneficial role of this molecule as a powerful tool for recognizing and neutralizing (inhibiting) the threatening factors shows great promise. These days the traditional methods for recognizing the biological factors based on immuno-affinity assays in which antibody molecules are used are being replaced with the new methods based on aptamer-affinity assays. The selected aptamers during the different stages of SELEX, with the ability of creating exclusive bonds and high tendency to interact with operational sites of biologic factors, in addition to having precise and fast recognition of this factors e.g. utilizing them in biological nano-sensors based on aptamer (nano-aptasensor), they could also be used in neutralizing and inhibiting the function of these threatening biological agents.

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ORIGINAL ARTICLES

Alteration of levels of thyroid hormones in acute ischemic stroke and its correlation with severity and functional outcome

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Abstract: *Introduction – With increasing life expectancy and changing trends in diseases, lifestyle diseases come forward and become a major concern. Ischemic stroke is an emerging public health issue leading to significant morbidity and mortality. To improvise positive outcome in acute ischemic stroke (AIS) and optimizing healthcare resources, accurate and timed prognostication of functional outcome is important. Various studies implied derangement of Hypothalamo-pituitary-thyroid (HPT) axis in AIS, thereby advocates neuroprotective properties to these hormones. This is, still, an open field for active research. Thyroid hormones can, therefore, be used as a surrogate tool for outcome predilection in AIS. This prospective observational cohort study aimed to investigate the alteration of levels of thyroid hormones in AIS and simultaneously correlate their levels in assessment of clinical severity and predicting the functional outcome.*

Materials and methods – We had collected data from 60 patients of, radiologically confirmed new cases of AIS who sought medical help within 72 hours of initiation of stroke symptom and had no previous history of thyroid disease.

Blood samples for thyroid function test [TSH, FreeT3 (FT3), and FreeT4 (FT4)] were collected within 24 hours after admission and subsequently at 7th and 30th day of discharge .On admission neurological clinical severity was assessed by using National Institute of Health Stroke Scale (NIHSS). Functional outcome was assessed by Modified Rankin Scale (mRS) at 7th and 30th day of discharge.

Result – Out of 60 AIS patients, 40 were having Non thyroidal illness Syndrome (NTIS). Low T3 was most common NTIS observed. Negative correlation was observed between FT3 levels and on admission NIHSS score and mRS score on follow up.

Conclusion – This study thereby concludes that low T3 levels were associated with clinical severity and poor functional outcome in AIS.

Keywords: *stroke, thyroid profile, correlation, NIHSS, mRS, NTIS*

INTRODUCTION

Stroke, described by the WHO, as the clinical syndrome of rapid onset of focal (or global, in SAH) neurological deficit, persisting more than 24 hrs or leading to death with no apparent cause other than a vascular one. [1]

It is an emerging health problem all over the world. Worldwide every year, 15 million people suffer a stroke. It is the fourth most common cause of death behind the disease of heart, cancer and chronic lower respiratory disease [2]. Globally, stroke is the second leading cause of death above the age of 60 years [3].

After dementia, CVA is the second chief cause of disability. Disability comprise of deprivation of speech and /or vision, confusion and paralysis [3].

Burden of stroke is increasing, which can be, due to

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increasing longevity, smoking, changing habits &lifestyles and type A personality. Beside these, other known risk factors include hypertension, obesity, diabetes mellitus and metabolic syndrome.

In comparison to developed countries, where the incidence of stroke has been decreased or reached a plateau, burden of stroke has been increasing in developing countries [3, 4, 5]. India, like other developing countries, is in the midst of epidemic of stroke.

In rural areas, the estimated adjusted stroke prevalence rate range 84-262/ 100,000 and 334-424/100,000 in urban areas. The incidence rate is 119-145/100,000 based on recent population based studies. [6] In India, nearly one fifth of patients with first ever stroke admitted to hospital is estimated to be of age 40 years or less [7]. Globally, a third of all strokes occur in 20-64 year age group [8].

Stroke can be classified into ischemic and hemorrhagic type. The Indian Collaborative Acute Stroke Study (ICASS) reported that up to 80% stroke were of the ischemic type [9]. The ICMR has advocated tobacco use, diabetes mellitus, hypertension and low concentration of hemoglobin as main risk factors for ischemic CVA [10].

Review of the literature reveals a significant derangement in thyroid hormones in patients with critical illness, specifically during the acute phase [11]. Euthyroid Sick Syndrome or Non Thyroidal Illness Syndrome (NTIS) is a condition in which derangement of thyroid hormones occurs in association with severity of disease and stress like surgeries [12] The most common pattern found under NTIS is low T3 level with normal level of T4 and TSH [13,14].

In acute ischemic stroke (AIS), HPT axis are significantly deranged. Various studies advocate low T3 levels as an indicator of poor prognosis in AIS [15, 16]. A reduction of serum T3 without elevation of TSH is associated with severity and worse clinical outcome in stroke [15, 17, 18]. Whether the prognosis is direr in AIS patients than those with the same disease but without the low T3 syndrome remain unknown. In AIS, implication of thyroid hormones is hence, controversial.

Low T4 syndrome, a condition found in severe illness, in which T3 and T4 levels are reduced while TSH level does not show expected pituitary thyroid axis reactivity [13, 14]

After acute stroke, better survivals were reported even when the cut-off point used was the median T3 levels [15], showing that lower T3, even it is in the normal range, may be associated with poorer prognosis. However, the association was not confirmed by other studies [19-21]. Therefore, it is not clear if, in ischemic CVA, lower T3 levels, especially within the normal range, on hospital admission provide worthy prognosticative information. A better understanding of this issue can be used for risk stratification in AIS and hence better healthcare resource utilization.

The basic aim of our study was to find out whether there occurs any alteration in thyroid hormone levels in AIS and also to correlate their levels in assessment of stroke severity and functional outcome as evaluated by using NIHSS and modified Rankin scale (mRS) respectively.

MATERIALS AND METHODS

This study was performed at the Department of Medicine at PGIMER (Post Graduate Institute of Medical Education and Research), Dr RML Hospital, New Delhi from November 2016 to March 2018. It was a prospective observational study in which a cohort of 60 patients of AIS was admitted and followed up at 7th and 30th day after discharge.

Inclusion criteria

Any patient of age 18 years or older & with no previous history of thyroid hormone abnormality, with first ever radiologically confirmed ischemic CVA presented within 72 hours of onset of stroke symptoms.

Exclusion criteria

1. Patients with known thyroid disease or biochemically defined overt thyroid disease,
2. Patients with transient ischemic attack, or past history of stroke,
3. Those using medications that can affect thyroid functions,
4. Those with cancer, severe renal or liver failure, inflammatory or infectious diseases and hematological diseases.

The enrolment of patients was done after informed consent. All the issues, including ethical issues of the study had been reviewed by the Institutional Review Board and approved.

The patient was first evaluated clinically which include a detailed history and examination.

Baseline characteristics, include demographic data such as age, gender, and concurrent diseases, thyroid disorder related medication use, risk factors of stroke, such as a history of diabetes mellitus, coronary artery disease hypertension, smoking and TIA.

Acute ischemic CVA was confirmed by using radiological modality NCCT head /CECT head/MRI brain was used.

On admission, stroke severity was assessed by using NIHS scale and was divided into 3 categories:-

- Mild NIHSS score <8
- Moderate NIHSS score 8-14
- Severe NIHSS score ≥ 14

Blood samples for assessment of the thyroid profile was drawn within the first 24hrs of presentation and then subsequently during follow up at 7th day and 30th day after discharge.

FT3, FT4 and TSH was measured by using chemiluminescent micro particle immunoassay.

Normal range in our laboratory is

- TSH 0.5-5.0 U/ml
- Free T3 (FT3) 2.0-4.4 pg/dl
- Free T4 (FT4) 0.7-2.0 ng/dl

On the basis of thyroid profile, the patients were divided into five categories:

1. NTIS was diagnosed as a low FT3, low or normal FT4, and low or normal TSH. Low T3 syndrome was defined as low FT3 levels with normal TSH and FT4 levels. [19, 22, 23, 28]

2. Euthyroid was diagnosed as normal FT3, FT4, and TSH. [19, 22, 23]

3. Subclinical hypothyroid was diagnosed as normal FT3, normal FT4, and high TSH. [24, 25, 26, 27, 29]

4. Hyperthyroid was diagnosed as low TSH with or without high FT4 and FT3 [22, 30, 31]

5. Hypothyroid was diagnosed as high TSH with low FT4 and normal FT3. [23, 30, 31]

Functional outcome was evaluated by using the Modified Rankin Scale (mRS) at the first follow up clinic visit at 7th day and subsequently 30th day after discharge. The mRS [32, 33, 34] is a scale to determine the disability after stroke. It has a score of 0-6, with 0 referring to no limitations or symptoms, 5 being severe disability requiring constant care and 6 being dead

On the basis of this scale, patients were divided into 2 outcome groups:

- Poor functional outcome (mRS > 2)
- Good functional outcome (mRS ≤ 2)

Data was analysed by using SPSS version 21.0 Qualitative & categorical or nominal variables were expressed as numbers (percent) or proportion. Quantitative & continuous or ordinal variables were expressed as medians or mean ± standard deviation (SD) Association and comparison of two categorical variables was done by using Chi square. The association between thyroid hormone levels and stroke severity and functional outcome was evaluated by Pearson correlation. The chi square and independent sample t test were used to compare respective categorical and continuous variables between different outcome groups. Variables with significant difference between different outcome groups were included in multiple logistic regression analysis. The criterion of statistical significance was set at p value < 0.05.

RESULTS

The mean age of the study population was 63.75 ±11.755 SD years with maximum age reported was 87 years and the minimum age was 38 years (Figure 1). Out of 60 study subjects 14 were female and 46 were male (Figure 2), suggesting ischemic stroke was more frequent in male (76.7%) as compared to female (23.3%) and male to female ratio was 3.28:1.

Figure 1.

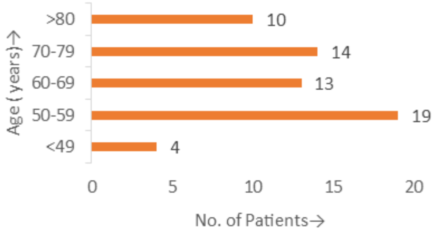

Figure 2.

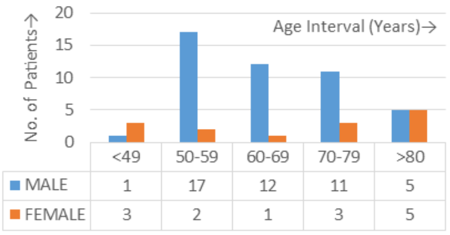

	<49	50-59	60-69	70-79	>80
■ MALE	1	17	12	11	5
■ FEMALE	3	2	1	3	5

The incidence of stroke increases with presence of risk factors. Common vascular risk factors, as shown in Table 1, included in the study were hypertension (n=40, 66.7%), diabetes (n=23, 38.3%), smoking (n=38, 63.3%) and dyslipidaemia (n=17, 28.3%).

Figure 3.

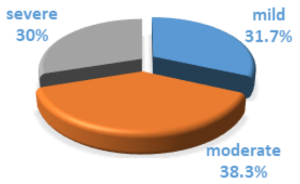

At admission, 19 patients (31.7%) had mild, 23 (38.3%) had moderate, whereas 18 (30%) had a severe stroke on NIHS scale (Figure 3). There was no statistical significant difference found between NIHSS score and age, gender, vascular risk factors for stroke studied (p value >0.05).

Table 1: Distribution of risk factors according to age group

Age (year)		<49	50-59	60-69	70-79	>80	Total	p-Value
Hypertension (HTN)	HTN	3	11	10	8	8	40	>0.05
	non HTN	1	8	3	6	3	20	
Diabetes (DM)	Diabetes	1	9	5	4	4	23	>0.05
	non DM	3	10	8	10	6	37	
Smoker	Smoker	1	12	8	11	6	38	>0.05
	non	3	7	5	3	4	22	
Dyslipidemia	Dyslipidemia	1	2	5	5	4	17	>0.05
	non	3	17	8	9	6	43	

Figure 4: Comparison between thyroid patterns observed

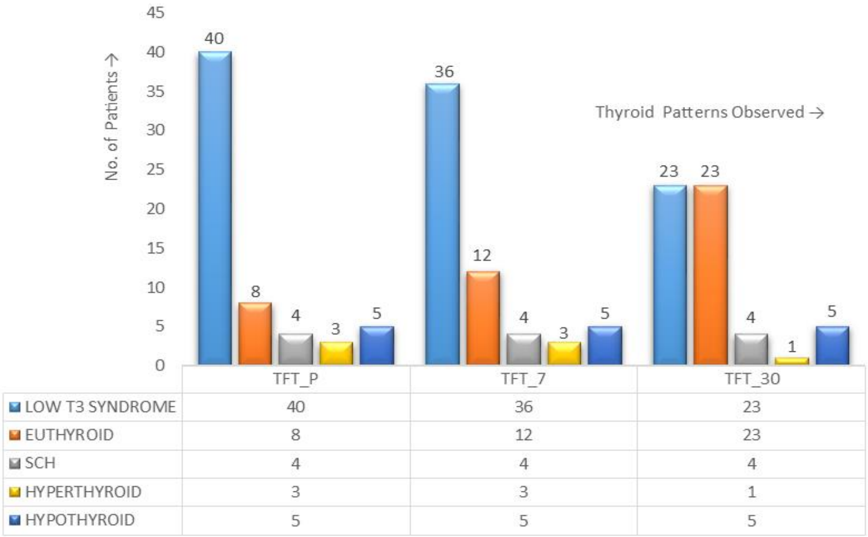

	TFT_P	TFT_7	TFT_30
LOW T3 SYNDROME	40	36	23
EUTHYROID	8	12	23
SCH	4	4	4
HYPERTHYROID	3	3	1
HYPOTHYROID	5	5	5

Figure 5: NIHSS score and thyroid dysfunction on admission

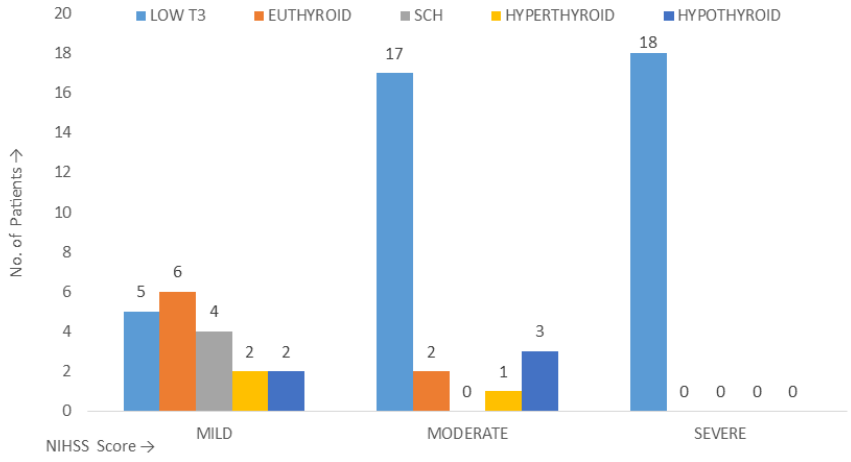

Table 2: Characteristics of AIS with low T3 & normal T3 values

	low T3 group (n=40)	normal T3 group (n=20)	p value
Clinical variables			
Age (years)	65.75	65.50	0.819
Sex (% male)	31 (77.5%)	15 (75%)	0.829
Hypertension	27 (67.5%)	13 (65%)	0.846
Diabetes	16 (40%)	7 (35%)	0.707
Smoking	25 (62.5%)	13 (65%)	0.850
Dyslipidemia	8 (20%)	9 (45%)	0.064
NIHSS (median)	11	6	0.000
Baseline NIHSS score	12.35±4.5	7.15±2.3	0.000
Distribution of NIHSS scores			
Mild	5 (12.5%)	14 (70%)	0.000
Moderate	17 (42.5%)	6 (30%)	0.000
Severe	18 (45%)	0	0.000
Thyroid function test (on admission)			
TSH	2.40±1.12	3.81±2.87	0.05
FT3	1.28±0.50	2.37±1.0	0.05
FT4	1.60±0.64	1.57±0.77	0.05
mRS_7 (median)	3	2	0.000
Pt with mRS_7 <2	8 (20%)	16 (80%)	0.000
mRS_30 (median)	3	2	0.000
Pt with mRS_ 30 <2	13	18	0.000

Thyroid pattern observed in this study (Figure 4) was low T3 syndrome (n=40, 66.7%), euthyroid (n=8, 13.3%), subclinical hypothyroid (SCH, n=4, 6.7%), hyperthyroid (n=3, 5%) & hypothyroid (n=5, 8.3%) during admission.

A statistically significant (r=0.436, p value <0.05, Figure 5) direct relationship found between NIHSS score and the number of patients with the low T3 syndrome.

Figure 6. Scatter plot graph between FT3 values and NIHSS scores on admission. Each circle represents a patient

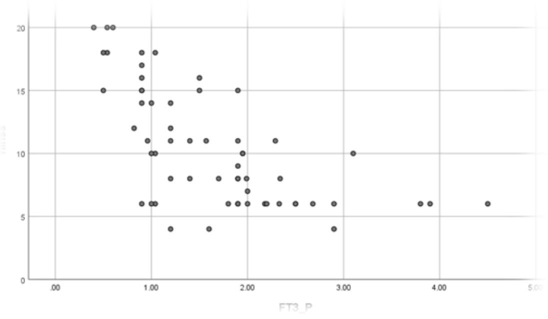

Table 3. Correlation between FT3 & NIHSS scores on admission

		NIHSS	FT3_P
NIHSS	Pearson Correlation	1	-0.712**
	Sig. (2-tailed)		0.000
	N	60	60
FT3_P	Pearson Correlation	-0.712**	1
	Sig. (2-tailed)	0.000	
	N	60	60

*** Correlation is significant at the 0.01 level (2-tailed).*
FT3_P: free T3 levels on presentation /admission

In order to study the impact of T3 levels of severity & outcome of stroke in our study, we divided cohort of 60 patients into two groups – low T3 and normal T3 group and also analyze the distribution of risk factors among them (Table 2). No significant difference observed between age, gender & vascular risk factors between these two groups.

There were significant negative correlations between free T3 (FT3) levels and NIHSS scores on admission (n=60, r= -0.712,

$r2=0.507$, $p=0.000$, 99%CI, Figure 6, Table 3) among all patients. This implies, after acute stroke, that the lower FT3 values are, the worse neurological impairment will be.

NIHSS Score was negatively correlated with TSH on admission as well as 7th and 30th day of discharge (Table 4 – correlation is significant at the 0.01 level 2-tailed). NIHSS Scores were shown a negative correlation with FT4 values only on follow up. Although the strength of the correlation was not as stronger as it was with FT3 levels.

Table 4: Associations between thyroid hormones and NIHSS score on admission and on 7th and 30th day of discharge

	NIHSS	Pearson Correlation (r)	r^2	Sig (2 –tailed)
TSH_P	-0.227	-0.227	0.051	0.041
FT3_P	-0.712	-0.712	0.507	0.000
FT4_P	-0.043	-0.043	0.002	0.747
TFT_P	-0.402	-0.402	0.162	0.001
TSH_7	-0.259	-0.259	0.101	0.044
FT3_7	-0.753	-0.753	0.567	0.000
FT4_7	-0.264	-0.264	0.070	0.042
TFT_7	-0.455	-0.455	0.207	0.000
TSH_30	-0.343	-0.343	0.117	0.007
FT3_30	-0.787	-0.787	0.619	0.000
FT4_30	-0.365	-0.365	0.133	0.004
TFT-30	-0.479	-0.479	0.229	0.000

ASSESSMENT OF FUNCTIONAL OUTCOME

Final outcomes were evaluated at 7th and 30th day of discharge and the mRS score so obtained was compared with on admission NIHSS Score as well as thyroid hormone levels measured concurrently.

Out of 60 patients, 36 patients (60%) had a poor functional outcome (mRS>2) at 7th day of discharge in comparison to 29 patients (48.3%) who still had a poor functional outcome at 30th day of discharge.

No mortality either in hospital or hospice was observed on first following up clinic visit. However, on second follow up clinic visit, 4 patients, among those with poor functional outcome had mRS Score 6 (dead) constituting 13.7 % of patients with mRS score>2. Retrospective analysis of these patients revealed that they had a severe stroke on NIHS scale. Analysis of thyroid hormone pattern on admission and at 7th day of discharge were suggesting that they belong to the low T3 syndrome. They had very low levels of free T3. TSH and free T4 levels were also low. These mortalities thereby emphasize severe derangement of hypothalamo-pituitary-thyroid axis.

Univariate analysis showed that mRS scores at first and second follow up visit, were not associated with age, gender and vascular risk factors like hypertension, smoking, diabetes & dyslipidaemia (p>0.05, Table 5).

Table 5: Demographic and vascular risk factors according to outcome at 7th & 30th day of discharge

Modified Rankin Scale (mRS)		Age (years)	Sex (male %)	Smoking (n, %)	HTN (n, %)	Diabetes (n, %)	Dyslipidemia (n, %)
TSH_P FT3_P FT4_P	mRS≤2 (n=24)	64±11.4	19 (79.2%)	20 (83.3%)	13 (54.1%)	07 (29.2%)	09 (37.5%)
	mRS>2 (n=36)	63.6±12.1	27 (75.0%)	18 (50.0%)	27 (75.0%)	16 (44.4%)	08 (22.2%)
	p value	> 0.05 (NS)					
TFT_P TSH_7 TFT-30	mRS≤2 (n=31)	61.4±12.6	23 (74.2%)	22 (70.9%)	19 (61.2%)	09 (29.0%)	10 (32.3%)
	mRS>2 (n=29)	66.2±10.3	23 (79.3%)	16 (55.2%)	21 (72.4%)	14 (48.3%)	07 (24.1%)
	p value	> 0.05 (NS)					

Table 6: Admission clinical and lab findings according to outcome at 7th & 30th day of discharge

	Modified Rankin scale at 7th day		**Modified Rankin scale at 30th day**		**P value**
	mRS_7≤2 (n=24)	mRS_7>2 (n=36)	mRS_30≤2 (n=31)	mRS_30>2 (n=29)	
Baseline NIHSS (median)	6	13	6	15	<0.05
Baseline NIHSS (mean±SD)	6.83±2.26	13.14±3.92	7.23±2.14	14.24±3.56	<0.05
Distribution of NIHSS score					
Mild (n)	17	2	19	0	<0.05
Moderate (n)	6	17	12	11	<0.05
Severe (n)	1	17	0	18	<0.05
Thyroid hormone levels at admission					
TSH	3.15±2.23	2.69±1.81	3.27±2.29	2.45±1.28	<0.05
FT3	2.16±0.83	1.30±0.72	2.08±0.87	1.18±0.59	<0.05
FT4	1.60±0.61	1.50±0.73	1.57±0.73	1.62±0.64	<0.05
Thyroid hormone levels at 7th day of discharge					
TSH	3.09±2.21	2.62±1.59	3.19±2.29	2.40±1.16	<0.05
FT3	2.20±0.84	1.33±.73	2.13±0.86	1.20±0.61	<0.05
FT4	1.56±0.65	1.47±0.64	1.60±0.72	1.42±0.45	<0.05
Thyroid hormone levels at 30th day of discharge					
TSH	3.17±2.05	2.66±1.47	2.42±1.36	2.40±1.23	<0.05
FT3	2.30±0.81	1.42±0.82	3.31±2.01	1.24±0.71	<0.05
FT4	1.58±0.61	1.35±0.54	2.30±0.81	1.24±0.43	<0.05

Patients with poor functional outcome were having high baseline & median NIHSS Score. The majority of patients with poor outcome had moderate to severe score on NIHS Scale (p value <0.05, Table 6). Free T3 levels were significantly lower in them during first as well second follow up visit (p value <0.05, Table 6).

Figure 7. Scatter plot between FT3 values and mRS_7 scores at 7th day of discharge

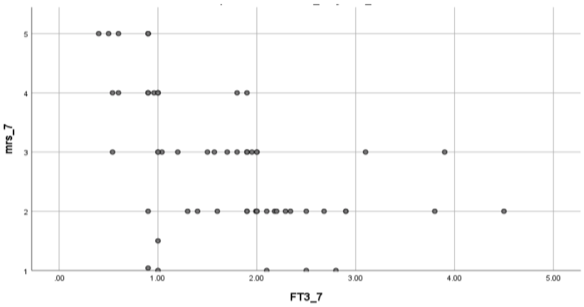

These findings, consistent with the findings reported by Xu and his colleagues [21], which is due to the fact that in patients with poor functional outcome there might be decreased peripheral conversion of FT4 to FT3 which lead to low levels of FT3.

Table 7. Correlation between FT3_7 values and mRS_7 scores at 7th day of discharge

		mRS_7	**FT3_7**
mRS_7	Pearson Correlation	1	-0.692**
	Sig. (2-tailed)		0.000
	N	60	60
FT3_7	Pearson Correlation	-0.692**	1
	Sig. (2-tailed)	0.000	
	N	60	60

***Correlation is significant at the 0.01 level (2-tailed).*
mRS_7: modified Rankin scale at 7th day of discharge
FT3_7: free T3 levels at 7th day of discharge

On the 7th day of discharge, there was a significant negative correlation between mRS scores and free T3 levels [correlation between mRS & FT3 on admission (n=60, r= -0.599, r^2=0.301, p<0.05, 99%CI, Table 8) and at 7th day of discharge (n=60, r= -0.692, r^2=0.479, $p<0.05$, 99% CI, Figure 7, Table 7)]

Table 8: Associations between thyroid hormones on admission & at 7th day of discharge and mRS scores at 7th day

	mRS _7 scores	Pearson Correlation (r)	r^2	Sig (2 –tailed)
TSH_P	-0.215*	-0.215*	0.046	0.009
FT3_P	-0.599**	-0.599**	0.301	0.000
FT4_P	-0.021*	-0.021*	0.0004	0.874
TFT_P	-0.384**	-0.384**	0.147	0.002
TSH_7	-0.242*	-0.242*	0.058	0.006
FT3_7	-0.692**	-0.692**	0.479	0.000
FT4_7	-0.150*	-0.150*	0.023	0.254
TFT_7	-0.445**	-0.445**	0.198	0.000

Table 9: Associations between mRS_30 scores and thyroid hormones on admission and on 7th and 30th day of discharge

	mRS_30 scores	Pearson Correlation (r)	r^2	Sig (2 –tailed)
TSH_P	-0.250*	-0.250*	0.063	0.054
FT3_P	-0.600**	-0.600**	0.360	0.000
FT4_P	-0.095*	-0.095*	0.009	0.570
TFT_P	-0.376**	-0.376**	0.141	0.003
TSH_7	-0.281*	-0.281*	0.466	0.029
FT3_7	-0.683**	-0.683**	0.028	0.000
FT4_7	-0.166*	-0.166*	0.042	0.204
TFT_7	-0.432**	-0.432**	0.186	0.001
TSH_30	-0.376*	-0.376*	0.141	0.003
FT3_30	-0.724**	-0.724**	0.524	0.000
FT4_30	-0.387*	-0.387*	0.149	0.002
TFT-30	-0.477**	-0.477**	0.228	0.000

***Significance of Correlation at the 0.01 level (2-tailed).*

** Significance of Correlation at the 0.05 level (2-tailed).*

mRS_30: mRS score at 30th day of discharge

On 2nd follow up clinic visit, similar trends of negative correlation was observed between mRS score and free T3 levels (Figure 8, Table 9).

This implies that AIS patients with the low T3 syndrome tend to have poor outcome at follow up. From Table 8, it was inferred that at first follow up visit, no statistically significant correlation found between mRS score and FT4 levels (neither an admission nor at 7th day of discharge). There were a statistically significant negative correlation between TSH levels (on admission, at 7th and 30th day of discharge) and mRS score at 30th day of discharge (p value <0.05, Table 9). No relation was seen between FT4 levels (on admission, & at 7th day of discharge) and mRS score at 2nd follow up visit (p value >0.05, Table 9). But a negative relationship seen between the two at 30th day of discharge (r = -0.387, CI 95%, p value <0.05, Table 9).

Figure 8. Scatter plot graph between FT3_30 values and mRS_30 scores at 30th day of discharge.

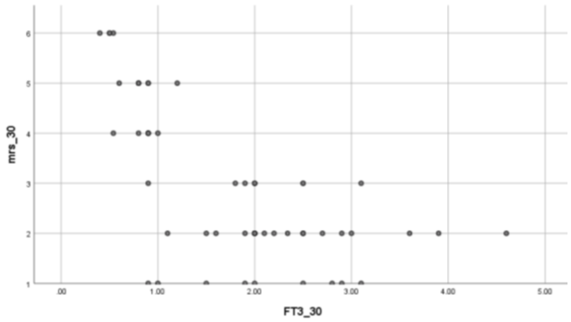

This findings can be explained by the fact that even with the severity of stroke, levels of T3 decrease immediately, but it requires some time for T4 levels to show changes in their levels.

On multiple logistic regression analysis (Table 10), lower FT3 concentration (odds ratio OR=1.166, 95%CI, p value <0.05) remained independently related to poor outcomes.

Table 10: Multiple logistic regression analysis

	NIHSS* OR (95% CI), p	TSH* OR (95% CI), p	FT3* OR (95% CI), p	FT4* OR (95% CI), p
7th day outcome mRS ≤2 vs mRS >2 or death	0.641 (0.457-0.845) P<0.05	0.936 0.661-1.326 P<0.05	1.166 (0.425-3.200) P<0.05	0.803 (0.289-2.229) P>0.05
30th day outcome mRS ≤2 vs mRS >2 or death	0.418 (0.241-0.725) p<0.05	1.125 (0.744-1.701) p<0.05	1.296 (0.276-6.087) p<0.05	0.255 (0.052-1.258) p>0.05

** values at the time of admission*

Table 11: ΔmRS score vs Δthyroid pattern during follow up

		Δthyroid pattern		Total	
		no change	euthyroid		
ΔmRS	improvement	10	4	14	Pearson chi square= 3.08 p value 0.215
	same statuts	27	11	38	
	detoriation	8	0	8	
Total		45	15	60	

ΔmRS = change in functional outcome (mRS_30 score - mRS_7 score)
Δthyroid pattern = change in thyroid pattern

Figure 9. Thyroid hormones pattern observed at various time interval in this study

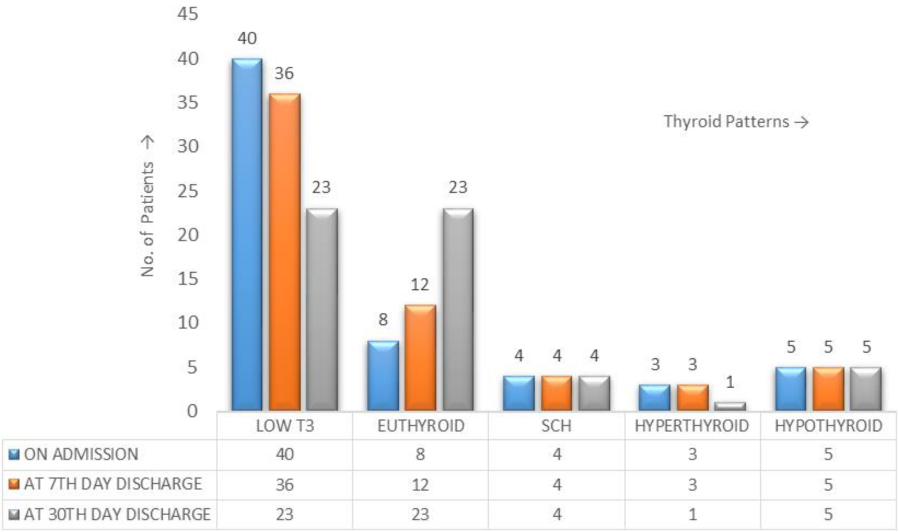

	LOW T3	EUTHYROID	SCH	HYPERTHYROID	HYPOTHYROID
ON ADMISSION	40	8	4	3	5
AT 7TH DAY DISCHARGE	36	12	4	3	5
AT 30TH DAY DISCHARGE	23	23	4	1	5

In addition to the FT3, the only other variables independently associated with poor functional outcome were NIHSS score (OR=0.641, 95% CI, p value <0.05) and TSH levels (OR=0.936, 95%CI, p value <0.05).

Of 40 patients with the low T3 syndrome, 13 patients became euthyroid on follow up (Figures 4 & 9, Table 11). This can be explained by the fact that during disease recovery, thyroid hormones and TSH concentrations return to normal [35]. This emphasize the need to follow up of such patients to confirm suspected cases of sick euthyroid. Similarly, on follow up, a very small percentage had shown improvement in their functional outcome. However, this finding was not statistically significant (p>0.05).

DISCUSSIONS

Conduction of this study was done with an objective to decipher the relationship between thyroid hormone levels and functional outcome in AIS. Observations recorded were similar to studies conducted by Alvezaki et al [17] and Zhang et al [19]. In hospitalized patients, especially in ICUs, the most common type of NTIS seen is low T3 [36, 37]. In ICH from either trauma or hemorrhagic CVA, low T3 has been reported [38, 39]. The present study showed thyroid hormones derangement in AIS and most common type of it was NTIS/low T3 syndrome.

An inverse relationship between T3 levels and NIHSS score was observed in our study. To follow up, patients with poor functional outcomes were found to have low free T3 levels. Thus, an inverse relationship observed between FT3 value and NIHSS score as well as mRS score, thereby strengthening its predictive role in estimating the severity and functional outcome in AIS.

The degree of decrease in T3 levels is related to worse neurological impairment. Thus, monitoring levels of FT3 could potentially be an easy, quick and surrogate marker in the future if other studies confirmed it further. The observations and findings delineated in the present study were similar to studies done by Alvezaki et al [17] which stated that low T3 is a possible independent predictor of stroke outcome.

In severe illness, T3 levels would decline to spare energy, considering it as an adaptive response to stressful conditions. [40-44].

Similar to other systemic severe diseases, a decline in serum T3 with no increment in TSH levels seem to be related to stroke severity and poor clinical outcome [17, 20, 45-47].

Possible mechanisms include the following:

(1) Change in the peripheral metabolism of thyroid hormones due to alterations in enzyme activity involved in peripheral T4 to T3 conversion, [42, 48]

(2) Involvement of pro-inflammatory cytokine action, [49]

(3) A disturbed shift in thyroid hormone distribution or change in binding proteins, [41, 49, 50]

(4) In severe illness, excessive glucocorticoids release, which lead to inhibition of hypothalamic-pituitary- thyroid axis activities and T4 to T3 conversion. [51]

Similar to various studies conducted in ICUs, AIS patients with low T3 or combination with low T3 and T4 found to have a worse prognosis. [15, 43, 48, 52-54]

Interestingly, studies showed that derangement in T3 levels is not related with regional distribution of stroke (anterior vs posterior circulation). This change in thyroid hormone levels can, therefore, be due to disturbance in their metabolism instead of any structural defect induced due to abnormality in blood supply to hypothalamic-pituitary-thyroid axis. [19, 23]

A significant negative correlation observed between NIHSS score and TSH (p value < 0.05) on admission and on follow up. On admission, no correlation was observed between NIHSS score and free T4, however, negative correlations observed between them only to follow up. Unlike, NIHSS score and FT3 levels, strength of association between NIHSS score and TSH & T4 levels were not strong.

After CVA onset, within hours, the decline in serum T3 levels and degree of alteration in its levels is associated with the severity of stroke.[48 ,55-57] In severely ill patients, levels of T4 also decrease [55, 57]. A drop in FT4 levels represents a continuum of the NTIS spectrum in which as illness progresses in its severity, levels of FT4 would decrease. Therefore, low FT4 levels also correlated with bad prognosis.

However, no relationship of NIHSS score with FT4 levels on admission can be explained by the fact that with the severity of stroke there occurs decrease in the peripheral conversion of T4 to T3 as a result of which T3 levels would decrease but T4 levels would either normal or increases. Similar findings were reported by Xu and his colleagues in their study [21] that T4 levels were found to be increased in patients with higher scores on NIHS scale and hence those with poorer prognosis can have higher or normal T4 levels.

On univariate analysis, during follow up, no relationship was observed between mRS score and baseline parameters, i.e. age, gender and vascular risk factors. It was observed that AIS patients with high moors scores had low levels of free T3 and also had a high NIHSS score, both baseline and median. These findings, consistent with the findings reported by Xu and his colleagues [21], which is due to the fact that in patients with poor functional outcome there might be decreased peripheral conversion of FT4 to FT3 which lead to low levels of FT3.

At first follow up clinic visit, only other thyroid parameters with which mRS score can be correlated was TSH. Similarly, on 2nd follow up visit, mRS score was negatively correlated with TSH levels. A negative correlation was observed between free T4 and 2nd follow up visit mRS score only at 30th day of discharge. To sum up, apart from free T3, TSH levels would be next important parameter to determine functional outcome in AIS. However, free T4 would not show any significant change in its level immediately, but ultimately its levels also decrease in those with severe stroke and poor outcome which would be observed only on follow up.

In the present study, no significant difference observed between age, gender and vascular risk factors among AIS patients with low T3 and those with normal T3 levels. Zhang et al [19] study, however, showed that low T3 group patients were slightly but not significantly older. Alvezaki et al [17] study showed that patients under low T3 group were significantly older. In elderly, baseline thyroid function is usually at the lower side and when stressful events like stroke occurs, they tended not to compensate fully. This was the explanation offered by his study.

Except low T3 subgroup, none of the other subgroups had shown any mortality. In this subgroup on follow up, 4 patients (10%) were expired. Retrospective analysis of these patients showed that they had a severe stroke on NIHS Scale and had very low levels of FT3.TSH and free T4 levels were also at the lower range. These mortalities emphasize a severe derangement in HPT axis. These findings were consistent with studies which advocate that long term survival is poor in patients with the low T3 syndrome and emphasized that ischemic stroke patients with the low T3 syndrome were at higher risk of poor outcome on follow-up that was arranged from 2 to 4 weeks after ischemic CVA and 1-year survival was remarkably worse in CVA patients with the low T3 syndrome. The outcomes were supported by other studies. [17, 19]

We, in the end, conclude that there is alteration in levels of thyroid hormones in AIS and low T3 levels are predictive of severity and functional outcome in these patients. However, the understanding of pituitary-thyroid axis, factors controlling action of thyroid hormone at the cellular level and explanation of different arrays of thyroid function tests may be considered cardinal in the management of ischemic CVA.

CONCLUSIONS

In acute ischemic stroke, there is the major impact of thyroid dysfunction. In such patients non thyroidal illness (NTIS) is not uncommon and reflect the increase in physical stress associated with the insult. The present study showed that severity of initial (functional) impairment measured by an NIH Stroke scale on admission, and free T3 & TSH levels had an independent predictive role in determining the functional outcome.

LIMITATIONS OF THE STUDY

Although the research was prepared carefully, there were some inevitable limitations such as small size of the sample, shortfall of long term follow up, selection biases, etc. Due to time constraint, this study was conducted on a small sample size, but it is advised to conduct it on large population so that better results and better comprehension of subjects can be made.

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ORIGINAL ARTICLES

Characteristics and complications of supernumerary permanent teeth in a sample of patients examined in a university pedodontics clinic

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Abstract: *Aim. The assessment of distribution of supernumerary permanent teeth (SPT) and the disturbances caused by them.*

Material and method. Cross-sectional study upon a sample of 85 children (58 boys) aged between 3 and 18 years (mean age=9.08±3.09 years) with at least one SPT. Were analyzed: patients' distribution according to gender, age and number of dental units; SPT distribution according to arch and topography and also the disturbances noticed.

Results. a) boys:girls ratio=2.14:1; b) the 85 patients had 107 SPT, mean number=1.25; c) 76.47% of patients had one SPT; d) 89.72% of SPT were on maxillary arch, maxilla:mandible ratio = 8.7:1; d) 92.52% of SPT were localized in anterior region, anterior:posterior region ratio = 12.3:1; e) mesiodens –71.96%, supernumerary lateral incisor – 19.62%; f) disturbances caused by SPT: ectopic eruption of permanent teeth – 36.47%; delay or failure of eruption of permanent teeth – 21.17%; crowding – 20%.

Conclusions. 1. Supernumerary teeth were most frequent noticed in boys, in maxillary anterior region. 2. Most often they caused eruption disturbances of permanent teeth.

Keywords: *supernumerary permanent teeth, distribution, complications*

INTRODUCTION

SPT are defined as any tooth or odontogenic structure that is formed from a tooth germ in excess of the usual number of teeth for any region of the dental arch [1, 2]. In permanent dentition, this dental anomaly is 5 times more frequent than in primary dentition, the prevalence values reported ranging from 0.1 to 6% [2, 3].

In Romania, there are few recent studies have been carried out on the SPT [4]. These focused more on the distribution of SPT, but less on local disturbances caused by their presence. This study is important because it helps to identify early signs of the presence of SPT and thus prevent loss of permanent teeth or further complications.

The aim was to analyze the distribution of SPT and to assess the disturbances produced by them, in a group of children examined and treated in a University Pedodontics Clinic.

MATERIAL AND METHODS

A retrospective cross-sectional study was conducted on a sample of 85 children (58 boys, 27 girls) aged 3 to 18 years (mean age = 9.08 ± 3.09 years), with at least one SPT. Children were examined in the Pedodontics Department,

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Faculty of Dental Medicine, Carol Davila University, Bucharest. Criteria for choosing the study sample were: patients aged up to 18 years old, without genetic diseases, chronic diseases or lip-palate clift. Pre-existing conditions, such as cardiac pathology, were evaluated [5-8].

Data on dental status were obtained from dental records of patients examined and treated by a single dentist. The diagnosis of the number anomaly was made on clinical and radiological examinations. For each patient diagnosed with this anomaly were noted: age, gender, permanent teeth erupted, presence, position and shape of the SPT and disturbances produced by them.

The data obtained were collected and processed using SPSS-version 20.0 for Windows (SPSS Inc, Chicago, IL, USA).

The aim of the research was to analyze: the distribution of patients with SPT according to sex, age and number of affected teeth; the distribution of SPT according to the affected arch and the topography of the tooth, and the disturbances produced.

RESULTS AND DISCUSSION

Sex distribution of the sample

The percentage of boys diagnosed with this anomaly was 68.23% and for girls - 31.77%. The boys:girls ratio was 2.14:1.

It was noted that in 3 male patients it was observed that the SPTs were associated with the congenital absence of other permanent teeth (Hyper-Hipodontia syndrome).

Age distribution of the sample

Most often, the number anomaly was noticed at the age of 9 years (Figure 1).

Patients' distribution according to the number of affected teeth

The 85 children had a total of 107 teeth, with an average number of 1.25 teeth. Most commonly patients had a single permanent tooth in excess – 65 (76.47%), 18 (21.17%) had 2 SPT and only 2 patients (2.35%) had 3 SPT.

Figure 1. Age distribution of the sample according to the age at which the anomaly was diagnosed

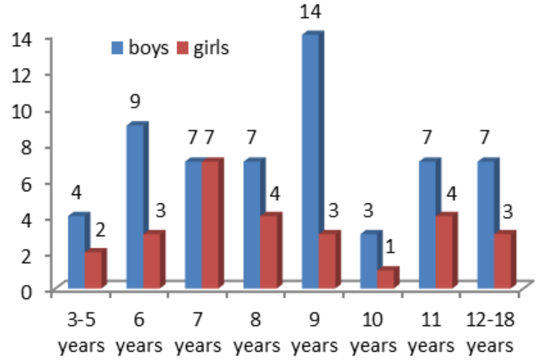

SPT distribution according to their topography

Most of the SPT were in the upper arch, in the front area (Table 1). Maxilla: mandible ratio was 8.7:1, and the anterior: posterior area ratio was 12.3:1. Note that multiple SPT have only met in the upper front area.

Table 1. Distribution of SPTs according to their topography

	Maxilla		Mandible		Total	
	n	%	n	%	n	%
Anterior	92	85.98	7	6.54	99	92.52
Posterior	4	3.74	4	3.74	8	7.48
Total	96	89.72	11	10.28	107	100

SPT distribution according to the type and shape of the teeth

The conical-shaped mesiodens was the most common oversized tooth, followed by lateral incisor with a similar shape to the normal-series tooth (Table 2).

Table 2. Distribution of SPT according to type and morphology

Shape	Supplementary	Conical shape	Tuberculate shape	Odontoma	Total	
					n	%
Mesiodens	9	47	17	4	77	71.96
Lateral incisor	19	1	1	0	21	19.62
Parapremolar	4	0	0	0	4	3.73
Paramolar	1	2	0	0	3	2.80
Distomolar	1	0	0	0	1	0.93
Canine	0	0	1	0	1	0.93
Total	33	50	19	4	107	100

Of the 77 mesiodens, 12 (15.58%) were inverted, being discovered by chance on the X-rays.

Distribution of the disorders caused by SPT

Most of mesiodens determined the deflection of the permanent tooth eruption from the normal series, while the lateral incisors caused or worsened the crowding (Table 3).

Table 3. Disorders caused by SPT

Disturbances	Mesiodens	Lateral incisor	Parapremolar distomolar	Para-molar	Canine	Total	
						n	%
Ectopic eruption	29	1	0	1	0	31	36.47
Failed eruption	16	2	0	0	0	18	21.17
Crowding	2	15	0	0	1	17	20
No consequences	8	2	0	0	0	17	20
It can't be appreciated	2	0	5	2	0	2	2.35
Total	77	21	5	3	1	85	100

DISCUSSIONS

In the literature there are numerous studies of this anomaly that analyze their frequency, shape variations and disturbances that can be associated with different population groups. Most studies were conducted on samples of patients from orthodontic clinics. But early diagnosis of SPT helps to achieve an appropriate treatment plan and to prevent further complications.

In permanent dentition, the prevalence of the disease varies between 0.1 and 2.1% in Caucasian, between 2.4 to 6% in Chinese, Japanese and black Americans [2, 5]. In Romania, the prevalence value was 3.04% [4].

Analyzing the distribution by sex, the boys:girls ratio of the SPT was 2.14:1. This result is similar to that reported by numerous studies conducted in international groups of children or adults [9-14]. Instead, Bäckman and Wahlin (2001) found a predilection of SPT in females [15], which was highlighted by Mitchell (1989), who found a female:male ratio of 2:1 [16]. Unlike them, Dominguez and Mendoza (1995) did not discover any difference between the sexes [17].

Rani et al. (2017) noted that SPT are more frequently diagnosed in people aged the first 3 decades of life [2]. In a study conducted by analyzing 2241 X-rays, Salcido-García et al. (2004) found that from the total number of patients with SPT 47.6% were in the third decade of life, followed by those in the first decade of life in a percentage of 28.5% [18]. This study, conducted on a group of children, showed that the average age to which this anomaly was diagnosed was 9 years. Also, Alberti et al. (2006) conducted a study on a lot of patients aged between 6 and 10 years and concluded that the interval for early diagnosis and prevention of malocclusions caused by hyperdontia is around the age of 9 [13]. More than ¾ between children had one extra tooth, resulting similar to values reported by other researchers [9-14].

Regarding the topography, the SPT were identified in the present study of approximately 90% in the upper jaw, a percentage similar to that reported by Zhu et al. [19] or Hattab et al. [20]. Instead, Fernández-Montenegro et al. (2006) [21] found that 74.5% of the SPT were at the maxilla, while Leco-Berrocal et al. (2007) – a percentage of 79.2% [9].

In the present study, 92.52% of the SPT were located in the front area. Other studies report in adult populations a higher frequency of SPT in the posterior jaw area. It is agree that in children the most frequent location is in the front area, and in adults in the posterior area [9].

In the group studied in the Pedodontics Clinic from Bucharest, mesiodens appeared in 71.96% of cases, followed by lateral incisor (19.62%). Also, Fernández-Montenegro et al. (2006) found that mesiodens was the most commonly diagnosed, followed by parapremolar and distomolar [21], Salcido-Garcia et al. (2004) found the order: mesiodens, parapremolar and lateral incisor [18] and Bereket et al.: mesiodens, distomolar, parapremolar [10]. On the other hand, in the study of Khandewal et al. (2018), parapremolars were most commonly met [14], and at Leco-Berrocal et al. (2007) [9] and Rani et al. (2017) – distomolars [2].

Most of the authors found that the conical shape is the most common, this fact also emerged in the present research [10-12, 14, 21, 22]. In second place, supplementary teeth were observed, as in the study conducted by Fernandez-

Montenegro et al. (2010) [21], while Shah et al., and Schmukli et al. (2010) found the tuberculate shape [11, 22].

As far as the consequences are concerned, in this study in most cases the deflection of the teeth eruption of the normal series was encountered, followed by the failed of permanent teeth eruption and crowding. Leco-Berrocal et al. reported that the most common complications were mechanical accidents, namely the deflection of adjacent teeth -39%, root resorption -12.5% and failed of eruption-4.1%, but also 19% follicular cysts [9]. Rajab and Hamdan were observed that 51.47% of meziodentes failed the eruption of permanent incisors [12].

No consequences were observed in 20% of cases. Instead, Fernández-Montenegro et al. (2006) and Bereket et al. (2015) found the lack of any consequences in a large percentage – 57.9%, respectively 61.64% [10, 21].

Most of the results from the present study were similar to the values reported in the literature. Differences may be caused by racial type and the mean age of the sample.

CONCLUSION

1. Hiperdontia was seen more frequently in boys compared to girls (2.14:1).
2. The SPT have been located most frequently in the upper front area.
3. The most common type of SPT was mesiodens (71.61%) followed by the upper lateral incisor (19.62%).
4. In most cases, the SPT resulted in the deflection of a tooth from the normal series.

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ORIGINAL ARTICLES

Development of quantitative real-time RT-PCR assay for detection and viral load determination of Crimean-Congo Hemorrhagic Fever (CCHF) virus

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Abstract: *Background and Aim: The CCHF (Crimean-Congo hemorrhagic fever) virus causes a severe disease in human with a case fatality rate of up to 50%. Since, there is no specific treatment or approved vaccine against CCHF viral infections, an accurate and early detection as well as a reliable surveillance and quantitative determination of viral load is necessary for patient improvement and case management. In this research, our aim was to develop a probe based one-step real-time reverse-transcription polymerase chain reaction (rRT-PCR) assay for in-house quantitative detection of CCHF virus.*

Methods: At first, the highly conserved S-fragment sequence of CCHF virus genome was adapted from GenBank and the specific probe and primers targeting this region were designed. Then, viral RNAs were extracted from 37 blood samples of different patients from east of Iran (Zahedan). The specificity and sensitivity of the probe and primers were also evaluated in positive blood samples, confirmed to have CCHF virus. A standard (PTG19-T vector containing S-fragment) for quantization was also constructed and the viral load was determined in some of positive samples.

Results: From a total of 37 suspicious blood samples, 15 samples were confirmed to be positive for CCHF virus by this probe based one-step rRT-PCR assay and no false-positive result was detected according to sequencing data. The predicted fragment of 176 bp was also confirmed in all positive samples by gel-based electrophoresis analysis. The assay was linear between 10 to 103 copy numbers per each microliter of extracted plasmid for this technique and the viral load determined in one of patient blood samples was 55,000 viral particles per each milliliter, for example. Bioinformatics and experimental evaluations approved the specificity of this assay. The LOD of the assay was 10 (or fewer) copy numbers of viral genome per each microliter of the extracted genome.

Conclusions: This research showed that the developed probe based one-step rRT-PCR assay is a specific, rapid, sensitive and the simple tool for detection and viral load determination of the CCHF virus.

Keywords: *CCHF Virus, Real-time RT-PCR, Quantitative, viral load, S-region*

INTRODUCTION

The CCHF (Crimean-Congo hemorrhagic fever) is a widespread disease caused by Nairovirus (a tick-borne virus) of the Bunyaviridae family. The CCHF virus causes a severe disease (a zoonotic viral hemorrhagic fever) in human and is endemic in Africa, the Balkans, Asia, and Europe [1-6]. In Iran, this virus has also caused serious challenges in the Sistan-va-Baluchestan province [7-15]. The fatality rate is about 10-50% [16, 17]. Since, there is no specific treatment or approved vaccine against CCHF viral infections, an accurate and early detection as well as a reliable surveillance and quantitative determination of viral load is necessary for case management, patient improvement, and protection of medical staffs.

Several different laboratory assays can be used for CCHFV diagnosis, including enzyme-linked immune sorbent assay (ELISA), serum neutralization, antigen detection, virus

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isolation by cell culture and reverse-transcriptase polymerase chain reaction (RT-PCR) assay [4, 18-21]. CCHFV RNA from different clinical samples is a proper diagnosis target during the acute phase of the infection or even before the beginning of disease when detection of antibody is untrustable or impossible [22-24].

Unlike the traditional two-step RT-PCR technique, the one-step real-time RT-PCR (rRT-PCR) assay does not need to pre-PCR (post-RT) sample handling, avoiding the PCR product dependent probable contamination transmission and resulting in more sensitive, rapid and also higher efficient assays. On the other hand, the probe based rRT-PCR assays have a very high specificity in addition to the mentioned features. Therefore, it has become an accepted recognition method for detecting many microbes [25]. However, in this research we developed a quantitative probe based one-step rRT-PCR assay for detection and viral load determination of the CCHF virus in suspected blood samples of the Sistan-va-Baluchestan prov¬ince of Iran. The aim of this experimental study was to develop a probe based one-step rRT-PCR assay for in-house quantitative detection of the CCHF virus.

METHODS

Collection of sample

To perform this study, we provided 37 blood samples of different CCHF suspicious patients from the Zahedan city in the southeast of Iran. A few samples had already been confirmed to be positive for CCHF viral RNA existence by rRT-PCR analysis (Pasteur Institute, Tehran, Iran) that were also used as the positive control and they had been stored at -70°C.

Extraction of viral genome

Viral genomes were extracted from 0.2 ml of blood samples using the High Pure Viral RNA Kit (Roche-Germany) in 30 minutes, according to manufacturer’s instructions. Then, each one of the extracted genomes were dissolved in 50 µl of RNase free water and stored at -70°C until rRT-PCR analysis (of which only 5 µl was required in each assay). It is noteworthy that all of manipulations were performed in a biological hood with class II biosafety.

Designing and synthesizing the specific probe and primers

The nucleotide sequence of S-region of the available CCHFV strains was adapted from the NCBI database (GenBank) and the highly conserved sequences in this region were determinated and specified from the alignment data analysis obtained from MEGA 7 software. Then the specific probe and primers targeting this region were designed using the Oligo software, version 7.0 (Molecular Biology Insights, Cascade, CO, USA) and GeneRunner (Hastings Software, Hastings, NY, USA).

The probe sequence designed for S-region internal sequence was FAM-5'-CAA AAC AGG ATC TAC ATG CAC CCT GCC-3'-BHQ as well as the forward and reverse oligonucleotide primers for S-region internal sequence were 5’-TGG GGK AAG AAR CTY TAT GAG CT-3’ and 5’-ATG GAC TTG GTR TGY CCA GAT CC-3’, respectively. K, R and Y are the wobble bases. Eventually, the probe and primers were synthesized by the Bioneer Company (Korea).

One-step real-time RT-PCR assay

The probe based one-step rRT-PCR assays were developed by use of QuantiTect® Probe RT-PCR Kit (Qiagen, Hilden, Germany) using both the Applied Biosystem (ABI) 7500 (USA) and Corbet (Rotor-Gene) 6000 (Germany) real-time PCR instruments. The amplification process was performed in 60 minutes within 20 µl reaction mixtures including 5 µl of the viral RNA template, 1 µl (10 pmol) of both primers (0.5 µM in the reaction) and 0.5 µl of the probe (0.25 µM in the reaction). The conditions of real-time cycler were as follows: two single steps of 30 minutes at 50°C (required for reverse transcriptase activity) and 15 minutes at 95°C (required for HotStarTaq DNA polymerase activation); followed by 50 cycles of 15 seconds at 94°C; and 60 seconds at 60°C (collecting the fluorescence data was accomplished at the end of each 60°C step). So, the real-time PCR products were detected trough an increase in the fluorescence intensity from cycle to cycle. Cycling curves were evaluated with respect to negative and positive controls (as the quality controls of the process) in the real-time analysis. The rRT-PCR products were then approved by gel-based electrophoresis.

Evaluation of sensitivity and specificity of the assay

Analytical sensitivity

To determine the LoD (limit of detection) of the assay, serial dilutions containing 1, 10, 100 and 1000 copies of the positive control sample (plasmid) per each microliter of the extracted sample were analyzed. The highest dilution of sample at which 100% of rRT-PCR replicates were positive was considered as the LoD of the assay. In this assay, the LOD was 10 or fewer copy numbers of viral genome per each microliter of the extracted genome (2500 or fewer copy numbers of viral genome per each milliliter of patient blood sample).

Analytical specificity via in silico prediction and rRT-PCR assy

As previously mentioned, none of the probe and primer sequences demonstrated genomic cross-reactivity with other viruses, human genome and other likely interfering genomes in a BLAST search analysis and only detected a 176 bp fragment of the CCHFV S-region in bioinformatics analysis (data not shown). On the other hand, in the experimental rRT-PCR assay, some of the accessible RNA viruses such as norovirus, flu A, HCV, WNV and CCHF (as the positive control) were also evaluated in terms of specificity of the assay.

Linearity range and repeatability of the assay

Linearity of the assay was evaluated by analysis of serial dilutions containing 10 to 105 copy numbers of positive control sample per each microliter of extracted plasmid and the assay was linear between 10 to 103 copy numbers per each microliter of extracted plasmid for this technique (Figure 1 – *Ct was plotted against the quantity of plasmid DNA (common logarithmic scale). Measurement of the copy number of CCHF virus was estimated from Ct by quantitative rRT-PCR< ■ [in Ct = 30.69] represents unknown patient sample and ■ [in Cts = 35.36, 31.87 and 28.42] represents the standard dilution*). These assays were evaluated and repeated by at least three different users and three times by each one of them on different days and the repeatability of the assays was approved. Also the assays were developed using both the Applied Biosystem (ABI) 7500 (USA) and Corbet (Rotor-Gene) 6000 (Germany) real-time PCR instruments.

Quantitative determination of viral load in positive samples

Construction of a standard for quantization: The fragment of 176 bp within the S-region sequence of CCHF virus genome was amplified by rRT-PCR and the amplicon was cloned into the PTG19-T vector using a T/A cloning Kit (Vivantis, Malaysia). The plasmid was then purified using a plasmid purification kit (Qiagen, Hilden, Germany). After the performance confirmation of cloning process by PCR, the accuracy of this process and the sequence of cloned fragment was confirmed by sequencing. Determination of recombinant plasmid concentration was then performed by a NanoDrop.

For preparation of a standard curve, the plasmid was diluted serially from 103 to 10 copy numbers per microliter as a master of standard positive control. As soon as optimizing the assay conditions, we obtained a standard curve with a linear range across at least three logs of DNA (recombinant plasmid) concentration, from which the Ct values can be referred to the virus copy number (Figure 1).

Figure 1. The standard curve with a linear range across three logs of different concentrations of the recombinant plasmid DNA containing the S-region sequence of CCHFV genome.

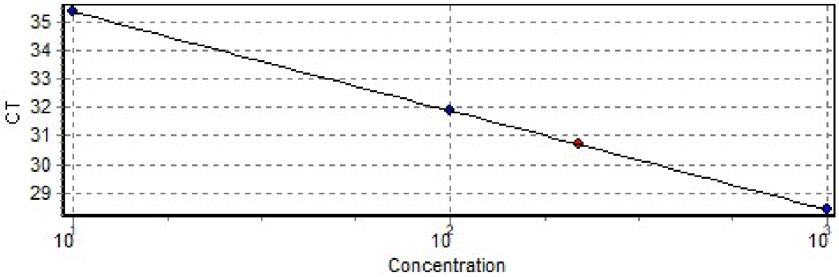

Determination of the Ct values and construction of the standard curve were performed using the Rotor-Gene 6000 software. In all real-time PCR assays, the correlation coefficient of the standard curve was greater than 0.980. Ct was plotted against the quantity of plasmid DNA (common logarithmic scale).

Measurement of the copy number of CCHF virus in unknown blood samples from different patients was estimated from Ct by quantitative rRT-PCR. Since, the viral genome was extracted from 200 µl of a patient blood sample, on the other hand, the extracted genome was dissolved in 50 µl of RNase-free water, as well as, 5 µl was only used in each quantitative assay, the final viral load in 1 ml of blood sample would be 50 folds.

RESULTS

Diagnostic assay of CCHF viral RNA in suspicious blood samples

From a total of 37 suspicious blood samples, 15 cases were approved to be positive and 22 were negative for CCHF viral RNA by use of this technique and no false positive was detected in the results of sequencing. The predicted length of 176 bp resulted by rRT-PCR amplification was approved in all the positive samples by electrophoresis analysis (Figure 2A). The amplification curve related to some of the suspicious blood samples evaluated is shown in Figure 2B.

Evaluation of analytical sensitivity of the assay

The serial dilutions prepared containing 1, 10, 100 and 1000 copies of the positive control sample (plasmid) per each microliter of the extracted genome were analyzed in rRT-PCR for the analytical sensitivity evaluation of the assay. As shown in table 1, the highest dilution at which 100% of rRT-PCR assays were positive (underlined) is considered as the LOD, which in this assay was 10 (or fewer) copy numbers of viral genome per each microliter of the extracted genome or 2500 (or fewer) viral particles per each milliliter of patient blood sample.

Figure 2. A) The predicted fragment with the length of 176 bp, resulted by rRT-PCR amplification. Lad is a 50 bp DNA Ladder (SinaClon, Iran).

B) The rRT-PCR amplification curve related to some of the suspicious blood samples considered (■ represents NTC [Not template control] or negative control sample, 🟥 [in Ct = 23.29] represents positive control sample, 🟦 and 🟪 represent negative patient sample, 🟫 [in Ct = 28.12] and 🟩 [in Ct = 26.85] represent positive patient sample)

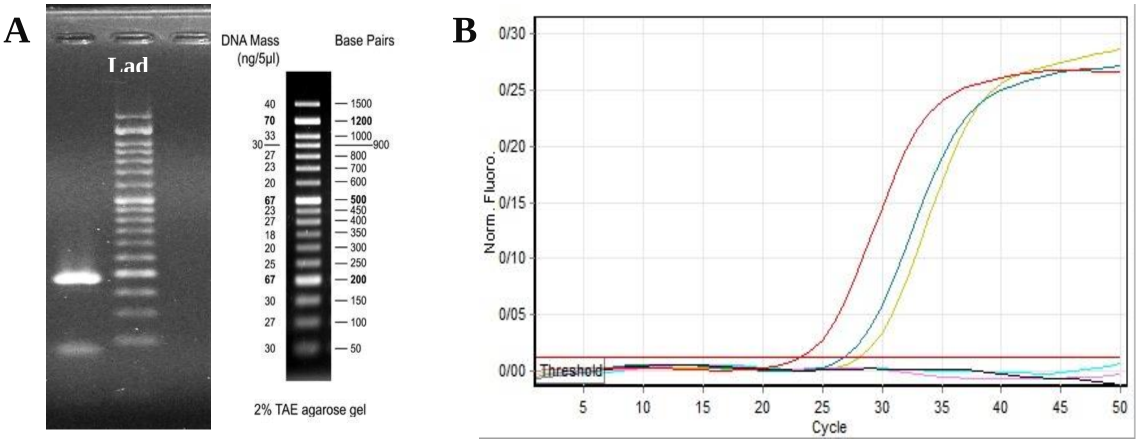

Table 1. CCHFV rRT-PCR assay limit of detection with positive control sample

Serial dilution *(copies/microliter)*	Positive tests (%) s – region
1	variable
10	100
100	100
1,000	100

Evaluation of analytical specificity via in silico prediction and rRT-PCR assay

As shown in Figures 3A and 3B, the highly conserved sequences (yellow) in the S-region were determinated from the alignment data analysis obtained from the MEGA 7 software which were quite specific for CCHF virus, as previously mentioned and the specific primers targeting this region were designed using the mentioned softwares (bioinformatics data for the probe is not shown).

Figure 3. The alignment data resulted from the MEGA 7 software related to the S-region of CCHFV. A) The Yellow zone is the highly conserved sequence used for designing the specific forward primer. B) The Yellow zone is the highly conserved sequence used for designing the specific reverse primer.

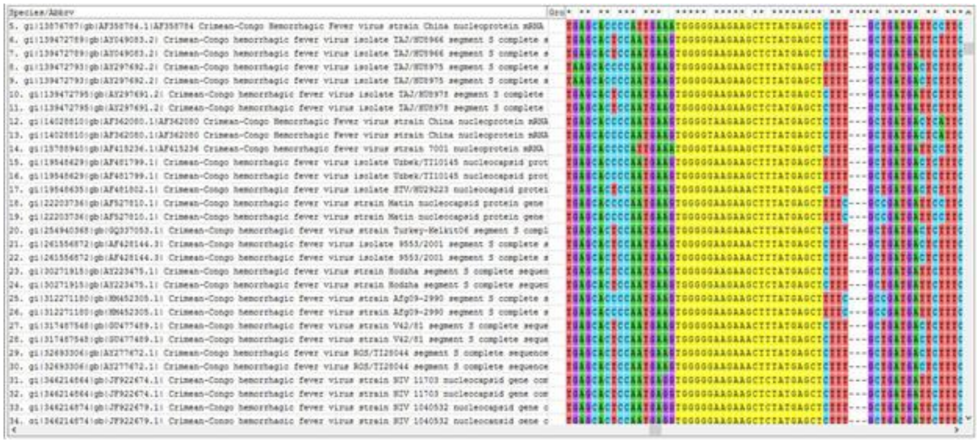

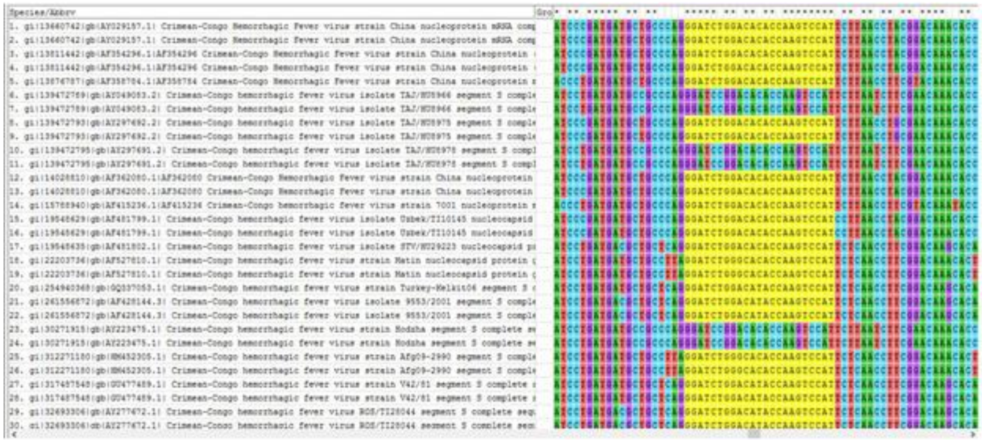

The results of experimental rRT-PCR assay on the accessible RNA viruses such as Norovirus, Flu A, HCV, WNV and CCHFV (as the positive control) showed that this assay was quite specific for CCHFV and other viruses were not detected by the designed probe and primers (Figure 4 - ■ represents NTC [Not template control] or negative control sample, 🟥 [in Ct = 21.44] represents the positive control [CCHFV] sample, 🟦 represents the negative result from Norovirus sample assay, ■ represents the negative result from Flu A sample assay, 🟪 represents the negative result from HCV sample assay and 🟥 represents the negative result from WNV sample assay).

Figure 4. The rRT-PCR amplification curve related to some of the accessible RNA virus samples

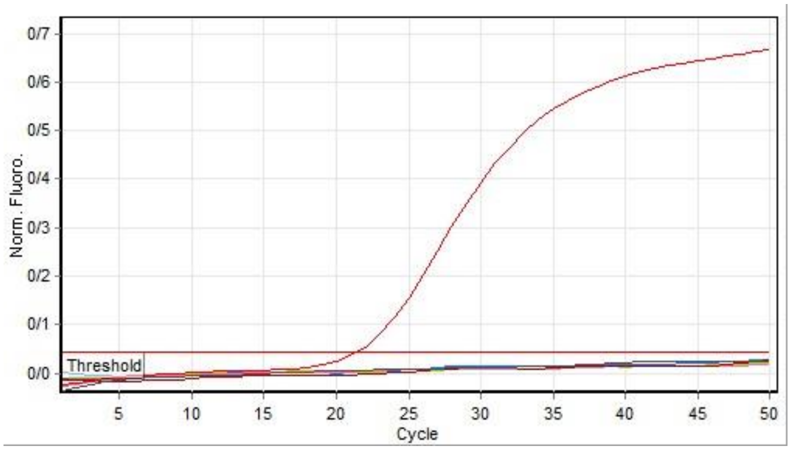

Figure 5. Representative amplification plot of the developed CCHFV quantitative rRT-PCR assay (quantitative curve) showing three ten-fold dilutions of the standards (Cts = 35.36, 31.87 and 28.42) and one unknown sample (Ct = 30.69).

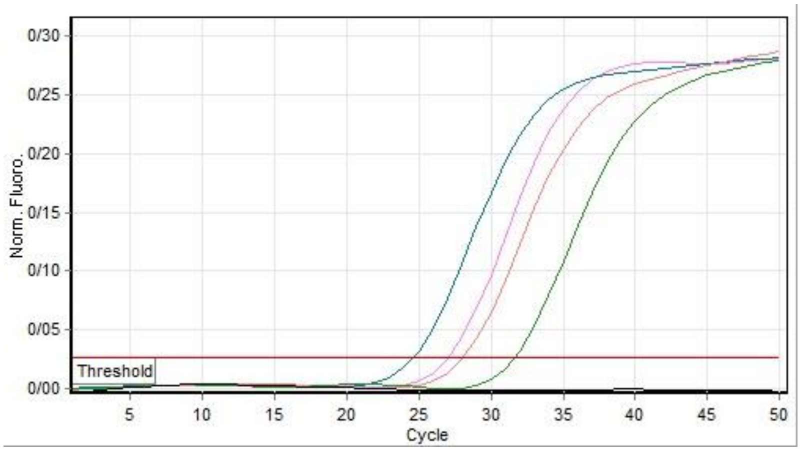

Quantitative determination of viral load in positive samples

The copy number of CCHF virus in different patient blood samples was estimated from Ct by the rRT-PCR assay (Table 2), based on the quantitative curve (Figure 5). For example, the viral load determined in patient blood sample-1 was 220 copy numbers of viral genome per each microliter of the extracted genome or 55,000 (250 folds) viral particles per each milliliter of patient blood sample.

It is notable that viral load decreased slowly in most of cases in subsequent samplings (severe form of disease) and in others (moderate form of disease) decreased very faster. The results of subsequent evaluations is not reported in this article.

Table 2. The calculated viral load of CCHF virus in several patient blood samples based on the quantitative curve and Ct, NTC is "not template control" or "negative control" sample

No.	Color	Name	Type	Ct	Given Conc. *(copy number/µl)*	Calc. Conc. *(copy number/µl)*	Calc. Conc. *(viral particle/ml)*
1	🟪	Sample 1	Unknown	30/69	------	220	55,000
2	🟦	Sample 2	Standard	28/42	1,000	994	248,500
3	🟥	Sample 3	Standard	31/87	100	101	25,250
4	🟩	Sample 4	Standard	35/36	10	10	2,500
5	⬛	Sample 5	NTC	------	------	------	------

DISCUSSION

The CCHF (Crimean-Congo hemorrhagic fever) is an acute widespread zoonotic illness, caused by Nairovirus (a tick-borne virus), which is characterized by a severe and often hemorrhagic course of disease, with a fatality rate of about 10-50% [16, 17].

The CCHF viral RNA from various clinical samples is a proper tracing target during the acute phase of the infection, or even before the beginning of the disease when detection of antibody is impossible or unreliable [22-24]. As there is no specific treatment or approved vaccine against CCHF virus, an accurate and early detection as well as a reliable surveillance and quantitative determination of viral load is necessary for case management, patient improvement, and protection of medical staffs.

Several different laboratory assays can be used for CCHFV diagnosis, including enzyme-linked immune sorbent assay (ELISA), serum neutralization, antigen detection, virus isolation by cell culture and reverse-transcriptase polymerase chain reaction (RT-PCR) assay [4, 18-21]. RT-PCR is the best molecular diagnostic techniques in clinical laboratories owing to its simplicity, high specificity and sensitivity [24]. Unlike the traditional two-step RT-PCR technique, the one-step real-time RT-PCR (rRT-PCR) assay does not need to pre-PCR (post-RT) sample handling, avoiding the PCR product dependent probable contamination transmission and resulting in more sensitive, rapid and also higher efficient assays. On the other hand, the probe based rRT-PCR assay has a very high specificity in addition to the mentioned features, compared with the SYBR Green based rRT-PCR assay that was also developed in our previous study [22]. Therefore, it has become an accepted diagnosis method for detecting many microbes [25] and also has greater repeatability when the need for post-amplification processing (analysis by agarose gel electrophoresis) is eliminated, thus avoiding the transfer contamination, difficulty and time-consuming [26, 27].

The assay was linear between 10 to 10^3 viral genomes per each microliter of the extracted genome for this technique and the bioinformatics and experimental evaluations approved high specificity of this assay. On the other hand, the LOD of the assay was 10 (or fewer) copy numbers of viral genome per each microliter of the extracted genome. Measurement of the CCHF virus copy number and quantitative determination of viral load in positive samples was estimated from Ct via the rRT-PCR on the basis of the quantitative curve. It is notable that construction of a standard for quantization of this assay is necessary and very difficult, which was devoted a lot of time in this study.

CONCLUSIONS

This research showed that the developed probe based one-step rRT-PCR assay is a specific, reliable, rapid, sensitive, repeatable and simple tool for detection as well as quantitative determination of viral load of the CCHF virus in various suspicious patient samples.

Acknowledgements

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ORIGINAL ARTICLES

Prioritisation in delivering health services: A military health system example

Ünal Demirtaş[1]

Abstract: *Objectives: The aim of this study is to use of available resources effectively and efficiently, to determine the situation of recource allocation, priority setting and to offer an insight into future planning in delivery of health services in Turkish Armed Forces (TAF).*

Study Design: This study was conducted using qualitative focus group method and it is a descriptive and qualitative study. Questions to be asked in the focus group discussions, the location and the time of discussions and the participants was determined by review of the literature.

Methods: Focus group interviews lasted between approximately 90-120 minutes, respectively, to each focus group, 9 chief phsycians, 13 doctors /dentists, 6 nurses, 8 health noncommissioned officers, 7 officers, 6 noncommissioned officers and 6 health administrators were attended.

Results: The result of focus group discussions; priority settings in military health services must be done and to preventive health care would be prioritised. The military hospitals in Internal Security Operation Area should be strengthened in terms of medical personnel and medical supplies and be full-fledged hospital. There was found that secondary care should be met from civilian hospitals in the other regions.

Conclusion: It is evaluated that the study will be contributed to set of priorities in TAF military health services take the investments in the public or private health sectors and operational requirements into consideration, the studies carried out by TAF Medical Command in order to restructure of the military hospitals, to be employed the recources allocated for health services and health personnel graduated from Gulhane Military Medical Academy such as physicians, health noncommissioned officer and nurses in real requirement positions.

Keywords: *Military hospital, prioritisation, primary health services, focus group, health personnel*

INTRODUCTION

Health services have great importance in protecting human health and according to the World Health Organization, they include all services necessary for diagnosis and treatment of all diseases, maintenance and improvement of health [1].

Primary purpose of health services is to protect the society from diseases and to ensure all individuals are healthy during their lifetimes within the scope of preventive health services; when it is not possible, to provide diagnosis and treatment services which are the secondary purpose of health services; and the third purpose is to give rehabilitation services to individuals who have not fully recovered [2].

Facilitating the access to health services, improving the scope and quality depend on resources such as money, personnel, material and medicine.

During the last 50 years, GPD expenditure on health in OECD countries has increased from 3.7% to 9.4%. Growth in health expenditures has nearly exceeded the economic growth

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nearly in all OECD countries in the last 15 years. Since current resources are insufficient compared to this growth and it is difficult to meet all health needs in an optimum level with current means, it is concluded that health services need to be limited and prioritization concepts in health services have begun to be studied [3].

Prioritisation in health services is a complicated decision process which includes many dynamics and decision-makers such as plans/plannings regarding which services, regions, programs, hospitals, patients or diseases the resources allocated to health service should be divided into primarily.

Prioritisation in health services is realized by receiving opinions of all sides and analysing data regarding service demands. These are findings regarding politicians and health managers, service providers (doctor and other health personnel), service recipients (society) and cost and efficiency of health needs and proper responses/treatments [4].

Methods such as public opinion survey, opinion researches, public meetings, focus group studies are used to determine the priorities in health services.

Prioritisation and resource allocation in health service can be used in many criteria such as respect for humans, necessity, individual responsibility, efficiency, the number of persons affected as well as fundamental criteria such as lifesaving, cost- effectiveness, cost- benefit and equality [5].

Determination of priority studies have been realized in many countries for many years. National Health Accounts (NHA) and National Burden of Disease (NBD) studies were realized for prioritisation in health services in Turkey [6], but no study regarding the prioritisation in national and international military health services has been found.

Military health services include controls for compatibility and suitability to the task and periodical or mandatory controls, health certificate control, prevention and removal of medical threats, discharge, treatment, health logistics, blood services and other needs with regards to military health [7].

The aim of military health system is to provide sustainability for soldiers to be healthy, prevent the weakening of combat power due to epidemics, discharging the wounded in time and successfully and saving the limbs and life of the injured. In short, it is to increase the desire and confidence for warfare of each soldier by facilitating well-coordinated and not-failing health system, keeping militant power fit [8].

Purpose of this study is to determine priorities regarding health services by taking military operational requirements and features into consideration, shedding light on studies conducted with regards to restructure of military hospitals, revealing data to take a good turn in terms of resources allocated for armed services. Also, it is to contribute to the employment of military health personnel, especially doctors, medical non-commissioned officers and nurses, in the areas where there is actual need.

MATERIALS AND METHODS

Study of prioritisation in delivering health services is realized through focus group meeting which is a qualitative method. Focus group meetings were realized from September 2014 to April 2015. The universe of this study consists of sides of health services which are service providers (doctors, nurses and medical non-commissioned officers), service recipients (commissioned officers, non-commissioned officers, specialized sergeants/privates and their families) and health managers (hospital chief surgeons, health administrators and headquarters planners). Participants are selected from chief surgeons and hospital administrators who work in different military hospitals; doctors, nurses and medical non-commissioned soldiers who have worked in different garrisons and have experience in terms of troop and headquarters, willing personnel of commissioned officers and non-commissioned officers who received health service from primary care and secondary care health organisations – who accept to participate to focus group meeting.

Focus group meetings were realized in four stages in this study. In the first stage, the subject of prioritisation in health services is presented through a wide scale literature review. In the second stage whom to be interviewed was decided. Groups who participated to the focus group meeting and their numbers are presented in Table 1.

After reviewing the literature, the questions to be asked in focus group meetings were prepared with contributions from the Department of Military Health Services, the Department of Biostatistics and Epidemiology faculty members who have worked in different positions including administration in different levels of health system. Which titles were to be used and which questions to be asked in which order during the meeting were determined beforehand, the questions were prepared by abstaining from directing the interviewee.

In the third stage, the place and time of the meeting was determined. Meetings were minimum 60 minutes and maximum 120 minutes. 9 chief surgeons, 6 doctors, 6 nurses, 8 medical non-commissioned officers, 7 commissioned officers, 6 non-commissioned officers and 7 doctors/dentists participated to the meeting, respectively.

Table 1. Focus groups and number of participants in group consultation.

Status		Female		Male		Total	
		N	*%*	*N*	*%*	*N*	*%*
Healthcare Providers	Chief Surgeon	0	0	9	100	9	100
	Administrative Chief	2	33.0	6	67.0	8	100
	Medical/Dentist Officer	2	15.3	11	84.7	13	100
	Nurse	7	100	0	0	7	100
	Health Non-commissioned Officer	0	0	8	100	8	100
	Total	**11**		**34**		**45**	**100**
Healthcare Takers	Officer	0	0	7	100	7	100
	Non-commissioned Officer	0	0	6	100	6	100
	Total	**0**	**0**	**13**	**100**	**13**	**100**

In the final stage, the meeting was summarised, content analysis was performed on the acquired data and the meeting results were presented. Data acquired in this study is reviewed and interpreted by way of content analysis method, researchers compared data acquired from each focus group with other focus groups. Opposing and similar views are tried to be distinguished by benefitting from the perspective of each group meeting.

FINDINGS

Distribution of participants who participated to focus group meetings by force are presented in Table 2 and Table 3.

Table 2. Distribution of participants who provide health service by force (*there is no force separation regarding nurses*).

Status	Land Forces		Naval Forces		Air Forces		Gendarmerie		Total	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Chief Surgeon	7	78.0	1	11.0	1	11.0	0	0	9	100
Administrative Chief	5	62.5	1	12.5	1	12.5	1	12.5	8	100
Medical/Dentist Officer	7	53.8	2	15.3	2	15.3	2	15.3	13	100
Health Non-commissioned Officer	3	37.5	1	12.5	3	37.5	1	12.5	8	100
Nurse									7	100

Table 3. Distribution of participants who received health service.

Status	Land Forces		Naval Forces		Air Forces		Gendarmerie		Total	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Officer	5	71.4	1	14.3	1	14.3	0	0	7	100
Non-commissioned Officer	3	50.0	0	0	1	16.6	2	33.4	6	100
Total	8	61.8	1	7.6	2	15.3	2	15.3	13	100

When the answers of participants are reviewed in focus group meetings, most participants stated that there is “a requirement for prioritization” to be able to present available resources and means in an effective and efficient way in terms of delivering health service, 1 of the nurses and 2 participants who receive health service stated that “all services must be provided without any prioritization”. Chief surgeons stated health care needs of armed forces as prioritization criteria; administrators and doctors stated operational requirements, civil health care means, cost-effectiveness status; nurses stated regional needs; medical non-commissioned officers stated regional needs, disease burden, frequent diseases whereas service recipients put emphasis on efficiency and cost-effectiveness.

When asked who should play a role in prioritization, people who provide healthcare service stated that especially doctors should play a role, opinions of those who receive service should be obtained, also opinions of patients who receive healthcare service and their families as service recipients must be received and they also stated that opinions of health personnel are important. Hospital administrators stated that the prioritization is needed to be realized according to operational requirements and opinions of generals who decide the operation are also needed to be received. A participant in the service recipient group stated that prioritization should be done according to data in the database by establishing a council for health science.

When asked if preventive health service, diagnosis-treatment services or rehabilitation services should be prioritized in terms of health care delivery of armed forces, health care providers stated their opinions that “preventive health service” and “primary health service delivered within unit” should be at the forefront by taking operational requirements into consideration since armed forces personnel mostly consist of able-bodied, young men and health service is given to a group healthier compared to the society. The opinion that duty of armed forces is to deliver primary health service and secondary and tertiary health are possible to receive from civil health organisations is stated especially by doctors and medical non-commissioned officers. Nurses who mostly work in hospitals and healthcare recipients stated that diagnosis-treatment services should be prioritized.

When the current structure of military hospitals is considered, chief surgeons stated that the most suitable thing is “to reduce the number of military hospitals and turn them into a general structure in terms personnel and medical devices” and to enable the existence of military hospitals only in the regions where there are operations against terrorism and “to enable other regions to receive secondary health service from civil hospitals”. Hospital administrators stated that the hospitals are currently inadequate and they are in no state to compete with civil health organisations in terms of service quality and delivered healthcare. Doctors, nurses and medical non-commissioned officers stated that actions should be in accordance with operational requirement, thus military hospitals outside of the region where there are operations against terrorism should be closed since secondary health service can be received from civil hospitals and military hospitals in the regions where operations against terrorism are realized should be fully-organised and in a level enough to compete with civil hospitals. Service recipients pointed out that military hospitals are not currently cost efficient and there is no high-quality health service in these hospitals, they also stated that there are only need to be military hospitals in the regions where operations against terrorism are realised so as to provide high-quality health service.

When asked which areas of expertise should be available in military hospitals, chief surgeons stated that “psychiatrists are essential” due to the increase in the number of personnel and specialized sergeants/privates who have psychological problems in the recent period, they also stated that there is a need for branches such as orthopaedics, general surgery, anaesthesia for operations; administrators presented their opinions that there should be evaluations specific to the regions, there is a need especially for psychiatry, emergency medicine, surgical branches and anaesthetists; doctors/dentists and nurses stated that there is a need for internal medicine, psychiatry, emergency medicine, anaesthesia and surgical branches; medical non-commissioned officers stated that there is no need for gynaecology and obstetrics and paediatrics, instead there should be surgical branches; some service recipients stated that all branches should be available and the others stated that there should be branches that can treat the wounded personnel.

RESULTS

When studies on this subject realized in countries such as USA, England, Germany, Holland and Sweden, it is determined that a basic benefit package is created to provide a balance between limited health resources and healthcare needs, to prevent parameters such as treatment costs, medicine and surgery expenses within the scope of general health insurance from imposing an excessive burden on national economy. Committees are formed to determine health services which are to be included in basic benefit packages. These committees consist of the representatives of healthcare providers (such as doctors, nurses) and healthcare recipients (society). Even though these committees differ in each country, they prepare guides in which priorities in delivering health services are determined by meeting with people from all levels of society and taking health data of countries into consideration. Whether disease-treatment (medicine, surgery etc.) expenses are to be covered by health insurances is decided according to these guides. Within this framework, studies such as International Health Accounts Household Health Expenses and Disease Burden Projects are realized in our country in the recent time.

Military health services are primary health services provided especially for operation. It is determined by all participants in the focus group meetings that primary health service should be prioritized. It is pointed out in the focus group meetings that there must be doctors and assistant health personnel in primary health organisations; it is not enough to have only health personnel and laboratory facilities and medical devices and materials should be re-enforced. Hospital administrators and doctor's point to the increase in the number of personnel and specialized sergeants/privates who have psychological problems in the recent years in troops, psychological counselling and guidance services which are mostly given by reserve officers could be more efficient if they are provided by regular personnel or civil psychologists.

In the studies realized by Wiseman and Rosén, transparency, equality, justice, efficiency, responsiveness of health system and health personnel's approach towards patients are stated as priority determination criteria in health services. In the study realized by Mamas, priority determination criteria in health services is stated as maintaining trust and accountability between patients and doctors and establishing patient-centered approach [9, 10, 11]. In some studies, criteria are determined as benefit of patient, cost effectiveness and severity of disease [12, 13]. In the PHD thesis by M. Top, the principles of equality, health need, cost- effectiveness are placed near the top [5]. In this study, prioritization criteria are listed as operational requirements of the region, status of civil health facilities in the garrison, benefit of patient and cost-effectiveness.

Parties who are required to take part in delivering health service are stated as doctors, patients and their families, respectively, by the doctors and as patients and their families, doctors and health authorities, respectively, by the patients who receive service in a study realized in Greece. Both groups stated that politicians should play no part [11]. In the study realized by Wiseman and others, it is stated that doctors, health service administrators and patients and their families should especially take part in determination of priorities [9]. In the studies realized by Bowling and Kneeshaw, the result was that doctors, local health authorities and public should be a great part of the prioritization studies [11]. In harmony with the literature, the opinion regarding health personnel and especially doctors is stated by service providers whereas service recipients had the opinion with regards to patients and their families and doctors in this study.

When military hospitals are evaluated according to their current state, it is seen that there are no specialists in many branches of many hospitals and there is only one doctor when there is a specialist in a branch. All participants who participated to the focus group meeting stated that secondary health service delivered in many military hospitals fall behind of civil health services and it would be suitable if military hospitals outside of the region where there are operations against terrorism should be closed and secondary health service is received from civil hospitals. It is emphasised that military hospitals in operation areas where fights against terrorism happen should be fully-organised with sufficient personnel, medical device and equipment and can provide service for 24 hours in a day with ambulance helicopters and health teams. The fact that many military hospitals are currently in an idle state and they can only provide service as polyclinics, the number of in-patients and surgeries are very low is exemplified. There are similar problems in the United States of America. Existence of military hospitals and how efficient and effective they are in their current state have become highly questionable. Pentagon military health administrations state that primary mission of hospitals is military needs, doctors and nurses must develop their abilities for combat requirements. Two thirds of USA Military Hospitals treated 30 or less patients daily in 2013 and less than one-third of them treated as many patients as civil hospitals treated. Nine hospitals treated 10 or less patients [14]. The military worked in more than half of domestic hospital network until today. Pentagon analysts suggested that 15 of 25 hospitals are to be reduced, but the list has been reduced to eight due to complaints by Land, Naval and Air Forces [15].

When criteria such as operational requirements of military hospitals, status of civil health organisations in the region are considered, it is stated that areas of expertise that are required to have in military hospitals first of all must be to meet the operational needs; afterwards, psychiatry, internal medicine and anaesthesia, surgical branches such as orthopaedics, general surgery are listed by the participants. Common opinion of all groups who participated to the focus group meeting is that there is no need for branches such as paediatrics, gynaecology and obstetrics in military hospitals in the regions where there are operations against terrorism and these services can be delivered by civil health organisations. When the literature is examined, it is seen that 66% of injuries happening in theater of operations are orthopaedics, 21% is general surgery, 6% are of head and neck injuries, 5% are of burn injuries [16]. In another study based on the experiences of English military health personnel got in Afghanistan, it is determined that 93 of 124 people who had been wounded in battle lost their lives because of injuries in upper thigh, inguen and pelvic. Most of the casualties are because of pelvic injuries (17). Almost 11% of 7,856 trauma patients who applied to English Role-3 Sahara Hospital in Afghanistan from 2003 to 2011 applied because of chest trauma and almost 14% of them lost their lives. Most of the wounded people whose systolic blood pressures were detected very low had head and abdominal injuries and cardiac arrest and they caused mortality [18]. In another study, 83% of 271 people who lost their lives because of war injuries died because of brain injuries. 80%

of 287 people who were potentially survivable, haemorrhage caused by major trauma caused mortality. When bleeding site was examined in these people, it is determined that 48% of bleeding happened in torso, 31% extremity and 21% neck, axilla and inguen [19]. In a study realized by Owens and others, extremity injuries in 1281 soldiers are examined and it is seen that 53% of them have penetrating soft tissue injury, 26% have fractures and these fractures are localized similarly in upper and lower extremities [20]. Of injuries except thoracic injuries of 258 patients treated for thoracic injuries happened with weapons with high kinetic energy between 1996 and 2005, 13% are intra-abdominal injuries, 5% are fractures, and 3.5% are major vascular injuries [21]. The most frequent injuries of patients who are brought to an emergency ward in a hospital in Turkey after getting injured in the civil war in Syria are extremity injuries, internal diseases; chest, abdominal and head injuries with similar rates and eye injuries, respectively [22].

In the light of this information, as stated by the participants, it is important to have specialists on surgical branches such as orthopaedics, general surgery, brain surgery, cardio-vascular surgery, thoracic surgery and branches such as anaesthesia, internal medicine, radiology to be able to treat the wounded people effectively and increase the motivation of the personnel in the military hospitals in the regions where there are operations against terrorism.

DISCUSSION

The following results are obtained through this study realized with focus group meetings to determine priorities in delivering health service to armed forces.

Almost all parties and participants who participated to the study pointed out the necessity for determining priority in military health system to use resources allocated to armed forces effectively and efficiently since resources are limit, requirements are many. While making prioritization, it is stated that criteria such as operational requirements, structure of civil health organisations in the region and military health organisations being utilized by personnel and their families is needed to be considered because of unique structure of armed forces.

As a result of focus group meetings, it is determined that the opinions of doctors especially are needed to be at the forefront during prioritisation as well as opinions of health personnel, hospital managers such as chief surgeon, assistant chief surgeon, hospital administrator, privates who receive service and civil patients.

It is emphasised that the priority of armed forces should be primary health services and preventive health services and primary health service is a must for armed forces. Considering regional features and operational requirements, it is common opinion of all parties that secondary health service can be received from civil health organisations. Health boards in military hospitals stated that specific health services such as health service for operational requirement must be focused on and it is more appropriate for rehabilitation services to continue as they are currently.

It is determined that military hospitals cannot be utilized in an efficient and effective way due to the fact that personnel and their families prefer civil health organisations, there is not sufficient specialists, medical devices and materials are lacking, physical conditions of hospitals are worse compared to civil hospitals, there are problems in logistics and the system responds to the needs very slowly.

The importance of providing efficient, correct and quick primary health service is emphasised. Within this scope, it is pointed out that there must be a doctor regarding primary service, but it is not enough only to have doctors, there should also be laboratory facilities which consist of complete blood, urine, roentgen devices and primary health services must be supported.

Recently, there is an increase in the number of personnel and specialized sergeants/privates who apply to Psychological Counselling and Guidance Centers and it is determined that to deliver more productive service to these patients, regular personnel or civil officers should work instead of reserve officers so that the service becomes more effective and efficient.

When military hospitals are evaluated according to their current state, it is seen that there are no specialists in many branches of many hospitals and there is only one doctor when there is a specialist in a branch, especially in the surrounding hospitals. Participants are in agreement regarding the fact that the military hospitals in the regions where there are operations against terrorism must be re-enforced and turned into hospitals which have a fully-organised structured and several doctors in each branch to continue delivering service, hospitals except this must be closed or turned into health centers suitable to the unit needs.

Parties that participated to the group meetings emphasised the importance of quick and efficient patient/wounded discharge regarding operations is emphasised. Afterwards, it is concluded that having an ambulance helicopter which have regular health personnel who provide service in accordance with 24-hour principle within military hospitals and having first aid and emergency medical technician working in units which take part in operations improve the efficiency of health services.

The following suggestions are developed in the light of these results:

Health system of armed forces is required to be restructured in a way that efficiency and economy criteria are taken into consideration, medical board processes specific to armed forces can be realized, diagnosis-treatment abilities and capacities focused on unit and operation needs, in accordance with national health legislation are increased without losing specific features and by re-evaluating vision, mission, current situation and the point the national health system has reached.

Health Services Policy Determination and Assessment Board should be organised to give scientific support to restructuring studies of Health System, one member from the Departments of Public Health, Military Health Services, General Surgery, Internal Medicine, Psychiatry, Infection Diseases and Family Medicine and Health Services Management and the Department of Epidemiology, 5 Chief Surgeons from Military Hospital and 3 general practitioners should be assigned.

Inpatient treatment facility and laboratory facilities which consist of complete blood, urine, roentgen devices should be provided especially for trainings units to deliver more efficient and effective primary health services and to make these services more suitable to unit needs.

For health board needs, regional hospitals, military hospitals outside of the region where there are operations against terrorism should be transformed into proper health centers, other military hospitals should be structured in accordance with the criteria of the Ministry of Health through personnel and medical device savings with this transformation.

As specialist quota is determined, planning should be made by considering operational requirements of armed forces, current status of staff in health board branches, especially branches such as general surgery, orthopaedics, anaesthesia, internal medicine, psychiatry, and radiology should be focused on.

When the fact that there is an increase in the number of personnel and specialized sergeants/privates who have psychological problems in the recent periods, regulations regarding regular personnel recruitment should be made to solve problems arising from conducting psychological counselling and guidance services in the troops especially with reserve officers.

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ORIGINAL ARTICLES

Why cancer/terminal ill diagnosis unsuccessful in India: a qualitative analysis

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Abstract: *The increasing of the modern technologies requires the setting up of multiple factories and industries that produce smokes and chemicals, which polluted the air, water, and the environment resulting in putting lives in dreaded conditions. In fact, the coming of the modern era and the modern lifestyle gives birth to numerous deadly diseases like cancer and its related diseases, which increasingly takes the lives of many in the country. The cold-blooded killer 'cancer' becomes the major public health concern in India as a whole. Among all the problems existed across the world, the problems or the issues associated with cancer or terminal illness becomes the worldwide phenomenon that produces several unwanted worst experiences. Human polluting the water, soil, and the air continued, and the global burden of cancer affected populations rapidly increasing year after year. Unfortunately, India turns out to be the world most densely cancer populated regions and a cancer hub. The reason behind the rapidly increasing of cancer illness and its mortality rates in India is mainly due to people lack awareness on cancerous factors, no proper preventive measures, and the late detection of the symptoms at the advanced stages when cure is not possible.Thus, concerning the helplessness conditions of the cancer effected people of in general, the current analytical study has been forms with an aim to spread awareness on the cold-blooded killer cancer across the country.*

Keywords: *cancer, terminal illness, awareness, mortality rates, diagnosis*

Objectives: The underlying aim of the study is to investigate the underlying reasons behinds the rapid growths of cancer populations and the cancer mortality rates in the present India.

The study emphasis mainly on the cancerous factors, the underlying barriers behind the unfruitfulness of the terminal diagnosis, and the propose solutions or preventive measures in traditional ways.

Methodology: The current study is an analytical study on the collected data and reports of the following reliable sources:

- The Population-Based Cancer Registries Data' of the Central and State Governments.
- The Data from National Cancer Registry and Regional Cancer Centers,
- National Family Health Survey of India (NFHS-3), NICPR-National Institute of Cancer Prevention and Research, ICMR-Indian Council of Medical Research, Indo-Asia News Service (IANS), and Zee Media Bureau. The study also utilized the available journals database, along with WHO database. The researcher also accesses to the government's data and hospitals documents on cancer statistics and their reports.

Results: The modern turns out to be a cancer hub and the world largest contributor to cancer mortality rates. The numbers of cancer effected people increases every year, while the government had minimal inputs towards the preventive measures against cancer/terminal illness.

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INTRODUCTION

Looking at the current cancer statistics, it is visible that Indian as a whole fought against the cold-blooded killer ‘terminal illness’ in the most unsuccessful way. The failures of the health care systems of the country in its terminal diagnosis resulted in making the larger groups of the cancer patients facing the worst ill experiences one had ever gone through in life. India is the world largest contributor to cancer mortality with around 500,000 deaths per year, mainly due to people unawareness of the cancer and its symptoms diagnosis policy. However, no proper preventives measures or actions had not been taken up by the government, though the country is densely populated with terminal illness. Moreover, 80% of the cancer populations in India lives in rural undeveloped areas with low economic status, who were not able to afford for their cancer diagnosis and on the other hand, higher diagnosis fees in India. Thus, undergoes painful terminal ill experiences and live a life of suffering hell without accesses to any hospitals or clinical diagnosis. The Indian Council of Medical Research, New Delhi, predicted that the numbers of terminal deaths in the country would increases significantly in the coming years. Stating by 2020 around 1,700,000 Indian would be diagnosing this cool-blooded killer and its related deadly disease. Moreover, the percentages of cancer patients would likely to be increases by 20% in India, if the government did not take up any immediate quality preventive measures against this cool-blooded killer.

INDIA IN THE BATTLE OF TERMINAL ILL ILLNESS: THE PRESENT SCENARIO’S

In the definitions of World Health Organization (WHO), cancer or terminal illness is the uncontrolled growth of deadly cells with unstoppable spreads, which destroys every portion of human body organ one after another. Once the deadly cells increase and affected the patient’s start losing the functioning of his/her body sites that usually leads to a paralyses condition. These deadly cancer cells expanded through invading the surrounding tissues and spreads to other parts of the body by metastasis leading to 8.2 million global deaths a year at present. In which India becomes the world top contributor to cancer mortality rates with around 556,400 terminal deaths per year.[1] The modern India turn out to be a cancer hub with 2.5 million cancer-affected people, which would be expected to increase by 50% in 2020, if no immediate action plan had been done by the governmental and non-governmental agencies. At present a premature death through non-communicable diseases is the leading causes of deaths in India like cardiovascular ailments, chronic respiratory problems, and diabetes. [2] The cervical and stomach cancer symptoms populations in the Indian state of Mizoram alone is equal with the total numbers of cancer affected populations of Japan and is the highest cancer populated region in the world.[3]

The Zee Media Bureau Report, among the 8.8 million global deaths, in which India is consider as the world largest contributor to cancer mortality rates.[4] In another finding, around 505,428 to 500,000 cancer patients in India died per year. The main reason for the high cancer mortality rates in India is mainly due to people unawareness of the treatment procedures of cancer and its symptoms. This being the reason the cancer affected people consulting their Oncologists mostly when they are in the terminal stages, which is in the fourth stage’ of cancer symptoms that is impossible to cure.[5] Thus, around 80% of the deaths from cancer’s cannot be prevents in India today.

However, consulting their clinicians in the second stages of cancer diagnosis patients could possibly have at least 60%, for cure, while the interventions of cancer diagnosis in its third stages the cure possible rates reduces to 30%, but with the possibility of stage four of cancer within a short time.

However, there are no chances for survivor when it comes to the stage four of cancer that gives a maximum life spends of 5-7 years. Basing on the latest findings of NICPR report, Breast Cancer and Cervix Uteri is the two most common Cancer killers among Indian women, while Lip/Oral Cavity and Lung Cancer becomes the two most common killers among Indian men, killing around 253,521 Indians every year.[6] The last 20 years were the periods where cancer mortality rates has seen rapidly increasing in the country, while the numbers of cancer incidence has been decreasing in many western countries. Out of all the cancer mortalities, the cervical cancer remains the leading cause of deaths among Indian men and women follow by Breast cancer mortality.[7] The following tableshows the five most common deadly cancer symptoms, its causes, and the numbers of terminal deaths in a year in India as a whole (Table 1).

Looking at the current cancer statistics, it is visible that India as a whole is fighting with the cold-blooded killer “Terminal Illness” in the most unsuccessful way. India at present is in critical conditions with rapid increasing rates of one-lakh cancer populations per year and minimal cancer care center. There are around only 300 regional cancer care centers, which is not enough even to treat the one thirds of the cancer populations in the country. Moreover, India today has only 1000 oncologists, which is in the ratio of 1:2000 (one oncologist per two thousand cancer patients). This constituted the underlying reason why modern India turned into a cancer hub. The fighting with cancer will continues as the numbers of affected people raises up every year.[8, 9]

The following statistic is formed to explain in detail about the current cancer status in India (Table 2).

Table 1: The common types of cancers and the numbers of death in a yearly basis. [4-6]

No.	Cancer symptoms	Numbers of death per year	Main causes
1	Gallbladder cancer	230,000	Unhealthy lifestyle, genetic, poor prevention
2	Breast cancer	5,522,000	Unhealthy lifestyle, genetic, alcohol
3	Head & neck cancer	575,000	Alcohol, tobacco, cigarettes
4	Throat cancer	115,200	Poor nutrition, smoking, alcohol, genetic syndromes
5	Lung cancer	22,900	Smoking, tobacco
6	Oral cancer	245,800	Smoking, tobacco, alcohol, hpv, sunlight, weak immune system
7	Cervical cancer	67,477	HPV, birth control pills, smoking, unhealthy sex

Table 2: Current cancer statistic in India. Adapted from NICPR statistical report on August 23, 2018.

No. of Cancer Affected	Yearly Cancer Registration	No. of Deaths In a Year	Common Age's of Death 39-60 Yrs	Women	Men	Percentage
2.5 Million	7 Lakh	556,400	395,400	195,300	200,100	71%

In India, at present, one woman dies of Cervical Cancer every 8 minutes and for every newly diagnosis with breast cancer one out of two women dies in India. Moreover, around 2,500 died per day due to the cancerous factors like tobacco and bitternut. While smoking, which is the most common practices in India cause 1 in 5 death amongst men and 1 in 20 death amongst women that constituted around 930,000 deaths in 2010. In which the most productive age period could be highlights in the ages between 30-60 years, which need a special consideration and these ages grouped need to be targeted the most.[6]

Why cancer/terminal diagnosis unsuccessful in India today?

The main cause of cancer is through the internal factors (inherited mutations, hormones, and immunes conditions) and external/environment factors (tobacco, insufficient diet, unhealthy food, organism, and chemical with radiations). Among all these components for the causes of cancer and its deadly symptoms, there is a closed link between unhealthy food and insufficient diets with cancer disease as observed by many experts.[2] Unhealthy lifestyles with alcohol and smoking are the second most common cancerous factors leading to chronic disease, cardiovascular, lung, kidney, throat, esophagus, and breast cancer. The third common factor for the causes of cancer is visible in excessive consumptions of red meats and salted fish leading to heart and breast cancer.[10] At present as per the National Tobacco Control Programmed, the tobacco related cancer illness like Heart Attack, Lung Diseases, and Stroke etc. were the common types of cancer in the country. The uses of tobacco cause 100% poor oral health with 90% of mouth cancer, 80% of lung cancer, 50% of all human cancers, 70% of lung diseases, and 60% of heart attacks.[11] The above mention cancerous factors were the most common practices in the Indian Sub-Continent at present, leading to the rapid growth of cancer populations in the country.

Another reason for India being the top cancer populated in the country is mainly due to people unawareness of cancerous components, its preventive measures, and the treatment policy. Due to the lack of awareness, majority of the cancer effected patients detected their cancer symptoms only when the physical pain becomes unbearable, which is in the terminal stages (third or fourth stages) that is impossible to cure. Late detection of cancer symptoms and late cancer diagnosis becomes the underlying reasons for the 80% of the cancer population failing to cure in India today. However, early detections of the symptoms in its first stage there is 80% chances to cure. On the other hand, undergoing cancer diagnosis in the second stages could possibly have 60%, chances for cure, and in the third stage the cure possibility rates reduce to 30%, but with the possibility of stage four of cancer within a short period of time. However, in the fourth stage, which is the terminal stage there is no chances to cure, rather to live with the painful symptoms for maximum life spends of 5-7 years.[9]

The general causes of stomach and cervical cancer symptoms is due to the excessive drinking of alcohol, cigarette smoking, and chewing bitternuts. While, lung and oral cancer that hugely affect the Indian men and women populations, is mainly due to the excessive uses of tobacco, smoking, hookah, and the consumption of local made alcohol. Another prevailing cancer symptom in India today is the nasopharyngeal cancer, which is a malignant cells disease form in the tissues of the nasopharynx. The nasopharyngeal cancer (NPC) is the rarest type of cancer around the world; accept in the South East Asia, North Africa, and Arctic. The NPC is claims to be a Chinese origin, which is largely affecting the North East Indian states of Nagaland, Manipur, Mizoram, and to some extend to Meghalaya. The state of Nagaland has the highest age adjusted with 19.5/100,000, followed by the state of Manipur. The nasopharyngeal cancer is mainly causing by excessive eating of meat, fish, salted fish, uses of firewood in the house and other environmental related factors like the eating of bitternuts (Kuwa or Komkuwa) with or without tobacco products. Mostly the women populations were mainly affected by nasopharyngeal cancer comparing to men. Moreover, within 15-20 years the nasopharyngeal affected women populations will be increasing, if the uses of tobacco, bitternuts, and the uses of firewood in cooking are not being under-controlled.[3, 10] The table below will clearly show the various factors that are responsible for the causes of cancer as a whole (Table 3).

Table 3: The cancerous factors in India as a whole.

Particles	Cultivations	Liquids Components	Environment	Components
polycyclic aromatic hydrocarbures pahs	metals or coated with metals	diesel exhaust particles	toxin from fungi	smoking & hookahs'
vinyl chloride and benzedrine	excessive meats	unhealthy drinking water	chewing of bitternuts	junk food

Apart from food habits, the population explosion, rapid industrialization, and genetics, which include mutations, hormonal and lack of immunity are also responsible for the rapid growth of terminal illness in India as a whole. If proper awareness had not given mainly to the rural undeveloped areas of the country, there is a possibility of increasing cancer populations to 19% in the next five years. In which women have more chances of affected with cancers than men in India, as per the findings of many. Moreover, the mortality rate of cancers in India is visible higher among the illiterate people groupin the rural areas of the country than the educated, and the maximum deaths in India are mainly due to poor prevention strategies and no proper diagnosis as a whole [13]

According to the latest NICRP reports, 122,844 women are diagnoses with cervical cancer every year out of which 67,477 women died from cervical cancer per year. In a population of 432.2 million women in India at present, those women who are at the age of 15 and above and or between 15- 40 years are at risk of developing cervical cancer.[14] The health scientists on the other hand, were not able to identify the processes of how the risk factors like genetic, hormonal, and environment factors works together to cause normal cells to become cancerous tumor for the cancer symptoms.[10] Thus, cancer becomes the leading causes of deaths in India, with 2.5 million cancer populations, with 1 million cancer patients added every single year. It could be predicting that the numbers of cancer patients by 2025 would be increases by five-fold in India. The rapid increasing rates of cancer in the country are mainly due to lifestyle risk factors like uses of tobacco, alcohol, low fiber in diet, increasing body weight, minimal physical activities, and the reproductive risk factors regarding age at first pregnancy, and higher numbers of children breastfeeding's. Out of many cancer mortalities, the two leading deaths are from cervical cancer (HPV), hepatitis C (liver), and gastric H. pylori (stomach).[15]

THE PROPOSE SOLUTIONS AND PREVENTIVE MEASURES

Terminal death is the top causes of Death in India, in the year 2000 India was in seventh position among the world cancer populated country. However, due to the rapid increasing of cancer populations from 2006-2018, India successfully stood as the highest contributor to world terminal fertility rates, in which the numbers of effected populations is currently visible increasing with 100,000 per year. In recent research finding, only 5-10% the cancer is from genetics, in which the other 90-95% of the cancer and its related deadly diseases are from hormonal and environment factors.[16] Which means, the 90% of the cancer and its related diseases can be prevented effectively through proper medical interventions in its early stages and hygienic lifestyle. Moreover, another 10% genetic cancerous symptoms can also be prevented through early detection and immediate diagnosis. In short, cancer of any types is curable if detected in its early stages and through proper diagnosis. The followings are the qualitative preventive measures in order to control the rapid growths of cancer in the country: [2, 10, 16]

1. Regular medical checked-up, though being in a healthy condition, negligees of regular medical checked-up is visible as the underlying reason for the rapid growths of cancer in the country for the past ten years.

2. Educating people with proper awareness strategies, offer effective public health concerned in the schools and organizing a social meeting for spreading cancer awareness. Especially in the rural areas of the country.

3. Early detection of the symptom and early medical intervention, because when it comes to third and fourth stages there is no possibility for cure.

4. Public awareness on active physical activities, exercise at least for 30 minutes per day, the healthier a person is, the cells in the body can effectively fight against the invader virus and bacteria. Regular exercise prevents oneself from colon and breast cancer too.

5. Minimizing the uses of alcohol products, smoking, tobacco, and chewing of bitternut products, which will minimize the risk of having lung, kidney, throat, esophagus, and breast cancer. It is better to develop a moderate way of consuming with 2-3 glass of alcohol products per day, which will keep oneself away from heart attacked.

6. Immunization against hepatitis B virus to the infants of one to sixth months old without failed. Neglecting medical treatment in hepatitis B & C can causes chronic illness and liver cancer.

7. Developing healthy and safe sexual practices to avoid cancer genesis, unhealthy sexual practices can give birth to cancerous cells.

8. Avoiding obesities, as being grossly fat or overweight have negative effects on health and the makes cells less effective.

9. Developing healthy diets, a healthy diet has scientifically proven with numerous health benefits and reducing the risk from chronic diseases.

10. Reducing occupational and environmental exposures, as excessive exposures to chemical and its related heavy metals produces ill health. It also effected for the future offspring and produces toxicity.

11. Avoiding excessive consumption of red meats, salt and long preserved food. High consumptions of red meat, salt and preserved food leads to diabetes, breast cancer, and obesity.

12. Developing the habits of eating fruits regularly, most fruits are less in calories, sodium, and fats. Fruits are the sources of essential nutrients like potassium, dietary fibers, vitamin C, and folate (folic acid), which prevents deficiency, birth defect, and helps a person to growth with healthy blood pressure.

13. In order to have fair skins some avoid exposing to sunlight, wearing protective clothing during 10 am-4 pm, in which the excessive uses of sunscreen cream need to be avoid. Avoiding of exposing to sun or UV resulted in many women diagnoses with skin cancer. On the other hand, excessive exposing to sunlight or UV ray is also dangerous, especially for those having genetic cancerous symptoms.

14. Immediate medical intervention on virus and bacterial infections, otherwise the bacteria diseases virus cells usually hide inside the cells and turning out to be a terminal virus

Moreover, the rapid increasing of industrializations and urbanizations are visible as the two factors leading to new lifestyle of many Indians, which resulting in increasing the cancer affected populations in the country. Concerning the current polluted environment, the burden of cancer incidences will gradually keep on increasing, as majorities of the Indians were not aware of cancerous preventive measures. The only ways to prevent the Indian men and women from the rapid growth of cancer and its deadly symptoms is to detect the symptoms at the early stages with immediate medical interventions. It is also important to prevent the water and the environment from polluted by industries and chemicals. However, it can only be possible only if the men and women in the rural areas were being educated on cancer awareness like preventive measures, and overall treatment policies, which needs multiple efforts from the government agencies and other non-governmental agencies. Through such awareness programs, men and women should realize the risk factor and to identify the symptom through screening by physical examination or by self-cancerous symptom examination, in which if the rick substances where found than certain carcinogenic substances need to be reduces or eliminated. Moreover, there are over 85,000 synthetic chemicals that were easily available in the market of the country today like cosmetic items to flame-retardants, plasticizers in water bottles to pesticides in fruits and vegetables' etc.[13, 16] In the findings of the researcher, 80% of the cancer patients in the country were associated with environment factors like exposes to contaminants, unhealthy lifestyle, food, and exposing to ionizing radiations. In the urban areas, the cleaning of contaminated drainages without any proper preventive measures, polluting the river to the maximum with many forms of chemical and eating those fishes from the river produces several cancerous symptoms. The using the polluted water from the factories or industries for agricultural farming also produces several cancerous components leading in rapid increasing of cancer populations through consuming theses agricultural products. Thus, maintaining healthy environment, healthy lifestyle, healthy food, proper diets, daily exercise, staying away from tobacco, and smoking, with decreasing alcohol consumptions can decreases the rates of cancer-affected populations. Moreover, in the rural villages the practice of using of those water which were uses in deeping hot metal or iron in the blacksmithfor washing hand and leg need to be avoided, as it contains the component that is cancerous.

CANCER TREATMENT POLICY AND TERMINAL DIAGNOSIS

The WHO (2017) stated that, the cancer patients need immediate access to the modern equipments like: 3D Conformal Radiation Therapy, Intensely Modulated and Therapy (IMRT), Image Guided Radiation (IGRT), VMAT and Rapid Arc-Volumetric Modulated Arc Therapy (VMAT), Low Dose Rate Brachyntherapy (LDR), High Dose Rate Brachyntherapy (HRD), Deep Inspiration Breath Hold (using the goggles or snorkel technique), and Stereotactic Radiation Therapy.[17] However, it is sad to say that, due to the unavailabilities of the above mention modern cancer treatment equipments, cancer patients in India could not undergo such treatments. Failing the interventions of modern equipments in terminal diagnosis, resulted in uneffective diagnosis in many cases. The quality treatment plan and policy are the core components for successful cancer metastasis diagnosis, which is also visible ineffective to the minimal. The main emphasis of a cancer diagnosis is to cure the symptom of the patient or to prolong the lives' of the patient through ensuring quality of life.[1] However, the greatest challenges in the Indian cancer diagnosis is the ensuring of the patient quality of life, which is not visible in the clinical practices of the country as a whole. Unnecessary prolonging of the patient lives with an aim to increases the numbers of days spend in the hospital ward for more bills. The patient's value and dignity were unconcern the most by the clinicians' and the patient undergoing treatment against their will/choices need to be rectifying immediately in the health care systems of the country as a whole.

The early detection and immediate treatment intervention are the two most effective way of cancer diagnosis to control the cancer metastasis and to deliver total cure. As per the above findings, the medical intervention in stages one and two has higher possibilities rates to cure, which has around 80% cure possibilities as per the finding. While detecting the cancer symptom and consulting the oncologists in stage three is visible in curtail conditions, having higher impossibility rates as the cancer metastasis accelerates in higher speeds from one body part to another. However, if the stage three patient being diagnosis by special medical team in a well to do clinical setting, it has around 60% possibility chances to cure. But does not guarantee a complete free from cancerous cells, as there is a possibility of being in terminal stage after three to five months. The reason being the cancer cells have the possibility of hiding under another cells that is hard to detect, resulting in leading one's life a disability-adjusted life years (DALY). Nevertheless, the detection of the cancer symptoms in the fourth stage have no possible way to cure by any means, which is term as the 'terminal stage' that is 100% impossible to cure in any clinical practices. Thus, resulting in leaving the patient with disability-adjusted life for five to seven years until the inevitable death strikes him/her.

These 5-7 years period of terminal experience is the most crucial moment for every terminal patients, which is also considers as the worst moment in a terminal experience with heartful of emotional sufferings and mindful of mental disharmony that needs special considerations to the most. The acknowledgment of the psycho-emotional symptoms alongside the treatments of physical pain is very essential in any terminal diagnosis in the clinical practices. However, at present India turns out to be the worst place to die or a place not to die. The reasons being the undergoing cancer/ terminal diagnosis core emphasis is on the physical pain treatment alone, leaving the psycho-emotional pain and suffering untreated. The psychological suffering and mental disharmony are the bi-products of the terminal illness undergoes by every terminal patients were considered the symptoms that need to be treated in the clinical practices in India. Thus, failing to acknowledge and leaving the psycho-emotional symptoms untreated resulted in worsening the patient's conditions. The psycho-emotional and mental well-being is for the successful treatment of the patient physical pain symptoms. (Suantak & Sisodia, 2018). Well being of the whole body requires a psycho-emotional and mental well-being to the most; a psychological well-being can give positive responses to physical pain treatment to the maximum.

In India as a whole, the clinicians tend to forgot that even when cure is not possible in terminal diagnosis, there is a possibility of delivering healing as an alternative to cure. However, only through a person centered meaning making psychotherapy in the clinical practices. The psychological self-reflective and life review therapeutic approach in terminal diagnosis can make oneself aware that he/she is still in the condition of limitless achievements. Which will in turn helps the patient to recreate his/her life goals, and set new life goal that could be achievable, also able to make beautiful memories with his/her loved ones that delivers the quality of life in terminal experience. Above all, it will make dying as normal as birth, which will deliver peaceful and meaningful death (Suantak & Sisodia, 2018). Thus, in terminal diagnosis, healing can be deliver as an alternative to cure, even when total cure is impossible in the clinical practices. However, sadly, in the clinical practices of India as a whole, the clinicians alone are the core medical team in the cancer/terminal diagnosis, in the absences of professional clinical psychologists and clinical social workers. The terminal diagnosis requires a multidisciplinary team to deliver quality of life and quality end-of-life care. The clinicians were responsible for treated the physical pain symptoms and its related, while the clinical psychologists and clinical social workers were responsible for the patient psycho-emotional suffering and mental disharmony treatments, which the trained medical doctors or nurses cannot handles in the clinical practices.

CHALLENGES AND CONCLUSION

Recently, Prime Minister Narendra Modi inaugurate "Ayushman Bharat Health Scheme" on September 23, 2018, which would be effective by 25 December 2018 in Ranchi with a primary aims to provide free health care to those underprivileged living in the rural areas, who are access not to any health care thus far. It means there are Indian who does not received any medical facilities thus far, which remain as the most challenging and root causes to maximum cancer/terminal illness in the country. As per the findings, 500,000 Indian died of cancer every single day and the government with the least concerned on the awareness programe and in establishing regional cancer care centers. This being the underlying reasons India successfully becomes the largest contributor to cancer mortality rate. Another reason for the rapid increasing mainly rates of cancer and its mortality rates is mainly due to people unawareness on the causes of cancerous factors, treatment policy, unhealthy lifestyles, and unhealthy food consumptions with no proper preventive measure interventions. Majority of the cancer patient in rural regions failed to maintain their diets, while proper dietary is the core to successful diagnosis. Moreover, eliminating the usages of tobacco, cigarettes and chewing of bitternuts can successfully reduces the rates of oral lip, breast, mouth, cervical, head and neck, and the nasopharyngeal (NPC) cancer into at least 40-50% as per the researcher findings. Another urgent need is to develop social awareness on the causes of cancer, and treatment policies by educating the people mainly those leaving in the rural areas of the country. Gearing up for health awareness and strengthening the health care team at the community level by addressing the cancer preventive measures will effectively reduces the growing cancer populations.

Hygienic living with healthy food awareness also the urgent requirement to fight against the deadly diseases, with the appointed staff, conducting seminar in each village at least once in a year. Training more oncologists in the country would also be an effective ways to successfully fight against the ongoing cancer mortality in the country. However, as of now the ratio of oncologists and cancer patients in the country is 1:2,000, and that is next to impossible for an oncologist treating 2,000 patients in a day. The Centre for Cancer Epidemiology in the rural areas of the country is at present minimal and majority of the cancer care centres are not functioning properly, whichis the major challenges for the government to take immidiate initial action. It is a high time for India to take up the necessary actions against cancer mortality as the rates of mortality increased year after years with 70-90% per 100,000 populations, which constituted around 2,500,000 (2.5 millions) with 800,000 new cases every year and 5,50,000 deaths in a year. Moreover, in the battle of terminal illness fighting back and defending people is the only option that India had. For the love of humanity, let the terminal diagnosis acknowledge the psycho-emotional suffering and mental disharmony of the patient alongside the physical pain symptoms to deliver the whole person treatment in the clinical practices.

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ORIGINAL ARTICLES

Simulation and dynamic analysis of military marching using lower limbs anthropometric data

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Abstract: *Background: Gait analysis is receiving increasing attention due to various applications in athletic performance, man-machine interfaces and especially in military services. This analysis involves the analysis of human locomotion augmented by body movements and biomechanics of joints. The kinematic motion of the body during a gait cycle capturing by cameras is then used as the desired target for modelling the motion of body segments. By taking advantage of gait analysis concept, this study aims to model the military marching, using anthropometric data with the focus on lower limbs while introducing top candidates with better healthy conditions in lower limb joints during a cycle of marching.*

Methods: Using 100 anthropometric data from military soldiers, equations of motion for the model are derived by applying Lagrangian methods in an inverse dynamic approach. In this model, the joints are simulated using springs and dampers while the actuators, simulated the muscles, acted like motors and applied enough torque on joints so that the model motion replicates normal military marching. Finally, all the springs and dampers coefficients are driven from optimization process.

Results: Hip, knee and ankle torques were calculated after the optimization process for all 100 soldiers and then 5 candidates among them were established with less suffering forces and torques in their joints.

Conclusions: In this study using biomechanics basics and anthropometry data at the same time, a standard could be evaluated to select the soldiers based on healthy condition of lower organs.

Keywords: *marching, anthropometric data, gait analysis, biomechanics, torque, equations of motion, optimization*

INTRODUCTION

Marching is a specific sample of daily human gait [1]. That being said, in order to simulate marching, simulation of human gait cycle is required in which we should benefit from priori biomechanical knowledge on human motion and anthropometric data at the same time [2]. The gait cycle consists of complex functional tasks requiring interaction between lower limb joints of the body [3]. Motion of body segments during this interaction is studied as a part of kinematics without considering any forces. Typically, camera systems and electromagnetic devices are being used to record the motion of the body during a cycle [4].

While the body segments are considered as a mechanism, joints in hip, knee and ankle provide degrees-of-freedom

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(DOF) of the system. The joint reaction forces and muscle net torques convert this problem into a dynamic analysis in which inverse dynamic method is being used commonly. Then, equations of motion relate the kinematics of the body motion to the forces and torques that are causing those motions [5]. Friction force calculation completes this set of equations as an external force. This force during a walking depends highly on the foot-ground contact simulation and modeling. Some of the studies used additional kinematic joint while other used temporal fixing to the support foot. But in some studies, realistic models for this section have been modeled which can simulate the reaction close to the real foot-ground contact [2, 6]. Some studies conduct the whole gait simulation by taking advantage of three dimensional models [7, 9] while others consider two dimensional models [3,10,11] for simulation [4,8]. Stability of such models is significantly important. Active feedback control systems [12] and simple PDI controller [10] are common methods used as a controller for the balance of gait models during a cycle.

Application of gait analysis in military services mainly limits to assessing the influence of boot [11, 13] and carrying a backpack of soldiers during a gait cycle. Rare studies like [1] demonstrated one of the first researches on marching locomotion. As other applications of gait analysis, there have been some studies about modeling the gait analysis in order to analyze the dynamic of prosthesis during a gait cycle [3, 10, 14, 18, 19, 20].

Towards simulation of marching, this paper conducts this analysis by computing the internal actuator forces and dynamic analysis of 100 soldiers. The major part of this simulation is to replicate normal kinematics of marching when subjected to normal muscle forces and joint torques during a cycle. In an inverse dynamic approach, using Lagrangian method the net joint torques is calculated from a sample military marching benchmark and external forces in a condition that muscles are the actuators of the musculoskeletal system. The optimization process then is needed to compute the forces and the torques in a way to generate the desired kinematic motion.

METHOD

From the engineering point of view, marching is in fact body segment movements in a mechanism way with couple of degrees of freedom (DOF). This mechanism consists of hip joint, knee joint and ankle joint. There is friction force between foot and ground which provides movement of the whole body. In this simulation, bones are considered as rigid elements while supporting the weight of the body and muscles act as elastic stimulators and displace the bones.

The appropriate method in studying the simulation of marching is by applying dynamic models. Using this method, the model was considered as a rigid body with several links. Considering exact and complete number of variables for dynamic simulation complicates the model and motion equations, therefore in this study some assumptions were employed to simplify the model. These assumptions include 1) the upper section of the body (head, trunk and hands) was considered as one link only, 2) friction was neglected in joints, 3) the weights of the elements were equally distributed, 4) modelling and solving equations of motion conducted in sagital plane (2D), 5) due to 2D modeling, joints were from hinge type and not spherical for hip joint.

The model for rigid body motion of a soldier contains seven links that include trunk, two thighs, two legs and two feet. The model was defined with nine generalized coordinates as consisting of seven links with vertical angles and hip coordinates in Cartesian coordinate system (Figure 1). In this model, the angles are trunk, right thigh, right leg, left thigh, left leg, right foot and left foot angles with vertical direction while the joints are hinge joints.

Figure 1. The model used in simulation with seven angles and hip coordinates

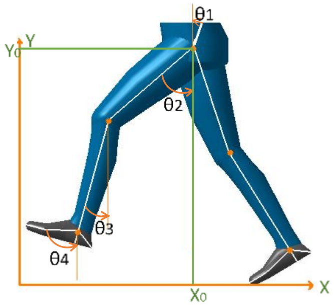

One of the most important issues in this dynamic simulation is modeling the reaction force of the ground to the body. The modeling of this section was developed based on using a spring and a damper in contact points of foot to the ground so that the equivalent of damper and spring forces reflects the ground reaction force to the body [16]. This estimation helps to calculate this reaction force during dynamic simulation and to simulate the viscoelastic contact of the foot. As it can be seen in Figure 2, a series of vertical springs and dampers were attached to the contact points to generate this vertical reaction force [10]. In the case of no contact between foot and ground, the spring and damper contacts are disconnected and no force is reacted and whenever the contact between foot and ground occurs again, the spring and damper contacts are connected and so the force is reacted.

Figure 2. Foot-ground contact force simulated by spring and damper

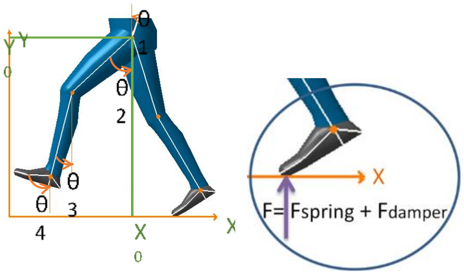

In this model, the muscles are neglected and instead, actuators are employed in hip, knee and ankle joints. The torque being applied on the joints is a function of current angle in joint, desired angle and their derivatives.

Indeed, springs and dampers are used in joints so that the actuators act as motors which are linked with these springs and dampers. Therefore, appropriate modelling is needed for calculating the optimized variables for springs and dampers. The general equation for torque in the joints is

$$\tau_i = K_i(\theta_i^m - \theta_i^d) + C_i(\dot{\theta}_i^m - \dot{\theta}_i^d)$$

In which K_i and C_i are constant coefficients, θ_i^m and $\dot{\theta}_i^m$ are angle and angular speed of links in each time frame, θ_i^d and $\dot{\theta}_i^d$ are desired angle and angular speed of the links.

Spring and damper are key factors in stability of the model during marching. If these factors are neglected, any small disturbance in the model at the contact time leads to instability of the whole system. On the other hand, calculating the optimized coefficients requires desired kinematic data and stability of whole model.

Table 1. Statistical parameters of all 100 anthropometric data of soldiers

Data	Waist height *(mm)*	Knee height *(mm)*	Weight *(kg)*	Height *(mm)*
Minimum	820	410	52	1,615
Maximum	1,152	602	78	1,875
Average	985.91	516.91	67.55	1,738.55
SD	58.12	35.94	7.07	60.17

In this study, anthropometric data of 100 military soldiers were analyzed through the dynamic simulation method. Table 1 shows the values of min, max, average and standard deviation parameters for all 100 soldiers. Using waist height and knee height, thigh length was calculated and based on the lengths of segments, the weights of segments were approached [15] (Table 2).

Table 2. Standards for mass calculation for body segments

Segment	Center of mass/segment length		Density *(Kg/l)*
	Proximal	Distal	
foot	0.5	0.5	1.1
leg	0.433	0.567	1.09
thigh	0.433	0.567	1.05
trunk	0.5	0.5	1.3

While the anthropometric data varies from one soldier to another, the kinematic data is the same for all soldiers. The kinematic data must be considered the same for all since the ending result of joint torques for the model varies for various kinematic data but this study targets to analyze the joint torques only based on anthropometric data, so that as a result a standard of anthropometric data could be concluded.

Therefore kinematic data of a marching benchmark (Figure 3) was considered for all soldiers.

Figure 3. Marching benchmark used in kinematic section of this study

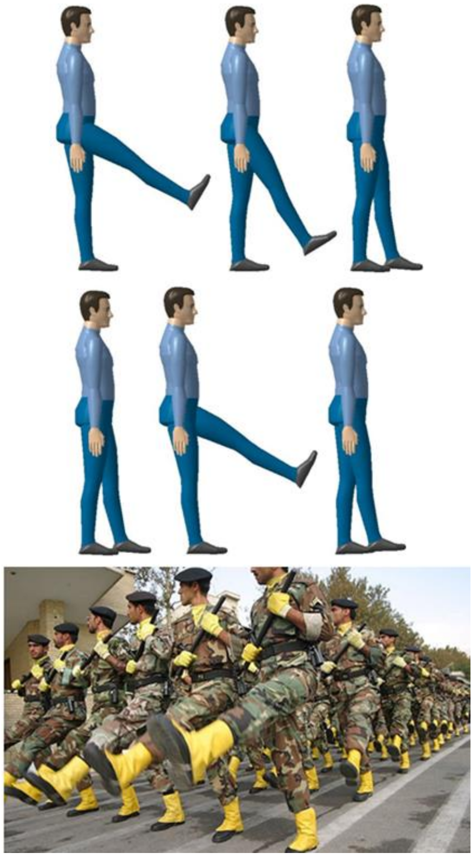

As this figure depicts, right thigh and leg are raised to specific height in an extension movement and then in a felxion movement, right foot makes contact with the ground and then left segments follow the same path alternatively. Knee angle was considered the same as hip angle which means that the knee by itself does not have any flexion-extension movement.

Kinematic data includes, 1) angular speed and angle of hip joint 2) angular speed and angle of knee joint 3) angular speed and angle of ankle joint 4) angular speed and angle of trunk. Figure 4 shows the angle of hip and ankle during one cycle of marching. Fourier function was used in order to generate a curve passing through acquired kinematic data points.

Figure 4. Hip and ankle angles during a gait cycle of the benchmark

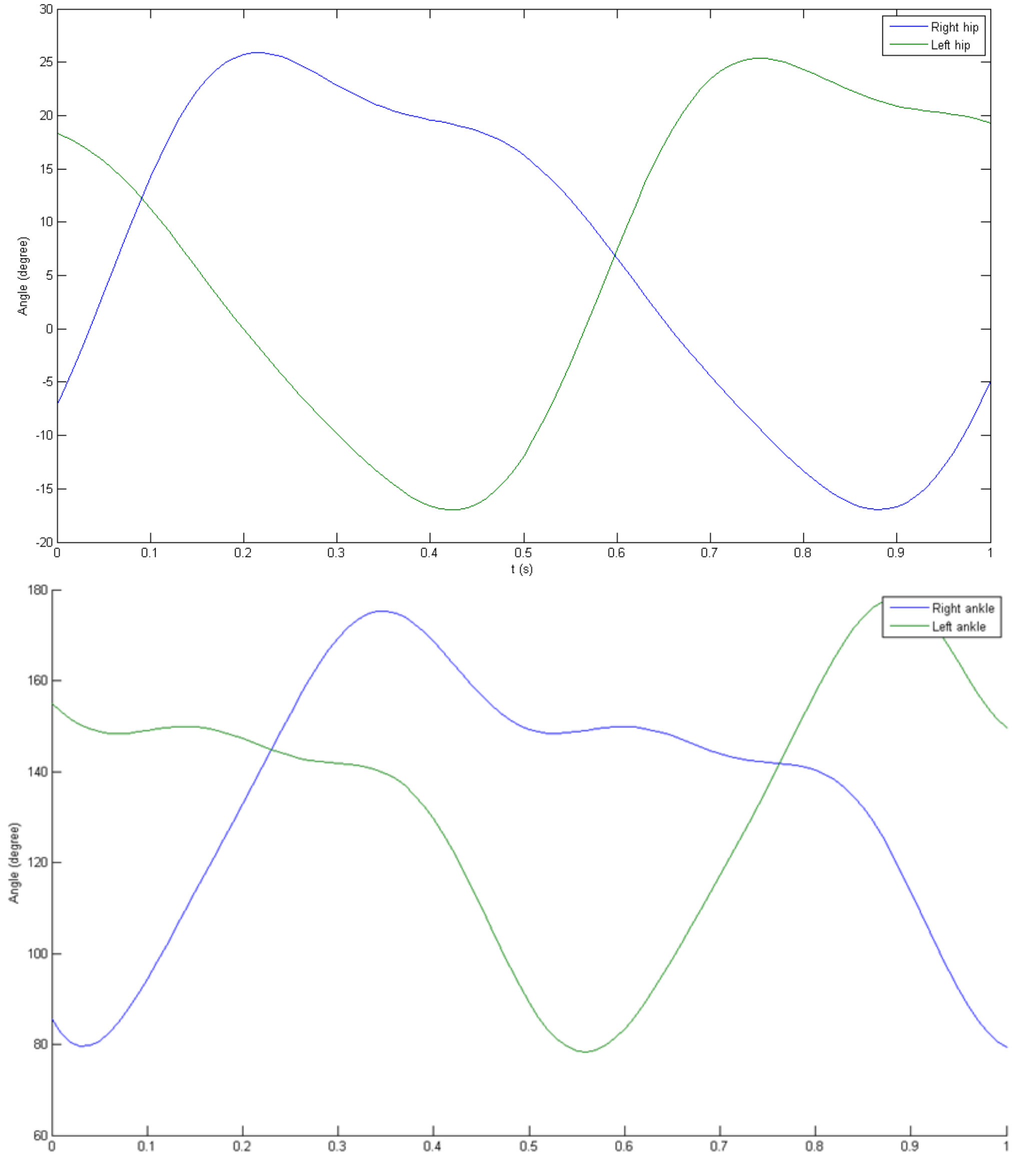

In order to complete the modeling of marching, adding a parameter to dynamic simulation is needed for keeping the balance of the model. To accomplish this step, a torsion spring and a linear damper are applied to the trunk as a function of simulated and desired kinematic of trunk. Whenever the model is contacting the ground half of the total torque in this torsion spring and damper is transferred to each hip joint to keep balance of the whole model.

As it was mentioned in previous section, the equations of motion for this 2D model follow Lagrangian method. General formulation of the Lagrangian method is

$$\frac{d}{dt}\left(\frac{\partial L}{\partial \dot{q}_k}\right)+\frac{\partial L}{\partial q_k}=Q_k$$

In which k is number of general coordinates which is 9 in this model, L is the difference between kinetic and potential energy of the whole system, Q_k reflects non-conservative forces. In order to use these equations, initially kinetic and potential energy of the whole system were calculated and then after 9 equations of the order of 2 were produced. For the dynamic simulation using this method, initial conditions and angular speed of the links were provided.

Constant coefficients in all joint torques (discussed in previous sections) could be generated in an optimization process. The goal of optimization is to calculate these coefficients in the condition that the discrepancy between flexion-extension angles of joints in simulated and desired circumstances is minimal. The desired (normal) values for this simulation were obtained from [17]. In order to conduct this optimization, genetic algorithm method was applied to decrease the difference between angles in desired and simulated modes. While in this optimization variables are the constant coefficients, the objective function is the summation squared of the difference between these angles. Below are the steps that the genetic algorithm takes to find the optimization coefficients:

✓ Initial parameters are provided as the initial condition of the problem,

✓ Initial guess of the spring and damper coefficients of the model is provided,

✓ Based on the values from steps 1 and 2, the equation of the motion is formed,

✓ From this equation of the motion, the angles function of the model is derived,

✓ By having the angles of the model and desired angles (from the literature), the objective function is found,

✓ The genetic algorithm comes to play and starts to minimize the objective function by finding the optimized values for spring and damper coefficients of the whole model

✓ Once the optimized coefficients are found from the step 6, the values are replaced with coefficients of step 2 until final optimized converged coefficients are obtained.

✓ Return to step 3.

RESULTS

The numerical programming for dynamic simulation of a soldier was accomplished in MATLAB software. After providing all necessary parameters such as initial conditions, kinematic data, equations of motion and optimization, the whole program focused to generate the constant coefficients using the optimization method for a sample anthropometric data. Then, whole 100 anthropometric data was given to the program to calculate the joint torques in hip, knee and ankle. In fact this torque value is equivalent to maximum total torques acting on that specific joint. Table 3 shows the statistic about resultant torque values.

Table 3. Resultant torques in hip, knee and ankle joints

Segment	Hip torque *(N.m)*	Knee torque *(N.m)*	Ankle torque *(N.m)*
Min	20.96	0.06	1.73
Max	22.94	1.37	3.50
Average	21.65	0.27	2.61
STD	0.33	0.19	0.37

Table 4. Top 10 candidates with lowest toque amount in hip joint

Hip torque *(N.m)*	Knee torque *(N.m)*	Ankle torque *(N.m)*	Waist height *(mm)*	Knee height *(mm)*	Weight *(Kg)*	Height *(mm)*
20.98	0.15	2.42	990	480	70	1,775
20.99	0.12	2.36	975	500	62	1,705
21.00	0.12	2.36	990	480	70	1,735
21.03	0.31	1.88	880	490	60	1,632
21.04	0.35	2.98	970	550	75	1,797
21.05	0.11	2.55	995	500	66	1,730
21.08	0.33	2.25	1,110	575	70	1,780
21.09	0.21	2.13	1,015	570	75	1,788
21.10	0.55	2.61	1,040	500	52	1,700
21.11	0.11	3.11	1,100	565	85	1,895

In the initial analysis of this huge data, we focused on hip data. Since in this study having healthy joints are more desirable, we sorted the data from small to large values and then just consider the first top 10 as good candidates for healthy joints (Table 4). This narrowing down of the results helps us to analyze the data more consistently.

Moreover, Figure 5 shows how the waist height and knee height vary by the torque in the hip. It is interesting that both heights increase by gradually increasing of the hip torque. This fact is confirming that higher waist and knee heights can lead to larger torques in the hip.

Figure 5. Variation of waist and knee heights by hip torque increasing in top 10 candidates with lowest torque amount in hip joint

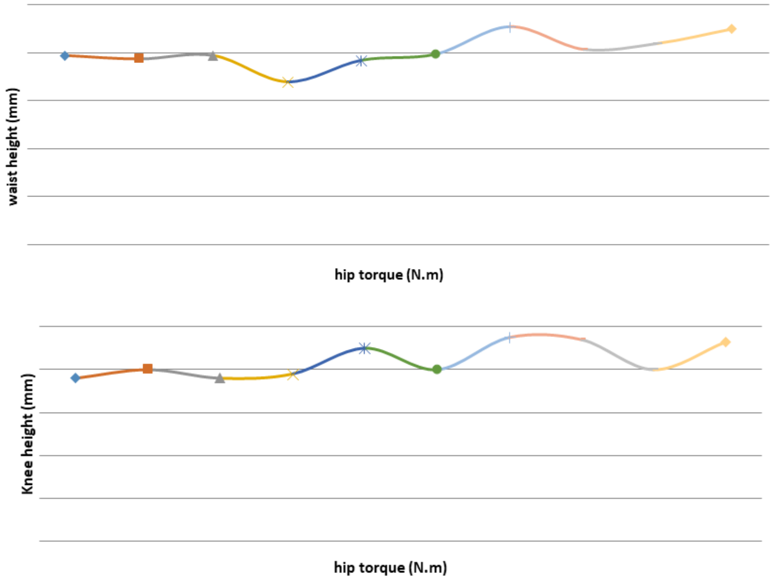

In the next step, knee torque was assessed by the numerical programming code in MATLAB and the results were again sorted from small to large and first 10 candidates were selected. This selection was done independently of previous selection for the hip. Indeed, this study approached the joint torques behaviour separately and then concludes all the resultant data together. The corresponding knee data can be found in Table 5.

Table 5. Top 10 candidates with lowest torque amount in knee joint

Hip torque *(N.m)*	Knee torque *(N.m)*	Ankle torque *(N.m)*	Waist height *(mm)*	Knee height *(mm)*	Weight *(Kg)*	Height *(mm)*
22.15	0.02	3.74	1,152	602	79	1,915
22.04	0.03	4.27	1,030	530	73	1,805
21.79	0.04	3.25	900	490	72	1,705
21.80	0.05	3.69	980	500	72	1,743
22.05	0.05	4.07	1,080	520	68	1,805
21.60	0.06	3.35	975	480	63	1,642
21.83	0.06	3.48	1,000	480	78	1,745
21.25	0.06	2.69	1,000	540	65	1,780
21.25	0.07	2.69	998	510	57	1,695
21.93	0.07	3.91	1,000	525	75	1,710

Comparing the torque values in knee joint to other two joints, it can be concluded that for this specific kinematic data for marching, the torque in knee is negligible. Therefore, for the ending result of this study, knee did not play a major role and that is because flexion – extension movement was not considered for the knee.

Ankle torques is the next criteria considering as a result. Table 6 represents the torques in ankle joint during a full cycle of marching for the top 10 candidates who their ankle torques were minimal independent from other joint torques.

Table 6. Top 10 candidates with lowest torque amount in ankle joint

Hip torque *(N.m)*	Knee torque *(N.m)*	Ankle torque *(N.m)*	Waist height *(mm)*	Knee height *(mm)*	Weight *(Kg)*	Height *(mm)*
21.22	0.08	1.75	1,070	520	78	1,835
21.03	0.31	1.88	880	490	60	1,632
21.36	0.47	1.95	1,148	600	75	1,900
21.63	0.11	1.98	994	500	71	1,766
21.63	0.50	2.08	945	475	72	1,680
21.09	0.21	2.13	1,015	570	75	1,788
22.07	0.13	2.15	1,040	530	56	1,708
21.58	0.35	2.18	930	480	69	1,720
22.01	0.22	2.23	1,010	535	80	1,758
21.08	0.33	2.25	1,110	575	70	1,780

Comparing the variation of the ankle torque versus waist and knee heights depicts the fact that ankle torque is highly dependent on the force being applied by the ground, since the waist and knee heights are almost constant with the variation of ankle torque (Figure 6). In fact the amount of ground force reaction plays the major role in the torque of the ankle.

Figure 6. Variation of waist and knee heights by ankle torque increasing in top 10 candidates with lowest torque amount in ankle joint

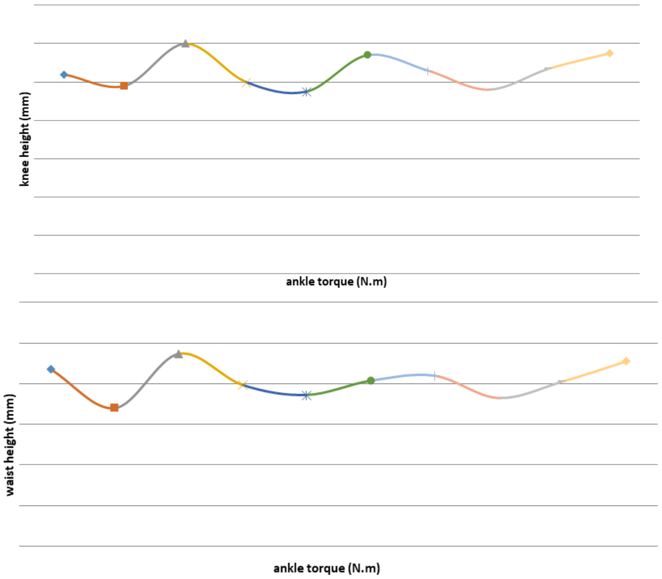

DISCUSSION

In order to assess the healthy joints under marching condition, 2 factors were considered in this study: 1) hip torque and 2) ankle torque. Then based on the torque data of each joint, the whole data was sorted from smallest to largest value three times. Then, first 10 candidates in each section were highlighted and analyzed. In the first look, it seems like 20 different candidates with identical anthropometric data have been introduced, therefore it is needed to define another factor in order to select from those candidates, those with better healthy conditions in terms of minimum torque value in their joints. Due to less torque rate in knee comparing to hip and ankle, a new factor called "Minimal Summation Torque" is defined for this study which follows below equation, considering the fact that the maximum torque in hip is 22.39 N.m and in ankle is 4.35 N.m.

$$minimal\ sumation\ torque\ = \frac{hip\ torque}{maximum\ hip\ torque} + \frac{ankle\ torque}{maximum\ ankle\ torque}$$

By using the maximum values of torques we normalized our torque data. Then, we needed to calculate this factor beside other factors for all 100 data and sort again based on minimum value of torques. Table 7 shows this result.

Table 7. Top 10 candidates with lowest Minimal Summation Torque

Hip torque *(N.m)*	Knee torque *(N.m)*	Minimal Summation Torque	Waist height *(mm)*	Knee height *(mm)*	Weight *(Kg)*	Height *(mm)*
21.22	1.75	1.35	1070	347	78	1835
21.03	1.88	1.37	880	325	60	1632
21.36	1.95	1.40	1148	325	75	1900
21.63	1.98	1.42	994	347	71	1766
21.09	2.13	1.43	1015	337	75	1788
21.63	2.08	1.44	945	336	72	1680
21.08	2.25	1.45	1110	348	70	1780
21.58	2.18	1.46	930	334	69	1720
21.34	2.26	1.47	980	296	72	1710
21.23	0.33	2.28	1005	330	66	1722

Analyzing Tables 4, 6, 7 reveals that there is a common point between all of them. There are some soldiers that exist in all those tables, showing that these candidates are common among all other candidates. This approves that this set of candidates contain a healthier body during a cycle of marching. We highlighted these candidates in all tables and allocated one colour to each of them. Table 8 represents all of them in one separate table containing corresponding anthropometric data.

Table 8. Top 5 candidates with healthier joint condition during a cycle of marching

Waist height *(mm)*	Knee height *(mm)*	Hip circumference *(mm)*	Knee circumference *(mm)*	Ankle circumference *(mm)*	Weight *(Kg)*	Height *(mm)*
880	490	990	416	230	60	1632
1148	600	990	460	230	75	1900
1015	570	940	380	250	75	1788
945	475	940	400	250	72	1680
1110	575	980	410	270	70	1780

In fact, in this study using biomechanics basics and anthropometry data at the same time, a standard could be concluded to select the soldiers based on healthy condition of lower organs.

This study is one of the first studies being conducted in the area of specifying a standard for soldiers based on their lower organ healthy condition during a cycle of marching.

Conflict of interest

The authors have no conflicts of interest.

Authors' contributions

Dr. Shakibaee provided assistance for all experiments and collecting anthropometric data. Dr. Asgari supervised this study and provided assistance in manuscript preparation. Dr. Kamal Mostafavi carried out the modelling, coordinated the study, completed the analysis, extracted the results and prepared the manuscript. Dr. Pourtaghti and Dr. Ebrahimpour assisted and coordinated the processing of data collection. All authors have read and approved the content of the manuscript.

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ORIGINAL ARTICLES

Drug allergies interpretation based on patient's history alone may have therapeutic consequences in hospital setting

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Abstract: *Background and aim. Adverse drug reactions (ADRs) are common events in medical practice, representing a matter of concern for both outpatient and in hospital care. Understanding and management of ADRs depend on their mechanism and clinical picture, which are complex and highly heterogeneous. Drug hypersensitivity reactions (DHRs) are immunologically-mediated ADRs, which are considered allergies after demonstrating evidence of either drug-specific antibodies or T-cells. The correct diagnosis of a drug allergy in hospital setting is essential for the outcome of treated disease and for the patient future in terms of therapeutic needs. Patients may be labeled as being drug allergic, based on their history and vague symptoms that do not correspond to a true allergic reaction. The aim of our study was to evaluate consequences of drug allergy labelling of female patients hospitalized in the department of obstetrics and gynecology from a university hospital during one year, in terms of medical attitude and therapeutic approach.*

Method and Results. We found that 159 out of 2395 patients hospitalized in the obstetrics-gynecology department declared a history of drug allergy on admission, but only 3 patients had medical documents and adequate recommendations from the allergist. Hospital medical attitude was significantly influenced in all declared allergic patients, mainly in terms of antimicrobial and anti-inflammatory therapy.

Conclusion. We concluded that overestimation of drug allergies in hospital care has important consequences in terms of current and future recommended therapy that should be based on standardized allergist evaluation.

Keywords: *drug allergies, hospital setting, patient's history, therapeutic errors*

INTRODUCTION

Adverse drug reactions (ADRs) are common events in medical practice, representing a matter of concern for both outpatient and in hospital care. The ADRs definition accepted by the World Health Organization is any noxious and unintended response to a medication that occurs at normal doses used for prophylaxis, diagnosis and/or treatment [1]. ADRs severity range from minor common side effects to potentially life-threatening medical conditions. The reported incidence of ADRs in general population varies between countries and may account for 2-3 % of all hospital admissions in some regions, as reported from Australia [2]. No reliable data about incidence of ADRs in Romania could be found. Understanding and management of ADRs depend on their mechanism and clinical picture, which are complex and highly heterogeneous [3].

The pharmacological classification of ADRs includes two major subtypes: type A which are dose-dependent and predictable reactions, with non-immunological mechanism,

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commonly termed intolerance and type B, which are unpredictable, not dose-dependent and have immunologic mechanism [4]. Drug hypersensitivity reactions (DHRs) are immunologically-mediated ADRs, considered allergies after demonstrating evidence of either drug-specific antibodies or T-cells [5]. Therefore it is recommended to use the term allergy only to describe reactions for which an immunological mechanism has been demonstrated. The majority of ADRs (>85%) are type A, while drug allergies comprise up to 15 % of ADRs [4]. It is considered that drug allergies affect more than 7 % of the general population [6]. The correct classification of an adverse drug reaction, by medical professionals, as allergy (due to immunological mechanism) or intolerance (non-immunological mechanism) has important medical implications [3]. Both underdiagnosis, due to underreporting and overdiagnosis, due to an overuse of the term „allergy" are common. There are still many controversies in drug allergy and recognized need for standardized and improved approaches of patients who report prior drug allergy [7]. Mislabeling of a drug allergy in patients records may result in unnecessary avoidance of an effective drug and medical recommendation of a second-line therapy, possibly less effective or more expensive. This situation is more frequent in case of antimicrobial drugs and may be also responsible for increased bacterial resistance to frequently used antimicrobials. Despite lack of reliable evidence, if an allergy label is noted into a patient medical record, it will remain for the rest of his life in most of the cases [8]. Maintaining the diagnosis of a drug allergy in hospital setting is essential for the outcome of treated disease and for the patient future in terms of therapeutic needs.

The aim of our study was to evaluate medical recording and consequences of drug allergy labeling of female patients hospitalized in the department of Obstetrics and Gynecology from a university hospital during one year, mainly in terms of current medical attitude, clinical outcome and therapeutic approach.

MATERIAL AND METHOD

We performed a retrospective analysis of hospital data over one-year period and we recorded diagnosis of drug allergies based on patient's history, as well as medical events considered as drug allergy and medical therapeutic attitude during hospitalization.

A total number of 2,395 female patients have been hospitalized during twelve months between January-December 2016 in the Obstetrics-Gynecology department of Nicolae Malaxa Clinical Hospital from Bucharest, with 1,817 patients hospitalized for childbirth and 578 patients hospitalized for gynecological diseases.

Patients were aged between 18-67 years, with a mean age of 33 years and about 80% lived in urban area. We recorded medical history documented in the patient file, including any type of reported drug allergies, written information regarding diagnosis confirmation and tolerated medication, concomitant diseases, other personal or familial allergies, current illness and medication, outcome and status at hospital discharge, particular recommendations regarding drug allergies. In cases who had medical events considered drug allergies during hospitalization, we noted type of reaction, severity, the possible culprit drug, relevant laboratory tests, therapeutic attitude and outcome.

RESULTS

We found that 159 out of 2395 patients, meaning 6.63% of female patients hospitalized in the Obstetrics-Gynecology department declared a history of drug allergy on admission. From this group, 116 were pregnant patients hospitalized for childbirth and 43 patients were hospitalized for gynecologic diseases.

We noticed that 26 patients out of 159 (16.35%) were current smokers. The possible culprit drugs based on patient's declaration were: antibiotics in 76 patients (47.79%), non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics in 41 patients (25.78%) and other drug allergies in 42 patients (26.41%) (Figure 1).

Only three patients out of 159 (1.88%) could show medical documents with diagnosis confirmation of drug allergy and clear recommendations to follow in case of future events.

Figure 1. Culprit drugs based on patient's declaration

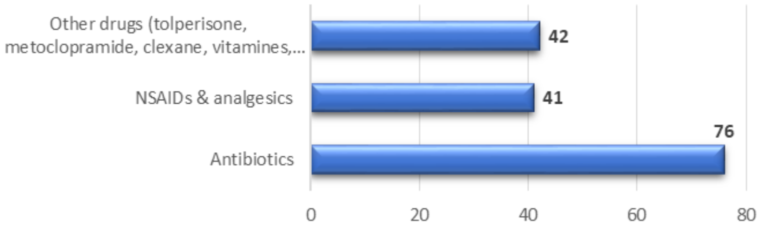

Clinical episodes considered drug allergies were recorded during hospitalization in another 9 patients from the total 2395 hospitalized group (0.37%), who have not declare any drug allergy on hospital admission. Eight patients had acute urticaria and one patient had respiratory symptoms. All these events have been successfully treated with antihistamines and systemic corticosteroids, based on allergist recommendation and resolved within short time. In all cases who declared history of drug allergy, the possible culprit drug was not administered and medication from another pharmacological class was considered. No allergological work-up could be performed during hospitalization, but this was recommended after hospital discharge. From the 76 cases who declared allergies to antibiotics, the incriminated classes were: beta-lactam antibiotics in 48 cases, macrolides in 9 cases, tetracyclines in 5, sulphonamides in 14 cases (Figure 2). The recommended antimicrobial therapy during hospitalization was quinolone in all cases (ciprofloxacin and levofloxacine).

Figure 2. Antimicrobials reported as culprit drugs

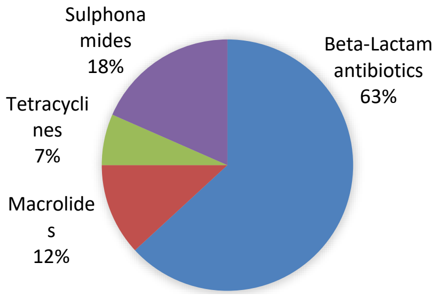

From the 41 cases who reported allergies to non-steroidal anti-inflamatory drugs (NSAIDs) and analgesics, the incriminated substances were: metamizole in 16 cases, aspirin in 8, acetaminophen in 3, diclofenac in 2, ibuprofen in 5, indomethacin in 3, nimesulide in 4 cases. (Figure 3)

Figure 3. NSAIDs and analgesics reported as culprit drugs

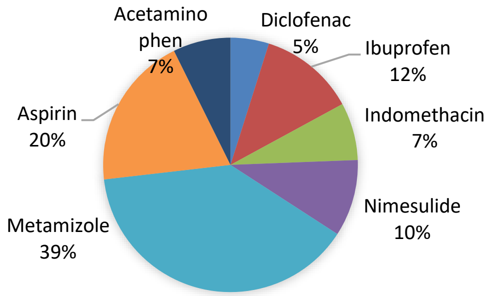

Other possible culprit drugs reported by patients who considered themselves to have drug allergies were: tolperisone, metoclopramide, clexane, vitamines, ergometrine, drotaverine.

The possible offending drug was avoided in all cases who reported drug allergies. No medical events attributable to anesthetics have been recorded.

In case of patients who declared allergy to NSAIDs, no drug from this class was administered, but also analgesics and antipyretics were avoided. No data referring to influence of drug allergy on duration of hospital stay could be found.

DISCUSSION

Our study showed that drug allergy is overestimated in hospital setting, based on patient's history alone and most of the patients have no evaluation or diagnosis confirmation from allergist. Hospital data referring to possible allergy were limited and some laboratory tests that might support atopy or certain symptoms of allergy, such as total serum IgE and blood eosinophilia were missing [9]. The main consequence for hospitalized patients who declare having drug allergy is change of the antimicrobial medication with a different class and refrain from any anti-inflammatory and painkiller medication. The situation might be more complicated and has to be more seriously considered for patients who declare multiple drug allergies to various and unrelated molecules.

The term „allergy" is frequently used and one study showed that more than 50% of reported allergies to antimicrobials have no immunologic pattern [10]. Generally, patients tend to overestimate adverse drug reactions and to interpret erroneously new symptoms or signs that occur during treating their disease.

Data from the literature showed that up to 25% of patient's self-report having drug allergies, most commonly induced by antimicrobials, mainly penicillins, followed by sulfonamides, macrolides and cephalosporines [11]. Beta-lactam allergy, recorded in up to 15 % hospitalized population and leading to increased use of broad-spectrum antibiotic use, is confirmed in only 5 % of patients [12].

Having a label of drug allergy or intolerance in the medical record may lead to withholding of first line therapy or even avoidance. It was proved that about 30% of patients having the label of drug allergy to at least one antimicrobial drug received another drug that did not reflect the standard of care [13]. Alternative antibiotic therapy may be less effective or may have less favorable safety profile, leading to treatment failure and increased risk of antibiotic resistance. Trends in antibiotic prescription rates show an increase use of broad-spectrum antibiotics, such as azithromycin and quinolones [14]. It was also proved that total hospital costs with medication were increased for patients caring the label of a drug allergy, with 63% higher antibiotic costs for patients with beta-lactam allergy [15]. Despite the potential deleterious consequences of selecting an alternate medication and the lack of validity of most allergy labels, many clinicians do not take time to complete a comprehensive drug allergy history before prescribing an antimicrobial. It was found that only 4% of patients who declared being allergic to antimicrobial medication on hospital admission had documentation detailing the specific reaction [16]. This finding may be due to the erroneous perception that therapeutic decisions based on an allergy label will not influence the patients' clinical outcome. The ability of health professionals to discriminate between allergy and intolerance, to evaluate the severity of an ADR and the degree of contraindication depends on their level of knowledge in the field of allergic diseases and was evaluated in recent studies and publications [17]. A published survey of physicians practice and knowledge in the field of drug allergies at a university medical center showed that those with any internal medicine training performed significantly better [18].

A recent study evaluating drug allergies in primary care practice in Romania concluded that the medical attitude and competence of family doctors in the field of drug allergies, as well as their collaboration with allergists are not standardized and updated according to guidelines and experts' recommendations [19]. Since primary care physicians play a key role in evaluating patients with possible drug allergies, there is a clear need for pre-and postgraduate allergy educational programs and training [20].

LIMITATIONS OF THE STUDY

Despite being the only study on drug allergy labeling performed in Romania, our study has some limitations. The main limitation is the retrospective analysis of medical records, with relative few and variable information about drug allergies, depending on the level of knowledge of doctors in training, students or nurses who filled in the patient's files. We had no way to verify the proportion of patients from our study with true versus false drug allergy label, based on standardized diagnosis criteria. No follow-up of the patients who experienced allergic reactions during hospitalization was performed.

CONCLUSIONS

We concluded that the impact of drug allergy label is underestimated in hospitalized patients and diagnosis is generally based on patient's history.

The allergy label is overused, most of the patients who self-report drug allergies have no confirmation from specialist and no medical documentation. The main consequences are switch of antimicrobial medication to another class, generally quinolones and avoidance of anti-inflammatory or analgesic medication.

We consider that information referring to drug allergy should be taken by medical staff with minimal training in this field and recorded more detailed in patient's files on hospital admission. Improved collaboration between specialists, including allergists and primary care physicians is needed.

Conflict of interest

The authors declare no conflict of interest in relation with this study and manuscript.

Disclosure statement

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ORIGINAL ARTICLES

New approaches regarding the protection forces' health against the effects of some toxic substances

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Abstract: *In the past, finding out the chemical resistance of the barrier materials designated for the production of individual protective equipment ofthe protection forces (policemen, firefighters, soldiers) was based on the need to respond to protection against weapons of mass destruction. Simple chemical methods based on indicating the colorimetric reactions were used. It was necessary to switch to more universal methods due to a change in the security of the environment and the need to focus on toxic industrial materials. The universality is due to using sensitive QCM sensors equipped with a special detection layer and some special carbon electrodes. The paper deals with new methods that are used to determine the chemical resistance of the barrier materials against the permeation of toxic substances, primarily in field conditions. However, they can also be used in stationary chemical laboratory conditions.*

Keywords: *Permeation, barrier material, PIEZOTEST, KONDUKTOTEST, SORPTEST*

INTRODUCTION

Public health is and must be a major interest for the policies and legislation of a country. Maintaining and improving it must be done by implementing appropriate measures and programs, by educating the population and by raising the standard of living. A particular sector within this field of health is represented by the health insurance of the protection forces (police, firefighters, soldiers). In addition to psychological training, physical training, proper nutrition, etc., they must be provided with the optimal equipment to cope with specific interventions. Exposure to various harmful factors (fire, extreme heat, chemicals, pollutants of all kinds, etc.) must take place in the optimum parameters, ensuring the best performing materials, with maximum level of protection [1]. Direct contact with these substances (drugs, chemicals, flammable substances, poisons, narcotics, various medical and pharmaceutical waste) [2-4] which can usually be found in nature and especially in cases of emergency (fire, war, explosions) can often be lethal, through harmful compounds that are released or which, in direct contact with the skin can cause severe burns, major infections, dizziness, fainting, headache, choking, serious pathologies as cancer [5, 6] etc., and finally – death.

The interest to produce protective materials impervious to possible contamination agents has increased in recent years. The political decision makers, as well as the economic agents and the manufacturing companies (that are producing harmful substances, pollutants, as well as those who's

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activity field involve obtaining the performant barrier materials) concentrate their activity more and more intensely on this aspect of the development of the protective equipment with high quality, reduced cost, with high demand on the market and which generate waste as little as possible following the production process [7-14].

In the last decade, there has been a shift from relatively simple chemical colorimetric methods for testing the resistance of the barrier materials against the permeation of toxic substances to sophisticated methods based on a principle of sensor technologies [15-21]. This change was called by a gradual re-evaluation of the security threats experienced by military professionals in military operations [22, 23]. Representatives of the North Atlantic Treaty Organization declared that high use of weapons of mass destruction is currently unlikely [24]. Industrial chemicals are now considered to be a significantly more important security threat because their leakage can cause many losses and can cause contamination of large areas of interest [25, 26]. New methods that enable the testing of the chemical resistance of the barrier materials against the permeation of toxic substances find their practical application also in testing of already established final products and also of the barrier materials that may find their use in the future [27, 28]. New methods also allow very fast evaluation of the permeation data found and meet all requirements set by military and European standards [29]. The concept of research, development, and production of permeation devices using sensor technologies has been designed to meet the requirements for their use in field conditions and to enable obtaining permeation data with minimal attendance and service [30-32].

All these aspects have led to a gradual decrease in the efficiency of work with colorimetric methods, applicable only for determining the chemical resistance of toxic substances (used in wars) under simulated laboratory conditions. These methods did not allow a detailed analysis at the level of the North Atlantic Treaty Organization nor at the European Union level.

PIEZOTEST method

The PIEZOTEST method uses a QCM (Quartz Crystal Microbalance) based piezoelectric sensor to detect permeated toxic substances. These sensors, which find their application in many technical and industrial applications, have been modified to detect the permeation of the toxic substances through the barrier materials by applying the appropriate detection layer to guarantee the functionality of this sensor as a gravimetric detection device. The 1 Hz sensor's response is reported to correspond to the detection of 1 ng of a toxic substance. This sensitivity is quite enough for operating the measuring device in both field and laboratory conditions. Application and software equipment, which is an integral part of the measuring equipment, allows on-line monitoring of a wide range of standardized parameters. The most important data is the breakthrough time (or also the resistance time) when the permeation mass's (cumulative amount) value is reached for a particular (subtracted) value based on the permeation mass (cumulative mass) versus time graph. However, it can also be specified as the normalized breakthrough time, but only if it relates to the normalized permeation weight (cumulated amount) specified by the relevant standard. For fast data evaluation without the need for application and software equipment, it is possible to use the value of protection time (chemical resistance time) expressed as lag-time, to which the value of diffusion coefficient is automatically recalculated. The time to reach the maximum permeated concentration, which is sometimes referred to as the time of half diffusion flow, is also significant for the purposes of assessing the capture efficiency of the test chemicals through barrier materials. The permeation calculator automatically recalculates the value of the respective diffusion coefficient to this value, which creates a space for comparing the penetration rate of the test chemical through the tested barrier material. The time to reach the maximum permeation rate (when the lag-time is reached) and the maximum reached permeation rate (when the steady state permeation rate is reached) is very important for the evaluation of the breathable barrier materials is very significant. Results of the calibration measurements showed that it is possible to achieve high-quality dependencies of the QCM sensor working frequency increase, thus the concentration of test chemical over time, even though this method was not primarily intended as analytical.

KONDUKTOTEST method

The KONDUKTOTEST method is based on the basic analytical method working on electrochemistry, thus dissociation of ions. It introduces practical knowledge of conductometry into the implementation testing practice and adapts them to the conditions for determining the chemical resistance of barrier materials against the permeation of toxic substances. A significant benefit of the used measuring device is the replacement of very expensive platinum electrodes with glass electrodes. These electrodes are characterized by a very good sensitivity and minimal maintenance and operation requirements. Their further advantage is that they do not experience problems with memory effect, which is an important and limiting factor for the electroanalytical methods. The elimination of this effect is based on the actual needs of measurement when working with very dilute solutions. In practice this means that a very low concentration of dissociated ions is sufficient to form a permeation curve, which serves as a basis for the reading of standard values. This concentration is so low that it does not produce the memory effect of the used carbon electrodes even after their repeated use. The KONDUKTOTEST method uses the dependence of electrical conductivity increaseover time to generate the resulting permeation data and evaluate the effectiveness of barrier materials to withstand to a direct contact with toxic substances. The other standard values are the same as in the previous case. The results of the calibration measurements confirmed the theoretical considerations associated with classical conductometry, which in practice means that high quality results have been achieved.

SORPTEST method

The SOPRTEST method suitably complements the abilities of the above two methods. Its primary use can be seen to determine the chemical resistance of barrier materials under dynamic conditions. Due to the combination of the conductometric measurement and the measurement based on the use of QCM sensors, a unique device has been constructed. It enables the combination of methods and used barrier materials. The permeation cells can be adapted to the particular type of measurement and the test material. In addition to breathable and insulating barrier materials, the sorption properties of the bulk sorbent layers can also be tested. By partial modification of the measuring system it is also possible to characterize filters of protective masks and thus to study their dynamic and static sorption capacity. The source of the gas phase of the test chemical is a test tube fitted with a Peltier´s cooler (condenser). The device enables continuous regulation of air flow during its use within calibration, actual measurement and desorption of QCM sensor. Before starting and during the measurement, it is possible to adjust the temperature, which ensures the transfer of the test chemical into the gas phase and the temperature of the Peltier´s cooler. Permeation measuring cells are tempered separately. A biological incubator has proved to be a suitable device. The SORPTEST measurement device allows the generation of the same standardized permeation data. This approach respects the principle of comparability of the observed permeation data across the older used measurement methods. Application and software equipment of this device enables creation of an output report from measurements. It includes results of calibration measurements and individual graphs characterizing the course of monitored standard quantities. The report also includes data concerning the chemical test that was used, its basic tabulated characteristics and toxicological data.

CONCLUSIONS

Obtaining permeation data characterizing barrier materials in accordance with European standards enables their unambiguous description not only at the national level but also at the European level.This approach will allow describing the barrier properties not only during their use, but also during their development and quality verification. The combination of methods for the implementation the knowledge of physics, sensor technologies, classical analytical chemistry and toxicology has resulted in the construction of very modern and sophisticated measuring systems that meet the highest demands on the quality of the obtained data while maintaining economic rationality.

Compliance with Ethics Requirements
The authors declare no conflict of interest regarding this article

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CLINICAL PRACTICE

Pregnancy outcomes in a patient with Fontan circulation for single ventricle congenital heart disease and aberrant praevia placental lobe

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Abstract: *Single ventricle heart (SVH) is a very rare congenital heart disease. However, since the introduction of Fontan's intervention, the survival rate of patients with SVH has been significantly improved, increasing the number of women who may become pregnant in this condition. It has been already reported, in isolated case presentations or in some series of patients, that the pregnacy in the context of SVH and Fontan circulation carries a very high risk for first trimester miscarriage, maternal cardiovascular or obstetric complications and for an increased rate of pre-term birth. Therefore, it is an open field of debate weather or not to allow pregnancy in this category of patients. We are presenting the case of a 22 years old patient who underwent two surgeries for SVH: a Glenn procedure when she was 2 years old and the implantation of a Fontan circuit at the age of 16 years. Since the last surgery she was under permanent internal cardiac stimulation. During the pregnancy the patient was hemodynamically stable, with a normal resting oxygen saturation and a good ejection fraction of the single ventricle. However, the evolution of this patient has become even more challenging due to an aberrant praevia placental lobe. This condition brought her to hospital for several episodes of metrorrhagia and uterine contractions. She delivered at 33 weeks of gestation by emergency Caesarian section with a good evolution until discharge, for mother and the newborn. This case is supporting the opinion that pregnancy can be tolerated without important cardiovascular events in patients with SVH and Fontan circulation, unless heart failure or cardiac arrythmia are associated. Nevertheless, the obstetrical complications remain an important problem in pregnancy overcome of patients with Fontan circulation for SVH that can be superimposed with other unfavorable conditions, like aberrant praevia placental lobe in this case. During the management of such particular clinical settings difficult decisions must be taken, including those addressing the antithrombotic therapy that will be covered in the presentation of this case.*

Keywords: *Pregnancy outcome; Cardiovascular pregnancy complications; Fontan procedure; Delivery, obstetrics*

INTRODUCTION

A single ventricle is a heart defect in which one lower chamber does not develop resulting in a one pumping ventricle only and cyanosis. Single ventricle heart (SVH) defects are rare. They occur in about five out of every 100 000 live births [1]. There have been distinguished more features of SVH, but the most frequently encountered is double-inlet left ventricle [2]. Since its description for the surgical management of tricuspid atresia in 1971, the Fontan procedure has become the definitive palliative surgical treatment of SVH. Its principal consists in the deviation of the systemic venous return directly to the pulmonary artery without passing through a subpulmonary ventricle. Contemporary cohorts of surviving patients with SVH in adulthood have undergone modified procedures compared with the original version, of which the most common is a Dacron by-pass connecting the inferior caval vein to the inferior aspect of the right pulmonary artery [3]. Since the

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introduction of Fontan's intervention, the survival rate of patients with SVH has significantly improved, increasing the number of women who may become pregnant in this condition. Overtime, patients with SVH and Fontan circulation are developing complications characterised by atrial arrhythmias, thrombo-embolic events and myocardial and even hepatic dysfunction [4-7]. In particular, the lack of subpulmonary ventricle predisposes to a low cardiac output state with pulmonary blood flow dependent on adequate preload [8]. This fragile balance can be overcome during pregnancy, when basal oxygen consumption increases by 50 mL/min at 40 weeks, requering a 30-80% increase in cardiac output [9]. Patients with Fontan circulation have limited resources to take over a volume overload or to increase adequately their cardiac output [10]. Moreover, it has been shown that pregnacy in the context of SVH and Fontan circulation carries a very high risk for first trimester miscarriage, maternal cardiovascular or obstetric complications and for an increased rate of pre-term birth [11]. Therefore, it is an open field of debate wether or not to allow pregnancy in this patients with SVH and Fontan circulation.

Patients with Fontan circulation have a high rate of thromboemebolic events, recorded in about 20% of cases, which seems to be similar in all variants of this operation, with most of the thrombs located in within the venous circulation [12]. However, current guidelines of the European Society of Cardiology adressed to cardiovascular diseases in pregnancy consider that therapeutic anticoagulation should be considered in pregnant women with Folan circulation, but balanced with the risk of bleeding [13].

CASE PRESENTATION

We are presenting the case of a 22 years old patient who underwent two surgeries for SVH: a Glenn procedure in order to connect the superior vena cava to the right pulmonary artery when she was 2 years old, and the implantation of a Fontan circuit at the age of 16 years. Since the last surgery she was under permanent internal cardiac stimulation.

Due to the fact that patients with Fontan circulation are considered as having high and very high risk in pregnancy (WHO risk class III or IV) (13) the patient was clearly informed of the possible complications that might occur. However, she gave her written consent for keeping the pregnancy, despite the repeated warnings of the cardiogists and obstetritions.

The patient had a double-inlet left ventricle with an ejection fraction of 57% and a small regurgitation of the right atrio-ventricular valve (Figure 1). Throughout of pregnancy the patient remained hemodynamically stable, with a normal resting oxygen saturation and a good function of the left ventricle, including during delivery. No arhythmia have been recorded. She received antiplatelet therapy with acetylsalicylic 100 mg per day, up to 31 weeks of pregnancy, in order to prevent thromboembolic events.

Figure 1. Transthoracic echocardiography indicating the one pumping chamber with double inlet left ventricle and a part of the Glenn-Fontan circuit

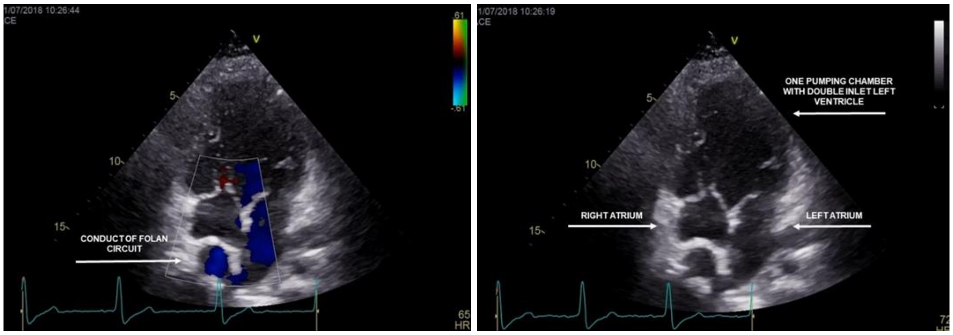

Based on echographic parameters in conjunction with maternal serum biochemistry, the estimated risk for trisomy 21, 18 and 13 was low. The thrombophilic work-up revealed MTHFR C677T homozygote mutation associated with PAI-1 4G/5G. An infection with Ureaplasma Urealyticum was detected, that required antibiotic treatment at 17 weeks of pregnancy.

However, the pregnancy overcome has become more challenging due to an aberrant praevia placental lobe. This condition brought her to hospital for several episodes of metrorrhagia and uterine contractions.

At 33 weeks of gestation the patient was admitted for uterine contractions and vaginal bleeding. She delivered by emergency Caesarean section for placenta praevia and pelvic presentation. The intervention was particularly difficult considering the emergency state of the procedure and the massive bleeding, but during delivery and afterwords, in the Intensive Care Unit, sufficient volume replacement was added in order to maintain adequate preload of Fontan circulation. The postoperative outcome was satisfactory, without further bleedings under prophylactic anticoagulation with low molecular weight heparines. After 12 days from the Cesarian section the patient was suspicioned for an intrauterine residual mass confirmed at echography (Figure 2) for which a guided instrumental revision was performed. The preterm neonate, weighting 1700 g, was registered with 7 on Apgar scale, but with good evolution until discharge.

Figure 2. Ultrasonographic aspect of intrauterine residual mass (transverse section), measuring 27 mm

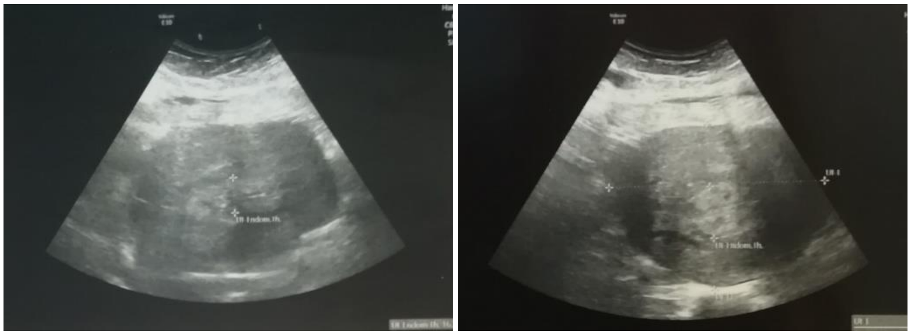

All the fragments extracted underwent an extensive histopathological analysis which concluded their placental origin with postpartum endometritis and decidual abcesses (Figures 3-5).

The patient received antibiotic therapy with Carbapenems for 10 days. She was discharged on post-operative day 25 in a good condition and without cardiovascular manifestations.

Figure 3. Acute villitis and intervillositis diffuse neutrophilic infiltration of intervillous space. HE 10x

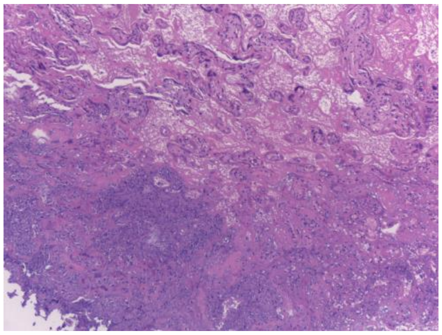

Figure 4. Massive inflamation and infection of the decidua. HE 20x

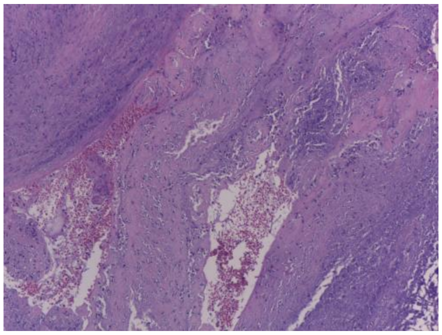

Figure 5. Postpartum endometritis leading to decidual abscess, massive inflamation and necrotic debris. HE 10x

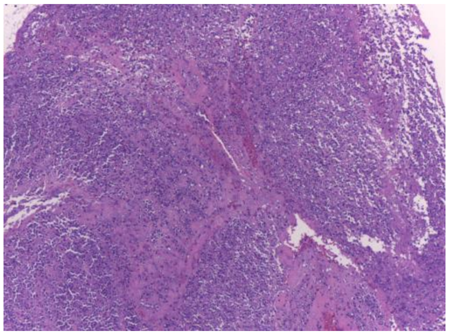

DISCUSSIONS

Since the introduction of Fontan's intervention, the survival rate of patients with SVH has significantly improved, increasing the number of women who may become pregnant in this condition. Based on a report from UK it is expected in the next decade a rise of the adults' number with this condition by 60% [14]. It has been already reported, in isolated case presentations or in some series of patients, that the pregnacy in the context of SVH and Fontan circulation carries a very high risk for first trimester miscarriage, maternal cardiovascular or obstetric complications, including perinatal haemorrhage and for an increased rate of pre-term birth or small for gestational age [15-20]. Due to these reports many authors advised against pregnancy in women with Fontan procedure for SVH. However, in some series of patients with this condition have been identified some categories of patients which might safely evolve during pregnancy, in particular those without heart failure or arrythmias and with a normal resting oxygen saturation [11, 21-23]. The presented case is supporting the opinion that pregnancy can be tolerated without important cardiovascular events in patients with SVH and Fontan circulation in selected situations. Age might have an important role in pregnancy overcome, as long as the risk of cardiovascular complications increases with the duration of time from the palliative intervention.

Antepartum haemorrhage complicates 2–5% of pregnancies, of which approximately one-third are caused by abnormal placental implantations [24]. Placenta previa is characterised by the partial or total covering of the internal os [25]. In certain situations, like in the presented case, only an aberrant lobe may have this aberrant location. Placenta praevia is an obstetric condition that can induce massive antepartum vaginal bleeding. The risk is even higher when accreta and percreta invasion are occuring [26]. It has been reported that the incidence of placenta praevia and abnormal placental invasion has increased in the last years, probably as a result of the large number of C-sections worldwide [25]. However, in our case the patient was primiparous and did not undergo any other previous surgical interventions, but the risk of antepartum bleeding was augmanted by the cardiovascular condition of this patient. Data accumulated over the two past decades are indicating that the maternal and fetal obtetrical complications are high in patients with Fontan circulation, even in those with an estimated low score of cardiovascular complications during pregnancy: a rate of about 50% for obsterical complications and between 70-80% of preterm delivery [11].

Patients with Fontan procedure have a high-risk of thromboembolic events. In pregnancy this risk can be augmented. For this reason our patient had an indication of therapeutic anticoagulation with low molecular weight heparine [13], but her pregnancy overcome was superimposed with an aberrant praevia placental lobe and repeated episodes of metrorrhagia. In this context, the obstetritions and the cardiologists involved in the management of this patient have chosen to give her acetylsalicylic 100 mg per day, because the risk of hemorrhages was too high under heparin therapy. This decision was taken according to the single multicenter, randomized, controlled study which has revealed no differencies in the thrombosis rate of Fontan patients between the use of aspirin and heparin/warfarin [27]. Moreover the postpartum period was complicated by the expulsion of an intrauterine mass. The extracted fragments proved to be placental and infected. Following the prompt guided instrumental revision and the antibiotic cure with Carbapenems, septic complications that could particularly affect Fontan's circulation have been avoided.

CONCLUSIONS

This case is supporting the opinion that pregnancy can be tolerated without important cardiovascular events in patients with SVH and Fontan circulation, unless heart failure or cardiac arrythmia are associated. Nevertheless, the obstetrical complications remain an important problem in pregnancy overcome of patients with Fontan circulation for SVH that can be superimposed with other unfavorable conditions, like aberrant praevia placental lobe in this case. During the management of such particular clinical settings difficult decisions must be taken, including those addressing the antithrombotic therapy, that must be carried on by a multidisciplinary team in order to reduce as much as possible the maternal and fetal morbidity and mortality.

Acknowledgment
All authors have participated equally in developing this study.

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VARIA

A simple approach for risk stratification in the plan of exercise in older adults

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Keywords: *aged; exercise; risk*

Letter to the Editor

The benefits of physical activity (PA) in decreasing morbidity and increasing life span have been proven at all ages, even in later life [1]. In addition, PA not only improves the quality of life but also contributes to maintenance of social and psychological well-being [2].

There are a lot of specific PA programs for adults aged over 65 years, with various recommendations and implementations. However, adherence and persistence in PA are generally not at desired levels in these individuals [3]. While numerous causes can decrease the compliance in PA, a set recommendations specific to person's condition and needs can successfully be used to individualize the exercise plan in older adults. Comorbid conditions and functional impairment can reduce engagement in PA. Therefore, these should be reviewed in the first step of a systematic approach by the physicians, especially in the primary care setting.

In order to improve the efficacy of PA plan offered to an older adult, we intended to test a novel approach in small sample of community dwelling elderly. The proposed tool classifies the subjects in three categories based on a geriatric evaluation that includes review of chronic diseases and 12-month fall history, assessment of cognitive status [assessed by Mini-Mental State Examination (MMSE), range 0-30], and screening for the functional status (assessed by KATZ index, range 0-6) [4, 5].

1. Low-risk individuals: Those with no or a single chronic disease, no cognitive impairment (MMSE score ≥ 28), no functional impairment (KATZ score ≥ 5), and no history of falls in past 12 months.

2. Medium-risk individuals: Those with two or more chronic diseases, mild cognitive impairments (MMSE score ≥ 24 and ≤ 27), mild functional disabilities (e.g., using a cane or walker) (KATZ index score 3 or 4) or positive history of falls without injury in past 12 months.

3. High-risk individuals: Those with two or more chronic diseases, significant cognitive impairment (MMSE score ≤ 23), advanced functional disability (KATZ index score ≤ 2), or positive history of falls with injury in past 12 months.

Table 1 suggests some exercise recommendations for the risk groups [6].

Encouraging older adults to participate in PA is an important issue at the primary care level. There are still problems to

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constitute an activity plan in this population. Therefore, comorbidities and physical activity status of older adults need to be assessed to recommend a suitable exercise plan and achieve reasonable outcomes.

Table 1. Examples of exercises according to the risk groups

Risk groups	Exercise types	Frequency	Intensity
Low	• Aerobic • Strength • Flexibility/Balance	• ≥ 30 min/day and ≥ 5 days/week • ≥ 2 days/week • ≥ 10 min and ≥ 2 days/week	• Moderate to high • Moderate to high • Moderate to high
Medium	• Aerobic • Strength (In selected subjects) • Flexibility/Balance (In selected subjects)	• ≥ 30 min/day and ≥ 5 days/week • 1-2 days/week • 1-2 days/week	• Low to moderate • Low to moderate • Low to moderate
High	• Active, active assistive, and passive (an individualized PA plan should be created)	• 20-30 min/day and ≥ 3 days/week	• Low

PA, physical activity.
Circle or check the boxes of the conditions that apply. "Low Risk" column refers to only individuals in Low Risk; "Medium Risk" column refers to only individuals in Medium Risk; "High Risk" column refers to only individuals in the High Risk (Please see the text).

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VARIA

Can we still manage the relationship with patients?

Liana Manolache[1]

Eighteen years ago, I started my out-patients' experience as specialist dermatologist. I worked from the very first day with National Health Insurance System. It was a chance to build a huge experience (more than 120,000 visits since then). Over time, relationships with patients have changed, influenced by many factors. To preserve good relationships we have to reflect and to use different approaches.

In 2001, my stamp and my signature were enough for a consultation. The cell phone era was at the beginning. Over the years, the process has become more and more complicated. Today, if the computer, internet, different types of applications, software, other devices (printer, card reader, etc.) are not working, my knowledge becomes absolutely necessary, but not sufficient for a consultation. So, the doctor is now, totally dependent on IT issues. Besides instant information, cell phones induce a lack of intimacy. Everyone could reach you everywhere, every moment. You are almost "forced" to give advice on whatsapp, e-mail, messenger and people expect you to answer on-site, otherwise you are not "reachable".

Time is the first pressure that we are feeling. Patients want and expect a consultation as soon as possible even you have appointments for the next few weeks. I try my best not to push later than 10 days, if it is a new ordinary consultation. Sometimes, it is possible for me to see the patient on-site (a child, an emergency, pregnant women, etc.). In the literature, time for appointment could vary from 7 working days in Brazil [1], to median 41 days in Canada (Ontario) [2] or median 45 days in US (Pennsylvania) [3] to a certain dramatic situation waiting list of 57 weeks in a unit in UK [4]. The number of consultations tends to increase. It is not only a perception, but a reality, revealed also by a French study (21% increase from 2000 to 2010) [5].

Time is also a burden for the doctor, because the real time for consultation has dramatically reduced in favor of bureaucratic issues. Official papers seem to be more important than people. It is a constant effort to remain emphatic and to really listen to patient's history. The stories that we are ignoring for lacking time could give us important clues for diagnosis, approach, could enforce the relationship and give trust, could relief the worries.

"You are the 5th dermatologist I am seeing"- a young woman told me the other day. I looked at her: she was around 20, some pimples on her jaw (not too many) and some tiny scars on her forehead covered by a lot of makeup. Not quite a good start for a relationship. Her expectations were not fulfilled by visiting the other doctors. Maybe the expectations were not realistic, maybe she was fed up trying different things. "How can I propose something new or miraculous when she tried probably "everything"? - I asked myself. I spent at least half an hour discussing about acne, therapeutic options and adjusting the expectations to our limits. It was more a counseling session than a dermatologic consultation.

Today, patients are coming in our office very informed. They "know" the diagnosis and sometimes even the treatment they want or need. After "Dr. Google" you can be a second opinion. Dermatology is underestimated even by other physicians that feel competent enough to prescribe medication inducing iatrogenesis [6]. So, what to expect from patients? It is a good thing to have an informed patient. It will save you a lot of time and energy in expanded explanations. But, there is also a lot of incorrect or

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misunderstood information, prejudices from other's experience shared on forums, incorrect auto-diagnosis or even incorrect auto-medication. It is our duty to listen and to correct, as much as we can. Patients have to be taught how to choose important information from the constant "soup of news feed".

The flux of information is huge, also for doctors as for patients. Thousands of opinions, articles, and new approaches invade our space daily. A good selection criteria for useful, relevant data is absolutely necessary.

A "happy" patient seems to be the one with a certain diagnosis, with clear treatment plan, including explaining side effects and with a contact number in case of recurrence [7]. The access of any information being so instant, patients expect a rapid response of the treatment. The result has to be now and definitive. The pressure of quick response is not very subtle, the doctor feeling it as a burden. It takes time to explain and to understand the progression of chronic illnesses.

Sometimes, the expectations are to get well with any treatment, eventually without any changes of their life style, even when doctor explains that some habits could aggravate or maintain the lesions. Taking responsibility for some adjustments is an important part to discuss with patient, as part of therapeutic approach. Paternal, omniscient doctor's image is no longer in actuality and patient is an important part of the relationship. Instead of an "infantile" patient, coming for any transitory rash or any mosquito bite, it is better to "grow" him/her as a self-confident "partner". The new and healthy bond has to transform patient from the passive, sometimes passive-aggressive role, into an assumed, informed, pro-active one. Cooperation is the key of healthy relation. Patient has the right to ask for explanations, to discuss therapeutic options, to refuse treatments. Patients have opinions that have to be respected and sometimes corrected if they are distorted. On the way to get therapeutic alliance and long-term cooperation, the doctor-patient relationship has to be personalized, giving value to it. This kind of relationship will make the difference in the end and even the direction is to involve more high tech and robots. Face-to-face relationship will not be replaced by anything and it will be highly appreciated after the "speed" condition passes. More social skills are often required, doctors not being known as the best communicators. Sometimes, doctors are not aware of patients' perception regarding communication [8]. I have got my social skills working day by day, no special courses were made during faculty, unfortunately. "We need fewer memorizers and more thinkers and communicators in modern medicine" is the most recent conclusion of Canadian Debate Series regarding medical students' selection [9].

Another pressure point is the constant fear of errors. With all the efforts of protocols for reducing the risk of mistakes or misconducts, unfortunately there are lots of gaps and debates. The fear of error and malpraxis leads to excess of medication and investigations, sometimes too expensive and useless.

Doctors should not be scared by the abundance of products, instruments, techniques, aggressively promoted. They have to be more flexible, more intuitive and more eager to try, making personal experience and not taking results for granted. Many of these products will not pass the test of time, even they are presented as "miraculous". Sometimes, patients' needs are the trigger for experimenting new methods and push us to progress faster.

Not only patients are in a rush, doctors too. The race for EMC points is making the doctor more informed, but we have to be careful not to become too superficial. Even a doctor is getting a diploma after a "3 days course", it doesn't mean that he/she will be an expert in that field, not even competent. It will take a lot of time and energy to really get the expertise, that course being only the very first step on the road. The "diplomas wall", real or virtual is a false goal. In the end, the real skills are more important than a sublime image and it will take time to get them.

Sometimes, vanity makes the teamwork harder. This will be unproductive for both doctors and patients. It is not a shame to refer the patient to an expert on a field when you feel that you have reached a limit.

People are hardly trying to change our state from patient to client, that new status being debatable. Being a client means to take some responsibilities as in a contract. That is the good part. But, fortunately, remaining a doctor means more than providing services. Practicing medicine is a state of knowledge, art, experience, intuition, with magic touches sometimes.

Fortunately, with all the changes during the last years, dermatologists seem to remain satisfied with their specialty. A recent Mexican study shows that 93% of dermatologists (with an average of 16 years of practice) were happy with their professional life, more than 98% choosing it once again [10].

Maintaining certain levels of professional and personal happiness, keeping informed and open-minded, avoiding burn-out, trying to fulfill patients' expectations, doctors are not in a battle, but in strong alliance with patients.

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The safety of genetic reserves in bioterrorism from the passive defense perspective

Hadi E.G. Ghaleh[1], Mohsen A. Farajzadeh[2], Mosa Jafari[3], Nader N. Shadbad[1]

Abstract: *Several studies have shown that some countries are among the main users of structural, molecular and physiologic information of human, animal, plant, and microorganisms cells (genetic and biological reserves) against living of organisms, especially humans. This method used as a mechanism for removing their rivals and opponents to achieve their goals. This descriptive-analytical study was designed to define the concept of bioterrorism, passive defense, and genetic resources and its historical development process, with emphasis on efforts to control these types of threats over time. In the present review, a wide range of literature through the search on Google Scholar, PubMed, Medline, and Scopus databases, extracted without regard to time constraints and focusing on the keywords listed above with three valid criteria and new sources of resources. The explanation of the material was based on the data obtained from the review of the sources and based on the inferences of the researchers from them. Given the importance of genetic information and the lack of clear prospective research in this area, the confidentiality of genetic data has been particularly respected among researchers. Eliminating data security can lead to unplanned advertising and costs. Therefore, the existence of laws and protective measures against coping with the misuse of enemies from genetic information is more than other country's prior demand.*

Keywords: *bioterrorism, passive defense, genetic resource, threat*

INTRODUCTION

Today, the hostile use of science and technology has led astray in the field of medical and human health that has led to the emergence of biological weapons for the purposes of biological assassinations [1]. The definition of bioterrorism is the misuse of microbial, viral, parasitic agents or their products for the purpose of intimidating or killing humans and destroying livestock or plants [2]. According to the International Police Bioterrorism definition, it is the release of biological or toxic agents with the intent to kill or harm humans, animals and plants with a prior intent to terrorize, threaten, or coerce a state or group of people. Acting or fulfilling political or social demands [3]. But with the advances in basic science and medicine, especially in the field of molecular biology, genetic engineering, biotechnology, synthetic biology, bioterrorism has recently taken on a new meaning [4]. In the meantime, we should not overlook the widespread developments of the biological sciences in the past few decades and the widespread scientific ability to manipulate and recombine genes and identify molecular pathogens to increase the virulence of deadly toxins [5]. The possibility of increasing drug resistance and antibiotic resistance and increasing the epidemic of disease has led hostile military researchers to research, procure, and produce hazardous agents in

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contravention of international laws and conventions prohibiting the development of biological weapons. Of course, they do so with justifications such as trying to make a vaccine or knowing the causes and pathogens or gathering scientific information [6]. On the other hand, genetic resources or hereditary flora and fauna are one of the most valuable national wealth and basic resources of any country. Considering that some countries are a rich country due to fossil fuel oil and gas reserves, the abundance of unique plant and animal genetic resources is also a major and strategic asset of countries and unfortunately has been neglected [7]. The economic function of genetic reserves is greater than that of other reserves in the countries, and these valuable reserves should be exploited. The genetic resources available in the countries are very rich and we can replace them with other resources and if we do not use them we will lose them in many ways. Even today in the world it is believed that genetic reserves in compared of gold and oil reserves are also more valuable and strategic [8]. Genetic reserve information is therefore the most important strategic part of a country that may be exploited if recently based on research conducted in the Netherlands and Japan, a new and modified strain of influenza virus has been studied for its respiratory transmission studies in many reflective laboratory animals [9]. And raised concerns among the scientific and security community about the potential for exploiting it in the production of dangerous biological agents. Finally, after long discussions and agreeing to publish it, the relevant researchers agreed that part of the technical knowledge and techniques of gene manipulation and production factors remove from this article [10, 11].

METHODS

This descriptive-analytical study explores the concept of bioterrorism, non-performing defense and its genetic reserves and its historical developmental process, with emphasis on efforts to control these threats over time. In this review study, a wide range of sources were extracted by searching Google Scholar, PubMed, Medline, and Scopus databases without considering time constraints and focusing on the keywords listed with three valid criteria and the newness of the sources. Explanation was based on the data obtained from the literature review and based on the researchers' inferences.

THEORETICAL FOUNDATIONS

Human Genetics Association, an organization called the Genetic Bank, announced that it would collect human genetic samples and data and store them for future scientific research [12]. Genetic specimens obtained from people with diseases such as cancer, cardiovascular disease, genetic diseases, diabetes, neurological disorders, etc. are a rich source for future research [13]. A collection of samples and genetic information related to clinical trials provides very good opportunities for analyzing genetic factors and helping to respond to treatment. Genetic information includes information on genes and genetic characteristics [4].

What are the goals of Genetic Banks?

Genetic banks generally pursue two basic goals:

✓ Storage and storage of biological substances such as blood, bone marrow, etc. for future therapeutic purposes

✓ Storing and keeping samples and genetic information of different individuals to identify disease-causing genes [14].

Uses of biological and genetic samples:
1. Diagnosis of genetic diseases and clinical care.
2. Genetic epidemiological studies.
3. Genetic mapping.
4. Applied Studies.
5. Development of diagnostic and therapeutic goals.
6. Drug design and pharmacogenomics.
7. Gene Therapy.

Genetic banks depend on the following organizations for various applications, including:
1. Forensic Medicine Organization;
2. Army;
3. Universities and study centers;
4. Business Organizations [5].

Biodiversity

One of the major threats in the discussion of threats is BioDiversity. The discipline is currently one of the safest biology disciplines in the world, and it is witnessed that there is no student admission to the field from third world countries. However, most of the funding in biology is devoted to this sector. In fact, the diversity within and between organisms in a region is considered to be the biodiversity of that region; the high biodiversity means the high diversity of genes that increases production, enhances product quality, and increases pest resistance [15]. This biodiversity has evolved over very long periods of time, and any human manipulation of it will alter and destroy the biological cycles of the creatures in an ecosystem that will have irreversible effects. For example, paddy fields in the north of the country have been destroyed by the introduction of fossils of Azolla by a Japanese researcher from Japan (16). Azola ferns in Japan make the rice unnecessary for nitrate fertilizer and multiply its production efficiency, but there is an insect that controls the population by eating ferns. The inability to prevent the growth of this fern in the northern fields and in fact the biomass of that region is different from the northern region and the lack of attention to the biodiversity of each region has had a major impact on domestic production [17]. Biodiversity is important in several respects, which is limited two resean:

First, high biodiversity leads to higher production. Today, the issue of food security and production is one of the most important issues in the world. The annual income of one Chinese medicinal plant, JinSingh, is about half of some countries annual oil sales. This high biodiversity can lead to more diverse crops as well as increased production [18].

Secondly, biodiversity is considered a source of human knowledge today. Biotechnology and future work on genes with insufficient genome resources, such as having proper knowledge of oil, but lacking oil storage [19]. India is the largest country to make huge profits from the blood trade. However, due to the lack of proper laws in some country, many plant, animal and microbial species go abroad for sequencing [20].

EFFECTIVENESS OF GENETIC RESOURCE CENTERS ACTIVITIES IN THE ECONOMIC AREA

Providing food security

Food security is a very complex issue, not only involving the production and processing of food products but also its accessibility for people to maintain a healthy and active life. Diversity is the basis of food security and today's production supplier and the prerequisite for ensuring tomorrow's production in the face of the rapidly changing world [21]. Achieving food security and ending hunger is one of the great challenges facing the world in the coming centuries. Population growth, resource depletion, and habitat degradation add to the deterioration [22]. The greater diversity of genetic resources in gene banks and their availability through an effective protection system helps ensure food security at lower costs. This will help provide the genetic feedstock for breeding activities to produce diverse and nutritious foods and increase access for poorer populations to healthier, more nutritious foods to combat malnutrition [23].

Climate Change Adaptation

Climate change will put unprecedented pressure on our ability to produce food, and these effects will be especially severe in developing countries. Research has shown that the trend of rising temperatures will continue in the next few centuries and the conditions for agriculture will be very different from the current situation, therefore, adaptation of agriculture to the conditions ahead is of particular importance and the need for a new variety that can with stand these challenges has been strongly considered. It is essential not only to reduce hunger but also to strengthen world food security in the medium and long term [24].

Biodiversity Conservation

Biodiversity loss is one of the most important environmental concerns today. According to some reports, as the current trend continues, half of the plant species will be at risk of extinction. Among the endangered species are wild ancestors of crops, which carry valuable traits that can be transferred to crops [25]. It is estimated that six percent of wild cereal ancestors (wheat, maize, rice, sorghum, etc), 18% of legume species (wild ancestors of beans, chickpeas and lentils) and 13 percent of vegetable and safflower species, including potatoes, tomatoes, eggplants and peppers, are at risk. Clever use of genetic diversity in breeding activities can be significantly effective in protecting the environment animal varieties that are susceptible to disease can reduce the need to use harmful drugs and increase production efficiency[26]. More varieties of plants with more branching ability can compete better with weeds and reduce the amount of herbicide needed. Drought-tolerant plants help conserve less water, varieties with deeper root systems can contribute to greater soil stability and stability and varieties with lower soil nutrient intake reduce fertilizer requirement [27].

Improving nutrition adequacy

Genetic diversity not only guarantees stability and stability in providing adequate amounts of feed (protein and energy), but also plays a key role in ensuring its quality. Diversity, which is a direct product of diversity in crops, nutritionists say it is optimal and the presence of vital nutrients (vitamins and minerals) can be enhanced by choosing the right genetic diversity [28]. New varieties can, through breeding, have higher nutritional quality in terms of vitamins, iron and other essential nutrients and have higher protein quality and less antifouling or toxic substances [29].

Poverty Reduction

Genetic diversity is one of the most powerful weapons to combat hunger and poverty. Agriculture is an economic infrastructure in many countries, and is a potential source of economic growth for developing countries agricultural growth is more beneficial for economic development and poorer and by providing affordable food, This benefits more than 70 percent of the world's poor, living in rural areas, And those people whose agriculture is their main livelihood [30].

Agricultural insurance can play a key role that requires a range of reforms, including the cultivation of higher value crops, the promotion of value-added activities, For example, improved product processing, expanding market access and lowering prices through increased production, higher processing and marketing efficiency[31]. The basis of all these possible solutions is genetic diversity, a variety that enables the farmer and breeder to produce higher yield varieties and higher quality. They can produce varieties that are tailored to specific processing methods and more portable and maintainable [32]. They can also produce varieties that are resistant to pests and diseases and environmental stresses, which protect the crop from damage and reduce the risk. The role of agriculture in combating poverty is very complex, but it cannot reach its potential without the proper exploitation of genetic diversity [33].

Sustainable Agriculture Guarantee

Harnessing the genetic diversity in plant and animal resources is an important strategy through which farmers can develop their farming systems with the least environmental impacts. The global off-site conservation system is a key component of the effort to conserve these resources and make them available to farmers in all countries [34]. In the 21st century, agriculture faces many challenges, as it needs to provide more food to meet the needs of a growing population living mainly in urban areas, while dependent on the shrinking rural labor force [35].

In addition, climate change is a threat that could increase the number of hungry people in the future and pose more complex agricultural challenges. While the effects of climate change are slowly becoming apparent, the future impacts will be significant if not taken properly [36]. Increased exploitation of plant genetic diversity is essential to address these threats and future challenges. Therefore, despite such strategic effects on genetic resources, the protection and security of such centers require special attention to prevent enemy misuse [9].

RECENT ADVANCES IN HUMAN GENETIC STUDIES

Human genetic studies have provided cognitive insights and mental disorders are the genetic basis of several inherited disorders, such as Down syndrome and Tay-Sachs disease, and other associations such as the role of APOE in Alzheimer's disease are still extensively studied [37]. However, despite advances in understanding the human genome, there are concerns about the privacy of genetic data and possible discrimination resulting from its disclosure, and there is incomplete monitoring of genetic testing [38].

NEW SCIENCE AND TECHNOLOGY AND NEW BIOLOGICAL FACTORS

Non-lethal biological agents

The some Department of Defense is already increasingly turning to non-lethal weapons (NLWs), these weapons designed specifically, the amount of deadly and serious damage to the low end and damage to the city, property and the environment and they don’t harm the environment some countries, of course, have been using crippling weapons for a long time. But these non-lethal weapons of biological origin can have different effects [39].

Non-microbial biomaterials in cells and organs of various animals (plants and animals) and humans that do not fall into the category of microorganisms and biological toxins, but despite their properties transmitted through an aerosol or through Respiratory and mucosal tissues absorbed If used in wars or bioterrorist operations, they can cause clinical signs and symptoms in humans, or cause economic damage to a country's strategic plants and animals [40].

These include a variety of brain regulators (Bio regulators) and some gastroenteritis-producing enterotoxins, as well as a variety of substances that affect the human senses, such as non-lethal bioweapons, calmative and malodorants Agents, etc. to be [41]. Other non-lethal biological agents can affect various materials and military and civilian equipment and cause them to corrupt, destroy or decompose in addition to causing economic damage to the country but also disrupting the political affairs and activities of the military. And becomes civilian the existence of oncogenes (carcinogenic genes) is no exception [42].

Recombinant and Synthetic Agents

By identifying the genome of different microorganisms or identifying a part of one's genome structure and using genetic engineering techniques of microbes' genes to increase their pathogenicity such as drug resistance, pathogenic genes, carcinogenic genes, and altered escape structure. The new recombinant germs are made from the diagnosis, vaccine-resistant that has created a new, recombinant weapon with special features [43].

Ethnic race pathogen agents

The susceptibility or resistance of different human races to different diseases, the abundance of hereditary diseases in specific human races, as well as research to discover and identify infectious substances and agents that can cause a particular race are bioterrorist efforts. Recently disclosed documents indicate a widespread program in the former of some countries racist regime to provide specific infectious black agents that could be used against those of the predominantly population [8,9].

Interfering RNA iRNA and SiRNA or Oligo Antisense Technology

Interfering RNAs silence genes, and are used in medicine by inactivating genes that cause diseases including cancer, viral infections and other diseases. Gene silencing methods are highly needed because of their ability to treat human diseases [44]. What puts the repressor RNA above all other competitors is its proprietary function in eliminating only inappropriate genetic information and that other healthy genes are immune to its effects[45]. Identification and synthesis of molecules that inhibit gene activity. This small fragment of RNA binds to or suppresses gene activity by binding to its complement locus in the process of gene activity. The technology is widely used in medicine as a vaccine. Also, using nanotechnology can produce bio-particles that are designed to stop vital activities of humans or other organisms, which can range from minor damage include a serious injury [46].

Genetic Engineering

Genetic engineering of food, one of the branches of biotechnology, involves targeted alterations to the genome of plants or animals. Most foods that are consumed today are either genetically modified to be termed GMOs or contain components derived from genetic engineering technology [47]. Genetic engineering and plant manufacturing and production of plants resistant to pests, diseases and the need for the use of dangerous pesticides have made a change in agriculture that is comparable only to the Green Revolution. Genetic engineering may also be engineered based on bioterrorism goals, which in turn require monitoring [48].

THE ROLE OF NEW TECHNOLOGIES IN PASSIVE DEFENSE AND CRISIS MANAGEMENT OF NEW THREATS

In the anticipation and prevention phase, it is attempted to provide the necessary reinforcements by training, developing laws and regulations, strengthening and using safety and security equipment prior to the threat. New information technologies and new equipment provide great help to passive defense, future research as well as disaster preparedness [49]. Production of detectors of biological, chemical and nuclear agents as well as installing biosensors in different parts of the country, the system will send the system information on the biological risk factors online to the relevant organization the equipment is built using modern technologies used in modern warfare and equipped with online information networks [50].

New achievements and development of various programs for the prevention and control of bioterrorism factors have been made, including:

- The existence of new vaccines preventing biological agents
- Antioxidants effective against toxicological biological agents
- The presence of potent antibiotics as drug prophylaxis in preparation,
- Developing a national, provincial, municipal and health care crisis response plan
- Provide vaccine equipment, medicines and other items and prepare for delivery
- Training as the most important element of preparation
- Using virtual training systems to train all forces
- Utilizing the ability of information networks to coordinate and execute maneuvers at the confrontation stage, new technologies play an important role in the control and crisis management of modern war events such as:
- New systems of rapid alert the threat of a new war quickly alerts crisis management and non-operational defense centers.
- The use of new technologies and new materials for disinfection that eliminates a large number of casualties, equipment and facilities without any adverse effects.
- Modern information systems are rapidly bringing forces and people into crisis.
- Crisis management operates much faster due to new information technologies.
- Use modern systems of field hospitals that can be installed in the shortest possible time and have the necessary facilities to help and treat modern war casualties.
- New medicines for treatment of modern warfare agents and new therapies
- The use of mobile laboratories and mobile vehicles to quickly detect and engage in modern warfare at the scene
- New management and information systems at the time of confrontation
- New systems for transporting the injured to health centers
- New clothes and equipment to protect the health care staff for action without contamination
- Reconstruction At this stage, given the facilities and equipment of the previous three stages, it is possible to return to pre-crisis mode in less time.
- Returning to the pre-crisis phase and control if the crisis has been well managed in the previous three phases [3].

Currently, some governments, following the approach and uses of genetic information, have enacted laws protecting this information, requiring that they establish a special protective location for genetic information to define this information. And to differentiate it from other forms of information, in particular, personal and health and medical information [10].

DISCUSSION

Well-known biological and bioterrorist agents, such as smallpox, plague, anthrax viruses, etc. are naturally pathogenic, but modern biotechnology and genetic engineering have enabled genetic modifications to control all pathogenic features of an organism that the organism is genetically manipulated is fully proportionate to its purpose[51, 52]. This technology of genetic manipulation can be exploited by the enemy if the human, animal, plant and even genome of a bacterium are identified. In general, recent biological capabilities in the field of weapons production are the result of advances such as the construction of engineered vectors, precise recognition, completion of the human genome project, and the drawing of human immunity, etc. [53]. Of course, new biological weapons production technologies provide the middle ground from very lethal to non-lethal weapons. And today, not only is the target of bio-weapons, but also the building of bio-weapons against the country's economic infrastructure to hit the country [54, 55].

So patients and researchers have long been concerned about the privacy of health information. In a study report participant’s collected medical information during a study, participants preferred (92%) to request permission before using their health information for any purpose other than medical treatment and 83% want research details before being allowed to use their health records.

This study shows that some issues such as family medical history, genetic disorders, mental illnesses, drug or alcohol related events, lists of previous cases, and current medications are highly sensitive. There are also ethical complaints about the ability of people with cognitive impairment to make informed consent or addicts to participate in studies involving substance use disorders. Tests to identify addicts or predict the risk of addiction raise concerns about privacy attacks, third-party misuse of data, and on the other hand, using data correctly will increase the power of the courts to identify the perpetrators by forcing them to perform such tests [56, 57].

The confidentiality of genetic data has also been addressed by researchers. Undermining data security can lead to unwanted advertising and costs [58, 59]. Some researcher write that to ensure that privacy laws do not indirectly replace observational studies using medical records, thoughtful decision-making by research ethics boards on the need for mandatory consent is necessary [11]. Attitudes and practices vary widely across societies. In an interview with Canadian university administrators, 47% of sites require individual consent to continue their studies, but 45% do not require consent or do not recommend the notification process and fix them [12]. One of the major challenges in the field of genetic banking related to national laws and international research policies. Therefore, in matters such as bioethics, harmonization of medical information privacy laws and the outcome of individuals, identical terms and interpretations, and how to obtain participant consent, in countries with access to genetic knowledge and genetic information, legislators should be considered to be [60, 61].

Therefore, passive defense measures should be put in place to enforce by laws and regulations on genetic information as well as how genetic banks operate and prevent any potential misuse by the enemy.

On the other hand, information networks as a platform for data collection and creation of databases on biological, chemical and radioactive and nuclear events, epidemic collection and information systems and biological events such as Medysis and Health map and a variety of human and animal and plant disease information systems have made it possible for the country and its experts to monitor and prepare the data online [62]. But there is a gap in protected data-collection systems and genetic reserves in the country. In addition, conservation of genetic stocks should also include measures to prevent breeding and genetic diversity of the country.

CONCLUSION

The results of the present study showed that the confidentiality of genetic data has been considered by researchers in different countries, and with the advances of molecular and genetic sciences, the possibility of using this science as a new bioterrorism is more likely. As a result, defensive measures in the area of non-biodegradable defense in order to preserve the genetic reserves and undiagnosed races by enemies are increasingly common.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Secure wireless system based on reconfigurable devices for human biomedical parameters monitoring

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Abstract: *Over the last decade the continuous improvements in sensor technologies, connected with recent hardware reconfigurable devices evolution, enable engineers to merge sensors and reconfigurable devices to develop new applications or to improve the existing ones. The miniaturization and integration of multiple sensors in one chip and the increase of precision, stability and power efficiency allow sensors to play an even more important role in medical technology with the main objective of building more accurate and smaller devices that help medical personal to monitor human biomedical parameters. This paper describes a secured wireless system design and implementation. The proposed system consists in one or more wearable sensor nodes that measure human biomedical parameters and then sends the collected data to a base station in order to be analyzed by qualified personal. This system can be used to monitor patient state-of-health or to supervise military personal in training or even in battles, because the new system uses secured transmission. The typical monitored parameters are body temperature, blood oxygen level, heart rate, respiratory rate, movement and position of the subject, but it can be extended and more other different sensors such as cameras or microphones can be added. Reconfigurable devices are used to process data in both sensor node and base station in an innovative environment.*

Keywords: *biomedical parameters, reconfigurable devices, sensor nodes, wireless*

INTRODUCTION

Nowadays, electronic industry and healthcare system are two modern domains, technologically advanced, working together to find solutions for many of the existing medical problems. Rapid diagnostic tests, helping patients after medical interventions and improving the lives of people with disabilities using sensors are authentic examples [1]. Many reviews in military health care system consider that "medicine is a filed in which evolution ignores borders, bureaucratic rules, customs and paradigms" [2] and innovative health solutions and technologies in medical activity are more than welcome, if the common goal is to save lives.

Heart failure is a major medical problem at global level, due to increased prevalence of cardiovascular diseases [3]. Rapid diagnosis of heart failure is almost impossible for human body subjected to high levels of stress (e.g. gym training workout, soldier training and in missions, free running and parkour, etc.).

In such cases diagnosis can be easily accomplished using wearable sensors, incorporated in many wearable devices like: chest belts, cloves, watches, wrist bands, finger clip and mobile phones. Many electronic integrated circuit manufacturers offer a variety of sensors capable to measure many biomedical parameters: body temperature, heart rate,

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oxygen saturation, electrocardiogram, etc. Furthermore, in the specific case of heart failure, medical personnel, supervisors or relatives can be alerted by communications devices integrated in wearable systems. These systems can also provide the exact location of the people in distress in order to be found and rescued, using GPS receivers, also integrated in a special designed wearable monitoring system.

The proposed system architecture is presented in the second section of this paper together with a short description of the system's main module and its components. In the next sections the proposed system operating mode in a real world scenario is presented together with encryption algorithms that can be implemented on the main controller in order to ensure different levels of security. In the last two sections implementation and experimental results are depicted. Conclusions and future work directions are also indicated, these will enhance the system overall performance and will enlarge application field.

This paper describes an improved solution to the existing health monitoring systems because its main functions are executed using reconfigurable devices such as CPLD or FPGA. Furthermore the new proposed system is secured using the strong advantage of reconfigurable devices capabilities, making it suitable for military applications.

SYSTEM ARCHITECTURE

The overall system architecture is presented in Fig. 1. The proposed system consists in three main modules: main controller, various biomedical sensors and a base station/gateway. Biomedical sensors and main controller can be integrated in a chest belt, wristband or a glove making it a wearable device, thus all the components of the wearable module must be low-power and small package devices, ideal for this kind of systems. The wristband and the glove are added to increase the systems performance in case of heart rate or oxygen saturation measurement, because in those areas the veins are located close to the surface of the skin and fingers are vascularized organs. The wearable module can be considered as a wireless sensor node and the base station as a gateway becoming a wireless sensor network.

Main controller

As the main part of the wearable module, it ensures the following functionalities:

- controls and configures the node;
- collect data from the sensors;
- establish and maintain communication with the base station/gateway or other sensor nodes;
- encrypts the acquired data and prepares it for transmission;
- decrypts and process the received data;
- wakes up the system and puts it back to sleep for power conservation.

Due to latest improvements in reconfigurable technology regarding power consumption, instead of classic microcontrollers, reconfigurable devices for both wearable module and base station are the best alternatives [4].

Figure 1. System architecture

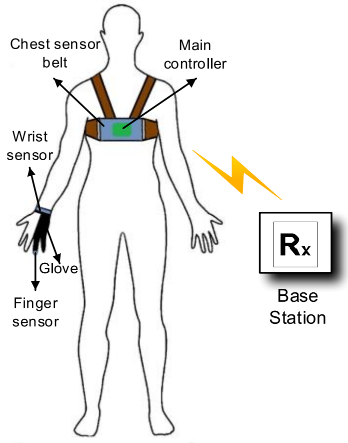

Biomedical sensor

In this system different biomedical sensor are used to measure and monitor human body relevant parameters in order determine its state of health or to prevent human health degradation (e.g. loss of consciousness, hypo or hyperthermia, physical fatigue, etc.) [5].

The new system presented in this paper is designed to monitor the fallowing physiological parameters: body temperature (T), electrocardiogram (ECG), photo-plethysmogram (PPG), heart rate (HR), peripheral capillary oxygen saturation (SpO_2). After studying numerous types of biomedical sensor taking into consideration power consumption, monitored parameters, chip dimension and sensor accuracy. As seen in Table 1 chosen sensors are built specially for wearable devices providing low power consumption and most common connection interface to communicate with the main controller. In the next paragraph a short description of this sensor is given:

- AFE4900 is an analog front-end (AFE) integrated circuit built by Texas Instruments designed mainly for (ECG) and (PPG) signal acquisition at data rates up to 1 KHz, but it can also be used for optical bio-sensing applications, such as heart-rate monitoring (HRM) and saturation of peripheral capillary oxygen (SpO2). The ADC codes from the PPG and ECG phases can be stored in a 128-sample first in, first out (FIFO) block and read out using either an I2C or a serial programming interface (SPI) interface;[6]

Table 1.

Part Number	Monitored Parameter	Average Power consumption		Interface
		Standby	Active	
AFE4900	ECG, PPG, HR, SpO_2	0μA	30μA	I^2C, SPI
MAX30205	T	1.65μA	600μA	I^2C
MAX86141	HR, SpO_2	0.6 μA	10 μA	SPI

- MAX30205 uses a sigma-delta analog-to-digital converter (ADC) with high resolution to convert temperature measurements to a digital form. It has a 16 Bit temperature resolution (0.0040°C) with 0.1 °C accuracy that meets clinical thermometry specification of the ASTM E1112 when soldered on the final PCB. The communication with the main controller is made through an I2C-compatible, 2-wire serial interface; [4, 7]

- MAX86141 is an ultra low-power optical data acquisition device designed for heart rate (HR) and oxygen saturation (SpO2) and optimized for wrist, finger or ear location.

This devices is also an analog front-end (AFE) integrated circuit with 19 bit analog to digital converter (ADC), ambient light cancelation and picket fence detect and replace algorithm. The device has an SPI interface to communicate with the main controller [8].

In Figure 2 a PPG and ECG measurement example is presented. The displayed data was captured using TIDA-01580 development board and its associated LabView application [9].

Figure 2. PPG and ECG measurement example

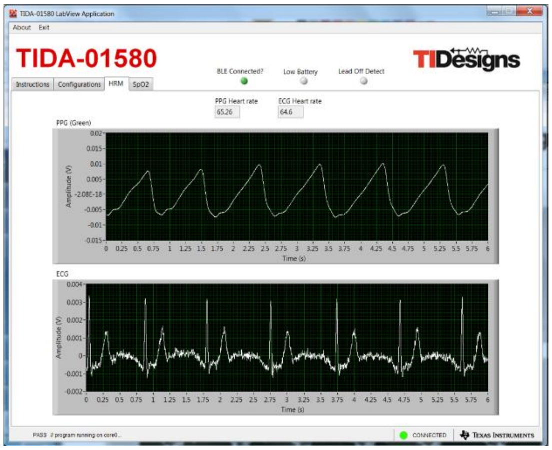

PPG Heart rate 65.26

ECG Heart rate 64.6

PASS // program running on core0...

Transceiver

The wearable module must be able to communicate with the base station and vice versa. To accomplish this, the license free ISM European 868 MHz band is chosen. Sub GHz wireless transceivers offers many advantages such as excellent propagation characteristic at low frequency, is easy to use [10] and consumes significant less power than the other communications modules (e.g. 2.4 GHz and 5 GHz WiFi module). In applications where so called sub GHz transceivers are used the battery life time is extended by 48% in comparison with applications where WiFi modules are used [11].

For the system presented in this paper the SPSGRF-868 low power programmable RF transceiver is preferred due to the low power characteristic and because it is delivered tiny form factor but providing a complete RF platform with integrated antenna. The module has the capability to alternate READY and SLEEP states in order to preserve power. Configuration and data transmit and receive is made through SPI interface. Both wearable module and base station is equipped with this transceiver.

Base station

In this case power consumption is not a key factor. For this reason a multitude of reconfigurable devices can be used, but after analyzing a set of this devices and taking into consideration system's requirements for data processing speed, reconfigurable resources, communication interface (e.g. SPI, I2C, CAN, etc), the best solution appears to be Xilinx Zynq XC7Z007S SoC.

This device has integrated a Single-core ARM Cortex-A9 capable of running at maximum frequency of 667 MHz and reconfigurable resources are equivalent to Artix-7 FPGA's. The base station has the following functions in the system:

- receive and process data from the sensor nodes;
- alerts the medical personnel when measured biomedical parameters are outside the defined threshold;
- encrypts and decrypts the transmitted or received data;
- configures the sensor node (threshold, id, etc);
- manage the encryption keys in the wireless sensor network.

Auxiliary components

In addition to the components and modules presented before, each sensor node has other components with less significant roles but without these components the system cannot function or satisfy the system requirements regarding power consumption. These components are:

- TCR2LFXX - low quiescent current (Iq) low dropout regulator (LDO);

- S25FL127S - 128 Mbit SPI flash memory used do store firmware in case of nodes with FPGA and other parameter like network id, node address, pre shared cryptographic keys, etc;
- 23LCV1024 - 1 Mbit Serial SRAM with Battery Backup use to store system flags, threshold and other parameters that system needs do store and then quickly access them when needed;
- SiT1533 - Ultra-Low Power 32.768 kHz Quartz XTAL to clock the system.

Figure 3. Operation Modes

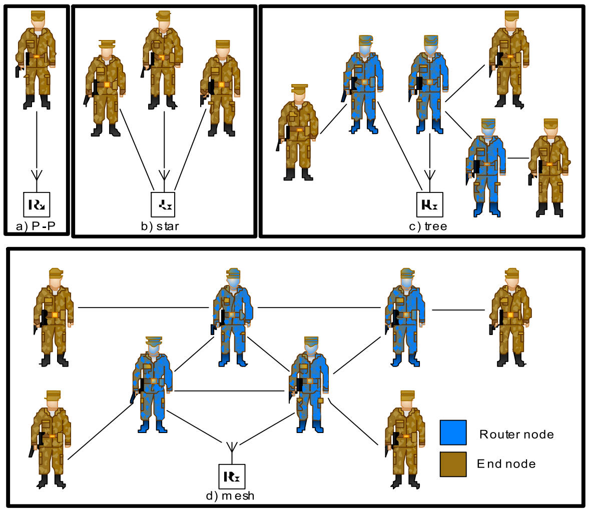

SYSTEM SECURITY

Due to the use of reconfigurable devices, security for this system can easily be achieved because this type of electronic circuits are notorious when comes to the implementation of cryptographic algorithms.

Depending on the desired level of security CPLD or Low-Power Spartan 6 FPGA can be used as main controller. While on CPLD a few low level cryptographic algorithms can be implemented (RC4, DES, Camellia, etc), on Spartan 6 all the advanced cryptographic algorithms can be implemented (AES, SHA, HMAC, RC6, ECC, etc.).

IMPLEMENTATION AND EXPERIMENTAL RESULTS

The use of reconfigurable devices was designated as the best choice for this design due to its flexibility in implementation of control functions and most common communication protocols (e.g. I2C, SPI, UART, CAN, etc.), [4] and due to latest advances in reconfigurable technology regarding power consumption. Synthesizable Verilog code has been preferred to implement the firmware running on CPLD and low-power Spartan 6 FPGA. The design was synthesized and simulated Xilinx ISE Design Suite 14.7 for CPLD and Spartan 6 and with Vivado Design Suite 2017.4 in the case of Zynq XC7Z007S SoC.

CPLD consumes only 16 µA at a running clock of 32.768 KHz and because in case of temperature measurement only five measurement are needed in 10 minutes of operation. The main power consummator in this design is the transceiver with 22 mA in TX mode at 11.6 dBm but in shutdown mode it consumes only 0.1 µA. Thus alternating ACTIVE and SHUTDOWN sates significantly reduces the overall power consumption.

In normal conditions Spartan 6 is a power consummator, making it inappropriate for our design but Xilinx introduced a low power version of Spartan 6 that consumes with 30-40% less power. In addition to this it too has the possibility to be put in to SLEEP mode furthermore reducing power consumption. After design implementation using AES-256, the following Spartan 6 part number XC6SLX9-L1TQG144C was chosen because it has the necessary logic resources needed for our design. The L later in the part number specifies the low power version of Spartan 6.

The wearable module can be powered from a 3.6V AA battery. Different voltages are adjusted from this power source in order to power up all the system's devices. The system needs the following voltages to function correctly: 1.8V for CPLD, 1V for Spartan 6 and 3.3V for all other devices. These voltages are obtained using LDO regulators with 2 µA quiescent current.

Table 2 estimates overall power consumption in different operating states. Battery life time is also indicated if a 2400 mA AA battery is used.

Table 2.

Device	Ready State	Shutdown State	Battery lifetime 5 s/min duty (days)
CPLD	11 mA	30 μA	≈142
Spartan 6	40 mA	30 μA	≈37

CONCLUSION AND FUTURE WORK

The design and implementation results presented in this paper provides a clear understanding of how engineering (e.g. electronics and telecommunications) and medical science can work together to design and built advanced biomedical monitoring systems using latest innovations in sensor technology advantages. It also proves the possibility to use reconfigurable devices in low power designs by alternating inactive and active operating states. Using this strategy, it becomes possible to integrate such systems in wearable devices, extending the battery life time for days or even weeks.

Adding new devices to the proposed system (e.g. GPS localization, CMOS cameras, microphones or other biomedical sensors) is also considered for future system developments.

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ADMINISTRATIVE ISSUES

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INTERNAL MEDICINE PAPERS

A case report of esophageal dismotility following laparoscopic sleeve gastrectomy

Andrada L. Popescu, Raluca S. Costache, Florin Savulescu, Mariana Jinga, Florentina Ionita-Radu

Laparoscopic sleeve gastrectomy (LSG) is becoming popular between bariatric procedures. There are few data available of LSG impact on esophagogastric physiology, some patients may have gastrointestinal symptoms such as reflux, dysphagia or odynophagia. This case report describes a 42-year-old female with esophageal dysmotility following LSG. She experienced globus sensation and minor GERD symptoms prior to the surgical intervention, the upper GI tract endoscopy revealed erosive esophagitis grade A (Los Angeles Classification), and high-resolution esophageal manometry (HRM) showed normal lower esophageal sphincter relaxation and distal esophageal spasm (> 20% premature contraction). HRM was analyzed according to the Chicago classification v3.0. One year after the procedure, the patient lost 40 kg, but she has mild dysphagia, early satiety, fullness and reflux. Her dysphagia could be associated with a structural cause or attributed to a "sleeve dysmotility syndrome." The result of upper gastrointestinal endoscopy was no esophagitis, normal gastric scar on the posterior wall, but on HRM more than 50% of contractions were ineffective swallows (ineffective motility), with normal relaxation of LES (IRP less than 15 mmHg). Also increased intragastric pressure (IIGP) after water swallows was observed in 30-40% of the ten swallows examined. A proposed alternative to pharmacological treatment to relieve reflux symptoms might be the gastric bypass, but it will not resolve the poor esophageal contractility.

Conclusions: Motility studies should be required for all patients planning to undergo a LSG to rule out preexisting esophageal dysmotility. Conversion to gastric bypass is not a universal method for managing esophageal dysmotility after LSG.

Optimizing diagnosis in pancreatic cystic neoplasms

Vasile Balaban, Andreea Zoican, Victor Mina, Raluca S. Costache, Petrut Nuță, Florentina Ioniță Radu, Mariana Jinga

With the widespread use of cross-sectional abdominal imaging for various indications, pancreatic cystic lesions (PCL) are being increasingly encountered in clinical practice. The stake is to differentiate neoplastic from non-neoplastic PCLs. Several guidelines have been proposed over the last years, balancing careful follow-up with need for surgery - misdiagnosis can lead to unnecessary, high-morbidity and mortality surgery or missing a potentially neoplastic lesion. In this setting, establishing a strong diagnosis becomes of utmost importance. Along with high-resolution imaging, endoscopic ultrasound plays an important role in the management of PCLs. Novel techniques such as cyst fluid analysis of molecular markers and cyst wall sampling can prove valuable in providing a definite diagnosis for a PCL. Also, innovative algorithms have been developed to aid clinicians in the management of PCLs.

Capsule endoscopy in Blue rubber bleb syndrome

Alexandra Lulache, Mariana Jinga, Florentina Ioniță Radu, Diana O. Preda, Săndica Bucurică, Andrada Popescu, Mihăiță Pătrășescu, Vasile Balaban, Andrei I. Gavrilă, Raluca S. Costache

Background: The small bowel evaluation remained unclear until 1996, when the first live pictures from pig gastrointestinal tract with wireless device was obtained. Hundreds of thousands patients have been examined since.

Nowadays it becomes the “key” method for evaluation of small bowel pathology.

Material and method: We present the case of young girl referred to our clinic for gastrointestinal bleeding with normal upper and lower endoscopy and severe anemia. At 14 years old she was diagnosed abroad with Blue Rubber Bleb Syndrom with duodenal and jejunal angiomas. We used the Olympus Endocapsule® System.

Results: The patient performed a standard small intestine complete examination, meaning the capsule reach the caecum. The result show the source of bleeding: multiple big angiomas of the small intestine.

Conclusion: The capsule endoscopy represents in our days the gold standard for small bowel tumor evaluations but has his own limitation – impossibility of therapeutic approach.

The role of transarterial chemoembolization (TACE) in the modern management of hepatocellular carcinoma

Florentina Ioniță Radu, Cezar Bețianu, Cezar Călin, Andrada L. Popescu, Alexandra I. Păun, Raluca M. Ștefan, Iulia Fulga, Maria M. Chereja, Anca Jianu, Alexandra I. Lulache, Andrei I. Gavrilă

Introduction: Our objective is to review current international guidelines for the management of hepatocellular carcinoma and to present our hospital`s experience with transarterial chemoembolization (TACE) for hepatocellular carcinoma.

Materials and methods: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. This type of malignant tumour has a very rich blood supply, mostly from the hepatic artery, while the rest of the liver tissue gets blood from the portal vein. This means that blocking the hepatic artery to cut off the blood supply to the tumour will not affect the rest of the liver.

Chemoembolization is a palliative type of treatment that delivers chemotherapy directly to the liver tumor through the hepatic artery and then the artery is blocked ("embolized") to cut of blood flow to the tumor.

TACE can be performed in patients who have a good liver function, without ascites or portal vein thrombosis and the tumour can’t be removed with surgery but it hasn’t spread to the major blood vessels in the liver, lymph nodes or other parts of the body. Another indication for this procedure (called “bridging” therapy) is to control the dimensions of HCC in patients waiting for liver transplant.

Results and conclusion: Despite ongoing efforts, there is no definitive consensus in the management of hepatocellular carcinoma. The Barcelona Clinic Liver Cancer (BCLC) system that is widely used nowdays has it`s limitations, meaning that the role of transarterial chemoembolization (TACE) in hepatocellular carcinoma management can be refined.

Topographic endomicroscopic morphology of normal stomach

Săndica Bucurică, Raluca S. Costache, Petruț Nuță, Mariana Jinga, Florentina Ioniță Radu

Normal stomach has a particular morpho- functional strategy to balance the secretion of acid and bicarbonate, and this is made by various mucosal cell types and component, with specific topography in different parts of the stomach. From histopathologic point of view there are two mucosal compartments: foveolar – that is superficial and glandular – in depth of mucosa, with different structure varying with the stomach site. The correspondence between this morphology and endomicroscopic appearance is very important in order to establish the subtle pathology changes that may not have endoscopic definite features and may benefit from endomicroscopic examination such as metaplasia or atrophy. The cardial aspect is characterized by branched glands (sometimes cystic, produce mucus), while the mucosa of the fundus has oxyntic glands that are straight, with higher proportion of glands/foveola comparative with antrum. In the antrum the mucus producing glands are also branched, but vacuolated, while the parietal cells are in the glands from fundus and body of the stomach, with function to produce acid, to bind the gastrin produced by antral cells and stimulate vagus nerve. Scattered in the fundus/body there are endocrine cells and chief cells that produce pepsinogen I/II are most in the fundus and body. So, having this various aspects, for the endomicroscopic examination of the stomach the key point is knowledge of normal mucosa. The Classification of gastric pit patterns by confocal endomicroscopy was proposed by Zhang who states that the pits patterns of stomach examined by confocal endomicroscopy are well correlated with histopathologic findings.

High-resolution esophageal manometry: interpretation in clinical practice

Andrada L. Popescu, Ionel P. Nuță, Raluca S. Costache, Săndica Bucurică, Bogdan Macadon, Mihaiță Pătrășescu, Mirela Chereja, Andrei I. Gavrilă, Mariana Jinga, Florentina Ioniță-Radu

Esophageal high-resolution manometry (HRM) defines the function of the lower esophageal sphincter and the esophageal body peristalsis, and is indicated to establish the diagnosis of suspected cases of achalasia, diffuse esophageal spasm, also for detecting esophageal motor abnormalities associated with systemic disease (connective tissue disease) or for placement of intraluminal devices (correct positioning of the probe for the 24-hour pH monitoring).

Esophageal high-resolution manometry (HRM) is the current state-of-the art diagnostic tool to evaluate esophageal motility patterns. HRM uses a high-resolution catheter to transmit intraluminal pressure data that are subsequently converted into dynamic esophageal pressure topography (EPT) plot. The pressure analysis is done according to the Chicago Classification, a formal analytic scheme for esophageal motility disorders, which is currently in version 3.0.

The standard HRM examination consists of a baseline phase and a series of 10 wet swallows in the supine position. In addition, in some cases, data from swallows in the seated position or provocative solid meal HRM is performed.

In our hospital this GI functional procedure is available since 2017 with the HR solid-state catheter and ManoScan ESO HR system (form Given Imaging), and recently with Unisensor HRIM catheters (from Isolab), with more than 170 procedures performed.

Conclusion: Esophageal HRM is a valuable and sophisticated clinical tool to evaluate esophageal motility patterns, useful in a case-by-case basis or to compare the physiological response among groups of individuals. Manometric screening for severe peristalsis dysfunction remains reasonable in specialized gastroenterology departments.

From Zenker diverticulum to a complex esophageal ailment

Mihăiță Pătrășescu, Săndica Bucurică, Raluca S. Costache, Vasile Balaban, Mariana Jinga, Florentina Ioniță Radu

Background: Zenker diverticulum is an out-pouching of mucosa and submucosa in Killian triangle which is a hypopharyngeal weak part between the fibers of inferior constrictor pharyngeal muscle and cricopharyngeal muscle. Pathophysiology may imply a disfunction of cricopharyngeal muscle which increases intraluminal pressure just above upper esophageal sphincter. The prevalence is very low (0.01-0.1%) but many cases go undiagnosed because of lack of symptoms.

Case report: I present a case of a 35-year-old patient who is admitted for dysphagia, halitosis, heartburn and regurgitation. The onset of symptoms was 10 years ago. The esophagogram shows a typical image of Zenker diverticulum. Upper digestive endoscopy was performed, and the surprise was the unmasking of two other esophageal pathologies: two areas of cervical esophageal “kissing” gastric patches and a short Barrett esophagus.

Conclusion: Zenker diverticulum is a rare ailment that may share symptoms with other diseases of esophagus (Barrett and gastric patches). These associations render a very important implication as diagnosis confounding factor and they may change the therapy management of patient.

The role of high-resolution esophageal manometry in achalasia

Andrada L. Popescu, Andrei I. Gavrilă, Mirela Chereja, Ionel P. Nuță, Raluca S. Costache, Săndica Bucurică, Bogdan Macadon, Mihăiță Pătrășescu, Vasile Balaban, Mariana Jinga, Florentina Ioniță-Radu

The role of this paper is to point out the importance of esophageal manometry in the diagnosis of achalasia.

Esophageal achalasia is a primary motility disorder characterized by impaired lower esophageal sphincter relaxation and absence of esophageal peristalsis leading to impaired bolus transit. The Eckardt score is used to assess the severity of achalasia symptoms and it is based on four major symptoms: dysphagia, regurgitations, chest pain and weight loss. The Eckardt score is also used to evaluate the efficiency of a treatment durring the follow-up, a score of less than 3 points is considered as remission of the disease.

The suspicion of the disease is clinical, completed by upper gastrointestinal tract endoscopy or barium swallow. The gold standard diagnostic tool for achalasia remains esophageal manometry. High-resolution manometry (HRM) of the esophagus is a new technique that provides a more precise assessment of esophageal motility than conventional techniques, using catheters with 36 solid-state sensors spaced 1cm apart. The main innovation of this method is the conversion of pressure data into a topographical plot. Thus we can evaluate the esophageal motor pattern, the contraction amplitude and LES pressure and function. HRM defines three manometric types of achalasia that seem to have different responsiveness to treatment. In our hospital this GI functional procedure is available since 2017 and we performed until now approximately 150 procedures.

Conclusions: HRM provide a detailed assessment of esophageal function and helps to identify distinct phenotypes of esophageal motility disorders, but more important HRM increased the recognition and diagnosis of achalasia.

Non-invasive diagnosis in hepatic fibrosis

Florentina Ioniță Radu, Raluca S. Costache, Mirela M. Chereja, Andrada l. Popescu, Anca Jianu, Alexandra I. Drugău-Păun, Raluca M. Ștefan, Iulia Fulga, Alexandra I. Lulache, Andrei I. Gavrilă

Introduction: Our objective is to review current non-invasive methods of assessing liver fibrosis, their availability and their utility in modern medical practice.

Materials and methods: The evaluation of the degree of liver fibrosis has been traditionally assessed by liver biopsy, an invasive “gold-standard” that, during the past 25 years, has been deemed imperfect; even according to its advocates, it is only “the best” among available methods.

Attempts at uncovering non-invasive diagnostic tools have yielded multiple scores and formulae based on biological parameters, and also multiple imaging techniques. All of them are better tolerated and more acceptable for the patient, safer and they can be repeated essentially as often as required. Another advantage is that they are much less expensive than liver biopsy. Consequently, their use is growing, and in most countries the number of liver biopsies performed, at least for routine evaluation of viral hepatitis (B or C), has declined sharply.

The major setback is that the accuracy and diagnostic value of most, if not all, of these methods remains debatable. The good news is that we may be witnessing the beginning of the end of the first phase of non-invasive markers development. Actual evidence suggests that they might be at least as good as liver biopsy. Novel experimental biomarkers and imaging techniques could produce a dramatic change in liver fibrosis diagnosis in the near future.

Results and conclusion: Liver biopsy has now been named the old standard for diagnosing liver fibrosis in most clinical settings and non-invasive diagnostic scores, formulae, and imaging modalities, all of which have several advantages compared to biopsy have been shown to be at least as good as liver biopsy in assessing the degree of liver fibrosis.

The importance of imaging: functional investigations in the diagnosis of gastrointestinal stromal tumors

Anca Minea, Iulia Enache, Alexandra Gheorghe, Raluca S. Costache, Petruț Nuță, Săndica Bucurică, Mihăiță Pătrășescu, Andrada Popescu, Vasile Balaban, Florentina Ioniță Radu, Mariana jinga, Bogdan Macadon

GISTs (gastrointestinal stromal tumors) are the most common mesenchymal neoplasms affecting the gastrointestinal tract - most often located in the anterior and posterior wall of the gastric fundus and proximal small intestine.

GISTs occur predominantly in middle-aged and older individuals (are rare in children or persons under the age of 40).

In adults are characterized by the expression CD117 antigen - synonymous with the KIT transmembrane receptor tyrosine kinase. In 95% of cases they occur sporadically, while in 5% of cases they are associated with syndromes such as Carney-Stratakis syndrome, neurofibromatosis type 1.

In 70% of cases the tumors are benign. The malignant ones do not metastasize in the lymph nodes, but they can disseminate in the liver and into the peritoneal cavity.

The clinical behavior of GISTs is highly variable; many are asymptomatic, being discovered incidentally - endoscopic or radiologic examination. When they become symptomatic, they are associated with nonspecific symptoms, unless they grow large enough to cause pain or obstruction, ulcerate, and bleed.

The endoscopic appearance is suggestive in the presence of an extramucosal structure, hemispherical in shape, with a normal overlying mucosa, which may present ulceration or bleeding.
Oral or intravenous contrast-enhanced computed tomography (CT) can characterize an abdominal mass, evaluate its extent, the presence or absence of metastatic disease.

Magnetic resonance imaging (MRI) may be preferred for GISTs affecting the rectum or the liver, especially when evaluating for surgery.

The endoscopic ultrasonography (EUS) is able to identify the layer of origin and enabling guided-tissue acquisition for diagnostic studies, including immunohistochemistry.

Positron emission tomography (PET) is sensitive for detecting tumors with a high glucose metabolism, including GIST. PET can be useful for resolving ambiguities from CT or detecting an unknown primary site.

Metaplasia: still a challenge-versatile endoscopic appearance of non-atrophic gastritis metaplasia

Săndica Bucurică, Mihaela Ailenei, Raluca S. Costache, Petruț Nuță, Mihăiță Pătrășescu, Bogdan Macadon, Andrei Gavrilă, Roxana Călin, Vasile Balaban, Andrada Popescu, Mirela Chereja, Mariana Jinga, Florentina Ioniță Radu

Introduction: Gastric atrophy and intestinal metaplasia are common findings from routine endoscopies and the earliest steps in gastric carcinogenesis. There is a real controversy regarding the correlation of macroscopic endoscopic appearance and histopathologic diagnostic.

Aim: The aim of this study is to determine the correlation between endoscopic diagnostic and the histopathologic result, also the implication of targeted or non-targeted biopsies in 2 selected groups of patients with macroscopic suspicion of gastric atrophy or intestinal metaplasia. One group had respected the Sydney non-targeted biopsy protocols and the second group had targeted biopsies from suspected lesions.

Methods: In this cross-sectional comparative study on the 54 patients from Gastroenterology Departament of University Central Military Hospital "Dr Carol Davila", Bucharest, we used for one group (n=25) the Sydney system for biopsy sampling protocol with 5 tissue specimens from the gastric mucosa (two from antral mucosa, two from corpus, and one sample from incisura angularis) and for the other comparative group (n=29) we took targeted biopsies from macroscopic suspicious lesions. We have classified endoscopic appearance into 6 phenotypes: whitish mucosa, hyperemic, erosions/ulceration, modified vascular pattern, elevation of mucosa and normal aspect. The atrophy status of each slide was determined as atrophic or non-atrophic and intestinal metaplasia as absent or present.

Results: As a particular finding, in most of the cases metaplasia was found in non-atrophic gastritis that could be a characteristic of the study group and needs to be verified in general population. In our study we found that metaplasia could take various forms of endoscopic expressions and those was not correlated with a specific phenotype, but for sure will not appear as normal($p<0.05$, adjusted R-squared =-2.3).

Among the 154 biopsies, 75 (48.7%) were classified by endoscopic phenotype as whitish mucosa, 16 (10.4%) as hyperemic, 12 (7.8%) as erosions/ulceration, 13 (8.4%) as modified vascular pattern, 16 (10.4%) as elevation of mucosa and 22 (14.3%) as normal. The incidence of the intestinal metaplasia was 16.9% (26 biopsies), higher in the targeted biopsies group (41.4%) than random biopsies group (12%). Metaplasia has moderate correlated with targeted biopsies($r=0.271$, $p<0.01$).

In the group with targeted biopsies ($p<0.01$) we found more cases with histological changes from the erodated and elevated lesions (adjusted R-squared=2.1 and 6.1) than other phenothypes described by endoscopists as whitish, hyperemic, but with no predominance of metaplasia. In all cases described being normal, the histology showed no changes (adjusted R-squared=2.4).

Metaplasia was not correlated with the grade of gastritis($r=0.042$, $p>0.01$), but it was associated ($p<0.01$) with the moderate grade of gastritis (adjusted R-squared=3.3), and negative associated with severe gastritis (adjusted R-squared=-2).

Conclusions: Metaplasia found in non-atrophic gastritis can take multiple endoscopic faces and could appear as: whitish mucosa, hyperemia, erosions/ulceration, elevation of mucosa, but none as normal. There is a bigger chance with statistical significance to find metaplasia by taking targeted biopsies. So, metaplasia does not have a specific endoscopic phenotype and its versatile appearance needs the support of additional or red flag techniques for endoscopic diagnosis and mapping of metaplasia.

The role of hepatitis C virus core antigen in the diagnosis and treatment algorithm of HCV infection

Corina Taubner, Clara Neguț

Chronic hepatitis C virus (HCV) infection is prevalent in approximately 1.1% of the world population and can lead to liver fibrosis, cyrrhosis, and hepatocellular carcinoma.

Currently, HCV diagnosis is a process that starts with screening assays for anti-HCV antibodies (HCV Ab). Such an assay can only inform about the exposure to the virus and can not diferentiate between acute and chronic infection, active replication and ongoing infection. On the one hand, even when a person cleared HCV or have been cured by treatment, HCV antibodies remain in a person's blood for years. On the other hand, the serological window for conversion to a positive antibody response is variabile with an average of 60 days and may remain negative for patients on haemodialysis or immunocompromised. Summarizing diagnosis of active HCV infection requires antibody testing and additional HCV RNA testing in order to confirm active HCV infection and to monitor antiviral therapy. An HCV RNA assay involving nucleic acid testing (NAT) is a confirmatory test, a highly sensitive and specific method, but requires expensive reagents and skilled personnel.

The hepatitis C core antigen (HCVc Ag) is a viral protein that forms the internal capsid which is highly conserved and antigenic and it can usually be found in the bloodstream two weeks after infection. Testing for HCV core antigen is a potential replacement for NAT as an indirect marker for HCV replication, as an atractive alternative due to the lower cost and short turnaround time. HCVc antigen can also be used to monitor the response to antiviral therapy.

Currently, seven tests for HCVc Ag detection are available: 1. The Abbott Architect HCV Ag assay; 2. The Fujirebio Otto HCV Ag test; 3. The Eiken Lumispot HCV Ag ; 4. The Hunan Jynda Bioengineering Group HCVc Ag ELISA; 5. The Ortho ELISA-Ag; 6. Bio-RAD Monolisa HCV Ag-an ULTRA; 7. HUNAN JYNDA HCV Core Ag ELISA.

At this moment, the immunology departament of our medical laboratory, analysis the opportunity of implementing both the two confirmatory tests for HCV infection, NAT and HCVc Ag testing.

Human dirofilariasis, an emerging zoonosis: case series in Romania

Valeriu Gheorghiță, Raluca M. Hrișcă, Adelina M. Radu, Ion Ștefan, Ruxandra Moroti

Background: The genus Dirofilaria (D.) includes vector-borne filarial nematodes, which are usually transmitted by mosquito bites. Human dirofilariasis is an unusual zoonotic infection, increasingly reported in recent years in many parts of the world. The European Mediterranean region is considered highly D. repens-endemic in animals. The objective of our report is to increase the level of awareness among physicians regarding the emergence of human dirofilariasis cases in Romania.

Methods: A retrospective analysis of the human dirofilariasis cases that we diagnosed in our centers in a sixteen years period (2003-August 2019)

Results: In our settings we recorded 10 patients diagnosed with dirofilariasis, one case in 2010, eight cases during 2012-2013 and the last case in the summer of 2019. Age distribution varied between 16-74 years (average 50.9) and the sex-ratio was 1:4 (male to female). Nine out of 10 patients are living in south-east and one in north-west of Romania, with an equal distribution for rural and urban areas (5:5). Eight out of 10 are living near puddles or swampy regions. All reported mosquitos' bites. Five patients had ocular dirofilariasis and another five had subcutaneous dirofilariasis. The diagnosis was misinterpreted as other diseases in 7 out of 10 patients such as bacterial orbital/soft tissues cellulitis and thoracic herpes zoster.

Conclusions: The diagnosis of human dirofilariasis does not require a high level of expertise in parasitic diseases, which is why we consider important to know the clinical features of this parasite, to avoid a misinterpretation of the patient anamnesis and a misdiagnosis.

The practical utility of ultrasonography of small parts and soft tissue

Mirela Oancea, Cătălin Mazilu, Raluca Mititelu, Carmen Tipar

Introduction: Currently, medical imaging presents a rapid development, at an exponential rate, on all the component branches (including ultrasonography).

The immediate value of the ultrasound results from the fact that it is a fast, cheap, non-invasive, non-irradiating imaging method, without contraindications or special previous training, which has developed a great deal and has been diversified in the different areas in which it is used.

An example is the ultrasound of superficial soft tissue, with great applicability including in the surgical specialties (general surgery, ENT surgery, and orthopedics).

Material and methods: Patient G.M., aged 54, admitted to the Surgery II section for a presternal localized mass that had been growing in size lately. For the purpose of excision the mass, the surgeon asked the problem of clarifying the relationships that the respective formation could have with the surrounding structures (thyroid, vascular and muscular planes).

In addition, the patient also had diffuse enlargement of the anterolateral cervical region.

Results: The ultrasound of the enlarged presternal region revealed a thickening of the subcutaneous cellular tissue with a diffuse lipomatous appearance, with the inclusion of the sternal and clavicular ends of the bilateral sternocleidomastoid muscle.

Also, at the level of the anterolateral and submentonal cervical region, has been viewed the same diffuse lipomatous aspect of the subcutaneous tissue and connective tissue between the musculotendinous planes.

In addition, it was also noted that all the surrounding structures (thyroid gland, vascular pedicles, tendons and muscles) were respected by this pathological transformation.

Conclusions/observations: Superficial soft tissue/small parts ultrasonography is a simple imaging method, with easy applicability and within the reach of any dedicated sonographer.

Also, because of its speed and low cost, it is an excellent method of solving some equivocal pathological situations in order to make the best surgical protocol decisions.

Role of radiotherapy in primary mediastinal B-cell lymphoma: case report

Răzvan G. Curcă, Alexandra T. Pasăre, Remus C. Stoica, Diana A. Mitrea, Ștefănel C. Vlad

Introduction. Primary mediastinal B-cell lymphoma is regarded as a subtype of diffuse large B-cell lymphoma. Due to the rarity of primary mediastinal B-cell lymphoma, there are few studies analyzing treatment efficacy, including radiotherapy. Considering the generous life expectancy of patients successfully treated, a good therapeutic intent is to minimize long and short term toxicity. Standard of care consists of chemotherapy combined with immunotherapy, the most common combination being R-CHOP. Radiotherapy of the mediastinum can be used to consolidate the treatment.

Materials and methods. We are presenting the case of a 45 year old man, diagnosed with primay mediastinal B-cell lymphoma, onset symptoms consisting in pain of the anterior thorax. After the initial diagnosis, the patient started with a course of R-CHOP, followed by 6 courses of R-dose adjusted EPOCH. The indication for radiotherapy was established after a PET-CT scan revealed residual metabolic disease.

Results and discussions. Standard treatment for primary mediastinal B-cell lymphoma consist of chemotherapy combined with immunotherapy. Modern treatment techniques show a good control of local disease and low recurrence rates. PET is the most recommended technique in the diagnosis, follow-up and post-treatment evaluation. Although recent data suggest that DA-EPOCH-R can obviate the need for routine mediastinal radiation, some patients require to undergo radiotherapy.

Anti-inflammatory interventions in major depressive disorder: status of research and therapeutic perspectives

Octavian Vasiliu, Daniel Vasile, A.G. Mangalagiu, B.M. Petrescu. C.A. Cândea, Corina Tudor, D. Ungureanu, M. Miclos, A. Drăghici, C. Florescu

Background: New data about the pathophysiology of major depression are arising based on preclinical models, and investigational drugs are being tested starting from these translational models. Inflammation has been considered a potential trigger for the lack of responsivity in a sub-group of patients diagnosed with major depression and anti-cytokines treatment has been suggested as a possible solution for these cases.

Objectives: To assess the current evidence that may support the recommendation of anti-inflammatory drugs as add-on in the treatment of major depressive disorder.

Method: A literature review was conducted in the main electronic databases (PubMed, EMBASE, CINAHL, Thomson Reuters/Web of Science) using the search paradigm “anti-inflammatory drugs” OR “anti-cytokines” OR “monoclonal antibodies” OR “TNF-alfa inhibitor” OR “IL-6 modulators” AND “major depressive disorder” OR “treatment-resistant depression”. All peer-reviewed papers published between 2000 and 2019 containing clinical trials, systematic reviews and meta-analyses were included in the first level of this study.

Results: A number of 23 papers remained after filtering the results according to the inclusion and exclusion criteria, in the second level of our study. Adalimumab decreased the severity of depressive symptoms after 12 weeks in patients with psoriazis, while etanercept confirmed its efficacy over the affective symptoms during an 84 week-extension trial in patients with the same dermatological pathology and comorbid depression. Infliximab decreased depression severity in patients with an increased initial level of inflammatory markers. Tocilizumab and sirukumab are studied as add-ons to antidepressat drugs, in severe cases of major depressive disorder, but the research has so far proved inconclusive.

Conclusions: Although antiinflammatory therapy is a relatively new intervention for affective disorders, it may be a promising intervention for major depression with partial response to classic therapeutic approaches, in a subgroup of patients with high levels of inflammatory markers.

Radiotherapy in thymoma: case report

Diana A. Mitrea, Remus C. Stoica, Maria Vlăsceanu, Gheorghe Bălașa, Ștefănel Vlad

Introduction. Invasive thymomas are rare tumors in the anterior mediastinum, representing 50% of the anterior mediastinal masses and about 20-30% of all mediastinal tumors. They are of unknown etiology. About 50% of patients with thymomas are diagnosed incidentally. Disease stage and completeness of resection have been found consistently to correlate with the duration of progression-free and overall survival.

Materials and method. This case study presents a 68 year-old man with complaints of breathlessness, nosea, vomiting, chest pain and fever. CT images revealed a defined heterogenous anterior mediastinal mass with evidence of infiltrations into the anterior mediastinal fat and an enlarged necrotic paratracheal lymph node. An incomplete resection was made and the findings of histopathological and imunohistochemistry analysis were consistent with thymoma type A. The patient underwent adjuvant radiotherapy.

Results and discussion. The standard primary treatment for this tumors is thymectomy. Modern external beam radiotherapy improves local control in adjuvant setting of incomplete resections. Studies and contouring guidelines regarding thymoma do not recommend elective nodal irradiation due to the fact that these tumors do not commonly metastasize to regional lymph nodes. An exception was made, in this case, for the paratracheal lymph node, considering its malignant appearances on the CT. IMRT technique improved dose distribution, decreasing toxicity to normal tissues.

Follow up appointments, after external beam radiotherapy, confirmed no evidence of local recurrence.

A rare cause of bone pain in a young patient

Oana Stancu, Mihai Șotcan, Adrian Anghel, Gabriel D. Stoicescu, Mihaela Iordache, Oana Ionescu, Lucian Ciobîcă

We present the case of a patient aged 40 years, without exposure to professional toxics and without significant pathological personal background that is presented in our service in order to establish the etiology of intense rashes with important functional impotence started about 4 months with progressive accentuation in the last weeks associated with unilateral eyelid ptosis suddenly installed a few days before hospitalization. He performed in an outpatient ophthalmological evaluation, neurological brain CT that did not establish the cause of the palpebral ptosis and also MRI that revealed bone infiltrative lesions in the lumbar segment. Biologically on admission: major inflammatory biologic syndrome, moderate normochromic anemia, high titration of alkaline phosphatase, high titration of lactate dehydrogenase. Taking into account the presence of bone lesions with possible tumor substrate, an extensive nonplastic balance was performed (tumor markers, endoscopic balance, serum protein electrophoresis – polyclonal hypergamaglobulinemia, immunogram and negative immunofixation). Also, a complete autoimmune-negative and infectious disease balance was performed, which revealed the presence of positive anti-HIV 1 and 2 type antibodies. The presence of antiretroviral antibodies has been confirmed by using a different method so this was the reason why the patient was guided in the Department of Infectious Diseases in order to complete the investigations. After thorough investigations, the diagnosis of retroviral infection was established with certainty and it was decided further, considering the increasing titration of alkaline phosphatase and of lactate dehydrogenase, the performance of a bone scintigraphy. This investigation revealed bone lesions with uncertain substrate - the most likely tumoral. An osteomedular biopsy was performed signaling the presence of Burkitt cells establishing the diagnosis of diffuse Burkitt lymphoma with large cells.

Providencia stuartii outbreaks and antibiotic susceptibility in a Romanian tertiary hospital

Raluca M. Hrișcă, Ion Ștefan, Constantin F. Ghiațău, Valeriu Gheorghiță

Background: Providencia stuartii (P. stuartii), the most common species of Providencia, is an opportunistic human pathogen with intrinsic resistance to polymyxin and tigecycline. The prevalence of carbapenem-resistant P.stuartii is increasing, and the selection of appropriate antibiotic therapy has become limited to a few antibiotics.

Methods: We retrospectively investigated the clinical features and the antibiotic susceptibility of P. stuarti strains isolated in our center between July 2017 and June 2019. In addition, we established the epidemiological links of the cases.

Results: We included 35 patients who had positive specimens with P. stuartii. Only one positive specimen from different sites was isolated in 28 patients: urine (n=11), blood (n=7), respiratory (n=5) and wound (n=5). In the other patients at least two positive samples were diagnosed during hospitalization in each case. We identified 18 cases with epidemiological linkage and two different outbreaks in the intensive care unit (ICU). The first between May-August 2018 and affected 6 patients, and the second between February-May 2019 and affected 11 patients from two departments (6 in the ICU, 5 in the coronary-ICU).The susceptibility profile showed high level of resistance to imipenem (77.8%), amikacin (43.9%) and aztreonam (29.8%). 4 isolates were pan-resistant to antibiotics.

Conclusions: P. stuartii represents a highly resistant and opportunistic pathogen causing severe infections especially in ICU debilitated patients with an increased ability to spread in a hospital environment probably as a result of an excessive use of colistin and tigecycline, the presence of multiple site-catheters and vascular lines and the cross-contamination between patients.

The usefulness of QuantiFERON®-TB Gold in diagnosis of active extrapulmonary tuberculosis: A comparative retrospective study between HIV positive and negative patients in a tertiary hospital

Valeriu Gheorghiță, Adelina M. Radu, Ion Ștefan, Adrian Streinu-Cercel

Introduction: Tuberculosis (TB) is the leading cause of death among people leaving with human immunodeficiency virus (HIV). The main objective of our study was to assess the clinical usefulness of the QuantiFERON®-TB Gold test in diagnosis of active extrapulmonary-TB in HIV-positive and HIV-negative patients.

Methods: We performed a comparative retrospective study on ganglionary-TB in HIV-positive patients versus non-HIV patients in a tertiary hospital from Bucharest, Romania, between October 2012 and June 2018. The main inclusion criteria were the definitive diagnosis of active ganglionary-TB in patients over 18 years old.

Results: A total of 50 patients were eligible for analysis, out of which 33 with HIV co-infection and 17 without HIV. The median age at the time of TB diagnosis was 32 years (IQR, 26.5-37.5) in the HIV-positive group and 58 years (IQR, 29-76) in the non-HIV group (p=0.003). The QuantiFERON®-TB Gold test was positive in 53% (n=9) of non-HIV patients and 27% (n=9) of the HIV-positive patients (p=0.07). In HIV-positive patients with positive QuantiFERON® test the median CD4 count was 230/mm3 (IQR, 178-259) versus 62/mm3 (IQR, 26-152) in those with negative test (p=0.01). The HIV-positive patients had more extensive TB (61% [n=22] had multicentric diseases) compared with HIV-negative patients (18% [n=3]) (p=0.006).

Conclusions: Although the rate of positive QuantiFERON®-TB Gold test in HIV co-infected individuals is lower compared with HIV-negative patients and active TB, it remain an useful tools especially for the diagnosis of latent TB than for active diseases in a population with an increased prevalence of TB.

The "immunological window" of anti-HCV antibodies and the relevance for the serological diagnosis of acute hepatitis C: a retrospective study in a tertiary hospital

Valeriu Gheorghiță, Adelina M. Radu, Ion Ștefan, Adrian Streinu-Cercel

Background: Acute HCV infection is often asymptomatic and is associated with higher rate of chronicity. The main objective of our study was to investigate the "immunological window" of anti-HCV antibodies (HCV-Ab) and the relevance for the serological diagnosis of acute hepatitis C. The secondary objectives were limited to define the epidemiological and clinical features of acute hepatitis C.

Methods: We performed a descriptive, retrospective analysis on all adult patients diagnosed with acute HCV infection during January 2010-December 2016 in the National Institute for Infectious Diseases "Prof. Dr. Matei Balș", Bucharest, Romania.

Results: We included 80 eligible patients with a median age of 47 years (IQR, 34-60) of which 58.8% (n=47) were male. The risk factors associated with infection were identified in 62.5% (n=50) of cases, of which 78% (n=39) were health-care associated infections, including surgical interventions in 74.3% (n=29). The median values of alanine aminotransferases, total bilirubin and prothrombin concentration were 1177 IU/L (IQR, 682-1865), 4.0 mg/dl (IQR, 1.35-9.25) and 96% (IQR, 83.9-109.5), respectively. The rate of positive HCV-Ab was 86.25% (n=69) with a median value of 11.3 (IQR, 6.02-21.5). 44 patients have available HCV-RNA at the baseline diagnosis. The median value was 1.054.334 IU/ml (IQR, 60.442-4.568.326), including 43.2% (n=19) with lower than 600.000 IU/ml.

Conclusions: The majority of patients have already developed seroconversion when hospitalized, which highlights the idea that, at least in symptomatic cases, the "immunological window" is transient and disappears rapidly, making HCV-Ab seroconversion one of the most reliable test for diagnosis of acute hepatitis C.

18F-FDG PET-CT in the evaluation of carcinoma of unknown primary

Raluca Mititelu, Cătălin Mazilu, Dragoș Cuzino, Magdalena Iriciuc, Carmen Tipar, M. Tudoran, T. Mititelu

Carcinoma of unknown primary (CUP) represents a heterogeneous group of cases with demonstrated metastatic disease but in which primary tumor was impossible to detect despite complete and extensive evaluation. This pathology is not rare and associates many difficulties in therapeutic management.

CUP is one of the 10 most frequent groups of cancer worldwide and represents the 4th most frequent cause of neoplasia related deaths in both men and woman. The management of these patients is complex, and usually includes high performance histopathology and imaging techniques. Most CUP are aggressive histopathologic forms and median survival rates reported in literature is 2-10 months from diagnosis.

Identifying of primary tumor is of great importance because it can determine optimization of therapy with significant improvement of outcome and survival.

At present 18F-FDG PET-CT is consider to have an important role in management of CUP and in identifying primary tumor. This is a non-invasive functional and metabolic method which complement anatomic information obtained from radiologic techniques (CT and MRI). Its utility comes from the fact that carcinomatous cells usually are glucose-avid, leading to a better efficiency in detecting the primary tumor.

This papers aims to review literature data concerning the use of 18F-FDG PET-CT in this pathology and to present our experience in the evaluation of these patients.

PET-CT in lung cancer: assessment of recurrence or residual disease

Cătălin Mazilu, Dragoș Cuzino, Carmen Tipar, Magdalena Iriciuc, Raluca Mititelu, M. Tudoran

Purpose: To present role of PET-CT fusion imaging in assessment of patients with lung cancer following surgery (pneumectomy, lobectomy, atypical resection) for primary tumor/lung metastases.

Matherial and method: Studied cases were represented by patients with pneumectomy, lobectomy, atypical resection for oncological disorders which were assessed between March 2018 – June 2019 in Nuclear Medicine Department of SUUMC “Dr. Carol Davila” for suspected local or distance recurrence, based on clinical, biological and radiological findings, at least 3 months following surgery. Acquisition of images was performed at 1 hr following iv injection of 2.5-3 MBq kg bw of F18-FDG, from tentorium to proximal 1/3 of thighs, also completed with planar details or vertex-plantar level scans when needed.

Results: Assessment of changes has includes and SUV lbm (standard uptake value lean body mass) using a cut-off of 2.5. Compared with standard post-surgery CT imaging, PET-CT scan has changed staging of disease by showing new lesions (mainly local disease recurrences and bone and lymph nodes metastases), changing the therapeutical approach, also excluding previously suspected CT recurrences.

Conclusion: Fused PET-CT imaging has excellent results in post-surgery assessment in patients with lung tumoral pathology.

Hepatitis E virus infection in Romania: a neglected cause of hepatitis

Valeriu Gheorghiță, Adelina M. Radu, Ion Ștefan, Ioana D. Olaru, Adrian Streinu-Cercel

Background: The seroprevalence of hepatitis E virus (HEV) infection throughout Europe varies between and within countries and over time. The primary goal of our study was to estimate the prevalence of autochthonous HEV infection in Romania. The secondary objectives were the identification of the risk factors associated with the HEV seropositive status.

Methods: Between January 2015 and December 2016, a total of 201 adult patients, were tested for anti-HEV-IgG. Multivariate logistic regression was used to examine for factors associated with a positive HEV-IgG test. The level of significance was set at $\alpha = 0.05$. The study was approved by the Hospitals’ Ethics Committees.

Results: The final analysis included 175 patients who followed the study protocol, of which 46 (26.3%) had positive, 121 (69.1%) had negative, and 8 (4.6%) had indeterminate HEV-IgG results. Patients with positive HEV-IgG were older [median age of 54.5 years (IQR 43-65)] compared to patients with negative HEV-IgG [median age of 37.5 years (IQR 28-57.5)], p <0.001. A positive HEV serology was more common in patients with history of blood transfusions [n=10 (22.7%) versus (vs) n=11 (9.4%), p=0.025], in those with immunosuppressive conditions [n=18 (40.9%) vs n=27 (23.1%), p=0.025] and in patients with positive HBs antigen (HBsAg) [n=14 (31.1%) vs n=10 (10.3%), p=0.002].

Conclusions: We identified that autochthonous HEV infection is common in the Romanian adult population. Thus, we strongly encourage the inclusion of HEV infection testing from the beginning in all cases of acute hepatitis, acute-on-chronic liver diseases and chronic hepatitis in immunosuppressed conditions.

IL 17 inhibitors a new therapeutic class for treatment of patients with psoriasis

Marcela Poenaru, Anton M. Ţilea, Monica S. Dărmănescu, Anca Ilean, Viorel Trifu

Psoriasis is a chronic, inflammatory disease, characterized by multiple recurrences that profoundly impact the patient's quality of life.

Due to the numerous pathogenic pathways and risk factors involved, psoriasis vulgaris represents a complex therapeutic challenge, usually requiring the combination of multiple treatment schemes, including: topical corticosteroids, sistemic retinoids, Methotrexate, biologic therapies. The characteristic inflammatory response in psoriasis is involving high levels of TNFα, IL-17, IL-23.

We present the cases of 2 patients with psoriasis vulgaris who were treated with Sekukinumab, an IL-17A inhibitor, which led to a significant improvement in both patients.

Hepatocellular carcinoma: Key points

Clara Ion, Romelia Sfetcu, Maria M. Iosif, Cezar Călin

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and is associated with high level of morbidity and mortality being the second most common cause of cancer-related death. Risk factors for HCC are chronic hepatitis B or C virus, alcohol abuse, aflatoxins, metabolic disorders and autoimmune-related disease. In up to 90% of cases, HCC develops in a cirrhotic liver. The pathogenesis of HCC is complex and involves chronic liver injury and genetic alteration. HCC has a poor prognosis if diagnosed at an advanced stage, with a 5 –year survival rate of 0-10%, that is why surveillance of patients at risk for HCC should be carried out by abdominal US every six months with or without workout of AFP.

Diagnosis of HCC is based on history, examination, laboratory tests, assessment of portal hypertension, imaging studies and tumour biopsy.

Staging system for HCC must take into account the next main factors: the assessment of tumour extent, AFP level, liver function, portal pressure, patient's physical status and effects of treatment.

Current management options in HCC depends on the stage of disease and liver function. Management of early and intermediate HCC are: liver resection, orthotopic liver transplantation and local ablation therapy and those are potentially curative treatment for patients with HCC.

Management of advanced disease – conventional chemotherapy regimens have not been found to be particularly effective in patients with HCC, and is not recommended.

Sorafenib is the standard of care for patients with advanced HCC and those with intermediate stage disease not eligible for, or progressing despite locoregional therapies. It is recommended in patients with well- preserved liver function and with an ECOG PS 0-2.

EMA recommends Lenvatinib as a front-line systemic treatment for patients with advanced HCC without main portal vein invasion and with ECOG PS 0-1. Guides recommends Regorafenib for patients with advanced HCC who have tolerated Sorafenib but progressed. Cabozantinib can be considered for patients who has progressive disease on one/two systemic therapies.

Pending approval from EMA for Ramucirumab in second-line patients with HCC and immunotherapy for patients who are intolerant or have progressed under TKI therapy. Further studies are needed.

Follow-up for patients with HCC includes: clinical evaluation for signs of liver decompensation, dynamic CT/MRI, mRECIST criteria.

The importance of multidisciplinary management of a patients with HCC is crucial and key specialities involved: primary care physician, diagnostic radiologist, hepatologist/ gastroenterologist, surgical oncologist/transplant surgeon, interventional radiologist, medical oncologist, palliative care physician.

The level of awareness of the general population regarding sun tanning beds

Irina G. Tănase, Mihaela Georgescu

Introduction: In order to assess the risk of skin cancer, patients should be counseled on the factors that play a major role in the development of this condition by their primary care physicians. UV radiations are used by artificial tanning devices, such as sun lamps and tanning beds. Despite the fact that these devices are thought to be carcinogenic, they are still sold as a safe alternative to UV exposure for both tanning and vitamin D biosynthesis.

Materials and methods: We created a Google form composed of 7 questions to assess the general knowledge of the population regarding sun tanning beds.

Results: A number of 128 persons responded to the Google form, 81.9% females and 18.9% males. The majority (63.3%) population is between 25-35 age interval. 25% admitted that they have used a sun tanning bed only for cosmetic purposes, but 6.3% confirmed using it also as a medical device. The majority of the population is aware that sun tanning beds cause melanoma. As alternatives to sun tanning beds people have used the sun (58.6%), special creams (13.3%), other devices, and a big portion (25.8%) did not use another alternative. A small percent of 3.9% consider a sun tan bed identical to the sun.

Conclusion: The general population should be made aware of the negative effects of sun tanning beds. This include National Health Care program, implication of the National Society of Dermatology and Government.

Comparative study on the use of 19G vs 22G needles used for EBUS-TBNA performed in the Department of Pulmonology, SUUMC

Ioana Ştefănescu, Elvis Firoiu, Florina Vasilescu

Between 2015-2019 in our Department of Pulmonology were performed approximately 100 EBUS-TBNA, 20 of these were performed using 19G biopsy needles. We made a comparative study regarding the use of 22G vs 19G biopsy needles. Given that the number of EBUS-TBNA with 19G needles was smaller than those used with 22G needles, the results cannot be interpreted as very relevant, we will return with details to future events, but we can draw the following conclusions: the lung cancer confirmation rate is comparable (about 85%), but since we use 19G biopsy needles, we had a 100% confirmation rate of Sarcoidosis with mediastinal lymph node involvement, while no suspicion of Sarcoidosis was confirmed when we used the 22G needles The risk of bleeding has increased about 3 times since we use 19G needles, but no bleeding required special therapeutic measures (transfusions, surgery, admission to ATI, etc.), all of which were controlled by bronchoscopic methods, cold serum administration, locally administered adrenaline dilution.

New pharmacological approaches for binge-eating disorder

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Background: Binge eating disorder (BED) is the most frequently diagnosed eating disorder among the US adults, and its worldwide incidence is almost double than the next most prevalent disorder, bulimia nervosa. Therefore, important research efforts at both basic and clinical levels have been reported in the last decades, with several drugs reaching the market, while still other investigational products are in the late phase of development.

Objective: To describe the current status of the pharmacological treatment in BED, based on the available data in the literature, and to hierarchize these therapeutic interventions according to their relevance for the clinical practice.

Methods: An electronic databases search was realized (PubMed, UpToDate, Thomson Reuters/Web of Science and clinicaltrials.gov) using as keywords “binge eating disorder” and “pharmacological treatment”. All published papers, including finalized or ongoing trials found between 2000 and 2019 were selected for the primary analysis.

Results: Lisdexamfetamine dimesylate is the only drug with enough evidence to support its recommendation for the treatment of patients diagnosed with BED. However, it may be associated with significant risk of addiction, which requires continuous monitoring during the entire duration of the therapy. Fluoxetine, sertraline, duloxetine, bupropion, atomoxetine, armodafinil, disulfiram, naltrexone+ bupropion, baclofen, zonisamide, topiramate, lamotrigine, samidorphan, chromium picolinate, liraglutide, orlistat and rimonabant have been associated with mixed results in clinical trials. GSK1521498 is an opioid antagonist that may be a solution for BED managment in the near future, and dasotraline, a serotonin-norepinephrine-dopamine reuptake inhibitor is in phase III of clinical trials, and it seems to be associated with significant efficacy.

Conclusions: A single molecule is nowadays available for clinical use in the treatment of BED, lisdexamphetamine dimesylate, based on solid evidence derived from pivotal trials. Many other drugs are evaluated for this indication, but unfortunately most of them seem to be associated with mixed results.

Correlations between hematoxylin & eosin stain photomicrographs and multiphoton microscopy images in encapsulated thyroid nodules

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Introduction: The diagnosis of malignancy must be confirmed by histopathology which has been the main tool used in the diagnosis of cancer. This technique has evolved from an era of diagnosis based on Hematoxylin and Eosin (H&E) and evaluation of tumors by immunohistochemistry (IHC) to confirm tumor histogenesis and histological subtype to the emerging multiphoton microscopy imaging which is a promising technique as an aid to traditional techniques. Second harmonic generation (SHG) microscopy is a promising technique for non-invasive in vivo high-resolution optical imaging. SHG signals are generated from specific endogenous molecules with a non-centrosymmetric structure, hence this contrast mechanism is intrinsic to collagen in living tissue. Investigating collagen distribution with SHG in tissues enables a precise and non-invasive assessment of extracellular matrix modifications, which represent a hallmark of many pathologies, including cancers.

Objective: The purpose of this study was to compare the accuracy of multiphoton microscopy images with H&E stain photomicrographs in the diagnosis of thyroid cancer to demonstrate some changes in the collagen structure and organization in papillary thyroid carcinoma (PTC) and follicular adenoma (FA) nodule capsules. For this purpose, multiphoton microscopy images might help the pathologist to diagnose thyroid cancer easier and faster.

Method: The SHG imaging was performed using a Leica TCS-SP confocal laser scanning microscope modified for nonlinear optical imaging. The microscope was equipped with a Ti:Sapphire laser (Chameleon Ultra II, Coherent) which was tuned at 860 nm, with ~140 fs pulses and a repetition rate of 80 MHz. A 10X magnification and 0.3 numerical aperture objective was used for focusing the laser beam on the sample and for collecting the backward-generated SHG signals. The forward generated SHG was collected using a 0.9 NA condenser. We acquired images with SHG microscopy on 4 μm thick tissue sections mounted on glass slides without H&E staining and assessed collagen organization of tumoral capsular tyroid nodules and we compared them with the H&E stain photomicrographs of the same capsular level of tyroid nodules (PTC, FA) respectively of the thyroid capsule with Leica microscop to gold standard H&E sample stained.

Results: Images were acquired on the same field of view with the optical microscope and the multiphoton microscope and the images were compared. Important features of PTC were the regions of interest (capsular invasion or capsular microangioinvasion). Images were also taken from the nodule capsule and its surroundings. It was noticed that SHG signals are very useful for the interpretation of the tissues state.

Conclusions: This study confirmed multiphoton microscopy images examination as a reliable and useful diagnostic procedure to demonstrate some changes in the collagen structure and organization in PTC. The results of our study demonstrate that multiphoton microscopy images is an accurate method which can aid in the diagnosis of thyroid cancer.

PET-CT in the management of infective endocarditis

Raluca Mititelu, Cătălin Mazilu, Silviu Stanciu, Dragoș Cuzino, Carmen Tipar, Magdalena Iriciuc, T. Mititelu, M. Tudoran

Infectious endocarditis is a serious condition, which has been increasing in recent years as a result of the growth in the number of surgical procedures involving implantation of prosthetic material in the heart. Infectious endocarditis is associated with a high mortality rate - 20% in-hospital and 40% at one year with maximum treatment.

The diagnosis of infectious endocarditis is mainly based on clinical and ultrasound criteria. However, there are many situations in which the severity of the phenomena and the multiple pathological associations prevent or delay the precise diagnosis. For this reason, it is necessary in many cases to perform imaging procedures withe higher specificity.

Increased glucose uptake at the site of infection represents the pathophysiological basis for using PET-CT with radiolabelled glucose in assessing infectious processes, including fever of unknown origin. Many researchers and clinicians have suggested the potential diagnostic role of 18F-FDG PET-CT in accurately diagnosis of infectious endocarditis, especially in prosthetic valves.

In normal conditions there is variable physiologic glucose uptake in the heart and for this reason it is very important an adequate preparation of the patient, in order to minimize FDG uptake in the normal heart tissue - low carbohydrate diet 24 hours before the investigation, fasting at least 6 hours before the investigation, the eventual administration of heparin iv which, according to many authors would further reduce the physiological uptake of glucose in the heart. All these measures have the role of increasing the specificity and accuracy of the diagnosis.

In this paper we will review the literature regarding the use of 18F-FDG PET-CT in the evaluation of patients with infectious endocarditis.

Coexistence of TIA and minor stroke in the same vascular territory with possible mixed hemodynamic and embolic mechanism

Cristina D. Ghizdavet, Florentina C. Pleșa, Sebastian Botezatu, Ionuț Caloianu, Alexandru Nistor, Carmen A. Sîrbu

Introduction: Transient ischemic attack (TIA) is a neurological emergency. 10% of patients with TIA or minor stroke will develop a stroke within the next 90 days, maximum risk being within the first 24 hours. Up to 80% of this risk could be prevented. The mechanisms of TIA are multiple, sometimes being intricate, therefore difficult to specify.

Case report: 65 years old male, presented right hemiparesis and aphasia, with total recover in less than one hour. He refused the hospitalization proposed by the ambulance crew. 24 hours after the first TIA, the symptomatology repeats. He arrives at ER and he is hospitalized. After the complete anamnesis, the neurological examination and the paraclinical investigations, ischemic lesions in left MCA territory and 70% left ICA stenosis were discovered. Left ICA stenosis has been evaluated at angiography as subocclusive stenosis and it has being stented.

Particularities: The hemodynamic mechanism has been confirmed during the angiography evaluation, when systolic blood pressure dropped from 180mmHg to 100 mmHg, resulting in a TIA recurrence (right hemiparesis and speech disorder for a few minutes). On the other hand, ultrasound images of atheroma and the presence of ischemic lesions in MCA territory (not in the border territories) suggest an arterio-arterial embolic mechanism.

Conclusion: The identification of the pathogenic mechanism is important for prophylactic therapy. There was no significant difference between minor stroke and TIA in prognosis. The treatment for preventing an ischemic stroke after TIA and recurrent stroke following a minor stroke are similar.

The anticoagulation in venous and arterial thromboembolic events associated with acquired thrombophilia

Maria A. Jercan, Lavinia Bârsan, Mihai Șotcan

There are three acquired thrombophilia that carry a high risk of arterial and venous thromboembolism. These acquired thrombophilia are antiphospholipid syndrome (APS) and its variant – catastrophic APS, heparin induced thrombocytopenia (HIT), and paroxysmal nocturnal hemoglobinuria (PNH).

The pathogenesis of thromboembolic disease in APS, HIT, and PNH is complex and includes cellular and/or complement - mediated mechanism.

The treatment of thrombotic events associated with these pathologies, the primary and secondary prophylaxis consist in anticoagulation and had some particularities. The gold standard of treatment in APS remains vitamin K antagonists (VKA), after initial overlap with unfractionated heparin (UNH) or low molecular weight heparin (LMWH). There are additional options in pregnancy associated with APS and in catastrophic APS, that includes the association of aspirin, plasmapheresis, corticosteroids, hydroxychloroquine to the anticoagulation.

The thromboembolism in HIT must be treated with nonheparin anticoagulant initially and after clinically resolution of thrombosis, we can switch the nonheparin anticoagulant to VKA.

PNH is a rare disease and the most important treatment is the eculizumab which stops the pathogenic mechanism. The anticoagulation treatment such as LMWH, of venous or arterial thrombosis associated with PNH must be started immediately and can be switch to VKA after clinically improvement.

Direct oral anticoagulants (DOACs) did not show a good effectiveness in this kind of pathologies, and their use is reserved for low risk acquired thrombophilia in prevention of thrombosis. Further studies are needed.

A rare tumor entity: Leiomyosarcoma of the mesenteric veins

Alexandra D. Radu, Raida T. Stănescu, Cezar Beţianu, Cristina Sandu, Teodor Voiosu, Elena Busuioc

Introduction: Vascular leiomyosarcoma is a rare clinical entity of malignant soft tissue tumours. The majority of reported cases arise from the inferior vena cava, followed by leiomyosarcoma of the main vessels of extremities, renal, hepatic and mesenteric veins.

Case report: A 76-year-old male patient with a history of class II congestive heart failure, ischaemic heart disease, stage II hypertension, paroxysmal atrial fibrillation, type 2 diabetes, stage III chronic kidney disease and hypothyroidism, was reffered to our clinic with diffuse abdominal pain. Blood tests revealed a slight elevation of serum transaminases and mild cholestasis (TGO, TGP and GGT twice the upper limit of normal). An abdominal ultrasound revealed a 10cm hypoechoic non-homogenous mass close to the pancreatic head, with invasion of the splenic vein and mesenteric artery at the origin from the abdominal aorta, with no exact determination of its origin. A contrast computed tomography of the thorax, abdomen and pelvis was performed, revealing a large hypodense non-homogenous tumour, probably with mesenteric vein origin. The patient underwent endoscopic ultrasound examination with fine needle biopsy that showed a heterogenous hypoechoic solid tumour with Doppler signal. The pathology and immunohistochemistry reports revealed the diagnosis of leiomyosarcoma.

Conclusion: We report a case of an unresectable leiomyosarcoma of the mesenteric vessels, which has no certain indication for neoadjuvant chemotherapy or radiotherapy because of its low incidence.

Mass casualty incident by bomb attack: multinational exercise Bucharest 2019

Bogdan C. Teușdea, Daniel Negoiță, R. David

Vigorous Warrior 2019 (VW19), was the biggest NATO medical exercise until now and was conducted in April 2019 in Romania. Representatives from Romania and 38 other nations took part in this exercise.

At the end of this international exercise a bomb attack at a subway station in the center of the capital was simulated and managed under military command.

The goal of the exercise was that the military and civilian entities, both national and multinational act in a coherent and effective way.

The forces involved in rescuing the simulant victims included: Romanian firefighters, Hungarian rescue teams, Romanian and foreign medical personnel, SMURD and civilian ambulances, and a large number of personnel, vehicles and medical systems from the Central Military Emergency Hospital Bucharest (SUUMC “Dr Carol Davila”).

Rescue actions included the extraction of over 200 victims from the subway tunnels and the provision of on-site triage and emergency care. A ROL1 hospital was deployed by the Central Military Emergency Hospital at the Academy of Economic Studies.

During the exercise 18 victims were evacuated to SUUMC “Dr Carol Davila”. Of these, 17 were simulated victims (12 red, 3 yellow and 2 green code of emergency) and one was a real case – one of the volunteers present in the subway station who presented medical problems requiring urgent medical attention.

Case managers from different countries supervised the interventions.

The staff involved in this exercise managed to work in a coherent way, even when crews were assembled of members from different countries and different institutions.

Radiotherapy in adrenocortical carcinoma: case report

Remus C. Stoica, Diana A. Mitrea, Răzvan G. Curcă, Alexandra T. Pasăre, Mihai Dumitrache, Ștefănel Vlad

Introduction. Neuroendocrine tumors are comprised from a broad tumor family, the most common of them are in the lungs, thymus, pancreas and gastrointestinal tract. Pituitary, adrenal, thyroid, parathyroid gland tumors are less common. Adrenocortical carcinoma is a rare malignancy with an estimated incidence of ~ 0.5 – 2 cases per million people per year.

Materials and Method. We are reporting a rare case of adrenocortical carcinoma in a 47 years old female patient, with uncontrolled systemic blood pressure and a left abdominal mass, which is displacing the surrounding structures. After imaging investigations, it was confirmed the malignant features of the left adrenal mass. In this clinical case report, the patient was treated with a complete surgical excision of the tumor, followed by adjuvant radiotherapy.

Results and Discussion. Given its rarity, adrenal carcinoma has been difficult to study and therefore treat. Surgery is considered to be the main approach for locoregional control in early stages, but systemic therapy and radiotherapy, in advanced tumors or with high-risk recurrence features, are useful as adjuvant treatments. Modern external beam radiotherapy, with its recent advances, improves local control in adjuvant setting.

Follow up appointments, after external beam radiotherapy, confirmed the normal blood pressure and no evidence of local recurrence.

Is there still a role for biomarkers in sepsis

Sebastian Dogaru, Bogdan C. Teușdea

The last definition of sepsis (Sepsis 3- 2016) as life-threatening organ dysfunction caused by a dysregulated host response to infection is based on qSOFA criteria in order to help the prehospital and ED teams. It was conceived as a tool for rapid diagnostic. Although a good working definition, after the initial enthusiasm, there are more and more experts saying that the threshold is too high. Waiting until the quick SOFA criteria should be met in order to diagnose sepsis it is sometime too late for an increasing number of patients that are growing older and with a complex adjacent pathology. Although very specific, the last definition is not very sensitive. The doctor’s goal should be the cure of the patient, not a specific diagnostic. The 2018 sepsis update states that the older 3 hour and 6 hour bundles (Sepsis 2) should be done in 1 hour, meaning that time is critical. Combining a not so specific score/ criteria with a specific biomarker in certain circumstances should buy the very necessary time in order that the patients’ status not to become critical, life-threatening. Faster the answer, better the prognosis.

This is why we think that the role of biomarkers in the field is not an obsolete one and the proactive intervention, early in the evolution of an infection, would save lives. A biomarker with good prognostic value should alert the doctor in charge that more proactive, more aggressive therapy should be applied earlier in order to prevent the degradation of patient status. Further research should be done.

An unusual case of chronic kidney disease with lymphadenopathy

Oana Stancu, Mihai Șotcan, Adrian Anghel, Gabriel D. Stoicescu, Oana Ionescu, Lucian Ciobîcă

We present the case of a patient aged 52 year, extensively investigated in the nephrology service for a moderate nitrogen retention syndrome with proteinuria and polyclonal hypergamaglobulinemia – with the exclusion of multiple myeloma. Patient is referred to our service for further investigations in the conditions of persistent nitrogen retention syndrome which is associated with the presence of adenopathies in the peripheral ganglion areas. Serum protein immunofixation is performed, K/lambda chain ratio – normal, immunogram – negative; increased rheumatoid factor. Biologically upon admission: moderate normochromic anemia – osteomedular biopsy was performed showing a nodular lymphoid infiltrate with small cells with nodular appearance. Autoimmune disease investigations was performed with negative results. At the CT scan, hepatosplenomegaly, peripheral and lumboaortic adenopathy, minimal bilateral pleurisy, osteocondensating vertebral lesions and lithic lesion of the left iliac wing are highlighted. Cardiac ultrasound shows minimal pericarditis and dilated right cavities with moderate pulmonary hypertension. The excision of an inguinal adenopathy with a histopathological examination that detects the lymphoganglionic Castelman's disease is decided. Immunohistochemical examination of the lymph node reveals Castelman Disease with the hyaline-vascular subtype; chronic sclerogenic reactive lymphadenitis with Castelman Disease changes. In order to complete the paraclinic investigations, an osteo-medullary biopsy is performed, revealing the presence of a plasmocitoid population, a positive variable at CD 56 invoking the differential diagnosis of multiple myeloma/POEMS and dysmegakariopoiesis at differential diagnosis with a myeloproliferative syndrome - diagnoses that could not be clinically supported. The final diagnosis was the one of Castelman's disease type lymphoproliferative syndrome for which there was a CHOP chemotherapy treatment in 3 sessions: initially with favorable evolution, later with relapse and deterioration of the general condition with massive pleurisy and aggravation of renal insufficiency.

Controversy in thrombolysis

Lorena F. Davidescu, Florentina C. Pleșa

Introduction: Stroke is the second leading cause of mortality worldwide after cardiovascular disease. In Romania, stroke is responsible for approximately 54,000 deaths annually.

Approved by FDA in 1996, intravenous thrombolysis with rtPA is addressed to patients with acute ischemic stroke within the first 4.5 hours after the onset of symptomatology, in the absence of contraindications.

Objective: Intravenous thrombolysis with rtPT has raised numerous controversies from the beginning to present and the aim of our study is to explore them.

Methods: We analised the AHA/ASA asociation and ESO Guidelines for the Early Management of Acute Ischemic Stroke and also the most recent PubMed articoles about controversies in trombolysis.

Results: We took into account the issues regarding the use of thrombolytic treatment such as: the correct identification of the acute stroke, considering clinical signs, severity and atypical presentations, the posibility of extending therapeutic window and the presence of relative contraindications. There is also the debate regarding brain imaging, because in difficult cases advanced neuroimaging techniques might be required.

Conclusions: Proper anamnesis, rigorous neurological clinical examination and complete paraclinical and imaging investigation ensure strict and adequate selection of patients with acute ischemic stroke eligible for Alteplase thrombolytic treatment with a favorable risk-benefit ratio.

Diabetic foot ulcers: new questions, the same problem

Ciprian Constantin, Laura Gagiu, Georgiana Constantin, Oriana Moraru, Cosmin Buzilă

Background: There are quite a number of treatments available for the management of diabetic foot ulcers (DFUs): local therapies, vasodilators, antibiotics, neuropathic and neurotrophic drugs, wound dressings, skin substitutes, growth factors and inflammatory modulators.

Material and methods: Study of regular Clinical Statements reveals that American Diabetes Association is the main professional association that aims to standardize the intervention to prevent this complication of diabetes mellitus, but the spread of this disease is the main reason for increasing incidence and prevalence of minor and major amputation caused by DFUs.

Results and conclusion: Lower-extremity peripheral artery disease (PAD) is a complication of diabetes and occurs in 4.3-29% of all patients, not only diabetics, worldwide.

A new solution is on his way to be aproved: Alpha2C adrenoceptor (A2Ca). A2Ca is a silent Gi-protein-coupled receptor stored in the endoplasmatic reticulum (ER) that implies: a translocation to the cell surface enhances catecholamine-signaling à vasoconstriction, a execution of translocation by external stressors (e.g. cold, endothelial dysfunction), alpha2C adrenoceptor signaling found preferentially in distal vascular smooth muscle cells, and could inhibits presynaptic release of norepinephrine and other neurotransmitters such as dopamine and serotonin.

Will be this A2Ca the solution for this multifactorial problem or will be a part of this problem?

How easily can the diagnosis of ruptured brain arteriovenous malformation be missed

Ionuț R. Dumitru, Carmen A. Sîrbu

Introduction: Arteriovenous malformations (AVMs) have a small rate of occurrence, 10% of them being localised to the posterior fossa (cerebellum). Even though the diagnosis is relatively easy to establish, an incomplete anamnesis along with a superficial neurological examination might lead us towards false conclusions.

Methods and Materials: We'll present the case of a 23-year-old man, who arrived at the ER with vomiting, imbalance, laterodeviation, mild cephalagia and diplopia. We will point out the errors in diagnosis, from both the prehospital stage (biliary dyskinesia) and the hospital one (labyrinthitis), and the way by which the patient, a medical student and volunteer with the ambulance service, had a decisive role in solving his own medical case.

Results: The cerebral CT performed showed a spontaneous hyperdense cerebellar lesion (identified as a tumour, with no hope in a therapeutic path for the patient!). The MRA, and especially the "4-vessel" angiogram found a cerebellar AVM with medium flow, arising from the superior left cerebellar artery, with drainage through a unique vein in the left transverse sinus. Considering the characteristics of this specific case, embolization of the newly formed structure has been chosen as the preferred course of action, with optimal results.

Discussion and conclusions: The essential aspect which raised the patient's doubts was diplopia. It was this symptom that caused the complex investigations that preceded the correct diagnosis. The clinical abilities based on vast medical knowledge lead to the rational use of paraclinical investigations and the establishment of correct diagnosis.

Fast-track: a new way for rapid evaluation in the ED

Bogdan C. Teușdea, Mihai Toma

Medical triage of the patients admitted into the ED is made on the basis of specific criteria established by the national laws taking into account their clinical condition, stability of vital functions, aggravating potential of their status, the necessity of starting a treatment or to carry out some investigations etc. setting up the priorities of the medical care. There are 5 triage categories/levels that establish patient's waiting time, from zero minutes – code red, to maximum 240 minutes the white code.

Fast-track – a new way for rapid evaluation in ED – Is a new concept that permits receiving the patients outside the ED, evaluation (eg. ECG, topical administrations of medications) and treatment of some types of emergencies (e.g fever, allergic reaction, dental problems).

Intricate presentation of a novel disease

Raida Stănescu, Alexandra Radu, Andreea Petridean, Florin Rusu, Elena Busuioc

Introduction: IgG4-related disease (IgG4RD) is a fibro-inflammatory condition that can affect nearly any organ. Common presentations include major salivary and lacrimal gland enlargement, orbital disease, autoimmune pancreatitis, and retroperitoneal fibrosis.

Case report: A 59-year-old female with a history of schizoaffective disorder was referred to our clinic for evaluation of elevated levels of serum creatinine. Her previous lab work also showed a long-standing normocytic, normochromic anemia, hypergammaglobulinemia and inflammatory syndrome with a high ESR. At the time of the referral the patient was unwell, with pale skin and mucous membranes. Blood work revealed a severe anemia (Hb-6.8g/dL), hyperkalemia (K-5.72mmol/L), high serum creatinine (Cr-6.8g/dL), inflammatory syndrome (ESR-70mm/h, CRP-43.32mg/L), hypergammaglobulinemia (gamma-24.8%), and high IgG levels (IgG-2374 mg/dL). Abdominal ultrasound displayed bilateral grade 3 hydronephrosis and a hypoechoic mass surrounding the aorta and inferior vena cava (IVC). Computed tomography of the chest, abdomen and pelvis, performed without administration of contrast material, demonstrated numerous enlarged retroperitoneal lymph nodes forming an extensive mass entrapping the aorta, IVC and both ureters. Bilateral double-J right ureteral catheters were placed with a good clinical outcome. Bone marrow aspiration and biopsy revealed 10% plasma cells, while radiographs of the skull, chest and pelvis showed no lytic lesions. Laparoscopic biopsy of the retroperitoneal mass was performed in a clinic in Italy demonstrating storiform fibrosis, lymphoplasmacytic infiltrate enriched with IgG4-positive plasma cells. A clinico-pathological diagnosis of IgG4RD was made. Methylprednisolone 48mg/day was started with favourable clinical and paraclinical evolution after one month (Hb-10.3g/dL, Cr-2.27mg/dL, IgG-1816mg/dL, gamma-22.3%).

Conclusion: The present study reports a case of IgG4RD presenting with hypergammaglobulinemia, lymphadenopathies and inflammatory syndrome. The diagnostic challenge of IgG4-RD is generated by the clinical and laboratory similarities with other hematologic diseases (lymphoma, plasma cell neoplasms).

The Ketogenic diet: is it suitable for everyone?

Laura Gagiu, Ciprian Constantin

Background: The Ketogenic diet has become very popular in the last period of time, among both people that are trying to lose weight but do not have any other comorbidities and among patients with different types of disease. The ketogenic diet is based on limiting very much the amount of carbohydrates (2-5%), increasing lipids (80-90%) and filling the rest with protein (8-15%).

Material and methods: There are various studies performed on the ketogenic diet published on NCBI and Medscape that have proved its benefits but also it's limits. It is well known that a diet with low carbohydrate intake can improve symptoms in epilepsy and that the ketogenic diet has been included by NICE (the National Institute for health and Care Excellence) as a treatment option in the Clinical Guidelines for Epilepsies. Also, recently it has been discovered that it is also safe and well tolerated in patients with relapsing multiple sclerosis by reducing proinflammatory adipokines.On the other hand low carbohydrate diets have been proven to increase both cardiovascular and cancer mortality data that has been presented on the ESC Congress in 2018.

Results and conclusion: Obesity is a worldwide problem that affects various aspects of a patient's life and should be considered as a disease that can also be prevented. When prevention fails, losing weight should be the first measure taken in order to improve the patient's quality of life. The ketogenic diet is one of the options that could be considered but when the decision is taken, all the comorbidities should be taken into consideration, and the balance between risks and benefits should lean towards benefits. For example in a diabetic patient,lowering the carbohydrate intake has been proven to reduce the glycemic levels but the excess fat has increased LDL levels which also increases its cardiovascular risk.

Even though there are both recommendations and restrictions regarding the ketogenic diet it depends very much on the patient and its associated diseases and also on how the doctor can make variations of the diet according to the patients needs.

suPAR: a biomarker in evaluation of patients with fever

Mihai Toma, Bogdan C. Teușdea

Background: Severe infection can cause sepsis that is a potentially fatal whole-body inflammation. Biomarkers are widely used in clinical practice and they are useful for monitoring the infectious process. Procalcitonin (PCT), lactate and C-reactive protein (CRP) have been most widely used, but even these have limited abilities to distinguish sepsis from other inflammatory conditions or to predict outcome.

The aim of our study was to investigate the role of new biomarkers – soluble urokinase-type plasminogen receptor (suPAR) in patients with fever.

Methods: Between January – July 2019, blood samples were taken after obtaining informed consent from 45 patients with fever. We determine five biomarkers: leucocyte, procalcitonin (PCT), lactate and suPAR. We obtained clinical data and calculate SIRS and qSOFA score. Statistical analysis was performed with StatDirect program.

Results: The mean values for the biomarkers were: leucocyte 16038.46/ml, procalcitonin 1.48 µg/ml, lactate 1.34 mmol/l and suPAR 5.68 ng/ml.

Discussion & conclusions: PCT and CRP are main markers used in clinical practice and are more useful to rule out infection. PCT is the most studied biomarker that guides early stopping of antibiotic therapy in adults. In our study elevated suPAR was associated with more hospitalization days, and was much better correlation with elevation of leucocyte than procalcitonin.

Relationship between HLA-B27 positivity and radiological damages in patients with Ankylosing Spondylitis

Daniela Anghel, Livia Otlocan, Raluca Bursuc, Elena Busuioc, Anca Manolache, Valeriu Smedescu, Maria M. Negru, Cristina F. Pleșa, Ciprian Jurcuț

Background: Axial spondyloarthritis (AxSpA) is a disease where back pain is the main symptom. The disease can cause axial-joint inflammation, erosion and new bone formation, leading to functional impairment. Traditional assessment tools include Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) which is designed to assess spondyloarthritis (SpA) disease activity and daily function. Although this tool is well validated, the relationships between disease activity, functional status, axial-joint inflammation and degree of syndesmophytes formation are more complicated. Studies showed that disease activity might not correlate with axial-joint inflammation and functional impairment could be caused by both axial-joint inflammation and structural damage of the spine.

Objectives: We are trying to find the prognostic factors for syndesmophytes formation. So far, the identified potential risk factors include male sex, human leukocyte antigen (HLA) B27 positivity, smoking, and an initially raised C-reactive protein (CRP). As some of the risk factors are potentially modifiable, it would be of interest to know if they will also apply in our local population. By categorizing a group of patients with AxSpA, we aimed to describe the clinical characteristics and their relationships with disease activity, functional status, and syndesmophytes formation.

Methods: This is an observational and descriptive study of 35 patients with AxSpA recruited from the Department of Internal Medicine II, "Dr. Carol Davila" Central Military Emergency University Hospital, from October 2018 to July 2019. Our study aims to determine the changes in magnetic resonance imaging (MRI) in patients with AxSpA.

Inclusion criteria included diagnosed AxSpA patients with current back pain. Exclusion criteria included pregnancy and inability to undergo MRI examination.

Clinical and blood parameters were collected. These included age, sex, smoking and drinking history, duration of back pain, location and characteristic of back pain, extraspinal features, associated medical history, HLA-B27 status, CRP, and erythrocyte sedimentation rate (ESR).

Patients completed a self-assessment questionnaire: BASDAI – to calculate the Ankylosing Spondylitis Disease Activity Score (ASDAS).

Radiological sacroiliitis based on the Modified New York criteria: grade 0, normal; 1, doubtful; 2, obvious; 3, fusion. Bilateral sacroiliitis grade 2 or above or unilateral sacroiliitis grade 3 or above was defined as radiological SpA. All patients had spine and sacroiliac joints MRI.

Results: 35 patients were recruited. Our cohort was characterized by long disease duration, high disease activity, moderate functional impairment and significant radiological damage.

Result 1: High disease activity (main BASDAI=4.28) and high radiological damages were found on 21 (60%) patients. HLA-B27 positivity was found in 70.0% of the population. Most of them were male (75.0%). There were 68% smokers. Average age was 44.5 year and mean CRP was 9.5 mg/dl.

Result 2: Low disease activity (BASDAI=3.34) and some radiological damages were found on 5 (14.29%) patients. All patients were found to have HLA-B27 positivity. All of them were male. All of population were smokers. Average age was 37.8 years and mean CRP was 0.75 mg/dl.

Result 3: High disease activity (BASDAI=4.87) and minimal radiological damages were found on 4 (11.43%) patients. All patients were found to have HLA-B27 positivity. All of them were female. None of the population were smoker. Average age was 43.5 years and mean CRP was 5.11 mg/dl.

Result 4: Highest BASDAI (BASDAI=5.41) and some radiological damages were found on 5 (14.29%) patients. All patients were HLA-B27 negative. Female patients were 61.9% of the study group. There was no smoker. Average age was 47.5 years and mean CRP was 5.46 mg/dl.

Conclusions: In a group of patients with long-standing SpA, we described the clinical characteristics in relation to disease activity, functional status and radiological damages.

We found that the group of patients with high axial-joint inflammation and structural damage of the spine were male smokers with HLA-B27 positivity. The findings correlate with poor prognostic factors.

In patients with HLA-B27 positive who do not have radiological changes, it is important to perform MRI for the diagnosis of ankylosing spondylitis.

By describing different characteristics of patients with SpA in relations to disease activity, functional status, and radiological damages, we hope to provide a clearer picture for rheumatologists to understand and interpret different clinical parameters and to provide the best management to patients.

The experience of the Central Military Hospital in the thrombolytic treatment of acute ischemic stroke

Oana C. Tache, Florentina C. Pleșa, Carmen A. Sîrbu, Gabriel Boeru, Cătălin Andrei, Gabriel Ștefănescu, Cristina L. Otavă, Simona Gheorghevici

Introduction: Thrombolytic medication is the Gold Standard treatment of acute ischemic stroke for eligible patients.

Objective: The purpose of this work is to evaluate the efficiency of thrombolysis in terms of operation time, teamwork, evolution, complications and to identify the dysfunctions, in order to optimize the therapy.

Methods: We analyzed a group of patients thrombolysed in our hospital, noticing their evolution during the hospitalization, at one and 3 months after hospitalization, appreciated by functionality scales. We have also followed the evolution of thrombolysis over time from its introduction as a treatment method for stroke.

The first thrombolysis was performed in 1958 when out of three patients treated only one presented improvement of symptoms. Intravenous thrombolysis was initiated in the clinical neurological activity more than 15 years ago.

Thrombolysis was introduced in the Central Military Hospital in March 2019 and now we have 10 patients who have benefited from this therapy.

Thrombolysis is possible with the fast and synchronous intervention of a team made out of: neurologist, emergency doctor and radiologist. Considering the hospital's compartmental structure, the team spirit is the binder that leads to obtaining the optimal times.

Thrombolysis had a beneficial effect on the majority of patients, the disability scores being significantly improved. The complications were reduced and in the case of death, it was mainly due to their comorbidities and complications, not to the thrombolytic treatment.

Conclusion: In conclusion, analyzing the thrombolysis cases from our hospital, we found a mortality rate of 30%, which was mainly due to the associated comorbidities. Being still at the beginning of the road, we cannot compare these results with those existing in the literature.

Thrombolysis role in the treatment of stroke

Mihaela M. Micu, Pleșa Andreea, Florentina C. Pleșa

Introduction: Stroke is one of the leading causes of morbidity and mortality worldwide. Stroke is the second leading cause of dementia and a common cause of depression and epilepsy in the elderly.

Objective: The purpose of this paper is to present a modern method for the treatment of stroke: thrombolysis. We will follow the whole procedure, starting with the onset of the symptoms and taking into account its indications, contraindications, risks and benefits.

Methods: We considered the National Protocol for Thrombolysis and emphasized the importance of correct diagnosis, proper indication and accurate procedure, everything happening in the therapeutic window.

The FAST method (Face, Arms, Speech, Time) plays an important role is early identification of a stroke. Clinical sings such as: facial asymmetry, inability to raise both arms and speech problems are warning signals and require a fast presentation at the emergency room.

Time lost means brain tissue lost. Hypoxia causes irreversible lesions in the center of the ischemic area. In contrast, the edges contain neurons that can be saved if the blood flow is restored timely. Consequently, thrombolysis has the highest success rate in the first hour, known as the "golden hour". However, therapeutic efficacy can be achieved up to 4.5 hours after the onset of symptomatology.

Conclusion: Finally, we would like to highlight that thrombolysis is a modern procedure for stroke treatment, proved to significantly improve patient evolution. Inter-clinical cooperation and promptness of intervention contribute to therapeutic success.

Efficient neuromotor recovery of a young patient with tetraparesis after traumatic vertebral meduller by road accident

Gigi Teodoru, Ovidiu C. Chiriac, Ovidiu S. Indrei

We present the case of a patient aged 35 year, patient immobilized in bed, with spastic tetraparesis after vertebro-medullary trauma by road accident, following the local clinical examination neuro-myo-arthro-kinetic functional, can make the transfers with help, cannot maintain the short sitting position at the edge of the bed and verticalization no was initiated.

For three weeks our medical collentive has progressively initiated verticalization and displacement with the help of a new generation device Robogait. Robogait are stationary gait rehabilitation systems. The Robogait is designed to be used with patients recovering from traumatic brain and spine injuries, stroke, neurological or orthopedic causes. This system provides a safe, controlled environment for gait rehabilitation over a treadmill. The speed of the treadmill is synchronized with the exoskeleton and the rehabilitation program.

The RoboGait reduces the number of physiotherapists required to work with a patient. The wearable robot increases the quantity of steps that can be achieved during each training session and ensures that they are all consistent. In these systems, the patient is suspended on the walking band and positioned perpendicular to the foot and connected to the robotic. The physiotherapist will decide after the examinations that the weight of the body will be reduced by the device and where the device will contribute to the walk. In the robotic walking system, the patient is executed according to the natural walking pattern on the walking band, while the receiver mounted on the patient's hips and knees evaluates the response of the body to the computer and reports to the computer which step is the problem. When the patient goes out of the normal walking pattern, the robot automatically corrects walking. Repetitive gait in robotic assisted walking therapy helps regain the impaired signal flow due to disease or injury between the cerebrospinal and muscles. Other benefits of this treatment include strengthening the muscles in bed-dependent and non-walking patients, providing circulation in the legs, reducing bone loss and fracture risk due to movement, and preventing bed sores and vascular occlusions.

After 9 sessions, the patient moves with help, metal frame, has considerably improved mobility.

SURGICAL PAPERS

Management of recurrent craniopharyngioma. Case presentation and literature review

O.M. Sîrbu, Alin V. Chirteș, A.M. Sîrbu, Adina Mazilu, Marian Mitrică

Craniopharyngioma, described by Cushing as ‘‘one of the most baffling problems which confront the neurosurgeon’’, account for less than 5% of all intracranial tumours. They are derived from remnants of Rathke's pouch along a line from the nasopharynx to the diencephalon. Although histologically benign they may be locally aggressive and their close proximity to vital structures such as the hypothalamic–pituitary axis, thalamus and optic apparatus makes them one of the most challenging pathologies in neurosurgery.

We present an ilustrative case and an updated literature review of the management options following unsuccessful initial surgery or recurrence.

Treatment options for recurrent craniopharyngioma include repeat surgery, radiotherapy or intracystic procedures.

Several small retrospective studies suggest that rates of obesity and diabetes insipidus related to hypotalamic injuries may be lower in patients treated with more conservative surgical approaches. Prospective studies with long-term follow-up are still needed.

Contemporary radiotherapie techniques (stereotactic radiotherapy, intensity-modulated radiation therapy, proton beam therapy) permit greater treatment precision and conformity but do not eliminate long-term toxicity.

With intracavitary chemotherapy experience is more limited. Bleomicin and interferon alfa have been reported in some centers with promising results.

An experienced multidisciplinary team (neurosurgery, radiotherapie, neuro-oncology, endocrinology, ophthalmology) is essential for the optimal management of craniopharingiomas. Future treatment with neoadjuvants chemotherapy and minimal surgical resection in order to preserve neurological status may improve the outcomes of these patients.

Approaches in anterolateral abdominal wall defects

Costin Duțu, Mădălina Vlad, A. Luchian, Ovidiu Albița, Cristian Mușat, Remus Nica, Teodor Rogin, Cristian Cîrlan, Florin Săvulescu

The treatment of anterolateral abdominal parietal defects is, in most cases, surgical. Surgery can be performed open or minimally invasively.

The open procedure consists in repairing parietal defect, followed by the reinforcement of the wall by placing a consolidation mesh (preaponeurotic / preperitoneal placement). In the last years, the laparoscopic minimally invasive procedure e-TEP has been developed, through which small incisions of maximum 10 mm are practiced and an intraperitoneal or retromuscular mesh is installed to prevent recurrence. The study analyzed data from 180 patients with abdominal wall defects whose surgical treatment was performed, through the different approaches already mentioned, during one year in the Second department of general surgery.

The age, weight, BMI, ASA score, defect size and placement, the size and nature of the mesh used, intraoperative blood loss, duration of the intervention, duration of hospitalization, association of diastasis or visceral lesions, the frequency and type of perioperative complications, the quality of the postoperative life, the time required for recovery and social reintegration were analyzed and compared.

The conclusions of the study revealed the advantages of minimally invasive procedures (rapid postoperative recovery, decreased postoperative complications, aesthetic role, and rapid recovery of social and professional activities. On the other hand, in case of bulky abdominal eventrations associated with a reduced medical education, the role of open procedure is important, especially in the emergency situations and associated lesions.

“Upside-down stomach”: a rare complication of hiatal hernia

Rareș Munteanu, Roni Gherghinoiu, Aurelian Sfetcu, Dumitru Lăcătușu

Hiatal hernia represents the stomach’s protrusion in the chest through the esophagus diaphragm Hiatal hernia can be: slip (cardia is in the chest), rolling (esogastric junction remains in the abdomen) or mixed form. A special form is hiatal hernia complicated with axial organ gastric (upside-down stomach). After a review of the specialized literature, we present the case of a 55-year-old patient who has emerged urgently in the emergency room for dysphagia, vomiting, nausea, palpitations. Standard radiological examinations with contrast substance and computer tomography revealed giant hiatal hernia mixed form and gastric volvulus (upside down stomach). After usual preparing the patient for surgery, the laparoscopic intervention occurred, the hernia was reduced, the diaphragm opening was recalibrated (pile suture), after which an antireflux procedure (Nissen fundoplication) was performed. The postoperator was subjected to radiological control with the contrast substance, the patient being exited 7 days postoperatively.

Enucleation of huge symptomatic liver hemangioma

Liviu Mosoia, Florin Macău, Traian Calu, Tanita Stancu

Objective: The cavernous hemangioma is the most common benign tumor of the liver and become symptomatic as it reaches a certain size. Giant hepatic hemangiomas greater than 20 cm in maximum diameter are often reported. Tumor resection or enucleation is tratment of choice for symptomatic hemangioma.

Materials & methods: A 41-year-old female presented with symptomatic huge hemangioma of the liver was treated by enucleation.

Results: The surgical indication was abdominal pain, complaints of a 4.5-kg weight loss, fullness in the right upper quadrant and increased abdominal girth. The tumor size was 24 x 18x 14cm.

Technical aspects were analysed.

The postoperative period was uneventful and the hospital stay was 5 days. Follow up imaging controls showed no recurrences.

Conclusion: Abdominal pain and enlargement are major surgical indications of symptomatic giant hemangiomas. Most of the symptoms disappear after the surgical treatment. Enucleation is the best surgical tehnique for management of symptomatic giant hemangioma.

eTEP in linea alba defects repair surgery

Costin Duțu, Mădălina Vlad, A. Luchian, C. Blăjuț, M. Iordache

eTEP (Extended-View Totally Extraperitoneal) technique was initially used for minimally invasive approach for inguinal hernia, but it was also applied for minimally invasive surgeries for linea alba defects, diastasis and ventral abdominal wall eventrations. We aim to present the initial results of patients with linea alba defects, diastasis and ventral abdominal wall eventrations for whom minimally invasive treatment was performed using eTEP technique.

A prospective analysis of cases with linea alba defects, diastasis and ventral abdominal wall eventrations with eTEP repair, made between October 2018 and May 2019 by two operating teams, in a single center was made. All patients were followed postoperatively for 2 months.

Patient history, defects characteristics, technical details of interventions, perioperative complications and quality of life outcomes were included in data analysis. The results of the case series were analyzed in detail.

The synthetic graft had dimensions between 10x10 cm and 20x30 cm. The mean duration of the intervention was 148 minutes, the intraoperative blood loss was 74.16 ml and the mean duration of hospitalization was 3.2 days. The peritoneum was opened in 80% of cases.

Only one case required conversion to laparoscopic surgery - the IPOM + technique. Postoperative complications were not significant and the quality of life was clearly improved after surgery (California Comfort Scale). Mean costs of intervention were lower in case of eTEP surgery than open or laparoscopic techniques. There were none readmissions in the first 30 days after the surgery.

The feasibility and advantages of this technique were highlighted on initial evaluation: decreased postoperative pain, low rates of postoperative complications, rapid recovery of social and professional activities and reduced mean costs than IPOM+ laparoscopic technique (due to the abandonment of using Dual Mesh graft) and open technique (due to decrease hospitalization duration). We intend to further analyze these data on a larger number of cases.

Glioblastoma multiforme (grade IV)

Cristian Năstase, Cristian Popescu

Introduction: The most malignant astrocytoma and the most common primary brain tumor. Histopathologycal pattern: areas of necrosis, neovscularisation with endothelial proliferation, gemistocytic astrocytes etc

Material and methods: We would like to present you our experience in treatment of this kindes of tumors. We operate more 1oo patients with GBM in our service and some of them survive more 3 years with adjuvant treatment (radiotherapy, chimiotherapy, etc).

Prognostic: Survival is highly dependent on condition at the time of surgery

Results: Anaplazic astrocitoma & GBM has severe prognosis; Neuroimaging: MRI – main tool; Mass effect: emergency intervention; Radiotherapy & chemotherapy: limited effect.

Breast cancer: where are we and what can we do

Remus I. Nica, Cristian J. Mușat, Doriana Vintilescu, Florina Vasilescu

Introduction. In Romania breast cancer accounts for 25% of all cancer cases and it is responsible for 1.56% of the total number of deaths. The genetic factors known to be involved in breast cancer risk comprise several high or moderate - penetrance genes and a high number of low-penetrance genes.

Aim. In this study we would like to present some representative cases of breast cancer that were encountered in our clinic and results regarding the interactions between low penetrance risk factors in the sporadic forms of invasive ductal carcinoma.

Materials and methods. Approximately 70% of investigated subjects had invasive ductal carcinoma. Women with invasive ductal carcinoma (n=125) and clinically healthy subjects without history of malignant disease (n=150) were selected for a case-control study. Mutations in six low-penetrance genes were determined for each sample. Multifactor Dimensionality Reduction analysis was used to investigate the epistatic relationships between the tested variables.

Results. The birth of the first child after age 30 ($p < 0.01$) was the main non-genetic risk factor associated with breast cancer. The simultaneous presence of TGFb -509T and IL6 -174C ($p < 0.01$) was a risk factor for invasive ductal carcinoma in the studied group.

Conclusions. The interaction between different categories of risk factors can increase the risk for sporadic forms of breast cancer. The birth of the first child after the age of 30 is a significant risk factor for the disease.

Combined dermato-surgical techniques in treating rhinophyma

Viorel Trifu, Monica Dărmănescu, Marcela Poenaru, Mihai Țilea, Răzvan Șerban

Rhinophyma is a slow evolving pathology that affects the nose (rhis' - nose; phyma - grows) and it represents the final stage of rosacea. It is characterized by a cauliflower-shaped, bulbous, thickened skin, erythematous, edematous, enlarged sebaceous glands accompenied by sebum plugs.

It mainly affects men starting at the age of 45 and can also affect other regions such as chin (gnatophyma), ears (otophyma), eyelids (blepharophyma) and forehead (metophyma)

Non-surgical treatment of rhinophyma consists of a prophylactic approach aimed at patients with rosacea but there are various systemic and topical treatments which can be used to lower the volume in the early stages of rhynophyma.

Surgical approach represents the gold-standard in treating Rhinophyma by using combined techniques in order to obtain a favorable result. Among the methods used we mention dermaplaning, dermabrasion, electrotherapy, CO2 Laser, Nd: YAG, Er: YAG, cryosurgery.

The cases selected in this presentation are of patients with rhinophyma of different severity grades who have been treated in the Dermatology Clinic.

Rhinophyma is a condition that affects the quality of life, especially regarding its social impact, as patients often report social isolation, especially because of their physical appearance, which can be corrected by proper dermatological and surgical treatment.

Remnant cystic duct: cause of post cholecystectomy syndrome

Ovidiu Albița, Teodor Rogin, R. Marin

Remnant duct pathology represents a rare evolution of the disease after cholecystectomy, caused by anatomo-clinical details at primary surgery, open or laparoscopically. Usually symptoms are discordant to imagistic exploration. However only a new operation, although technically demanding, provides very good results.

Foreign body in the hypopharynx: surgical approach

Claudiu Nistor, Daniel Pantile, Adrian Ciuche

Introduction: While pharyngeal foreign bodies are a common complaint in children, for adults this is a relatively rare condition, which can be resolved endoscopically in a majority of cases.

Material and method: We present the case of a young patient with complete dysphagia, sialorrhea, odynophagia, and a sensation of foreign body. The symptomatology appears after ingesting a foreign body on an otherwise normal esophagus.

Paraclinical investigations (cervico-thoracic X-Ray, cervical and thoracic CT scan) highlight some foreign body in the hypopharynx. Superior endoscopy and laryngoscopy do not visualize the foreign body, only some erythema on the hipopharinx.

Endoscopic extraction of the foreign body has been tried, with no results. Considering this situation, the surgical approach has been decided, through a slightly atypical approach – right side approach, the foreign body being on the right side of the esophagus.

Results: Intraoperative, the foreign body is identified in the right esophageal wall, puncturing the esophageal mucosa. With favorable postoperative outcome, the patient showed no signs of mediastinitis or esophageal fistula.

Discussion: For most cases of foreign bodies stuck in the hypopharynx, superior endoscopy is 80-100% efficient in extracting the foreign body. In the presented case, nor the endoscopy or laryngoscopy succeeded extracting it. The surgical intervention was the final, salvatory, solution.

Conclusions: Endoscopic treatment remains the mainstay treatment for most ingested foreign bodies. This patient required surgical intervention because of the impossibility to visualize the foreign body through an endoscopic procedure.

A rare case: vulvar extra-mammary Paget's disease

Alexandra T. Pasăre, Răzvan G. Curcă, Remus C. Stoica, Ștefănel C. Vlad

Introduction. Paget's disease of the vulva is a rare vulvar neoplasm most commonly seen in Caucasian postmenopausal women. Probably because of its multicentric nature, Paget's disease has a chronic and relapsing course. Surgery is the treatment of choice, but the disease can extend from the visible lesion and the surgical margins are frequently positive.

Materials and methods. This is the rare case of a 50 year old patient, known with vulvar Paget's disease since 2010. She underwent large wide excision of the left labia, with clear surgical margins and a good postoperative evolution. After nine years, the disease relapsed and the proposed treatment was left hemivulvectomy, followed by adjuvant external beam radiotherapy.

Results and discussions. The standard of treatment for vulvar Paget's disease has been surgery with the control of the surgical margins, but given the fact that the positive margins and recurrence rates are greater than 50%, combined treatments have been proposed. Radiotherapy can be used as a curative treatment or in an adjuvant setting to prevent local recurrence. After the combined treatment, the follow up showed minimal adverse effects after radiotherapy, with good local control of the disease.

Giant left ovarian fibroma: Demons-Meigs Syndrome in a 69-year-old female patient

Ioana A. Negoiță, Nicolae Niculescu, Bogdan Panaite, Florin Năftănăilă-Mali, Ioana Niculescu, Ovidiu V. Nicodin

Goals: Among the different types of ovarian tumours, ovarian fibromas are mostly silent tumours, without clinical impact (especially when they are small in size), being non-functional tumours. There are cases that can show sterility, compression disorders, dysmenorrhea, secondary amenorrhea, and in cases of solid ovarian tumours, they may associate Demon-Meigs Syndrome. The diagnostic orientation is based on clinico-biological, imaging and anatomo-pathological elements. Demons-Meigs Syndrome, first described in 1937 by Professor Joe V. Meigs, is defined as an association of a benign ovarian tumor (ovarian fibroma or thecoma) complicated by hydrothorax (most commonly on the right side) and ascites in large quantities. Surgical removal of ovarian formation leads to disappearance of ascites and hydrothorax.

Summary: The authors present the case of a 69-year-old female patient, with multiple pathology (cardiac, pulmonary, diabetes mellitus, gastroduodenal), who came to the ED of the SUUMC for general condition worsened in the last days, showing severe dyspnea, increased volume of the abdomen, epigastralgia. It will be presented the manner of approaching this case, from hospitalization and its evolution.

To be retained: 1. Meigs Syndrome is a challenge in establishing differential and certainty diagnosis. 2. The therapy of choice remains surgical resection, either ovariectomy/adnexectomy/tumorectomy or, at postmenopausal ages, total hysterectomy with bilateral adnexectomy. 3. The ascites and the hydrothorax remit after surgical treatment. 4. The prognosis in Demons-Meigs Syndrome is a very good one.

Liver resection for huge hepatocellular carcinoma in non-cirrhotic liver

Liviu Mosoia, Florin Macău, Traian Calu, Tanita Stancu, Marian Vasile

Objective: HCC in non-cirrhotic liver has low prevalence and tumor size may be large at an advanced stage as surveillance is not performed in a non cirrhotic liver. Tumor resection is the only curative treatment if resecability criteria are achieved.

Materials & Methods: Three patients with huge HCC (18, 20 and 23 cm) underwent liver resection in our department. In one patient, due to the insufficient future liver remnant, we performed right portal vein ligation (PVL) in the first instance, to convert the unresectable tumor to resectable for potential cure. Technical aspects were analysed.

Results: One patient underwent right hepatectomy after 7 weeks after PVL; the others underwent right hepatectomy resection. In the patient with the biggest tumor (23/20 cm) we used anterior approach and the hanging manoeuvre due to the huge volume of the tumor. The postoperative period was uneventful and the hospital stay were 7 to 9 days. Histopathology revealed in one patient a mixed off HCC with cholangiocarcinoma and all the patients are alive, disease free currently.

Conclusion: Surgical resection for huge HCC in non cirrhotic liver is possible even in elderly patients or even if initially, the future liver remnant does not allowed surgical therapy.

Minimally invasive approach for anterior flail chest

Claudiu Nistor, Dragoș Marin, Aurora Fera, Daniel Pantile, Adrian Ciuche

Introduction: Anterior flail chest is a life-threatening medical condition that occurs when a segment of the rib cage breaks due to trauma and becomes detached from the rest of the chest wall. Over time several stabilizing methods have been imagined.

Material and method: We present a method of stabilization for anterior flail chest using the minimally invasive repair procedure for pectus excavatum – Nuss procedure.
This procedure consists of a concave stainless-steel bar slipped under the sternum fixed by a stabilizer fitted around the bar and into the ribcage.

Preoperative CT scan with 3D bone reconstruction helps the surgeon decide the best position for the stainless-steel bar. The surgical intervention is performed under thoracoscopic control.

Results: The results were very good in both patients. The patients were extubated immediate postoperative, followed by respiratory recovery.

Discussion: Although our experience is limited to just two cases, postoperative results were encouraging in both cases. Each time patients were extubated immediately postoperative, and the paradoxical movement of the rib cage disappeared. The stainless-steel bar was removed 2-4 months after surgery, with a good quality chest wall stabilization.

Conclusions: The results obtained using this method of chest wall stabilization were encouraging. Patients' recovery is quick and of good quality. By being a minimally invasive procedure, esthetic results are far superior, and patients' mobilization can begin early.

Functional and reconstructive otologic surgery in private medical centers

Vasile Ciuchi, Oana I. Popa

Purpose: Otologic surgery without hospitalization or involving one day hospitalization applied in external and middle ear pathology, except oncologic disease, represent a new approach in the practice of E.N.T. speciality.This new approach in functional and reconstructive otologic surgery became widespread due to the occurrence of private medical centers which offers possibilities of investigation and modern treatment.
Methods: The authors present the experience of 12 years and 8 months of activity in two private medical centers, where they performed numerous surgical interventions of external and middle ear. They present the most commonly addressed diseases as well as surgical techniques and some useful conclusions for otologic surgeons in training.

Imagery in diagnosis of anterior segment pathology

Mihail Zemba, Ovidiu Mușat, Cornel Ștefan

Purpose: to show the usefulness of imagery

Methods: some clinical cases are presented, insisting on how imagery was essential in establishing the diagnosis or the therapeutic attitude

Results: lack of imagery would have done establishing of a proper diagnosis much more difficult

Conclusions: though clinical examination is simple, some particular cases with anterior segment pathology need additional investigations, every methods with its indications and limits

Therapeutical approach for right pulmonary artery aneurysm: case presentation

Claudiu Nistor, Olivia Batog, Daniel Pantile, Adrian Ciuche

Introduction: Aneurysms of the pulmonary artery are rare and often underdiagnosed. Pulmonary artery aneurysms generally occur in a younger age group than aortic aneurysms with an equal sex incidence, affecting main branches of the pulmonary artery.

Material and method: The authors present a case of a patient admitted with small efforts dyspnea for a round, well defined lung tumor, located in the right lower lobe. Paraclinical investigations are described and the surgical intervention has been recommended in order to remove the tumor.

Intraoperative, the surgical team has identified the tumor as a pulmonary artery aneurysm and an arterio-arterial shunt between the systemic and pulmonary circulation. Right lower lobectomy has been performed with the removal of the aneurysm.

Massive hemorrhage was imminent until the aneurysm has been removed.

Results: The postoperative outcome was favorable, with the remission of dyspnea.

Discussion: Despite modern diagnostic methods, there is no clear guideline for the best therapeutic approach, and there is limited experience because of the infrequency of the disease. Overall, idiopathic pulmonary artery aneurysm seems to be a relatively benign condition, and clinical manifestations are dominated by refractory dyspnea.

Conclusions: Pulmonary artery aneurysms seldom occur, are rarely diagnosed, and do not present with distinct symptoms. Although there are no clear guidelines on the optimal treatment for patients with pulmonary artery aneurysms because of the small number of cases, surgical treatment remains an important approach, especially in symptomatic patients.

Thoracic oulet syndrome: case presentation

Daniel Pantile, Adrian Iordache, Claudiu Nistor

Introduction: The cervical rib represents the elongated costal element of the seventh cervical vertebra. It’s an important cause of thoracic outlet syndrome. Bilateral cervical ribs are very rare, with an incidence of less than 1%. We chose to present this case to highlight the possibility of bilateral cervical ribs as a cause for thoracic outlet syndrome.

Material and method: We present the case of a 32-year-old patient, referred to our department with pain in right shoulder and arm, debuted a year prior to her presentation.

The cervical X-Ray revealed bilateral cervical ribs; on the right side the rib being fixed to the first rib. Doppler ultrasound shows bilateral subclavian shunt, more important on the right side. MRI imaging showed right brachial plexus compression.

The surgical intervention was performed under general anesthesia, through an anterior cervical approach. Intraoperative findings: cervical rib, fixed to the first thoracic rib; the cervical rib has been completely removed.

Results: Postoperative outcome was satisfactory, the patient began recovery the third postoperative day. Patient’s symptomatology has decreased gradually, and 5 months postoperative the patient’s sole complain was a mild right arm paresthesia. Doppler ultrasound performed postoperative shows great improvement in subclavian artery blood flow.

Discussion: Although a congenital condition, the cervical rib becomes symptomatic at the age of 20-25 years old. The cervical rib is more frequent in women, on the left side; the right cervical rib is more often symptomatic – especially in right-handed people. After the resection of the cervical rib, the symptomatology gradually decreases.

Conclusions: While a rare condition, bilateral cervical ribs must be considered in differential diagnosis of upper member neurological pain. Sometimes a cervical rib can mimic cervical radiculopathy and myelopathy.

High index of suspicion and detailed clinical evaluation is needed to prevent inappropriate cervical spine surgery!

Proliferative diabetic retinopathy: surgical treatment

Ovidiu Mușat, Cornel Ștefan, Mihail Zemba, Monica Armegioiu, Liliana Pulbere, Horațiu Manole, Laura Macovei, Cătălin Cornăcel

This paper presents the case of a patient with neglected type I Diabetus Mellitus and Proliferative Diabetic Retinopathy.

Posterior vitrectomy was practiced with peeling of membranes and silicone oil endotamponade. Postoperative evolution was favorable.

We will also discuss several treatment options for this type of cases.

Corneo-conjunctival tumor: diagnostic and therapeutic challenges

Mihail Zemba, Ovidiu Mușat, Cornel Ștefan, Alexandra Calu

Purpose: to show the diagnosis, preoperator assessment and surgical treatment for a corneoconjunctival tumor

Methods: there is a review of clinical and paraclinical arguments for the diagnosis; surgical solution is shown in a video film

Results: two months postoperatively there is good functional recovery; 4 months postoperatively there is no sign of relapse of the tumor

Conclusions: the main treatment for corneoconjunctival tumor is surgical; in very large tumors the surgeon must be able to cover the area of conjunctival excision and use solutions to prevent relapse.

Malignant insulinoma: Therapeutical approach

Tanita Stancu, Augustin Dima, Cezar Bețianu, Florina Vasilescu, Aurelian Ranetti

Introduction: Malignant insulinoma is very rare (1-4/100,000) and the most common pancreatic neuroendocrine tumor. Originated from B-pancreatic cells which synthesize and secrete insulin. Diagnostic is based on clinical symptoms and signs (Whipple triad) and it is confirmed by CT Scan/RMI.

DESCRIPTION: We will report the case of a young man 44 y.o. BMI: 27 kg/m^2, diagnosed by classical signs and symptoms, confirmed by CT Scan, with a large tumor in the body of the pancreas, with metastatic lymph nodes, without any distant metastasis. The patient underwent minimally invasive surgical treatment (laparoscopic distal spleno-pancreatectomy with extensive lymph node dissection)

The postoperative course was uneventful and the patients was discharged in the the tenth postoperative day. The anatomopathological report confirmed the malignant insulinoma with multiple lymph nodes metastasis and R0 resection.

Conclusion: The surgical treatment is the only curative treatment with a good survival rate at 5-10 years. Minimal invasive approach is recommended and feasible in specialized centers in pancreatic surgery.

Laparoscopic hepatic left lateral sectionectomy: How I do it

Augustin Dima, Tanita Stancu, Șerban Spiratos, Cezar Bețianu

We present a video case of a 72 y.o. man admitted to our surgical unit for left hepatic lobe hepatoceular carcinoma. Patient has HCV cirrhosis Child-Pugh A diagnosed in 2010 for which he was treated with Viekirax, Exviera and Rivabirin between Oct. 2016 - Jan. 2017. AFP is not elevated. Hepatic ultrasound with enhanced contrast and CT scan strongly suggests an HCC located on the lateral section of the left liver. No previous abdominal surgery. According to our multidisciplinary team is proposed laparoscopic left lobectomy. Pringle maneuver was not performed, blood transfusion was not required. Postoperative course was uneventful. In our experience, minimally invasive resection in cirrhotic liver should be performed in selective patients and by an experienced team in hepatic surgery.

Radical antegrade modular pancreatosplenectomy for borderline resectable pancreatic cancer

Augustin Dima, Tanita Stancu, Cezar Bețianu, Delia Mateescu

Introduction: Radical antegrade modular pancreato-splenectomy (RAMPS) was first reported by Strasberg in 2003 and since then has attracted increasing attention in the treatment on left sided pancreatic cancer. There is not enough data in literature to sustain the efficacy of RAMPS vs. standard approach.

Discussion: We report a 72 y.o man BMI: 24kg/m2, diagnosed with border-line resectable left side pancreatic adenocarcinoma who underwent before surgery 6 courses of neoadjuvant chemotherapy based on FOLFIRINOX regimen, with decreased tolerance. We performed RAMPS with en block mezenteric-portal vein confluence resection and venous reconstruction by interposition of left renal vein graft. The postoperative course was uneventful. The anatomopathological report concluded the R0 resection without any lymph node metastasis.

Conclusion: The patient was able to resume postoperative chemotherapy with the same regimen at two months after surgery. The RAMPS procedure for the treatment of border-line resectable left sided pancreatic cancer can achieve high rates of R0 resection and is the best option to choose when vascular resection is required.

Locally advanced/border-line resectable pancreatic cancer: A challenge for the surgeon

Augustin Dima, Tanita Stancu, Șerban Spiratos, Cezar Bețianu, Theodor Voiosu

Introduction: Pancreatic cancer is the fourth leading cause of cancer death in the world. Median survival for patients treated by surgical resection is 15-23 months, with a five year survival <20%. Even though over the past decade improvements in diagnostic, imaging and treatment, the overall survival rate remains poor.

Discussion: In both border-line and locally advanced pancreatic cancer, the tumor is localized to the pancreas but adheres to or invades adjacent vascular structures, including the celiac axis vessels, superior mesenteric artery, superior mesenteric vein, and portal vein. Patients with border-line and locally advanced pancreatic cancer represent a special subset of patients who are not candidates for primary surgical resection and there is now emerging consensus that a subgroup of patients' poor candidates for surgery may become eligible for resection after neoadjuvant chemotherapy.

Conclusion: Current studies suggest that 25-30% of initially border-line respectable pancreatic cancer may become candidates for resection following neoadjuvant chemotherapy. Lack of consensus definition and the paucity of randomized trials in therapeutic algorithms have delayed the progress of proposing good practice guidelines for diagnosis and treatment.

Reconstruction of the forehead and temporal area

Viorel Trifu, Monica Dărmănescu, Țilea Mihai, Marcela Poenaru, Răzvan Șerban

Surgical approach of the forehead and temporal area poses several unique challenges for the dermatologic surgeon. A thorough understanding of the anatomic structures of the forehead and temporal area is paramount to optimal surgical result.

A case series of regional reconstruction of forehead and temporal area is presented, with a variety of reconstructive techniques. The anatomy of the region is reviewed; it is important to preserve the function of motor and sensory nerves, although aesthetic concerns are of great importance in forehead and temple reconstruction. For optimal operative results, flaps must be properly sized in all dimensions, including thickness. In forehead and temporal reconstruction with skin flaps, the surgeon should anticipate secondary motion accurately, as not distorting anatomic free margin. Respecting the normal position of facial landmarks such as the eyebrows and hairline can be challenging during reconstruction.

The goals of reconstruction include hiding incisions in natural rhytids, maintaining natural hairlines and eyebrows and preserving motor and sensory function.

The reliability of the radial forearm free flap for intraoral reconstruction

Dragoș Muraru, Iulia Muraru, Ioana Tuhar, Ionuț Guzganu, Paul Ionescu, Liliana Moraru, Adrian Gabără, Ana Căruntu, Bogdan M. Marinescu

Introduction: Head and neck defects after tumor resection can be difficult to reconstruct using autogenous tissue without utilizing a free flap. The radial forearm free flap is one of the workhorse flaps used for reconstruction of intraoral defects after resections due to malignancy.

Objective: The aim of this paper is to emphasize the reliability of the radial forearm free flap, by presenting our clinical experience and results.

Methods: Three patients with intraoral carcinoma have been reviewed, one with floor of the mouth and buccal mucosa defect and two with hemiglossectomies. They underwent immediate reconstruction using fascio-cutaneous free flaps from the radial forearm. In all cases we used one of the radial comitant veins for the venous T-T anastomosis with the facial vein.

Results: There weren't any microvascular failures, the intraoral healing time was reduced to 10 days on average and hospitalization was less than two weeks. Two donor sites with width less than 4cm underwent direct closure and one was grafted with an autograft harvested from the ipsilateral thigh. The donor site healed uneventfully in all patients.

Conclusion: The radial forearm free flap is a very effective method for reconstruction after resection of intraoral malignancies.

Postmastectomy breast reconstruction with autologous tissue in previously irradiated patients

Bogdan M. Marinescu, Ioana Tuhar, Ionuț Guzganu, Paul Ionescu, Dragoș Muraru

Introduction: Breast cancer represents the most common cause of cancer death among women worldwide. Mastectomy is an important tool in breast cancer therapy, in order to prevent any possibility of disease recurrence. After mastectomy, we can rebuild the shape and the volume of the breast, by using various techniques of breast reconstruction, including implants, skin expanders, reconstruction with autologous tissue (flaps), combination of implants and flaps. The reconstructive procedure can be made at the same time as the mastectomy or later on.

Material and method: In these paper, we will present our experience by using latissimus dorsi and transverse rectus abdominis myocutaneous flaps in breast reconstruction. In the last 2 years, 15 patients underwent breast reconstruction with 10 LD flaps – 8 unilateral and 1 bilateral, 7 TRAM flaps – 5 unilateral and 1 bilateral. In mastectomy patients reconstructed with LD flap, we use implants underneath the muscle flaps, in order to obtain adequate volume for the reconstructed breast. All the patients received adjuvant radio-therapy before reconstruction. We perform in all patients delayed breast reconstruction. Donor sites were closed primarily.

Results: In our case series all flaps survived. There was just one case of partial flap loss (TRAM flap group). The reconstructed breasts have a natural shape, volume and contour. Donor sites morbidity were acceptable with no significant functional loss. There are no major complications and the patient satisfaction was high.

Conclusions: A large variety of techniques are now available for breast reconstruction. Among the possibilities for breast reconstruction, the transfer of autologous tissue from adjacent regions to the breast, keeping up the original blood supply is a good option for previously irradiated patients.

Cataract following penetrating keratoplasty

Mihail Zemba, Ovidiu Mușat, Cornel Ștefan

Purpose: to discuss about ethiology and specific issues of surgical technique in a case of cataract after keratoplasty

Methods: evolution and surgical solutions for management of the case, starting with deep corneal ulcer

Results: after four surgeries, visual acuity is 0.5

Conclusions: multiple surgeries may be necessary for a good visual result

Corneal transplantation: permanent challenge for ophthalmologist surgeon

Mihail Zemba, Ovidiu Mușat, Cornel Ștefan

Corneal transplantation is a complex and multi-step surgical procedure, it is a permanent challenge for ophthalmologist surgeon. Most often the procedure is done for optical purposes. Corneal transplantation is constantly undergoing changes in surgical technique. In Romania corneal transplantation has a long history and in 1983 the first corneal transplant was performed in a child of one and a half years old. We will present this clinical case after 35 years.

The monitoring and treatment center of the glaucoma: the computerized program

Ovidiu Mușat, Cornel Ștefan, Mihail Zemba, Horațiu Manole, Liliana Pulbere, Laura Macovei, Monica Armegioiu, Cătălin Cornăcel, Elena Gosav, Nicolae Alexe, Cristina Timaru

The monitoring and treatment center of the glaucoma in Central University Emergency Military Hospital is a necessity because Glaucoma is increasing in number of cases, early onset glaucoma (when the disorder appears before the age of 40) and the methods of diagnosis and treatment are constantly upgraded.
The computerized program is useful for building the database, interconnecting diagnostic terminals including imaging with end-scopes - determining the algorithm of treatment.

In the modern era of AAA treatment is still open surgery the gold standard

Ionel Droc, Mihai Dumitrașcu, Cosmin Buzilă, Tudor Păduraru

Endovascular repair has emerged as an alternative to open repair for patients with abdominal aortic aneurysm. Although the method’s safety and efficacy have been established, challenging anatomy and especially inadequate landing zones create limitations to its application. Stent grafts, fenestrated and branched, were developed to overpass these anatomic restrictions. Contrast-enhanced Ultrasound (CEUS) is investigated as a novel, noninvasive technique that can be employed to characterize endoleak type and consequently prescribe appropriate treatment in the follow up of this procedures.

Open repair is a safe and durable procedure. But it has some long term complications as graft thrombosis, infection or paraanastomotic aneurysms. Aorto enteric fistula is a rare but difficult to treat complication. Also open surgery is used for graft explantation for graft failure or infection. The overall mortality for open procedures is about 4.5%.

In conclusion, open surgery for AAA remains the gold standard in young and low risk surgical patiets. Endovascular procedures are of first choice in all high risk patients, even in emergency situations.

The endovascular treatment of thoracic aortic aneurysm – case presentation

Ionel Droc, Daniel Nita, Elena Dumitru, Ioana Vlad, Liviu Stan

Thoracic Aortic Aneurysms are serious conditions with risks for potentially lethal complications. The decision regarding the treatment options for a TAA is made according to the age of the patient, the risk assessment, and the vessel anatomy. Nowadays, in Romania, TAAs are mostly treated with open surgery – clamping, removing the affected aortic segment, and replacing it with a graft. Endovascular treatment of TAAs is an infrequent treatment solution in our country, but with increasing average. We have had few patients treated with TEVAR in our clinic.

We present the case of a TAA treated in our clinic with thoracic endovascular aortic repair (TEVAR). 45-year-old male patient admitted to our hospital with post-traumatic thoracic aortic aneurysm underwent endovascular stent grafting with prior carotid-subclavian by-pass and proximal ligation of the subclavian. Following the procedures the patient's outcome was uncomplicated. CT scan 6 months after discharge shows a reduction in both the internal and external diameters of the thoracic aortic aneurysm.

Thoracic endovascular aortic repair (even when covering the origin of the left subclavian artery) is an effective and safe treatment option for patients in Romania.As the current international recommendations, in patients for whom TEVAR is accompanied by the debranching of the LSA, we suggest mandatory surgical revascularizarization of LSA.This operation should be performed prior to the endovascular procedure in order to prevent the ischemic or neurologic complications associated with LSA occlusion. The long-term prognosis of such cases is not known and remains to be determined in the future.

Surgery for intramiocardial wall foreign body (bullet) – case presentation

Ionel Droc, Dragos Trandafir, Alin Toader, Vasile Murgu

We present a very rare case of a 37 year old male patient diagnosed with an intramiocardial wall fordeign body (bullet) – resting in the diaphragmatic wall of the right ventricle. It was operated with success three months after the accident. The patient was shot parasternally, in the left third intercostal space, with a high-velocity military AK 47 projectile. The shot was indirect – the bullet hit the patient after two rebounds on the metallic struts of the car he was driving. Shortly thereafter, the patient was examined in a hospital. A diagnosis of antero-inferior mediastinal foreign body (bullet) was established then.We examined the patient 3 months after the acccident. The plain X-Ray confirmed the diagnosis. We performed a thoracic CT scan which showed that the bullet was „most probably located above the diaphragm, in the lower anterior mediastinum, 1.5 cm behind the sternum". We also performed a fluoroscopy, which showed that the bullet was moving synchronously with the heart and, therefore, we asked for a cardiac echography in order to rule out a cardiac/pericardial location of the bullet. The trans-thoracic cardiac echography did not establish the presence of the bullet in the myocardiun or pericardium, nor has it shown indirect signs of cardiac or pericardial wounds. We therefore performed a subxyfoidian approach. We examined the anterior mediastinum but we failed to find the bullet, although we palpated a structure feeling very much like the bullet. We then opened the pericardium as in a pericardial window and we found the bullet embedded in the lower aspect of the right ventricular wall.The patient was operated under extracorporeal circulation and the bullet was extracted from the right venticle.The resting orifice was closed with separete sutures on daflon patches using also reinforcement of the tissue with bioglue.The postoperateve evolution was simple.No infection or bleeding were noted. In conclusion the intramural ventricular foreign bodies (extremely rare non fatal) can be safely treated surgically under extracorporeal circulation.

NURSES PAPERS

The hydro-electrolyte balance and the acid base balance: interpretation of the ASTRUP

Mariana Ene, Nicoleta Tudora

The water balance: the role in bio-chemical processes, electrolyte and acid base balances, transmission of nerve impulse, transmission of genetic information, etc. Electrolytic balance: it strictly refers to the balance of salts of the blood plasma. Acid base balance: human organism, for a proper function, must maintain the balance between acidity and alkalinity of its fluids. The utility of the blood test: the evaluation and assesing the severity of patient's condition. The technique of collection of blood. The interpretation and recognition of the main imbalances

Scintigraphy: preparation of the patient and the fear of radiation

Maria A. Gherman, Eugenia Rusu, Constanța Bandulea, Mariana Ciaușescu

In order to achieve the best scintigraphic images it is very important to know how to prepare the patient for investigation. With the large variety of organs that can be seen with this investigation, the preparation differs in function of the organ that is desired to be seen, in function of the kinetics inside the cell of the specific organ, the medication that the patient is taking and the quality of the image that is being taken (eliminating artifacts). Since Chernobyl and Fukushima, the word radiation brings up the idea that something bad is happening or that we shouldn't get in touch with it. But, in the case of nuclear medicine, the word radiation means the improvement in detecting cancers and other malfunctions of the organs. Taken into consideration that there are some dose limits, that the patients are advised what to do after a nuclear medicine procedure in order to minimize the population exposure and that the scintigraphic investigation is being taken under the ALARA principle there should not be any fear of radiation.

Medullar transplant

Adriana A. Andrei, Florența M. Nicolescu; Mihai A. Șotcan

The transplant of hematopoietic stem cells implies reconstructing the haematopoiesis by transferring stem cells (from a donor or from the patient). Unlike the transfusion of blood components (where the effects are temporary due to limited viability of the transfused cells), transplanting progenitor strain cells causes a long-term effect through its capacity to proliferate in the medulla. The source of hsc may be the hematopoietic bone marrow, the peripheral blood or the umbilical cord. The donor may be a healthy person (allotransplant) or the patient himself/herself (auto-transplant). Currently the HSC transplantation is widely used in a variety of hematologic and non-hematologic impairments. Yet the procedure is not harmless, entailing high morbidity and mortality due to its complications. Therefore the indication of transplant shall be discussed for each particular case and a decision shall be made only after considering the risk and benefit for each particular case.

Malign diseases: acute lymphoblastic leukaemia, acute myeloblastic leukaemia, chronic granulocytic leukaemia, Hodgkin's disease, multiple myeloma, solid tumours – breast, ovary, lung (auto-transplant only). Non-malign diseases: congenital and acquired aplastic anaemia, autoimmune diseases and hemoglobinopathies.

Purpose: Suppressing the patient's immune system, suppressing the bone marrow and destroying the tumour cells. The treatment consists in post-transplant radiotherapy and chemotherapy.

Impact of nurses involvement in fundraising and social activities: RxRun, we are running for our patients

Ani Ivan, Andrei Toma, Norocel Bocănială, Ştefănel C. Vlad

The impact that a trained nurse or RTT can have on their community in Romania is huge. It is so much more than just a job in the hospital. The work they do, caring for their patients will have an immeasurably large reach and change the lives of countless vulnerable people, such as patients with cancer in advanced stages. People decide to run long distances for all sorts of reasons. Some do it for exercise and health benefits, because they love running. We do it for our patients, we put our passion for a cause: to help patients with incurable disease to have good quality palliative care in Hospice Casa Sperantei. We started the fundraising activities in patient's benefit in 2016, as RxRun, a team of runners, one doctor and three nurses working in the Radiotherapy Department of the Central University Emergency Military Hospital "Dr. Carol Davila", Bucharest. Another two young radiotherapy doctors joined the team in 2018 and 2019. We have participated in numerous races, crossing distances from few kilometers to marathon and half-marathon. With the help of friends who have sustained our cause we succeed in fundraising important amounts, representing palliative care services for many hospice-treated cancer patients from Hospice Casa Sperantei. It is important for any nurse involved in oncological field to be part of social activities, to highlight the difficulties faced by many patients with cancer to receive good quality care and to deal with their disease.

Renal lithyasis patient care

Manuela Flămânzeanu

Kidney stones are small, hard deposits that form in one or both kidneys. The stones are made up of minerals or other compounds found in urine. Kidney stones vary in size, shape and color. To be cleared from the body or ''passed '', the stones need to travel through ducts that carry urine from the kidneys to the bladder (ureters) and be excreted. Depending of their size, kidney stones generally take days to weeks to pass out of the body and can cause severe pain. Kidney stones can cause abdominal or back pain (renal colic). This pain usually begins sporadically but then becomes constant and can lead to nausea and vomiting. The nurse administers painkillers, anti-inflammatory and anti-vomiting drugs at the doctor's advice and she write everything in the care plan. They can be extremely painful and can lead to kidney infections or the kidney not working properly if left untreated.

Nurse's role in taking care for the patient with pleural effusion

Marcela Brighiu, Ionela Paraschiv

A pleural effusion is defined by the accumulation of a fluid inside the pleural cavity, with or without the inflammation of the pleura. A pleural effusion usually accompanies other local or systemic affections. The presence of pleural fluid inside the pleural cavity has a major impact on the respiratory and circulatory function.

The authors present several measures needed to be taken by the nursing personnel for patients with pneumothorax. Some nursing care plans are described and explained in order to fully understand what is needed when taking care for a patient with a pleural effusion.

Carefully nursing a patient with a pleural effusion (usually a patient with associated health problems) has very good results if the nursing plans are respected. Reducing the postoperative pain and improving the respiratory function are essential for patients' evolution.

The goals of the nursing plans include relief of pain, adherence to prescribed pharmacological regimen, establishment of a normal, effective respiratory pattern as evidenced by absence of cyanosis, and demonstration of increase in perfusion. If these goals are achieved, the patient can be safely discharged.

The nurse's role in taking care for the patient with a pleural effusion is very important. The nurse helps the patient reach several goals during the hospital stay. Good nursing means better quality of life for the patient!

Challenges in the determination of the blood group and compatibility

Anca L. Mogoi, Cristiana Chiriță, Carmen B.Ș. Jianu, Gabriela Vasile, Carmen Măcău

The human body contains approximately 4- 6 L of blood, depending on the body mass of the individual. The red blood cells (RBC), haematids, which represent 45% of the blood volume, have a crucial role in the oxygen transportation to the tissues, and implicitly in maintaining the organism alive. On the red blood cells membranes, there are proteins that carry the blood group antigens. These were discovered by Landsteiner, who classified them in the ABO system. Nowadays, more than 300 RBC antigens are identified and categorized into 36 major systems. The determination of the ABO/Rh blood group is based on a relatively simple test that can be performed in 5-10 minutes depending on the technique used. However, there are situations in which the determination of the blood group may involve additional techniques that require more time and foremost knowledge on the medical history of the patient. The aim of the compatibility tests is to highlight the interactions between the donor's antigen and the patient's antibodies. Selecting a compatible blood unit is often challenging due to the presence of a multitude of antigens and antibodies with high immunogenicity or unexpected antibodies (auto- or irregular erythrocyte antibodies), cases in which time-consuming supplementary testing is required.

This paper describes the most common particular cases encountered in our practice when performing blood group and compatibility determination.

Headache in neurology disorders

Cătălin Dinoiu, Alexandru Gila, Claudia Lia

A headache is pain or discomfort in the head or face area. Headaches vary greatly in terms of pain location, pain intensity, and how frequently they occur. Headache syndromes can be associated with focal neurological symptoms or signs. Good knowledge of primary headaches, a detailed history and a thorough clinical examination are prerequisites for their differential diagnosis. The neurological symptoms produced by the migraine aura are the most characteristic and recognisable. However, structural lesions, such as vascular malformations, can produce similar symptoms to migraine with aura, which highlights that paraclinical investigations are necessary in most patients with headache and focal neurological symptoms.

The different types of primary headaches are: migraines, tension headaches and cluster headaches. The full extent of the problem may not be understood immediately, but may be revealed with a comprehensive medical evaluation and diagnostic testing. The diagnosis of a headache is made with a careful history, physical examination and diagnostic tests.

The ultimate goal of treatment is to stop headaches from occurring. Adequate headache management depends on the accurate identification of the type of headache.

Some headaches may require immediate medical attention including hospitalization for observation, diagnostic testing, or even surgery. Treatment is individualized depending on the underlying condition causing the headache. Full recovery depends on the type of headache and other medical problems that may be present.

The conflict

Giorgeta Grosu

A conflict is a disagreement between two or more people whose attitudes, beliefs, values, feelings or needs differ and it is, at the same time, a part of every working environment, medical care organizations included. Needs, perceptions, power, values, feelings and emotions are all involved within a conflict. In the past, conflicts from the working environment were seen as dysfunctional issues and they were supposed to solve by themselves or to be solved having a winner and a loser. Nowadays, conflicts are expected to occur in a dynamic organizational environment and are not considered harmful anymore. Medical staff may face four levels of organizational conflicts: intrapersonal, interpersonal, intragroup and intergroup, however most organizational conflicts occur at interpersonal and intergroup levels, substantial and personalized conflicts being the most common two types of conflicts. Supervisors and managers who mediate conflicts need to stay calm, to take measures in order to promptly put an end to negative conflicts and to stay positive until a final resolution is reached. It is essential to separate the problem from the person, to be impartial, to look for creative solutions, to discuss with all parties involved in the conflict so that people feel they are involved in the process and will contribute to the final result. Confrontation, compromise, collaboration, admittance and avoidance are the five common approaches to solve conflicts, each of them having its own specific characteristics.

Challenges in IMRT: RTTs point of view

Andrei Toma, Norocel Bocănială, Mihai Dumitrache, Ștefănel C. Vlad

Within the radiation oncology department of the Military Emergency and University Central Hospital “Dr. Carol Davila”, Bucharest we are using 2 techniques of treatment delivery: 3D conformal and intensity-modulated radiation therapy (IMRT).

IMRT is an advanced type of high-precision radiotherapy, that allows for the radiation dose to conform more precisely to the 3D shape of the tumor by modulating the intensity of the radiation beam in multiple small volumes. Due to its high complexity and the need of confirmation of the dose distribution to the target volume, the positioning of the patient has to be highly accurate and it has to be checked daily.

It should be noted that the duration of treatment using IMRT technique is higher than 3DCRT, therefore the number of patients that are being treated is limited. Taking into consideration the high number of patients coming to the department, we had to extend the working schedule in order to maintain a lower waiting time compared to other public clinics in the country. It should be also mentioned that the radiotherapy installation is being overused, which may lead to the wear of some components.

These issues are important, but also motivating, due to the importance of IMRT technique in modern approach of oncological cases, because it allows higher radiation doses to be focused on the tumor while minimizing the dose to surrounding normal critical structures.

Implantable ports used in chemotherapy

Maria L. Berbece, Casandra Marin

Many times during cancer treatment, the medical team in charge of the patient must have access to the patient’s veins be that to administer the cytostatic treatment or for other intravenous perfusions or injectable antibiotics. For these procedures to be as comfortable as possible for the patient, the oncologist recommends implanting a special device called implantable port (port-a-cath) which allows repeated administrations of cytostatic treatment during a long period of time. The port is attached in the operating theatre by a medical team consisting of the surgeon and the anaesthesiologist, experienced in performing this kind of procedures. The intervention takes place in the operating room under local anaesthesia and/or mild sedation. Conclusions: the implanting is performed only once during the entire period of treatment, the procedure is mildly invasive and performed under local anaesthesia, the access to blood veins is easy, the toxic effect of cytostatic on the veins is significantly reduced, the cytostatic treatment may be administered continuously or intermittently, the period of usage is long, the device has a good aesthetic appearance and allows exposure to water, nevertheless, the recommendation to implant such a port must come after discussing the matter with the oncologist.

Patient care with acute subdural hematoma

Florina C. Tănăsescu, Andreea Ciurea

The care process involves five stages, each stage having a number of key components, as follows: Data collection/Their analysis and interpretation/Care planning. Pre and postoperative care: the preoperative care includes:/The physical and mental preparation of the pacient; /General preparation;/Preparation for surgery. Postoperative care: Post-operative surveillance of the patient starts from the time of completion of the surgery. It can be said that the pacients are divided into two categories: the first – those who return to the ward and the second- the patients who are admitted to the intensive care unit; During transport the following will be observed: skin coloration, breathing, pulse, the functioning of the various devices used for monitoring, perfusion. Sudden movements will be avoided. Positioning of the patient in bed varies depending on the type of surgery and anesthesia.The most common position is the dorsal decubitus.Positive diagnosis for acute subdural hematomas is mainly based on advanced radiogical investigations which highlight the acute subdural collections which, correlated with the clinical findings, confirm the clinical diagnosis.

Menopause: between physiology and pathology

Nicoleta Mavriș, Mihaela Moscaliuc, Ancuța Istrate, Carmen Miron, Aglaia Nedelcu, Tatiana Onofrei, Florentina Rogojină, Andreea Serafim, Nicoleta Vale, Corina Enache, Luminița Crăciun

Menopause and menopausal transition are physiologic, but symptoms are frequent and often require medical attention. Approximately 60% to 80% of menopausal women experience hot flashes at some point during menopausal transition. They are more likely to develop anxiety symptoms at perimenopause and postmenopause and depressive symptoms or clinical depressive disorders in late perimenopause. Sleep disturbances are frequent. Vaginal dryness and genital atrophy can cause sexual dysfunction. Lately, postmenopausal osteoporosis leads to increased morbidity and mortality as well as an important reduction of the quality of life as a consequence of fractures. We present our experience with menopausal patients seeking medical attention in our department.

Patient care after cardiac pacemaker implantation

Elena M. Dumitrescu, Cecilia Pătru

The pacemaker is a special device that is implanted in the prepectoral and subclavicular level. The device generates electrical impulses at the heart, determining to work in optimal parameters. Recently, the cardiac pacemaker has gained a crucial importance in the life of patients with pathologies such as: symptomatic sinus bradycardia, complete atrio-ventricular block (grade III), sinus node disease, etc. In present, it is estimated that annually.

Approximately 200,000 people benefit from a cardiac pacemaker. In this context, efficient management of postimplant pacemaker care lead to an increase in the quality of life of the patient as well as to a substantial reduction of the complications that may occur postoperatively. We note that overinfection of the wound is one of the most important complications. The present paper aims to highlight the most important aspects of care for the patient post device implantation so that he / she will benefit from the best medical care and his recovery will be fust and properly.

In fact, a very important aspect of care of the post-implant patient is his education, according to the lifestyle that he must adopt. Upon discharge from the hospital, the patient is taught how to care and protect the wound. Also, the patient is educated on the activity that can be performed during the recovery: the patient will limit the use of the arm for a period of 1.5-3 months and will strictly respect the indications received from the medical team.

Utilization of ATP bioluminescence assay in monitoring medical hand hygiene compliance in healthcare workers

Raluca M. Hrișcă, Tudorița Marinescu, Adriana Ruță, Daniel Păun

Healthcare associated infections (HCAI) are an important problem in terms of the associated morbidity, mortality and cost. Hand hygiene is an important part of quality care, and it is the simplest, low cost and evidence supported method to reduce the HCAI. We want to evaluate the healthcare workers hand hygiene from our settings. Retrospective study conductes in our setting between 1st May 2019- 31 July 2017, based on the swab sample collected from healthcare workers hands using Adenosine Triphosphate Hygiene Monitoring System. We set 60 RLU as the upper limit for a proper hand hygiene (≤ 60 RLU -PASS, > 60 RLU –FAIL). While collecting the samples we conduct an interview about the reasons that prevent them from practicing hand hygiene regularly. We collect 416 sample, 46.63% PASS and 53.34% failed the test. The main 4 reasons given for failing was: cleaning hands causes skin irritation and dryness, the accessibility (the disinfectant vial no longer matches with the dispenser), sink location and the lack of time. Conclusions: We must insist on the hand hygiene policies; the hospital units must be properly equipped; we have to use less skin aggressive disinfectants and to use hand cream for prevention of skin dryness and irritations.

Fournier gangrene diagnosed patient’s care

Georgiana Niculae

Etiology: Fournier gangrene is a form of fulminant fasciitis necrotizing of perianal region, perineal and genital.

Determining factors are: aerobic germs (E-Coli), anaerobic germs (Bacterioides).

Other factors are: Proteus, Staphylococcus, Enterococcus, Klebsiella, and Clostridium.

Favoring factors are: diabetes, alcoolism, obesity, old age, AIDS

Clinical diagnosis: The onset is sudden or preceded by a prodromal syndrome with: fever, digestive disorders, and genital pruritus.

Subsequent will appear painful erythematous edema with cyanosis and tissue necrosis.

Treatment: Patient resuscitation, broad-spectrum antibiotics, debridement, removing of the necrotizing tissue are required.

Preoperative it is necessary hemodynamic equilibration, glycemia, anemia, coagulation disorders.

Conclusions: Fournier gangrene is a major urological emergency, with rapid escalation, with high risk mortality up to 30%.

Presenting time to the hospital, age, and associative disorders are key factors in this disorder.

Characteristics of care for patients with autonomic regulation therapy

Carmen M. Bosincianu, Silviu Dumitrescu, Mihai Dumitrașcu

Introduction: Any form of structural heart disease that impairs ventricle functional ability to fill or to eject blood according to the needs of the tissue may cause heart failure syndrome. Many people are diagnosed with heart failure syndrome and in spite of prescribed medical treatments they continue to suffer from fatigue and shortness of breath. Trying to handle heart failure syndrome in terms of surgical approach is based on the vagus nerve stimulation through device implantation which sends an electrical impulse from the brain to the heart.

Objectives: implanting the device is an innovative treatment BAT(baroreflex activation therapy) which restores the equilibrium of the autonomic nervous system, leading to enhanced cardiac performance, improving the pumping capacity of the heart, reducing the risk of malignant ventricular arrhythmias associated with heart failure and ultimately improving the quality of life and increasing the rate of survival.

Material and method: we studied 5 patients who were operated between January- July 2019

Results: we analysed the postoperative evolution, showing the special nursing cares.

Conclusions: the study marks out distinguished particularities of nursing patients with the vagus nerve stimulation. The nurses' role, besides medical treatment given by physicians, involves continuous monitoring during hospitalization and educating the patients on how to lead a healthy life.

Dudenopancreatectomy in the eyes of medical assistance

Imola Grigore

Introduction: Cephalic duodenopancreatectomy is one of the most complex operations in abdominal surgery, reserved mainly for surgeons with experience in hepato-biliopancreatic pathology. If in the past it was used sporadically, today it has become a routine intervention.

Objectives: The main indication of CPD is the pancreatic cephalic neoplasm, along with the periampullary neoplasms (distal choledochial cancer, pulmonary ampuloma, duodenal cancer). DPC remains a difficult, complex intervention, due to vascular relations (portal vein, mesenteric vessels) and restoration of bile, pancreatic and digestive continuity. Two types of operations are used: Whipple and TraversoLongmire. Improvement of surgical techniques and progress in anesthesia, intensive care and nutritional support have led to decreased postoperative mortality in CPD but also radical intervention with increased survival.

Material and method: we will study 2 patients with CPD performed in our clinic this year.

Results: we will analyze the post-operative evolution of the cases, especially from the point of view of the care offered by the nurse.

Conclusions: To date, surgical resection is the only effective therapy that offers the prospect of a significant prolongation of the survival and healing potential of patients with cancer in the pancreatic-biliary sphere.

The role of the nurse is very important in the postoperative care by administering the medication indicated by the doctors and continuous monitoring during the hospitalization as well as by educating the patient to adopt a healthy lifestyle, which will extend his life.

Thrombolysis therapy in acute ischemic stroke

Olguța Preda-Carsote, Oana Rada, Violeta Gâlcă, Nicoleta Balint

Stroke is a major cause of mortality and thrombolysis has served as a catalyst for major changes in the management of acute ischaemic stroke. Modern management of stroke includes rapid assessment and admission protocols, thrombolysis for acute ischaemic stroke, early specialist management in a stroke unit and appropriate physiological monitoring. Thrombolysis restores cerebral blood flow in some patients with acute ischemic stroke and may lead to improvement or resolution of neurologic deficits. The most important factor in successful reperfusion therapy of acute ischemic stroke is early treatment with Alteplase (Actylise). Intravenous alteplase (actylise) is the only approved thrombolitic agent at present indicated for acute ischaemic stroke. While the licensed time window extends to three hours from symptoms onset, recent data suggest that the trial window can be extended to four and a half hours with overall benefits. Identification of viable penumbra based on computed tomography/magnetic resonance imaging may allow future extension of the time window. Intracranial haemorrhage is the major complication associated with thrombolysis, and the key factors increasing risk of haemorrhage include increasing age, high blood pressure, and diabetes and stroke severity. Some people with ischaemic stoke are eligible for a clot-busting drug. Actylise aims to disperse the clot and return the blood supply to the brain. For most people thrombolysis needs to be given within four and a half hours since the stroke symptom started.

Emergency nurse and acute trauma evaluation

Ion M. Lefter

The Advanced Trauma Life Support (ATLS) was developed in response to a perceived need to identify a safe, consistent, standardized, and effective way to initially evaluate and resuscitate patients with multiple injuries.
The principles of ATLS and triage need to be known by all personal, included nurses.

Nurse’s role in esophageal manometry

Elena Zanfir, Mariana Moldoveanu, Corina Marcovici-Petu, Aurica Avram, Cristiana Tablan, Nicoleta Helmis, Andrei Crișu, Liliana Radu, Raluca S. Costache, Andrada L. Popescu

Introduction: In this paper we want to point out the nurse role in performing esophageal manometry - a procedure used to measure the strength and function of the esophagus as food and liquids pass from the mouth to the stomach.

Esophageal manometry is done to evaluate the cause of stomach reflux back up into the esophagus, to determine the cause of dysphagia, and the cause of non-cardiac chest pain. Esophageal manometry is indicated to establish the diagnosis of suspected cases of achalasia or diffuse esophageal spasm, also for detecting esophageal motor abnormalities associated with systemic disease or for correct positioning of the probe for the 24-hour pH monitoring.

The nurse is preparing the patient, informing him about the procedure, about the time needed and the steps of it. The nurse will verify that the patient had nothing by mouth in the last 6 hours prior to the test. After the sterilization of the probe and calibration, the nostril and throat is numbed with a topical anesthetic, then the probe is inserted in a sitting upright position while the patient swallows water. The patient will lie down and the nurse will give him small sips of water to record the progression of the swallow. The contractions of the esophageal muscle will be measured at rest and during swallows.

Conclusion: In our hospital this GI functional procedure is available since 2017, and we performed until now approximately 150 procedures. Manometric screening for severe peristalsis dysfunction remains reasonable in specialized gastroenterology departments.

Management of operative wound infections

Maria Stoian, Carmen Florea

Introduction: All the surgical wounds are microbially contaminated, but only a small part of them can become infected. In most cases surgical wounds are considered to be clean or contaminated clean. The main factors recognized as influencing the evolution of the wound (healing as well as the aesthetic aspect) are quantitative and qualitative contamination.

Objectives: The paper presents: the risk factors in surgical wound contamination, diagnostic criteria for the infection of the wound, the treatment and the prophylactic measures preoperative, intraoperative and postoperative.

Materials and methods: We present our experience on 2000 patients operated in 2018 of which approximately:

- 40% of the surgery wounds were clean (hernias, classic or laparoscopic; other interventions, classic or laparoscopic);
- 30% of the surgery wounds were contaminated clean (classic and laparoscopic interventions on the cholecyst, stomach, small intestine, colon, rectum);
- 30 % surgery wounds 100% septic (abscesses, phlegm, gangrene, peritonitis).

The evolution of clean surgery wounds was favorable in all cases for the contaminated clean and the contaminated ones. The evolution was favorable in proportion of 80%, the rest of 20% was unfavorable due to other diseases (diabetes, malnourished patients, and neoplasic patients)

Conclusions: The correct application of the aseptic and antiseptic techniques, the observance of the guides and protocols regarding the surgical techniques and the antibiotic prophylaxis, the observance of the hygiene rules for both the patient and the medical personnel are essential conditions, absolutely necessary for the favorable evolution of the surgical wounds.

Heart attack

Delia Spătaru, Ramona Oancea

A heart attack occurs when the flow of blood to the heart is blocked. The blockage is most often a buildup of fat, cholesterol and other substances, which form a plaque in the arteries that feed the heart (coronary arteries). The plaque eventually breaks away and forms a clot. The interrupted blood flow can damage or destroy part of the heart muscle. A heart attack, also called a myocardial infarction, can be fatal, but treatment has improved dramatically over the years. It's crucial to call 211 or emergency medical help if you think you might be having a heart attack. In the CTIC department of January 2018 so far, over 700 patients with heart attack have been admitted, and over 200 patients have come into the RO-STEMI program. In the absence of adequate treatment: heart attack mortality 50%. Between 50% years produce in the first 2 hours of debut and 40% long in the first months of heart attack debut.

When to see a doctor

Act immediately. Some people wait too long because they don't recognize the important signs and symptoms. Take these steps:

• Call for emergency medical help. If you suspect you're having a heart attack, don't hesitate. Immediately call 112 or your local emergency number. If you don't have access to emergency medical services, have someone drive you to the nearest hospital. Drive yourself only if there are no other options. Because your condition can worsen, driving yourself puts you and others at risk.

• Take nitroglycerin, if prescribed to you by a doctor. Take it as instructed while awaiting emergency help.

• Take aspirin, if recommended. Taking aspirin during a heart attack could reduce heart damage by helping to keep your blood from clotting.

Aspirin can interact with other medications, however, so don't take an aspirin unless your doctor or emergency medical personnel recommend it. Don't delay calling 112 to take an aspirin. Call for emergency help first.

What to do if you see someone who might be having a heart attack

If you see someone who's unconscious and you believe is having a heart attack, first call for emergency medical help. Then check if the person is breathing and has a pulse. If the person isn't breathing or you don't find a pulse, only then should you begin CPR to keep blood flowing. Push hard and fast on the person's chest in a fairly rapid rhythm — about 100 to 120 compressions a minute. If you haven't been trained in CPR, doctors recommend performing only chest compressions. If you have been trained in CPR, you can go on to opening the airway and rescue breathing.

Cryoultrasound therapy

Laura Iosif, Daniela E. Iuga

Cryoultrasound therapy exploits the action of two different therapeutic physical modalities: cryotherapy and therapeutic ultrasound. Cryotherapy is generally regarded as a basic component of most injury management strategies and has proved its effectiveness in reducing edema and short-term pain. Ultrasound consists of high-frequency sinusoidal sound waves with a peak pressure of 0.5 bar, approximately 1000 times lower than shock wave Therapeutic ultrasound may induce thermal and nonthermal effects in the tissues. Thermal effects include a rise in blood flow, reduction of muscle spasm, increase in the extensibility of collagen fibers and a pro-inflammatory response. Non-thermal effects are cavitation and acoustic microstreaming that induce stimulation of fibroblast activity, increase in protein synthesis and in blood flow, tissue regeneration, and bone healing. It has been suggested that the non-thermal effects of ultrasound are more important in the treatment of soft tissue lesions than are thermal effects. Cryoultrasound therapy is a new technology that could combine the benefits of therapeutic ultrasound with those of cryotherapy, which could reinforce both, avoiding the possible complications of the thermal heating effect of the ultrasound therapy and improving its mechanical and biological therapeutical effects. In fact, a temperature decrease in deep tissues permits an enhancement in waves density in the tissue itself, increases the mechanical effect and, notably, reduces the thermic effect of the ultrasound. Recently, cryoultrasound therapy has been tested for musculoskeletal and musculotendinous disorders.

Lung diffusing capacity

Gabriela Drilea

This year our department was endowed with a pulmonary diagnosis line. The device comprises the module for spirometry, for measuring lung volumes of Nitrogen washout and for Lung Diffusion Capacity.

Measuring diffusion in pulmonary function laboratories can provide information about the transfer of gas between the alveoli and the pulmonary capillary blood. Measurement of the diffusing capacity is usually performed by using an inspired gas containing Carbon Monoxide because of its high affinity for Hemoglobin.

This is a simple non-invasive procedure and consists of instructing the patient to carry out a maximal inspiration and an 8-10 second breath (during which the expiratory valve is closed), followed by a slow exhalation to the level of Residual Volume.

This investigation is indespensable in the diagnosis of diffuse interstitial pneumopathy , diffuse pulmonary emphysemia, vasculitis, neuro-vascular disease, thoracic deformities, is recommended for the surveillance of people at high risk of disease (exposure to asbestos, beryllium, aluminum powders, patients with extrapulmonary collagenosis or extrinsic alveolitis, patients treated with certain drugs), useful in the evaluation of emphysematous component or the global evaluation of the gas exchanges at COPD, can evaluate the therapy administered , can appreciate the prognosis of lung diseases, can determine the degree of functional disability.

The Lung Diffusing Capacity CO (DLCO) test is very useful in diagnosing lung disease coming to the aid of many patients.

A few considerations about fever and nursing in fever

Dana Sorica, Carmen Hornea

Fever is defined as the elevation of core body temperature above normal; in normal adults, the average oral temperature is 37°C (98.6°F). In medical practice, a single temperature of more than 38.3°C (101°F) or three readings (at least 1 hour apart) of more than 38°C (100.4° F) are considered significant. Fever of an unknown origin (FUO) is defined as a febrile illness lasting more than 3 weeks, with temperatures exceeding 38.3°C on several occasions, and lacking a definitive diagnosis after 1 week of evaluation in the hospital.

The febrile response, of which fever is but one component, is a complex physiologic reaction to disease involving a cytokine-mediated rise in body temperature, generation of acute-phase reactants, and activation of numerous physiologic, endocrinologic, and immunologic systems.1 The temperature of the body is dependent on maintaining a balance between the production and dissipation of heat.

Goals of care: The presence of fever is associated with potential metabolic consequences, including dehydration and increased oxygen consumption and metabolic rate. If prolonged, fever may be associated with an increase in nutritional demands, which may be problematic if the patient already is compromised nutritionally. Prolonged fever may also lead to debilitating fatigue Suppression of fever may help to alleviate uncomfortable, constitutional symptoms of fatigue, myalgias, diaphoresis, and chills. In addition to constitutional symptoms, focal findings related to the etiology of fever may contribute to the burden of symptoms. For example, the formation of an abscess is often associated with pain, whereas uncomfortable dyspnea and cough may be related to pneumonia. For instance, individuals seeking comfort-oriented care exclusively may decline parenteral antibiotic treatment of pneumonia to avoid hospitalization and remain at home. For others, treating the underlying etiology of fever with more aggressive interventions, such as surgical drainage of a painful abscess, will offer symptom palliation and potentially contribute to the prolongation of life.

Multiple sclerosis

Georgiana Drăghici

Multiple sclerosis (MS) involves an immune-mediated process in which an abnormal response of the body's immune system is directed against the central nervous system (CNS). The CNS is made up of the brain, spinal cord and optic nerves. Within the CNS, the immune system causes inflammation that damages myelin — the fatty substance that surrounds and insulates the nerve fibers — as well as the nerve fibers themselves, and the specialized cells that make myelin.There are several types of MS: Clinically isolated syndrome (CIS), Relapsing-remitting MS (RRMS), Primary progressive MS (PPMS) and Secondary progressive MS (SPMS).

Treatments for MS attacks

Disease-modifying therapies (DMTs) are designed to slow disease progression and lower the relapse rate. In 2017, the Food and Drug Administration (FDA) approved the first DMT for people with PPMS. This injectable drug is called ocrelizumab (Ocrevus), and it can also be used to treat RRMS.

The bottom line

MS is a lifelong condition. The patient face unique challenges that can change over time.They should focus on communicating concerns with the doctor, learning all they can about MS, and discovering what makes them feel their best. Many people with MS even choose to share their challenges and coping strategies through in-person or online support groups.

Pages from the history of blood transfusion: old Romanian press reflection

Elena I. Milea, Nicoleta M. Băltărețu, Florența N. Borhan, Camelia Tudor, Laura M. Constantin

More and more researchers appeal to old press to investigate their chosen subject. Newspapers and magazines, historical image of the time when they were printed, are work instruments for researchers. While trying to find out as much novelties as possible about blood transfusion history, I have researched the Romanian Academy's Library newspapers and magazines published between 1881 and just after the Second World War. Among the researched magazines, I would like to mention:"Bulletin de l'Academie de Medicine" (1881 – 1944).

Pages from old newspapers and magazines describe the first transfusion performed from animal to human, from human to human, but also the first blood transfusion performed in Romania, quite late when compared to Western Europe. After an unsuccessful blood transfusion performed in 1853 by Carol Davila, the next blood transfusion was performed in Romania in 1912 – 1914.

The press of the time highlighted the great discoveries regarding blood transfusion. Lots of pages have been dedicated to indirect blood transfusion, veno-venous transfusion performed with the aid of a device (during the World War I – Oehlecker), or to the discovery of blood types. On the agenda were also International Transfusion Congresses, the appearance of blood banks, flourishing businesses or great scandals when patients died due to blood transfusion.

Old newspapers and magazines are archives of the daily life of those times. Only by reading them one can acknowledge the progress of medicine and of blood transfusion, progress preparing the events of today, as today's progress prepares us for the events of tomorrow.

Chronic pancreatitis

Adina Bălan, Viorica Gogor, Mihaela Vasile, Cătălina Oana

Chronic pancreatitis is a chronic inflammatory disease of the pancreas, with progressive evolution towards exocrine and endocrine pancreatic destruction, leading to pancreatic insufficiency. Symptom for pancreatitis are dbdominal pain - the dominant symptom epigastric, periumbilical, triggered by heavy meals or alcohol consumption. Steatoreea - a late sign, when malabsorption occurs bulky, pasty chairs, with a rancid smell; always accompanied by weight deficit. History and clinical picture of chronic alcoholism - recognized or not (collateral history, in the relatives). The most important cause of PC (over 90% of cases) necroptic > 45% of alcoholics showed morphological changes of PC, in the absence of clinical signs of disease toxic dose of pure alcohol. Some patients may have ethanolic hepatopathy (steatosis, alcoholic hepatitis or even ethyl hepatic cirrhosis)

Treatment: Dietary: complete and definitive abolition of alcohol consumption; avoiding abundant meals rich in lipids and proteins; in episodes of PA on PC background dietary rest, parenteral nutrition, naso-gastric well. Drug: pain relievers (Algocalmin, Piafen, Tramal); high lipase preparations (Creon, Panyztrat, Festal, Cotazym, Nutryzym, Digestal) even in the absence of malabsorption; when there is malabsorption, minimum 20,000 U lipase/mass; if the preparation is not gastroprotected, before the meal, an antisecretory is given before 30 minutes.

Nursing applied to a thrombophlebitis patient

Cristina Filimon, Cecilia Pătru

Thrombophlebitis is an inflammatory process that causes a blood clot to form and block one or more veins, usually in the legs. The affected vein may be near the surface of the skin or deep within a muscle. Causes include trauma, surgery or prolonged inactivity. The three main factors that are causing this pathology are the endothelial lesions, the inflammation localised on the vein's wall and the hypercoagulation process. Thrombophlebitis frequently occurs in cardiac insufficiency cases and diseases followed by venous stasis as a result in prolonged inactivity. The primary physiopathological alteration is the formation of a blood clot that is the mechanical obstructive element. Symptoms include warmth, tenderness and pain in the affected area, as well as redness and swelling. Complications- from thrombophlebitis are rare. However, complications might include: pulmonary embolism- if part of a deep vein clot becomes dislodged, it can travel to the lungs, where it can block an artery and become potentially life-threatening, postphlebitic syndrome- this condition also known as post-thrombotic syndrome, can develop months after a person had DVT. This can cause lasting and possibly disabling pain, swelling and a feeling of heaviness in the affected leg. For superficial thrombophlebitis, the doctor might recommend applying heat to the painful area, elevating the affected leg and possibly wearing compression stockings. Another recommendation is the treatment with blood-thinning medications, such as heparin or clot-dissolving medication. Prescription strenght compression stockings help prevent swelling and reduce the chances of complications of DVT.

Clostridium difficile infection: a nurse's point of view

Mariana Bogriceanu, Grațiela Olteanu, Liliana Bulat

Introduction: Our objective is to review current guidelines in managing Clostridium difficile colitis and our department`s experience in this field.

Materials and methods: Clostridium difficile is a Gram-positive spore-forming anaerobe that was identified, beginning with 1978 as the causative pathogen in the majority of antibiotic-associated diarrhea and colitis cases. It is believed that a small percent of healthy individuals have this bacterium in their colon, but it is more often found in the ground, water, animal or human feces, C. difficile being a growing cause for nosocomial disease.

The most common risk factor for C. difficile infection is the use of antibiotics, as they can disrupt the normal microbial balance in the colon.

This bacterium is dangerous because it can secret two toxins (A and B) that cause inflammation of the colonic mucosae, which can lead to moderate diarrhea, pseudomembranous colitis (most frequent form) or fulminant colitis. If the physician suspects a C. difficile infection, it is the nurse`s task to explain to the patient how to collect a stool sample and take it as fast as possible to the laboratory.

After infection is confirmed, it is up to the nurse to assure contact precautions: isolate the patient with single use gowns and gloves being available at room entrance; environmental disinfection; explaining the importance of hand hygiene and of course making sure that the patient takes his medication correctly, according to the physician`s recommendations.

Results and conclusion: C. difficile colitis has become the most frequent infectious cause of healthcare-associated diarrhea, causing high morbidity and mortality, mainly because of the use of broad-spectrum antibiotics.

Immunoglobulin therapy: the election treatment for primary immunodeficiency diseases

Nicoleta Voinea

The term “immunoglobulin” refers to the fraction of blood plasma that contains immunoglobulins. Immunoglobulin therapy is a purified product prepared from human plasma donors. Its main therapeutic indications are primary and secondary immunodeficiences. Immunodeficiences are diseases with variable genetic transmission. Immunoglobulin therapy is generally administered subcutaneously, intramuscularly or intravenously. In our hospital the most used is the intravenous administration and the product used is Intratect. The systemic adverse reactions of immunoglobulin therapy are usually determined by the patient’s intolerability to a rapid infusion (headache, nausea, general altered state, fatigue, dispnea). The adverse reactions which occur during subcutaneous administration are localized skin reactions at the site of the injection: swelling, inflammation, rash, and induration. Immunoglobulin therapy is a complex therapy which can lengthen many patients’ life span.

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Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

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(3) Open to all prospective registrants free of charge or at minimal cost;
(4) Validates registered information;
(5) Identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

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- *Evolution of mortality rates in ST elevation myocardial infarction*
- *Gingival involvement of oral non-tumoral mucosal diseases*
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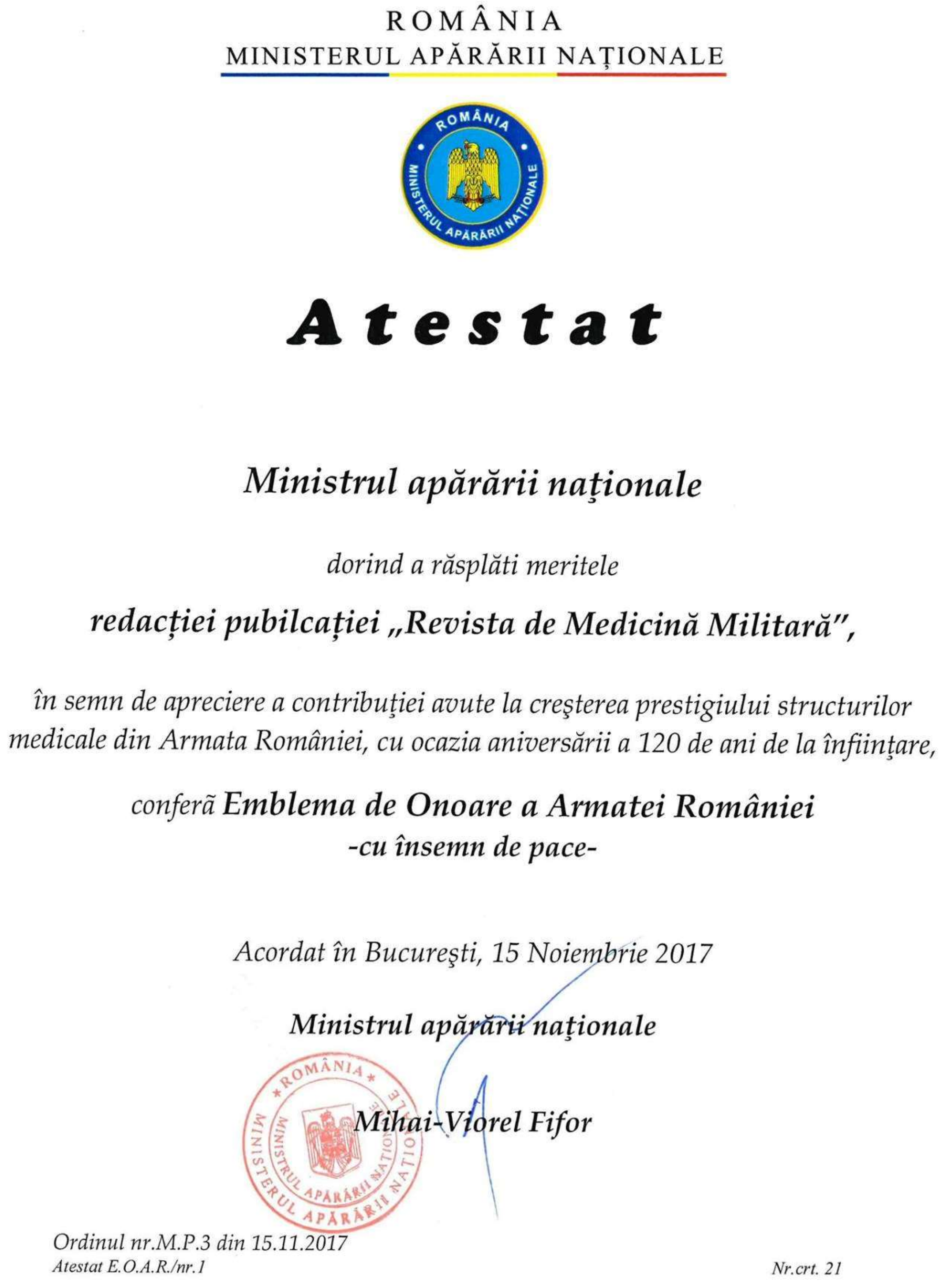

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dorind a răsplăti meritele

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Mihai-Viorel Fifor

Ordinul nr.M.P.3 din 15.11.2017
Atestat E.O.A.R./nr.1

Nr.crt. 21

EDITORIAL

The Military Medical Institute: its past and its future

Col. Lect. MIHAIL TUDOSIE

Commander in Chief, Military Medical Institute, Bucharest, Romania

Mihail Tudosie

The Military-Medical Institute was founded in 1884 through High Royal Decree number 2824 published in Official Monitor number 2/23.01.1884, and was initially led by general academician Zaharia Petrescu. For a long period of time, the institute functioned based on models such as similar Austrian and Prussian institutes, but with a strong French influence. In those times, as well, the students attended both professional training courses at the Faculty of Medicine of Bucharest and military medical training within the Institute.

Through a regrettable decision of the authorities, the courses of the institute are suspended in 1987, military medicine being deeply prejudiced. During the following years, up to 1990, military medical staff was recruited indirectly.

In 1990, the Government of Romania brings moral justice, by adopting Government Decision 585, through which the Military-Medical Institute of Bucharest is re-established as a superior institute of military medical education, with a Military-Medical Training Section based in Târgu-Mureş.

The present activity of the Institute relies on these traditions, which ensure military medical training of all future doctor officers of the Romanian Army, the Ministry of Internal Affairs and the National Administration of Prisons.

For the continuous improvement of military doctors in the structures of the Romanian Army, career classes are being organized and held in the Military-Medical Institute with a curricula that is compatible with NATO standards within the operational medicine field.

The teaching personnel of the Institute is permanently preoccupied with promoting academic elitism. Students must find models to follow. Every day, the teaching personnel of the Institute must pass the test of education optimization and of increasing the quality of the educational act. In this regard, steps are being taken at the ministry's management level for the recruitment of teaching personnel. The quality of the educational act can be improved only through the recruitment of personnel with a high level of professional training and, also, through the equipment of classrooms with video projection systems and highly performing apparatus in both Bucharest and Târgu-Mureş,

Because mobility nowadays is not only desirable, but also a necessity, we are doing all that is in our power to achieve this scope. Our students participate in Erasmus+ programs offered by the two Universities of Medicine and Pharmacy – "Carol Davila" of Bucharest and that of Târgu-Mureş – with whom we form a partnership. These scholarships ensure our students' preparation for a whole semester at first class

European medicine universities. Thus, students connect “live” to medicine in other countries. At the national level, they participate in the experience exchange program held between civil universities – TransMed. Military students are amongst the most disciplined and diligent, representing a true heraldry of the Army in the civil universities’ environment.

Also, regarding operational military medicine, our students have participated and continue to participate in different national and international exercises, where they learn from the experience of NATO allied countries. A lot of students of the Institute work during their studying years as volunteers at emergency medicine services, such as S. M. U. R. D.

In order to improve scientific performances, the Military Medicine Students’ Association, in collaboration with the Military-Medical Institute, annually organizes a scientific presentations event, in partnership with military students of other academies. In a similar manner, UMF Tg. Mures annually organizes an international congress, which includes a special military medicine session.

A reason for pride is also represented by the consistent participations of the institutes’ students at numerous meetings, symposiums and congresses organized by the civil medicine universities or by the army: The Balkan Medicine Congress or the congresses organized by the academies representing the three forces of the Romanian Army. Thus, young military students are connected to a flow of information pertaining to university science, showing a striking inclination towards research work.

Another aspect pertaining to priority preoccupations is represented by the participation in sports activities organized between military education institutes or between the various components of the National Defense System, Public Order System and National Security System, where the students of the Institute always win a leading place.

Taking into consideration the actual recruiting demands of the Army, regarding the increase of our defense capability, the tuition figures for 2018 have doubled. Thus, 100 places were approved for Bucharest and another 100 places for Tg. Mures. Of these, general medicine represents 60 places in Bucharest and 90 in Tg. Mures. For dental medicine, 13 places are approved, and 15 for pharmacy, only in Bucharest. The other beneficiaries have their places approved for general medicine and dental medicine as follows: Ministry of Internal Affairs – 12 places (7 in Bucharest and 5 in Tg. Mures), and National Administration of Prisons - 8 places for general medicine (5 places in Bucharest and 3 in Tg. Mures), 2 places for dental medicine. As tuition figures rise, by the order of the Ministry of National Defense, in 2018, the institute has 20 places approved for doctors who will become officers through indirect activation.

For all the conducted activities, we enjoy an excellent collaboration with military hospitals and operative military units, as well as with civil universities. Thus, we can form and perfect military doctors for our hospitals and units, who are always ready to take part in international missions.

It is known that the performing armies of the world have never hesitated to show their skill, equipment and combative force. We also adhere to this direction, going out in public space every time we have the chance.

The image of the Institute if promoted by all means (press, radio, television, Internet). The students of the Institute participate in military ceremonies, charity events or blood donating activities, and activities which promote the military doctor profession in civil and military high-schools.

In the future, we wish to maintain and even ascend this standard of medical and military training through which our students have always stood out. We will stress the importance of developing the capacity and appetite for learning, of creativity, and of creating the profile of a military doctor that is receptive to all that is new, always willing to be involved, ready for action, competent and competitive. The future belong to these young people and for this, we do all that is in our power to form them as medical doctors of great value for the Romanian Army.

REVIEW ARTICLE

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Evolution of mortality rates in ST elevation myocardial infarction

Alice E. Munteanu[1,2], Cristina M. Calcan[1], Irina Florescu[2]

Abstract: *The purpose of this paper is to provide information about mortality rate in patients undergoing primary angioplasty for STEMI. Cardiovascular diseases lead to one third of the deaths worldwide, surpassing the mortality rate produced by neoplasia, acute respiratory failure and diabetes mellitus all together. In the world, approximately 17 million die annually because of cardiovascular disease and every 5 seconds one is suffering from a myocardial infarction.*

In 2010, in our country was implemented a national program (RO-STEMI) to offer rapid interventional treatment for patients with STEMI, aligned to ESC Guidelines. At the beginning only small parts of the patients were transferred to the hospital in charge for STEMI, so, we chose to study patients from the second year of the program and from last year. We made a retrospective study, including patients with STEMI that reached our hospital for primary angioplasty in the first 12h after the debut of the symptoms, aiming to make a parallel between the in-hospital mortality rates at maximum one month after the primary PCI. In order to be included, the patients must present ST elevation and another 1 out of 3 criteria (clinical, echocardiographic and biological changes suggestive for myocardial infarction).

The main target was to evaluate the evolution of early mortality rates post primary PCI after the changes in the ESC Guidelines and the progresses in the medical field. A secondary target was to correlate mortality rates with the presence of comorbidities or risk factors.

INTRODUCTION

Alexander Muirhead is reported to have attached wires to a feverish patient's wrist to obtain a record of the patient's heartbeat in 1872 at St Bartholomew's Hospital.[1]

Another early pioneer was Augustus Waller, of St Mary's Hospital in London.[2] His electrocardiograph machine consisted of a Lippmann capillary electrometer fixed to a projector. The trace from the heartbeat was projected onto a photographic plate that was itself fixed to a toy train. This allowed a heartbeat to be recorded in real time.

An initial breakthrough came when Willem Einthoven, working in Leiden, the Netherlands, used the string galvanometer (the first practical electrocardiograph) he invented in 1901.[2]

This device was much more sensitive than both the capillary electrometer Waller used and the string Galvanometer that had been invented separately in 1897 by the French engineer Clément Ader.[3]

Einthoven had previously, in

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1895, assigned the letters P, Q, R, S, and T to the deflections in the theoretical waveform he created using equations which corrected the actual waveform obtained by the capillary electrometer to compensate for the imprecision of that instrument. Einthoven also described the electrocardiographic features of a number of cardiovascular disorders. In 1924, he was awarded the Nobel Prize in Medicine for his discovery.[3] In 1937, Taro Takemi invented the first portable electrocardiograph machine.[4]

Cardiovascular diseases represent the main cause of death worldwide, 30% of them being caused by coronary artery disease. Myocardial infarction (MI) is one of the five main manifestations of coronary heart disease, namely stable angina pectoris, unstable angina pectoris, MI, heart failure and sudden death.[5]

Myocardial Infarction (MI) is defined by myocardial cell necrosis due to significant and sustained ischemia. Most frequently it is an acute manifestation of atherosclerosis-related coronary heart disease. MI results from either coronary heart disease, which implies obstruction to blood flow due to plaques in the coronary arteries or, much less frequently, to other obstructing mechanisms (e.g. embolism, spasm of plaque-free arteries).[6]

The clinical presentation of MI varies from a minor coronary event to life-threatening clinical situations or sudden death.[2] The criteria for STEMI are well known – specific, prolonged >15-20 min pain, specific site, and it is not ameliorated by nitroglycerin intake. The ECG shows persistent ST elevation or new onset of LBBB. Specific biomarkers are represented by high-sensitive Troponin I, CK/CK-MB, which have a specific dynamic curve.[7]

In 2010 in our country debutantes a national program named ”RO-STEMI”. In this program all patients with STEMI, from a well-defined area, are send to a certain hospital for being coronarographic evaluated. Due to this program more than 63% of patients' beneficiated of myocardial reperfusion and the mortality rate dropped all over the country from 13 to 7%.[8]

MATERIALS AND METHODS

It's been realized a retrospective study in STEMI patients in our hospital, on all patients from 2012 and 2016. All the patients included in this study respected STEMI criteria for diagnosis. We took 252 patients from 2012, out of which 29% were women and 71% were men, and 136 from 2016, with a non-significant difference in gender balance (28% women and 72% men). The number of patients is smaller in 2016 than in 2012 because in the beginnings of the national program there were only a small number of hospitals being part of it. In present the number of STEMI hospitals has increased so STEMI patients are distribute to more STEMI centers than during the past years.

RESULTS

During this period of time, the median age of suffering an infarction was 60 years for men, with no significant difference between years, but for women the age evolved, so now, usually women suffer a myocardial infarction at about 63, compared with 66 years old in 2012.

The onset can be seen as a predictor for evolution and a gravity factor for STEMY patients.

A cardiorespiratory stop onset represents a high gravity risk factor. We noticed that in 2016 were twice more patients, than 2012, with cardiorespiratory stop on onset of STEMI. Knowing that, it is expected that the mortality rate being higher in 2016 that 2012.

Figure 1 Distribution of patients by cardiorespiratory stop onset.

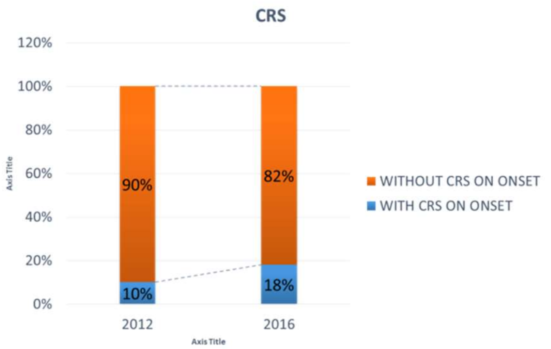

The distribution of modifiable risk factors is the same for the 2 groups, the change consists of the number of risk factors present at the same patient, which was definitely higher last year (in average 2 risk factors per patient in 2012 vs. average 3.5 risk factors/patient in 2016). Even if we strongly advise the patients to quit smoking, and there is also a huge implication of the government in this, the number of smokers increased, but the good news is that their mortality has a decreasing trend over the years.

The median time from pain to cath-lab has lowered from 12h in 2012 to 9h in 2016. This improvement could be in one side due to well function of RO-STEMI program, and in other side due to improvement in social health education, the patients being better informed about myocardial infarction symptom-tology which decrease pain-call time.

If we take a look to the coronary lesions, we can see that first affected artery is left anterior descending artery (LAD), followed by right coronary artery (RCA), circumflex artery and marginal artery.

A very important observation is the growth of the number of patients with right coronary artery involvement, with can be associated with right ventricle infractions and an extremely poor prognosis. Also right coronary artery involvement means a higher risk of malignant arrhythmias and sudden cardiac death. 45% of STEMI patients had only one affected vessel, 40% had 2 affected vessels and only 15% had three coronary arteries affected most of them with CABG indication.

Only 15% of the patients in 2012 were thrombolysed before PCI, while in 2016 the use of t-lyse increase to more than 30% of the patients, this measure helps physicians to buy time for them to reach the cath lab.

Figure 2 Rate of deceased after thrombolysis

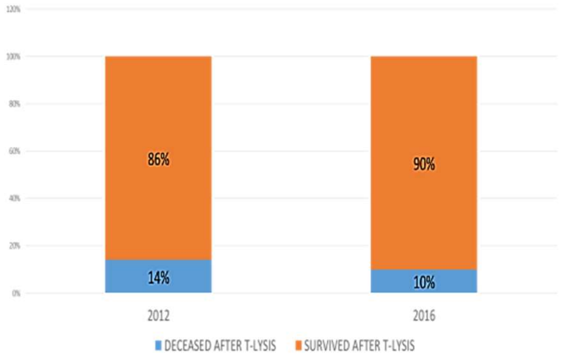

From the number of those thrombolised, only a small amount has died, but none because of a bleeding. All died because of malignant arrhythmias or asystolia secondary to the myocardial infarction. Notice that in 2016, even though the number of thrombolised patients was bigger than in 2012, the rate of deceased patients after thrombolysis was smaller in 2016 than in 2012. The reason for this difference could be a better triage of elected patients and the evolution of thrombolytic agents.

At the beginning, drug eluded stents were extremely rare and expensive, nowadays we use much more frequently DES then bare metal stents, in 2016 73% of used stents were DES and the target is using only DES, as the indication in ESC guideline.[7] This made the rate of early in stent restenosis to go from almost 20% to 0%.

Figure 3 Mortality rate in the two groups of patients

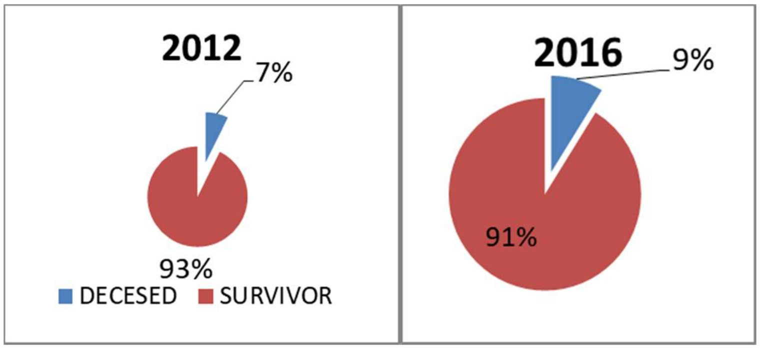

Due to the shortening of the time from symptoms-onset to revascularization and from associating more frequently thrombolysis with PCI, the number of those with ventricular dysfunction has dropped significantly.

Even though the number of thrombolysis raised, and the time to cath lab is shorter, the number of patients who didn`t make it to the cath lab is increasing, the mortality rate has increased from 2012 to 2016. If we consider that the rate of cardiorespiratory stop as onset as a high gravity factor and if we see that the patients in 2016 were more complex than in 2012, then the high mortality rate is expected. Another factor suggests that the gravity of cases was higher in 2016 is that the number of deceased patients without reperfusion was higher in 2016.

CONCLUSIONS

The National Program RO-STEMI became more effective by increasing the number of involved hospitals.

During time the mortality rate have had a slightly increasing trend due to a growth of the number of risk factors/patient, an increase in the severity of the coronary lesions, an increase in the number of the lesions in the right coronary artery, and a poorer prognosis in echocardiography.

The time from symptoms onset to cath lab decreased and by associating more frequently thrombolysis for “buying” more time, so the chances for the ventricle to remodel remain low.

By respecting the ESC guidelines for each period of time, even though the early mortality rate seems to have an ascending trend, the overall mortality rate and longtime prognosis of the STEMI patients are getting better.

It is very important to make such an analysis from time to time, in order to make thinks go better and see where some changes could be made.

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REVIEW ARTICLE

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Gingival involvement of oral non-tumoral mucosal diseases

Elena C. Tofan[1], Ioanina Părlătescu[1], Elena Coculescu[1], Şerban Ţovaru[1], Carmen Nicolae[1], Bogdan I. Coculescu[2]

Abstract: *Gingival conditions are frequently encountered in dental clinical practice and they can raise diagnosis and treatment problems. The diagnostic is variable from normal conditions to autoimmune diseases or even malignancy. Some of them cause disturbances such as pain, burning sensation and have an impact on patient's quality of life.*

INTRODUCTION

Although oral cavity is an area easy to explore by visual exam, there is a large variety of conditions and disorders which can be encountered. We present a general overview of gingival lesions frequently associated with oral mucosal diseases. Dental plaque-induced gingivitis and all gingival enlargements (reactive or tumorous with benign, cystic or malignant variants) are not the subject of this article.

DEFINITION OF SPECIFIC TERMS AND LESIONS

The gingiva is a "first line" mechanical barrier defined as the keratinized mucosa that covers the teeth and the alveolar bone. The periodontium is made of gingiva with the alveolar bone, periodontal ligament and cementum. The mucogingival junction separates the gingiva from alveolar mucosa [1]. In healthy persons the gingiva has a pink color (Figure 1), or it can have pigmented areas depending on the individual skin color (Figure 2).

Histologically, gingiva is composed of a thick stratified, keratinized epithelium and the underling connective tissue made of collagen and elastin fibers, fibroblasts, melanocytes and immune cells [2]. The epithelium cell-to-cell adhesion is done through desmosomes and adherence junctions while the adhesion to the basement membrane is done by hemidesmosomes and focal contacts [3].

Superficial lesions affect epithelium and underlying connective tissue. Depending on the clinical appearance, evidenced by visual examination, they can be divided into: keratosis or white lesion (Figure 3A and 3B), atrophic (Figures 4A, 4B, 4C), ulcers resulting from vesicles (Figure 5A) or bulla (Figure 5B).

The gingival diseases are presented in the periodontal diseases classification elaborated by Armitage in 1999 [4] which is universally

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accepted for both periodontal and gingival conditions.

Figure 1. Normal gingiva

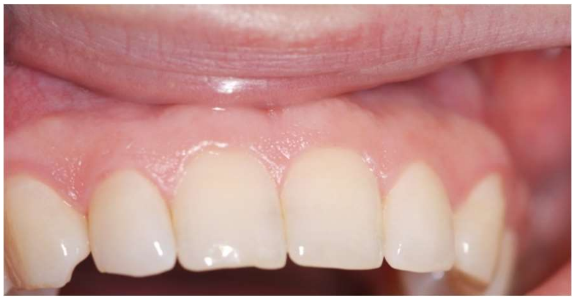

Figure 2. Pigmented gingiva

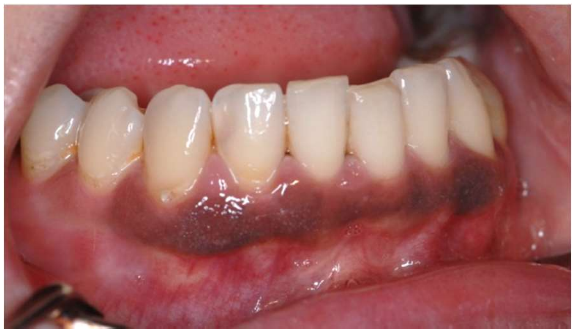

Figure 3A. Gingival white lesion. Extended homogenous plaque of unknown etiology. Diagnosis: leukoplakia

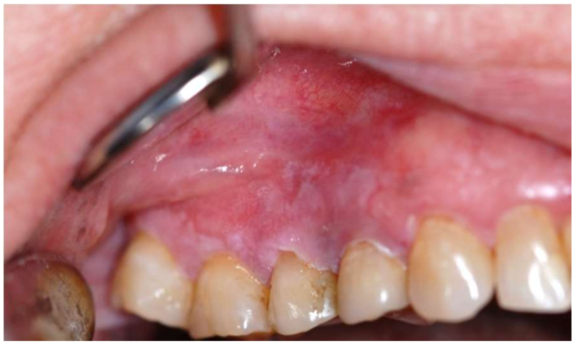

Figure 3B. Gingival white lesion. Diagnosis: traumatic keratosis (associated with aggressive tooth-brushing technique)

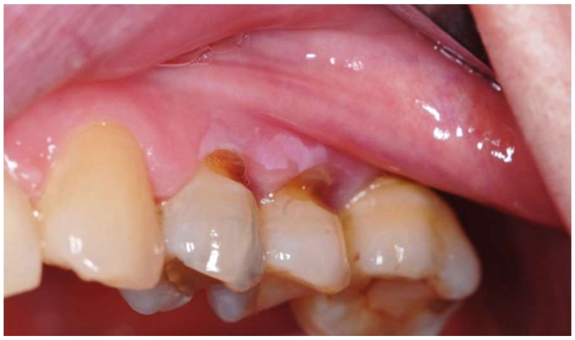

Figure 4A. Mild atrophy. Diagnosis: desquamative gingivitis in oral lichen planus

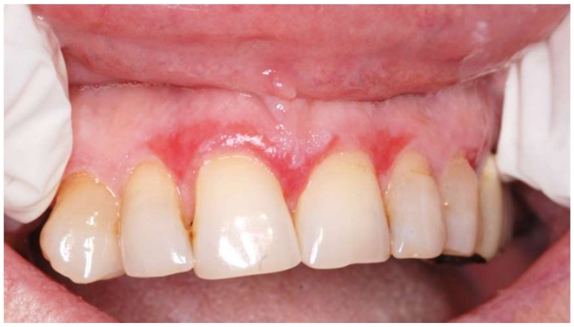

Figure 4B. Moderate atrophy. Diagnosis: desquamative gingivitis in oral lichen planus

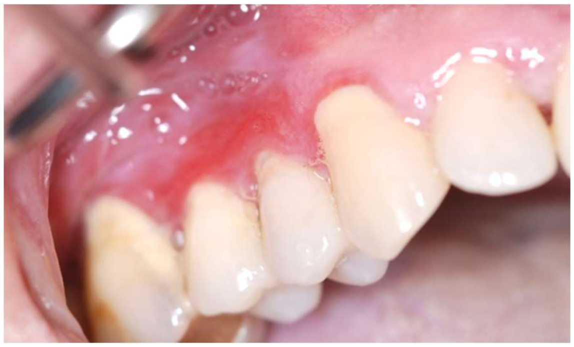

Figure 4C. Severe atrophy and reticular keratosis. Diagnosis: desquamative gingivitis in oral lichen planus

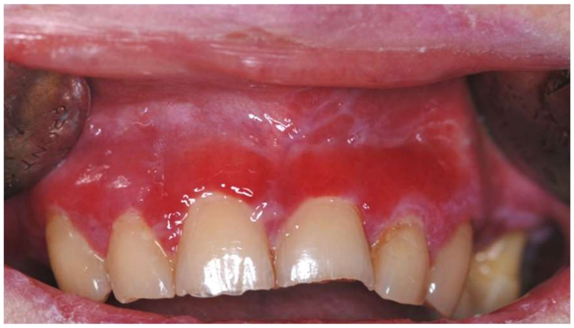

Figure 5A. Small gingival ulcers following vesicles. Diagnosis: primary herpetic gingivitis

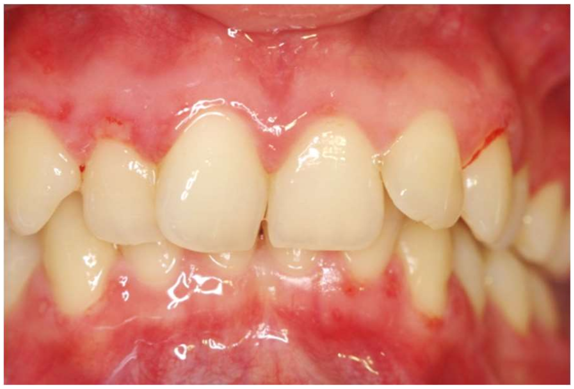

Figure 5B. Extended ulceration after bullae rupture. Diagnosis: desquamative gingivitis in bullous dermatosis

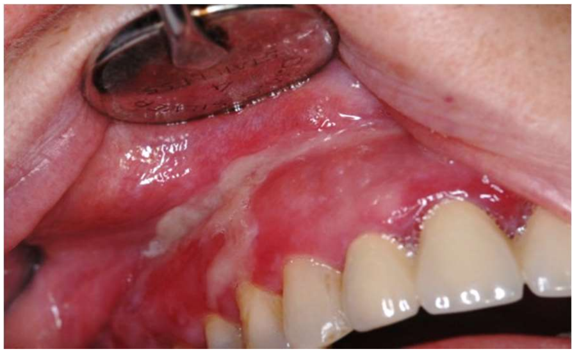

The gingival disorders are presented in Table 1.

CLINICAL FEATURES AND THE BASIC THERAPEUTICALLY APPROACH OF ORAL MUCOSAL DISEASES WITH GINGIVAL INVOLVEMENT

We reviewed and summarized the clinical characteristics of the most frequent oral diseases. The oral manifestations of general diseases cover a large range of conditions (Table 2). The mainly local signs usually affects more than one mucosal area, so the gingival lesions must be interpreted in the whole context.

Table 1. The gingival diseases classification [4]

Periodontal diseases classification. Section gingival diseases		
A. Dental plaque-induced gingival diseases	1. Gingivitis associated with dental plaque only	
	2. Gingival diseases modified by systemic factors	
	3. Gingival diseases modified by medications	
	4. Gingival diseases modified by malnutrition	
B. Non-plaque-induced gingival lesions	1. Gingival diseases of specific bacterial origin	
	2. Gingival diseases of viral origin	
	3. Gingival diseases of fungal origin	
C. Gingival lesions of genetic origin	1. Hereditary gingival fibromatosis	
	2. Other	
D. Gingival manifestations of systemic conditions	1. Mucocutaneous disorders	1) lichen planus
		2) pemphigoid
		3) pemphigus vulgaris
		4) erythema multiforme
		5) lupus erythematosus
		6) drug-induced
		7) other
	2. Allergic reactions	
E. Traumatic lesions (factitious, iatrogenic, accidental)	1. Chemical injury	
	2. Physical injury	
	3. Thermal injury	
F. Foreign body reactions		

Oral lichen planus is a disease with chronic evolution and immune mediated pathogenesis [5]. The clinical signs and symptoms are variable depending on the lesions associated [2]. The lesions are multiple, associated, merged and coexist and symmetrically distributed. The gingival involvement, commonly encountered in these patients is described as desquamative gingivitis. This shows various lesions from white, keratotic to atrophy, ulcerations and bullae. The diagnostic criteria universally accepted includes the histological confirmation (with hydropic degeneration and band-like T-cells infiltrate), although there are microscopic features similar to other mucosal diseases [6].

The therapy is not curative but it intends to control acute lesions and symptoms. The agents used cover a large spectrum of drugs: topical or systemic corticoids, hydroxychloroquine, azathioprine, mycophenolate mofetil, methotrexate, dapsone, thalidomide, biological agents etc. [6].

Mucous membrane pemphigoid is a chronic autoimmune blistering disease which presents subepithelial vesicles and bullae. Any area of the oral mucosa can be affected. The gingival lesions present as desquamative gingivitis which simulates oral lichen planus and are noticed in 60% of cases [6]. The diagnosis is established by histopathological analysis and direct immunofluorescence which shows along the basal membrane linear deposits of immune-globulin IgG, IgM, IgA and complement [6]. The therapy is mainly long-term immunosuppressive treatment used local and systemic.

Table 2. General data on oral mucosal diseases, clinical features [1-3]

Oral conditions	Disease	Oral mucosa manifestations
Autoimmune diseases	Oral lichen planus	Variable presentation, generally symmetrical lesions (reticular, popular, plaque form, atrophy, ulcers, bulla)
	Pemphigus vulgaris	Small intraepithelial bulla and secondary erosion
	Mucous membrane pemphigoid	Desquamative gingivitis, shallow ulcer after multiple blisters affecting the oral mucosa
	Linear IgA dermatosis	Annular vesicular-bullous lesions
	Allergic reaction to dental materials and/oral health products	Variable clinical features: inflammation, reddish or white areas
Infectious diseases		
Viral	Primary herpetic infection Secondary herpetic infection	Ulcerative lesions following short duration vesicles
	Herpes zoster	Unilateral ulcers following vesicles, located along nerve distribution, mainly in older patients
Bacterial	Necrotizing ulcerative gingivitis	Painful, purulent ulcers of the gingival papillae; mostly in teenagers and young adults
Traumatic lesions	Caused by excessive tooth brushing Mucosal burn caused by substances held in close contact for a long period of time Local radiotherapy	Ulcers, atrophy and keratosis
Pigmented lesions	Rasial pigmentation Melanotic macule Metallic tattoo	Pigmented areas on the gingiva
White lesions	Leukoplakia	Predominantly white lesion of unknown or tobacco etiology

Oral pemphigus vulgaris is a lifelong autoimmune bullous disease which affects the epithelium with autoantibodies directed against the desmosomal cadherine – desmoglein 3 causing acantholysis and blistering [7]. Oral mucosa is the initial site of the lesions in 80% of the patients [7]. The painful, irregular-shaped erosions and blisters affect the buccal mucosa, the tongue and gingiva. In these patients the desquamative gingivitis shows ulcers following bullae. The diagnosis is established by clinical features, histopathology, immunopathology, and serology. Direct immunofluorescence shows intercellular deposition of immunoglobulin G and complement 3 [7]. Treatment is complex and includes a variety of immunosuppressive and anti-inflammatory steroids in order to get disease control.

In patients with desquamative gingivitis there is an increased risk periodontal tissue breakdown mainly caused by the plaque deposits and the pain associated with tooth-brushing [8].

Herpetic oral viral infection is caused by two types of herpes simplex virus. The primary infection which affects mainly children or young adults shows a wide range of manifestations varying from an extended oral vesicular-ulcerative eruption to a reduced, asymptomatic condition [2, 9]. The primary eruption may be associated with general signs and symptoms such as fever, malaise, lymphadenopathy and dysphagia. The secondary infective episode usually involve gingival mucosa and the hard palate. The lesions are vesicles which break quickly and leave painful ulcers. These heal in 7-10 days without scars [9] in healthy and immunologically competent patients. Infections diagnosed early show a good response to antiviral medication. Also local symptomatic suspensions will ease the discomfort in both primary and secondary herpetic eruption.

For **pigmented lesions of the oral cavity**, more than for other conditions the diagnosis is challenging even for an experienced clinician [10]. On the gingiva the physiological pigmentation and the melanotic macules are frequently seen but this does not exclude a melanoma or Kaposi sarcoma diagnosis. The physiological pigmentation is caused by an increased melanin pigmentation of the basal layer or incontinent melanin and/or melanophages in the superficial lamina propria and the melanotic macules are a result of a increased melanin production from the basal melanocytes. In these cases, the diagnosis is appreciated after size, shape, or color, location together with the anamnestic data [10]. Generally a biopsy is needed to determine a definitive diagnosis and to exclude risky conditions.

Oral leukoplakia is defined as a predominately white lesion of the oral mucosa, lesion of questionable risk having excluded (other) known diseases or disorders that carry no increased risk of cancer [11]. It can be met in any area of the oral mucosa, including gingiva. At present about this lesion, the literature states that neither histology nor clinical factors can reliably predict the behavior [12]. Proliferative verrucous leukoplakia is one of the most aggressive form described which shows a predominant gingival involvement.

CONCLUSIONS

These gingival conditions can share similar clinical features which may lead to misdiagnosis or underdiagnosed situations. Dentists and general practitioners need to be familial to the gingival disorders. In general a definitive diagnosis is based on biopsy of the lesional tissue but also the anamnestic data should be taken to account.

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REVIEW ARTICLE

Emerging concepts regarding the molecular profile of breast carcinoma: one-year experience in a University Center

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Abstract: *Breast carcinoma represents the leading cause of oncologic mortality for women. Due to therapeutic and diagnostic advances, the mortality and morbidity in the last decade declined, but breast cancer still has a great impact on quality of life and also on medical service cost. In the light of these facts, an integrated approach, considering histopathology features, molecular profiles and corroboration with clinical and imagistic data is necessary. A descriptive retrospective one-year study analyzed the breast cancer heterogeneity in 121 cases registered in the Pathology Department of the University Emergency Hospital in Bucharest, Romania. Our purpose was to evaluate histopathological, immunohistochemically, clinical and imagistic aspects of breast cancer considering the current molecular classification (Luminal A, Luminal B, HER2 enriched and triple negative/basal like).*

Our assay revealed that most prevalent histotype was NST (no special type) followed by invasive lobular carcinoma. Considering the molecular pattern, the most common was luminal B. Triple negative basal like and HER 2-enriched were correlated with an aggressive morphologic pattern and lymph-nodes positivity. Considering the imagistic acquisitions, mammography proved to be the most accurate technique for measuring the dimension of NST.

In conclusion, breast carcinoma is a heterogeneous disease that needs an integrated approach and personalized treatment based on the histopathologic and molecular features. Considering the great number of advanced stages as diagnosis, a national screening program for breast cancer is imperiously needed.

Keywords: *breast cancer, NST, ILC, IHC, molecular subtypes, biopsy*

INTRODUCTION

According to the most recent statistic database of WHO 2015, breast carcinoma represents the main cause of mortality for Romanian women [1]. The decline of breast cancer mortality is a trend that, according to the most recent epidemiologic estimation will continue [2]. This direction is mostly due to advancement in diagnosis and treatment [3].

A better understanding of pathogeny and a more accurate diagnostic approach of breast cancer led to several updates in guidelines for classification and treatment. Since 2003, for grading breast carcinomas, the Elston Ellis semiquantitative method modified score was used according to the 3rd edition of WHO guidelines for classification the breast tumours. The most recent WHO diagnos-

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tic guideline (4th edition, 2014) considers that for prognostic and predictive factors a panel of antibodies is needed for a further classification into molecular subtypes (luminal A, luminal B, Her2 enriched and triple negative basal-like). This approach is supported by multiple studies and summarized in the most important European or American conferences or guidelines. For example, the 14th St. Gallen International Breast Cancer Conference, 2015 [4], considers breast carcinoma a heterogeneous pathology according to its intrinsic molecular types whereas the 8th edition of AJCC (American Joint Committee on Cancer) states that breast malignant tumours should be staged according to the prognostic group, based on population that had been offered and mostly treated with appropriate endocrine or systemic therapy which includes anatomic T, N and M plus grade and status of HER2, ER, PR. [5]

MATERIAL AND METHOD

The aim of this retrospective analysis was to offer an exhaustive perspective of breast cancer considering clinical and imagistic presentation, morphological and molecular profiles of the main mortality factor for women. We analyzed the breast cancer heterogeneity in 121 cases registered as mastectomies, lumpectomies, quadrantectomies or biopsies in the Pathology Department of the University Emergency Hospital in Bucharest, performed over a period of 1 year (1st January 2017-31st of December 2017). The diagnostic varied from benign lesions (fibroadenoma, fibroadenomatoid mastopathy) to the most common malignancies, NST carcinoma and even rare cases like carcinoma with medullary features, mucinous, adenosquamous, papillary carcinoma or Phyllodes tumor.

Table 1. Frequency of the surgical procedures used for the histopathological diagnoses in the analyzed population

Type of lesion	Mastectomy + axillary in dissection	Lumpectomy	Core needle biopsy	Total
NST	40	19	19	78
NST with papillary features		1		1
Invasive lobular carcinoma	4	1	3	8
Medullary carcinoma	1			1
Adenosquamous carcinoma	1			1
Phyllodes tumour	1			1
Adenomioepithelioma		1		1
Metastasis of B cell lymphoma		1		1
Benign lesions, non-inflammatory	5	5	1	11
Fibroadenomas	1	8	2	11
Benign lesions, inflammatory	1	3		4
Metastasis of breast carcinoma			3	3
Total	54	39	28	121

For clinical data and imagistic interpretation, we used the hospital electronic database. The histologic specimens were studied using both conventional histopathological examination with HE stains and also immunohistochemically methods, classifying them further in the main 4 surrogate-molecular subtypes: luminal A, luminal B, HER2-neu non-luminal and basal-like/triple-negative. For diagnosing the main histologic subtypes, the last WHO guideline criteria were applied. For data collection and processing Microsoft Office Excel 2010 was used.

RESULTS

This one-year retrospective study analyzed 121 cases female patients registered in Pathology Department of the University Emergency Hospital in Bucharest with diagnostic of benign lesions and also with malignant pathologies. Those included carcinomas, with NST histotype being the most common (78 cases), ILC (8 cases) and also rare types. Detailed information is found in Table 1.

We focused our attention on the most common types of breast carcinoma: NST and ILC. In our study the most common histotype of breast carcinoma was NST, with a predominance of G2 grade of differentiation (59%), followed by an equal percentage of G1 and G3 (8% for each one).

In the absence of precise data about menstrual status, we considering the climax age at 50 years old. With this presumption, most women were postmenopausal (79%), and the average age at presentation was 61.48 years, varying from 35 to 86 years old. Distribution of the analyzed patients according to their age, histotype and molecular profile of their tumor is detailed in Table 2.

Table 2. Age distribution according to the main histotype, grade of differentiation and molecular profile of the most important histotypes of breast carcinoma

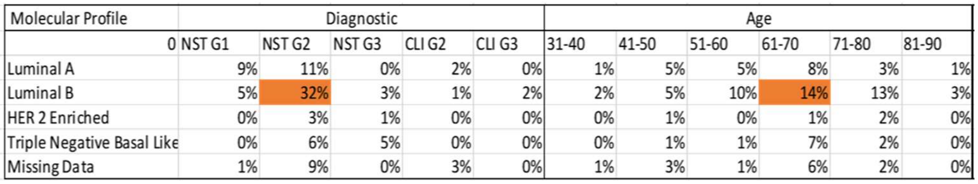

Molecular Profile	Diagnostic					Age					
0	NST G1	NST G2	NST G3	CLI G2	CLI G3	31-40	41-50	51-60	61-70	71-80	81-90
Luminal A	9%	11%	0%	2%	0%	1%	5%	5%	8%	3%	1%
Luminal B	5%	32%	3%	1%	2%	2%	5%	10%	14%	13%	3%
HER 2 Enriched	0%	3%	1%	0%	0%	0%	1%	0%	1%	2%	0%
Triple Negative Basal Like	0%	6%	5%	0%	0%	0%	1%	1%	7%	2%	0%
Missing Data	1%	9%	0%	3%	0%	1%	3%	1%	6%	2%	0%

Most patients addressed to surgery or Obstetrics-Gynecology Departments of the University Emergency Hospital in Bucharest for painless tumor masses. We also noticed sporadic cases of ulcerated bleeding tumors. A single patient presented for asymptomatic tumor incidental discovered with mammographic screening.

A demographic brief analysis revealed that most patient came from urban areas, with a percentage of 56% for the latter and 44% for rural areas; taking a closer look, we observed that the majority of advanced stages (III and IV) were more frequent in women with urban provenience (10.7% for stage III respective 4.7% for stage IV) in comparison to rural population that addressed to our hospital (1.2% stage III and 0.6% for stage IV).

Regarding clinical data obtained from hospital database, we noticed that the metabolic profile of a majority of analyzed women was impaired, most of them being overweight and obese, only a quarter bearing a normal weight, as the Figure 1 reveals. More than half of studied patients suffered dyslipidemia and experienced cardiovascular complications (essential hypertension and atherosclerosis were most noted). A subgroup also registered a history of gynecologic or endocrine pathology but not enough for a trustable correlation.

Figure 1. BMI of analyzed cases

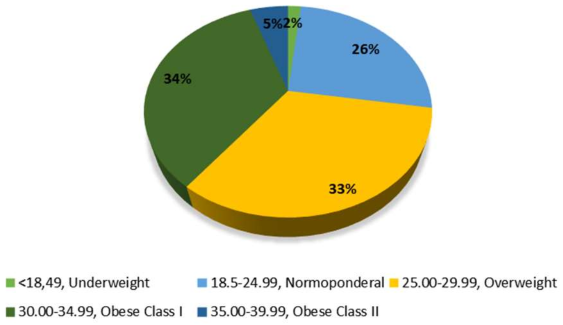

When we put side by side the matric data of different types of investigations (ultrasonography, mammography at CT) with histopathological measurement (macroscopically and microscopically evaluation) we noticed that the highest accuracy in appreciating the dimension of breast lesions is radiologic examination (mammography and CT), followed by ultrasonography which tend to underestimate most of lesions. Considering the main histotypes, the most difficult breast tumor to be precisely measured in conventional imagistic methods is invasive lobular carcinoma, in accordance with its manner of infiltrating the mammary tissue.

With the available data, we observed that invasive breast carcinomas were all scored with BIRADS 5 or 6, a single case of NST G1, luminal A, T1c being underappreciated as 3.

Figure 2. Molecular profile of breast carcinoma according to histotype (%)

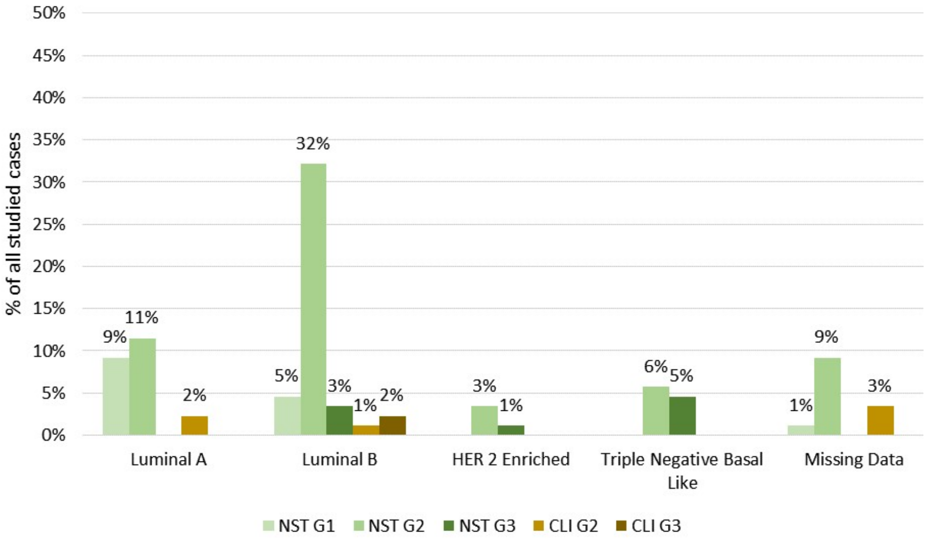

In relation to histologic examination of breast cancer, formalin fixed-paraffin-embedded tissue were studied using both conventional histopathological examination including HE stains and also immunohistochemically methods, classifying them further in the 4 main surrogate-molecular subtypes: luminal A, luminal B, HER2-neu non-luminal and basal-like/triple-negative. For diagnosing the main histologic subtypes, the last WHO guideline criteria were used.

When we evaluated breast cancer histotypes and their molecular pattern, we observed that the most prevalent is luminal B with a pick incidence in NST grade 2, followed by luminal A, also most prevalent for NST G2.

The oncologic stage according to WHO 2014 for HER 2 enriched and triple negative varied between IIB and IV. We did not find any single case in stage I. We also observed that for luminal A category the majority of cancer were in stage I and II. An interesting aspect is registered for luminal B, the most frequent molecular type of our statistic. We can easily observe that it has the highest frequency in most of cases, but not in stage I of disease. A more explicative illustration of the preponderance of tumor stages according to the 7th edition of UICC for TNM classification of malignant tumors, in relation to their molecular profile is highlighted in figure 3.

Figure 3. Molecular profiles of tumor stages related to the molecular profile

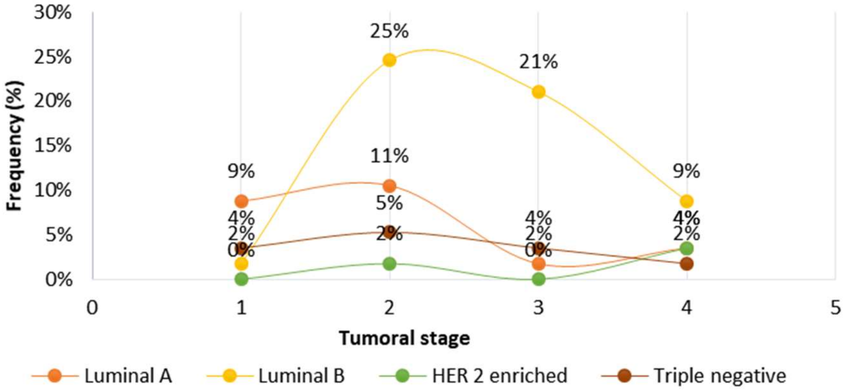

Figure 4. Regression between ER expression and KI 67 in luminal A and luminal B breast carcinomas

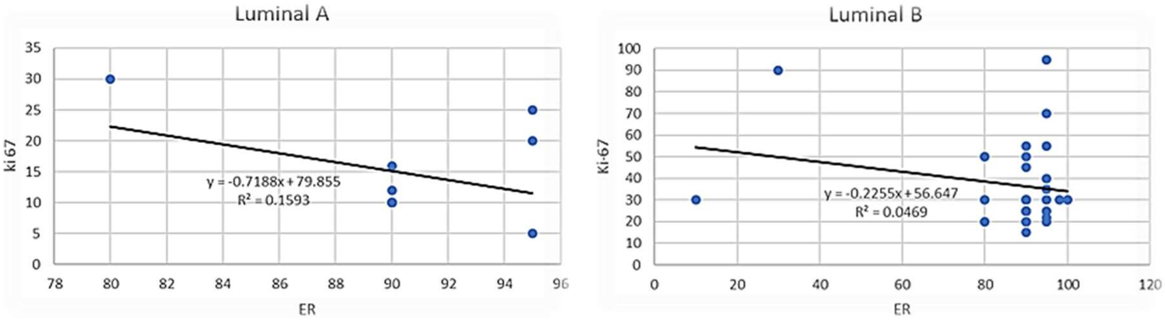

Considering the hormonal expression in the luminal type carcinomas and their proliferation index, we observed the regression between ER expression and the mitotic index evaluated by Ki 67 in luminal molecular profiles. For luminal A there is a better correlation between a higher expression of ER and a lower Ki 67 (R2=0.159) comparing to luminal B (R2=0.046), as in the Figure 4 can be easily seen. The juxtapose of the two types was not only useful for understanding the variation of hormonal variation regarding the proliferation rate, but also necessary considering the limited number of analyzed cases.

In our study, no invasive lobular breast carcinoma was identified among non-luminal carcinomas. We registered more triple negative than HER 2 enriched molecular subtype, and their prevalence were highest in climax women. As expected, their histologic features were more aggressive, with large areas of necrosis, infiltrative pattern of growth, and large numbers of mitoses, none of those being graded as well differentiated.

DISCUSSION

Breast carcinoma is not a single disease, it has a large panel of shapes, clinical manifestations, imagistic and histologic representations. The current WHO guidelines for breast carcinoma counts 37 different coded histotypes, but this classification it is far from being adequate for proper treatment [6]. Tumors with identical clinical and pathological presentations may have different behaviors. Beside the TNM classification and grading method (today the Nottingham histologic score system is universally accepted), the interest for treating in the most efficient and precise way each particular biological form of disease gave birth to the molecular subtype classification. This procedure evaluates the hormonal expression of tumor tissue (ER, PR), presence or absence of HER2 protein and levels of Ki-67.

We started our analysis by looking on the preferred diagnostic procedure, and in some cases, it was similar with the surgical treatment. The method of choice for the majority of invasive breast carcinoma clinically diagnosed is represented by mastectomies with axillary lymph node dissections were. Most of these procedures started with a frozen section for certifying the malignant nature of the lesion. After the frozen section, surgical interventions ended with mastectomy and lymph node dissection. Less than half of analyzed women received a neoadjuvant chemo-radio-therapy after core needle biopsy and came back for completing the surgical treatment.

The concordance of core needle biopsy and surgical excision specimen for diagnosing the histotype and hormonal profile of the tumor was proofed to be safe in large studies [7, 8]. According to ESMO, assessment of primary tumor includes: physical examination, mammography, breast ultrasound for lymph node determination, breast MRI and core biopsy with pathology determination of histotype, grade, ER, PR, HER-2 and Ki67 status. If preoperative systemic therapy is planned, a core needle biopsy is mandatory to ensure a diagnosis of invasive disease and assess biomarkers (recommendation IIIA) [9].

Figure 5. NST HER2 enriched carcinoma a. Numerous atypical mitosis HE 40X; b. perineural invasion, HE 40X; c. Intravascular tumor emboli; d. Lymph node invasion, HE 10X; e. HER2 intensely positive, scored 3+, 20X; HER2 negative, score 0, 20X.; f. Bone metastases with GATA 3 positivity suggesting breast origin, 20X.

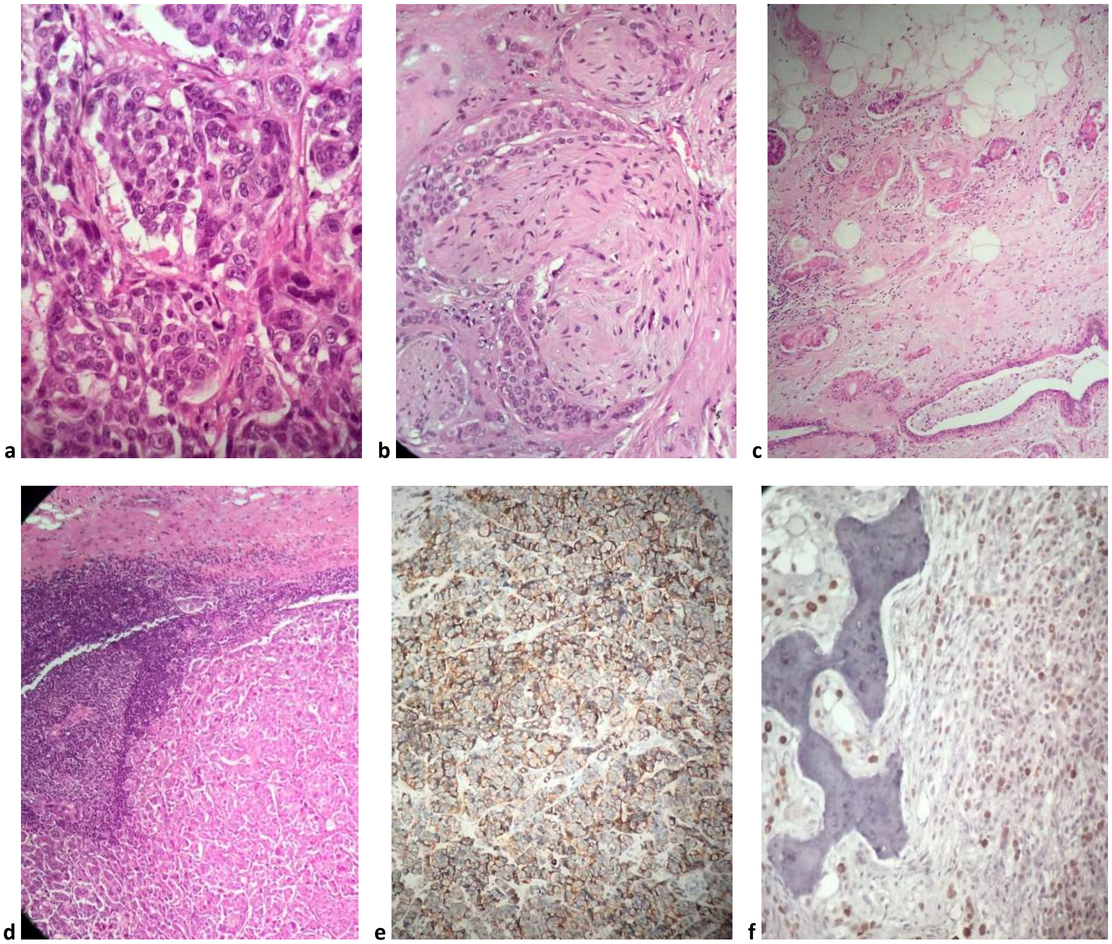

Since it is not yet a standardized method, CNB does not represent a IA indication for non-operative diagnostic. Even if the Royal College of Pathology [10], the WHO guidelines for breast cancer and AJCC 8th edition [5] enclose certain recommendation for reporting the results of a CNB, this practice is controversial regarding the rate of metastasis [11], the reliability of histological grade considering the dimensions of collected fragments [12], the limitation in diagnosing certain subtypes of carcinoma (e.g. mucinous carcinoma) [13], and even rise the problem of "vanishing carcinoma", when tumor cells are present on CNB but absent in the surgical specimen [14];

We focused our attention on the most frequent histotypes of breast tumors, NST and ILC. The rare types of malignancies like papillary carcinoma, medullary carcinoma, Phyllodes tumor or metastasis to the breast are not well represented in this one-year study and for the sake of statistic relevance, we choose not to include them in our calculation. Also, the benign lesions (fibroadenoma, adenomyoepithelioma, abscesses or fibrocystic disease) were not relevant for the heterogeneity of breast carcinoma.

The interesting demographic results that we obtained underlines that the majority of severe stages of disease (stage III and IV) were addressed from urban population. This trend is not observed in other studies made in Australia [15], Africa [16] or even in multiethnic and multiracial population [17], but in our opinion, these unexpected results may appear because of many reasons: probably the most important one is the limited number of cases that were included in our analysis. Other reasons may consider the higher addressability of urban women to private health system, the migration of urban population to the country side or a better surveillance of patients from rural area by their family doctor. On a deeper assessment, it is easily noticed that most of our luminal B cases had a high Ki 67 index (above 20%), only a third of them being HER2 positive or equivoque on IHC. A possible explanation could be the absence of a -ISH method in our department for a precise assessment of HER 2 mutation. Anyway, considering the limited period and number of analyzed cases, for a more accurate distribution of luminal subtypes more studies are needed.

Taking into account hormonal expression, using regressions, we observed that for luminal A molecular subtype the higher the estrogen receptor expression is, the lower the proliferation index is (quantified by Ki 67); in comparison, for luminal B subtype p-value was only 0.0469.

Regarding the age, the highest amount of breast cancers appeared in the 6th decade of life, with a considerable delimitation between pre- and post-menopausal age. Even so, in the 3rd decade we registered only luminal subtypes of cancer (considered to have a better prognosis) but in the 4th decade we registered all the molecular subtypes.

Regarding clinical correlation, we became aware of the impaired weight of our studied group, most patients being overweight, with a medium BMI of 27.73. Also, we noticed that a great proportion of studied women who underwent a diagnostic biopsy and returned after approximately 3 months for surgical treatment gained extra kg, their BMI being augmented. This observation could be an alarm signal for a better nutritional surveillance and psychologic management of oncologic patients, considering that obesity it is an important risk factor for malignancy, comorbidities and mortality [18 - 20].

Unfortunately, our clinical correlation was limited because we did not had access to variables like family and history of menstruation, oral contraceptive use, age of first full-term pregnancy, number of children, duration of lactation or hormonal replacement therapy.

CONCLUSIONS

In our study, primary malignant epithelial tumors of the breast were NST with a moderate grade of differentiation with an age of incidence ranging from 3rd to 8th decade, being most prevalent in the 6th decade. Regarding molecular classification, luminal B was more common than others, with a wide clinical and histologic appearance; it was found in all analyzed histotypes and in all pathologic stages of disease. Because of its predominance, most of severe cases we registered (metastatic breast disease, ulcerated tumors) were also luminal B subtype. We admit the limitations of our study (few cases in a single year in a single hospital with emergency profile) but we are convinced that for a better breast cancer management, keeping in mind the most important aim, a lower mortality and morbidity, a proper national screening program is of an imperative necessity.

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SYSTEMATIC REVIEW

Analysis of risk factors for antipsychotic-resistant schizophrenia in young patients – A retrospective analysis

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Abstract: *Treatment resistant schizophrenia (TRS) is a severely disabling disorder, which decreases dramatically the quality of life and overall functionality, while it increases the rate of hospital admissions and overall healthcare costs. The main objective of this research was to evaluate the risk factors for TRS in a group of patients based on a retrospective analysis. The secondary objective was to design an algorithm for initial evaluation in patients with schizophrenia, in order to detect the candidates at risk for developing TRS. Medical charts and consultation records of all patients aged between 18 and 30, diagnosed with TRS, evaluated during 1-year in our department, were selected for analysis. The most significant risk factors for TRS found in univariate model were younger age at schizophrenia onset, male gender, living in rural areas, co-morbid drug dependence, lower therapeutic adherence, and premorbid personality disorder. Marginally significant were higher Positive and Negative Syndrome Scale (PANSS) scores at previous admissions, higher scores on PANSS negative symptoms sub-scale, and lower educational background. In the multivariate model, TRS was still significantly predicted ($p<0.05$) by younger age at the disease onset, addictive co-morbidity, and lower therapeutic adherence. An algorithm based on these risk factors is suggested, based on (a) structured PANSS evaluation using SCI-PANSS and Informant Questionnaire for PANSS, (b) a scale for the detection of co-morbid drug dependence (i.e. Inventory of Drug Taking Situations, IDTS), (c) an interview for detecting premorbid personality disorders (i.e. Structured Clinical Interview for DSM IV – Axis II Disorders, SCID-II), and (d) Treatment Satisfaction Questionnaire for Medication (TSQM) for therapeutic adherence monitoring. Also, the inclusion of several pharmacogenetic parameters (at least CYP450 2D6 panel for detection of poor/ultrarapid metabolizers) could be useful when establishing an adequate therapeutic management, and may help in decreasing the rate of non-response due to variations in antipsychotics plasma levels.*

Keywords: *antipsychotics, treatment-resistant schizophrenia, pharmacogenetics, therapeutic adherence, co-morbidity*

TREATMENT-RESISTANT SCHIZOPHRENIA IN YOUNG PATIENTS – CURRENT STAGE OF RESEARCH

Young age is an important negative prognosis factor in patients diagnosed with schizophrenia, and individuals aged 26 or less at their first psychotic episode have a higher risk to be treatment-resistant [1]. This phenomenon has significant impact over patients and their caregivers functionality, but also involves higher costs for society, and especially for the healthcare system.

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Treatment resistant schizophrenia (TRS) is defined by the lack of significant clinical improvement despite therapy with two different antipsychotics from at least two different chemical classes (at least one is an atypical agent) in the previous 5 years at recommended doses for at least 2-8 weeks each drug [2]. Other definitions are more inclusive, stating that resistance is observed when the patient has inadequate response to at least two antipsychotic drugs at the maximally tolerated dose within the recommended therapeutic range in trials of at least 6 weeks [3].

Pseudo-resistance may be attributed to a large spectrum of factors, i.e. lack of therapeutic adherence, co-morbid psychiatric and/or somatic disorders, pharmacokinetic interactions, and pharmacodynamic negative impact of concomitant medication. Also, from a pragmatic perspective, cases of partial response or lack of response may be related to changes of generic antipsychotics, i.e. in patients who take different products (although with the same non-proprietary names) during successive months, due to economic or administrative issues. This observation is supported by at least a literature review which confirms clinical deterioration, adverse effects, and changes in pharmacokinetics associated with generic substitution of several psychotropics, antipsychotics included, while generics do not always lead to anticipated monetary savings and raise compliance issues [4].

To make things more complicated, certain pharmacological strategies could produce ambiguous results, i.e. combining two or more antipsychotic agents is a practice observed in more than 40% of patients with schizophrenia [5]. It's very difficult in these cases to differentiate the effect of each drug, and to monitor the clinical evolution of such patients.

A re-evaluation of the diagnosis is granted in all cases of non-response, and various psychological factors that could worsen the patient's overall status should be addressed by the case manager (i.e. housing instability or homelessness, lack of integration in the medical health system, familial conflicts). Integration of these factors in the therapeutic management of schizophrenia as possible factors for decreasing the response rate should be considered good practice.

Currently it is assumed that one third of the patients who has schizophrenia don't respond to an adequate treatment [6]. Even if these patients are switched on clozapine, after two failed trials with different antipsychotics, up to 40% of them will experience only partial remission, and full functional recovery is impossible for more than half of these individuals [7].

Various predictors for poor treatment response were reported, i.e. severity of negative symptoms, or low level of premorbid functioning [8]. Treatment-resistant patients present glutamatergic abnormalities, a lack of dopaminergic abnormalities, higher familial loading, and significant decrease in grey matter compared to responsive patients, which raises the question if a distinct type of schizophrenia could be detected from the very beggining [8]. An analysis of adult patients with TRS (N=8624) included in the Danish National Registry showed that higher rates of treatment-resistance were observed in younger individuals, living in rural or provincial areas, with lower education level, hospitalised over 30 days in the year before first schizophrenia diagnosis, who were in-patients at first schizophrenia diagnosis, with paranoid subtype, comorbid personality disorder, and/or previous suicide attempt, who used multiple psychotropic drugs (antipsychotics, antidepressants, or benzodiazepines) [9].

Pharmacogenetic studies detected associations between genes polymorphisms in pharmacokinetic (i.e. cytochrome P450 1A2 and 2D6 isoenzymes) and pharmacodynamic (i.e. D2, D4, and 5HT2A receptors) factors, and response to antipsychotics [10]. These genes are involved in various stages of processing and action of antipsychotics, from the hepatic metabolism to generation of various adverse events. If all these factors could be defined on large-scale trials, they could improve the efficacy and tolerability of antipsychotic treatment, in a future personalised medicine. Until then, several studies and case reports linked functionally 5HT3 receptors gene single-nucleotide polymorphisms (SNP) to the clozapine responsivity, and CYP1A2*1F genetic polymorphism to the clozapine metabolism [11,12]. Both these observations could explain several cases of TRS, but larger trials are needed to confirm these reports.

A predictive model for therapeutic response in schizophrenia, based on logistic regression analyses, was suggested, and it showed that 76% of the patients carrying combination of four SNPs will have a lower response to atypical antipsychotic monotherapy [13].

Another interesting suggestion is classifying schizophrenia in different subtypes, based on biological markers, clinical staging (illness severity, prognosis, and therapeutic options), and treatment response [14]. Antipsychotic responsive schizophrenia, clozapine responsive schizophrenia, and clozapine resistant schizophrenia are the subtypes suggested by this model [14]. This classification may have important prognostic value and could help clinicians in formulating an adequate therapeutic management. Unfortunately, no good quality data exist for the next step in the treatment of clozapine-resistant patients, although co-prescribing a second atypical antipsychotic, a selective serotonin reuptake inhibitor, lithium or divalproex, glycine or D-cycloserine, and memantine has been investigated with low to moderate success [15].

Table 1. Demographic and disorder-related variables at the evaluation visit

Variables	Values	Observations
Current age	26.1 (range 18-30)	Only young patients were included in the analysis
Age at onset of the disorder	22.9 (range 18-29)	
Gender		
Female	8	53.3%
Male	7	46.7%
Environment		
Rural	7	46.7%
Urban	8	53.3%
Marital status		
Single (including unmarried and divorced)	11	73.3%
Married	4	26.7%
Social status/income		
High	1	6.7%
Medium	4	26.7%
Low	10	66.6%
Independent living	14	This proportion could be due to the addressability of our department
Institutionalized	1	
Number of previous psychotic episodes	0.6 (range 0-3)	Most of the patients were at their second psychotic episode
Substance related disorders present (nicotine dependence included)	11	9%
Personality disorders present	8	53.3%
PANSS overall score	91.8 (range 86-122)	
PANSS-negative Scale	30.5 (range 15-40)	
PANSS-positive Scale	32.6 (range 20-42)	
Length of the current episode (weeks)	12.5 (range 6-30)	

OBJECTIVE AND METHODS

The main objective of this research was to evaluate the risk factors for TRS in a group of patients based on a retrospective analysis.

The secondary objective was to design an algorithm for initial evaluation in patients with schizophrenia, in order to detect the candidates at risk for developing TRS.

Medical charts and consultation records of all patients aged between 18 and 30, diagnosed with TRS, evaluated during 1-year in our department, were selected for analysis. A number of 15 TRS cases were detected from a total of 68 patients diagnosed with schizophrenia. The diagnosis of TRS was based on DSM IV TR criteria and the World Federation of Societies of Biological Psychiatry Guidelines for Schizophrenia criteria [2].

A logistic regression analysis using SPSS 22.0 was performed on 25 demographic, clinical, and therapeutic variables, in a univariate, followed by a multivariate model. No pharmacogenetic variables could be identified in the selected medical documents.

RESULTS

The most significant risk factors for TRS in univariate model were younger onset age ($p<0.01$), male gender ($p<0.01$), living in rural areas ($p<0.05$), co-morbid drug dependence ($p<0.05$), lower therapeutic adherence ($p<0.05$), and premorbid personality disorder ($p<0.05$).

Marginally significant in the same univariate model were higher Positive and Negative Syndrome Scale (PANSS) score at previous admissions ($p=0.056$), higher score on PANSS negative symptoms scale ($p=0.058$), and lower educational background ($p=0.061$).

In the multivariate model, TRS was still significantly predicted ($p<0.05$) by younger age at onset ($p=0.022$), addictive co-morbidity ($p=0.042$), and lower therapeutic adherence ($p=0.048$).

Table 2. Risk factors for TRS in young adults

Factors	Values	
	Spearman's ρ	Linear regression p
Younger age at the current episode onset	0.467	<0.01
Gender – male	0.592	<0.01
Substance-related disorder comorbidity	0.320	<0.05
Living in rural areas	0.367	<0.05
Lower therapeutic adherence	0.322	<0.05
Premorbid personality disorder	0.298	<0.05
PANSS overall score at previous admission	0.155	0.056
PANSS-Negative Scale current score	0.152	0.058
Lower educational background	0.142	0.061

An algorithm based on these risk factors is suggested, based on:

(a) Structured PANSS evaluation based on SCI-PANSS and Informant Questionnaire for PANSS, because the previous PANSS overall scores and current Negative Scale score are correlated with the patients risk for developing TRS;

(b) A scale for the detection of co-morbid drug dependence (i.e. Inventory of Drug Taking Situations, IDTS), because current drug-related disorders should be integrated in the therapeutic management, and it has prognostic value;

(c) An interview for detecting premorbid personality disorders (i.e. Structured Clinical Interview for DSM IV – Axis II Disorders, SCID-II), because of the correlation between the risk for TRS and several premorbid disorders (especially cluster A disorders);

(d) Treatment Satisfaction Questionnaire for Medication (TSQM) for the monitorisation of therapeutic adherence, because a low therapeutic adherence is a major risk factor for developing treatment-resistance

Also, the inclusion of several pharmacogenetic parameters (at least CYP450 2D6 panel for detection of poor/ultrafast metabolizers) could be useful when establishing an adequate therapeutic management, and may help in decreasing the rate of non-response due to variations in antipsychotics plasma levels. This could have important pharmacoeconomic impact, since many antipsychotics undergo hepatic metabolism, not to mention the risk of potential pharmacokinetic interactions between antipsychotics, or between antipsychotics and other co-administered drugs.

CONCLUSIONS

Analysis of risk factors for TRS should be included in an evaluation algorithm for all patients diagnosed with schizophrenia. While some risk factors could not be influenced therapeutically, still others can be addressed, with targeted intervention for co-morbid addictive disorders, personality disorders, negative symptoms, or with techniques for increasing therapeutic adherence.

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Blending between occultism and scientism in Vasile Voiculescu's short stories

Mirela Radu[1]

Abstract: *Vasile Voiculescu (1884-1963), besides being a well-known writer, was also a prominent, much respected physician who practiced between the two world wars. He attended the courses of the Faculty of Letters and Philosophy in Bucharest for a year (1902-1903), but gave up on them in order to attend the Faculty of Medicine. The literary debut took place in 1912 in the journal Convorbiri literare. He published the first volume of lyrical poems in 1916 and in 1918 he was granted the Academy Award for lyrical volume From the Aurochs Country and other poems. In 1935 he became a member of the Academy of Sciences of Romania and in 1941 he received the National Poetry Prize. The following volumes of poems are: Ripeness (1921), Poems with Angels (1927), Destiny (1933), Ascent (1937), Gleams (1939). His work also includes a short story: The Demiurge (1943). Shakespeare's last imaginative sonnets in Vasile Voiculescu's translation (1964), and the novel Zahei-The blind (1966), Sentimental Gymnastics (1972) are published posthumously. The present article is aimed at revealing the world of Voiculescu's short stories.*

Keywords: *medical studies, abyssal psychology, scientism, mystery, obscurity*

Although the most analyzed part of Voiculescu's work seems to be his poetry, he proved his worth in the same manner in storytelling. His creative enthusiasm needed several means of expression as Voiculescu the poet was sometimes haunted by a "spirit of storytelling manifested as a spontaneous eruption." [1]

His inspiration manifested itself as prose also because creative adaptability seems to grow with age: "As the man seemed to «bend under the weight of the years», the creator received new powers, as a sum of his life's and artistic experience by a high level of expression, in a veritable eruption if poetry, in verses and prose." [2] The surprise comes from the chameleonic nature of the author who took on prose with different motives than those in his poetry, coming out as a writer who was: "(…) a perfect master of his own means, not related in any way with the poet." [3] Voiculescu's stories are that much more appealing as they give the sensation of combining some fantasies from the folkloric supernatural, amusing stories, hunting adventures but also "(…) oddities to be told at midnight, parapsychological medical cases etc., which the author attributes a narrative density, emphasizes their equivoque and gives them a superior meaning through the means of literature." [4]

What characterizes the doctrinal physicist's stories are miraculous happenings populated by divine characters

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with an occult composition, “(...) in which «the efficiency of symbols» is not alien to the writer's anthropomorphic manner.” [5] Some of his stories seek to demystify believes: the conviction that the shadow building can lead to death (The shadow, 1935), even to the actualization of some pseudo-scientific procedures of rejuvenation fashionable at the time, among which the method of doctor Sergiu Voronoff for grafting of some glands called by him interstitial, a method that was widely debated in the periodicals of the time [6] (The Demiurge, 1943) [7]. The connection between certain life forms represents the main theme in the physician’s novellas. The influence of medical studies is present due to the fact that Voiculescu combines the folkloric preconceptions with scientific revelations and with: “the hypothesis and conclusions of modern experimental psychology (…). [8] The reason for his leaning towards folkloric heresies was the fact that, being in a permanent search for answers, the literate-physician did find them even in practiced medicine. Unlike other prose writers with folkloric roots, Voiculescu’s characters are fantastic with occult origins. Some critics noticed his short stories astonishing capability of entwining the new with the old, and of “discovering pathways of the archaic in the modern, some depths of existence itself, this causing Voiculescu to write some of his best prose.” [9]

Supernatural, mystery and obscurity were what fascinated him, ticking his interest even before his medical studies in :”(…) heresies, magical symbols, in one word the occult part of existence.” [10] Unavoidably, the permanent dispute in his soul between objectivity and belief can be seen in his work. A proof in this this sense is represented but the fact that in his short stories, the author uses characters that, if not belonging to the medical world, at least belong to the scientific one, meant to shed an accessible perspective of fantastic events to the reader. These characters can be considered an alter-ego of the author, his nature of undressing every fantastic element by finding a clear explanation, or are either introduced in order for the reader to decipher the message. In a world in which the occult loses ground to the prosaic, these characters anchored in the real world have the task of opening doors through which a new reality can be either seen or understood.

The separation of social and personal life is impossible. This can be seen in Voiculescu’s work. The contaminations from one to another: living scientifically but sensing aesthetically can be found in written pieces: “(…) the diagnostician and the therapist can be found, in way or another in writing (…) but the physician-writer relationship can reside in other places too and can be the subject of a study on man, as a professional in medicine but also in prose, who borrows aspects of the life and work of the former.” [11] This combination can only occur by presenting bizarre medical cases, almost always pathological.

To prove this deduction, Florentin Popescu brings the example of the short story The cursed cow (written by the literate-physician in only one day, 27th February 1947) which portraits the morbid case of zoophilia between a spoon maker who satisfies his sexual urges with the cow, Dumana. The short story Dead season (2nd June 1948) from the volume Magical love appeals to psycho-analysis. This time, the character dominated by rationalism- physician Charles and his dog Azor are preyed upon by a mysterious force. Azor mates with a fox and the medic with a peasant. Everything comes back to normal when the mystical element, the fox, is slain. The explanation that the rational man-Charles-tries to attribute to the semi-hypnotic event is insolation and the effect that the moon had on him: “Meaning that his brain was struck by the too powerful light of the moon. Like others get insolation, there are some more sensible to the magnetic waves of the moon.” [12] Some exegetes saw this piece as a case of “ecological Freudiansim” [13], a way of setting loose the sexual restraints. The same as in other stories of Voiculescu, the deeds are described by a narrator which seeks to be objective, doctor X, “… medic and poet in his free time.” [14] This same narrator has he role of arguer, even if he doesn’t acknowledge his ability to psycho-analyze the events “I am not a psychologist and less a Freudian (…) I never sought an explanation for humanism that erupted in him covering the self-imposed artificially to himself.” [15] The writer does not judge, and nor does he incline the scale towards cabalistic or rationalism, he just offers facts left for the reader to judge and decipher. “His interest goes towards a magical underlay in the peasant’s spirituality, which he does not judge or condemn.” [16]

Dramaturgy represents another aspect under which the inner torments of the Voiculescu, the physician-literate, developed on paper. Among his plays are The wanderer, Sentimental Gymnastics and The Demiurge. In Sentimental Gymnastics, a commoner, Ion Ionescu, comes in touch with psychological elements from William James’ doctrine when he receives training, from an ex-colleague – Puiu Moldovian, in repressing affective emotions. The purpose of this training is that of freeing the character from doubts and boosting his self-esteem. If professionally this turn of events is favorable to him, sentimentally it ends in a failure as he becomes too attractive to the women in his town who will fall prey to his charm.

The influence of Expressionism can be seen in the short story On the miracle doorstep (1934) also named dramatic poem in which the issues of religion and fantasy are exposed. Itic Holzfleisch carries the burden of introducing a new religion to mankind. Because of his lack of credibility he is arrested. In prison he hangs himself behind the door where passers could not see him. Because of his supposed disappearance Itic is thought to indeed possess supernatural powers and can become invisible. The miracle though is not fully completed, hence the key in the title – “on the doorstep” – meaning on the edge. Ovid. S. Crohmălniceanu identifies a Verkundigung Drama in this short story. By not respecting any canons, this short story presents itself as an “authentic modern «mystery».” [17] Religious in appearance, the short story could be labeled in the template set by Gândirea, but by shedding light on the main character’s inner feelings, the critic perceives it as expressionistic. The same Ovid. S. Crohmălniceanu considers another two short stories belonging to the same literary current, The shadow and The bear’s girl, both published in 1930 and then included in 1943 into The Earth’s spirit.

The short story The Demiurge (1943) precedes the nightmare that Europe would live three years later: the second worldwide conflagration and the Nazis’ experiments for perfecting the pure race. This time, the main character is yet again a medic: Muşatin. Descendant of an old family he seeks to refresh his family’s genealogy by gene amelioration. In order to do this, he focuses on experiments, at first on animals, and later on human beings. Lucian, his son, does not agree with his father’s conceptions and becomes his victim, having his passion and sexual characteristics removed. The critics saw this short story not only as a S.F. one, but also as a mockery, from a scientific standpoint, of certain theories that would later become a nightmare, thus providing: “a virulent and convincing satire on the dangerous fascist theory of race amelioration and dramatically-beyond value, resistance in the face of critics and posterity- probes the lucid, realistic and healthy position of a physician in the face of some aberration which humanity has lived at a certain time in history.” [18]

To narrow down The Demiurge to either the categorization of a satire or a S.F. short story is a narrow angle of analysis. The short story represents a new phase for the political drama. The utilization of science to the damage of mankind and the certainty that a scientist can replace God make this short story an outpost of Romanian novellas with political connotations, resembling with “The physicists by Durenmatt” [19] in its intrigue. Just like Faust, Muşatin defies morality, makes a pact in which his own son will fall: the physician transplants his son’s genital organs to an elderly person. Other critics claimed to Romanticism the doctor’s ability to fuse the real with the fantastic, by embracing revelry and creation. Belonging also to Romanticism is his approach on national history, as an“(…) altar on which he issued canonicals.” [20]

Voiculescu’s characters take on magical properties, through spells and calling upon magic being able to access a world separated from the perceivable knowledge. This can be seen in the short story In the middle of the wolves (written 2nd May 1947). This time the reasoning main character is a magistrate that collects folkloric elements. To his astonishment, people around him think greatly of him because of his belief of having supernatural powers. The character meets Lupar, the one that used wolves instead of dogs. The influence of the studies attended by the author can be seen in the characterization of Lupar, which resembles the physiognomy of the criminal noted by Cesare Lombroso, Italian physician and criminalist. In order to get closer to this bizarre character, the magistrate admits that he is no stranger to magical practices, as he practiced spiritism. The physician-literate, as Eugen Simion also noted, has the ability to reiterate mythical elements, through refinement, he refreshes them without fatiguing the reader: “V. Voiculescu uses folkloric syncretism which grants him the ability to update the myths without seeming outdated or suggesting their social death and without sociologism and ethnography.” [21]

If it is natural to appreciate the pages which send you towards the abyssal psychology thanks to the knowledge Voiculescu gained in his years of medical study, other critics see those writing that forego scientism and appeal to the fabulous as having a real value: “The storyteller Voiculescu finds his own space in those stories in which he steps in not as a psychologist or a social-moralist but as a character in contingency with the unnatural and supernatural.” [22] These short stories filled up with an electrifying combination of the profane and sacred, a world which the prose writer does not reveal to the reader but rather lets him discover it, make the author a homo religious. [23]

This effort to reveal the intangible needs to be appreciated even more so knowing that behind the author hides a rational nature, a man of science dominated by practicality and who values systems of rational thinking and deductive judgment: “The positivism of the physician and the rationalism as the love of beings and things of the spiritual man impregnated Voiculescu’s stories with the resin that makes objects durable, giving them good clerical fragrance.” [24]

Originating from an urban environment, the characters from Voiculescu’s short stories are townsfolk which fight a battle of regaining the right to dream, opposing the civilized world which estranges them from their fabulous origins, as the society from which they originate alienates the human being “through the abolishing of humanity.” [25] These urbanites fall prey to devouring love, as humanly as possible, and the magic is given off by “beings with a htonic power.” [26] Voiculescu remodels reality enriching the means of sensible knowledge: “Sooner a systematic try of apprehending reality through abundancy of sensations, though the equity of perception.” [27] As any men of science, Voiculescu is inclined towards explaining in a somewhat scientific manner, in a rational way all deeds that appear irrational.” [28] And these explanations are given through the prism of psychoanalysis and psychology.

Treated by some critics as epigrammatic dystopia, Prefrontal Lobo coagulation appears in 1948, meant as a caustic response, a counterweight to the work of the American psychiatrist Benjamin Karpman, entitled Prefrontal Lobes, enemies of humanity. The work references a practice which would end up serving communism through the psychiatric reprogramming of individuals, of creating “new” people and through eugenics. Voiculescu’s short story has the merit of constructing itself in a “revelatory text specifically for Voiculescu’s harsh perspective and for the pioneering that is represented by his work.” [29] Written on the 27th of July 1948, the short story will be printed only later in the journal Transylvania, in its 9th number of the year 1966. Another source of the short story is found in an article signed by Pierre Wertheimer published on the 10th of July 1948 in the French periodical Medical Presses. Voiculescu’s short story is not just a S.F. story, even though many a people identified it as such, but also a ridicule of such deviant practices which were used by the Nazi as well.

The new social order is imposed by the Perfect Permanent Presidium of the Nations for Peace and the newborns are subject to an operations that have as a goal the removal of the prefrontal cerebral lobes, whose physiological function is the adjustment of personality. Initially, this intervention has as subjects, those who belong to certain classes, such as: “the sick, anxious, melancholic, suicidal, perfectionists, schizophrenics, mad men, which often times brought them back to normal.” [30] The goal was that of neutralizing possible revolutionary elements though the “affective sterilization, aiming especially for the nervous centers of unrest and revolt.” For a bigger impact the medic Voiculescu goes through with the description of the inhuman procedure: “But then it was a barbaric procedure. The medic would make two holes in the cranium and then search blindly and cut the roots of the required fibers.” [31] Later they would resort to a less invasive method, automated radio coagulation, which is applied to all people, striving for the numbing of emotions.

To the stupor of all scientists who saw the operation as the perfect way of relieving people from sentiments and emotions, the youth would first present physiological secondary effects such as: “dyspepsia, problems with digestion, lack of appetite, anemia, and especially constipation” followed by reactions that danger the procedure itself: “the disorders took a psychological turn among the youth, the cases of soul sufferings started to stain the once impeccable epithelium of the P.P.P.P.P and the wanderings started to multiply.” [32] People become more and more independent and are surprised by waves of feelings, daring to feel and even love.

The danger proved way greater: people try to be conscious of spirit. Although these manifestations of revolt are harshly punished, scientists try to answer the question if: “not somehow those blasted fibers had awakened some other hidden branch, untouched by the coagulator?” The answer seems to be found in the pineal gland, responsible for sexual impulses and as such it is the next organ which will be annihilated through radio coagulation. In vain, as people continue to be affected by affection among which “dreaming, sloth, glittered with names such as meditation, thinking, the work of inner searching, the inclination toward sadness, jealousy, the fight against competitors for a woman, for a job, all kinds of shortcomings, and most of all the fear for the future, all the metaphysical fears, and the sentimental complications took all kinds of shapes, as never seen before” [33] The search for the source of evil continues and end on the conclusion that the hearth modifies itself physically obtaining a new cerebelous structure because:” (...) it changed not only its aspect, form and weight, but also its histological architecture. It was heavier, redder, more globulous, less muscular, and full of nervous fibers and autonomous ganglions” and more striking is the fact that “in the upper part, on its globe, underneath the exit of the aorta, there were some curly buds that resembled brain buds.” [34] Voiculescu ends his story in a more optimistic way and once again proves the power of his medical studies even in building good quality literature.

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ORIGINAL ARTICLES

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Research for the development of logistics planning information support in health protection against biological agents

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Abstract: *Making medical countermeasures requires documentation to assess vulnerabilities, threats and operational risks and bioterrorism through the collection and processing of specific multidisciplinary data in human medicine, veterinary, phytosanitary, the environment, defense and national security, etc. Based on retrospective data and late information (medical intelligence), we can estimate the prospective risk to public health, respectively for troops and civilian population in a given area, the area of operations.*

The biological military or bioterrorist attack, whether overtly or masqueraded as human or animal epidemics can cause a major biological crisis for troops and the civilian population, for domestic and wild animals, for crops or wild plants or for the environment, which remains contaminated.

Therefore, it is important to constantly monitor the situation of infectious diseases in the area of responsibility (national territory, operations theaters) to assess vulnerability, threat and bioterrorist risk but also for making medical anti-bioterrorist countermeasures, which can only be effective by genuine cooperation between all specialized structures of the Ministry of Defense, other departments and the civil society.

Keywords: *biological attack, bioterrorism, medical countermeasures, logistics support, medical treatment*

INTRODUCTION

Medical protection logistics planning against biological warfare agents (ABR) is based on interdisciplinary studies and specific calculations.

The complexity of this approach involves processing a database of medical, pharmaceutical and logistics that can only be operated in real time by using a suitable IT support. It consists in hardware (IBM compatible standard configuration computer), software (Windows based NATO specific program), specialized personnel (NATO computer operators, specialists in military medicine, logistics, CBRN defense, etc.) and properly equipped and secure space.

Scenarios that simulate hypothetical situations reported in the territory, will be tested by constructive simulation as functional experimental models of "war games ".[1]

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EXPERIMENTAL MODELS

Making the experimental model of the information system for constructive simulation for medical protection against biological weapons and bio-terrorism and testing it as a military medical exercise demonstrated and validated the scientific data, using modeling and constructive simulation, together with UNAp/CISM, the current Center for War Games and doctrinal Experiments (CJRED) of the General Staff. [2] It was awarded a large database that can be exploited in different complementary approaches. Of all bacteria, Bacillus anthracis has been chosen for being used in the constructive simulation experiment, it is known as the biologically bacterial agent most used for biological attacks. [3]

Medical countermeasures must be known and individualized for each situation and appropriately applied, with appropriate forces and means. This requires an adequate and efficient logistic support because the scale of medical needs (medicines, sanitary materials, reagents, accommodation, transport, etc.) exponentially increases during an epidemic. As a result, at any level, health-pharmaceutical materials are rapidly becoming inadequate. The unintended consequence is that we cannot prevent illnesses or treat patients, so the affected communities are no longer capable of fighting and working and there is a risk of over mortality. Therefore, the medical protection against biological weapons requires a proper logistic planning that is also based on statistical analysis of some relevant information for this purpose. The exploitation of such data requires a dedicated computer system, with suitable software, trained personnel, quick and secure media and communications, etc. Because the computer system for the surveillance of infectious diseases caused by combat biological agents and by bioterrorism to be reliable, it must be implemented in stages, with feedback in the form of custom scenarios for biological attack or epidemic outbreak.

Achieving this pilot experiment in the Romanian Army, demonstrated the usefulness of extending simulation tests on other categories of warfare and on specific logistics for biological crisis. The system used was JCATS – Joint Conflict and Tactical Simulation. The information was delivered in the form of screen-shots that required image processing, computer analysis and interpretation. [5]

The data was processed and a computer media has been made for logistics planning of medical protection against biological warfare and bioterrorism agents through statistical analysis of the resulting data bank. To estimate the number of contaminants analysis were carried out on the three simulated hypothesis: without sensors, with military sensors and alarm on short (in barracks) and with military sensors and alarm on short distance (in barracks) together with a sensors system of the local Inspectorate for Emergency Situations (ISU) and alarm in the city. We have obtained information that was collated for each simulation, in numerical tables and graphs illustrating the impact such an undesirable event can have. [6]

The obtained data allowed the dynamic evaluation (an estimate every 5 minutes) on the effects of a biological attack with anthrax on cities with different number of inhabitants and military forces.

The post exercise analysis showed that in terms of exposed population, where a relatively small number of people, the presence of the sensor is useful, the number of people being affected (civilian and military) decreased after they triggered the alarm.

In a similar situation, in the case of larger cities it has been observed that activation of military sensors does not cause diminishing the number of affected civilians, but decreases the number of infected soldiers.

The comparative analysis of the post-biological attack, for the soldiers of the sea shows that there are a huge number of people affected in the absence of any alarm sensor (over 50%) as their protection or travel ability is restricted to the soldiers on land. Also, the percentage of affected soldiers who do not benefit from the ship protection is higher (70%), if they go out on the deck.

The major differences recorded between the high percentage of affected civilians and that of the military, leading to the idea that it is useful and necessary for the civilian population to be at least more informed about how they should act when triggering an alarm of any kind. [7]

Following statistical analysis of the database resulting from constructive simulation system experimentation resulted in an acquisition, storage, retrieval and information-processing algorithm as an interactive database. [8]

SANITARY LOGISTICS

The statistical analysis conduct to information somehow surprising by the extremely high number of people affected, at least from the civilian population, which is not trained to respond to any alarm. Regarding the military, which is a segment of the relatively "trained" population compared to civilians, we found that the number of those affected drastically decreases, especially when special sensors for detection exist. The analysis of relevant simulation for the purpose of the experiment showed that a possible attack with anthrax spores, even if it is not repeatable, it could result in a significant number of contaminated, many of which will develop the disease, and some will die. The evolution of the epidemic will depend on the capacity of the health system (military and civilian) to promptly and adequately act with the necessary forces and means and with the appropriate logistic support, depending on the epidemiological situation of the troops and the civilian population. [8]

This means that all exposed persons should be medically supervised: rapid laboratory analyzes for different areas/neighborhoods, nasal/throat exuding for identifying B. anthracis.

The contaminated must be prophylactic treated, also with antibiotics according to the therapeutic scheme Guide for 60 days and under medical supervision. Patients should be isolated and treated for 60 days. For those with severe forms (pulmonary anthrax) intensive oxygen therapy must be also applied, etc. [9, 10, 11]

The deceased must be disinfected and handed over to and Funeral Services (the civilians) or the Logistics department (military) for transport and burial, in accordance with epidemic provisions. Incineration is contraindicated due to the large number of corpses. Repatriation is not allowed because of the contagion risk. [12]

Overall, the medical forces and means are overburdened and there is an urgent need for specific additional procurement for a 7 week period.

For such need, specific measures are necessary because sanitary-pharmaceutical materials are rapidly becoming inadequate and the only way to overcome the situation involves cooperation with all own and allied military forces as well as civil-military cooperation (CIMIC).

CONCLUSION

- Medical countermeasures against biological or bioterrorist attack are complex and require specialized forces and means, in very large quantities and in real time.
- By medical intelligence the real situation must be known and the short, medium and long term development must be assessed in order to correctly estimate the forces and means necessary for the medical countermeasures.
- By appropriate application of the logistics assurance principles and CIMIC cooperation the specific logistical support can be achieved, necessary to carry out the medical countermeasures for the prevention of diseases and treating patients.
- The logistical support for medical countermeasures against biological or bioterrorist attack must be coherent, efficient and fast.

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ORIGINAL ARTICLES

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Modern methods to differentiate benign thyroid nodules from malignant ones

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Abstract: *Objective: The purpose of this study is to compare the capsular structure appearing in follicular adenoma and papillary thyroid carcinoma to differentiate the benign and malignant thyroid nodules.*

Methods: Second Harmonic Generation (SHG) Microscopy was used to image collagen distribution in the capsules of several types of nodules. The tissue fragments were formalin-fixed, paraffin-embedded but without H&E staining, with 4-5 microns thick sections. From the same sample, two consecutive sections were made. The first was colored with H&E and capsule images were acquired with an optical microscope. The second was not colored with H&E. On the uncolored sample, type I collagen from the capsule was visualized by SHG microscopy. In addition, a parameter was used to study the orientation of collagen fibers, obtained from the Fast Fourier Transform (FFT) analysis of 2D-images.

Results: SHG microscopy images were acquired to assess the collagen organization of tumoral capsular thyroid nodules previously diagnosed as benign or malignant by conventional H&E staining. Different degree of collagen fibers orientation was observed from the two kinds of capsules and quantified using FFT analysis.

Conclusion: These above described microscopy method can be used to distinguish between benign and malignant thyroid nodules, based on different degree of the capsular collagen fibers orientation.

Keywords*: follicular adenoma, papillary thyroid carcinoma, benign and malignant thyroid nodules, capsular collagen fibers*

INTRODUCTION

The clinical and imagistic diagnosis of malignancy must be confirmed in pathology departments.

Although histopathology and cytopathology are the main tools for diagnosing cancer, there are some difficult cases that pathologists may encounter during routine diagnosis procedures, where the differentiation between a benign tumor proliferations from a malignant one is very difficult to achieve. In such situations complementary examination procedures are required (eg. serial sections from formalin-fixed, paraffin-embedded tissue blocks, mounted on glass slides and usual stained with H&E, immunohistochemistry, molecular studies, electron microscopy, etc.).

Another method which can be used in thyroid pathology for diagnostic purposes is Second Harmonic Generation (SHG) Microscopy.

SHG microscopy is a label-free laser scanning

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microscopy technique that exploits a nonlinear optical effect – the generation of the second harmonic in samples illuminated by an intense laser field [1, 2]. SHG can occur only from materials that do not have a center of symmetry, thus analyzing absolute molecular structure in organized organic environments, such as collagen fibrils, microtubules and the myosin chain of muscle cells. SHG microscopy has been proven to be a useful tool in biomedical tissue imaging [2] because it can be used for imaging endogenous structures like type I collagen [1] as well as for the characterization of collagen organization [3-5] and has been recognized as a promising approach for the diagnostics of different cancer types [6-8], by detecting collagen structure modifications [7].

The thyroid gland is wrapped in a thin fibrous capsule, from which originate numerous fibrous septae that penetrate the thyroid parenchyma and divide it into incomplete lobules (the so-called thyromeres). Macroscopically, the thyroid capsule seems complete, but it is incomplete from the microscopic point of view, as it adheres tightly to adjacent structures (laryngeal thyroid cartilage and trachea) [9].

Nodular enlargement of the thyroid gland comprises those situations in which one or more nodules are present. This presence of nodular enlargement is the most common in the case of non-toxic nodules, also known as adenomatous goiter or colloidal goiter.

Polinodular goiter with different-sized follicles can be a clinical manifestation of a wide range of different pathologies and not the expression of a single disease [10].

The usual classification of neoplasms of follicular epithelial cells are in benign (adenomas) and malignant (carcinomas) tumor proliferation. The benign ones are called follicular adenomas [11], which are encapsulated, non-invasive tumors showing evidence of thyroid follicular cell proliferation without the specific nuclear characteristics of thyroid papillary carcinoma [12].

Papillary Thyroid Carcinoma (PTC) is a malignant epithelial tumor, usually invasive, showing evidence of abnormal proliferation of thyroid follicular cells with characteristic nuclear changes: irregular contours and nuclear pseudoinclusions, nuclear membrane irregularities, and chromatin marginalization. For a correct diagnosis of the classical PTC, the papillary fibrovascular axes, the invasive character or the characteristic cytological features are required. [12].

The encapsulated variant of PTC is a type of proliferation with typical cytological and architectural changes, entirely surrounded by a thin, fibrous capsule that may be intact or infiltrated only focally by the tumor. If the capsule is invaded by the proliferation of malignant tumors, we are dealing with an aggressive form of PTC.

The encapsulated variant represents about 10% of all cases of PTC and has an excellent prognosis. Differential diagnosis is made with follicular adenoma with papillary hyperplasia [12].

Over the years, diagnostic criteria for follicular and papillary thyroid carcinomas have changed, but the two names have been retained. There have been several variants of papillary carcinoma for which the primary diagnostic criterion is based on nuclear features [11].

Some thyroid encapsulated neoplasms may present diagnostic difficulties due to the fact that only nuclear changes are sufficient to justify and diagnose PTC but there is the uncertainty about the capsular or vascular invasion. Encapsulated follicular tumors lacking the PTC nuclear characteristics are difficult to differentiate. For example, follicular carcinoma (a malignant tumor) can be distinguished from follicular adenoma (a benign tumor) only by demonstrating invasive character in the nodule capsule or capsular vessels [12].

We started from the idea that SHG can be used to observe three-dimensional structures of a tissue sample without the need for staining.

In this way, this method helps us in the correct diagnosis of encapsulated tumor entities that require complementary examinations from the pathologist or in order to determine a possible capsular structural change before the onset of malignant transformation of a benign encapsulated tumor.

OBJECTIVE

The purpose of this study was to use the collagen structure in the nodule capsule to obtain information on the follicular adenoma capsule and encapsulated papillary thyroid carcinoma (PTC) capsule for differentiating between benign and malignant thyroid nodules. The study was based on optical microscopy images and SHG microscopy, with the latter used for the visualization of type I collagen. We also show that the FFT analysis of the SHG images can provide information about the collagen fiber orientation.

MATERIALS AND METHODS

A batch composed of encapsulated-PTC and follicular adenoma tissue from 24 sections tissue samples (12 for Encapsulated-PTC capsules and 12 for follicular adenoma capsules) was used for this study. Sections of 4-5 µm were made from each paraffin tissue block. They were mounted on glass blades and stained with Hematoxylin and Eosin (H&E) and visualized with a light field microscope (Leica DM3000). The tissue architecture of the normal, benign and malignant regions of interest and the capsule surrounding the nodules have been identified. Unstained pairs of the H&E stained tissue sections were used for SHG imaging for collagen organization assessment.

SHG imaging was performed using a Leica TCS-SP confocal laser scanning microscope modified for nonlinear optical imaging [13]. We have used a mode-locked Ti:Sapphire laser operating at 780 nm and with ~150 fs pulses. A 40X, 0.75 numerical aperture objective was used to focus the laser beam on the sample, while the SHG signals were collected in the forward direction through a condenser lens. The average power reaching the sample plane was kept less than 15 mW and no photodamage was observed on the samples.

SHG images were used to estimate the collagen fiber orientation by FFT analysis using ImageJ software. For an image containing aligned fibers, we expect an FFT image with higher values along the direction orthogonal to the direction of the fibers and with an elliptic intensity plot. On the other hand, for an image with randomly oriented fibers, the FFT image intensity plot should be close to a circle. The FFT power spectra images were first binarized and then fitted with an ellipse. A collagen orientation index was calculated based on the length of the minor (S) and major (L) axes of the ellipse: $N = 1 - S/L$ [14]. Collagen orientation can thus be represented by an index ranging from 0 (random fibers) to 1 (aligned fibers).

RESULTS

Optical microscopy images of H & E stained sections do not give us information about the ultrastructure of the studied tumoral nodules capsule. The capsule appears as a fibrous structure surrounding the tumoral nodule. It looks identical to the follicular adenoma nodule and to the Encapsulated-PTC nodule. So this optic method is limited in terms of capsule study.

On the other side, the SHG helps to study the ultrastructural changes of the capsule. SHG highlights type I collagen in the capsule structure. So we can see if there are qualitative changes of this type of collagen from the two kinds of capsules. In addition, using FFT analysis of SHG images we can bring precious differentiation information of the two types of capsules: the follicular adenoma nodule or the Encapsulated-PTC nodule. We have noticed that in the Encapsulated-PTC nodule capsule there appears to be a collagen fiber organization as if they were trying to isolate malignant epithelial proliferation.

We acquired images with Second Harmonic Generation Microscopy which were used to assess the collagen orientation of tumoral capsular thyroid nodules previously diagnosed as benign or malignant by conventional H&E staining.

We compute the FFT spectra for the SHG images acquired for the considered pathologies. One can see that the average collagen orientation (N) computed from the FFT spectrum is higher for the follicular adenoma capsule ($N = 0.69$) than for the encapsulated-PTC capsule ($N = 0.4$). The FFT spectra were calculated for the entire images. We consider that if the spectra are computed for smaller regions of interest (ROIs), a distribution of values can be obtained, depending on the degree of alignment for the collagen fibers in the considered ROI. Averaging over a larger area, which is also the case of the whole area analysis, it results in a lower N. Even though N was calculated on whole areas, the obtained results show a difference in collagen organization between the two capsules.

Figure 1. Follicular adenoma capsule

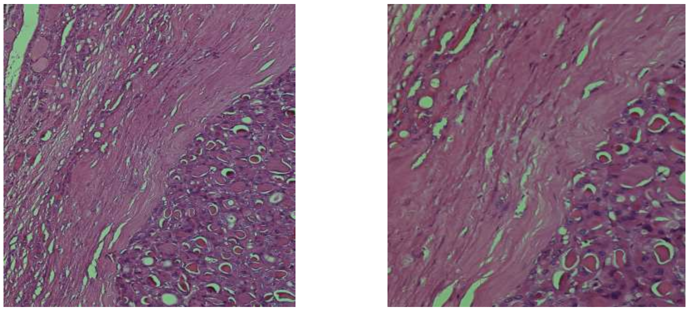

a). H&E 100x

b). H&E 200x

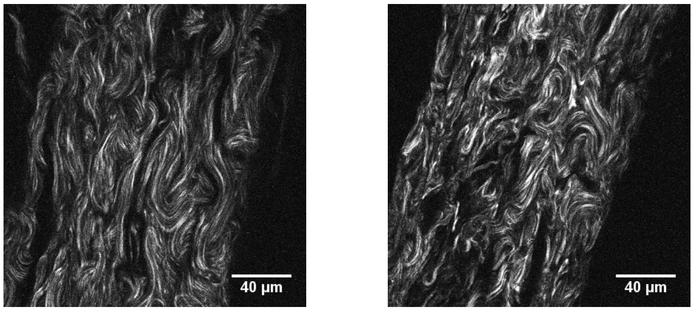

c). SHG image 1

d). SHG image 2

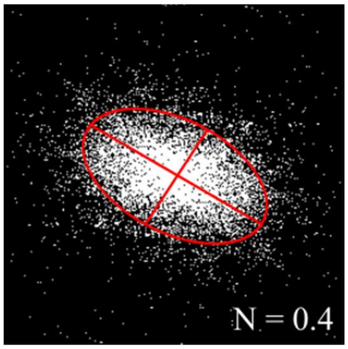

e). The FFT power spectra images - Follicular Adenoma Capsule

Figure 2. Encapsulated-PTC capsule

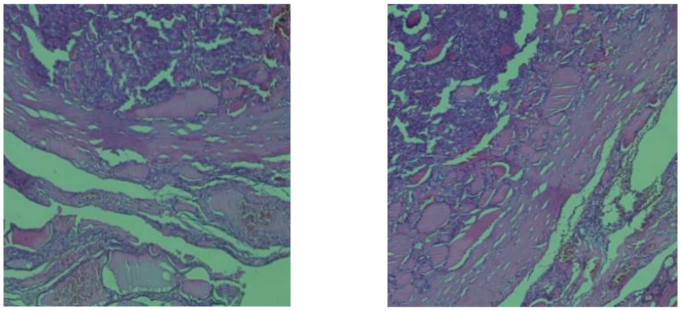

a). H&E 100x

b). H&E 200x

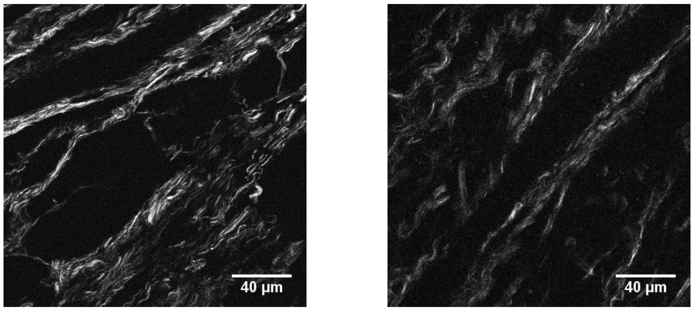

c). SHG image 1 d). SHG image 2

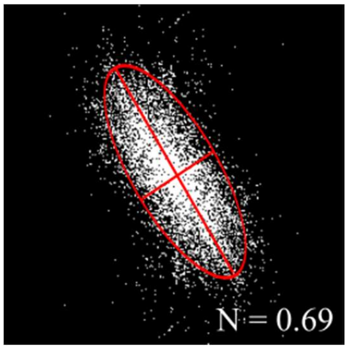

e). The FFT power spectra images – Encapsulated-PTC Capsule

CONCLUSIONS

We found differences in the orientation of collagen fibers between the capsules surrounding benign nodules (follicular adenoma) and malignant ones (Encapsulated-PTC) by using SHG microscopy images and FFT analysis of these images.

Our research indicates that different collagen structures can be detected in the malignant nodules capsule (PTC) compared to the benign nodule capsule and the thyroid capsule.

Higher orientation was detected for the malignant capsule but that does not mean the collagen fibers are organized or that the malignant tissue organizes the fibers. We believe it is rather a defense mechanism that goes from the level of the thyroid adjacent stroma of tumoral nodules.

Future plans

The present study deals with the differential diagnosis between follicular adenoma versus encapsulated – PTC, since both are clear cases, thus making it easier to prove the validity of the theory regarding the reorganizing of the collagen fibers within the two types of nodules, as proven with SHG Microscopy. Having completed that, the research may advance to analyzing less obvious situation:

1. Differential diagnostic between:
- Follicular adenoma versus follicular carcinoma;
- "Non-invasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) versus encapsulated papillary thyroid carcinoma with capsular invasion;
- Adenomatous nodule vs follicular adenoma.

2. Early detection of papillary carcinomas developed on follicular adenomas.

The SHG images can be developed as markers for use in clinical applications – a complementary method to early detection of suspected malignant nodules.

These above described microscopy methods help us distinguish between benign or malignant thyroid nodules, based on the capsular collagen organization.

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Association of rare disease and acute intoxication – case report

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Abstract*: Benzodiazepine overdose has various clinical manifestation, mainly regarding the central nervous system (CNS), cardiac and respiratory side effects, but rarely results in significant morbidity and mortality. Acute benzodiazepine poisoning results in dizziness, ataxia, nystagmus, dysarthria, hypoxia, hypothermia, bradycardia, hypotension, apnea, pulmonary aspiration, respiratory depression, coma, cardiopulmonary arrest and death. Anyway, deep nonresponsive coma should be investigated for additional etiologies. On the other hand, human transmissible prion disease has a fatal outcome with no specific treatment.*

Keywords: *prion disease, benzodiazepine intoxication, opisthotonus*

Human transmissible prion diseases are neurodegenerative disorders characterized by infectious pathogens accumulation with a long incubation period and devastating outcomes. A prion is defined as a small infectious agent lacking of nucleic acid, but containing protein [1]. It has an important feature regarding resistance to a number of decontamination procedures including processes affecting nucleic acids such as hydrolysis [2]. Up to present, human pathology recognize five related entities: Creutzfeldt-Jakob disease (CJD), Kuru, variant Creutzfeldt-Jakob disease (vCJD also known as new variant CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) [3].

Prionopathies result from abnormal isoforms accumulation in the brain that leads to neuron apoptosis and important brain damage. Infective prion is a product resulting from defective folding of the normal prion, having the gene located on the short arm of the chromosome 20 [4]. Unfortunately, the prognosis is very poor in these patients, despite all efforts and sustained interventions.

Benzodiazepines are commonly used against various disorders such as insomnia and anxiety. They also are preferred agents for drug overdose, although their acute toxicity is low. Oral benzodiazepine intoxication rarely results in

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significant morbidity and mortality. The body develops tolerance against such effects in the course of time and most common side effects are sedation and ataxia. Anxiolytic effects is rarely implicated in tolerance development. Acute benzodiazepine poisoning may result in dizziness, ataxia, nystagmus, dysarthria, hypoxia, hypothermia, hypotension, bradycardia, apnea, pulmonary aspiration, respiratory depression, coma, cardiopulmonary arrest and death.

CASE PRESENTATION

We present the case of a young white female, aged 26, admitted in our Toxicology – Intensive Care Unit of Clinical Emergency Hospital Bucharest from Bucharest, the capital of Romania. She was referred to us after deliberate ingestion of 200 mg benzodiazepine associated with 500 mg metoprolol. Ten months before this event the patient experienced complaints such as mood disorders, memory loss, irritability, inability every day social behavior and insomnia; initially, the condition was assessed as depression but no certain treatment was recommended. We couldn’t find any history of visual hallucinations or tremor. Interview emphasized multiple study visits in the United Kingdom and occasionally intravenous abuse substances dependence. Any other past medical data were unremarkable. The first medical examination revealed a conscious patient, mild agitated, stable vital signs, with unusual hyperosmia and visual impairment. Toxicology assessment was positive for benzodiazepine in the urine, detected with fluorescence polarization immunoassay method; toxicological screening was negative for other drugs. She had a rapid unfavorable evolution the following hours after admission experiencing degenerative progression of psychomotor and cognitive functions, lack of hand-eye and alternating hand coordination, left hand tremor and left lower extremity weakness and in the end, ten hours after ingestion, she developed impressive opisthotonus and deep coma that required mechanical ventilation. The evolution was unexplained by her toxicological status and occurred unexpectedly. Brain tomography was in normal ranges (Figure 1). She was performed lumbar puncture and the cerebral fluid features included clear aspect, normotensive, with glucose and electrolyte levels within normal limits and no type of cell detection. Regarding highly suspicion of tetanus, due to her occasionally intravenous drug administration, the infectious disease evaluation indicated anti-tetanus serum therapy, but clinical evolution wasn’t improving. After 48 hours of nonresponsive evolution a new brain CT scan identified massive cerebral edema. Cranial MRI showed marked cerebral atrophy in frontoparietal areas, DWI hyperintensities (cortical ribboning) in basal ganglia bilaterally and diffusion restriction in these areas suggesting progressive multifocal leukoencephalopathy, whereas putamen and caudate nucleus were free of signal intensity (Figure 3). Considering these aspect, we performed PCR for the detection of JC virus which was negative. A prionic disease was highly suspected and 14-3-3 protein immunoassay on CSF was positive. The patient died after 2 months in Critical Care Unit due to multiple dysfunctions, with the diagnostic of Creutzfeldt-Jakob disease.

DISCUSSION

As a part of the prionic disease family, CJD evolution is characterized by rapid evolution and neurological deterioration. The majority of cases are represented by sporadic human prion diseases, while 1-2% of the remaining are infectious forms acquired from an established source with the prion disease and 5-15% is represented by the autosomal dominant type [1]. The presented case exhibits most of the sporadic CJD clinical features with rapid cognitive decline and mioclonus; MRI was an indispensable tool for differential diagnosis considering that DWI hyperintensities in the putamen, caudate and cortex have a high specificity for sporadic prion disease [6]. However, the short dramatic course of the disease and detection of protein 14-3-3 in CSF adds value to diagnostic criteria. The literature shows that the mean age of onset is around 65 years of age with a very short median survival time around four months [7], but we report an extreme age (eg. 26 years old).

CONCLUSIONS

The case presented in this paper was meant to describe the clinical progression of a rapid evolving encephalopathy in a patient admitted for benzodiazepine ingestion. It also underlines the importance of keeping an open mind when dealing with unexpected and unusual evolution of acute intoxication.

Figure 1. The first CT scan within normal ranges

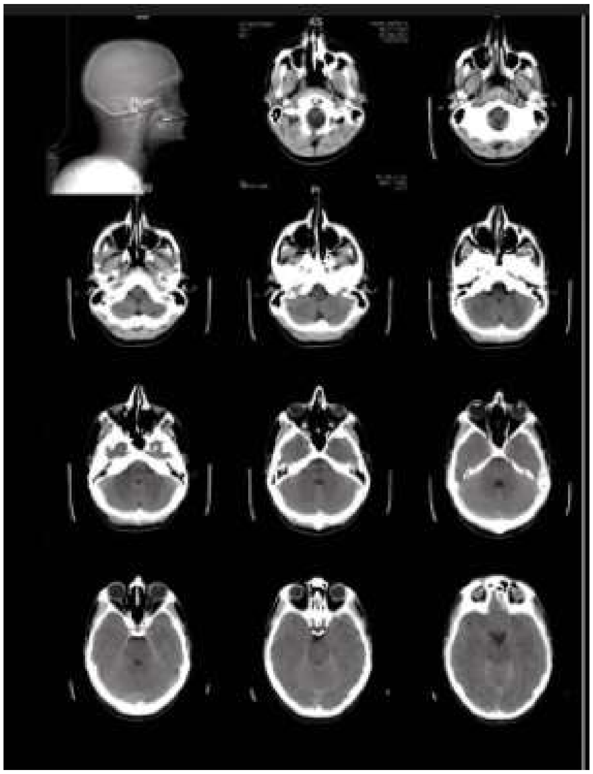

Figure 2. Cranial MRI showing cerebral atrophy, DWI hyperintensities (cortical ribboning) in basal ganglia bilaterally and diffusion restriction in these areas suggesting progressive multifocal leukoencephalopathy, whereas putamen and caudate nucleus were free of signal intensity

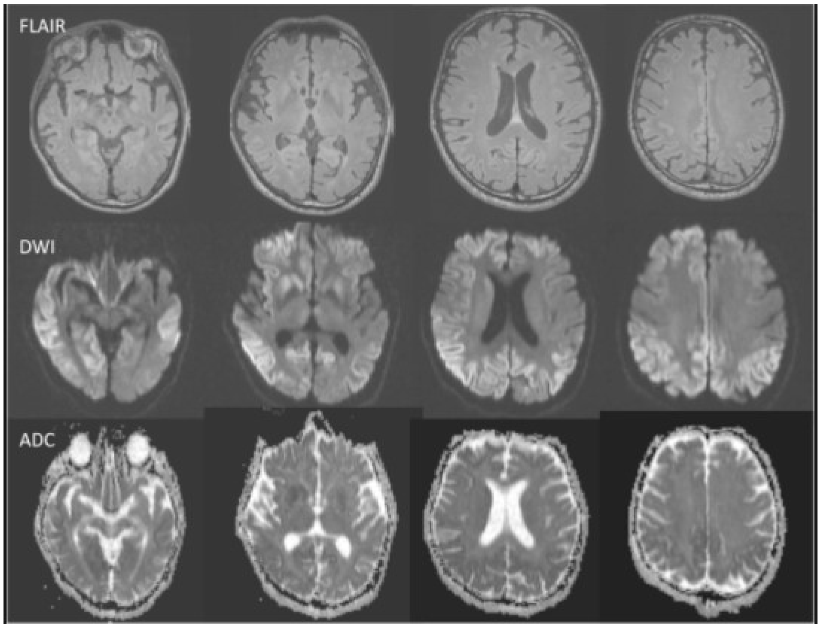

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Clinical aspects in implant fixed prosthodontics oral rehabilitation. Case report

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Abstract*: Benzodiazepine overdose has various clinical manifestation, mainly regarding the central nervous system (CNS), cardiac and respiratory side effects, but rarely results in significant morbidity and mortality. Acute benzodiazepine poisoning results in dizziness, ataxia, nystagmus, dysarthria, hypoxia, hypothermia, bradycardia, hypotension, apnea, pulmonary aspiration, respiratory depression, coma, cardiopulmonary arrest and death. Anyway, deep nonresponsive coma should be investigated for additional etiologies. On the other hand, human transmissible prion disease has a fatal outcome with no specific treatment.*

Keywords: *implants, osseous integration, staging, fixed prosthesis*

INTRODUCTION

Each year endoosseous dental implants gain greater acceptance among clinicians and patients. This has come about for several reasons, including excellent success rates published in long-term studies, improvements in fixture and abutment designs, more predictable surgical placement techniques, and increased consumer desire to have tooth replacement. Regardless of the implant system, the placement and functional success of endo osseous implants is greater than 90%) [1]. Fixed prosthodontic on implants is the most desirable treatment for the patients among the treatments on implants. When transforming a partially edentulous patient in a complete edentulous one, the transition difficult period can be avoided by keeping some teeth for fixed provisional, until the osseous integration of the implants is accomplished and the final prosthetic can be done [3]. In this regard, the importance of restoring patients with functionality during the implant healing period has grown in recent decades [7].

This paper presents a staged protocol of the patient's treatment that made the case easier to manage and offered a better comfort in the transition period.

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CASE REPORT

Material and method

T.S., a 66 years old female patient, with a general medical history of chronical C hepatitis, presented with a mucocell in the sinus cavity and a T,L-T maxillary edentation and L-T mandibular edentation, with inadequate fixed restorations, both in upper and lower jaw. An accurate and complex clinical examinations wad performed, including the prosthodontic, implant – surgical assessment, panoramic radiograph and computed tomograms for both jaws. The intermaxillary relationships were carefully examined and the OVD was recorded (5.7 cm).

A comprehensive treatment plan was proposed and decided, following the conclusions from the diagnosis findings. Thus, all the remaining teeth were in a poor dental status and needed to be extract, but in a staged approach, as in figure 1.

Figure 1: Initial dental status

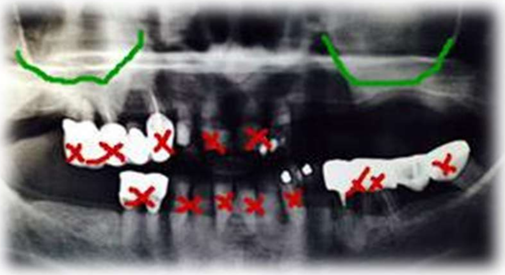

The 1st step was the surgical one on the maxillary arch. So, after CT, Rx measurements and evaluation of bone volume and density related to prosthetic planning dental implants position was decided the placement of 3 implants (Superline, Dentium) (2.2/2.4/2.6). Prior to this was performed the left sinus mucocel evacuation. Meantime was performed also the internal sinus lifting with mineral bone grafting (Osteon, Dentium).

The 2nd surgical step. After the healing period (2 months), right sinus lifting was performed with same materials and were placed 3 implants (1.4/1.5/1.6).

By the time of implants osseous integration, the 11 and 21 were kept in order to provide patient's minimal aesthetic and functional comfort. Provisionals, immediate loading – nor fixed or removable couldn't be used due to bilaterally sinus lifting with bone grafting materials and the bone density. (Figure 2)

Figure 2: The clinical situation before the 3rd step

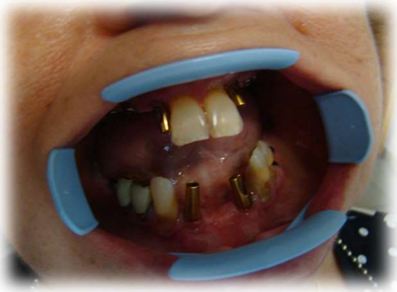

Meanwhile the 6 upper jaw implants osseous integration, the 3rd surgical step, consisted in the placement, on the lower jaw, of 2 implants (4.6/4.7) and 1 implant in the upper jaw (1.2), all of them prosthetic driven. After the evaluation of the occlusal vertical dimension (OVD) that we recorded and preserved, the vertical space was assessed as well as mandibular implants positioning in order to obtain correct occlusal relationships.

In the 4th surgical step the insertion of 2 frontal mandibular implants (3.2/4.2) and 2 left lateral mandibular implants (3.4/3.6) was performed. 3.3/ 3.4/4.3/4.4/4.5 for fixed provisional. The extracted teeth were: 1.2/1.3/1.5/1.6/2.2 initially and just before the implants uncovering, 1.1 and 2.1, in the upper jaw and 3.1/3.2/3.5/4.1/4.2/4.6/4.8 in the lower jaw and 3.3/3.4/4.3/4.4/4.5 were used for provisionals. (Figure 3)

Figure 3: Panoramic radiograph after the implant's placement

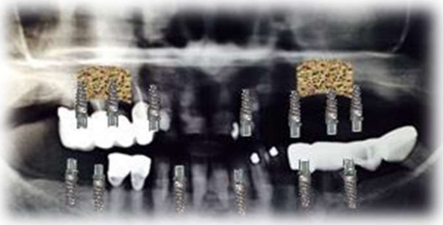

RESULTS AND DISCUSSIONS

Due to the case complexity, because of the surgical aspects (13 implants insertion), bilaterally internal sinus lifting with bone grafting and the evacuation of a mucocell, related to the prosthodontic ones, meaning the complete fixed bimaxillary rehabilitation, have made the treatment to last 1,5 years until the final prosthetic rehabilitation that we will describe next.

Due to the staged surgical approach, there was no possibility to split the final prosthetic work in more segments, nor in maxilla or mandible, so we produced 2 full – arch restorations on implants.

The idea of the final prosthetic treatment was to rehabilitate simultaneously the 2 jaws in order to split optimally the vertical prosthetic space. So, after all implant's uncovering and the last teeth extraction the healing abutments were applied and after 10 days the impression was taken. Monopren transfer (Kettehbach) was used as impression material, in an open individual tray technique, using transfer abutments.

The work casts were obtained with the analogue implants in their position. On this casts, occlusion rims were made for the intermaxillary relationship registration. All the steps were performed in order to establish accurately the occlusal and intermaxillary relationships: the vestibular curve of the upper occlusion rim was esthetical and phonetic established.The occlusal plane's level and direction were established using the usual methods and also the patient s youth photo, that provided a lot of useful information, such as the visibility of the 6 frontals. The initial correct OVD was kept and the 2 rims were fixed in the correct CR position. The information about the facial midline, the smile line and canine lines, together with other information regarding the teeth colour, size, form, arrangement and positioning were transmitted to the laboratory.

After the intermaxillary relationship registration, the dental lab produced the metal frame of the prosthetic work. The trial of the metallic part was done related to the axial and cervical marginal fit. With the patient s agreement, the last esthetical decision were taken, regarding the colour and form of the artificial teeth, to provide the dental lab all the information for the ceramic application. In the dental lab Vita ceramic layers were applied.

The try-in of the prosthetic work was done, aesthetic, phonetic and occlusal relationships and intermaxillary, horizontally and vertical. Also, occlusal adjustments were performed. Then the 2 fixed prostheses were glazed and polished.

The final work was provisional cemented and then definitively (Figure 4).

Figure 4: Final result

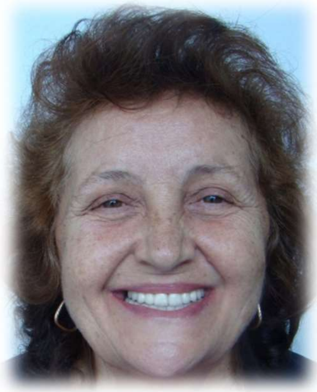

Figure 5: A 3 year follow up

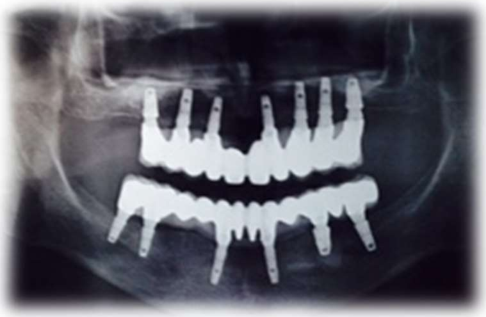

Figure 6: Final occlusal relationship

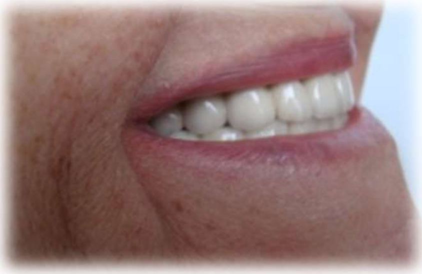

The follow-up was done, after 6 months and then after 3 years, and the results can be observed on the panoramic radiograph and indicates a very good situation.

CONCLUSIONS AND CLINICAL IMPLICATIONS

For many patients, as for the patient s case that we described previously, the transition between the dentate state and the completely edentulous state, with removable provisional is difficult to accept. That ▯s why we choose a staged approach for our case, with strategic extractions and implants applied in 4 surgical different phases. The provision prosthetic was a fixed one, the major advantage of this treatment protocol. The treatment planning in such complex cases represents a challenge for the implantologist and prosthodontist and for the patient and our presented protocol facilitates the transition from a destroyed dentition to a full-arch reconstruction.

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Occipital radionecrosis and neuroplasticity in astrocytoma

Carmen A. Sirbu[1,2], Octavian M. Sirbu[3], Alexandra E. Bastian[4,5]

Abstract*: Background: Late-delayed complications after radiotherapy (RT) results from an imbalance between cell lesions and the protective capacities of the CNS. Temozolomide (TMZ) after RT may represent a potent radiosensitizing regimen. Although radionecrosis (RN) of the anterior visual pathway has been documented, in our opinion this is the first report of reversible visual field loss associated with occipital lobe radionecrosis.*

Case observation: We report a patient who suffered a left lateral homonymous hemianopia one year after radiochemotherapy (RCT) for an infiltrative low grade fibrillary astrocytoma. The visual field deficit was completely reversible after one month. Visual field defects have been described after the use of conventional external beam therapy for lesions near the anterior visual pathway. The cortex is relatively spared after RT.

Conclusions: Given the new scientific data, we suppose that neuroplasticity may play a role in the reversibility of visual field deficit. At this time there is no proven treatment of radionecrosis. Homonymous hemianopia caused by occipital lesions are attributable to vascular disease and tumors, but take into account radiation effects. The risk of neurogenic visual loss must be factored into the decision to irradiate the whole brain.

Keywords: *fibrillary astrocytoma, temozolomide, radiation necrosis, visual recovery, neuroplasticity*

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INTRODUCTION

Radiation therapy is an integral part of modern therapy in gliomas. Early or delayed side effects are taken into account in determination of the therapeutic algorithm of each patient. The combination of radiotherapy and chemotherapy further increases the vulnerability to side effects. In this context, we present the case of a young woman diagnosed with fibrillary astrocytoma. One year after combined radiotherapy and chemotherapy with Temozolomide, right occipital lobe radionecrosis with impaired vision appeared.

Although such cases cited in literature are irreversible, in this case, both visually campimetric deficit and occipital brain lesion were fully reversible.

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MATERIAL AND METHODS

A 21 years old, right-handed Caucasian female with mild idiopathic thrombocytopenia presented to the hospital with right sensory-motor hemiparesis. MRI revealed a subcortical low grade glioma in left rolandic region, extended to basal frontal and ascending parietal white matter regions, invading the dominant arcuate fasciculus, confirmed by brain biopsy.

Given the infiltrative character of the tumor and its deep location, a surgical intervention is considered risky (the tumor invades left pyramidal fibers, thalamocortical fibers and superior longitudinal fascicle), especially without perioperative functional cortico-subcortical cartography procedures. Our patient undergoes 64Gy (photon teleradiation) radiotherapy for 6 weeks, followed by chemotherapy with Temozolomide (TMZ), 250 mg/day, 5 days/month for 6 months. Conventional MRI control reveals stationary aspect of the tumor (figure 1).

Figure 1

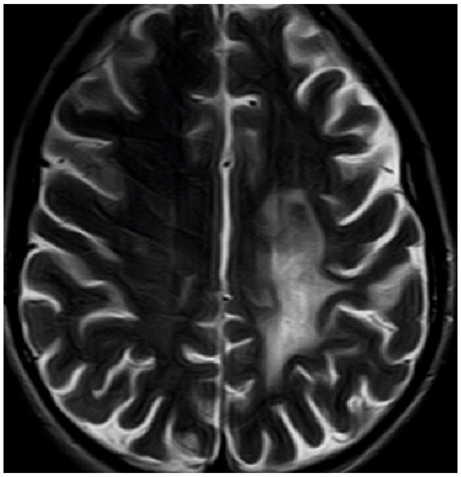

One year after chemotherapy, the patient insidiously develops left lateral homonymous hemianopia. A new conventional brain MRI shows a new 26/10 mm, irregular, non-homogenous, imprecisely defined right occipital lesion with hyperintense T2/Flair and isointense T1 signals, without contrast media enhancement. Fluorodeoxiglucose (FDG) PET-CT scan allows the differential diagnosis between tumor growth or radionecrosis and it reveals significantly decreased FDG uptake in the right occipital region, including the visual cortex (figure 2).

Figure 2

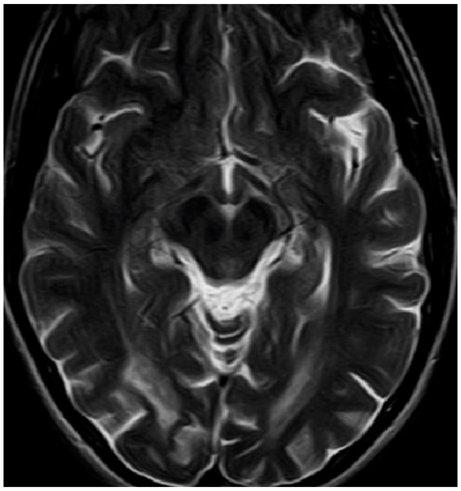

RESULTS

We conclude that the visual field deficit is the result of cerebral radionecrosis. The patient started a short period of corticoid treatment, with no improvements. One month from the first appearance of the hemianopia, it completely remitted and occipital right lesion were not visible.

Worsening deficit in patients with cerebral tumors may suggest either tumor progression or its recurrence [1]. Imagistic methods reveal edema and enhanced lesions near the tumor area. On the other hand, radiotherapy may determine effects after months or years and these are called late-delayed reactions [2].

Figure 3

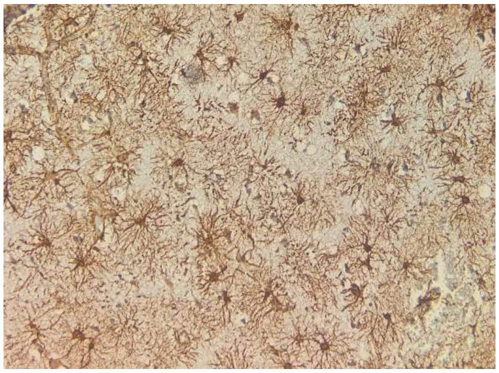

One of them is radionecrosis that occurs by vascular changes and ischemia, increase of capillary permeability and cerebral edema. Due to capillary permeability disturbances, adverse reactions of chemotherapy appear earlier and are more severe (figures 3, 4). Necrosis can be a result of radio and chemotherapy combination [3].

Figure 4

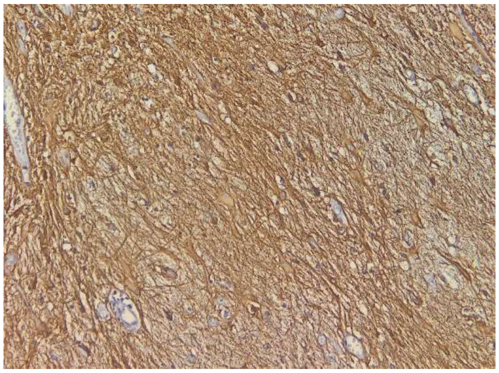

Temozolomide (TMZ) amplify the answer to radiations through DNA modifications and apoptosis [4]. TMZ combined with radiotherapy, enhances blood-brain barrier damage, leading to increased concentrations of therapeutic agents [5].

DISCUSSIONS

Few things are known about radionecrosis because it is difficult to differentiate from the tumor relapse with imaging techniques [6] and because the rate of surgical secondary interventions and brain autopsies are low in these patients [7].

In a study of 426 patients presenting glioma, Ruben and co. prove that the average time for the first appearance of necrosis is 11.6 months [8]. Also, adjuvant chemotherapy rises to over 4 times the cerebral necrosis risk. Peterson and co. reported a 2.5% incidence of radionecrosis, 8-31 months post-radiation, in a study including 200 patients with radiated and chemo-treated cerebral tumors [9]. So, as many have already demonstrated, chemotherapy has an amplifying effect in cerebral radionecrosis development. The incidence of radionecrosis is slightly raised in anaplastic oligodendroglial tumors than anaplastic astrocytomas and this is an argument for glial theory and its role in radionecrosis occurrence.

Things are even more complicated because the edema associated to this pseudoprogression is as symptomatic as the tumor actually progresses [10]. Conventional MRI (T2 and T1 with contrast) is limited in differentiating between tumor relapse and RT induced necrosis. Unfortunately, in many Gad positive lesions that occur after combined treatment, there are necrosis lesions and viable tumor cells and molecular magnetic resonance imaging of endogenous proteins and peptides are not for current use [11]. Diffusion MRI measures the mobility of water from tissues into the cells. After treatment, tumor cells are dehydrated and have a high apparent diffusion coefficient (ADC). Decreases of ADC indicate high cellularity [12]. Magnetic Resonance Spectroscopy detects tumor metabolites from tissues (choline, creatinine, lactate, lipids, N-acetyl aspartate) and is more sensible [13]. Dimensional spectroscopy has superior sensitivity indicating a relapse based on raised choline/NAA and choline/creatinine ratios [14].

18-Fluorodeoxyglucose PET scan is useful for differentiating between radionecrosis and recurrence and is 81-86% sensitive and 40-94% specific [15]. Still, false positive results have been encountered in non-malignant inflammatory processes, electric epileptic seizures and healing processes up to 3 months post-surgery. Even though, radionecrosis activates healing mechanisms and raises the glucose metabolism. This is why MRS is superior. There are some mechanisms that can explain postradiation necrosis: DNA modifications, coagulation factors imbalance with thrombosis facilitation [16]. The main mechanism of radionecrosis is associated with oligodendroglia injury or vascular endothelial damage (figures 5, 6).

Figure 5

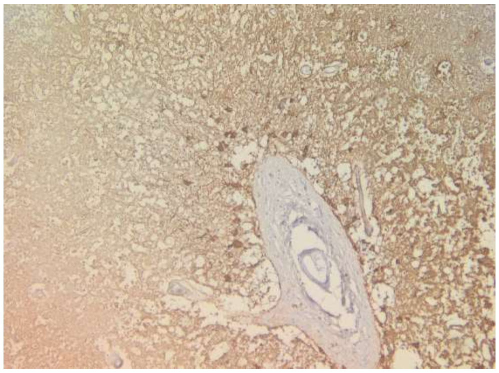

As a result, white matter tissue is often affected than gray matter tissue. Cytotoxic edema and tissue necrosis are the consequence of imbalance between tissue plasminogen activator and urokinase plasminogen activator.

But an autoimmune mechanism may be also involved.

Figure 6

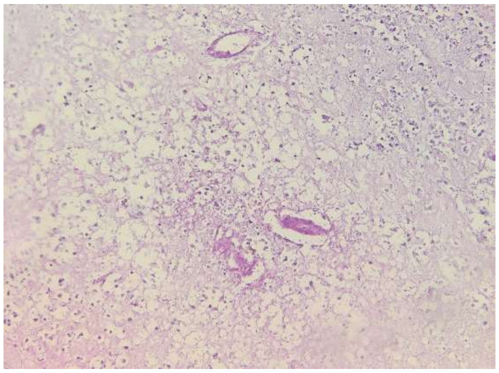

Anterior visual pathways necrosis was well documented, especially in pituitary tumors, craniopharyngioma, parasellar meningiomas or optic nerve tumors [17]. In generally, visual field defects occur after one or more years after the radiotherapy stopped. Corticosteroids, surgery, bevacizumab and Hyperbaric Oxygen Therapy (HBO) are all possible good treatment options for symptomatic RN. The treatment goal is to provide the patient with resolution of neurologic symptoms, with the least toxicity and invasiveness [18]. Corticosteroid therapy was used to diminish the cerebral edema and cytokine release after radiation. Hyperbaric oxygen is considered to be useful in raising the tissular oxygen supply and for initiating tissue healing.

Diffuse, infiltrative, low grade gliomas (LGG) represent a clinical, histopathological and molecular distinct group with a very controversial management [19]. LLG patients may survive up to 20 years from the moment they were diagnosed, but there is the risk of tumor evolution into higher grades, eventually leading to neurological deficits and death [20]. Diffuse astrocytomas include the fibrillary, gemistocytic and protoplasmic types [21]. The fibrillary one is the most widespread and it presents a homogenous cellular population and moderate uncharacteristic nuclear modifications. Mitosis rate in grade II OMS astrocytomas is very small [22]. Clinically, the patient presents epileptic seizures, focal deficits occurred after many years and intracranial high pressure, especially in the subtentorial and intraventricular location. Conventional MRI is useful for guiding the biopsy and the resection, for radiotherapy and treatment monitoring. The usual method is MRI without contrast media, but when we add contrast it reveals changes in tumor grade transformation, better. In LGG spectroscopy shows high choline (high turn-over) and low NAA, reflecting neuronal loss [23]. Even though, similar results can be seen in non-tumor lesions. MRI is used rather for guiding the biopsy in an intense active area than for monitoring the evolution. 18 Fluorodeoxyglucose PET scan has a limited value as long as LGG indicates a low caption comparative to normal cortex [24]. The role of PET-FDG is, however, demonstrated in differentiating radionecrosis from tumor recurrence. More frequently used are [11C] methionine-PET scans given the fact that methionine is captured by tumor cells showing their proliferation process, thus giving a better contrast. PET-MET is also used for differentiating LGG from non-tumor lesions [25].

Neuroplasticity defines the ability to regain function after a neuronal lesion due to neuronal structures that take over the function of the damaged area and to nervous tissue reorganization (that is based on formation of new connections between neurons and/or new cell synthesis). The theory imposed in 1920 by S. Ramon y Cajal (which stated that once the development stopped, growth and regeneration of the axon and dendrites irrevocably stop) is torn down discredited by present scientific data [26]. Altman and co. showed the ability of some brain areas (such as hippocampus and olfactory bulb) to generate new neurons and they called this process neurogenesis [27]. Later, proves appeared that shown this process is influenced by factors like age, stress, physical condition [28].

Multipotent stem cells divide periodically in our brain giving birth to other stem cells or to adult cells. The newly formed stem cells move into areas that allow maturation. Approximatively 50% of them will die without reaching maturity. The others will become glial cells or neurons depending on the necessities of the brain area where they migrated. Fred H. Gage, emphasized in his review, that the necessary time for a stem cell to become functional and adapted to brain is about a month [29]. In this way we could explain full recover of the patient with left lateral homonymous hemianopia a month after the debut. A series of growth neuronal factors play an important role in keeping stem cells alive, mature and functional. Regaining cortical functions can be somehow explained by reorganization of cortical areas [30]. This process takes also place in the areas near the lesion or near the contralateral homologous cortex. Visual cortex reorganization after focal lesions implies plasticity mechanisms accompanied by excitation/ inhibition imbalances and changes in dendritic and axonal divisions [31]. Studies demonstrated a decrease in GABA inhibition and a growth of glutamate answer mediated by NMDA receptors in adjacent areas with the afflicted visual cortex [32].

CONCLUSIONS

Combined radiation and chemotherapy rises many times the cerebral necrosis risk, which may occur months or years later. Patient management should use specific and sensitive diagnostic tests. The differential diagnosis between tumor recurrence and radionecrosis is very important. Based on this differentiation, therapeutic attitude is different. For diagnosis certainty, tumor molecular biology tests are very useful, especially that current imaging techniques still cannot make a clear distinction. Low-grade gliomas should be better supervised by complete excision of the tumor, where the situation allows. Other therapeutic interventions, like chemo and radiotherapy, especially in the early years of evolution, should be used with caution, given their unpleasant effects. Radionecrosis should be treated promptly with corticosteroids, surgery, hyperbaric oxygen or bevacizumab.

Also, we need a plan for neurorehabilitation that assist in the neuroplasticity. All these compete to increase life expectancy and quality of life.

Conflict of interests

The authors declare that they have no conflict of interests.

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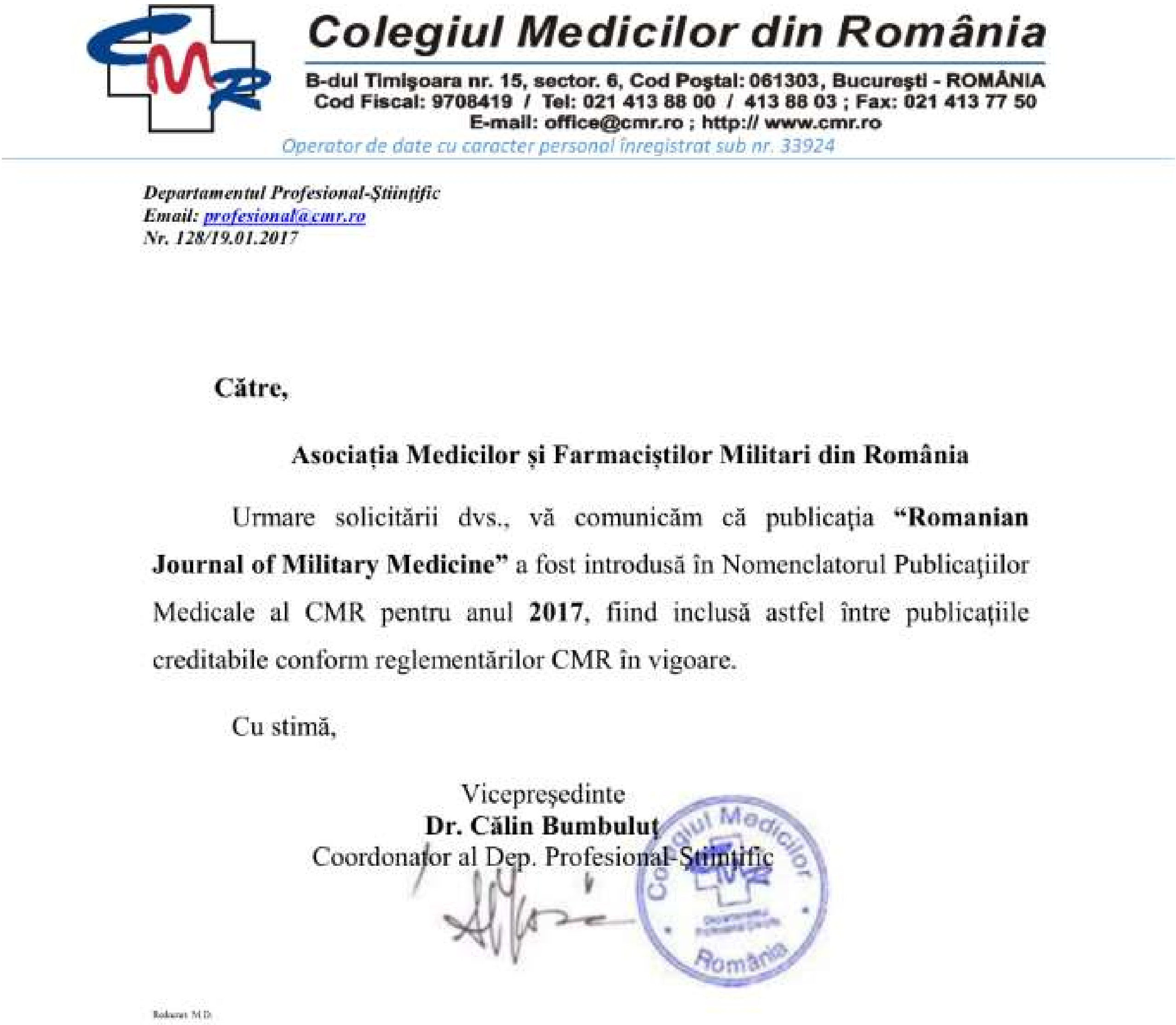

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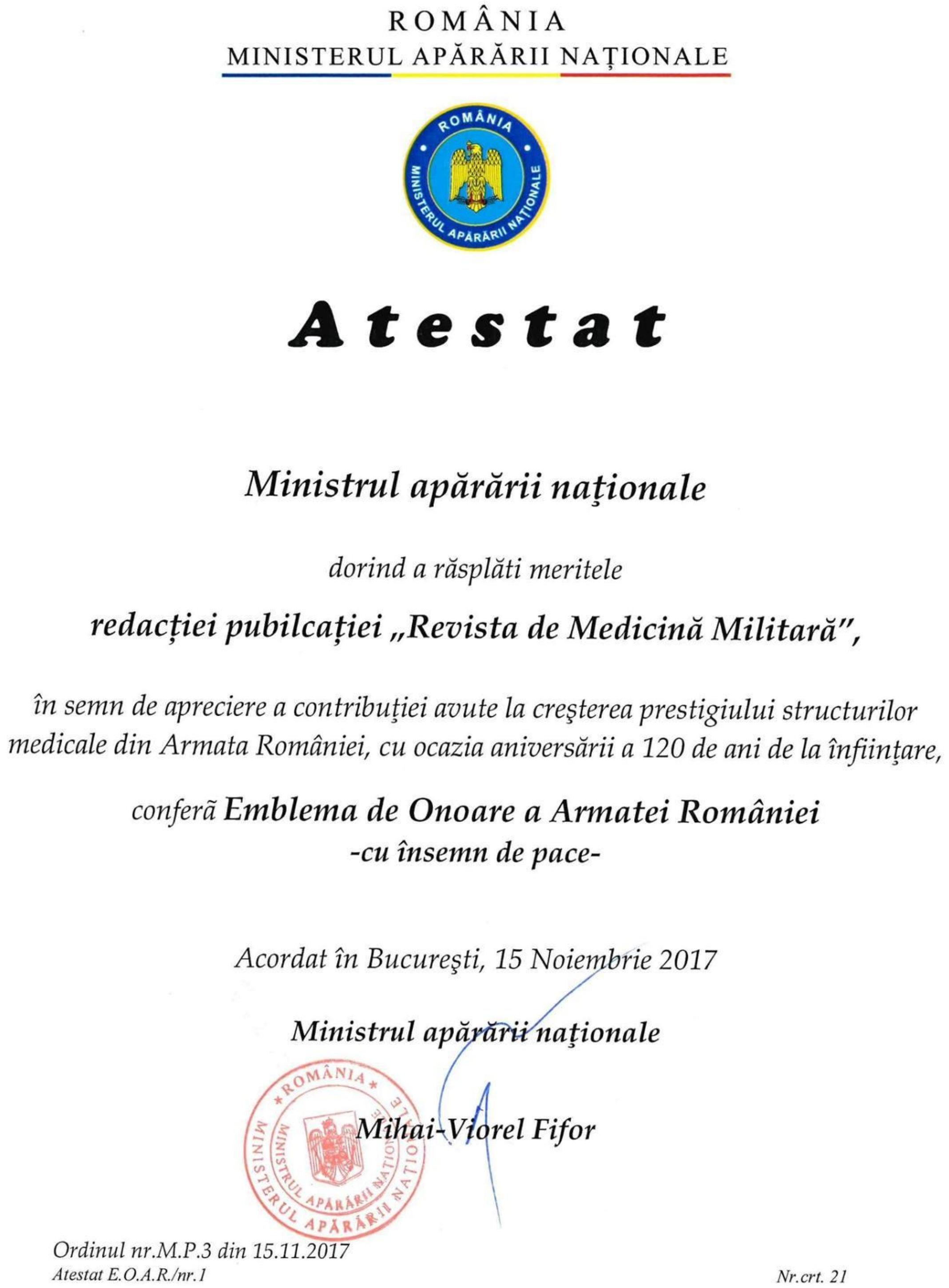

ROMÂNIA
MINISTERUL APĂRĂRII NAȚIONALE

Atestat

Ministrul apărării naționale

dorind a răsplăti meritele

redacției publcației „Revista de Medicină Militară",

în semn de apreciere a contribuției avute la creşterea prestigiului structurilor medicale din Armata României, cu ocazia aniversării a 120 de ani de la înființare,

conferă ***Emblema de Onoare a Armatei României***
-cu însemn de pace-

Acordat în Bucureşti, 15 Noiembrie 2017

Ministrul apărării naționale

Mihai-Viorel Fifor

Ordinul nr.M.P.3 din 15.11.2017
Atestat E.O.A.R./nr.1

Nr.crt. 21

EDITORIAL

ROMÂNIA
1918-2018 | SĂRBĂTORIM ÎMPREUNĂ

Brief notes about the Great War, Romanian military doctors and the Great Union

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Motto: "L'Histoire, c'est la rencontré d'une volonté et d'une évènement" – Charles de Gaulle, 1890-1970

This short notice, largely iatrohistoric, appear in the 100th year since the Great Union out of the desire to know more about what has happened.

In Romania, there was a lot of talk about the First World War. The Germans remember this war under the name of "der Erste Weltkrieg worde von 1914 bis 1918 in Europa, in Naken Osten, in Africa, Ostasien and auf dez ozeanen gefurt". Obviously this first conflagration was the army of "Zweiter Weltkrieg"! The British preferred the denomination of the "European War" or, more correctly, they named it "the Great War".

It appears that this name is slowly but surely penetrating our literature, following "World War I" which, referring to the title of this editorial only makes us Romanians remind that we have also had a Small Union (1859), followed by the Great Union of 1918.

It must be remembered that the Romanian literature between 1948-1989 wrote about the Great War in an abbreviated manner, because of two reasons: the Eastern neighbors had "turned history" – things did not happen as planned and the contribution and participation of the Romanian Royalty to the final victory was extremely important but also very embarrassing that it had to be silenced.

The Great War began in Sarajevo in 1914 and ended at Versailles in 1949 in the Mirror Hall, with multiple European implications...

Then, at Versailles in the Grand Trianon Palace, a treaty was signed on June 4, 1920 between 16 allied states (including Romania) and the successor state of the Austro-Hungarian Empire. At the beginning of the war, Romania, a "very small country", in the form of "L", had 137,000 km^2 and a population of 7.2 million inhabitants, and after Trianon, it was reunited and became „The Great Romania", with an area of 295,000 km2 and a nation of 18 million people. It is certainly why, in the collective mentality of a neighboring nation, that this situation is perceived as unacceptable even after 100 years!

After the assassination of the crown Prince of Austria-Hungary – Franz Ferdinand on June 28, 1914 the actors, both big and small, began to enter the stage: Central Powers – Germany, Austria-Hungary, Turkey, Bulgaria and Antanta or the Triple Alliance, England, Russia followed by Italy, Romania, USA...

The Kingdom of Romania had passed through a recent, unforgettable experience for the army and especially for the military doctors. In 1913, during the Second Balkan War, the Romanian troops that had easily entered the northern half of Bulgaria lost 1,600 lives due to the cholera epidemic – a fearful "enemy".

Evidently, the accusations have risen, obviously committees for dysfunctions research have been named, obviously the responsible military doctors – Constantin Papilian (1852-1917) and Senator G-ral (r) Prof. Dr. Athanase Demosthen (1846-1925) informed I.C. Bratianu – Prime Minister and Minister of War about all this.

Certainly, the two years of neutrality have chosen better and more efficient organizational lines, as "stage sanitation, semi-hospital evacuation, evacuation hospitals, auxiliary hospitals, and infirmary station ".

Just after two years of "armed expectancy" war began, for Romania as well as for other nations, how all wars start "suddenly and unprepared!". We do not insist in geostrategic and political-economic details. We present only the result and brief considerations about military doctors truly involved in the "Perpetual Drama of War".

What Prof. Dr. Vasile Sârbu, a Templar Knight of Romanian Surgery and Iatrohistory, presented with his known erudition a few months ago, is perfectly true: "In this war, 400 military doctors died out of 2,800 participants." 2,400 health workers have also died out of 14,000 participants, as well as 14 pharmacists and 20 students of the Military Health Institute. These numbers do not say much. If we compare them with the other "weapons", we will be surprised to learn that this group of people is on the 2nd place after the infantry, which made King Ferdinand to offer them the right to wear the "combatant weapon" badge.

"This was the result!..."

Among the personalities, the first name to be quoted with gratitude and piety is that of Prof. Dr. Ion Cantacuzino – Jean Cantacuzen for the French, descendant of Byzantine emperors, a medical school creator, graduate of the French medical school, born in 1863 in Bucharest, student of Ilia Mecinikov, founder of the Romanian School of Immunology and Experimental Pathology, doctor of medicine with a thesis on the destruction of the vibrio cholera. The subject of the thesis, supported in 1894, and its findings will prove useful in almost 20 years, as in the novels of Alexandre Dumas.

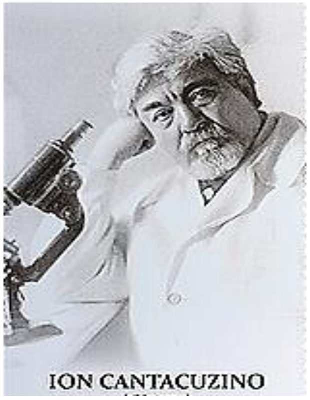

He became Professor of Experimental Medicine at the Faculty of Medicine in Bucharest at the age of 38 and was appointed in 1908 as General Manager of the Health Service to "Effectively fight epidemics, set up isolation hospitals and pavilions, rural infirmaries and bacteriological laboratories". In the Bulgarian campaign he successfully ordered the vaccination in an epidemic environment, called and known as "the great Romanian experience."

He conducted the Civil Public Health and Military Public Health Directorate during the Great War, a true Ministry of Health, which allowed him to organize anti-choleric vaccination and fight against exanthematic typhus, typhoid fever and smallpox – having the rank of Col. Dr. of the Romanian Army.

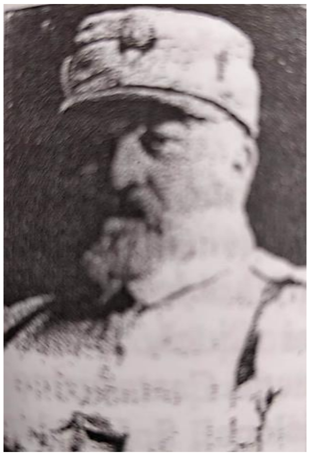

In 1920, together with Nicolae Titulescu and Mihai Ciucă, his student, participated, as the Romanian state delegate, at the Treaty of Trianon. He enjoyed a high prestige, he had an important word to say, was even a friend of French Prime Minister Georges Clemenceau, a distinguished neurologist... On April 1, 1921, as a result of his unrelenting thought, effort, and determination, he founded the "Serum and Vaccine Institute", by royal decree, which will then bear its name.

O tempora, o mores!...

In 1911 the General Dr. Nicolae Vicol (1861-1936), as Director of the Health Department of the Ministry of War, organizes two preparatory sanitary maneuvers around Bucharest that have proven to be beneficial in the future. In August 1916, when he signed the troops mobilization he followed the General Constantin Prezan – the head of the General Headquarters, unfortunately not having total decision-making power and being obliged to listen to the Minister Constantin Angelescu. Since February 1917, when the Public Health Directorate was founded, led by the supreme authority in the field – Colonel Prof Dr Ion Cantacuzino, he starts a great collaboration with him.

The General Dr. Iacob Potarca (1866-1942), a graduate of the Bucharest Faculty of Medicine, specialized in general surgery in Paris, physician colonel in 1916, head physician of the First Army’s Corp, general in 1917, then Sanitary Inspector of the First Army – who fought in the Mărăști-Mărășești sector, was not only an illustrious military physician in the war. In 1924 he becomes General Inspector of the Army's Sanitary Service, but it is worth mentioning that he is the first Romanian surgeon to have operated the esophagus, having other remarkable surgical researches quoted by Professor Dan Setlacec in his formidable monograph "Romanian Medicine, European medicine".[3]

The drama on the battlefront at the end of 1917 – the beginning of 1918, was almost at its peak. In absolute anarchy a single thought seemed to be clear! The thought of the Great Union!

In August 1917, at Mărășești, there were "many other doctors from the old country, young, learned able-bodied: Victor Papilian, Titu Vasiliu, Odiseu Apostol, Grigore T. Popa, Constantin, Mihail Kerubach, and many, many others”.[4]

The name Col (r) Prof. Iacob Iacobovici (1879-1959) is worth mentioning from the beginning, not only for being the founder of the Surgery School in Cluj, after the Great Union and of the first Emergency Hospital in Romania, the one in Bucharest, but also an involved participant in the Bulgarian campaign and the commander of the 7th Evacuation Hospital of the Second Army in Bacau in 1917.

Professor Iuliu Moldovan (1882-1966) attended the Faculty of Medicine in Vienna and Prague, and then, what a few know (4), he worked as a military doctor at the Department of Dermatovenerology and at the

Central Laboratory of Bacteriology of the Austro-Hungarian Army.

In July 1914 he was mobilized and appointed the head hygienist of one of the Austro-Hungarian armies, effectively engaging in the eradication of some epidemics. On the 1st of December 1918 he took part in the works of the Great National Assembly in Alba-Iulia. Between 1919 and 1920, he became the professor of the Department of Hygiene and Social Hygiene of the Faculty of Medicine in Cluj, general secretary of the Social Protection Resort of the Transylvanian Conducting Council and he also organized the Transylvanian Medical Service.

I think it is worth mentioning the contribution of other illustrious physicians to the Great Union just to contradict Albert Camus, who said with cynicism: "Forgetting is the first faculty of man!"

Iuliu Hatieganu (1885-1959) the first professor of a Medical Clinic, dean of the new Faculty of Medicine in Cluj, "Magnificus rector", precursor, visionary, called the "Hippocrates of the Romanians", was also a participant in the Great National Assembly in Alba-Iulia.

The last, but not the last, because the number of the unknown is overwhelmingly large, is Dr. Alexandru Vaida Voievod (1872-1950).

He was a graduate of the Faculty of Medicine in Vienna, doctor of medicine, who established in Carlsbad where he trained as an intern and balneologist, later attracted to the political activity, debuted in the Chamber of Budapest and the one who has read, on the 18th of October 1918 in the Hungarian Chamber, the Declaration of Self-Determination of the Romanian People from Transylvania. He was a member of the ministry cabinet of Ion I.C. Brătianu, he also joined the Peace Conference delegation in Paris and formed and led the first Government of the United Romania.

When Romania was finally united, things seemed to be on an upward trend.

The Romanian military doctors, as well as the civilian physicians, great personalities or unknown remarkable people, have fulfilled their duty.

Regarding "The Map of Great Romania in 1924" we have only one comment: the year 1924 was the year in which, in Germany, the ideology of Nazism has started to blossom.[5]

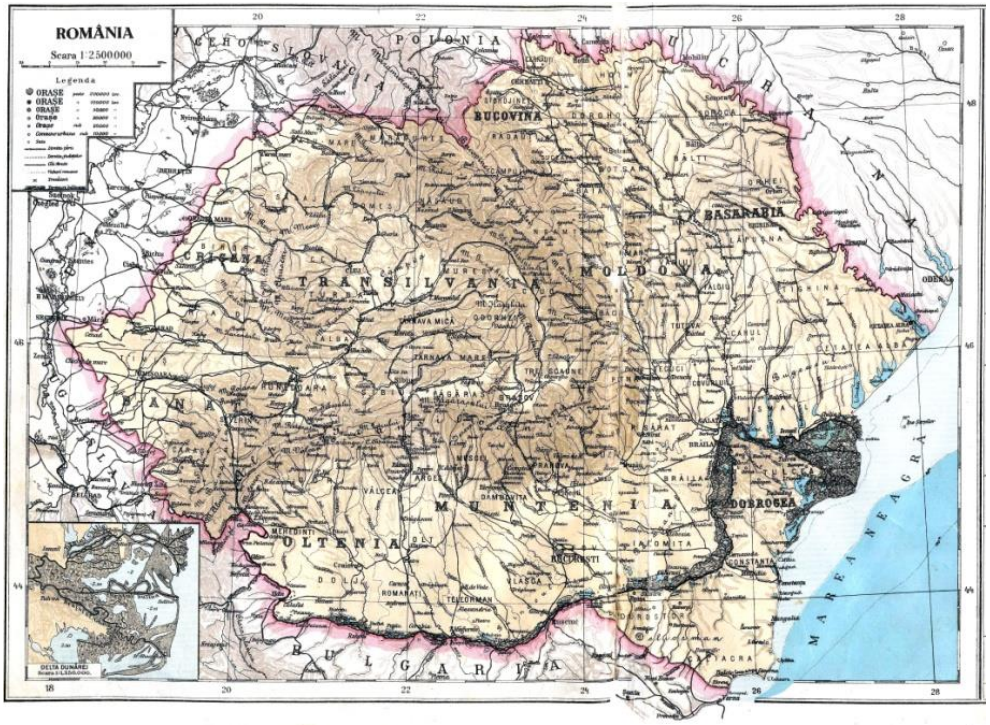
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BUCOVINA
CRIŞANA
BANAT
OLTENIA
MUNTENIA
DOBROGEA
BULGARIA
POLONIA
UCRAINA
MAREA NEAGRĂ
DELTA DUNĂREI

Our history, of the Romanian people, has implied over the years a steady climb with a lot of difficulties. The Great Union was accomplished in stages, all springing up from the ideal of unity of our nation, never forgotten.

The unification of the Romanian states is the nation's greatest act, which configures and fully certifies our existence among other nations of the world.

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REVIEW ARTICLE

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Reactive nitrogen species and cardiovascular diseases

Gabriel Gorecki[1], Elena Rusu[1], Horaţiu Moldovan[1,2], Ioan S. Tudorache[1]

Abstract: *Oxidative stress plays a major part in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. Cardiovascular disease is the leading cause of death in the United States and Europe and is poised to become the most significant health problem worldwide. Reactive nitrogen species are involved in the regulation of cardiovascular motor tone, modulation of myocardial contractility, control of cell proliferation and inhibition of platelet activation, aggregation, and adhesion. Cellular constituents of our body are altered in oxidative stress conditions, resulting in various disease states. The oxidative stress can be effectively neutralized by enhancing cellular defenses in the form of antioxidants. To understand the mechanism of action of antioxidants, it is necessary to understand the generation of free radicals and their damaging reactions.*

Keywords: *Oxidative stress, ROS, antioxidants, CVD*

INTRODUCTION

Normal biochemical reactions, increased exposure to the environment, and higher levels of dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are responsible for the oxidative stress in different pathophysiological conditions.

Cellular constituents of our body are altered in oxidative stress conditions, resulting in various disease states.

Oxidative stress plays a major part in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [1].

Free radicals are defined as "any chemical species capable of independent existence that contains one or more unpaired electrons". This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical.

An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, has been defined "oxidative stress".

It soon appeared that nitric oxide (NO) plays a key role in the physiological regulation of the cardiovascular system, since abnormalities in its productions and/or bioavailability accompany or even

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precede diseases such as hypertension, atherosclerosis and angiogenesis-associated disorders. Cardiovascular disease (CVD) is the leading cause of death in the United States and Europe and is poised to become the most significant health problem worldwide.

Free radicals are generated from either endogenous or exogenous sources. Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer and aging. Exogenous free radicals result from air and water pollution, cigarette smoking, alcohol, heavy metals, certain drugs (cyclosporine, tacrolimus), industrial solvents, cooking and radiation.

ROS and RNS products can bring about reversible or irreversible chemical changes (oxidation, nitrosylation and nitrosation) in proteins, lipids, and DNA, resulting in diminished biochemical functions [2]. The greater the amounts of ROS and RNS, the more extensive the chemical changes in these targets. ROS and RNS can induce adducts to DNA, leading to DNA fragmentation [3].

Reactive nitrogen species (RNS) are free radicals which are associated with the nitrogen atom: nitric oxide (NO), nitrogen dioxide (NO_2) and peroxy-nitrite ($ONOO^-$). Reactive species are produced by regulate enzyme such as nitric oxide synthase (NOS), and isoforms of NADPH oxidase, or as by-products from not so well regulated sources, such as mithocondrial electron-transport chain.

Nitric oxide is a biatomic free radical containing an unpaired electron. Until now have been described three forms of NO, nitrosonium cation (NO^+), nitric oxide ($NO^{\cdot}$), and nitroxyl anion (NO^-) with nitrogen oxidation number +3, +2, and +1, respectively. NO can react with oxygen free radical to form peroxynitrate ($ONOO^-$). This last molecule is involved in protein oxidation reaction under physiological conditions.

CARDIOVASCULAR DISEASES

Cardiovascular diseases are prevalent in human population and most of them are related to diet but genetic lipid abnormalities such as hypercholesterolemia, hypertriglyceridemia, HDL metabolism disorders, and combined hyperlipidemias are more severe. Cardiovascular disease is one of the major causes of mortality and morbidity worldwide and the costs that involve handling this disorder are huge. The 2008 overall rate of death attributable to cardiovascular disease was 244.8 per 100 000 individuals and this rate is critically growing [4]. Recent evidence demonstrates that cardiovascular disorders are usually associated with increased level of stress hormones [5, 6].

Cardiovascular risks such as defects in angiogenesis/ vasculogenesis or vessel repair are major complications of coronary artery disease (CAD) which are mostly seen in aged people. Similarly, CVD risks have also increased in women during pregnancy which is an important issue for management of their cardiovascular health [7]. Conventional risk factors such as cigarette smoking, diabetes, hyperlipidemia, and hypertension are absent in 15-20% of patients with CVD. Atherosclerosis is the main cause of death in the world through causing ischemic heart disease. It is peripheral arterial disease, most prevalent, morbid, and mortal disease. It is one of the most common disorders among the elderly, because of depression prevailed in the old age and rates of very high atherosclerosis. Atherosclerosis is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intimae of the walls of large and medium size arteries.

Abnormal proliferation of vascular smooth muscle is implicated in various pathological situations including atherosclerotic lesions, restenosis after balloon angioplasty, and vascular wall thickening in hypertension. NOS may play protective role by inhibiting proliferation of vascular smooth muscle cell [8]. For example, leiomyosarcoma, which is an aggressive mesenchymal tumor with differentiation toward smooth muscle tissue, represents up to 9% of all primary malignant tumors. Some cases of leiomyosarcoma presumed to be infective endocarditis [9].

THE CHEMISTRY OF RNS

Nitric oxide ($NO^{\cdot}$) is a small molecule generated in biological tissues by specific nitric oxide synthase (NOS) which metabolizes arginine and citrulline with the formation of $NO^{\cdot}$ via a five electron oxidative reaction [10]. Nitric oxide synthase utilize L-arginine as the substrate, and molecular oxygen and reduced nicotinamide-adenine-dinucleotide phosphate – NADPH as co-substrat.

NO is involved in the regulation of cardiovascular motor tone, modulation of myocardial contractility, control of cell proliferation and inhibition of platelet activation, aggregation, and adhesion [11].

Hypertension is also associated with NO synthesis [12].

The enzyme nitric oxide synthase produce reactive nitrogen species (RNS), such as nitric oxide ($NO^{\cdot}$) from arginine.

$$\text{L-Arg} + O_2 + \text{NADPH} \rightarrow NO^{\cdot} + \text{citruline}$$

An inducible nitric oxide synthase (iNOS) is capable of continuously producing large amount of $NO^{\cdot}$, which act as a $O_2^{\cdot-}$quencher. The $NO^{\cdot}$ and $O_2^{\cdot-}$ react together to produce peroxynitrite ($ONOO^{-}$), a very strong oxidant, hence, each can modulate the effects of other. Although neither $NO^{\cdot}$ nor $O_2^{\cdot-}$ is a strong oxidant, peroxynitrite is a potent and versatile oxidant that can attack a wide range of biological targets. Peroxynitrites can interact with several cellular components and are implicated in NO signaling mechanisms involving protein modifications.

$$NO^{\cdot} + O_2^{\cdot-} \rightarrow ONOO^{-}$$

In aqueous aerobic solutions NO predominantly forms nitrite (NO_2^-). In the presence of oxyhemoglobin and oxymioglobin, NO is completely oxidized to nitrate (NO_3^-). Covalent interactions of NOS with cellular macromolecules are responsible for its many physiological and pathological effects. Protein containing iron and thiol groups are the major cellular target of NOS [13].

There are three types of NOS, neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS which plays a very important role in the vascular homeostasis) and inducible nitric oxide synthase (iNOS; it is found in myocytes, macrophages and ECs and is activated by immunological and inflammatory stimuli). Under septic conditions iNOS and nNOS are upregulated in endothelial and muscle cells, respectively, leading to over-production of NO in the microvasculature and arteriolar dysfunction.

Neuronal NOS is constitutively expressed in specific neurons of the brain and its enzymatic activity is regulated by Ca^{+2} and calmodulin. This NOS isoform has been identified also in the spinal cord, in the sympathetic nerves, in epithelial cells of various organs, in pancreatic islet cells, in the vascular smooth muscle and in the skeletal muscle [14, 15].

Endothelial NOS is mostly expressed in endothelial cells; Ca^{2+}-activated calmodulin is important for the regulation of eNOS activity because Ca^{2+} induces the binding of calmodulin to the enzyme. Endothelial NOS appears to be a homeostatic regulator of numerous essential cardiovascular functions and also controls the expression of genes involved in atherogenesis. The blood vessel wall NO is mainly produced from l-arginine by endothelial NOS.

Nitric oxide as a key endothelial vasodilator also directly affects metabolism by competing with mithocondria for oxygen and consequently inhibiting switching the metabolism to some other pathways. Also, some studies suggested that NO is implied in the response of *Candida albicans* species to the oxidative stress and also against some azoles drugs. *Candida albicans* is a commensal species of the human gastrointestinal tract, in which it lives without adverse effects on the host, but yeast-to-hypha transition has been associated with increased virulence, mucosal invasiveness and biofilm formation. Candidemia and invasive candidiasis are frequently associated with high morbidity and high mortality rates [16].

ANTIOXIDANTS AND DEFENSE MECHANISMS

Overproduction of ROS (arising either from mitochondrial electron-transport chain or excessive stimulation of NADPH) results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA. In contrast, beneficial effects of ROS/RNS (e.g. superoxide radical and nitric oxide) occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as for example in defense against infectious agents, in the function of a number of cellular signaling pathways, and the induction of a mitogenic response [2]. Cellular constituents of our body are altered in oxidative stress conditions, resulting in various disease states. The oxidative stress can be effectively neutralized by enhancing cellular defenses in the form of antioxidants. Low levels of antioxidants have been associated with the heart disease and cancer.

When ROS/RNS are generated in vivo, their actions are opposed by intricate and coordinated antioxidant lines of defense systems. These include enzymatic and non-enzymatic antioxidants that keep in check ROS/RNS level and repair oxidative cellular damage. The antioxidant enzymes reduce the levels of lipid hydroperoxide and H_2O_2, thus they are important in the prevention of lipid peroxidation and maintaining the structure and function of cell membranes.

The major enzymes, constituting the first line of defense, directly involved in the neutralization of ROS/RNS are: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). SOD is a cytoplasmic and mitochondrial enzyme, which accelerate the dismutation of superoxide. They are present in almost all aerobic cells and in the extracellular fluids. They contain metal ions that can be copper, zinc, manganese or iron. In humans, the copper/zinc superoxide dismutase is present in the cytosol, while manganese superoxide dismutase is present in the mitochondria. CAT, an exclusively peroxisomal enzyme in most tissues, converts H_2O_2 to water and O_2. However, the most important H_2O_2-removing enzymes are the selenoprotein GPx enzymes. GPx enzymes remove H_2O_2 by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Glutathione peroxidase also catalyses the reduction of unstable hydroperoxides at the expense of GSH [17].

The nonenzymatic antioxidants are of two types, the natural antioxidants and the synthetic antioxidants. Vitamin C, vitamin A and plant phytochemicals like phenolics that inhibit the oxidation chain initiation and prevent chain propagation represented the second line of defense. Vitamin A has a vital antioxidant contribution in protecting human LDL against copper stimulated oxidation. Lipid-soluble antioxidants such as α-tocopherol localize mainly to membranes and lipoproteins where they serve to limit lipid peroxidative damage. Vitamins E and C have been demonstrated to reduce the progression of atherosclerosis. Vitamin E (α-tocopherol) is the most important lipid-soluble antioxidant and protects cell membranes against oxidation by reacting with the lipid radicals produced in the lipid peroxidation chain reaction and removing the free radical intermediates. Phenolics are therefore an integral part of the diet, with significant amounts being reported in vegetables, fruits, teas and traditional plants. Epidemiological evidence indicates that consumption of fruit, vegetables and teas may reduce the risk of cardiovascular disease and it is increasingly suggested that this may due to their antioxidants that include ß-carotene, vitamin C, vitamin E and polyphenolics. Dietary antioxidant phenolics may quench reactive oxygen and nitrogen species and, hence potentially modify pathogenic mechanisms relevant to cardiovascular disease [18]. Vitamin C regenerates vitamin E in cell membranes in combination with glutathione or compounds capable of donating reducing equivalents.

Low levels of antioxidants have been associated with the heart disease and cancer. Antioxidants provide protection against a number of disease processes such as aging, allergies, algesia, arthritis, asthma, atherosclerosis, autoimmune diseases, broncho-pulmonary dyspepsia, and cancer. The other disorders to which antioxidants provide protection are cataract, cerebral ischemia, diabetes mellitus, eczema, gastrointestinal inflammatory diseases, and genetic disorders.

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REVIEW ARTICLE

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Ethical limits between aesthetic and cosmetic dentistry

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Abstract: *Esthetics is the "new trend" in dental medicine as a natural consequence of the development of modern society, with implications in practice and training. Like any rule in art, but also within the medical field, esthetics must be known and addressed in relation to other medical or non-medical principles (dental cosmetic), respect the ethics rules.*

Aim. Literature study designed to focus on the current problems that modern dentistry is facing, in relation to esthetic requirements. The literature search strategy in electronic databases: EBSCO Data Base, Dentistry & Oral Sciences Source, Pub Med indexed articles, used Boolean Operators.

As a conclusion, the dentist must be familiar with the differences between esthetic and dental cosmetic, must minimize the subjective component of the examination, identify the reasons of presentation, guide the patient in choosing the optimal treatment, including obtaining the desired esthetic results, within the ethical boundaries of the noble medical profession.

Keywords: *ethics, esthetics, cosmetic dentistry*

INTRODUCTION

Nowadays, more and more frequently, within dental, practical or training activities, we are dealing with matters related to esthetics. Patients often require esthetic restorations without being able to specify most of the time, what exactly they would like. Students show an increasing interest in esthetic dentistry aspects.

As professionals we are flooded with an information influx both through scientific publications and dental materials producers, with a dental esthetics value. After the implant, esthetics is the "new trend" in dental medicine as a natural consequence of the development of modern society.

Esthetic concerns existed since forever, from the first protagonist of scientific esthetics Pythagoras, who defined the "golden ratio", combined with dynamic symmetry discovered in 1920 by Jay Hambridge and Sir D'Arcy Thompson who explained how natural beauty can be quantified and how it can be reproduced in art, architecture and other crafts.

For dentistry, as terminology, in the Glossary of

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Prosthodontic terms, esthetic dentistry is defined as the part which studies beauty, creating harmonious results through prostheses, and ethics is a moral principle or a set of moral values of an individual or group of individuals, in our case – the ones involved in the treatment (doctors, technicians). Cosmetic dentistry is not a term indexed by GPT, its definition being present in the Collins dictionary, like maneuvers aimed to beautify without purpose or functional form. The medical profession has ethical obligations [1] centered to prevent and treat diseases, in order to rehabilitate the dento-masticatory apparatus functionality, namely mastication, phonation, and the patient's physiognomy.

Questions related to dental esthetics are: What are the ethical boundaries of the esthetic trends in dentistry? Can anything be done from a medical standpoint for the sake of obtaining an esthetic outcome? Are we ready for this new challenge as physicians who took the Hippocratic Oath for the “primum non nocere” principle? Are we trained as trainers, academics, in order to educate students so that they become true professionals in esthetic dentistry? What are the limits of esthetic dentistry and dental cosmetic, as a new term in our vocabulary?

These are some of the questions that have led us to write this paper. This study is a literature one designed to focus the current problems that modern dentistry is facing in relation to esthetic requirements. The original aspect of this work is related to the definition (both for patient and doctor) of these two terms, their character being a little bit confusing, also being capable to lead to legal aspects, even malpractice.

MATERIAL AND METHOD

A comprehensive literature study was completed in October 2015. There were selected publications in English, peer reviews, articles from academic publications, dated January 2000 to December 2015. There was obtained a total of 1248 articles, including full text criteria, of which 580 articles were retained, and after applying the selection criteria only 14 publications remained. Identified as directions of interest were: (a) dental esthetics as part of dentistry – boundaries; (b) the difference between esthetic and dental cosmetic, from an ethical point of view; (c) esthetics, the reason for treatment and clinical examination; (d) medical training, scientific publications, patient information, consent. The search strategy was conducted using EBSCO Data Base Dentistry & Oral Sciences Source with the aid of Boolean Operators. The following keywords were combined: ethics, esthetic, and cosmetic dentistry. The search was limited to English peer reviewed articles, full text and years limitation January 2000 - October 2015 academic journals.

RESULTS

There was obtained a total of 1248 articles, including full text criteria, of which 580 articles were retained, matching the search criteria requested. After applying the search criteria 10 publications became relevant. Furthermore, there was done a manually electronic search on themed websites. In the end, 14 publications that included the search criteria were selected. Among the issues raised by the retained publications, there were identified 4 axes of interest:

1. Dental esthetics as part of dentistry – boundaries.

2. Difference between esthetics and dental cosmetic from the ethics point of view.

3. Esthetics, as a reason for treatment and clinical examination.

4. Training the physicians, scientific publications, informing the patient – consent.

DISCUSSION

Dental Esthetics as Part of Dentistry – Boundaries

At this point two issues detach themselves – functionality and bias. As noted in the introduction, there is a definition of dental esthetics in the GPT, however this is rather vague, making reference to “beauty, following the art’s rules and principles''. In dentistry, the therapeutic dental restorations are not only esthetic, but they should primarily ensure the dento-masticatory apparatus and the dental occlusion functionality. For example, dental fillings can be done medically with physiognomic or non-physiognomic materials, both having advantages and disadvantages, the dominant criteria for the physician must be based on clinical experience, choosing the optimal method of treatment in order to meet the basic medical principle, “primum non nocere”. [2]

The medical profession bases its treatment on clinical, objectively examination of the patient. When regarding the esthetics dental problems, subjectivity might occur; hence, the need to establish clear criteria, both objective and subjective, for the examination in dental esthetics. Indeed esthetic sense is not a criterion for graduation from the Faculty of Dentistry; it has a great variability from person to person, from clinician to clinician, as well as from patient to clinician. Given these difficulties related to the subjective issues, it was suggested a hierarchy of the esthetic aspects in dentistry, starting from basic esthetic rules where the smile’s coordinates comply to the classical principles of the golden ratio, symmetry, dental and dento-facial proportions, smooth line smiling.

The next level is represented by the cultural and regional subjective aspects, for example in the United States the so-called Hollywood smile is a social standard, the whiteness and perfect alignment of teeth being associated with wealth, social and financial success. At last, the latest level is the so-called virtual level – the one that a computer program sets as ideal for the patient, from the esthetic point of view. [3]

Is dental esthetics a part of the patient’s general health state? Yes, it was clinically proven that the esthetic restoration brings an important psychological benefit to the patient. [3]

Difference between Esthetics and Dental Cosmetic from the Ethics Point of View

Within the last years, appearing the dental cosmetic term, that was medically not registered otherwise, there have been many misunderstandings between this term and the esthetic dentistry, both among patients and practitioners.

Traditionally, dental medicine as a medical specialty is centered, ethically speaking, on the prophylaxis and the treatment of the dental tissues in order to ensure a good health state, respecting, of course, the dento-masticatory functions. So, we are talking about affected tissues, or with such potential. [4]

Dental cosmetic seeks only embellishment, often without any consideration for functionality, interfering with healthy tissues, without clinical impairment for beautifying intentions. Often, these maneuvers contradict even the esthetics concept – defined as being an integration concept of beauty in natural proportions, with a humane dimension. Is it esthetically the completely unnatural pure white smile of an 80 years old lady? Is it not against the physiological processes of aging teeth, with natural tooth staining due to time passage? Therefore, the difference between cosmetic and esthetic dentistry must be properly ethically and medically differentiated [5,6]. Moreover, some cosmetic maneuvers might damage a healthy dental tissue – for example when applying veneers, esthetic crowns, excessive grinding, applying adhesive – without pulp protection – can lead to tooth loss – defined as disfigurement, from the ethical point of view. [3]

One must respect, from the ethical point of view, the principle of minimally invasion; the so-called enamel sacrifice on the altar of vanity [7, 8, 9] does not correspond to the principles of medical ethics. On long-term, the biological implications of the maneuvers consequences that were performed only for cosmetic purposes should be correctly assessed by practitioners. [10] Esthetic would mean beauty, form and function – and cosmetic only beauty. [2]

Esthetics, as a reason for treatment and clinical examination

As shown, although the boundary between esthetic and cosmetic maneuvers may seem “too fine” sometimes, the practitioner disposes of objective criteria when deciding the treatment plan. [11]

Patients who address the dentist for solving the esthetic problems divide into two categories – among these reasons are dental crowding, discoloration, unsightly tooth discoloration, missing teeth, multiple teeth with coronal restorations. The patients’ reasons may be esthetic ones, but after a properly conducted clinical examination, the dentist will establish the functional problems – occlusion problems, migration and others that, from an objective medical point of view, should be rehabilitated in order to restore the morpho – functional, esthetic, masticatory and phonetic balance. The dentist will decide the patient's treatment plan, single or multidisciplinary orthodontics (teeth alignment through braces), conservative treatment (bleaching, esthetic restorations, and ceramic veneers), and dental prosthetic (crowns coverage, dental implants). A second category refers to patients without enough arguments – patients suffering from narcissism, personality disorder, patients who can't accept their age. As in the first group, the dentist is the one that will make a "proper diagnose" considering the medical history and clinical examination.

Patients with such presentation reasons will permanently be unsatisfied with the treatment outcome. Unlike the ones with consistent esthetical grounds that will be satisfied once the esthetic problem is solved, for the second class the result will not be acceptable even if it has improved the esthetic aspect. [2] These are the most common candidates for dental cosmetic, for whom the "primum non nocere" principle must be respected from the ethical viewpoint. [10]

And not least, after the clinical examination, if the dentist is in a doubtful situation, he should, according to "when in doubt, it is probably not ethical" [7] test himself with "The Daughter Test" – Would I proceed with this intervention on my daughter? [8,12]

Training the physicians, scientific publications, informing the patient – consent

Another important aspect is the dentists training, in addition to the fundamental principles of dental esthetics already learned in college; the profession currently faces numerous specialty publications in which the so-called academic articles are praising esthetic results obtained – the ethical aspect of the presented cases being often questionable from the fairness of the dimension's vertical occlusion point of view, occlusion stability and durability of these restorations. Publishing some insufficiently and superficially documented cases – medically speaking, designed only to beautify, can be really dangerous, especially for young doctors who didn't benefit from enough clinical experience and being pressured by patients in order to obtain esthetic results, can guide their therapeutic conduct, based on good faith. [10]

In the modern age, consumer society pushes dentists to features, such as advertising, with the temptation for many dentists to promise spectacular results with a negative impact on the professionalism of the entire profession [6], we must not forget the fundamental nature of our medical profession profile, namely the professional doctor [13] and not the beautifying one. In this context it is important, ethically speaking, the doctor-patient communication regarding the dental esthetic issues – the doctor is required in this type of treatment to inform the patient in order to receive his consent over long-term implications (especially in younger patients). Communication must be made in terms that the patient will be able to understand (not necessarily medical terms), assisted by pictures, drawings, suggestive dental casts. It is also required to present to the patient, where appropriate, one or more treatment alternatives, including the less esthetic alternative, before signing the informed consent [3]. Esthetic dentistry requires less accommodation, incorporates acceptable biologic technology for long-term survival, functions suitably, and mimics the pristine state of the natural dentition. Cosmetic and esthetic dentistry are different in definition, concept, and execution [14].

CONCLUSIONS

As a result of this extensive literature study on a very actual dentistry issue – ethical considerations of esthetics and dental cosmetic, we came up with some interesting conclusions intended to clarify the often encountered confusion regarding these terms. Dental Esthetics regroups several dental maneuvers, often interdisciplinary, aiming the morfo-functional rehabilitation of the dento-masticatory apparatus, following universal esthetic principles harmoniously integrated into the overall health and harmony of the human body as part of dentistry. Dental cosmetic is a set of maneuvers that, although have a medical character, do not seek the reconstruction of the maxillary device functionality, just have a beautifying character, intervening on healthy tissues without any prophylactic role, often with disabling long-term implications. In terms of bioethics, the "primum non nocere" principle is not respected within these maneuvers.

Therefore, it is important for the dentist to know the differences, the fundamental dental esthetic concepts, in order to minimize the subjective component of the examination, to succeed the clinical examination with identification of the presentations reasons, to be trained for all medium- and long-term treatment implications, in order to be able to present to his patient all treatment alternatives and guide him to choose the optimal treatment option for obtaining the desired esthetic results within the ethical boundaries of this noble profession. The theme being new and the boundary between esthetics and cosmetic dentistry being quite subjective, no doubt that they still have to be studied, there are needed further studies and research that will clarify the differences between them on an evidence-based scientific system.

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REVIEW ARTICLE

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History of medicine on the border between philosophy and science

Mirela Radu [1]

Abstract: *Physicians have represented a long time the main transmitters of knowledge as they were real scholars. If Renaissance promoted the study of the human body anatomy and physiology, the next step made by practitioners of medicine was to spread the enlightenment. That meant the shift of the very purpose of their profession: from passive opposition to ailments towards an active involvement into the lives of the impoverished. In order to change the odds in the battle against diseases, physicians had the great burden to enlarge the cultural horizons of those whose health was in their hands. Therefore, one way of imparting knowledge was by publishing and spreading their attainments to the general public in a comprehensible way. Once people gained awareness of the dangers entailed by bad hygiene, the physicians' role in society switched towards more cultural realms. At the beginning of the 20th century health care professionals achieved the next step in the becoming of medicine: setting up a new science to link humanities with pure science. In Romania, the main promoters of this new border science were Victor Gomoiu and Valeriu Bologa and they co-opted other intellectuals.*

Keywords: *philosophy, science, history of medicine, alchemy, folklore*

The new involves acknowledging the past, transforming it and bypassing mistakes. The 20th century met the expectations of those who wanted to know this history by setting up the *Institute of History of Medicine* in 1921 in Cluj. "More and more are those who pretend to have a spiritual imitation in the past to save the intellectual character of modern medicine. This postulate translates practically into the multiplication of medical-historical literature and giving a growing importance to the history of medicine."[1] One of the first teachers to honor the Romanian institute was the French Jules Guiart (1870-1965) who taught for three years this subject. Those who strongly supported him were Valeriu Bologa and Emil Racoviţă. Guiart, fascinated by what he had discovered on the Romanian realm, would also work as an ethnographer, travelling intensively and gathering various ethnographic materials and photos from all corners of our country.

The Romanian physician Valeriu Bologa (1892-1971) is the exponent of a whole caste: that of doctors aware of the modeling power of culture. He dedicated himself to the study of natural sciences (at the University of Jena) and, afterwards, he was attracted to the medical studies in Austria and Cluj. The pride he felt for the art

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of healing practiced by the Romanians led him to lay the foundations of a new branch of science: the history of medicine. Between 1949-1971 he presided the International Society of Medicine History. Feeling that the progress can only be heard through the knowledge of the past, Bologa devoted many books to the facts of the medicine in the past. One of the first important papers signed by the Romanian physician focused on the special situation of the Hippocratic profession practiced by the Transylvanian Romanian doctors who had to face not only the lack of material means but also the political repression: *Contributions to the history of medicine in Transylvania* (1927). Three years later, Bologa signed a second monograph, *The Beginnings of Romanian Scientific Medicine* for which he would receive in 1931 the V. Adamachi Prize of the Romanian Academy. But until 1927, the physician signed only a studies with great historical significance, dedicated to some of the most diverse themes – from midwifery, to the forerunners of doctors, from ophthalmology to medical lexicology formation: *Spells, old women and midwives today and the past* (1921); *New data for Ioan Molnar* (1925); *About Romanian Occultists* (1925); *Medicine in Moldavia* (1925); *Between physiology and medicine* (1925); *Romanian Medical Terminology of doctor I. Molnar* (1926).

Furthermore, Bologa dedicates himself to the construction and endowment of a museum dedicated to medical science in Romania. The Romanian scholar was particularly fond of two sections of the museum: *Old Romanian Medicine* and *Medicine in the Transylvanian past*. The great importance he gave to the knowledge of the old times of the profession he revered could be felt from the appreciation with which he emphasizes the importance of those early times, but also the respect he had for his ancestors. For the reader of any age is visible the attachment and esteem that doctor Bologa carries to those who have done medical pioneering work, especially in the Transylvanian region: “From this rich Romanian medical library can be reconstituted the hard work of the first gatherers of new roads in Romanian science. It is possible to see the influences from the outside, it can be seen how gradually a Romanian medical terminology was formed, it can be noticed how, from the great Davila, our medicine goes from the assimilation phase to the one of creation, as more and more characteristically forms a Romanian medical current. The old Transylvanian medical literature was represented equally well at the beginning – from the 16th century – by the works of the German doctors, later with those of the Hungarians, finally from the 18th century and with the first Romanian medical translations.”[2]

But Bologa was not the only one who fought for this new branch of medicine. He was helped in his efforts by the fellow surgeon Victor Gomoiu (1882-1960) who founded museums dedicated to the history of medicine in Târgu-Jiu and Craiova. Gomoiu, in turn, published a monograph entitled *From the History of Medicine and Romanian Medical Education* (1923) and during the interwar period he was elected president of the International Society of Medicine History (1936). Gomoiu was also the one who signed the first *History of the Medical Press in Romania* (1936), the work of collecting and organizing numerous medical papers and writings. But Gomoiu was not just an encyclopedic spirit. He also actively contributed to the struggle that doctors used to do with illnesses whose mortality reaches worrying odds. Director of the Osteoarticular Tuberculosis Sanatorium for Children in Techirghiol, eventually Gomoiu would practice surgery in Bucharest. His surgical work is quantified by the large number of innovative articles he has written, by implementing the term solarectomy (resection of lymph nodes), initiated the inguinal approach of varicocele (Gomoiu-Phocas method). Intransigent character, Gomoiu was removed from academic education. His merit in the history of medicine is to insist on the Romanian contribution to the international folk medicine fund. This brought, at least historically, the Romanian medicine at the level of the other countries reducing the gap. A proof of his ideal and his effort to bring medicine to the Western level are the three works published by the Romanian physician in 1938: *La Croix dans la Folclor medical roumain*, *Histoire du Folclore medical en Roumanie* and *Medicine in the Romanian folk prose*.

Bologa also corresponded intensely with Mircea Eliade, whom he intended to co-opt in his work at the

Institute of Medical History. Eliade, despite the fact he had no necessary time for active participation in this approach, had a special esteem for the intellectual physician, as is also apparent from the lines written by the philosopher in an epistle addressed to the physician-historian, a letter dated 23 October 1928: “I testify that whenever I skim through your work and meditate on the situation of the history of sciences in our country – I am remorseful that I do not write more often, warmer and harder, in the papers that are at my fingertips. I know that, personally, for the scientific history studies I'm interested in – I have to thank you. Not to mention what others owe you, especially doctors and historians. The Institute makes «environment» scientific history, we, isolated ones, can at most, make the atmosphere. If an association for such studies can be woken up, I always think that the courage of the achievements has been with the production of the Cluj Institute.“[3] Even in India, Eliade maintains contact with the Romanian physician for whom he does not hesitate to admit he has a great cult of his extraordinary work of a huge volume: “The passion of science – that is, the slow, precise, technical sorting of the material our culture provides us – is the great temptation that brings me closer to you .” (Letter dated 16.02.1930, Calcutta)[4]

The reason why Eliade particularly appreciated Bologa resides in the philosopher's aspiration to write a few stories on traditional Indian medicine branches. Eliade admired the founder of medical history the ability to synthesize the huge volume of works, objects and manuscripts. It was the systematization work that occupied the author of *Religious History Treaty* and *History of Religious Beliefs and Ideas* all the time. At the same time he was better equipped to understand the enormous sacrifice of time and resources involved in ordering, ranking, and organizing such amount of information. Frustrated by the huge volume of notes, contact with Bologa developed philosopher's rationalization and ability to think more rigorously. Eliade's interest in medicine crystallizes in 1936 when, following a lecture held at an International Congress of History, Eliade publishes *History of Medicine in Romania*. The affection borne by the philosopher of the religions to this new emerging branch stems from the support given to the history of medicine which he perceives as a means of producing: “real services to the humanism of our age.”[5] Folk medicine is viewed with reverence by Eliade because it represents the immaterial and immutable connection with the ancestors of the nation. Since 1926, Eliade collaborated with Aldo Mieli, who was the publisher of *Archeion* magazine, producing short studies of the history of various sciences, medicine and folklore. That's how Eliade got to correspond with Bologa. The latter wanted to develop a collaboration with Miel's *Archeon* by making contributions in the form of articles devoted to Italian influences on Romanian medicine.

For Eliade, the whole science represents, at least in the initial phase, a single corpus. Subsequently, science has specialized and subspecialized over time. What could bring back all these disparate fragments to one place would be the philological field. In fact, even Bologa was aware that his scientific approach was far more philological. This is how one can explain the help that he Bologa asked from the philosopher. Another connection between the two, Bologa and Eliade, was the scientific curiosity to study botany. As a small child, Eliade devoted much energy to catching, studying, analyzing and cataloging various insects. At the age of fourteen, Eliade published a study titled *Silkworm's Enemy*, under the pen name Eliade Gh. Mircea, which showed the passion he has for insect biology. *The marvelous journey of the five beetles in the land of the red ants*-sketch of the novel – was written in the same period. More the outline of a teenager fascinated by the world of gangs, behind the modest mise-en-place is hiding a satire, an annoyance of the enemy (ants) by five elite bettles. It is a mockery of the human society reduced to the microcosm of insects.

The step to science would come when Eliade participated and won a contest that proposed the literary approach of a scientific subject. The title of the essay (*How I found the philosopher's stone*) is an epiphany of the future path that the teenager Eliade would take. The essay written by a youngster seems to have amazed the author himself when, over the years, he said, “How much I would like to be able to reread this story now, understand what that mysterious character revealed to me, what alchemical operations he assisted! I had found the philosophical stone in my dream ... I could only understand, decades later, after I read Jung, the meaning of this oniric symbolism!"[6] The short story, though a fantastic text loaded with supernatural, has as its starting point in Eliade's interest in chemistry and alchemy. Although he had promised Bologa that he would make his contribution to writing a history of Romanian medicine, Eliade's departure to India would break this momentum. Though time did not allow him, for the young Eliade was trying to absorb the new information that was crowded him, the philosopher gathers medical material inspired by yoga practices and even offers to write to the Romanian physician an article about Ayurvedic medicinal products, as we find out from a letter dated February 6, 1930: "I have a considerable number of facts on pharmaceutical medicine and magic in India, some of them astounding, such as those relating to *vagus nerve* control."[7]

For Eliade, alchemy is the gate open to an occult form of practice. Alchemy is the first type of objective report that leave leave, over the history of humanity, truly scientific discoveries; a kind of ancestor of rational knowledge. This preparatory, pseudo-scientific phase, the first attempt of structuring scientific knowledge was the one that attracted Eliade from his youth because of its esoteric character. In 1928 Eliade wrote an article (*Marcelin Berthelot and alchemy*) dedicated to a French chemist and biologist who imposed his name in the field of thermodynamics. Conscious of the enormous gap between Romanian and Western science at the beginning of the 20th century, Eliade sensed, from the philologist and philosopher point of view, the need to systematize the totality of historiographical material in order to be saved from oblivion: "We cannot wait until Romanian science reaches a European level to promote the validity of historical-scientific studies. There is no discipline that can be postponed."[8] The philosopher's insight was to build a methodology in this vast field of history of medicine. The history of science would be appropriate, with a takeover from a chemist and American historian Sarton, a new form of intellectual movement that would put man and science in the center: "Eliade understands a new interpretation or vision of man not derived from philological studies (textual), as it was Renaissance humanism, but in the history of science - understood as «any systematized knowledge» (Sarton), therefore more than «positive sciences»."[9]

Eliade, great admirer of George Sarton (1884-1956), intuited in the Belgian chemist the innovative spirit. Sarton embraced the history of science as a branch of gnoseology and aimed at linking science and humanism to a comprehensive one: the philosophy of science. Eliade was in the current with the theories of the American and hence the enthusiasm at the moment when a homologous branch was formed on the Romanian realm. The only ones of sufficient intellectual scope that Valeriu Bologa could count on were Mircea Eliade and Nicolae Iorga.

If alchemy was the gate open to science, popular creation and ancestral healing practices were the preamble of modern science. And Eliade felt this correlation, especially as the prose was anchored in folklore: "In his writings, the folkloric elements intertwine with those of the history of religions or ethnology. His stories take place in *illo tempore*, somewhere outside of physical time, and the characters have supernatural powers, their existence enrolling in an eternal present, and the facts being predetermined in advance. Witches, queens, beautiful women who make pact with the evil, curative herbs and charm plants, here are some of the ingredients with which Eliade sows his writings inspired by folklore."[10]

The pioneering work of building a frontier science in our country like the history of science did not frighten Eliade. We find out from a letter addressed to Bologa that, on the contrary, ostentatiously, he protects this new branch of knowledge, although he is aware of the weight of action in a rebellious society to the new: "I defend a science against the envy and imbecility of our intellectuals. I do not even think that our science will soon become popular. But it must not be ignored and, above all, dishonored by the elite to which it is de jure and de facto aimed at."[11]

What brought together a physician (Valeriu Bologa) and a philosopher (Mircea Eliade) were the folk traditions with application in medicine. Apparently two opposing personalities collaborated efficiently and discovered the common denominator, the unspoken binder between a scholar spirit and a metaphysical one, for “the research of Valeriu Bologa met the interests of Mircea Eliade and although they did not sign articles or books, the mere fact that they shared their opinions meant much for the later developments of both.”[12]

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ORIGINAL ARTICLE

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Therapeutic management of schizophrenia and substance use disorders dual diagnosis – clinical vignettes

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Abstract: *Patients with schizophrenia are frequently diagnosed with addictive comorbidities, and data in the literature support a 10 to 70% prevalence of this dual diagnosis. Nonetheless, substance use disorders can be missed during the initial interview with a psychotic patient, if the clinician is focused only on the more obvious manifestations. Therefore, using psychometric scales and structured interviews in patients with schizophrenia is strongly encouraged because the case manager will base his/her therapeutic decisions on quantifiable data about these patients' symptoms and functional status. Clinical management in dual diagnosis cases must address both conditions simultaneously, as the delay in the initiation of substance withdrawal treatment may hinder the recovery from a psychotic episode. An important issue is represented by the potential pharmacologic interactions between drugs administered for schizophrenia and those targeting substance withdrawal and substance dependence. Other important aspects refer to (1) the therapeutic adherence, which can influence the prognosis of both conditions, (2) the negative impact of residual psychotic symptoms and substance-related disorders over the patient quality of life and daily functioning, (3) the necessity to integrate variables like the patient's specific needs, lifestyle, and psychological resources in the therapeutic decision. These clinical vignettes are focused on clinical, biological, psychometric, and pharmacological dimensions, supporting the formulation of treatment recommendations based on monitoring both psychiatric and biological profiles.*

Keywords: *schizophrenia, substance use disorders, antipsychotics, dual diagnosis, cannabis, nicotine, alcohol dependence*

BACKGROUND

Substance-related disorders are very common throughout the course of schizophrenia, and this phenomenon is responsible for poorer quality of life, higher impairment of daily functioning, lower rate of treatment response, lower therapeutic adherence, leading to a worse prognosis in these patients.

Prevalence of dual diagnosis (substance use disorder and psychotic disorders) ranges from 10 to 70% in a large-scale trial for schizophrenia [1].

Many hypotheses about the link between *cannabis use* and schizophrenia are still tested, cannabis being considered an independent risk factor for psychosis and a variable that may worsen prognosis in schizophrenia patients [2]. A cannabinoid hypothesis of schizophrenia has been suggested, based on the observed alteration of endocannabinoid system (abnormalities in cannabinoid type 1 receptor binding properties and modified levels of anandamide in the cerebrospinal fluid) [2]. Cannabis use was associated

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with an earlier onset of schizophrenia, more severe forms of disorder, higher rates of relapse, and longer hospitalizations [3-5]. Longitudinal studies report that cannabis use in childhood and adolescence doubles the risk of psychosis onset later in life, which supports a causal role of this drug in the development of schizophrenia [6]. Certain alleles of the type 1 cannabis receptor gene (CNR1) may confer susceptibility to schizophrenia [7].

Also, the overlap of *nicotine dependence* and schizophrenia has been debated as a form of self-medication for schizophrenia-related cognitive deficits, based on the fact that the nicotine receptors activation increases the release of dopamine in cortical and subcortical areas [8,9]. Still, cigarette smoking decreases the bioavailability of many psychotropics that are metabolized through the CYP450 1A2 isoenzymes and consequently may diminish the clinical effect of these drugs and delay the patients recovery [10]. Multiple genes have been linked to both conditions, e.g. binding protein genes, protein modification genes, and energy production genes involved in cognitive functions and neuronal plasticity [11].

Alcohol use disorder was found in 33.7% of patients diagnosed with schizophrenia or schizophreniform disorder in the Epidemiologic Catchment Area study [12]. A dysregulation of the dopamine transmission has been suggested as common neurological basis, but shared genetic vulnerability factors have also been investigated (e.g. KPNA3, or alcohol dehydrogenase variants) [13-16]. A review of the current evidence for common risk factors in alcohol use disorders and schizophrenia supports a highly polygenic model, with rare penetrant alleles and frequent alleles with small effects [16].

Patients diagnosed with schizophrenia tend to abuse *anticholinergic* drugs. These agents are often used for the treatment of antipsychotic-induced extrapyramidal symptoms, and a national database analysis showed that patients with schizophrenia took 20 times more frequently antiparkinsonian agents than patients with Parkinson disease [17]. Trihexyphenidyl abusers may claim this drug improve their daily functioning and their affect, and a possible pharmacological explanation is that this agent has a structural similarity with phencyclidine [18,19]. The risk of biperiden and orphenadrine abuse was relatively small in a large database analysis [17].

CLINICAL VIGNETTES

The first patient, M.S., is a 29-year old male, diagnosed since 2015 with schizophrenia according to the DSM-5 criteria [20], currently at his third psychotic episode. He was hospitalized after he presented at the Emergency Department with delusions of persecution and auditory hallucinations ("there are people who want me dead because of my soul, they want to collect my psychic energy", "I can hear them through the walls, day and night, they are plotting against me, and they are saying bad things about my family", "They are forcing me to do evil things, like cursing strangers with no reason"). These manifestations led to changes in his behavior, he became reclusive, didn't go out of his house for weeks and spoke with his family only by phone, refusing to see them ("I can protect them if I'm not seen with them"). He recently abandoned his job as a salesman and didn't want to see her girlfriend anymore because of the belief that she was in cahoots with the persecutors who want him dead.

The pharmacological history in this case included olanzapine 20 mg/day as the main treatment for his first psychotic episode. After hospital discharge, he received the same antipsychotic for 8 months, then he dropped out and relapsed after about 6 months. The overall clinical status during the second admission was similar to the first episode of psychosis, with persecutory delusions and auditory hallucinations (both conversing and imperative voices) and induced defensive behavior- the patient refused to go out by himself because of the fear of being watched and plotted against. Olanzapine was re-initiated, but shortly after this the patient was switched on aripiprazole 30 mg/day due to concerns related to his metabolic status (240 mg/dl for the total cholesterol, 150 mg/dl for LDL-cholesterol, and 250 mg/dl for triglycerides). The evolution was favorable during the hospitalization and the patient was recommended to work in a controlled environment and to participate in occupational therapy. However, after 7 months he discontinued treatment and soon relapsed, so that a new hospitalization was required. This time the patient was stabilized on aripiprazole, but for the maintenance phase the long acting injectable form of aripiprazole 400 mg every 4 weeks was selected, in order to diminish the risk of therapeutic non-adherence.

The patient was also diagnosed during the current psychotic episode with alcohol use disorder, moderate, based on the DSM-5 criteria, admitting a daily intake of 8 drinks, consisting mainly in beer and wine for more than 12 months. Also, he is smoking 20 cigarettes daily, with a value of 10 pack-year. Biochemistry panel reflected the liver damage, with values for gamma-GT, GOT and GPT of 156 U/l, 70 U/l, and 67 U/l, respectively. No abnormalities were detected on his chest X-ray and abdominal ultrasound exam (except for hepatic steatosis).

The psychological evaluation realized during the initial visit for the third episode showed a 98 score on PANSS [21], with high values on both positive and negative sub-scales. CRDPSS [20] score was 17, based mainly on hallucinations, delusions and abnormal psychomotor behavior. AUDIT [22] score was 14, reflecting a moderate risk of harm related to the alcohol use, and the severity of nicotine dependence was high, as supported by the FTND [23] score of 9. GAF score at admission was 45, based on symptoms severity and functional impairment, while the CGI-S score was 5, which means a "markedly ill" clinical status.

Therapeutic challenges analysis: This patient presented a history of therapeutic non-adherence which triggered two relapses. He was diagnosed with two substance use disorders (alcohol and nicotine dependence), which were not therapeutically approached during his two previous psychotic episodes, and this could be also a factor that may contribute to relapse in schizophrenia [24]. There is a lack of social and professional insertion in this case, related to both positive and negative symptoms. The patient lacks familial support and he discontinued occupational therapy. Moreover, the metabolic profile and the hepatic functional status were abnormal. All these negative prognosis factors have been evaluated by the case manager when the therapeutic strategy was formulated. Aripiprazole was preferred because of its good metabolic profile [25], and a long acting injectable formula was selected because of the more stable plasma concentrations and lower risk of discontinuation. The patient received counselling for his addictive behavior, and he participated in 4 individual sessions focused on smoking cessation and alcohol use relapse prevention. Alcohol withdrawal symptoms were mild-to-moderate and remitted after B-vitamin therapy, parenteral rehydration, and oral lorazepam 3 mg/day for 7 days, with gradual dose reduction. Naltrexone, 50 mg/day, was initiated for alcohol dependence after the withdrawal symptoms remission, and nicotine replacement therapy was suggested, but the patient refused. There are no data reported about pharmacokinetic interactions between aripiprazole and naltrexone in the literature, which supports this therapeutic recommendation.

Follow-up visits: The patient was monitored for 4 months, using psychometric instruments, in order to document psychotic symptoms, severity of addictions, and overall clinical status evolution under treatment. Global functioning improved once the psychotic positive symptoms remitted, although the negative symptoms persisted at a lower level of severity (as reflected by PANSS and CRDPSS scores).

Table 1. Psychologic evaluations during the first patient's initial visit

Clinical scale	Results
PANSS	98
CRDPSS	17
GAF	45
CGI-S	5
FTND	9
AUDIT- Interview Version	14

Alcohol use disorder had a favorable evolution and the AUDIT scores diminished gradually, but the nicotine dependence persisted and the mean number of cigarettes increased with 25%, while the FTND score increased with 10%. Biochemistry panel reflected an improvement of the liver status after 4 months, with values for gamma-GT, GOT and GPT of 56 U/l, 23 U/l, and 37 U/l, respectively, and the metabolic parameters improved, also: 190 mg/dl for the total cholesterol, 120 mg/dl for LDL- cholesterol, and 170 mg/dl for triglycerides.

Conclusion: Addictive behaviors must be approached as soon as possible by the case manager in patients with schizophrenia, because the risk of therapeutic non-adherence, somatic complications, and reduced functionality is higher if these conditions are left untreated or if the appropriate treatment is delayed. In this particular case, naltrexone was efficient in the treatment of alcohol use disorder, and the patient had also significant decrease of the psychotic symptoms. However, nicotine dependence could not be addressed pharmacologically because the patient refused, and he participated only in a few counseling sessions, which led to the persistence of his substance related condition.

Fig.1. Evolution of the clinical variables during the first 4 months of treatment

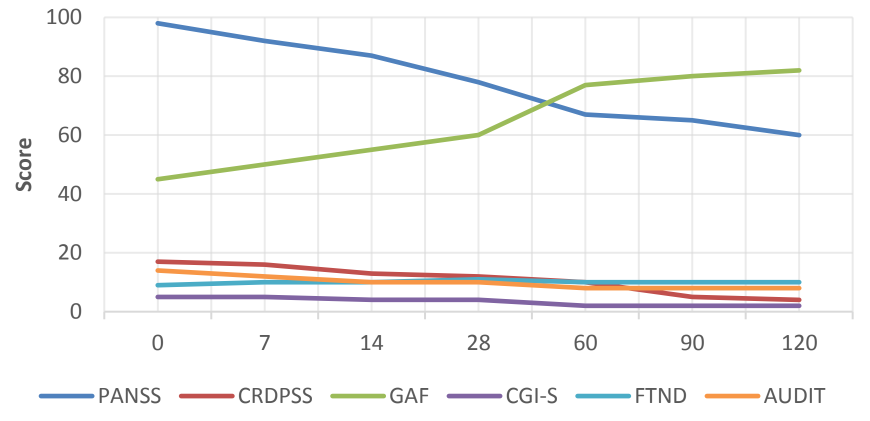

The second patient, E.D., is a 30-year old female, diagnosed with schizophrenia for 6 years, currently in a partial remission, who presented to her psychiatrist asking for a therapeutic change because of galactorrhea and irregular periods. She attributed these symptoms to risperidone, which was initiated by the psychiatrist during her latest psychotic relapse, about 3 months ago.

This patient had 4 psychotic episodes since the onset of her disease at age of 22 and received for her first episode haloperidol 15 mg/day for 2 months, followed by amisulpride 800 mg/day for 10 months; for her second episode, she was treated with olanzapine 15 mg/day, for 16 months; during her third episode she received haloperidol 20 mg/day for the acute phase, and again olanzapine 15 mg/day for an indefinite period of time, and for the last episode she received risperidone 6 mg/day maintenance dose. Changes in the antipsychotic regimen were determined by adverse events- extrapyramidal symptoms during haloperidol treatment, hyperprolactinemia during amisulpride administration, and weight gain during olanzapine therapy.

This patient presented also criteria for nicotine and cannabis use disorder, both of moderate severity, according to the DSM-5 criteria. She was on cannabis for more than 2 years, with very few short periods of abstinence, and regarding nicotine use she admitted she was smoking 15 cigarettes daily for at least 8 years. She admitted she did not recognized cannabis addiction in front of her psychiatrist until the current visit. She was never offered nicotine replacement therapy or any other type of treatment targeting nicotine dependence.

The psychological evaluation during her third episode sh owed a PANSS score of 69, with low values on both positive and negative sub-scales. CUDIT-R [26] score was 16, supporting severe cannabis dependence, and the severity of nicotine dependence was high, as reflected by the FTND score of 7. GAF score at admission was 60, based on symptoms severity and functional impairment, while the CGI-S score was 4, which means “moderately ill” clinical status. EuroQoL (EQ-5D-5L) [27,28] visual analogic scale score was 67, which seems to be correlated more with her substance-related symptoms than with her psychotic manifestations. Quality of life domains that seemed more affected by her current status were anxiety/depression (a score of 4) and usual activities (a score of 3).

Her somatic status was good, with no abnormalities on CBC or serum biochemistry panel. Also, her ECG and chest X-ray didn’t suggest any abnormalities.

Therapeutic challenges analysis: This patient has a history of adverse events to several antipsychotics (haloperidol, amisulpride, olanzapine) which were severe enough to grant changes in the antipsychotic treatment. The patient received a new antipsychotic, ziprasidone 160 mg/day, which has been associated with low risk for hyperprolactinemia, weight gain, and extrapyramidal syndrome [29]. A gradual switch was preferred due to the different pharmacodynamic profiles of risperidone and ziprasidone [29-31]. ECG monitoring was initiated, and periodic measurement of metabolic parameters was continued throughout the duration of the antipsychotic therapy. The presence of cannabis use disorder raises an important question because there is no pharmacological treatment with clear evidence of efficacy in patients diagnosed with this disorder, while data about psychotherapy effects are still debatable [32]. However, gabapentin and N-acetylcysteine have been suggested as possible therapies [32], and gabapentin was preferred in this case because of its positive effect on anxiety and low risk of pharmacokinetic interactions [33]. Nicotine replacement therapy with nicotine patch 25 mg/16h for 8 weeks, followed by gradual dose reduction, combined with psychological counselling, was accepted by the patient.

Follow-up visits: The evolution of the psychotic symptoms was favorable, as reflected in the PANSS (-10%) and CRDPSS (-11%) scores. The overall functionality increased significantly (+33%) compared to baseline, and this improvement seems related to the decrease in both FTND and CUDIT scores, with 71% and 75%, respectively. Also, the quality of life improved, both on the visual analogic scale (+17%), and on its subscales (depression/anxiety -50% and usual activities -33%). The Clinical Global Impression-Severity improved with 50%, and the patient was considered after 4 months “borderline mentally ill”. Minimal QTc prolongation was detected on ECG after 4 months (+1.3%), but it didn’t reach the level of significance (considered to be 460 msec in women, after correction with Fredericia’s formula). No metabolic abnormalities were detected during the monitoring period, and the BMI decreased with 2.1% compared to baseline.

Conclusion: Targeting the cannabis and nicotine use disorders may improve the overall functionality and patient’s quality of life, reducing further schizophrenia-associated symptoms, like depression, apathy, anhedonia or anxiety. In this case, the patient was compliant to the therapeutic suggestions, and participated in counselling sessions focused on substance use relapse prevention, while being adherent to the pharmacologic treatment. Her evolution was favorable and the therapeutic switch from risperidone to ziprasidone was well tolerated. No pharmacokinetic interactions were anticipated between the treatment for nicotine use disorder (replacement therapy), cannabis use disorder (gabapentin) and schizophrenia (ziprasidone).

Table 2. Psychologic evaluations during the second patient’s initial visit

Clinical scale	Results
PANSS	69
CRDPSS	9
GAF	60
CGI-S	4
FTND	7
AUDIT – R	16
EuroQoL	
Visual analogic scale	67
Mobility	1
Self-care	2
Usual activities	3
Pain/discomfort	2
Anxiety/depression	4

Fig.2. Evolution of the clinical variables during the first 4 months of treatment

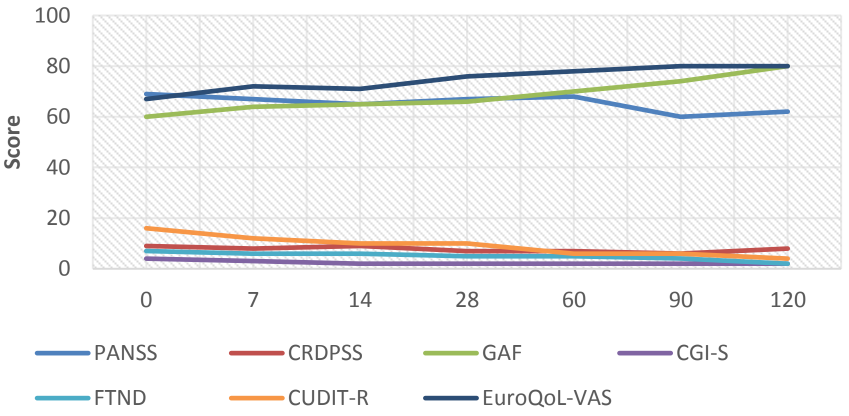

The third patient, S.G., was diagnosed for the first time with acute psychotic disorder 3 months ago. Her symptoms at hospital admission consisted in psychomotor agitation, grandiose ("I am very powerful, and I can make any wish come true, like Santa Claus, only better") and persecutory ("there is a group of forces trying to kill me and take my powers") delusions, auditory and visual hallucinations ("I can hear them talking about me and trying to make me feel miserable… they are cursing me and telling lies about me and my family", "They are moving through the light, I can see them… they are like some green shadows"), disorganized behavior, and diminished emotional expression. She was 27 years old when she was first admitted in hospital, and her psychotic symptoms had an insidious onset over at least 4 months. First, she was initiated on risperidone 6 mg/day, and her response was good, but discontinued oral treatment because she had to take this drug twice a day. The patient developed positive symptoms of psychosis after one month of no treatment, and she was readmitted in the Psychiatry Department. The selected drug for clinical stabilization was risperidone because of her previous good response. She was informed that a long acting injectable form of this antipsychotic exists, which requires administration every two weeks. Also, she was informed that paliperidone, the active metabolite of risperidone, has two long acting injectable formulations, with administration of one dose every 4 weeks, and after stabilization, the drug may be administrated every 12 weeks. She agreed to be initiated on paliperidone long acting after stabilization of acute symptoms.

The patient was smoking 30 cigarettes daily, with a value of 9 pack-years. She fulfilled the DSM-5 criteria for nicotine use disorder and accepted treatment for this condition. She received nicotine replacement therapy, but she declined the invitation to join a group therapy focused on abstinence.

Her ECG was normal, as were chest X-ray, cerebral CT-scan, CBC and serum biochemistry panel. The toxicology exam was also negative.

Table 3. Psychologic evaluations during the third patient's initial visit

Clinical scale	Results
PANSS	88
CRDPSS	14
GAF	35
CGI-S	5
FTND	9

Therapeutic challenges analysis: This patient is still in the early phase of disease, as her diagnosis of schizophrenia was just established. She met the necessary criteria for this diagnosis- time (more than 6 months including pre-hospitalization period of active symptoms), clinical manifestations, functionality, and differentials. The challenge is to select a treatment regimen that could be more readily accepted by a young and active person (she has to travel often because she has contracts with different enterprises), while targeting both schizophrenia and nicotine addiction symptoms. One advantage in this case is the insight of the patient and her willingness to continue the treatment. She understood the therapeutic options her psychiatrist presented, and she has chosen the treatment which allows her less time for administration and medication-supplying procedures (visits to her GP, treating psychiatrist, and local pharmacy). Therefore, paliperidone was considered the most appropriate option for her, and after stabilization with oral medication, she was switched on paliperidone palmitate (PP1M) 100 mg monthly as maintenance dose for 4 months, and paliperidone palmitate (PP3M) 350 mg every 3 months after 4 months. Regarding her nicotine use disorder, she received 25mg/16 h nicotine patches and nicotine spray administered prn, in case of withdrawal symptoms, with gradually dose reduction, and termination after 3 months. The nicotine spray was recommended because the patient is a heavy smoker, and because she had no asthma, chronic sinusitis, or other related diseases. Paliperidone is not a substrate for CYP1A2, therefore its plasma concentrations are not expected to be modified by cigarette smoking, in case substance use disorder treatment fails.

Follow-up visits: The evolution of psychotic symptoms was favorable, as reflected in the PANSS and CRDPSS scores, which decreased with 40% and 65%, respectively. The favorable trend maintained even after switching on the long-acting formulae (PP1M and PP3M). The slower rate of improvement after day 36 is related to the stabilization of the clinical status, which is a condition for switching on long-acting antipsychotic formula. The patient reported that she could return to her job after 6 weeks of treatment and her professional performances were fair. The cigarette use declined during the first 4 weeks, but she admitted she smoked during nicotine replacement therapy and after its discontinuation. Therefore, after 11 months her FTND score reflected a moderate dependence. She refused a new trial of nicotine replacement therapy and counselling sessions, as she states “smoking is not a problem for me anymore… I’m only smoking when I’m feeling nervous”. Her BMI increased with 3.5% compared to baseline, but no significant alterations in plasma lipids, blood glucose, hepatic enzymes or QTc were reported.

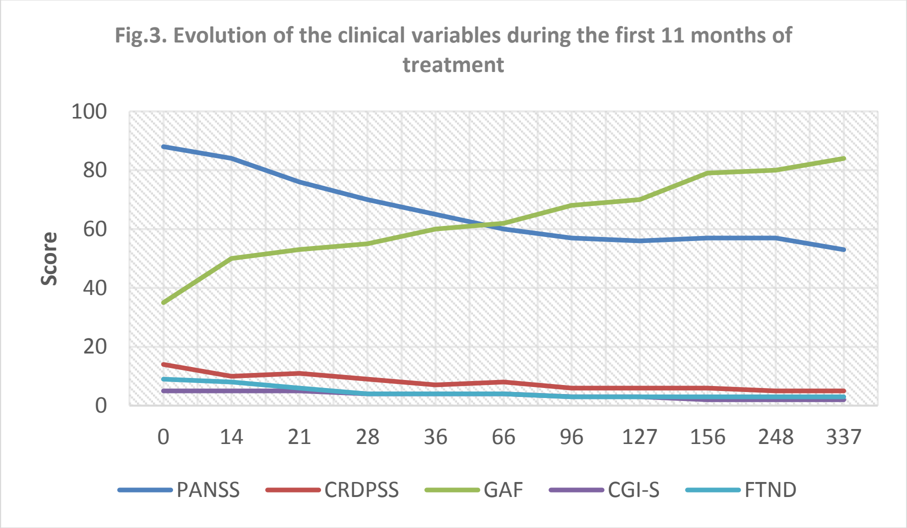

CONCLUSIONS

In young patients who experience first episode of psychosis establishing therapeutic relationship could be a difficult challenge. Communication between the psychiatrist and the patient is crucial in order to assure an adequate level of therapeutic adherence. The psychiatrist should consider the lifestyle of the patient, her psychological resources and specific needs, and to formulate the most appropriate therapeutic strategy. In this case a long-acting formula of an atypical antipsychotic was preferred because of the active lifestyle of the patient, and her expressed preference for a treatment which could be easily administered. The treatment for nicotine dependence has been a challenge, as the patient did not quit completely smoking, but only diminished it. Paliperidone could be useful in patients who smoke because it is not metabolized through CYP1A2, and its plasma concentrations remain stable even if this isoenzyme gene is induced by the polycyclic aromatic hydrocarbons of the tobacco smoke [34].

Abreviations list

AUDIT = Alcohol Use Disorders Identification Test
BMI = Body mass index
CBC = Complete blood count
CGI-S = Clinical Global Impression- Severity
CRDPSS= Clinician-Rated Dimensions of Psychosis Symptoms Severity
CUDIT-R = Cannabis Use Disorders Identification Test – Revised
EuroQoL 5D-3L= EuroGroup Quality of Life Scale
FTND = Fagerstrom Test for Nicotine Dependence
GAF = Global Assessment of Functioning
PANSS = Positive and Negative Syndrome Scale
PP1M = paliperidone palmitate with monthly administration
PP3M = paliperidone palmitate administered every 3 months
prn = *pro re nata*

Disclaimer

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ORIGINAL ARTICLES

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Patient reported outcome measures and joint replacement

Alexandra Sopu [1]

Abstract: *PRO (Patient Reported Outcome) is a clinically based questionnaire filled directly by patients, and other variant types of measures, in clinics and hospitals that gather patients' stance on their conditions in treatment. PRO is different from Patient Based Outcomes whereby the latter addresses the patient's concerns but do not necessarily enquire from them. However, PRO gather strictly from patients themselves through interviews, self-administered questionnaires and other available measures. The patient's perspective on issues that is significant in enacting certain particular clinical policies and regulations such as approval of a medication/drug. Most PROM constitutes one (one-dimensional) or more underlying assessments (multidimensional) connoted as constructs, which bear several levels of scale to assess degree.*

Keywords: *PROMs, orthopaedics, patients, hip replacement, knee replacement, healthcare system*

OBJECTIVE

The questionnaire or interview used to gather information is referred to as *measures, tools or instruments.* Commonly, there are two types of PROM questionnaires.

Generic PROMs, which are used to assess generally across numerous diseases in a broad spectrum perspective, and condition-targeted PROMs that are developed for a particular medical condition [1].

This paper critically examines patient-reported outcome measures (PROMs) and joint replacement from a broad perspective.

METHODS

Most PROMs measure aspects such as Quality of Life (QoL) that is fulfilment of needs and impact of restrictions on emotional wellbeing, and drug side effects. Others include symptoms/impairments that is pain and depression, functioning during disability, locomotion, daily living activities and personal care. In addition, Health Related Quality of Life (HRQoL), health status, general health experience and rating of healthcare facilities and operations [2].

Analysis of PROMs is usually conducted using approved analysis tools for proper interpretation such as Rasch analysis or confirmatory factor analysis. PROMs are often validated using particular tools and methodologies, such as Linguistic validation for population's differences and others to ensure that they are effective in gathering relevant information. Patient grouping too

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should be reliable and conform to ideal scaling, development and psychometric standards. Examples of renowned PROMs include the SF-36 Health Survey, EuroQol (EQ-5D), SF-12 Health Survey, Profile, Quality of Well-Being Scale, Health Utilities Index and Consumer Assessment of Healthcare Providers and Systems (CAHPS) [3].

These are examples of generic PROMs. Ideal examples of condition-targeted PROMs include Adult Asthma Quality of Life Questionnaire (AQLQ), Seattle Angina Questionnaire (SAQ), Kidney Disease Quality of Life Instrument, Epilepsy Surgery Inventory, National Eye Institute Visual Functioning Questionnaire, Ankylosing Spondylitis Quality of Life questionnaire (ASQoL) and Migraine Specific Quality of Life (MSQOL) [4].

With the advent of PROMs and the role they play in medicine, individual countries such as England's National Health Service (NHS) have made it a prerequisite for particular surgical operations to provide non-compulsory PROMs before the procedure and following the procedure (ideally three months after procedure); these include hip, knee and other joint replacements, hernia surgery and varicose vein surgeries.

England used the PROMs to assess the effectiveness and effects of the surgeries on its national a patients and deduced that the frequency of operations/ surgeries should be maintained. Due to their efficiency and importance in quality health service, PROMs are updated monthly as a policy in most developed countries. PROMs are currently used to grade health facilities with scores parameters according to patient satisfaction. In England, HES (Hospital Episode Statistics) use PROMs to rate hospital services across the state and their use are gaining impetus across the global health sector [5].

There is a general dataset that PROMs include in questionnaires; Generic and condition-specific measures of self-reported health status. Patient-identifiable information included in the PROMs, which is used for relation purposes, is strictly not availed for wider analysis, due to confidentiality. Additional questions inquiring into the patient's health status include whether they have antecedent conditions such as diabetes or arthritis [6]. The outcomes of a health procedure can be ascertained from the patient's perspective, through self-reported symptoms and functional status, by comparing and determining the differences in data between the pre-operative and post-operative PROMs.

However, the PROMs are not compulsory for patients to fill. More so, consent from patients who participate in the PROMs has to be sought before their data is used for analysis [7]. The patient's identifiable information is only used to electronically fetch for his or her National Health Number in government database during analysis of PRO data. The rest of the data is transferred to a database, such as the HES in England, from where the PRO analysis consequently occurs. Pre-operative and post-operative PROMs from the same patients are identifiable in the dataset since they possess similar serial numbers from which they are linked. After analysis, data in the HES is pseudonymised before it is made available to the public for download for analysis and scrutiny and hospital/clinical scoring [8]. Other uses of PROMs include: allows managers and clinicians to benchmark their own performance with regards to others, they are used for research purposes and draw relations to effectiveness and cost-effectiveness of health procedures to care. It is also used to compare implications of presence and absence of the treatment or rather alternative treatment, searching for healthcare inequalities, and research on relationship between pre-existing health and social conditions and risk of deterioration after procedure. Other than the anonymised data that is availed to the public for scrutiny and further personalized analysis, PROMs can be availed to service providers of patient care through provider level extract only with patient's approval. More so, extract service of particular requested data sub-subs by customers can be availed at an administrative fee depending on complexity of the request [9].

There are variant methodologies in which PROMs are used to score and rate health facilities. Some examples of standardised PROMs that are analysed by specific methodologies include the five-dimensional descript-tive system EQ-5DTM health questionnaire and the

EuroQol Group's visual analogue scale (EQ-VAS) [10]. Most PROMs used for joint replacement procedure, such as hip and knee replacement, are condition-targeted. Most common post-operative PROM questions include an inquiry into the patient's health status after procedure such as their state of mobility or other operative complications.The EQ-5D is a simple, generic measure of health for clinical and economic appraisal [11].

The PROM with single indexing values for health status and an unsophisticated descriptive profile is widely used in economic and clinical evaluation of healthcare and in health surveys of populations. The EQ-5D provided in joint replacements contains a descriptive system with issues on mobility, discomfort /pain, self-care e.g. washing and dressing, anxiety and depression and normative activities e.g. work, housework, study, family or leisure activities etc. Each of these five dimensions has several level statements which the patients tick against the most appropriately descriptive of his or her condition.

Each dimension has a score digit for each level statement hence every patient has five string scores thence the connotation '5D' [12]. Using a formula, the five string score are converted into a singular summary index, referred to as the 'social preference weights' assigned to each statement in the dimensions. The value of full health is assigned to value one (or state 11111, in EQ-'5D') from which reference is sought. EQ-VAS index scores range from 0 to 100, least and best health respectively. The patients mark, within the range, his or her relevantly perceived state of health [13].

Other PROMs used in England for joint replacement include the condition-targeted Oxford Knee Scores (OKS) and Oxford Hip Scores (OHS). The PROMs contain twelve multiple choices, assigned later with scores, about the patients state of mobility, pain, ease of joint movement, ease of partaking normal chores and activities. The scores in the PROM are such that the less the scores the poorer the patient's condition with zero for greatest severity. For each multiple choice, 4 is the greatest score for best patient condition. Hence, the total score for every patient in the PROM have a maximum limit of 48 for ideal patient condition while zero indicates worst severity [14].

Postoperative Recovery Profile in condition-targeted PROMs with recovery specific questions is used to determine the quality of joint replacement procedures in health facilities and person-centeredness of clinical services [15]. This is common procedure for persons with arthritis. The USA, Norway, Denmark, Sweden, New Zealand, Canada and England operate 77-153 and 66-143 hip and knee replacement per 100 people in prevalence rate. With advances in biomedical operations and medication intended to shorten or alleviate the post-operative recovery period especially since recovery takes place in the vicinity patient's home, PROMs are significant and effective method of evaluating these procedures on patients [16]. Traditionally, assessment of joint replacement were assessed by drawing connections between different intervention methods such as variant joint replacement procedures. The types joint replacement include prosthetics, implants, surgical techniques. Relations of these types of joint replacements were drawn to revision rates, complications and post-operative medications. With increased impetus on the use of PROMs, this evaluation is augmented thus allowing for an improvement of healthcare services [17]. While EQ-5D, EQ-VAS, OHS and OKS are important instruments in PROMs, they do not comprehensively provide adequate information important of the requisite important aspects that allow for quick recovery. Recovery-Specific Instruments have been devised to bridge this gap. Swedish healthcare PRP (Post-operative Recovery Profile) PROMs on joint replacement patients has comprehensive data on patient's problems, medical interventions and outcomes of treatments such that it has gained global recognition [18]. The Swedish methodologies of PROMs and their analytical tools are being replicated across the developed world since they include measurements on different groups of patients and can be altered for different purposes in the variant PROM areas [19]. The PRP has additionally incorporated global-, dimensional- and item levels in scoring not only for sole patients but more so for every group of patients. The global score is significant in deducting the recovery rate of a population-based profile.

An embodiment of a PROM is one in Sweden that was conducted on joint replacement (hip and knee) operations patients whereby the PROM questionnaire was provided the day before the procedure, three days after the procedure and one month later after the procedure. The peri-operative variables included sex, surgical procedure, American Society of Anaesthesiologists' (ASA) guided physical classification, age, duration of surgery, length of postoperative stay, blood loss and marital status [20]. PRP was included into the PROM and 19 *constructs* collected included physical functions and symptoms, psychological, social and activity measures.

Response category for assessments, from which scores would be assigned, included: none, mild, moderate, and severe symptoms. Recovery profiles for every individual and group on each item and dimension were provided by the PRP. The 19 item responses account for a detailed individual response profile over the recovery dimensions and the item frequency distributions reports on the item response profile of the group [21]. *Fully recovered* score in the group ideally would have indicator score of 19, 15-18 indicator sum for *almost full recovered* , 8-14 indicator sum for *partly recovered* , 7 indicator score for *slightly recovered* and below 7 *not at all recovered.*

Using methodological tools of frequency distribution analysis, out of the 75 patients who voluntarily participated in the PROMs assessment after undergoing primary knee and hip replacement due to osteoarthritis, 23 patients indicated the same level of pain on both the 3 day and one month follow-up [22]. The remainder showed a decrease in pain after the one month follow-up. Significance in RP values was used to assess the systematic change in recovery of groups. Individual variations within groups and between groups can thereafter be scrutinized.

RESULTS

Besides pain, other score categories included muscle weakness and re-establishment of everyday life. On physical symptoms and function's frequency distribution, for three days the frequency for the *none* assessment ranged between 62% and 7% while one month later, 72% to 25% [23]. Systematic group changes, unchanged assessments, individual variability in all dimensions were analysed. For global level assessments for day 3, 11(score for partly recovered) was the median while 13(score for partly recovered) was median after one month follow-up [24].

This information was used to ascertain whether the surgical intervention or rehabilitation in the joint replacement procedures and therapy were appropriate for individual patients or for groups of patients [25]. The PROMs data was also used to identify particular risks in particular groups (categorized by demographics) associated with the hip and knee replacements. While the analysis of the PROMs indicated homogenous recovery changes in the groups, certain assessments were unchanged for both the 3day and one month assessment; muscle weakness and pain.

However, great individual variations on the two categories were found to result to this. Using this data, Sweden was able to determine the best treatment and therapy techniques to render to joint replacement patients for a quick recovery [26]. It was also found out that a standardized treatment method for groups that exhibited great variations in individuals was not necessarily the remedy to the situation. Extensive use of PROMs in Sweden has allowed the country to increase its knowledge on the best healthcare practices hence an improvement in their healthcare delivery and high score/ratings of their hospitals internationally [27].

Based on the PROMs Swedish healthcare system is able to establish expected recovery within junctures in recover period. This can be sued to grade other treatments as set-backs and gains with regards to the expected outcome and therefore facilitate the overall recovery and create awareness of the recovery process. PROMs have also been used to enhance, as a clinical tool, the manner of clinical relationship and contact in follow-up visits between physicians, nurses and their patients. In joint replacement, resumption of normative daily activity and functionally capacity were usually found to be unsatisfactory.

The level of satisfactions was greatly influenced by pain, mental functioning and fulfilled expectations regarding postoperative pain [28]. The psychological dimension rated higher for those who concomitantly scored 13 in physical functioning and pain. Longer period assessment such as after 6 months have been shown to record greater recovery scores. Nonetheless, the Swedish HealthCare is keen on shortening the recovery period for joint replacement thus the focus of PROMs of short recovery periods. For long period PROMs, categories of assessment such as Quality of Life (QoL) and Health Related Quality of Life (HRQoL) are incorporated [29].

These PROM scores by patients who visit variant clinical and hospital facilities allow for the grading of hospitals/clinics too. Using relevant and respective methodological tools, scores from patients attended in various hospitals/clinics can assist in the national grading and scoring of hospitals. This initiates competition for better healthcare provision concurrently improving the healthcare of a country. Data has established that 70% of countries with esteemed healthcare systems have national policies on the use of PROMs in their HealthCare facilities.

CONCLUSION

Facilities that exhibit consistently low scores could be sanctioned and enquiries instigated into their medical practices. This allows for the monitoring of healthcare [30]. PROMs therefore are ideal instruments for the improvement of healthcare provision. They assist in determining the treatment and medication that ideal for patients in quick recovery mode. They create a patient-centred healthcare system. Hospital ranking/scoring on the other hand allow for benchmarking of clinical performances thus generally improving the provision of healthcare in a country [31].

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ORIGINAL ARTICLES

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Physical effort – an underused preventable method in colorectal cancer

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Abstract: *Colorectal cancer prevalence is increasing worldwide. Modifiable risk factors are responsible for almost 50 % of cases and this could imply a huge potential of preventability. Among these factors the level of physical activity is of paramount importance. Physical activity has a positive impact on health status in general and it decreases the prevalence of various cancers including colorectal cancer. Physical activity decreases the prevalence of benign colorectal adenomas and it prolongs the disease free interval after surgery in colorectal cancer, thus increasing survival. The mechanisms involved are multiple: decreasing bowel transit time, regulating energy balance, decreasing peripheral insulin resistance, decreasing hyperinsulinism, antiinflamatory effects, increasing vitamin D production.*

Keywords: *colorectal cancer, physical activity, obesity, lifestyle modifications*

Physical activity is a major and potentialy modifiable component of life style, which may be able to highly influence the risk of main cancers. Hence, there are convincing evidence that an important benefit may be derived concerning risk reduction in endometrial cancer, colorectal cancer, breast cancer, prostate cancer, lung cancer and ovary cancer. It is estimated that in Europe in 2008 between 150000 and 300000 cases of cancer could have been prevented only by the way of maintaining a resonable level of physical effort in general population.[1]

A series of convincing observational data suggest that regular physical activity, be it ocupational type or recreational type, protects against colorectal cancer (CRC)[1,2]. Around 60 studies have been published till 2010 concerning the issue of physical activity and CRC.[2] A metaanalysis that included 21 studies stated a significant reduction of CRC risk by 27% in the group of subjects that performed vigurous physical activity as comparing with the group of sedentary subjects (RR 0.73, 95% CI 0.66-0.81).[3] The mechanism that may provide an explanation for the

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relative protection of physical effort is currently unknown. There are no interventional type studies published yet to support the role of regular physical effort as preventive method in CRC.

In 2007 the results of a cohort study (Nurses' Health Study) has been published that enrolled 80,000 female subjects from 1986 with a 16 years follow-up. There have been diagnosed approximately 500 cases of CRC. A multivariate analysis that controlled the confounding factors represented by other risk factors for CRC concluded that there was a proportionate reversed relationship between physical effort and distal colonic cancer and, to a lesser extent, with proximal colonic cancer. Women situated in the highest percentile of recreational physical activity had a reduction of distal CRC risk by half as compared with women situated in the lowest percentile (RR=0.54, 95% CI 0.34-0.84). Risk reduction did not vary with body mass index (BMI), although former studies had suggested that physical activity had the greatest impact on CRC only in high BMI subjects. The level of physical effort to produce prophylactic benefits may be only minimal, as this study demonstrated. As such, even an hour of slight walking a week may reduce the risk of CRC by 31% (RR=0.69, 95% CI 0.45-1.03) as compared with women who do not report any kind of physical activity. This protective effect of slight walking reached a plateau at 2 hours a week (RR 0.64, 95% CI 0.41-1.00) as opposed to moderate and vigorous physical effort that was characterized by very clear dose-response relationship. The more alert slight walking rendered greater protective effect than slower slight walking (RR=0.43, 95% CI 0.17-1.05). Furthermore, 4 hours a week of moderate/ vigorous physical effort may reduce the risk of CRC by 44% comparing with 1 hour a week (RR= 0.56, 95% CI 0.33-0.94). Physical activity lessened the risk of CRC regardless of the impact on BMI: this idea may imply that physical activity protects against CRC by a mechanism independent of that involved in resolution of obesity. The protective effect of physical effort validates at distance. The actual reduction of CRC risk is considerable only after several years. In conclusion, the authors of this study suggest that even minor physical effort may derive benefit on CRC risk reduction. Several mechanisms have been proposed. Thus, physical activity regulates energetic balance and intervenes in reduction of hyperinsulinism and peripheral resistance to insulin. Physical activity may intervene also through anti-inflammatory mechanisms. Moreover, the positive effects of physical effort may also be explained by reduction of obesity in spite of the data that demonstrated that physical activity may reduce the risk of CRC independent of the effect on obesity. Another proposed mechanism involves accelerating the peristalsis which reduces the contact time between intraluminal carcinogens and colonic mucosa. As a matter of fact, it is well known data that physical active individuals are more prone to sun exposure for longer periods of time which facilitates production of vitamin D that is associated with lessening the risk of CRC.[4]

Figure 1: Mechanisms involved in protective effect of physical effort on colorectal cancer risk

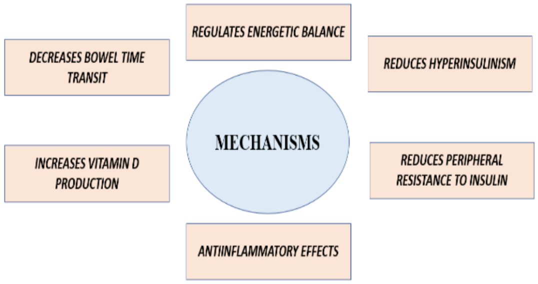
REGULATES ENERGETIC BALANCE
DECREASES BOWEL TIME TRANSIT
REDUCES HYPERINSULINISM
MECHANISMS
INCREASES VITAMIN D PRODUCTION
REDUCES PERIPHERAL RESISTANCE TO INSULIN
ANTIINFLAMMATORY EFFECTS

An epidemiologic study published in 2008 (NIH-AARP Diet and Health Study) shows interesting observations regarding the periods of an individual life when the

physical effort has the utmost impact on the risk of CRC. Thus, if the physical activity is performed in the age group 15-30 years the impact on CRC risk reduction will be minimal; on the other hand, if the physical activity is performed in the age group 30-39 years or throughout the whole life of an individual the reduction of CRC life-time risk will be maximal.[5]

Sedentarism is a globally important public health issue, especially in developed countries, in women, in old people and in low income individuals. Lack of physical activity is responsible of the increase in mortality rates especially from diabetes mellitus and heart diseases. To a comparable extent physical activity of moderate and vigorous intensity is associated with certain benefits regarding health status, including reduction of obesity risk, cardiovascular risk, stroke risk, risk of some types of cancers and decreasing in global mortality rate. Physical activity increases the probability to cease smoking, delays cognitive decline in old individuals, alleviates the adverse effects of stress, anxiety and depression. A study published in 2016 regarding the issue of physical activity status in the group of more than 50 years old individuals in USA the date are worrisome: 27% do not report any kind of physical activity outside working place in the last month; the prevalence of inactivity increases with age, reaching 35.3% in age group after 75 years; sedentarism is more prevalent in women then in men, in Afro-Americans then in Caucasians. Also, the prevalence of inactivity is decreasing with increasing in educational level and with decreasing in BMI.[6]

Lack of physical activity is the main cause of CRC being responsible of 14% of cases of CRC in USA; 12% of cases may be attributable to western diet, 12% to lack of daily administration of aspirin and 8% may be related to a family history of CRC.[6]

Sedentarism, especially that kind related to spending time in front of TV, is independently associated with increasing CRC risk. Hence, if one spends 9 hours in front of TV, as comparing with 3 hours or less, the risk of CRC will rise significantly by a RR=1.61 (95% CI=1.14-2.27).[5]

The relationship between physical activity, sedentarism and BMI is not to be changed even if one may exclude the contribution of age, race, family history of CRC, smoking and western diet.[6]

Several studies shows contradictory results concerning the issue of rectal localization of CRC. Cancer Prevention Study II indicates that moderate/vigorous physical activity in men and in women reduces the risk of rectal cancer by 30 %.[7] Many cohort studies did not find any kind of association between physical activity and rectal localization of CRC.[8,9]

A meta-analysis published in 2010, which included 20 studies on physical activity and colorectal benign adenoma, concluded that there was 16% reduction of the risk of these benign precursors of CRC if we compared active population with less active populations. Risk reduction was even more significant if we took into consideration polyps bigger then 1cm (31% risk reduction). It has been demonstrated in that way that physical effort might decrease the risk of CRC earlier in the stage of precursor lesions of oncogenic process.[10]

The role of physical activity as a protection factor in CRC is hardly known in general population. A study developed in USA that included 2000 subjects showed that only 15% of them are aware of this benefit of physical activity.[6] One similar study from Europe that included 21 countries indicated a 30% level of knowledge concerning this topic.[11] Several studies stated also that there was a close connection between the level of information concerning prophylactic benefits of physical effort in CRC and the increasing of the motivational status to produce life style changes that, in the end, will decrease the prevalence of CRC.[12,13,14]

Physical exercises represent a form of human activity that may benefit health more then it may inflict side effects. The most common side effects are musculo-skeletal injuries. The least common side effects (sometimes more severe) are: cardiac arrhythmias, heart arrest and myocardial infarction. Generally, we may appreciate that the potential benefits of physical exercises highly surpass the potential risks. Moreover, it is considered that it is unnecessary to screen for coronary diseases prior to initiating physical activity if

the subject was asymptomatic and included in the low cardiovascular risk group.

One may consider mandatory that all healthy adult individuals to include in their life style moderate or vigorous physical exercises. The majority of authors agrees that the highest health benefits are provided by 150 minutes a week of moderate physical activity or by 75 minutes a week of vigorous physical activity. Nevertheless, adults that have a limited physical activity capabilities should remain active because it has been noticed that even if a modest amplitude of physical effort is exercised regularly health benefits will be significant.

Another epidemiologic studies suggest that physical activity may influence not only the risk of CRC but also it may prevent the recurence of CRC after curative surgical treatment. All the data available resulted from observational type of studies; randomized and interventional studies are not published. Nevertheless, American Society of Oncology (ASCO) recently recomended that the surviveours of CRC should maintain an optimal weight, should perform daily physical exercises and should follow a healty diet.[14] Futher on, there are some interesting results of a study published in 2006 that included 832 patients suffering from CRC stage III surgicaly treated and that followed a program of chemotherapy. It has been demonstrated that moderate physical activity perfomed for at least 300 minutes a week has increased the free disease interval with 45% and has improved by 29-36% the mortality rate of any cause.[16] The benefits have been dose dependent.

In an observational study (unpublished data) that I have conducted in 2016, concerning the topic of CRC and its relationship with diabetes mellitus and other risk factors an important conclusion has been drawn.

A multivariate analisys in which the most acknowledged CRC risk factors have been included showed the statistical significance ($p<0.05$) have been reached for smoking and physical activity only. This implies that by the way of increasing physical activity levels in general populations and by giving up smoking CRC „epidemics” could be fairly prevented.

Table 1: Multivariate analysis of risk factors in CRC

Variabile	*Coeficient*	*Standard error*	*t Stat*	*p*
Age (years)	0.0014	0.0035	0.4087	0.68
BMI(Kg/m2)	0.0031	0.0055	0.5650	0.57
Smoking	0.1273	0.0647	1.9658	**0.05**
Daytime nap (hours)	0.0150	0.0440	0.3404	0.73
Physical activity	-0.1334	0.0601	-2.2181	**0.02**

In conclusion, the preventable potential of CRC is high through the way of regular physical exercise and this may represent a very approachable solution to decrease the global burden of the disease. It is very important to stress that the target of decreasing the prevalence of CRC by physical exercise does not necessarily imply an impact on obesity, the benefits on CRC being independent from the benefits on BMI. A good level of motivation in general population through health politics is mandatory because the changes in life-style (level of physical activity, diet and smoking) are otherwise impossible to be reached.

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The communication and promotion policies of the medical organizations in the marketing of Romanian healthcare services

Bogdan I. Coculescu[1,2], **Victor L. Purcărea**[3], **Elena C. Coculescu**[4]

Abstract: *The interdisciplinarity of the marketing department is due to the application of concepts, methods and marketing technics specific both to service and to social marketing. In addition to this fact, the attempt of the social services to satisfy the patient's needs places the health care domain at the border between social and economic, between profit and non-profit orientation. However a lot of the notions from the marketing field (competition, promotion, strategy, need, supply, cost etc.) acquire new meaning when used for defining the rivalry between the distinct medical organizations, the advance of the health care services, the development and implementation policies in medical marketing, the increasingly acute demand for treatment, the use and the supply of health care services as well as the cost that it requires..*

Conclusion: These above described microscopy method can be used to distinguish between benign and malignant thyroid nodules, based on different degree of the capsular collagen fibers orientation.

Keywords*: communication policy, promotion policy, marketing mix strategy, Romanian healthcare services*

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INTRODUCTION

Medical organizations communication policy towards the health care market through constructive and favorable relationships are an important objective that every health care provider should promote. Primarily communication strategies target the following aspects:

- Promoting the service offering of medical organizations in order to attract new potential clients;

- Persuasion of the potential clients for the necessity of purchasing these services by presenting the positive advantages of the respective health care procedures. Communications possess an important role in the buying process, taking part both at the pre-sale and sale, and also post sale stages [1-4].

DISCUSSION

Communication is a constituent of a great importance in the marketing mix (product – cost – distribution – development) aiming at establishing and maintaining

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a steady relationship with the patients. It represents the tool with which an entity participates in the informational exchange with the different business field's components, to inform about its presence, the products and services it provides, to create a positive attitude and to stimulate consumers to buy products and services.

Figure 1: Communications and healthcare services marketing [5].

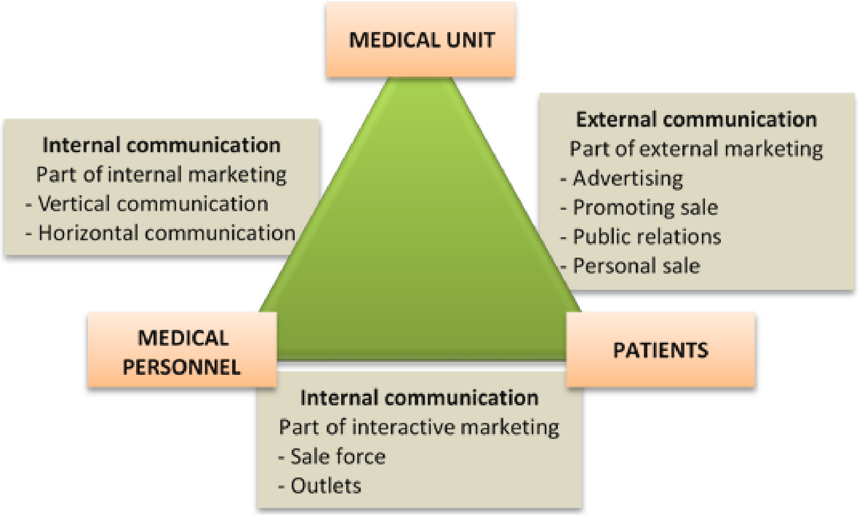
MEDICAL UNIT
Internal communication
Part of internal marketing
- Vertical communication
- Horizontal communication
External communication
Part of external marketing
- Advertising
- Promoting sale
- Public relations
- Personal sale
MEDICAL PERSONNEL
PATIENTS
Internal communication
Part of interactive marketing
- Sale force
- Outlets

The instruments of the communicational mix are based particularly on interpersonal communication, yet at the same time on the adaptation and enrichment of the classical techniques via the concept of marketing integrated communication resulting in complex communicational programs. The marketing mix in the health care services sector includes as well staff politics, represented by two segments which must be approached differentially as follows: the employees of the company providing services and the consumers.

The principal methods and ways to communication, that can be adopted by a medical organization in order to orientate the patient in their referring to a certain health care service or to build and reinforce a favorable image of that sanitary unit on the market, constitute the promotional mix of the medical institution.

The achievement of an optimal promotional mix, which satisfies the patients' needs and fulfill the best objectives of the promotional communication, is one from the key points in obtaining the attributions distinctive for a marketing specialist.

There are two ways by whom a medical organization can communicate with its patients (Figure 1):

a. On the outside through:

- advertising
- promoting sales
- public relations
- direct marketing (inclusive online)

b. On the inside through the employed medical personnel during the specific activities.

The advertising activity has clear purpose in preparing the target public for favorable receiving of the medical unit's offer. Three types of marketing objectives are to be distinguished from one another depending on their purpose as follows: informing, convincing and reminding. To advertise and broadcast the commercial message the medical organization can make use of different communicational channels: newspapers, magazines, the press, printing materials (flyers, brochures, catalogues), external publications (billboards, posters, leaflets, stickers), internet, radio, television etc. Through advertising activities the medical organization succeeds in informing the potential patients about its work and services with the aim of influencing their decision for adherence to this services.

The direct relationship between the medical staff and the patients favors a particular manner of communication: personal sale – the potential patients can be notified and persuaded to subscribe for services at the medical unit. Consequently the medical contact personnel plays an important role for increasing the sell's volume in the medical system. They must be very well prepared professionally and respond promptly to the patients expectations. Additionally the immediate connection with patients represents the main way for informing them about the benefits, offers, promotions, advantages, performance conditions, health care services prices etc. of the medical organization they belong.

A lot of the authors specify a clear distinction from the concept of "team" and of "teamwork". The "team" concept is referring to persons who work together for common purpose, while the "teamwork" concept – to a certain environment from a larger organization, which creates and sustain relations of trust, support, respect, interdependence and collaboration.

It must be mentioned that in sanitary organizations – particularly in the hospitals – team communication possess an increasingly important role. A good team communication, understanding the advantages, disadvantages, "principles" of teamwork, contributes to identifying the proper solutions to the inherent problems. In multiple cases where it is necessary to work as a team, encouragement and orientation of the team members can improve the sanitary organization's results via: their motivation, use of the team member's ideas and personal capabilities, acquiring support from their side, improving the performance. Through guidance, the quality of health employee's performance can be made better, while the tasks are accomplished properly by them.

Promoting is one of the forms of communication. The difference between the two notions are made at the level of the sent message. So that promoting to have the desired effect, the messages received by the patients must be clear and reflect what the organization has to offer.

Promoting, as a variable from the marketing mix, occupies place apart in the case of medical services, because it is essential for the development and maintenance of durable relations with the target public. Marketing politics aims to inform the target public as much as possible about the health activities and the services offered by the respective medical organization, but at the same time also for the information received by them to have a positive impact.

A particular case of promoting of medical organization is represented by spreading the "mouth to mouth" advertisement, from patient to patient, extremely productive, as demonstrated fact in the practice of health care service with results in growth of consumers addressability in these services ("one satisfied patient brings more patients").

Client services in the field of health care relates to the benefits offered to the patients – or the public in general – further than the product itself, including its nontechnical and nonclinical aspects. The connection between client services and other elements of the marketing mix is one of completion and support. The efficient client services reduce the cost due to patients and improves the access to health services [4-6].

CONCLUSIONS

Promoting sales imposes the use of all procedures and stimulation techniques and increasing the sales of medical services of the organization. If advertising has a role in offering purchase motivation, conversely the sell promotion has a role in the sells' stimulating process for the potential patients by the means of consecrate methods in promoting sales, in the form of promotional presents (watches, calendars, agendas, pens, notebooks, umbrellas with inscription of the medical organization etc.), with the aim of image promoting on the target market.

Public relations have a part in setting trust relations with the patients, ensuring protection, planning, organizing and controlling the whole actions unfolded by a medical organization for achieving its objectives. The methods used in the activity of public relations for obtaining the marketing goals (informing the patients about the advantages of the organization, stimulating the sales volume, keeping the investment in promotional materials at minimal level) consists in organizing events with scientific subject (congresses, conferences), giving interviews, publishing brochures, profile publications, promoting through press conferences, participating in medical markets and exhibitions etc. The coordination of a public relations project with the other elements from the promotional mix can be beneficial for the increase in prestige of the medical organization.

The core of the marketing strategy in the field of health care is presented by the quality of services, quality which in its turn results from: precision of performance, promptitude and professionalism of the employees, kindness and politeness towards the patients. Creation and implementation of a coherent and productive medical marketing strategy as well as defining the value system of a sanitary organization consists a vital necessity for the organizations, whose purpose is executing top health services.

In the field of health care services the marketing strategy represents actually the attitude of the sanitary organization towards the marketing environment and simultaneously its behavior in regard to its components.

Communication of the organization with the marketing environment is an essential condition for achievement of its activity objectives. The fulfilment of the organization mission assumes concentrating marketing efforts in the direction of achieving a permanent and efficient communication with the external surroundings, with the market and with the patients.

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Medical applications of the GC/MS method in the acute intoxication with dimethoate – clinical case

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Abstract*: Mass spectrometry is a chemical analytical method of determining organic substances by comparing their mass spectrum with mass spectra found in system libraries. In the case of biological products, substances of interest, like organophosphorus compounds, must be separated and identified for rapid and good medical measures (antidotism procedures) in acute intoxication case. A gas chromatograph coupled with a Varian mass spectrometer (GC-MS), was used to develop the application. The proposed objective is presenting the medical applicability in acute organophosphorus compounds intoxication management of the GC/MS method (gas chromatography coupled with mass spectrometry) as a separation and identification method for these compounds and their metabolites in urine samples.*

Keywords: *dimethoate, GC/MS, urine, acute intoxication*

INTRODUCTION

Acute intoxications represent a worldwide problem that tends to gain more amplitude each year.

Each intoxication presents certain characteristics which stem from the degree of socio-economic development of each country.

The organophosphorus compounds are mainly used to fight pests, as an alternative to chlorinated hydrocarbons, which persist much longer in the environment. Yet these substances are very toxic for humans too.[6] Because of this, measures were taken both to limit their utilization and to control the contamination of the environment.

The most efficient, but also most toxic substances utilized as pesticides are cholinesterase inhibitors (through reversible or irreversible mechanism). For both mammals and insects, the major effect of these substances is the inhibiting of acetylcholinesterase through the phosphorylation of the esterase site. The signs of symptoms which characterize the acute intoxication are caused by the inhibition of this enzyme and the accumulation of acetylcholine. Some of these substances possess a direct cholinergic activity.[4]

The absorption of organophosphorus compounds can be realized through three methods: inhalation, digestive and through the skin. One of their main characteristics is the fixation at hair level, where they enter through the skin, thus representing a permanent source of intoxication. Through the inhalation way, the intoxication is the most rapid. Through direct action on

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the bulbar respiratory center (muscarinic phenomenon) and the paralysis of respiratory musculature (nicotinic phenomenon), respiratory arrest occurs. Organophosphorus compounds are rapidly absorbed through the skin or digestively in the thin intestine. They bind well to the plasmatic proteins. High concentrations can be attained in the body within hours.[7]

Organophosphorus compounds represent a group of compounds with liposolubility or hydrosolubility, with high distribution volume. They are rapidly distributed in the liver, lungs, kidneys, heart and brain but do not accumulate.

This type of organophosphorus compounds is liposoluble, accumulating in the lexophile tissues of the organism, being a source for metabolic conversion to very toxic compounds. Thus, the intermediate syndrome can be explained (it appears between the 5th and 18th day from the intoxication). The toxicity of these compounds is manifested through the direct inhibition of cholinesterase, probably through direct poly enzymatic inhibition.[7]

The metabolizing of organophosphorus com-pounds takes place rapidly in the body, and as such they do not accumulate. Compounds such as parathion, malathion, phenthion, chlorpyrifos, normally inactive, upon entering the body they transform at liver microsomal level, through oxidation, in highly active compounds (paraoxon, malaoxon).

This transformation takes place under the action of paraoxonase. Their degradation takes place through hydrolytic and oxidative ways, through liver and kidney enzymes. The erythrocyte-origin cholinesterase remain blocked for the remainder of the red blood cell's life. Their regeneration takes place slowly (0.5-1% per day), remaining below normal level for over 3 months in severe intoxications.[8]

Organophosphorus compounds are eliminated through urine as such or as metabolites.

In the current global situation, it is very probable that these substances will be used in wars, conflicts, terrorist attacks. In such scenarios, they are used as extremely toxic agents and thus continuously represent severe threats to humans, animals and the fauna.[2]

The dimethoate, patented and introduced in the 50s, is a acetylcholinesterase inhibiting organo-phosphorus compound, it is not volatile, it is water soluble and it is not mobile in soil, where it degrades with a half-life of approximately 2-4 days, depending on the conditions. Dimethoate and omethoate urine levels reflect recent exposure.

Once having entered the body, organo-phosphorus compounds are metabolized to dialkylphosphates, which are eliminated through urine. Their metabolizing takes place rapidly in the body and as such they don't accumulate.

Compounds such as parathion, malathion, phenthion, chlorpyrifos, normally inactive, enter the body and transform at liver microsomal level, through oxidation, into highly active compounds (paraoxon, malaoxon). Identification of these metabolites in the urine may be an indicator of exposure to organophosphorus compounds and can be performed through a GC/MS analytical method with an ion trap and electronic ionization.

The dimethoate is rapidly metabolized, mainly through the initial splitting of the C-N bond to obtain dimethoate carboxylic acid and, eventually, a number of tiophosphate and phosphate esters. The minor quantitative elimination way involves the oxidative metabolism to produce the oxygen analogue of dimethoate, omethoate. The parent compounds represents 1-2% of the dose excreted in the urine.[5]

MATERIALS AND METHOD

The research was performed on a GC-MS Saturn 2000 Varian system composed of gas chromatograph model Varian CP – 3800 and mass spectrometer Varian Saturn – 2000.

Establishing optimal working conditions and functional parameters for the development of a GC/MS method are important steps in the development of a GC/MS method for the separation and spectral identification of dimethoate in urine.

ANALYTICAL CONDITIONS

GC – an instrument equipped with a ion trap detector; GC – gas-cromatograph has the role of taking in the sample and separate the mixture of substances composing it.

GC/MS parameters

Injector temperature: 300° C
Carrier gas: He • Column flow: 1.2 ml/min
Separation time: 50 min
Ionization mode: electron impact (70eV)
Ionization current: 20 µA
Ionization temperature: 170° C
Detection mode: full scan
Manifold temperature: 80° C
Ion trap temperature: 170° C
Interface temperature GC-MS: 260° C

The separation of the compounds was realized by the active layer of the DB-5MS capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm). The optimal conditions for chromatographic separation and detection were established following the study on the compound's retention time's dependency on the structures of the said substances.

The temperature program of the gas-chromatograph's column's furnace is presented in Table 1.

Table 1: The column gas furnace temperature program.

Temperature (°C)	Rate (°C/min)	Hold (min)
140	0.00	1
290	5.00	19.00

MS – mass spectrometer **–** has the role of analysing molecules that come out of the gas-chromatograph through their unique mass spectre. Thus, the molecules that come out of the GC can be identified by the user. Mass spectrometry established the relative abundance of ions resulted from the ionization process of an organic molecule. The method is used in chemical analyses, in the analysis of some quantum processes or in the separation of certain chemical elements. The determined mass represents the m/z ratio (mass to charge) of the atom or group of atoms from which the ions resulted.

Mass spectrometer parameters

Manifold temperature = 800º C
Ion trap temperature = 1700º C
Ionization current = 20 µA
Acceleration tension = 70 eV
Working times
  0 - 5 min – closed filament
  5 - 37 min acquisition in mass domain 50 - 450 amu.
Acquisition domain 50 – 400 amu
Segment setpoint
Scan time = 1 sec/scan
Multiplier offset = 0 V
Emission current in FS 10 µA
Ion threshold 1 count

Scanning parameters for ions formed in the trap are presented in Table 2.

Table 2. Scanning parameters for ions formed in the trap.

Low Mass (m/z)	High Mass (m/z)	Ionization Storage Level (m/z)	Ionization Time Factor (%)
10	99	48.0	100
100	249	48.0	100
250	399	48.0	100
400	650	48.0	100

Normally, the mass spectrometer operates in the domain in which the analyzed substances will be found. When coupling it with a gas-chromatograph, substances no greater than 450 amu will be sent to the mass spectrometer, as those with greater molecular weight cannot be vaporized in the chromatographic column.

Thus, the maximum acquisition domain will be 50 – 450 amu, as below 50 amu an acquisition is not typical, since the atmosphere in the apparatus has a rich spectre up to 44 amu.

The use of the full scan technique (FS) is very good, since following the obtaining of the mass spectre, it can be compared with mass spectres already existant in dedicated spectral libraries. In this case though, the duration of a ion's analysis is of 4ms, in case it is used as an acquisition time for a scan of 1 s.

Confirming the identity of the compounds relies on comparing the mass specter and ratio of reference ions' abundance for each analyzed substance identified in the sample with those of standards using the mass specter library. To identify the obtained specters, the following were used: Pfleger – Maurer – Weber specter library (PMW), specialized for compounds of interest in toxicology as well as specter libraries NIST2000 and Wiley6. If a mass spectre obtained from the sample cannot be compared with mass spectres in specialize spectral libraries, the identification of these compounds in biological matrices would not be possible to perform.

RESULTS AND DISCUSSIONS

Dimethoate has moderate acute toxicity for mammals (for example, DL50 in mice and rats is 150 and 400 mg/kg of bodyweight) (IPCS, 1989). Omethoate is approximately 10 times more toxic and a stronger cholinesterase inhibitor than dimethoate. Dimethoate is well distributed in the body's tissue and metabolized in the liver to omethoate (most probably through the enzyme system of P450 cytochrome) which is then quickly transformed into multiple dialkyl methyl phosphate metabolites, which are eliminated in urine within 1-2 days. Dimethoate is considered mutagenic, but it is not teratogenic. [1,10].

In order to verify the developed methods, they were applied on biological samples obtained from patients in the ATI II Clinical Toxicology Section, patients suspected of acute organophophorus compounds intoxication. The urine (aprox. 25 ml) undergoes liquid-liquid extraction procedures in order for the sample to be analyzed through the GC/MS system. For example, we present the medical applicability of this method in the case of an acute dimethoate intoxication.

The separation and identification of dimethoate or omethoate in urine corroborate with the enzymatic activity determinations for serum pseudo-cholinesterase and lead to the establishment of an analytical diagnosis in case of an acute dimethoate intoxication and the initiation of specific therapies for this type of intoxication (such as antidotes). Identifications are performed through the comparison of the mass specter obtained through the analysis of urinary extract samples with mass specters already existent in the database. This specter is obtained based on the molecular mass of the compound of interest which, following fragmentation, gives birth to specific spectral lines. These are shown in tables 3 and 4.

CASE REPORT

C.P., male, aged 19, no occupation

Admission reasons
- Coma
- Acute respiratory insufficiency
- Muscular fasciculation

APP – no significant case history

History – patient with no significant case history is found by his parents in a coma with respiratory dysfunctions and muscular fasciculation, symptoms that follow the voluntary ingestion of an organophosphorus pesticide. Near the young man, the parents found an unlabeled bottle containing a liquid with a smell particular to insecticides. They call the ambulance and the young man is transported to the Clinical Emergency Hospital.

In the Major Emergencies Department, the patient is in a coma with severe dyspnea and muscular fasciculation. The oropharyngeal secretions are vacuumed and orotracheal intubation and Ruben balloon ventilation are applied.

Table 3: Specific ionic fragments and spectral lines for dimethoate

Compound	Spectral line (m/z)	Chemical formula of ionic fragment
Dimethoate C5H12NO3PS2 (M=229)	157	– [M-72] - (CH3O)2PS.S+
	143	– [M-86] - (CH3O)(HO)PS.S+
	125	– [M-104]- (CH3O)2PS+
	93	– [M-136]- (CH3O)2P+

Figure 1: Mass spectre for dimethoate (Nist98 library)

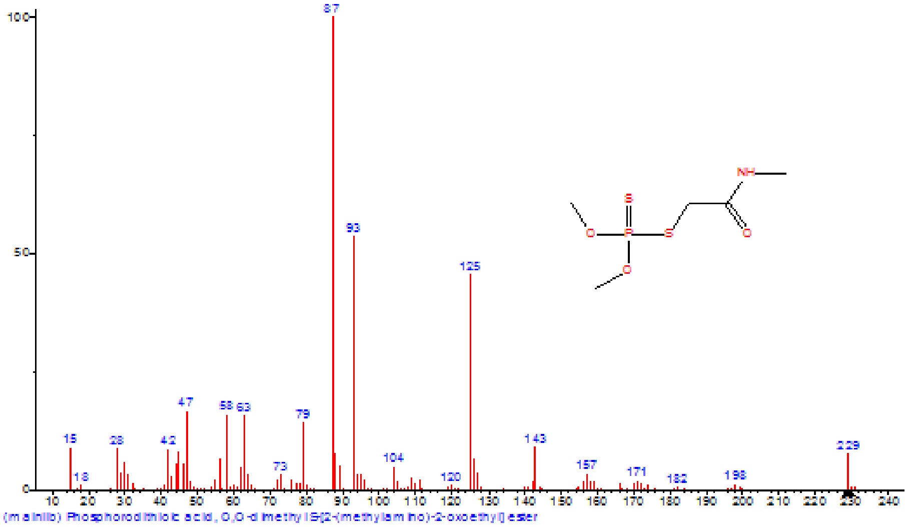

Figure 2: Mass spectre for omethoate (Nist98 library)

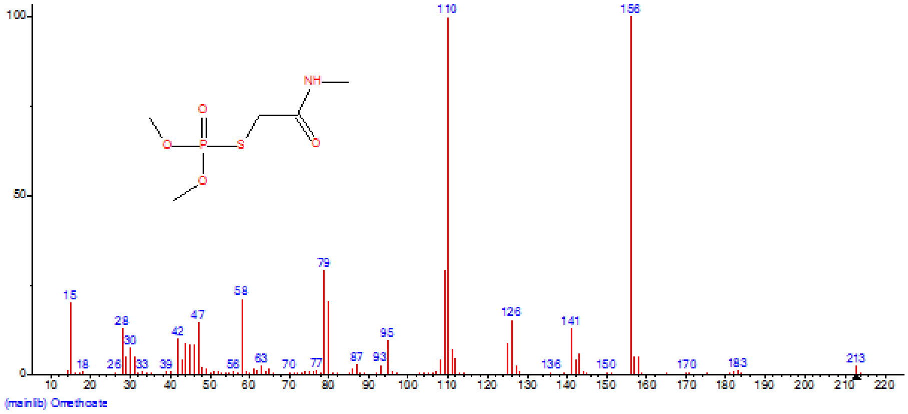

Table 4: Specific ionic fragments and spectral lines for omethoate

Compound	Spectral line (m/z)	Chemical formula of ionic fragment
Omethoate C5H12NO4PS (M=213)	156	– [M-57] - CH3NCO
	141	– [M-72] - (CH3O)2P=O.S+
	126	– [M-87] - (CH3O)2(HS)P+
	110	– [M-103] - (CH3O)2(HO)P+
	109	– [M-104] - (CH3O)2P=O+
	79	– [M-134] - (CH3O)(HO)P+

The determination of pseudocholinesterase activity detects a high degree of inhibition of 0.5 UI/ml. A urine sample is being taken for the toxicological examination and is sent to the Analytical Toxicology Laboratory. The patient is hospitalized in the ATI Toxicology Section.

Objective examination upon admission:

- Severe general condition;
- Reed IV coma; non-reactive;
- Pale, sweaty skin;
- Cyanotic extremities;
- Miotic, equal pupils, with slow photomotor pupil

reflex;

- Strong reflexes in all four members, with subintrant muscular fasciculation;
- At pulmonary level, bronchial rales bilaterally diffuse;
- Does not efficiently ventilate on IOT probe, so mechanical ventilation is applied;
- Diarrhea;
- Arterial Tension = 80/60 mm Hg;
- Subfebrile (t^o = 37.5).

ECG upon admission records sinus bradycardia (44/min) without conduction or repolarization.

The CT brain scan and lumbar puncture exclude a vascular etiology of the coma.

The analytical toxicological GC/MS examination of the urine:

Following the analysis of the urine, the total ion chromatogram that is shown in Figure 3 was obtained. In it, besides dimethoate, its metabolites can also be identified.

The substances identified through the above GC/MS method used for the urine analysis are shown in table 5.

The cardiopulmonary radiography detects a homogenous opacity situated in the inferior right pulmonary field.

Therapeutic measures

Stabilization

- Vacuuming tracheobronchial secretions
- Support ventilation
- Nasogastric intubation; gastric lavage;
- Central sub-clavicle catheter
- Parenteral fluids
  - o glucose solution;
  - o Ringer solution;
  - o isotonic saline solution;
  - o Gelofusine;

Support therapy

- Specific antidote – atropine under clinical surveillance
- Mucolytic – acetyl cysteine;
- Bronchodilators – miofilin;
- Antibiotherapy in combinations (penicilin G 1 milion UI/6 hours + gentamicin 80 mg/day + metronidazol 500 mg/day);
- Plasma – 4 units/day, for 3 days and 2 units/day for 2 days;
- Monitoring vital functions;
- Monitoring pseudocholinesterase activity.

The applied therapeutic measures, respiratory and general nursing are continued.

Clinical evolution

4 days from admission, the evolution is favorable; the patient is conscious; he is taken off the mechanical ventilation apparatus; he breathes spontaneously with the intubation probe and is extubated. His oral cavity is washed.

The cholinesterase activity is measured: 2.1 UI/ml.

The antibiotherapy is continued 3 days from the extubation to solve his pulmonary affection.

The evolution is favorable. The patient is released 7 days from admission with no neurological damage.

Table 5: The substances identified in the urinary extract that was analyzed through the GC/MS method and their specific spectral lines.

Compound	MW	EI fragment ions (m/z)
Dimethoate	229	87, 125, 93, 79, 229
Omethoate	214	156, 126, 110
Dimethoate M (HOOC-) ME	230	93, 125, 198, 230
Phosphoditioic acid – O,S,S trimethyl ester	172	93, 109, 125

Figure 3: Dimethoate and its metabolites – omethoate, dimethoate M (HOOC-) ME and the phosphoditioic acid – O,S,S trimethyl ester. Omethoate mass specter.

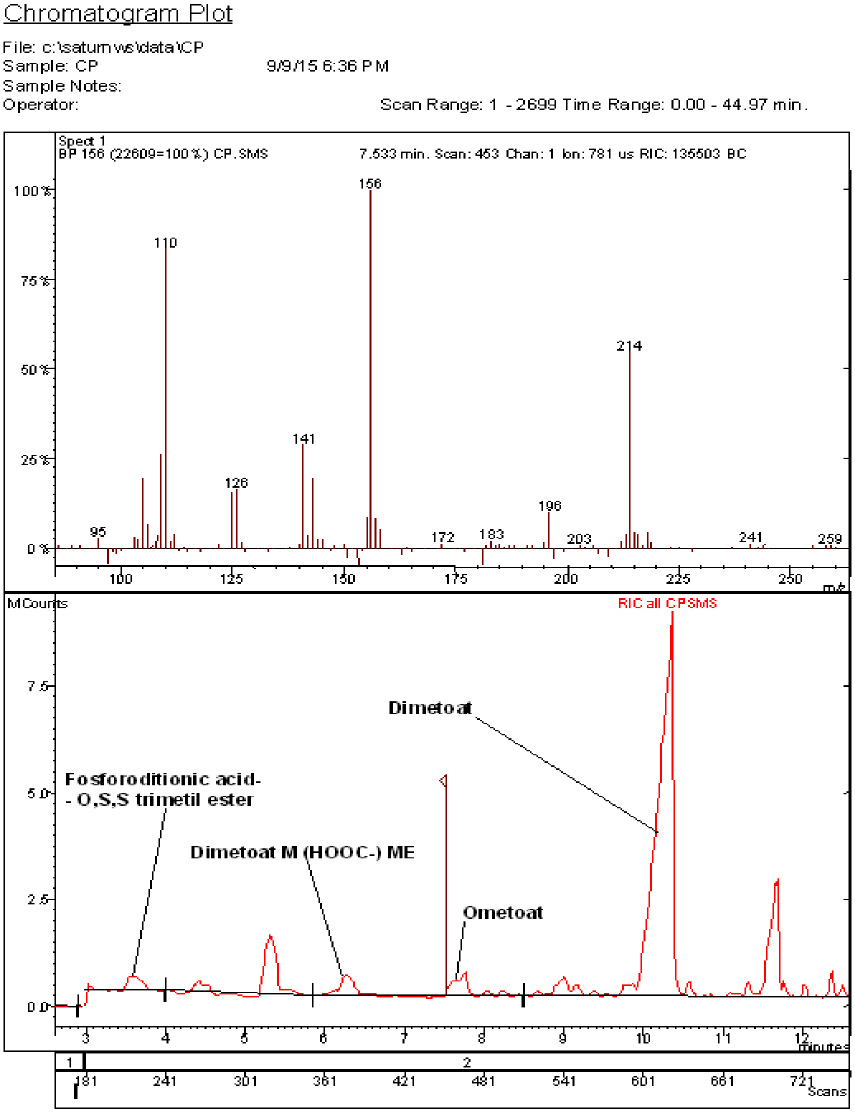

Chromatogram Plot

File: c:\saturnws\data\CP
Sample: CP 9/9/15 6:36 PM
Sample Notes:
Operator: Scan Range: 1 - 2699 Time Range: 0.00 - 44.97 min.

CONCLUSIONS

The GC-MS method is the only method that can determine organophosphorus compounds in unknown matrices for the quality control of water, the environment and foods or to establish the analytical diagnosis in case of contamination/intoxication with organophosphorus compounds.

The mass spectrometer can acquire data through various methods. In "full scan" method (FS), the mass spectre obtained through electron impact ionization can be compared with mass spectres found in specialized libraries, making possible the identification of these substances in unknown matrices.

To reduce the number of false positive or false negative results, analytical procedures based on precision, accuracy, detection limits, error source identification are needed but also the expanding of existent spectre databases.

A quick and correct analytical diagnosis influences the quickness and correctness of specific therapy measures that can be applied in such a context.

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Rare case of Stevens-Johnson-TEN overlap syndrome caused by mycotoxins

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Abstract*: Mushroom poisoning is rarely associated with skin involvement. Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening mucocutaneous reactions, characterized by extensive necrosis. SJS/TEN overlap includes patients with skin exfoliation between 10 to 30 percent of the body surface area. We report the case of a patient that was assumed to have ingested one type of toxic mushroom within the twelve hours prior to the appearance of skin lesions typical for SJS/TEN overlap syndrome.*

Keywords: *Amatoxin, skin involvement, SJS/TEN overlap, MODS*

INTRODUCTION

Picking wild mushrooms is a very popular activity in European countries, therefore mushroom poisoning is a constant and serious health issue [1-3]. More than fifty species of toxic mushrooms are known, which are usually very similar to edible mushrooms.[4,5]

The most severe cases of mushroom poisoning are mainly caused by ciclo-peptides – toxins contained by Amanita mushrooms.[2,5,6] The most frequent cause of death in mushroom poisoned patients is *Amanitaphalloides,* also called ”the death cap”.[5-8] Toxic effects of *Amanitaph.* are determined by phallatoxins and amatoxins.[6,9] Phallatoxins are heptapeptides with severe toxic systemic effects that cannot be absorbed from the digestive tract.[10] However these toxins can induce gastrointestinal symptoms through lesions of the enterocytes .[1]

α-amanitin, the most important amatoxin, is resistant to all gastrointestinal fluids and after absorption it mainly locates in the hepatocytes.[10,11] After reaching the liver cells, α-amanitin binds DNA-dependent ribonucleic acid (RNA) polymerase II, with high specificity, causing protein synthesis inhibition.[11,12] Its toxic effects are also increased through the enterohepatic circulation of this toxin.[1,11]

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Histopathological results of these effects are initially represented by nuclear lipid and carbohydrate deposits and finally by hepatic centrolobular necrosis.[1,12] α-amanitin also affects other metabolically active tissues like the kidney or the gastrointestinal tract.[7,12,13] No cases of skin involvement secondary to *Amanita ph.* poisoning have been reported so far except erythromelalgia – a disease that is characterized by erythema and pain especially in the extremities – that was sometimes associated with some species of mushroom intoxication, but never with *Amanita ph*. Poisoning.[14,15]

Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but highly severe disorders that can affect patients of all ages.[16,17]

Caused by a variety of drugs, infections and rarely by toxins, SJS and TEN are defined as a hypersensitive cutaneous reaction that produces dermato-bullous skin lesions.[16,18-20] Pathogenesis of these conditions is controversial and involves genetic susceptibility (haplotypes like HLA B*1502, HLA B12 etc.), immune cells (especially T lymphocytes CD 8+), cytokines and mediators of cell death.[16,19,21]

Although initially was thought that SJS and TEN are separate entities, today it is considered that they are varying degrees of severity of the same disease.[22] The difference between these two forms of disease is represented by the percentage of the affected body surface area (BSA) – SJS detachment of <10 % BSA and TEN detachment of >30% BSA.[17,22]

Overlapping SJS/TEN includes cases with detachment between 10-30% BSA.[22,23] Regardless of the size of the affected area erythematous and macular lesions may be associated.[17]

Apart from skin lesions, mucosal (respiratory, gastrointestinal, urinary) and other organs (liver, lungs and kidneys) involvement can occur.[20,24-26] Despite numerous attempts of identifying an effective curative therapy SJS/TEN has a mortality rate from 5 to 30%.[27]

Furthermore a variety of long-term sequelae can be encountered in surviving patients.[24]

CASE PRESENTATION

We present the case of a 49 year old female patient who was transferred to the Anesthesiology – Toxicology – Intensive Care Unit of the Clinical Emergency Hospital in Bucharest from a regional Hospital Unit. She was suspected to have ingested a sort of poisonous mushroom within the 12 hours prior to admission. She presented to the emergency room after mushroom consumption after picking them from a local forest. During the night the patient presented abdominal pain, nausea, vomiting and diarrhea, for which she administered no treatment. In the morning she decided to go to the emergency room, although clinical signs became milder. Her medical records revealed no pathological findings, except an untreated dyslipidemia. Clinical examination revealed a mediocre general condition, pale skin, gingivitis, tachycardia and mild abdominal pain. Furthermore she noticed appearance of cough and rhinorrhea in the last two hours. After volume and electrolyte rebalancing, she was transferred to our Department.

On admission the patient presented an altered general state, she was conscious and feverish (38.6°C). Apart from cough and rhinorrhea she also presented dysphagia, myalgia and arthralgia especially in the lower limbs. Physical examination revealed jaundice, diffuse erythema on hands and feet, tachypnea, tachycardia (HR=113bpm), hepatomegaly. Preliminary laboratory results indicated hepatic cytolysis (ALAT = 9,412 U/L, ASAT = 6,720 U/L), hyperbilirubinemia (4 mg/dl), decreased serum potassium (3.1), elevated creatinine level (2 mg/dl) and blood urea nitrogen (BUN = 49.4 mg/dl). Electrocardiography showed no abnormality, except the abovementioned tachycardia. Superior digestive endoscopy indicated diffuse erythematous lesions in the pharynx and esophagus.

Although according to the description of the ingested mushroom we suspected an *Amanita ph.* poisoning, rapid Meixner test could not be performed due to the lack of gastric material when endoscopy was performed. Therefore mushrooms leftovers from the meal were sent to a specialized laboratory.

Considering the high suspicion of developing Steven-Johnson syndrome and the liver insufficiency secondary to mushroom poisoning, initial therapy management was carefully selected. Oxygen therapy, fluid replacement was initiated in combination with gastric protection, antiemetic and diuretic therapy. Vitamin supplementation, corticotherapy (prednisone 138 mg/day) and antioxidant therapy (N-acetyl-cysteine 1,800 mg/day and alpha lipoic acid 900 mg/day) were also added. We performed continuous digestive decontamination by administering 25 mg mannitol p.o, 20 g lactulose and Ricinus communis oil 15 mg. Moreover activated charcoal administration was started in order to interrupt mushroom toxins enterohepatic recirculation.

Twelve hours after admission the patient presented altered mental status, dyspnea, tachypnea and a peripheral oxygen saturation of 80% under oxygen mask. Because of that she was intubated and mechanically ventilated. Despite volume controlled ventilation hypoxemic index could not raise above 100. Furthermore over the erythematous areas of the feet and hands atypical irregular lesions with darker centers were observed. These lesions evolved within the next twenty four hours to vesicles and bullae (Nikolsky sign and Asboe-Hansen sign positive) (Figures 1 and 2).

Figure 1

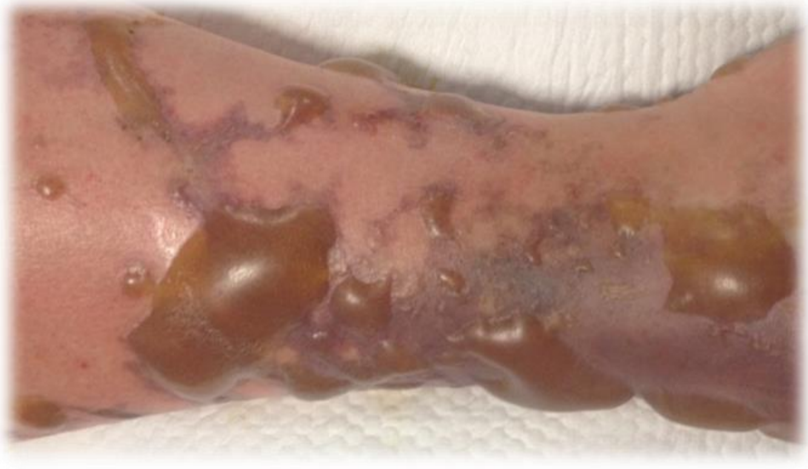

Figure 2

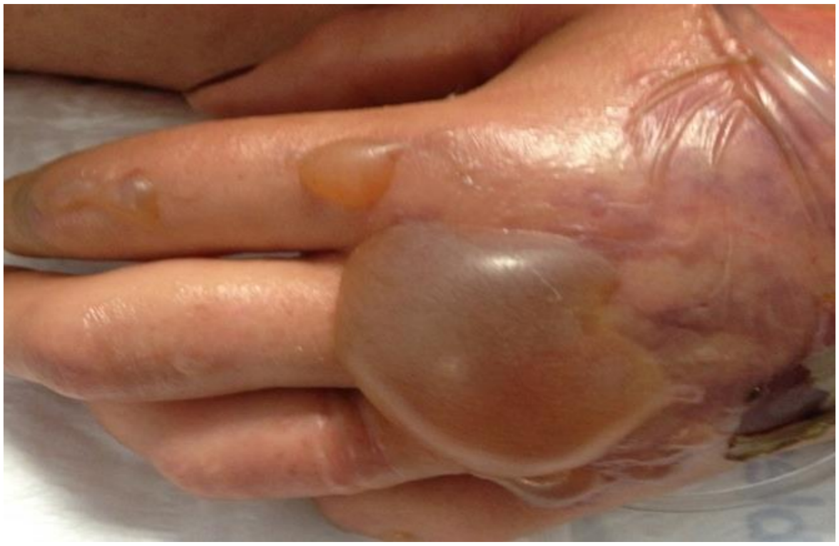

Figure 3

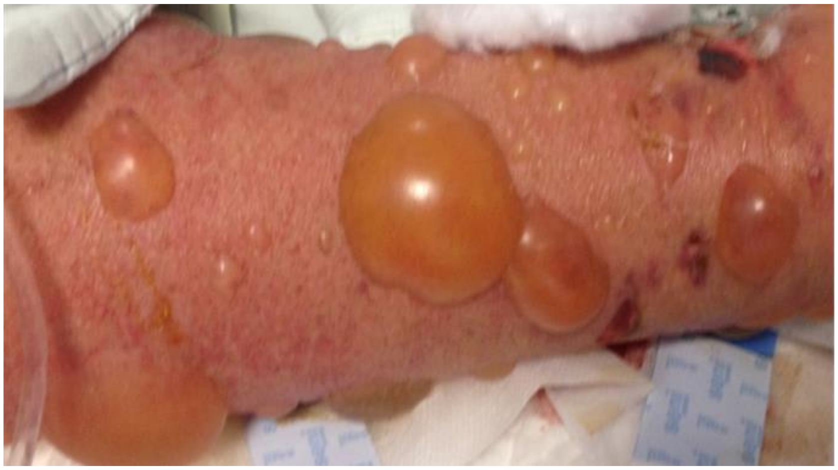

Skin lesions extended in the next few hours on both forearms (Figure 3) and legs thus evaluating the affected body surface, this case was classified as Steven-Johnson – TEN overlap syndrome.

Gynecological exam also revealed vulvar bullae. In this conditions SCORTEN score indicated a mortality risk of 58.3%.

Four days from admission *Amanita ph.* toxins were identified in the laboratory.

Since *Amanita ph*. poisoning is not a specific cause of Steven-Johnson syndrome, other causes were thoroughly investigated.

Usually associated medication which is a trigger for Steven-Johnson was excluded through detailed anamnesis of the patient and her family. Viral and bacterial causes like HIV, Cytomegalovirus or Mycoplasma pneumonie were excluded after the PCR and/or serological tests were negative.

Although liver insufficiency was remitted after one week of treatment the evolution of the patient was severe. Twelve days after admission the patient died with multisystem organ failure.

DISCUSSION

Numerous studies are dedicated to the toxic effects of mushroom poisoning. A considerable percentage of fatal mushroom poisoning cases occur after ingestion of *Amanita ph.*[1] Although amatoxins induce massive liver cell necrosis, not all patients develop fatal acute liver failure.[4]

Moreover, various treatment strategies proposed in the literature decreased the mortality rates in these patients.

Delayed onset of signs and the polymorphic symptoms due to amatoxin poisoning may aggravate liver toxicity, in the absence of early decontamination treatment.[2] Besides liver failure, amatoxin was associated with nephrotoxicity.[7] However there is no report in the literature about the amatoxin's toxic effects on skin.

SJS/TEN is an acute severe mucocutaneous disease caused by a variety of drugs, infections or malignant comorbidities.[21] In the case presentation, the patient did not receive any medication potentially associated to SJS/TEN, neither in our unit nor in the regional hospital. Moreover she was not on any chronic treatment or suffered from any infectious or malignant disease. However, several serological tests were performed in order to exclude the most frequent infectious causes of SJS/TEN.

SJS/TEN is often preceded by a prodrome characterized by fever, headache and pharyngitis.[16,17] In this case report after the gastrointestinal phase of the amatoxin poisoning, the patient developed rhinorrhea, malaise, dysphagia and fever.

SJS/TEN may be associated with multisystem organ failure.[16] In addition to the hepatic failure caused by the amatoxin poisoning, soon after admission the patient developed respiratory dysfunction, requiring mechanical ventilation. Secondary to the respiratory and hepatic dysfunction, neurological dysfunction developed. Despite adequate volume repletion, cardio circulatory dysfunction appeared requiring continuous vasopressor therapy.

There are no recommendations regarding treatment of the acute phase of SJS/TEN.[16] Considering that there is no specific treatment for SJS/TEN, minimizing the therapy with risk of aggravation of SJS/TEN was tried and corticotherapy was initiated. Despite the maximal supportive treatment and liver function improvement, the patient died from multisystem organ failure.

CONCLUSIONS

From our knowledge this is the first case report on Stevens-Johnson/TEN overlap syndrome induced by mushroom poisoning. This case presentation aims to highlight the polymorphic manifestations of severe amatoxin intoxication as well the difficulties of managing such a patient. Clinicians should be aware of the systemic involvement in mushroom poisoning.

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CLINICAL PRACTICE

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Uncommon giant sphenoidal tumor. Case report

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Abstract*: The authors will present a case report of a woman that presented a giant sphenoidal tumor with endocranially extension and compression of the cerebral trunk. The patient was already presented in a neurosurgical service where due to the tumor volume, the high-risk surgical elements involved was sent to our ENT department to try to perform an endoscopic biopsy.*

Using the endoscopic optical and mechanical ensemble the authors performed trans nasally a biopsy. The histopathologic result was a surprise and was confirmed with three different immune-histochemistry exams.

Keywords: *sphenoidal tumor; hypophysis*

INTRODUCTION

Sphenoidal sinus is located in the middle of the skull, and is one of the most difficult sinuses to be attacked.[1]

The surgical risk elements are the internal carotid artery, optic nerve; optic chiasma; hypophysis and skull-base.[2,3]

The tumors located in the sphenoidal sinus are difficult to access and due to the vicinity of vital anatomical structures.

A biopsy must be performed to have a histopathological result and based on that the patient will have a treatment scheme.

In some cases, even biopsy's hard to complete, and high-risk factors are involved.[4,5]

The patient must be informed preoperatively about the risk involved in taking the biopsy, why is necessary to perform that biopsy and what are the risks of not having a biopsy and treatment.

Multiple biopsies must be performed to have a histopathologic result.

MATERIAL AND METHODS

We present a care, it was admitted a 52 years old woman with the following symptoms: headache, bilateral nasal obstruction, right abducens nerve

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paralysis, right exophthalmia, and diplopia.

Anamnestic we have found out that the symptoms started to develop six months ago gradually.

The patient was already consulted in a neurosurgical department. It was performed a CT scan, and the result was: expansive tumor which was located at, the level of sphenoidal body, extending anterior to rhino pharynx (nearly totally blocked) and posterior intracranial, with emphasizing compression of the cerebral trunk (pons and bulb).

Figure 1: CT scan exam expansive tumor which was located at the level of sphenoidal body, extending anterior to rhino pharynx (nearly totally blocked) and posterior intracranial, with emphasizing compression of the cerebral trunk (pons and bulb).
The intracranial fragment has T1 signal, suggesting intratumoral hemorrhage.
The cranian invasion extends to abducens nerves both sides.

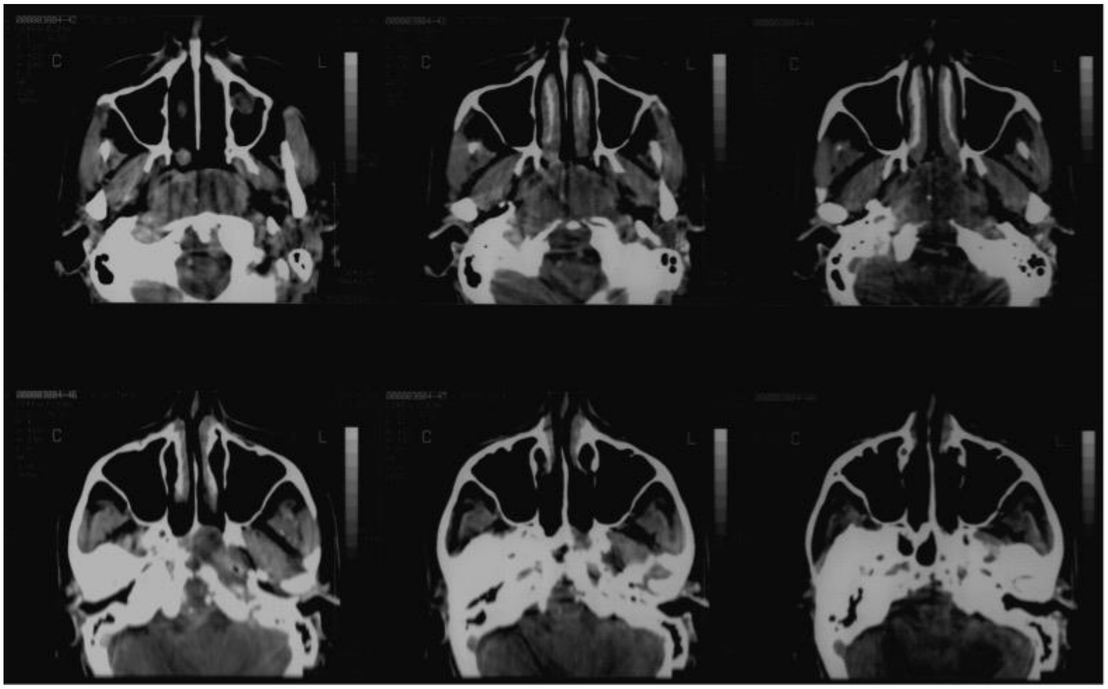

The CT scan exam conclusion was: tumor located at the level of the sphenoidal body, invading rhino pharynx and breaking into posterior fossa compressing cerebral trunk.

Possible diagnostic taken into account was chordoma, sarcoma, primitive tumor of rhino pharynx invading sphenoid sinus.

The patient was sent to our ENT department to be performed a transnasal endoscopic biopsy to see the histological nature of the tumor. Depending on the histological nature of the tumor the skull base team (neurosurgeon and ENT surgeon) will decide the best treatment schema for the patient.

In our hospital first, we have performed a fiber optic exam transnasally and transorally retrograde, where we were able to see the tumor that destroys the anterior and inferior wall of the sphenoid sinus, protruding into the rhino pharynx that was blocked near totally.

The surface of the tumor was smooth, and the mucosa of the posterior wall of the rhino pharynx was not destroyed, it was pushed from beneath. The tumor had a rich vessels supply. The patient had a rhino sinusitis secondary to the nasal obstruction produced by the tumor.

We have started to prepare the patient for the endoscopic trans nasally biopsy.

First, we have treated the rhino sinusitis with antibiotics, in steroids anti-inflammatory drugs and we have cleaned the nasal fossa every day. We wanted to obtain a clean surgical field because of the communication that will be created with the endocranial cavity.

Figure 2: Optical and mechanical ensemble to endoscopically expose the rhinopharynx

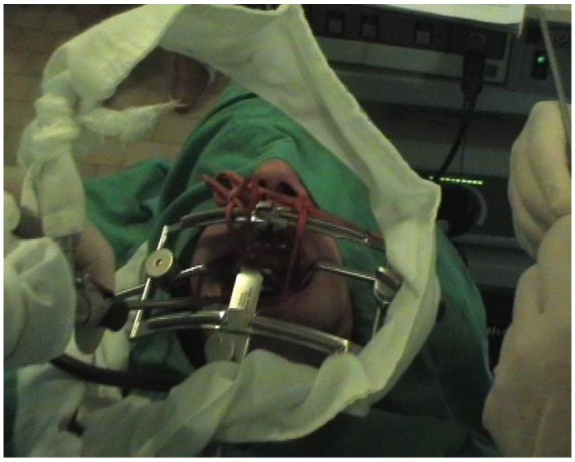

Figure 3: Video fiber optic trans orally exam. The tumor block near totally the rhinopharynx

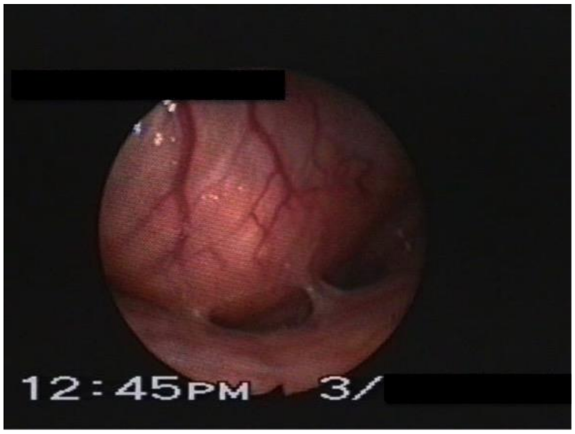

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First, we have performed a trans nasal video contact endoscopy exam on the tumor. Video contact endoscopy is an endoscopic technique that allows the surgeon to study, after staining the tissue with methylene blue, the desired tumoral area as an in vivo histological exam. The surgeon can examine the histological superficial epithelial layer, observing histologic abnormalities and the vessels of tumors.

After one week we started to perform the biopsy under general anesthesia using both trans orally and trans nasal corridor.

Figure 4: Video contact endoscopy of the rhinopharynx. Locating a low vascular area

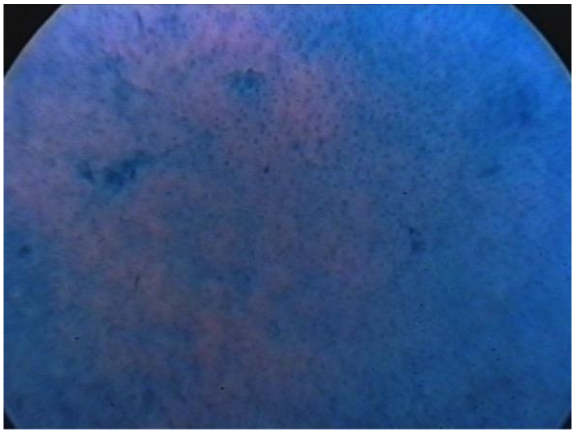

Figure 5: Targeted biopsy performed trans nasally

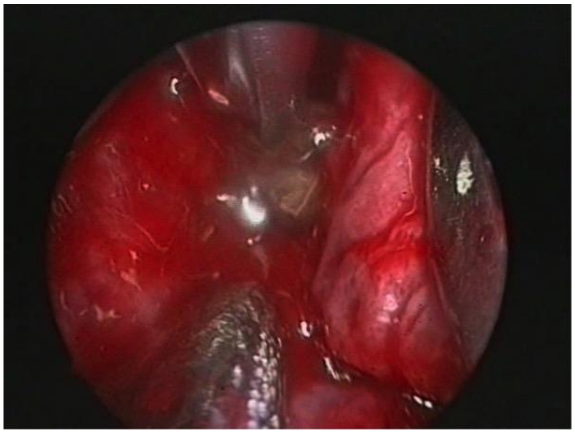

In our case, we did not discover any character of malignity at the superficial layer of the tumor, and we have discovered rich areas of vessels that fed the tumor.

We have started to gentle perform a targeted biopsy from the tumor in an area where the vascular network was limited because we wanted to prevent an important hemorrhage.

We have used cutting instruments because we did not want to perform traction maneuvers on a tumor that can have adherence on the cerebral bulb and ponds.

RESULT AND DISCUTIONS

We have obtained the bioptic material, and we have aspirate an important quantity of liquid. We had no CSF leak, we have packed the cavity with Gelfoam. The patient was packed for 48 hours.

The evolution of the patient was good, and the headache ceased, right abducens nerve paralysis started to reduce and disappear in 3 months.

The histopathological exam was a surprise, and it was confirmed using three different labs and immunochemistry tests. The sphenoidal tumor was a giant non-functionary pituitary adenoma.

The patient received medical and radiotherapy treatment after two months. The symptomatology disappeared.

CONCLUSION

In conclusion, trans nasal and trans oral corridor provides the surgeon with a minimally invasive route to preform biopsy and surgery.

The use of video contact endoscopy helped the surgeon to perform targeted biopsy from a low vascular area and to observe that we are not dealing with malignancy.

We underline the role of the multidisciplinary neurosurgical and ENT team. We have chosen a minimal invasive surgery to minimize the surgical risks (vascular and neurosurgical), and we have achieved an uncommon histological finding that changed the prognostic and the therapeutically route of the patient.

Acknowledgement
All authors have contributed equally to this paper.

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We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

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RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

Education and Imaging

The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as ‘What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?’ Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

MANUSCRIPT PREPARATION

Style

Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors’ revised ‘Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication’, as presented at http://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster’s Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader’s task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi)

acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

Text

Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

References

The Vancouver system of referencing should be used. In the text, references should be cited using superscript Arabic numerals in the order in which they appear. If cited only in tables or figure legends, number them according to the first identification of the table or figure in the text. In the reference list, the references should be numbered and listed in order of appearance in the text. Cite the names of all authors when there are six or less; when seven or more list the first three followed by et al. Names of journals should be abbreviated in the style used in MEDLINE. Reference to unpublished data and personal communications should appear in the text only.

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Tables

Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals, and cited in consecutive order in the text. Each figure should be supplied as a separate file, with the figure number incorporated in the file name.

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A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

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If the above items are not included in the cover letter, manuscripts cannot be sent for review.

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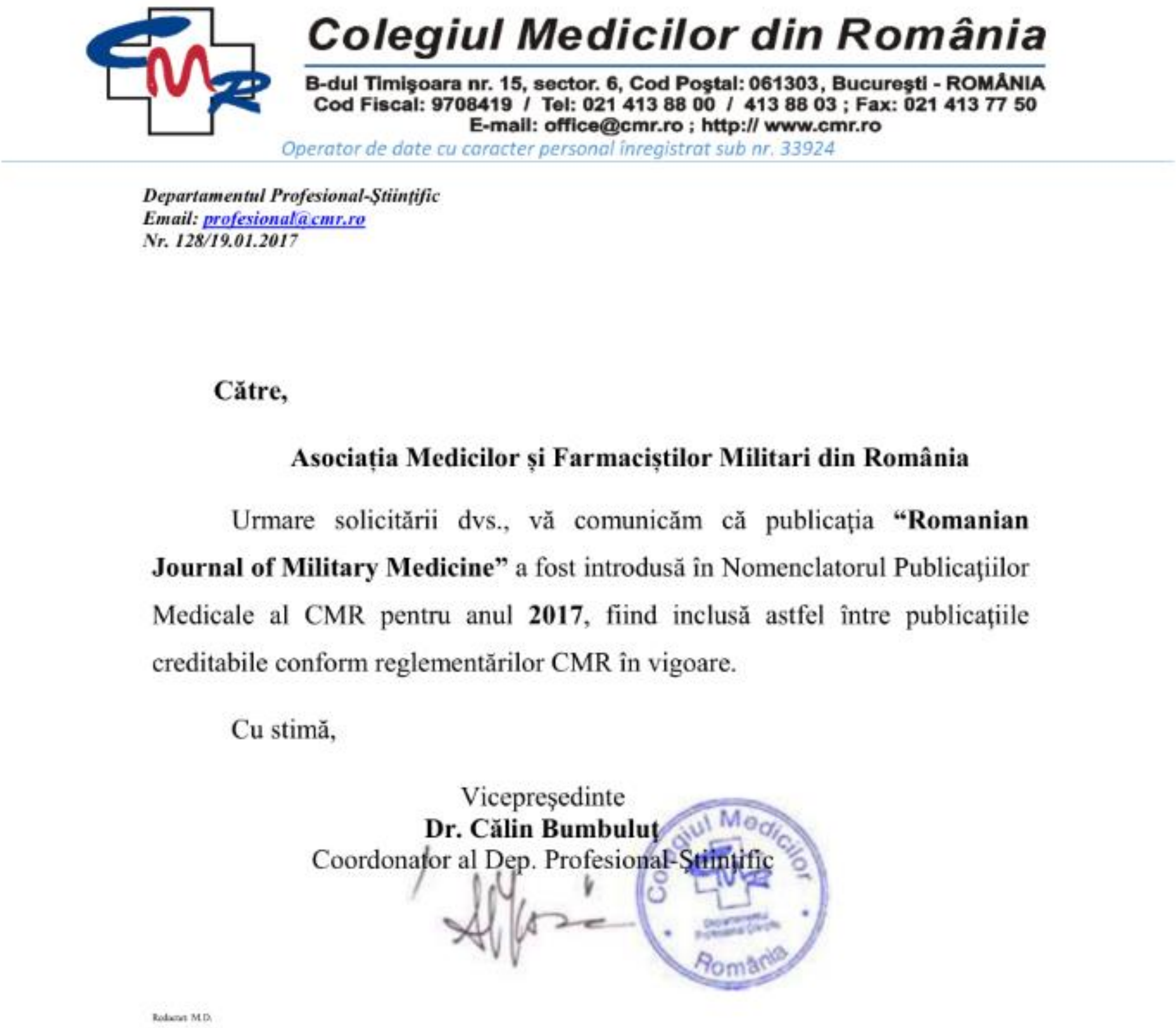

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Către,

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Urmare solicitării dvs., vă comunicăm că publicaţia **"Romanian Journal of Military Medicine"** a fost introdusă în Nomenclatorul Publicaţiilor Medicale al CMR pentru anul **2017**, fiind inclusă astfel între publicaţiile creditabile conform reglementărilor CMR în vigoare.

Cu stimă,

Vicepreşedinte
Dr. Călin Bumbuluț
Coordonator al Dep. Profesional-Ştiinţific

Redactat M.D.

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EDITORIAL

Brief notes about the Great War, Romanian military doctors and the Great Union

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Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Motto: "L'Histoire, c'est la rencontré d'une volonté et d'une évènement" – Charles de Gaulle, 1890-1970

This short notice, largely iatrohistoric, appear in the 100th year since the Great Union out of the desire to know more about what has happened.

In Romania, there was a lot of talk about the First World War. The Germans remember this war under the name of "der Erste Weltkrieg worde von 1914 bis 1918 in Europa, in Naken Osten, in Africa, Ostasien and auf dez ozeanen gefurt". Obviously this first conflagration was the army of "Zweiter Weltkrieg"! The British preferred the denomination of the "European War" or, more correctly, they named it "the Great War".

It appears that this name is slowly but surely penetrating our literature, following "World War I" which, referring to the title of this editorial only makes us Romanians remind that we have also had a Small Union (1859), followed by the Great Union of 1918.

It must be remembered that the Romanian literature between 1948-1989 wrote about the Great War in an abbreviated manner, because of two reasons: the Eastern neighbors had "turned history" – things did not happen as planned and the contribution and participation of the Romanian Royalty to the final victory was extremely important but also very embarrassing that it had to be silenced.

The Great War began in Sarajevo in 1914 and ended at Versailles in 1949 in the Mirror Hall, with multiple European implications...

Then, at Versailles in the Grand Trianon Palace, a treaty was signed on June 4, 1920 between 16 allied states (including Romania) and the successor state of the Austro-Hungarian Empire. At the beginning of the war, Romania, a "very small country", in the form of "L", had 137,000 km^2 and a population of 7.2 million inhabitants, and after Trianon, it was reunited and became „The Great Romania", with an area of 295,000 km2 and a nation of 18 million people. It is certainly why, in the collective mentality of a neighboring nation, that this situation is perceived as unacceptable even after 100 years!

After the assassination of the crown Prince of Austria-Hungary – Franz Ferdinand on June 28, 1914 the actors, both big and small, began to enter the stage: Central Powers – Germany, Austria-Hungary, Turkey, Bulgaria and Antanta or the Triple Alliance, England, Russia followed by Italy, Romania, USA...

The Kingdom of Romania had passed through a recent, unforgettable experience for the army and especially for the military doctors. In 1913, during the Second Balkan War, the Romanian troops that had easily entered the northern half of Bulgaria lost 1,600 lives due to the cholera epidemic – a fearful "enemy".

Evidently, the accusations have risen, obviously committees for dysfunctions research have been named, obviously the responsible military doctors – Constantin Papilian (1852-1917) and Senator G-ral (r) Prof. Dr. Athanase Demosthen (1846-1925) informed I.C. Bratianu – Prime Minister and Minister of War about all this.

Certainly, the two years of neutrality have chosen better and more efficient organizational lines, as "stage sanitation, semi-hospital evacuation, evacuation hospitals, auxiliary hospitals, and infirmary station ".

Just after two years of "armed expectancy" war began, for Romania as well as for other nations, how all wars start "suddenly and unprepared!". We do not insist in geostrategic and political-economic details. We present only the result and brief considerations about military doctors truly involved in the "Perpetual Drama of War".

What Prof. Dr. Vasile Sârbu, a Templar Knight of Romanian Surgery and Iatrohistory, presented with his known erudition a few months ago, is perfectly true: "In this war, 400 military doctors died out of 2,800 participants." 2,400 health workers have also died out of 14,000 participants, as well as 14 pharmacists and 20 students of the Military Health Institute. These numbers do not say much. If we compare them with the other "weapons", we will be surprised to learn that this group of people is on the 2nd place after the infantry, which made King Ferdinand to offer them the right to wear the "combatant weapon" badge.

"This was the result!..."

Among the personalities, the first name to be quoted with gratitude and piety is that of Prof. Dr. Ion Cantacuzino – Jean Cantacuzen for the French, descendant of Byzantine emperors, a medical school creator, graduate of the French medical school, born in 1863 in Bucharest, student of Ilia Mecinikov, founder of the Romanian School of Immunology and Experimental Pathology, doctor of medicine with a thesis on the destruction of the vibrio cholera. The subject of the thesis, supported in 1894, and its findings will prove useful in almost 20 years, as in the novels of Alexandre Dumas.

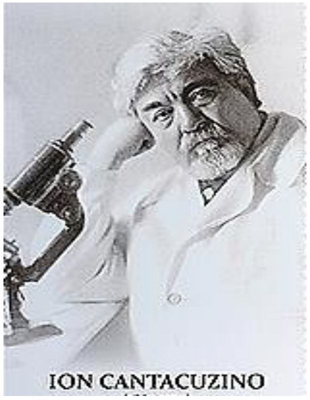

He became Professor of Experimental Medicine at the Faculty of Medicine in Bucharest at the age of 38 and was appointed in 1908 as General Manager of the Health Service to "Effectively fight epidemics, set up isolation hospitals and pavilions, rural infirmaries and bacteriological laboratories". In the Bulgarian campaign he successfully ordered the vaccination in an epidemic environment, called and known as "the great Romanian experience."

He conducted the Civil Public Health and Military Public Health Directorate during the Great War, a true Ministry of Health, which allowed him to organize anti-choleric vaccination and fight against exanthematic typhus, typhoid fever and smallpox – having the rank of Col. Dr. of the Romanian Army.

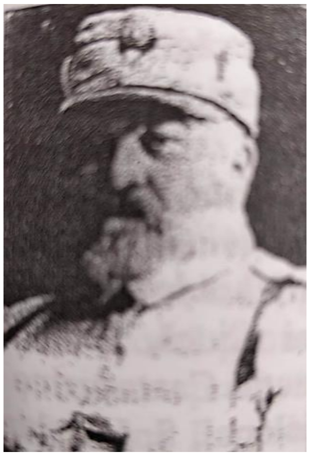

In 1920, together with Nicolae Titulescu and Mihai Ciucă, his student, participated, as the Romanian state delegate, at the Treaty of Trianon. He enjoyed a high prestige, he had an important word to say, was even a friend of French Prime Minister Georges Clemenceau, a distinguished neurologist... On April 1, 1921, as a result of his unrelenting thought, effort, and determination, he founded the "Serum and Vaccine Institute", by royal decree, which will then bear its name.

O tempora, o mores!...

In 1911 the General Dr. Nicolae Vicol (1861-1936), as Director of the Health Department of the Ministry of War, organizes two preparatory sanitary maneuvers around Bucharest that have proven to be beneficial in the future. In August 1916, when he signed the troops mobilization he followed the General Constantin Prezan – the head of the General Headquarters, unfortunately not having total decision-making power and being obliged to listen to the Minister Constantin Angelescu. Since February 1917, when the Public Health Directorate was founded, led by the supreme authority in the field – Colonel Prof Dr Ion Cantacuzino, he starts a great collaboration with him.

The General Dr. Iacob Potarca (1866-1942), a graduate of the Bucharest Faculty of Medicine, specialized in general surgery in Paris, physician colonel in 1916, head physician of the First Army’s Corp, general in 1917, then Sanitary Inspector of the First Army – who fought in the Mărăști-Mărășești sector, was not only an illustrious military physician in the war. In 1924 he becomes General Inspector of the Army's Sanitary Service, but it is worth mentioning that he is the first Romanian surgeon to have operated the esophagus, having other remarkable surgical researches quoted by Professor Dan Setlacec in his formidable monograph "Romanian Medicine, European medicine".[3]

The drama on the battlefront at the end of 1917 – the beginning of 1918, was almost at its peak. In absolute anarchy a single thought seemed to be clear! The thought of the Great Union!

In August 1917, at Mărășești, there were "many other doctors from the old country, young, learned able-bodied: Victor Papilian, Titu Vasiliu, Odiseu Apostol, Grigore T. Popa, Constantin, Mihail Kerubach, and many, many others”.[4]

The name Col (r) Prof. Iacob Iacobovici (1879-1959) is worth mentioning from the beginning, not only for being the founder of the Surgery School in Cluj, after the Great Union and of the first Emergency Hospital in Romania, the one in Bucharest, but also an involved participant in the Bulgarian campaign and the commander of the 7th Evacuation Hospital of the Second Army in Bacau in 1917.

Professor Iuliu Moldovan (1882-1966) attended the Faculty of Medicine in Vienna and Prague, and then, what a few know (4), he worked as a military doctor at the Department of Dermatovenerology and at the

Central Laboratory of Bacteriology of the Austro-Hungarian Army.

In July 1914 he was mobilized and appointed the head hygienist of one of the Austro-Hungarian armies, effectively engaging in the eradication of some epidemics. On the 1st of December 1918 he took part in the works of the Great National Assembly in Alba-Iulia. Between 1919 and 1920, he became the professor of the Department of Hygiene and Social Hygiene of the Faculty of Medicine in Cluj, general secretary of the Social Protection Resort of the Transylvanian Conducting Council and he also organized the Transylvanian Medical Service.

I think it is worth mentioning the contribution of other illustrious physicians to the Great Union just to contradict Albert Camus, who said with cynicism: "Forgetting is the first faculty of man!"

Iuliu Hatieganu (1885-1959) the first professor of a Medical Clinic, dean of the new Faculty of Medicine in Cluj, "Magnificus rector", precursor, visionary, called the "Hippocrates of the Romanians", was also a participant in the Great National Assembly in Alba-Iulia.

The last, but not the last, because the number of the unknown is overwhelmingly large, is Dr. Alexandru Vaida Voievod (1872-1950).

He was a graduate of the Faculty of Medicine in Vienna, doctor of medicine, who established in Carlsbad where he trained as an intern and balneologist, later attracted to the political activity, debuted in the Chamber of Budapest and the one who has read, on the 18th of October 1918 in the Hungarian Chamber, the Declaration of Self-Determination of the Romanian People from Transylvania. He was a member of the ministry cabinet of Ion I.C. Brătianu, he also joined the Peace Conference delegation in Paris and formed and led the first Government of the United Romania.

When Romania was finally united, things seemed to be on an upward trend.

The Romanian military doctors, as well as the civilian physicians, great personalities or unknown remarkable people, have fulfilled their duty.

Regarding "The Map of Great Romania in 1924" we have only one comment: the year 1924 was the year in which, in Germany, the ideology of Nazism has started to blossom.[5]

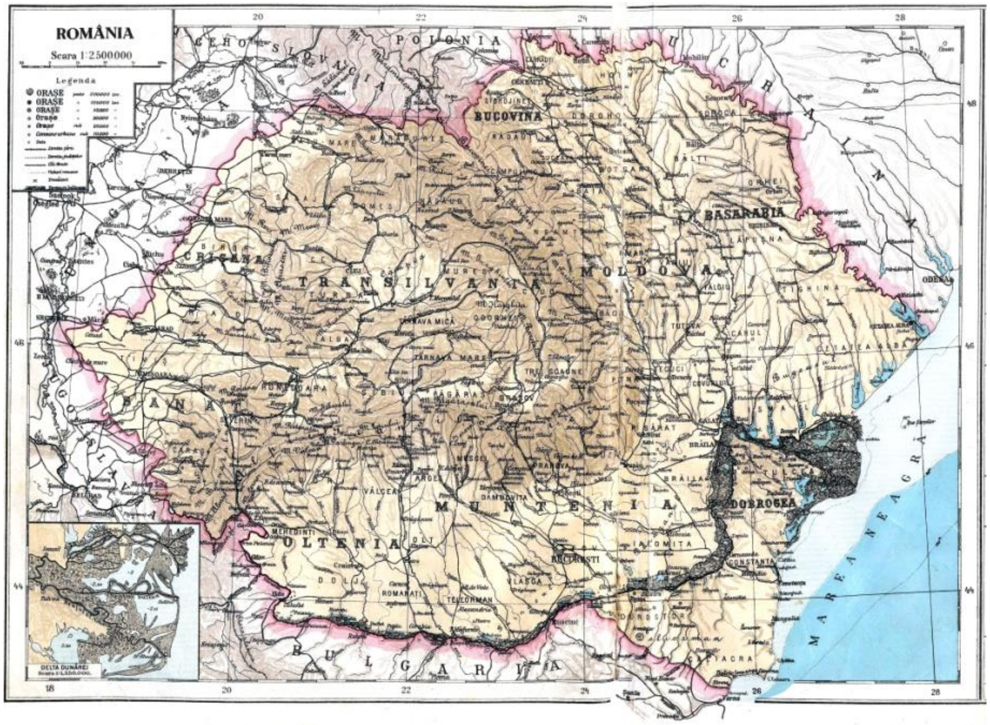
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DELTA DUNĂREI
BUCUREȘTI

Our history, of the Romanian people, has implied over the years a steady climb with a lot of difficulties. The Great Union was accomplished in stages, all springing up from the ideal of unity of our nation, never forgotten.

The unification of the Romanian states is the nation's greatest act, which configures and fully certifies our existence among other nations of the world.

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REVIEW ARTICLE

Article received on February 09, 2018 and accepted for publishing on May 28, 2018.

Ethical limits between aesthetic and cosmetic dentistry

Marina Melescanu Imre[1], Elena Preoteasa[1], Ana Maria C. Tancu[1], Cristina T. Preoteasa[1], Mihaela Pantea[1], Paula Perlea[1]

Abstract: *Esthetics is the "new trend" in dental medicine as a natural consequence of the development of modern society, with implications in practice and training. Like any rule in art, but also within the medical field, esthetics must be known and addressed in relation to other medical or non-medical principles (dental cosmetic), respect the ethics rules.*

Aim. Literature study designed to focus on the current problems that modern dentistry is facing, in relation to esthetic requirements. The literature search strategy in electronic databases: EBSCO Data Base, Dentistry & Oral Sciences Source, Pub Med indexed articles, used Boolean Operators.

As a conclusion, the dentist must be familiar with the differences between esthetic and dental cosmetic, must minimize the subjective component of the examination, identify the reasons of presentation, guide the patient in choosing the optimal treatment, including obtaining the desired esthetic results, within the ethical boundaries of the noble medical profession.

Keywords: *ethics, esthetics, cosmetic dentistry*

INTRODUCTION

Nowadays, more and more frequently, within dental, practical or training activities, we are dealing with matters related to esthetics. Patients often require esthetic restorations without being able to specify most of the time, what exactly they would like. Students show an increasing interest in esthetic dentistry aspects.

As professionals we are flooded with an information influx both through scientific publications and dental materials producers, with a dental esthetics value. After the implant, esthetics is the "new trend" in dental medicine as a natural consequence of the development of modern society.

Esthetic concerns existed since forever, from the first protagonist of scientific esthetics Pythagoras, who defined the "golden ratio", combined with dynamic symmetry discovered in 1920 by Jay Hambridge and Sir D'Arcy Thompson who explained how natural beauty can be quantified and how it can be reproduced in art, architecture and other crafts.

For dentistry, as terminology, in the Glossary of

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Prosthodontic terms, esthetic dentistry is defined as the part which studies beauty, creating harmonious results through prostheses, and ethics is a moral principle or a set of moral values of an individual or group of individuals, in our case – the ones involved in the treatment (doctors, technicians). Cosmetic dentistry is not a term indexed by GPT, its definition being present in the Collins dictionary, like maneuvers aimed to beautify without purpose or functional form. The medical profession has ethical obligations [1] centered to prevent and treat diseases, in order to rehabilitate the dento-masticatory apparatus functionality, namely mastication, phonation, and the patient's physiognomy.

Questions related to dental esthetics are: What are the ethical boundaries of the esthetic trends in dentistry? Can anything be done from a medical standpoint for the sake of obtaining an esthetic outcome? Are we ready for this new challenge as physicians who took the Hippocratic Oath for the “primum non nocere” principle? Are we trained as trainers, academics, in order to educate students so that they become true professionals in esthetic dentistry? What are the limits of esthetic dentistry and dental cosmetic, as a new term in our vocabulary?

These are some of the questions that have led us to write this paper. This study is a literature one designed to focus the current problems that modern dentistry is facing in relation to esthetic requirements. The original aspect of this work is related to the definition (both for patient and doctor) of these two terms, their character being a little bit confusing, also being capable to lead to legal aspects, even malpractice.

MATERIAL AND METHOD

A comprehensive literature study was completed in October 2015. There were selected publications in English, peer reviews, articles from academic publications, dated January 2000 to December 2015. There was obtained a total of 1248 articles, including full text criteria, of which 580 articles were retained, and after applying the selection criteria only 14 publications remained. Identified as directions of interest were: (a) dental esthetics as part of dentistry – boundaries; (b) the difference between esthetic and dental cosmetic, from an ethical point of view; (c) esthetics, the reason for treatment and clinical examination; (d) medical training, scientific publications, patient information, consent. The search strategy was conducted using EBSCO Data Base Dentistry & Oral Sciences Source with the aid of Boolean Operators. The following keywords were combined: ethics, esthetic, and cosmetic dentistry. The search was limited to English peer reviewed articles, full text and years limitation January 2000 - October 2015 academic journals.

RESULTS

There was obtained a total of 1248 articles, including full text criteria, of which 580 articles were retained, matching the search criteria requested. After applying the search criteria 10 publications became relevant. Furthermore, there was done a manually electronic search on themed websites. In the end, 14 publications that included the search criteria were selected. Among the issues raised by the retained publications, there were identified 4 axes of interest:

1. Dental esthetics as part of dentistry – boundaries.

2. Difference between esthetics and dental cosmetic from the ethics point of view.

3. Esthetics, as a reason for treatment and clinical examination.

4. Training the physicians, scientific publications, informing the patient – consent.

DISCUSSION

Dental Esthetics as Part of Dentistry – Boundaries

At this point two issues detach themselves – functionality and bias. As noted in the introduction, there is a definition of dental esthetics in the GPT, however this is rather vague, making reference to “beauty, following the art’s rules and principles''. In dentistry, the therapeutic dental restorations are not only esthetic, but they should primarily ensure the dento-masticatory apparatus and the dental occlusion functionality. For example, dental fillings can be done medically with physiognomic or non-physiognomic materials, both having advantages and disadvantages, the dominant criteria for the physician must be based on clinical experience, choosing the optimal method of treatment in order to meet the basic medical principle, “primum non nocere”. [2]

The medical profession bases its treatment on clinical, objectively examination of the patient. When regarding the esthetics dental problems, subjectivity might occur; hence, the need to establish clear criteria, both objective and subjective, for the examination in dental esthetics. Indeed esthetic sense is not a criterion for graduation from the Faculty of Dentistry; it has a great variability from person to person, from clinician to clinician, as well as from patient to clinician. Given these difficulties related to the subjective issues, it was suggested a hierarchy of the esthetic aspects in dentistry, starting from basic esthetic rules where the smile’s coordinates comply to the classical principles of the golden ratio, symmetry, dental and dento-facial proportions, smooth line smiling.

The next level is represented by the cultural and regional subjective aspects, for example in the United States the so-called Hollywood smile is a social standard, the whiteness and perfect alignment of teeth being associated with wealth, social and financial success. At last, the latest level is the so-called virtual level – the one that a computer program sets as ideal for the patient, from the esthetic point of view. [3]

Is dental esthetics a part of the patient’s general health state? Yes, it was clinically proven that the esthetic restoration brings an important psychological benefit to the patient. [3]

Difference between Esthetics and Dental Cosmetic from the Ethics Point of View

Within the last years, appearing the dental cosmetic term, that was medically not registered otherwise, there have been many misunderstandings between this term and the esthetic dentistry, both among patients and practitioners.

Traditionally, dental medicine as a medical specialty is centered, ethically speaking, on the prophylaxis and the treatment of the dental tissues in order to ensure a good health state, respecting, of course, the dento-masticatory functions. So, we are talking about affected tissues, or with such potential. [4]

Dental cosmetic seeks only embellishment, often without any consideration for functionality, interfering with healthy tissues, without clinical impairment for beautifying intentions. Often, these maneuvers contradict even the esthetics concept – defined as being an integration concept of beauty in natural proportions, with a humane dimension. Is it esthetically the completely unnatural pure white smile of an 80 years old lady? Is it not against the physiological processes of aging teeth, with natural tooth staining due to time passage? Therefore, the difference between cosmetic and esthetic dentistry must be properly ethically and medically differentiated [5,6]. Moreover, some cosmetic maneuvers might damage a healthy dental tissue – for example when applying veneers, esthetic crowns, excessive grinding, applying adhesive – without pulp protection – can lead to tooth loss – defined as disfigurement, from the ethical point of view. [3]

One must respect, from the ethical point of view, the principle of minimally invasion; the so-called enamel sacrifice on the altar of vanity [7, 8, 9] does not correspond to the principles of medical ethics. On long-term, the biological implications of the maneuvers consequences that were performed only for cosmetic purposes should be correctly assessed by practitioners. [10] Esthetic would mean beauty, form and function – and cosmetic only beauty. [2]

Esthetics, as a reason for treatment and clinical examination

As shown, although the boundary between esthetic and cosmetic maneuvers may seem “too fine” sometimes, the practitioner disposes of objective criteria when deciding the treatment plan. [11]

Patients who address the dentist for solving the esthetic problems divide into two categories – among these reasons are dental crowding, discoloration, unsightly tooth discoloration, missing teeth, multiple teeth with coronal restorations. The patients’ reasons may be esthetic ones, but after a properly conducted clinical examination, the dentist will establish the functional problems – occlusion problems, migration and others that, from an objective medical point of view, should be rehabilitated in order to restore the morpho – functional, esthetic, masticatory and phonetic balance. The dentist will decide the patient's treatment plan, single or multidisciplinary orthodontics (teeth alignment through braces), conservative treatment (bleaching, esthetic restorations, and ceramic veneers), and dental prosthetic (crowns coverage, dental implants). A second category refers to patients without enough arguments – patients suffering from narcissism, personality disorder, patients who can't accept their age. As in the first group, the dentist is the one that will make a "proper diagnose" considering the medical history and clinical examination.

Patients with such presentation reasons will permanently be unsatisfied with the treatment outcome. Unlike the ones with consistent esthetical grounds that will be satisfied once the esthetic problem is solved, for the second class the result will not be acceptable even if it has improved the esthetic aspect. [2] These are the most common candidates for dental cosmetic, for whom the "primum non nocere" principle must be respected from the ethical viewpoint. [10]

And not least, after the clinical examination, if the dentist is in a doubtful situation, he should, according to "when in doubt, it is probably not ethical" [7] test himself with "The Daughter Test" – Would I proceed with this intervention on my daughter? [8,12]

Training the physicians, scientific publications, informing the patient – consent

Another important aspect is the dentists training, in addition to the fundamental principles of dental esthetics already learned in college; the profession currently faces numerous specialty publications in which the so-called academic articles are praising esthetic results obtained – the ethical aspect of the presented cases being often questionable from the fairness of the dimension's vertical occlusion point of view, occlusion stability and durability of these restorations. Publishing some insufficiently and superficially documented cases – medically speaking, designed only to beautify, can be really dangerous, especially for young doctors who didn't benefit from enough clinical experience and being pressured by patients in order to obtain esthetic results, can guide their therapeutic conduct, based on good faith. [10]

In the modern age, consumer society pushes dentists to features, such as advertising, with the temptation for many dentists to promise spectacular results with a negative impact on the professionalism of the entire profession [6], we must not forget the fundamental nature of our medical profession profile, namely the professional doctor [13] and not the beautifying one. In this context it is important, ethically speaking, the doctor-patient communication regarding the dental esthetic issues – the doctor is required in this type of treatment to inform the patient in order to receive his consent over long-term implications (especially in younger patients). Communication must be made in terms that the patient will be able to understand (not necessarily medical terms), assisted by pictures, drawings, suggestive dental casts. It is also required to present to the patient, where appropriate, one or more treatment alternatives, including the less esthetic alternative, before signing the informed consent [3]. Esthetic dentistry requires less accommodation, incorporates acceptable biologic technology for long-term survival, functions suitably, and mimics the pristine state of the natural dentition. Cosmetic and esthetic dentistry are different in definition, concept, and execution [14].

CONCLUSIONS

As a result of this extensive literature study on a very actual dentistry issue – ethical considerations of esthetics and dental cosmetic, we came up with some interesting conclusions intended to clarify the often encountered confusion regarding these terms. Dental Esthetics regroups several dental maneuvers, often interdisciplinary, aiming the morfo-functional rehabilitation of the dento-masticatory apparatus, following universal esthetic principles harmoniously integrated into the overall health and harmony of the human body as part of dentistry. Dental cosmetic is a set of maneuvers that, although have a medical character, do not seek the reconstruction of the maxillary device functionality, just have a beautifying character, intervening on healthy tissues without any prophylactic role, often with disabling long-term implications. In terms of bioethics, the “primum non nocere” principle is not respected within these maneuvers.

Therefore, it is important for the dentist to know the differences, the fundamental dental esthetic concepts, in order to minimize the subjective component of the examination, to succeed the clinical examination with identification of the presentations reasons, to be trained for all medium- and long-term treatment implications, in order to be able to present to his patient all treatment alternatives and guide him to choose the optimal treatment option for obtaining the desired esthetic results within the ethical boundaries of this noble profession. The theme being new and the boundary between esthetics and cosmetic dentistry being quite subjective, no doubt that they still have to be studied, there are needed further studies and research that will clarify the differences between them on an evidence-based scientific system.

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REVIEW ARTICLE

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History of medicine on the border between philosophy and science

Mirela Radu [1]

Abstract: *Physicians have represented a long time the main transmitters of knowledge as they were real scholars. If Renaissance promoted the study of the human body anatomy and physiology, the next step made by practitioners of medicine was to spread the enlightenment. That meant the shift of the very purpose of their profession: from passive opposition to ailments towards an active involvement into the lives of the impoverished. In order to change the odds in the battle against diseases, physicians had the great burden to enlarge the cultural horizons of those whose health was in their hands. Therefore, one way of imparting knowledge was by publishing and spreading their attainments to the general public in a comprehensible way. Once people gained awareness of the dangers entailed by bad hygiene, the physicians' role in society switched towards more cultural realms. At the beginning of the 20th century health care professionals achieved the next step in the becoming of medicine: setting up a new science to link humanities with pure science. In Romania, the main promoters of this new border science were Victor Gomoiu and Valeriu Bologa and they co-opted other intellectuals.*

Keywords: *philosophy, science, history of medicine, alchemy, folklore*

The new involves acknowledging the past, transforming it and bypassing mistakes. The 20th century met the expectations of those who wanted to know this history by setting up the *Institute of History of Medicine* in 1921 in Cluj. "More and more are those who pretend to have a spiritual imitation in the past to save the intellectual character of modern medicine. This postulate translates practically into the multiplication of medical-historical literature and giving a growing importance to the history of medicine."[1] One of the first teachers to honor the Romanian institute was the French Jules Guiart (1870-1965) who taught for three years this subject. Those who strongly supported him were Valeriu Bologa and Emil Racoviţă. Guiart, fascinated by what he had discovered on the Romanian realm, would also work as an ethnographer, travelling intensively and gathering various ethnographic materials and photos from all corners of our country.

The Romanian physician Valeriu Bologa (1892-1971) is the exponent of a whole caste: that of doctors aware of the modeling power of culture. He dedicated himself to the study of natural sciences (at the University of Jena) and, afterwards, he was attracted to the medical studies in Austria and Cluj. The pride he felt for the art

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of healing practiced by the Romanians led him to lay the foundations of a new branch of science: the history of medicine. Between 1949-1971 he presided the International Society of Medicine History. Feeling that the progress can only be heard through the knowledge of the past, Bologa devoted many books to the facts of the medicine in the past. One of the first important papers signed by the Romanian physician focused on the special situation of the Hippocratic profession practiced by the Transylvanian Romanian doctors who had to face not only the lack of material means but also the political repression: *Contributions to the history of medicine in Transylvania* (1927). Three years later, Bologa signed a second monograph, *The Beginnings of Romanian Scientific Medicine* for which he would receive in 1931 the V. Adamachi Prize of the Romanian Academy. But until 1927, the physician signed only a studies with great historical significance, dedicated to some of the most diverse themes – from midwifery, to the forerunners of doctors, from ophthalmology to medical lexicology formation: *Spells, old women and midwives today and the past* (1921); *New data for Ioan Molnar* (1925); *About Romanian Occultists* (1925); *Medicine in Moldavia* (1925); *Between physiology and medicine* (1925); *Romanian Medical Terminology of doctor I. Molnar* (1926).

Furthermore, Bologa dedicates himself to the construction and endowment of a museum dedicated to medical science in Romania. The Romanian scholar was particularly fond of two sections of the museum: *Old Romanian Medicine* and *Medicine in the Transylvanian past*. The great importance he gave to the knowledge of the old times of the profession he revered could be felt from the appreciation with which he emphasizes the importance of those early times, but also the respect he had for his ancestors. For the reader of any age is visible the attachment and esteem that doctor Bologa carries to those who have done medical pioneering work, especially in the Transylvanian region: "From this rich Romanian medical library can be reconstituted the hard work of the first gatherers of new roads in Romanian science. It is possible to see the influences from the outside, it can be seen how gradually a Romanian medical terminology was formed, it can be noticed how, from the great Davila, our medicine goes from the assimilation phase to the one of creation, as more and more characteristically forms a Romanian medical current. The old Transylvanian medical literature was represented equally well at the beginning – from the 16th century – by the works of the German doctors, later with those of the Hungarians, finally from the 18th century and with the first Romanian medical translations."[2]

But Bologa was not the only one who fought for this new branch of medicine. He was helped in his efforts by the fellow surgeon Victor Gomoiu (1882-1960) who founded museums dedicated to the history of medicine in Târgu-Jiu and Craiova. Gomoiu, in turn, published a monograph entitled *From the History of Medicine and Romanian Medical Education* (1923) and during the interwar period he was elected president of the International Society of Medicine History (1936). Gomoiu was also the one who signed the first *History of the Medical Press in Romania* (1936), the work of collecting and organizing numerous medical papers and writings. But Gomoiu was not just an encyclopedic spirit. He also actively contributed to the struggle that doctors used to do with illnesses whose mortality reaches worrying odds. Director of the Osteoarticular Tuberculosis Sanatorium for Children in Techirghiol, eventually Gomoiu would practice surgery in Bucharest. His surgical work is quantified by the large number of innovative articles he has written, by implementing the term solarectomy (resection of lymph nodes), initiated the inguinal approach of varicocele (Gomoiu-Phocas method). Intransigent character, Gomoiu was removed from academic education. His merit in the history of medicine is to insist on the Romanian contribution to the international folk medicine fund. This brought, at least historically, the Romanian medicine at the level of the other countries reducing the gap. A proof of his ideal and his effort to bring medicine to the Western level are the three works published by the Romanian physician in 1938: *La Croix dans la Folclor medical roumain*, *Histoire du Folclore medical en Roumanie* and *Medicine in the Romanian folk prose*.

Bologa also corresponded intensely with Mircea Eliade, whom he intended to co-opt in his work at the

Institute of Medical History. Eliade, despite the fact he had no necessary time for active participation in this approach, had a special esteem for the intellectual physician, as is also apparent from the lines written by the philosopher in an epistle addressed to the physician-historian, a letter dated 23 October 1928: “I testify that whenever I skim through your work and meditate on the situation of the history of sciences in our country – I am remorseful that I do not write more often, warmer and harder, in the papers that are at my fingertips. I know that, personally, for the scientific history studies I'm interested in – I have to thank you. Not to mention what others owe you, especially doctors and historians. The Institute makes «environment» scientific history, we, isolated ones, can at most, make the atmosphere. If an association for such studies can be woken up, I always think that the courage of the achievements has been with the production of the Cluj Institute.“[3] Even in India, Eliade maintains contact with the Romanian physician for whom he does not hesitate to admit he has a great cult of his extraordinary work of a huge volume: “The passion of science – that is, the slow, precise, technical sorting of the material our culture provides us – is the great temptation that brings me closer to you .” (Letter dated 16.02.1930, Calcutta)[4]

The reason why Eliade particularly appreciated Bologa resides in the philosopher's aspiration to write a few stories on traditional Indian medicine branches. Eliade admired the founder of medical history the ability to synthesize the huge volume of works, objects and manuscripts. It was the systematization work that occupied the author of *Religious History Treaty* and *History of Religious Beliefs and Ideas* all the time. At the same time he was better equipped to understand the enormous sacrifice of time and resources involved in ordering, ranking, and organizing such amount of information. Frustrated by the huge volume of notes, contact with Bologa developed philosopher's rationalization and ability to think more rigorously. Eliade's interest in medicine crystallizes in 1936 when, following a lecture held at an International Congress of History, Eliade publishes *History of Medicine in Romania*. The affection borne by the philosopher of the religions to this new emerging branch stems from the support given to the history of medicine which he perceives as a means of producing: “real services to the humanism of our age.”[5] Folk medicine is viewed with reverence by Eliade because it represents the immaterial and immutable connection with the ancestors of the nation. Since 1926, Eliade collaborated with Aldo Mieli, who was the publisher of *Archeion* magazine, producing short studies of the history of various sciences, medicine and folklore. That's how Eliade got to correspond with Bologa. The latter wanted to develop a collaboration with Miel's *Archeon* by making contributions in the form of articles devoted to Italian influences on Romanian medicine.

For Eliade, the whole science represents, at least in the initial phase, a single corpus. Subsequently, science has specialized and subspecialized over time. What could bring back all these disparate fragments to one place would be the philological field. In fact, even Bologa was aware that his scientific approach was far more philological. This is how one can explain the help that he Bologa asked from the philosopher. Another connection between the two, Bologa and Eliade, was the scientific curiosity to study botany. As a small child, Eliade devoted much energy to catching, studying, analyzing and cataloging various insects. At the age of fourteen, Eliade published a study titled *Silkworm's Enemy*, under the pen name Eliade Gh. Mircea, which showed the passion he has for insect biology. *The marvelous journey of the five beetles in the land of the red ants*-sketch of the novel – was written in the same period. More the outline of a teenager fascinated by the world of gangs, behind the modest mise-en-place is hiding a satire, an annoyance of the enemy (ants) by five elite bettles. It is a mockery of the human society reduced to the microcosm of insects.

The step to science would come when Eliade participated and won a contest that proposed the literary approach of a scientific subject. The title of the essay (*How I found the philosopher's stone*) is an epiphany of the future path that the teenager Eliade would take. The essay written by a youngster seems to have amazed the author himself when, over the years, he said, “How much I would like to be able to reread this story now, understand what that mysterious character revealed to me, what alchemical operations he assisted! I had found the philosophical stone in my dream ... I could only understand, decades later, after I read Jung, the meaning of this oniric symbolism!"[6] The short story, though a fantastic text loaded with supernatural, has as its starting point in Eliade's interest in chemistry and alchemy. Although he had promised Bologa that he would make his contribution to writing a history of Romanian medicine, Eliade's departure to India would break this momentum. Though time did not allow him, for the young Eliade was trying to absorb the new information that was crowded him, the philosopher gathers medical material inspired by yoga practices and even offers to write to the Romanian physician an article about Ayurvedic medicinal products, as we find out from a letter dated February 6, 1930: "I have a considerable number of facts on pharmaceutical medicine and magic in India, some of them astounding, such as those relating to *vagus nerve* control."[7]

For Eliade, alchemy is the gate open to an occult form of practice. Alchemy is the first type of objective report that leave leave, over the history of humanity, truly scientific discoveries; a kind of ancestor of rational knowledge. This preparatory, pseudo-scientific phase, the first attempt of structuring scientific knowledge was the one that attracted Eliade from his youth because of its esoteric character. In 1928 Eliade wrote an article (*Marcelin Berthelot and alchemy*) dedicated to a French chemist and biologist who imposed his name in the field of thermodynamics. Conscious of the enormous gap between Romanian and Western science at the beginning of the 20th century, Eliade sensed, from the philologist and philosopher point of view, the need to systematize the totality of historiographical material in order to be saved from oblivion: "We cannot wait until Romanian science reaches a European level to promote the validity of historical-scientific studies. There is no discipline that can be postponed."[8] The philosopher's insight was to build a methodology in this vast field of history of medicine. The history of science would be appropriate, with a takeover from a chemist and American historian Sarton, a new form of intellectual movement that would put man and science in the center: "Eliade understands a new interpretation or vision of man not derived from philological studies (textual), as it was Renaissance humanism, but in the history of science - understood as «any systematized knowledge» (Sarton), therefore more than «positive sciences»."[9]

Eliade, great admirer of George Sarton (1884-1956), intuited in the Belgian chemist the innovative spirit. Sarton embraced the history of science as a branch of gnoseology and aimed at linking science and humanism to a comprehensive one: the philosophy of science. Eliade was in the current with the theories of the American and hence the enthusiasm at the moment when a homologous branch was formed on the Romanian realm. The only ones of sufficient intellectual scope that Valeriu Bologa could count on were Mircea Eliade and Nicolae Iorga.

If alchemy was the gate open to science, popular creation and ancestral healing practices were the preamble of modern science. And Eliade felt this correlation, especially as the prose was anchored in folklore: "In his writings, the folkloric elements intertwine with those of the history of religions or ethnology. His stories take place in *illo tempore*, somewhere outside of physical time, and the characters have supernatural powers, their existence enrolling in an eternal present, and the facts being predetermined in advance. Witches, queens, beautiful women who make pact with the evil, curative herbs and charm plants, here are some of the ingredients with which Eliade sows his writings inspired by folklore."[10]

The pioneering work of building a frontier science in our country like the history of science did not frighten Eliade. We find out from a letter addressed to Bologa that, on the contrary, ostentatiously, he protects this new branch of knowledge, although he is aware of the weight of action in a rebellious society to the new: "I defend a science against the envy and imbecility of our intellectuals. I do not even think that our science will soon become popular. But it must not be ignored and, above all, dishonored by the elite to which it is de jure and de facto aimed at."[11]

What brought together a physician (Valeriu Bologa) and a philosopher (Mircea Eliade) were the folk traditions with application in medicine. Apparently two opposing personalities collaborated efficiently and discovered the common denominator, the unspoken binder between a scholar spirit and a metaphysical one, for “the research of Valeriu Bologa met the interests of Mircea Eliade and although they did not sign articles or books, the mere fact that they shared their opinions meant much for the later developments of both.”[12]

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Medical applications of the GC/MS method in the acute intoxication with dimethoate – clinical case

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Abstract*: Mass spectrometry is a chemical analytical method of determining organic substances by comparing their mass spectrum with mass spectra found in system libraries. In the case of biological products, substances of interest, like organophosphorus compounds, must be separated and identified for rapid and good medical measures (antidotism procedures) in acute intoxication case. A gas chromatograph coupled with a Varian mass spectrometer (GC-MS), was used to develop the application. The proposed objective is presenting the medical applicability in acute organophosphorus compounds intoxication management of the GC/MS method (gas chromatography coupled with mass spectrometry) as a separation and identification method for these compounds and their metabolites in urine samples.*

Keywords: *dimethoate, GC/MS, urine, acute intoxication*

INTRODUCTION

Acute intoxications represent a worldwide problem that tends to gain more amplitude each year.

Each intoxication presents certain characteristics which stem from the degree of socio-economic development of each country.

The organophosphorus compounds are mainly used to fight pests, as an alternative to chlorinated hydrocarbons, which persist much longer in the environment. Yet these substances are very toxic for humans too.[6] Because of this, measures were taken both to limit their utilization and to control the contamination of the environment.

The most efficient, but also most toxic substances utilized as pesticides are cholinesterase inhibitors (through reversible or irreversible mechanism). For both mammals and insects, the major effect of these substances is the inhibiting of acetylcholinesterase through the phosphorylation of the esterase site. The signs of symptoms which characterize the acute intoxication are caused by the inhibition of this enzyme and the accumulation of acetylcholine. Some of these substances possess a direct cholinergic activity.[4]

The absorption of organophosphorus compounds can be realized through three methods: inhalation, digestive and through the skin. One of their main characteristics is the fixation at hair level, where they enter through the skin, thus representing a permanent source of intoxication. Through the inhalation way, the intoxication is the most rapid. Through direct action on

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the bulbar respiratory center (muscarinic phenomenon) and the paralysis of respiratory musculature (nicotinic phenomenon), respiratory arrest occurs. Organophosphorus compounds are rapidly absorbed through the skin or digestively in the thin intestine. They bind well to the plasmatic proteins. High concentrations can be attained in the body within hours.[7]

Organophosphorus compounds represent a group of compounds with liposolubility or hydrosolubility, with high distribution volume. They are rapidly distributed in the liver, lungs, kidneys, heart and brain but do not accumulate.

This type of organophosphorus compounds is liposoluble, accumulating in the lexophile tissues of the organism, being a source for metabolic conversion to very toxic compounds. Thus, the intermediate syndrome can be explained (it appears between the 5th and 18th day from the intoxication). The toxicity of these compounds is manifested through the direct inhibition of cholinesterase, probably through direct poly enzymatic inhibition.[7]

The metabolizing of organophosphorus com-pounds takes place rapidly in the body, and as such they do not accumulate. Compounds such as parathion, malathion, phenthion, chlorpyrifos, normally inactive, upon entering the body they transform at liver microsomal level, through oxidation, in highly active compounds (paraoxon, malaoxon).

This transformation takes place under the action of paraoxonase. Their degradation takes place through hydrolytic and oxidative ways, through liver and kidney enzymes. The erythrocyte-origin cholinesterase remain blocked for the remainder of the red blood cell's life. Their regeneration takes place slowly (0.5-1% per day), remaining below normal level for over 3 months in severe intoxications.[8]

Organophosphorus compounds are eliminated through urine as such or as metabolites.

In the current global situation, it is very probable that these substances will be used in wars, conflicts, terrorist attacks. In such scenarios, they are used as extremely toxic agents and thus continuously represent severe threats to humans, animals and the fauna.[2]

The dimethoate, patented and introduced in the 50s, is a acetylcholinesterase inhibiting organo-phosphorus compound, it is not volatile, it is water soluble and it is not mobile in soil, where it degrades with a half-life of approximately 2-4 days, depending on the conditions. Dimethoate and omethoate urine levels reflect recent exposure.

Once having entered the body, organo-phosphorus compounds are metabolized to dialkylphosphates, which are eliminated through urine. Their metabolizing takes place rapidly in the body and as such they don't accumulate.

Compounds such as parathion, malathion, phenthion, chlorpyrifos, normally inactive, enter the body and transform at liver microsomal level, through oxidation, into highly active compounds (paraoxon, malaoxon). Identification of these metabolites in the urine may be an indicator of exposure to organophosphorus compounds and can be performed through a GC/MS analytical method with an ion trap and electronic ionization.

The dimethoate is rapidly metabolized, mainly through the initial splitting of the C-N bond to obtain dimethoate carboxylic acid and, eventually, a number of tiophosphate and phosphate esters. The minor quantitative elimination way involves the oxidative metabolism to produce the oxygen analogue of dimethoate, omethoate. The parent compounds represents 1-2% of the dose excreted in the urine.[5]

MATERIALS AND METHOD

The research was performed on a GC-MS Saturn 2000 Varian system composed of gas chromatograph model Varian CP – 3800 and mass spectrometer Varian Saturn – 2000.

Establishing optimal working conditions and functional parameters for the development of a GC/MS method are important steps in the development of a GC/MS method for the separation and spectral identification of dimethoate in urine.

ANALYTICAL CONDITIONS

GC – an instrument equipped with a ion trap detector; GC – gas-cromatograph has the role of taking in the sample and separate the mixture of substances composing it.

GC/MS parameters

Injector temperature: 300° C
Carrier gas: He • Column flow: 1.2 ml/min
Separation time: 50 min
Ionization mode: electron impact (70eV)
Ionization current: 20 μA
Ionization temperature: 170° C
Detection mode: full scan
Manifold temperature: 80° C
Ion trap temperature: 170° C
Interface temperature GC-MS: 260° C

The separation of the compounds was realized by the active layer of the DB-5MS capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm). The optimal conditions for chromatographic separation and detection were established following the study on the compound's retention time's dependency on the structures of the said substances.

The temperature program of the gas-chromatograph's column's furnace is presented in Table 1.

Table 1: The column gas furnace temperature program.

Temperature (°C)	Rate (°C/min)	Hold (min)
140	0.00	1
290	5.00	19.00

MS – mass spectrometer **–** has the role of analysing molecules that come out of the gas-chromatograph through their unique mass spectre. Thus, the molecules that come out of the GC can be identified by the user. Mass spectrometry established the relative abundance of ions resulted from the ionization process of an organic molecule. The method is used in chemical analyses, in the analysis of some quantum processes or in the separation of certain chemical elements. The determined mass represents the m/z ratio (mass to charge) of the atom or group of atoms from which the ions resulted.

Mass spectrometer parameters

Manifold temperature = 800º C
Ion trap temperature = 1700º C
Ionization current = 20 μA
Acceleration tension = 70 eV
Working times
  0 - 5 min – closed filament
  5 - 37 min acquisition in mass domain 50 - 450 amu.
Acquisition domain 50 – 400 amu
Segment setpoint
Scan time = 1 sec/scan
Multiplier offset = 0 V
Emission current in FS 10 μA
Ion threshold 1 count

Scanning parameters for ions formed in the trap are presented in Table 2.

Table 2. Scanning parameters for ions formed in the trap.

Low Mass (m/z)	High Mass (m/z)	Ionization Storage Level (m/z)	Ionization Time Factor (%)
10	99	48.0	100
100	249	48.0	100
250	399	48.0	100
400	650	48.0	100

Normally, the mass spectrometer operates in the domain in which the analyzed substances will be found. When coupling it with a gas-chromatograph, substances no greater than 450 amu will be sent to the mass spectrometer, as those with greater molecular weight cannot be vaporized in the chromatographic column.

Thus, the maximum acquisition domain will be 50 – 450 amu, as below 50 amu an acquisition is not typical, since the atmosphere in the apparatus has a rich spectre up to 44 amu.

The use of the full scan technique (FS) is very good, since following the obtaining of the mass spectre, it can be compared with mass spectres already existant in dedicated spectral libraries. In this case though, the duration of a ion's analysis is of 4ms, in case it is used as an acquisition time for a scan of 1 s.

Confirming the identity of the compounds relies on comparing the mass specter and ratio of reference ions' abundance for each analyzed substance identified in the sample with those of standards using the mass specter library. To identify the obtained specters, the following were used: Pfleger – Maurer – Weber specter library (PMW), specialized for compounds of interest in toxicology as well as specter libraries NIST2000 and Wiley6. If a mass spectre obtained from the sample cannot be compared with mass spectres in specialize spectral libraries, the identification of these compounds in biological matrices would not be possible to perform.

RESULTS AND DISCUSSIONS

Dimethoate has moderate acute toxicity for mammals (for example, DL50 in mice and rats is 150 and 400 mg/kg of bodyweight) (IPCS, 1989). Omethoate is approximately 10 times more toxic and a stronger cholinesterase inhibitor than dimethoate. Dimethoate is well distributed in the body's tissue and metabolized in the liver to omethoate (most probably through the enzyme system of P450 cytochrome) which is then quickly transformed into multiple dialkyl methyl phosphate metabolites, which are eliminated in urine within 1-2 days. Dimethoate is considered mutagenic, but it is not teratogenic. [1,10].

In order to verify the developed methods, they were applied on biological samples obtained from patients in the ATI II Clinical Toxicology Section, patients suspected of acute organophophorus compounds intoxication. The urine (aprox. 25 ml) undergoes liquid-liquid extraction procedures in order for the sample to be analyzed through the GC/MS system. For example, we present the medical applicability of this method in the case of an acute dimethoate intoxication.

The separation and identification of dimethoate or omethoate in urine corroborate with the enzymatic activity determinations for serum pseudo-cholinesterase and lead to the establishment of an analytical diagnosis in case of an acute dimethoate intoxication and the initiation of specific therapies for this type of intoxication (such as antidotes). Identifications are performed through the comparison of the mass specter obtained through the analysis of urinary extract samples with mass specters already existent in the database. This specter is obtained based on the molecular mass of the compound of interest which, following fragmentation, gives birth to specific spectral lines. These are shown in tables 3 and 4.

CASE REPORT

C.P., male, aged 19, no occupation

Admission reasons
- Coma
- Acute respiratory insufficiency
- Muscular fasciculation

APP – no significant case history

History – patient with no significant case history is found by his parents in a coma with respiratory dysfunctions and muscular fasciculation, symptoms that follow the voluntary ingestion of an organophosphorus pesticide. Near the young man, the parents found an unlabeled bottle containing a liquid with a smell particular to insecticides. They call the ambulance and the young man is transported to the Clinical Emergency Hospital.

In the Major Emergencies Department, the patient is in a coma with severe dyspnea and muscular fasciculation. The oropharyngeal secretions are vacuumed and orotracheal intubation and Ruben balloon ventilation are applied.

Table 3: Specific ionic fragments and spectral lines for dimethoate

Compound	Spectral line (m/z)	Chemical formula of ionic fragment
Dimethoate $C_5H_{12}NO_3PS_2$ (M=229)	157	– [M-72] - $(CH_3O)_2PS.S^+$
	143	– [M-86] - $(CH_3O)(HO)PS.S^+$
	125	– [M-104]- $(CH_3O)_2PS^+$
	93	– [M-136]- $(CH_3O)_2P^+$

Figure 1: Mass spectre for dimethoate (Nist98 library)

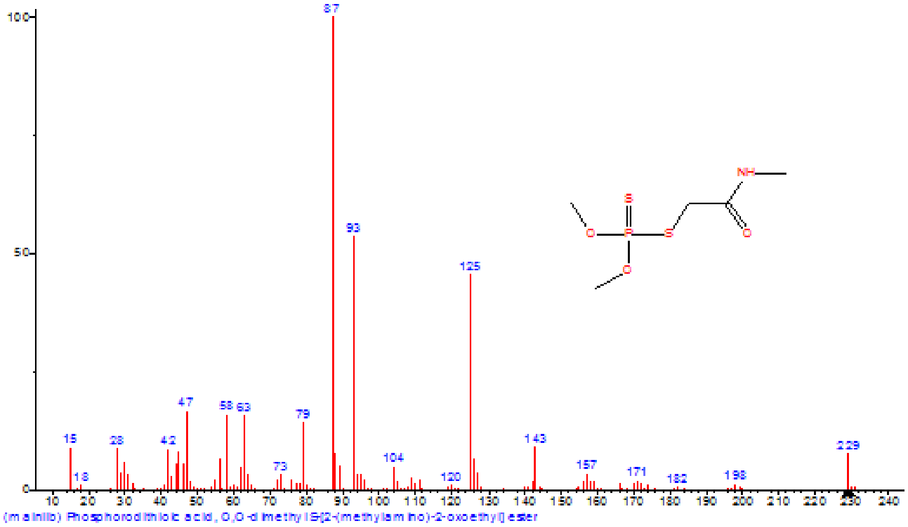

Figure 2: Mass spectre for omethoate (Nist98 library)

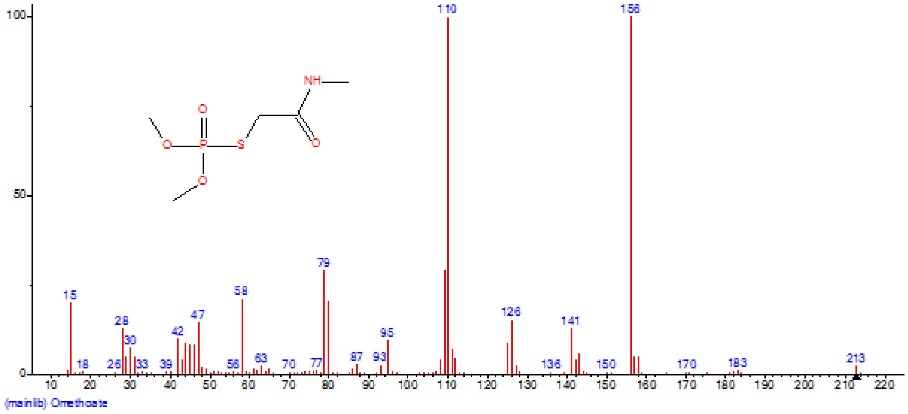

Table 4: Specific ionic fragments and spectral lines for omethoate

Compound	Spectral line (m/z)	Chemical formula of ionic fragment
Omethoate $C_5H_{12}NO_4PS$ (M=213)	156	– [M-57] - CH_3NCO
	141	– [M-72] - $(CH_3O)_2P{=}O.S^+$
	126	– [M-87] - $(CH_3O)_2(HS)P^+$
	110	– [M-103] - $(CH_3O)_2(HO)P^+$
	109	– [M-104] - $(CH_3O)_2P{=}O^+$
	79	– [M-134] - $(CH_3O)(HO)P^+$

The determination of pseudocholinesterase activity detects a high degree of inhibition of 0.5 UI/ml. A urine sample is being taken for the toxicological examination and is sent to the Analytical Toxicology Laboratory. The patient is hospitalized in the ATI Toxicology Section.

Objective examination upon admission:

- Severe general condition;
- Reed IV coma; non-reactive;
- Pale, sweaty skin;
- Cyanotic extremities;
- Miotic, equal pupils, with slow photomotor pupil

reflex;
- Strong reflexes in all four members, with subintrant muscular fasciculation;
- At pulmonary level, bronchial rales bilaterally diffuse;
- Does not efficiently ventilate on IOT probe, so mechanical ventilation is applied;
- Diarrhea;
- Arterial Tension = 80/60 mm Hg;
- Subfebrile (t^o = 37.5).

ECG upon admission records sinus bradycardia (44/min) without conduction or repolarization.

The CT brain scan and lumbar puncture exclude a vascular etiology of the coma.

The analytical toxicological GC/MS examination of the urine:

Following the analysis of the urine, the total ion chromatogram that is shown in Figure 3 was obtained. In it, besides dimethoate, its metabolites can also be identified.

The substances identified through the above GC/MS method used for the urine analysis are shown in table 5.

The cardiopulmonary radiography detects a homogenous opacity situated in the inferior right pulmonary field.

Therapeutic measures

Stabilization
- Vacuuming tracheobronchial secretions
- Support ventilation
- Nasogastric intubation; gastric lavage;
- Central sub-clavicle catheter
- Parenteral fluids
    - o glucose solution;
    - o Ringer solution;
    - o isotonic saline solution;
    - o Gelofusine;

Support therapy
- Specific antidote – atropine under clinical surveillance
- Mucolytic – acetyl cysteine;
- Bronchodilators – miofilin;
- Antibiotherapy in combinations (penicilin G 1 milion UI/6 hours + gentamicin 80 mg/day + metronidazol 500 mg/day);
- Plasma – 4 units/day, for 3 days and 2 units/day for 2 days;
- Monitoring vital functions;
- Monitoring pseudocholinesterase activity.

The applied therapeutic measures, respiratory and general nursing are continued.

Clinical evolution

4 days from admission, the evolution is favorable; the patient is conscious; he is taken off the mechanical ventilation apparatus; he breathes spontaneously with the intubation probe and is extubated. His oral cavity is washed.

The cholinesterase activity is measured: 2.1 UI/ml.

The antibiotherapy is continued 3 days from the extubation to solve his pulmonary affection.

The evolution is favorable. The patient is released 7 days from admission with no neurological damage.

Table 5: The substances identified in the urinary extract that was analyzed through the GC/MS method and their specific spectral lines.

Compound	MW	EI fragment ions (m/z)
Dimethoate	229	87, 125, 93, 79, 229
Omethoate	214	156, 126, 110
Dimethoate M (HOOC-) ME	230	93, 125, 198, 230
Phosphoditioic acid – O,S,S trimethyl ester	172	93, 109, 125

Figure 3: Dimethoate and its metabolites – omethoate, dimethoate M (HOOC-) ME and the phosphoditioic acid – O,S,S trimethyl ester. Omethoate mass specter.

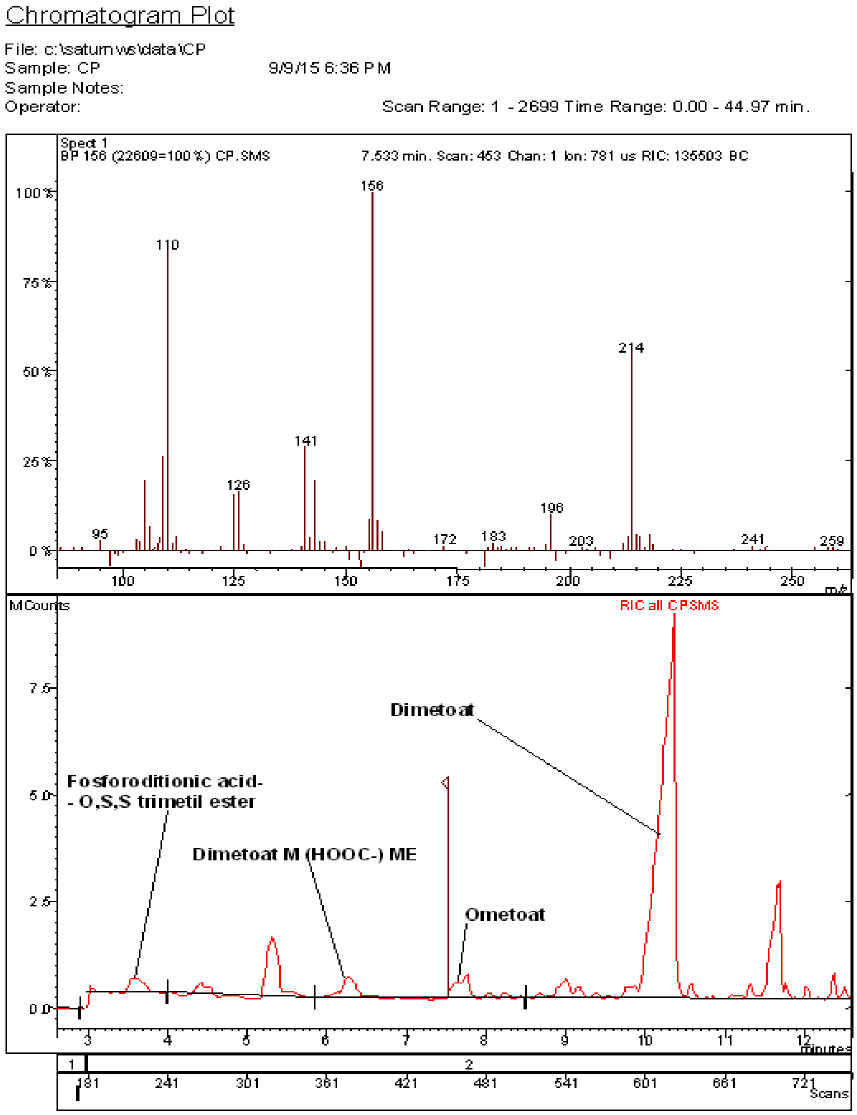

Chromatogram Plot

File: c:\saturnws\data\CP
Sample: CP 9/9/15 6:36 PM
Sample Notes:
Operator: Scan Range: 1 - 2699 Time Range: 0.00 - 44.97 min.

Spect 1
BP 156 (22609=100%) CP.SMS 7.533 min. Scan: 453 Chan: 1 Ion: 781 us RIC: 135503 BC

CONCLUSIONS

The GC-MS method is the only method that can determine organophosphorus compounds in unknown matrices for the quality control of water, the environment and foods or to establish the analytical diagnosis in case of contamination/intoxication with organophosphorus compounds.

The mass spectrometer can acquire data through various methods. In "full scan" method (FS), the mass spectre obtained through electron impact ionization can be compared with mass spectres found in specialized libraries, making possible the identification of these substances in unknown matrices.

To reduce the number of false positive or false negative results, analytical procedures based on precision, accuracy, detection limits, error source identification are needed but also the expanding of existent spectre databases.

A quick and correct analytical diagnosis influences the quickness and correctness of specific therapy measures that can be applied in such a context.

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ORIGINAL ARTICLES

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Patient reported outcome measures and joint replacement

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Abstract: *PRO (Patient Reported Outcome) is a clinically based questionnaire filled directly by patients, and other variant types of measures, in clinics and hospitals that gather patients' stance on their conditions in treatment. PRO is different from Patient Based Outcomes whereby the latter addresses the patient's concerns but do not necessarily enquire from them. However, PRO gather strictly from patients themselves through interviews, self-administered questionnaires and other available measures. The patient's perspective on issues that is significant in enacting certain particular clinical policies and regulations such as approval of a medication/drug. Most PROM constitutes one (one-dimensional) or more underlying assessments (multidimensional) connoted as constructs, which bear several levels of scale to assess degree.*

Keywords: *PROMs, orthopaedics, patients, hip replacement, knee replacement, healthcare system*

OBJECTIVE

The questionnaire or interview used to gather information is referred to as *measures, tools or instruments.* Commonly, there are two types of PROM questionnaires.

Generic PROMs, which are used to assess generally across numerous diseases in a broad spectrum perspective, and condition-targeted PROMs that are developed for a particular medical condition [1].

This paper critically examines patient-reported outcome measures (PROMs) and joint replacement from a broad perspective.

METHODS

Most PROMs measure aspects such as Quality of Life (QoL) that is fulfilment of needs and impact of restrictions on emotional wellbeing, and drug side effects. Others include symptoms/impairments that is pain and depression, functioning during disability, locomotion, daily living activities and personal care. In addition, Health Related Quality of Life (HRQoL), health status, general health experience and rating of healthcare facilities and operations [2].

Analysis of PROMs is usually conducted using approved analysis tools for proper interpretation such as Rasch analysis or confirmatory factor analysis. PROMs are often validated using particular tools and methodologies, such as Linguistic validation for population's differences and others to ensure that they are effective in gathering relevant information. Patient grouping too

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should be reliable and conform to ideal scaling, development and psychometric standards. Examples of renowned PROMs include the SF-36 Health Survey, EuroQol (EQ-5D), SF-12 Health Survey, Profile, Quality of Well-Being Scale, Health Utilities Index and Consumer Assessment of Healthcare Providers and Systems (CAHPS) [3].

These are examples of generic PROMs. Ideal examples of condition-targeted PROMs include Adult Asthma Quality of Life Questionnaire (AQLQ), Seattle Angina Questionnaire (SAQ), Kidney Disease Quality of Life Instrument, Epilepsy Surgery Inventory, National Eye Institute Visual Functioning Questionnaire, Ankylosing Spondylitis Quality of Life questionnaire (ASQoL) and Migraine Specific Quality of Life (MSQOL) [4].

With the advent of PROMs and the role they play in medicine, individual countries such as England's National Health Service (NHS) have made it a prerequisite for particular surgical operations to provide non-compulsory PROMs before the procedure and following the procedure (ideally three months after procedure); these include hip, knee and other joint replacements, hernia surgery and varicose vein surgeries.

England used the PROMs to assess the effectiveness and effects of the surgeries on its national a patients and deduced that the frequency of operations/ surgeries should be maintained. Due to their efficiency and importance in quality health service, PROMs are updated monthly as a policy in most developed countries. PROMs are currently used to grade health facilities with scores parameters according to patient satisfaction. In England, HES (Hospital Episode Statistics) use PROMs to rate hospital services across the state and their use are gaining impetus across the global health sector [5].

There is a general dataset that PROMs include in questionnaires; Generic and condition-specific measures of self-reported health status. Patient-identifiable information included in the PROMs, which is used for relation purposes, is strictly not availed for wider analysis, due to confidentiality. Additional questions inquiring into the patient's health status include whether they have antecedent conditions such as diabetes or arthritis [6]. The outcomes of a health procedure can be ascertained from the patient's perspective, through self-reported symptoms and functional status, by comparing and determining the differences in data between the pre-operative and post-operative PROMs.

However, the PROMs are not compulsory for patients to fill. More so, consent from patients who participate in the PROMs has to be sought before their data is used for analysis [7]. The patient's identifiable information is only used to electronically fetch for his or her National Health Number in government database during analysis of PRO data. The rest of the data is transferred to a database, such as the HES in England, from where the PRO analysis consequently occurs. Pre-operative and post-operative PROMs from the same patients are identifiable in the dataset since they possess similar serial numbers from which they are linked. After analysis, data in the HES is pseudonymised before it is made available to the public for download for analysis and scrutiny and hospital/clinical scoring [8]. Other uses of PROMs include: allows managers and clinicians to benchmark their own performance with regards to others, they are used for research purposes and draw relations to effectiveness and cost-effectiveness of health procedures to care. It is also used to compare implications of presence and absence of the treatment or rather alternative treatment, searching for healthcare inequalities, and research on relationship between pre-existing health and social conditions and risk of deterioration after procedure. Other than the anonymised data that is availed to the public for scrutiny and further personalized analysis, PROMs can be availed to service providers of patient care through provider level extract only with patient's approval. More so, extract service of particular requested data sub-subs by customers can be availed at an administrative fee depending on complexity of the request [9].

There are variant methodologies in which PROMs are used to score and rate health facilities. Some examples of standardised PROMs that are analysed by specific methodologies include the five-dimensional descript-tive system EQ-5DTM health questionnaire and the

EuroQol Group's visual analogue scale (EQ-VAS) [10]. Most PROMs used for joint replacement procedure, such as hip and knee replacement, are condition-targeted. Most common post-operative PROM questions include an inquiry into the patient's health status after procedure such as their state of mobility or other operative complications.The EQ-5D is a simple, generic measure of health for clinical and economic appraisal [11].

The PROM with single indexing values for health status and an unsophisticated descriptive profile is widely used in economic and clinical evaluation of healthcare and in health surveys of populations. The EQ-5D provided in joint replacements contains a descriptive system with issues on mobility, discomfort /pain, self-care e.g. washing and dressing, anxiety and depression and normative activities e.g. work, housework, study, family or leisure activities etc. Each of these five dimensions has several level statements which the patients tick against the most appropriately descriptive of his or her condition.

Each dimension has a score digit for each level statement hence every patient has five string scores thence the connotation '5D' [12]. Using a formula, the five string score are converted into a singular summary index, referred to as the 'social preference weights' assigned to each statement in the dimensions. The value of full health is assigned to value one (or state 11111, in EQ-'5D') from which reference is sought. EQ-VAS index scores range from 0 to 100, least and best health respectively. The patients mark, within the range, his or her relevantly perceived state of health [13].

Other PROMs used in England for joint replacement include the condition-targeted Oxford Knee Scores (OKS) and Oxford Hip Scores (OHS). The PROMs contain twelve multiple choices, assigned later with scores, about the patients state of mobility, pain, ease of joint movement, ease of partaking normal chores and activities. The scores in the PROM are such that the less the scores the poorer the patient's condition with zero for greatest severity. For each multiple choice, 4 is the greatest score for best patient condition. Hence, the total score for every patient in the PROM have a maximum limit of 48 for ideal patient condition while zero indicates worst severity [14].

Postoperative Recovery Profile in condition-targeted PROMs with recovery specific questions is used to determine the quality of joint replacement procedures in health facilities and person-centeredness of clinical services [15]. This is common procedure for persons with arthritis. The USA, Norway, Denmark, Sweden, New Zealand, Canada and England operate 77-153 and 66-143 hip and knee replacement per 100 people in prevalence rate. With advances in biomedical operations and medication intended to shorten or alleviate the post-operative recovery period especially since recovery takes place in the vicinity patient's home, PROMs are significant and effective method of evaluating these procedures on patients [16]. Traditionally, assessment of joint replacement were assessed by drawing connections between different intervention methods such as variant joint replacement procedures. The types joint replacement include prosthetics, implants, surgical techniques. Relations of these types of joint replacements were drawn to revision rates, complications and post-operative medications. With increased impetus on the use of PROMs, this evaluation is augmented thus allowing for an improvement of healthcare services [17]. While EQ-5D, EQ-VAS, OHS and OKS are important instruments in PROMs, they do not comprehensively provide adequate information important of the requisite important aspects that allow for quick recovery. Recovery-Specific Instruments have been devised to bridge this gap. Swedish healthcare PRP (Post-operative Recovery Profile) PROMs on joint replacement patients has comprehensive data on patient's problems, medical interventions and outcomes of treatments such that it has gained global recognition [18]. The Swedish methodologies of PROMs and their analytical tools are being replicated across the developed world since they include measurements on different groups of patients and can be altered for different purposes in the variant PROM areas [19]. The PRP has additionally incorporated global-, dimensional- and item levels in scoring not only for sole patients but more so for every group of patients. The global score is significant in deducting the recovery rate of a population-based profile.

An embodiment of a PROM is one in Sweden that was conducted on joint replacement (hip and knee) operations patients whereby the PROM questionnaire was provided the day before the procedure, three days after the procedure and one month later after the procedure. The peri-operative variables included sex, surgical procedure, American Society of Anaesthesiologists' (ASA) guided physical classification, age, duration of surgery, length of postoperative stay, blood loss and marital status [20]. PRP was included into the PROM and 19 *constructs* collected included physical functions and symptoms, psychological, social and activity measures.

Response category for assessments, from which scores would be assigned, included: none, mild, moderate, and severe symptoms. Recovery profiles for every individual and group on each item and dimension were provided by the PRP. The 19 item responses account for a detailed individual response profile over the recovery dimensions and the item frequency distributions reports on the item response profile of the group [21]. *Fully recovered* score in the group ideally would have indicator score of 19, 15-18 indicator sum for *almost full recovered* , 8-14 indicator sum for *partly recovered* , 7 indicator score for *slightly recovered* and below 7 *not at all recovered.*

Using methodological tools of frequency distribution analysis, out of the 75 patients who voluntarily participated in the PROMs assessment after undergoing primary knee and hip replacement due to osteoarthritis, 23 patients indicated the same level of pain on both the 3 day and one month follow-up [22]. The remainder showed a decrease in pain after the one month follow-up. Significance in RP values was used to assess the systematic change in recovery of groups. Individual variations within groups and between groups can thereafter be scrutinized.

RESULTS

Besides pain, other score categories included muscle weakness and re-establishment of everyday life. On physical symptoms and function's frequency distribution, for three days the frequency for the *none* assessment ranged between 62% and 7% while one month later, 72% to 25% [23]. Systematic group changes, unchanged assessments, individual variability in all dimensions were analysed. For global level assessments for day 3, 11(score for partly recovered) was the median while 13(score for partly recovered) was median after one month follow-up [24].

This information was used to ascertain whether the surgical intervention or rehabilitation in the joint replacement procedures and therapy were appropriate for individual patients or for groups of patients [25]. The PROMs data was also used to identify particular risks in particular groups (categorized by demographics) associated with the hip and knee replacements. While the analysis of the PROMs indicated homogenous recovery changes in the groups, certain assessments were unchanged for both the 3day and one month assessment; muscle weakness and pain.

However, great individual variations on the two categories were found to result to this. Using this data, Sweden was able to determine the best treatment and therapy techniques to render to joint replacement patients for a quick recovery [26]. It was also found out that a standardized treatment method for groups that exhibited great variations in individuals was not necessarily the remedy to the situation. Extensive use of PROMs in Sweden has allowed the country to increase its knowledge on the best healthcare practices hence an improvement in their healthcare delivery and high score/ratings of their hospitals internationally [27].

Based on the PROMs Swedish healthcare system is able to establish expected recovery within junctures in recover period. This can be sued to grade other treatments as set-backs and gains with regards to the expected outcome and therefore facilitate the overall recovery and create awareness of the recovery process. PROMs have also been used to enhance, as a clinical tool, the manner of clinical relationship and contact in follow-up visits between physicians, nurses and their patients. In joint replacement, resumption of normative daily activity and functionally capacity were usually found to be unsatisfactory.

The level of satisfactions was greatly influenced by pain, mental functioning and fulfilled expectations regarding postoperative pain [28]. The psychological dimension rated higher for those who concomitantly scored 13 in physical functioning and pain. Longer period assessment such as after 6 months have been shown to record greater recovery scores. Nonetheless, the Swedish HealthCare is keen on shortening the recovery period for joint replacement thus the focus of PROMs of short recovery periods. For long period PROMs, categories of assessment such as Quality of Life (QoL) and Health Related Quality of Life (HRQoL) are incorporated [29].

These PROM scores by patients who visit variant clinical and hospital facilities allow for the grading of hospitals/clinics too. Using relevant and respective methodological tools, scores from patients attended in various hospitals/clinics can assist in the national grading and scoring of hospitals. This initiates competition for better healthcare provision concurrently improving the healthcare of a country. Data has established that 70% of countries with esteemed healthcare systems have national policies on the use of PROMs in their HealthCare facilities.

CONCLUSION

Facilities that exhibit consistently low scores could be sanctioned and enquiries instigated into their medical practices. This allows for the monitoring of healthcare [30]. PROMs therefore are ideal instruments for the improvement of healthcare provision. They assist in determining the treatment and medication that ideal for patients in quick recovery mode. They create a patient-centred healthcare system. Hospital ranking/scoring on the other hand allow for benchmarking of clinical performances thus generally improving the provision of healthcare in a country [31].

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ORIGINAL ARTICLES

Physical effort – an underused preventable method in colorectal cancer

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Abstract: *Colorectal cancer prevalence is increasing worldwide. Modifiable risk factors are responsible for almost 50 % of cases and this could imply a huge potential of preventability. Among these factors the level of physical activity is of paramount importance. Physical activity has a positive impact on health status in general and it decreases the prevalence of various cancers including colorectal cancer. Physical activity decreases the prevalence of benign colorectal adenomas and it prolongs the disease free interval after surgery in colorectal cancer, thus increasing survival. The mechanisms involved are multiple: decreasing bowel transit time, regulating energy balance, decreasing peripheral insulin resistance, decreasing hyperinsulinism, antiinflamatory effects, increasing vitamin D production*.

Keywords: *colorectal cancer, physical activity, obesity, lifestyle modifications*

Physical activity is a major and potentialy modifiable component of life style, which may be able to highly influence the risk of main cancers. Hence, there are convincing evidence that an important benefit may be derived concerning risk reduction in endometrial cancer, colorectal cancer, breast cancer, prostate cancer, lung cancer and ovary cancer. It is estimated that in Europe in 2008 between 150000 and 300000 cases of cancer could have been prevented only by the way of maintaining a resonable level of physical effort in general population.[1]

A series of convincing observational data suggest that regular physical activity, be it ocupational type or recreational type, protects against colorectal cancer (CRC)[1,2]. Around 60 studies have been published till 2010 concerning the issue of physical activity and CRC.[2] A metaanalysis that included 21 studies stated a significant reduction of CRC risk by 27% in the group of subjects that performed vigurous physical activity as comparing with the group of sedentary subjects (RR 0.73, 95% CI 0.66-0.81).[3] The mechanism that may provide an explanation for the

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relative protection of physical effort is currently unknown. There are no interventional type studies published yet to support the role of regular physical effort as preventive method in CRC.

In 2007 the results of a cohort study (Nurses' Health Study) has been published that enrolled 80,000 female subjects from 1986 with a 16 years follow-up. There have been diagnosed approximately 500 cases of CRC. A multivariate analysis that controlled the confounding factors represented by other risk factors for CRC concluded that there was a proportionate reversed relationship between physical effort and distal colonic cancer and, to a lesser extent, with proximal colonic cancer. Women situated in the highest percentile of recreational physical activity had a reduction of distal CRC risk by half as compared with women situated in the lowest percentile (RR=0.54, 95% CI 0.34-0.84). Risk reduction did not vary with body mass index (BMI), although former studies had suggested that physical activity had the greatest impact on CRC only in high BMI subjects. The level of physical effort to produce prophylactic benefits may be only minimal, as this study demonstrated. As such, even an hour of slight walking a week may reduce the risk of CRC by 31% (RR=0.69, 95% CI 0.45-1.03) as compared with women who do not report any kind of physical activity. This protective effect of slight walking reached a plateau at 2 hours a week (RR 0.64, 95% CI 0.41-1.00) as opposed to moderate and vigorous physical effort that was characterized by very clear dose-response relationship. The more alert slight walking rendered greater protective effect than slower slight walking (RR=0.43, 95% CI 0.17-1.05). Furthermore, 4 hours a week of moderate/ vigorous physical effort may reduce the risk of CRC by 44% comparing with 1 hour a week (RR= 0.56, 95% CI 0.33-0.94). Physical activity lessened the risk of CRC regardless of the impact on BMI: this idea may imply that physical activity protects against CRC by a mechanism independent of that involved in resolution of obesity. The protective effect of physical effort validates at distance. The actual reduction of CRC risk is considerable only after several years. In conclusion, the authors of this study suggest that even minor physical effort may derive benefit on CRC risk reduction. Several mechanisms have been proposed. Thus, physical activity regulates energetic balance and intervenes in reduction of hyperinsulinism and peripheral resistance to insulin. Physical activity may intervene also through anti-inflammatory mechanisms. Moreover, the positive effects of physical effort may also be explained by reduction of obesity in spite of the data that demonstrated that physical activity may reduce the risk of CRC independent of the effect on obesity. Another proposed mechanism involves accelerating the peristalsis which reduces the contact time between intraluminal carcinogens and colonic mucosa. As a matter of fact, it is well known data that physical active individuals are more prone to sun exposure for longer periods of time which facilitates production of vitamin D that is associated with lessening the risk of CRC.[4]

Figure 1: Mechanisms involved in protective effect of physical effort on colorectal cancer risk

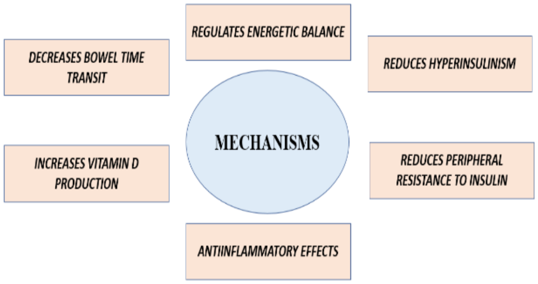
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Sedentarism is a globally important public health issue, especially in developed countries, in women, in old people and in low income individuals. Lack of physical activity is responsible of the increase in mortality rates especially from diabetes mellitus and heart diseases. To a comparable extent physical activity of moderate and vigorous intensity is associated with certain benefits regarding health status, including reduction of obesity risk, cardiovascular risk, stroke risk, risk of some types of cancers and decreasing in global mortality rate. Physical activity increases the probability to cease smoking, delays cognitive decline in old individuals, alleviates the adverse effects of stress, anxiety and depression. A study published in 2016 regarding the issue of physical activity status in the group of more than 50 years old individuals in USA the date are worrisome: 27% do not report any kind of physical activity outside working place in the last month; the prevalence of inactivity increases with age, reaching 35.3% in age group after 75 years; sedentarism is more prevalent in women then in men, in Afro-Americans then in Caucasians. Also, the prevalence of inactivity is decreasing with increasing in educational level and with decreasing in BMI.[6]

Lack of physical activity is the main cause of CRC being responsible of 14% of cases of CRC in USA; 12% of cases may be attributable to western diet, 12% to lack of daily administration of aspirin and 8% may be related to a family history of CRC.[6]

Sedentarism, especially that kind related to spending time in front of TV, is independently associated with increasing CRC risk. Hence, if one spends 9 hours in front of TV, as comparing with 3 hours or less, the risk of CRC will rise significantly by a RR=1.61 (95% CI=1.14-2.27).[5]

The relationship between physical activity, sedentarism and BMI is not to be changed even if one may exclude the contribution of age, race, family history of CRC, smoking and western diet.[6]

Several studies shows contradictory results concerning the issue of rectal localization of CRC. Cancer Prevention Study II indicates that moderate/vigorous physical activity in men and in women reduces the risk of rectal cancer by 30 %.[7] Many cohort studies did not find any kind of association between physical activity and rectal localization of CRC.[8,9]

A meta-analysis published in 2010, which included 20 studies on physical activity and colorectal benign adenoma, concluded that there was 16% reduction of the risk of these benign precursors of CRC if we compared active population with less active populations. Risk reduction was even more significant if we took into consideration polyps bigger then 1cm (31% risk reduction). It has been demonstrated in that way that physical effort might decrease the risk of CRC earlier in the stage of precursor lesions of oncogenic process.[10]

The role of physical activity as a protection factor in CRC is hardly known in general population. A study developed in USA that included 2000 subjects showed that only 15% of them are aware of this benefit of physical activity.[6] One similar study from Europe that included 21 countries indicated a 30% level of knowledge concerning this topic.[11] Several studies stated also that there was a close connection between the level of information concerning prophylactic benefits of physical effort in CRC and the increasing of the motivational status to produce life style changes that, in the end, will decrease the prevalence of CRC.[12,13,14]

Physical exercises represent a form of human activity that may benefit health more then it may inflict side effects. The most common side effects are musculo-skeletal injuries. The least common side effects (sometimes more severe) are: cardiac arrhythmias, heart arrest and myocardial infarction. Generally, we may appreciate that the potential benefits of physical exercises highly surpass the potential risks. Moreover, it is considered that it is unnecessary to screen for coronary diseases prior to initiating physical activity if

the subject was asymptomatic and included in the low cardiovascular risk group.

One may consider mandatory that all healthy adult individuals to include in their life style moderate or vigorous physical exercises. The majority of authors agrees that the highest health benefits are provided by 150 minutes a week of moderate physical activity or by 75 minutes a week of vigorous physical activity. Nevertheless, adults that have a limited physical activity capabilities should remain active because it has been noticed that even if a modest amplitude of physical effort is exercised regularly health benefits will be significant.

Another epidemiologic studies suggest that physical activity may influence not only the risk of CRC but also it may prevent the recurence of CRC after curative surgical treatment. All the data available resulted from observational type of studies; randomized and interventional studies are not published. Nevertheless, American Society of Oncology (ASCO) recently recomended that the surviveours of CRC should maintain an optimal weight, should perform daily physical exercises and should follow a healty diet.[14] Futher on, there are some interesting results of a study published in 2006 that included 832 patients suffering from CRC stage III surgicaly treated and that followed a program of chemotherapy. It has been demonstrated that moderate physical activity perfomed for at least 300 minutes a week has increased the free disease interval with 45% and has improved by 29-36% the mortality rate of any cause.[16] The benefits have been dose dependent.

In an observational study (unpublished data) that I have conducted in 2016, concerning the topic of CRC and its relationship with diabetes mellitus and other risk factors an important conclusion has been drawn.

A multivariate analisys in which the most acknowledged CRC risk factors have been included showed the statistical significance ($p<0.05$) have been reached for smoking and physical activity only. This implies that by the way of increasing physical activity levels in general populations and by giving up smoking CRC „epidemics" could be fairly prevented.

Table 1: Multivariate analysis of risk factors in CRC

Variabile	*Coeficient*	*Standard error*	*t Stat*	*p*
Age (years)	0.0014	0.0035	0.4087	0.68
BMI(Kg/m2)	0.0031	0.0055	0.5650	0.57
Smoking	0.1273	0.0647	1.9658	**0.05**
Daytime nap (hours)	0.0150	0.0440	0.3404	0.73
Physical activity	-0.1334	0.0601	-2.2181	**0.02**

In conclusion, the preventable potential of CRC is high through the way of regular physical exercise and this may represent a very approachable solution to decrease the global burden of the disease. It is very important to stress that the target of decreasing the prevalence of CRC by physical exercise does not necessarily imply an impact on obesity, the benefits on CRC being independent from the benefits on BMI. A good level of motivation in general population through health politics is mandatory because the changes in life-style (level of physical activity, diet and smoking) are otherwise impossible to be reached.

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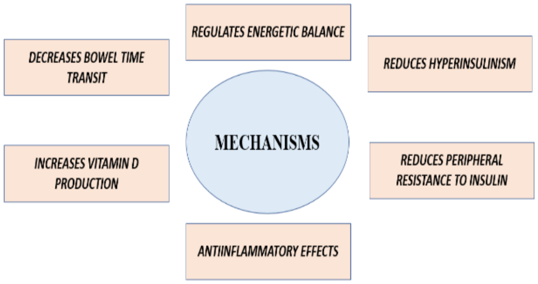
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Rare case of Stevens-Johnson-TEN overlap syndrome caused by mycotoxins

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Abstract*:* *Mushroom poisoning is rarely associated with skin involvement. Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening mucocutaneous reactions, characterized by extensive necrosis. SJS/TEN overlap includes patients with skin exfoliation between 10 to 30 percent of the body surface area. We report the case of a patient that was assumed to have ingested one type of toxic mushroom within the twelve hours prior to the appearance of skin lesions typical for SJS/TEN overlap syndrome.*

Keywords: *Amatoxin, skin involvement, SJS/TEN overlap, MODS*

INTRODUCTION

Picking wild mushrooms is a very popular activity in European countries, therefore mushroom poisoning is a constant and serious health issue [1-3]. More than fifty species of toxic mushrooms are known, which are usually very similar to edible mushrooms.[4,5]

The most severe cases of mushroom poisoning are mainly caused by ciclopeptides – toxins contained by Amanita mushrooms.[2,5,6] The most frequent cause of death in mushroom poisoned patients is *Amanitaphalloides,* also called ”the death cap”.[5-8] Toxic effects of *Amanitaph.* are determined by phallatoxins and amatoxins.[6,9] Phallatoxins are heptapeptides with severe toxic systemic effects that cannot be absorbed from the digestive tract.[10] However these toxins can induce gastrointestinal symptoms through lesions of the enterocytes .[1]

α-amanitin, the most important amatoxin, is resistant to all gastrointestinal fluids and after absorption it mainly locates in the hepatocytes.[10,11] After reaching the liver cells, α-amanitin binds DNA-dependent ribonucleic acid (RNA) polymerase II, with high specificity, causing protein synthesis inhibition.[11,12] Its toxic effects are also increased through the enterohepatic circulation of this toxin.[1,11]

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Histopathological results of these effects are initially represented by nuclear lipid and carbohydrate deposits and finally by hepatic centrolobular necrosis.[1,12] α-amanitin also affects other metabolically active tissues like the kidney or the gastrointestinal tract.[7,12,13] No cases of skin involvement secondary to *Amanita ph.* poisoning have been reported so far except erythromelalgia – a disease that is characterized by erythema and pain especially in the extremities – that was sometimes associated with some species of mushroom intoxication, but never with *Amanita ph*. Poisoning.[14,15]

Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but highly severe disorders that can affect patients of all ages.[16,17]

Caused by a variety of drugs, infections and rarely by toxins, SJS and TEN are defined as a hypersensitive cutaneous reaction that produces dermato-bullous skin lesions.[16,18-20] Pathogenesis of these conditions is controversial and involves genetic susceptibility (haplotypes like HLA B*1502, HLA B12 etc.), immune cells (especially T lymphocytes CD 8+), cytokines and mediators of cell death.[16,19,21]

Although initially was thought that SJS and TEN are separate entities, today it is considered that they are varying degrees of severity of the same disease.[22] The difference between these two forms of disease is represented by the percentage of the affected body surface area (BSA) – SJS detachment of <10 % BSA and TEN detachment of >30% BSA.[17,22]

Overlapping SJS/TEN includes cases with detachment between 10-30% BSA.[22,23] Regardless of the size of the affected area erythematous and macular lesions may be associated.[17]

Apart from skin lesions, mucosal (respiratory, gastrointestinal, urinary) and other organs (liver, lungs and kidneys) involvement can occur.[20,24-26] Despite numerous attempts of identifying an effective curative therapy SJS/TEN has a mortality rate from 5 to 30%.[27]

Furthermore a variety of long-term sequelae can be encountered in surviving patients.[24]

CASE PRESENTATION

We present the case of a 49 year old female patient who was transferred to the Anesthesiology – Toxicology – Intensive Care Unit of the Clinical Emergency Hospital in Bucharest from a regional Hospital Unit. She was suspected to have ingested a sort of poisonous mushroom within the 12 hours prior to admission. She presented to the emergency room after mushroom consumption after picking them from a local forest. During the night the patient presented abdominal pain, nausea, vomiting and diarrhea, for which she administered no treatment. In the morning she decided to go to the emergency room, although clinical signs became milder. Her medical records revealed no pathological findings, except an untreated dyslipidemia. Clinical examination revealed a mediocre general condition, pale skin, gingivitis, tachycardia and mild abdominal pain. Furthermore she noticed appearance of cough and rhinorrhea in the last two hours. After volume and electrolyte rebalancing, she was transferred to our Department.

On admission the patient presented an altered general state, she was conscious and feverish (38.6°C). Apart from cough and rhinorrhea she also presented dysphagia, myalgia and arthralgia especially in the lower limbs. Physical examination revealed jaundice, diffuse erythema on hands and feet, tachypnea, tachycardia (HR=113bpm), hepatomegaly. Preliminary laboratory results indicated hepatic cytolysis (ALAT = 9,412 U/L, ASAT = 6,720 U/L), hyperbilirubinemia (4 mg/dl), decreased serum potassium (3.1), elevated creatinine level (2 mg/dl) and blood urea nitrogen (BUN = 49.4 mg/dl). Electrocardiography showed no abnormality, except the abovementioned tachycardia. Superior digestive endoscopy indicated diffuse erythematous lesions in the pharynx and esophagus.

Although according to the description of the ingested mushroom we suspected an *Amanita ph.* poisoning, rapid Meixner test could not be performed due to the lack of gastric material when endoscopy was performed. Therefore mushrooms leftovers from the meal were sent to a specialized laboratory.

Considering the high suspicion of developing Steven-Johnson syndrome and the liver insufficiency secondary to mushroom poisoning, initial therapy management was carefully selected. Oxygen therapy, fluid replacement was initiated in combination with gastric protection, antiemetic and diuretic therapy. Vitamin supplementation, corticotherapy (prednisone 138 mg/day) and antioxidant therapy (N-acetyl-cysteine 1,800 mg/day and alpha lipoic acid 900 mg/day) were also added. We performed continuous digestive decontamination by administering 25 mg mannitol p.o, 20 g lactulose and Ricinus communis oil 15 mg. Moreover activated charcoal administration was started in order to interrupt mushroom toxins enterohepatic recirculation.

Twelve hours after admission the patient presented altered mental status, dyspnea, tachypnea and a peripheral oxygen saturation of 80% under oxygen mask. Because of that she was intubated and mechanically ventilated. Despite volume controlled ventilation hypoxemic index could not raise above 100. Furthermore over the erythematous areas of the feet and hands atypical irregular lesions with darker centers were observed. These lesions evolved within the next twenty four hours to vesicles and bullae (Nikolsky sign and Asboe-Hansen sign positive) (Figures 1 and 2).

Figure 1

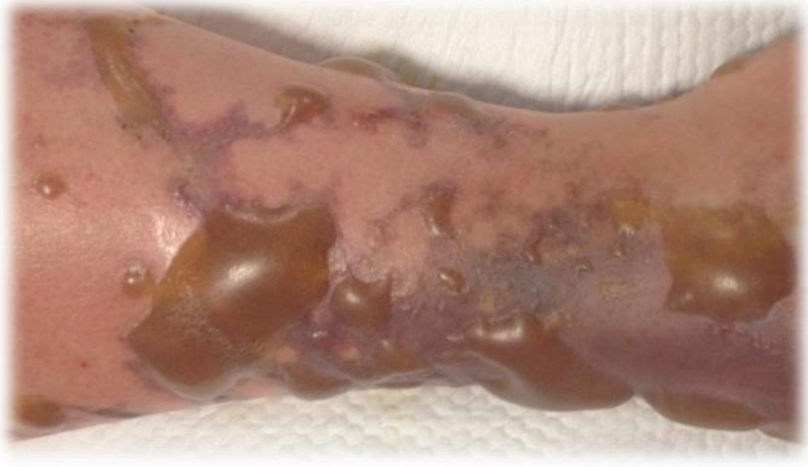

Figure 2

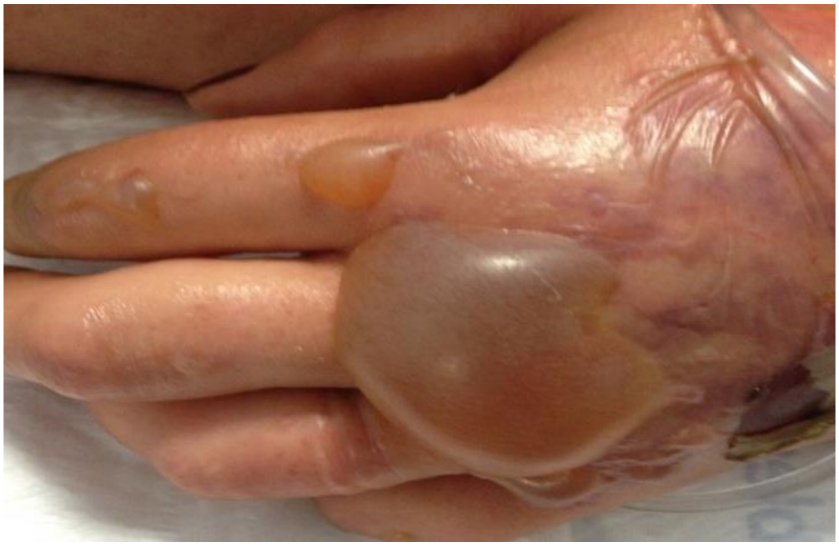

Figure 3

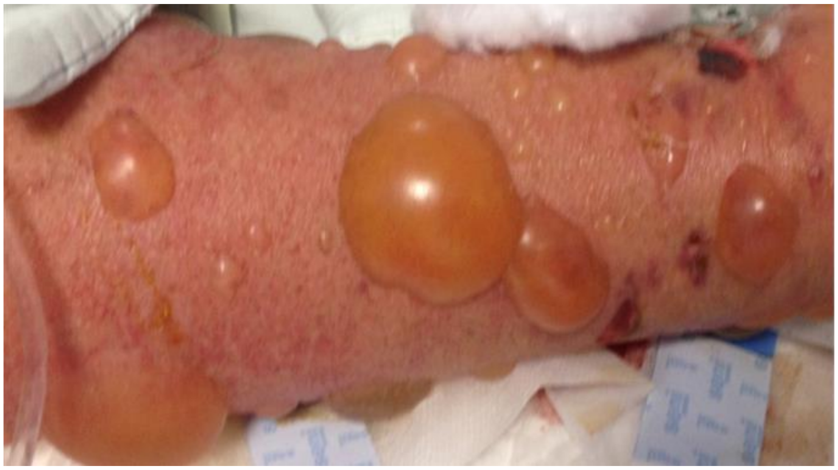

Skin lesions extended in the next few hours on both forearms (Figure 3) and legs thus evaluating the affected body surface, this case was classified as Steven-Johnson – TEN overlap syndrome.

Gynecological exam also revealed vulvar bullae. In this conditions SCORTEN score indicated a mortality risk of 58.3%.

Four days from admission *Amanita ph.* toxins were identified in the laboratory.

Since *Amanita ph*. poisoning is not a specific cause of Steven-Johnson syndrome, other causes were thoroughly investigated.

Usually associated medication which is a trigger for Steven-Johnson was excluded through detailed anamnesis of the patient and her family. Viral and bacterial causes like HIV, Cytomegalovirus or Mycoplasma pneumonie were excluded after the PCR and/or serological tests were negative.

Although liver insufficiency was remitted after one week of treatment the evolution of the patient was severe. Twelve days after admission the patient died with multisystem organ failure.

DISCUSSION

Numerous studies are dedicated to the toxic effects of mushroom poisoning. A considerable percentage of fatal mushroom poisoning cases occur after ingestion of *Amanita ph.*[1] Although amatoxins induce massive liver cell necrosis, not all patients develop fatal acute liver failure.[4]

Moreover, various treatment strategies proposed in the literature decreased the mortality rates in these patients.

Delayed onset of signs and the polymorphic symptoms due to amatoxin poisoning may aggravate liver toxicity, in the absence of early decontamination treatment.[2] Besides liver failure, amatoxin was associated with nephrotoxicity.[7] However there is no report in the literature about the amatoxin's toxic effects on skin.

SJS/TEN is an acute severe mucocutaneous disease caused by a variety of drugs, infections or malignant comorbidities.[21] In the case presentation, the patient did not receive any medication potentially associated to SJS/TEN, neither in our unit nor in the regional hospital. Moreover she was not on any chronic treatment or suffered from any infectious or malignant disease. However, several serological tests were performed in order to exclude the most frequent infectious causes of SJS/TEN.

SJS/TEN is often preceded by a prodrome characterized by fever, headache and pharyngitis.[16,17] In this case report after the gastrointestinal phase of the amatoxin poisoning, the patient developed rhinorrhea, malaise, dysphagia and fever.

SJS/TEN may be associated with multisystem organ failure.[16] In addition to the hepatic failure caused by the amatoxin poisoning, soon after admission the patient developed respiratory dysfunction, requiring mechanical ventilation. Secondary to the respiratory and hepatic dysfunction, neurological dysfunction developed. Despite adequate volume repletion, cardio circulatory dysfunction appeared requiring continuous vasopressor therapy.

There are no recommendations regarding treatment of the acute phase of SJS/TEN.[16] Considering that there is no specific treatment for SJS/TEN, minimizing the therapy with risk of aggravation of SJS/TEN was tried and corticotherapy was initiated. Despite the maximal supportive treatment and liver function improvement, the patient died from multisystem organ failure.

CONCLUSIONS

From our knowledge this is the first case report on Stevens-Johnson/TEN overlap syndrome induced by mushroom poisoning. This case presentation aims to highlight the polymorphic manifestations of severe amatoxin intoxication as well the difficulties of managing such a patient. Clinicians should be aware of the systemic involvement in mushroom poisoning.

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REVIEW ARTICLE

Reactive nitrogen species and cardiovascular diseases

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Abstract: *Oxidative stress plays a major part in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. Cardiovascular disease is the leading cause of death in the United States and Europe and is poised to become the most significant health problem worldwide. Reactive nitrogen species are involved in the regulation of cardiovascular motor tone, modulation of myocardial contractility, control of cell proliferation and inhibition of platelet activation, aggregation, and adhesion. Cellular constituents of our body are altered in oxidative stress conditions, resulting in various disease states. The oxidative stress can be effectively neutralized by enhancing cellular defenses in the form of antioxidants. To understand the mechanism of action of antioxidants, it is necessary to understand the generation of free radicals and their damaging reactions.*

Keywords: *Oxidative stress, ROS, antioxidants, CVD*

INTRODUCTION

Normal biochemical reactions, increased exposure to the environment, and higher levels of dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are responsible for the oxidative stress in different pathophysiological conditions.

Cellular constituents of our body are altered in oxidative stress conditions, resulting in various disease states.

Oxidative stress plays a major part in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [1].

Free radicals are defined as "any chemical species capable of independent existence that contains one or more unpaired electrons". This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical.

An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, has been defined "oxidative stress".

It soon appeared that nitric oxide (NO) plays a key role in the physiological regulation of the cardiovascular system, since abnormalities in its productions and/or bioavailability accompany or even

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precede diseases such as hypertension, atherosclerosis and angiogenesis-associated disorders. Cardiovascular disease (CVD) is the leading cause of death in the United States and Europe and is poised to become the most significant health problem worldwide.

Free radicals are generated from either endogenous or exogenous sources. Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer and aging. Exogenous free radicals result from air and water pollution, cigarette smoking, alcohol, heavy metals, certain drugs (cyclosporine, tacrolimus), industrial solvents, cooking and radiation.

ROS and RNS products can bring about reversible or irreversible chemical changes (oxidation, nitrosylation and nitrosation) in proteins, lipids, and DNA, resulting in diminished biochemical functions [2]. The greater the amounts of ROS and RNS, the more extensive the chemical changes in these targets. ROS and RNS can induce adducts to DNA, leading to DNA fragmentation [3].

Reactive nitrogen species (RNS) are free radicals which are associated with the nitrogen atom: nitric oxide (NO), nitrogen dioxide (NO_2) and peroxy-nitrite ($ONOO^-$). Reactive species are produced by regulate enzyme such as nitric oxide synthase (NOS), and isoforms of NADPH oxidase, or as by-products from not so well regulated sources, such as mithocondrial electron-transport chain.

Nitric oxide is a biatomic free radical containing an unpaired electron. Until now have been described three forms of NO, nitrosonium cation (NO^+), nitric oxide ($NO^·$), and nitroxyl anion (NO^-) with nitrogen oxidation number +3, +2, and +1, respectively. NO can react with oxygen free radical to form peroxynitrate ($ONOO^-$). This last molecule is involved in protein oxidation reaction under physiological conditions.

CARDIOVASCULAR DISEASES

Cardiovascular diseases are prevalent in human population and most of them are related to diet but genetic lipid abnormalities such as hypercholesterolemia, hypertriglyceridemia, HDL metabolism disorders, and combined hyperlipidemias are more severe. Cardiovascular disease is one of the major causes of mortality and morbidity worldwide and the costs that involve handling this disorder are huge. The 2008 overall rate of death attributable to cardiovascular disease was 244.8 per 100 000 individuals and this rate is critically growing [4]. Recent evidence demonstrates that cardiovascular disorders are usually associated with increased level of stress hormones [5, 6].

Cardiovascular risks such as defects in angiogenesis/ vasculogenesis or vessel repair are major complications of coronary artery disease (CAD) which are mostly seen in aged people. Similarly, CVD risks have also increased in women during pregnancy which is an important issue for management of their cardiovascular health [7]. Conventional risk factors such as cigarette smoking, diabetes, hyperlipidemia, and hypertension are absent in 15-20% of patients with CVD. Atherosclerosis is the main cause of death in the world through causing ischemic heart disease. It is peripheral arterial disease, most prevalent, morbid, and mortal disease. It is one of the most common disorders among the elderly, because of depression prevailed in the old age and rates of very high atherosclerosis. Atherosclerosis is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intimae of the walls of large and medium size arteries.

Abnormal proliferation of vascular smooth muscle is implicated in various pathological situations including atherosclerotic lesions, restenosis after balloon angioplasty, and vascular wall thickening in hypertension. NOS may play protective role by inhibiting proliferation of vascular smooth muscle cell [8]. For example, leiomyosarcoma, which is an aggressive mesenchymal tumor with differentiation toward smooth muscle tissue, represents up to 9% of all primary malignant tumors. Some cases of leiomyosarcoma presumed to be infective endocarditis [9].

THE CHEMISTRY OF RNS

Nitric oxide ($NO^·$) is a small molecule generated in biological tissues by specific nitric oxide synthase (NOS) which metabolizes arginine and citrulline with the formation of $NO^{\cdot}$ via a five electron oxidative reaction [10]. Nitric oxide synthase utilize L-arginine as the substrate, and molecular oxygen and reduced nicotinamide-adenine-dinucleotide phosphate – NADPH as co-substrat.

NO is involved in the regulation of cardiovascular motor tone, modulation of myocardial contractility, control of cell proliferation and inhibition of platelet activation, aggregation, and adhesion [11].

Hypertension is also associated with NO synthesis [12].

The enzyme nitric oxide synthase produce reactive nitrogen species (RNS), such as nitric oxide ($NO^{\cdot}$) from arginine.

$$\text{L-Arg} + O_2 + \text{NADPH} \rightarrow NO^{\cdot} + \text{citruline}$$

An inducible nitric oxide synthase (iNOS) is capable of continuously producing large amount of $NO^{\cdot}$, which act as a $O_2^{\cdot-}$quencher. The $NO^{\cdot}$ and $O_2^{\cdot-}$ react together to produce peroxynitrite ($ONOO^{-}$), a very strong oxidant, hence, each can modulate the effects of other. Although neither $NO^{\cdot}$ nor $O_2^{\cdot-}$ is a strong oxidant, peroxynitrite is a potent and versatile oxidant that can attack a wide range of biological targets. Peroxynitrites can interact with several cellular components and are implicated in NO signaling mechanisms involving protein modifications.

$$NO^{\cdot} + O_2^{\cdot-} \rightarrow ONOO^{-}$$

In aqueous aerobic solutions NO predominantly forms nitrite (NO_2^-). In the presence of oxyhemoglobin and oxymioglobin, NO is completely oxidized to nitrate (NO_3^-). Covalent interactions of NOS with cellular macromolecules are responsible for its many physiological and pathological effects. Protein containing iron and thiol groups are the major cellular target of NOS [13].

There are three types of NOS, neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS which plays a very important role in the vascular homeostasis) and inducible nitric oxide synthase (iNOS; it is found in myocytes, macrophages and ECs and is activated by immunological and inflammatory stimuli). Under septic conditions iNOS and nNOS are upregulated in endothelial and muscle cells, respectively, leading to over-production of NO in the microvasculature and arteriolar dysfunction.

Neuronal NOS is constitutively expressed in specific neurons of the brain and its enzymatic activity is regulated by Ca^{+2} and calmodulin. This NOS isoform has been identified also in the spinal cord, in the sympathetic nerves, in epithelial cells of various organs, in pancreatic islet cells, in the vascular smooth muscle and in the skeletal muscle [14, 15].

Endothelial NOS is mostly expressed in endothelial cells; Ca^{2+}-activated calmodulin is important for the regulation of eNOS activity because Ca^{2+} induces the binding of calmodulin to the enzyme. Endothelial NOS appears to be a homeostatic regulator of numerous essential cardiovascular functions and also controls the expression of genes involved in atherogenesis. The blood vessel wall NO is mainly produced from l-arginine by endothelial NOS.

Nitric oxide as a key endothelial vasodilator also directly affects metabolism by competing with mithocondria for oxygen and consequently inhibiting switching the metabolism to some other pathways. Also, some studies suggested that NO is implied in the response of *Candida albicans* species to the oxidative stress and also against some azoles drugs. *Candida albicans* is a commensal species of the human gastrointestinal tract, in which it lives without adverse effects on the host, but yeast-to-hypha transition has been associated with increased virulence, mucosal invasiveness and biofilm formation. Candidemia and invasive candidiasis are frequently associated with high morbidity and high mortality rates [16].

ANTIOXIDANTS AND DEFENSE MECHANISMS

Overproduction of ROS (arising either from mitochondrial electron-transport chain or excessive stimulation of NADPH) results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA. In contrast, beneficial effects of ROS/RNS (e.g. superoxide radical and nitric oxide) occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as for example in defense against infectious agents, in the function of a number of cellular signaling pathways, and the induction of a mitogenic response [2]. Cellular constituents of our body are altered in oxidative stress conditions, resulting in various disease states. The oxidative stress can be effectively neutralized by enhancing cellular defenses in the form of antioxidants. Low levels of antioxidants have been associated with the heart disease and cancer.

When ROS/RNS are generated in vivo, their actions are opposed by intricate and coordinated antioxidant lines of defense systems. These include enzymatic and non-enzymatic antioxidants that keep in check ROS/RNS level and repair oxidative cellular damage. The antioxidant enzymes reduce the levels of lipid hydroperoxide and H_2O_2, thus they are important in the prevention of lipid peroxidation and maintaining the structure and function of cell membranes.

The major enzymes, constituting the first line of defense, directly involved in the neutralization of ROS/RNS are: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). SOD is a cytoplasmic and mitochondrial enzyme, which accelerate the dismutation of superoxide. They are present in almost all aerobic cells and in the extracellular fluids. They contain metal ions that can be copper, zinc, manganese or iron. In humans, the copper/zinc superoxide dismutase is present in the cytosol, while manganese superoxide dismutase is present in the mitochondria. CAT, an exclusively peroxisomal enzyme in most tissues, converts H_2O_2 to water and O_2. However, the most important H_2O_2-removing enzymes are the selenoprotein GPx enzymes. GPx enzymes remove H_2O_2 by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Glutathione peroxidase also catalyses the reduction of unstable hydroperoxides at the expense of GSH [17].

The nonenzymatic antioxidants are of two types, the natural antioxidants and the synthetic antioxidants. Vitamin C, vitamin A and plant phytochemicals like phenolics that inhibit the oxidation chain initiation and prevent chain propagation represented the second line of defense. Vitamin A has a vital antioxidant contribution in protecting human LDL against copper stimulated oxidation. Lipid-soluble antioxidants such as α-tocopherol localize mainly to membranes and lipoproteins where they serve to limit lipid peroxidative damage. Vitamins E and C have been demonstrated to reduce the progression of atherosclerosis. Vitamin E (α-tocopherol) is the most important lipid-soluble antioxidant and protects cell membranes against oxidation by reacting with the lipid radicals produced in the lipid peroxidation chain reaction and removing the free radical intermediates. Phenolics are therefore an integral part of the diet, with significant amounts being reported in vegetables, fruits, teas and traditional plants. Epidemiological evidence indicates that consumption of fruit, vegetables and teas may reduce the risk of cardiovascular disease and it is increasingly suggested that this may due to their antioxidants that include ß-carotene, vitamin C, vitamin E and polyphenolics. Dietary antioxidant phenolics may quench reactive oxygen and nitrogen species and, hence potentially modify pathogenic mechanisms relevant to cardiovascular disease [18]. Vitamin C regenerates vitamin E in cell membranes in combination with glutathione or compounds capable of donating reducing equivalents.

Low levels of antioxidants have been associated with the heart disease and cancer. Antioxidants provide protection against a number of disease processes such as aging, allergies, algesia, arthritis, asthma, atherosclerosis, autoimmune diseases, broncho-pulmonary dyspepsia, and cancer. The other disorders to which antioxidants provide protection are cataract, cerebral ischemia, diabetes mellitus, eczema, gastrointestinal inflammatory diseases, and genetic disorders.

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ORIGINAL ARTICLES

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The communication and promotion policies of the medical organizations in the marketing of Romanian healthcare services

Bogdan I. Coculescu[1,2], **Victor L. Purcărea**[3], **Elena C. Coculescu**[4]

Abstract: *The interdisciplinarity of the marketing department is due to the application of concepts, methods and marketing technics specific both to service and to social marketing. In addition to this fact, the attempt of the social services to satisfy the patient's needs places the health care domain at the border between social and economic, between profit and non-profit orientation. However a lot of the notions from the marketing field (competition, promotion, strategy, need, supply, cost etc.) acquire new meaning when used for defining the rivalry between the distinct medical organizations, the advance of the health care services, the development and implementation policies in medical marketing, the increasingly acute demand for treatment, the use and the supply of health care services as well as the cost that it requires..*

Conclusion: These above described microscopy method can be used to distinguish between benign and malignant thyroid nodules, based on different degree of the capsular collagen fibers orientation.

Keywords*: communication policy, promotion policy, marketing mix strategy, Romanian healthcare services*

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INTRODUCTION

Medical organizations communication policy towards the health care market through constructive and favorable relationships are an important objective that every health care provider should promote. Primarily communication strategies target the following aspects:

- Promoting the service offering of medical organizations in order to attract new potential clients;

- Persuasion of the potential clients for the necessity of purchasing these services by presenting the positive advantages of the respective health care procedures. Communications possess an important role in the buying process, taking part both at the pre-sale and sale, and also post sale stages [1-4].

DISCUSSION

Communication is a constituent of a great importance in the marketing mix (product – cost – distribution – development) aiming at establishing and maintaining

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a steady relationship with the patients. It represents the tool with which an entity participates in the informational exchange with the different business field's components, to inform about its presence, the products and services it provides, to create a positive attitude and to stimulate consumers to buy products and services.

Figure 1: Communications and healthcare services marketing [5].

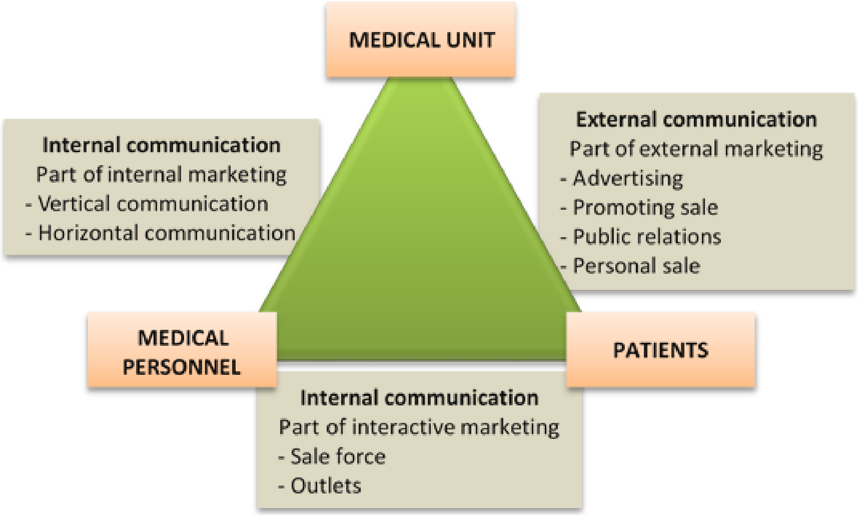
MEDICAL UNIT
Internal communication
Part of internal marketing
- Vertical communication
- Horizontal communication
External communication
Part of external marketing
- Advertising
- Promoting sale
- Public relations
- Personal sale
MEDICAL PERSONNEL
PATIENTS
Internal communication
Part of interactive marketing
- Sale force
- Outlets

The instruments of the communicational mix are based particularly on interpersonal communication, yet at the same time on the adaptation and enrichment of the classical techniques via the concept of marketing integrated communication resulting in complex communicational programs. The marketing mix in the health care services sector includes as well staff politics, represented by two segments which must be approached differentially as follows: the employees of the company providing services and the consumers.

The principal methods and ways to communication, that can be adopted by a medical organization in order to orientate the patient in their referring to a certain health care service or to build and reinforce a favorable image of that sanitary unit on the market, constitute the promotional mix of the medical institution.

The achievement of an optimal promotional mix, which satisfies the patients' needs and fulfill the best objectives of the promotional communication, is one from the key points in obtaining the attributions distinctive for a marketing specialist.

There are two ways by whom a medical organization can communicate with its patients (Figure 1):

a. On the outside through:

- advertising
- promoting sales
- public relations
- direct marketing (inclusive online)

b. On the inside through the employed medical personnel during the specific activities.

The advertising activity has clear purpose in preparing the target public for favorable receiving of the medical unit's offer. Three types of marketing objectives are to be distinguished from one another depending on their purpose as follows: informing, convincing and reminding. To advertise and broadcast the commercial message the medical organization can make use of different communicational channels: newspapers, magazines, the press, printing materials (flyers, brochures, catalogues), external publications (billboards, posters, leaflets, stickers), internet, radio, television etc. Through advertising activities the medical organization succeeds in informing the potential patients about its work and services with the aim of influencing their decision for adherence to this services.

The direct relationship between the medical staff and the patients favors a particular manner of communication: personal sale – the potential patients can be notified and persuaded to subscribe for services at the medical unit. Consequently the medical contact personnel plays an important role for increasing the sell's volume in the medical system. They must be very well prepared professionally and respond promptly to the patients expectations. Additionally the immediate connection with patients represents the main way for informing them about the benefits, offers, promotions, advantages, performance conditions, health care services prices etc. of the medical organization they belong.

A lot of the authors specify a clear distinction from the concept of “team” and of “teamwork”. The “team” concept is referring to persons who work together for common purpose, while the “teamwork” concept – to a certain environment from a larger organization, which creates and sustain relations of trust, support, respect, interdependence and collaboration.

It must be mentioned that in sanitary organizations – particularly in the hospitals – team communication possess an increasingly important role. A good team communication, understanding the advantages, disadvantages, “principles” of teamwork, contributes to identifying the proper solutions to the inherent problems. In multiple cases where it is necessary to work as a team, encouragement and orientation of the team members can improve the sanitary organization's results via: their motivation, use of the team member's ideas and personal capabilities, acquiring support from their side, improving the performance. Through guidance, the quality of health employee's performance can be made better, while the tasks are accomplished properly by them.

Promoting is one of the forms of communication. The difference between the two notions are made at the level of the sent message. So that promoting to have the desired effect, the messages received by the patients must be clear and reflect what the organization has to offer.

Promoting, as a variable from the marketing mix, occupies place apart in the case of medical services, because it is essential for the development and maintenance of durable relations with the target public. Marketing politics aims to inform the target public as much as possible about the health activities and the services offered by the respective medical organization, but at the same time also for the information received by them to have a positive impact.

A particular case of promoting of medical organization is represented by spreading the “mouth to mouth” advertisement, from patient to patient, extremely productive, as demonstrated fact in the practice of health care service with results in growth of consumers addressability in these services (“one satisfied patient brings more patients”).

Client services in the field of health care relates to the benefits offered to the patients – or the public in general – further than the product itself, including its nontechnical and nonclinical aspects. The connection between client services and other elements of the marketing mix is one of completion and support. The efficient client services reduce the cost due to patients and improves the access to health services [4-6].

CONCLUSIONS

Promoting sales imposes the use of all procedures and stimulation techniques and increasing the sales of medical services of the organization. If advertising has a role in offering purchase motivation, conversely the sell promotion has a role in the sells' stimulating process for the potential patients by the means of consecrate methods in promoting sales, in the form of promotional presents (watches, calendars, agendas, pens, notebooks, umbrellas with inscription of the medical organization etc.), with the aim of image promoting on the target market.

Public relations have a part in setting trust relations with the patients, ensuring protection, planning, organizing and controlling the whole actions unfolded by a medical organization for achieving its objectives. The methods used in the activity of public relations for obtaining the marketing goals (informing the patients about the advantages of the organization, stimulating the sales volume, keeping the investment in promotional materials at minimal level) consists in organizing events with scientific subject (congresses, conferences), giving interviews, publishing brochures, profile publications, promoting through press conferences, participating in medical markets and exhibitions etc. The coordination of a public relations project with the other elements from the promotional mix can be beneficial for the increase in prestige of the medical organization.

The core of the marketing strategy in the field of health care is presented by the quality of services, quality which in its turn results from: precision of performance, promptitude and professionalism of the employees, kindness and politeness towards the patients. Creation and implementation of a coherent and productive medical marketing strategy as well as defining the value system of a sanitary organization consists a vital necessity for the organizations, whose purpose is executing top health services.

In the field of health care services the marketing strategy represents actually the attitude of the sanitary organization towards the marketing environment and simultaneously its behavior in regard to its components.

Communication of the organization with the marketing environment is an essential condition for achievement of its activity objectives. The fulfilment of the organization mission assumes concentrating marketing efforts in the direction of achieving a permanent and efficient communication with the external surroundings, with the market and with the patients.

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ORIGINAL ARTICLE

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Therapeutic management of schizophrenia and substance use disorders dual diagnosis – clinical vignettes

Octavian Vasiliu[1]

Abstract: *Patients with schizophrenia are frequently diagnosed with addictive comorbidities, and data in the literature support a 10 to 70% prevalence of this dual diagnosis. Nonetheless, substance use disorders can be missed during the initial interview with a psychotic patient, if the clinician is focused only on the more obvious manifestations. Therefore, using psychometric scales and structured interviews in patients with schizophrenia is strongly encouraged because the case manager will base his/her therapeutic decisions on quantifiable data about these patients' symptoms and functional status. Clinical management in dual diagnosis cases must address both conditions simultaneously, as the delay in the initiation of substance withdrawal treatment may hinder the recovery from a psychotic episode. An important issue is represented by the potential pharmacologic interactions between drugs administered for schizophrenia and those targeting substance withdrawal and substance dependence. Other important aspects refer to (1) the therapeutic adherence, which can influence the prognosis of both conditions, (2) the negative impact of residual psychotic symptoms and substance-related disorders over the patient quality of life and daily functioning, (3) the necessity to integrate variables like the patient's specific needs, lifestyle, and psychological resources in the therapeutic decision. These clinical vignettes are focused on clinical, biological, psychometric, and pharmacological dimensions, supporting the formulation of treatment recommendations based on monitoring both psychiatric and biological profiles.*

Keywords: *schizophrenia, substance use disorders, antipsychotics, dual diagnosis, cannabis, nicotine, alcohol dependence*

BACKGROUND

Substance-related disorders are very common throughout the course of schizophrenia, and this phenomenon is responsible for poorer quality of life, higher impairment of daily functioning, lower rate of treatment response, lower therapeutic adherence, leading to a worse prognosis in these patients.

Prevalence of dual diagnosis (substance use disorder and psychotic disorders) ranges from 10 to 70% in a large-scale trial for schizophrenia [1].

Many hypotheses about the link between *cannabis use* and schizophrenia are still tested, cannabis being considered an independent risk factor for psychosis and a variable that may worsen prognosis in schizophrenia patients [2]. A cannabinoid hypothesis of schizophrenia has been suggested, based on the observed alteration of endocannabinoid system (abnormalities in cannabinoid type 1 receptor binding properties and modified levels of anandamide in the cerebrospinal fluid) [2]. Cannabis use was associated

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with an earlier onset of schizophrenia, more severe forms of disorder, higher rates of relapse, and longer hospitalizations [3-5]. Longitudinal studies report that cannabis use in childhood and adolescence doubles the risk of psychosis onset later in life, which supports a causal role of this drug in the development of schizophrenia [6]. Certain alleles of the type 1 cannabis receptor gene (CNR1) may confer susceptibility to schizophrenia [7].

Also, the overlap of *nicotine dependence* and schizophrenia has been debated as a form of self-medication for schizophrenia-related cognitive deficits, based on the fact that the nicotine receptors activation increases the release of dopamine in cortical and subcortical areas [8,9]. Still, cigarette smoking decreases the bioavailability of many psychotropics that are metabolized through the CYP450 1A2 isoenzymes and consequently may diminish the clinical effect of these drugs and delay the patients recovery [10]. Multiple genes have been linked to both conditions, e.g. binding protein genes, protein modification genes, and energy production genes involved in cognitive functions and neuronal plasticity [11].

Alcohol use disorder was found in 33.7% of patients diagnosed with schizophrenia or schizophreniform disorder in the Epidemiologic Catchment Area study [12]. A dysregulation of the dopamine transmission has been suggested as common neurological basis, but shared genetic vulnerability factors have also been investigated (e.g. KPNA3, or alcohol dehydrogenase variants) [13-16]. A review of the current evidence for common risk factors in alcohol use disorders and schizophrenia supports a highly polygenic model, with rare penetrant alleles and frequent alleles with small effects [16].

Patients diagnosed with schizophrenia tend to abuse *anticholinergic* drugs. These agents are often used for the treatment of antipsychotic-induced extrapyramidal symptoms, and a national database analysis showed that patients with schizophrenia took 20 times more frequently antiparkinsonian agents than patients with Parkinson disease [17]. Trihexyphenidyl abusers may claim this drug improve their daily functioning and their affect, and a possible pharmacological explanation is that this agent has a structural similarity with phencyclidine [18,19]. The risk of biperiden and orphenadrine abuse was relatively small in a large database analysis [17].

CLINICAL VIGNETTES

The first patient, M.S., is a 29-year old male, diagnosed since 2015 with schizophrenia according to the DSM-5 criteria [20], currently at his third psychotic episode. He was hospitalized after he presented at the Emergency Department with delusions of persecution and auditory hallucinations ("there are people who want me dead because of my soul, they want to collect my psychic energy", "I can hear them through the walls, day and night, they are plotting against me, and they are saying bad things about my family", "They are forcing me to do evil things, like cursing strangers with no reason"). These manifestations led to changes in his behavior, he became reclusive, didn't go out of his house for weeks and spoke with his family only by phone, refusing to see them ("I can protect them if I'm not seen with them"). He recently abandoned his job as a salesman and didn't want to see her girlfriend anymore because of the belief that she was in cahoots with the persecutors who want him dead.

The pharmacological history in this case included olanzapine 20 mg/day as the main treatment for his first psychotic episode. After hospital discharge, he received the same antipsychotic for 8 months, then he dropped out and relapsed after about 6 months. The overall clinical status during the second admission was similar to the first episode of psychosis, with persecutory delusions and auditory hallucinations (both conversing and imperative voices) and induced defensive behavior- the patient refused to go out by himself because of the fear of being watched and plotted against. Olanzapine was re-initiated, but shortly after this the patient was switched on aripiprazole 30 mg/day due to concerns related to his metabolic status (240 mg/dl for the total cholesterol, 150 mg/dl for LDL-cholesterol, and 250 mg/dl for triglycerides). The evolution was favorable during the hospitalization and the patient was recommended to work in a controlled environment and to participate in occupational therapy. However, after 7 months he discontinued treatment and soon relapsed, so that a new hospitalization was required. This time the patient was stabilized on aripiprazole, but for the maintenance phase the long acting injectable form of aripiprazole 400 mg every 4 weeks was selected, in order to diminish the risk of therapeutic non-adherence.

The patient was also diagnosed during the current psychotic episode with alcohol use disorder, moderate, based on the DSM-5 criteria, admitting a daily intake of 8 drinks, consisting mainly in beer and wine for more than 12 months. Also, he is smoking 20 cigarettes daily, with a value of 10 pack-year. Biochemistry panel reflected the liver damage, with values for gamma-GT, GOT and GPT of 156 U/l, 70 U/l, and 67 U/l, respectively. No abnormalities were detected on his chest X-ray and abdominal ultrasound exam (except for hepatic steatosis).

The psychological evaluation realized during the initial visit for the third episode showed a 98 score on PANSS [21], with high values on both positive and negative sub-scales. CRDPSS [20] score was 17, based mainly on hallucinations, delusions and abnormal psychomotor behavior. AUDIT [22] score was 14, reflecting a moderate risk of harm related to the alcohol use, and the severity of nicotine dependence was high, as supported by the FTND [23] score of 9. GAF score at admission was 45, based on symptoms severity and functional impairment, while the CGI-S score was 5, which means a “markedly ill” clinical status.

Therapeutic challenges analysis: This patient presented a history of therapeutic non-adherence which triggered two relapses. He was diagnosed with two substance use disorders (alcohol and nicotine dependence), which were not therapeutically approached during his two previous psychotic episodes, and this could be also a factor that may contribute to relapse in schizophrenia [24]. There is a lack of social and professional insertion in this case, related to both positive and negative symptoms. The patient lacks familial support and he discontinued occupational therapy. Moreover, the metabolic profile and the hepatic functional status were abnormal. All these negative prognosis factors have been evaluated by the case manager when the therapeutic strategy was formulated. Aripiprazole was preferred because of its good metabolic profile [25], and a long acting injectable formula was selected because of the more stable plasma concentrations and lower risk of discontinuation. The patient received counselling for his addictive behavior, and he participated in 4 individual sessions focused on smoking cessation and alcohol use relapse prevention. Alcohol withdrawal symptoms were mild-to-moderate and remitted after B-vitamin therapy, parenteral rehydration, and oral lorazepam 3 mg/day for 7 days, with gradual dose reduction. Naltrexone, 50 mg/day, was initiated for alcohol dependence after the withdrawal symptoms remission, and nicotine replacement therapy was suggested, but the patient refused. There are no data reported about pharmacokinetic interactions between aripiprazole and naltrexone in the literature, which supports this therapeutic recommendation.

Follow-up visits: The patient was monitored for 4 months, using psychometric instruments, in order to document psychotic symptoms, severity of addictions, and overall clinical status evolution under treatment. Global functioning improved once the psychotic positive symptoms remitted, although the negative symptoms persisted at a lower level of severity (as reflected by PANSS and CRDPSS scores).

Table 1. Psychologic evaluations during the first patient’s initial visit

Clinical scale	Results
PANSS	98
CRDPSS	17
GAF	45
CGI-S	5
FTND	9
AUDIT- Interview Version	14

Alcohol use disorder had a favorable evolution and the AUDIT scores diminished gradually, but the nicotine dependence persisted and the mean number of cigarettes increased with 25%, while the FTND score increased with 10%. Biochemistry panel reflected an improvement of the liver status after 4 months, with values for gamma-GT, GOT and GPT of 56 U/l, 23 U/l, and 37 U/l, respectively, and the metabolic parameters improved, also: 190 mg/dl for the total cholesterol, 120 mg/dl for LDL- cholesterol, and 170 mg/dl for triglycerides.

Conclusion: Addictive behaviors must be approached as soon as possible by the case manager in patients with schizophrenia, because the risk of therapeutic non-adherence, somatic complications, and reduced functionality is higher if these conditions are left untreated or if the appropriate treatment is delayed. In this particular case, naltrexone was efficient in the treatment of alcohol use disorder, and the patient had also significant decrease of the psychotic symptoms. However, nicotine dependence could not be addressed pharmacologically because the patient refused, and he participated only in a few counseling sessions, which led to the persistence of his substance related condition.

Fig.1. Evolution of the clinical variables during the first 4 months of treatment

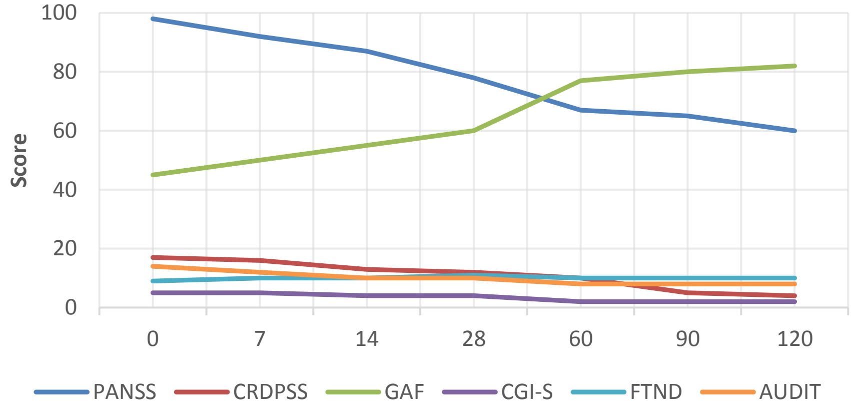

The second patient, E.D., is a 30-year old female, diagnosed with schizophrenia for 6 years, currently in a partial remission, who presented to her psychiatrist asking for a therapeutic change because of galactorrhea and irregular periods. She attributed these symptoms to risperidone, which was initiated by the psychiatrist during her latest psychotic relapse, about 3 months ago.

This patient had 4 psychotic episodes since the onset of her disease at age of 22 and received for her first episode haloperidol 15 mg/day for 2 months, followed by amisulpride 800 mg/day for 10 months; for her second episode, she was treated with olanzapine 15 mg/day, for 16 months; during her third episode she received haloperidol 20 mg/day for the acute phase, and again olanzapine 15 mg/day for an indefinite period of time, and for the last episode she received risperidone 6 mg/day maintenance dose. Changes in the antipsychotic regimen were determined by adverse events- extrapyramidal symptoms during haloperidol treatment, hyperprolactinemia during amisulpride administration, and weight gain during olanzapine therapy.

This patient presented also criteria for nicotine and cannabis use disorder, both of moderate severity, according to the DSM-5 criteria. She was on cannabis for more than 2 years, with very few short periods of abstinence, and regarding nicotine use she admitted she was smoking 15 cigarettes daily for at least 8 years. She admitted she did not recognized cannabis addiction in front of her psychiatrist until the current visit. She was never offered nicotine replacement therapy or any other type of treatment targeting nicotine dependence.

The psychological evaluation during her third episode sh owed a PANSS score of 69, with low values on both positive and negative sub-scales. CUDIT-R [26] score was 16, supporting severe cannabis dependence, and the severity of nicotine dependence was high, as reflected by the FTND score of 7. GAF score at admission was 60, based on symptoms severity and functional impairment, while the CGI-S score was 4, which means "moderately ill" clinical status. EuroQoL (EQ-5D-5L) [27,28] visual analogic scale score was 67, which seems to be correlated more with her substance-related symptoms than with her psychotic manifestations. Quality of life domains that seemed more affected by her current status were anxiety/depression (a score of 4) and usual activities (a score of 3).

Her somatic status was good, with no abnormalities on CBC or serum biochemistry panel. Also, her ECG and chest X-ray didn't suggest any abnormalities.

Therapeutic challenges analysis: This patient has a history of adverse events to several antipsychotics (haloperidol, amisulpride, olanzapine) which were severe enough to grant changes in the antipsychotic treatment. The patient received a new antipsychotic, ziprasidone 160 mg/day, which has been associated with low risk for hyperprolactinemia, weight gain, and extrapyramidal syndrome [29]. A gradual switch was preferred due to the different pharmacodynamic profiles of risperidone and ziprasidone [29-31]. ECG monitoring was initiated, and periodic measurement of metabolic parameters was continued throughout the duration of the antipsychotic therapy. The presence of cannabis use disorder raises an important question because there is no pharmacological treatment with clear evidence of efficacy in patients diagnosed with this disorder, while data about psychotherapy effects are still debatable [32]. However, gabapentin and N-acetylcysteine have been suggested as possible therapies [32], and gabapentin was preferred in this case because of its positive effect on anxiety and low risk of pharmacokinetic interactions [33]. Nicotine replacement therapy with nicotine patch 25 mg/16h for 8 weeks, followed by gradual dose reduction, combined with psychological counselling, was accepted by the patient.

Follow-up visits: The evolution of the psychotic symptoms was favorable, as reflected in the PANSS (-10%) and CRDPSS (-11%) scores. The overall functionality increased significantly (+33%) compared to baseline, and this improvement seems related to the decrease in both FTND and CUDIT scores, with 71% and 75%, respectively. Also, the quality of life improved, both on the visual analogic scale (+17%), and on its subscales (depression/anxiety -50% and usual activities -33%). The Clinical Global Impression-Severity improved with 50%, and the patient was considered after 4 months "borderline mentally ill". Minimal QTc prolongation was detected on ECG after 4 months (+1.3%), but it didn't reach the level of significance (considered to be 460 msec in women, after correction with Fredericia's formula). No metabolic abnormalities were detected during the monitoring period, and the BMI decreased with 2.1% compared to baseline.

Conclusion: Targeting the cannabis and nicotine use disorders may improve the overall functionality and patient's quality of life, reducing further schizophrenia-associated symptoms, like depression, apathy, anhedonia or anxiety. In this case, the patient was compliant to the therapeutic suggestions, and participated in counselling sessions focused on substance use relapse prevention, while being adherent to the pharmacologic treatment. Her evolution was favorable and the therapeutic switch from risperidone to ziprasidone was well tolerated. No pharmacokinetic interactions were anticipated between the treatment for nicotine use disorder (replacement therapy), cannabis use disorder (gabapentin) and schizophrenia (ziprasidone).

Table 2. Psychologic evaluations during the second patient's initial visit

Clinical scale	Results
PANSS	69
CRDPSS	9
GAF	60
CGI-S	4
FTND	7
AUDIT – R	16
EuroQoL	
Visual analogic scale	67
Mobility	1
Self-care	2
Usual activities	3
Pain/discomfort	2
Anxiety/depression	4

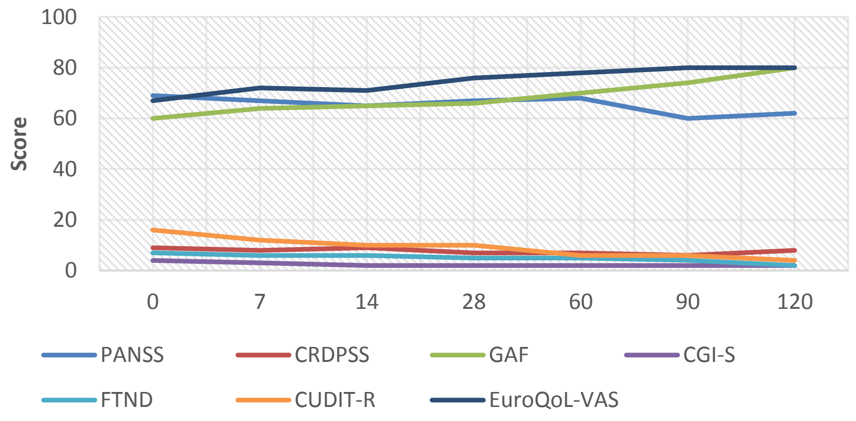

Fig.2. Evolution of the clinical variables during the first 4 months of treatment

The third patient, S.G., was diagnosed for the first time with acute psychotic disorder 3 months ago. Her symptoms at hospital admission consisted in psychomotor agitation, grandiose ("I am very powerful, and I can make any wish come true, like Santa Claus, only better") and persecutory ("there is a group of forces trying to kill me and take my powers") delusions, auditory and visual hallucinations ("I can hear them talking about me and trying to make me feel miserable… they are cursing me and telling lies about me and my family", "They are moving through the light, I can see them… they are like some green shadows"), disorganized behavior, and diminished emotional expression. She was 27 years old when she was first admitted in hospital, and her psychotic symptoms had an insidious onset over at least 4 months. First, she was initiated on risperidone 6 mg/day, and her response was good, but discontinued oral treatment because she had to take this drug twice a day. The patient developed positive symptoms of psychosis after one month of no treatment, and she was readmitted in the Psychiatry Department. The selected drug for clinical stabilization was risperidone because of her previous good response. She was informed that a long acting injectable form of this antipsychotic exists, which requires administration every two weeks. Also, she was informed that paliperidone, the active metabolite of risperidone, has two long acting injectable formulations, with administration of one dose every 4 weeks, and after stabilization, the drug may be administrated every 12 weeks. She agreed to be initiated on paliperidone long acting after stabilization of acute symptoms.

The patient was smoking 30 cigarettes daily, with a value of 9 pack-years. She fulfilled the DSM-5 criteria for nicotine use disorder and accepted treatment for this condition. She received nicotine replacement therapy, but she declined the invitation to join a group therapy focused on abstinence.

Her ECG was normal, as were chest X-ray, cerebral CT-scan, CBC and serum biochemistry panel. The toxicology exam was also negative.

Table 3. Psychologic evaluations during the third patient's initial visit

Clinical scale	Results
PANSS	88
CRDPSS	14
GAF	35
CGI-S	5
FTND	9

Therapeutic challenges analysis: This patient is still in the early phase of disease, as her diagnosis of schizophrenia was just established. She met the necessary criteria for this diagnosis- time (more than 6 months including pre-hospitalization period of active symptoms), clinical manifestations, functionality, and differentials. The challenge is to select a treatment regimen that could be more readily accepted by a young and active person (she has to travel often because she has contracts with different enterprises), while targeting both schizophrenia and nicotine addiction symptoms. One advantage in this case is the insight of the patient and her willingness to continue the treatment. She understood the therapeutic options her psychiatrist presented, and she has chosen the treatment which allows her less time for administration and medication-supplying procedures (visits to her GP, treating psychiatrist, and local pharmacy). Therefore, paliperidone was considered the most appropriate option for her, and after stabilization with oral medication, she was switched on paliperidone palmitate (PP1M) 100 mg monthly as maintenance dose for 4 months, and paliperidone palmitate (PP3M) 350 mg every 3 months after 4 months. Regarding her nicotine use disorder, she received 25mg/16 h nicotine patches and nicotine spray administered prn, in case of withdrawal symptoms, with gradually dose reduction, and termination after 3 months. The nicotine spray was recommended because the patient is a heavy smoker, and because she had no asthma, chronic sinusitis, or other related diseases. Paliperidone is not a substrate for CYP1A2, therefore its plasma concentrations are not expected to be modified by cigarette smoking, in case substance use disorder treatment fails.

Follow-up visits: The evolution of psychotic symptoms was favorable, as reflected in the PANSS and CRDPSS scores, which decreased with 40% and 65%, respectively. The favorable trend maintained even after switching on the long-acting formulae (PP1M and PP3M). The slower rate of improvement after day 36 is related to the stabilization of the clinical status, which is a condition for switching on long-acting antipsychotic formula. The patient reported that she could return to her job after 6 weeks of treatment and her professional performances were fair. The cigarette use declined during the first 4 weeks, but she admitted she smoked during nicotine replacement therapy and after its discontinuation. Therefore, after 11 months her FTND score reflected a moderate dependence. She refused a new trial of nicotine replacement therapy and counselling sessions, as she states “smoking is not a problem for me anymore… I’m only smoking when I’m feeling nervous”. Her BMI increased with 3.5% compared to baseline, but no significant alterations in plasma lipids, blood glucose, hepatic enzymes or QTc were reported.

Fig.3. Evolution of the clinical variables during the first 11 months of treatment

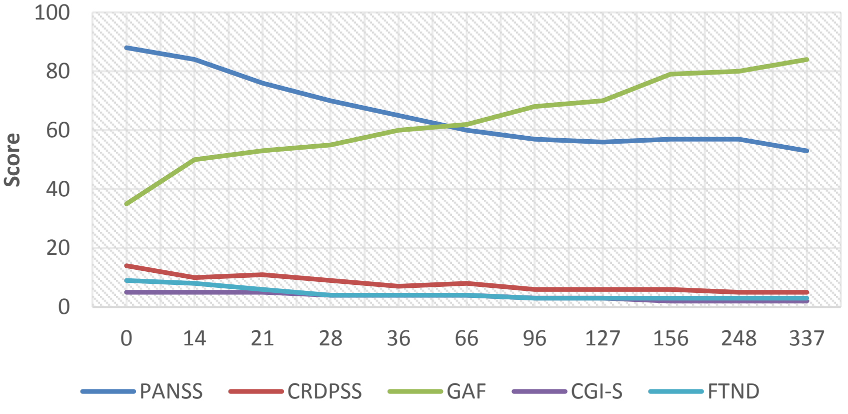

CONCLUSIONS

In young patients who experience first episode of psychosis establishing therapeutic relationship could be a difficult challenge. Communication between the psychiatrist and the patient is crucial in order to assure an adequate level of therapeutic adherence. The psychiatrist should consider the lifestyle of the patient, her psychological resources and specific needs, and to formulate the most appropriate therapeutic strategy. In this case a long-acting formula of an atypical antipsychotic was preferred because of the active lifestyle of the patient, and her expressed preference for a treatment which could be easily administered. The treatment for nicotine dependence has been a challenge, as the patient did not quit completely smoking, but only diminished it. Paliperidone could be useful in patients who smoke because it is not metabolized through CYP1A2, and its plasma concentrations remain stable even if this isoenzyme gene is induced by the polycyclic aromatic hydrocarbons of the tobacco smoke [34].

Abreviations list

AUDIT = Alcohol Use Disorders Identification Test
BMI = Body mass index
CBC = Complete blood count
CGI-S = Clinical Global Impression- Severity
CRDPSS= Clinician-Rated Dimensions of Psychosis Symptoms Severity
CUDIT-R = Cannabis Use Disorders Identification Test – Revised
EuroQoL 5D-3L= EuroGroup Quality of Life Scale
FTND = Fagerstrom Test for Nicotine Dependence
GAF = Global Assessment of Functioning
PANSS = Positive and Negative Syndrome Scale
PP1M = paliperidone palmitate with monthly administration
PP3M = paliperidone palmitate administered every 3 months
prn = *pro re nata*

Disclaimer

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CLINICAL PRACTICE

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Uncommon giant sphenoidal tumor. Case report

R. Hainăroșie[1,2], Irina Ioniță[1,2], Cătălina Pietroșanu[1,2], S. Pițuru[1], Mura Hainăroșie[1,2], V. Zainea[1,2]

Abstract*: The authors will present a case report of a woman that presented a giant sphenoidal tumor with endocranially extension and compression of the cerebral trunk. The patient was already presented in a neurosurgical service where due to the tumor volume, the high-risk surgical elements involved was sent to our ENT department to try to perform an endoscopic biopsy.*

Using the endoscopic optical and mechanical ensemble the authors performed trans nasally a biopsy. The histopathologic result was a surprise and was confirmed with three different immune-histochemistry exams.

Keywords: *sphenoidal tumor; hypophysis*

INTRODUCTION

Sphenoidal sinus is located in the middle of the skull, and is one of the most difficult sinuses to be attacked.[1]

The surgical risk elements are the internal carotid artery, optic nerve; optic chiasma; hypophysis and skull-base.[2,3]

The tumors located in the sphenoidal sinus are difficult to access and due to the vicinity of vital anatomical structures.

A biopsy must be performed to have a histopathological result and based on that the patient will have a treatment scheme.

In some cases, even biopsy's hard to complete, and high-risk factors are involved.[4,5]

The patient must be informed preoperatively about the risk involved in taking the biopsy, why is necessary to perform that biopsy and what are the risks of not having a biopsy and treatment.

Multiple biopsies must be performed to have a histopathologic result.

MATERIAL AND METHODS

We present a care, it was admitted a 52 years old woman with the following symptoms: headache, bilateral nasal obstruction, right abducens nerve

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paralysis, right exophthalmia, and diplopia.

Anamnestic we have found out that the symptoms started to develop six months ago gradually.

The patient was already consulted in a neurosurgical department. It was performed a CT scan, and the result was: expansive tumor which was located at, the level of sphenoidal body, extending anterior to rhino pharynx (nearly totally blocked) and posterior intracranial, with emphasizing compression of the cerebral trunk (pons and bulb).

Figure 1: CT scan exam expansive tumor which was located at the level of sphenoidal body, extending anterior to rhino pharynx (nearly totally blocked) and posterior intracranial, with emphasizing compression of the cerebral trunk (pons and bulb).
The intracranial fragment has T1 signal, suggesting intratumoral hemorrhage.
The cranian invasion extends to abducens nerves both sides.

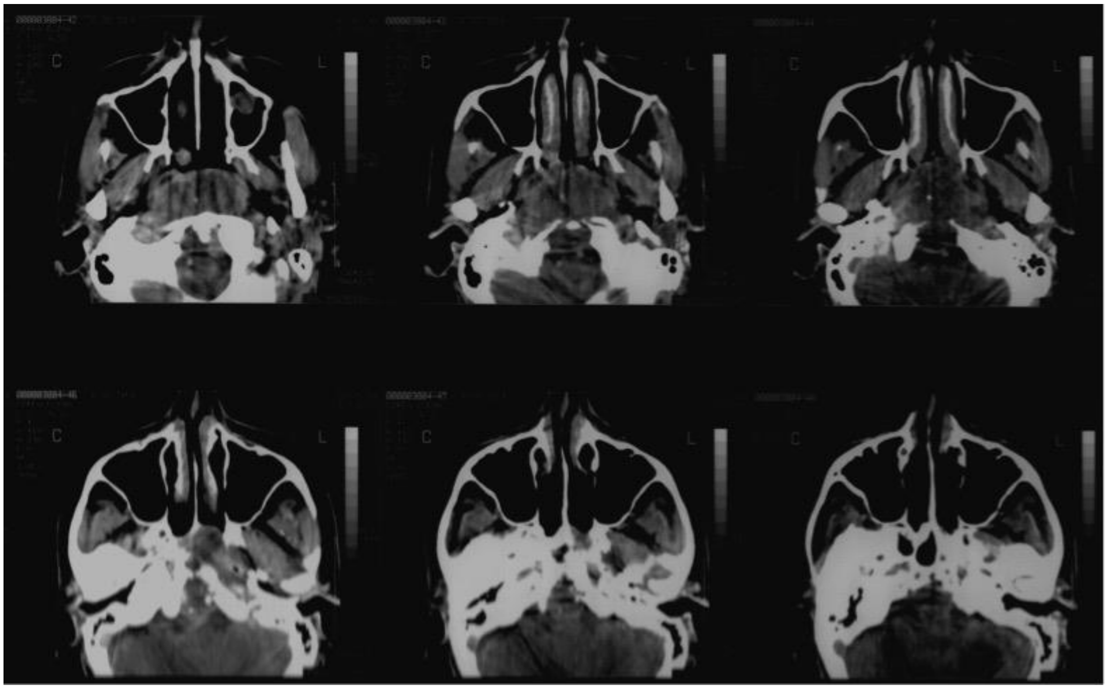

The CT scan exam conclusion was: tumor located at the level of the sphenoidal body, invading rhino pharynx and breaking into posterior fossa compressing cerebral trunk.

Possible diagnostic taken into account was chordoma, sarcoma, primitive tumor of rhino pharynx invading sphenoid sinus.

The patient was sent to our ENT department to be performed a transnasal endoscopic biopsy to see the histological nature of the tumor. Depending on the histological nature of the tumor the skull base team (neurosurgeon and ENT surgeon) will decide the best treatment schema for the patient.

In our hospital first, we have performed a fiber optic exam transnasally and transorally retrograde, where we were able to see the tumor that destroys the anterior and inferior wall of the sphenoid sinus, protruding into the rhino pharynx that was blocked near totally.

The surface of the tumor was smooth, and the mucosa of the posterior wall of the rhino pharynx was not destroyed, it was pushed from beneath. The tumor had a rich vessels supply. The patient had a rhino sinusitis secondary to the nasal obstruction produced by the tumor.

We have started to prepare the patient for the endoscopic trans nasally biopsy.

First, we have treated the rhino sinusitis with antibiotics, in steroids anti-inflammatory drugs and we have cleaned the nasal fossa every day. We wanted to obtain a clean surgical field because of the communication that will be created with the endocranial cavity.

Figure 2: Optical and mechanical ensemble to endoscopically expose the rhinopharynx

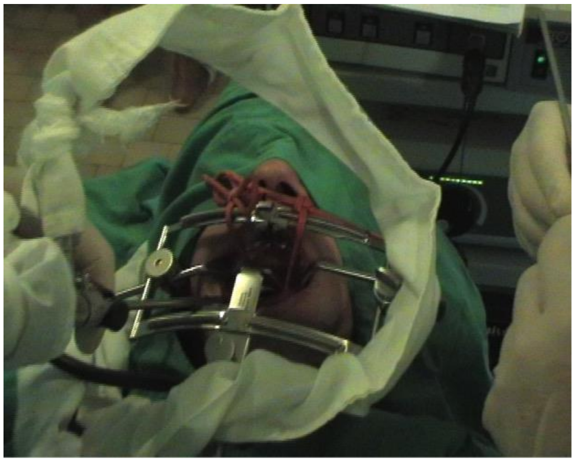

Figure 3: Video fiber optic trans orally exam. The tumor block near totally the rhinopharynx

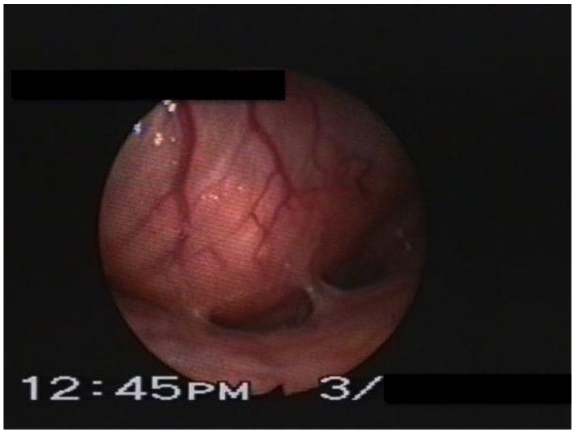

First, we have performed a trans nasal video contact endoscopy exam on the tumor. Video contact endoscopy is an endoscopic technique that allows the surgeon to study, after staining the tissue with methylene blue, the desired tumoral area as an in vivo histological exam. The surgeon can examine the histological superficial epithelial layer, observing histologic abnormalities and the vessels of tumors.

After one week we started to perform the biopsy under general anesthesia using both trans orally and trans nasal corridor.

Figure 4: Video contact endoscopy of the rhinopharynx. Locating a low vascular area

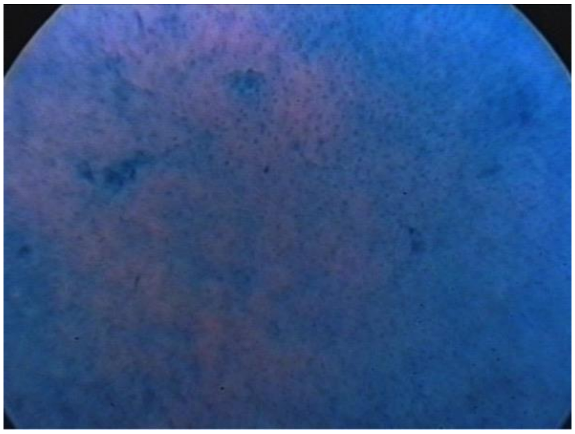

Figure 5: Targeted biopsy performed trans nasally

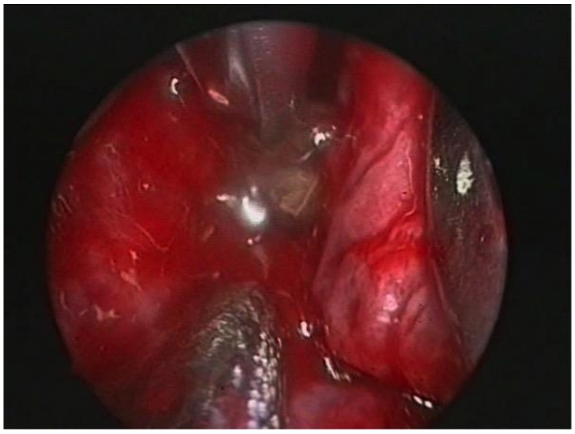

In our case, we did not discover any character of malignity at the superficial layer of the tumor, and we have discovered rich areas of vessels that fed the tumor.

We have started to gentle perform a targeted biopsy from the tumor in an area where the vascular network was limited because we wanted to prevent an important hemorrhage.

We have used cutting instruments because we did not want to perform traction maneuvers on a tumor that can have adherence on the cerebral bulb and ponds.

RESULT AND DISCUTIONS

We have obtained the bioptic material, and we have aspirate an important quantity of liquid. We had no CSF leak, we have packed the cavity with Gelfoam. The patient was packed for 48 hours.

The evolution of the patient was good, and the headache ceased, right abducens nerve paralysis started to reduce and disappear in 3 months.

The histopathological exam was a surprise, and it was confirmed using three different labs and immunochemistry tests. The sphenoidal tumor was a giant non-functionary pituitary adenoma.

The patient received medical and radiotherapy treatment after two months. The symptomatology disappeared.

CONCLUSION

In conclusion, trans nasal and trans oral corridor provides the surgeon with a minimally invasive route to preform biopsy and surgery.

The use of video contact endoscopy helped the surgeon to perform targeted biopsy from a low vascular area and to observe that we are not dealing with malignancy.

We underline the role of the multidisciplinary neurosurgical and ENT team. We have chosen a minimal invasive surgery to minimize the surgical risks (vascular and neurosurgical), and we have achieved an uncommon histological finding that changed the prognostic and the therapeutically route of the patient.

Acknowledgement

All authors have contributed equally to this paper.

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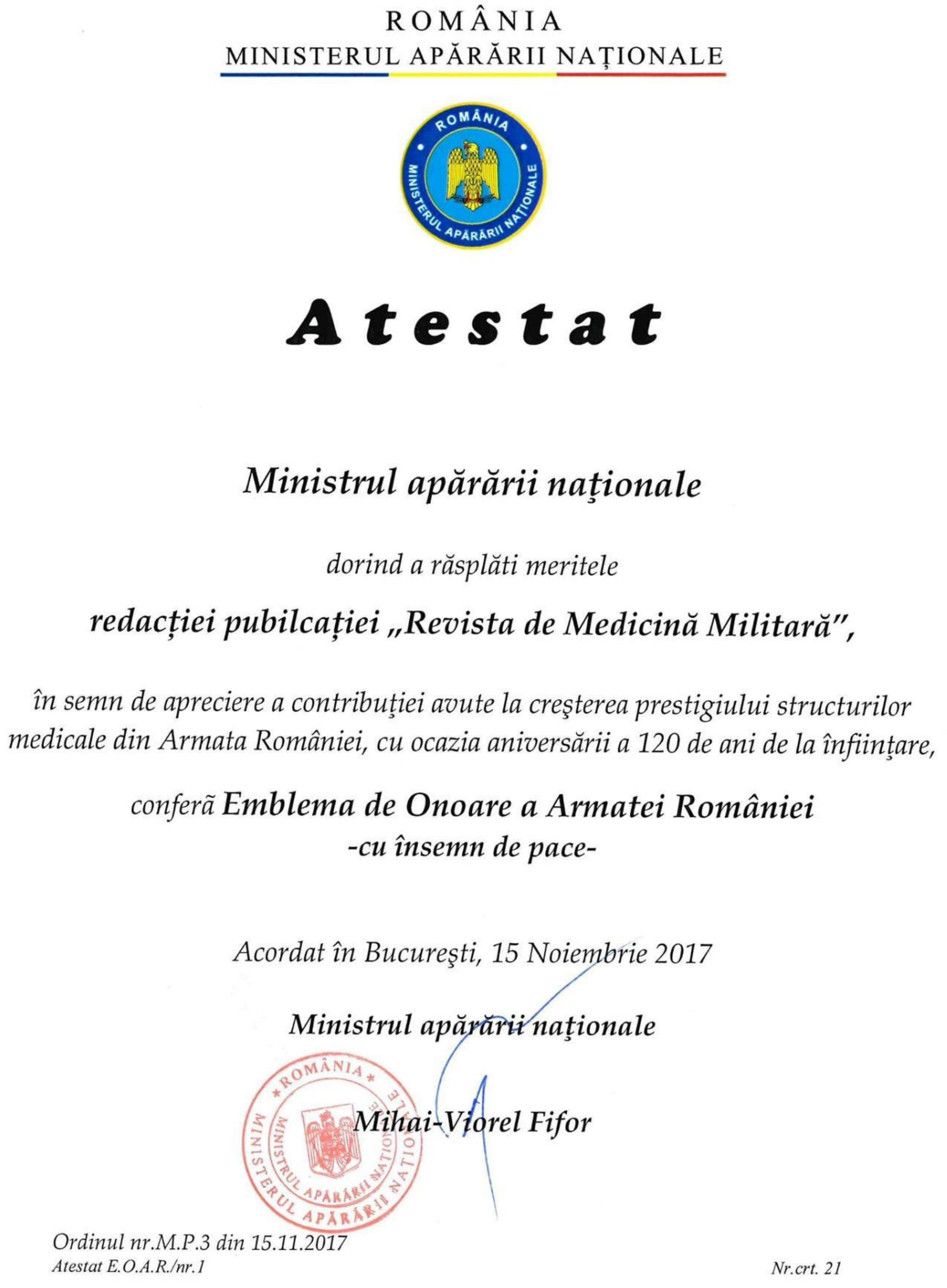

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redacției pubilcației „Revista de Medicină Militară",

în semn de apreciere a contribuției avute la creșterea prestigiului structurilor medicale din Armata României, cu ocazia aniversării a 120 de ani de la înființare,

conferă ***Emblema de Onoare a Armatei României***
-cu însemn de pace-

Acordat în București, 15 Noiembrie 2017

Ministrul apărării naționale

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EDITORIAL

ROMÂNIA
1918-2018 | SĂRBĂTORIM ÎMPREUNĂ

The history of military medicine in the last 100 years xxx

Paul Oprea

Gral Brig PAUL OPREA
Chief of Romanian Army Medical Directorate

The begining of the medical military service in Romania is related to the set up of the public institutions with military regime: gendarmerie and military subunits (pedestrians and riders), military firefighters, whose purpose was to provide sanitary services.

Due to the different evolution of Romanian regions, the history of military-medical structures was different.

The unification of the Romanian Principalities under the ruler Alexandru Ioan Cuza - modernist and good administrator - was an important leap in the development of the medical assistance structures of the armies, with the increase, diversification and specialization of the military units. It is the time when battalion infirmaries grow in size, the number of stationary beds for officers and troop's increases, more doctors are employed and therapeutic methods are diversified.

The arrival of King Carol I, one of the founders of modern Romania, in the country, has generated progress and development of the medical-military network.

After the conquest of Romania's state independence, the development of the institutions of the new state under the leadership of Carol I and of a political class of liberal and nationalist orientation recorded a momentum not yet encountered. Consequently, the first military hospitals were set up in addition to those in Bucharest, the period between 1880 and 1900 being a period of massive construction.

In addition to the Central Military Hospital in Bucharest, hospitals were built in Craiova, Piteşti, Galaţi, Focsani and the hospital from Iaşi was resized. Fortunately for the Romanian people, the beginning of the 20th century found the Romanian Army at a good level of organization, endowment and with enough staff.

In the Armed Forces, the medical service has developed to a great extent: there are specialists in surgery, internal medicine, dermatology, laboratory, etc. Hospitalization conditions are modern, both in the medical departments and in laboratories, the latter being managed by well-trained medical staff. In this period, there is a rise of military-medical professors, some of which turned out to be excellent specialists, role models for the next generations.

With the general physician Carol Davila, the first command (planning / administration / coordination) structure of the Army's medical service appears. He is the first chief in the history of the current Medical Directorate.

Having central activity as support, medical structures - infirmaries, hospitals - are standardized as structure, organization chart and endowment.

The period 1914-1916 until Romania's entry into war provided a partial modernization of the Army. Entry into World War I was an act of courage and sacrifice for all the services of the Army. The medical service was well prepared and managed to provide comprehensive health care in the operations theatres. Unfortunately, the Army's sanitary material conditions were not at an appropriate level, making it possible for the outbreak of typhus epidemic, which has ravaged thousands of lives among the Army's military. The intervention of the medical service, benefiting from the consistent support of volunteer women, coordinated by the new queen of Romania, eventually juggled the epidemic, improving the level of healthcare insurance - especially in terms of providing care for the wounded and sick.

The victorious outbreak of war and the creation of the Romanian national state - Great Romania - allying Transylvania, Basarabia and Bucovina - is the greatest moment in our history. However, the Romanian military continued military operations aimed at imposing order and compelling Hungary to accept the Versailles peace treaty.

On the occasion of the reintegration, the Army's medical care network has expanded accordingly, including hospitals from Timisoara, Sibiu, Brasov, Cluj-Napoca, Oradea, Cernauti and Chisinau. The newly established military units organized unit and garrison infirmaries. One of the Army's sanitary warehouses was organized in an old artillery unit, in Sebes, in addition to the one in Bucharest.

After the great global conflagration of 1914-1918, the principles and norms of organization and functioning of the campus hospitals (mobile and fixed - of the inner area) organized in school buildings, mansions, administrative buildings, etc. - were established. During the war, campus hospitals provided medical services to save the lives of the wounded and sick military.

The interwar period was a period of development of the Armed Forces medical network not only in numerical / quantitative but also qualitative terms: the medical units / departments and medical specialties, as well as the therapeutic resources and the means of diagnostics diversified, the imaging (Rx) developed consistently. In these twenty years, hospitals in Romania (including the military ones) evolved, developed, offering diagnostic and treatment methods similar to those available in Western Europe.

Romanian medical education has matured through the activity of the Medical Faculties in Bucharest, Cluj-Napoca, Timisoara and Iasi, generating a plethora of high-performance medical practitioners and pharmacists (some of the world's recognition personalities).

The interwar evolution of international relations that gradually brought Romania to Hitler's Germany led to the conclusion of the assistance protocol on the military line between Germany and Romania. Due to the offensive actions in particular, the functioning of the medical service has been rethought in order to ensure the triage and saving of life in the battlefield, in the conditions of multiple victims, tactical and strategic medical evacuation, ensuring the continuity of medical care along the hospital network (rehabilitation of wounded / sick military personnel so that it could be used again in combat), storage of functional and reserve stocks, use of blood transfusion (for the first time in history).

During the years 1940-1945, Romanian military medical network (both internal military hospitals and civil ones, as well as campus military hospitals) were tested. The Romanian sanitary trains, used as modern means of medical evacuation - during the First World War, demonstrated the logistic capacity held by the Romanian Army, allowing the strategic medical evacuation to be carried out by placing combat actions far away from the country.

The period 1945-1950 represented, in addition to the introduction of Russian communism dogma in the country, a period of radical transformation of the Army and, implicitly, of its medical service. There has been a general and dramatic regression by the exclusion from military physicians of leading personalities, who were considered counterrevolutionary and irredentist. These people were replaced by less prepared and competent, but "healthy" staff, unable to provide proper quality of the military-medical activities. The performance of the entire Romanian education system decreased consistently, therefore there was a decrease in the quality of the medical-military education as well.

After the 1960, the number and size of the military units increased by reorganizing the Army of the Romanian People's Republic (the beginning of the Cold War between Western Capitalist Europe and Eastern European Communist Europe).

The number of unit infirmaries and company hospitals increased and the number of military hospitals was decided, as follows: Bucharest Military Hospital, Cluj-Napoca Military Hospital, Craiova Military Hospital, Constanta Military Hospital, Focşani Military Hospital, Galati Military Hospital, Iasi Military Hospital, Oradea Military Hospital (transferred to the Ministry for Internal Affairs after 1989), Pitesti Military Hospital, Sibiu Military Hospital, Timisoara Military Hospital.

Military dispensaries were organized in big garissons: Bucharest (became CMDTA Acad. Ştefan Milcu), Bacău, Braila, Buzău, Ploieşti, Targoviste, Caracal, Alba Iulia and others. At Sebeş the central sanitary storage center was reorganized, as well as the pharmaceutical storage center in Bucharest. The Center for Preventive Medicine and the Armed Forces Transfusion Center were set up within the Central Military Hospital in Bucharest.

In view of the new concept of reaction to weapons of mass destruction (microbiological, chemical, radiological and nuclear means), the Army's Medical-Military Scientific Research Center was established in 1970-1978. Also, in 1975, the Faculty of Military Medicine was established.

After 1960 - with the improvement of the political and social situation in the country, the access to education of all young people and the organization of a fair promotion system are also present within the Armed Forces medical system, thus creating the premises for a series of excellent professionals: doctors and pharmacists.

The school of sanitary petty officers was founded in 1966 in Focsani. It prepares the sanitary petty officers that work in management and medical planning structures and provide medical care within unit infirmaries and campus hospitals.

However, the responsibility of relations with the medical education system from the Western Europe, which was evolved and emancipated by arbitrary restrictions, generated a period of regress in the performance of medical service. The provision of medical logistics was poor by reducing the import of medical devices: the only medical devices factory (ITM Bucharest) and several other medicine factories provided mediocre quality goods for the requirements of medical service. However, at the level of infrastructure, progress was made by building new structures and setting up medical services, diversifying the range of medical services by introducing new medical specialties in the surgical field (cardiovascular surgery, thoracic-pulmonary surgery, neurosurgery, maxillofacial surgery, orthopedics, urological surgery, gynecological surgery and pediatrics surgery) but also in the field of internal medicine (neurology, psychiatry, cardiology, pulmonology, gastroenterology and hematology, endocrinology and nutritional diseases, geriatrics, obstetrics, etc.).

After 1989, the medical-military system went through a regression, between the years 1990-1995, generated by deficiencies in planning, budgeting and supply, but that was outreached. Since 1994, medical-military education has started in an institutional framework again, within the Medical and Military Institute from Bucharest (with two sections: one in Bucharest at Carol Davila University of Medicine and Pharmacy and the other in Targu Mures - University of Medicine and Pharmacy). Although the number of trainees is much lower than the generations prior to 1989, tradition continues.

In accordance to the other institutions of the Romanian State, the Army registered a reduction of budgets and investments in the military field between 1990 and 2000. Military units were reduced in number and size progressively. Military medicine from the level of different forces also declined.

Military hospital units registered a real progress in the years 1997-2000 through the modernization of medical devices (centralized purchase of medical goods worth US $ 114 million). This strategic budgetary allocation has allowed the development of a network of military hospitals providing superior quality medical services which managed to impose at national level.

The reorganization of the Romanian Armed Forces during the period 2000-2005, which anticipated the entry into the North Atlantic Alliance, generated a regress in the Army's medical system by giving up the Military Hospital from Oradea to the Ministry of Internal Affairs, the retreat of many excellent doctors (introduction of mandatory retirement at the age of 55 - age corresponding to top performance in the medical career), abolition of the campus hospitals network, infirmary of large units, etc.

Medical-military scientific research is reduced because of inadequate budgets (a trend at national level), suspending the production of antidotes and salts used in CBRN interventions - implemented from 1978-1980.

Gradually, from 2007 to 2010, the specialized human resources were fewer and fewer, some of the causes were: low addressability of admission to the military system, migration of work force to the West, lack of vision at the level of the Medical Directorate regarding the future of the military medical system, legislative modifications in the health field, etc.

In 2008 there is an unfortunate event: the reduction of military functions at the level of military hospitals. This event associated with the transition to the self-financing system of military hospitals (public hospital quality) and defense funding gap - generated a major imbalance in the Army's medical network: each hospital evolved separately - depending on the period of medical services in which it was located, the performance of the medical staff, the management and organizational skills of the different managers, lack of vision and involvement at the level of the Medical Directorate, etc.

The foundation of the Health Insurance House of the Army, Public Order, National Security and Judicial Authority was a saving and integrative action in the national health system, establishing a favorable attitude towards maintaining the quality of services, fulfilling the accreditation standards, implementation of systematic management, providing for hygiene and sanitary-veterinary conditions of operation, ensuring respect for patients' rights and life security, etc.

From 2017, the Romanian Army benefits from a budget allocation of 2% of GDP. This financial support has enabled the Medical Directorate of the Ministry of National Defense to develop several projects in line with the major medical insurance objectives of the Army:

1. Modernizing medical and military education and training;

2. Standardization of medical structures (mobile and fixed);

3. Providing operational medical support - according to the training needs, resilience forces functioning and defense of the territory in times of crisis and war;

4. Ensure medical logistics of forces and territory in case of peace (crisis) and war.

REVIEW ARTICLE

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Complications of systemic lupus erythematosus: A review

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Abstract: *Systemic lupus erythematosus (SLE) is a complex autoimmune condition, which often evolves with severe complications. In SLE, autoantibodies appear, with systemic inflammation and multiple tissue destructions. SLE is the most common form of lupus and is considered more severe than other forms. SLE can affect many parts of the body, including the kidneys, heart, lungs, brain, blood, and skin. Symptoms vary among patients. SLE can follow an unpredictable pattern of remissions (symptoms improve) and flares (symptoms worsen). Renal involvement is one of the most severe complications in SLE patients. The process of depositing the immune complexes in the kidneys could lead to the appearance of lupus nephritis. If the treatment is early and treat-to-target, it can change the course of the renal disease.*

Keywords: *systemic lupus erythematosus, renal complications, target treatment*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune condition, characterized by an exaggerated activity of the immune system, which often evolves with severe complications. In SLE, autoantibodies appear, with inflammation and multiple tissue destructions.

EPIDEMIOLOGY OF SLE

SLE is a disease that appears particularly in young people, aged between 15 and 45 years, mainly young women. 10-15 times more women than men have SLE. The black population is more commonly affected by the disease, 2-3 times more than the white population, and develop more aggressive forms [1]. The incidence of the disease has increased significantly over the last decades, probably due to the development of diagnostic methods. [2]

ETIOLOGY OF SLE

The etiology of SLE includes genetic, environmental, hormonal and immune factors, which have a strong importance in triggering the disease.

Genetic factors plays an important role in triggering SLE. The monozygotic twins` disease risk is about 57% higher compared to heterozygote twins' risk.[3]

The existence of genetic susceptibility is also supported

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by the presence of other autoimmune diseases, such as autoimmune thyro-iditis, hemolytic anemia, idiopathic thrombocytopenic purpura [4,5]. SLE is associated with a multigenic determinism. The correlation with the major histocompatibility complex (MHC) on the short arm of the chromosome 6 has a particular importance in the development of the autoimmune processes in SLE [6]. Clinical studies have demonstrated the association of certain haplotypes of HLA DR (DR2 and DR3), DQ (DQ6), B8, components of the complement system (C2 and C4) with various clinical and immunological manifestations. Other genes, like C1q, the Fcγ receptor gene may be involved.

The *environmental factors* involved in the SLE are: ultraviolet rays (UV), that can trigger the autoimmune processes, justifying the occurrence or exacerbation of clinical manifestations after exposure to sun; both viral and bacterial infections: antibodies anti Epstein-Barr virus are present in the serum of SLE patients; bacterial triggers (bacterial DNA, membrane lipopoly-saccharides, endotoxins) were described as factors that can induce an exaggerated immune response; medications that may cause SLE or "lupus-like" phenomena: hydralazine, procainamide, isoniazide, chlorpromazine, methyldopa, penicillamine, minocycline, TNF alpha inhibitors, alpha-interferon; other possible environmental factors: nutritional, vitamin D deficiency, smoking.[7-10]

Hormonal factors: the increased prevalence of the disease in women is supported by increased levels of estrogen and prolactin. Dihydroepiandrosterone has low values in SLE patients.[11] Birth control and substitution therapy hormone increases the risk of developing SLE in the women with a certain genetic susceptibility.[12]

Immune factors. Lymphocytes T and B, monocytes, macrophages and neutrophils have increased production of autoantibodies in SLE patients.[13] The formation of autoantibodies has a central role in the appearance of SLE complications. The main antibodies are antinuclear antibodies (ANA). Anti-DNA antibodies (Ac anti-ADNdc) are the most specific for diagnosis, being correlated with disease' activity. Anti-histone antibodies are associated with drug-induced SLE. Other types of antibodies are: anti-Chromatin; antibodies to RNA structures and ribonucleoproteins; anti Smith antibodies, only described in SLE; antiprotetic P ribosomal antibodies.[14]

Autoantibodies in SLE participate in lesions of various organs through the formation of immune complexes (by immune response of type III) or by the direct action of these antibodies (by type II cytotoxic reaction) [15]. Pathogenic alterations can be secondary to deposition of circulating immune complexes, but also the formation in situ of immune complexes, especially at renal, cutaneous, vascular, nervous system level, causing inflammatory phenomena because of activation of the complement system.[16]

Lupus has different forms: systemic, cutaneous, drug-induced, and neonatal.

The systemic form is the most severe and complex, leading to kidney, heart, lung, brain, blood and skin complications. Symptoms vary among patients. SLE can follow an unpredictable pattern of remissions (symptoms improve) and flares (symptoms worsen).

Cutaneous lupus is a form of lupus that involves only the skin and causes rashes. These rashes may appear anywhere, but are usually found on the face, neck, and scalp. When the cutaneous lesions had an atypical localization, a differential diagnosis is needed [17-19]. This type of lupus usually does not involve the internal organs, but a number of patients living with cutaneous lupus will develop systemic lupus.

Drug-induced lupus can occur after the intake of high doses of certain drugs. The symptoms are similar to systemic lupus, but usually disappear when the drug is stopped or can manifest six months to one year. The antinuclear antibody (ANA) test may remain positive for years.

Neonatal lupus is present in newborns of a woman with lupus or another autoimmune disease. This condition can cause skin rashes, anemia or liver complications. Symptoms usually disappear after a few months and do not cause permanent damage. Some babies with neonatal lupus can be born with a severe heart defect.[18]

COMPLICATIONS OF SLE

SLE is a complex, multifactorial and polymorphic disease that may lead to severe complications.

Muscular complications. Muscular pain, commonly encountered in many diseases, may occur frequently in SLE patients with progressive evolution and it can often lead to a misdiagnose [20,21]. It is often located in the scapular-humeral belt and symptoms like myalgia and muscle weakness often appear.[19] Finally, franc myositis is associated with the increase in serum muscle enzymes, aminotranspherases or creatine kinase levels.[22] The pain is, in many cases, very severe an requires multimodal treatment.

Osteoarticular complications. Arthritis in SLE resembles that of rheumatoid arthritis, symmetrically affecting the small joints of the hands. In the case of SLE, arthritis is non-erosive. A special form of SLE arthritis is Jaccoud arthropathy, with deformations "swan neckline" type. When erosive arthritis occurs in SLE, it is called rhupus. Osteoporosis can occur frequently in SLE patients and is directly related to the high risk of fractures [23]. The surgical management of these fractures is complicated, especially due to bone fragility, needing limited periosteum stripping in order to prevent further damage of the bone [24]. Osteoporosis can also be associated with the adverse effects of cortisone therapy in these patients.

Cardio-vascular complications. The most common are pericarditis, which may range from asymptomatic to accumulation of moderate fluid, and myocarditis, with global hypokinesia at echocardiography.[25] A typical lesion that may occur in SLE patients is Libman-Sacks verrucous endocarditis, with thickening of the mitral and aortic valve [26-28]. The presence of antiphospholipid antibodies appears to be directly related to valvular damage.[29]

Pulmonary complications. Pleural effusion is the most common, is bilateral, and associated with the presence of antinuclear antibodies. In patients with SLE, impairment may be at the level of parenchyma, with lupus pneumonia or alveolar hemorrhage, or at the pulmonary vessels level, with pulmonary hypertension.[30,31]

Gastrointestinal complications may vary from mesenteric vasculitis or intestinal obstruction syndromes to peritonitis, pancreatitis or inflammatory bowel syndromes [32,33]. The most common symptoms are abdominal pain, nausea, anorexia, and vomiting [34,35]. Hepatomegaly and hepatic enzyme elevations are found in 30% of patients, hepatorenal syndrome can appear [36], and Budd Chiari syndrome may also be associated with the presence of antiphospholipid antibodies.[37-39]

Neuro-psihiatric complications are the least understood complications. They may involve either the central nervous system, causing aseptic meningitis, seizures, anxiety syndrome, psychosis, or the peripheral nervous system, with myasthenia gravis, mononeuritis, autonomic neuropathy or polyneuropathy. Endothelial dysfunction may be responsible for the passage of autoantibodies to the nervous system and the occurrence of the lesions.[40]

Renal complications are one of the most serious complications in SLE patients [41-43]. The process of depositing the immune complexes in the kidneys could lead to the appearance of lupus nephritis.[44] The classification of ISN RPS 2004 of lupus nephritis is universally accepted:

- Class I: Minimal mesangial lupus nephritis, with normal glomeruli by microscopic evaluation, but mesangial immune deposits at immunofluorescence.
- Class II: Mesangial proliferative nephritis, with mesangial hyperplasia at the optical microscope, but mesangial immune deposits on immunofluorescence.
- Class III: Focal proliferative nephritis, involving less than half of the glomeruli, with or without mesangial involvement. Subgroups: IIIA (active lesions); IIIA/C (active and chronic lesions); IIIC (inactive lesions).
- Class IV: Diffuse proliferative nephritis, involving more than half of the glomeruli.
- Class V: Membranous nephritis.
- Class VI: Sclerotic nephritis with no active lesions.

Other less common forms of lupus renal disease include interstitial nephritis, drug-induced and vascular disease, when the renal vessels are affected.[40,44,45]

Paraclinical, the parameters evaluated are: 24-h proteinuria, with values above 0.5 g/24 h; active urinary sediment with diffractional red blood cells and cell cylinders; serum creatinine above the upper limit of the laboratory, low creatinine clearance, low complement and high titer of double-stranded DNA antibodies.[46]

Usually, about 25–50% of lupus patients have abnormalities of the renal function, starting early or during the evolution of the disease. The renal complications seem to be discovered in the first three years after the diagnosis of SLE.[47,48] Regarding the ethnic origin, different studies revealed that renal impairment is significantly worse in black subjects than in white patients with lupus nephritis.[49-51]

Proteinuria is considered to be the essential piece in the lupus nephritis structure. In a review on lupus nephritis, proteinuria was reported in 100% of patients, with nephrotic syndrome in 45–65%.[47] Microscopic hematuria was found in about 80% of patients during the disease course.[46]

A recent retrospective study, exploring the determinants of earlier renal disease in patients with SLE, found that features like young age, non-European origin and male sex have a great impact on the disease [52]. Approximately 10–15% of patients with lupus nephritis, despite treatment, progress to end-stage renal failure.[49] Features predictive of end-stage renal disease in patients with severe lupus nephritis included higher baseline serum creatinine level and failure to obtain remission, with increased level of potassium, which can lead to arrhythmic events53. In this stage of disease evolution, the anesthetic risk for this patients is high, being necessary a strictly perioperative management in order to prevent further complications.[54]

Anti-phospholipidic syndrome (APS) is a disorder associated with recurrent arterial or venous thrombotic events and pregnancy morbidity, along with the presence of antiphospholipid antibodies (anticardiolipin antibodies and/or lupus anticoagulant) [55]. Renal disease in APS is characterized by interstitial tubular or glomerular injury due to obstruction of the vessels. Renal manifestations include thrombotic microangiopathy, renal vein thrombosis, renal infarction, renal artery stenosis or malignant arterial hypertension.[56] APS nephropathy was found to be statistically correlated with arterial thrombosis, fetal loss, and the presence of lupus anticoagulant. These characteristics seem to be linked to an increased prevalence of hypertension, raised serum creatinine, and interstitial renal fibrosis.[57]

The therapy of renal disease in SLE is a challenge for the practitioner. An early and targeted intervention can influence the disease' course. The mortality of SLE decreased considerably after the introduction of cyclophosphamide as the major weapon against lupus nephritis, the renal disease no longer impairing the survival rates of these patients. The results of a recent study sustain the role of combined therapy with intravenous pulses of cyclophosphamide and methylprednisolone in the improvement of lupus nephritis [58,59]. However, the long-term use of cyclophosphamide can cause major side effects, such as bone marrow suppression, hemorrhagic cystitis [60-63], gonadal toxicity and, eventually, the development of neoplastic disorders or can complicate some surgical procedures [64-66], with subsequent infections.[67-70] On the other hand, studies have very good results on long-term remission rates and the ability to achieve a second remission with currently recommended intravenous cyclophosphamide regimens. Concerning the new therapeutic options [71,72], it seems that biological agents, such as blockers of other co-stimulatory pathways (for example, CTLA4-Ig), monoclonal antibody against CD20, anticomplement (anti-C5b) and anti-cytokine treatment, induction of T and B cell tolerance, and hormonotherapy, could be involved in the management of lupus nephritis, but these therapies still have no sustainable evidence.[73-75]

CONCLUSIONS

SLE is an autoimmune disease in which self-tolerance is lost, with polymorphic manifestations that represent a real challenge, both for diagnosis and for treatment. SLE often involves severe complication in the various systems of the body, causing a decrease in the patient's quality of life. Renal impairment is one of the most serious complications and could lead to end-stage renal failure. Lately, the focus is on early diagnosis and targeted-treatment for the good management of these patients.

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REVIEW ARTICLE

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Heart failure with preserved ejection fraction: A review

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Abstract: *Heart failure is the final stage of evolution of a large number of cardiovascular diseases and represents a health problem worldwide, due to increased prevalence of cardiovascular diseases. The patients with heart failure with preserved ejection fraction (HFpEF) represent about a half of the patients with heart failure and the prevalence of HFpEF is on the rise. HFpEF is mainly a disease of the elderly patients, who have numerous cardiovascular diseases – hypertension, myocardial ischaemia, atrial fibrillation, valvular disease, and also non-cardiovascular comorbidities, such as obesity, diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, and obstructive sleep apnoea. HFpEF is more prevalent in women than in men, but women have a better prognosis than men. The pathophysiological changes that appear in HFpEF are: ventricular stiffening, cardiomyocite hypertrophy and hypercontractility, myocardial fibrosis and inflammation, which lead to abnormal diastolic function with delayed relaxation and inappropriate filling, in the presence of a normal systolic function of the left ventricle. The diagnosis of HFpEF is based on clinical, echocardiographic and biological criteria. In contrast to HFrEF, there is no effective treatment for HFpEF. The treatment of HFpEF includes diuretics for clinical improvement and treatment of comorbidities. The prognosis of patients with HFpEF is similar with those with HFrEF. Currently, the main objectives of the treatment in patients with HFpEF are to improve clinical status and decrease hospitalizations. Further studies are needed to establish an effective treatment to increase survival in these patients. Therefore, HFpEF continues to be a challenge for clinicians regarding the optimal therapy..*

Keywords: *heart failure with preserved ejection fraction, diastolic dysfunction, myocardial fibrosis, natriuretic peptides*

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INTRODUCTION

Heart failure is the final stage of evolution of a large number of cardiovascular diseases. Heart failure is a major health problem worldwide, due to increased prevalence of cardiovascular diseases and prolonged survival of some categories of patients with cardiovascular diseases, after successful treatment of their conditions.

The most frequent causes of heart failure are: myocardial ischaemia, valvular diseases, arrhythmias, atrio-ventricular conduction disorders, myocarditis, pericarditis. Often, heart failure has more than one cause. It is extremely important to determine the aetiology of heart failure in order to establish the specific therapeutic options (for exemple to reduce

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heart rate in tachyarrhythmias, to replace a valve in valvular disease, revascularization of the coronary arteries in patients with myocardial ischaemia, etc.) [1].

Currently, heart failure is classified into heart failure with reduced ejection fraction (less than 40%) - HFrEF,mid-range ejection fraction (between 40-49%) – HFmrEF, and preserved ejection fraction (≥ 50%) – HFpEF1. HFmrEF is a new category of heart failure, first introduced in the classification of heart failure in 2016, in the European Society of Cardiology Guidelines [1].

HFmrEF is usually studied in trials along with HFpEF, so in this paper we will use the terminology of HFpEF, including both entities. Until now, the majority of clinical trials regarding the treatment of heart failure have focused on patients with HFrEF. While in the case of HFrEF, it had been proven that the treatment can reduce both morbidity and mortality, in the case of HFpEF there is no drug which can reduce mortality or morbidity. This is why HfpEF is more challenging for the clinician regarding the diagnosis and treatment.

Patients with HfpEF associate many comorbidities, such as hypertension, diabetes mellitus, vascular disease, atrial fibrillation, metabolic syndrome, chronic kidney disease, which complicate even more the evolution of HFpEF and increase the risk of mortality.

PREVALENCE OF HFpEF

In the last years, the prevalence of HfpEF increased, because of the demographic changes and the changes in the risk factors prevalence, such as the increasing prevalence of hypertension, atrial fibrillation and diabetes mellitus that are all risk factors for HFpEF [2]. HFpEF currently affects more than 7 million people in Europe and is the only cardiovascular condition that has increasing incidence and prevalence [3]. The increasing prevalence of HfpEF can also be a result of the changes in clinicians' awareness over time and of the fact that the diagnostic methods have evolved during the last decades.

The patients with HFpEF represent about a half or even more of the patients with heart failure and the prevalence of HFpEF is on the rise [4-8]. HFpEF is mainly a disease of the elderly, who associate numerous cardiovascular and non-cardiovascular comorbidities [9].

HFpEF is more prevalent in women than in men, with a ratio women to men of 2:1, explained by the different sex cardiomyocite remodeling pattern in women than men [10,11]. In men, the eccentric left ventricle remodeling is more frequent, while in women the concentric remodeling is more frequent, as a response to aortic stenosis or hypertension [10]. Due to the concentric remodeling, women have a smaller left ventricle cavity and an increased wall thickness, with less collagen deposition [10]. Consequently, women have a better contractility and ejection fraction of the left ventricle, with the predominance of HFpEF. Women with HFpEF are more likely to be obese, hypertensive or to have renal disease or diabetes mellitus, while men are more likely to have coronary artery disease, atrial fibrillation, chronic obstructive pulmonary disease, anemia [12]. Even if HFpEF is more common in women, they have a better prognosis, with a rate of hospitalization with 20% less than men and a lower risk of cardiovascular and non-cardiovascular events. [12]

RISK FACTORS

Traditional cardiovascular risk factors, such as smoking, obesity, diabetes mellitus, hypertension, precede the onset of both heart failure with reduced and preserved ejection fraction, but there are specific risk factors for each of them. While male sex, myocardial infarction, left bundle branch block or high potassium level represent major risk factors for HFrEF, the risk factors for HFpEF are female sex, higher systolic blood pressure, right bundle branch block and atrial fibrillation [13-15].

Hypertension is common in patients with HFpEF and increases the risk of developing heart failure. The optimal treatment of hypertension improves diastolic filling, leads to the regression of left ventricular hypertrophy and may reduce the progression of heart failure.

Atrial fibrillation (AF) and HFpEF are related, and each of the diseases predisposes to the other. AF occur in approximately two thirds of the patients with HFpEF and contribute to the progression of the illness, due to the uncontrolled ventricular rates and the loss of atrial contraction and its contribution to the left ventricle filling, leading to a shorter filling time and diastolic dysfunction of the left ventricle [16]. When present together, HFpEF and AF lead to a worse outcome than each condition separately [17]. Patients with HFpEF and AF have a higher risk of stroke and mortality. All patients with HFpEF and AF should receive an oral anticoagulant and should have a good control of the ventricular rate.

Myocardial ischemia may contribute to HFpEF, too, by altering the diastolic filling. Significant coronary artery disease is present in more than half of the patients with HFpEF. Patients with HFpEF and myocardial ischemia have a greater risk of ventricular dysfunction and mortality than those without myocardial ischemia. Coronary revascularization can improve the prognosis of patients with HFpEF and myocardial ischemia.

Regarding patients with diabetes mellitus, particularly patients with insulin-dependent diabetes mellitus have an increased risk to develop major adverse cardiovascular events, including cardiovascular mortality, hospitalization for heart failure, non-fatal myocardial infarction, non-fatal non-hemorrhagic stroke, aborted cardiac arrest. Therefore, patients with HFpEF and diabetes have a worse quality of life and poorer outcomes than those without diabetes mellitus.

Comorbidities such as obesity, diabetes mellitus, chronic kidney disease, anemia, chronic obstructive pulmonary disease, and obstructive sleep apnea are related to the worsening of heart function in HFpEF. The treatment of these comorbidities improves the prognosis of heart failure and reduces mortality [18-20].

PATHOPHYSIOLOGY OF HEART FAILURE WITH PRESERVED EJECTION FRACTION

HFpEF is characterized by a normal systolic function, but abnormal diastolic function, with inappropriate filling of the left ventricle in the presence of normal pressure, initially. In more advanced stages, it associates also elevated filling pressures, especially during physical exercise. High filling pressures are considered the primordial cause of dyspnea and exercise intolerance [21]. The abnormalities encountered in HFpEF include delayed relaxation, stiffening of the myocardium and inappropriate filling dynamics.

The changes that occur in the cardiomyocites are the key to understand the dysfunction that appears in HFpEF and consequently to discover a specific treatment. An important element is cardiac hypertrophy. The extent of hypertrophy is a major predictor for HFpEF outcome. Other elements that contribute to HFpEF are fibrosis, left ventricle filling pressure, left ventricle end-diastolic volume, systemic inflammation, and metabolic disorders [22].

HFrEF is characterized by a diminished contractile response of the cardiomyocite, reduced availability of calcium activator and inadequate storage of calcium in the sarcoplasmic reticulum [22]. Knowing these mechanisms, HFrEF may benefit of a specific treatment which acts at the level of renin-angiotensin-aldosterone system and beta-adrenergic receptors. The treatment for HFrEF is ineffective in HFpEF, because of the different pathophysiological mechanisms.

In the case of HFpEF, there are no animal models, which is a major problem to a complete understanding of the pathophysiological mechanisms, in order to establish a specific therapy.

A study published in June 2018 suggests a new theory regarding HFpEF: hypertrophic cardiomyocytes develop also hyperfunction and elevated availability of systolic calcium activator [23]. In vivo, it is present a diastolic dysfunction and in vitro, hypercontractility of the cardiomyocytes. The researchers also noticed discrete, focal areas of fibrosis and in vivo, propensity to arrhythmias, caused by the instability of the calcium from the sarcoplasmic reticulum, and sudden death [23]. Apparently, the hypercontractility due to the increase of the L-type calcium channel density is responsible for the normal systolic function [23]. On the other hand, the high level of calcium limits the relaxation and promotes the stiffness of the ventricle walls. It was noticed also a deficit in cardiomyocytes, which leads to the compensatory hypertrophy of surviving myocytes [23]. Therefore, when failure occurs, diastolic dysfunction is predominant, but ejection fraction is preserved and end-diastolic volume is normal. There have been also observed the hypertrophy of the cardiomyocytes, the stiffness of the ventricle walls and focal interstitial fibrosis.

Arrhythmias can occur in HFpEF and they are related with the high levels of operational calcium, which confer instability, and with spontaneous release of calcium from the sarcoplasmic reticulum during diastole. Arrhythmias increase the risk of sudden cardiac death [23].

Myocardial fibrosis contributes to diastolic dysfunction. Patients with HFpEF have a profibrotic state. Fibrosis is organized in focal areas, observed especially near the interventricular septum. Fibrosis increases the stiffness of the walls. Collagen type I and III are elevated in HFpEF, collagenase and metalloproteinase-1 are reduced, but tissue inhibitor of matrix metalloproteinase-1 is increased, changes which contribute to fibrosis [23]. In tissue biopsy of patients with HFpEF, inflammatory cells have been also found. Hypertrophy is related to a diminished population of cardiomyocytes at an early-stage of growth. Hypercontractility predisposes to cardiomyocytes rupture and substitution with fibrotic tissue. Consequently, the integrity of the cardiac muscle is affected by focal fibrosis and the change of the ventricle geometry. In time, as the failure progresses, the diastolic function is totally compromised, but the systolic function, measured by the ejection fraction, is conserved [23].

Ventricular stiffening is associated with vascular stiffening in HFpEF. Vascular stiffening increases with age and is related to hypertension, chronic kidney disease, diabetes mellitus and obesity [24-26]. Vascular stiffening requires increased cardiac output to fill the rigid arteries that contributes to ventricular stiffening. Thus, ventricular-vascular stiffening is a common association in patients with HFpEF.

Inflammation has an important role in HFpEF. Comorbidities, such as renal disease, diabetes mellitus, may contribute to the proinflammatory state [27]. Several proinflammatory cytokines are increased in HFpEF, such as interleukin-6, tumor necrosis factor α, pentraxin 3 and soluble ST2 [27]. These patients have systemic inflammation and endothelial dysfunction, caused by the elevated expression of vascular cell adhesion molecules: VCAM-1, E-selectin, reactive oxygen species (ROS) [20]. The high level of ROS leads to the reduction of nitric oxide and consequently aggravates cardiomyocyte stiffness, hypertrophy and fibrosis [20,26].

DIAGNOSIS OF HFpEF

Heart failure can be initially suspected based on clinical criteria. For a definite diagnosis, it is mandatory to perform an echocardiography.

At echocardiography, the ejection fraction of the left ventricle, the thickness of the ventricular walls, the cavities' geometry and dimensions, the associated pathologies, such as valvular stenosis or insufficiency, are evaluated.

The clinical and paraclinical elements necessary for the diagnosis of HFpEF/HFmrEF are:

- The presence of symptoms and signs of heart failure.
- A left ventricle ejection fraction ≥ 50% for HFpEF and 40-49% for HFmrEF.
- Increased levels of natriuretic peptides: BNP > 35 pg/mL; NT-proBNP > 125 pg/mL.
- Objective elements of structural and/or functional disorders of the heart as a possible cause for the clinical presentation.
- If the diagnosis is uncertain, a stress test or invasive measurement of increased left ventricle filling pressure may be necessary [1].

The specific symptoms of heart failure are breathlessness at variable degrees of effort or at rest and exercise intolerance. Dyspnea interferes with the daily activities and impairs the quality of life. On physical examination, patients with heart failure have pulmonary crackles, bilateral ankle edema, jugular venous dilatation, laterally displaced apical beat.

Echocardiography is the conclusive method for the diagnosis of heart failure. The left ventricle ejection fraction (LVEF) is the most important parameter used to determine the systolic function of the left ventricle and a powerful predictor of death in patients with reduced ejection fraction [27]. LVEF is an important predictor for fatal and nonfatal cardiovascular events in patients with heart failure, including heart failure-related death, sudden cardiac death, hospitalization, myocardial infarction, especially in patients with reduced ejection fraction [28]. A 10% reduction of the ejection fraction is associated with a 39% higher risk of cardiovascular mortality [27].

In patients with HFpEF, echocardiography reveals a normal or near-normal ejection fraction and inadequate diastolic relaxation and left ventricle filling.

The structural elements of HFpEF are the left atrial volume index > 34 mL/m^2, a left ventricular mass index ≥ 115 g/m^2 for males and ≥ 95 g/m^2 for females [1].

The diastolic dysfunction has 3 stages that can be evaluated by echocardiography. The first stage is delayed relaxation, with an E/A ratio under 0.8, deceleration time of E wave > 200 ms, E/e’ medium ratio less than 8. The second stage is pseudonormal function, with E/A ratio between 0.8 and 1.5, deceleration time of E wave between 160 ms and 200 ms, E/e’ medium ratio between 9 and 12. The last stage is the restrictive pattern, with E/A ratio more than 2, deceleration time of E wave less than 160 ms, E/e’ medium ratio more than 13 [28].

If echocardiography at rest is inconclusive, the diastolic stress test, using a semi-supine bicycle to measure the pulmonary artery pressures and cardiac output changes with exercise, may be necessary [1]. Another paraclinical test is invasive measurement of the filling pressures – the pulmonary capillary wedge pressure ≥ 15 mmHg, left ventricular end-diastolic pressure ≥ 16 mmHg [1].

In the presence of atrial fibrillation, the echocardiographic diagnosis of HFpEF is more difficult, because the left atrial volume index is higher, the functional parameters of diastolic disorder are not well established. Also, the values of natriuretic peptides are higher in patients with atrial fibrillation. On the other hand, atrial fibrillation can be a mark of HFpEF. Patients with HFpEF and atrial fibrillation may have more advanced heart failure than those with HFpEF and sinus rhythm [1].

Biomarkers measured with highly specific and sensitive assay play an important role in the diagnosis of heart failure and its risk stratification [27]. The Brain Natriuretic Peptide (BNP) is higher in patients with HFpEF than in patients without heart failure, but lower than in patients with HFrEF [28]. BNP is associated with diastolic left ventricle walls stress and pressure. Biomarkers of extracellular matrix and fibrosis (soluble ST2, galectin-3, type I procollagen C-terminal pro-peptide, type I and II collagen amino-terminal pro-peptide, collagen telopeptides, matrix metallo-proteinases – MMP-1, MMP-2, MMP-8, MMP-9, tissue inhibitor of metalloproteinases – TIMP-1, TIMP-4, osteopontin) can be increased in patients with HFpEF [6]. Renal biomarkers, such as cystatin C and urinary albumin, and cardiac troponins are also elevated6. All these biomarkers may sustain the diagnosis of HFpEF, but have a poor prognostic role and, excepting natriuretic peptides, cannot be used to guide the treatment [6]. The natriuretic peptides values are related to the severity of the disease, therefore higher levels of natriuretic peptides are linked with more advanced HFpEF [6].

TREATMENT OF HFpEF

There is no specific or effective treatment for patients with HFpEF, in order to reduce the mortality rate [29]. In clinical practice, fewer patients with HFpEF appear to receive beta-blockers, diuretics, angiotensin-converting enzyme inhibitors, angiotension receptor blockers and aldosterone antagonists than patients with HFrEF and if they receive these drugs, it may be to treat comorbidities, such as hypertension, coronary artery disease, atrial fibrillation [29,30].

Renin-angiotensin-aldosterone system blockers have been studied in patients with HFpEF, due to their effects on hypertension, fluid retention and fibrosis. Also, beta-adrenergic blockers were studied due to their effects of prolonging diastole and increasing the filling time of the left ventricle. Despite their well-known benefits in patients with HFrEF, in those with

HFpEF beta blockers offer only a modest symptomatic improvement and do not reduce mortality [31,32]. Some studies revealed a decreased hospitalization rate with angiotensin converting enzyme inhibitors/ angiotensin receptors blockers [6]. The study „The Effects of Candesartan in Patients with Heart Failure and Preserved Left-Ventricular Ejection Fraction" concluded that patients with HfpEF who received candesartan had fewer hospitalizations for decompensated heart failure compared with placebo, but without influence on the mortality rate [6]. Probably, renin-angiotensin-aldosterone-system does not have such an important role in HFpEF as in HFrEF, this is why its blockers have much less benefits [6].

The use of aldosterone antagonists was also tested in HFpEF. The results of the study ALDO-DHF, published in 2013, showed an improvement of the diastolic function, but no improvement was noticed in the maximal exercise capacity, clinical symptoms and quality of life [6]. The TOPCAT study, whose results have been published in 2014, also did not achieve its primary outcomes – cardiovascular mortality or aborted cardiac arrest, only a small decline in hospitalization rate has been observed [6,33].

Sildenafil, a phosphodiesterase-5 inhibitor, was tested in RELAX study and showed no advantage on mortality or symptomatic relief [6].

Statins can be prescribed in patients with HFpEF and have shown a significant impact on survival, due to their pleomorphic anti-inflammatory effects [6].

Considering the lack of results on mortality rate with beta-blockers use, ivabradine was also studied. Ivabradine slows the sinus node rate, but does not affect contractility and peripheral vasculature, in contrast to beta-adrenergic blockers [6]. Studies on mice revealed an improvement in the left ventricle function and lowering of aortic stiffness and fibrosis after four weeks of therapy with ivabradine [6]. A clinical trial, including 61 patients with HFpEF, has found an improvement in exercise capacity and decreased exercise-induced E/e' ratio, which represents diastolic pressure index, with ivabradine versus placebo [6]. No side effects appeared. Ivabradine cannot be used in patients with bradycardia or chronotropic incompetence and neither in patients with restrictive type of diastolic disorder, because filling occurs early and rapidly in these patients and decreasing heart rate would worsen cardiac output even more [6].

Introduction of device therapy was a great progress for patients with HFrEF, with pacemakers, implantable cardioverter defibrillators, and resynchronization therapy. In patients with HFpEF, asynchrony is rarer than in HFrEF, thus device therapy has no applicability in HFpEF [6].

No treatment has yet been found to diminish morbidity and mortality in patients with HFpEF and HFmrEF. An important objective of the treatment in these patients is to improve symptoms and the quality of life. Diuretics are prescribed to reduce pulmonary and systemic congestion signs and symptoms. As mentioned before, aldosterone antagonists and beta-blockers do not improve symptoms in patients with HFpEF, only a relative improvement in NYHA class with candesartan was observed [1].

Regarding hospital admission, patients with HFpEFin sinus rhythm may benefit from the use of nebivolol, spironolactone and candesartan, which decrease hospital admission1. For patients who associate atrial fibrillation, beta-blockers are ineffective and digoxin has not yet been studied [1].

Arterial hypertension should be carefully treated in patients with HFpEF/HFmrEF, who have often systolic arterial hypertension. The antihypertensive drugs recommended are diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and aldosterone antagonists [1]. Beta-blockers appear to be less effective [1].

The specific therapy for comorbidities is also important, for example losing weight in obese patients, oral anticoagulants for atrial fibrillation or other arrhythmias, medical and/or interventional treatment for coronary artery disease, treatment of diabetes mellitus, preferably with metformin [1], renal disease treatment [34-37], treatment of anemia, chronic obstructive pulmonary disease and obstructive sleep apnea.

The most challenging situation is the treatment of HFpEF when different kinds of surgical procedures are required [38-40]. Either is abdominal surgery [41-44] or pelvic interventions [45-47], in males [48-51] or females procedures [52-55], the management of these comorbidities involves complex treatment strategies from surgeons, cardiologists or anesthesiologist.

Thus, the management of HFpEF includes diuretics, which reduce systemic congestion and improve the filling pressure of the left ventricle, and treatment of comorbidities, which improves considerably the clinical outcome [56].

PROGNOSIS OF PATIENTS WITH HFpEF

Overall, the prognosis of patients with HFpEF is similar with those with HfrEF [57]. Patients with HFpEF have more frequent a non-cardiovascular cause of death or hospitalization, in contrast to the patients with HFrEF, who have commonly a cardiovascular cause for hospitalization or death [1]. Renal involvement in patients with HF is a redutable complication, especially in patients with increased blood pressure [58-61].

The left ventricle hypertrophy and elevated left ventricle filling pressure correlate with hospitallizations for heart failure [62,63]. Recurrent hospitalizations are as frequent in HFpEF as in HFrEF and are associated with higher mortality rate [62].

There are sex-related differences between the risk factors for HFpEF. Hypertension, obesity and renal disease are more common in women, while ischemia, atrial fibrillation, chronic obstructive pulmonary disease and anemia are more frequent in males [12]. Women have a 20% lower risk of hospital admission than men, and also a lower risk of cardiovascular and non-cardiovascular events [12]. Women have an overall better prognosis than men [12]. The prognosis is modified by the presence of atrial fibrillation, stable angina pectoris, and renal disease in both sexes [12].

CONCLUSIONS

Patients with heart failure with preserved/mid-range ejection fraction represent approximately a half of the patients with heart failure.

The diagnosis of HfpEF is based on clinical signs and symptoms, echocardiography, natriuretic peptides levels and stress tests or invasive measurement of filling pressures and left ventricular end-diastolic pressure, if necessary.

Overall, the prognosis of patients with HFpEF is similar with those with HFrEF, with the difference that in these patients the death of non-cardiovascular cause is more common, in contrast to the patients with HFrEF, in whom the cardiovascular death is more frequent.

There has not yet been found a specific therapy for HFpEF that may reduce morbidity and mortality, in contrast to HFrEF. The main objective of the treatment in patients with HFpEF is to improve clinical status and decrease hospitalizations.

Further studies are needed to establish an effective treatment, to increase survival in these patients. In conclusion, HFpEF continues to be a challenge for clinicians regarding the optimal treatment.

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REVIEW ARTICLE

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Gut microbiota – new insight in colorectal cancer pathogenesis

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Abstract: *Gut microbiota is a superorganism involved in homeostasis and in pathogenesis. Microbiota composition is influenced by several factors such as: type of birth (cesarean or natural), role of age, role of diet. Pathological consequences of certain type of diet (especially western type of diet) may, in fact, be mediated by gut microbiota alteration. Also few studies have investigated the issue of gut microbiota composition in patients suffering of colorectal cancer, several reports notice relevant differences. Certain pathogenic bacteria in gut microbiota have been extensively studied in relation to their role in colorectal cancer. Thus, Fusobacterium nucleatum, which is abundant in colorectal cancer patient's colon, may initiate the progression from adenoma to adenocarcinoma. A suggested pattern of oncogenesis in colorectal cancer may be represented by this: dysbiosis-inflammation-oncogenesis. A metanalysis that has been published in 2006 concluded a protective role of probiotics in colorectal cancer and in colonic adenoma.*

Keywords: *colorectal cancer, gut microbiota, probiotics*

Human gut harbors a complex and abundant microbial community which is symbiotically linked with host organism and plays a major role in maintaining the homeostasis by the way of fulfilling various functions such as: limiting the number of intraluminal pathogens, functionnal optimization of immunity system, and important contribution to maintaining energy balance.

It is estimated that there are around 1,014 microorganisms in the colon (mostly bacteria) which is 10 fold above the number of eukaryote cell of human body and that contains a 100 fold richer genome than human genome [1]. As a matter of fact we may acknowledge the existence of a true superorganism whose implications in physiology and pathology of human body are continuously revealed by a great pool of scientific data.

Microbiota composition is influenced by several factors such as: type of birth (cesarean or natural), role of age, role of diet.

Pathological consequences of certain type of diet (especially western type of diet) may, in fact, be mediated by gut microbiota changing [2].

It has been noticed that there are significant differences between European children that eat a western diet and African children that eat rural diet rich in vegetal fibers and poor in meat, as concerning the

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composition of gut microbiota.

Firmicutes genera is prevalent in European children and Bacteroidetes genera is depleted as opposed to African children in whom the ratio is reversed [3]. The real significance of those changes is to be better defined in the near future. Medical literature is abundant in information on this topic, especially about the composition of microbiota in various pathological situations, but the studies published are mostly animal studies and the implications in understanding of pathological mechanisms and in medical practice are ill defined.

The role of gut microbiota played in certain inflammatory ailments (such as inflammatory bowel diseases – IBD) is well known [1]. Important practical consequences may be derived from these data: stool transplant and probiotics treatment [4]. Chronic local inflammation is a risk factor for colorectal cancer (CRC). As a matter of fact patients suffering from IBD have a yearly risk of CRC of 1% after 10 years from the onset of inflammatory disease [4]. It is an answered question as to what extent the composition of gut microbiota is implicated in CRC susceptibility.

Also a few studies have investigated the issue of gut microbiota composition in patients suffering of CRC [1], several reports notice relevant differences. For instance, a study indicated an increased prevalence of Bacteroidetes and Prevotella genera than in general population [5]. Another study which investigated bacterial populations on the surface of colonic mucosa as opposed to luminal bacteria showed that there was an abundance of Faecalibacterium and Dorea species in patients suffering of adenomatous colonic polyps as opposed to those without polyps [6].

Certain pathogenic bacteria in gut microbiota have been extensively studied in relation to their role in CRC. Thus, Fusobacterium nucleatum, which is abundant in CRC patients' colon, may initiate the progression from adenoma to adenocarcinoma [7]. Rubinstein showed that the species of Fusobacterium which produced the virulence factor FadA stimulated epithelial cell proliferation, promoted inflammation and initiated oncogenesis [8]. Similar studies have been published about other components of gut microbiota such as: E. Coli, B. fragilis, S. Gallolyticus (former S. bovis particularly associated with CRC) [8,9,10,11]

Also it is obvious that there is a change in CRC patients gut microbiota composition, it is not clear if those changes are already present before the diagnosis of CRC suggesting a role of risk factor or those changes reflect the consequences of the disease.

From another point of view, the CRC risk factors modify gut microbiota and may elicit the oncogenic process by this route. Several studies have indicated that a meat diet, by the way of microbiota composition alteration, may play an important role in oncogenesis [12,13].

Obesity is a major risk factor in CRC. Many studies showed a certain pattern of gut microbiota change in obese subjects, in animal as well as in human studies [14,15]. Gut bacteria have an impact on peripheral resistance to insulin, systemic inflammatory status and on systemic adiposity by the way of interaction with intestinal epithelial cells. Those interactions are very complex and mutually beneficial. Thus, gut bacteria metabolize various nutrients such as digest-resistant carbohydrates which results in production of short chain fatty acids (mainly butyrate). Gut epithelial cells uses as a main source of energy those fatty acids. Moreover, butyrate may be involved in anti-oncologic effects [16]. Backend noticed, in a mice study, that germ-free subjects experienced a 60% increase in adiposity and a significant increase in peripheral resistance to insulin after 14 days from stool transplant performed from lean mice, in spite of a restrictive diet. Stool transplant from obese mice elicits an even greater increase in total fat mass, which may render a reasonable demonstration about the role played by microbiota in obesity and this may further suggest a role in colonic oncogenesis [15].

A suggested pattern of oncogenesis in CRC may be represented by this: dysbiosis – inflammation – oncogenesis. Alteration of gut microbiota (dysbiosis), as a response to an unidentified trigger, has been studied in animal experimental models with regard to collective or individual role of bacteria in development of inflammation and CRC [17]. The pathogenic

mechanism is still unclear but we may appreciate an involvement of certain receptors of innate immune system responsible for microbial detection [18].

Indirect data on the topic of gut microbiota in CRC are derived from the acknowledged protective role of probiotics. The term of probiotics has been used for several decades but World Health Organization has issued a definition only in 2001: „ live microorganisms that render health benefits when they are administered in proper doses” [19]. Lactic bacteria and Bifidobacteria are among the most known types of probiotics. A metanalysis that has been published in 2006 concluded a protective role of probiotics in CRC and in colonic adenoma [20]. The authors of this metanalysis suggested a cautious use of these data as the interventional type of studies were still not enough to render an unequivoque positive answer.

Recently, Faecalibacterium prausnitzii, a commensal bacterium in gut microbiota that has anti-inflammatory properties, has also been indicated as a probiotic to decrease the risk of CRC [21].

Mechanisms engaged by probiotics in the process of oncogenesis are multiple: cell cycle influences, oxygen reactive species, apoptosis, specific bacterial enzymes, influences on human host metabolome [1]. Probiotics have an important contribution to the development of mucosal associated immune system as it modulates unspecific inflammatory response and it dampens down mucosal inflammation. Probiotics exert also certain effects on dendritic cell, colonic epithelial cells, lamina propria T cells, thus having an impact on adaptive immune system. Perdigon noticed in a mice study, that joghurt (rich in lactic bacteria) hampered the growth of colonic tumors which had been previously induced by mice exposure to 1,2 dimetilhidralazine [22]. Urbanska reached a similar conclusion in another mice study. He showed that yoghurt might reduce the volume of the tumor. Induction of apoptosis may be credited as a major mechanism that mediates the beneficial effects of probiotics against oncogenesis of colonic tumors. Hence, the induction of apoptosis could be seen as a phenomenon of oncologic screening [23].

In spite of all these experimental data from animal models that indicate the fact that probiotics have the potential to stop the development of CRC, systematic studies to show the same benefits in humans are very limited. In a human study about the prophylactic effect of Lactobacillus casei in CRC the conclusion was that two years of continuous administration significantly reduced the incidence of CRC as compared to control group [24]. Recently, in a cohort study that included 45000 subjects over a period of 12 years it has been noticed that large daily amounts of yoghurt reduced the incidence of CRC, suggesting a major prophylactic benefit from long term administration of joghurt [25,26]. Future clinical studies should consider, also, the standardization of probiotics composition, as well as the control of diet, time and frequency of probiotics administration. They should identify the proper biomarkers that may allow monitor of long term effects.

In conclusion, a large and significant pool of data indicates a major role of gut microbiota in CRC oncogenesis as well as in CRC prophylaxis. The majority of studies are, still, animal studies. Those and the few human studies are, never the less, highly suggestive. Unfortunately, we do not know the definition of „normal” composition of gut microbiota, yet. The mechanisms that link dysbiosis and CRC are complex and are related to local and systemic chronic inflammation. Some of the modifiable risk factors in CRC (obesity, diabetes mellitus, and western type of diet) may act, also, through alteration of gut microbiota. The prophylactic role of probiotics may render a powerful mean to significantly reduce the incidence of CRC in the future.

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ORIGINAL ARTICLE

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Diagnosis and management of cases with deep infiltrating endometriosis affecting the urinary tract

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Abstract: *Objective: The goal of this paper is to present the management of cases of deep endometriosis that affects the bladder, the ureters and the kidney.*

Materials and method: In this review we analyzed the sensitivity and specificity of imaging techniques for diagnosing deep infiltrating endometriosis, the optimal surgical technique and the surgical approach.

Results: In patients with pelvic endometriosis in advanced stages the urinary tract is frequently involved. Preoperative work-up of patients with deep infiltrating endometriosis is aimed at evaluating the extension of the lesions in order to assess the complexity of the intervention and to choose the optimal approach.

Conclusions: Laparoscopic and robotic management of these cases are good therapeutic alternatives. Ultrasound, sonovaginography with gel and MRI are useful imaging techniques for evaluating patients with deep infiltrating endometriois. The surgical management of patients with deep infiltrating endometriosis that involves the urinary tract consists of partial bladder resections, ureterolysis, ureteral resection and anastomosis and ureteral reimplantation..

Keywords: *bladder endometriosis, deep infiltrating endometriosis, ureterolysis*

INTRODUCTION

Endometriosis is a benign pathology of the female reproductive tract, characterized by the presence of endometrial glands or stroma outside the uterine cavity [1]. The presence of endometrial tissue at the level of the bladder will be stimulated during the menstrual cycle. In response to the cyclical stimulation of hormones throughout the menstrual cycle, some patients may even present with cyclic hematuria [2,3].

Bladder endometriosis can be defined as “primary” or “secondary”. Primary bladder endometriosis is represented by lesions associated with deep infiltrating endometriosis.

Secondary bladder endometriosis is represented by lesions that have occurred iatrogenically after pelvic surgery, caesarean section or hysterectomy [4,5].

The distribution of the lesions is multifocal, affecting the bladder trigone and dome from the serosa to the mucosa [6,7].

The ectopic endometrial tissue is subjected to stimulation during the normal cycle creating hormonal changes that result in

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bleeding, inflammation and adhesions [8]. The two main manifestations of this disease are chronic pelvic pain of varying intensity and infertility [9,10].

Table 1: The prevalence of urinary tract endometriosis [11]

Kidney	4 % of all urinary tract locations
Ureter	9 % of all urinary tract locations
Bladder	85 % of all urinary tract locations
Urethra	2 % of all urinary tract locations
Prevalence	0,3 – 12 % of all cases of endometriosis

DIAGNOSIS

The diagnosis of bladder endometriosis is based on the presence of ectopic endometrial glands and stroma located at the level of the detrusor muscle. Patients presenting with bladder endometriosis often present nonspecific urinary symptoms. In some cases, patients will present with dysuria, urgency to void, frequent micturition and abdominal pain [12,13]. The main aspect that dominates the clinical picture is exacerbation of these symptoms during the menstrual period [14]. Due to the fact that the symptomatology of bladder endometriosisis is nonspecific, many women will not seek medical attention in the first few years. The symptomatology can vary depending on the site and the dimension of the lesion [15]. The various degrees of abdominal or pelvic pain associated with bladder endometriosis will not prompt patients to seek additional work-up for this syndrome [16]. Thus, an average of 6 years will pass from the onset of the symptomatology to the diagnosis of endometriosis [17].

Urinary tract endometriosis can present with a non-specific symptomatology [18-20]. The presence of endometrial tissue at the level of ureteral orifices can produce hydronephrosis, with subsequent renal failure [21,22]. If the ureteral obstruction is acute, the symptoms may mimic a renal colic that requires a form of drainage [23-25].In chronic urinary obstruction, the lumbar pain can be absent and the renal insufficiency could appear like arterial hypertension, due to a bad regulation of blood pressure, thus involving the delay of a right treatment [26-28]. The medical history and accurate identification of the patient's symptoms can guide the clinician in choosing the correct imaging investigations based on the presenting symptoms.

Imaging diagnosis

Ultrasonography is the first line imaging investigation used in the work-up for patients with endometriosis. The bladder and the kidneys can be evaluated using transvaginal and abdominal ultrasound. The patient's bladder should be in semirepletion for a correct evaluation. (Figure 1)

Figure 1: Vesical endometriosis

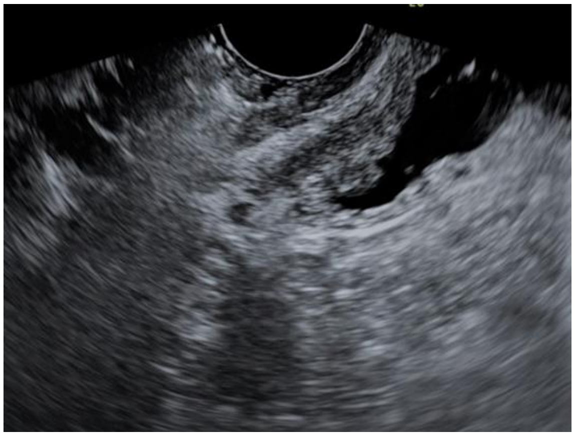

Abdominal ultrasound can be used in cases of suspected lesions of the bladder, abdominal wall and umbilical endometriosis [29]. During the initial work-up of a patient with deep infiltrating endometriois, an abdominal ultrasound must be performed to evaluate the impact on the ureters and kidneys. Endometriotic lesions can affect the ureter extrinsically or intrinsically. This can have an impact on the kidney causing hydronephrosis or ureterohydronephrosis. Any other causes of ureteral extrinsec compression must be excluded [30-33]. If surgical excision of the lesions is not performed this may affect the kidney leading to loss of function [19-25].

There are some authors that suggest that ureteral endometriosis should be suspected in all cases of deep infiltrating endometriosis [34].

Sonovaginography with ultrasound gel is a useful diagnostic option in cases with deep infiltrating endometriosis. This method is useful for identifying endometriotic lesions in the posterior compartiment as well as in the anterior compartment. An acoustic window is created by introducing 20 ml of ultrasound gel into the vagina. The technique initially used saline solution [35]. Using this technique, we observed a high diagnostic accuracy for the following locations (transvaginal ultrasound – TVS sonovaginography with gel – SVG) – Table 2.

Table 2. The diagnostic accuracy for transvaginal ultrasound and sonovaginography with gel in the anterior and posterior compartiment [36].

Endometriotic lesion	Ultrasound	Sensitivity	Specificity	PPV	NPV
Ovaries	TVS	94.8 %	95.6 %	92.5 %	94 %
	SVG	95.5 %	97 %	92.6 %	93 %
Urinary bladder	TVS	65.5 %	98.6 %	66.6 %	98.5 %
	SVG	67 %	97 %	98 %	98 %
Utero-sacral ligaments	TVS	69.7 %	95 %	84.2 %	87 %
	SVG	78.5 %	96 %	89 %	91 %
Vagina	TVS	58.5 %	99 %	94.3 %	95.4 %
	SVG	79 %	99 %	92 %	96 %
Recto-vaginal septum	TVS	64.8 %	99.4 %	81.5 %	97.8 %
	SVG	94 %	97 %	82 %	93.5 %
Pouch of Douglas	TVS	81.5 %	99 %	95 %	97 %
	SVG	81 %	98 %	91 %	97 %
Recto-sigmoid	TVS	91 %	97 %	95 %	96 %
	SVG	94 %	95.5 %	91 %	97.2 %

In the anterior compartment the transvaginal ultrasound and sonovaginography with gel have a similar sensitivity and specificity (80.1 % vs 81.2 % and 97.1% vs 97%). However, the positive predictive value is in favour of the latter (79.5 % vs 95.3 %). In cases of with deep infiltrating endometriosis the association between magnetic resonance imaging and computer tomography- based virtual colonoscopy has a sensitivity of 98.36 % for nodule identification. The association of these two investigations allows a superior mapping of the endometriotic lesions at the level of the posterior compartment. This aspect is important in the preoperative work-up and planning for the surgery [37].

TREATMENT

Medical treatment

Medical treatment using progestins, oral contraceptives and GnRH agonists can be used in the treatment of patients with endometriosis [38-40]. The contraindications of medical treatment in patients with urinary tract endometriosis are: evidence of the kidney being affected by hydronephrosis, the patient's desire and contraindications for the treatment [41,42]. The imunohistochemical aspects of patients treated with progesterone can be useful in choosing the optimal medical treatment after the surgical intervention [43,44]. The expression of the anti-apoptotic markers (Bcl-2), cell proliferation markers (Ki-67), estrogen and progesterone receptors offers anexplanation for the different outcomes after medical treatment for patients with endometriosis [45,46].

Surgical treatment

Surgical treatment of urinary tract endometriosis must be performed when the patient does not respond to medical treatment, if the patient suffers from infertility or if the lesion has an impact on the kidney (ureteral obstruction, hydronephrosis or uretero-hydronephrosis) [47,48]. Due to the fact that the obstruction of the ureter is due to fibrosis, patients will not respond well to medical treatment [49,50]. During the initial work-up and planning for the surgical intervention, the symptoms exhibited by the patient and the imaging investigations can guide the therapeutic conduct and anticipate the complexity of the case [51,52]. This is a key aspect in deciding the optimal intervention for the patient and anticipate whether a multidisciplinary approach is in order (gynaecologist, urologist and colorectal surgeon) [53,54]. The treatment for bladder endometriosis is represented by the surgical excision of the lesion.

Figure 2: Robotic ureterolysis (*Personal collection of Prof. Elvira Brătilă*)

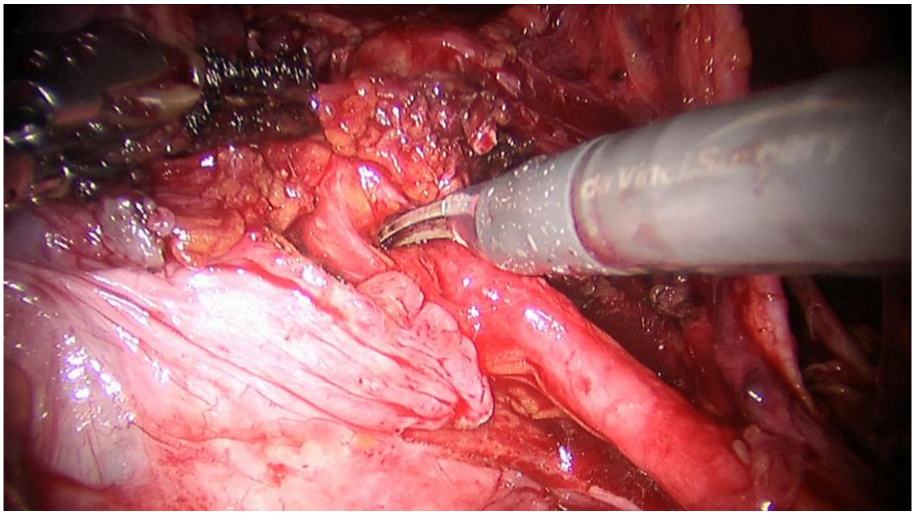

The surgical approach can be performed by laparotomy, laparoscopy or robotic surgery (Figure 2) [55-57]. The treatment for ureteral endometriosis consists of the surgical excision of the lesions involving the ureters in order to prevent ureteral compression (Figure 3).

Figure 3: Endometriotic lesion of that affected the ureter extrinsically at the level of the vesicoureteral junction (*Personal collection of Prof. Elvira Brătilă*)

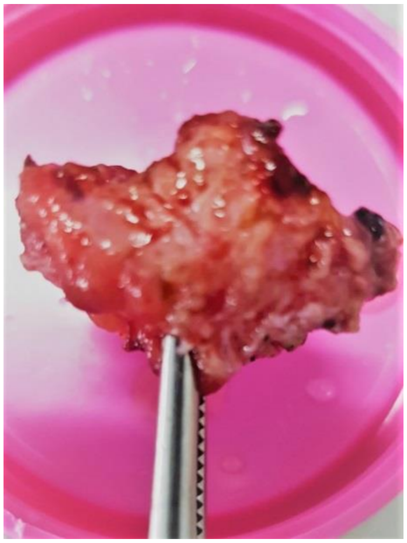

Ureterolysis is the first procedure performed when the lesion affecting the ureter is extrinsic. During the laparoscopic and robotic surgery, the transperitoneal approach way allows a thorough evaluation of the extension of the disease.

When the ureters are affected intrinsically, the ureteral segment can be infiltrated by the endometriotic lesions. In this case, a resection of the affected segment and reimplantation can be an alternative if ureterolysis cannot be performed [58,59].

After the completion of the ureterolysis (dissection of the tissue up to the level of the cardinal ligament), a thorough assessment of the remaining segment is required [60]. This must take into account the possibility of another obstruction of the ureter. The level at which the ureter is affected represents a factor for the intraoperative decision to perform ureterolysis, ureteral resection and anastomosis or ureteroneocystotomy [61,62]. If the lesion is located in the distal part of the ureter, the affected segment can be resected and the ureter can be reimplanted in the bladder. If the lesion is located in the upper third of the ureter, the affected segment can be resected and repaired with an uretero-ureteral anastomosis. The choice between ureterolysis and resection cannot be made preoperatively.

CONCLUSIONS

In patients with pelvic endometriosis in advanced stages, the urinary tract is frequently involved. Laparoscopic and robotic management of these cases is a good therapeutic alternative [63,64]. Preoperative work-up of patients with deep infiltrating endometriosis is aimed at evaluating the extension of the lesions in order to assess the complexity of the intervention and to choose the optimal approach [65,66]. Ultrasound, sonovaginography with gel and MRI are useful imaging techniques for evaluating patients with deep infiltrating endometriosis [67]. The surgical management of patients with deep infiltrating endometriosis that involve the urinary tract consists of ureterolysis, ureteral resection and anastomosis and ureteral reimplantation [68-70].

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The strategic importance of vaccination for national defense and security

Viorel Ordeanu[1]

Abstract: *Public health is the most important power factor of a nation, from which all other factors derive. Infectious diseases are the most important cause of morbidity and mortality in humans. Vaccination is the most important method of prophylaxis of infectious-contagious diseases. Mass implementation of vaccination prevents the occurrence of epidemics, epizootic diseases or homonymous pandemics, and at the individual level ensures the specific active immunity (total or partial). So the vaccination of the population and/or the risk groups is of strategic importance to the nation, both in peacetime and in time of war.*

The national antidote production along with anti-infectives (vaccines, therapeutic serums, immunomodulators, antimicrobial chemotherapies and pesticides) has a strategic importance for the health of the population and of the troops. Biological crisis situations are cases of force majeure, where the Medicines Law provides the exception for manufacturing and use, even without a "marketing authorization".

The military scientific research could make micro-production of specific drugs (antidotes, antiinfectives) for the needs of the army and the risk groups of the population. An example would be the preparation of vaccines for the prophylaxis of diseases caused by BWA and bioterrorism as niche products. Nothing hinders the preparation of any medication, such as magistral and galenical preparations, or pilot batch, provided there is adequate space, equipment, staff and procedures according to the law or the exception.

Keywords: *vaccination, public health, national defense, national security, military production*

INTRODUCTION

We live in turbulent times, and the current international context is volatile. Even in our east-westerly vecinity, hybrid wars, prolonged for many years and without the clear prospect of their cessation, take place. These exacerbate the risk for national defense. The social unrest, stimulated both domestically and internationally, amplifies the risk for national security. This context also causes direct or indirect risks to the population, and economic, social and other disruptions reduce the standard of living.

Not only does war and uprising implicitly cause a polytrauma epidemic, but it also profoundly affects the public health. Premises for epidemics (right at this time, the measles outbreak is happening in the EU) and epizootic diseases (right at this time, the African swine fever epizootic is happening in the EU) are created, extending as far as biological attacks, bioterrorism and bio-crime. And if we attempt to interpret the military-medical health situation in the

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country, we can think that the current epidemics are favored by the propaganda of the antivaccinists, whose hidden interests and their coordinator still remain unknown.

As far as the current epizootic is concerned, it is a viral disease for which there is no vaccine for prophylaxis or specific antiviral treatment, and the economic effects can be catastrophic. The distribution of outbreaks on the map shows that the virus has penetrated the country at the Hungarian border and spreads to the interior of Transylvania (a major producer and exporter of pork). A second wave followed, shortly after, at the Ukrainian border, extending to northern Dobrogea and eastern Bărăgan, gradually reaching Bucharest and the border with Bulgaria. Until now, over 1,000 outbreaks of African swine fever have been identified by Romanian veterinarians in domestic (farm or house) pigs in different places in the country, but not in wild boars who were initially believed to have carried the virus. Therefore, how did the disease come to exist and how does it spread? The breeders assume that through forages (including by industrial premixes); however, these are not a supportive environment for the preservation or multiplication of viruses. The only possible explanation would be the intentional contamination with this virus, thus, a likely new bioterrorist attack with an economic goal. But the hypothesis has to be confirmed with scientific evidence, by laboratory analysis and molecular epidemiology; otherwise, it shall only resemble the "conspiracy theories" partly projected by the press. Until now, the secret services have found nothing (being probably occupied with other issues) and the military medicine has not received samples for analysis, although it has advanced microbiological diagnostic capabilities and has the ability and expertise to test possible biological attacks.

THE PUBLIC HEALTH

The public health is the most important power factor of a nation (large or small), from which all other factors derive. In Romania, it was effectively supported by the territorial healthcare system introduced after World War I by Prof. Ioan Cantacuzino (at that time the Minister of Health), according to the most advanced Western model imported from France. The sanitary facilities monitored all people (not just citizens or policyholders) and all businesses in the assigned territory, so that no man and no square meter in Romania remained outside the sanitary system: prophylaxis, diagnosis and treatment. In parallel, the veterinary districts supervised domestic and wild animals. The County Health Directorates and Departments, as well as the Sanitary-Veterinary Directorates centralized the activity and reported it to the central authorities, to establish the necessary measures to be implemented, locally, nationally or internationally.

As an example, in the 1990s, the Medical Directorate of the Ministry of National Defence organized in Sinaia the first working meeting between Romanian and NATO military doctors. We presented the arrangement of the anti-epidemic surveillance in Romania, integrating human, military, veterinary, phytosanitary and environmental medicine, which now represents the One health concept. When we made the statement that "In Romania the anti-epidemic surveillance is not of sound quality..." we were interrupted by the American representative who stated that "It is our case as well!". However, we continued our idea as follows: "In Romania, the anti-epidemic surveillance is not of sound quality, but of very high-quality: we have 50 Health Directorates, 41 Sanitary Veterinary Directorates, 41 Agricultural Directorates, 41 County Environmental Inspectorates, 5 Medical University Centers, various Institutions and Central Laboratories, we are members of the World Health Organization, and the Cantacuzino Institute is part of the Pasteur Institute network, etc." The approval that came from the present audience closed the topic. However, in the meantime, the Sanitary Reform has altered some of the problem's data.

The public health was also effectively supported by the Romanian pharmaceutical industry by providing the national needs and exports of medicines, vaccines, reagents, parapharmaceuticals, pesticides and medical devices, to which the Cantacuzino Institute has contributed significantly since 1921. Until recently, persisted the moment of glory of the Romanian medicine where, with the existing forces and means and with reasonable funding, major progress was made in the public health sector, the eradication or quasi-eradication of infectious-contagious diseases, the decrease of infant mortality, the improvement of the quality of life, the extension of the duration of life and labour.

However, the public health in Romania has been partially undermined by the abolition of the territorialisation in primary healthcare and the drastic reduction of the pharmaceutical industry, in such a way that from the status of exporters of medication we have become importers, and the medication crisis is current. Although the new Constitution of the country still provides for the country's obligation to ensure the public health, this role has been significantly reduced. A system where the health is highly expensive and the foreign investors are exaggeratedly gaining through medical insurance and trade in medicines and pharmaceuticals. However, the results of medical assistance do not seem to be superior to those of previous years, and even in the field of infectious and contagious diseases there is a worsening of the situation, and the press publicly presents this situation. At times, the doctors are being tried as natural persons as an explanation for the inefficiency of the current healthcare system and sanitary reform. Overall, the increase in costs has not been accompanied by an increase in efficiency, and public health is declining, according to statistics from the National Institute of Statistics.

VACCINATION

Infectious diseases are the most important cause of morbidity and mortality in humans. Diseases caused by parasitic living beings (bacteria, viruses, prions, fungi, protozoa and parasitic animals) have prevailed since ancient times and are still the most common in underdeveloped countries. But also in developed countries (including Romania), where these diseases are officially ranked third (after cardiovascular diseases and cancers), there are many diseases that actually have a known infectious origin (such as gastric and duodenal ulcers, which are caused by a bacterium, or some cancers caused by certain viruses, etc.) or not yet known (unidentified bacterial endocarditis, supposed infectious dementia etc.); thus, these are equally infectious diseases.

Vaccination is the most important method of prophylaxis of infectious and contagious diseases. The massive application of vaccination prevents the occurrence of the epidemic, epizootic or homonymous pandemic, and at the individual level ensures specific active immunity (total or partial). Therefore, the vaccination of the population and/or the risk groups is of strategic importance to the nation, both in time of peace as well as in time of armed conflict.

The history of vaccinology begins in the Middle Ages, with the empirical immunization and continues in the Modern Age with scientific immunization. The first mass vaccinations were made in the 1800s, when Napoleon ordered the compulsory vaccination of the Great Army (hundreds of thousands of soldiers) as prophylaxis in military campaigns. From antiquity to the contemporary era, many battles and even wars have been lost due to epidemics that have weakened the combatants' ability to fight. Prof. N. Iorga once said "read history to know what shall come" [10]; and, the history of medicine is a component of history and partially overlaps with military history. It is not the intention of this article to present a history of vaccinology in general, so we will limit ourselves to the chronological presentation of the vaccine production in Romania and the national development of vaccination as a medical protection against infectious diseases.

In the last century, the appearance of sulphamides and antibiotics led to antibacterial chemotherapy which, along with vaccination and hygiene, has rapidly improved the public health to its current level and the population of the planet is growing exponentially. It is the most important demographic success of the human species, and it is mainly based on the advances of medicine in regards to vaccinology and anti-infective therapy. Vaccinology saved more lives than surgery.

After the discoveries in biology of the chemist Pasteur, the founder of modern microbiology, Romanian scientists were also attracted by this field. Victor Babeş studied in the West and published in French the first microbiology handbook in the world. Ioan Cantacuzino worked at the Pasteur Institute in Paris, under the guidance of Prof. Mecinikov, and returned to Bucharest as a professor at the Faculty of Medicine, where in the Experimental Medicine Laboratory (which he created in 1901) prepared sera and anti-infective vaccines. These were successfully used for the protection of the population and the Romanian Army during the Balkan Wars (he was also the Head of the Army's Sanitary Service, ranked as Colonel) and during World War I, including the "great Romanian experience", during which he ended the terrible plague epidemic, and later (in 1921) the Institute that was named after him was founded, and was led by him until the moment of his death in 1936.

Prof. Al. Slatineanu, along with ŞL C-tin. Ionescu Mihăeşti and Dr. Mihai Ciucă ("laboratory assistant for serum preparation", the future professor and director of the Cantacuzino Institute), published in 1915 the first Military Epidemiology Handbook: "Notions in epidemiology and serovaccinations (with special application in the campaign)", The laboratory of experimental medicine, Bucharest, State Printing House, 95 p. [12]. This article is based on the medical experience during the Balkan Wars, which has been useful during the First World War, when the Romanian Army faced major epidemics. The four vaccines produced at that time are also presented here: the anti-vitiligo, anticholeric, anti-plague and antivarioulous vaccine.

In 1939, Prof. C. Ionescu-Mihaesti and Prof. M. Ciuca, together with 27 specialists from the Cantacuzino Institute, published the first "Handbook of infectious disease with prevention concepts, serotherapy and vaccinations, drawn up by the Cantacuzino Institute", the Foundation for literature and art "King Carol II", the National Printing House, Bucharest, 1940, 313p. [9]. The work is based on the World War I medical experience and the advances in inter-war medicine, being useful in the Second World War, when the Romanian Army experienced no more epidemics. The main infectious and contagious diseases, 18 vaccines, 16 therapeutic sera, 6 in vivo reagents, immunotransfusion therapies, with bacteriophages, with sulfamides, etc., are presented in a total of 43 biological drugs. For comparison, “Formulaire ASTIER, 9-e Edition, Vade-mecum de Medecine Pratique”, Paris, 1942, 1306 p (representing the most advanced medicine of the time) describes 21 vaccines, 19 therapeutic sera and some biological therapeutic methods. [1]. Romania was at a European level even before being a member of the European Union.

In 1944, the Sera and Vaccines Institute "Dr. I. Cantacuzino" publishes the "Guidelines for the Use of Sera and Vaccines, 1944 Edition", Bucharest, George Ionescu – Pharmacist, 143 p. [3]. The work could be considered as the first handbook of vaccinology in Romanian. There are 21 vaccines, 13 therapeutic sera, 4 bacteriophages, 14 biological reagents, 2 biological dressings, a total of 54 biological drugs. During the same year, appears the second edition of the Handbook of Infectious Diseases, 320 p. [9], based on the experience of the World War II. 23 vaccines, 17 therapeutic sera, 4 bacteriophages, 6 biological reagents, the immunotransfusion and sulfamido-therapy are presented.

Prof. C. Ionescu-Mihaesti and Prof. M. Ciuca, as students and followers of Prof. Ioan Cantacuzino, who further developed the Institute, published in 1945, in English, the first International Handbook on Infectious Diseases under the auspice of the League of Nations: “League of Nations. Handbook of infectious diseases with notes on prophylaxis, serum treatment and vaccination” by the Staff of the Cantacuzene Institute, under the direction of Professors C. Ionescu-Mihaesti and M. Ciuca” Series of League of Nations, Publications III Health, Geneva, 1945. III. 1. 331p. [8]. The handbook also addresses the field of immunological medicinal products: 23 preventive and therapeutic vaccines, 19 therapeutic sera, 10 diagnosis sera, phage therapy, immunotransfusions, sulfamidotherapy and, for the first time in a handbook, antibiotherapy – in chap. IX Anti-biotic products of microbic origin (fungi, bacteria).

After the regime change, "R.P.R. Ministry of Health, Institute of Sera and Vaccines Dr.I. Cantacuzino, Guidelines for the Use of Sera and Vaccines" State Printing House, Medical Literature, Bucharest, 1949, 144 p. [2]. The work describes 11 vaccines, 4 anatoxins, 7 therapeutic sera, 2 bacteriophages and 17 biological reagents, a total of 41 anti-infective biological products. In 1952, the Ministry of Health and Social Welfare publishes the "The Prophylaxis of communicable diseases", re-published in 1961 [4] etc. In 1962, MSPS publishes "Vaccines, Sera and Biological Diagnosis Products", 227 p. which features 87 assortments. [7]

After the military-political shock of 1968, the "Dr. I. Cantacuzino" Institute is revitalized and publishes "Vaccines, serums, biological diagnosis products, culture media and laboratory animals" Medical Printing House, 1969, 285 p., which describes 251 biological products, of which 18 vaccines. In total, from 75 assortments in 1947, 340 were produced after 1969. [5].

However, after the 1989 Revolution, the Cantacuzino Institute gradually lost its importance, and gave up a part of its staff (by restructuring, retirement, relocation, unemployment, etc.), its External Departments of the province (Iaşi, Timişoara, Dârvari etc.), part of its portfolio of anti-infectives and microbiological diagnosis products. According to the latest INCDMI "Cantacuzino" Product Catalog for 2009, of the 802 products previously in the portfolio were produced 13 vaccines, 2 therapeutic sera, etc. and in 2013 only one vaccine (flu), no therapeutic sera and only one part of the other microbiological products. [6] Then followed: the financial crisis, the cancellation by the MS/ANMDM of production authorizations and the crisis of authority and competence, which led to the complete cessation of the production of vaccines and therapeutic sera. There was a need for drastic measures to relaunch the production of biological medicine, including the takeover by the Ministry of National Defence, so that the Cantacuzino National Military-Medical Development Research Institute could be run by the military and concrete results are expected.

The evolution of the production of vaccines and sera for human use in Romania (incrementa et decrementa), exemplified by specialized medical publications, shows the following dynamics: 1901 = 0; 1915 = 4; (1921 establishment of IC); 1939 = 34; (1942 = 40 in France); 1944 = 40; 1945 = 42; 1949 = 22; 1969 = 25; 2009 = 15; 2013 = 1; 2014 = 0; and currently = 0, but there are good prospects.

During those periods of production, vaccinations were free of charge (material and labor) and compulsory, according to the Annual vaccination schedule of the Ministry of Health for the civilian population, including children, of the Ministry of National Defence for the Military and of the Ministry of Agriculture for animals.

The safety of vaccinations

The Romanian production of sera and vaccines for human use (with circulating strains in the country) has protected Romanians from infectious-contagious diseases and epidemics for 113 years (armed with between 100-500 thousand soldiers and populations between 11-22 million people), also achieving a very rich vaccination experience, with no major medical incidents.

The testing base on millions of people of all ages and over a century provides a large-scale epidemiology and pharmacovigilance study confirming the effectiveness of vaccinations and the quality of vaccines prepared by the Romanian Pharmaceutical Industry, primarily at Cantacuzino Institute. It would be interesting to have a retrospective scientific research on vaccination in Romania that would quantify the data objectively and extract the appropriate conclusions. When the theory, or the Law of Medicine, does not fit the practice, the theory should be corrected (if there are no hidden interests).

Vaccinology is an important concern of global medical research, of the epidemiology and the pharmaceutical industry; new vaccines, new regulations, new forms of administration have been created worldwide, but we still do not have vaccines for all infectious-contagious diseases. The antivaccinist current is also added to the existing challenges, which, out of ignorance or bad faith, opposes vaccination for various reasons: the fear of supposed adverse effects, the desire to let nature take its course, the belief that sickness and health come from God, the antisocial attitude, financial interests etc. Some adverse effects exist with varying degrees of severity, but are very rare and the risk/ effectiveness ratio is considerably favorable, particularly in social terms. No medication, including the vaccine, is granted the "marketing authorization" if it does not meet the Efficacy and Safety conditions set by the drug legislation. The World Health Organization (WHO), as a specialized UN forum, recommends the necessary vaccinations anywhere in the world and at any time, and national authorities comply, but sometimes only within the limits of financial possibilities, through self production and/or imports.

WHO List 2017 Essential Medicines Vaccines Section: BCG, Diphtheria, Hemophilus Type B, Hepatitis B, HPV, Measles, Pertussis, Pneumococcal, Polio, Rotavirus, Rubella, Tetanus, Japanese Encephalitis, Yellow Fever, Tick-borne encephalitis, Cholera, Hepatitis A, Meningococcal, Rabies, Typhoid, Influenza (Seasonal), Mumps, Varicella.

NATO's List of Biological War Agents (BWA) in conformity with STANAG 4632:

- Bacteria Bacillus anthracis, Yersinia pestis, Francisella tularensis, Vibrio cholerae*, Brucella melitensis, Burkholderia mallei, Coxiella burnetti;
- Viruses VEE virus, Ortopox virus, Yellow fever virus*
- Toxins Ricin, Saxitoxin, Botulinum toxin, SEB, T2 mycotoxin

Note: Only the two BWAs marked with * are found in the WHO List, meaning that the civilian medicine is not particularly interested in the biological warfare; therefore, implicitly, this task rests with the military medicine.

The European Medicines Agency has published the List of Biological Agents Usable in Bioterrorism (equivalent to CDC USA): EMA BA 2002, 2007 and 2014, presenting in summary: the disease, treatment and prophylaxis. It should be noted that for some diseases there is treatment, for others there is no "recommended treatment", for some diseases there is a vaccine (which is or is not available), for others there is no vaccine, and is or is not undergoing research/ authorization. An example could be the recent Ebola epidemic, a disease caused by a long-known virus, but in the epidemic still did not exist: a diagnostic reagent, a prophylactic vaccine, and no specific antiviral treatment. Only the existence of the epidemic has prompted scientific research to produce them. As a result, the international collaboration has had unsatisfactory results, and additional intervention by a U.S. Army Medical Brigade, which applied general medical countermeasures combined with military discipline to enforce anti-epileptic precautions, was needed.

The niche production for vaccines of military-medical interest (WBA + Bioterrorism)

The military-medical scientific research also aims at maintaining health and combat and labor capacity (troops and population) as well as their recovery in the case of common or specific illnesses (eg CBRN). The Center for Scientific Military-Medical Research (CCSMM), established in 1967/1968/1969, permanently collaborated with the Cantacuzino Institute (1921/2017), the national producer of serums and vaccines, for the medical protection against biological weapons, including for vaccinology (the Dermojet automated injector, lyophilized intradermal vaccines, specific tests, etc.).

Medical countermeasures are based on: diagnosis, prophylactic treatment, pre-, intra- and post-exposure prophylaxis, recovery treatment for combat, work or life. It is well known that the best military recovery results for the battle were obtained by the German army (on the principle of phased evacuation), and the best recovery results for labour or life were obtained by the US army (on the principle of evacuation by destinations). In fact, the two principles of evacuation of sanitary losses apply in different situations of conducting military operations, namely classical front or expeditionary actions.

The effect of the disseminated biological agent may differ depending on many factors and their association: pathogenicity, virulence, toxigenesis, resistance to the environment, resistance to treatment, unprotected population, specifically non-vaccinated, without pre-exposure prophylaxis, without intra-exposure prophylaxis, without post-exposure prophylaxis, without anti-infective treatment, cases of illness and outbreaks, as well as sanitary-pharmaceutical logistics: forces, means, money, etc., the result being expressed in terms of financial and social costs.

The national antidote production along with antiinfectives (vaccines, therapeutic serums, immune-modulators, antimicrobial chemotherapies and pesticides) has a strategic importance for the health of the population and of the troops. Biological crisis situations are cases of force majeure, where the Medicines Law provides the exception for manufacturing and use, even without a "marketing authorization". Public health must be an obligation of the state towards its citizens and not a business of producers or importers.

The military scientific research could make micro-production of specific drugs (antidotes, antiinfectives) for the needs of the army and the risk groups of the population. An example would be the preparation of vaccines for the prophylaxis of diseases caused by BWA and bioterrorism as niche products, in order to avoid the competition of the large multinational pharmaceutical producers, whose turnover exceeds the state budget of Romania. Nothing hinders the preparation of any medication, such as magistral, galenical preparations, or pilot batch, provided there is adequate space, equipment, staff and procedures, according to the rules: of good pharmaceutical practice, good manufacturing practice (GMP), good laboratory practice (GLP), good practice for safety check and the exception of force majeure. [11]

CONCLUSION

Vaccination is very important for national defence and security. Vaccine production started in 1901, developed since 1921, had a glory period before the financial crisis of 2009 (regardless of the regime), went into decline and is currently only declared intent. The military-medical scientific research could resume the production of vaccines, complying with specific conditions, especially as niche production, possibly through national and/or international partnerships. The vaccines production must continue.

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ORIGINAL ARTICLES

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The role of metatarsophalangeal joint arthroscopy in hallux rigidus treatment: technique and early results

Ieronim O. Crișan[1]

Abstract: *Introduction: The hallux rigidus is a term that signifies a mobility limitation of the metatarsophalangeal joint of the big toe due to osteoarthritic degeneration. Several factors are incriminated in the appearance of this pathology but without being able to establish the cause. The cartilaginous erosion of the metatarsal head and / or the base of the phalange, the dorsal osteophyte of the head of the first metatarsal were found during cadaveric studies. The main reason for consultation is pain and limitation of the dorsiflexion of the big toe during the step.*

Hypothesis: Arthroscopic debridement of the first metatarsophalangeal joint improves the early postoperative clinical score compared to isolated percutaneous treatment.

Material and method: 11 patients divided into 2 groups were assessed clinically at 3 months postoperatively. All patients were hospitalized on an outpatient basis.

Results: There was a gain in mobility at 3 months compared to the preoperative assessment in both groups, as well as an improvement in pain without being able to demonstrate the superiority of the additional arthroscopy to the percutaneous debridement. Hypothesis reversed..

Keywords: *hallux rigidus, orthopedics, arthroscopy, osteophyte*

INTRODUCTION

Pathogenesis

Hallux rigidus is a term invented by Cotterill in 1888, refers to a limitation in mobility of the metatarsophalangeal joint of the hallux. Although he did not call it hallux rigidus, in 1887 Davies-Colley reported for the first time the resection of the base of the first phalanx for this pathology, which he called hallux flexus because of the flexion attitude of the metatarsal-phalangeal joint and the limitation of extension of this joint. Moreover, the understanding of this pathology has advanced thanks to radiological techniques, but the pathogenesis of the hallux rigidus is not yet defined.

The evolution towards the aggravation of the destruction is on the other hand well known. The cartilagenous lesion is supposed to initiate synovitis, which itself supports cartilage destruction, accompanied by osteophyte formation and subchondral bone involvement. [1]

The degenerative process can begin in adolescence when a traumatic event at the metatarsophalangeal

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[1] Centre Hospitalier Colmar and Guebwiller, France

joint damages the articular surface of the head of the first metatarsal. Repetitive strain injuries can also cause cartilage damage. Other causes are mentioned: osteochondritis dissecans of the head of the first metatarsal secondary of an osteochondral fracture at the level of the dorsal convexity of the articular surface, the hyperextension of the first metatarsal, an excessive length of the first metatarsal and a severe pronation of the foot. The hallux rigidus of the adult is most often caused by degenerative arthropathy or osteoarthritis of the metatarsophalangeal joint, while in adolescents the hallux rigidus results from a localized cartilaginous lesion of the head of the first metatarsal most post traumatic time [1,2]

A system for classifying the severity of hallux rigidus takes into account passive mobility, pain, and radiographic assessment to assign a stage from 0 to 4. This classification of Coughlin and Shurnas has been reported to be reliable in predicting the outcome of surgical treatment. [1]

Despite the fact that the metatarsus primus elevatus (dorsal positioning of the first metatarsal on a lateral X-ray in load of the foot) has been incriminated as a primary cause in the pathogenesis of hallux rigidus, this was not proved. No association was identified between hallux rigidus and primus elevatus, hypermobility of the first ray, the first long metatarsal and stiffness of the Achilles tendons, abnormal posture of the foot, symptomatic hallux valgus, shoes or professional occupation. It is more frequently associated with hallux valgus interphalangeal, bilateral involvement in patients with family history, unilateral involvement in patients with traumatic history, female gender. [2]

McMaster reported that the most common finding in seven patients with hallux rigidus was cartilaginous cleavage at the head of the first metatarsal without subchondral bone detachment and that the earliest radiological change was a small depression in the dome of the first metatarsal head. The cleavage is always between the top of the dome and the dorsal edge of the articular surface. McMaster believes that the exquisite pain and dorsal range of motion limitation can be explained by the osteophytic lesion on the dorsal metatarsal head. When hallux is in extension, the arrival of the first phalanx against the metatarsal cleavage site produces pain and reflex flexion of the joint, limiting extension. Then the disease progresses, an osteophyte at the level of the dorsal articular margin of the head of the first metatarsal produces a stop during great toe extension. This is the initial image of the patient: osteoarthritic changes in or around the metatarsophalangeal joint of the hallux. Often the first metatarsal is in the dorsal forcing position, decreasing its angle of plantar inclination on the profile X-rays (primus elevatus). This elevation of the first metatarsal is rather secondary to metatarsophalangeal osteoarthritis than the cause of this one. [1,2,3]

Clinical

The main reason for consultation is the progressive onset of pain and the limitation of the articular amplitudes of the hallux which is either fixed in plantar flexion or limited in dorsiflexion due to osteophytes present around the head of the first metatarsal, especially on the dorsal surface. [1]

This is not the case on the plantar surface where there is a normal mobility in flexion. The severity of the degenerative changes is directly dependent on the duration of the symptomatology. [2]

Muscular cramps in the extensor hallucis long tendon without mechanical conflict can be found early in the course of the degenerative process. Gradually the hallux becomes fixed in plantar flexion and the patient is forced to walk on the outer face of the foot. [2]

Unilateral involvement is the most common despite Nilson who postpones bilateral involvement in 60% of his cases. The aesthetic factor (the intolerance of wearing shoes in the heel) gives a preponderance to the female sex. Although Smith reported small series of older patients suffering from hallux rigidus, in the literature the age of the first symptoms were adolescence. [2]

Radiological characteristics

The flattening of the head of the first metatarsal, the joint narrowing, the presence of osteophytes of the dorsal, medial and lateral faces of the metatarsal head and the base of the phalanx, subchondral sclerosis and the formation of cysts at the level of the head of the first metatarsal are common elements in the elderly population. Sometimes the proximal phalanx is held in plantar flexion due to posterior conflict, and stress images can confirm that the osteophyte block prevents extension of the metatarsophalangeal joint. An osteochondral lesion is occasionally observed in the metatarsal head in adolescents according to Goodfellow and McMaster. [1,2]

Conservative treatment

For most patients, surgical correction is necessary to relieve pain and improve function; however, Yee and Lau, in a review of the hallux rigidus literature, have determined that non-operative measures such as foot orthotics, shoe modifications, and corticosteroid and hyaluronic acid infiltrations prior to surgery should be considered. [2]

Surgical treatment

Despite several recommended surgical procedures for the treatment of hallux rigidus, (including the dorsal subtraction osteotomy of the base of the first phalanx or the distal portion of the first metatarsal, the Keller operation or metatarsophalangeal arthrodesis) no procedure has proved its superiority. The indication for the metatarsophalangeal arthrodesis is the articular narrowing objectivized not only on the frontal and lateral incidences but also on the oblique incidence which shows that the remaining articular space is absent on the frontal and lateral incidences. Modern fixation techniques using compression screws or dorsal plates result in high melting rates (between 94% and 98%) and acceptable clinical outcomes for patients.

Gait studies showed significant improvements in propulsive force, foot load, and walking stability after arthrodesis for hallux rigidus. [2]

The justification for metatarsophalangeal debridement is the pain relief due to mechanical conflict suppression between the dorsal osteophyte of the first metatarsal and the base of the phalanx and the boot. The debridement is performed by resection of the osteophyte and other bone growths on each side of the joint with the aim of a dorsal flexion of the hallux of at least 70°. The success rate varies between 56% and 92%, with better results for stage 1 and 2 and for patients over 60 years of age. The gait analysis showed that there is an overload in the lateral part of the head of the first metatarsal after joint debridement and that these changes can lead to future joint degeneration. [1,2]

The modified Keller procedure (arthroplasty-resection), in which the short extensor hallux tendon and the capsule were used as interposition materials, has been described for the treatment of severe hallux rigidus, but the results vary.[2]

Interposition arthroplasty was also modified by oblique resection of the proximal phalanx. [2]

Silicone-rubber interposition arthroplasty was a short term solution because of silicone synovitis. The hemiarthroplasty of the proximal phalanx with a metallic component was also described, with good results, but there are no long-term studies to evaluate this technique. A study of 23 hemiarthroplasties reported that 91% of patients were satisfied with the results at 5.7 years and that the scores of the American Society of Foot and Ankle were significantly improved. [1,2]

Arthroscopic treatment

The first author to describe the arthroscopy of the hallux was Watanabe in 1972. The procedure gained clinical importance in the 1990s when several authors described the technique. The advantage of arthroscopy at this level is to have a fast recovery and professional reintegration, reduction of postoperative pain, less scar problems and a mobility and strength of the metatarsophalangeal satisfactory. [3,4,5]

The working hypothesis: Arthroscopic treatment improves mobility and decreases pain compared to isolated percutaneous debridement under image intensifier.

MATERIAL AND METHOD

The study is retrospective.

The inclusion criteria was the hallux rigidus stages 1 and 2, without limitation of the walking perimeter.

The exclusion criteria was the diabetic foot and the neurological foot.

Results evaluation was simple based on subjective pain (0-10 where 0 signifies absence of pain and 10 signifies insupportable pain) and local range of motion (hallux extension).

Between November 2015 and March 2016 at Centre Hospitalier Colmar and Guebwiller (France) 11 patients, 6 women and 5 men were undergo surgical treatment of hallux rigidus. Seven patients underwent arthroscopy debridement (group 2) and 4 patients underwent isolated percutaneous debridement (group 1), among them 1 patient had to have a bilateral hallux rigidus surgery at 5 months interval. Patients were evaluated at 3 months postoperatively.

The main symptom was the pain of the hallux when walking, and the additional assessment includes standard loading X-ray of the front foot, anterior-posterior, lateral-lateral and three quarters (figure 1).

Figure 1: Hallux rigidus, stage 2, presence of the dorsal osteophyte on the lateral and three quarters X-Ray

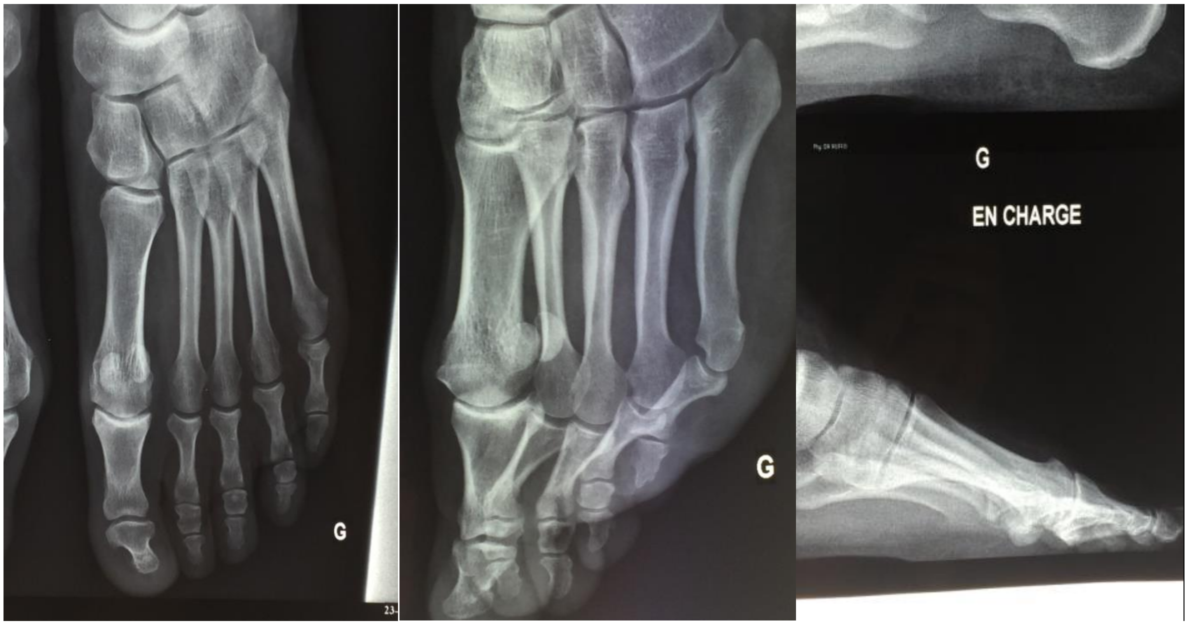

Percutaneous articular debridement was accomplished using a specific Shannon TM drill 2.0x8 millimeters under image intensifier within regional anesthesia, no tourniquet needed.

Metatarsal-phalangeal hallux arthroscopy was performed under regional anesthesia (popliteal block) with a calf tourniquet in all patients.

The patient is placed supine, heel in the vacuum, with a pad under the ipsilateral buttock to ensure a strictly vertical position of the foot. The surgeon is positioned at the end of the table, scrub nurse left of the surgeon (left-handed surgeon), arthroscopy column next to the patient's head (figure 2).

Hallux flexion-extension movements helps identifying metatarsophalangeal space. A needle is placed in the joint, laterally or medially to the long extensor tendon of the great toe, image intensifier serves in checking the correct intraarticular positioning. To avoid dorsal sensory branches damage the incision is placed slightly plantar affecting the skin only, while a Kelly or Halsted forceps is used for deep dissection. To prevent cartilage damage a small cannula is introduced. A 2.4 mm scope is used and placed preferably postero-medial while the instruments are placed postero-lateral. In doubt X-Ray checking is desirable to confirm the correct intra-articular instruments positioning.

Articular distraction gap is performed by a “Japanese fingerstall [3]” or by pulling out the hallux with a Velpeau band (figure 3). By pulling the toe with the hand and placing the probe within the joint, the anatomical structures can be explored during flexion-extension movements (sesamoid and head of the first metatarsal) (figure 4).

Figure 2: Patient positioning, the hardware and the operating room display. Artisanal traction by Velpeau band.

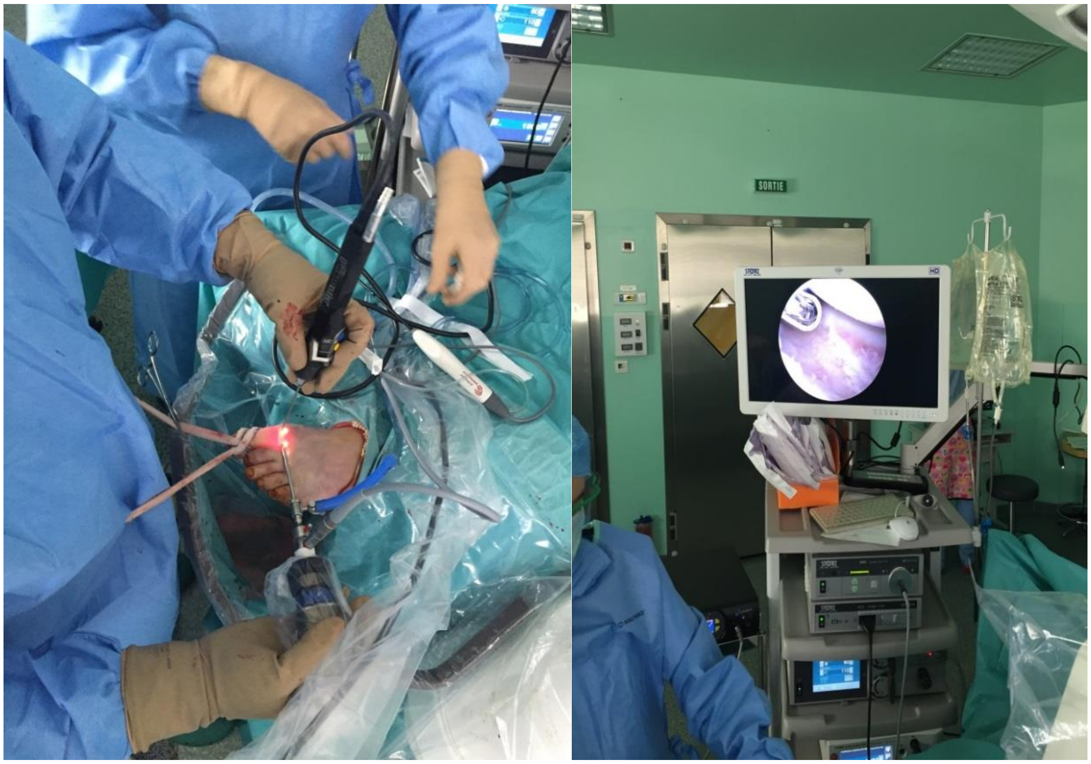

Often the posterior synovium and the lateral and medial gutters must be cleaned along using a Shaver TM to obtain a good visualization of the joint, paying attention to the extensor tendon of the hallux (figure 5). [3] [4] [5]

Figure 3: Hallux arthroscopic approach

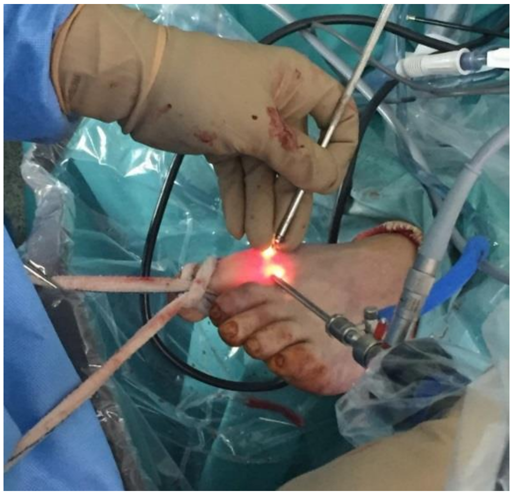

In first and second stages, a cartilage lesion is usually found centrally or dorsally on the first phalanx or on the dorsal metatarsal head, while the subchondral bone remains intact (Figure 6).

Figure 4: The medial sesamoid

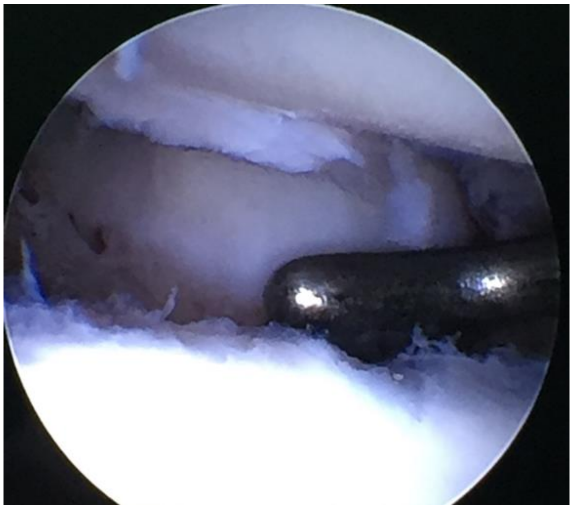

Cartilaginous valves are regularized with a curette and the debridement and osteophytes resection continues with the shaver or radiofrequency probe. If the subchondral bone is exposed, micro fractures by a 15 millimeters diameter Kirschner wire are produced to stimulate neo cartilage induction.

Figure 5: Hallux extensor tendon

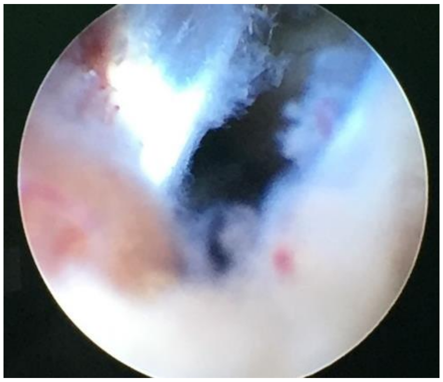

Figure 6: Cartilaginous valve and head of the metatarsus

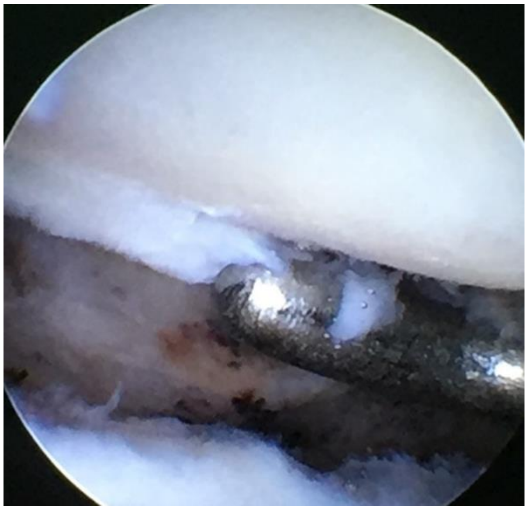

The pre- and postoperative dorsiflexion are measured by laterally X-Ray checks, allowing us to note in the operative report the mobility gain (figure 7).

Figure 7: Left image: 45° of dorsal flexion on preoperative X-Ray; right image: 80° dorsal flexion of the great toe on postoperative x-ray

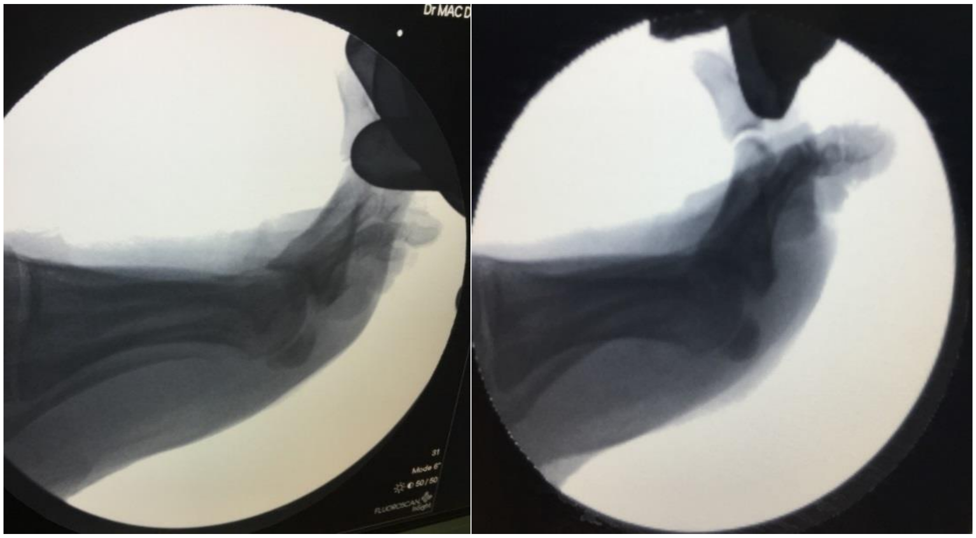

At the end of the procedure, the release of the tourniquet enables bleeding control, the skin is stitched with resorbable sutures, and a compressive dressing is realized.

Postoperatively walking without crutches is allowed the next day and the wearing of a medical soft padded shoe is recommended for the next two weeks.

In all cases, patients needed one day hospital stay.

RESULTS AND DISCUSSION

The average age for group 1 was 53.2 years and 56.8 years for group 2.

The preoperative mobility for group 1 was 26° of extension and 17.5° of flexion on average; for group 2 the hallux extension was at 47° for 15° flexion. At the end of the procedure, the dorsiflexion obtained was at least 80°. The average duration of the percutaneous and arthroscopic procedure was 64 minutes, no adverse events were reported.

Operative follow-up was simple with minimal residual pain (VAS = 1) for both groups and one return to work at 3 months with one exception, 1 patient who had 4 months off work due to persistent pain (VAS = 4).

At 3 months of the operation the group 1 had 77° extension of the hallux for 8° of flexion while for the group 2 one notes 85° of extension for 15° of flexion. In terms of increased mobility at 3 months, arthroscopy has not yet shown superiority over isolated percutaneous articular pruning, but in terms of subjective satisfaction, group 2 reported better results in terms of pain. Only one patient underwent a bilateral pruning, and he declared himself more satisfied on the side operated by arthroscopy plus percutaneous pruning than by the side operated by isolated percutaneous pruning.

Our working hypothesis was therefore invalidated.

Weaknesses of the study: retrospective study with a small series of patients, no long-term results.

The literature is poor in this field, no comparison was made.

CONCLUSION

The arthroscopy of the hallux makes it possible to achieve an accurate joint assessment and a suitable treatment. In our very short experience and to confirm the arthroscopic debridement and the synovectomy complementary to the percutaneous pruning improves the subjective score of the treatment of the early hallux rigidus, but it remains to define the result and in particular the gain of mobility at a distance and at to quantify the progression towards osteo-phalangeal osteoarthritic degeneration.

Abreviations

VAS: Visual Analog Scale

Acknowledgements

There are no conflict of interest.

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Women's facial attractiveness nowadays – results from analysis of public figures

Cristina T. Preoteasa, Sabina Iordache, Marina Imre, Paula Perlea, Ana Maria C. Tancu, Elena Preoteasa

Abstract: *Aim. To identify the main facial features associated to facial attractiveness in women nowadays.*

Method. A descriptive study was conducted on a sample of 20 female public figures, very famous and successful women, most frequently actresses, singers or models, considered very beautiful nowadays. Their selection followed the analysis of several recent tops that aimed identifying most beautiful women, in which raters were lay persons. Data was collected by analyzing photos from frontal and lateral view, during rest position and smiling.

Results. The persons analyzed registered diverse facial traits. Most obvious trends, encountered in more than 75% of them, included: Caucasian race; straight and long hair; average nasal tip protrusion; maxillary dental midline concordant to facial midline; a thicker lower lip compared to the upper lip; exposure of only maxillary incisors during smile; decreased or absent buccal corridor; maxillary central incisors of oval/rectangular shape; absence of maxillary teeth crowding. Other frequently encountered features were: American nationality; brown hair; eyes with medium size, almond shape, wide set, of green color; nose with an average width; face with a decreased lower third and a slightly convex profile; a thick lower lip and an average upper lip; exposure during smiling till the first molar, with a normal smile line.

Conclusions. The public beautiful women nowadays register features that are generally associated to an increased facial attractiveness. Knowledge of trends in facial beauty is useful in dental practice, in order to understand better patient's need in terms of esthetic expectations.

Keywords*: esthetics; beautiful; dentistry*

INTRODUCTION

Nowadays, in the modern society physical appearance and facial esthetics play an important part in communication in everyday life, which associated changes of patients' perception on various aspects related to them, including dental treatments. Exposure to mass media (television, internet, movies) influenced perception of human attractiveness, which registered in time changes, some of the considerable. Consequently, more and more in the dental field preoccupations that target providing esthetic outcomes can be observed. In this context, the dentist must know and understand what is considered attractive nowadays, in order to

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explain to the patient what are the possibilities and limits of the interventions provided by him. The diversity of clinical situation require dental treatment individualization, and a specific plan in order to enhance facial attractiveness. Considering beauty has a biological inherent dimension, each treatment should be adapted according to ones features. One particular intervention that had a good outcome from an esthetic point of view in one person, does not necessary have a similar good outcome in another.

The aim of this study was to identify the main facial features associated to facial attractiveness in women nowadays.

METHOD

A descriptive study was conducted on a sample of female public figures, very famous and successful women, most frequently actresses, singers or models, considered very beautiful nowadays. Their selection followed the analysis of several recent tops that aimed identifying most beautiful women nowadays, in which raters were lay persons that voted online, of different ages and nationality. In the study sample was targeted to be included 20 women, most frequently encountered in these tops, as being considered very beautiful and attractive.

Data upon facial characteristics was collected by analyzing photos from frontal and lateral view, during rest position and smiling. Considering the inability to personally examine the subjects, an extensive search was conducted in order to identify as many photos as possible. Two persons evaluated each person independently, and for discrepancies between them consensus was achieved together with a third evaluator afterword. Study variables included personal data (age, sex, nationality, race), general features of the face, hair, eyes and nose, lips, teeth, some registered during rest or smile position.

Data on medical interventions with impact on face attractiveness was also searched, main being triggered two directions – dental treatment (e.g, prosthetic restorations, orthodontic treatment, tooth whitening) and plastic surgery or other interventions to the face, nose or lips. Data was collected through photo inspection aided by metric and angular measurements performed in Adobe Photoshop software.

Data analysis was mainly based on descriptive statistics, targeting describing facial attractiveness patterns by describing most frequent encountered characteristics in females considered very beautiful and attractive. Microsoft Excel and SPSS (Statistical Package for the Social Sciences) were used.

RESULTS

The persons analyzed registered diverse facial traits. Of the 20 persons, for one was not found any picture while smiling. Most obvious trends, encountered in more than 75% of them, included the following: Caucasian race; straight and long hair; average nasal tip protrusion; maxillary dental midline concordant to facial midline; a thicker lower lip compared to the upper lip; exposure of only maxillary incisors during smile; decreased or absent buccal corridor; maxillary central incisors of oval/rectangular shape; absence of maxillary teeth crowding; presence of teeth whitening.

Figure 1: Aspect of the lips of the women analyzed

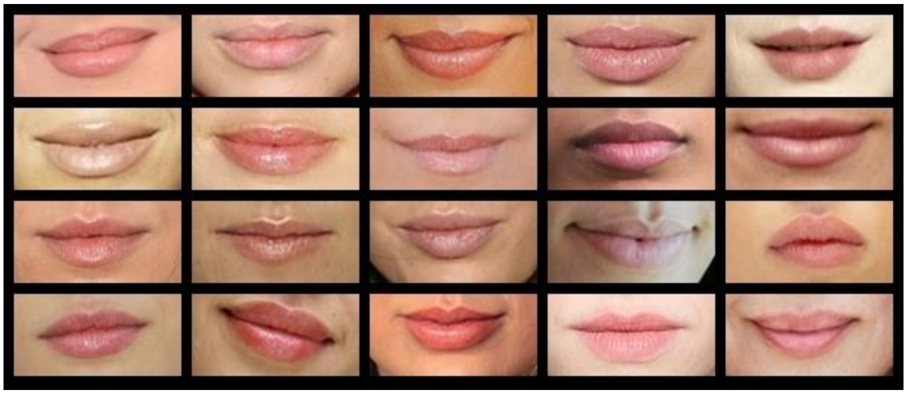

Other frequently encountered features, found in more than 50% of the analyzed persons, were: American nationality; brown hair; eyes with medium size, almond shape, wide set, of green color; nose with an average width; face with a decreased lower third and a slightly convex profile; a thick lower lip and an average upper lip; exposure during smiling till the first molar, with a normal smile line (Table 1, Figure 1 and 2).

Table 1: General and facial characteristics of the beautiful women analyzed

Age (years)	mean: 31; median: 29.5; range: 24-49
Nationality	American (n=11); Indian (n=2); English (n=2); Ukrainian (n=2)
Race	Caucasian (n=18); African-American (n=2)
Hair	color: brown (n=11); blond (n=8); brunette (n=1) aspect: straight (n=16); curly (n=4) length: long (n=17); short (n=3)
Eyes	size: medium (n=13); big (n=4); small (n=3) shape: almond eyes (n=10) position: wide set eyes (n=11); average set eyes (n=7); close set eyes (n=2) color: green (n=10); brown (n=6); blue (n=4)
Nose	nose width: average (n=12); narrow (n=5); wide (n=3) nasal tip protrusion: average (n=15); protruded (n=4); retruded (n=1) nasolabial angle: increased (n=12); medium (n=6); decreased (n=2)
Face shape	pentagon (n=7); oval (n=6); square (n=3); round (n=2); rectangular (n=2)
Dental midline to facial midline	concordant (n=17); slightly deviated (n=2)
Vertical facial proportion	decreased lower third of the face (n=12); similarity of lower and middle third (n=5); increased lower third (n=3)
Profile	slightly convex (n=14); straight (n=6)
Lips	upper lip volume average (n=12); thick (n=7); thin (n=1) lower lip volume: thick (n=13); thin (n=7) comparative evaluation: thicker lower lip (n=18); thicker upper lip (n=2)
Tooth exposure while smiling	only maxillary teeth (n=16); maxillary and mandibular teeth (n=3) till the first molar (n=10), second premolars (n=8), canines (n=1) normal exposure of maxillary central incisors/smile line (n=13); lower exposure of maxillary central incisors (n=3); gummy smile (n=4)
Buccal corridor	decreased or absent (n=14)
Central maxillary incisors	form: oval/rectangular (n=15); triangular (n=4) size: average (n=15); bigger (n=4)
Maxillary teeth crowding	absent (n=16); mild (n=3)
Interventions with impact on facial esthetics	dental prosthesis (n=2); teeth whitening (n=14); orthodontic treatment (n=2); interventions to the nose (n=3); interventions to the chin (n=1)

DISCUSSION

This study's results, by the high percentages observed in many facial traits, suggest that in the public opinion there is a facial pattern of female attractiveness. Some of them (e.g. facial symmetry, normal smile line) are in accordance to general norm that define a beautiful face.

In this study the majority of the persons were of American nationality and Caucasians. This may be primarily linked to the way study sample was formed, more precisely of actresses, singers and models. The American movie and music industries are very well developed and mediatized worldwide, through this probably influencing lay person perception of facial attractiveness.

The age of the persons included in this study sample was variable, from 29 to 49 years old. In this regard, should be noticed that it can be said that beautiful and attractive women in current society does not equal young women, but also middle-aged ones.

Figure 2: Aspect of the smile of the women analyzed

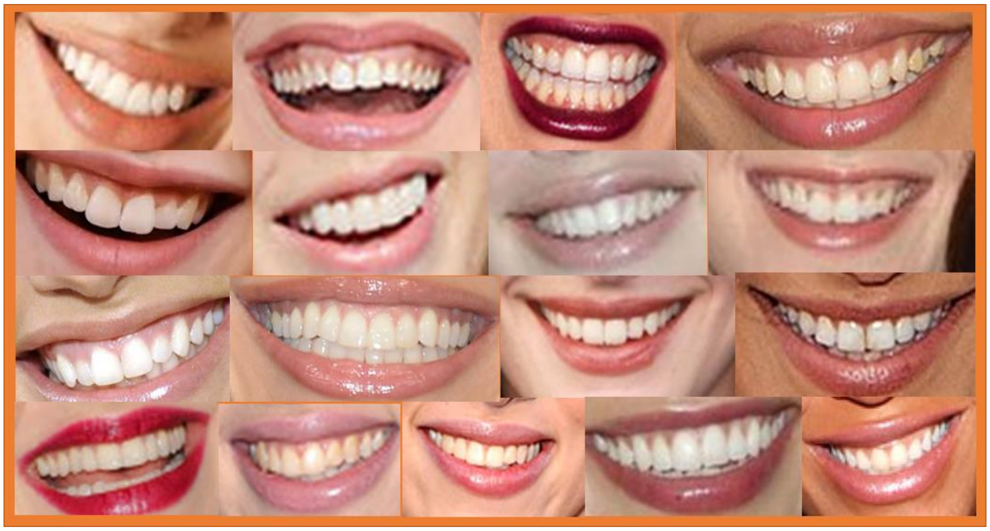

Oval and pentagonal face forms are generally considered as being most attractive form face [1], these being the most frequently observed in this study sample. Also, symmetry is another aspect that is known to influence facial attractiveness [2], which is also supported by the results of this study. In our sample of women considered very attractive there were found most frequently persons with a reduced lower facial third, and very few with an increased lower facial third, results that are supported by the findings of numerous studies [3].

Lips are considered to play an important role in facial attractiveness, one important parameter being lips' thickness [4,5]. In females analyzed in this study were generally found to have thick lips, most frequently the lower lip being thicker than the upper lip. Generally an equal thickness of upper and lower lip is perceived as unaesthetic, this situation not being encountered in this study.

Smile is considered as greatly influencing facial attractiveness, dental treatments being often solicited for remediating some problems as perceived by patients, therefore an accurate evaluation of it being recommended [6]. Aspects as color of teeth and gingival display are well known to play an important role in smile appearance [7,8]. This aspect is confirmed by this study results as most persons had normal smile line, and an important part tooth whitening. Also, most of the persons presented an increased smile width and a decreased or absent buccal corridor, which is confirmed by some studies to be related to an increased facial attractiveness [9,10].

An attractive facial profile includes often, beside ones natural traits, also artificial improvements through interventions, some medical, as the dental ones. In this study was tried to identify medical interventions made in order to increase facial esthetics. This aspect is difficult to analyze but, even so, by comparing nowadays to previous photos, there were identified several dental interventions, namely presence of fixed prosthetic restoration, tooth whitening and orthodontic treatment, for the previous one pictures of the persons or/and their own statements being used. Considering that, it is important for the dentists of various specialty (e.g., orthodontists, prosthodontist) to know and understand lay persons' perception on facial attractiveness, in order to deliver most appropriate interventions, in accordance to patient's need and expectations [11-13].

Among study limitation can be mentioned the method of data collection, considering the difficulties in finding adequate photos for a correct analysis and difficulties in assessing prior intervention made to enhance facial esthetics. Even so, there were mainly registered variables that were relatively easy to be assessed. Also, another limitation is the relative small sample size, considering the numerous beautiful women that are public figures nowadays.

CONCLUSIONS

The public beautiful women nowadays register features that are generally associated to an increased facial attractiveness. Knowledge of trends in facial beauty is useful in dental practice, in order to understand better patient's need in terms of esthetic expectations, and for adapting to his practice by including newest treatment that aim improving esthetics. The dentist can also use example of public beautiful women in order to explain and argument his recommendations regarding certain treatment conduct, from an esthetic point of view.

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CLINICAL PRACTICE

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The semi-centenary of a prestigious medical unit: Military-Medical Research Center

Viorel Ordeanu[1,2]

Abstract*: The Military Medical Research Center (CCSMM) is a health research unit, subordinated to the Medical Directorate of the Ministry of National Defence. The overall objective is represented by conducting researches on the medical protection against weapons of mass destruction (ADM) that use chemical, biological, radiological and nuclear (CBRN) agents, on the accidents involving CBRN agents and on the military-medical expertise in the field. The CCSMM specialists develop methods and techniques for diagnosis, prophylaxis, treatment and recovery to optimize medical countermeasures during peace, war, or crisis situations. The activity is complementary to the one carried by the "Cantacuzino" National Military-Medical Institute for Research and Development which deals with the diagnosis of infectious diseases (microbiology and epidemiology) and the production of reagents and biological medicinal products for infectious diseases.*

In conclusion, CCSMM is a unique healthcare unit nationwide, by its field of activity, having a tradition of over half a century, during which it has formed specialists and has had outstanding achievements in the medical protection against CBRN weapons and agents. There are prerequisites for the further development and visibility of the institution.

Keywords: *military medical research, CBRN medical protection, biological warfare agents, bioterrorism, microbiology*

INTRODUCTION

The Military Medical Research Center (CCSMM) is a health research unit, subordinated to the Medical Directorate of the Ministry of National Defence. The overall objective is represented by conducting researches on the medical protection against weapons of mass destruction (WMD) that use chemical, biological, radiological and nuclear (CBRN) agents, on the accidents involving CBRN agents and on the military-medical expertise in the field. The CCSMM specialists develop methods and techniques for diagnosis, prophylaxis, treatment and recovery to optimize medical countermeasures during peace, war, or crisis situations. The activity is complementary but distinct to the one carried by the "Cantacuzino" National Military-Medical Institute for Research and Development (INCDMM) which deals with the diagnosis of infectious diseases (microbiology and epidemiology) and the production of reagents and biological medicinal products for infectious diseases. It also cooperates with the CBRN Defence and Ecology Scientific Research Center (CCSACBRNE) which seeks the CBRN non-medical protection. Each of the three research institutions addresses the issue of the protection of troops and the civilian population from another perspective, without overlapping

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but being complementary. As a result, they must cooperate.

HISTORICAL BACKGROUND

In the military-political context of the Cold War, there was also the danger of nuclear attack. The Romanian Ministry of Health set up a research healthcare unit in 1967, which functioned on the platform of Fundeni Hospital in Bucharest, called the "Center of Radiobiology and Molecular Biology". This unique institution in Romania did not happen by chance. We remind that at that time on Romanian territory was prohibited the stationing of nuclear weapons, so as not to be considered a pretext for a nuclear attack on the country. The international context was strained: the "missile crisis" had just ended in which the US nuclear missiles from Incirlik (Turkey) had been directed to Moscow, and the Soviet ones from Cuba had been directed towards Washington, and the "mid-range missiles" problem was debated in Europe from the east and west coast of the continent.

As a result of the events of August 1968, when the USSR invaded Czechoslovakia, and Romania was in danger of being invaded, the Romanian Army was reorganized and this Center was taken over from the Ministry of Health by the Ministry of Armed Forces (which later became the Ministry of National Defence) by HCM no. 2423/31.10.1968. Starting with December 1st, 1968 it functioned as a military unit called the "Radiobiology Center". Its aim was scientific research in the field of medical protection against the effects of the nuclear weapon.

In 1969, through the Order of the Chief of Staff, the Center's attributions were extended to the medical protection against the other WMDs as well, and the name which it still preserves was changed to the "Military Medical Research Center". The unit had an independent brigade rank subordinated to the Medical Directorate, and the commanding ranks were at Division level of Lieutenant General, M.D. and Major General, M.D. The three component laboratories corresponded to the NBC classification (nuclear, biological and chemical), being ranked regimental, and were run by Colonels – medical doctors, who were also scientific scholars. The laboratories were made up of wards and work stations. To the personnel recruited from the Ministry of Health were added the military and civilian specialists, and from the Military Hospitals in the territory were selected the most valuable specialists, who became scientific scholars. The ranking was made for real personal merits and professional experience, not by “competition”.

CLASSIFICATION AND ENDOWMENT

The first commander, since December 1st 1968, the Center’s founder, was the late Colonel Alexandru Popescu M.D., an exceptional microbiologist with experience in exotic and tropical diseases, and who also participated in the fight against the plague and cholera epidemics in India. He would later become Head of the Medical Directorate, Major General Professor A.G. Popescu, PhD.

Other personalities of military medicine were also in charge: the Colonel Laurenţiu Dragomirescu M.D., Colonel Aurel Andrieş M.D., Colonel Tudor Toma M.D., Colonel Ion Urseanu M.D. A special stage in the evolution of CCSMM began during the mandate of the commander Colonel Victor Voicu M.D., who was to become Head of the Medical Directorate, Major General Acad. Professor Victor A. Voicu, M.D., PhD former Secretary General and current Vice President of the Romanian Academy and President of the Department of Medicine of the Romanian Academy, member of the Medical Academies in Bucharest and Paris etc. Colonel Mihail Silviu Tudosie M.D. (currently the commander of the Military-Medical Institute) and Colonel Radu Gabriel Hertzog M.D., the current CCSMM commander, followed.

During this period, the CCSMM gradually benefited from several workplaces, suitable for the scientific research activity: the Bratianu villa from 37 C.A. Rosetti Street (Pavilion A for Command and Administration and Pavilion B for the Toxicology Laboratory), a radiobiology pavilion at Fundeni Hospital, subsequently completed with vivarium and the underground pavement of the Accelerator for Cancer Therapy; the Microbiology Laboratory at the Cantacuzino Institute (1st Ward), 7th floor and another at the Cernica Fort (2nd Ward), the Department of Clinical Toxicology at the Floreasca

Emergency Hospital, the Psychophysiology and the Psycho-pharmacology Laboratory at the Central Military Hospital (in a pavilion originally designed and built to belong to CCSMM), etc. But as a result of successive restructurings, since 1986 and those following the December 1989 Revolution, the staff has been progressively reduced and the rank of the unit has fallen to regimental level. After the annulment of the Warsaw Treaty, it was assumed that there would be no more wars (!!) and the Center was in danger of being completely abolished. Several spaces were successively lost, the remaining staff and research equipment were and will probably be deployed.

THE SCIENTIFIC RESEARCH ACTIVITY

The unit was certified as a scientific research institution (IP) through GD no. 551/2007 and ANCS Decision no. 9806/2008. Currently, Romania, being a member of the European Union and NATO, grants due importance to national defense and security, including to the medical protection against the CBRN WMD. In this context, CCSMM is in the period of renewal of staff, premises, endowment and work procedures, for modernization and openness to new trends in military medicine and military scientific research.

The results of the scientific research activity have materialized through processes, methods of treatment or diagnosis, and experimental development of new protective compounds and antidote products, protective equipment, etc. These have been endorsed, received, approved, and some have been patented as inventions. CCSMM had at its former headquarters a permanent exhibition of experimental models and prototypes of reagents, medicines, antidotes, complete kits and models developed within the research projects. Many of them were also presented at military exhibitions: The permanent exhibition of military technology from ACTTM Clinceni, EXPOMIL 2011 and 2013, BSDA 2014, 2016 and 2018, the National Exhibition of Inventors 2012 and 2013 (where the Golden Medal and Diplomas of Excellence were obtained), etc. Some of them have been practically used on missions in the country and abroad (Saudi Arabia, Iraq, Somalia, Angola etc.).

SPECIFIC MILITARY ACTIVITIES

CCSMM has also been directly involved in military activities such as national and international exercises and applications, participating in the Persian Gulf War (1991), in Somalia and Angola, providing military-medical expertise in CBRN incidents, expertise of suspected objects brought by the Romanian Intelligence Service (e.g. “biological bomb” discovery or “anthrax letter” of U.S. Embassy), MAI, the Military Police (e.g. control for aircontainers), DIR, BOS (e.g. Afghanistan suspect probes), CBRN equipment testing in laboratory and in field, postal biological control (letters and box) etc. which constitute situational experiments for validating research and training in real terms.

PUBLISHING ACTIVITY

The CCSMM staff actively participate in the scientific life through communications to national and international scientific events, publishing of books, chapters and articles (including hundreds of ISI articles indexed in the Web of knowledge), inventions, didactics and publicity in the military and civilian press. In 1998, Major General Acad. Professor Victor A. Voicu, M.D., PhD organized a NATO scientific event, in Bucharest, entitled “Proceeding of the NATO Advanced Research Workshop on Antidotes, Protectors and Decorporators”, the papers of which were published by the Kluwer Publishing House in 1999. Scientific papers have been published in the Journal of Military Medicine, the National Defense University Bulletin, Strategic Impact, the Military Science Journal, Strategic Universe, NBC Defense, the Military Observatory, Medical Life, etc. Books were published by the Military Publishing House, the Medical Publishing House, the "Carol Davila" University Publishing House, as well as own publishing. Through these activities, the scientific knowledge of interest disseminates and the visibility of CCSMM increases, both in the country and abroad.

EDUCATIONAL ACTIVITIES

CCSMM specialists are also involved in academic and university activities, at the Military-Medical Institute, the "Carol Davila" University of Medicine and

Pharmacy in Bucharest, the "Titu Maiorescu" University, Bioterra University, the Medical School of Non-comissioned Officers in Focşani, etc. The CCSMM researchers have benefited from recognition of their work: excellence diplomas, awards, decorations, biographies mentioned in encyclopedias, etc.

PROSPECTS FOR DEVELOPMENT

The military-medical scientific research, which seemed to be forgotten by decision-makers in recent years, is reviving. The military-political world situation, which is "volatile", brings to the forefront the importance of military medicine and, implicitly, of military-medical scientific research, primarily in regards to the medical protection against CBRN weapons/agents. The prospects for the quantitative and qualitative development of CCSMM materialize and, as consequence, the context favorable for the cooperation with an old collaborator occurs, the “Cantacuzino” Military-Medical Research-Development National Institute, which was also taken over from the Ministry of Health by the Ministry of National Defense.

The current situation also allows the area of scientific preoccupations to be extended in order to cover other areas of medical and military interest, as well as the formation of a new generation of specialists to take over for the future. Besides the main field of scientific research and expertise for medical protection against CBRN/WMD, new and related areas are also successfully addressed, which are concerned with specific military pathology. Thus, in May 2018, an original work of regenerative medicine was awarded a prize at the 23rd Congress of the Balkan Military Medical Committee (BMMC) in Antalya, Turkey. The operationalization of the Integrated Platform of Scientific Research and Expertise of CBRN agents for medical protection will support the work of CCSMM and will allow new research directions to be addressed within existing laboratories. The latest participation is of 16th Medical Biodefense Conference in Munich, 23-31 October 2018.

CONCLUSIONS

CCSMM is a national military-medical unit, unique in its field of activity with a tradition of more than half a century, during which it has formed exceptional specialists and has had outstanding achievements in the scientific research on medical protection against the CBRN weapons and agents. There are prerequisites for the further development and visibility of the institution.

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CLINICAL PRACTICE

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Black hairy tongue due to antibiotic intake

Catalina E. Lavric[1], Silviu V. Dumitrescu[2]

A 65-year-old man presented to our clinic for a sudden onset of black hairy tongue. The patient who has no significant medical history was explored 9 days before for non-specific gastrointestinal symptoms which had progressively accentuated in the past two months. Physical examination showed epigastric tenderness and bloating. Blood tests were within normal ranges but stool antigen test revealed Helicobacter pylori infection. Triple therapy was initiated with 1 gram amoxicillin, 500 mg clarithromycin and 30 mg lansoprazole, all recomanded twice daily for 14 days. In the 9th day he presented with a black hairy tongue which appeared "overnight".

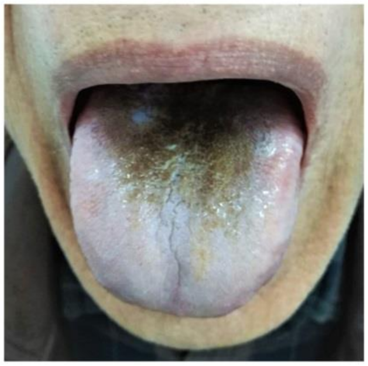

Black hairy tongue represents a defective desquamation of the filiform papillae on the dorsal surface of the tongue, usually due to a lack of mechanical stimulation and debridement [1]. A range of medications [2] have been linked to hairy tongue including amoxicillin, penicillin, bismuth, linezolid. This condition is reported more often in males and the main problem is its unsighty appearance [3].

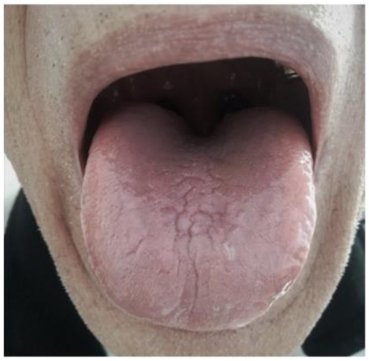

Sometimes patients may complain of a burning sensation on the tongue and halitosis. Black hairy tongue doesn't typically require medical treatment [4].

In this case the patient stopped the antibiotic intake on his own initiative and was encouraged to have a good oral hygene. He continued taking 30 mg of lansoprazole for three more weeks. One month later the tongue recovers its normal characteristics. Reevaluation of the stool test showed the efficiency of the short course of treatment, Helicobacter pylori being eradicated.

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CLINICAL PRACTICE

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Atypical case of achalasia

Andreea Grigore[1], Bianca Săndulescu[1], Alexandra Lulache[1], Andrada Popescu[1,2], Săndica Bucurică[1,2], Mihai Șotcan[1], Florina Vasilescu[1], Petruț Nuță[1], Mariana Jinga[1,2], Florentina Ioniță Radu[1,3], Daniel O. Costache[1], Raluca S. Costache[1,2]

INTRODUCTION

Esophageal cancer, represented by squamous carcinoma and adenocarcinoma, is ranked 8th in incidence among all the types of neoplasm in the world (456,000 new cases in 2012, 3.2% of total cancers), and is characterized by a significant lethal potential, being the 6th cause of cancer-induced mortality (400,000 in 2012, 4.9% of total cancers) [1]. 5 years survival after diagnosis is less than 5%, as esophageal cancer causes symptoms when more than 60% of the esophagus is infiltrated by tumor [2].

CASE REPORT

A 48-year-old patient presented for progressive dysphagia to solids, weight loss of about 15 kg and diffuse abdominal pain for the last 3 months. In addition, the patient noticed moderate dyspnea and intense pain localized at the right shoulder, without relation to movements. Two months before presentation, the patient performed a superior digestive endoscopy, which diagnosed him with ulcerative esophagitis, but without improvement of the symptoms despite recommended treatment. The patient is known with two surgical procedures, a Billroth I anastomosis for perforated gastric ulcer 12 years ago, and a cholecystectomy in 2018. Moreover, the patient had a stroke in 2014 and in 2015 was diagnosed with hepatic cirrhosis of unspecified etiology. The patient was a smoker with a 30 pack per year history and didn't work in a toxic environment.

The patient was underweight (BMI = 18.0 kg/m^2), but had a satisfactory overall condition. The abdomen was dense and painful on palpation.

The patient had hepatomegaly, was cardio-respiratory balanced and did not show any significant changes in the other organs and systems.

At the time of admission, stage diagnostics included dysphagic syndrome, abdominal algic syndrome, underweight, post-gastrectomy status, sequelae of stroke, liver cirrhosis in observation.

It is to be mentioned the inflammatory syndrome (fibrinogen = 484 mg/dL), cholestasis (total bilirubin = 1.47 mg/dL, direct bilirubin = 0.45 mg/dL, alkaline phosphatase = 145 U/L, GGT = 322 U/L), hyposideremia (Fe = 58 micrograms/dL) without anemia (Hb = 14.2 g/dL, Ht = 41.2%, RBC count 4.70 million/mm^3). During

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hospitalization, hemoglobin, hematocrit, and RBC count decreased but did not indicate iron deficiency anemia (hemoglobin = 13.6 g/dL, hematocrit = 39.5, number of erythrocites = 4.5 million/mm^3). The patient's hormonal profile indicated euthyroidism. The patient was tested for HIV infection, hepatitis B and C virus infection, Kohn bacillus infection, but the results refuted those diagnostics. CEA and CA 19-9 had elevated values (CEA = 58.21 ng/mL, CA19-9 = 47.53 U/mL) and α-fetoprotein was slightly above the normal range (AFP = 1.1 ng/mL).

Abdominal ultrasound and thoracic radiography did not reveal pathological changes in the first phase. At upper digestive endoscopy fluid debris were present in the esophagus and stomach that prevented visualization of the digestive tract. At the repeated upper digestive endoscopy, white deposits were attached to the normal mucosa at the level of the esophagus, characteristic of mycotic esophagitis. Initially, we couldn't overcome the cardia with the normal endoscope, which is why we turned to babyscope. There were no pathological changes in the stomach and duodenum I and II, and the mouth of anastomosis from the perforated ulcer surgery was supple. Due to these endoscopic findings, the patient underwent esophageal manometry, and was diagnosed with type 2 achalasia (panesophageal pressurization 100%, IRP = 80.2 mmHg).

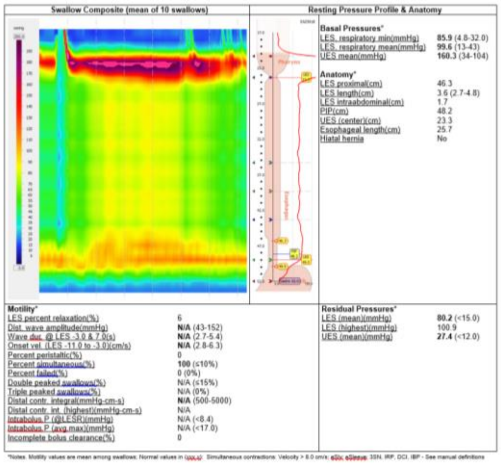
Swallow Composite (mean of 10 swallows)
Resting Pressure Profile & Anatomy

Basal Pressures*	
LES, respiratory min(mmHg)	**85.9** (4.8-32.0)
LES, respiratory mean(mmHg)	**99.6** (13-43)
UES mean(mmHg)	**160.3** (34-104)

Anatomy*	
LES proximal(cm)	46.3
LES length(cm)	3.6 (2.7-4.8)
LES intraabdominal(cm)	1.7
PIP(cm)	48.2
UES (center)(cm)	23.3
Esophageal length(cm)	25.7
Hiatal hernia	No

Motility*	
LES percent relaxation(%)	6
Dist. wave amplitude(mmHg)	**N/A** (43-152)
Wave dur. @ LES -3.0 & 7.0(s)	**N/A** (2.7-5.4)
Onset vel. (LES -11.0 to -3.0)(cm/s)	**N/A** (2.8-6.3)
Percent peristaltic(%)	0
Percent simultaneous(%)	**100** (≤10%)
Percent failed(%)	0 (0%)
Double peaked swallows(%)	N/A (≤15%)
Triple peaked swallows(%)	N/A (0%)
Distal contr. integral(mmHg-cm-s)	**N/A** (500-5000)
Distal contr. int. (highest)(mmHg-cm-s)	N/A
Intrabolus P (@LESR)(mmHg)	N/A (<8.4)
Intrabolus P (avg max)(mmHg)	N/A (<17.0)
Incomplete bolus clearance(%)	0

Residual Pressures*	
LES (mean)(mmHg)	**80.2** (<15.0)
LES (highest)(mmHg)	100.9
UES (mean)(mmHg)	**27.4** (<12.0)

*Notes: Motility values are mean among swallows; Normal values in (xxx.x). Simultaneous contractions: Velocity > 8.0 cm/s; eSix, eSleeve, 3SN, IRP, DCI, IBP - See manual definitions

Taking into account the persistent symptoms and the changes of the above mentioned laboratory analyzes, computerized thoracic and abdominal tomography with contrast substance was performed. Computed tomography revealed pulmonary diffuse multiple micronodular lesions of millimetric dimension. At the eso-gastric junction a tumor block was described, with exophytic growth, with polylobate form and iodophyllic aspect, with a maximum diameter of about 5.5/3.8 cm, with small necrosis included, causing distension and stasis in the upstream esophagus. Additionally, adenopathy blocks in the small gastric curvature and celiac trunk were present, measuring up to 6 cm in diameter, the largest being without boundaries of demarcation with the celiac and pancreatic trunks. The liver was increased in size, with a maximum cranio-caudal diameter of 19.5 cm, with irregular contour, and irregular inhomogeneous structure due to the presence of multiple nodular lesions, randomly developed in the entire parenchyma that measured up to 1.8 cm, iodophyllic, some with a tendency to conflate, compatible with secondary lesions. Intrahepatic peripheral bile ducts in the left lobe exhibited slight dilation, most likely by infiltration generated by secondary lesions while extrahepatic bile ducts were unchanged. No pathological changes were detected in the abdominal vein circulation as well as in the spleen, pancreas, kidneys or adrenal glands.

Concluding the tomographic examination, it showed that the patient had a esogastric junction tumor with bilateral pulmonary and hepatic metastasis and abdominal adenopathy. Later on, the oncological consult confirmed the suspicion of eso-gastric tumor with metastases and recommended biopsy of the hepatic nodules in order to perform the pathological anatomy and immunohistochemical examinations. Hepatic tissue fragments were thus obtained by ultrasound-guided biopsy puncture and the result were: small fragments of liver tissue with neoplastic infiltrates of poorly differentiated carcinoma which is arranged in nests of different sizes. Tumoral cells were cytokeratin7 positive and CDX2 positive. The immune-histochemistry and histopathologic aspects sustain the diagnosis of hepatic metastasis of poorly differentiated gastric adenocarcinoma.

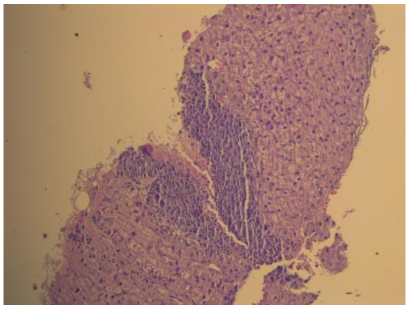

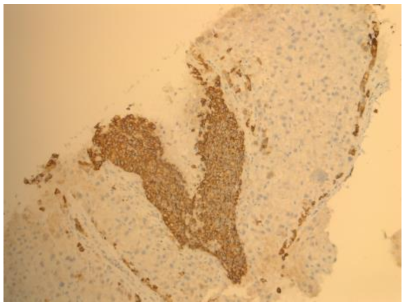

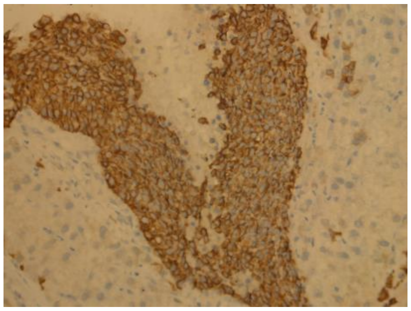

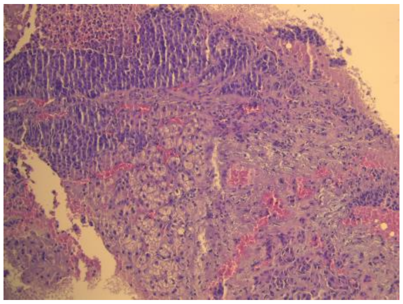

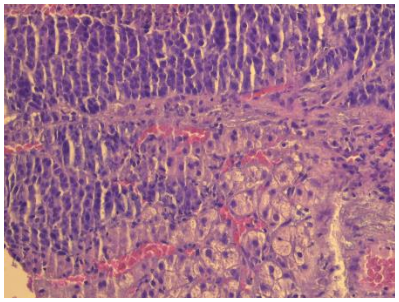

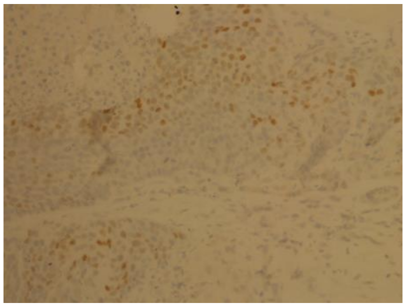

The patient was then referred to a thoracic surgical consult which stated that the tumor had no surgical indication and in the event of total dysphagia the therapeutic indication was to place an esophageal stent or a gastrostomy.

An esophageal stent was place and the patient remains under oncologic surveillence.

DISCUSSIONS

The differential diagnosis in this case started from dysphagia, the symptom which was the most intense and disturbing for patient. One type of dysphagia is the oropharyngeal dysphagia (diseases of the mouth, hypopharynx, esophagus above) in which case the cause can either be neuromuscular (SLA, central nervous system tumors, multiple sclerosis, myasthenia gravis) or structural (carcinoma, throat infections, Zenker's diverticulum) [3]. However, in this type of dysphagia, coughing and choking are described immediately after swallowing, the symptoms are often located precisely in the neck and in some cases food and fluids follow the path of the trachea or the nose. Another type of dysphagia is esophageal that can be caused by primary disorders of motility (achalasia, esophageal spasm, esophageal hypercontractility or other disorders of the peristalsis) or secondary disorders of motility (Chagas' disease, dysfunction of gastroesophageal reflux disease, scleroderma), or may be due to intrinsic structural pathologies (benign or malignant tumor, diverticulum, eosinophilic esophagitis, esophageal rings, foreign bodies, peptic stricture) or extrinsic ones (mediastinal tumors, osteophytes spinal, vascular compression) [3].

Considering the patient's features of the dysphagia, progressive dysphagia for solids accompanied by weight loss, there was an initial suspicion of a mechanical cause with a higher likelihood of malignant tumor formation. In general, peptic strictures caused by gastroesophageal reflux disease are accompanied by chronic heartburn and do not cause weight loss. The normal endoscopic examination of the esophageal mucosa initially directed the attention to a motility disorder, the most common being achalasia, a pathology characterized by poor relaxation of the lower esophageal sphincter in swallowing and lack of peristalsis in the smooth esophageal muscles. Dysphagia in this disease is progressive, occurs both for solid and liquid foods and is frequently associated with regurgitation, chest pain, weight loss symptoms that usually progress insidiously within about 2 years [4]. Although manometry diagnosed the patient with achalasia, the other symptoms, namely diffuse abdominal pain, dyspnea, and shoulder pain required the continuation of the investigations, the problem raised being if all the patient's symptoms were in the context of the same disease or another disease overlapping the esophageal pathology. From paraclinical examinations, elevated tumor markers have turned attention to a possible malignant tumoral pathology at the abdominal level that required CT examination, which was the one that brought the most important information to the case.

The particularity of this case is that although the patient was initially diagnosed with type II achalasia, the manometry examination showed pseudoachalasia in the context of esophageal cancer. Data from literature suggest that such presumptive results in any manometry examination are not specific to achalasia and under the conditions of sudden symptoms in less than 1 year, with a weight loss of more than 7 kg in a patient over 50, the suspicion of pseudoachalasia is raised. Thus, 5% of the manometric results indicating achalasia are actually given by tumor pseudoachalasia [5]. Such a case has been published by Segal et al, a case of a 50-year-old UK patient with the same symptomatology as the one of the patient in our center where the diagnostic process indicated achalasia in the first phase, but metastatic adenocarcinoma was eventually detected [6].

An interesting aspect to be mentioned is that the relationship between achalasia and cancer may be a causal one, which is already known in medical practice. Patients with achalasia are at risk of developing esophageal cancer, with the predominance of the squamous cell cancer and the adenocarcinoma in some situations. On one hand, achalasia causes esophageal stasis with bacterial overpopulation and production of various chemical compounds, while causing chronic inflammatory changes in the esophageal mucosa and dysplasia. Although this complication of achalasia is reduced in frequency, the prevalence of cancer in achalasia being variable according to several studies, there were several reported cases in literature [7]. Rios-Galvez et al published a case of a patient, known with achalasia for more de 15 years, whose symptomatology had significantly aggravated within 6 months and who, after following investigations, is diagnosed with squamous carcinoma of the esophagus [8]. What is different in our case is precisely the absence of the personal pathological history, achalasia requiring a prolonged period of illness for the esophageal malignancy to occur. On the other hand, esophageal adenocarcinoma occurs more frequently in the context of gastroesophageal reflux disease as a complication of the interventional treatment of achalasia by esophageal dilatation and myotomy [7].

CONCLUSION

In conclusion, dysphagia may occur in many benign or malignant conditions, but in a patient with alarming signs such as age, marked weight loss, even under the condition of a diagnosis of neuromuscular motility disorder, the suspicion of a malignant process must always be raised and we should act accordingly, following paraclinical investigations that can confirm or refute it.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.

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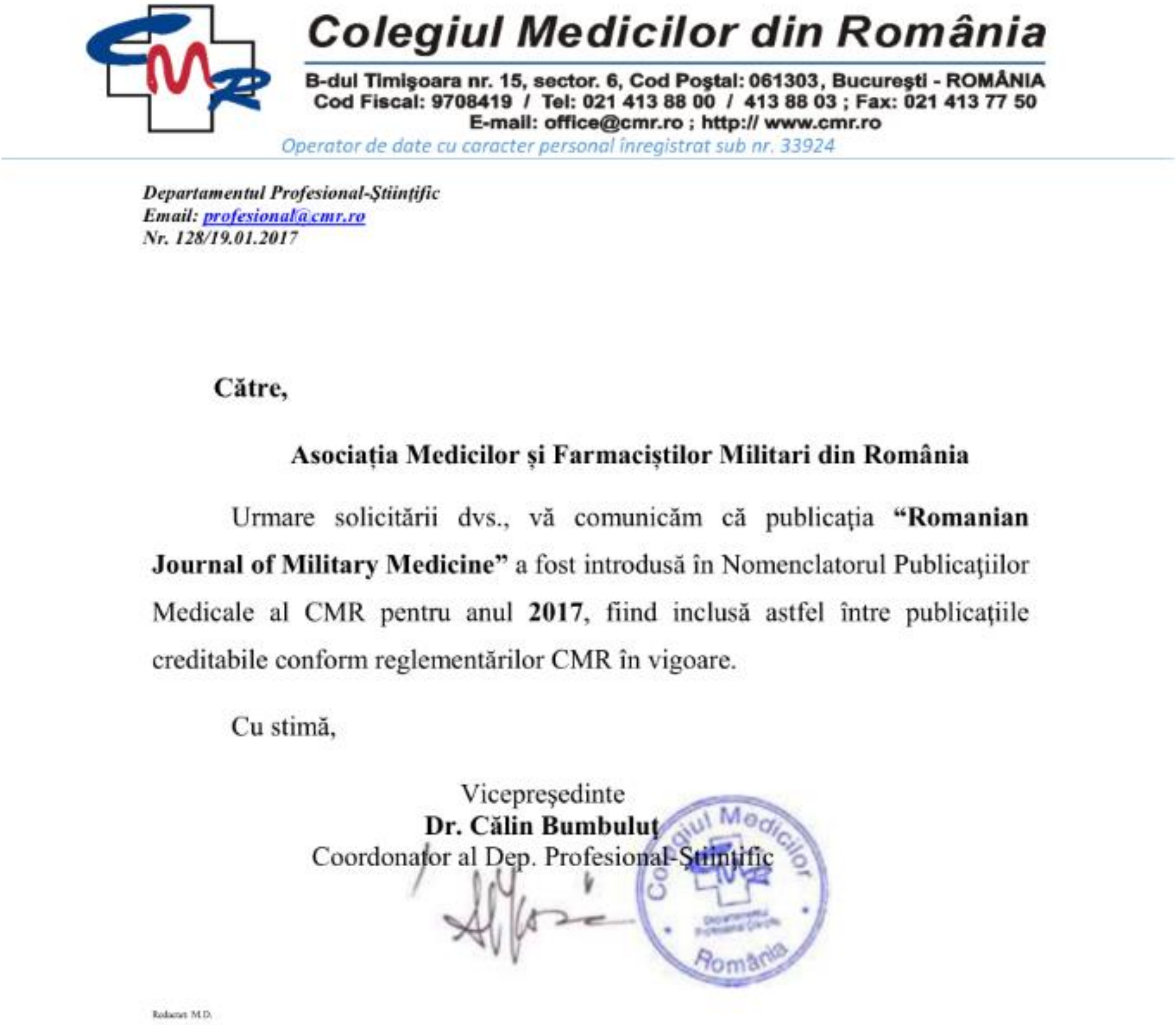

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REVIEW

Leadership skills in surgeons: a review of the literature

Alexandra Sopu[1], Juergen Apel[1]

Abstract: *Clinicians, professors of medicine in general, students, and professionals from the health sciences tend to focus their efforts on technical training related to their discipline in practice as well as activities that produce some benefit direct or personal satisfaction. It is unusual to devote time and effort to training in areas such as teaching or expand their skills in sociological and organizational aspects. Would you like to take a course on strategic planning if not a director or officer of the hospital? Manage time in your schedule for a workshop on communication and conflict management? Have room in your schedule for educational activities on leadership, organizational change, and diffusion of innovations, science or science complexity of implementation? Generally, doctors and teachers do not spend much time on these activities (at least voluntarily) because we feel that what we have learned from these topics on personal experience is enough [1].*

Effective teamwork, not only in the operating room but also throughout the perioperative route is considered an important and critical component of the safety and effectiveness of surgical care and leads to good results and quick recovery of surgical patients. Therefore, considerable efforts have been made to understand how teams work within the route of surgical care and to improve teamwork [2].

The simple act of bringing together some experts does not guarantee that turned out an expert team. Given the evidence suggests that teamwork in the operating room sometimes is far from harmonious and effective, the importance of training the surgical team to work effectively is receiving international acceptance as a key strategy to maximize safety in surgery.

This systematic literature review aims to present a review of literature that focuses on the importance and role of leadership skills in the surgeon and how these skills can play a significant role in increasing the quality of care and patients' safety in operating room (OR) settings.

Keywords: *leadership, surgeons, operating room, qualities, leader, surgical team, conflict*

INTRODUCTION

Clinicians, professors of medicine in general, students, and professionals from the health sciences tend to focus their efforts on technical training related to their discipline in practice as well as activities that produce some benefit direct or personal satisfaction. It is unusual to devote time and effort to training in areas such as teaching or expand their skills in sociological and organizational aspects. Would you like to take a course on strategic planning if not a director or officer of the hospital? Manage time in your schedule for a workshop on communication and conflict management? Have room in your schedule for educational activities on leadership, organizational change, and diffusion of innovations, science or science complexity of implementation? Generally, doctors and teachers do not spend much time on these activities (at least voluntarily) because we feel that what we have learned from these topics on personal experience is enough [1].

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The way to see the errors and patient safety has changed radically over the last 15 years. Effective teamwork, not only in the operating room but also throughout the perioperative route is considered an important and critical component of the safety and effectiveness of surgical care and leads to good results and quick recovery of surgical patients. Therefore, considerable efforts have been made to understand how teams work within the route of surgical care and to improve teamwork [2].

During the past 15 years, the conceptualization of security in surgery has changed substantially. There is a growing awareness that the skills required to consistently achieve security in surgery go far beyond the knowledge and technical skills of the surgeon [3]. Teamwork, defined in terms of "a set of behaviors, actions, cognitions, and attitudes that facilitate the work to be done" [4], is increasingly seen as an essential component of safe surgery. Work crews are essential when "errors can result in serious consequences; when the complexity of the activity exceeds the capacity of one person; when the working environment is ill-defined or ambiguous or stressful; when you need to make many decisions quickly, and when the lives of others depend on the collective view of the members'. The importance of teamwork to the safety and efficiency of operations has been recognized for some time and, therefore, is an integral part of the education, training, and evaluation of workers in many industries. It seems that, finally, the health community begins to embrace the concept of "work teams are a source of security" [5].

The simple act of bringing together some experts does not guarantee that turned out an expert team. Given the evidence suggests that teamwork in the operating room sometimes is far from harmonious and effective, the importance of training the surgical team to work effectively is receiving international acceptance as a key strategy to maximize safety in surgery. The development of training interventions to provide surgical team knowledge, skills, and attitudes that serve as a foundation for effective performance has become a dominant theme in the literature of surgery, anesthesia, and nursing.

OBJECTIVE

This systematic literature review aims to present a review of literature that focuses on the importance and role of leadership skills in the surgeon and how these skills can play a significant role in increasing the quality of care and patients' safety in operating room (OR) settings.

METHODS

A systematic review of the available literature was performed on leadership skills in the operating room. The search was made on the PubMed, JSTOR, and Cochrane library databases on June 10th, 2020 using the following formula: (Leadership skills AND Surgeons AND Operating Room). First, the articles were screened by title and abstract. The following inclusion criteria for relevant articles were used during the initial screening of titles and abstracts: must focus on leadership skills of surgeons, must focus on models of leadership for surgeons, and must focus on skills required by surgeons to be good leaders for the OR team. Other exclusion criteria were: studies published before 2010, studies other than the English language, studies conducted in under-developed countries, studies focusing on non-technical skills other than leadership skills.

In the second step, the full texts of the selected articles were screened, with further exclusions according to the previously described criteria. Moreover, articles not reporting clinical results were excluded. Relevant data were then extracted and collected in a single database with a consensus of the two observers to be analyzed for the present manuscript.

Table 1 provides the list of articles that are included in the review.

Table 1: List of articles included in the review.

Author (year)	Title
Yue-Yung Hu, Sarah Henrickson Parker, Stuart R Lipsitz, Alexander F Arriaga, Sarah E Peyre, Katherine A Corso, Emilie M Roth, Steven J Yule, Caprice C Greenberg (2016).	Surgeons' Leadership Styles and Team Behavior in the Operating Room
Arora, S., Hull, L., Fitzpatrick, M., Sevdalis, N. and Birnbach, D. (2015).	Crisis Management on Surgical Wards.
Balch, C., Shanafelt, T., Sloan, J., Satele, D. and Freischlag, J. (2019).	Distress and Career Satisfaction Among 14 Surgical Specialties, Comparing Academic and Private Practice Settings.
Culley, D., Fahy, B., Xie, Z., Lekowski, R., Buetler, S., Liu, X., Cohen, N. and Crosby, G. (2014).	Academic Productivity of Directors of ACGME-Accredited Residency Programs in Surgery and Anesthesiology.
Danis, D., Klinkner, G., Malec, A. and Rees, S. (2007).	Improving Patient Safety by Standardizing Handoff Communications.

Author (year)	Title
Dominguez, D., Garcia, C. and LaFrance, K. (2013).	Developing A New Graduate Program In Healthcare Management: Embracing The Transformation Of Healthcare Management Education On A Pathway To Success.
Doumouras, A., Keshet, I., Nathens, A., Ahmed, N., and Hicks, C. (2012).	A Crisis of Faith? A Review of Simulation in Teaching Team-Based, Crisis Management Skills to Surgical Trainees.
Graafland, M., Schraagen, J. and Schijven, M. (2012).	Systematic review of serious games for medical education and surgical skills training.
Hudspeth, R. (2008).	Complex Healthcare Regulation.
Khatrouch, I., Kaddes, M., Kermad, L., Boujelbene, Y. and ElMhamedi, A. (2013).	New approach for building multidisciplinary teams: case of surgical teams.
Kothari, S., Brethauer, S., Rosenthal, R., Nguyen, N. and Morton, J. (2015).	Response to “Ensuring Excellence in Centers of Excellence Programs”.
McKean, S. (2012).	Principles and practice of hospital medicine.
Mitchell, L., Flin, R., Yule, S., Mitchell, J., Coutts, K. and Youngson, G. (2011).	Thinking ahead of the surgeon. An interview study to identify scrub nurses’ non-technical skills.
Mumghamba, E. (2013).	Integrating a Primary Oral Health Care Approach in the Dental Curriculum: A Tanzanian Experience.
Parker, S., Flin, R., McKinley, A. and Yule, S. (2019).	The Surgeons' Leadership Inventory (SLI): a taxonomy and rating system for surgeons' intraoperative leadership skills.
Parker, S., Yule, S., Flin, R. and McKinley, A. (2012).	Surgeons' leadership in the operating room: an observational study.
Patel, V., Warren, O., Humphris, P., Ahmed, K., Ashrafian, H., Rao, C., Athanasiou, T. and Darzi, A. (2010).	What does leadership in surgery entail?
Siassakos, D., Fox, R., Bristowe, K., Angouri, J., Hambly, H., Robson, L. and Draycott, T. (2020).	What Makes Maternity Teams Effective and Safe? Lessons From a Series of Research on Teamwork, Leadership, and Team Training.
Sittig, D. (2011).	Defining Health Information Technology–Related Errors.
Stevens, L., Cooper, J., Raemer, D., Schneider, R., Frankel, A., Berry, W. and Agnihotri, A. (2017).	Educational program in crisis management for cardiac surgery teams including high realism simulation.
Thomas, E., Sexton, J. and Helmreich, R. (2003).	Discrepant attitudes about teamwork among critical care nurses and physicians*.
Valentine, M., Nembhard, I. and Edmondson, A. (2014).	Measuring Teamwork in Health Care Settings.
Winlaw, D., Large, M., Jacobs, J. and Barach, P. (2011).	Leadership, surgeon well-being and non-technical competencies of pediatric cardiac surgery.
Yeung, J., Ong, G., Davies, R., Gao, F. and Perkins, G. (2012).	Factors affecting team leadership skills and their relationship with quality of cardiopulmonary resuscitation
Yule, S. and Paterson-Brown, S. (2012).	Surgeons’ Non-technical Skills.

RESULTS

Importance of Leadership and Teamworking for Surgeons

From the technological point of view, surgery is highly complex and is a practice that faces a constantly changing scenario. The surgeon works in the Department of Surgery, often in a specialized unit, and participates with interdisciplinary teams, and develops their daily clinical practice with other specialists and technicians working in different settings (such as operating room, ward) [6]. The primary objective of a surgeon is to provide the best patient care, with greater safety. When required today in most prestigious forums, safe surgery for the patient, it must be remembered that the complications that arise during treatment (about 45% in surgical patients) are rarely the result of the failure of a single individual, but in over 80% of cases they are related to poor planning of patient care systems, or with failures in teamwork [7].

A significant number of studies draw attention to the importance of effective teamwork, which by nature must be interdisciplinary, to get quality treatment over the surgical patient stays. Among them are two: the one entitled “To Err is Human: Building a Safer Health System” [8] published by the Institute of Medicine in 2000, in which the high percentage of errors detected was evaluated in the Health System of the USA and the well-known Kennedy Report in May, which analyzed the very high mortality of pediatric cardiovascular surgery service in Bristol, UK. As a result of the Surgery Quality Improvement Project with which has reduced mortality by a third and two-fifths in morbidity in all the services of surgery [9]. They participated in June. The overall analysis has led to a clear conclusion: the causes of human errors and their adverse consequences for the patient are deficiencies related to teamwork, as well as the communication between its members.

While the topical figure of an individualist and arrogant surgeon, and even violent with his surgical environment, has already been exceeded, it is not uncommon that surgeons think they work together, when in fact, most do in the group [10]. Team working requires leadership essentially, to ensure that decisions making is done by consensus, analyze the reasons for the disagreements and try to fix them, ensure that the objectives are understood and accepted by the team, encourage all members to contribute their ideas, require the operation of the equipment is periodically reviewed [11]. It can only be made possible by the work of each of its members to appreciate, and finally, that leadership is shared, as necessary. Teamwork is not easy, but with good leadership and proper training of its members in cognitive abilities, and personal relationships, it can be achieved.

The results of recent research, advise that surgical services training programs to develop teamwork in specific tasks (operating room, emergency department, hospital ward), as has already been done, and successfully in other high-risk professions, such as aviation, by applying the methodology called crew resource management (CRM). The key elements of this methodology that can be applied to the work of the surgeons are: the "briefing" (a previous discussion of the action plan), the question between senior member and juniors team of decisions, and take "de-briefing" sessions (understood as the discussion of what happened afterward). Compliance with these requirements can only be achieved in an atmosphere of work and communication between members of a team in which everyone is always willing to learn [12].

The "Non-technical skills for surgeons' project, designed by the University of Aberdeen, has identified several cognitive skills and personal relationships, which must be the essential complement to the expertise of the surgeon, to keep the OR, high levels of quality and safety [13]. These skills include the surgeon's ability to exercise leadership, the state of permanent alert, the ability to take the right decision at all times, and the ability to stimulate communication and teamwork. It is currently available instruments to assess, on stage at the theatre, the teamwork, and skills that are not just technical.

Several studies have shown a relationship between teamwork (surgeons, anesthetists, and instrumentalists) [14], the "performance" and safety in the operating room. It is estimated that up to 1/3 of the duration of the activity in an operating room, communication failures occur in the team. 33% of these failures originate delays, workarounds an unsolved problem ("workaround"), increased environmental stress, unnecessary expense, and disturbance to the patient. A recent study assessed for the first time, the impact that a training course in non-technical skills (CRM methodology) had on the results of two types of surgery, showing that the improvement observed in the teamwork produced the best technical results. These data confirm, albeit in a different field, the results of the multicenter project MedTeams [15], in the US, where the application of CRM in the emergency services model was evaluated and found to a reduction in clinical errors (30.9 to 4.4%) and improved teamwork and attitudes of its members [16]. This is the clearest example of how improved teamwork can avoid complications. It is possible that system-based training, as well as patient simulators that allow the practice of specific behaviors, can avoid the need for real instructors.

However, since efficient teamwork does not arise spontaneously, should be encouraged as soon as possible the development of new hospital culture, for the hospital administration and the Department of Surgery must provide the training tools to help its implementation. But above all, it is essential to have well-trained surgical leaders, able to motivate and change, for the good behavior of team members, with the ultimate goal, to always be the best possible result for the patient. It would be desirable that scientific societies and the NHS itself develop programs similar to the Royal College of Surgeons of England, aimed at the specific training of leaders, and their corresponding surgical equipment, improving, no doubt, patient safety [17].

Leadership in Surgeons

Leadership is one of the most important qualities for a successful surgeon. Surgical outcomes depend critically on a surgical team rather than on an individual; therefore, the role of surgeons is essential for optimal management of patients in a surgical service [18]. There are different kinds of leadership: one who asks the group to follow him, the group encouraged to follow you, or those who are forced to follow. It is important to remember that the most effective leader is using different styles at different times. One should be aware of the strengths and weaknesses in him/her as a leader and recognize what kind of leadership is most often used, as must implement leadership strategies in line with the situation [19].

Several points should be present in every surgeon in assuming the leadership role within a surgical team and in OR [20]:

- One must learn the art of conflict resolution: it is important to remember that there is a conflict over a point of view and always more of a perception of truth. The surgeon should try to understand each different point and perspective, but it has not always solved them, has to determine the ideal to maintain the cohesion of the group and its mission.

- Role model: is defined as a guide; they are people showing the right path to be followed, through their actions and behaviors. It is a huge responsibility and a great opportunity to demonstrate their attitudes and skills. They can demonstrate such using a suitable treatment with the patient, excellent academic foundation, and good relationships.

- Counsellor: young interns and residents come daily to the surgeon with conflicts, patient care questions, personal problems, etc. In these situations, the surgeon should be a director and confidant. He/she should be affable, confident ear, who can provide advice on mental and physical health for the residents. Also, he/she helps monitor the stress level of the residents.

- It is the right program director to indicate the stress level of the residents; remember the old phrase of "praise in public and criticize in private". Feedback should attentively, timely, and honest, but critical. Compliments are an effective way to encourage positive behavior, academic, interpersonal, and professional effort among residents.

CONCLUSIONS

In conclusion here are some final tips for excellent leadership development for surgeons:

1. Be seen as someone approachable and trustworthy; residents gain a better understanding as people.

2. Time Management: schedule daily activities; new things appear daily, so the surgeon should learn to prioritize the moment they happen.

3. Surgeons must take time for personal growth and professional development.

4. Surgeons must create a relationship with their boss, additional close work with the program director.

Limitations of this Study

Since this is a systematic literature review, therefore, the main limitation of this study is that it lacks primary research. This is a purely secondary research study although the research reviewed in this review does contain primary research evidence.

Also, the author of this review could only conduct searches in only three databases. Another limitation of this review is that it includes studies in English only and therefore studies published in another language have been excluded.

The scope of the review is also limited because it focuses on the leadership skills of surgeons only and does not focus on the leadership skills of other staff in the operating room. Although there are several limitations in this review yet as explained in previous sections the search strategy is comprehensive and systematic which ensures reliability and validity of the results and findings.

Implications of this review

The main implication of this review is for surgeons and students. They can understand the importance of leadership skills and include the development of leadership skills in their development plans.

Besides, the management and administration of hospitals can also benefit from this review and motivate surgeons and other staff to develop leadership skills and ultimately maximize the quality of care and patients' safety in their hospitals.

The review stresses the importance of leadership skills and therefore it recommends that administrations should plan and implement training courses for surgeons and OR staff to enhance their leadership skills.

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ARTICLE REMOVED ON REQUEST OF THE AUTHORS

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ORIGINAL ARTICLES

The effect of Dexmedetomidine on sedation of patients undergoing open-heart surgery in a military hospital

Seyed M.A. Zavareh[1], Marzieh Lak[2]

Abstract: *Background: Dexmedetomidine facilitates patient communication with the ICU nurses, and is a valuable drug for use in patients undergoing mechanical ventilation due to a reduction in the duration of mechanical ventilation and prevalence of delirium.*

Objectives: In this study, investigators aimed to compare the effect of Dexmedetomidine with placebo on sedation in patients undergoing open-heart surgery.

Methods: In this double-blind clinical trial, 69 patients with open-heart surgery who underwent a cardiopulmonary bypass pump, were randomly assigned into two groups of Dexmedetomidine (group D) and placebo (group P). At the end of the operation, patients in groups D and P respectively received 1 µ / kg infusion of Dexmedetomidine and normal saline in 10 minutes. In case of a lack of proper sedation, midazolam with the doses of 1 mg titration was used up to reaching the goal of sedation. Also, for pain relief, morphine titrated at a dose of 1 mg was Injected, and dosage was recorded in both groups. In each shift, patients were examined for delusions by the nurse involved in their care, using the "Confusion Assessment Method for the ICU (CAM-ICU)". For patients with delirium, 1 to 5 mg of Haloperidol was used. Also, the length of stay in the ICU was recorded.

Results: There was no significant difference between the two groups in systolic blood pressure, diastolic blood pressure, and heart rate at different stages of treatment ($p<0.05$). comparison of the two groups in terms of the length of stay in ICU, duration of mechanical ventilation, staff satisfaction, and occurrence of Delirium after surgery indicated that there were significant differences between the two groups ($p<0.05$). There was a significant difference between the two groups in the dose of morphine and midazolam ($p<0.05$).

Conclusion: This study showed that sedation with Dexmedetomidine significantly reduced the incidence of postoperative delirium, the length of stay in ICU, the duration of mechanical ventilation, the satisfaction rate of ICU staff, and the dose of analgesic and sedative medication. As there were no reported hemodynamic complications as well, it seems that it can be indicated as a safe and appropriate drug for sedation of patients in the ICU.

Keywords: *Dexmedetomidine, Intensive care unit, Open heart surgery, sedation*

BACKGROUND

Propofol and Dexmedetomidine are preferred drugs for sedation and short-term sleep in ICU [1]. The use of Dexmedetomidine in ICU has a similar cost and prognosis to Propofol [2]. Dexmedetomidine is a potent and selective agonist of alpha-2 receptors, which has sedative, anxiolytic, and analgesic effects. The effects of sleepy sleep are similar to normal sleep, so patients taking Dexmedetomidine easily

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wake up and cooperate [3]. The rate of respiratory depression is at least [3, 4]; and can be safely used in patients who are not mechanically ventilated [4]. In 2003, the use of Dexmedetomidine was approved by the FDA [3]. Dexmedetomidine is preferable to routine drugs for up to 24 hours for mild to moderate sedation [5] and is easy to use in patients undergoing mechanical ventilation in ICU. It reduces the duration of mechanical ventilation compared to midazolam [6, 7], and the time of extubation compared to midazolam and propofol [5, 6]. The use of Dexmedetomidine alone has led to less anxiety after extubation [7]. Among sedative and analgesic drugs, Dexmedetomidine is a unique drug due to brain protection. Its properties make it possible for ICU patients to be used and reduce the complications of ICU admission, such as cognitive impairment [8]. Sedation by Dexmedetomidine reduces the risk of developing delirium [4, 6, 7, 9], and can even treat delirium [4]. Compared to Propofol, Dexmedetomidine decreases the prevalence and duration of delirium after cardiac surgery in the elderly [10].

Dexmedetomidine facilitates patient communication with ICU nurses [6]. It is a valuable drug for use in patients undergoing mechanical ventilation due to a reduction in the duration of mechanical ventilation, length of stay in the ICU, and prevalence of delirium [11]. However, it is reported that using guidelines to limit the use of Dexmedetomidine is cost-effective [12]. The previous study recommended limiting the dose of Dexmedetomidine to 0.7 µg/kg/hour, the use of bolus fentanyl doses before the onset of infusion of Dexmedetomidine, and the use of certain criteria for non-intubated patients [13].

In this study, the authors aimed to compare the effect of Dexmedetomidine with placebo on sedation in patients undergoing open-heart surgery.

MATERIALS AND METHODS

In this double-blind clinical trial study, 69 patients with open-heart surgery who underwent a cardiopulmonary bypass pump, who were referred to Baqiyatallah Hospital, were randomly assigned into two groups of Dexmedetomidine (group D) and placebo (group P). Emergency patients, patients with liver failure, renal failure, grade 2 or 3 heart block, systolic blood pressure below 90 mmHg, history of neurological and psychiatric illnesses, use of psychiatric drugs, opiate addicts, redo surgery, and patients requiring re-surgery Were excluded.

To induce anesthesia, investigators used fentanyl at a dose of 500-700 µg and diazepam at a dose of 10 mg. To facilitate endotracheal intubation, cisatracurium was used at a dose of 0.15 mg/kg. To maintain anesthesia, infusion of fentanyl, midazolam, and cisatracurium was used and propofol infusion was considered if needed. With the help of TNG and inotropic drugs, systolic blood pressure was maintained in the range of 95 to 115 mmHg. During CPB, blood pressure was maintained at 60 to 80 mmHg and hematocrit between 20 to 22%. When patients found appropriate conditions for acid and base, electrolytes and hematocrit (22% or more), they were separated from the pump.

In group D, at the end of the operation in the operating room, when the patient was completely monitored and mechanically ventilated, patients received 1 µ/kg infusion of Dexmedetomidine in 10 minutes and were transferred to ICU. In ICU, the dose of 0.2 µg/kg/h was used for the initiation, and depending on the patient's need, to achieve Richmond agitation-sedation scale (which was scored from −5 [unresponsive] to +4 [combative], −2 to +1 (lightly sedated to restless)), the dosage was increased up to 0.7 µg/kg/h, Increased. After reaching the extubation condition, the patient was extubated and the dose of Dexmedetomidine was continued to achieve RASS= 0 (calm and alert).

In group P, after the completion of the surgery, a placebo (normal saline) was infused over 10 minutes and the patients were transferred to the ICU. In ICU, placebo infusion continued and the purpose of sedation was reaching to mechanical ventilation, RASS= -2 to + 1, and after extubation, RASS = 0.

For the lack of proper sedation, midazolam with a dose of 1 mg titration was used up to reaching the goal of sedation, and the dosage was recorded. Also, for pain relief, morphine titrated at a dose of 1 mg was Injected, and dosage was recorded in both groups.

The patient was extubated under acceptable conditions in terms of hemodynamics, acid and base, and alertness. The mechanical ventilation period was indicated from the end of the surgery to the extubation of the patient. During the period of mechanical ventilation, continuous monitoring of the vital signs was considered for both groups; and in case of hypotension (systolic blood pressure less than 90 mmHg or 30% reduction in blood pressure relative to the initial pressure) and also in case of bradycardia (heart rate Under 40/min), infusion of the drugs was discontinued, and in the absence of improvement, they were treated with ephedrine at a dose of 10 mg and atropine at a dose of 0.1 mg/kg, respectively.

In each shift, patients were examined for delusions by the nurse involved in their care, using the "Confusion Assessment Method for the ICU (CAM-ICU)”. For patients with delirium, 1 to 5 mg of Haloperidol was used. From the arrival of the patient to the ICU until the discharge from the ICU, it was recorded as the length of stay. At the time of the patient's discharge from the ICU, the satisfaction rate of three of the personnel involved in patient care was assessed using the Visual Analogue Scale (VAS) scoring method. Patients were monitored for ECG, and any new-onset arrhythmia was recorded. Data were gathered by a form including age, sex, type of surgery, CPB time, Cross Clamp time, systolic blood pressure, diastolic blood pressure, and heart rate. Investigators examined the effect of Dexmedetomidine on patients' hemodynamics, delirium, the length of stay in ICU, the duration of mechanical ventilation, and the dose of sedative medications. P-value < 0.05 was considered statistically significant.

Ethical Considerations: This research project was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences (number: IR.BMSU.REC.1398.090 date: 2019-06-25) and it was registered in the Iranian registry of clinical trials (IRCT20161022030421N4). Consent letters were obtained from participants.

RESULTS

In this study, 69 patients in two groups were evaluated consisting of 34 patients in the group D and 35 patients in group P. There was no significant difference between the two groups based on age, sex, and type of surgery (Table 1) (p=0.514, p=0.555, p=0.143 respectively).

Table 1: Demographic and type of surgery base on groups

		Case (N=34)		Control (N=35)		P-Value
		Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	
Age		59.8±12.04	21-78	61.6±11.15	38-79	0.514[1]
		N (%)		**N (%)**		
Gender	Female	15 (44.1)		13 (37.1)		0.555[2]
	Male	19 (55.9)		22 (62.9)		
Type of Surgery	CABG	23 (67.6)		29 (82.9)		0.143[2]
	Valvular	11 (32.4)		6 (17.1)		

1: Independent Sample T-Test; 2: Pearson Chi-Square Test

According to preoperative factors, there was no significant difference between the two groups (Table 2) (p<0.05). CPB time was 59.7±18.87 minutes in group D and 61.6±18.69 minutes in group P and no significant difference was noted (p=610).

Table 2: Comparison of the two groups based on pre-operative factors

		Case (N=34)		Control (N=35)		P-Value
		Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	
Clinical factors	Ejection fraction	46.6±8.5	30-60	47.3±8.26	20-55	0.797[1]
		N (%)		**N (%)**		
	Hypertension	26 (76.5)		25 (71.4)		0.633[3]
	Diabetes mellitus	17 (50)		22 (62.9)		0.281[3]
	Kidney disease	5 (14.7)		3 (8.6)		0.477[4]
	Lung disease	5 (14.7)		9 (25.7)		0.256[3]
	Smoker and Opioid addiction	9 (47.4)		10 (52.6)		0.845[3]

1: Mann-Whitney Test; 2: Independent Sample T-Test; 3: Pearson Chi-Square Test; 4: Fisher Exact Test

Also, Cross Clamp time was 46.8±17.15 minutes in group D and 50.4±19.6 minutes in group P, and no significant difference was shown between groups (p=501). There was no significant difference between the two groups in systolic blood pressure, diastolic blood pressure, and heart rate at different stages of treatment (Tables 3-5) (p<0.05).

Table 3: Comparison of the two groups based on systolic blood pressure (mmHg) in different stages

	Case (N=34)		Control (N=35)		P-Value
	Mean±SD	Min-Max	Mean±SD	Min-Max	
Primary (Before drug)	110.3±18.93	80-161	113.9±21.07	74-175	0.47[1]
During bolus dose infusion	116.2±14.49	80-143	117.8±15.65	92-150	0.669[1]
During mechanical ventilation	118.3±12.9	99-145	116.5±14.41	91-149	0.577[1]
After mechanical ventilation in ICU	118.2±12.01	94-148	120.3±12.6	100-146	0.485[1]

1: Independent Sample T-Test

Table 4: Comparison of the two groups based on diastolic blood pressure (mmHg) in different stages

	Case (N=34)		Control (N=35)		P-Value
	Mean±SD	Min-Max	Mean±SD	Min-Max	
Primary (Before drug)	65.8±13.65	45-99	66.2±15.37	33-101	0.893[1]
During bolus dose infusion	68.5±10.53	51-95	67.8±11.19	50-100	0.789[1]
During mechanical ventilation	67.7±8.78	51-90	66.9±10.23	51-99	0.529[1]
After mechanical ventilation in ICU	66±7.58	53-87	68.5±9.29	51-96	0.198[2]

1: Independent Sample T-Test; 2: Mann-Whitney Test

Table 5: Comparison of the two groups based on heart rate (N) in different stages

	Case (N=34)		Control (N=35)		P-Value
	Mean±SD	Min-Max	Mean±SD	Min-Max	
Primary (Before drug)	83.8±14.43	60-110	88.2±13.83	59-113	0.206[1]
During bolus dose infusion	82.4±14.89	55-108	89.7±17.78	53-138	0.073[1]
During mechanical ventilation	83±16.2	55-118	90.3±15.54	51-126	0.067[1]
After mechanical ventilation in ICU	83±14.04	53-115	90.7±15.49	50-122	0.037[1]

1: Independent Sample T-Test

In terms of the prevalence of delirium after surgery; in group D, 5 patients (14.7%) and group P, 16 patients (45.7%) were delirious and the comparison between the two groups showed a significant difference (p=005).

The duration of mechanical ventilation in group D was 8.2±4.32 hours, and in the group, P was 12.7±3.94 hours. There was a significant difference between groups (p<0.001). Comparison of the two groups regarding the length of stay in ICU showed that in group D, length of stay in the ICU was 3.2±0.48 hours and in the group, P was 3.8±0.65 hours, and the difference was significant (p<0.001).

The satisfaction rate of three nurses involved in patient care showed that the VAS score was 9.2±1.2 in group D and 6.9±1.77 in group P and a significant difference was noted (p<0.001).

Regarding the opioid dose in the two groups: The mean dose of morphine in the D group was 1.18±2.7 mg (Min-Max 0-13) and in the P group 8.5±4.12 mg (Min-Max 0-15), with a significant difference between the two groups (p=0.001). Comparison of the dose of the sedative drug in the two groups: The mean dose of midazolam in the D group was 1.5±2.75 mg (Min-Max 0-13) and in the P group 3.7±3.88 mg

(Min-Max 0-13), the difference between the two groups being significant (p=0.004). New-onset atrial fibrillation occurred in one patient in group D, and four patients in group P.

DISCUSSIONS

In this study, investigators evaluated patients undergoing open-heart surgery and transferred to intensive care units with mechanical ventilation, in two groups of Dexmedetomidine and placebo. There was no significant difference in systolic and diastolic blood pressure and heart rate between the two groups at different stages. Mechanical ventilation time and length of stay in ICU in the Dexmedetomidine group were significantly reduced. The prevalence of delirium in group D was also significantly decreased. The satisfaction of ICU nurses was significantly higher in group D. Patients in group D required significantly lower doses of morphine and midazolam. New-onset atrial fibrillation occurred in one patient in group D and four patients in group P.

Fish and colleagues implemented an updated sedation and analgesia guideline, which was provided by the community of critical care medicines (SCCM) on their patients. They compared the prognosis of the patients, one to five years after the implementation of the protocol, with one year before proceeding with the protocol. Their protocols included 1 - Using a bolus dose of analgesic and sedative medicine before starting the continuous infusion; 2 - Using concomitant sedatives and analgesics; 3 - Using Propofol and Dexmedetomidine instead of midazolam, when needing the continuous infusion of sedative, and 4 - measuring pain in patients in the intensive care unit. They concluded that with this guideline, the duration of mechanical ventilation, the length of stay in the ICU, and the duration of admission to the hospital were decreased, without any increase in mortality. It was also economically feasible [14].

Diaz and colleagues at Northwest Hospital assessed patients taking Dexmedetomidine. The evaluation data included the primary diagnosis of the disease, the indication of the use of Dexmedetomidine, the duration of treatment, the number of consumed needs, the infusion rate, the recorded CAM-ICU score, the amount of Propofol, opiate and the benzodiazepine supplement, the length of stay in the ICU and the duration of staying in the hospital. They appropriately prescribed Dexmedetomidine for all patients admitted to ICU and had CAM-ICU and RASS in over 92% of patients. Their goal was to achieve light sedation; the dose was not higher than 1.2 µg/kg/hour, and the inappropriate use of the drug was longer than 48 hours when the Propofol was used simultaneously [15].

Furthermore, Elzohry and colleagues used Dexmedetomidine alone to induce anesthesia in the endoscopy of the gastro-intestinal tract with bolus dosage of one microgram per kilogram and then 0.8 micrograms per kilogram per hour, concluding that, this drug can provide adequate analgesia and sedation, with rapid onset and rapid recovery in these patients, and the occurrence of complications such as bradycardia and hypotension was not serious and could be controlled [16].

Previous studies [1, 14, 15] were the confidence point for the current study to use this drug with a dose of 1 µg/kg for patients transferred to the ICU, and the results of the study showed that using Dexmedetomidine at this dose was not associated with hemodynamic disorders and had no adverse effects.

Morelli and colleagues in a cross-over study in patients with septic shock treated patients with Propofol, switched Propofol to Dexmedetomidine for 4 hours and re-set Propofel for 8 hours, and evaluated the degree of sedation and the dose of Norepinephrine at the end of these hours. They concluded that changing the drug from Propofol to Dexmedetomidine would reduce the dose of norepinephrine in patients with septic shock [17].

The same with the current study, Morelli, and colleagues addressed anxiety in using this drug. However, when it was used in patients with septic shock without complications, it seems that it can be used with less concern in ICU patients.

Guo and his colleagues assessed the patients undergoing mechanical ventilation randomly in two groups. In the control group, the sedation score was, 3 to -4 (RASS, 3 to -4), and in the intervention group, the sedation score was, 0 to -1 (RASS, 0 to -1) (that is, the condition that the patient was awake, be comfortable and collaborative). They concluded that in the intervention group, the duration of mechanical ventilation and the length of stay in the ICU was shorter, the prevalence of pneumonia due to mechanical ventilation, and delirium was lower, and the dose of sedative medication decreased [18].

In the current study, investigators attempted to put the patient's sedation around, -2 to +1 RASS and did not have any complications at this level. In a study on 498 non-surgical and surgical patients admitted to the ICU, Schrader and colleagues examined two Propofol and Dexmedetomidine drugs for hemodynamic disorders. For the first time, hemodynamic instability and frequency of hemodynamic instability were the benchmarks for comparing two drugs. They concluded that hemodynamic instability did not differ significantly between the two groups of Propofol and Dexmedetomidine in terms of duration and frequency [19].

This study, consistent with all of the studies mentioned, demonstrated the safety and the appropriateness of the use of Dexmedetomidine for the sedation of patients in the intensive care unit. In confirming Baylon's study, Dexmedetomidine should probably be a counterpart to sedative medications, especially in patients admitted to ICU [20].

In a retrospective study, Brianna Thompson and colleagues compared the effects of Propofol and Dexmedetomidine on the incidence of atrial fibrillation after cardiac surgery. They noted no significant difference in the prevalence of atrial fibrillation between the two groups because of the significant difference in prophylactic use of amiodarone [21]. In the current study, new atrial fibrillation occurred in one patient in the sample group and four patients in the control group in the ICU. Therefore, more research is needed to assess the effect of Dexmedetomidine in the prevention of new-onset arrhythmia.

CONCLUSION

This study showed that sedation with Dexmedetomidine significantly reduced the incidence of postoperative delirium, length of stay in ICU, duration of mechanical ventilation, the satisfaction rate of ICU staff, and the dose of sedative medication. As there were no reported hemodynamic complications as well, it seems that it can be indicated as a safe and appropriate drug for sedation of patients in the ICU.

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ORIGINAL ARTICLES

The assessment of the vitamin D deficiency between the patients with acute coronary syndrome and those with stable coronary artery disease in a military hospital

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Abstract: *Background: A few studies compared the vitamin D deficiency condition between the patients with the acute coronary syndrome (ACS) and those with stable coronary artery disease. The present comparative study aimed to assess the vitamin D deficiency status between the patients with ACS and those with stable coronary artery disease.*

Methods: This cross-sectional study was performed on 200 consecutive patients with the diagnosis of ACS or stable coronary artery disease. Serum Vitamin D level was measured by immunoassay and its serum level was categorized as normal (> 30 ng/ml), insufficient (20 to 30 ng/ml), and deficient (< 20 ng/ml).

Results: Overall, 103 patients were categorized as stable angina group, 74 had unstable angina, 10 had STEMI, and 13 had NSTEMI. There was no difference in serum vitamin D3 level across the four groups including stable angina group (13.81 ± 15.13 ng/ml), unstable angina group (15.13 ± 11.11 ng/ml), STEMI group (21.58 ± 19.87 ng/ml), and NSTEMI group (18.67 ± 13.07 ng/ml) (p = 0.276). No difference was also found in the mean vitamin D3 level between the groups with new MI (15.98 ± 12.44 ng/ml versus 14.01 ± 15.31 ng/ml, p = 0.318). In total, 8.5% had normal vitamin D levels and 9.0% had vitamin D insufficiency, while the majority of patients (82.5%) were vitamin D deficient. The vitamin D deficiency was comparable in the patients with stable angina compared to those in ACS groups. According to ROC curve analysis, measuring serum vitamin D3 had a moderate value to discriminate stable angina from ACS (AUC = 0.651). In this regard, the best cutoff point for serum vitamin D3 level to discriminate stable angina from ACS was 9.0 ng/ml yielding a sensitivity of 70.1% and a specificity of 57.3%.

Conclusion: The measurement of vitamin D and thus the presence of vitamin D deficiency have no sufficient power to discriminate ACS from stable coronary artery disease.

Keywords: *vitamin D deficiency, coronary syndrome, artery disease*

INTRODUCTION

Acute coronary syndrome (ACS) has been now known as the major cause of death whole of the world, especially in industrial countries. Potentially, the presence of various genetic, metabolic, and environmental risk factors can increase the risk for mortality and disability in affected patients [1]. In this regard, some metabolic and endocrinological factors have been identified to link the progression and severity of coronary artery disease (CAD). Within the last few years, a significant association between deficiency of some vitamins and minerals and CAD has been found [2-4]. In this regard, an association of vitamin D deficiency and different cardiovascular risk factors and

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diseases has been extensively evaluated. Some prospective and observational studies have addressed the possible linkage of vitamin D deficiency and the development of CAD and its risk factors [5, 6]. Thus, vitamin D deficiency not only has a major role in bone metabolism, homeostasis regulation, and immune pathways, but also the presence of vitamin D receptors on cardiomyocytes, endothelial cells, and vascular smooth muscle cells suggests the role of vitamin D in vitamin D-mediated cardiovascular system [7-9]. It has been shown that cardiac muscle functioning depends on the circulating blood concentration of calcitriol [10]. Calcitriol affects the growth, proliferation, and morphology of murine cardiomyocytes. In detail, treatment with calcitriol increased the expression of the cardiac muscle protein myotrophin. The link between vitamin D deficiency and cardiovascular risk factors has been also shown [11]. According to the Framingham Offspring Study [5], individuals with low activated vitamin D faced more with incident cardiovascular disease such as myocardial infarction, coronary insufficiency, and heart failure compared to those with a normal level of vitamin D. Thus, treatment with vitamin D supplementation could reduce cardiovascular death and length of hospitalization in ACS patients; however, a few studies compared the vitamin D deficiency condition between the patients with ACS and those with stable coronary artery disease. The present comparative study aimed to assess the vitamin D deficiency status between the patients with ACS and those with stable coronary artery disease.

METHODS

This cross-sectional study was performed on 200 consecutive patients with the diagnosis of ACS or stable coronary artery disease. The ACS was diagnosed according to the ACS criteria defined based on clinical symptoms, ECG changes, and cardiac enzyme rising. Stable coronary artery disease was defined as typical exertional chest pain relieved by rest or nitroglycerin administration and a positive exertional ECG test. The exclusion criteria were history of surgery within 6 months ago or having a hematologic disease or any hemoglobinopathy or coagulopathy, history of chronic clinical conditions such as liver or kidney disease or malignancies, or history of cerebrovascular disease or other simultaneous cardiovascular disorders.

Baseline characteristics and clinical data of participants including demographics, anthropometric parameters (weight, height, and body mass index), cardiovascular risk factors (current smoking, hypertension, hyperlipidemia, and opium addiction) as well as laboratory parameters (fasting blood sugar, hemoglobin A1c, lipid profile, thyroid functional hormones, and serum creatinine) were collected from the hospital recorded files. Serum Vitamin D level was measured by immunoassay and its serum level was categorized as normal (> 30 ng/ml), insufficient (20 to 30 ng/ml), and deficient (< 20 ng/ml). The primary endpoint was to assess the relation between the type of ACS and the severity of vitamin D deficiency. The secondary endpoint was to determine the best cutoff point for vitamin D to differentiate ACS from stable coronary artery conditions. The ethical committee of Kerman University of Medical Sciences approved the study research proposal all procedures were in accord with the standards of the World Medical Association Declaration of Helsinki for research involving human subjects. Informed consent was obtained from all study participants.

Results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Continuous variables were compared using t, and one-way ANOVA tests, or non-parametric Mann-Whitney U, or Kruskal-Wallis tests whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the groups. The ROC curve analysis was also used to determine the value of vitamin D measurement to predict the presence of ACS and also to determine the best cutoff value of this vitamin to discriminate ACS from stable angina. For the statistical analysis, the statistical software SPSS version 16.0 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

RESULTS

Overall, 103 patients were categorized as stable angina group, 74 had unstable angina, 10 had STEMI, and 13 had NSTEMI. Comparing baseline characteristics and clinical data (Table 1) across the groups showed that the patients with stable angina were older than the patients in other groups.

Also, hypertension was more frequent in those with STEMI, while hyperlipidemia was more prevalent in those with stable angina. Family history of CAD was found less and opium addiction was revealed more in the NSTEMI group when compared to other groups. Statin use, aspirin use as well as the history of prior PCI was all more in a group with stable angina than in other groups, while prior CABG was scheduled more in the NSTEMI group.

Meanwhile, there was no difference across the four groups in terms of gender, mean BMI, history of diabetes, smoking, alcohol use, thyroid dysfunction, prior SK use, and left ventricular dysfunction.

Table 1: Baseline characteristics and clinical data of study population

	Stable angina (N = 103)	Unstable angina (N = 74)	STEMI (N = 10)	NSTEMI (N = 13)	P-value
Male gender	60 (58.3)	46 (62.2)	7 (70.0)	10 (76.9)	0.555
Age, year	64.29 ± 8.85	59.92 ± 11.25	60.70 ± 12.09	61.92 ± 8.78	0.058
BMI, kg/m^2	27.92 ± 3.82	27.54 ± 2.93	26.43 ± 2.31	26.45 ± 3.94	0.333
Hypertension	87 (84.5)	46 (62.2)	10 (100)	9 (69.2)	0.002
Diabetes	34 (33.0)	33 (45.6)	5 (50.0)	6 (46.2)	0.480
Hyperlipidemia	96 (93.2)	17 (23.0)	0 (0.0)	3 (23.1)	< 0.001
Family history	44 (42.7)	31 (41.9)	4 (40.0)	3 (23.1)	0.035
Smoking	29 (28.2)	12 (16.2)	2 (20.0)	2 (15.4)	0.260
Opium use	7 (6.8)	5 (6.8)	1 (10.0)	2 (15.4)	0.033
Alcohol use	9 (8.7)	2 (2.7)	0 (0.0)	0 (0.0)	0.210
Hyperthyroidism	3 (2.9)	1 (1.4)	0 (0.0)	0 (0.0)	0.963
Hypothyroidism	7 (6.8)	1 (1.4)	0 (0.0)	0 (0.0)	0.611
CCU admission	69 (67.0)	29 (39.2)	5 (50.0)	5 (38.5)	0.011
Statin use	99 (96.1)	56 (75.7)	7 (70.0)	9 (69.2)	< 0.001
Aspirin use	90 (87.4)	53 (71.6)	8 (80.0)	9 (69.2)	0.023
NSAIDS use	2 (1.9)	2 (2.7)	0 (0.0)	0 (0.0)	0.883
Prior PCI	96 (93.2)	15 (20.3)	1 (10.0)	3 (23.1)	< 0.001
Prior SK receive	11 (10.7)	3 (4.1)	1 (10.0)	0 (0.0)	0.475
Prior CABG	3 (2.9)	13 (17.6)	3 (30.0)	2 (15.4)	0.013
LVEF	48.99 ± 8.18	47.58 ± 10.46	44.80 ± 10.29	43.85 ± 5.67	0.152

Regarding laboratory parameters (Table 2), mean serum FBS was significantly lower in the group with stable angina, while serum HDL level was higher among those with stable angina than other patients. The highest liver enzymes were revealed in those with STEMI. The patients with stable angina had a significantly higher level of T3 than other ones.

Table 2: Baseline laboratory indices in the study population

	Stable angina	Unstable angina	STEMI	NSTEMI	P-value
Hemoglobin	14.77 ± 2.89	13.89 ± 1.60	14.73 ± 1.12	14.16 ± 2.20	0.106
Hematocrit	48.61 ± 36.91	40.85 ± 4.46	41.78 ± 3.46	41.09 ± 6.48	0.258
Platelet	226.21 ± 65.72	218.84 ± 58.57	195.70 ± 48.04	223.54 ± 73.51	0.495
ESR	15.17 ± 6.46	16.14 ± 15.67	13.10 ± 7.56	14.69 ± 15.00	0.848
CRP	0.97 ± 8.36	0.32 ± 0.47	0.50 ± 0.52	0.62 ± 0.51	0.918
FBS	115.27 ± 36.98	143.47 ± 61.22	170.70 ± 68.86	166.85 ± 71.98	< 0.001
Creatinine	3.25 ± 14.27	1.33 ± 0.97	1.14 ± 0.21	1.19 ± 0.20	0.613
Uric acid	7.83 ± 11.78	5.59 ± 1.93	4.74 ± 1.44	6.45 ± 2.09	0.317
Triglyceride	157.69 ± 71.86	148.07 ± 86.70	137.50 ± 68.42	105.87 ± 93.43	0.153
Cholesterol	183.37 ± 50.72	171.80 ± 52.83	170.10 ± 40.04	170.23 ± 50.33	0.433
LDL	98.46 ± 30.33	105.40 ± 42.09	100.34 ± 25.09	105.91 ± 42.84	0.609
HDL	46.43 ± 30.16	36.38 ± 8.52	36.90 ± 7.91	34.54 ± 10.22	0.018
AST	21.36 ± 7.96	24.38 ± 19.29	65.40 ± 42.45	34.38 ± 26.31	< 0.001

	Stable angina	Unstable angina	STEMI	NSTEMI	P-value
ALT	26.89 ± 17.69	28.46 ± 24.30	52.00 ± 33.04	34.46 ± 22.99	0.005
ALKP	181.25 ± 73.84	185.70 ± 60.70	170.40 ± 30.67	225.92 ± 79.55	0.144
Calcium	13.56 ± 19.05	8.98 ±0.61	8.86 ± 0.33	9.16 ± 0.47	0.141
Phosphorus	4.16 ± 1.25	3.99 ± 0.58	3.38 ± 0.71	3.83 ± 0.72	0.091
PTH	30.48 ± 30.51	47.81 ± 21.74	43.29 ± 11.42	49.96 ± 15.40	0.227
T3	2.99 ± 3.86	0.89 ± 0.97	0.70 ± 0.14	0.68 ± 0.17	< 0.001
T4	45.44 ± 47.98	62.71 ± 19.19	65.74 ± 11.80	61.28 ± 11.44	0.102
TSH	4.19 ± 14.30	2.64 ± 4.32	2.14 ± 1.59	1.62 ± 1.18	0.698
HbA1C	6.33 ± 1.36	14.88 ± 70.03	7.99 ± 2.01	7.61 ± 2.63	0.622

There was no difference in serum vitamin D3 level across the four groups including stable angina group (13.81 ± 15.13 ng/ml), unstable angina group (15.13 ± 11.11 ng/ml), STEMI group (21.58 ± 19.87 ng/ml), and NSTEMI group (18.67 ± 13.07 ng/ml) ($p = 0.276$). No difference was also found in the mean vitamin D3 level between the groups with new MI (15.98 ± 12.44 ng/ml versus 14.01 ± 15.31 ng/ml, $p = 0.318$).

In total, 8.5% had normal vitamin D levels and 9.0% had vitamin D insufficiency, while the majority of patients (82.5%) were vitamin D deficient. As shown in Figure 1, the vitamin D deficiency was comparable in the patients with stable angina compared to those in other ACS groups ($p = 0.338$). However, vitamin D deficiency was more prevalent in the old MI group compared to the new MI group (87.0% versus 78.0%, $p = 0.011$).

Figure 1: Vitamin D deficiency in ACS subgroups

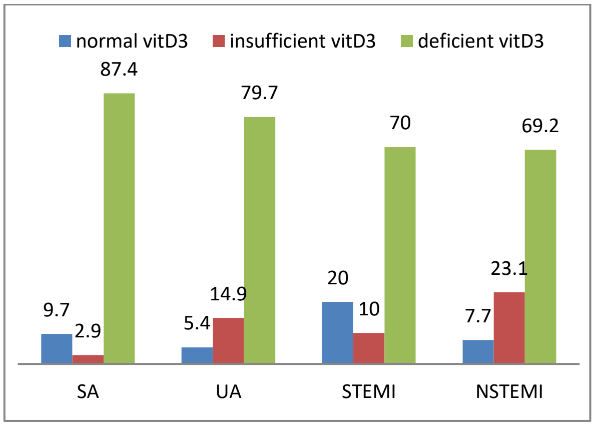

The level of vitamin D was similar in the patients with and without hypertension (15.04 ± 14.56 ng/ml versus 14.88 ± 11.93 ng/ml, $p = 0.9450$), the groups with and without diabetes mellitus (13.46 ± 4.17 ng/ml versus 15.35 ± 15.08 ng/ml, $p = 0.760$), and those with and without hypertriglyceridemia (13.29 ± 4.02 ng/ml versus 15.03 ± 12.49 ng/ml, $p = 0.714$).

According to ROC curve analysis, measuring serum vitamin D3 had a moderate value to discriminate stable angina from ACS (AUC = 0.651, 95% CI: 0.573 – 0.728, $P < 0.001$) (Figure 2). In this regard, the best cutoff point for serum vitamin D3 level to discriminate stable angina from ACS was 9.0 ng/ml yielding a sensitivity of 70.1% and a specificity of 57.3%.

Figure 2: ROC curve analysis to determine the value of vitamin D level to discriminate stable angina from ACS

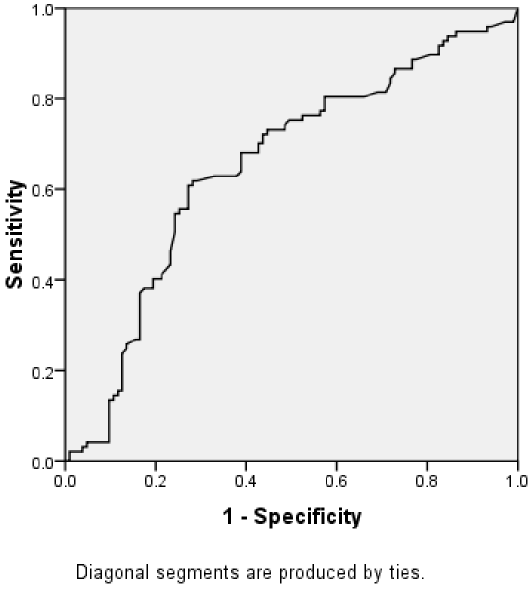

DISCUSSIONS

There is modest evidence that lower vitamin D levels are associated with an increased risk of CAD. However, few studies have researched CAD as a separate disease entity from cardiovascular disease. It has been now suggested that people with low vitamin D levels had twice the risk of developing a heart attack compared to those with adequate vitamin D levels, but it has remained uncertain the effect of vitamin D levels on CAD risk particularly in variant angina.

Hence, we attempted to compare the value of vitamin D measurement and thus vitamin D deficiency in differentiating stable coronary conditions from ACS variants. In this regard, we could demonstrate a similar incidence of vitamin D deficiency in stable coronary arteries and those without this condition. Even, using ROC curve analysis, it was shown that the measuring vitamin D level could discriminate stable coronary involvement and ACS variants with moderate power. However, a higher incidence of vitamin D3 might be predicted in those with old MI than in patients that newly suffered MI. In a study by Raina et al [12] in India, it was suggested a significant correlation between vitamin D deficiency and chronic stable angina. In India, results similar to the pointed were seen by Sanjeev Kumar et al. [13] who studied 100 patients undergoing CAG. They found that the mean 25(OH) D level was 14.8 ng/mL; vitamin D deficiency was present in 80% and only 7% had an optimal 25(OH) D level that was near to our result. More interestingly, another study that divided 2910 patients with coronary artery disease history (acute myocardial infarction – unstable angina – stable angina) into three groups based on their angiography results showed that although vitamin D deficiency is prevalent in all groups, it is more prevalent in patients with stable angina and also it is indicative of a worse prognosis [14] that was numerically near to our results.

The similarity in incidence of vitamin D deficiency in our study subgroups may be due to this fact that the majority of patients included into the study had vitamin D deficiency so 82.5% were vitamin D deficient that is a considerable finding. Another reason for the insignificant association between vitamin D level and the ACS variants could be related to no significant association between the level of vitamin D and various cardiovascular risk subgroups. As we assessed this association, the level of vitamin D was similar in cardiovascular risk subgroups. In fact, because of a probable causative relation between cardiovascular risk factors and vitamin D deficiency, our finding can be explained. Another reason for paradoxical results across the studies may be due to the different cutoff points for defining vitamin D deficiency. During a Framingham–Off spring study on 1739 participants who were followed up within 5.4 years Wang and colleagues [15] revealed that vitamin D deficiency is associated with cardiovascular disease. However in the mentioned study levels < 15 ng/ml were considered as vitamin D deficiency. Also, in Siadat et al study [16], it was considered levels lower than 30 ng/ml as vitamin D deficiency, while in our study, vitamin D deficiency was defined as the levels of lower than 20 30 ng/ml.

It seems that the effect of vitamin D on cardiovascular risk can be mediated by some mechanisms including reduction of the risk for underlying metabolic disorders such as diabetes and high blood pressure, lowering the thickening of the arterial walls, reduces the risk of respiratory infections, suppression of inflammatory pathways, and reduction the risk of arterial calcification or hardening [17-21]. Furthermore, some studies are indicative of a relation between vitamin D deficiency and cardiovascular disease, increased blood pressure, increased insulin resistance, heart failure, and fatal strokes [22-26] that can explain the association between CAD risk and vitamin D deficiency; however, this association was not proved in our study population.

As a new result, the best cutoff value for differentiating stable angina from ACS conditions was 9.0 that considerably lower than the cutoff values defined for vitamin D deficiency. We could not find a similar study on determining the best cutoff values for vitamin D to predict stable angina and thus the obtained result on this subject should be more assessed in further studies.

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ORIGINAL ARTICLES

An organizational chart of an Emergency Health Operation Center

Athanasios Zafeirakis[1], Panagiotis Efstathiou[2]

Abstract: *Nowadays, a wide range of natural and man-made disasters, disease outbreaks and pandemics give rise to health emergencies at a global level. Thus, a health security strategy is urgently required through central planning. At the national level, that vision can be implemented with a properly organized and fully functional Emergency Health Operation Center (EHOC). The main executive sections of an EHOC are Command, Planning, Operations, Logistics, Administration, Communications/Intelligence, and Reporting/Briefing. Each of the above sections has several functions and sub-functions, all operating according to a concrete legal basis and detailed operating procedures.*

Keywords: *emergency planning, public health, operation center, health crisis*

INTRODUCTION

In our epoch, due to the extreme complexity of the modern age, natural and man-made threats the management of health crises should imply an effective and integrated action plan, qualified with characteristics that define the effectiveness and success of such a system. An EHOC is such a system that can lead and coordinate the overall national medical response in every emergency incident, especially when involving mass casualties that either exceeds the management capabilities of the nation's health mechanism or disorganize the existing procedures planned for routine conditions. A successful EHOC should be based on two pillars: Legal authority and organizational structure; both will be herein briefly discussed.

DISCUSSION

Legal authority

The legal authority of an EHOC refers to the development of a concrete legislation directive for the public health authorities as regards roles, responsibilities, and coordination with national disaster management resources for legal health operations. That legislation should establish policy guidance, officials of the government and involved organizations, and other executive officers and professionals tasked to provide leadership at a strategic level. The legal authority of an EHOC varies among countries, while the key governmental agencies usually involved are unfortunately often scattered in various Ministries. The long list of those national agencies includes, but is not limited to the following: Fire Brigade; Police; Ministry of National Defence; Coast Guard; Ministry of Transport and Communications; Civil Airlines; Medical School Laboratories; National Public Health Organization; National Center for Emergency Assistance (NCEA); Hospital units (public and private); National Atomic Energy Committee; Forensic Services; Ministry of Foreign Affairs; Non-governmental healthcare and humanitarian responders [1]. The concomitant spread of leadership centers combined with defective multi-ministerial coordination has been proved devastatingly ineffective in the last decades in many environmental or man-made disasters throughout the world. Hence, an EHOC

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must be set under a unified command, a sort of an all-hazard ministry or deputy ministry "of Interior Threats", or "of Civil Protection"; that Ministry can substitute roles, authorities, personnel, and infrastructure of other Ministries and also replace at the highest level both the GSCP and all sorts of emergency committees, mechanisms and operation centers of other Ministries.

Organizational structure

An EHOC should operate within the framework of a thoroughly planned national incident management system, with dedicated personnel and a consolidated operational terminology. Organizational prerequisites for such a system are described in detail in relative handbooks [2-4] and involve at minimum the following executive sections:

1. Command
2. Planning
3. Operations
4. Logistics
5. Administration
6. Communications/Intelligence
7. Reporting/Briefing

The function of an EHOC should be based on competent personnel and equipment installed in a capacious building, consisting of at least an operation-command room, a communication room, and a multipurpose meeting room. The operational doctrine of an EHOC is based on the C4-I principle, i.e. Command – Control – Communication – Coordination – Intelligence. These procedures are prioritized for the planning and response to potential public health risks, albeit they do not replace the daily operational control of resources and services. The goal of C4-I is operational cross-disciplinary coordination at the regional, national and international level and is an integral part of the Concept of Health Operations [5]. Each of the above sections has several functions and sub-functions, (data illustrated in Figure 1), operating according to relevant response actions, called Standard Operating Procedures (SOPs), as described below:

1. Command Section: The EHOC director exerts ex officio the vertical, unified command. He/she is a senior medical officer, with documented expertise in the field of Health Crises and with vast experience in managing complex health operations. The EHOC director will be subordinated to the GSCP and will be supported at his command by a deputy director. Two branches are straight subordinated to the EHOC director:

a) A *Legal Operations Unit* (LOU), staffed by dedicated legal advisers, supports the EHOC leader in taking complicated decisions without unacceptably high legal risks (mainly in emergencies, when some regulations of normal life might need to be temporarily revised or suspended for EHOC operations to be carried out).

b) The *Ancillary Services Unit* (ASU), a supportive bundle of services. Apart from full secretarial support, that unit covers personnel's health, safety and security, cleaning, and janitorial affairs of buildings.

Apart from managing, coordinating, and collaborating skills, the EHOC director must be able to set and follow priorities and optimize results based on available resources. A thorough presentation of the required qualifications of an EHOC director is described by Efstathiou et al [6]. The EHOC director is responsible for the following specific functions:

- The overall operational function and legal responsibility of the EHOC.

- The application of effective command systems, which clearly define roles and responsibilities for all EHOC staff.

- The final responsibility for verification of any possible health event, mobilization of the national mechanism, and information of government. That highly demanding decision is accomplished through the systematic gathering of relevant information from various sources, such as operational contacts, expert networks, printed reports, and media information.

- The coordination (called "interoperability") among EHOC's sections and also between EHOC as a concrete system and any participant organization, including local and private agencies, when a major health incident/operation takes place. As major participants, apart from the national agencies are considered the following international stakeholders:

- Center for Disease Control and Prevention (CDC), with worldwide action; a European-CDC, as well as national CDCs also exist (Planning Section, bellow).
- Monitoring and Information Center (MIC): The operational heart of the United Nations Health Security Committee; mobilizes international aid for mass disaster incidents.
- World Health Organization (WHO): Provides the ultimate health assistance worldwide.
- North Atlantic Treaty Organization (NATO): An intergovernmental military alliance, also providing some specific health standardization agreements (STANAGs).

2. Planning Section: It emphasizes coordination, interoperability, and cooperative team working through relevant SOPs, with the following branches and sub-functions:

Figure 1: Organizational structure of an EHOC

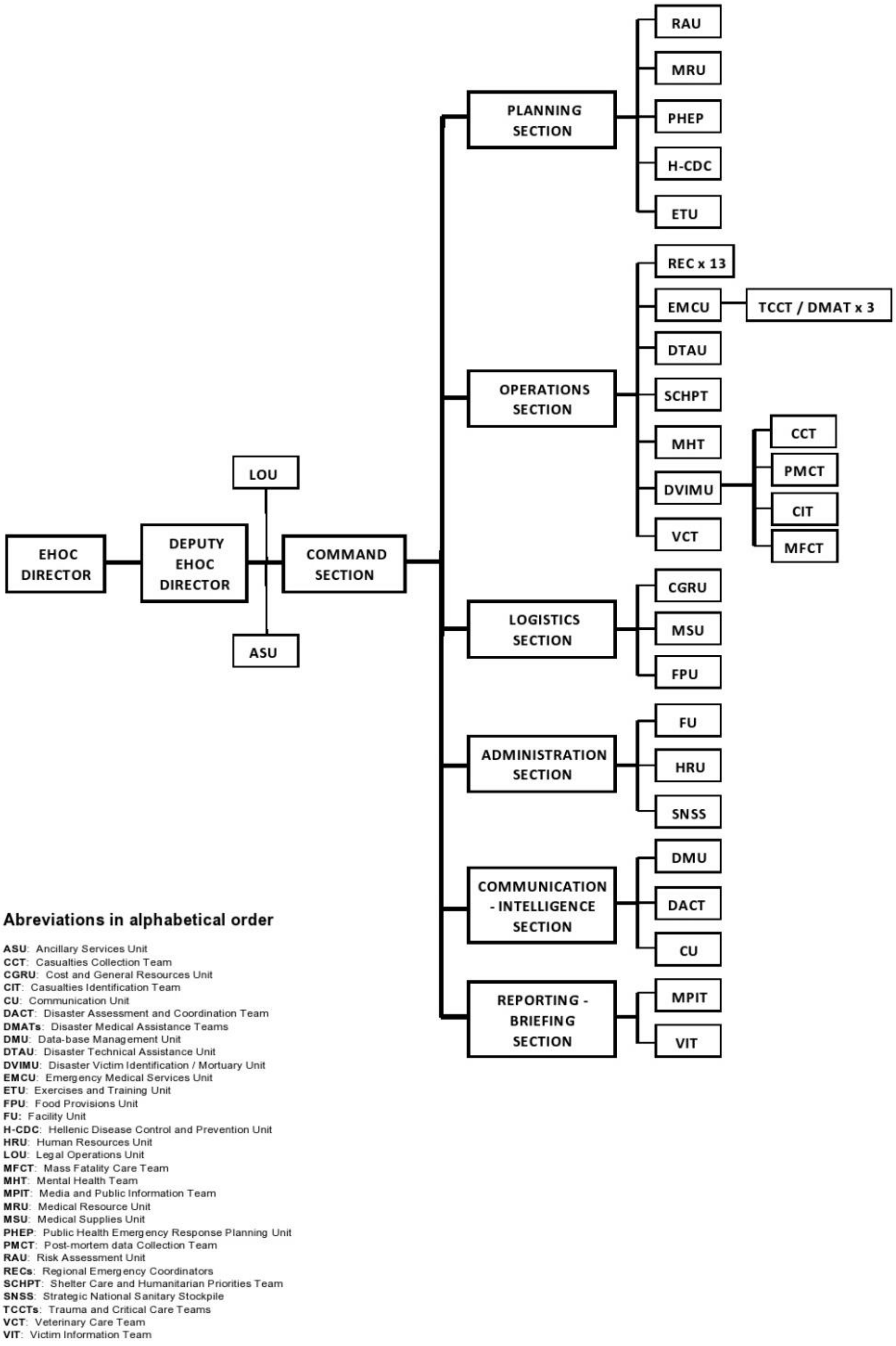
EHOC DIRECTOR
DEPUTY EHOC DIRECTOR
LOU
ASU
COMMAND SECTION
PLANNING SECTION
RAU
MRU
PHEP
H-CDC
ETU
OPERATIONS SECTION
REC x 13
EMCU
TCCT / DMAT x 3
DTAU
SCHPT
MHT
DVIMU
CCT
PMCT
CIT
MFCT
VCT
LOGISTICS SECTION
CGRU
MSU
FPU
ADMINISTRATION SECTION
FU
HRU
SNSS
COMMUNICATION - INTELLIGENCE SECTION
DMU
DACT
CU
REPORTING - BRIEFING SECTION
MPIT
VIT

Abreviations in alphabetical order

ASU: Ancillary Services Unit
CCT: Casualties Collection Team
CGRU: Cost and General Resources Unit
CIT: Casualties Identification Team
CU: Communication Unit
DACT: Disaster Assessment and Coordination Team
DMATs: Disaster Medical Assistance Teams
DMU: Data-base Management Unit
DTAU: Disaster Technical Assistance Unit
DVIMU: Disaster Victim Identification / Mortuary Unit
EMCU: Emergency Medical Services Unit
ETU: Exercises and Training Unit
FPU: Food Provisions Unit
FU: Facility Unit
H-CDC: Hellenic Disease Control and Prevention Unit
HRU: Human Resources Unit
LOU: Legal Operations Unit
MFCT: Mass Fatality Care Team
MHT: Mental Health Team
MPIT: Media and Public Information Team
MRU: Medical Resource Unit
MSU: Medical Supplies Unit
PHEP: Public Health Emergency Response Planning Unit
PMCT: Post-mortem data Collection Team
RAU: Risk Assessment Unit
RECs: Regional Emergency Coordinators
SCHPT: Shelter Care and Humanitarian Priorities Team
SNSS: Strategic National Sanitary Stockpile
TCCTs: Trauma and Critical Care Teams
VCT: Veterinary Care Team
VIT: Victim Information Team

a) *Risk Assessment Unit* (RAU); as a component of a comprehensive risk management program, RAU conducts hazard-specific vulnerability analyses according to the following flow-chart: Information gathering – data analysis – assessment – prioritization of actions – follow up of resultant outcome.

b) *Medical Resource Unit* (MRU); it predicts the local needs in medical resources, supervises hospital operation in emergency status, and registers medical devices, pharmaceutical stock, beds and bedding, laboratory capabilities, supplies and reserves, personal safety equipment, bio-safety facilities, etc.

c) *Public Health Emergency Planning Unit* (PHEP); it devises hazard generated procedures, priorities and risks, preparedness programs, and all relevant SOPs, both hazard- and response-specific, in a form of an operation manual or handbook. Expected activities are described in flow-chart diagrams and regard all actions to take place from the first hour up to several weeks after the incident. Such SOPs exist for all levels of response and describe, among others, personnel, equipment, supplies and also define operational time points. In their standard form, SOPs include a basic plan describing current legislation, operational phases, and appendices describing incident functions, response specific and deactivation plans, checklists, templates, job cards, resources, and relevant literature [7].

d) *Disease Control and Prevention Unit* (CDC); the national link to the European-CDC.

e) *Exercises and Training Unit* (ETU) for continuous and efficient training of the EHOC staff according to international standards [8]; ETU also conducts preparatory planning for future emergency response scenarios, reviews existing national and international regulations, frameworks, and planning guidelines, and organizes periodically full-scale exercises on a realistic base. A final goal for ETU is an overall improvement of awareness, competence, operational readiness, and continuous updating of plans and procedures.

3. Operations Section: The cardinal EHOC branch comprises preparedness and response activities provided by trained personnel capable of responding within two hours after identification of a major health incident. Specific SOPs are:

- Gathering and analyzing all relevant pieces of information.
- Stepwise analysis and evaluation of all factors affecting the mission; in these are included not only medical factors but also general ones (environment, weather conditions, available time, humanitarian implications, cooperation reluctance or conflict of interest among various public and private participants, staff security, etc).
- Consideration of all alternative case-based actions; that is called mitigation, i.e. threat/hazard/disease-specific, on occasion, actions to minimize health-related impact after hazard occurrence.
- Activation of preparedness levels, from Level 3 (lowest) up to Level 1 (highest), according to the existing situation after interaction with bio-surveillance laboratories network and with the national response framework given by GSCP. The preparedness SOPs include a detailed and continuously updated catalog of involved personnel, methods of fast recall on a 24h/7d basis, key-persons, and all other necessary details of an early warning protocol.
- Flexibility, surge capacity, i.e. ability to respond in multiple emergency health incidents, surge capability, i.e. ability to cope with unusual/special medical needs, and adequate durability, i.e. long-lasting response [9].
- Close collaboration and engagement with scientific entities in strategic preparedness and tactical operations, such as associates from the academic world, seismologists, meteorologists, building engineers, forensic scientists, laboratory experts, anthropologists, epidemiologists, etc.
- Linkage and integration of healthcare volunteers, non-governmental organizations, and major humanitarian responders, e.g. Red Cross.
- Recovery actions at multiple levels, which restore the stricken environment to as close to former “normal” status after a major health incident.
- Promotion of environmental health, social engagement, mobilization, and solidarity of the community.

Hence, according to the above functions the Health Operations Section acts in the following response stages: Incident recognition – Notification – Activation – Mobilization – Operations – Demobilization - Transition to recovery. The Operations Section is divided into several branches [9], as follows:

a) *Regional Emergency Coordinators* (RECs), acting as operational liaisons between central EHOC and the corresponding regional departments of Civil Protection for each of the administrative districts of the nation’s territory; for each REC there should be established a mobile (in a vehicle) operation center with satellite intercommunication with the regional Civil Protection liaison.

b) An *Emergency Medical Services Unit* (EMCU); which is a field-level rapid deployment medical force subdivided into three (3) Trauma and Critical Care Teams (TCCTs). Depending on the incident, these teams can be reorganized in equipment and staffing to Disaster Medical Assistance Teams (DMATs) [10]. DMATs provide primary and acute care, triage of mass casualties, initial resuscitation and stabilization, advanced life support, and preparation of sick or injured for evacuation. All victims are managed by use of the internationally standardized procedure of colored cards which illustrate a brief medical history and mainly the medical priority for each victim (by the respective color). After prompt evacuation from the disaster area (when it is such), a rapid pre-hospital triage, followed by emergency initial stabilization and then by urgent transport of casualties constitutes the operational “holy trinity” for these teams. At the disaster point the TCCT/DMAT leader should be the first official responder who will undertake the following SOPs:

- Rapid estimation of hazard situation on the operation field along with an estimation of a probable number of casualties.
- Prompt notification to his/her superiors about urgent requirements in staff and equipment (ambulances, bedding, paramedics, body bags for fatalities, cold storage facilities, drinking water supply, etc.).
- Together with the (non-medical) on-site delegate of Civil Protection, they immediately delineate operational disaster zones around the disaster point.
- Outside the “hot zone” the above two operational delegates minister to the needs of less injured victims; the TCCT/DMAT leader, in particular, activates the operational teams of sub-paragraphs (c-g) of the EHOC Operation Section (described below).
- Remains in the “hot zone” and coordinates the rescue teams until he/she receives a replacement order.

c) A *Disaster Technical Assistance Unit* (DTAU), consisting of non-medical technical specialists who will provide, support, and repair mechanical and electrical equipment of operation.

d) A *Shelter Care and Humanitarian Priorities Team* (SCHPT); it coordinates humanitarian affairs, especially of the more vulnerable population groups, and prioritizes on-site non-medical, but still life-threatening needs that can heavily affect a disaster area, such as shelter, covers, safety, nutrition, drinkable water, etc. This unit cooperates with the Construction/Engineering Branch of the Civil Protection for shelters and sanitation systems construction.

e) A *Mental Health Team* (MHT), consisting of psychiatrists, psychologists, and social workers. They assess early post-disaster stress within the affected population and responders and support their mental health status by interventional and/or pharmaceutical practices and by cooperation with corresponding church/religion representatives.

f) A *Disaster Victim Identification/Mortuary Unit* (DVIMU); provides expertise, technical assistance, and support for casualties and human remains documentation, in collaboration with the Reporting/Briefing Section of the EHOC (described below). The unit further develops four operational branches; the Casualties Collection Team (employs laborers), the Post-mortem data Collection Team (collects and evaluates fingerprints, DNA/dental samples, etc, for forensic medicine purposes), the Casualties Identification Team, and the Mass Fatality Care Team (offers disaster mortuary services).

g) A *Veterinary Care Team* (VCT); offers care to animal casualties and to operating animals (e.g. rescue dogs), using veterinary medical support, equipment, supplies, and pharmaceuticals.

4. Logistics Section: It determines and supervises the provision of emergency resource needs for the unceasing operational function of the EHOC on a 24h/7d basis. It is divided into the following branches:

a) *Cost and General Resources Unit* (CGRU); it monitors cash flow and expenses and coordinates all sources of funding. Additionally, it can provide direct cash from a special account for immediate access in urgent needs of high priority, thus avoiding time-consuming bureaucracy.

b) *Medical Supplies Unit* (MSU); it monitors needs and provides required quantities of medical and pharmaceutical supplies as well as personal protective equipment for frontline health care workers.

c) *Food Provisions Unit* (FPU); it provides all services, resources, and supplies regarding nutrition to operation needs and also ensures that operating EHOC personnel have sufficient supplies of food and drinkable water.

5. Administration Section: It provides facility systems and supports the overall physical infrastructure (premises, furniture, equipment, etc.) of the EHOC; The section is divided into the following branches:

a) *Facility Unit* (FU); it organizes building and sectional layout of EHOC units in dedicated, purpose-built facilities, with adequate space for its staff, physically and environmentally secure, accessible, and survivable in the event of a threat or disaster.

b) *Human Resources Unit* (HRU); it formulates job descriptions and classifies staff requirements based on the knowledge, skills, and abilities required for the role and function of each person in the EHOC.

c) *Strategic National Sanitary Stockpile* (SNSS); it develops and deals with the national repository of basic life-support medications (antibiotics, antidotes, etc) and surgical supplies for any nation-wide event of a mass health emergency.

6. Communications/Intelligence Section: The equipment of that sector includes high technology infrastructure (computers, servers, internet stations, software, etc.) and all tools available to support an effective, all-weather-resistant continuous communication and data management according to high-level privacy and security standards. Of utmost importance is the timely, efficient, and accurate information flow on a 24 hour/7 day basis, between the Command section and each of the corresponding field-units from the Operations sector. For that to be accomplished a sophisticated web-based digital tool allowing fast, real-time information sharing to multiple recipients is constantly in function. Some specific sub-functions of that section are:

- A continuous surveillance system, for gathering and exchanging information about sensitive civil national territories (e.g. cities and forest areas), by use of a broad camera-surveillance network and by electronic processing of the data-flow from an advanced, interactive mapping application. The relevant SOPs address all kinds of environmental and man-made threats, monitoring procedures, and actions according to certain hazard thresholds.
- An internet monitoring and medical intelligence/analysis system that verifies information to prevent duplication/ contradictory data collection.
- A rapid early warning/alert system triggering the coordinated rapid recall of staff back to their positions.
- A classified and secure web-based priority system for high-level communication and providing of information among national and transnational decision-makers.

The Communications/Intelligence sector is divided into the following branches:

a) *Data-base Management Unit* (DMU); it controls and centralizes information distribution to appropriate stakeholders for coordinated resource mobilization and correct decision-making to a wide range of emergencies.

b) *Disaster Assessment and Coordination Team* (DACT); it links the abovementioned data with relevant colleagues and experts from both country-level contacts and from the European Monitoring and Information Centre (MIC), as well as from UN/WHO.

c) *Communication Unit* (CU), equipped with telecommunication infrastructure and emergency communication services for sharing urgent relevant information among operational delegates from national and European governmental and private health agencies.

7. Reporting/Briefing Section: Chief is a senior Public Information Officer with pronounced communication skills to prevent inaccurate information spreading and panic diffusion, thus public composure and confidence to be preserved. The chief of the section applies uniform medical reporting SOPs according to the basic requirements: Who – when – where - what and how to report. The Reporting / Briefing section is divided into two branches:

a) *Media and Public Information Team* (MPIT), for regular management of the media and consequently of public expectations for the incident, by the timely transmission of valid information, carefully filtrated for any confidential or higher classified evidence.

b) *Victim Information Team* (VIT); it acts as the liaison between the afflicted population (victims or their families) and state authorities to serve their needs, to manage the list of missing persons, and to provide discreet information, in cooperation with DVIMU and MFCT of the Operations Section. The VIT also formulates antemortem medical records for the deceased and provides forensic support by coordination with local and national law enforcement agencies.

CONCLUSIONS

Albeit the proper function of an EHOC depends on numerous factors, qualifications, and principles, only two ones can be regarded as primary; first, the political will to radically reform the national and trans-national administrative “status quo” at the highest level, so that various authorities to be centralized under the same command through an appropriate legislative framework and second, a properly structured and fully functional organizational plan of an exemplary EHOC.

Disclosure statement

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ORIGINAL ARTICLES

Correlation between FibroScan and AST/ALT ratio and splenic size in NASH patients in a tertiary care center

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Abstract: *Background: Non-Alcoholic Steatohepatitis (NASH) is an aggressive form of Non-Alcoholic Fatty Liver disease (NAFLD), with liver inflammation and scarring. Due to a lack of clinical biomarkers and asymptomatic nature, NASH is often under-diagnosed. It is the most common cause of chronic liver disease in the USA. Liver biopsy is the gold standard to diagnose NASH, but it is invasive and life-threatening and histologic evaluation of a liver biopsy sample is imperfect as a reference because of sampling variability due to the irregular distribution of fibrosis. Aim: Validating AST/ALT ratio as a stand-alone scoring system in NASH is scarce and so this study aims to establish a correlation between FibroScan values and AST/ALT ratio. Methodology: All NASH patients, who underwent FibroScan were included. Their demographics, FibroScan, AST, ALT values were recorded in M S Excel and Pearson correlation between FibroScan values and AST/ALT ratios of 150 NASH patients was calculated using SPSS. Results: Out of 150 NASH patients, 72% were males and 57.33% belonged to 40-50 years age group. FibroScan values and AST/ALT ratio showed positive Pearson correlation of 0.245, (p value=0.003). FibroScan values and splenic size also showed a positive Pearson correlation of 0.289 (p value<0.001). Conclusion: Males of 40-50 years age group had higher distribution of NASH, so middle aged males should be screened routinely as they are at a higher risk. FibroScan value with AST/ALT ratio and splenic size showed a positive correlation, thus showing that AST/ALT ratio and splenic size increases with increase in liver stiffness.*

Keywords: *NAFLD, NASH, FibroScan, Elastography, AST/ALT*

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a broad term used for a range of liver conditions affecting people who drink little to no alcohol, and is the most common cause of chronic liver disease in the United States of America [1], affecting approximately 90% of the obese population and 15-40% of the general population [2]. As the name implies, the main characteristic of NAFLD is excessive fat stored in the hepatic cells of the liver [1]. It consists of a spectrum of conditions: benign steatosis or non-alcoholic fatty liver (NAFL), steatosis accompanied by inflammation and fibrosis or nonalcoholic steatohepatitis (NASH), and finally cirrhosis [3].

The rising prevalence of NAFLD globally may be accounted for by changes in dietary habits and an increase in sedentary lifestyle [4]. Insulin resistance and oxidative stress play an important role in NAFLD development and progression [5]. Due to the lack of clinical biomarkers as well as its asymptomatic nature, NASH is often under-diagnosed and most commonly found incidentally during routine Ultrasound of the abdomen [3]. No highly sensitive and specific tests are available to differentiate NASH from simple steatosis, but diagnostic accuracy can be improved by combining blood biomarkers [6]. It is an aggressive form of fatty liver disease, marked histologically by liver

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inflammation and may progress to advanced scarring (cirrhosis) and liver failure, which is similar to the damage caused by heavy alcohol use. The number of people at risk for NAFLD is even greater given the increasing prevalence of obesity, diabetes and metabolic syndrome [1]. Although steatosis can be a significant cofactor in progression of established CLD, or even hepatocellular carcinoma (HCC), only steatohepatitis indicates the presence of progressive liver disease in patients with NAFLD. Benign and malignant liver tumors have been more frequently reported with the increasing prevalence of obesity and diabetes [7]. Early identification and targeted treatment of patients with NASH are needed to improve patient outcomes, directing patients toward intensive lifestyle modification to promote weight loss as well as for referral for bariatric surgery as indicated [8]. In NAFLD, HCC frequently develops in a not-yet cirrhotic liver; ss there are no surveillance programs for these patients, diagnosis often occurs at a tumor stage beyond curative options, further marking the importance of its early diagnosis [9]. According to a recent study done by Edenvik et al. [10], NAFLD and alcoholic liver disease were more commonly associated with deficient surveillance for HCC, and therefore early diagnosis was missed.

Although liver biopsy is considered the gold standard to diagnose NASH, only those patients with a high risk of NASH or advanced fibrosis require this evaluation, since it is an invasive and potentially life threatening procedure. Non-invasive tools for diagnosis and disease staging are required, reserving liver biopsy for those patients where it offers clinically relevant additional information only [11]. The use of non-invasive tests to stage the severity of liver disease (ie. scarring) is now well established in the management of patients with chronic liver disease since the assessment of liver scarring provides prognostic information and establishes treatment priorities [12]. Techniques such as transient elastography, magnetic resonance elastography and acoustic radiation force imaging are becoming more established in detecting fibrosis in a variety of chronic liver conditions in addition to NAFLD, as reviewed by Fitzpatrick E et al. [13]

One such technique, transient elastography (TE), is a simple, safe and efficient way to estimate liver scarring which is done using FibroScan, and is the most popular non-invasive device used to assess liver 'hardness' (or stiffness) via TE. Liver hardness is evaluated by measuring the velocity of a vibration wave (also called a 'shear wave') generated on the skin [12]. The main clinical indication for liver elastography is fibrosis staging of chronic liver disease and detect advanced fibrosis. Histologic evaluation of a liver biopsy sample is an imperfect reference standard because some degree of sampling variability is inevitable due to the irregular distribution of fibrosis in chronic liver disease.

FibroScan can be easily performed at the bedside or in the outpatient clinic with immediate results for the diagnosis of significant fibrosis and cirrhosis in nonalcoholic fatty liver disease chronic hepatitis C, in recurrence of hepatitis C after liver transplantation, in co-infected HIV-HCV patients, in chronic hepatitis B, in chronic cholestatic diseases, in alcoholic disease [14]. FibroScan evaluates liver stiffness, inflammation and portal hypertension and the values have to be interpreted according to clinical, biological and morphological data. FibroScan® results range from 2.5 kPa to 75 kPa. Between 90–95% of healthy people without liver disease will have a liver scarring measurement <7.0 kPa (median is 5.3 kPa) [12].

The usual observed biochemical pattern in hepatic steatosis due to NAFLD is of increased levels of transaminases, with alanine aminotransferase (ALT) levels exceeding those of aspartate aminotransferase (AST). This classical pattern differentiates between hepatic steatosis from NAFLD and alcoholic liver injury, with the latter normally associated with a high AST/ALT ratio [15]. Generally, the AST to ALT ratio increases with the severity of the necroinflammatory and fibrotic changes [16].

Data validating the use of the AST/ALT ratio as a stand-alone scoring system in NASH is scarce. This study aims to establish a correlation between FibroScan values and AST/ALT ratio. Given the high prevalence of NAFLD in the general population, these noninvasive methods could be used in clinical practice to screen patients with NAFLD [17]. Lifestyle modifications to reduce weight are a major factor in the treatment of NAFLD. No pharmacological substances are as yet approved for the indication NASH [18].

MATERIALS AND METHODS

Out of a total of 2000 patients diagnosed with NASH over a period of 3 years (January 2017 to December 2019) in Amrita Institute of Medical Sciences and Research Centre, Kochi, 150 patients who underwent Transient Elastography Scan (FibroScan) is selected. Inclusion criteria consists of abnormal liver function tests, fatty liver infiltration on ultrasonography (US), undergone FibroScan etc. Exclusion Criteria are patients with a history of alcohol intake, hepatitis B and C, history of hepatotoxic drug intake and other liver diseases. Ethics Comittee of Amrita Institute of Medical Sciences has approved the study.

The mean of 10 elastography values is taken as the FibroScan value. Patient details like their demographics, USG findings, AST/ALT ratio, Neutrophil/Lymphocyte ratio, LFT etc were obtained using their hospital numbers from Amrita Health Information System and recorded in MS Excel.

Pearson correlation of FibroScan values with AST/ALT ratio and splenic size and Receiver Operative Characteristic (ROC) is used to find out optimal cut off for AST/ALT and Splenic diameter, as well as their sensitivity, specificity, and predictive values, using SPSS.

RESULTS

The majority of cases were seen among males (Figure 1). 74% (111) of cases were above 40 years (Table 1 and Figure 2).

Table 1: Distribution of age

Age Group (years)	Frequency	Percent Distribution
11-20	1	0.6
21-30	15	10
31-40	23	15.3
41-50	38	25.3
51-60	48	32
61-70	22	14.6
71-80	3	2
Total	150	100

Figure 1: Distribution of gender

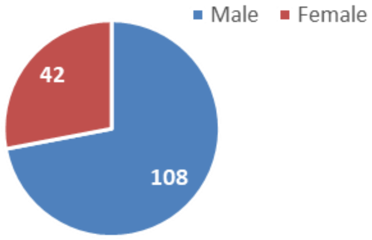

Figure 2: Distribution of age

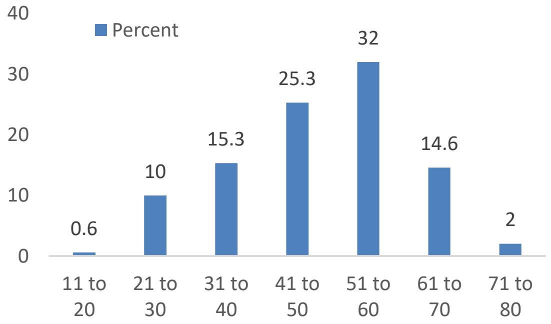

Mean laboratory values are given in Table 2.

Table 2: Demographics and laboratory characteristics

Mean Age	48.4 years
Gender (Males)	108 (72%)
Mean HDL	46.68 mg/dl
Mean LDL	127.34 mg/dl
Mean TG	136.52 mg/dl
Mean AST	72.18 IU/L
Mean ALT	103.72 IU/L
FibroScan	7 kPa
Splenic Size	9.7 cm
Neutrophil	54.12%
Lymphocytes	35.5%
NEU/LYM	2.02

In order check the correlation between FibroScan and AST/ALT (Figure 3), Pearson correlation was used. Positive correlation of 0.245 with a statistically significant P value of 0.003 was obtained. Positive correlation of 0.298 with a statistically significant P value of <0.001 was obtained between FibroScan and Splenic Diameter (Figure 4).

Figure 3: Scatter Plot of FibroScan and AST/ALT

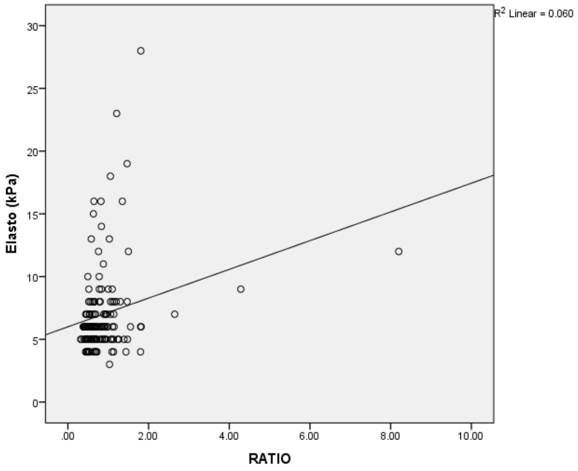

Figure 4: Scatter Plot of FibroScan and splenic size

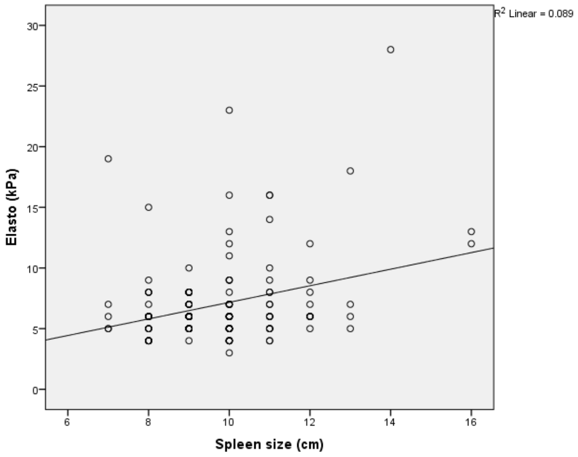

Table 3: Demographics and laboratory characteristics

		AST/ALT	Splenic size
Fibroscan	Pearson corrrelation	0.245	0.298
	p-value	0.003	<0.001

ROC curves were used to check the diagnostic accuracy of AST/ALT (Figure 5) which showed an AUROC of 0.641, ASTAST/ALT cut off 0.32 (P value 0.01), with a sensitivity and specificity of 97.3% and 29.2%. ROC for Splenic Diameter (Figure 6) showed an AUROC of 0.60, cut off of 9.5cm (P value 0.06), with a sensitivity and specificity of 62% and 51.3%.

Figure 5: ROC curve for AST/ALT

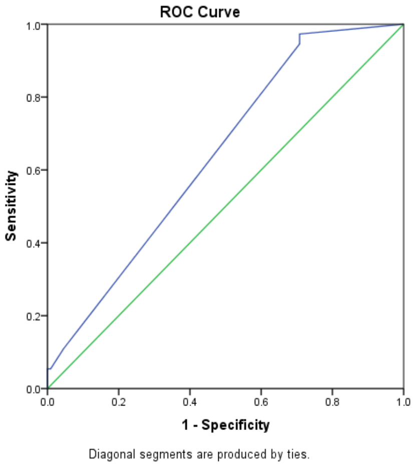

Area	0.641
AST/ALT	0.32
P value	*0.01*
Sensitivity	97.3%
Specificity	29.2%

Figure 6: ROC curve for Splenic Size

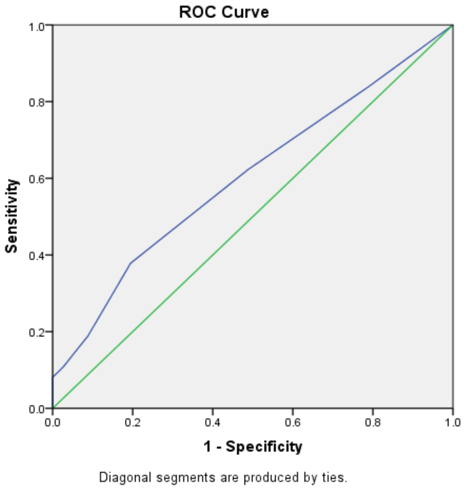

Area	0.603
SS	9.50
P value	*0.061*
Sensitivity	62%
Specificity	51.3%

CONCLUSION

Males of age more than 40 years had higher distribution of NASH, so middle aged males should be screened routinely as they are at a higher risk for developing NASH. FibroScan value with AST/ALT ratio and splenic size showed a statistically significant positive correlation, thus showing that AST/ALT ratio and splenic size increases with increase in liver stiffness. Thus our study shows that AST/ALT ratio can also be used for prompt diagnosis and management of NASH patients.

Contribution of authors

Varsha Somakumar, V S Srikanth, Cibi Manoharan – Concepts, design, definition of intellectual content, literature search, data acquisition, data analysis, Manuscript preparation, editing and review; Vishal Marwaha, VR Mujeeb, M.G.K.Pillai - Concepts, design, definition of intellectual content, Manuscript editing and review.

The manuscript has been read and approved by all the authors, and each author believes the manuscript represents honest work. There is no conflict of interest among the authors.

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ORIGINAL ARTICLES

The value of lipid profile in asthma-obstructive sleep apnea overlap

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Abstract: *The asthma obstructive sleep apnea overlap is a clinical entity relatively new and the association with a dysfunctional plasma lipid profile is not known.*

Aim. In this study, we compared the serum lipid profile in asthma-obstructive sleep apnea overlap syndrome to those in asthma and obstructive sleep apnea patients.

Methods. A cross-sectional study of 97 cases of which 26 patients had only asthma, 51 were diagnosed with OSA, and 20 were identified with A-OSA overlap. The lipid profile included the total cholesterol, HDL-cholesterol, triglycerides, the LDL-cholesterol. The Casteli indexes I and II and the atherogenic plasma index were calculated.

Results. The patients with asthma- obstructive apnea overlap were older (55.55 years vs 53,73 years in obstructive sleep apnea and 51,04 years in asthmatics), mostly obese (95%), and had a significantly higher average of BMI (38.11 vs. 35.64 and 28.8, respectively). Asthma- obstructive apnea overlap was at higher risk of cardiovascular disease than asthmatics, in respect to the lipid profile: the levels of HDL-cholesterol were lower, triglycerides were higher, and the values of Castelli index I and II were higher.

Conclusions. Based on our results, we confirm that age and BMI contribute to the risk of A-OSA overlap. The major differences in the common markers of the serum lipid profile are lower HDL-C and higher triglycerides. As a clinical implication, both Castelli indexes could provide additional value for cardiovascular risk and should be monitored in A-OSA overlap.

Keywords: *asthma, obstructive sleep apnea, HDL-cholesterol, triglycerides*

INTRODUCTION

The number of studies describing the asthma-obstructive sleep apnea overlap (A-OSA overlap), also known as "asthma-obstructive sleep apnea phenotype" is constantly increasing. The prevalence of obstructive sleep apnea (OSA) in asthmatic patients ranges from 19-60%. The prevalence is higher in older patients, with more than 10 years duration of asthma, in poorly controlled and in obese asthmatics [1, 2].

There is also a higher risk for the development of asthma in obese OSA patients. The coexistence of asthma in OSA aggravates the desaturation during the night [3].

A pro-atherogenic serum lipoprotein profile in OSA, reported by many studies [4, 5], and broadly accepted is dyslipidemia, one of OSA's comorbidities [6, 7]. There is some clinical evidence that dyslipidemia improves after CPAP therapy [7]. The metabolic effects and, particularly, the lipid profile in asthma are less investigated. However, a systematic review showed an increased level of LDL-cholesterol in this category of patients [8].

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As more and more cases of A-OSA overlap are reported, the alterations of the lipid profile become more suggestive for additional cardiovascular comorbidity. In this respect, we developed a study to compare the serum lipid profile in patients with asthma-obstructive sleep apnea overlap syndrome compared to asthma and OSA, as a unique diagnosis.

METHODS

An observational, cross-sectional study was conducted in two Romanian centers from Bucharest and Constanta city.

From March to June 2019, patients with occupational asthma, that agreed to participate, were screened by Stop Bang and Epworth testing in the Clinic Department of Occupational Diseases in Colentina Hospital of Bucharest. Those asthmatics, with high scores, underwent a polygraphy test. OSA's diagnosis was based on apnea-hypopnea index (AHI) higher than 5/h.

The Constanta group of patients, previously diagnosed with asthma, were investigated by a similar methodology for OSA's diagnosis, in the Sleep Laboratory of Constanta Clinic Pneumophthisiology Hospital.

The study was approved by the Ethic Committee of the Constanța Clinic Pneumophthisiology Hospital.

Both research teams recorded data about demographics (age, sex), the body mass index (BMI), fasting glycemia, total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels. BMI was calculated by dividing weight by the height squared. The current smoking habit was assessed. If the cessation of smoking was more than 1 year long, the subject was classified as a former smoker.

The blood profile of total cholesterol, triglycerides, and HDL-c cholesterol was directly measured by standardized methods, while LDL-C was calculated according to the Friedewald formula: LDL-C = TC − HDL-C − (TG/5).

The Castelli risk index I was computed by the ratio of total cholesterol/HDL-cholesterol. The risk values for primary prevention were considered (e.g. >5 for men and >4.5 for women) [9]. For the LDL-C to HDL-C ratio (Castelli risk index II), the levels associated with high were higher than 3.5 in men and 3 in women [9].

Atherogenic plasma index, as the cut-off for the cardiovascular diseases risk, was considered the logarithm of the ratio triglycerides/HDL-cholesterol higher than 0.5.10

Consolidated data were processed in a StatPLus for Mac software. Numerical data were compared by Kruskal Wallis test, while categorical ones required a Chi-square test. Regression was used to verify the relationship between variables. For the statistical significance, the threshold was set to estimate a 95% probability.

RESULTS

Among 97 cases, mean aged 53.38±6.76-year-old, mostly males (n=67), 26 patients had only asthma, 51 were diagnosed with OSA, without having a personal medical history of asthma, 20 were identified with A-OSA overlap, which made a point prevalence of asthma inside all OSA patients of 28.17%.

The main characteristics of these groups are presented in Table 1.

Table 1: Main characteristics of the patients

	All (mean ± SD)	**Asthma** (mean ± SD)	**OSA** (mean ± SD)	**Asthma-OSA overlap** (mean ± SD)
Age (years)*	53.38 ± 6.76	51.04 ± 4,87	53.73 ± 7.52	55.55 ± 6.16
Gender (F/M)*	30/67	13/13	12/39	5/15
Smoking (% active smokers/total)	17.53%	19.23%	23.53%	0%
BMI (kg/m^2)**	34.29 ± 7.06	28.8 ± 4.99	35.64 ± 6.59	38.11 ± 6.53
Total cholesterol (mg/dL)	210.78 ± 50.44	208.04 ± 28.27	207.82 ± 59.71	221.3 ± 43.66
HDL-cholesterol (mg/dL)**	50.2 ± 18,84	66.95 ± 25.76	44.42 ± 12.36	47.05 ± 13.44
LDL-cholesterol (mg/dL)	126.59 ± 42.99	116.9 ± 29.62	127.1 ± 51.22	135.5 ± 29.61
Triglycerides mg/dL)*	158.95 ± 142.03	122.45 ± 92.33	187.1±193.22	172.19 ±114.21
Castelli 1 index (TC/HDL)**	4.86 ± 2.8	3.51 ± 1.32	5.32 ± 3.27	5.14 ± 2.26
Castelli 2 (LDL/HDL)**	2.83 ± 1.4	2.04 ± 0.97	3.05 ± 1.53	3.12 ±1.18
API	0.48 ± 0.41	0.35 ± 0.31	0.6 ± 0.52	0.49 ± 0.32
Fasting glycemia (mg/dL)	118.06 ± 43.82	102.8 ± 16.5	125.65 ± 52.26	106.35 ± 18.62

*OSA = obstructive sleep apnea; F=female; M=male; BMI = body mass index; HDL cholesterol = high density lipoprotein cholesterol; LDL cholesterol = low density lipoprotein cholesterol; API= atherogenic plasma index; *statistically significant difference between all groups (p<0.05); ** high statistically significant difference* *between all groups (p<0.01)*

Most patients were in their 5th and 6th decade of age. The asthma-OSA association was significantly reported in the oldest patients than in the asthma group (H= 5.31, p = 0.02).

There was also a male predominance in the OSA group and A-OSA.

There was no difference by gender distribution between asthmatics and patients with A-OSA overlap (chi2 = 3.59, p=0.058).

The majority of patients were obese (73%) or overweighting (21%), especially in the A-OSA overlap group (38.11±6.53), with major differences among groups according to BMI categories (42% obese, 42% overweight, 12% normal weight, and 4% underweight, among asthmatics, compare to A-OSA overlap group with 95% obese and 5% overweight). The prevalence of obesity in the OSA patients was an intermediary between the prevalence encountered in the asthmatics and A-OSA overlap group (Figure 1).

Figure 1: Distribution of cases according to diagnosis and BMI categories

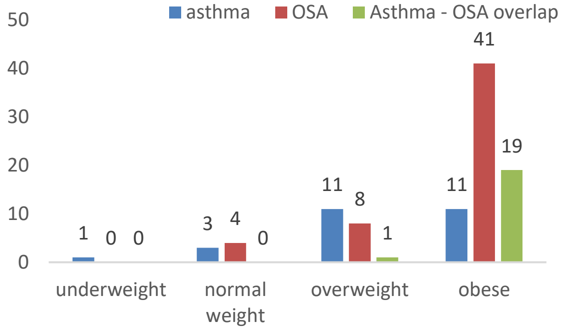

OSA= obstructive sleep apnea

Concerning smoking status, the highest percentage of active smokers was in the OSA group (23.53%), followed by 19.23% in asthmatics. However, this difference was not significant (chi2 =0.18, p=0.66).There were no active smokers in A-OSA overlap.

There was a direct correlation between BMI and triglycerides blood levels (Rho = 0.48, p=0.0001) and a reverse, significant correlation with HDL-C (Rho= -0.28, p=0.007).

The serum LDL-C showed no relation with BMI. However, the differences between groups maintained their statistical significance independent of the BMI.

The lipid profile was dominated by a high level of triglycerides and a low level of HDL-C in OSA and A-OSA overlap as compared to the asthma group.

The LDL-C had the highest values in A-OSA-overlap.

The triglycerides and LDL-C were significantly higher in A-OSA overlap as compared to asthma (H=3.98, p=0.04, and H=4.52, p=0.03, respectively) while the HDL-C was significantly lower (H=8.31, p=0.003).

No significant differences were noted between the triglycerides, the LDL-C and HDL-C serum levels in OSA and A-OSA overlap (H=0.02, p=0.89; H=0.75, p=0.38, and H=0.25, p=0.62, respectively).

The average Castelli index was in the risk range values both in OSA and in A-OSA overlap groups. When measured individually, a few asthmatics had risk values than OSA patients. The highest percentage of risk values was found in the A-OSA overlap group (Figure 2).

Figure 2: Distribution of the Castelli index I among the patients with asthma, obstructive sleep apnea, and asthma-obstructive sleep apnea overlap

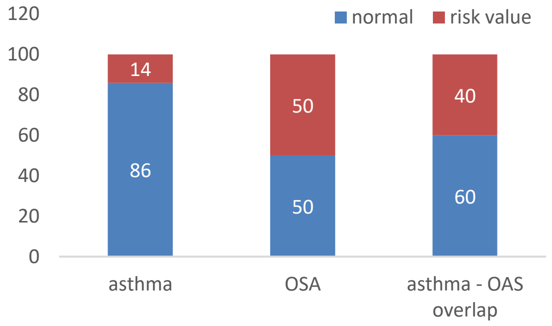

OSA= obstructive sleep apnea

The number of patients with abnormal Castelli index in A-OSA overlap was higher than in the asthma group, but without statistical significance (chi2 =3.77, p=0.05), as the risk distribution between OSA and A-OSA overlap (chi2 = 0.21, p=0.65).

The majority (90%) of asthmatics had the ratio LDL/HDL in the normal values range.

The higher level of risk, according to this index, was among OSA patients, without significant difference compare to A-OSA overlap (chi2 = 0.02, p=0.89), as well as between asthma and A-OSA overlap (chi2 = 1.32, p=0.25) (Figure 3).

The percentage of patients with atherogenic plasma index (API) >0.5 was almost equally distributed among groups; 33% in asthma, 37% in OSA, and 38% in A-OSA overlap (chi2 =0.88, p=0.64). However, there is a significant difference between the API values in asthma as compared to the OSA group (H=3.89, p=0.04).

Figure 3: Distribution of the Castelli index II among the patients with asthma, obstructive sleep apnea and asthma-obstructive sleep apnea overlap

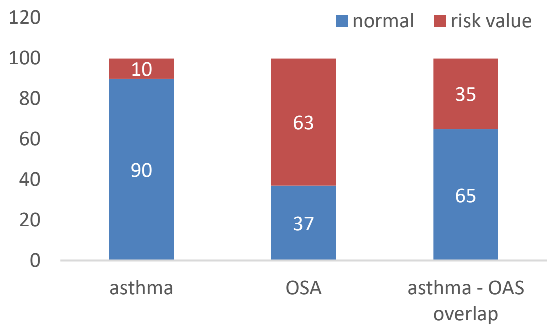

OSA= obstructive sleep apnea

DISCUSSION

In this study, we report differences in the serum lipid profile between asthma, OSA, and the co-existence of these two clinical entities referred to as asthma-obstructive sleep apnea overlap. The main findings are higher risk values for the Castelli index I and II in patients with A-OSA overlap compare to asthma patients.

Patients with A-OSA overlap were older, mostly obese (95%), with a significantly higher average BMI. These findings are from previous literature data [1, 2, 11]. The progression to A-OSA overlap requires a longer duration of time [12], considering the convergent effect of prolonged persistent chronic airways inflammation and aging.

Obesity contributes as well to the inflammatory status and increases the collapsibility of the upper airways and it is commonly accepted as a major risk factor for OSA [13]. In what concerns asthma, obese patients are reporting more severe symptoms, have a greater prevalence of co-morbidities [14], and a higher number of hospitalizations due to exacerbations [15]. The risk for A-OSA overlap might increase by higher corticoid doses that contribute to the deposition of fat in the neck region [1], another cause of upper airways resistance. OSA could be an explanation, as CPAP therapy in these patients reduces asthma reported symptoms [16].

Between the three groups, and between asthma and A-OSA overlap, the most significant difference in the serum lipid profile concerns the HDL-C. The presence of OSA seems to be the most important factor, as patients in this group had a lower blood level of HDL-C. Several studies have found the same reduction in HDL-C levels in OSA [4]. In asthma, a meta-analysis revealed no association between asthma and HDL-C levels in adults, but an increase in the LDL-C [17]. These studies support our hypothesis that the reduction in HDL-C levels in A-OSA overlap is mainly a consequence of the intermittent hypoxia related to chronic nocturnal repeated desaturation. As the A-OSA overlap is a relatively newly described entity, we could not directly compare our results with other studies. To the best of our knowledge, neither Medline nor Scopus databases give any result of a study specifically looking to this relation.

Despite the reproducible association in large cohorts of the increased cardiovascular risk related to low HDL-C level, the current clinical guidelines did not found enough evidence that raising HDL-C in serum would be of any benefit for these patients [18].

The latest evidence shows that not only the quantity but also the type of HDL particulate matters. The HDL particles are very heterogeneous and carry more functions than reverse cholesterol transport. Apolipoprotein A I and A-II are the major protein components in HDL. The HDL particle also contains a variety of lipids (phospholipids, cholesterol, in not esterified or esterified forms, and triglycerides) according to the maturation stage. It is a plasma vehicle for many other biologically active molecules, such as fat-soluble vitamins, steroid hormones, bile acids, bioactive lipids, haptoglobin, α-1 antitrypsin, transferrin, micro RNA) [19] and enzymes with antioxidant properties that would protect for reactive oxygen species and reactive nitrogen species damaging the endothelium [20]. To assure the esterification of the cholesterol, HDL contains lecithin cholesterol acyltransferase (LCAT) and to exchange esterified cholesterol and triglycerides with other lipoproteins, cholesteryl ester transfer protein (CEPT) [21].

The nomenclature of the HDL-C varies according to the method utilized for detection, and the degree of correspondence is far from perfect [22], leading to some confusion in the interpretation of results coming from different studies. Briefly, HDL is synthesized in the liver and the small intestines [23] as lipid poor preβ-HDL (the nascent HDL). In peripheral tissues, including macrophages foam cells, this particle interacts with ATP-binding cassette transporter A1 (ABCA1) to initiate the reverse transport of cholesterol to the liver and transforms to small HDL. Esterification of cholesterol inside the small HDL by LCAT and the exchange of esterified cholesterol with triglycerides from VLDL, IDL, and LDL-C, via the CEPT, end up in the formation of the mature HDL-C particle (the large HDL). Finally, HDL binds to the HDL receptor in the liver, and, through endocytosis, it is removed from circulation [24]. The triglyceride content of HDL promotes its clearance [23]. This permanent exchange, between the lipoproteins, in the bloodstream, influences the types of HDL particles in circulation.

In theory, both small and large particles might point towards a certain risk: the predominance of the small ones could show an ineffective/diminished potential for reverse cholesterol transport, while the predominance of the large ones could indicate a high burden of cholesterol in blood circulation. In epidemiological studies, a high level of large HDL seems to be protective for atherosclerosis [25], while, in already confirmed cardiovascular disease, high levels of small HDL are found [26] with a certain positive prognosis potential [27].

The techniques used to define the types of HDL particles are currently available mainly for research purposes, although their clinical value in assessing the risk and adapting therapy is of major value. Without this availability, different ratios of the commonly determined plasma lipoproteins and lipids were proposed to better characterize the atherogenic risk.

The estimation of the particle size is partially fulfilled by the API and the 2 Castelli indexes.

API was initially described to assess the atherogenic risk. Indeed, high levels were found in apparently healthy individuals in correlation with other biochemical markers of subclinical vascular diseases [28-29] and it was increased in subjects with cardiovascular disease compared to healthy controls [30, 31]. However, this marker was not confirmed as a predictable tool for stroke prediction [32]. In our study, the API did not have a discriminative value between the 3 groups. As there is no other study to compare with, this result should be cautiously interpreted and further investigated.

The Castelli risk I was a better estimation of the cardiovascular risk than individual lipoprotein particles measurement in the initial large cohort studies, as the Framingham study or the LRCP one [9], reflecting mostly the particles are responsible for the peripheral transport of cholesterol (the LDL and VLDL). It was, later, related to insulin resistance [33]. Even in normal weighted subjects, this marker had significant prognostic value for cardiovascular disease [34]. Castelli risk I show a direct relationship with the large HDL particles and indirect relation with the HDL 2b (small particles) and the small LDL [35].

Castelli index II describes the ratio between the HDL and LDL particles and is generally well associated with the Castelli index I and non-HDL cholesterol. It has been also used to differentiate the risk of atherosclerosis in chronic renal disease [36] or metabolic syndrome [37].

It is important to notice a high negative correlation between BMI and HDL with HDL-C in our study population. This finding is important, because, in certain circumstances, as in a chronic, persistent, inflammatory milieu that characterizes obesity, HDL particles become dysfunctional, losing their capacity for cholesterol efflux, and their anti-inflammatory and antithrombotic potential [38]

The low HDL-C and the high-risk Casteli index were not influenced by smoking, as all the A-OSA overlap patients were non-active smokers. It is assumed that, after smoking cessation, an improvement of the serum lipid profile manifests, mainly, on the elevation of HDL-C [39]. Even if the smoking effects would have been present, it was probably opposed by other pathogenic mechanisms. The groups of asthma and OSA had similar numbers of active smokers. Therefore, even if a certain influence of smoking on the lipid profile cannot be excluded, the significant difference in HDL-C noted between the groups should have another explanation.

We are aware of certain limitations of this study. Respiratory nocturnal polygraphy might underestimate the number of OSA patients inside the asthma population, because the majority of respiratory events are hypopneas, with arousals, that are more accurately counted only by polysomnography study [11]. Even so, the asthma patients were actively interviewed for OSA –related symptoms and none of them would clinically qualify for the diagnosis.

Another limitation of our study consists in the moderate number of patients, but from the perspective of no existing studies, we do consider that these results should be shared with the research community to promote further investigation in this domain. Despite these limitations, the results presented from this study add valuable information for the domain and substantiate the need for larger studies.

CONCLUSION

Based on our results, we confirm that age and BMI contribute to the risk of A-OSA overlap.

The major differences in the common markers of the serum lipid profile are lower HDL-C and higher triglycerides. As a clinical implication, both Castelli indexes could provide additional value for cardiovascular risk and should be monitored in A-OSA overlap.

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ORIGINAL ARTICLES

The effect of the transversus abdominis plane block on postoperative analgesia and patient comfort in patients having abdominal surgery with general anesthesia

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Abstract: *Background: This study aimed to investigate the effects of tranversus abdominis plane (TAP) block placed postoperatively using ultrasound guidance for patients with caesarean section, myomectomy, and hysterectomy under general anesthesia by eliminating additional pain caused by surgical incision.*

Methodology: The study has been planned to investigate the postoperative analgesia of 50 patients with TAP blocks applied and non-applied between the ages of 18-65, who will pass abdominal surgery. The study has been conducted in a prospective, randomized and controlled manner. Patients have been classified as those who have undergone TAP block (group: I) and those without block (group: II).

Results: Mean age of the patients in the TAP block group was 35.52 years, the mean age of the other group was 32 years and there was no statistically significant difference. The patients' visual analog scale (VAS) was evaluated at the 30th minute, 1st, 2nd, 6th, 12th, and 24th hours in the postoperative period. For patients with TAP blocks, the VAS score was found to be lower for all hours than patients without TAP blocks (p<0.05). Beginning at the 30th minute and 1st-hour analgesic needs of the block patients were compared with the other group (p<0.05).

Conclusion: TAP, that can be easily and safely placed provides effective analgesia and reduces the additional disruptions caused by the pain.

Keywords: *transversus abdominis plane block, postoperative pain, Bupivacaine, ultrasound-guided regional anesthesia*

INTRODUCTION

The transversus abdominis plane (TAP) block is a regional anesthesia technique described in recent years that provides analgesia for the anterior portion of the abdominal wall after abdominal surgery. With this method, local anesthetic injection of the area between the internal oblique and transversus abdominis muscles will block the afferent nerves of the abdominal wall.

TAP is an attractive method of block simplicity and safety, and it has been shown to provide effective analgesia, reducing the response to postoperative stress and speeding up post-surgery improvement [1].

TAP block can be used in abdominal lower region operations such as hernia repair, appendectomy, abdominal hysterectomy. The most common operation where TAP is used is after cesarean operations. It is used for postoperative pain control in cesarean operations, reduction of analgesic drug consumption, reduction of side effects that may occur

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after the operation, and side effect profile due to analgesics used [2, 3].

The purpose of this study is to investigate the effects of the TAP block, which is performed with ultrasound after surgery, on patients with abdominal surgery such as cesarean, myomectomy, and hysterectomy under general anesthesia, on post-surgery pain and patient comfort.

MATERIALS AND METHODS

The study was conducted following the Helsinki Declaration Principles (rev.2013) and with the approval of the local ethical board. After the written and signed approval of the patients, it has been planned to investigate the postoperative analgesia and comfort of a total of 50 patients The study examined the postoperative pain levels after abdominal surgery with and without TAP block. Subjects were between the ages of 18-65 who will have abdominal surgery with transverse incision (cesarean section, hysterectomy (benign), myomectomy) and the American Society of Anesthesiologists Physical Status Classification System (ASA) I-II. Our study is planned to be prospective, randomized, and controlled. The individuals to be engaged were divided into two groups in a randomized form. Randomization was based on computer-generated code. In the first group, a total of 25 patients were admitted with 16 cesareans, 6 myomectomies, and 3 hysterectomies applied to the TAP block. In the second group, a total of 25 patients who did not undergo TAP block, no needle intervention, 18 cesareans, 5 myomectomies, and 2 hysterectomies were included as the control group. Those with skin infections, patients with organomegaly (hepatomegaly and splenomegaly), people who did not give consent to the method, and individuals with allergies to drugs to be used in the intervention area where Transversus abdominis plane is to be conducted were not included in the study.

0.9% NaCl solution at a rate of 2 ml/kg/hour was administered to the individuals whose oral intake was discontinued 8 hours before the surgery. The individuals to be included in the study were not given any sedative agent on the morning of their surgery. Before the procedure, 6-8 ml/kg volume of liquid replacement with crystalloid was applied to the individuals to be included in the study. 10 cm visual analog scale (VAS) for measuring the pain level was explained in detail to all patients before the procedure. All individuals to be included in the study were followed up with an anesthesia monitor, heart rate (CAH), electro-cardiography (ECG) (lead II), systolic blood pressure (SKB), and diastolic blood pressure (DBP) in the preoperative and preoperative period. After induction of anesthesia with 1 µg/kg fentanyl, 0.5 mg/kg rocuronium bromide, and 5 mg/kg thiopental sodium, 4-6% Desflurane and 50% N2O in Oxygen were used to maintain anesthesia following endotracheal intubation. Differently in general anesthesia management at cesarean operations, 5 mg/kg thiopental sodium and 0.5 mg/kg rocuronium bromide were administered in the induction and 4-6% Desflurane, (50% N2O+50% Oxygen) and 1 µg/kg fentanyl are added as inhalation anesthesia after umbilical cord clamping in maintenance. The maintenance dose of rocuronium bromide was 0.15 mg/kg for all cases. A necessary fluid replacement was performed during the operation. At the end of the operation, TAP blocks were placed on the 1st group of patients.

After the necessary sterilization conditions were established, the linear ultrasound probe was sterilely coated and placed in the middle of the iliac crest with the end limit of the ribs. Starting with skin, the layers in descending order, subcutaneous adipose tissue, external oblique muscle, internal oblique muscle, transversus abdominis muscle, and peritoneum were identified. As the tip of the 21Gx100 mm needle (visible cannula by ultrasound, with facet tip) passed through the muscular layers and fascia, a fascial click was felt and the needle was advanced with ultrasound in a controlled manner. After receiving the second click sensation (passage of the fascia of the internal oblique muscle), after a 0.5-1 ml test dose, the location of the needle was fixed and frequently aspirated and applied to the bilateral neurophysical plan so that 20 ml of 0.25% bupivacaine was applied to one side.

The first group of patients was awakened after TAP block treatment and reversal of muscle relaxant with atropine sulfate and neostigmine; the control group was awakened after reversal of muscle relaxant with atropine sulfate and neostigmine at the end of the operation. When VAS>3 in the postoperative period, 1g of paracetamol was administered as an intravenous infusion over 10 minutes in terms of the first analgesic requirement.

Systolic and diastolic arterial pressures, heart rates of all patients were monitored immediately before and postoperatively at 30th minute and 1st, 2nd, 4th, 6th, 12th, and 24th hours after block administration. The pain levels of the patients were measured at the 30th minute, 1st, 2nd, 4th, 6th, 12th, and 24th postoperative VAS values. The need for nausea, vomiting, and initial analgesia was recorded. In this study, none of the patients underwent any invasive procedures other than routine procedures.

When parametric test counts were fulfilled (Kolmogorov-Simirnov) by uploading the data obtained from our study to SPSS (ver:22.0) program, significance test of the difference between the two averages, variance analysis in repeated measurements, Bonferroni test, Mann Whitney U test, Wilcoxon test, Freidman test, and Chi-square test were used when parametric test assumptions were not fulfilled. When α is taken as 0.05, β: 0.10, 1-β: 0.90, 25 individuals were included in each group and the strength of the test was p= 0.90388.

Our data were reported as the arithmetic mean, standard deviation, several individuals, and the level of error was taken as 0.05.

RESULTS

The age of the patients who underwent the TAP block was 35.52 ± 9.60 years; the age of the patients in the group without block was 32.00 ± 8.10 years. There was no difference in age compared to both groups (p>0.05). The duration of the case was 43.60 ± 11.41 minutes in the block group and 45.00 ± 12.24 minutes in the group without block. The difference between the groups in terms of case duration is insignificant (t: 0.41, p<0.678, p>0.05).

When the heart rate values of the groups were compared; the difference between the groups at 30th minute, 1st, 2nd, 4th, 6th, and 24th postoperative hours was found to be significant and the heart rate was lower in the block group compared to the other group (p<0.05) (Table 1).

Table 1: Comparison of groups in terms of heart rate (times/min)

Postop. tıme (hour)	**TAP group** (mean ± SD)	**Control group** (mean ± SD)	**Result**
0	80.50 ± 15.29	80.12 ± 13.85	t: 0.48 p: 0.96
½	86.84 ± 12.09	94.52 ± 14.42	t: 2.04 p: 0.047*
1	81.80 ± 9.30	92.72 ± 9.96	t: 4.00 p: 0.001*
2	80.44 ± 5.60	86.84 ± 6.74	t: 3.64 p: 0.001*
4	78.56 ± 6.31	86.48 ± 7.56	t: 4.01 p: 0.001*
6	77.0 ± 5.82	81.28 ± 5.19	t: 2.74 p: 0.009*
12	75.64 ± 7.40	78.44 ± 5.21	t: 1.54 p: 0.129
24	73.72 ± 4.71	76.56 ± 5.14	t: 2.03 p: 0.047*

**p<0.05 significant; SD – standard deviation*

When comparing the rest Visual Analogue Scale (VAS) values assessed at various times of patients in the two groups of study, the difference was significant (p<0.05). The resting VAS values measured at different periods of the individuals in the TAP block group were lower (Table 2).

Table 2: Comparison of resting values of groups

Postop. tıme (hour)	**TAP group** (mean ± SD)	**Control group** (mean ± SD)	**Result**
½	5.04 ± 1.59	8.88 ± 0.83	p: 0.001*
1	4.36 ± 0.86	8.12 ± 0.66	p: 0.001*
2	4.0 ± 0.95	7.40 ± 0.81	p: 0.001*
4	3.20 ± 0.95	6.92 ± 0.90	p: 0.001*
6	2.80 ± 0.76	5.64 ± 0.81	p: 0.001*
12	1.84 ± 0.74	4.0 ± 0.85	p: 0.001*
24	1.24 ± 0.43	3.44 ± 0.76	p: 0.001*

**p<0.05 significant; SD – standard deviation*

When the systolic blood pressure (SBP) values measured at different times were compared, the difference was found to be significant when the individuals in both groups were compared at the 1st hour and 4th hour postoperatively (p<0.05). SBP values of the individuals who received block at 1st and 4th hours are lower. A significant difference between the groups at 30th minute, 2nd, 6th, 12th, and 24th postoperative hours was not found (p>0.05) (Table 3).

Table 3: Comparison of groups in terms of systolic blood pressure (mm/Hg)

Postop. tıme (hour)	TAP group (mean ± SD)	Control group (mean ± SD)	Result
0	112.4 ± 10.9	111.24 ± 10.94	t: 0.48 p: 0.96
½	128.4 ± 15.18	137.2 ± 19.26	t: 1.79 p: 0.079
1	122.8 ± 10.21	132.8 ± 14.86	t: 2.77 p: 0.008*
2	119.6 ± 10.59	126.8 ± 14.92	t: 1.96 p: 0.055
4	115.6 ± 10.83	126.0 ± 12.24	t: 3.18 p: 0.003*
6	114.4 ± 11.93	113.24 ± 23.92	t: 0.21 p: 0.829
12	116.0 ± 9.57	110.8 ± 10.37	t: 1.84 p: 0.072
24	116.6 ± 7.57	112.8 ± 9.79	t: 1.45 p: 0.429

**p<0.05 significant; SD – standard deviation*

When comparing the diastolic blood pressure (DBP) values of the patients with and without TAP block, a statistically significant difference was found between the two groups at the 30th minute, 1st, 2nd, and 4th hour postoperatively (p<0.05). DBP values of the individuals who received block at these hours are lower. No difference between the two groups at the 6th, 12th, and 24th hours was found (p>0.05) (Table 4).

Table 4: Comparison of groups in terms of diastolic blood pressure (mm/Hg)

Postop. tıme (hour)	TAP group (mean ± SD)	Control group (mean ± SD)	Result
0	65.4 ± 8.1	67.8 ± 9.27	t: 0.77 p: 0.335
½	76.4 ± 8.6	83.8 ± 11.03	t: 2.77 p: 0.009*
1	77.2 ± 7.37	84.0 ± 10.0	t: 2.73 p: 0.009*
2	71.6 ± 6.87	80.0 ± 10.4	t: 3.36 p: 0.002*
4	70.8 ± 8.12	78.8 ± 11.66	t: 2.81 p: 0.007*
6	70.8 ± 7.59	72.8 ± 8.42	t: 0.88 p: 0.382
12	71.2 ± 8.32	71.6 ± 8.5	t: 0.16 p: 0.867
24	66.4 ± 8.10	68.8 ± 9.27	t: 0.77 p: 0.335

**p<0.05 significant; SD – standard deviation*

When comparing the need for analgesic drugs for the groups in the first 30 minutes, the difference was significant (p<0.05). Fewer patients in the TAP block group reported the need for analgesic drugs. When comparing the need for analgesic drugs for the groups at 1st hour, the difference was found to be significant (p<0.05). As can be seen, 8 patients in the TAP block group within the first postoperative hour and 25 patients in the control group within the first postoperative hour stated that they needed analgesics. Individuals without block application stated that they needed analgesics at the 30th minute and 1st hour, while two people in the block group needed analgesics at later hours (Table 5).

Table 5: Comparison of resting values of groups

Postop. tıme (hour)	TAP group (mean ± SD)	Control group (mean ± SD)	Result
½	8 patients (32%)	16 patients (64%)	p: 0.024*
1	0 patient (0%)	9 patients (100%)	p: 0.001*
12	2 patients (11.7%)		p: 0.49

**p<0.05 significant; SD – standard deviation*

When comparing postoperative nausea and vomiting (PONV) according to the groups, a statistically significant difference was found between the two groups at the 30th minute postoperatively ($p<0.05$). Nausea was never seen in the block group, whereas nausea was seen in 10 of the individuals who did not receive the block. No difference in nausea compared to both groups at the first hour was found ($p>0.05$). 4 patients (%16) in the non-block group indicated they had nausea. While 100% of the two groups did not have nausea at the 2nd hour, no difference was found between the two groups at the 4th hour ($p>0.05$). Nausea was observed in two individuals who did not receive block at the 4th hour. Individuals in both groups did not experience nausea at the 6th, 12th, and 24th hours.

When comparing the two groups in terms of vomiting, the difference at the 30th minute was significant ($p<0.05$). No vomiting was observed in the TAP block group, while vomiting was observed in 9 patients (36%) in the non-TAP group. No difference in vomiting compared to both groups at the first hour was found ($p>0.05$). Vomiting was observed in 4 patients (16%) in the non-block group, while no vomiting was observed in the other group. No vomiting was observed at the 2nd, 6th, 12th, and 24th hours. There was no difference between the groups at the 4th hour ($p>0.05$). Vomiting was observed in 2 patients (8%) without block application.

DISCUSSION

Today, pain is one of the frequent causes for doctor visits and is held responsible for a significant part of the loss of the labor force [4, 5]. Among the negative effects and complications; pain includes thromboembolic complications due to immobility and prolonged hospitalization time [4, 5].

It has been reported that with postoperative pain treatment using regional anesthesia methods, analgesic drug need is reduced and the recovery process of patients is faster [6]. The use of effective regional anesthesia methods in postoperative pain treatment also reduces the side effects of systemic analgesic drugs [7, 8].

TAP block is a regional anesthesia technique performed to provide analgesia after abdominal operations [9]. TAP block is used for retropubic prostatectomy [10], abdominal operations with bowel resection [11, 12], gynecological malignancy operations, total abdominal hysterectomy operations [13], cholecystectomy [14], and appendectomy operations [15] postoperative analgesia. Some of these studies were performed using the TAP block, a petite triangle, and a part of the study using the loss of resistance technique, followed by ultrasound. During the TAP block, the literature has been reported to damage the inner body of the abdomen, both in blind technical blocks and in blocks in the ultrasound guide [16]. Intraperitoneal injections, hematoma in the intestines, and temporary femoral nerve damage are among other complications [17]. It is important to remember the local anesthetic toxicity due to the increased local anesthetic volumes after bilateral TAP block placement. Therefore, careful aspiration is required to avoid intravascular local anesthetic injection [17]. Our study did not show any complications to the group where bilateral TAP block was applied.

Pain evaluations of the individuals included in the study were evaluated with VAS scoring at 1st, 2nd, 12th, and 24th hours. VAS score was found to be significantly lower in the TAP group compared to the control group at all evaluation times. With these results, very close results were obtained when compared to a study describing the petite triangle in TAP block [11]. In our study, unlike this study, the first analgesic needs of the patients were evaluated and it was determined that the TAP block group needed the first dose analgesic less frequently. Also, our study addressed additional complaints such as nausea and vomiting of patients.

The most commonly used local anesthetics in TAP block administration are Bupivacaine [10, 15, 18], Ropivacaine [13], and Levobupivacaine [9]. In our study, TAP block was applied to each site as 20 ml with 0.25% Bupivacaine, which we think has a longer duration of action. This application we made in our study complies with the current literature.

A study [12] included 40 patients who underwent colorectal surgery under general anesthesia, applied 0.25% Bupivacaine to the TAP group and SF to the control group as 20 ml bilaterally at the end of the surgery. A 65% decrease was observed in total morphine consumption for 24 hours compared to the control group in the TAP group. Although the need for initial analgesia was similar in both groups, subsequent doses were required longer in patients in the TAP group [12]. Another study [14] divided the patients including 54 patients that have undergone laparoscopic cholecystectomy as randomized 3 groups. The first group was the control group, the second group was the TAP group with 0.25% levobupivacaine and the third group was the TAP group with 0.5% levobupivacaine. The need for postoperative analgesia was found to be lower in the TAP groups. No significant difference was found between the 2nd and 3rd groups, and no complications related to TAP block were observed [14].

According to a study [15], 52 patients who underwent appendectomy under general anesthesia, divided the patients into two groups as randomized double-blind and applied TAP block with Bupivacaine accompanied by ultrasound to the second group. Total morphine dose and VAS scores for the first 24 hours were found to be significantly lower in the TAP group and no complications occurred in the TAP group [15]. In contrast to these studies, a conducted research [13] on 13 patients who underwent gynecological cancer surgery in 2010; no difference was found between the groups with and without TAP block in terms of analgesic drug consumption and patient comfort. In our work, the TAP block was applied to individuals after the end of the operation.

In a meta-analysis examining the studies for postoperative pain control after cesarean section, 524 patients were evaluated in a total of 9 studies, and the main purpose of these individuals who underwent TAP block after spinal anesthesia (7 studies) and general anesthesia (2 studies) is to investigate pain control and analgesic consumption. It has been emphasized that after spinal anesthesia, TAP block application without intrathecal opioid is a significant contribution to the control of postoperative pain and that after spinal anesthetic using opioid, you need to work further, although unclear [19].

Some studies have compared TAP blocks to epidural analgesia. Recently, a group of researchers conducted a retrospective matched case-control study comparing continuous TAP block catheters (posterior and subcostal approaches; n = 15) to thoracic epidural analgesia (n = 15) [20]. Except for assessments in the post-anesthesia care unit, there was no appreciable difference in pain scores between the two groups over a 3-day follow-up period. While patient satisfaction was similar between groups, the TAP block group required a significantly higher amount of breakthrough fentanyl over the study period. The therapeutic failure rate was higher in the epidural group (patchy block in 4 patients) versus the TAP catheter group (unilateral block in 2 patients). Hypotension was reported in 2 patients from the epidural group [20].

Also, an increasing number of reports indicate that TAP blocks can be a safe alternative to neuraxial blockade in patients with coagulation disorders [21, 22].

In our study, postoperative patient-controlled analgesia was not studied as a dose and the first analgesic requirement was recorded as time; heart rate, systolic, and diastolic blood pressure were found to be lower in the patient group who underwent TAP block compared to the other group. No nausea-vomiting was observed in the TAP block group. Thus, we believe that the likelihood of nausea-vomiting complications is reduced.

CONCLUSION

In our study by applying TAP blocks, the need for the first postoperative supplemental analgesia was delayed in the TAP group, thus reducing any side effects that may develop due to these analgesics. Although in our study, in the patient group who underwent TAP block; we have achieved significant and positive results, such as less postoperative pain, less initial analgesic need and additional convergence, more stable vital signs, more comprehensive investigations are necessary to routinely implement this practice.

Tap blocks are relatively easy to place and appear to be associated with little risk when placed with ultrasound guidance by trained practitioners. This kind of block provides effective analgesia and reduces the additional disruptions caused by the pain.

Contribution of authors

Study Design: Nagihan Yuksek, Ahmet Cemil Isbir; Data Collection: Nagihan Yuksek; Statistical Analysis: Iclal Ozdemir Kol; Data Interpretation: Ahmet Cemil Isbir; Manuscript Preparation: Onur Avci, Ahmet Cemil Isbir; Literature Search: Kenan Kaygusuz, Sinan Gursoy

Registered at ClinicalTrials.gov, ID: NCT04364477.

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ORIGINAL ARTICLES

Assessment of complications and outcomes of mechanical bowel obstruction in a military hospital

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Abstract: *Aims: Mechanical bowel obstruction is a frequent surgical emergency and a frequently confronted burden in abdominal operation. This study aimed to find out the model of mechanical bowel obstruction in the Iranian population.*

Methods and materials: In this study, all patients with clinical and radiological evidence of mechanical bowel obstruction that admitted to the Department of Surgery of Baqiyatallah Hospital with a diagnosis of mechanical bowel obstruction from January 2012 to December 2015 were included. The age, gender, symptoms operative details, postoperative complications, outcome, and mortality, were recorded.

Results: 193 patients with mechanical bowel obstruction were admitted in our study. Regarding the clinical presentation of the patients, the absence of passage of flatus and/or feces with abdominal pain (65.8%) were the most common presenting symptoms. Adhesions, colonic volvulus, hernias, different types of colorectal cancer were the most frequent causes of obstruction (54.9%, 15.54%, 7.2%, and 9.8%, respectively). Twenty-one patients died, resulting in a mortality rate of 10.9%. In the 19 patients with colorectal cancer, 7 (31.6%) patients have died and cause of death in all them related to cancer disease.

Conclusion: The results showed that the most common mechanisms of intestinal obstruction were adhesions, volvulus, cancers, and hernias, respectively, and the most common risk factors were appendectomy history, herniation surgery history, and cholecystectomy history, respectively. About 21 patients (10.8%) underwent obstruction surgery with a diagnosis of malignancy, which was confirmed in 19 cases of tumor pathology, of which about one third had recurrence within 3 years.

Keywords: *mechanical bowel obstruction, adhesions, colorectal cancer*

INTRODUCTION

Mechanical bowel obstruction is a frequent surgical emergency and a frequently confronted burden in abdominal operation [1-3]. Intestinal obstruction relates to extremely severe circumstances, demanding a fast and accurate diagnosis as well as critical, rational, and efficient treatment [4].

The diagnosis of mechanical bowel obstruction is not always simple, and an implication of operation requires a vast indication of suspicion [5, 6]. Comprehensive past and precise clinical exams are important to obtain a practical diagnosis and preparation therapy [7]. There are several cardinal characteristics, i.e. colic, distension, vomiting, and constipation, but the influence of each of these is affected by the position and kind of obstruction [5-9]. The administration needs early diagnosis and therapy with meticulous fluid, electrolyte balance, and timely operational intervention [10]. The complications can be decreased by

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early diagnosis and immediate surgical intervention [11].

Emergency surgery for malignant bowel obstruction is correlated with a mortality incidence of up to 30 percent [12]. It also results in considerable morbidity with prolonged hospital stay, delay at the beginning of chemotherapy and many stoma organizations, with a disorder of quality of life [12-14].

Also, malignant bowel obstruction has a major affected on health costs given that these cases are hospitalized and may need multiple mediations [13]. Medical administration has lower short-term mortality but may be correlated with low resolve or recurrence of the obstruction [14]. Because of the enormous problem of MBO in cases with cancer and the meaningful discrepancies in the morbidity of surgical versus medical treatments, it is necessary to characterize the predictors of survival and the impact of therapy modality on case results [15]. However, MBO may be due to the primary tumor, metastatic disease, or past surgical intervention leading to adhesions or an internal hernia [16, 17]. Albeit, many studies showed that MBO in the setting of colon cancer, the actual incidence, and the results remain unknown [9, 12-16].

In a study showed that 10-50% of patients with advanced cancer stage would develop MBO during their illness and experience from intractable abdominal pain, vomiting, which result in a decreased quality of life, mental and emotional problems [18]. Various therapies, including operation, palliative radiotherapy, chemotherapy and total parenteral nutrition formulas, may be used to reduce the symptoms in MBO patients; however, there is currently no agreement about the optimal treatment approach and no substantial evidence supporting the effectiveness of any treatment in enhancing the quality of life and prolonging survival [16-18]. Varying the pattern of the disease from time to time needs periodic studies to assess the etiological factors and behavior of the disease [12-18]. Also, as well as regional differences in the pattern of bowel obstruction and changes in the illness pattern over the years, are well documented in the previous studies [18]. This study aimed to find out the model of mechanical bowel obstruction in the Iranian population.

METHODS AND MATERIALS

In this study, all patients with clinical and radiological evidence of mechanical bowel obstruction that admitted to the Department of Surgery of Baqiyatallah Hospital with a diagnosis of mechanical bowel obstruction from January 2012 to December 2015 were included. The enrollment of the patients in the study was approved by the ethics committee of the Baqiyatallah University of Medical Sciences and written informed consent was obtained from all patients. Patients with paralytic ileus were excluded from our study.

The age, gender, symptoms operative aspects, post-operative complications, result, and death were recorded. Following clinical investigations, every six hours with the same attending surgical unit were conducted in all cases to assess the patients' symptoms. All cases had been operated on by the authors in the general surgery departments of the Baqiyatallah hospitals.

The diagnosis was based on clinical judgments, history, and supported by ultrasonography and radiological studies (plain x-rays) in all cases. The final diagnosis was presented at exploratory laparotomy, which was endeavored following thorough first evaluation, examinations, and resuscitation. The operation was determined as required in those patients where the operation was conducted in thirty-six hours.

Chi-square test and Fisher's exact test was utilized for the relationship between qualitative variables. The non-parametric Mann-Whitney test made a comparison among the groups. The Kolmogorov–Smirnov test was used for the normality of the data. The data was used by SPSS 20 software. The significance level for the tests was considered less than 0.05.

RESULTS

193 consecutive patients with mechanical bowel obstruction were included. The average age was 58.1 ± 19.0 years while women comprised 32.64% of the group. The most of the patients (60.1%) presented in above 59 years old.

Regarding the clinical presentation of the patients, the absence of passage of flatus and/or feces with abdominal pain (65.8%) were the most common presenting symptoms, only abdominal pain (27.5%) was the second frequent physical finding on clinical examination abdominal.

Regarding the types of previous operations, 35 patients (23.33%) had undergone an appendectomy, 27 (18.0%) hernia procedures, 24 (16.0%) had a cholecystectomy, and 86 (43.3%) had other surgical procedures.

Adhesions, colonic volvulus, hernias, different types of colorectal cancer were the most frequent causes of obstruction (54.9%, 15.54%, 7.2%, and 9.8%, respectively). There was no significant difference in the cause of obstruction regarding gender and age levels. An evaluation of 193 cases of mechanical bowel obstruction from the standpoint of etiology, age, and sex distribution, is presented.

Types of colorectal cancer were overrepresented accounting for 8 (42.1%) with obstruction due to sigmoid cancer, whereas six (31.6%) patients had a colon cancer, 3 (15.8%) had a cecum cancer, and one (5.2%) had a rectum cancer.

Regarding the clinical presentation of the patients and cause of obstruction, Adhesions and abdominal pain (56.08%) with were the most common presenting symptoms and cause of obstruction. The absence of passage of flatus and/or feces and Adhesions (33.9%) were the second frequent presenting symptoms and cause of obstruction.

There was a significant association between the cause of obstruction and history of surgery (P<0.001).

109 patients (56.4%) of the total study group were safely and more efficiently treated.

Eighty-four cases of the 193 patients sustained complications; 28 (33.3%) suffered from recurrence adhesion along with obstruction, 15 (17.9%) had abdominal pain, 15 (17.9%) had constipation. In the 19 patients with colorectal cancer, six (31.6%) patients had a recurrence.

Twenty-one patients expired, following in a death rate of 10.9%. In the 19 patients with colorectal cancer, 7 (31.6%) patients have died and cause of death in all them related to cancer disease.

DISCUSSIONS

In the past, the most common cause of intestinal obstruction was a strangulated hernia, but today, due to the higher prevalence of surgery than in the past, the most common cause of intestinal obstruction (75-80% of cases) is adhesions due to previous surgeries [7, 9, 11, 12]. These patients present with clinical signs of obstruction including colic abdominal pain, abdominal distension, and nausea and vomiting, and constipation. The complete obstruction causes intestinal dilation with an accumulation of water and gas. Radiological signs are visible 3-5 hours before obstruction [11-13]. In the present study, the most common postoperative diagnoses were adhesions in 106 patients (54.9%), volvulus in 30 cases (15.5%), colorectal cancers in 19 patients (9.8%) and hernias in 14 patients (7.2%), respectively. While in the study of Saber, the diagnosis of the cause of obstruction was observed in most cases with sigmoid volvulus and inguinal hernia, each with 18% and the site of obstruction in the small intestine with 55%. The most common symptoms in the present study were abdominal pain, which was seen in more than 95% of cases, and the second most common symptom was lack of gas or stool, which was seen in more than 65% of cases. In the study of Markogiannakis et al., the most common symptoms were flatulence (90%) followed by bloating and abdominal pain (65.3%). Adhesion was observed in 64.8% of cases, incarcerated hernia in 14.8%, and colon cancer in 13.4% [3].

Füzün et al. (2005), which looked at the causes of mechanical intestinal obstruction, found that intestinal adhesions were the most common cause of obstruction with 44%, followed by hernias with 23.9% and volvulus with 12.7%. Colon carcinoma was 10.1% of the causes of intestinal obstruction [9]. Our study is consistent with the above study in terms of 4 common factors and only slightly different in order after the most common cause, which is adhesion in both studies. The prevalence of colorectal cancers is very close in the two studies.

Regarding the clinical presentation of our cases, the lack of passage of feces or/and flatus with abdominal pain were the many common presenting symptoms and abdominal pain was the second most prevalent physical conclusion on clinical research [12-14]. The results, even though some variations are marked, are in agreement with the studies. Especially, Cheadle et al. showed abdominal vomiting 82%, pain 92%, distention 59%, and abdominal tenderness 64% as the most frequent signs [19]. While abdominal distension, bilious vomiting, absolute constipation, and abdominal pain were the main signs and symptoms in another study. In research on 100 patients with adhesive small bowel obstruction and recognized that the most common symptoms were vomiting 77%, colicky abdominal pain 68%, and absence of passage of flatus and/or feces 52% while abdominal distension developed the most common clinical symptom with a rate of 56% [20]. In a study of patients with bowel obstruction due to large bowel volvulus, the most common symptom of sigmoid volvulus was distension 79% and the most common symptoms were pain 58% and obstipation 55%, while most patients with cecal volvulus presented with pain 89% [21]. Moreover, in a review study of patients with obstruction because of small and large bowel intussusception, abdominal pain, nausea, vomiting, and abdominal distension were the frequent symptoms and signs, respectively [22].

Adhesions, colonic volvulus, hernias, different types of colorectal cancer were the most common causes of obstruction [23]. These results were also mentioned in the current research.

Furthermore, adhesions were the most common etiology of obstruction in and the total study group. Several studies propose that adhesions are responsible for 32%-74% of bowel obstruction and are the leading cause of small intestinal obstruction representing 45%-80% of it [20-23]. The large majority of the cases with adhesive obstruction have experienced previous abdominal procedures [23]. In the present study, this was agreement with previous studies.

The model of mechanical intestinal obstruction shows a difference in various areas. The obstructed herniae were observed as the 3rd common condition in the current research, whereas it was the second common condition of the other research, which almost is comparable with the other investigations carried in the country [14-15]. As for the types of surgeries in our cases, appendectomies, and hernia surgeries, cholecystectomies were more prevalent. This results also in agreement with the literature [9, 16].

However, the proper administration of adhesive obstruction is still uncertain, a large portion of these cases, varying from 35% to 65% in some investigations, can reliably and efficiently be managed with operation administration as it was also noted in our cases [23, 24].

The growing performance of adhesions as a condition of acute intestinal obstruction requires a higher requirement for regular precautionary standards toward adhesion formation [25]. Some effective actions are now supported through elective abdominal surgery to reduce the increase of adhesions that might consequently produce intestinal obstruction [26].

In previous studies showed sigmoid cancer is the etiology of obstruction that cases with high intestinal obstruction with a percentage of 40%-90% [27]. A study by Baloch et al., of 252 patients with mechanical bowel obstruction, showed due to the regional epidemic, the most common cause of obstruction was abdominal tuberculosis, which was observed in 30% of cases, and unlike our study and most other studies, adhesion was the second most common cause of obstruction. Also, in this study, the hernia was observed in only 17.5% of patients [15].

In our research, complication and mortality percentage were almost small. In the previous studies, complication proportion varies from 6 to 47 percent while death ranges from 2 to 19 percent [25, 28]. In our study, there was the association between mortality rate and colorectal cancer disease.

CONCLUSION

The results showed that the most common mechanisms of intestinal obstruction were adhesions, volvulus, cancers, and hernias, respectively, and the most common risk factors were appendectomy history, herniation surgery history, and cholecystectomy history, respectively. About 21 patients (10.8%) underwent obstruction surgery with a diagnosis of malignancy, which was confirmed in 19 cases of tumor pathology, of which about one third had recurrence within 3 years.

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The nuclear accident at Chernobyl: Immediate and further consequences

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Abstract: *The accident at Chernobyl occurred in April 1986 at the Chernobyl Nuclear Power Plant in Soviet Union. The incident occurred during a scheduled safety test. A combination of inherent reactor design flaws and operators' mistakes resulted in reactor's No.4 disaster and the emission of a large quantity of radiation. The immediate actions involved the fire extinguishing, the cleanup of radioactive residues and the prevention of a new explosion. For this purpose, plenty of people worked with self-sacrifice. The people who lived nearby were removed. As far as the socio-economic impact for the Soviet Union is concerned, it was quite serious. Moreover, the environmental and human health consequences were also alarming with thyroid cancer being the most studied. Useful conclusions, especially for the safety both of reactors and nuclear power, as well as for the impact of radiation at ecosystems have been drawn. The debate about the use of nuclear power has remained open ever since.*

Keywords: *nuclear power, thyroid cancer, RBMK reactor, radiation, radioactivity, liquidators*

INTRODUCTION

The Chernobyl nuclear accident occurred on 26 April 1986 in the light water graphite moderated reactor No 4 at the Chernobyl Nuclear Power Plant, close the town of Pripyat, in Ukrainian Soviet Socialist Republic Soviet Union, roughly 100km of the city of Kiev [1].

The reactor exploded during a safety test that was inappropriately made, with the operators losing control. This was due to a design defect of the RBMK reactor, which made it unstable when performed at low power. The outcome was the destruction of the reactor and the leakage of a huge amount of radiation into the environment [2, 3].

During the accident two deaths were caused because of explosion. Over the coming weeks a number of emergency staff was hospitalized with acute radiation sickness (ARS), while 28 firemen and employees finally died. The Chernobyl accident is considered the most damaging nuclear power plant accident in history. The Chernobyl and the Fukushima accident are the two nuclear accidents classified as a level 7 (the maximum classification) on the International Nuclear Event Scale [4].

CHRONOLOGY

The city of Chernobyl is located in Ukraine at a distance of 104km northeast of Kiev and 16km from the border with Belarus. Before the evacuation, it had about 14,000 inhabitants. Closer to the plant was the larger city Pripyat (about 49,000), which was built to accommodate the employees of the nuclear plant and their families [1]. The Chernobyl Nuclear Power Plant began to operate in 1977 with the first reactor of a total of four. Gradually, three more

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reactors began to operate by 1983. They were boiling water reactors with enriched uranium, type RBMK. The plant had a total capacity of 4000 MW electric power. The combination of graphite as a moderator and water as coolant is not found in other types of reactors [5].

THE ACCIDENT

When the reactor operates, it has an integrated cooling system. When it is shut down, the radioactive decay continues and heating of the reactor continues too. As a result, further cooling is required even after the reactor has been switched off. However, the three diesel engines which were used for this purpose needed 60-75 sec interval to achieve the required performance. In case of emergency, the reactor would be left without adequate cooling for this period, which was a very serious risk [6]. To remedy this problem, it was suggested the use of the kinetic energy of the steam turbine which was produced during the deactivation of the reactor.

After three failed tests (1982, 1984 and 1985) and some changes in design, a new test was designed to become in April 1986, when the Reactor No 4 was deactivated for scheduled maintenance. The test ended up in the tragic accident that occurred in 01:26 on Saturday, April 26, 1986. After a sequence of events during the test (which are not relevant to the present paper) it finally resulted in an uncontrolled increase of power. Simultaneously, the steam pressure in the reactor increased which eventually led to the first burst. The reactor's building was destroyed and a significant amount of radioactive materials were released into the atmosphere. A few seconds later, a second stronger explosion followed. Several fires broke out while the reactor itself started to flare [7].

Causes of the accident

The accident at Chernobyl was the result of a combination of unauthorized manipulations, human errors and design imperfections of the type RBMK reactor. According to the reappraisal of the accident by International Nuclear Safety Advisory Group at 1997 [8] the factors that contributed were:

- Unlike to most nuclear reactors which have a negative void coefficient, the RBMK had a dangerously high positive void coefficient.
- The use of graphite at the end of the control bars resulted in lowering the reactor power, had as a result the reactor power to be increased for a few seconds. This behavior is counter-intuitive and was not known to the reactor operators.
- The design of RBMK reactor had also other defects and was not consistent with the minimum acceptable safety rules for nuclear reactors.
- There was deficient safety culture in the design, organization and operation of nuclear power by the Soviet Union.
- There was inefficient and inadequate transmission of important information, both between operators and between operators and designers.
- There was inadequate safety analysis.
- The test was scheduled to take place before the night shift from well informed and familiar with the process employees. However, due to an unexpected ten-hour delay, the test was made during the night from less experienced staff.

The first hours

During the accident two workers were killed because of the explosion. There have been many fire focuses, the main of them were on the roof of the station and the reactor No 4. Initially, firemen were unaware of the risk of smoke and debris and did not receive any additional protection measures. So they received high doses of radioactivity. Although the fire on the roof had extinguished until 05:00 o'clock, the fire inside the reactor was still active. Within the next few days about 5,000 tons of chemical materials and sand were thrown by military helicopters to absorb the radiation.

The levels of radioactivity reached 20.000 Roentgens (R) per hour (the leather dose is 500R within 5 hours). In the first hours, both workers and supervisors in the control room were unaware of the actual levels of radioactivity. The one dosimeter did not work and the second one was not accessible due to the explosion. The remaining dosimeters had a measurement limit of 0,001R/s, so they showed “off scale” [9]. Due to faulty readings, the head of the station, Alexander Akimov, believed that the reactor was not affected by the explosions. Akimov and his assistants remained in the reactor building until the morning without taking any protection measures. Most of them, including the Akimov, died from exposure to radiation within the next three weeks.

IMMEDIATE CONSEQUENCES – CRISIS MANAGEMENT

After the extinguishing of fireplaces, a number of problems and potential risks had to be addressed:

I. Steam explosion risk

The floors under the reactor were filled with water. At the same time, smoldering graphite, fuel and other materials fed to the reactor began to mix with molten concrete from the reactor walls resulting in a semi-liquid material that resembled a lava [10]. If this mixture succeeded in penetrating the reactor floor and coming into contact with water, it could cause a more disastrous explosion. To prevent this danger it was considered necessary the water pools to be drained. This hazardous mission was carried out by engineers Alexei Ananenko and Valeri Bezpalov and the shift supervisor Baris Baranov. After the valves had been opened a large amount of extremely radioactive water was pumped.

II. Radioactive debris removal

The next challenge was the removal of the radioactive particles which were dispersed around. The main part of this effort shouldered the army. The plan included the transport and disposal of radioactive materials within the reactor core. Wearing lead protective clothing, the “liquidators”, as the workers were called, either transplanted radioactive materials manually, or handled heavy machines which did the same job. Many of them received cumulatively high doses of radiation throughout the procedure [11]. Furthermore, a large number of remote-controlled machines were used in order to be avoided the exposure of workers to high doses of radioactivity.

III. Announcement of the accident

At 21:02 on 28 April, about 67 hours after the accident, a 20-second announcement was read on a TV news program: “There has been an accident at the Chernobyl Nuclear Power Plant. One of the nuclear reactors was damaged. The effects of the accident are being remedied. Assistance has been provided for any affected people. An investigative commission has been set up”. However, attempting not to hurt the prestige of the regime, the Soviet authorities initially had tried to hide the incident. The above announcement occurred only when the morning of the 28 April, in routine control, personnel of the Swedish Nuclear Power Plant at Fosmark recorded high levels of radioactivity. The Swedish government came into contact with the Soviet Union and the latter was forced to admit that an accident had occurred [12]. Although, the Soviet government, attempting to downplay the event, issued a statement that there was no reason to postpone the celebrations and parade for the 1 May International Worker’s Day in Kiev.

IV. Evacuation of Pripyat

After the incident, the residents of Pripyat, completely ignored what had happened. A special committee was created a few hours later to investigate the accident. The committee arrived at the plant on the afternoon of April 27 and soon had enough evidence of the seriousness of the situation seriousness. So, in the early hours of April 27, 36 hours after the accident, they decided to evacuate Pripyat. It was initially decided that the evacuation would only be for 3 days but shortly was decided the permanent implementation of this measure.

With a statement issued by the city council the residents were informed that they would displace the city for 3 days by buses started at 14:00, on 27 April. The evacuation would be under the supervision of the police and the city officials. The residents had also been informed to take with them only their documents and entirely essential personal belongings. It was also announced that all assets would be kept by the police after the evacuation. Except for the police officers who would remain in the city for safety, there was a list of employees who were required to remain for the continuity of the plant operation. Around 15:00 a total of about 45.000 residents were transferred to different villages in the Kiev region [12].

V. Set up of exclusion zone

On May 2, a government committee decided –rather arbitrarily- a 30km area from the damaged reactor as the designated evacuation zone. This area was also divided into three sub-zones in which, both the protection measures of those who arrived, and the activities which took place, were different: The area around the reactor, a second with a radius of about 10km, and the rest zone until the 30km.

Later, this distinction was made according to the revised dose limit of 100mSv [13]. So there were:

- the “Black Zone” (over 200 μsv/h)
- the “Red Zone” (50-200 μsv/h)
- the “Blue Zone” (30-50 μsv/h)

In the first place the inhabitants would never return. In the second they may be back when the radiation levels would be reduced. In the third only children and pregnant women would have to be removed. Although the evacuation of these areas was not immediate, a total of 116,000 people were eventually removed. However, there were few residents, mainly elderly, who refused to remove. After repeated efforts at expulsion, authorities finally accepted their presence giving them an unofficial permission to stay.

The boundaries and the status of the zone and sub-zones have been amended many times in the coming years.

FURTHER CONSEQUENCES

I. Financial consequences

It is very difficult to determine the total cost of the Chernobyl accident. It afflicted, and continues to, both the Soviet Union (and the countries which have arisen since its breakup), and many other countries. According to the Chernobyl Forum Report (2005) the 22% of the national budget of Belarus in 1991 was connected to Chernobyl (decreased at 6% in 2002), while in Ukraine 5-7% of the government spending continues to be related to the accident [14]. A great part of the cost is still about Chernobyl-related social benefits in approximately seven million people in Russian, Ukrainian and Belarus. Another important economic factor for the years after the accident was the removal of 1,900,000 acres of agriculture land, as well as 1,700,000 acres of forest from production.

Finally, a large number of countries, mainly Europeans, have been forced to spend resources to check the amount of radioactivity in soils, food and water, while International Organizations, such as the World Bank, have financed both the rehabilitation work and the building of the new reactor shelter (New Safe Confinement).

II. Political and social consequences.

Before the accident M. Gorbachev had implemented the political "glasnost" with purpose to enhance the transparency of politics. However, the accident led to return to past secretive politics. At the same time, distrust of international community against the Soviet regime was grown up. The mistrust of the citizens towards the regime was also high enough, especially among nearby areas residents and those who needed to move.

Combined with the economic consequences, the lack of confidence, both in international and national level, is believed to have contributed to the fall of the Soviet Union, a few years later. Apparently, except for economic impact, political and social consequences continued to affect the 3 countries in this region, even the fall of the Union [14]. On the other hand, the need for bio-scientific cooperation helped forge closer relationship between Soviet Union and US at the end of the Cold War.

III. Environmental effects:

a) Spread of radioactive substances

It is estimated that Chernobyl released about four times the amount of radioactivity of the two atomic bombs in Hiroshima and Nagasaki. More than 100,000 km^2 were significantly contaminated even though the areas around the plant were the most overburdened. Lower levels of radiation were detected in different regions in Europe. The radioactivity dispersion was incongruous since it depended on both weather conditions and water paths. Soviet and Western scientists claim that 60% of radioactivity contaminated Belarus. Also, according to the 2006 TORCH report, half of the volatile particles came out of the Soviet Union [15].

b) Residual radioactivity

The Chernobyl Nuclear Power Plant is located next to the river Pripyat, which at that time was watering the broader area of Kiev. Although water purification measures were initially taken, the supply of water to Kiev began to become from the river Desna, two months later. Also, in some lakes in Russia and Belarus the concentrations of radioactivity in certain species of fish far exceeded the acceptable limits, even the period 1990-1992 [16]. On the contrary, excluding the exclusion zone, the groundwater does not seem to be affected.

c) Flora and fauna

After the accident, 4 km^2 of the neighboring pine trees were died, giving a characteristic red color that gave the name of the "Red Forest". Several of the wild animals in the most affected areas, either died or stopped breeding (most of the domestic animal in exclusion zone were removed) [17]. A typical example was the animals of a small island in the river Pripyat, where, the horses died and the beasts either died or were extremely weak. All of them due to significant thyroid malfunction.

In the following years, increased levels of radioactivity were detected in domestic animals both inside and outside of the Soviet Union. Several European countries have carried out checks on animal products in order to ensure consumer safety, for many years after the accident. As reported, were contaminated in total over 19.000 km^2 of agriculture land. Of these 2.640 km^2 will never be cultivated. Also about 17.000 km^2 of forest, mainly in the Ukrainian lands, were infected [14].

IV. Human impact

a) General

After the accident, totally 273 people suffered from acute radiation sickness, 31 of whom were died within the next three months. According to the Chernobyl Forum Report (2005), 28 of the "liquidators" died of Acute Radiation Syndrome, while 15 died of thyroid cancer within the coming years [14].

The same report states that it is extremely difficult to be calculated the total number of cancer due to the Chernobyl accident. The four most harmful radionuclides spread from Chernobyl were iodine-131 (half-lives: 8.02 days), caesium-134 (2.07 years), strontium-90 (28.8 years) and caesium-137 (30.2 years). Iodine, despite the small half-life, is the most unstable and appears to have caused the most important health problems up to now [18]. It tends to become concentrated in thyroid and milk glands and cause thyroid cancer. Caesium tends to accumulate in vital organs such as the heart. Finally, strontium accumulates in bones increasing the risk of cancer.

According to WHO and independent researchers who studied the children of emergency workers, there was no statistically significant difference in mutation frequencies among children conceived before and after their father’s exposure [19]. However, due to fear, distrust to the authorities and poor media information, an increase in the number of abortions in -other normal- pregnancies has been observed in some European countries. In Greece, although radioactivity measurements did not exceed the safety limits, there was an observed 2,500 excess of otherwise wanted pregnancies being terminated [20]. Furthermore, in countries such as Sweden and Finland, where there were not noticed more abortions, there was not increase in the incidence of congenital malformations [21]. The same conclusion is reached by Frank Castranovo study, of the Harvard Medical School in 1999, which has, among other things, analyzed the data from the two largest maternity clinics in Kiev [22]. He says that there is no evidence that the accident has increased the rate of birth defects.

b) Cancer assessments

The estimates of the number of cancers that have occurred or will arise in the future as a result of the accident vary enormously. This is due to both the lack of solid scientific data and the different methodologies used to quantify mortality. The Chernobyl Forum predicts that the total number of deaths could reach 4,000. However, these deaths are expected to be mainly among the most exposed people (emergency workers, evacuees, residents of the most contaminated areas) [14]. The Risk Projection suggests that Chernobyl can be responsible for about 1,000 cases of thyroid cancer and about 4,000 cases of other cancer, in Europe (0.01% of all cancer after the accident) but, as stated, the estimates are subjective. The most tightly associated with the accident cancer is thyroid cancer, due to the well-documented influence of the iodine on this gland.

According to the Forum, thyroid cancer is one of the main health impacts from the Chernobyl. On the contrary there has not been evidence for an increase of the number of other kinds of cancer or leukemia. The United Nations Scientific Committee on the Effects of Atomic Radiation ends up to similar conclusion. It finds an increase in the incidence of thyroid cancer, particularly among children and adolescents exposed to radiation, but no other indications of major health impacts [23]. Finally, the International Atomic Energy Agency indicates that there has been no increase in the incidence of solid cancer as a consequence of Chernobyl accident [14]. On the contrary, there are estimates such as the International Physicians for the Prevention of Nuclear War that refers to about 10.000 cases of thyroid cancer and 50.000 cases which are expected in the future [24].

c) Health disorders

One other important impact of the accident, especially among the directly involved populations, has to do with mental health. Both the incident itself and the initial attempt to be downgraded it had as a result to create exaggerated fears about the risks of radiation exposure. The IAEA supports that the designation of the affected people as “victims” rather than “survivors” has fed them to perceive themselves as weak, helpless and lacking control over their future [14]. Further health effects may have been caused due to this belief.

Other surveys show that humans remain largely unsure of what the effects of radiation actually are. For example, some teenagers or young adults who had received modest or small amount of radiation feel condemned to be ill and thus believe that there is no downside to have unprotected sex or use drugs.

The research and assessment of long-term effects of radiation on human health are continuous by many organizations, such as the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) [25].

DISCUSSION

The review of the causes and consequences of the Chernobyl accident had as a result many important conclusions to have drawn and, at the same time, there was a matter of intense concern.

The weaknesses and pathogens of the Soviet regime were clearly showed up. The authorities initially tried to conceal and then to devalue the gravity of the incident. For this reason, the decision to evacuate the much burden areas was delayed. This has resulted in the distrust and dispute of the citizens towards the authorities, which had as a result the citizens not to comply with the instructions of the following period.

Questions have also been raised about the safety, both the RBMK reactor and the nuclear reactors in general. The accident led to be made corrections to the design and operation of Soviet reactors but also of the reactor in West [26]. Simultaneously, a culture of safety was dealt with in the handling of nuclear energy. The combination of reactor faulty manufacturers and human errors that led to the Chernobyl accident resulted in the persistence in safety of both governments and scientists as well as the leaders and staff of the nuclear power plants. A repetition of the Chernobyl accident is now virtually impossible, according to report of the German Nuclear Safety Agency [27].

Furthermore, cooperation between East and West has improved in terms of reactor improvement and safety. Since 1989 there have been many reciprocal visits from the former Soviet Union and the West. In 1989 the World Association of Nuclear Operators (WANO) was founded, which has under the auspices 130 operators of nuclear power plants in more than 30 countries [26].

The Chernobyl nuclear accident also helped to understand the impact of radiation on the environment, ecosystem and humans. Scientists had the opportunity to study short and long term radioactive contamination as well as to relate specific exposure levels with corresponding results. Also, knowledge and experience on the appropriate measures to protect against radioactivity have increased.

Despite the intense fear and concern it seems that the only sufficiently documented impact that accident had at the human health (excluding emergency workers who have received high doses of radiation during the first days from the accident) was thyroid cancer [28].

Finally, the debate on the use of nuclear energy remains open. There is a continuing dispute between those who argue that the use of nuclear power is dangerous, and those who claim it is necessary and sufficiently secure. The growing public awareness about climate change and the critical role that carbon dioxide and methane emissions play have increased the intensity of the debate. The use of nuclear energy is environmentally friendly and reduces fossil fuel consumption. However, if anything goes wrong the consequences can be devastating to humans and to the environment. The debate remains open today between those who fear the power of nuclear and those who fear what will happen to the planet if humanity does not use nuclear power.

Disclosure statement

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VARIA

Comparative study on the independent learning activity of young people in the bachelor's and master's programs

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Abstract: *During the late adolescence, it can be reached the highest level of mental operative functions and other intelligent human manifestations. Abstract mental operations can be done at this level: synthesis of syntheses, generalization of generalizations, abstraction of abstractions. Students go through many attempts to find which is their personal style of learning new materials, new contents. The study was conducted on a number of 31 participants. The group was divided into two subgroups: 16 students in the 5th year at Faculty of General Medicine and 15 master students in the second year at University of Sports, Sports Performance program. The used instrument consists of 21 questions (11 questions are about the individual studying process of the participants and 10 are about their beliefs about the teaching process of academics in courses), along with 4 more questions related to personal data. It is an opinion questionnaire. The teaching style of academics differs: those of Faculty of General Medicine are focusing on a lecture type style (75%), and University of Sports on an interactive style (67%). We discovered differences in the process of self-learning: between continuous and discontinuous style. For medical students, there was a longer distribution of the self-taught learning process; instead, University of Sports students organize their learning time in the last part of the semester. University of Sports students are more concerned with on the development and refinement of their personal practical skills, but also of the subjects they work with (kids or athletes), their goal being to accumulate their own experience as rich as possible from working with as many individuals and less to take from the experience of other colleagues. The coach-athlete/sports teacher-pupil relationship is more personalized and longer-lasting than the doctor-patient relationship.*

Keywords: *laser Doppler vibrometry, middle ear*

INTRODUCTION

1. Learning activity and its role in summative assessment

1.1. Learning activity for young people - generalities

During the adolescence period (considered between 14/15 and 20 years), the individual seeks his own identity and begins the stage of abstract thinking. Hayford [1] highlights the student period of 18/20-24/25 years as a prolonged adolescence. The young adult age is between 20-40 years and assumes that the most important aspects are the intellectual skills that will be used in professional life and the foundation of intimate and personal life.

Around the age of 17-18, the highest level of mental functioning and the manifestation of human intelligence is reached. The operations of thinking are fully liberated from the informational contents to which they were initially applied, they are generalized, they are transferred and they become formal. Thus, the second-degree operations are

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successful: syntheses of syntheses, generalizations of generalizations, abstractions of abstractions.

Piaget describes the stage of concrete operations as ending around the age of 12-13 years old, when adolescents are able to perceive the constancy of objects in terms of transformations, regarding structure, volume, in a nutshell, the ability to understand the fact that objects are constant in relation to the notion of flexibility. After the age of 13, up to 17-18 years, the stage of formal operations is completed, when young people manage to think inferentially, including the possible, with the issuance of assumptions and the definition of specific pathways for each option seen separately. It defines the logic, the concepts of contradiction, certain grammatical structures of the “double negation” type, the detection of the grammatical concepts of circumstantiality and attributiveness and the optional-conditional, which could be seen differently. Also, an important role is played by the concept of “perceiving the absurd” or of the possibility to operate on the determinants, of the empty set, in the sense of superior mathematics.

During late adolescence, there is a strong conflict related to the balance between maturity and childhood, the balance between needs and how to meet them, due to the relationship between aspirations and possibilities and the conflict between dependence and independence.

Some authors [2] consider that adolescence is a period of confusion, but also a period of future projections that the adolescent makes. The desire for self-improvement appears, emotions and feelings are amplified, moral autonomy and self-awareness are developed, the adolescent has the feeling of independence. There are feelings of disagreement and rejection of ideas imposed by other people.

The period of youth, between 20-24 years [3] is considered a period of transition to adulthood. The acquisition of economic autonomy begins, leading to greater independence. Levinson [4] calls the period from 17 to 22 years - the period of young adulthood and considers that it is characterized by the coexistence of the adolescent and the young adult status. He considers that it is a period of transition dominated by the transition of the young man from the state of child-man to that of young man.

Young people begin to become autonomous, move away from the family environment, from the model imposed by their childhood, create their own social system and thus structure themselves as individuals and part of their generation. The aspirations, the ideal about the world and life, the value system begin to particularize and become constant, proper in the young person's life, giving coherence to his/her actions.

Learning is the main activity of young people. They have a very high capacity to assimilate information, to incorporate it. They also show an increased availability for this activity. The preoccupation for learning comes from an internal motive towards self-improvement, continuous development, search for existential meanings and senses. They are beginning to lose the importance of the school assessment, grading and ranking component, focusing on the activity of acquiring the necessary knowledge in the desired field. According to Erikson [5], the adult stage involves: amplifying social identity, employment and involvement in social life, intense feeling of love, the beginning of family life and the development of intimacy, the oscillation between isolation – intimacy - the tendency to link relationships with others groups.

Knowledge and, in general, the intellect has a wider notional area than the psychic process of memory. In turn, memorization is only the first part that defines memory as a high psychic function, the other two aspects being the preservation of information and updating (which can be in the form of recognition or reproduction). The intellect is the superior form to which the definition of the personality of young people accesses, this including the peaks of the "iceberg" represented by each function or psychic process. In the absence of memory, life would be a "ball of sensory impressions", structuring the continuity of the psyche leaving "gaps" in the network in which students submit their information. If we refer strictly to the psychic process of memorization, we can describe the concept of "buffer", in which many students run, more or less information, depending on individual abilities, some being those who learn early and cope with information exams stored in long-term memory, others having as a favorite profile the loading of the buffer (this being a capacity, an ability in itself) and having remarkable results with the information stored here. The persistence over time and the effectiveness of this type of long-term learning in relation to valid and valuable expression in the future profession is to be discussed.

The overall mental operations are developed and many specific algorithms are formed. The motivation for vocational learning is strong, especially practical work-related learning. Another important aspect is that learning becomes strongly selective, conscious and voluntary. The formation of hypothetical-deductive thinking appears when the young person has the capacity to abstract, to separate the notion of object from a concrete reality and the possibility to analyze his own thoughts. He/she begins to understand and deduce the cause-effect, singular-particular-general connections. The ability to apply in practice with an individual character is also formed - the young person has the tendency and ability not only to acquire new knowledge, but also to use it to create something new, integrates it much better [6].

Young people go through new challenges once they enter the university/vocational education system related to important exams, different classification system, different grading system, different type of examination, university structure and so on.

1.2. Self-training

Youth has an emphasis on the need and desire to learn more on their own, with autonomous effect. A style that focuses on the need for continuous renewal of one's knowledge through a constant personal effort is preferred, an effort that opens the perspective of developing the skills to learn alone, of autonomous and independent training. These self-taught systems will gain ground, because they respond better to self-motivations, an internal motivation for self-improvement and training, for self-completion, each individual having the opportunity to cultivate and better value their own abilities. and skills. Thus, the individual becomes his own "manager" of his intellectual, cultural and professional progress and plays an active role in the development of his cognitive function and his own training.

Self-training is defined as an integral part of self-education [7], which refers to the enrichment of knowledge and independent intellectual training. It involves a motivational impulse and the acquisition of tools of independent intellectual work. Individuals create their own self-training system, with personal, adapted techniques, which are based on his style. These involve: choosing the necessary information, systematizing it, processing the learning content, using the content to solve some learning tasks (exercises that help to integrate the learned content), self-control and so on.

Self-training is considered a form of manifestation of cognitive activism [8], a difficult and complex process that is not summarized only during university studies, but throughout the life of the individual. It is considered that the self-taught activity requires great efforts, ability to organize, to organize his/her own personal space, concentration on the task, developed motivational sphere, permanent control and self-improvement. This type of learning is common in high school. It is based on awareness and formation of personal appreciation of the domain taught. Metacognition is the theoretical foundation behind self-training. Self-regulated learning is based on self-responsibility.

The following question also arises: why a person cannot have a self-regulated learning throughout all learning experiences. Other theorists [9] believe that, when they are young, children cannot be self-regulators during learning in any formal way. In addition, comes Piaget's theory [10] that explains that against self-learning learning comes the egocentrism of the young child. And Vygotsky [11] emphasizes the importance of young children's inability to use inner language in guiding roles.

It is important to mention that the self-training activity must in turn be learned and repeated. Students go through many attempts in order to find out what is their personal style of deepening the teaching courses, their contents. Dordea [12] states that there are three sub-competencies that students must master in order to succeed in the process of self-teaching: self-appreciation of the personalized strategy, realization/implementation of that strategy, self-evaluation of results. Basically, the student has to "learn how to learn".

The individual learning project can be improved through two main ways: 1) indirectly, through experience; and 2) directly, through training. Thus, individual study can be encouraged through recurrent positive experiences (students notice that self-assessment is positive and that self-instruction increases the accuracy of learning), similar to the theory of positive reinforcement.

It is important that adolescents and young people acquire self-regulatory skills during their development. As the time passes, they need to process an ever-expanding volume of information and operate with knowledge at increasingly abstract levels. An essential feature of self-regulated learning is the dynamic approach of the topics to be learned. Thus, the student can analyze the teacher's comments and compare them with his own perceptions of the learning situation. Can self-learning lead to a burn-out syndrome due to the pressure and informational volume? Recent scientific research based on evidence-based medicine is highlighting the increased number of young people that are developing burn-out syndrome, sometimes due to medical conditions (neuroendocrine for example) that leads, in the severe form, up to depression [13].

The situation of students who become pregnant at a young age should also be discussed as a separate perspective (even if they have a lower frequency in the University of Medicine or Sports, given the specifics of learning or activities/ practical works). However, their number should not be neglected, especially in terms of pregnancy hormones, which cause mood swings, which can influence the ability of learning, given the motivational-volitional links with those of mnesic-prosexic processes and learning, especially in the amygdala area and the hippocampus area, brain areas significantly involved in both emotional motivational regulation and superior gnostic functions, so that they are active at the optimal level in terms of supporting the effort to complete a university curriculum [14, 15].

1.3. Adult education

The educational process of adults must respond to the challenges posed by today's society, which is changing its rhythm very quickly. Giddens [16] proposes a series of characteristics of late modernity, as typical elements for contemporary western society: industrial capitalism, reflexivity, individual responsibility. Modernization has accentuated digital education, with the help of digital technologies in many fields, from trade to education, medicine, teachers [17].

Adult education in Romania takes place in the context of another type of modernity than that of the western countries, namely tendentious modernity [18]. This concept refers to the fact that there are differences between the style in our country and the western one, due to some older concepts in our system, which makes the modernization to be partial and unfinished.

Adult education occurs only by virtue of personal own vision of the project of developing society, with all the implications of the coexistence of the old with the new, of progress with conservatism, of the modern with the traditional. It aims to improve existing skills and/or acquire new ones. It also aims to assimilate system values that have previously proven social performance and put them into practice. Adult education asserts itself in different forms of education: continuing education, non-school education, non-formal education.

There are a number of phenomena that prevent the formation of adults: inequality of opportunities in education, inequalities between levels of education (between educational units from the same area, between students), inequalities of access between faculties. In several cases these inequalities have a huge impact on the developing personality of an adult person even more if this personality closes the borderline type or reaches the psychiatric connotations [19]. There are several causes that are against unitary access to educational levels: poor material endowments, underfunding of educational units, differences between schools in urban and rural areas, problems related to human resources in schools, school dropout, repetition, the existence of a segment of the population that does not take advantage of the educational system. The higher the level of schooling, the higher the value of the additional costs of education that the family has to cover, discussing here for example: the fee for college, food expenses, textbooks, rent [20]. Nowadays, recent strategies involving the cost of education are trying to keep the step with full on-line education system, additional costs covered by the family have changed their levels: for example, a higher investment in modern communication devices. In the same time, the educational system redirects its costs based on this new on-line strategy.

Lifelong learning involves everything a person does throughout life to complete his/her knowledge, learn new things, acquire skills or develop his/her talents in formal, non-formal and informal contexts.

It is important to note that education, from one level onwards, is optional. Post-school training is often a personal choice. Unfortunately, there are no mechanisms in society to encourage adults to learn continuously. They may limit themselves to the knowledge gained in school due to routine, inability to cope with change, difficulty in economic and financial management, disinterest in performance.

It is observed that young people who have obtained a higher education have: information processing capacity that can be extended to other cognitive areas, the operability of thinking is kept at high levels, the young person's thinking is wide, deep, systematic, rigorous, very good mental adaptability to specific professional. Young people have critical thinking [21].

1.4. Evaluation - types of evaluation

Evaluation is a component part of the educational process. It informs us about the effectiveness of teaching-learning strategies and methods and appreciates the correct choice of operational objectives on lessons. The teaching-learning-evaluation relationship presupposes a cause-effect circle, in which each component conditions the other. If we are thinking at the western model, the evaluation should not be considered a control method that would punish those who fail it. Students must understand the learning content, not only memorize it, and the given task must be contextualized, in which the subject can express his/her learning style.

Evaluation can be of several types. It differs depending on the type of classification. According to the criterion of the moment at which it is performed, the evaluation can be:

- initial (in the initial phase of the instructive-educational process) - aims to see what is the level from which to start,
- continue (throughout the training program) - aims to observe the improvements along the way and to achieve the optimization of the educational process,
- summative (at the end of a session/module).

The evaluation can also be: formal and informal. The formal one presupposes well-defined criteria, previously outlined and it is associated with standardized tests, it presupposes a certain level of expertise of the one who performs it (License type exams, Baccalaureate, National Assessment etc.). Informal assessment is based on intuitive criteria and involves tests created by the teacher.

There is another classification of evaluation: internal or external. The internal one is made by the internal teacher (the one who designed the learning content is the one who evaluates it). In this case, the evaluator knows the particularities of the students, is familiar with them, knows their progress along the way. The external evaluation is performed by a teacher who has not been involved in the instructional-educational process and has the advantage that it is objective.

The evaluation can also be of normative and criterion type. The normative one has the following characteristics: the performance of the subjects is related to the group norm, to the reference group (class, year, respective series) and compares the students, it ranks them. Criterion evaluation presupposes that performance is related to an absolute criterion, to a standard. Evaluate absolute success in evaluation.

According to some other authors [22-24], evaluation can be: comparative (has the role of classifying subjects, relating them to each other, according to their level of success), based on a criteria (has the role of providing functional information, allowing subjects to be in in relation to achieving the objectives common to all subjects), corrective (wants to provide the subject with additional information depending on the difficulties he encountered), conscious or formative (oscillates between cognition and metacognition and promotes active participation and autonomy of the subject so that he/she, subsequently, takes control of his/her own transformation, becoming aware of its shortcomings and difficulties).

The traditional assessment methods are: oral tests, written tests and practical tests. There are also complementary, more modern methods, among which: systematic observation of behavior, investigation, project, portfolio, self-assessment [25].

Sometimes the psychological and physical overload based on a frail personality structure could lead to an interpretative phenomenon of key components of an evaluation, that, for the student, seems to be debatable. Risks will lead later on to anxiety and depression sometime at psychiatric levels [26, 27].

1.5. Summative evaluation

It is also called cumulative evaluation. The act of evaluation involves three stages: measurement, assessment of results and adoption of improvement measures [28]. It is performed at the end of a session/module. It has the purpose of accreditation/decision to improve future activities. It is performed by an internal or external evaluator, is done at long intervals and aims to see the final results of the students. It tries to test the final and accumulated knowledge that the students have acquired. It is important because of its dual role. It is useful for the trainer, to see if the chosen means and methods are useful, effective, if the information has arrived correctly and as quantitatively as possible. It is the feedback he receives from students. It is also useful for students to objectify what information they understood, what should have been clarified in more detail, etc.

It is a type of balance sheet assessment, global, at the end of relatively large activity segments. The disadvantage of this type of evaluation is that it does not allow the regulation/improvement of the didactic process; it is possible only after relatively long periods or never. Thus, trainers cannot change the teaching-training style to improve the results of their students, because they cannot make a second summative evaluation. However, it highlights the level at which the activity took place and its results, recognizes the efforts made by the subjects and the interest shown by them. It also offers the satisfaction of the social recognition of the subjects' competencies. At the level of society, these cumulative assessments certify that subjects are able to cope with certain tasks and requirements and that they can play certain roles in social life.

It is well known that grades, often, do not exactly reflect the true level of performance of the subjects and as a result, are not stimulating and do not provide sufficient data on the effectiveness of the training program. It is advisable to use it together with other types of assessment, such as continuous assessment, to rely on continuous feedback to cooperate the teacher with the students [29].

MATERIALS AND METHODS

The present research aimed to highlight some differences in the learning-teaching process of young people at the level of students at the Faculty of General Medicine, compared to those at the National University of Physical Education and Sports. We tried to observe what is the time that young people aged 18-25 allocate for individual study in their preparation for the summative assessment and the level of awareness of the need to be involved in the self-taught process. We highlighted the perception of students in relation to the teaching style of academics. We also sought to highlight possible psychological motivational explanations for interpreting the results.

The tool used was a questionnaire that consists of 21 questions (11 questions are related to the individual study process of young people in the group, and 10 are about the teaching process sustained by the professors at courses), along with four more questions to highlight the factual data. It is an opinion questionnaire. The participants received the appropriate training to complete the answers and were assured of the confidentiality of the data, their results being used only for the purpose of this research. The questionnaire took about two minutes to complete.

The tool was uploaded in google forms and sent to the participants to form the database. The data were collected between April 15-25, 2020.

The study was performed on a number of 31 participants. The group was divided into two groups: 16 students in the 5th year at the University of Medicine and Pharmacy- Faculty of General Medicine and 15 master students in the second year at The National University of Physical Education and Sports, the Motor Performance program.

The ages of the participants ranged from 22 to 26 years. There was female (18) and male (13) participants.

RESULTS

Regarding the methods of education and training in young people, we highlighted the teaching style of the university staff from the two faculties and we noticed differences between the style of them, one activating in the field of health and the other one in the field of sports (Figure 1).

Figure 1: Teaching style of academics (percentage of teachers)

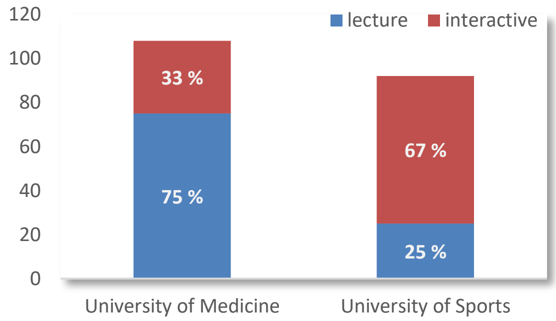

Although, currently, the teachers from the medical university are increasingly open to a more interactive teaching style, it is important that a well-structured lecture is based on a diagnostic tree, and the mastery of logical thinking by the medical student implies, in for the most part, the acquisition of diagnostic tree-type thinking. This is the reason why many medical teachers prefer a lecture style because it is better suited to the large learning content, compared to those in the field of sports that emphasize the practical part of the theorized content.

Medical professors focus more on teaching "thick materials" in a lecture-style method when they are addressing to a homogeneous group of young people, with preliminary and extensive concepts from the pre-clinical years and for whom the information presented makes sense and it is easy to understand. For more difficult subjects, students prefer a direct teaching method, in which they manage to write down the information as accurately as possible, in order to have the chance of a second or third passage through the materials represented by their own notes, in the idea that the information it was so abundant that the student (less familiar with information gaps from previous years) could not be assimilated. For such students, who have become more accustomed to the learning required to go to the University of Medicine, the lecture-method makes it less difficult for students to be less thorough, as they need accurate exposure of information and at a slower pace, so that the lesson can be more easily assimilated.

Regarding the presentation style of the information in the courses, the total distribution at the level of both faculties is presented in Figure 2 – presentation style. We consider that it is a too high percentage of teachers who read the presentations in the courses, being more a monologue type course, than a bidirectional one in which the information is incorporated by the student. It is true that of this 48%, 66% of respondents were medical students. This suggests, again, that the need for knowledge, the dictionary that future doctors must master, is much greater than that of sports specialists. Thus, it is absolutely necessary for health teachers to exhaustively expose, perhaps sometimes arid and dull, medical terms that students must master.

Figure 2: Presentation style

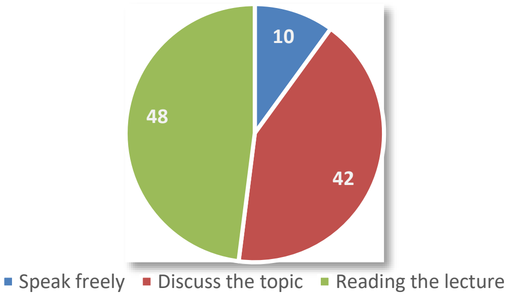

This research can show the need for certain teaching materials that must be presented in a readable and slow tempo. This need is related to a psychological concept regarding the teacher's ability to establish a connection between the unconscious mind of the students (who gradually enter in a learning state) and the unconscious mind of the teacher (the later having a more systematized information). The slow tempo prepares the student to enter the scientific atmosphere in a preliminary psychic state, kind of an awaiting state of mind for the "key" information, which comes gradually. The student must receive the information with the same curiosity, interest and respect for the simple information. These will represent clues for the future job, because the causal links cannot be understood exactly if the students expect only interesting and spectacular information and they pay attention only when these are exposed.

Most students (81%) of both groups estimated that a course has about 10-20 pages. All subjects accepted that they reopened the materials for the information taught in their practice.

To the question that probed the need to individually read and understand the content of the courses, the students from the sports university answered, in a percentage of 100%, yes. On the other hand, in the group of medicine students, the distribution was uneven. This shows us that at the university of medicine is a much more detailed and exhaustive content that requires explanations from teachers, while at the sports university is more emphasis on the practical side, students having the necessary knowledge to understand the content of the courses. The explanation can be seen in the Figure 3.

Figure 3: The need to extract the main information individually from the course content (number of students)

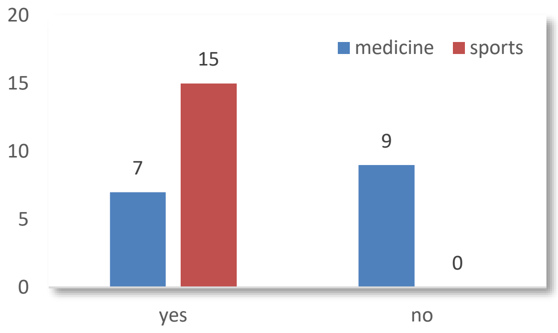

Regarding the information presented at either of the two faculties, if we refer to the high-level learning, more exactly, the preparation for master's or doctorate for UNEFS students versus the preparation for bachelor's or residency for medical students, it is necessary to explain the detailed information in time, for longer periods in terms of dosing effort. It is well known that for grade 7, students must go through the subject once, for grade 9, twice, and for grade 10, three times. This is especially important for difficult materials, which include arid parts (and less practical demonstrations), the preclinical subjects in the first years of the Faculty of Medicine or the theoretical parts of some less publicized sports. Also, the preparation of any important exam involves dosing the effort, thus, in the first days one learns about four-five hours a day, and in the last ten to fourteen days, in the memory has to run for the last time the whole subject, with the intelligence to focus on the key concepts and logical causal links.

The results showed that most medical students' courses have more than 7 pages, which makes them spend more time learning the content needed for summative assessment. It should be noted that the material to be learned from the Faculty of General Medicine follows a general pattern that involves a predetermined order: prevalence and incidence of the phenomenon/disease, etiological aspects, pathophysiological aspects, characteristic symptoms, their grouping in syndromes, main diagnosis, differential diagnoses, evolution and prognosis (positive factors, negative factors), treatment options. It is known that most of the time is spent understanding the pathophysiological mechanisms behind the diseases, but in the field of health it is absolutely necessary to go through these arid mini-chapters to understand the phenomenon in full, compared to specialists in sports, where the accent is on practical application. In addition to changing/developing learning skills in relation to the age and experience in student years, learning styles also change in relation to the years spent in a particular faculty, with the involvement in adapting to the specifics of that faculty. This capacity of adapting involves flexibility of thought and intellect and, of course, experience. Once familiarized with the most important concepts in the field of activity, after about five years in a faculty, students, master students, doctoral students or residents achieve the performance of reading about 20-30 pages of scientific content with which they are familiar, without feeling the effort, with pleasure, interest and the possibility of thinking in parallel for developing their own opinions. In the last category, the teaching-learning mechanism is reversed, starting from practice to conceptualization, which is more attractive for students because they enjoy the results of concrete work faster (Figure 4 – the learning processes).

At the level of both educational structures, students similarly reported the need to take notes during the courses, but two respondents from the medicine university noted that most courses receive written support which made them not need to take notes. We emphasize here the importance of distributing written course support to students.

Figure 4: The learning processes.

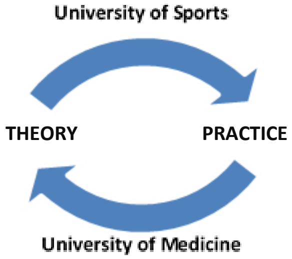

We consider that the educational instructional process is much more effective in the case of the existence of written materials after which the student can be guided later, during the individual study process. The main activity of the student during the course should be to focus attention on the topic of the course, on the information that the teacher has to offer. We are trying to adhere to a western education system, in which the modernization of the process with the help of technologies is desirable. We consider it appropriate to emphasize the link between taking notes and the facilitation of the active memorization, later learning and reproduction the material. There are students who manage to go through large materials (hundreds or thousands of pages) through active learning and using a specified type memory, the one from which the information drains unconsciously in a passive way. Others are focused on compiling their own notes, on taking notes, failing to store information if it is not processed and systematized in their personal way. We do not comment on the superiority of any of these techniques, but we want to emphasize different ways of how the mind function. The personal notes, as the individual dedicates himself to the profession, can become more and more schematic, becoming understandable only by the one who writes them. These personal notes can exclusively define causalities, "key" concepts, inferences, connections and fewer mnemonic formulas (these being typical for an earlier stage learning).

Another aspect to note is the desire for the lecture not to be completely similar to the course support and it proved to be more efficient to listen to the lecture, when the student does not focus on finding those notions in the written material, but on understanding the explanations given by the teacher and following the logical thread of the lecture, proving much more effective than the situation in which the student reads the learning content beforehand. The notions described in medical courses are difficult, imply new and unknown terms to the student, which makes it important to lecture and especially to be accompanied by explanations, examples that make the learning content more accessible. That said, the lecture is a material more understandable to the student than some course notes in medical terminology. In this case, the role of the lecture is to lower the level of understanding from abstract to concrete, from general to particular. Instead, in the sports environment, the provision of specialized materials is more easily accessible at first reading even to an audience unfamiliar with the field, but the difficulty lies in the practical applicability of the theoretical content.

In both Universities, students rated similarly on a Likert scale from 1 to 10 the "weight" – the difficulty of a course, somewhere at a grade of 7-8. This suggests that the teaching content is reported to the type of students taking part in the course. Thus, the participants do not have a comparison model, all the courses are of the same similar level within his university. At the both faculties we suggest the need to introduce courses with graded difficulty, thus the difficult subject is exposed at the, approximately, seventh-eighth meeting with students, after they have acquired interest in the studied information and have sufficient knowledge to be interested in deepening the subject.

We wanted to probe the students' conscientiousness about the self-instruction process for summative assessment. The Figure 5 shows the difference between continuous learning (throughout the year) and discontinuous learning (exhaustive period during the session, in which they only prepare for assessments). For medical students, there was a longer distribution of the self-taught learning process, instead, the sport university students organize their learning time in the last part of the semester.

Figure 5: Distribution of the self-training process over a longer period of time (number of students)

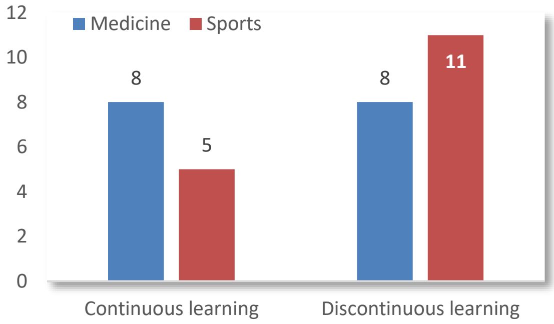

Figure 5 shows that the distribution of medical students' conscientiousness is divided equally between those who study continuously and discontinuously, while in the category of students at sports university, most of them learn the theory for the exam shortly before it.

Among the explanations, we thought about the aspect that, in both situations, the practice of that profession has two components: the practical part (working with patients versus working with students) and the conceptualization part which involves talking and theorizing what you do. For the doctor, we refer to the composition of the observation sheet, the possibility to explain the case and the scientific research; and for sport coaches and teachers, it involves the lesson plan, the training plan, the monitoring through observational feedback. Medical students, from year 3, go to hospitals, see clinical cases that arouse their interest, receive partial or incomplete explanations from their seminarians about solving the case. Thus, the interest to learn more is aroused, and with the help of modern forms of learning (internet, for example), their curiosity makes them look for information and learn more constantly through the challenges from practice to theory. This learning is not necessarily a systematized one, but it puts the student in a greater contact with the learning content.

The students at the sport University are concerned with everything related to the development and refinement of personal practical skills, but also of the subjects they work with, their goal being to accumulate their own experience as rich as possible from working with as many individuals and less to take from the experience of other colleagues, because the coach-athlete/teacher-student relationship is more personalized and longer lasting than the doctor-patient one (especially in the emergency medical branches and not in chronic diseases). However, there are also some long-term doctor-patient relationships, such as these in chronic diseases (such as psychosomatic, rheumatological, oncological diseases, which require time dosing the treatment regimens and proper management, both somatic and psychological). Such relationships are of the adult to adult type (when the patient is responsible for dose adjustment depending on the acute symptoms present), parent to child relationships (which are sometimes necessary when the patient himself is a doctor and such an attitude becomes useful for making the patient in question listen), friendship-type relationships (where the doctor goes from being an “enemy of the disease” to being a friend of the patient, a counselor, a trusted person who helps the patient not feeling the limitations imposed by disease). Similarly, the athlete-coach relationship can: define the coach’s domination (in terms of his personality and executive function) or transform the coach into an advisor of training, shifting the responsibility to the athlete. In the later type of relationship, the coach becomes guide for optimal and efficient development of his athlete. The coach can be a mentor, a friend, an advisor, an authoritarian superego, who controls the desires and objectives of his athlete and the dosage of efforts, but always maintains the high-performance standard.

We objectified that most medical students give for the learning process over 5 days before an exam, while those at the sports University give 1-5 days. The uneven distribution can be seen in the Figure 6.

Figure 6: Distribution of days granted for learning process, compared between the two universities (percentage of the students)

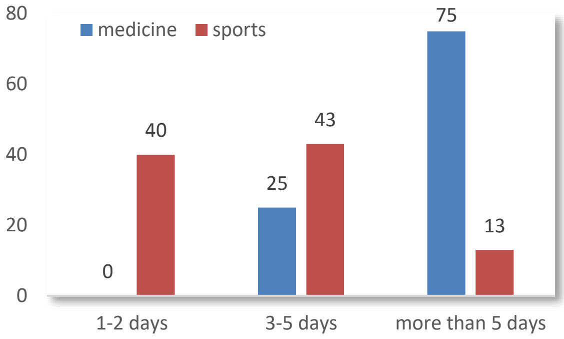

An explanation of the phenomenon could be that those medical students who give more than 5 days are those who, over time, have formed a structured thinking, to learn in steps, from micro aspects, to macroscopic aspects, from pathophysiology and etiology towards the logical understanding of the formation of the symptoms characteristic of the respective disease. In another category are those medical students who believe that diseases should be acquired from the clinic to understand the etiology and thus, they intentionally omit the arid part of the learning content, believing that it operationalizes learning. Many of these "more efficient" students can get high grades in practical exams, maybe even at oral ones, where the teacher requires only the clinical conceptualization of the case, but still a deeper approach will reveal the gaps in understanding the etiopathogenic phenomenon

We sought to probe the maximum time a young person can stay focused in a learning activity in late adolescence and whether there are differences between the training and the profession they choose, differences that will influence their ability to concentrate. From the collected data, we did not discover significant differences, which leads us to think that this capacity depends on certain internal factors and on the stage of personal development. This can be seen in the Figure 7.

The psychological explanation of this phenomenon can be in the concept of endurance and the development of long-term endurance in terms of staying longer and longer in contact with notions, concerns, problems, conceptualizations of the chosen profession. The faculty and the acquisition of information during it are only the preamble, endurance itself is obtained with the practice of the profession, important roles having curiosity, self-improvement, desire to be up to date with news, desire to interact and exchange information with other professionals. We consider that the age range of 18-25 years is the one in which the foundations of endurance are barely laid and still different students are looking for the right techniques for acquiring information, sweeping between what would be easy from theory to practice or to practice in theory ("learning to learn").

Figure 7: Concentration endurance

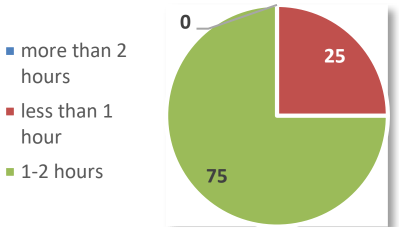

We found that there are significant differences between group samples in terms of the maximum number of hours per day spent for individual study. The percentage of those who spend more than 5 hours a day for individual study is in medical students' area. This demonstrates that they make greater efforts to self-train, have a greater ability to organize, focus on the task, a higher motivational motive, but also the desire for self-improvement more developed than in the field of physical education. The distribution can be seen in the Figure 8.

Figure 8: The maximum number of hours per day spent for self-training (percentage of the students)

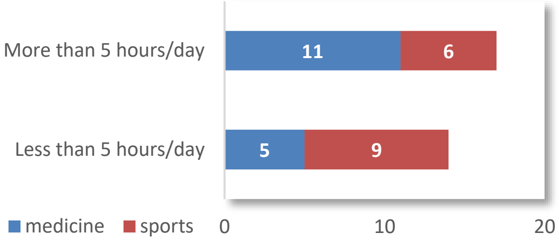

We also probed the importance of the self-taught process, by asking the need to study at home the material presented in the course to pass the final exam. We wanted to emphasize that medical students need a period of self-improvement, a period of in-depth assimilation of the information presented to the course or practical work, compared to those from a sports University, who managed to pass the exams based only on the knowledge assimilated to the courses (Figure 9).

Figure 9: The process of passing the exams based only on the knowledge assimilated to the course (percentage of students)

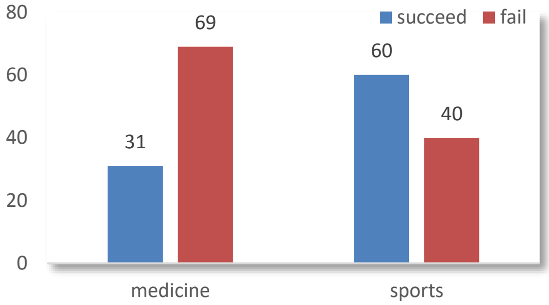

DISCUSSION

There is a major difference between the style of teaching knowledge for students in the two faculties - the mechanism for medical students is from theory to practice; while for students in the field of physical education, the learning mechanism is reversed, from practice to the formulation of theories.

The field of medicine has been developed for thousands of years, and during all this time, specialists have learned to work simultaneously: in one plan to diagnose and treat, and in the other to make explicit, theorize, generalize, argue, and develops an inferential and dichotomous thinking. They have developed their own style of communication between specialists to convey key information in operationalized and synthetic concepts. Thus, 66% of medical students perceive that the teaching style of teachers regarding the medical dictionary must be mastered as being much focused on the presentation of information and less on practical applicability. Instead, sports coaches or teachers have recently started to focus on the conceptualization of their work, on quantification, on operationalization, on ways of exchanging information (to external sources such as: psychologist, athlete's family, school leader, etc. or between them as specialists).

100% of the students from the sports University consider that they need to inspect the learning material themselves, but it is very important that they have the necessary information to understand it individually. In the field of coaches and sport teachers, the emphasis was on the practical side, on streamlining the educational-instructional process, on refining personal skills, on developing the means and methods of teaching the learning content.

Teachers in both faculties should focus on lecture-type teaching, but not identical to the written material; they have to include examples, follow a red thread (message to take home) and ensure that it has been passed on to the student.

Along these lines, students should be considered active partners in the teaching process even if their level of instruction is much lower than the teacher’s one and even if there are significant differences between participating students in terms of previous level of knowledge, involvement, curiosity and motivation. The teacher's role is to modulate the "global intelligence of the audience", encouraging the obviously participatory students and stimulating the less interested ones. It would be a didactic mistake to have the preconception that the overall intelligence of the audience is low, which may lead the teacher to lower the level of the lecture or consider that it is no longer necessary to provide complete information, omitting examples and features or not presenting parts considered more difficult. Students similarly rate the weight of the courses on grades 7-8, which demonstrates that the learning material is hard enough to keep them focused and grasp in their understanding, but easy enough not to lose them and to be at their level of knowledge.

Among the teacher's tasks should be to increase the student's endurance (making him forget how time passes), involving him in active listening in which the student's thinking becomes a partner of the teacher's thinking in understanding the chain of information explained (even if it not perceived). We notice that in young people aged 18-25, endurance is limited to 1-2 hours mostly, but it is acquired by stimulating logical thinking, memorization through vivid representations with reference to clinical cases and practical applications. It is also known that during this period, students are still learning how to improve themselves, how to learn information more effectively, learn how to learn. At the end of the lecture, the student must remain in a still active state, with the desire and curiosity to look for a minimum of personally discovered information regarding the topic of the course presented. Also, the student's thinking must remain during the lecture able to compare the newly received information with a previous knowledge database, which helps him to make his entire previous notional system richer and supported on a more solid basis.

According to the scientific literature, the period of late adolescence is considered a maximum in terms of the ability to assimilate new information, as well as curiosity and openness to it, the desire to filter and systematize it according to personal experience. Therefore, the teacher must teach them a material appealing to the maximum capacity that he intuits as being present in these young people, crediting them with motivation, life choices, determination for the profession, the belief that they will never give up self-improvement.

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Authors’ contribution:

AIT and RTB contributed in all the stages of the article, they designed the article and revised the manuscript for important intellectual content. AIT acquired the data and AIT and RVC drafted the work. RVC contributed to the conception of the work and revised the language.

All authors read and approved the final manuscript.

Ethics approval and consent to participate:

For this study, the agreement was obtained from the Ethics Committee of Teaching Staff Training Department of The National University of Physical Education and Sports. The subject data were completely confidential and they approved the publication of data.

Patient consent for publication: Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

The information generated and analyzed during the current study is available from the corresponding author on reasonable request.

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VARIA

Evaluation of systemic second-line therapy in recurrent or metastatic esophageal cancer

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Abstract: *Background: More than half of patients with locally advanced esophageal cancer are unresponsive to first-line chemotherapy. To date, there is no standard second-line regimen; new agents, such as immune-checkpoint inhibitors or anti-Neurotrophic Tropomyosin-Related Kinas agents, have only been recently recommended. This study aimed to assess the benefits of classical second-line chemotherapy in terms of survival and tolerance. Materials and Methods: This retrospective analysis assessed the benefits and tolerance of different second-line regimens of chemotherapy in progressive, locally advanced or metastatic oesophageal cancer treated at the department of Radiotherapy-Oncology at the Institute of Oncology between January 2011 and January 2017. Results: The analysis found 87 patient files with a median age 59.5 years (ranging between 41 and 84), of which 40.2% (35 patients) failed the first-line platinum and 5-fluorouracil combination. Histology favoured adenocarcinomas (63.2%). The patients were in good clinical condition, with ECOG 0–1 in 86.1% (74 patients), and less than five percent had more than three metastatic sites (3.4%, 3 patients). The regimens were mostly two-drug cytotoxic (53.9%, 47 patients): FOLFIRI in 18 patients (20.7%), FOLFOX in 18 patients (20.7%), vinorelbine–platinum combination in 11 patients (12.6%), and docetaxel in 40 patients (38.7%). The median treatment duration was 7.74 weeks (range 1 to 12). The general response rate was 47.1% (41 patients), including stable disease. The median time to progression was 7.61 weeks, with a range between 1.6 and 8.6 weeks, with no differences in this respect among the fourth type of treatment received (p = 0.170). Grade 3–4 toxicities, including neutropenia, gastrointestinal problems, and metabolic disorders, were observed in 44 patients (50.6%). The median event-free survival was 2 months (range between 1 and 6.3), and 63.2% (55 patients) subsequently received a third line of chemotherapy. Conclusion: Second-line chemotherapy is still the first-choice recommendation in all fit patients with advanced oesophageal cancer. The new agents are efficacious only in cases with PD-L1 positive or high microsatellite instability.*

Keywords: *second-line chemotherapy, locally advanced/metastatic oesophageal cancer, survival benefit, toxicity*

INTRODUCTION

Esophageal cancer has two histopathological subtypes: squamous carcinoma found in the superior and upper-two-thirds regions [1], and adenocarcinoma, which develops at deep inferior and junctional mucosal glands. Both have high similarities in their pattern of metastasis [2]. Esophageal cancer is the sixth most common cause of specific deaths worldwide [3]. The peak of incidence is reported in the sixth decade of age, and cancer occurs more frequently in men [4].

Approximatively half of the patients have advanced disease at diagnosis. Three-quarters of resectable disease will relapse, of which more than half will be candidates for

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second-line systemic therapy [5]. Overall five-year survival is less than 10% [3].

Regarding the first line, for adenocarcinoma, the regimen used is similar to those used in gastric cancer, fluoropyrimidine-based chemotherapy while in squamous esophageal cancer the regimen is cisplatin-based and in general, regardless the histology, the median overall survival (OS) does not exceed nine months [6].

Currently, there is no established second-line systemic treatment for esophageal cancer [7]. Subsequent lines of chemotherapy have recently been proposed by NCCN (National Comprehensive Cancer Network) guidelines for locally advanced or metastatic esophageal and oesophagogastric junction cancer [8]. New treatment options include ramucirumab and paclitaxel which give a 2-month benefit in median overall survival (9.6 months versus 7.4 months for paclitaxel only, p = 0.017) [9]. Pembrolizumab is a category 1 recommendation anti-PD1 (programmed cell death protein) antibody for esophageal PD-L1 (programmed death-ligand 1) positive or for any histology displaying MSI-H (microsatellite instability-high) or d-MMR (deficient mismatch repair) status [8] based on a benefit of 1 month (8.2 vs. 7.1 months, HR = 0.78, P = 0.0095) versus classical chemotherapy taxanes or irinotecan in the squamous cell carcinoma subgroup [10]. With a 2B level of recommendation, entreticnib is proposed for a median 10 months' duration of response, 95% CI [7.1 - NE] [11], and larotrectinib is proposed in NTRK (Neurotrophic Tropomyosin-Related Kinase) fusion-positive esophageal tumours [12].

MATERIALS AND METHODS

Medical files from the institutional database of patients diagnosed with metastatic invasive esophageal squamous cell carcinoma (SCC) or adenocarcinoma (AC), referred to our institution for second-line chemotherapy were analysed. The main inclusion criteria were age >18 years, clinical-stage IV according to AJCC 8th edition [15], treatment by at least one regimen of chemotherapy or radiotherapy and assessed for response.

Patients were excluded if they were eligible for salvage surgery or had synchronous cancer or severe comorbidities that might impair our results. The study was approved by Ethycal Committee of Insitute of Oncology "Prof. Dr. Alexandru Trestioreanu", Bucharest, Romania no 1597/17th December 2010.

The primary outcome was time to progression, evaluated between the start of second-line chemotherapy and first progression or death by any cause. Secondarily, treatment benefit was evaluated using CT scan, and, in terms of tolerance, the rate and duration of grade III-IV adverse events (AE) classified according to CTCAE v. 4.03 [16].

Data were analysed using SPPS version 23 (IBM Corporation, Armonk, NY, USA). Non-parametric tests were used to compare subgroups. The chi-squared test determined the correlation between response to treatment and demographic and clinical variables. Fisher's exact test was employed when there were fewer than five cases in any cell of the 2×2 table. The associations between variables and survival parameters were analysed by logistic regression analysis, and survival curves were generated using the Kaplan–Meier method and compared using the log-rank test. Hazard ratio (HR) and 95% confidence interval (CI) for variables were calculated using the Cox proportional hazard model. All statistical tests were two-sided, and a p-value < 0.05 was considered significant.

RESULTS

3.1. Patient characteristics

During the period of analysis, a total of 87 patients were treated in our department by chemotherapy for esophageal cancer, with a mean age 59.5 (range between 41 and 84). Among them, 37 patients were treated for recurrent disease, 29 patients had metastatic disease at initial presentation, and 21 patients received the second-line treatment for a locally advanced disease with no indication of surgery or radiotherapy.

The principal histology was adenocarcinoma (63.2%, 55 patients), and the primary lesion was typically situated in the lower and junction of the esophagus (48.9%, 51 patients). One-third of patients had liver metastasis, but almost all had no more than two sites of secondary lesions (96.6%, 84 patients) and were in generally good condition, with ECOG 0–1 in 85.1% (74 patients) (Table 1).

Table 1: Patients characteristics in target population

Characteristics	n	%
Age (years): median 59.5; range [41–84]		
40–50	14	16.1
51–70	63	72.4
>70	10	11.5
Gender		
Male	58	66.7
Female	29	33.3
Location of primary lesion		
Upper	14	16.1
Middle	22	11.5
Lower	25	35.6
Junction	26	33.3

Characteristics	n	%
Histopathological sub-type		
Squamous cell carcinoma	32	36.8
Adenocarcinoma	55	63.2
The initial stage of disease		
I	17	19.5
II	10	11.5
III	31	35.6
IV	29	33.3
Metastatic site		
Lymph node	18	20.7
Lung	18	20.7
Liver	30	34.5
Bone	12	13.8
Other	9	10.3
Number of metastatic sites		
1	50	57.5
2	34	39.1
3 and more	3	3.4
ECOG		
0	44	50.6
1	30	34.5
2	13	14.9

Abbreviations: CHT = chemotherapy, ECOG = Eastern Cooperative Oncology Group Performance Status, FU-Pl: 5-fluorouracil–platinum regimen, LV5FU2CDDP =5-fluorouracil 1600 mg/m2 + leucovorin 200 mg/m2 + cisplatin 50 mg/m2q2w, 5FU-CDDP=5-fluorouracil 1000 mg/m^2 day 1-4 + cisplatin 100 mg/m^2 day 1 q3w, PR = partial remission , PD = progressive disease, SD = stable disease.

3.2. Treatment characteristics

The first-line combination of cisplatin plus 5-FU (5-fluorouracil) was most typical in localized and metastatic esophageal cancer (40.2%, 35 patients). This combination involves 5-fluorouracil 1000 mg/m^2 per day for 4 days and cisplatin 100 mg/m^2 on day 1 every 21 days (17) or, in the so-called LV5-FU2 – cisplatin two-week regimen, cisplatin 50 mg/m^2, calcium folinate 200 mg/m^2, 5-FU 400 mg/m^2 on day 1 and 5-FU 1200 mg/m^2 over a 46-hour continuous infusion (18). Other protocols used were FOLFIRI (39 pts, 44.8%) vinorelbine-cisplatine based (10 pts, 11.5%) and taxane based (3 pts, 3.4%).

Patients who received FOLFOX regimen, as second line treatment modified they represented 20.7% (18 patients), 46% (40 patients) received a docetaxel regimen, 20.7% (18 patients) received a FOLFIRI regimen, and 12.6% (11 patients) received a vinorelbine–cisplatin regimen. Details regarding the protocols used in second line are listed in the Table 2.

Table 2: Second line chemotherapy protocols summary.

Second line regimen	Protocol summary	Number patients	Response rate	Reference
FOLFOX6	oxaliplatin 85 mg/m^2, leucovorin 200 mg/m^2, 5-FU push 400 mg/m^2, 5FU 2400 mg/m^2 for 46 hours, q2w	18 (20.7%)	83.3% (15 pts)	(19)
NVB-CDDP	vinorelbine 25 mg/m^2 day 1+ 8, cisplatin 80 mg/m^2 q3w	11 (12.6)	45.5% (5 pts)	(20)
FOLFIRI	irinotecan 150–180 mg/m^2, 5-FU 400 mg/m^2 push, leucovorin/ calcium folinate acid, 125mg/m^2, 5-FU 2400 mg/m^2/46 hours, q2w	18 (20.7%)	50% (9 pts)	(21)
DTX	docetaxel 75mg/m^2, q3w	40 (46%)	12 (30%)	(22)

Abbreviations: FOLFOX6 = Oxaliplatin 85 mg/m^2+ leucovorin 200 mg/m^2 + 5 FU 2800 mg/m^2 q2w, FOLFIRI = Irinotecan 150–180 mg/m^2 + leucovorin 125 mg/m^2 + 5-FU 1600 mg/m^2 q2w, NVB-CDDP = Vinorelbine 25 mg/m^2 day 1 + 8 + cisplatin 80mg/m^2 q3w, DTX = docetaxel 75 mg/m^2 q3w.

The median time of chemotherapy was 7.74 weeks, with a range between one and twelve weeks. The mean number of cycles was 2.36, with a range between one and seven cycles and no significant differences was registered between the medians of any regimen ($p = 0.065$). Choice of chemotherapy regimen was correlated with the initial stage of disease, histological subtype, the number of metastatic sites, site of metastasis and performance status ($p < 0.0001$). More than half of the patients included in the study were able to benefit from the third line of treatment (55 pts, 63.2%) (Table 3).

3.3. Benefit

The response rates that were obtained were as follows: 28.7% (25 patients) had partial remission, 18.4% (16 patients) stable disease and 52.9% (46 patients) recorded either clinical or radiological progression.

Table 3: Second line chemotherapy protocols characteristics and toxicity.

Characteristics	n	%
Number of cycles: median 2.87, range [1–7]		
< 2	28	32.2
2–4	45	51.7
> 4	14	16.1
CHT duration (weeks): median 7.74, range [1–12]		
< 6	20	22.9
6–8	52	59.7
> 8	15	17.3
Grade 3–4 toxicities		
Without	44	50.6
Haematological	30	34.5
Gastrointestinal	10	11.5
Metabolic disorders	3	3.4

The difference between the response to different second line regimen of chemotherapy were not statistically significant (p=0.042).

We estimated that the median OS on the basis of the results of several studies of second-line chemotherapy [26-29].

Response was directly correlated with primary tumour location (p = 0.022) and histopathological subtype (p = 0.017) and with tumour burden and type of chemotherapy regimen (p < 0.0001).

The median time to progression was 7.61 weeks, ranging between 1.6 and 8.6 weeks, with no statistical difference between the fourth type of treatment received (p = 0.170).

Time to progression was associated with the number of metastatic sites (HR 2.886, p = 0.004) but not with the type of chemotherapy regimen (p = 0.373). By contrast, the log-rank test showed significant differences in time to progression by type of chemotherapy (chi-square 22.1852, p < 0.0001) (Figure 1).

Figure 1: Kaplan–Meier curves in locally advanced or metastatic oesophageal cancer patients according to the second-line chemotherapy regimen.

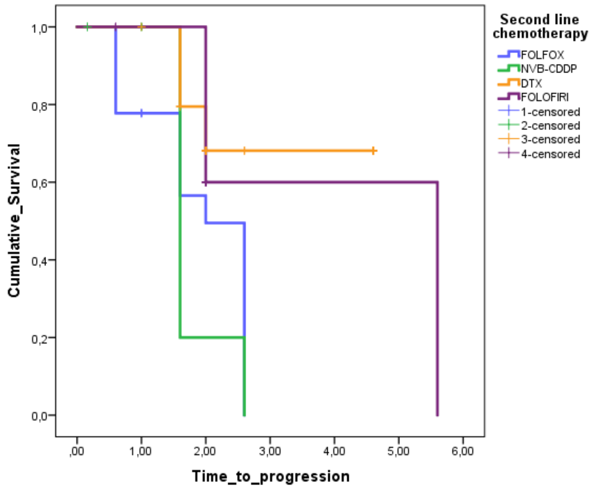

Fifty-five patients (63.2%) could benefit from a subsequent, third line of chemotherapy.

The median event-free survival for all patients was two months, with a range between 1 and 6.3 months.

3.4 Toxicity

Forty-four patients (50.6%) displayed no grade III-IV toxicities. Seven patients displayed haematological and gastrointestinal disorder together, and all patients presenting metabolic disorder also had febrile neutropenia.

DISCUSSION

Esophageal cancer are a challenging disease for which there are limited treatment effective options. Despite research, the only treatment with clinical benefit and more accessible in the second-line setting remains chemotherapy.

The population in our study was in relatively good clinical condition: 85.1% with ECOG 0 or 1, almost all without more than two sites of metastasis (96.6%), younger than 70 and, in more than 60%, bearing adenocarcinoma as histological subtype. In this analysis, the most-used (53.9% of cases, 47 patients) were two-agent regimens: FOLFIRI, or cisplatin–vinorelbine, and FOLFOX. The median treatment duration was 7.74 weeks, and most had received 5-fluoropyrimidine in the first line (85.1%, 74 patients).

The general benefit was reflected by a 47.1% (41 patients) response rate, including cases with disease stabilisation, the median time to progression of 7.61 weeks (range 1.6 to 5.6 weeks) and a median time to death of 2 months (range 1 to 6.3 months). Chemotherapy was generally tolerated, though almost half of patients developed grade III-IV toxicities; one-third of these were mostly non-complicated grade III anaemia or thrombopenia.

Several earlier retrospective or phase II studies have evaluated whether second-line chemotherapy might be useful for oesophageal cancer patients.

Regarding irinotecan, in a small phase II study, 29 patients (27 with adenocarcinoma) were given capecitabine 2,000 mg/m2 day on days 1–14 and irinotecan 250 mg/m2 every 3-week cycle. The study showed an objective response of 17% (five patients) and median progression-free survival of 3.1 months 95% CI [2.2–4.1] and median OS of 6.5 months 95% CI [6–7.1] with a 10% rate of febrile neutropenia [24].

In another phase II study, irinotecan combined with cetuximab in 63 cases of platinum-refractory gastro-esophageal carcinoma, led to an 11% (6 patients) partial response rate and 37% (23 patients) rate of stabilization, with median progression-free survival of 2.8 months, median overall survival of 7.1 months and a 32% rate of grade III-IV toxicities [25].

Even as a single agent, irinotecan showed a survival advantage, compared to best supportive care, in 40 patients with metastatic or locally advanced adenocarcinoma gastro-esophageal of the junction. Irinotecan given of 250 mg/m2 with the possibility of an increase to 350 mg/m2 if well-tolerated, gave a 53% rate of stable disease and 50% clinical benefit versus 7% and a median survival of 4 months 95% CI (3.6–7.5) versus 2.4 months 95% CI (1.7–4.9) and 0.48 HR for death 95% CI (0.25-0.92) [26].

In untreated metastatic gastro-esophageal adenocarcinoma (N = 220 patients), by substituting oxaliplatin for cisplatin in a FOLFOX-like regimen (fluorouracil 2,600 mg/m^2 over 24-hour infusion, leucovorin 200 mg/m^2 and oxaliplatin 85 mg/m^2 every 2 weeks) there was no statistically difference in terms of progression-free survival (5.7 vs. 3.9 months, $p = 0.077$), response rate (35% versus 20%) or median overall survival (10.7 vs. 9.8 months). Adverse events were significantly fewer (9% vs. 19%) and treatment showed better responses in patients aged over 65 years old compared to cisplatin: 41.3% response rate versus 16.7% ($p = 0.012$), time to treatment failure of 5.4 versus 2.3 months ($p < 0.001$) and progression-free survival of 6.0 versus 3.1 months ($p = 0.029$) [19]. A later meta-analysis (N =1294 patients in three randomized trials), regarding the meme subject, showed improved progression-free survival (HR = 0.88, $p = 0.02$) and overall survival (HR 0.88, $p = 0.04$) in advanced, unresectable gastric cancer [27].

In a monocentric retrospective study of 94 patients with metastatic esophageal cancer, a modified FOLFOX 6 regimen comprising fluorouracil 2400 mg/m^2 over 48 hours after a bolus of 400 mg/m^2, leucovorin 400 mg/m^2 and oxaliplatin 85 mg/m^2 gave an objective response rate of 41.4% (39 patients), of whom 6.3% (6 patients) with a complete response and 21.3% (20 patients) with stable disease. Any grade of hematologic toxicity was found in 69.1% of cases [28].

Regarding taxane-based chemotherapy, the UK COUGAR-2 phase III study(N=168) confirmed the benefit of second-line chemotherapy by docetaxel 75 mg/m^2 every 3 weeks compared to best supportive care by a median OS of 5.2 versus 3.6 months (HR 0.67, 95% CI [0.49–0.92]; $p = 0.01$) in patients with a good performance status [22].

In a retrospective study which assessed in 163 patients the clinical benefit of second-line taxane therapies, docetaxel at 70 mg/m^2 every 3 weeks versus paclitaxel 100 mg/m^2 weekly for 6 weeks of 8 weeks cycle, survival without progression was identical (2.3 months) for both regimens the overall survival was 6.1 versus 5.3 months, with 32.6% and 16.1% rates, respectively, of grade III-IV toxicities [29].

Only a few randomized studies have compared the benefits of different second-line chemotherapy regimens, mainly in gastric cancer and in Asian patients.

In 202 Korean patients with less than two prior lines of chemotherapy, randomized in a 2:1 ratio treated with docetaxel or irinotecan compared with supportive treatment,was recorded a superior overall survival (5.3 vs. 3.8 months; HR 0.66, $p = 0.007$) while no survival difference was recorded between docetaxel and irinotecan. The tolerance was good, the hematologic toxicities manageable and the rates of non-haematologic toxicities were comparable in both groups but there was with no significant differences in terms of survival between two regimens of chemotherapy [30].

In another Asian study, in 223 Japanese patients randomized to received paclitaxel alone or irinotecan alone as second line treament, the median overall survival was comparable (9.5 vs. 8.4 months, $p = 0.38$), although less toxicity appeared in the paclitaxel arm and more of those patients went on to receive third-line chemotherapy [31].

In a meta-analysis of 28 studies (N = 4810 patients), mostly retrospective, the second line showed no difference between taxane- and irinotecan-based regimens in terms of overall survival (HR 0.94, CI [0.78–1.13]) or survival without progression (HR 0.84, CI [0.69–1.03]), nor was there any difference for any single-agent regimen compared to best supportive care (HR 0.65, 95% CI [0.53–0.79]. Median survival gain ranged from 1.4 to 2.7 months among the individual studies considered. Doublet chemotherapy based on taxane or irinotecan plus platinum and fluoropyrimidine did not display different survival but showed increased toxicity compared to taxane or irinotecan monotherapy [6].

As far as new agents, despite the failures of several clinical trials involving molecularly targeted agents, several have shown some benefits worth mentioning that represent the basis of NCCN recommendations [8].

Ramucirumab, an antibody that targets the vascular endothelial growth factor receptor 2, the expression of which might be a mechanism of the aggressiveness of gastric and gastro-esophageal junction adenocarcinoma, led in pre-treated patients with esophagogastric cancer significant survival benefit, 5.8 months compared with 3.8 months for placebo (HR 0.776, 95% CI [0.603–0.998]), a similar benefit were seen in the COUGAR-02 trial assessing the benefit of docetaxel [32].

By adding paclitaxel to ramucirumab, in another phase III trial, it was reported a significant benefit in terms of median OS 9.6 months versus 7.4 months, HR 0.807, 95% CI [0.678–0.962] ($p = 0.017$). In terms of adverse events, the association was mainly marked by hypertension (14%, 46 patients vs. 2%, 8 patients), fatigue (12%, 39 patients vs. 5%, 18 patients) and abdominal pain (6%, 20 patients vs. 3%, 11 patients) [9].

Regarding the role of immune checkpoint inhibitors, in the KEYNOTE 181 trial (N = 628 patients), the response rates with pembrolizumab were double or even triple those achieved with chemotherapy at the investigator's choice (paclitaxel, docetaxel, or irinotecan), though the median duration of response was longer in the group with PD-L1 CPS (Combined Positive Score) equal or higher than 10 (9.3 vs 7.7 months with chemotherapy HR 0.69; 95% CI [0.52–0.93]; $p = 0.0074$); 12-month overall survival rate in CPS ≥10 patients was 43% compared to 20%. With pembrolizumab, fewer patients had drug-related adverse events of any grade (64% vs. 86%) or grades 3 to 5 (18% vs. 41%) compared with chemotherapy. The most common events were fatigue (11.8%), hypothyroidism (10.5%), loss of appetite (8.6%), nausea (7.0%) and diarrhoea (5.4%) [10]. The phase III KEYNOTE-590 study (ClinicalTrials.gov identifier NCT03189719), assessing the efficacy and tolerance of pembrolizumab plus chemotherapy as first-line therapy for locally advanced or metastatic esophageal carcinoma, is ongoing [33].

HER 2 (human epidermal growth factor receptor) expression was not considered in the study due to the fact that it was not frequently found in the study population, and Trastuzumab treatment is not approved in Romania for second-line treatment [23, 34].

The time to disease progression was quite difficult to track due to the fact that several patients have continued their treatment at other institutions or have been transferred to a palliative care unit without the possibility to obtain their date of death, because in Romania there is not a computerized death reporting system and this is a limitation of the study.

The limitations of our study were the retrospective character, small number by each subgroup of treatment patients and lack of the subgroup of patients treated with both active and passive immunotherapy.

CONCLUSIONS

Immunotherapy using immune checkpoint inhibitors has revolutionized the cancer treatment and becomes the new standard of second line treatment in non-operable advanced or metastatic esophageal cancer.

Moreover, the new agents seem to be efficacious only in cases where the expression PD-L1 is positive or high microsatellite instability is present.

The old chemotherapy regimen has a similar efficacy and relatively good tolerance and still might the first-choice recommendation in fit patients

This analysis showed a generally good response to systemic treatment, comparable to the responses reported in other studies.

Our study did not show a difference in clinical benefit depending on the treatment regimen.

Moreover, the incidences of adverse events such as grade 3 or higher leukopenia and febrile neutropenia was observed in all patients. Forty-four patients (50.6%) displayed no grade III-IV toxicities. Seven patients displayed haematological and gastrointestinal disorder together.

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All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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The study was approved by Ethical Committee of Institute of Oncology "Prof. Dr. Alexandru Trestioreanu", Bucharest, Romania.

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Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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VARIA

Therapeutic decision of laparoscopy and its benefits as a gold standard in acute cholecystectomy

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Abstract: *Background: Acute cholecystitis is the most frequent complication of cholelithiasis and counts for one third of surgical emergencies.*

Aim: The study evaluates the outcomes and limits of laparoscopic cholecystectomy in acute cholecystitis.

Materials and methods: A retrospective study was performed for 2 years, between 01.01.2016-31.12.2017 in the 2nd Surgery Department of the Sibiu County Emergency Clinical Hospital, on hospitalized patients with acute cholecystitis, who underwent surgery. The severity of acute cholecystitis was analyzed using the Tokyo Guidelines (TG13 / TG18) severity risk scale. The preoperative evaluation of the anesthetic-surgical risk was based on American Society of Anesthesiologists Physical Status Classification (ASA PS) and Charlson Comorbidity Index (CCI) (9). Statistical analysis was performed to analyze the outcomes and limits of laparoscopic cholecystectomy and the risk factors for conversion and open surgery.

Results: Out of the 262 patients in the study group, most of the patients (61%) were diagnosed with moderate acute cholecystitis, while 67 patients (26%) were diagnosed with mild form and 34 (13%) with severe acute cholecystitis. Laparoscopic cholecystectomy was performed in 96.1% of cases with no conversion to open surgery. The postoperative complications were ligature slippage (1.9%), main bile duct injury (1.9%), postoperative hemorrhage (3.9%) and surgical site infections (2.4%), most of them being managed conservatory.

Conclusions: Laparoscopic cholecystectomy can be performed nowadays with minimal morbidity in acute cholecystitis. Knowledge of various factors predicting possible conversion helps in adequate pre-operative selection and counseling for open procedure with further reduction in the overall morbidity of laparoscopic cholecystectomy.

Keywords: *laparoscopic cholecystectomy, acute cholecystitis, risk factors, inflammation*

INTRODUCTION

Acute cholecystitis is the most frequent complication of cholelithiasis and counts for one third of surgical emergencies. Blocked stones at the level of the infundibulum or cystic duct generate inflammatory phenomena of variable intensity, from mild to severe, with organ failure and septic condition. It is considered to be the second source of complicated intra-abdominal infection (18.5%), according to the World Society of Emergency Surgery complicated intra-abdominal infections Score study [1]. Laparoscopic cholecystectomy has become the “gold standard” in treatment of biliary lithiasis due to its undeniable advantages in reducing pain and postoperative complications. However, there are still controversies regarding the management of patients with acute cholecystitis. In the early days, after the first laparoscopic

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cholecystectomy, acute cholecystitis was considered as an absolute and later, a relative contraindication for this surgery [2] due to the possible risk of increased morbidity, especially common bile duct injuries [3].

The current concept is in favor of emergency intervention in the first 72 hours after hospitalization, as there is no evidence that delaying the intervention after resolving the acute outbreak would benefit in reducing intraoperative complications, especially major biliary tract injuries, due to local fibrous remodeling [4-7]. The current guidelines recommend surgery as soon as possible because evidenced-based clinical studies confirmed that an early treatment reduces the total hospital stay and does not increase the complication or conversion rates [8].

The study evaluates the outcomes and limits of laparoscopic cholecystectomy in acute cholecystitis.

MATERIALS AND METHODS

A retrospective study was performed for 2 years, between 01.01.2016-31.12.2017 in the 2nd Surgery Department of the Sibiu County Emergency Clinical Hospital, on hospitalized patients with acute cholecystitis. The inclusion criteria were:

- hospitalization diagnosis of “Acute non-/lithiasis cholecystitis”
- histopathological confirmation of the acute character of the inflammation of the gallbladder wall;
- surgical treatment of the pathology during hospitalization.

Patients with acute biliary pathology, but who were not treated surgically, and patients who did not show acute inflammatory signs in the gallbladder were excluded.

Observation sheets and operating protocols were analyzed. The collected data documented age, sex, associated pathology, preoperative clinical and paraclinical data (hemoleukogram, fibrinogen, AST, ALT, bilirubin, amylase, INR, urea, creatinine, abdominal ultrasound), type of intervention, postoperative complications, appearance histopathological examination of the gallbladder specimen, duration of hospitalization. The severity of acute cholecystitis was analyzed using the Tokyo Guidelines (TG13 /TG18) severity risk scale. According to this classification, Grade III (severe acute cholecystitis was considered the acute cholecystitis associated with organ/system (renal, cardiovascular, hepatic, respiratory, neurologic, hematologic) dysfunction, Grade II (moderate) acute cholecystitis associates clinical signs of acute cholecystitis with white blood cells (WBC)>18000/mmc, palpable tender mass in the right upper abdominal quadrant, marked local inflammation and the onset of symptoms of more than 72 hours before presentation. Grade I (mild) acute cholecystitis is defined as acute cholecystitis which does not meet criteria for grade II or III [7, 8].

The preoperative evaluation of the anesthetic-surgical risk was based on American Society of Anesthesiologists Physical Status Classification (ASA PS) and Charlson Comorbidity Index (CCI) [9]. Statistical analysis was performed to analyze the outcomes and limits of laparoscopic cholecystectomy and the risk factors for conversion and open surgery.

Table 1: Preoperative evaluation of the patients in the study group: demographic data and associated comorbidities

General characteristics in the study group	
	Total (n=262)
Gender	
Female	171 (65.26%)
Male	91 (34.73%)
Age	
21 - 30 years	20 (8%)
31 – 40 years	44 (17%)
41 – 50 years	68 (26%)
51 – 60 years	47 (18%)
61 – 70 years	34 (13%)
71 – 80 years	31 (12%)
>80 years	18 (7%)
Comordibities	
Diabetes mellitus	29 (11.06%)
Hypertension	120 (45.8%)
Coronary diseases	70 (26.7%)
Valvular diseases	7 (2.6%)
Respiratory diseases	22 (8.3%)
Obesity	41 (15.6%)
Previous abdominal surgery	9 (3.4%)
CCI*	
0	107 (41%)
1	73 (28%)
2	23 (9%)
3	23 (9%)
4	16 (6%)
5	10 (4%)
≥6	10 (3%)
ASA PS**	
I	59 (22%)
II	92 (25%)
III	75 (29%)
≥IV	36 (14%)

**CCI: Charlson comorbidity index*

***ASA PS: American Society of Anesthesiologists Physical Status Classification*

RESULTS

Of the total number of patients (361) who presented at the Sibiu County Hospital, Surgery Clinic, with acute biliary pathology during the analyzed period, 262 (73%) presented inclusion criteria in the study. Demographic characteristics of the study group (Table 1) confirm the predominance of pathology in females (65.26%), and middle adulthood (40-60 years). In 73% of cases, the presentation was late, after more than 72 hours after the onset of painful abdominal symptoms.

The main associated comorbidities were: cardiovascular disease, obesity and diabetes. Assessment of surgical anesthetic risk by calculating the Charlson comorbidity score (CCI) and placing it in the risk classes of the American Society of Anesthesiologists Physical Status Classification (ASA PS) system (Table 1).

Routine preoperative evaluation consisted of clinical evaluation, abdominal ultrasound, chest X-ray, EKG, as well as evaluation of biochemical and hematological parameters, characteristics to assess the severity of infection (hemoleukogram, fibrinogen, ESR), but also liver function (AST, ALT, bilirubin), renal (urea, creatinine), coagulation (INR). According to TG13/TG18 severity risk scale, most of the patients (61%) were diagnosed with moderate acute cholecystitis, while 67 patients (26%) were diagnosed with mild form and 34 (13%) with severe acute cholecystitis.

The management of acute cholecystitis was according to Tokyo Guidelines, based on the severity of symptoms, ASA and CCI index. Patients with ASA≥3 and CCI≥6 or sepsis, underwent fluid rebalance and general supportive care before surgery could be performed safely. Emergency laparoscopic cholecystectomy in the first 72-96 hours after admission was performed. Large spectrum intravenous antibiotherapy was used in all cases.

The operative moment was carefully planned after attentive clinical and paraclinical evaluation of the patient and rebalancing of associated comorbidities. Most of the patients (252; 96%) underwent laparoscopic cholecystectomy. Open surgery was used in 10 patients. There were no conversions in the study group, all intraoperative incidents and accidents were managed by laparoscopic approach.

The main reason for deciding from the beginning the classic intervention is the complex adhesion syndrome, but also the suspicion of phlegmon, representing 34% of the number of patients in this category (4 patients). Others include perforation, with biliary peritonitis, postoperative eventration, ascites and peritoneal carcinomatosis associated with colon neoplasm (1 patient each) (Figure 1).

A comparative analysis of the histopathological aspect of the gallbladder for the 2 groups in the study group (laparoscopic and classic) shows that the form of catarrhal inflammation of the gallbladder wall was 87% in the laparoscopic group and 60% in the classically operated group.

Figure 1: Factors for open cholecystectomy in the study group (percentages)

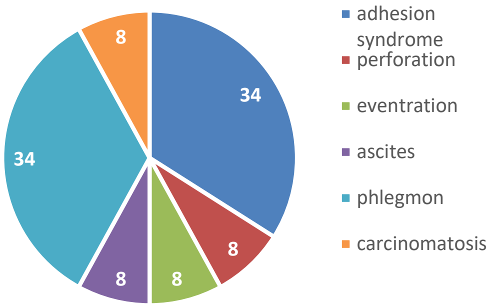

Although less frequent in the laparoscopically operated group, the presence of the difficult forms, such as gangrenous cholecystitis, piocholecystitis, pericholecystic plastron, is not a contraindication for the laparoscopic approach being successfully resolved in this way. On the other hand, the presence of peritoneal carcinomatosis, ascites, eventration, or extensive postoperative adhesions in the upper abdominal floor are absolute or relative contraindications for laparoscopy (Table 2).

Table 2: Histopathological aspects of the removed cholecyst: laparoscopic vs open cholecystectomy

Histopathological aspect of the removed cholecyst	Laparoscopic Cholecystectomy	Open Cholecytectomy
pericholecystic plastron	1 (1%)	1 (10%)
piocholecystitis	3 (1%)	0
pericholecystic fibrosis	5 (2%)	1(10%)
gangrenous cholecystitis	17 (7%)	1(10%)
hydrops	6 (2%)	1(10%)
cataral inflammatory changes	220 (87%)	6 (60%)

A particular aspect as a difficulty in surgical tactics is the association of acute cholecystitis with primary gallstones in 13 cases and angiocolitis in 2 cases.

Main bile duct lithiasis resolution was achieved by ERCP, in 15% preoperatively and in 85% postoperatively.

The outcome was favorable with no complications, in most cases (88%) operated laparoscopically and in 50% of cases operated classically, which is correlated with the degree of difficulty and associated comorbidities increased in the group of 10 patients who were treated by classic cholecystectomy.

The average hospital stay was 5.7 days in the laparoscopic group and 11.2 days in the classically operated group (Figure 2).

Figure 2: Postoperative hospital stay in open and laparoscopic cholecystectomy

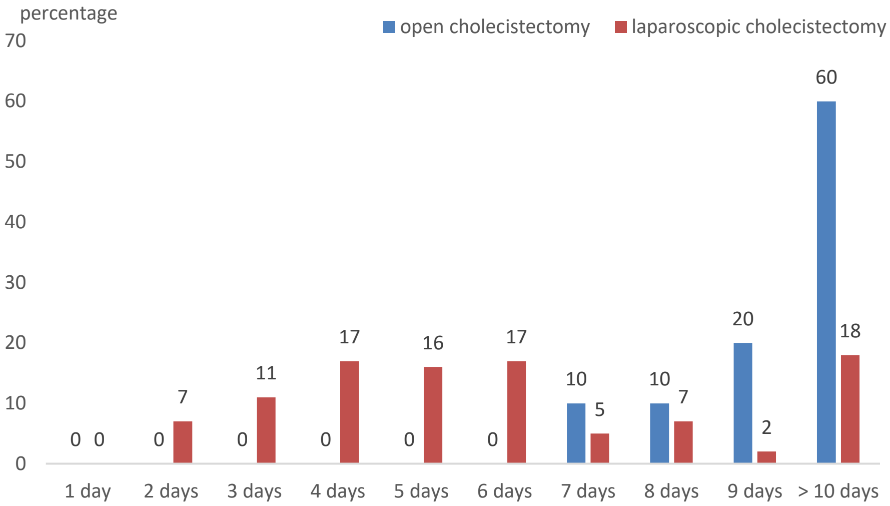

The postoperative complications are presented in Tables 3 and 4. Following the study we determined the most common postoperative complications due to the surgical technique, these being: ligature slippage, main bile duct injury, postoperative hemorrhage and surgical site infections. It can be seen that the most common complication in the study is postoperative hemorrhage, caused by: either skidding of the cystic artery ligation, or by inefficient hemostasis (3.9%), followed by skidding of the cystic ligature (1.9%).

Table 3. General postoperative complications in open and laparoscopic cholecystectomy

General postoperative complications	Open cholecystectomy		Intervenție laparoscopică	
	No	*percentage*	*No*	*percentage*
Death	1	10 %	5	2 %
Stroke	0	0 %	1	1 %
Acute miocardial infarction	0	0 %	4	2 %
Malign arterial hypertension	0	0 %	2	1 %
Ascitis	1	10 %	3	1 %
Nosocomial infection	3	30 %	5	2 %
Sepsis	0	0 %	3	1 %
Others	0	0 %	6	2 %

The reasons for the skidding of the ligature are multiple and we will give some examples: the skidding of the metal clip, being the edematous and friable cyst, the skidding of the metal clip being cut to its laughter, etc. On the 3rd place, but very important due to their severity, is the damage of the main bile duct during the maneuvers (1.9%) (Tables 3, 4).

Table 4. Surgery related postoperative complications in open and laparoscopic cholecystectomy

Surgery related postoperative complications	Open cholecystectomy		Intervenție laparoscopică	
	No	*percentage*	*No*	*percentage*
Skidding of the cystic ligature	2	20 %	5	1.9 %
Lesions of the main bile duct	0	0 %	5	1.9 %
Postoperative hemmorhages	1	10 %	10	3.9 %
Surgical site infections	3	30 %	6	2.4 %

The resolution of surgical complications was done in most cases by conservative management, 2 cases requiring reintervention, 1 for postoperative hemorrhage and resolution by ERCP approach of CBP lesion.

DISCUSSION

Laparoscopic cholecystectomy has become the "gold standard", due to its undeniable advantages in reducing pain and postoperative complications, as well as the length of hospital stay. Even during the COVID-19 pandemic era, when laparoscopy was firstly regarded with extreme prudence, the general surgical opinion was that we must not step back from the achievements of modern surgery, but reinforce the safety measure regarding the ultrafiltration of pneumoperitoneum [10].

Together with the development of anesthesia and intensive care skills and techniques, the safety limit for performing laparoscopy also increased nowadays towards the age of 80-85 years [8, 11], and even of 91 years old in our study group, if not severe ASA risk is associated. In the study group there was no case of conversion. Careful preoperative assessment of the comorbidities and attentive patient clinical evaluation is extremely important before choosing between open and laparoscopic surgery. Conversion to open surgery is associated in several studies with negative effects, including prolonged operation time and increased bleeding which is particular important in patients with increased anesthetico-surgical risk [12].

Another conclusion in the study was that the surgeons should be aware of the significant risk of severe cardiovascular acute events following both laparoscopic and open cholecystectomy, especially in cases with severe sepsis and inflammation or high risk associated comorbidities. These complications should be included in the reasonable disclosure, when the patient is informed about the procedure and the associated possible risks and complications. Informed consent process must be the base of a partnership between doctor and patient, in sharing the inherent risks associated to surgical procedures and understanding what the expectations should be [13].

The severity of local inflammatory changes is a major risk factor for intra operatory and postoperative associated complications. Hyperbilirubinemia significantly increases the likelihood of finding common duct stones in patients with acute cholecystitis, but it also occurs in patients with acute cholecystitis without common duct stones [8]. Hyperbilirubinemia is also a biomarker of intraperitoneal inflammation, along with lipopolysaccharide binding protein (LBP) and Il-6, which increase as a response of liver disfunction associated to bacterial aggression. There are evidences that Escherichia coli and Bacteroides fragilis, common pathogens of the biliary and intestinal wall, were supposed to interfere with hepatocyte microcirculation, inducing sinusoidal lesions. LPSs activate monocytes and macrophages to secrete inflammatory cytokines (tumor necrosis factor alpha (TNF-α) and IL-1, etc.) and other potent mediators [14] by an intracellular signal amplification pathway. These mediators, in turn, act on additional target cells to produce cardiovascular shock, multisystem organ failure, and septic shock [15], one of the major causes of death in intensive care units [16]. In recent studies, a raised concentration in bile but also a more pronounced immunohistochemical expression of cytokines, likeTNF-α, IL-1α, IL-6 was found in the cholecystic wall in both acute and chronic cholecystitis, which may suggest the role of these cytokines in pathogenesis of inflammation and cholelithiasis [17, 18].

Severe inflammation is associated with increased risk of main bile duct during laparoscopic dissection of gallbladder, one of the most serious complications of cholecystectomy. Recently, near infrared (NIR) fluorescence Indocyanine Green was employed in challenging cases to allow a better visualization of the biliary tree. The molecule of ICG is a polymethylic cianyne, which can be administered intravenously, with an excellent safety profile. It binds with circulating albumins and lipoproteins and is excreted into the bile almost unchanged after hepatic extraction [19]. Several studies proved the beneficial use of ICG, which may ensure the surgeon to perform LC with confidence, even in the presence of severe inflammation [20, 21].

Laparoscopic cholecystectomy can be performed nowadays with minimal morbidity in acute cholecystitis [23]. Knowledge of various factors predicting possible conversion helps in adequate pre-operative selection and counseling for open procedure [24] with further reduction in the overall morbidity of laparoscopic cholecystectomy.

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VARIA

Medical causes and diseases leading to early permanent disqualification in IRIAF pilots based on their service categories

Mohammad Darvishi[1], Hamze Shahali[1]

Abstract: *Background and Aim: Efficient manpower is the most important capital and studies have shown the relationship between health and performance.Understanding the factors leading to disability has preventive importance.Our aim is determine the causes and diseases leading to early permanent medical disqualification of the Islamic Republic of Iran Air Force pilots based on their service categories.*

Methods: This is a descriptive, cross-sectional, retrospective study with target population of the Islamic Republic of Iran Air Force pilots with early and permanent medical disqualification from 1986 to 2016.

Results: Out of 508 cases of early medical discharges, 239 items were considered medical disqualification, 17 war captives, 34 service exchange and the remaining 218 was for on service killed or missed persons.The main medical causes were neurosurgery, cardiovascular and psychiatric, while common diseases include lumbar discopathy, myocardial infarction and cardiac arrhythmia.The lost service years was 3233 person year and the average was 12.63 person year per individual.

Conclusions: Based on the previous similar studies, it was expected that the common cause include neurosurgery, cardivascular and psychiatric with more prevalence in fighter, transportation, and helicopter service categories.At the end, we found that our results were in line with initial expectations.

Keywords: *disabilities, medical disqualification, health, prevention, performance*

INTRODUCTION

Human resources are the most important capital of armed forces, and many studies have shown the strong links between personal health and organizational performance. In order to increase productivity, organizations have adopted numerous measures not only to evaluate pre-employment health status, but also monitor staff health during their service life. Nonetheless, health risks remain the most important reducing factor of personal productivity. Disability is defined as the effect of a disorder on physical, mental, and social activity of a person where the work environment and the family status are also effective elements [1, 2, 3, 4]. According to statistics, effective prevention of cardiovascular risk factors in aviation population has a significant role in improving the professional health index of aviators (pilots). The study of aviator's work environment and their lifestyle, active intervention, periodic regular examinations and screening tests for early detection of diseases are among the basic measures [10].

These days, the economic, mental and social problems caused by disability in armed forces are critical burdens. Regardless of the huge cost to run and maintain a powerful army, the supportive burden of victims can be devastating for any society [22]. In 1993, the United States (U.S) army paid 500 million dollars to compensation newly recruited armed forces [7, 8] In a study between 1980 and 1994 on the

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causes of disability in U.S. air and sea forces, it has been shown that disability in armed forces had led to an excessive cost of about 1.5 billion dollar in form of a compensation [2].

The preparation of military pilot volunteers to enter professional trainings and the provision of optimal services in the form of well-trained pilots require a huge amount of resources, both physical and intellectual [1]. The ultimate goal of present study was to determine the causes and diseases which led to early permanent medical disqualification (EPMD) of Islamic Republic of Iran Air Force (IRIAF) pilots based on their service categories from 1986 to 2016. Other objectives include; determine the causes and diseases which led to EPMD in IRIAF pilots who captured during the war, killed or missed in actions or have been forced to change their service category due to medical reasons, their lost service years (LSY) and its average and provision of scientific and practical solution to prevent the EPMD of IRIAF pilots, all based on their service categories.

METHODS

This is a descriptive-cross sectional-retrospective research which ethically approved by the ethics committee of the Aerospace and Sub-aquatic Medical Faculty in Aja University of Medical Sciences (with registration No# 10167118) and was conducted by the personal expenses of the corresponding author. However, like other up-to-date military articles, we are reluctant to disclose a number of military confidential information (i.e. total number of assessed cases).

All the IRIAF pilots(which all are male) with EPMD who had relevant official records from 1986 to 2016 was included in the study and those who had medical disqualification due to physical or mental illness and death in non-occupational accidents, non-medical reasons, personal requests or disciplinary punishments were excluded. In this study, all pilots were captured during the war, killed or missed in actions, and those who confronted with service exchange were classified as permanent disqualification. However, this is one of the study advantages and all data was illustrated in detail separately. In order to observe the principle of confidentiality a non-disclosed random ID code were assigned for every person.

Main disease of the participants was classified based on the ICD-10 (10th version of international classification of diseases) and registered in to the Microsoft excel-based pre-designed electronic data sheet. Other recorded information included; date of entry into the service, date of departure from the service and service categories. Indeed, the total lost service years and average lost service years were calculated for each service category. Finally, this data was analyzed and displayed as graphs and figures for better presentation.

RESULTS

During the period of 1986 to 2016, 508 cases of early permanent discharges occurred in IRIAF pilots which contained; 239 medical disqualification, 218 killed or missed in actions, 34 service exchange and 17 war captives (Table 1).

Table 1: Study data

Service pilots categories / Status	Fighter	Transportation		Helicopter	Total
		light	heavy		
Medical disqualification	171	20	26	22	239
War captives	16	0	0	1	17
Killed or missed in actions	159	7	30	22	218
Service exchange	28	0	3	3	34
	374	27	59	48	508
		86			

The most prevalent medical causes of EPMD in IRIAF pilots were neurosurgery, cardiovascular and psychiatric (Figure 1).

A gynecological and urological consultation was requested for a non-functioning urethral-vesical catheter of a 60 years-old female patient admitted in the Orthopedic Department that underwent surgery with external fixation 2 days prior for a complex pelvic fracture (transverse fracture of the right acetabulum, left obturator ring fracture, left sacral fracture, fifth lumbar transverse apophysis fracture with paralysis of the external left sciatic nerve) (Figure 1).

The most common diseases led to EPMD in IRIAF pilots included; lumbar discopathy, myocardial infarction, cardiac arrhythmia, generalized anxiety disorder and post-traumatic stress disorder (Table 2).

Figure 1: Causes lead to the EPMD of IRIAF pilots based on their service categories

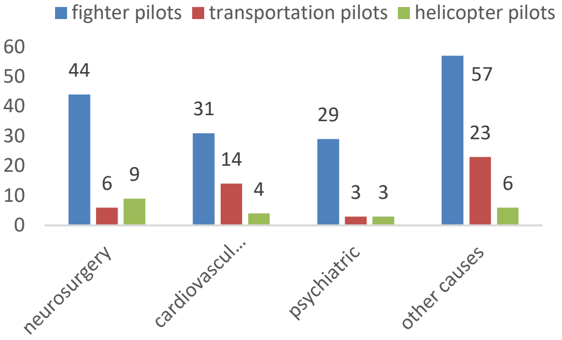

Table 2: Diseases lead to the EPMD of IRIAF pilots based on their service categories

Service pilots categories Status	Fighter	Transport	Helicopter
Lumbar discopathy	18	5	5
Myocardial infarction	12	6	2
Cardiac arrhythmia	10	2	1
Generalized anxiety disorder	10	0	1
Post-traumatic stress disorder	10	0	0
Other diseases	111	33	13

The most prevalent medical causes of EPMD in war captive IRIAF pilots were neurosurgery, psychiatry and orthopedics (Figure 2).

Figure 2: Causes lead to the EPMD of war captive IRIAF pilots based on their service categories.

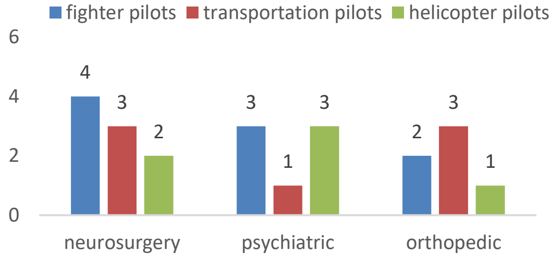

The most common diseases led to EPMD in war captive IRIAF pilots included; post-traumatic stress disorder, lumbar vertebral fracture with fusion and complicated bone fracture (Table 3).

The most prevalent medical causes of permanent medical service exchange (PMSE) in IRIAF pilots were ear, nose and throat (ENT), neurosurgery and orthopedics (Figure 3).

Table 3: Diseases lead to the EPMD of war captive IRIAF pilots based on their service categories

Service pilots categories Status	Fighter	Transport	Helicopter
Post-traumatic stress disorder	3	1	1
Lumbar vertebral fracture with fusion	2	0	1
Complicated bone fracture	1	0	1
Lumbar discopathy	1	1	0
Cervical discopathy	1	0	1
Other diseases	3	0	0

Figures 3: CT-scan showing complex pelvic fracture and the communication between the bladder and the left labia.

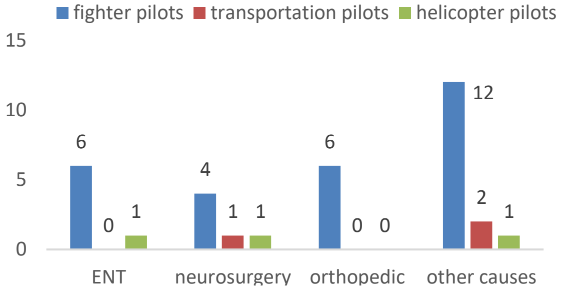

The most common diseases led to PMSE in IRIAF pilots included; motion sickness, complicated bone fracture, lumbar discopathy (Table 4).

Table 4: Diseases lead to the PMSE in IRIAF pilots based on their service categories

Service pilots categories Status	Fighter	Transport	Helicopter
Motion sickness	6	0	0
Complicated bone fracture	4	0	0
Lumbar discopathy	2	1	0
Cervical discopathy	2	0	1
Generalized anxiety disorder	2	0	0
Other diseases	12	2	2

As 3233 LSY, 2457 (%76) was belong to fighter pilots, 459 (%14.4) to transportation pilots and 317 (%9.8) to helicopter pilots. The most prevalent medical causes of LSY in IRIAF pilots with EPMD were neurosurgery, psychiatry and cardivascular (Figure 4).

The most LSY in IRIAF pilots with EPMD were belong to lumbar discopathy, generalized anxiety disorder and cardiac arrhythmia (Table 5).

Figure 4: Causes lead to the most LSY in IRIAF pilots with EPMD based on their service categories.

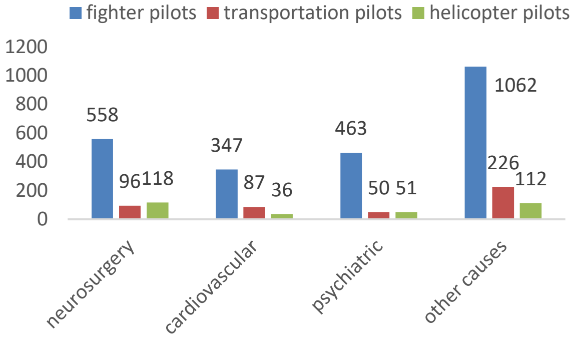

The average of LSY in IRIAF pilots with EPMD was 12.63 person year per individual (13.14 in fighter, 9.98 in transportation and 13.78 in helicopter pilots).

The most prevalent medical causes of LSY average in IRIAF pilots with EPMD were psychology, ophthalmology and neurosurgery (Figure 5).

Table 5: Diseases lead to the most LSY in IRIAF pilots with EPMD based on their service categories

Service pilots categories **Status**	**Fighter**	**Transport**	**Helicopter**
Lumbar discopathy	225	80	56
Generalized anxiety disorder	187	0	12
Cardiac arrhythmia	136	19	18
Complicated bone fracture	151	0	0
Post-traumatic stress disorder	151	0	0
Other diseases	1067	360	231

Figure 5: Causes lead to the most LSY in IRIAF pilots with EPMD based on their service categories.

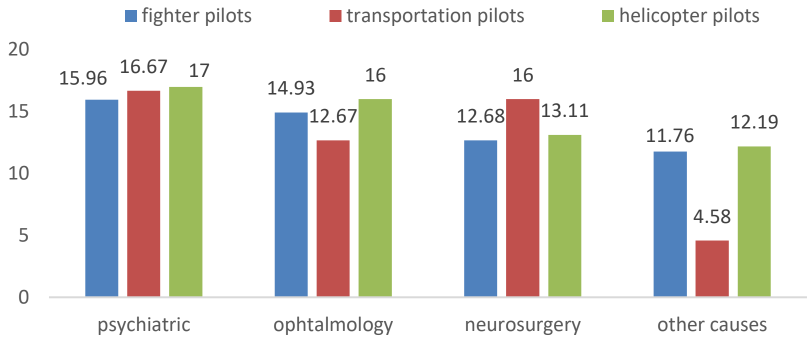

The diseases resulted in the highest average of LSY in IRIAF pilots with EPMD included; migraine, cardiac arrhythmia and lumbar discopathy (Table 6).

Table 6: Diseases lead to the most LSY average in IRIAF pilots with EPMD based on their service categories

Service pilots categories **Status**	**Fighter**	**Transport**	**Helicopter**
Migraine	13	16	23
Cardiac arrhythmia	13.6	9.5	18
Lumbar discopathy	12.5	16	11.2
Generalized anxiety disorder	18.7	0	12
Epilepsy	14	12	0
Other diseases	11.92	6.58	11.56

DISCUSSION

In the study of Whitton-1984 on United State Air Force (USAF) pilots and navigators, the common causes were cardivascular and neurologic [17], while in the same study of Mc Crary-2002, the most common causes in 1995 to 1999, include cardivascular, musculoskeletal, neurologic and endocrinology [12]. Also, in the study of Montazeri-2005, the most common causes of EPMD in IRIAF pilots included cardivascular and musculoskeletal [20]. In the study of Ghazizade-2010 on the same population in 1992 to 2003, cardivascular, nervous-skeletal and gastrointestinal were the most common causes [19].

In the study of Dark-1986 with the help of The Federal Aviation Administration (FAA), on medical disqualification of American airlines pilots in 1983 and 1984, the most commonly reported causes were cardiovascular and Neuropsychiatric events [18]. In the Nakanishi study on permanent disability of 260 Japanese crews, significant causes include malignancies, neurodegenerative, cardiovascular, gastrointestinal and musculoskeletal disorders [9]. In the review of trained pilots on Commonwealth countries from 1994 to 2004, the causes of cardiovascular were prevalent [19]. In the Arva study on 257 Norwegian civil pilots, the main causes were cardiovascular, neurologic, musculoskeletal and psychiatric disorders [11]. In the studies of Mitchell-2004 and Evan-2006 on sudden incapacitation of British civil pilots, 36 cases have been diagnosed and half of them were due to cardiovascular, cerebrovascular and psychiatric disorders [13, 14]. In the Nezami study on 200 civil pilots of Vnukovo airport in Moscow, cardivascular, neurologic and digestive have been the main causes of medical disqualification [10].

In the Mc crary's study, the more common diseases were ischemic heart diseases, high blood pressure, back pain, inter vertebral disc disorders and migraine [12]. In the study of Nezami, ischemic heart diseases, myocardial infarction, high blood pressure, cerebrovascular sclerotic lesions and peptic ulcer have been common [10]. FAA has conducted a great comprehensive study in 2014 on the causes of medical disqualification of pilots listing fifteen major causes, including; pectoris angina, bipolar mood disorder, cardiac valve replacement, coronary artery diseases, diabetes mellitus, loss of consciousness with unknown reason, seizure, cardiac replacement, myocardial infarction, permanent pace maker, disabling personal disorder, psychosis, drug abuse and dependency and lack of neural control, in order of significance [16].

In the study of Montazeri, the mean LSY of IRIAF retired with EPMD was 6.5 person year per individual [20]. In the Ghazizade study, the mean LSY was 6.14 and the highest was 10 person year per individual [19].

The present study has limitations that include: 1) missing details due to the lack of presence of a comprehensive electronic system for recording personal medical records; 2) the possibility of oriented malingering in disorders where specific objective diagnostic methods are not available and were mostly diagnosed subjectively, such as motion sickness, migraine and etc, and 3) flying device factors (ergonomics and exhaustion), operational and airbase factors (work and rest schedule), Personal factors (life style, physical and psychological capabilities, socioeconomic state, use of legal and illicit drugs and habits), medical factors (medical and psychological advices and hygiene education) are important items which have effective influence on pilots health.

The current investigation has important advantages such as: 1) so far, in terms of quantity and quality, there is a limited number of similar studies have been conducted about military pilot's health; 2) it has the longest cross-sectional period (35 years), the closest was performed during a 10-years period [18]; 3) express the statistics of IRIAF pilots which captured during the war, killed or missed in actions and those who confronted with service exchange, and 4) Records given were sorted according to the service categories.

CONCLUSIONS

More studies should be conducted on military and civil aviator's (pilots) health in the future. We believe that the life style modifications, regular fitness training, hygiene and health education and use of medical and psychological counseling for preventing the EPMD in military pilots are necessary preventive steps to take. Also, use of up-to-date comprehensive electronic system, upgrading on-the-job examinations, sufficient periodic in-service examination in occupational health centers are recommend by authors.

List of Abbreviations

U.S = United States; IRIAF = Islamic Republic of Iran Air Force; EPMD= Early and Permanent Medical Disqualification; LSY= Lost Service Years; ICD-10 = 10th International Classification of Diseases; PMSE= Permanent Medical Service Exchange; ENT = Ear, Nose and Throat; USAF= United State Air Force; FAA= Federal Aviation Association

Authors' contribution:

MD – data collector and supervisor, statistical analyzer and literary editor; HS – main owner of the idea, scientific supervisor, executive author and editor.

Ethics approval and consent to participate:

The ethical approval of this study was issued with registration No#10167118 by the ethics committee of the Aerospace and Sub-aquatic Medical Faculty in Aja University of Medical Sciences.

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Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

All the data of this study is available and could be accessible for publication by that valuable military journal.

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VARIA

Golden hour of sepsis: Can we do more?

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Abstract: *Introduction: Sepsis is a life-threatening organ dysfunction due to a dysregulated host response to an infection1 and also an important worldwide health problem. Recent progresses in understanding pathogenesis are reflected in increasing emphasis put on time (Sepsis Update 2018 decreased the time for diagnosis and treatment from 3- 6 hs Sepsis-2 to 1 hour). The aim of the study was to combine an early warning score (increased sensitivity- NEWS2) and a biomarker (increased specificity- presepsin) in order to detect earlier an acute illness (infection) before becoming life-threatening.*

Materials and methods: It was a retrospective, single-center observational cohort study of 125 consecutive patients who were diagnosed with systemic inflammatory response syndrome between July 2016 and July 2018 at Emergency Department of "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania in order to determine sooner, which patient will further develop a sepsis. We used the area under receiver–operator characteristic curves (AUC) to assess the overall discriminatory power of NEWS2, MEDS and presepsin in detecting sepsis, septic shock and the probability of death on admission and compared them with combined scores between NEWS2 and presepsin.

Results: Using a combined score and a simple algorithm, the NEWS2 score had changed (12,8%) for 16 patients. For 10 out of 95 (10,52%) without sepsis and 6 out of 30 (20%) of septic patients, more points were added to NEWS2 meaning that more septic patients were diagnosed before an infection become life-threatening

Conclusions: In the present study, we have tried to assess the impact of a monitoring score and a rapid POCT biomarker, in a condition when time is of critical importance. Using a verified early warning score, NEWS2, with increased sensibility and low specificity and presepsin, POCT biomarker with increased specificity, we diagnosed sepsis earlier in a number of cases.

Keywords: *sepsis, early warning score, NEWS2, combined score, presepsin*

INTRODUCTION

Sepsis is a life-threatening organ dysfunction due to a dysregulated host response to an infection [1] and also an important worldwide health problem. Nowadays the global sepsis estimates are more than double than previous calculations, with 11 million sepsis deaths and 48.9 million incident sepsis cases in 2017 with a global trend of decreasing sepsis [2]. Although Sepsis-3 definition is designed to put a diagnosis in prehospital settings, battlefield and Emergency Department (ED) on arrival there is much to be done to further improve diagnostic of patients with sepsis. The specificity of the Sepsis-3 criteria is great, but sometimes the sepsis diagnostic is put too late for a specific patient.

Rapid clinical evaluation on arrival in ED and as soon as possible paraclinical evaluation are realized with POCT (point of care testing) devices which allow a more rapid targeted response (complete blood count, acid-base analysis, targeted biomarkers, etc.) to gain time for the patient and to prevent and fight against further deterioration of a patient in a not an optimal state. Sepsis-2 [3] emphasized the fact

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that time management is of critical importance. Early targeted therapy and management bundle put an algorithm at hand for doctors and 2018 Sepsis update [4] indirectly stressed out that rapid medical intervention is paramount because what it should have been done in the first 3-6 hours, from that moment on, it must be done in one hour. For greater accuracy and tailored treatment more exhaustive laboratory analysis usually arrive in at least 30-40 minutes.

By 2015 over 36 Early Warning Scores were used and evaluated for accurately forecast the prognosis [5]. Developed as specific tools, in different environments (different wards, ED, ICU), some scores are nowadays obsolete or of limited use [5, 6] but some are still used and revised (NEWS2).

A possible research direction in sepsis might be to combine an early warning sign/score with increased sensibility, low specificity and a specific biomarker which will increase the specificity to buy time for the patient and ultimately to save more lives with increased cost-results efficiency.

Conceived in 2012, verified on different wards for several years and revised in 2017, National Early Warning Score 2 is nowadays used in United Kingdom with great success [7].

Used in different environments, presepsin is one of the biomarkers which passed the proof the time, as a valuable tool in POCT, being used for more than a decade now [8, 9, 10, 11, 12, 13]. Presepsin increases in patients developing infections in a severity-dependent manner [14, 15, 16]. Previous studies showed that presepsin levels performed when used for early diagnosis, risk stratification and prognosis [17, 18].

The aim of the study was to find new ways in which to combine these recently developed tools, an early warning score (NEWS2) and a specific biomarker (presepsin) in order to diagnose more cases of severe infections, before developing sepsis, preventing further deterioration of an already altered patient with multiple comorbidities. The proposed score is easy to calculate, rapidly available and allowed us to markedly decrease the time for intervention for patients with altered medical status before becoming life-threatening.

MATERIAL AND METHODS

Study design

This was a retrospective, single-center observational cohort study of 125 consecutive patients who were diagnosed with systemic inflammatory response syndrome between July 2016 and July 2018 at Emergency Department of “Dr. Carol Davila” Central Military Emergency University Hospital, Bucharest, Romania in order to determine sooner, which patient will further develop a sepsis. We developed a simple algorithm for earlier diagnose of sepsis and compared the results with Sepsis-3 criteria, NEWS2 and MEDS scores. All patients were above 18 years old and have agreed to sign the informed consent. The study was approved by the ethical committee at “Dr. Carol Davila” Central Military Emergency University Hospital, Bucharest, Romania.

Blood sample analysis

Presepsin concentrations were measured using a chemiluminescent enzyme immunoassay (PATHFAST™ Presepsin), performed on the PATHFAST point-of-care analyzer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Measuring range is 20–20,000 ng/L; manufacturer's URL (95th percentile) is 320 ng/L. Correlation between heparinized and EDTA plasma was Y = 1.00× + 1.53 (r=0.999, n=23) [19].

Developing a tool for earlier diagnosis of sepsis

National Early Warning Score (NEWS) 2 is recommended by the NHS (National Health System) UK to determine the degree of illness of a patient, to prompt critical care intervention and recommends the NEWS 2 in the following settings: Emergency (for initial assessment, serial monitoring and assessment for triage), ward (for initial inpatient assessment and serial monitoring), prehospital (for communication of illness severity to receiving hospitals). Respiratory rate, hypercapnic respiratory failure/room or supplemental O_2, temperature, systolic BP (mmHg), pulse (beats per minute) and consciousness are assessed.

In order to try other combination of NEWS2 and PSP we have obtained other scorings to better predict the prognosis and earlier detection of sepsis.

We have created a new score NEWS2PSP: for PSP between 0-1000 pg/ml we granted 1 point; between 1000-2000 points=2 points, and so on. Adding those values to NEWS2 score lead us to the new score NEWS2 PSP.

Another compounded score was tested: for PSP<200 pg/ml we have given 1 point, 2 points for PSP=200- 300 pg/ml, 3 points for PSP=300-500 pg/ml, 4 points for PSP=500-1000 pg/ml and 5 points for PSP>1000 pg/ml. The new score was NEWS2PSP2.

NEWS2PSP3 resulted from granting 1 point if PSP>1000 and 0 points if PSP<1000.

Granting 0 points for PSP<5000 pg/ml, 1 point for PSP=5000-10000 pg/ml and 2 points for PSP>10000 and adding these points to NEWS2 lead us to NEWS2PSP4.

Statistical analysis

Descriptive statistics was used to summarize the study population. Baseline and follow-up characteristics were described by means and standard deviations (SD) or by median and interquartile range (IQR) for continuous variables, as appropriate, and by percentages for categorical variables. We performed Chi2 statistics or Fisher's exact tests when appropriate for qualitative variables, and the Wilcoxon/Mann–Whitney test for continuous variables with skewed distributions to compare baseline patient characteristics and study outcomes. Categorical variables are presented as counts and percentages. We used the area under receiver–operator characteristic curves (AUC) to assess the overall discriminatory power of NEWS2, MEDS and presepsin in detecting sepsis, septic shock of the probability of death on admission. The IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

RESULTS

There were 125 consecutive patients which were considered to have SIRS (systemic inflammatory response syndrome) and included in the study. When 2 or more SIRS criteria were found, presepsin (PSP) was assessed. From these 30 were diagnosed as having sepsis (Sepsis-3 definition, with 2 or more qSOFA criteria) and 11 were diagnosed as having septic shock. During the study 27 patients have died from different causes, some of them being sepsis or septic shock. The mean age was of 70.88 years with standard deviation of 15.74 years; they ranged from 18 to 96 years. Of the total study population, 79 (63.2%) were men. Table 1 provides the baseline characteristics demographic data, the calculated scores, selected comorbidities and the site of infection.

Table 1: Baseline characteristics, used scores, selected comorbidities and the site of infection

Demographics	All patients (n=125)	Sepsis (n=30)	Septic shock (n=11)	Exitus (n=27)
Age (mean, SD)	70.888 (15.74406)	73.4666 (15.3139)	72.2727 (15.3391)	75.6296 (14.4277)
Sex (male, %)	79 (63.2)	23 (29.11)	10 (12.65)	20 (74.07)
Weight (mean, SD)	71.472 (12.6835)	70.7333 (9.2805)	72.2727 (9.3525)	70.5185 (15.8637)
GCS (mean, SD)	14.264 (2.1841)	12.8666 (2.9970)	12.4545 (2.6064)	13.8148 (2.4651)
QSOFA (mean, SD)	0.672 (0.9779)	2.2666 (0.4422)	2.5454 (0.4979)	1.1481 (1.1123)
NEWS2 (mean, SD)	4.768 (3.6532)	9.7333 (2.8511)	12 (2.3741)	6.5925 (4.0208)
MEDS (mean, SD)	9.568 (4.3512)	14.0333 (2.9606)	15.8181 (2.5873)	12.9259 (4.2681)
PSP (mean, SD)	2594.608 (3063.226)	3582.8 (4306.524)	2961.545 (2163.594)	3272.593 (4373.756)
Selected comorbidities				
Malignancy (%)	35 (28)	10 (28.57)	2 (5.71)	8 (22.85)
Diabetes melitus (%)	27 (21.6)	9 (33.33)	4 (14.81)	6 (22.22)
Cardiac failure (%)	49 (39.2)	9(18.36)	4 (8.16)	13 (26.53)
Kidney failure (%)	36 (28.8)	7 (19.44)	3 (8.33)	11 (30.55)
Respiratory failure (%)	20 (16)	8 (40)	3 (15)	8 (40)
Institutionalized patient (%)	7 (5.6)	3 (42.85)	1 (14.28)	6 (85.71)
Site of infection				
Abdominal (%)	26 (20.8)	6 (23.07)	3 (11.53)	8 (30.76)
Cutaneous (%)	15 (12)	1 (6.66)	0	3 (20)
Endocard (%)	1 (0.83)	0	0	1 (100)
Pulmonary (%)	20 (16)	6 (0.3)	2 (10)	5 (25)
Kidney (%)	28 (22.4)	11 (39.28)	5 (17.85)	5 (17.85)
Unspecified (%)	35 (28)	6 (17.14)	1 (2.85)	5 (14.28)

GCS= Glasgow Coma Scale, qSOFA= quick Sequential Organ Failure Assessment, NEWS2= National Early Warning Score 2, MEDS= Mortality in Emergency Department Score, PSP= presepsin

Patients who met the Sepsis-3 criteria (2 or more qSOFA points) were treated accordingly to the current guidelines for sepsis. Only 30 patients were diagnosed with sepsis and 11 with septic shock.

Table 2 shows the Area Under the Receiver Operating Characteristics and confidence intervals for the different scores and compound scores.

NEWS2 9.7±2.9, MEDS 14.0±3.0 and PSP (presepsin) 3582.8±4380.1 showed increased values for sepsis with statistical significance for NEWS2 $p<0.001$ MEDS $p<0.001$; of note the statistical significance for PSP was $p=0.043$. For sepsis NEWS2 has the best AUROC (Area under ROC) 0.962

(95%CI 5.54-7.51), MEDS 0.875 (95%CI 4.39-7.36), PSP 0.603(95%CI 41.32-2559.17) according to Sepsis-3 criteria as depicted in Figure 2. ROC curves are the same for septic shock NEWS2 0.974 (95%CI 6.11-9.74), MEDS 0.913(95%CI 4.39-9.3) and respectively for PSP 0.626 (95%CI 1526.19-2330.88) (Figure 3).

Table 2: AUROC and 95%CI calculated for different combined scores in sepsis, septic shock and mortality

	Sepsis		Septic shock		Mortality	
	AUROC	**95%CI**	**AUROC**	**95%CI**	**AUROC**	**95%CI**
NEWS2	0,962	5,54- 7,51	0,974	6,11- 9,74	0,670	0,79- 3,85
MEDS	0,875	4,39- 7,36	0,913	4,39- 9,3	**0,762**	2,55- 6,01
PSP	0,603	41,32- 2559,17	0,626	-1526,19- 2330,88	0,561	-454,79- 2184,34
NEWS2PSP	0,z924	6,36- 9,45	0,949	5,66- 11,20	0,656	1,21- 5,39
NEWS2PSP2	0,953	5,69- 7,84	**0,975**	6,31- 10,19	0,676	0,94- 4,17
NEWS2PSP3	0,956	5,95- 7,65	**0,975**	6,22- 9,96	0,672	0,90- 4,03
NEWS2PSP4	**0,971**	5,69- 7,65	0,972	6,07- 9,76	0,670	0,82- 3,91

Figure 1: Receiver-operating characteristics (ROC) curves for NEWS2, MEDS, PSP and for derived scores (NEWS2PSP, NEWS2PSP2, NEWS2PSP3, NEWS2PSP4) in sepsis (AUROC 0,971 %95 CI 5,69- 7,65 for NEWS2PSP4)

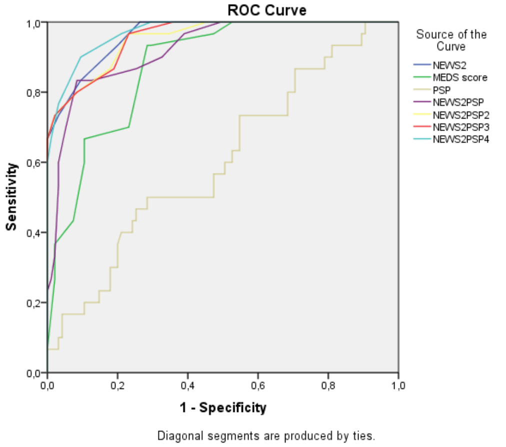

Figure 2: Receiver-operating characteristics (ROC) curves for NEWS2, MEDS, PSP and for derived scores (NEWS2PSP, NEWS2PSP2, NEWS2PSP3, NEWS2PSP4) in septic shock (AUROC 0,975 %95 CI 6,31- 10,19 for NEWS2PSP2 respectively AUROC 0,975 %95 CI 6,22- 9,96 for NEWS2PSP3)

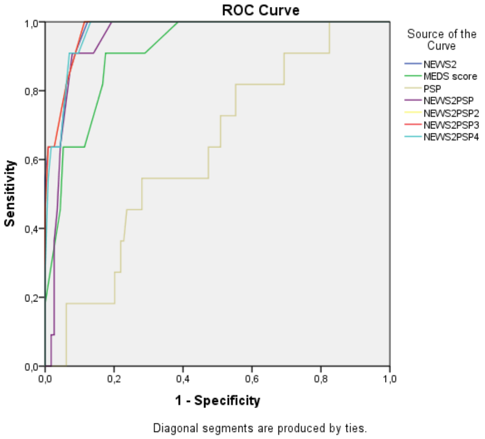

Figure 3: Receiver-operating characteristics (ROC) curves for NEWS2, MEDS, PSP and for derived scores (NEWS2PSP, NEWS2PSP2, NEWS2PSP3, NEWS2PSP4) in mortality (AUROC 0,762 %95 CI 2,55- 6,01 for MEDS score)

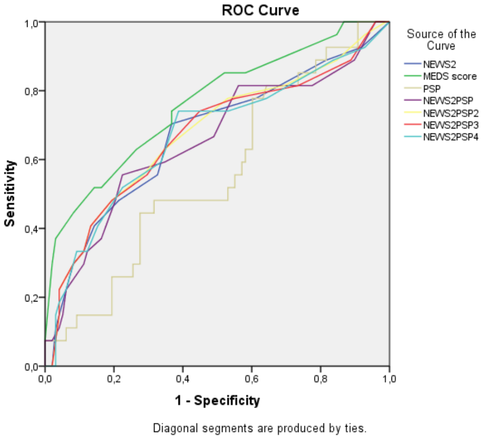

We created a new score, NEWS2PSP, transforming PSP values as follows: between 0-1000 pg/ml = 1 point; 1000-2000 pg/ml = 2 poits, and so on; adding these points to NEWS2 score resulted the new score NEWS2PSP; AUROC for sepsis and septic shock were not superior to NEWS2: AUROC NEWS2PSP in sepsis 0.924 (95%CI 6.36- 9.45), in septic shock 0.949 (95%CI 5.66-11.20), compared to AUROC NEWS2 in sepsis 0.962 (95%CI 5.54-7.51) or in septic shock 0.974 (95%CI 6.11-9.74). MEDS, a specific designed score was superior to NEWS2PSP in predicting mortality AUROC MEDS 0.762 (95%CI 2.55-6.01), AUROC NEWS2PSP 0.656 (95%CI 1.21-5.39).

Another compounded score was tested: for PSP<200 pg/ml we have given 1 point, 2 points for PSP=200- 300 pg/ml, 3 points for PSP=300-500 pg/ml, 4 points for PSP=500-1000 pg/ml and 5 points for PSP>1000 pg/ml. Those values were added to NEWS2 and formed NEWS2PSP2 which has no more added value to NEWS2 for predicting sepsis (AUROC NEWS2 0.962 (95%CI 5.54-7.51), AUROC NEWS2PSP2 0.953 (95%CI 5.69-7.84), minimal in septic shock (AUROC NEWS2 0.974 (95%CI 6.11-9.74), AUROC NEWS2PSP2 0.975 (95%CI 6.31-10.19). MEDS kept an advantage in predicting death over NEWS2PSP2 (AUROC MEDS 0.762 (95%CI 2.55-6.01), AUROC NEWS2PSP2 0.676 (95%CI 0.94-4.17).

NEWS2PSP3 resulted from granting 1 point if PSP>1000 and 0 points if PSP<1000- with no better prognostic value over NEWS2 for predicting sepsis ((AUROC NEWS2 0.962 (95%CI 5.54-7.51), AUROC NEWS2PSP3 0.956 (95%CI 5.95-7.65) and kept a slightly advantage over NEWS2 in septic shock AUROC NEWS2 0.974 (95%CI 6.11-9.74), AUROC NEWS2PSP3 0.975 (95%CI 6.22-9.96). MEDS kept an advantage in predicting death over NEWS2PSP3 (AUROC MEDS 0.762 (95%CI 2.55-6.01), AUROC NEWS2PSP3 0.672 (95%CI 0.90-4.03).

Granting 0 points for PSP<5000 pg/ml, 1 point for PSP=5000-10000 pg/ml and 2 points for PSP>10000 and adding these points to NEWS2 lead us to NEWS2PSP4 which is superior to NEWS2 (AUROC NEWS2PSP4 0.971 (95%CI 5.69-7.65) compared to AUROC NEWS2 0.962 (95%CI 5.54-7.51). Using a cut-off value of 9.5 for NEWS2PSP4 we can diagnosis of sepsis with a sensitivity of 60%. (for a NEWS2PSP4 compound score >6.5 sepsis is diagnosed with a sensitivity of 90% and 90.5% specificity).

Using this simple algorithm, for 16 patients the NEWS2 score had changed (12.8%). For 10 out of 95 (10.52%) without sepsis and 6 out of 30 (20%) of septic patients, more points were added to NEWS2 meaning that more septic patients were diagnosed before an infection become life-threatening.

For septic shock NEWS2PSP4 was a reliable score; AUROC values were as follows: AUROC NEWS2 0.974 (95%CI 6.11-9.74), and NEWS2PSP4 0.972 (95%CI 6.07-9.76) (very close), while MEDS kept the advantage in predicting mortality (similar AUROC for NEWS2 and NEWS2PSP4) (AUROC NEWS2 0.670 (95%CI 0.79-3.85), MEDS 0.762 (95%CI 2.55-6.01), NEWS2PSP4 0.670 (95%CI 0.82-3.91). Figures 1, 2 and 3 represent receiver-operating characteristics (ROC) curves for NEWS2, MEDS, PSP and the derived scores for sepsis/ septic shock/mortality as above.

DISCUSSION

In our study we proved that adding a specific biomarker, presepsin, to NEWS2 we can put a sepsis diagnosis earlier, reliable, with increased accuracy, avoiding further deterioration. Studies have proved that clinical deterioration is often forecasted by changes in vital signs up to 6 to 24 hours [20, 21]. This can buy time for a specific patient, but in fact also for the system to recover.

To be remembered, although an increased specificity sign, ST elevation, did not decrease mortality per se. Only after angina pectoris was combined with a specific biomarker, troponin, combined with proactive actions, coronary stenting, markedly decreased the mortality.

Immunity against a microorganism relies primarily on the activity of monocytes, macrophages and granulocytes that recognize pathogen-associated molecular patterns that activate downstream pathways and participate to bacteria clearance. This is partly done through a cluster-of-differentiation marker protein 14 (CD14), which has an immediate response against lipopolysaccharides (LPS) [14, 22]. After binding of LPS to CD14 through the LPS-binding protein (LBP), a subtype of soluble CD14 (sCD14-ST, or presepsin, a 13 kDa protein that is a truncated N-terminal fragment of CD14 [15, 16] is released to the blood flow.

Different scores were used as specific tools in specific circumstances. The more versatile ones are the ones that can be used in as many as possible situations, with minimum of resources, as fast as possible. National Early Warning Score 2 (NEWS2) is released after 5 years of trials after the first version of evaluation in hospitals in UK. This score advocates a system to standardize the assessment and response to acute illness and its efficacy was proven in time. Using six simple physiological parameters already recorded in routine practice, NEWS2 is a pragmatic approach with a key emphasis on system-wide standardization

Rapid diagnostic on ED saves lives. Acute myocardial infarction, stroke and sepsis are medical conditions when short time to diagnosis is of paramount importance, that cannot be overemphasized.

NEWS2 can be used as a point of departure for assessing an acute medical illness, but to accurately diagnose a patient a specific tool is needed (ST elevation for acute myocardial infarction on EKG, CT scan for stroke and nowadays qSOFA criteria for sepsis).

As Sepsis-3 stated, a sepsis is declared when an infection becomes life-threatening. This puts a rapid diagnose with minimal resources. Having some preliminary results can gain time to earlier diagnose a life-threatening condition and to prevent further deterioration. When we have the Sepsis-3 criteria, the diagnosis is obvious.

We proved that with minimal resources, monitoring vital parameters and assessing a score and when suitable a POCT biomarker, in 15 minutes we raised the suspicion of sepsis, before becoming manifest, with increased accuracy, gaining at least 45 minutes. We found that a biomarker, presepsin, can accurately earlier diagnose sepsis used in correlation with a verified score, NEWS2 which is used for a patient to access the appropriate level of care at a certain moment. We also took into account MEDS as a level of reference, for its power to predict the prognosis due to sepsis (Mortality in Emergency Department Score).

Of note is that the presepsin values were used to help diagnose sepsis after Sepsis-2 definition, but in our study, we tried to use the presepsin for earlier detection of sepsis, after Sepsis-3 definition, before the clinical life-threatening criteria to become manifest (SBP≤100 mmHg, ≥22 breaths per min, GCS<15).

With an altered mental state, incomplete anamnesis to be taken from relatives or the lack of proper documents, increasing number of patients on admission and too often not enough personnel, an accurate, proper assessment on admission is mandatory. After the initial evaluation, vital functions monitoring and blood samples are taken, before more complete laboratory analysis to be available – with a median of one hour, the Point-Of-Care-Testing devices can give you a more comprehensive understanding of the actual medical state in approximatively 15 minutes. POCT biomarkers, arterial gases analysis and complete blood count with multiple scores applied, offer a more comprehensive assessment of a patient and sometimes prioritize the subsequent investigations. While a MEDS score needs not so easy to get information (rapid terminal comorbid illness, neutrophil bands, lower respiratory infection or nursing home resident) NEWS2 is a reliable, verified score intended to be used for monitoring, with increased sensitivity. Based on initial evaluation, taking presepsin (available in 17 minutes) only to patients with SIRS and NEWS2 assessment, taken together increased the number of the patients to be earlier diagnosed with sepsis with 10%. Presepsin was the ideal biomarker due to the fact that it has earlier response compared with procalcitonin after the onset of an infection (peak level<2 h) plus a short half-time (0,5-1 h) compared with those of procalcitonin (2-3 h and 20-24 h, respectively) and CRP (6 h and 4-6 h, respectively) lead us to use this biomarker. The lack of specificity as procalcitonin increases also in surgical interventions, cardiogenic shock, thermal shock, graft-versus-host disease, immunotherapy, burns, acute pancreatitis lead us to consider using presepsin as specific biomarker [21, 23, 24].

To our knowledge this was the first report of PSP and NEWS2 score used in combination for sepsis diagnosis. Nevertheless, although this association is better for earlier detection of sepsis, there no added advantages in predicting septic shock or mortality.

Sepsis is not a completely understood pathology. Surviving Sepsis Campaign with the periodic reevaluation (even the Sepsis, Sepsis-2, Sepsis-3 definition suggests the dynamics and the major concern involved) still leaves room for refinement and improvement. Our study reflects our approach for earlier diagnose of sepsis. Having a patient with clear criteria for sepsis accordingly to qSOFA leaves no room for doubts and the nowadays guidelines stress the importance of earlier diagnosis and rapid measurements to be taken (2018 Sepsis Uptated) in the first hour.

Sepsis defined as life-threatening organ dysfunction caused by a dysregulated host response to infection does not shed light over the dysregulation. Is it due to the increased aggressivity of the pathogen, to the amount of the pathogen, to the particularities of the injured organ/system, of the comorbidities which decrease the defense mechanisms, of the defense of the defense mechanisms itself? In order to achieve timely treatment for a possible lethal condition, a sensitive screening tool is more important than a specific one [25, 26] and identifying the poor prognostic factors may have a role [27].

Although qSOFA has an increased specificity and minimal resources to be used, lacks the sensibility and consequently the diagnosis of sepsis is often put too late. Even one point granted on initial evaluation does not imply that subsequent evolution is not going to be worse, to further deteriorate without becoming life-threatening. So, more refined approach is sometimes necessary.

Study limitations and strengths.

The present study has some limitation. First, being a single center retrospective, observational study we may not have a comprehensive view of this complex pathology. Second, being a tertiary hospital, may have had more complex patients which may have biased in generalizing the conclusions. Future studies should include more patients from different sites. Third, sepsis defined as life-threatening condition, may alter the health status by itself- an out-control infection might bias the renal function by itself or by aggravation of an already altered renal function as a comorbidity in a more general health status. Fourth, the limited number of patients studied, although significant, are a mere part of the patients with sepsis, so the drawn conclusions should be further verified. Fifth, the size of the cohort might have not enough power requested to draw final conclusions. Finally, although already described in literature, the correlation between presepsin/ biomarker with renal clearance levels and kidney function in patients with life-threatening condition should be studied further.

As strengths in our study we assessed the impact of an earlier intervention over a life-threatening condition in which time is of critical importance. Although widely used, the scores are sometimes inadequately applied. Making distinction between the goals and the moment to be applied to, a score is not a goal in itself. Using a monitoring, verified score as NEWS2, high sensitivity, low specificity combined with a specific biomarker with short time until peak values are attained, lead us to suggest that before becoming dysregulated and life threatening, the sepsis might have an earlier, more optimized approach.

CONCLUSIONS

In the present study, we have tried to assess the impact of a monitoring score and a rapid POCT biomarker, in a condition where time is of critical importance. We acknowledged that in a not completely understood lethal pathology as sepsis, different approaches might be tried in order to earlier detect a life-threatening condition. Using a verified monitoring score, NEWS2, with increased sensibility and low specificity and presepsin, POCT biomarker result within 17 minutes, we diagnosed sepsis earlier.

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VARIA

The effect of multimedia training on social function of burn patients in Shahid Motahhari Hospital, Tehran: A clinical trial study

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Abstract: *Introduction: Burn is a tissue injury and affects social functioning and relationships. Complications of burns lead to disruption of social relationships and consequently social dysfunction.*

Objective: Aim of this study was to determine the effect of multimedia training on social functioning of burn patients in Shahid Motahhari hospital in Tehran.

Methods: This clinical trial study was performed on 100 burned patients. The intervention group received a multi-media self-care discharge training on a CD in addition to the common education. Social function of quality of life was examined in both groups before intervention, 3 months and 6 months after intervention.

Conclusion: Results showed that before intervention the mean score of social function of quality of life in intervention and control group was 1/55± 0/46, 1/92± 0/6 respectively which was statistically significant (p <0.001). Mean and standard deviation of social function of quality of life in the intervention and control groups three and six months after intervention were 2/47± 0/56, 4/05± 0/77, 2/15±0/39, 3/29 ± 0/95 respectively which was statistically significant (p <0.001).

Keywords: *self-care, multimedia, patient discharge, social adjustment, burns*

INTRODUCTION

Burn have been described as one of the most devastating disasters on the human body [1] which seriously damages one's life and health and is considered to be the fourth most common injury [2]. The World Health Organization estimates that the incidence of burns Severe is one percent of life expectancy and more than 300,000 people die from burns worldwide each year [3]. According to the Forensic Medicine Organization statistics, in the first quarter of this year, 379 people died from burns in the country. Of these, 213 were men and 166 were women [4], so burn injuries are one of the most dangerous health incidents in Iran. Over the past decade, advances in health care have made patients with more severe burns survive [5]. Even if urgent management is successful, burn injuries can create many obstacles for patients. In addition to their physical problems, they also suffer from social problems and ultimately their quality of life is affected [6]. Patients suffer from severe seizures at the gate, which can live longer. High-end living is particularly attractive in most places where a lot of space for cabins can be attractive. And the place of marriage, relationships, places of residence, in their lives, and ultimately cause them to become overwhelmed by anxiety and anxiety [7]. People

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have trouble meeting new people and dating, worrying about developing relationships and exhibiting various reactions, such as shyness, aggression, or extreme social avoidance. Therefore, it is necessary to design appropriate supportive programs to improve their quality of life [8]. The only thing that will help the patient is how to live with this situation and learn to meet their own needs and to be less in need of others and to have the attitude that they can play with these shortcomings and that It will be nothing but training and learning and rehabilitation [9]. The philosophy of patient education is to apply the information and skills learned to control and cope better with the disease performed by the care team, especially the nurses [10]. The role of nurses in the last few years as the most important member of the health care team has undergone a historical transformation, from promoting patient-centered health education to empowering patients to self-care and achieve health. Informing the patient and contributing to decision-making speeds recovery and reduces hospital stay and reduces hospital readmission [11]. This is a key challenge here in achieving donation. Education is a traditional teaching tool and disability [12], because lecture-based training requires a great deal of time and expense and, on the other hand, a patient with a burn accident during hospitalization due to mental and physical injuries, physical injuries. Physical weakness, painful daily activities, intellectual discomfort, and lack of focus on decision making may make you less prepared to learn and remember education. The empowerment and self-care is not [9]. In recent decades, traditional approaches to learning with the advent of new technologies such as multimedia virtual education have undergone dramatic changes [13]. The purpose of multimedia application is to make meaningful learning happen, and meaningful learning occurs when the learner can make meaning to the material presented by constructing a coherent mental image from multiple sources of information [14]. It seems that learning will be better if the patient is able to carry out a self-care program using a comprehensive audiovisual CD to suit any time and circumstances they wish [9]. Also, due to the gradual process of rehabilitation in patients, education during discharge for these patients to return to the community must be sufficient and carefully planned [15].

OBJECTIVES

Since humans are social beings and communicating with others is an important factor in life and patients with burns suffer from this, the researchers sought to investigate the impact of multimedia training on the social functioning of burn patients. At the Shahid Motahhari Hospital in Tehran.

MATERIALS AND METHODS

Setting

This research was a randomized clinical trial with a control group that was conducted in the hospitalization wards of Shahid Motahari Burn Center, Tehran, Iran in from 2016 to 2017. The study population consisted of all burn patients who were admitted to Shahid Motahari Burn Center and were participated in the study based on the inclusion criteria.

Inclusion and exclusion criteria

The criteria for participating in the study were the patients` age, people who were between 18 to 60 years, and had the ability to use audiovisual compact discs (CDs), their burning percentage to be 10-45%, degrees of 1, 2 and 3, those having a minimum reading and writing literacy, and understanding of Persian language. Also those who lacked sensory and motion problems and brain and mental disorders, mental retardation, those who were living in Tehran and the suburbs of Tehran, people whose burns were due to accident and non-self-immolation, non-burning with electricity were included in the current study. The exclusion criteria were the withdrawal of continued study and severity of disease, disability and death of patient.

Sampling method

The sampling method was at convenience and continuous study; the patients were randomly assigned into intervention and control groups. According to the studies carried out in this regard, the effect of educational interventions with 95% of confidence and 80% of test capacity was considered on the number of samples needed for each group and taking into account 10 scores of difference in the quality of the psychological dimension of life of the two groups. So the population was estimated to be 55 people based on the following formula in a way that each group included 50 subjects, considering 10% of the probability of not participating. Finally, 100 samples were considered to be participated in the study with the formula $n=2(z1-\alpha/2+z1-\beta)2s2/(\mu1-\mu2)2$. In this formula $z1-\alpha/2=1.96$, $z1-\beta=0.84$, $s=9$ and $\mu1-\mu2=5$.

Measures

Two questionnaires were used in the recent study. The demographic information questionnaire and the status of the disease including some questions about gender, age, occupation, marital status, burning agent or source of heat (gasoline, gas, flame, hot liquids, oil, hot food, etc.), level of education, grade and percentage of burns, burning area, city, location of incident and economical status. This questionnaire was selected by the patient and a research associate on the first day so that the samples inclusion in the study was completed. The next instrument was a questionnaire of burn patient's Quality of Life [BHS-B (Burning Specific Health Scale). The social dimensions of this questionnaire were used.

The questionnaire included 40 questions about skin sensitivity to heat, body image, hand performance, care for burnt areas, communication, ability to perform simple activities, sexual function and psychological dimension with the options of high, moderate, low and never, which had been scored from 1 to 5, respectively. Each questionnaire had at least one and maximally five scores. Based on this questionnaire, the quality of life in each dimension or domain was determined separately and in all domains. From 40 questions of the questionnaire, 18 questions were related to the physical dimension of quality of life, 11 questions were about the psychological dimension of quality of life and 11 questions revealed the social dimension.

Demographic and disease information questionnaire was given to 10 faculty members of the faculty of medical sciences in terms of validity and content validity, and then their opinions were applied as the reliability and validity of the BHS-B were measured by Kildal et al. in 2001 using its dimensional analysis [16] In Iran, Pishnamaazi et al. (2009) had measured its validity and reliability by Alpha Cronbach of 94% in the burn patients in Shahid Motahari and Hazrat Fatemeh hospitals [17] and at Qotboddin Shirazi Hospital [18], calculated the reliability of this tool with Alpha Cronbach of 98%. In our study, Alpha Cronbach was measured 94%.

Education and treatment program

Based on the implementation of this method, the researcher referred to the Burn Medical Educational Center of Shahid Motahari Hospital after receiving the study confirmation from Iran University of Medical Sciences and the ethical code from the university's ethics committee (93-02-28-24922-106366 on 8/12/2014 and registered in a clinical trial with the code IRCT 2014112920145).

After introducing the principal investigator and the collaborators of the research and the research objectives to the hospital's officials and obtaining permission, he referred to the departments and, while introducing himself and the colleagues of the research and the study objectives to the departmental authorities, the samples were randomly provided according to the conditions of inclusion as the control or intervention group. After explaining the procedure and ensuring the anonymity of the samples, a written informed consent was obtained from each participant. They were announced that the transportation cost and the cost of left work to be compensated at 3 and 6 months by the researcher. Before the intervention, the demographic information and burn characteristics questionnaire was completed by the patient with the help of a research associate and using medical records. Then the intervention and control groups received face-to-face routine trainings, however, the intervention patients, in addition to routine trainings, received the self-care discharge education of a burnt patient given at the time of discharge in an educational CD containing text, slide, film and recorded sound; then the researcher gave this CD to the patients to perform at home. In educational session they used CDs and answered questions for 30-60 minutes at the time of discharge.

Educational content was prepared based on the sources of self-care educations of the burn patients. The questionnaire of the quality of life of burnt patients in the psychological, physical and social dimensions was completed by the patient before the intervention on the day of discharge and 3 and 6 months after the intervention; the telephone number, email address, researcher's telegram number were given to patients to call if necessary. The researcher conducted a weekly phone contact with the patients in the intervention and control groups to follow up and ensure the preservation of the samples. After 3 and 6 months of intervention, the patients in both the control and intervention groups were contacted by phone to complete the questionnaire. Patients completed the questionnaires in the manner of the self-report. At the end of the research, the educational CD was provided to the control group for observing ethics in the research.

Ethical consideration

This study was approved by the Ethics Committee of Iran University of Medical Sciences and the Ethics Committee of the place where research was conducted (Ethic code: 93-02-28-24922-106366).

The clinical trial was approved by the Iranian Registry of Clinical Trials (IRCT) under No: IRCT2014112920145N1. The CONSORT checklist was used to report the study.

Statistical analysis

After collecting raw data for the analysis, the descriptive and inferential statistics (Chi-square and independent and paired t tests for the distribution of normal variables), Fisher's exact test, nonparametric tests such as Mann-Whitney, Wilcoxon and Friedman test and Dunn test, with Bonferroni's correction, Spearman correlation coefficient were used by SPSS software (version 21, Chicago, IL, USA). It should be noted that all of the participants were included in the process and no one was excluded during the investigation.

RESULTS

Among the participants of this study, 56% of the subjects were male and 56% were female. Only 34% of them in the intervention group were in the age range of 39-48 years and 44.9% of the control group`s members were in the age range of 29-38 years.

According to the statistics, most of them (44% in the intervention group and 79.6% in the control group) were married. In the intervention group, 48% and in the control group, 62.5% were employed; 52.1% had diploma in the intervention group and 66.7% had diploma educational level in the control group.

Moreover, 36% in the intervention group and 34% in the control group were burned by fire flame and 60% in the intervention group and 64% in the control group had a degree of burns of 1, 2 and 3. Furthermore, 24% in the intervention group had burning percentage of 15-20% and in the control group, 36% had burning percentage of 21-26%. About 46% in the intervention group had burn in the trunk, hand, and foot and 47.9% in the control group had burn in the whole body.

The majority of patients in the intervention and control group (58.1%, 79.2%, respectively) were resident in Tehran. The majority of patients in the intervention and control group (58.1% and 53.5%, respectively) were burned at home. In the intervention group, 56.5% were on average economical level and 37.8% were in weak economic level.

The Mann-Whitney test showed that before intervention, the mean of social function in intervention and control groups was 1.92±0.6 and 1.55±0.46, which was statistically significant (p<0.001). The mean difference of the score of social function in both intervention and control groups at the time before intervention was statistically significant and the mean score of social dimension in the intervention group was slightly higher than the control group.

Mean and standard deviation of social function score in intervention and control groups three months after intervention were 3.29±0.95 and 2.15±0.39, respectively and six months after intervention, the mean and standard deviation of intervention and control group were 4.05±0.77and 2.47±0.56, which were statistically significant (p<0.001) (Table 1).

Table 1: Comparison of mean and standard deviation of social function score in intervention and control groups one month before and three and six months after intervention

	Intervention group	Control group
Mean and standard deviation before intervention	1/92 ± 0/6	1/55 ± 0/46
Test result	Z = -2/98 P value < 0/001	
Mean and standard deviation three months after intervention	3/29 ± 0/95	2/24 ± 0/4
Test result	Z = -6/83 P value < 0/001	
Mean and standard deviation six months after intervention	77/0 ± 05/4	2/75 ± 0/58
Test result	Z = -7/74 P value < 0/001	

Considering the chi-square value (=95.14) and the value of significance level (p<0.001) in Table 2, since the level of significance was less than 0.05, the assumption of the equality of the mean scores of social function during three periods was rejected statistically, that is, the average score of social function varied at least in two periods of the three ones. Therefore, in order to determine which of the two periods had a significant difference, the Dunn follow-up test was used.

The results of the test were presented in Table 2 and 3, while the mean score of each period had significant differences with other periods because the corrected significance level value was less than 0.05.

Figure 1 shows that the social dimension score before intervention in the intervention group was slightly higher than the control group, but after 3 and 6 months of intervention, the social function score of the intervention group demonstrated a significant difference compared to the control group (p<0.001).

Table 2: Results of comparison of mean and standard deviation of social function score before intervention, 3 and 6 months after intervention in intervention group using Friedman test in intervention group

Time	Number	Mean	Standard deviation	Test result
Before intervention	50	1/92	0/6	Chi-square = 95/14 df = 2 p-value<0/001
Three months after intervention	50	3/29	0/95	
Six months after intervention	50	4/05	0/77	

Table 3: Comparison results of social function score in 3 periods with Dunn pairwise test with Bonferroni's correction in intervention group

Difference	Standardized test statistic	Corrected significance level
Before intervention - 3 months later	-5/4	<0/0.001 adj. p-value
Before the intervention - 6 months later	-9/45	<0/0.001 adj. p-value
3 months later - 6 months later	-4/05	<0/0.01 adj. p-value

Table 4: Comparison results of social function score in 3 periods with Dunn pairwise test with Bonferroni's correction in control group

Difference	Standardized test statistic	Corrected significance level
Before intervention - 3 months later	-5/25	<0/0.001 adj. p-value
Before the intervention - 6 months later	-8/4	<0/0.001 adj. p-value
3 months later - 6 months later	-3/15	<0/0.01 adj. p-value

Figure 1: Comparison of the trend of changes in the mean score of social function 3 and 6 months before and after intervention in two groups

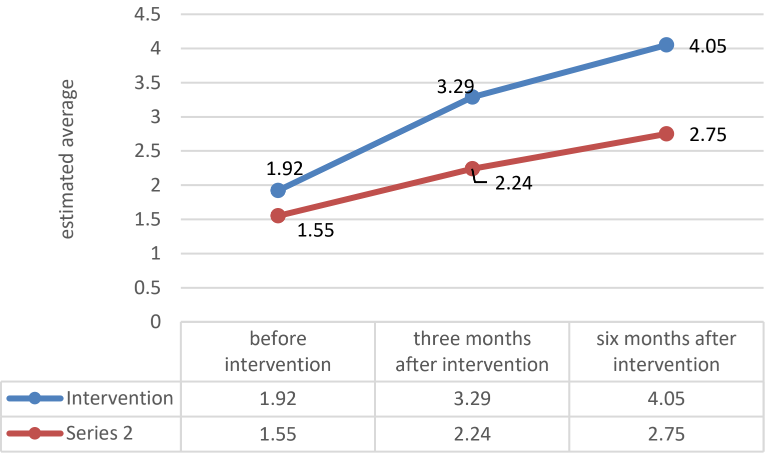

	before intervention	three months after intervention	six months after intervention
Intervention	1.92	3.29	4.05
Series 2	1.55	2.24	2.75

DISCUSSION

The results of this study showed that multimedia virtual self-care discharge education improves social functioning of burn patients. This finding is consistent with the study by Li et al. In their study, they found that social functioning of burn patients 5 weeks after rehabilitation was better than the control group [19]. Tang et al. (2015) study also showed that the use of rehabilitation interventions and self-care measures increased the social function dimension of patients three months after intervention, which is consistent with the present study [20]. Radwan et al. (2011), who found that running a 7-day rehabilitation program for 2 weeks improved social performance in the experimental group, also consistent with our study [21]. Burn injuries affect one's ability to cope with life's stresses and interact with the outside world [8]. These people feel ashamed and embarrassed about being in the community and communicating with others due to the apparent changes caused by burns and burns, and the look and sometimes excitement of others suggests that they feel compassion and compassion. For this reason, they lack communication and social skills and require intervention [23]. Hojati et al.'s study showed that psychosocial interventions had a significant effect on life satisfaction, occupational activity, mental health, physical health, quality of life, and social relationships.

Therefore, these interventions increase patients' life satisfaction and social relationships [24]. The results of the Fatimid study showed that patients' quality of life in the social dimension was relatively undesirable, so it recommended that patients be taught communication skills [25]. The consequence of education in the community is maintaining and promoting health and will have many benefits, including reducing the duration of illness, accelerating client independence and maintaining self-confidence in self-care [26]. A study by Elalem et al. (2018) showed that self-care nursing intervention was effective in burn patients.

And self-care intervention led to active participation of patients in their treatment and led to a significant improvement in quality of life as well as self-esteem, which is consistent with the present study which correlates with self-esteem and quality of life later. Social has had a significant impact [27]. Hospital discharge is associated with stress and anxiety and an increased need for patients to receive information. Information training is essential for the well-being of patients because patients experience discomfort after awareness discharge [28]. Discharge does not mean the end of treatment for burn patients, but discharge means that the patient and his or her family must resume the responsibility of managing their lives without assistance from hospital staff. Burn patients need to adapt to new situations that include self-care at home, lifestyle change and return to society [29].

Limitation and recommendation

One of the limitations of the present study was the mental state of the patient which could be effective in answering the questions. It was the researcher who emphasized the importance of the subject in the result of the research and asked the samples to fully comply with the CDC's recorded care instructions and followed up the samples by telephone.

CONCLUSIONS

Given the findings of the present study, it is important to provide virtual and multimedia education and to institutionalize a self-care culture. This allows the patient to self-care and engage in self-care. Nurses and caregivers in burn centers need to know that these patients will be socially isolated and isolated, and that they need to return to the community, and nurses are one of those who treat patients' behaviors and attitudes more than others. So they can help them get back into the community with proper education, but education through lectures does not meet their educational need because they are involved in the mental illness of their illness and burn accident, especially while they are still in hospital. And maybe the ability to understand and learn training in that situation So they need virtual training to use it in the right conditions and in a comfortable place. That is, self-care education should be continuous, accessible, follow-up, and economically viable. So using educational CDs, which is a virtual teaching method, can be a good option. It is recommended that nurses use this method in clinical centers.

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Conflict of interests

It is not declared by the authors.

Ethics approval and consent to participate

Clinical trials with the issue number IRCT 2014112920145, ethical code number 93-02-28-24922-106366 on 18/09/1393.

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VARIA

The use of IPACK and the continuous adductor canal block in the perioperative management of total knee prosthesis

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Abstract: *Background: Although locoregional anesthesia techniques in total knee arthroplasty (TKA) have progressed steadily, the emergence of the adductor canal block representing a step forward for postoperative evolution in preservation of the quadriceps muscle strength, analgesia of the posterior territory of the knee still remains a challenge. The emergence of IPACK, in association with earlier techniques, offers promising preliminary results in terms of its contribution to a favourable evolution and to the satisfaction of TKA patients.*

Materials and methods: Prospective trial performed on a group of 28 patients undergoing a total knee arthroplasty intervention; analgesia control in the postoperative period was performed either with opioid and nonopioid systemic analgesics, or by associating the continuous adductor canal block with IPACK and systemic analgesics. The primary objective is pain control at rest/on mobilization, while the secondary purpose is analysis of opioid use, their side effects and recovery of joint mobility.

Results: There is a significantly better pain control in the group of patients with locoregional anesthesia, both at rest and on mobilization ($p<0.005$), accompanied by a decrease in opioid use ($p<0.00001$) and the related adverse reactions; at the same time, there is an improvement in the functional recovery of the knee joint, quantified by the flexion degree at 24 and 48 hours after surgery (61.5/71.12 degrees for continuous adductor canal block with IPACK versus 45.14/55.42 degrees for general anaesthesia)

Conclusions: The introduction of peripheral nerve blocks in the multimodal analgesia regimen after TKA results in better control of postoperative pain, both at rest and on mobilization, in a decrease in opioid use and their related adverse reactions and in an improved recovery of joint range of motion, as well.

Keywords: *continuous adductor canal block, IPACK, VAS, TKA*

INTRODUCTION

Total knee prosthesis is currently one of the most common surgeries in the field of orthopedics, the number of such interventions performed annually being on an accelerated upward trend. On the other hand, the pain stimulus that occurs in total postarthroplasty of the knee is often very intense, TKA being known as one of the most painful orthopedic surgeries , pain control is a major component in perioperative management of TKA [1].

The pain occurring in the postoperative period, as well as the systemic inflammatory response initiated following the tissue trauma will have significant consequences for the

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postoperative evolution of the patients, including prosthesis infection which can compromise the joint functionality and complicate postoperative evolution [2]. It has been shown that activating an exacerbated systemic inflammatory response leads to increased rates of local and systemic complications, increased perioperative morbidity and mortality, increased length of hospitalization and patient dissatisfaction. In view of the above mentioned, the existence of a standardized and complex protocol for the perioperative management of patients with TKA becomes an absolute requirement in order to improve the postoperative evolution, decrease the duration of hospitalization and increase patient satisfaction [3]. The multidisciplinary approach is decisive in determining the prognosis both in case of primary TKA, as for modular endoprosthetic reconstruction, as well [4].

MATERIAL AND METHOD

After getting the agreement of the Ethics Commission of the Bucharest University Emergency Hospital (SUUB), a prospective monocentric trial was performed on 28 patients undergoing unilateral total knee arthroplasty surgery in the SUUB Orthopedics and Traumatology Clinic between May 2020 and March 2021.

The patients included in the trial belonged to ASA risk classes 1-3; patients with ASA risk class > IV, chronic opioid users prior to presentation, obese patients with BMI>40 kg/sq.m., known allergies to analgesic medication included in the protocol, contraindications for neuraxial anesthesia, chronic kidney disease or pre-existing peripheral neuropathy were excluded from the trial. Prior to surgery, patients were informed extensively with regard to the protocol, the technique of anesthesia and analgesia, the related risks and benefits, and the patients gave their informed consent.

The total knee prosthesis surgery was performed by experienced surgeons of the Orthopedics and Traumatology Clinic of SUUB with a parapatellar anteromedial approach, with bicompartmental prosthesis and using the hemostatic band [5].

The trial was performed on 2 groups of 14 patients each, divided according to the anesthetic management and the perioperative analgesia approach. The first group comprised of patients that received balanced anesthesia with volatile pivot and intravenous multimodal postoperative analgesia. In the second group, the anesthetic technique consisted of spinal anesthesia associated with IPACK and the continuous adductor canal block. All patients (both group 1 and group 2) received preemptive analgesia, paracetamol 1 g, Gabaran 300 mg and Celecoxib 200 mg one hour before surgery; the treatment was continued for the first 72 hours postoperatively (paracetamol 1 g every 6 hours under ASAT/ALAT control, Gabaran 600 mg/day in 2 doses, Celecoxib 200 mg twice daily). The locoregional anesthesia maneuvers were performed by anesthetists of the Anesthesia-Intensive Care Department of SUUB.

Patients in group 2 were admitted to the preoperative ward approximately one hour before the time of surgery for performing the 2 peripheral nerve blocks. The patient is placed in supine position, a peripheral venous line is inserted and the patient receives standard monitorization (according to the standards required by the Romanian Anesthesia and Intensive Care Society). To perform the adductor canal block, the patient is positioned with the thigh in slight abduction and external rotation; the patient's thigh is disinfected with 2% chlorhexidine solution, the high frequency linear probe (Philips Cx50 transducer L 12-5 50 mm) is used, which is placed transversely on the thigh in its anteromedial portion approximately at the junction between the medial and distal thirds of the thigh, and the saphenous nerve located anterolaterally to the femoral artery, under the sartorius muscle is identified; under ultrasound guidance (the in-plane approach) the Touhy needle from the Contiplex Touhy Ultra-B. Braun set is advanced under careful ultrasound visualization, from the lateral to the medial area up to the vicinity of the femoral artery. After aspiration and the prior injection of 1-2 ml in order to check the position of the needle, 20 ml of ropivacaine 0.5% is infiltrated into the upper part of the artery (Figure 1).

Figure 1: Infiltration area

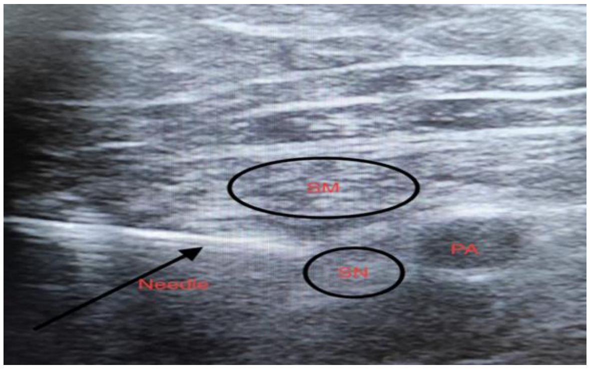

SM sartorious muscle, SN safenous nerve, PA popliteal artery

A plexus catheter is inserted in the adductor canal; the catheter is secured at 11-13 cm with Histoacryl and Tegaderm to prevent dislocation and extravasation of local anesthetic. A mixture of ropivacaine 0.2% with Fentanyl 2-gamma/ml with Easy Pump B. Braun, with a volume of 125 ml and a constant administration rate of 5 ml/hour, is administered postoperatively, allowing for the easy mobilization of the patient. The catheter is retracted 72 hours postoperatively.

The second peripheral nerve block performed is IPACK. The patient is in a supine position with his knee slightly bent. The high frequency linear probe (Philips Cx50 transducer L 12-5 50 mm) is used, which is positioned transversely in the popliteal fossa for the visualization of the femoral condyles, the popliteal artery, the tibial nerve and the common peroneal nerve; the transducer is advanced slightly proximally until the posterior edge of the femur is visualized. After local anesthesia with 1-2 ml of lidocaine 1%, a Stimuplex Ultra 360 20G-100 mm needle is inserted, under ultrasound guidance (the in-plane approach), in the space between the popliteal artery and the posterior edge of the femur, from the lateral to the medial area, and the local anesthetic is infiltrated, 20 ml of ropivacaine 0.5% in a single injection (Figure 2).

Figure 2: Infiltration area for second peripheral nerve block

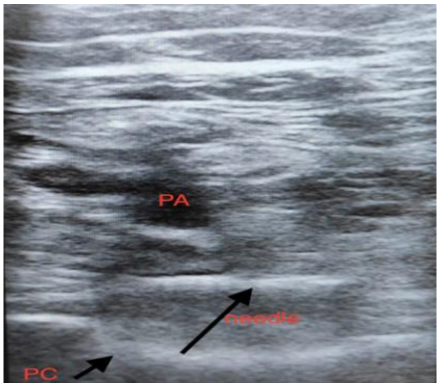

PA popliteal artery, PC posterior capsule

Postoperatively the patients are admitted to the postoperative intensive care unit for the first 24 hours. The patients in group 1 (general anesthesia) enter the multimodal analgesia protocol with morphine combined with paracetamol 1 g/6 hours, Gabaran 300 mg/12 hours and Celecoxib 200 mg/12 hours. Postoperative analgesia of patients in group 2 (spinal anesthesia with peripheral nerve blocks) is ensured by the administration of local anesthetic via adductor canal catheter, supplemented by the administration of paracetamol 1 g/6 hours, Gabaran 300 mg/ 12 hours and Celecoxib 200 mg/12 hours.

The primary objective of the trial is to record the pain score by means of the visual analogue scale (VAS) both at rest and in activity, while the secondary objectives that we followed are the consumption of opioids in the two groups, their adverse effects and the degree of mobility of the joint that correlates directly with a quick postoperative rehabilitation of the joint.

Statistical analysis

The data recorded is processed using descriptive statistics, Student t-test. The nature of the hypotesis testing was two-tailed, and P<0.005 was considered statistically significant.

RESULTS

From a demographic point of view, no significant differences are observed between the two groups of patients (tabel 1). The preoperative pain score at rest is also comparable, and in terms of chronic analgesic use it is observed that only 4 patients in group 1 and 3 patients in the group 2 were chronically using analgesics of the NSAID category (in the last 3 month) and one patient analgesics of the weak opioids class.

Table 1: Demographic data, surgical information (values are presented as mean or number and percent)

Treatment group	General anesthesia n=14	IPACK/cACB n=14
Age(y)	62 (49-70)	68 (59-74)
Gender, male, n	4 (14)	5 (14)
BMI (kg/m²)	34 (25-39)	32 (25-38)
Surgical time(min)	117 (70-170)	130 (80-180)
VAS rest at home	3 (3-4)	4 (3-5)
NSAID consumption at home (last 3 month)	4 (14)	3 (14)
No opioid consumption at home	13 (14)	14 (14)

IPACK, infiltration of local anesthetic between posterior artery and capsule of the knee; cACB, continuous adductor canal block; BMI, body mass index; VAS, Visual Analogue Scale ; NSAID, Nonsteroidal anti-inflammatory drugs

VAS score at rest and at work on day 1 and day 2 showed significantly (p<0.005) better values in locoregional anaesthesia group (lot 2) compared to general anaesthesia group (lot 1). VAS score at rest after 8 hours postoperatively also showed significantly (p<0.025) better values in locoregional anaesthesia group compared to general anaesthesia (Figure 3).

The mean ranges of movement on days 1 and 2 postoperatively for locoregional anaesthesia group (61.5 degrees and 71.12 degrees, respectively) were significantly better (p< 0.0005) than ranges of movement on day 1 and 2 for group with general anaesthesia (45.14 degrees and 55.42 degrees).

Similary, the opioid usage was lower (P<0.00001) on day 1 for pacients who received adductor canal blockade combined with IPACK.

Figure 3: Pain numerical rating scale at rest

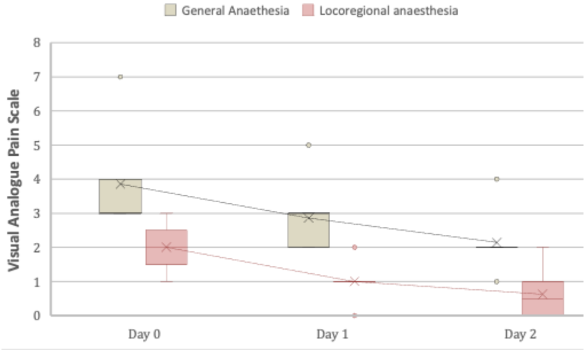

Boxes represent 25th-75th percentiles interval. X marks mean value

DISCUSSION

Because effective analgesia is largely responsible for blocking this metabolic breakdown called systemic inflammatory response, much of the efforts undertaken by clinicians have focused on controlling postoperative pain [6]. Recent decades have witnessed the wide implementation of the concept of multimodal analgesia, a protocol in which, in order to ensure effective analgesia, conventional analgesia methods (administration of opioids, paracetamol, nonsteroidal anti-inflammatory drugs, etc.) are associated with new locoregional anesthesia techniques. This makes it possible to reach an effective analgesia threshold while significantly reducing the use of opioids and analgesic drugs, therefore decreasing the adverse reactions related to their excessive consumption [7].

The role of locoregional anesthesia in the management of the TKA patient is already well established. Numerous studies have shown that the association of different peripheral nerve blocks with the administration of systemic analgesics in the multimodal analgesia protocol (the femoral nerve block and more recently the adductor canal block) makes an important contribution to the perioperative management of such patients [8, 9]. Unlike the femoral nerve block, which often results in quadriceps muscle fatigue and increases the perioperative risk of accidental fall, the adductor canal block preserves the muscular strength of the quadriceps muscle, reduces the risk of in-hospital fall and significantly improves the functional recovery of the joint [10-12]. However, it seems that the inclusion of the femoral nerve block or the adductor canal block in the perioperative management plan does not necessarily eliminate the need for opioid administration in the perioperative period [13]. This can be explained by the fact that both variants of the femoral nerve block – the classic one and the adductor canal block – will ensure the analgesia of the anterior and mediolateral territory of the knee joint, so that the posterior area innervated by branches detaching from the sciatic nerve is completely free of analgesia, hence the need to supplement with analgesics postoperatively [14].

In this sense, different solutions have been searched to ensure efficient analgesia in this territory, without affecting the motor performances that play an important role in the rapid postoperative functional recovery. Thus, the emergence of the IPACK block (infiltration between the popliteal artery and the capsule of the posterior knee) appears as a promising solution in terms of ensuring analgesia for the posterior territory of the knee joint [15, 16]. The innervation of the posterior territory of the knee joint is ensured by articular branches detaching from 3 main trunks: the tibial nerve (whose branches represent the main source of innervation of the knee posterior capsule), the common peroneal nerve (that sends terminal branches to the anterolateral and posterolateral portion of the capsule) and the obturator nerve (3 branches ensuring the innervation of the superomedial portion of the capsule) [17]. In essence, IPACK blocks the terminal sensitive branches detached from the popliteal plexus and the obturator nerve, thus ensuring analgesia of the posterior capsule of the knee, without involving motor branches and therefore without affecting the process of functional recovery of the joint. Sparing the motor branches of the common peroneal and tibial nerves and covering the posterior territory of the knee joint are two features that can make IPACK the ideal solution, along with the adductor canal block, with a view to ensuring effective analgesia of the knee joint in the postoperative period [18, 19]. From a technical point of view, this is achieved by the infiltration of a local anesthetic into the area located between the popliteal artery and the posterior capsule of the knee, an ultrasonographically guided maneuver. So far, experience with the use of the combined technique consisting in locoregional anesthesia, adductor canal block associated with IPACK, has been limited in terms of the functional recovery of the joint, the analgesic efficiency and the opioid use.

Starting from the idea that the new techniques described in locoregional anesthesia represent a major step forward in the perioperative management of TKA patients, the trial described here will analyze the efficacy of the continuous adductor canal block associated with IPACK in improving pain control and functional recovery, in reducing opioid use and the incidence of side effects, as well as in reducing the duration of hospitalization [19].

From a demographic point of view, no significant differences are observed between the two groups of patients. The preoperative pain score at rest is also comparable, and in terms of chronic analgesic use it is observed that only 4 patients in group 1 and 2 patients in the group 2 were chronically using analgesics of the NSAID category and one patient analgesics of the weak opioids class.

As regards the intensity of pain on the day of surgery, patients in the group with postoperative analgesia based on the combination of analgesics of the opioid class and nonopioids had a higher VAS score than those for whom locoregional anesthesia with systemic analgesics of the weak opioids class and nonopioids was used in the multimodal analgesia protocol. Also, there is a better control of pain in group 2, including days 2 and 3 postoperatively, both at rest and when mobilizing the knee joint with the help of kinetics.

Following the functional recovery of the joint, we notice that the patients in group 2 were able to suffer a higher degree of flexion of the joint in terms of pain, compared to patients without locoregional anesthesia. At the same time, the use of opoids was significantly higher in patients with systemic analgesia only.

During the trial, only one major adverse event occurred, namely one case of respiratory depression following opioid administration; adverse reactions such as nausea and vomiting were more common in patients with opioids, while in the group of patients with locoregional anesthesia no adverse effects were reported of the type systemic toxicity of the local anesthetic, extravasation of the anesthetic at the site of catheter insertion or in-hospital fall due to decreased muscle strength of the quadriceps muscle.

Although locoregional analgesia in the perioperative management of TKA is supported by the results of the trial, it has certain limitations. First of all, the trial is not a randomized one. Secondly, the follow-up of the patients was performed only in the first 72 hours postoperatively, until the removal of the adductor canal catheter, therefore we cannot provide any data on the further developments in terms of pain intensity and analgesic requirements after locoregional analgesia is suppressed. Further studies are needed in order to determine whether the benefit of locoregional anesthesia (joint mobilization, pain score) is maintained after its discontinuation. Thirdly, despite significantly higher opioid use in patients in group 1, postoperative pain is more difficult to control, therefore comparing the degree of joint mobility of the two groups may not be relevant, the intensity of the pain being an important limiting factor of the mobility degree.

In a prospective, triple-blind trial conducted by Kim D. et al in 2017 on a group of 86 patients undergoing primary unilateral knee arthroplasty, the association between IPACK and the adductor canal block at the periarticular infiltration of the local anesthetic provides a high level of analgesia and helps reduce opioid use compared to the joint infiltration associated with the systemic multimodal analgesia regimen. This trial supports the introduction of IPACK and the adductor canal block in a standardized multimodal analgesia protocol for patients undergoing orthopedic knee joint prosthesis surgery [20].

Another prospective trial by Patterson ME. et al. on 69 patients enrolled during the period 2016-2018 shows that the association of IPACK with the multimodal analgesia regimen improves pain control at rest in the postoperative period, but without bringing a significant clinical improvement (no significant differences were found between the pain in conditions of physical exertion, distance walked, length of hospitalization or opioid use), therefore the trial did not recommend the introduction of IPACK in the routine management of knee arthroplasty [21].

CONCLUSIONS

Although the results of the clinical trials conducted so far are controversial and long-term research is needed on this topic, the trial conducted in our center supports that the introduction of peripheral nerve blocks in the multimodal analgesia regimen after total knee prosthesis results in a better control of the postoperative pain both at rest and on mobilization, in the decrease of opioid use and implicitly of the incidence of the related adverse reactions. Moreover, the IPACK association seems to bring an additional benefit consisting in the control of the painful stimuli at the back of the knee joint, with a faster rehabilitation and increased joint mobilization, which in the long run will lead to a lower rate of complications caused by prolonged immobilization and to a higher degree of patient satisfaction.

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VARIA

Couvelaire-Uterus: literature review and case report

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Abstract: *Uteroplacental apoplexy or Couvelaire uterus is a rare complication in the spectrum of the life-threatening placental abruption.*

We report a 29-year-old pregnant woman who had a placental abruption complicated with the rare condition of Couvelaire uterus, with an delivery per uterus-sparring cesarean section.

The early diagnosis and proper medical management of placental abruption through uterus-sparring cesarean section present the best outcome for mother and child.

Keywords: *uterus Couvelaire, placental abruption, uteroplacental apoplexy*

INTRODUCTION

Utreroplacental apoplexy or Couvelaire uterus is a rare manifestation which complicates aprox. 5% of placenta abruptions and was described for the first time in 1911 by French obstetrician Dr. Alexandre Couvelaire [1, 2]. The placenta abruption complicates ~1% of pregnancies [3] and the maternal mortality can reach up to 5%. The uteroplacental apoplexy is a rare, nonfatal complication of the placenta abruption [4].

A literature search for the last 10 years was conducted on 25.11.2020 using the PubMed database and having the key words ‘Couvelaire uterus’. This resulted in 27 articles published in the last 10 years. The Couvelaire uterus is often underreported and underestimated. The etiology is unknown and the diagnosis is done through inspection or histological examination [4].

This mechanism of placenta abruption can be seen as physiological after the fetus delivery and as pathological in the pregnancy with the fetus still in uterus. The acute condition of placenta abruption is the result of a compromise of the vascular structures supporting the placenta, having as outcome a retroplacental hematoma. This process may represent the starting point for Couvelaire uterus. The hemorrhage from pathologic vascular damage within the placenta seeps into the decidua basalis causing a separation of the placenta. Before the second stage of labour is finished, the blood created by bleeding resulting from the early placenta abruption infiltrates into the myometrium finding its way into the peritoneal cavity. Occasionally, such effusions of blood extend in the parametrium. As a result of the described mechanism we notice the macroscopic aspect of Couvelaire uterus which displays lesions characterized by violet to black ecchymoses [5, 6, 7]. The Couvelaire uterus sometimes loses its contractile power but usually responds well to intravenous oxytocin [6, 8]. The uterine ability to contract is preserved, particularly after amniotomy and decompression to allow constriction of spiral arteries [9].

Among the risk factors for placenta abruption are early

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pregnancy bleeding, hypertension or thrombophilia, or abdominal trauma [10]. The diagnose of placenta abruption is made clinical and is completed by the ultrasound scan and CTG. The patients present a painful, tender and rigid uterus, may have uterine contractions, vaginal bleeding is also common. The finding of a retroplacental clot on ultrasound scan is a risk factor for placenta abruption, the ultrasound scan plays an important role correlated to the clinical situation in the diagnose of the placenta abruption [3, 10, 11].

The delay of the diagnose of placenta abruption increase the risk of intrauterine fetal death, hypoxic brain injury, bleeding, coagulopathy with increased maternal and fetal morbidity and mortality [12, 13]. The incidence of coagulopathy in placenta abruption is reported to be 5.8% [3]. In the case of Couvelaire uterus the disseminated intravascular coagulation can be induced through the infusion of thromboplastic material in maternal circulation [14].

Couvelaire uterus was associated in different reports with placenta abruption, placenta previa, amniotic fluid embolism, and preeclampsia [4, 15]. Cesarean section in the patient's history is a risk factor for uterine rupture, abnormal placental implantation, placental abruption and uterine scar dehiscence in subsequent pregnancies [16]. Some authors consider that the incidence of Couvelaire uterus is increased by the increase in cesarean deliveries [4].

The management of the antenatal and postpartum hemorrhage plays a key role, on one side in the control of the bleeding and blood transfusion, and on the other side aims to reduce fetal and maternal morbidity and mortality [4].

In the past hysterectomy was performed as a routine therapy of Couvelaire uterus. Nowadays, is recommended to opt for the conservative management [4, 8]. The proper management of this pathology reduces the high maternal and fetal risks. The literature reports cases of fetal demise, but also hysterectomy as a result of severe disseminated intravascular coagulopathy [3, 4, 17]. The early decision to perform a hysterectomy taken before the patient's condition is extreme serious is required in order to minimize the morbidity and mortality [18, 19]. On the other hand, before deciding for more aggressive procedures, like hysterectomy, B-Lynch technique should be taken into consideration in cases of postpartum haemorrhage as a result of abruptio placentae followed by development of Couvelaire uterus [19, 20].

In a study on placenta abruption poor perinatal outcome associated with Couvelaire uterus, pregnancy induced hypertension and disseminated intravascular coagulopathy is reported [18].

CLINICAL EXAMPLE

A 29-year-old gravida two, para one, in the 37th (36+6) week of an uncomplicated pregnancy, with a history of an uneventful spontaneous delivery, was admitted to the obstetric ward with symptoms of nephrolithiasis and urinary infection for further therapy. The obstetrical check-up was uneventful. The admission symptoms were alleviated, discharge could have been possible but she developed irregular and poorly coordinated uterine contractions. She developed uterine tenderness without relaxation between contractions, with wooden hardness by palpation. Vaginal examination revealed acute hemorrhage from the uterine cavity. The patient was hemodynamically stable. The cervix was 2 cm long and 1 cm dilated. CTG was normal. Cesarean delivery was indicated by suspicion of placental abruption. Intraoperatively massive hemoperitoneum and the diagnose of placenta abruption with Couvelaire uterus was confirmed (Figure 1).

A healthy boy was delivered. Uterus-sparring surgery was performed.

Postoperative recovery was uneventful.

Figure 1: Couvelaire uterus as a result of acute intradecidual hemorrhage produced by the rupture of the uterus–placental spiral arterioles which produces ecchymosis discoloration, secondary to extravasation of blood into the myometrium and serosa, can lead to hemoperitoneum.

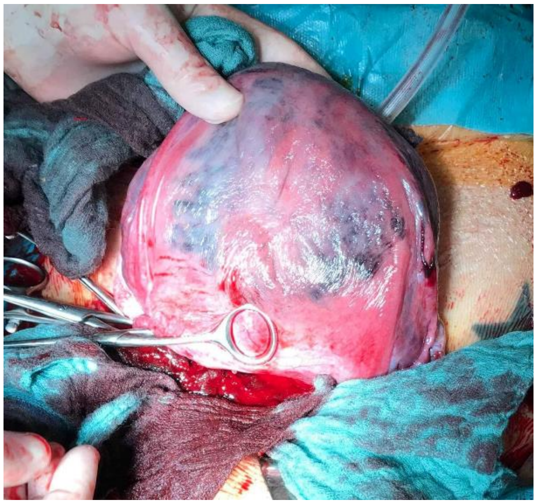

DISCUSSION AND CONCLUSION

Placental abruption represents the early separation of the placenta from the uterus wall developing a retroplacental hematoma which is an acute intradecidual hemorrhage produced by the rupture of the uterus–placental spiral arterioles. Couvelaire uterus or uteroplacental apoplexy is the result of blood extravasations in the tissues, occasionally the infiltrations which reach the peritoneal cavity creating hemoperitoneum [1, 2, 5].

In our case, the first intraoperative view after opening the peritoneal cavity was the massive hemoperitoneum and Couvelaire uterus with dark purple and copper color patches with ecchymosis discoloration and hemorrhagic infiltration in the lateral portions of the uterus.

Diagnosis is made through direct inspection or histological examination [2]. The management of this complication of the placenta abruption is conservative through uterus-sparring surgery, hysterectomy is usually not required and should be discouraged [2, 6]. Couvelaire uterus is a rare complication of placental abruption, with an underreported incidence and it is often associated with placenta previa, amniotic fluid embolism, and preeclampsia [4, 15] .

Often Couvelaire uterus is reported with massive placenta abruption requiring blood transfusion [4]. In our reported case blood transfusion was not required. Antenatal fetal and maternal monitoring, but also the delivery on time, plays an important role in the delivery outcome, otherwise due to bleeding it can come to fetal demise [15, 21]. Placental abruption has an increased maternal morbidity risk of haemorrhage, hysterectomy and consumptive coagulopathy [3]. The life-threatening condition of disseminated intravascular coagulation should not be underestimated [3, 18] .

In the reported case the patient was in the hospital under monitoring and the prompt medical decisions reduced the maternal and fetal risks.

The early diagnosis and proper medical management of placental abruption through the uterus-sparring cesarean section provide the best outcome for mother and child.

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VARIA

The role of the communication in changing health behaviors

Dodu G. Petrescu[1], Raluca Răducu[1], Cristina Soare[1], Victor L. Purcărea[1]

Abstract: *The communication in the field of health can be defined as a communication strategy used to inform and influence individual and community decisions with the aim of improving and enriching personal and community behaviors and public health practices. It includes studying public health attitudes and behaviours to determine the type, frequency and format of the messages.*

Keywords: *attitudes, behavior, change, communication, health*

Social communication and behavioral change are at the heart of any successful public health initiative. The purpose of communication is to make someone to think in a certain way, to do something or to act, in other words is the exchange of emotions, ideas and opinions in the form of information between sender and receiver [1]. It is the most important aspect of the educational process aimed at changing behaviour and attitude and improving the level of knowledge [2].

The communication has several functions [3]:
- instrumental function – to achieve something;
- information function – to find out or explain something;
- social contact function – to make a pleasant environment;
- control function – to cause someone to behave in a certain way;
- expression function – to express feelings or to present themselves in a certain way;
- role-related function – for the given situation;
- educational function – to convey knowledge;
- stimulation function– to bring interest;
- entertainment function – to provide leisure activity;
- cultural promotion function – to strengthen cultural rites.

According to John Fiske-1982, [4] there are two complementary schools with different approaches, in the study of the communication, the school "process" (communication as a transmission of messages; communication analysis models: Lasswell - 1948, Shannon and Weaver - 1949, Newcomb - 1953, Gerbner - 1956, Westley and MacLean - 1957, Jakobson - 1960, etc.) and the semiotian school (communication as production and exchange of meanings; founders and contributors: Charles S. Peirce and Ferdinand de Saussure , as well as C.K. Ogden and I.A. Richards, Louis Trolle Hjelmslev, Roland Barthes, Pierre Gouriaud); There are many schemes of the communication process, but the most complete belongs to Philip Kotler, who managed to integrate all the elements of the process, as can be seen in the Figure 1.

The Healthy People 2010 Report defines the communication in public health as "the art and technique of informing, influencing and motivating individuals, institutions, general public on the important public health issues [6].

The term health communications was first used in 1961, when the U.S. National Health Council held a National Health Forum to discuss the challenges facing health intelligence communications [7]. The term was used again in 1962, when the surgeon Luther Terry organized a conference on health

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communications to discuss how different communication techniques can be used to make health information available to the public [8]. The term was adopted by members of an interest group at the International Communication Association (ICA) in 1975 [9].

Figure 1: Kotler's model.

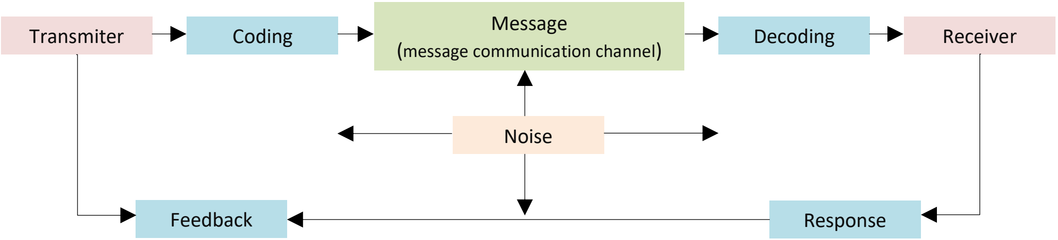

Source: Adaptation by Kotler, Ph., Principles of Marketing, III. Developing the Marketing Mix. 13. Integrated Marketing Communication Strategy, Pearson, Canada, pag. 546, accesibil online la: http://www.pearsoned.ca/highered/divisions/virtual_tours/kotler/kotler_ch13.pdf [5]

Health communication is a hybrid field that derives from communication, marketing, journalism and public relations studies and overlaps with health education and health promotion [10].

The health education is used to make people aware of the patterns of healthy behavior and its importance. The health education programmes related to behavioral change are considered to be a pillar of medical activity [11].

The health education is a social science that relies on medical, biological, physical, environmental and psychological sciences to prevent the occurrence of the diseases and promote health through behavior-changing activities based on the education.

Health campaigns for the prevention of the communicable diseases, for the promotion of maternal and child health, for the promotion of the immunisation, for education about family planning methods and other preventive health services have a long history [12].

The health education is linked to changes in people's feelings, knowledge and behaviour. It is the process carried out by actively involving people who aim to initiate healthy behavior and to know the prejudices and practices of people who are discouraged towards health in order to achieve the health goal [13].

The health communication is an extensive area that includes health education, risk communication, health and policy promotion, focus communication, patient and supplier communication and health literacy. Health communication strategies enable to the doctors and nurses, community health centres, hospitals and hospital administrators, nursing homes, health educators, non-profit organisations, government agencies and others to disseminate information that can positively influence personal health behaviors and choices.

Using informed marketing tactics and sociological data to lead to decision-making, the health communication is used to promote health campaigns in order to influence and educate the public on health issues [14].

The success of the health communication or health education programmes depends on people's interest. It is important for them to be actively involved, the change cannot be imposed, people must be motivated to adopt healthy behavior [15].

Communication related to the behavior change can take different forms, to appeal at the individual or group level, in order to change behavior towards a specific health problem. Health communication includes interpersonal communications, such as individual meetings between a healthcare provider and the patient [16], as well as community-focused communications such as public meetings, advertisements in local newspapers, the distribution of brochures and leaflets or educational events.

The media plays a key role in health communication, serving as a channel of health information from the government and specialized health organizations to the public. With their extensive coverage through radio and television commercials, the media can persuade the general public to adopt new healthy behaviors and recall information on preventive health care, disease prevention and treatment, immunizations and more.

The Internet is now disseminating information on an unprecedented scale, but at the same time, false and unsafe information has become more common than ever, posing risks to the health of uninformed users. This situation can cause an unjustified panic among those who receive the messages and can be a problem as technology continues to move forward. Therefore, public health providers must communicate the risks to the public in a responsible and transparent way and identify and eliminate misleading notions [17].

The decision to choose a particular communication channel must be based first on the research of the target audience and then on its usefulness and coverage. Thus, some technologies are not particularly effective when used by a small number of people or are too complicated to be operated by ordinary people.

Health communication issues have become a major topic of large-scale research programs sponsored by various foundations, health care corporations and government agencies.

Communication to change behavior is based on research and involves interaction with individuals and communities and personalizes messages that meet the needs of a particular target audience. Messages are then disseminated through different communication channels to bring about behavioral changes in specific problems and achieve the desired positive results.

The message must have an impact and it should disseminate appropriate health content defined by [6] :

- accuracy (the content is valid and without errors of interpretation or judgment);

- availability (the content must be placed where the public can access it);

- balance (to present both the benefits and risks of potential actions);

- consistency (remains consistent over time and it is consistent with information from another source);

- cultural competence (it must take into account ethnic, educational, linguistic issues); - relevant scientific evidence;

- coverage (to be available to the largest possible number of people in the target population);

- reliability (the source of the content is credible and the content is up-to-date);

- repetition (the delivery of the message must be continuous and repeated over time).

The development and management of social and behavioral change programmes involves the building of the partnerships at the national and local level, using new technologies and techniques of social and behavioral change and the incorporating of the social and behavioural change activities into programming the provision of services.

The utilisation of the new communication technologies for processing and dissemination of the health information has given rise to a new field, called health informatics. As information technologies advance, there are a variety of the new tools and information media for disseminating and accessing health information.

The access to relevant and in useful time at health information can help consumers participate in decision-making and encourage cooperation and collaboration between providers and consumers.

The health communication can help solve ways in which consumers and providers can most profitably inform themselves about relevant health problems, identify the best ways to develop and present high-quality health information to the target audience, and encourage decision-making in modern efforts to adopt healthy behaviors.

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VARIA

Planning a hospital to respond to the COVID-19 outbreak: experience from a Romanian reference unit

Gabriela Hofer[1], Dodu G. Petrescu[1], Victor L. Purcărea[1]

Abstract: *During the COVID outbreak, many hospitals were nominated as a support hospital for the treatment of positive tested patients infected with SARS CoV-2 virus. "Dr. Nicolae Ruşdea" Pneumophtiziology Hospital Baia Mare carried out an exceptional activity compared to the previous year considering the current epidemiological context. Major changes were made in strategies, procedures and protocols. The staff had to adapt to the new epidemiological situation through dedication and devotion. In 2020, there were no COVID outbreaks within the unit, as separate compartments were made, intended to protect the medical personnel, but also to ensure the health of the patients.*

Keywords: *COVID plan, hospital, Romanian reference unit*

INTRODUCTION

"Dr. Nicolae Ruşdea" Pneumophtiziology Hospital Baia Mare is a specialized hospital providing medical services in the specialties of pneumology for adults and children. Starting with April 2020, it provides hospital care for patients tested positive for the SARS-CoV-2 virus, including intensive care. In 2020, the hospital served the entire county of Maramureș for patients tested positive for SARS-CoV-2 virus. In the intensive care department, patients from Maramureș County received health care, mainly from the country, depending on the places available at the request of the Committee for Emergency Situations. Throughout this period, the fight against pulmonary tuberculosis continued through the national control and surveillance program as well as ensuring the continuity of integrated ambulatory care services for patients with non-Covid pneumological diseases and the follow-up of post-Covid patients. Compared to the previous year, considering the current epidemiological context, which led to major changes in the way of working, procedures and protocols. In order to provide the resilience of health systems during the COVID-19 pandemic, we describe, at the hospital level, the key elements to provide the medical staff sand patients safety, including the challenges and issues to anticipate following the experience of our center

CLINICAL EXAMPLE

The aim was to establish synergies between health worker safety and patient safety policies and strategies, developing linkages between occupational health and safety, patient safety, quality improvement, infection prevention and control programmes [1]. It was necessary to review and upgrade the standards, guidelines and codes of practice in order to assure the health worker and patient safety [1]. The executive board consisting of a multidisciplinary team: general manager, epidemiologist and the technical-administrative staff, planned the separation of the hospitals' circuits in COVID and NON-COVID. During the pandemic, the hospital changed his usual structure, consisting of 2 departments of medical specialties (pneumology, intern medicine, cardiology, urology, metabolic diseases,

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neurology), one section of surgical specialties, one pediatric department, a department ICU (intensive care unit), blood transfusion units, palliative care and an operating room. The rest of the structure was maintained: farmacy, sterilization, medical analysis laboratory, radiology department, tuberculosis dispensary, reception desk, evaluation and medical statistics department (Figure 1).

Figure 1: Plan for new structure of the hospital to augment the health system during the pandemic.

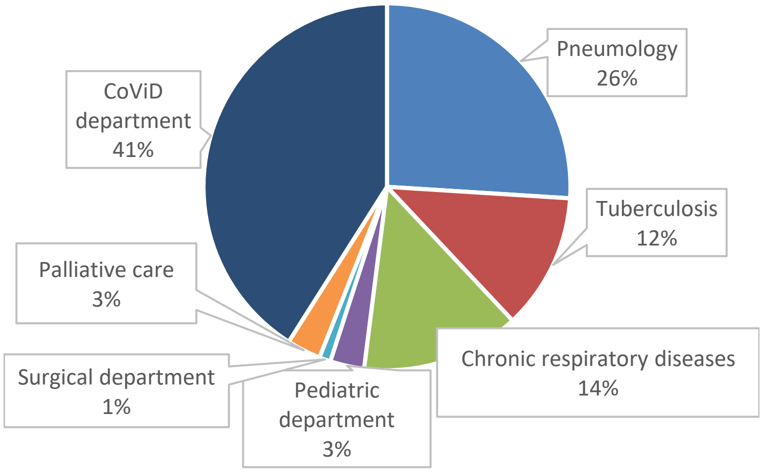

After the State of emergency in the coronavirus pandemic, depending on the local epidemiological evolution, the hospitalizations and the scheduled surgical interventions can be resumed, as well as the activity in the outpatient clinics. Being a 4-storey Hospital, a level was created for the green zone, non-COVID, and the 2nd and 3rd floor were allocated for the COVID section - red zone, served by an elevator: at each level a separate filter for dressing and undressing the protective equipment; the 4 elevators and 6 exits of the hospital had separate destinations: a red zone entrance, one for staff, one for drugs and one for food. A special elevator for patients, one for the staff and the elevators on the opposite side of the building are used for waste and medical staff. A separate circuit was intended for the ICU section, and a separate one for the rest of the staff. In the green area, the staff was separated, depending on the section, so as not to be conglomerated by people. Radiology department: provides a separate circuit for non-COVID patients hospitalized, and a dedicated department for tuberculosis dispensaries. Also a sequential circuit for computer tomography access. The organization of the COVID departments provides a separate circuit for access to the Covid patients section with a dedicated elevator only for COVID-positive patients. The medical personnel circuit with clothing filter area, decontamination area, green area for rest and document preparation (common for COVID and ATI section). The contaminated waste had a special circuit - collection and transport on a circuit intended only for waste (all waste generated in the COVID section are considered hazardous waste). The food circuit, consisting in disposable dishes, are collected as infectious medical waste. Assistance to acute and chronic non-COVID pneumology patients was assured. When entering the hospital, all people will go through an epidemiological triage, depending on the symptoms presented, the suspicious cases will be hospitalized in special isolation areas (buffer-zones), organized at the 1st floor, to be tested by RT-PCR for SARS-CoV-2 infection (according to the recommendations of the National Institute of Public Health). Separate circuits are provided for these patients.

DISCUSSIONS

With all the circuits, red-zone and green-zone, the hardest part was teaching the staff to overcome fear, but the idea that you have some completely separate safe circuits made the staff trustful and work, and accept the new rules imposed. Moreover, having 4 training teams that teaches the use of the protective equipment, the existence of a supervisor in the first 3 months to monitor the dressing and undressing the equipment, in order to avoid mistakes. As a result, there was no outbreak in the hospital for 12 months, with only isolated cases coming from outside of the hospital.

CONCLUSIONS

The coronavirus disease (COVID-19) outbreak is a global public health problem. Medical personal is an important at-risk population that can get COVID-19 due to close contact with patients2. The healthcare reform, with subsequent amendments and additional changes, caused a new reorganization and restructuring of health units, depending on the needs, in order to ensure the isolation conditions and the functional circuits that aim at the prevention and control of the SARS-COV-2 infection3,4. The reorganization and restructuring of the health units subordinated to the local public administration authorities will be done with their consultation. The reason for these changes is mostly because of the epidemiological evolution of the SARS-COV-2 virus pandemic. In addition, there is an increased addressability of patients with pneumological problems including patients with pulmonary diseases after SARS-COV-2 infection that need recovery. Also, the need to carry out the activities of the tuberculosis prevention and control programs.

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VARIA

Copper and its role in the human body – the importance of establishing copper concentrations in the body

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Abstract: *Copper is essential to all living beings, as a food mineral, being an essential component of the cytochromoxidase enzymes complex. It is also an essential component of several systems involved in the production of hemoglobin, of sugar metabolism, of catecholamines' synthesis and in the formation of cross-links between collagen, elastin and keratin fibers within the hair. The dysregulation of copper through impaired absorption or excretion, either genetically based or in acute or chronic poisoning, results in deficiency or toxicity. For this reason, the measurement of copper levels in various biological samples: urine, blood, or hair, provides the physician with information regarding contamination, poisoning, or a metabolic disease. Due to low concentrations in most heavy metals' and metalloids' cases, finding traces in biological samples is a difficult problem. Classical methods (staining, flame photometry, reagent kits, electrochemical methods) can only be used for a limited number of elements, such as sodium, potassium, calcium, magnesium, copper, iron and zinc. The analysis of biological samples is the most important use of graphite furnace atomization. The advantages of using this technique are its great sensitivity and the very low quantity of sample necessary for performing the measurement.*

In modern medicine, determining microelements is very important in diagnosing diseases and/or in treating them. The GF-AAS method is quick, reproductible and has very good sensitivity.

Keywords: *copper, toxicity, metabolism disorders, Wilson disease, determination methods, spectrophotometry, GF-AAS*

INTRODUCTION

A great number of chemical elements in nature are present in the body, either as standalone components, or bound one to another, forming organic and inorganic substances that facilitate or create various disorders in the human body. A part of them (Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, Sr and V) are essential to life.

Other elements (As, Ba, Be, Cd, Li, Hg, Pb, Sb, Sn) are considered toxic, and their concentrations in any given biological product is preferred to be close to nil. When such concentration is different from zero, the value is considered normal if it is lower than the limit established by toxicology authorities around the world.

Copper is a chemical element that has been used for thousands of years in alloyes. Its compounds are frequently found as copper (II) salts, which usually yield the blue or green colours of minerals, such as azurite or turquoiuse, and have been largely used as pigments. Copper sulfate forms are bright blue crystals containing five molecules of water [$CuSO_4 \cdot 5H_2O$]. It is commonly known as the "Blue Vitriol" or "Blue Stone" [1] and it is used mainly for agricultural purposes as a pesticide, and in the leather industry [2, 3].

Biological Roles of Copper

Copper is essential to all living beings, as a food mineral, as

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it is an essential component of the cytochromoxidase enzymes' complex. It is found in the liver in vertebrates. In high concentrations, copper compounds are toxic for evolved organisms, and are used as bacteriostatics and fungicides.

Copper alloys have intrinsical natural properties that kill a large number of microorganisms, such as E. coli O157: H7, methicillin-resistant Staphylococcus aureus (MRSA), Clostridium difficile, influenza virus A, adenovirus, fungi [4, 5]. Some copper 355 alloys have been shown to kill more than 99.9% of germs within two hours, when properly cleaned [4]. The United States Environment Protection Agency (EPA) has authorized these copper alloys as „antimicrobial materials with public health benefits" [4]. Recent biotechnological researches use copper ions coupled with indocyanine and proteins in targeted cancer treatments [6].

Proteins which contain copper have several biological roles in electron and oxygen transport, processes in which interconversion of Cu (I) and Cu (II) easily takes place [7]. The biological role of copper began the moment oxygen appeared in our planet's atmosphere [8].

In the cytochrome-c oxidase, which is necessary for aerobic respiration, copper and iron work together to reduce oxigen. Copper is found in superoxide-dismutase, which catalyzes the breakdown of superoxides, through conversion to oxygen peroxide and hydrogen:

$$2\ HO_2 \rightarrow H_2O_2 + O_2$$

Copper is an essential component of several systems involved in the production of hemoglobin, in sugar metabolism, in catecholamine biosynthesis, and in forming cross-links between collagen, elastin and keratin fibers within hair.

Copper deficits leads to growth and behavioural disorders, especially in children [9]. Copper deficiency is an etiology of anemia, neutropenia, and bone marrow dysplasia that may be under-recognized, being a possible cause of medical errors in clinical practice [10, 11]. The mechanism of anemia in copper deficiency may be related to the role of copper-dependent enzymes, such as ceruloplasmin and cytochrome-c oxidase, in iron metabolism and transportation [12]. Bone marrow findings of marked vacuolization of both erythroid and myeloid precursors have been consistently reported, and ring sideroblasts are also occasionally reported [13].

Kinetics of Copper

Copper intake is mainly achieved through diet : it is easily absorbed, but its absorbtion is limited by homeostatic mechanisms, after requirements have been met. The human body contains aproximately 1.4-2.1 mg/kg of copper [14]. Copper is absorbed in the gut, then transported to the liver bound to albumin [15]. After being processed in the liver, it is then distributed to other tissues. Copper transportation involves a protein called ceruloplasmin, which also transports copper excreted in milk [16]. The body is able to excrete excessive levels of copper, if necessary, through the bile. The excreted excess is not reabsorbed by the intestine [17, 18]. In catalytic oxidation processes, metalloproteins containing copper are also necessary, as they intervene in oxygen metabolism. Also, copper also acts in oxidative phosphorylation reactions. Copper intake from food can also prevent certain diseases or deficiencies, such as allergies, hair loss, AIDS, leukemie, osteoporosis and gastric ulcer. Together with iron, copper helps in red blood cells' synthesis.

Copper metabolism disorders

The dysregulation of copper through impaired absorption or excretion results in deficiency or toxicity, as illustrated by two rare genetically based diseases.

Wilson's disease was first described in 1912 by Kinnear Wilson as "progressive lenticular degeneration". Wilson's disease can manifest itself at birth, but signs and symptoms only appear when copper accumulates in the brain, liver, or another organ. The signs and symptoms vary with the body part that is affected by the disease and can include fatigue, loss of appetite or abdominal pain, jaundice, brown-golden Kayser-Fleischer rings, muscular stiffness, lack of motor coordination, speech impairment and others.

Menkes' disease, when the body cannot stock copper, leading to its deficiency; it is also known and the uncombable hair syndrome (hair lacks in color, is sparse, wiry and rough, with occasional nodosities) or Menkes' kynky hair disease. It is a rare hereditary X-linked transmitted disease, described by Menkes in 1962 as a severe degenerative disease of the nervous system. Biologically, serum copper and ceruloplasmin levels markedly decrease. Some of the clinical manifestations pertaining to this disease are facial dysmorphia, signs of neuropsychic involvement, hypothermia, skeletal manifestations similar to those in scurvy, and the irregular lumen of blood vessels.

Clinical signs in copper intoxication

In acute intoxication, serum copper reaches very high values, ceruloplasmin and hepatic copper are within normal limits, and urine copper is increased.

In chronic intoxication, serum, urinary copper and ceruloplasmin are increased, and hepatic copper reaches very high values. The acute toxicity of copper in humans is likely due to the generation of reactive oxygen species, which deteriorate DNA [10]. A minimal dietary value of at least 3 ppm was reported for healthy growth in rabbits [20]. However, high concentrations of copper (100 ppm, 200 ppm, sau 500 ppm) in their diets can favorably alter their growth rates [21].

Copper toxicity is determined by the balance between copper intake and excretion. Voluntary copper salts intake, copper intake from food and beverages, inhaling dust which contains copper or aerosols which contain copper salts, through manual spraying of insecticides or fungicides, represent the main risks of exposing the human body to copper contamination.

The main actions of copper on the human body are gastrointestinal irritation, liver and renal disorders, intravascular hemolysis and shock. Acute intoxication following ingestion of copper salts targets the GI tract, cardiovascular and circulatory systems, hematopoietic system, liver, kidneys and nervous system.

The respiratory effects following inhalation are: acute exposure to copper vapors targets the respiratory pathways and chronic exposure to copper salts, seen in those who spray plants, targets the lungs.

The local, irritating effects, through cutaneous or eye contact with copper salts, are seen at skin and eye level.

Other diseases in which abnormalities in copper metabolism appear to be involved include Indian childhood cirrhosis (ICC), endemic Tyrolean copper toxicosis (ETIC), and idiopathic copper toxicosis (ICT), also known as non-Indian childhood cirrhosis. ICT is a genetic disease recognized in the early twentieth century primarily in the Tyrolean region of Austria and in the Pune region of India [22].

DETERMINING COPPER CONCENTRATIONS IN BIOLOGICAL PRODUCTS

Due to low concentrations in most heavy metals' and metalloids' cases, finding traces in biological samples is a difficult problem. Classical methods (staining, flame photometry, reagent kits, electrochemical methods) can only be used for a limited number of elements, such as sodium, potassium, calcium, magnesium, copper, iron and zinc.

Modern methods replace these types of tests. Elements can be determined through atomic absorbtion spectrometry AAS with flame atomization FAAS or graphite furnace atomization GTAAS, inductively coupled plasma and atomic emission spectrometry ICP-AES or inductively coupled plasma and mass spectrometry ICP-MS. ICP-MS is the most sensitive method for determining metals and metalloids, but it also has the highest cost. For determining metals and metalloids in biological samples, the GTAAS method is recommended. For Na+, K+, Cl-, Ca2+ it is much more cost effective to use machines which only measure blood or urine concentrations of these ions, through electrochemical methods.

The analytical method used to determine the concentration of studied elements, from the analyzed matrices, is the atomic absorbtion spectrometry coupled with graphite furnace atomization GF-AAS (Graphite Furnace Atomiser – Atomic Absorbtion Spectrometry). Atomic absorbtion spectrometry is an analytical method used to quantitatively measure metals or metalloids in a sample, based on the quantity of light that is absorbed or emitted by atoms, from a line that is characteristic for the specter of light the atom emits in an excited state.

The first method that was used to bring atoms to an excited state was heat emanated by a flame. The temperature of this flame is approximately 1200 – 13000C. Due to the low temperatures that can be achieved with this method, the sensitivity of this type of atomization is not useful in determining the majority of elements in biological samples.

Graphite furnace atomization has become very useful in analytical chemistry, as a technique which is very good for determining trace elements in a wide variety of sample types. The principle of this method, of achieving a controlled temperature, using electrical current, makes it perfectly reproductible, compared to flame atomization. The greatest advantage, however, consists in the high atomization temperatures, which can never be attained with a flame, which makes analyzing trace elements in biological samples possible.

The number of replicates (repeatedly performing the same determination) is very important for obtaining quality results. Thus, the working program allow real-time quality control of obtained results, by verifying the standard deviation of replicates. If the value of the square mean deviation (standard deviation) of values measured in the same sample exceeds the pre-established value, the system performs the determination once more. The usually admitted standard deviation is 4-5%. The increase of replicates' number increases the duration of a given determination, wears down the graphite furnace, but improves the accuracy of the results. Due to the marked stability of the machine, it is possible to use the minimum number of two replicates.

The absorbance signal has its own noise due to the variations in emitted light, due to its transmission through the optical chain, due to variations caused by the photomultiplier and the electronic system which amplifies the electric signal. This type of noise can be removed by mathematical methods. The methods which may be applied are either in 3, 5, or 9 points. The calibration method is extremely important for the accuracy of the results. For this reason, choosing an optimal calibration method is one of the most important decisions in establishing the method. For standard calibration, the software automatically chooses the liniar calibration. The calibration curbe may or may not pass through point zero. When measured levels are very low, this curve must pass through point zero. Quality control is mandatory to establish the accuracy of the obtained results. Quality control also involves checking: the standard deviation of replicates; the correlation coefficient of the calibration curve; the detection limit of the method and the apparatus.

Table 1: Concentrations of some essential trace elements in blood and urine (mg/l).

Element	Serum (μg/L)	Blood (μg/L)	Urine (μg/L)
Chromium	0.5	0.5	10
Cobalt	0.2	2.0	100
Copper	1100	1200	60
Iron	1200	-	180
Magnesium	0.5	5	300
Molibdenum	1.0	100	100
Nickel	5.0	5.0	85
Selenium	100	100	30
Strontium	50	-	150
Vanadium	0.5	4.0	16

In urine spot samples, there was a great variation in copper content, which is why collecting 24h urine and sampling from the total volume is recommended. The conventional level taken as diagnostic of Wilson's Disease is >100 mg/24 hours in symptomatic patients [23].

A method to determine the concentration of an element in the matrix involves establishing the working conditions of the spectrometer, of the graphite furnace atomizer, of the sample dispenser and the conditions for the automatic quality control of the test.

The calibration curves of the elements, absorbance as a function of concentration, are calculated with the following equation:

$$A/C = a + b * A + cA^2$$

where: A – is the sample absorbance, C – is the sample concentration, A, b, c, d – are coefficients of the calibration curve.

The determination of copper in biological samples is usually carried out for diagnosing Menkes or Wilson disease or for monitoring the distribution and the amount of copper removed during dialysis procedures of patients with chronic renal disease [24, 25].

In order to develop a method for determining direct GF-AAS copper in biological samples (blood and urine) the establishment of operating parameters for atomic absorption spectrometer system – AAS 880 Spectra Varian and for graphite tube atomizer – GTA 100 is of major importance.

The utilized wavelength must be a part of the absorbtion specter of copper and depends on the type of sample. It may be 327.4 nm or as alternative wavelengths, 324.8 nm or 217.9 nm or 218.2 nm. The correct choice is made with the purpose of eliminating the non-atomic absorbtion which may appear during sample analysis. The width of the used diaphragm is of 0.2 or 0.5 nm and its size depends on the type of analyzed sample.

Method for determining the level of copper in whole blood samples

The samples were analyzed with a system formed from the following components:

- Atomic absorbtion spectrometer AAS-880.
- Graphite tube atomizor GTA-100.
- Programmable sample dispenser – PSD.
- Water cooler – Neslab CFT 22.
- Nitrogen generator - Dominick Hunter.
- Gas cylinder (Argon) 99.9999% purity

Optimized working parameters for determining copper levels in whole blood

In order to determine the Cu concentration in the blood samples taken from patients (2 ml of venous blood, taken on anticoagulants), the following method was used:

- Pipetting method – automixing;
- Quantity of injected sample – 10 μl;
- Measurement method – peak height;
- Concentration calibration;
- Smoothing – 9 points;
- Number of replicates - 2 for standard and for sample;
- Cathode tube current – 4 mĂ;

- Wavelength – 327.4 nm;
- Slit width - 0.5 nm;
- Background correction – deuterium lamp;
- Drying – 1200C;
- Calcination - 8000C;
- Atomization - 23000C;
- Cleaning - 25000C;
- Three-point calibration curve – 0, 50 and 100 µg/l;
- Recalibration rate – 15;
- Method detection limit <1.0 µg/l.
- Apparatus detection limit <0.04 µg/l.

Automatic quality control – dispersion <10 %; correlation coefficient of calibration curve >0.998.

The optimization of the temperature program must provide optimal conditions for the measurement of Cu signals by GF-AAS, free of interferences from the background which is a very common problem in the analysis of biological samples (26). The application of the optimized temperature program made possible to eliminate the whole matrix of the sample before the atomization step, as confirmed by the low background signals observed in the measurement of Cu. No chemical modifiers were added. However, biological samples must undergo a deproteinization process, which is meant to eliminate non-atomic absorbance.

Table 2: Established and optimized steps for GTA 100

Step	Temp C	Time s	Flow L/min	Gas Type	Read	Signal Storage
1	40	5	3	N_2	No	No
2	80	10	3	N_2	No	No
3	105	5	3	Ar	No	No
4	105	10	3	Ar	No	No
5	800	20	3	Ar	No	No
6	800	5	3	Ar	No	No
7	800	2	0	Ar	No	Yes
8	2650	0.9	0	Ar	Yes	Yes
9	2650	2	0	Ar	Yes	Yes
10	2650	3	3	Ar	No	Yes
11	40	30	3	N_2	No	No
12	40	5	0	N_2	No	No

Copper stock standard solution containing 1000 mg/L copper was diluted with a premixed solution of distilled water and analytical grade concentrated nitric acid to provide working standards of various concentrations in 0,1% (w/v) HNO3. The calibration blank solution used throughout was a 0.1% w/v HNO3 solution. For copper, the working standards were 100 and 50 µg/L.

Table 3: Performance parameters of linear regression analysis

Conc. ug/L	Abs	Abs. Average	STDEV	RSD %
0	0.0193	0.0183	0.0014	7.55
0	0.0167			
0	0.0188			
50	0.3996	0.4001	0.0009	0.23
50	0.4012			
50	0.3996			
100	0.7214	0.7178	0.0034	0.47
100	0.7147			
100	0.7174			

The whole blood sample (200 µL) was treated with 800 µL solution 5% antifoam B (Sigma) and with 1000 µL of 1.6 M HNO3 solution. The mixture was maintained for 20 minutes and centrifuged with 5000 rpm for 5 min. The supernatant was analyzed by GF-AAS. The results are obtained in µg/dL. A linear relationship was found between the absorbance at 327.4 nm and the concentration of cooper in the range of 0.0 to 100 µg/L. The representative linear equation was y = 0,007x + 0,029 where: y is the absorbance, x is lead concentration (µg/L), calculated by the least squares method. The regression coefficient (r) standard curve was 0.9972, indicating good linearity (r > 0.99).

The performance parameters of linear regression equation are presented in Table 3. The parameters of the GF-AAS analysis method of lead are presented in Table 4. LOD and LOQ were calculated based on the standard deviation and the slope of the regression line.

Table 4: Validation parameters

Standard error	0.0173
R Square	0.9972
Intercept	0.0290
Slope	0.0070
SE of intercept	0.0091
LOD (µg/L)	***4.29***
LOQ (µg/L)	***13.01***

LOD is the lowest concentration of an analyte that can be detected while LOQ is defined as the lowest concentration of an analyte that can be determined at the acceptable level of precision and accuracy and were calculated according to the formula below: LOD = 3,3*(SD of intercept/Slope) and LOQ = 10*(SD of intercept/Slope)

The established method can be easily used to perform copper measurements in the laboratory, both in urine and blood samples taken from patients suspected for copper intoxication. Copper deficiency and toxicity can be either of genetic or non-genetic origin. The study of copper's genetic diseases, which are the focus of intense international research activity, has shed insight into how human bodies use copper, and why it is important as an essential micronutrient.

The evolution of the copper concentration in urine or whole blood provides very useful information for poisoning diagnostic when the history is not clear [27, 28].

CONCLUSIONS

Microelements play an important role in the normal course of life. The variation of their blood levels within the body can lead to metabolic disturbances. For example, copper deficiency is found in Menkes syndrome or ,,uncombable hair disease”, and copper excess leads to Wilson’s disease. The rise in blood levels of copper can also be due to acute intoxication, for example, as a result of spraying plants with copper sulphate.

For these reasons, measuring Cu concentrations in various biological samples, urine, blood, or hair, provides the physician with information regarding contamination, intoxication or the existence of a metabolic disease.

The analysis of biological samples is the most important use of graphite furnace atomization. The advantages of this technique are its high sensitivity and the very low quantity of sample necessary for the measurement.

In modern medicine, determining trace elements is very important in diagnosing various diseases and/or in treating them. The GF-AAS is a quick, reproductible and highly sensitive method.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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The article was received on January 26, 2021, and accepted for publishing on May 05, 2021.

REVIEW

Manipulation of the gut microbiome may be a promising pathogenic approach in rheumatic inflammatory diseases – A review

Daniela Anghel[1,2], Oana G. Petrache[1], Mădălina E. Hoinoiu[1], Florentina Ioniţă Radu[1,3], Raluca S. Costache[1,3]

Abstract: *A symbiotic association between humans and microbes has been established over time. Disruptions in gut homeostasis have been linked to immune-mediated inflammatory diseases.*

Factors such as genetics, environment, diet, age, and medication play a major role in shaping the intestinal microbiome. An altered gut microbiome seems to modulate the progression and severity of inflammatory rheumatic diseases. Disruptions in the eubiotic state of the intestinal microbiome might promote intestinal and extraintestinal autoimmune and inflammatory disorders, although the mechanisms involved are not well understood.

In this review, we provide a general overview of several studies concerning intestinal microbiome perturbations and their implications in rheumatoid arthritis, ankylosing spondylitis, and systemic lupus erythematosus. Furthermore, we discuss the relationship between diet, intestinal microbiota, and inflammation.

Keywords: *intestinal microbiome, intestinal dysbiosis, probiotics, rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus*

INTRODUCTION

The human digestive tract is inhabited by somewhere between 300 and 1000 different species of bacteria. Microorganisms like bacteria, yeast, and viruses compose the gut microbiota. Bacteria are classified into phyla, classes, orders, families, genera, and species. [1]

There are four dominant bacterial phyla: Bacteroides spp., Firmicutes spp., Actinobacteria spp., and Proteobacteria spp. The Firmicutes phylum contains more than 200 genera such as Lactobacillus spp., Bacillus spp., Clostridium spp. (95% of the Firmicutes), Enterococcus spp., and Ruminicoccus spp. The Bacteroidetes phylum consists of predominant genera such as Bacteroides and Prevotella. Actinobacteria phylum is less abundant and represented by the Bifidobacterium genus. [2]

The relationship between humans and gut flora is a symbiotic one. The microbial colonization occurs before birth and stabilizes around the age of three. The intestinal microbiome has various roles: fermentation and degradation of dietary fibers, modulation of antibody production, defense against pathogenic bacteria colonization, production of vitamins, and maintenance of intestinal epithelium integrity.

The most important end products of microbial fermentation processes are short-chain fatty acids (SCFA), acetate, propionate, and butyrate. These molecules promote the immunity of the intestinal mucosa and protect it from pathogens. [3]

Only 1% of bacteria can be grown in cultures. New

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techniques like the extraction of DNA and the amplification of the 16S ribosomal RNA gene (rRNA) allowed researchers to analyze nucleic acids (DNA and RNA) from the stool. The Human Microbiome Project (HMP) focused on identifying, studying, and cataloging uncultivated bacteria.

The intestinal microbiome is influenced by diet and the environment. People who eat more carbohydrates and simple sugars have more Prevotella, while Bacteroides are associated with saturated fats and proteins. Plant-based diets are associated with a greater abundance of Prevotella and a greater diversity of microbes [4, 5].

It is believed that high-fiber diets ameliorate autoimmune, metabolic, and allergic diseases mainly through their microbiota dependent fermentation to SCFAs that promote tissue barrier integrity, mucus production, immune serum globulin (IgA) secretion, and regulatory lymphocyte T (Treg) differentiation, sustaining an anti-inflammatory environment [6].

Some microbes, such as Bacteroides fragilis, can drive the immune system to create anti-inflammatory cytokines while some segmented filamentous bacteria favor the production of pro-inflammatory cytokines. Bacteroides promote the production of immuno-regulatory T cells that suppress immune responses to self and bacterial antigens and stimulate epithelial repair. [7]

Manipulation of the intestinal microbiota through diet and probiotics may be the target for future strategies and preventive interventions in rheumatic diseases.

METHODS

Relevant literature relating to the gut microbiome and autoimmune diseases was selected through searching online medical databases. Search terms used were: “intestinal microbiome”, “gut microbiota”, “intestinal dysbiosis”, “manipulation of the gut microbiome”, “rheumatic diseases and intestinal microbiome”, “probiotics”. Only English-written studies published from 2011 to date were selected and analyzed.

RHEUMATOID ARTHRITIS (RA)

Rheumatoid arthritis is a chronic, autoimmune disease that primarily affects joints and the first study that links intestinal microbiome to arthritis was published in the late 1970s, suggesting that bacterial flora may be a stimulus for induction of cell-mediated immunity and may also have suppressive effects on the disease evolution. [8]

The patient microbiome plays a big role in the progression of rheumatoid arthritis. Together with the lymphoid tissue, the microbiome helps maintain immune homeostasis. Changes at this level can damage the intestinal mucosa and the systemic immune system, triggering the onset of autoimmune diseases.

Using zonulin as a serological marker, some recent studies have demonstrated the increased gut permeability in rheumatoid arthritis patients. This finding has been shown in about 1/3 of pre-RA patients. [9]

In rheumatoid arthritis, studies have shown that patients present a dysbiotic state, with the depletion of gram-negative bacteria and an increase of gram-positive bacteria. Furthermore, these patients present a higher concentration of Collinsella and Eggerthella lenta than normal individuals. [10]

As well, these patients have a high level of Prevotella copri and a low level of Bacteroides. P. copri pathogenicity was explained through its capacity of stimulating Th1 cells. Some patients produce antibodies against P. copri (IgA, IgG). Moreover, an over-abundance of P. copri has been identified in individuals at risk of rheumatoid arthritis (patients without arthritis but anti-citrullinated protein antibody – ACPA/ rheumatoid factor – RF positive). [11, 12, 13]

A study showed that a Prevotella strain, Prevotella histicola, suppresses arthritis in humanized HLA-B27 mice. [14] P. histicola treatment can reduce pain and improve motor function as a response to an increase in T regulatory cells and a decrease in IL-6 levels. [15]

Lactobacillus salivarius is more abundant in rheumatoid arthritis patients but its effects are unclear. Administration of L.salivarius in mice showed a significant improvement of collagen-induced arthritis and it may help reduce interleukin-17 and increase anti-inflammatory interleukin-10. Thus, it may help reduce clinical manifestations in RA patients. [16]

A study conducted on 60 women with rheumatoid arthritis, showed the beneficial role of Lactobacillus casei. Used as adjuvant therapy, it reduces the symptoms, lowers the pro-inflammatory cytokines, and lowers serum C - reactive protein and DAS28 score. [17]

A more recent study, conducted on P. histicola treated mice, showed a decrease in the incidence and severity of arthritis. P. histicola decreases the intestinal permeability and lowers the pro-inflammatory IL-17 cytokine and increases IL-10. [14] Because P. histicola is normally present in the gut, the treatment as a probiotic in humans is beneficial, with minimum adverse effects.

A study conducted in China found a greater abundance of

Enterobacteriaceae spp. and Klebsiella spp. and a lower abundance of Bifidobacterium spp. in rheumatoid arthritis patients with high levels of TNF-α or IL-17A. Moreover, ACPA-positive patients had a greater abundance of Blautia spp., Akkermansia spp. and Clostridiales spp. then patients with negative ACPA. In conclusion, patients with RF/ACPA seropositivity exhibited a different microbial composition than RF/ACPA negative patients, suggesting an association between intestinal microbiome alteration and disease activity. [18]

Porphyromonas gingivalis, a periodonthopathogenic bacteria that exist in dental plaque can be swallowed with saliva and can affect the composition of the gut microbiome. Several studies have shown a strong correlation between P. gingivalis infection and systemic disease. Intestinal dysbiosis by oral inoculation of P. gingivalis has been shown to contribute to the progression of arthritis. [19, 20]

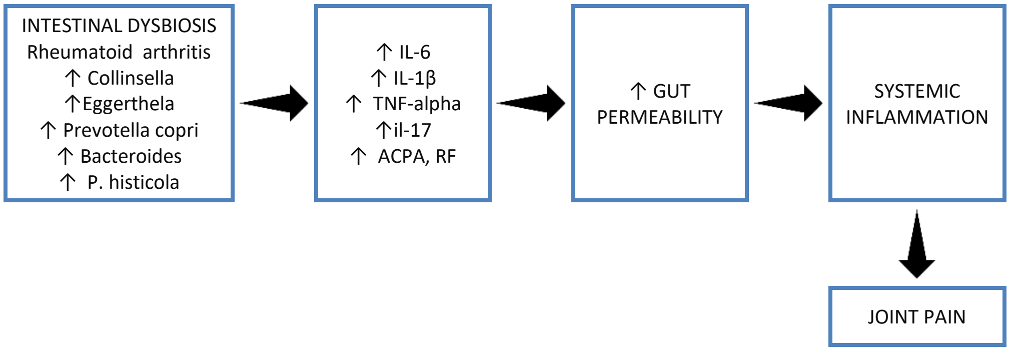

Figure 1: Associated microbiome in rheumatoid arthritis

Rheumatoid arthritis treatment and intestinal microbiota

The use of disease-modifying anti-rheumatic drugs (DMARDs) can modulate the intestinal microbiome and has the potential to shift the intestinal flora towards a healthy microbiome.

Two studies showed a significant decrease in Bacteroides fragilis and an increase in Prevotella maculosa after treatment with Methotrexate (MTX). Dysbiosis associated with RA was partially resolved after treatment. [21, 22] Another study suggests that patients that were treated with Methotrexate showed increased species richness and diversity compared to other patients receiving different treatments. [23] These studies suggest that MTX could correct intestinal dysbiosis.

Some studies concluded that pharmacological treatment in rheumatoid arthritis increased the Firmicutes and Bacteroidetes phyla. MTX induced a significant growth in Firmicutes over the Bacteroidetes that was associated with improved drug response and reduced inflammation. [24, 25] In a study conducted in 2018, the microbial profile of Etanercept treated patients (in monotherapy or combination with Methotrexate) was compared to normal individuals. There has been reported a significant decrease of Faecalibacterium compared to healthy individuals. In Etanercept treated patients there has been reported a great abundance of Cyanobacterium compared to patients without treatment.

Some species of Cyanobacterium produce some metabolites and bioactive substances that have cytotoxic properties and are involved in gastroenteritis. On the contrary, some metabolites have anti-inflammatory and immunosuppressive properties that are beneficial in rheumatoid arthritis. [26]

ANKYLOSING SPONDYLITIS (AS)

Ankylosing spondylitis is a chronic, autoimmune disease characterized by long-term inflammation of the sacroiliac joints and spine with or without HLA-B27. But the most important genetic factor in ankylosing spondylitis, HLA-B27, could change the intestinal microbiome, contributing to multisystemic inflammation and the production of IL-23 and other proinflammatory cytokines.

HLA-B27 positive patients had changes in Faecalibacterium prausnitzii, Bacteroides vulgares, and Akkermansia muciniphila. An abundance of Bacteroides vulgatus has been discovered in HLA-B27 rats, suggesting that HLA-B27 may alter the intestinal microbiome composition. [27]

In ankylosing spondylitis, 87% of the patients have an altered microbiome (dysbiosis). Their microbiome was characterized by an abundance of species of Proteobacteria spp., Enterobacteriaceae spp., Bacilli spp., Streptococcus spp., Actinobacteria spp. and a low number of Bacteroides and Lachnospiraceae. [28] Dysbiosis is also associated with disease activity.

Akkermansia muciniphila, of the phylum Verrucomicrobia spp., resides in the mucus layer of the large intestine. It is implicated in maintaining intestinal integrity and can cause the degradation of the mucin in the gut epithelium. [29]

Prevotella can increase Th17-mediated immune response in the intestinal mucosa. Increased Prevotella abundance stimulates the production of IL-1, IL-6, IL-8 and also stimulates neutrophil recruitment. The mucosal inflammation caused by Prevotella leads to systemic dissemination of inflammatory mediators and promotes chronic inflammation. [30]

Some microbes, such as Faecalibacterium prausnitzii, administered to mice, have been found to protect them against induced intestinal inflammation, thus concluding that F. prausnitzii has anti-inflammatory effects. [31] A decreased abundance of F. prausnitzii was observed in fecal samples of patients with ankylosing spondylitis. [28, 32]

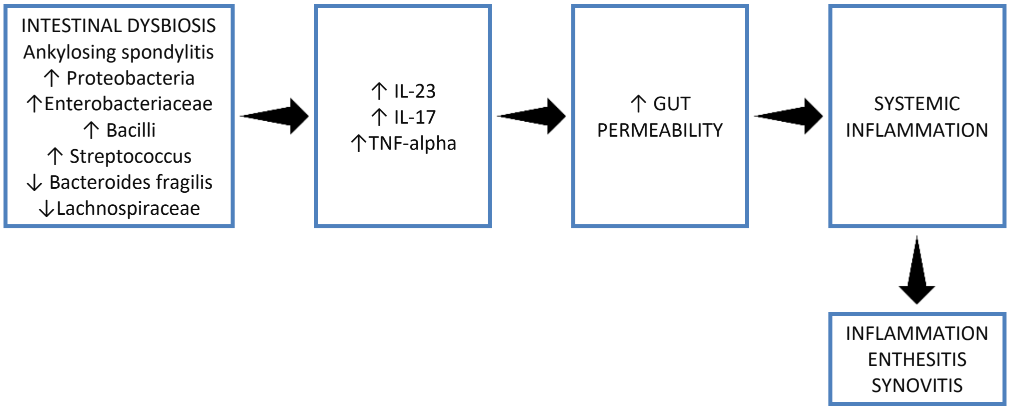

Figure 2: Associated microbiome in ankylosing spondylitis

Ankylosing spondylitis treatment and intestinal microbiota

Patients with ankylosing spondylitis treated with biological treatment have shown a greater abundance in Proteobacteria spp. (especially the Enterobacteriaceae family) and Veillonella genus compared to patients without treatment. [33]

A perturbation of the intestinal fungal microbiome (mycobiome) has been observed in patients with ankylosing spondylitis after biological treatment. An increase in the abundance of Saccharomycetales was found after anti-TNF and anti-IL17 treatment. [34]

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Systemic lupus erythematosus is an autoimmune disease that is characterized by severe inflammation that can affect almost all organs of the body. Lopez et al. observed the influence of the microbiota over T lymphocyte differentiation in healthy and SLE groups. These authors found a rise in lymphocyte activation and differentiation toward Th17 in SLE patients. [35]

There were some studies on SLE patients which demonstrated that the abundance of Firmicutes is decreased and that of Bacteroidetes is increased compared to healthy control groups. If the intestinal epithelial barrier is damaged, Enterococcus gallinarum and Lactobacillus reuteri, possible gut pathobionts, can translocate into the systemic tissue, inducing autoantibody production. In addition, Ruminococcus gnavus can trigger an anti-double-stranded DNA antibody response. [36, 37]

A study conducted in Spain has proved that there is a gut microbial dysbiosis related to SLE in Caucasian female patients. SLE patients developed intestinal dysbiosis with a decrease within the Firmicutes spp. and a rise in the Bacteroides spp. It also showed that SLE patients with inactive lupus disease had significantly lower Firmicutes/ Bacteroidetes ratios than healthy control individuals did. [38]

A study conducted by Van der Meulen et al also showed that in SLE patients, the intestinal microbiota composition is characterized by lower bacterial richness, lower Firmicutes/ Bacteroidetes ratio, and a greater abundance of Bacteroides species. [39]

Similarly, a study by Greiling et al concluded that the Firmicutes/Bacteroidetes ratio in SLE patients decreased compared to healthy controls. In addition, commensal bacteria which contain Ro60 orthologs could induce the production of autoantibodies that cross-react with human Ro60, therefore triggering autoimmune diseases like SLE. [40, 41]

On the contrary, a study concluded that Firmicutes/

Bacteroidetes ratio is not significantly different between SLE patients and healthy controls. Also, this study concluded that Gram-negative bacteria, like Proteobacteria spp. and Blautia spp. were increased and Odoribacter spp. was decreased in SLE patients. [42]

A study explored whether the alteration of the intestinal microbiome in SLE patients from China was according to the intestinal dysbiosis of SLE patients from Spain. The depletion of Firmicutes spp. and the abundance of Bacteroidetes spp. in SLE patients from China was in keeping with the SLE patients from Spain. This study also showed that nine genera of intestinal microbiota were SLE-related microorganisms: Rhodococcus spp., Eggerthella spp., Klebsiella spp., Prevotella spp., Eubacterium spp., Flavonifractor spp. and Incartae spp. sedis were found in great abundance but Dialister spp. and Pseudobutyrivibrio spp. were depleted in SLE subjects. These nine genera have the potential to distinguish SLE patients from healthy controls. Also, the gut microbiome profiles of SLE patients were found to be more influenced by disease than ethnicity. [43]

Another study conducted in China concluded that the genera Streptococcus spp., Campylobacter spp., Veillonella spp. and the species Anginosus spp. and Dispar spp. were in direct correlation to lupus activity, while the genus Bifidobacterium spp. was in negative correlation to the disease activity. [44]

A study conducted in New York, on SLE female patients, investigated the fecal microbiota for possible pathobionts. Patients with a high disease activity index had a greater abundance of Ruminococcus gnavus. The highest levels of serum anti-Ruminococcus gnavus strain-restricted antibodies were found in patients with active nephritis. These findings showed evidence of altered fecal commensal taxa and increased immunoglobulin levels and other biomarkers, as evidence of altered gut barrier function. The study concluded that some strains can contribute to the immunological pathogenesis of lupus nephritis. [45]

Treatment with probiotics composed by Lactobacillaceae members has been used as anti-inflammatory therapy. Studies found the up-regulation of anti-inflammatory cytokine genes and down-regulation of the pro-inflammatory ones.

Moreover, the probiotic-treated group exhibited higher anti-oxidant activities and low levels of lipid peroxides. CRP levels were found to be in the normal range in comparison with the untreated group which levels were elevated.

Immunohistochemistry found high levels of IL-10 and low levels of IL-6, demonstrating the anti-inflammatory properties of L. mucosae and L.fermentum. [46, 47]

Lactobacillus rhamnosus and Lactobacillus delbrueckii have been found beneficial in the management of newly diagnosed SLE patients. [48] L. rhamnosus stimulates IL-12 and IL-10 production. Moreover, it secretes soluble factors that decrease TNF-α production, a potent pro-inflammatory cytokine. [49]

Although there is still no clarity about the role of microbiota within the development of SLE, the evidence suggests that the dysbiosis observed within SLE patients might be associated with this disease.

Figure 3: Associated microbiome in systemic lupus erythematosus

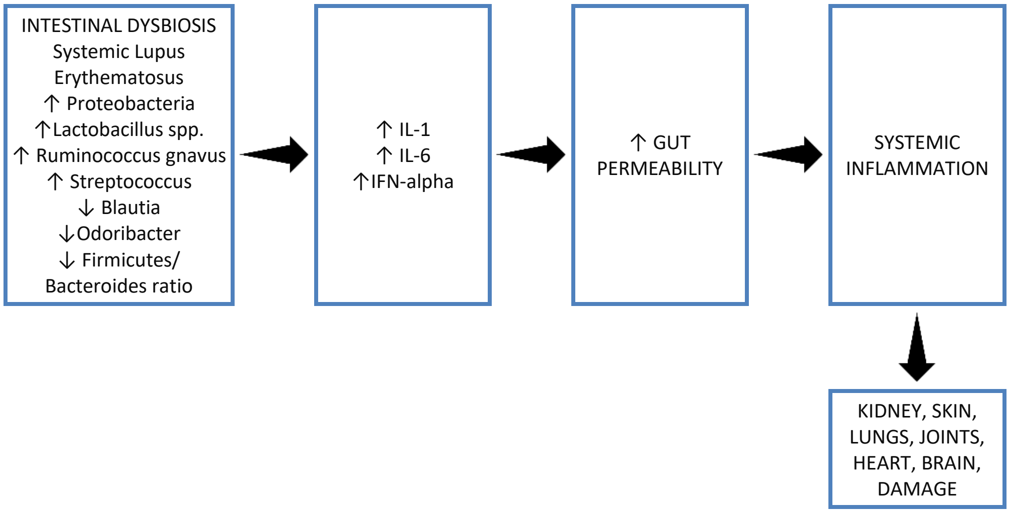

Systemic lupus erythematosus treatment and intestinal microbiota

Nonselective immunosuppressive therapies, like Dexamethasone and azathioprine, in SLE patients, may have vast effects on the intestinal microbiome. It is believed that at the same time as immunosuppressive medications are acting directly onto suppressing inflammation, these medications can also help in the formation of a microbiome that promotes the regulation of local inflammation within the bowels and potentially at distant sites within the body. A study showed that the gut microbiota tended to be more diverse after Dexamethasone treatment. Also, a greater abundance of lactobacilli was found after the treatment with Dexamethasone. [42]

On the contrary, no significant results were found between gut microbiota and methotrexate use in a recent study within the British TwinsUK cohort. [39] Chloroquine and hydroxychloroquine are reported to inhibit the intracellular growth of bacteria in vitro, but these drugs do not seem to own any antibacterial activity or effect on extracellular bacterial growth. Regarding the use of NSAIDs, a study observed no significant effect on the gut microbiome. Also, oral corticosteroid use didn't significantly affect the gut microbiome. [50]

DIET

Diet and the intestinal microbiota may be the target for future treatments and preventive interventions in rheumatic diseases. Diet can modulate the symptoms in rheumatoid arthritis through the metabolites produced by the intestinal microbiome. The intestinal microbiome has the capacity of modifying the circulating pro and anti-inflammatory mediators. [51]

Western diets are characterized by increased consumption of refined carbohydrates, red meat, and salt, which can induce a pro-inflammatory state and trigger the onset of autoimmune diseases. [52, 53]

Patients with a positive rheumatoid factor or anti-citrullinated protein antibody had a decreased concentration of omega-3 fatty acids in the red blood cell membranes, suggesting the potential beneficial effect in individuals with genetic susceptibility to rheumatoid arthritis. [54]

Some studies suggest that there might be a link between high salt consumption and the increasing incidence of autoimmune diseases, especially rheumatoid arthritis. A high concentration of sodium chloride can induce the activation of some signaling pathways, the activation of Th1, the overproduction of pro-inflammatory cytokines (IFN-gamma, IL-17), and the differentiation of macrophages. [55, 56]

For example, trimethylamine-N-oxide, a pro-inflammatory metabolite that derives from choline and carnitine, found in red meat, eggs, and dairy, is produced by Prevotella copri. [57][58]. Prevotella copri was found in great concentrations in newly diagnosed patients with rheumatoid arthritis. [59]

Probiotic use of Lactobacilli spp. improves intestinal immune function and stimulates anti-inflammatory cytokines (IL-10).

In general, Lactobacillus spp. and Bifidobacterium spp. are considered beneficial. Both species produce catabolites that decrease intestinal permeability and have anti-inflammatory and antioxidant properties, with the enforcement of the intestinal barrier function. Fermented foods that contain Bifidobacterium spp. and Lactobacillus spp. genera can have a protective role against immune-mediated diseases. [60]

A study evaluated the effects of L. casei supplementation on disease activity and inflammatory markers levels in rheumatoid arthritis females. A decrease in CRP, DAS28, and inflammatory cytokines was observed. [17]

The microbial degradation of complex carbohydrates will increase short-chain fatty acids that are beneficial for the intestinal immune response. [61] Several foods (highly refined flours, gluten, saturated and trans fats, red meat, dairy) have been identified as proinflammatory foods. Some vegetables as tomatoes, eggplants, and potatoes, contain solanine and increase intestinal permeability.

Foods high in omega 3 (chia seeds, flax seeds, sesame seeds, avocados), fruit that contain enzymatic proteins (pineapple, mango, papaya), turmeric, black pepper, green tea, and legumes offer multiple benefits.

To decrease inflammation, an ITIS diet has been suggested. It consists of a modified Mediterranean diet with several changes: the separation of carbohydrates and proteins, gluten-free foods, fruit with enzymes, and other anti-inflammatory foods mentioned above. [62]

A study regarding intestinal microbiome and Mediterranean diet observed that an increased ratio of Firmicutes/ Bacteroides was related to lower adherence to the Mediterranean diet. A lower animal protein intake was associated with an abundance of Bacteroides. Moreover, high consumption of sugars, saturated fats can affect the intestinal microbiota diversity. A plant-based diet was related to higher bifidobacterial counts. [63]

A case report discussing a fecal matter transplant (FMT) for a rheumatoid arthritis patient found that HAQ-DI (Health

Assessment Questionnaire – Disability Index) dropped seven days after FMT. [64] Therefore, fecal matter transplant may be an effective treatment for rheumatoid arthritis through the restoration of beneficial microbiota.

DISCUSSIONS

The treatment in rheumatology has made great advances with the potential to significantly impact the evolution of inflammatory diseases. A promising area of active research is the targeted manipulation of the microbiome to improve the outcome of rheumatic diseases.

The Fecal Matter Transplant in rheumatic diseases is not studied enough and more data is needed to achieve durable responses. Extensive donor testing is mandatory to guarantee patients' safety.

The use of prebiotics and probiotics can promote the growth of beneficial microbes. Probiotics supplements may be used as adjunctive therapy for rheumatic patients.

The microbiome composition can be used for prognosis and diagnosis, but most importantly, the manipulation of the intestinal microbiome through diet may be a promising future therapeutic approach. Large, long-term clinical trials are needed for specific dietary recommendations to rheumatic patients.

CONCLUSIONS

Current literature shows the link between the intestinal microbiome and the activity and severity of rheumatic diseases.

The imbalance of bacterial phylum, especially Firmicutes spp. and Bacteroidetes spp., is believed to be one of the most important causative factors for autoimmune diseases.

Although a lot of progress had been made in the past years regarding the intestinal microbiome, the research still requires more data. Targeted manipulation of the intestinal microbiome is needed for better treatment and management of inflammatory rheumatic diseases. Although 16S rRNA sequencing provides information about microbial identity, its function remains unknown.

Fecal matter transplant may have a good therapeutic effect on rheumatic diseases through its capacity of restoring and maintaining eubyosis.

Diet has been shown to influence the evolution and severity of autoimmune diseases. The use of pre and probiotics may be useful. The use of Lactobacillus species, as an adjuvant treatment, has been found beneficial.

Dietary or targeted manipulation of the intestinal microbiome may restore homeostasis and restrain disease-promoting pathobionts.

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ORIGINAL ARTICLES

Diagnostic predictability of using portal vein diameter in confirming portal hypertension

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Abstract: *Objective: To find an accurate cut-off value for Portal vein diameter as a diagnostic tool for Portal hypertension on comparing with Doppler ultrasound, CT abdomen, or endoscopy of collateral circulation.*

Materials and methods: This was a cross-sectional study, with a sample size of 100 patients. Inclusion criteria were patients above the age of 15 years who underwent routine USG abdomen for various conditions in the Radiology Department of a tertiary hospital. Distribution of age, gender, chronic liver disease, portal hypertension, and portal vein diameter of all the patients were measured and recorded. Portal hypertension was diagnosed using the presence of collaterals either on upper endoscopy (if done), Doppler USG, or CT multiphase abdomen.

Results: Portal vein diameter of 10.5mm was found to have a sensitivity of 61.22%, specificity of 60.78%, a positive predictive value of 60.00%, a negative predictive value of 62.00%, and accuracy of 61.00%. 45 out of 59 (76.3%) and 37 out of 49 (75.5%) patients of the 50-70 years age group had chronic liver disease and portal hypertension respectively. 45 out of 65 (69.2%) males 37 out of 65 (56.9%) males developed CLD and PHTN.

Conclusion: Using portal vein diameter as a diagnostic indicator of PHTN has an accuracy of %, so it can be used in routine USG abdomen of CLD patients to prevent the dreaded complications of PHTN and treat it early. Male gender and patients above the fifth decade were increasingly predisposed to both CLD and PHTN.

Keywords: *portal hypertension, chronic liver disease, portal vein diameter, collateral circulation*

INTRODUCTION

Portal hypertension is defined as portal venous pressure more than 12 mm of Hg [1]. It is measured as the hepatic venous pressure gradient (difference of wedge hepatic pressure and free hepatic venous pressure), which is normally 4-6 mm Hg. More than 10 mmHg is portal hypertension, and more than 12 mmHg causes clinically significant variceal bleeding. [2]

Portal hypertension is major pathophysiology in patients with cirrhosis. Portal pressure is the gold standard to evaluate the severity of portal hypertension, and radiological intervention is the only procedure for pressure measurement [3].

Causes of portal hypertension are pre-sinusoidal (portal vein thrombosis, extrinsic compression of portal vein, Schistosomiasis (S. mansoni or S. japonicum)), sinusoidal (cirrhosis) and post-sinusoidal (Budd-Chiari syndrome, hepatic venous-occlusive disease, congestive cardiac failure) [1]

Portal hypertension leads to dilatation of portal vein,

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splenomegaly, and formation of portal-systemic collaterals at different sites. The portal system and the systemic venous circulation are connected at several locations. Collaterals develop in areas where anatomic connections exist between the portal venous and systemic circulation. These are vascular channels that are functionally closed in normal conditions but become dilated in portal hypertension as a consequence of increased intravascular pressure and blood flow. These gastro-oesophageal varices are responsible for the main complications of portal hypertension and massive upper GI bleeding [4].

The complications of portal hypertension (gastrointestinal hemorrhage, hepatic encephalopathy, hepatorenal syndrome, and ascites) continue to be the cause of significant morbidity and mortality. Portal hypertension remains one of the most serious sequelae of chronic liver disease [2].

To prevent bleeding from occurring, an appropriate early diagnosis of oesophageal varices at risk of bleeding should be achieved. Upper digestive tract endoscopy is the gold-standard method for diagnosing the presence of gastroesophageal varices and identifying signs of risk of bleeding. Universal endoscopic screening of oesophageal varices was recommended in all patients newly diagnosed with cirrhosis until 2015. However, due to the increase in the proportion of patients with early cirrhosis achieved by non-invasive diagnostic methods, this strategy proved to lead to a large number of unnecessary endoscopies that would eventually decrease the patient’s compliance and increase the health system costs. In the last 10 years, increasing evidence regarding non-invasive methods accumulated and proved useful for stratifying the risk of carrying varices and varices requiring treatment in patients with CLD [5].

60-80% of patients with cirrhosis of the liver are associated with esophageal varices and 25-30% of them have got a risk of bleeding. Every year incidence of esophageal varices increases by 5% and 5-10% of patients with small varices progress to large varices [6].

Endoscopy is the standard diagnostic approach in patients with portal hypertension. But it is an invasive technique. Noninvasive imaging techniques are preferred for early diagnosis and prevention of complications. Portal vein diameter, peak systolic velocity, presence of collaterals, splenomegaly, etc can be found using Doppler USG and CT multiphase abdomen. The overall sensitivity for detection of coronary-gastroesophageal collaterals using USG was 80% and 64%, respectively, in a study done by Subramanyam et al. [7]. Doppler USG is frequently obtained in patients with chronic liver disease, suspected portal hypertension, and known portal hypertension. Among the many findings of portal hypertension is the enlargement of the portal vein, decreased portal vein velocity, increased congestion index, development of portosystemic collaterals, and reversal of portal vein flow. Using all of these, it is possible to diagnose portal hypertension with a high degree of sensitivity and specificity non-invasively [8].

It is possible to rule out clinically significant portal hypertension in patients with cirrhosis (i.e., hepatic venous pressure gradient ≥ 10 mmHg) with a level of clinically acceptable accuracy by combining measurements of liver stiffness, spleen stiffness, and Doppler ultrasound evaluation. The combination of these methods allows for the identification of patients with the most serious degree of portal hypertension as seen by Bolognesi M et al. [9].

Despite the safety, affordability, and widespread use of ultrasound, there is a paucity of literature on ultrasonographic assessment of the portal vein (PV) diameter in adult patients in our local environment [10]. According to a study done by Nestaĭko OV [11], real-time ultrasound investigation could provide important information and should be used as a non-invasive and harmless investigation in hepatological studies. Splenic Stiffness can also be used for assessing and monitoring PHTN in hepatitis C induced cirrhosis according to the study by Colecchia et al. [12].

The cutoff point for portal vein diameter differs from study to study despite the existence of a normal mean portal vein diameter between 10–15 mm in different settings. This implies the existence of limited evidence on the exact portal vein diameter which can help diagnose portal hypertension early [13].

This study will help find an exact cutoff point of portal vein diameter which can help rule out false positives and find out false negatives and provide prompt detection and treatment and prevention of progression of a chronic liver disease patient to portal hypertension, using a routine non- invasive USG scan.

MATERIALS AND METHODS

This is a cross-sectional study carried out in the Radiology department of AIMS, Kochi. Since this is a pilot study, a total of 100 patients above the age of 15, referred from various other departments to the Department of Radiology for routine USG of the abdomen are included. Those patients who underwent imaging studies elsewhere, patients having thromboembolic problems, etc are excluded. Portal vein diameter and Doppler study of spleen for splenic collaterals are carried out. MRD numbers of these patients are collected to note down their age, history of CLD, Portal hypertension, and the presence of collaterals in the CT scan of the abdomen or upper endoscopy (if taken previously) is tabulated in MS Excel. The cut-off for Portal vein Diameter is found using Receiver Operator Characteristic Curve in SPSS. Qualitative variables are expressed as percentages and quantitative variables are expressed as mean with standard deviation. Informed consent was obtained from all the patients. The Ethics Committee of Amrita Institute of Medical Sciences has approved the study.

RESULTS

The mean age of the sample population was 54.9 +/- 13.89 years, median age 56 years (range of 17-84 years), and mode 67 years. The majority (61, 61%) of the patients were of the 50-70 years age group (Figure 1).

Figure 1: Distribution of age

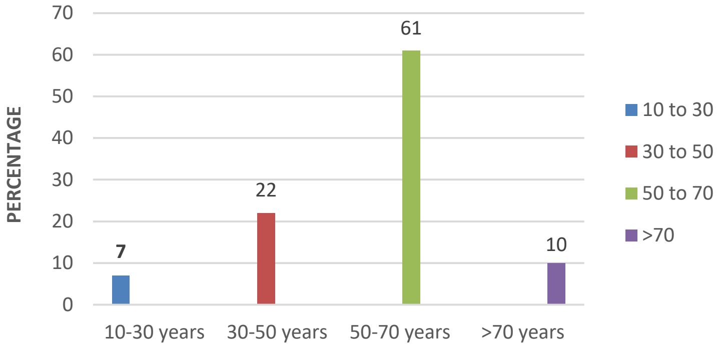

35 (35%) of patients were females and 65 (65%) were males (Figure 2).

Figure 2: Distribution of gender

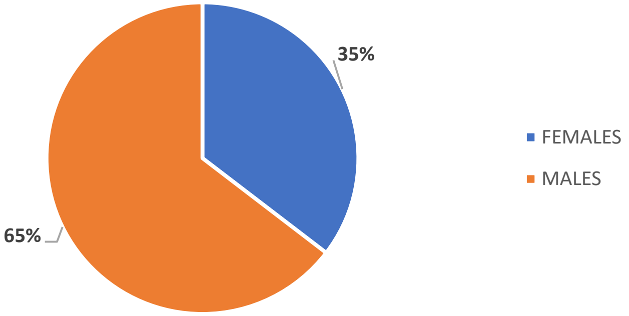

Of the 100 patients who underwent USG abdomen, 59 (59%) patients were found to have ultrasonographic features of chronic liver disease, while 41 (41%) patients did not have CLD.

49 (49%) patients were found to have collateral circulation, seen either on Doppler USG, or CT Multiphase abdomen, or Upper endoscopy (if done previously for any other indications), and 51 (51%) patients did not have any collaterals (Table 1).

Thus 49 (49%) patients had portal hypertension. Among the 50-70 years age group, 60.6% (37) of patients had PHTN, while 0 patients had PHTN in the 10-30 years age group, which clearly showed that PHTN was more common after the fifth decade of life (Figure 3).

Table 1: Distribution of portal hypertension (PHTN)

Collaterals	Frequency	Percent
Present	49	49.0
Absent	51	51.0
Total	100	100.0

More Males (56.9%) had PHTN as compared to females (34.2%), which was statistically significant, p-value <0.05 (Figure 4).

ROC curve to find out the portal vein diameter cut off had an Area 0.676, diameter 10.5 mm, with a sensitivity of 61.2%

and specificity of 60.8%, p-value of 0.002 (Figure 5).

Figure 3: Comparison of age and PHTN

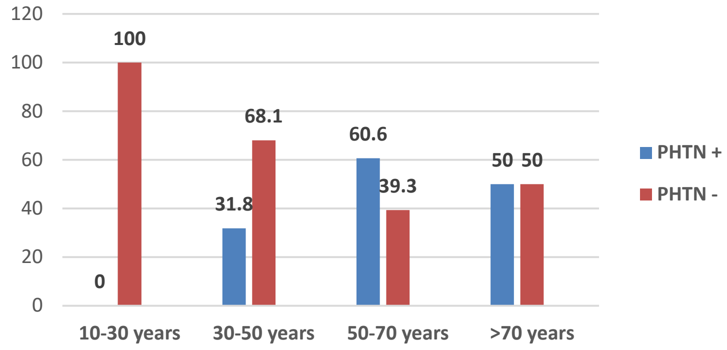

Figure 4: Comparison of gender and PHTN

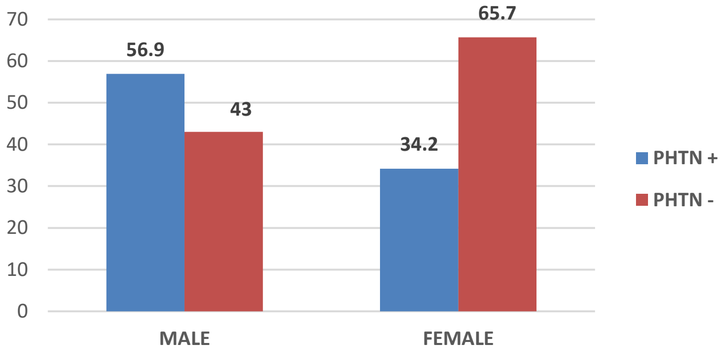

Figure 5: ROC curve of portal vein diameter and PHTN

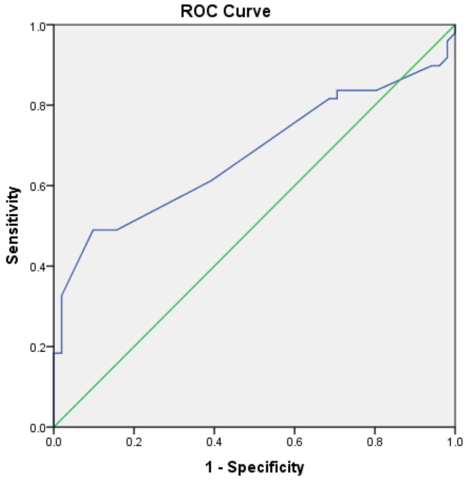

Diagonal segments are produced by ties.

AUROC	0.676
PVD	10.5 mm
p value	*0.002*
Sensitivity	61.2%
Specificity	60.8%

DISCUSSION

Portal hypertension is the most common complication and also one of the important causes of death in chronic liver diseases. Increased resistance to portal blood flow due to alteration of the hepatic architecture leads to dilatation of portal vein, splenomegaly, and formation of oesophageal and gastric varices, variceal hemorrhage, ascites, hypersplenism, encephalopathy, etc [4]. The overall sensitivity for detection of coronary-gastroesophageal collaterals using USG was 80% and 64%, respectively, in a study done by Subramanyam et al. [7].

The mean age of the sample population was 54.9 +/- 13.89 years, the median age was 56 years (range of 17-84 years) and the mode of the age was 67 years. Most of the patients (61.0%) were in the 50-70 years age group, similar to the findings of Gaduputi et al. [14]. In a similar study done by Gunda et al. [15], it was found that the odds of having esophageal varices were independently higher among patients with increased age (Median age of 51 vs. 45 years; OR:1.02; 95%CI: 1.0–1.04; p = 0.030)

Out of the 100, 35 (35.0%) of patients were females and 65 (65.0%) were males. This pattern of male predominance is comparable to the study done by Sharma et al. [16] which had 86.1% of male patients. In the study by Akande et al. [17], 81% of males and a mean age of 44+12.6 years were seen among the cases.

More patients, i.e 73.7% and 60.6% of patients in the 50-70 years age group was found to have chronic liver disease and portal hypertension respectively; which clearly shows that both CLD and subsequently the development of PHTN is more common after the fifth decade of life. The same was the case with the male gender, i.e, 69.2% and 56.9% of males had CLD and PHTN respectively; which again shows that males have an increased association with CLD and subsequent development of PHTN. In a similar study done by Manik Mahajan et al. [18], it was found that the majority of the patients (32%) belonged to the 6th decade, with 66% of male and 34% of female patients having CLD and PHTN.

10.5 mm was found to be the cut off for Portal vein diameter in our study using ROC curve, with an area of 0.676, p-value 0.002, comparable to that found by Bhattarai S et al. [19], Sarwar et al. [20], and Guo et al. [21]. Collateral circulation was found in 49% of patients, which was seen either during Doppler ultrasound, CT multiphase abdomen, or endoscopy. Out of these 30, (60.0%) of the patients had a portal vein diameter of more than 10.5 mm. These were the true positive cases. Among these, 72% were in the 50-70 years age group. A similar study done by Gemecho et al. [13] revealed a normal mean portal vein diameter of 10.6 mm ±1.8 SD with a respirophasic variation of 25.6% and in the study by Ekta Gupta et al. [22] in North India, mean normal portal vein diameter measurement in male subjects was 10.33 mm ± 1.004 & in female 9.41 mm ± 1.16.

PVD > 10.5 mm was found in 20 (40.0%) of patients, but they did not have any collaterals; i.e these were the false-positive cases. CLD was found in 6 (30%) of these patients while 1 (0.5%) patient had portal vein thrombosis which explains the increase in diameter. Higher portal vein diameter was seen in those patients with esophageal varices in the study by Sangma MA [23] and Li FH et al. [24], as well as in cirrhotic children by Riahinezhad et al [25]. Diameter tended to increase in Child-Pugh scoring initially, before getting stenosed at higher scores, as found by Zhoy et al. [26].

The true negative cases were 31 (62.0%) of patients, who had a portal vein diameter less than 10.5 mm. The false-negative cases, i.e, 19 (38.0%) patients with PVD<10.5 mm but with the presence of collaterals, were long-standing chronic liver disease patients with well-established collateral circulation and a grossly shrunken liver, which led to the stenosis of the portal vein, leading to the decrease in PVD. Normal mean portal vein diameter in Ethiopian patients was found to be 10.0+1.8 mm (range 8.2 to 11.8) with a 21.5% reduction with the phases of respiration, in a similar study done by Scabel et al. [27]

In another study done by Lopamudra et al. [4], the average portal vein diameter of patients without gastro-oesophageal varices was 11.545 ± 1.514 mm and of patients with varices 13.998 ± 1.123 mm, which was slightly higher than the PVD found in our study, so was the normal mean portal vein diameter found in a study by Jefferey W et al. [28], which was 11 + 2 mm in patients aged between 21-40 years.

Thus, portal vein diameter of 10.5 mm had a sensitivity of 61.22%, specificity of 60.78%, the positive predictive value of 60.00%, the negative predictive value of 62.00%, and accuracy of 61.00%, in correctly diagnosing portal hypertension. In a similar study done by Johana Prihartini et al. [29], PVD of 11.5 mm had a sensitivity of 75% and a specificity of 54.5%. The sensitivity of using portal vein calibrations was found to be only 41.8% in the study done by Bolondi L et al. [30].

Limitations of our study: Portal vein diameter can vary in various physiological conditions, like inspiration, expiration, duration of liver pathology, etc. In our study, the diameter was taken at the hilum without taking into account the respiratory changes or the duration of CLD. Portal vein diameter can also vary according to age. There are wide variations in the age groups of the patients. More specific indicators like splenic size, peak systolic velocity, the direction of flow in the portal vein could have been included in the study.

CONCLUSIONS

Thus, portal vein diameter of 10.5 mm was the cut-off above which portal hypertension could develop, as was shown in the study. Though of limited sensitivity and specificity, it can be used to lookout for portal hypertension in chronic liver disease patients and prevent it as early as possible, before its dreaded complications set in. Both CLD and PHTN had a high incidence after the fifth decade of life, so screening for either disease is recommended after 50 years of age.

Contribution of authors

Varsha Somakumar, Cibi Manoharan, V S Srikanth – Concepts, design, the definition of intellectual content, literature search, data acquisition, data analysis, Manuscript preparation, editing, and review

Rajesh Kannan, Chitra Tomy, Anagha Vasant, Vishal Marwaha, VR Mujeeb - Concepts, design, the definition of intellectual content, Manuscript editing and review.

This has been presented as a poster at AMRITACON 2019 at Amrita Institute of Medical Sciences and Research Center by Miss Anagha Vasant.

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ORIGINAL ARTICLES

Health sector organization to respond to a bioterrorist attack

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Abstract: *Bioterrorism constitutes a major health threat to global communities, with many specific features that carry the potential to extraordinary stress the Public Health Sector (PHS) of any country, because of the needs of resources and of a coordination network required for a successful response to such incidents. Bioterrorism incidents of the near past prove that public mortality and morbidity can be further reduced if a functional protocol would allow first responders, hospitals, and officials of the PHS to steer more efficiently the public expectations. At the national level, the PHS director is responsible to mobilize and coordinate that network of the critical human, financial and logistical resources during the preparedness and response phases after a bioterrorist attack to mitigate its effects. Both these phases include many specific functions and responsibilities, which will be beneath analyzed.*

Keywords: *bioterrorism, public health sector, health preparedness*

BACKGROUND

Bioterrorism is the deliberate use of biological agents to disseminate life-threatening diseases to threaten or devastate a population [1]. The bioterrorist threat consists of a rapidly growing and perhaps the most frightening menace to world health security [2]. The weaponized application of pathogens to disorder populations has been established throughout history [3]. However recently, the profound development of bio-molecular engineering combined with the worldwide proliferation of high-level biosafety laboratories has aroused concern about the increased accessibility of biotechnology [4] and potential misuse of biological agents [5]. Bioterrorism manufacture requires just culture samples, the means to grow, purify, stabilize and disseminate them [6].

The motives underlying bioterrorist incidents of the last years have been nationalistic objectives, revenge, or a mix of paranoid – conspiratorial – apocalyptic prophecy [7]. Additionally, large terrorist organizations such Al-Qaida would always seek ways to acquire biological weapons [8].

The biological agents considered suitable for terrorism should have high inter-human infectiousness, high morbidity and/or lethality, ease of manufacture, storage, and delivery in adequate quantities, environmental stability after dispersion, and absence of effective treatment [9].

Most of the abovementioned characteristics are easily achievable and that raises concerns about the devastating power of biological weapons, because of various reasons: they disseminate in various ways, extremely small quantities of them are lethal, manufacture information is readily obtainable, and a bioterrorist attack can initially be covert since the appearance of symptoms and illness will lag for several hours or days [10].

Bioterrorism agents are classified into bacteria and viruses and yet into three categories; A, B, and C, depending on the ease of dissemination, severity of illness caused, and ability

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to cause death [11]. Of those, microorganisms of Category-A (i.e. anthrax, botulism, plague, smallpox, and viral hemorrhagic fevers), carry the potential to extraordinary stress the PHS, because:

1. The initial release of the bioterrorist agent could go undetected until the onset of disease, creating a sudden influx of severe illness;

2. Incidents that result in impairment of food and water supplies, as well as transportation and communication barriers, may gravely affect the national stability;

3. Contamination risks and subsequent fears of exposure may further complicate recovery operations and cause civil disorder [12].

Up to now, bioterrorism remains a challenge for the PHS of every nation, mainly due to the need for resources and a coordination network required for a successful response to an incident [13]. At the national level, the PHS director is responsible to mobilize and coordinate that network of the critical human, financial and logistical resources during the preparedness and response phases after a bioterrorist attack. Both these phases include specific functions and responsibilities of PHS, which will be beneath analyzed.

PREPAREDNESS PHASE

Risk Assessment

Among weapons of mass destruction, biological agents are much more destructive than chemical weapons, including nerve gases, whilst under certain conditions, biological weapons can be equally devastating to nuclear ones. For example, according to a traditional disaster simulation model [14], a few kilograms of anthrax in a dense urban area can kill as many people as a Hiroshima-size atomic bomb, or cause even more casualties when considering the current suburban living situation of a western Metropolis, combined with favorable upwind conditions [15].

Risk assessment is a multidisciplinary activity, aiming at identifying those who may wish to use biological weapons against the population, the agents that may be used, and the circumstances under which they may be used. Risk assessment of biological threats often involves modeling approaches based on certain risk assumptions. To understand these risks, planners assess them as a function of their likelihood to occur, their potential consequences, and the possible measures that could be taken to control them, based on an understanding of existing surveillance data, the literature, past experiences, and opinions of experts [16].

Intelligence

Preparedness for bioterrorist attacks includes international collaboration for the secure exchange of sensitive information regarding the capabilities of terrorists [2]. Accurately predicting potential biologic threats is difficult without the implementation of intelligence data, adequate funding [17], strategies of data- and cyber-security, and expanding use of electronic information, reporting and surveillance systems [18]. Furthermore, networked video cameras and advanced software designed to verify important intelligence information have become powerful tools for counterterrorism operations [19]. Notification to WHO is required for all cases of sufficient seriousness, such as diseases with severe public health impact, risk of international spread, cases of unusual or unexpected nature, and of course all cases of category A diseases, which constitute a "public health emergency of international concern" [20].

Disease Surveillance

The cause of a bioterrorist disease may be difficult to determine, especially if the initial cases are few. Because of rapid progression to illness and the potential for dissemination of bioterrorist agents, authorities of the PHS should apply accurate, sensitive, and specific surveillance systems to promptly recognize non-specific disease patterns that might indicate the early manifestations of a biological attack. That can be accomplished through two pillars; epidemiological and syndromic surveillance.

Epidemiological surveillance

Following a confirmed case of biological exposure or clusters of unusual symptoms or diseases detected by PHS surveillance, forensic epidemiology tracks back to identify the population at risk. This procedure is called the epidemiological investigation and uses descriptive, analytical, and molecular epidemiologic tools to identify the source and possibly to prevent further events [21, 22]. For that to be accomplished, epidemiological models encompass the potential agent to be used, the specific geographic location of an attack, the level of preparedness, and early intervention on a local and national level. Epidemiologic principles must be used to assess whether a patient’s presentation is typical of an endemic disease or is an unusual event that should raise concern. An epidemiological alert system should include multiple sub-systems regarding disease reporting, laboratory network surveillance for specific pathogens, sentinel hospital surveillance for infectious diseases based on primary healthcare data, and a rapid response system with a 24 h/7 d epidemic response team [23].

Syndromic surveillance

The concept involves real-time collecting and analyzing statistical data in a given population, focusing on symptoms rather than laboratory confirmation of diagnosis. Syndromic surveillance systems register suspicious or confirmed cases reported by physicians, and the data are used to monitor the progress of a bioterrorism outbreak [2]. The technique may be affected by various factors, such as data selection criteria, seasonality, size and dispersion of the infected population, the incubation period, selection of statistical detection thresholds, duration of follow-up, access to diagnostic practices or reference laboratories, and reporting procedures; all these results in potentially false alarms and overuse of resources to investigate them [24]. Specificity for distinguishing random variations of ordinary illnesses from bioterrorist attacks may be low when the early symptoms of bioterrorism-related illness overlap with those of many common infections, whilst respective sensitivity may also be low if detection thresholds are reduced to enhance specificity [25]. Lastly, syndromic surveillance is planned to enhance, rather than replace, existing diagnostic tools to epidemic detection [26].

Early Warning and Response System (EWRS)

Numerous biological agents may be used in bioterrorism; terrorists might use combinations of them, common or new, drug-resistant or genetically engineered pathogens, or attack in more than one location simultaneously. Early detection and response to highly contagious biological agents are hence crucial. That can be accomplished through an EWRS, i.e. a rapid web-based alert system used for exchanging information on health threats resulting from the deliberate release of a chemical, biological and radiological agents, but mostly for monitoring epidemics. A major benefit of global collaboration at a technical level is the development of trust networks among experienced scientists and clinicians, for both natural and intentional outbreaks [27].

Emergency Planning

Terrorist attacks are more frequently covert compared to bombings or even chemical attacks. That is an intrinsic feature of bioterrorism, due to the incubation period of pathogens that brings a delay between exposure and onset of symptoms and consequently, between the attack and the involvement of PHS and civil security agencies [28]. The importance of emergency planning lays in the fact that public health infrastructure should be prepared to confront a rapid sequence of emergency events, including a delayed massive wave of illness, rapid dissemination in the population, and the need for prevention strategies, analyzed below.

Laboratory Preparedness

A solid laboratory network would enable quick and accurate detection and subsequent response to biological threats. The response capacity of those laboratories is classified into four Biosafety Levels (BSL); from BSL-1 (clinical laboratories with minimal biosafety requirements), up to BSL-4 (implementing the highest biosafety standards), according to their scientific background, expertise, and infrastructure to support the diagnostic needs of a highly hazardous biological event [4]. During such, a proper laboratory network enables the following functions:

1. Activating all BSL laboratories nationwide.

2. Prioritizing and sharing information among sentinel laboratories and PHS officials on an emergency, 24/7 day basis, to ensure that the public received precise and prompt messages [18].

3. Recognizing and responding to any covert event involving the collection, preservation, transport, and testing of highly infectious human specimens according to uniform guidelines for handling samples of pathogens of public health interest [29].

Food and Water

Biological agents constitute a major concern regarding the safety of food [30, 31]; contamination of water with biological agents should be also considered, albeit that is improbable to be the major target of bioterrorism, due to chlorination, dilution, and the need for large quantities of the agent to cause a significant biological threat [32]. Nevertheless, quantities far less than a kilogram of botulinal toxin, for example, in the main water supply of a city may cause 28-40,000 deaths, depending on how early symptoms will be recognized and remedial measures will be applied [14].

Stockpiling

The strategic medical stockpile of a country is a national repository of vaccines, drugs (antibiotics, antidotes, etc), and general medical supplies that can be deployed to areas affected by various sorts of public health disasters, including bioterrorism. In the case of the dissemination and subsequent detection of a biological agent, strategic stockpiles of respective medication against the agent should be provided to the affected area within less than 24 hours; subsequently, the medical countermeasures should be appropriately dispensed in the affected community within another 24 hours [33]. Strategic stockpiling has to be considered within the framework of cost-effectiveness;

stockpiling of pharmaceuticals with a sort expiration date may be costly and problematic since the turnover inventory would require a significant monetary amount to maintain stockpiling facilities for continuous checking and renewing [34].

Training

Improved awareness and readiness during a bioterrorist attack include among others education of all medical personnel, especially primary-care providers and first responders to patients affected by a biological attack35. Training inserts knowledge about basic epidemiologic principles, disease patterns, clinical information on diagnosing and treating infected patients [36] and should be refreshed periodically with large-scale exercises to ensure that skills remain up-to-date [37]. Additionally, analysis of actual previous incidents provides precious information regarding proper response. Most importantly, the lessons learned should be incorporated into future planning to identify areas of improvement. Finally, education of health departments and the community on biosafety items, including information for crisis-phone hotlines, contributes to mitigating the negative health effects of a bioterrorist attack [38].

RESPONSE PHASE

Detection-Verification

Prompt diagnosis should be urgent in a probable bioterrorist incident, because for both health and security reasons [39]. The earlier a dissemination is detected, the faster the response and the lower the morbidity and mortality rates. Apart from significant awareness by the PHS, this also requires advanced diagnostic technology. Highly sensitive and specific and yet portable laboratory diagnostic systems are nowadays available to a greater extent than a decade ago, enabling a faster, cheaper, and more accurate verification of the infectious agent. Moreover, increased networking and collaboration of higher-level biosafety laboratories also improve response to biohazards [40]. The critical factors in reducing mortality following a biological incident were found to be early detection of exposure, registering of cases, and dispensing capacity at local and national levels [41, 42]. The verification of a possible bioterrorist attack should fulfill most of the following epidemiological criteria [43]:

1.Occurrence of a specific epidemic disease with similar symptoms in a discrete population;

2.Single case infection caused by an uncommon or class A biological agent;

3.Unusual disease for a specific geographic area or a specific age group;

4.Unusual season of transmission;

5.Many unexplained disease or death incidents;

6.Unusual high level of disease severity with resistance or response failure in standard treatment;

7.Unusual exposure routes of a “known” pathogen;

8.Higher incidence of occurrence in certain area zones, depending on whether the biological agent was released in- or outdoors;

9.Relevant claims by terrorists of a bioterrorism attack or existing intelligence for a potential threat.

Coordination

Biodefense is a complex procedure requiring a multidisciplinary approach. In any bioterrorist incident, interoperation mechanisms between major local, national and international, health- and non-healthcare agencies must be applied. Coordination should be reinforced and biodefense protocols effectively integrated into existing plans [10]. Government stakeholders called to react and collaborate to a bioterrorism incident include policy makers, law enforcement officials, emergency responders of PHS, special centers from scientific, research, forensic, and professional societies and technicians, along with the commercial branches of medical, safety, and decontamination equipment suppliers [28]. In any case, the management of a large-scale bioterrorism outbreak is well beyond the resources and capabilities of most countries; thus many lives can be saved in bioterrorist outbreaks by early mobilization of large-scale international assistance by W.H.O. Furthermore, many national armies afford special Chemical-Biological-Radiological (CBR) units, able to respond to a bioterrorism event; these have the means and often the war expertise to detect, verify and neutralize biohazard threats and to decontaminate victims and areas exposed to such agents.

Rapid Response

Following alleged or genuine dissemination of biological agents, a large number of people will probably seek medical care. Fear and panic are expected in symptomatic patients, yet also in involved health personnel of public hospitals and private clinics, who may be the first to recognize a biohazard outbreak and initiate a response. A broad network of communication towards many stakeholders must then be rapidly activated [44]. Moreover, a plan dealing in detail with the possibility of great numbers of people who concurrently might seek medical care, along with means for psychological support and medical treatment is of utmost importance in the triage rapid response process conducted by various health care professionals. These should operate according to the national SOPs regarding response to the outbreak of a biological agent [18]. Unfortunately, the mobilization of a specialized CBR unit can never match the 24-hour availability and general emergency-management experience of existing emergency response and public health services, whilst the use of CBR protective equipment also requires special training and the adaptation of existing procedures for emergency management [45]. Furthermore, in case of risk for international dissemination of the disease, the International Health Regulations (IHR) should be implemented to prevent the worldwide spread of diseases [46].

Mass Prophylaxis/Infection Control

For most biohazard agents, specialized testing is necessary by PHS specialists or laboratories. Then, vaccination and/or treatment with antibiotics or antitoxins must be started early for viral agents and bacterial diseases. Before undertaking a mass prophylaxis plan, the PHS should identify the population at risk, the sort and the availability of medication to be applied, and the mechanism of administration of such a plan. The decision to apply a mass prophylaxis plan is based on various factors, such as data from epidemiological analysis, hospital bed availability, availability and determination of the quantities of drugs required to support the plan, immunity delays till antibodies development, side-effects of a massive immunization and the estimated number of exposed or ill people [47]. For starting mass prophylaxis, the following admissions should be considered:

1. An adequate stockpile of vaccines and medications is maintained at the national level;

2. The biohazard situation is anticipated to (or already did) overwhelm the regular public health care system;

3. There are shortfalls in local capacity to dispense disease-specific health care services;

4. Multiple biohazard incidents in many locations are already involved with, or without parallel requests for specialized health care provision;

5. A request for international aid has already proceeded.

Vaccination or prophylactic antibiotic treatment of healthcare providers may also be sound, especially in the biological threat of category A biohazard agents, since the occurrence of infection in that cohort will not only severely compromise the ability of PHS to manage the incident, but will also lead the population to the erroneous perception that health care personnel constitute a high-risk source of infection [48].

Personal Protection/Decontamination Issues

During the spread of an aerosolized biological agent, the primary route of exposure will be via the airways and respiratory tract. Respiratory protection will then be the most important component of physical protection. Furthermore, all patients in healthcare facilities with suspected or confirmed bioterrorism-related illnesses should be managed to utilize standard precautions; these are designed to reduce transmission from both recognized and unrecognized sources of infection in healthcare units [49].

Adequate procedures for routine cleaning, disinfection, contaminated waste disposal, and sterilization of equipment and environment should also be available [50]. For category, agents additional precautions may be needed to reduce the likelihood of transmission. Since in most bioterrorism scenarios anthrax is considered the most likely agent to be dis¬seminated, it is worth noticing that anthrax spores can persist in the environment for months or even years, which requires careful evaluation of the decontamination process [51].

Protection of health-care personnel against infection remains particularly problematic; personal protective equipment should be affordable, effective, and available to use with minimal training and under harsh environments [52]. If agents of contagious diseases are released, standard precautions include basic hygiene and infection-control measures, e.g. gloves, hands-washing (whether or not gloves are worn), PPE masks with eye protection (or face shield), and disposable gowns. In parallel, avoiding as possible direct contact with secretions from infected individuals, keeping exposed persons away from public places, and isolating suspected or symptomatic cases, may be essential in limiting secondary spread. These guidelines may though be impractical in case the number of patients presenting to a healthcare facility is too large to allow routine isolation strategies. The fact that in a mass bioterrorism incident many victims may go to hospitals on their initiative has important implications for the application of triage and decontamination protocols. Grouping affected patients who present with similar syndromes into a designated section of a clinic may be a vital alternative to the dissemination of infection. Community containment strategies, including isolation and quarantine, are the ultimate measures to prevent further transmission. Isolation and quarantine are optimally performed voluntarily, but basic legal authority should be in place for mandatory isolation and quarantine of people and communities when necessary to protect public health [53].

Communication in crisis

Public preparedness and response capability relies greatly on efficient information dissemination in a clear, reassuring, and unemotional language. That chain of mostly non-confidential intelligence contains readily apprehensible, explicit, and comprehensive instructions of clinical and epidemiological information and communication channels, discouraging the parallel overreaction of the public after an incident. The population should also be told what signs and symptoms to watch out for and whom to call or where to go if they appear. If specific preventive measures are available to mitigate exposure and infection, the public must be clearly and rapidly informed. Failure to provide a clear public information flow-line may enhance anxiety, fear, and misinterpretations, mainly among citizens who feature non-specific symptoms of exposure to a possible biological agent and that may result in local health facilities becoming overwhelmed by uninfected or panicked patients. Convincing and trustable spokespersons, including respected clinicians, skilled scientists, and public authorities must be able to represent the PHS at all stages of the crisis, also taking into account that the global distribution of social networking increases the dissemination of false or misleading information [54].

Mass Fatality care

During a bioterrorist outbreak, laboratories that receive or dispose of suspicious human body specimens must be informed of relevant clinical and operational protocols. Autopsies should be conducted under full personal protective measures, especially when there is an increased risk of aerosolization or splatter of infectious liquids [44]. Concerning management of bioterrorism fatalities, morgue and funeral protective planning should also be implemented along with prompt and accurate electronic reporting details [18].

Psychological Aspects

The psychological impact, even from a minor bioterrorism-related event may be disproportionately strong55. After such an attack, panic can well affect both victims and healthcare personnel. Psychological reactions following a bioterrorist attack include fear, anger, panic, inappropriate or unrealistic worry about possible contagion or illness, social withdrawal, depression, or demoralization. To handle patient and public fears, the psychological preparedness for bioterrorism should aim at minimizing panic by offering a clear explanation of risks and medical support, and by avoiding unnecessary isolation or quarantine. Furthermore, excessive anxiety in unexposed individuals, experiencing objective but irrelevant clinical symptoms should be treated with non-medical psychological support or on occasion with anxiolytic medication administration. Concerning in particular the healthcare workers, their fears can be managed by bioterrorism readiness education, protection plans, and encouragement of participation in acting field-level roles (e.g. disaster triage volunteering) [56].

CONCLUSION

Increased preparedness, response planning, investments, training, and exercises constitute perhaps the best defense of PHS to mitigate the effects of bioterrorist attacks. Most importantly, citizens must always be aware of a possible deliberate biological contamination and how to respond appropriately. Bioterrorism incidents of the near past prove that public mortality and morbidity can be further reduced if a functional protocol would allow first responders, hospitals, and officials of the PHS to steer more efficiently the public expectations.

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ORIGINAL ARTICLES

Ovarian cancer under the magnifying glass

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Abstract: *Cancer is a pathology generating problem to the public health systems all over the world.*

The etiology and pathogenesis of ovarian cancer are still partially unknown. It is still a challenge when it comes to the origin, the molecular basis of ovarian carcinogenesis, tumor progression, and therapy options of ovarian cancer.

This paper brings an overview of ovarian cancer, starting with the pathogenesis, histopathological forms, and molecular biology of ovarian cancer, and follows the growth factors as molecular targets for specific therapy.

Keywords: *ovarian cancer, pathogenesis, molecular biology, growth factors*

INTRODUCTION

Ovarian cancer is a pathology that throughout history has sparked both controversy and questions, some of which remain unanswered to this day. Over time we have been working hard towards laying the foundation for the current therapeutic standard in addressing existing ovarian tumors.

Following the epidemiological data and the steps taken gradually by our forerunners in their pioneering work in both surgical as well as drug therapy, but also the new findings in the field and the literature developed, overall representing a rich scientific dowry, we realize that we are faced with pathology that over time rose many difficulties for clinicians, researchers, and patients under therapy.

EPIDEMIOLOGY AND PATHOGENESIS DATA

Cancer is a pathology generating problem to the public health systems in Romania, its incidence being among the lowest in Central and South-East Europe, whilst having a higher mortality rate [1]. Both body functions and immune response are involved in the pathogenesis of cancer and angiogenesis, hypoxia and inflammation can be identified at the cellular level [2].

The etiology and pathogenesis of ovarian cancer are still partially unknown [3, 4].

When determining the origin of ovarian cancer, there are several theories including the theory of the de novo ovarian cancer [5], the hypothesis of the inclusion of cysts [6, 7, 8] based on a Mullerian phenotype causing the malignant transformation of the mesothelial ovarian cortex corresponding to the serous, endometroid, mucinous, clear cell, transition types, but also to the Mullerian epithelium found outside the uterus, fallopian tube, cervix [7, 8, 9, 10, 11]. The fallopian tube may be more or less involved in the origin of serous ovarian cancer, both through the proliferation of fallopian secretion cells and through its vicinity, causing dysplastic lesions, the inclusion cysts formed at the time of ovulation near the fimbria causing the

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formation of endosalpingiosis [6, 12, 13], the transformation of epithelial ovarian inclusions, of the serous adenomatous cyst into an ovarian borderline serous tumor which may become invasive carcinoma [14, 15, 16].

PECULIARITIES OF HISTOPATHOLOGICAL FORMS OF OVARIAN CANCER

Ovarian cancer has the following histological types according to WHO (World Health Organization): serous, endometrioid, mucinous (most common), clear cell, transitional cell, squamous cell, and undifferentiated and mixed epithelial. Tumors can be benign, malignant, or borderline, and, depending on the subtype, they can be low grade or high grade (depending on nuclear atypia and mitosis) [17, 18]. Borderline tumors are considered potentially indolent and weakly malignant [19, 20, 21].

Ovarian cancer can be classified as follows: type I consisting of low-grade carcinomas -serous, endometrioid, clear cell, mucinous, and Brenner tumors, they are generally indolent, may have originated in borderline tumors and endometriosis, showing molecular entities such as KRAS, BRAF, ERBB2, PTEN, CTNNB1, PIK3CA, and type II consisting of high-grade carcinomas, serous, endometrioid, mixed mesodermal malignant tumors (carcinosarcomas) and undifferentiated carcinomas, they are generally aggressive, characterized by genetic instability, may have originated in intraepithelial carcinoma of the uterine tube, their molecular entity presented as mutations at the TP53 level [19, 20, 21, 22, 23, 24]. Both types may have originated in the extra ovarian tissue that can be implanted in the ovary [25].

Following the changes made in the FIGO staging, a new staging resulted. As recommended by FIGO, it is indicated that histology, including the grading, should be part of the staging process and also that the primary site (ovary, fallopian tube, peritoneum) should be mentioned where possible, and if this is not possible, this also must be mentioned. The damage to the retroperitoneal lymph nodes must be proven histologically or cytologically and stage I tumors with dense adhesions can be classified as stage II if malignant cells are proven to be histologically present [6, 26]. According to the International Agency for Research on Cancer (IARC), from histology's point of view, we can differentiate between carcinomas (serous carcinomas, mucinous carcinomas endometrioid carcinomas, clear cell carcinoma, NOS adenocarcinoma, other specific carcinomas, nonspecific carcinomas), sex-cord stromal tumors, germ cells tumors, other specific cancers (these including malignant Brenner tumors, carcinosarcomas, mixed Mullerian tumors), non-specific cancers [27, 28].

MOLECULAR BIOLOGY OF OVARIAN CANCER

The molecular basis of ovarian carcinogenesis and progression are not yet fully elucidated, considering the genetic and epigenetic alterations that translate into alterations of the molecular signaling pathways involved in survival, proliferation and cell death, adhesion, and angiogenesis.

Carcinogenesis involves inadequate adjustment mechanisms of the signaling pathways governing cell cycle, differentiation, apoptosis, expression of oncogenes and/or antioncogens, the action of transcription, and growth factors [29, 30, 31].

Amongst the RB family members, the RB gene (retinoblastoma gene) is involved in ovarian carcinogenesis. The majority of malignant ovarian tumors have had a strong expression of the pRb about a normal ovary where the protein is barely detectable [32, 33]. It was demonstrated that overall survival was positively influenced by the positive expression of p21 and p27 in patients with negative p53 only [34]. The p53 is the most relevant oncosupressor gene. Inactivation of the p53 oncosupressor gene's function appears to be an early event in hereditary ovarian cancer induction characterized by germ mutations of the BRCA1 tumor suppressor gene. The somatic mutation of p53 is the most common molecular change that occurs in epithelial ovarian cancer. The immune-histochemical expression of p53 protein was associated with the highly differentiated serous type, positive cytology, residual tumor, and stage of disease [4, 35, 36, 37, 38].

Studies in the specific literature suggest that the expression of Bcl-2 and Bax is an important prognostic marker [39].

The ovary is subject to cyclical changes where the angiogenesis process is continuous [3]. VEGF (vascular endothelial growth factor) is a growth factor that stimulates the angiogenesis process and increases microvascular permeability [40]. Ovarian cancer causes the release of VEGF in response to conditions of hypoxia and acidity, the serum and ascitic fluid VEGF values being elevated with malignant tumors as opposed to benign [40, 41, 42 43]. Type I and II ovarian tumors differ in molecular-genetic evidence just as much as they differ morphologically [44].

High-grade serous ovarian cancer, which is part of type II tumors, has frequently known mutations of TP53 (in more than 80% of cases) and amplification of CCNE1, which are characteristic of type II tumors, but rarely mutations that characterize type I tumors such as KRAS, BRAF, ERBB2, PTEN, CTNNB1 and PIK3CA [44, 45, 46, 47]. Type I tumors are more stable genetically and show distinctive pattern mutations that happen in a particular cell type, as opposed to type II which present morphological and molecular homogeneity, are genetically unstable with a high-frequency mutation of TP53, thus suggesting that ovarian tumors develop on different molecular pathways [44, 48]. The mutations in the KRAS or BRAF lead to subsequent activation of the MAPK signaling pathway (mitogen-activated protein-kinase) [49, 50]. It was shown that some inflammatory markers, glucose protein transporter 1 (Glut-1), iNOS, cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), nuclear factor kappa B (NF-kB) are involved in the initiation and progression of cancer [51, 52]. Immunohistochemical analyses showed statistically increased levels of expression of Glut-1, iNOS, COX-1, COX-2 in type II ovarian tumors, but no statistical difference of expression of NF-kB in ovarian tumors type I and II [52].

Researchers have identified five molecular subtypes presenting clinicopathological characteristics and survival rates for ovarian epithelial cancer, which are: Epi-A, Epi-B, Mes and Stem-A, and Stem-B. Epi-B and Stem-A subtypes are independent prognosis factors [53].

The BRCA1 gene was often studied because of the proven implications in the transmission of hereditary breast and ovarian cancer. In molecular terms, we distinguish as follows: low-grade serous ovarian carcinoma presents KRAS and BRAF mutations, and the high-grade present mutations of TP53 and abnormalities of BRCA. Serous high-grade ovarian carcinoma shows an increased nuclear expression of Ki-67 and has immunoreactions for p53, BRCA1, WT1, p16. Mucinous ovarian carcinoma presents KRAS mutations in its tumorigenesis, which are immunoreactive for CK7 and CK20. Endometrioid ovarian carcinoma shows mutations of the β-catenin (CTNNB1), PTEN, and ARID1A. Endometrioid ovarian carcinoma is immunoreactive for vimentin, CK7, CK20, the epithelial membrane antigen, estrogen, and progesterone receptors. Ovarian clear cell carcinoma presents ARID1A mutations, lack of protein BAF250, is positive for HNF1-β, and is negative for ER and WT1 [5, 54, 55, 56, 57, 58].

GROWTH FACTORS AS MOLECULAR TARGETS FOR SPECIFIC THERAPY

Knowing that VEGF is involved in tumor growth, invasion, and dissemination to the lymph nodes, liver, lungs, numerous drug agents are being used with the target role occupied by VEGF in ovarian cancer, especially when peritoneal carcinomatosis and/or ascites are present. One of the agents used in neutralizing the VEGF is bevacizumab, a recombinant humanized monoclonal antibody that stops the binding of VEGFRs on all the VEGF isoforms, thus preventing tumor growth and ascites production. Paclitaxel can be used in combination with bevacizumab acting synergistically and with the same effects. Bevacizumab used in combination with cisplatin increases the survival rate [59, 60, 61].

Agents acting as inhibitors of tyrosine kinase receptors are counted as VEGFR antagonists (Ramucirumab, Cediranib, Semaxanib) and agents that have as target multiple tyrosine kinase receptors (Sunitinib, Sorafenib, Vatalanib, Intedanib, Pazopanib, Motesanib, Vandetanib, AEE788) [62, 63].

VEGF overexpression in ovarian cancer makes it a therapeutic target, thus aiming at the inhibition of neo-vascularization, but also anti-angiogenic agents. VEGF is a dimeric protein, a member of the VEGF/PDGF, including VEGF-B, VEGF-C, VEGF-D, PIGF, and PDGF, involved in angiogenesis, proliferation, survival, migration of endothelial cells and stimulated by hypoxic conditions [12, 60, 64, 65].

Anti-VEGF therapy has been shown to play an important role in female patients with primary and recurrent ovarian cancer [60, 61, 66]. The use of bevacizumab can have aggressive complications that can lead to death, the risk is even higher when used in combination with chemotherapy, particularly platinum-based (cisplatin, carboplatin, oxaliplatin) or taxanes (paclitaxel, docetaxel) [66, 67, 68, 69]. As standard therapy in advanced ovarian cancer, cytoreduction surgery followed by platinum-based chemotherapy is used with a fairly good response, but sometimes resulting in tumor recurrence within 5 years and therapeutic resistance [66]. In case of resistance to platinum other chemotherapeutics are used such as metronomic paclitaxel, docetaxel, topotecan, liposomal doxorubicin, etoposide, gemcitabine, but the response is quite limited with a fairly low survival rate [50]. Attempts have been made to develop different therapies aiming at the interruption of angiogenesis, molecular target, inhibition of the Poly- (ADP) Ribose polymerase function (PARP), growth factors such as insulin-like growth factor (IGF) [70, 71]. Treatments often target the vascular endothelial growth factor pathway (using bevacizumab or aflibercept), the mechanisms of DNA repair (using iniparib or olaparib), or are guided against folate ways (using pemetrexed farletuzumab, vintafolid) [61].

Oral therapy with olaparib is currently being used as maintenance therapy, this being a PARP inhibitor used on patients with recurrent serous ovarian cancer sensitive to the platinum-based therapy, where the existence of mutations in BRCA has been proven and where the patient had two or more courses of platinum-based chemotherapy and showed a partial or complete response to the most recently used platinum-based chemotherapy [22, 71, 72, 73, 74, 75, 76].

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ORIGINAL ARTICLES

Comparison of the effect of intravenous ketamine and midazolam as a premedication in children undergoing cochlear implantation at the time of separation from parents

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Abstract: *Background and aim: Preoperative anxiety and fear of separation from parents are some of the most important preoperative problems for children with deaf muteness. Here, the effect of intravenous administration of ketamine and midazolam was compared on sedation in children who candidates for choral cochlear implantation at the time of separation from parents.*

Patients and methods: This double-blind, prospective clinical study was performed on 74 children with deaf-muteness before cochlear implantation. Patients were randomly divided into two groups, midazolam (0.1 mg /kg) and ketamine (1 mg/kg). The sedation anxiety score scale (SASS) form was filled in to assess the anxiety and sedation status for each child. The children's relaxation score from recovery was assessed by the PACU sedation score scale (PACUSSS).

Results: The average of the children's anxiety score after the injection of both drugs during the separation from their parents was 3, and there was no significant difference in the mean of SASS between the two groups (p>0.05). The mean PACU sedation score scale in both ketamine and midazolam groups was 1.08 ± 0.89 and 1.32 ± 1.01, respectively (p = 0.27). The mean of extubation and recovery times in the ketamine group were 10.44 ± 4.47 and 30.27 ± 6.87, respectively, while the mean extubation and recovery times in the midazolam group were 11.83 ± 3.33 and 29.81 ± 6.31 minutes, respectively.

Conclusion: Both ketamine and midazolam caused equal sedation scores in children with deaf muteness at the time of separating from their parents.

Keywords: *deaf-muteness children, cochlear implantation, ketamine, midazolam, sedation, anxiety*

INTRODUCTION

According to the world health organization (WHO), more than 250 million people worldwide suffer from disabling hearing loss and 34 million of which are children [1, 2]. Pediatrics hearing loss, which often results from genetic defects, can be associated with multiple problems. Children with hearing impairment not only have difficulty talking, but also they do not respond to sounds [2]. Therefore, early diagnosis and treatment of this complication are very important for the disease improvement and quality of their life.

Cochlear implantation remains the only reliable therapeutic option for patients with hearing loss and deaf-mutism [3, 4]. However, this surgery is time-consuming and requires general anesthesia which may be associated with

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emergence agitation, restlessness, disorientation, and anxiety in children [5, 6]. Deaf-muteness children like healthy children recognize environmental events and preoperative anxiety and the fear of separation from parents are some of the most important preoperative problems for these children [5, 7]. Therefore, sedation or premedication is often needed for deaf-muteness children during their separation from parents and entrance to the waiting room.

Midazolam and ketamine are commonly used as premedication in children undergoing medical procedures [8]. Midazolam is an imidazole benzodiazepine that has depressant effects on the central nervous system and provides sedation, muscle relaxation, and amnesia before procedures [9, 10]. It has a rapid onset of action following oral, intravenous, intramuscular, nasal, and rectal administration [10]. A great number of studies have used midazolam as a safe premedication without any serious adverse effects for sedation of children during procedures [11, 12]. It has been also shown that preoperative oral midazolam reduces separation anxiety and emergence agitation in children following anesthesia [6].

Ketamine is a general anesthetic with sedative and analgesic properties which is commonly used as a premedication for sedation in children [13]. Its favorable effects on cardiovascular and pulmonary parameters are a reason by which physicians use this drug as a sedative [14, 15]. Therefore, these data indicated that both midazolam and ketamine are safe effective sedative premedication agents which can be used in children undergoing painful producers [16]. Although many studies have investigated the effect of midazolam and ketamine for sedation of children following medical surgery, no study evaluated the preoperative effect of these drugs on sedation in children with hearing loss and deaf mutism. Therefore, we designed this study, for the first time, to compare the preoperative effect of midazolam and ketamine as premedication on sedation in deaf-mutism children during their separation from parents and before cochlear implantation.

PATIENTS AND METHODS

Patients

This double-blinded, prospective clinical trial study was conducted on 74 children with deaf-muteness who were referred to Baqiyatallah Hospital for cochlear implantation from June 2019 to November 2019.

This study was registered in the Iranian Registry of Clinical Trials under no. IRCT20161022030421N3 cod. The protocol of the study was confirmed by the ethics committee of Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1397.031).

Written informed consents were signed by all parents. A questionnaire containing demographic data of all patients (e.g. age, sex, weight, and history of any previous illness) was filled before surgery. Children aged from 6 months to 5 years old, and American Society of Anesthesiology (ASA) Class I and II were included in the study. Children who met the following criteria were excluded from the study: (i) sensitivity to ketamine or midazolam, (ii) history of treatments with neuropsychiatric drugs, (iii) age less than 6 months, (iv) increased intracranial pressure (ICP), (v) history of seizure, and (vi) history of chronic diseases such as diabetes, liver disease, and upper and lower respiratory tract infection.

Intervention and measurements

After preliminary considerations and patient selection based on inclusion and exclusion criteria, using a computer-generated randomization list, children were divided into two groups of midazolam (n=37) and ketamine (n=37). Intravenous (IV) access was performed using local anesthetic cream 1 hour before their admission in the operating room. The sedation anxiety score scale (SASS) questionnaire was filled out by an observer who was unaware of the study groups. The questionnaire was filled out based on children's behavior in the pre-operative preparation room when they were with their parents. The SASS score ranged from 1 to 5 based on the child's status (Table 1).

Table 1: The SSAS scoring questionnaire

Score	Status	More details
1	Unconscious	Sleepy, no response to mild motor stimulations
2	Sleepy	Sleepy, may have closed eyes but react to mild stimulations
3	Normal	Calmly, comfortable sited or sleeping with eyes open
4	Anxious	Looks afraid, no adhesion to parents, without crying
5	Very rest less	Crying, stomping, or adhesion to parents

After SASS scoring, midazolam (0.1 mg/kg) or ketamine (1 mg/kg) drugs were intravenously injected into children by another person who was unaware of the study groups. Following the injection of the drug, pulse oximetry monitoring was provided for patients. Furthermore, the SASS form was filled out again following the drug's injection just after children felt calming and when they were separating from their parents. Children were then transferred to an operating room and the observer measured the extent of child tolerance during the anesthetic induction by SASS.

Pethidine (1 mg/kg) and atropine (0.2 mg/kg) were injected into children as premedication and then anesthesia induction was performed with propofol and atracurium. Sevoflurane was applied to maintain the anesthesia. Cefazolin, hydrocortisone, and dexamethasone were injected into all children. All children also received ondansetron (1 mg/kg). Children were transferred to the recovery room after surgery and the observer assessed the sedation rate by the post-anesthesia care unit (PACU) sedation score scale (PACUSSS) (Table 2). The PACUSSS score ranged from 0 to 4 based on the child's status. When children obtained the safe discharge criteria from the recovery room, they were transferred to the section. The observer also recorded any postoperative side effects or complications such as nausea, vomiting, emergence agitation, and decreases in arterial oxygen saturation below 92%. The duration of surgery, extubation time, and recovery time was also recorded.

Table 2: The PACU sedation score scale questionnaire

Score	Status
0	Slept, calmly, and without crying
1	Whining, Not rest less
2	Crying, Slightly rest less
3	Continuous crying

Statistical analysis

The results of this study were analyzed by SPSS software (IBM, version 19). Quantitative data were analyzed using the descriptive program and presented as Mean ± SD. Crosstabs and Chi-Square tests were used to compare the percentages or frequencies between the two groups. The comparison of the mean of parametric data between midazolam and ketamine groups was compared by independent student samples-test. In this study, p< 0.05 was considered statistically significant.

RESULTS

In this study, 74 patients were entered into the survey. 32 patients were boys (43.2%) and 42 patients (56.8%) were girls. There was no significant difference in the percentage of male and female genders between midazolam and ketamine groups (p=0.24; Table 3).

The mean age of all participated patients was 29.55 ± 16.92 months. There was no significant difference in the mean age of patients between the ketamine and midazolam groups (p=0.94; Table 3).

The mean weight of all participated patients was 13.18 ± 3.74 kg. There was no significant difference in the mean weight of patients in the ketamine and midazolam groups (p=0.72; Table 3).

Table 3: Demographic characteristics of patients in both groups

Parameters	Midazolam	Ketamine	p-value
Age (months)	29.7 ±17.68	29.4 ±16.36	0.94
Gender			
Male (%)	18 (48.64%)	14 (37.83%)	0.24
Female (%)	19 (51.35%)	23 (62.16%)	
Weight (kg)	13.33 ± 4.3	13.02 ± 3.13	0.72

A comparison of the mean of SASS between the ketamine and midazolam groups is shown in Table 4. There was no significant difference in the mean of each score between the two groups. The mean score of children status in the waiting room with their parents was approximately 4, indicating they were anxious. The mean score of children's status after drug injection, when separating from their parents was decreased to around 3, which suggests the normal status of the patients in both groups. Furthermore, the mean score of the patient's condition at the anesthetic induction time ranged from 2.5 to 3, which indicates they had sleepy or normal conditions.

Table 4: Comparison of the mean of SASS between ketamine and midazolam groups

Parameters	Midazolam	Ketamine	p-value
In the waiting room with parents	4 ± 0.78	3.89 ±0.9	0.58
After drug injection, when separating from parents	2.91 ± 0.49	2.83 ± 0.55	0.5
Anesthetic induction time	2.62 ± 0.68	2.59 ± 0.68	0.86

The prevalence of each SSAS item in the waiting room before pre-medication is presented in Table 5. There was no significant difference in the frequency of each item in patients between the two groups (p=0.6). Most of the children (40.54% in the ketamine group and 48.64% in midazolam groups) had the anxious condition before premedication. Furthermore, 27.02% of children in the both ketamine and midazolam groups were very restless. The percentage of normal conditions in children in the midazolam and ketamine groups was 21.62% and 29.72%, respectively.

The prevalence of each SSAS item after drug injection, when separating from their parents is presented in Table 5. No significant difference was found in the frequency of each item between the two groups (p=0.68). Most of the patients (67.56% in the ketamine group and 75.67% in midazolam groups) had the normal condition. 24.32% of children in ketamine and 16.21% of patients in the midazolam group were sleepy. Only 8.1% of patients in both groups had anxiety conditions.

The percentage of each SSAS item at the time of anesthetic induction is shown in Table 5. There was no significant difference in the frequency of each item between the two groups (p=0.69). Most of the patients (54.05% in the ketamine group and 48.64% in midazolam groups) had the normal condition at the time of anesthetic induction, while 40.54% of children in ketamine and 45.94% of patients in the midazolam group were sleepy. Only 2.1% of patients in both groups were very resting less.

Table 5: The prevalence of pediatrics SSAS in different conditions

Parameters	Midazolam	Ketamine	p-value
Before pre-medication			
Unconscious	0	1 (2.7%)	
Sleepy	1 (2.7%)	0	
Normal	8 (21.62%)	11 (29.72%)	0.6
Anxious	18 (48.64%)	15 (40.54%)	
Very rest less	10 (27.02%)	10 (27.02%)	
Separating from parents			
Unconscious	0	0	
Sleepy	6 (16.21%)	9 (24.32%)	
Normal	28 (75.67%)	25 (67.56%)	0.68
Anxious	3 (8.1%)	3 (8.1%)	
Very rest less	0	0	
Anesthetic induction time			
Unconscious	0	1 (2.7%)	
Sleepy	17 (45.94%)	15 (40.54%)	
Normal	18 (48.64%)	20 (54.05%)	0.69
Anxious	1 (2.7%)	0	
Very rest less	1 (2.7%)	1 (2.7%)	

The prevalence of children's relaxation scores during the recovery time can be seen in Table 6. There was no significant difference in the prevalence of children's condition between the two groups (p=0.64). Most of the patients (37.83% in ketamine and 32.43% in midazolam groups) had to whine without rest less, whereas 29.72% of patients in ketamine and 24.32% in midazolam groups were kept without crying. Only 5.4% of children in ketamine and 13.5% in midazolam were continuously crying.

Table 6: The prevalence of children relaxation scores during the recovery time

Children status	Midazolam	Ketamine	p-value
Slept, calmly and without crying	9 (24.32%)	11 (29.72%)	0.64
Whining, Not rest less	12 (32.43%)	14 (37.83%)	
Crying, Slightly rest less	11 (29.72%)	10 (27.02%)	
Continuous crying	5 (13.51%)	2 (5.4%)	

There was no significant difference in the mean of extubation (p=0.2) and recovery times (p=0.76) between the two groups. The mean of extubation and recovery times in the ketamine group were 10.44 ± 4.47 and 30.27 ± 6.87 minutes, respectively, while the mean extubation and recovery times in the midazolam group were 11.83 ± 3.33 and 29.81 ± 6.31 minutes, respectively.

We didn’t observe any significant side effects from both studies' drugs. In the waiting room, no child showed a drop in SPO2 below 92% and there was no need for mask ventilation for these patients. Nausea and vomiting were the most important complications after recovery. Three patients (8.1%) in the ketamine group had nauseous and 10 (27.02%) in the midazolam group suffered from nausea (p=0.023). Also, one patient in the midazolam group had vomiting. None of the patients in both groups experienced emergence agitation and decreased arterial oxygen saturation in recovery time.

Interestingly, our findings showed that 37.03% of children in the midazolam group start laughing after receiving premedication (Figure 1), but this condition was not observed in patients in the ketamine group.

Figure 1: Children's reaction after premedication with midazolam. About 37% of children in the midazolam group start laughing after receiving this premedication, but this status was not observed for children in the ketamine group

DISCUSSION

In this study, we compared the intravenous administration effect of ketamine and midazolam as a premedication in children undergoing cochlear implants at the time of separating from their parents. To the best of our knowledge, the use of intravenous midazolam and ketamine for sedation of children with deaf-muteness before cochlear implantation has not been investigated yet. Our data have revealed that there was no significant difference in the mean anxiety score between the ketamine and midazolam groups. Children were anxious (mean sedation anxiety score of 4) before premedication with ketamine and midazolam, while they reached normal status (mean sedation anxiety score of 3) after premedication. Furthermore, most children had sleepy and normal status after premedication at the time of anesthetic induction. There was no significant difference in the mean PACU sedation score between the ketamine and midazolam groups. Furthermore, we didn't find any serious adverse effects in each group; however, nausea occurred more frequently in the midazolam group. More importantly, we found that 37.03% of children in the midazolam group started laughing after receiving the premedication, while this condition was not observed in patients in the ketamine group. However, this mechanism is unclear.

A great number of studies compared the effect of ketamine and midazolam as premedication in the prevention of anxiety, pain score, and emergence agitation in children and adults before entering the operating room. For example, Jeong et al. [17] demonstrated that intravenous administration of ketamine (1 mg/kg) before entering the operating room significantly reduces separation anxiety, postoperative pain score, and also the incidence of emergence agitation in children following ophthalmic surgery. Ozdemir et al. [18] reported that intravenous, oral, and rectal administration of ketamine and midazolam had equal sedation effects in children with malignancy. Several studies compared the combination of ketamine and midazolam as premedication in different groups. For example, Golpayegani et al. [19] compared the oral administration of ketamine and midazolam as sedative agents in pediatric dentistry. They found that the Ketamine-Midazolam combination provides sufficient sedative effect in children's behavior. In another study, Wathen et al. [20] found that intravenous ketamine and its combination with midazolam had equal effective sedation in children. They also found that vomiting occurred more frequently in the ketamine group, while oxygen desaturation occurred more frequently in the ketamine-midazolam group. Barkan et al. [21] evaluated the efficacy of oral midazolam alone and oral midazolam-ketamine combination in 60 children requiring laceration repair. They didn't find a significant difference in the mean pain score between the two groups; however, midazolam and ketamine combination caused deeper sedation than midazolam alone. Additionally, no clinically significant adverse effects were found in either group which was in agreement with the results of our study. In another study, the effect of high dose oral ketamine (10 mg/kg) with midazolam (0.7 mg/kg) was evaluated in children during laceration repair [12]. The results showed better tolerance to local anesthetics and deeper sedation in children treated with ketamine with no serious adverse events in both groups [12]. Kim et al. [6] investigated the intravenous effect of ketamine (1 mg/kg) and midazolam (0.1 mg/kg) on emergence agitation in children at the postanaesthetic care unit. They showed that premedication with these drugs significantly decreases preoperative anxiety. However, they found that premedication with ketamine was more effective than midazolam in the prevention of early post-operative emergence agitation. Similarly, in this research, we used the same doses of ketamine and midazolam for the improvement of preoperative anxiety and sedation in children. Our data showed that premedication with ketamine and midazolam significantly improves anxiety and sedation in children without any serious side effects.

Our findings have also revealed that there is no significant difference in the mean of extubation and recovery times between the two groups. Similarly, Kim et al. [6] didn't found a significant difference in the mean of extubation and recovery times between children in midazolam and ketamine groups. Ozdemir et al. [18] reported that ketamine and midazolam are equally effective in recovery time improvement. Recent evidence has proposed that the recovery time in patients receiving ketamine and midazolam is dependent on multiple factors such as drugs dosage, kind of surgery, and the physician on call. For example, previous studies didn't observe a significant improvement in recovery times when children were treated with higher doses of ketamine (2.5-3 mg/kg) and midazolam (0.25-0.5 mg/kg). Several studies reported higher recovery times in children treated with higher doses of midazolam (0.5 mg/kg) and ketamine (5 mg/kg) [21]. Abdolahi et al. [22] reported that low dose administration of ketamine (0.5 mg/kg) during anesthesia induction did not affect postoperative pain score, postoperative nausea, and vomiting, as well as perioperative additional analgesic requirements in ophthalmic surgery. However, premedication with ketamine improved the extubation time. Based on these data, in our study, children were treated with 0.1 mg/kg midazolam and 1 mg/kg ketamine. Therefore, these data suggest that both drugs are equally effective for the prevention of anxiety in children before surgery and separating from their parents. However, the dose of drugs and kind of surgery are important factors affecting sedation and anxiety which should be considered before administration.

CONCLUSION

In summary, the results of the current clinical study indicated that both midazolam and ketamine are equally safe with desirable sedation effects on children with deaf-muteness before separating from their parents for cochlear implantation. Premedication with 0.1 mg/kg midazolam or 1 mg/kg ketamine decreases preoperative anxiety in these cases. Both drugs improved recovery and extubation times without any serious adverse effects. However, nausea occurred more frequently in the midazolam group. More importantly, children in the midazolam group start laughing after premedication with this drug which caused their parents felt well, while children in the ketamine group didn’t show this reaction. Further studies with a larger sample size should be conducted to determine the optimal dose and to establish the safety of the combination therapy.

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ORIGINAL ARTICLES

Evaluation of in-vitro bactericidal activity and anticancer effects of venom fractions of *Pseudocerastes persicus* snake

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Abstract: *Introduction: It was reported that snake venom contains a wide mixture of proteins and peptides with various toxicological and pharmacological effects. In recent years, the isolation of effective molecules from snake venom as a drug to treatment of some incurable diseases is considered by many biological research centers. This investigation aimed to determine electrophoretic and chromatographic patterns of Pseudocerastes persicus snake venom and in-vitro study of bactericidal and anti-cancer properties of its fractions in a human hepatocellular liver carcinoma cell line (HepG2).*

Materials and methods: The fractions of venom were isolated and collected using preparative RP-HPLC. The Bactericidal activity of fractions in 20 µg/ml of protein concentration was investigated toward Gram-negative and Gram-positive bacteria. Antibacterial susceptibility tests were done using MTT and MIC assays. Furthermore, cell viability was measured by MTT reduction assay and confirmed with Neutral red uptake assay following exposure of HepG2 cancer cells to 20 and 40 µg/ml of protein concentrations of fractions having more antibacterial properties. Apoptotic effects were investigated using the comet assay.

Results: SDS-PAGE pattern of the crude venom revealed 10 major bands with molecular weight ranging from 13.2 to 99.25 kDa and the single-step separation of different fractions from the venom by HPLC resulted in isolation and collection of 11 fractions. The results of this study showed that two fractions of snake venom have significant antibacterial activity against Gram-positive as compared to Gram-negative bacteria. On the other hand, our findings indicated that one of them is not toxic to the HepG2 cells.

Discussion: We for the first time reported bactericidal and anticancer effects of isolated fractions of Persian horned viper venom. These properties make components of these fractions a favorable source for isolation of molecule(s) with antibacterial and antitumor activities.

Keywords: *snake venom, Pseudocerastes persicus, antibacterial, cytotoxicity, cancer*

INTRODUCTION

One of the serious problems of the medical community in the treatment of infectious diseases caused by bacterial agents, many bacteria are resistant to common antibiotics. These bacteria are becoming a serious clinical problem around the world (Jami al Ahmadi et al., 2010). This has

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prompted pharmaceutical firms to explore new antibacterial drugs or optimize antibiotics to help them produce new and effective drugs. Nowadays poison of poisonous animals is considered by many researchers as one of the most important natural sources for the isolation and identification of antimicrobial molecules and it has been a great success.

Reports indicate that, despite that the oral and tooth smears of poisonous snakes in a wide range of pathogenic bacteria, there is a small incidence of bacterial infection in the bites of the injured patients due to snakebite (Talan et al., 1991). These observations have led the researchers to point out that antibacterial compounds are found in Snake (Deivy et al., 2005).

The first report on antibacterial activity in the venom poison the families of Alpidea and Vipride was published in 1948 and 1968 respectively (Ferreira, 1956). Also Skarnes in 1970, for the first time reported antimicrobial activity in some of the enzymes found in the venom of snakes (Aloof-Hirsch et al., 1968). Studies of Zey et al. in 2000 also led to the isolation of peptides from Naja atra, which in vitro inhibited the growth of resistant bacteria of Mycobacterium tuberculosis and showed inhibitory activity (Xie et al., 2003).

Studies show that molecular compositions and especially proteins present in the veniphil genus of the Viperidae variety are very diverse. Important compounds of these toxins include serine protease, metalloproteinase, phospholipase A2 (PLA2), C-type lectin, acetylcholinesterase, L-aminoacids, and detergents (Markland, 1997).

Recent studies have shown that many of these compounds have antimicrobial properties (Jami al Ahmadi et al., 2010; Stabeli et al., 2004; Lu et al., 2002).

In this study, the antibacterial effects of protein fractions isolated from Pseudocerastes persicus snake and the anti-cancer effects of fractions with antibacterial properties were studied.

MATERIALS AND METHODS

Venom preparation

To prepare the venom from the snake, the snake venom safety tips were placed directly into the venom collection jar, and the venom was briefly injected into the venous gland. The toxic solution was kept at minus 20 °C until lyophilization until use in the experiments.

Microorganisms and cell lines used

Bacillus subtilis subsp. spizizenii a gram-positive and non-pathogenic bacteria (ATCC 6633), bacteria Escherichia coli a gram-negative bacteria (ATCC 25922), and pathogen Staphylococcus aureus a gram-positive bacteria (ATCC 25923) were prepared from the Persian Type Culture Collection (PTCC) of Iran and liver cancer cells (HepG2, C158) of Pasteur Institute of Tehran cell bank.

Studying the crude venom electrophoresis pattern using SDS-PAGE method

To study the electrophoresis pattern of crude venom of Pseudocerastes persicus snake, 12-15% gel (Chellapandi and Jebakumar, 2008) was applied to each well of 5-10 microliters of toxic solution (containing 20 to 15 micrograms of protein) denatured with an appropriate volume of sample buffer was injected.

Standard protein was used to estimate the molecular weight of protein bands.

Electrophoresis first started at 50 V and after passing the dyeing gel through the concentrating gel, electrophoresis continued at 150V until the color of the tracing reached about 1 cm from the end of the gel cassette. After staining the gel with coma blue and removing excess dye from the gel surface, the estimated molecular weight of crude venom protein bands was determined.

Isolation of crude venom fractions using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

Fragile venom of Pseudocerastes persicus snake using RP-HPLC preparation with UV detector (220 nm absorbance), C18 SUPELCOSIC TM Pcc-18 (59185) 250mm×21.2mm, 12µm] column and Gradient Linear Application [buffer A (acetonitrile) 20% containing 0.1% TFA) and buffer B (80% acetonitrile containing 0.1% TFA)] separated in 70 minutes. In the reverse phase method, a polar mobile and non-polar stationary phase are used and the material is separated by hydrophobicity (Steuten et al., 2007).

For the separation of crude venom fractions, 40 mg of dried powder in 20 ml of buffer (water). Containing 0.01% TFA containing 5% acetonitrile and dissolved for 2 hours at 4°C. After centrifugation, the insoluble material was removed from the suspension for 5 min at 10,000 rpm at 4°C. The supernatant was used for chromatographic patterning and fractions separation by HPLC. The corresponding fractions of each peak were collected manually in perfectly clean glassware free of any protein contamination and stored at -80°C after lyophilization. The retention time of each peak was recorded for each peak.

Protein assay

For antibacterial and cytotoxicity tests, some of the lyophilized venoms were dissolved in sterile distilled water twice. The amount of protein in the toxic solution was determined using Bradford (Bradford 1976) method and the protein-free fractions were excluded from further studies.

Examination of antibacterial properties

Culture media and antibiotics: MH (Mulberry Hinton) Company, Quelab, Canada, was used for bacterial culture. Tetracycline (Sigma, USA) at a concentration of 50 µg/ml was used to compare the effect of the venom with standard antibiotics.

Evaluation of the antibacterial effect of fractions using MTT assay

MTT assay is a colorimetric assay based on the reduction and breakdown of tetrazolium yellow crystals by the succinate dehydrogenase enzyme present in the cytoplasm and ultimately the formation of insoluble blue crystals. These crystals are soluble by the addition of DMSO (Hengwei et al., 2010). The bacterium was cultured in the Muller Hinton broth medium and then after 5 h when it reached the absorbance was equivalent to the absorbance of 0.5-McFarland (at 600 nm with an absorbance of 0.08 to 0.1) (Husniye et al., 2013). 5 µL per 96 plate sterile wells were cultivated. Fractions were added to wells at a concentration of 20 µg/ml and the final volume of each well was made up to 100 µL using a liquid culture medium (Muller Hinton Broth). In this experiment, tetracycline (50µg/ml) was used as a positive control, bacterial suspension and culture medium as a negative control, and non-bacterial culture medium as blank. The plate was incubated in the incubator for 23h at 37°C. Then 5µl MTT (5µg/ml concentration) was added to all wells. The plate was incubated at 37°C under dark conditions for one hour. Then 100 µL of DMSO was added to each well. After 2 hours of incubation in dark conditions, the absorbance of the wells at 595 nm was measured using a plate reader (Biotek, USA). The above experiment was performed 3 times and 3 wells (3 times repeated) were considered for each concentration. Bacterial viability after exposure to different concentrations of venom was calculated using the following formula (Husniye et al., 2013).

100 × (a/b) = Bacterial vital percent

a = Optical Absorption Sample - Blank Optical Absorption
b = Negative optical absorption - Blank optical absorption

Evaluation of antibacterial effect of fractions using MIC assay

In this study, we aimed to determine the inhibitory effects of crude venom on bacteria. The steps of the minimum inhibitory concentration test (Shebl et al., 2012) were similar to the MTT assay but after incubating the bacteria with different concentrations of the venom at 37 ° C for 24 hours without adding any biomarkers, the optical absorption of the wells at the wavelength of 605 nm was measured using a plate reader (Biotek, USA). The experiment was repeated 3 times and for each concentration 3 wells (3 times). The percent inhibitory effect of the standard venom and antibiotic effect was calculated using the following formula (Shebl et al., 2012).

100 × (a/b)]-1[= Inhibitory percentage of bacteria

a = Optical Absorption Sample - Blank Optical Absorption
b = Negative optical absorption - Blank optical absorption

Anti-cancer properties of fractions

Cell culture:

To eliminate possible contamination in the toxic solution required by cellular tests, 1% antibiotic-antimycotic (Invitrogen, USA) was added and used in experiments at 4°C overnight.

DMEM-F12 medium (Gibco, USA) containing 10% FBS (Fetal Bovin Serum) prepared from the company (Gibco, USA) was used for HepG2 cell culture. These cells were cultured in flasks containing 25-50 ml cube and stored at 37°C and 5% carbon dioxide. Replace the old culture medium every 2–3 days, and after providing the cell population required for each experiment using trypsin-EDTA prepared from Sigma-Aldrich, USA, isolated from the flask and after counted with neobars lam were used.

Evaluation of cytotoxicity of fractions by MTT assay

This procedure was performed according to the method of Zargan et al. (Zargan et al., 2011). For this test, 3×104 cells were cultured in 96 well plates containing 100 µl serum-free culture. After overnight incubation at 5% CO2, humidity about 80%, and 37°C, the old culture medium was discharged and the new serum-free medium containing different concentrations of 20 and 40 µg/ml of the fraction (With antibacterial properties) were added to the wells. The plate was incubated for 23 hours after incubation with the venom cell. Then 5 µL of MTT solution (5 mg/ml) was added to each well. After the formation of dark blue crystals (Formazone), the contents of each well were removed and after washing with PBS, 100 µL of DMSO was added. In this test, the culture medium was used as control and the cell culture medium as control. Optical absorption of wells at 570 nm was measured using a plate reader (Biotek, USA). The experiment was performed 3 times and 3 wells (3 times) for each concentration. The percentage of cell viability after exposure to different concentrations of venom was calculated using the following formula (Shebl et al., 2012).

% Viable cell = (a/b) × 100

a = The absorbance of treated cells - The absorbance of the blank
b = The absorbance of the control - The absorbance of the blank

Evaluation of cytotoxicity of fractions by Neutral red uptake assay

The steps of Neutral red uptake assay (Abdul et al., 2013) were similar to MTT assay but after incubating the cell adhered to the toxic solution for 23 h, one microliter of Neutral red solution (5 mg/ml) was replaced with MTT solution. Ml) was added to each well. The plate was then incubated under dark conditions at 37°C, with 5% CO_2 and about 80% moisture until red crystals (neutral red with cell lysosome surface). Then, the solution in each well was drained and after washing each well with PBS twice, 100 µl of fixative buffer (37% formaldehyde, 10% purchase). After one minute, this buffer was evacuated and 100 µL of the solvent buffer (0.5% acetic acid) was added. Plates were then incubated on a shaker for 20 minutes in dark conditions at laboratory temperature and optical absorption of wells at 540 nm was measured using a plate reader (Biotek, USA). The experiment was performed 3 times and 3 wells (3 times) for each concentration. Inhibitory percentages induced by different concentrations of venom on cell growth were calculated using the following formula.

100× (a/b)]-1 [= Percentage of cell death

a = The absorbance of treated cells - The absorbance of the blank
b = The absorbance of the control - The absorbance of the blank

Evaluation of apoptosis induction by fractions using comet assay

Comet assay or single-cell gel electrophoresis is one of the best tests for DNA fragmentation in the cell (Mir et al., 2013). For this experiment, 12×104 cells were cultured in a sterile 24-well plate containing 300 µl of serum-free medium. Plates were incubated overnight under 5% CO2, 80% humidity, and 37°C. In this test, the culture medium was used as blank and the cell culture medium was used as control. The primary culture medium was then added to each well of the test well, drained, and 300µL of a new medium (without serum) containing different concentrations of 20 and 40µg/ml of fractions (with antibacterial properties). The plate containing the cell was incubated for 24 hours at 37°C, 80% humidity, and 5% CO2. After the end of the mentioned time, cells in each well were separated by trypsin and collected in a 1.5ml tube. The microtubes containing the cells were centrifuged for 4 min at 4°C at 1500 rpm and the supernatant was discharged. The cells were washed with 400 µl of PBS (pH = 7.4) and washed after centrifugation with the supernatant and then added to 200 µl of PBS. The slides required for testing were coated with normal melting point agarose (NMA% 1), and the cell suspension with low melting point agarose (LMA% 1) was compared to one to one. Two mixtures were poured onto the slides, one slide was placed on each slide to create a cell layer, and all slides were lysed in the lysis buffer to lyse the cell and nucleus membranes. (Fresh 2.5M NaCl, 100mM EDTA, 10mM Tris, 0.2M NaOH and Triton X-100 1% pH=10) were cooled and cooled in the refrigerator for 16–18 h. After removing the sliding buffer, slides 20 the cells were washed for 2 min with electrophoresis buffer (300 mM NaOH, 1 mM EDTA, and pH> 13) and then incubated in cold electrophoresis buffer for 40 min and then incubated in the refrigerator. The electrophoresis was carried out in an electrophoretic tank containing buffer and electrophoresis was performed at 4 ° C for 45 minutes under 25 V and 300 mA current. Samples were placed in a neutral buffer (Tris 400mM, pH =7.5) for 10 min to neutralize the playing environment. For staining, cells were added to each slide with 100 µL of ethidium bromide solution at a concentration of 20µg/ml for 10 min at room temperature. The slides were then washed twice with distilled water for 10 minutes and studied by fluorescent microscopy. At least 100 cells of DNA were obtained for each sample and the results were statistically analyzed.

Statistical analysis

The results of each test were reported as Mean ± SD and the data were analyzed using software (GraphPad InStat). Different concentrations of venom were compared to the control group and compared to each other by one-way ANOVA and Tukey tests. P<0.05 was considered significant in all tests. Draw charts in the Microsoft Excel software (2013 version).

RESULTS

Results of crude venom electrophoresis and chromatography results

The molecular weights of the bands were determined using standard protein diagrams. SDS-PAGE (15% gel) results for 10 protein bands with molecular weight of 13.2, 19.6, 24.2, 27.91, 39.8, 54.8, 65.35, 75.4, 98.32 and 99.25 KDa Showed (Figure 1). The results of RP-HPLC separation of crude venom fractions showed 11 distinct peak peaks (Figure 2). The fractions collected were named F1 to F11, respectively, at the time of departure from the column. The fractions corresponding to each peak were collected in perfectly clean and non-contaminated glass and stored at -80 ° C after lyophilization. The retention time of the product corresponding to each courier is given in Table 1.

Figure 1: SDS-PAGE pattern (15%) of venom of the Pseudocerastes persicus snake

Column 1: Standard Protein; Column 2: the venom of the Pseudocerastes persicus snake

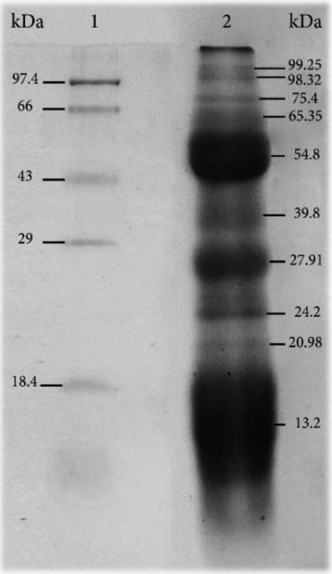

Protein assay: The results showed that fractions 1, 2, and 11 lacked proteins that were excluded from subsequent studies (Table 2).

Results of antibacterial tests

Results of antibacterial effect of fractions using MTT assay:

The results showed that fractions 3, 4, 7, and 9 had no inhibitory effect on Escherichia coli. But fractions 5, 6, 8, and 10 showed antibacterial effects, respectively, with the bacterial viability percentage being 81.55, 81.2, 69.87, and 71.45 % respectively. Statistical analysis showed that recent fractions had a significant inhibitory effect on bacterial growth compared to the negative control (normal culture medium containing bacteria) but their mortality rate was not significant compared to tetracycline as a positive control (Figure 3).

Figure 2: Chromatogram of unicorn raw venom of the Pseudocerastes persicus snake based on the results of R-HPLC Fractions of raw venom of Pseudocerastes persicus snake using R-HPLC preparation with a UV detector (220 nm absorbance), C18 column, and linear gradient program (buffer A: acetonitrile 20% containing 0.1% TFA and buffer B: acetonitrile 80% containing 0.1% TFA percentage was separated. The fractions corresponding to each peak were manually collected in the non-contaminated glass and the retention time of each peak was also recorded. The fractions collected were named F1 to F11 at the time of exit from the column, respectively.

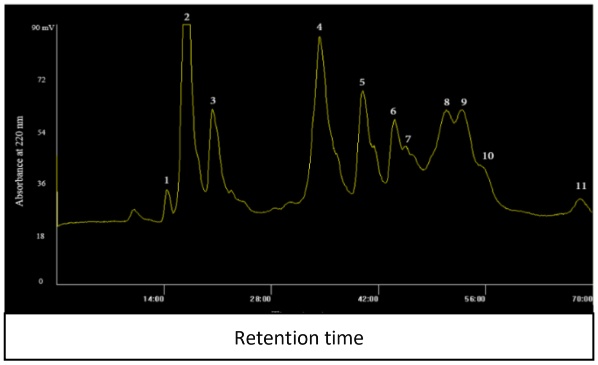

Table 1: Exit time of each component from column (in minutes)

Start time and end of peak	Fractions										
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Start	13:50	15:36	19:22	31:14	38:27	52:51	45:01	47:50	51:59	54:56	67:06
End	15:01	19:22	24:35	38:27	42:51	45:01	47:50	51:59	54:56	59:18	69:78

Table 2: Protein content of fractions isolated from the venom

Protein concentration (mg/ml)											
Fractions	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Protein concentration	0	0.0075	1.55	11.77	10.77	6.29	1.93	12.85	11.35	4.5	0

Also, test results on Bacillus subtilis showed that its vital percentage in media containing fractions 3, 4, 5, 6, 7, 8, 9, and 10 were 78.41, 0.35, 99.91, 81.27, 100, 98.94, 81.15, and 97.49 % respectively. Fractions 3, 4, 6, and 9 had a significant inhibitory effect on the growth of this bacterium compared to negative control of growth inhibition, among which Fraction 4 had more inhibitory effect than standard antibiotic (tetracycline) (Figure 3).

The viable percentages of Staphylococcus aureus in media containing fractions 3, 4, 5, 6, 7, 8, 9 and 10 were 91.58, 69.23, 70.47, 81.38, 100, 96, 99.76, and 95.76 % respectively.

Statistical analysis of data showed that fractions 4, 5, and 6 had a significant inhibitory effect on bacterial growth compared to the negative control. The highest inhibitory effect of fractions isolated from crude venom belonged to fraction 4 with a 30.77% inhibitory effect, which was significantly less than that of tetracycline (Figure 3).

Figure 3: Critical percentages of Escherichia coli, Bacillus subtilis, and Staphylococcus aureus after exposure to 20 µg/ml protein concentration from different raw venom Pseudocerastes persicus snake fractions based on MTT assay. The effects of fractions were evaluated in comparison with the negative control group

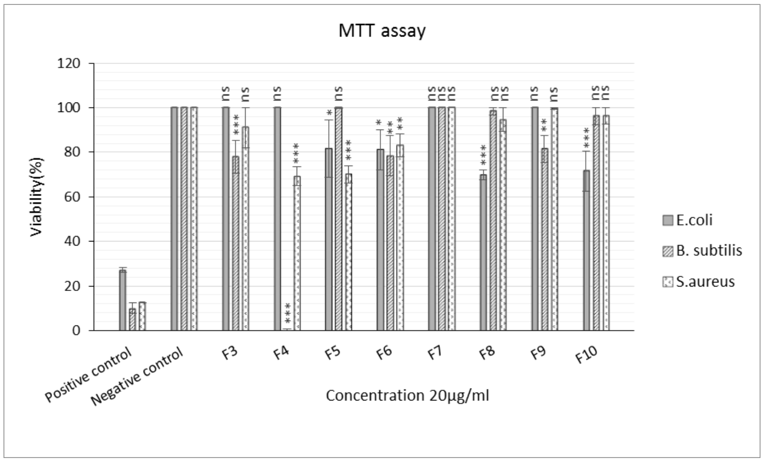

Results of antibacterial effect of fractions using Minimum Inhibitory Concentration Method (MIC assay)

The inhibitory effects of the fractions isolated at 20 µg/ml on bacterial growth showed that fractions 3 and 9 did not affect Escherichia coli, but fractions 4, 5, 6, 7, 8, and 10, respectively 5.21, 3.48, 25.2, 17.71, 17.8 and 13.06 % had an inhibitory effect on bacterial growth. Statistical analysis showed that fraction 6 had more inhibitory effect than other fractions but this effect was not significant compared to standard antibiotics (Figure 4).

Figure 4: Percentage of Inhibitory Effect of Protein Fractions (20 µg/ml Concentration) of raw venom Pseudocerastes persicus snake on Escherichia coli, Bacillus subtilis, and Staphylococcus aureus by MIC Test. The effects of fractions were evaluated in comparison with the negative control group

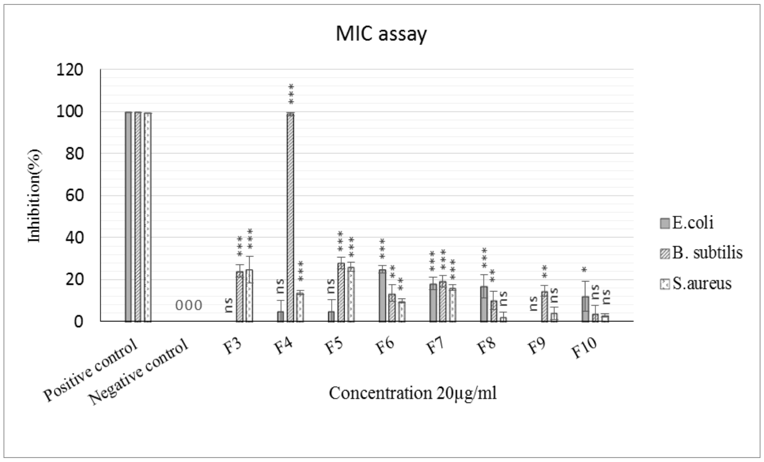

The results showed that fractions Nos. 3, 4, 5, 6, 7, 8, 9, and 10 in Bacillus subtilis were 24.06, 98.46, 28.77, 13.14, 17.88, 12.30, 13.76, and 2.61, respectively produced inhibitory effects. Statistical analysis showed that mortality caused by the effect of fractions 3, 4, 5, 6, 7, 8, and 9 on bacteria was significant compared to the negative control. It is noteworthy that the inhibitory effect of fraction 4 on the growth of this bacterium was much greater than that of the positive control (tetracycline), but this effect was not significant (Figure 4).

Also, fractions 3, 4, 5, 6, 7, 8, 9, and 10 were found to be effective on the growth of Staphylococcus aureus and were

25.46, 13.85, 25.88, 9.85, 15.88, 2.28, 4.74, and 3.03%, respectively. percent inhibitory effect on it. It is noteworthy that the inhibitory effect of fractions Nos. 3, 4, 5, 6, and 7 on this bacterium was significant compared to negative control but not significant compared to standard antibiotics (Figure 4).

Results of anti-cancer properties of fractions

Summary of the results of the antibacterial effects of the fractions isolated from the crude venom of Pseudocerastes persicus snake and statistical analysis of data showed that fractions 4 and 8 had more inhibitory effects on the tested bacteria compared to the other fractions. Therefore, in this study, the anticancer effects and their cytotoxicity were considered.

MTT assay for cytotoxicity test results

Evaluation of cytotoxic effects of fractions 4 and 8 isolated from crude venom on the growth of liver cancer cells at two concentrations of 20 and 40 μg/ml showed that viable percentage of cells affected by fraction 4 at two concentrations compared to control, respectively, 51.55% and 50.62%. However, cell viability after exposure to fraction 8 increased at above concentrations and reached 82.29 and 74.81, respectively. Statistical analysis of data showed that the effects of fractions Nos. 4 and 8 on cell growth inhibition at two concentrations of 20 and 40 μg/ml were significant compared to control, but the inhibitory effects of the two fractions were not significant (Figure 5).

Figure 5: Vital percentages of liver cancer cells after 24 h exposure to 20 and 40 μg/ml protein concentrations of raw venom Pseudocerastes persicus snake fractions based on MTT assay. The effects of fractions were evaluated in comparison with the negative control group

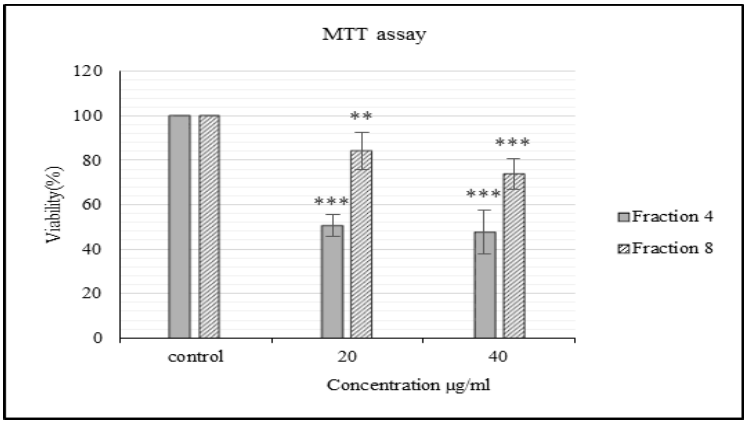

Evaluation of cytotoxicity of fractions by Neutral red uptake assay

The results of the cytotoxicity study of fractions 4 and 8 using Neutral red assay showed that mortality from fraction 4 at two concentrations of 20 and 40 μg/ml in cells was 35.19 and 50.93, respectively. The percentages in the case of fraction 8 were 82.29 and 74.81 percent, respectively, which were significant compared to the control (Figure 6).

Figure 6: Mortality rate of liver cancer cells after 24 h exposure to different concentrations of raw venom Pseudocerastes persicus snake based on Neutral red uptake dye method. Mortality from the effect of each concentration was compared to the control group

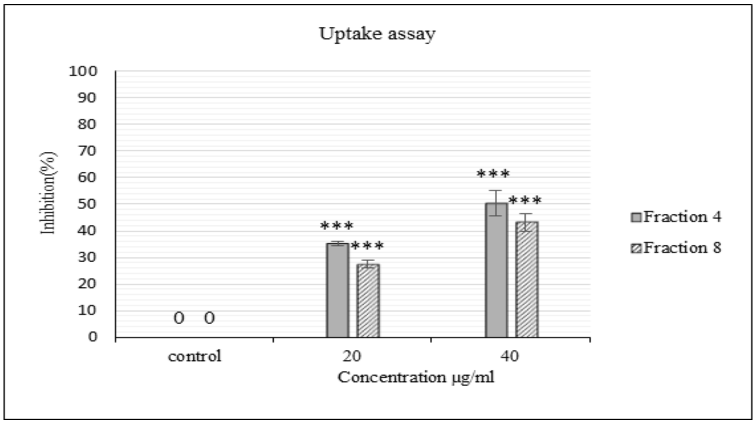

Results of fractions induction of apoptosis using comet assay

The results of the comet assay showed that fraction 4 in two concentrations of 20 and 40 μg/ml had 39.31% and 38.97% and fraction 8 in the above two concentrations was 24.73% and 25.34% apoptotic, respectively. Created liver cancer cell that was significant compared to control (Figure 7–9).

Figure 7: Apoptosis rate induced by Pseudocerastes persicus snake Fragment Fraction No. 4 in Liver Cancer Cells Based on Comet Assay Test Results. The effect of each concentration was evaluated in comparison with the control

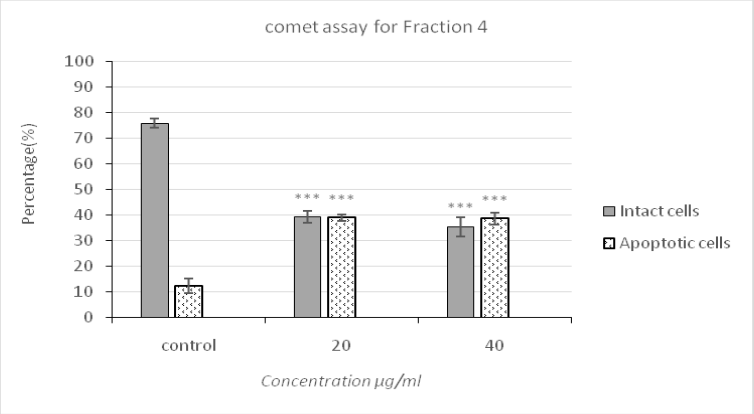

Figure 8: Apoptosis rate induced by a fraction of Pseudocerastes persicus snake number 8 in liver cancer cells based on Comet assay results. The effect of each concentration was evaluated in comparison with the control

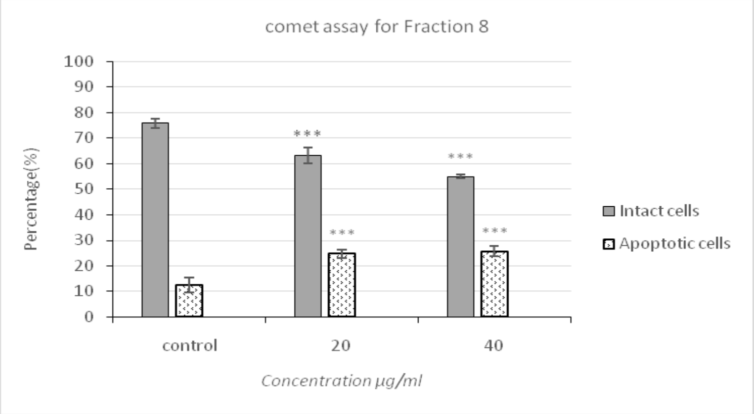

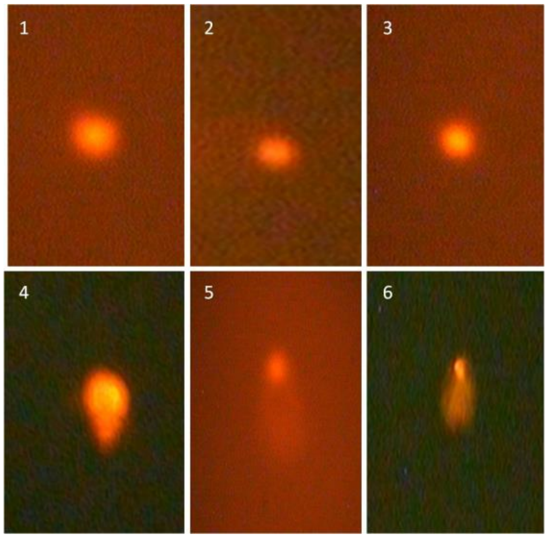

Figure 9: Morphological changes of DNA (40%) in liver cancer cells exposed to raw venom Pseudocerastes persicus snake fractions compared to control cells based on comet assay. [1-3: DNA image of healthy cells (40%), 4-6: DNA image of an apoptotic cell (40%)]

DISCUSSION AND CONCLUSION

Snake venom is a complex of molecules with different biological activities, which can be of great interest in medical and biological research (Suntravat et al., 2010). It was first carried out by researchers over 40 years ago (Master and Rao, 1961). Since the proteins and peptides present in snake venom have a wide range of molecular size as well as biochemical properties, proteomic analysis can be very important in the identification and isolation of these compounds (Phizicky et al., 2003). In the present study, a combination of electrophoretic and chromatographic methods was used to investigate the protein profile of venom. The chromatographic pattern of the venomous snake venom was studied in 2004 by Mehriyar Amini Inst using Sephadex G-50 gel filtration and 6 fractions were isolated from the raw venom (Amininasab et al., 2004).

In this study, the RP-HPLC method was used to evaluate the toxicity of the chromatographic pattern. Finally, 11 fractions were isolated and collected. Protein assay results showed that only 8 fractions of the separated fractions contained protein. In addition, our findings showed 10 protein bands in the electrophoretic pattern (SDS-PAGE). The discrepancy in the results of the electrophoresis pattern with the chromatography pattern is probably because some proteins are close to each other and are collected under a fractionation during separation.

Previous studies have shown that the pattern of electrophoresis and chromatography of different snakes is different (Nawarak et al., 2003; Rioux et al., 1998; Fry et al., 2003; Mehdizadeh-Kashani et al., 2012; Bhaskar et al., 2011). In many of these studies, by separating different components of the venom, proteins and peptides were isolated. Have been shown to have excellent therapeutic properties that can have broad implications for medicine (Barker et al., 2000; Zhao et al., 2001; Marcinkiewicz et al., 2003; Shuting et al., 2004).

The alarming growth of infectious diseases caused by bacterial resistance has prompted more research in natural resources, including the venom of various toxic animals such as snakes to discover more effective and newer antibiotics.

Based on the results of this study, it was found that the raw venom of Pseudocerastes has antibacterial properties. Also, the results showed that the raw venom of this snake against high gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus) has high inhibitory effects. And against Escherichia coli only the highest concentration used (400 µg/ml) has inhibitory effects. Therefore, in this part of the study, we tried to investigate the components of raw venom, isolation, and activity of each fraction against the mentioned bacteria. MTT redaction and MIC assay methods were used for this purpose.

The results showed that fraction 4 had the most inhibitor effects on the growth of gram-positive bacteria. The inhibitory effect of this fraction was 99% on Bacillus subtilis and 31% on Staphylococcus aureus. Fraction No. 8 with 30% inhibition had the most inhibitor effect on the growth of Escherichia coli.

These results are quite consistent with the data obtained from the study of the antibacterial effects of the raw snake venom listed on these bacteria. If these fractions were selected as antibiotics for use in animals or humans, their cytotoxicity on the cell should be assessed. Therefore, the cytotoxic effects of selected fractions (with antibacterial properties) were evaluated using MTT assay, Neutral red uptake assay, and Single cell gel electrophoresis (Comet assay) on liver cancer cell line (HepG2 cell line). This part of the study showed that fraction 8 induced the least toxicity in the cell. This property introduces this fraction as a suitable candidate for the isolation of effective molecules with antibacterial properties that have the least side effects on human cells. Fraction No. 4 also induced the greatest effect on cancer cells, which, according to Comet assay results, is most likely via induction of apoptosis. Therefore, this fraction is a suitable candidate for use in drug discovery. Will be anti-cancer. Previous studies on snake venom have also shown that their venom has anti-cancer properties (Yamileth et al., 2003).

In summary, it can be concluded that the results of the present study have the potential of fraction 8 for further study as an antibiotic and fraction 4 as an anti-cancer drug.

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ORIGINAL ARTICLES

Evaluation of epidemiological characteristics, treatments, and prognosis of patients with COVID-19 hospitalized in the Intensive Care Unit

Seyed M.A. Zavareh[1], Seyed A.A. Zavareh[2], Razieh Bakhtiari[3], Marzieh Lak[4]

Abstract: *Introduction: Intensive care facilities may play an important role in reducing corona mortality. This study aimed to assess the epidemiological features, treatments, and prognosis of corona patients that admitted to the Intensive Care Unit (ICU) of Baqiyatallah Hospital.*

Methods: All patients with a corona that admitted to the ICU of Baqiyatallah Hospital from March 5 to May 20, 2020, were included. The epidemiological characteristics, pharmacological, non-pharmacological, and supportive therapies and prognosis were assessed. Underlying diseases, smoking, and severity of lung involvement based on CT scan findings (mild, moderate, severe) and length of hospital stay in the ICU were recorded in a questionnaire. Non-invasive ventilation (NIV) or tracheal intubation, ventilator ventilation, and duration of endotracheal intubation, if needed, were recorded in the questionnaire. Meanwhile, whether tracheostomy was performed, the time of the procedure, and its duration were also recorded.

Results: Overall, 82 patients were included in the study that 68 (82.9%) of them were males. The mean age was 57.96±14.08 years. The mortality rate was 53 cases (64.6%). There were 57 subjects (73.1%) at a severe lung involvement and 21 subjects (26.9%) at a moderate lung involvement. There was a significant relationship between severity of lung involvement and mortality (Odds ratio= 14.3, P<0.001).

There was a significant relationship between mortality and Armadile use (Odds ratio= 2.9, P=0.04). There was a significant relationship between mortality and tracheal intubation (Odds ratio= 27.2, P<0.001). There was no significant difference between mortality and discharge of patients regarding the intubation period (P=0.26). There was not a significant relationship between tracheostomy, NIV, and mortality status in the patients (P=0.14 and 0.33 respectively). Also, there was no significant difference between mortality and discharge of patients regarding tracheostomy and NIV period (P=0.51 and 0.17). There was no significant difference in age between severe and moderate lung involvement cases (P=0.63). There was a significant difference in the duration of ICU hospitalization between severe and moderate lung involvement cases (P=0.004).

Conclusion: The results showed none of the factors of age, sex, underlying diseases other than hypertension, pharmacological methods other than Armadile, and non-pharmacological methods such as hemoperfusion, plasmapheresis, IVIG, hyperimmune plasma injection, and stem cell as well as Ozone therapy does not affect corona patient's mortality. Only the severity of lung involvement leading to endotracheal intubation and mechanical ventilation is related to patients' mortality

Keywords: *Covid-19, Mortality, ICU*

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INTRODUCTION

In December 2019, the world faced a great challenge. A respiratory disease caused by the coronavirus that causes severe acute respiratory syndrome (SARS) in patients and endangers the health of people around the world [1].

Treatment of these patients is supportive and usually respiratory failure is the main cause of death [2]. The clinical features and CT scan images help in the early diagnosis and treatment of this disease [3].

This disease's mortality has been stated 3.6% in China and 1.2 to 1.7% outside of China. the difference between these statics is because of not considering patients with mild illness and taking diagnostic tests. The actual number of deaths is unknown [4].

Intensive care facilities may play an important role in reducing the risk of COVID-19 death [5].

In this retrospective study, we surveyed the epidemiological features, treatments, and prognosis of patients with coronavirus admitted to the ICU of Baqiyatallah Hospital, that we may identify preventable factors associated with the patient recovery and reduce mortality of these patients.

METHODS

In a retrospective study from March 5 to May 20, 2020, all patients with COVID-19admitted to ICU of Baqiyatallah Hospital were evaluated for their epidemiological characteristics, pharmacological, non-pharmacological, and supportive therapies, and prognosis. "Baqiyatallah Hospital is one of the important referral medical centers for corona patients in Iran."

The diagnostic criteria for Corona Patients are clinical symptoms, positive PCR test, and CT scan findings in this center. Hospitalized patients with respiratory distress or SPO2 with oxygen and storage bag below 90% in general wards and emergency departments were transferred to ICU.

Epidemiological characteristics of patients including age, sex, underlying diseases, smoking and Drug abuse, the severity of lung involvement based on CT scan findings (mild, moderate, severe), and length of hospital stay in the ICU were recorded in a questionnaire.

All drugs used in the treatment of patients, as well as non-pharmacological methods including antibody therapy, plasmapheresis, hemofiltration with Jeffron filter, hyperimmune plasma, IVIG, CRRT with cytosorb filter, stem cell, and other methods, were recorded in the questionnaire.

NIV or tracheal intubation, ventilator ventilation, and duration of endotracheal intubation, if needed, were recorded in the questionnaire. Meanwhile, whether tracheostomy was performed, the time of the procedure, and its duration were also recorded.

The data were analyzed by SPSS-22 software. Quantitative data were analyzed using descriptive software and displayed as Mean ± SD. Chi-Square tests were used to compare percentages or frequencies. A P-value less than 0.05 was considered statistically significant.

RESULTS

Overall, 82 patients were included in the study that 68 (82.9%) of them were males. The mean age was 57.96±14.08 years. The mortality rate was 53 cases (64.6%). Table 1 showed the distribution of age, sex, duration of hospitalization in the ICU, and history of diabetes mellitus (DM), hypertension (HTN), kidney disease, liver disease, rheumatic disease, smoking, opium addiction, corticosteroid use, and ARBS regarding mortality of the patients.

The mean age was 59.59±13.85 years in mortality cases and 55.03±14.24 years in discharge patients. There was no significant difference in age between mortality and discharge of patients (P=0.16). Also, there was not a significant relationship between gender and mortality (P=0.76(.

There was not a significant relationship between mortality and DM, kidney disease, lung disease, liver disease, and rheumatic disease in the patients (Table 1). However, there was a significant relationship between mortality and HTN (Odds ratio= 2.99, P=0.04) (Table 1).

There was not a significant relationship between mortality and smoking, opium addiction, corticosteroids, and ARBS in the patients (Table 1).

Table 2 showed the distribution of pharmacological treatment regarding the mortality status of the patients. There was a significant relationship between mortality and Armadile use (Odds ratio= 2.9, P=0.04) (Table 2).

Table 3 showed the distribution of tracheal intubation, intubation period, tracheostomy, tracheostomy period, NIV, NIV period regarding the mortality status of the patients.

There was a significant relationship between mortality and tracheal intubation (Odds ratio= 27.2, P<0.001) (Table 3). The mean intubation period was 3.50±2.83 days in mortality cases and 2.81±2.22 days in discharge patients. There was no significant difference between mortality and discharge of patients regarding the intubation period (P=0.26).

Table 1: Distribution of age, sex, duration of hospitalization in the ICU, DM, HTN, kidney disease, liver disease, rheumatic disease, smoking, drug use, corticosteroids, and ARBS regarding mortality status of the patients

Mortality status		Death	Discharge	Total	P-value
Number (%)		53 (64.6%)	29 (35.4%)	82	---
Sex	male	43 (81.1%)	25 (86.2%)	68 (92.9%)	0.76
	female	10 (19.9%)	4 (13.8%)	14 (17.1%)	
Age, years, mean (±SD)		59.59±13.85	55.03±14.24	57.96±14.08	0.16
Duration of ICU hospitalization, days, mean (±SD)		13.28±8.72	14.17±1.30	13.59±10.06	0.83
DM	Yes	22 (41.5%)	9 (31.0%)	31 (37.8%)	0.35
	No	31 (58.5%)	20 (69.0%)	51 (62.2%)	
HTN	Yes	23 (43.4%)	6 (20.7%)	29 (35.4%)	0.04*
	No	30 (56.6%)	23 (79.3%)	53 (64.6%)	
kidney disease	Yes	5 (9.4%)	3 (10.3%)	8 (9.8%)	0.58
	No	48 (90.6%)	26 (89.7%)	74 (90.2%)	
Lung disease	Yes	4 (7.5%)	1 (3.4%)	5 (6.1%)	0.65
	No	49 (92.5%)	28 (96.6%)	77 (93.9%)	
Liver disease	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	No	53 (100%)	29 (100%)	82 (100%)	
Rheumatic disease	Yes	0 (0.0%)	1 (3.4%)	1 (1.2%)	0.35
	No	53 (100%)	28 (96.6%)	81 (98.8%)	
Smoking	Yes	3 (5.7%)	0 (0.0%)	3 (3.7%)	0.54
	No	50 (94.3%	29 (100%)	79 (96.3%)	
Opium addiction	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	No	53 (100%)	29 (100%)	82 (100%)	
Corticosteroids	Yes	3 (5.7%)	2 (6.9%)	5 (6.1%)	0.58
	No	50 (94.3%	27 (93.1%)	77 (93.9%)	
ARBS	Yes	13 (24.5%)	4 (13.8%)	17 (20.7%)	0.35
	No	40 (75.5%)	25 (86.2%)	65 (79.3%)	

** P-value is significant at the 0.05 level, SD=Standard deviation*

Table 2: Distribution of pharmacological treatment regarding the mortality status of the patients

Mortality status		Death	Discharge	Total	P-value
Tamiflu	Yes	28 (52.8%)	16 (55.2%)	44 (53.7%)	0.51
	No	25 (47.2%)	13 (44.8%)	38 (46.3%)	
Interferon beta	Yes	12 (22.6%)	8 (27.6%)	20 (24.4%)	0.61
	No	41 (77.4%)	21 (72.4%)	62 (75.6%)	
Hydroxychloroquine	Yes	48 (90.6%)	27 (93.1%)	75 (91.5%)	0.52
	No	5 (9.4%)	2 (6.9%)	7 (8.5%)	
Remdesivir	Yes	6 (11.3%)	3 (10.3%)	9 (11%)	0.60
	No	47 (88.7%)	26 (89.7%)	73 (89%)	
Ribavirin	Yes	35 (66%)	18 (62.1%)	53 (64.6%)	0.71
	No	18 (34%)	11 (37.9%)	29 (35.4%)	
Favipiravir	Yes	20 (37.7%)	11 (37.9%)	31 (37.8%)	0.98
	No	33 (62.3%)	18 (62.1%)	51 (62.2%)	
Kaletra	Yes	51 (96.2%)	28 (96.4%)	79 (96.3%)	0.71
	No	2 (3.8%)	1 (3.4%)	3 (3.7%)	
Anticoagulant	Yes	47 (88.7%)	24 (82.8%)	71 (86.6%)	0.45
	No	6 (11.3%)	5 (17.2%)	11 (13.4%)	
Azithromycin	Yes	49 (92.5%)	28 (96.6%)	77 (93.9%)	0.41
	No	4 (7.5%)	1 (3.4%)	5 (6.1%)	

Mortality status		Death	Discharge	Total	P-value
Plasmapheresis	Yes	6 (11.3%)	4 (13.8%)	10 (12.2%)	0.73
	No	47 (88.7%)	25 (86.2%)	72 (87.8%)	
Corticosteroids therapy	Yes	47 (88.7%)	27 (93.1%)	74 (90.2%)	0.70
	No	6 (11.3%)	2 (6.9%)	8 (9.8%)	
Hemoperfusion	Yes	29 (54.7%)	25 (51.7%)	44 (53.7%)	0.79
	No	24 (45.3%)	14 (48.3%)	38 (46.3%)	
Naproxen	Yes	45 (84.9%)	25 (86.2%)	70 (85.4%)	0.57
	No	8 (15.1%)	4 (13.8%)	12 (14.6%)	
CRRT	Yes	0 (0.0%)	1 (3.4%)	1 (1.2%)	0.35
	No	53 (100%)	28 (96.6%)	81 (98.8%)	
Cinnora	Yes	3 (5.7%)	2 (6.9%)	5 (6.1%)	0.58
	No	50 (94.3%)	27 (93.1%)	77 (93.9%)	
IVIG	Yes	26 (49.1%)	9 (31.0%)	5 (42.7%)	0.11
	No	27 (50.9%)	20 (69.0%)	47 (57.3%)	
Armadile	Yes	20 (37.7%)	5 (17.2%)	25 (30.5%)	0.04*
	No	33 (62.3%)	24 (82.8%)	75 (69.5%)	
Actemra	Yes	14 (26.4%)	5 (17.2%)	19 (23.2%)	0.34
	No	39 (73.6%)	24 (82.8%)	63 (76.8%)	
AB therapy	Yes	45 (84.9%)	24 (82.8%)	69 (84.1%)	0.79
	No	8 (15.1%)	5 (17.2%)	13 (15.9%)	
Plasma hyper immune	Yes	20 (37.7%)	12 (41.4%)	32 (39.0%)	0.74
	No	33 (62.3%)	17 (58.6%)	50 (61.0%)	
Stem cell	Yes	6 (11.3%)	2 (6.9%)	8 (9.8%)	0.70
	No	47 (88.7%)	27 (93.1%)	74 (90.2%)	
Ozone therapy	Yes	2 (3.8%)	0 (0.0%)	2 (2.4%)	0.53
	No	51 (96.2%)	29 (100%)	80 (97.6%)	

** P-value is significant at the 0.05 level.*

Table 3: Distribution of tracheal intubation, intubation period, tracheostomy, tracheostomy period, NIV, NIV period regarding mortality status of the patients

Mortality status		Death	Discharge	Total	P-value
Tracheal intubation	Yes	50 (94.3%)	11 (37.9%)	61 (74.4%)	<0.001*
	No	3 (5.7%)	18 (62.1%)	21 (25.6%)	
Intubation period, days, mean (±SD)		3.50±2.83	2.81±2.22	3.37±2.72	0.26
Tracheostomy	Yes	29 (54.7%)	11 (37.9%)	40 (48.8%)	0.14
	No	24 (45.3%)	18 (62.1)	42 (51.2)	
Tracheostomy period, days, mean (±SD)		11.29±7.26	12.81±5.82	11.69±7.19	0.51
NIV	Yes	13 (24.5%)	10 (34.5%)	23 (28.0%)	0.33
	No	40 (75.5%)	19 (65.5%)	59 (72.0%)	
NIV period, days, mean (±SD)		3.63±2.20	2.54±1.43	3.09±1.90	0.17

** P-value is significant at the 0.05 level.*

There was not a significant relationship between tracheostomy, NIV, and mortality status in the patients (P=0.14 and 0.33 respectively). Also, there was no significant difference between mortality and discharge of patients regarding tracheostomy and NIV period (P=0.51 and 0.17) (Table 3).

Table 4 showed the distribution of mortality, age, tracheostomy, duration of ICU hospitalization, tracheal intubation, intubation period, tracheostomy, tracheostomy period, NIV, NIV period regarding the severity of the disease of the patients.

There were 57 subjects (73.1%) at a severe lung involvement and 21 subjects (26.9%) at a moderate lung involvement (Table 4). There was a significant relationship between severity of lung involvement and mortality (Odds ratio= 14.3, P<0.001).

The mean age was 58.05±13.43 years in severe lung involvement cases and 55.19±15.15 years in moderate lung involvement cases. There was no significant difference in age between severe and moderate lung involvement cases (P=0.63).

The mean duration of ICU hospitalization was 15.59±10.61 days in severe lung involvement cases and 8.85±5.78 days in moderate lung involvement cases. There was a significant difference in the duration of ICU hospitalization between severe and moderate lung involvement cases (P=0.004).

49 cases (86.0%) in severe lung involvement had tracheal intubation There was a significant relationship between the severity of diseases and tracheal intubation (P<0.001). The mean intubation period was 3.30±2.32 days in severe cases and 4.12±4.96 days in moderate cases. There was not a significant difference in the intubation period between severe and moderate lung involvement cases (P=0.86).

Also, 34 cases (59.6%) in severe lung involvement had a tracheostomy. There was a significant relationship between the severity of diseases and tracheostomy (P<0.001). There was not a significant difference in the tracheostomy period between severe and moderate lung involvement cases (P=0.74).

There was not a significant relationship between NIV and the severity of disease in the patients (P=0.15). The mean NIV period was 3.44±1.91 days in severe lung involvement cases and 1.50±0.57 days in moderate lung involvement cases. There was a significant difference in the NIV period between severe and moderate lung involvement cases (P=0.026) (Table 4).

Table 4: Distribution of morality, age, duration of ICU hospitalization, tracheal intubation, intubation period, tracheostomy, tracheostomy period, NIV, NIV period regarding the severity of lung involvement of the patients

The severity of lung involvement		Moderate	Severe	Total	P-value
Number (%)		21 (26.9%)	57 (73.1%)	82	---
Mortality	Yes	6 (28.6%)	43 (75.4%)	49 (62.8%)	<0.001*
	No	15 (71.4%)	14 (24.6%)	29 (37.2%)	
Age, Years, mean (±SD)		55.19±15.15	58.05±13.43	57.27±13.87	0.63
Duration of ICU hospitalization, days, mean (±SD)		8.85±5.78	15.59±10.61	13.78±9.98	0.004*
Tracheal intubation	Yes	8 (38.1%)	49 (86.0%)	57 (73.1%)	<0.001*
	No	13 (61.9%)	8 (14.0%)	21 (26.9%)	
Intubation period, days, mean (±SD)		4.12±4.96	3.30±2.32	3.42±2.79	0.86
Tracheostomy	Yes	4 (19.0%)	34 (59.6%)	38 (48.7%)	0.001*
	No	17 (81.0%)	23 (40.4%)	40 (51.3%)	
Tracheostomy period, days, mean (±SD)		10.50±4.79	11.75±7.08	11.62±6.84	0.74
NIV	Yes	3 (14.3%)	19 (33.3%)	22 (28.2%)	0.15
	No	18 (85.7%)	38 (66.7%)	58 (71.8%)	
NIV period, days, mean (±SD)		1.50±0.57	3.44±1.91	3.09±1.90	0.026*

** P-value is significant at the 0.05 level*

DISCUSSION

Overall, 82 patients were included in this study that 68 (82.9%) of them were males. The mean age was 57.96±14.08 years. The mortality rate was 53 cases (64.6%).

There was a significant relationship between mortality and history of HTN, Ardamadil use for treatment, the severity of lung involvement, and tracheal intubation. There was no significant relationship between NIV and the severity of lung involvement in the patients. There was a significant difference in the NIV period between severe and moderate lung involvement cases.

The mean duration of ICU hospitalization was 15.59±10.61 days in severe cases and 8.85±5.78 days in moderate cases. There was a significant difference in the duration of ICU hospitalization between severe and moderate cases

Daniel E L Promislow and colleagues concluded that aging is associated with increased complications of corona and that men of all ages have more serious complications than women [6].

Qirong Ruan and colleagues in a study of hospitalized corona patients concluded that age had a significant effect on mortality and gender had no significant effect. Underlying diseases, secondary infections, and high levels of inflammatory factors in the blood are predictors of high mortality [7].

However, in this study, patient's age and sex did not have a significant relationship with mortality. Maybe the reason that gender didn't have a significant relationship with mortality in this study was that this study included patients admitted to the ICU and patients with critical situations were being transferred to this sector., and this statistic is different from the general mortality statistics in the society.

As in previous studies, Mandeep R. et al. Concluded heart diseases were associated with increased mortality in hospitalized corona patients. In their study, the use of ACEIs and ARBs was not associated with an increase in mortality [8].

In a meta-analysis of 1576 patients with COVID 19, JingYang and colleagues concluded that underlying diseases such as hypertension, heart disease, and respiratory disease were more common in critically ill patients than in less critically ill patients [9].

Marcello Covino et al. concluded that the use of ACEIs or ARBs in corona hypertensive patients was not associated with increased mortality [10].

In this study, in line with Mandeep R. and JingYang studies, blood pressure had a significant relationship with mortality. Also, in line with the study of Mandeep R. and Marcello Covino, the use of ACEIs and ARBs had no significant relationship with mortality.

Thomas C. Hanff and colleagues stated that corona hypertensive patients treated with ACEIs should not change their treatment regimen [11].

While Lei Fang and colleagues found that diabetes, heart disease, and hypertension were factors associated with corona disease severity, they suggested replacing calcium channel blockers with ACEIs or ARBs [12].

Li-Lin Liang and colleagues found that higher mortality rates could be due to fewer testers. Also, an older population, weak government, and a small number of beds can increase mortality [13].

A study by YUNPENG JI et al. In China concluded that the reason for the high mortality rate in HUBEI (2.9%) compared to other Chinese provinces (0.7%) was probably due to the rapid increase in the number of infections around the outbreak center. Which has led to insufficient health resources [14].

CONCLUSION

According to the results of this study, none of the factors of age, sex, underlying diseases other than hypertension, pharmacological methods other than Armadile, and non-pharmacological methods such as hemoperfusion, plasmapheresis, IVIG, hyperimmune plasma injection, and stem cell as well as Ozone therapy does not affect corona patient's mortality. Only the severity of lung involvement leading to endotracheal intubation and mechanical ventilation is related to patients' mortality, so according to studies by Li-Lin Liang and YUNPENG JI, it can be concluded that The most important factor in reducing patient mortality is the management of the disease epidemic, which makes adequate treatment resources available to patients and no patient dies due to lack of facilities in severe epidemics. And in epidemics, special attention should be paid to hypertensive patients.

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ORIGINAL ARTICLES

Endoscopic treatment in carpal tunnel syndrome

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Abstract: *Carpal tunnel syndrome is currently the most common peripheral nerve compression neuropathy that affects approximately 1% of the population [7,8,9]. The incidence of CTS seems to be increasing. The diagnosis is based on clinical symptoms and electrodiagnostic studies.*

Keywords: *endoscopic, carpal tunnel, median nerve, compression*

INTRODUCTION

Definition

Carpal tunnel syndrome is the compression of the median nerve in the wrist (radiocarpal joint and palm) and the most common neuropathy in the upper limb. Paresthesias, especially nocturnal pain, numbness and weakness of the hand, tingling, atrophy of the thenar muscles are the most common clinical signs of CTS in addition to the Tinel sign, the Phalen and Durkan test, ultimately leading to severe damage to the hand and forearm [1].

Anatomy

The Carpal tunnel consists of the carpal bones and the transverse carpal ligament that forms the roof of the tunnel and extends from the hamat bone (hook bone) and triquetrium (pyramidal bone) on the ulnar side to the scaphoid and trapezium on the radial side. The carpal bones are located in 2 rows (from radial to ulnar); proximal row: scaphoid, lunate, triquetrium, and pisiform bone, distal row: trapezium, trapezoid, capitate, and hamat. Through this canal pass the median nerve which is accompanied by the artery of the median nerve wrapped in a "perinerv" sheath and the flexor tendons which are wrapped by the synovial sheath which helps the proper functioning of the flexor apparatus under normal conditions. (long thumb flexor, four superficial flexors of the fingers, and four deep flexors of the fingers).

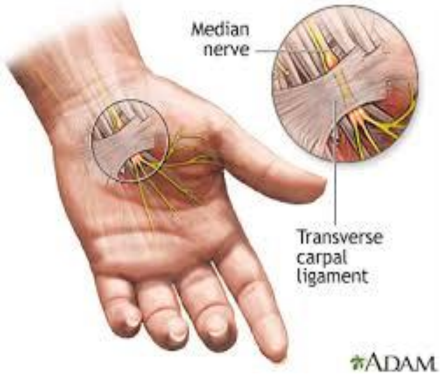

Also at the wrist, there is a canal where the ulnar vascular-nervous package passes called the Guyon canal, which may or may not be affected at the same time as the carpal tunnel syndrome.

Etiology

Among the causes of CTS are: idiopathic, hereditary,

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infectious, endocrinological, metabolic, hematological, renal, rheumatic, traumatic, tumor.

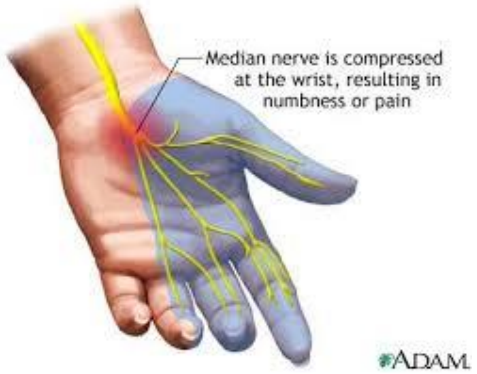
Median nerve is compressed at the wrist, resulting in numbness or pain
ADAM.

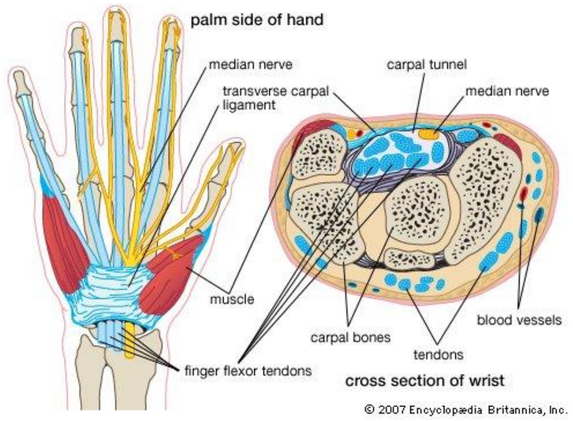

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Diagnostic

It is put in principle on the clinical signs, the Tinel sign, the Phalen and Durkan test, then it is passed to the paraclinical analyzes: electromyography (EMG), nerve conduction speed, radiology, ultrasound, MRI, laboratory blood tests.

The costoclavicular Adson and Wright tests are used to exclude chest compression syndrome that can mimic CTS. Cervical examination, including the Spurling test, is essential to exclude cervical radiculopathy. The percussion of all the primary peripheral nerves can lead us to an unexpected area of compression [2].

A study of nerve conduction speed and electromyography is performed. They help us to confirm the diagnosis of CTS and to exclude other patho-logy[3].

Basic x-rays of the wrist are useful in detecting unexpected pathology.

TREATMENT

Over time, several treatment methods have been tried; conservative by rest and immobilization, treatment with topical or infiltrated anti-inflammatory drugs in the carpal tunnel (in rare cases), and surgical treatment by a classical or endoscopic technique that have proven effective compared to conservative treatment which is considered a way of delaying surgery; Surgical treatment by classical technique involves a curved longitudinal incision parallel to the tenerian crease for prolonged sometimes proximal zigzag to forearm through the flexion creases of the wrist to relax the forearm fascia, external or internal neurolysis, sinevoectomy or remove any formations that can compress the median nerve, sectioning TCL from proximal to distal on the ulnar side to avoid injury to the thenar motor branch, hemostasis control, skin suture, dressing, and immobilization. Risks including pillar pain, weakness in the hand, sensitivity to scarring, delayed return work and slower return to office, relatively long recovery period, and the risk of adhesions. This technique has proven good over time, but because medicine is evaluating and we are continuing our search for new techniques and procedures to minimize risk and recovery time, we will present endoscopic surgical treatment.

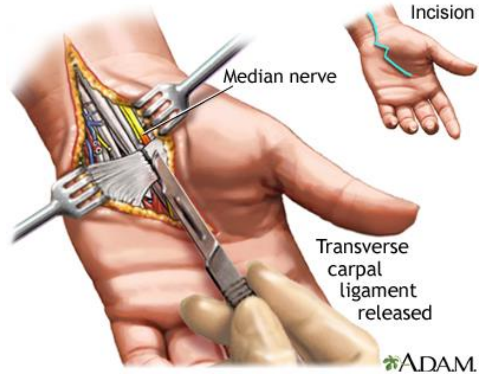

History of carpal tunnel endoscopy

Interest in less invasive open procedures for treating CTS developed in the early 1980s. Paine [4], a neurosurgeon, used a retinaculatom to divide TCL through a small transverse incision in one of the creases of the wrist. However, he found a 2.5% incidence of the incomplete division of the TCL. There were four cases of palmar hematoma, most likely developed due to superficial palmar arch injury. Pagnanelli [5] reported the use of retinaculatom in 577 patients and had only two incomplete divisions (0.3%) of TCL with no median or vascular nerve injuries.

In the history of carpal tunnel endoscopy, 2 main techniques for relaxing the carpal tunnel have been described; "One portal procedure" and "duel portal procedure". The first endoscopic technique was introduced in the relaxation of the carpal tunnel "one portal procedure" in 1986-1987 by Okutsu, a Japanese orthopedist, and in 1989 the technique

"Duel portal procedure" was introduced by Chow, an orthopedist. the two techniques had modifications and developments by other doctors (Agee, Menon, Worseg, Brown, Tsai Resnik, and Miller...) but remained in 2 categories: one portal procedure and duel portal procedure.

Indications

The indications are similar to those of classical technique and include the severity of symptoms, duration of symptoms, ineffective response to conservative treatment.

Relative contraindications

Include patients with severe CTS with evidence of muscle atrophy, rheumatoid arthritis, previous surgical treatment of repeated CTS !!, significant loss of wrist extension, proliferative synovitis, and lesions invading the carpal tunnel [2].

Endoscopic surgical technique

One portal procedure (Agee's technique modified):

Following the inflation of a tourniquet with the initial recommendation of general or regional anesthesia, then with the evolution of the surgeons' experience, they started to use local anesthesia with or without sedation, a 2-3 cm incision is made in one of the flexion creases of the wrist between ulnar carpal flexor and radial carpal flexor. Longitudinal subcutaneous dissection exposes the forearm fascia.

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A U-shaped incision is made on the forearm fascia, creating a rectangular flap based distally on the TCL. While the flap is raised, a synovial lift is placed under the TCL [6]. An instrument (a kind of dilator) is inserted into the carpal tunnel to create a path for the blade assembly. The blade endoscope assembly is then inserted into the carpal tunnel and is passed distally to the distal edge of the TCL. A few steps are usually required to obtain an adequate definition of TCL. After defining the distal edge of the ligament, the tip of the instrument (along with the blade) is placed distal to the edge of the TCL. After checking the correct positioning, the trigger mechanism is activated and the blade is engaged and raised to 3.5 mm above the assembly at an angle of 80° [6]. The instrument is retracted, and under direct view, the TCL is split in a distal to proximal direction. With the blade retracted, several passes can be made to check the ligament transection. If necessary, the blade can be coupled and the remaining TCLs can be sectioned. Then, a proximal volar antebrachial fasciotomy is performed, followed by standard closure of the wound.

Dual portal procedure (Chow's technique and its modifications):

His original technique involved a transbursaral approach to the carpal tunnel. The surgeon and the nurse should sit in front of each other at the hand table. A TV monitor is placed behind each surgeon so that both can have a complete view of the procedure. A transverse incision of 1 cm is made at 0.25 cm proximal and 0.25 cm radially from the pisiform bone. Chow later modified his technique by changing the landmarks of the proximal incision [3, 7, 8]. He recommended that the proximal portal be made by drawing a transverse line of 1 to 1.5 cm radially from the proximal pole of the pisiform bone. A second line is longitudinally marked 0.5 cm from the end of the first line. A third line is transverse 1 cm to the radial from the end of the second line to indicate the entrance portal. A longitudinal incision is made on the forearm fascia, taking care to protect the artery and the ulnar nerve. The flexor tendons are exposed (trans-bursal) and retracted towards the radial part, finding the space between the ulnar neurovascular bundle and the flexor tendons. A trocar is inserted, and the wrist is placed and held in hyperextension by a special hand frame. Using the tip of the trocar, feel the base of the harness hook, then lift it and feel it, if you feel the cannula means it is above the TCL or in the Guyon channel and must be removed and reinserted into the channel under the TCL. A second incision (the palmar one) is made is distal to the distal edge of the carpal transverse ligament along the long axis of the ring finger. The endoscope is inserted proximally into the trocar, which has an end that is immediately below the TCL. Steps are taken to ensure that only the transverse fibers that makeup TCL are visible and not tendons or nerves. The endoscope is inserted into the proximal incision and advanced to the distal edge of the TCL. A knife inserted through the second incision (palmar incision) is used to cut the distal edge in a proximal direction up to half the ligament. To complete the release of the TCL, the endoscope is inserted into the distal opening. The knife is used to cut the proximal edge of the TCL, and the retrograde knife is inserted into the middle section and advanced proximally to complete the release. The wounds are sutured, a simple dressing is applied, and the sutures are removed after one week postoperatively. Because the transbursive technique frequently produced ulnar neuropaxia, either due to withdrawal pressure or the cannula, the technique was modified by Resnick and Miller [9] in a subligamental or extracurricular approach. In this technique, the forearm fascia is incised proximal to the TCL, and the dissection is made under the fascia and in the carpal tunnel under the ligament, avoiding the flexor tendon bursa. The rest of the operation is performed in a similar way to the Chow method.

In our clinic, we use the endoscopic technique "one portal procedure” (Figures 1 to 6).

Figure 1: Surgical instruments for carpal tunnel endoscopy @ byDr.HazimElayan

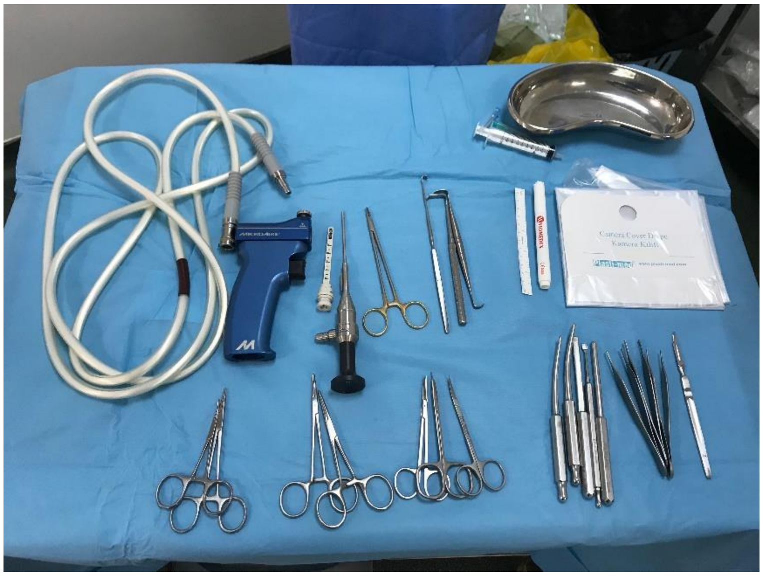

Figure 2: Preoperative marking lines @ byDr.HazimElayan

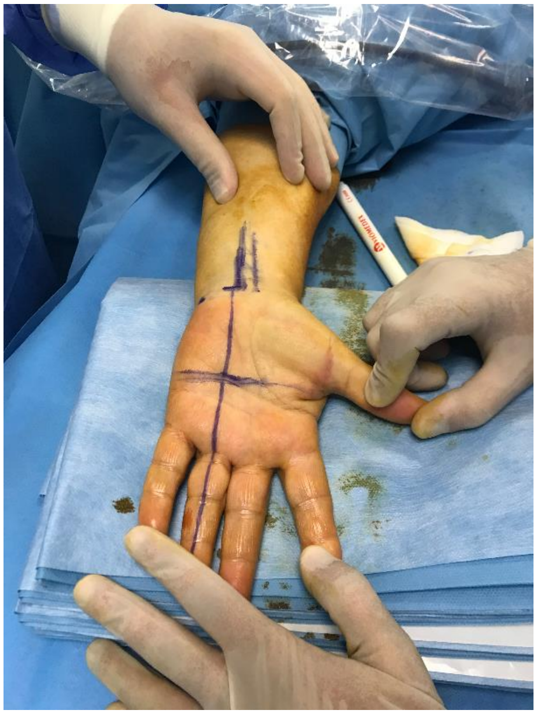

Figure 3: Endoscope inserted into the carpal tunnel through an incision of about 1.5 cm @ byDr.HazimElayan

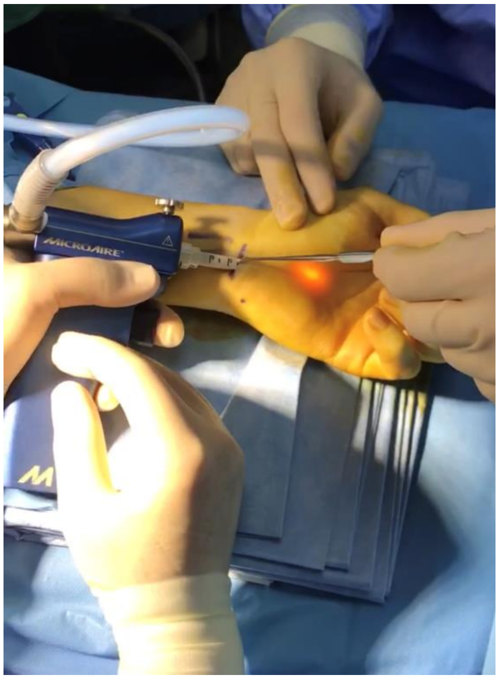

Figure 4: Transverse carpal ligament visualized on the monitor through the endoscope @ byDr.HazimElayan

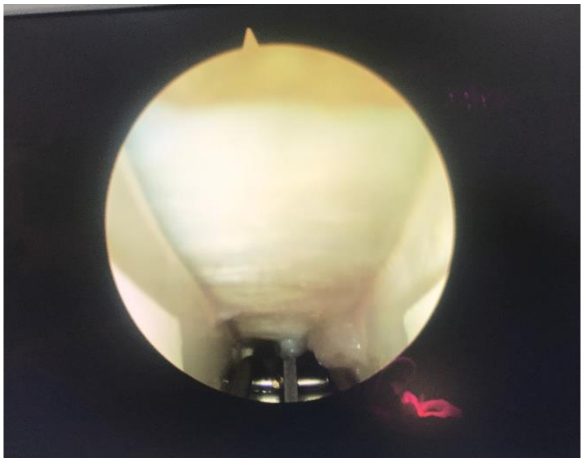

Figure 5: Sectioning the transverse carpal ligament, the remains of fibers that are sectioned by repeating the endoscope blade 2-3 times until complete sectioning @ byDr.HazimElayan

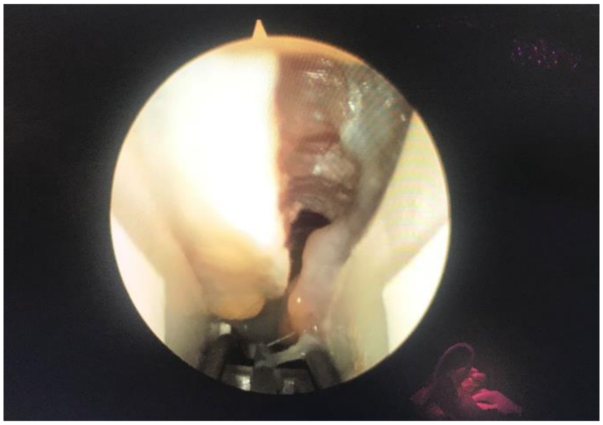

Figure 6: The end of the intervention, skin suture. @ byDr.HazimElayan

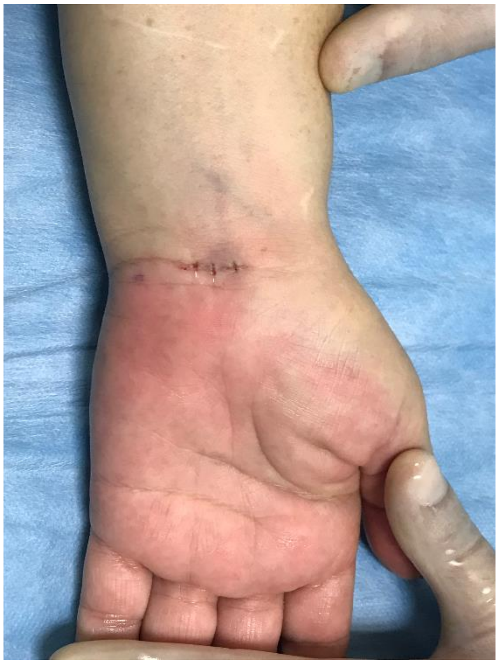

COMPLICATIONS

Endoscopic carpal tunnel release-specific adverse events have been described and are listed here to avoid these possible complications.

1. Nerve injury:

This usually involves the digital nerve of the middle and/or ring finger, which are in a straight line coinciding with the insertion of the endoscope [10, 11, 12].

2. Lesion of the superficial palmar arch:

Because the distance between the superficial palmar arch and the distal edge of the transverse carpal ligament is approximately 9.4±3.5 mm [11, 13], insertion of the endoscope too distally should be avoided.

3. Flexor tendon injury:

This is due to an absence of experience using the endoscope and most commonly affects the superficial flexor tendon of the ring finger, typically a risk in the one-portal procedure [11, 12].

4. Penetration of the Guyon Canal:

This can injury the nerve or the ulnar artery [11].

5. Incomplete sectioning of the TCL

The only recognition of this may be the persistence of carpal tunnel syndrome symptoms in the postoperative period [11].

Benefits (advantages)

Minor postoperative pain, faster recovery, no immobilization of the hand, minimal scarring, faster return to work.

Disadvantages

Higher cost. inability to excise the formation or other structures that may compress the median nerve.

CONCLUSIONS

1.It is a modern technique that requires a special professional experience of the surgeon.

2.It is a minimally invasive technique with the protection of noble elements (vessels, nerves, and tendons) at this level (from the radiocarpal joint and palmar).

3.Recovery of the hand-operated much faster with endoscopic technique (does not require immobilization, rapid socio-professional reintegration).

4.The duration of surgery in an experienced surgeon is much shorter than with the classical technique.

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ORIGINAL ARTICLES

The application of social marketing principles in health care campaigns

Ligia Pop (Micle)[1], Norbert D. Stenczel[1], Victor L. Purcărea[2], Iuliana R. Gheorghe[2], Laura Tribus[3], Gabriela Hoffer Oniga[1]

Abstract: *Social Marketing offers new insight into the design of health care programs that focus on the comprehensive vision of understanding the target audience. As there have been several confusions between the Health Care Social Marketing and education or preventive health care programs, the characteristics refer to the social good and behavior change on voluntary grounds. This paper aimed to elaborate some guidelines for Health Care Social Marketing campaigns to increase the chances of success and trigger the expected behavioral change in the target audience. To ensure the successful implementation of a Social Marketing campaign in health care, a plan that describes the necessary steps to respond in terms of efficiency and effectiveness to the public health campaign outcomes needs to be elaborated. The proposed model has three levels of intervention, as found in the scientific literature: Diagnosis of social problems, Benefits, and social change, and Social Marketing Mix.*

Keywords: *social marketing, health care campaigns, Health Care Social Marketing*

INTRODUCTION

Since its beginnings, Social Marketing has been a controversial concept, without having a formally approved definition. However, in 1971, Kotler and Zaltman considered that Social Marketing could be defined as “the design, implementation, and control of programs calculated to influence the acceptability of social ideas and involving considerations of product planning, pricing, communication, distribution, and marketing research” [1]. Similarly, in 1989, Kotler and Roberto described Social Marketing as “a program planning process that promotes the voluntary behavior of target audiences by offering benefits they want, reducing barriers they are concerned about, and using persuasion to motivate their participation in program activity” [2]. Moreover, a more recent perspective offered by Kotler and Lee stated that Social Marketing should be “a process that applies marketing principles and techniques to create, communicate and deliver value to influence target audience behaviors that benefit society as well as the target audience” [3]. These definitions have highlighted some common elements of Social Marketing, such as social good, behavior, the impact of Social Marketing on the target audience. In a broader sense, Social Marketing is based on the following orientations [4]:

- Client orientation, which is the identification of the people’s needs, aspirations, values, and priorities;
- Creative orientation in finding imaginative methods to engage the target audience;
- Collective orientation is shaped by the recognized social context matters of a target audience;
- Competitive orientation represents the critical addressing

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to the competition through active strategic measures, for instance, brand competition, product competition, enterprise competition, and generic competition.

Thus, in practice, Social Marketing should be used when there is a voluntary behavior exchange and it is helpful when applied from the start in the planning process of a campaign. Social Marketing principles may be applied in any field. For example, Social Marketing offers a new approach to the design of health care programs due to its more comprehensive focus on the target audience, namely, the audience orientation [5]. An audience-oriented perspective is materialized under a bottom-up philosophy in contrast to the traditional approach [6].

Consequently, Social Marketing is more suitable to be used in the health care field, as it: (a) guides consumers from the beginning of the planning process, having a consumer orientation; (b) improves the management, implementation, and evaluation of intervention programs using efficient marketing activities through the development of a plan, lined up to a target audience; (c) promotes the message of campaigns through Mass-Media following the target audience; (d) improves public satisfaction using marketing research to have an accurate perspective on the desired outcomes; (e) helps individuals identify the need for behavior change [7].

Although most Health Care Social Marketing campaigns focus on the operationality of integrating the Marketing Mix to encourage the target audience to change their behavior, they do not provide the expected results not because the Marketing principles have been inappropriate, but rather because there has been an incomplete analysis of some elements at the design stage [8]. Therefore, this article aimed to elaborate some guidelines for Health Care Social Marketing campaigns to increase the chances of success and trigger the expected behavioral change in the target audience

THE HEALTH CARE SOCIAL MARKETING ORIENTATION

The Health Care Social Marketing orientation is a complex concept because it is not easily understood and usually confusion may occur because most specialists focus on promotion instead of the individual's behavioral change process. Put it in simple words, Health Care Social Marketing should guide the target audience towards the action and the behavior change by using health policies and decreasing the assumptions, constraints, and barriers encountered in the external and internal environment of the target audience. The desired outcome of a Social Marketing campaign in the health care field takes place when the target audience identifies and recognizes the benefits of the change.

In practice, there is a refined version of what a Health Care Social Marketing campaign is and where interventions should be applied. Using the guidelines of French and Blair-Stevens in the shape of several benchmarks, the empirical challenges have been solved, once a Health Care Social Marketing initiative has been designed [9]. These benchmarks refer to the following elements as presented in Figure 1.

Figure 1: The Benchmarks of Health Care Social Marketing

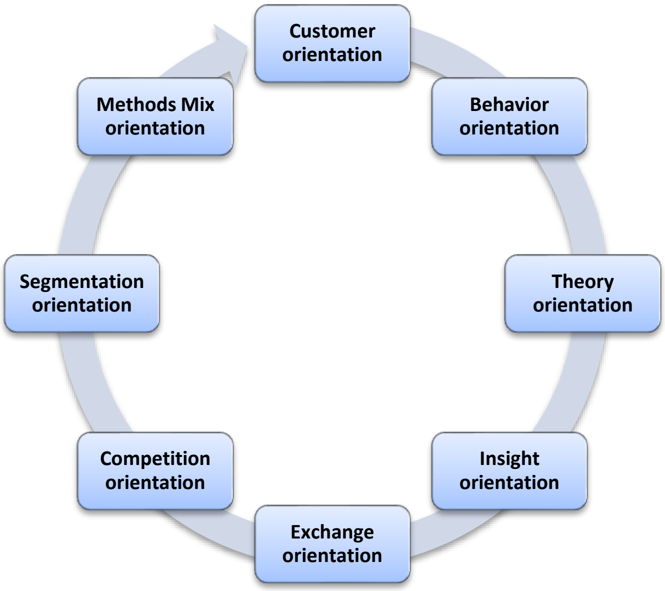

- Customer orientation - Develops a robust understanding of the audience, based on the features of a market and consumer research;
- Behavior orientation - Has a clear focus on behavior, based on a strong behavioral analysis, with a specific emphasis on behavioral goals;
- Theory orientation - Drawing the information from an integrated theoretical framework is fundamental for behavior change;
- Insight orientation - Based on developing a deeper insight approach - it promotes behavioral changes and motivations;
- Exchange orientation integrates an exchange analysis, suggesting the understanding of what the person must give to get the proposed benefits;
- Competition orientation includes a competitive analysis to understand what competes with the time and attention of the audience;
- Segmentation orientation uses a developed segmentation approach;
- Methods mix orientation identifies an appropriate mix of methods, using the de-CIDES intervention framework [10] that may help in the selection, coordination, and combination of different intervention types. The de-CIDES interventions relate to the following:
  - Inform/communicate: inform/communicate/prompt/trigger/remind/reinforce/awareness/explain;
  - Educate: enable/engage/train/skill development/inspire/encourage/motivate;
  - Support: service provision/practically assist/promote access/social networks/social mobilization;
  - Design: alter/physical/product/social/organizational/systems/technology;
  - Control/regulate: control/rules/require/constrain/restrict/enforce/regulate/legislate.

HEALTH CARE SOCIAL MARKETING PLANNING

As there have been many confusions between what Health Care Social Marketing is and what is not, with a specific reference to education and promotion, many scholars felt the need to reaffirm the main points of interest when planning a health care campaign. Therefore, Health Care Social Marketing is a sort of utility package related to the activities, processes, and efforts to deliver benefits to individuals, namely, it is more than communication, and education, it is a plan that focuses on new opportunities to trigger ideas and behavior in health care consumers [11]. Accordingly, Continho Da Silva and Mazzon [8] proposed a plan that described the necessary steps to respond in terms of efficiency and effectiveness to the public health campaign outcomes (Figure 2). This model has three levels of intervention, as it follows: (i) diagnosis - research, collect, and analyze market data and information to identify social problems; (ii) benefits and social change - design an appropriate benefit to the target audience and influence the social change to them; (iii) the Social Marketing Mix - provide and ensure mechanisms and instruments to measure, analyze, and assess the results of the health care campaign.

Figure 2: The steps of a Health Care Social Marketing plan

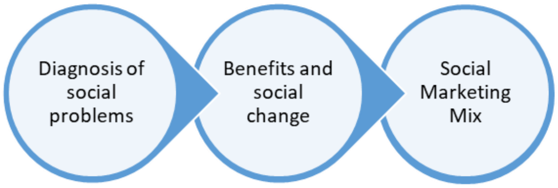

1. Level 1: Diagnosis of social problems

At this level there are 3 steps:

- Identifying the mission in terms of strategic intent, organization values (moral and ethical assumptions), ability and skills (performance), the market definition using consumer-oriented activities and processes, and positioning, meaning the image of the benefits of the campaign.
- The environment of the Social Marketing campaign refers to the outside environment, made up of the demography, economy, nature, legal, technology, and competition, as well as to the inside environment that concentrates on the resources and competencies, culture, suppliers, publics, and channels.
- The data and information collections use the research and the Marketing Information System (MIS). The scope of MIS is to provide relevant information for social marketers to take decisions on time.
- The factors of the target audience's behavior are divided into external factors, such as the skills necessary to perform a particular behavior, the access to the promoted services, the policies, laws and regulations, the culture in terms of customs, values, and lifestyles, as well as the internal factors that refer to the knowledge, and attitudes of the target audience. The internal factors are shaped by the target audience's perceived self-efficacy, risks, social norms, consequences, and intentions.
- Identifying the social problem (situation analysis) provides a SWOT-like approach by analyzing both the internal and

external environments of the Health Care Social Marketing campaign. The internal environment refers to the strengths and weaknesses that the social campaign might produce, and, respectively, the external environment, made up of the opportunities and threats that may influence the health care campaigns.

2. Level 2: Benefits and social changes

The specific steps at this level are the following:

• Defining the goal of the Social Marketing campaign that should be SMART, meaning measurable. Moreover, it should be a reference, specific, have a date and a location, set realistic goals, be clear, and be known to all the stakeholders involved in the Social Marketing campaign.

• Identifying the social change by being a collective action on a long period, having a permanent nature and complexity arising from a social problem, and, respectively, combining the change with the idea of progress. Following several social change theories as well as behavior change models. This step refers to the cognitive, action, behavior, and value at different behavioral change stages such as the pre-contemplative, contemplative, preparation, action, maintenance, and termination. Most Health Care Social Marketing consumers are in the pre-contemplative or contemplative stages.

• Informing the change benefits to the target audience to achieve the potential cognitive consequences that will trigger a changing behavior.

• The target audience's reaction and their demographic characteristics are very important in determining the needs and the wants, their perceptions in attitudes and shared values as well as in the readiness to adopt the change.

• Positioning is the goal of every Health Care Marketing campaign as it can make the target audience reflect on the possibilities of change, mainly, on providing attractive benefits.

3. Level 3: Social Marketing Mix

The Marketing Mix is the operational part of the Social Marketing campaign and it focuses on the strategies applied for a target audience.

In the scientific literature, there have been many variants of Social Marketing Mix but in the health care context, it is made up of the classic Marketing Mix components such as the product, price, place, promotion, and of the specific Social Marketing Mix parts as People, Processes, and Performance.

Briefly, the Product is usually elaborated around the identified social problem and its solution arises from the educational and cultural levels of the population, as well as its popularity, which will be debated by the effects and the benefits on a long period.

The Price refers to all the costs involved in an act of change since there are more non-monetary costs such as physical, time, social and psychological. In fact, in Health Care Social Marketing, the costs are materialized in barriers for individuals in adopting a certain behavior.

Place suggests where, when, and how a behavioral change is required, after providing the necessary information to the target audience, whereas Promotion suggests communication strategies and techniques to persuade the target audience to embrace the benefits of the change rather than the costs. In Health Care Social Marketing, represents the promotion of awareness and provides knowledge about the attitudes, intentions, and behavior change expected from the target audience, and ensure a sustainable relationship between the social marketers and the target audience.

The process encompasses both the visible and non-visible activities involved in a Social Marketing campaign. An efficient process must be constantly adapted to the dynamic evolution of the environment, with the scope of avoiding failures, by excluding steps that do not bring value to the target audience and have several orientations, for instance, service quality delivery, productivity, suitable physical evidence and elaborate a blueprint of the processes.

Professional refers to the skills of the staff team that should focus on the knowledge of the entire cycle of service processes from the start to the finish to deliver qualitative services, manage conflicts between different entities and the target audience and respect the ethical patterns encountered in Social Marketing environments. In the health care context, Social Marketing professionals should be able to provide support and knowledge concerning the processes involved.

Performance is used to measure the quality of the Social Marketing campaign, in the shape of breaking the barriers and delivering several benefits, at the same time, respecting some ethical guidelines. As performance may also be considered a quantitative coefficient, the Health Care Social Marketing campaign suggests responsiveness, helpfulness, security, credibility, proactivity, and empathy.

Program is reflected in the above-mentioned elements of the Social Marketing Mix, and it may be part of a plan that will help professionals in mapping the solutions to the process failures, introduce the consumer-oriented measures and instruments that would ensure the benefits, as well as prioritize sustainable actions and control the performance of a Social Marketing campaign.

EXAMPLES OF HEALTH CARE SOCIAL MARKETING CAMPAIGNS

Social marketing has opened new areas for the expertise of marketers and has intrigued public health advocates.

The early health applications of Social Marketing emerged as part of the international development effort, under the umbrella of "development communication", and were implemented in the Third World countries between 1960 and 1970 [12]. As such, programs promoting immunization, family planning, various agricultural reforms, and nutrition were implemented in many countries in Africa, Asia, and South America in the 1970s. These were based on some of the agricultural development experiences supported by the U.S. Agency for International Development (USAID), as well as community-based family planning programs initiated by the Ford Foundation and the Population Council in the late 1960s.

Soon, international programs began to incorporate marketing techniques, including radio, media, and advertising, on a larger scale [13]. Hence, family planning program designers, for example, initially started distributing contraceptives in pharmacies and small stores and, subsequently, developed systems for market segmentation, mass communication, and product placement to achieve a wider and more efficient distribution of discounted contraceptives.

Peter King from the Indian Institute of Management, in collaboration with the Indian Government's Central Family Planning Board conducted the first national program focused on family planning and the Ford Foundation funded the project, as USAID supplied condoms to the program without cost.

Further, USAID increased the funding for social contraceptive marketing projects in the 1970s, supporting projects in Kenya, Colombia, Sri Lanka, and Jamaica. Much of the important research and developments to advance social health marketing applications have taken place internationally in the area of family planning. In the same vein, Bill Smith at U.S. Academy for Educational Development began using Social Marketing in several countries around the world to improve child survival rates through oral rehydration, breastfeeding, and childhood immunization [14].

Several heart disease prevention programs have also been developed in the U.S. In 1971, the Stanford University Heart Disease Prevention Program undertook a three-community study to discover a risk reduction method that could be generally applicable [15]. Another program was the National High Blood and Heart Health Program Pawtucket (National High Blood Pressure Prevention Program, and the Pawtucket Heart Health Program). Risk factors were high blood pressure, increased blood cholesterol, obesity, smoking cigarettes, and sedentary life. To assess the program, cross-sectional and cohort risk factor surveys were conducted, together with mortality and morbidity surveillance, both in Pawtucket, Rhode Island, and in the non-intervention comparison city of New Bedford, Massachusetts [16]. Some of these programs used the term Social Marketing and openly declared to apply and adapt several marketing concepts or approaches without fully embracing the concept of marketing [17].

The Swiss Federal Office for Public Health and the Swiss AIDS Foundation has promoted AIDS prevention campaigns. A multi-media information campaign with the main slogan "STOP-AIDS" began in February 1987 and is still ongoing. The campaign and its objectives have reached the general population and target groups.

In its evolution, the application of Social Marketing principles has raised many community issues but has also solved them by using instruments and techniques accordingly.

After the 1990s, worldwide, government or non-profit organizations used Social Marketing techniques to implement programs to promote a change in health-beneficial behavior, such as increasing fruit and vegetable consumption, decreasing fat consumption, promoting physical activity, reducing alcohol and tobacco consumption, special programs for pregnant women, infants, and children, behavioral problems related to environmental protection [18]. At the international level, among other facts, Social Marketing has been used to improve access to drinking water [19], eradicate leprosy in Sri Lanka [20], increase adherence to tuberculosis medicines [21], promote immunizations, and universal iodine legislation [22].

Despite being heavily used, even in the pandemic context, Health Care Social Marketing, most of the time, does not have the desired outcome, namely, the behavioral change in individuals, as it needs control and monitoring instruments, as well as elaborating the messages sent differently, for instance, applying cues to trigger a certain emotion. In a study about smoking cessation, using shock advertising messages proved to be inefficient, and maybe changing the message's embedded emotion could trigger the expected result, that is instead of using fear, what should be used is humor [23].

CONCLUSION

Health Care Social Marketing has been a controversial concept, as the application of Social Marketing principles in the medical field has been difficult and many scholars confused the activities specific to Social Marketing with the promotion of health and prevention of health. Having an empirical perspective, Social Marketing has many particularities among which are voluntary behavior exchange, a consumer orientation, and a competitive behavioral change. Moreover, elaborating a complex plan of a Health Care Social Marketing campaign may be truly successful and it should include the following three steps: Diagnosis of social problems, benefits, and social change, and Social Marketing Mix.

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ORIGINAL ARTICLES

Innovative solution for SARS-COV2 epidemic management

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Abstract: *The SARS-CoV-2 pandemic challenged the authorities into taking measures that limit the spread of the virus in the community. TAMEC project (Advanced Community Approaches to Epidemic Management) developed a system that detects the contacts of patients infected with the SARS-CoV-2 virus and alerts them using mobile applications. It allows real-time analysis of confirmed patients and identified contacts, early detection of new outbreaks, and provides decision-making information to prevent viral transmission. The system uses its patent for homomorphic encryption, a method that allows to process and manipulate data in an encrypted format, without having access to the original data. Thus, the solution proposed in the TAMEC project fully respects the privacy rules of citizens imposed by the EU, using the concept of privacy preservation. Our solution offers the possibility to identify and validate a risk score that could become an extremely helpful tool in the stratification of COVID-19 patients. The TAMEC system is innovative in its simplicity and ability to facilitate the prevention of the spread of the SARS-CoV-2 virus in the community*

Keywords: *SARS-CoV-2 virus, prevention, privacy, artificial intelligence*

The SARS-CoV-2 virus pandemic challenged the authorities into taking measures that slow and control the spread of the virus and limit the disease among the population. Prompt identification of contacts and possible outbreaks is essential so that normal activities could be resumed. Researchers from Google and Oxford [1] concluded that if 15% of the population would benefit from a digital alert system, the number of infections and deaths could be reduced by 8% and 6%, respectively. Their studies concluded that 90% of people alerted would voluntarily start quarantine for 14 days, with an abandonment rate of 2% per day. One could assume that the benefits would be even greater if the participating population in such a system were more than 15%.

Epidemiological research is crucial in limiting the spread of the SARS-CoV-2 virus. It aims to identify and alert people at risk who have been in contact with confirmed infected patients. Currently, these surveys are carried out by Public Health Care (DSP) employees and include interviewing each infected person. Data collected are used in compiling a list of people in contact with the infected person within a period determined by epidemiologists. Each possible contact is then alerted and informed of the following procedure by a Public Health Care employee (DSP). This is time-consuming and involves significant human resources. Also, the collected data are sometimes incomplete.

Thus, we have developed a system that detects the contacts of patients infected with the SARS-CoV-2 virus and alerts them using mobile applications [2, 3, 4]. It aims to help limit the spread of the disease among the population and to reduce the health care employees' epidemiological research. This system can be extended for use in other

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contagious diseases, such as seasonal influenza.

An essential part of the TAMEC project (Advanced Community Approaches to Epidemic Management) [5] is the TAMEC Mobile Application which enables the detection and rapid warning of people who had been in contact with patients infected with the SARS-CoV-2 virus. The application provides a high degree of confidentiality for all users' data, thus protecting their privacy [6, 7].

The TAMEC project developed two systems that limit the spread of the virus in the community in two different effective ways:

- TAMEC Mobile System is responsible for early detection and notification of people who have been in contact with an infected person and who are possibly infected with contaminants.
- TAMEC Map System which is a real-time display of the distribution of confirmed cases and possible infected contaminants according to different parameters such as age, gender, profession, geographical location. This system analyses case distribution in different geographical areas and the time evolution of cases in different subgroups. This analysis allows early detection and geographical location of new outbreaks and provides decision-making information to prevent viral transmission.

To date, many such mobile applications rely on collecting location data or interactions of Bluetooth users. These approaches have drawn much criticism and concern for the safety of users' privacy. To solve this problem, the TAMEC Mobile application has been designed using the concept of privacy preservation. Thus, CERTSIGN has used its patent for homomorphic encryption [7]. This is a method that allows a third party to process and manipulate data in an encrypted format without having access to the original data (unencrypted data). This method allows a server to directly evaluate the encrypted data and obtain a result that is also encrypted, without the ability to decrypt the result or initial data. Thus, users will be assured that private information will not be misused, nor accessed without their explicit consent. If a user of the TAMEC mobile application has contact with an infected person, it is entirely their decision to inform the health care employees and to follow specific procedures such as testing or treatment. Also, a user notified by the mobile application as being in contact with an infected person will not be able to identify the infected person, nor the time of contact.

With all measures to secure data and preserve privacy, we are confident that users will be convinced the application is safe. The way the application is launched and promoted might determine the number of people who choose to use it. In addition to alerting the population through a mobile application such as the TAMEC Mobile system, it is also necessary to inform them to increase awareness in combating the spread of the SARS-Cov-2 virus. By knowing the evolution of the virus, one can have feedback and be motivated in adhering to the epidemiological strategies issued by the authorities.

Researchers such as epidemiologists and specialists from public health departments need well-structured and detailed data to determine key factors of the epidemic trend in the community. Accurate, concise, and easy-to-interpret data available for health care specialists enable the identification of outbreaks and risk areas. Thus, the authorities will be able to adopt prompt measures to prevent the spread of the virus from possible outbreaks.

TAMEC Mobile device application uses a system for detecting and alerting the contacts of confirmed infected patients. The system can preserve data privacy, revealing both authorities and users only the minimum information required. The core of this solution is generated by a homomorphic encryption scheme patented by CERTSIGN partner [7], the only Romanian patent of its kind. The system also includes existing digital contact tracing solutions already in use worldwide [8, 9].

The developed system is a modular one that includes six important components, namely:

- TAMEC mobile application. The TAMEC mobile application prototype is dedicated to Android operating system devices with 8 or newer Bluetooth mode versions. The detection of the contact was done using only Bluetooth technology, not GPS location, following the European Commission recommendation published shortly after drafting the project. The mobile app TAMEC is intended to be used by citizens, whom we will also call users. The application allows identifying contacts with confirmed infected patients, with sufficiently good precision in terms of distance and exposure time, using Bluetooth technology available on users' devices. The list of identified contacts that meet the case definition criteria is stored in the phone's memory (14 days for each contact). Periodically, this list is compared to the list of infected people from the Public Health Care System (DSP), using the backend components for anonymization. If a person is identified on both lists, the user is notified, without knowing who the infected person was or when the contact occurred in the last 14 days.
- The DSP web service is a backend component that has the role of ensuring the management of infected people and providing to the Computing Server component the list of

anonymized IDs of infected users. In the case of a production system, this component should be located and managed by the National Centre for Contagious Disease Surveillance and Control/Ministry of Health.

- The KMS web service (Key Management System) is also a backend component that has the role of managing the cryptographic keys necessary to ensure the confidentiality of the data from the system. In the case of a production system, this component should be located and managed by another state entity (e.g. STS);

- CS web service (Computing Server) is another backend component that has the role of performing the cryptographic calculations required by the system, for example, the intersection of anonymized mobile devices users list with the anonymized list provided by the DSP server to identify contacts with infected people. In the case of a production system, this component could be located and managed by a civil entity. This entity has also the role of giving trust to the users and ensuring that no state entity will be able to access, use, and manipulate confidential data of users.

- The CC web service (Client Configuration) is a component of the backend that has the role of centralizing mobile application configurations, depending on the phone type on which it is installed.

- The DSP-WebApp application is a web application that will be used by DSP employees in the territory for contacts management, respectively of the persons notified by the mobile application on the fact that they came in contact with an infected person, and report this and follow the necessary steps (isolation, testing, treatment, etc.). This web application can be hosted by the DSP, even on the DSP server that hosts the corresponding backend component (see the TAMEC Mobile application diagram in Figure 1).

Figure 1: TAMEC Mobile application graphic

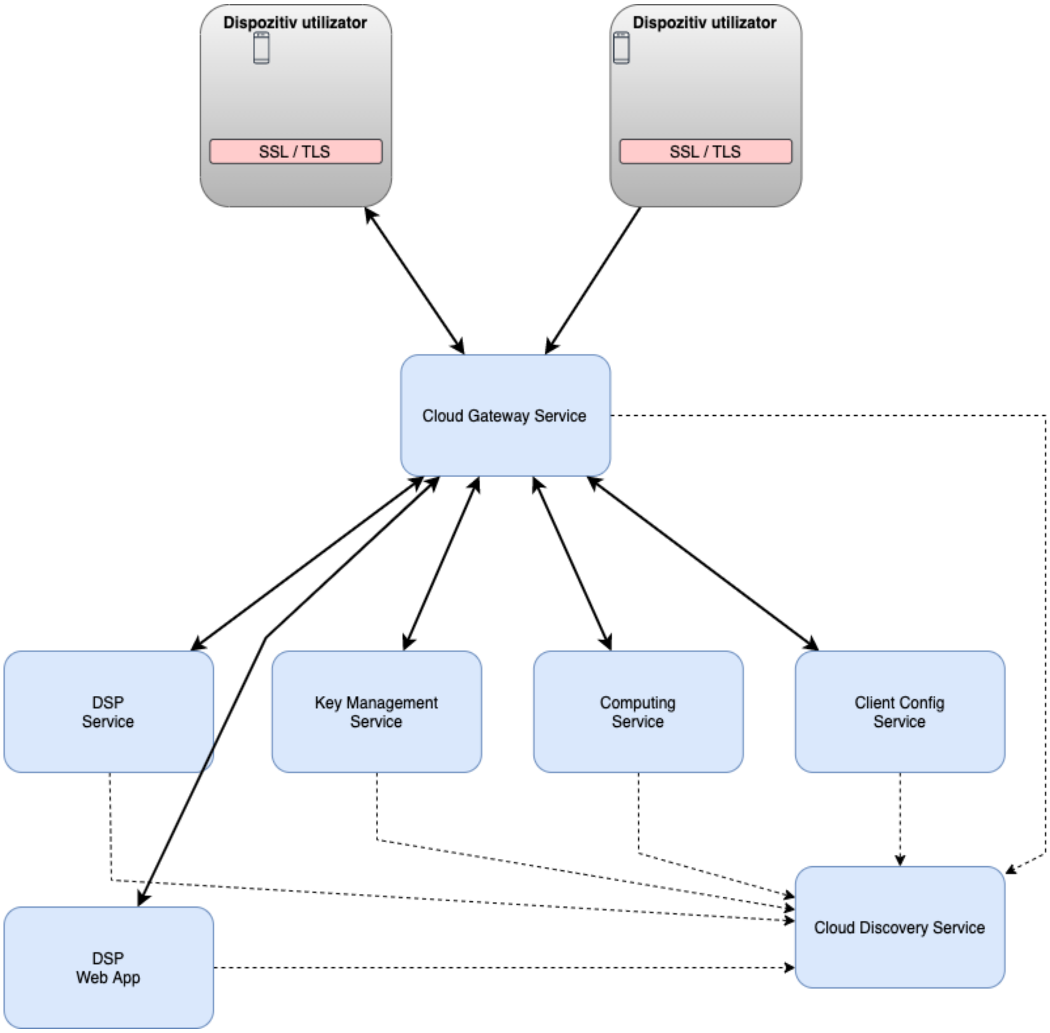

- The management system of all confirmed cases and possibly infected contacts detected by the system for detecting and warning the contacts of patients confirmed with SARS-CoV-2, TAMEC Mobile, was named TAMEC Map.

- The developed TAMEC Map system uses the software infrastructure already created in the first phase for the TAMEC Mobile system. The main components of the system are:

- TAMEC Map system database required to present the detailed map with the real-time distribution of all confirmed cases and possibly infected contacts. For each confirmed case or contact this includes the location, form of disease and severity, comorbidities, age, sex, professional category, etc. External data sources are used for this database, such as data available in the Corona-Forms system for confirmed cases, as well as data stored in the DSA WebApp database for contacts. From a technical point of view, the TAMEC Map system database is an extension of the DSA WebApp database developed in the first phase of stage I.

- The graphical interface of the TAMEC Map system is a web application that extends the DSP-WebApp application previously developed with the components necessary for the management and real-time analysis of confirmed cases and contacts and the display of this information, both on a map and in other graphical formats as well. This graphical web interface includes a public area used for dissemination related to the evolution of the pandemic in Romania, as well as a private area used by DSP and MS specialists to analyze the geographical evolution of the virus according to selected parameters, early identification of potential new outbreaks and making the best decisions based on the local situation. This web application can be hosted by the DSP, even on the DSP server that hosts the corresponding backend component.

Geolocations for confirmed or potential cases are obtained from the addresses provided by the Corona-Forms application using a service provided by Google, the Geocoding API. https://cloud.google.com/maps-platform/ terms/maps- service-terms # 3.-geocoding-api.

This database is consumed by a graphical interface (called a dashboard) that allows real-time analysis of confirmed patients and identified contacts according to available criteria. Among the information available on this map, a series of necessary data will be identified and counted, such as the location of the diagnosed persons, age group, sex, comorbidities, occupational category, and severity of the disease. This database will not contain personal data of individuals, but only anonymized IDs that can allow only the relevant authority (DSP) to find the identity for a given ID, if necessary. From the graphical interface, it will be possible to easily generate and export reports regarding all the information from the database, depending on the search and reporting criteria. This information can be very helpful for the Public Health Care System to analyze the population epidemic impact for quick and adequate epidemiological measures initialization and to allocate the hospital resources where hospitalization and treatment are required according to medical recommendations.

Also, this type of representation allows real-time mapping of infected population characteristics in each territory, rapid triage, and classification into a risk category according to certain variables (age, sex, associated diseases, stage of disease, pathogenic type of the virus, biological and immunological status of the host). Thus, our solution opens a wide field of epidemiological and clinical research, offering the possibility to identify and validate a risk score that would become an extremely helpful tool in the stratification of COVID-19 patients.

The application also includes tools that allow the dissemination of certain types of reports on social media and indicators that measure their audience.

To conclude, the solution proposed in the TAMEC project is innovative in its simplicity and ability to facilitate the prevention of the spread of the SARS-CoV-2 virus in the community and fully respects the privacy rules of citizens imposed by the EU. The application will allow the development of statistical analysis components, adaptive filtering, and automatic prediction based on artificial intelligence. Starting from the existing information in the system database this can perform their automatic analysis, discover and predict critical developments (e.g. the emergence of new outbreaks), and generate alerts to epidemiologists, accompanied by intervention recommendations.

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ORIGINAL ARTICLES

Obesity impact on symptomatology associated with pelvic floor dysfunction

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Abstract: *The increasing incidence of obesity, a significant health problem worldwide, represents a major socio-economic burden with a high impact on the quality of life. It is considered a risk factor for multiple medical conditions. More than half of women from developed countries are overweight or obese, this comorbidity can affect them from puberty through the reproductive period and then in the menopausal period, involving multiple medical and surgical specialties. It has a significant impact on the onset of puberty, the menstrual cycle, ovulation, fertility rate, and pregnancy outcome. In postmenopausal women, obesity represents an important risk factor for breast cancer, endometrial cancers or polyps, fibroids, pelvic organ prolapse, or stress urinary incontinence in addition to a higher risk of post-operative complications and also to some types of fragility fractures, especially if diabetes mellitus is associated. Pelvic floor disorders include pelvic organ prolapse, urinary or fecal incontinence. Obese patients seem more affected compare with normal-weighted females. We introduce a single-center, retrospective study on 74 patients (admitted between 2016 and 2019) with pelvic organ prolapses that were referred for surgical correction, and further on they were evaluated after one month, respective after 6 months since procedure. On admission, the subjects, aged between 41-82 years (median of 57 years) showed a strong correlation between the symptomatology caused by the prolapse degree and obesity (40.54% of them), as it appears that the discomfort and symptoms are significantly higher in the women with higher body mass index. In the reevaluation form, the patients with obesity complaint more frequently compared to the non-obese females about urinary and colorectal distress and low sexual desire and interest. The use of surgical treatment for pelvic organ dysfunctions is a contributor to improved quality of life in adult obese females.*

Keywords: *pelvic organ prolapse dysfunction, obesity, prolapse degree, body mass index, menopause, reproductive age*

INTRODUCTION

Obesity is one of the most severe health issues in developed and developing countries contributing to more than 4 million deaths each year [1]. It is by far the most frequent comorbidity severe pathologies and represents a major socio-economic burden with a high impact on the quality of life because of the disease itself, associated conditions, and impair the outcome of apparently non-connected conditions like abdominal surgery or procedures for gynecological diseases [2]. Excessive weight in the female population of reproductive age interferes with puberty, menstrual cycle, ovulation, fertility rate, abortion rate, pregnancy outcome, perinatal complications, and increased risk of severe infections [3-6]. Also, it is a risk factor for endometrial cancer, breast cancer, and uterine fibroids [7, 8]. It might

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represent a poor prognosis factor in other malignancies like ovarian or color-rectal types [9, 10]. Most cases of obesity are associated with elements of metabolic syndrome like dyslipidemia, high blood pressure, and type 2 diabetes mellitus, also associating high serum uric acid, chronic inflammatory syndrome, anomalies of bone turnover markers, damage of coagulation profile, and mineral metabolism including hypovitaminosis D [11-14]. Obesity also may be secondary to endocrine tumors like adrenal – and pituitary neoplasia (for instance, Cushing syndrome, acromegaly, etc.), also associating anomalies of glucose profile, higher risk of some other tumors, poor wound healing, etc. [15]. Other causes of obesity and glucose profile anomalies are related to pancreatic tumors (somatostatinoma, glucagonoma, etc.) with heterogeneous manifestations [16-18]. Recently, obesity has been linked with a higher risk of fragility fractures, even though estrogens (including those derived from adipose tissue, not only of ovarian origin) are traditional elements of bone protection and that bone mineral density is directly correlated with body mass index [19, 20].

Pelvic floor dysfunction affects both females of reproductive age and post-menopausal, and it includes pelvic organ prolapse and urinary incontinence are significantly influenced by obesity [21]. Urinary incontinence is associated with increased intra-abdominal pressure caused by central obesity and is related to the period that women had had excessive weight [22]. Systematic studies regarding the effect of obesity on urinary incontinence and the results of surgery on this pathology showed that one of the main causes of decreased surgery effectiveness is represented by excessive weight [23,24].

Similarly, many studies had found a higher prevalence of pelvic organ prolapse among women with excessive weight especially morbidly obese and severely obese patients [25]. Unfortunately, the tissue damage from the pelvic floor caused by excessive weight is irreversible, as the cystocele, rectocele, or uterine prolapse does not reduce after weight loss [26]. Pelvic organ prolapse progresses with aging and has a low progression rate after menopause [27]. Some authors consider that the incidence of pelvic disorders will increase by 46% by 2050 [28].

Several surgical treatments for pelvic organ prolapse and stress urinary incontinence are known but many variables can influence the outcome. These are represented by prolapse degree, symptomatology, lifestyle, age, body mass index, sexual activity. This type of pathology, the treatment required, and the correlated risk factors have an important impact on the quality of life.

Obesity is particular comorbidity that is found in many patients with pelvic floor disorders. Obese patients can associate severe life-threatening pathologies but in the meantime, they can present sexual dysfunctions, pelvic organ prolapse, modified bowel movement, or urinary incontinence [29]. Pelvic floor dysfunctions are correlated by themselves with poor quality of life, modified bowel movement, modified urinary pattern, or sexual dysfunction. Obesity is correlated with an increased risk of urinary incontinence between 20 and 70% proportional with the body mass index [30]. Urinary stress incontinence is, without doubt, more frequent in obese women and it is demonstrated that bodyweight loss will lead to decreasing in the number of episodes [31, 32]. In the literature are reported studies in which up to 40% of patients admitted for pelvic organ prolapse presented with a high body mass index [33, 34]. Persisting and progressive obesity were associated with progressing prolapse degree but weight loss was not associated with significant improvement of the prolapse degree [35].

Studies have shown that obesity influences postoperative evolution. A poor outcome with anatomical and functional failure after surgery was associated with obesity, multiple vaginal deliveries, and the intensity of the symptoms before surgery [36].

MATERIALS AND METHODS

This study aimed to evaluate the influence of obesity on women with pelvic floor dysfunction that underwent surgery in our clinic between January 2016 and December 2019. It was designed as a retrospective study in women with pelvic organ prolapses who had clinical post-operative follow-up at 1 month and 6 months. The patient data collected included age, body mass index, number of births, hormonal status. The patients agreed to anonymously use their medical data during hospitalization for surgical procedures and during follow-up.

The pelvic floor disorder was evaluated before intervention for every patient with the Pelvic Floor Impact Questionnaire (PFIQ 7) which evaluates three aspects: Urinary Impact (UIQ-7), Colorectal-Anal Impact (CRAIQ-7), and Pelvic Organ Prolapse Impact (POPIQ-7) and with Pelvic Floor Distress Inventory-20 (PFDI-20) [19]. The women responded to the questionnaire in the presence of the medical team and the responses were corroborated with the patient's history.

RESULTS

There were evaluated 74 patients that underwent surgery and had completed the questionnaires before the procedure. We included all 74 women aged between 41 and 82 years with a median of 57 years to participate in this study. Most of the patients were above 50 years at the moment of surgery (70.27%) and only 29.73% under 50 years. Only 6 of the 74 patients were nulliparous, 27 had one child and 33 had 2 children. Only 8 patients had more than 3 children. The majority of the women had one or two children (44 patients). Of these women, 59.46% had a body mass index lower than 30 and 40.54% were obese with a body mass index higher than 30. The majority of women reached menopause (48 patients), of which 30 were non-obese. Stage I and II pelvic organ prolapse was presented in 26.67% of the obese patient and 56.81% of the non-obese patients. Stage III and IV were present in 73.33% of the obese women and 43.18% of the non-obese women. The mean body mass index for patients with pelvic organ prolapse III or IV was 30.95 (±0.83) in obese patients and 25.64 (±1.96) in non-obese patients. The demographic data are presented in Table 1.

Table 1: Demographic data

	Non-obese n=44	**Obese n=30**
Age	40 – 77 (mean 59)	41 – 82 (mean 52.5)
IMC	20.65 – 29.39 (mean 25.33)	30.07 – 33.26(mean 30.83)
Menopause	30	18
Parity - nulliparous - 1 birth - 2 births - 3 and more than 3 birts	 4 17 19 4	 2 10 14 4
Pelvic organ prolapse - I or II - III or IV	 25 19	 8 22

The symptomatology was evaluated with PFIQ-7, PFDI-20, and FSFI for sexual life evaluation. A high result in PFIQ-7 and PFDI-20 represents an increased discomfort (scales 0 to 300). These patients also have poorer sexual function. For the PFIQ-7 the mean value was 191.73 (±60.00) for obese women and 152.72 (±69.16) for non-obese women (p = 0.0034, t = 3.0248, df = 72). The PFDI-20 the mean value was 192.5 (±43.12) for obese women and 156.84 (±64.33) for non-obese women (p = 0.0097, t = 2.6535, df = 72). These values suggest a strong correlation in our patients between the symptomatology caused by the prolapse degree and obesity, as it appears that the discomfort and symptoms are significantly higher in women with higher body mass index. Women with high scores on PFQI and PFDI had high scores on sexual function FSFI. Sexual desire and interest were low on 56%, sexual arousal reduced by 61% and 71% reached orgasm less than half of the time or rarely. The data collected is shown in Table 2.

Table 2: Pelvic Floor Distress Inventory-20 and Pelvic Floor Impact Questionnaire – scores in obese and non-obese patients

Variable	Non-obese (n=44)	Obese (n=30)	P value
PFIQ-7	Mean = 152.72 S.D.=69.16	Mean = 191.73 S.D.=60.00	p = 0.0034 t = 3.0248 df = 72
PFDI-20	Mean = 156.84 S.D.=64.33	Mean = 192.5 S.D.=43.12	p = 0.0097 t = 2.6535 df = 72
Prolapse degree	I-II = 25 III-IV= 19	I-II = 8 III-IV= 22	

PFIQ = Pelvic Floor Impact Questionnaire (scale 0-300)
PFDI = Pelvic Floor Distress Inventory (scale 0 -300)

The 74 women were evaluated 1 month after surgery, with a period of 4-6 weeks of sexual abstinence. The reevaluation was realized 6 months after surgery. At the reevaluation, the patients with obesity (30%) complaint more frequently compared to the non-obese females (18%) about urinary and colorectal distress and low sexual desire and interest.

There was 55.41 % with high pelvic organ prolapse degree (III or IV), from which most were women with body mass index above 30 (22 patients, compared to 19 non-obese patients). The high pelvic organ prolapse degree does not correlate with the body mass index (p=3.7, df =39).

DISCUSSION

The prevalence of obesity in developed and developing countries is continuously increasing. Although it can be managed with many options, it remains one of the most difficult comorbidity to correct. In our study, obesity was not correlated with stage III or IV prolapse, but pelvic floor symptoms were associated with a body mass index higher than 30.

The literature that evaluates the association between pelvic floor symptoms and obesity contains various results. The main cause of different results can result from the methodology of symptoms evaluation and defining the prolapse degree. In our study, one of the limits is represented by the small lot of patients that reduces the power of the study.

Hendrix et all [34] included in their study 16,616 women, from which 14.2 had uterine prolapse, 34.4% cystocele, and 18.6% rectocele, and found a strong association between this pathology and parity and obesity. Whitcomb et al [37, 38] enrolled 1,155 women in their study and concluded that the prevalence of any type of pelvic organ dysfunction was highest in morbidly and severely obese patients (57% and 53%) compared to obese women (44%), but the degree of discomfort was not influenced by the degree of obesity. Another large study [26] that included 16,608 patients concluded that obesity is associated with the progression of the pelvic organ prolapse and the damage might be irreversible. Both obesity and pelvic floor dysfunctions are associated with low quality of life.

CONCLUSIONS

Although the data can be conflicting, the practitioner must evaluate the patient and discuss the optimal approach to obesity-related complications. Managing obesity, even if it is not always a priority, it must be taken into consideration. Results take time, but in correlation with surgical treatment of pelvic organ dysfunctions, the overall quality of life of these patients will be significantly improved.

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Authors' contribution:

MCD drafted the manuscript and critically revised the final form, AEN is the corresponding author and revised the literature, NA researched the literature, AMO critically revised the manuscript, AP drafted the manuscript and revived the references, CGN researched the literature and approved the final form. All authors read and approved the final manuscript.

All authors read and approved the final manuscript.

Ethics approval and consent to participate:

For this study, the agreement was obtained from the Ethics Committee. The subject data were completely confidential and they approved the publication of data.

Patient consent for publication: All patients consented that the data declared could be used for educational purposes and statistical studies.

Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

The information generated and analyzed during the current study is available from the corresponding author on reasonable request.

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ORIGINAL ARTICLES

Negative-pressure wound therapy - advantages and disadvantages

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Abstract: *The applications of negative-pressure wound therapy (NPWT) have achieved remarkable diversification. Initially used in the therapy of soft tissue wounds related to open fractures, this technique led to the understanding of the complex mechanisms underlying it. This paper aims to analyze the evolutionary scenarios of a group of patients with heterogeneous lesions under negative pressure aspiration treatment. We aimed to identify and analyze various associated pathologies that have the potential to negatively influence the healing process under negative pressure treatment and implicitly analyze the particularities of representative cases for the group whose evolution under treatment proved to be unfavorable. Retrospective, observational study, type series of cases focused on a group of 17 hospitalized patients, who received treatment by negative aspiration, between 2015-2019 in the Surgery Department of the University Emergency Hospital Bucharest. Regarding the obtained results, diabetes mellitus, obliterating arteriopathy, and anemia had a prevalent distribution in the group with unfavorable evolution. The glycemic value can predict 17% of the evolutionary scenario of a patient (favorable/unfavorable) being necessary an integrative therapeutic conduct. Concluding, although an extremely promising therapeutic option, NPWT can prove to be disappointing given its impact in the background of suboptimally managed comorbidities.*

Keywords: *negative pressure therapy, negative prognosis, comorbidities, amputation, wound complications, lower limb obliterative arteriopathy*

INTRODUCTION

The negative-pressure wound therapy (NPWT) method involves the application of a negative (subatmospheric) pressure to the wounds in a controlled manner [1]. Using a system consisting of a vacuum source, a porous material (filler) arranged inside the wound that respects its limits, a polyurethane adhesive film, which is transparent, permeable to water vapor, and impermeable to bacteria, this therapy method ensures tightness [2]. The first indications of negative pressure aspiration therapy cited in the literature were in the management of soft tissue lesions associated with open fractures and for closing dermatofasciotomy wounds [3]. Currently, after numerous studies have elucidated many complex gears underlying the mechanism of action and it was possible to understand how the overall therapeutic effect is influenced by these mechanisms, the range of therapeutic indications has gained remarkable proportion [4].

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There are many ways in which negative wound pressure suction therapy promotes healing [5]. The suction force acting on the lesion drains the exudate and causes mechanical stress at the edges of the wound [6]. This mechanical stress alters the local tissue perfusion, consequently stimulating angiogenesis and the development of granulation tissue [7].

The changes in tissue perfusion induced by NPWT are a consequence of multiple determinism. Angiogenesis is responsible for a part of the perfusion changes [8]. In addition, pressure modifications from the wound and the physical changes of the existing blood vessels, which in turn initiate alterations in blood rheology, are other important components of the angiogenesis process [9]. Various studies have concluded that these perfusion changes induced by NPWT depend on several factors [10]. The first factor is the regime of application of negative pressure (continuous or intermittent) [11]. Then, the pressure regime used (-50mmHg - -200mmHg), the condition of the tissue (healthy/ ischemic), the type of tissue, as well as its proximity to the area on which the negative pressure acts [12].

The healing process can be influenced by modifying various functional and material parameters [13]. Thus, the pressure level, the application of the pressure regime (continuous/ intermittent), the type of filler material (foam/gauze), the presence of additional layers in contact with the wound, installation, etc., represent the therapeutic variables that must be agreed with the patient's particularities. Respectively, the patient's pathology, the type of wound, even its shape are factors to consider [14]. All these data play a key role in the therapeutic equation, whose ultimate goal is to obtain the maximum therapeutic benefit and minimize the disadvantages as much as possible [15].

Numerous studies on different types of wounds have revealed that NPWT is a more economical treatment method compared to conventional ones [16]. This involves the use of fewer dressings, and the frequency with which other reconstructive options are used is lower [17]. The healing time of wounds is shorter, and the overall duration of treatment and hospitalization is remarkably reduced [18]. Although the materials used in NPWT are at the beginning more expensive than those required for conventional treatment, in the long run, the costs of conventional therapy outweigh those of NPWT [19].

NPWT was introduced as a preventing technique for post-cesarean delivery wound morbidity, especially for obese women. The reported results are controversial; the heterogeneity of the results being summarized in two suggestive meta-analyses [20]. The background for using preventive NPWT consists in the actual rising trend of obesity and cesarean delivery among pregnant women and the compelling data regarding the results of this therapy in non-obstetrical surgical specialties [21]. However, at this moment, there are no valid studies to demonstrate that the use of NPWT is beneficial as a prevention strategy for wound infections and complications in the obstetric population; it is also not justified in the matter of economical features [22].

MATERIALS AND METHODS

The retrospective study used data collected from the computer database of the hospital, respectively the discharge summaries of the patients. The data collection process focused on the following: demographic data, hospitalization duration, and the main diagnosis. Special attention was given to the associated pathologies that may influence the evolution of lesions of interest under treatment with negative pressure (lower limb obliterative arteriopathy – LLOA, anemia, atherosclerosis, type II diabetes). In addition, other factors were registered, such as laboratory tests (complete blood count, biochemistry analyses, laboratory tests for hemostasis), general clinical examination data, paraclinical investigations (radiography, Doppler ultrasound, angiography, CT (computed tomography), angio-CT), the nature of the primary and subsequent intervention, clinical evolution on the period of hospitalization under negative pressure therapy, the post-vacuum aspect of the lesions, the clinical condition of the patient and the aspect of the lesions of interest at the post-discharge controls.

The centralized data were analyzed descriptively using Microsoft Excel 2019. SPSS Statistics software was used to perform logistic regressions. After the first session of NPWT, the division of patients into the two groups reflecting the general evolution (favorable/unfavorable) and the post-vacuum aspect of the lesions of interest was considered.

Objectives

We aimed to analyze the prognosis of a group of patients with heterogeneous lesions undergoing NPWT. Our objectives included the identification and division of cases according to the favorable/unfavorable evolution of lesions of interest. It also included the appreciation of the distribution among patients of various associated comorbidities significant for the targeted population; according to literature, they have the potential to negatively impact the healing process, even under NPWT (diabetes, obliterating arteriopathy, anemia). We also looked into the analysis of the particularities of the representative cases from the study group whose evolution under treatment proved to be unfavorable.

RESULTS

According to the inclusion criteria, a group of 17 patients was identified (5 female patients and 12 male patients), aged between 47 and 80 years, the average age being 62 years between 01.01.2015 - 31.12.2019, within the General Surgery III Department of the University Emergency Hospital of Bucharest. The situation of inpatient diagnoses, as well as the prognosis balance, are presented in a more schematic framework in Table 1.

Table 1: Inpatient diagnoses of the studied group

Diagnosis at admission	Patients no.	Prognosis
Gangrene in the lower limbs with various locations	7	Favorable – 5 Unfavorable – 2
Foot trophic ulcer	2	Unfavorable
Secondary purulent peritonitis	2	Unfavorable
Local complications of postoperative wound	5	Favorable – 4 Unfavorable – 1
Necrotizing fasciitis and cellulite of the abdominal wall	1	Favorable

Regarding the prognosis scenarios under NPWT, 7 patients (2 women and 5 men) showed an unfavorable course, the remaining 10 patients (3 women and 7 men) presenting an improved aspect of the lesions of post-vacuum interest (Table 2) (Figure 1).

Following the centralization of the number of hospitalization days, it was possible to outline a distribution of patients, respectively, the minimum number of hospitalization days (six), as well as the maximum number of days of hospitalization (seventy-five). Intuitively, the minimum duration of hospitalization was recorded in the favorable outcome group. Somewhat more edifying, it turned out to be the average number of days of hospitalization, calculated for each group. Therefore, an average of 22 days of hospitalization corresponded to the group with a favorable outcome, while for the group with an unfavorable prognosis, the average number of days of hospitalization was 40 days, with an increase of approximately 45% of the average time spent in the hospital and all its related costs.

Regarding the associated pathologies studied, respectively type II diabetes, anemia and LLOA, were identified in both groups, but overall there was a greater aggregation of these comorbidities within the group with an unfavorable prognosis.

Figure 1: A. Myxofibrosarcoma of the thigh; B. Wound dehiscence after hip disarticulation; C. Wound dehiscence treated with NPWT; D. improved lesion post vacuum therapy

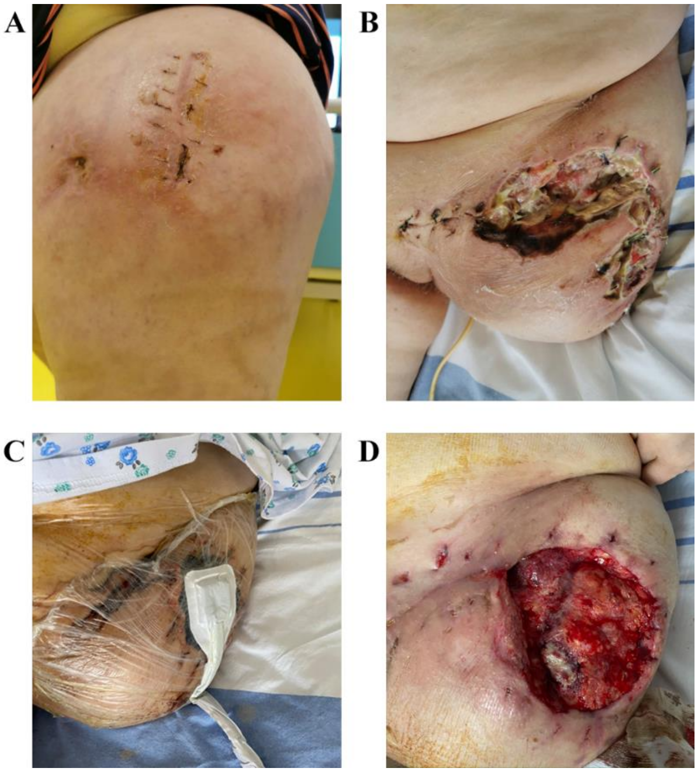

Table 2: The detailed situation of the study group situation regarding the evolution of the diagnosis and the number of hospitalization days.

Diagnosis at admission	Post NPWT	Hospitalization days
Post-operatory wound suppuration	Improved	6
Leg gangrene	Improved	11
Gangrene at the level of the stump after amputation of the toe	Gangrene at the level of the stump	11
Forefoot gangrene	Improved	12
Post-operatory wound seroma	Improved	15
Stump gangrene after amputation of fingers II-V	Improved	15
Post-operatory wound suppuration	Improved	19
Secondary purulent peritonitis (strangulated eventration)	Wound seroma	24
Finger V gangrene	Improved	25
Calf gangrene	Improved	27
Post-operatory wound suppuration	Wound suppuration	31
Necrotizing fasciitis, abdominal wall cellulite	Improved	36
Heel trophic ulcer	Trophic ulcer	40
Leg gangrene	Gangrene at the level of the stump	52
Secondary purulent peritonitis (perforated gastric ulcer)	Wound dehiscence	53
Post-operatory skin necrosis	Improved	56
Toe trophic ulcer	Gangrene at the level of the stump	75

The particularities of the distribution of comorbidities in the studied group of patients are as follows:

- 5 patients diagnosed with LLOA; 4 belong to the group with unfavorable evolution;
- 9 patients diagnosed with anemia; 5 belong to the group with unfavorable evolution;
- 12 patients diagnosed with type II diabetes; all 7 patients constituting the group with unfavorable evolution had type II diabetes.

Regarding the blood glucose values at admission, out of the 12 known patients with type II diabetes, the only values corresponding to the normal range (70 - 110 mg/dl) were recorded in 2 patients belonging to the group with favorable outcome, respectively 85 and 93 mg/dl. The rest of the patients, more precisely the 3 remaining patients of the group with favorable outcomes and the totality of the patients that formed the group with unfavorable prognosis registered glycemic values higher than the normal range.

The tendency was of aggregation of high glycemic values, with small exceptions, in the group with unfavorable evolution.

Upon admission, 9 of the 17 hospitalized patients presenting with the inflammatory syndrome. Of these, 5 patients had a favorable follow-up.

DISCUSSION

The heterogeneity of the studied group lies both in the variety of types of lesions for which vacuum therapy was chosen, and their pathological personal history.

It was noted that the shortest duration of hospitalization, as well as the lowest value of blood glucose at admission, were found in patients belonging to the group with favorable evolution, while their maximum values belonged to patients in the group with unfavorable evolution. This similar pattern of distribution of maximum and minimum values can offer a prediction of the number of hospitalization days based on blood glucose at admission. However, the result obtained was not statistically significant, the value of blood glucose at admission explaining only 0.5% of the number of days of hospitalization and the hypothesis that the value of blood glucose at hospitalization is a predictor of the prognosis of patients has not been confirmed. On the other hand, the statistical results obtained suggest that the glycemic value explains 17% of a patient’s evolution.

For a better understanding of the factors involved in guiding the evolution towards a favorable or unfavorable outcome, the second part of the study focused on a succession of 5 case presentations. All the selected 5 patients presented lesions with persistent or even worse follow-up under NPWT, the evolution as a whole of each case lying on the background of various comorbidities.

Regarding the first 3 cases, we present the outcome of 3 male patients with the ages of 53, 58, and 47 years, respectively. All these cases presented chronic lesions located in the lower limbs, as well comorbidities with significant resonance on blood perfusion: type II diabetes, LLOA, anemia (case 1 and case 3). In all 3 cases, the presence of LLOA is an important issue in the healing process of the lesions, which is why its solution by intervention or surgery plays a crucial role in the management process along with other surgical interventions and NPWT.

Unlike the cases presented above, case 4 is characterized by the vital risk of the associated pathology with which the patient presents, namely generalized peritonitis secondary to a revived, imprisoned multisacular eventration, complicated by strangulation, torsion, and necrosis of the intestinal loops. The patient's prognosis is impacted by the purulent post-operative wound, with a dragging character despite the NPWT applied. Regarding the unfavorable outcome of this case, we have to mention that in addition to the multiple comorbidities (type II diabetes, stage II hypertension, osteoporosis, bilateral gonarthrosis) and multiple personal pathological surgical histories (thyroidectomy, appendectomy, myomectomy, intestinal occlusion) in this case, the particularity emerges from the coexistence of short bowel syndrome and anorexia nervosa. Short bowel syndrome is the prerogative of an extensive surgical bowel section that involved subtotal enterectomy [23]. Anorexia nervosa is a complex behavioral and psychiatric pathology [24]. It is impressive how the two pathologies are intertwined and maintained an altered biological status of the patient, burdened by dehydration and electrolyte and acid-base disorders. All these overlapped with metabolic disorders caused by an unbalanced type II diabetes lead to a status of severe malnutrition. This context, associated with anemia, deprives the whole body, including the postoperative wound of nutrients needed for healing. One mechanism by which NPWT promotes the healing process is by increasing local blood flow, so implicitly the supply of oxygen and nutrients, but the beneficial effect is canceled if the blood flow to the wound is poor in oxygen and deprived of nutrients [25].

In selected case 5, the patient presents for a parietal suppuration in the right inguinal region after surgery with alloplastic materials for the right inguinal hernia. The patient's personal history includes an evacuated inguinal parietal abscess, which occurred after the surgical correction of the right inguinal hernia. In terms of comorbidities, the pathologies representative for the group with unfavorable evolution, respectively diabetes and anemia were noted. In addition, cardiovascular abnormal findings and a prostate adenoma were diagnosed. In the management process, an abscess underlying the pubic symphysis is identified. Surgical treatment aimed to remove all the debris and a cartilaginous fragment, subsequently applying NPWT to the semi-open wound. The patient was discharged healed and in good general condition.

At the first follow-up visit, the patient presents with a dehiscent wound with a visible granulation process and with a good general condition. However, at the second reevaluation, the biological and clinical condition of the patient was significantly worse (presence of fever and inflammatory signs), the right inguinal region appeared pasty and edematous, with the suppuration area located at the inferior area of the wound. Imaging results (inflammatory phenomenon extended to the muscle groups adjacent to the suppurative process, multiple lymphadenopathies, densification of subcutaneous inguinoscrotal and bilateral gluteal tissue, the osteolytic appearance of pubic bones) corroborated with other clinical and paraclinical investigations outlined the diagnosis of pubic osteitis. This diagnosis placed the case on a higher hierarchy in terms of gravity. It is to note that under conservative treatment the symptomatology diminished.

In an attempt to understand the unfavorable evolution of the patient, certain aspects should be discussed. First, the patient was the oldest from the study group (80 years) and the specialty literature mentions the alteration of healing processes in the advanced senescence process [4, 20, 21]. Secondly, there were various comorbidities, of which poorly controlled diabetes, which is responsible for the management difficulties of traumatic wounds. In addition, the infectious factor must be brought into question - staphylococcus aureus was isolated from the patient's wound – a virulent pathogen that causes treatment difficulties, even in people without a significant amount of comorbidities.

There are several limitations of this study that should be mentioned. One of these is the small number of patients included. Also, the study was retrospective and the data were collected from indirect sources, namely the computer database of the hospital, with no information available regarding the materials (filler material, wound contact layers) and the functional parameters applied (pressure level, pressure regime, vacuum source). Certain biological parameters crucial for documenting the prognosis of the case could not be identified, for which reason it was not possible to include them in the statistical analysis.

CONCLUSIONS

According to our study results, the majority of patients for which negative pressure wound treatment was applied presented a favorable outcome.

In order of frequency, the diagnoses at admission for patients included in our study were as follows: gangrene with various localizations in the lower limb, local complications of postoperative wound, legs trophic ulcers, secondary purulent peritonitis, necrotizing fasciitis, and parietal-abdominal cellulitis.

The average number of hospitalization days for the group with unfavorable outcomes was almost double compared to that of the group with favorable evolution, which engaged higher costs.

Pathologies such as diabetes, lower limb obliterative arteriopathy, and anemia had a prevalent distribution in the group with unfavorable evolution. According to the statistical analysis, the glycemic value can predict 17% of a patient's prognosis scenario (favorable/unfavorable).

The cases presented in the study mean to highlight the importance of an immediate, integrative and exhaustive therapeutic approach. Although an extremely promising therapeutic option, NPWT can prove to be disappointing, given its impact in the background of suboptimally managed comorbidities.

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Authors' contribution:

GP - design of the paper; CIM - drafting of the paper; VAB - analysis of data; NT - interpretation of data; MMC - revising of the paper; BC - final approval of the paper; CC - final approval of the paper All authors read and approved the final manuscript.

Ethics approval and consent to participate:

The study was done following the ethical standards stipulated by the Helsinki Declaration.

Patient consent for publication: Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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ORIGINAL ARTICLES

Assessment of traumatic injuries in 1028 patients referred to the Ayatollah Taleghani Hospital from the Sarpul-Zahab earthquake

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Abstract: *Introduction: Investigating and recognizing the epidemiological structure of earthquake damages in earthquake-prone countries is of great importance. The aim of this study was to evaluation of traumatic injuries in1028 patients referred to the Ayatollah Taleghani Hospital from the Sarpul-Zahab earthquake (November 12, 2017).*

Method: This study is a cross-sectional study that includes 1028 traumatic patients from the Kermanshah earthquake. The patients, including outpatients in the emergency department, admitted in orthopedic, general surgery and neurosurgery, or even patients who needed to be transferred to other treatment centers.

Result: Of the 1,028 patients, 488 were men (47.5%). The number of men with high injury severity was 51 (10.4%) and the number of women with high severity of the injury was 58 (10.7%). The average age of the injured was 33.04±15.01 years. The mean ISS measure in this study was 8.9±1.6. A total of 109 (10.6%) had scores above 17. The number of cases with head and neck injury was 328 (24%), 233 cases (17%), chest 114 (8.3%), The abdomen or pelvis is 190 (13.9%), the spine is 200 patients (14.6%). After examining the type of trauma in 1791 injuries, 1074 injuries (59.9%) had penetrating trauma and 717 injuries (40.1%) of blunt trauma were calculated. Of patients with severe trauma, 83 patients suffered severe injuries (7.7%) and in patients with blunt trauma, 68 patients suffered severe injuries (9.4%). The mortality rate was 5 (0.5%).

Conclusion: This study established a fracture pattern in the referred patient of the Sarpul-Zahab earthquake to the Ayatollah Taleghani Hospital and is practical for clinicians to increase understanding of this natural disaster and permits superior selection of the triage management and therapeutic process.

Keywords: *earthquake, disaster, trauma, Kermanshah*

INTRODUCTION

Trauma is the most common cause of morbidity and mortality in the first third of life in human societies [1-3]. In recent years, trauma has always been one of the three main causes of injury and general mortality [4]. The distribution of incidence of natural disasters is different in the various regions of the world. There are more than a few potent geographical regions for trauma experiences, such as earthquakes, organized violence, terrorism, combats, and other natural disasters [5-6]. In the past few decades, Iran has experienced more than a few earthquakes, which caused severe and massive damages. Therefore, investigating and recognizing the epidemiological structure of earthquake damages in earthquake-prone countries such as Iran is of great importance. On November 12, 2017, an earthquake

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take place in Sarpul-Zahab and Sallatha Babajani cities, Kermanshah Province, Iran, with a 6.9-magnitude on the Richter scale. The urban and population density areas were exposed due to this earthquake for significant severe and massive damages. The fracture pattern in injured patients due to earthquakes is different in the various regions of the world, for example, in the earthquake of Armenia, the head and neck injury rate was 22% [7]. In the Wenchuan earthquake, the highest number of injuries has been seen in those who suffered damage to the external surface of the body [8-9]. Generally, the fracture pattern of an earthquake can be including simple morbidity, to even mortality. Therefore, characterization of the mechanism and epidemiologic variables of these traumatic events is necessary for the management of proper initial triage [10].

The aim of this study was to evaluation of traumatic injuries in1028 patients referred to the Ayatollah Taleghani Hospital from the Sarpul-Zahab earthquake (November 12, 2017) regarding different levels of demographic variables (age and gender), pathology of the disease (anatomical location of the injury, type of trauma, triage level, etc.) and the stages of treatment (how the patient transmits, operating care, etc).

METHODS

This study is a cross-sectional study that includes traumatic patients from the earthquake in Kermanshah province on November 12, 2017, referred to the Ayatollah Taleghani Hospital of Kermanshah as the largest treatment center in the west of Iran. The study population included 1028 patients, including outpatients in the emergency department, admitted in orthopedic, general surgery, and neurosurgery, or even patients who needed to be transferred to other treatment centers.

Data analysis including frequency varying levels, the percentage of damage to the severity of the trauma for variables such as age, sex, type of trauma, and injury as well as the correlation between trauma severity and anatomical location of some of the variables. After the initial studies, the patients' medical records were filled in for two months.

The severity of trauma by ISS method according to the AIS criteria for traumatic patients with a proper pre-study was carried out. To calculate the patient ISS, the body is divided into six parts as follows:

Head and neck
Face
Chest
Abdomen or pelvis
Spinal cord
Limbs

To calculate the ISS, the highest severity of AIS is considered in the three areas of the body that have seen the most damage, then we squeeze three AIS scores together to calculate the severity of the ISS damage. AIS scoring is based on the amount of injury and the anatomical location of the damage between 1 and 6, which is 1 indicates minor damage and 6 is the number of fatal injuries. Hence, the severity of the ISS damage is between 1 and 75, since by eliminating deadly injuries with a score of 6, the sum of the squares of other AIS levels is 75 (25 + 25 + 25 = 75), 75 (25 + 25 + 25), the injuries that the IIS is 6, 75 are considered [6, 13, 14]. Registered demographic data allow the proportion of male and female patients or the age range of patients to be identified in terms of the severity of the injury. The structure of the damage is also known by its characteristics, such as trauma, triage, and anatomical location. The protocol of the study was approved by the ethical committee of Baqiyatallah University of medical sciences.

Data analysis was performed using SPSS-20 software. Descriptive statistics such as frequency, percent, mean and standard deviation were presented.

RESULTS

1028 patients transferred to Ayatollah Taleghani hospital were included in the study. Table 1 depicts patients' characteristics regarding transition status, sex, age levels, Anatomic location of the injury, Triage level, Type of trauma, Duration of admission, ISS, and outcomes (Table 1). Of the 1,028 patients, 488 were men (47.5%). The number of men with high injury severity was 51 (10.4%) and the number of women with high severity of the injury was 58 (10.7%). The ratio of women with high severity of the injury was 5.6% for all patients and 4.9% for men. The average age of the injured was 33.04±15.01, range from 1 to 95 years.

Table 1: Patients' characteristics regarding transition status, sex, age levels, Anatomic location of the injury, Triage level, Type of trauma, Duration of admission, ISS, and outcomes

Variable	N	%
Transition status		
Emergency	354	34.4
Personal car	420	40.9
Other ways	254	24.7
Sex		
Male	488	47.5
female	540	52.5
Age		
0-4	56	5.4
5-14	59	5.7
15-24	136	13.2
25-44	410	39.9
45-64	242	23.5
>65	125	12.3

Variable	N	%
Anatomic location of the injury		
Head and neck	328	18.3
Face	233	13
Chest	114	6.3
Abdomen and pelvis	190	10.7
Spine and Spinal cord	200	11.2
Limb	726	40.5
Triage level		
1	2	0.2
2	470	45.7
3	534	52.0
4	22	2.1
5	0	0.0
Type of trauma		
Penetrating	1074	59.9
Blunt	717	40.1
Duration of admission		
1 day	676	65.8
1-7 day	328	31.9
7-30 day	22	2.1
>30 day	2	0.2
Operational care		
Emergency clearance	527	51.3
Orthopedic surgery	349	33.9
General surgery	109	10.6
Neurological surgery	43	4.2
The outcomes		
Partial recovery	733	71.3
Die	5	0.5
Transfer to another hospital	189	18.4
Other	101	9.8
ISS		
1-7	578	56.2
8-16	341	33.2
17-25	71	6.9
>25	38	3.7

The mean ISS measure in this study was 8.9±1.6, ranging from 1 to 75. A total of 109 (10.6%) had scores above 17.

The total of injuries for 1028 patients was 1,791 injuries, according to the anatomical status of injury, the number of cases with head and neck injury was 328 (24%), 233 cases (17%), chest 114 (8.3%), The abdomen or pelvis is 190 (13.9%), the spine is 200 patients (14.6%). The organs are 726 cases (53.3%) (Table 2).

Table 2: Frequency of different anatomic location groups of damage in the ISS levels

		ISS				
		1-7	8-16	17-25	>25	Total
Anato	Head and neck	136	99	54	39	328
	Face	104	98	21	10	233
	Chest	43	50	14	7	114
	Abdomen or pelvis	66	62	30	32	190
	Spinal cord	83	62	25	30	200
	Limbs	489	161	51	25	726
	Total	921	532	195	143	1791

The division of patients in terms of the age group showed that the highest number of patients was in the age group of 25-44 years, and the lowest number of patients was in the age group of 0-4 years. This severity of injury shows that the severity of injury in the age group of 15 to 24 years has the highest proportion compared to other groups (Table 3).

Table 3. Frequency of different age groups and frequency of severe injuries in groups

		Frequency	Frequency percent	Percentage of damage
Age	0-4	56	5.4	3.5
	5-14	59	5.7	6.7
	15-24	136	13.2	12.5
	25-44	410	39.9	11.4
	45-64	242	23.5	8.6
	>65	125	12.3	14.4

After examining the type of trauma in 1791 injuries, 1074 injuries (59.9%) had penetrating trauma and 717 injuries (40.1%) of blunt trauma were calculated. Of patients with severe trauma, 83 patients suffered severe injuries (7.7%) and in patients with blunt trauma, 68 patients suffered severe injuries (9.4%). The mortality rate was 5 (0.5%).

DISCUSSION

Earthquakes are administered in different methods, according to particular regions and countries [11]. In the past few decades, Natural disasters have affected 800 million people, and have caused over $500 billion of economic costs [12-14].

In recent decades, various methods have been used to evaluate the severity of the trauma, including ISS, TRISS, etc. In this study, the ISS method was used and we examined the severity of trauma with other variables. Being the benchmark used in this article, ISS is a global traumatic survey criterion defined based on the sum of the three injuries with the highest AIS score. ISS is usually divided into 6 areas per person and is also used for patients with more than one injury. The numerical value of this numerical criterion is between 1 and 75, with scores less than 7.5 categorized in the group of weak injuries, scores between 7.5 to 17 categorized in the group of moderate injuries, and scores more than 17 categorized in the group of severe injuries. Traumatic studies and epidemiological structure of the trauma caused by the earthquake led to the classification of vulnerable groups and the identification of vulnerable anatomical parts of the body in injured people. This knowledge informs us about the necessary steps to prevent further problems and to deal with the damage that has been caused.

In this study, five mortality (2 female and 3 male patients) were seen that have multiple trauma and blunt abdominal trauma. 60 % of the dead were between the ages of 25-45. The severity of injury for all of them was higher than 25, with an average injury severity of 38.4. this mortality can reduce if the management of triage was done with the standard method, for example, Sonography in Trauma (FAST) can be regarded in the administration of disaster management, that it can reduce mortalities due to blunt abdominal and multiple traumas, as Kakaei et al. [15] reported on the value of FAST as a primary diagnostic method for revealing of blunt abdominal and multiple trauma. Therefore, conducting FAST is a primary screening with high speed that can use in situations of disaster. The impotence of the FAST as a primary diagnostic method has been confirmed in the previous studies for primary screening [16].

The administration of triage of patients is one of the most important episodes of disaster management, hence, it has been indicated that triage of injured patients in disastrous events, particularly earthquakes, can decrease considerably mortality and morbidity. The accessibility of hospitals is very significant in disaster administration after earthquakes, and this subject is related to the structure of the hospital and building standards [17]. In this study, we have few mortality (5 cases), because the administration of triage of patients was done correctly, and the hospital was accessible and all the patients were established on the same crisis day. Although, in the Bam earthquake, the patients were triaged during the night following the disaster, since the two main hospitals of Bam were destroyed [18].

In this study, the demographic results established that trauma was more common in females (52.5% for females and 47.5% for males), and 39.3% of the patients have 25-44 years. The several studies indicated comparable outcome with these in the different regions of the world however In the Pakistan earthquake, 60.7% of the patient was male and 39.3% were female and 49% of patients have 25-44 years [19] because the most population of this region are in the third decade of life.

In this study, the age range of the patients was 1-95 years with a mean of 33.4 years. In the Marmara earthquake, Turkey, the age range of the patients was 11-74 years with an average of 34 years.

Limb injuries are the most common trauma in earthquake injuries, in this study, 42% of patients have presented limb injuries that the previous studies indicated similar outcomes, as 39% of the patients suffered head and neck injuries in the Armenia earthquake [8-11]. In our study, the most frequent area exposed for injury was limbs (42%) and followed by head and neck (18%), face (12.7%), abdomen and pelvis (10.5%), chest (5.9%). Also, the most common area exposed for injury was limbs (38.5 %) and followed by chest (21.4%), abdomen and pelvis (15.2%), spine (9.5%), head and neck (7.9%), face (1.3%) in the Wenchuan earthquake. In the Bam earthquake, 68% of the organs were damaged, compared to 42% in our study. Because the Bam earthquake occurred more time than this earthquake, also the strength of the Bam earthquake was higher due to the surface of the earthquake center [12, 20-24].

CONCLUSION

This study allows us to assess and manage the crisis, trauma due to earthquake, epidemiology of earthquake trauma. The results of this study confirmed with studies of other earthquakes in the world, however, some results different since this study was conducted to determine the epidemiological structure of the trauma, the issues which haven't been discussed in the literature. Consequently, this study established a fracture pattern in the referred patient of the Sarpul-Zahab earthquake to the Ayatollah Taleghani Hospital and is practical for clinicians to increase understanding of this natural disaster and permits superior selection of the triage management and therapeutic process.

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Tonsillar neoplasm: experience of the 2nd Otorhinolaryngology Clinic from Cluj-Napoca

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Abstract: *Introduction. The primary tonsillar neoplasm is a common malignancy of the head and neck, alcohol and/or cigarette smoke and Human Papilloma Virus is the main etiological agent. This study offers a retrospective analysis of the tonsillar neoplasm cases in a tertiary medical center from Romania.*

Patients and methods. We present a 4-year (2015-2018) retrospective study on 72 patients diagnosed with tonsillar neoplasm in our clinic, previously untreated patients. The study was included only the patients diagnosed through histopathological evaluation with tonsillar neoplasm. We searched the cases in our clinic database, and we collected data about age, gender, symptoms on admission, findings of the objective physical examination, oro-pharyngoscopy, medical imaging examination results, histopathological results, stage of the disease, therapeutic management.

Results. The group composition is: 75% men and 25% women, 62.5% of them are smokers. Dysphagia/swallowing discomfort was the most common symptom identified, and the non-keratinized squamous cell carcinoma was the most common histopathological form. Stage I tonsil carcinoma was diagnosed in 25% of the cases, stage II in 15.3%, while 47.2% of the cases had stage III or IV. None of the patients presented distant metastases. Lymphoma was diagnosed in 12.5% of the cases.

We performed for all patients in stages I and II underwent total unilateral tonsillectomy. For the patients in stages III and IV, we performed surgery after radiotherapy-chemotherapy treatment or only radiotherapy-chemotherapy.

Conclusions. In Romania, smoking is still the most important risk factor for tonsil cancer. The majority of patients have visited a doctor only when dysphagia and, above all, palpable cervical adenopathy, were present, and the tumor was in the advanced stage. In Romania, the ENT-oncology integrated medical services are few, in most cases patients are lost from otorhinolaryngological follow-up.

Keywords: *tonsillar neoplasm, palatine tonsil, squamous cell carcinoma*

INTRODUCTION

Head and neck cancer is the fifth most frequent form of cancer worldwide [1]. The primary tonsillar neoplasm is the third most common malignancy of the head and neck, after thyroid and larynx cancer [2]. Every year, around 264,000 cases of oral cavity cancer are diagnosed in the world. At the time of diagnosis, approximately 50% of patients have lymphatic dissemination [3].

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Within five years, 15% of patients with a tonsillar neoplasm present a secondary carcinoma in the head-neck area, especially those patients who are chronically exposed to the carcinogenic factors of the upper aero-digestive tract [3].

The risk factors for the development of tonsillar cancer are alcohol and/or cigarette smoke, and, especially for young patients without these risk factors, Human Papilloma Virus (HPV) may be an etiological agent [4]. Irrespective of the etiology, T1 or T2 stage tonsillar primary lesions can be treated successfully with acceptable loco-regional control and good survival rates by using either surgery or primary radiotherapy. The therapeutic success rates for T3 or T4 stage disease are less encouraging regardless of the treatment modality or medical center [5].

This study aims to make a retrospective analysis of this pathology, based on 72 cases diagnosed during 2015-2018, in a tertiary medical center from Romania.

PATIENTS AND METHODS

The study design is a retrospective observational study. It included patients admitted into the clinic for tonsillar neoplasm between 1 January 2015 – 31 December 2018. The inclusion criteria: histopathological diagnosis of tonsillar neoplasm during this period. The search for cases in the database was conducted based on the main diagnosis at discharge from the hospital, i.e. malignant tonsil tumor and disease code. The exclusion criteria: patients whose data were inaccessible, incomplete, or did not fulfill the inclusion criteria.

The statistical basis and clinical records were examined by the same investigator; the following data were collected: age, sex, symptoms on admission, findings of the objective physical examination, oro-pharyngoscopy, medical imaging examination results, histopathological results, stage of the disease, therapeutic management. The patient's privacy and confidentiality were protected under the laws in force.

All cases in the study were biopsied and all the specimens were fixed in 10% (v/v) buffered formalin and sent for the histopathological evaluation. For the classical histopathological examination, hematoxylin-eosin (HE) staining was used, while for the immunohistochemical (IHC) tests we used the antibodies: CD20 (cloneL26), CD10 (cloneSP67), bcl2 (clone124), CD5 (cloneSP19), bcl6 (cloneL26), CD3 (clone 2GV6), Ki67 (clone30-9) - Ventana Medical Systems, Tucson, AZ, on Benchmark XT Ventana-Roche platform.

None of the patients had been tested for HPV.

The patients were staged following the TNM classification of the American Joint Committee on Cancer (stage groups for non–HPV-mediated (p16 -) oropharyngeal cancer) [6].

The study protocol was approved by the University Clinical Hospital of Railway Company Ethics Council under the No. 11/09.12.2020.

RESULTS

Of the 72 patients, 75% (n=54) were men and 25% (n=18) women. The average age was 62.8 (range 42-79 years) in the men subgroup and 56.3 (range 37-69 years) in women. The number of patients from rural and urban areas was about equal. The newly diagnosed cases each year were relatively constant.

Figure 1: Advanced stage tonsillar neoplasm – macroscopic aspect

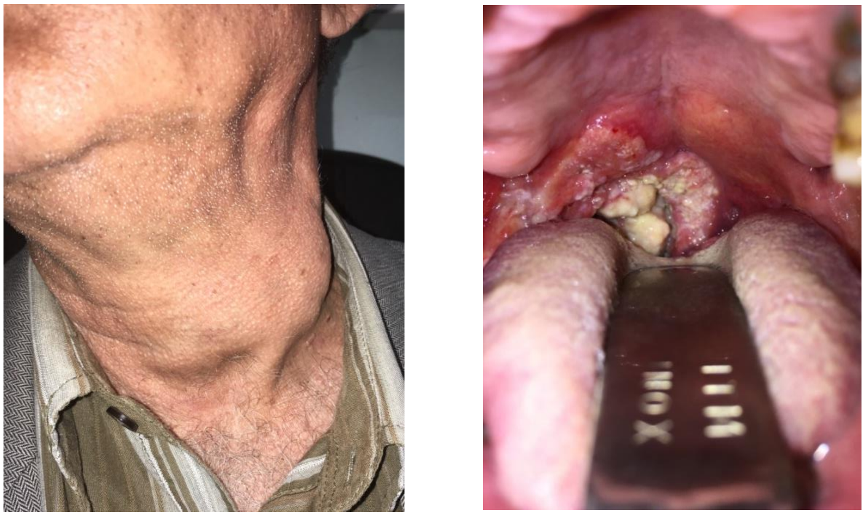

A: Lateral-cervical adenopathic block in a patient with tonsillar neoplasm

B: Vegetative tumor with a starting point in the right tonsil (keratinized squamous-cell carcinoma).

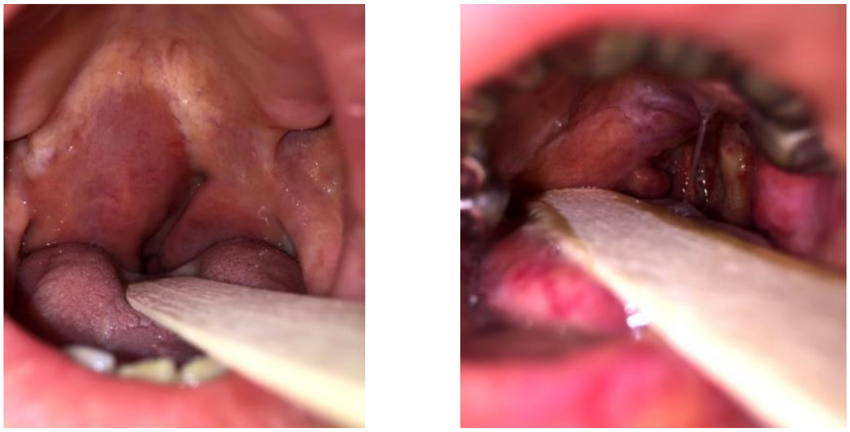

C, D: Infiltrative tumor with a starting point in the right tonsil (non-keratinized squamous-cell carcinoma).

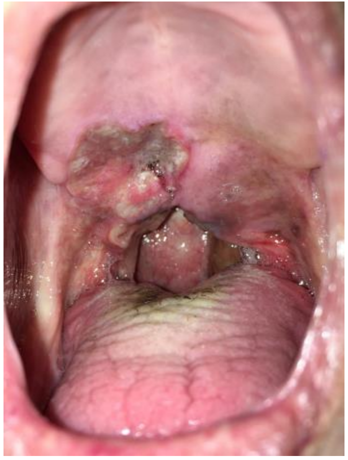

E: Ulcerated-infiltrative tumor with a starting point in the left tonsil. Bucco-nasal fistula (non-keratinized squamous-cell carcinoma).

Risk factors. Smokers accounted for 62.5% (n=45, 35 men and 10 women), while 29.2% admitted to concomitant alcohol and tobacco consumption (n=21, 18 men and 3 women).

Clinical evaluation. The symptoms on admission to the hospital were: dysphagia/swallowing discomfort in 63.8% (n=46), palpable homolateral adenopathy (Figure 1A) in 20.8% (n=15), negative body weight curve in 36.1% (n=26), reflex otalgia 9.7% (n=7), trismus 8.3% (n=6), and pain in the oropharynx, tolerable without analgesic medication in 4.1% (n=3).

The macroscopic aspect of the tonsil tumor indicated a vegetative form in 34.7% (n=25) - Figure 1B, infiltrative in 30.5% (n=22) - Figure 1C-D, ulcero-infiltrative in 26.4% (n=19) - Figure 1E, while occult forms with metastatic lateral cervical adenopathies were present in 8.4% (n=6) of the patients, in which a triple biopsy (palatine tonsil, rhino pharynx, tongue base) was performed, with positive results at the tonsillar level.

Histopathology. The non-keratinized squamous cell carcinoma (Figure 2A) was evidenced in 58.3% (n=42) of the cases, keratinized squamous cell carcinoma (Figure 2B) in 29.2% (n=21) of cases, and B cell lymphoma (Figure 3) in 12.5% (n=9) of cases. The G2 histologic grade (moderately differentiated) was identified in the majority of cases (71%, n=51).

Figure 2: Squamous tonsillar carcinoma

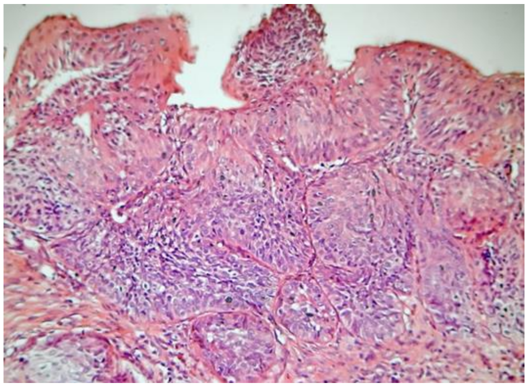

A – non-keratinized, moderately differentiated (G2) HE staining: ×100;

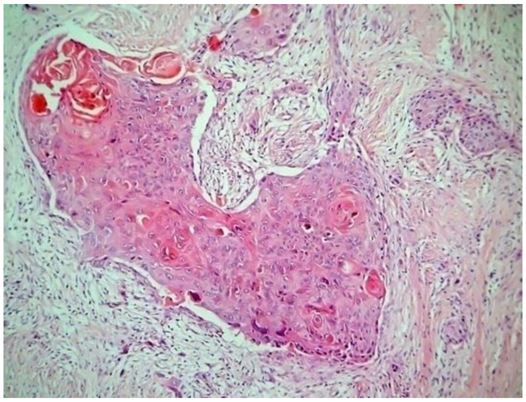

B – keratinized, moderately differentiated (G2) HE staining: ×200

Figure 3: B cell tonsillar lymphoma

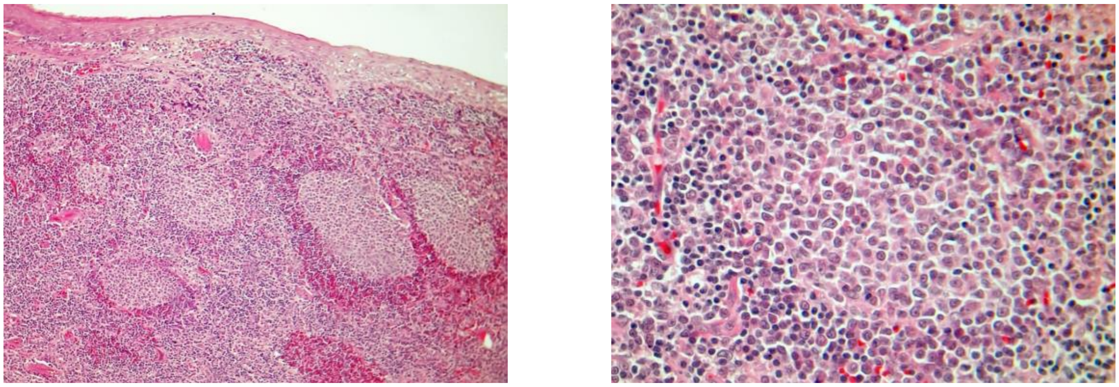

A - Different dimension follicles with no tingible bodies. HE staining: ×200

B - Small follicle made of centrocytes and centroblasts without mantle zone. HE staining: ×400

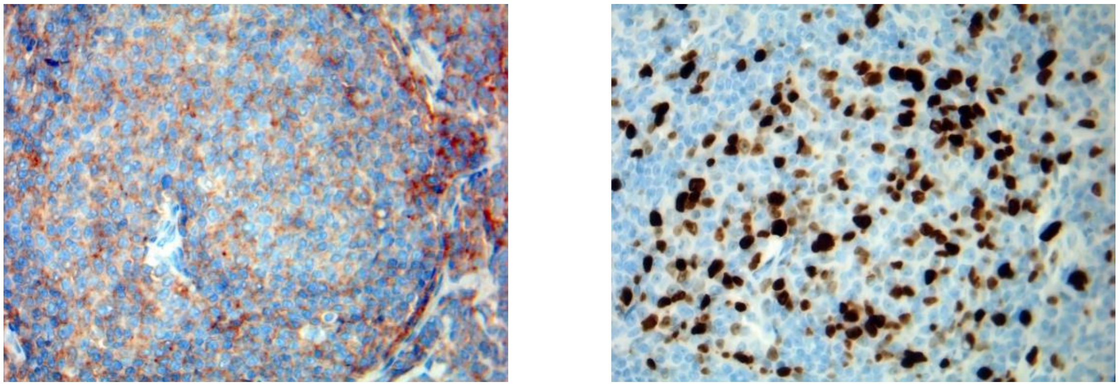

C - CD10 positive in and outside the germinal center. ×400

D - Low ki67 positivity in the germinal center. ×400

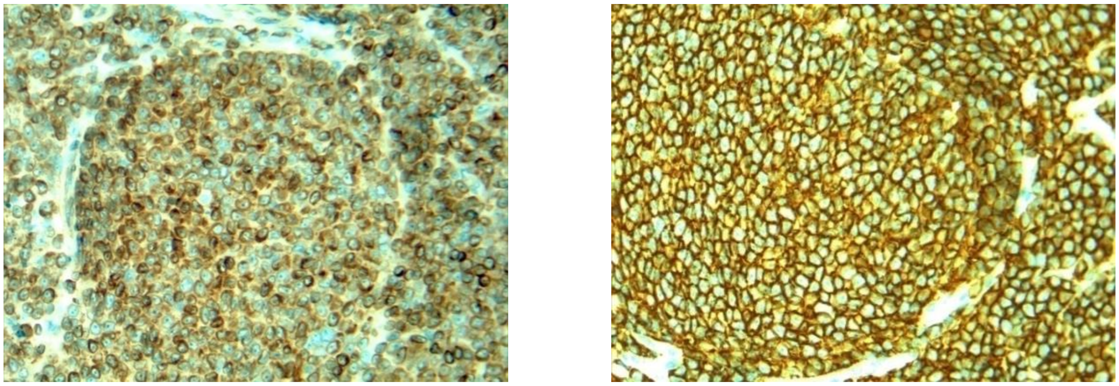

E - BCL2 positive in the germinal center. ×400

F - CD20 positive in and outside the germinal center. ×400

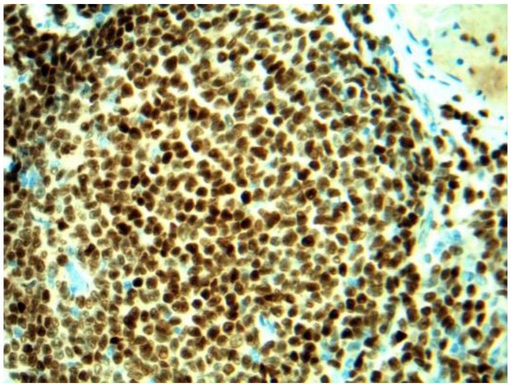

G - BCL6 positive in and outside the germinal center. ×400

Imaging investigations. After the confirmation of the histopathological diagnosis, a contrast-enhanced computer tomography (CT) was performed (Figure 4A) in 70 patients, while positron emission tomography (PET-CT) was performed in one patient (Figure 4B).

Figure 4: CT images of the tonsillar neoplasm

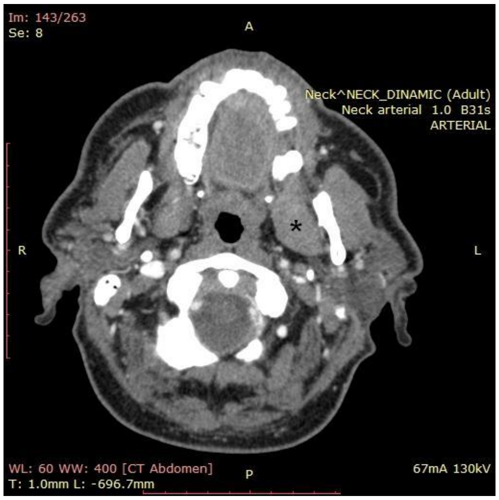
Im: 143/263
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A
Neck^NECK_DINAMIC (Adult)
Neck arterial 1.0 B31s
ARTERIAL
*
R
L
WL: 60 WW: 400 [CT Abdomen]
T: 1.0mm L: -696.7mm
P
67mA 130kV

A - Contrast-enhanced CT, transversal section: left tonsil tumor (marked by *).

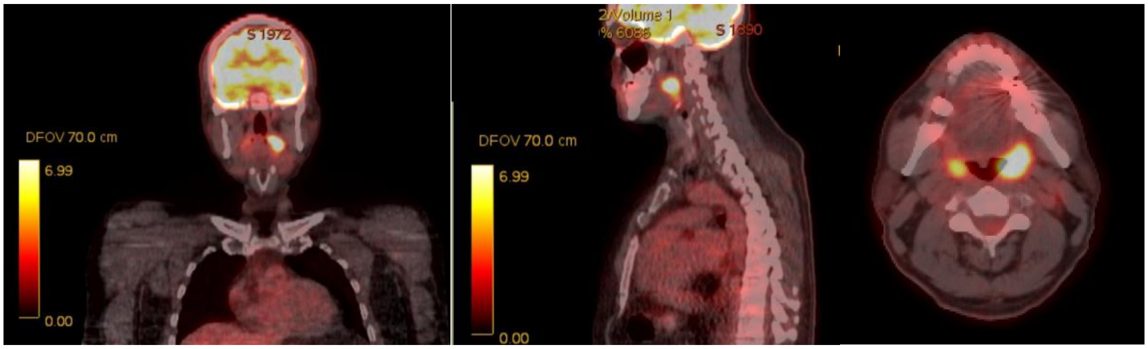
S 1972
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B - PET-CT, frontal, sagittal, and transversal sections: high perfusion tumor of the left tonsil.

Treatment. All patients in stages I and II, and the lymphoma cases underwent total unilateral tonsillectomy through a direct transoral approach (Figure 5A).

For the patients in stages III and IV (surgical cases), we performed, after radiotherapy-chemotherapy treatment, pharyngectomy with partial glossectomy in 2 cases (Figure 5B), tonsillectomy, and partial palatectomy in 9 cases.

In all cases operated, lateral-cervical lymph node ablation was performed: for N0 cases – selective supra omohyoid neck dissection, and for N+ cases – radical neck dissection modified (Figure 5C-E).

Figure 5: Intraoperative aspects

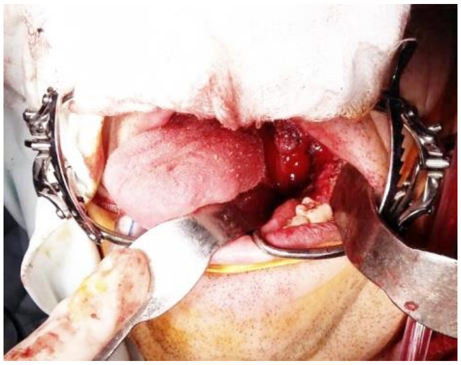

A - Transoral extended left tonsillectomy (T2N0M0).

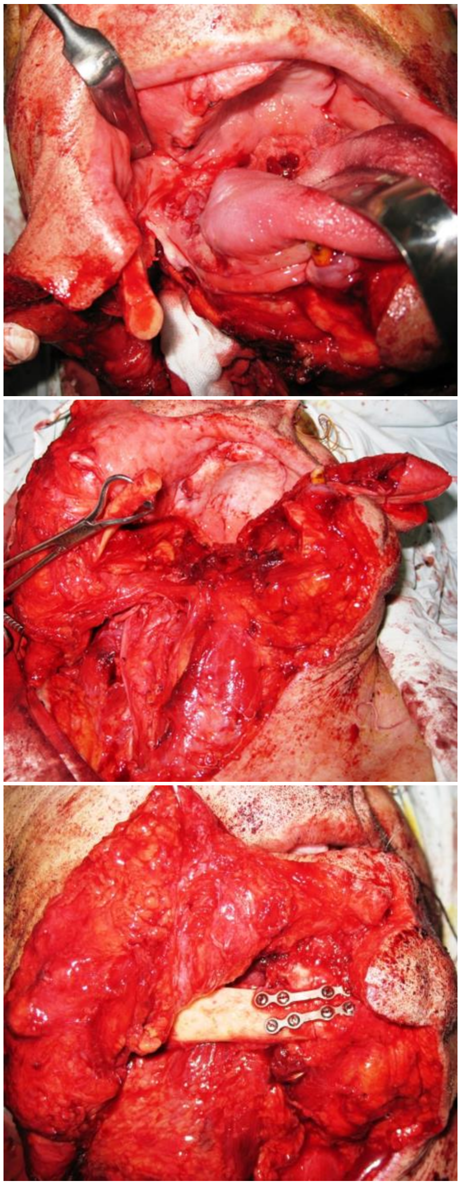

B1-3 - Transmandibular pharyngectomy with pectoralis major reconstruction.

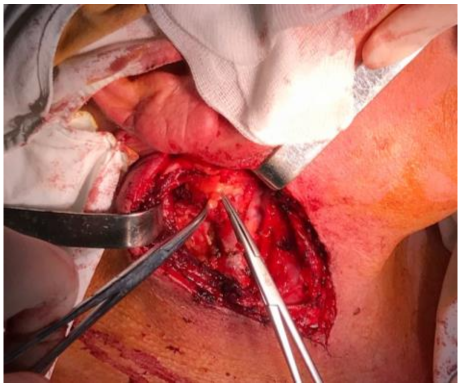

C - Right lateral-cervical lymphadenectomy.

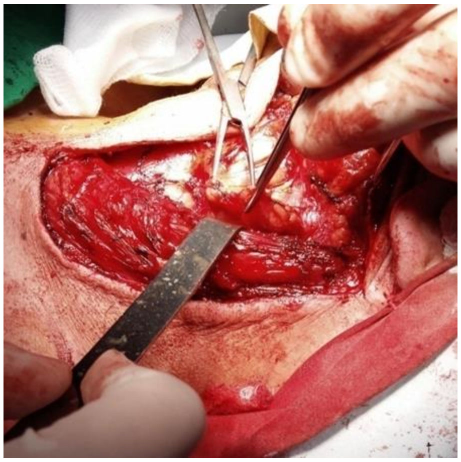

D1: Dissection below the sternocleidomastoid muscle.

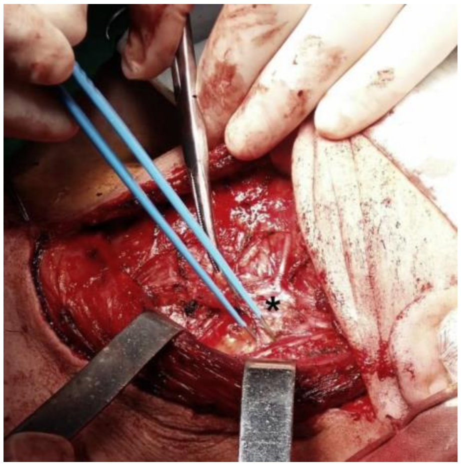

D2: Exposure of the right internal jugular vein (marked by *).

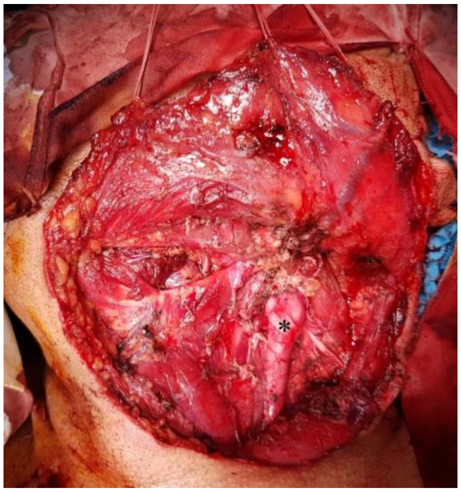

E - Radical dissection of the neck in a case with T4bN2M0. The tumor includes the left secondary carotid arteries (carotid glomus marked by *).

The cases considered beyond surgical therapy were referred only to radiotherapy (Figure 6), chemotherapy.

Figure 6: A, B CT - based treatment plans for a patient with right tonsil squamous carcinoma cT4N2cM0, stage IVB (Collection of Chiricuta Institute of Oncology Cluj-Napoca)

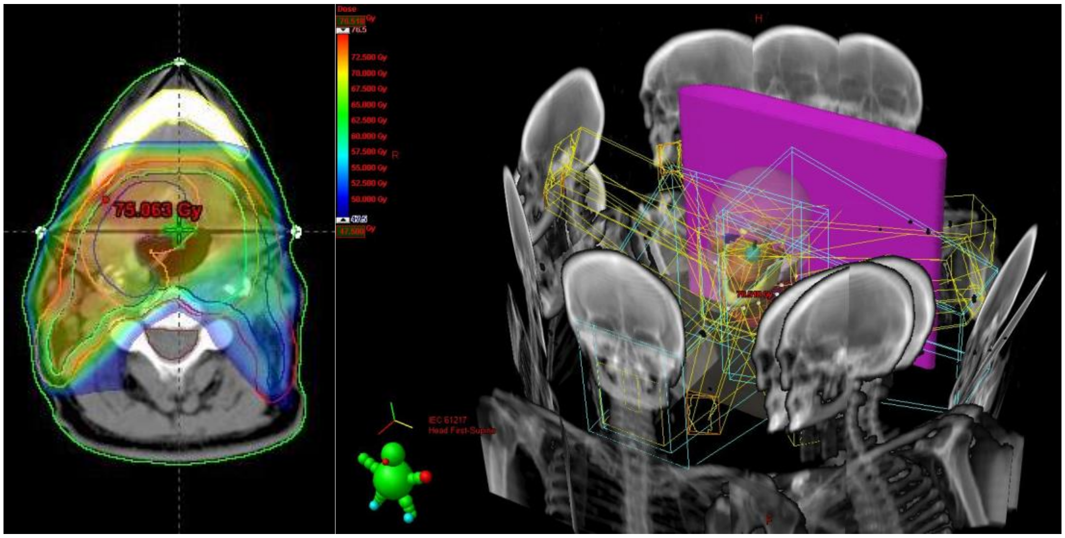

In Table 1 we present the distribution of cases according to Stage Groups for Non–HPV-Mediated (p16 -). At the time of imaging tests, we did not evidence metastases of the tonsillar neoplasm to other organs, so no patient was identified in stage IV C.

Table 1: TNM distribution of cases

Stage	Number of cases	Percent
Lymphoma	9	12.5%
I (T1N0M0)	18	25%
II (T2N0M0)	11	15.3%
III (T2N1M0)	9	12.5%
III (T3N1M0)	8	11.1%
IV A (T4aN2M0)	5	6.9%
IV A (T3N2M0)	5	6.9%
IV B (T4bN2M0)	4	5.6%
IV B (T4aN3M0)	3	4.2%

DISCUSSION

Tonsillar cancer was already recognized in the 19thcentury and attempts were made to characterize it [7].

Risk factors. Tobacco and alcohol remain the major risk factors for tonsillar neoplasm, especially for squamous cell carcinoma [8-9]. In our study 62.5% of the patients were smokers, and 29.2% took alcohol and tobacco concomitantly. There is a high probability that some patients did not admit to having these habits (especially in the case of alcohol). The percentage of about 60% for smokers was also reported in the United States of America, but it presents variations in region and sex [10].

Tobacco is a risk factor independent of alcohol drinking, as demonstrated by studies on individuals who have never consumed alcohol [11]. There is an interaction between tobacco and alcohol well established for oral cavity cancers. The studies show that most cases are attributed to tobacco alone or a combination of tobacco and alcohol, while very few cases would be attributed to alcohol alone [12].

In high-income countries, tobacco prevalence has been decreasing in the last decades, due to the antismoking campaigns, which proves their importance [10].

Brandsma and Abramson found HPV in tonsillar squamous cell carcinomas in 1989, and subsequent studies have confirmed that HPV, especially the oncogenic subtype 16, increases the risk for developing tonsillar neoplasm [13-16]. The most frequent neoplasm sites associated with HPV infection in the head-neck area are the base of the tongue and palatine tonsils [13]. These patients have distinctive risk factors, such as a large number of sexual partners. Today, the link between oral sex behavior and oropharyngeal HPV infection is well known, HPV infection being the most common sexually transmitted disease in the United States [8]. The patients are usually younger, do not use cigarette smoke or alcohol, and have a better prognosis [17-18].

The HPV status of the tumor can be revealed by PCR or immunohistochemical staining [8, 19]. In our study, HPV viral analyses of the tumors had not been performed.

Clinical evaluation. The early injury, frequently asymptomatic, is mostly detected accidentally – 15% of our patients were found in stage 1. The patient may have no symptoms even in stage 2, due to submucosal tumoral development or deep formations in the tonsillar crypts [3]. However, in most cases the patients go to the doctor later, when dysphagia, cervical lymphadenopathy, and tumoral local extension are present – 54% of our patients were diagnosed in stages 3-4. The tonsillar asymmetry, palpable firmness, the visible lesion in the tonsil, cervical lymphadenopathy, unexplained weight loss, history of head-neck cancer can be local signs for a tonsillar neoplasm [20].

As well, the trismus (caused by pterygoid involvement), and otalgia (referred pain from the ninth and 10th cranial nerves) may be encountered [21].

The most frequently found symptom in our case series was dysphagia (63.8%).

Histopathology. A tissue biopsy can generally be performed with local anesthesia and is an essential step for confirming the suspect diagnosis.

The most frequent tonsillar cancer type is squamous cell carcinoma (75% of tonsillar neoplasm cases and 10% of head and neck malignancies [22]), followed by lymphoma [4], though other, rare histological forms have also been reported (small cell neuroendocrine carcinoma [23], mucoepidermoid carcinoma [24], small cell cancer of the tonsil [25]). The majority of tonsillar lymphomas are of B-cell origin. The association of B-cell tonsillar lymphoma with Epstein-Barr Virus infection is uncommon [26]. In our study, we evidenced squamous cell carcinoma in 80.6% of cases (51.4% non-keratinized, 29.2% keratinized), and B cell lymphoma in 18% of the cases.

Pediatric tonsil cancer is rare, with non-Hodgkin lymphoma accounting for the majority of pediatric tonsillar neoplasms. It raises suspicion in children who present with relatively rapid tonsil enlargement, tonsillar asymmetry characterized by a difference in the tonsillar size of ≥2 degrees on the Brodsky scale, or simultaneous cervical lymphadenopathy [27].

A meta-analysis on 54901 patients has shown that in current practice, the incurred cost of routine histological examination of tonsillectomy and adenoidectomy specimens is not warranted. The histopathological exam is warranted only when patients demonstrate findings associated with malignancy: tonsillar asymmetry, history of cancer, cervical adenopathy, tonsil injuries, weight loss [28]. Through biopsies, false-negative results may arise if the tumor is localized in the submucosa or deep crypts [29]. A tonsillar cancer is more likely to be missed by biopsies than by tonsillectomy [3].

Metastases of tonsillar neoplasm. The head-neck neoplasms usually extend firstly to the regional lymph nodes, but sometimes may metastasize to distant sites. The metastasis to distant lymph node groups is a rarely [30]. Furthermore, delayed multiple metastases without local recurrence are uncommon [31]. Approximately 15-20% of oropharyngeal carcinomas develop distant metastases, most commonly in the lung, liver, and bone [32-33].

The cutaneous metastasis from tonsillar cancer is rare but should be considered; new skin lesions should be searched and biopsied because the morphology of the cutaneous metastases from tonsillar squamous cell carcinoma is variable [22]. Cutaneous metastases result either from the local spread of cancer to overlying skin, (from direct extension through contiguous tissue planes), or more distant spread through either dermal lymphatics or blood vessels [34].

The metastases to tonsils are also rare, accounting for 0.8% of all tonsillar tumors [35]. The extension is due to the hematogenous dissemination and is associated with advanced-stage disease [36-37]. We did not identify metastases of tonsillar neoplasm or metastases to tonsils in this study.

Imaging investigations. The computed tomography or magnetic resonance imaging (MRI) is the best suited for the evaluation of oropharynx neoplasm. The cervical lymph node metastases are optimally evaluated by contrast CT. MR imaging is preferred for soft tissue assessment because of the greater resolution [38-39].

PET-CT scanning is indicated in search of an unknown primary tumor in patients who have a neck mass secondary to carcinoma, if a recurrent carcinoma may be present when there are metastatic with N0 cervical lymph nodes, or CT is inconclusive [39]. By ultrasound, we can perform an initial indicative TNM staging of the case before other expensive imaging studies such as CT and MRI [40].

In our study all the patients were examined by head-neck contrast-enhanced CT, with one exception – a patient requested a PET-CT in another unit.

Treatment. The treatment decision for tonsil cancer should be made by a multidisciplinary team.

According to the guidelines [41], early-stage tonsil cancer may be treated by transoral primary surgery [42] or open resection of the primary tumor, with or without neck dissection or definitive radiotherapy [43], the difference in the therapeutic benefit being insignificant.

The direct transoral approach provides the quickest and most direct way with the least potential of morbidity. However, advanced cancer or trismus, kyphosis, dental obstruction can limit access [44]. Transoral robotic surgery may be an effective alternative to open surgery or radiotherapy, especially for early-stage oropharyngeal squamous cell carcinoma [45].

Either Intensity–Modulated Radiation Therapy or 3D conformal radiotherapy of the primary tumor (Figure 6) and the involved lymph nodes with a total dose up to 70Gy is recommended, to minimize the irradiation to critical structures [46]. The addition of systemic therapy to radiotherapy should be considered for HPV-negative N1 disease and HPV-positive T2 disease and the involvement of a single node ≤3 cm [47].

Though the local-regional control and survival are similar following surgery or radiotherapy, primary surgery can be accomplished with minimal functional morbidity while reserving radiation for recurrent disease. Surgical salvage after radiotherapy may be difficult technically, being associated with a significant increase of complications [4]. Tonsil resection before radiation therapy is associated with improved survival in low-stage tonsil cancer [48].

For locally advanced resectable tonsil cancer the recommendations do not differ between the HPV status [41]. The treatment can be made either by giving concurrent systemic therapy and radiotherapy [49], or transoral or open resection of the primary and neck with appropriate adjuvant therapy, meaning systemic therapy concomitant with radiotherapy or radiotherapy alone, or as a third option induction chemotherapy followed by radiotherapy or concomitant systemic therapy with radiotherapy. Surgery and postoperative radiotherapy continue to provide a superior outcome in locally advanced tonsil squamous cell carcinoma in patients with the surgically resectable disease [50].

When concurrent systemic therapy/radiotherapy for the locoregionally advanced cancer of the tonsil is administered, the high-dose cisplatin is the preferred systemic agent. As induction chemotherapy, the preferred regimen consists of taxane/cisplatin/5-fluorouracil, known as TPF [51] or paclitaxel/cisplatin/5-fluorouracil [52].

Though data are scarce regarding the prevalence of bilateral synchronous tonsil cancer (the first report in 1971 [53]), certain authors maintain that this form of the disease is under-reported, and they recommend bilateral tonsillectomy in all patients with suspected or proven unilateral tonsil cancer and in patients with cervical carcinoma metastasis from an unknown primary tumor [54]. The recommendation for a bilateral tonsillectomy may be found in the literature but without application in clinical practice or integration into guidelines [3, 55]. On the other hand, unanimity exists for ipsilateral tonsillectomy.

Due to the deficiency of integrated services, many patients are lost from otorhinolaryngological follow-up. The most of our patients in the advanced stage never returned to our clinic after the start of oncological treatment. Because of this, we cannot show survival rates for our patients.

CONCLUSIONS

In Romania, smoking is still the most important risk factor for tonsil cancer, and HPV detection analysis is not available in public hospitals.

Most patients have visited a doctor when dysphagia and, above all, palpable cervical adenopathy, were present, but the tumor was in an advanced stage, which means that the therapeutic management requires close collaboration between otorhinolaryngologist and the radiotherapist-oncologist.

In Romania, the ENT-oncology integrated medical services are few, in most cases patients are lost from otorhinolaryngological follow-up.

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VARIA

A review of the need to provide hyperbaric medical research infrastructure for the treatment of combat-related injuries

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Abstract: *HBOT has been used quite extensively in the last couple of decades. But there are not many centers that offer this treatment, even in civilian situations. This review has described the rationale and clinical evidence for equipping medical centers with HBO systems, especially in military areas.*

A narrative review of all the literature available on HBOT in combat-related injuries, studies were retrieved from systematic searches on Science Direct, PubMed, SCOPUS, ISC, Google Scholar, and by cross-referencing until 2020.

First, the etiological mechanisms of action of HBOT were discussed. Furthermore, we have provided an overview of 117 studies that clinically investigated the effect of HBOT in the last couple of decades. Therefore, based on the number of studies showing a positive effect, to speed up the treatment process, especially in war-related injuries, it is better to provide its infrastructure and equip hospitals and centers with HBO.

Keywords: *hyperbaric, hyperbaric therapy, trauma, war trauma*

INTRODUCTION

The trauma of firearms has caused many casualties in human societies since world wars military facility up to date with the advancement of military facilities, and a large part of the medical staff has always been dedicated to treating and improving the disability of injuries caused by war trauma [1]. Many studies have been done in this regard, and the beneficial effect of hyperbaric treatment has been proven [2].

Hyperbaric Oxygen therapy is the treatment of patients with oxygen respiration in a high-pressure treatment room. Increasing oxygen and delivering it to tissues has beneficial effects for various diseases. Some of these injuries are related to combat-related injuries [3]. Although the number of hyperbaric oxygen therapy sessions varies depending on the type of disease, and there is no absolute definition for the duration or pressure of oxygen in each treatment session, when hyperbaric oxygen therapy timely and properly is done, the evolution and the final result will be improved [4]. However, due to the technical limitations of treatment such as the need for a high-pressure treatment room, adequate oxygen, and compressed air supply, medical and paramedical staffs, the centers that provide this treatment are not numerous, even in civilian situations [5]. Hyperbaric Therapy Working Group investigates the possible military applications of hyperbaric oxygen therapy and defines the terms of its use. The amount of damage caused by war traumas inside or outside the city varies depending

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on the geographical location, which necessitates a comprehensive study to provide a suitable solution to prevent trauma-related injuries [1]. Although it is not possible to set up hyperbaric treatment centers near the operation site, it seems possible to organize medical evacuation routes. It appears that this collection of information in the form of a review paper will benefit to help shorten the overall treatment time of combat-related injuries and optimize the final recovery by building the right infrastructure [6, 7]. It appears that this collection of information in the form of a review paper will benefit to help shorten the overall treatment time of combat-related injuries and optimize the final recovery by building the right

METHOD

To find available data and information in the literature on the role of Hyperbaric oxygen therapy (HBOT) induced combat-related injuries via vasoconstriction and Increase oxygen supply after trauma and we performed a search in multiples data banks such as Science Direct, PubMed, SCOPUS, ISC, Google Scholar, and the paper published Until 2020 were reviewed.

It should be mentioned that the search terms and keywords for evaluation of all published papers in this manner were "Hyperbaric Oxygen Therapy", "Refractory Osteomyelitis ", "Intracranial Abscess", "Radiation Injuries", "Burns", "Wound", "Traumatic Wound", "Traumatic Brain injury", "crush injury", "military Medicine" and "inflammation", "Acoustic trauma "Also, we used MESH term "OR" operator between synonyms keywords and "AND" operator between independent keywords for finding articles and papers as well as articles with the most consistency with our goal. For a review of all papers, we had inclusion criteria, and in 203 published papers which in them the hyperbaric oxygen therapy in combat-related injuries, 117 papers were selected for review and citation of all published papers.

DISCUSSIONS

The effects of hypoxia vary depending on the cause, whether the condition is acute or chronic, as well as the overall health of each individual. Cellular hypoxia may occur in multiple organ failure syndromes due to increased tissue oxygen demand or due to the ability of oxygen to break down at the cellular level; to repair, oxygen transfer and metabolic support are important components of treatment.

Definition and mechanism of action of HBOT

Since the 1960's HBO has been used for the treatment of various diseases and illnesses [8]. HBO involves the respiration of pure oxygen under high air pressure [8]. HBOT often use in complex trauma. Lamm [9] and Appaix [10] are credited for their pioneering work on the role of HBOT in these two conditions

According to the laws of physics and physiology, breathing oxygen at high pressure increases alveolar oxygen pressure and pharmacological effects with increased arterial O_2 content leading to improvement of ischemic state due to lack of oxygen such as chronic unhealed wounds, causes vasoconstriction and special antibacterial effect against anaerobic bacteria on the body [11].

In this procedure, the patient is completely enclosed in a special chamber, and by breathing 100% oxygen, the pressure inside the chamber gradually increases to more than one atmosphere [12]. So HBOT is a relatively time-consuming therapy for patients because patients must be physically present in the oxygen chamber [13]. HBOT is considered a safe therapy; however, side effects do occur in some studies. Contraindications to hyperbaric oxygen therapy are low, including untreated pneumothorax and some chemotherapy drugs such as bleomycin, which can lead to pulmonary fibrosis [14].

The effect of HBOT on the treatment of war traumas

Acoustic trauma

Acoustic trauma is damage to the auditory structures in the inner ear due to loud noise. Hearing loss and tinnitus are two of the most common disabilities caused by military veterans. Reports indicate a worrying increase in these complications in the military population [13]. Recent war scenes have seen soldiers face numerous dangers that lead to these effects, such as explosions, ototoxic chemicals, and loud noises [15]. Damage to the auditory mechanism can be caused by an explosion near the ear, shooting, or prolonged exposure to loud noises. Hearing loss, the fourth leading cause of medical visits to soldiers [16]. Of the more than 5,000 troops stationed in Iraq and Afghanistan who underwent audiometry, 1550 people have been reported to have been exposed to acute trauma of a sound explosion; of these, 72% have lost their hearing. Of all deployed personnel who underwent audiometry, 28% had a degree of hearing loss. More than two-thirds of English soldiers who have returned from Afghanistan, from injuries suffered severe and permanent hearing loss [5]. Animal experiments showed that 60 hours after acoustic trauma injury, the number of morphologically damaged inner ear sensory cells was lower in animals treated with HBO than in those treated without HBO [17, 18]. The best therapeutic effect on noise-induced hearing loss was obtained with a combination of HBO and prednisolone [19]. Animal studies in rats [20], pigs [18], and chickens [21] all showed that the HBO-treated group had a significantly reduced signal-to-noise ratio. Also in human studies, victims of acoustic trauma in military operations were treated with HBO [22]. There was a significant improvement in hearing loss. Therapeutic results confirmed that 65% of patients who were not treated with various methods had improved hearing [23]. Based on studies performed at different times and conditions of hearing loss compared to other treatments, it can be concluded that HBO is recommended within 3 months after the onset of the disorder, and treatment with it is guaranteed [24].

Soft tissue infection

Soft-tissue infections, occur after trauma and around the foreign body, and even spontaneously [25]. A common factor, tissue hypoxia that suppresses the immune defense mechanisms [26]. In animal models (mice, rats, rabbits), mortality was significantly lower during treatment with antibiotics and HBO. But (depending on the specific type of bacteria) seen a series of adverse effects was treated with HBO, the streptococcal infection worsened [27]. The level of evidence for soft tissue infections and HBO is low. There are only case reports and retrospective studies, and no RCTs are available. Almost all patients studied were endangered hosts [28-30].

In the study of war-related organ injuries including type 3 combat fractures and the description of the effect of HBOT on wound healing and complications of infection, it was shown that the reduction of infectious complications in patients who have used HBOT in the treatment process (31). Recent studies aimed at determining the effect of HBOT in the treatment of acute surgical wounds and trauma have shown a lack of quality clinical trial studies and pointed to the need for further studies [32-34].

Crush Injury

Crushing causes severe tissue damage and calls into question tissue survival. These injuries lead to local hypoxia. Ischemia, on the other hand, causes edema due to direct vascular trauma, fluid secretion, or compartment syndrome. Edema is either vasogenic or cytogenic and increases the distance of oxygen delivery to the cells. Edema also leads to capillary collapse, thereby increasing the severity of hypoxia [35]. The main purpose of using hyperbaric oxygen in crushing injuries is to cause excessive tissue oxidation.

Animal models (rodents, dogs) Crush injury studies have shown significant effects of HBO treatment [36]. HBO also regulates inflammatory gene expression in endothelial cells and can reduce the expression of apoptosis.

In human studies, most data are obtained from case reports or series of up to 35 patients. And there is only one RCT that compares crush injuries with and without HBO treatment [37], which improved treatment and significantly reduced surgery. Patients over the age of 40, in particular, benefited greatly from HBO treatment. From the available data, experts conclude that the adjuvant use of HBO in crushing injuries may lead to a favorable outcome for selected patients [34, 38].

Burns

Burns are followed by hypoxia and tissue ischemia [39], which increase edema with loss of capillary wall integrity (endothelial cell contraction). This fluid loss, by changing the oncotic pressure gradient in the capillary wall, further reduces the intra-capillary fluid pressure and thus increases stasis [40]. As a result, areas of second-degree superficial burnt tissue (minor burns), develop in the first 24 hours after the burn, it progresses to a deep second or third-degree burn, which requires early surgery and a transplant to heal [41].

In all of these pathophysiological mechanisms, hypoxia plays a pivotal role. Oxygenation of ischemic tissues should be performed rapidly and in large volumes, to reduce tissue damage with ischemic-reperfusion. Hyperbaric oxygenation is the only treatment that can ensure that the treatment is successful [42, 43].

Several reports show the help of HBOT in the treatment of severe burns [44]. The use of HBOT in severe burns in a war scene may reduce the need for surgery and length of hospital stay [45], and possibly reduce mortality [46].

When soft tissues (such as skin and muscle) are burned, blood flow to the affected area is severely reduced. This causes the area to swell, and the swelling spreads from the affected area in all directions. The swelling may reach deep into the muscles and surrounding skin, causing more damage than the original injury. HBO should be used very soon to limit initial debridement and gradual tissue destruction [47].

The theoretical beneficial effect of HBOT on burn wounds is in the antibacterial effects of possible infections, in reducing edema and systemic inflammatory response, in limiting the size of burned tissue, and in increasing collagen formation. It seems that the best results from starting hyperbaric treatment in the first hours of injury. However, studies indicate a low and unclear role of HBOT in the management of severe burns. A randomized study supports the use of HBOT to evaluate the potential positive effects of HBOT on experimental burn wound healing [48]. In this study, the authors demonstrated a beneficial effect on HBOT wound healing and hypothesized that such an effect would be determined by reducing edema and maintaining microcirculation. Although the quantity and quality of evidence-based studies on the potential benefits of HBOT in patients with burns are poor, its potential use in medicine, war is worthy of further investigation. To achieve the best result, careful selection and screening of patients are recommended [47].

Special attention has been paid to the use of HBO in respiratory injuries. There is currently concern that HBO may worsen lung damage, especially in patients with high levels of inhaled O_2 [49]. Studies have not reported any evidence of oxidative stress in HBO-treated burn patients who used lipid peroxidation exhalation products as a symptom [50].

However, a series of compelling prospective and retrospective studies have been published [47, 51] that show less need for surgery, reduced mortality, shorter hospital stays, and reduced treatment costs when using HBO concomitantly with conventional therapy. But so far no prospective placebo-controlled trials have been published that explicitly prove the effectiveness of HBO treatment [52, 53]. Therefore, acceptance of HBO as a valuable adjunctive therapy is limited to those burn centers that can provide early treatment of HBO without adding additional risks (infectious, hemodynamic) to the patient's condition [54, 55].

Frostbite

Frostbite-like thermal burns a profound impact on local microcirculation that it can be cold or an injury or an acute and prolonged exposure to cold be defined. It has been shown that after heating, a long-term damage phase occurs, which is very similar to the ischemia-reperfusion injury observed in thermal burns. At least, in theory, hyperbaric oxygen therapy could have the following benefits in frostbite injury.

Animal studies (especially rabbit studies) have been conducted sporadically over different years [56, 57], with each study showing different results [58-60]. These different results suggest that deep and rapid freezing damage can indicate several important differences in pathogenesis and tissue damage from slow to moderate freezing.

The experiments carried out on animals, the early application of HBO during or immediately after the heat re-use. Some sporadic case reports suggest that HBO may be helpful, even long after warming up. Because these are uncontrolled and isolated reports, the evidence supporting these claims is not very strong. In 2001, he reported a case in which HBO was used after only 4 days for a young boy who had both hands frozen, and was able to save all his fingers [61]. Similarly, cases have been reported, 12 and 22 days after frost damage and the use of HBO led to the rescue of the individual fingers [62, 63].

Although the experience is relatively limited because HBO is generally not recognized as a complementary treatment standard for frostbite; clinically, HBO appears to provide early, good and rapid separation of growing tissue from non-growing tissue. MR angiography, one week after the onset of the injury, looks promising in estimating the likelihood of resuscitation of the fingers [64].

Spinal cord injury

Many studies have examined the mechanism of action of HBOT in SCI [65, 66]. Some studies have shown the protective effect of HBOT in the treatment of SCI [67, 68] and others recommended a review of the care process [66]. The number of treatment sessions for patients with spinal cord injuries is determined by the patient's condition and response to treatment, but according to studies, at least 30 sessions of standard HBO treatment 5 sessions per week were recommended to begin treatment [69].

Carbon monoxide poisoning

Because lipid peroxidation is an important aspect of CO-induced neuronal damage, inhibition of neutrophil O_2 integrins at the cellular level by HBO therapy has been identified as a significant advantage of that treatment. HBO relieves cerebral edema through vasoconstriction [70]. HBO therapy is unique in increasing oxygen delivery while causing blood vessels to constrict.

Experimental studies have shown that HBO treatment restores mitochondrial oxidative metabolism in addition to eliminating tissue hypoxia [71] and prevent lipid peroxidation [72] and prevents endothelial leukocyte adhesion [73]. Although many studies have talked about the healing process and preventing the disease from getting worse [74-76], In some studies, the authors failed to demonstrate a significant advantage of an HBO treatment session over NBO treatment in patients with loss of consciousness. Similarly, treatment with two sessions of HBO showed no advantage over one session of HBO in patients who were in a coma [77].

Decompression sickness (DCS)

Decompression sickness (DCS) occurs when atmospheric pressure decreases too rapidly. During this syndrome, bubbles of inert gas dissolved in blood or tissues lead to blood supply blockage, direct mechanical damage, or biochemical actions. DCS can be expected after scuba diving, ascent with flying, or hypobaric or hyperbaric exposure [78, 79].

The endothelium of vessels can be injured by the bubbles causing DCS. This damage induces platelet aggregation, lipoproteins denaturation, and leukocytes activation. These events eventually lead to capillary leaks and proinflammatory events [80, 81].

Hyperbaric oxygen therapy (HBOT) by using an oxygen gradient and also through pressure is ale to reduce the size of the bubbles. According to Boyle's law, pressure increases can decrease the volume of the bubble. The bubble causing DCS is thought to be composed of nitrogen.

In the process of DCS, when a tissue ascends to a decreased atmospheric pressure, bubbles derived from nitrogen seep out of blood, tissue, or both. By breathing 100% oxygen in HBOT, the blood becomes rich in oxygen and poor in nitrogen that leads to a gradient of nitrogen between the blood and the bubble. So nitrogen goes from the bubble into the bloodstream which makes the bubble smaller [79].

Recompression is the choice of treatment. The greatest success from recompression, as the definitive treatment, occurs when treatment is done as soon as possible but no exclusionary time from symptom onset has been established [79, 81]. Assessment patient's symptom response to therapy determines the time a patient is kept at 60 or 30 fsw [82].

Recovery therapy

Military response is now accepted around the world to assist civil society in natural disasters. Most of the time, military support is provided only in large-scale disasters. In contrast, local resource capacity can be used in small-scale disasters.

On the other hand, due to the proposed vascular etiology, many other treatment modalities were proposed and experimentally tested. Examples of treatments are blood flow-promoting agents, dextran, vasodilators, and pentoxifylline. However, many of these studies have did not show effectiveness within the clinical setting [83-87].

According to some authors, contraction of the prostheses caused by HBOT treatment reduces tissue edema and secretion of acute post-traumatic inflammatory factors, which can have an analgesic effect on acute pain [88, 89]. So we conclude that, at least from a pathophysiological point of view, HBO should be started as soon as possible, so HBO delayed effects can be considered reasonable, even so, it is impossible to define a period in which HBO can be considered anything more than a tool [90].

Recommendations

For future studies, we recommend starting therapy as early as possible, preferably within 48 h and to use combination therapy. Furthermore, we recommend investigating the relationship between the effects of HBO and age, sex, and underlying disease. In future studies, randomization and having a control group also seem necessary.

CONCLUSIONS

HBOT has been used quite extensively in the last couple of decades. Even though most studies were not randomized controlled trials, we think that based on the number of studies showing a positive effect, HBOT should be discussed with patients (shared decision making) as optional therapy.

This narrative review has described the rationale and clinical evidence for equipping medical centers with HBO systems, especially in military areas. Equipping military hospitals with this treatment system can perform the first stage of treatment for the individual before the transfer and speed up the treatment process, especially in combination therapies.

Therefore, centers can be classified based on belonging to different categories of hospitals, and for each pathology, a suitable hospital category can be defined. Given that so far no formal coordination has taken place between the military hyperbaric centers of different countries, interoperability facilitated the organization of cooperation in the field of multinational operations.

We emphasize international cooperation on the applications of hyperbaric medicine in military medicine. At the International Congress of Military Medicine (ICCM) held in Iran in 2018, a panel on hyperbaric medicine was held with the participation of hyperbaric medical professors from the participating countries. In this research panel, Dr. Shahram Oliaei, hyperbaric medical researcher, emphasized research and educational cooperation to further coordinate the hyperbaric medical centers of the member countries.

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VARIA

Updates in teenage acute intentional self-poisonings

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Abstract: *Acute intentional poisonings represent an important part of emergency pediatric pathology, as well as a psychiatric one, with an escalation tendency over the past years. The current paper consists of a descriptive prospective study, which took place over 12 months, and included 342 children within the age range 6-18 years, that presented in the Emergency Unit, being subsequently treated as in-patients of the Pediatric Poisoning Center of "Grigore Alexandrescu" Emergency Clinical Hospital for Children. The number of pediatric acute intentional poisonings hospitalized within the time frame of the study was 819 cases. Of these, 342 were intentional, which constitutes a percentage of 41.75%, and 477 were accidental, i.e. 58.24%. The etiology of the acute intentional poisonings is varied; however, medication (54.38%), ethanol (28.65%), and drugs of abuse (12.28%) were predominant. A higher frequency of acute intentional poisonings was noticed in girls (65.49%) as compared to boys (34.50%), probably due to the particularities the psychological profile of this sex shown at this age, i.e. higher emotional instability and display tendency. Out of the total number of acute intentional poisonings, we identified 20 cases of suicide attempts, which represent 5.83% of the total acute voluntary poisonings. Moreover, some of these cases constitute a repeated suicide attempts. The studied group included 30 cases of chronic substance abuse and 6 chronic alcohol abuse cases, with ages within the 13-17 years range, 12 of which were females and 24 males. Out of the 36 chronic substance/alcohol abuse patients, 4 were social cases. Pediatric acute intentional self-poisoning is an important public health issue, alarming through its consequences and its hidden neuropsychiatric and behavioral substrate. This is because adolescence is a period of marked emotional fragility, sensitive to all sorts of influences.*

Keywords: *self-poisoning, teenager, psychiatric disorders, alcohol, substances of abuse*

INTRODUCTION

The prevalence of teenage acute intentional self-poisoning is high since adolescence is a period of profound changes with visible effects on an individual's physical aspect, behavior, and relationships with the surrounding world. It features a personality focus on acquisitions and social statuses related to school life, family life, friend circle.

Adolescence constitutes a time frame of intense development, marked by a series of profound changes with visible effects on physical appearance, behavior, and relationships with the surrounding environment. Resorting to an extreme gesture, such as acute intentional self-poisoning, which puts one's life at risk, is often a potential exacerbation of a chronic psycho-social disorder [1, 2].

Teenage years are marked by intense developmental

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processes, on a sexual level included. Affectivity, relationships of all kinds begin to include the sexual identity and its integration in the self-image.

Drug abuse, juvenile delinquency, poor learning achievements, teenage pregnancy, sexually transmitted diseases, feelings of despair are frequent causes that lead teenagers to resort more and more often to suicide attempts through various acute self-poisonings. Between the ages of 13 and 16 years, adolescents are confronted with frequent and unexpected mood swings. Both the regression and the progression of the psychological age represent explanations regarding the unpredictability of teenagers, as well as the difficulty to sometimes understand them. Behavioral disorders occur, the greater part of which is induced by disturbances in the emotional development caused by family issues, parental conflicts, and splitting [3].

The lack of affection does not refer strictly to its absence concerning the adolescent, but also its misguidance in a direction that ignores the affective needs of the child. The lack of education prevents the adolescent from acquiring necessary social life skills. All these family issues are the source of teenager disorders that lead to powerful social inadequacy behaviors and antisocial acts [4]. The adolescent becomes introverted and develops inferiority complexes. It is important that parents combine proofs of affection with exigencies and demand respect, as well as teach their children the affinity towards imitating behaviors of individuals who distinguish themselves through moral and intellectual qualities [5].

Acute voluntary self-poisoning is a dynamic medical pathology that is often the result of a potential exacerbation of a chronic psychosocial disorder; frequently, patients have a personal history of various psychiatric disorders, such as emotional, behavioral, and personality disorders, depression, mental retardation [6].

The purpose of the study is to determine the frequency of acute intentional self-poisoning cases, to identify the most frequently used substances as the etiology of the self-poisoning, to plot the distribution according to sex, age, the month of the year, the presence of suicide-associated psychiatric pathology and to identify the age interval associated with the highest risk for this pathology.

Acute intentional self-poisoning is often associated with school dropouts or the patients are social cases. However, there are cases when the child is left unsupervised or cases when a teenager will abuse certain substances in a moment of emotional turmoil triggered by a conflict with her/his parents, guardians, or life partner [7].

A particular intentional self-poisoning in the 12-13 year age group (adolescents) is due to ethanol. Ethanol is one of the most frequent causes of acute intentional self-poisonings in teenagers. The abuse is usually acute and within a social context, unlike adult cases, where the most common causes of acute intentional poisoning are psychotropic medication and medication acting on the cardiovascular system. In addition, another objective of our study was the identification of the particularities of alcohol consumption in teenagers living in our country compared to European teenagers [3].

PATIENTS AND METHODS

The personal study was undertaken in the Pediatric Poisoning Center of “Grigore Alexandrescu” Emergency Clinical Hospital for Children, in Bucharest.

The present research is a descriptive, prospective study that took place for 12 months (1st of January 2017-31st December 2017), and included 342 children with ages in the 6-18 years range, who presented in the Emergency Unit and were subsequently treated as in-patients in the Pediatric Poisoning Center of “Grigore Alexandrescu” Emergency Clinical Hospital for Children.

The clinical cases selected for the study were treated as in-patients in the Pediatric Poisoning Center for acute voluntary self-poisoning with various substances. Out of the 2457 hospital admissions in the clinic, 342 cases fulfilled the inclusion criteria for the study.

Inclusion criteria for the study group:
- patients with acute intentional self-poisoning;
- under 18 years of age, or younger than 19, but still students.

Exclusion criteria:
- patients with accidental acute self-poisoning;

Studied parameters:
- patient identification data (age, sex);
- substances used as the etiology of acute intentional self-poisoning;
- motive for the acute intentional self-poisoning;
- number of suicide attempts;
- various chronic substances abuse;
- clinical picture;
- associated psychiatric disorders;

RESULTS

The number of acute poisonings in children treated as in-patients in the studied time frame was 819 cases. Out of these, 342 were intentional, representing 41.75%, and 477 were accidental, i.e. 58.24%.

In the studied group, which contained 342 patients, the etiology of acute voluntary self-poisonings is varied; however, medication (54.38%), ethanol (28.65%), and substances of abuse (12.28%) were predominant (Table 1). Statistical data withstand comparison to literature data - a study performed between 2003 and 2013 in the same clinic - the most common cause remains acute voluntary self-poisoning with medication [8].

Table 1: Case distribution as a function of the etiology of the acute intentional self-poisoning

Etiology	Cases	Percentage
Medication	186	54.38 %
Alcohol	98	28.65 %
Coffein, energy drinks	4	1.16 %
Tabacco	1	0.29 %
Substances of abuse	42	12.28 %
Household toxic substances	7	2.04 %
Pesticides/Insecticides	2	0.58 %
Caustic substances	1	0.29 %
Plants/mushrooms	1	0.29 %

As far as the distribution regarding the months of the year, the study pointed out a higher frequency of cases of acute intentional self-poisonings in January (11.69%), June (11.98%), November (10.88%), but also February (9.35%), March (8.47%), December (9.64%). A lower frequency was noted in April (4.97%), July (6.14%), August (5.55%), and September (6.14%). Most cases of acute intentional self-poisoning were noted in June, which coincides with the end of the school year, and the least cases were noted in the months which correspond to the summer school break.

A higher frequency of acute intentional self-poisoning was noted for females (224 cases, which represents 65.49%), as compared to males (118 cases - 34.50%), probably due to the particularities of the psychological profile of the gender shown at this age, i.e. higher emotional instability and display tendency [9, 10].

In the study group, we noticed that for female cases, the most frequent etiology for acute intentional self-poisoning was medication (70.5% of intentional self-poisonings in females), while for males, the most common etiology was alcohol (49.1% of the total self-poisonings in males), while the second most common one consisted of substances of abuse (23.7%) (Table 2).

A predominance of acute intentional self-poisonings in children was noticed for the age interval 15-16 years (50.58%), which corresponds to adolescence, a period of marked emotional fragility towards all kinds of influences (Table 3).

Table 2: Case distribution according to etiology and sex

Etiology	Female	%	Male	%
Medication	158	46.19%	28	8.18%
Alcohol	40	11.69%	58	16.95%
Coffein, energy drinks	4	1.16%	0	0%
Tabacco	0	0%	1	0.29%
Substances of abuse	14	4.09%	28	8.18%
Household toxic substances	7	2.04%	0	0%
Pesticides/ insecticides	2	0.58%	0	0%
Caustic substances	0	0%	1	0.29%
Plants	1	0.29%	0	0%

Table 3: Case distribution concerning age

Age	No. of Cases	Percentage
<10 years	2	0.58%
10-14 years	100	29.23%
15-16 years	173	50.58%
17-18 years	67	19.59%

The study group (342 cases) comprised patients with ages within the range of 6 to 18 years. For both sexes, a predominance in the age interval 15-16 years was noted: females (32.74%), males (17.83%) (Table 4).

Table 4: Case distribution according to age and sex

Age	Female	Percentage	Male	Percentage
<10 y.	0	0%	2	0.58%
10-14 y.	75	21.92%	25	7.30%
15-16 y.	112	32.74%	61	17.83%
17-18 y.	37	10.81%	30	8.77%

There were 100 patients in the 10-14 years age group. 75% were girls.

There were 173 patients in the 15-16 years age group. 65.73% were girls.

There were 67 patients in the 17-18 years age group. 55.22% were girls.

The highest incidence in girls was noted in the 10-14 years age group and we noticed that as adult age is approached, the sex distribution tends to be equal for girls and boys.

In the study group, 79 children (42.47%) presented medication used in the treatment of CNS disorders as the etiology of acute intentional self-poisoning. 49 children ingested Acetaminophen (26.34%) and 19 children (10.21%) used medication acting on the cardiovascular system for intentional self-poisoning (Table 5). Out of the medication acting on the CNS, patients used carbamazepine, benzodiazepine, valproic acid, levetiracetam, sertraline, risperidone, extraveral, cipralex (escitalopram).

Table 5: The distribution of cases according to medication types as etiology for acute intentional poisonings

Medication class	Cases	Percentage
Medication used in the treatment of cardiovascular disorders	19	10.21%
Medication used in the treatment of central nervous system disorders	79	42.47%
Aspirin	11	5.91%
Acetaminophen	49	26.34%
Antibiotics	10	5.37%
Oral antidiabetics	4	2.15%
Opioids (Morphine)	1	0.53%
Antiemetics	5	2.68%
Vitamins	4	2.15%
Antidiarrheals	3	1.61%
Anti-acne medication (Roaccutane)	1	0.53%

In our study, we noted that, concerning medication self-poisonings, single-medication poisonings were the most frequent type: 63.44%, while multiple-medication poisonings represented 36.55%. Among the combinations encountered, we mention acetaminophen + CNS-acting medication, aspirin + cardiovascular acting medication or blood pressure-lowering medication, carbamazepine + euthyrox + omeprazole, aspirin + acyclovir.

Pediatric acute intentional self-poisoning of alcohol-related etiology was caused by the consumption of vodka (20 cases), wine (17 cases), and whiskey (9 cases). Distilled drinks are predominant in the etiology of alcohol ingestion in the adolescents included in the study (Table 6). Moreover, the study showed several 26 cases of unknown etiology (the substance of abuse was either unknown or undeclared). Our results are different from those reported by other European countries [11].

Most of the cases of alcohol ingestion took place in a social context, most often in the company of friends, but also with family.

As far as the context in which the acute intentional poisoning took place, most often socializing in a public space or at home with friends determined the occurrence of the poisoning, according to literature as well [12, 13]. In 46 cases, the reason for the ingestion/abuse remained unknown or could not be classified in one of the previously mentioned categories. In 7 of the cases, the etiology of the acute intentional self-poisoning was the combination of medication and alcohol (4 girls/3 boys), and in other 7 cases, the poisoning occurred through the consumption of both alcohol and substances of abuse (3 girls/4 boys).

Table 6: Case distribution according to the etiology of acute intentional alcohol self-poisonings

Etiology	No. of cases	Percentage
Wine	17	17.34%
Vodka	20	20.40%
Whiskey	9	9.18%
Beer	6	6.12%
Champagne	2	2.04%
Distilled drinks	7	7.14%
Fruit-flavored distilled drinks	4	4.08%
Tequila	2	2.04%
Medical-use alcohol	1	1.02%
Ethylenglycol-antifreeze	4	4.08%
Unspecified etiology	26	26.53%

In the studied group: 98 of the cases of acute intentional self-poisoning presented alcohol as etiology; 60 cases showed no coma; 17 cases were complicated by 2nd-degree coma; 12 cases showed 1st-degree coma and only 9 cases showed 3rd-degree coma.

Out of 38 cases that presented with coma, 15 were females (39.47%) and 23 were males (60.52%).

Out of 98 cases of an acute intentional poisoning with alcohol, most cases ingested a quantity of alcohol below 500 ml, 8 cases ingested between 500 and 1000 ml (Table 7). Only one case ingested 3,000 ml of alcohol (wine).

Table 7: Case distribution according to the quantity of alcohol ingested

Quantity of alcohol ingested	No. of cases	Percentage
<500 ml	10	10.2%
500-1000 ml	8	8.16%
1000-3000 ml	1	1.02%
Not specified	79	80.61%

Coma occurred most often with the consumption of wine or vodka. In 7 cases, the type of alcohol ingested could not be precisely determined (Table 8).

In the case of acute intentional poisonings with substances of abuse, the study showed that the most frequently used substances were cannabis and the new psychoactive substances (Table 9). The consumers of substances of abuse were in the 11-18 years old range, the ratio of girls over boys being 1/2.

Acute intentional self-poisonings, especially in the case of adolescents, occur most often with display purposes (parasuicide) or suicide purposes in the context of family disputes. Most are meant to attract attention, few are actual suicide attempts [14, 15].

Table 8: Case distribution according to the type of alcohol consumed that precipitated the occurrence of coma

Substance	No. of cases	Percentage
Distilled drinks	4	10.52%
Wine	10	26.31%
Vodka	8	21.05%
Whiskey	6	15.78%
Beer	2	5.26%
Fruit-flavored distilled drinks	1	2.63%
Unspecified	7	18.42%

Table 9: Case distribution according to the abuse-substances used as etiology in acute intentional self-poisonings

Substance of abuse	No. of cases	Percentage
Canabis/Marijuana	24	57.14%
Glue	4	9.52%
Heroin	1	2.38%
New psychoactive substances	13	30.95%

In this study, we have noted 20 cases of suicide attempts. We noted 10 cases at the 2nd suicide attempt, 6 cases at the 1st suicide attempt, 2 cases at the 5th attempt (Table 10).

Tabel 10: Case distribution according to the number of suicide attempts, as a function of the total number of patients

No of suicide attempts	No of cases	Percentage
1	6	1.75%
2	10	2.92%
3	1	0.29%
4	1	0.29%
5	2	0.58%

Out of the total number of acute intentional self-poisonings, we pointed out 20 cases of suicide attempts in our study, which represent 5.83% of the total cases. Moreover, we noticed a part of these were not the 1st attempt. The age of patients with a suicide attempt was in the 14-18 years interval; the ratio of female/male was 3/1 (15 girls/5 boys). The majority of patients suffered from psychiatric disorders such as depressive disorder (4 cases), behavior disorders (5 cases), personality disorders (2 cases), conversion disorders (1 case), emotional disorders (5 cases).

The etiology of suicide attempts consisted of multiple drugs in 9 cases, single-drug in 10 cases (CNS-acting drugs, acetaminophen, ibuprofen, blood pressure-lowering drugs), and in one case the etiology was chlorine ingestion.

54.39% of the patients with acute intentional poisonings could be placed in a category of psychiatric disorders, with the help of the extended team of a psychologist and a psychiatrist, after stabilizing the patient. The rest, 45.61% of the patients, were cases of acute intentional poisonings with no psychiatric involvement (Table 11).

Tabel 11: Case distribution according to associated psychiatric disorders

Psychiatric disorder	No. of cases	Percentage
Behaviour disorder	30	8.77%
Depression	40	11.69%
Conversion disorder	1	0.29%
Emotional disorder	101	29.53%
Mental retardation	3	0.87%
Personality disorder	10	2.92%
Tourette syndrome	1	0.29%
No psychiatric disorder	156	45.61%

Out of the 342 acute intentional poisonings, 36 cases of chronic consumers of substances of abuse/alcohol were noted, representing 10.5% (Table 12). This is a worrisome percentage, bearing in mind the age group, i.e. less than 18 years. It is an alarm signal, which imposes the application of urgent measures, on a national level.

Table 12: Case distribution according to the chronic consumption of different substances

Chronic consumption	No. of cases	Percentage
Alcohol	6	1.75%
Substances of abuse	30	8.77%

The study group contained 30 cases of chronic consumers of substances of abuse and 6 cases of chronic alcohol consumption, with ages in the interval 13-17 years, 12 female patients, and 24 male patients. Out of the 36 cases of chronic substances of abuse/alcohol consumption, 4 were social cases.

The clinical study showed 30 social cases (low economical status, neglected child, disorganized families, domestic violence, children left in the care of relatives or institutionalized) and 11 patients who dropped out of school.

In 2 cases, patients aged 16, presented with evolving pregnancy. The etiology of the poisoning was medication-related: vitamins and ketoprofen were the culprits.

DISCUSSION

In the studied group, consisting of 342 patients, the etiology of acute voluntary self-poisonings varied, but medication, ethanol, and substances of abuse were predominant. Statistical data withstand comparison to literature data - a study performed between 2003 and 2013 in the same clinic; the most common cause remains acute voluntary self-poisonings with medication.

Out of the 342 children with acute intentional self-poisoning, the ratio of girls/boys is 1.89/1. A higher frequency of acute intentional self-poisoning was noted in females (65.49%), as compared to males (34.50%), probably due to the psychological profile of this gender at this age, i.e. higher emotional instability and display tendency. The psycho-behavioral features of the adolescents of the new generation make them vulnerable to life problems.

In the study group, we noticed that for female cases, the most frequent etiology for acute intentional self-poisoning was medication (70.5% of intentional self-poisonings in females), while for males, the most common etiology was alcohol (49.1% of the total self-poisonings in males) and the second most common one consisted of substances of abuse (23.7%).

A predominance of acute intentional self-poisonings in children was noticed for the age interval 15-16 years (50.58%), which corresponds to adolescence, a period in which the changes produced by growth, biological function changes; not only does it affect the behavior, but also the inner feelings of the adolescent. Poisoning is a common reason for presentation to hospitals and one of the top five acute medical presentations. The peak age groups are teenagers and young adults.

The highest incidence in girls was noted in the 10-14 years age group and we noticed that as adult age approaches, the sex distribution tends to be equal for girls and boys. This can be explained through the emotional particularities concerning age in girls. To lower the incidence of acute poisonings, educational prophylactic measures must be applied in schools, beginning at the age of 10, an age that corresponds to the fifth year within the gymnasium educational period.

In the study group, 42.47% of cases presented medication used in the treatment of CNS disorders as the etiology of the acute intentional self-poisoning. 26.34% of children ingested Acetaminophen and 10.21% used medication acting on the cardiovascular system for intentional self-poisoning. Out of the medication acting on the CNS, patients used carbamazepine, benzodiazepine, valproic acid, levetiracetam, sertraline, risperidone, extraveral, cipralex (escitalopram). Medication self-poisonings were the most frequent type of acute intentional self-poisonings (63.44%).

Pediatric acute intentional self-poisoning of alcohol-related etiology was caused by the consumption of vodka (20.4%), wine (17.3%), whiskey (9.1%). Distilled drinks are predominant in the etiology of alcohol ingestion in the adolescents included in the study. The results of the study performed in our country are different from the data obtained in other European adolescent populations [10]. According to literature, the drink preferred by European adolescents is beer (47%), followed by wine and distilled drinks (37-38%), cocktail drinks (32%), and cider (27%). As far as the context in which the acute intentional poisoning took place, most often socializing in a public space or at home with friends determined the occurrence of the poisoning.

In 7 of the cases, the etiology of the acute intentional self-poisoning was the combination of medication and alcohol (4 girls/3 boys), and in 7 other cases, the poisoning occurred through the consumption of both alcohol and substances of abuse (3 girls/4 boys). In the study group, acute intentional poisonings using substances of abuse were most often determined by the consumption of cannabis (57.14%) and new psychoactive drugs (30.95%), less often heroin (2.38%) or glue (9.52%). Out of the 42 cases, 14 were females and 28 were males, with ages within the 11-18 years interval, the ratio of girls/boys was 1/2.

Acute intentional self-poisonings, especially in the case of adolescents, occur most often with display purposes (parasuicide) or suicide purposes in the context of family disputes. Most are meant to attract attention, few are actual suicide attempts. Out of the total number of acute intentional self-poisonings, we pointed out 20 cases of suicide attempts in our study, representing 5.83% of the total acute intentional poisonings. Moreover, we noticed a part of these was not the first attempt. The age of patients with a suicide attempt was in the 14-18 years interval; the ratio of female/male was 3/1 (15 girls/5 boys). The majority of patients suffered from psychiatric disorders such as depressive disorder, behavior disorders, personality disorders, conversion disorders, emotional disorders. Adolescents and young adults are the age group at highest risk for the first onset of commonly occurring mental disorders. This life stage has also been identified as a critical period for the onset of the first suicide attempt [16].

54.39% of the patients with acute intentional poisonings could be placed in a category of psychiatric disorders, with the help of the extended team of a psychologist and a psychiatrist, after stabilizing the patient. The fact that there are patients with recurrent suicide attempts proves the inefficacy of the psychiatric treatment and the lack of psychological support after the period of hospitalization.

CONCLUSIONS

The clinical study showed a lot of social cases (low economic status, neglected child, disorganized families, domestic violence, children left in the care of relatives or institutionalized) and 11 patients who dropped out of school, 30 cases of chronic consumers of substances of abuse, with ages within the 11-18 years interval and 6 cases of chronic alcohol consumption. In addition, for two of the female patients, a diagnosis of evolving pregnancy was established. Reducing socio-economic deprivation and its associated problems may be an important strategy in the prevention of suicidal behavior, especially in young people.

Out of the 342 acute intentional poisonings, 36 cases of chronic consumers of substances of abuse/alcohol were noted, representing 10.5%. This is a worrisome percentage, bearing in mind the age group, i.e. less than 18 years. It is an alarm signal, which imposes the application of urgent measures, on a national level.

Pediatric acute intentional self-poisoning is an important public health issue, alarming through its consequences and its hidden neuropsychiatric and behavioral substrate. This is because adolescence is a period of marked emotional fragility, sensitive to all sorts of influences [17, 18].

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Author's contribution

SS contributed to the conception and design of the study, the acquisition, analysis, and interpretation of data of the study. She also contributed to the drafting of the work and its critical revision for important intellectual content. IB contributed to the acquisition, analysis, and interpretation of data of the study, to the drafting of the work, and its critical revision for important intellectual content. HTS contributed to the conception and design of the study, the acquisition, analysis, and interpretation of data of the study, contributed to the drafting of the work and its critical revision for important intellectual content. CD contributed to the conception and design of the study, the acquisition, analysis, and interpretation of data of the study, contributed to the drafting of the work and its critical revision for important intellectual content. MM contributed to the design of the study and the drafting of the study and its critical revision for important intellectual content. ACT contributed to the analysis and interpretation of data of the study, to the drafting of the work, and its critical revision for important intellectual content. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interests

It is not declared by the authors.

Ethics approval and consent to participate

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital, "Grigore Alexandrescu" Clinical Emergency Hospital for Children (Bucharest, Romania).

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Evaluation of the accuracy of E-FAST ultrasound in blunt trauma patients referred to the Emergency Department

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Abstract: *Introduction: Trauma is one of the most common causes of death in the world. E-Fast ultrasound can detect the haemoperitoneum, intravenous pneumothorax, and hemothorax. In this study, we evaluated the accuracy of E-FAST focused ultrasound in blunt trauma patients referred to the Emergency Department.*

Methods: In this study 167 blunt trauma patients were included. E-FAST is performed from four standard spaces in terms of free fluid, chest in terms of hemothorax, and pneumothorax. Ultrasound is performed on the trauma patient and the obtained results were compared with the result of the standard gold CT scan test. All patient information including age, sex, mechanism of trauma, patient ISS, E-FAST result, and CT scan result were registered.

Results: Overall, 174 patients were included that 45 (25.9%) of them were females. The mean age of the patients was 38.39±12.31 years. E-FAST had a sensitivity of 66.7 and a specificity of 100% in the diagnosis of hemothorax. Also, a positive predictive value of 100% and a negative predictive value of 99.4% were detected for 120 people in both normal tests.

Conclusion: The results show E-FAST is a suitable alternative to CT scan in the diagnosis of hemothorax, pneumothorax, and free fluid in the abdomen, vulva, and pericardial cavity. Due to portability, safe body, no need for contrast, fast performance, as well as cheapness, and reproducibility, it will be a great help in diagnosing trauma-related lesions and prompt and timely treatment. CT scan should be used only in limited cases and special indications.

Keywords: *E-Fast ultrasound, blunt trauma, accuracy*

INTRODUCTION

Trauma is one of the most common causes of death in the world. Sometimes due to accompanying trauma and distracting pain, our findings in physical examinations are limited. In trauma patients, internal bleeding is life-threatening and requires prompt and timely action [1-3].

E-Fast ultrasound can detect the haemoperitoneum and intravenous pneumothorax and hemothorax. It can be performed in traumatic patients with symptoms of hemorrhagic shock or evidence of intra-abdominal injury [1]. The features of FAST have led to this practice being adopted as an international standard of care in most developed countries of the world [2].

The benefits of this method can be noninvasive, portable; the low cost can be done in less than 5 minutes, repeatable without the need for radiation and counter, and can be performed by an emergency medicine specialist or surgeon [3-5].

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Studies have shown that E-Fast has a sensitivity of between 99-86% compared to other imaging and diagnostic modalities in the presentation of haemoperitoneum [7]. Laparotomy requires early diagnosis to minimize mortality and morbidity, and FAST can also be a reliable surrogate for CT scans in the diagnosis of intra-abdominal fluid [8-13]. The use of CT is limited due to its high cost and its harms.

In this study, we evaluated the accuracy of E-FAST focused ultrasound in blunt trauma patients.

METHODS

The study was performed on 167 blunt trauma patients referred to the three emergency departments of Baqiyatallah, Shohadae 7th Tir and Imam Hussein Hospital. The statistical population was Multiple Traumatic Blunt Patients and referred to those three emergency departments. E-FAST is performed from four standard spaces in terms of free fluid, chest in terms of hemothorax, and pneumothorax. Ultrasound is performed on the patient's bed and the obtained results are compared with the result of the standard gold CT scan test.

All patient information including age, sex, mechanism of trauma, patient ISS, E-FAST result, and CT scan result are entered in the questionnaire form. In this study, patients are examined for the presence of free fluid in the pelvic abdomen, pericardium, and the presence of free fluid in the pleural cavity on both sides. Patients who are admitted to the emergency room as blunt triage trauma, which includes car accident, motor accident, and fall from a height, and a fight, all-penetrating trauma, were excluded from the study. At the beginning of hospitalization and in the primary survey stage, the E-FAST blunt trauma patients were operated on from the pelvic abdomen and chest (Morrison Patch, Splenorenal free fluid around the bladder, pericardium, and pleural cavity on both sides).

Its positive and negative results were recorded in a specific form. All patient characteristics by trauma time, "time of visit", age, sex, "trauma mechanism", are recorded in the patient's specific form and patient records.

ISS under 16 is excluded from the study because they do not need a CT scan. Patients with ISS above 16 are visited by a surgeon after the initial procedures. If the patient is hemodynamically unstable and undergoes laparotomy and thoracotomy without a CT scan, the patient's laparotomy and thoracotomy results are compared with the E-FAST result. For other patients, if they had a CT scan of the abdomen and chest, the results would be recorded in the desired form. Finally, only 176 patients who had a CT scan of the abdomen and chest, or a laparotomy and thoracotomy are included in this study. Their results are compared with the E-FAST result

Data presented as frequency, percent, mean and standard deviation. An Independent T-test was used for comparing data between various levels. Finally, accuracies indices such as sensitivity, specificity, positive and negative predictive values were assessed for determining the accuracy of E-FAST diagnostic value compared to CT scan as a standard gold test. P-value <0.05 was considered significant.

RESULTS

Overall, 174 patients were included that 45 (25.9%) of them were females. The mean age of the patients was 38.39±12.31 years. Among the total number of people, 61 (35.1%) referred to pedestrians due to a vehicle accident. The relationship between gender and the mechanism of trauma was statistically significant (P<0.001) (Table 1).

Table 1: Distribution subject respect to the mechanism of trauma and sex

			Mechanism of trauma						Total
			Car accident	**Vehicle accident with pedestrians**	**Car overturning**	**Falling from a height**	**Falling from a motorcycle**	**Conflict**	
Gender	Female	Count	5	29	2	4	5	0	45
		% of Total	2.9%	16.7%	1.1%	2.3%	2.9%	0.0%	25.9%
	Male	Count	24	32	26	13	33	1	129
		% of Total	13.8%	18.4%	14.9%	7.5%	19.0%	0.6%	74.1%
Total		Count	29	61	28	17	38	1	174
		% of Total	16.7%	35.1%	16.1%	9.8%	21.8%	0.6%	100.0%

The mean ISS in all patients was 29.65±10.25. The mean ISS was 28.15±10.93 in females and 30.05±9.99 in males

(P=0.28).

E-FAST showed normalized outcomes in 125 patients (711.8%), 2 patients (1.1%) hemothorax, 4 patients (2.3%) pneumothorax and 18 patients (3.10% morison pouch fluid, 13 cases (7.7%), 5% splenorenal free fluid, 11 cases (3.6%) of free fluid in pelvis.

One case of free fluid in the pericardial was observed that was not confirmed by CT scan. Two patients were diagnosed with hemothorax in both tests and 3 patients were diagnosed with pneumothorax in both tests.

Out of 176 patients, CT-SCAN results for 133 (76.4%) normal, 3 (1.7%) hemothorax, 4 (2.3%) pneumothorax, 31 (17.8%) free fluid in the abdominal and the pelvis, and 3 patients were found to have retroperitoneal fluid (Table 2).

Table 2: Distribution of results according to E-FAST and CT-SCAN tests

		CT.SCAN						Total (%)
		Hemothorax	Pneumothorax	Free fluid in the abdominal and pelvic		Retroperitoneal fluid	Normal	
E-FAST	Hemothorax	2	0	0		0	0	2 (1.1%)
	Pneumothorax	0	3	0		0	1	4 (2.3%)
	Pericardial effusion	0	0	0		0	1	1 (0.6%)
	Morison pouch fluid	0	0	14	31	0	4	18 (10.3%)
	Splenorenal free fluid	0	0	9		0	4	13 (7.5%)
	Free fluid in the pelvic	0	0	8		2	3	11 (6.3%)
	Normal	1	1	3		1	120	125 (71.8%)
Total (%)		3 (1.7%)	4 (2.3%)	31 (17.8%)		3 (1.7%)	133 (76.4%)	174

E-FAST had a sensitivity of 66.7 and a specificity of 100% in the diagnosis of hemothorax. Also, a positive predictive value of 100% and a negative predictive value of 99.4% were detected for 120 people in both normal tests (Table 3).

Table 3: Sensitivity, specificity, positive predictive value, and negative E-FAST predictive value

Items	Sensitivity	Specificity	Positive predict value	Negative predictive value
Hemothorax	66.7%	100%	100%	99.4%
Pneumothorax	75.0%	99.4%	75.0%	99.4%
Normal	90.2%	87.8%	96.0%	73.4%
Free fluid in the abdominal and pelvic	91.1%	88.0%	63.2%	97.7%

DISCUSSION

In this study, 174 patients were included. The mean ISS in all patients was approximately 30 and the mean was not significant in both sexes. The mean ISS indicates the severity of considerable. In this study, E-FAST had a sensitivity of 91% and a specificity of 88% in the diagnosis of normal and normal individuals. E-FAST obtained 66% sensitivity and 100% positive predictive value and 4.99% negative predictive value in the diagnosis of hemothorax. In this study, this test had a sensitivity of 75% and a specificity of 2.99% in the diagnosis of pneumothorax, and a positive predictive value of 74% and a negative predictive value of 4.99% were obtained. Results showed all items are almost equal and even better in some respects. The results indicate that FAST is a very good alternative to CT scan in the diagnosis of hemothorax, pneumothorax, free fluid in the abdomen, vulva, and pericardium. There were several cases of retroperitoneal bleeding that could not be detected by FAST but were detected by CT scan.

In a study by Porter et al. on a large number of patients (2576 patients), FAST achieved a sensitivity of 93% and a specificity of 90% in the diagnosis of hemoperitoneum. In another study by MT.MILLER on 359 patients Sensitivity was 68%, specificity was 98%, positive predictive value was 67% and negative predictive value was 93% in the diagnosis of hemoperitoneum [14].

In another study by Porter et al., It was observed that among 1631 people with abdominal trauma, fast ultrasound is one of the main methods with high sensitivity and specificity. This sonography can discuss the complications of the disease [15].

In another report by McKenney et al., 899 ultrasounds of abdominal trauma were performed, of which 101 were positive and 783 were negative. Among the negative cases, 768 patients were confirmed by CT scan, surgery, and subsequent follow-up. Among the positive cases, 95 patients were confirmed. In this report, 86% sensitivity and 99% specificity were calculated [16].

However, it seems that in our study, due to the high accuracy of E-FAST in intra-abdominal, pelvic, and thoracic lesions in blunt trauma patients, it is recommended that more training be given to emergency medicine students and surgeons by a radiologist. Now it is preferable to use high-quality methods and high resolution and at frequent and regular intervals without hasty action.

Also, if E-FAS are performed at regular intervals (4-6) hours for hemodynamically stable trauma patients, its results will be much better, but its false positive and negative cases will be minimized and its sensitivity will be more than 98. And due to its advantages such as portability, safe body, no need for contrast, fast performance, as well as cheapness and reproducibility will help in the diagnosis of trauma lesions and prompt and timely treatment. And CT scans should be used only in limited cases and special indications.

CONCLUSIONS

The results show E-FAST is a suitable alternative to CT scan in the diagnosis of hemothorax, pneumothorax, and free fluid in the abdomen, vulva, and pericardial cavity. The reason for decreased sensitivity and specificity of E-FAST in the diagnosis of abdominal bleeding in this study was mostly due to several cases of retroperitoneal bleeding that could not be detected by FAST but were observed by CT scan. Due to portability, safe body, no need for contrast, fast performance, as well as cheapness, and reproducibility, it will be a great help in diagnosing trauma-related lesions and prompt and timely treatment. CT scan should be used only in limited cases and special indications.

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VARIA

Colorectal cancer epidemiology and risk factors

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Abstract: *Colorectal cancer is an important health problem, being the third most frequent cancer pathology both in men and women. Programmes have been developed to decrease CRC incidence and mortality, including primary prevention strategies focusing on population education regarding diet and physical activity and secondary prevention programmes such as screening. Modifiable risk factors are known to be diet, obesity, smoking, alcohol and read meat consumption. Non-modifiable risk factors are age, inflammatory bowel disease, genetic syndromes and family history of colorectal cancer.*

Keywords: *colorectal cancer, risk factors, genetic syndromes, Lynch syndrome, diet, obesity, diabetes*

Colorectal cancer is an important cause for mortality and morbidity all over the globe and recent data from the United States of America report it as the third most frequent cancer pathology both for women and men. [1]

According to American Cancer Society, the risk for developing colorectal cancer during lifetime is 1:23 for men and 1:15 for women. [2] In industrialised western countries, the lifetime risk for such malignant pathology is approximately 5%. [3]

World Health Organization reports approximately 1.9 million new cases every year. [4] Incidence and morality due to colorectal cancer increase with age, most of the cases being diagnosed around 70 years of age. [5] During last years, it has been reported an increasing in incidence with 1 % for people 50 to 64 years of age and by 2 % for people younger than 50 years. [6] Because of increasing life expectancy, people migration and changes in daily habits, it is expected that by 2035, there will be 2.4 million new colorectal cancer cases world wide. [5]

In Romania, the incidence of CRC is 10.1/100000 in men and 7.3/100000 in women. [4]

All over the world, strategies have been developed in order to reduce colorectal cancer incidence and mortality. These strategies cover primary prevention such as educational programmes for healthy life style and secondary prevention, such as screening programmes. [7, 8]

Regarding the risk factors involved in colorectal cancer pathogenesis, they can be divided into two major groups:

1. Non-modifiable risk factors: age, sex, personal history of adenomas, personal history on inflammatory bowel disease, family history of colorectal cancer or genetic syndromes

2. Modifiable risk factors: diet, physical activity, weigh gain, medication, alcohol consumption and smoking

1. Age is an important non-modifiable risk factor, as colorectal cancer risk starts to increase after the age of 40, and most of cancers are diagnosed after 50 years, with a

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maximum of incidence between 60 and 70 years of age. [9] Latest reports show an increase in CRC incidence in 20-49 years of age persons and so it becomes one of 10 most frequent cancers for this group. [6, 10]

As for differences between sexes, it appears that colorectal cancer is more frequent in men that in women, regardless of the age group, with 4.4% risk precent in men and 4.1% in women. [11] There is an interesting discussion regarding the distribution of precursor lesions in men and women. In women, adenomas are more frequent in proximal colon while in men, adenomas have a distal grow pattern. [12] This information in useful in choosing the best method for screening both in men and in women. [13]

During lifetime, the risk of developing a tubular or villous colorectal adenoma is approximately 19%. [14] 95% of sporadic CRC develop from adenomas, as they are known to be precursor lesions. [15] Interestingly, not all adenomas evolve in colon cancer. Most of them will regress. It usually takes approximately 10 years for a polyp to transform in a malignant tumor. [16] So there is an opportunity window in order to discover precursor lesions through screening. This is why people with personal history of adenomas should be monitored with colonoscopy, in order to decrease the risk of developing colon cancer. [17] Also, an important topic to debate in this case is the role of modifiable risk factors that maybe influence in a positive manner by reversing the process of transformation from adenoma to carcinoma. If it is really so, it would be quite difficult to demonstrate it.

Another important risk factor for developing colorectal cancer is the personal history of inflammatory bowel disease, meaning Crohn disease and ulcerative colitis. The severity of endoscopic and histologic inflammation, the duration and the extent of colorectal lesions are important elements that increase the risks of neoplasia. [18, 19, 20] Patients with inflammatory bowel disease have a sixth time higher risk of developing colorectal cancer, comparing to normal population, and the risk is even higher for those with extensive colitis. [21]

Although approximately 75% - 80% of colorectal cancers are sporadic forms, the rest of 20-25% are diagnosed in persons with familial history of colorectal cancer [22] especially in first degree relatives of patients with colorectal cancer diagnosed before 60 years of age [23]. In these familial cases, genetic factors and life style work together.

Genetic syndromes are involved in 5-10% of diagnosed colorectal cancer. The best known and studied genetic syndromes are:

- Lynch syndrome - hereditary non-polyposis colon cancer (HNPCC)

- Familial adenomatous polyposis (FAP) with its subtypes: attenuated FAP (AFAP), Gardner syndrome and Turcot syndrome

- MUTYH - associated polyposis (MAP)

- Peutz-Jeghers syndrome (PJS)

Lynch syndromes is the most common inherited CRC syndromes as it accounts for approximately 2-4% of cases. [24] The risk of developing colorectal cancer during lifetime is about 70-80% and the median age at diagnosis is usually around 40 years of age. [25] This hereditary syndromes is causes by a mutation detected in the genes responsible for DNA repair (for example MLH1, MSH2 or MSH6). People with this condition can also develop gastric, pancreatic or uterine cancers. [26]

In the case of familial adenomatous polyposis (FAP), the risks of CRC cancer is given by the germinal mutation of APC gene from chromosome 5q21. [27] In this case, the patient is diagnosed with hundreds or even thousands of polyps in colon and rectum having a major risk of transformation in colon or rectal cancer before the age of 20 years. [28] This disease is diagnosed in less than 1% of population. [27]

People with AFAP are diagnosed with less than 100 polyps during colonoscopy and the higher risk of colorectal cancer is around the age of 40. [29] Regarding the other attenuated forms of FAP, the high risk for colorectal cancer remains and is associated with other forms of non-cancerous tumours of skin, bones or soft tissue as in Gardner syndrome or with medulloblastoma as in Turcot syndromes. [24, 28]

In MUTHY-associated polyposis, the risk of cancer is also reported at a young age. In this case, colon polyps appear because of MUTHY gene mutation, a gene that is involved in "proofreading" the DNA. People with this condition also have an increased risk of thyroid and other gastrointestinal cancers. [29]

Peutz-Jeghers syndrome appear because of STK11gene mutation. Is a very rare syndrome. It is associated with increased risk of hamartomas in digestive tract and breast, ovary and pancreatic cancer, beside colon cancer. [30]

2. Taking into consideration that 75-80% of colon cancer are sporadic forms, it is considered that environmental, dietary, cultural, educational and social factors play an important role in this disease occurrence and, by educational programmes, it is a preventable cancer. This statement is supported by population studies focused on health of migrants and their children. It was observed that in migrants that came from low incidence colorectal cancer countries,

the incidence of this neoplasia starts to increase during lifetime and their children have the same incidence of colorectal cancer as the people in the adoptive country [31, 32]. Also, the incidence is higher in urban area than in rural area. [32]

For many years, a large number of studies and meta-analysis tried to pinpoint the risk factors for colorectal cancer. The conclusion was that high consumption of read meat or processed meat, heavy alcohol consumption and smoking, high mass index and lack of physical activity are clear factor that can raise the risk ok colon cancer. [33-35] Data show that by consuming 5 portions of red meat per week, one can have a 13% increased risk of developing colon cancer. [28] In contrast with this data, fruit and vegetables can reduce the risk of colorectal cancer by 15% if consumed for as many as 3 portions per day. [36]

Alcohol and smoking are considered to produce inflammatory damage of normal colon mucosa, leading to formation of polyps. Although the exact mechanism by which alcohol affects colorectal mucosa is not completely understood, in the case of cigarette, it is considered that carcinogens are the ones to be incriminated in this process.[37] Studies showed that there is a 3 fold higher risk for smokers to develop colon polyps. [38]

Weight gain and maintaining a high body mass index can promote inflammatory state through inflammatory cytokines and adipokines. [39] This process favours angiogenesis and colon carcinogenesis. In this case, the protective role of NSAID, in reducing inflammation, especially Aspirin is quite easily to be understood. [40]

The EPIC study demonstrated that by adopting a healthy life style, meaning weight control, sustained physical activity, lack of smoking, low consumption of alcohol and read meat can actually decrease the risk of developing colorectal cancer. [41]

Given the important role of diet, constant exercise and maintaining a normal body index in preventing colorectal cancer and consistent with EPIC study results, World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR listed a number of recommendations for healthy population and also for colorectal cancer survivors [42] A summary of these recommendations is:

- Body mass index - one should maintain as thin as possible without becoming underweight

- Physical activity - at least 30 minutes per day

- Restrict as much as possible sugary drinks

- Consume vegetables and fruits

- Limit red meat and avoid processed one

- Limit alcohol intake

- Limit salt consumption

- It is preferred to have all nutrients mostly from diet and less from supplements

- Breastfeeding for women

An interesting discussion is linked to patients with diabetes. It appears that they have a higher risk of colon cancer, although this can be related to increased body mass and sedentary, studies demonstrated that even with correct diet and physical activity, they still have an increased risk of CRC than that of general population. [34]

Also, the use of insulin in the treatment of diabetes it appears to be related to a higher risk of CRC diagnosis and a worst prognosis than the patients with diabetes treated with metformin. [43]

Much discussion takes place in the last 10 years regarding microbiota role in colorectal cancer carcinogenesis. It is certain that in patients with colorectal cancer, colonic dysbiosis is reported. Several bacterial species might play a role in CRC such as Streptococcus bovis, Enterococcus faecalis, Fusobacterium spp or Bacteroides fragilis [44-46] Pro-carcinogenic results of the presence of these micro-organisms are described to be: genotoxicity, inflammation, oxidative stress, host defence regulation [47] More studies are in progress analysing the correlation between microbiota composition changes and molecular pathways of colorectal cancer

CONCLUSIONS

Taking into account all the modifiable risk factors, the need for primary prevention programmes is clear in order to decrease the burden of colorectal cancer incidence.World Health Organisation listed recommendations to be taken into consideration by general population regarding diet, physical activity and weight control.

Regarding non-modifiable risk factors, the secondary prevention programmes must be considered crucial for people with family history of colon cancer or for those diagnosed with inherited familial syndromes.

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VARIA

Evaluating the effects of medication reconciliation by pharmacists on patient safety in hospitalized patients

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Abstract: *Backgrounds: Medication errors are one of the most common errors that are associated with adverse effects on patients and may even lead to mortality. One of the methods for determining patient safety in hospitals is assessing the prevalence of medication errors. In this regard, medication reconciliation is used to improve drug safety in the treatment process. The present study was conducted on 7564 patients in the emergency department of one of the hospitals in Tehran, Iran.*

Methods: In total, 256 adult patients with a mean of 45 years, who had been admitted to the emergency department of a hospital in the past six months and had used at least three drugs before admission, were entered into the study. In this study, the effect of medication reconciliation on admitted patients was assessed to prevent drug errors at the time of admission by pharmacists. The main outcome measure was Type and Frequency Percentage of Drug Differences Observed during Medication Reconciliation were measured.

Results: According to the results, the presence of a trained pharmacist was significantly effective in medication reconciliation for enhancing patient safety and treatment process accuracy. In addition, it was concluded that age, the condition of hospitalization, and medical center personnel had a direct relationship with the frequency of medication discrepancies.

Conclusion: The presence of a trained pharmacist to perform the medication reconciliation process significantly affected the improvement of patient safety and the accuracy of the treatment process.

Keywords: *medication reconciliation; pharmacists; patient safety*

INTRODUCTION

Medication errors are quite prevalent and are the fourth leading cause of death all over the world. These errors may occur through dug prescription, preparation, distribution, or administration [1, 2, 3]. Some patients like the elderly, are at higher risk of medication errors because they always suffer from different comorbidities and use complex drug regimens [4]. A study in one of our teaching hospitals indicated the high rate of medication errors especially the missed dose errors [5]. It is one of the most important goals of all health care systems to prevent different types of medication errors.

The medication reconciliation process is a method to identify the most accurate list of all medications a patient is taking, to improve patient safety. It is always performed on

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admission, to compare patient drug history with what is ordered in the hospital, and discharge as well as transferring from one ward to another [6]. It was first incorporated as a National Patient Safety Goal (NPSG) in 2005 by the Joint Commission. WHO and collaborators have designed a project for its implementation in a range of countries [7]. This process can effectively reduce the rate of different types of medication errors in hospitals [8, 9, 10]. It can also decrease the rate of readmission and its related costs [10].

Medication reconciliation is known as an important tool to prevent drug-related problems and their related burden. This process includes four stages, as follows:

- Preparing and recording the medication history of patients upon admission
- Comparing the medication history of patients with drug orders in the hospital and identifying and resolving discrepancies
- Providing a written list of medications upon the discharge of patients
- Teaching drug regimens to patients upon discharge [11].

Evaluating the medication reconciliation process supports the role of pharmacists as key members dealing with patients' medications, and collaboration between pharmacists and other healthcare team members is crucial in preventing drug-related errors and promoting patient safety especially in high-risk patients [12]. Medication reconciliation is a difficult and time-consuming process and is affected by factors like drug resources, the discharge process, and hospital policies. Distinguishing different barriers will contribute to the improvement of the exact drug information available between different health care settings [13].

Considering the impact of the medication reconciliation process on patient safety and the fact that this process used to be managed by doctors, medical interns, or nurses in our hospitals and is recently reformed with emphasis on the role of pharmacists, we designed this study to evaluate the following objectives in one of the hospitals in Tehran:

- Determining the difference between drug history taken by pharmacists and nurses
- Determining the frequency of identified errors in the reconciliation process
- Determining the frequency of identified errors related to dangerous medications (high risk)
- Determining the time spent for each medication reconciliation (time of comparison of BPMH and the current medication prescription inserted into the patients' file by the physician), resources used for history taking, the mean amount of medications used before admission, and time spent for corrective measures.

Ethics Approval

This study was approved by the Guilan University of Medical Sciences Ethics Committee.

MATERIALS AND METHODS

The impact of admission medication reconciliation by pharmacists on the rate of medication errors was assessed in this study.

During the six months from September 2019 to March 2019, 256 patients who were admitted to the emergency department of one of the general private hospitals in Tehran, were included in this study. The hospital had 350 approved beds and 300 active beds with an admission rate of 50 % in the emergency ward.

Inclusion criteria:

- Age over 45 years old,

- History of taking at least 3 medications at home

Medication reconciliation was conducted by trained hospital pharmacists from 8 a.m. to 8 p.m. each day. Patients who were admitted after 8 pm were visited by pharmacists the following day.

During the study period, all patients who were admitted to the emergency department were referred to the pharmacist for medication reconciliation if met the inclusion criteria. The pharmacist collected the best medication history (BPMH) from the patients or their caregivers as well as other information sources like patient GP or local pharmacy if required, using effective communication skills. Information sources were specified in medication reconciliation forms (MRFs). Insurance company records were also used to determine the details about drugs that were covered by insurance. Patients were also asked to bring all the medications they were using at home, if there were any, to provide more details. All the following information were entered in medication reconciliation forms:

- History of chronic diseases (e.g., diabetes, hypertension, hyperlipidemia, metabolic and genetic disorders, seizure, history of myocardial infarction (MI), and stroke)
- Any weight loss or weight gain diet and related medications
- Any herbal drugs in the form of topical, tea, and distillates

- Any prescribed and non-prescribed drugs
- Any high-risk medication as an anticoagulant, chemotherapy, antic arrhythmia, anti-convulsion, immunosuppressant, insulin
- Generic and brand name, dosage, frequency, and route of administration as well as how long days were on that medication were specified about each medication.
- History of smoking, alcohol, and narcotics
- Pregnancy and breastfeeding status
- History of any drug and food allergy

Then MRFs were compared with the medication history recorded in patient's files who were routinely taken by nurses. It was also compared with doctor's drug orders on admission. Any differences observed at this stage that required correcting the doctor's order were considered as clinical interventions.

The time spent by pharmacists and nurses to provide the BPMH were also recorded for each patient,

Finally the following were evaluated

- The differences between medication histories taken by the pharmacists and nurses
- The frequency of medication errors during the reconciliation process
- The frequency of medication errors related to high-risk drugs
- The time spent on each medication reconciliation
- Information sources used to take the medication histories and an average number of medications used before admission.

RESULTS

About 46% of our study population were male and 54% were female with a mean age of 61 (±16) years, they were taking 3- 10 medications before hospital admission (average of 6 drugs). Our results showed that from 256 patients who entered the study, 92 patients required some kind of clinical intervention. These interventions were approved by medical doctors in 87 cases, that 15.2% of these interventions were related to high-risk drugs.

In addition, we observed at least one difference between MRFs and medication history by nurses in 98.7% of cases. (1025 cases) which 13.4 % of these differences were related to high-risk drugs. Details about these differences are described in Table 1. Drug omission was the most prevalent difference we observed in this study. The second and third most prevalent differences were about dose and frequency of drug administration.

Table 1: Type and Frequency of differences Observed between MRFs and nurses medication histories

Type of observed medication error	Frequency percentage of error
Drug omission	48%
Dose	21%
Drug regimen	20%
Drug name	5%
Drug duplication	3%
Dosage form	2%
Other cases	1%

Regarding the time spent taking BPMH, we observed that pharmacists spent an average of half an hour to feel an MRF compare to 3 minutes, for taking a medication history, by nurses. In addition, the average time spent on comparing MRFs with doctors' drug orders was 12 minutes and it took a median of 9 minutes for pharmacists to complete their clinical intervention.

Information regarding medication reconciliation was obtained from the patients in 84 cases (32.8%), their caregivers in 61 cases (23.82%), patients' home medication bags in 43 cases (16.79%), their insurance records in 58 cases (22.65%) and other sources in 4 cases (1.56%) (Figure 1).

Figure 1: Source of information for medication reconciliation

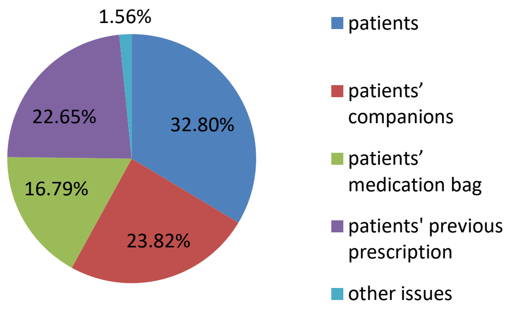

DISCUSSION

The impact of medication reconciliation by pharmacists on the rate of medication errors was evaluated in this study. Our results showed that medication histories taken by pharmacists were more comprehensive than routine drug histories taken by nurses. We also observed that pharmacist interventions can have a positive impact on the rate of medication errors.

Results of various studies indicate the high that rate of different types of medication errors and related burden in hospitalized patients especially through admission or discharge process as well as transferring from one unit or facility care to another. These errors are mainly the result of miscommunication and lack of required information and can lead to serious complications [5, 14, 15, 16]. Our results showed a high rate of medication errors in our study population, especially during the discharge process. We observed that errors related to the dose of patient medications were the most common errors. While in some other studies various types of medication errors, as well as errors in drug administration, were observed with almost the same frequencies [19].

Medication reconciliation, as a method for identifying and correcting medication errors, is widely used in health care settings in different countries [17]. The results of a systemic review showed that medication reconciliation can significantly decrease the frequency of different types of medication errors (17 from 17 studies), and drug side effects (2 from 2 studies) [18]. Different studies indicate that a large number of patients admitted to hospitals, experience medication discrepancies that can be prevented by careful medication reconciliation by pharmacists [20, 21]. We also observed in the present study, that different types of medication errors can be avoided by performing a careful medication reconciliation by a trained pharmacist especially when a patient is admitted to the hospital. This can prevent any discrepancies between what has been taken at home and what is ordered in the hospital.

About two-thirds of the complications that occurred after discharge are due to medication-related problems. Generally, medication reconciliation at discharge could be a strategy to reduce medication errors and improve patient safety even after discharge from the hospital [22].

CONCLUSION

In the present study, the impact of medication reconciliation by a pharmacist, on the rate of medication errors was evaluated. The results showed a significant difference between the history taken by the pharmacists and nurses. It should be noted that although the process of medication reconciliation by pharmacists is time-consuming and need considerable human and financial resources that may be difficult to afford now, its positive impact on direct and indirect costs of medication errors cannot be dismissed. Our findings also indicated that the involvement of a trained pharmacist in patients' treatment teams can significantly improve their safety profiles.

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Conflict of interests

The authors have no conflicts of interest to declare.

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VARIA

The effect of lowering the dialysate temperature on the stabilization of hemodynamic indices of diabetic patients admitted to Ali ibn Abi Talib Hospital, Zahedan: A clinical trial

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Abstract: *Introduction: One of the basic pillars of hemodialysis is its adequacy, which is achieved by stabilizing hemodynamic parameters. This is especially important in the hemodialysis adequacy of diabetic patients. The current study aims to determine the effect of lowering the dialysate temperature on hemodynamic indices and dialysis adequacy of diabetic patients.*

Materials and methods: This is a clinical trial that was performed on 32 diabetic patients admitted to the Dialysis Ward of Ali ibn Abi Talib Hospital affiliated with Zahedan University of Medical Sciences in 2017. The research subjects were selected using the convenience sampling method from eligible patients. Each patient underwent hemodialysis twice; i.e. routine dialysate temperature (37° C) and cold dialysate temperature (36 °C). Except for lowering the dialysate temperature to (36 °C), all other conditions were the same during the study. Blood pressure (BP) and pulse rate of patients were monitored before and after dialysis and during the first, second, and third hours after dialysis; however, the body temperature and weight of patients were monitored before and after each dialysis session. Patients' blood samples were collected at each stage before and after dialysis to evaluate urea and calculate dialysis adequacy. Data analysis was carried out using descriptive statistics, paired t-test, and one-way repeated measures ANOVA in SPSS Ver. 21. P-value <0.05 was considered as the significant level.

Results: Systolic, diastolic, and mean arterial blood pressure among patients who underwent hemodialysis with normal dialysate temperature were significantly lower than those who underwent hemodialysis with cold dialysate temperature (p<0.05). There were no significant differences between cold and routine dialysis in terms of changes in body temperature before and after dialysis. The dialysis adequacy at 36 °C showed a significant increase as compared to 37° C.

Conclusion: The results of the present study showed that hemodialysis with cold solution (36 °C) stabilized hemodynamic variables and improved dialysis adequacy among diabetic patients, therefore, this treatment is recommended in hemodialysis wards.

Keywords: *cold dialysis solution, hemodynamic indices, dialysis adequacy, hemodialysis, diabetic patients, chronic renal failure*

INTRODUCTION

Type 2 diabetes is one of the most common non-communicable diseases in the world and one of the 10 leading causes of death in many countries [1].

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In terms of the severity of the disease, 35% of dialysis patients with chronic renal failure are diabetic [2].

Chronic kidney disease is the most common and important complication of diabetes, which is associated with a range of different pathological processes and leads to an irreversible decrease in renal function. Among these pathological processes, end-stage renal disease (ESRD) is one of the most important and common life-threatening diseases, which is associated with severe or complete renal insufficiency in the excretion of waste products, urine concentration, and regulation of electrolytes [4]. Studies show a growing increase in the prevalence and incidence of ESRD patients in Iran, reaching from 24,000 in 2008 to 58,000 at the end of 2016, of which about 29,200 (95%) are undergoing hemodialysis and 1624 are undergoing peritoneal dialysis [5, 6]. Considering the prevalence of diabetes and hypertension, it is predicted that the number of kidney patients, dialysis patients, and kidney transplants will be doubled and reach at least 90,000 by 2022 [6]. Aghighi et al. reported that the number of these patients increased by more than 130% in Iran during 1997-2006, which indicates the need to pay attention to this disease [7].

Considering the average 7%growth in the number of these patients in Iran, hemodialysis is expected to remain the most important method of dialysis in the treatment of these patients and increase the life expectancy of these patients, however, hemodialysis-related complications and problems also affect the health of these patients [7, 8].

Despite many technological and technical advances in hemodialysis and its improvement, there are still many pre- and post-dialysis problems. Intradialytic hypotension (IDH) is the most common serious complication of hemodialysis, occurring in 5%-40% of hemodialysis treatments. Hypotension widely increases the overall mortality of patients and not only limits fluid removal during dialysis, but can also cause severe adverse vascular effects on mesentery, cerebral infarction, and ischemia. This condition increases the need for nursing care and has negative effects on their quality of life, which is especially important among diabetic dialysis patients [2]. Diabetic patients are at higher risk for IDH, which is due to the autonomic dysfunction and cardiovascular problems in these patients.

One of the effective solutions proposed to prevent IDH, which has also been used, is to reduce the temperature of the dialysate [11-16]. Hemodialysis patients, who are usually hypothermic, gain extra heat during the treatment session. It seems that the increase in body temperature is due to increased sympathetic activity and decreased ability to dissipate heat due to severe contraction of peripheral arteries and dilation of central arteries, and ultimately reduced cardiac output [17, 18].

A slight decrease in dialysate temperature of about 1-2 °C helps reverse these changes by increasing central venous artery tonicity, venous return, and cardiac output [20]. Mojerloo et al. used cold and routine dialysate and found that generally, hypotension rate was 18% and 5% among the dialysis groups treated with routine temperature and cold dialysate, respectively, and hypotension rate was significantly lower in the dialysis group with cold dialysate [21].

Because hemodialysis patients experience many problems due to the specific physical and mental conditions arising from their disease, and dialysis quality is an important factor and effective in reducing these problems, and one of the important parameters to improve dialysis adequacy is to stabilize hemodynamic indicators [16], there is a need to adequate standard hemodialysis by reducing side effects to reduce referrals among patients (especially diabetic patients) for hemodialysis. Considering the importance of the foregoing and the results of several studies, the use of cold dialysate is a simple and inexpensive method that can reduce the frequency of hypertension, stabilize hemodynamic indices, improve the hemodialysis quality, and thus improve quality of life and general health of diabetic patients undergoing hemodialysis [22-24]. Since there has been no similar study in the province, this study aimed to determine the effect of lowering the dialysate temperature on hemodynamic indices and dialysis adequacy of patients with type 2 diabetes admitted to Ali Ibn Abitaleb Hospital of Zahedan University of Medical Sciences in 2017.

METHODS

This quasi-experimental one-group study was carried out on 32 eligible patients with type 2 diabetes who referred for hemodialysis using convenience sampling. Inclusion criteria included being on dialysis for at least 3 months, non-use of antihypertensive drugs, weight gain up to three kg between hemodialysis sessions, and no anemia, malignant disease, and thyroid disorders. Information record form was made available to patients who expressed their consent to participate in the study. This form consisted of two parts; the first part included individual and clinical characteristics and the second part included calculating and recording the dialysis adequacy of each patient in each hemodialysis session.

The sample size was estimated at 14 people using the following sample size formula and taking into account the numerical values of the variables related to the study by

Mousavi et al. (2014), 95% confidence interval, and power= 95%. To increase the reliability of the results, the sample size was increased to 30 people; finally, it was increased to 32 people considering the possible drop-out.

$$n = \frac{(Z_1 - \frac{\alpha}{2} + Z_1 - \beta)^2 \sigma_\delta{}^2}{\delta^2} + \frac{Z_1^2 - \frac{\alpha}{2}}{2}$$

x̄1=83.94 Z1- β=1.64 Z1- α/2 = 1.96 S2=19.26

The study was performed in two stages. All patients underwent hemodialysis with a dialysate temperature of 37°C (routine dialysate) before the intervention. They then underwent hemodialysis with a dialysate temperature of 36°C (cold solution) in the next session. In other words, all patients underwent hemodialysis once with a routine dialysis solution (37°C) and once with a cold dialysis solution (36 °C) during two consecutive sessions. A sequence of dialysis with cold or routine dialysate for each patient was performed using random allocation where blue and green balls were used. The patient was asked to pick up a ball from the box containing 16 blue balls and 16 green balls. Blue and green balls represented the cold and routine dialysate in the first session, respectively. In the next session, the dialysis method was shifted. Hemodialysis variables were the same in both cases except for the solution temperature. All patients underwent hemodialysis 3 times a week for 3-4 hours each time. The mean body temperature of patients before hemodialysis was determined in the range of 37 °C and R5 polysulfane dialyzers made by the same company with the same ultrafiltration coefficient (High-flux) and bicarbonate dialysate (bicarbonate powder 650 g 35 Meql/lit, Pharmed Medical Industries Company), which was fixed during the study, was used for all patients. The equipment used, including dialysis machines equipped with an ultrafiltration control system (Fresenius model 4008-B), scales (Seca), and pressure gauges were investigated and confirmed. The dialysate flow rate was constant (500 Ml min).

The dialysate removal rate was calculated and determined for each patient based on the difference between pre-dialysis weight and dry weight (after dialysis). Blood flow rate (300 ml/min) was determined and 16-gauge needles were used. All parameters were constant on the machine until the end of the dialysis session. To measure pre-dialysis BUN level, an arterial blood sample was taken immediately before hemodialysis and after the hemodialysis session. To prepare a blood sample after dialysis, first, the pump speed was reduced to 100 cc per minute and another sample was taken from the arterial route 15 seconds later. All samples taken in the hospital laboratory were examined and the results were received. After measuring the research samples, the dialysis adequacy was calculated by KT/V method using the DaugirdasII formula, which is an internationally acceptable formula. Systolic blood pressure (SBP) and diastolic blood pressure (DBP), and mean arterial blood pressure (ABP) was calculated and recorded before, during (first, second and third hours) and at the end of hemodialysis. The patient's body temperature and heart rate (HR) were also measured before and after hemodialysis. KT/V index ≥ 1.2 and < 1.2 show dialysis with good and low adequacy, respectively. KT/V index values greater and lower than 1.2 indicate more and lower adequacy of dialysis.

Finally, the obtained data were entered into SPSS ver. 21 and analyzed. Descriptive analysis was carried out using mean, frequency, standard deviation, and percentage. A dependent t-test was used to evaluate the effect of cold dialysis on dialysis adequacy. P-value< 0.05 was considered as the significant level.

RESULTS

Of the 32 patients included in the study, 1 patient was excluded from the study due to being on a trip. The majority of people were female (45.2%) and married (83.9%), and 61.5% had the underlying disease. Mean ± SD of patients' age, duration of diabetes, duration of treatment, and weight gain was 60.5 ± 9.21 years, 12.07 ± 7.27 years, 21.82 ± 27.74 days, and 1.64 ±0.68 kg, respectively.

The results of comparing dialysis adequacy with cold and routine dialysate temperature (Table 1) showed a statistically significant difference between the two methods (p<0.001) and dialysis adequacy was higher in cold dialysis.

Table 1: Results of dialysis adequacy in two cold dialyzes and dialysis with routine temperature among hemodialysis patients

Dialysis method or group	Mean and standard deviation	Test result
Cold dialysis	1.14±0.34	P=0.001
Routine dialysis	0.98±0.20	P=0.001

DISCUSSIONS

The results of the present study on the mean systolic blood pressure in hemodialysis with cold and routine dialysate temperatures, before dialysis, 1, 2, and 3 hours of dialysis, and after dialysis showed no statistically significant differences between the two groups in terms of systolic blood pressure in the above five measurement periods except after the intervention (P =0.003).

The lack of a clear drop in SBP after intervention in the cold dialysis group indicates the effectiveness of cold dialysate in preventing a drop in SBP during dialysis.

The results of the present study are consistent with the study by Mojerloo et al. (2015) [21]. In the study entitled "The effect of lowering the dialysate temperature on the stability of hemodynamic variables in patients prone to hemodynamic instability under hemodialysis", Mousavi et al. (2014) showed that reducing the dialysate temperature to (35 °C), as compared to the standard dialysate temperature (37° C), prevents a drop in systolic, diastolic, and mean arterial blood pressure 2 and 3 hours of hemodialysis [25].

In a similar study conducted by Azar et al. (2009) in Egypt, patients were evaluated during 6-dialysis periods and results showed no change in systolic blood pressure after hemodialysis with cold dialysate [9].

Ayoob et al. (2004) carried out a study on two groups of patients, including 5 patients with periods of IDHs and 5 patients with stable BP during hemodialysis. Each patient underwent hemodialysis for three periods with cold dialysate (35 °C) and then three periods with standard dialysate (36.5 °C). The results showed that the mean arterial blood pressure during and after dialysis with dialysate was significantly high in the hypotensive group and did not change in the group with a stable BP [22].

The findings of the present study showed that lowering the dialysate temperature to (36 °C) compared to routine dialysate temperature (37 °C) could prevent SBP at the end of hemodialysis, although SBP did not decrease significantly during 1, 2 and 3 hours of dialysis. The results showed that hemodialysis with routine dialysate temperature reduces BP in patients during dialysis until the end of it.

Findings of the present study showed that the use of cold dialysate for diabetic patients prone to hypotension significantly prevents hypotension during and at the end of dialysis, which is consistent with the results of the study by Ayoob et al. (2004) [22].

The results of the present study are consistent with the results of similar studies conducted in Iran [17, 27]. Therefore, it can be concluded that IDH is more stable in hemodialysis with cold dialysate, and reduces the frequency of IDH. Also, the results showed no drop in DBP at different hours of dialysis in the cold dialysis group, however, there is an evident drop in DBP in the routine group, as we approach the last hours of dialysis, although the statistical tests did not show a significant difference in this regard. The results of the present study are consistent with the study by Mousavi et al. (2014). The results of their study showed a statistically significant difference between cold dialysate and routine dialysate in terms of DBP level suggesting that cold dialysate prevents pressure drop in DBP [25]. Therefore, it can be concluded that the cold dialysate can prevent a reduction in DBP.

The present study also investigated the mean ABP in routine and cold dialysate temperatures, before dialysis, during 1, 2, 3 hours of dialysis, and after dialysis. The results showed a significant difference between the two methods in terms of mean ABP. Although no statistically significant difference was observed between the two methods during hemodialysis in terms of mean ABP, it was relatively higher in the cold dialysate than routine dialysate ($p<0.001$), however, mean ABP was higher in the cold dialysate group than routine dialysate group.

The results of the present study are consistent with the study by Mousavi et al. (2014). They also found that routine dialysate led to a reduction in mean ABP [25]. Ayoob et al. also showed that the use of cold dialysate stabilizes blood pressure [22]. Considering the foregoing, it can be concluded that the use of cold dialysate stabilizes systolic and diastolic blood pressure and mean arterial pressure during and after hemodialysis. Cold dialysate causes hemodynamic stability during dialysis through various mechanisms, which include keeping the body temperature constant, increasing the activity of the sympathetic nervous system, and thus increasing the cardiac muscle contractile strength, and peripheral vascular resistance, stimulation of alpha-1 receptor in visceral arteries, followed by increased central circulatory volume and increased cardiac output [28-30]. Other studies also concluded that lowering the dialysate temperature reduced nitric oxide synthesis and thus reduced the frequency of hypotension [28].

The present study also investigated the mean HR in routine and cold dialysis methods, before dialysis, during 1, 2, 3 hours of dialysis, and after dialysis. Results showed no difference between the two methods in terms of HR changes, which are completely consistent with the study by Mousavi et al. (2014) [25], but inconsistent with the results of the study by Azar et al. (2009) [9] who found a significant difference in this variable. One of the reasons for the difference in the results may be attributed to the fact that bicarbonate-containing solutions are currently used, all of which contribute to the stability of patients' hemodynamic conditions and heart rate.

The present study also investigated the body temperature of patients who underwent hemodialysis with routine and cold dialysate before and after dialysis. Results showed that the body temperature was not significantly different before and after dialysis, but there was a significant difference in the mean changes of body temperature after hemodialysis. That is, the mean body temperature of people undergoing hemodialysis with routine dialysate was higher than that of people undergoing hemodialysis with cold dialysate (P = 0.02).

The findings of the above study are consistent with the study by Borzoo et al. (2015). They found changes in clinical parameters with changes in dialysate temperature and the mean body temperature of patients decreased with decreasing dialysate temperature, meaning that the mean body temperature of patients who underwent hemodialysis with normal dialysate temperature (37 °C) was 0.12 and 0.27 higher than those underwent hemodialysis with at dialysate temperatures 36 °C and 35 °C, respectively [24]. Another important finding of the present study is the mean body temperature of patients in the two groups before dialysis, which is 36.9°C in the cold dialysis group and 36.8°C in the routine dialysis group, but the statistical tests did not show significant differences (p=0.11). These findings are not consistent with the results of previous studies, which indicate that the body temperature of dialysis patients is lower than the body temperature of normal people [24]. Anemia often leads to a decrease in internal body temperature, which leads to chills, especially in the extremities such as legs, arms, ears, and nose, even when the person is in a warm room. Therefore, using a solution with a temperature higher than body temperature can not only lead to the destabilization of cardiovascular conditions [9, 18, 24] but also causes discomfort in patients [31].

Considering the results of the current study suggesting the positive effect of cold dialysate on the dialysis adequacy and comfort of patients, it seems that the time has come for all patients not to be permanently dialyzed with a single dialysate temperature and the dialysate temperature to be determined according to patients' clinical conditions. The use of cold dialysate, which is both inexpensive and readily available, can prevent hemodynamic instability during hemodialysis and reduce the periodic occurrence of IDH. However, it should be noted that all patients may not benefit from this method, therefore, people who have a lower pre-dialysis temperature can benefit more from this method.

CONCLUSIONS

Considering the positive effects of cold dialysis on the hemodialysis adequacy and stabilization of hemodynamic variables, it is suggested that if there is no prohibition in this treatment, cold dialysate replaces the standard dialysate. It is recommended to carry out further studies on the effect of cold hemodialysis on hemodialysis adequacy in other groups, including non-diabetic patients.

Ethical considerations

The present study was registered in the Ethics Committee of Zahedan University of Medical Sciences with the number (IRZaums.REC.1396.292) and in the Iranian Registry of Clinical Trials with the code IRCT20171009036675N2.

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VARIA – Letter to Editor

Challenging of COVID-19 crisis in the Emergency Department

Hassan Goodarzi[1], Sardollah Mahmoodi[1]

Dear editor,

The outbreak of coronavirus disease 2019 (COVID-19) is quickly spreading in the world [1]. The COVID-19 has negatively affected the standard services presented in the emergency department (ED). The ED must provide health services to large populations with various health problems. COVID-19 conditions raised pressure on the limited resources in ED and caused interruptions in preliminary treatment. Recently, evidence verified that there are increased rates in emergency healthcare services usage because of the raising traumatic injuries. In the COVID-19 crisis, the statistics and causes of trauma injuries patients were dramatically changed and because of the attendance of further family memberships at household domestic injuries and burning in children were increased [2].

Healthcare services overcrowding has negative consequences to the patients, the healthcare services, and the population. Overcrowding of the healthcare services could also hurt standards services preparation [2]. Moreover, in an outbreak of a communicable disease such as COVID-19, overcrowding has specific negative consequences on controlling and prevention of expansion of diseases. Overcrowding in ED could be the trigger to spread COVID-19 more rapidly. Furthermore, the structure in many EDs is insufficient for facing with overcrowding of patients, causing unsatisfactory situations while people are anticipated to have prolonged for suitable treatment in ED. Also, in many EDs maybe there are inadequate clinical spaces to facilitate evaluation and therapy of cases with confidentiality. Also, there are a high number of emergency patients and the seriousness of their conditions has hindered the timely admissions of emergency patients and cause overcrowding of healthcare services. The increased high non-urgent patients that were scared from coronavirus flow to the hospitals were other identified factors that contributed to the hospital crowding. These circumstances have adversely affected the quality of services and performance.

Following the quick spread of the COVID-19 crisis in the world, unfortunately, there is a limitation to access to supplies such as gloves, medical masks, respirators, hand washers, etc. However, a shortage of personal protective equipment could endanger the general population and as well as healthcare workers. Governments and healthcare administrators must performance rapidly to provide supplies.

Healthcare workers involved with COVID-19 cases in ED were commonly under high pressure. Maintaining the mental health of Healthcare workers in ED is very crucial to manage COVID-19 cases. Administrators must have care to the work stress and the associated causes of the Healthcare workers.

A suitable registry system in ED could help for better management and follow-up of COVID 19 cases and also it could help researchers for better understanding and studying about various aspects of COVID 19 during a long time follow-up after treatment.

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We look forward to your submission.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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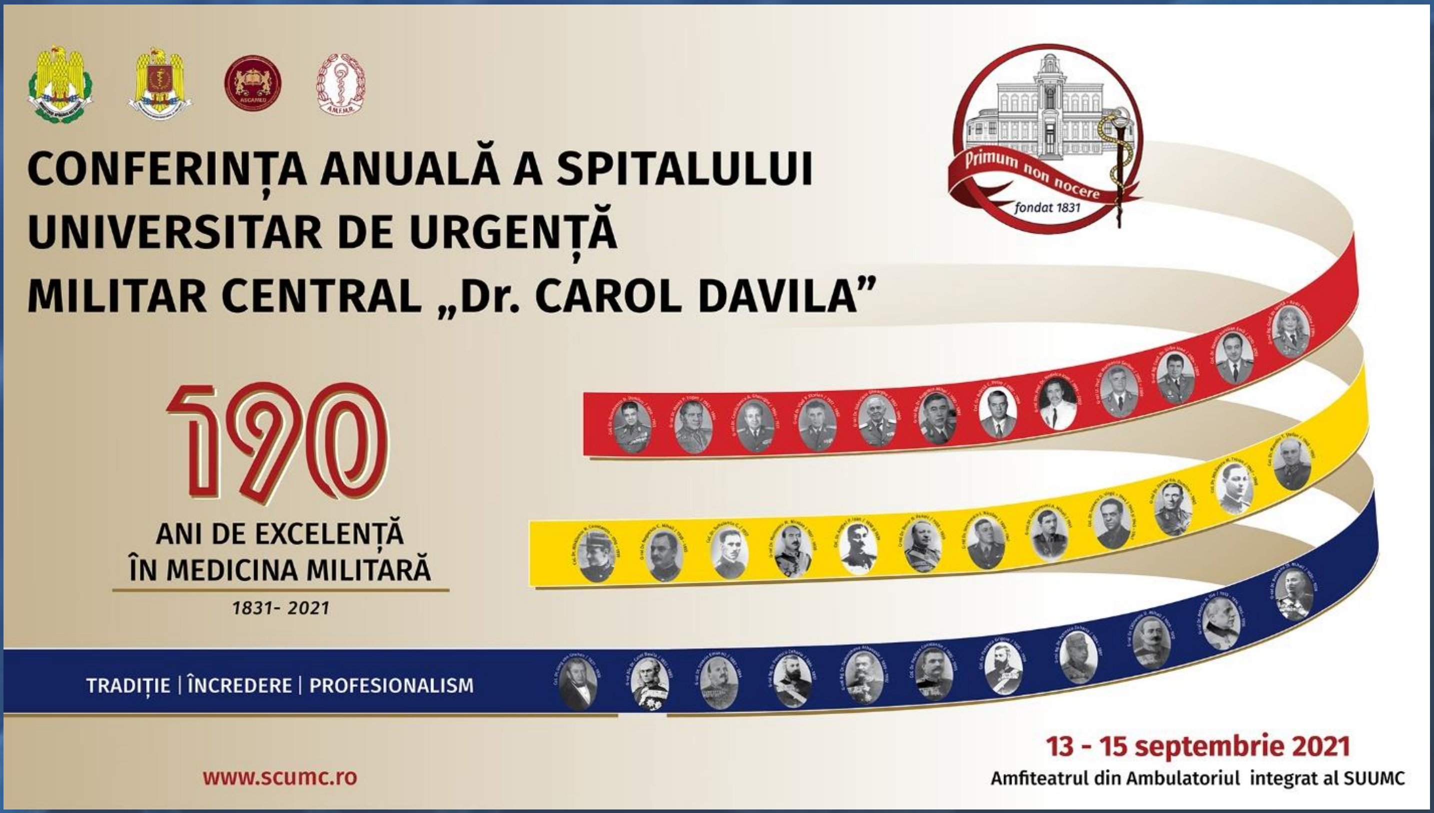

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Emergency Department in the context of the COVID-19 pandemic

SARS COV2/COVID 19 testing dynamics – Emergency Department response

C.B. Teusdea, S. Dogaru

ED is always in the first line. As emergency physicians, we are always facing the unknown and trying to manage the unpredictable. The COVID-19 is only the newest threat, but in fact a small part of our daily routine. As professionals we did what we were taught and followed the procedures, as humans we were confronted with a new contagious disease with a potentially ominous course.

Wearing an uncomfortable protection suit in an already overcrowed space, we witnessed the increase of the incidence of COVID-19 cases, faced the aggravation of the medical status for some of those patients while waiting for an ICU bed to be freed. We also kept under close supervision critical patients with complex comorbidities and acute pathology while waiting the results for COVID-19 testing in order to admit the patient in the proper zone (with or without COVID-19).

In this review we will present the experience of the Emergency Department during the pandemics regarding COVID 19 testing dynamics.

Ventilation of COVID-19 patients in ED

C.B. Teusdea, M. Toma

The COVID-19 pandemic determine an increase of number of patients in need of hospital care that exceeded the availability of medical care. Many COVID-19 patients experience ALI (Acute Lung Injury) and Acute Respiratory Distress Syndrome (ARDS), conditions that can be treated with mechanical ventilation.

In overcrowded period of pandemic, the numbers of patients that need mechanical ventilation, inclusive noninvasive ventilation, push the resources of Emergency Department to the limits, due to the large number of patients stationed in ED (lack of Intensive Care admission places) who required mechanical ventilation.

In this review we will present the experience of the Emergency Room during the pandemic regarding the burden that we have to overcome in terms of long-term ventilation of Covid-19 patients.

Functional circuits of COVID-19 patients in Emergency Department

C.B. Teusdea, M. Toma, C. Stanciu

SARS-CoV-2 is responsible for the largest pandemic facing humanity since the Spanish flu pandemic in the early twentieth century. The COVID-19 has taken the world by storm and ravaged almost every country in the world.

The biggest challenge was the emergency department modeling to manage the COVID-19 outbreak. The Emergency Department (ED) was in this period of time the frontlines, having an essential role in identifying these patients, isolating them, and providing urgent medical care.

In this presentation we notice the challenges faced and the measures taken by our Emergency Department in response to the COVID-19 outbreak.

Emergency Department in the context of the COVID-19 pandemic – Role 2 experience

F. Costea, G. Ifrim

In a small town in China, in December 2019, a new member of the coronaviride family, the SARS-CoV-2 virus, was identified. It has evolved and spread rapidly, leading to a rapid increase in the number of cases of disease. Thus, the pressure on the already overcrowded medical system has increased.

In the Emergency Department the flow of patients is very high all over the world, because the health system is constantly changing and the number of patients presenting is increasing. At the same time, the Emergency Department represented during this period, for most of the patients, the first place where they addressed at the appearance of symptoms, substantially supplementing the number of presentations.

However, the medical staff of ED showed professionalism, involvement and perseverance by participating in all activities carried out since the first case. Their vast experience in field medicine was an important point of support in the commissioning the first ROL2 Military Hospital COVID-19, located inside the National Institute of Gerontology and Geriatrics "Ana Aslan". With the hospital activation, new challenges began to appear, some caused by extremely capricious weather, others by extensive legislation, but insufficient to solve the problems, the pandemic context being a test for all countries. All these impediments could be overcome due to close cooperation between medical staff and Ministry of National Defense structures, which continues today.

In conclusion, in the absence of concrete information on the diagnosis and treatment of SARS-CoV2 infection, providing healthcare was a major challenge. The medical staff of the Emergency Department was involved in many actions carried out by the Emergency Military Hospital, including the proper functioning of the ROL2 Military Hospital COVID-19.

COVID-19 pandemic – experience for the future

F. Costea, S. Ionescu, G. Iancu, G. Ifrim, I.M. Staicu, A. Salcianu

Starting with December 2019, people around the world have started a seemingly endless war with a tiny and unknown enemy, the SARS-CoV2 virus. In Romania, the first case of illness with this virus appeared on February 26, 2020 and so we joined this unknown war. In the first line, this time, it was no longer the military, but the medical staff who did not hesitate and fought non-stop for the good of the patients.

Being a new virus, whose origin, mechanism of action, effects on living organisms were not known, finding effective methods of treatment or prevention was a real challenge and thus led to an increase in pressure on the health system. Military medicine was an important point of support, because the vast experience of military doctors in field medicine led to the construction of the first ROL2-COVID19 Level Campaign Hospital, located within the National Institute of Gerontology and Geriatrics "Ana Aslan”. It was the first functional modular hospital in the country, dedicated exclusively to treating military and civilian patients infected with the new SARS-cov-2 virus. During the activity carried out, from the design to the present, there have been numerous challenges represented by the vagaries of the weather or the lack of sometimes the appropriate legislative settlements. All these impediments have been overcome due to a close cooperation between the medical staff and the MApN structures, which continues today.

Medical personnel are constantly exposed to the risk of contamination with viruses, bacteria or parasites. This risk is accentuated during a global pandemic, thus increasing the predisposition for the development of burnout syndrome in health personnel. The fast pace of work, special conditions (requiring special protective equipment), the multitude of uncertainties since the beginning of the pandemic regarding the mode of transmission, the effectiveness of treatments and prevention methods have created an additional level of stress and anxiety for medical staff.

Therefore, overcoming this war in peacetime is possible only through a collective effort, with medical staff at the forefront, who try to save as many lives as possible and stop the attack of this small and cunning virus.

Medical 1

The many faces of acute distal ischemia – are we thinking outside of the box?

M. Casian, I.T. Ionita, L. Chiriac, A.E. Munteanu

Introduction – Acute distal ischemia demands an accurate and timely diagnosis. When the surgical act can be delayed, it is vital to identify the real pathophysiological mechanism. Acute ischemia with distal pulse is a particular clinical entity, with a variable prognosis depending on the substrate, being associated with rheumatological (vasculitis), hematological (multiple myeloma, Waldenstorm macroglobulinemia), and even infectious (COVID finger) disorders.

Material and method – We present the case of a 67 year-old female patient, with multiple cardiovascular risk factors (hypertension, dyslipidemia), with a history of extensive carotid atheromatosis, multiple ischemic transitory attacks, cerebral lacunarism and recurrent syncopal episodes, who develops progressive necrosis of the distal phalanx of the 3rd left finger. The physical exam reveals a normal radial and ulnar pulse in both hands. Given the patient's history, the following mechanisms are considered: extensive atheromatosis or cardiac embolism (paradoxical, atrial fibrillation, valvular vegetation). However, the differential diagnosis will include other extra-cardiac potential pathologies.

Result – The ECG and 72 hour continous ECG monitoring did not reveal any particular aspects, such as atrial fibrillation, pathological pauses or other major arrhythmia. Transthoracic echocardiography showed mild left ventricular hypertrophy, with a normal ejection fraction, no significant valvular disease and no pulmonary hypertension. Transesophageal echocardiography did not reveal any intracavitary masses or valvular vegetations and the interatrial septum was intact. Laboratory tests showed marked dyslipidemia, severe inflammatory syndrome and positive COVID19 antibodies, in the absence of any documented infection. Selective angiography of the left upper limb confirmed the normal flow, while no lesions were identified. Serum protein electrophoresis was. The tilted table test was negative. The necrotic area expanded despite the anticoagulant treatment. The patient required amputation of the distal phalanx. The histopathological exam was not specific, revealing areas of inflammation and thrombosis.

Discussions – Therefore, we are faced with the case of an acute distal ischemia in a patient with multiple cardiovascular risk factos, in which no embolic sources are identified. We are considering COVID finger as a plausibile diagnosis, given the positivity for COVID19 antibodies. However, their presence may be purely coincidental. After amputation, a new area of necrosis appeared on the ipsilateral index finger. The antinuclear antibodies complete profile revealed high titers of anti-centromere antibodies. Hence, we formulated the diagnosis of systemic sclerosis. However, the patient lacked the involvement of other organs (lung, esophagus), or other clinical elements at the time of diagnosis (Raynaud syndrome, pulmonary hypertension). The patient began treatment with iloprost with a favourable course. At around 4 months after the diagnosis, the patient developed other clinical elements (Raynaud sydrome, hardening of the skin).

Conclusions – The particular aspect of the case consists in the fact that the distal ischemia was the first manifestation of systemic sclerosis, even though we initally expected another cause, such as atheromatosis or cardiac embolism. Furthermore, the patient was found to have COVID19 antibodies. False positive results have been reported in patients with autoimmune diseases (such as vasculitis or antiphospholipid syndrome). Despite the correct diagnosis, the initial area of necrosis expanded and the phalanx had to be amputated. However, the second finger affected by a similar necrotic lesion was saved with the adequate therapy. Therefore, when faced with acute ischemia with distal pulse it is mandatory to consider other potential causes, since the prognostic of the affected segment is related to the primary disease.

Opioid treatment of pain

C. Calin

In current medical practice, pain is a common morbidity, regardless of specialty. The correct treatment of pain can be done only through the extensive knowledge of pain syndrome and therapeutic options. This paper aims to concisely present the basics needed for effective pain therapy.

Pain assessment is the first step in treatment and is done through several measuring instruments, applied recurrently, given that pain is a subjective symptom. The most commonly used is the visual analog pain scale. Depending on this, pain is classified as mild pain (VAS <4), moderate pain (VAS 4-6), severe pain (VAS 7-10). Depending on the severity of the pain, analgesics will be chosen, taking in consideration the classification as stage I analgesics (neopioids, eg NSAIDs, paracetamol), stage II opioids (eg codeine, tramadol) and stage III opioids (morphine, fentanyl).

Besides the severity of the pain, the prescription of the correct dose of opioids is made taking into account the patient's comorbidities, the pharmacology of the drug, the side effects, and the route of administration and last but not least the legal provisions (law 339/2005).

The correct choice of opioid and its correct administration decreases the human resources, emotional and economic burden, both of the patient and of the medical system.

Coffee: healthy or not?

A.E. Ranetti, I.D. Cojocaru

Coffee, green tea or energy drinks? Many times, these are consumed to increase wakefulness and work productivity thanks to their high content in caffeine. Besides coffee and tea, there is a variety of plants that contain caffeine (for example cocoa beans), but it can also be synthesized and added to foods and beverages, making caffeine the most widely consumed psychoactive agent.

Coffee and tea are an important part of people’s traditions and socializing. Therefore, there were numerous concerns regarding the risk of cardiovascular disease and cancer associated to the consumption of coffee. Multiple studies have been conducted since, searching the effect of coffee on different organs and systems and the mortality associated. A key element of coffee is the content of hundreds of biologic active phytochemicals, but also modest amounts of vitamin B3, potassium and magnesium, elements with a favorable effect on the organism. Coffee is involved in glucose and lipidic metabolism, improve the gut microbiome and can reduce oxidative stress.

By binding to the adenosine receptors, caffeine has multiple effects on the brain: it increases vigilance and mental performance, it may reduce the risk of depression and amplify the analgesic effect of NSAIDs used in the treatment of headaches and other causes of pain. Caffeine is said to reduce the risk of Parkinson disease. On the other hand, caffeine can lead to insomnia and can induce anxiety, especially when consumed in high doses and by susceptible persons. Interpersonal differences in the effects of caffeine on sleep and anxiety are large. In addition, there are several medications from a range of drug classes that can reduce caffeine clearance, increasing its half-life because they are metabolized by the same liver enzymes. The absorption of caffeine is nearly complete within 45 minutes after ingestion and the peaked levels are present after 15 minutes to 2 hours. Then, in the liver, caffeine is metabolized by cytochrome P-450 enzymes.

It is proven that quitting caffeine can lead to withdrawal symptoms that include headaches, fatigability, and depressed moods as well as influenza-like symptoms. There appear at approximately 24-48 hours after cessation of intake and can persist up to 9 days. Caffeine can also induce toxic effects, when consumed in very large amounts (up to 75-100 standard cups of coffee) in a short time for the dose to be fatal.

Regarding the cardiovascular risk, it was observed that consuming caffeine can have a protective role. The ingestion of coffee is not associated with an increased risk of arterial hypertension in the long term, though in the short-term caffeine intake raises epinephrine levels and blood pressure. An inverse association has been observed between coffee consumption and coronary artery disease. Cohort studies have demonstrated there is no association between caffeine and atrial fibrillation. However, unfiltered coffee and espresso-based coffee can aggravate dyslipidemias by increasing the levels of LDL-cholesterol, predicting an estimated 11% higher risk of major cardiovascular events. In contrast, this risk was not demonstrated in persons that consume filtered coffee, so overall limiting consumption of unfiltered coffee may help control serum cholesterol levels.

From a metabolic point of view, caffeine improves the energetic balance by reducing the appetite and by increasing the basal metabolic rate and food-induced thermogenesis, probably through stimulation of the sympathetic nervous system. Furthermore, there are studies which proved that coffee consumption has been consistently associated with a reduced risk of type 2 diabetes, both for caffeinated and decaffeinated coffee.

Caffeine also has effects on the liver: it reduces the risk of fibrosis, cirrhosis, the risk of gallstones and liver and gallbladder cancer, with a stronger association for caffeinated coffee than for decaffeinated one, implying that caffeine may be the element responsible for the protective role. Like mentioned above, coffee may prevent cholesterol gallstone formation by stimulating gallbladder contraction and by increasing the cholecystokinin secretion.

On the kidneys, high doses of coffee can have a diuretic effect, but without affecting the hydration status.

Although it was initially disputed, consumption of coffee is not associated with mortality caused by cancer. On the contrary, the intake of coffee slightly reduces the risk of melanoma, nonmelanoma skin cancer, breast cancer, prostate cancer, and endometrial cancer. For the last one, the associations were similar with both caffeinated and decaffeinated coffee.

However, coffee consumption also has some risks. For example, while pregnant, caffeine intake, regardless of its origin, has been associated with lower birth weight and a higher risk of pregnancy loss. Caffeine can pass the placenta and by increasing blood levels of catecholamine levels in the mother and fetus, induce uteroplacental vasoconstriction and therefore hypoxia. Coffee drinkers are more likely to smoke, while energy drinks, energy shots and supplements based on caffeine bring an exaggerated contribution of glucose.

Though there is an interpersonal variability when regarding the quantity of coffee consumed and the times needed for its metabolization, for the majority of adults who are not pregnant, lactating or do not have specific health conditions, coffee consumption can be part of a healthy lifestyle.

Impact of homocysteine level on carotid intima-media thickness in rheumatoid arthritis patients with anti-TNF therapy

D. Anghel, O.G. Petrache, E. Busuioc, A.R. Bursuc, A.E. Manolache, V. Smedescu, C. Jurcuț, I. Copaci, C.A. Sîrbu

Objective: To assess if anti-TNF therapy and homocysteine level influence carotid intima-media thickness in rheumatoid arthritis patients.

Methods: Assessments were realized to determine the evolution of ITM and homocysteine levels at patients with biological treatment from 64 patients with RA. The group of patients was divided into approximately three equal parts, each being treated with a different biological product, respectively adalimumab, etanercept, and infliximab.

Results: In the 3 groups, after 12 months of anti-TNF alfa therapy IMT2 progression was significantly reduced compared to baseline. There weren't significant differences between the three groups of treatment. A strong correlation was observed between IMT1-IMT2 in Etanercept group ($p<0.001$, $r=0.758$) in Adalimumab group ($p<0.001$, $r=0.761$) and in Infliximab group ($p<0.001$, $r=0.829$).

The low level of Hcy2 after 12 months of anti-TNF alfa therapy was significantly correlated with a decrease in ITM2 ($p<0.001$) on patients who had a high level of Hcy and ITM>0.9 mm at baseline.

Conclusion: Biological treatment and low level of homocysteinemia reduces the cardiovascular risk in RA, regardless of the treatment chosen (infliximab, adalimumab or etanercept). Results from this study support the use of carotid ultrasonography as a fast and easy tool for the prediction of cardiovascular events in rheumatoid arthritis patients.

Just an end stage of a common disease? A case presentation

M. Iordache, O. Stancu, O. Ionescu, Stoicescu G.D., Anghel A., Ciobica M.L.

There are three acquired thrombophilia that carry a high risk of arterial and venous thromboembolism. These acquired thrombophilia are antiphospholipid syndrome (APS) and its variant – catastrophic APS, heparin induced thrombo-cytopenia (HIT), and paroxysmal nocturnal hemoglobinuria (PNH).

The pathogenesis of thromboembolic disease in APS, HIT, and PNH is complex and includes cellular and/or complement - mediated mechanism.

The treatment of thrombotic events associated with these pathologies, the primary and secondary prophylaxis consist in anticoagulation and had some particularities. The gold standard of treatment in APS remains vitamin K antagonists (VKA), after initial overlap with unfractionated heparin (UNH) or low molecular weight heparin (LMWH). There are additional options in pregnancy associated with APS and in catastrophic APS that includes the association of aspirin, plasmapheresis, corticosteroids, and hydroxychloroquine to the anticoagulation.

The thromboembolism in HIT must be treated with nonheparin anticoagulant initially and after clinically resolution of thrombosis, we can switch the nonheparin anticoagulant to VKA.

PNH is a rare disease and the most important treatment is the eculizumab which stops the pathogenic mechanism. The anticoagulation treatment such as LMWH, of venous or arterial thrombosis associated with PNH must be started immediately and can be switch to VKA after clinically improvement.

Direct oral anticoagulants (DOACs) did not show a good effectiveness in this kind of pathologies, and their use is reserved for low risk acquired thrombophilia in prevention of thrombosis. Further studies are needed.

Inflammatory bowel disease in a young man with primary immunodeficiency and arthritis – therapeutic challenges?

M. Casian, A. Șerban, D.M. Chițimuș, A. Manolache, D.V. Balaban, C. Jurcuț

Introduction – Primary immunodeficiencies (PID) are a rare group of diseases characterized by an increased susceptibility to infections, as well as an increased frequency of other diseases, including autoimmune diseases.

Material and method – We present the case of a 24-year-old male patient from our adult PID cohort. He has been diagnosed with PID since childhood and has been receiving immunoglobulin replacement therapy (IRT) periodically, as well as long-term low-dose corticosteroid therapy and methotrexate for reccurent arthritis of the right knee, which was initially labelled as JIA. He was admitted to our department with digestive symptoms (abdominal pain, diarrhea) and systemic symptoms (fever, weight loss, fatigue). Clostridioides difficile infection was diagnosed and treated accordingly. After a period of symptomatic remission, the digestive symptoms recurred, including sanguinolent stools. The Clostridioides difficile tests were negative, the fecal calprotectin levels were increased and the lower endoscopy revealed ulcerative lesions in a skip-lesion pattern. The histopathological features of the biopsies were characteristic of Crohn's disease. The patient was started on adalimumab with a marked improvement of the digestive and articular symptoms, as well as the normalization of the inflammatory markers.

Conclusion – Cases of PID complicated by Crohn's-like disease have been previously described in the literature. Articular involvement in Crohn's disease is well documented. However, it is unusual for arthritis to occur years before the digestive symptoms, as in our patient. Several similar cases have been described in the literature and treatment with adalimumab seems to be the method of choice in addition to IRT and other immunosuppressant drugs.

A diagnostic and therapeutic challenge

B.A. Sandulescu, O. Ionescu, G. Stoicescu, A. Anghel, O. Stancu, M. Iordache, L.M. Ciobica

Introduction: Nonspecific symptoms are often a main reason of presentation in the Internal Medicine departments. We would like to present you a case that for a month was searching a diagnosis and our fulfillment, as internists, was to establish it quickly and correctly, in a race for life.

Case presentation: A 39-year-old man was admitted to our clinic with a 1-month history of dyspnea, pain in the left hypochondrium, intermittent fever, marked physical asthenia and weight loss (~ 10 kg). The patient was previously pneumological and hematological evaluated and, after multiple investigations, related diseases of these specialties have been disproved. The clinical examination shows pallor, tachycardia and marked dyspnea. Biologically, a significant inflammatory syndrome, leukocytosis with neutrophilia, cholestasis and mild normochromic anemia. ECG and heart-lung X-ray without significant changes, but the abdominal ultrasound shows acute calculous cholecystitis, splenomegaly and a hypoechoic splenic area of ~ 6 cm located subcapsularly, suggestive appearance for a splenic infarction. In this context, an emergency cardiac ultrasound was performed highlighting a bicuspid aortic valve with a vegetative formation of ~ 2 cm and severe aortic regurgitation. 4 sets of blood cultures were taken, 2 worked in the ultra-fast system, and empirical broad-spectrum antibiotic therapy was initiated. Despite the favorable clinical evolution, cardiac ultrasound reveals the progression of the valve lesion so emergency surgery was decided and the best option for the patient was forming a multidisciplinary surgical team with sequential removal of the gallbladder, spleen and later, aortic prosthesis intervention. The postoperative evolution was slowly favorable.

Conclusion: This case emphasizes a life-threatening condition and the importance of a good interdisciplinary collaboration, both with medical specialties (cardiovascular laboratory, microbiology laboratory) and surgical ones (general surgery, cardiovascular surgery) from diagnosis to the optimal treatment and moment of intervention.

Management of acute upper gastrointestinal bleeding in COVID 19 patients: SUUMC experience

M. Grigore, R.S. Costache, A. Gavrila, M. Chirvase, I. Enache, A. Popescu, B. Macadon, V. Balaban, M. Patrasescu, P. Nuta, F. Ionita Radu, M. Jinga, S. Bucurica

Introduction: The therapeutic approach of upper gastrointestinal bleeding during COVID pandemic represented a real challenge both in terms of the urgency and the lack of previous protocols to guide the conduct of medical staff.

Methods: This study carries out a retrospective analysis of COVID 19 (+) patients with upper gastrointestinal bleeding that received endoscopic emergency treatment between March 2020 and June 2021 at SUUMC. The study aims to realise a characterization of the types of lesions encountered in COVID 19 (+) patients, as well as an analysis of the number of interventions, the type of hemostasis and the protocol followed within the SUUMC to minimize the exposure of medical personnel at the time of endoscopic intervention.

Results: The types of lesions encountered did not vary significantly compared to the general population.

Conclusion: Upper gastrointestinal bleeding may be among the clinical presentation modalities of COVID 19 patients, the causal relationship between the 2 still being studied. Management of upper digestive bleeding in COVID 19 (+) patients requires a careful selection of patients in order to minimize the exposure of medical staff to a procedure that generates an increased amount of aerosols and to maximize the risk-benefit ratio for patients.

Endoscopic ultrasound during the COVID-19 pandemic

Condrache V., Balaban D.V., Bucurică S., Pătrășescu M., Macadon B., Popescu A.L., R.S. Costache, Nuță I.P., Ioniță-Radu F., Jinga M.

Introduction – The COVID-19 pandemic has led to significant changes in endoscopy services, with upper endoscopic digestive explorations being aerosol-generating manoeuvres. The aim of the present research was to evaluate the practice of endoscopic ultrasound (EUS) in our service during the COVID-19 pandemic.

Methods – We conducted a retrospective analysis of endoscopic explorations performed in our clinic during the first year of the COVID-19 pandemic (03/2020 - 03/2021) compared to the same period of the previous year (03/2019-03/2020) in terms of number of procedures, indications and procedure quality indicators.

Results – The number of EUS examinations decreased by 32% during the COVID-19 pandemic, mainly on account of the first 2 months of the pandemic (275 vs. 187). Excluding the state of emergency period, the decline was 15% during the pandemic period compared to the previous year. In terms of indications for EUS examination, we found an increase in the frequency of biliopancreatic tumour pathology during the pandemic compared to the previous period (51.3% vs 47.65). There was no difference in the rate of periprocedural complications.

Conclusions – Despite the restrictions caused by the COVID-19 pandemic, EUS practice was carried out under similar conditions to the previous year, with an increase in the frequency of biliopancreatic neoplasms.

Retrospective study on immunomodulation strategies used in patients with severe and critical forms of Covid-19 disease treated in the ICU department of the Role 2 Military Hospital

Tănase N, Asandi R, Tuță A, Șerbu A, Filimon C, Ipate D, Chițoi D, Simion D, Avram A, Moisa E, Corneci D

Introduction: Immunomodulatory treatments used in the management of severe and critically ill patients with COVID-19 disease have been shown to reduce mortality in these patients, in whom the extent of the cytokine storm correlates with the severity of multisystemic impairment.

Study hypothesis: To what extent the immunomodulatory therapies used in the ICU of the ROL 2 Military Hospital have improved survival in COVID-19 patients with hyperinflammatory forms of the disease.

Material and method: We undertook a retrospective analysis of all patients (n = 510) hospitalized in the ICU of MH ROL 2 during 28.04.2020-10.07.2021. Severe and critical forms of COVID-19 disease have been defined according to the recommendations of the MoH.

Patients receiving immunomodulatory therapies were divided into 5 categories: (1) dexamethasone standard dose 6 mg, (2) dexamethasone high-dose, (3) methylprednisolone, (4) tocilizumab plus corticotherapy (either dexamethasone or methiprednisolone), (5) anakinra plus corticotherapy. The main outcome of the study was hospital mortality; the secondary objectives were to assess the length of stay in ICU for each category of patients.

Results: Of the 510 patients analyzed, 477 met the inclusion criteria. The mortality recorded among the patients treated in the ward was 47%. The lowest mortality was reported in the tocilizumab plus corticosteroid group. The length of stay in the ICU did not show statistical differences among the five groups.

Conclusions: In this retrospective cohort study performed in patients with critical and severe forms of COVID-19 disease treated in the ICU of MH ROL 2, the combination of tocilizumab-corticotherapy was shown to provide superior survival compared to corticosteroid therapy (monotherapy) or anankira-corticotherapy combination.

Hemostasis disorders associated with SARS-CoV-2 infection in intensive care patients

Latiș M, Zaciu A, Zahiu V, Apalaghiei T, Mirciulescu C, Vișanu L, Popa Ș, Moisa E, Corneci D

Introduction: An important feature of COVID-19 is the high frequency of thrombosis, particularly in patients who require admission in ICU units due to respiratory failure. The physiopathology of the disease in not completely understood, but the hyperinflammatory state plays an important role in the endothelial disfunction and thrombotic complications.

Although the mechanism of thrombosis is unclear, anticoagulation with high doses of heparin has been proposed for these patients. Heparin induced thrombocytopenia (HIT) is a drug-associated complication more frequently observed in COVID-19 patients, one of the possible causes of the high incidence could be the immune dysregulation associated with COVID-19 disease.

Objective: The objective of this study was to analyze the incidence of Heparin Induced Thrombocytopenia in critically ill COVID-19 patients from our population.

Methods: We retrospectively reviewed all cases of HIT among COVID-19 ICU patients hospitalized in our ward during April 2020 to July 2021. Diagnosis was confirmed by the presence of antibodies to PF4/Heparin. We compared the HIT positive population to the HIT negative population by sex, BMI, exposure to unfractioned heparin or low-molecular weight heparin and mortality.

Results: From all the 477 hospitalized COVID-19 patients studied 35 were HIT positive, thus the incidence of HIT in our population was 7.33% compared to 0.1-5% in non-COVID-19 ICU patients. The percentage of patients exposed to unfractioned heparin was 54.28% in HIT positive patients compared to 35.06% in HIT negative population. There were no differences between the two populations in terms of BMI and gender. Having in mind that HIT is a severe life-threatening complication, the mortality in the HIT positive population was 80% compared to 44.3% in HIT negative population.

Conclusion: Thrombocytopenia and thrombosis are commonly observed complications in ICU patients. Rapid detection of antibodies to PF4/heparin is necessary in patients with COVID-19 to avoid misdiagnosis.

Microbiological profile of patients with SARS-CoV-2 infection hospitalized in the ICU I and ICU Role 2 wards of SUUMC between 1 May 2020-30 June 2021

Zahiu V, Latiș M, Apalaghiei T, Mirciulescu C, Vișanu L, Popa Ș, Ruscu C, Costan C, Moisa E, Corneci D

Corticotherapy and severe forms of SARSCov-2 disease are risk factors for opportunistic bacterial infections. We performed a retrospective study of the critical patients treated in ICU 1 and ICU ROL2 wards in 1 May- 30 June 2020, studying the bacterial cultures found in the hospital data base , the prevalence of certain risk factors(such as age, obesity, certain treatments), some biologic parameters related to infection-leucocytosis, inflammation factors.

We have gathered information of 477 patients, of whom 199 had at least one bacteria determined. The information suggestive for colonization/contamination. There has been a high prevalence of Gram-negative bacteria (Acinetobacter baumanii 28.7 %, Klebsiella pneumoniae 26.4%, Pseudomonas aeruginosa 18.6%,, rarely Providencia and other species) and a smaller one for Gram-positive (MRSA 6.9%). Fungal infection was determined predominately by Candida albicans species. Most often (39% of total infections) were pulmonary-detected germs (39%), followed by hemocultures (25.7%).

Bacterial infections, mainly Gram-negative, are an important factor of morbidity and mortality for SARS CoV-2 patients. Prevention and targeted antibiotic treatment are deciding factors for the evolution of the patients.

The psychological impact of the Covid-19 pandemic on ICU staff in SUUMC

Zahiu V, Zaciu A, Chițoi D, Zamfir D, Corneci D

Burn-out in a syndrome of exhaustion, increased mental distance from one’s job and reduced professional efficacy, resulting from chronic workplace stress. Psychologists suggest in recent studies an epidemic of burn-out and post-traumatic stress for health-caregivers implied in treating COVID-19 patients. At present, in Romania, there are only disparate studies (e.g. Timisoara), signaling a high percentage of burn-out.

A questionnaire consisting in 25 questions has been administered to ICU nurses and residents, voluntarily and anonymously, as an electronic sheet, in June-July 2021. Each question had a result from 1 to 5. Results have been analyzed statistically, allowing the stratification in different risk levels regarding burn-out.

Results have shown that 12 % of responders are at increased level of burn-out, 47 % are in burn-out and 42% are at real danger with their physical and psychical state.

We need active measures to improve the psychological condition of health-caregivers of critical patients.

Therapeutic difficulties in obese patients with SARS CoV-2 infection – case presentation

Tuță A, Latiș M, Șerbu A, Filimon C, Ipate D, Corneci D

Introduction: Until the beginning of 2019, obesity was already known as an independent risk factor for respiratory infections. Its evolution towards severe form of disease, such as the acute respiratory distress syndrome, contributed the notion of “obesity paradox”, more precisely the risk of death being inversely proportional with the body mass index. However, the data gathered following the pandemic show that pneumonia COVID 19 is more likely to be lethal to overweight or obese patients, who take up a third of the global population.

Case presentation: Patient known with Class 2 obesity comes to the hospital for: dry cough, fever and dyspnea, symptoms which debuted four days ago. Bioumoral: RT-PCR SARS CoV2 positive, inflammatory syndrome, hepatic cytolysis syndrome, degradation products of fibrin increased and lymphopenia. Imaging findings: areas of interstitial infiltration in “matte window” which occupy approximately 40% of both lung fields.

Management and results: We proceeded with non-invasive mechanical ventilation through a high flow nasal cannula, administration of antiviral therapy (Remdesivir), immune-modulatory drugs (Tocilizumab and convalescent plasma) and anticoagulant therapy (Enoxaparin). Due to the high respiratory effort and decrease in the oxygenation parameters we decided in favor of airway prosthesis and invasive mechanical ventilation in a controlled mode, followed by the sedation and neuromuscular blockade of the patient and turning him in prone position. In the 10th day of hospitalization venous-venous ECMO treatment is established. Survival percentage is evaluated at 33%, having the RESP score (Respiratory ECMO Survival Prediction Score) -2. During day 14 the patient dies despite all the medical interventions.

Conclusion: Obesity played a major role in the outcome of this patient. Weight gain is already known for its impact on ventilation, secondary to deformation of the rib cage and high intra-abdominal pressure, but visceral adiposity appears to be associated with severe COVID 19 outcome, through its role of reservoir of inflammatory factors.

Patient with morbid obesity and SARS CoV-2 infection: therapeutic particularities – case presentation

Tudose A, Perju C, Serban T, Bonto D, Popescu M, Corneci D

Obesity represents a major risk factor for patients who develop severe SARS-COV-2 infections. In this presentation we will focus on both mechanical ventilation strategies (non-invasive, invasive, and weaning) and evolutive particularities (inflammatory syndrome rebound on tapering systemic corticotherapy) in a 41 year old patient with morbid obesity.

Viral ARDS followed by bacterial ARDS in a patient with SARS CoV-2 infection – case presentation

Ivașcu R, Pleșa C, Tudorache I, Hostiuc L, Miron M, Silea S, Mătăsaru C, Colesnic L, Corneci D

Treating SARS-CoV-2 virus infection has been a real challenge for the medical system for the last 2 years. A percentage of 5% of diagnosed patients end up in intensive care with the diagnosis of acute respiratory failure.

We herein report a case of a 41-year-old patient with no significant personal pathological history who was diagnosed with SARS-CoV-2 infection, and a week later he presented in our clinic for acute respiratory failure. During the hospitalization, the patient developed two episodes of respiratory distress syndrome, the first being of viral cause and the second of bacterial cause.

After approximately 2 months of treatment in the intensive care unit, the patient's condition improves, being transferred to a medical recovery department.

Pandemic lessons

The patterns of clinical evolution for COVID-19

B. Circiumaru

Introduction: Due to the epidemiological emergency induced by the COVID-19 pandemics, during April 2020 to May 2021, I have performed medically duties to the Infectious Diseases Ward at the ROL-2 Military Field Hospital in Otopeni.

Methods: I have clinically and epidemiologically observed and noticed, hundreds of COVID-19 patients, most of them with medium-severe diseases, describing their clinical evolution patterns.

Results: Most of the inpatients (85%), have had self-limiting disease; 10% of them having had hypoxemia - severe disease, whilst 5% had critical disease (MODS, ARDS, sepsis). Most of the patients presented post-COVID sequelae, needing prolonged/iterative multi-disciplinary admissions.

Conclusions: The acquired experience as well as the COVID-19 clinical evolution patterns description, was helpful to streamline the therapeutical approach, thus providing quality medical care; being an ongoing support for the future.

Pandemic lessons – Infectious Diseases Department experience in the management of COVID-19 infection

I. Ștefan

Introduction: Tuberculosis (TB) is the leading cause of death among people leaving with human immunodeficiency virus (HIV). The main objective of our study was to assess the clinical usefulness of the QuantiFERON®-TB Gold test in diagnosis of active extrapulmonary-TB in HIV-positive and HIV-negative patients.

Methods: We performed a comparative retrospective study on ganglionary-TB in HIV-positive patients versus non-HIV patients in a tertiary hospital from Bucharest, Romania, between October 2012 and June 2018. The main inclusion criteria were the definitive diagnosis of active ganglionary-TB in patients over 18 years old.

Results: A total of 50 patients were eligible for analysis, out of which 33 with HIV co-infection and 17 without HIV. The median age at the time of TB diagnosis was 32 years (IQR, 26.5-37.5) in the HIV-positive group and 58 years (IQR, 29-76) in the non-HIV group (p=0.003). The QuantiFERON®-TB Gold test was positive in 53% (n=9) of non-HIV patients and 27% (n=9) of the HIV-positive patients (p=0.07). In HIV-positive patients with positive QuantiFERON® test the median CD4 count was 230/mm3 (IQR, 178-259) versus 62/mm3 (IQR, 26-152) in those with negative test (p=0.01). The HIV-positive patients had more extensive TB (61% [n=22] had multicentric diseases) compared with HIV-negative patients (18% [n=3]) (p=0.006).

Conclusions: Although the rate of positive QuantiFERON®-TB Gold test in HIV co-infected individuals is lower compared with HIV-negative patients and active TB, it remain an useful tools especially for the diagnosis of latent TB than for active diseases in a population with an increased prevalence of TB.

Evolution of antibiotic consumption in COVID-19 era

C.G. Carp, R.M. Hrișcă, A. Ghiță, E.M. Munteanu

Background: Overuse and misuse of antibiotics is the most important risk factor for antimicrobial resistance. In 2019 WHO published a list of top ten threats to global health, antimicrobial resistance is one of them. This study aimed to compare antibiotic consumption in Central Military Emergency Dr. Carol Davila Hospital in different years.

Methods: For all department in our hospital we analyzed antibiotic consumption in accordance with "ATC/DDD, DDD/100 obd" (Anatomical Therapeutic Chemical/Defined Daily Doses, DDD/100 occupied bed days) methodology supported by World Health Organization.

Results: Results are presented as DDD/100 occupied bed days. Total annual consumption of drugs group J – Antibacterials for systemic use in the evaluated years are increased from 57.815 DDD/100obd in 2019 to 74.696 DDD/100 obd in 2020. The consumption of carbapenems increased from 5.071 DDD/100obd in 2019, to 7.851

DDD/100obd, quinolones increased from 4.457 DDD/100obd in 2019, to 5.216 DDD/100obd in 2020, 3rd generation cephalosporins increased from 18.779 DDD/100obd in 2019, to 21.217 DDD/100obd in 2020, polymyxins increased from 1.054 DDD/100obd in 2019, to 1.575 in 2020.

Conclusion: The obtained data reveals that consumption of antibiotics in the evaluated period increased significantly in 2020 compared with 2019.

Screening of the employees from the "Dr Carol Davila" Central Universitary Military Emergency Hospital for SARS-CoV-2, and monitoring during the infection

R. Hrișcă, A. Rotaru, C. Carp, F. Ioniță-Radu

Introduction: COVID-19 is the name given to the infection caused by the new coronavirus, SARS-CoV-2. The people infected with SARS-CoV-2 can be symptomatic or asymptomatic. Keeping in mind that asymptomatic people discover often the disease by chance, the percentage of asymptomatic people infected is unknown, but according to epidemiological studies that took place, it seems like these people can spread the virus as well.

The Central Military Emergency University Hospital "Dr Carol Davila" (SUUMC) started the screening of the SUUMC personnel at the ending of March 2020, firstly with the people that had direct contact with people that had COVID-19, secondly with the people that were sent to ROLE 2 field hospital, people that came back from vacation, and once the quick tests were internationally recognized, the screening took place weekly to the entire personnel of SUUMC that were not vaccinated, or people that were not naturally immunized. The screening procedure was updated periodically according to intercommunity spread and the capacity of the testing laboratory, according to the type of test used.

Objectives: Calculation of the percentage of SUUMC personnel that got tested with the PCR method or quick antigenic SARS-CoV-2 positive.

Method: The retrospective cohort study for the tested SUUMC personnel through RT-PCR-SARS-CoV-2 or quick antigenic during the period between April 30th 2020 and June 2021. The data has been taken from the Laboratory app for the fore mentioned period and the SUUMC personnel have been selected. 18539 RT-PCR-SARS-CoV-2 tests had been taken on the SUUMC personnel, 17709 of which came out negative, 50 came out inconclusive and 780 of which came out positive. 4525 quick antigenic tests were taken, 4416 of which came out negative, and 109 came out positive. A 30.98% of the employed people were positive for SARS-CoV-2, 8 of which had gotten the infection once again.

Conclusion: Considering the low percentage of natural immunized people, to prevent the spread of the virus on the workplace, there is a big importance to increase the percentage of vaccinated employees, or maintaining the number of tests for the unimmunized personnel when the community spread is high.

The impact of the SARS CoV-2 pandemic on SPIAAM activity

E. Munteanu, C. Carp, R. Hrisca, D. Paun, A. Ruta, T. Marinescu, A. Bratu, N. Ciongaru

The Department for the Prevention of Healthcare Associated Infections (SPIAAM) has been established within the Central Military Emergency University Hospital Dr. Carol Davila, in January 2017, in accordance to the Order of the Ministry of Health no. 1101/2016, and its responsibilities are clearly defined in the previously mentioned Order.

The Endpoint of the study was to identify the impact of the SARS CoV-2 pandemic on SPIAAM activity.

Methods: The direct comparison of the main activities carried out according to the activity plan for two periods: January-December 2019, respectively in January-December 2020.

Results: The study identified a high number of medical information uploaded by SPIAAM on the local website regarding: briefings and procedures, training activities with reference to the proper use of personal protective equipment, the methods for prevention of the air-borne infections, epidemiological triage and investigations. The number of daily and weekly reports to the Ministry of National Defense and the Ministry of Health has increased considerably. The number of approvals solicited by the Army Preventive Medicine Center for the optimum functioning of the Hospital, for the: permanent vaccination center, temporary vaccination centers as well as for the ROLE-2 Field Hospital has increased.

Conclusions: The increase in workload generated an overload of tasks for the employees and the appearance of the burn-out phenomenon.

SUUMC contribution in the Vaccination Campaign

COVID-19 vaccination campaign in Romania: challenges and results

V. Gheorghiță

Introduction: The COVID-19 (coronavirus disease) pandemic caused by the rapid worldwide spread severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has generated the largest public health crisis of the past 100 years, with enormous medical, economic and social consequences. Access to safe and effective vaccines is critical to ending the COVID-19 pandemic. Thus, Romania started COVID-19 vaccination campaign on the 27th of December, 2020.

Objectives: The main objective of COVID-19 vaccination strategy was to ensure a safe and efficient environment for vaccination, as an effective policy to prevent SARS-CoV-2 infection in order to achieve heard immunity level estimated at more than 70% of the Romanian population. The secondary objectives were as follow: supporting the implementation of vaccination strategy at the highest political level and through implication of all relevant actors in the vaccination activities; increasing the populations awareness regarding the benefits, risks and importance of the vaccination; ensuring the supply chain and the distribution system for the vaccines according to different cold chain; identifying the necessary human resources; developing information technology tools for scheduling vaccinations and for registering vaccinated people; ensuring the protection of the personnel involved in the vaccination activities; managing medical waste; evaluating and monitoring the vaccination progress; documenting the lessons learned during the vaccination campaign that could be further useful in other vaccination programs.

Methods: An inter-ministerial committee has been set up to coordinate the COVID-19 vaccination campaign in an integrated manner. The COVID-19 vaccination strategy comprises three phases: the first is addressed to the medical staff, the second addressed to vulnerable people and essential activities and the third for the entire eligible population. We optimized the previous National Electronic Vaccination Register for COVID-19 vaccines. A national electronic system has been developed to ensure a coherent programming process of people for vaccination. There were organised fix and mobile vaccination centres, and subsequent the vaccination process has been supported by the drive-through centres, vaccination in the medical units and general practitioner’s cabinet and mobile vaccination teams, especially for long term care facilities, dialysis centres, rural areas, private economic operators and prisons.

Results: The vaccine storage and distribution network has been established at national level, being created seven regional centres that ensure the appropriate conditions for the cold chain of -80°C, -20°C and +2 - +8°C. Since the beginning of the vaccination campaign, 9.4 million doses of all four types of vaccine authorized in the European Union have been administered. Thus, 4.95 million people have been vaccinated with at least one dose; 96% of those people being fully vaccinated. One third of the eligible population have been vaccinated with at least one dose. The vaccination coverage for medical and social staff is more than 80% of the eligible population; the vaccination rate of residents in long-term care units is higher than 70%; in dialysis centres the vaccination rate is estimated at 70%. Vaccination activities with mobile vaccination teams have been organised in more than 80% of villages.

Conclusions: Currently, we have already implemented several vaccination options and developed a larger vaccination capacity, which could very easily ensure the access of the population to the COVID-19 vaccination.

Musculoskeletal manifestations of covid-19 – clinical experience

D. Anghel, O.G. Petrache, E. Busuioc, A.R. Bursuc, C.A. Sîrbu, A.E. Manolache, V. Smedescu, I. Copaci, C. Jurcuț

Objective: To determine the incidence of musculoskeletal manifestations in patients with COVID-19 infection.

Methods: The study was retrospective and included 35 patients with COVID-19 infection, diagnosed through RT-PCR SARS-CoV-2. Musculoskeletal manifestations were present in 24 patients (68.5%) during and after the infection. The study followed not only the debut but also the duration of the symptoms after the infection.

Results: In our study group, from 35 patients, 24 (68.6%) presented musculoskeletal manifestations and 10% were men. The mean age was 49+11.2 years. We followed the type of musculoskeletal manifestations and the persistence of these after the acute SARS CoV-2 infection. The musculoskeletal manifestations were present as following: 12 patients had costosternal enthesitis (34.3%), 5 patients had arthralgia (14.3%), 5 patients myalgia (14.3%) and 2 patients had bilateral Achillean enthesitis (5.7%).

The persistence of these symptoms after COVID-19 infection showed that costosternal enthesitis lasted 3 months, arthralgia disappeared after 2 weeks, and myalgia disappeared after 2 weeks and Achillean enthesitis after 6 weeks.

All patients presented respiratory symptoms at the onset of the COVID-19 infection. The musculoskeletal manifestations appeared 1-2 weeks after the respiratory manifestations.

We observed a strong correlation between costosternal enthesitis and myalgia ($p<0.001$). All patients were treated with anti-inflammatory and antialgic therapy. 90% of the patients responded. 10% of patients required medium dose of corticotherapy for the remmision of musculoskeletal symptoms.

Conclusion: The most diagnosed manifestation and the longest duration until symptom remission was found in costosternal enthesitis. Arthralgia and myalgia disappeared more quickly than enthesitis. The association between anti-inflammatory drugs and antialgic therapy controlled the musculoskeletal manifestations.

Successful management of post COVID-19 severe recurrent Clostridium difficile colitis, from Vancomycin to fecal microbiota transplantation

B.A. Sandulescu, O. Ionescu, M. Iordache, G. Stoicescu, A. Anghel, O. Stancu, L.M. Ciobica

Introduction: In the last year, the COVID-19 experience has brought us many unknowns, related both to the disease itself but also to its complications. One of these is Clostridium Difficile colitis.

Case presentation: A 61-year-old patient presented to our clinic with diffuse abdominal pain and persistent acceleration of intestinal transit. He had a recent medical history of SARS COV 2 infection (a month ago) with subsequently severe Clostridium difficile colitis. Recurrence of digestive symptoms was 7 days ago, with no response to oral Vancomycin 3 days before presentation. At clinical examination, we saw a patient severely dehydrated, with an enlarged abdomen, sensitive to palpation and respiratory distress. The laboratory findings showed severe sepsis, marked inflammatory syndrome, metabolic acidosis, hypoalbuminemia and renal failure. CT scan showed a wide colon with thickened wall, suggestive appearance for pancolitis. After serial blood cultures were collected, aggressive volume resuscitation and antibiotic therapy with Vancomycin, Metronidazole, Tygacil and Rifaximin were instituted as a matter of urgency. Despite the partial improvement of biological parameters, diarrhea persisted. Three days later, surgical exploration is performed with drainage of the peritoneal cavity and preserving for the moment the integrity of the digestive tract. We escalated the therapy by adding Fidaxomycin and administrating intravenous Immunoglobulin. The evolution was undulating, partially favorable, so it was decided to perform the recolonization of the digestive tract, material provided by Matei Bals Institute. After this, the evolution was clearly favorable.

Conclusion: A case of severe recurent colitis in which the classical therapeutic algorithms were not enough, requiring a prompt and aggressive approach, the administration of immunoglobulins and culminating with the fecal microbiota transplantation, performed for the first time in our hospital, in collaboration with Matei Bals Institute.

Management of severe COVID-19 pneumonia in a patient with serial negative RT-PCR for SARS COV2

Ionescu O.P., Stancu O.F., Sandulescu B.A., Iordache M., Anghel A., Stoicescu G.D., Ciobica L.M.

The diagnosis of SARS COV2 infection is mainly based on the positivity of the real-time PCR test, but there have been a lot of false negative tests that have made the management of the COVID-19 disease difficult.

We report the case of a 66-year-old woman, hospitalized in our department for dyspnea, low fever, chills, dry cough, fatigue and loss of appetite for the last 3 days. She did not mention any contact with positive patients before coming in our hospital. Clinical laboratory examinations showed significant inflammatory syndrome, normal values of lymphocytes, negative procalcitonin, multiple RT-PCR tests and negative antibodies against SARS COV2. The multiple bilateral pulmonary opacities shown on the chest x-ray suggested SARS COV2 pneumonia. Computed tomography showed pulmonary consolidation with ground glass opacity involving approximately 80% of the lung parenchyma with an aerial bronchogram on the upper and lower lobes of the right lung which confirmed the diagnosis of a viral pneumonia.

Even if the samples of nasopharyngeal/oropharyngeal exudates were repeatedly negative, a presumed diagnosis of COVID-19 disease was made. Thus, the patient was isolated and treated with oxygen therapy, antibiotics, anticoagulants in curative doses, corticosteroids, antivirals, probiotics and proton-pump inhibitors. The evolution was slowly favorable, with an improvement in clinical symptoms, a decrease in oxygen demand and resorption of the infiltrates on the control computed tomography. On the 6th day of hospitalization, to confirm the suspected diagnosis, our patient was tested for serological antibodies. Both IgM and IgG SARS-COV-2 antibodies showed serological positive results.

Complications of SARS CoV2 infection found in the Pulmonology Department

I. Erhan, I. Stefanescu, E. Firoiu, I. Jascu, C. Ionita, C. Popovici, G. Derscariu

In the Pulmonology Department, severe cases of SARS CoV2 infection were treated since the beginning of the pandemic, cases that associated respiratory failure. 3 categories of complications were reported: Various infectious complications, occurring against the background of low immunity on the one hand of the infection and on the other hand of the immunosuppressive treatment (corticosteroids, immunotherapy); complications related to coagulation disorders, either states of hypercoagulability that led to various thrombotic events (pulmonary thromboembolism, stroke, etc.), or hypocoagulability, which led to spontaneous intramuscular hematomas; The third category of complications was related to the appearance of spontaneous pneumomediastinum and pneumothorax, unrelated to noninvasive ventilation. Most of the patients who presented such complications had an unfavorable evolution.

The value of bronchoscopy in times of COVID 19 pandemic

I. Erhan, I. Stefanescu, E. Firoiu, I. Jascu, C. Ionita, C. Popovici, G. Derscariu

The diagnosis of COVID-19 is mainly based on typical symptoms, history of exposure to an infected person, bilateral “ground glass” infiltrates on chest X-rays or CT scan, biological inflammatory syndrome and positive serology (IgM and/ or IgG) for SARS COV2.

Usually it is confirmed by a positive nucleic acid test for SARS-CoV-2 from oropharyngeal and nasopharyngeal swabs.

However, it remains a category of patients with high suspicion based on clinical, imaging and biological findings, with more than one negative nasal/oropharyngeal swab.

In this situation, it is important to decide if it is an active SARS-COV2 infection with great risk for public health or it is an alternative diagnosis which may require a patient-appropriate treatment.

Bronchoscopy is an important tool that we have used in patients with COVID19 suspicion to perform bronchial wash or bronchoalveolar lavage which were analyzed for SARS-CoV-2 using RT-PCR.

In the last year (April 1st 2020 and May 1st 2021) we performed bronchoscopy with bronchial wash in 46 patients with high suspicion and two negative RT-PCR SARS-CoV2 tests (nasal and oropharyngeal swabs or sputum and a nasal and oropharyngeal swab).

We found:
- 19 patients with two negative RT-PCR SARS-CoV2 tests and also negative serology (IgM and Ig G for SARS-CoV2). In 7 patients (36.84%) the bronchial wash was positive for SARS-CoV2, using RT-PCR technique
- 15 patients with two negative RT-PCR SARS-CoV2 tests and positive serology for SARS-CoV2 (only IgM, only IgG or both IgM and IgG). All patients with IgM antibodies had positive RT-PCR test from bronchial wash. All patients with IgG antibodies and 80% of those

patients with IgM and IgG antibodies had negative RT-PCR test from bronchial wash.

In the same period of time we perform bronchoscopy in ICU Department (ATI-COVID19 Role 2) in 19 patients on mechanical ventilation due to COVID ARDS.

The main indications were:
- Clinical and imaging suspicion of obstructive atelectasis;
- Suspected concomitant lower respiratory tract infections;
- Suspected tracheal lacerations in patients mechanically ventilated with subcutaneous emphysema and/or pneumomediastinum;
- Hemoptysis;
- Tracheostomy complications.

Conclusion: To our knowledge, this is the largest number of bronchoscopies performed in Romania in order to confirm or rule out SARS CoV2 infection in patients with positive or negative antibodies, two previous negative RT-PCR test (nasal and oropharyngeal swabs or nasal and oropharyngeal swab and sputum) and high clinical, imaging and biological suspicion.

Also, we are leaders in performing bronchoscopies in ICU COVID-19 patients, thick hypersecretion with risk of atelectasis being the most common complication encountered.

There was no infection in medical team (doctor or nurse) due to bronchoscopy in COVID 19 patients.

Bronchoscopy with adequate precaution and standard protocols could be useful, in selected cases, for the diagnostic of SARS-COV2 infection and management of complication in critically ill, mechanically ventilated patients with COVID-19.

High-flow oxygen therapy and noninvasive ventilation for severe COVID-19 cases

Jascu I., Stefanescu I., Ionita C., Firoiu E., Popovici C., Erhan I., Derscariu G.

The pandemic period, in the last year and a half, was a very difficult period for the health system, with many attempts and sacrifices, especially for the medical staff in the front line of an unequal fight with this new enemy, which gradually revealed its weapons.

In the time interval November 2020 - May 2021 the Pulmonology Section of SUUMC „Dr. Carol Davila” hospitalized and treated patients with COVID-19 moderate and severe forms with lung damage and hypoxemic respiratory failure.

Operating as a respiratory intermediate therapy department, our section has provided complex respiratory care services such as high-flow oxygen therapy on the nasal cannula (HFNC) or noninvasive ventilation (NIV) for COVID-19 cases with severe and critical evolution.

During this time, our department cared for 179 patients with SARS-COV2 infection, of which almost half needed high-flow oxygen therapy and non-invasive ventilation. We recorded that only about 15 percent of this cases needed intensive care unit (ICU) later.

SARS-CoV-2 infected patients with acute hypoxaemic respiratory failure can benefit from HFNC or NIV outside an ICU. The techniques appear to be safe for healthcare workers and could well liberate critical ICU resources.

Clostridium difficile infection in the patient with COVID 19

Derscariu A.G.

Clostridium difficile is an anaerobic, gram positive, sporulated, non-invasive and opportunistic bacterium. This causes an infection that îs transmitted through the digestive tract, fecal-oral, and the bacterium being present in the feces of symptomatic or asymptomatic patients.

Transmission is made directly from a patient with diarrhea or an asymptomatic carrier to another patient, relatives or medical staff and indirectly through contact with spores (infected surfaces, medical equipement, dirty hands).

The main factors favoring the onset of Clostridium difficile infection are: increased oral antibiotic use, chemotherapy belts, prolonged administration of gastric acid inhibitors, prolonged constipation, excessive diarrhea, surgery on the digestive tract.

The diagnosis of Clostridium difficile infection is based on the presence of diarrhea (more than three unformed stools per day, more than two days, and no other known cause), stool detection of toxin A or B by PCR or culture, Elisa or GDH test. For severe forms, a CT scan of the abdomen can be used to diagnose pseudomembranous colitis and an abdominal x-ray to diagnose toxic megacolon.

Treatment consists of general measures and specific antibiotic therapy. Out of a total of 181 patients confirmed with COVID 19 in the Pneumology Department, 11 patients were detected with Clostridium difficile infection.

The incidence of Clostridium difficile infection increased after administration of antivirals (Favipiravir, Tocilizumab), high-dose corticosteroids, gastric acid inhibitors and antibiotic therapy for associated infections (azithromycin, quinolones, cephalosporins). A patient with a severe form of SARS COV 2 who had initially received Favipiravir, and subsequently needed Tocilizumab, had a favorable clinical course. The rest of the patients received Favipiravir as an antiviral. The evolution of the patients was favorable în 9 patients who had the associated infection with Clostridium difficile, and 2 patients died. It should be noted that approximately 30% of those with COVID 19 had diarrhea, but no Clostridium difficile infection was detected.

Clostridium difficile enterocolitis is and will be a permanent challenge în patients with COVID 19, representing one of the dreaded complications of COVID/postCOVID patients, because it can produce severe diarrheal syndromes and trigger an intestinal disease.

Cardiac arrhythmias and COVID-19 Infection

L. Chiriac, A. Munteanu, C. Patru, V. Indries, L. Demiras, L. Iorescu

Medical triage of the patients admitted into the ED is made on the basis of specific criteria established by the national laws taking into account their clinical condition, stability of vital functions, aggravating potential of their status, the necessity of starting a treatment or to carry out some investigations etc. setting up the priorities of the medical care. There are 5 triage categories/levels that establish patient's waiting time, from zero minutes – code red, to maximum 240 minutes the white code.

Fast-track – a new way for rapid evaluation in ED – Is a new concept that permits receiving the patients outside the ED, evaluation (eg. ECG, topical administrations of medications) and treatment of some types of emergencies (e.g fever, allergic reaction, dental problems).

Massive pulmonary embolism – a frequent and long term complication of COVID-19

Ioniță I.T., M. Casian, Chiriac L., Munteanu A.E.

Introduction – Pulmonary embolism is the presence of a clot in any vascular structure of the pulmonary arterial system, which can be life threatening in the absence of treatment. The origin of the blood clot is most frequently due to the deep vein thrombosis of the lower limbs, but there are other predisposing factors such as those related to vascular wall injury, blood stasis and increased viscosity, as well as major trauma, prolonged bed rest, malignancies or pregnancy.

Material and method – We present the case of a 76-year-old obese and hypertensive female patient who presented with acute dyspnea with orthopnea, atypical chest pain, fatigue and palpitations.

Results – Upon admission D dimer levels were increased while the levels of hs-troponin were normal. COVID-19 antibodies were positive (the patient was not vaccinated), with no history of infection. Echocardiographically, severe tricuspid regurgitation was evident, with right ventricular dysfunction and paradoxical septal movement. Given the high suspicion of PE, emergency contrast CT scan was performed and the presumptive diagnosis of PE was confirmed. No other plausible causes were detected, except for the COVID-19 antibodies.

Discussions – Therefore, a patient with multiple cardiovascular risk factors develops massive PE, without a clear etiology. Although asymptomatic, the COVID-19 antibodies indicate that patient was infected.

Conclusions – The particular aspect of this case is given by the long term life-threatening repercussions of COVID-19, even in patients who were asymptomatic.

Dyspnea in a young patient in the Covid Era

Demiras L., Sebastian O., A.M. Demiras, R. Bolohan, L. Chiriac, C. Grigore

Introduction – Myocarditis is an inflammatory disease of the heart characterized by inflammatory infiltrates and myocardial injury without an ischemic cause.

The most commonly identifiable cause of myocarditis is viral. The viral RNAs of Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV, which are close relatives of SARS-CoV-2, were found in the heart tissues of infected animals, suggestind that these coronaviruses possess cardiotropism.

Material and method – We present the case of a 52 year old obese, hypertensive, diabetic male patient who presented with dyspnea, fatigue and palpitations.

Results – Results of blood tests show elevated levels of lactate, C-reactive protein, and other inflammatory markers. Baseline cardiac enzymes (troponin and N-terminal pro-B type natriuretic peptide – NT-proBNP) were elevated on admission. Also positive Covid-19 antibodies, with history of mild infection, 1.5 month ago, without need for hospitalization.

ECG-Atrial fibrillation. Transthoracic echocardiography showed left ventricular disfunction, depressed ejection fraction, moderat mitral valve regurgitation. From the history of the patient we mention that he had a coronary angiography 2 years ago which was normal, and normal ejection fraction at that time.

Given the high suspicion of myocarditis, do to recent viral infection with Covid-19 the patient had an MRI which is considerd the first tool for noninvasive assessment of patients with suspected myocarditis, thanks to its unique ability to directly image myocardial necrosis, fibrosis and edema.

The MRI comfirmed the diagnostic showing at T2-weighted images mark interstitial edema, besides lower ejection fraction and wall motion abnormalities.

Discussions – Therefore, a patient with multiple cardiovascular risk factors develops on set atrial fibrillation, with symptoms of left-side heart failure and left ventricular dysfunction, depressed ejaction fraction, 1 month after having viral pneumonia with covid 19.

Conclusion – The particular aspect of the case consists in the fact that a patient with preexisting cardiovascular disease that has covid 19 - viral pneumonia with mild symptoms, whitout any need of hospitalization, only at-home specific treatment develops arrhythmia and heart failure within 1 month of contracting the virus.

With current insights into the knowledge of progression of AM to CM or DCM with life-threatening symptoms, we emphasise the importance of a correct diagnosis of myocarditis. This can be done noninvasively with a high diagnostic accuracy, by using CMR.

Cardiac pathology in the COVID era

C. Patru, L. Chiriac, L. Iorescu, Mocanescu E., S. Grigore

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified to cause severe respiratory distress syndrome at the end of 2019 when it was isolated in Wuhan.

COVID-19 is an enveloped positive-stranded RNA virus with nucleocapsid. While it primarily affects the respiratory system, further evidence has shown that it can also damage multiple other organs. Cardiac manifestations may include myocarditis, life threatening arrhythmias, acute coronary syndrome, systolic heart failure, pulmonary embolism and cardiogenic shock. The presenting cardiovascular symptoms include chest pain, dyspnoea, and palpitations.

The proposed mechanisms of myocardial injury based on current knowledge are the direct entry of the virus through the ACE2 receptor into the cell, systemic inflammation, hypoxia, cytokine storm, interferon-mediated immune response, and plaque destabilization.

The reported cardiac injury may be a result of the direct viral invasion of cardiomyocytes with consequent unopposed effects of angiotensin II, increased metabolic demand, immune activation or microvascular dysfunction. Thromboembolic events have been widely reported in both the venous and arterial systems, and have attracted intense interest in the underlying mechanisms. These could potentially be due to endothelial dysfunction secondary to direct viral invasion or inflammation. Additionally, thromboembolic events may also be a consequence of an attempt by the immune system to contain the infection through immunothrombosis and neutrophil extracellular traps. Cardiac arrhythmias have also been reported with a wide range of contributory factors, ranging from direct viral myocardial injury, as well as other factors, including at-risk individuals with underlying inherited arrhythmia syndromes. Heart failure may also occur either as a result of viral cardiac injury, the initiation or withdrawal of certain drugs, or the accumulation of des-Arg9 -bradykinin (DABK) with excessive induction of pro-inflammatory G protein coupled receptor B1 (BK1).

In conclusion, we must carefully follow the evolution of our COVID 19 patients due to the diversity of viral pathogenetic mechanisms and the multiple organ dysfunctions they cause.

Medical 3

New Treatments for Major Neurocognitive Disorder due to Alzheimer's disease

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Background: Active or passive immunization is a new way to approach Alzheimer's disease (AD) treatment and many hopes are related to the possibility of stopping or even reversing the pathogenic processes of this disorder. Antibodies targeting tau protein or amyloid-beta42 have been investigated in the last two decades in both translational and clinical trials, with variable proofs of efficacy and certain concerns about severe neurological adverse events. In June 2021, FDA approved aducanumab using the accelerated pathway for the treatment of AD, a decision that started controversies in the scientific community.

Objectives: To assess the current level of evidence that may support the recommendation for the immune therapy in AD treatment.

Method: A literature review was conducted through the main electronic databases (PubMed, EMBASE, Cochrane Database of Systematic Reviews, Thomson Reuters/Web of Science) using the search paradigm "tau immunotherapy" OR "amyloid-β42 immunotherapy" OR "monoclonal antibodies" OR "active/passive immunization" AND "Alzheimer's disease". All peer-reviewed papers published between January 2000 and June 2021 containing clinical trials, systematic reviews and meta-analyses were included in this review.

Results: Aducanumab is a recombinant human IgG1 antibody targeting high molecular weight soluble oligomers of amyloid-beta and insoluble fibrils, and its efficacy is based on the results of 3 double-blind, randomized, placebo-controlled trials with 3482 patients diagnosed with Alzheimer's disease. AADvac1 and ACI-35.030 are active anti-tau vaccines in early phases of development, while gosuranemab, zagotenemab, tilovenemab, semorinemab, and bepranemab are antibodies targeting tau proteins. Anti-amyloid immunization variants have been also studied, both active- AN1792, CAD106, Lu AF20513, ACC-001, or ABvac40, and passive- bapineuzumab, crenezumab, solanezumab, gantenerumab, lecanemab, and donanemab. The rapport between efficacy and tolerability is not yet convincing for any of these of these investigational products.

Conclusions: Passive and active immunization targeting tau protein and amyloid-beta are continuously developing, but only aducanumab has gathered enough evidence for being release into clinical practice in USA, but not yet for the European Medicines Agency approval.

COVID-19-related psychiatric disorders: A case series

O. Vasiliu, I.A. Alexandru, R.E. Manolache, I.E. Ghenoiu, M. Gionea, F. Gainaru, R.G. Vlaicu, I. Amanolesei, A. Vlaic, D. Bitineanu, A. Andrei

Background: Psychiatric or neurological disorders have been diagnosed in the aftermath of the Covid-19, within six months of infection, especially anxiety and mood disorders, strokes and neurocognitive disorders. Both SARS-CoV-2 infection and the psychological stress associated with the hospitalization may be related to the onset of several psychiatric disorders.

Objectives: To analyze three cases of Covid-19-related psychiatric disorders in patients without prior clinical problems.

Method: Patients admitted in our department were screened for Covid-19 infection in personal history in the last 6 months prior to their admission. Patients who were diagnosed and treated for Covid-19 in the past were further evaluated in order to detect any possible causal relationship between this pathology and their current psychiatric disorder.

Results: The first patient was a female, 44 years old, diagnosed with psychotic disorder, somatic type, after 5 months since her discharge from hospital for Covid-19, her main symptom being the delusion of still being infected with SARS-CoV-2. The second patient was a male, 34 years old, diagnosed with panic disorder without agoraphobia, with onset at 6 months after the resolution of Covid-19 symptoms, and the patient still thought he can suffocate due to unproven respiratory sequelae. The third patient was a female, 76 years old, diagnosed with neurocognitive disorder, possibly related to the Covid-19 disease, after 6 months since the remission of the infectious disease, as she was in a coma for 10 days during the SARS-CoV-2 infection. All patients were tested prior to their admission and during their current hospitalization and were negative for SARS-CoV-2 infection. No significant organic dysfunctions were identified during hospitalization, although from all these patients blood tests

were collected and specific respiratory investigations were performed.

Conclusions: Psychosocial stressors associated with the Covid-19 disease, neurobiological changes possibly related to the SARS-CoV-2 infection, undetermined prior vulnerability and other pathogenic factors may be related to the onset of new psychiatric disorders in patients who overcame Covid-19.

Acute ischemic stroke therapy in the COVID pandemic 19 – The role of the multidisciplinary team

Sirbu C.A., Pleșa F.C., Botezatu A.S.

Introduction: Initiating reperfusion therapy for eligible patients with ischemic stroke as soon as possible, reduces morbidity and mortality, with beneficial effects on quality of life and costs. Studies covering the pandemic period found a reduction of hospitalized cases of acute ischemic stroke and thrombolysis. These cases had a higher NIHSS score on presentation, so they were severe cases. A small but significant delay in intravenous thrombolysis was found, but no significant delay in thrombectomy time metrics.

Method and results: The pre-hospital stage is essential for inclusion in the 4.5-hour therapeutic window. The UPU team, already announced, is waiting for the patient. The door-to-needle time (DNT), used to evaluate the quality of acute stroke care, must be a maximum of 60 minutes. At the same time, blood constants, anamnesis, time of onset, neurological examination, NIHSS score, native brain CT examination to exclude cerebral haemorrhage or stroke mimics, checklist for thrombolytic treatment eligibility, patient/relatives' consent are obtained. In the SUUMCB neurology clinic, in 2019 a number of 21 patients with an average NIHSS score of 11, were thrombolyzed, compare with 17 patients with an average NIHSS score of 14, during the pandemic, between March 15, 2020, and March 14, 2021.

Conclusions: Our data are consistent with those in the literature. Ischemic stroke is considered an emergency as great as acute myocardial infarction, its treatment involving a joint effort of emergency physicians, radiologists, neurologists, laboratory, and interventionists.

Successful thrombaspiration after intravenous thrombolysis for ischemic stroke under anticoagulant treatment – Case report

Dumitru R, Pleșa CF, Caloianu I, Sirbu AC, Botezatu S

Introduction. Ischemic stroke (stroke) is a major medical emergency, being the second cause of death worldwide, but also the leading cause of disability in developed countries. Most ischemic strokes are due to cardioembolism or thrombosis, other less often mechanisms being hemodynamic or vasospasm. Intravenous thrombolysis performed in a 4.5-hour therapeutic window is the first-line indication for ischemic stroke and can significantly improve patients' prognosis. Thrombaspiration and thrombectomy, recently introduced in therapeutic guidelines, preceded by thrombolysis, is the current standard of treatment for large vessel occlusions.

Materials and methods. In the following, we present the case of a 73-year-old patient, known with FiA, undergoing treatment with OAC, who presents to the emergency department for left hemibody paresis, symptoms that began approximately 2 hours before the presentation in the emergency room.

Results. Emergency brain imaging reveals a hyperdensity suggestive of proximal occlusion of the right middle cerebral artery (MCA), so it is decided to perform thrombolysis (ineffective - NIHSS score remaining unchanged), then successfully followed by thrombaspiration.

Conclusions. Thrombolysis is effective in only 1/3 of these cases, and thrombaspiration/thrombectomy is one of the most effective treatments known in medicine, with a success rate of up to 80%. It should not be forgotten that 1/5 of patients still have anatomical difficulties that may make thrombectomy impossible.

Neuroendovascular treatments during COVID-19 pandemic – Case report

Ivan R, Sirbu CA, Plesa CF, Botezatu AS

Background and aims. Internal carotid artery (ICA) aneurysms account for about 2-9% of all cerebral aneurysms. The association between this pathology and ICA stenosis is rare, but not impossible, giving the common risk factors that they share. The treatment possibilities for intracranial aneurysms include the removal of the aneurysm from circulation and the occlusion of the origin artery, together with the aneurysm.

Methods. We present the case of a hypertensive patient, with a history of ischemic stroke and ruptured and embolized ICA aneurysm, with subsequent proximal permeabilization. She presented to the hospital for left persistent parieto-occipital headache, diplopia, balance disorders, and left parietal dysesthesias, with progressive worsening over the last month. The clinical-paraclinical evaluation and the medical history imposed the performance of cerebral arteriography. Results. The images revealed a partially embolized sacciform aneurysm in the cavernous segment of the left ICA, with important recanalization and pre-aneurysmal stenosis, and with irrigation of the anterior cerebral arteries from the right ICA.

Conclusions. We must emphasize the fact that despite the previous aneurysm embolization, its diameter grew, and it became permeable again (a so-called “malignant aneurysm”). This, together with the severe pre-aneurysmal stenosis made the risks of performing a new intervention outweigh the benefits. The best endovascular method is the implantation of a flow diversion stent and not a coiling method, but its risk of immediate thrombosis is too big, considering the local anatomy and vascular stenosis. The particularity of this case was the temporary lack of therapeutical meanings, caused by the overlap of pathologies, leaving the vascular wall vulnerable to the turbulent blood flow.

Advanced Parkinson’s disease management during COVID-19 pandemic – Case reports

Georgescu R, Sirbu CA, Plesa CF, Bucurică SN

Background: Parkinson’s disease (PD) is a chronic progressive neurodegenerative disease, with a negative impact on the quality of life, whose prevalence increases with age. PD is clinically characterized by asymmetrical motor symptoms resulting from dopamine deficiency in striatum.

Orally administered Levodopa, absorbed exclusivelly in the jejunum, is the most effective symptomatic treatment of motor symptoms in PD, focusing on the stimulation of dopaminergic receptors. As the disease progresses, Levodopa-induced motor complications, in particular motor fluctuations and dyskinesia, become gradually more frequent due to delayed gastric emptying and intestinal absorbtion. Represents an efficient therapy option whose main purposes are to increase daily ON time, reduce OFF time and ameliorate dyskinesia and motor fluctuations, thus improving the quality of life.

Methods: We present two clinical cases of advanced Levodopa-responsive Parkinson’s Disease patients during the COVID-19 pandemic. Despite the multiple adjustments of conventional treatment, they did not have adequate control of debilitating motor fluctuations and dyskinesia. Hence, intra-intestinal infusion of Levodopa/Carbidopa gel has been administered.

Results: In the first case, the surgical procedure has been succesfully performed with no complications. Daily ON periods have significantly increased with improvement of motor fluctuations and dyskinesia. Although in the second case the motor symptoms had a good response to enterally administered Levodopa/Carbidopa using a naso-jejunal tube, the surgical intervention was impossible to perform due to an anatomical variation.

Conclusions: This case series ilustrates the benefits and limitations of enterally administered Levodopa/Carbidopa gel therapy, highlighting the importance of multidisciplinary teams in advanced PD treatment.

Multidisciplinary therapeutic approach of the patient with thyroid storm

Ranetti A.E., Dascalu A.N., Ranetti R.C.

Hyperthyroidism or thyrotoxicosis is referring to the manifestations of abnormal high quantities of the thyroid hormones, even if most of patients with thyrotoxicosis do not need hospitalization and generally they have had symptoms months before presentation, there are few patients that present with a decompensated form of thyrotoxicosis called thyroid storm.

The thyroid storm is more frequent among patients with an undiagnosed thyroid pathology that become ill (infections, trauma etc.), who are undergoing non-thyroid surgical procedures or after external beam radiation to the neck. It can also be associated with ingestion of sympathomimetic drugs in a thyrotoxic patient or with accidental overdoses of thyroid hormone.

The mortality rate of the thyroid storm is 7% up to 30%, even when properly treated.

Typically the patient is agitated, delirious and tremulous with flushed skin due to vasodilation. Dehydration and hypovolaemia may also occur due to diarrhoea.

Clinical manifestations almost always include temperature higher than 38 Celsius degrees, rapid sinus tachycardia and tachyarrhythmias, such as atrial fibrillation, and frequently can result in congestive heart failure.

The diagnosis of thyroid storm includes clinical history of thyroid disease, thyroid hormone or iodine ingestion and goiter or stigmata of Graves’ disease at physical examination.

Differential diagnosis for the patient presenting with this symptoms includes: severe infection, neuroleptic malignant syndrome, malignant hyperthermia and acute mania with life-threatening catatonia.

The major lines of thyroid storm treatment are: the therapy of the underlying illness (digoxin, diuretics, antibiotics, IV fluids with B-complex vitamins and insulin for the patients with diabetic ketoacidosis), supportive care (cooling blanket, antipyretics) blocking the peripheral effects if thyroid hormone (beta-adrenergic blocking drugs such as Propranolol, Metoprolol etc.) and inhibition of thyroid hormone synthesis and release (Propylthiouracil, Methilmazole).

Ultrasound of salivary glands in patients with Sjögren's syndrome: the role of inflammatory markers

A. Mihai, D. Opris-Belinski, D. Anghel, C. Jurcut, R. Ionescu

Background – Echographic evaluation of the salivary glands in patients with Sjogren's syndrome (SS) is a necessity nowadays. However, predictive parameters associated with the severity of echographic features are still incomplete evaluated.

Objectives – The aim of this study was to evaluate the predictors for severe echographic involvement in patients with SS followed in a tertiary care center.

Methods – We included 63 patients with SS (mean age: 52.3±11.9; 59 female). The complete laboratory workup, clinical manifestations and treatment were reviewed and the EULAR Sjögren's syndrome disease activity index (ESSDAI) was calculated for each patients. We performed the standard echographic evaluation of salivary gland in all patients and used a 4 grade system for severity staging.

Results – The distribution of echographic grade was: no any echographic features – 4 pts (6.3%); grade 1 - 24 pts (38.1%); grade 2 – 20 pts (31.7%); grade 3 – 10 pts (15.9%); grade 4 – 5 pts (7.9%). The ESSDAI and the hydroxychloroquine use were similar in these subgroups. We didn't find differences regarding CRP and fibrinogen and echographic features. The age of the patients, the anti-SSA and anti-SSB, ESR, total protein, IgA, IgG and rheumatoid factor levels were significantly higher and lymphocyte count was lower in patients with echographic severity above grade 2 when compared with patients with no or mild echographic features. However, using ANOVA test and post-hoc analysis, the only parameters associated with the severity of echographic features were high ESR (53 vs 17 in grade 4 vs 1, p=0.02), IgA (363 vs 190 in grade 4 vs 1, p=0.004) and IgG (1985 vs 1191 U/l in grade 4 vs 1, p=0.001) levels.

Conclusions – Parameters linked to polyclonal hypergammaglobulinemia (IgA and IgG levels; and ESR) seem to be linked to the severity of echographic appearance of salivary gland in patients with SS.

Inedited features of lithiasis of common biliary duct – the view of the ERCP specialist

Pătrășescu M, R.S. Costache, Balaban V., Nuță P., Bucurică S., Borcoși C., Almasri A., Ioniță F., Jinga M

Background: The lithiasis of common biliary duct is a frequently encountered ailment in gastroenterology practice. It is mostly associated with lithiasis of gallbladder, issuing as a complication of it, but not infrequently it appears in patients that had suffered cholecystectomy. For many decades, the solution has no longer been surgery but an endoscopic biliary sphincterotomy by one of the most complex technique of interventional digestive endoscopy: ERCP (endoscopic retrograde cholangio-pancreatography). The greatest challenges raised by this technique are represented by the effort to establish the proper indication and by the treatment of complications. The high rate of complications, even in tertiary centers, mandates by necessity the optimization of the efforts to rigorously select the cases to benefit from ERCP.

Conclusion: I shall present a series of cases, in which I have performed this technique in our department in 2021, in plain COVID 19 pandemic, trying to show several groups of facets of endoscopic and gastroenterological practice challenges, in order to shed some light over some difficult problems that are being raised to clinicians by the patients suffering of lithiasis of common bile duct.

A very particular clinical presentation in a group of patients with triple positive antiphospholipid antibody profile: a case series

M. Casian, A. Ciotoracu, A. Șerban, D.M. Chițimuș, M. Șotcan, C. Jurcuț

Introduction – Antiphospholipid syndrome (APS) is a clinical entity that associates circulating antiphospholipid antibodies with recurrent thrombotic or obstetrical events. A triple positive antiphospholipid antibody profile is a high-risk situation even in asymptomatic patients with no arterial, venous, or obstetric events. Several distinct APS types are described, such as primary APS, secondary APS in the case of a coexisting autoimmune disorder (such as SL), or catastrophic APS. An intermediate form of APS has also been described in patients with lupus-like manifestations (especially specific laboratory parameters) who fail to meet the criteria for SLE.

Material and method – We report five cases of patients with triple-positive circulating antiphospholipid antibodies, hypocomplementemia, and positive antinuclear and anti-DNA antibodies who initially lacked the clinical manifestations generally associated with this immunological profile. The first patient was diagnosed with APS after an unprovoked episode of PE. The second patient tested positive for antiphospholipid antibodies after routine blood tests revealed a prolonged APTT. Shortly after his evolution was complicated by PE. Both patients progressively developed other SLE-associated clinical manifestations. The third case is of a completely asymptomatic female who had a triple positive antiphospholipid antibody profile and lupus-specific autoantibodies. The fourth patient was investigated after routine blood tests showed a prolonged APTT. He later developed lupus-specific cutaneous lesions, but no arterial or venous thrombotic events. The fifth case is of a male patient who was diagnosed with APS after an unprovoked episode of PE. As before, the prolonged APTT warranted further investigations.

Conclusions – A triple-positive antiphospholipid antibody profile should always prompt further immunological tests, even in asymptomatic patients. Closely monitoring these patients, as shown by our cases, is prudent and advisable since other clinical manifestations develop over time. Last but not least, we need to emphasize that in our small group, this pattern was more frequently observed in male patients.

Recurrent fever of unexpected etiology

A. Şerban, M. Casian, D.M. Chiţimuş, D.V. Balaban, M. Pătrăşescu, A. Ciuche, C. Jurcuţ

Introduction – Lymph node tuberculosis counts among the more common forms of extrapulmonary tuberculosis, often presenting in a nonspecific manner, thus making it difficult to differentiate this infection from other consumptive diseases.

Materials and methods – We present the case of a 44-year-old male, with a history of achalasia, pulmonary tuberculosis and chronic kidney disease. The patient was admitted to our clinic for recurrent fever (daily episodes for the last 8 years), nonspecific musculoskeletal pain, weight loss (approximately 8 kilograms in the last 45 days) and bilateral enlargement of cervical and supraclavicular lymph nodes. The blood work on presentation showed significant inflammatory syndrome, leukocytosis and moderate anemia. The computed tomography revealed a severely dilated esophagus. Therefore, an upper endoscopy was performed, which raised suspicion of a patent cardial fistula. The patient was started on meropenem, with a sudden and dramatic improvement of symptoms and inflammatory markers within the next 24 hours. The fistula was not confirmed on a later oral contrast CT scan. Supraclavicular lymph node resection and histopathological examination were performed, which established the diagnosis of lymph node tuberculosis. The patient was referred to a territorial tuberculosis center and received tuberculostatic therapy, with a good response to treatment on telephonic follow-up.

Conclusion – Lymph node tuberculosis is thought to account for 20-30% of all extrapulmonary tuberculosis cases. In this specific instance, the diagnostic delay was years, thus emphasizing the fact that the clinical presentation of this particular kind of tuberculosis is rather vague. The history of achalasia and suspicion of cardial fistula only added to the particularity of this case, given that both cardial fistula and tuberculosis are compatible with the clinical presentation of our patient.

Laboratory diagnosis in SARS-CoV-2 infection – The experience of the Carol Davila Military Hospital medical laboratory during the pandemic context

I. Butte, C.F. Ghiatau, A.R. Gireada, C.M. Gilca, L.I. Grecu, C. Ignat, C. Negut, C.L. Taubner

Starting March 2020, the laboratory diagnosis of SARS-CoV-2 infection has become a priority for the medical world in general and for colleagues in our hospital in particular.

During the first month and a half, the National Institute of Research and Development for Microbiology and Immunology "Cantacuzino" performed, through an outsourced service, real-time RT-PCR analysis for patients and hospital staff. During that time our molecular biology laboratory was established (space arrangement, high-performance technical equipment, purchase of reagents and consumables, training the team of specialists).

The first SARS-CoV-2 RT-PCR tests performed in our laboratory were reported on 28.04.2020. Up to now, more than 80,000 tests have been performed on nasopharyngeal exudate samples, sputum, broncho-alveolar lavage, stool and wastewater and the consulting skills of our team have improved. The quality of the results was tested by comparison with reference laboratories and external quality control.
As soon as they became commercially available, IgM and IgG anti-SARS-CoV-2 antibodies were introduced in serological diagnosis being performed by ELISA method, immunochromatographic method and on CMIA-Abbott Alinity platforms. The results quality was tested by external quality control. A quantitative test for IgG anti-spike antibodies is currently available.

In addition to the diagnosis for COVID-19, the laboratory provided high-performance quantitative methods for different parameters of interest like D-dimers, ferritin, PCT, troponin, NT pro-BNP and, last but not least, interleukin 6.

An alternative to RT-PCR for etiological diagnosis of COVID-19 was the SARS-COV-2 antigen determination implemented in early 2021 wich allowed up to 1000 diagnostic tests per day (RT-PCR and antigen) reported in CORONAFORMS.

SARS-CoV-2 Infection Detection in Carol Davila University Central Military Emergency Hospital's patients

R Hrișcă, C Taubner, C Carp, E Munteanu, A. Rotaru

Background – Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the name given to the 2019 novel coronavirus. This virus has not been previously identified in humans. COVID-19 is the name given to the disease associated with the virus. The infectious period may begin around two days before symptoms appear and it is estimated 20 to 40% of patients have few of no symptoms. For these reason, from April 2020 in our hospital we began screening patients in an effort to reduce asymptomatic or presymtomatic transmission according with our internal guidance. These guidance was updated periodic taking into account factors such our laboratory capability, national guidance and local SARS-CoV-2 transmission.

Objective and methods – A retrospective study was performed in order to finding out the number of patients admitted in our hospital wards who had at least one PCR or rapid tests for SARS-CoV-2 antigen detection was positive. The study period was from April 2020 to June 2021.

Results – In this period a total of 39294 RT-PCR tests were performed in our hospital wards, in the study period. Among these, 1103 (2.8%) had a positive result, 36 (0.09%) had an inconclusive RT-PCR result, the rest was negative. A total of 12,233 rapid tests for SARS-CoV-2 antigen detection were performed, of which 83 (0.67%) were positive, 27 inconclusive (0.22%) and the rest were negative. Following the tests performed a number of 882 SARS CoV-2 patients were identified, most of them having an asymptomatic or with mild symptoms.

Conclusion – Inpatient SARS-CoV-2 screening is important when intra-community transmission is extended to prevent COVID-19 associated infections.

Imaging aspects in differential diagnosis of the pulmonary lesions in COVID 19

D. Cuzino, F. Naftanaila, C. Radulescu

Background – The pandemic of COVID-19 is an on-going public health emergency. The main clinical symptoms extensively reported derive from upper respiratory tract and lungs involvement. Since alterations in coagulation tests have emerged in COVID-19 infection, there has been an increased warning of potentially critical systemic complications.

Findings – As soon as first evidence in literature has shown that SARS-CoV-2 infection may predispose to both venous and arterial thrombotic complications, pulmonary embolism (PE) emerged as one of the most frequent. Moreover, it should be highlighted that microangiopathy plays a key role in the pulmonary pathobiology of COVID-19 and microthrombi, undetectable at Computed Tomography Angiography, are consistently observed in pulmonary arterioles and alveolar capillaries. Patients' positive for PE had an important lung disease involvement, with extended parenchymal consolidations.

Conclusion – Covid-19 disease is an unprecedented challenge to the world, in particular to the medical community. We now understand that Covid-19 is a multisystem disease with involvement of brain, lungs, gastro-intestinal system, hepatobiliary system, vascular system and kidneys. The mainstay of diagnosis emains RT-PCR test, Radiology however plays an important role in early diagnosis of the disease and its complications, useful in reducing the morbidity and mortality associated with the disease.

Imaging aspects of the thromboembolic diseases complications after COVID 19

S. Capisizu, D. Cuzino, F. Naftanaila

Background – The SARS-CoV-2 infection can be spread outside the respiratory system and reach the central neural system (which remains one of the most sources of morbidity and disability) and other sytems of the body. We notice that COVID 19 is a predisposing factor that affects the coagulopathy status. The patient can be presented as a sequela of vascular thrombosis all over the body. One of the complications of the infection can affect the vessels and present with small or large vessel occlusion, thromi or hematoma.

Findings – We selected COVID 19 pacient who underwent CT/MRI scan of the brain and other imaging procedures. We considered positive cases when we have detected an acute lesion whether it is hemorrhagic or ischemic in other parts of the body including also the lungs.

Conclusion – Covid-19 patients are at higher risk for acute brain infarction, whether it is ischemic or hemorrhagic and those patients should be in close monitoring for stroke assessment. We also have to monitor the pacients for other possible sites of the lesions.

Differential diagnosis of thyroid nodule's capsule based on pixel-level collagen angular distribution

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Background & Objectives – The aim of this study is to demonstrate the changes in the collagen structure and organization of the capsule of benign (follicular thyroid adenoma) or malignant (papillary thyroid carcinoma) thyroid nodules.

Materials and Methods – We selected the cases in which, on the same section of the thyroid tissue, from the same patient, we identified a malignant transformed nodule found among the other benign nodules. The distribution of collagen was evaluated with the acquisition of images by Second Harmonic Generation (SHG) microscopy namely the Forward SHG (FSHG) and Backward SHG (BSHG).

Results – We calculated several parameters that evaluate the angular distribution of collagen fibers from the capsules of the thyroid nodules and we noticed that there are statistically significant differences between the angular distributions of the collagen fibers in the benign nodule capsule compared to the malignant nodule capsule. Namely, the collagen fibers in the malignant nodule capsule are tighter, uniform, and those in the benign nodule capsule are wavier, coiled.

Conclusion – The results of FSHG and BSHG microscopy demonstrates that the collagen distribution can be different in an objective manner. These results can be further used in future examinations to help in the differential diagnosis of apparent encapsulated thyroid nodules.

MINOCA in CoViD-19: the role of multimodality imaging

Indrieș V.D., Casian M., Țoc F., Stanciu S., Chiriac L.

Introduction: Acute coronary syndrome with ST-segment elevation is a cardiological emergency. In the case of permeable coronary epicardial arteries, the patients are labeled as MINOCA. Further investigations are warrented in order to establish a final diagnosis and appropriate therapy.

Methods: We present the case of a 56-year-old female patient, who associates multiple cardiovascular risk factors, with a history of atypical angina pain who is transferred to our hospital in the RO-STEMI program with ECG changes in the lateral leads and elevated tropinin values. Upon presentation, the patient developed cardiogenic shock, requiring inotropic support (EF of 25%). Angiography did not detect significant coronary lesions.

Results: The patient's evolution was favorable, without the recurrence of angina symptoms, the inotropic support being stopped at 72 hours after the onset. Paraclinically, the patient developed an important inflammatory syndrome, moderate eosinophilia and high troponin values (completely remitted at discharge). Cardiac ultrasound showed the recovery of the ejection fraction, without segmental hypokinesia and the formation of a small pericardial effusion. Speckle-tracking ultrasonography revealed normal GLS, with lower than normal values in the lateral wall. The causes could be: vasospasm in the territory of the circumflex artery, coronary thrombosis, myocarditis and stress cardiomyopathy. The arguments for the ischemic hypothesis are: ECG and movement abnormalities in the lateral territory, troponin values rise and fall and a history of atypical anginal pain. The emotional context is characteristic of stress cardiomyopathy. Cardiac MRI is performed, which detects cavities with normal volumes and normal kinetics, the LGE pattern was suggestive of myocarditis. COVID-19 antibodies are positive, without vaccination.

Conclusions: Therefore, we present the case of a patient with fulminant cardiogenic shock, inflammatory syndrome and pericardial effusion, with a rapid recovery of the ejection fraction in which the diagnosis of myocarditis was formulated based on multimodality imaging. In the context of positive COVID-19 antibodies, myocarditis could be a long term complication of COVID-19.

The imaging appearance of aggressive angiomyxomas

G.S. Toma

Background: Aggressive angiomyxomas are rare, benign, mesenchymal tumors that arise from connective tissue, but have an aggressive behavior due to local recurrence, rarely metastasizing.

Objective: To demonstrate the CT and MRI appearance of aggressive angiomyxomas as an incidental finding, that it’s important to recognise

Material and methods: We used a MRI scanner Siemens AERA of 1.5 T with body coil and a 128 GE CT scanner.

Results: It is the case of a patient, that first had a CT examination for another pathology and it was depicted an anomaly of the soft tissues near the pubic bone and symphysis. At CT it was found a proliferative mass, with calcifications included, with moderate, progressive, inhomogeneous enhancement and collateral vessels. At MRI it was confirmed a proliferative mass in the vulvar perineal region, which protrudes interpubically with associated collection and pubic osteitis. It has a laminated and swirled appearance, with high T2 signal of the myxoid component and linear hyposignal T2 of the collagen fibrils. Enhancement is also progressive in the myxoid tissue and there is no enhancement of the cystic component.

Conclusions: Even if aggressive angiomyxomas are rare and benign, because of their aggressive behavior, due to local recurrence, it is important to diagnose.

Avascular osteonecrosis, a long term complication in COVID-19 patients treated with corticosteroids – imaging findings

Toma G.S., Mircea G., Anghel A., Costea R., David O., Graur A., Micu M., Tita M.

Background: Avascular osteonecrosis (AVN) is a well-recognized condition of unknown etiology whereby the circulation of the blood to an area of bone is impaired, followed by necrosis. COVID-19 can affect different body systems, skeletal system being one of them. At present, avascular necrosis (AVN) as a certain sequalae of COVID-19 has yet not been documented, but there are data that suggest a link between the corticosteroid use in COVID-19 patients and AVN. The association between COVID infection, steroid use and AVN has been stated since the SARS epidemic from 2002-2003.

Objective: To demonstrate the MRI appearance of AVN that occurred after a few months in COVID - 19 patients treated with corticosteroids.

Material and methods: We used a MRI scanner Siemens AERA of 1.5 T with dedicated coils for knee and ankle. The patients we included had a history of COVID -19 infection and corticosteroid treatment

Results: We selected two patients with a history of COVID 19 infection and corticosteroid treatment. One patient had pain in both ankles and both knees, so the clinician indicated MRI for all these joints. After MRI one knee had AVN and one had sequelae from an older knee injury and both ankles had AVN of the talus. The other patient had knee pain and MRI was performed for this joint only and demonstrated AVN. The AVN areas were large and geographic shaped involving both distal femur and proximal tibia in the knee joint. The talus involved had also a large area of necrosis.

Conclusions: In this new shaped era from COVID 19 infections we have to keep in mind the potential development of bone lesions, and especially AVN, in patients that had COVID and also received corticosteroid treatment. The early diagnosis of AVN is important, as proper treatment may arrest the progress of the condition and prevent subsequent bone collapse. MRI is the most sensitive diagnostic technique for the diagnosis of AVN at any stage, particular in the early stage.

MR Enterography for patients with inflammatory bowel disease

Toma G.S., David O., Anghel A., Costea R., Graur A., Mircea G., Micu M., Tita M.

Background: MR enterography (MREg) has become the primary imaging modality in the assessment of inflammatory bowel disease (IBD), in both children and adults, primarily due to its non-invasiveness, superior soft tissue contrast, and lack of ionizing radiation.

IBD primarily refers to two chronic diseases that cause chronic inflammation of all or part of the digestive tract: ulcerative colitis (UC) and Crohn’s disease (CD). CD is a transmural chronic inflammatory disorder that can affect any part of the gastrointestinal tract in a discontinuous distribution. UC is a mucosal and submucosal chronic inflammatory disease that typically originates in the rectum and may extend proximally in a continuous manner. The diagnosis of this two entities is made based of morphological changes seen on MREg, in conjunction with endoscopy and histopathologic analysis.

Objective: To ilustrate the appearance of bowel through MR Enterography and the diagnostic input in cases of inflammatory bowel disease (IBD)

Material and methods: We used an MRI scanner Siemens Aera of 1.5 T. The patients prior to the examination were given a solution of diluated Manitol in 30 minutes. The examination is done in a prone position to reduce the respiratory movements and artefacts. There are some special sequences that are different from the usual abdominal MRI imaging techinque. The latest MREg techniques uses diffusion-weighted imaging (DWI) and Cine-MR sequences, that enable the differential diagnosis between edema and fibrosis (that represents a key issue in patients who suffer from IBD) and provide even functional information, allowing the visualization and analysis of bowel motility (additionally increasing the lesion detection rate).

Results: In this presentation we included one case with active Chron disease, one with chronic presentation of Chron and others that showed colitis or normal appearance and ruled out active bowel inflamatory disease. Because of chronic, unpredictable relapsing and remitting pattern of IBD, MREg is used to rule out complications such as strictures and abcesses requiring surgical intervention and also assess disease activity.

Conclusions: MR Enterography is a modern non invasive way of contributing to the diagnosis for bowel inflamatory disease in correlation witht other laboratory findings.

The imaging diagnosis of patients with digestive tract tumors

Toma G.S., Graur A.M., Anghel A., Costea R., David O., Mircea G., Micu M., Tita M.

Background: Gastrointestinal cancer refers to malignant conditions of gastrointestinal tract. The symptoms relate to the organ affected and can include obstruction (leading to difficulty swallowing or defecating), abnormal bleeding or other associated problems. The treatment depends on the location of the tumor, as well as the type of cancer cell.

Objective: The paper aims to present some cases of digestive tract tumors and how we can recognize their imaging characteristics. Imaging has an important role in depicting and staging the disease.

Material and methods: We selected some cases of digestive tract tumors examined from 1 April to 31 June 2021 in the Laboratory II Radiology and Medical Imaging of Central Military Emergency University Hospital “Dr.Carol Davila”, Bucharest. The patients were examined at the CT scanners available in our department, 128 GE CT scanner and a 256 CT Philips scanner.

Results: Three types of cases were characterized: gastric cancer, small intestine cancer and colorectal cancer. In this short period, a large number of cases of gastrointestinal tumors have been reported, therefore clinicians, radiologists and pathologists must be aware of the importance of this type of cancer. The increased incidence can also be explained by the fact that patients avoided going to the doctor and delayed further investigations due to the covid-19 pandemic which limited the possibility of depicting them earlier.

Conclusions: It is important to diagnose, treat early and follow up all cancers because by not doing this we see more advanced cancers with poor prognosis and limited treatment options.

Bile duct strictures: Role of CT imaging and MR Cholangiopancreatography in characterization

Toma G.S., Anghel A.I., Costea R., David O., Mircea G., Micu M., Tita M.

Background: Biliary stricture is a fixed narrowing of a focal segment of the bile duct that usually results in proximal biliary dilatation and clinical features of obstructive jaundice. There is a wide spectrum of benign and malignant pathologic conditions that can result in the development of biliary strictures. The most common benign etiologies include iatrogenic causes, cholangitis, pancreatitis, choledocholithiasis, infections and ischemia, while ampullary and periampullary carcinomas, pancreatic carcinoma and cholangiocarcinoma are usually the malignant causes for biliary strictures.

Objectives: We emphasize the role of imaging techniques in providing an accurate assessment of the location and extent of biliary strictures, as they create a map of the biliary tree and help guide endoscopic therapy, particularly when endoscopic retrograde cholangiopancreatography (ERCP) is indicated. Also, we discuss the aid of imaging studies in differentiating malignant from benign strictures, since their treatment and prognosis vary.

Materials and Methods: The patients with suspected biliary stricture were evaluated in the Laboratory II of Radiology and Medical Imaging department of Central Military Emergency Hospital “Dr Carol Davila”, at a 128 GE CT scanner and a 256 CT Philips scanner, as well as a MRI scanner Siemens Aera of 1.5 T. The advantages of magnetic resonance cholangiopancreatography (MRCP) over CT include the lack of ionizing radiation, along with the ability to provide high quality cholangiograms. MRCP is also especially appropriate for the assessment of congenital pancreatic anomalies since it depicts ductal anatomy rapidly and noninvasively, without the risk of acute pancreatitis. MR Cholangiopancreatographic techniques involve the use of heavily T2-weighted sequences to accentuate the high signal from relatively static fluid in the biliary tract while suppressing the signal from background tissues, including flowing blood. The ability to obtain a cholangiogram without injection of contrast into the biliary tree is significant, since the injection of contrast medium can sometimes result in cholangitis. However, while abdominal imaging helps to identify the level of obstruction and provides a ‘road map' for further endoscopic investigations, histopathologic diagnosis is usually needed to make a definitive diagnosis.

Results: Narrowing of the biliary duct was demonstrated by imaging in all patients, CT scanning being usually followed by MRCP which brought additional information and valuable clues regarding the etiology and the nature of the stenosis. The bile duct strictures proved to be of either benign causes, such as periductal inflammation secondary to necrotizing pancreatitis, or a malignancy suspicion was raised, such as ampullary carcinoma.

Conclusions: As biliary strictures frequently represent a challenge in terms of diagnosis, awareness of the various causes of bile duct strictures and familiarity with their features using the most adequate imaging techniques are important for an accurate diagnosis, for patient follow-up and for the optimal patient management.

Surgical 1

The impact of COVID-19 pandemic on surgical activity of our department

Săvulescu AF, Albița IO, Mușat JC, Duțu C, Nica RI, Rogin TD, Cîrlan C, Blăjuț FC, Orosan DL, Dumitru C, Sima O, Coajă F, Vintilă AI, Vintilescu D, Marin R, Iordache-Petrescu MI, Iordache M, Ionescu BA, Vlad M, Stanciu D, Popa L, Sbărnea EI, Călina O, Florea I, Șipoteanu A, Perșinaru D, Matei R, Katih N, Sabha A, Surdu D, Mouhanad AA

In the current epidemiological context of the SARS-CoV-2 pandemics our department of general surgery had to provide safe and effective care with specific guidelines. We had to triage the urgency of our daily surgical procedures and consider non-surgical management options where possible. Our department worked with a capacity of 13 beds, 3 operating theaters for non-COVID cases and 2 separate operating theaters for COVID cases. We managed 1292 patients from March 2020 until June 2021, 24 cases COVID-19 positive. The mortality rate increased from 1.39% (2019) to 3.75% (2020). Most of our patients suffered from colorectal cancer, gastric cancer, breast cancer, acute cholecystitis, abscesses, incarcerated hernias, bowel perforations and obstructions, acute mesenteric ischemia. The interventions were open procedures and minimally invasive surgery (laparoscopic and robotic surgery). With specific guidelines implemented and followed we were able to continue providing safe and effective care for our patients during the COVID-19 pandemic.

Involvement of Surgery I Departament in the general effort of Central University Emergency Military Hospital during the SARS-COV 2 pandemic

Macau F., Mosoia L., Pruna M., Stefanescu V., Dima A., Artenie T., Dragan C., Mitru C., Tanase M.

Included in SUUMC's overall health care effort during the SARS-COV 2 pandemic, the SURGERY I department was faced with special circumstances, less common or unusual until now. Starting with the much discussed and repeated trainings – removal and disposal of protective equipment, in order to come in contact with positive COVID patients, we ensured the circuits in the surgical ward as well as the scheduling of on-call shifts and other missions, which were added to the challenges that we had to overcome.

The commissioning of a COVID operating room for all surgical departments in the hospital was another objective that SURGERY I department had to fulfill.

A part of the team of nurses and orderlies as well as auxiliary staff participated in the fitting of the ROL 2 Hospital and of the ATI COVID Departments established during the pandemic.

The paper highlights the strengths and weaknesses that we found and improved during the SARS-COV 2 pandemic. When it comes to strengths, we pride ourselves with the fact that we strictly obeyed the rules and no staff member was contaminated with the SARS-COV 2 virus during active duty in the department.

Large scale surgery in the SARS - COV 2 pandemic

Dima A., Mosoia L., Prună M., Stefanescu V., Macau F., Artenie T., Dragan C., Mitru C., Tanase M.

This paper is a comparative study of the large scale surgery evolution between the first 15 months of the SARS COV 2 pandemics and the previous 15 months before the pandemics. We focused our attention on highlighting the difficulties related to the general conditions in the country, to patients and the exact situation from Central University Emergency Military Hospital and the Surgery I departament:

1. State of emergency
2. The patients transport to and from the hospital
3. RT-PCR and SARS-COV 2 testing;
4. Organization of the operating room;
5. The limitation of surgery to vital emergencies
6. The aggravation of patients' condition until the time of the curative surgery
7. The strong and the weak points in the organization of the Surgery I department;
8. The support received from SUUMC in the gradual resuming of the surgical activity in the treatment of large scale cases.

The conclusions are presented at the end of the paper.

Laparoscopically assisted vaginal hysterectomy in large uterus

N. Niculescu, I. Cobani, B. Panaite, C. Pirvulescu, M. Serban, M. Nicodin

Background: If there are specific situations in laparoscopically assisted vaginal hysterectomy in the case of large uteruses and ways to resolve difficult situations, making this intervention feasible.

Methods: LAVH was evaluated and performed on 50 patients diagnosed with fibromatous uterus. The weight of the uterus was estimated between 300 - 1000g. The interventions were carried out between 2010 and 2016. Clinical parameters and baseline operators were recorded for analysis.

Results: The weight of the uterus, the loss of blood, the duration of the intervention, the reconversion of the approach path, the intra and postoperative complications, the administration of blood, the number of days of hospitalization are analyzed,

Conclusions: LAVH is a safe and feasible intervention with low morbidity.

Why we choose the least invasive path in hysterectomy

Nicodin O., Niculescu N., Panaite B., Pirvulescu C., Serban M., Cobani I., Nicodin M.

Hysterectomy is one of the most common surgeries (first in 1813) performed on patients in our clinic for common benign conditions. Minimally invasive surgical techniques are reviewed that offer many benefits to the patient: decreased scarring, better cosmetics, reduced pain, decreased number of days of hospitalization and a faster return to normal activities in general, faster integration into society. The rate of intra and postoperative complications is low. The original minimally invasive hysterectomy was vaginal hysterectomy, LAVH, robotic hysterectomy, (Davinci) effective, with low morbidity. With the advent of laparoscopic surgical techniques and growing technology, minimally invasive hysterectomy methods have evolved over time to avoid laparotomy. This paper will analyze the minimally invasive hysterectomy surgical methods in the gynecology department during 1990-2020 including the tools and techniques currently available.

The advantages and disadvantages of each approach will be discussed. An algorithm for evaluating patients and the criteria that should be used in choosing the appropriate surgical approach for each patient will also be outlined.

Why methods of reducing uterine volume in vaginal hysterectomy are safe

M. Nicodin, N. Niculescu, C. Pirvulescu, M. Serban, I. Cobani, A. Popescu, A. Bodac, A. Negoita, O. Nicodin

Objective: The aim of this paper is to evaluate methods for reducing the uterus in vaginal hysterectomy and their impact on intra- and postoperative complications.

Methods: A number of 350 selected patients aged 41-50 years were included in the evaluation. Selected with the diagnosis of leiomyomas and uterus approximately up to 1200 g, which were subjected to vaginal hysterectomy. For comparison, a number of 400 abdominal hysterectomies, with similar uteruses in size. The procedures were performed between January 2010 and December 2016

Results: They are analyzed: operating time, intraoperative blood loss was higher in patients operated on abdominally, the average weight of uteruses removed vaginally and abdominally (820 g vs 970 g), hospitalization was shorter in patients operated on vaginal route 2.7 days vs. 4.5 days.

The methods of reduction of the large uterus are analyzed: morcelation, intramiometrial coring, hemisection, multiple myomectomies, and amputation of the cervix.

There were 2 patients reconverted to abdominal hysterectomy (adhesions caused by pelvic endometriosis).

Conclusion: Vaginal hysterectomy is a minimally invasive technique compared to the abdominal hysterectomy in patients with large uteri (weighing up to 1200g). Morbidity did not increase in patients with total vaginal hysterectomy.

Uterine size is not a contraindication for vaginal hysterectomy.

Uterine morcelation, hemisection, coring, is a safe method for removing large uteruses in selected cases.

Priapism – A urological emergency

Marcu D, Iorga L, Spinu D, Amza A, Andrei R, Cherciu A, Budur M, Mischianu D, Bratu O

Priapism is a complex and a challenging urological emergency that requires a fast diagnosis and immediate treatment in order to prevent the onset of local ischemic changes and subsequent erectile dysfunction.

Anamnesis, clinical examination and Doppler ultrasound examination are the main elements that can help us establish the type of priapism, therefore guiding the therapeutic approach. The duration of priapism is of great importance, especially in the case of ischemic priapism, being known that a duration between 48 and 72 hours will eventually associate erectile dysfunction.

While in the case of non-ischemic priapism the treatment is mainly conservative, in patients with ischemic priapism a form of treatment should be rapidly initiated in order to avoid the onset of local irreversible structural changes and erectile dysfunction.

Failure to recognize the urgency of diagnosis and treatment in the case of priapism can have unfavorable consequences, both from a medical point of view for the patient and also from a legal point of view for the medical staff involved in the evaluation and the management of such patients.

Multidisciplinarity approach in surgical treatment of pheocromocytoma

Marcu D, Iorga L, Spinu D, Amza A, Andrei R, Bratu I, Cherciu A, Budur M, Mischianu D, Madan V, Bratu O

Introduction – Pheochromocytoma is a medullary adrenal catecholamine-secreting tumor consisting of chromaffin cells, usually benign; the malignant form being diagnosed only by the presence of chromatin metastases at the bone, liver, lung or lymph nodes.

Material and method – This paper aims to present surgical treatment as the only method of curative treatment in terms of existing therapeutic modalities and the contribution of all specialties that are included in this pathology such as endocrinology, oncology, radiology, nuclear medicine, otorhinolaryngology, surgery general, urology and pathological anatomy.

Results – Surgical treatment remains the only curative method of treatment of pheochromocytoma, depending on the location at the medullary adrenal level involving different laparoscopic methods (minimally invasive adrenalectomy) or classical (tumors exceeding 6 cm or invasive), diagnosis and preoperative treatment being established in a multidisciplinary manner.

Conclusions – Pheochromocytoma is a pathology that requires a multidisciplinary approach, with multiple therapeutic methods, but only the surgical approach is the only potentially curative therapeutic modality, with different techniques for each body segment that has been affected.

Maximizing HPV detection in prostate pathology: a comparison between p16 detection and PCR – a pilot study

Amza A, Marcu D, Iorga L, Spinu D, Andrei R, Cherciu A, Budur M, Mischianu D, Bratu O

Human Papilloma Virus (HPV) is the most common cause of sexually transmitted diseases (STDs) of viral etiology worldwide, and prostate adenocarcinoma continues to be the second most common cancer affecting the male population, thus calling into question the correlation between the two and especially if HPV has a role in PCa oncogenesis.

Material and method – The aim of this study is to review the comparison between the detection of HPV by PCR and the immunohistochemical expression of p16-INK4A in biopsied prostate tissue, following studies performed on considerable numbers of patients.

Results – It was found that in the case of PCR-HPV-positive samples where koilocytes (with pathognomonic significance) were detected, p16-INK4A is overexpressed compared to PCR-HPV-negative samples, indirectly suggesting the presence of E7 oncoprotein and the link between HPV and the role that it has it in the development of PCa.

Conclusions – There are multiple ways to diagnose HPV in prostate cancer, but it comes to the detection through PCR and the expression of p16-INK4A through immunohistochemistry we find that whenever koilocytes are found p16 is overexpressed.

Giant ovarian tumor neglected as presentation in the context of the COVID 19 pandemic

I. Florea, M. Vlad, C. Duțu, A. Zaciu, D. Stanciu, R. Marin, D. Perșinaru, O. Călina

Within the Surgery II section of the Central Military Emergency University Hospital, a special surgery was performed, both from a surgical and anesthetic point of view, which consisted in the extraction of a giant cystic tumor formation, which belonged to the right ovary, neglected in social context, but also epidemiologically - in the current SARS Cov 2 pandemic.

The patient, aged 40, presented for an increase in size of the abdomen, with an onset of about 5 years, accompanied in recent weeks by diffuse lower back and abdominal pain, nausea and dyspnea.

The CT aspect of the formation was a giant abdomino-pelvic mass, with dimensions of 55/35 cm, with mass effect on the intra-abdominal organs. The patient was hospitalized in the Surgery II section for specialized surgical treatment. Considering the young age of the patient and the benign characters of the formation - biological, imaging and intraoperative, as well as the slow evolution of the disease, it was decided to practice right annexectomy with tumorectomy, under general anesthesia.

Intraoperatively, the giant cystic formation (55 cm in diameter, 40.7 kg) was found, with serous content and right annexal affiliation, with compression on the neighboring structures and pushing the entire visceral mass into the right and interhepatophrenic hypochondrium. The formation had a relatively thick capsule and was extracted in its entirety. The tumor was punctured and sent to the histopathological examination that confirmed the choice made (ovarian papillary multilocular serous cystadenoma). It is worth mentioning the very rare nature of the size and weight of the formation (almost half of the preoperative weight of 91kg of the patient).

Postoperatively, the patient was carefully monitored and her evolution was favorable, afebrile and normal transit and digestive tolerance, gradual mobilization and volume stabilization in the context of vascular bed decompression. The patient was discharged in good condition, with a normal-looking wound, being healed.

The case presented highlights the impact that the current pandemic has on patients with other pathologies, non-COVID 19- delayed diagnosis and treatment, worsening of the patient's condition and increased risk of complications.

Optimizing surgeon-patient communication in the context of Covid-19 pandemic

M.A. Vlad, I.A. Florea, C. Duțu

The current pandemic of COVID-19 has changed doctor-patient relationship because of SARS-CoV-2 transmission risk, which raised the need to wear personal protective equipment, to maintain physical distance and to use telemedicine. These measures affect in a negative way the communication with the patient.

In surgery, the impact of the pandemic on the communication and interaction between the surgeon and patient is noticeable, in terms of some particularities. Thus, for doctor, the intraoperative contact with the patient and the exposure to blood and other bodily fluids, and for patient, the fear of infection in the hospital and the delayed presentation to the doctor. Decreased confidence in the doctor-patient relationship can lead to decreased adherence to the treatment and unfavorable subsequent evolution.

Adapting communication and interpersonal skills can allow the doctor to acquire important information for establishing the diagnosis, formulating medical recommendations and increasing the patient's confidence in the medical act. Decrease in non-verbal communication possibilities affects the doctor's transmission of empathy through facial expression and haptic communication to the haptic (such as, simple handshake), to which increased anxiety of the patient and the difficile communication with patient family have been added.

Among the methods used to compensate for surgeon-patient communication we list the increase of eye contact, the use of paralinguistics, listening to the patient and encouraging him to transmit emotions.

A special chapter is online consultation, whose advantages and disadvantages are exposed in the article.

COVID-19 made us reconsider the way we communicate with the patient and makes it difficult for us to find a method of interaction for building trust in the doctor-patient relationship. All those methods, implemented in the pandemic context, can be useful from now on.

Corneal transplantation activity at the beginning of COVID 19 pandemic

Zemba M., Musat O., Manole H., Macovei L., Pulbere L., Stefan C.G.

The corneal transplantation activity has not been stopped when the pandemic started.

The paper shows the difficulties that appeared with eight surgeries that have been performed in the first two months of the pandemic.

Some epidemiologic measures made the activity more difficult: the need to test the doctors who prelevate the tissue and the need to test the receiver of the cornea, activities that take a lot of time.

Due to conjunctival transmission of SARS-COV 2, testing of the donor has been introducedand that has dropped the number of donors.

Because of logistical problems the time to use a fresh cornea has decreased dramatically. This situation has shown more and more clearly the need of a corneal bank.

Benefits of robotic approach in recurrent hiatal hernia repair

Savulescu F, Blajut C, Cirlan C, Vintila I, Iordache-Petrescu M, Iordache M, Ionescu B, Stanciu D, Popa L, Lutenco C.

Introduction: Recurrent hiatal hernia repair may be indicated in cases where symptoms are uncontrolled with medications coupled with an anatomic recurrence. Up to 15% of patients may develop a recurrent hiatal hernia on imaging at 1 year postoperatively, but only 10% of these patients are symptomatic. The size of the index hernia is an independent predictor of recurrence, and although mesh may decrease short-term recurrence, it has not been found to impact long-term recurrence rates.

Methods: Current studies in this field describe robotic assistance of significant value when performing complex, technically demanding procedures. Although conventional laparoscopy is nowadays the common approach for hiatal hernia repair and anti-reflux surgery, it can be difficult, especially in case of recurrent hiatal hernia repair. Mediastinal dissection of large diaphragmatic hernias, a history of multiple abdominal surgeries, or redo surgery constitute a technical challenge, often influencing the surgeon's decision to favor an open procedure over a laparoscopic approach. In these cases, the wrist-like motion of instruments and enhanced visualization provided by robotic systems can extend the possibilities of minimally invasive surgery while retaining the advantages of conventional laparoscopy such as reduced blood loss, lower postoperative morbidity and mortality, and shorter recovery period when compared to open repair. The favorable ergonomic and working position of the surgeon during the complex phase of the intervention may also be of importance.

Results: Our experience consist of 45 robotic hiatal hernia repairs out of which 2 patients had a recurrent hiatal hernia.

Conclusion: In our experience, robotic surgery, with benefits of improved visualization and precisely controlled dissection, provides a safe and effective approach to challenging recurrent hiatal hernia repairs. The robot facilitates an extended dissection needs into the mediastinum and stapler angles that can be challenging for a laparoscopic approach to these patients.

Robotic repair of iatrogenic ureteral injury during robotic hysterectomy

Blajut C, Savulescu F, Cirlan C, Vintila I, Iordache-Petrescu M, Iordache M, Ionescu B, Stanciu D, Popa L.

Study objective Iatrogenic ureteral injuries, more than half of which occur during gynecologic surgery, may have devastating consequences for both patients and physicians. The objective is to present a case of iatrogenic ureteral injury during robotic hysterectomy and its repair.

Material and methods: We present the case of a 61 year old woman who was referred to us for robotic hysterectomy for a benign uterine mass. During the dissection and section of the left lateral vaginal wall a ureteral injury was produces.

Results: We repaired the ureteral injury by introducing a "double J" stent and performing a ureteral suture with the help of robotic platform. The guide wire and the stent were introduced via the vaginal port. A drainage tube was placed afterwards. Postoperative course was uneventful, and the hospital stay was 2 days. The urinary catheter and the drainage tube were removed after 14 days, and the "double J" stent was removed after 1 month.

Conclusions: Early repair of iatrogenic ureteral injuries is mandatory in order to decrease the rate of outpatient complications or stricture recurrence. We suggest that, if possible and appropriate, effort should be made to identify and subsequently repair iatrogenic ureteral injuries early to minimize morbidity for the patient as well as avoid a potentially increasingly complex ureteral reconstruction.

The experience of robotic surgery at the SCUMC Urology Clinic

Arbunea D., Păvălean M., Bratan I., Chirilă L., Aungurenci A., Săvulescu F., Mădan V., Dinu M., Mischianu D.

Between February 2017 and June 2021, within the Urology Clinic of SCUMC, with the support of the General Surgery Department II, 116 radical prostatectomies, 33 partial nephrectomies, 17 pyeloplasties were performed.

Methods: We analyzed the evolution of the operative time, of the hospitalization duration, the intraoperatively and postoperatively complications.

Results: It has been noted a decrease in the prepering preoperative time(within 4 years it shortened with 50%), an improvement of the overall duration of the surgical intervention (intraoperative time has been reduced with 30% comparing with first interventions) and a decreased number of hospitalization days, all in the context of increasingly complex cases.

Conclusions: Considering in one hand that the daVinci System from the Central Military Emergency University Hospital (SUUMC) is one of the fewest functional robots provided by the Public Health System and, probably, the only one used for urological interventions and on the other hand, considering the important costs associated with these interventions, solving cases with a high degree of difficulty and shortening hospitalization times with much faster reintegration back into society life allowed us to categorize this system as being extremely effective.

How can we maximize the degree of brain tumor resection and the implications on quality of life?

O Sirbu, AV Chirtes, V Mitrica, O Zamfir, M Mitrica

The incidence of brain tumours is 1% - 3% in all cancer types. The treatment of these patients is a complex, multimodal, multidisciplinary procedure bringing together neurologists, neurosurgeons, radiologists, radiotherapists, oncologists, and physiotherapists. In most cases, the initial treatment is based on neurosurgical tumour resection. The degree of tumour resection is correlated in most cases with an extended lifespan and increased quality of life. For infiltrative tumors it is well known that the appearance or consistency of the tumour does not allow the neurosurgeon a clear delimitation of the lesion from healthy brain tissues. The same difficulties can be encountered even in more circumscribed lesions like cerebral metastases. We are reviewing the main intraoperative techniques used in achieving gross total resection and try to highlight the importance of intraoperative MRI, 5 aminolevulinic acid, fluorescein and cerebral ultrasonography in neurooncology.

Prognostic factors for poor outcome in suboccipital decompressive craniectomy for cerebellar ischemia

O.M. Sîrbu, V. Gilard, A. Didier-Laurent, A. Triquenot-Bagan, P. Hannequin, S. Derrey

Suboccipital decompressive craniectomy is the treatment of choice for patients with cerebellar stroke who deteriorate secondary to posterior fossa edema. Functional outcomes, mortality or adverse effects are not well defined in the current guidelines. Therefore, we share our experience of cerebellar stroke craniectomy together with the identified factors for an unfavorable outcome. All patients treated with SDC for cerebellar infarction in our department between 2002 and 2018 were retrospectively analyzed. Demographic, clinical and radiological data, surgical technique and outcome at one-year follow-up were gathered. A poor long-term outcome was defined as a modified Rankin Score at one-year check-up ≥3. We analyzed the following prespecified factors: age≥65, Glasgow Coma Score (GCS) <9, brainstem infarction, bilateral infarction, time between onset of symptoms and surgical intervention, surgical technique. Eleven suboccipital decompressive craniectomy were performed. The mean age at diagnosis was 54.73 ± 12.84 years old. Mean GCS at presentation was 13.7±2.4. The most frequently affected vessel was Posterior Inferior Cerebellar Artery. A bilateral distribution of the infarcted zone was seen in 45.5%. In 63.6% an external ventricular drain was inserted just before decompressive craniectomy. Mortality rate at three months was 27.2%. A poor outcome was associated with brainstem ischemia and a bilateral distribution of the stroke. Emergent surgical posterior fossa decompression is the treatment of choice in patients with cerebellar ischemia with clinical deterioration due to posterior fossa edema with overall good long-term results. However, patients with accompanied brain stem infarcts or bilateral stroke have an unfavorable prognosis.

The effects of the pandemic on neurosurgical activity: The experience of the Neurosurgery Departement of SUUMC

O Sirbu, C Popescu, O Zamfir, AV Chirtes, I Cusciac, C Nastase, E Popa, D Maris, M Mitrica

The challenges of patient management during the 2020 COVID-19 worldwide pandemic are immense and never-before-seen in our generation. We present the University Emergency Central Military Hospital (Bucharest, Roumania) Departement of Neurosurgery institutional experience in managing neurosurgical emergencies, elective spine surgeries or neurooncological procedures. Being a tertiary university trauma center in a dense populated capital city and addressing patients form a radius of nearly 300km numerous challenges had to be overcome. Futhermore, being a military hospital, medical personnel were deployed in first line of combat with the virus in Covid 19 Intensive care units. Protocols were constantly modified in order to adapt to a continuously changing healthcare environment. The Neurosurgical Departement efforts in protecting the medical personnel while providing best therapeutic management to our patients and their results are described. A comprehensive comparison with the same period one year prior the pandemic is analyzed.

Preservation rhinoseptoplasty in deviated noses

Anghel G.A., Anghel I., Urs R., Matasaru M., Bunea A., Tanase D., Bichir C., Anghel O.A.

Material, methods: In cases with deviated noses, the nasal septum is deviated and distorted.

Results: It is presented the experience in 31 cases with preservation septoplasty.

In classic techniques, the surgical correction consist in open approach, and may require extracorporeal septoplasty.

In preservation septorhinoplasty performed a modified “swinging door”technique that consist in preserve the septal cartrilage, integrity and vitality.

Conclusions: Devited Nose represent excellent modification nose for preservation septo-rhinoplasty.

Presevation will be applied to septplasty as well following the modified “swinging door”principle.

Thoracic surgery in complicated SARS-CoV2 pneumonia

Nistor C.E., Ilias M., Istrate A., Iordache A.M., Marin D., Pantile D., Gogolan G., Fera A., Ciuche A.

Introduction: SARS-COV 2 infection, a new clinical entity that has spread all over the world at the end of 2019, can lead to air-leak complications such as pneumothorax, pneumomediastinum, and/or subcutaneous emphysema, requiring a good collaboration between the thoracic surgeon and all the other specialists for their management.

Materials and method: A thorough analysis of all the 32 patients admitted over 9 months in our hospital for SARS-COV 2 pneumopathy complicated with pneumothorax, pneumomediastinum, and/or subcutaneous emphysema, as single entities or associated, was performed. In pneumothorax patients pleural drainage was performed, while in patients with pneumomediastinum associated or not with subcutaneous emphysema, subcutaneous needle insertion, and angiocatheter needles along with manual cutaneous decompressive massage were used.

Results: In most of the cases, despite initial improvement following surgery, evolution was unfavorable, especially in intubated patients, mostly due to both comorbidities and dynamic evolution of these patients towards ARDS.

Conclusions: Survival depends mostly on the degree of severity of the pulmonary lesions. SARS-COV2 infection and SARS-COV 2 pneumopathy must be included in the etiopathogenic classification of secondary spontaneous pneumothorax.

Minimally invasive approach in malignant pleuropericardial effusions – a retrospective study

Nistor C.E., Pantile D., Fera A., Gogolan G., Găvan C., Cucu A.P., Ciuche A.

Objective: From 2016 to 2019 a total of 146 patients admitted for malignant pleuropericardial effusions (MPPE) who underwent minimally invasive pleuropericardial drainage were included in a 3-years retrospective study.

Materials and methds: Ultrasound or CT-guided pericardiocentesis was performed before the actual surgical procedure in patients presenting with cardiac tamponade. In all selected cases patients underwent minimally invasive surgery. Pericardial window by either thoracoscopic or subxiphoid pericardiostomy was performed. Our surgical approaches allowed for both pericardial biopsy sampling and the creation of a pericardial window allowing pericardial fluid drainage. A hybrid technique using an electric scalpel and endoGIA endostapler was used for window creation. Talc powder or a combination of mechanical and chemical pleurodesis with betadine was used for the creation of pleural adhesions.

Results: We present comparative preoperative imaging, endoscopic operative images, and postoperative results in significant cases of MPPE included in this study.

Conclusions: Minimally invasive thoracic surgery is currently the best approach for MPPE, allowing for both an accurate diagnosis, and adequate treatment with low postopaerative morbidity and mortality compared to open surgery.

Modern technology in thoracic surgery

Nistor C.E., Marin D., Iordache A.M., Găvan C., Cucu A.P., Ciuche A.

Objective: This paper presents the positive influence of modern technology in thoracic surgery on of safety, extent, and duration of the intervention.

Materials and methods: We present imaging studies and pre-/intra- and postoperative images from several selected cases. Surgical technique was optimized by the use of modern technology-electrical vascular seal (LigaSure® & ENSEAL®), by ultrasound (HARMONIC®) and vascular staplers allowing safe surgical maneuvers and shortening of operating time.

Results: The use of modern technology enabled us to perform complex surgery, thus avoiding complications that might have endangered the patients' lives. Current stapling and bipolar devices allow easy and safe vessel stapling and sealing, in both minimally invasive and open surgery. Adaptive tissue technology permits enhanced surgical precision and performance across tissue variability.

Conclusions: The use of modern technology in thoracic surgery allows increased safety and efficacy of surgery, increased precision in complex surgery, and reduction of intraoperative and postoperative complications.

Treatment options in cervicothoracic chylous effusions

Nistor C.E., Ilias M., Istrate A., Gavan C., Cucu A.P., Ciuche A.

Objective: Chilous effusion have always been a real challenge for the thoracic surgeon, from the perspective of both conservative and surgical treatment

Materials and methods: Patients with various conditions, both traumatic and non-traumatic, complicated with chylothorax or cervical chylous fistula were treated in the Thoracic Surgery Clinic of our hospital. Metabolic and hydro electrolytic complications, as well as therapeutic strategies, were analyzed.

Results: Conservative treatment was reserved for selected cases, while the association of surgical treatment was necessary for most of the patients. Increased chylous output, despite optimal nutritional and pharmacological treatment, imposed the surgical treatment.

Conclusions: Minimally invasive surgery is currently essential for the treatment of cervical thoracic chylous effusions since conservative treatment becomes inefficient in high-output chyle leaks.

How easily can the diagnosis of ruptured brain arteriovenous malformation be missed?

I.R. Dumitru, C.A. Sîrbu

Introduction: Arteriovenous malformations (AVMs) have a small rate of occurrence, 10% of them being localised to the posterior fossa (cerebellum). Even though the diagnosis is relatively easy to establish, an incomplete anamnesis along with a superficial neurological examination might lead us towards false conclusions.

Methods and Materials: We'll present the case of a 23-year-old man, who arrived at the ER with vomiting, imbalance, laterodeviation, mild cephalagia and diplopia. We will point out the errors in diagnosis, from both the prehospital stage (biliary dyskinesia) and the hospital one (labyrinthitis), and the way by which the patient, a medical student and volunteer with the ambulance service, had a decisive role in solving his own medical case.

Results: The cerebral CT performed showed a spontaneous hyperdense cerebellar lesion (identified as a tumour, with no hope in a therapeutic path for the patient!). The MRA, and especially the "4-vessel" angiogram found a cerebellar AVM with medium flow, arising from the superior left cerebellar artery, with drainage through a unique vein in the left transverse sinus. Considering the characteristics of this specific case, embolization of the newly formed structure has been chosen as the preferred course of action, with optimal results.

Discussion and conclusions: The essential aspect which raised the patient's doubts was diplopia. It was this symptom that caused the complex investigations that preceded the correct diagnosis. The clinical abilities based on vast medical knowledge lead to the rational use of paraclinical investigations and the establishment of correct diagnosis.

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We look forward to your submission.

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Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as ‘What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?’ Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’ industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

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The Vancouver system of referencing should be used. In the text, references should be cited using superscript Arabic numerals in the order in which they appear. If cited only in tables or figure legends, number them according to the first identification of the table or figure in the text. In the reference list, the references should be numbered and listed in order of appearance in the text. Cite the names of all authors when there are six or less; when seven or more list the first three followed by et al. Names of journals should be abbreviated in the style used in MEDLINE. Reference to unpublished data and personal communications should appear in the text only.

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REVISTA DE MEDICINĂ MILITARĂ

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- *Towards an integrated medical system for radiological medical imaging investigations*
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EDITORIAL

Being a surgeon – A terrible and fascinating job

Dan Mischianu

Gral (R) Prof DAN MISCHIANU

Chief of Urology Clinic, Carol Davila Central Emergency Military Hospital
Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

At first it was a simple job: a barber, the surgeon, the "țirulic" (from Turkish language), the "gerah" (from Turkism "cerrah"), a job that did not dare to compare with the more "chosen" profession of "doctor/ physician". Doctors represented something else, they were much esteemed in the public consciousness, and the society really appreciated them. Later, people learned and found that being a surgeon (term surgeon derives from the Greek "cheirurgos" means one that does the job with their own hands!), is a noble, very noble profession, perhaps simply because its practitioners live "risky lives"!

Surgery is quite different from being a "doctor/ physician" and I assume this dichotomy trying to explain to the reader, in the most simple way: surgeons practice a risky job and live in this regard, particularly "dangerous"!

When I chose to serve in this profession I knew almost nothing about what it will happen to me during my professional life. I thought, as a medical student, that being a surgeon is a profession or a specialty as all the others, something that means to have a job and to go to "punch in", to sit in front of the "bench" (such as smiths!) or in front of your computer (if you are IT-ist!) and at H hour (usually 15:00) to get up from the desk, to close "the issue of the day" and leave never forgetting to "punch out". I never ever wanted something like this and God helped me in this regard! Now, in adulthood, I think I would not have been able to be part of such a "procedure"...

Then, shortly, nearly 40 years ago, I learned that being a surgeon really is something else…

Surgery has valences of the ineffable.

I stayed in the hospital even for 2-3 days, and when the Revolution came I got home on 04/01/1990, after 19 days of alarm. I have gathered in my life as a surgeon, eight and a half days of shifts performed in our homeland hospitals. I think know what I want to share...

I wondered, often, who can become a true surgeon.

You do not get to become a surgeon because you want, you become a surgeon by chance. You were a painstaking student, you've prepared well for the secondary exam (old style) or the residency (new style), you get an efficient score and choose a surgery specialty. You thought you can endure this challenge... "Absolutely by chance" – as George Topârceanu's verse from the poem "Jealousy"... Many of these future "surgeons" will regret this decision for the rest of their lives!

Let me explain: you will not become a good surgeon unless you have a vocation for this job. He who chooses the job of resident surgeon because he has passed the residency exam will curse his uninspired choice when he will find out he has no vocation for it.

I met such surgeons. They ended up withdrawing in resignation "pigmented" by completely different concerns.

And therefore I find that being a surgeon is a vocational profession, such as the athletes or airplane pilots.

Because I remembered two other noble professions, have you ever asked the question how "consumptive" is being a surgeon? How challenging is it – pre and postoperatively, how much rigor is needed to practice it in the fairest way possible and how many years it shortens surgeon's life in comparison with other doctors? I am absolutely convinced that you know at what age is over the "working life" of great athletes like Nadia Comaneci, Gheorghe Hagi and, to answer your previous question, the surgeon life is shortened for an average of ten years compared to other colleagues.

I dare to mention two other issues. There are, in present times, surgeons who know a "relative anatomy" of the human body. Professor Dr. Eugen Târcoveanu specified 12 years ago, in a fabulous article, one simple thing: "Although today there is no compulsory internship anatomy, anatomical knowledge are indispensable to any surgical careers. Clinical training must precede technical training and manuality. This apprenticeship has changed since surgical specialization has become a necessity. This is beneficial considering the complexity of some surgical fields. However, the formation of a surgeon must keep an overall basic surgery knowledge." (E. Țârcoveanu – A fi chirurg în zilele noastre – Journal of surgery, Vol 10, No 3, 2014, Iași ISSN 1584-9341)

I think it is a pity of the legacy of William Harvey! These surgeons usually do not even get to practice this profession. They fail in peculiar professions like politics, diplomacy, business, etc.

There's also a "second group" of surgeons: those who do not like the clinical surgery. I do not think it is conceivable that after you operate a human being, you dare, having all the knowledge and diplomas, not to examine, to consult, to mend and talk with that man. Observation of man by man gives us some truths that we can not learn by phone, computer or "skype"! Perhaps only this simple fact makes and will always make surgery a terribly fascinating job!

I wrote these lines in the first decade of December 2016 with great sorrow in my heart about what happens to us doctors, especially to those born and not made surgeons. I wrote these lines with hope for humanity and respect for hardworking values! Before they became values! And I can only repeat myself. I wrote it before. Stephen King wrote in "The Shawshank Redemption" (translated to us as "The angels prison"): "Hope is a good thing, maybe the best of all, because hope dies last!..." But dies in torment I allowed myself to add, with undisguised cynicism!

REVIEW ARTICLE

Article received on November 20, 2016 and accepted for publishing on January 12, 2017.

Towards an integrated medical system for radiological medical imaging investigations

Lidia Dobrescu[1], Silviu Stanciu[2], Cezar Pleșca[3], Armand Ropot[3]

Abstract: *The continuously increasing number of medical investigations using radiological methods imposes the strong necessity of informing patients about benefits and risks regarding radiation absorbed doses. Tracking the radiation doses absorbed by patients must be a future challenge of any medical system. The effective doses received by patients in many types of medical investigations must be calculated, transformed, recorded and cumulated. Doctors and patients must be very responsible in prescribing or demanding new radiological medical investigations. Radiological standards, legislation, guidelines, programmers and practice supervised by international commissions on radiology protection must include new specific measures for patients' cumulated doses. A pilot Romanian project had tried to accomplish some of these tasks.*

INTRODUCTION

Radiation is commonly associated with nuclear accidents or modern irradiative artificial sources such as: mobile phones, great antennas, satellites, food irradiation, and radioactive building materials.

Generally, radiation is associated with all kind of human activities.

As a huge surprise, in fact, radiation is quite a natural phenomenon in our lives. There are many radioactive substances in air, water and soil that are called natural background radiation. During a long airplane flight one can also receive an important radiation dose. Typically doses from natural sources are measured in mSv. The Sievert (Sv) is the International System of Units (SI) derived unit for radiation dose.

There are many other different units for absorbed radiation and transformations can be made in order to compare them.

A recent United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) publication [1] estimates worldwide distribution of radiation exposure:

- 13% from cosmic sources
- 16% from soil
- 9% from food
- 20% from medical investigations
- 42% from radon sources

Radon-222 is a radionuclide in the form of a gas that normally emanates from the soil. Radon is a colorless,

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odorless, radioactive gas. Most exposure to radon comes from being indoors in buildings. A strong interaction between smoking and radon has been proven, so radon is considered as a primary cause of lung cancer, smokers being more vulnerable [2]. There are no widely available medical tests to measure radon exposure. So radon belongs to natural sources of radiation.

The increased doses of radiation in our climate and habitat is surveyed by national or international organizations, there are standards, policies and strategies for general radiation protection.

International commissions on radiology protection are worldwide active for radiological standards, legislation, guidelines, programmers, and practice.

The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) highlights the levels and effects of exposure to ionizing radiation [1].

The International Commission on Radiological Protection (ICRP) has published a lot of reports on generally human protection and the specific protection of patients exposed to ionizing radiation [3].

In Romania, CNCAN is the national competent authority in nuclear field "having responsibilities of regulation, authorization and control stipulated in this Law is the National Commission for Nuclear Activities Control, public institution of national interest, legal entity, with the head office in Bucharest, being headed by a President having the rank of State Secretary, coordinated by the Prime Minister [4].

RADIOLOGICAL MEDICAL INVESTIGATIONS

Medical imaging is a complex process of visualization of the interior of a human body. A lot of techniques and engineering tools are used in order to obtain a visual representation:

- Radiology using X-rays;
- Computed Tomography (CT scan) using also uses special X-ray equipment;
- Nuclear medicine, using internal radioactive substances including scintigraphy that uses radio-isotopes travelling to organs, positron emission tomography (PET) or Single-photon emission computed tomography (SPECT) ;
- Mammography as a specific type of breast imaging that uses low-dose x-rays;
- Magnetic resonance imaging using the property of hydrogen atoms from human body to absorb or emit radiofrequency energy when placed in an external magnetic field;
- Medical ultrasonography or ultrasound, a diagnostic imaging technique based on the application of ultrasound;
- Endoscopy using an instrument inserted in the human organ;
- Thermography, using thermographic cameras and infrared imaging techniques;
- Elastography;
- Tactile imaging or mechanical imaging.

Only the first four investigations imply radiological methods in medical investigations. The last ones do not imply radiological risks, even there are scientific debates on SAR (specific absorption rate) as a dosimetric quantity for Electromagnetic Fields bio effects [5].

Patient exposure to ionizing radiation can be made externally, internally nor both. CTs doses are usually externally absorbed, while in angiography, fluoroscopy or scintigraphy investigations, a radioactive substance is ingested. Contrast media are also used on radiography and CT to visualize vessels or tissues. Iodine is a modern intravenous contrast agent, but barium is also used. Usually iodine contrast media is used intravenously, it can also be used intra arterially. Barium sulfate is usually swallowed. Magnetic Resonance Imaging or CTs also use contrast agents.

Differences between exposure and contamination can also be debated [6]. Exposure to a radiation source does not imply that this person becomes a new radioactive source. Contamination incorporates the radioactive agent in the body, such as in thyroid gland.

Exposure to X- rays increases the future incidence of cancer, but quantitative models predicting the level of risk are still not worldwide accepted. Different methods for radiation risk calculus are available. The

linear no-threshold (LNT) model assumes the proportionality between cancer risks versus absorbed dose. The relationship between dose and DNA damage is considered linear. The International Commission on Radiological Protection (ICRP) recommends the use of the LNT model [7].

Significant uncertainties are related to dose assessment, low dose and low dose-rate extrapolation approaches, transfer of risks from a population for which health effects data are available to one for which such data are not available [8]. Adaptive responses of human cells have also been observed at low doses [9].

Classical radiographies, computed tomographies (CTs) and scintigraphies are worldwide used medical radiological investigations that expose the patients to significant doses of radiation.

The actual volume of medical prescription, the contrast substances used in order to increase their accuracy, the overlapped investigations are general causes for a strong increasing of the cumulative radiation dose absorbed by patients. Patients and doctors usually excess in demanding, using and prescribing medical investigations in spite of harmful effects associated with radiation delivery.

The prescribed medical investigations total volume, starting from dental radiographies and ending at multiple regions CT scans prove a gap in monitoring and tracking of the cumulative radiation doses in many health services all over the world. The received radiation doses by patients are quit impossible to be cumulated in spite of the fact that in our modern world, 20 % of absorbed radiation dose is due to medical investigation.

The patients travel from one hospital to another, all over the country or all over the world.

An integrated system must be implemented. In order to cumulate all the absorbed doses by a patient in different types of radiological investigations, the doses measurement units must be the same and the recorded doses must be kept together in a single patient monitoring unit.

RADIATION DOSE RISK AND DIAGNOSTIC BENEFIT [10]

The treating physician and the radiologist can prescribe and carry out radiological imaging procedures. Several objectives and subjective elements are implied in a prescription:

- Medical request
- Imaging investigation algorithm
- The personal experience of the examiner
- Patient history
- The results of any other previous investigations

All the time a correct judgment concerning the benefit/risk ratio must be performed. Medicine and the radiation risks knowledge must be well handled. The aim of managing radiation exposure is to minimize the irradiative risk without sacrificing, or unduly limiting, the obvious benefits in the prevention, diagnosis and also in effective cure of diseases (optimization).

When too little radiation is used for diagnosis or therapy there is an increase in risk. Too low an amount of radiation in diagnosis will result in an image that does not have enough information to make a correct diagnosis. There are several ways that will minimize the risk without sacrificing the valuable information that can be obtained for patients' benefit. A possible measure is to carefully justify the examination before sending a patient to the radiologist or nuclear medicine physician. Repetition should be avoided in recently investigations performed into another clinic or hospital. An investigation may be seen as a useful one if its outcome – positive or negative – influences management of the patient. Another factor, which potentially adds to usefulness of the investigation, is strengthening confidence in the diagnosis. Most common examples of unjustified examinations include: routine chest radiography at admission to a hospital or before surgery in absence of symptoms indicating cardiac or pulmonary involvement (or insufficiency); skull radiography in asymptomatic subjects of accidents; lower sacrolumbal radiography in stable degenerative condition of the spine in the 5th or later decade of life, but there are of course many others [11]. From medical experience, the diagnosis

benefit is a priority. For a lung Rx examination any suspected image is repeated or supplementary investigated in a Computed Tomography.

Many times, the personal experience of the radiologist is the dominant factor in high radiation doses investigations:

- urgent exam such as trauma and polytrauma, fractured skull or spine where CT exams are a real benefit for the patient and an economic benefit for the hospital. They can shorten the patient’s hospital stay.
- scintigraphic investigation in thyroid diseases
- repeated mammography
- bone disease with seemingly benign tumors requiring an examination for differential diagnosis.

RADIATION MEASUREMENT UNITS

„The International Commission on Radiological Protection (ICRP) recommends that the public limit of artificial irradiation should not exceed an average of 1 mSv effective dose per year, not including medical and occupational exposures. ICRP limits for occupational workers are 20 mSv per year, averaged over defined periods of five years, with the further provision that the dose should not exceed 50 mSv in any single year“ [12].

The Sievert (Sv) is a derived unit in the International System of Units (SI) used for equivalent absorbed radiation dose measurement. This must be the central unit of a tracking medical system.

There are many different units for absorbed radiation used on a large scale. Becquerel (Bq) and Curie (Ci) as SI units are used for released radioactivity, while Coulomb/Kilogram (C/kg) and Roentgen (R) are used for the dose travelling through the air, Gray (Gy) and Rad, used with quantities of absorbed dose. A lot of Internet sites provide convertors between them.

The biological effects of the absorbed amount of radiation can be described with Roentgen equivalent man (rem) and Sievert (Sv). They are specific measurement units.

The Sievert can better describe the effective equivalent dose absorbed by biological tissues while the Gray can describe the absorbed dose of any material.

Table 1: Radiation Measurement Units

Type	Measurement unit		
Released radioactivity	Curie (Ci)	Non-SI	**1 curie = 3.7x10^{10} radioactive decays per second**
	Becquerels (Bq)	SI unit Derived	One becquerel represents the activity of a quantity of radioactive material in which one nucleus decays per second – **1 Bq = 1 s^{-1}**
Dose travelling through air (Exposure)	Coulomb/Kilogram (C/kg)	SI unit	**1 C/kg = 3876 R**
	Roentgen (R)	Non-SI	**1 C/kg = 3876 R**
Absorbed Radiation dose	Gray (Gy)	SI unit Derived	It is defined as the absorption of one joule of radiation energy per kilogram of matter **1Gy=1J/Kg=1m^2/s^2**
	Rad	Deprecated, replaced by the Gray	**1 rad = 0.01 Gy = 0.01 J/kg**
Dose equivalent (the biological effects of the absorbed amount of radiation)	Röntgen equivalent man (rem)	Non-SI	**1 rem=0,01Sv** **1 mrem/h = 8766 mrem/yr**
	Sievert (Sv)	SI unit Derived	represents the equivalent biological effect of the deposit of a joule of radiation energy in a kilogram of human tissue

Modern radiological apparatus for computerized tomographies or scintigraphies can provide the radiation doses during a particular investigation, but the recorded doses’ types and the radiation

measurement units in different types of investigations are not the same.

In the classic radiological investigation an important topic for data management is the measurement and the calculation of radiation dose expressed in Dose area product (DAP), expressed in (Gy*cm2). The dose length product (DLP) was chosen as input data for the system in CTs investigations and it is expressed in mGy*cm. The DLP must be converted to mSv in order to unify the results.

REQUIREMENTS OF A DESIGNED TRACKING SYSTEM

A tracking proposed system must include computers, smartcards for storing data and suitable software applications for recording, retrieving and alert. The effective doses received by patients must be unified and cumulated.

The system provides the replication of the information stored in central databases, local databases and patient cards to cover all the following possible situations:

- The patient goes to the doctor without the patient card. In this case, the system provides the data corresponding to the patient based on the local database, if the patient has been investigated in that hospital unit; if the patient is new, the system must provide the data from the central database to the local database, so as to take the optimal decision in recommending the type of investigation. After the investigation, the system must store the new local database accumulated radiation dose to the patient, the information arriving in the central database. Later, when the patient goes to the doctor for further investigations with the card, the system must ensure synchronization between the information stored on the card and information stored in the local and central database.
- The patient goes to the doctor with no card and the hospital unit's information system does not have access to the central database (ex: for mobile laboratories). If the local database contains the information corresponding to the patient, the doctor must be able to use them for recommending a particular type of investigation. After the investigation, the system must store in the local database the new radiation doses accumulated by the patient. Later, when the local system can access the central system, the information corresponding to the patient must be synchronized between the two databases.
- The patient presents the card to the doctor but the doctor does not have access to any database (local or central). In this case the doctor, using a computer with a card reader can access the history of investigations and the doses received by the patient, as the current cumulative dose calculated and can recommend the most appropriate investigation. After investigation, the appropriate dose must be recorded on the patient's card and next time when the patient goes to the doctor with the card, this information must be stored in both in the local and the central database.

Additionally the system provides applications for:

- Viewing in real time the history of investigations, of the doses delivered to the patient and of the current cumulative calculated dose expressed in mSv.
- Aiding the medical staff in taking the adequate decisions regarding the indication of investigations according to the current calculated cumulative doses and the maximum doses allowed for the risk and age groups.
- Performing of various periodic reports in order to take different types of decisions related with the existing radioprotection rules.

The proposed system includes the following modules:
a) Applications running on smart cards, called on card applications.
b) Off-card applications that are running on medical stations. They include smart card readers and writers in order to record and retrieve the information about the type of investigations and the specific emitted doses
c) A data base records all the necessary information in order to replicate a lost or destroyed card but also this database must provide the possibility of collecting data about the patients on several criteria, it must provide the possibility of standard or customized reports' creation.
d) The security solutions such as public key infrastructure PKI in order to achieve a high level of

security of recording and retrieving data. A public-key infrastructure (PKI) is a set of hardware, software, people, policies, and procedures needed to create, manage, distribute, use, store, and revoke digital certificates. A PKI establishes and maintains a trustworthy networking environment by providing key and certificate management services that enable encryption and digital signature capabilities across applications — all in a manner that is transparent and easy to use. Digital certificates are electronic credentials that are used to assert the online identities of individuals, computers and other entities on a network. They are issued by certification authorities (CAs) that must validate the identity of the certificate-holder both before the certificate is issued and when the certificate is used.

The entire system must be designed around the two types of cards: the patients' cards and the medical cards and it ensure many requirements ensuring secured communications.

Two different data flows must be implemented:

- Electronic cards flow management that includes: smart cards issuance, their renewal in case of loss, damage or theft.
- Operational cards flow management including: patients and doctors authentication using unique PINs.

Figure 1: Designed system

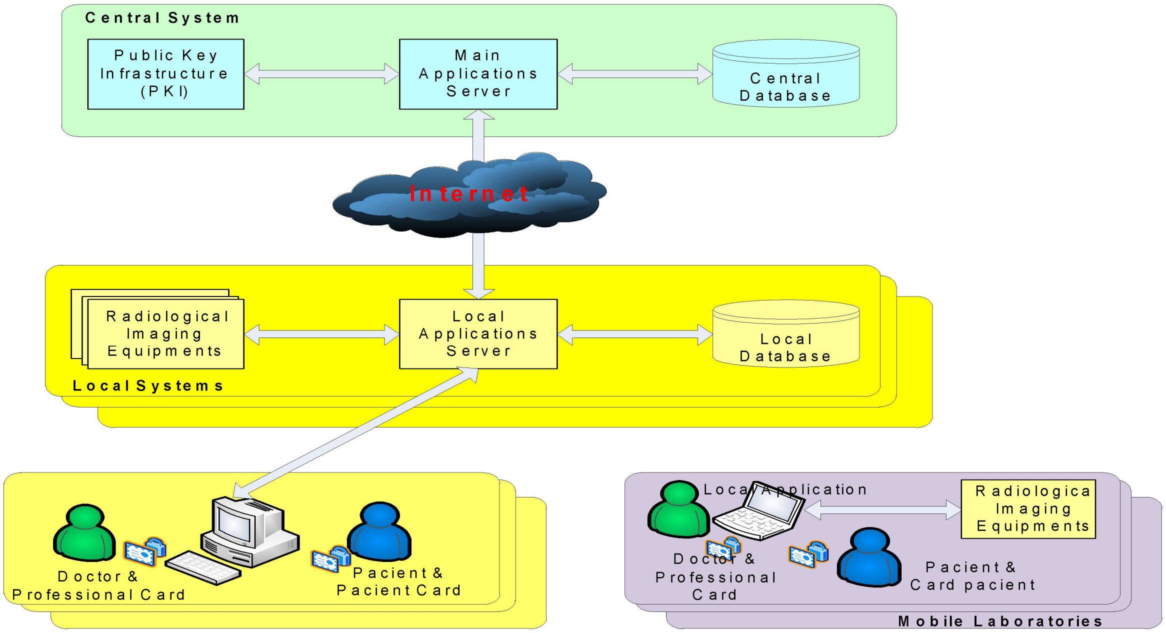
Central System
Public Key Infrastructure (PKI)
Main Applications Server
Central Database
Internet
Radiological Imaging Equipments
Local Applications Server
Local Database
Local Systems
Doctor & Professional Card
Pacient & Pacient Card
Local Application
Radiological Imaging Equipments
Doctor & Professional Card
Pacient & Card pacient
Mobile Laboratories

DESIGNED SYSTEM

A Romanian pilot study has been implemented the International System of Units uses the Sievert (Sv) as a derived unit in order to estimate the effective dose.

The new Romanian system is based on smart cards and Public Key Infrastructure and its structure is represented in figure 1. The system was implemented in Dr. Carol Davila Central Military Universitary Emergency Hospital, Bucharest, Romania.

The whole designed system and software application was the main result of the 187/2012 SRSPIRIM project in a Romanian National Partnerships Program, Collaborative Applied Research Projects – PCCA 2.

The system includes the following components:

- smart cards dedicated to patients: Citizen Radiation Safety Card (CRSC) and
- professional Radiation Safety Card (PRSC), designed for medical and investigation laboratories personnel. Among the last ones, one or more Administrator Radiation Cards are also designed and used in order to enroll patients in the application.

A common patient card and a doctor card are shown

in Figure 2.

Figure 2: Types of cards

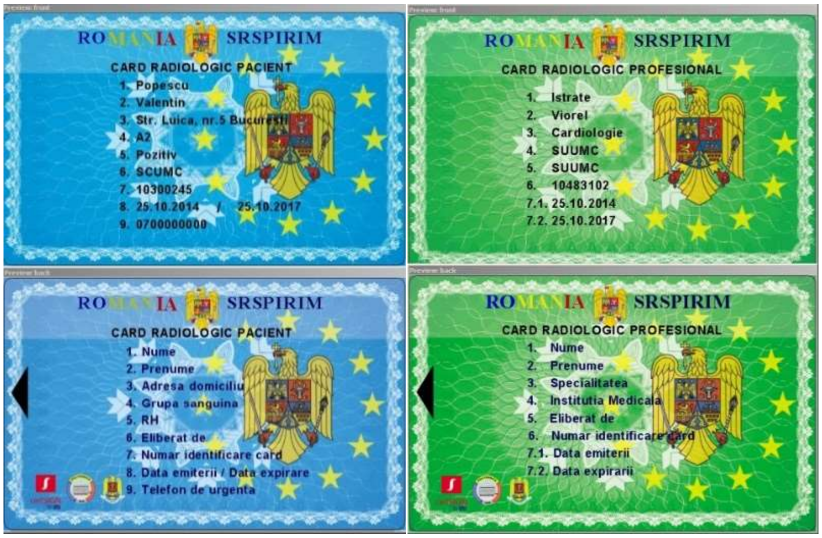

ROMANIA SRSPIRIM
CARD RADIOLOGIC PACIENT
1. Popescu
2. Valentin
3. Str. Luica, nr.5 Bucuresti
4. A2
5. Pozitiv
6. SCUMC
7. 10300245
8. 25.10.2014 / 25.10.2017
9. 0700000000

ROMANIA SRSPIRIM
CARD RADIOLOGIC PROFESIONAL
1. Istrate
2. Viorel
3. Cardiologie
4. SUUMC
5. SUUMC
6. 10483102
7.1. 25.10.2014
7.2. 25.10.2017

ROMANIA SRSPIRIM
CARD RADIOLOGIC PACIENT
1. Nume
2. Prenume
3. Adresa domiciliu
4. Grupa sanguina
5. RH
6. Eliberat de
7. Numar identificare card
8. Data emiterii / Data expirare
9. Telefon de urgenta

ROMANIA SRSPIRIM
CARD RADIOLOGIC PROFESIONAL
1. Nume
2. Prenume
3. Specialitatea
4. Institutia Medicala
5. Eliberat de
6. Numar identificare card
7.1. Data emiterii
7.2. Data expirarii

The hardware part of the system consists in computers, smart cards and card readers in order to record and retrieve the information about the type of investigations and the specific emitted doses.

The software includes a data base that records the commonly information on doctors, patients, types of medical procedures but also designed applications in order to replicate a lost or destroyed card.

Figure 3: Central database

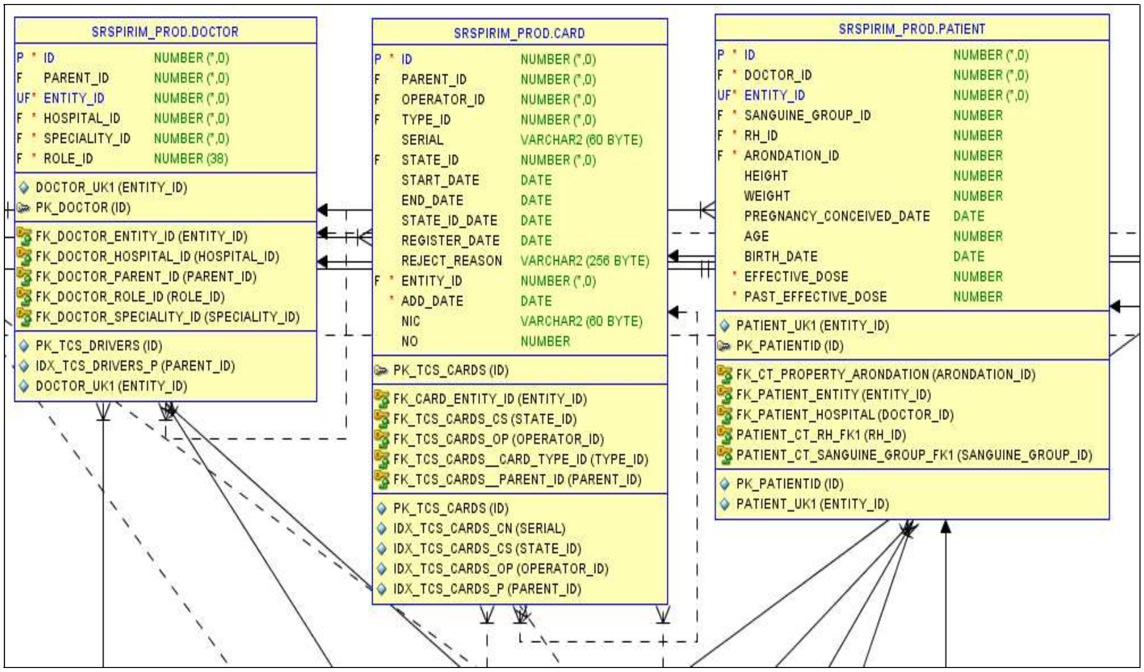

SRSPIRIM_PROD.DOCTOR

P *	ID	NUMBER (*,0)
F	PARENT_ID	NUMBER (*,0)
UF*	ENTITY_ID	NUMBER (*,0)
F *	HOSPITAL_ID	NUMBER (*,0)
F *	SPECIALITY_ID	NUMBER (*,0)
F *	ROLE_ID	NUMBER (38)

DOCTOR_UK1 (ENTITY_ID)
PK_DOCTOR (ID)

FK_DOCTOR_ENTITY_ID (ENTITY_ID)
FK_DOCTOR_HOSPITAL_ID (HOSPITAL_ID)
FK_DOCTOR_PARENT_ID (PARENT_ID)
FK_DOCTOR_ROLE_ID (ROLE_ID)
FK_DOCTOR_SPECIALITY_ID (SPECIALITY_ID)

PK_TCS_DRIVERS (ID)
IDX_TCS_DRIVERS_P (PARENT_ID)
DOCTOR_UK1 (ENTITY_ID)

SRSPIRIM_PROD.CARD

P *	ID	NUMBER (*,0)
F	PARENT_ID	NUMBER (*,0)
F	OPERATOR_ID	NUMBER (*,0)
F	TYPE_ID	NUMBER (*,0)
	SERIAL	VARCHAR2 (60 BYTE)
F	STATE_ID	NUMBER (*,0)
	START_DATE	DATE
	END_DATE	DATE
	STATE_ID_DATE	DATE
	REGISTER_DATE	DATE
	REJECT_REASON	VARCHAR2 (256 BYTE)
F *	ENTITY_ID	NUMBER (*,0)
*	ADD_DATE	DATE
	NIC	VARCHAR2 (60 BYTE)
	NO	NUMBER

PK_TCS_CARDS (ID)

FK_CARD_ENTITY_ID (ENTITY_ID)
FK_TCS_CARDS_CS (STATE_ID)
FK_TCS_CARDS_OP (OPERATOR_ID)
FK_TCS_CARDS__CARD_TYPE_ID (TYPE_ID)
FK_TCS_CARDS__PARENT_ID (PARENT_ID)

PK_TCS_CARDS (ID)
IDX_TCS_CARDS_CN (SERIAL)
IDX_TCS_CARDS_CS (STATE_ID)
IDX_TCS_CARDS_OP (OPERATOR_ID)
IDX_TCS_CARDS_P (PARENT_ID)

SRSPIRIM_PROD.PATIENT

P *	ID	NUMBER (*,0)
F *	DOCTOR_ID	NUMBER (*,0)
UF*	ENTITY_ID	NUMBER (*,0)
F *	SANGUINE_GROUP_ID	NUMBER
F *	RH_ID	NUMBER
F *	ARONDATION_ID	NUMBER
	HEIGHT	NUMBER
	WEIGHT	NUMBER
	PREGNANCY_CONCEIVED_DATE	DATE
	AGE	NUMBER
	BIRTH_DATE	DATE
*	EFFECTIVE_DOSE	NUMBER
*	PAST_EFFECTIVE_DOSE	NUMBER

PATIENT_UK1 (ENTITY_ID)
PK_PATIENTID (ID)

FK_CT_PROPERTY_ARONDATION (ARONDATION_ID)
FK_PATIENT_ENTITY (ENTITY_ID)
FK_PATIENT_HOSPITAL (DOCTOR_ID)
PATIENT_CT_RH_FK1 (RH_ID)
PATIENT_CT_SANGUINE_GROUP_FK1 (SANGUINE_GROUP_ID)

PK_PATIENTID (ID)
PATIENT_UK1 (ENTITY_ID)

This database provides the possibility of collecting data about the patients on designed criteria and future designed reports. Central database diagram, containing the recorded medical information are stored in many tables. A part of the design of the complex relationships between these tables is presented in Figure 3.

The security solutions include a Public Key

Infrastructure base (PKI), in order to achieve a high level of security of recording and retrieving data. A public-key infrastructure (PKI) is a set of hardware, software, people, policies, and procedures needed to create, manage, distribute, use, store, and revoke digital certificates. A PKI establishes and maintains a trustworthy networking environment by providing key and certificate management services that enable encryption and digital signature capabilities across applications — all in a manner that is transparent and easy to use. The application ensures a secured authentication of doctors and patients using digital certificates and cryptographic hardware. The whole logic application is stored in the central server. The clients use the minimum functional software. The applications users have access through a web browser. The system uses cryptographic libraries FIPS 140-2 or CC EAL4. The keys are RSA 1024 bits minimum length. The cryptographic hardware must support PKCS#11, PKCS#15 and Microsoft CryptoAPI. The cryptographic hardware must ensure the recovery of the cryptographic context. The certification system ensures roll–over facilities. The PKI system has an own relational database storing all the emitted certificates.

Figure 4: Effective dose calculus in radiological investigations

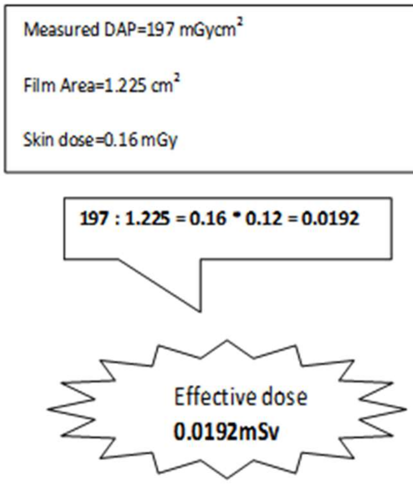

EXPERIMENTAL IMPLEMENTING

In the classic radiological investigation the calculus for radiation dose uses Dose Area Product (DAP), expressed in (Gy*cm^2) as an indicator of the total risk of inducing cancer. It also has the advantages of being easily measured, with a DAP meter on the X-ray set. In order to obtain the effective dose measured in Sv, the DAP is divided by the film area and then it is multiplied by the tissue factor [13] as shown in Figure 4. A sensible situation was determined by CT-s registered dose. More related CT radiation doses are available on CT consoles. Dose length Product DLP was chosen as input data for the system in CTs investigations but the required reported data is the effective dose. Conversion factors were used. The conversion factors can slightly vary from different manufacturers. Conversion factors for normalized effective dose per DLP depend only on the region of the body being scanned. All CT manufacturers provide tables with such conversion factors.

Conversion factors are also available for children of various ages, but this project does not include children [14]. Conversion problems were detected for multiple CT scans in one single examination, because more DLP are measured in the same examination and recording them becomes a long procedure.

EXPERIMENTAL RESULTS

Different studies were performed.
During a starting one month study in the hospital, the maximum cumulative dose has been overpass The CT exams are the most concerning prescribed imaging investigations that can lead to high cumulative doses.

The initially Figure 5 reveals some aspects from the initial study at the beginning of the project. The initial proposed threshold radiation dose of 20 mSv was frequently over passed and maybe a higher dose may be accepted for medical investigations in the future.

During a three years study in the hospital, different studies were performed.

The database contains 1125 records with 333 patients. From these records 42 patients were rejected in the final analysis because incomplete data.

These records contain all the investigations performed and all patients.

A lot of patients were examined in more than one investigation, so the system enrolled only 291 unique patients.

Figure 5: Cumulative radiation doses received by patients in the initial study

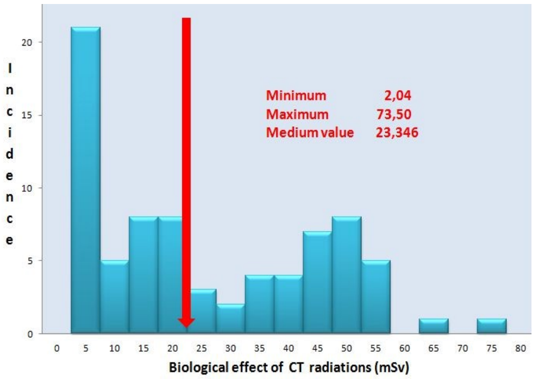

The recorded cumulated effective doses are shown in figure 6 and their distribution on decades is shown in figure 7.

Figure 6: Recorded doses in the project

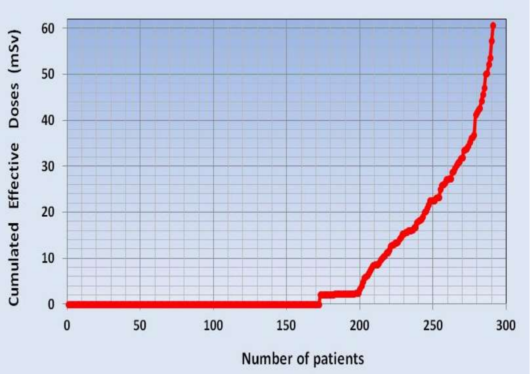

Figure 7: Recorded doses on decades

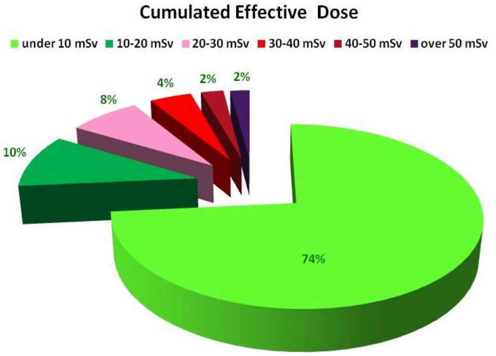

From these 291 patients, shown in figure 8 by gender, many of them have performed multiple investigations as in figure 9 and from them, in 47 cases, the received cumulated dose exceed the 20 mSv threshold dose, meaning 16,5% of cases.

Figure 8: Patients recorded in the project

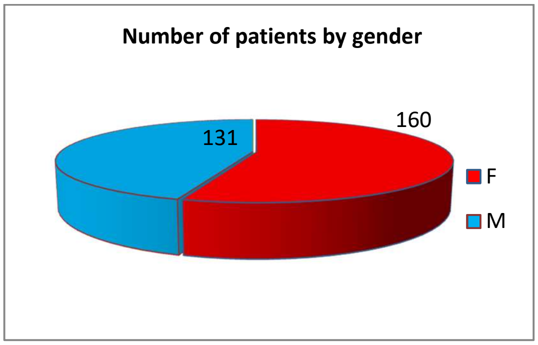

Figure 9: Patients recorded in the project

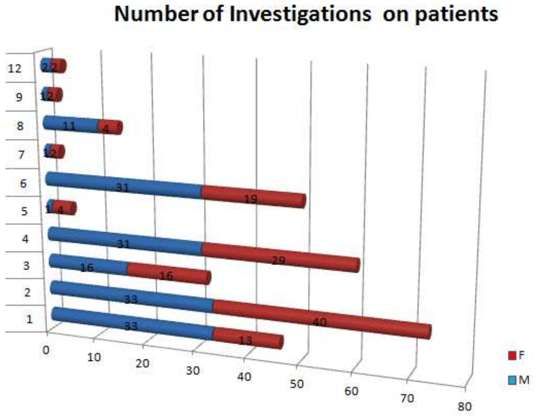

ACKNOWLEDGMENT

The results were obtained from the SRSPIRIM project 187/2012, in Romanian Partnerships Program, Collaborative Applied Research Projects Subprogram. The authors wish to address thanks to all the persons involved in this project for their support, work and ideas.

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REVIEW ARTICLE

General considerations regarding the infections with the Escherichia coli pathogen

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Abstract: *Escherichia coli is the species of the genus Escherichia with the greatest epidemiological impact. Escherichia coli infections are found mainly in places with poor hygiene; the infants with ages between 1 and 3 years old are included in the category with the highest risk. It is a "fecal-oral" transmission mechanism as a result of consumption of contaminated food or water, or by "dirty hands". The foods most commonly implicated in the transmission of the infection are unpasteurized milk and milk products, beef, especially the one insufficiently cooked, unpasteurized fruit juice, lettuce and insufficiently washed vegetables. The disease has been reported worldwide, being described numerous episodes of infection with Escherichia coli that caused multiple illnesses and deaths. Escherichia coli has three types of antigens: antigen "O" (somatic), antigen "H" (flagella) and antigen "K" (capsular). Clinical manifestations are present in the form of non-specific diarrhea, a dysentery form of enteritis, choleriform enteritis, hemorrhagic colitis and hemolytic uremic syndrome (HUS). The Escherichia coli infection diagnosis is made by identifying the etiologic agent and/or by highlighting the VTI toxin in the feces. The treatment consists in precautionary antibiotherapy, hydrodynamics and electrolyte rebalancing, blood transfusions and dialysis, if in the case of renal failure. The prevention of infections with Escherichia coli is achieved by personal hygiene, food hygiene and work hygiene.*

Keywords: *Escherichia coli, infection, toxin, treatment, hygiene*

INTRODUCTION

Escherichia coli is the species of Enterobacteriaceae, of the genus Escherichia, with the greatest epidemiological impact. Escherichia coli infections are found mainly in places with poor hygiene; infants are included in the category with the highest risk. Also, adults can develop severe forms of infections, especially in regions where underdevelopment, poverty and lack of hygiene are the dominant picture. The disease can occur in particular through the "fecal-oral" transmission mechanism as a result of consumption of contaminated food or water, or by "dirty hands". The foods most commonly implicated in the transmission of the infection are unpasteurized milk and milk products, beef, especially the one insufficiently cooked, unpasteurized fruit juice, lettuce

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and insufficiently washed vegetables. Processing raw food can contaminate kitchen utensils, especially if these are not separately used for each food category and are not cleaned between uses. Contamination can occur through contact with carrier animals, if the basic rules of personal hygiene are not complied with, such as washing hands with soap and water.

In recent years, in nearly all parts of the world have been reported numerous episodes of infection with Escherichia coli, resulting in numerous illnesses and even deaths. We recall the tragedy of Walkerton (Ontario/Canada), when approximately 5,000 people from the community showed symptoms specific to the digestive infection, the etiological agent being Escherichia coli [1]. In 2011, in Germany broke one of the largest outbreaks of acute gastroenteritis with hemolytic uremic syndrome (HUS) caused by the E. coli O104: H4 strain (German hemolytic uremic syndrome/ enterohemorrhagic E. coli - GHUSEC). The outbreak has had an unusual epidemiological origin only partially resolved. Investigations conducted by authorities showed that the illnesses occurred amid the consumption of contaminated water by agricultural runoff into a water well and subsequently there was the groundwater contamination [2].

The latest outbreak of the infection with Escherichia coli is evolving in Romania with this disease being contracted by 12 children (so far), aged between 1 and 3 years, all coming from localities in Argeș County.

The particularly severe infection resulted in the death of 3 children and even though the competent bodies through their health authorities have sought to find the source of the outbreak, they have not been able until now to find the source of the infection.

After the latest information, this seems to have been about a false "outbreak of diarrheal disease with uremic syndrome"; in reality the infectious etiology is varied, as confirmed by the Cantacuzino Institute.

In conclusion, due to the lack of a common source for the contagion, there cannot be a question of an epidemic or outbreak as the cases are isolated; however, due to the media pressure the incident could be termed as a "media outbreak".

ETIOLOGY

The genus Escherichia is of the Enterobacteriaceae family which comprises of 44 genera, 25 of which are found in human pathology. Most of these genres include commensal bacteria very abundant in nature (soil, water, etc.) [3], their natural habitat being the gastrointestinal tract of humans and warm-blooded animals, their feces containing more than 1010 enterobacteria/gram [4]. The constant presence of Escherichia coli strains in human and animal intestines and implicitly in the feces, determined that this bacterium represents the indicator of fecal pollution of the environment, especially of the water. Within the genus, are known 6 species: Escherichia coli, Escherichia fergusonii, Escherichia hermannii, Escherichia vulneris, Escherichia albertii and Escherichia blattae, the latter being isolated due to the fact that it only comes from specific species of insects.

Figure 1: Escherichia coli
(http://www.telegraph.co.uk 620 × 387)

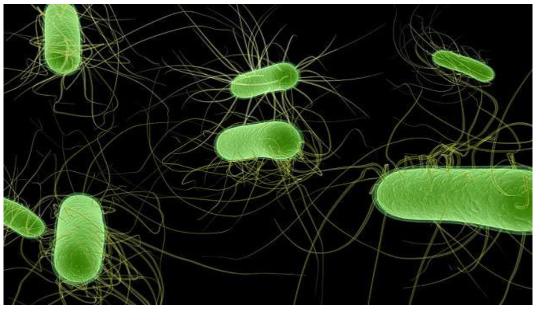

The most important representative of the genus is Escherichia coli, bacterium discovered by Austrian pediatrician Theodor Escherich. In 1886, after a hard clinical and laboratory activity he has presented at the Faculty of Medicine in Munich and subsequently published in Stuttgart, a monograph on the link between intestinal bacteria and the physiology of digestion in children entitled "Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung" [5]. In 1919, Castellani and Chalmers proposed that the name of the isolated bacillus from the colon (Bacterium coli commune), by the renowned Austrian pediatrician Theodor Escherich be changed in Escherichia coli, in his honor, a name that was officially approved in 1958 [6]. Bacteria are present in the form of straight or slightly curved bacilli with rounded ends,

the presence of flagella and therefore the mobility being constant in most species (Figure 1). It has a size between 2-5 µm/0.5-1 µm and from a tinctorial point of view is Gram-negative.

It grows on simple culture media (simple glucose broth, nutrient agar and blood agar) developing specific Type S colonies, round, domed, shiny with a smooth surface (Figure 2) and regular edges or with dry R colonies with irregular edges.

Figure 2: Escherichia coli culture on blood agar (http://www.wzzm13.com 534 × 401)

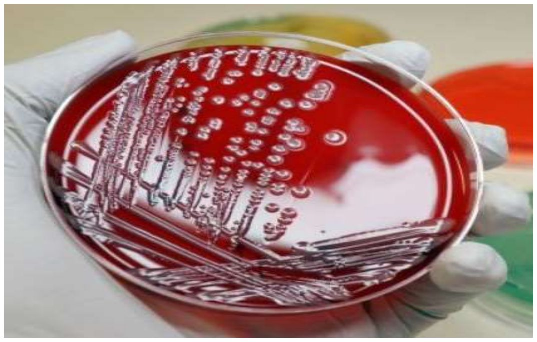

Biochemically, is fermenting the glucose with the production of gas, it produces indol, β-galactosidase and ferments lactose, characteristic that is allowing the differentiation from other enterobacteriaceae. Escherichia coli has an aerobic or optionally anaerobic metabolism and develops at an optimum temperature of 370C and pH between 7.2-7.8 [7], but differs from other coliform bacteria by increasing to 44^0C.

THE ANTIGENIC STRUCTURE

Escherichia coli has three types of antigens that can be highlighted by serologic antigen-antibody reactions:

- The O antigen (somatic) represented by a lipopolysaccharide surface antigen, thermostable, common to all S forms of the Enterobacteriaceae family;
- The H antigen (flagella) is a proteinaceous antigen (flagellin), heat-labile present in bacteria flagella;
- The K antigen (capsular) which may be of a polysaccharide or protein (fimbriae) nature. The O and H antigens are transmitted via plasmids and can be found together with the K antigens that are chromosomally determined.

Up until now, have been identified more than 170 types of the O antigen, more than 50 types of H antigen, 90 types of K antigen and several types of fimbriae antigens (F antigens). Combining all these antigenic variants, results in more than 1,000 antigenic types of Escherichia coli.

CHARACTERISTICS OF PATHOGENICITY

Colonic microflora in healthy persons (normal intestinal microbiota) is dominated by strains of Escherichia coli that live in a symbiotic relationship with the host. These infections may occur only if the host is immunocompromised or when it crosses the intestinal barrier and reaches areas which would normally be sterile. There have also been described pathogenic strains of Escherichia coli which have an exacerbated virulence with the possibility of being able to multiply, triggering infectious processes in immunocompetent individuals. Currently, have been described 6 intestinal Escherichia coli pathotypes involved in the EPEC diarrheal syndrome (intestinal pathotypes) and a pathotype involved in extraintestinal infections (urinary tract infections, meningitis, septicemia), ExPEC [8]. It is the most common etiologic agent in urinary infections, being incriminated in over 80% of urinary tract infections (UTI).

The health of the host (diabetes, immunodeficiency, colon malformations, obstructive uropathies, etc) is predisposing factors for infection with Escherichia coli.

Intestinal pathotypes are: enteropathogenic Escherichia coli (EPEC), enterotoxigenic Escherichia coli (ETEC), enteroinvasive Escherichia coli (EIEC), verotoxin producing Escherichia coli (VTEC), enteroaggregative Escherichia coli (EAEC) and diffuse adhesion Escherichia coli (DAEC). Within the VTEC pathotype stands out, mainly to highlight the association with severe clinical manifestations (haemorrhagic colitis), the subset of strains of the enterohaemorrhagic Escherichia coli (EHEC), whose prototype is the O157: H7 serotype. The pathogenic Escherichia coli strains produce virulence factors that contribute and affect the processes undertaken at cell level in the host (the protein synthesis the cytoskeleton's function, the cell division, ion secretion,

the mitochondrial function, apoptosis). Adhesins are adhesion structures located on the fimbriae/pili (P fimbriae from the uropathogenic strains of Escherichia coli, the CFA colonization factors from the ETEC strains) or on the surface of the outer bacteria membrane (Afa afimbriae adhesins from DAEC strains). They are important virulence factors that allow bacteria to colonize areas of the human body (the small intestine, the urinary tract, the vagina), in which it does not normally reside. Toxins are another category of virulence factors. Endotoxins are part of the structure of the outer membrane (the A lipid from the LPZ complex) and are present in all Gram-negative bacteria. Released upon the elimination of the bacterial cell wall, they manifest their toxic effects being involved in the occurrence of the fever and the toxic shock from septicemia. Exotoxins are synthesized in the processes of the bacterial metabolism, being specific to the various pathotypes of Escherichia coli. Currently, are known the structure and the functions of many toxins responsible for the pathogenicity of strains of Escherichia coli (e.g., heat-labile enterotoxin produced by ETEC strains, verotoxins 1 and 2 synthesized by the VTEC strains, enteroaggregative Escherichia coli heat-stable enterotoxin 1 of EAEC strains etc.). Another important component of the pathogenicity of the species is the capsule. The main role of the capsule is to inhibit the action of the serum complement and the phagocytosis, thus imparting bacterial resistance. The K1 capsular polysaccharide antigen is present in 80% of Escherichia coli strains, isolated from the cases of neonatal meningitis.

THE DISEASE

Most of the Escherichia coli outbreaks were associated with the presence of the O15: H7 (VTEC) serotype. The incubation period of the disease varies between 1 and 9 days, averaging 3-5 days. Clinical manifestations are extremely varied ranging from non-specific diarrhea, dysentery form of enteritis (with an invasion of the colon), choleriform enteritis (with the invasion of the jejunum-ileum mucosa) to hemorrhagic colitis and consecutively the hemolytic uremic syndrome (HUS). The hemolytic uremic syndrome is characterized by acute renal failure, thrombocytopenia and angiopathic hemolytic anemia. The most common form of HUS is associated with diarrhea and has the highest incidence in children, being the major cause of acute renal failure in children [9, 10]. The factor responsible in HUS manifestations occurred after the VTEC infection and the Shiga dysentery is a protein enterotoxin called the Shiga toxin or VTI. Initially, the hemolytic uremic syndrome mortality rate reached to 50%, but the evolution of medicine and modern treatment techniques have reduced the mortality rate to 3-5%, indicating that approximately 30% of the healed patients remain with long-term sequelae, such as acute kidney failure [8]. Escherichia coli is the cause in 80% (ExPEC pathotype) of ITU (urethral cystitis, cystitis, nephritis, pyelonephritis, etc.). Contamination may be downward (blood-borne) or upward, this route being the most common. Infection is favored by the existence of obstructive congenital lesions, post gonococcal infection strictures, urinary stasis via decubitus or pregnancy. Once the bladder mucosa is invaded, subsequently occurs the upward progression to the kidney and the infection stabilizes at this level as a result of slow mobilization of the phagocytes [11].

Lab diagnosis

The diagnosis of the Escherichia coli infection is made by addressing two strategies namely, identifying and highlighting the etiological agent of the VTI toxin. The usual method used by most diagnostic laboratories is the selective isolation of the etiologic agent (Escherichia coli O157H7; O157H), on the MacConkey with sorbitol culture media from stool [12]. The method’s principle is that the strains of Escherichia coli O157: H7 do not ferment sorbitol in 24 hours and the colonies appear colored on the culture, characteristic that differentiates them from the other strains of Escherichia coli. If the isolated strains are suspected as belonging to the serotype O157, but ferment the sorbitol in 24 hours, then will be used the specific antisera for titration and the agglutination tests [13]. The detection of the VTI toxin can be achieved through the cytotoxicity method, cultivation of isolated strains from feces on Vero or HeLa cell lines. The genetic methods (PCR) are based on the detection of specific sequences of polynucleotides that encode the synthesis of VTI. A new and very precise method is MALDI TOF, namely the proteomics analysis through

mass spectrometry of the specific proteins extracted from the ribosomes [14]. The principle of the method consists in measuring the mass of the weights of the polypeptides from the sample to be examined, generating and comparing its spectrum with the spectrum from the data base of the mass spectrometer (figure 3).

Figure 3: Maldi TOF operating flow (after Bruker Daltonix)

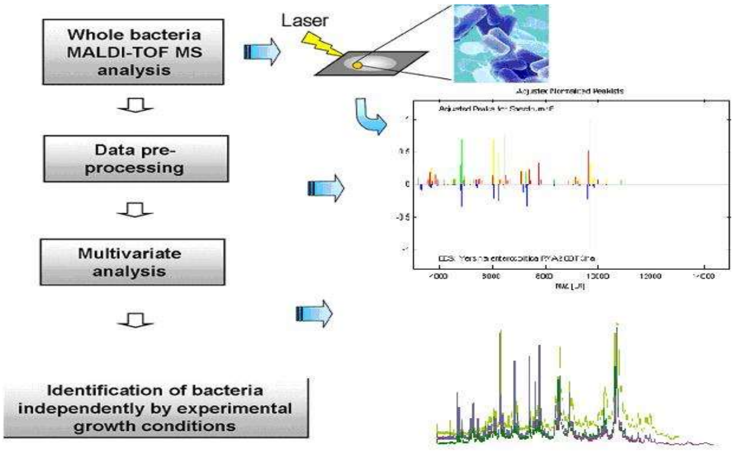
Whole bacteria MALDI-TOF MS analysis
Laser
Data pre-processing
Multivariate analysis
Identification of bacteria independently by experimental growth conditions

Other lab diagnostic techniques that are being used are the ELISA, for the detection of the O 157 antigen from feces and the serological methods which, however, are of limited value because Escherichia coli does not always develop serum antibodies against the VTI toxin. Also, the LPS antigen used may develop cross-reactions with other biological agents, such as Brucella abortus.

Treatment and Prevention

The treatment of the infection with Escherichia coli O157:H7 is often symptomatic and consists in the treatment of the complications arising due to massive fluid loss, and consequently of the acute diarrhea condition. As a result of dehydration and permeabilization of the blood vessels, pathological changes take place in the kidney function with renal impairment and severe anemia, that may become complicated with hemolytic uremic syndrome (HUS), as apparently is the case in the current outbreak in Romania, since winter-spring 2016. The therapeutic management must take into account the avoidance of the abuse of antibiotics that can foster the development of strains of Escherichia coli, avoiding the administration of antispasmodic drugs with effect in slowing bowel, thus maintaining more toxins in the digestive tract and amplifying its harmful effects. It recommended avoiding administering products based on salicylates and ibuprofen that can increase digestive bleeding. The symptomatic treatment must include hydrodynamics and electrolyte rebalancing, blood transfusions and, if in severe forms of renal failure, dialysis. Prevention of disease is achieved by non-specific measures such as: washing hands with soap and water before and after eating; processing animal products and by-products; washing fruits and vegetables before consumption or industrial processing; avoid the consumption of raw animal products (milk) or semi-raw (hamburger); avoid the consumption of water from bacteriologically unverified sources; washing utensils that came in contact with meat or raw meat products; avoiding the use of unpasteurized juices, in particular for children, etc. In serious cases, potentially fatal, especially in young children, may be needed the treatment from the Intensive Care with perfusions, resuscitation and even peritoneal dialysis.

CONCLUSIONS

- Escherichia coli is from the Enterobacteriaceae species present in the colonic microflora of a healthy human, which has the largest epidemiological impact.
- The transmission mechanism of the infection is

"fecal-oral" as a result of the consumption of contaminated food or water, or by "dirty hands".

- The disease has been reported worldwide, being described numerous episodes of infection with Escherichia coli that caused multiple illnesses and sometimes even deaths (Walkerton Ontario/Canada, GHUSEC - Germany).

- Escherichia coli has three types of antigens: antigen "O" (somatic) antigen "H" (flagella) and antigen "K" (capsular) that can be highlighted by serologic antigen-antibody reactions. Combining all these antigenic variants theoretically result in 1,000 antigenic types of Escherichia coli.

- There are six known pathotypes of Escherichia coli involved in the EPEC diarrheal syndrome (intestinal pathotypes) and one pathotype involved in extraintestinal infections (urinary tract infections, meningitis, septicemia), ExPEC.

- Toxins are the most important category of virulence factors in Escherichia coli (heat-labile and heat-stable enterotoxin produced by ETEC strains, verotoxins 1 and 2 synthesized by VTEC strains, enteroaggregative heat-stable enetrotoxin of EAEC strains etc.).

- Clinical manifestations are extremely varied ranging from non-specific diarrhea, dysentery form of enteritis (with an invasion of the colon), choleriform enteritis (with the invasion of the jejunum-ileum mucosa) to hemorrhagic colitis and consecutively the hemolytic uremic syndrome (HUS).

- The Escherichia coli infection diagnosis is made by identifying the etiologic agent (biochemical tests, agglutination reactions, PCR, mass spectrometry) and by highlighting the VTI toxin (cell culture cytotoxicity assay, immuno-chromatographic methods, ELISA).

- The treatment is symptomatic and includes hydrodynamics and electrolyte rebalancing, blood transfusions and, if in severe forms of renal failure, dialysis.

- The prevention of infections with Escherichia coli is achieved by non-specific measures such as: washing hands with soap and water before and after eating; processing animal products and by-products; washing fruits and vegetables before consumption or industrial processing; avoid the consumption of raw animal products (milk) or semi-raw (hamburger); avoid the consumption of water from bacteriologically unverified sources.

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REVIEW ARTICLE

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Clinical features of oral angiolipoma – A review

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Abstract: *The aim of this paper was to analyze the data obtained from case reports of oral angiolipoma in terms of age and gender distribution, site of occurrence, nature (intraosseous/ extraosseus) and infiltration. an internet search using Google scholar and Pubmed engine was carried out using search terms 'angiolipoma' and 'oral'/'mouth'. English literature full text articles and abstracts of oral angiolipoma obtained from 1976-2016 were analyzed for clinical data and presented in this article.*

Keywords: *angiolipoma, oral, mouth*

INTRODUCTION

Angiolipoma (AL) is rare variant of lipoma which consists of both fatty and vascular elements. [1] ALs were first describe by Bowen in the year 1912. [2] The first published report of an oral angiolipoma was by Davis et al in 1976. [3] Angiolipomas account for 5 to 17% of all the lipomas occurring in the oral cavity, lipomas themselves account for 1-5% of all oral benign tumors [1]. Since oral angiolipoma is rare benign tumor, published reports are mainly restricted to case reports. [4,5]

CLASSIFICATION OF ANGIOLIPOMAS

Based on their studies Gonzalez-Crussi et al. classified angiolipomas into infiltrating and non-infiltrating. angiolipomas.[6] The non-infiltrating angiolipomas are encapsulated, whereas infiltrating angiolipomas lack this capsule.[6] Among the two, infiltrating angiolipoma (IAL) is a rare lesion typically characterized by infiltration of the surrounding structures, especially skeletal muscle.[7] Based on whether the angiolipoma occur in bone or soft tissues they can be classified as intra-osseous or extra-osseous types.[8] The intra-osseous variety is extremely rare in comparison to the extraosseus variety. [8,9]

METHODOLOGY FOR ANALYSIS OF THE CASE REPORTS

An analysis of the data obtained from case reports of oral angiolipoma in terms of age and gender distribution, site of occurrence, nature (intraosseous/ extraosseus) and infiltration. An internet search using Google scholar and pubmed engine was carried out using search terms 'angiolipoma' and 'oral'/'mouth'. Full text and abstracts of oral angiolipoma cases obtained from 1976-2016 were analyzed. (Table 1)

GENDER PREDICTION OF ORAL ANGIOLIPOMA

Fregnani ER et al (2003) stated that male to female

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ratio from these intraoral angiolipomas was 1.4:1, in contrast to cutaneous angiolipomas and intraoral lipomas as a group, which either shows no sex predilection or a slight female predilection.[10]

Table 1: Clinical data obtained from full texts and abstract of oral angiolipoma case reports published from the year 1976-2016

Researcher	Year of publication	Type infiltrating/ non-infiltrating	Gender	Age group	Site	Extraosseous/ intraosseous
Davis et al [3]	1976	-	-	-	hard palate	
Polte HW et al [30]	1976	-	-	-	mandible	intraosseous
Weitzner et al [35]	1978	-	male	4-year-old	cheek	
Campos et al [34]	1980	-	-	-	cheek	
Lewis DM et al [31]	1980	-	-	-	mandible	intraosseous
Brahney CP et al [21]	1981	-	-	-	tongue	
Flaggert et al [19]	1986	-	female	8-year-old	palate	
Reilly et al [15]	1988	-	female	infant	parotid	
Lin SC et al [17]	1989	-	male	49-year-old	tongue	
Manganaro AM et al [32]	1994	-	-	-	mandible	intraosseus
Ali MH et al [36]	1996	-	-	-	cheek	
Sugiura J et al [16]	1999	infiltrating	-	-	muco-buccal fold	
Ida-Yonemochi H et al [12]	2005		male	69-year-old	buccal mucosa	
Altug HA et al [11]	2009	non-infiltrating	male	22-year-old	cheek	
Gerard N et al [20]	2009	-	-	-	upper lip	
Thakur B eta al [37]	2010	non-infiltrating	female		cheek	
Palaia G et al [38]	2011	non-infiltrating	female	66-year-old	cheek	
Yanase S et al [13]	2011	non infiltrating	male	76-year-old	cheek	
Sah K et al [4]	2012	non infiltrating	female	9-year-old	upper lip	
Hemavathy S et al [9]	2012	-	female	21-year-old	mandible	intraosseus
Silva-Junior GO et al [5] 2 cases	2013	non-infiltrating	male	57-year-old	cheek	
	2013	non-infiltrating	female	29-year-old	masseteric region	
Shahi AK et al[14]	2014	infiltrating	female	9-month-old	cheek	
Mohiuddin SA et al [39]	2014	non-infiltrating	male	46 year old	mandibular buccal vestibule	
Ohnishi Y et al [7]	2015	infiltrating	female	74 year old	lower lip	
Chandrasekaran D et al [1]	2016	non infiltrating	female	55-year-old	palate	

However most of the other researchers have stated that no gender predilection. [1,7,11,12] According to the data obtained from the analysis of the reports, seven cases were in males, ten cases occurred in females. However we could not obtain enough data regarding gender in 9 case reports.

AGE PREDILECTION OF ORAL ANGIOLIPOMA

Fregnani ER et al (2003) stated that the mean age of the affected patients was 37 years (range, 4 to 81 years),which was in contrast to other variants of lipomas occurring in the oral cavity that occur in the 50-60 year age group.[10] On analysis of the available case reports we found that occurrence of oral angiolipoma ranged from a 4-month-old to 76-year-old individual.[13,14,15] Infiltrating angiolipomas are usually diagnosed in older individuals.[7, 16, 17, 18]

SITE PREDILECTION OF ORAL ANGIOLIPOMA

Angiolipoma have a prediction of occurrence in the

buccal mucosa, followed by lip.[5] Occurrence in palate has been reported in few cases.[1,3,19]. Upper lip involvement is more common than lower lip.[4, 20] Few cases have also been reported in tongue.[17, 21] However many of the cases of angilipoma of tongue have the muscle component along with fat and vasculature and thus are termed as angiomyolipoma.[22,23, 24, 25]. The occurrence of angiolipoma in the parotid has also been reported.[15] The benign angilipomas occurring the salivary gland have glandular component apart from adipose and vascular components and are called as sialoangiolipoma.[26] Such cases have been reported to have been observed in parotid and submandibular gland.[27, 28] A rare case of a lipoma with vascular and salivary gland components has been reported in palate also.[29]

INTRAOSSEOUS ANGIOLIPOMA

The first case of intraosseous angiolipoma was by Polte in the year 1976.[30] Mandible is the most common site of occurrence of intraosseous angiolipoma.[9,31,32] Intraosseous lipoma may present a range of clinical manifestations such as swelling, or presence of neurologic signs (hypesthesia or paresthesia) or can be totally asymptomatic.[9] On radiographic examination the lesion usually presents as well-circumscribed radiolucency.[9] Extraction related trauma is believed to be cause for intraosseous angiolipoma of the jaws. Fatty metamorphosis of a central hemangioma and possible variation osteoporotic bone marrow defect are other suspected etiologies. [33]

INFILTRATING ANGIOLIPOMA

Infiltrating angiolipomas lack encapsulation and are typically characterized by infiltration of the surrounding structures.[6,7,14,16] In contrast to the non-infiltrating angiolipomas are well encapsulated. [34,35,36,37,38]. Infiltrating angiolipoma usually occur in older individuals but a report of 9-month-old diagnosed with infiltrating angiolipoma has recently been published.[14] Infiltrating angiolipomas are also associated with symptoms muscular pain and neural deficits.[39,40]

CONCLUSION

In this review we have made an attempt to analyze the clinical features of oral angiolipoma in terms of its gender predilection, site predilection, infiltrative nature, intra/extraosseus types. The information obtained from this review could aid the clinicians in diagnosis this extremely rare benign lesion.

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SYSTEMATIC REVIEW

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Hepatocellular carcinoma – A review

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Hepatocellular carcinoma is a major public health issue, occupying the fifth place of the most common cancers and the third leading cause of cancer in the world. The incidence of HCC has increased in the past decade thanks to higher rates of HCV infection and improvements in the management of cirrhotic patients. The regions with the highest incidence of HCC are, in descending order: Eastern Asia, sub-Saharan Africa, South-East Asia and southern Europe. It has been observed that etiological factors which lead to HCC have a heterogeneous distribution; thereby, in Asia and Africa the most common risk factor is HBV infection, increased by ingestion of aflatoxin B1-contaminated food that is associated with a specific mutation in the p53 tumor suppressor gene. HCC may develop earlier in these regions, most common in a noncirrhotic liver. In developed countries, the principal risk factors are HCV infection and alcohol-related cirrhosis [1].

RISK FACTORS

The main risk factors involved in the development of HCC are chronic viral infections, hemochromatosis and alcoholic cirrhosis. Other risk factors which may lead to HCC are cirrhosis secondary to autoimmune hepatitis and Wilson disease. In the last years, diabetes was suggested as HCC risk factor in numerous studies. However, it is difficult to study the possible association between the two concepts, because diabetes is a risk factor for nonalcoholic liver disease and nonalcoholic steatohepatitis , which may lead to the appearance of liver fibrosis, of cirrhosis, and finally to HCC, being known the fact that the principal risk factor is the presence of cirrhosis, no matter of the cause. On the other hand, end stage liver disease may herself determine glucose intolerance and diabetes. Studies show that >90% of patients with cirrhosis have glucose intolerance and 30% have diabetes [2].

Cirrhosis is a premalignant statement independent to the etiology. Liver cell dysplasia is probably the intermediate step. Patients with cirrhosis and high rates of cellular proliferation are at high risk to develop hepatocellular carcinoma.

HBV and HVC. It has been shown that there is an interval of 15-40 years between the development of chronic hepatitis and the appearance of hepatocellular carcinoma and the distance is shorter for the virus C infection. For hepatitis B, is important to know the age at which the infection occurred- patients infected in the prenatal period or in childhood are at high risk to develop chronic hepatitis or to become chronic carriers and at the same time to develop cirrhosis and hepatocellular carcinoma.

HBV is responsible for almost 80% of hepatocellular

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carcinoma. Vaccination against HBV and antiviral treatment are the most efficient ways for prevention. HBV seems to be directly and indirectly carcinogenic. There is a connection between the level of replication of virus B (viral load) and the risk of hepatocellular carcinoma. HBV integrates into the genome host and the viral load precedes almost always the development of hepatocellular carcinoma, leading to mutations at the place of integration and high levels of TNF-α. HBx protein inhibit the p53 tumor suppressor gene and activate genes implied in the control of cellular growth. Patients with HB antigen positive infected with delta virus have a reduced incidence of hepatocellular carcinoma, probably because delta virus suppress HBV.

HCV, despite HBV, is an ARN virus which does not have reverse transcriptase and can't integrate into the genome host. The manner of carcinogenesis production is uncertain, but probably is generated by chronic hepatitis and cirrhosis. Persistent hepatitis with high levels of alanine aminotransferase is a semnificative predictor of hepatocellular carcinoma development. Accelerated cellular turnover is implied in the pathogenesis of cirrhosis; at the same time, it has been demonstrated that patients with persistent hepatic injury develops a sort of HCV species that metamorphoses in time, meanwhile patients with normal aminotransferase levels have a stabile infection with a single species of HCV. It seems that during this process are produced species more ,,oncogenic" of HCV core protein. HCV reacts with cellular genes that regulates the cellular growth and differentiation. P53 activity is suppressed and tumor suppressor functions are disabled. HCV core proteins are probably oncogenic [3].

Alcohol intake increases four time the risk of hepatocellular carcinoma development, especially in older patients; cirrhosis is always present. The risk of hepatocellular carcinoma is highest in patients with chronic hepatitis and persistent hepatocellular injury. Patients who stop the alcohol intake reduce considerably the risk of hepatocellular carcinoma development. The enzyme induction mediated by alcohol increases the conversion of co-carcinogens in carcinogens.

Aflatoxin B1, produced by Aspergillus flavus and Aspergillus parasiticus is a pro-carcinogen which is converted in the liver into a metabolite by the cytochrome P450 system at the 8,9-vinyl bond with the formation of an unstable reactive intermediate, aflatoxin B1-8,9-epoxide, which can bind covalently to DNA. The result is the formation of Aflatoxin B1-guanine adducts and protein- aflatoxin B1-albumin and other protein adducts. In addition, aflatoxin B1 acts as a carcinogen by mechanisms that include the formation of reactive oxygen species leading to increased hepatic damage [4].

Sex and genetic predisposition. Globally, hepatocellular carcinoma is 3 times more frequent in males than females. In regions with increased incidence, familial history of hepatocellular carcinoma is an important factor. It is possible to exist a genetic predisposition for hepatocellular carcinoma or a familial predisposition for an extended phase of HBV replication.

Other factors. Hepatocellular carcinoma is a rare complication of autoimmune hepatitis and primary biliary cirrhosis; it may occur frequently in hemochromatosis (in almost 45% of cases, due to excessive levels of free iron submitted in tissues, carcinogenic by the formation of reactive oxygen species). α1-antitrypsin deficiency, tyrosinosis, porphyria cutanea tarda, Clonorchis sinensis infection may also increase the risk of hepatocellular carcinoma development.

There have been identified certain factors which may decrease the risk of HCC in patients with cirrhosis : high serum retinol levels, high coffee consumption, alcohol cessation, clearance of HCV virus with treatment, iron depletion of hemochromatosis patients [5].

PATHOGENESIS

The pathogenesis of HCC is not fully understood, but most probably HCC results from activation of proto-oncogenes, deactivation of tumor suppressor genes (p53, pRb), changes in growth factors signaling processes (insulin-like growth factor-IGF, transforming growth factor-TGF), changes in telomeric length and

activity or microsatellite instability. Genetic injury may result from a variety of sources, such as HBV and HCV viruses. HBV is associated with a relatively high frequency of mutations as it replicates through RNA-mediated reverse transcription and the HBV reverse transcriptase (HBV-RT) does not have proofreading function. Unlike HBV, HCV is a single-stranded non-retroviral RNA virus which does not integrate into the host genome.

Although there have been identified multiple genetic abnormalities in the development of HCC, it is probably that the process is complex and genetic studies are continuing [1].

DIAGNOSIS

Hepatocellular carcinoma should be suspected in patients with previously compensated cirrhosis who develop decompensation, such as encephalopathy, ascites, jaundice or variceal bleeding associated with the extension of the tumor into the hepatic or portal veins or arteriovenous shunt induced by the tumor. Other uncommon presentations include: para-neoplastic syndromes – hypercalcemia (by PTH-like synthesis), erythrocytosis (by EPO-like synthesis), carcinoid syndrome, porphyria cutanea tarda, vascular angioma.

Diarrhea may be the reason for presentation to hospital, caused by cholestasis or the production of prostaglandins by the tumor.

Cutaneous manifestations include pemphigus foliaceus, dermatomyositis, Leser-Trelat sign (suddenly installed seborrheic keratosis, accompanied by freckles, very itchy).

Acute presentation is determined by spontaneous rupture of the tumor into the peritoneum or invasion of the liver capsule. In this case, the preferred treatment is the chemoembolization of the bleeding artery. Capsule invasion is causing severe pain, similar to biliary colic, lasting less than 24 hours.

In end stages, patients may present consumptive syndrome with impaired general condition, anorexia, weight loss and fatigue.

Physical examination can detect an abdominal mass and a bruit related to arterioportal shunting may be heard. A supraclavicular lymphadenopathy (Virchow-Troisier), Sister Mary Joseph or axillar lymph-adenopathies are rarely present [6].

Laboratory studies can show high levels of aminotransferase, but can be also normal. Alkaline phosphatase may be elevated in infiltrative tumor or if there is biliary obstruction due to malignancy. α-fetoprotein, the tumor marker most frequently associated with hepatocellular carcinoma (a glycosylated protein expressed in proliferating hepatocytes) has a sensitivity of 41-65% and specificity of 80-94% when the cut-off value is 20 ng/ml; levels higher than 400 ng/ ml are likely to be caused by hepatocellular carcinoma. AFP>1000 ng/ml associated with a liver mass is diagnostic for hepatocellular carcinoma. However, AFP level is elevated in only 60-70% of patients and false-positive results may occur during pregnancy, in active liver disease, certain gastrointestinal tumors and embryonic tumor. Additional studies of AFP have revealed that AFP has three glycoforms (AFP-L1, AFP-L2, AFP-L3) according to their binding ability to the lectin lents agglutinin (LCA). It has been shown that AFP-L3 exists only in the serum of patients with hepatocellular carcinoma at a cut-off value of 15%, with a sensitivity and specificity of 96.9% and 92%, respectively, in detecting hepatocellular carcinoma [7].

Des-gamma carboxyprothrombin (DCP-prothrombin,a protein induced by vitamin K-absence, PIVK-II) has been described as a protein produced by malignant hepatocytes. Different studies have shown than a combined analysis of AFP and DCP can lead to a better prediction in early stages of hepatocellular carcinoma [8]. Glypican-3, a member of the glypican famlily, has been revealed as a diagnosis marker, having the capacity to differentiate hepatocellular carcinoma from cirrhotic liver or benign lesion. It has been demonstrated that Glypican-3 and AFP together increase sensitivity and specificity for the diagnosis of small and unicentric HCC, respectively [9].

Heat shock protein 70 (HSP) – a protein produced by cells as a response to exposure to stressful conditions, may also be used as a marker for diagnosis in hepatocellular carcinoma [10].

The International Consensus Group of Hepatocellular Neoplasia recommends to define a pathological diagnosis of hepatocellular carcinoma if at least 2 markers are positive.

Imaging diagnosis

A minimum of 2 imaging exploration is required for staging and to confirm/exclude vascular invasion (ultrasound, CT with contrast, MRI); additionally is performed chest CT and bone scintigraphy if taken into account liver transplantation. In case of portal vein thrombosis associated with a small hepatic lesion, Doppler ultrasound is performed and thrombus punction ultrasound guided; the presence of thrombus exclude the possibility of radical treatment. Arterial signal into the thrombus indicate the tumoral etiology.

Abdominal ultrasonography has a 50% sensitivity in hepatocellular carcinoma detection for liver with advanced cirrhosis. Lesions under 2 cm can usually be detected.

Ultrasound appearance is variable. Lesions are usually hypoechoic. In large hepatocellular carcinoma, hyperechoic or heterogenic appearance is due to necrosis, hemorrhage or fat infiltration. Small hepatocellular carcinomas can be homogeneously hyperechoic and can be similar to hemangioma (due to a large portion of fat being present in the tumor) or can appear hypoechoic. Doppler ultrasonography can be used in the detection of portal vein, hepatic artery or inferior vena cava invasion. Portal venous invasion is more common in hepatocellular carcinoma, but hepatic vein invasion is more specific.

Small hyperechoic masses seen on ultrasound require further examination, because they can represent hemangioma (most frequently), metastatic disease or, less likely, hepatocellular carcinoma. Further imaging with CT scanning or MRI during dynamic contrast enhancement shows the typical peripheral, nodular contrast enhancement pattern of hemangioma. MRI or CT scanning can further characterize many nonspecific hepatic masses seen on ultrasonography.

CT scan – dynamic CT, spiral CT is the investigation of election for hepatocellular carcinoma diagnosis. The CT appearance of hepatocellular carcinoma is variable, depending on image phase and tumor size. The most common attenuation pattern is iso-hyper-iso-attenuation on prephase, arterial phase and venous phase, respectively; however, this pattern is common to other hepatocellular nodules, including regenerative and dysplastic nodules.

Unenhanced CT typically shows an iso-hypodense mass; for large masses central areas of necrosis may be seen.

In the hepatic arterial phase, nodules appear hyperdense (relative to hepatic parenchyma), as a result of hepatic arterial supply. Larger tumors may have necrotic central regions that are usually hypodense during this imaging phase. Neovascularity indicate the presence of inconspicuous lesions.

In the portal venous phase, small nodules may appear isodense or hypodense and larger nodules with necrotic regions remain hypodense.

In the delayed-postcontrast phase, small nodules may be inconspicuous on late phases.

Spiral-CT evidentiate 17% of lesions <1 cm, 29% of lesions between 1 and 2 cm and 63% of lesions higher than 2 cm.

MRI has higher sensitivity for focal hepatic lesions than CT.

Precontrast and postcontrast MRI has a 70-85% chance of detecting a solitary mass of HCC.

MRI can differentiate cirrhotic nodules from HCC: if the mass is bright on T2-weighted images, it is HCC until proven otherwise; if the mass is dark on T1- and T2-weighted images, it is a siderotic regenerative nodule or siderotic dysplastic nodule; if the mass is bright on T1-weighted images and dark or isointense on T2-weighted images, it is a dysplastic nodule or low-grade HCC [11].

Hepatic angiography.

Hepatocellular carcinoma is vascularized by hepatic artery and selective arteriography of superior mesenteric artery or celiac artery may identify the lesion. This is an invasive procedure used for hepatic chemoembolisation.

Liver biopsy.

For small lesions detected on ultrasound or CT, histological confirmation is very important. Biopsy is performed with ultrasound or CT guidance. There is a possibility of sowing on the needle path.

In present, the diagnosis of hepatocellular carcinoma is established by clinical examination associated with typical imaging aspect, with/without increased α-fetoprotein, histological confirmation not being absolutely necessary.

EASL 2016 recommend to perform liver biopsy for lesions with atypical imaging aspect and in non-cirrhotic patients [12].

DIFFERENTIAL DIAGNOSIS OF HEPATOCELLULAR CARCINOMA IN THE PRESENCE OF CIRRHOSIS

The major problem consist in the differentiation between hepatocellular carcinoma and a dysplastic nodule. Hepatocellular carcinoma appear hyper-vascularised compared to surrounding tissues.

For lesions higher than 2 cm identified in 2 different imaging studies, in the presence of cirrhosis, the diagnosis of hepatocellular carcinoma can be established. Also, if the lesion is identified in 2 different imaging studies and α-fetoprotein level is higher than 400 ng/ml, the diagnosis is very probable and histological confirmation is not really necessary [1].

SCREENING

Hepatocellular carcinoma screening is recommended in patients at high risk, such as male patients, HBV, HCV viruses, age > 40 years and chronic liver disease, especially cirrhosis and high grade dysplasia.

EASL 2016 clinical practice guidelines on the management of hepatocellular carcinoma recommend screening for patients with chronic HBV infection, even without cirrhosis, Asian males >40 years, females >50 years, patients with family history of hepatocellular carcinoma, African Americans >20 years, primary biliary cirrhosis stage 4, hemochromatosis, α1-antitrypsin deficiency.

Recommended screening methods are abdominal ultrasound combined with α-fetoprotein every 6 months. In cirrhotic patients, a nodule <1 cm detected must be supervised every 4 months for 1 year, than every 6 months.

For nodules 1-2 cm in diameter, the diagnosis is established by non-invasive criteria or histological examination.

Nodules higher than 2 cm may be diagnostic for hepatocellular carcinoma in case of typical aspect; for atypical aspect, histological examination is required [12].

TREATMENT OPTIONS

The treatment is established using the Barcelona-Clinic Liver Cancer (BCLC) staging system which defines very early stage cancer (single nodule,<2 cm, Child-Pugh A) as stage 0, early stage cancer (1-3 nodules, <3 cm each, Child-Pugh A-B) as stage A, intermediate stage for multinodular HCC (stage B), advanced stage which involves vascular invasion or extrahepatic spread (stage C) and terminal stage at patients with Child-Pugh C cirrhosis. Resection, ablation and transplantation is recommended for stage 0 and A, transcatheter arterial chemoembolization for stage B, Sorafenib – stage C and best supportive care for terminal stage.

Surgical treatment

Resection is curative, but limited in patients without cirrhosis or in the presence of cirrhosis and without portal hypertension. It is accepted as treatment for localized hepatocellular carcinomas. Intrahepatic recurrence is common, because the potential of neoplastic liver remain unchanged. The rate of recurrence at 5 years is almost 52 %. Partial hepatectomy is indicated in the absence of cirrhosis or in Child-Pugh stage A with normal portal pressure and normal bilirubin. The best results are obtained in patients with unicentrical disease, without vascular invasion, under 5 cm and with liver disease relatively inactive (5% of hepatocellular carcinomas). 5-year survival rate for curative treatment (resection, transplantation and local ablation therapy) is almost 80%.

Liver transplantation – the technique is successful in

selected patients, but it requires immunosuppressive treatment for life. It is indicated in patients with advanced cirrhosis (Child-Pugh B and C) who cannot survive on surgical resection. Liver transplantation can be applied independently on the liver stage disease and treat both hepatocellular carcinoma and neoplastic potential of cirrhotic liver.

Liver transplantation is indicated for unique tumors under 5 cm or <3 nodules (Milano criteria), each less than 3 cm.

It is difficult to elect between resection and transplantation for a tumor <5 cm. Altered liver function is in favor of transplantation.

Nonsurgical treatment

Chemotherapy

Sorafenib, a Raf-kinase and tyrosine kinase inhibitor, is indicated in patients with normal liver function (Child-Pugh A) and portal vein thrombosis, extrahepatic tumors and failure of other therapies. In patients with altered liver function, only best supportive care is indicated.

Local ablation-is potentially curative in patients with small tumors, <3-5 cm that are not candidates for resection or transplantation.

TACE (Transarterial chemoembolisation) – hepatic artery catheterization via the femoral artery or celiac axis allows the nutrient vessel embolization of tumor, and chemotherapeutic agents can be delivered in high concentrations. (Cisplatinum, Doxorubicim, foam gel, Epirubicin). Chemoembolization is indicated in unresecable tumors, patients with Child-Pugh A-B. The result is partial/total tumoral necrosis. The most frequent side effects are fever, nausea, encephalopathy, ascites and high levels of aminotransferase.

Percutaneous injection of ethanol (PEI) is efficient in 2-3 cm tumors (<5 cm), usually less than 3 nodules, even in the presence of advanced cirrhosis. The technique suppose the ethanol injection under ultrasound/CT guidance; outpatient treatment may be carried out 2 times per week, 3-15 sessions, using 2-16 ml of absolute alcohol. 3-year survival rate is 71% in patients with Child-Pugh A and 41% in Child-Pugh B.

Radiofrequency ablation (RFA) is a treatment that uses imaging guidance to place a needle electrode through the skin into a liver tumor. High-frequency electrical currents are passed through the electrode, creating heat that destroys the cancer cells. RFA is more effective than PEI in larger tumors (3-5 cm) and it requires fewer sessions. Recurrence rates are higher than in resection, but survival rate is similar to resection.

Trans-catheter arterial chemoembolization uses angiography and allows the nutrient vessel embolization of tumor. TACE is recommended in palliative treatment for unresecable tumors and in patients on transplantation list; the effectiveness of the method depends on the tumor dimension. TACE it is not recommended in the treatment of large and unresecable tumors.

CONCLUSION

In conclusion, hepatocellular carcinoma remain a fatal disease. All treatments used increases survival. Some studies have shown that there were no big differences between the results given by resection, transplantation and arterial embolization [13].

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ORIGINAL ARTICLES

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Lipid profile and comorbidities in patients with psoriasis vulgaris

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Abstract: *Background and Aim: Psoriasis is an inflammatory skin disorder with important influence on the lipid profile, insulin resistance and cardiovascular disease. The aim of this study is to compare the lipid profile of classical therapy versus anti-TNFs patients and asses the associated diseases.*
Methods: We conducted a prospective study. Patients' data were collected from two Romanian Dermatology Clinics, during the period January 2015 – June 2016. The patients were divided into two groups: study group (on TNF inhibitors) and control group (on classical treatment).
Results: The subjects in the study group had more associated diseases and much more patients suffered from dyslipidemia. There were not statistically significant differences in the lipid profile between the two groups. But eliminating patients treated with Acitretin and Methotrexate, a p-value of 0.0259 (significant statistically) was observed when comparing the values of triglycerides in the two groups.
Conclusions: We did not observe a beneficial effect of the TNF inhibitors therapy in psoriatic patients on the lipid profile. Subjects on biological treatment suffer from a more severe form of psoriasis vulgaris and associate much more comorbidities.

Keywords: *lipid profile, comorbidities, anti-TNF agents*.

INTRODUCTION

Psoriasis is a chronic, inflammatory skin disorder [1]. The inflammation predisposes to a pro-atherogenic lipoprotein profile, large vessel inflammation, and adipokine dysregulation [1] thus leading to insulin resistance and cardiovascular diseases [2]. There is a strong link between psoriasis and risk factors for metabolic syndrome (central obesity, insulin resistance [3], abnormal lipid metabolism [4], hypertension [5]. Patients with psoriasis have a more increased risk of developing atherosclerosis due to inflammation and dyslipidemia [4]. Moreover, lipid abnormalities may play an important role in the development of psoriasis [4].

The inflammation mediated changes at lipids and lipoproteins levels have more likely a role in protecting the host. The treatment of psoriasis was shown to lead to a normalization of the lipid levels [6]. TNF induces a reduction in cholesterols (especially HDL) by interfering with LDL receptors, Apolipoprotein A and B, and 7A1 and 7B1 cytochromes with no changes in HMG-Co reductase action [7].

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In Romania, however, the inclusion criteria for biological therapies require previous two systemic non-biological therapies (retinoids, systemic glucocorticoids, methotrexate, PUVA, UVB narrow band).

Moreover, most of these therapies are known as lipid modifiers [4].

The aim of this study was to examine two groups of patients (biological versus non-biological therapy psoriatic patients) in terms of comorbidities and lipid profile.

MATERIAL AND METHODS

Study design

We carried out a prospective study. Patients' data were collected from two Romanian Dermatology Clinics: Dermatology Department, Carol Davila Emergency Military Hospital, Bucharest, (January – June 2016) and Dermatology Department, Mureş County Clinical Hospital, (January – December 2015).

All subjects had a physical examination. Body max index (BMI) was calculated as weight (kg)/height (cm²) (normal weight: 18.5–24, 9; overweight: 25–29, 9; class I obesity: 30–34, 9; class II obesity: 35–39, 9; class III obesity: ≥ 40). Each patient completed a DLQI form and a dermatologist filled the PASI form.

The data collected included (i) patients' demographic characteristics: date of birth, gender, weight, height, BMI; (ii) psoriasis-related data: type of psoriasis, current therapies (topical treatment, Methotrexate/ MTX, Acitretin, PUVA, UVB TL01, anti-TNFs: Infliximab original and biosimilar, Adalimumab, Etanercept); (iii) comorbidities, with an accent on metabolic syndrome (hypertension, diabetes mellitus, obesity); (iv) cholesterol/cho and triglycerides/TG levels.

Patients were divided into two groups: study group (biological therapy) and control group (topical treatment, Methotrexate, Acitretin, PUVA, UVB TL01).

All participants gave written informed consent.

Statistical analysis

Data were analyzed using 2017 GraphPad Software.

RESULTS

At the end of the follow-up period, 102 subjects were included for the analysis: study group – 53 patients: 39 males (73.58%) and 14 females (26.42%) and control group – 49 patients: 25 females (51.02%) and 24 males (48.98%).

The biological therapy patients were distributed as follows: Infliximab (9, 16.98%), Infliximab biosimilars (4, 7.54%), Entanercept (17, 32.07%), Adalimumab (23, 43.39%). Four subjects had a switch of anti-TNF therapy, two changed Infliximab for Etanercept, the other two switched Infliximab for Adalimumab. Only 16 (15.68%) patients out of the 102 achieved both PASI and DLQI equal to zero during the study.

Biological patients were screened for associated diseases. Table 1 includes the number/(%) and comorbidities.

Table 1: Comorbidities of biological patients

Associated diseases	Number of patients /(%)
HTA	10/(18.86%)
Diabetes mellitus type 2	3/(5.66%)
Obesity	40/(77.35%)
Latent tuberculosis	5/(9.43%)
Hepatitis VHB	1/(1.88%)
Herpes zoster	1/(1.88%)
BPOC	1/(1.88%)
Chronic bronchitis	2/(3.77%)
Hepatic steatosis	1/(1.88%)
Glaucoma	1/(1.88%)
Arthropathy	15/(28.3%)
Synovitis right knee	1/(1.88%)
Disc hernia	1/(1.88%)
Anemia	1/(1.88%)
Allergy to penicillin and erythromycin	1/(1.88%)
Renal litiasis	1/(1.88%)
Hidradenitis suppurativa	3/(5.66%)
Bipolar disorder	1/(1.88%)
Amputation of right police	1/(1.88%)
Thyroid nodule operation	1/(1.88%)
Cholecystectomy	3/(5.66%)
Appendectomy	2/(3.77%)

The subjects with criteria of metabolic syndrome were

41 (77.35%) with obesity, 3 (5.66%) had diabetes mellitus type 2, 10 (18.86%) with hypertension. Other comorbidities were previous surgery (cholecystectomy, thyroid nodule operation, appendectomy), infection (latent tuberculosis, hepatitis VHB, herpes zoster), accidents (amputation of right police), pulmonary disease (BPOC, chronic bronchitis), inflammatory disease (hidradenitis suppurativa), hepatic disease (hepatic steatosis), renal disease (renal litiasis), ocular disorder (glaucoma), skeletal problems (arthropathy, synovitis right knee, disc hernia), hematological disease (anemia), psychiatric disorder (bipolar disease), allergy to drugs (allergy to penicillin and erythromycin).

The classical therapy patients associated obesity – 20 (40.81%), diabetes mellitus type 2 – 3 (6.12%), hypertension – 4 (8.16%). Other associated diseases were: hematological (anemia, thalassemia), skin cancer, arthropathy, osteoporosis, Cushing disease, venous disease, operated pituitary gland (table 2).

Table 2: Associated diseases of control group

Associated diseases	Number of patients
HTA	4
Diabetes mellitus type 2	3
Obesity	20
Anemia	1
Thalassemia	1
Skin cancer	2
Arthropathy	9
Osteoporosis	1
Cushing disease	1
Operated pituitary gland tumor	1
Chronic venous insufficiency	1

Patients with psoriasis have an important weight problem. The normal weight patients in the study group represented 12 (22.64%) versus 16 (32.65%) control group patients. Increased BMI was found in 74 (72.54%) psoriatic patients. (Table 3)

Some patients on anti-TNFs required additional drugs for disease control. 10 (18.86%) biological therapy patients used only topical treatment versus the majority of control group – 45 (91.83%).

Table 3: BMI for the two groups

BMI	Study group	Control group
Normal weight	12	16
Overweight	20	20
Obesity class I	15	13
Obesity class II	5	-
Obesity class III	1	-

Methotrexate was prescribed to 6 (11.3%) patients in the study group and to 4 (8.16%) patients on classical therapy.

Retinoid (Acitretin) was used only in 1 (1.88%) biological patient versus 2 (4.08%) patients in the control group. (Tables 4, 5)

Table 4: Additional therapy of anti-TNFs patients

Patient	Additional therapy
1	Acitretin
2	Methotrexate
3	Methotrexate
4	Methotrexate
5	Calcipotriol and betamethasone gel
6	Clobetasol propionate 0.0525% ointment
7	Calcipotriol and Betamethasone gel
8	Calcipotriol and Betamethasone gel
9	Methotrexate
10	Calcipotriol and Betamethasone gel
11	Calcipotriol and Betamethasone gel
12	Calcipotriol and Betamethasone gel
13	Keratolytic creams, Dermatocorticoids
14	Calcipotriol and Betamethasone gel
15	Calcipotriol and Betamethasone gel
16	Dermatocorticoids, Methotrexate
17	Methotrexate
18	Methotrexate

Table 5: Therapies of patients in the control group

Patient	Therapy
1	calcipotriol and betamethasone gel, 15% urea cream, UVB TL01
2	0.1% mometasone furoate cream, vitamin A, emollient, cream with salicylic acid, UVB TL01
3	topical corticosteroid, calcipotriol and betame-thasone gel, keratolytics, emollient cream, Methotrexate, UVB TL01
4	clobetasol propionate 0.0525% cream, Methotrexate, PUVA
5	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, PUVA
6	0.1% mometasone furoate cream, UVB TL01
7	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, UVB TL01
8	0.1% mometasone furoate cream, Methotrexate
9	betamethasone dipropionate and salicylic acid cream, clobetasol propionate 0.0525% cream, vitamin A, vitamin E,UVB TL01
10	15% urea cream
11	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, 15% urea, vitamin A, vitamin E, PUVA
12	0.1% mometasone furoate cream, emollient cream, keratolytic cream, UVB TL01
13	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, Vitamin E, PUVA
14	topical corticosteroid, UVB TL01
15	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, Vitamin E, PUVA
16	clobetasol propionate 0.0525% cream, calcipotriol and betamethasone gel, Vitamin E, Vitamin A, salicylic acid cream, PUVA
17	clobetasol propionate 0.0525% cream, Vitamin E, Vitamin A, UVB TL01
18	0.1% mometasone furoate cream, emollient cream, betamethasone dipropionate and salicylic acid cream, UVB TL01
19	0.1% mometasone furoate cream, emollient cream
20	clobetasol propionate 0.0525% cream, Vitamin E, Vitamin A, UVB TL01
21	calcipotriol and betamethasone gel, Vitamin E, Vitamin A
22	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, emollient cream, Vitamin E, Vitamin A, PUVA
23	vitamin E, 30% urea cream
24	clobetasol propionate 0.0525% cream, emollient cream, UVB TL01
25	calcipotriol and betamethasone gel, 15% urea cream, PUVA
26	calcipotriene hydrate and betamethasone solution, 0.1% mometasone furoate cream, Vitamin A, Vitamin E, Methotrexate, PUVA
27	0.1% mometasone furoate cream, Desloratadine cp, UVB TL01
28	0.1% mometasone furoate cream, clobetasol propionate 0.0525% cream, Desloratadine cp, PUVA
29	clobetasol propionate 0.0525% cream, emollient cream, vitamin A, vitamin E, PUVA
30	0.1% mometasone furoate cream, emollient cream, Methotrexate
31	0.1% mometasone furoate cream, emollient cream, salicylic acid cream, Clarithromycin
32	0.1% mometasone furoate cream, emollient cream, UVBTL01
33	clobetasol propionate 0.0525% cream, Ciprofloxacin cp, UVB TL01
34	clobetasol propionate 0.0525% cream, salicylic acid cream, UVB TL01

35	calcipotriol and betamethasone gel, vitamin E, Vitamin A, UVB TL01
36	0.1% mometasone furoate cream, UVB TL01
37	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, vitamin A, vitamin E, PUVA
38	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, vitamin A, vitamin E, UVB TL01
39	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, silver nitrate solution, vitamin A, vitamin E, UVB TL01
40	UVB TL01
41	Methotrexate
42	0.1% mometasone furoate cream, calcipotriol and betamethasone gel
43	0.1% mometasone furoate cream, Desloratadine cp, UVB TL01
44	clobetasol propionate 0.0525% cream, UVB TL01
45	0.1% mometasone furoate cream, UVB TL01
46	calcipotriol and betamethasone gel
47	0.1% mometasone furoate cream, 15% urea cream, emollient cream, Methotrexate
48	0.1% mometasone furoate cream, 15% urea cream, emollient cream, Methotrexate
49	calcipotriol and betamethasone gel, 15% urea cream, vitamin A, vitamin E, Methotrexate, PUVA

The lipid profile was screened in both groups of patients. But there was no difference between the two groups of patients. The p-value comparing the two groups of cholesterol (study vs. control) was 0.8674, and for the triglycerides groups (study vs. control) was 0.218. On the other hand, a significant statistically p-value of 0.0259 was observed when comparing the values of triglycerides in the 2 groups, but after eliminating the patients on Acitretin and Methotrexate.

We compared the cholesterol and triglycerides values from each group for patients treated only Methotrexate or Acitretin and the ones on anti-TNFs and Methotrexate or Acitretin, but no significant p-values were found.

We observed high triglycerides values in patients treated with Infliximab – 7 (58.33%) followed by Etanercept – 5 (35.71%) and Adalimumab – 4 (22.22%). The highest levels of cholesterol were observed in the Etanercept and Adalimumab subgroup 11 (78.57%). The others subgroups contained only small samples of patients. (Table 6)

The study group was divided into subgroups of years on biological therapy and the levels of cholesterol and triglycerides were compared.

Table 6: Patients with high lipid values divided into systemic therapies

Therapy	High cholesterol No/(%)	High triglycerides No/(%)
Infliximab (original + biosimilar)	2 (16%)	7 (58.33%)
Etanercept	11 (78.57%)	5 (35.71%)
Adalimumab	11 (61.11%)	4 (22.22%)
Adalimumab + Methotrexate	3 (75%)	3 (75%)
Etanercept + Methotrexate	2 (100%)	0 (0%)
Adalimumab + Acitretin	1 (100%)	0 (0%)
Methotrexate	2 (50%)	3 (75%)
Acitretin	0 (0%)	0 (0%)

The subgroup 4-6 years had the highest level of dyslipidemia as it can be observed in Table 7.

DISCUSSION

Analyzing the two groups, the patients on biological therapy seemed to have more associated comorbidities than the control group. Cardiovascular events appear more often in patients with severe (8) and therapy resistant psoriasis like the ones treated

with anti-TNFs.

The two groups showed a difference in the frequency of hypertension (study group – 18 (86%) vs. control group – 8 (16%)). Even though, in normotensive and rheumatoid arthritis individuals, Infliximab reduced blood pressure levels, we observed in our study that the anti-TNFs do not improve alone the blood pressure values in psoriatic hypertensive patients [9].

Table 7: Levels of cholesterol and triglycerides divided into subgroup of years of anti-TNF therapy

Years of biological therapy	High cholesterol	High triglycerides	Cholesterol %	Triglycerides %
≤ 3.5 years	7	4	13.20	7.54
4-6 years	22	15	41.50	28.30
7-8 years	2	2	3.70	3.70

Many studies observed that high blood pressure is more often noted in patients with psoriasis compared to the non-psoriatic population [10], especially psoriatic patients who were male, middle-aged and elderly people [11] as our study group (39 males (73.58%) versus 14 females (26.42%)). Men are more predispose to severe forms of psoriasis [12].

High blood pressure is independent associated with psoriasis [8].

Apparently, in psoriasis the pathophysiologic mechanisms for high blood pressure are related to the underlying autonomic nervous system dysfunction [13], elevated plasma renin activity, elevated angiotensin converting enzyme activity, and elevated endothelin-1, TNFG308A polymorphism [14].

Psoriasis is thought to promote insulin resistance by overproduction of Th1 cytokines [10]. An association can be observed between high levels of TNFα, IL6, IL17, leptin, and C-reactive protein, and increases in BMI, which contribute to alterations in insulin biochemical pathways, leading to insulin resistance, type II diabetes [15]. Our patients are overweight, dyslipidemic and topical corticosteroid users for long periods of time, more over they have an inflammatory disease which is pro-atherogenic. Although only 6 patients out of 103 were diagnosed with diabetes mellitus type 2, the screening is continuous, because psoriasis is an independent risk factor for type 2 DM, the risk being greater in severe disease. (Marius Irimie, 2015).

Most of our patients with psoriasis vulgaris are above normal weight (72.54%). It has been reported that psoriasis favors weight increase and obesity [15]. In obesity there is an unbalanced production of pro- and anti-inflammatory adipokines in obesity, leading to a chronic low-grade inflammation state, which seems to favor worsening of psoriasis lesion and a poorer response to treatment [16].

Although we did not evaluate the weight gain before and after anti-TNF treatment, several studies have reported an increase in weight and body mass index (BMI) with the use of anti-TNF agents (adalimumab, etanercept and infliximab) [9, 17]. Most of our patients in the study group are overweight (77.35%).

On the other hand, weight loss (greater than or equal to 5% of the initial weight) is associated with a higher success rate in achieving control of disease activity in patients with overweight or obesity with psoriatic arthritis treated with anti-TNF-α [9]. A study with patients on low-energy diet lost weight which led to significant reductions of several cardiovascular risk-associated factors, moreover the weight loss improved HbA1c levels by 0.7%, equivalent to reductions achieved by treatment with an anti-diabetic drug [18]. Moreover, the psoriatic patients with the lowest monounsaturated fatty acid (MUFA) intake presented the highest values of PASI score and inflammation marker [19].

The mean age of psoriasis patients in our study was 52.07 years similar to international studies, thus confirming the average age of type 2 psoriasis patients worldwide [20]. Moreover the biological patients are the ones that do not respond to classical therapy

suggesting the severe form. The equal distribution in the classical therapy group can be due only to a mild form of disease.

Between the two groups there were not statistically significant differences in the lipid profile. Our results are similar with those of [21], their psoriatic patients treated with etanercept, adalimumab, or infliximab (each patient serving as their own control), did not present a significant change in total cholesterol, LDL and triglycerides. On the other hand, a p-value of 0.0259 (significant statistically) was observed when comparing the values of triglycerides in the two groups (eliminating the patients on Acitretin and Methotrexate).

We discovered that patients with 4-6 years of anti-TNF therapy were the majority group with dyslipidemia. However, the study of Ehsani et al. demonstrated an insignificant 5% increase in LDL and triglycerides after 24 weeks of treatment with anti – TNF agents [7], but all the changes in lipid profile by biological therapy are temporary, and the negative feedback will balance these changes over time [7]. Others have proven that patients with long term psoriasis have an increase in the serum total cholesterol levels and triglyceride levels [22]. Several authors found increased cholesterol levels (more than 5 years), but the lipid profile changes from the early stage to the late chronic stage of the disease. In contrast, Jorge Santos-Juanes et al. did not find a relationship between lipid levels and disease duration [23]. Maybe lipid profile abnormalities might be genetically determined rather than acquired [8].

Comparing only patients on MTX or Acitretin versus patients on anti-TNFs and MTX or Acitretin we did not observe a significant statistically important difference. But in similar studies there was a significant increase in triglycerides in patients treated with TNF inhibitors + MTX compared with patients treated with MTX alone. In our study, the highest triglycerides levels had patients on Infliximab – 7 (58.33%), followed by Etanercept – 5 (35.71%) and Adalimumab – 4 (22.22%). Concerning cholesterol levels were higher in Adalimumab and Etanercept – 11 subjects (61.11%; 78.57%). The patients on systemic classical treatment (Methotrexate or Acitretin) or on anti-TNF + MTX/ Acitretin were too few to have statistical significance [21].

Our control group (excluding MTX and Acitretin) showed increased cholesterol (26/53.06%) versus triglycerides levels (6/12.24%). Other studies showed that TG levels were significantly higher in psoriatic subjects than control groups [24].

We did not exclude patients on Methotrexate and Acitretin.

These may cause or influence the dyslipidemia. Recent studies proved that retinoid acid treatment could produce unsaturated fatty acids and induce triglyceride breakdown, bile acid secretion, lipolysis, and retinoids elimination. But, retinoid x receptor α deficiency might induce the synthesis of saturated fatty acids, triglyceride, cholesterol, bile acids, and retinoids [25]. Retinoids, especially Acitretin increase triglycerides levels [26], but also a mild and transient reduction in HDL levels and insulin sensitivity [27].

Regarding MTX, there are conflicting studies on the effect of Methotrexate on the lipid metabolism. The HDL profile function is favorable influenced by Methotrexate [28], Methotrexate has an anti-atherogenic potential [29] with cardioprotective properties [30]: tendency to decrease the risk of heart failure [9], a lower incidence of vascular disease (cardiovascular, cerebrovascular and arteriosclerosis) [17]. But it has been proven that cholesterol, LDL cholesterol and HDL cholesterol are increased short-termed after the initiation of Methotrexate therapy [31].

CONCLUSION

Our study did not show a positive effect of anti-TNF therapy in psoriatic patients on the lipid profile. Most likely, the abnormalities of the lipids are due to genetically inherited mechanisms. Our patients on biological therapy have a more severe form of psoriasis vulgaris, with many comorbidities.

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ORIGINAL ARTICLES

Management of burning mouth syndrome (BMS) in patients with diabetes mellitus

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Abstract: *Unlike the therapy of primary BMS, the treatment of secondary BMS benefits, in addition to symptomatic therapy, from the possibility of using means and methods to correct biological disturbances and/or morphological, psycho-emotional changes respectively. The use of these therapeutic means in secondary BMS is recommended to be established as a first step of therapeutic conduct. Their success is reflected in homeostasis restoring, correcting morphological and functional changes of the field, which frequently leads to no longer use prescription of symptomatic treatment.*

DEFINITION

The International Association for the Study of Pain (IASP) defines burning mouth syndrome (BMS) as a "distinct nosological entity" characterized by "oral sensation of burning or pain, persistent and non-remitting in time in the absence of clinical objective changes of the mouth mucosa" [1]. A relatively similar vision is supported by Grushka (2014). BMS defining a sensory sensation, the author admits that this is manifested mainly by: a) subjective characteristics: changes in taste perception; bilateral burning sensation associated with altered taste mouth; sensation of "dry mouth", although salivary flow is not reduced; perceived foreign body in the oropharynx and b) objective criterion: the oral tissues with clinically normal aspect. The author specifically mentions that the 4 subjective lists, encountered in BMS come forth from the absence of results after an objective clinical exam, a defining element for this condition [2].

EPIDEMIOLOGY

Setting the epidemiology of BMS is difficult and imprecise because there is no universally accepted definition. The various epidemiological studies often refer to different clinical entities in which oral mucosal lesions lack [3]. This disease has a high rate that varies according to the studies from 0.7% to 14.8 - 15% [3,4,5]. The prevalence of painful syndrome in the Service of Oral Pathology of the Faculty of Dental Medicine - "Carol Davila" University of Medicine and Pharmacy, Bucharest (Romania) is 16.23% [6].

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ETIOLOGY. PREDISPOSING FACTORS

Pathogenic, sex, age, menopause can be considered contributing factors. Therefore:
1. Although it was observed in any age group between 27-87, BMS has a clear predisposition related to age. The average age of reporting the disease is 61 [2]. BMS rarely occurs before the age of 30 [7].
2. Sex is also placed between contributory factors. The women are 2.5 to 7 times more frequently affected than men.
3. The body functioning as a whole, at menopause, besides circulating electrolyte changes, it suffers from other disturbances such as:
a) Lipid disorders with consequences in the development of atherosclerosis;
b) exacerbated production of ROS/RNS, including liquid in the gingival sulcus (where ROS = reactive oxygen species, and RNS = reactive nitrogen species);
c) changes in the hormonal constellation at menopauses the quantity of estrogen become responsible for the development of female cardiovascular pathologies same as men.

Data from the literature considers that up to 90% of the female patients with BMS are around menopause, the onset of symptoms characteristic of peri-menopausal period taking place three years before and 12 years after climax [8].

The explanation for BMS distribution predominantly in women during perimenopause, according to Woda et al. (2009) is that BMS is the result of hormonal changes (hypo-estrogenism) in women during menopause, which often associates with metabolic disorders and neuropsychological chronic anxiety or stress. Mental disorder in menopausal women explains anxiety and depression from BMS [9].

4. Another pathogenic explanation takes into consideration the interrelationship taste-saliva-pain. Laboratory tests showed that female patients with disorders in climax present a growth of salivary phosphate concentrations, proteins, Na, K+, Ca2+ and Mg2+. The referred electrolyte disturbances trigger the disruption of the membrane potential, including those of the cells involved in collecting taste excitations or cells responsible for saliva secretion [10].

Certain hormonal changes (hypoestrogenemia, diabetes mellitus, hypothyroidism, thyroid dysfunction type) and functional morphological and immunological disorders affecting the endocrine glands were also among factors described as possible cause of this disorder [11]. Regarding the relationship between diabetes and BMS various assumptions were formulated, including the existence of metabolic changes in the mouth due to peripheral neuropathy, which can generate among other disturbances the hypo-function of the salivary glands, thus reducing the secondary salivary flow [3,12,13].

The mode of action of psycho-neuro-endocrine factor is even more difficult to explain since it has been shown that patients presenting BMS have a greater tendency to somatization and to develop psychiatric symptoms [11]. For this reason the theory was accepted that burning mouth syndrome is a form of neuropathic pain [14]. The neuropathy mechanism in the pathogenesis of BMS is still controversial, the expert literature admitting the possibility of dysfunction via peripheral or central reflex arc and cortical excitation [8,10].

BMS SYMPTOMS

Subjectively, patients describe the syndrome BMS as the presence of oral mucosa burning sensation, the most common manifestation present in the disease. Other features of the pain in this syndrome are itching sensation, numbness, impaired taste (taste “spicy”), dry mouth etc. As topography, the symptom is located almost constantly close to the lingual mucosa (especially in the front third) and the lip. Recently, Demarosi (2013) stresses however that it is not possible that all the oral mucosa could be involved without being identified any precise anatomic distribution [3].

Even since 2003, Scala et al. underlined the semiotic nature of BMS as being more complex, consisting of main and associated symptoms:
a) The main symptom is a triad, consisting of:
1. Pain in the oral mucosa: burning, stinging, tingling, swelling;

2. Dysgeusia (altered taste): the persistence of a certain flavor/taste perception alteration;
3. Xerostomia, with dry mouth.
b) Other associated symptoms: thirst, headache, pain in the temporomandibular joint (TMJ), tenderness/ pain during mastication and/or in some regions of the neck suprahyoid or shoulder level muscles [15].

The amount of positive clinical examination in the diagnosis and any associated distress, which act as causative factors in BMS syndrome

BMS diagnosis is essentially one of exclusion [16]. It is based on a historic and very thorough clinical examination. An accurate diagnosis requires careful investigation of BMS medical history of each patient (such as a preexisting condition at the oral mucosa level, malnutrition, cerebral dysfunctions or drug intoxication) [17,18].

1. The contribution of general clinical examination in the diagnosis of BMS

At their first visit at the dentist's, patients frequently report present disorders and diseases already diagnosed under therapy. When there is a suspicion of disease present and undiagnosed, the patient is advised to consult a physician in the respective domain [19].

2. The role of local clinical examination in the diagnosis of BMS

Local clinical examination often does not reveal any changes. Sometimes clinical examination can detect minor changes or variations of normality: fissured tongue, exfoliative glossitis, depapillations of various origins, geographic tongue or coated tongue [16].

If physical examination reveals the absence of clinically obvious lesions on the oral mucosa, it is reasonable to suspect that intraoral burning is a possible indicator of a systemic disorder [3].

Identifying the possibility of the existence of systemic diseases associated with or responsible for installing the BMS syndrome is by clinical examination and by performing general laboratory or laboratory tests [16].

THE MAIN THERAPIES EMPLOYED IN THE BMS

Based on a comprehensive assessment of the patient's disease state that integrates general clinical examination results, psychiatric/psychological balance and the local dental examination, a therapy in BMS is set. The characteristic of glossodynia treatment is its individualization and adapting to each individual case [19].

Primary stage in establishing BMS therapy requires the differentiation of the primary from the secondary. This is because if the balance sheet allowed the identification of predisposing and etiologic factors, therapy will target their harmful action through appropriate specific methods.

Clinical attitude to be adopted to establish the diagnosis and clinical form of BMS is summarized in figure 1. Schematic representation is useful because it allows to facilitate the understanding of therapeutic medical conduct to follow. According to Demarosi (2013), clinical, biochemical and hematological test results represent two phases of special importance in the etiological diagnosis of the BMS [3].

The therapy goals of the BMS syndrome are administered to target a triple purpose:
1) Improvement of symptoms;
2) Correction of biological and/or morphological disturbances (if there are), commonly referred to as correction therapy;
3) Changes in psycho-emotional therapy [3].

Symptomatic treatment

Symptomatic treatment is similar in both cases of BMS (idiopathic or secondary). While pain is a symptom of many chronic diseases, persistent pain should not be regarded simply as a symptom. Pain itself can become a disease where pain signals origins fail to be controlled due to deterioration of the pain alarm system, leaving the patient with persistent pain. Whatever the cause, chronic pain can transcend ordinary physical pain. Persistent pain interferes with daily life, other important relationships and puts a mark on the mind, body and spirit of the BMS patient. The pain and related problems (e.g., medication side effects, depression and anxiety) can be best managed

using a complex program of integrative treatments tailored to each patient. The name of integrative treatment derives from the means/method by which it is achieved, the approach of a "multi-modal" one.

Figure 1: The current clinical attitude in the presence of a case with BMS [3]

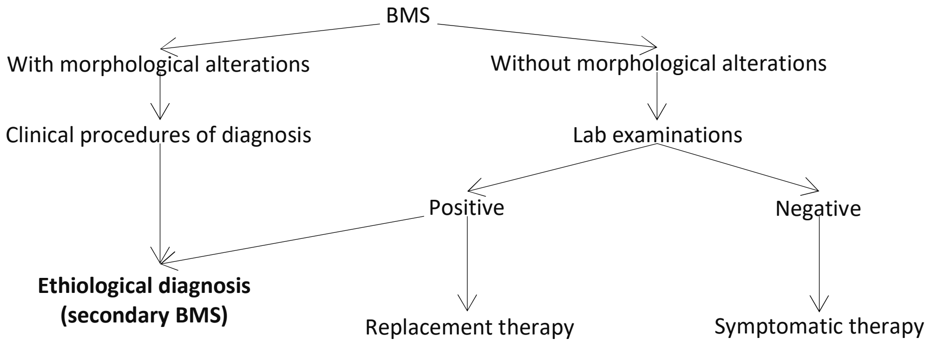
BMS
With morphological alterations
Without morphological alterations
Clinical procedures of diagnosis
Lab examinations
Positive
Negative
Ethiological diagnosis (secondary BMS)
Replacement therapy
Symptomatic therapy

The method involves combining treatments from conventional medicine with those of complementary and alternative therapies. Scientifically, for the efficiency of the latter, evidence have been provided for both the quality and safety of the therapy. In this category, the treatment of patients with BMS can use homeopathic products, cognitive-behavioral methods, aromatherapy, herbal extracts, vitamins, dietary supplements, some combination of minerals etc. [21,22]. Also, acupuncture as an adjunctive therapy method is referred to in the expert literature as being beneficial for the relief of symptoms in patients with BMS [14].

Generally speaking, it is acceptable that the treatment of idiopathic BMS is essentially a symptomatic treatment. In the context of such therapy, the dentist has available a number of symptomatic therapies that can be administered by the general or local means with topical use. Some of these are summarized in the table shown above. The purpose of a symptomatic therapy aims at either improving the intensity of symptoms or halting the clinical manifestations. The results are uncertain, including failure.

Therapeutic strategies include benzodiazepines (clonazepam), tricyclic antidepressants (amitriptyline), opioids (gabapentin), selective inhibitors of serotonin receptors (paroxetine and sertraline), capsaicin topical/systemic, alpha-lipoic acid (neurological antioxidant) hydrochloride, benzydamine 0.15%, hormone replacement therapy, vitamin supplementation and/or zinc, iron and psycho-cognitive therapy [3].

Tricyclic antidepressants (amitriptyline/doxepin/ nortriptyline) are extremely useful for patients with chronic pain. Although developed for the treatment of depression, they have a spectrum of biological activities depending on the dose, including inducing analgesia in a variety of clinical conditions. Although the mechanism is not known, the analgesic effect of antidepressants have a more rapid onset and occurs at a dose lower than that typically necessary to treat depression. The maximum effect is achieved by tricyclic antidepressants in patients with chronic pain who are not depressed, reduced pain being a certainty. Anticonvulsants (carbamazepine) and tranquilizers (clonazepam) are particularly useful for patients with neuropathic pain [23].

Capsaicin, a substance extracted from red peppers, is used for its revulsive and analgesic action. Capsaicin is specifically detected by the "transient receptor potential vanilloid receptor" (TRPV1) distributed in the oral mucosa. The explanation is that the capsaicin molecule is a selective agonist vanilloid receptors TRPV1. Binding means stimulating the receptor, which is found in the nociceptors (pain receptors) in the oral cavity. Overstimulation of TRPV1 receptors make them become "desensitized", no longer able to respond to the stimuli that normally cause pain in patients with peripheral neuropathic pain [24].

Topical application of capsaicin has been shown to sometimes be useful in some pain syndromes, in particular for relieving the symptoms of herpes neuralgia or diabetic polyneuropathy hyperalgesia [25].

Similar conclusions on the effectiveness of topical application of capsaicin (0.02%/0.002%) in BMS are made by Silvestre et al. (2012) and Spanemberg et al. (2011) [26,27].

Other authors speak of the efficacy of capsaicin. Spadari et al. (2014) reported a success rate of over 70% for each control visits after 1, 3, 6 and 12 months of topical therapy (as by rinsing the mouth with a solution of capsaicin 3 times/day) [28].

Among the most widely used symptomatic therapies for BMS are salivary substitutes, from the most simple and economical specific physiological solution to gel. Their use is motivated by the fact that xerostomia is a frequent syndrome in patients with BMS [3].

Grushka (2014) suggests that treatment involves a combination of several BMS drugs that act together, increasing each other's effect (they are given together as dose so that a low-dose should enhance the action). From such an attitude, "low dose" can achieve favorable results on major symptoms present in patients. Achieving success involves on the one hand an individualized (personalized) therapy, on the other successive explorations, to identify the effective drug combination. Based on the results of his studies, Grushka (2014) admits that ⅔ of the patients respond to the treatment aimed at suppressing the effects of benzodiazepine receptor agonists group GABA-A and inhibition of pain. The combination drug likely to complete such a success is a triad of medications: clonazepam 0.5 mg before bed + 10 mg amitriptyline + gabapentin 100-300 mg 3-4 times +/or 25-75 mg Pregabalin 3-4 times daily. Again Grushka (2014) states that zinc therapy is effective in taste changes, prescribing zinc gluconate 50 mg 1-3 times a day for a period of 3-4 months [2].

In conclusion, according to the expert literature, the current level of knowledge about the disease does not include any certainly effective treatment for primary BMS.

Treatment of secondary BMS

According to Ţovaru et al. (2015), for the treatment of secondary BMS the major objectives are:

- detection and treatment of general factors involved in the emergence of glossodynia (anemia, vitamin B deficiency, diabetes, autoimmune disorders of the digestive tract, disorders during menopause, and esophageal reflux caused by gastric hyperacidity);
- detection and removal of the local causes of BMS (incorrect prostheses, changed vertical dimension of the lower floor of the face, improper dental alloys, chronic mucosal infections – for example, Candida – contact allergies, dry mouth, migratory glossitis;
- detection and treatment of coexisting psychiatric disorders (anxiety neurosis or depression, with stress, chronic states of conflict, emotional pressure, cancer-phobia) [19].

Achieving this objectives requires expert advice and patient's hospitalization for the neuropsychological disorder, often lasting for years.

Etiological treatment

A priori, BMS secondary treatment is etiologic, addressed to fight against the general and local factors that maintain the syndrome. The success of etiologic therapy is dependent on the precision with which the causative factor was identified [8,19]. Thus, in the case of the presence of allergic contact reactions, simply removing the suspected allergen (e.g., the material / dental alloy) determines remission of the symptoms of BMS.

If the necessary examinations revealed the presence of anemia, therapy should be prescribed to correct hemoglobin assets and deposits of iron and vitamin B12. Completion of these deposits is mandatory because suppressing the substitute anti-etiological treatment after restoration of erythrocyte hemoglobin level, allows a recurrence of anemia after a certain period of time.

Etiological treatment is required in other cases of BMS secondary diseases such as diabetes mellitus, gastric ulcer, gastro-esophageal reflux disease, mycosis, and the burning mouth is one of the manifestations of

these disorders [3].

Psychological and psychiatric treatment

The second place in the treatment of secondary BMS is psycho-pharmaceutical correction. Frequently, symptomatic treatment [3] is no longer resorted to.

Psychological and psychiatric treatment in the syndrome of secondary BMS is applied not only to the patients with psychiatric disorders or behavioral disorders diagnosed during the first medical checkup, but also to patients who do not respond to dental treatment. For this purpose, patients are directed to consult a specialist who would in fact perform a reassessment if the psychotherapeutic treatment is not appropriate to be set [3,29].

Specialized treatment is achieved by two means:

a) psychotherapy (psychological exam);

b) with drugs, therapy monitored by a psychiatrist;

Psychological treatment aims at controlling and alleviating the mental disorders that occurred. Typically, psychological treatment complements the psychiatric one, but it is the first to start with, especially in patients who have problems with mental BMS, such as those with dental treatment failure. Results of consequent studies have concluded that pharmacological products such as benzidamine-HCl solution, sucralfate, antihistamines, lidocaine, capsaicin have not shown significant improvement statistically [2,3,16].

Frequently followed conduct is the psychoanalytic association with administration of specific psychotropic medication (anxiolytic, antidepressant). The usefulness of psychotherapy is to reduce symptoms, if there is a psychological component to glossodynia [19].

Counselling treatment

BMS syndrome treatment conduct includes counselling, preferably applied by a physician demonstrating empathy for the patient. The purpose of counselling is to provide the patient information and explanations about the sickness, benignity of the lesion, its correlation with notions of its correlation with field (age and sex). These patients should always know that their disorders are often related to stress and that if they make it go away, at least in part their suffering can diminish [3].

The doctor should always have the status of patients with BMS, the suffering and disease, so they have to devote the necessary time to communicate and discuss the patients' problems. This is because one of the major concerns of patients with BMS is to undergo a treatment to alleviate the symptoms [3].

During counselling sessions the BMS patient will be informed about the need to respect therapeutic indications, even if obvious improvements do not occur. Also, we will clarify that for the relief of symptoms, duration of treatment is for months/years.

Starting from his own medical experience, Demarosi (2013) states that patients rarely comply with prescribed BMS treatment, the recommended therapy is often performed incorrectly and doctor visits are random. Simultaneously, the patient will be clearly advised that the outcome of the treatment consisting in improvement or even the disappearance of the disease is often only temporarily effective [3].

Since the treatment of BMS is generally unsatisfactory even if it is appropriate, and the pain is chronic, it is necessary that patients be counselled with correct information on expectations that are to be understood realistically. Otherwise, the patient loses confidence in his regular doctor and becomes psychotic, going from doctor to doctor.

EVOLUTION AND PROGNOSIS IN BMS SYNDROME

Sardella et al. (2006) in a retrospective study of 18 months showed that 28.3% of the BMS (basic form) cases registered moderate improvement, 49% not any significant change, 18.9% of the cases presented a worsening of symptoms in patients who have not received any treatment. Spontaneous complete remission is rare and has been reported by the same team in 3% of cases investigated over a period of 5 years [30,31].

Cure rates measured in glossodynia of type I and II were almost comparable (61% versus 59%). 70% of all

cases that have not responded to treatment present associated psychiatric disorders [19].

Idiopathic nonspecific painful syndromes are most rebellious to treatment. Some cases even exclude any possibility of allopathic treatment, in which case additional treatment, such as acupuncture is resorted to (14). Frequently, the results of acupuncture are not always stable, and in some cases a patient can get addicted to this kind of treatment [19].

In other words, where allopathic treatment + psychological + counseling fail, acupuncture may be used with relative efficacy and limited duration, which requires to repeat the complementary procedure.

In another study, a group of 91 patients with BMS symptoms were analyzed and it was concluded that in a small number of patients symptoms may resolve spontaneously within five years from the onset. Moreover, a partial improvement was observed in 38.4% of these cases under various treatments (for example, clonazepam in local administration). This rate of improvement is greater (up 61.7%) when patients were administered clonazepam locally associated with psychotherapy [32].

Fenelon et al. (2014), evaluating the results of short-term therapy with clonazepam versus amitriptyline in the pain relief of BMS highlighted that after 6 weeks of treatment there was an improvement in the symptoms of pain in both situations, noting that the effect of amitriptyline was lower than that of clonazepam. To explain such particularity of the therapy, the authors imply a slow onset of action of the first drug. After 3 months, the results were similar in the two groups of patients evaluated [33].

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ORIGINAL ARTICLES

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Dosimeters: measuring occupational UV exposure's involvement in skin cancer development

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Abstract: *Introduction: Solar exposure is a major causative factor for multiple skin pathologies. Even though UV radiation is the most important factor implicated in skin cancer pathogenesis, still, few European countries recognize UV occupational-induced skin cancer as an occupational disease, Romania not being part of this category. The objectives of this study were to evaluate the UV exposure doses in agriculture and construction workers and to establish efficient protective measures.*

Materials and methods: In our Department, a prospective, observational, analytical study was conducted, including six outdoor workers (in construction and agriculture), over a period of three summer months (July, August, September). Subjects were asked to wear personal dosimeters and the following parameters were analyzed: profession, duration of exposure, UV exposure doses, skin aspect, preexisting preventive measures.

Results: The wearing of dosimeters was well tolerated by all subjects, without any interference with daily professional activities. Daily UV radiation doses ranged from 1.79 SED (tractor driver) to 19 SED (agriculture worker). Maximum UVR doses were recorded between 10:00 am and 4 pm. Clinical examination was made after measurements, with no detectable skin changes.

Conclusion: Even though workers in agriculture and construction in Romania are exposed to significant UV doses, with 40% more than the general population, these professions are not recognized as high-risk professions for developing skin tumors. More prospective and retrospective studies are necessary for more significant statistical results and for demonstrating the necessity of introducing primary, secondary and tertiary preventive measures.

Keywords: *ultraviolet radiation, occupational exposure, skin cancer*

INTRODUCTION

Solar exposure is a major causative factor for multiple skin pathologies. Most frequently it is the case of cutaneous tumors, both precancerous and malignant lesions (actinic keratoses, basal cell carcinomas, squamous cell carcinomas and melanomas). [1]

There are numerous professions that imply working

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outdoors, from which we concentrated our study on agriculture and construction workers. Occupational ultraviolet (UV) exposure can be measured with UV dosimeters.

Personal UV dosimeters are electronic devices that are able to record data that permits analysis of UV exposure dose on working days. Modern dosimeters are small in size, therefore easy to wear at the workplace, permitting personal UV measurements. Even more so, multiple dosimeters can be used, recording data from different parts of the body. They are relatively new devices used more and more frequently in UV exposure-related studies. [2-5]

Ultraviolet radiations represent segments of the electromagnetic specter and are classified in UVC (100-290 nm), UVB (290-320 nm) and UVA (320-400 nm).

UV radiation is very important in dermatological practice, being able to determine both exacerbations and remissions of different skin disorders and of course playing a major role in skin cancer development. [6]

Even though UV radiation is the most important factor implicated in skin cancer pathogenesis and World Health Organization introduced UV radiation on the carcinogens agents list of group I, still, few European countries recognize UV occupational-induced skin cancer as an occupational disease, Romania not being part of this category. [7]

The lack of studies in this area in Romania suggests the fact that more analysis should be made for a better understanding of this aspect in our country.

Objectives: to evaluate UV exposure doses in agriculture and construction workers and to establish efficient prevention and protection measures.

MATERIALS AND METHODS

In our Department, a prospective, observational, analytical study was conducted, including six outdoor workers (in construction and agriculture), over a period of three summer months (July, August, September).

Subjects were selected based on their profession. Our study took place in Bordusani, Ialomita, Romania, with the geographical coordinates 44°28’21”N 27°53’59”E for the agriculture outdoor workers and in Bucharest, Romania, with the geographical coordinates 44°25’57”N 26°06’1 for the construction workers.

Subjects were asked to record in personal diaries information regarding: profession, location, date, start time and end time of their schedule, lunch breaks and weather conditions.

The following parameters were analyzed: profession, duration of exposure, UV exposure doses, skin aspect, preexisting preventive measures.

For the agriculture workers, we’ve selected irrigation system manufacturer, tractor driver and beans manufactures and for the construction site we’ve selected road workers.

Subjects were asked to wear personal dosimeters for several working days. Sun exposed areas involved the face, neck, forearms and dorsum of hands, the rest of the areas were covered by equipment.

Clinical examination was performed before and after measurements to detect any skin changes.

The personal dosimeter used in this study was X-2012-10 Personal UV Irradiance Dosimeter from Gigahertz-Optik and a “Velcro” fastening system was used to attach it on the subject’s forearm.

Data was processed using Microsoft Office Excel 2013.

RESULTS

The wearing of dosimeters was well tolerated by all subjects, without any interference with daily professional activities.

Daily UV radiation doses ranged from 1.79 SED (tractor driver) to 19 SED (agriculture worker).

Maximum UVR doses were recorded between 10:00 am and 4 pm. Apart from standard equipment, no protective measures were applied.

Clinical examination was made after measurements, with no detectable skin changes.

DISCUSSION

Sunlight is composed of a continuous spectrum of electromagnetic radiation divided into three major groups: ultraviolet, visible and infrared. [8] Ultraviolet radiation is a major causative factor in skin cancer development. [9]

Even though UVA has less energy than UVB and penetrates deeper, into the dermis, UVB radiation is most implicated in carcinogenesis. UVA is responsible for carcinogenesis through immunosuppression: it determines production of reactive oxygen species that cause damage in the deoxyribonucleic acid, therefore gene mutations and damage in the epidermal keratinocytes and dermal connective tissue.

Examples of gene mutations include PTCH and p53 tumor suppression gene in basal cell carcinomas, activated mitogenic ras pathway and p53 in squamous cell carcinomas and ras pathway, inactive p16 and p53 in melanomas. [10]

Also, the duration and intensity of UV exposure are factors implicated in skin cancer development. For example, cumulative, long-term UV exposure is related to chronic actinic skin damage, therefore implicated in the appearance of actinic keratoses and squamous cell carcinomas, since intermittent, high intensity UV exposure especially at young ages would most likely cause basal cell carcinomas and melanomas. [11]

For measuring UV exposure doses on an outdoor worker, irradiance should be determined - the flux of radiant energy per unit (W/m2). But this applies only for one moment in time, so for a larger period (e.g. one working day) the term "exposure dose" (J/m2) is used. [12] For this, a standardized unit – SED – Standard Erythema Dose is applied, where 1 SED is equivalent with 100J/m2. [13]

The annual exposure dose for the general population in Germany is 130 SED. [14] Considering our results, with a daily UV exposure dose of 19 SED (15% daily of the annual exposure dose), we have to acknowledge the fact that both agriculture and construction professions in Romania are professions with high-risk of developing solar exposure related pathologies.

Even more so, by German standards, if the outdoor worker was exposed to a cumulative UVR dose with 40% higher than the general population, than the nonmelanoma skin cancer is considered to be of “occupational origin”. [14]

Therefore these professions should be placed in the occupational skin diseases category.

It is very important to take into consideration that different parts of the body absorb different UV doses depending on body geometry, therefore involve different risks. Multiple personal dosimeters should be used, calculating UV doses on different body sites. [16]

Horizontal body locations seem to receive 2x - 2,5x more UV that the vertical ones. [17]

Also, UV exposure dose depends on the reflection of UV radiation on surrounding structures. For example, UV reflection for construction workers surrounded by metallic structures is different to that of fishermen that are surrounded by water. This is a significant additional benefit of personal dosimeters, being able to record UV doses from different parts of the body and not taking into consideration only the direct, vertical radiation.

For example, even if an outdoor worker has correct equipment, with a broad-brimmed hat, UV reflection from surrounding structures could still reach him. Therefore additional protective measures should be implemented, such as protective sun creams. Also, personal dosimeters, being able to measure UV radiation received by the subject, these measurements could be compared with those stated by meteorological departments. [18]

Even more so, occupational exposure to UV radiation can determine specific features of the tumors involved. For example, basal cell carcinoma appears more frequently in the "mask area" of the face in occupational exposed workers and also histopathologically appears to be more aggressive in evolution. [19]

All of this important features reinforce the necessity of implementing strict preventive and protective measures for outdoor workers, including protective sun creams (SPF 30 or more), special sun protective

equipment (long pants and long sleeves, broad-brimmed hats or helmets, sunglasses), multiple breaks in indoor areas and rotating shifts. [15] Also, considering the fact that the maximum UVR dose was detected between 10 am and 4 pm, outdoor workers should avoid working outside in this timeframe.

Even if by the end of our study no pathological skin changes were expected, we have to keep in mind that most UV skin related pathologies depend on a cumulative UVR dose with long term exposure. More interventional and observational studies are necessary for a better understanding of UVR effects on outdoor workers in Romania.

CONCLUSION

Even though workers in agriculture and construction in Romania are exposed to significant UV doses, with 40% more than the general population, these professions are not recognized as high-risk professions for developing nonmelanoma skin cancer or melanomas. More prospective and retrospective studies are necessary for more significant statistical results and for demonstrating the necessity of introducing primary, secondary and tertiary preventive measures.

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CLINICAL PRACTICE

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A case of acne fulminans

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Abstract: *Background and aim: Acne fulminans is a rare and severe form of acne which usually appears in young men with a personal history of mild to moderate acne. It is characterized by a sudden onset with nodules and ulcerative lesions mostly located on the thorax and extracutaneous manifestations (fever, weight loss, osteoarticular damage). The pathophysiologic mechanism is represented by a type III or IV hypersensitivity reaction to Propionibacterium acnes.*

Patient: We present the case of a 16-year old patient with acne fulminans. The lesions were accompanied by an impaired general condition with fever and weight loss. Paraclinically an inflammatory syndrome was revealed without osteoarticular changes. It was treated with prednisone 40 mg/day, erythromycin 2g/day and local antiseptic solutions. A gradual tapering of the prednisone dose (5 mg/week) and a dermatological revaluation after 3 weeks to introduce systemic treatment with retinoids were recommended at discharge.

Conclusions: Acne fulminans is a severe form of acne with systemic manifestations and corticotherapy is the first line treatment.

Keywords: *acne fulminans, corticotherapy*.

INTRODUCTION

Acne fulminans is a rare and severe form of acne which usually appears in young men with a personal history of mild to moderate acne. It is characterized by a sudden onset with nodules and ulcerative lesions mostly located on the thorax and extracutaneous manifestations (impaired general condition, fever, bone pain, anorexia, splenohepatomegaly) [1-3]. Osteoarticular impairment is common, it being reported in about half of the cases [4,5]. The pathophysiologic mechanism is represented by a type III or IV hypersensitivity reaction to Propionibacterium acnes [6]. The involvement of drugs such as anabolic steroids and retinoids are also quoted [7,8]. The treatment of choice are corticosteroids with initial dosing of 1 mg/kg/day followed by a gradual tapering and retinoids introduction from the 4th week [9].

MATERIALS AND METHODS. CASE PRESENTATION

We present the case of a 16 years old patient who required a dermatological consult for a cutaneous eruption located on the face and the anterior and posterior thorax.

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The skin examination showed comedones, papules, pustules and ulcerative nodules with necrotic crust. The patient reported a personal history of untreated acne vulgaris.

He described the onset of the rash 14 days prior to admission. Lesions were accompanied by anorexia, mild fever (unchecked at home). He has lost 4 kg from the appearance of the eruption.

Laboratory results have revealed an increased ESR (64 mm/h), leukocytosis (16,340/mm^3).

The bacteriological examination of the lesions was negative. The radiography of the large joints (elbows, knees, lumbosacral) has revealed no pathological changes.

The patient was treated with prednisone (40 mg/day), antibiotics (cefuroxime, erythromycin), antiseptics and benzoyl peroxide with clindamycin.

The evolution was favourable after 7 days of treatment with a decrease in the skin lesions and the inflammatory markers.

Figure 1

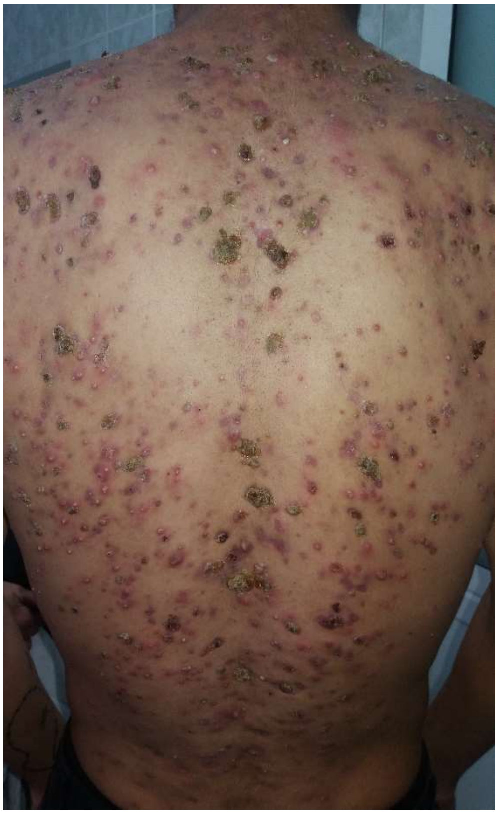

Figure 2

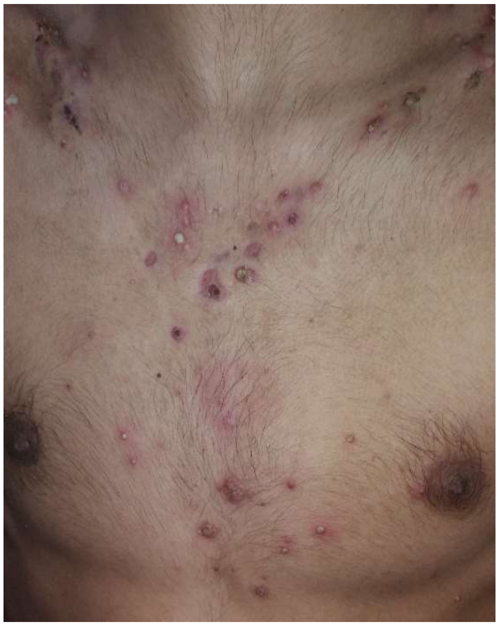

Figure 3

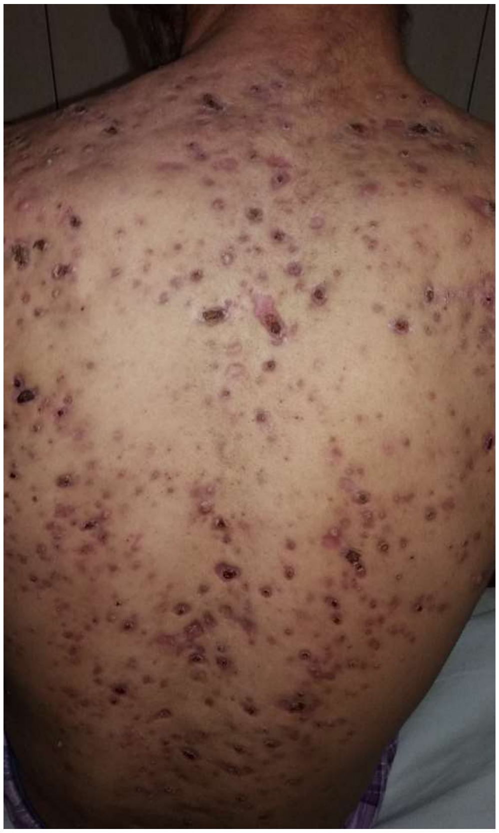

A gradual tapering of the prednisone dose (5 mg/week) and a dermatological revaluation after 3 weeks to introduce systemic treatment with retinoids were recommended at discharge.

DISCUSSION

In the literature acne fulminans is presented either as a stand-alone entity or as a manifestation within SAPHO syndrome (Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis). Also cited are the association of acne fulminans with inflammatory bowel disease, ankylosing spondylitis, the appearance of it after an intake of anabolic steroids and paradoxically after isotretinoin administration.

Rajaee et al presented the case of a 16 years old boy with acne fulminans with positive antinuclear antibodies and positive HLA-B27 antigen. The radiological examinations of the sacroiliac joints and chest revealed no changes and that of the right knee revealed effusion and soft tissue swelling [10].

Also interesting is the association of acne fulminans with Crohn's disease and ulcerative colitis. Pecova et al. describe the case of a 30 years old patient with known Crohn's disease and papulopustular acne from the age of 17. He had the first episode of acne fulminans at the age of 21 after discontinuation of methylprednisolone therapy utilized for primary disease. Five years later the same patient developed a new flare of acne fulminans due to the introduction of biological therapy (infliximab) [11]. Another case is that of a 19 years old man treated for ulcerative colitis for 2 years, who developed acne fulminans and was successfully treated with prednisone and diaminodiphenyl sulfone orally [12]. Regarding the association of acne fulminans with the administration of testosterone, an interesting case is that of the twins suffering from Kallman syndrome who from the age of 17 received testosterone substitution treatment for delayed puberty. Nine months after the substitution therapy started the patients developed acne fulminans [13].

A case of acne fulminans was reported in a young bodybuilder who used anabolic steroids (testosterone enanthate, trenbolone acetate, drostanolone propionate and methandrostenolone) for muscle growth. Shortly after the cessation of the anabolic steroids use he developed acne fulminans with debilitating arthralgias [14]. Mayerhausen and Riebel presented in a 1,989 article the case of a 23 years old man, javelin thrower, who developed acne fulminans after taking anabolic steroids and vitamin B preparations. [15]

SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis, osteitis) is a rare inflammatory disorder that associates osteoarticular lesions with pustular disorders such as pustular psoriasis, palmoplantar pustulosis, acne fulminans and hidradenitis suppurativa. The axial skeleton, the sternoclavicular and sternocostal joints are more commonly affected than the peripheral joints [16]. Lakshmi Divya et al presented the case of an 18 years old man with a 9 months old painful nodulocystic acne. The patient had also severe pain in the wrists and lower back, chills and stiffness for 30 days. An MRI scan of the right hand joint revealed osteolytic lesions. A scintigraphy with technetium 99m indicated increased capture in the bilateral distal radial metaphyses, sacroiliac joints, vertebral plateaus and the 7th costovertebral joint. The patient was treated orally with cefpodoxime, doxycycline and colchicine 1 mg/day. 4 weeks later systemic therapy with isotretinoin 20 mg/day was started with remission of the lesions at 12 weeks [17].

Acne fulminans can be paradoxically induced by isotretinoin. Some authors consider that its appearance is dose dependent. Pereira et al. presented a case of acne fulminans induced by low-dose isotretinoin (20 mg/day). The patient was treated with oxacillin, prednisone and oral isotretinoin in the same dose with a favorable evolution [18]. MacKenzie describes another case of isotretinoin induced (20 mg/day) acne fulminans one month after the initiation of therapy. Following treatment with prednisone (60 mg/day) and erythromycin (2 g/day) the lesions diminished after a month. Isotretinoin treatment was then reintroduced with gradually increased dosages from 10 mg/day to 70 mg/day with full remission of the lesions after 11 months [19]. Zanelato et al presented the case of a 14 years old boy that was initiated on isotretinoin 0.5 mg/kg/day and prednisone 0.1 mg/kg/day. 12 days later ulcero-necrotic lesions started developing on the face and trunk. The patient had also musculoskeletal involvement (bilateral sacroileitis, scapulohumeral, chondrocostal, jaw, ankles and lumbar intervertebral joints). Isotretinoin was stopped and the patient was started on prednisone with a favorable evolution. 30 days from the interruption, isotretinoin was reintroduced in doses of 0.2 mg/kg/day with subsequent dose increase after 2 months to 0.5 mg/kg/day while corticosteroid therapy was tapered. [20].

CONCLUSION

Acne fulminans represents a severe form of acne with systemic manifestations. The mechanism of the disorder is not yet elucidated and corticotherapy is the first line treatment.

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Col. Richard Belcescu MD, turns 90 – A portrait

Adrian M. Nistor

I am a human being to whom very few opportunities have been offered in an entire lifetime or maybe I was not able to turn them to account when they popped up. But meeting retired Colonel Richard Belcescu MD, was for me what may be called a "chance," a "stroke of luck."

Immediately after graduating from the dentistry school of Bucharest, I was assigned to work in the surgery where Dr. Richard Belcescu worked busily, on the seventh floor of the Carol Davila Central Military Hospital. A soft-eyed middle-aged man came to meet me, dressed up to the nines, who, making no fuss over the whole thing, helped me get acquainted with the activity of the section. I was to discover subsequently that my then boss was one of the most outstanding human beings I was destined to meet. He tried to polish the numerous and serious irregularities of the personality of a young lieutenant doctor, who had just finished school. As a matter of fact he retained his capacity as a polishing creator of medical but also human personalities all along his stay at the OMFS section, exerting it on many young doctors.

Colonel Richard Belcescu, MD, was not very well known in public life: he gave no resounding interviews to the propaganda newspapers of the 70s and the 80s, and did not appear on TV. However, thanks to his qualities, he asserted himself as an opinion leader all the hospital staff held in high esteem.

Being side by side with him made you feel calm and protected at that time of turmoil. Endowed with much earnestness in all the aspects of his professional, but also personal, life, Dr. Richard Belcescu was certainly the one colleague that "should be found in any clinic." He used to solve everything inherent in a surgical team: he used to commit himself to solving some cases that required a special relation between the doctor and the patient besides surgery as such.

We kept in touch even if life and the difference of age made us stay apart from each other. He used to pay regular visits to me at various stages of my professional career, but he always tried to be as discreet and warm-hearted as possible, with no trace of conceit whatsoever. He was familiar with everything going on in our section but also in the whole hospital, getting emotionally involved in an extremely sincere manner.

I learnt by mere chance that he is in an old people's home where his family have taken him very affectionately because of the marked difficulties due to old age. His being there certainly makes him benefit from remarkably good palliative care, which nobody else can better give.

Extremely impressed, I came near to the bed where Dr. Richard Belcescu lay: "Hi, Adrian! How are you? We haven't seen for ages!" Frankly speaking, I was almost at a loss in that clean tidy room where one of my mentors was bedridden because of the lapse of time. We had a short genial conversation during which I found it difficult to suppress my emotion. All the unforgettable recollections of the events that kept us close together for many years came to my mind.

I left the sunlit room in which Dr. Richard Belcescu lives now and promised to come and visit him from time to time. Being 90 – is it a long or a short time of one's life? On the way to my surgery, where he came to visit me and where I had the honor of treating him, I remember part of the moments spent by the side of this wonderful man. This is why I seem to hear again the words previously spoken: "In every clinic there must be a Dr. Belcescu" as a benchmark for medical rigors but also for the consummate behavior of a colleague.

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We look forward to your submission.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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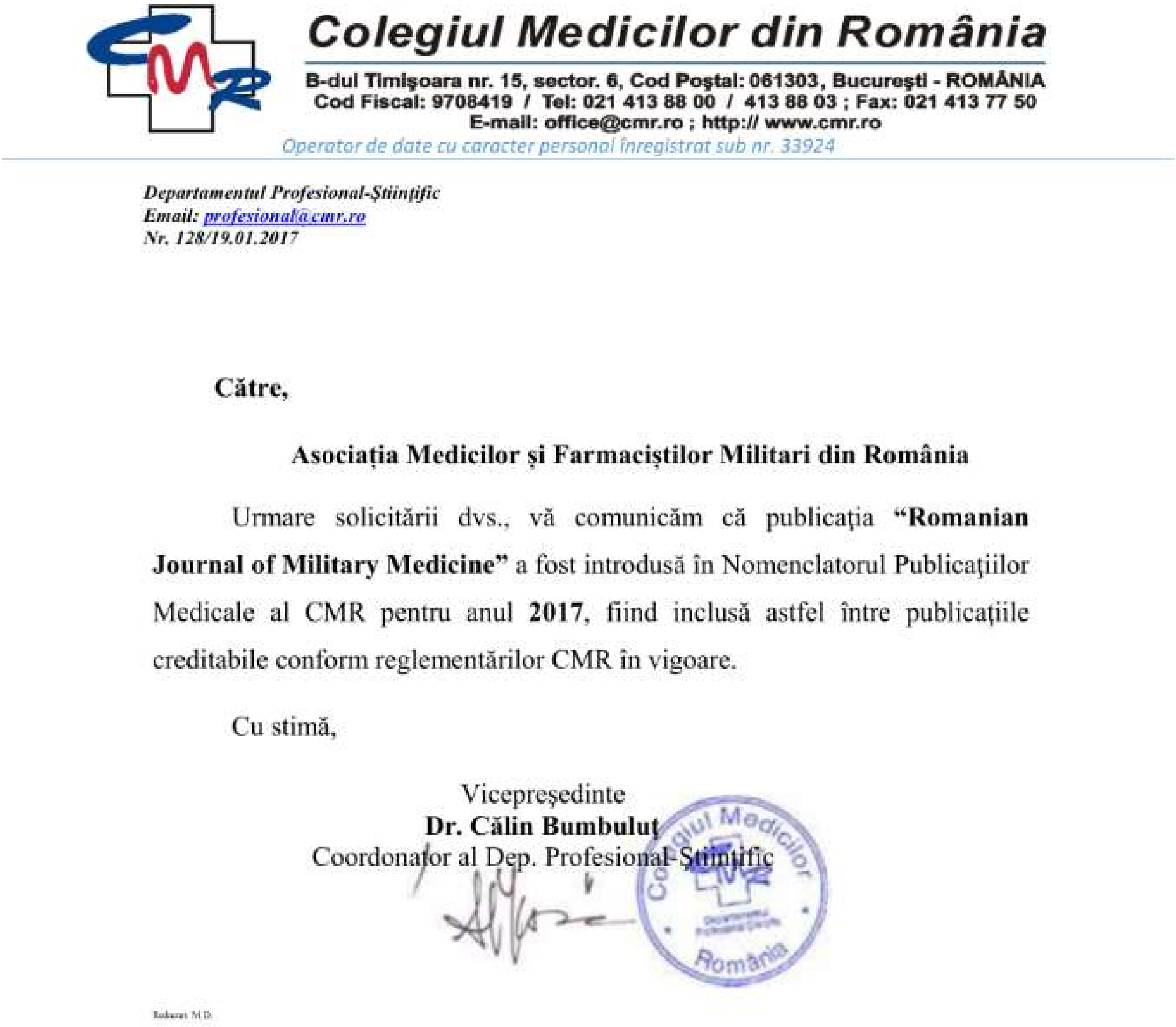

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The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’

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EDITORIAL

Being a surgeon – A terrible and fascinating job

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Gral (R) Prof DAN MISCHIANU

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At first it was a simple job: a barber, the surgeon, the "țirulic" (from Turkish language), the "gerah" (from Turkism "cerrah"), a job that did not dare to compare with the more "chosen" profession of "doctor/ physician". Doctors represented something else, they were much esteemed in the public consciousness, and the society really appreciated them. Later, people learned and found that being a surgeon (term surgeon derives from the Greek "cheirurgos" means one that does the job with their own hands!), is a noble, very noble profession, perhaps simply because its practitioners live "risky lives"!

Surgery is quite different from being a "doctor/ physician" and I assume this dichotomy trying to explain to the reader, in the most simple way: surgeons practice a risky job and live in this regard, particularly "dangerous"!

When I chose to serve in this profession I knew almost nothing about what it will happen to me during my professional life. I thought, as a medical student, that being a surgeon is a profession or a specialty as all the others, something that means to have a job and to go to "punch in", to sit in front of the "bench" (such as smiths!) or in front of your computer (if you are IT-ist!) and at H hour (usually 15:00) to get up from the desk, to close "the issue of the day" and leave never forgetting to "punch out". I never ever wanted something like this and God helped me in this regard! Now, in adulthood, I think I would not have been able to be part of such a "procedure"...

Then, shortly, nearly 40 years ago, I learned that being a surgeon really is something else...

Surgery has valences of the ineffable.

I stayed in the hospital even for 2-3 days, and when the Revolution came I got home on 04/01/1990, after 19 days of alarm. I have gathered in my life as a surgeon, eight and a half days of shifts performed in our homeland hospitals. I think know what I want to share...

I wondered, often, who can become a true surgeon.

You do not get to become a surgeon because you want, you become a surgeon by chance. You were a painstaking student, you've prepared well for the secondary exam (old style) or the residency (new style), you get an efficient score and choose a surgery specialty. You thought you can endure this challenge... "Absolutely by chance" – as George Topârceanu's verse from the poem "Jealousy"... Many of these future "surgeons" will regret this decision for the rest of their lives!

Let me explain: you will not become a good surgeon unless you have a vocation for this job. He who chooses the job of resident surgeon because he has passed the residency exam will curse his uninspired choice when he will find out he has no vocation for it.

I met such surgeons. They ended up withdrawing in resignation "pigmented" by completely different concerns.

And therefore I find that being a surgeon is a vocational profession, such as the athletes or airplane pilots.

Because I remembered two other noble professions, have you ever asked the question how "consumptive" is being a surgeon? How challenging is it – pre and postoperatively, how much rigor is needed to practice it in the fairest way possible and how many years it shortens surgeon's life in comparison with other doctors? I am absolutely convinced that you know at what age is over the "working life" of great athletes like Nadia Comaneci, Gheorghe Hagi and, to answer your previous question, the surgeon life is shortened for an average of ten years compared to other colleagues.

I dare to mention two other issues. There are, in present times, surgeons who know a "relative anatomy" of the human body. Professor Dr. Eugen Târcoveanu specified 12 years ago, in a fabulous article, one simple thing: "Although today there is no compulsory internship anatomy, anatomical knowledge are indispensable to any surgical careers. Clinical training must precede technical training and manuality. This apprenticeship has changed since surgical specialization has become a necessity. This is beneficial considering the complexity of some surgical fields. However, the formation of a surgeon must keep an overall basic surgery knowledge." (E. Târcoveanu – A fi chirurg în zilele noastre – Journal of surgery, Vol 10, No 3, 2014, Iași ISSN 1584-9341)

I think it is a pity of the legacy of William Harvey! These surgeons usually do not even get to practice this profession. They fail in peculiar professions like politics, diplomacy, business, etc.

There's also a "second group" of surgeons: those who do not like the clinical surgery. I do not think it is conceivable that after you operate a human being, you dare, having all the knowledge and diplomas, not to examine, to consult, to mend and talk with that man. Observation of man by man gives us some truths that we can not learn by phone, computer or "skype"! Perhaps only this simple fact makes and will always make surgery a terribly fascinating job!

I wrote these lines in the first decade of December 2016 with great sorrow in my heart about what happens to us doctors, especially to those born and not made surgeons. I wrote these lines with hope for humanity and respect for hardworking values! Before they became values! And I can only repeat myself. I wrote it before. Stephen King wrote in "The Shawshank Redemption" (translated to us as "The angels prison"): "Hope is a good thing, maybe the best of all, because hope dies last!..." But dies in torment I allowed myself to add, with undisguised cynicism!

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REVIEW ARTICLE

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Towards an integrated medical system for radiological medical imaging investigations

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Abstract: *The continuously increasing number of medical investigations using radiological methods imposes the strong necessity of informing patients about benefits and risks regarding radiation absorbed doses. Tracking the radiation doses absorbed by patients must be a future challenge of any medical system. The effective doses received by patients in many types of medical investigations must be calculated, transformed, recorded and cumulated. Doctors and patients must be very responsible in prescribing or demanding new radiological medical investigations. Radiological standards, legislation, guidelines, programmers and practice supervised by international commissions on radiology protection must include new specific measures for patients' cumulated doses. A pilot Romanian project had tried to accomplish some of these tasks.*

INTRODUCTION

Radiation is commonly associated with nuclear accidents or modern irradiative artificial sources such as: mobile phones, great antennas, satellites, food irradiation, and radioactive building materials.

Generally, radiation is associated with all kind of human activities.

As a huge surprise, in fact, radiation is quite a natural phenomenon in our lives. There are many radioactive substances in air, water and soil that are called natural background radiation. During a long airplane flight one can also receive an important radiation dose. Typically doses from natural sources are measured in mSv. The Sievert (Sv) is the International System of Units (SI) derived unit for radiation dose.

There are many other different units for absorbed radiation and transformations can be made in order to compare them.

A recent United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) publication [1] estimates worldwide distribution of radiation exposure:

- 13% from cosmic sources
- 16% from soil
- 9% from food
- 20% from medical investigations
- 42% from radon sources

Radon-222 is a radionuclide in the form of a gas that normally emanates from the soil. Radon is a colorless,

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odorless, radioactive gas. Most exposure to radon comes from being indoors in buildings. A strong interaction between smoking and radon has been proven, so radon is considered as a primary cause of lung cancer, smokers being more vulnerable [2]. There are no widely available medical tests to measure radon exposure. So radon belongs to natural sources of radiation.

The increased doses of radiation in our climate and habitat is surveyed by national or international organizations, there are standards, policies and strategies for general radiation protection.

International commissions on radiology protection are worldwide active for radiological standards, legislation, guidelines, programmers, and practice.

The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) highlights the levels and effects of exposure to ionizing radiation [1].

The International Commission on Radiological Protection (ICRP) has published a lot of reports on generally human protection and the specific protection of patients exposed to ionizing radiation [3].

In Romania, CNCAN is the national competent authority in nuclear field “having responsibilities of regulation, authorization and control stipulated in this Law is the National Commission for Nuclear Activities Control, public institution of national interest, legal entity, with the head office in Bucharest, being headed by a President having the rank of State Secretary, coordinated by the Prime Minister [4].

RADIOLOGICAL MEDICAL INVESTIGATIONS

Medical imaging is a complex process of visualization of the interior of a human body. A lot of techniques and engineering tools are used in order to obtain a visual representation:

- Radiology using X-rays;
- Computed Tomography (CT scan) using also uses special X-ray equipment;
- Nuclear medicine, using internal radioactive substances including scintigraphy that uses radio-isotopes travelling to organs, positron emission tomography (PET) or Single-photon emission computed tomography (SPECT) ;
- Mammography as a specific type of breast imaging that uses low-dose x-rays;
- Magnetic resonance imaging using the property of hydrogen atoms from human body to absorb or emit radiofrequency energy when placed in an external magnetic field;
- Medical ultrasonography or ultrasound, a diagnostic imaging technique based on the application of ultrasound;
- Endoscopy using an instrument inserted in the human organ;
- Thermography, using thermographic cameras and infrared imaging techniques;
- Elastography;
- Tactile imaging or mechanical imaging.

Only the first four investigations imply radiological methods in medical investigations. The last ones do not imply radiological risks, even there are scientific debates on SAR (specific absorption rate) as a dosimetric quantity for Electromagnetic Fields bio effects [5].

Patient exposure to ionizing radiation can be made externally, internally nor both. CTs doses are usually externally absorbed, while in angiography, fluoroscopy or scintigraphy investigations, a radioactive substance is ingested. Contrast media are also used on radiography and CT to visualize vessels or tissues. Iodine is a modern intravenous contrast agent, but barium is also used. Usually iodine contrast media is used intravenously, it can also be used intra arterially. Barium sulfate is usually swallowed. Magnetic Resonance Imaging or CTs also use contrast agents.

Differences between exposure and contamination can also be debated [6]. Exposure to a radiation source does not imply that this person becomes a new radioactive source. Contamination incorporates the radioactive agent in the body, such as in thyroid gland.

Exposure to X- rays increases the future incidence of cancer, but quantitative models predicting the level of risk are still not worldwide accepted. Different methods for radiation risk calculus are available. The

linear no-threshold (LNT) model assumes the proportionality between cancer risks versus absorbed dose. The relationship between dose and DNA damage is considered linear. The International Commission on Radiological Protection (ICRP) recommends the use of the LNT model [7].

Significant uncertainties are related to dose assessment, low dose and low dose-rate extrapolation approaches, transfer of risks from a population for which health effects data are available to one for which such data are not available [8]. Adaptive responses of human cells have also been observed at low doses [9].

Classical radiographies, computed tomographies (CTs) and scintigraphies are worldwide used medical radiological investigations that expose the patients to significant doses of radiation.

The actual volume of medical prescription, the contrast substances used in order to increase their accuracy, the overlapped investigations are general causes for a strong increasing of the cumulative radiation dose absorbed by patients. Patients and doctors usually excess in demanding, using and prescribing medical investigations in spite of harmful effects associated with radiation delivery.

The prescribed medical investigations total volume, starting from dental radiographies and ending at multiple regions CT scans prove a gap in monitoring and tracking of the cumulative radiation doses in many health services all over the world. The received radiation doses by patients are quit impossible to be cumulated in spite of the fact that in our modern world, 20 % of absorbed radiation dose is due to medical investigation.

The patients travel from one hospital to another, all over the country or all over the world.

An integrated system must be implemented. In order to cumulate all the absorbed doses by a patient in different types of radiological investigations, the doses measurement units must be the same and the recorded doses must be kept together in a single patient monitoring unit.

RADIATION DOSE RISK AND DIAGNOSTIC BENEFIT [10]

The treating physician and the radiologist can prescribe and carry out radiological imaging procedures. Several objectives and subjective elements are implied in a prescription:

- Medical request
- Imaging investigation algorithm
- The personal experience of the examiner
- Patient history
- The results of any other previous investigations

All the time a correct judgment concerning the benefit/risk ratio must be performed. Medicine and the radiation risks knowledge must be well handled. The aim of managing radiation exposure is to minimize the irradiative risk without sacrificing, or unduly limiting, the obvious benefits in the prevention, diagnosis and also in effective cure of diseases (optimization).

When too little radiation is used for diagnosis or therapy there is an increase in risk. Too low an amount of radiation in diagnosis will result in an image that does not have enough information to make a correct diagnosis. There are several ways that will minimize the risk without sacrificing the valuable information that can be obtained for patients' benefit. A possible measure is to carefully justify the examination before sending a patient to the radiologist or nuclear medicine physician. Repetition should be avoided in recently investigations performed into another clinic or hospital. An investigation may be seen as a useful one if its outcome – positive or negative – influences management of the patient. Another factor, which potentially adds to usefulness of the investigation, is strengthening confidence in the diagnosis. Most common examples of unjustified examinations include: routine chest radiography at admission to a hospital or before surgery in absence of symptoms indicating cardiac or pulmonary involvement (or insufficiency); skull radiography in asymptomatic subjects of accidents; lower sacrolumbal radiography in stable degenerative condition of the spine in the 5th or later decade of life, but there are of course many others [11]. From medical experience, the diagnosis

benefit is a priority. For a lung Rx examination any suspected image is repeated or supplementary investigated in a Computed Tomography.

Many times, the personal experience of the radiologist is the dominant factor in high radiation doses investigations:

- urgent exam such as trauma and polytrauma, fractured skull or spine where CT exams are a real benefit for the patient and an economic benefit for the hospital. They can shorten the patient's hospital stay.
- scintigraphic investigation in thyroid diseases
- repeated mammography
- bone disease with seemingly benign tumors requiring an examination for differential diagnosis.

RADIATION MEASUREMENT UNITS

„The International Commission on Radiological Protection (ICRP) recommends that the public limit of artificial irradiation should not exceed an average of 1 mSv effective dose per year, not including medical and occupational exposures. ICRP limits for occupational workers are 20 mSv per year, averaged over defined periods of five years, with the further provision that the dose should not exceed 50 mSv in any single year“ [12].

The Sievert (Sv) is a derived unit in the International System of Units (SI) used for equivalent absorbed radiation dose measurement. This must be the central unit of a tracking medical system.

There are many different units for absorbed radiation used on a large scale. Becquerel (Bq) and Curie (Ci) as SI units are used for released radioactivity, while Coulomb/Kilogram (C/kg) and Roentgen (R) are used for the dose travelling through the air, Gray (Gy) and Rad, used with quantities of absorbed dose. A lot of Internet sites provide convertors between them.

The biological effects of the absorbed amount of radiation can be described with Roentgen equivalent man (rem) and Sievert (Sv). They are specific measurement units.

The Sievert can better describe the effective equivalent dose absorbed by biological tissues while the Gray can describe the absorbed dose of any material.

Table 1: Radiation Measurement Units

Type	Measurement unit		
Released radioactivity	Curie (Ci)	Non-SI	**1 curie = $3.7x10^{10}$ radioactive decays per second**
	Becquerels (Bq)	SI unit Derived	One becquerel represents the activity of a quantity of radioactive material in which one nucleus decays per second – **$1 Bq = 1 s^{-1}$**
Dose travelling through air (Exposure)	Coulomb/Kilogram (C/kg)	SI unit	**1 C/kg = 3876 R**
	Roentgen (R)	Non-SI	**1 C/kg = 3876 R**
Absorbed Radiation dose	Gray (Gy)	SI unit Derived	It is defined as the absorption of one joule of radiation energy per kilogram of matter **$1Gy=1J/Kg=1m^2/s^2$**
	Rad	Deprecated, replaced by the Gray	**1 rad = 0.01 Gy = 0.01 J/kg**
Dose equivalent (the biological effects of the absorbed amount of radiation)	Röntgen equivalent man (rem)	Non-SI	**1 rem=0,01Sv** **1 mrem/h = 8766 mrem/yr**
	Sievert (Sv)	SI unit Derived	represents the equivalent biological effect of the deposit of a joule of radiation energy in a kilogram of human tissue

Modern radiological apparatus for computerized tomographies or scintigraphies can provide the radiation doses during a particular investigation, but the recorded doses' types and the radiation

measurement units in different types of investigations are not the same.

In the classic radiological investigation an important topic for data management is the measurement and the calculation of radiation dose expressed in Dose area product (DAP), expressed in (Gy*cm2). The dose length product (DLP) was chosen as input data for the system in CTs investigations and it is expressed in mGy*cm. The DLP must be converted to mSv in order to unify the results.

REQUIREMENTS OF A DESIGNED TRACKING SYSTEM

A tracking proposed system must include computers, smartcards for storing data and suitable software applications for recording, retrieving and alert. The effective doses received by patients must be unified and cumulated.

The system provides the replication of the information stored in central databases, local databases and patient cards to cover all the following possible situations:

- The patient goes to the doctor without the patient card. In this case, the system provides the data corresponding to the patient based on the local database, if the patient has been investigated in that hospital unit; if the patient is new, the system must provide the data from the central database to the local database, so as to take the optimal decision in recommending the type of investigation. After the investigation, the system must store the new local database accumulated radiation dose to the patient, the information arriving in the central database. Later, when the patient goes to the doctor for further investigations with the card, the system must ensure synchronization between the information stored on the card and information stored in the local and central database.
- The patient goes to the doctor with no card and the hospital unit's information system does not have access to the central database (ex: for mobile laboratories). If the local database contains the information corresponding to the patient, the doctor must be able to use them for recommending a particular type of investigation. After the investigation, the system must store in the local database the new radiation doses accumulated by the patient. Later, when the local system can access the central system, the information corresponding to the patient must be synchronized between the two databases.
- The patient presents the card to the doctor but the doctor does not have access to any database (local or central). In this case the doctor, using a computer with a card reader can access the history of investigations and the doses received by the patient, as the current cumulative dose calculated and can recommend the most appropriate investigation. After investigation, the appropriate dose must be recorded on the patient's card and next time when the patient goes to the doctor with the card, this information must be stored in both in the local and the central database.

Additionally the system provides applications for:

- Viewing in real time the history of investigations, of the doses delivered to the patient and of the current cumulative calculated dose expressed in mSv.
- Aiding the medical staff in taking the adequate decisions regarding the indication of investigations according to the current calculated cumulative doses and the maximum doses allowed for the risk and age groups.
- Performing of various periodic reports in order to take different types of decisions related with the existing radioprotection rules.

The proposed system includes the following modules:

a) Applications running on smart cards, called on card applications.

b) Off-card applications that are running on medical stations. They include smart card readers and writers in order to record and retrieve the information about the type of investigations and the specific emitted doses

c) A data base records all the necessary information in order to replicate a lost or destroyed card but also this database must provide the possibility of collecting data about the patients on several criteria, it must provide the possibility of standard or customized reports' creation.

d) The security solutions such as public key infrastructure PKI in order to achieve a high level of

security of recording and retrieving data. A public-key infrastructure (PKI) is a set of hardware, software, people, policies, and procedures needed to create, manage, distribute, use, store, and revoke digital certificates. A PKI establishes and maintains a trustworthy networking environment by providing key and certificate management services that enable encryption and digital signature capabilities across applications — all in a manner that is transparent and easy to use. Digital certificates are electronic credentials that are used to assert the online identities of individuals, computers and other entities on a network. They are issued by certification authorities (CAs) that must validate the identity of the certificate-holder both before the certificate is issued and when the certificate is used.

The entire system must be designed around the two types of cards: the patients' cards and the medical cards and it ensure many requirements ensuring secured communications.

Two different data flows must be implemented:

- Electronic cards flow management that includes: smart cards issuance, their renewal in case of loss, damage or theft.
- Operational cards flow management including: patients and doctors authentication using unique PINs.

Figure 1: Designed system

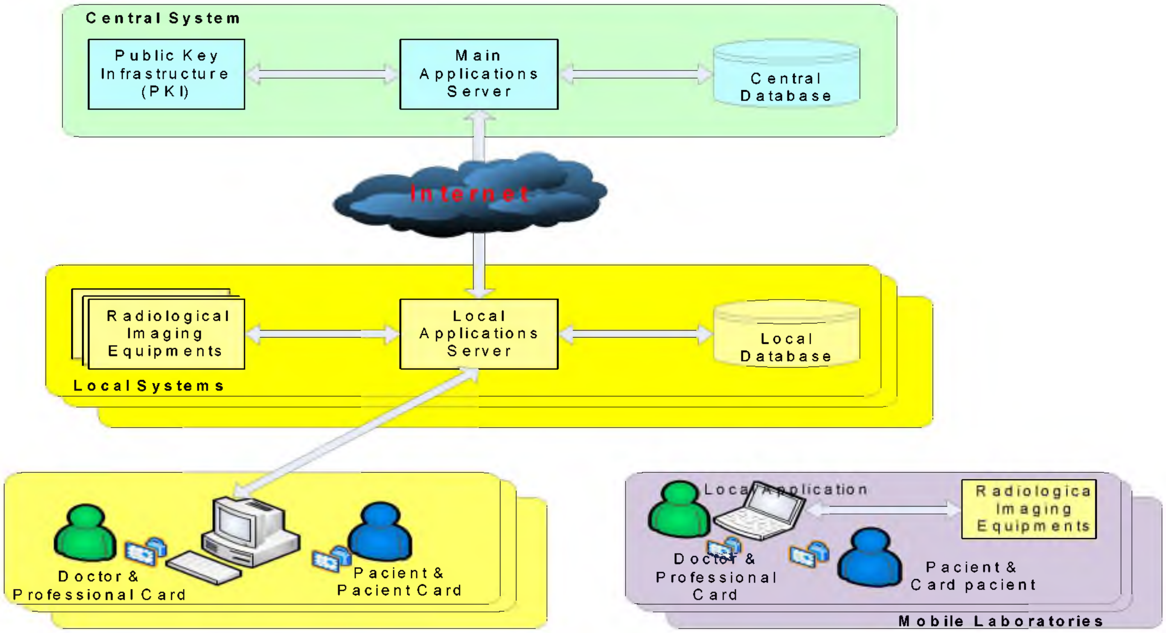
Central System
Public Key Infrastructure (PKI)
Main Applications Server
Central Database
Internet
Radiological Imaging Equipments
Local Applications Server
Local Database
Local Systems
Doctor & Professional Card
Pacient & Pacient Card
Loca Application
Radiologica Imaging Equipments
Doctor & Professional Card
Pacient & Card pacient
Mobile Laboratories

DESIGNED SYSTEM

A Romanian pilot study has been implemented the International System of Units uses the Sievert (Sv) as a derived unit in order to estimate the effective dose.

The new Romanian system is based on smart cards and Public Key Infrastructure and its structure is represented in figure 1. The system was implemented in Dr. Carol Davila Central Military Universitary Emergency Hospital, Bucharest, Romania.

The whole designed system and software application was the main result of the 187/2012 SRSPIRIM project in a Romanian National Partnerships Program, Collaborative Applied Research Projects – PCCA 2.

The system includes the following components:

- smart cards dedicated to patients: Citizen Radiation Safety Card (CRSC) and
- professional Radiation Safety Card (PRSC), designed for medical and investigation laboratories personnel. Among the last ones, one or more Administrator Radiation Cards are also designed and used in order to enroll patients in the application.

A common patient card and a doctor card are shown

in Figure 2.

Figure 2: Types of cards

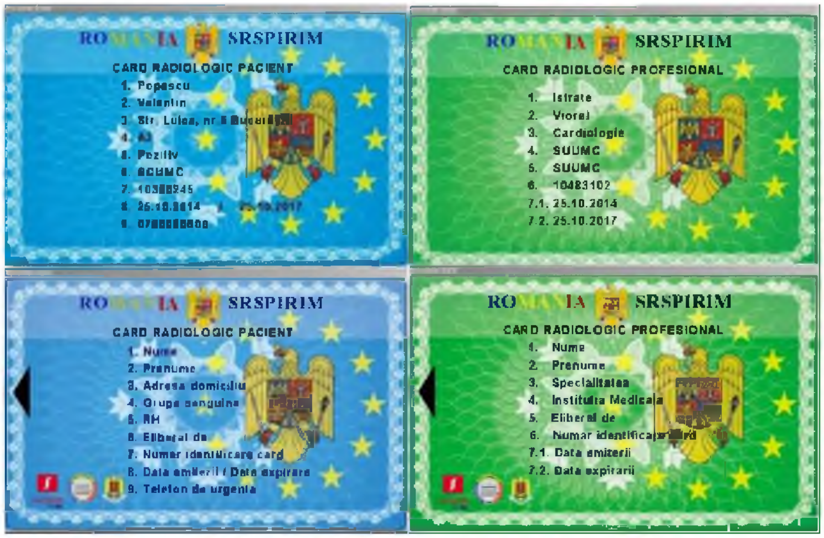

ROMANIA SRSPIRIM
CARD RADIOLOGIC PACIENT
1. Popescu
2. Valentin
3. Str. Luiea, nr 5 [redacted]
4. [illegible]
5. Pozitiv
6. SCUMC
7. 103[redacted]945
8. 25.10.2014 / 25.10.2017
9. 07[redacted]

ROMANIA SRSPIRIM
CARD RADIOLOGIC PROFESIONAL
1. Istrate
2. Viorel
3. Cardiologie
4. SUUMC
5. SUUMC
6. 10483102
7.1. 25.10.2014
7.2. 25.10.2017

ROMANIA SRSPIRIM
CARD RADIOLOGIC PACIENT
1. Nume
2. Prenume
3. Adresa domiciliu
4. Grupa sanguina
5. RH
6. Eliberat de
7. Numar identificare card
8. Data emiterii / Data expirare
9. Telefon de urgenta

ROMANIA SRSPIRIM
CARD RADIOLOGIC PROFESIONAL
1. Nume
2. Prenume
3. Specialitatea
4. Institutia Medicala
5. Eliberat de
6. Numar identificare card
7.1. Data emiterii
7.2. Data expirarii

The hardware part of the system consists in computers, smart cards and card readers in order to record and retrieve the information about the type of investigations and the specific emitted doses.

The software includes a data base that records the commonly information on doctors, patients, types of medical procedures but also designed applications in order to replicate a lost or destroyed card.

Figure 3: Central database

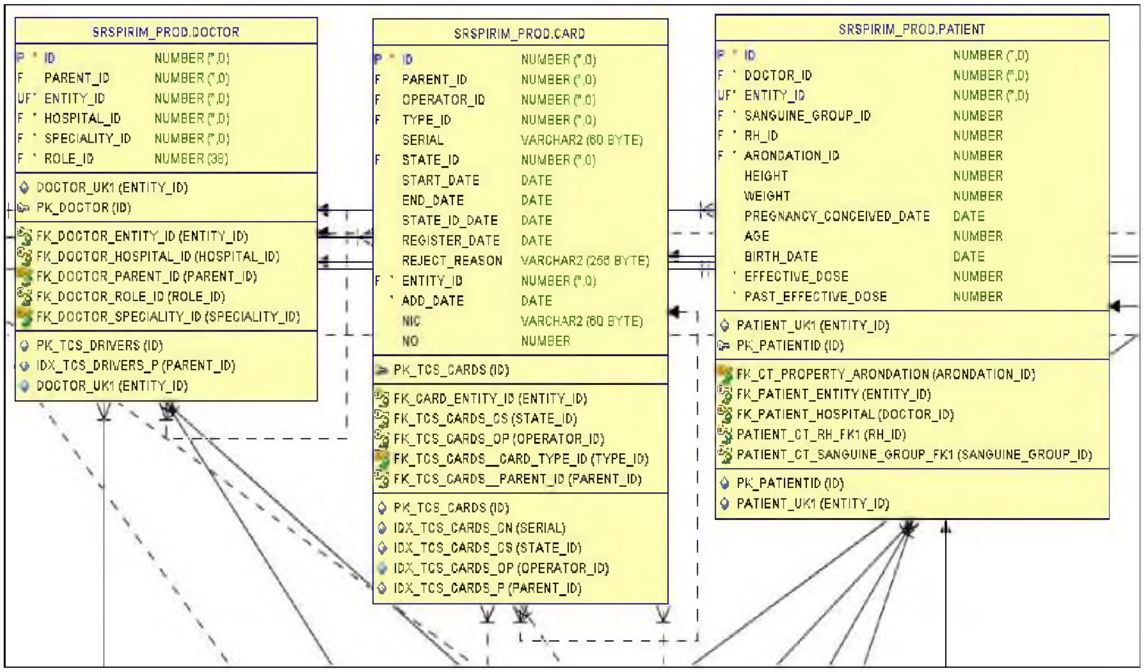

SRSPIRIM_PROD.DOCTOR

P *	ID	NUMBER (*,0)
F	PARENT_ID	NUMBER (*,0)
UF*	ENTITY_ID	NUMBER (*,0)
F *	HOSPITAL_ID	NUMBER (*,0)
F *	SPECIALITY_ID	NUMBER (*,0)
F *	ROLE_ID	NUMBER (38)

DOCTOR_UK1 (ENTITY_ID)
PK_DOCTOR (ID)

FK_DOCTOR_ENTITY_ID (ENTITY_ID)
FK_DOCTOR_HOSPITAL_ID (HOSPITAL_ID)
FK_DOCTOR_PARENT_ID (PARENT_ID)
FK_DOCTOR_ROLE_ID (ROLE_ID)
FK_DOCTOR_SPECIALITY_ID (SPECIALITY_ID)

PK_TCS_DRIVERS (ID)
IDX_TCS_DRIVERS_P (PARENT_ID)
DOCTOR_UK1 (ENTITY_ID)

SRSPIRIM_PROD.CARD

P *	ID	NUMBER (*,0)
F	PARENT_ID	NUMBER (*,0)
F	OPERATOR_ID	NUMBER (*,0)
F	TYPE_ID	NUMBER (*,0)
	SERIAL	VARCHAR2 (60 BYTE)
F	STATE_ID	NUMBER (*,0)
	START_DATE	DATE
	END_DATE	DATE
	STATE_ID_DATE	DATE
	REGISTER_DATE	DATE
	REJECT_REASON	VARCHAR2 (256 BYTE)
F *	ENTITY_ID	NUMBER (*,0)
*	ADD_DATE	DATE
	NIC	VARCHAR2 (60 BYTE)
	NO	NUMBER

PK_TCS_CARDS (ID)

FK_CARD_ENTITY_ID (ENTITY_ID)
FK_TCS_CARDS_CS (STATE_ID)
FK_TCS_CARDS_OP (OPERATOR_ID)
FK_TCS_CARDS__CARD_TYPE_ID (TYPE_ID)
FK_TCS_CARDS__PARENT_ID (PARENT_ID)

PK_TCS_CARDS (ID)
IDX_TCS_CARDS_CN (SERIAL)
IDX_TCS_CARDS_CS (STATE_ID)
IDX_TCS_CARDS_OP (OPERATOR_ID)
IDX_TCS_CARDS_P (PARENT_ID)

SRSPIRIM_PROD.PATIENT

P *	ID	NUMBER (*,0)
F *	DOCTOR_ID	NUMBER (*,0)
UF*	ENTITY_ID	NUMBER (*,0)
F *	SANGUINE_GROUP_ID	NUMBER
F *	RH_ID	NUMBER
F *	ARONDATION_ID	NUMBER
	HEIGHT	NUMBER
	WEIGHT	NUMBER
	PREGNANCY_CONCEIVED_DATE	DATE
	AGE	NUMBER
	BIRTH_DATE	DATE
*	EFFECTIVE_DOSE	NUMBER
*	PAST_EFFECTIVE_DOSE	NUMBER

PATIENT_UK1 (ENTITY_ID)
PK_PATIENTID (ID)

FK_CT_PROPERTY_ARONDATION (ARONDATION_ID)
FK_PATIENT_ENTITY (ENTITY_ID)
FK_PATIENT_HOSPITAL (DOCTOR_ID)
PATIENT_CT_RH_FK1 (RH_ID)
PATIENT_CT_SANGUINE_GROUP_FK1 (SANGUINE_GROUP_ID)

PK_PATIENTID (ID)
PATIENT_UK1 (ENTITY_ID)

This database provides the possibility of collecting data about the patients on designed criteria and future designed reports. Central database diagram, containing the recorded medical information are stored in many tables. A part of the design of the complex relationships between these tables is presented in Figure 3.

The security solutions include a Public Key

Infrastructure base (PKI), in order to achieve a high level of security of recording and retrieving data. A public-key infrastructure (PKI) is a set of hardware, software, people, policies, and procedures needed to create, manage, distribute, use, store, and revoke digital certificates. A PKI establishes and maintains a trustworthy networking environment by providing key and certificate management services that enable encryption and digital signature capabilities across applications — all in a manner that is transparent and easy to use. The application ensures a secured authentication of doctors and patients using digital certificates and cryptographic hardware. The whole logic application is stored in the central server. The clients use the minimum functional software. The applications users have access through a web browser. The system uses cryptographic libraries FIPS 140-2 or CC EAL4. The keys are RSA 1024 bits minimum length. The cryptographic hardware must support PKCS#11, PKCS#15 and Microsoft CryptoAPI. The cryptographic hardware must ensure the recovery of the cryptographic context. The certification system ensures roll–over facilities. The PKI system has an own relational database storing all the emitted certificates.

Figure 4: Effective dose calculus in radiological investigations

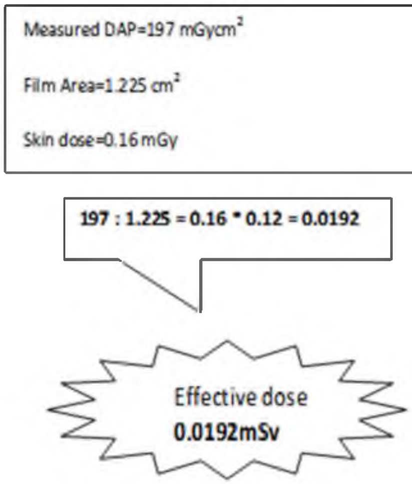

EXPERIMENTAL IMPLEMENTING

In the classic radiological investigation the calculus for radiation dose uses Dose Area Product (DAP), expressed in (Gy*cm^2) as an indicator of the total risk of inducing cancer. It also has the advantages of being easily measured, with a DAP meter on the X-ray set. In order to obtain the effective dose measured in Sv, the DAP is divided by the film area and then it is multiplied by the tissue factor [13] as shown in Figure 4. A sensible situation was determined by CT-s registered dose. More related CT radiation doses are available on CT consoles. Dose length Product DLP was chosen as input data for the system in CTs investigations but the required reported data is the effective dose. Conversion factors were used. The conversion factors can slightly vary from different manufacturers. Conversion factors for normalized effective dose per DLP depend only on the region of the body being scanned. All CT manufacturers provide tables with such conversion factors.

Conversion factors are also available for children of various ages, but this project does not include children [14]. Conversion problems were detected for multiple CT scans in one single examination, because more DLP are measured in the same examination and recording them becomes a long procedure.

EXPERIMENTAL RESULTS

Different studies were performed.

During a starting one month study in the hospital, the maximum cumulative dose has been overpass The CT exams are the most concerning prescribed imaging investigations that can lead to high cumulative doses.

The initially Figure 5 reveals some aspects from the initial study at the beginning of the project. The initial proposed threshold radiation dose of 20 mSv was frequently over passed and maybe a higher dose may be accepted for medical investigations in the future.

During a three years study in the hospital, different studies were performed.

The database contains 1125 records with 333 patients. From these records 42 patients were rejected in the final analysis because incomplete data.

These records contain all the investigations performed and all patients.

A lot of patients were examined in more than one investigation, so the system enrolled only 291 unique patients.

Figure 5: Cumulative radiation doses received by patients in the initial study

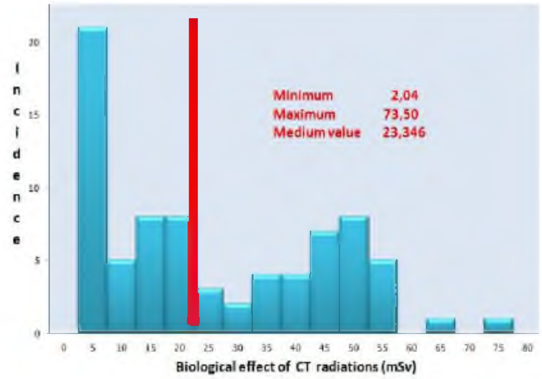

The recorded cumulated effective doses are shown in figure 6 and their distribution on decades is shown in figure 7.

Figure 6: Recorded doses in the project

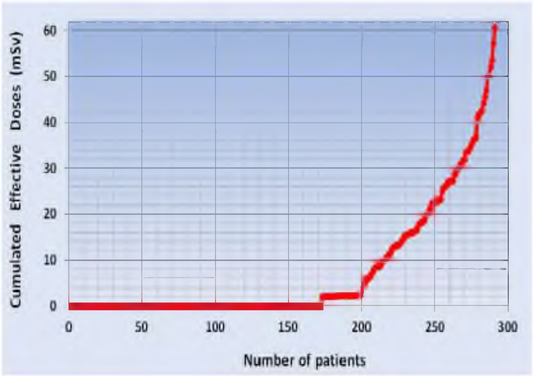

Figure 7: Recorded doses on decades

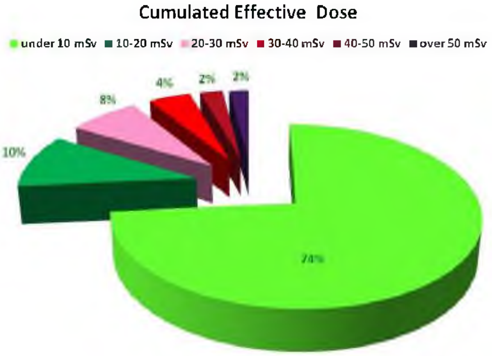

From these 291 patients, shown in figure 8 by gender, many of them have performed multiple investigations as in figure 9 and from them, in 47 cases, the received cumulated dose exceed the 20 mSv threshold dose, meaning 16,5% of cases.

Figure 8: Patients recorded in the project

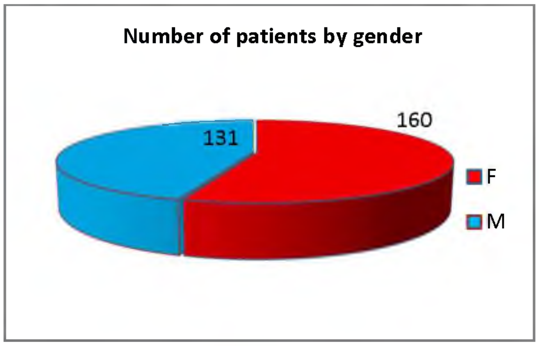

Figure 9: Patients recorded in the project

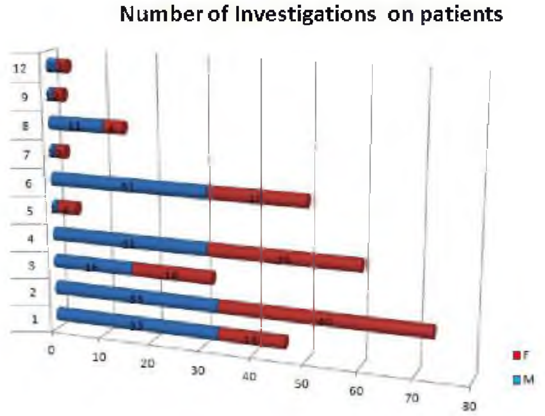

ACKNOWLEDGMENT

The results were obtained from the SRSPIRIM project 187/2012, in Romanian Partnerships Program, Collaborative Applied Research Projects Subprogram. The authors wish to address thanks to all the persons involved in this project for their support, work and ideas.

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REVIEW ARTICLE

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General considerations regarding the infections with the Escherichia coli pathogen

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Abstract: *Escherichia coli is the species of the genus Escherichia with the greatest epidemiological impact. Escherichia coli infections are found mainly in places with poor hygiene; the infants with ages between 1 and 3 years old are included in the category with the highest risk. It is a "fecal-oral" transmission mechanism as a result of consumption of contaminated food or water, or by "dirty hands". The foods most commonly implicated in the transmission of the infection are unpasteurized milk and milk products, beef, especially the one insufficiently cooked, unpasteurized fruit juice, lettuce and insufficiently washed vegetables. The disease has been reported worldwide, being described numerous episodes of infection with Escherichia coli that caused multiple illnesses and deaths. Escherichia coli has three types of antigens: antigen "O" (somatic), antigen "H" (flagella) and antigen "K" (capsular). Clinical manifestations are present in the form of non-specific diarrhea, a dysentery form of enteritis, choleriform enteritis, hemorrhagic colitis and hemolytic uremic syndrome (HUS). The Escherichia coli infection diagnosis is made by identifying the etiologic agent and/or by highlighting the VTI toxin in the feces. The treatment consists in precautionary antibiotherapy, hydrodynamics and electrolyte rebalancing, blood transfusions and dialysis, if in the case of renal failure. The prevention of infections with Escherichia coli is achieved by personal hygiene, food hygiene and work hygiene.*

Keywords: *Escherichia coli, infection, toxin, treatment, hygiene*

INTRODUCTION

Escherichia coli is the species of Enterobacteriaceae, of the genus Escherichia, with the greatest epidemiological impact. Escherichia coli infections are found mainly in places with poor hygiene; infants are included in the category with the highest risk. Also, adults can develop severe forms of infections, especially in regions where underdevelopment, poverty and lack of hygiene are the dominant picture. The disease can occur in particular through the "fecal-oral" transmission mechanism as a result of consumption of contaminated food or water, or by "dirty hands". The foods most commonly implicated in the transmission of the infection are unpasteurized milk and milk products, beef, especially the one insufficiently cooked, unpasteurized fruit juice, lettuce

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[2] Titu Maiorescu University, Bucharest

and insufficiently washed vegetables. Processing raw food can contaminate kitchen utensils, especially if these are not separately used for each food category and are not cleaned between uses. Contamination can occur through contact with carrier animals, if the basic rules of personal hygiene are not complied with, such as washing hands with soap and water.

In recent years, in nearly all parts of the world have been reported numerous episodes of infection with Escherichia coli, resulting in numerous illnesses and even deaths. We recall the tragedy of Walkerton (Ontario/Canada), when approximately 5,000 people from the community showed symptoms specific to the digestive infection, the etiological agent being Escherichia coli [1]. In 2011, in Germany broke one of the largest outbreaks of acute gastroenteritis with hemolytic uremic syndrome (HUS) caused by the E. coli O104: H4 strain (German hemolytic uremic syndrome/ enterohemorrhagic E. coli - GHUSEC). The outbreak has had an unusual epidemiological origin only partially resolved. Investigations conducted by authorities showed that the illnesses occurred amid the consumption of contaminated water by agricultural runoff into a water well and subsequently there was the groundwater contamination [2].

The latest outbreak of the infection with Escherichia coli is evolving in Romania with this disease being contracted by 12 children (so far), aged between 1 and 3 years, all coming from localities in Argeş County.

The particularly severe infection resulted in the death of 3 children and even though the competent bodies through their health authorities have sought to find the source of the outbreak, they have not been able until now to find the source of the infection.

After the latest information, this seems to have been about a false "outbreak of diarrheal disease with uremic syndrome"; in reality the infectious etiology is varied, as confirmed by the Cantacuzino Institute.

In conclusion, due to the lack of a common source for the contagion, there cannot be a question of an epidemic or outbreak as the cases are isolated; however, due to the media pressure the incident could be termed as a "media outbreak".

ETIOLOGY

The genus Escherichia is of the Enterobacteriaceae family which comprises of 44 genera, 25 of which are found in human pathology. Most of these genres include commensal bacteria very abundant in nature (soil, water, etc.) [3], their natural habitat being the gastrointestinal tract of humans and warm-blooded animals, their feces containing more than 1010 enterobacteria/gram [4]. The constant presence of Escherichia coli strains in human and animal intestines and implicitly in the feces, determined that this bacterium represents the indicator of fecal pollution of the environment, especially of the water. Within the genus, are known 6 species: Escherichia coli, Escherichia fergusonii, Escherichia hermannii, Escherichia vulneris, Escherichia albertii and Escherichia blattae, the latter being isolated due to the fact that it only comes from specific species of insects.

Figure 1: Escherichia coli
(http://www.telegraph.co.uk 620 × 387)

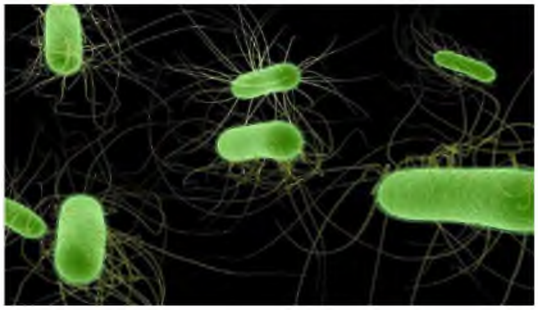

The most important representative of the genus is Escherichia coli, bacterium discovered by Austrian pediatrician Theodor Escherich. In 1886, after a hard clinical and laboratory activity he has presented at the Faculty of Medicine in Munich and subsequently published in Stuttgart, a monograph on the link between intestinal bacteria and the physiology of digestion in children entitled “Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung” [5]. In 1919, Castellani and Chalmers proposed that the name of the isolated bacillus from the colon (Bacterium coli commune), by the renowned Austrian pediatrician Theodor Escherich be changed in Escherichia coli, in his honor, a name that was officially approved in 1958 [6]. Bacteria are present in the form of straight or slightly curved bacilli with rounded ends,

the presence of flagella and therefore the mobility being constant in most species (Figure 1). It has a size between 2-5 µm/0.5-1 µm and from a tinctorial point of view is Gram-negative.

It grows on simple culture media (simple glucose broth, nutrient agar and blood agar) developing specific Type S colonies, round, domed, shiny with a smooth surface (Figure 2) and regular edges or with dry R colonies with irregular edges.

Figure 2: Escherichia coli culture on blood agar (http://www.wzzm13.com 534 × 401)

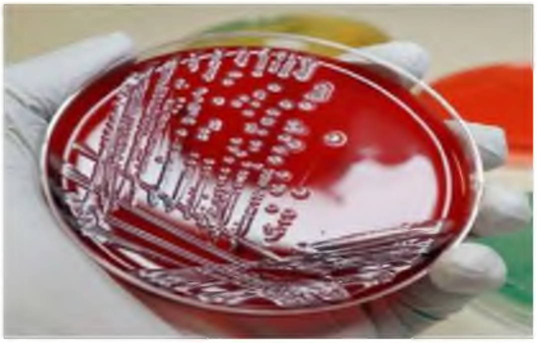

Biochemically, is fermenting the glucose with the production of gas, it produces indol, β-galactosidase and ferments lactose, characteristic that is allowing the differentiation from other enterobacteriaceae. Escherichia coli has an aerobic or optionally anaerobic metabolism and develops at an optimum temperature of 370C and pH between 7.2-7.8 [7], but differs from other coliform bacteria by increasing to 44^{0}C.

THE ANTIGENIC STRUCTURE

Escherichia coli has three types of antigens that can be highlighted by serologic antigen-antibody reactions:

- The O antigen (somatic) represented by a lipopolysaccharide surface antigen, thermostable, common to all S forms of the Enterobacteriaceae family;
- The H antigen (flagella) is a proteinaceous antigen (flagellin), heat-labile present in bacteria flagella;
- The K antigen (capsular) which may be of a polysaccharide or protein (fimbriae) nature. The O and H antigens are transmitted via plasmids and can be found together with the K antigens that are chromosomally determined.

Up until now, have been identified more than 170 types of the O antigen, more than 50 types of H antigen, 90 types of K antigen and several types of fimbriae antigens (F antigens). Combining all these antigenic variants, results in more than 1,000 antigenic types of Escherichia coli.

CHARACTERISTICS OF PATHOGENICITY

Colonic microflora in healthy persons (normal intestinal microbiota) is dominated by strains of Escherichia coli that live in a symbiotic relationship with the host. These infections may occur only if the host is immunocompromised or when it crosses the intestinal barrier and reaches areas which would normally be sterile. There have also been described pathogenic strains of Escherichia coli which have an exacerbated virulence with the possibility of being able to multiply, triggering infectious processes in immunocompetent individuals. Currently, have been described 6 intestinal Escherichia coli pathotypes involved in the EPEC diarrheal syndrome (intestinal pathotypes) and a pathotype involved in extraintestinal infections (urinary tract infections, meningitis, septicemia), ExPEC [8]. It is the most common etiologic agent in urinary infections, being incriminated in over 80% of urinary tract infections (UTI).

The health of the host (diabetes, immunodeficiency, colon malformations, obstructive uropathies, etc) is predisposing factors for infection with Escherichia coli.

Intestinal pathotypes are: enteropathogenic Escherichia coli (EPEC), enterotoxigenic Escherichia coli (ETEC), enteroinvasive Escherichia coli (EIEC), verotoxin producing Escherichia coli (VTEC), enteroaggregative Escherichia coli (EAEC) and diffuse adhesion Escherichia coli (DAEC). Within the VTEC pathotype stands out, mainly to highlight the association with severe clinical manifestations (haemorrhagic colitis), the subset of strains of the enterohaemorrhagic Escherichia coli (EHEC), whose prototype is the O157: H7 serotype. The pathogenic Escherichia coli strains produce virulence factors that contribute and affect the processes undertaken at cell level in the host (the protein synthesis the cytoskeleton’s function, the cell division, ion secretion,

the mitochondrial function, apoptosis). Adhesins are adhesion structures located on the fimbriae/pili (P fimbriae from the uropathogenic strains of Escherichia coli, the CFA colonization factors from the ETEC strains) or on the surface of the outer bacteria membrane (Afa afimbriae adhesins from DAEC strains). They are important virulence factors that allow bacteria to colonize areas of the human body (the small intestine, the urinary tract, the vagina), in which it does not normally reside. Toxins are another category of virulence factors. Endotoxins are part of the structure of the outer membrane (the A lipid from the LPZ complex) and are present in all Gram-negative bacteria. Released upon the elimination of the bacterial cell wall, they manifest their toxic effects being involved in the occurrence of the fever and the toxic shock from septicemia. Exotoxins are synthesized in the processes of the bacterial metabolism, being specific to the various pathotypes of Escherichia coli. Currently, are known the structure and the functions of many toxins responsible for the pathogenicity of strains of Escherichia coli (e.g., heat-labile enterotoxin produced by ETEC strains, verotoxins 1 and 2 synthesized by the VTEC strains, enteroaggregative Escherichia coli heat-stable enterotoxin 1 of EAEC strains etc.). Another important component of the pathogenicity of the species is the capsule. The main role of the capsule is to inhibit the action of the serum complement and the phagocytosis, thus imparting bacterial resistance. The K1 capsular polysaccharide antigen is present in 80% of Escherichia coli strains, isolated from the cases of neonatal meningitis.

THE DISEASE

Most of the Escherichia coli outbreaks were associated with the presence of the O15: H7 (VTEC) serotype. The incubation period of the disease varies between 1 and 9 days, averaging 3-5 days. Clinical manifestations are extremely varied ranging from non-specific diarrhea, dysentery form of enteritis (with an invasion of the colon), choleriform enteritis (with the invasion of the jejunum-ileum mucosa) to hemorrhagic colitis and consecutively the hemolytic uremic syndrome (HUS). The hemolytic uremic syndrome is characterized by acute renal failure, thrombocytopenia and angiopathic hemolytic anemia. The most common form of HUS is associated with diarrhea and has the highest incidence in children, being the major cause of acute renal failure in children [9, 10]. The factor responsible in HUS manifestations occurred after the VTEC infection and the Shiga dysentery is a protein enterotoxin called the Shiga toxin or VTI. Initially, the hemolytic uremic syndrome mortality rate reached to 50%, but the evolution of medicine and modern treatment techniques have reduced the mortality rate to 3-5%, indicating that approximately 30% of the healed patients remain with long-term sequelae, such as acute kidney failure [8]. Escherichia coli is the cause in 80% (ExPEC pathotype) of ITU (urethral cystitis, cystitis, nephritis, pyelonephritis, etc.). Contamination may be downward (blood-borne) or upward, this route being the most common. Infection is favored by the existence of obstructive congenital lesions, post gonococcal infection strictures, urinary stasis via decubitus or pregnancy. Once the bladder mucosa is invaded, subsequently occurs the upward progression to the kidney and the infection stabilizes at this level as a result of slow mobilization of the phagocytes [11].

Lab diagnosis

The diagnosis of the Escherichia coli infection is made by addressing two strategies namely, identifying and highlighting the etiological agent of the VTI toxin. The usual method used by most diagnostic laboratories is the selective isolation of the etiologic agent (Escherichia coli O157H7; O157H), on the MacConkey with sorbitol culture media from stool [12]. The method’s principle is that the strains of Escherichia coli O157: H7 do not ferment sorbitol in 24 hours and the colonies appear colored on the culture, characteristic that differentiates them from the other strains of Escherichia coli. If the isolated strains are suspected as belonging to the serotype O157, but ferment the sorbitol in 24 hours, then will be used the specific antisera for titration and the agglutination tests [13]. The detection of the VTI toxin can be achieved through the cytotoxicity method, cultivation of isolated strains from feces on Vero or HeLa cell lines. The genetic methods (PCR) are based on the detection of specific sequences of polynucleotides that encode the synthesis of VTI. A new and very precise method is MALDI TOF, namely the proteomics analysis through

mass spectrometry of the specific proteins extracted from the ribosomes [14]. The principle of the method consists in measuring the mass of the weights of the polypeptides from the sample to be examined, generating and comparing its spectrum with the spectrum from the data base of the mass spectrometer (figure 3).

Figure 3: Maldi TOF operating flow (after Bruker Daltonix)

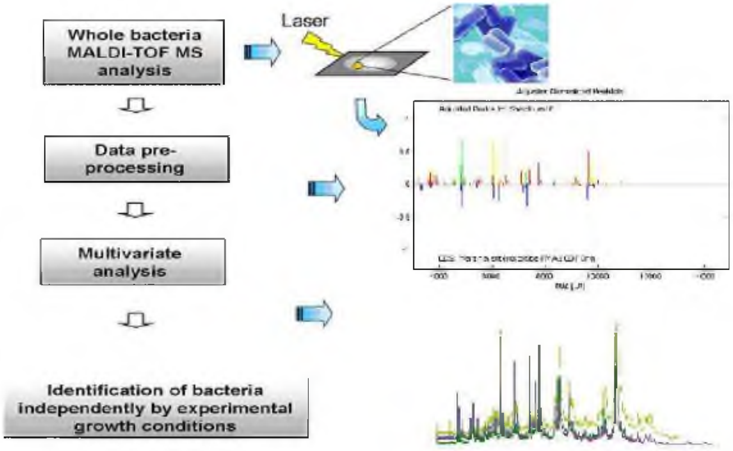
Whole bacteria MALDI-TOF MS analysis
Laser
Data pre-processing
Multivariate analysis
Identification of bacteria independently by experimental growth conditions

Other lab diagnostic techniques that are being used are the ELISA, for the detection of the O 157 antigen from feces and the serological methods which, however, are of limited value because Escherichia coli does not always develop serum antibodies against the VTI toxin. Also, the LPS antigen used may develop cross-reactions with other biological agents, such as Brucella abortus.

Treatment and Prevention

The treatment of the infection with Escherichia coli O157:H7 is often symptomatic and consists in the treatment of the complications arising due to massive fluid loss, and consequently of the acute diarrhea condition. As a result of dehydration and permeabilization of the blood vessels, pathological changes take place in the kidney function with renal impairment and severe anemia, that may become complicated with hemolytic uremic syndrome (HUS), as apparently is the case in the current outbreak in Romania, since winter-spring 2016. The therapeutic management must take into account the avoidance of the abuse of antibiotics that can foster the development of strains of Escherichia coli, avoiding the administration of antispasmodic drugs with effect in slowing bowel, thus maintaining more toxins in the digestive tract and amplifying its harmful effects. It recommended avoiding administering products based on salicylates and ibuprofen that can increase digestive bleeding. The symptomatic treatment must include hydrodynamics and electrolyte rebalancing, blood transfusions and, if in severe forms of renal failure, dialysis. Prevention of disease is achieved by non-specific measures such as: washing hands with soap and water before and after eating; processing animal products and by-products; washing fruits and vegetables before consumption or industrial processing; avoid the consumption of raw animal products (milk) or semi-raw (hamburger); avoid the consumption of water from bacteriologically unverified sources; washing utensils that came in contact with meat or raw meat products; avoiding the use of unpasteurized juices, in particular for children, etc. In serious cases, potentially fatal, especially in young children, may be needed the treatment from the Intensive Care with perfusions, resuscitation and even peritoneal dialysis.

CONCLUSIONS

- Escherichia coli is from the Enterobacteriaceae species present in the colonic microflora of a healthy human, which has the largest epidemiological impact.
- The transmission mechanism of the infection is

"fecal-oral" as a result of the consumption of contaminated food or water, or by "dirty hands".

- The disease has been reported worldwide, being described numerous episodes of infection with Escherichia coli that caused multiple illnesses and sometimes even deaths (Walkerton Ontario/Canada, GHUSEC - Germany).

- Escherichia coli has three types of antigens: antigen "O" (somatic) antigen "H" (flagella) and antigen "K" (capsular) that can be highlighted by serologic antigen-antibody reactions. Combining all these antigenic variants theoretically result in 1,000 antigenic types of Escherichia coli.

- There are six known pathotypes of Escherichia coli involved in the EPEC diarrheal syndrome (intestinal pathotypes) and one pathotype involved in extraintestinal infections (urinary tract infections, meningitis, septicemia), ExPEC.

- Toxins are the most important category of virulence factors in Escherichia coli (heat-labile and heat-stable enterotoxin produced by ETEC strains, verotoxins 1 and 2 synthesized by VTEC strains, enteroaggregative heat-stable enetrotoxin of EAEC strains etc.).

- Clinical manifestations are extremely varied ranging from non-specific diarrhea, dysentery form of enteritis (with an invasion of the colon), choleriform enteritis (with the invasion of the jejunum-ileum mucosa) to hemorrhagic colitis and consecutively the hemolytic uremic syndrome (HUS).

- The Escherichia coli infection diagnosis is made by identifying the etiologic agent (biochemical tests, agglutination reactions, PCR, mass spectrometry) and by highlighting the VTI toxin (cell culture cytotoxicity assay, immuno-chromatographic methods, ELISA).

- The treatment is symptomatic and includes hydrodynamics and electrolyte rebalancing, blood transfusions and, if in severe forms of renal failure, dialysis.

- The prevention of infections with Escherichia coli is achieved by non-specific measures such as: washing hands with soap and water before and after eating; processing animal products and by-products; washing fruits and vegetables before consumption or industrial processing; avoid the consumption of raw animal products (milk) or semi-raw (hamburger); avoid the consumption of water from bacteriologically unverified sources.

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REVIEW ARTICLE

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Clinical features of oral angiolipoma – A review

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Abstract: *The aim of this paper was to analyze the data obtained from case reports of oral angiolipoma in terms of age and gender distribution, site of occurrence, nature (intraosseous/ extraosseus) and infiltration. an internet search using Google scholar and Pubmed engine was carried out using search terms 'angiolipoma' and 'oral'/'mouth'. English literature full text articles and abstracts of oral angiolipoma obtained from 1976-2016 were analyzed for clinical data and presented in this article.*

Keywords: *angiolipoma, oral, mouth*

INTRODUCTION

Angiolipoma (AL) is rare variant of lipoma which consists of both fatty and vascular elements. [1] ALs were first describe by Bowen in the year 1912. [2] The first published report of an oral angiolipoma was by Davis et al in 1976. [3] Angiolipomas account for 5 to 17% of all the lipomas occurring in the oral cavity, lipomas themselves account for 1-5% of all oral benign tumors [1]. Since oral angiolipoma is rare benign tumor, published reports are mainly restricted to case reports. [4,5]

CLASSIFICATION OF ANGIOLIPOMAS

Based on their studies Gonzalez-Crussi et al. classified angiolipomas into infiltrating and non-infiltrating. angiolipomas.[6] The non-infiltrating angiolipomas are encapsulated, whereas infiltrating angiolipomas lack this capsule.[6] Among the two, infiltrating angiolipoma (IAL) is a rare lesion typically characterized by infiltration of the surrounding structures, especially skeletal muscle.[7] Based on whether the angiolipoma occur in bone or soft tissues they can be classified as intra-osseous or extra-osseous types.[8] The intra-osseous variety is extremely rare in comparison to the extraosseus variety. [8,9]

METHODOLOGY FOR ANALYSIS OF THE CASE REPORTS

An analysis of the data obtained from case reports of oral angiolipoma in terms of age and gender distribution, site of occurrence, nature (intraosseous/ extraosseus) and infiltration. An internet search using Google scholar and pubmed engine was carried out using search terms 'angiolipoma' and 'oral'/'mouth'. Full text and abstracts of oral angiolipoma cases obtained from 1976-2016 were analyzed. (Table 1)

GENDER PREDICTION OF ORAL ANGIOLIPOMA

Fregnani ER et al (2003) stated that male to female

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ratio from these intraoral angiolipomas was 1.4:1, in contrast to cutaneous angiolipomas and intraoral lipomas as a group, which either shows no sex predilection or a slight female predilection.[10]

Table 1: Clinical data obtained from full texts and abstract of oral angiolipoma case reports published from the year 1976-2016

Researcher	Year of publication	Type infiltrating/ non-infiltrating	Gender	Age group	Site	Extraosseous/ intraosseous
Davis et al [3]	1976	-	-	-	hard palate	
Polte HW et al [30]	1976	-	-	-	mandible	intraosseous
Weitzner et al [35]	1978	-	male	4-year-old	cheek	
Campos et al [34]	1980	-	-	-	cheek	
Lewis DM et al [31]	1980	-	-	-	mandible	intraosseous
Brahney CP et al [21]	1981	-	-	-	tongue	
Flaggert et al [19]	1986	-	female	8-year-old	palate	
Reilly et al [15]	1988	-	female	infant	parotid	
Lin SC et al [17]	1989	-	male	49-year-old	tongue	
Manganaro AM et al [32]	1994	-	-	-	mandible	intraosseus
Ali MH et al [36]	1996	-	-	-	cheek	
Sugiura J et al [16]	1999	infiltrating	-	-	muco-buccal fold	
Ida-Yonemochi H et al [12]	2005		male	69-year-old	buccal mucosa	
Altug HA et al [11]	2009	non-infiltrating	male	22-year-old	cheek	
Gerard N et al [20]	2009	-	-	-	upper lip	
Thakur B eta al [37]	2010	non-infiltrating	female		cheek	
Palaia G et al [38]	2011	non-infiltrating	female	66-year-old	cheek	
Yanase S et al [13]	2011	non infiltrating	male	76-year-old	cheek	
Sah K et al [4]	2012	non infiltrating	female	9-year-old	upper lip	
Hemavathy S et al [9]	2012	-	female	21-year-old	mandible	intraosseus
Silva-Junior GO et al [5]	2013	non-infiltrating	male	57-year-old	cheek	
2 cases	2013	non-infiltrating	female	29-year-old	masseteric region	
Shahi AK et al[14]	2014	infiltrating	female	9-month-old	cheek	
Mohiuddin SA et al [39]	2014	non-infiltrating	male	46 year old	mandibular buccal vestibule	
Ohnishi Y et al [7]	2015	infiltrating	female	74 year old	lower lip	
Chandrasekaran D et al [1]	2016	non infiltrating	female	55-year-old	palate	

However most of the other researchers have stated that no gender predilection. [1,7,11,12] According to the data obtained from the analysis of the reports, seven cases were in males, ten cases occurred in females. However we could not obtain enough data regarding gender in 9 case reports.

AGE PREDILECTION OF ORAL ANGIOLIPOMA

Fregnani ER et al (2003) stated that the mean age of the affected patients was 37 years (range, 4 to 81 years),which was in contrast to other variants of lipomas occurring in the oral cavity that occur in the 50-60 year age group.[10] On analysis of the available case reports we found that occurrence of oral angiolipoma ranged from a 4-month-old to 76-year-old individual.[13,14,15] Infiltrating angiolipomas are usually diagnosed in older individuals.[7, 16, 17, 18]

SITE PREDILECTION OF ORAL ANGIOLIPOMA

Angiolipoma have a prediction of occurrence in the

buccal mucosa, followed by lip.[5] Occurrence in palate has been reported in few cases.[1,3,19]. Upper lip involvement is more common than lower lip.[4, 20] Few cases have also been reported in tongue.[17, 21] However many of the cases of angilipoma of tongue have the muscle component along with fat and vasculature and thus are termed as angiomyolipoma.[22,23, 24, 25]. The occurrence of angiolipoma in the parotid has also been reported.[15] The benign angilipomas occurring the salivary gland have glandular component apart from adipose and vascular components and are called as sialoangiolipoma.[26] Such cases have been reported to have been observed in parotid and submandibular gland.[27, 28] A rare case of a lipoma with vascular and salivary gland components has been reported in palate also.[29]

INTRAOSSEOUS ANGIOLIPOMA

The first case of intraosseous angiolipoma was by Polte in the year 1976.[30] Mandible is the most common site of occurrence of intraosseous angiolipoma.[9,31,32] Intraosseous lipoma may present a range of clinical manifestations such as swelling, or presence of neurologic signs (hypesthesia or paresthesia) or can be totally asymptomatic.[9] On radiographic examination the lesion usually presents as well-circumscribed radiolucency.[9] Extraction related trauma is believed to be cause for intraosseous angiolipoma of the jaws. Fatty metamorphosis of a central hemangioma and possible variation osteoporotic bone marrow defect are other suspected etiologies. [33]

INFILTRATING ANGIOLIPOMA

Infiltrating angiolipomas lack encapsulation and are typically characterized by infiltration of the surrounding structures.[6,7,14,16] In contrast to the non-infiltrating angiolipomas are well encapsulated. [34,35,36,37,38]. Infiltrating angiolipoma usually occur in older individuals but a report of 9-month-old diagnosed with infiltrating angiolipoma has recently been published.[14] Infiltrating angiolipomas are also associated with symptoms muscular pain and neural deficits.[39,40]

CONCLUSION

In this review we have made an attempt to analyze the clinical features of oral angiolipoma in terms of its gender predilection, site predilection, infiltrative nature, intra/extraosseus types. The information obtained from this review could aid the clinicians in diagnosis this extremely rare benign lesion.

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SYSTEMATIC REVIEW

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Hepatocellular carcinoma – A review

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Hepatocellular carcinoma is a major public health issue, occupying the fifth place of the most common cancers and the third leading cause of cancer in the world. The incidence of HCC has increased in the past decade thanks to higher rates of HCV infection and improvements in the management of cirrhotic patients. The regions with the highest incidence of HCC are, in descending order: Eastern Asia, sub-Saharan Africa, South-East Asia and southern Europe. It has been observed that etiological factors which lead to HCC have a heterogeneous distribution; thereby, in Asia and Africa the most common risk factor is HBV infection, increased by ingestion of aflatoxin B1-contaminated food that is associated with a specific mutation in the p53 tumor suppressor gene. HCC may develop earlier in these regions, most common in a noncirrhotic liver. In developed countries, the principal risk factors are HCV infection and alcohol-related cirrhosis [1].

RISK FACTORS

The main risk factors involved in the development of HCC are chronic viral infections, hemochromatosis and alcoholic cirrhosis. Other risk factors which may lead to HCC are cirrhosis secondary to autoimmune hepatitis and Wilson disease. In the last years, diabetes was suggested as HCC risk factor in numerous studies. However, it is difficult to study the possible association between the two concepts, because diabetes is a risk factor for nonalcoholic liver disease and nonalcoholic steatohepatitis , which may lead to the appearance of liver fibrosis, of cirrhosis, and finally to HCC, being known the fact that the principal risk factor is the presence of cirrhosis, no matter of the cause. On the other hand, end stage liver disease may herself determine glucose intolerance and diabetes. Studies show that >90% of patients with cirrhosis have glucose intolerance and 30% have diabetes [2].

Cirrhosis is a premalignant statement independent to the etiology. Liver cell dysplasia is probably the intermediate step. Patients with cirrhosis and high rates of cellular proliferation are at high risk to develop hepatocellular carcinoma.

HBV and HVC. It has been shown that there is an interval of 15-40 years between the development of chronic hepatitis and the appearance of hepatocellular carcinoma and the distance is shorter for the virus C infection. For hepatitis B, is important to know the age at which the infection occurred- patients infected in the prenatal period or in childhood are at high risk to develop chronic hepatitis or to become chronic carriers and at the same time to develop cirrhosis and hepatocellular carcinoma.

HBV is responsible for almost 80% of hepatocellular

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carcinoma. Vaccination against HBV and antiviral treatment are the most efficient ways for prevention. HBV seems to be directly and indirectly carcinogenic. There is a connection between the level of replication of virus B (viral load) and the risk of hepatocellular carcinoma. HBV integrates into the genome host and the viral load precedes almost always the development of hepatocellular carcinoma, leading to mutations at the place of integration and high levels of TNF-α. HBx protein inhibit the p53 tumor suppressor gene and activate genes implied in the control of cellular growth. Patients with HB antigen positive infected with delta virus have a reduced incidence of hepatocellular carcinoma, probably because delta virus suppress HBV.

HCV, despite HBV, is an ARN virus which does not have reverse transcriptase and can't integrate into the genome host. The manner of carcinogenesis production is uncertain, but probably is generated by chronic hepatitis and cirrhosis. Persistent hepatitis with high levels of alanine aminotransferase is a semnificative predictor of hepatocellular carcinoma development. Accelerated cellular turnover is implied in the pathogenesis of cirrhosis; at the same time, it has been demonstrated that patients with persistent hepatic injury develops a sort of HCV species that metamorphoses in time, meanwhile patients with normal aminotransferase levels have a stabile infection with a single species of HCV. It seems that during this process are produced species more ,,oncogenic" of HCV core protein. HCV reacts with cellular genes that regulates the cellular growth and differentiation. P53 activity is suppressed and tumor suppressor functions are disabled. HCV core proteins are probably oncogenic [3].

Alcohol intake increases four time the risk of hepatocellular carcinoma development, especially in older patients; cirrhosis is always present. The risk of hepatocellular carcinoma is highest in patients with chronic hepatitis and persistent hepatocellular injury. Patients who stop the alcohol intake reduce considerably the risk of hepatocellular carcinoma development. The enzyme induction mediated by alcohol increases the conversion of co-carcinogens in carcinogens.

Aflatoxin B1, produced by Aspergillus flavus and Aspergillus parasiticus is a pro-carcinogen which is converted in the liver into a metabolite by the cytochrome P450 system at the 8,9-vinyl bond with the formation of an unstable reactive intermediate, aflatoxin B1-8,9-epoxide, which can bind covalently to DNA. The result is the formation of Aflatoxin B1-guanine adducts and protein- aflatoxin B1-albumin and other protein adducts. In addition, aflatoxin B1 acts as a carcinogen by mechanisms that include the formation of reactive oxygen species leading to increased hepatic damage [4].

Sex and genetic predisposition. Globally, hepatocellular carcinoma is 3 times more frequent in males than females. In regions with increased incidence, familial history of hepatocellular carcinoma is an important factor. It is possible to exist a genetic predisposition for hepatocellular carcinoma or a familial predisposition for an extended phase of HBV replication.

Other factors. Hepatocellular carcinoma is a rare complication of autoimmune hepatitis and primary biliary cirrhosis; it may occur frequently in hemochromatosis (in almost 45% of cases, due to excessive levels of free iron submitted in tissues, carcinogenic by the formation of reactive oxygen species). α1-antitrypsin deficiency, tyrosinosis, porphyria cutanea tarda, Clonorchis sinensis infection may also increase the risk of hepatocellular carcinoma development.

There have been identified certain factors which may decrease the risk of HCC in patients with cirrhosis : high serum retinol levels, high coffee consumption, alcohol cessation, clearance of HCV virus with treatment, iron depletion of hemochromatosis patients [5].

PATHOGENESIS

The pathogenesis of HCC is not fully understood, but most probably HCC results from activation of proto-oncogenes, deactivation of tumor suppressor genes (p53, pRb), changes in growth factors signaling processes (insulin-like growth factor-IGF, transforming growth factor-TGF), changes in telomeric length and

activity or microsatellite instability. Genetic injury may result from a variety of sources, such as HBV and HCV viruses. HBV is associated with a relatively high frequency of mutations as it replicates through RNA-mediated reverse transcription and the HBV reverse transcriptase (HBV-RT) does not have proofreading function. Unlike HBV, HCV is a single-stranded non-retroviral RNA virus which does not integrate into the host genome.

Although there have been identified multiple genetic abnormalities in the development of HCC, it is probably that the process is complex and genetic studies are continuing [1].

DIAGNOSIS

Hepatocellular carcinoma should be suspected in patients with previously compensated cirrhosis who develop decompensation, such as encephalopathy, ascites, jaundice or variceal bleeding associated with the extension of the tumor into the hepatic or portal veins or arteriovenous shunt induced by the tumor. Other uncommon presentations include: para-neoplastic syndromes – hypercalcemia (by PTH-like synthesis), erythrocytosis (by EPO-like synthesis), carcinoid syndrome, porphyria cutanea tarda, vascular angioma.

Diarrhea may be the reason for presentation to hospital, caused by cholestasis or the production of prostaglandins by the tumor.

Cutaneous manifestations include pemphigus foliaceus, dermatomyositis, Leser-Trelat sign (suddenly installed seborrheic keratosis, accompanied by freckles, very itchy).

Acute presentation is determined by spontaneous rupture of the tumor into the peritoneum or invasion of the liver capsule. In this case, the preferred treatment is the chemoembolization of the bleeding artery. Capsule invasion is causing severe pain, similar to biliary colic, lasting less than 24 hours.

In end stages, patients may present consumptive syndrome with impaired general condition, anorexia, weight loss and fatigue.

Physical examination can detect an abdominal mass and a bruit related to arterioportal shunting may be heard. A supraclavicular lymphadenopathy (Virchow-Troisier), Sister Mary Joseph or axillar lymph-adenopathies are rarely present [6].

Laboratory studies can show high levels of aminotransferase, but can be also normal. Alkaline phosphatase may be elevated in infiltrative tumor or if there is biliary obstruction due to malignancy. α-fetoprotein, the tumor marker most frequently associated with hepatocellular carcinoma (a glycosylated protein expressed in proliferating hepatocytes) has a sensitivity of 41-65% and specificity of 80-94% when the cut-off value is 20 ng/ml; levels higher than 400 ng/ ml are likely to be caused by hepatocellular carcinoma. AFP>1000 ng/ml associated with a liver mass is diagnostic for hepatocellular carcinoma. However, AFP level is elevated in only 60-70% of patients and false-positive results may occur during pregnancy, in active liver disease, certain gastrointestinal tumors and embryonic tumor. Additional studies of AFP have revealed that AFP has three glycoforms (AFP-L1, AFP-L2, AFP-L3) according to their binding ability to the lectin lents agglutinin (LCA). It has been shown that AFP-L3 exists only in the serum of patients with hepatocellular carcinoma at a cut-off value of 15%, with a sensitivity and specificity of 96.9% and 92%, respectively, in detecting hepatocellular carcinoma [7].

Des-gamma carboxyprothrombin (DCP-prothrombin,a protein induced by vitamin K-absence, PIVK-II) has been described as a protein produced by malignant hepatocytes. Different studies have shown than a combined analysis of AFP and DCP can lead to a better prediction in early stages of hepatocellular carcinoma [8]. Glypican-3, a member of the glypican famlily, has been revealed as a diagnosis marker, having the capacity to differentiate hepatocellular carcinoma from cirrhotic liver or benign lesion. It has been demonstrated that Glypican-3 and AFP together increase sensitivity and specificity for the diagnosis of small and unicentric HCC, respectively [9].

Heat shock protein 70 (HSP) – a protein produced by cells as a response to exposure to stressful conditions, may also be used as a marker for diagnosis in hepatocellular carcinoma [10].

The International Consensus Group of Hepatocellular Neoplasia recommends to define a pathological diagnosis of hepatocellular carcinoma if at least 2 markers are positive.

Imaging diagnosis

A minimum of 2 imaging exploration is required for staging and to confirm/exclude vascular invasion (ultrasound, CT with contrast, MRI); additionally is performed chest CT and bone scintigraphy if taken into account liver transplantation. In case of portal vein thrombosis associated with a small hepatic lesion, Doppler ultrasound is performed and thrombus punction ultrasound guided; the presence of thrombus exclude the possibility of radical treatment. Arterial signal into the thrombus indicate the tumoral etiology.

Abdominal ultrasonography has a 50% sensitivity in hepatocellular carcinoma detection for liver with advanced cirrhosis. Lesions under 2 cm can usually be detected.

Ultrasound appearance is variable. Lesions are usually hypoechoic. In large hepatocellular carcinoma, hyperechoic or heterogenic appearance is due to necrosis, hemorrhage or fat infiltration. Small hepatocellular carcinomas can be homogeneously hyperechoic and can be similar to hemangioma (due to a large portion of fat being present in the tumor) or can appear hypoechoic. Doppler ultrasonography can be used in the detection of portal vein, hepatic artery or inferior vena cava invasion. Portal venous invasion is more common in hepatocellular carcinoma, but hepatic vein invasion is more specific.

Small hyperechoic masses seen on ultrasound require further examination, because they can represent hemangioma (most frequently), metastatic disease or, less likely, hepatocellular carcinoma. Further imaging with CT scanning or MRI during dynamic contrast enhancement shows the typical peripheral, nodular contrast enhancement pattern of hemangioma. MRI or CT scanning can further characterize many nonspecific hepatic masses seen on ultrasonography.

CT scan – dynamic CT, spiral CT is the investigation of election for hepatocellular carcinoma diagnosis. The CT appearance of hepatocellular carcinoma is variable, depending on image phase and tumor size. The most common attenuation pattern is iso-hyper-iso-attenuation on prephase, arterial phase and venous phase, respectively; however, this pattern is common to other hepatocellular nodules, including regenerative and dysplastic nodules.

Unenhanced CT typically shows an iso-hypodense mass; for large masses central areas of necrosis may be seen.

In the hepatic arterial phase, nodules appear hyperdense (relative to hepatic parenchyma), as a result of hepatic arterial supply. Larger tumors may have necrotic central regions that are usually hypodense during this imaging phase. Neovascularity indicate the presence of inconspicuous lesions.

In the portal venous phase, small nodules may appear isodense or hypodense and larger nodules with necrotic regions remain hypodense.

In the delayed-postcontrast phase, small nodules may be inconspicuous on late phases.

Spiral-CT evidentiate 17% of lesions <1 cm, 29% of lesions between 1 and 2 cm and 63% of lesions higher than 2 cm.

MRI has higher sensitivity for focal hepatic lesions than CT.

Precontrast and postcontrast MRI has a 70-85% chance of detecting a solitary mass of HCC.

MRI can differentiate cirrhotic nodules from HCC: if the mass is bright on T2-weighted images, it is HCC until proven otherwise; if the mass is dark on T1- and T2-weighted images, it is a siderotic regenerative nodule or siderotic dysplastic nodule; if the mass is bright on T1-weighted images and dark or isointense on T2-weighted images, it is a dysplastic nodule or low-grade HCC [11].

Hepatic angiography.

Hepatocellular carcinoma is vascularized by hepatic artery and selective arteriography of superior mesenteric artery or celiac artery may identify the lesion. This is an invasive procedure used for hepatic chemoembolisation.

Liver biopsy.

For small lesions detected on ultrasound or CT, histological confirmation is very important. Biopsy is performed with ultrasound or CT guidance. There is a possibility of sowing on the needle path.

In present, the diagnosis of hepatocellular carcinoma is established by clinical examination associated with typical imaging aspect, with/without increased α-fetoprotein, histological confirmation not being absolutely necessary.

EASL 2016 recommend to perform liver biopsy for lesions with atypical imaging aspect and in non-cirrhotic patients [12].

DIFFERENTIAL DIAGNOSIS OF HEPATOCELLULAR CARCINOMA IN THE PRESENCE OF CIRRHOSIS

The major problem consist in the differentiation between hepatocellular carcinoma and a dysplastic nodule. Hepatocellular carcinoma appear hyper-vascularised compared to surrounding tissues.

For lesions higher than 2 cm identified in 2 different imaging studies, in the presence of cirrhosis, the diagnosis of hepatocellular carcinoma can be established. Also, if the lesion is identified in 2 different imaging studies and α-fetoprotein level is higher than 400 ng/ml, the diagnosis is very probable and histological confirmation is not really necessary [1].

SCREENING

Hepatocellular carcinoma screening is recommended in patients at high risk, such as male patients, HBV, HCV viruses, age > 40 years and chronic liver disease, especially cirrhosis and high grade dysplasia.

EASL 2016 clinical practice guidelines on the management of hepatocellular carcinoma recommend screening for patients with chronic HBV infection, even without cirrhosis, Asian males >40 years, females >50 years, patients with family history of hepatocellular carcinoma, African Americans >20 years, primary biliary cirrhosis stage 4, hemochromatosis, α1-antitrypsin deficiency.

Recommended screening methods are abdominal ultrasound combined with α-fetoprotein every 6 months. In cirrhotic patients, a nodule <1 cm detected must be supervised every 4 months for 1 year, than every 6 months.

For nodules 1-2 cm in diameter, the diagnosis is established by non-invasive criteria or histological examination.

Nodules higher than 2 cm may be diagnostic for hepatocellular carcinoma in case of typical aspect; for atypical aspect, histological examination is required [12].

TREATMENT OPTIONS

The treatment is established using the Barcelona-Clinic Liver Cancer (BCLC) staging system which defines very early stage cancer (single nodule,<2 cm, Child-Pugh A) as stage 0, early stage cancer (1-3 nodules, <3 cm each, Child-Pugh A-B) as stage A, intermediate stage for multinodular HCC (stage B), advanced stage which involves vascular invasion or extrahepatic spread (stage C) and terminal stage at patients with Child-Pugh C cirrhosis. Resection, ablation and transplantation is recommended for stage 0 and A, transcatheter arterial chemoembolization for stage B, Sorafenib – stage C and best supportive care for terminal stage.

Surgical treatment

Resection is curative, but limited in patients without cirrhosis or in the presence of cirrhosis and without portal hypertension. It is accepted as treatment for localized hepatocellular carcinomas. Intrahepatic recurrence is common, because the potential of neoplastic liver remain unchanged. The rate of recurrence at 5 years is almost 52 %. Partial hepatectomy is indicated in the absence of cirrhosis or in Child-Pugh stage A with normal portal pressure and normal bilirubin. The best results are obtained in patients with unicentrical disease, without vascular invasion, under 5 cm and with liver disease relatively inactive (5% of hepatocellular carcinomas). 5-year survival rate for curative treatment (resection, transplantation and local ablation therapy) is almost 80%.

Liver transplantation – the technique is successful in

selected patients, but it requires immunosuppressive treatment for life. It is indicated in patients with advanced cirrhosis (Child-Pugh B and C) who cannot survive on surgical resection. Liver transplantation can be applied independently on the liver stage disease and treat both hepatocellular carcinoma and neoplastic potential of cirrhotic liver.

Liver transplantation is indicated for unique tumors under 5 cm or <3 nodules (Milano criteria), each less than 3 cm.

It is difficult to elect between resection and transplantation for a tumor <5 cm. Altered liver function is in favor of transplantation.

Nonsurgical treatment

Chemotherapy

Sorafenib, a Raf-kinase and tyrosine kinase inhibitor, is indicated in patients with normal liver function (Child-Pugh A) and portal vein thrombosis, extrahepatic tumors and failure of other therapies. In patients with altered liver function, only best supportive care is indicated.

Local ablation-is potentially curative in patients with small tumors, <3-5 cm that are not candidates for resection or transplantation.

TACE (Transarterial chemoembolisation) – hepatic artery catheterization via the femoral artery or celiac axis allows the nutrient vessel embolization of tumor, and chemotherapeutic agents can be delivered in high concentrations. (Cisplatinum, Doxorubicim, foam gel, Epirubicin). Chemoembolization is indicated in unresecable tumors, patients with Child-Pugh A-B. The result is partial/total tumoral necrosis. The most frequent side effects are fever, nausea, encephalopathy, ascites and high levels of aminotransferase.

Percutaneous injection of ethanol (PEI) is efficient in 2-3 cm tumors (<5 cm), usually less than 3 nodules, even in the presence of advanced cirrhosis. The technique suppose the ethanol injection under ultrasound/CT guidance; outpatient treatment may be carried out 2 times per week, 3-15 sessions, using 2-16 ml of absolute alcohol. 3-year survival rate is 71% in patients with Child-Pugh A and 41% in Child-Pugh B.

Radiofrequency ablation (RFA) is a treatment that uses imaging guidance to place a needle electrode through the skin into a liver tumor. High-frequency electrical currents are passed through the electrode, creating heat that destroys the cancer cells. RFA is more effective than PEI in larger tumors (3-5 cm) and it requires fewer sessions. Recurrence rates are higher than in resection, but survival rate is similar to resection.

Trans-catheter arterial chemoembolization uses angiography and allows the nutrient vessel embolization of tumor. TACE is recommended in palliative treatment for unresecable tumors and in patients on transplantation list; the effectiveness of the method depends on the tumor dimension. TACE it is not recommended in the treatment of large and unresecable tumors.

CONCLUSION

In conclusion, hepatocellular carcinoma remain a fatal disease. All treatments used increases survival. Some studies have shown that there were no big differences between the results given by resection, transplantation and arterial embolization [13].

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Lipid profile and comorbidities in patients with psoriasis vulgaris

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Abstract: *Background and Aim: Psoriasis is an inflammatory skin disorder with important influence on the lipid profile, insulin resistance and cardiovascular disease. The aim of this study is to compare the lipid profile of classical therapy versus anti-TNFs patients and asses the associated diseases.*

Methods: We conducted a prospective study. Patients' data were collected from two Romanian Dermatology Clinics, during the period January 2015 – June 2016. The patients were divided into two groups: study group (on TNF inhibitors) and control group (on classical treatment).

Results: The subjects in the study group had more associated diseases and much more patients suffered from dyslipidemia. There were not statistically significant differences in the lipid profile between the two groups. But eliminating patients treated with Acitretin and Methotrexate, a p-value of 0.0259 (significant statistically) was observed when comparing the values of triglycerides in the two groups.

Conclusions: We did not observe a beneficial effect of the TNF inhibitors therapy in psoriatic patients on the lipid profile. Subjects on biological treatment suffer from a more severe form of psoriasis vulgaris and associate much more comorbidities.

Keywords: *lipid profile, comorbidities, anti-TNF agents.*

INTRODUCTION

Psoriasis is a chronic, inflammatory skin disorder [1]. The inflammation predisposes to a pro-atherogenic lipoprotein profile, large vessel inflammation, and adipokine dysregulation [1] thus leading to insulin resistance and cardiovascular diseases [2]. There is a strong link between psoriasis and risk factors for metabolic syndrome (central obesity, insulin resistance [3], abnormal lipid metabolism [4], hypertension [5]. Patients with psoriasis have a more increased risk of developing atherosclerosis due to inflammation and dyslipidemia [4]. Moreover, lipid abnormalities may play an important role in the development of psoriasis [4].

The inflammation mediated changes at lipids and lipoproteins levels have more likely a role in protecting the host. The treatment of psoriasis was shown to lead to a normalization of the lipid levels [6]. TNF induces a reduction in cholesterols (especially HDL) by interfering with LDL receptors, Apolipoprotein A and B, and 7A1 and 7B1 cytochromes with no changes in HMG-Co reductase action [7].

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In Romania, however, the inclusion criteria for biological therapies require previous two systemic non-biological therapies (retinoids, systemic glucocorticoids, methotrexate, PUVA, UVB narrow band).

Moreover, most of these therapies are known as lipid modifiers [4].

The aim of this study was to examine two groups of patients (biological versus non-biological therapy psoriatic patients) in terms of comorbidities and lipid profile.

MATERIAL AND METHODS

Study design

We carried out a prospective study. Patients' data were collected from two Romanian Dermatology Clinics: Dermatology Department, Carol Davila Emergency Military Hospital, Bucharest, (January – June 2016) and Dermatology Department, Mureş County Clinical Hospital, (January – December 2015).

All subjects had a physical examination. Body max index (BMI) was calculated as weight (kg)/height (cm^2) (normal weight: 18.5–24, 9; overweight: 25–29, 9; class I obesity: 30–34, 9; class II obesity: 35–39, 9; class III obesity: ≥ 40). Each patient completed a DLQI form and a dermatologist filled the PASI form.

The data collected included (i) patients' demographic characteristics: date of birth, gender, weight, height, BMI; (ii) psoriasis-related data: type of psoriasis, current therapies (topical treatment, Methotrexate/ MTX, Acitretin, PUVA, UVB TL01, anti-TNFs: Infliximab original and biosimilar, Adalimumab, Etanercept); (iii) comorbidities, with an accent on metabolic syndrome (hypertension, diabetes mellitus, obesity); (iv) cholesterol/cho and triglycerides/TG levels.

Patients were divided into two groups: study group (biological therapy) and control group (topical treatment, Methotrexate, Acitretin, PUVA, UVB TL01).

All participants gave written informed consent.

Statistical analysis

Data were analyzed using 2017 GraphPad Software.

RESULTS

At the end of the follow-up period, 102 subjects were included for the analysis: study group – 53 patients: 39 males (73.58%) and 14 females (26.42%) and control group – 49 patients: 25 females (51.02%) and 24 males (48.98%).

The biological therapy patients were distributed as follows: Infliximab (9, 16.98%), Infliximab biosimilars (4, 7.54%), Entanercept (17, 32.07%), Adalimumab (23, 43.39%). Four subjects had a switch of anti-TNF therapy, two changed Infliximab for Etanercept, the other two switched Infliximab for Adalimumab. Only 16 (15.68%) patients out of the 102 achieved both PASI and DLQI equal to zero during the study.

Biological patients were screened for associated diseases. Table 1 includes the number/(%) and comorbidities.

Table 1: Comorbidities of biological patients

Associated diseases	Number of patients /(%)
HTA	10/(18.86%)
Diabetes mellitus type 2	3/(5.66%)
Obesity	40/(77.35%)
Latent tuberculosis	5/(9.43%)
Hepatitis VHB	1/(1.88%)
Herpes zoster	1/(1.88%)
BPOC	1/(1.88%)
Chronic bronchitis	2/(3.77%)
Hepatic steatosis	1/(1.88%)
Glaucoma	1/(1.88%)
Arthropathy	15/(28.3%)
Synovitis right knee	1/(1.88%)
Disc hernia	1/(1.88%)
Anemia	1/(1.88%)
Allergy to penicillin and erythromycin	1/(1.88%)
Renal litiasis	1/(1.88%)
Hidradenitis suppurativa	3/(5.66%)
Bipolar disorder	1/(1.88%)
Amputation of right police	1/(1.88%)
Thyroid nodule operation	1/(1.88%)
Cholecystectomy	3/(5.66%)
Appendectomy	2/(3.77%)

The subjects with criteria of metabolic syndrome were

41 (77.35%) with obesity, 3 (5.66%) had diabetes mellitus type 2, 10 (18.86%) with hypertension. Other comorbidities were previous surgery (cholecystectomy, thyroid nodule operation, appendectomy), infection (latent tuberculosis, hepatitis VHB, herpes zoster), accidents (amputation of right police), pulmonary disease (BPOC, chronic bronchitis), inflammatory disease (hidradenitis suppurativa), hepatic disease (hepatic steatosis), renal disease (renal litiasis), ocular disorder (glaucoma), skeletal problems (arthropathy, synovitis right knee, disc hernia), hematological disease (anemia), psychiatric disorder (bipolar disease), allergy to drugs (allergy to penicillin and erythromycin).

The classical therapy patients associated obesity – 20 (40.81%), diabetes mellitus type 2 – 3 (6.12%), hypertension – 4 (8.16%). Other associated diseases were: hematological (anemia, thalassemia), skin cancer, arthropathy, osteoporosis, Cushing disease, venous disease, operated pituitary gland (table 2).

Table 2: Associated diseases of control group

Associated diseases	Number of patients
HTA	4
Diabetes mellitus type 2	3
Obesity	20
Anemia	1
Thalassemia	1
Skin cancer	2
Arthropathy	9
Osteoporosis	1
Cushing disease	1
Operated pituitary gland tumor	1
Chronic venous insufficiency	1

Patients with psoriasis have an important weight problem. The normal weight patients in the study group represented 12 (22.64%) versus 16 (32.65%) control group patients. Increased BMI was found in 74 (72.54%) psoriatic patients. (Table 3)

Some patients on anti-TNFs required additional drugs for disease control. 10 (18.86%) biological therapy patients used only topical treatment versus the majority of control group – 45 (91.83%).

Table 3: BMI for the two groups

BMI	Study group	Control group
Normal weight	12	16
Overweight	20	20
Obesity class I	15	13
Obesity class II	5	-
Obesity class III	1	-

Methotrexate was prescribed to 6 (11.3%) patients in the study group and to 4 (8.16%) patients on classical therapy.

Retinoid (Acitretin) was used only in 1 (1.88%) biological patient versus 2 (4.08%) patients in the control group. (Tables 4, 5)

Table 4: Additional therapy of anti-TNFs patients

Patient	Additional therapy
1	Acitretin
2	Methotrexate
3	Methotrexate
4	Methotrexate
5	Calcipotriol and betamethasone gel
6	Clobetasol propionate 0.0525% ointment
7	Calcipotriol and Betamethasone gel
8	Calcipotriol and Betamethasone gel
9	Methotrexate
10	Calcipotriol and Betamethasone gel
11	Calcipotriol and Betamethasone gel
12	Calcipotriol and Betamethasone gel
13	Keratolytic creams, Dermatocorticoids
14	Calcipotriol and Betamethasone gel
15	Calcipotriol and Betamethasone gel
16	Dermatocorticoids, Methotrexate
17	Methotrexate
18	Methotrexate

Table 5: Therapies of patients in the control group

Patient	Therapy
1	calcipotriol and betamethasone gel, 15% urea cream, UVB TL01
2	0.1% mometasone furoate cream, vitamin A, emollient, cream with salicylic acid, UVB TL01
3	topical corticosteroid, calcipotriol and betame-thasone gel, keratolytics, emollient cream, Methotrexate, UVB TL01
4	clobetasol propionate 0.0525% cream, Methotrexate, PUVA
5	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, PUVA
6	0.1% mometasone furoate cream, UVB TL01
7	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, UVB TL01
8	0.1% mometasone furoate cream, Methotrexate
9	betamethasone dipropionate and salicylic acid cream, clobetasol propionate 0.0525% cream, vitamin A, vitamin E,UVB TL01
10	15% urea cream
11	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, 15% urea, vitamin A, vitamin E, PUVA
12	0.1% mometasone furoate cream, emollient cream, keratolytic cream, UVB TL01
13	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, Vitamin E, PUVA
14	topical corticosteroid, UVB TL01
15	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, Vitamin E, PUVA
16	clobetasol propionate 0.0525% cream, calcipotriol and betamethasone gel, Vitamin E, Vitamin A, salicylic acid cream, PUVA
17	clobetasol propionate 0.0525% cream, Vitamin E, Vitamin A, UVB TL01
18	0.1% mometasone furoate cream, emollient cream, betamethasone dipropionate and salicylic acid cream, UVB TL01
19	0.1% mometasone furoate cream, emollient cream
20	clobetasol propionate 0.0525% cream, Vitamin E, Vitamin A, UVB TL01
21	calcipotriol and betamethasone gel, Vitamin E, Vitamin A
22	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, emollient cream, Vitamin E, Vitamin A, PUVA
23	vitamin E, 30% urea cream
24	clobetasol propionate 0.0525% cream, emollient cream, UVB TL01
25	calcipotriol and betamethasone gel, 15% urea cream, PUVA
26	calcipotriene hydrate and betamethasone solution, 0.1% mometasone furoate cream, Vitamin A, Vitamin E, Methotrexate, PUVA
27	0.1% mometasone furoate cream, Desloratadine cp, UVB TL01
28	0.1% mometasone furoate cream, clobetasol propionate 0.0525% cream, Desloratadine cp, PUVA
29	clobetasol propionate 0.0525% cream, emollient cream, vitamin A, vitamin E, PUVA
30	0.1% mometasone furoate cream, emollient cream, Methotrexate
31	0.1% mometasone furoate cream, emollient cream, salicylic acid cream, Clarithromycin
32	0.1% mometasone furoate cream, emollient cream, UVBTL01
33	clobetasol propionate 0.0525% cream, Ciprofloxacin cp, UVB TL01
34	clobetasol propionate 0.0525% cream, salicylic acid cream, UVB TL01

35	calcipotriol and betamethasone gel, vitamin E, Vitamin A, UVB TL01
36	0.1% mometasone furoate cream, UVB TL01
37	0.1% mometasone furoate cream, calcipotriol and betamethasone gel, vitamin A, vitamin E, PUVA
38	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, vitamin A, vitamin E, UVB TL01
39	clobetasol propionate 0.0525% cream, betamethasone dipropionate and salicylic acid cream, silver nitrate solution, vitamin A, vitamin E, UVB TL01
40	UVB TL01
41	Methotrexate
42	0.1% mometasone furoate cream, calcipotriol and betamethasone gel
43	0.1% mometasone furoate cream, Desloratadine cp, UVB TL01
44	clobetasol propionate 0.0525% cream, UVB TL01
45	0.1% mometasone furoate cream, UVB TL01
46	calcipotriol and betamethasone gel
47	0.1% mometasone furoate cream, 15% urea cream, emollient cream, Methotrexate
48	0.1% mometasone furoate cream, 15% urea cream, emollient cream, Methotrexate
49	calcipotriol and betamethasone gel, 15% urea cream, vitamin A, vitamin E, Methotrexate, PUVA

The lipid profile was screened in both groups of patients. But there was no difference between the two groups of patients. The p-value comparing the two groups of cholesterol (study vs. control) was 0.8674, and for the triglycerides groups (study vs. control) was 0.218. On the other hand, a significant statistically p-value of 0.0259 was observed when comparing the values of triglycerides in the 2 groups, but after eliminating the patients on Acitretin and Methotrexate.

We compared the cholesterol and triglycerides values from each group for patients treated only Methotrexate or Acitretin and the ones on anti-TNFs and Methotrexate or Acitretin, but no significant p-values were found.

We observed high triglycerides values in patients treated with Infliximab – 7 (58.33%) followed by Etanercept – 5 (35.71%) and Adalimumab – 4 (22.22%). The highest levels of cholesterol were observed in the Etanercept and Adalimumab subgroup 11 (78.57%). The others subgroups contained only small samples of patients. (Table 6)

The study group was divided into subgroups of years on biological therapy and the levels of cholesterol and triglycerides were compared.

Table 6: Patients with high lipid values divided into systemic therapies

Therapy	High cholesterol No/(%)	High triglycerides No/(%)
Infliximab (original + biosimilar)	2 (16%)	7 (58.33%)
Etanercept	11 (78.57%)	5 (35.71%)
Adalimumab	11 (61.11%)	4 (22.22%)
Adalimumab + Methotrexate	3 (75%)	3 (75%)
Etanercept + Methotrexate	2 (100%)	0 (0%)
Adalimumab + Acitretin	1 (100%)	0 (0%)
Methotrexate	2 (50%)	3 (75%)
Acitretin	0 (0%)	0 (0%)

The subgroup 4-6 years had the highest level of dyslipidemia as it can be observed in Table 7.

DISCUSSION

Analyzing the two groups, the patients on biological therapy seemed to have more associated comorbidities than the control group. Cardiovascular events appear more often in patients with severe (8) and therapy resistant psoriasis like the ones treated

with anti-TNFs.

The two groups showed a difference in the frequency of hypertension (study group – 18 (86%) vs. control group – 8 (16%)). Even though, in normotensive and rheumatoid arthritis individuals, Infliximab reduced blood pressure levels, we observed in our study that the anti-TNFs do not improve alone the blood pressure values in psoriatic hypertensive patients [9].

Table 7: Levels of cholesterol and triglycerides divided into subgroup of years of anti-TNF therapy

Years of biological therapy	High cholesterol	High triglycerides	Cholesterol %	Triglycerides %
≤ 3.5 years	7	4	13.20	7.54
4-6 years	22	15	41.50	28.30
7-8 years	2	2	3.70	3.70

Many studies observed that high blood pressure is more often noted in patients with psoriasis compared to the non-psoriatic population [10], especially psoriatic patients who were male, middle-aged and elderly people [11] as our study group (39 males (73.58%) versus 14 females (26.42%)). Men are more predispose to severe forms of psoriasis [12].

High blood pressure is independent associated with psoriasis [8].

Apparently, in psoriasis the pathophysiologic mechanisms for high blood pressure are related to the underlying autonomic nervous system dysfunction [13], elevated plasma renin activity, elevated angiotensin converting enzyme activity, and elevated endothelin-1, TNFG308A polymorphism [14].

Psoriasis is thought to promote insulin resistance by overproduction of Th1 cytokines [10]. An association can be observed between high levels of TNFα, IL6, IL17, leptin, and C-reactive protein, and increases in BMI, which contribute to alterations in insulin biochemical pathways, leading to insulin resistance, type II diabetes [15]. Our patients are overweight, dyslipidemic and topical corticosteroid users for long periods of time, more over they have an inflammatory disease which is pro-atherogenic. Although only 6 patients out of 103 were diagnosed with diabetes mellitus type 2, the screening is continuous, because psoriasis is an independent risk factor for type 2 DM, the risk being greater in severe disease. (Marius Irimie, 2015).

Most of our patients with psoriasis vulgaris are above normal weight (72.54%). It has been reported that psoriasis favors weight increase and obesity [15]. In obesity there is an unbalanced production of pro- and anti-inflammatory adipokines in obesity, leading to a chronic low-grade inflammation state, which seems to favor worsening of psoriasis lesion and a poorer response to treatment [16].

Although we did not evaluate the weight gain before and after anti-TNF treatment, several studies have reported an increase in weight and body mass index (BMI) with the use of anti-TNF agents (adalimumab, etanercept and infliximab) [9, 17]. Most of our patients in the study group are overweight (77.35%).

On the other hand, weight loss (greater than or equal to 5% of the initial weight) is associated with a higher success rate in achieving control of disease activity in patients with overweight or obesity with psoriatic arthritis treated with anti-TNF-α [9]. A study with patients on low-energy diet lost weight which led to significant reductions of several cardiovascular risk-associated factors, moreover the weight loss improved HbA1c levels by 0.7%, equivalent to reductions achieved by treatment with an anti-diabetic drug [18]. Moreover, the psoriatic patients with the lowest monounsaturated fatty acid (MUFA) intake presented the highest values of PASI score and inflammation marker [19].

The mean age of psoriasis patients in our study was 52.07 years similar to international studies, thus confirming the average age of type 2 psoriasis patients worldwide [20]. Moreover the biological patients are the ones that do not respond to classical therapy

suggesting the severe form. The equal distribution in the classical therapy group can be due only to a mild form of disease.

Between the two groups there were not statistically significant differences in the lipid profile. Our results are similar with those of [21], their psoriatic patients treated with etanercept, adalimumab, or infliximab (each patient serving as their own control), did not present a significant change in total cholesterol, LDL and triglycerides. On the other hand, a p-value of 0.0259 (significant statistically) was observed when comparing the values of triglycerides in the two groups (eliminating the patients on Acitretin and Methotrexate).

We discovered that patients with 4-6 years of anti-TNF therapy were the majority group with dyslipidemia. However, the study of Ehsani et al. demonstrated an insignificant 5% increase in LDL and triglycerides after 24 weeks of treatment with anti – TNF agents [7], but all the changes in lipid profile by biological therapy are temporary, and the negative feedback will balance these changes over time [7]. Others have proven that patients with long term psoriasis have an increase in the serum total cholesterol levels and triglyceride levels [22]. Several authors found increased cholesterol levels (more than 5 years), but the lipid profile changes from the early stage to the late chronic stage of the disease. In contrast, Jorge Santos-Juanes et al. did not find a relationship between lipid levels and disease duration [23]. Maybe lipid profile abnormalities might be genetically determined rather than acquired [8].

Comparing only patients on MTX or Acitretin versus patients on anti-TNFs and MTX or Acitretin we did not observe a significant statistically important difference. But in similar studies there was a significant increase in triglycerides in patients treated with TNF inhibitors + MTX compared with patients treated with MTX alone. In our study, the highest triglycerides levels had patients on Infliximab – 7 (58.33%), followed by Etanercept – 5 (35.71%) and Adalimumab – 4 (22.22%). Concerning cholesterol levels were higher in Adalimumab and Etanercept – 11 subjects (61.11%; 78.57%). The patients on systemic classical treatment (Methotrexate or Acitretin) or on anti-TNF + MTX/ Acitretin were too few to have statistical significance [21].

Our control group (excluding MTX and Acitretin) showed increased cholesterol (26/53.06%) versus triglycerides levels (6/12.24%). Other studies showed that TG levels were significantly higher in psoriatic subjects than control groups [24].

We did not exclude patients on Methotrexate and Acitretin.

These may cause or influence the dyslipidemia. Recent studies proved that retinoid acid treatment could produce unsaturated fatty acids and induce triglyceride breakdown, bile acid secretion, lipolysis, and retinoids elimination. But, retinoid x receptor α deficiency might induce the synthesis of saturated fatty acids, triglyceride, cholesterol, bile acids, and retinoids [25]. Retinoids, especially Acitretin increase triglycerides levels [26], but also a mild and transient reduction in HDL levels and insulin sensitivity [27].

Regarding MTX, there are conflicting studies on the effect of Methotrexate on the lipid metabolism. The HDL profile function is favorable influenced by Methotrexate [28], Methotrexate has an anti-atherogenic potential [29] with cardioprotective properties [30]: tendency to decrease the risk of heart failure [9], a lower incidence of vascular disease (cardiovascular, cerebrovascular and arteriosclerosis) [17]. But it has been proven that cholesterol, LDL cholesterol and HDL cholesterol are increased short-termed after the initiation of Methotrexate therapy [31].

CONCLUSION

Our study did not show a positive effect of anti-TNF therapy in psoriatic patients on the lipid profile. Most likely, the abnormalities of the lipids are due to genetically inherited mechanisms. Our patients on biological therapy have a more severe form of psoriasis vulgaris, with many comorbidities.

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ORIGINAL ARTICLES

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Management of burning mouth syndrome (BMS) in patients with diabetes mellitus

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Abstract: *Unlike the therapy of primary BMS, the treatment of secondary BMS benefits, in addition to symptomatic therapy, from the possibility of using means and methods to correct biological disturbances and/or morphological, psycho-emotional changes respectively. The use of these therapeutic means in secondary BMS is recommended to be established as a first step of therapeutic conduct. Their success is reflected in homeostasis restoring, correcting morphological and functional changes of the field, which frequently leads to no longer use prescription of symptomatic treatment.*

DEFINITION

The International Association for the Study of Pain (IASP) defines burning mouth syndrome (BMS) as a "distinct nosological entity" characterized by "oral sensation of burning or pain, persistent and non-remitting in time in the absence of clinical objective changes of the mouth mucosa" [1]. A relatively similar vision is supported by Grushka (2014). BMS defining a sensory sensation, the author admits that this is manifested mainly by: a) subjective characteristics: changes in taste perception; bilateral burning sensation associated with altered taste mouth; sensation of "dry mouth", although salivary flow is not reduced; perceived foreign body in the oropharynx and b) objective criterion: the oral tissues with clinically normal aspect. The author specifically mentions that the 4 subjective lists, encountered in BMS come forth from the absence of results after an objective clinical exam, a defining element for this condition [2].

EPIDEMIOLOGY

Setting the epidemiology of BMS is difficult and imprecise because there is no universally accepted definition. The various epidemiological studies often refer to different clinical entities in which oral mucosal lesions lack [3]. This disease has a high rate that varies according to the studies from 0.7% to 14.8 - 15% [3,4,5]. The prevalence of painful syndrome in the Service of Oral Pathology of the Faculty of Dental Medicine - "Carol Davila" University of Medicine and Pharmacy, Bucharest (Romania) is 16.23% [6].

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ETIOLOGY. PREDISPOSING FACTORS

Pathogenic, sex, age, menopause can be considered contributing factors. Therefore:

1. Although it was observed in any age group between 27-87, BMS has a clear predisposition related to age. The average age of reporting the disease is 61 [2]. BMS rarely occurs before the age of 30 [7].
2. Sex is also placed between contributory factors. The women are 2.5 to 7 times more frequently affected than men.
3. The body functioning as a whole, at menopause, besides circulating electrolyte changes, it suffers from other disturbances such as:

a) Lipid disorders with consequences in the development of atherosclerosis;

b) exacerbated production of ROS/RNS, including liquid in the gingival sulcus (where ROS = reactive oxygen species, and RNS = reactive nitrogen species);

c) changes in the hormonal constellation at menopauses the quantity of estrogen become responsible for the development of female cardiovascular pathologies same as men.

Data from the literature considers that up to 90% of the female patients with BMS are around menopause, the onset of symptoms characteristic of peri-menopausal period taking place three years before and 12 years after climax [8].

The explanation for BMS distribution predominantly in women during perimenopause, according to Woda et al. (2009) is that BMS is the result of hormonal changes (hypo-estrogenism) in women during menopause, which often associates with metabolic disorders and neuropsychological chronic anxiety or stress. Mental disorder in menopausal women explains anxiety and depression from BMS [9].

4. Another pathogenic explanation takes into consideration the interrelationship taste-saliva-pain. Laboratory tests showed that female patients with disorders in climax present a growth of salivary phosphate concentrations, proteins, Na, K+, Ca2+ and Mg2+. The referred electrolyte disturbances trigger the disruption of the membrane potential, including those of the cells involved in collecting taste excitations or cells responsible for saliva secretion [10].

Certain hormonal changes (hypoestrogenemia, diabetes mellitus, hypothyroidism, thyroid dysfunction type) and functional morphological and immunological disorders affecting the endocrine glands were also among factors described as possible cause of this disorder [11]. Regarding the relationship between diabetes and BMS various assumptions were formulated, including the existence of metabolic changes in the mouth due to peripheral neuropathy, which can generate among other disturbances the hypo-function of the salivary glands, thus reducing the secondary salivary flow [3,12,13].

The mode of action of psycho-neuro-endocrine factor is even more difficult to explain since it has been shown that patients presenting BMS have a greater tendency to somatization and to develop psychiatric symptoms [11]. For this reason the theory was accepted that burning mouth syndrome is a form of neuropathic pain [14]. The neuropathy mechanism in the pathogenesis of BMS is still controversial, the expert literature admitting the possibility of dysfunction via peripheral or central reflex arc and cortical excitation [8,10].

BMS SYMPTOMS

Subjectively, patients describe the syndrome BMS as the presence of oral mucosa burning sensation, the most common manifestation present in the disease. Other features of the pain in this syndrome are itching sensation, numbness, impaired taste (taste “spicy”), dry mouth etc. As topography, the symptom is located almost constantly close to the lingual mucosa (especially in the front third) and the lip. Recently, Demarosi (2013) stresses however that it is not possible that all the oral mucosa could be involved without being identified any precise anatomic distribution [3].

Even since 2003, Scala et al. underlined the semiotic nature of BMS as being more complex, consisting of main and associated symptoms:

a) The main symptom is a triad, consisting of:

1. Pain in the oral mucosa: burning, stinging, tingling, swelling;

2. Dysgeusia (altered taste): the persistence of a certain flavor/taste perception alteration;
3. Xerostomia, with dry mouth.
b) Other associated symptoms: thirst, headache, pain in the temporomandibular joint (TMJ), tenderness/ pain during mastication and/or in some regions of the neck suprahyoid or shoulder level muscles [15].

The amount of positive clinical examination in the diagnosis and any associated distress, which act as causative factors in BMS syndrome

BMS diagnosis is essentially one of exclusion [16]. It is based on a historic and very thorough clinical examination. An accurate diagnosis requires careful investigation of BMS medical history of each patient (such as a preexisting condition at the oral mucosa level, malnutrition, cerebral dysfunctions or drug intoxication) [17,18].

1. The contribution of general clinical examination in the diagnosis of BMS

At their first visit at the dentist's, patients frequently report present disorders and diseases already diagnosed under therapy. When there is a suspicion of disease present and undiagnosed, the patient is advised to consult a physician in the respective domain [19].

2. The role of local clinical examination in the diagnosis of BMS

Local clinical examination often does not reveal any changes. Sometimes clinical examination can detect minor changes or variations of normality: fissured tongue, exfoliative glossitis, depapillations of various origins, geographic tongue or coated tongue [16].

If physical examination reveals the absence of clinically obvious lesions on the oral mucosa, it is reasonable to suspect that intraoral burning is a possible indicator of a systemic disorder [3].

Identifying the possibility of the existence of systemic diseases associated with or responsible for installing the BMS syndrome is by clinical examination and by performing general laboratory or laboratory tests [16].

THE MAIN THERAPIES EMPLOYED IN THE BMS

Based on a comprehensive assessment of the patient's disease state that integrates general clinical examination results, psychiatric/psychological balance and the local dental examination, a therapy in BMS is set. The characteristic of glossodynia treatment is its individualization and adapting to each individual case [19].

Primary stage in establishing BMS therapy requires the differentiation of the primary from the secondary. This is because if the balance sheet allowed the identification of predisposing and etiologic factors, therapy will target their harmful action through appropriate specific methods.

Clinical attitude to be adopted to establish the diagnosis and clinical form of BMS is summarized in figure 1. Schematic representation is useful because it allows to facilitate the understanding of therapeutic medical conduct to follow. According to Demarosi (2013), clinical, biochemical and hematological test results represent two phases of special importance in the etiological diagnosis of the BMS [3].

The therapy goals of the BMS syndrome are administered to target a triple purpose:
1) Improvement of symptoms;
2) Correction of biological and/or morphological disturbances (if there are), commonly referred to as correction therapy;
3) Changes in psycho-emotional therapy [3].

Symptomatic treatment

Symptomatic treatment is similar in both cases of BMS (idiopathic or secondary). While pain is a symptom of many chronic diseases, persistent pain should not be regarded simply as a symptom. Pain itself can become a disease where pain signals origins fail to be controlled due to deterioration of the pain alarm system, leaving the patient with persistent pain. Whatever the cause, chronic pain can transcend ordinary physical pain. Persistent pain interferes with daily life, other important relationships and puts a mark on the mind, body and spirit of the BMS patient. The pain and related problems (e.g., medication side effects, depression and anxiety) can be best managed

using a complex program of integrative treatments tailored to each patient. The name of integrative treatment derives from the means/method by which it is achieved, the approach of a "multi-modal" one.

Figure 1: The current clinical attitude in the presence of a case with BMS [3]

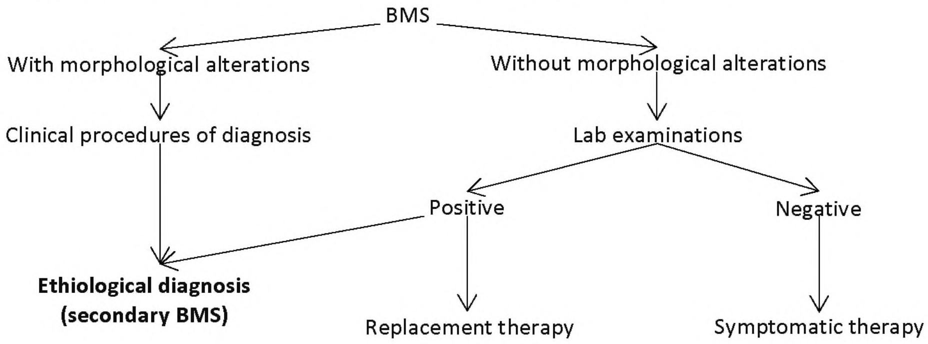

The method involves combining treatments from conventional medicine with those of complementary and alternative therapies. Scientifically, for the efficiency of the latter, evidence have been provided for both the quality and safety of the therapy. In this category, the treatment of patients with BMS can use homeopathic products, cognitive-behavioral methods, aromatherapy, herbal extracts, vitamins, dietary supplements, some combination of minerals etc. [21,22]. Also, acupuncture as an adjunctive therapy method is referred to in the expert literature as being beneficial for the relief of symptoms in patients with BMS [14].

Generally speaking, it is acceptable that the treatment of idiopathic BMS is essentially a symptomatic treatment. In the context of such therapy, the dentist has available a number of symptomatic therapies that can be administered by the general or local means with topical use. Some of these are summarized in the table shown above. The purpose of a symptomatic therapy aims at either improving the intensity of symptoms or halting the clinical manifestations. The results are uncertain, including failure.

Therapeutic strategies include benzodiazepines (clonazepam), tricyclic antidepressants (amitriptyline), opioids (gabapentin), selective inhibitors of serotonin receptors (paroxetine and sertraline), capsaicin topical/systemic, alpha-lipoic acid (neurological antioxidant) hydrochloride, benzydamine 0.15%, hormone replacement therapy, vitamin supplementation and/or zinc, iron and psycho-cognitive therapy [3].

Tricyclic antidepressants (amitriptyline/doxepin/ nortriptyline) are extremely useful for patients with chronic pain. Although developed for the treatment of depression, they have a spectrum of biological activities depending on the dose, including inducing analgesia in a variety of clinical conditions. Although the mechanism is not known, the analgesic effect of antidepressants have a more rapid onset and occurs at a dose lower than that typically necessary to treat depression. The maximum effect is achieved by tricyclic antidepressants in patients with chronic pain who are not depressed, reduced pain being a certainty. Anticonvulsants (carbamazepine) and tranquilizers (clonazepam) are particularly useful for patients with neuropathic pain [23].

Capsaicin, a substance extracted from red peppers, is used for its revulsive and analgesic action. Capsaicin is specifically detected by the "transient receptor potential vanilloid receptor" (TRPV1) distributed in the oral mucosa. The explanation is that the capsaicin molecule is a selective agonist vanilloid receptors TRPV1. Binding means stimulating the receptor, which is found in the nociceptors (pain receptors) in the oral cavity. Overstimulation of TRPV1 receptors make them become "desensitized", no longer able to respond to the stimuli that normally cause pain in patients with peripheral neuropathic pain [24].

Topical application of capsaicin has been shown to sometimes be useful in some pain syndromes, in particular for relieving the symptoms of herpes neuralgia or diabetic polyneuropathy hyperalgesia [25].

Similar conclusions on the effectiveness of topical application of capsaicin (0.02%/0.002%) in BMS are made by Silvestre et al. (2012) and Spanemberg et al. (2011) [26,27].

Other authors speak of the efficacy of capsaicin. Spadari et al. (2014) reported a success rate of over 70% for each control visits after 1, 3, 6 and 12 months of topical therapy (as by rinsing the mouth with a solution of capsaicin 3 times/day) [28].

Among the most widely used symptomatic therapies for BMS are salivary substitutes, from the most simple and economical specific physiological solution to gel. Their use is motivated by the fact that xerostomia is a frequent syndrome in patients with BMS [3].

Grushka (2014) suggests that treatment involves a combination of several BMS drugs that act together, increasing each other's effect (they are given together as dose so that a low-dose should enhance the action). From such an attitude, "low dose" can achieve favorable results on major symptoms present in patients. Achieving success involves on the one hand an individualized (personalized) therapy, on the other successive explorations, to identify the effective drug combination. Based on the results of his studies, Grushka (2014) admits that ⅔ of the patients respond to the treatment aimed at suppressing the effects of benzodiazepine receptor agonists group GABA-A and inhibition of pain. The combination drug likely to complete such a success is a triad of medications: clonazepam 0.5 mg before bed + 10 mg amitriptyline + gabapentin 100-300 mg 3-4 times +/or 25-75 mg Pregabalin 3-4 times daily. Again Grushka (2014) states that zinc therapy is effective in taste changes, prescribing zinc gluconate 50 mg 1-3 times a day for a period of 3-4 months [2].

In conclusion, according to the expert literature, the current level of knowledge about the disease does not include any certainly effective treatment for primary BMS.

Treatment of secondary BMS

According to Ţovaru et al. (2015), for the treatment of secondary BMS the major objectives are:

- detection and treatment of general factors involved in the emergence of glossodynia (anemia, vitamin B deficiency, diabetes, autoimmune disorders of the digestive tract, disorders during menopause, and esophageal reflux caused by gastric hyperacidity);
- detection and removal of the local causes of BMS (incorrect prostheses, changed vertical dimension of the lower floor of the face, improper dental alloys, chronic mucosal infections – for example, Candida – contact allergies, dry mouth, migratory glossitis;
- detection and treatment of coexisting psychiatric disorders (anxiety neurosis or depression, with stress, chronic states of conflict, emotional pressure, cancer-phobia) [19].

Achieving this objectives requires expert advice and patient's hospitalization for the neuropsychological disorder, often lasting for years.

Etiological treatment

A priori, BMS secondary treatment is etiologic, addressed to fight against the general and local factors that maintain the syndrome. The success of etiologic therapy is dependent on the precision with which the causative factor was identified [8,19]. Thus, in the case of the presence of allergic contact reactions, simply removing the suspected allergen (e.g., the material / dental alloy) determines remission of the symptoms of BMS.

If the necessary examinations revealed the presence of anemia, therapy should be prescribed to correct hemoglobin assets and deposits of iron and vitamin B12. Completion of these deposits is mandatory because suppressing the substitute anti-etiological treatment after restoration of erythrocyte hemoglobin level, allows a recurrence of anemia after a certain period of time.

Etiological treatment is required in other cases of BMS secondary diseases such as diabetes mellitus, gastric ulcer, gastro-esophageal reflux disease, mycosis, and the burning mouth is one of the manifestations of

these disorders [3].

Psychological and psychiatric treatment

The second place in the treatment of secondary BMS is psycho-pharmaceutical correction. Frequently, symptomatic treatment [3] is no longer resorted to.

Psychological and psychiatric treatment in the syndrome of secondary BMS is applied not only to the patients with psychiatric disorders or behavioral disorders diagnosed during the first medical checkup, but also to patients who do not respond to dental treatment. For this purpose, patients are directed to consult a specialist who would in fact perform a reassessment if the psychotherapeutic treatment is not appropriate to be set [3,29].

Specialized treatment is achieved by two means:

a) psychotherapy (psychological exam);

b) with drugs, therapy monitored by a psychiatrist;

Psychological treatment aims at controlling and alleviating the mental disorders that occurred. Typically, psychological treatment complements the psychiatric one, but it is the first to start with, especially in patients who have problems with mental BMS, such as those with dental treatment failure. Results of consequent studies have concluded that pharmacological products such as benzidamine-HCl solution, sucralfate, antihistamines, lidocaine, capsaicin have not shown significant improvement statistically [2,3,16].

Frequently followed conduct is the psychoanalytic association with administration of specific psychotropic medication (anxiolytic, antidepressant). The usefulness of psychotherapy is to reduce symptoms, if there is a psychological component to glossodynia [19].

Counselling treatment

BMS syndrome treatment conduct includes counselling, preferably applied by a physician demonstrating empathy for the patient. The purpose of counselling is to provide the patient information and explanations about the sickness, benignity of the lesion, its correlation with notions of its correlation with field (age and sex). These patients should always know that their disorders are often related to stress and that if they make it go away, at least in part their suffering can diminish [3].

The doctor should always have the status of patients with BMS, the suffering and disease, so they have to devote the necessary time to communicate and discuss the patients' problems. This is because one of the major concerns of patients with BMS is to undergo a treatment to alleviate the symptoms [3].

During counselling sessions the BMS patient will be informed about the need to respect therapeutic indications, even if obvious improvements do not occur. Also, we will clarify that for the relief of symptoms, duration of treatment is for months/years.

Starting from his own medical experience, Demarosi (2013) states that patients rarely comply with prescribed BMS treatment, the recommended therapy is often performed incorrectly and doctor visits are random. Simultaneously, the patient will be clearly advised that the outcome of the treatment consisting in improvement or even the disappearance of the disease is often only temporarily effective [3].

Since the treatment of BMS is generally unsatisfactory even if it is appropriate, and the pain is chronic, it is necessary that patients be counselled with correct information on expectations that are to be understood realistically. Otherwise, the patient loses confidence in his regular doctor and becomes psychotic, going from doctor to doctor.

EVOLUTION AND PROGNOSIS IN BMS SYNDROME

Sardella et al. (2006) in a retrospective study of 18 months showed that 28.3% of the BMS (basic form) cases registered moderate improvement, 49% not any significant change, 18.9% of the cases presented a worsening of symptoms in patients who have not received any treatment. Spontaneous complete remission is rare and has been reported by the same team in 3% of cases investigated over a period of 5 years [30,31].

Cure rates measured in glossodynia of type I and II were almost comparable (61% versus 59%). 70% of all

cases that have not responded to treatment present associated psychiatric disorders [19].

Idiopathic nonspecific painful syndromes are most rebellious to treatment. Some cases even exclude any possibility of allopathic treatment, in which case additional treatment, such as acupuncture is resorted to (14). Frequently, the results of acupuncture are not always stable, and in some cases a patient can get addicted to this kind of treatment [19].

In other words, where allopathic treatment + psychological + counseling fail, acupuncture may be used with relative efficacy and limited duration, which requires to repeat the complementary procedure.

In another study, a group of 91 patients with BMS symptoms were analyzed and it was concluded that in a small number of patients symptoms may resolve spontaneously within five years from the onset. Moreover, a partial improvement was observed in 38.4% of these cases under various treatments (for example, clonazepam in local administration). This rate of improvement is greater (up 61.7%) when patients were administered clonazepam locally associated with psychotherapy [32].

Fenelon et al. (2014), evaluating the results of short-term therapy with clonazepam versus amitriptyline in the pain relief of BMS highlighted that after 6 weeks of treatment there was an improvement in the symptoms of pain in both situations, noting that the effect of amitriptyline was lower than that of clonazepam. To explain such particularity of the therapy, the authors imply a slow onset of action of the first drug. After 3 months, the results were similar in the two groups of patients evaluated [33].

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ORIGINAL ARTICLES

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Dosimeters: measuring occupational UV exposure's involvement in skin cancer development

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Abstract: *Introduction: Solar exposure is a major causative factor for multiple skin pathologies. Even though UV radiation is the most important factor implicated in skin cancer pathogenesis, still, few European countries recognize UV occupational-induced skin cancer as an occupational disease, Romania not being part of this category. The objectives of this study were to evaluate the UV exposure doses in agriculture and construction workers and to establish efficient protective measures.*

Materials and methods: In our Department, a prospective, observational, analytical study was conducted, including six outdoor workers (in construction and agriculture), over a period of three summer months (July, August, September). Subjects were asked to wear personal dosimeters and the following parameters were analyzed: profession, duration of exposure, UV exposure doses, skin aspect, preexisting preventive measures.

Results: The wearing of dosimeters was well tolerated by all subjects, without any interference with daily professional activities. Daily UV radiation doses ranged from 1.79 SED (tractor driver) to 19 SED (agriculture worker). Maximum UVR doses were recorded between 10:00 am and 4 pm. Clinical examination was made after measurements, with no detectable skin changes.

Conclusion: Even though workers in agriculture and construction in Romania are exposed to significant UV doses, with 40% more than the general population, these professions are not recognized as high-risk professions for developing skin tumors. More prospective and retrospective studies are necessary for more significant statistical results and for demonstrating the necessity of introducing primary, secondary and tertiary preventive measures.

Keywords: *ultraviolet radiation, occupational exposure, skin cancer*

INTRODUCTION

Solar exposure is a major causative factor for multiple skin pathologies. Most frequently it is the case of cutaneous tumors, both precancerous and malignant lesions (actinic keratoses, basal cell carcinomas, squamous cell carcinomas and melanomas). [1]

There are numerous professions that imply working

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outdoors, from which we concentrated our study on agriculture and construction workers. Occupational ultraviolet (UV) exposure can be measured with UV dosimeters.

Personal UV dosimeters are electronic devices that are able to record data that permits analysis of UV exposure dose on working days. Modern dosimeters are small in size, therefore easy to wear at the workplace, permitting personal UV measurements. Even more so, multiple dosimeters can be used, recording data from different parts of the body. They are relatively new devices used more and more frequently in UV exposure-related studies. [2-5]

Ultraviolet radiations represent segments of the electromagnetic specter and are classified in UVC (100-290 nm), UVB (290-320 nm) and UVA (320-400 nm).

UV radiation is very important in dermatological practice, being able to determine both exacerbations and remissions of different skin disorders and of course playing a major role in skin cancer development. [6]

Even though UV radiation is the most important factor implicated in skin cancer pathogenesis and World Health Organization introduced UV radiation on the carcinogens agents list of group I, still, few European countries recognize UV occupational-induced skin cancer as an occupational disease, Romania not being part of this category. [7]

The lack of studies in this area in Romania suggests the fact that more analysis should be made for a better understanding of this aspect in our country.

Objectives: to evaluate UV exposure doses in agriculture and construction workers and to establish efficient prevention and protection measures.

MATERIALS AND METHODS

In our Department, a prospective, observational, analytical study was conducted, including six outdoor workers (in construction and agriculture), over a period of three summer months (July, August, September).

Subjects were selected based on their profession. Our study took place in Bordusani, Ialomita, Romania, with the geographical coordinates 44°28’21”N 27°53’59”E for the agriculture outdoor workers and in Bucharest, Romania, with the geographical coordinates 44°25’57”N 26°06’1 for the construction workers.

Subjects were asked to record in personal diaries information regarding: profession, location, date, start time and end time of their schedule, lunch breaks and weather conditions.

The following parameters were analyzed: profession, duration of exposure, UV exposure doses, skin aspect, preexisting preventive measures.

For the agriculture workers, we’ve selected irrigation system manufacturer, tractor driver and beans manufactures and for the construction site we’ve selected road workers.

Subjects were asked to wear personal dosimeters for several working days. Sun exposed areas involved the face, neck, forearms and dorsum of hands, the rest of the areas were covered by equipment.

Clinical examination was performed before and after measurements to detect any skin changes.

The personal dosimeter used in this study was X-2012-10 Personal UV Irradiance Dosimeter from Gigahertz-Optik and a “Velcro” fastening system was used to attach it on the subject’s forearm.

Data was processed using Microsoft Office Excel 2013.

RESULTS

The wearing of dosimeters was well tolerated by all subjects, without any interference with daily professional activities.

Daily UV radiation doses ranged from 1.79 SED (tractor driver) to 19 SED (agriculture worker).

Maximum UVR doses were recorded between 10:00 am and 4 pm. Apart from standard equipment, no protective measures were applied.

Clinical examination was made after measurements, with no detectable skin changes.

DISCUSSION

Sunlight is composed of a continuous spectrum of electromagnetic radiation divided into three major groups: ultraviolet, visible and infrared. [8] Ultraviolet radiation is a major causative factor in skin cancer development. [9]

Even though UVA has less energy than UVB and penetrates deeper, into the dermis, UVB radiation is most implicated in carcinogenesis. UVA is responsible for carcinogenesis through immunosuppression: it determines production of reactive oxygen species that cause damage in the deoxyribonucleic acid, therefore gene mutations and damage in the epidermal keratinocytes and dermal connective tissue.

Examples of gene mutations include PTCH and p53 tumor suppression gene in basal cell carcinomas, activated mitogenic ras pathway and p53 in squamous cell carcinomas and ras pathway, inactive p16 and p53 in melanomas. [10]

Also, the duration and intensity of UV exposure are factors implicated in skin cancer development. For example, cumulative, long-term UV exposure is related to chronic actinic skin damage, therefore implicated in the appearance of actinic keratoses and squamous cell carcinomas, since intermittent, high intensity UV exposure especially at young ages would most likely cause basal cell carcinomas and melanomas. [11]

For measuring UV exposure doses on an outdoor worker, irradiance should be determined - the flux of radiant energy per unit (W/m2). But this applies only for one moment in time, so for a larger period (e.g. one working day) the term "exposure dose" (J/m2) is used. [12] For this, a standardized unit – SED – Standard Erythema Dose is applied, where 1 SED is equivalent with 100J/m2. [13]

The annual exposure dose for the general population in Germany is 130 SED. [14] Considering our results, with a daily UV exposure dose of 19 SED (15% daily of the annual exposure dose), we have to acknowledge the fact that both agriculture and construction professions in Romania are professions with high-risk of developing solar exposure related pathologies.

Even more so, by German standards, if the outdoor worker was exposed to a cumulative UVR dose with 40% higher than the general population, than the nonmelanoma skin cancer is considered to be of "occupational origin". [14]

Therefore these professions should be placed in the occupational skin diseases category.

It is very important to take into consideration that different parts of the body absorb different UV doses depending on body geometry, therefore involve different risks. Multiple personal dosimeters should be used, calculating UV doses on different body sites. [16]

Horizontal body locations seem to receive 2x - 2,5x more UV that the vertical ones. [17]

Also, UV exposure dose depends on the reflection of UV radiation on surrounding structures. For example, UV reflection for construction workers surrounded by metallic structures is different to that of fishermen that are surrounded by water. This is a significant additional benefit of personal dosimeters, being able to record UV doses from different parts of the body and not taking into consideration only the direct, vertical radiation.

For example, even if an outdoor worker has correct equipment, with a broad-brimmed hat, UV reflection from surrounding structures could still reach him. Therefore additional protective measures should be implemented, such as protective sun creams. Also, personal dosimeters, being able to measure UV radiation received by the subject, these measurements could be compared with those stated by meteorological departments. [18]

Even more so, occupational exposure to UV radiation can determine specific features of the tumors involved. For example, basal cell carcinoma appears more frequently in the "mask area" of the face in occupational exposed workers and also histopathologically appears to be more aggressive in evolution. [19]

All of this important features reinforce the necessity of implementing strict preventive and protective measures for outdoor workers, including protective sun creams (SPF 30 or more), special sun protective

equipment (long pants and long sleeves, broad-brimmed hats or helmets, sunglasses), multiple breaks in indoor areas and rotating shifts. [15] Also, considering the fact that the maximum UVR dose was detected between 10 am and 4 pm, outdoor workers should avoid working outside in this timeframe.

Even if by the end of our study no pathological skin changes were expected, we have to keep in mind that most UV skin related pathologies depend on a cumulative UVR dose with long term exposure. More interventional and observational studies are necessary for a better understanding of UVR effects on outdoor workers in Romania.

CONCLUSION

Even though workers in agriculture and construction in Romania are exposed to significant UV doses, with 40% more than the general population, these professions are not recognized as high-risk professions for developing nonmelanoma skin cancer or melanomas. More prospective and retrospective studies are necessary for more significant statistical results and for demonstrating the necessity of introducing primary, secondary and tertiary preventive measures.

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CLINICAL PRACTICE

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A case of acne fulminans

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Abstract: *Background and aim: Acne fulminans is a rare and severe form of acne which usually appears in young men with a personal history of mild to moderate acne. It is characterized by a sudden onset with nodules and ulcerative lesions mostly located on the thorax and extracutaneous manifestations (fever, weight loss, osteoarticular damage). The pathophysiologic mechanism is represented by a type III or IV hypersensitivity reaction to Propionibacterium acnes.*

Patient: We present the case of a 16-year old patient with acne fulminans. The lesions were accompanied by an impaired general condition with fever and weight loss. Paraclinically an inflammatory syndrome was revealed without osteoarticular changes. It was treated with prednisone 40 mg/day, erythromycin 2g/day and local antiseptic solutions. A gradual tapering of the prednisone dose (5 mg/week) and a dermatological revaluation after 3 weeks to introduce systemic treatment with retinoids were recommended at discharge.

Conclusions: Acne fulminans is a severe form of acne with systemic manifestations and corticotherapy is the first line treatment.

Keywords: *acne fulminans, corticotherapy*.

INTRODUCTION

Acne fulminans is a rare and severe form of acne which usually appears in young men with a personal history of mild to moderate acne. It is characterized by a sudden onset with nodules and ulcerative lesions mostly located on the thorax and extracutaneous manifestations (impaired general condition, fever, bone pain, anorexia, splenohepatomegaly) [1-3]. Osteoarticular impairment is common, it being reported in about half of the cases [4,5]. The pathophysiologic mechanism is represented by a type III or IV hypersensitivity reaction to Propionibacterium acnes [6]. The involvement of drugs such as anabolic steroids and retinoids are also quoted [7,8]. The treatment of choice are corticosteroids with initial dosing of 1 mg/kg/day followed by a gradual tapering and retinoids introduction from the 4th week [9].

MATERIALS AND METHODS. CASE PRESENTATION

We present the case of a 16 years old patient who required a dermatological consult for a cutaneous eruption located on the face and the anterior and posterior thorax.

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The skin examination showed comedones, papules, pustules and ulcerative nodules with necrotic crust. The patient reported a personal history of untreated acne vulgaris.

He described the onset of the rash 14 days prior to admission. Lesions were accompanied by anorexia, mild fever (unchecked at home). He has lost 4 kg from the appearance of the eruption.

Laboratory results have revealed an increased ESR (64 mm/h), leukocytosis (16,340/mm^3).

The bacteriological examination of the lesions was negative. The radiography of the large joints (elbows, knees, lumbosacral) has revealed no pathological changes.

The patient was treated with prednisone (40 mg/day), antibiotics (cefuroxime, erythromycin), antiseptics and benzoyl peroxide with clindamycin.

The evolution was favourable after 7 days of treatment with a decrease in the skin lesions and the inflammatory markers.

Figure 1

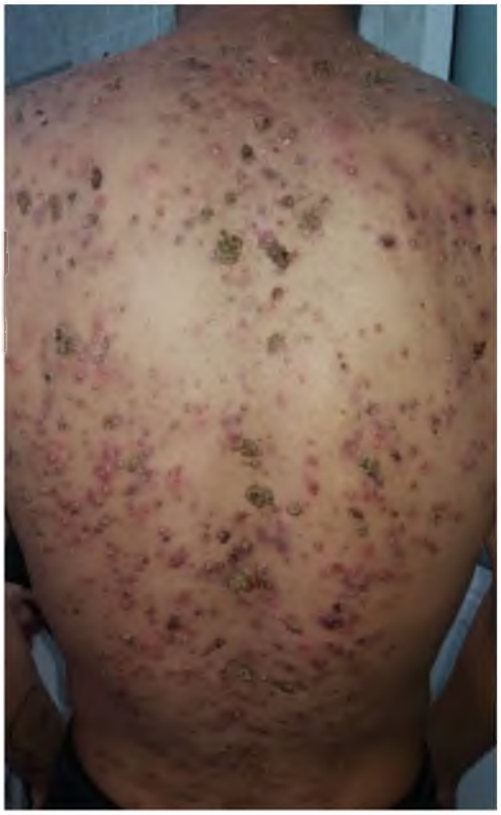

Figure 2

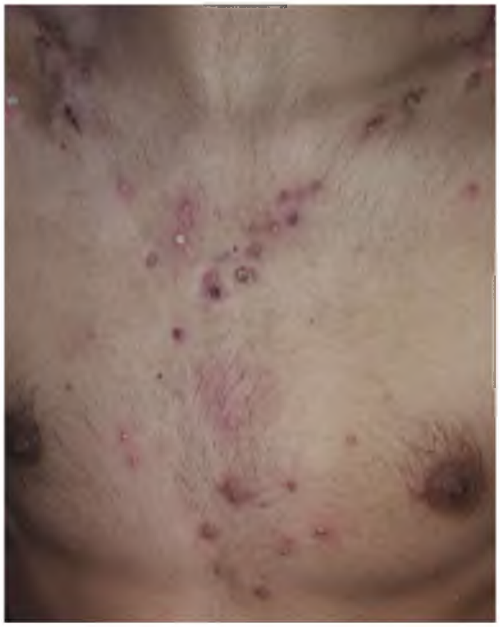

Figure 3

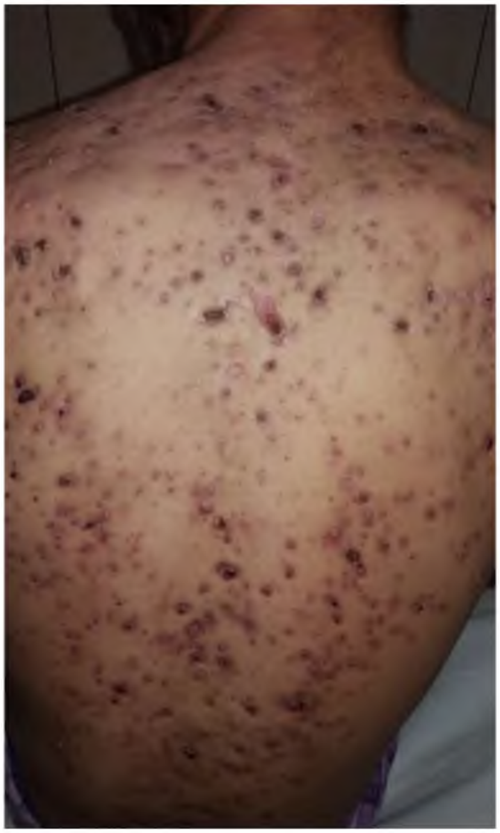

A gradual tapering of the prednisone dose (5 mg/week) and a dermatological revaluation after 3 weeks to introduce systemic treatment with retinoids were recommended at discharge.

DISCUSSION

In the literature acne fulminans is presented either as a stand-alone entity or as a manifestation within SAPHO syndrome (Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis). Also cited are the association of acne fulminans with inflammatory bowel disease, ankylosing spondylitis, the appearance of it after an intake of anabolic steroids and paradoxically after isotretinoin administration.

Rajaee et al presented the case of a 16 years old boy with acne fulminans with positive antinuclear antibodies and positive HLA-B27 antigen. The radiological examinations of the sacroiliac joints and chest revealed no changes and that of the right knee revealed effusion and soft tissue swelling [10].

Also interesting is the association of acne fulminans with Crohn's disease and ulcerative colitis. Pecova et al. describe the case of a 30 years old patient with known Crohn's disease and papulopustular acne from the age of 17. He had the first episode of acne fulminans at the age of 21 after discontinuation of methylprednisolone therapy utilized for primary disease. Five years later the same patient developed a new flare of acne fulminans due to the introduction of biological therapy (infliximab) [11]. Another case is that of a 19 years old man treated for ulcerative colitis for 2 years, who developed acne fulminans and was successfully treated with prednisone and diaminodiphenyl sulfone orally [12]. Regarding the association of acne fulminans with the administration of testosterone, an interesting case is that of the twins suffering from Kallman syndrome who from the age of 17 received testosterone substitution treatment for delayed puberty. Nine months after the substitution therapy started the patients developed acne fulminans [13].

A case of acne fulminans was reported in a young bodybuilder who used anabolic steroids (testosterone enanthate, trenbolone acetate, drostanolone propionate and methandrostenolone) for muscle growth. Shortly after the cessation of the anabolic steroids use he developed acne fulminans with debilitating arthralgias [14]. Mayerhausen and Riebel presented in a 1,989 article the case of a 23 years old man, javelin thrower, who developed acne fulminans after taking anabolic steroids and vitamin B preparations. [15]

SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis, osteitis) is a rare inflammatory disorder that associates osteoarticular lesions with pustular disorders such as pustular psoriasis, palmoplantar pustulosis, acne fulminans and hidradenitis suppurativa. The axial skeleton, the sternoclavicular and sternocostal joints are more commonly affected than the peripheral joints [16]. Lakshmi Divya et al presented the case of an 18 years old man with a 9 months old painful nodulocystic acne. The patient had also severe pain in the wrists and lower back, chills and stiffness for 30 days. An MRI scan of the right hand joint revealed osteolytic lesions. A scintigraphy with technetium 99m indicated increased capture in the bilateral distal radial metaphyses, sacroiliac joints, vertebral plateaus and the 7th costovertebral joint. The patient was treated orally with cefpodoxime, doxycycline and colchicine 1 mg/day. 4 weeks later systemic therapy with isotretinoin 20 mg/day was started with remission of the lesions at 12 weeks [17].

Acne fulminans can be paradoxically induced by isotretinoin. Some authors consider that its appearance is dose dependent. Pereira et al. presented a case of acne fulminans induced by low-dose isotretinoin (20 mg/day). The patient was treated with oxacillin, prednisone and oral isotretinoin in the same dose with a favorable evolution [18]. MacKenzie describes another case of isotretinoin induced (20 mg/day) acne fulminans one month after the initiation of therapy. Following treatment with prednisone (60 mg/day) and erythromycin (2 g/day) the lesions diminished after a month. Isotretinoin treatment was then reintroduced with gradually increased dosages from 10 mg/day to 70 mg/day with full remission of the lesions after 11 months [19]. Zanelato et al presented the case of a 14 years old boy that was initiated on isotretinoin 0.5 mg/kg/day and prednisone 0.1 mg/kg/day. 12 days later ulcero-necrotic lesions started developing on the face and trunk. The patient had also musculoskeletal involvement (bilateral sacroileitis, scapulohumeral, chondrocostal, jaw, ankles and lumbar intervertebral joints). Isotretinoin was stopped and the patient was started on prednisone with a favorable evolution. 30 days from the interruption, isotretinoin was reintroduced in doses of 0.2 mg/kg/day with subsequent dose increase after 2 months to 0.5 mg/kg/day while corticosteroid therapy was tapered. [20].

CONCLUSION

Acne fulminans represents a severe form of acne with systemic manifestations. The mechanism of the disorder is not yet elucidated and corticotherapy is the first line treatment.

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Col. Richard Belcescu MD, turns 90 – A portrait

Adrian M. Nistor

I am a human being to whom very few opportunities have been offered in an entire lifetime or maybe I was not able to turn them to account when they popped up. But meeting retired Colonel Richard Belcescu MD, was for me what may be called a "chance," a "stroke of luck."

Immediately after graduating from the dentistry school of Bucharest, I was assigned to work in the surgery where Dr. Richard Belcescu worked busily, on the seventh floor of the Carol Davila Central Military Hospital. A soft-eyed middle-aged man came to meet me, dressed up to the nines, who, making no fuss over the whole thing, helped me get acquainted with the activity of the section. I was to discover subsequently that my then boss was one of the most outstanding human beings I was destined to meet. He tried to polish the numerous and serious irregularities of the personality of a young lieutenant doctor, who had just finished school. As a matter of fact he retained his capacity as a polishing creator of medical but also human personalities all along his stay at the OMFS section, exerting it on many young doctors.

Colonel Richard Belcescu, MD, was not very well known in public life: he gave no resounding interviews to the propaganda newspapers of the 70s and the 80s, and did not appear on TV. However, thanks to his qualities, he asserted himself as an opinion leader all the hospital staff held in high esteem.

Being side by side with him made you feel calm and protected at that time of turmoil. Endowed with much earnestness in all the aspects of his professional, but also personal, life, Dr. Richard Belcescu was certainly the one colleague that "should be found in any clinic." He used to solve everything inherent in a surgical team: he used to commit himself to solving some cases that required a special relation between the doctor and the patient besides surgery as such.

We kept in touch even if life and the difference of age made us stay apart from each other. He used to pay regular visits to me at various stages of my professional career, but he always tried to be as discreet and warm-hearted as possible, with no trace of conceit whatsoever. He was familiar with everything going on in our section but also in the whole hospital, getting emotionally involved in an extremely sincere manner.

I learnt by mere chance that he is in an old people's home where his family have taken him very affectionately because of the marked difficulties due to old age. His being there certainly makes him benefit from remarkably good palliative care, which nobody else can better give.

Extremely impressed, I came near to the bed where Dr. Richard Belcescu lay: "Hi, Adrian! How are you? We haven't seen for ages!" Frankly speaking, I was almost at a loss in that clean tidy room where one of my mentors was bedridden because of the lapse of time. We had a short genial conversation during which I found it difficult to suppress my emotion. All the unforgettable recollections of the events that kept us close together for many years came to my mind.

I left the sunlit room in which Dr. Richard Belcescu lives now and promised to come and visit him from time to time. Being 90 – is it a long or a short time of one's life? On the way to my surgery, where he came to visit me and where I had the honor of treating him, I remember part of the moments spent by the side of this wonderful man. This is why I seem to hear again the words previously spoken: "In every clinic there must be a Dr. Belcescu" as a benchmark for medical rigors but also for the consummate behavior of a colleague.

Founded 1897 • New Series
Vol. CXX • No. 2/2017 • August

Romanian Journal of Military Medicine

REVISTA DE MEDICINĂ MILITARĂ

Celebrating 120 years of MEDICAL EXCELLENCE

- *The story of a journal – The 120th anniversary of the Romanian Journal of Military Medicine*
- *The concept of operationalization of an integrated platform for scientific research and expertise of war and bioterrorism biological agents*
- *Intellectual mobility in medical higher education system*
- *The influence of homocysteine on osteoporosis*
- *Efficacy and tolerability of calcium channel alpha-2-delta ligands in psychiatric disorders*
- *Medicine versus philosophy*
- *Incidence of peripheral trophic disorders determined by vein thrombosis of the lower limbs correlated with risk factors by age*
- *New synthesized oximes active in nerve agents' hazards*
- *Ethical considerations in sudden unexpected death in epilepsy (SUDEP)*
- *Pericardium – An editorial success*

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RJMM
Romanian Journal of Military Medicine

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Edited by the Romanian Association of Military Physicians and Pharmacists.

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EDITORIAL

The story of a journal – The 120th anniversary of the Romanian Journal of Military Medicine

Dan Mischianu

Gral (R) Prof DAN MISCHIANU

Chief of Urology Clinic, Carol Davila University Central Emergency Military Hospital Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

The Military Medicine Magazine, later became the Romanian Journal of Military Medicine (RJMM)… well, I assure you that it did not come out of thin air!

It appeared on September 15, 1897, 120 years ago, on the initiative of military medical professionals.

I think few of us know that on January 12, 1897, Army Corps General Professor Dr. Athanase Demosthen was elected correspondent member of the Medical Academy of Paris.

Those who celebrated him for success, mostly his students and contemporaries, doctors, pharmacists and veterinarians (military veterinarians, the cavalry did not disappear – as a fighting weapon!) decided, according to the French example, to create a magazine with independent statute and organization, and objectives that do not go beyond our present understanding. Namely: "maintenance of scientific activity and emulation among the members of the sanitary body, establishment of the collegiality links between the sanitary officers, as well as the preservation of the scientific and moral prestige that they should enjoy in the army and in society, the culture of all scientific and technical knowledge among the members of the military health body related to the medical, pharmaceutical and veterinary profession" (*Revista Sanitară Militară, 1972, nr. 4-5, pg. 411*).

The "Steering Committee" met three months later, on April 12, 1897, and established that under the leadership of General Prof. Dr. Athanase Demosthen – as chairman, a new magazine called "Military Sanitary Review" will be born with the previously mentioned status. The companions of General Demosthen for this enterprise were the generals Dr. I. Şerbănescu, N. Zorileanu, the army pharmacist M. Marinescu, first class regiment physician I.M. Călinescu, second class regiment physician Iacob Potârcă (a memorable name in Romanian surgery – versus Whitehead procedure!), pharmacists C. Dumitrescu-Parepa and Constantin Merișanu (whose bust guards a hospital alley).

The magazine appeared on 15 September 1897 and the photocopy presented reproduces the first cover of the Military Health Magazine (*Revista Sanitară Militară, 1972, nr. 4-5, pg. 409*).

The "Military Health Magazine" wanted to be, from the very beginning, "the depository of the work and activity of the health officers in the realm of veterinary and human medicine and military pharmacy" (*Oameni și Fapte din Istoria Medicinii Militare Românești, Gral brig (r) dr. Mircea Diaconescu, vol II, pg. 229*).

The Military Health Magazine records a great performance in our medical literature: it appeared in the same year as the Surgery Magazine – 1897, went through the same "trials", did not have the same audience but resisted... An absolutely remarkable fact!...

ANUL I SEPTEMBRE 1897 No. 1

REVISTA
SANITARĂ MILITARĂ
APARE O DATĂ PE LUNĂ

SUMARUL

DIRECȚIUNEA ȘI REDACȚIUNEA LA SPITALUL MILITAR CENTRAL
BUCUREȘTI
ADMINISTRAȚIUNEA LA CASIERUL CASEI DE DOTAȚIE A OASTEI

ADRESA

D-lui

BUCURESCI
TIPOGRAFIA «DREPTATEA», STRADA CÂMPINEANU 15. – TELEFON No. 15
1897

Before continuing the "story of the Magazine", I think it is worth telling you "my story – vs the Magazine".

I may be subjective, and actually I am.

The system, the organization of that time, had assigned me after graduating from the Faculty as a "trainee intern" since December 1979. I was assigned, together with my colleague, to the 2nd Medical Department, the current Clinic of Internal Medicine and Gastroenterology of the Central Military Hospital. We have been extraordinarily well received and grateful to those people.

I knew about the Magazine, I had read it "en passant", then there were no "student" magazines, I did not even dream to publish an article in a magazine with such background.

Well, those very formidable men – the "workers" from the editorial office of any long-term magazine, that is, Col. Dr. Cristea Neculescu and Mr. Nicolae Dragoi – editorial secretary – considered an absolute "insignificant" article in time – but perhaps pompously titled: "Diagnostic significance of the atrial fibrillation wave amplitude" by D. Mischianu, V. Andrei, Military Health Magazine, 1980, 1, pg 71-75, may receive the "good fortune"! It was an article that sumed up what we, presented to the Students Session at the Bucharest Medical and Pharmaceutical Institute in the April 1979 session.

I leave aside my memories and come back to the historical reality, of which nobody understands at all.

The magazine has gone through chaotic and sad moments.

It had "ups and downs," a sort of "crises" as they are told today. Most of the causes were of financial origin, which led to changes in the typographic format and the continuity of occurrence.

The first syncope is in 1903, so that between 1903-1905 the magazine no longer appears.

In April 1905, everything goes back to normal the first exchanges of journals began with other armies: Italian, English, French, German and everything went flawless until 1908. The number 2 of 1906 was a jubilee number; ten years had passed from the gorgeous initiative of 1897, the number being dedicated to "the great and mighty King Carol I".

Unfortunately after the numbers 3 and 4 of 1908, the second syncope is recorded. The Military Health Magazine ceases to appear until 1913 (*Oameni și Fapte din Istoria Medicinii Militare Românești, Gral brig (r) dr. Mircea Diaconescu, vol II, pg. 230*).

The magazine is coming back shortly. On June 28, 1914, Archduke Franz Ferdinand and his wife were assassinated in Sarajevo, the three kings – blood cousins: Kaiser Wilhelm II of Germany, Tsar Nicholas II of Russia, and King George V of The United Kingdom of Great Britain, all three good English speakers were not able to come to terms (they were grandchildren of the Queen Victoria of Great Britain), the Great War (or the First World War as it is known today) begins, our magazine has its third syncope: 1915-1919 .

With all these vicissitudes, in 1917 and 1918, in Iasi and Bacau appear "the Comptes bulletins from the Société medico-surgical du russo-roumain, namely Comptes vendus des seinces from the medical reunion of II-ème armee, presenting medico-military communications with participants from Russian and French allied armies " (*Revista Sanitară Militară, 1972, nr. 4-5, pg. 412*).

There is also a fourth syncope, after the first numbers appeared in 1919. In fact, the magazine really revived in 1928, when things were settled, the country seemed to have emerged from the crisis. It is Miron Costin's word: "There are not the times under the man, but the poor man under the times!"

In the review, there are reports of the "continuous medical education" sessions – that is the sessions of the Romanian Military Doctors' Association with the subsequent subsidiaries from all the existing Military Hospitals, homage numbers regarding the personalities of the Romanian medicine who came from or not from the same "medical strain": General dr. N. Zorileanu (dermatovenerologist), professor dr. V. Babeş (bacteriologist), professor dr. Dimitrie Gerota (surgeon, radiologist), professor dr. Al. Costiniu (ENT), etc. Articles in French and German also appear (*Revista Sanitară Militară, 1972, nr. 4-5, pg. 413*).

The fifth syncope, hopefully the last, is also the longest: 1949-1957. The times were like they were, everything was changing, and who needed the written word of the Romanian military doctors?

We publish the cover of a Magazine published in 1938 in which, in the end, lieutenant physician Dr. Eugen Mareş publishes an obituary of a generation colleague (*Oameni și Fapte din Istoria Medicinii Militare Românești, Gral brig (r) dr. Mircea Diaconescu, vol II*).

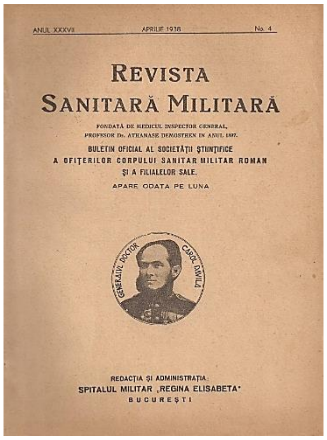

ANUL XXXVII APRILIE 1938 No. 4

REVISTA
SANITARĂ MILITARĂ

FONDATĂ DE MEDICUL INSPECTOR GENERAL,
PROFESOR Dr. ATHANASE DEMOSTHEN IN ANUL 1897.
BULETIN OFICIAL AL SOCIETĂȚII ȘTIINȚIFICE
A OFIȚERILOR CORPULUI SANITAR MILITAR ROMAN
ȘI A FILIALELOR SALE.
APARE ODATA PE LUNA

REDACȚIA ȘI ADMINISTRAȚIA
SPITALUL MILITAR „REGINA ELISABETA"
BUCUREȘTI

The same man, the same true Romanian military doctor who I personally met, when he was at the peak of his profession – Deputy Minister of Health in socialist Romania and at the end of his life, with a typical Oltenian perseverance, "revives" the Military Health Magazine in 1957.

In 1972, when the magazine was celebrated for "three quarters of a century of existence in the service of the protection of the health of our soldiers", the technical box of Magazine Sanitary Military Magazine no. 4-5/ 1972, which we reproduce in the facsimile, mentions his name, along with the name of another military doctor dear to my soul – General Professor Iuliu Șuteu – editor-in-chief of the magazine at that time.

REVISTA
SANITARĂ
MILITARĂ
1897 1972

4-5
1972

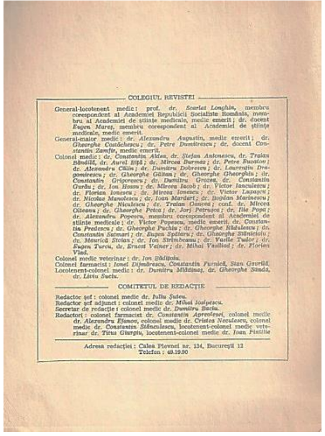

COLEGIUL REVISTEI

General-locotenent medic: prof. dr. *Scarlat Longhin*, membru corespondent al Academiei Republicii Socialiste România, membru al Academiei de științe medicale, medic emerit; dr. docent *Eugen Mareș*, membru corespondent al Academiei de științe medicale, medic emerit.
General-maior medic: dr. *Alexandru Augustin*, medic emerit; dr. *Gheorghe Costăchescu*; dr. *Petre Dumitrescu*; dr. docent *Constantin Zamfir*, medic emerit.
Colonel medic: dr. *Constantin Aldea*, dr. *Ștefan Antonescu*, dr. *Traian Băndilă*, dr. *Aurel Bîță*; dr. *Mircea Burnaz*; dr. *Petre Buzatou*; dr. *Alexandru Călin*; dr. *Dumitru Dobrescu*; dr. *Laurențiu Dragomirescu*; dr. *Gheorghe Găitan*; dr. *Gheorghe Gheorghiu*; dr. *Constantin Grigorescu*; dr. *Dumitru Grozea*, dr. *Constantin Gurău*; dr. *Ion Hossu*; dr. *Mircea Iacob*; dr. *Victor Ianculescu*; dr. *Florian Ionescu*; dr. *Mircea Ionescu*; dr. *Victor Lapașcu*; dr. *Nicolae Manolescu*; dr. *Ioan Mardari*; dr. *Bogdan Marinescu*; dr. *Gheorghe Niculescu*; dr. *Traian Oancea*; conf. dr. *Mircea Olteanu*; dr. *Gheorghe Petca*; dr. *Jorj Petrușca*; dr. *Ilie Popa*; dr. *Alexandru Popescu*, membru corespondent al Academiei de științe medicale; dr. *Victor Popescu*, medic emerit, dr. *Constantin Predescu*; dr. *Gheorghe Puchia*; dr. *Gheorghe Rădulescu*; dr. *Constantin Satmari*; dr. *Eugen Spătaru*; dr. *Gheorghe Stănicioiu*; dr. *Mauriciu Stoian*; dr. *Ion Strîmbeanu*; dr. *Vasile Tudor*; dr. *Eugen Turcu*, dr. *Ernest Vainer*; dr. *Mihai Vasiliad*; dr. *Florian Vlad*.
Colonel medic veterinar: dr. *Ion Bădițoiu*.
Colonel farmacist: *Ionel Dijmărescu*, *Constantin Furnică*, *Stan Gavrilă*.
Locotenent-colonel medic: dr. *Dumitru Mădăraș*, dr. *Gheorghe Sandă*, dr. *Liviu Suciu*.

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Adresa redacției: Calea Plevnei nr. 134, București 12
Telefon: 49.19.90

I also want to remember another name dear to me – General Lt. Academician Gheorghe Niculescu, for many years, editor-in-chief and "living spirit" that agglutinated the energies and "pencils", stimulating and promoting those who really had something to say in this field.

After 1990, fearful, as observed to another journal "Surgery", changed its name. It became the Journal of Military Medicine.

REVISTA SANITARA MILITARA
75 ANI DE LA APARITIE
ROMANIA
1897
1972

Probably the term "sanitary" was appropriate in the beginning, the term "medicine" is common to all military doctors because we all come from the same strain – the School of Medicine created by Carol Davila, who arrived on Romanian soil as a French civilian and became a Romanian general and physician!

Revista de
Medicină
Militară
ANUL CIX
Nr. 1/2006

He has escaped, in this way, of a "complex".

In time there have been other Romanian medico-military publications.

However, the Romanian Journal of Military Medicine (RJMM) remains unique and emblematic after five major "syncopes", having in its portfolio "a valuable scientific and informational forum not only for military doctors but for all Romanian medicine" (*Oameni și Fapte din Istoria Medicinii Militare Românești, Gral brig (r) dr. Mircea Diaconescu, vol II, pg. 232*).

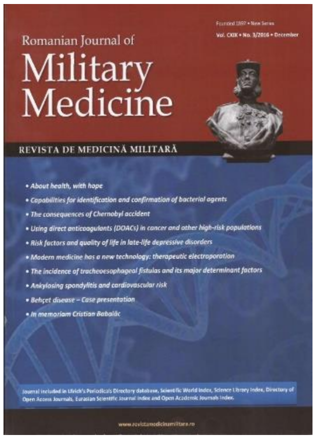
Romanian Journal of
Military
Medicine
REVISTA DE MEDICINĂ MILITARĂ

The magazine had the power, and those who were close to it knew how to do it, to reborn always like the Phoenix bird.

120 years after the first appearance, the Romanian Journal of Military Medicine remains a magazine that has its own program, unaltered by the passing of the ages, even when the inter-war or post-war political factor implied perhaps another orientation.

The Romanian Journal of Military Medicine wishes to bring up-to-date information to the servants of this noble profession, and hopes that the puzzling of the information on the internet, which can make anyone out of the question, is presented on-line or printed (I confess that I deeply dislike these English barbarisms!) after they have been read and corrected (not censored!) by a true scholar in the field.

I am absolutely convinced that all Romanian, military or civilian medical officers, descendants of our common ancestor – General Professor Dr. Carol Davila – will adhere to this profession of faith and will continue as long as it is needed!

Happy Birthday Journal of Military Medicine!

Happy Birthday dear readers!

REVIEW ARTICLE

Article received on February 11, 2017 and accepted for publishing on June 12, 2017.

The concept of operationalization of an integrated platform for scientific research and expertise of war and bioterrorism biological agents

Viorel Ordeanu[1,2], Marius Necşulescu[1], Diana M. Popescu[1], Lucia E. Ionescu[1], Simona N. Bicheru[1], Gabriela V. Dumitrescu[1], George Corlan[1]

Abstract: *The international situation requires a strengthening of the national security measures, including in the field of CBRN and public health. The upgraded microbiology laboratories from DM/MND must be operated at full capacity for the operationalization of an integrated Platform for the research and expertise of biological war and bioterrorism agents. This is necessary for reasons of national security, for CBRN defense and for public health, in the context of biological agents of 3 and 4 risk groups' epidemics.*

The existing upgraded objectives should be operationalized in order to meet the established scope: scientific research and expertise of biological agents and biological weapons, a laboratory for in vitro analysis and a bio-base for in vivo analysis. The highly secure lab allows working with any high-risk agents: biological, genetic, chemical, radiological, etc., being provided with a special room for the insertion/removal of equipment and their decontamination.

Following an increase in the capability requirements concerning laboratories, we have provided in the design concept new technical parameters of the platform and the integration of new compartments with related activities of toxicology, pathology, neuro-psycho-pharmacology, bio-pharmacy, micro-pharmaceutical, specific testing activities, etc.

Creating the integrated platform and its operationalization is necessary in order to meet the requirement of the national security strategy as a collective CBRN defense/protection facility and military-medical scientific research for CBRN medical protection.

Keywords: *biological agents, bioterrorism, medical protection, microbiology laboratory, scientific research, integrated platform, CBRN*

INTRODUCTION

The international situation requires a strengthening of the national security measures, including in the field of CBRN defense (chemical, biological, radiological and nuclear) and public health. The upgraded microbiology laboratories from the Medical Department of the Ministry of National Defense (MD/MND) must be operated at full capacity, for the operationalization of an integrated Platform for the scientific research and expertise of biological war and bioterrorism agents. The project is necessary for national security purposes, for CBRN defense and for the public

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health, in the context of biological agents of 3 and 4 risk groups epidemics, and it responds to current threats. In risk group 3, are listed extremely dangerous bacteria and other pathogens such as warfare biological agents (WBA). In the maximmum risk group 4, are included 8 species of extremely dangerous viruses such as Ebola or WBA viruses.[1,2,3]

Current regulations take into account the provisions of biosafety (such as internal protection for operators), of biosecurity (as protection against facility external hazards) and of bioprotection (protection of the sample to be analyzed). As a result, laboratories are properly classified, depending on the different level of protection. In French literature they are referred to as P1-4 (Protection).

The key features are: P1 with open work areas for education; P2 with biosafety hoods, for medical purposes; P3 special biosafety equipment for the extremely dangerous bacteria; P4 for the extremely dangerous viruses, with the maximum level of biosafety. In English, they are called Biosafety Laboratory (BSL1-4).[4] In Romanian, they appear as Basic Labs with a level 1 and 2 of biosafety, Highly Secured Laboratory with a level 3 of biosafety, and a highly secure laboratory with a level 4a of biosafety (with collective protection equipment) and 4b (with special equipment for individual protection). In the absence of proper facilities, the biological agents classified in the risk categories cannot be legally worked with. [2]

STATE-OF-THE-ART

Nationally, the Ministry of Health has modernized a microbiology lab at the National Research Institute “Cantacuzino” as a P3 laboratory and one (under construction) at the Bucharest Hospital for Infectious Diseases "Babeș". The Ministry of Agriculture has two P3 microbiology laboratories at the Bucharest Institute for Animal Diagnosis and Health. Currently, in Romania, a P4 highly secure microbiology laboratory does not exist yet.

Due to the fact that in Romania scientific research for medical protection against CBRN agents belongs to MND, it is mandatory to have a proper facility. According to Biosafety in medical laboratories Guidelines (World Health Organization 2004 and Ministry of Health 2006) the bacterial biological agents are expertised in the P3 laboratory, and the viral agents in a P4 laboratory.

The international context shows that eventhough the risk of biological warfare has decreased as a result of the Geneva Convention (BTWC 1972), that was signed and ratified by more than 90% of the world's countries, the risk of bioterrorism is as real as the risk of pandemic, of zooantroponosys, of exotic and tropical infectious and contagious diseases, etc., as it is with the recent epidemic of Ebola.[5] As a result, the WHO, the EU and NATO recommend concrete measures for decreasing those risks and for increasing the response capacity of each country, of the alliance and of the international community.

Given the EU's recommendations to operationalize at least 40 P4 laboratories within the member countries, to be designed and operated under standardized WHO conditions, the project responds to international demands and also to the national interest.

The operationalization of an integrated research and expertise platform for biological warfare and bioterrorism agents is relevant to the approached scientific field, following the **state of the art** (current stage) character of the research in the field of protection against biological agents, by implementing internationally used modern techniques. The project is based on implementing **cutting edge** technologies for diagnostic, prophylactic pre-exposure and post-exposure, cure and recovery, aspects regarding the action mechanism in infectious diseases, the therapeutic means to counteract the effects of biological agents and the epidemiological surveillance.[6]

Internationally, there are concerns regarding the achievement of a coherent system of epidemiological warning and intervention, the project being in line with the world situation. Designing and equipping the platform according to international recommendations determines that the level of performance of the proposed infrastructure must be internationally competitive and must take an active role in the global effort of health protection against biological agents.[7]

Our concept for the operationalization of the Integrated Platform of scientific research and expertise of war and bioterrorism biological agents.

Creating the Integrated Platform will allow the defensive scientific research and medical expertise for weapons/biological agents and for the diagnosis of people infected with particularly dangerous biological agents, and for the medical intervention in epidemics or biological attacks.[8,9,10] It involves, in the first stage, monitoring the continuation and completion of the upgrading and rehabilitation of buildings, installations and perimeter security measures, including strengthening the capacity of health protection against biological weapons, bioterrorism and particularly dangerous biological agents.

It is important to liaise with specialized companies (selected not only by public tenders) within the allocated funds, for the execution of the Integrated Platform, for doing the reception at the time of commissioning the modernized Laboratory of Microbiology (declared at the UN as being under construction) for in vitro analysis, and for doing the reception at the time of commissioning the modernized Biobase, for in vivo analysis as well as for complementary analysis of analytical and experimental toxicology, neuro-psycho-pharmacology, bio-pharmacy and the related logistical and security support.[8]

Taking into account the unique nature of this facility on national and regional level, as well as the direct and indirect costs related to the spatial planning, maintenance, and exploitation of this advanced technology objective, we rethought the configuration and the operation of the component objectives for maximum efficiency.[8] Thus, the facility can provide, through its equipment and operation, not only the scientific research and expertise for ABR, bioterrorism and biocrime, but it can also become practically involved in the epidemics/ pandemics or zoonoses with exotic and tropical diseases, that can occur as major emergencies, as is the recent Ebola outbreak. This facility with a maximum level of biosafety can also serve for other specific activities with major risk level; for example, with chemical warfare agents (CWA) or accidents with toxic industrial chemicals (TIC), with radiological warfare agents (RWA) or accidents with radioactive substances, or for any other extremely dangerous substances (HazMat). Changing destination can be achieved operationally, whenever necessary, even if it is one day to another, by replacing the work team with specialists from the DM/MND structures, according to the new destination and by replacing the specific equipment: removing from the highly secured working chamber of the equipment and materials which are no longer needed (through the decontamination airlock outlet) and inserting those that are necessary to the new mission. However, inside the work chamber, materials deposits are not stored for in vitro analysis. These are found in the “hand deposit” of the laboratory, inside the units’ deposit or are purchased by emergency from different sources.[8,9,10] At the same time, the Biobase for test animals can serve all military medical laboratories due to the fact that it is conceived to immediately adapt to new requirements for different animal species. In terms of the annex facilities and amenities, they are basically for general purposes, for every type of medical laboratory and they inclusively ensure the proper functioning of the entire facility in which the integrated Platform is found. The platform can be adapted without structural changes to the CBRN agents of any kind, either separately or combined.

The integrated platform for the scientific research and expertise of bioterrorism and biological warfare agents contributes to strengthening the capacity of scientific research of DM/MND for the expertise of WBA/CBRN agents. During biological crisis situations, it can strengthen the National Healthcare System in terms of providing a microbiological diagnostic to the sick or suspected to be sick or to the animals, as well as for the detection, identification and confirmation of CBRN agents under biosafety and biosecurity conditions.[11,12] The facility, as a medical-military objective, can be useful interdepartmentally: Ministry of National Defense (MND), Ministry of Health (MH), Ministry of Agriculture and Rural Development (MARD), Ministry of Internal Affairs (MIA), Ministry of Justice (MJ), Ministry of Environment, Water and Forests (MEWF) and Romanian Intelligence Service (RIS).

Studies and procedures for the operationalization and integration of the Laboratory of Microbiology and of the Biobase.

The integrated platform consists of complementary elements acting in algorithm: the Microbiology laboratory for in vitro analysis, the Biobase for in vivo analysis, the Mobile team for biological intervention (EMI-Bio) for field activities, as well as the annex utilities for the proper functioning of the objective.

The design, the feasibility study and the outline design for the Integrated platform of the scientific research and expertise of war and bioterrorism biological agents also includes the update of the previously modernized objectives, during 2007-2009 (Laboratory of Microbiology and Biobase), as well as the utilities and Annex facilities. The commissioning of the installed equipment and staff training with accredited supplier firms must be supplemented by specific new latest technology purchases. The explanatory memoranda and the feasibility study for the operationalization of the integrated platform are necessary because the financial burden is very high and must be quantified because the investment proposal submitted to the policy makers needs to be supported by the necessity and the opportunity of the investment.[8]

The activity of verifying the conditions related to the authorisation, the qualification, the certification and the accreditation of the Integrated platform for scientific research and expertise of biological agents

- The activity of verifying the conditions related to the authorisation of the Integrated platform. The process of modernizing the two objectives was carried out under the guidance of the National Authority for Scientific Research and Innovation (ANCSI) after attending the PNCDI Capacities national competition and after obtaining government funding through the Ministry of Education in 2007. As required by the investor, existing complementary objectives were selected: a laboratory of virology and the vivarium. ANCSI monitored the design, development and the endowment with installations and equipment of modernized objectives and performed the final inspection for the reception of the works, in 2009. The report prepared by ANCSI concerns only the fulfillment of the specific research and acquisitions objectives. Depending on the technical characteristics of the newly installed equipment, the owner must provide the utilities: electricity, cold water, hot water, distilled water, sewage, lighting, ventilation, heating, security, specific supplies and specialised staffing.

After the rehabilitation works to the building and installations have been completed, the Verbal Proceeding for the works reception will be issued. Next, follows the sanitation stage of the premises, of authorization and of commissioning the specific equipment and devices, by specialized companies and suppliers. Next, will be purchased consumables, reagents and inventory objects for the endowment of laboratories, depending on the tasks.

After establishing work teams with qualified and specialized staff follows the stage of drawing up the Verbal Proceeding for the functioning status of the Laboratory (through self-assessment and internal auditing). The Technical file for the authorization of the objective that contains data about the space, the endowment, about the staff and the work procedures is submitted to the Territorial Center for Preventive Medicine (TCPM/DM) in view of the sanitary authorization inspection of the objective.

After obtaining the sanitary functioning authorization, that involves the prior solving of the rehabilitation at the level of the entire facility, immediately will be performed a recheck of the qualification, certification and accreditation conditions of the Integrated platform for scientific research and expertise of biological agents.

- The verification of the qualification conditions of the Integrated platform, according to the Guidelines head. 7, p. 44-45: "Recommendations for the qualification of the laboratory and its facilities." The qualification of the laboratory/its facilities may be defined as a systematic process of examination and documen-tation, demonstrating that the structural elements of the laboratory and of the systems and/or of the system components have been installed, inspected and tested, in terms of their operation, in conformity with the national and international standards.[2]

The laboratories designed to correspond to the Biosafety Levels 1 to 4, have different qualification requirements, with increasing levels of complexity. The geographical and climatic conditions, such as moisture or extreme temperatures, can also affect the laboratory structure, and thereby, its qualification demands. Once the qualification process has been completed, the significant structural components and the related systems will be subject to various conditions, including working conditions, under imposed conditions, logically possible, that will only thereafter be approved.

The qualification process and the acceptance criteria will have to be established from the design phase, of construction and/or renovation. By knowing the qualification requirements from the very beginning, the staff (the architects, engineers, the staff responsible for the safety and health assessment, as well as the staff of the laboratory) will be able to better understand the performance that has to be achieved by the laboratory.

The qualification process provides the institution and the community within which it operates a higher degree of confidence, given the fact that the structural elements, the electrical systems, the mechanical and drainage systems, the insulation and decontamination systems, as well as the security and alarm systems will function as initially designed, ensuring secure handling in the laboratory or in the biobase of any potentially dangerous microorganism.

The qualification activities are generally performed, from the design stage, continuing as such during the construction and installation of the laboratory and its facilities and during the warranty period, which should cover at least one year after its entry into service.

The agent assessing the qualification, acts as a guide for the institution that is building and renovating the laboratory and must be considered as a member of the concept team, his early involvement in the project design being essential. The institution may act as its own qualification agent, if it has a trained auditor. In the case of more complex laboratory facilities, with biosafety level 3 or 4, the institution may use the services of a qualification agent outside the institution, with proven experience in the successful implementation of the qualification of laboratories and biobases with complex levels of biosafety. When referring to a freelancer qualification agent, representatives of the institution will also participate as team members: the safety officer at institution level, the project manager, the program manager and a representative of the technical service’s maintenance and intervention.

A list will exist to work with, consisting of the laboratory’s systems and of the components that will be included in the qualification plan, for testing the functionality correlated with the degree of securing the facilities to be built or renovated. This list is not exhaustive, being adapted to the laboratory specifics. Clearly, the actual qualification plan must reflect the complexity of the respective laboratory.

- The verification of the certification conditions of the Integrated platform according to the Guideline head. 8 p. 45-60: "Recommendations for laboratory certification and its facilities"; it is similar to the qualification, but is run by a committee of national experts approved (the Ministry of Health, RENAR, the National Association of Medical Laboratories etc.). The WHO model questionnaire list, shall be completed during the certification inspection, in the presence of the institution’s representatives, who know the objective.[2]

- Verification of the accreditation conditions of the Integrated Platform.

The Ministry of Health is able to grant accreditation only up to the P3 level; so, after reaching this level, the next step would be to resort to an international organization (WHO, EU, ECDC, NATO etc.), if an accreditation at maximal level is required and if all the required conditions are fulfilled and whether there is adequate funding.[7]

The complexity of running operations in a laboratory with maximal biosafety, exceeds the scope of the Biosafety Guidelines. More details and information can be found in the O.M.S. Biosafety Programme (according to the Biosafety Guidelines, Annex 3). The available information related to the training courses and to the profile information materials can be obtained by written request, for example from the Biosafety programme, Department of Communicable Disease Surveillance and Response, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (http://www.who.int/csr/).

Basically, the activity of verifying the conditions related to the authorisation, the qualification, the certification and the accreditation of the Integrated platform for scientific research and expertise of biological agents ascertains whether the objectives satisfy the requirements and proposes the accreditation. If the requirements are only partially met, then the nonconformities will be recorded and the inspection will be effectively resumed when all rehabilitation works to the platform's components will be completed. Only then, the laboratory may be declared fully functional at the highest level of biosafety. If all the imposed requirements cannot be met, or in the meantime the requirements for biosafety are amplified and the facility no longer meets the new requirements, then the facility can be accredited to a lower level, adding additional equipment for biological protection or other appropriate measures, whenever necessary.

OBSERVATION

Following an increase in the capability requirements concerning laboratories, we have provided in the design concept new technical parameters of the platform and the integration of new compartments with related activities of toxicology, pathology, neuropsycho-pharmacology, biopharmacy, micro pharmaceutical, specific testing activities, etc. However, the new facility does not allow, in terms of spaces and technological flows, their permanent dislocation in the space of the integrated Platform. If the dislocation of any other laboratories is needed, other spaces must be designed, built and purchased that correspond to international standards in the field, disseminated by the Ministry of Health.

Executing the integrated platform, its operationalization and, implicitly, the investment's financing is required in order to meet the requirement of the E6218 Capability Target, as a collective CBRN defense/ protection facility and military-medical scientific research for CBRN medical protection.

The original concept of ABR and bioterrorism scientific research and expertise was completed over time, in consultation with the colleagues from other specialties who are involved in CBRN medical protection and related areas of expertise that are complementary to the basic activity, in order to work under biosafety conditions, in full compliance with international norms.

CONCLUSIONS

The existing upgraded objectives should be operationalized so as to fulfill the targeted purpose: scientific research and expertise of biological agents and biological weapons, for in vitro and in vivo analysis. A laboratory with a maximal biosafety level allows working with any high risk agents, being versatile. We also provided new compartments with related activities to toxicology, radiobiology, anatomic pathology, neuro-psycho-pharmacology, biopharmacy microproduction and specific testing etc. The general concept enables the collaboration/cooperation of all CBRN medical protection laboratories and the cooperation with other military, civilian, national and international entities: NATO, EU or CIMIC.

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REVIEW ARTICLE

Article received on February 12, 2017 and accepted for publishing on May 4, 2017.

Intellectual mobility in medical higher education system

Iulia Alecu[1], Horia Mocanu[2], Ioan E. Călin[1]

Abstract: *Intellectual mobility brings change, there is the primary factor in the way of progress and optimal premise of human being development from theoretic and practice regards. Medical Higher Education, worldwide, is generally similar in structure and consistency, but different in typology of presentation, teaching, learning and assessment. In fact, general medicine, as a subject refers to the same biological body, but presented differently depending on culture, space and under various methods of teaching and learning.*

The idiom of intellectual mobility is not new, but according to globalization, which we live at the present times, brought the mobility in the main plan of Europeanization, a new plan, with continues sustainable development and maybe of success. By institutional mobility, both for students and for academic staff, an exchange means a period of one academic year or a semester, for students and, for two days to several months for academic staff, into a foreign university. These stages of study, practice, and teaching take place most frequently within the Erasmus + framework, have been of 30 years in Europe and 20 years in Romania. Also, there are other programs that can perform intellectual mobility, but the most well-known is Erasmus program, where European Commission has allocated the biggest legal and financial budget framework. Overall activity program features has a variety of tools to be deployed and an inter-institutional framework with qualified staff to manage it.

Keywords: *medical education, recognition, higher education, mobility, Erasmus program*

INTRODUCTION

General Medicine as an important branch of study has a special status in the conduct of mobility as importance of the field and also under emotional aspect.

Intellectual mobility takes a certain mental patterns generated by education and a native predisposition, regardless of the field. Along with this added dynamic appeal cases handled by the abstract representations. In the field of medicine is worked with a high degree of abstraction. Or spatial relocation and mobility means mental derived including concrete way of their operationalizing in the two phases of the medical profession: correct diagnosis and treatment of the patient, not the disease. Appeal to intellectual mobility, is done often in the holistic approach to the patient. The doctor represents, on the one hand a mechanic and on the other hand a sociologist. This would be the condition of being a successful doctor. There is also the assumption that both treatment and technique coordinate the doctor to a successful performing.

Casuistry with high risk requires determination,

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curiosity and professional beliefs. However, it is based on a dominant personality characteristic correlated with a predominantly emotional intelligence, and then on IQ. But there is a less explored area of the mind of a physician or a future doctor, a student, how to manage frustration generated by failure in high-risk cases, but not only. Failure, in any other domain generates lessons learned that are grouped in a simple taxonomy:

1. Lessons indicating approaches “know how";
2. Lessons to refute or confirm hypotheses absolutely absurd;
3. Lessons to optimize cases generally valid but wrong managed.

Thus, intellectual mobility in general, depends of subjective perception of reality itself the objective of playfulness and cognitive operators with which man is accustomed routinely to operational abstractions. Medical education has the same topology and provides almost the same bibliographic regarding symptoms of the disease; unfortunately get to treat the disease using methods quite invasive.

Such patterns of study are known in medical education through student mobility from one education system to another. Exchange of experience, for a period from one university to another can bring new knowledge, new methods of learning, but also a personal self, psycho-emotional development.

Erasmus mobility can bring competitive doctors on the labor market and ensure a quality structure, but the real problem is the distribution on the labor market.

As it is well known Romanian doctors prefer to work in European hospitals or beyond Europe, which is not bad, on the one hand, but on the other hand it led to a destabilization of the health system in Romania. So Erasmus mobility tended by a medical mass migration.

Naturally, in this context a basis is economically and socially disadvantaged in Romanian hospitals and moral degradation of the system.

MEDICAL HIGHER EDUCATION IN DRESDEN, ROME AND BUCHAREST

A brief history

A brief history of medicine proves that it was practiced of ancient times from trained professional. History and times prove how the society have changed and it is also in a continuous changes in the approach to sickness and disorder from ancient beginnings

It is well known in the world that medical services were provided for the poor people in monastic hospitals. The care was rudimentary way and rather palliative. As we can observe also medical services and school education, in any domains started from the monastic area, in churches. As just a thin remembering it can be named that culture and civilization started around the human necessity of norms and rules issued by the spirituality.

In the 9th century there were some medical schools in Italy. The influence from other nations as: Greek, Latin, Arabic and Hebrew gave an international dimension. Students learn three years as preliminary courses and five years of medical schools. Nowadays they study five, six or seven years in Europe and in SUA more than ten years.

Italy is the place where medical universities were founded, after came France and England which developed medical schools. So we can see from the beginning the health science started in an internationally manner and mobility and migration are quit ancient and were very important in the development of it.

Today according to the progress of technology, techniques and information system mobility is very used and normal in the society.

Recognition of studies

In order to define studies in Erasmus Program we have to analyze its framework. The main problem is the recognition in making Erasmus.

Although there is a desired of full recognition of Erasmus studies, according to the Erasmus Charter, it is not possible, discussing the case by case,

depending on the curriculum and structure. Of course, that is an ideal situation that a degree program can be accepted fully recognized, but there are features that can be dealt with individually. Although Erasmus Charter directs the full recognition and, in general, universities are trying to respect this principle, even though at the end of the program, there are people involved in Agencies of Recognition and Accreditation of Studies from all over Europe, not easily accept that Erasmus has a special pattern, easily convertible by ECTS and also has all instruments and forms provided. We will see a simple case which presents two distinct situations of curricula on a few subjects of study. We'll see how a curriculum in three different states differ and how to work through the transformation to studies from a curriculum to another, by grades, ECTS obtained both from practical or clinical training and courses. Quantification of studies must be easy and in interest of students. Of course it is of great important qualitative component, especially in the field of medicine. But always should take into account the socio-cultural characteristics, adjusting the student in a new cultural space and psycholinguistics barrier.

In the context of Europeanization and for exemplifying the above situation exposes three major universities with medical schools, in Europe.

Universitatsklinikum "Carl Gustav Carus" Dresden Technische Universität of Germany, especially the ENT discipline; Universita Degli Studi di Sapienza, Rome, Italy, with examples on general surgery and internal medicine and Titu Maiorescu University in Bucharest, Romania, as a university of origin/home university, which makes recognition and equivalence studies.

Germany is a country of art, technique and study continuously, so the team from ENT created a standardized teaching maneuvers examination by a small guide, so that students can observe organized clinical examination of Otolaryngology. Consideration is made all the standardized by checking maneuvers in a form, for a period of 6 minutes of examining a patient. This calculates a score of the exam, and the form can be filled by two examiners for the compared results.

Such an experience has brought by an academic staff that has benefited of teaching stage in Dresden. Today, the ENT method was implemented in Bucharest for teaching and assessment the subject. One such example is enlightening to harmonize the methods of teaching and assessment, job shadowing lead to the development of new skills.

Regarding the Recognition of Studies facts are the discussions become slightly rigid and austere although the program is provided with all the necessary tools.

A student who chose as subjects of study maxillofacial surgeon, a course of a single module named Head has 1 ECTS, but the workload is the same as that of a course of otolaryngology at Bucharest, which has 4 ECTS. Also, the grading system is different. In Germany the scale grades are from 1 to 5, and in Italy from 18 to 30; 1 ECTS has 25 hours of workload. In Romania, at faculty of medicine 1 ECTS has approximately 14 hours of workload. Depending on the workload is denoted by ECTS, 30 for a semester and 60 per academic year, which should be equivalent between higher education systems, but they are not. Module Thorax includes the following disciplines: Cardiology, Angiology, Pulmonology, Vascular Surgery, Thoracic and Cardiac has a volume of 10 ECTS, if studied together, if it divides, then the number of ECTS is divided too, in Romania they are separate disciplines. In Italy, Internal Medicine and General Surgery is a module that measures 12 ECTS together, and in Romania internal Medicine has 6 ECTS for the first semester and for the second semester it has 5 ECTS, in the end there are 11 ECTS, just Internal Medicine. General Surgery is another subject and it has 4 ECTS for the first semester and 4 ECTS for the second semester; they are totally different from one system to other. Such recognition is based on workload and always is made in the favour of the student, or should be.

In Germany the focus is on clinical stages, more than in Italy. In Romania, specialized practice/training is done since of the first year of study, according to the students' testimony.

Although there are differences in the three systems

of teaching and learning, Erasmus has provided the tools; study contract/Learning Agreement, and students can choose their subjects to be studied and disciplines which will be equate to return. Studies are, or should be recognized, integrum, full recognition under the Erasmus Charter. If the student does not fulfill the learning agreement he/she will support additional exams from local education until completion of ECTS number needed to pass the academic year. These are predetermined patterns of procedure and related methods for classification and institutionalization of each institution. What becomes interesting is the prospect of personal development of each individual differs from person to another depending on operators and cognitive education.

There are three factors that can prevent full recognition, as following:

1. Changing subjects of study during mobility, Erasmus Learning Agreement provides that rule can be changed, only in the first 14 days of mobility. Thus, changing the curriculum content, can prevented full recognition procedure because of the time period from the moment of making the new choice of new disciplines and to the approval by the academic tutor from home institution.

2. A second factor that keeps the procedure and otherwise representing a procedural error is soliciting approvals for the recognition and equivalence studies to the professors who are tutor of the disciplines. Thus, the holder of course, may be not sufficiently informed and decide in the detriment of the student. Erasmus Rule requires the application of Charter based on acceptance of Erasmus in function. So is forbidden that a tutor can decide regarding his/her discipline.

3. Finally a third factor, which prevents full recognition, is negative influence of the party who decide subjective and would not assumes the recognition of Learning Agreement. These are isolated cases, and in recent years almost no longer exist. The procedure for recognition and equivalence is the essential characteristic of students in the decision to go in Erasmus mobility.

According to the magazine Prime 2010, only 19% of students surveyed are convinced that it will not benefit from an exchange. The rest of the students who responded to the questionnaire in the same magazine argued that regardless of the recognition of studies, mobility itself and experience are more important than the recognition of studies, thus they assuming full academic exchange activities. Of course that always the activity must be tried separately according to each case. If the student wishes on its own initiative to have examinations in the subjects of home university, it is not prevented, or if the student did not follow important disciplines for future examinations of competence, then it will have them at the return from the mobility without charge of any fee.

There is also a risk that the student take courses that are done in the near future/years of study in the home university and through full recognition, the student would be forced to repeat subject mobility in the coming years. As such, the choice of subjects, from a curriculum structured around six years can be challenging even for academic tutor. This happens because the curricula are not similar, nor how to be similar. Bologna process does not seek to standardize the Higher Education, but seek to a better harmonization of curricula, a socio-cultural and economic uniformity.

If the student is studying disciplines in the curriculum of the home university is doing in a upper year, the University, study case of this research, recognizes full program of study at the partner university and mention the time spent abroad in the Diploma Supplement, and recognizes discipline by discipline in the years that match the local program, and to promote appropriate student take exams in the subjects of study sessions legal up, and in special cases can be organized special sessions for Erasmus students. Whatever, the situation of recognition and equivalence of studies is made, only in students' favor without affect the merit place at home university.

Despite these shortcomings of procedures for recognition of studies, students wishing to repeat the experience to the extent permitted by the Erasmus program.

Most often students after followed a study program they would like to follow a clinical internship. From the experience of the university concerned, they apply after completing their studies in residency programs in the world. University receives per few times a year, forms requesting certification of studies by agencies of recognition of medical studies in the USA and Canada.

Table 1: Example of a model of recognition studies, in Germany
(it can be observed that the students pass more than 5 ECTS at home university)

Name of the subject	ECTS	Grade in Germany	Grade in Romania
Maxillofacial Surgery	1	1,5	10
Cardiology, Angiology, Pulmonology, Vascular Surgery, Thoracic and Cardiac	10	2	9
Dermatology & Venereology	3	4	5
Rheumatology	2	attended	
Pathophysiology & Medical Biochemistry and Laboratory Diagnostics	9	4	5

It is mention were equated the subjects studied in Germany in the V th year, semester 1 of the study, under the Erasmus Charter, after the student has pass the remaining number of 5 ECTS, as follows:

Name of the subject	ECTS	Grade in Romania
Pneumology	4	7
Occupational diseases	5	8
Radiology	5	9
Gerontology		10
TOTAL	14	

Analyzing the situation of a student who was Erasmus, in the fifth year of study at the University of Dresden, after she came back home observed willingness to continue the study and mobility training, such as she obtained a period of 2 months in a hospital in Germany, combining theoretical and practical work with a clinical internship. Besides the recognition and equivalence of procedural, student mobility has made a qualitative leap in medical education, as well as psycholinguistic improving linguistic competence and went beyond customary in the sphere of national education.

CONCLUSION

Mobility in medical education can make steady progress in what is called globalization, harmonization of curricula and skills in the labor market. The main objective of globalization and mobility function is preventing and overcome poverty and habits that can hinder knowledge and human progress. The internationalization of education is a process of development and a better preparation of students for a globalized society, based on knowledge and skills. Higher education institutions have the main role to prepare graduates for the labor market in the line with international policies of globalization. Mobility brings extra value to Europe and is a sine qua non of human development in relation to the labor market, the practical problem that remains in the knowledge era is the economic and political, social and moral worldwide crisis, otherwise unmanageable. Now it is felt the effects of the global turmoil, but concrete results are likely to be known around 2050.

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REVIEW ARTICLE

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The influence of homocysteine on osteoporosis

Elena Rusu[1]

Abstract: *Osteoporosis is a major health problem, and the economic costs are expected to rise due to an increase in life expectancy throughout the world. Its major consequence is fractures, and especially hip fractures are associated with institutionalization and increased mortality. Homocysteine is an amino acid intermediate formed during the metabolism of methionine. Homocysteinuria is a rare autosomal recessive biochemical abnormality which causes elevated plasma concentrations of homocysteine and severe occlusive vascular disease. In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. The high prevalence of osteoporosis among patients with homocysteinuria suggests that high levels of plasmatic homocysteine may also increase the risk of fractures. Nutritional factors such as vitamins B12, B6, and folate are cofactors in homocysteine metabolism, and vitamin intakes may inversely affect plasma homocysteine levels.*

Keywords: *osteoporosis, homocysteine, hip fracture*

INTRODUCTION

Osteoporosis is a major health problem, and the economic burden is expected to rise due to an increase in life expectancy throughout the world. Its major consequence is fractures, and especially hip fractures are associated with institutionalization and increased mortality. The prevalence of osteoporosis increases with age due to an imbalance in the rate at which bone is removed and replaced during the bone remodeling cycle, which is an important physiological process that is essential for maintenance of a healthy skeleton.

Pharmacological interventions may prevent 30-60% of fractures in patients with osteoporosis. Common sites for osteoporotic fracture are the spine, hip, distal forearm and proximal humerus. The remaining lifetime probability in women at the menopause of a fracture at any one of these sites exceeds that of breast cancer (approximately 12%), and the likelihood of a fracture at any of these sites is 40% or more in developed countries [1].

The level of bone mass can be assessed with adequate precision by measuring bone mineral density using dual X-ray absorptiometry. It has

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been suggested that bone strength may be reflected, independently of bone mineral density level, by ultrasonic measurements of bone and by measuring bone turnover using specific serum and urinary markers of bone formation and resorption.

Physical activity as a way to prevent osteoporosis is based on evidence that it can regulate bone maintenance and stimulate bone formation including the accumulation of mineral, in addition to strengthening muscles, improving balance, and thus reducing the overall risk of falls and fractures. It is well known the important influence of hormones as well as dietary and specific nutrient abundance on bone, growth and health is emphasized and premature bone loss associated with dietary restriction and estradiol withdrawal in exercise-induced amenorrhoea [2].

OSTEOPOROSIS

It is becoming increasingly clear that there is a relationship between growth and development in early childhood and bone health in old age. In fact, suboptimal bone development leads to a reduction in peak bone mass, and a higher risk of osteoporotic fracture later in life. Osteoporosis is a skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility. Preventative strategies against osteoporosis can be aimed at either optimizing the peak bone mass obtained, or reducing the rate of bone loss.

One of the largest risk factors for fractures is a reduction in bone mineral density. Risk factors for fracture can be purely skeletal-related affecting bone mass, bone geometry, bone micro-architecture and bone turnover, or solely fall-related such as neuromuscular dysfunction, poor balance, cognitive impairment, cardiovascular instability, reduced visual acuity and sedative medications. Others risks are both skeletal and fall related such as age, genotype, and family history of fracture, weight, weight change and mobility [3]. Falls history is a further independent risk factor for fracture, in particular in men [4]. About 50% of white women and 20% of men will have an osteoporosis-related fracture in their lifetimes. Fractures of the hip and spine may be disabling and are associated with mortality rates that are about 20% greater than that of an age-matched population. The goal of any treatment for osteoporosis is to improve bone strength, thereby decreasing fracture risk.

Bone remodeling is the result of two opposite activities, the production of new bone matrix by osteoblasts and the destruction of old bone by osteoclasts. The rates of bone production and destruction can be evaluated either by measuring predominantly osteoblastic or osteoclastic enzyme activities or by assaying bone matrix components released in the bloodstream and excreted in the urine [5].

Factors which influence negatively the osteogenetic potential are age, nutrition diseases and endocrinopathy, ionized radiations treatments and different toxic factors. It has been scientifically proved the fact that elder patients have decreased general biological potential. Regarding the bone system, there occur structural changes which weaken the resistance, to which we can add a diminishing of the response potential towards harmful factors. These lacks are considered to belong to multiple causes: a diminishing of the vitamin D action and of calcitonin, hyperactivity of the parathyroid hormones with osteoblast inhibition, etc. [6] As a result, osteolysis-osteo-synthesis dynamics is reversed, resorbing processes become dominant: osteodystrophy, osteoporosis, senile osteopenia, metabolic and endocrine osteopathy, which, affecting the very bone structure, are the main causes of diminish in the osteogenic potential in elderly patients. Nutrition diseases and endocrinopathies seem to have the most harmful effects on osteogenesis and the repairing processes of the bone tissue.

The evaluation of biochemical markers of bone turnover has been useful in clinical research. However, the predictive factor of these

measurements is not defined clearly, and these findings should not be used as a replacement for bone density testing [7]. There is a high prevalence of calcium, protein and vitamin D insufficiency in the elderly. Calcium and vitamin D supplements decrease secondary hyperparathyroi-dism and reduce the risk of proximal femur fracture, particularly in the elderly living in nursing homes. Sufficient protein intakes are necessary to maintain the function of the musculoskeletal system, but they also decrease the complications that occur after an osteoporotic fracture.

HOMOCYSTEINE

Sulfur is the seventh most abundant element measurable in the human body and is supplied mainly by the intake of methionine, an indispensable amino acid found in plant and animal proteins. Inhibition of cystathionine-β-synthase activity causes the upstream sequestration of homocysteine and the downstream drop in cysteine and glutathione [8]. Homocysteine is an aminoacid which contains a thiol group formed by methionine intracellular demethylation (alpha amino gama methylthio butyric). In plasma, homocysteine is found free in oxidized or disulphidic form, linked to proteins. Homocysteine has two ways to be metabolised, a metabolic path is represented by transsulfuration to cysteine through cystatin synthetasis, enzymes which need vitamin B6 as co-factor (proving the need of vitamin B6 administration during the treatment). Synthesis of cysteine as a product of the transsulfuration pathway can be viewed as part of methionine or homocysteine degradation, with cysteine being the vehicle for sulfur conversion to end products (sulfate, taurine) that can be excreted in the urine. Transsulfuration of homocysteine to cysteine is catalyzed by two pyridoxal 5′-phosphate-dependent enzymes, cystathionine β-synthase and cystathionine γ-lyase. The transsulfuration pathway is responsible for catabolism of the carbon chain of methionine, release of the amino nitrogen in a form that can be funneled into pathways of nitrogen excretion, and transfer of Met sulfur to serine to synthesize cysteine [9].

Another metabolic path is remethylation to methionine in the presence of methylentetra-hydrofolate reductase (MTHFR) and methionine synthesis in the presence of folic acid as an under layer for vitamin B12 as co-enzyme (proving the need of folic acid and vitamin B12 administration during the treatment). There are studies which proved that the level of homocysteine in blood is inversely proportional with folate levels, vitamin B12, vitamin B6 and oxygen intake induced by these vitamins [10].

Homocysteinuria is a rare autosomal recessive biochemical abnormality which causes elevated plasma concentrations of homocysteine and severe occlusive vascular disease. In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. Blood levels of total homocysteine increase throughout life in men and women. Prior to puberty, both sexes enjoy optimally healthy levels (6 μmol/L). During puberty, levels rise, more in males than women, reaching, on average, almost 10 μmol/L in men and more than 8 μmol/L in women. As we age, mean values of homocysteine continue to rise and the concentrations usually remain lower in women than in men. Adults without homocysteinuria who have high homocysteine levels are also at risk for fractures. Thus, elevated plasma homocysteine concentrations (>14 μmol/L) are associated with osteoporosis and may increase the risk of hip fracture, in both men and women. These can lead to substantial disability, high medical costs, and death [11]. Elevated plasma homocysteine concentrations are associated with reduced physical performance and muscle strength in older women [12].

Hyperhomocysteinemia may contribute to the development of osteoporosis. High hyper-homocysteine and low vitamin B12 concentrations were significantly associated with low bone turnover markers, high markers of bone turnover, and increased fracture risk [13]. Folate and vitamins B12 and B6 are major determinants of homocysteine concentrations in older persons [14]. The B vitamins folate, B12, and B6 are important cofactors in homocysteine metabolism, and low status of these

nutrients is the primary determinant of elevated plasma homocysteine concentrations in elders. Patients with pernicious anemia have decreased bone mineral density at the lumbar spine, and in comparison with the general population they have almost double the risk of hip fracture. Gene polymorphisms related to homocysteine metabolism also may result in high homocysteine levels. Thus, nutritional factors such as B12/folate deficiency and genetic factors may affect homocysteine levels and contribute to fracture risk.

Hyperhomocysteinemia is regarded as a risk factor for ischemic stroke and for hip fractures in Parkinson's disease patients receiving levodopa [15]. The high prevalence of osteoporosis among patients with homocysteinuria suggests that hyperhomocysteine may also increase the risk of fractures [16]. Higher plasma levels of total homocysteine and folate were independent predictors of coronary heart disease [17]. In the Framingham study authors had shown that plasma total homocysteine concentration is inversely related to the intake and plasma levels of folate and vitamin B6 as well as vitamin B12 plasma levels. Almost two-thirds of the prevalence of high homocysteine is attributable to low vitamin status or intake. Elevated homocysteine concentrations in plasma are a risk factor for prevalence of extracranial carotid artery stenosis of at least 25% in both men and women [18]. Some researchers want to determine if there is a possibility that plasma total homocysteine may serve as an indicator of the status and perhaps the intake of a number of vitamins, including folic acid, vitamin B12, and vitamin B6. This possibility derived from the large number of studies that implied that methionine metabolism is tightly regulated and from other studies that showed that deficiencies in the above vitamins are often associated with hyper-homocysteinemia.

It was shown that premenopausal women have lower homocysteine levels than men and postmenopausal women. Higher plasma levels of total homocysteine concentrations in men than in women may be explained by differences in muscle mass, hormone and vitamin status [19]. Factors that influence total homocysteine plasma levels in the general population include diet, in particular folate intake, blood levels of folate, vitamin B12, and betaine, renal function, and the MTHFR 677C>T polymorphism [20]. There are studies to establish that increasing evidence that plasma total homocysteine is inversely associated with bone health. It has been speculated that moderately elevated total homocysteine levels could contribute to osteoporotic changes, based on the fact that osteoporosis is a common phenomenon in homocysteinuria. High total homocysteine and low vitamin B12 concentrations are significantly associated with high levels of markers of bone turnover, and relations have been reported between total homocysteine and markers of bone resorption [13].

Elevated plasma total homocysteine, deficiencies of folate and vitamin B12 are associated with risk of osteoporosis and fracture. In some studies, there were examined whether plasma levels of elevated plasma total homocysteine, folate, and vitamin B12 predicted hip fracture [21]. They found that elevated plasma total homocysteine is a predictor for hip fracture among elderly men and women.

CONCLUSION

In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. The high prevalence of osteoporosis among patients with homocysteinuria suggests that hyperhomocysteine may also increase the risk of fractures. Nutritional factors such as vitamins B12, B6, and folate are cofactors in homocysteine metabolism, and vitamin intakes may inversely affect plasma homocysteine levels.

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SYSTEMATIC REVIEW

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Efficacy and tolerability of calcium channel alpha-2-delta ligands in psychiatric disorders

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Abstract: *Matching drugs with anxiolytic properties- but without the potential of inducing dependence or abuse- with clinical manifestations of various affective disorders is a very important challenge for psychiatrists. Although the first line of pharmacologic treatment for anxiety disorders remains antidepressants with serotoninergic properties, calcium channel alpha-2-delta ligands are adjuvant agents which could be useful for augmenting antidepressant agents' clinical effects. Unfortunately, calcium channel alpha-2-delta ligands efficacy and tolerability are not very well known, due to a lack of large scale, randomized, placebo-controlled trials focused on psychiatric disorders. Data regarding pregabalin and gabapentin pharmacology and clinical effects are reviewed and conclusions with pragmatically impact based on the discovered evidence are formulated accordingly.*

Keywords: *calcium channel alpha-2-delta ligands, generalized anxiety disorder, fibromyalgia, social anxiety disorder, pregabalin, gabapentin*

PHARMACOLOGICAL PROPERTIES OF CALCIUM CHANNEL α2δ-LIGANDS

Alpha-2-delta subunits of voltage-gated calcium channels (VGCC) have an important role in modulation of the calcium currents and participate in important cellular and inter-cellular phenomena like muscular excitability, neurotransmission, re-gulation of gene expression etc. As a consequence, drugs with alpha-2-delta VGCC antagonist properties could improve symptoms of fibromyalgia and neuropatic pain, but they are also used for treatment of several psychiatric disorders, the most extensive researched being the field of anxiety disorders.

Pregabalin is a structural analog of the inhibitory neurotransmiter γ-aminobutyric acid (GABA). This drug reduces the synaptic release of several neurotransmitters through binding to the alpha2-delta type 1 protein and demonstrated anticonvulsant, analgesic, and anxiolytic properties in preclinical models [1,2].

Pregabalin is rapidly absorbed after oral administration, with a bioavailability value higher than 90%; peak plasma concentrations are reached after 1-1.5h, steady-state concentrations are achieved within 24-48h after repeated administration, and if used with food there is no clinically significant

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effect on the drug's absorption; pregabalin half-life is about 6 hours, it does not bind to plasma proteins, and 90% of a dose is eliminated unchanged in urine [3].

Gabapentin is derived from gamma-aminobutyric acid (GABA) by addition of a cyclohexyl group and crosses several lipid membrane barriers through L-amino acid transporters system [4]. Gabapentin has a bio-availability that varies inversely with dose (between 35% and 60%), a volume of distribution of 0.6-0.8 l/kg, cerebrospinal fluid concentrations are 20% of plasma concentrations and brain tissue values are 80% the plasma level; gabapentin is not metabolized in humans and is eliminated unchanged in the urine [5].

CLINICAL PHARMACOLOGY OF GABAPENTIN AND PREGABALIN

Agents from alpha-2-delta ligands class have a large number of indications in psychiatry and neurology, but this paper focused only on the first area of interest. Regarding the second domain, pregabalin and gabapentin are used for diabetic neuropathy, post-herpetic neuralgia, pain associated with spinal cord lesions, diverse types of seizures etc [6]. We also included in this paper trials focused on fibromyalgia, since this pathology usually requires an interdisciplinary approach, and psychiatrists are included in the consultation teams.

Pregabalin combined with duloxetine lead to good results in depression associated with fibromyalgia, according to a randomized, double-blind trial, which reported lower final Beck Depression Inventory–II (BDI-II) scores compared to placebo ($p<0.05$) [7]. Another randomized, placebo-controlled trial targeting fibromyalgia patients with associated affective symptoms showed significant improvement on Hospital Anxiety and Depression Scale – Anxiety and Depression ($p<0.001$) [8].

Pregabalin used as augmentation agent to amitriptyline, venlafaxine, or paroxetine in old age patients with fibromyalgia improved overall symptoms, including depressive scores on Center for Epidemiological Studies Depression Scale (CESDS), and the highest tolerability was detected in pregabalin+ paroxetine group [9].

Pregabalin had a good therapeutic effect in anxious depression, according to a case series [10]. Adjunctive pregabalin to conventional antidepressants in partial responders with major depressive disorder and residual anxiety decreased Hamilton Depression Rating Scale overall scores and anxiety/somatization subscale scores after 8 weeks with 65% response rate and 35% remission rate [11]. Therefore, pregabalin could be recommended as add-on agent in unipolar depressed patients with significant levels of anxiety and 49.1% of overall consecutive diagnosed outpatients that received pregabalin had a diagnosis of mood disorder, followed by 21.9% generalized anxiety disorder [10,12].

A review of 3 randomized controlled trials that compared efficacy and safety of pregabalin with placebo in social anxiety disorder- generalized form revealed a good efficacy in patients who couldn't tolerate or didn't respond well enough to SSRIs or SNRIs, which recommend pregabalin as either alternative to antidepressants or add-on agent to pharmacotherapy or cognitive-behavioural therapy [13].

Pregabalin is considered by expert opinion a safe and efficacious agent for generalized anxiety disorder, with favourable properties like absence of active metabolites and no interactions with CYP450 enzymes [14]. A pooled analysis of 6 studies confirmed the efficacy of pregabalin in depressive symptoms associated with generalized anxiety disorder, and the most beneficial response was detected at 300-450 mg daily dose [15]. A recent post-hoc analysis of a multicenter, prospective, 6-month study evaluated the effectiveness of pregabalin in resistant generalized anxiety disorder and severe depressive symptoms and concluded reduced with more than 50% Hamilton Anxiety Scale total score, and Montgomery-Asberg Rating Scale score, when combined with antidepressants and/or benzodiazepines [16].

Pharmaco-economic analysis of augmentation SSRIs treatment with pregabalin versus switch to pregabalin in treatment resistant generalized anxiety disorder reported significantly health-care costs reductions at 6 months in both treatment algorithms [17]. Pregabalin was associated with significantly higher QALY gain in refractory generalized anxiety disorder when compared to usual care in a cost-effectiveness model based on data derived from a large scale trial [18]. Also, pregabalin was superior to SSRI and SNRI in benzodiazepine-refractory generalized anxiety disorder in terms of QALY gain, but increased health-care costs and drug costs [19].

Treatment augmentation with pregabalin in patients with combat-related chronic posttraumatic stress disorder (PTSD) was efficient in a 6-week placebo-controlled trial as it improved PTSD Check List-Military Version scores ($p<0.05$), although severity of depression, anxiety and quality of life parameters didn't differ significantly between the two groups [20]. An open label pilot study with accident-related posttraumatic stress disorder showed pregabalin augmentation to antidepressant treatment as an effective and well tolerated option [21]. However, a retrospective analysis of US service members who suffered burns didn't detect differences in posttraumatic stress disorder onset rate in patients that received pregabalin or gabapentin after trauma for pain, compared to patients who didn't receive this kind of drugs [22].

Pregabalin proved itself efficacious and well tolerated in a single-blind randomized trial with active control (clonidine) that targeted opioid withdrawal symptoms [23]. In alcohol dependence, pregabalin was efficacious, by decreasing of the craving and withdrawal symptomatology [24]. A review showed positive results for pregabalin in both treatment of alcohol dependence and benzodiazepine dependence [25].

Although the use of pregabalin as add-on agent in bipolar disorder maintenance treatment has not been extensively investigated, some authors suggest its potential use for this indication [26]. Pregabalin could increase the response to quetiapine in acute mania [27], and also could help in decreasing affective symptoms in treatment-resistant manic episodes [28]. An open trial of pregabalin as adjunctive treatment in acute and maintenance phase of resistant bipolar disorder showed mood-stabilizing effect, antidepressant effect or antimanic effect in the acute phase, and also with good efficacy on long term [29].

Data extracted from a metaanalysis focused on dopaminergic and non-dopaminergic medications in restless legs syndrome found 11 studies with α2δ-ligands supporting good efficacy for gabapentin, gabapentin enacarbil, and pregabalin [30].

Use of pregabalin, as well as buspirone, added to antipsychotics in cases of schizophrenia with anxiety (almost 65% of patients with schizophrenia have anxiety symptoms), could be considered as an efficient therapeutic option [31]. Case reports suggest efficacy of pregabalin in treatment-resistant insomnia (in a patient who didn't respond to benzodiazepines, antidepressants with sedative properties, or antipsychotics) [32], and Charles Bonnet syndrome associated visual hallucinations [33], but also an enhancement of sexual desire in overdose [34].

Gabapentin significantly improved abstinence rates and heavy drinking in patients with current alcohol dependence, during a 12-week, double-blind, placebo-controlled trial, with linear dose-effects relation in mood, sleep, and craving domains [35]. Abstinence rates in this trial corresponded to a NNT value of 8 for 1800 mg daily, while lack of heavy drinking corresponded to a NNT value of 5 for the same dose [35]. Gabapentin reduced the stress-induced GABA activation in amygdala that is associated with alcohol dependence and therefore could be useful in this addiction [36].

Patients with opioid withdrawal that received adjunctive treatment with gabapentin in addition to methadone for 3 weeks reported significant improvement of general status as reflected by Subjective Opiate Withdrawal Scale (SOWS) and doses of 1600 mg/day were significantly superior to medium doses of 900 mg/day in decreasing symptoms' severity [37].

A Cochrane analysis of gabapentin efficacy in fibromyalgia associated pain didn't found good evidence to support or contradict the recommendation of this drug in daily doses of 1200-2400 mg [38]. However, the quality of evidence was rated as very low due to the fact that only one trial corresponded to the inclusion/exclusion criteria established by authors [38].

Gabapentin proved itself efficient in reducing symptoms of social anxiety disorder and it was also well tolerated [39].

Gabapentin wasn’t efficient in bipolar depression, according to a large randomized controlled trial which compared standard mood stabilizers versus gabapentin 600-3600 mg/day [40].

A cross-over, double-blind study, showed gabapentin is efficient in treatment of sensory and motor symptoms in restless legs syndrome, improving also sleep architecture in periodic leg movements during sleep [41].

CONCLUSION

Pregabalin and gabapentin are efficient anxiolytics, pregabalin being more supported by evidence in social anxiety disorder, generalized anxiety disorder, depression with significant anxiety, and also in posttraumatic stress disorder, including cases of combat-related PTSD.

Pregabalin is a well supported indication for fibromyalgia treatment, with a high efficacy-to-adverse-effects ratio. Pregabalin had a significant impact not only over pain, as the core fibromyalgia symptom, but also over affective symptoms associated with this disorder.

Gabapentin recommendation is more supported by evidence than pregabalin in alcohol dependence and opioid withdrawal, but pregabalin has some support in the treatment of benzodiazepine dependence.

None of the alpha-2-delta ligands were associated with high quality positive evidence in bipolar disorder.

In restless legs syndrome pregabalin and gabapentin are efficient for primary symptoms and associated clinical manifestations, and gabapentin improved sleep architecture in sleep related periodic leg movements.

Occasional reports of pregabalin efficacy in schizophrenia associated anxiety, treatment resistant insomnia or Charles-Bonnet syndrome need further exploration in randomized clinical trials.

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Medicine versus philosophy

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Abstract: *The ancient Greek medicine was based on the principle that philosophy influences all natural sciences as a whole. The doctor had, first of all, a humanistic formation followed by study of applied sciences specific to medicine. If humanism is purely theoretical, medicine is an applied science and the two-philosophy and medical knowledge, despite the apparent antinomy are able to create a union to the benefit of humanity. Medicine is the art of treating patients, identifying diseases and malady prevention. In its endeavor, medicine is based on the findings of numerous other fields such as physics, chemistry, anatomy, physiology, etc. Philosophy, on the other hand, can be defined as an attempt to understand human life as a whole. It is inevitable that the two ways of dealing with human beings to have influenced each other and the history of mankind. Both forms of knowledge have a major impact and influence on the world. Philosophy, understood in its older meaning, urged towards the prophylaxis and treatment of diseases of the soul whereas medicine, relying on philosophical teachings is aimed at healing the body and study its psychosomatic features.*

Keywords: *medicine, physician, philosophy, methodology, metaphysics*

Medicine and philosophy have influenced each other along mankind's history.

The present article aims and present the way in which renowned physicians have blended the strict knowledge of medical science with the more humanistic philosophical approach.

Attempts of demonstrating how philosophy clout over medicine have existed since Ancient times. Our paper is just a glimpse in the outstanding synthesis between the two apparently incongruent areas of knowledge.

Claudius Galenus of Pergamum (129-216) is one of the first physicians who sensed the need of a philosophical foundation of clinical practice. The beginnings of his scientific training originate in studying Aristotle's texts. Galen, who stated that a good physician should first of all be a philosopher, is the one who realized the necessity of associating medicine with the philosophy in order to achieve better results in human treatment. Personal physician for Marcus Aurelius, Galen received his medical expertise in a gladiators' school. His name links to the first cataract surgeries, our knowledge of the spinal cord and the functioning of the kidneys. He divided the intercellular fluid into humors: blood, bile, lymph and spleen.

What brought Galen brought new during his times was an experimentalist approach. But he abandoned his original humanist-philosopher formation, after

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having a dream, for the practical practice of medicine, “intensively dealing with philosophy yet, confident peripatetic, he is still a syncretistic, which is better seen in his logic.” [1]

During a great fire large part of his writings were destroyed yet his Institutio Logica was kept. His contribution to philosophy consists in demarcation made between logic and philosophy by postulating the theory of equivalence and by introducing of the fourth figure in syllogism.

Abu Bakr Muhammad ibn Zakariya' al-Razi (865-925), known in the western world as Rhazes, was of Persian origin and promoted experimental medicine bringing significant contributions in pediatrics, neurosurgery, nephrology and ophthalmology. In his early life, Al-Razi was more interested in studying music and alchemy. Rhazes, physician at the court and a hospital director in Baghdad, was prolific in the scientific field through the books he wrote. Some of the most important papers signed by the Arab scientist include Medicine Treaty for Mansur (dedicated to the governor of Rayy containing ten chapters for diseases which was to be translated in Latin, by scholar Gerard of Cremona, under the name Liber ad Almansoris), the study Smallpox and measles, works on applied medicine (About surgery), books meant to popularize medicine (The book to one who cannot reach the doctor), as well as doctors’ guide aimed at his fellow physicians (The guide of the nomad physician and Royal medicine).

Al-Razi believed that in serious cases of leprosy and cancer, the doctor cannot blamed or kept responsible for the inability of curing the patient. Furthermore, the physician-philosopher wrote extensively about medical ethics. His area of interest included medical chemistry, in which he made experiments with mercury, sulfuric acid, alcohol, and paraffin. In the field of pharmacy, Al-Razi made his mark by introducing devices such as mortar, vials and spatula.

Neither the field of metaphysics and philosophy were foreign to him as his papers in these fields recommend his as an inborn scholar. Relying on Aristotelian system and Plato’s philosophical thinking, Al-Razi wrote an ambitious paper ambitious in nine volumes, Virtuous life, book in which he accused Galen of not supporting his medical findings on too many case studies. Another important work with great impact, this time on the general public, The book to one who cannot reach the doctor, has the merit of explaining some diseases and associated treatments on the simple men’s understanding. The book talks about some of the most encountered afflictions such as headaches, colds, coughing, piles, diabetes, and other gastric ailments such as dysentery, ophthalmic and ear conditions, which were associated to medical treatments in order to be healed. He was the first physician who associated allergic rhinitis to the scent of flowers.

Al-Razi believed that a physician cannot really be a good practitioner unless he was a philosopher. The Persian doctor was a follower of the Euclidean theory of the space considered homogeneous and isotropic, regardless of the spatial distribution of matter. For al-Razi, to this absolute space and mechanical time corresponds the world to which man is reported. Also, Democritus’ theory, that the world is composed of atoms structured into matter and vacuum was adopted by the philosopher of Arab origin. His metaphysical system is based on the belief that the soul is intelligent and that the three-dimensional reality consists of time, space and matter. Al-Razi believed in afterlife.

In order to overcome the fear of nothingness, people, in his opinion, should instruct in areas such as religion and esotericism. Among his works on the border between medicine, philosophy and religion we can mention Spiritual medicine, Philosophical approach, Metaphysics, Small treatise on deism, Modern philosophy, etc.

Avicenna (980-1037), philosopher, physician and writer of Persian origin, in his paper Canon Medicinae (1025) set out methods of understanding, differentiation and variability of phenomena, considered methodology, which currently is regarded as vital in inductive logic and scientific methodology. In another treatise, Sanatio (1027), the Persian thinker brought criticism to the Aristotelian methods of inference, because they had, according to him, an absolute value. As such, the Arab polymath developed a complex of examination and testing methods meant to meet scientific challenges.

Francis Bacon (1561- 1626) has influenced science, in general and medicine, in particular. Renowned writer, philosopher and scientist he was the originator of empiricism as a way to test all the scientific achievements. He initiates controlled experiments. In his New Organon, Bacon stances diametrically opposed to the deductive, Aristotelian thinking. For Bacon induction is one that takes precedence as it is meant to "substitute once for all idealistic Scolastico-medieval one based on syllogistic deduction (..)." [2] Bacon talked about mind and soul as tantamount notions. Efficacious treatment of the body in medicine, according to the English thinker, implies a thorough study of the organism.

If practitioners of medicine fail in achieving their goals is due to lack of visionary perspective: the body is a complex mechanism which cannot be treated on parts, rather as a whole. In order to learn as much as possible about the human body implies clinical observation, along with the analogy of different bodies, vivisections and careful scrutiny of pathological changes. The physician has a double role: to reestablish the well-being of the diseased as well as to reduce suffering of those who are terminally-ill. What Bacon brought new to the field of medicine is the larger perspective of this field. He made no distinction between medical area and natural sciences. Philosophy of medicine is just a particular type of the more general knowledge of philosophy.

Jean-Paul Marat (1743-1793) was born in Boudry, nowadays Switzerland, but played an important role during the French Revolution. During his teenage period, he left home searching for fame and to build a stable financial situation. Marat studied medicine in Paris but failed to obtain a diploma in this area. However, he published a study about the way he treated his friends for gonorrhea, which propelled him in the medical world. In 1773, Marat published a paper entitled Essay on human philosophy. This work has the ambition to present, as scientifically as possible, the relationship between the human body and the soul and the way in which the two are interrelated. Thus, Marat believed that the soul and body were separate entities, which could, however, affect each other through the liquid within the nervous system. Marat, in this essay, analyzed the way in which, physiologically, the body can respond to emotional experiences through the excitement of the cardiac plexus.

Marat's work has the merit to connect the two planes – physiological and spiritual – into a unitary whole. The author analyzes, through his knowledge of human anatomy and physiology, how the body folds onto affects. The bodies can influence, in the physician-philosopher's opinion, the mode of existence of the soul. Thus, there are what we call qualities such as wisdom, stupidity, prudence, reason, imagination, memory, delicacy, sagacity and genius. The influence of his medical studies can be seen by how Marat relates and, therefore, builds his philosophical edifice on diseases such as spina bifida and microcephaly. The essay remarks itself by references the author made to areas of knowledge such as history, literature and botany.

In 1775, Marat obtained from the University St. Andrews references necessary to work effectively as a physician which would bring him, two years later, his appointment as the guard physician for Count d'Artois, who would become Charles X. In London, Marat published a paper dedicated to eye diseases and their way of treating: Enquiry into the Nature, Cause, and Cure of a Singular Disease of the Eye. His reputation grows and the financial situation is improving. With the money earned, he established a laboratory marquise l'Aubespine's house.

His experiments would end in his work Marat's findings related to fire, electricity and light (1779). Based on experiments, his scientific work continues. A year later, in 1780, he published About the physics of fire. Unfortunately, the Academy of Sciences did not approve his work since Marat had had the courage to call into doubt some of Newton's conclusions about refraction. His growing influence increase within scientific circles and personalities in the field recognized his value. They include Benjamin

Franklin and Goethe. In 1788 he published another work based on experimental method: Research on the physics of light. Fascinated by the subject, meanwhile, the French philosopher published essays related to electricity and medical applications of optics Memories on medical electricity (1783) and Basic optical notions (1784).

But his exuberant personality did not keep him away from politics. Enlightened spirit and with an incisive tone, Marat advocated equality of men. His political beliefs had to find a place in the newspaper The people's friend, originally named Publiciste parisien, which he edited beginning with 1789. In 1782, due to his radical views, inspired by Rousseau and Cesare Beccaria, Marat was publishing Public plan in criminal legislation that supported the idea that the death penalty should apply regardless of social status, advocating the idea of an ombudsman institution. Member of the Jacobin movement, which played an important role during the reign of terror Marat would end assassinated by stabbing, in his bath by Charlotte Corday Marie-Anne d'Armont.

Arthur Schopenhauer (1788-1860), German philosopher who would influence numerous other philosophers such as Nietzsche, Freud, Bergson, Ludwig Wittgenstein and Cioran, had a great impact on the literature of psychological character, writers such as Tolstoy, Eminescu, Proust and Thomas Mann. One of his main works was The world as will and representation (1818). Initially, in 1809 he entered the Medicine University of Göttingen which he would abandon in favor of philosophy, becoming a doctor with a thesis on the fundamental principles of thinking: The quadruple root of the principle of sufficient reason (1813). The reason for which the philosopher had as first option the study of medicine was that he wanted to know the world objectively, scientifically before starting his theoretical speculation.

Incidentally, later, Schopenhauer concludes that absolute knowledge can only take place in the presence of solid knowledge about natural sciences. The courses he attended as a medical student were extremely varied: physics, chemistry, mathematics, botany, mineralogy, physiology, comparative anatomy, humanities as well as humanistic sciences such as linguistics, ethnography, history. The great biologist and anatomist Johann Friedrich Blumenbach, who connected the human being with the study of natural sciences and linked his name to identifying five human race, would play an important role in the shaping of the future philosopher.

Just that anatomist's love towards animals made Schopenhauer reluctant to vivisections and determined him to step towards more academic areas. During his medical studies period, Schopenhauer himself had a poodle with whom he used to take long walks. During the second half of the first year in medicine, the one who was to influence generations of philosophers discovered the writings of Plato, Kant, Schelling and Upanishads. During his second year of medicine Schopenhauer concluded that he needed to change his studies, by choosing philosophy.

His philosophical system, set up during his medical studies, is based on the principle that, the basis of reality, is suffering. The first argument is that happiness is an illusion, life being nothing but a permanent deception. Yet, reality may be dominated by will. The very basis of the will is a necessity. Schopenhauer considers individual goals to have no rational basis because everything is evanescent, the only certainty being only death. According to the German philosopher, there are two teleologies: an external one (human beings' goals) and internal (understanding the purpose of life) and the one that should be taken into consideration is non-existence.[3]

Although tempted to teach in the University of Berlin, Schopenhauer renounces to academic life aspirations. In 1839 he becomes member of the Norwegian Society of Sciences. His true recognition occurs following the publication of a volume of philosophical essays Parerga and Paralipomena (1851).

Not being a religious spirit, the German philosopher feels attracted more towards oriental doctrines, such as Hinduism and Buddhism, the mystical practices and Theurgy than to Christianity. Although Freud denied Schopenhauer's influence in his clinical work, there were others who stated a close correlation between hard practice and Freudian theories of Schopenhauer. It is about Freudian theories of repression and sexuality that coincide with the ideas of the German philosopher.[4]

Karl Theodor Jaspers (1883-1969) saw daylight in Lower Saxony, in Oldenburg. Although initially prepared to study law, Jaspers chose, in 1902, medical studies which he completed seven years later. His work in the hospital of Heidelberg as a psychiatrist brought him dissatisfaction with the state of psychiatry at the time, therefore, in 1913, when he had the chance, he would choose a teaching career at the University of Heidelberg. In time, Jaspers began to connect the knowledge acquired clinically with the study of philosophy. The work that propelled him in psychology was Treaty of general psychopathology.

In 1932, more and more concerned with the study of philosophy, Jaspers signed another reference work (Philosophy) that subscribes to the existentialist current. Like Schopenhauer, Jaspers felt more attracted to Eastern religions than to Western theological doctrines. Consciousness of guilt (1946) came to blame the indifference and even the moral guilt of the people whose citizen he was, representing a wake-up call “partly utopian toward self-analysis in order to overcome the chaos, a sense of guilt that seemed to be put under possession of souls Germans.”[5] The guilt of the German population is the complicity to the Nazi’s genocide. In 1947 Jasper signed a book that seems to reveal the link between the scientific and the spiritual side of its author: Philosophical logic. Jaspers's influence in the medical field translates into identifying premises of psycho-pathology: descriptive principle, that of comprehension and of causality. Jaspers’ methodology proposes a clear, clinical, study of mental pathologies such as psychosis and schizophrenia.

If in modern society the connection between medicine and psychology becomes a more profound by the fact that medical psychology brings together knowledge from various areas of science such as the fields of border such as sociology, anthropology, psychopathology, holistic, experiential and dynamic psychology, psychoanalysis, chronobiology, ethology and neurophysiology, in the past this connection almost did not exist. Psychology has made the junction between medicine and philosophy.

Medicine as a branch of scientific knowledge is a form of cognitive understanding while philosophy is a science although it has only partially cognitive aspiration to become an area of scientific knowledge. Psychology became the first bond between the two. Gradually, both medical science and philosophy have found in common their nomological value. And the merit of physicians is to succeed, and not in few cases, to make the junction between these areas so apparently opposing.

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ORIGINAL ARTICLES

Incidence of peripheral trophic disorders determined by vein thrombosis of the lower limbs correlated with risk factors by age

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Abstract: *Introduction: Venous thromboembolism (VTE), in its clinical spectrum, includes both deep venous thrombosis (DVT) and pulmonary embolism (PE). It is a disease with high incidence and morbidity in hospital and community settings. Venous thromboembolism has various risk factors and there are studies proving that the risk of increasing the incidence of the disease is proportional to the risk factors.*

Diagnosis, treatment and complications of lower limb deep vein thrombosis (DVT) depend on the anatomical location and extent of the process. The post-thrombotic syndrome (PTS) is the most common complication of deep vein thrombosis (DVT) and clinically it is characterized by chronic pain, edema, enlarged veins, skin induration and other signs of the affected limb, while, in severe cases, it can develop venous ulcers. The incidence of peripheral trophic disorders by age and the prevalence of risk factors for deep vein thrombosis of the lower limbs were examined in this regard.

Materials and method: A retrospective study (January 2013 - December 2015) was conducted by collecting data from medical documents available in "Floreasca" Emergency Hospital Bucharest, Romania.

The patients diagnosed with deep vein thrombosis, on the basis of Doppler ultrasound, were divided into two groups, according to age: group A (59 patients aged ≤50 years) and group B (130 patients aged> 50 years). A number of data from the medical anamnesis, along with clinical and paraclinical data were collected by us and we were interested in the incidence of peripheral trophic disorders caused by deep vein thrombosis of the lower limbs correlated with the risk factors.

The study showed the incidence of deep venous thrombosis in a certain age and a certain environment of origin. The incidence of patients who have had a VTE history is half the patients with deep vein thrombosis who have had prophylactic anticoagulant therapy before hospitalization. The incidence of patients who have had prophylactic anticoagulant therapy before hospitalization is 61.1% of the patients with deep vein thrombosis and a VTE history. The incidence of trophic disorders caused by deep vein thrombosis of the lower limbs in patients who have had prophylactic anticoagulant therapy before hospitalization and in patients who also had a history of VTE is higher in those over 50 years old. The study showed the association of some risk factors for venous thrombosis with an age-related factor.

Conclusions: Improving preventive strategies and an optimally efficient utilization of these strategies for patients at risk of venous thrombosis can lead to improved clinical outcomes in practice and also to the post-thrombotic syndrome prevention. Taking into consideration the risk factors by age group and a better understanding of epidemiology and the risk factors for the first or recurrent venous thrombosis can lead to optimal use of prophylactic strategies and improved quality of life. DVT affects all age groups and the incidence associated with PTS is high, therefore the prevalence of PTS in general population is considerable.

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Thrombosis is also associated with impaired quality of life, especially when post-thrombotic syndrome develops. To assess the overall risk of VTE in every patient, individual risk factors or combinations of these should be carefully analyzed, an aspect that may have important implications for the type and duration of appropriate prophylaxis.

Keywords: *peripheral trophic disorders, post-thrombotic syndrome, venous thrombosis, risk factors, age groups*

INTRODUCTION

Deep vein thrombosis (DVT) is characterized by the formation of blood clots (thrombi) in the deep veins and usually affects the deep veins of the legs or the deep veins of the pelvis [1]. Venous thromboembolism (VTE) is manifested as deep venous thrombosis (DVT) or pulmonary embolism (PE) and occurs at an incidence of approximately 1 per 1,000 annually in adult populations [2].

About two-thirds of the episodes manifest themselves as DVT and a third as PE, with or without DVT. [3]. VTE is a very common medical problem that occurs either in isolation or as a complication of other diseases or procedures [4]. It is predominantly a disease of older adults and has a slight preponderance of males [1]. To prevent potentially fatal acute complications of pulmonary embolism (PE) and long-term complications of post-thrombotic syndrome and pulmonary hypertension, an accurate diagnosis of DVT is extremely important.

It is also important to avoid unjustified anticoagulant therapy in patients diagnosed with high risk of bleeding [5]. DVT prevention through prophylaxis, recognition in due time and DVT treatment and prevention of recurrent DVT will continue to have the greatest impact on reducing the global burden of post-thrombotic syndrome. Despite considerable progress in the diagnosis and treatment of deep vein thrombosis (DVT) of the lower extremities, one in every 2-3 patients will develop post-thrombotic sequelae within two years, which are severe in about 10% of cases and produce considerable socio-economic consequences [6].

DVT affects all age groups and the incidence associated with PTS is high, therefore the population prevalence of PTS is considerable [7]. Thrombosis is also associated with impaired quality of life, especially when the post-thrombotic syndrome develops [8.9].

MATERIALS AND METHOD

The study was retrospective (January 2013 - December 2015) and the data were collected from medical documents available in "Floreasca" Emergency Hospital Bucharest, Romania. The method used in this paper is the observational, non-experimental, descriptive study. In the study group there were included patients diagnosed with deep vein thrombosis of the lower limbs, based on the Doppler ultrasound, hospitalized in various wards of the Emergency Hospital, such as, internal medicine, orthopedics, cardiology and general surgery wards. The Doppler ultrasound determined the presence of chronic venous insufficiency, the type of venous thrombosis - deep or superficial and its location - proximal and distal.

The group of patients with deep vein thrombosis (DVT) comprises 189 patients, of which 54 have superficial vein thrombosis (SVT). According to their age, we divided the patients into two groups: group A (59 patients aged ≤50 years) and group B (130 patients aged> 50 years). For each patient we collected general data (age, gender, origin), and clinical and paraclinical data. The clinical data have identified the presence of unilateral leg edema or the entire leg edema and the presence of peripheral trophic disorders (erythema, infiltration, skin induration, cellulitis and venous ulcers).

From the anamnesis data we identified the presence of comorbidities and risk factors, namely immobilization before hospitalization, a history of venous thromboembolism (VTE) and pulmonary thromboembolism (PE), anticoagulation prior to hospitalization, various medical conditions, a history of surgical conditions (orthopedic, gynecological, urological, abdominal), neoplasm and antineoplastic treatment, cerebrovascular accident associated with motor deficiency, congestive heart failure, renal disease (renal lithiasis, nephrotic syndrome, hydronephrosis, chronic kidney disease, enlarged prostate), obesity, diabetes, hypertension, a history of heart attack, fractures before admission, alcohol consumption, smoking.

We used SPSS, version 15.0, to statistically analyze the data. For some additions to the statistical analysis we used the MedCalc program. Some of the graphics were done with Excel 2007 and other graphics with SPSS. The vast majority of the data were nominal (Yes, No); for these we did the analysis using the Chi square test. For the type of numeric data we did an ANOVA analysis. An OR (Odds Ratio) risk assessment was calculated for risk factors with the Mantel-Haenszel test. We also used binary logistic regression. The statistical differences and dependencies were statistically significant for Sig <0.05.

RESULTS

The group of patients with deep vein thrombosis (DVT) comprises 189 patients, of which 54 (28.6%) have superficial vein thrombosis (SVT). We divided patients into two groups: patients aged ≤50 years (31.22%) and patients aged> 50 years (68.78%). Applying ANOVA with a variable depending on age and an independent variable belonging to one of the two groups, we obtained as a result the average age of the patients of the first group as 37.71 years old with SD = 8,445, and for those of the second group as 68.50 with SD = 11,133. The difference between the two means is statistically significant (Sig <0.001). In the first group we have 23 (38.98%) women and 36 (61.02%) men and in the second 62 (47.69%) women and 68 (52.31%) men.

In the first group we have 9 (15.25%) patients in rural areas and 50 (84.75%) patients in urban areas, and in the second we have 30 (23.08%) patients in rural areas and 100 (76 92%) patients in urban areas. Therefore, the prevalence of patients in urban areas is very high, about 80%. By using Chi square analysis and an OR estimation performed with SPSS and MedCalc, we analyzed the relationship between variables and there are 72 patients taking anticoagulants before admission, namely 24 (40.68%) in the first group and 48 (36, 92%) in the second group. According to the test, there is no statistically significant link (Sig = 0.622) between the presence in one of the two groups and the existence of anticoagulant prior to admission. The OR estimation is not statistically significant because Sig = 0.622. From the group of patients with deep vein thrombosis the incidence of patients who had prophylactic treatment with anticoagulants before admission is 38.09% and among those the incidence of the patients who had a history of VTE is 50%.

The incidence rates of a history of VTE, of PE at admission and in the personal history for these 72 patients are presented in Table 1.

Table 1. The incidence rates of a history of VTE, of PE at admission and in the personal history

Sex	≤ 50		> 50		Total	
	N	%	N	%	N	%
History of VTE	12	50.0%	24	50.0%	36	50.0%
PE at admission	2	8.3%	1	2.1%	3	4.2%
History of PE	2	8.3%	2	4.2%	4	5.6%

From the group of patients with deep vein thrombosis, the incidence of VTE in patients who had a history of VTE (58 patients) is 30.68% and were distributed as follows: 19 (32.2%) in the first group and 39 (30.0%) in the second group. Among those patients who had a history of VTE, 38.9% had a history of VTE without Prophylactic anticoagulation treatment and 61.1% had a history of VTE with prophylactic anticoagulant treatment. The incidence rate of PE at admission and in the personal history among these 58 patients are presented in Table 2.

Table 2. The incidence rates of PE at admission and in the personal history

History of VTE	≤ 50		> 50		Total	
	N	%	N	%	N	%
PE at admission	2	10.5%	2	5.1%	4	6.9%
History of PE	3	15.8%	3	7.7%	6	10.3%

Taking this aspect into consideration enables us to optimally use the prophylactic strategies against venous thromboembolism. A better understanding of epidemiology and the risk factors for the first and the recurrent venous thrombosis can lead to improved clinical outcomes in practice. To assess the overall risk of VTE in every patient, individual risk factors or combinations of these should be carefully analyzed, an aspect that may have important implications for the type and duration of appropriate prophylaxis.

The incidence of trophic disorders caused by venous thrombosis of the lower limbs in patients who had prophylactic anticoagulant therapy before hospitalize-tion is 23.6% venous ulcers, 93% edemas, 83.3% different trophic disorders (reddish–brown cutaneous depigmentation, indurated fibrous skin, redness, irritation or dermatitis) and 12.5% cellulitis. Statistical analysis by age group reveals that the incidence is higher in patients over 50 years old, namely 24.5% venous ulcers, 93.9% swelling, 91.83% different trophic disorders and 14.28% cellulitis. In patients who are under 50 years old there was an incidence of21.73% venous ulcers, 91.30% edemas, 65.21% various trophic disorders and 8.7% cellulitis.

The incidence of trophic disorders caused by venous thrombosis of the lower limbs in patients who had prophylactic anticoagulant therapy before hospitalize-tion and history of VTE by age group reveals that the incidence is higher in patients over 50 years old, namely 45.83 % venous ulcers, 100% swelling, 91.66% various trophic disorders and 25% cellulitis. In patients under 50 years old there was an incidence of 41.6% venous ulcers, 100% swelling, 66.7% various trophic disorders and 16.7% cellulitis.

According to the data provided by Chi square analysis and OR estimation performed with SPSS and MedCalc test, there is a statistically significant link (Sig = 0.003) between the presence in one of the two groups and the presence of chronic venous insufficiency. There are 78 patients with chronic venous insufficiency: 15 (25.42%) in the first group and 63 (48.46%) in the second group. Cont. Coef. = 0.212 is the strength of that link. Sig = 2.758 and OR = 0.003, therefore it is statistically significant. Chronic venous insufficiency is more present in the second group.

By using Chi square analysis and OR estimation performed with SPSS and MedCalc, we analyzed the relationship between variables, specifically the risk factors present in the database, considered to be risk factors for DVT, for each group separately (Table 3).

We obtained statistically significant values:

○ For Varicose veins, there is a statistically significant link (Sig = 0.016) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.173 is the strength of that link. OR = 2.471 and Sig = 0.018, therefore it is statistically significant. Varicose veins are more common in the second group.

○ For Congestive heart failure, there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.352 is the strength of that link. OR = 63.328 and Sig = 0.004, therefore it is statistically significant. Congestive heart failure is present only in the second group.

○ For Fractures before admission, there is a statistically significant link (Sig = 0.006) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.197 is the strength of that link. OR = 0.326 and Sig = 0.007, therefore it is statistically significant. Fractures before admission are more common in the first group.

○ For Smoking, there is a statistically significant link (Sig = 0.027) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.159 is the strength of that link. OR = 0.494 and Sig = 0.028, therefore it is statistically significant. Smokers are more present in the first group.

○ For HTN (hypertension), there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.499 is the strength of that link. OR = 53.833 and Sig <0.001, therefore it is statistically significant. HTN is present almost entirely in the second group.

Table 3. Analysis of risk factors by age group.

Risk factors	≤ 50 years		> 50 years		Chi square analysis		Odds ratio	
	No N (%)	Yes N (%)	No N (%)	Yes N (%)	Cont coef	Sig	OR	Sig
Immobilization before admission/hospitalization	47 (79.66%)	12 (20.34%)	88 (67.69%)	54 (41.54%)	0.122	0.091	1.869	0.094
Varices	48 (81.36%)	11 (18.64%)	83 (63.85%)	47 (36.15%)	0.173	0.016	2.471	0.018
History of VTE	40 (67.80%)	19 (32.20%)	91 (70.00%)	39 (30.00%)	0.022	0.761	0.902	0.761
PE at admission	53 (89.83%)	6 (10.17%)	124 (95.38%)	6 (4.62%)	0.105	0.147	0.427	0.157
History of PE	55 (93.22%)	4 (6.78%)	125 (96.15%)	5 (3.85%)	0.064	0.380	0.550	0.386
Obesity	35 (59.32%)	24 (40.68%)	84 (64.62%)	46 (35.38%)	0.051	0.485	0.799	0.485
Congestive heart failure	59 (100.00%)	0 (0.00%)	85 (65.38%)	45 (34.62%)	0.352	<0.001	63.328	0.004
COPD or pulmonary cond.	49 (83.05%)	10 (16.95%)	111 (85.38%)	19 (14.62%)	0.030	0.680	0.839	0.680
Anemia	44 (74.58%)	15 (25.42%)	81 (62.31%)	49 (37.69%)	0.119	0.099	1.774	0.101
Fractures prior to admission	44 (74.58%)	15 (25.42%)	117 (90.00%)	13 (10.00%)	0.197	0.006	0.326	0.007
Smoking	30 (50.85%)	29 (49.15%)	88 (67.69%)	42 (32.31%)	0.159	0.027	0.494	0.028
HTN	57 (96.61%)	2 (3.39%)	45 (34.62%)	85 (65.38%)	0.499	<0.001	53.833	<0.001
Peripheral artery disease	59 (100.00%)	0 (0.00%)	113 (86.92%)	17 (13.08%)	0.207	0.004	18.348	0.044
Type 2 diabetes	56 (94.92%)	3 (5.08%)	85 (65.38%)	45 (34.62%)	0.300	<0.001	9.882	<0.001
Lipid alterations	39 (66.10%)	20 (33.90%)	67 (51.54%)	63 (48.46%)	0.135	0.062	1.834	0.063
Alcohol consumption	45 (76.27%)	14 (23.73%)	114 (87.69%)	16 (12.31%)	0.143	0.046	0.451	0.050
Extensive distal localization (calf)	11 (18.64%)	48 (81.36%)	9 (6.92%)	121 (93.08%)	0.174	0.015	3.081	0.019
Extensive proximal DVT localization	25 (42.37%)	34 (57.63%)	36 (27.69%)	94 (72.31%)	0.144	0.045	1.920	0.047
Patients with medical conditions	47 (79.66%)	12 (20.34%)	25 (19.23%)	105 (80.77%)	0.500	<0.001	16.450	<0.001
Patients with a history of surgical conditions	51 (86.44%)	8 (13.56%)	76 (58.46%)	54 (41.54%)	0.266	<0.001	4.530	<0.001
History of major gynecological surgical interventions	58 (98.31%)	1 (1.69%)	115 (88.46%)	15 (11.54%)	0.162	0.024	7.565	0.053
History of major urological surgical interventions	50 (84.75%)	0 (0.00%)	114 (87.69%)	16 (12.31%)	0.201	0.005	17.149	0.049
Hip or knee arthroplasty. Hip surgery	48 (81.36%)	11 (18.64%)	112 (86.15%)	18 (13.85%)	0.062	0.396	0.701	0.398
History of heart attacks	59 (100.00%)	0 (0.00%)	120 (92.31%)	10 (7.69%)	0.157	0.029	10.369	0.108
Renal conditions (CKD. Hydronephrosis renal lithiasis)	55 (93.22%)	4 (6.78%)	109 (83.85%)	21 (16.15%)	0.127	0.078	2.649	0.087
Neoplasia	57 (96.61%)	2 (3.39%)	95 (73.08%)	35 (26.92%)	0.265	<0.001	10.500	0.002
CVA (mainly associated with motor deficiency)	59 (100.00%)	0 (0.00%)	121 (93.08%)	9 (6.92%)	0.149	0.038	9.305	0.127

○ For Peripheral arterial disease, there is a statistically significant link (Sig = 0.004) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.207 is the strength of that link. OR = 18.348 and Sig = 0.044, therefore it is statistically significant. Peripheral artery disease is present only in the second group.

○ For Type 2 diabetes, there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and risk factors analyzed. Account. Coef. = 0.300 is the strength of that link. OR = 9.882 and Sig <0.001, therefore it is statistically significant. Type 2 diabetes is now almost entirely present in the second group.

○ For Alcohol consumption, there is a statistically significant link (Sig = 0.046) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.143 is the strength of that link. OR = 0.451 and Sig = 0.050, therefore it is statistically significant. Alcohol consumption is more present in the first group.

○ For Extensive distal location (calf), there is a statistically significant link (Sig = 0.015) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.174 is the strength of that link. OR = 3.081 and Sig = 0.019, therefore it is statistically significant. Extensive distal location (calf), is more present in the second group.

○ For Extensive proximal DVT localization, there is a statistically significant link (Sig = 0.045) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.1144 is the strength of that link. OR = 1.920 and Sig = 0.047, therefore it is statistically significant. Extensive proximal DVT localization is more present in the second group.

○ For Patients with medical conditions, there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.500 is the strength of that link. OR = 16.450 and Sig <0.001, therefore it is statistically significant. Medical conditions are highly present in the second group.

○ For Patients with a history of surgical conditions, there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.266 is the strength of that link. OR = 4.530 and Sig <0.001, therefore it is statistically significant. Surgical conditions are more present in the second group.

○ For History of major gynecological surgical interventions, there is a statistically significant link (Sig=0.024) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.162 is the strength of that link. OR = 7.567 and Sig=0.053, therefore it is statistically significant. The major gynecological surgical interventions are more present in the second group.

○ For History of major urological surgical interventions, there is a statistically significant link (Sig=0.005) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.201 is the strength of that link. OR = 17.149 and Sig=0.049, therefore it is statistically significant. The major urological surgical interventions are present only in the second group.

○ For History of heart attacks (myocardial infarctions), there is a statistically significant link (Sig=0.029) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.157 is the strength of that link. OR = 10.369 and Sig=0.108, therefore it is statistically significant. The heart attack is present only in the second group.

○ For Neoplasms, there is a statistically significant link (Sig<0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.265 is the strength of that link. OR = 10.500 and Sig=0.002, therefore it is statistically significant. Neoplasms are more present in the second group.

○ For CVA (mainly associated with motor deficiency), there is a statistically significant link (Sig=0.038) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.149 is the strength of that link. OR = 9.305 and Sig=0.127, therefore it is statistically significant. CVA is present only in the second group.

A logistic regression for age was performed with all these factors as covariates and the following result present in table 4 was obtained.

Table 4. Logistic regression for age

	B	S.E.	Wald	df	Sig.	OR
Immobilization before admission	1.020	0.997	1.047	1	0.306	2.774
Varices	0.179	0.678	0.070	1	0.791	1.197
History of VTE	-0.401	0.644	0.387	1	0.534	0.670
PE at admission	-0.186	1.098	0.029	1	0.865	0.830
History of PE	-1.460	2.001	0.532	1	0.466	0.232
Obesity	-0.602	0.647	0.867	1	0.352	0.548
Congestive heart failure	17.980	4725.744	0.000	1	0.997	6E+007
COPD or pulmonary conditions	***-2.264***	***1.010***	***5.024***	***1***	***0.025***	***0.104***
Anemia	-0.286	0.686	0.174	1	0.677	0.751
Fractures before admission	***-4.041***	***1.886***	***4.591***	***1***	***0.032***	***0.018***
Smoking	0.037	0.558	0.004	1	0.947	1.038
HTN	***4.046***	***1.528***	***7.011***	***1***	***0.008***	***57.163***
Peripheral artery disease	20.165	7631.510	0.000	1	0.998	6E+008
Type 2 diabetes	0.917	1.050	0.763	1	0.382	2.502
Lipid alterations	-0.479	0.730	0.430	1	0.512	0.620
Alcohol consumption	-0.635	0.739	0.738	1	0.390	0.530
Extensive distal location (calf)	0.021	0.496	0.002	1	0.965	1.022
Extensive proximal DVT location	0.088	0.544	0.026	1	0.872	1.092
Patients with medical conditions	1.028	0.832	1.527	1	0.217	2.797
Patients with a history of surgical conditions	1.953	1.083	3.250	1	0.071	7.048
History of major gynecological surgical interventions	-0.482	2.100	0.053	1	0.819	0.618
History of major urological surgical interventions	16.465	8232.574	0.000	1	0.998	1E+007
Hip or knee arthroplasty; hip surgery	-0.753	1.615	0.217	1	0.641	0.471
History of heart attacks	17.258	8314.948	0.000	1	0.998	3E+007
Renal conditions (CKD, hydronephrosis, renal lithiasis)	-0.120	0.882	0.019	1	0.892	0.887
Neoplasia	0.720	1.482	0.236	1	0.627	2.055
CVA (mainly associated with motor deficiency)	-2.435	12313.405	0.000	1	1.000	0.088

The Cox-Snell's multiple coefficient of determination R2 was 0.587 and Nagelkerke's was 0.782, which signifies that the model explains 78% of the variation in covariates distribution by age. The Hosmer and Lemeshow's test showed the significance Sig = 0.910, which means that the calculated model fits the included variables.

COPD (Sig = 0.025, OR = 0.104) and fractures before admission (Sig = 0.031, OR = 0.018) occur mainly in patients under 50 years old, while hypertension (Sig = 0.008, OR = 57.163) manifests especially in patients over 50 years old.

A logistic regression for SVT was performed by age, gender, origin, venous ulcers, edemas, various peripheral trophic disorders (brown or reddish skin depigmentation, fibrous and indurated skin, redness, irritation or dermatitis), diffuse cellulitis and chronic venous insufficiency as covariates and the following result was obtained and are presented in Table 5.

Table 5. A logistic regression for SVT was performed by age, gender, origin, venous ulcers, edemas, various peripheral trophic disorders

	B	S.E.	Wald	df	Sig.	OR
Age	***-0.800***	***0.339***	***5.580***	***1***	***0.018***	***0.449***
Sex	-0.275	0.355	0.601	1	0.438	0.760
Origin	0.459	0.426	1.161	1	0.281	1.582
VU	0.738	0.491	2.256	1	0.133	2.091
Edemas	-1.059	0.682	2.413	1	0.120	0.347
Various trophic disorders	0.221	0.522	0.180	1	0.672	1.247
Cellulitis	0.854	0.705	1.469	1	0.225	2.349
CVI	***1.781***	***0.434***	***16.827***	***1***	***0.000***	***5.938***

The Cox-Snell's multiple coefficient of determination R2 was 0.316 and Nagelkerke's was 0.422, which means that the model explains 42% of the variation in covariates distribution by SVT. Hosmer and Lemeshow's test showed the significance Sig = 0.464 which means that the calculated model fits the included variables. The age (Sig = 0.018, OR = 0.449) shows that SVT is manifested especially in patients under 50 years old. CVI (Sig <0.001, OR = 5.938) occurs predominantly in patients with SVT.

DISCUSSION

Thrombosis, as described by Virchow's triad, occurs as a result of hypercoagulability, endothelial damage or stasis, or a combination of these all. The risk of VTE for each patient should be individually assessed when prophylaxis is needed. Universal preventive guidelines were made with difficulty due to differences between individuals, such as age, medical history and social history [10]. DVT prevention by using thrombo-prophylaxis, effective in patients with high risk of recurrence and the minimization of the risk of DVT reduce the frequency of post-thrombotic syndrome (PTS). The identification of the patients at high risk for PTS, the assessment of the role of thrombolysis in preventing PTS and the optimal evaluation of compression stockings in preventing and treating PTS are issues that should be the concern for future research, as well as researching and evaluating the new therapies for treating PTS. The post-thrombotic syndrome (PTS) is the most common complication of deep vein thrombosis (DVT), it is cumbersome and expensive for patients and for the community, because of its high prevalence, severity and chronicity and has received little attention from clinicians and researchers [11].

Initially, venous thromboembolism (VTE) was perceived as a complication of hospitalization for major surgery or was associated with late-stage terminal illness, but studies have shown a risk of VTE in hospitalized patients with medical conditions comparable to those seen after major general surgery. The epidemiological studies have shown that between one quarter and one half of all symptomatic VTE, clinically recognized, occur in people who are not either hospitalized or recovering from a major illness, which involves expanding the populations at risk that could benefit from prophylaxis and challenges doctors to carefully examine the risk factors for VTE [12].

Clinical evaluation for the diagnosis of venous thrombosis in itself cannot be invoked for patient management but remains useful in determining the need for further testing, namely impedance plethysmography, which is particularly useful in excluding DVT in patients with suspicious signs and symptoms. The medical history for identifying risk factors for VTE is as important as physical examination [13]. When clinical probability is estimated before the diagnostic tests, the diagnostic accuracy for DVT is improved. The patients who experience low clinical probability of DVT have a prevalence of less than 5% and the diagnosis of DVT can be excluded without using ultrasound, while for the patients with clinical suspicion of DVT the results should not affect clinical decisions [14].

Patients with symptomatic DVT may present an increase in volume of the affected limb, spontaneous pain or on palpation in the calf or thigh muscles, increased pain on the flexion of the foot (the Homans sign), high temperature and purplish coloration of the affected lower limbs. Less than 10% develop severe symptoms including thrombophlebitis, pain, swelling, leg ulcers or skin induration [15].

The present study has shown that DVT is more common in patients over 50 years old and it is more frequent in men and in patients who have the urban environment as origin.

Despite the anticoagulation treatment, VTE recurs frequently in the first few months after the initial event, with a recurrence rate of ≈7% at 6 months. Death occurs in ≈6% of DVT cases and 12% of PE cases within 1 month of diagnosis. The seasons of the year may affect the occurrence of VTE, with a higher incidence in winter than in summer. Early mortality after VTE is strongly associated with the presentation as PE, advanced age, cancer and underlying cardiovascular disease [2]. Despite universal thromboprophylaxis, patients with critically severe surgical or medical conditions remain at risk for deep vein thrombosis of the lower limbs [1].

VTE is rare in adolescents and it is predominantly a disease of old age. The incidence of deep vein thrombosis and pulmonary embolism increases exponentially with age [16]. Venous thromboembolism is a major national health problem, especially among the elderly. While the incidence of DVT remains the same for men and increases for older women, the incidence of pulmonary embolism has decreased over time [17]. The incidence rates after the age of 45 years old are generally higher in males, while the incidence rates in women are somewhat higher during the reproductive years. For both sexes, with increasing age, PE represents a growing proportion of VTE [17]. The annual rate of venous thromboembolism events has increased despite the progress made in the identification, prevention and treatment. These increases may be due to the increased sensitivity of diagnostic methods, especially for PE. This fact implies that the current prevention and treatment strategies are less than optimal [18].

Currently, the prophylaxis is both mechanical and pharmacological. The goals of the treatment are to prevent the spread of thrombosis, pulmonary embolism, thrombosis recurrence and the development of complications such as post-thrombotic syndrome and pulmonary hypertension.

CONCLUSIONS

Thromboprophylaxis could have a tremendous potential if it was efficiently and optimally administered to patients at risk of venous thrombosis. Understanding the risk factors and epidemiology of the first and recurrent venous thrombosis enables the optimal and efficient use of prophylactic strategies against VTE and would prevent post-thrombotic syndrome and improve clinical outcomes in practice.

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ORIGINAL ARTICLES

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New synthesized oximes active in nerve agents' hazards

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Abstract: *Object: The aim of the study is to select the most active new imidazolium-quinuclidinum-oxime, from some similar chemical compounds synthesized in our chemistry department, with sufficient efficacy to decrease the acute toxicity of neurotoxic organophosphates known as nerve agents. Method: The experimental study consist in vivo testing the antidotal efficacy of obidoxime and of selected imidazolium oximes synthesized in our chemistry department. Each oxime was included, by equimolar replacing the obidoxime, in an antidotal formula, which also contains atropine. The above mentioned formula containing atropine and obidoxime was used as reference. The protective ratio, defined as the ratio between the lethal median dose of the poisoned and treated study group and the median lethal dose (LD50) of the poisoned and untreated study groups was one of the used parameters in order to select a new active chemical structure in counteracting the neurotoxic organophosphorus compounds acute toxicity. Another studied parameter was the erythrocyte acetylcholinesterase value measured in whole blood 24 hours after exposure. Results: The protective ratio against an organophosphorus compound were the follow: obidoxime chloride: 2; 1,3-dimethyl-2-hydroxyethyl-imidazolyliodide: 1,75;3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl]quinuclidin-dichl-oride: 2,5; 1-methyl-quinuclidin-3-iodide: 1,5. The erythrocyte acetycholinesterase main values were the following: the unpoisoned and untreated study group:3,45 ±0,13mmol/dl; the poisoned and untreated study group: 0,89 ±0,09 mmol/dl; the poisoned and 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl]quinuclidindichloride treated study group:2,89 ±0,11 mmol/dl; the poisoned and obidoxime treated study group: 2,53±0,15 mmol/dl. Conclusions: 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl] quinuclidindichloride synthesized in our chemistry department, has shown a better protective ratio and a more prolonged surviving time than the reference (obidoxime). It has shown the best AChE reactivation of all the synthetized compounds. This compound can be a cheap and good option for replacing obidoxime in the antidotal formula active in nerve agent exposure.*

Keywords: *obidoxime chloride; 1,3-dimethyl-2-hydroxyethyl-imidazolyl-iodide;3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl] quinuclidin dichloride; 1-methyl-quinuclidin-3-on-iodide; organophosphorus compounds; nerve agents*

INTRODUCTION

Strategic expert analyzes have shown that in the next period, there may be international conflicts, amplifying the risk of attempts to use highly toxic chemicals as weapons of mass destruction.[1]

Romania through its geostrategic position, being the member of NATO, can provide a better potential target of such actions.

Neurotoxic organophosphorus compounds (sarin, soman, tabun, most insecticides) are chemical compounds, phosphonic acid esters with central and

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peripheral properties, acetyl-cholinesterase and butyryl-cholinesterase irreversible inhibitors with toxic effects on smooth and striated muscles and central nervous system.[2-4]

They were registered on the list of substances to be destroyed in accordance with international regulations (Convention on the Prohibition of Development, Production and Stockpiling Chimice-Geneva).

Although banned by international conventions, these toxic substances have been used in terrorist attacks against civilians (Tokyo 1995 Halabja in 1987, Anfal-1988). In this context geostrategic and due to soaring use of organophosphorous insecticides, development of medical measures to protect against the lethal toxicity is one of the priorities of research within specialized programs of NATO (NBC – Science for Peace) and European Union (Chemical Program Weapons, Monitoring and Protection, created in the OPCW).

The acute toxicity of organophosphorus compounds known as nerve agents mainly result from their action as irreversible inhibitors of acetylcholinesterase (AChE). They suffer some conformational and chemical changes, resulting a phenomenon known as "aging", whose speed of appearance is directly proportional to the toxicity of compounds.[5,6]

Accumulation of acetylcholine stimulation leads to persistent cholinergic muscarinic receptors that trigger the syndrome whose symptoms include miosis, salivation, bronchial hypersecretion, bradycardia, bronchoconstriction, hypotension and diarrhea.[7]

Another effect of organophosphate anticholinesterases is the desensitization of nicotinic receptors followed by overstimulation, translated by skeletal muscle twitching and subsequent paralysis.[8] Central nervous system toxic effects include anxiety, restlessness, confusion, ataxia, tremors, convulsions, paralysis, cardiorespiratory effects and coma [9].

The treatment of nerve agents poisoning is based on an antimuscarinic agent (atropine), and an acetylcholinesterase reactivator called oxime according to its chemical structure. Atropine blocks the effects of accumulated acetylcholine resulting overstimulation of muscarinic receptors.[9,10]

Acetylcholinesterase reactivators dephosphorylate the acetylcholinesterase – organophosphorus complex, reactivating the enzyme activity.[11] The early appeared seizures were counteracted by benzodiazepines.

Currently available oximes (pralidoxime, obidoxime), have been shown to be less effective against one of the most toxic nerve agents (soman tabun). There is a strong interest in developing new, more potent acetyl cholinesterase reactivators with oxime structure.[12]

The present paper represents an in vivo screening study in selecting more potent chemical compounds, active in counteracting the nerve agents acute toxicity. The paper describes an experimental test of antidotal efficiency of some compounds containing imidazolium, or quinuclidinium rings, which equimolar replace obidoxime chloride in the antidoltal formula.

The results were expressed as the protective ratio representing the ratio between the DL50 of the neurotoxic compound administred to the poisoned and treated rat study group and the DL50 administered to the organoposphate compound of the poisoned and untreated study group.

MATERIAL AND METHODS

Chemicals

- obidoxime chloride (CAS number 111-90-9);
- atropine sulphate (CAS number 5908-99-6) were purchased from Sigma Aldrich;
- diclorvos (PESTANAL CAS number 62-73-7) were purchased from Sigma Aldrich.
- the experimentally tested quinuclidinium-imidazolium oximes were synthetized in the chemistry department of Medical Military Research Center.

Animals

Male Wistar rats (150-200g) were maintained on rice husk in polypropylene cages. Wistar free access to water and rodent pellet food. The study was

approved by the Ethical Committee on Animal Experimentation.

Acetycholinesterase measurement method: Elisa kit.

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Acetylcholinesterase in human serum, plasma and other biological fluids.

Target Information: Acetylcholinesterase hydrolyzes the neurotransmitter, acetylcholine at neuromuscular junctions and brain cholinergic synapses, and thus terminates signal transmission. It is also found on the red blood cell membranes, where it constitutes the Yt blood group antigen.

Figure 1: The chemical structure of the in vivo tested compounds as acetycholinesterase reactivators

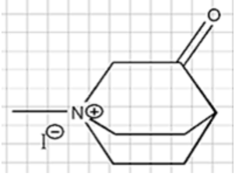

1methyl-quinuclidine-3-on-iodide

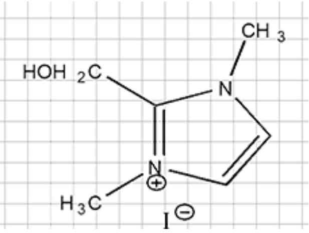

1,3-dimethyl-2-hidroxyethyl-imidazolyl-iodide

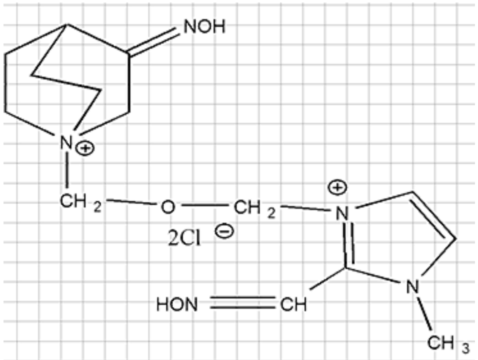

3oxim[3(2-hidroxy-iminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride

Treatments

120 Wistar rats were divided into 12 groups, each, including ten rats as follows:

- 1st group: control group unpoisoned, untreated
- 2nd group: poisoned with diclorvos (1,5 DL50) and untreated;
- 3rd group: poisoned with diclorvos (1,5 DL50,) after one minute, were administered the antidotal formula including atropine (2mg) and obidoxime chloride (250 mg);
- 4th group: poisoned with diclorvos (1,5 DL50), after one minute, were administered the antidotal formula including atropine (2 mg) 1methyl-quinuclidine 3-on iodide in an equimolar dose with obidoxime chloride (78,5 mg);
- 5th group: poisoned with diclorvos (1,5DL50,) after one minute were administered the antidotal formula including atropine (2 mg), 1,3- dimethyl-2-hidroxy-ethylimidazolyl iodide in an equimolar dose with obidoxime chloride (61,94 mg/kg);
- 6th group: poisoned with diclorvos (1,5 DL50), after one minute, were administered the antidotal formula including atropine (1,5 mg) and 3oxim[3(2-hidroxy-iminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride (118 mg/kg).

The above mentioned oximes were experimentally tested against 1.5, 1.75, 2, 2.5, DL50.

24 hours after poisoning mortality, protective ratio was registered and erythrocyte achetylcolinesterase activity values were measured.

RESULTS

The aim of the study is to evaluate the most active newly synthesised imidazolquinuclidine-oxime with the great efficacy in reactivating the phosphorylated acetylcholinesterase through some similar chemical compounds synthesized in our chemistry department, able to decrease acute neurotoxic compounds toxicity.

The protective ratio of the obidoxime and experimentally tested formulas correlated with their efficacy as acetylcholinesterase reactivators are represented in the Table 1.

The 3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2-oxapropyl] quinuclidine dichloride protective ratio correlated with its acetylcholinesterase reactivator activity is greater than the obidoxime, resulting a

better antidotal efficacy.

Statistical analysis Student's t-test probability associated with statistically significant differences between mean values of erythrocyte acetyl-cholinesterase between the control study group and the poisoned and treated study groups is represented in tables 2-7.

The poisoned and untreated group statistically significantly differs from normal in terms of the acetylcholinesterase inhibition.

Neurotoxic organo-phosphorous compounds cause in the intoxicated group and untreated study group an acetylcholinesterase inhibition of 75.08%, incompatible with survival.

Obidoxime and new synthesized imidazolium-quiniclidinium oximes highlighted new capabilities of acetylcholinesterase reactivation correlated with the protection index.

3-oxime compound [3-(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl]-quinuclidine-dichloride-quinuclidine 3-on iodide revealed a protection index bigger than other obidoxime and other imidazolium compounds studied.

Explanation of this is that the quinuclidine ring, by its position intensifies allosteric oxime group (Table 1).

Tables 2-7 reveals that the intoxicated untreated control group differs statistically significantly from normal and so the intoxicated study groups were treated with different therapeutic formulations.

Table 1: The protective ratio of the antidotal formulas containing the experimentally tested imidazolium oximes as AChE reactivators

Study group	**Acetylcholinesterase reactivator**	**Dose of reactivators** (mg/kg)	**Protective ratio** (DL_{50})	**Acetylcholinesterase reactivation due to oxime** (%)
2	-	-	-	
4	1methyl-quinuclidine 3-on iodide	78.58	1.5	47.82
8	1,3-dimethyl-2-hidroxyethyl-imidazolyl iodide	61.94	1.75	60.28
10	obidoxime chloride	100	2	73.33
12	3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride	118.88	2.5	82.31

Table 2: Statistical analysis of the probability P (T test) associated with average values of erythrocyte AchE of unpoisoned and poisoned and untreated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
1	3.45 ± 0.13	0.003	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 1: unpoisoned and untreated study group;
Study group 2: paraoxon poisoned and untreated study group

Table 3: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and obidoxime treated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
10	2.53 ± 0.15	0.007	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 10: paraoxon poisoned and obidoxime treated study group;
Study group 2: paraoxon poisoned and untreated study group.

Table 4: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and 1,3-dimethyl-2-hidroxyethylimidazolyl iodide treated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
8	2.01 ± 0.07	0.004	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 8: paraoxon poisoned and 1,3-dimethyl-2-hidroxyethylimidazolyl iodide treated study group;
Study group 2: paraoxon poisoned and untreated study group.

Table 5: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and 1methyl-quinuclidine 3-on iodide study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
4	1.65 ± 0.03	0.009	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 4: paraoxon poisoned and iodide treated study group;
Study group 2: paraoxon poisoned and untreated study group

Table 6: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and 3-oxim[3(2-hidroxyiminomethyl-1- imidazolyl)-2oxapropyl] quinuclidine dichloride- quinuclidine 3-on iodide treated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
12	2.84 ± 0.05	0.0002	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 12: paraoxon poisoned and 3-oxim[3(2- hidroxyiminomethyl-1- imidazolyl)-2oxapropyl] quinuclidine dichloride- quinuclidine 3-on iodide treated study group;
Study group 2: paraoxon poisoned study group

Table 7: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, obidoxime treated and 3-oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride-quinuclidine 3-on iodide treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
12	2.84 ± 0.05	0.01	P ≤ 0.05 statistically significant difference between groups
10	2.53 ± 0.15		

Legend:

Study group 12: paraoxon poisoned and 3-oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride-quinuclidine 3-on iodide treated study group;

Study group 10: paraoxon poisoned and obidoxime treated study group

Table 8: The correlation between administered dose of acetylcholinesterase reactivator and the pharmacodynamic effect

Study group.	Reactivator dose used in the antidotal formula (mg)	AChE Mmol/ml	Correlation coeficicient
4	61,94	1,65	0,9927
8	78,58	2,01	
10	100	2,53	
12	118,88	2,84	

Legend:

Study group 4: poisoned study group and 1methyl-quinuclidine 3-on iodide treated;

Study group 8: poisoned study group and 1,3-dimethyl-2-hidroxyethyl-imidazolyl iodide treated;

Study group 10: poisoned study group and obidoxime chloride treated;

Study group 12: poisoned study group and 3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride treated;

From a medical standpoint, an organophosphoric nerve poisoning that caused inhibition of acetyl-cholinesterase survival limit was applied.

This intoxication was further antagonized with different formulas containing the synthesized imidazoliumquinuclidinium oximes and obidoxime as antidotes.

They showed mean values of acetylcholinesterase significantly different from the poisoned and untreated group. The studied imidasolium quinuclidinium oximes showed average values of acetylcholinesterase statistically significantly different between them, thus emphasizing that the doses used may cause significant variations in therapeutic effect.

Table 8 highlights the so-called "dose finding", the correct correlation between dose and pharmaco-dynamic effect. The correlation coefficient of 0.9927 demonstrates that doses of acetylcholinesterase reactivators used in the antidotal formulas are correct, being those that cause maximum pharmacodynamic effect.

CONCLUSION

- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride synthesized in the Medical Military Research Centre Chemistry Department has shown a better protective ratio and a more prolonged surviving time than obidoxime considered as reference.
- It has shown the best acetylcholinesterase reactivation of all the synthetised compounds and obidoxime
- The very good correlation dose-effect highlights that the correct dose of acetylcholinesterase reactivator was chosen in order to obtain the better pharmacodynamic effect.

- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride can be considered an efficient antidote in neuroparalytic organophosphorous hazards.

- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride can be a cheaper and better option for replacing obidoxime in the antidotal formula active in nerve agent poisoning.

- Thus one can conclude that the result of the experimental study is consistent with the proposed object.

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Ethical considerations in sudden unexpected death in epilepsy (SUDEP)

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Abstract*: Epilepsy is one of the world's oldest diseases. Social stigma, misunderstanding and thus, discrimination have surrounded patients and their families from the beginnings until nowadays. Approximatively up to 80% of epilepsy cases worldwide are found in developing regions. The risk of premature death is two to three times higher than for the general population. There is contradictory evidences concerning the question of whether to inform patients about the possibility of sudden unexpected death in epilepsy (SUDEP). Actual guidelines states that individuals with epilepsy and their families or careers should be given access to information on SUDEP. We have information about how, when and what to say to the patients and families about SUDEP. But it's a delicate subject, and some patients do not want to know that they are at risk for this.*

Keywords: *epilepsy, SUDEP, ethics*

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INTRODUCTION

Despite age, racial, social, geographic or national boundaries, epilepsy remain a prevalent chronic neurological disorder.

The incidence of epilepsy was estimated at 24-53 per 100,000 people.

World Health Organization (WHO) estimates that around 50 million people worldwide have epilepsy, 80% from developing regions. Epilepsy is charactererized by recurrent seizures due to excessive electrical discharges in a group of different parts of the brain cells. It is consider that up to 10% of people worldwide have one seizure during their lifetimes. Epilepsy is defined by two or more unprovoked seizures. Only one fourth of affected people in developing countries get the treatment they need and only 70% of these respond to medication. Mortality is higher in patients with epilepsy than in general population. People with epilepsy and their families can suffer from stigma and discrimination in many parts of the world. For example in China and India, epilepsy is a reason for prohibiting or annulling marriages.

In the United Kingdom, a law forbidding people with epilepsy to marry was repealed only in 1970. In the USA, until the same years, it was illegal that people with seizures have access to restaurants, theatres,

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recreational centers and other public buildings. Even nowadays patients have reduced access to health and life insurance, to obtain a driving license, to get a job or have other limitations.[1-5] Still now a person may be identified as an ‘‘epileptic” rather than ‘‘a person with epilepsy”.[6] SUDEP is the second death cause in epilepsy after status epilepticus. Unusually, the diagnostic of SUDEP is retrospective. Is defined as sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death in patients with epilepsy, with or without evidence of a seizure and excluding documented status epilepticus, in which postmortem examination does not reveal a toxicological or anatomical cause of death. [7] It is responsible for 7.5–17% of all epilepsy deaths and has an incidence among adults between 1:500 and 1:1000 patients per year. [8] Epileptologists agreed that SUDEP is mainly, but not exclusively, a problem for patients with refractory epilepsy. Epilepsy-related mortality is a significant risk in pregnancy, 1:1000 women died from epilepsy (mostly SUDEP) during or shortly after pregnancy. [9] SUDEP has an estimated annual incidence rate of 0.81 cases per 100000 population, or 1.16 cases per 1000 patients with epilepsy. Comparing years of potential life lost from SUDEP with selected other neurologic diseases, SUDEP ranks second only to stroke. [4] There are some risk factors like: generalized tonic-clonic seizures (GTCS), nocturnal seizures, variability of EEG records, and duration of the disease ranging from 15 to 20 years, early onset of epilepsy, poly-medications, cold temperatures, alcohol abuse, and street drugs. [10-14] It has been suggested that the most common pathogenic mechanism underlying SUDEP is heritable arrhythmogenic syndromes, or cardiac channelopathies, such as familial long QT syndrome (LQTS).[15,16] LQTS associated with syncope, seizures and sudden cardiac death is caused by mutations in more than 10 genes, encoding potassium and sodium ions channels.

Among cardiac arrhythmias, respiratory dysfunction, neurogenic pulmonary edema and dysregulation of systemic and cerebral circulation are other proposed pathophysiological events implicated in SUDEP.[17-19]

ETHICS ON SUDEP

There is a reasonable question about SUDEP: must it be discussed with all patients with epilepsy who are at risk of SUDEP, or not? The Task Force of the American Epilepsy Society and the Epilepsy Foundation have guidelines concerning what, how, when SUDEP should be discussed with patients, their families and caregivers. In Europe, the National Institute of Clinical Excellence (NICE) has the same attitude. [3]

The topic of SUDEP involves many moral dilemmas, most of which do not have absolute solutions. Advocates of universal SUDEP counseling cite the “right to know,” but others point to the “right not to know”. There is neither empirical evidence nor consensus on the question of whether to inform patients and parents about the possibility of SUDEP.[20]

Arguments to inform patients about SUDEP

In recent times there has emerged a debate regarding the obligation to warn even newly diagnosed patients of the risk of SUDEP. If there is a reasonable chance of preventing SUDEP, it must be discussed with all patients with epilepsy who are at highest risk of SUDEP. A few strategies that patients with epilepsy and their careers can take to reduce death risk could then be share by the physician.

On the other hand, the term “unexpected” is improper used because in some patients with risk factors SUDEP could be expected.[7] Precise definition and classification of SUDEP is necessary to scientific and medical communication, and is also important from a legal point of view. The new classification consists of six classes and definitions:
1. Definite SUDEP
1a. Definite SUDEP Plus (preexisting condition could have contributed to the death),
2. Probable SUDEP, (meets criteria for SUDEP, but no postmortem examination was done to exclude another pathologic process)
3. Possible SUDEP (postmortem examination may have identified drowning)
4. Near SUDEP (cardiorespiratory arrest that is successfully resuscitate),

5. Not SUDEP when a clear cause of death is known.
6. Unclassified: incomplete information available; not possible to classify

The patient suffering from epilepsy is, in many respects, no different to any other patient being treated by medical practitioner. [21] The ethical principle of patient autonomy in health care involve the patient's right to know about medical condition and prognosis. Information should be provided promptly to patients, their families and caregivers if they ask about the potential adverse consequences of the seizures or about the mortality risk associated with epilepsy.

In learning about SUDEP, parents expressed a need to be informed of the risk of that. There was a consensus that it should be the parents' decision as to whether or not the child should be present at the meeting or when to inform the child about the risk of SUDEP.[22]

In some cases the risk of SUDEP may need to be emphasized to encourage compliance with medical and surgical therapy for epilepsy. Recent evidence from a meta-analysis of randomized clinical trials of adjunctive AEDs at efficacious doses provides strong support for AED treatment as mono- or polytherapy to increase seizure control and protect against SUDEP in patients with refractory epilepsy.

For patients for whom seizure control is unattainable, supervision or monitoring may prevent SUDEP, though this has never been formally tested. [15] Increasing awareness of SUDEP may facilitate improved seizure control and possibly decreasing SUDEP incidence.[23]

Furthermore, SUDEP discussion can be encouraging to patients with very low SUDEP risk. Patients with absence epilepsy or benign epilepsy syndromes must know that their risk of SUDEP is negligible.

Arguments for not informing patients about SUDEP

By low, failure to discuss SUDEP with a patient suffering from epilepsy cannot constitute negligence because the outcome cannot be as a consequence of the actions of the doctor based upon current knowledge.

Mainly, if the patient has not asked about his distress, there is not the basis for litigation against the doctor who chooses not to discuss this topic.[20]

Until now, no interventional measures are known to stop the outcome, so we get nothing by warning of SUDEP. To admit the SUDEP, it may seriously deteriorate quality of life. In this case the doctor may deliberately omit information to avoid patient fear and anxiety, respecting in this way the right „do not harm”. The negative influence on quality of life may represent a form of negligence.

It may be possible to show causal connection between impaired quality of life and the doctor divulging information that the patient did not explore. Polymedication is an important risk factor for SUDEP, but a necessary intervention for the epilepsy management. So many patients requiring polytherapy have been refused it for fear of SUDEP.[14]

CONCLUSIONS

Duty of care dictates open and frank discussion if the patient wishes information about mortality and epilepsy. The big dilemma concerning ethics considerations in SUDEP is: the ‘‘right to know’’ or the converse which is the ‘‘right not to know’’?

This conflict, places the clinician in a serious ethical difficulty because it requires the balancing of these diametrically opposed concepts and demands a value judgement on the part of the clinician. SUDEP is essentially unpredictable for any individual patient. It also confirms that ‘‘never’’ and ‘‘always’’ are dangerous terms when used by doctors.

To assure the patient of something which cannot be assured may represent another form of negligence and failure of duty of care. The doctor provide the patient with accurate and informed response to questions raised .Where the detailed information will not alter the outcome for the patient, failure to provide it cannot be deemed to represent negligence.

The ethical consideration is met if the doctor tries to ascertain what the patient wants to know and responds accordingly by providing that information which the patient requests. Thus, each case must be managed individually and doctors are advised to document the decision-making process. The patient care is like a balance between what the career offers, and what the patient accepts.

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Pericardium – An editorial success

Teodor Horvat[1]

PERICARDIUM. Anatomy, physiology, pathophysiology, pathology and surgery

By Teodor Horvat and Daniel Fudulu

Why did I write this book? I keep asking myself this question... Why did I write it? Maybe because cardiovascular surgery was my first rotation during my residency that I started after graduating with a first honors degree (gold medal), from the Faculty of Medicine Bucharest in 1975.

My destiny in the winter of 1976, was to be allocated to start my first rotation in the newly formed Military Department of Cardiovascular Surgery, Fundeni Clinical Hospital, under the supervision of Lieutenant Colonel (Lt Col) Dr Vasile Cândea. Because this unit was not fully functional, I started working under Professor Ioan Pop D. Popa and then in the Vascular Surgery Department where I had the opportunity to meet a brilliant, master surgeon – Professor Tiberiu Ghițescu. Finally, I started working in the Military Department of Cardio-vascular Surgery in August 1976, 7 months after the completion of the official paperwork certifying the opening of this new department.

At the beginnings, we were just a few of us, Lieutenant Colonel (Lt Col) Dr Vasile Cândea and Lieutenant Dr Horvat. Later, Dr Richard Florescu, a non-military doctor and my medical school colleague joined us. He immigrated later to Germany. After a few months Captain Dr Ion Țintoiu – cardiologist, Captain Dr Alexandru Popa – anesthetist, Major Dr Ioan Condor surgeon and Captain Dr Ioan Mociorniță – surgeon joined us. Our senior scrub nurse was Mrs Rodica Poreceanu and she was an exceptional first assistant. I've rarely seen such manual dexterity that she had. So, I started as a second assistant while she was the first assistant for Dr Cândea. I have learned a lot from her. She was unique. I have never met a

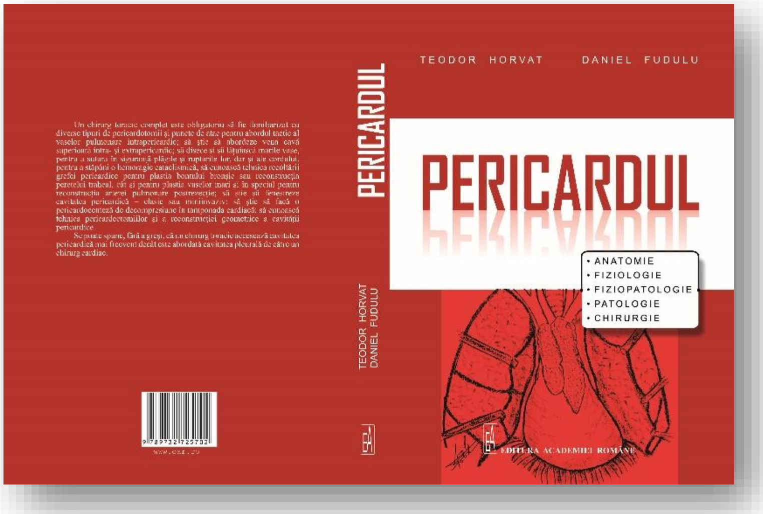

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clone of her.

Week by week our department grew and this culminated with the first open heart operation – an atrial septal defect that was corrected in the February of 1977. The operation was a success. All of us were very cheery until the great disaster came – March the 4th, 1977 – The Great Earthquake.

Both the old and new Fundeni Hospital buildings were affected; the old building was in a terrible state. I remember our department being affected very badly, the interior walls were full of cracks because this was located on level 2 where the flexion and extension of the walls happened. Not even level 3 – Professor Ghițescu's Vascular Surgery Department was spared from these cracks. Above level 4, there was less damage. The old building was beyond any description. No medical treatments and of course no surgery could be performed there. All the departments were moved, in the end, into the new building, the most robust out of all.

The Department of General Surgery led by Professor Dan Setlacec had to move too. Here, in the new building, at floor level, in the building with three levels, I saw The Professor for the first time. He was going to supervise me later as general surgery resident. I've spotted Professor Dan Setlacec in a big group of young surgeons. I could see only his head, his short haircut and his bushy, unmissable eyebrows. That's what I could only see!

After the rubble was scooped and some renovation was undertaken, I went to see Lt Lieutenant Colonel (Lt Col) Cândea and I asked his permission to leave his department. He asked the reasons for leaving. I've had to explain: "I started building the "surgical house" with the roof rather than the" foundation". I have started in the "super-specialty" of Cardiovascular Surgery without having a basic surgical foundation. In other words, I have started without completing a general surgical rotation.

So, I left. We ended up our collaboration in good terms, and we kept in touch until today. I learned a lot from surgeon Cândea, both about surgery but also about interpersonal relations. I have seen and heard a lot in these 14 months not only amongst military doctors but more amongst civilians. They are only a few cardiac surgeons within this specialty but they there is an intense rivalry and hate between them. If I would put aside the parasites who used to copy their personalities, you had what to learn from these remaining doctors – both surgery and medicine.

I have worked directly, as an intern, both at the bedside and in the operating theatre with doctors outside the Military Departments such as: Professor Pop D. Popa, Clinical Lecturer, Dr Dan Făgărăsanu towards the end and with Dr Ilie Pavelescu who was going to become later a professor in Timisoara. I also worked with Dr Martin Constantinescu, a great loss for Romanian Surgery by his immigration, Dr Tiberiu Ghițescu – the great master, Dr Traian Stefănescu, Dr Francisc Proinov – Fundeni Hospital Medical Director, Dr Dan Mogoș, specialty doctor, later Professor of Surgery in Craiova, Dr Vasile Sârbu – specialty doctor later Professor of Surgery and Dean of Constanța Faculty of Medicine, Dr Radu Nemeș – specialty doctor, currently Professor of Surgery at Craiova, Dr Șoimaru – specialty doctor, Dr Roth – specialty doctor who later immigrated to Germany and many others.

I remember our top cardiologists: Dr Daniel Constantinescu, Dr Tudorică Popa, Dr Ion Ținotoiu, Dr Sichitiu and our devoted anaesthetists: Dr Aurelia Bălan ("Tanti"), Dr Teodora Petrilă, Dr Radu Făgarașanu and Dr Alexandru Popa – they have all taught me a lot.

I was in close relationship with the Department of Haemotology led by Professor Ștefan Bereceanu. I used to be very happy when my expertise was needed by: Dr Dan Coliță later Professor and Clinical Lead of the Haematology Department, by Dr Adriana Coliță – later clinical lecturer in Haematology, by clinical lecturer Dr Elena Butoianu, the second in the department hierarchy. I was happy because they were asking for my help, whom I was a beginner at that time, a non-initiated in the art of surgery. They used to refer patients for lymph node biopsies and this is how I became an operator, by doing lymph node, muscle and skin biopsies. Over the years, our work collaboration grew and this time they were no longer referring lymph node biopsies but complex mediastinal tumors with haematological implication.

But let us get back to my career story. I left Fundeni Hospital in March 1977, intern in surgery, and I returned specialty doctor in general surgery, to build up the foundations of my surgical house.

I have met Professor Dan Setlacec in November 1977. In January 1978, the General Surgical Department moved to the old building where I worked until the spring of 1980 when I moved to the Central Military Hospital, General and Thoracic Surgery Department II, led by Professor General Dr Traian Oancea. I first visited this department on Thursday, 9th of October 1969, when I was a first-year medical student, but I will write this story in another book.

In Professor Setlacec's department everyone was focused on work, on performance. There was no rivalry amongst the surgeons, it was a true school of general surgery that was overseen by The Professor. He was The Master, the conductor of the surgical orchestra. He was producing "true" surgeons. Here, I assisted daily in operations. Not one operation, but two, three even four major operations. We had no minor or simple procedures. I was the operator's "second hand ", the "first assistant" in English language. I have learned a lot from the surgeons in the old building. Here I started to build the foundations of my surgical school. I believe that every surgeon, regardless of the pursued specialty needs to learn the basic surgical skills, the principles of surgery in general surgery not in an "super-specialty", like I have started. Luckily, I have realized I made a mistake and I repaired it. I like to believe I made the right choice and that I have succeeded!

Because Professor Traian Oancea was also doing general thoracic surgery not only general surgery I was attracted by this specialty. Thoracic Surgery was also performed by other two surgeons there: Colonel Dr Therdor-Stefănescu Galați and Major Dr Gheroge Voicu – later promoted to lieutenant colonel and then colonel. Both of them were working in the department of thoracic surgery located on the 5th floor of the surgical building. I have never returned to the field of cardiovascular surgery, however, I have encountered and treated in my practice surgical conditions at border between the thoracic and cardiac surgery. I have always cultivated and consolidated my relations with the cardiovascular surgeons and anesthetists.

Now, let me return to my first question. Why did I write this book? Well… because sometimes I feel guilty and regret I have abandoned and never returned to cardiovascular surgery – it is an argument that we must consider. But, the most powerful reason is because as a pure thoracic surgeon I often used to cross the "pericardial border" during major lung or mediastinal surgery but also when addressing surgically diseases of the thoracic wall or the diaphragm.

We also need to take into consideration the malignant pericardial effusions that were associated to pleural effusions (both conditions having a common cause) that required concomitant surgical treatment, under the same anesthesia. Not only the associated pericardial effusions but also the isolated pericardial effusion caused by a malignant a condition are mainly treated within my specialty – thoracic surgery.

In addition to the points mentioned above, it is mandatory for a "complete" thoracic surgeon to be familiarized with the pericardiotomy approaches, to know how to approach the superior vena cave both intra- or extra-pericardially; he has to know how to dissect and isolate the major heart vessels in order to safely repair the various vascular or cardiac injuries but also to be able to control a cataclysmic surgical bleeding; he needs to know how to harvest a pericardial flap to reinforce the bronchial stump, to reconstruct the tracheal wall but also to do a patch repair of the major vessels – particularly patch angioplasty of the pulmonary artery; they have to know how to perform a pericardial window via classical or minimally invasive approach; they have to know how to perform pericardiocentesis to decompress a cardiac tamponade and finally to master the technique of pericardiectomy and "geometric" pericardial cavity reconstruction.

I can confirm, without doubts, that a thoracic surgeon approaches the pericardial cavity more often than a cardiac surgeon approaches the pleural cavity.

From what I remember seeing in my early years of surgical practice, there were thoracic surgeons that never crossed the pericardium. In other words, they used to declare tumors inoperable even if there was slight involvement of the extrapericardial pulmonary vessels. I have always asked myself asked myself why they do not dare to cross the pericardium; the explanation is in their early days of training. In Romania, thoracic surgery, as stated by Professor Alexandru Boțianu from Târgu Mureș, was born because of Professor Dr Cărpinișan and a disease of his time – tuberculosis. In such circumstances, thoracic surgery was born with a ʼʼlimpʼʼ, without the esophageal surgery but with the pericardial surgery for the relief of pericardial constriction due to tuberculosis. Unfortunately, after the founder of thoracic surgery passed away, in Romania, the interest in the pericardium had almost disappeared amongst his descendants.

In the year of 1986, while I was working at Filaret Hospital, under Prof Dr Constantin Coman, to complete my general thoracic surgery fellowship (e.g. after completion of my general surgery training), I have never witnessed the pericardium being opened to approach the intrapericardial pulmonary vessels.

I remember a day in 1987 when I was working in the Central Military Hospital and I had to scrub to assist Professor Traian Oancea to perform a left pneumonectomy for a lung cancer. The tumor appeared unresectable. Despite this, I persuaded my ex-boss and Professor to open the pericardium. After a moment of reflection, he turned to me and asked: ʼʼHave you ever been inside the pericardium?ʼʼ I responded without hesitation: ʼYes! I've been there before!ʼʼ He replied: ʼʼOK then, let's open it!ʼʼ It was the first intrapericardial dissection of the pulmonary vessels that I ever assisted and observed. The operation was a success.

Anyone who had seen Professor Oancea operating, remained deeply impressed, like me, of his delicate surgical gestures, of his control of the situation and his safeness and many others skills and attributes that I do not have enough space here to enumerate.

The years have passed and the work volume in thoracic surgery increased exponentially, with cases that were more and more difficult to tackle. However, I have never seen again a case being declared inoperable based on extrapericardial involvement. The majority of the pneumonectomies were performed using an intrapericardial approach.

Pericardial resections for lung tumors invading the pericardium had followed. In selected cases, we even performed atrial, mediastinal, chest wall or diaphragmatic resection associated with geometric pericardial reconstructions.

We admitted more and more cases of malignant pericardial effusions either isolated or associated to unilateral or bilateral pleural effusions and even peritoneal effusions.

In the Thoracic Surgery Department at the Central Military Hospital, that I led for 15 years, we were the first ones to perform nationally: pericardial-peritoneal fenestration, the pericardial-pleural window performed with the use of a endostapler (right and left), VATS pericardoscopy (right and left) and subxiphoid pericardoscopy.

I was unpleasantly surprised by a certain event, in addition to many other disappointments or harassments choreographed by the Central Military Hospital management. The first assistant who helped me perform the first subxiphoid pericardoscopy followed by a VATS pericardial resection, published this novel procedure under his own signature, in the ʻʻJournal of Military Medicineʼʼ – the case operated by me. I am going to say only this – I was helped by Dr Cornel Savu and this is the end of the story, and it is not worth expanding on this.

Certainly, all thoracic surgeons who trained in the Thoracic Surgery Department at the Central Military Hospital learned all the various pericardial procedures that they use when needed. These surgeons are the ones who either have not moved: Dr Claudiu Nistor, Dr Adrian Ciuche, Dr Constantin Grozavu, or the ones that work in other parts: Professor Ioan Cordoș, Dr Codin Saon Dr Mihnea Orghidan, Dr Radu Matache, Dr Cornel Savu from Filaret Hospital, Professor Alexandru Nicodin from Timișoara, Dr Cezar Pavelescu, Dr Laurentiu Marinescu from Floreasca Emergency Hospital, Dr

Madalina Grigoroiu and Dr Codruț Stănescu at Fundeni Hospital, Dr Constantin Mitrofan at Iași, Dr Emanuel Palade who worked in Wagen, Germany, then Freiburg and at present in Lubeck – Clinical Lead, Dr Marius Paraschiv at Bagdasar-Arsene Hospital, Dr Cristescu and Dr Brânză from Brașov, Dr Hugoi from Oradea, Dr Demetrian and Dr Dobrinescu from Craiova and others. All these thoracic surgeons will teach the new comers to work on the land of thoracic surgery. They will teach them about the pericardial border, how to approach and go pass it without risks, incidents or accidents.

Like them, I had to move to another hospital along with Dr Cezar Motaș, Dr Mihnea Davidescu, Dr Natalia Motaş, Dr Corina Bluoss, Dr Rus Ovidiu, Dr Daniel Fudulu, Dr Andrei Bobocea. We arrived at the Institute of Oncology, on the Fundeni platform. For me it was like in the fairy tales, I have returned at old age where I used to work when I was young but in another building.

Here pericardial diseases are more frequently referred than at the Military Hospital. All the surgical pericardial conditions used to be referred to our neighbors, across the fence, to the Cardiovascular Department at Fundeni, before our arrival.

I cannot remember the day and the month, but I remember the year of 2007 when I went to visit the thoracic surgery theatres at the Military Hospital. The younger surgeons were not allowed to discuss with me or request my help for the various cases at that time. This was imposed by the new clinical lead that was supported by the management. So, I used to go uninvited. I used to go like I used to do in the previous years. During that day, Dr Cezar Motaş was assisted by Dr Natalia Motaş – his wife. Dr Motaş told me he was operating a malignant pericardial effusion and that he approached the pericardium from the left side of xiphisternum. I said: ''Well done!'' and left. After a few steps, I came back to him and told him: ''This was never done, take some pictures! It is an original procedure'' It was and it is indeed the ''left paraxiphoid approach to the pericardial cavity''. The procedure was published in the ''Interactive Journal of CardioVascular and Thoracic Surgery'' in January 2010 under the heading: ''New Ideas in Oncology''. The idea was applied and published by a Romanian thoracic surgeon – Dr Cezar Motaş who unfortunately passed away too early in the year of 2013, month October, the 30th.

Because we had a lot of clinical and operative experience in pericardial surgery accumulating throughout the years I thought this will be a very good topic to study by a young medical student, future doctor and future thoracic surgeon. Therefore, more than 10 years ago, a young medical student from Bucharest approached me and asked my permission to observe a thoracic surgical procedure. He proved to be a responsible and serious student and later a thoracic surgery trainee and sponsored PhD student. His name is Daniel Fudulu.

His graduation diploma thesis was based on the surgical experience of the Thoracic Surgery Clinic II, University of Medicine and Pharmacy ''Carol Davila'' in the treatment of pericardial diseases. The above academic surgical department functioned until 2009 at the Military Hospital and from October 2009 was moved to the Institute of Oncology Bucharest.

The plan was for this subject to be further explored and researched in the form of a PhD thesis for which Dr Fudulu successfully obtained sponsorship of his tuition fees. He had to abandon his PhD when he immigrated to England in 2010.

This distance was not an obstacle or a reason to abandon the writing of the monograph ''The Pericardium – Anatomy, Physiology, Pathophysiology, Pathology and Surgery''. Therefore, we decided to write this book. Some of the drawings are done by Dr Fudulu while others are done by a young and talented drawer – Eugen Tudorache. I want to thank them both for the detailed and exceptionally clear illustrations.

Together with Dr Fudulu I also published on CTSNet – ''Pericardial Reconstructions in Thoracic Surgery''. Thank you to Professor Mark Ferguson from Chicago for accepting to publish our work and all his editing work and advice. The article was published online on December 2010.

In this chapter, I have told you the story of my career path and the motivations behind writing this book. This monograph is written from the point of view of a senior thoracic surgeon and of a thoracic surgery trainee – we have nothing against the cardiac surgeons! On the contrary, it is our meeting point at the border between the thoracic and cardiac surgery. This book is written for anyone who has the desire to read and learn regardless of their position. It is intended for students, junior trainees, registrars, consultants, academics and no academics.

I do hope it will prove useful!

Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine.* Please read the complete Author Guidelines carefully prior to submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

ETHICAL CONSIDERATIONS

Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) Accessible to the public at no charge;

(2) Searchable by standard, electronic (Internet-based) methods;

(3) Open to all prospective registrants free of charge or at minimal cost;

(4) Validates registered information;

(5) Identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

Plagiarism Detection

The journal employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

Committee on Publication Ethics

The journal subscribes to the principles of the Committee on Publication Ethics (COPE).

MANUSCRIPT CATEGORIES AND SPECIFICATIONS

All articles, with the exception of Editorials, must contain an abstract of no more than 250 words. Abstracts for original articles should be formatted into subheadings, as detailed below. Titles must not be longer than 120 characters (including spaces).

Editorials

These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

Review Articles

RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related “mini-series” of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

Meta-Analyses or Systematic Reviews

RJMM particularly welcomes submission of Meta-Analyses and Systematic Reviews, which underpin evidence-based medicine. Guidelines for preparation of Meta-Analysis and Systematic Reviews are similar to other reviews, and articles are subject to the usual peer review process. Meta-Analyses and Systematic Reviews have a word limit of 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

Original Articles (including clinical trials)

RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

Education and Imaging

The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as ‘What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?’ Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

MANUSCRIPT PREPARATION

Style

Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors’ revised ‘Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication’, as presented at http://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster’s Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader’s task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’

industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/ mesh/meshhome.html).

Text

Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

References

The Vancouver system of referencing should be used. In the text, references should be cited using superscript Arabic numerals in the order in which they appear. If cited only in tables or figure legends, number them according to the first identification of the table or figure in the text. In the reference list, the references should be numbered and listed in order of appearance in the text. Cite the names of all authors when there are six or less; when seven or more list the first three followed by et al. Names of journals should be abbreviated in the style used in MEDLINE. Reference to unpublished data and personal communications should appear in the text only.

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Tables

Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals, and cited in consecutive order in the text. Each figure should be supplied as a separate file, with the figure number incorporated in the file name.

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, publication requires high quality images to prevent the final product being blurred or fuzzy.

SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at rjmilmed@yahoo.com

A cover letter containing an authorship statement should be included.

The cover letter should include a statement covering each of the following areas:

1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
2. Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.
3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research

must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.

4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

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If the above items are not included in the cover letter, manuscripts cannot be sent for review.

Please also note that the cover letter does not require a detailed or lengthy description of the content or structure of the manuscript itself.

Two Word-files need to be included upon submission: A title page file and a main text file that includes all parts of the text in the sequence indicated in the section 'Parts of the manuscript', including tables and figure legends but excluding figures which should be supplied separately.

The main text file should be prepared using Microsoft Word, doubled-spaced. The top, bottom and side margins should be 30 mm. All pages should be numbered consecutively in the top right-hand corner, beginning with the first page of the main text file.

Each figure should be supplied as a separate file, with the figure number incorporated in the file name. For submission, low-resolution figures saved as .jpg or .bmp files should be uploaded, for ease of transmission during the review process. Upon acceptance of the article, high-resolution figures (at least 300 d.p.i.) saved as .eps or .tif files will be required.

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Accepted papers will be passed to production team for publication. The author identified as the formal corresponding author for the paper will receive an email, being asked to complete an electronic license agreement on behalf of all authors on the paper.

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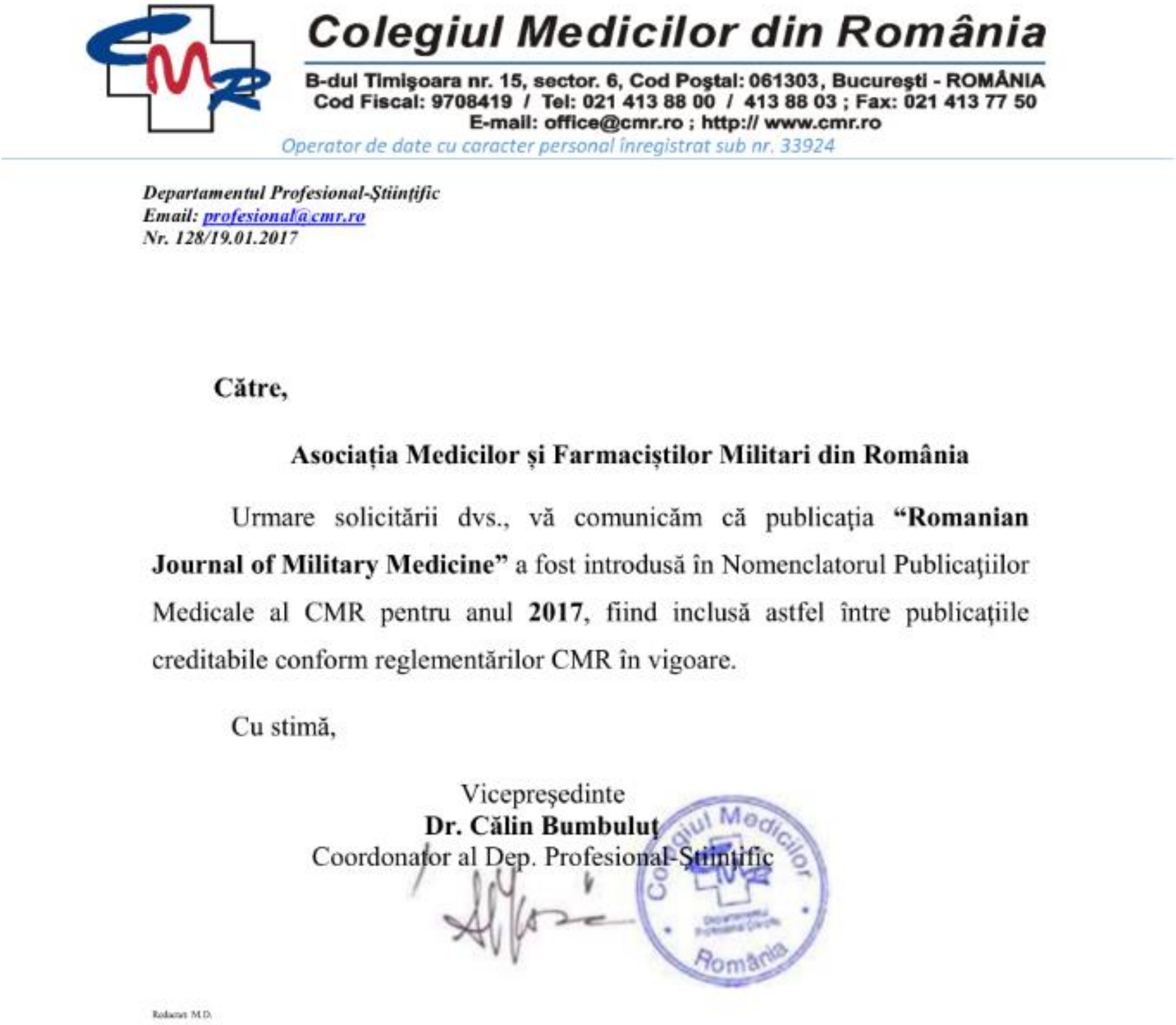

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Către,

Asociația Medicilor și Farmaciștilor Militari din România

Urmare solicitării dvs., vă comunicăm că publicaţia **"Romanian Journal of Military Medicine"** a fost introdusă în Nomenclatorul Publicaţiilor Medicale al CMR pentru anul **2017**, fiind inclusă astfel între publicaţiile creditabile conform reglementărilor CMR în vigoare.

Cu stimă,

Vicepreşedinte
Dr. Călin Bumbuluţ
Coordonator al Dep. Profesional-Ştiinţific

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EDITORIAL

The story of a journal – The 120th anniversary of the Romanian Journal of Military Medicine

Gral (R) Prof DAN MISCHIANU

Chief of Urology Clinic, Carol Davila University Central Emergency Military Hospital Faculty of General Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Dan Mischianu

The Military Medicine Magazine, later became the Romanian Journal of Military Medicine (RJMM)... well, I assure you that it did not come out of thin air!

It appeared on September 15, 1897, 120 years ago, on the initiative of military medical professionals.

I think few of us know that on January 12, 1897, Army Corps General Professor Dr. Athanase Demosthen was elected correspondent member of the Medical Academy of Paris.

Those who celebrated him for success, mostly his students and contemporaries, doctors, pharmacists and veterinarians (military veterinarians, the cavalry did not disappear – as a fighting weapon!) decided, according to the French example, to create a magazine with independent statute and organization, and objectives that do not go beyond our present understanding. Namely: "maintenance of scientific activity and emulation among the members of the sanitary body, establishment of the collegiality links between the sanitary officers, as well as the preservation of the scientific and moral prestige that they should enjoy in the army and in society, the culture of all scientific and technical knowledge among the members of the military health body related to the medical, pharmaceutical and veterinary profession" (*Revista Sanitară Militară, 1972, nr. 4-5, pg. 411*).

The "Steering Committee" met three months later, on April 12, 1897, and established that under the leadership of General Prof. Dr. Athanase Demosthen – as chairman, a new magazine called "Military Sanitary Review" will be born with the previously mentioned status. The companions of General Demosthen for this enterprise were the generals Dr. I. Şerbănescu, N. Zorileanu, the army pharmacist M. Marinescu, first class regiment physician I.M. Călinescu, second class regiment physician Iacob Potârcă (a memorable name in Romanian surgery – versus Whitehead procedure!), pharmacists C. Dumitrescu-Parepa and Constantin Merișanu (whose bust guards a hospital alley).

The magazine appeared on 15 September 1897 and the photocopy presented reproduces the first cover of the Military Health Magazine (*Revista Sanitară Militară, 1972, nr. 4-5, pg. 409*).

The "Military Health Magazine" wanted to be, from the very beginning, "the depository of the work and activity of the health officers in the realm of veterinary and human medicine and military pharmacy" (*Oameni și Fapte din Istoria Medicinii Militare Românești, Gral brig (r) dr. Mircea Diaconescu, vol II, pg. 229*).

The Military Health Magazine records a great performance in our medical literature: it appeared in the same year as the Surgery Magazine – 1897, went through the same "trials", did not have the same audience but resisted... An absolutely remarkable fact!...

REVISTA
SANITARĂ MILITARĂ
APARE O DATĂ PE LUNA
SUMARUL

Before continuing the "story of the Magazine", I think it is worth telling you "my story – vs the Magazine".

I may be subjective, and actually I am.

The system, the organization of that time, had assigned me after graduating from the Faculty as a "trainee intern" since December 1979. I was assigned, together with my colleague, to the 2nd Medical Department, the current Clinic of Internal Medicine and Gastroenterology of the Central Military Hospital. We have been extraordinarily well received and grateful to those people.

I knew about the Magazine, I had read it "en passant", then there were no "student" magazines, I did not even dream to publish an article in a magazine with such background.

Well, those very formidable men – the "workers" from the editorial office of any long-term magazine, that is, Col. Dr. Cristea Neculescu and Mr. Nicolae Dragoi – editorial secretary – considered an absolute "insignificant" article in time – but perhaps pompously titled: "Diagnostic significance of the atrial fibrillation wave amplitude" by D. Mischianu, V. Andrei, Military Health Magazine, 1980, 1, pg 71-75, may receive the "good fortune"! It was an article that sumed up what we, presented to the Students Session at the Bucharest Medical and Pharmaceutical Institute in the April 1979 session.

I leave aside my memories and come back to the historical reality, of which nobody understands at all.

The magazine has gone through chaotic and sad moments.

It had "ups and downs," a sort of "crises" as they are told today. Most of the causes were of financial origin, which led to changes in the typographic format and the continuity of occurrence.

The first syncope is in 1903, so that between 1903-1905 the magazine no longer appears.

In April 1905, everything goes back to normal the first exchanges of journals began with other armies: Italian, English, French, German and everything went flawless until 1908. The number 2 of 1906 was a jubilee number; ten years had passed from the gorgeous initiative of 1897, the number being dedicated to "the great and mighty King Carol I".

Unfortunately after the numbers 3 and 4 of 1908, the second syncope is recorded. The Military Health Magazine ceases to appear until 1913 (*Oameni și Fapte din Istoria Medicinii Militare Românești, Gral brig (r) dr. Mircea Diaconescu, vol II, pg. 230*).

The magazine is coming back shortly. On June 28, 1914, Archduke Franz Ferdinand and his wife were assassinated in Sarajevo, the three kings – blood cousins: Kaiser Wilhelm II of Germany, Tsar Nicholas II of Russia, and King George V of The United Kingdom of Great Britain, all three good English speakers were not able to come to terms (they were grandchildren of the Queen Victoria of Great Britain), the Great War (or the First World War as it is known today) begins, our magazine has its third syncope: 1915-1919 .

With all these vicissitudes, in 1917 and 1918, in Iasi and Bacau appear "the Comptes bulletins from the Société medico-surgical du russo-roumain, namely Comptes vendus des seinces from the medical reunion of II-ème armee, presenting medico-military communications with participants from Russian and French allied armies " (*Revista Sanitară Militară, 1972, nr. 4-5, pg. 412*).

There is also a fourth syncope, after the first numbers appeared in 1919. In fact, the magazine really revived in 1928, when things were settled, the country seemed to have emerged from the crisis. It is Miron Costin's word: "There are not the times under the man, but the poor man under the times!"

In the review, there are reports of the "continuous medical education" sessions – that is the sessions of the Romanian Military Doctors' Association with the subsequent subsidiaries from all the existing Military Hospitals, homage numbers regarding the personalities of the Romanian medicine who came from or not from the same "medical strain": General dr. N. Zorileanu (dermatovenerologist), professor dr. V. Babeş (bacteriologist), professor dr. Dimitrie Gerota (surgeon, radiologist), professor dr. Al. Costiniu (ENT), etc. Articles in French and German also appear (*Revista Sanitară Militară, 1972, nr. 4-5, pg. 413*).

The fifth syncope, hopefully the last, is also the longest: 1949-1957. The times were like they were, everything was changing, and who needed the written word of the Romanian military doctors?

We publish the cover of a Magazine published in 1938 in which, in the end, lieutenant physician Dr. Eugen Mareş publishes an obituary of a generation colleague (*Oameni și Fapte din Istoria Medicinii Militare Românești, Gral brig (r) dr. Mircea Diaconescu, vol II*).

REVISTA
SANITARĂ MILITARĂ

BULETIN OFICIAL AL SOCIETĂȚII ȘTIINȚIFICE
A OFIȚERILOR CORPULUI SANITAR MILITAR ROMÂN
ȘI A FILIALELOR SALE
APARE ODATĂ PE LUNĂ

REDACȚIA ȘI ADMINISTRAȚIA
SPITALUL MILITAR „REGINA ELISABETA"
BUCUREȘTI

The same man, the same true Romanian military doctor who I personally met, when he was at the peak of his profession – Deputy Minister of Health in socialist Romania and at the end of his life, with a typical Oltenian perseverance, "revives" the Military Health Magazine in 1957.

In 1972, when the magazine was celebrated for "three quarters of a century of existence in the service of the protection of the health of our soldiers", the technical box of Magazine Sanitary Military Magazine no. 4-5/ 1972, which we reproduce in the facsimile, mentions his name, along with the name of another military doctor dear to my soul – General Professor Iuliu Șuteu – editor-in-chief of the magazine at that time.

REVISTA
SANITARĂ
MILITARĂ
1897 1972

4-5
1972

I also want to remember another name dear to me – General Lt. Academician Gheorghe Niculescu, for many years, editor-in-chief and "living spirit" that agglutinated the energies and "pencils", stimulating and promoting those who really had something to say in this field.

After 1990, fearful, as observed to another journal "Surgery", changed its name. It became the Journal of Military Medicine.

REVISTA SANITARA MILITARA
75
ROMANIA
1897
1972

Probably the term "sanitary" was appropriate in the beginning, the term "medicine" is common to all military doctors because we all come from the same strain – the School of Medicine created by Carol Davila, who arrived on Romanian soil as a French civilian and became a Romanian general and physician!

Revista de
Medicină
Militară

He has escaped, in this way, of a "complex".

In time there have been other Romanian medico-military publications.

However, the Romanian Journal of Military Medicine (RJMM) remains unique and emblematic after five major "syncopes", having in its portfolio "a valuable scientific and informational forum not only for military doctors but for all Romanian medicine" (*Oameni și Fapte din Istoria Medicinii Militare Românești, Gral brig (r) dr. Mircea Diaconescu, vol II, pg. 232*).

Romanian Journal of
Military
Medicine
REVISTA DE MEDICINĂ MILITARĂ

The magazine had the power, and those who were close to it knew how to do it, to reborn always like the Phoenix bird.

120 years after the first appearance, the Romanian Journal of Military Medicine remains a magazine that has its own program, unaltered by the passing of the ages, even when the inter-war or post-war political factor implied perhaps another orientation.

The Romanian Journal of Military Medicine wishes to bring up-to-date information to the servants of this noble profession, and hopes that the puzzling of the information on the internet, which can make anyone out of the question, is presented on-line or printed (I confess that I deeply dislike these English barbarisms!) after they have been read and corrected (not censored!) by a true scholar in the field.

I am absolutely convinced that all Romanian, military or civilian medical officers, descendants of our common ancestor – General Professor Dr. Carol Davila – will adhere to this profession of faith and will continue as long as it is needed!

Happy Birthday Journal of Military Medicine!

Happy Birthday dear readers!

REVIEW ARTICLE

Article received on February 11, 2017 and accepted for publishing on June 12, 2017.

The concept of operationalization of an integrated platform for scientific research and expertise of war and bioterrorism biological agents

Viorel Ordeanu[1,2], Marius Necşulescu[1], Diana M. Popescu[1], Lucia E. Ionescu[1], Simona N. Bicheru[1], Gabriela V. Dumitrescu[1], George Corlan[1]

Abstract: *The international situation requires a strengthening of the national security measures, including in the field of CBRN and public health. The upgraded microbiology laboratories from DM/MND must be operated at full capacity for the operationalization of an integrated Platform for the research and expertise of biological war and bioterrorism agents. This is necessary for reasons of national security, for CBRN defense and for public health, in the context of biological agents of 3 and 4 risk groups' epidemics.*

The existing upgraded objectives should be operationalized in order to meet the established scope: scientific research and expertise of biological agents and biological weapons, a laboratory for in vitro analysis and a bio-base for in vivo analysis. The highly secure lab allows working with any high-risk agents: biological, genetic, chemical, radiological, etc., being provided with a special room for the insertion/removal of equipment and their decontamination.

Following an increase in the capability requirements concerning laboratories, we have provided in the design concept new technical parameters of the platform and the integration of new compartments with related activities of toxicology, pathology, neuro-psycho-pharmacology, bio-pharmacy, micro-pharmaceutical, specific testing activities, etc.

Creating the integrated platform and its operationalization is necessary in order to meet the requirement of the national security strategy as a collective CBRN defense/protection facility and military-medical scientific research for CBRN medical protection.

Keywords: *biological agents, bioterrorism, medical protection, microbiology laboratory, scientific research, integrated platform, CBRN*

INTRODUCTION

The international situation requires a strengthening of the national security measures, including in the field of CBRN defense (chemical, biological, radiological and nuclear) and public health. The upgraded microbiology laboratories from the Medical Department of the Ministry of National Defense (MD/MND) must be operated at full capacity, for the operationalization of an integrated Platform for the scientific research and expertise of biological war and bioterrorism agents. The project is necessary for national security purposes, for CBRN defense and for the public

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health, in the context of biological agents of 3 and 4 risk groups epidemics, and it responds to current threats. In risk group 3, are listed extremely dangerous bacteria and other pathogens such as warfare biological agents (WBA). In the maximmum risk group 4, are included 8 species of extremely dangerous viruses such as Ebola or WBA viruses.[1,2,3]

Current regulations take into account the provisions of biosafety (such as internal protection for operators), of biosecurity (as protection against facility external hazards) and of bioprotection (protection of the sample to be analyzed). As a result, laboratories are properly classified, depending on the different level of protection. In French literature they are referred to as P1-4 (Protection).

The key features are: P1 with open work areas for education; P2 with biosafety hoods, for medical purposes; P3 special biosafety equipment for the extremely dangerous bacteria; P4 for the extremely dangerous viruses, with the maximum level of biosafety. In English, they are called Biosafety Laboratory (BSL1-4).[4] In Romanian, they appear as Basic Labs with a level 1 and 2 of biosafety, Highly Secured Laboratory with a level 3 of biosafety, and a highly secure laboratory with a level 4a of biosafety (with collective protection equipment) and 4b (with special equipment for individual protection). In the absence of proper facilities, the biological agents classified in the risk categories cannot be legally worked with. [2]

STATE-OF-THE-ART

Nationally, the Ministry of Health has modernized a microbiology lab at the National Research Institute “Cantacuzino” as a P3 laboratory and one (under construction) at the Bucharest Hospital for Infectious Diseases "Babeş". The Ministry of Agriculture has two P3 microbiology laboratories at the Bucharest Institute for Animal Diagnosis and Health. Currently, in Romania, a P4 highly secure microbiology laboratory does not exist yet.

Due to the fact that in Romania scientific research for medical protection against CBRN agents belongs to MND, it is mandatory to have a proper facility. According to Biosafety in medical laboratories Guidelines (World Health Organization 2004 and Ministry of Health 2006) the bacterial biological agents are expertised in the P3 laboratory, and the viral agents in a P4 laboratory.

The international context shows that eventhough the risk of biological warfare has decreased as a result of the Geneva Convention (BTWC 1972), that was signed and ratified by more than 90% of the world's countries, the risk of bioterrorism is as real as the risk of pandemic, of zooantroponosys, of exotic and tropical infectious and contagious diseases, etc., as it is with the recent epidemic of Ebola.[5] As a result, the WHO, the EU and NATO recommend concrete measures for decreasing those risks and for increasing the response capacity of each country, of the alliance and of the international community.

Given the EU's recommendations to operationalize at least 40 P4 laboratories within the member countries, to be designed and operated under standardized WHO conditions, the project responds to international demands and also to the national interest.

The operationalization of an integrated research and expertise platform for biological warfare and bioterrorism agents is relevant to the approached scientific field, following the **state of the art** (current stage) character of the research in the field of protection against biological agents, by implementing internationally used modern techniques. The project is based on implementing **cutting edge** technologies for diagnostic, prophylactic pre-exposure and post-exposure, cure and recovery, aspects regarding the action mechanism in infectious diseases, the therapeutic means to counteract the effects of biological agents and the epidemiological surveillance.[6]

Internationally, there are concerns regarding the achievement of a coherent system of epidemiological warning and intervention, the project being in line with the world situation. Designing and equipping the platform according to international recommendations determines that the level of performance of the proposed infrastructure must be internationally competitive and must take an active role in the global effort of health protection against biological agents.[7]

Our concept for the operationalization of the Integrated Platform of scientific research and expertise of war and bioterrorism biological agents.

Creating the Integrated Platform will allow the defensive scientific research and medical expertise for weapons/biological agents and for the diagnosis of people infected with particularly dangerous biological agents, and for the medical intervention in epidemics or biological attacks.[8,9,10] It involves, in the first stage, monitoring the continuation and completion of the upgrading and rehabilitation of buildings, installations and perimeter security measures, including strengthening the capacity of health protection against biological weapons, bioterrorism and particularly dangerous biological agents.

It is important to liaise with specialized companies (selected not only by public tenders) within the allocated funds, for the execution of the Integrated Platform, for doing the reception at the time of commissioning the modernized Laboratory of Microbiology (declared at the UN as being under construction) for in vitro analysis, and for doing the reception at the time of commissioning the modernized Biobase, for in vivo analysis as well as for complementary analysis of analytical and experimental toxicology, neuro-psycho-pharmacology, bio-pharmacy and the related logistical and security support.[8]

Taking into account the unique nature of this facility on national and regional level, as well as the direct and indirect costs related to the spatial planning, maintenance, and exploitation of this advanced technology objective, we rethought the configuration and the operation of the component objectives for maximum efficiency.[8] Thus, the facility can provide, through its equipment and operation, not only the scientific research and expertise for ABR, bioterrorism and biocrime, but it can also become practically involved in the epidemics/ pandemics or zoonoses with exotic and tropical diseases, that can occur as major emergencies, as is the recent Ebola outbreak. This facility with a maximum level of biosafety can also serve for other specific activities with major risk level; for example, with chemical warfare agents (CWA) or accidents with toxic industrial chemicals (TIC), with radiological warfare agents (RWA) or accidents with radioactive substances, or for any other extremely dangerous substances (HazMat). Changing destination can be achieved operationally, whenever necessary, even if it is one day to another, by replacing the work team with specialists from the DM/MND structures, according to the new destination and by replacing the specific equipment: removing from the highly secured working chamber of the equipment and materials which are no longer needed (through the decontamination airlock outlet) and inserting those that are necessary to the new mission. However, inside the work chamber, materials deposits are not stored for in vitro analysis. These are found in the “hand deposit” of the laboratory, inside the units’ deposit or are purchased by emergency from different sources.[8,9,10] At the same time, the Biobase for test animals can serve all military medical laboratories due to the fact that it is conceived to immediately adapt to new requirements for different animal species. In terms of the annex facilities and amenities, they are basically for general purposes, for every type of medical laboratory and they inclusively ensure the proper functioning of the entire facility in which the integrated Platform is found. The platform can be adapted without structural changes to the CBRN agents of any kind, either separately or combined.

The integrated platform for the scientific research and expertise of bioterrorism and biological warfare agents contributes to strengthening the capacity of scientific research of DM/MND for the expertise of WBA/CBRN agents. During biological crisis situations, it can strengthen the National Healthcare System in terms of providing a microbiological diagnostic to the sick or suspected to be sick or to the animals, as well as for the detection, identification and confirmation of CBRN agents under biosafety and biosecurity conditions.[11,12] The facility, as a medical-military objective, can be useful interdepartmentally: Ministry of National Defense (MND), Ministry of Health (MH), Ministry of Agriculture and Rural Development (MARD), Ministry of Internal Affairs (MIA), Ministry of Justice (MJ), Ministry of Environment, Water and Forests (MEWF) and Romanian Intelligence Service (RIS).

Studies and procedures for the operationalization and integration of the Laboratory of Microbiology and of the Biobase.

The integrated platform consists of complementary elements acting in algorithm: the Microbiology laboratory for in vitro analysis, the Biobase for in vivo analysis, the Mobile team for biological intervention (EMI-Bio) for field activities, as well as the annex utilities for the proper functioning of the objective.

The design, the feasibility study and the outline design for the Integrated platform of the scientific research and expertise of war and bioterrorism biological agents also includes the update of the previously modernized objectives, during 2007-2009 (Laboratory of Microbiology and Biobase), as well as the utilities and Annex facilities. The commissioning of the installed equipment and staff training with accredited supplier firms must be supplemented by specific new latest technology purchases. The explanatory memoranda and the feasibility study for the operationalization of the integrated platform are necessary because the financial burden is very high and must be quantified because the investment proposal submitted to the policy makers needs to be supported by the necessity and the opportunity of the investment.[8]

The activity of verifying the conditions related to the authorisation, the qualification, the certification and the accreditation of the Integrated platform for scientific research and expertise of biological agents

- The activity of verifying the conditions related to the authorisation of the Integrated platform. The process of modernizing the two objectives was carried out under the guidance of the National Authority for Scientific Research and Innovation (ANCSI) after attending the PNCDI Capacities national competition and after obtaining government funding through the Ministry of Education in 2007. As required by the investor, existing complementary objectives were selected: a laboratory of virology and the vivarium. ANCSI monitored the design, development and the endowment with installations and equipment of modernized objectives and performed the final inspection for the reception of the works, in 2009. The report prepared by ANCSI concerns only the fulfillment of the specific research and acquisitions objectives. Depending on the technical characteristics of the newly installed equipment, the owner must provide the utilities: electricity, cold water, hot water, distilled water, sewage, lighting, ventilation, heating, security, specific supplies and specialised staffing.

After the rehabilitation works to the building and installations have been completed, the Verbal Proceeding for the works reception will be issued. Next, follows the sanitation stage of the premises, of authorization and of commissioning the specific equipment and devices, by specialized companies and suppliers. Next, will be purchased consumables, reagents and inventory objects for the endowment of laboratories, depending on the tasks.

After establishing work teams with qualified and specialized staff follows the stage of drawing up the Verbal Proceeding for the functioning status of the Laboratory (through self-assessment and internal auditing). The Technical file for the authorization of the objective that contains data about the space, the endowment, about the staff and the work procedures is submitted to the Territorial Center for Preventive Medicine (TCPM/DM) in view of the sanitary authorization inspection of the objective.

After obtaining the sanitary functioning authorization, that involves the prior solving of the rehabilitation at the level of the entire facility, immediately will be performed a recheck of the qualification, certification and accreditation conditions of the Integrated platform for scientific research and expertise of biological agents.

- The verification of the qualification conditions of the Integrated platform, according to the Guidelines head. 7, p. 44-45: "Recommendations for the qualification of the laboratory and its facilities." The qualification of the laboratory/its facilities may be defined as a systematic process of examination and documentation, demonstrating that the structural elements of the laboratory and of the systems and/or of the system components have been installed, inspected and tested, in terms of their operation, in conformity with the national and international standards.[2]

The laboratories designed to correspond to the Biosafety Levels 1 to 4, have different qualification requirements, with increasing levels of complexity. The geographical and climatic conditions, such as moisture or extreme temperatures, can also affect the laboratory structure, and thereby, its qualification demands. Once the qualification process has been completed, the significant structural components and the related systems will be subject to various conditions, including working conditions, under imposed conditions, logically possible, that will only thereafter be approved.

The qualification process and the acceptance criteria will have to be established from the design phase, of construction and/or renovation. By knowing the qualification requirements from the very beginning, the staff (the architects, engineers, the staff responsible for the safety and health assessment, as well as the staff of the laboratory) will be able to better understand the performance that has to be achieved by the laboratory.

The qualification process provides the institution and the community within which it operates a higher degree of confidence, given the fact that the structural elements, the electrical systems, the mechanical and drainage systems, the insulation and decontamination systems, as well as the security and alarm systems will function as initially designed, ensuring secure handling in the laboratory or in the biobase of any potentially dangerous microorganism.

The qualification activities are generally performed, from the design stage, continuing as such during the construction and installation of the laboratory and its facilities and during the warranty period, which should cover at least one year after its entry into service.

The agent assessing the qualification, acts as a guide for the institution that is building and renovating the laboratory and must be considered as a member of the concept team, his early involvement in the project design being essential. The institution may act as its own qualification agent, if it has a trained auditor. In the case of more complex laboratory facilities, with biosafety level 3 or 4, the institution may use the services of a qualification agent outside the institution, with proven experience in the successful implementation of the qualification of laboratories and biobases with complex levels of biosafety. When referring to a freelancer qualification agent, representatives of the institution will also participate as team members: the safety officer at institution level, the project manager, the program manager and a representative of the technical service's maintenance and intervention.

A list will exist to work with, consisting of the laboratory's systems and of the components that will be included in the qualification plan, for testing the functionality correlated with the degree of securing the facilities to be built or renovated. This list is not exhaustive, being adapted to the laboratory specifics. Clearly, the actual qualification plan must reflect the complexity of the respective laboratory.

- The verification of the certification conditions of the Integrated platform according to the Guideline head. 8 p. 45-60: "Recommendations for laboratory certification and its facilities"; it is similar to the qualification, but is run by a committee of national experts approved (the Ministry of Health, RENAR, the National Association of Medical Laboratories etc.). The WHO model questionnaire list, shall be completed during the certification inspection, in the presence of the institution's representatives, who know the objective.[2]

- Verification of the accreditation conditions of the Integrated Platform.

The Ministry of Health is able to grant accreditation only up to the P3 level; so, after reaching this level, the next step would be to resort to an international organization (WHO, EU, ECDC, NATO etc.), if an accreditation at maximal level is required and if all the required conditions are fulfilled and whether there is adequate funding.[7]

The complexity of running operations in a laboratory with maximal biosafety, exceeds the scope of the Biosafety Guidelines. More details and information can be found in the O.M.S. Biosafety Programme (according to the Biosafety Guidelines, Annex 3). The available information related to the training courses and to the profile information materials can be obtained by written request, for example from the Biosafety programme, Department of Communicable Disease Surveillance and Response, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (http://www.who.int/csr/).

Basically, the activity of verifying the conditions related to the authorisation, the qualification, the certification and the accreditation of the Integrated platform for scientific research and expertise of biological agents ascertains whether the objectives satisfy the requirements and proposes the accreditation. If the requirements are only partially met, then the nonconformities will be recorded and the inspection will be effectively resumed when all rehabilitation works to the platform's components will be completed. Only then, the laboratory may be declared fully functional at the highest level of biosafety. If all the imposed requirements cannot be met, or in the meantime the requirements for biosafety are amplified and the facility no longer meets the new requirements, then the facility can be accredited to a lower level, adding additional equipment for biological protection or other appropriate measures, whenever necessary.

OBSERVATION

Following an increase in the capability requirements concerning laboratories, we have provided in the design concept new technical parameters of the platform and the integration of new compartments with related activities of toxicology, pathology, neuropsycho-pharmacology, biopharmacy, micro pharmaceutical, specific testing activities, etc. However, the new facility does not allow, in terms of spaces and technological flows, their permanent dislocation in the space of the integrated Platform. If the dislocation of any other laboratories is needed, other spaces must be designed, built and purchased that correspond to international standards in the field, disseminated by the Ministry of Health.

Executing the integrated platform, its operationalization and, implicitly, the investment's financing is required in order to meet the requirement of the E6218 Capability Target, as a collective CBRN defense/ protection facility and military-medical scientific research for CBRN medical protection.

The original concept of ABR and bioterrorism scientific research and expertise was completed over time, in consultation with the colleagues from other specialties who are involved in CBRN medical protection and related areas of expertise that are complementary to the basic activity, in order to work under biosafety conditions, in full compliance with international norms.

CONCLUSIONS

The existing upgraded objectives should be operationalized so as to fulfill the targeted purpose: scientific research and expertise of biological agents and biological weapons, for in vitro and in vivo analysis. A laboratory with a maximal biosafety level allows working with any high risk agents, being versatile. We also provided new compartments with related activities to toxicology, radiobiology, anatomic pathology, neuro-psycho-pharmacology, biopharmacy microproduction and specific testing etc. The general concept enables the collaboration/cooperation of all CBRN medical protection laboratories and the cooperation with other military, civilian, national and international entities: NATO, EU or CIMIC.

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REVIEW ARTICLE

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Intellectual mobility in medical higher education system

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Abstract: *Intellectual mobility brings change, there is the primary factor in the way of progress and optimal premise of human being development from theoretic and practice regards. Medical Higher Education, worldwide, is generally similar in structure and consistency, but different in typology of presentation, teaching, learning and assessment. In fact, general medicine, as a subject refers to the same biological body, but presented differently depending on culture, space and under various methods of teaching and learning.*

The idiom of intellectual mobility is not new, but according to globalization, which we live at the present times, brought the mobility in the main plan of Europeanization, a new plan, with continues sustainable development and maybe of success. By institutional mobility, both for students and for academic staff, an exchange means a period of one academic year or a semester, for students and, for two days to several months for academic staff, into a foreign university. These stages of study, practice, and teaching take place most frequently within the Erasmus + framework, have been of 30 years in Europe and 20 years in Romania. Also, there are other programs that can perform intellectual mobility, but the most well-known is Erasmus program, where European Commission has allocated the biggest legal and financial budget framework. Overall activity program features has a variety of tools to be deployed and an inter-institutional framework with qualified staff to manage it.

Keywords: *medical education, recognition, higher education, mobility, Erasmus program*

INTRODUCTION

General Medicine as an important branch of study has a special status in the conduct of mobility as importance of the field and also under emotional aspect.

Intellectual mobility takes a certain mental patterns generated by education and a native predisposition, regardless of the field. Along with this added dynamic appeal cases handled by the abstract representations. In the field of medicine is worked with a high degree of abstraction. Or spatial relocation and mobility means mental derived including concrete way of their operationalizing in the two phases of the medical profession: correct diagnosis and treatment of the patient, not the disease. Appeal to intellectual mobility, is done often in the holistic approach to the patient. The doctor represents, on the one hand a mechanic and on the other hand a sociologist. This would be the condition of being a successful doctor. There is also the assumption that both treatment and technique coordinate the doctor to a successful performing.

Casuistry with high risk requires determination,

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curiosity and professional beliefs. However, it is based on a dominant personality characteristic correlated with a predominantly emotional intelligence, and then on IQ. But there is a less explored area of the mind of a physician or a future doctor, a student, how to manage frustration generated by failure in high-risk cases, but not only. Failure, in any other domain generates lessons learned that are grouped in a simple taxonomy:

1. Lessons indicating approaches “know how";
2. Lessons to refute or confirm hypotheses absolutely absurd;
3. Lessons to optimize cases generally valid but wrong managed.

Thus, intellectual mobility in general, depends of subjective perception of reality itself the objective of playfulness and cognitive operators with which man is accustomed routinely to operational abstractions. Medical education has the same topology and provides almost the same bibliographic regarding symptoms of the disease; unfortunately get to treat the disease using methods quite invasive.

Such patterns of study are known in medical education through student mobility from one education system to another. Exchange of experience, for a period from one university to another can bring new knowledge, new methods of learning, but also a personal self, psycho-emotional development.

Erasmus mobility can bring competitive doctors on the labor market and ensure a quality structure, but the real problem is the distribution on the labor market.

As it is well known Romanian doctors prefer to work in European hospitals or beyond Europe, which is not bad, on the one hand, but on the other hand it led to a destabilization of the health system in Romania. So Erasmus mobility tended by a medical mass migration.

Naturally, in this context a basis is economically and socially disadvantaged in Romanian hospitals and moral degradation of the system.

MEDICAL HIGHER EDUCATION IN DRESDEN, ROME AND BUCHAREST

A brief history

A brief history of medicine proves that it was practiced of ancient times from trained professional. History and times prove how the society have changed and it is also in a continuous changes in the approach to sickness and disorder from ancient beginnings

It is well known in the world that medical services were provided for the poor people in monastic hospitals. The care was rudimentary way and rather palliative. As we can observe also medical services and school education, in any domains started from the monastic area, in churches. As just a thin remembering it can be named that culture and civilization started around the human necessity of norms and rules issued by the spirituality.

In the 9th century there were some medical schools in Italy. The influence from other nations as: Greek, Latin, Arabic and Hebrew gave an international dimension. Students learn three years as preliminary courses and five years of medical schools. Nowadays they study five, six or seven years in Europe and in SUA more than ten years.

Italy is the place where medical universities were founded, after came France and England which developed medical schools. So we can see from the beginning the health science started in an internationally manner and mobility and migration are quit ancient and were very important in the development of it.

Today according to the progress of technology, techniques and information system mobility is very used and normal in the society.

Recognition of studies

In order to define studies in Erasmus Program we have to analyze its framework. The main problem is the recognition in making Erasmus.

Although there is a desired of full recognition of Erasmus studies, according to the Erasmus Charter, it is not possible, discussing the case by case,

depending on the curriculum and structure. Of course, that is an ideal situation that a degree program can be accepted fully recognized, but there are features that can be dealt with individually. Although Erasmus Charter directs the full recognition and, in general, universities are trying to respect this principle, even though at the end of the program, there are people involved in Agencies of Recognition and Accreditation of Studies from all over Europe, not easily accept that Erasmus has a special pattern, easily convertible by ECTS and also has all instruments and forms provided. We will see a simple case which presents two distinct situations of curricula on a few subjects of study. We'll see how a curriculum in three different states differ and how to work through the transformation to studies from a curriculum to another, by grades, ECTS obtained both from practical or clinical training and courses. Quantification of studies must be easy and in interest of students. Of course it is of great important qualitative component, especially in the field of medicine. But always should take into account the socio-cultural characteristics, adjusting the student in a new cultural space and psycholinguistics barrier.

In the context of Europeanization and for exemplifying the above situation exposes three major universities with medical schools, in Europe.

Universitatsklinikum "Carl Gustav Carus" Dresden Technische Universität of Germany, especially the ENT discipline; Universita Degli Studi di Sapienza, Rome, Italy, with examples on general surgery and internal medicine and Titu Maiorescu University in Bucharest, Romania, as a university of origin/home university, which makes recognition and equivalence studies.

Germany is a country of art, technique and study continuously, so the team from ENT created a standardized teaching maneuvers examination by a small guide, so that students can observe organized clinical examination of Otolaryngology. Consideration is made all the standardized by checking maneuvers in a form, for a period of 6 minutes of examining a patient. This calculates a score of the exam, and the form can be filled by two examiners for the compared results.

Such an experience has brought by an academic staff that has benefited of teaching stage in Dresden. Today, the ENT method was implemented in Bucharest for teaching and assessment the subject. One such example is enlightening to harmonize the methods of teaching and assessment, job shadowing lead to the development of new skills.

Regarding the Recognition of Studies facts are the discussions become slightly rigid and austere although the program is provided with all the necessary tools.

A student who chose as subjects of study maxillofacial surgeon, a course of a single module named Head has 1 ECTS, but the workload is the same as that of a course of otolaryngology at Bucharest, which has 4 ECTS. Also, the grading system is different. In Germany the scale grades are from 1 to 5, and in Italy from 18 to 30; 1 ECTS has 25 hours of workload. In Romania, at faculty of medicine 1 ECTS has approximately 14 hours of workload. Depending on the workload is denoted by ECTS, 30 for a semester and 60 per academic year, which should be equivalent between higher education systems, but they are not. Module Thorax includes the following disciplines: Cardiology, Angiology, Pulmonology, Vascular Surgery, Thoracic and Cardiac has a volume of 10 ECTS, if studied together, if it divides, then the number of ECTS is divided too, in Romania they are separate disciplines. In Italy, Internal Medicine and General Surgery is a module that measures 12 ECTS together, and in Romania internal Medicine has 6 ECTS for the first semester and for the second semester it has 5 ECTS, in the end there are 11 ECTS, just Internal Medicine. General Surgery is another subject and it has 4 ECTS for the first semester and 4 ECTS for the second semester; they are totally different from one system to other. Such recognition is based on workload and always is made in the favour of the student, or should be.

In Germany the focus is on clinical stages, more than in Italy. In Romania, specialized practice/training is done since of the first year of study, according to the students' testimony.

Although there are differences in the three systems

of teaching and learning, Erasmus has provided the tools; study contract/Learning Agreement, and students can choose their subjects to be studied and disciplines which will be equate to return. Studies are, or should be recognized, integrum, full recognition under the Erasmus Charter. If the student does not fulfill the learning agreement he/she will support additional exams from local education until completion of ECTS number needed to pass the academic year. These are predetermined patterns of procedure and related methods for classification and institutionalization of each institution. What becomes interesting is the prospect of personal development of each individual differs from person to another depending on operators and cognitive education.

There are three factors that can prevent full recognition, as following:

1. Changing subjects of study during mobility, Erasmus Learning Agreement provides that rule can be changed, only in the first 14 days of mobility. Thus, changing the curriculum content, can prevented full recognition procedure because of the time period from the moment of making the new choice of new disciplines and to the approval by the academic tutor from home institution.

2. A second factor that keeps the procedure and otherwise representing a procedural error is soliciting approvals for the recognition and equivalence studies to the professors who are tutor of the disciplines. Thus, the holder of course, may be not sufficiently informed and decide in the detriment of the student. Erasmus Rule requires the application of Charter based on acceptance of Erasmus in function. So is forbidden that a tutor can decide regarding his/her discipline.

3. Finally a third factor, which prevents full recognition, is negative influence of the party who decide subjective and would not assumes the recognition of Learning Agreement. These are isolated cases, and in recent years almost no longer exist. The procedure for recognition and equivalence is the essential characteristic of students in the decision to go in Erasmus mobility.

According to the magazine Prime 2010, only 19% of students surveyed are convinced that it will not benefit from an exchange. The rest of the students who responded to the questionnaire in the same magazine argued that regardless of the recognition of studies, mobility itself and experience are more important than the recognition of studies, thus they assuming full academic exchange activities. Of course that always the activity must be tried separately according to each case. If the student wishes on its own initiative to have examinations in the subjects of home university, it is not prevented, or if the student did not follow important disciplines for future examinations of competence, then it will have them at the return from the mobility without charge of any fee.

There is also a risk that the student take courses that are done in the near future/years of study in the home university and through full recognition, the student would be forced to repeat subject mobility in the coming years. As such, the choice of subjects, from a curriculum structured around six years can be challenging even for academic tutor. This happens because the curricula are not similar, nor how to be similar. Bologna process does not seek to standardize the Higher Education, but seek to a better harmonization of curricula, a socio-cultural and economic uniformity.

If the student is studying disciplines in the curriculum of the home university is doing in a upper year, the University, study case of this research, recognizes full program of study at the partner university and mention the time spent abroad in the Diploma Supplement, and recognizes discipline by discipline in the years that match the local program, and to promote appropriate student take exams in the subjects of study sessions legal up, and in special cases can be organized special sessions for Erasmus students. Whatever, the situation of recognition and equivalence of studies is made, only in students' favor without affect the merit place at home university.

Despite these shortcomings of procedures for recognition of studies, students wishing to repeat the experience to the extent permitted by the Erasmus program.

Most often students after followed a study program they would like to follow a clinical internship. From the experience of the university concerned, they apply after completing their studies in residency programs in the world.

University receives per few times a year, forms requesting certification of studies by agencies of recognition of medical studies in the USA and Canada.

Table 1: Example of a model of recognition studies, in Germany
(it can be observed that the students pass more than 5 ECTS at home university)

Name of the subject	ECTS	Grade in Germany	Grade in Romania
Maxillofacial Surgery	1	1,5	10
Cardiology, Angiology, Pulmonology, Vascular Surgery, Thoracic and Cardiac	10	2	9
Dermatology & Venereology	3	4	5
Rheumatology	2	attended	
Pathophysiology & Medical Biochemistry and Laboratory Diagnostics	9	4	5

It is mention were equated the subjects studied in Germany in the V th year, semester 1 of the study, under the Erasmus Charter, after the student has pass the remaining number of 5 ECTS, as follows:

Name of the subject	ECTS	Grade in Romania
Pneumology	4	7
Occupational diseases	5	8
Radiology	5	9
Gerontology		10
TOTAL	14	

Analyzing the situation of a student who was Erasmus, in the fifth year of study at the University of Dresden, after she came back home observed willingness to continue the study and mobility training, such as she obtained a period of 2 months in a hospital in Germany, combining theoretical and practical work with a clinical internship. Besides the recognition and equivalence of procedural, student mobility has made a qualitative leap in medical education, as well as psycholinguistic improving linguistic competence and went beyond customary in the sphere of national education.

CONCLUSION

Mobility in medical education can make steady progress in what is called globalization, harmonization of curricula and skills in the labor market. The main objective of globalization and mobility function is preventing and overcome poverty and habits that can hinder knowledge and human progress. The internationalization of education is a process of development and a better preparation of students for a globalized society, based on knowledge and skills. Higher education institutions have the main role to prepare graduates for the labor market in the line with international policies of globalization. Mobility brings extra value to Europe and is a sine qua non of human development in relation to the labor market, the practical problem that remains in the knowledge era is the economic and political, social and moral worldwide crisis, otherwise unmanageable. Now it is felt the effects of the global turmoil, but concrete results are likely to be known around 2050.

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REVIEW ARTICLE

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The influence of homocysteine on osteoporosis

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Abstract: *Osteoporosis is a major health problem, and the economic costs are expected to rise due to an increase in life expectancy throughout the world. Its major consequence is fractures, and especially hip fractures are associated with institutionalization and increased mortality. Homocysteine is an amino acid intermediate formed during the metabolism of methionine. Homocysteinuria is a rare autosomal recessive biochemical abnormality which causes elevated plasma concentrations of homocysteine and severe occlusive vascular disease. In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. The high prevalence of osteoporosis among patients with homocysteinuria suggests that high levels of plasmatic homocysteine may also increase the risk of fractures. Nutritional factors such as vitamins B12, B6, and folate are cofactors in homocysteine metabolism, and vitamin intakes may inversely affect plasma homocysteine levels.*

Keywords: *osteoporosis, homocysteine, hip fracture*

INTRODUCTION

Osteoporosis is a major health problem, and the economic burden is expected to rise due to an increase in life expectancy throughout the world. Its major consequence is fractures, and especially hip fractures are associated with institutionalization and increased mortality. The prevalence of osteoporosis increases with age due to an imbalance in the rate at which bone is removed and replaced during the bone remodeling cycle, which is an important physiological process that is essential for maintenance of a healthy skeleton.

Pharmacological interventions may prevent 30-60% of fractures in patients with osteoporosis. Common sites for osteoporotic fracture are the spine, hip, distal forearm and proximal humerus. The remaining lifetime probability in women at the menopause of a fracture at any one of these sites exceeds that of breast cancer (approximately 12%), and the likelihood of a fracture at any of these sites is 40% or more in developed countries [1].

The level of bone mass can be assessed with adequate precision by measuring bone mineral density using dual X-ray absorptiometry. It has

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been suggested that bone strength may be reflected, independently of bone mineral density level, by ultrasonic measurements of bone and by measuring bone turnover using specific serum and urinary markers of bone formation and resorption.

Physical activity as a way to prevent osteoporosis is based on evidence that it can regulate bone maintenance and stimulate bone formation including the accumulation of mineral, in addition to strengthening muscles, improving balance, and thus reducing the overall risk of falls and fractures. It is well known the important influence of hormones as well as dietary and specific nutrient abundance on bone, growth and health is emphasized and premature bone loss associated with dietary restriction and estradiol withdrawal in exercise-induced amenorrhoea [2].

OSTEOPOROSIS

It is becoming increasingly clear that there is a relationship between growth and development in early childhood and bone health in old age. In fact, suboptimal bone development leads to a reduction in peak bone mass, and a higher risk of osteoporotic fracture later in life. Osteoporosis is a skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility. Preventative strategies against osteoporosis can be aimed at either optimizing the peak bone mass obtained, or reducing the rate of bone loss.

One of the largest risk factors for fractures is a reduction in bone mineral density. Risk factors for fracture can be purely skeletal-related affecting bone mass, bone geometry, bone micro-architecture and bone turnover, or solely fall-related such as neuromuscular dysfunction, poor balance, cognitive impairment, cardiovascular instability, reduced visual acuity and sedative medications. Others risks are both skeletal and fall related such as age, genotype, and family history of fracture, weight, weight change and mobility [3]. Falls history is a further independent risk factor for fracture, in particular in men [4]. About 50% of white women and 20% of men will have an osteoporosis-related fracture in their lifetimes. Fractures of the hip and spine may be disabling and are associated with mortality rates that are about 20% greater than that of an age-matched population. The goal of any treatment for osteoporosis is to improve bone strength, thereby decreasing fracture risk.

Bone remodeling is the result of two opposite activities, the production of new bone matrix by osteoblasts and the destruction of old bone by osteoclasts. The rates of bone production and destruction can be evaluated either by measuring predominantly osteoblastic or osteoclastic enzyme activities or by assaying bone matrix components released in the bloodstream and excreted in the urine [5].

Factors which influence negatively the osteogenetic potential are age, nutrition diseases and endocrinopathy, ionized radiations treatments and different toxic factors. It has been scientifically proved the fact that elder patients have decreased general biological potential. Regarding the bone system, there occur structural changes which weaken the resistance, to which we can add a diminishing of the response potential towards harmful factors. These lacks are considered to belong to multiple causes: a diminishing of the vitamin D action and of calcitonin, hyperactivity of the parathyroid hormones with osteoblast inhibition, etc. [6] As a result, osteolysis-osteo-synthesis dynamics is reversed, resorbing processes become dominant: osteodystrophy, osteoporosis, senile osteopenia, metabolic and endocrine osteopathy, which, affecting the very bone structure, are the main causes of diminish in the osteogenic potential in elderly patients. Nutrition diseases and endocrinopathies seem to have the most harmful effects on osteogenesis and the repairing processes of the bone tissue.

The evaluation of biochemical markers of bone turnover has been useful in clinical research. However, the predictive factor of these

measurements is not defined clearly, and these findings should not be used as a replacement for bone density testing [7]. There is a high prevalence of calcium, protein and vitamin D insufficiency in the elderly. Calcium and vitamin D supplements decrease secondary hyperparathyroi-dism and reduce the risk of proximal femur fracture, particularly in the elderly living in nursing homes. Sufficient protein intakes are necessary to maintain the function of the musculoskeletal system, but they also decrease the complications that occur after an osteoporotic fracture.

HOMOCYSTEINE

Sulfur is the seventh most abundant element measurable in the human body and is supplied mainly by the intake of methionine, an indispensable amino acid found in plant and animal proteins. Inhibition of cystathionine-β-synthase activity causes the upstream sequestration of homocysteine and the downstream drop in cysteine and glutathione [8]. Homocysteine is an aminoacid which contains a thiol group formed by methionine intracellular demethylation (alpha amino gama methylthio butyric). In plasma, homocysteine is found free in oxidized or disulphidic form, linked to proteins. Homocysteine has two ways to be metabolised, a metabolic path is represented by transsulfuration to cysteine through cystatin synthetasis, enzymes which need vitamin B6 as co-factor (proving the need of vitamin B6 administration during the treatment). Synthesis of cysteine as a product of the transsulfuration pathway can be viewed as part of methionine or homocysteine degradation, with cysteine being the vehicle for sulfur conversion to end products (sulfate, taurine) that can be excreted in the urine. Transsulfuration of homocysteine to cysteine is catalyzed by two pyridoxal 5′-phosphate-dependent enzymes, cystathionine β-synthase and cystathionine γ-lyase. The transsulfuration pathway is responsible for catabolism of the carbon chain of methionine, release of the amino nitrogen in a form that can be funneled into pathways of nitrogen excretion, and transfer of Met sulfur to serine to synthesize cysteine [9]. Another metabolic path is remethylation to methionine in the presence of methylentetra-hydrofolate reductase (MTHFR) and methionine synthesis in the presence of folic acid as an under layer for vitamin B12 as co-enzyme (proving the need of folic acid and vitamin B12 administration during the treatment). There are studies which proved that the level of homocysteine in blood is inversely proportional with folate levels, vitamin B12, vitamin B6 and oxygen intake induced by these vitamins [10].

Homocysteinuria is a rare autosomal recessive biochemical abnormality which causes elevated plasma concentrations of homocysteine and severe occlusive vascular disease. In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. Blood levels of total homocysteine increase throughout life in men and women. Prior to puberty, both sexes enjoy optimally healthy levels (6 μmol/L). During puberty, levels rise, more in males than women, reaching, on average, almost 10 μmol/L in men and more than 8 μmol/L in women. As we age, mean values of homocysteine continue to rise and the concentrations usually remain lower in women than in men. Adults without homocysteinuria who have high homocysteine levels are also at risk for fractures. Thus, elevated plasma homocysteine concentrations (>14 μmol/L) are associated with osteoporosis and may increase the risk of hip fracture, in both men and women. These can lead to substantial disability, high medical costs, and death [11]. Elevated plasma homocysteine concentrations are associated with reduced physical performance and muscle strength in older women [12].

Hyperhomocysteinemia may contribute to the development of osteoporosis. High hyper-homocysteine and low vitamin B12 concentrations were significantly associated with low bone turnover markers, high markers of bone turnover, and increased fracture risk [13]. Folate and vitamins B12 and B6 are major determinants of homocysteine concentrations in older persons [14]. The B vitamins folate, B12, and B6 are important cofactors in homocysteine metabolism, and low status of these

nutrients is the primary determinant of elevated plasma homocysteine concentrations in elders. Patients with pernicious anemia have decreased bone mineral density at the lumbar spine, and in comparison with the general population they have almost double the risk of hip fracture. Gene polymorphisms related to homocysteine metabolism also may result in high homocysteine levels. Thus, nutritional factors such as B12/folate deficiency and genetic factors may affect homocysteine levels and contribute to fracture risk.

Hyperhomocysteinemia is regarded as a risk factor for ischemic stroke and for hip fractures in Parkinson's disease patients receiving levodopa [15]. The high prevalence of osteoporosis among patients with homocysteinuria suggests that hyperhomocysteine may also increase the risk of fractures [16]. Higher plasma levels of total homocysteine and folate were independent predictors of coronary heart disease [17]. In the Framingham study authors had shown that plasma total homocysteine concentration is inversely related to the intake and plasma levels of folate and vitamin B6 as well as vitamin B12 plasma levels. Almost two-thirds of the prevalence of high homocysteine is attributable to low vitamin status or intake. Elevated homocysteine concentrations in plasma are a risk factor for prevalence of extracranial carotid artery stenosis of at least 25% in both men and women [18]. Some researchers want to determine if there is a possibility that plasma total homocysteine may serve as an indicator of the status and perhaps the intake of a number of vitamins, including folic acid, vitamin B12, and vitamin B6. This possibility derived from the large number of studies that implied that methionine metabolism is tightly regulated and from other studies that showed that deficiencies in the above vitamins are often associated with hyper-homocysteinemia.

It was shown that premenopausal women have lower homocysteine levels than men and postmenopausal women. Higher plasma levels of total homocysteine concentrations in men than in women may be explained by differences in muscle mass, hormone and vitamin status [19]. Factors that influence total homocysteine plasma levels in the general population include diet, in particular folate intake, blood levels of folate, vitamin B12, and betaine, renal function, and the MTHFR 677C>T polymorphism [20]. There are studies to establish that increasing evidence that plasma total homocysteine is inversely associated with bone health. It has been speculated that moderately elevated total homocysteine levels could contribute to osteoporotic changes, based on the fact that osteoporosis is a common phenomenon in homocysteinuria. High total homocysteine and low vitamin B12 concentrations are significantly associated with high levels of markers of bone turnover, and relations have been reported between total homocysteine and markers of bone resorption [13].

Elevated plasma total homocysteine, deficiencies of folate and vitamin B12 are associated with risk of osteoporosis and fracture. In some studies, there were examined whether plasma levels of elevated plasma total homocysteine, folate, and vitamin B12 predicted hip fracture [21]. They found that elevated plasma total homocysteine is a predictor for hip fracture among elderly men and women.

CONCLUSION

In patients with homocysteinuria, there is an increased prevalence of skeletal deformities, including osteoporosis, which is a primary risk factor for hip fracture. The high prevalence of osteoporosis among patients with homocysteinuria suggests that hyperhomocysteine may also increase the risk of fractures. Nutritional factors such as vitamins B12, B6, and folate are cofactors in homocysteine metabolism, and vitamin intakes may inversely affect plasma homocysteine levels.

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SYSTEMATIC REVIEW

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Efficacy and tolerability of calcium channel alpha-2-delta ligands in psychiatric disorders

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Abstract: *Matching drugs with anxiolytic properties- but without the potential of inducing dependence or abuse- with clinical manifestations of various affective disorders is a very important challenge for psychiatrists. Although the first line of pharmacologic treatment for anxiety disorders remains antidepressants with serotoninergic properties, calcium channel alpha-2-delta ligands are adjuvant agents which could be useful for augmenting antidepressant agents' clinical effects. Unfortunately, calcium channel alpha-2-delta ligands efficacy and tolerability are not very well known, due to a lack of large scale, randomized, placebo-controlled trials focused on psychiatric disorders. Data regarding pregabalin and gabapentin pharmacology and clinical effects are reviewed and conclusions with pragmatically impact based on the discovered evidence are formulated accordingly.*

Keywords: *calcium channel alpha-2-delta ligands, generalized anxiety disorder, fibromyalgia, social anxiety disorder, pregabalin, gabapentin*

PHARMACOLOGICAL PROPERTIES OF CALCIUM CHANNEL α2δ-LIGANDS

Alpha-2-delta subunits of voltage-gated calcium channels (VGCC) have an important role in modulation of the calcium currents and participate in important cellular and inter-cellular phenomena like muscular excitability, neurotransmission, re-gulation of gene expression etc. As a consequence, drugs with alpha-2-delta VGCC antagonist properties could improve symptoms of fibromyalgia and neuropatic pain, but they are also used for treatment of several psychiatric disorders, the most extensive researched being the field of anxiety disorders.

Pregabalin is a structural analog of the inhibitory neurotransmiter γ-aminobutyric acid (GABA). This drug reduces the synaptic release of several neurotransmitters through binding to the alpha2-delta type 1 protein and demonstrated anticonvulsant, analgesic, and anxiolytic properties in preclinical models [1,2].

Pregabalin is rapidly absorbed after oral administration, with a bioavailability value higher than 90%; peak plasma concentrations are reached after 1-1.5h, steady-state concentrations are achieved within 24-48h after repeated administration, and if used with food there is no clinically significant

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effect on the drug's absorption; pregabalin half-life is about 6 hours, it does not bind to plasma proteins, and 90% of a dose is eliminated unchanged in urine [3].

Gabapentin is derived from gamma-aminobutyric acid (GABA) by addition of a cyclohexyl group and crosses several lipid membrane barriers through L-amino acid transporters system [4]. Gabapentin has a bio-availability that varies inversely with dose (between 35% and 60%), a volume of distribution of 0.6-0.8 l/kg, cerebrospinal fluid concentrations are 20% of plasma concentrations and brain tissue values are 80% the plasma level; gabapentin is not metabolized in humans and is eliminated unchanged in the urine [5].

CLINICAL PHARMACOLOGY OF GABAPENTIN AND PREGABALIN

Agents from alpha-2-delta ligands class have a large number of indications in psychiatry and neurology, but this paper focused only on the first area of interest. Regarding the second domain, pregabalin and gabapentin are used for diabetic neuropathy, post-herpetic neuralgia, pain associated with spinal cord lesions, diverse types of seizures etc [6]. We also included in this paper trials focused on fibromyalgia, since this pathology usually requires an interdisciplinary approach, and psychiatrists are included in the consultation teams.

Pregabalin combined with duloxetine lead to good results in depression associated with fibromyalgia, according to a randomized, double-blind trial, which reported lower final Beck Depression Inventory–II (BDI-II) scores compared to placebo ($p<0.05$) [7]. Another randomized, placebo-controlled trial targeting fibromyalgia patients with asociated affective symptoms showed significant improvement on Hospital Anxiety and Depression Scale – Anxiety and Depression ($p<0.001$) [8].

Pregabalin used as augmentation agent to amitriptyline, venlafaxine, or paroxetine in old age patients with fibromyalgia improved overall symptoms, including depressive scores on Center for Epidemiological Studies Depression Scale (CESDS), and the highest tolerability was detected in pregabalin+ paroxetine group [9].

Pregabalin had a good therapeutic effect in anxious depression, according to a case series [10]. Adjunctive pregabalin to conventional antidepressants in partial responders with major depressive disorder and residual anxiety decreased Hamilton Depression Rating Scale overall scores and anxiety/somatization subscale scores after 8 weeks with 65% response rate and 35% remission rate [11]. Therefore, pregabalin could be recommended as add-on agent in unipolar depressed patients with significant levels of anxiety and 49.1% of overall consecutive diagnosed outpatients that received pregabalin had a diagnosis of mood disorder, followed by 21.9% generalized anxiety disorder [10,12].

A review of 3 randomized controlled trials that compared efficacy and safety of pregabalin with placebo in social anxiety disorder- generalized form revealed a good efficacy in patients who couldn't tolerate or didn't respond well enough to SSRIs or SNRIs, which recommend pregabalin as either alternative to antidepressants or add-on agent to pharmacotherapy or cognitive-behavioural therapy [13].

Pregabalin is considered by expert opinion a safe and efficacious agent for generalized anxiety disorder, with favourable properties like absence of active metabolites and no interactions with CYP450 enzymes [14]. A pooled analysis of 6 studies confirmed the efficacy of pregabalin in depressive symptoms associated with generalized anxiety disorder, and the most beneficial response was detected at 300-450 mg daily dose [15]. A recent post-hoc analysis of a multicenter, prospective, 6-month study evaluated the effectiveness of pregabalin in resistant generalized anxiety disorder and severe depressive symptoms and concluded reduced with more than 50% Hamilton Anxiety Scale total score, and Montgomery-Asberg Rating Scale score, when combined with antidepressants and/or benzodiazepines [16].

Pharmaco-economic analysis of augmentation SSRIs treatment with pregabalin versus switch to pregabalin in treatment resistant generalized anxiety disorder reported significantly health-care costs reductions at 6 months in both treatment algorithms [17]. Pregabalin was associated with significantly higher QALY gain in refractory generalized anxiety disorder when compared to usual care in a cost-effectiveness model based on data derived from a large scale trial [18]. Also, pregabalin was superior to SSRI and SNRI in benzodiazepine-refractory generalized anxiety disorder in terms of QALY gain, but increased health-care costs and drug costs [19].

Treatment augmentation with pregabalin in patients with combat-related chronic posttraumatic stress disorder (PTSD) was efficient in a 6-week placebo-controlled trial as it improved PTSD Check List-Military Version scores ($p<0.05$), although severity of depression, anxiety and quality of life parameters didn't differ significantly between the two groups [20]. An open label pilot study with accident-related posttraumatic stress disorder showed pregabalin augmentation to antidepressant treatment as an effective and well tolerated option [21]. However, a retrospective analysis of US service members who suffered burns didn't detect differences in posttraumatic stress disorder onset rate in patients that received pregabalin or gabapentin after trauma for pain, compared to patients who didn't receive this kind of drugs [22].

Pregabalin proved itself efficacious and well tolerated in a single-blind randomized trial with active control (clonidine) that targeted opioid withdrawal symptoms [23]. In alcohol dependence, pregabalin was efficacious, by decreasing of the craving and withdrawal symptomatology [24]. A review showed positive results for pregabalin in both treatment of alcohol dependence and benzodiazepine dependence [25].

Although the use of pregabalin as add-on agent in bipolar disorder maintenance treatment has not been extensively investigated, some authors suggest its potential use for this indication [26]. Pregabalin could increase the response to quetiapine in acute mania [27], and also could help in decreasing affective symptoms in treatment-resistant manic episodes [28]. An open trial of pregabalin as adjunctive treatment in acute and maintenance phase of resistant bipolar disorder showed mood-stabilizing effect, antidepressant effect or antimanic effect in the acute phase, and also with good efficacy on long term [29].

Data extracted from a metaanalysis focused on dopaminergic and non-dopaminergic medications in restless legs syndrome found 11 studies with $\alpha 2\delta$-ligands supporting good efficacy for gabapentin, gabapentin enacarbil, and pregabalin [30].

Use of pregabalin, as well as buspirone, added to antipsychotics in cases of schizophrenia with anxiety (almost 65% of patients with schizophrenia have anxiety symptoms), could be considered as an efficient therapeutic option [31]. Case reports suggest efficacy of pregabalin in treatment-resistant insomnia (in a patient who didn't respond to benzodiazepines, antidepressants with sedative properties, or antipsychotics) [32], and Charles Bonnet syndrome associated visual hallucinations [33], but also an enhancement of sexual desire in overdose [34].

Gabapentin significantly improved abstinence rates and heavy drinking in patients with current alcohol dependence, during a 12-week, double-blind, placebo-controlled trial, with linear dose-effects relation in mood, sleep, and craving domains [35]. Abstinence rates in this trial corresponded to a NNT value of 8 for 1800 mg daily, while lack of heavy drinking corresponded to a NNT value of 5 for the same dose [35]. Gabapentin reduced the stress-induced GABA activation in amygdala that is associated with alcohol dependence and therefore could be useful in this addiction [36].

Patients with opioid withdrawal that received adjunctive treatment with gabapentin in addition to methadone for 3 weeks reported significant improvement of general status as reflected by Subjective Opiate Withdrawal Scale (SOWS) and doses of 1600 mg/day were significantly superior to medium doses of 900 mg/day in decreasing symptoms' severity [37].

A Cochrane analysis of gabapentin efficacy in fibromyalgia associated pain didn't found good evidence to support or contradict the recommendation of this drug in daily doses of 1200-2400 mg [38]. However, the quality of evidence was rated as very low due to the fact that only one trial corresponded to the inclusion/exclusion criteria established by authors [38].

Gabapentin proved itself efficient in reducing symptoms of social anxiety disorder and it was also well tolerated [39].

Gabapentin wasn't efficient in bipolar depression, according to a large randomized controlled trial which compared standard mood stabilizers versus gabapentin 600-3600 mg/day [40].

A cross-over, double-blind study, showed gabapentin is efficient in treatment of sensory and motor symptoms in restless legs syndrome, improving also sleep architecture in periodic leg movements during sleep [41].

CONCLUSION

Pregabalin and gabapentin are efficient anxiolytics, pregabalin being more supported by evidence in social anxiety disorder, generalized anxiety disorder, depression with significant anxiety, and also in posttraumatic stress disorder, including cases of combat-related PTSD.

Pregabalin is a well supported indication for fibromyalgia treatment, with a high efficacy-to-adverse-effects ratio. Pregabalin had a significant impact not only over pain, as the core fibromyalgia symptom, but also over affective symptoms associated with this disorder.

Gabapentin recommendation is more supported by evidence than pregabalin in alcohol dependence and opioid withdrawal, but pregabalin has some support in the treatment of benzodiazepine dependence.

None of the alpha-2-delta ligands were associated with high quality positive evidence in bipolar disorder.

In restless legs syndrome pregabalin and gabapentin are efficient for primary symptoms and associated clinical manifestations, and gabapentin improved sleep architecture in sleep related periodic leg movements.

Occasional reports of pregabalin efficacy in schizophrenia associated anxiety, treatment resistant insomnia or Charles-Bonnet syndrome need further exploration in randomized clinical trials.

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Medicine versus philosophy

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Abstract: *The ancient Greek medicine was based on the principle that philosophy influences all natural sciences as a whole. The doctor had, first of all, a humanistic formation followed by study of applied sciences specific to medicine. If humanism is purely theoretical, medicine is an applied science and the two-philosophy and medical knowledge, despite the apparent antinomy are able to create a union to the benefit of humanity. Medicine is the art of treating patients, identifying diseases and malady prevention. In its endeavor, medicine is based on the findings of numerous other fields such as physics, chemistry, anatomy, physiology, etc. Philosophy, on the other hand, can be defined as an attempt to understand human life as a whole. It is inevitable that the two ways of dealing with human beings to have influenced each other and the history of mankind. Both forms of knowledge have a major impact and influence on the world. Philosophy, understood in its older meaning, urged towards the prophylaxis and treatment of diseases of the soul whereas medicine, relying on philosophical teachings is aimed at healing the body and study its psychosomatic features.*

Keywords: *medicine, physician, philosophy, methodology, metaphysics*

Medicine and philosophy have influenced each other along mankind's history.

The present article aims and present the way in which renowned physicians have blended the strict knowledge of medical science with the more humanistic philosophical approach.

Attempts of demonstrating how philosophy clout over medicine have existed since Ancient times. Our paper is just a glimpse in the outstanding synthesis between the two apparently incongruent areas of knowledge.

Claudius Galenus of Pergamum (129-216) is one of the first physicians who sensed the need of a philosophical foundation of clinical practice. The beginnings of his scientific training originate in studying Aristotle's texts. Galen, who stated that a good physician should first of all be a philosopher, is the one who realized the necessity of associating medicine with the philosophy in order to achieve better results in human treatment. Personal physician for Marcus Aurelius, Galen received his medical expertise in a gladiators' school. His name links to the first cataract surgeries, our knowledge of the spinal cord and the functioning of the kidneys. He divided the intercellular fluid into humors: blood, bile, lymph and spleen.

What brought Galen brought new during his times was an experimentalist approach. But he abandoned his original humanist-philosopher formation, after

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having a dream, for the practical practice of medicine, "intensively dealing with philosophy yet, confident peripatetic, he is still a syncretistic, which is better seen in his logic." [1]

During a great fire large part of his writings were destroyed yet his Institutio Logica was kept. His contribution to philosophy consists in demarcation made between logic and philosophy by postulating the theory of equivalence and by introducing of the fourth figure in syllogism.

Abu Bakr Muhammad ibn Zakariya' al-Razi (865-925), known in the western world as Rhazes, was of Persian origin and promoted experimental medicine bringing significant contributions in pediatrics, neurosurgery, nephrology and ophthalmology. In his early life, Al-Razi was more interested in studying music and alchemy. Rhazes, physician at the court and a hospital director in Baghdad, was prolific in the scientific field through the books he wrote. Some of the most important papers signed by the Arab scientist include Medicine Treaty for Mansur (dedicated to the governor of Rayy containing ten chapters for diseases which was to be translated in Latin, by scholar Gerard of Cremona, under the name Liber ad Almansoris), the study Smallpox and measles, works on applied medicine (About surgery), books meant to popularize medicine (The book to one who cannot reach the doctor), as well as doctors' guide aimed at his fellow physicians (The guide of the nomad physician and Royal medicine).

Al-Razi believed that in serious cases of leprosy and cancer, the doctor cannot blamed or kept responsible for the inability of curing the patient. Furthermore, the physician-philosopher wrote extensively about medical ethics. His area of interest included medical chemistry, in which he made experiments with mercury, sulfuric acid, alcohol, and paraffin. In the field of pharmacy, Al-Razi made his mark by introducing devices such as mortar, vials and spatula.

Neither the field of metaphysics and philosophy were foreign to him as his papers in these fields recommend his as an inborn scholar. Relying on Aristotelian system and Plato's philosophical thinking, Al-Razi wrote an ambitious paper ambitious in nine volumes, Virtuous life, book in which he accused Galen of not supporting his medical findings on too many case studies. Another important work with great impact, this time on the general public, The book to one who cannot reach the doctor, has the merit of explaining some diseases and associated treatments on the simple men's understanding. The book talks about some of the most encountered afflictions such as headaches, colds, coughing, piles, diabetes, and other gastric ailments such as dysentery, ophthalmic and ear conditions, which were associated to medical treatments in order to be healed. He was the first physician who associated allergic rhinitis to the scent of flowers.

Al-Razi believed that a physician cannot really be a good practitioner unless he was a philosopher. The Persian doctor was a follower of the Euclidean theory of the space considered homogeneous and isotropic, regardless of the spatial distribution of matter. For al-Razi, to this absolute space and mechanical time corresponds the world to which man is reported. Also, Democritus' theory, that the world is composed of atoms structured into matter and vacuum was adopted by the philosopher of Arab origin. His metaphysical system is based on the belief that the soul is intelligent and that the three-dimensional reality consists of time, space and matter. Al-Razi believed in afterlife.

In order to overcome the fear of nothingness, people, in his opinion, should instruct in areas such as religion and esotericism. Among his works on the border between medicine, philosophy and religion we can mention Spiritual medicine, Philosophical approach, Metaphysics, Small treatise on deism, Modern philosophy, etc.

Avicenna (980-1037), philosopher, physician and writer of Persian origin, in his paper Canon Medicinae (1025) set out methods of understanding, differentiation and variability of phenomena, considered methodology, which currently is regarded as vital in inductive logic and scientific methodology. In another treatise, Sanatio (1027), the Persian thinker brought criticism to the Aristotelian methods of inference, because they had, according to him, an absolute value. As such, the Arab polymath developed a complex of examination and testing methods meant to meet scientific challenges.

Francis Bacon (1561- 1626) has influenced science, in general and medicine, in particular. Renowned writer, philosopher and scientist he was the originator of empiricism as a way to test all the scientific achievements. He initiates controlled experiments. In his New Organon, Bacon stances diametrically opposed to the deductive, Aristotelian thinking. For Bacon induction is one that takes precedence as it is meant to "substitute once for all idealistic Scolastico-medieval one based on syllogistic deduction (..)." [2] Bacon talked about mind and soul as tantamount notions. Efficacious treatment of the body in medicine, according to the English thinker, implies a thorough study of the organism.

If practitioners of medicine fail in achieving their goals is due to lack of visionary perspective: the body is a complex mechanism which cannot be treated on parts, rather as a whole. In order to learn as much as possible about the human body implies clinical observation, along with the analogy of different bodies, vivisections and careful scrutiny of pathological changes. The physician has a double role: to reestablish the well-being of the diseased as well as to reduce suffering of those who are terminally-ill. What Bacon brought new to the field of medicine is the larger perspective of this field. He made no distinction between medical area and natural sciences. Philosophy of medicine is just a particular type of the more general knowledge of philosophy.

Jean-Paul Marat (1743-1793) was born in Boudry, nowadays Switzerland, but played an important role during the French Revolution. During his teenage period, he left home searching for fame and to build a stable financial situation. Marat studied medicine in Paris but failed to obtain a diploma in this area. However, he published a study about the way he treated his friends for gonorrhea, which propelled him in the medical world. In 1773, Marat published a paper entitled Essay on human philosophy. This work has the ambition to present, as scientifically as possible, the relationship between the human body and the soul and the way in which the two are interrelated. Thus, Marat believed that the soul and body were separate entities, which could, however, affect each other through the liquid within the nervous system. Marat, in this essay, analyzed the way in which, physiologically, the body can respond to emotional experiences through the excitement of the cardiac plexus.

Marat's work has the merit to connect the two planes – physiological and spiritual – into a unitary whole. The author analyzes, through his knowledge of human anatomy and physiology, how the body folds onto affects. The bodies can influence, in the physician-philosopher's opinion, the mode of existence of the soul. Thus, there are what we call qualities such as wisdom, stupidity, prudence, reason, imagination, memory, delicacy, sagacity and genius. The influence of his medical studies can be seen by how Marat relates and, therefore, builds his philosophical edifice on diseases such as spina bifida and microcephaly. The essay remarks itself by references the author made to areas of knowledge such as history, literature and botany.

In 1775, Marat obtained from the University St. Andrews references necessary to work effectively as a physician which would bring him, two years later, his appointment as the guard physician for Count d'Artois, who would become Charles X. In London, Marat published a paper dedicated to eye diseases and their way of treating: Enquiry into the Nature, Cause, and Cure of a Singular Disease of the Eye. His reputation grows and the financial situation is improving. With the money earned, he established a laboratory marquise l'Aubespine's house.

His experiments would end in his work Marat's findings related to fire, electricity and light (1779). Based on experiments, his scientific work continues. A year later, in 1780, he published About the physics of fire. Unfortunately, the Academy of Sciences did not approve his work since Marat had had the courage to call into doubt some of Newton's conclusions about refraction. His growing influence increase within scientific circles and personalities in the field recognized his value. They include Benjamin

Franklin and Goethe. In 1788 he published another work based on experimental method: Research on the physics of light. Fascinated by the subject, meanwhile, the French philosopher published essays related to electricity and medical applications of optics Memories on medical electricity (1783) and Basic optical notions (1784).

But his exuberant personality did not keep him away from politics. Enlightened spirit and with an incisive tone, Marat advocated equality of men. His political beliefs had to find a place in the newspaper The people's friend, originally named Publiciste parisien, which he edited beginning with 1789. In 1782, due to his radical views, inspired by Rousseau and Cesare Beccaria, Marat was publishing Public plan in criminal legislation that supported the idea that the death penalty should apply regardless of social status, advocating the idea of an ombudsman institution. Member of the Jacobin movement, which played an important role during the reign of terror Marat would end assassinated by stabbing, in his bath by Charlotte Corday Marie-Anne d'Armont.

Arthur Schopenhauer (1788-1860), German philosopher who would influence numerous other philosophers such as Nietzsche, Freud, Bergson, Ludwig Wittgenstein and Cioran, had a great impact on the literature of psychological character, writers such as Tolstoy, Eminescu, Proust and Thomas Mann. One of his main works was The world as will and representation (1818). Initially, in 1809 he entered the Medicine University of Göttingen which he would abandon in favor of philosophy, becoming a doctor with a thesis on the fundamental principles of thinking: The quadruple root of the principle of sufficient reason (1813). The reason for which the philosopher had as first option the study of medicine was that he wanted to know the world objectively, scientifically before starting his theoretical speculation.

Incidentally, later, Schopenhauer concludes that absolute knowledge can only take place in the presence of solid knowledge about natural sciences. The courses he attended as a medical student were extremely varied: physics, chemistry, mathematics, botany, mineralogy, physiology, comparative anatomy, humanities as well as humanistic sciences such as linguistics, ethnography, history. The great biologist and anatomist Johann Friedrich Blumenbach, who connected the human being with the study of natural sciences and linked his name to identifying five human race, would play an important role in the shaping of the future philosopher.

Just that anatomist's love towards animals made Schopenhauer reluctant to vivisections and determined him to step towards more academic areas. During his medical studies period, Schopenhauer himself had a poodle with whom he used to take long walks. During the second half of the first year in medicine, the one who was to influence generations of philosophers discovered the writings of Plato, Kant, Schelling and Upanishads. During his second year of medicine Schopenhauer concluded that he needed to change his studies, by choosing philosophy.

His philosophical system, set up during his medical studies, is based on the principle that, the basis of reality, is suffering. The first argument is that happiness is an illusion, life being nothing but a permanent deception. Yet, reality may be dominated by will. The very basis of the will is a necessity. Schopenhauer considers individual goals to have no rational basis because everything is evanescent, the only certainty being only death. According to the German philosopher, there are two teleologies: an external one (human beings' goals) and internal (understanding the purpose of life) and the one that should be taken into consideration is non-existence.[3]

Although tempted to teach in the University of Berlin, Schopenhauer renounces to academic life aspirations. In 1839 he becomes member of the Norwegian Society of Sciences. His true recognition occurs following the publication of a volume of philosophical essays Parerga and Paralipomena (1851).

Not being a religious spirit, the German philosopher feels attracted more towards oriental doctrines, such as Hinduism and Buddhism, the mystical practices and Theurgy than to Christianity. Although Freud denied Schopenhauer's influence in his clinical work, there were others who stated a close correlation between hard practice and Freudian theories of Schopenhauer. It is about Freudian theories of repression and sexuality that coincide with the ideas of the German philosopher.[4]

Karl Theodor Jaspers (1883-1969) saw daylight in Lower Saxony, in Oldenburg. Although initially prepared to study law, Jaspers chose, in 1902, medical studies which he completed seven years later. His work in the hospital of Heidelberg as a psychiatrist brought him dissatisfaction with the state of psychiatry at the time, therefore, in 1913, when he had the chance, he would choose a teaching career at the University of Heidelberg. In time, Jaspers began to connect the knowledge acquired clinically with the study of philosophy. The work that propelled him in psychology was Treaty of general psychopathology.

In 1932, more and more concerned with the study of philosophy, Jaspers signed another reference work (Philosophy) that subscribes to the existentialist current. Like Schopenhauer, Jaspers felt more attracted to Eastern religions than to Western theological doctrines. Consciousness of guilt (1946) came to blame the indifference and even the moral guilt of the people whose citizen he was, representing a wake-up call “partly utopian toward self-analysis in order to overcome the chaos, a sense of guilt that seemed to be put under possession of souls Germans.”[5] The guilt of the German population is the complicity to the Nazi’s genocide. In 1947 Jasper signed a book that seems to reveal the link between the scientific and the spiritual side of its author: Philosophical logic. Jaspers's influence in the medical field translates into identifying premises of psycho-pathology: descriptive principle, that of comprehension and of causality. Jaspers’ methodology proposes a clear, clinical, study of mental pathologies such as psychosis and schizophrenia.

If in modern society the connection between medicine and psychology becomes a more profound by the fact that medical psychology brings together knowledge from various areas of science such as the fields of border such as sociology, anthropology, psychopathology, holistic, experiential and dynamic psychology, psychoanalysis, chronobiology, ethology and neurophysiology, in the past this connection almost did not exist. Psychology has made the junction between medicine and philosophy.

Medicine as a branch of scientific knowledge is a form of cognitive understanding while philosophy is a science although it has only partially cognitive aspiration to become an area of scientific knowledge. Psychology became the first bond between the two. Gradually, both medical science and philosophy have found in common their nomological value. And the merit of physicians is to succeed, and not in few cases, to make the junction between these areas so apparently opposing.

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ORIGINAL ARTICLES

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Incidence of peripheral trophic disorders determined by vein thrombosis of the lower limbs correlated with risk factors by age

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Abstract: *Introduction: Venous thromboembolism (VTE), in its clinical spectrum, includes both deep venous thrombosis (DVT) and pulmonary embolism (PE). It is a disease with high incidence and morbidity in hospital and community settings. Venous thromboembolism has various risk factors and there are studies proving that the risk of increasing the incidence of the disease is proportional to the risk factors.*

Diagnosis, treatment and complications of lower limb deep vein thrombosis (DVT) depend on the anatomical location and extent of the process. The post-thrombotic syndrome (PTS) is the most common complication of deep vein thrombosis (DVT) and clinically it is characterized by chronic pain, edema, enlarged veins, skin induration and other signs of the affected limb, while, in severe cases, it can develop venous ulcers. The incidence of peripheral trophic disorders by age and the prevalence of risk factors for deep vein thrombosis of the lower limbs were examined in this regard.

Materials and method: A retrospective study (January 2013 - December 2015) was conducted by collecting data from medical documents available in "Floreasca" Emergency Hospital Bucharest, Romania.

The patients diagnosed with deep vein thrombosis, on the basis of Doppler ultrasound, were divided into two groups, according to age: group A (59 patients aged ≤50 years) and group B (130 patients aged> 50 years). A number of data from the medical anamnesis, along with clinical and paraclinical data were collected by us and we were interested in the incidence of peripheral trophic disorders caused by deep vein thrombosis of the lower limbs correlated with the risk factors.

The study showed the incidence of deep venous thrombosis in a certain age and a certain environment of origin. The incidence of patients who have had a VTE history is half the patients with deep vein thrombosis who have had prophylactic anticoagulant therapy before hospitalization. The incidence of patients who have had prophylactic anticoagulant therapy before hospitalization is 61.1% of the patients with deep vein thrombosis and a VTE history. The incidence of trophic disorders caused by deep vein thrombosis of the lower limbs in patients who have had prophylactic anticoagulant therapy before hospitalization and in patients who also had a history of VTE is higher in those over 50 years old. The study showed the association of some risk factors for venous thrombosis with an age-related factor.

Conclusions: Improving preventive strategies and an optimally efficient utilization of these strategies for patients at risk of venous thrombosis can lead to improved clinical outcomes in practice and also to the post-thrombotic syndrome prevention. Taking into consideration the risk factors by age group and a better understanding of epidemiology and the risk factors for the first or recurrent venous thrombosis can lead to optimal use of prophylactic strategies and improved quality of life. DVT affects all age groups and the incidence associated with PTS is high, therefore the prevalence of PTS in general population is considerable.

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Thrombosis is also associated with impaired quality of life, especially when post-thrombotic syndrome develops. To assess the overall risk of VTE in every patient, individual risk factors or combinations of these should be carefully analyzed, an aspect that may have important implications for the type and duration of appropriate prophylaxis.

Keywords: *peripheral trophic disorders, post-thrombotic syndrome, venous thrombosis, risk factors, age groups*

INTRODUCTION

Deep vein thrombosis (DVT) is characterized by the formation of blood clots (thrombi) in the deep veins and usually affects the deep veins of the legs or the deep veins of the pelvis [1]. Venous thromboembolism (VTE) is manifested as deep venous thrombosis (DVT) or pulmonary embolism (PE) and occurs at an incidence of approximately 1 per 1,000 annually in adult populations [2].

About two-thirds of the episodes manifest themselves as DVT and a third as PE, with or without DVT. [3]. VTE is a very common medical problem that occurs either in isolation or as a complication of other diseases or procedures [4]. It is predominantly a disease of older adults and has a slight preponderance of males [1]. To prevent potentially fatal acute complications of pulmonary embolism (PE) and long-term complications of post-thrombotic syndrome and pulmonary hypertension, an accurate diagnosis of DVT is extremely important.

It is also important to avoid unjustified anticoagulant therapy in patients diagnosed with high risk of bleeding [5]. DVT prevention through prophylaxis, recognition in due time and DVT treatment and prevention of recurrent DVT will continue to have the greatest impact on reducing the global burden of post-thrombotic syndrome. Despite considerable progress in the diagnosis and treatment of deep vein thrombosis (DVT) of the lower extremities, one in every 2-3 patients will develop post-thrombotic sequelae within two years, which are severe in about 10% of cases and produce considerable socio-economic consequences [6].

DVT affects all age groups and the incidence associated with PTS is high, therefore the population prevalence of PTS is considerable [7]. Thrombosis is also associated with impaired quality of life, especially when the post-thrombotic syndrome develops [8.9].

MATERIALS AND METHOD

The study was retrospective (January 2013 - December 2015) and the data were collected from medical documents available in "Floreasca" Emergency Hospital Bucharest, Romania. The method used in this paper is the observational, non-experimental, descriptive study. In the study group there were included patients diagnosed with deep vein thrombosis of the lower limbs, based on the Doppler ultrasound, hospitalized in various wards of the Emergency Hospital, such as, internal medicine, orthopedics, cardiology and general surgery wards. The Doppler ultrasound determined the presence of chronic venous insufficiency, the type of venous thrombosis - deep or superficial and its location - proximal and distal.

The group of patients with deep vein thrombosis (DVT) comprises 189 patients, of which 54 have superficial vein thrombosis (SVT). According to their age, we divided the patients into two groups: group A (59 patients aged ≤50 years) and group B (130 patients aged> 50 years). For each patient we collected general data (age, gender, origin), and clinical and paraclinical data. The clinical data have identified the presence of unilateral leg edema or the entire leg edema and the presence of peripheral trophic disorders (erythema, infiltration, skin induration, cellulitis and venous ulcers).

From the anamnesis data we identified the presence of comorbidities and risk factors, namely immobilization before hospitalization, a history of venous thromboembolism (VTE) and pulmonary thromboembolism (PE), anticoagulation prior to hospitalization, various medical conditions, a history of surgical conditions (orthopedic, gynecological, urological, abdominal), neoplasm and antineoplastic treatment, cerebrovascular accident associated with motor deficiency, congestive heart failure, renal disease (renal lithiasis, nephrotic syndrome, hydronephrosis, chronic kidney disease, enlarged prostate), obesity, diabetes, hypertension, a history of heart attack, fractures before admission, alcohol consumption, smoking.

We used SPSS, version 15.0, to statistically analyze the data. For some additions to the statistical analysis we used the MedCalc program. Some of the graphics were done with Excel 2007 and other graphics with SPSS. The vast majority of the data were nominal (Yes, No); for these we did the analysis using the Chi square test. For the type of numeric data we did an ANOVA analysis. An OR (Odds Ratio) risk assessment was calculated for risk factors with the Mantel-Haenszel test. We also used binary logistic regression. The statistical differences and dependencies were statistically significant for Sig <0.05.

RESULTS

The group of patients with deep vein thrombosis (DVT) comprises 189 patients, of which 54 (28.6%) have superficial vein thrombosis (SVT). We divided patients into two groups: patients aged ≤50 years (31.22%) and patients aged> 50 years (68.78%). Applying ANOVA with a variable depending on age and an independent variable belonging to one of the two groups, we obtained as a result the average age of the patients of the first group as 37.71 years old with SD = 8,445, and for those of the second group as 68.50 with SD = 11,133. The difference between the two means is statistically significant (Sig <0.001). In the first group we have 23 (38.98%) women and 36 (61.02%) men and in the second 62 (47.69%) women and 68 (52.31%) men.

In the first group we have 9 (15.25%) patients in rural areas and 50 (84.75%) patients in urban areas, and in the second we have 30 (23.08%) patients in rural areas and 100 (76 92%) patients in urban areas. Therefore, the prevalence of patients in urban areas is very high, about 80%. By using Chi square analysis and an OR estimation performed with SPSS and MedCalc, we analyzed the relationship between variables and there are 72 patients taking anticoagulants before admission, namely 24 (40.68%) in the first group and 48 (36, 92%) in the second group. According to the test, there is no statistically significant link (Sig = 0.622) between the presence in one of the two groups and the existence of anticoagulant prior to admission. The OR estimation is not statistically significant because Sig = 0.622. From the group of patients with deep vein thrombosis the incidence of patients who had prophylactic treatment with anticoagulants before admission is 38.09% and among those the incidence of the patients who had a history of VTE is 50%.

The incidence rates of a history of VTE, of PE at admission and in the personal history for these 72 patients are presented in Table 1.

Table 1. The incidence rates of a history of VTE, of PE at admission and in the personal history

Sex	≤ 50		> 50		Total	
	N	%	N	%	N	%
History of VTE	12	50.0%	24	50.0%	36	50.0%
PE at admission	2	8.3%	1	2.1%	3	4.2%
History of PE	2	8.3%	2	4.2%	4	5.6%

From the group of patients with deep vein thrombosis, the incidence of VTE in patients who had a history of VTE (58 patients) is 30.68% and were distributed as follows: 19 (32.2%) in the first group and 39 (30.0%) in the second group. Among those patients who had a history of VTE, 38.9% had a history of VTE without Prophylactic anticoagulation treatment and 61.1% had a history of VTE with prophylactic anticoagulant treatment. The incidence rate of PE at admission and in the personal history among these 58 patients are presented in Table 2.

Table 2. The incidence rates of PE at admission and in the personal history

History of VTE	≤ 50		> 50		Total	
	N	%	N	%	N	%
PE at admission	2	10.5%	2	5.1%	4	6.9%
History of PE	3	15.8%	3	7.7%	6	10.3%

Taking this aspect into consideration enables us to optimally use the prophylactic strategies against venous thromboembolism. A better understanding of epidemiology and the risk factors for the first and the recurrent venous thrombosis can lead to improved clinical outcomes in practice. To assess the overall risk of VTE in every patient, individual risk factors or combinations of these should be carefully analyzed, an aspect that may have important implications for the type and duration of appropriate prophylaxis.

The incidence of trophic disorders caused by venous thrombosis of the lower limbs in patients who had prophylactic anticoagulant therapy before hospitalize-tion is 23.6% venous ulcers, 93% edemas, 83.3% different trophic disorders (reddish–brown cutaneous depigmentation, indurated fibrous skin, redness, irritation or dermatitis) and 12.5% cellulitis. Statistical analysis by age group reveals that the incidence is higher in patients over 50 years old, namely 24.5% venous ulcers, 93.9% swelling, 91.83% different trophic disorders and 14.28% cellulitis. In patients who are under 50 years old there was an incidence of21.73% venous ulcers, 91.30% edemas, 65.21% various trophic disorders and 8.7% cellulitis.

The incidence of trophic disorders caused by venous thrombosis of the lower limbs in patients who had prophylactic anticoagulant therapy before hospitalize-tion and history of VTE by age group reveals that the incidence is higher in patients over 50 years old, namely 45.83 % venous ulcers, 100% swelling, 91.66% various trophic disorders and 25% cellulitis. In patients under 50 years old there was an incidence of 41.6% venous ulcers, 100% swelling, 66.7% various trophic disorders and 16.7% cellulitis.

According to the data provided by Chi square analysis and OR estimation performed with SPSS and MedCalc test, there is a statistically significant link (Sig = 0.003) between the presence in one of the two groups and the presence of chronic venous insufficiency. There are 78 patients with chronic venous insufficiency: 15 (25.42%) in the first group and 63 (48.46%) in the second group. Cont. Coef. = 0.212 is the strength of that link. Sig = 2.758 and OR = 0.003, therefore it is statistically significant. Chronic venous insufficiency is more present in the second group.

By using Chi square analysis and OR estimation performed with SPSS and MedCalc, we analyzed the relationship between variables, specifically the risk factors present in the database, considered to be risk factors for DVT, for each group separately (Table 3).

We obtained statistically significant values:

○ For Varicose veins, there is a statistically significant link (Sig = 0.016) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.173 is the strength of that link. OR = 2.471 and Sig = 0.018, therefore it is statistically significant. Varicose veins are more common in the second group.

○ For Congestive heart failure, there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.352 is the strength of that link. OR = 63.328 and Sig = 0.004, therefore it is statistically significant. Congestive heart failure is present only in the second group.

○ For Fractures before admission, there is a statistically significant link (Sig = 0.006) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.197 is the strength of that link. OR = 0.326 and Sig = 0.007, therefore it is statistically significant. Fractures before admission are more common in the first group.

○ For Smoking, there is a statistically significant link (Sig = 0.027) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.159 is the strength of that link. OR = 0.494 and Sig = 0.028, therefore it is statistically significant. Smokers are more present in the first group.

○ For HTN (hypertension), there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.499 is the strength of that link. OR = 53.833 and Sig <0.001, therefore it is statistically significant. HTN is present almost entirely in the second group.

Table 3. Analysis of risk factors by age group.

Risk factors	≤ 50 years		> 50 years		Chi square analysis		Odds ratio	
	No N (%)	Yes N (%)	No N (%)	Yes N (%)	Cont coef	Sig	OR	Sig
Immobilization before admission/hospitalization	47 (79.66%)	12 (20.34%)	88 (67.69%)	54 (41.54%)	0.122	0.091	1.869	0.094
Varices	48 (81.36%)	11 (18.64%)	83 (63.85%)	47 (36.15%)	0.173	0.016	2.471	0.018
History of VTE	40 (67.80%)	19 (32.20%)	91 (70.00%)	39 (30.00%)	0.022	0.761	0.902	0.761
PE at admission	53 (89.83%)	6 (10.17%)	124 (95.38%)	6 (4.62%)	0.105	0.147	0.427	0.157
History of PE	55 (93.22%)	4 (6.78%)	125 (96.15%)	5 (3.85%)	0.064	0.380	0.550	0.386
Obesity	35 (59.32%)	24 (40.68%)	84 (64.62%)	46 (35.38%)	0.051	0.485	0.799	0.485
Congestive heart failure	59 (100.00%)	0 (0.00%)	85 (65.38%)	45 (34.62%)	0.352	<0.001	63.328	0.004
COPD or pulmonary cond.	49 (83.05%)	10 (16.95%)	111 (85.38%)	19 (14.62%)	0.030	0.680	0.839	0.680
Anemia	44 (74.58%)	15 (25.42%)	81 (62.31%)	49 (37.69%)	0.119	0.099	1.774	0.101
Fractures prior to admission	44 (74.58%)	15 (25.42%)	117 (90.00%)	13 (10.00%)	0.197	0.006	0.326	0.007
Smoking	30 (50.85%)	29 (49.15%)	88 (67.69%)	42 (32.31%)	0.159	0.027	0.494	0.028
HTN	57 (96.61%)	2 (3.39%)	45 (34.62%)	85 (65.38%)	0.499	<0.001	53.833	<0.001
Peripheral artery disease	59 (100.00%)	0 (0.00%)	113 (86.92%)	17 (13.08%)	0.207	0.004	18.348	0.044
Type 2 diabetes	56 (94.92%)	3 (5.08%)	85 (65.38%)	45 (34.62%)	0.300	<0.001	9.882	<0.001
Lipid alterations	39 (66.10%)	20 (33.90%)	67 (51.54%)	63 (48.46%)	0.135	0.062	1.834	0.063
Alcohol consumption	45 (76.27%)	14 (23.73%)	114 (87.69%)	16 (12.31%)	0.143	0.046	0.451	0.050
Extensive distal localization (calf)	11 (18.64%)	48 (81.36%)	9 (6.92%)	121 (93.08%)	0.174	0.015	3.081	0.019
Extensive proximal DVT localization	25 (42.37%)	34 (57.63%)	36 (27.69%)	94 (72.31%)	0.144	0.045	1.920	0.047
Patients with medical conditions	47 (79.66%)	12 (20.34%)	25 (19.23%)	105 (80.77%)	0.500	<0.001	16.450	<0.001
Patients with a history of surgical conditions	51 (86.44%)	8 (13.56%)	76 (58.46%)	54 (41.54%)	0.266	<0.001	4.530	<0.001
History of major gynecological surgical interventions	58 (98.31%)	1 (1.69%)	115 (88.46%)	15 (11.54%)	0.162	0.024	7.565	0.053
History of major urological surgical interventions	50 (84.75%)	0 (0.00%)	114 (87.69%)	16 (12.31%)	0.201	0.005	17.149	0.049
Hip or knee arthroplasty. Hip surgery	48 (81.36%)	11 (18.64%)	112 (86.15%)	18 (13.85%)	0.062	0.396	0.701	0.398
History of heart attacks	59 (100.00%)	0 (0.00%)	120 (92.31%)	10 (7.69%)	0.157	0.029	10.369	0.108
Renal conditions (CKD. Hydronephrosis renal lithiasis)	55 (93.22%)	4 (6.78%)	109 (83.85%)	21 (16.15%)	0.127	0.078	2.649	0.087
Neoplasia	57 (96.61%)	2 (3.39%)	95 (73.08%)	35 (26.92%)	0.265	<0.001	10.500	0.002
CVA (mainly associated with motor deficiency)	59 (100.00%)	0 (0.00%)	121 (93.08%)	9 (6.92%)	0.149	0.038	9.305	0.127

○ For Peripheral arterial disease, there is a statistically significant link (Sig = 0.004) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.207 is the strength of that link. OR = 18.348 and Sig = 0.044, therefore it is statistically significant. Peripheral artery disease is present only in the second group.

○ For Type 2 diabetes, there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and risk factors analyzed. Account. Coef. = 0.300 is the strength of that link. OR = 9.882 and Sig <0.001, therefore it is statistically significant. Type 2 diabetes is now almost entirely present in the second group.

○ For Alcohol consumption, there is a statistically significant link (Sig = 0.046) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.143 is the strength of that link. OR = 0.451 and Sig = 0.050, therefore it is statistically significant. Alcohol consumption is more present in the first group.

○ For Extensive distal location (calf), there is a statistically significant link (Sig = 0.015) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.174 is the strength of that link. OR = 3.081 and Sig = 0.019, therefore it is statistically significant. Extensive distal location (calf), is more present in the second group.

○ For Extensive proximal DVT localization, there is a statistically significant link (Sig = 0.045) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.1144 is the strength of that link. OR = 1.920 and Sig = 0.047, therefore it is statistically significant. Extensive proximal DVT localization is more present in the second group.

○ For Patients with medical conditions, there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.500 is the strength of that link. OR = 16.450 and Sig <0.001, therefore it is statistically significant. Medical conditions are highly present in the second group.

○ For Patients with a history of surgical conditions, there is a statistically significant link (Sig <0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.266 is the strength of that link. OR = 4.530 and Sig <0.001, therefore it is statistically significant. Surgical conditions are more present in the second group.

○ For History of major gynecological surgical interventions, there is a statistically significant link (Sig=0.024) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.162 is the strength of that link. OR = 7.567 and Sig=0.053, therefore it is statistically significant. The major gynecological surgical interventions are more present in the second group.

○ For History of major urological surgical interventions, there is a statistically significant link (Sig=0.005) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.201 is the strength of that link. OR = 17.149 and Sig=0.049, therefore it is statistically significant. The major urological surgical interventions are present only in the second group.

○ For History of heart attacks (myocardial infarctions), there is a statistically significant link (Sig=0.029) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.157 is the strength of that link. OR = 10.369 and Sig=0.108, therefore it is statistically significant. The heart attack is present only in the second group.

○ For Neoplasms, there is a statistically significant link (Sig<0.001) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.265 is the strength of that link. OR = 10.500 and Sig=0.002, therefore it is statistically significant. Neoplasms are more present in the second group.

○ For CVA (mainly associated with motor deficiency), there is a statistically significant link (Sig=0.038) between the presence in one of the two groups and the presence of the analyzed risk factors. Cont. Coef. = 0.149 is the strength of that link. OR = 9.305 and Sig=0.127, therefore it is statistically significant. CVA is present only in the second group.

A logistic regression for age was performed with all these factors as covariates and the following result present in table 4 was obtained.

Table 4. Logistic regression for age

	B	S.E.	Wald	df	Sig.	OR
Immobilization before admission	1.020	0.997	1.047	1	0.306	2.774
Varices	0.179	0.678	0.070	1	0.791	1.197
History of VTE	-0.401	0.644	0.387	1	0.534	0.670
PE at admission	-0.186	1.098	0.029	1	0.865	0.830
History of PE	-1.460	2.001	0.532	1	0.466	0.232
Obesity	-0.602	0.647	0.867	1	0.352	0.548
Congestive heart failure	17.980	4725.744	0.000	1	0.997	6E+007
COPD or pulmonary conditions	***-2.264***	***1.010***	***5.024***	***1***	***0.025***	***0.104***
Anemia	-0.286	0.686	0.174	1	0.677	0.751
Fractures before admission	***-4.041***	***1.886***	***4.591***	***1***	***0.032***	***0.018***
Smoking	0.037	0.558	0.004	1	0.947	1.038
HTN	***4.046***	***1.528***	***7.011***	***1***	***0.008***	***57.163***
Peripheral artery disease	20.165	7631.510	0.000	1	0.998	6E+008
Type 2 diabetes	0.917	1.050	0.763	1	0.382	2.502
Lipid alterations	-0.479	0.730	0.430	1	0.512	0.620
Alcohol consumption	-0.635	0.739	0.738	1	0.390	0.530
Extensive distal location (calf)	0.021	0.496	0.002	1	0.965	1.022
Extensive proximal DVT location	0.088	0.544	0.026	1	0.872	1.092
Patients with medical conditions	1.028	0.832	1.527	1	0.217	2.797
Patients with a history of surgical conditions	1.953	1.083	3.250	1	0.071	7.048
History of major gynecological surgical interventions	-0.482	2.100	0.053	1	0.819	0.618
History of major urological surgical interventions	16.465	8232.574	0.000	1	0.998	1E+007
Hip or knee arthroplasty; hip surgery	-0.753	1.615	0.217	1	0.641	0.471
History of heart attacks	17.258	8314.948	0.000	1	0.998	3E+007
Renal conditions (CKD, hydronephrosis, renal lithiasis)	-0.120	0.882	0.019	1	0.892	0.887
Neoplasia	0.720	1.482	0.236	1	0.627	2.055
CVA (mainly associated with motor deficiency)	-2.435	12313.405	0.000	1	1.000	0.088

The Cox-Snell's multiple coefficient of determination R2 was 0.587 and Nagelkerke's was 0.782, which signifies that the model explains 78% of the variation in covariates distribution by age. The Hosmer and Lemeshow's test showed the significance Sig = 0.910, which means that the calculated model fits the included variables.

COPD (Sig = 0.025, OR = 0.104) and fractures before admission (Sig = 0.031, OR = 0.018) occur mainly in patients under 50 years old, while hypertension (Sig = 0.008, OR = 57.163) manifests especially in patients over 50 years old.

A logistic regression for SVT was performed by age, gender, origin, venous ulcers, edemas, various peripheral trophic disorders (brown or reddish skin depigmentation, fibrous and indurated skin, redness, irritation or dermatitis), diffuse cellulitis and chronic venous insufficiency as covariates and the following result was obtained and are presented in Table 5.

Table 5. A logistic regression for SVT was performed by age, gender, origin, venous ulcers, edemas, various peripheral trophic disorders

	B	S.E.	Wald	df	Sig.	OR
Age	***-0.800***	***0.339***	***5.580***	***1***	***0.018***	***0.449***
Sex	-0.275	0.355	0.601	1	0.438	0.760
Origin	0.459	0.426	1.161	1	0.281	1.582
VU	0.738	0.491	2.256	1	0.133	2.091
Edemas	-1.059	0.682	2.413	1	0.120	0.347
Various trophic disorders	0.221	0.522	0.180	1	0.672	1.247
Cellulitis	0.854	0.705	1.469	1	0.225	2.349
CVI	***1.781***	***0.434***	***16.827***	***1***	***0.000***	***5.938***

The Cox-Snell's multiple coefficient of determination R2 was 0.316 and Nagelkerke's was 0.422, which means that the model explains 42% of the variation in covariates distribution by SVT. Hosmer and Lemeshow's test showed the significance Sig = 0.464 which means that the calculated model fits the included variables. The age (Sig = 0.018, OR = 0.449) shows that SVT is manifested especially in patients under 50 years old. CVI (Sig <0.001, OR = 5.938) occurs predominantly in patients with SVT.

DISCUSSION

Thrombosis, as described by Virchow's triad, occurs as a result of hypercoagulability, endothelial damage or stasis, or a combination of these all. The risk of VTE for each patient should be individually assessed when prophylaxis is needed. Universal preventive guidelines were made with difficulty due to differences between individuals, such as age, medical history and social history [10]. DVT prevention by using thrombo-prophylaxis, effective in patients with high risk of recurrence and the minimization of the risk of DVT reduce the frequency of post-thrombotic syndrome (PTS). The identification of the patients at high risk for PTS, the assessment of the role of thrombolysis in preventing PTS and the optimal evaluation of compression stockings in preventing and treating PTS are issues that should be the concern for future research, as well as researching and evaluating the new therapies for treating PTS. The post-thrombotic syndrome (PTS) is the most common complication of deep vein thrombosis (DVT), it is cumbersome and expensive for patients and for the community, because of its high prevalence, severity and chronicity and has received little attention from clinicians and researchers [11].

Initially, venous thromboembolism (VTE) was perceived as a complication of hospitalization for major surgery or was associated with late-stage terminal illness, but studies have shown a risk of VTE in hospitalized patients with medical conditions comparable to those seen after major general surgery. The epidemiological studies have shown that between one quarter and one half of all symptomatic VTE, clinically recognized, occur in people who are not either hospitalized or recovering from a major illness, which involves expanding the populations at risk that could benefit from prophylaxis and challenges doctors to carefully examine the risk factors for VTE [12].

Clinical evaluation for the diagnosis of venous thrombosis in itself cannot be invoked for patient management but remains useful in determining the need for further testing, namely impedance plethysmography, which is particularly useful in excluding DVT in patients with suspicious signs and symptoms. The medical history for identifying risk factors for VTE is as important as physical examination [13]. When clinical probability is estimated before the diagnostic tests, the diagnostic accuracy for DVT is improved. The patients who experience low clinical probability of DVT have a prevalence of less than 5% and the diagnosis of DVT can be excluded without using ultrasound, while for the patients with clinical suspicion of DVT the results should not affect clinical decisions [14].

Patients with symptomatic DVT may present an increase in volume of the affected limb, spontaneous pain or on palpation in the calf or thigh muscles, increased pain on the flexion of the foot (the Homans sign), high temperature and purplish coloration of the affected lower limbs. Less than 10% develop severe symptoms including thrombophlebitis, pain, swelling, leg ulcers or skin induration [15].

The present study has shown that DVT is more common in patients over 50 years old and it is more frequent in men and in patients who have the urban environment as origin.

Despite the anticoagulation treatment, VTE recurs frequently in the first few months after the initial event, with a recurrence rate of ≈7% at 6 months. Death occurs in ≈6% of DVT cases and 12% of PE cases within 1 month of diagnosis. The seasons of the year may affect the occurrence of VTE, with a higher incidence in winter than in summer. Early mortality after VTE is strongly associated with the presentation as PE, advanced age, cancer and underlying cardiovascular disease [2]. Despite universal thromboprophylaxis, patients with critically severe surgical or medical conditions remain at risk for deep vein thrombosis of the lower limbs [1].

VTE is rare in adolescents and it is predominantly a disease of old age. The incidence of deep vein thrombosis and pulmonary embolism increases exponentially with age [16]. Venous thromboembolism is a major national health problem, especially among the elderly. While the incidence of DVT remains the same for men and increases for older women, the incidence of pulmonary embolism has decreased over time [17]. The incidence rates after the age of 45 years old are generally higher in males, while the incidence rates in women are somewhat higher during the reproductive years. For both sexes, with increasing age, PE represents a growing proportion of VTE [17]. The annual rate of venous thromboembolism events has increased despite the progress made in the identification, prevention and treatment. These increases may be due to the increased sensitivity of diagnostic methods, especially for PE. This fact implies that the current prevention and treatment strategies are less than optimal [18].

Currently, the prophylaxis is both mechanical and pharmacological. The goals of the treatment are to prevent the spread of thrombosis, pulmonary embolism, thrombosis recurrence and the development of complications such as post-thrombotic syndrome and pulmonary hypertension.

CONCLUSIONS

Thromboprophylaxis could have a tremendous potential if it was efficiently and optimally administered to patients at risk of venous thrombosis. Understanding the risk factors and epidemiology of the first and recurrent venous thrombosis enables the optimal and efficient use of prophylactic strategies against VTE and would prevent post-thrombotic syndrome and improve clinical outcomes in practice.

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ORIGINAL ARTICLES

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New synthesized oximes active in nerve agents' hazards

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Abstract: *Object: The aim of the study is to select the most active new imidazolium-quinuclidinum-oxime, from some similar chemical compounds synthesized in our chemistry department, with sufficient efficacy to decrease the acute toxicity of neurotoxic organophosphates known as nerve agents. Method: The experimental study consist in vivo testing the antidotal efficacy of obidoxime and of selected imidazolium oximes synthesized in our chemistry department. Each oxime was included, by equimolar replacing the obidoxime, in an antidotal formula, which also contains atropine. The above mentioned formula containing atropine and obidoxime was used as reference. The protective ratio, defined as the ratio between the lethal median dose of the poisoned and treated study group and the median lethal dose (LD50) of the poisoned and untreated study groups was one of the used parameters in order to select a new active chemical structure in counteracting the neurotoxic organophosphorus compounds acute toxicity. Another studied parameter was the erythrocyte acetylcholinesterase value measured in whole blood 24 hours after exposure. Results: The protective ratio against an organophosphorus compound were the follow: obidoxime chloride: 2; 1,3-dimethyl-2-hydroxyethyl-imidazolyliodide: 1,75;3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl]quinuclidin-dichl-oride: 2,5; 1-methyl-quinuclidin-3-iodide: 1,5. The erythrocyte acetycholinesterase main values were the following: the unpoisoned and untreated study group:3,45 ±0,13mmol/dl; the poisoned and untreated study group: 0,89 ±0,09 mmol/dl; the poisoned and 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl]quinuclidindichloride treated study group:2,89 ±0,11 mmol/dl; the poisoned and obidoxime treated study group: 2,53±0,15 mmol/dl. Conclusions: 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl] quinuclidindichloride synthesized in our chemistry department, has shown a better protective ratio and a more prolonged surviving time than the reference (obidoxime). It has shown the best AChE reactivation of all the synthetized compounds. This compound can be a cheap and good option for replacing obidoxime in the antidotal formula active in nerve agent exposure.*

Keywords: *obidoxime chloride; 1,3-dimethyl-2-hydroxyethyl-imidazolyl-iodide;3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl] quinuclidin dichloride; 1-methyl-quinuclidin-3-on-iodide; organophosphorus compounds; nerve agents*

INTRODUCTION

Strategic expert analyzes have shown that in the next period, there may be international conflicts, amplifying the risk of attempts to use highly toxic chemicals as weapons of mass destruction.[1]

Romania through its geostrategic position, being the member of NATO, can provide a better potential target of such actions.

Neurotoxic organophosphorus compounds (sarin, soman, tabun, most insecticides) are chemical compounds, phosphonic acid esters with central and

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peripheral properties, acetyl-cholinesterase and butyryl-cholinesterase irreversible inhibitors with toxic effects on smooth and striated muscles and central nervous system.[2-4]

They were registered on the list of substances to be destroyed in accordance with international regulations (Convention on the Prohibition of Development, Production and Stockpiling Chimice-Geneva).

Although banned by international conventions, these toxic substances have been used in terrorist attacks against civilians (Tokyo 1995 Halabja in 1987, Anfal-1988). In this context geostrategic and due to soaring use of organophosphorous insecticides, development of medical measures to protect against the lethal toxicity is one of the priorities of research within specialized programs of NATO (NBC – Science for Peace) and European Union (Chemical Program Weapons, Monitoring and Protection, created in the OPCW).

The acute toxicity of organophosphorus compounds known as nerve agents mainly result from their action as irreversible inhibitors of acetylcholinesterase (AChE). They suffer some conformational and chemical changes, resulting a phenomenon known as "aging", whose speed of appearance is directly proportional to the toxicity of compounds.[5,6]

Accumulation of acetylcholine stimulation leads to persistent cholinergic muscarinic receptors that trigger the syndrome whose symptoms include miosis, salivation, bronchial hypersecretion, bradycardia, bronchoconstriction, hypotension and diarrhea.[7]

Another effect of organophosphate anticholinesterases is the desensitization of nicotinic receptors followed by overstimulation, translated by skeletal muscle twitching and subsequent paralysis.[8] Central nervous system toxic effects include anxiety, restlessness, confusion, ataxia, tremors, convulsions, paralysis, cardiorespiratory effects and coma [9].

The treatment of nerve agents poisoning is based on an antimuscarinic agent (atropine), and an acetylcholinesterase reactivator called oxime according to its chemical structure. Atropine blocks the effects of accumulated acetylcholine resulting overstimulation of muscarinic receptors.[9,10]

Acetylcholinesterase reactivators dephosphorylate the acetylcholinesterase – organophosphorus complex, reactivating the enzyme activity.[11] The early appeared seizures were counteracted by benzodiazepines.

Currently available oximes (pralidoxime, obidoxime), have been shown to be less effective against one of the most toxic nerve agents (soman tabun). There is a strong interest in developing new, more potent acetyl cholinesterase reactivators with oxime structure.[12]

The present paper represents an in vivo screening study in selecting more potent chemical compounds, active in counteracting the nerve agents acute toxicity. The paper describes an experimental test of antidotal efficiency of some compounds containing imidazolium, or quinuclidinium rings, which equimolar replace obidoxime chloride in the antidoltal formula.

The results were expressed as the protective ratio representing the ratio between the DL50 of the neurotoxic compound administred to the poisoned and treated rat study group and the DL50 administered to the organoposphate compound of the poisoned and untreated study group.

MATERIAL AND METHODS

Chemicals

- obidoxime chloride (CAS number 111-90-9);
- atropine sulphate (CAS number 5908-99-6) were purchased from Sigma Aldrich;
- diclorvos (PESTANAL CAS number 62-73-7) were purchased from Sigma Aldrich.
- the experimentally tested quinuclidinium-imidazolium oximes were synthetized in the chemistry department of Medical Military Research Center.

Animals

Male Wistar rats (150-200g) were maintained on rice husk in polypropylene cages. Wistar free access to water and rodent pellet food. The study was

approved by the Ethical Committee on Animal Experimentation.

Acetycholinesterase measurement method: Elisa kit.

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Acetylcholinesterase in human serum, plasma and other biological fluids.

Target Information: Acetylcholinesterase hydrolyzes the neurotransmitter, acetylcholine at neuromuscular junctions and brain cholinergic synapses, and thus terminates signal transmission. It is also found on the red blood cell membranes, where it constitutes the Yt blood group antigen.

Figure 1: The chemical structure of the in vivo tested compounds as acetycholinesterase reactivators

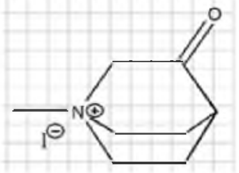

1methyl-quinuclidine-3-on-iodide

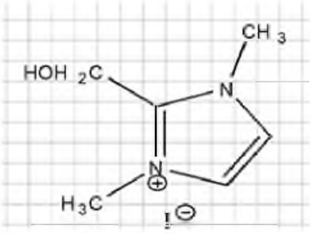

1,3-dimethyl-2-hidroxyethyl-imidazolyl-iodide

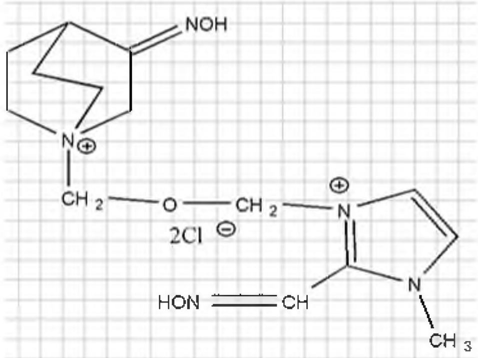

3oxim[3(2-hidroxy-iminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride

Treatments

120 Wistar rats were divided into 12 groups, each, including ten rats as follows:

- 1st group: control group unpoisoned, untreated
- 2nd group: poisoned with diclorvos (1,5 DL50) and untreated;
- 3rd group: poisoned with diclorvos (1,5 DL50,) after one minute, were administered the antidotal formula including atropine (2mg) and obidoxime chloride (250 mg);
- 4th group: poisoned with diclorvos (1,5 DL50), after one minute, were administered the antidotal formula including atropine (2 mg) 1methyl-quinuclidine 3-on iodide in an equimolar dose with obidoxime chloride (78,5 mg);
- 5th group: poisoned with diclorvos (1,5DL50,) after one minute were administered the antidotal formula including atropine (2 mg), 1,3- dimethyl-2-hidroxy-ethylimidazolyl iodide in an equimolar dose with obidoxime chloride (61,94 mg/kg);
- 6th group: poisoned with diclorvos (1,5 DL50), after one minute, were administered the antidotal formula including atropine (1,5 mg) and 3oxim[3(2-hidroxy-iminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride (118 mg/kg).

The above mentioned oximes were experimentally tested against 1.5, 1.75, 2, 2.5, DL50.

24 hours after poisoning mortality, protective ratio was registered and erythrocyte achetylcolinesterase activity values were measured.

RESULTS

The aim of the study is to evaluate the most active newly synthesised imidazolquinuclidine-oxime with the great efficacy in reactivating the phosphorylated acetylcholinesterase through some similar chemical compounds synthesized in our chemistry department, able to decrease acute neurotoxic compounds toxicity.

The protective ratio of the obidoxime and experimentally tested formulas correlated with their efficacy as acetylcholinesterase reactivators are represented in the Table 1.

The 3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2-oxapropyl] quinuclidine dichloride protective ratio correlated with its acetylcholinesterase reactivator activity is greater than the obidoxime, resulting a

better antidotal efficacy.

Statistical analysis Student's t-test probability associated with statistically significant differences between mean values of erythrocyte acetylcholinesterase between the control study group and the poisoned and treated study groups is represented in tables 2-7.

The poisoned and untreated group statistically significantly differs from normal in terms of the acetylcholinesterase inhibition.

Neurotoxic organo-phosphorous compounds cause in the intoxicated group and untreated study group an acetylcholinesterase inhibition of 75.08%, incompatible with survival.

Obidoxime and new synthesized imidazolium-quiniclidinium oximes highlighted new capabilities of acetylcholinesterase reactivation correlated with the protection index.

3-oxime compound [3-(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl]-quinuclidine-dichloride-quinuclidine 3-on iodide revealed a protection index bigger than other obidoxime and other imidazolium compounds studied.

Explanation of this is that the quinuclidine ring, by its position intensifies allosteric oxime group (Table 1).

Tables 2-7 reveals that the intoxicated untreated control group differs statistically significantly from normal and so the intoxicated study groups were treated with different therapeutic formulations.

Table 1: The protective ratio of the antidotal formulas containing the experimentally tested imidazolium oximes as AChE reactivators

Study group	Acetylcholinesterase reactivator	Dose of reactivators (mg/kg)	Protective ratio (DL_{50})	Acetylcholinesterase reactivation due to oxime (%)
2	-	-	-	
4	1methyl-quinuclidine 3-on iodide	78.58	1.5	47.82
8	1,3-dimethyl-2-hidroxyethyl-imidazolyl iodide	61.94	1.75	60.28
10	obidoxime chloride	100	2	73.33
12	3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride	118.88	2.5	82.31

Table 2: Statistical analysis of the probability P (T test) associated with average values of erythrocyte AchE of unpoisoned and poisoned and untreated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
1	3.45 ± 0.13	0.003	$P \leq 0.05$ statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 1: unpoisoned and untreated study group;
Study group 2: paraoxon poisoned and untreated study group

Table 3: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and obidoxime treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
10	2.53 ± 0.15	0.007	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 10: paraoxon poisoned and obidoxime treated study group;
Study group 2: paraoxon poisoned and untreated study group.

Table 4: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and 1,3-dimethyl-2-hidroxyethylimidazolyl iodide treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
8	2.01 ± 0.07	0.004	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 8: paraoxon poisoned and 1,3-dimethyl-2-hidroxyethylimidazolyl iodide treated study group;
Study group 2: paraoxon poisoned and untreated study group.

Table 5: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and 1methyl-quinuclidine 3-on iodide study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
4	1.65 ± 0.03	0.009	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 4: paraoxon poisoned and iodide treated study group;
Study group 2: paraoxon poisoned and untreated study group

Table 6: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and 3-oxim[3(2-hidroxyiminomethyl-1- imidazolyl)-2oxapropyl] quinuclidine dichloride- quinuclidine 3-on iodide treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
12	2.84 ± 0.05	0.0002	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 12: paraoxon poisoned and 3-oxim[3(2- hidroxyiminomethyl-1- imidazolyl)-2oxapropyl] quinuclidine dichloride- quinuclidine 3-on iodide treated study group;
Study group 2: paraoxon poisoned study group

Table 7: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, obidoxime treated and 3-oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride-quinuclidine 3-on iodide treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
12	2.84 ± 0.05	0.01	P ≤ 0.05 statistically significant difference between groups
10	2.53 ± 0.15		

Legend:

Study group 12: paraoxon poisoned and 3-oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride-quinuclidine 3-on iodide treated study group;

Study group 10: paraoxon poisoned and obidoxime treated study group

Table 8: The correlation between administered dose of acetylcholinesterase reactivator and the pharmacodynamic effect

Study group.	Reactivator dose used in the antidotal formula (mg)	AChE Mmol/ml	Correlation coeficicient
4	61,94	1,65	0,9927
8	78,58	2,01	
10	100	2,53	
12	118,88	2,84	

Legend:

Study group 4: poisoned study group and 1methyl-quinuclidine 3-on iodide treated;

Study group 8: poisoned study group and 1,3-dimethyl-2-hidroxyethyl-imidazolyl iodide treated;

Study group 10: poisoned study group and obidoxime chloride treated;

Study group 12: poisoned study group and 3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride treated;

From a medical standpoint, an organophosphoric nerve poisoning that caused inhibition of acetylcholinesterase survival limit was applied.

This intoxication was further antagonized with different formulas containing the synthesized imidazoliumquinuclidinium oximes and obidoxime as antidotes.

They showed mean values of acetylcholinesterase significantly different from the poisoned and untreated group. The studied imidasolium quinuclidinium oximes showed average values of acetylcholinesterase statistically significantly different between them, thus emphasizing that the doses used may cause significant variations in therapeutic effect.

Table 8 highlights the so-called "dose finding", the correct correlation between dose and pharmacodynamic effect. The correlation coefficient of 0.9927 demonstrates that doses of acetylcholinesterase reactivators used in the antidotal formulas are correct, being those that cause maximum pharmacodynamic effect.

CONCLUSION

- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride synthesized in the Medical Military Research Centre Chemistry Department has shown a better protective ratio and a more prolonged surviving time than obidoxime considered as reference.
- It has shown the best acetylcholinesterase reactivation of all the synthetised compounds and obidoxime
- The very good correlation dose-effect highlights that the correct dose of acetylcholinesterase reactivator was chosen in order to obtain the better pharmacodynamic effect.

- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride can be considered an efficient antidote in neuroparalytic organophosphorous hazards.
- 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride can be a cheaper and better option for replacing obidoxime in the antidotal formula active in nerve agent poisoning.
- Thus one can conclude that the result of the experimental study is consistent with the proposed object.

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Ethical considerations in sudden unexpected death in epilepsy (SUDEP)

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Abstract: *Epilepsy is one of the world's oldest diseases. Social stigma, misunderstanding and thus, discrimination have surrounded patients and their families from the beginnings until nowadays. Approximatively up to 80% of epilepsy cases worldwide are found in developing regions. The risk of premature death is two to three times higher than for the general population. There is contradictory evidences concerning the question of whether to inform patients about the possibility of sudden unexpected death in epilepsy (SUDEP). Actual guidelines states that individuals with epilepsy and their families or careers should be given access to information on SUDEP. We have information about how, when and what to say to the patients and families about SUDEP. But it's a delicate subject, and some patients do not want to know that they are at risk for this.*

Keywords: *epilepsy, SUDEP, ethics*

INTRODUCTION

Despite age, racial, social, geographic or national boundaries, epilepsy remain a prevalent chronic neurological disorder.

The incidence of epilepsy was estimated at 24-53 per 100,000 people.

World Health Organization (WHO) estimates that around 50 million people worldwide have epilepsy, 80% from developing regions. Epilepsy is characterized by recurrent seizures due to excessive electrical discharges in a group of different parts of the brain cells. It is consider that up to 10% of people worldwide have one seizure during their lifetimes. Epilepsy is defined by two or more unprovoked seizures. Only one fourth of affected people in developing countries get the treatment they need and only 70% of these respond to medication. Mortality is higher in patients with epilepsy than in general population. People with epilepsy and their families can suffer from stigma and discrimination in many parts of the world. For example in China and India, epilepsy is a reason for prohibiting or annulling marriages.

In the United Kingdom, a law forbidding people with epilepsy to marry was repealed only in 1970. In the USA, until the same years, it was illegal that people with seizures have access to restaurants, theatres,

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recreational centers and other public buildings. Even nowadays patients have reduced access to health and life insurance, to obtain a driving license, to get a job or have other limitations.[1-5] Still now a person may be identified as an ‘‘epileptic” rather than ‘‘a person with epilepsy”.[6] SUDEP is the second death cause in epilepsy after status epilepticus. Unusually, the diagnostic of SUDEP is retrospective. Is defined as sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death in patients with epilepsy, with or without evidence of a seizure and excluding documented status epilepticus, in which postmortem examination does not reveal a toxicological or anatomical cause of death. [7] It is responsible for 7.5–17% of all epilepsy deaths and has an incidence among adults between 1:500 and 1:1000 patients per year. [8] Epileptologists agreed that SUDEP is mainly, but not exclusively, a problem for patients with refractory epilepsy. Epilepsy-related mortality is a significant risk in pregnancy, 1:1000 women died from epilepsy (mostly SUDEP) during or shortly after pregnancy. [9] SUDEP has an estimated annual incidence rate of 0.81 cases per 100000 population, or 1.16 cases per 1000 patients with epilepsy. Comparing years of potential life lost from SUDEP with selected other neurologic diseases, SUDEP ranks second only to stroke. [4] There are some risk factors like: generalized tonic-clonic seizures (GTCS), nocturnal seizures, variability of EEG records, and duration of the disease ranging from 15 to 20 years, early onset of epilepsy, poly-medications, cold temperatures, alcohol abuse, and street drugs. [10-14] It has been suggested that the most common pathogenic mechanism underlying SUDEP is heritable arrhythmogenic syndromes, or cardiac channelopathies, such as familial long QT syndrome (LQTS).[15,16] LQTS associated with syncope, seizures and sudden cardiac death is caused by mutations in more than 10 genes, encoding potassium and sodium ions channels.

Among cardiac arrhythmias, respiratory dysfunction, neurogenic pulmonary edema and dysregulation of systemic and cerebral circulation are other proposed pathophysiological events implicated in SUDEP.[17-19]

ETHICS ON SUDEP

There is a reasonable question about SUDEP: must it be discussed with all patients with epilepsy who are at risk of SUDEP, or not? The Task Force of the American Epilepsy Society and the Epilepsy Foundation have guidelines concerning what, how, when SUDEP should be discussed with patients, their families and caregivers. In Europe, the National Institute of Clinical Excellence (NICE) has the same attitude. [3]

The topic of SUDEP involves many moral dilemmas, most of which do not have absolute solutions. Advocates of universal SUDEP counseling cite the “right to know,” but others point to the “right not to know”. There is neither empirical evidence nor consensus on the question of whether to inform patients and parents about the possibility of SUDEP.[20]

Arguments to inform patients about SUDEP

In recent times there has emerged a debate regarding the obligation to warn even newly diagnosed patients of the risk of SUDEP. If there is a reasonable chance of preventing SUDEP, it must be discussed with all patients with epilepsy who are at highest risk of SUDEP. A few strategies that patients with epilepsy and their careers can take to reduce death risk could then be share by the physician.

On the other hand, the term “unexpected” is improper used because in some patients with risk factors SUDEP could be expected.[7] Precise definition and classification of SUDEP is necessary to scientific and medical communication, and is also important from a legal point of view. The new classification consists of six classes and definitions:
1. Definite SUDEP
1a. Definite SUDEP Plus (preexisting condition could have contributed to the death),
2. Probable SUDEP, (meets criteria for SUDEP, but no postmortem examination was done to exclude another pathologic process)
3. Possible SUDEP (postmortem examination may have identified drowning)
4. Near SUDEP (cardiorespiratory arrest that is successfully resuscitate),

5. Not SUDEP when a clear cause of death is known.
6. Unclassified: incomplete information available; not possible to classify

The patient suffering from epilepsy is, in many respects, no different to any other patient being treated by medical practitioner. [21] The ethical principle of patient autonomy in health care involve the patient's right to know about medical condition and prognosis. Information should be provided promptly to patients, their families and caregivers if they ask about the potential adverse consequences of the seizures or about the mortality risk associated with epilepsy.

In learning about SUDEP, parents expressed a need to be informed of the risk of that. There was a consensus that it should be the parents' decision as to whether or not the child should be present at the meeting or when to inform the child about the risk of SUDEP.[22]

In some cases the risk of SUDEP may need to be emphasized to encourage compliance with medical and surgical therapy for epilepsy. Recent evidence from a meta-analysis of randomized clinical trials of adjunctive AEDs at efficacious doses provides strong support for AED treatment as mono- or polytherapy to increase seizure control and protect against SUDEP in patients with refractory epilepsy.

For patients for whom seizure control is unattainable, supervision or monitoring may prevent SUDEP, though this has never been formally tested. [15] Increasing awareness of SUDEP may facilitate improved seizure control and possibly decreasing SUDEP incidence.[23]

Furthermore, SUDEP discussion can be encouraging to patients with very low SUDEP risk. Patients with absence epilepsy or benign epilepsy syndromes must know that their risk of SUDEP is negligible.

Arguments for not informing patients about SUDEP

By low, failure to discuss SUDEP with a patient suffering from epilepsy cannot constitute negligence because the outcome cannot be as a consequence of the actions of the doctor based upon current knowledge. Mainly, if the patient has not asked about his distress, there is not the basis for litigation against the doctor who chooses not to discuss this topic.[20]

Until now, no interventional measures are known to stop the outcome, so we get nothing by warning of SUDEP. To admit the SUDEP, it may seriously deteriorate quality of life. In this case the doctor may deliberately omit information to avoid patient fear and anxiety, respecting in this way the right „do not harm". The negative influence on quality of life may represent a form of negligence.

It may be possible to show causal connection between impaired quality of life and the doctor divulging information that the patient did not explore. Polymedication is an important risk factor for SUDEP, but a necessary intervention for the epilepsy management. So many patients requiring polytherapy have been refused it for fear of SUDEP.[14]

CONCLUSIONS

Duty of care dictates open and frank discussion if the patient wishes information about mortality and epilepsy. The big dilemma concerning ethics considerations in SUDEP is: the ''right to know'' or the converse which is the ''right not to know''?

This conflict, places the clinician in a serious ethical difficulty because it requires the balancing of these diametrically opposed concepts and demands a value judgement on the part of the clinician. SUDEP is essentially unpredictable for any individual patient. It also confirms that ''never'' and ''always'' are dangerous terms when used by doctors.

To assure the patient of something which cannot be assured may represent another form of negligence and failure of duty of care. The doctor provide the patient with accurate and informed response to questions raised .Where the detailed information will not alter the outcome for the patient, failure to provide it cannot be deemed to represent negligence.

The ethical consideration is met if the doctor tries to ascertain what the patient wants to know and responds accordingly by providing that information which the patient requests. Thus, each case must be managed individually and doctors are advised to document the decision-making process. The patient care is like a balance between what the career offers, and what the patient accepts.

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Pericardium – An editorial success

Teodor Horvat[1]

PERICARDIUM. Anatomy, physiology, pathophysiology, pathology and surgery

By Teodor Horvat and Daniel Fudulu

Why did I write this book? I keep asking myself this question... Why did I write it? Maybe because cardiovascular surgery was my first rotation during my residency that I started after graduating with a first honors degree (gold medal), from the Faculty of Medicine Bucharest in 1975.

My destiny in the winter of 1976, was to be allocated to start my first rotation in the newly formed Military Department of Cardiovascular Surgery, Fundeni Clinical Hospital, under the supervision of Lieutenant Colonel (Lt Col) Dr Vasile Cândea. Because this unit was not fully functional, I started working under Professor Ioan Pop D. Popa and then in the Vascular Surgery Department where I had the opportunity to meet a brilliant, master surgeon – Professor Tiberiu Ghițescu. Finally, I started working in the Military Department of Cardio-vascular Surgery in August 1976, 7 months after the completion of the official paperwork certifying the opening of this new department.

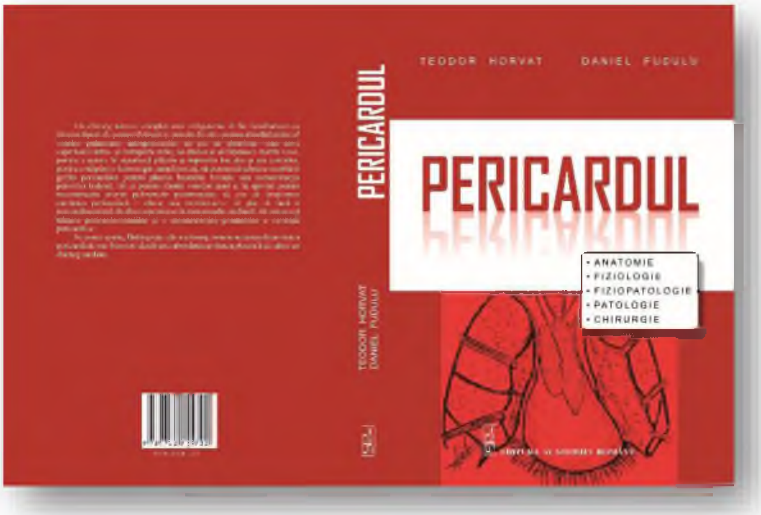

At the beginnings, we were just a few of us, Lieutenant Colonel (Lt Col) Dr Vasile Cândea and Lieutenant Dr Horvat. Later, Dr Richard Florescu, a non-military doctor and my medical school colleague joined us. He immigrated later to Germany. After a few months Captain Dr Ion Țintoiu – cardiologist, Captain Dr Alexandru Popa – anesthetist, Major Dr Ioan Condor surgeon and Captain Dr Ioan Mociorniță – surgeon joined us. Our senior scrub nurse was Mrs Rodica Poreceanu and she was an exceptional first assistant. I've rarely seen such manual dexterity that she had. So, I started as a second assistant while she was the first assistant for Dr Cândea. I have learned a lot from her. She was unique. I have never met a clone of her.

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Week by week our department grew and this culminated with the first open heart operation – an atrial septal defect that was corrected in the February of 1977. The operation was a success. All of us were very cheery until the great disaster came – March the 4th, 1977 – The Great Earthquake.

Both the old and new Fundeni Hospital buildings were affected; the old building was in a terrible state. I remember our department being affected very badly, the interior walls were full of cracks because this was located on level 2 where the flexion and extension of the walls happened. Not even level 3 – Professor Ghițescu's Vascular Surgery Department was spared from these cracks. Above level 4, there was less damage. The old building was beyond any description. No medical treatments and of course no surgery could be performed there. All the departments were moved, in the end, into the new building, the most robust out of all.

The Department of General Surgery led by Professor Dan Setlacec had to move too. Here, in the new building, at floor level, in the building with three levels, I saw The Professor for the first time. He was going to supervise me later as general surgery resident. I've spotted Professor Dan Setlacec in a big group of young surgeons. I could see only his head, his short haircut and his bushy, unmissable eyebrows. That's what I could only see!

After the rubble was scooped and some renovation was undertaken, I went to see Lt Lieutenant Colonel (Lt Col) Cândea and I asked his permission to leave his department. He asked the reasons for leaving. I've had to explain: "I started building the "surgical house" with the roof rather than the" foundation". I have started in the "super-specialty" of Cardiovascular Surgery without having a basic surgical foundation. In other words, I have started without completing a general surgical rotation.

So, I left. We ended up our collaboration in good terms, and we kept in touch until today. I learned a lot from surgeon Cândea, both about surgery but also about interpersonal relations. I have seen and heard a lot in these 14 months not only amongst military doctors but more amongst civilians. They are only a few cardiac surgeons within this specialty but they there is an intense rivalry and hate between them. If I would put aside the parasites who used to copy their personalities, you had what to learn from these remaining doctors – both surgery and medicine.

I have worked directly, as an intern, both at the bedside and in the operating theatre with doctors outside the Military Departments such as: Professor Pop D. Popa, Clinical Lecturer, Dr Dan Făgărăsanu towards the end and with Dr Ilie Pavelescu who was going to become later a professor in Timisoara. I also worked with Dr Martin Constantinescu, a great loss for Romanian Surgery by his immigration, Dr Tiberiu Ghițescu – the great master, Dr Traian Stefănescu, Dr Francisc Proinov – Fundeni Hospital Medical Director, Dr Dan Mogoș, specialty doctor, later Professor of Surgery in Craiova, Dr Vasile Sârbu – specialty doctor later Professor of Surgery and Dean of Constanța Faculty of Medicine, Dr Radu Nemeș – specialty doctor, currently Professor of Surgery at Craiova, Dr Șoimaru – specialty doctor, Dr Roth – specialty doctor who later immigrated to Germany and many others.

I remember our top cardiologists: Dr Daniel Constantinescu, Dr Tudorică Popa, Dr Ion Țintoiu, Dr Sichitiu and our devoted anaesthetists: Dr Aurelia Bălan ("Tanti"), Dr Teodora Petrilă, Dr Radu Făgarașanu and Dr Alexandru Popa – they have all taught me a lot.

I was in close relationship with the Department of Haemotology led by Professor Ștefan Bereceanu. I used to be very happy when my expertise was needed by: Dr Dan Coliță later Professor and Clinical Lead of the Haematology Department, by Dr Adriana Coliță – later clinical lecturer in Haematology, by clinical lecturer Dr Elena Butoianu, the second in the department hierarchy. I was happy because they were asking for my help, whom I was a beginner at that time, a non-initiated in the art of surgery. They used to refer patients for lymph node biopsies and this is how I became an operator, by doing lymph node, muscle and skin biopsies. Over the years, our work collaboration grew and this time they were no longer referring lymph node biopsies but complex mediastinal tumors with haematological implication.

But let us get back to my career story. I left Fundeni Hospital in March 1977, intern in surgery, and I returned specialty doctor in general surgery, to build up the foundations of my surgical house.

I have met Professor Dan Setlacec in November 1977. In January 1978, the General Surgical Department moved to the old building where I worked until the spring of 1980 when I moved to the Central Military Hospital, General and Thoracic Surgery Department II, led by Professor General Dr Traian Oancea. I first visited this department on Thursday, 9th of October 1969, when I was a first-year medical student, but I will write this story in another book.

In Professor Setlacec's department everyone was focused on work, on performance. There was no rivalry amongst the surgeons, it was a true school of general surgery that was overseen by The Professor. He was The Master, the conductor of the surgical orchestra. He was producing "true" surgeons. Here, I assisted daily in operations. Not one operation, but two, three even four major operations. We had no minor or simple procedures. I was the operator's "second hand ", the "first assistant" in English language. I have learned a lot from the surgeons in the old building. Here I started to build the foundations of my surgical school. I believe that every surgeon, regardless of the pursued specialty needs to learn the basic surgical skills, the principles of surgery in general surgery not in an "super-specialty", like I have started. Luckily, I have realized I made a mistake and I repaired it. I like to believe I made the right choice and that I have succeeded!

Because Professor Traian Oancea was also doing general thoracic surgery not only general surgery I was attracted by this specialty. Thoracic Surgery was also performed by other two surgeons there: Colonel Dr Therdor-Stefănescu Galați and Major Dr Gheroge Voicu – later promoted to lieutenant colonel and then colonel. Both of them were working in the department of thoracic surgery located on the 5th floor of the surgical building. I have never returned to the field of cardiovascular surgery, however, I have encountered and treated in my practice surgical conditions at border between the thoracic and cardiac surgery. I have always cultivated and consolidated my relations with the cardiovascular surgeons and anesthetists.

Now, let me return to my first question. Why did I write this book? Well... because sometimes I feel guilty and regret I have abandoned and never returned to cardiovascular surgery – it is an argument that we must consider. But, the most powerful reason is because as a pure thoracic surgeon I often used to cross the "pericardial border" during major lung or mediastinal surgery but also when addressing surgically diseases of the thoracic wall or the diaphragm.

We also need to take into consideration the malignant pericardial effusions that were associated to pleural effusions (both conditions having a common cause) that required concomitant surgical treatment, under the same anesthesia. Not only the associated pericardial effusions but also the isolated pericardial effusion caused by a malignant a condition are mainly treated within my specialty – thoracic surgery.

In addition to the points mentioned above, it is mandatory for a "complete" thoracic surgeon to be familiarized with the pericardiotomy approaches, to know how to approach the superior vena cave both intra- or extra-pericardially; he has to know how to dissect and isolate the major heart vessels in order to safely repair the various vascular or cardiac injuries but also to be able to control a cataclysmic surgical bleeding; he needs to know how to harvest a pericardial flap to reinforce the bronchial stump, to reconstruct the tracheal wall but also to do a patch repair of the major vessels – particularly patch angioplasty of the pulmonary artery; they have to know how to perform a pericardial window via classical or minimally invasive approach; they have to know how to perform pericardiocentesis to decompress a cardiac tamponade and finally to master the technique of pericardiectomy and "geometric" pericardial cavity reconstruction.

I can confirm, without doubts, that a thoracic surgeon approaches the pericardial cavity more often than a cardiac surgeon approaches the pleural cavity.

From what I remember seeing in my early years of surgical practice, there were thoracic surgeons that never crossed the pericardium. In other words, they used to declare tumors inoperable even if there was slight involvement of the extrapericardial pulmonary vessels. I have always asked myself asked myself why they do not dare to cross the pericardium; the explanation is in their early days of training. In Romania, thoracic surgery, as stated by Professor Alexandru Boțianu from Târgu Mureș, was born because of Professor Dr Cărpinișan and a disease of his time – tuberculosis. In such circumstances, thoracic surgery was born with a ''limp'', without the esophageal surgery but with the pericardial surgery for the relief of pericardial constriction due to tuberculosis. Unfortunately, after the founder of thoracic surgery passed away, in Romania, the interest in the pericardium had almost disappeared amongst his descendants.

In the year of 1986, while I was working at Filaret Hospital, under Prof Dr Constantin Coman, to complete my general thoracic surgery fellowship (e.g. after completion of my general surgery training), I have never witnessed the pericardium being opened to approach the intrapericardial pulmonary vessels.

I remember a day in 1987 when I was working in the Central Military Hospital and I had to scrub to assist Professor Traian Oancea to perform a left pneumonectomy for a lung cancer. The tumor appeared unresectable. Despite this, I persuaded my ex-boss and Professor to open the pericardium. After a moment of reflection, he turned to me and asked: ''Have you ever been inside the pericardium?'' I responded without hesitation: 'Yes! I've been there before!'' He replied: ''OK then, let's open it!'' It was the first intrapericardial dissection of the pulmonary vessels that I ever assisted and observed. The operation was a success.

Anyone who had seen Professor Oancea operating, remained deeply impressed, like me, of his delicate surgical gestures, of his control of the situation and his safeness and many others skills and attributes that I do not have enough space here to enumerate.

The years have passed and the work volume in thoracic surgery increased exponentially, with cases that were more and more difficult to tackle. However, I have never seen again a case being declared inoperable based on extrapericardial involvement. The majority of the pneumonectomies were performed using an intrapericardial approach.

Pericardial resections for lung tumors invading the pericardium had followed. In selected cases, we even performed atrial, mediastinal, chest wall or diaphragmatic resection associated with geometric pericardial reconstructions.

We admitted more and more cases of malignant pericardial effusions either isolated or associated to unilateral or bilateral pleural effusions and even peritoneal effusions.

In the Thoracic Surgery Department at the Central Military Hospital, that I led for 15 years, we were the first ones to perform nationally: pericardial-peritoneal fenestration, the pericardial-pleural window performed with the use of a endostapler (right and left), VATS pericardoscopy (right and left) and subxiphoid pericardoscopy.

I was unpleasantly surprised by a certain event, in addition to many other disappointments or harassments choreographed by the Central Military Hospital management. The first assistant who helped me perform the first subxiphoid pericardoscopy followed by a VATS pericardial resection, published this novel procedure under his own signature, in the ''Journal of Military Medicine'' – the case operated by me. I am going to say only this – I was helped by Dr Cornel Savu and this is the end of the story, and it is not worth expanding on this.

Certainly, all thoracic surgeons who trained in the Thoracic Surgery Department at the Central Military Hospital learned all the various pericardial procedures that they use when needed. These surgeons are the ones who either have not moved: Dr Claudiu Nistor, Dr Adrian Ciuche, Dr Constantin Grozavu, or the ones that work in other parts: Professor Ioan Cordoș, Dr Codin Saon Dr Mihnea Orghidan, Dr Radu Matache, Dr Cornel Savu from Filaret Hospital, Professor Alexandru Nicodin from Timișoara, Dr Cezar Pavelescu, Dr Laurentiu Marinescu from Floreasca Emergency Hospital, Dr

Madalina Grigoroiu and Dr Codruț Stănescu at Fundeni Hospital, Dr Constantin Mitrofan at Iași, Dr Emanuel Palade who worked in Wagen, Germany, then Freiburg and at present in Lubeck – Clinical Lead, Dr Marius Paraschiv at Bagdasar-Arsene Hospital, Dr Cristescu and Dr Brânză from Brașov, Dr Hugoi from Oradea, Dr Demetrian and Dr Dobrinescu from Craiova and others. All these thoracic surgeons will teach the new comers to work on the land of thoracic surgery. They will teach them about the pericardial border, how to approach and go pass it without risks, incidents or accidents.

Like them, I had to move to another hospital along with Dr Cezar Motaș, Dr Mihnea Davidescu, Dr Natalia Motaș, Dr Corina Bluoss, Dr Rus Ovidiu, Dr Daniel Fudulu, Dr Andrei Bobocea. We arrived at the Institute of Oncology, on the Fundeni platform. For me it was like in the fairy tales, I have returned at old age where I used to work when I was young but in another building.

Here pericardial diseases are more frequently referred than at the Military Hospital. All the surgical pericardial conditions used to be referred to our neighbors, across the fence, to the Cardiovascular Department at Fundeni, before our arrival.

I cannot remember the day and the month, but I remember the year of 2007 when I went to visit the thoracic surgery theatres at the Military Hospital. The younger surgeons were not allowed to discuss with me or request my help for the various cases at that time. This was imposed by the new clinical lead that was supported by the management. So, I used to go uninvited. I used to go like I used to do in the previous years. During that day, Dr Cezar Motaş was assisted by Dr Natalia Motaş – his wife. Dr Motaş told me he was operating a malignant pericardial effusion and that he approached the pericardium from the left side of xiphisternum. I said: ''Well done!'' and left. After a few steps, I came back to him and told him: ''This was never done, take some pictures! It is an original procedure'' It was and it is indeed the ''left paraxiphoid approach to the pericardial cavity''. The procedure was published in the ''Interactive Journal of CardioVascular and Thoracic Surgery'' in January 2010 under the heading: ''New Ideas in Oncology''. The idea was applied and published by a Romanian thoracic surgeon – Dr Cezar Motaş who unfortunately passed away too early in the year of 2013, month October, the 30th.

Because we had a lot of clinical and operative experience in pericardial surgery accumulating throughout the years I thought this will be a very good topic to study by a young medical student, future doctor and future thoracic surgeon. Therefore, more than 10 years ago, a young medical student from Bucharest approached me and asked my permission to observe a thoracic surgical procedure. He proved to be a responsible and serious student and later a thoracic surgery trainee and sponsored PhD student. His name is Daniel Fudulu.

His graduation diploma thesis was based on the surgical experience of the Thoracic Surgery Clinic II, University of Medicine and Pharmacy ''Carol Davila'' in the treatment of pericardial diseases. The above academic surgical department functioned until 2009 at the Military Hospital and from October 2009 was moved to the Institute of Oncology Bucharest.

The plan was for this subject to be further explored and researched in the form of a PhD thesis for which Dr Fudulu successfully obtained sponsorship of his tuition fees. He had to abandon his PhD when he immigrated to England in 2010.

This distance was not an obstacle or a reason to abandon the writing of the monograph ''The Pericardium – Anatomy, Physiology, Pathophysiology, Pathology and Surgery''. Therefore, we decided to write this book. Some of the drawings are done by Dr Fudulu while others are done by a young and talented drawer – Eugen Tudorache. I want to thank them both for the detailed and exceptionally clear illustrations.

Together with Dr Fudulu I also published on CTSNet – ''Pericardial Reconstructions in Thoracic Surgery''. Thank you to Professor Mark Ferguson from Chicago for accepting to publish our work and all his editing work and advice. The article was published online on December 2010.

In this chapter, I have told you the story of my career path and the motivations behind writing this book. This monograph is written from the point of view of a senior thoracic surgeon and of a thoracic surgery trainee – we have nothing against the cardiac surgeons! On the contrary, it is our meeting point at the border between the thoracic and cardiac surgery. This book is written for anyone who has the desire to read and learn regardless of their position. It is intended for students, junior trainees, registrars, consultants, academics and no academics.

I do hope it will prove useful!

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Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/ mesh/meshhome.html).

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

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Romanian Journal of Military Medicine

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- *Hyphema – from the effect to the cause*
- *Extra-pulmonary tuberculosis in immunocompetent patients: Case series and mini review of the literature*
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EDITORIAL

One health

Victor Purcărea

Life, freedom, and pursuit of happiness are mentioned in the Declaration of Independence and engraved in the quote "***think big, you can do anything***" that defines the American spirit. Health is the element without which (sine qua non) obtaining all these would not be possible, and which is essential in the longevity and quality of life. Health is a priceless good in the economic and social development and in supporting the economies and powerful democracies, both at the individual and at the population level. Moreover, health is important for the security and prosperity of the nation. "We hope that, during the elections for the Congress, in 2018, the candidates will first think about the American citizens, thus re-launching the American tradition "think big, you can do anything", by applying the spirit of the winner in the reform of the authentic healthcare services", affirmed the famous professor Eliot Sorel (Senior Scholar Healthcare Innovation & Policy Research, GWU School of Medicine & School of Public Health and Founder Conflict Management Section WPA) in his article "*Health: its importance in Life, Freedom and Pursuit of Happiness*", which was published online on April, 14 2017.

Health of people represents a priority both for the European Union. The EU's main objective regarding the public health is the improvement of the health state of the population and the prevention of the diseases. This objective is mentioned in article 168 of the Treaty regarding the Functioning of the European Union (the Treaty of Lisbon), which also offers examples of activities which the EU cannot undertake in order to achieve the objectives. Among these, the following can be mentioned: controlling epidemics, studying their causes and their prevention. EU also supports drugs abuse control, as well as the efforts of the member states in the fight against smoking and of excessive alcohol consumption. Another objective is the increase in the degree of complementarity of the healthcare services in the border areas.

Prof Eng VICTOR PURCĂREA

Executive Editor

The remarkable progress of healthcare doubled by better life conditions continuously lead to a raise in the life expectancy of the people everywhere worldwide.

In contrast to this positive evolution, the diseases threatening the health of the people keep on developing, diversifying and, implicitly, the expenses and the effort to preserve an appropriate state of health continually raise and use a huge amount of resources that are hardly sustained.

In this context, a new global medical concept, "One Health", started being organized worldwide, at the same time trying to find the causes for the evolution of the diseases and their spreading, looking to recommend the adequate solutions in the fields in which the human health is more and more assaulted.

Among the accomplishments in this field, the International Center for Food Chain and Networks

Research – **FoodNetCenter Bonn**, founded in 2006 in the University of Bonn, can be reminded. The center's main objectives are the following: interdisciplinarity, contribution to the evolution of the university profile, research, and development.

Moreover, it has three *Focus Groups*, which are base units for the organizational structure, fully integrated in the University: *Food Waste*, *"One Health"* and *Responsible system innovation.* The main common problem of all the three Focus Groups is the fact that the food request will double until 2050 and, the interdisciplinary groups of interest could try to solve the problem by contributing to a responsible and solid production, processing, and food consumption.

The University of Bonn (the International Center for Food Chain and Networks Research – FoodNetCenter Bonn) has involved from the beginning in founding, in 2015, the Education and Qualification Alliance – ***EQA,*** which acts at the level of the network by supporting the knowledge suppliers, the standardization and certification organisms in promoting quality, food safety, sustainable development, social and corporate responsibility and, ***"One Health",*** only one Health for People, animals and the Environment, represents the new global concept that reunites preoccupations of the specialists in all the fields that threaten health, including Food, Nutrition and Occupation, with two main action directions: Primary Prevention of Life and Life Post-Therapy.

From the initiative, giftedness, and generosity of some important personalities in the social, cultural, and scientific life, the Association **"ONE HEALTH-New Medical Concept"** Romania was founded in Romania in May 2015, being an associated member of the ***Federation*** of the ***European Academies of Medicine*** (FEAM) (www.onehealth.ro).

In our country, as well as in others, the *"One Health"* concept implies the summing of the efforts and the achievements that *Human Medicine, Veterinary Medicine, Environment Medicine, Food and Nutrition Medicine, Occupational Medicine, Compared Medicine (zoonoses and compared oncology) record with the Primary prevention of life, promoted as a major vector*, together with their application in medicine, while desiring a strong health state with costs as low as possible.

The objectives of the activity are the following: creating a national network of research and development and implementation in the field of One Health, which is internationally affiliated; promoting and developing scientific research projects; establishing professional and organizational relationships with research-development and education structures; educating the people who work in the field of compared medicine, food safety, environment medicine and bioeconomy, as well as cooperation in the scientific academic field; organizing events and activities that would favor the exchange of scientific ideas in the field of compared medicine, food safety, environment medicine and bioeconomy; implementing new scientific guidelines of the involvement of "life environment" in the "one health"-human medicine-veterinary medicine-environment medicine relationship, in order to achieve the concept of eco-prevention and eco-therapy in the initiative of developing the strategy of primary prevention of diseases in humans and animals; promoting postgraduate education in the field of compared medicine and stimulating the activity of technological transfer in the field of compared medicine.

In order to achieve these goals, an efficient and continuous implementation and international recognition, the Association founded departments on specialties such as *Department of Management, Bioeconomy and Law; Department of Compared Medicine; Department of Human Medicine; Department of Veterinary Medicine; Department of Environment Medicine; Department of Food Safety; Department of Zoonoses; Department of Pharmaceutical Production and Biological Drugs; Department of Scientific Research; Department of Education; Department of Non-Conventional Therapies; Department of Compared Oncology; Department of Radiology in Medicine; Department of Phytopathology* and *Department of Accreditation*.

ONE HEALTH New Medical Concept Association has already organized its first prestigious scientific meeting, One Health National Conference 2017, in Târgu-Mureș, with the support of the University of

Medicine and Pharmacy in Târgu-Mureș, the Academy of Medical Sciences in Romania, Spiru Haret University, Mureş County Council, College of Physicians in Romania and ***Romanian College of Veterinarians***, the President of Honor being Andre Laurent Parodi – Academy of Medicine in France, and, the executive presidents being Acad. Nicolae Manolescu – President of OHNMC Romania, Prof. Leonard Azamfirei, MD, PhD – Rector of Târgu-Mureș University of Medicine and Pharmacy and Prof. Irinel Popescu, MD, PhD – President of the Academy of Medical Sciences in Romania. Next year, the Association will also organize "ONE HEALTH - 2nd European Inter-regional Conference, 5–8 September 2018, under the high patronage of the Government of Romania. The organizers of the event will be Spiru Haret University, Bucharest; ONE HEALTH - New medical concept Romania and the University of Veterinary Medicine Hungary – Budapest. The area of interest will be the following: Environmental medicine; Antimicrobial resistance; Comparative medicine; Food safety and nutrition medicine; Comparative oncology and Zoonoses. The Presidents of Honor will be Prof. George GRIFFIN, MD, PhD, President of the Federation of European Academies of Medicine (FEAM) and Member of the British Academy of Medicine; Prof. Bernard CHARPENTIER, MD, PhD, Past President of the Federation of European Academies of Medicine (FEAM) and Member of the French Academy of Medicine, Prof. André-Laurent PARODI, DVM, PhD, Honorary President of the French Academy of Medicine and Honorary Director of Alfort National Veterinary School, France, Prof. René CARLSON, DVM, PhD, Past President of the World Veterinary Association AVMA and Director of International Affairs, and the Executive presidents will be Prof. Nicolae MANOLESCU, DVM, PhD, Correspondent Member of the Romanian Academy, Correspondent Member of the French Academy of Medicine and President of the Romanian One Health New Medical Concept Association; Prof. Stefan CONSTANTINESCU, MD, PhD, Correspondent Member of the Romanian Academy, Professor at the Université Catholique de Louvain, Brussels, Belgium, Vice President of the Federation of European Academies of Medicine (FEAM) and Vice President of the Romanian One Health New Medical Concept Association; Prof. Peter SOTONYI, MVD, PhD, Rector of the University of Veterinary Medicine, Budapest, Hungary; Assoc. Prof. Aurelian A. BONDREA, PhD, Rector of Spiru Haret University, Bucharest, Romania; Cheryl STROUD, MD, PhD, Executive Director of One Health Commission, and Prof. Florian POPA, MD, PhD.

REVIEW ARTICLE

Article received on April 21, 2017 and accepted for publishing on September 18, 2017.

Hyphema – from the effect to the cause

Raluca D. Georgescu[1], Ovidiu Mușat[1], Corina Cernat[1], Diana Colța[1], Ana M. Boariu[1], Ioana Popescu[1], Tatiana Tănase[1], Lucian Alexandru[1]

Abstract: *The purpose of this review is to describe the etiology (traumatic, iatrogenic, spontaneous causes), clinical and paraclinical findings and the management of hyphemas.*

The clinical appearance of hyphema is variable and is influenced by the volume of blood and the amount of time erythrocytes are present in the anterior chamber. When hyphema is evident, a complete history should be obtained and a thorough physical examination performed to direct the initial selection of diagnostic tests. Secondary complications of hyphema include glaucoma, synechiae, cataract formation, blood-staining of the cornea, and blindness.

Early diagnosis and treatment can minimize or prevent the installation of secondary complications and improve the prognosis.

INTRODUCTION

Hyphema is blood collection in the anterior chamber of the eye (delimited anteriorly by the cornea, posteriorly by the lens and iris and circumferentially by the angle between iris and cornea).[1]

Depending on the blood volume present in the anterior chamber, hyphema is classified as (Figure 1):

- Grade 0: circulating erythrocytes (microhyphema)
- Grade 1: <1/3 anterior chamber volume
- Grade 2: 1/3 -1/2 anterior chamber volume
- Grade 3: >1/2 anterior chamber volume
- Grade 4: total anterior chamber volume (”eight ball hyphema”)

The symptoms that bring the patient to the doctor may vary depending of etiology, but the most common symptoms are blurred vision, pain and photophobia.

The most important thing in finding the etiology of hyphema is a detailed medical history of the patient. It’s important to ask the patient if he suffered a trauma, what was the mechanism and if an object was involved, because hyphema in some cases may be accompanied by the presence of an intraocular foreign body. Other questions will be related to certain diseases such as diabetes, sickle cell anemia or other blood disorders and treatment with antiplatelet agents or anticoagulants.

After a complete medical history, physical exam will include: biomicroscopy, FO exam, gonioscopy, ocular ultrasonography, ultrasound biomicroscopy, radiographic examination or CT (when there is severe orbit damage or the doctor suspects the presence of an intraocular

[1] Carol Davila University Central Emergency Military Hospital, Bucharest

foreign body).[3]

Figure 1: Hyphema grading system
(American Academy of Ophthalmology)[2]

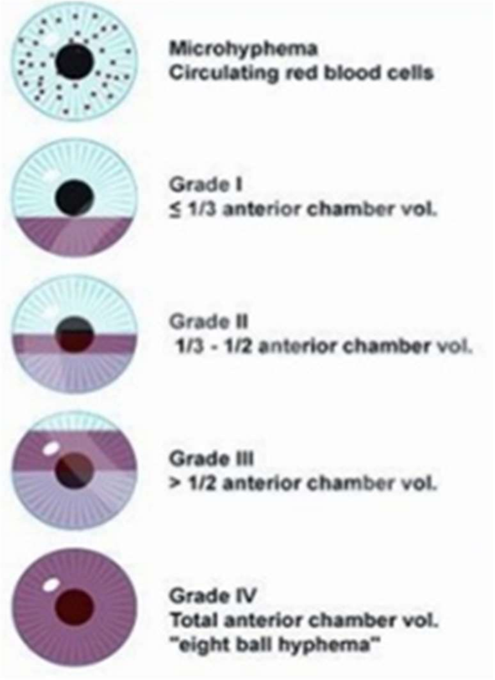
Microhyphema
Circulating red blood cells
Grade I
≤ 1/3 anterior chamber vol.
Grade II
1/3 - 1/2 anterior chamber vol.
Grade III
> 1/2 anterior chamber vol.
Grade IV
Total anterior chamber vol.
"eight ball hyphema"

ETIOLOGY

Most frequently hyphema is caused by trauma, followed by intraocular surgery and spontaneous hyphema: abnormal growth of blood vessels – neovascularization (diabetes, ischemia), intraocular tumors (iris melanoma, juvenile xanthogranuloma, retinoblastoma), infections (herpetic keratouveitis), blood dyscrasias (sickle cell anemia, leukemia), anticoagulant therapy.[4]

Traumatic hyphema is most common in young males. Ocular trauma may be blunt or lacerating. The mechanisms of developing traumatic hyphema are:

- In blunt trauma occurs a rapidly anteroposterior compression of the eye with equatorial expansion and increased intraocular pressure, which leads to shearing forces that tear the vessels of the iris and ciliary body with accumulation of red blood cells within the anterior chamber.

- In lacerating trauma, traumatic agent causes direct damage of the vessels

Studies have shown that more than 50% of the cases, hyphema occupies less than 1/3 of the anterior chamber volume and less than 10% of the cases hyphema occupies the total volume of the anterior chamber.

In the case of a trauma, hyphema may be accompanied by other injuries such as angle recession (seen in 85% of traumatic hyphemas and can be associated with the development of glaucoma), traumatic iritis (presence of inflammatory cells in the anterior chamber and presence of pigment on the anterior capsule – Vossius ring – Figure 2), fixed dilated pupil in approximately 10 % of the cases, iridodialysis (disinsertion of the iris from the scleral spur), cyclodialysis cleft (separation of the ciliary body from the scleral spur, creating a direct connection between the anterior chamber and the suprachoroidal space, leading to ocular hypotony), corneal changes (from minor abrasion to corneal endothelium damage), cataract, lens subluxation, posterior segment injuries (vitreous hemorrhage, retinal edema/hemorrhages/ holes/tears, choroidal rupture etc.).[1]

Hyphema caused by ocular surgery can occur both intraoperatively and postoperatively. Intraoperatively, has been described in cataract surgery by direct damage of iris vessels, in peripheral iridectomy, cyclodialysis, and intervention on the posterior pole. Amsler sign is the presence of blood in the anterior chamber in patients with Fuchs heterochromic cyclitis, following rapid pressure change after anterior chamber paracentesis.

Postoperatively, the Uveitis Glaucoma Hyphema syndrome may occur after cataract surgery, caused by mechanic trauma of the iris by the IOL, more common in anterior chamber IOL.[5]

Spontaneous hyphema may occur secondary to iris neovascularization (proliferative diabetic retinopathy, ocular ischemic syndrome, retinal vein occlusion, chronic choroidal detachment), chronic uveitis (herpes zoster), tumors (uveal melanoma, retinoblastoma, lymphoma, leukemia, metastasis etc.), drugs (both anticoagulant and antiplatelet agents), blood dyscrasias (hemophilia, thrombophilia, von Willebrand disease, immune thrombocytopenic purpura).[6,7]

Figure 2: Vossius ring [11]

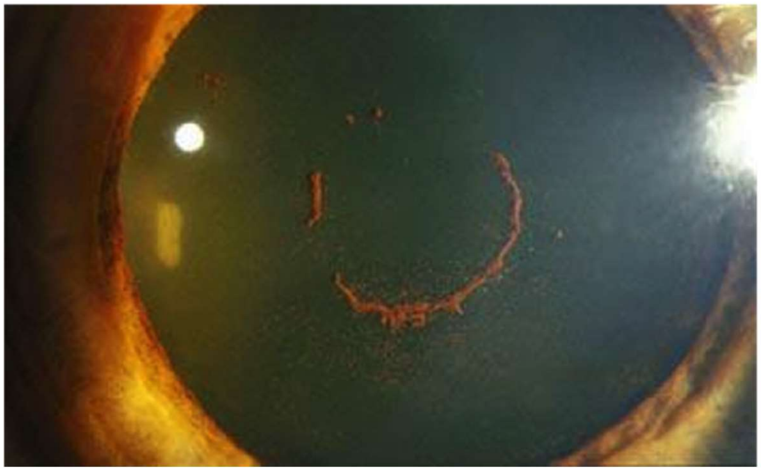

COMPLICATIONS

Obstruction of the trabecular meshwork with increased intraocular pressure is one of the complications that can result from untreated or improperly treated hyphema. This occurs in about 1/3 of hyphema cases. Studies have shown that 25% of patients develop intraocular pressure >25 mmHg and 10% of patients develop intraocular pressure > 10 mmHg. Usually, patients with a larger volume of blood in the anterior chamber have an increased risk in developing elevated intraocular pressure. Another risk factor would be the appearance of the recurrent bleeding. Intraocular pressure should be monitored daily. An untreated intraocular pressure > 35 mmHg for more than 5-7 days can lead to irreversible optic nerve damage.[8]

Recurrent bleeding or secondary hemorrhage (Figure 3) is another complication that can occur. It is described as increase of blood volume or appearance of a new layer of fresh blood in the anterior chamber. Most commonly occurs 3-5 days after the initial injury as a result of blood clot lysis. Recurrent bleeding increase the risk of other complications such as: increased intraocular pressure, corneal blood staining, optic atrophy, and peripheral anterior synechia. This complication is often associated with a weak prognosis or need for surgery.[9]

Figure 3: Recurrent bleeding [12]

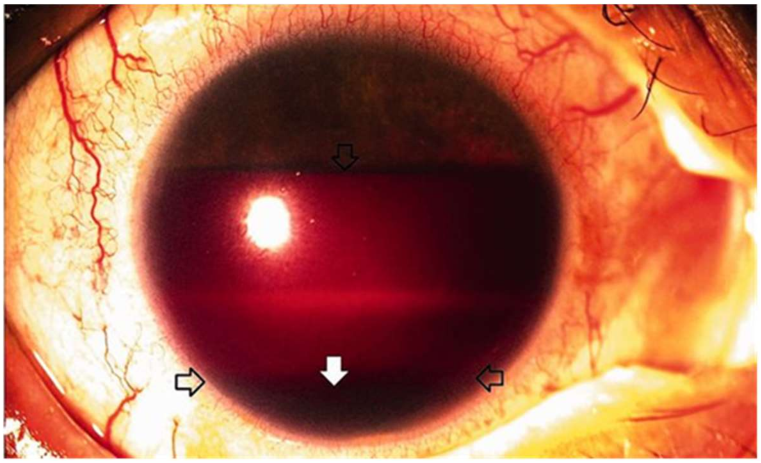

Glaucoma followed by optic atrophy occurs in 10-20 % of patients with traumatic hyphema due to trabecular meshwork damage or due to peripheral anterior synechia which leads to secondary open angle glaucoma.

Another complication that can occur in an untreated hyphema is synechiae formation (Figure 4), both posterior synechia and peripheral anterior synechia. Peripheral anterior synechia are more common and occur after inflammation or persistent blood clot. Patients with hyphema dating more than 7 days have a higher risk of developing synechiae.[10]

Figure 4: Peripheral Anterior Synechiae [13]

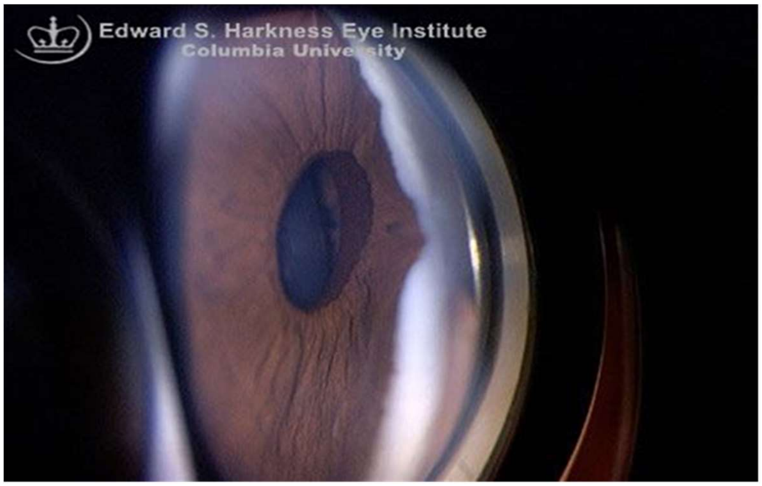

Corneal blood staining (Figure 5) is the corneal impregnation with intra and extracellular hemoglobin and hemosiderin particles. This occurs in 2-11% of cases, particularly in cases of total hyphemas, increased intraocular pressure, recurrent bleeding, long persistent of a blood clot, corneal endothelial cell dysfunction. The first sign of corneal damage is the presence of fine, deep, yellow line in the stroma, especially central, followed by extension in 24 -36 hours. After the resolution of hyphema, cornea gradually clarify, in months or even years, from the periphery to the center.[15]

Figure 5: Corneal blood staining [14]

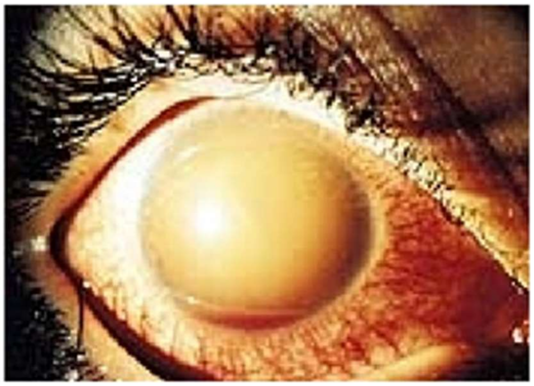

MANAGEMENT

The goals of the treatment are:
- clearing the hyphema
- reducing the bleeding rate
- treating the associated tissue lesions
- minimizing the long-term sequelae

Hyphema, unaccompanied by any complications, should be treated conservatively with a protective patch, limited activity, elevate the head of the bed at an angle of 30-45 degrees, so that the hyphema can settle out inferiorly and avoid obstruction of vision and limit the exposure of cornea to blood. The patient should be closely monitored during the first days when the risk of bleeding recurrence and increased intraocular pressure is higher.

Hospitalization is recommended in cases of noncompliant patients, patients with blood dyscrasias, with severe ocular and orbital damage, and patient with an increased intraocular pressure and sickle cell anemia.[16]

The medical treatment consists of administration of topical corticosteroids or, in severe cases, systemic corticosteroids to decrease inflammation associated with hyphema, topical cycloplegics (atropine 1% for 5 days: 1 drop/day in children, 1 drop x 3/ day in adults) to stabilize the blood-aqueous barrier, to decrease pain by relaxing the ciliary spasm and prevent the formation of posterior synechiae, topical aqueous suppressants (beta-blockers and alpha-agonists) or systemic carbonic anhydrase inhibitors and hyperosmotic agents (acetazolamide or mannitol – 1,5 mg/body weight) if topical management fails to control the pressure, antifibrinolytic agents (aminocaproic acid – 50-100 mg/body weight every 4 hours, up to a maximum of 30 g/day, for 5 days, tranexamic acid) to reduce the risk of recurrent bleeding.[4,15,17]

Surgical intervention is required in approximately 5% of cases with the following indications: IOP elevation > 50 mmHg for 5 days, IOP elevation > 35 mmHg for 7 days to avoid optic nerve damage, IOP elevation > 25 mmHg for 5 days in cases of total hyphema to prevent corneal blood staining, IOP elevation > 25 mmHg for more than 24 hours at patients with sickle cell anemia, large stagnant clots persisting for more than 10 days to prevent peripheral anterior synechia formation.[16]

Surgical techniques are paracentesis/ AC washout for liquid blood – is the safest and simplest method able to evacuate circulating red blood cells, can be repeated, avoid affecting the conjunctiva in case of a possible future filtration surgery, provide control of intraoperative bleeding and fast reduction of intraocular pressure, and bimanual cutting/aspiration for clotted hyphema, using the vitrectomy probe. Other surgical interventions that may become necessary include: peripheral iridectomy and trabeculectomy for glaucoma, cyclodiathermy and ultrasonic emulsification and aspiration.[10]

PROGNOSIS

Prognosis for visual recovery is related to the following factors: amount of associated damage to other ocular structures (choroidal rupture, retinal detachment, posttraumatic macular edema, lens opacification, vitreous hemorrhage, and angle-recession glaucoma), whether secondary hemorrhage or complications such as glaucoma, corneal blood staining or optic atrophy occur.

Studies have demonstrated that more than 75% of patients have a good prognosis, so approximately 80% of patient with hyphema <1/3 AC regain visual acuity of 20/40 (6/12) or better, 60% of patient with hyphema >1/2 AC but less than total of AC regain visual acuity of 20/40 (6/12) or better, while only 35% of those with total hyphema have good visual results. Poor visual outcome in traumatic hyphema can be directly attributed to the hyphema in 11% of patients; the poor visual outcome is usually the result of secondary hemorrhage associated with optic atrophy or corneal bloodstaining.[10,16]

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REVIEW ARTICLE

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Extra-pulmonary tuberculosis in immunocompetent patients: Case series and mini review of the literature

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Abstract: *Introduction: Tuberculosis (TB) is still one of the most frequent infectious diseases, causing 2 million deaths a year worldwide. The prevalence of TB has increased in both immunocompetent and immunocompromised and virtually, it can affect any organ. Up to 25% of tuberculosis cases present extra-pulmonary involvement. The diagnosis of extra-pulmonary TB (EPTB) is difficult, often requiring biopsy.*

Material and methods: We present two cases of EPTB in immunocompetent young women. The first case is about a 15-years old female who presented with persistent high fever, ascites and an increased CA-125 serum level. Biochemical analysis of ascites fluid revealed a high value of adenosine deaminase (ADA), negative Ziehl-Neelsen stain and undetectable Mycobacterium tuberculosis at PCR assay. The peritoneal biopsy confirmed the diagnosis of peritoneal TB. The second case, a 29-years old female with a seven-month history of cervical lymph nodes swelling, with drainage and excision biopsy performed, misdiagnosed as cats-scratch disease based on the histological description as granulomatous lymphadenitis, which turned out to be tuberculous lymphadenitis at the second histopathological opinion.

In conclusion, these cases highlight the need for tuberculosis consideration in the differential diagnosis of extra-pulmonary involvement, even in immunocompetent individuals, in order to decrease the misdiagnoses and complications rates.

Keywords: *extra-pulmonary tuberculosis, immunocompetent, peritoneal tuberculosis, lymphadenitis, challenging diagnosis*

INTRODUCTION

Tuberculosis (TB) can be considered an ancient disease which is still causing 2 million deaths a year worldwide. According to global statistics, one person is newly infected with TB bacilli every second world-wide and one third of the global population is currently infected with TB. TB ranks alongside HIV as a leading cause of death from an infectious disease. The prevalence of TB has increased in both immunocompetent and immunecompromised and virtually, it can affect any organ.

It is now seen that extra-pulmonary presentations form a major proportion of new cases. Between 2002 and 2011, extra-pulmonary TB (EPTB) accounted for 19.3% of all TB cases throughout Europe. EPTB is more common in children, women and minorities. Based on Romanian Tuberculosis Notification Data,

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12,498 new TB cases (a rate of 6.4 cases per 100,000 individuals) were registered in our country during 2014, which is higher compared with a rate of 5.2 cases per 100,000 individuals throughout Europe. Extra-pulmonary involvement occurred in 8% of the new cases. Among the extra-pulmonary TB (EPTB) cases, 30.1% were lymph node TB, 13.3 % osteo-articular TB, 6.3% genitourinary TB and 47.3% in other anatomical sites.

Lymphadenitis is the most common extra-pulmonary presentation of TB. Abdominal TB is also one of the most prevalent forms of EPTB. In particular, peritoneal TB is the sixth most common location of EPTB, with an incidence between 0.1% and 0.7% of all TB cases worldwide.

In EPTB, the diagnosis is challenging because of nonspecific symptoms and low sensitivity of classical diagnostic methods, such as microscopy and culture and it is often requiring biopsy. Delay in diagnosis and treatment initiations lead to poor prognosis and sequelae in up to 25% of cases.

MATERIAL AND METHOD

Case 1

A 15-year-old young female patient presented at the territorial hospital with abdominal distension, low abdominal pain, fever (40 degrees) with shiver, excessive sweating and loss of appetite. No other previous health problems were reported. All vaccines provided in universal vaccination schedule were performed. She denied smoking, drug use or alcohol consumption, and she was sexually inactive. The tests performed led to the diagnosis of ascites of unknown etiology. Despite the empirical antibiotic treatment with ceftriaxone, she continued to present fever, being subsequently referred to our clinic for further investigations.

At admission, the clinical status of the patient revealed a relatively good general condition, without fever, no abnormalities on pulmonary examination and normal vital signs. The abdomen was enlarged, with shifting dullness and positive ascitic wave, diffuse pain on palpation, without signs of peritoneal irritation. No masses or organomegaly were present on clinical examination. Blood tests showed lymphopenia (880/mm^3), mild microcytic anemia, elevated inflammatory markers (erythrocyte sedimentation rate of 58 mm/1h, Fibrinogen of 709 mg/dl, C-reactive protein of 112 mg/dl) with negative procalcitonin and normal biochemistry. Chest X-ray did not reveal any infiltrates. Abdominal ultrasonography showed the ascites in large quantity. Transthoracic echocardiography described minimal pericardial effusion (20-30 millimeters).

Further immunology and virology lab tests [rheumatoid factor, antinuclear antibodies, anti-double stranded DNA antibodies (Ab), complement fractions C3, C4, hepatitis B surface antigen, hepatitis C virus Ab, and Human Immunodeficiency Virus 1 and 2 Ab] were negative. The patient underwent paracentesis with ascitic fluid analysis. The gross appearance of the fluid was pale yellow, with exudative features, based on the biochemistry, a total nucleated cell count of 600/mm3 with predominance of polymorphic lymphocytes (72%). The ascites fluid ADA level reached a high value of 140 IU/l, normal value being <33 IU/L. The microscopy for non-specific bacteria and acid fast bacilli, using Gram and Ziehl-Neelsen stains, was negative. Moreover, we performed a broad-spectrum molecular microbial identification from the ascites (IRIDICA BAC BSI® Assay platform for bacteria and fungi) and MTB-DNA amplification (GeneXpert® platform), both of them being undetectable. The serum tumor markers such as CEA, CA19-9 and CA15-3 were within normal limits, apart from CA 125, which was significantly higher (189.5 IU/ml). Serum gamma-interferon specific to MTB antigens produced by T cells (QuantiFERON®-TB Gold) was positive.

In the meantime, we started empiric antibiotic treatment based on oral clarithromycin 1g/day for 3 days, with fever persistence. Then we switched to intravenous (iv) ertapenem 1g/day. After 5 days of ertapenem treatment, the fever has disappeared, with minimal ascites fluid regression. Abdominal and pelvic magnetic resonance imaging (MRI) showed the presence of ascites and described irregular masses with peripheral contrast enhancement near the ovarian fossa, similarly to epiploic fringes.

The patient was transferred to a pediatric general surgery department for exploratory laparoscopy and peritoneal biopsy. The laparoscopic findings described thickened peritoneum with disseminated miliary white nodules on the surface and intestinal wall congestion, being highly suggestive of PTB (Figure 1).

Figure 1. Laparoscopic findings with thickened peritoneum, diffuse congestion, disseminated milliary white nodules on the surface of peritoneum highly suggestive for TB.

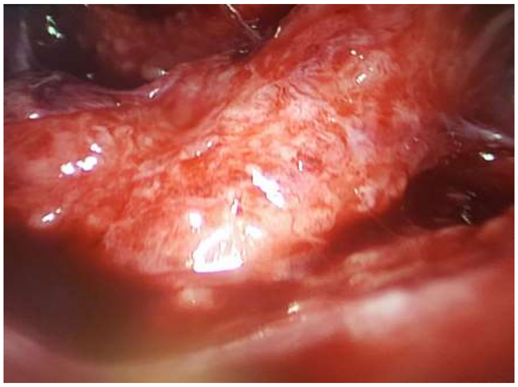

Figure 2. Multiple foci of granulomatous inflammation with multinucleated giant cells, epithelioid histiocytic cells and peripheral lymphocytes are seen on Periodic acid-Schiff (PAS) staining (100X).

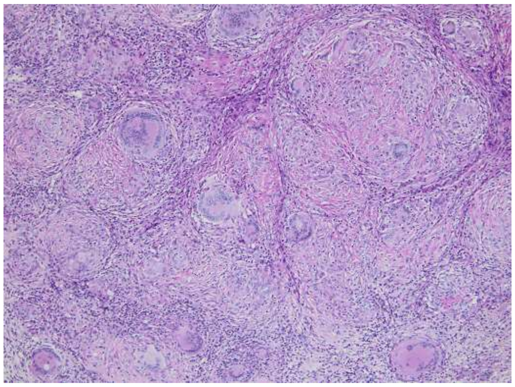

Multiple tissue biopsies were collected. Based on clinical and biological features, along with laparoscopic findings, we decided to start antituberculous therapy with isoniazid 200 mg, rifampicin 450 mg, pyrazinamide 1,500 mg, ethambutol 1,200 mg daily, and oral pyridoxine vitamin 250 mg/day. After 10 days of antituberculous treatment, the symptoms of the patient have regressed, serum C-reactive protein level decreased from 153 mg/dl to 8.6 mg/dl, with no ascites presence at the abdominal ultrasound.

The histopathological results confirmed the diagnosis of peritoneal tuberculosis (Figure 2). In addition, MTB-DNA from the biopsy sample was detected, with no detection of genetic mutations associated with rifampicin resistance.

Case 2

A previously healthy, 30 year-old Caucasian female, smoker (10 pack/year), presented to our department for laterocervical and supraclavicular lymph nodes enlargement, slightly painful on palpation. She also complained of fatigue and dry cough. She was not on any medication, no histories of allergies, all vaccines provided in universal vaccination schedule were performed and she has not travelled during the past year outside of Romania. She has a cat which is an indoor pet.

The symptoms began about 7 months ago with a red, painful swelling of about 4-5 cm, in her left laterocervical region, which she noticed several weeks ago and continued to enlarge. She went to the surgical department where was performed the incision of the lymph node with drainage and lavage. The cultures provided from the procedure were sterile at 48 hours.

5 months later, she presented to the thoracic surgery department with suppurated supraclavicular lymph nodes. All the laboratory tests performed were normal (blood count, coagulation, biochemistry (glucose, creatinine, ALT, AST, GGT, Alkaline phosphatase, sodium, potassium). The patient was evaluated by cervical and thoraco-abdominal CT and the images showed multiple extended outbreaks of pulmonary infiltrates with tree in bud pattern in the apical-dorsal segment of the superior left lobe and in the dorsal segment of the superior right lobe and some isolated centimeter nodal images and mediastinal, supra-clavicular, and bilateral laterocervical lymph nodes. They continued the investigations with bronchoscopy with GenExpert test, direct microscopic examination and culture for acid fast bacilli in bronchial aspiration and sputum. They performed an excision biopsy of the supraclavicular lymph node and the histopathological findings described as confluent epithelioid granulomas

with central clotting necrosis have been interpreted as specific lesions for cat scratch disease. The patient was referred to a department of infectious diseases for additional investigations and specific treatment. Meanwhile, the GenExpert test and the direct microscopic examination for acid fast bacilli were negative with cultures in progress.

When she came in our department, she had good general state. On physical examination we noticed in the left laterocervical area two scars with redness surrounding and several firm, tender, lymph nodes about 1 to 3 cm in diameter, palpable in the left laterocervical and supraclavicular area. The initial laboratory tests were normal, the test for antibody to human immunodeficiency virus (HIV) was also normal, with no other immunity issue found. The serology for Bartonella henselae infection was negative for both antibodies Ig M and Ig G. The patient was referred to the National Institute of Pneumophtisiology for reevaluation. Based on the high clinical and histopathological suspicion of tuberculosis, the biopsy blades were re-analyzed and by performing Ziehl-Neelsen coloration, were discovered few acid-fast bacilli (Figure 3). The histopathological description with necrotizing granulomatous epithelioid inflammation was also pathognomonic for tuberculosis.

Figure 3. Acid-fast bacilli on Ziehl–Neelsen smear (100X)

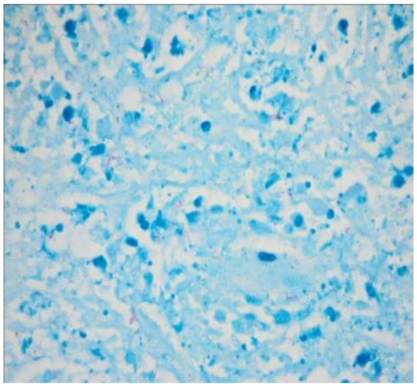

The patient was put on anti-tubercular drugs with good evolution. The cultures for mycobacterium tuberculosis read at 2 months were positive.

DISCUSSIONS

TB is still one of the most frequent infectious disease and worldwide strategies to eradicate it have met only with partial success. According to global tuberculosis report, in 2015, there were an estimated 10.4 million new TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. People living with HIV accounted for 1.2 million (11%) of all new TB cases. There were an estimated 1.4 million TB deaths in 2015, and an additional 0.4 million deaths resulting from TB disease among people with HIV. Although the number of TB deaths fell by 22% between 2000 and 2015, TB remained one of the top 10 causes of death worldwide in 2015.

Although it is well known that TB occurs mainly in immunocompromised host, it seems that the prevalence of TB has increased both in immune-competent and immunocompromised patients. Nowadays extra-pulmonary presentations form a major proportion of new cases of TB. Among them, lymphadenitis is the most common clinical presentation, with a major predilection for cervical lymph nodes.

Abdominal TB is also one of the most prevalent forms of EPTB and the peritoneal involvement has been observed in about 1-2% of all cases of TB. Pelvic TB is common in developing countries, mainly in young, fertile women aged between 15 to 25 years old. The common clinical features include ascites, fever, diffuse abdominal pain and weight loss. Therefore, peritoneal TB is often misdiagnosed, usually as carcinomatous peritonitis. The lacking of medical awareness of pelvic TB could lead to mutilating surgical treatment like adnexectomy for many young, fertile, women.

Our patient presented with a clinical picture highly compatible with peritoneal TB. However, we had to continue the investigations because the conventional methods, like microscopy and MTB-DNA amplification from ascitic fluid, failed to establish the positive diagnosis. In addition to clinic-biological findings and radiological characteristics, the accuracy of early diagnosis was increased by measuring the ascites ADA and serum CA125 levels.

The studies showed a significant correlation between

ADA and CA-125 in patients with peritoneal TB. The tests are simple, non-invasive, cheap, and it seems to have enough power to confirm or exclude the diagnosis of peritoneal tuberculosis and it is a good approach to begin the empirical treatment while waiting for the histopathological result or cultures.

The diagnosis of peritoneal TB can also be suggested by characteristics findings at laparoscopy or laparotomy; however, these are invasive examination methods and should be recommended with caution in clinical practice.

Lymphadenitis is the most common extra-pulmonary presentation of TB. It is seen in nearly 35 per cent of extra-pulmonary TB which constituted about 15 to 20 per cent of all cases of TB. Although previously considered a childhood disease, lymphadenitis has a peak age of onset of 20-40 years. The most common site of involvement is represented by cervical lymph nodes, reported in 60% to 90% patients, followed by mediastinal and axillary lymph nodes6.

Cervical lymph nodes can be involved via various pathological processes, including inflammatory diseases, lymphomas and even metastases. It is important that a high index of suspicion for tuberculous lymphadenopathy should be kept in mind because it mimics other pathologic processes and yields inconsistent physical and laboratory findings.

TB lymphadenitis usually presents with gradually painless swelling of one or more lymph nodes. Initially the nodes are firm, mobile, later they become matted with inflamed overlying skin and with abscesses formation in the advanced stages. Some patients may associate systemic symptoms such as fever, weight loss, fatigue, dry cough.

In our case, the patient had a seven-month history of cervical lymph nodes gradually swelling, which become matted with redness of the overlying skin. She also associated systemic symptoms represented by dry cough and fatigue.

The diagnosis of tuberculous lymphadenitis is difficult due to low sensitivity of mycobacterial isolation, prolonged time of the isolation process and multiple differential diagnosis that need to be ruled out. In endemic areas, for faster diagnosis, has been used fine-needle aspiration cytology (FNAC), combined with polymerase chain reaction for detecting Mycobacterium tuberculosis, in the absence of compatible cytological findings or negative acid-fast bacilli smears. Acid fast bacilli may be present in 25-50% of smears and the mycobacterium may be isolated in up to 70% of cases where there is a high suspicion of tuberculosis. Caseating granulomas are seen in almost all the biopsies and in 77% cases of the FNAC's. Typically, epithelioid cell granulomas, multinucleated giant cells and caseation necrosis are features of tuberculous lymphadenitis but these are neither specific nor sensitive and the diagnosis remains a challenge, necessitating a high index of suspicion, since granulomatous lymphadenopathy has an extensive differential diagnosis [13].

Cat scratch disease (CSD) is an infectious disease caused by gram-negative bacteria Bartonella henselae (B. henselae) and/or Bartonella quintana. It is represented by regional suppurated lymphadenitis, the lymph nodes affected are cervical (33%), axillary (27%) and inguinal (18%). The disease affects children and youths under 18 years old. Epidemiologic studies from the United States, Europe, Israel, Australia, and Japan, suggest that CSD has a worldwide distribution. In the United States, a retrospective study of patients younger than 65 years of age identified 13,273 patients with a diagnosis of CSD from 2005 to 2013. The diagnosis is based on the following: a) history of contact with a cat; b) checking of injury sites; c) regional lymph node swelling; d)characteristic histology of lymph node; e)skin test for CSD, replaced in the last years by immunohistochemically staining with antibodies to B. henselae in formalin-fixed paraffin–embedded tissue sections and polymerase chain reaction.

The histopathologic findings are nonspecific and depend upon the stage of the disease. Lymphoid hyperplasia is present initially, followed by the development of stellate granulomas. The centers are acellular and necrotic with histiocytes and peripheral lymphocytes surrounding these areas. Microabscesses develop and may become confluent at a later stage. Although these findings support a diagnosis of CSD, they should not be considered definitive.

Based on the granulomas found on the histological examination, our patient was first misdiagnosed as cat scratch disease without staining for acid-fast bacilli or PCR identification for Mycobacterium tuberculosis on the biopsy specimens.

CONCLUSIONS

Tuberculosis still remains a worldwide public health issue. It is still a common disease in developing countries, even in immunocompetent individuals. It should be kept in mind in the differential diagnosis of extra-pulmonary manifestations in order to avoid misdiagnosing and further complications. Surgical biopsy leaves unwanted scars, causing esthetic problems and also the possibility of long-term drainage problems through the incision line.

In young, fertile, women, presenting with febrile ascites, often mimicking adnexal malignancy, surgeons should be aware of peritoneal TB, avoiding unnecessary mutilating surgery leading to increase complications, such as infertility.

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***Conflicts of interest**: All authors – none to declare*

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SYSTEMATIC REVIEW

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Early in-stent restenosis after revascularization in patients with ST-elevation myocardial infarction

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Abstract: *Background and aim: Obstructive coronary disease is one of the most important causes of death worldwide. Percutaneous coronary intervention is one of the most important treatments of this pathology. Using of coronary artery stents represented a major advance in interventional cardiology. While bare metal stents (BMS) set the reference point for improved safety over balloon-angioplasty, first-generation drug-eluting stents demonstrated significant improvements in efficacy, but not necessarily safety, and further technologic developments have focused on optimizing both.*

Methods: We studied 228 patients with STEMI in the last 6 months, divided in 2 groups – in the first group are 122 patients (53.5%) who developed in stent restenosis and the second group has 106 patients had no signs of restenosis (46.5%). The mean time of performing the angiographic reevaluation was 111 days for the first group and 154 days for the second group. The clinical signs that indicate the need of coronary reevaluation was stable angina (116, 50.87%), acute coronary syndrome (58, 25.4%) and asymptomatic patients (64, 28.9%). The cardiovascular risk factors correlate with high risk of restenosis was diabetes (24.3% in first group and 12.2% in the second one, p=0.004), active smoking (p=0.010) and metabolic syndrome (p=0.003). The stent's length >28mm, stent's diameter ≤ 2.5 mm and chronic occlusion correlate with high risk of in-stent restenosis in BMS.

Conclusion: Understanding the importance of risk factors control, will reduce the risk of restenosis, of other cardiovascular event, even sudden death.

Keywords: *stent, early restenosis, STEMI, revascularization*

INTRODUCTION

Cardiovascular disease represents the first cause of morbidity and mortality world-wide.

ST-elevated myocardial infarction (STEMI) represents a challenge for any cardiologist interventionist team who has to decide the best treatment option based on short, medium and long-time prognostic and possible complications.

The first successful percutaneous transluminal coronary angioplasty (PTCA) was performed using a balloon catheter.[1] Due to high rate of coronary occlusion during PTCA and of high rate of in-stent restenosis bare metal stent (BMS) was developed. First studies have shown that BMS are very effective in treating or preventing acute blood vessel obstruction, thus avoiding emergency surgical bypass interventions. Two randomized trials – the Benestend and STRESS (Stent Restenosis

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Study) have shown that stenting lesions de novo on native coronary have reduced angiographic restenosis by about 30% compared to conventional balloon angioplasty.[1,2,3]

Implanting stents leads to a luminal diameter larger than balloon angioplasty, both right after the surgery and in the follow up, thus to a lower restenosis rate.

Using BMS was compared with coronary artery bypass grafting (CABG) in the treatment of obstructive multi-coronary disease in ARTS trial (Arterial Revascularization Therapies Study). The 1 year follow up showed that the mortality, myocardial infarct and stroke rate was similar in the two groups. Survival rate without cardiovascular events was higher in CABG group (87.8% vs 73.8%). Also less surgery patients needed a second revascularization procedure (3.5% vs 16.8%).[4]

Stone et al, studying the safety and effectiveness of BMS and DES on 3006 patients with STEMI with immediate PCI noticed that in DES group the restenosis in stent and recurrent ischemia needing revascularization procedure in 1 year follow up was significant lower than in BMS group.[5] Although, mortality and in stent thrombosis rate were similar for the 2 groups.

Drug eluting stents are a newer coronary stent category, superior to BMS due to a significant reduction of in-stent restenosis, a significant decrease of major adverse cardiac events, as well as target lesion revascularization in those patients which benefited from DES at 6 month follow up.[6, 7] Results at 5 years have demonstrated a similar rate of in-stent thrombosis between the two groups, but a significant lower incidence of major adverse cardiac events in the DES group.[6]

The beneficial using DES is not absolute. DES are superior in reduction of in-stent restenosis incidence especially in high risk lesions. For low risk restenosis patients or for those with high risk of acute in-stent thrombosis due to need of early dual antiplatelet therapy abortion the using of BMS has an important indication.[8,9] Although, lot of research show that using BMS is an alternative for uncomplicated patients or for those with long term dual antiplatelet therapy contraindication, in high diameter vessels, in venous graft lesions or in patient with acute coronary syndrome – STEMI.[10-14]

Despite last decades progresses, in stent restenosis stays an unsolved problem. Revascularization needs in 1 year is 6.7% in DES using, and 11% in BMS, frequently due to restenosis.[15]

METHODS

This study is a unicentric, retrospective, non-randomized trial. The patient from this study were patients of "Academician Vasile Candea" Emergency Clinical Center of Cardiovascular Diseases of the Army Bucharest. We included in the study patients diagnosed with STEMI who underwent myocardial revascularization in less than 24 hours from the appearance of chest pain, who followed the medical treatment according to ESC Guideline, and who underwent angiographic reevaluation in the first 6 months after PTCA. The patients were divided in 2 groups: the study group who developed in-stent restenosis in less than 6 months from PTCA, and the control group who has no signs of restenosis. We evaluate local factors (type of stent, the length and the diameter of stent, the affected vessel and the type of restenosis – focal or diffuse restenosis) and general factors (genetic factors, demographic factors, smoke, and comorbidities – arterial hypertension, dyslipidemia, diabetes, metabolic syndrome, history of stoke, peripheral arterial disease).

RESULTS

We studied 228 patients with STEMI in the last 6 months, divided in 2 groups – in the first group are 122 patients (53.5%) who developed in stent restenosis, from minimal restenosis (24 patients, 20%) to occlusion (16 patients, 13.3%), and the second group has 106 patients which had no signs of restenosis (46.5%). The mean time of performing the angiographic reevaluation was 111 days for the first group and 154 days for the second group.

Most patients came from urban areas (67% for both groups – without significant differences between groups). In the study group the most patients are men (82 patients, 68.3%), while in the other group only 54%

were men (OR=2.476; CI 95% 1.465-3.780; p<0.003). The clinical signs that indicate the need of coronary reevaluation was stable angina (116, 50.87%), acute coronary syndrome (58, 25.4%) and asymptomatic patients (64, 28.9%).

The cardiovascular risk factors correlated with high risk of restenosis were diabetes (24.3% in first group and 12.2% in the second one, p=0.004), active smoking (p=0.010) and metabolic syndrome (p=0.003). The influence of arterial hypertension and dyslipidemia over the risk of in-stent restenosis is hard to appreciate. Prior PTCA, peripheral arterial disease, genetic impregnation also have little correlation with restenosis risk.

The most frequent location of stent implantation in left anterior descending artery (LAD), followed by right coronary artery and circumflex artery, but the stent location doesn't correlate this risk of restenosis.

If we talk about stent's characteristics which can interfere with evolution and prognosis of the patients, the stent's length >28mm (OR 2.77; CI 95% 1.45-4.44; p<0.001), stent's diameter ≤ 2.5 mm (OR 2.45; CI 95% 1.54-3.46; p<0.001), and chronic occlusion (OR 3.78; CI 95% 2.03-7.35; p<0.001) correlate with high risk of in-stent restenosis in BMS. Although, the stent's diameter ≥3.25 mm (OR 0.34; CI 95% 0.24-0.49; p <0.001), the length ≤15 mm (OR 0.58; CI 95% 0.42-0.81; p=0.001) correlate with further restenosis.

DISCUSSION

Our results shows that diabetes mellitus, metabolic syndrome, prior interventional myocardial revascularization, longer stent length and smaller stent diameter are important factors that correlate with risk of in-stent restenosis. Prior PTCA, peripheral arterial disease and genetic factors correlate with restenosis risk. Our research was design to study the risk factors for early in-stent restenosis. Thus, we compared our result with many available researches that studied risk of long term and very long term in-stent restenosis, and results are similar. [16-19] It is known that diabetes, history of myocardial infarction and/or PTCA, different stent and lesion factors correlate with risk of long term and very long term in-stent restenosis, even on BMS or DES.[20-22] Despite rigorous risk factors control and correct medical treatment some patients develop in-stent restenosis, many factors could be incriminated in this phenomenon according with clinical trials. The factors incriminated in in-stent restenosis development, in both BMS and DES, are biological factors (drug resistance, hypersensitivity), mechanical factors and technical factors.[23]

The drugs in DES have a cytostatic (Sirolimus and its analogs) or a cytotoxic effect (Paclitaxel). Statistic data indicate that patients genetic mutations can develop resistance to Sirolimus or Paclitaxel.[24, 25]

Other biological factor is hypersensitivity of compound element in stents. For BMS, release of nickel and molybdenum, and for DES, cobalt releasing, are potential triggers for in-stent restenosis due to local allergic reaction, which can accelerate the atherogenic process.[26, 27]

The mechanical factor responsible of in-stent restenosis are stent underexpansion during the procedure, which is undetectable angiographically, nonuniform drug distribution due to local blood flow alterations, damage of the polymer, stent fracture resulting an important decrease in local drug delivery.[28-31]

Technical factors potentially responsible for increased risk of restenosis include barotrauma outside the stent, randomized clinical trials indicate that restenosis occurred at the proximal margin of stent and uncovered residual plaque, which correlate with increased risk of myocardial infarction at 1 year.[32-33]

Clinical presentation of patient with in-stent restenosis was represented by stable angina in more than half of patients and a quarter of patients were completely asymptomatic. Also, almost ¼ of patients presented acute coronary syndrome, in which smoking and prior myocardial infarction are clinical predictor factors.

Thus, is very important for our patients to understand the importance of risk factors control, reducing the risk of restenosis, of other cardiovascular event and sudden death. Also, there are some biological factors which are difficult to evaluate and some mechanical and technical issues that should be consider in patients with in stent restenosis. Further, we could study the impact of subclinical factors which could interfere with evolution and prognostic, early identifying technics and ways for prevention complications for increase the quality of live and decrease the costs of long term managing of patients.

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Study of the type and distribution of tumor infiltrating lymphocytes in 27 cases of lung adenocarcinoma

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Abstract: *Pathologists observed the presence of tumor infiltrating lymphocytes (TILs) in solid tumors many years before the research focus shifted from cancer cells to the tumoral microenvironment. In lung adenocarcinoma TILs vary in number, phenotype and distribution. Our study investigate the type and distribution of TILs regarding the stage of disease and histological subtype in 27 cases of lung adenocarcinoma. By immunohistochemistry we evaluated semi-quantitatively CD3, CD8 and cyclin D1. We have found that CD3+CD8- lymphocytes prevail at the invasive margin and in tumoral stroma. Cyclin D1 was diffusely positive in tumoral cells in all the five cases of lepidic type of lung adenocarcinoma. In other histological types cyclin D1 was focally positive, most at the margin of the tumor irrespective of the presence or absence of TILs.*

Keywords: *lung adenocarcinoma, tumor infiltrating lymphocytes (TILs), CD3, CD8 and cyclin D1*

INTRODUCTION

In solid tumors, tumoral stroma acts as an interface between the body and cancer and its effectors are fibroblasts and inflammatory cells. Inflammatory cells are mainly lymphocytes (tumor infiltrating lymphocytes/TILs) and few macrophages, dendritic cells and neutrophils. In line with step-wise modulated activity of immune cells, it is a deductive reason that tumor infiltrating lymphocytes have impaired activity caused by peculiar biochemical condition in tumoral microenvironment. Types of lymphocytes are defined by expression of markers that underlie immune competence or suppression, such as CD3, CD8, Foxp3, PD1 and CTLA4. Lymphocytes have a variable presence in lung adenocarcinomas and they vary considerably in number, phenotype and function [1,2,3].

The prognostic value of lymphocytes in cancers demonstrate an organ restriction efficiency of TILs, in few tumors it was shown that presence of TILs has positive impact on evolution, as in melanoma, colon cancer with micro-satellite instability or renal cancer [6,7,8]. Increased level of TILs has been positive correlated with survival [2] and it was shown to be an independent prognostic marker for lung adenocarcinoma patients [3].

Topographical distribution of lymphocytes and their phenotype suggest different triggers for intratumoral lymphocytes and peritumoral lymphocytes at the invasive front. The molecular structure of the “tumoral

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antigen" is still elusive, while the dynamic balance of simultaneous activator and inhibitory signals in the tumoral microenvironment interfere with non-self-recognition capacity of the lymphocytes.

MATERIAL AND METHOD

In this study we investigated by immunehistochemistry the type and distribution of lymphocytes that infiltrate tumoral stroma and peritumoral areas in 27 cases of lung adenocarcinomas. These cases were selected from 43 lung adenocarcinomas that have been diagnosed and operated in Emergency University Hospital Bucharest between January 2013 and June 2016. CD3 and CD8 expression of tumor infiltrating lymphocytes were evaluated by immunohistochemistry on paraffin-embedded tissue.

Additionally, we investigated the cyclin-D1 mediated DNA synthesis in tumoral cells looking for patterns of positivity by immunohistochemistry for cyclin-D1 in relation the presence and density of lymphocytes in vicinity and homogeneity versus clusters of positivity.

Table 1

	Age	Gender	Stage of disease	Histological type	Grade of differentiation	Follow-up period* (*months*)
1	43	M	IV	mixt	G1	19
2	57	M	IA	lepidic	G3	73
3	47	M	IIA	lepidic	G3	16
4	81	M	IA	lepidic	G3	4
5	61	M	IIB	mixt	G1	13
6	67	M	I A	mixt	G2	22
7	48	M	IIB	mixt	G3	34
8	61	M	IA	mixt	G3	1
9	62	M	III	mixt	G2	36
10	59	F	IB	mixt	G2	3
11	75	F	IA	mixt	G2	1
12	65	M	IIIB	mixt	G3	13
13	64	M	-	lepidic	G2	18
14	64	M	IB	acinar	G1	22
15	56	M	IA	mixt	G1	1
16	62	M	-	mixt	G3	1
17	67	M	IA	lepidic	G3	18
18	57	M	IB	mixt	G2	2
19	62	F	IIB	mixt	G2	7
20	46	M	IA	mixt	G2	9
21	62	M	IB	acinar	G2	10
22	70	F	IA	acinar	G1	12
23	59	F	IA	mixt	G2	3
24	70	M	IV	acinar	G2	1
25	56	M	I	acinar	G2	1
26	68	M	IIA	acinar	G2	1
27	70	M	IV	acinar	G2	6

**Follow-up period was measured as the interval from diagnosis and the last medical record for the patient in the hospital database*.

Age, gender, stage of disease, histological type of tumor, grade of differentiation (G) and follow-up period from hospital records were taken into account.

The selection of cases for immunehistochemistry investigation was made by evaluating hematoxylin-eosin slides of each case for the presence of tumor infiltrating lymphocytes. Positive criteria were 20 lymphocytes at the invasive front or in tumoral stroma or between tumoral cells present at least on 6 high power fields (HPF). Cases with massive necrosis were excluded, although some of these showed limited areas of abundant infiltrating lymphocytes.

In Table 1 details of the each patient are shown.

The mean age of diagnosis was 61 years, between 43 and 81. 81.5% were male and 18.5% were female. The mean follow-up period was 13 months.

RESULTS

Examination of the hematoxylin-eosin slides showed a topographic variation of the constitutive elements. In the center of the tumor prevails desmoplastic reaction and/or areas of necrosis, while tumoral cells form small groups and collapsed tubes surrounded by cancer associated fibroblasts (CAFs), collagen fibers and rare inflammatory cells. Necrosis rarely appear at less than 0.5 cm from the margin of the tumor and it was absent in lepidic type of lung adenocarcinoma as we found in our five cases of this type. Necrosis appear at the margin of tumor in the invasive front in cases diagnosed in advanced stages, III and IV.

Table 2

	Histological type			Grade of differentiation			Stage of disease					
	acinar	mixt	lepidic	G1	G2	G3	I	IIA	IIB	IIIA	IIIB	IV
N (%)	7 (26)	15 (55)	5 (19)	5 (19)	14 (52)	8 (29)	15 (55)	2 (7)	3 (11)	1 (4)	1 (4)	3 (11)

Figure 1A. CD3 positive in most of the TILs. 20X. B. Same area, CD8 positive in rare TILs; 20x.

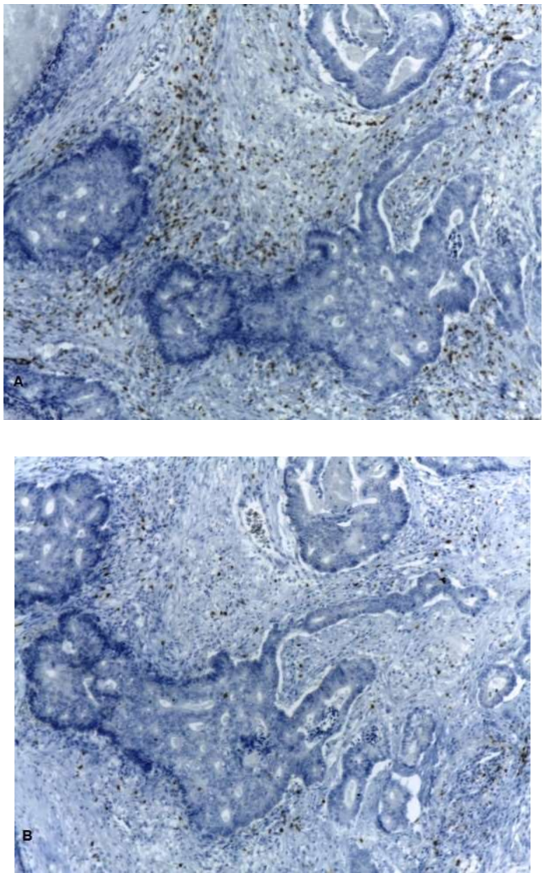

We identified three patterns of morphologic distribution of tumor infiltrating lymphocytes (TILs) as: intratumoral TILs – when infiltrate among tumoral cells (in proximity with them), stromal TILs – when infiltrate in connective stroma between acini and groups of tumoral cells, and peritumoral lymphocytes – when surround discrete or continuously the tumor at the invasive front. Two or all three of these patterns coexist in each tumor.

An inconstant finding was the presence of tertiary lymphoid structures in normal tissue in vicinity of tumor at 0.5-1 cm.

Depending on histological subtype of lung adenocarcinoma, lepidic type showed the following peculiar aspects: two out of five cases showed homogeneous moderate nuclear positivity for cyclin D1 in>80% of tumor cells irrespective of distance from the margin of the tumor, necrosis was absent and lymphocytes were found to have scarce stromal and discrete peritumoral distribution, and they were predominantly CD3+/CD8-.Stromal TILs were discontinuous, from absent to 50 lymphocytes on HPF, peritumoral TILs were discrete, intratumoral TILs were absent.

Figure 2. Cyclin D1 diffuse positive in lepidic lung adenocarcinoma; 10x.

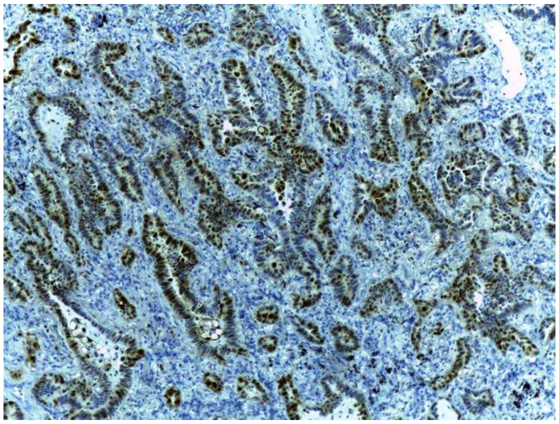

The other types of lung adenocarcinoma included in this study, acinar and mixt, (the mixt type was represented by predominantly acinar plus other pattern, solid type being identified most of the time), showed important areas of necrosis and desmoplastic reaction in the center of the tumor.

When necrosis was near the margin, it was also found unexpected presence of intratumoral TILs dispersed among tumoral cells with immunophenotype CD3+/CD8+ and increased peritumoral TILs.

Regarding the stage of disease, we have found that in the later stages of disease (stage III and IV) peritumoral lymphocytes are increased, in continuous distribution, with no significant variation regarding stromal or intratumoral lymphocytes, and that CD3+CD8-/ CD3+CD8- ratio always favor CD3+CD8-.

We interpreted that cyclin-D1 showed a gland cluster distribution when positive signals are present in most of the cells in the same gland and we suppose that those cells are in the same developmental stage.

Positivity of cyclin D1 at the margin of tumor is increased compared to the center of the tumor, irrespective to the presence or absence of peritumoral lymphocytes.

TILs in lung adenocarcinoma are predominant CD3+CD8-, they are frequently found at the invasive front, they are variable present in desmoplastic stroma and scarce to absent among tumoral cells.

DISCUSSIONS

Because of their potential cytotoxic ability, TILs are regarded as promising anticancer therapeutic targets. PD1 and CTLA-4 are the former immune-checkpoint with inhibiting effect on T-cell after their ligands binding [11]; in clinical practice, monoclonal antibodies with PD1 and CTLA-4 blocking activity show inconstant efficiency.

The variable presence of TILs could be due to variable immunogenity of tumor, due to the process of positive and negative clonal selection of lymphocyte during their maturation in lymphoid organs when tolerance cover a variable spectrum of “self” and “non-self” molecules that may be native or altered proteins, which produces variation among individuals and also resistance to cancer, or due to contradictory signals in tumor microenvironment, both proinfla-mmatory and inhibitory cytokines.

The variable distribution of TILs, mainly in stroma and peritumoral, and rarely in direct contact with tumoral cells, make the stroma an agent of sequestration for immune cells, or, from the other side, an agent of compartmentalization of tumoral cells in order to escape the immune system. In tumoral stroma, the inhibiting effect on TILs is orquestrated by CAFs (cancer associated fibroblasts) which secrete soluble cytokines which interfere with all the immune cells in the milieu.

Anterior studies predicted CD4+/CD8- phenotype predominance of TILs [1, 2]. We have sustaining results regarding CD3+ and CD8- lymphocytes. Confirmation of the presence of TILs is the first step in promoting immune-checkpoints targeted therapy. The actual role of the pathologist may be to attest the presence of TILs and to quantify the signal of the target molecule (PD1/PD-L1) in tumoral tissue.

As known, cyclin D1 has a role in promoting cell-cycle progression from G1 to S phase, when the DNA duplicate. In cancers cyclin D1 may be a driving factor (as in mantle cell lymphoma) and sometimes just a secondary event. Cyclin D1 was shown to have role also in genome stability [12].

The cluster positivity of cyclin D1 could suggest that cells derived from the same progenitor have a cell-cycle coherence; we could speculate that this is maintained until disproportionate access to nutrients favor clonal selection for expansion. The lack of correlation between TILs and tumoral cells expressing cyclin D1 apparently exclude a mitogenic effect of infiltrating lymphocytes on cancer cells as we might presume if TILs appear to express more inflammatory then cytotoxic qualities.

CONCLUSIONS

TILs are potential allies in anti-cancer therapeutic strategy. CD8 positive TILs predict a better outcome in lung adenocarcinoma.

Determinants of the variable presence and topographical distribution of TILs have yet to be explained.

Cyclin D1 is variably express among histological types of lung adenocarcinoma, with increased expression in lepidic type, and is not correlated with the presence or absence of TILs.

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ORIGINAL ARTICLES

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Autologous versus synthesis materials used in the surgical closure of CSF leak

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Abstract: *The evolution of the endoscopic skull base surgery in the last ten years had led to the development of new surgical reconstructive techniques. In the endoscopic reconstructive surgery of the CSF leaks both synthesis and natural materials. The advancement of the endoscopic reconstructive surgery was closely linked with the production of new synthesis materials. The aim of the paper is to compare the efficacy of synthesis versus natural materials in the closure of CSF leaks and to point out certain rules of when and where to use a synthesis or natural material.*

INTRODUCTION

In the reconstructive surgery of the skull base, the surgeon can use both natural and synthesis materials.

The developments of the endoscopic surgery of the skull base had led to removing bigger tumors from high-risk areas that were not suitable for endoscopic surgical approach up to now. The endoscopic surgical approaches tend to replace the external approaches even in malignant tumors of the skull base in selected cases.

Because of that, the surgeon started to encounter more defects at the level of the skull base with or without CSF leaks.

New surgical reconstructive techniques started to be developed in close connection with the production of new surgical reconstructive materials and adhesives.[1]

The materials used in the reconstruction of the skull base defect can be divided in autologous tissue and synthesis reconstructive materials.

Autologous tissues that are used in the reconstructive skull base surgery are: fascia, cartilage, bone, fat, free mucosal grafts, vascularize flaps and free flaps with micro anastomosis.

Fat can be used to obliterate dead space or to plug a CSF leak.[1]

Fascia lata or fascia temporalis can be placed in an underlay technique between the dura and the skull base or overlay technique in the sinus cavities. Multilayered fascia lata was effective especially in the management of open cisterns or in cases of another type of high-pressure CSF leak.[2]

Bone or septal cartilage are placed in the underlying tech-

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nique and are used to support the larger reconstruction of the skull base.[3]

The free mucosal grafts can be used in the closure of small skull base defects with or without CSF leak.

The vascularized flaps can be used to reconstruct large defect with a high rate of success.

When loco regional vascularized flaps are not available, and we are dealing with large defects, we can use free flaps with micro anastomosis.[3]

Autologous tissue is popular because their availability, biocompatibility and does not involve a cost. We have to take into account the morbidity of the donor site and the additional time that it will be spent in the operating room. All the autologous materials can be harvest with minimal difficulty, and the harvesting process can be planned.[4,5]

One of the advantages of the autologous materials is the rapid incorporation in the surrounding tissue. Hoseman demonstrated that the free middle turbinate mucosa graft was fixed after six days and the re-epithelization had been seen microscopically after 12 days. The autologous grafts do not produce an important inflammatory response, an important quality for patients that do require follow up endoscopic or imagistic exam because unnecessary biopsies will be avoided.

Disadvantages of autologous grafts are the limited tissue availability, and the additional incision is created. The major complication is not reported, but the donor site can sometimes associate pain, minor complications and require additional healing time.

The synthesis materials use in the skull base surgery can be divided into two categories: acellular human dermis and engineered collagen products.

Acellular human dermis (Alloderm) is used in many anatomical areas in the head and neck surgery in the last ten years. Multiple layers reconstruction at the level of anterior and middle cranial fossa had proved to be effective. Studies have been shown a successful closure of the CSF leak in 90-100% of cases on the first attempt. Additional surgical harvesting time is not required. The material has to be soaked for ten minutes before use. The acellular human dermis is a low skink age material. Crusting may occur for about eight weeks. Some authors have raised the theoretical risk of transmitting prion-mediated diseases, despite the fact that the skin is not known to be a reservoir of infectious material as Creutzfeldt Jacob disease. A recent study has shown that in the USA more than two million patients had undergone reconstruction surgery using acellular dermis grafts without any case of infection.

The other category of synthesis materials are the engineered collagen products that are the result of advancement in bioengineering. Those type of products is collagen-rich sponges from the animal that were processed to remove all cells and antigens. The animal collagen matrix is chemically treated to induce a cross-linking among the collagen fibrils. The result is a porous matrix of collagen. Those products are designed to fully incorporate into native tissue using remodeling collagen technology with minimal surrounding tissue inflammatory response. Histopathologic studies have shown a fibroblast infiltration starting with four weeks after surgery a full incorporation in surrounding dura by 16 weeks after surgery.

Studies reported that the bigger the pore size it is (100 μm) the fastest incorporation we have. For matrixes with a pore size between 10-20 μm the fibroblast population started to grow after six months. In cases of small pore size (5 μm), no fibroblast migration was seen after six months from the surgical reconstruction. Severe inflation is rarely seen when using collagen-based products.

The skull base surgery also needs a rigid support in cases of important defects. Synthesis materials used for creating the rigid support are poly-methylmethacrylate, hydroxyapatite, titanium mesh or plates and polytetrafluoroethylene.

Methylmethacrylate products are easier to use comparing the hydroxyapatite products due to the faster hardening time (10 versus 30 minutes). Another important quality of the methyl-methacrylate is the ability to hardening in a wet field. Studies have shown that after using them in reconstructive surgery, both materials give a minimal inflammatory response.

Titanium mesh and plates are malleable, are easy to see on imaging exams are quite safe to use in skull base reconstruction. Esposito found migration or dislodgement of the titanium mesh in 3 from 378 patients.

The surgeon must present the risk of a skull-base during dissecting the tumor and all the related risk to the procedure and the risks of not having the surgical treatment and the alternatives to the surgical treatment.

The surgeon is obliged to present to the patient all the reconstructive alternatives techniques, materials, biological glues used in the skull-base reconstruction.

Advantages, disadvantages, surgical risks and risks that occur if the reconstruction is not performed.

Table 1 – Synthesis and natural materials used in reconstructive surgery of the skull base in period 2003-2013

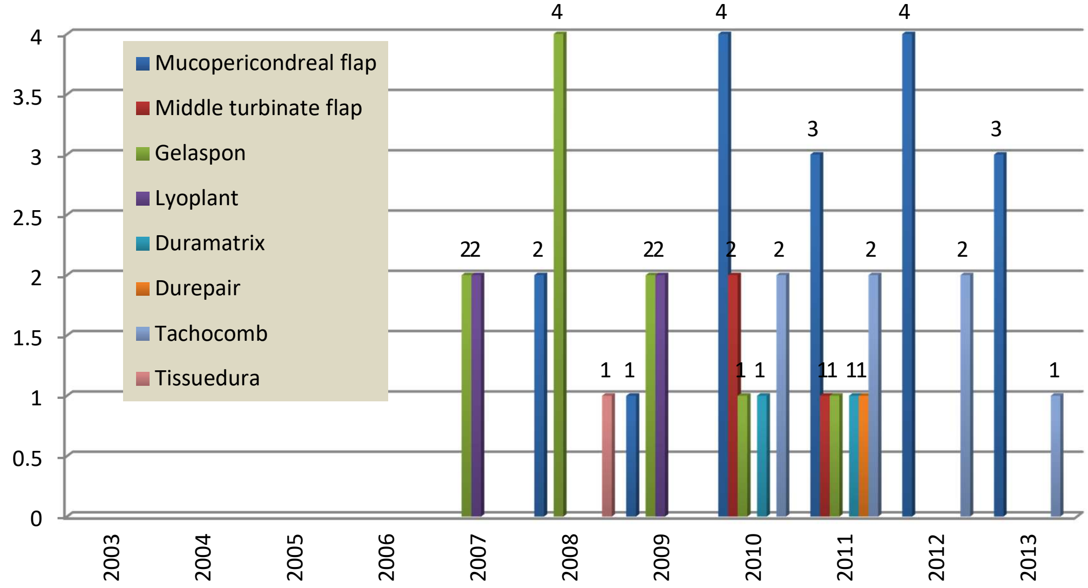

MATERIAL AND METHOD

We have used in our practice as synthesis materials collagen-based products. We have used starting with the year 2007 the following collagen products: Gelaspon, Lyoplant, Duramatrix, Durepair, Tachocomb, Tissuedura.

First, we have used those type of products to reconstruct small skull base defects with or without CSF leak in underlay and underlay technique. The important inflammatory response was not encountered. The results were good, and the primary closure of the CSF leak was 100%. The advantages of the synthesis products are the high availability, an important quantity of material availability and total integration in the host dura. The main disadvantage is the price of those products. We recommend using collagen-based materials in small skull base defects.

When we are dealing with important defects of the skull the reconstruction using solely collagen-based product is not recommended, in that cases, the collagen products have to be used with vascularized intranasal or extra nasal flaps with rigid support.

RESULTS AND DISCUSSION

The natural materials that we have used in skull base reconstruction are fascia, cartilage, bone, fat, free mucosal grafts, vascularize flaps and free flaps with micro anastomosis. The main vascularized flaps that we have used are the mucoperichondrial septal flap described by Haddad and the middle turbinate flap.

CONCLUSION

We consider that naso-septal Haddad flap type to be the best option in skull base reconstruction due to its good vascular supply and versatility. The vascular supply of that flap is the septal branch of the sphenopalatine artery. We have never encountered necrosis of the flap even after radiotherapy. In case of recurrence the flap can be reused, it can be taken down, preform ablative surgery and reposition the flap. The surgeon can cover defects up to 25 cm^2. The surgeon will have a limitation in pediatric population up to 13 years due to the development of the nasal septum that can lead to the insufficient surface of the flap. The flap can include the mucosa from the nasal fossa floor; it can be prepared bilateral or contra-lateral.

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ORIGINAL ARTICLES

Media, medical intelligence and hybrid war

Oana M. Ciobanu[1]

Abstract: *Contemporary hybrid war is a novelty in that it is taken to populated conflict zones, with the participation of the population, under the eyes of the international community. Thus, according to many authors, the most serious threats to the United States, including to its NATO dimension and to its partnership with Europe, would come from extremist groups and their ideology, fueled by poor urban areas of the world, where youth faces great economic and social problems, and is militating for a change. These unconventional actors can operate beyond state control by circumventing international borders, norms and agreements. Some of these belong to global terrorist networks, and others co-operate with criminal elements to destabilize certain areas. The hybrid confrontation with these groups will become "a clash beyond the physical elements of the conflict, in which media manipulation, the use of the internet and the integration of information operations with strategic communication programs are as important as weapon systems on the battlefield."*

Crises generated in the context of a hybrid war are events characterized by low probability and important consequences that threaten an organization for its most profound purposes. Because of their low probability, these events defy any interpretation and require a sustained effort to understand and adapt the reactions. Crises affecting social groups differ in their causes and duration. Obviously, because of the multitude of types of crises, human reason can only play a limited role in their development. However, they trigger human action, which can turn small deviations into major crises.

Crisis and conflict management involves very complex systems of political, diplomatic, social, economic, cultural, international, military and other actions.

Such a system could be structured on political, diplomatic, social, economic, informational, military, psychological and cultural components and could include the following types of action: on the causes; on effects; on the phenomenon; on value systems; on crisis-generating strongpoints (critical areas); on vulnerable sites; on the leaders.

METHODS

The analysis of open sources of information and of the experience gained during the activity in the field of communication and public relations, concretized through the elaboration and implementation of both the communication management strategies and of the projects included in the field of defense and national security.

In hybrid war, opponents (states, state-supported or independent movements) will exploit access to modern military capabilities and support insurgent, terrorist and criminal movements as well as the intervention of states that can combine high-tech military capabilities with terrorist actions and cyberwar operations against financial targets.

The analysis of available sour-

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ces suggests that the likelihood of a hybrid warfare is in direct connection with local population's satisfaction and the stability of states. Experts believe that, in failed or failing states, population security and services are often provided by dissident groups, armed factions, and criminal gangs. These areas become a fertile ground for terrorist, fundamentalist, extremist groups with regional or global influence and seriously affect the world economic system by facilitating illegal activities.

Contemporary hybrid war is a novelty in that it is taken to populated conflict zones, with the participation of the population, under the eyes of the international community. Thus, according to many authors, the most serious threats to the United States, including to its NATO dimension and to its partnership with Europe, would come from extremist groups and their ideology, fueled by poor urban areas of the world, where youth faces great economic and social problems, and is militating for a change. These unconventional actors can operate beyond state control by circumventing international borders, norms and agreements. Some of these belong to global terrorist networks, and others co-operate with criminal elements to destabilize certain areas. The hybrid confrontation with these groups will become "a clash beyond the physical elements of the conflict, in which media manipulation, the use of the internet and the integration of information operations with strategic communication programs are as important as weapon systems on the battlefield."

The issue emerging in the hybrid war resides in the fact that the enemy is trying to circumvent the military victory by focusing on long-term political victory by encouraging and maintaining tensions, crises and conflicts on a small scale, with implications at regional and sub-regional level, using the media handling concept with increasing intensity. Almost every person has come to have their own perception about manipulation, but without a complete conscious understanding of its content.

In the public debate, confusion is often made between information, adhesion, influence and manipulation.

Through the means of mass communication, the meaning of the war creates, metaphorically, a war of the meaning, of information, that conveys and / or prevents opponents from accessing information to influence their emotions, motives or goals, aiming at obtaining behaviors and actions favoring initiators.

The influencing action – whether by coercive or non-coercive strategies – has the purpose of altering the behavior of the people it is exercising upon. Regardless of the type of influence exerted on the individual, it produces its effects only through the human psyche.

In other words, any action of influence on an individual – be it propaganda, threat, promise, persuasion, intimidation, blackmail, noise, annoyance, surprise or harassment, etc. – it achieves its effects through the human psyche.

All definitions given to communication, regardless of the schools of thought to which they belong or the guidelines they enclose, present at least the following common elements: communication is the process of transmitting information, ideas, opinions, opinions from one individual to another, or from one group to another; conscious communication is an attribute of the human species; activities, events, processes and decisions involving people cannot be conceived beyond communication.

Any communication process has the following components (Zorlentan, T., 2001): Issuer; Receiver; Message; Context; Communication channels. An efficient communication process calls for both categories of channels to be considered, knowledge of how they operate, and the benefits and drawbacks that exist in their use and control [1].

Any human behavior, however, cannot be determined directly, but indirectly by actions on the elements that determine its appearance.

Misinformation consists of any intervention on the basic elements of a communicative process that deliberately modifies the messages being circulated in order to trigger in the target groups certain attitudes, reactions, actions desired by the opponent.

Vladimir Volkoff [2] defines misinformation as a "manipulation of public opinion for political purposes, using information subjected to hijacked means", as well as several ways in which it could be used: discrediting; undermining leaders' reputation; ridiculing traditions.

He also sets out twelve ways of effectively using misinformation to change the attitude of public opinion and to force it to act in the proposed direction of the manipulators: denial of deeds; reversing the facts; the mixture of truth and lies; changing the motif; changing circumstances; blurring; camouflage; interpretation; generalization; illustra-tion; unequal parts; equal parts. [3]

Disinformation as a weapon carefully conceals its origin and camouflages its intention of transforming the target into a manipulated object, refusing its right to specific traits and behaviors.

"Manipulation is the action of determining a social actor (person, group, community) to think and act in a way that is compatible with the interests of the initiator, instead of his interests, by using persuasion techniques that deliberately distort the truth, leaving the impression of freedom of thought and decision (...) The true intentions of the person who conveys the message remain insidious to the recipient. From a political point of view, manipulation is a form of imposing the interests of this class, group, community, not by means of coercion, power, but by ideological ones, by deception." [4]

In defining the notion of manipulation, it is necessary to specify a few specific elements [5]:

- The dissemination of a large amount of detailed information that cannot be verified while also concealing the real aspects of a case;

- The obsessive repetition of information with a negative trend, to be fixed in the memory of the listeners;

- <embargo> on the real information;

- Exploiting the fact that people are hungry with rumors.

New information technologies often separate the information as such from the identity of the person who initiated the news, and thus from the particular social context that would give it a certain credibility and a certain meaning. From this point of view, the power is directed towards credible communicators, towards those who can also offer a frame in an understandable context, easily recognizable by public opinion.

The context in which the news is located is very important – the reputation and credibility of the communicator matters. Type/brand becomes extremely important as a result. If 50 years ago, the battle was to gain control over the means of transmitting information, today the battle is being done to build/destroy credibility. The illusion of the need for information to be provided "readily interpreted" has been created, that is why the credibility of the source is a guarantee of a good interpretation. Global transparency, driven by new media opportunities and low-priced technologies, is now setting the stage for media outreach.

Thus, it is necessary to communicate very clearly to the target which will be its personal benefit by adopting a certain behavior or attitude, instead of the national, pan-national or regional benefit it will receive. The promised benefit in the future for that person must be personalized.

As for the brand image (it can be, for example, the US one), particular attention should be paid to the five basic dimensions of a brand: presence; relevance; differentiation; credibility; image and target. [6,7]

Crises are events characterized by low probability and important consequences that threaten an organization in its most profound purposes. Because of their low probability, these events defy any interpretation and require a sustained effort to understand and adapt reactions.

As a consequence, we can state that the crisis is an acute phenomenon which, left uncontrolled, can become chronical – that is, it can degenerate into conflict, therefore the dynamics of the decision-making process is particularly complex and it is carried out in most situations under uncertainty and stress.

Analyzing the phenomenon of crisis, we express the opinion that any crisis of any nature, has certain characteristics, such as: Threat; Expedite; Surprise; Time pressure; increased uncertainty and intensity.

Crisis and conflict management involves very complex systems of political, diplomatic, social, economic, cultural, international, military and other actions.

Such a system could be structured on political, diplomatic, social, economic, informational, military, psychological and cultural components and could include the following types of action: on the causes; on effects; on the phenomenon; on value systems; on crisis-generating strongpoints (critical areas); on vulnerable sites; on the leaders

Case Study: Ebola, Romania, 2015 [8]

The hypothesis of biological warfare, using the hemorrhagic fever virus, Ebola, as a weapon, was brought to the attention of the specialized public by military intelligence analysts, but also by the medical academics.

Although insignificant in size and proportions, the media crisis caused by the presence of Ebola virus in Romania raised concern at the level of state structures actively involved in the early detection, isolation and treatment of these patients, which led to the development of an action and communication strategy, following the premises mentioned in this article, which has also been concretized through an evaluation mission of the European Union experts.

Thus, during March 19-21, 2015, at the invitation of Dr. Raed Arafat, State Secretary and Head of the Emergency Situations Department of the Ministry of Internal Affairs, an expert team led by ECDC visited Romania. The main objectives of the visit were to provide specialized support to public institutions with tasks and responsibilities in the field of health, in order to review and verify the effectiveness of operational emergency intervention procedures, to verify the mechanisms of national inter-institutional cooperation in the context of management, under maximum safety and security conditions, of the cases imported by Ebola.

The main finding of the report was that Romania took the threat of Ebola seriously and initiated and established a wide range of measures as soon as possible, being able to ensure the rapid detection of infected persons, transport and specialized medical care, under conditions of maximum security for the staff involved in this procedural chain. Immediately after the World Health Organization Statement of October 2014 disclosing the threat to the Ebola virus, which was categorized as an emergency of international public health, the National Emergency Situation Committee of Romania created a national network, dedicated to managing this threat.

The role of the Ebola National Committee is to coordinate the interventions and measures needed to prevent and combat Ebola virus infections. It is chaired by the Head of the Department for Emergency Situations (Secretary of State within the Ministry of Internal Affairs), Vice-President - Secretary of State within the Ministry of Health.

In the Committee there are governmental decision making actors, namely within the Ministry of Health, the Ministry of Internal Affairs (Department for Emergency Situations), the Ministry of Foreign Affairs, the Ministry of National Defense, the Ministry of Education and Research, the Ministry of Transport, as well as key institutions, subordinated to these ministries, such as the Public Health Institute, the National Institute of Infectious Diseases "Matei Balş" and the Central Military Hospital "Dr. Carol Davila".

Throughout this period, the Ebola National Committee has worked intensively to develop a coherent national strategy for preparing for the safe identification and management of possible cases of Ebola and has consequently adopted seven decisions in this area, covering various issues related to early detection, triage, management and prevention of future spread of the Ebola hemorrhagic fever virus, as well as the creation of a dedicated budget to support this activity.

According to the Report, public health activity at the local level is carried out at the level of 42 public health authorities (VATA), and centralized data are provided to the National Public Health Institute (NPHI), which works under the aegis of the Ministry of Health. The Institute has five branches in the main cities:

Timişoara, Iaşi, Cluj-Napoca, Târgu-Mureş and Sibiu, and monitors all epidemiological situations with potential for national and international impact, along with other relevant institutions, being involved in activities aimed at increasing the degree of awareness. The site of the institute is the most complete and complex source of information, updated for both professionals and the general public. Although not mentioned in the Ebola National Committee's decisions, the NPHI also has the role of conducting and implementing population training campaigns, while maintaining institutional dialogue with regional public health authorities (VATA) as regards the evolution of relevant epidemiological issues, as well as contributing to the training and professional development of public health specialists.

In addition, CNAS and the Ministry of Health provide the early warning and response system to threats to national and, implicitly, European health security, assuming its responsibilities and commitments to WHO to address issues related to the implementation of the International Health Regulation (IHR).

At European level, key actors from other sectors of activity (e.g.: international affairs, civil protection, etc.) have been actively involved in discussions and in the start of inter-sector collaboration protocols that have taken place. In addition, a number of EU/EEA Member States have extensively participated in international efforts to counter the African epidemic by providing expertise, logistical and financial support.

Although for many countries the danger has passed, some European countries may still be at risk of exposure to the Ebola threat, given the migration phenomenon of residents from countries on the African continent, especially those in the West, severely affected by this disease.

The EU Health Security Committee continues to work to ensure effective communication at Member State level, in particular as regards the development and establishment of a joint action plan of addressing this challenge, further enhancing the dimension of the concept of preventing the spread of the disease by examining cross-sectoral and cross-border issues, as well as by checking capacities and capabilities for responding to biological incidents and accidents with extremely violent public health impact.

The lessons learned by Romania in this context have brought back a number of issues on the public agenda, such as the fragility of the healthcare and public health infrastructure; the high exposure to infection, given that our country is a gateway to Europe for the African population.

National vulnerabilities: lack of specialized medical personnel (in 2007 out of a total of 45 000 doctors, only 16 000 remained in Romania, others preferring other Member States, the phenomenon of labor migration is acutely felt in the intensive care, epidemiology and infectious diseases departments), the management of bio-waste, the lack of joint training drills in case of biological danger, the possible difficulties in the organization of medical evacuation - at this moment in Romania, there is no generic preparedness plan for a rapid response to eradication of outbreaks of infectious diseases, although it would be relatively easy to establish one as a result of the Pandemic Influenza Preparedness Plan used during 2009-2010-, the blocking of public procurement for advanced products/devices to support activities in the medical field as well as research-innovation and health system development.

An important aspect is also the need to ensure the functionality of a public health lab, of vital importance both for the national preparedness to respond to the threat of infectious diseases and for mandatory reporting at EU level through the ECDC. Not even today did the analyzes carried out by the Ion Cantacuzino National Research and Development Institute for Microbiology and Immunology surpass the threat of the uncertainty of activity financing, a key component of the public health system, which entails a whole chain of vulnerabilities that converge significantly to creating a state of health insecurity.

CONCLUSIONS

That is why medical intelligence, defined in Joint Pub 1-02 as "a type of information products resulting from the collection, evaluation, analysis and interpretation of medical, biological and scientific data as well as from the climate-related data, represent a special category, essential to national strategic planning, whether we are talking about the military or civilian environment, the planning of the fighting force conservation operations or the performance of medical capabilities assessments of rapid intervention."

"The role of medical intelligence structures is often confused with that of preventive medicine. Although the two disciplines have no common ground, they have become complementary over time, even in a relationship of interdependence.

While preventive medicine attempts to identify those events that constitute medical threats to subsequently assess the risks posed by exposure to such threats, while ensuring at the same time that decision-makers are advised on the adoption of preventive strategies, the medical intelligence is the component which ensures the permanent upgrading of the prevention process by identifying new types of non-conventional medical risks and threats, described in the published literature, in the possession of potential aggressors, depending on the changes in the security climate and the geostrategic map of influence (state actors or terrorist militarized formations), assesses new trends and medical technologies, especially in the dual-use industry." [9]

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CLINICAL PRACTICE

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Oncological follow-up after radical prostatectomy

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Abstract: *Radical prostatectomy is the gold standard treatment for patients with localized prostatic cancer, but often the pathological diagnosis reveals a locally advanced stage.*

The aim of this paper is to present the oncological follow-up after radical prostatectomy on a group of 42 patients, patients which have been diagnosed after surgery with a more aggressive stage, locally advanced PCa. Over a period of four years 136 patients have undergone radical prostatectomy. The pathological examination has established that 42 patients presented extracapsular invasion and/or lymph node invasion. 18 patients (pT3-4N0M0) have undergone radiotherapy alone and 24 patients (8 pN1 and 16 pT3-4N1M0) have received androgen deprivation therapy in association with radiotherapy. Over the next two years 15 patients presented biochemical recurrence. For these patients chemotherapy was initiated, but in two cases PSA increase has been observed during periodic evaluation. Although it is not uncommon that postoperatively the histopathological examination establishes a more aggressive disease stage than that established before surgery, radical prostatectomy remains the solution for the localized prostate cancer, solution that can be boosted by radiotherapy and/or androgen deprivation therapy.

Keywords: *prostate cancer, oncological follow-up, complications*

INTRODUCTION

Prostate cancer is one of the most frequent, if not the most frequent neoplasic pathology encountered in men with ages over 60 years, its incidence increasing with age. The steady growth of life expectancy, due to a healthier life-style that more and more people tend to adopt, will automatically lead in the fallowing decades to a higher rate of prostate cancer detection [1-3].

Since its introduction, more than 20 years ago, prostate specific antigen (PSA) screening has significantly increased the number of patients diagnosed with localized prostate cancer.

The discovery of this marker at the beginning of the 1970s by Dr. Ablin and his colleagues has radically changed, in a good manner, the prognostic and the lives of the patients who suffer from this disease, but it took almost two decades to approve its use as a screening marker.

Before the FDA approval and introduction of the PSA as a tool to evaluate the treatment response and disease recurrence in 1986 and later in 1994 as a screening test for prostate cancer, the patients were usually diagnosed in advanced stages, with metastatic disease, associating a poor prognostic, less than 5% of the patients being curable [4].

Currently, due to early detection of prostate cancer,

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the curability rate of this pathology has significantly improved, the patients being diagnosed more frequently with lower disease stages, thanks to PSA screening. Radical prostatectomy among other therapeutical options (radiotherapy, brachytherapy) offers good disease control and even good cure rates, with low rates of local recurrence.

Radical prostatectomy is the most frequent therapeutical option used in the management of patients diagnosed with localized prostate cancer and with a life expectancy that is estimated to be higher than 10 years, being considered the gold standard treatment for this stage of disease [5]. Often, despite the fact that the preoperative evaluation establishes a localized stage, the postoperative pathological examination reveals a more advanced stage of prostate cancer.

The purpose of this article is to present the oncological follow-up in a group of patients diagnosed after radical prostatectomy with a locally advanced prostate cancer stage, after initially establishing a localized stage during the preoperative evaluation.

MATERIAL AND METHODS

We have retrospectively analyzed the experience of our clinic regarding open radical prostatectomy for localized prostate cancer and we have encountered that over a period of four years, from January 2013 to January 2016, we have performed 136 radical prostatectomies. Only the patients diagnosed during the preoperative evaluation with localized prostate cancer have been included in this study.

We have analyzed the PSA value, the result of the pathological examination after prostatic biopsy, pre- and postoperatory staging and Gleason score and as well as the short and long term oncological results and complications.

The preoperative diagnostic of prostate cancer was established by transrectal ultrasound prostate biopsy, this procedure being done in patients who presented repeated PSA values above the upper limit of the PSA reference interval or for the patients who presented a clinical suspicion of prostate cancer during digital rectal examination. After the pathological examination confirmed the cancer diagnostic all the patients have undergone pelvic contrast MRI and bone scan.

The prostatectomy was performed 6 to 8 weeks after the transrectal biopsy and 12 weeks after transurethral prostatic resection (TUR-P), for the patients diagnosed with prostate cancer after the pathological examination.

The postoperative evaluation was done at one month after the surgery and afterwards periodically at three months in order to evaluate the PSA level, the urinary continence status and erectile function. Biochemical recurrence was defined as two consecutive increases of the PSA level over 0.2 ng/mL.

RESULTS

The mean age of the patients included in the study was 63.09 years, varying between 51 and 78 years. The number of the prostatic tissue fragments harvested during prostatic biopsy puncture ranged between 6 and 12, being higher in saturation biopsy (24 to 32 fragments).

The mean operative time was 130 minutes, ranging between 100 and 300 minutes. Intraoperative bleeding has decreased over time, due to increased surgical experience, from 1500mL (in the first prostatectomies) to less than 200 mL in more recent surgeries. No major complications have occurred during the procedure.

As early postoperative complications we report macroscopic hematuria in less than 10% of the cases, wound infection (4%), prolonged lymphatic drainage in 8% of the cases and anastomotic fistula in 3 patients. Regarding the late postoperative complications (one year after the surgery) we have encountered: urinary incontinence, erectile dysfunction and lower urinary tract symptoms (LUTS).

The degree of urinary incontinence was evaluated periodically at one month after the surgery and afterwards at 3, 6 and 12 months using the International Continence Society male questionnaire. The degree of urinary continence was established by the number of urinary pads used during 24 hours. Out of 136 patients who have undergone radical prostatectomy only 3 patients presented total urinary incontinence, during the postoperative follow-up, 38 patients being partially continent and 95 patients being completely continent. Complete urinary continence was defined as the absence of the need for urinary pads during 24 hours and as well as the absence of urinary leakage during daily activities.

Erectile function was evaluated using the IIEF- 5 questionnaire. It was noticed that 78% of the patients for whom we have practiced bilateral nerve sparing surgery and 30% of the patients with unilateral nerve sparing presented satisfactory erections. For the patients with erectile dysfunction we have recommended phosphodiesterase 5 inhibitors.

9% of the patients presented moderate or severe LUTS, due to urethral stricture or due to bladder neck sclerosis. These postoperative complications were managed endoscopically.

42 patients presented after the pathological examination extracapsular invasion and/ or lymph node invasion. Therefore these patients presented in fact locally advanced prostate cancer and not the localized stage that was established during the preoperative evaluation. 18 patients out of 42 with extracapsular invasion (pT3-4N0M0) have undergone radiotherapy alone and 24 patients with lymph node invasion (8 patients with pN1 and 16 patients with both extracapsular and lymph node invasion) have undergone radiotherapy and androgen deprivation therapy (complete androgen blockade for two years).

15 patients presented successive increases of the PSA value at more than 2 years after treatment initiation, cases for which chemotherapy was recommended. 2 patients presented castration-resistance prostate cancer. Their therapeutical management consisted in bilateral orchiectomy and anti-androgen therapy. Two patients out 15 were resistant to the chemotherapy.

DISCUSSIONS

PSA detection has proven over the years to be a vital, cheap and reliable investigation for the detection of prostate cancer. Early PSA screening has led to a significant increase of the rate of patients diagnosed with localized prostate cancer, nowadays approximately 90% of this patients being curable [6].

Several studies have compared over the years the efficacy of radical prostatectomy and radiotherapy in patients with localized prostate cancer and have concluded that both therapeutical options provide good results regarding the recurrence free survival, cancer specific survival, overall survival and biochemical recurrence, especially for the low risk and intermediate risk patients [7-10]. In a study comparing the efficacy of radical prostatectomy versus intensity-modulated radiotherapy, Aizer concluded that for the high risk patients the radiotherapy approach provided better results when compared to the radical prostatectomy group. No significant differences were found between these two types of treatment for the low risk and intermediate risk patients [11].

Schreiber et all reported a better biochemical control for the patients with high risk localized prostate cancer for whom radiotherapy was the treatment of choice when compared to the radical prostatectomy group after a follow-up period of 6 years (67.4% RT vs 41.3% RRP). Regarding the low and intermediate risk groups he reported no significant differences between radiotherapy and radical prostatectomy, despite the fact that in both risk groups radiotherapy presented initially better results. The rates of biochemical control after 6 years for low risk and intermediate risk were 90.3% and 82.6% without any salvage therapy, while for the radical prostatectomy group the rates were 85.6% and 59.7% without salvage radiotherapy and 90.3% and 74% with salvage radiotherapy [7]. Petrelli reported in a 2015 meta-analysis, a better overall and cancer specific survival in high risk patients treated with radical prostatectomy when compared to radiotherapy [12].

In a 2015 study regarding the comparative outcomes between radical retropubic prostatectomy, high dose rate brachytherapy (HDRBT), cryoablation and high-intensity focused ultrasound (HIFU) for localized prostate cancer it was concluded that HDRBT presented the worst outcomes (cancer control, biochemical recurrence, salvage treatment free rate and metastases free rate) when compared to the alternatives [13].

CONCLUSIONS

Radical retropubic prostatectomy is the gold standard in the treatment of localized prostate cancer and in some cases, in the locally advanced prostate cancer.

Radical prostatectomy provides a significant improvement of the survival rate, for the cases where the surgical indication and technique have been correctly established and carried out.

Although it is not uncommon that postoperatively the histopathological examination establishes a more aggressive disease stage than that established before surgery, radical prostatectomy remains the a solution for the localized prostate cancer, solution that can be boosted by radiotherapy and/or androgen deprivation therapy.

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CLINICAL PRACTICE

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Diagnostic difficulties in c-kit negative gastrointestinal stromal tumors: report of four cases

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Abstract: *Introduction: The gastrointestinal stromal tumors (GIST) are dominated by KIT and PDGFRA mutation. The immunohistochemical detection of CD117, a protein express by KIT gene, is essential for the diagnosis and those that are negative always represented a diagnostic challenge*

Case reports: In this article we present a series of 4 cases of CD117 negative GIST tumors, diagnosed and surgically resected in Fundeni Clinical Institute and an overview of the histogenesis, diagnostic problems and management of c-kit negative GIST. All patients were males and the tumors were located in the stomach and small bowel.

Conclusion: It is important for the pathologists to beware of the fact that a CD117 negative in the context of a typical morphological appearance does not exclude a GIST tumor and also the oncologist must be aware not to exclude the therapy with imatinib based on the negativity of CD117.

Keywords: *GIST, gastrointestinal stromal tumors, c-kit negative, CD117, DOG1, immune-histochemistry*

INTRODUCTION

GISTs are rare tumors accounting for less than 1% of all gastrointestinal tumors but they are the most common mesenchymal tumors of the GI tract.[1] The incidence in Europe and USA is 7-10 cases/1,000,000 [2] and in Korea is 16-22/1,000,000 [3]. Large population based studies from Iceland, Netherlands and Sweden found the incidence to be 11, 12.7 and 14.5 cases/1,000,000 [4,5,6]. However there are studies that explain this increased incidence by a better diagnosis using modern diagnosis criteria rather than a real increase [7].

The most common age of the diagnosis is between 50 to 60 years old (a mean age of 65) and a peak incidence between 70 and 79, before 20 years old being quite rare ≈ 1% [7,8]

GIST affects both sexes equally [6] though some authors found them to be more common in men [7,9]. They arise most often in the stomach (50-60%) and small intestine (30-35%) but it can occur in any segment of the gastrointestinal tract only with a lower frequency.[10]

Gain of function mutation in KIT or PDGFRA oncogenes lead to ligand-independent kinase activation [11] which gives rise to the majority of GIST. Immunohistochemistry is useful for diagnosis and

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typically detects c-kit protein expression, but if the tumor is negative for c-kit, a final diagnosis can be challenging.

In this article we present a series of 4 cases of c-kit negative GIST tumors, diagnosed and surgically resected in Fundeni Clinical Institute and an overview of the histogenesis, diagnostic problems and management of c-kit negative GIST.

CASE REPORTS

In the pathology archives of Fundeni Clinical Institute, between 2004 and 2017 we have traced four cases of c-kit negative GIST. We have examined available clinical data from the hospital database and also HE slides. Immunohistochemically investigations were performed with biotin-streptavidin method [12] in order to assess the phenotype and provide a definitive diagnosis.

Antibody suppliers were Novocastra, and Labvision/Thermo Fisher Scientific as previously reported [13] and the dilutions respected the manufacturer recommendation. The stratification risk according to Miettinen&Lasota (2006) was used [2]. For the mitotic rate we counted the number of mitosis on 50 high power fields (HPF). Tumor cellularity was divided in three categories: spindle, epithelioid and mixed.

Case no. 1

A 47 years old male who presented with lower gastrointestinal bleeding, was investigated with capsule-endoscopy procedure and found with a tumor on the ileum, close to the ileocecal valve. A segmental enterectomy was performed. The surgical specimen was 12 cm long and presented centrally a well circumscribed white nodule of 4/3/4 cm, expanded in the submucosa and muscularis propria with mucosal ulceration. Histologic examination revealed a tumor consisting of spindle cells with a mitotic rate of 1-2/50 hpf. Immunohistochemical stains showed negativity for CD34, CD117, PDGFRA, Desmin, a weak positivity for SMA and a diffuse cytoplasmic positivity for DOG1. The prognostic group was 2, and 7 years later the patient is alive with no recurrent disease.

Figures case 1

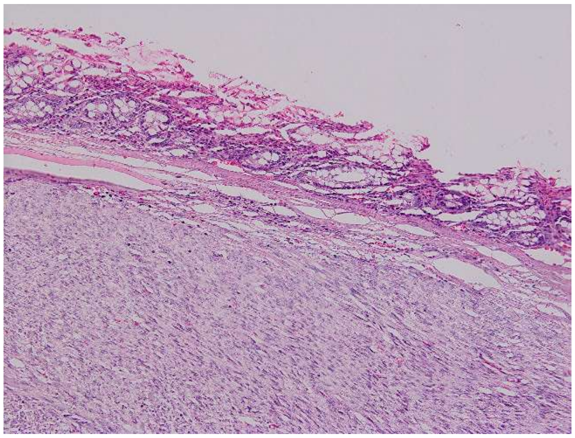

HEx100

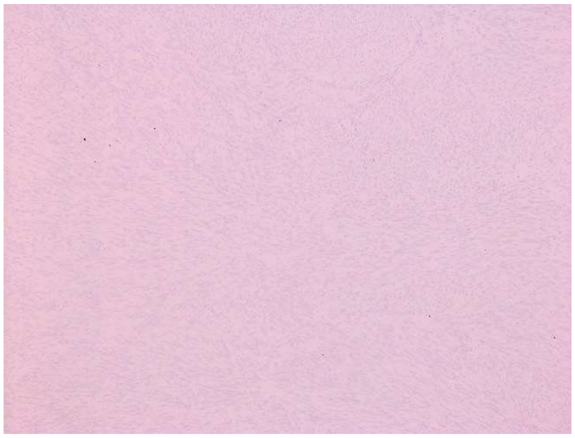

CD117x100

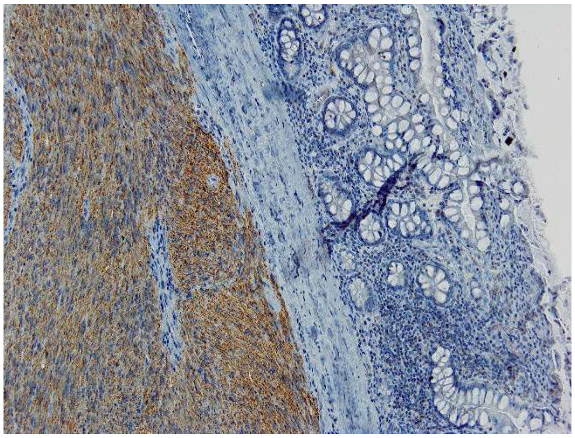

DOG1x100

Case no. 2

A 69 years old male presented with abdominal pain, nausea and vomiting for 12 hours before admission. The abdominal CT scan revealed a pelvic tumoral mass on the left paramedian side, developed behind the abdominal wall that involved a small intestinal loop. A segmental enterectomy was performed. The surgical specimen was 30 cm long and presented a solid white tumor with irregular contour 8.5/3.5/2cm with predominantly intramural and subserosal development and focal mucosal ulceration.

Microscopic examination reveal a mixt morphology with spindle and epithelioid cells, with mild pleomorphism, intratumoral hemorrhagic foci and a mitotic rate of 2/50 HPF.

Immunohistochemical stains were negative for SMA, Desmin and CD117 and positive for DOG1. CD34 and PDGFRA were not done.

Figures case 2

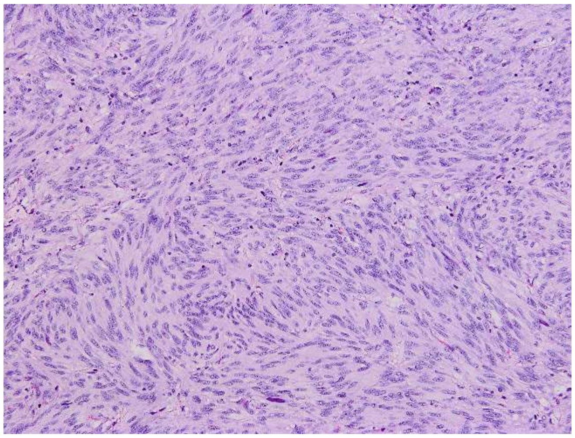

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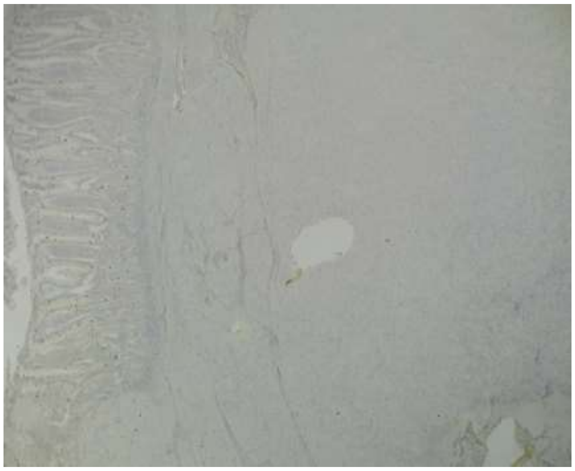

CD117x100

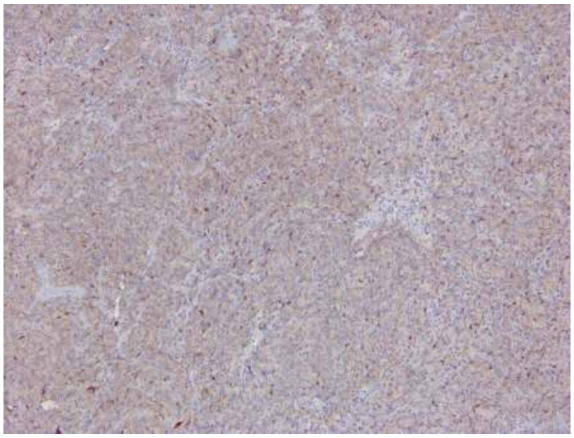

DOG1x100

On the bases of these results the tumor was diagnosed as a c-kit negative GIST with moderate risk (group 3a) and survival from the time of the diagnosis until death was 33 months.

Case no. 3

A 67 years old male was admitted to the hospital with melena, fatigue and weight loss. An esophagogastroduodenoscopy was performed, revealing a 1.5 cm lesion in the gastric fornix.

The tumor biopsy showed a stromal gastric hemorrhagic proliferation associated with a chronic gastritis with Helicobacter Pylori infection.

A local excision was carried out and on the surgical specimen was found a firm rubbery white intramural nodule with a diameter of 3/3/3 cm.

Microscopy revealed a tumor proliferation with spindle cell morphology, without mucosal involvement, with 1-2 mitotic figure/50HPF.

Immunohistochemistry showed negativity for CD117, Actin, Desmin and positivity for DOG1. CD34 and PDGFRA were not done.

The diagnosis of c-kit negative GIST with low risk (group 2) was established and the patient is alive with no recurrence.

Figures case 3

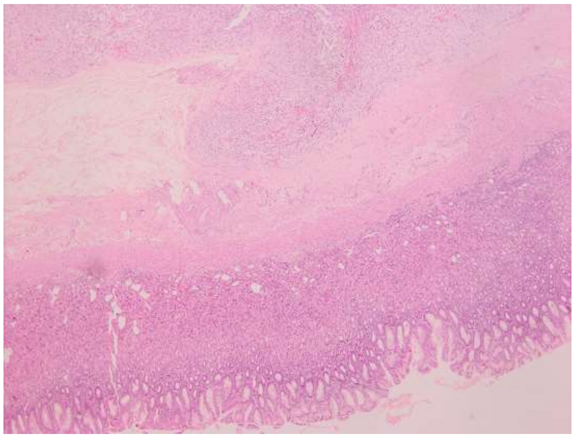

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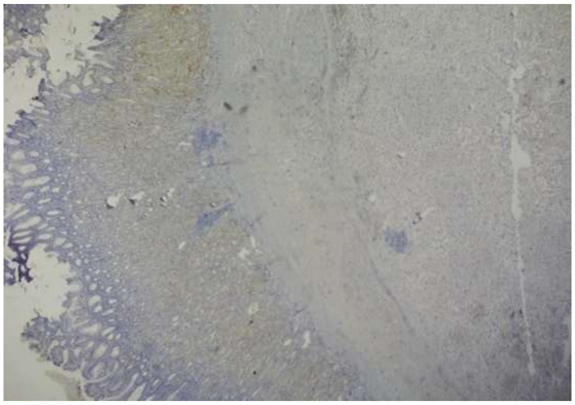

CD117x100

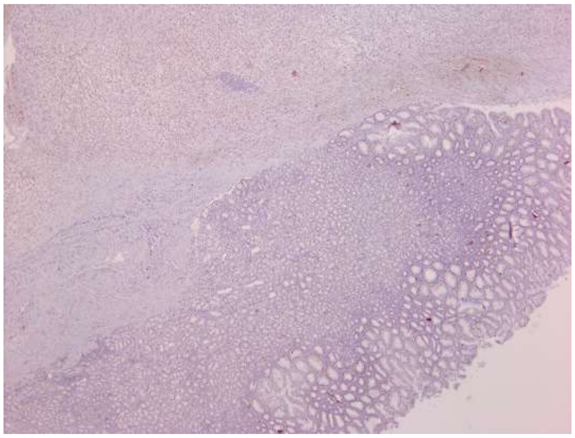

DOG1x100

Case no. 4

59 years old male with loss of appetite, abdominal pain, weight loss, is admitted to the hospital for investigations.

Esophagogastroduodenoscopy revealed an ulcerated tumoral proliferation on the body of the stomach and an ultrasound discovered multiple localized proliferations in left and right hepatic lobes.

A total gastrectomy was performed and the surgical specimen showed a12 cm white grey tumor with intramural development and cystic degeneration. On the microscopic examination it showed an epithelioid and spindle cell morphology with fascicles that intersect and intertwine at various angles, with more than 10 mitotic figures/50 hpf Immunohistochemistry showed negativity for CD117 and Desmin, and positivity for SMA and DOG1.

A diagnosis of GIST with high risk of recurrence (group 6b) was established and the patient survived time from the initial diagnosis was for 9 months.

Figures case 4

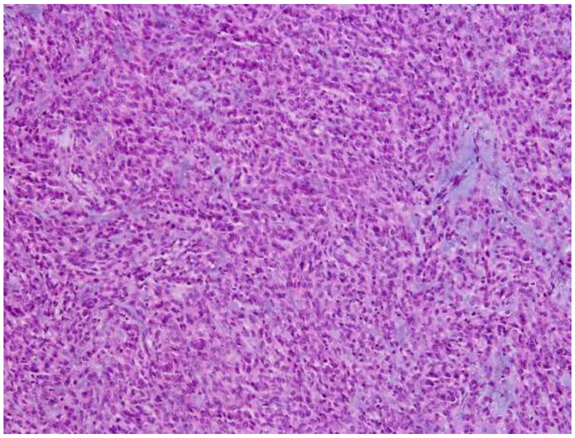

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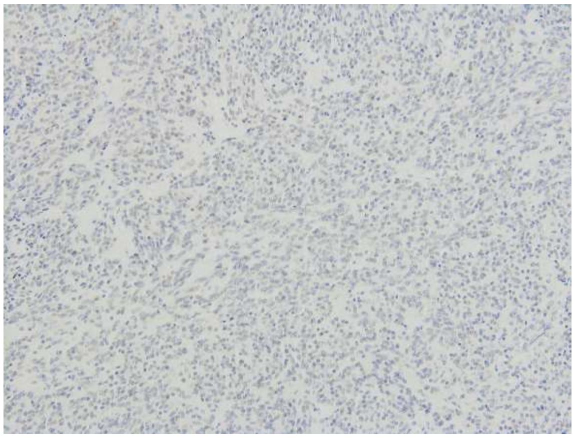

CD117x100

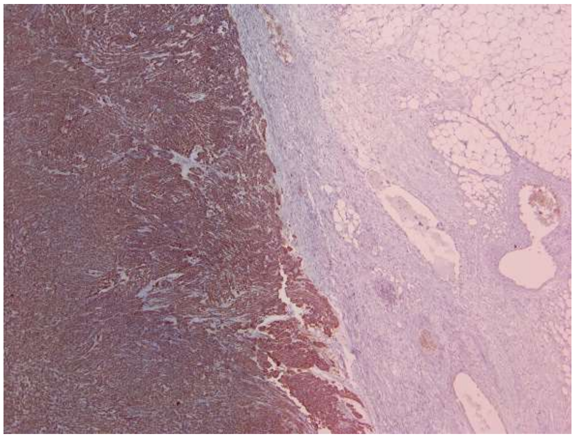

DOG1x100

DISSCUSSION

Arising in the interstitial cell of Cajal, GIST tumors are an entity dominated by mutations in KIT receptor tyrosine kinase which accounts for almost 80% of all cases. The histopathological diagnosis of such tumors is built upon the microscopic morphology and immunohistochemically stains.

The detection of CD117, a protein express by KIT gene, is essential for the diagnosis and we can find it in approximately 95% of GIST [8, 14].

Table 1: Clinical-pathological characteristics of the four cases presented

Case no.	Age	Location	Metastasis	Tumor diameter	Mitosis/ HPF	Cell type	Group	Death	Survival months	Resection
1	47	SI	no	4.0	<5	fusiform	2	no	84	R0
2	69	SI	peritoneum lung	8.5	<5	mixt	3a	yes	33	R1
3	67	S	no	3.0	<5	fusiform	2	no	135	R0
4	60	S	liver peritoneum	12.0	>5	mixt	6b	yes	9	R1

Table 2

	CD117	DOG1	Desmine	SMA
Clone Dilution	T595 1:40	K9 1:100	DE-R-11 1:100	αsm-1 1:50
Case no.1	negative	positive	negative	positive
Case no.2	Negative	positive	negative	negative
Case no.3	Negative	positive	negative	positive
Case no.4	negative	positive	negative	positive

However, there are 4-5% of GISTs that are negative for CD117 and in these cases immunomarkers like DOG1 (ANO1) are very useful for the confirmation of the diagnosis [9,15,16].

DOG1 shows higher prevalence of positivity in gastric epithelioid GISTs, which are often KIT negative.[17]

Depending of the antibody used, the positivity of DOG1 in KIT-negative GISTs varies: with clone DOG1.1, the positivity is 36% [18] and with clone K9, the positivity is 50–76%. [17,19]. We also used K9 clone in our cases, which we found very helpful.

Other immunohistochemical markers used, that can guide the diagnosis are CD34 (with a much higher positivity in the stomach-80% then in the small intestine-35%) together with SMA Desmin, Vimentin. CD34 was done only in one of our cases, a small intestine GIST and it was negative so the only reliable marker was DOG1.

Most of the GISTs are reported in the stomach (50-60%), jejunum and ileum (30-35%), duodenum (5%), colorectal (4%), and rarely in the esophagus and appendix (<1%) [10, 20]. Primary tumors outside the GI tract have been reported in small numbers in omentum [21,22], mesenteries and retroperitoneum [23,24]. The location of c-kit negative GIST is more frequently reported in the stomach [25, 26, 27] and less in the small intestine [28,29]. Two of our cases were located in the stomach but the other two located in the small intestine gave rise to diagnostic suspicion so we had to pay more attention at the differential diagnosis due to the rarity of the tumor site.

Location and size of the tumor influence the apparition of symptoms. Small tumors are usually silent and are being discovered incidentally during investigations or surgical procedures for other disease However if a GIST becomes symptomatic, it usually cause non-specific symptoms like weight loss, fatigue, nausea, bowel obstruction or overt or occult gastrointestinal bleeding due to mucosal ulceration or tumor rupture[30,9]. Two of our patients presented with nonspecific symptoms like abdominal pain, nausea, fatigue, weight loss, and the other two with bleeding signs. Non-specific symptoms delays the diagnosis and we can see from Table 1 that those two cases have also the largest diameter (8.5 cm in the small intestine and 12 cm in the stomach).

The prognosis and predictive factors for survival of patients with C-kit negative GISTs are still unclear and difficult to assess because of insufficient data. The tumor diameter, metastatic disease, incomplete resection is associated in our cases with a shorter survival rate (Table1).

DOG1 especially clone K9 is an invaluable marker of the diagnosis but mutational status comes in hand in the treatment management. Complete resection and inhibitors of tyrosine kinase are considered for the treatment as in c-kit positive GIST [31].

However when we encounter a c-kit negative GIST we need to take into consideration:
- The antibody that we use, the clone and the laboratory technique
- The fixation methods of the surgical specimen may influence the reaction
- The CD117 negativity does not exclude a GIST diagnosis and other markers like DOG1 are necessary to complete the immunohistochemical diagnostic panel; we also must think at GIST with other mutations like PDGFRA
- For a possible treatment with inhibitors of tyrosine kinase (imatinib) we must have in mind mutation analysis as well
- GIST wild type is a constantly changing concept, at first considered a KIT negative GIST, now is described as GIST with no identified gain of function mutation. Besides the KIT gene, GISTs may present mutations in another receptor tyrosine kinase - the PDGFRA which accounts for less than 10% of all GISTS [32].

According to most studies, activating mutations in KIT or PDGFRA are present in 85 - 90% and are mutually exclusive [33]. Other driver mutations studied, like BRAF, RAS, PIC3K, SDHA, NF1 need to have other genetic event in order to develop and progress [34,33, 35,36]

CONCLUSIONS

Kit negative GIST is a problematic entity which makes the diagnostic difficult.

In conclusion, we report four cases of CD117 negative, DOG1 positive GIST located in the stomach and small intestine, all being male patients.

It is important for the pathologists to beware of the fact that a CD117 negative in the context of a typical morphological appearance does not exclude a GIST tumor and also the oncologist must be aware not to exclude the therapy with imatinib based on the negativity of CD117.

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Conflict of interests

The authors declare no conflict of interests.

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CLINICAL PRACTICE

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The role of calcium hydroxide in the treatment of chronic apical periodontitis in patients with type 2 diabetes – a case report

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Abstract: *From an anatomical perspective, the dental pulp is a connective tissue with a specific microcirculatory system with repairing abilities in order to preserve the vitality of the pulp15. Different therapeutic methods in treating the affected dental pulp can be compromised by different factors and can lead us to failure. Due to disorders emerged in the microcirculatory system, the treatment of the affected dental pulpin patients with type 2 diabetes is an additional challenge. Type 2 diabetes can impact different functions of the patients' immune system, with a predisposion to chronic inflammation, progressive tissue degradation and reduced tissue repairing15. Regarding the endodontic therapy of the affected dental pulp in patients with diabetes mellitus (DM), an important role in the treatment of pulpal complications is played by the calcium hydroxide which is considered „the golden standard" because of its excellent properties, the very alkaline pH being one of the most important.*

Keywords: *diabetes mellitus, chronic apical periodontitis, calcium hydroxide*

INTRODUCTION

Diabetes mellitus is one of the most frequent metabolic disorders affecting 366 million patients of all ages, according to the statistical data of the year 2011 [26,1, 21]. It is a disease that is chronic, complex, progressive and crippling and that has no current treatment, diabetes being characterized by a partial or total deficiency of insulin production [5].

Patients with diabetes mellitus have a tendency to develop oral complications such as: cavities [5,27, 22], pulpal and periapical pathology [5,10,14,23] and, very often, periodontal disease [2,10,4,14,12,16,20, 1,7], which is considered the sixth complication of DM and the most chronic affection [20,1].

Many materials have been used in the endodontic treatment but unfortunately none of them managed to succeed in satisfying the complete requirements of an ideal sealer [11,19].

The pure calcium hydroxide paste has an increased pH value of approximately 12.5-12.8 [9] and it is chemically

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classified as a strong base. Its main actions are accomplished through the dissociation of Ca^{2+} and OH^- ions affecting the vital tissues, inducing the deposition of hard dental tissue and possessing antibacterial properties [24].

$Ca(OH)_2$ is an antimicrobial agent with a bactericidal effect, and by using it in the course of the endodontic treatment prevents the apical periodontium from becoming infected [25]. Moreso, it is being used to reduce inflammation (thus decreasing the pain), helping eliminating the apical exudate in order to control the root resorption and to prevent the contamination of the apical periodontium between treatment sessions [9].

MATERIALS AND METHOD

Patients diagnosed with type 2 diabetes have participated in this study. During the radiologic exam we have ascertained that these patients presented radiologic modifications of the periapical tissues with obvious signs of root and bone resorption.

These patients have been informed about the treatment they were about to receive and have signed an informed consent agreement to participate in our study.

Inclusion criteria for participation in the study: 1. patients over 18 years old diagnosed with type 2 diabetes; 2. patients with mature teeth that presented an apical radio translucency at the radiologic exam.

All patients have been submitted to an orthograde endodontic treatment. The teeth have profited by an intermediary session when a paste with calcium hydroxide and radio opacifying agent has been applied in the root canals.

For every patient we have used dam isolation – a diga. The endodontic therapy has been conducted using rotary instrumentation, the crown-down technique and by successively applying endodontic irrigating solutions such as NaOCl 5.25% and citric acid 40%.

Between the therapy sessions the root canals were filled with a calcium hydroxide based paste and for the obturation of the tooth crown we have used glass ionomer cement.

The calcium hydroxide based paste was introduced using a Lentulo spiral filler and condensed using paper cones as pluggers. In the next therapy session the $Ca(OH)_2$ paste is removed using alternative irrigation with NaOCl 5.25% and citric acid 40%,using ultrasonic activation, NaOCl 5.25% and EDTA 17% and manual instrumentation for the working length.

An intermediate radiography was conducted before the obturation of the root canals with gutta-percha and the warm compaction technique.

RESULTS

Clinical case no. 1

The patient M.C. presents a chronic apical periodontitis in 1.7, following an incomplete endodontic treatment (Figure 1).

The patient had several recurrent acute episodes that induced a right odontogenic sinusitis that has been diagnosed some years ago.

Following the last otorhinolaryngology checkup the patient was sent for an oral and dental evaluation and also for a radical sinus surgery.

We have opted for a non-surgical conservative endodontic treatment (orthograde approach) finalized in the second therapy session.

Figure 1: Initial radiological aspect of 1.7

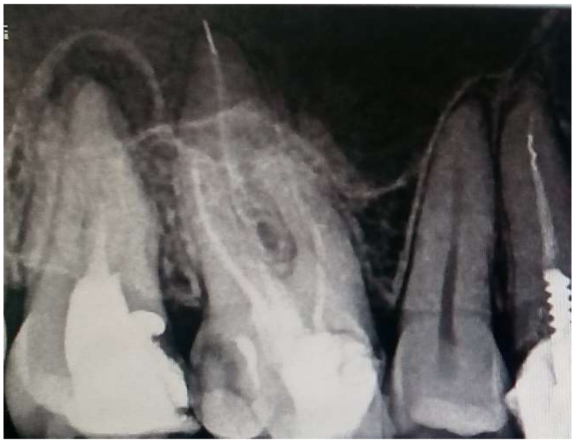

As an intermediate root obturation we have used a calcium hydroxide based paste and an opacifying agent (Figure 2).

Figure 2: The radiological aspect of 1.7after root obturation with $Ca(OH)_2$ as intermediary therapy (after 4 months).

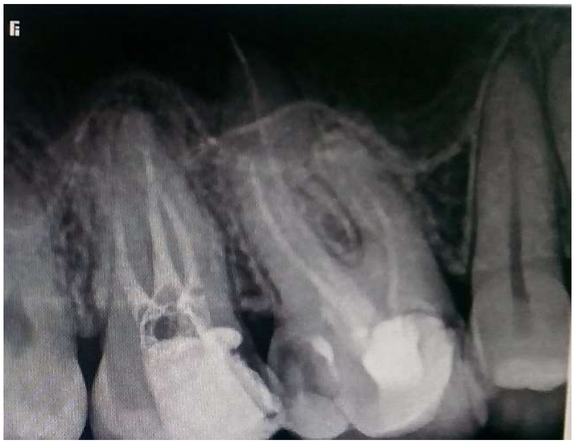

Figure 3: The radiological aspect of 1.7. The gutta-percha root canal obturation.

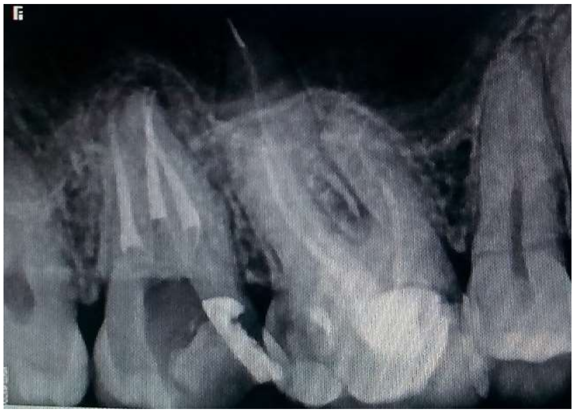

The apical radio translucency has decreased significantly and the patient's clinical status has improved (Figure 3). The subjective signs of sinusitis have progressively amended.

Clinical case no. 2

The patient V.N. came in our clinic for periapical bone lesions in 3.6 following an apical resection procedure after endodontic treatment (Figure 4).

We made the decision to conduct a non-surgical conservative treatment (orthograde approach) finalized in 2 treatment sessions.

For the intermediate root canal obturation we have used $Ca(OH)_2$ paste and opacifying agent for a better radiographyic imaging (Figure 5).

Figure 4: The initial radiological aspect of 3.6

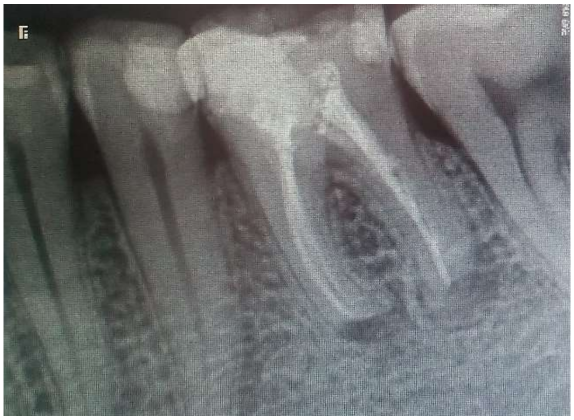

Figure 5: The radiological aspect of 3.6 with its roots canals intermediately obturated with $Ca(OH)_2$ (after 3months)

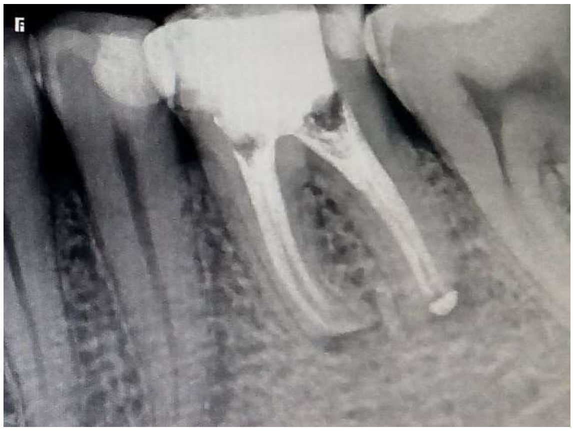

Figure 6: The radiological aspect of 3.6. Verifying the root canal obturation with gutta-percha.

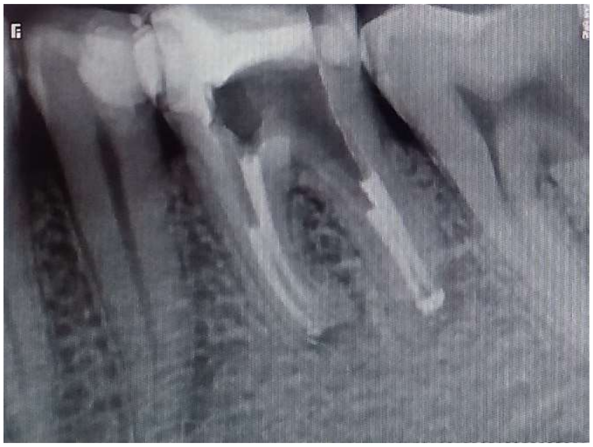

After the decrease of the apical translucency, 3.6's roots were permanently obturated using the warm vertical compaction technique (Figure 6).

DISCUSSION

The success criteria in endodontic treatment were established in 1994 by the European Society of Endodontics and include the absence of pain, inflammation and fistula, maintaining the dental function, the presence of radiological evidence to show a proper periodontal ligament space and the absence of apical periodontitis and root resorption [8].

The endodontic treatment conducted was non-surgical and conservative using the orthograde approach under Diga isolation and benefiting abundant irrigation with endodontic irrigation solutions.

We have varied the time in which the calcium hydroxide paste was maintained in the root canal accordingly.

In both cases we can observe the initiation of the periradicular bone remineralization process.

The ceasing of the resorption of the external radicular necrotic pulp can be exclusively attributed to the elimination of the necrotic tissue and to the conducted antibacterial intracanalar treatment [18].

The diffusion of the OH-ions through the dentin depends upon the duration of the medication, upon the diameter of the dentinal tubules (cervical vs apical) and upon the ablation of the layer smear (for an increased diameter of the dentinal tubules). Moreso, the diffusion of the OH-ions into the root resorption areas where the pH is acid has a positive effect on the progression of the root's inflammation and resorption [17].

The $Ca(OH)_2$ based paste can be used successfully for the management of perforations, horizontal radicular fractures and radicular resorptions [17].

Gergely JM. et al. have shown that calcium hydroxide is frequently recommended in the treatment of periapical abscess because of its strong alkaline pH (approximately 12). The antibacterial effect is due to the dissociation of $Ca(OH)_2$ into OH^- and Ca^{2+}, that also has a healing effect on the damaged tissues [13, 6].

The endodontic treatment in patients with diabetes and infections of the radicular canal can be correlated with a decrease of the treatment's success and therefore the dispensarization of these patients is mandatory [3].

CONCLUSIONS

In case of patients with recurrent treatments it is advisable to perform a non-surgical conservative treatment.

Regarding the evaluation of the success of the examined endodontic treatments we have concluded that the success rate in type 2 diabetes patients is high only when the treatment is thoroughly conducted.

We cannot say that the type 2 diabetes patients have an increased predisposition for developing periradicular lesions because the endodontic treatment in these patients was incorrectly conducted because of incomplete root canal treatment.

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Yolk sac tumor in a 20 years old patient

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Abstract: *Yolk sac tumor or endodermal sinus tumor is a rare malignant ovarian germ cell tumor (is a borderline tumor) diagnosed in young females up to 25 years old. Histopathological it resembles the mesenchyme of the primitive yolk sac. Microscopically it presents the following triad: Schiller-Duval bodies, reticular aspect and PAS+ hyaline droplets (α-fetoprotein).*

The diagnosis of Yolk sac tumor is made by dosing serum α-fetoprotein, ultrasound and MRI – DWI imaging.

It requires surgical treatment, followed by chemotherapy (new therapies – platin or carboplatin, etoposide, bleomycin) [1]. Survival prognosis at 5 years is of 80% for stage I.

Differential diagnosis is with Brenner tumor, ovarian clear cell carcinoma, dysgerminoma, malignant teratomas, androblastoma, dermoid cyst.

Keywords: *Yolk sac tumor, AFP, MRI, DWI, abdominal-pelvic pain*

INTRODUCTION

Yolk sac tumor (endodermal sinus tumor) is an ovarian germ-like, extremely rare, (borderline) malignant tumor, which occurs very often at ages < 25 years. Schiller-Duval glomeruloid bodies are specific [2]. They are composed of a papilla with a conjunctive-vascular axis, delimited by round cells; the papilla predominates within a cyst delimited by embryonic tumor cells.

CASE PRESENTATION

The 20 years old patient, R.S., from rural area, is admitted within our service for abdominal-pelvic pain, predominately on the right flank, with onset of approximately 7 days and with progressive intensification. The patient is nulliparous, nuligest, with irregular menstrual cycles, of normal quantity. From her pathological personal history, we retain appendectomy in 2012.

The general objective examination upon admission reveals a patient of good general state, afebrile, of normoponderal condition. The local objective exam reveals a slightly enlarged volume of the abdomen, mobile with breathing, painful spontaneously and upon palpation, predominantly in the right flank, with no abdominal guarding; nulliparous cervix, in axis, closed external cervical orifice, without macroscopic modifications, uterus in anteversoflexion position, of normal consistency and volume, left adnexal area slightly sensible upon palpation, right adnexal area modified by presence of a tumor with an approx. 20 cm diameter. There is no liquid in the Pouch of Douglas.

Biological tests, EKG, pulmonary radioscopy do not

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show pathological changes.

Transvaginal ultrasound shows uterus in anteversoflexion, with diameters of 45/38/42 mm, smooth contour, homogeneous myometrium, linear cavity, endometrium of 3 mm. Voluminous abdominal-pelvic mass extended cranial-caudally on approximately 19 cm and axially on 18 cm, transonic, multi-septated, including hypo-/hyperechoic heterogeneous confluent areas, especially in the caudal portion. There is contact with the uterine wall, without demarcation line with the adnexal areas. Thin layer of fluid in the Pouch of Douglas.

The high-performance imaging examination – MRI with diffusion weighted images (DWI) shows:

- The mass presents areas of restricted diffusion, with hyper-intense aspect on the ADC map (Figure 1), suggestive of benign aspect.

Figure 1: ADC map

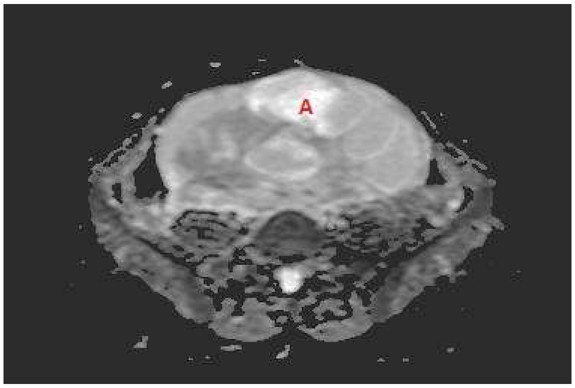

The mass is well delimitated, with predominantly hyper-signal T2-weighted, presents small areas of restricted diffusion (A), with hypo intense aspect on the ADC map (B), suggestive of "malignant foci" within a larger tumor.

- Small areas of restricted diffusion can be appreciated on the DWI image (Figure 2).

The native and contrast-enhanced abdominal MRI examination shows voluminous cystic multiloculated tumoral mass, with abdominal-pelvic development, of 21.5/18.3/7.9 cm maximum diameters, with adnexa as starting point, connecting both adnexa, including multiple solid areas, with gadolinium uptake and which is developed superiorly up to the inferior renal third, manifesting mass effect on the adjacent structures, to which it maintains the interface of demarcation.

Figure 2: DWI measurement

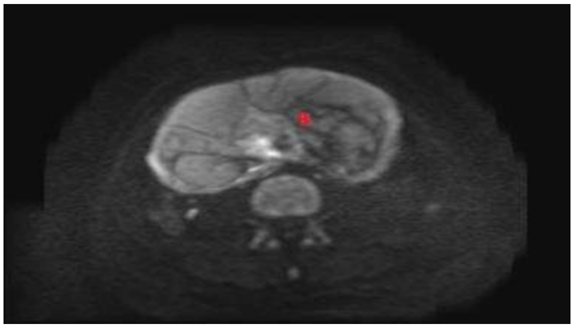

Uterus of normal size, net, regulated contours and homogeneous structure. There is no sign of abdominal-pelvic adenopathies. Minimum perihepatic ascites and in the paracolic gutters. Liver, cholecyst, spleen, kidneys, pancreas with morphology and MRI signal within normal limits.

Figure 3: T2-weighted image in sagittal plane. Voluminous abdominal-pelvic mass. Sagittal T2-weighted image with predominantly liquid signal, multiseptate, including solid areas.

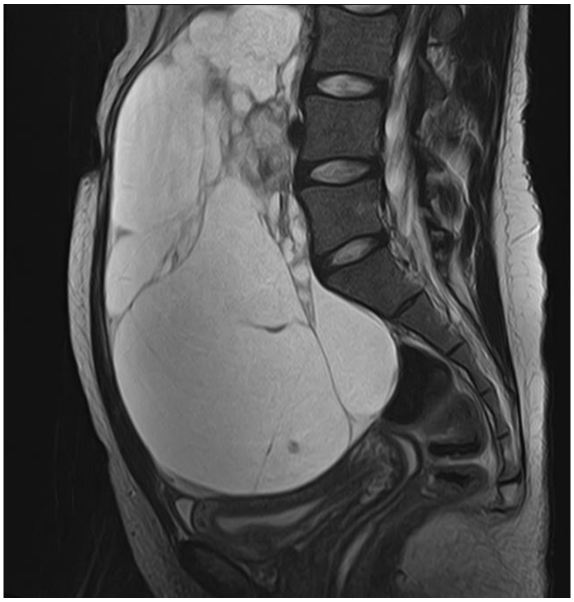

After a proper pre-surgical preparation, surgery is performed under general anesthesia, with orotracheal intubation. Upon exploration serosanguineous fluid of approximately 300 ml is found, which is collected and sent for HP examination. Right adnexa with tumoral transformation, of approximately 20/19 cm, of mixed consistency, with multiple septa, presents on its surface two continuity solutions at capsule level, by which serous, citrine fluid is exteriorized.

Figure 4: T1 FS weighted images – MRI; T1 FS weighted images following intravenous administration of gadolinium contrast shows parietal enhancement also at the level of internal septa.

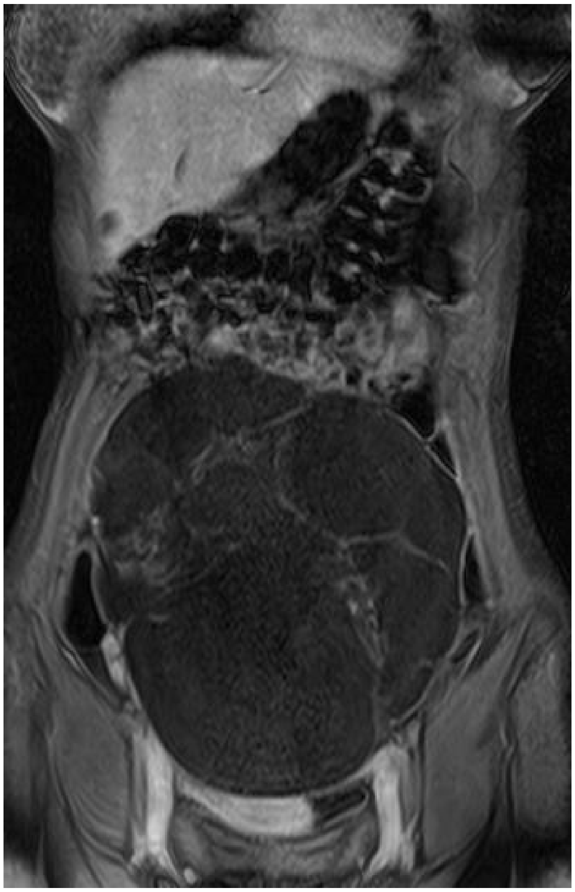

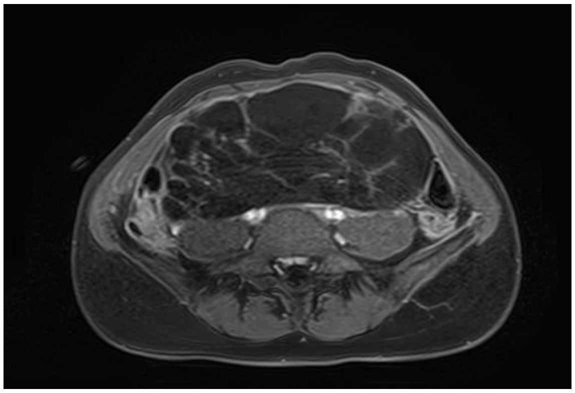

Left ovary with tumoral transformation, by presence of a 2/5 cm cyst, of serous content. Uterus of normal macroscopic aspect. Right adnexectomy, left cystectomy are performed, as well as hemostasis control, drainage, and parietoraphy in anatomical layers. The evolution following the surgery was favorable under antibiotic, antalgic, anticoagulant treatment, with discharge on the fourth day following the surgery.

Figure 5: Right adnexal tumor of approximately 20/19 cm, of mixed consistency, with multiple septa, presents on its surface two solutions of continuity at capsule level.

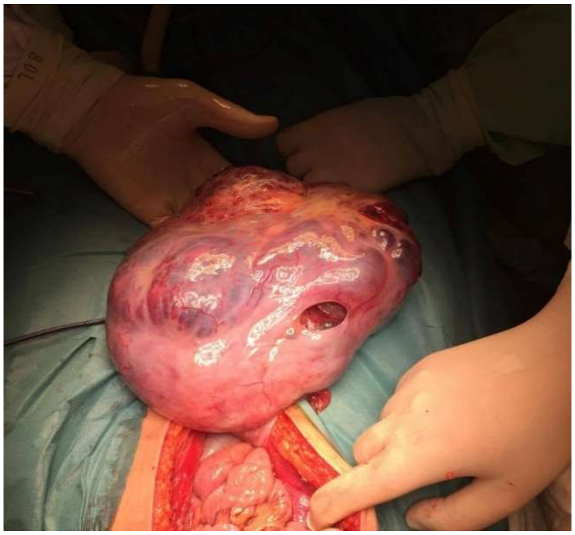

Figure 6: Intraoperative image following right adnexectomy and left cystectomy

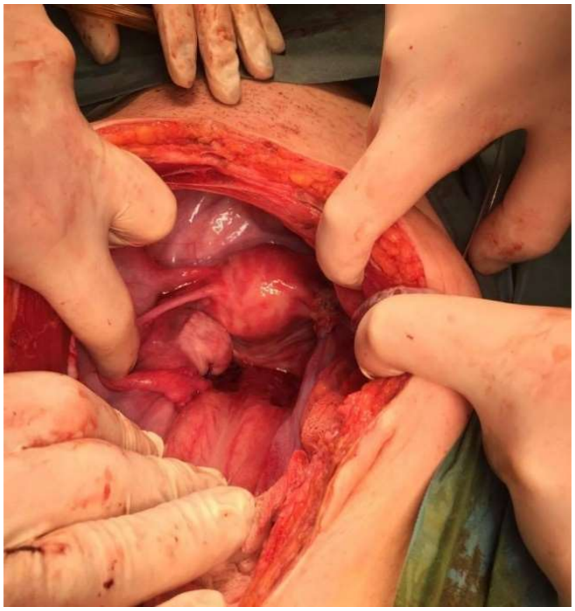

The histopathological examination of the macroscopic piece shows a solid mass of 20/18/6 cm, with cystic areas, necrotic and hemorrhagic areas. The microscopic examination on the piece shows the following: tumoral mass consisting in linear channels of atypical epithelial cells, islands with clear cells and aspect of Schiller-Duval bodies, myxoid hypocellular stroma and areas of tumoral necrosis; aspects suggestive of Yolk tumor; salpinges without tumoral infiltrates.

Immunohistochemical study is recommended for confirmation and differential diagnosis with ovarian clear cell carcinoma. Upon immunohistochemical study the diagnosis was of Yolk sac tumor.

DISCUSSIONS

Yolk sac tumor is also known as endodermal sinus tumor and represents an ovarian germ-like tumor. It is an extremely rare malignant tumor that appears predominantly in the second decade of life. Yolk sac tumor may occasionally arise from a pre-existing dermoid cyst. An increased value of AFP can be relevant for this diagnosis. Symptomatology is contoured by intense abdominal-pelvic pain.

Bilateral presence of this tumor is extremely rare. It is described as a pelvic mass that can also be extended abdominally, is complex and contains both solid and cystic portions. Cystic areas are often composed of epithelial cysts produced by the tumor or by coexisting mature teratomas.

Ultrasound in Yolk sac tumor describes echoic and hypoechoic areas.

Magnetic resonance imaging demonstrate on DWI sequences, presents small areas of restricted diffusion, with hypo-intense aspect on the ADC map, suggestive of "malignant foci" within a larger tumor [4]

Yolk sac tumor increases quite rapidly in size, the symptomatology being especially described in intense abdominal-pelvic pain. Lymphatic dissemination may occur early in some cases.

The treatment is surgical, followed by chemotherapy (platinum or carboplatin, bleomycin, etoposide)..

CONCLUSIONS

There are difficulties in the diagnosis and surgical staging of ovarian tumors of low malignant potential. AFP utility together with ultrasound examination and magnetic resonance imaging with DWI sequence helps in reaching an accurate diagnosis in Yolk sac tumoral masses.

Being a borderline tumor, a prompt diagnosis represents a success in the long-term prognosis of the disease. Transabdominal surgical approach is of choice in endodermal sinus tumors.

The success of subsequent therapy is largely determined by the initial surgical procedure. Surgical treatment is the therapeutic method of first choice, which can lead to healing in the early stages I-a and I-b and which decisively influences the results of complementary treatments, chemo- and radio-therapy.

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In memoriam – Brigadier General Mihai Nicolae Bînă

Dan Mischianu[1]

Now, in February 2017 as I write this, I find it appropriate to revive the dormant memory of the reader to remember about Prof. Mihai Nicolae Bina, MD.

The future General, Physician and Professor of Urology Mihai Nicolae Bina was born on April 2, 1942 in the city of Craiova and passed away on the day of Saturday, March 3, 2012, when he still had certainly much to say and decide in our profession.

He did not like to remember and to celebrate his birthday, because, unfortunately, he had lived in his youth, as a medical student, a memorable drama: on the day of April 2, on the same ward of the Central Military Hospital, in the same clinical unit which, over the years, he will lead with professionalism, skill and dedication, his mother died – the distinguished professor Olimpia Bina. I remember that sometimes on the eve of that day, he avoided to enter in that reserve.

His father, General Professor Nicholas Bina, was a distinguished professor at the Military Academy ("Carol I" National Defense University, new style).

He came, therefore, from a family with an application for the study and the profession of arms so that, from the outset, the stars lined up to become an excellent professional of military medicine.

After graduating "Dimitrie Cantemir" high school from Bucharest, in 1959 he became a student of the Faculty of Medicine of the Medical and Pharmaceutical Institute in Bucharest and also the "pupil" of the Medico-Military Institute (this was the denomination for those who do not know). He once told me with sorrow and frustration as he went, in boots, from the Military Medical Institute Street near North railway Station to the college. There were incomprehensible times...

He completed his university studies, he graduated first of his generation in 1965.

His professional excursus was, from the outset placed under the best possible circumstances and almost obvious under today's ARU slogan: "Labor omnia vincit improbus" – Virgil, Georgice.

He was "extern" by competition (1963-1964), "intern" by competition (1964-1967), a brief period of teaching assistants at the Military School in Focsani, then secondary physician urologist, managed with the highest scores in the country.

[1] Carol Davila University Central Emergency Military Hospital, Bucharest

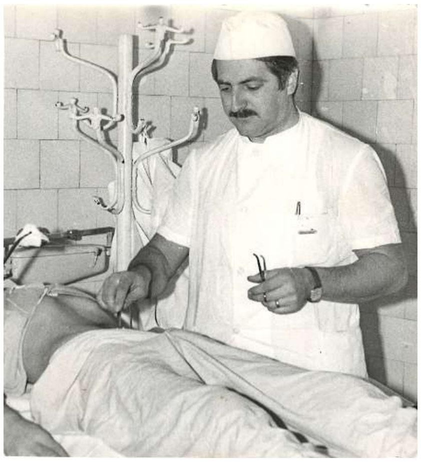

I got to make a confession.

Military doctors came, then and now from the same "strain" – the School of General Professor Carol Davila. After the Second World War, given the times and their understanding and approach their respective professional elite, felt, certainly some frustration in relation to their "civil" academic peers.

This "embarrassment" was not shared by the elite of the Romanian military medicine: Generals dr. Ion Atanasiu, Scarlat Longhin, Iuliu Şuteu, Gheorghe Niculescu, Traian Oancea, Academicians Victor Voicu and Ioanel Sinescu and many others, which through perfect and fair relationships with civilian colleagues have proven their worth and have integrated in the academic medical landscape of Bucharest as an unshakable symbiotic part. Future General Professor dr. Mihai Nicolae Bina had the same status!

Dr. Mihai Nicolae Bina became a specialist urologist (1971-1979) and consultant urologist in 1979 in one of the last competitions of its kind conducted in Communist Romania, because, then things have broken down altogether.

In fact, until 1991 the contest for "primary physician"/ consultant (new style!) was no longer conducted.

After a brilliant internship in general surgery in which he learned much and already mastered this "type" of specialty he confessed with some nostalgia the "impact" it had on him the first "seen" prostatectomy in Fundeni Hospital which was performed by his first urologic "mentor" – Professor Dr. Gheorghe Olanescu. Particularly bleeding, not followed by rigorous hemostasis as in abdominal surgery, continued by placing a hemostatic mesh, he seemed quite marked after three decades by this motivational experience.

Professor Bina was, and has been throughout his terrestrial existence, a great fighter.

And to dispel the "embarrassment" that we mentioned earlier he elaborated under the leadership of Professor dr. Docent Valentin Neagu – incomparable personality of Romanian urology (2017 is the 100th anniversary of his birth: who knows this?) his hard worked PhD thesis, entitled 'Readjustment of the upper urinary tract after dezobstruction of the bladder neck in benign prostate hyperplasia" – held in 1976.

In 1979 he became consultant urologist.

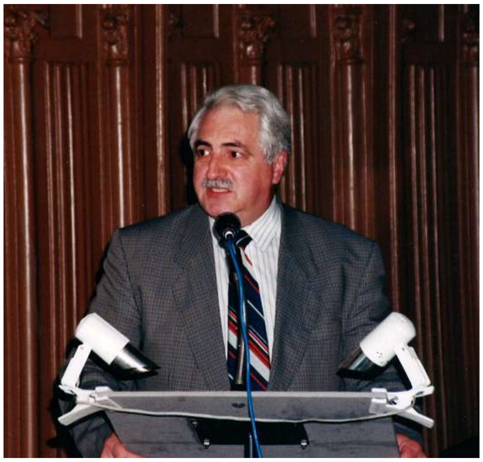

Meanwhile, in an absolutely dumb decade, he had carried out studies of “Emergencies in Urology" and "Urologic Oncology" (1979), under the mentorship of the Academician Eugen Proca and an International Course for young military doctors (Macolin, Switzerland), which he told me about often.

Post-revolutionary era found him prepared for future missions. He was the head physician of Clinical Urology Department of the Central Military Hospital between December 1990 and November 2004.

I was a doctor on duty on the day of November 30, 1991 when, he arrived from the Ministry of Defense, and he told me that on December 1, 1994 will be raised to the rank of Brigade General. I kissed him on both cheeks and I was proud that urology in this regard has its continuity.

This is a unique moment in the life of a man – after a lifetime of work and struggle.

This memory can certainly be connected to those written by the great writer Marin Preda, in a novel, once: "What has meaning for a civilian, for a soldier has a totally different one!"

In 1994 he accomplished his universitary dream: he became Senior Lecturer and later, in 2000, Professor of Urology of the Department of Urology, Faculty of Specialization and Training, Medico-Military Institute, University of Medicine and Pharmacy "Carol Davila" Bucharest.

Since 1995 he was a member of the Academy of Medical Sciences.

As I wrote five years ago in an ever undesirable "In Memoriam": "Hollow data fail to surprise and to outline the man that was Mihai Bina".

At Professor Mihai Bina I think everyone who knew him admired his sharp observation spirit, his gentleness, surgical talent, intelligence and diligence in all that which he has undertaken in earthly life.

Without Professor Mihai Bina, without his delicacy when advising or criticizing, without his professional experience and humanity, life looks very sad!...

To me, I confess, the work effort and way of thinking of Professor Mihai Bina was an example and an impulse.

ADMINISTRATIVE ISSUES

Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine.* Please read the complete Author Guidelines carefully prior to submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

ETHICAL CONSIDERATIONS

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) Accessible to the public at no charge;

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(3) Open to all prospective registrants free of charge or at minimal cost;

(4) Validates registered information;

(5) Identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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Committee on Publication Ethics

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All articles, with the exception of Editorials, must contain an abstract of no more than 250 words. Abstracts for original articles should be formatted into subheadings, as detailed below. Titles must not be longer than 120 characters (including spaces).

Editorials

These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

Review Articles

RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published reviews are in response to editorial invitation, including thematically related "mini-series" of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication', as presented at http://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

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Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

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The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

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Acknowledgments

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As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

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Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

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Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

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Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

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If the above items are not included in the cover letter, manuscripts cannot be sent for review.

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Two Word-files need to be included upon submission: A title page file and a main text file that includes all parts of the text in the sequence indicated in the section 'Parts of the manuscript', including tables and figure legends but excluding figures which should be supplied separately.

The main text file should be prepared using Microsoft Word, doubled-spaced.

The top, bottom and side margins should be 30 mm. All pages should be numbered consecutively in the top right-hand corner, beginning with the first page of the main text file.

Each figure should be supplied as a separate file, with the figure number incorporated in the file name. For submission, low-resolution figures saved as .jpg or .bmp files should be uploaded, for ease of transmission during the review process. Upon acceptance of the article, high-resolution figures (at least 300 d.p.i.) saved as .eps or .tif files will be required.

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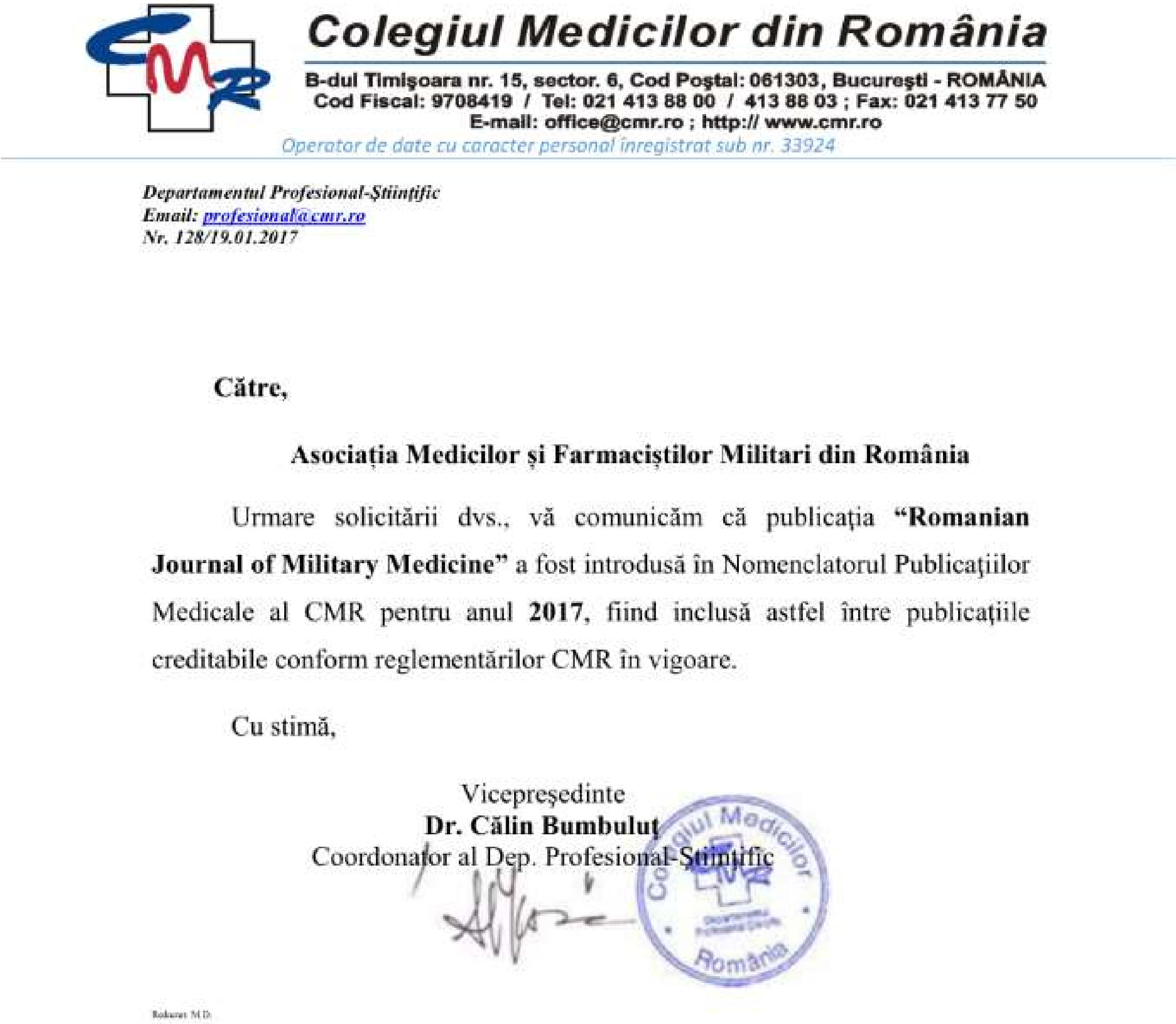

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EDITORIAL

The 7th Edition of Carol Davila Central University Emergency Military Hospital National Conference

Florentina Ioniță Radu

Dear partners, dear colleagues and friends,

We approach the starting line of the national, scientifically marathon known as National Conference of the "Dr. Carol Davila" University Emergency Central Military Hospital, event that succeded this year, with the support of everyone involved, to celebrate its 7th Edition.

Since its beginings, from the draft stage of the event, we envisioned it to be flexible but coherent, strong as institutional message, but sensitive and representative of the concerns, the mission and values of military medicine. That is why, now, we can talk about a solid form of cooperation and dialogue between experts from both military and civilian medical domain, and our partners in academical and universitary world.

Alongside traditional collaborators, we intend this year to organize an event able to illustrate, in a definitory way, current standards defining the performance of our teams, but also the goals that we want to meet, on short and medium term. Unlike other scientific events, SUUMC National Conference brings together, first of all, the colleagues from National Defence Medical Corps, but also those from similar institutions pertaining the National Security System.

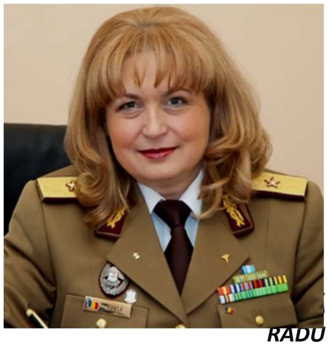

RADU
Commander, Central Emergency Military Hospital, Ministry of National Defence, Romania
Associate Professor, Titu Maiorescu University, Bucharest

If last year's addressed theme has brought rise to a series of medical events, supported by the presentation of some recent diagnostic and therapeutic approaches from the high quality patient care perspective, this year's Conference aims to come up with a blast new amplification and substantiation of new concepts applied in modern medicine, the concepts that will be debated in the framework of round tables, courses, workshops, interactive sessions and through broadcasts live.

As in every year, we are pleased to have with us as speakers, thought leaders from both Romanian and medicine from abroad, but also younger colleagues, who managed to impose, through their expertise, in the field of academic and scientific pursuits.

I wouldn't want to finish before I express the hope that all those listed above, but also relational history of us are strong arguments that will determine you to model your program, so can honor the invitation to participate in this suite of scientific events, in order to create together a new point of reference for Romanian medicine.

OP session infectious diseases, endocrinology

Superbugs: clinical, epidemiological and therapeutical peculiarities

B. Circiumaru

Background: The antibiotic resistance became a global crisis, as the "superbugs", resistant to nearly all current available anti-infectious treatments spread worldwide, causing a serious burden of morbidity and mortality.

Is there anything we could do considering the actual situation and could we improve that by any means?

Materials and methods: I ambispectivelly studied the multi-drug resistant infections in many hospitals in Bucharest, and I characterized the clinical evolution, the epidemiological patterns, and the therapy provided.

I consulted the actual literature: the treaties, the guidelines, as well as the quality internet resources and I tried to synthetized them.

Results: I described two forms of clinical evolution: unimodal-induced by out-hospital infections, and bimodal: characteristic for nosocomial infections.

I emphasized the role of epidemiological tracking, and I discussed the therapeutically challenges, as there still are undefined optimal anti-infectious strategies. Finally, I suggested recommendations to improve the case management.

Conclusions: The nosocomial infections are an unwanted reality, as the superbug's presence is getting worse; thus, some actions are useful.

Identifying those infections on: clinical, epidemiological and biological (e.g. antibiograms) grounds, the spreading of good medical practices as: routinely culture the patients admitted in the ICU`s, the epidemiological tracking to characterize the bacterial strains, and providing anti-infectious therapy according to the guidelines but, based on the clinical judgement, are the main measures that would improve the current situation.

Autoimmune diseases in women and infertility-short review and algorithm proposal

A.E. Ranetti, Anca P. Cucu

The reproductive axis is susceptible to various factors in both sexes, the immune-mediated processes interfering at various levels. Although immune infertility is described mostly in male, due to specific antibodies, autoimmune diseases in women are underestimated. The mucosal immune system in women is under hormonal control; also a periodic growth and remodelling is characteristic.

Material and method: Autoimmune endocrine diseases with a major impact on fertility include polycystic ovarian disease, occult primary ovarian insufficiency, premature ovarian senescence, multiple sclerosis, Hashimoto thyroiditis, Addison's disease, type 1 diabetes mellitus, hyper-prolactinemia, but also rare conditions such as lymphocytic hypophysitis, the resistant ovary syndrome or autoimmune polyglandular syndromes.

Also antiphospholipid syndrome or other factors (antisperm antibodies, antinuclear antibodies or anti gliadin antibodies) can result in infertility.

Endometriosis, a benign gynecological condition, carries out an autoimmune component in its pathogen, is linked to female infertility. We present a short review of autoimmune diseases effects on female fertility and we propose a diagnostic algorithm proposal.

Metformin versus myo-inositol effects on ovarian morphology in women of reproductive age with PCOS

Anca P. Cucu, A.E. Ranetti

Background: Polycystic ovarian syndrome (PCOS) is the most common metabolically determined gynecologic disorder with an incidence of 4 to 184% in women of reproductive age. PCOS diagnosis is set based on Rotterdam criteria, including hyperandrogenism, chronic anovulation and typical ultrasound appearance. The current ultrasound criteria include 12 or more follicles of 2-9 mm diameter in each ovary +/- increased ovarian volume.

Material and method: 20 non-diabetic women between 25-35 years old, with PCOS untreated prior to presentation, with a BMI>30 kg/m^2, were included in a pilot study. Ovarian ultrasound (morphology, volume), BMI, FSH, LH, DHEA-S were evaluated before and after the treatement. 10 women were treated with myo-inositol along with diet (non-MET-group) and 10 started a 6 months therapy with diet and metformin administered daily on a weekly increased dose up to a total of 2 g/day (Met-group). None of the patients were on a meat and dairy free diet.

Results: The Met-group had a significant weight reduction and a decreased ovarian volume, as well as improvement of ovarian morphology compared to non-Met; ovulation was similar in both groups.

Conclusions: Metformin, a biguanide used in treatment of type II diabetes mellitus, as well as myo-inositol, could be for reducing ovarian volume and improvement of ovarian morphology. For an accurate compared efficiency of the two, further studies including larger cohorts of patients are needed.

The treatment with aromatase inhibitors and osteoporosis for women with breast cancer

Simona V. Lia, Adina Mazilu

Background: Breast neoplasm is the first cause of female cancer mortality and early diagnosis leads to a favorable evolution.

Aromatase is an enzyme involved in the production of estrogen acting by catabolizing the conversion of Testosterone into Estradiol. Aromatase inhibitor (AI) therapy has shown superior efficacy compared to Tamoxifen in postmenopausal women and is quickly becoming the therapy of choice in this context. However, adjuvant AI therapy depletes residual estrogen and is associated with rapid bone loss and increased fracture risk.

Breast cancer patients receiving adjuvant AI therapy will require specialized management strategies to identify and treat patients at high risk for fracture. Randomized clinical trials in postmenopausal women indicate that bisphosphonates prevent bone loss and accelerated turnover associated with AI therapy and are a promising strategy for the prevention and treatment of osteoporosis in this context. Bisphosphonates, along with a healthy lifestyle and adequate intake of calcium and vitamin D are the treatments of choice to prevent bone loss.

Methods: The current method of determining bone density and its resistance is DXA, a method especially used to track osteoporosis.

Discussion: Following a study of 120 women after treatment with AI, 29% were diagnosed with osteoporosis, 59% with osteopenia, and 13% with fractures.

Conclusion: Adjuvant hormonal therapy for a post-menopausal woman with receptor-positive breast cancer includes an aromatase inhibitor as initial therapy or after treatment with tamoxifen. Women with breast cancer and their physicians must weigh the risks and benefits of all therapeutic options because this could pose a risk of developing osteoporosis.

Large Cabergoline dose induced complete macroprolactinoma necrosis

Adina Mazilu, M. Mitrica, C. Nastase, A. Chirtes, M. Stefanescu

Introduction: Large Cabergoline doses up to 3 mg/day were used to treat prolactinomas, especially those resistant to dopamine-agonist agents. They appeared to be safe, valvulopathy was seen more in patients that needed Cabergoline for Parkinson's disease treatment. Our previous experience included a malignant macroprolactinoma that was completely cured to empty sella syndrome after 24 months of Cabergoline 3.5 mg/week and transition from macro to microprolactinoma in a 20 years aged woman with clinical and biological remission.

Material: A 22 years male was examined prior to surgery.He had severe cachexia with gonadal, thyroid and GH defficiency, high prolactin levels.

Method: TSH, free T4, prolactin, FSH, LH, testosteron, cortisol levels were measured by ELISA imunoassays. Enhanced Gadolinium pituitary MRI revealed a large macroprolactinoma.

Cabergoline was started at 1 mg/week, associated with Levothyroxine 50 mcg/day and Testim gel 5 g/day.

Results: After 1 month prolactin levels were reduced, but not within normal limits. Dosage of Cabergoline was increased to 3.5 mg /week, 0.5 mg/day, for 4 months, then reduced again to 1 mg/week 1 months. MRI evaluation at 6 months showed complete tumoral necrosis with empty sella syndrome.

Conclusions: large Cabergoline doses for short-term treatment, with rapid escalation, might be beneficial in macroprolactinomas, avoiding unnecessary surgery, especially in young patients. This might lead to a better control of macroprolactinomas, but large RCT are needed.

Malignant atrophic papulosis associated with stage IV thyroid carcinoma – case report

Mihaela Georgescu, D.A. Chiriţă, V. Trifu, M. Dumitrescu, M.A. Ţilea

Introduction: Malignant atrophic papulosis (MAP; also known as Degos' disease) is a rare, multisystem obliterative vasculopathy of unknown etiology. It has a purely cutaneous variant and a systemic variant with cutaneous manifestations. Both have similar cutaneous eruptions.

Case presentation: We present the case of a 55 years old male patient that came for a consultation presenting with 2-15 mm scars with central, porcelain-white atrophic centres. Purely cutaneous MAP is a benign condition that can be life-long. Also, the patient had a stage IV thyroid cancer which was excised then treated with radiotherapy.

A skin biopsy revealed a wedge-shaped degeneration of collagen is present with a prominent interface reaction with squamatization of the dermoepidermal junction, melanin incontinence and epidermal atrophy. The patient was recommended Pentoxifylline 400 mg, 2 tbs daily and emollients on a daily basis.

After half of year the patient developed generalized peritonitis due to a postradial enteric fistula. He was operated with a very slow recovery.

The rest of the general examination was normal.

Discussion: Systemic MAP has a grim prognosis, but is not uniformly fatal. The cause of death is usually intestinal perforation, but in our case the patient was quickly operated. Unfortunately, no treatment has been shown to be effective in the treatment of MAP.

Conclusion: Our case represents the first report of such association, but most likely the finding is incidental. The patient's prognostic is affected due to the stage IV thyroid carcinoma.

Attention to melanoma! Clinical and histo-pathological aspects

Alexandra Calu, L. Eftimie, M. Curea, Florina Vasilescu, Liana Toma, Rodica Bulata, M. Dumitrescu

Introduction: The incidence of melanoma, the most aggressive type of skin cancer has continued to increase over the past few years in our country, therefore starting with 2017 a National Electronic Registry for patients with melanoma will be created.

In this context, the paper aims to present some of the clinical and pathological features of malignant melanoma.

Materials and methods: For this purpose we studied the cases of malignant melanoma registred from 01 January to 31 July 2017 in the Pathology Department of Central Military Emergency University Hospital "Dr. Carol Davila".

Three subtypes of cases were characterized: primary cutaneous melanoma (the most common), uveal melanoma and metastatic melanoma.

We analyzed the distribution of cases by age, gender, location, clinical aspects and histopathological features (such as growth pattern, Breslow thickness - which is the most important prognostic factor for melanoma, depth of invasion - Clark level, ulceration)

Conclusions: During this short period, a high number of cases of malignant melanoma was registred therefore the clinicians and pathologists must realize the importance of this type of cancer and must be aware of its great clinical and pathological variability.

Most cases were diagnosed in men, with a peak of incidence around the sixth decade of life and commonly affecting the skin on the trunk. More than half pacients have been diagnosed at an advanced stage of the disease, with an average value of the Breslow index of 3,92mm. This aspect suggest the importance of setting up some national screening programs for melanoma in an attempt to diagnose this disease in an early stage.

OP session emergencies, cardiology

Hypertensive urgencies and emergencies

Alice E. Munteanu, Irina Florescu, Cristina M. Calcan

Arterial hypertension represents a worldwide health issue remaining the main cause of death. 1-2% of chronic hypertensive patients at some time will develop hypertensive urgencies or emergencies. Hypertensive emergencies can be defined severe elevation of blood pressure – SBP>180 mmHg and/or DBP>120 mmHg – in the presence of acute organ damage.

Acute coronary syndromes, dissecting aortic aneurisms, acute pulmonary edema, hypertensive encephalopathy, acute cerebral infarction, intracerebral hemorrhage, or acute arterial bleeding or eclampsia represent clinical conditions in which an immediate blood pressure reduction is needed to prevent the progression of target-organ damage.

The patient evaluation is crucial for identification of acute target organ damage. The first step is a complete history (with particular attention paid to pre-existing hypertension and comorbidities like diabetes mellitus, coronary artery disease and cerebrovascular disease) and an accurate physical examination (including fundoscopic examination), electrocardiogram, selected laboratory studies such as urinalysis, creatinine, urea, electrolytes, a full blood count, and a chest radiogram should be performed.

When a secondary form of hypertension is suspected a sample for plasma renin activity, aldosterone, and catecholamines should also be drawn. Further investigation which should be performed according to the clinical presentation are echocardiography, brain CT scan, abdominal ultrasonography, thoracic-abdominal CT scan, vascular ultrasound.

Cardiovascular events may occur in context of hypertensive urgencies and emergencies are myocardial infarction, stroke, acute heart failure, acute pulmonary edema, aortic dissection and arrhythmias.

Patients should be admitted to an intensive care unit for clinical surveillance and continuous BP monitoring. Aggressive treatment with parenteral drugs is the preferred approach.

In the presence of severe elevations of blood pressure identification of acute target organ damage is very important. Treatment should be started promptly in the emergency department. The initial goal of treatment is rapidly reduction of blood pressure, but a precipitous fall in BP should be avoided.

Involvement of ACE and HSPG gene polymorphisms and risk for metabolic syndrome

M. Toma, Oana A. Alexiu, Anne M. Crăciun, Lavinia Berca, I. Radu, D. Cimponeriu

Metabolic syndrome (MS) is a syndrome characterized by association of five medical conditions: abdominal obesity (central), increased blood pressure, increased fasting glucose, high triglycerides and low HDL levels.

The aim of our study was to investigate possible relationships between polymorphism in five candidate genes (TGF beta, HSPG, insulin, IGF2 and ACE) and hypertension in patients with metabolic syndrome.

Blood samples were taken after obtaining informed consent from 34 patients with metabolic syndrome and hypertension and 80 healthy controls. Genomic DNA was extracted from peripheral blood using a commercial kit and the five polymorphisms were genotyped by PCR -RFLP. Statistical analysis was performed with StatDirect program.

For the HSPG BamH1 polymorphism, GG genotype (OR 9.27, $p<0.05$) and G allele (OR 2.87, $p<0.05$) are associated with increased risk of disease. For the ACE ID polymorphism, DD genotype (OR 4.54, $p<0.05$) and D allele (OR 3.33, $p<0.05$) have been associated with disease risk. We have not identified statistically significant associations between other polymorphisms and metabolic syndrome or hypertension.

These results support that HSPG BamH1 and ACE ID polymorphisms may contribute to the risk for patients with metabolic syndrome and hypertension.

Mitral valve involvement in STEMI

Alice E. Munteanu, D. Nita, Cristina M. Calcan, Irina Florescu

The purpose of this paper is to provide information about the influence of mitral regurgitation on mortality rate in pacients undergoing primary angioplasty for STEMI. Cardiovascular diseases lead to one third of the deaths worldwide, surpassing the mortality rate produced by neoplasia, acute respiratory failure and diabetus mellitus all together. In the world, approximately 17 millions die annually because of cardiovascular desease and every 5 seconds one is suffering from a myocardial infarction.

There are a number of publications and data-bases which have centralized informations about STEMI pacients for almost 20 years now, enhancing a serious lowering of the mrtality rate. Central Military Hospital became part of the RO STEMI national programme in 2004. We made a retrospective study in which we included pacients with STEMI in 2016 that reached our hospital in the first 24 hours since the beginnings of symptoms in order to benefit from emergency interventional therapy. Including criteria are the classic ones for STEMI: clinical, electrical and biological features.

There was recorded the presence of preexinting valvulopathies, evolution of their severity after interventional myocardial revascularization, the presence and the importance of comorbidities and associated risk factors.

The aim of this study is to establish the relationship between mitral regurgitation, the evolution and short-term prognosis, to establish early mortality rate after an acute arterial state and also the influence of risk factors and comorbidities in our clinic for the chosen period of time.

After final results are obtained, a parallel with mortality rates declared in the first years of this programme and with those declared for STEMI pacients admitted in cardiology departament of our hospital in 2011.

Post-STEMI evolution – the experience of our hospital

Alice Munteanu, L. Chiriac, D. Nita, Irina Florescu, Cristina Calcan

The major limitations of balloon angioplasty have been represented by the acute obstruction and restenosis of blood vessels. Early studies of intracoronary stents have shown that these devices are very effective in treating or preventing acute blood vessel obstruction, thus avoiding emergency surgical bypass interventions. Two randomized trials – the Benestend and STRESS (Stent Restenosis Study) have shown that stenting lesions de novo on native coronary have reduced angiographic restenosis by about 30% compared to conventional balloon angioplasty. Implanting stents leads to a luminal diameter larger than balloon angioplasty, both right after the surgery and in the follow up, thus to a lower restenosis rate.

Indications for interventional myocardial revascularization are: STEMI, NON-STEMI, stable angina, angina equivalent – dyspnea, arrhythmias, syncope, asymptomatic patients or moderately symptomatic with a medium-large surface of viable myocardia, or moderately-severe myocardial ischemia on noninvasive tests, angiographic – hemodynamically significant lesions on myocardia arteries which irrigate a viable myocardia and have a diameter higher than 1.5 mm.

Relative contraindications for interventional myocardial revascularization are: relevant comorbidities, small diameter coronaries or venous grafts with diffuse stenosis, LAD stenosis in a patient with CABG recommendation (surgery remains the preferred therapy method for such cases), PCI unsuitable coronary anatomy.

Contraindications for interventional myocardial revascularization are: patients with contraindications for antiplatelet or anticoagulant therapy, coronary lesions which prevent fully inflating the balloon and properly implanting the stent.

Vasile Candea Emergency Clinical Centre for Cardiovascular Diseases is part of the national program RO-STEMI for 5 years. During this period 3453 patients were admitted with STEMI. 2952 patients received primary PCI.

The results regarding procedures performed were: 14.5% were not admitted to angiography, 4.4% received only PCI without stent, 52.6% of patients received one stent, 21.5% 2 stents, 6.4% 3 stents and 0.6% 5 stents. The vessel responsible for STMI was in 57% of cases LAD, in 30.2% was RCA, in 11% was CXA and in 1.7% was not specified. The rate of early complications after angiography was 9.3%: 3.5% of patients suffered acute stent thrombosis, 3.2% of patients had pseudoaneurysm, 1.7% of patients had procedural failure and 0.9% had coronary dissection. At discharge 71.5% of patients hadn't simptoms of heart failure. 25% of patients showed signs of left heart failure and 3.5% of patient admitted in programme died.

Hospital management of mass casualty incident

B. Teușdea, F. Costea, M. Toma, S. Dogaru

Mass casualty incident with a large influx of victims are inevitable. A correct and careful risk assessment and an efficient planning of activities and resources will allow the impact of these situations to be reduced to the basic activity of the hospital and the quality of healthcare. Much of these situations can be managed using resources that work redundantly to mitigate the expected effects of the emergency.

Beyond the proper management of risks, the introduction of an alert and evaluation system is extremely important. These systems, together with precautionary measures related to unidentifiable risks, will help prevent traps that can occur after a crisis has occurred.

Communication systems in the event of disasters or in major emergencies may be affected. Under these circumstances, the White Plan sets out the main means of communication that must/can be used.

WHITE PLAN represents the response plan of bedside units in the event of a massive influx of patients following a collective accident, a calamity, epidemic or pandemic. The WHITE PLAN allows the management of emergencies outside the hospital, requiring additional human, logistical, financial, and appropriate training.

The course addresses to medical specialists and nurses and intend to make introduction to the White Plan's overall framework, explain what does the WHITE PLAN mean, present the crisis cell at the ED and at the hospital level, and organization of receiving multiple emergencies, explain the Triage of Patients and Triage Card, and finally present some concept regarding to decontamination in case of nuclear, chemical and biological accidents.

Civilian and military trauma – the same, although different

B. Teușdea, M. Toma, S. Dogaru

The first triage system was conceived and developed on the battlefield, but the ATLS (Advanced Trauma Life Support) course was developed by a civilian.

Nowadays, military patterns of injury are increasingly seen in the civilian setting as a result of terrorist attacks.

Common principles can be applied to trauma resuscitation meaning there is one system for all patients and all injury mechanism. This is the <C>ABC system.

In the military setting there are four definable levels of clinical capability: care under fire, tactical field care, field resuscitation and advanced resuscitation.

The differences between military and civilian trauma are also related to injury pattern, population at risk, physical factors, security, equipment and drugs, people and focus.

Seeing trauma care in dynamics from ATLS courses versus military trauma rules remain an interesting and open discussion.

Multiple trauma management in a fighter pilot

D.D. Negoiță, C.B. Teușdea

Major trauma management is well described in the literature, for example ATLS courses. Military personnel that suffer major trauma must be evaluated after having received complex treatment for the suitability to further serve in the military.

We would like to present the case of a fighter jet pilot who had to eject himself, suffered a major trauma, was stabilized at the nearest emergency hospital, and then referred to our hospital for advanced treatment.

The patient was further investigated in the emergency room, his pain was alleviated, supportive treatment was continued, and then he was admitted to neurosurgery, operated the next day, and later on underwent physical rehabilitation with good results.

This case illustrates the peculiarities of the work in the emergency department of a tertiary hospital.

Civil/private versus military emergency medical systems for aeromedical evacuation

L.M. Lupu

Introduction and objectives: Emergency medical systems are wide organizations and need a lot of resources and knowledge to perform. The objective is to compare the organizational principles, C3 (command, control and communication), methods, protocols and resources between civil and military emergency system.

Materials & methods: This paper provides a review of the most important aspects of planning and execution of patient movement within the two systems.

Results: It’s described the result of managing medical cases by the two systems.

Conclusion: A close interaction between the systems, including methods of training and working together can be an option. The aim to create a single National Emergency System for Aeromedical Evacuation with civil, military and private component is debatable.

Clinical toxicology laboratory a half century of experience

Sandra M. Forje, Dida Ardeleanu, Cristina M. Șerban

Military Medical Research Center is a pillar of excellence in the field of Toxicology for nearly half a century.

The Clinical Toxicology Department within the Military Medical Research Center, performes scientific research activities for the development and application of methods and means to improve medical first aid and specifically treatment in various acute poisoning by studying the mechanisms of pathogenesis of acute intoxications with Chemical Warfare and other substances with toxic potential.

As part of the preparedness and capacity of intervention in situations involving the personnel of the Ministry of National Defense, Clinical Toxicology Laboratory provides analytical laboratory investigations for diagnosis of certainty for intoxications cases from the Emergency Hospital Bucharest, as well as other Health Units.

The performing of toxicological screening through analytical techniques Fluorescence Polarization Immunoassay and Gas Chromatography – Mass Spectrometry is ensured that for a large number of xenobiotics and their metabolites, as organochlorine compounds, organophosphorus compounds herbicides, fungicides and various drugs. A distinct group of cases of intoxications are represented by psychotomimetics substances excess consumption.

From the recent years studied cases the autors will present a selection of assays that helped fisicians from ”Carol Davila” University Central Emergency Military Hospital in establishing the diagnosis of certainty.

OP session internal medicine

Changes in lipid levels associated with initiation of TNF inhibitor therapy in patients with ankylosing spondylitis

Daniela Anghel, M.L. Ciobîca, C.V. Jurcut, Livia Otlocan, Maria M. Negru, Andreea I. Lungu, G.D. Stoicescu, A. Anghel

Background: The increased risk for cardiovascular diseases (CVD) is associated with high levels of total cholesterol (TC), low density lipoproteins (LDL) and triglicerides (TG) and low levels of high density lipoproteins (HDL). In ankylosing spondylitis (SA) patients the risk of CVD is greater than the general population.

Objectives: To compare lipid levels in ankylosing spondylitis (SA) patients and osteoarthritis (OA) patients and to determine the changes in lipid levels in ankylosing spondylitis patients after 6 months of initiation of anti-TNF therapy.

Methodes: The study is retrospective and we followed-up adults with ankylosing spondylitis or OA with no other inflammatory diseases. We determined to this patients a lipid profile – TC, LDL, HDL, TG- levels at baseline and after 6 months.

The patients don’t intake a lipid-lowering medication (LLM). All patients with SA received anti-TNF alfa therapy for the first time.

Results: The study included 43 patients with SA with anti-TNF alfa initiation and 35 patients with OA who met eligibility criteria of diagnosis. The patients diagnosed with hyperlipidemia was signiticantly higher in ankylosing spondylitis group than in OA group (58% vs. 42%, $p<0.0001$). Mean age it was 55 years and 78% were women.

At baseline OA patients had lower TC (196 mg% vs. 209 mg% $p<0.0001$) than the SA patients. Significantly lower proportions of ankylosing spondylitis vs. OA patients had borderline or lower TC and LDL levels.

Ankylosing spondylitis patients after 6 months of the initiation of anti-TNF inhibitors had a significant increase in TC level (5 mg% $p<0.0001$) and LDL level (3 mg% $p=0.0005$) and a decreased HDL level (0.5 mg% $p=0.001$).

Conclusion: Results of study indicate that ankylosing spondylitis patients have a higher level of total cholesterol and low density lipoproteins than OA patients.

In ankylosing spondylitis patients with initiation of alfa TNF inhibitors therapy we observed an increased TC and LDL levels vs. OA and HDL levels was similar to OA patients.

A good management of dyslipidemia and reduced systemic inflammation with anti-TNF therapy may reduce the risk of cardiovascular diseases in ankylosing spondylitis patients.

Dermatomyositis with severe Raynaud syndrome

G.D. Stoicescu, L.M. Ciobica, A. Anghel, Daniela Anghel, S.M. Stanciu

Idiopathic inflammatory myopathies (IIM) are autoimmune disorders with an annual incidence of 2 to 10 cases/million. Main clinical and biological features include symmetric proximal muscle weakness, heliotrope rash, and an increase in skeletal muscle enzymes, myositic specific antibodies, and characteristic abnormal electromyografic pattern and bioptic evidence of myositis

A 43 year–old NCO male was first admitted in our clinic in 2012 and diagnosed with dermatomyositis according with Bohan and Peter criteria.

Treatment was started and continued according to the Romanian guide for diagnosis and treatment of IIM (established in 2010). We used high-dose systemic corticoterapy, which was tapered according to pacient evolution. During tapering there were 2 relapses of myositic features (one severe and one mild) – thus necessitating the restart of high-dose corticoterapy. A stable and persistent remission of the disease was achieved after 1 year. Azathioprine was used as the steroid sparing agent and is now the main current treatment of the pacient.

Systemic corticoterapy generated secondary Cushing features and steroid osteoporosis with vertebral fractures. Steroid replacement and bisphosphonates were used for control.

Severe Raynaud syndrome with spontaneous symptoms and partial response to therapy was present from the beginning of the clinical manifestations and is the main feature now. Due to the absence of myositic specific antibodies and the presence of myositis-associated autoantibodies an overlap with systemic sclerosis will be explored.

Treatment in IIM is often efficient in controlling the disease, but the pacient’s outcome is determined by the secondary effects of the steroids or immunosupresives used and by the existence of other immune or nonimmune disorders.

Sarcoidosis – from non-therapeutic intervention to systemic immune suppresion

L. Ciobîcă, Iolanda Sîrbu, Ana M. Dumitru, Andreea Săvulescu

Sarcoidosis is a rare disorder with an incompletely discovered etiopathogenic mechanism. Symptomatology and presentation patterns are generally polymorphic, which constantly generates differential diagnosis problems, especially in the initial stages of the disease. Also, the therapeutic approach is very variable, from non-therapeutic intervention and follow-up to systemic immunosuppression.

We chose two clinical cases, diagnosed and monitored in the Central Military Emergency Clinical Hospital. Both were diagnosed with pulmonary sarcoidosis. The diagnostic and therapeutic approach of these cases require imagistic support and interdisciplinary collaboration, taking into account the polymorphism of clinical manifestations and possible organ damage.

All these were reasons for updating and presenting modern data on the management of sarcoidosis.

Associated autoimmune diseases

L. Ciobîcă, S. Stanciu, D.G. Stoicescu, Iolanda Sîrbu, Ana M. Dumitru, Andreea Săvulescu

Ankylosing spondylitis (AS) is a chronic, inflammatory and rheumatic disease from the group of spondyloarthropathies (SPAs), which primarily targets the sacroiliac joint and spine, and less frequently, the peripheral joints. It can also present with non-joint involvement. The disease frequently affects young adult males

We present the case of a 33-year-old patient suffering from ankylosing spondylitis, who came in for perioral paraesthesia and paresthesia on the right-hand side which started 5 days ago. Background disease began 5 years ago when, after a gastrointestinal infectious episode, the patient’s knee arthritis was unresponsive to NSAID treatment but with favorable progression following treatment with sulfasalazine.

Biologically, laboratory tests show moderate leukocytosis, moderate inflammatory syndrome, and low sideremia. The neurological examination, supported by the outcome of the cerebral MRI, raises the suspicion of multiple sclerosis, but does not exclude the diagnosis of toxocarosis.

Immunological evidence is being processed now.

Although the most common autoimmune diseases associated with ankylosing spondylitis are inflammatory bowel disease or psoriasis, unknown etiopathogenic mechanism and polymorphic symptomatology should always raise questions about any new emerging symptom, the diagnostic and therapeutic approach of these cases requiring imagistic support and interdisciplinary collaboration.

More than an immune deficit…

Oana Stancu, Roxana Diaconu, Denise A. Mardale, Theodora Militaru, V. Smedescu, Elena Busuioc, A. Zugravu, E. Mandache, I. Copaci, C. Jurcuț

We present the case of a 52-year-old patient who was hospitalized in our clinic for evaluation and treatment of newly discovered hypogammaglobulinemia. The disease started with a persistent diarrheal syndrome, being previously diagnosed and treated as an inflammatory bowel disease, with unfavorable evolution through multiple complications: repeated respiratory tract infections, urinary tract infections, bronchiectasis, severe malabsorption syndrome with malnutrition and multiple metabolic deficiencies (vitamin D with secondary osteoporosis, vitamin B12, folic acid and iron secondary anemia). Upon admission, the patient accuses physical asthenia and weight loss. The clinical exam revealed a generally mediocre state, cachexia, teguments and pale mucous. Laboratory tests confirmed metabolic deficits and low IgA, IgM and IgG values. Digestive endoscopies invalidated the diagnosis of intestinal inflammatory disease and excluded celiac disease. Treatment for correction of metabolic defects was initiated and monthly intake of intravenous gammaglobulins was initiated. After several months, the patient accused nausea and vomiting, was diagnosed with gastric calcinoma and was operated with favorable oncological evolution, but subsequently he returned for edematous syndrome with significant proteinuria. Immunofixation of serum proteins was within normal limits, renal biopsy determined the diagnosis of amyloidosis and high serum amyloid A thus suggesting AA type of amyloidosis.

Hypogammaglobulinaemia has various clinical manifestations and presents an increased risk for neoplasia, so early diagnosis and treatment are essential.Systemic amyloidosis type A is a rare complication, which is explained by chronic inflammation induced by recurrent infections.

Modern methods to differentiate benign thyroid nodules from malignant ones

L. Eftimie, R. Hristu, A. Calu, M. Curea, Florina Vasilescu, Liana Toma, Rodica Bulata, M. Sajin, Mariana Costache, S. Stanciu, G. Stanciu, M. Dumitrescu

Objective: The purpose of this study was to compare the ultrastructural capsular changes appearing in follicular adenoma, papillary and follicular thyroid carcinoma for differentiating between benign and malignant thyroid nodules.

Methods: Second Harmonic Generation (SHG) Microscopy was used to image collagen distribution in the capsules of several types of nodules. The tissue fragments were formalin-fixed, paraffin-embedded but without H&E staining, with 4-7 microns thick sections. Collagen organization was evaluated using different parameters such as the collagen organization coefficient based on the Fast Fourier Transform (FFT) of 2D-images and the Gray-Level Co-Occurrence Matrix (GLCM) angular second moment (ASM) used as a measure of textural uniformity and entropy which is a parameter of disorder in a micrograph.

Results: SHG microscopy images were acquired to assess the collagen organization of tumoral capsular thyroid nodules previously diagnosed as benign or malignant by conventional H&E staining.

Different degree of collagen fibers organization was observed and quantified using FFT and GLCM. A higher degree of structural variation was observed for the thyroid capsule compared to nodular capsules resulting in lower value of ASM and higher entropy.

Conclusion: These above described microscopy method help us distingues between benign or malignant thyroid nodules, based on the capsular colagen parameters, especially in patients treated with antithyroid drugs.

“Living with” mesenteric panniculitis

Anca Ghiatau, V. Smedescu, Anca Manolache

Mesenteric panniculitis is a rare, benign and chronic fibrosing inflammatory disease that affects the adipose tissue of the mesentery of the small intestine and colon with no specific etiology. The diagnosis is suggested by computed tomography and is confirmed by surgical biopsies- rarely necessary. The disease is more common in men (3:1)

An 88-year-old male patient presented to our care for a chronic history of abdominal discomfort and mechanical lumbar pain. His past medical history was significant for hypertension, prostatic adenoma- for which he was operated, pancreatic lipomatosis and significant cerebral atrophy. He also had a history of an imagistic examination 8 years before presentation in which pancreatic lipomatosis and mesenteric lipodystrophy was described.

Upon physical examination, he had stable vital signs and a slight tenderness upon deep palpation of the epigastric region with a tendency towards constipation. Laboratory data revealed a normal blood count, hyperglycemia (237mg/dl with a glycated hemoglobin of 9.5%) and slight inflammatory syndrome.

Abdominal ultrasound reveled a hyperechogenic mass in the epigastric region resembling the echostructure of the liver and raising suspicion for a retroperitoneal tumor. Computed tomography of the abdomen was performed without intravenous contrast administration (due to slightly elevated creatinine), which showed a focal increase in density of the mesenteric fat in the epigastric and mesogastric region, compatible with a mesenteric panniculitis. He was examined by a general surgeon which suggested oral anti-inflammatory treatment.

The clinical response was good, with the regression of pain, therefore we decided to postpone other possible treatment options (steroids, thalidomide, cyclophosphamide, colchicine, tamoxifen, antibiotics, and radiotherapy, surgery in the case of bowel obstruction or perforation) for the possibility of clinical aggravation. Our patient "had been living with" mesenteric panniculitis for at least 8 years now; therefore we expect a good short and long-term prognosis.

Pancoast Tobias syndrome due to a plasmacytoma

Anca Ghiatau, M. Sotcan, Mihaela Iordache, C. Jurcut

Pancoast syndrome is the result of a malignant neoplasm of the superior sulcus of the lung which leads to destructive lesions of the thoracic inlet and involvement of the brachial plexus and cervical sympathetic nerves, it mainly consists of severe pain in the shoulder region radiating toward the axilla and scapula and Horner syndrome. Plasmacytoma is a plasma cell dyscrasia in which a plasma cell tumor grows within soft tissue or within the axial skeleton. Extramedullary plasmacytomas most often occur in the upper respiratory tract (85%).

We present the case of a 64 year old man from the rural region who was admitted in our clinic for severe right shoulder pain and epistaxis.

His recent history included admission in the pneumology and thoracic surgery wards where he was diagnosed with right upper lobe lung cancer with invasion in the thoracic wall and T1 vertebral body without endobronchial proliferation at bronchoscopy.

Biopsies were taken from the laterocervical lymph nodes- the extemporaneous examination suggesting a neuro-endocrine tumor – the imunohistochemistry was not ready at the time of admission.

His recent – 2 weeks before our admission – blood tests revealed a normal creatinine (1.13 mg/dl) and a mild normochythic anemia (10.6 g/dl N=12-18).

On admission he presented with moderate normochytic anemia (7.9 g/dl), moderate thrombocytopenia (89.000/ul), elevated BUN and creatinine (5.26 mg/dl), elevated calcium (12.10 mg/dl). Given the high suspicion of multiple myeloma (CRAB criteria fulfilled) a protein electrophoresis was performed which revealed a 61% monoclonal gamma component.

Medullary aspiration confirmed a plasmacytic infiltration of ~70% and the patient was transferred in the hematology ward for specific treatment.

Despite aggressive fluid resuscitation the creatinine and calcium remained elevated and the patient suffered a cardiorespiratory arrest soon after the first chemotherapy dose.

In conclusion, this is the case of a pulmonary plasmacytoma presenting as Pancoast Tobias syndrome, the main and first manifestation of this case of multiple myeloma.

Multiple myeloma – a devastating disease

M. Șotcan, Lavinia Bârsan, E. Dănăilă

A 55 year-old man presented to our clinic in June 2015 for low back pain and inflamatory syndrome. Complex imagistic and biological tests sustained the diagnosis of multiple myeloma stade IIIA with sacral plasmocitoma. The patient was treated with 2 VAD and 6 Cybord chemotherapy protocols – with VGPR. Than, in august 2016 he received autotransplant.

The treatment followed with beweekly bortezomib and dexametazone until February 2017 when he accused sacral pain. The MRI revealed reccurence of the sacral tumor. Abdominal ultrasound also diagosed a left hepatic lobe tumor. The tumor was biopsied under ultrasound guidance and the diagnosis was hepatic plasmocitoma. The patient was treated with VTD protocol but the diseased progresed even more; the computed tomography revealed disseminated disease. He is now on rescue therapy with lenalidomide-dexametazone; but the prognosis is bad.

Hyperleukocytosis in acute myeloid leukemia

M. Șotcan, Lavinia Bârsan, E. Dănăilă

Hyperleukocytosis (HL) is a laboratory abnormality, commonly defined by a white blood cell count >100000/microL, caused by leukemic cell proliferation. Not the high blood count itself, but complications such as leukostasis, tumor lysis syndrome and disseminated intravascular coagulation put the patient at risk and require therapeutic intervention. The risk of complications is higher in acute then in chronic leukemias, and particularly leukostasis occurs more often in acute myeloid leukemia (AML) for several reasons. Only a small proportion of AML patients present with HL, but these patients have a particularly dismal prognosis because of a higher risk of early death resulting from hyperleukocytosis complications and a higher probability of relapse and death in the long run. Hyperleukocytosis at initial diagnosis must be considered a hematologic emergency and requires rapid action of the admitting physician in order to prevent early death.

Cognitive rehabilitation from theory to practice

D. Nedelescu, A. Iliuta, Paulina Vintila, Simona Ionescu

Neurocognitive or Cognitive Rehabilitation is a cognitive skills therapy system designed for the rehabilitation of individuals who have experienced cognitive impairment as a result of traumatic brain injury, stroke and other neurological insults.

Cognitive Rehabilitation Therapy is designed to develop and enhance cognitive functions across the following domains: attention skills; executive skills; memory skills; visuospatial skills; problem solving skills; communication skill.

The approach to rehabilitation draws on the expertise and experience of specialists from many disciplines who work together to address each patient's individual physical, emotional, behavioral and social challenges. Theoretically - team members include: certified rehabilitation vocational counselor; cognitive therapist; neuropsychologist; nurse case manager; occupational therapist; physical therapist; speech-language pathologist.

However, rehabilitation is not limited to improving physical disability. Cognitive rehabilitation attempts to enhance functioning and independence in patients with cognitive impairments as a result of brain damage or disease, most commonly following traumatic brain injury or stroke. As with physical rehabilitation, cognitive rehabilitation may include interventions that aim to lessen impairments, or interventions that aim to lessen the disabling impact of those impairments. Interventions are applied through technology and other compensatory strategies that may allow the individual with cognitive impairment to accomplish important life activities and more fully participate in society.

Because of the variability in patients and the Cognitive Rehabilitation they may receive, research studies, to date, have not identified a single most effective treatment. In some cases, reports of limited research about Cognitive Rehabilitation has led private health plans to deny Cognitive Rehabilitation. Many reports calls for more research on Cognitive Rehabilitation, but recognizes the difficulty in obtaining conclusive results.

The heterogeneous array of treatments available, as well as the lack of a unified theoretical framework for defining and quantifying them, makes definitive evaluation of their effectiveness particularly challenging.

OP session gastroenterology

Choledochoduodenostomy causing liver abscesses: case report of a delayed complication

Georgiana C. Robu, V.D. Balaban, C. Bețianu, Raluca S. Costache, P. Nuță, Andreea Zoican, Marina Ciochină, Sandica Bucurică, M. Pătrășescu, M. Tănase, Giorgiana Păvăloiu, Florentina Ioniță-Radu, Mariana Jinga

Introduction: Liver abscesses are relative rare septic complications within the digestive system. Recently, outcomes in patients with liver abscesses have improved as a result of advances in radiological diagnosis and percutaneous treatment options.

Case description: We report the case of a 78 year-old female who was admitted to our department for right upper quadrant pain, nausea and fever, symptoms that had been lasting for about four weeks.

Her past history revealed cholecystectomy 30 years ago and benign common bile duct stenosis 20 years previously.

On physical examination we noticed tenderness and a palpable mass in the right quadrant of the abdomen.

Laboratory evaluation revealed marked leukocytosis, moderate anemia, and cytolysis and kidney failure.

The last upper endoscopy showed choledochoduodenostomy.

Computer tomography revealed a liver abscess in segments

V-VIII.

We started systemic, broad-spectrum antibiotic therapy and a percutaneous drainage was performed. Evolution was favorable at the begining, but after three days it has been worsened.Blood cultures were negative.CT showed a new, complex liver abscess near the drainage tube. Patient was transferred to the surgery deparment where laparoscopic drainage was done.

After 7 days of postoperative recopevery, the patient was discharged with resolution of symptoms, leukocytosis, improvement in liver and kidney function.

Conclusions: Liver abscess can be considered a pathology of connection between gastroenterology, radiology and surgery and that's why it is important to have a multidisciplinary team.

Focal nodular hyperplasia in a patient with viral hepatitis – a case report

Mihaela Iordache, S.M. Stanciu, G.D. Stoicescu, A. Anghel, Dana Anghel, L.M. Ciobica, Raluca Costache, P. Nuta, Mariana Jinga, Florentina Ionita Radu

Hepatic nodules found in patients with viral hepatitis usually are hepatocellular carcinomas. Focal nodular hyperplasia (FNH) is a regenerative mass lesion of the liver and, the second most common benign liver lesion after cavenoud haemangioma. FNH is believed to occur as a result of a localized hepatocyte response to an underlying congenital arteriovenous malformation. It is not seen in patients with cirrhosis.

Differential diagnosis of new-onset hepatic nodules based on imaging features remains the great concern for physicians. To our knowledge, no cases of new-onset FNH in patients with hepatitis C virus have been reported, therefore we present a case of hepatitis C virus infected female with a new-onset FNH during follow-up after interferon therapy.

Atypical adult Celiac disease – decompensated liver cirrhosis: case report

Andrada L. Popescu, A.I. Gavrilă, P. Nuță, Raluca S. Costache, Mariana Jinga, Sandica Bucurică, B. Macadon, M. Pătrășescu, Mirela Chereja, Florentina Ioniță-Radu

Introduction: Celiac Disease (CD) is a disease in which the mucosal lining of the small intestine is damaged in response to ingestion of gluten and similar proteins, which are found in wheat, oats, rye, barley and other grains. Adult CD has a wide variety of presentations and symptoms.

We are presenting the case of a 39-year-old male transferred to our unit after 2 weeks for further investigations and management, diagnosed with decompensated cirrhosis with recent worsening of his symptoms and suspicion of spontaneous bacterial peritonitis. He had a history of alcohol consumption, negative viral markers, fatigue, and clinical signs of portal hypertension, ascites, and large peripheral edema.

Additional history revealed flatulence and occasional episodes of diarrhea without blood or mucous. On laboratory investigation, he had important inflammatory syndrome, leukocytosis with neutrophilia, low protein and albumin level.

We performed paracentesis with peritoneal fluid testing (cell count, gram stain, culture, SAAG), and look for other possible infection. We raised the suspicion of protein losing enteropathy after excluding other causes, the upper GI endoscopy showed loss of kerckring folds in the descending duodenum and the histopathology revealed villous atrophy. The serology markers were positive for anti-tissue transglutaminase antibody. Based on the above findings, the diagnosis of celiac disease was strongly suspected and the patient was managed with gluten- free diet. He responded nicely and all his symptoms and clinical signs improved, with complete remission of edema and ascites.

Conclusion: This case report highlights the polymorphic nature of celiac disease, than can mimic another disease. In conclusion, a high index of suspicion is needed to identify the atypical forms of the disease.

Familial adenomatous polyposis and liver metastases

Mihaela Iordache, S.M. Stanciu, G.D. Stoicescu, A. Anghel, L.M. Ciobica, Raluca Costache, P. Nuta, Mariana Jinga, Florentina Ionita Radu

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder caused by mutation of the adenomatous polyposis coli (APC) gene. It is mainly characterized by the presence of a large number of polyps in the gastrointestinal tract, more than 100 colonic polyps, with extra-colonic manifestations in the soft tissue and eye lesions.

Neuroendocrine tumors (NETs) are also a rare condition. They are occasionally observed in patients with familial adenomatous polyposis (FAP), suggesting a role for the adenomatous polyposis coli/β-catenin pathway.

We present the case of a male diagnosed with FAP and liver metastases. Histological analysis revealed metastases to be secondary to a neuroendocrine tumor. To date, only a few cases showing the simultaneous occurrence of the two entities have been published.

Clostridium difficile infection in end-stage-liver disease-current challenges – case report

Florentina Ioniță-Radu, Andrada L. Popescu, P. Nuță, Mariana Jinga, Raluca S. Costache, Sandica Bucurică, B. Macadon, M. Pătrășescu, A.I. Gavrilă, Mirela Chereja

Introduction: Clostridium difficile is very common in patients with end-stage liver disease. The diagnosis in cirrhotic patients is difficult due to the background of a partial systemic inflammatory response syndrome (SIRS) and atypical presentation. This condition is very often followed by prolonged hospitalization, further nosocomial infections and de-listing from liver transplantation. MATERIALS AND Methods: We present a case of recurrent Clostridium difficile infection in a 40 year old men with end-stage liver disease due to alcohol consumption, with atypical presentation and negative immunoassay enzyme tests for Clostridium toxins A and B.

The patient presented in our clinic with anasarca, encephalopathy and intermittent diarrhea. No history of fever was identified. Biological findings showed elevated protrombin time, leukocytosis, macrocytic anemia, severe hypoproteinemia and dyselectrolytemia. No SIRS or elevated procalcitonin level were identified. The patient had no proteinuria, multiple negative hemocultures, coprocultures, negative Clostridium difficile immunoassay enzyme tests and also no abnormalities on sternal puncture or evidence of venous thrombosis. A PCR for Clostridium difficile was lately performed and was positive. Oral vancomicin treatment was started and resumed with tapered doses after remission. Metronidazol treatment was withheld as it worsen liver failure. Despite the treatment the patient had multiple recurrences with admissions in therapy unit, followed by severe nosocomial infections and two resuscitated cardiac arrests.

Results and conclusion: Clostridium difficile infection is associated with a high rate of mortality in cirrhotic patients and should be excluded as soon as possible in all decompensated patients even there is no typical presentation or cogent immunoassay tests.

Controlled attenuation parameter for assessment of hepatic steatosis in chronic liver disease

Raluca S. Costache, Florentina Ioniță-Radu, Mariana Jinga, P. Nuță, Samdica Bucurică, B. Macadon, M. Pătrășescu, Andrada L. Popescu, V. Balaban, Gaudia Mănescu-Avram, Georgiana Robu, A.I. Gavrilă, D.O. Costache

Introduction: In developed countries liver steatosis became nowadays a major health problem affecting about 30% of population, with increased incidence in population with high rates of obesity, type 2 diabetes, alcohol consumption or those receiving certain drugs as steroids, amiodarone. Hepatic steatosis is also close related with hepatitis B and C infection

Based on ultrasound attenuation principle a new noninvasive technique was developed to quantify the liver steatosis along with liver fibrosis: controlled attenuation parameter (CAP)

Material and method: We evaluate 264 patients using FibroScan 502 Touch (Echosens, France) with M or XL probe divided in three groups: 141 patients with virus C infection, 72 patients with virus B infection and 52 patients with hepatic steatosis.

Results: The mean age was 57.64, 50.73 and 52.78 years respectively for the three groups of patients. Male to female ratio was 1.08 (137/127). We use for the CAP cut-off values the median resulting from a meta-analysis on 11 studies on 12 cohorts. For the three groups of patients we evaluate the CAP values according with fibrosis grade, ALT, AST, glycemic values, cholesterol and triglycerides with high level of concordance.

Conclusions: CAP as a noninvasive method for detection of hepatic steatosis is an objective, reliable and easy to perform, suitable for daily practice.

Ménétrier disease – a rare cause of recurrent upper gastrointestinal bleeding

D.V. Balaban, Georgiana Robu, C. Marina, A. Zoican, I. Moraru, M. Tănase, L. Eftimie, P. Nuță, Raluca S. Costache, Florentina Ioniță-Radu, Mariana Jinga

Introduction: Ménétrier disease (hypertrophic gastropathy) is rarely found in clinical practice. Complications such as gastrointestinal bleeding may occur and usually originate from superficial erosions of the mucosa. We herein report the case of a patient with recurrent upper GI bleeding, with occult source, who was finally diagnosed with Ménétrier disease.

Case report: A 78 years-old male, with a past medical history of spherocytosis and gastric resection for bleeding ulcer (20 years previously), presented to the Emergency Department with intermittent melena for about two weeks.

The patient was hemodynamically stable and had a hemoglobin level of 4.2 g/dl. After hematologic correction of anemia, endoscopy was performed and showed diffuse bleeding at the anastomosis, without a clear source, which was stopped by epinephrine injection.

Second day the patient had massive hematemesis and endoscopy revealed a spurting hemorrhage on the posterior wall of the stomach, and successful hemostasis with epinephrine injection and clips was performed.

The patient continued to pass melenic stools intermittently for the next three weeks, with consecutive drop in hemoglobin levels, and multiple, repeated endoscopies were negative.

Anterograde enteroscopy and ileocolonoscopy were also negative; selective angiography did not show any bleeding sources, except congestion at the level of left gastric artery.

Of note, the patient had severe hypoalbuminemia during the entire length of hospitalization, for which substitution therapy was prescribed.

We suspected intermittent gastric bleeding and completion gastrectomy was performed, with remission of melena and increase in hemoglobin values. The pathology report from the surgical specimen showed features consistent with Ménétrier disease.

The patient later died of cardiopulmonary complications.

Discussions: Occult gastrointestinal bleeding can sometimes represent a diagnostic and therapeutic challenge. Our case report highlights the difficulty in reaching a final diagnosis and the need for multidisciplinary team decisions when routine protocols are non-diagnostic.

Physical effort – the prodigal son of prophylaxis in colorectal cancer

M. Pătrășescu, M. Diculescu, P. Nuță, Raluca S. Costache, Sandica Bucurică, B. Macadon, Andrada Popescu, V. Balaban, Mariana Jinga, Florentina Ioniță-Radu

Introduction: Physical activity is a major and potentially modifiable component of life style that may reduce the risk of some cancers (endometrial, breast, colon, prostate, pulmonary, ovary).

Material: A series of observational studies (around 60 till now) suggests that physical activity decreases the risk of colorectal cancer (CRC).

A very comprehensive meta-analysis that included 21 studies reports a significant 27% risk reduction of proximal CRC and 26% of distal CRC comparing highly active vs. sedentary individuals.

There are not yet published interventional studies concerning this issue.

It has been noticed that physical activity may reduce the risk of CRC regardless of the effect on weight, suggesting an independent mechanism of action as opposed to that represented by decreasing weight. A minimum of physical activity such as one hour per week of walking may sufficiently reduce the risk of CRC by 31%. There is a dose-response concerning heavier physical effort.

The proposed protective mechanisms may be: decreasing hyperinsulinism, increasing peripheral sensitivity to insulin, anti-inflammatory effects, controlling obesity, increasing intestinal peristalsis that may decrease the contact time between intraluminal carcinogens and colonic mucosa.

The physical activity renders the maximum CRC prophylactic benefit if it was performed in the age group of 30-39 years as opposed to no benefit for this issue in the age group of 15-30 years.

A relevant pool of literature data shows that physical activity has also a positive impact at the level of early CRC lesions (adenomas) and it ensures a prolonged survival after CRC diagnosis and treatment.

In spite of all these information the benefits of physical activity on CRC are not largely acknowledged.

Conclusion: Encouraging physical activity in the general population may be a very effective way of decreasing the global burden of CRC.

Experience in colonoscopy of fellows in training of the Department of Gastroenterology at Carol Davila University Emergency Military Central Hospital

Georgiana C. Robu, Gaudia Mănescu-Avram, A. Lungu, A.I. Gavrilă, Mirela Chereja, Iulia Enache, R. Mateescu, T. Anghel, Geanina Spulber, Roxana Călin, Andrada Popescu, V.D. Balaban, Raluca S. Costache, Sandica Bucurică, P. Nuță, M. Pătrășescu, Florentina Ioniță-Radu, Mariana Jinga

Introduction: Department of gastroenterology is a university clinical in which 14 fellows in training in gastroenterology develop their activity.

The aim of our study is to share the experience of fellows in training in colonoscopy

Materials and methods: We enrolled in the study third and fourth year residents from Dr Carol Davila University Emergency Central Military Hospital who have performed lower gastrointestinal endoscopy during the whole year 2017. We have tried to establish indicators for quality colonoscopy such as: cecal intubation, withdrawal time, bowel preparation and adenoma detection rate.

Results: From the begining of the 2017 till August 129 colonoscopies were performed.

Fellows in training achieved the cecum in 78% of the colonoscopies, the rest of 22% failed to reach to cecum due to: 58% of patient's intolerance, 16% of tumoral stenosis, 16% of acute diverticulitis, 10% other causes. In 100% of examinations, the withdrawal time was over 6 minutes.The ileal intubation rate was 12% of colonoscopies.

Conclusions: All the procedures performed by residents were assisted and overseen by their seniors and indicators for quality colonoscopy were achieved in the majority of cases.

OP session cardiology

Particularities in interventional treatment in STEMI patients

S. Botezatu, A. Iancu, D. Niţă

ST segment elevation myocardial infarction most commonly occurs when thrombus formation results in complete occlusion of a major epicardial coronary vessel. The most serious form of acute coronary syndromes, STEMI is a life-threatening, time-sensitive emergency that must be diagnosed and treated promptly via coronary revascularization, usually by percutaneous coronary intervention.

Thrombectomy for the treatment of ST elevation myocardial infarction (STEMI) is a simple and intuitive idea. However, the favorable results with thrombo-aspiration in STEMI were subsequently called into question by data indicating not only a lack of efficacy, but a risk of potentially deleterious complications. From our experience thromboaspiration is a safe procedure and very usefull in patients with STEMI and total thrombus oclusion.

ST-segment elevation (STEMI) myocardial infarction due to critical stenosis or abrupt occlusion of the ULMCA (unprotected left main coronary artery) is a catastrophic situation with a very high in-hospital and long-term mortality. Many cases are never reported because of pre-hospital death. Although coronary artery bypass grafting (CABG) remains a class I recommendation for LM revascularization in European and American guidelines, percutaneous coronary intervention (PCI) is becoming an attractive option in patients with acute myocardial infarction and ULMCA as an infarct-related artery, especially when in cardiogenic shock.

A minority of patients presenting with ST elevation myocardial infarction (STEMI) have angiographically normal coronary arteries. There is a small but definite incidence of angiographically normal coronary arteries in patients presenting with STEMI. While the eventual aetiology remains uncertain in most patients, long-term outcomes appear favourable.

Endovascular treatment of brain aneurysm

S. Botezatu, A. Iancu, D. Niţă

1. What is brain Aneurysm?

A brain aneurysm, also referred to as a cerebral aneurysm or intracranial aneurysm, is a weak bulging spot on the wall of a brain artery very much like a thin balloon. As the artery wall becomes gradually thinner from the dilation, the blood flow causes the weakened wall to swell outward. This pressure may cause the aneurysm to rupture and allow blood to escape into the space around the brain.

2. Treatment options for Brain Aneurysms:

- Surgical clipping
- Endovascular
- Parent artery sacrifice with or without bypass surgery
- Conservative management (no treatment)

3. Endovascular treatment for brain aneurysms:

- Coiling of aneurysm

- Stent assisted coiling
- Balloon assisted coiling
- Flow diverters

4. Coiling of aneurysm: Endovascular embolization, or coiling, uses the natural access to the brain through the bloodstream via arteries to diagnosis and treat brain aneurysms. The goal of the treatment is to safely seal off the aneurysm with thin wires made by platinium and stop further blood from entering into the aneurysm and increasing the risk of rupture or possibly rebleeding.

5. Balloon assisted coiling: In slightly wide necked aneurysms balloon assistance is taken to coil an aneurysm in order to prevent prolapse of coil mass into the artery.

6. Stent assisted coiling: For wide necked aneurysms stent can be placed to hold coil mass inside the aneurysm.

7. Flow diverter: Flow diverter is a kind of stent which can be used without use of coils. They divert the blood flow to relief the pressure inside the aneurysm and favors in time the thrombosis.

8. Cases

An anxious doctor at risk of sudden death

S. Cecoltan, R. Boingiu, S. Dodoiu, Magdalena M. Gurzun, I. Hănţulie, A. Nicolae, S.I. Dumitrescu

Overview: Sudden death – non-traumatic, unexpected fatal event occurring within 1 hour of the onset of symptoms in an apparently healthy subject. It is a major problem of public health, represents 25% of all cardiovascular deaths, among the young people with an incidence of 0.46-3.7 cases/ 100,000 persons/year, gaining a dramatic psychological and social burden. As a second etiology, after coronary artery disease, is cardiomyopathy. HCM – the disease is an autosomal dominant trait caused by mutations in cardiac sarcomere protein genes, consequently appear structural and functional abnormalities of the ventricular myocardium.

Method: We present the case of a 43-year-old patient, without noticeable cardiovascular risk factors, without a family and personal pathological cardiac history, extremely anxious, who presented for fatigability at sustained efforts and an episode of fast-paced palpitations.

Results: Objective exam, laboratory tests, ECG, echocardiography – where all within normal limits, which led to an initial diagnosis of anxiety related disorder. Conversely, a 24-hour ECG Holter monitoring revealed a NSVT episode and the surprise came at the second comprehensive echocardiography exam, made during the hospitalization in our clinic, when we detected posterior wall hypertrophy with the maximum thickness at the level of the papillary muscles – approximately 26 mm, without intraventricular gradient at rest, but the Valsalva maneuver showed a gradient of 64 mmHg, and on the stress test >92 mmHg, normal myocardial velocities and grade 1 diastolic dysfunction. MRI exam confirmed the diagnosis of HCM.

Conclusions: The peculiarities of the case are lack of ECG changes described in very rare HCM forms with this location, the presence of normal myocardial velocities, the difficulty of identifying the hypertrophy with this location and the importance of Valsalva maneuver in detecting an intraventricular gradient. The caveat of the case is that overexpression of anxiety without obvious reasons could lead to underdiagnoses of severe disease.

The surprises of a „common" hypertensive pulmonary edema

Maria Manea, Oana Tăutu, S.I. Dumitrescu, Maria Dorobanţu

We are presenting the case of a man, 56 years old, smoker, known with dyslipidemia and high blood pressure, who was admitted for aggravated dyspnea to dyspnea at rest in a few minutes, the symptomatology being suggestive for cardiogenic acute pulmonary edema in context of high blood pressure. Physical examination performed at emergency room shown that the patient was conscious, cooperant, and afebrile. The auscultation revealed rhytmic heart sounds, with HR 100 b/min, BP 240/110 mm Hg and pulmonary crackles bilateral basal. The blood tests revealed leucocytosis, hyperglicemia, dyslipidemia and increased creatinine. The ECG showed synus rhythm, with negative T waves in lateral teritory of the heart. The chest x-ray revealed confluated alveoli opacities localised at the right pulmonary parenchima.

Transthoracic ecocardiography showed the surprise: excessive trabeculation of the left ventricle, which raised the suspicion of non-compaction of the left ventricle. Also, ecocardiography showed dilatation of the left ventricle and severe systolic dysfunction (LVEF 35%). In this context, it was performed a cardiac MRI which confirmed the diagnosis of LVNC.

In the context of systolic dysfunction of the left ventricle, it was performed a coronarography which shown that the coronary arteries have no atherosclerotic lessions, so the ischemic cause was excluded.

ECG Holter/24 h did not record significant arrythmias. Also, the arterial pressure was recorded with a Holter which revealed high blood pressure by night, which raised the suspicion of obstructive sleep apnea.

The patient was treated according to the heart failure and high blood pressure guidelines: betablocant, statin, loop diuretic. It was initiated treatment with antialdosteronic diuretic and ACEi, but hyperpotasemia was noted.

The patient"s evolution was favourable, with normal values of blood pressure and asymptomatic regarding heart failure. The patients need reevaluation every 6 months with ecocardiography and Holter ECG to record and treat promptly eventual arrythmias.

This case is particular because a complex pathology was hidden behind a simple crisis of high blood pressure: non-compaction of the left ventricle, a rare congenital cardiopathy, evolving heart failure, arrythmias and emboli.

Ascites after liver transplant – cardiologist's opinion

Cecilia Pătru, C. Calin, P. Platon, I. Popescu, Carmen Ginghina

We present the case of a patient of 32 years old with congenital cardiac malformation (ventricular septal defect with bidirectional shunt, sever pulmonary stenosis and foramen ovale patent) operated 8 years before: closure with Dacron patch of the defect and pulmonary comisurotomy. After approximately one year from the intervention it was established a diagnosis of constrictive- effusive pericarditis and it was done pericardiolysis with release of both cava veins and dranaige of 250 ml blood.

After 7 years from closure of the septal defect, the patient was diagnosed with liver cirosis of hepatitis B and he had indication of liver transplant.The induction and maintenance of immunosuppression was achieved with tacrolimus, mycophenolate mofetil and corticosteroids. After transplant, the patient presents persistent ascites, although both the biological and the tomographic computer function of the graft is normal, HBV antigen is undetectable after 5 months post-transplant.

Echocardiographie, computer tomography and cardiac catheterization revealed an aspect of constrictive pericarditis.

The peculiarities of the case:

Sudden onset of the symptoms for right heart failure immediately post liver transplant

Limited therapeutic resourses.

OP session general surgery

Anterior approach of the lombar vertebral column, multidisciplinary team

M. Mitrica, A. Dima, A. Chirtes

The paper presents the constitution of a mixed team l neurosurgery - general surgery capable to approach complex pathology situated at the anatomic line between the two specialities.

We present two cases who were treated with this approach and we hope to be the beginning of a constant collaboration, beneficial for the pacients of our institution.

The presented cases are pacients with disc infections with anterior extension in the psoas muscle.

Which requires an anterior transperitoneal approach. This approach isn't specific for the neurosurgery, being neccesary the presence of the visceral surgeon.

Ventriculo-cardiac drainage

C. Nastase, M. Marinescu, M. Mitrica

Definition: Ventriculoatrial shunt placement enables cerebrospinal fluid (CSF) to flow from the cerebral ventricular system to the atrium of the heart.

Objectives: The primary consideration in any patient who may undergo ventriculoatrial shunt placement is whether the patient has symptomatic hydrocephalus that necessitates CSF diversion. We have to rule out the peritoneal space as an acceptable location before selecting the cardiac atrium as the site for the distal catheter

Material and methods: We have treated in our service two pacients, with multiple revised operations of ventriculo-peritoneal shunts. It was necessary to convert VPS in VCS, because the peritoneum lost the capacity to resorbe the CSF.

Conclusions: The both pacients have good results and VCS remains an alternative way for CSF derivation in all hydrocephalus cases.

Abreviation: VPS – ventriculo-peritoneal shunt; VCS – ventriculo-cardiac shunt; CSF – cerebro-spinal fluid

Retroperitoneal abscess – A 6-year review

C. Mușat, R. Nica, O. Albița, C. Duțu, T. Rogin, D. Orosan, C. Cîrlan, Elena Starcu, A. Lică, A. Luchian, C. Blăjuț, C. Bețianu, Madalina Piron, Lavinia Răboj, R. Dragoș, F. Săvulescu

Introduction: Retroperitoneal abscesses are rarely encountered in the usual medical practice, due to the scarce manifestation of the early stages, more often the patients' refferring to the E.R. with full onset of the symptoms and signs. The condition is usually severe and the diagnosis is late, resulting in sepsis and high morbidity and mortality rates.

Methods: Between Jan-2010 and Jun-2017, 21 patients were admitted for retroperitoneal abscesses in our department. The most common causes were pancreatic and colorectal, while a few cases (3) remained of unknown origin. The approach was by open surgery in 9 cases, minimally invasive in 7 cases and both – in 5 cases.

Results: All the patients have been discharged. There was no in-hospital mortality. The readmittance rate was 23% (5 patients). The approach methods and clinical outcome are dicussed.

Conclusions: Retroperitoneal abscesses remain a clinical and therapeutical challenge due to the late diagnosis and the severe manifestations – both general (sepsis, debilitated patients) and local. Open surgery and percutaneous drainage are analyzed and compared regarding the indication, timing and outcome.

Abdominal hemangiopericytoma – case report

R. Nica, Florina Vasilescu, A. Zaciu, Cristina Sandu, R. Dragoș, Madalina Piron, Lavinia Răboj, F. Săvulescu, C. Mușat

Introduction: Hemagiopericite is a type of vascular malignancy that are arising from mesenchymal cells with pericytic differentiation. Diagnosis is often controversial because the immunohistochemical profile is uncertain. Differential diagnosis is done with fibrous histiocytoma, mesenchymal chondrosarcoma or synovial sarcoma.

Abdominal hemangiopericomatosis is a very rare tumor, with the incidence being highest in adults in the 5th age and is rare in children (less than 10% of all cases of hemangiopericytomas).

Material and methods: The authors present the case of a multiple patient operated for colorectal abdominal hemangiopericitosis in the period 1992-2016 (patient 43 years of age at the time of first surgery). Serious tumor tumors were performed at variable distances with recurrences at decreasing periods.

Results: Between 1992 and 2016 surgical treatment was accompanied by chemo and radiotherapy. It is remarked that relapses occurred at increasingly small periods, and the character of tumor metastases was increasingly aggressive, with occlusive and haemorrhagic tendencies requiring emergency surgeries. Although the most frequent place of remote metastasis is lung and bone, our patient presented metastases both enterocolic occlusive-haemorhagic, peritoneal, and hepatic (large) metastases.

Conclusion: Abdominal Hemangiopericytoma is a malignant, multi-relapse tumor, surgically accessible occlusive-haemorhagic tumor, but resistance to radio and chemotherapy.

Saphenoperitoneal shunt role in abdominal wall pathology of cirrhotic patient

O. Albița, T. Rogin, O. Sima, V. Vârjoghe

Chronic liver disease, especially cirrhosis, complicates with ascites. The degree varies from mild to severe, intractable ascites with no response to classic diurethic drugs. When this pacients develops an incisional hernia or umbilical hernia, with incarceration symptoms, the surgeon faces new challenge in surgical treatment. Saphenoperitoneal shunt is regarded as an excellent, cheap and effective method to lower the ascites pressure and to obtain best conditions before the surgical treatment. Patency of long saphenian vein and competence of saphenofemoral junction are mandatory. We treated 5 pacients with severe ascites and abdominal wall pathology. All pacients presented good results, with progressive abdominal pressure lowering, followed by an adequate surgical treatment one month later.

Surgical treatment in complicated colonic diverticulitis

F. Săvulescu, C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin

Colonic diverticulosis is one of the most common diseases of developed western countries. Although most people who have diverticular disease remain asymptomatic, up to a quarter of patients with diverticulosis ultimately progress to diverticulitis, and about 15 % of diverticulitis patients develop severe complications, such as abscess, phlegmon, fistula, obstruction, bleeding, or perforation; all these conditions generally require surgical treatment. Hinchey's classification of acute diverticulitis goes from a phlegmon (stage Ia), localized abscesses (stages Ib and II), free perforation with purulent (stage III) to feculent peritonitis (stage IV) .Management of the diverticular disease of the colon has seen progressive success owing to the advances of the diagnostic methods, intensive care settings, minimal access techniques and surgical experience. The optimal treatment of acute complicated diverticulitis is a matter of debate and has undergone significant changes. Currently, the main focus of surgical treatment concepts is on controlling the emergency situation triggered by acute complicated colonic diverticulitis through interventional and minimally invasive measures. Treatment goals for diverticulitis, whether the patient is stable or with an acute episode, are to reduce the symptoms, prevent the recurrence and, whenever possible, to perform elective rather than emergency treatment.

Conclusion: Due to diversity in disease presentation, in many cases, optimal surgical treatment of acute diverticulitis remains unclear with regard to patient selection, timing, and technical approach in both elective and urgent settings. This review addresses the current treatment recommendations for surgical management of diverticulitis, highlighting technical aspects and patterns of care.

Total mesorectal excision in the treatment of rectal cancer

F. Săvulescu, C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin

Total mesorectal excision (TME) is a common procedure used in the treatment of colorectal cancer. TME addresses earlier treatment concerns regarding adequate local control of rectal cancer when an anterior resection is performed. The introduction of total mesorectal excision (TME) for rectal cancer has reduced local recurrence rates and improved oncologic outcomes, although complication rates such as anastomotic leak have also been a consequence. Another benefits of TME are increased sparing of the anal sphincter, with a concomitant decrease in permanent stomas from abdominoperineal resection (APR) and that TME is a nerve-sparing dissection, less likely to lead to bladder and sexual dysfunction.

Conclusion: Total mesorectal excision is now the standard surgical approach to rectal cancer. Since its first descriptions, the ensuing discussion among surgeons and in training programs over what constitutes proper surgical technique for proctectomy with rectal cancer has led to a widely accepted and systematic approach to the surgery. This, in turn, has produced better oncologic outcomes compared with nonstandardized surgery, while maintaining acceptable morbidity and mortality rates. With the advent of neoadjuvant therapy for rectal cancer, many are questioning how this development may change the role of TME.

Transanal Endoscopic Microsurgery (TEM) in the surgical treatment of rectal cancer

F. Săvulescu, C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin

Rectal cancer treatment is complex, multidisciplinary and includes, in addition to radical resection techniques (gold standard), local excision techniques, derived products as well as specific oncological treatment.

Transanal endoscopic microsurgery (TEM) can be used as a curative operation for rectal polyps that are not amenable to colonoscopic resection. In addition, it may be used in selected patients with rectal cancer.

The following indications have been recommended by various surgical societies, including the American Society of Colon and Rectal Surgeons (ASCRS): lesions smaller than 3 cm mobile lesions, polypoid lesions, anatomically accessible lesions localized to the bowel wall (T1N0), lesions confined to the extraperitoneal region of the rectum, lesions occupying less than 40% of the circumference of the bowel lumen (however, the use of TEM for giant circumferential rectal adenomas has been reported), well-differentiated or moderately differentiated lesions and lesions not associated with lymphovascular invasion.

Its main feature is to avoid laparotomy, the opening of the abdominal cavity and the passage through it.

Conclusion: In summary we can define TEM as a truly minimal invasive surgical technique that, by utilizing a natural orifice, allows access to the rectum by avoiding the transperitoneal approach and the abdominal surgical scar.

This access route involves a substantially painless postoperative course, a lower risk of infection, a rapid recovery of normal physiological functions, a more early return to home and work activity.

The incidence of direct inguinal hernia in the era of minimally invasive treatment

C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin, F. Săvulescu

Over time, the data regarding the incidence of inguinal hernia have revealed that 25% of men and 2% of women develop a form of hernia. This data was obtained based on imaging investigations, on clinical findings and on clinical data collected after the surgical treatment. Minimally invasive surgery of inguinal hernias, besides the technical advantages for the pacient and for the surgical team, allows inspection of the bilateral abdominal and pelvic wall. This last aspect determined the change of statistical data regarding the incidence of different type of inguinal hernia.

In this paper we will present the statistical data obtained in the Second General Surgery Department of Central Military Hospital for 3 years.

Management of ventral incisional hernia with Ramirez procedure

O. Albița, T. Rogin, O. Sima, V. Vârjoghe

Incisional hernia may occur in more than 50% of median laparatomies, leading to giant wall defect. Several techniques have been proposed, with or without mesh but the main goal is to achieve low intraabdominal pressure. Ramirez's procedure (component separation) offers a solution to translate anterior wall flaps for a distance of 10-15 cm and to obtain reduced tension of suture line; various mesh placement is at surgeon disposal.

Patient – doctor communication and the medical internet

C. Dutu, O. Albita, C. Musat, R. Nica, T. Rogin, C. Carlan, C. Blajut, Elena Starcu, A. Luchian, F. Savulescu

Patient – doctor relationship is the basis of any medical act, being grounded primarily in direct communication between them. Not less than 30 percent of malpractice cases are based on a communication error. There are "non technical skills" (leadership, communication and decision making) and cognitive capacities whose development must be followed in addition to technical ones. Ignoring human communication in the communications era can cause serious harm not only to the patient but also to the physician.

The paper reviews the principles underlying the communication between the doctor and the patient (confidence in the surgeon, his competence, the patient's compliance and the agreement on the treatment to be applied) and their application in conditions where at least 90 percent of the patients approach Internet before a visit to a doctor.

Internet use by patients as a source of information on health and disease is rapidly expanding with obvious effects on the doctor-patient relationship. Medical websites and daily Internet health-related information searches allow for enormous and rapid change in health behavior. The present article reviews this impact from different angles, based on self-administered questionnaires.

This impact includes incorrect information, commercial interests, unnecessary tests and treatments resulting from patient's Internet search and language and cultural differences.

Using the Internet as the first source of information can only be useful as a first informational stage in patient-to-doctor communication and not as a substitute for it.

Training of surgeon – from disciple to resident

C. Duțu, C. Tiu, Andreea Gherghe

The last 20years has fundamentally changed the character of general surgery and the experience of residency graduates. Curent issues with general surgical residency are: inadequate surgical exposure in medical school, highly variable teaching effectiveness at different instructions: significant dependence of training outcomes on program size, reduced breath, complexity and techniques of resident operative experience.

This paper is a part of the „Surgical Train the Trainers" project. Institutes in Spain, Germany, Hungary and Romania are studying the methods of education of surgical residents in their countries organized by a consortium to analyze the different methods of surgical education and provide the most successful procedures for practical application for the surgical community in their countries.

Following the european working time directive we have a shorter working week (48 hours – 2009 vs 56 hours -2007, vs 58 hours -2004)- less than 17,500 hours for all residency - 60% less than 10years ago, with inevitable consequences:

decreased operative experience and diminution of trainer/trainee relationship.

Other challenges for residents are: reduced operating lists, increased cost of OR time, altered casemix and complex technology, changing public expectations.

Residents are educated by consultants, mentors, tutors according to their own pedagogical abilities – and need uniformed, structured methods.

In conclusion, the practice of general surgery is undergoing significant change.

The rapid adoption of new technologies, the integration of traditional and minimally invasive operations and the exponential expansion of the knowledge and variety of procedures that trainees must learn dramatically alter the landscape of surgery.

Testing the risk factors for breast cancer

R. Nica, J.C. Mușat, V. Zahiu, Alexandra Mihai, Florina Vasilescu, Mădălina Piron-Dumitrașcu, Lavinia Răboj, R. Dragoș, Silvia Nica, F. Săvulescu, D. Cimponeriu

Introduction. Tumors of the mammary gland are a heterogeneous group of diseases at the genetic and phenotypic levels. The main genetic risk factors for these diseases are represented by mutations in proto-oncogenes or tumor-suppressor genes.

Aim. The purpose of this study was to test the association of different risk factors with mammary tumors in Romanian women

Materials and methods. This study included women diagnosed with mammary gland tumors (n = 100) or considered clinically healthy (n = 100). DNA was extracted for each subject from 500 µl of peripheral blood. Mutations in three tumor -suppressor genes (e.g. BRCA1, BRCA2 and TP53) was tested with the PRONTO® BRCA kit and by indirect methods (e.g. high resolution melting curve analysis, SSCP or heteroduplex analysis).

Results. The most common types of tumors identified in this study were invasive ductal carcinoma (71%) and mixed ductal and lobular carcinoma (10%). In one samples from a patient with invasive ductal carcinoma was identified BRCA1 5382insC mutation with the PRONTO® BRCA kit and then was confirmed by indirect methods of analysis. In all other samples these methods of analysis did not reveal mutations in the investigated regions.

Conclusions. Invasive ductal carcinoma (71%) and mixed lobular and ductal carcinoma (10%) were the most common types of mammary tumors identified in this study were. The frequency of mutations with high penetrance in our patients with different types of mammary tumor tumors is reduced (1%).

OP session urology, ophtalmology

Laparoscopic approach for benign pathology of horseshoe kidney

A. Aungurenci, V. Mădan, F. Rusu, O. Pacu, A. Iliescu, G. Picu, C. Stănescu, C. Iatagan, A. Rădulescu, V. Botea, L. Chirilă, M. Dinu

Introduction: Horseshoe kidney is a rare congenital malformation, with an incidence of 0.25% in global population. Due to some anatomic specific features, stone disease and ureteropelvic junction obstruction syndrome are frequently encountered in patients that have this kind of malformation. In the last 2 decades the laparoscopic approach on horseshoe kidney had started to replace the classic open surgery with similar functional results and short postoperative recover period.

Materials and methods: Between January and March 2017 in our clinic were admitted 2 patients with benign pathology on horseshoe kidney: a female patient with left ureteropelvic junction obstruction syndrome and a male patient diagnosed with a right junction obstructive calculus of 16/26 mm, with grade III hydronephrosis. Both patients were operated by laparoscopic approach: pyeloplasty respectively pyelo-lithotomy.

Results: Operative time for laparoscopic operations ranged between 160 and 240 minutes. We have not encountered intraoperative complications that could require the conversion to open surgery. Postoperative recovery was favorable for all patients, with rapid recover of bowel function and rapid discharge in 3-5 days postoperative. Functional recovery of the kidney after pyeloplasty was assessed by scintigraphy.

Conclusion: The laparoscopic approach for benign conditions in horseshoe kidney may be regarded as an efficient alternative for open surgery, with similar functional results and rapid postoperative recovery.

Definitive radiotherapy for patients with low and intermediate risk prostate cancer

G. Ricu, M. Matei, G. Balasa, M. Dumitrache, Alina Tanase, Vasilica Balabaneanu, Maria Vlasceanu, S. Vlad

Introduction: Radiotherapy treatment protocols use prognostic factors to stratify patients into low-, intermediate-, and high-risk groups. These risk groups include patients with the same correlation between pathologic stage of disease and risk of recurrence. Material and Method: We present the indication for exclusive radiotherapy in low-risk prostate cancer patients (PSA < 10 ng/ml, Gleason score <6 and cT <T2a) and in patients with intermediar risk prostate cancer (PSA = 10-20 ng/ml or Gleason 7 or cT2b-T2c score) using modern radiotherapy technique with total doses between 74 and 78 Gy, with image guidance on intraprostatic fiducial gold markers.

We evaluate the results and acute/late toxicity obtained with modern radiation techniques, involving dose escalation and image guided radiotherapy.

Results and conclusions: External irradiation is an effective treatment option for patients with localised prostate cancer in low and intermediate risk group with an acceptable toxicity profile. The treatment is safe and well tolerated. Injecting a transperineal "spacer" gel between the prostate and the anterior wall of the rectum can limit acute and late rectal toxicity.

Ovarian-type epithelial tumors: a rare case of papillary serous tumour of low malignant potential of the testis

Florina Vasilescu, Rodica Bulata, M. Dumitrescu, M. Curea, L. Eftimie, Alexandra Calu, C. Mehotin, Liana Toma

Serous papillary tumours of the testis and adjacent tissues are very rare neoplasms similar to the surface epithelial tumours of the ovary, representing a surprising and challenging finding in diagnostic procedures.

Method: We present the case of a 71 year old man with a left testicular mass measuring 4.5 cm.Under the assumption of a testicular malignant tumour, inguinal orchiectomy was performed.

Results: Histopathological examination revealed testicular parenchyma presenting a cystic lession with intracystic blunt papillae lined by columnar epithelium, with hyperchromatic nuclei, areas of hobnail cells and rare mitoses. A focus of microinvasion was present. The underlying stroma had a fibrilar ovarian-like aspect. Immunohistochemistry presents the expression of CK7, EMA, PLAP, WT1, CA 125 and ER, calretinin positive in isolated cells and CEA, MOC 31, CK20 negative; Ki 67 was 10%. Final diagnosis was serous papillary tumour of the testis with low malignat potential-ovarian type.

Conclusion: Serous papillary tumour of ovarian type of the testis should be considered when the diagnosis of malignant mesothelioma is suspected. The microscopic appearance combined with immunohistochemistry are helpful for differential diagnosis.

Challenging cases in cataract surgery

H.T. Stanca

Purpose: To show various intraoperative aspects of challenging cataract cases.

Methods: We are presenting several difficult cataract cases successfully operated by ultrasound phacoemulsification and other adjuvant instruments and maneuvers.

Results: We reached the therapeutical aim for all cases - to take out the opacified lens without corneal decompensation restoring the visual function and also the anatomical aspect of the globe.

Conclusion: The ultrasound phacoemulsification is the gold standard in cataract surgery, but challenging cases need an extensive armamentarium, the surgeon’s experience being the most important tool.

New trends in Optical Coherence Tomography

H.T. Stanca

Purpose: To value the Optical Coherence Tomography (OCT) as an essential diagnostic tool in ophthalmology and to show the new trends in OCT evaluation.

Methods: The optical coherence tomography (OCT) is a laser interferometry based equipment which is able to analyze the multiple layers that constitute the vitreous face, retina, RPE, Bruch’s membrane, inner choroid and the optic nerve. The OCT had become a standard diagnostic tool for various diseases of the retina and the optic nerve in the last fifteen years and nowadays the technology is facing us with fantastic acquisition speeds and great accuracies. We are presenting our experience with the spectral domain (SD) OCT.

Results: We are discussing different cases running through age-related macular degeneration, diabetic macular edema, macular dystrophies, glaucoma and We are showing how to use the OCT data and the new algorithms in daily clinical activity.

Conclusions: The OCT is a must have equipment in any ophthalmology unit and the new acquisitions in technology and interpretation have to be mastered in order to achieve the best results for our patients.

Perforated corneal ulcer with intraocular complications

M. Zemba, O. Musat, C. Stefan

Purpose: to show the surgical solution for a cataract after a perforated corneal ulcer

Methods: surgical solution is shown in a video film

Results: visual acuity has improved from hand movement perception to 0.9

Conclusions: “step by step” approaching of a complictated case may allow us an easier solution than we think at first assessment

Corneoconjunctival tumor – diagnostic and thrapeutical difficulties

M. Zemba, O. Musat, C. Stefan

Purpose: to show the diagnosis, preoperator assessment and surgical treatment for a corneoconjunctival tumor

Methods: there is a review of clinical and paraclinical arguments for the diagnosis; surgical solution is shown in a video film

Results: two months postoperatively there is total functional recovery; 6 months postoperatively there is no sign of relapse of the tumor

Conclusions: the main treatment for corneoconjunctival tumor is surgical; in very large tumors the surgeon must be able to realize partial excision of the wall of the globe, especially near the limbus and it is necessary to have solutions to cover the area of conjunctival excision

High dose rate interstitial brachytherapy treatment for oral cancer

Alina Tănase, M. Dumitrache, M. Matei, C. Dumitru

Introduction: High-dose rate (HDR) brachytherapy is a type of internal radiation therapy that delivers high doses of radiation from implants placed close to, or inside the tumour. Brachytherapy results in better dose distribution compared with other treatments because of steep dose reduction in the surrounding normal tissues. This therapy has been used for treating many types of cancer such as gynecological cancer, breast cancer, and prostate cancer but also head & neck cancer.

Materials: To enhance the role of this therapy in treatment of head & neck lesions, we will present our experience in 3D conformational interstitial brachytherapy planning for an oral cancer case, using BrachyVision software, a single 192Ir source and a GammaMed Plus afterloader. The procedure was performed in a team, involving the effort of the surgeon, radiation oncologist, pathologist, medical physicist and radiation therapy technician.

Methods: The case was a single-plan implant of 4 catheters, placed parallel to the incision. 3D treatment planning required CT simulation. The clinical target volume and the organ at risk (tongue, mandible, parotids glands, teeth, and skin) were defined. Source loading was performed 9 days after catheters implant and wound closure. A dose of 3.0 Gy/fraction were delivered once daily, during 6 days. Before brachytherapy, the patient has also undergone external radiation therapy with a dose of 58 Gy.

Results: The patient follow-up after six month reveal that the clinical response to treatment was very good, with no major complications. Nevertheless, patient complains about minor difficulty in swallowing and dry mouth.

Conclusion: For brachytherapy technique a meticulous planning is esesential, to ensure adequate dose coverage of the tumor/surgery bed.With HDR brachytherapy, radiation is deposited inside the body, in the area of the tumor, thereby delivering a maximum dose while minimizing exposure to the surrounding healthy tissue. This is especially useful for advanced buccal mucosal tumors, or when the external radiotherapy is not longer an option. High-dose rate brachytherapy can also minimize the side effects that we may experience during standard radiation therapy.

OP session thoracic surgery, orthopedy, medical therapy

Minimally invasive surgical treatment for malignant pleural effusions

C. Nistor, M. Iliaș, D. Marin, Olivia Batog, A. Ciuche

Introduction: Malignant pleural effusions (MPEs) represent a common complication which can occur in any neoplastic disease.

Materials and method: We performed a retrospective study aimed to establish the adequate treatment for malignant pleural effusions. 306 patients were included in this study, during the last 6 years, diagnosed and treated in the Thoracic Surgery Department of the Central Military Emergency University Hospital.

Results: Most patients underwent chemical pleurodhesis with talcum powder (through thoracoscopic surgery or through a chest drainage tube), while for the rest of the patients, betadine was chosen as chemical agent for pleurodhesis (through thoracoscopic surgery or through a chest drainage tube). The authors compare the results obtained with each chemical agent.

The most frequent cancers with secondary MPEs were lung cancers. The efficiency of the two methods was the same. The most used chemical pleurodhesis procedure was the thoracoscopic surgery. The most used agent for pleurodhesis was talcum powder.

Conclusions: Chemical pleurodhesis with betadine is a safe procedure, which can be used intraoperative (thoracoscopic surgery) or through a chest drainage tube (for patients with major surgical risk). Chemical pleurodhesis with betadine plays an important role in addressing MPEs because of its high therapeutic efficiency and lower complications rate when compared to other agents (talcum powder).

Pancreatic pseudocyst – a rare etiology for bilateral pleural effusions

Aurora Fera, N. Tănase, M. Iliaș, D. Pantile

Introduction: Pancreatic pseudocysts usually develop inside the abdomen and in close relation with the pancreas. Under the action of the negative pressure inside the pleural cavity, these cysts can migrate inside the mediastinum and mediastinal-pleural fistulae may appear. The association of acute and chronic, local and general inflammatory phenomena generate a complex, sometimes dramatic, clinical status.

Materials and method: We present the case of a 53-year-old male admitted with severe general status and severe dyspnea, symptoms installed over the last week before presentation to the hospital. Several paraclinic investigations were performed: CT scan revealing bilateral pleural effusion, paraesophageal fluid accumulation with no direct connection with the pancreatic duct, laboratory exam finding leucocytosis and normal pancreatic enzyme levels.

Results: Surgical treatment has been performed, in several stages: both pleurae were drained with chest tubes (minimal pleurotomy), followed by the drainage of the migrated pancreatic pseudocyst through video assisted thoracic surgery. Medical treatment (rehydration, parenteral nutrition and antibiotherapy) had a major contribution to the patient’s favourable outcome.

Conclusions: Medical and surgical treatment for migrated pancreatic pseudocysts require a multidisciplinary team. When establishing the therapeutic conduct, several factors must be considered: biological status, the type and stage of the pancreatic disorder, and the presence of abdominal, thoracic or general complications.

Therapeutic options in primary spontaneous pneumothorax

C. Nistor, A.M. Iordache, G. Gogolan, Olivia Batog, D. Pantile

Introduction: Primary spontaneous pneumothorax is likely due to the formation of small sacs of air (blebs) in lung tissue that rupture, causing air to leak into the pleural space. Air in the pleural space creates pressure on the lung and can lead to its collapse.

Materials and method: The authors present several methods of surgical treatment for primary spontaneous pneumothorax. 82 patients were included in this study.

The most frequent surgical therapeutic options were minimal pleurotomy, lung apex resection and blebs resection, mechanical and chemical pleurodhesis and limited parietal pleurectomy, except for the minimal pleurotomy, all other surgical procedures were performed using minimally invasive thoracic surgery (video assisted thoracic surgery).

Results: Observing the postoperative outcome, the authors have elaborated several algorithms for surgical treatment for this condition (primary spontaneous pneumothorax).

Conclusions: Minimally invasive thoracic surgery is the most utilized surgical procedure for primary spontaneous pneumothorax, giving the best results with the shortest hospital stay.

Stabilization of post traumatic flail chest and fractured sternum using nuss procedure for pectus excavatum

A. Ciuche, D. Pantile, D. Marin, C. Năstase

Introduction: We present the case of a patient with a massive flail chest sustained in a car accident. Multiple rib fractures and a sternal transverse fracture were observed. We will describe a flail chest stabilization by minimally invasive repair of pectus excavatum (MIRPE), employing a Nuss bar.

Materials and method: A 40-year-old male patient suffering from shock, splenic rupture, post traumatic C5-C6 and C6-C7 vertebral disc hernia and flail chest resulting from a car accident was brought to the emergency department. After emergency splenectomy has been performed in another hospital, the patient has been transferred in our hospital. The intubated patient was taken to the intensive care unit (ICU).

The flail chest resulted in dyspnea, which was treated by positive end-expiratory pressure on ventilator. As the patient could not be weaned from the ventilator, chest stabilization was deemed appropriate.

We decided on reconstruction of the flail chest by MIRPE.

Results: A three-dimensional computed tomography scan was taken to locate the fractured ribs and the sternum fracture. A Nuss bar was designed before surgery. The reconstruction operation was performed on the 5th day. With the help of a video-assisted thoracoscopic surgery, an introducer was inserted through the fifth intercostal space into the thorax.

The cervical lesions were addressed in the same surgical session, performing C5-C6 and C6-C7 discectomy, as well as C6 corpectomy and rahisinthesys with plate, spacer and titan screws.

The patient was extubated after surgery and was discharged from the hospital on the 14th postoperative day without any complications.

Conclusions: We conclude that surgical stabilization of severe flail chest and fractured sternum with Nuss bar by MIRPE is a safe and effective therapy in properly selected patients.

This procedure can be performed in multidisciplinary surgical teams for polytrauma patients.

The modern treatment of wartime-like osteocartilaginous defects

M. Moga, Mihaela S. Lăpușneanu, M.E. Pogărășteanu, A.G. Barbilian

Introduction: In military medicine, wartime wounds are a main concern for any surgeon. Without the context of theatre of operations situations, preparation for such events and expertise are gathered through the treatment of wartime-like injuries.

Matherials and methods: This being said, we present the case of a patient who sustained a high-energy impact with a blunt metallic object (a steel pipe) at the level of his ankle. After initial treatment, the patient was left with a large osteocatillagenous defect at the level of the talar dome, along with a great amount of metallic debris. This impaired his ability to walk and run, and he was left with a painful joint that in time would evolve towards arthrosis and, in the end, a total loss of joint function. The decision was made to perform a mosaicplasty, a surgical technique in which we harvested bone and cartilage cylinders of various size from the patient's knee (non-weight bearing area), and used them to fill in the defect in his talar dome, thus restoring its original shape. During the same procedure we extracted most of the metallic debris.

Results: Following the surgery the patient was able to return to a normal pain-free life, and with a much better prognosis for the future.

Discutions: The mosaicplasty surgical technique, although primarily developed and used to repair the osteo-cartilaginous defects in the osteocondritis dissecans disease, may prove to be valuable for the military orthopedic surgeon, in the context of wartime traumatic lesions.

Rehabilitation in total paralysis of brachial post-traumatic plexus in adult – case report

D. Nedelescu, R. Alexandru

Objective: The total plex brachial paralysis is a condition that can become invalid if not treated properly and on time. The aim of this case report is to underline the pozitive functional outcome and importance of rehabilitation in the total plex brachial paralysis.

Method: We present the case of a 71-year-old hypertensive male with a history of ischemic cerebellar stroke (2013), prostate adenoma, who presented (12.12.2016) with motor deficiency of left upper limb, paresthesia at the same level, brachial-forearm hypotonicity and amyotrophy, vasculo-trophic hand disorders and severe functional impotence.

We mention that the symptomatology occurred after a trauma (06/10/2016) by falling from the same level, not being associated with other lesions.

Examining EMG (24.10.2016): Advanced axonal lesion of the entire left brachial plexus with the lack of voluntary activity in most of the examined sites; poor activity only in axillary nerve and median stg.

After an extensive functional evalation, the patient was integrated in a complex physical rehabilitation program, based mostly on kinesitherapy that initially followed stimulation of the paralyzed, denervated muscular tonus in order to prevent muscle atrophy by passive, passive exercises, exercises to promote stretch-reflex. With the increase in muscle contraction capacity, it was desired to increase muscle strength by using passive-active exercises, analytical exercises for each muscle, isometric and isotonic exercise with progressive resistance to achieve this goal. Of great importance was the prevention and treatment of vasculotrophic disorders. As a last resort, kinetotherapy has proposed to regain the functionality and ability of the upper limb.

Also very useful were electrotherapy sessions – electro-stimulation low frequency exponential currents for the main affected muscular groups and toning massage.

The patient had 3 admissions (December 2016, February and July 2017), 11 days each, in which it was possible to monitor the evolution and adapt the physiokinetotherapy program. During the complex rehabilitation program, the patient continued the symptomatic and neurological treatament.

Results: Following the rehabilitation program initiated, it was aimed at improving the proximal-distal volunteer motor control, increasing muscle tone and muscular strength, improving vasculotrophic disorders and significantly improving the strength and left hand fineness.

Examination of EMG (27.04.2017): Net improvement of neuromuscular function in electrophysiological and needle testing compared to previous examination.

For a favorable functional prognosis, at each discharge we recommended daily exercise.

Conclusion: The goal of rehabilitation in total brachial plexus paralysis is to improve and maintain functional independence, social reintegration and improvement of patient's quality of life.

OP session students 1

Specific actions of thyroid hormones on systems and organs

Anca Andrei

Introduction. Thyroid hormones have a very important role in the human body because they raise the basal metabolism and the consumption of energy, they stimulate neurogenesis and myelinisation, cortical tonus; they also have a role in morphogenetic processes of growth and differentiation, both cellular and tissular, this action being pregnant in the nervous system.

Analysis. As far as their functions are concerned, thyroid hormones have the following roles: they raise the metabolic actions in all the tissues in the body, thus being able to increase the basal metabolism with 60-100%; they stimulate the rate of protein synthesis and catabolism. Thyroid hormones also stimulate the growth and development of the brain during the fetal-life and first years of postnatal life. Moreover, they increase the intestinal absorption of glucose, hepatic degradation of glycogen, the synthesis of glycogen, but also the peripheral use of glucose. The global effect is hyperglycaemia. The increase of thyroid secretion lowers the concentration of cholesterol, phospholipids and triglycerides in the plasma; the oxidation process of fats in the cells is accelerated. The increase of tissular metabolism stimulates the production of heat, which leads to the necessity of eliminating it, thus being accelerated the cutaneous blood flow.

Conclusion. To sum up, the thyroid hormones main functions on systems and organs are: the hyper-secretion of thyroid hormones raise the force and the frequency of myocardial contractions, it stimulates the pulmonary ventilation and the respiration rate, due to the increase of basal metabolism and the consumption of oxygen. In addition to this, the high secretion of thyroid hormones stimulates the appetite, the secretion of digestive juice and the motility of gastro-intestinal tract.

Physiology and pathophysiology of physical activity

D. Cojocaru, M.C. Chițu, F.R. Badea, G. Pariza, M. Simion

We consider this subject is accessible to a student who has just finished the first year of Medical School, so this is why I chose to write about it. In this way, I would like to make my colleagues and those who will watch this presentation aware of the importance of having a healthy lifestyle and of the changes that occur in the human body while exercising.

In this project I will classify the efforts, the organ systems involved, but i will focus more on the muscular system, because it is of utmost importance in matters of the extent of metabolic processes. I will also present the characteristics of the muscle fibers depending on the type of physical effort.

A very important part is the modification of the structures and functions of skeletal and cardiac muscle induced by physical effort, which has mostly positive effects, but also negative effects.

Finally, I will insist on two of the most common pathologies amongst athletes who suddenly stop their activity that also affect common people who practice different sports for maintenance or for pleasure: Varicose Veins and Pectoral Angina.

The dietary supliments consumtion for military students versus civil students

Denisia E. Dabija, Iulia M. Staicu, B. Savu

Introduction: In medical literature there are many studies about nutritive supplements consumption for military. Nevertheless there are no conclude dates about consumption for military or civil students. The aim of this study is to evaluate the prevalence of using dietary supplements for military students in comparison with civil students.

Materials and methods: A questionnaire will be handled to military and civil students alike. The dates obtained through this method will be processed by EPIINFO program.

Results: The 30 military and 30 civil students will fill the questionnaire so that the frequency of use for nutritive supplements and the expected effects will be followed. The results and the dates from medical literature will be compared, and afterwards will be established how many students used dietary supplements before high grade admission and how many began to use them after, and also which are the most frequent motivations to use this kind of products.

Conclusions: After completion of the study, we will draw conclusions and they will be present in the conference.

Significance of B12 hypervitaminemia: clinical and pathophysiological aspects

Andreea Mutu, M.B. Maftei, Marina Oțelea

Hypercobalaminemia (high serum vitamin B12 levels) is a little understood anomaly, being considered until recently irrelevant from a clinical point of view, most of the studies having been focused on vitamin B12 defficiency. Ironically, hypercobalaminemia can be associated with vitamin B12 deficiency's signs and symptoms; in fact, it's about a functional deficiency, secondary to qualitative defects or tisular uptake.

The aetiological spectrum of high serum cobalamin levels comprises diseases with high morbidity and mortality, for which it is vital that we diagnosed them early: colon neoplasms, prostate neoplasms, hepatocarcinomas, and haematological malignancies (chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, primary hypereosinophilic syndrome, acute leukemias), liver and kidney diseases.

The degree of elevation of vitamin B12 has been correlated in some studies with the size of certain solid neoplasms. In stage IV cancers in palliative care, prognostic value has been attributed to B12/C reactive protein index (BCI). A BCI greater than 40000 was associated with 90% mortality at 3 months.

In this paper we are going to review the actual data regarding B12 hypervitaminemia, the most frequent aetiologies and their pathophysiological mechanisms that lead to B12 hypervitaminemia and also the diagnostic approach of hypercobalaminemia in order to identify and exclude the aforementioned pathologies.

The effects of chronic intake of alcohol and the evolution to hepatocellular carcinoma

Denise M. Dina, Monica Grancea-Iancu

Introduction: Europe is the region with the largest intake of alcohol per person, in the world. The alcohol induced hepatic disease has multiple manifestation levels of severity that often can coexist in the same pacient.

Case report: 47 years old patient is admitted with morning and postprandial sickness, characterized by vomiting, lack of appetite and loss of approximately 5 kilograms, in the last month.

On the clinical exam, the patient has a good general state, afebrile but with subicterical sclera. The abdomen is soft, and pain responsive in the right hypochondrium and epigastrium.

The liver has its inferior border at approximately 8 cm. under the costal arch.

The laboratory exams indicate elevated result of hepato-cellular carcinoma tumoral markers.

There are also modifications that diagnose pancytopenia and hyperbilirubinemia.

Results: While hospitalized, the treatment of choice consisted of Ranitidina, Gastrofait and Arginina, the general condition of the patient having turned a more favorable direction than in the moment of admittion. Alarmingly, the patient experienced episodes of withdrawl as a result of the lack of alcohol intake.

Conclusions: Patient's evolution is currently monitorised. The correspondence between the alcohol intake history and the hepatocellular carcinoma is made with the help of the alpha-feto protein, the most significant tumoral marker for this pathology, with elevated results in this patient s case.

Kainic acid intracerebellar repeated administration – A chronic mouse model of generalized dystonia

V. Morozan, Denise C.M. Zahiu, Laura Georgescu, Ioana Georgescu, A. Steopoaie, A. Pana, Ana M. Zagrean, Daniela Popa

Dystonia is a neurological disorder involving abnormal cerebellar signaling, as well as dysfunctions in the basal ganglia and in the cortico-thalamo-cerebellar loops. Acute or 48 hours continuous intracerebellar administration of kainic acid was proved to induce dystonic motor behavior. We demonstrated in our experiment that repeated applications of kainic acid into cerebellar vermis of mice for 5 consecutive days generate constant and reproducible dystonic motor behavior.

No epileptiform activity was recorded on electrocorticogram during the dystonic postures or movements. This new mouse model of dystonia can be used to assess the role of the cerebellum, as well as the role of motor and sensory cortex in the pathogenesis of dystonia.

Pulmonary thrombembolism

Filofteia A. Ghilencea, B. Savu

Introduction: Pulmonary embolism is the clinical entity, determined by the embolization of venous or right cord thrombi in the pulmonary arteries.

Material and method: The study of the specialized literature allowed the identification of the latest pulmonary embolism diagnostic algorithm based on exclusion and confirmation tests. It also follows from this study that the treatment of pulmonary embolism (produced in 90% of cases of deep vein thrombosis (DVT) located in the lower limbs or pelvis) should be guided by stratification of the risk of death and consideration should be given to the other comorbidities.

Conclusions: Pulmonary embolism is difficult to diagnose. There is now a safer diagnostic algorithm. Anticoagulation is the central therapeutic element, and thrombolysis is indicated in life-threatening pulmonary trombembolism.

The diagnosis and the prevention of sudden cardiac death in hypertrophic cardiomiopathy

Adriana E. Rosca

Background: Hypertrophic cardiomiopathy (HCM) is the most frequent hereditary heart disease, with a prevalence of 1:500.

Methods: In this scientific work, we summarize the information regarding the diagnosis and the prevention of sudden cardiac death, using a selection of articles and guidelines relevant to the clinical practice.

Results: Over 1400 mutations on 27 genes determine the phenotype of HCM. Two thirds of the cases are hereditary and genetic testing may be used as screening in families with HCM. The mutations affect most frequently the sarcomeric structures, leading to the specific histopathological aspect of fibrosis with myocardial fiber “dissaray”. The diagnosis is based on echocardiography and/or cardiac IRM discovery which can differentiate between the two forms of HCM: a more common, obstructive type (OHCM- 70%) and the non-obstructive type (NOHCM - 30%). The main symptoms are: dyspnea, angina pectoris, palpitations, dizzines and sometimes syncope. Sudden heart death may be the first sign of HCM, especially in young asymptomatic adults.

Conclusions: A correct diagnosis and the risk stratification in regard to the need of an implantable cadiac defibrillator (ICD) may be of life-saving importance.

The aesthetic treatment of dental dyschromias

Cozmina M. Cîrdei, Cristina Gurau

Introduction: Dental dyschromias are a well defined clinical entities, commonly encountered, caused by a multitude of local and systemic factors which can pose problems of physiognomy, the specialist being called to find out the causes and to solve the problem. For treating discolourations, multiple procedures can be indicated, from simple manual or professional brushing to teeth whitening or the application of composite resins veneers.

Material and method: This paper presents the current techniques used to treat dental dyschronomias:

- the current teeth whitening techniques, the factors affecting it, patient's perception, indications, contra-indications and its possible adverse effects. Teeth whitening is a conservatory solution for solving mild and moderate dental dyschromias occurring on vital or devitalised teeth.

- the veneer procedure which is used to solve some of the aesthetic defects by covering the entire vestibular face of the tooth with a physiognomic veneer. This method requires a minimum removal of the tooth enamel from the vestibular surface of the teeth.

- the manual and professional brushing which has a positive influence on the dental-periodontal structures, its objectives being to remove and interrupt the dental plaque formation and maturation cycle, to remove food scraps from dental surfaces and extrinsic colouring materials.

Objectives: This paper aims at orienting the patients towards choosing a correct treatment and with a minimal risk of dental dyschromias depending on its aetiology, depending on its dental periodontal status, raising awareness of the need for applying this type of treatment, its possible adverse effects.

Discussions and conclusions: The data provided attest the safety factor of the treatment followed strictly under medical supervision and once the importance of this safety factor is established, the success of a treatment for dental dyschromias by whitening is guaranteed. Teeth veneers made of composite resins and ceramic masses started to play an important role in Dental Cosmetics, the number of the situations suitable for this type of interventions being pretty good.

Multiple Casualties Incidents – The importance of early specific training of military medical personnel

T.C. Mureș, Maria M. Ancuța, R.A. Zamfir, B.S. Zamfir

Introduction: MCI has always been a challenge even for well-developed health systems. The geo-climatic and socio-economic current conditions clearly predispose us to increased incidence and magnitude of such events. Moreover, the current trend of the evolution of armed conflict to asymmetric and unconventional confrontations causes increasingly frequent incidents which involves civilians in armed military conflicts, representing an additional risk factor. In this context, the contribution of military medicine at the management of civil MCI can be particularly valuable both in human terms and in terms of capabilities.

Material: The sudden and unexpected onset, the major destructive effects, unpredictable developments and major impact both physical and psychological on the intervention personnel are the main features of the MCI. These characteristics superimposed over a high level of current activity destabilize the health systems. To ensure an adequate emergency response, procedures are conducted after specific and complex operation plans that differ substantially from those used in the current work routine. The knowledge and application of these procedures accurately by the staff involved is essential to ensure actional interoperability between the structures participating in the intervention.

Conclusion: Aquiring, maintaining and improving knowledge and skills needed to perform, in conditions of extreme physical and mental stress, the complex activities provided in emergency response procedures require completion of a gradual and continuous program of specific training. Ideally, this program will begin as early as possible in the medical career.

Basic medical attention in military operations

S. Politic, B.M. Cornea

In this project I will present the main stages of the TCCC (Tactical Combat Casualty Care) and how the medical staff reacts when a victim appears in an armed conflict. On the battlefield, the pre-hospital period is the most important time to care for any combat casualty. A significant percentage of deaths are potentially avoidable with a good treatment.

For a better understanding I will strat to explain the term TCCC and what does it mean, the 3 major targets: treat the casualty, prevent additional casualties, complete de mission. The medical treatment in the combat zone suffers some modifications from the civil one due to extrem conditions. The tactical decisions are combined with the medical.

I will present elementary notions about how to stop haemorrhage, stabilization of the victim for transportation, defensive actions to limit the number of casualties and methods for victim transportation.

We will insist on discussing about the medical actions necessary for victim stabilization till it will be transportated to the next medical echelon, tactical actions which comes to ensure the medical treatment and at the same time to limit the numer of the victims.

OP session students 2

Diagnosis and treatment of chylothorax

F.M. Caplea, D. Pantile

Introduction: The chylothorax defines a type of pleurisy resulting from the accumulation of chill fluid in the pleural cavity. The main lymphatic collector is the thoracic duct, the shedding of which occurs at the confluence of internal jugular veins and subclavicular veins. The lymphatic system originates from the lateral mesoderm, as a result of the development of the 6 lymphatic buds, originating in the inner layer of the nearby veins.

Material and method: This presentation is a synthesis of the works in the medical literature, which aims to highlight the importance of a correct treatment of the chylothorax, in order to maintain the essential function of the lymphatic system in the homeostasis of the body. Diagnosis of chylothorax is based on specific and non-specific investigation methods, of the lymphatic system, and their association with a simple biochemical and cellular analysis can guide us to establish the diagnosis of chylothorax.

The treatment of chill overflows varies depending on the cause and magnitude of lymph loss, which is why it is established in three stages: conservative, hormonal and surgical.

Objectives: The purpose of this work is to understand the importance of the correct diagnosis and treatment of the chylothorax, both for the relief of respiratory and cardio-circulatory phenomena, as well as to prevent the occurance of repercussions from the immune and nutritional point of view.

Discussions and conclusions: Although there is a variety of paraclinical investigations able to guide us to the diagnosis of chylothorax, its incidence has remained unchanged, some authors claiming a slight increase due to iatrogenic trauma.

Risk factors, diagnosis and surgical treatment of colon cancer

Corina M. Poşircă

Introduction: The colon cancer represents uncontrolled malignant transformation of the mucosal epithelial cells which tap the internal colonial lining face.

Diagnostic methods: At the bases of colon cancer diagnosis are different predisposing factors and some examples would be: colorectal polyps, various genetic factors (familial colonic polyposis, Lynch syndrome), nutritional factors (abuse of animal fat and proteins, red meat, alcohol, excessive caloric intake), inflammatory bowel disease (Crohn’s disease, ulcero-hemorrhagic rectocolitis).

Nowadays, the colon cancer represents the second cause of cancer death in civilized countries. The election method for colon cancer detection is colonoscopy. With the colonoscope the rectum, colon and also the last portion of small intestine can be examined. Besides colonoscopy, screening can also be completed using sigmoidoscopy, computer tomographic colonograpy and nuclear magnetic resonance.

Treatment: Surgical treatment of colon cancer is determined by tumor localization, local and distantly tumor spreading rate, the patient status and the associated comorbidities. Depending on the localization of the cancer, the possible surgical procedures include: left hemicolectomy, transverse colectomy, right hemicolectomy, sigmoidectomy, subtotal colectomy.

Evolution: Early diagnosis of this type of cancer means a favorable evolution for the patient. Lifestyle modifications, learning about genetic factors and the early discovery of colorectal polyps are also current preventive methods of reducing the risk of colon cancer.

Synchronous rectal and gastric adenocarcinomas. Case presentation

Anca I. Chetroiu, M.C. Chițu, Florina R. Badea, G. Pariza, M. Simion

Objectives: The development of synchronous colon and stomach cancer is particularly rare, accounting 3 to 5% of gastric cancer patients. The incidence of multiple primary adenocarcinomas in most of the studies was under 4.3% of gastrointestinal tumors.

The aim of this presentation is to review the management of patients with synchronous lesions, and to draw conclusions regarding their optimal diagnosis, treatment and evolution.

Methods: We report the case of a 67 year-old patient who presented lower gastrointestinal bleeding, transit disorders (constipation) and weight loss. Colonoscopy revealed a bleeding rectal mass of 6-7 centimeters, at 12-13 centimeters distance from the anus.

An abdomino-pelvic computer tomography was performed and no other pathological changes were identified in the gastrointestinal tract. An anterior protectomy with end-to-end colorectal mechanic anastomosis was decided in view of the diagnosis of rectal cancer.

During the operation, inspection of the abdominal cavity incidentally revealed the presence of gastric cancer and a liver tumor of 2/2 centimeters with a hemangioma aspect, on the underside of the left hepatic lobe. Subtotal gastrectomy with Billroth II end-to-side gastrojejunostomy and an Omega loop and Braun entero-enterostomy were performed.

Results: Histopathological examination revealed a moderately differentiated gastric adenocarcinoma (pT3N2) and a well differentiated rectal adenocarcinoma (pT2N0), both with lymph nodes metastases, and a hepatic hemangioma.

Nine months after the surgery, there is no evidence of recurring disease.

Conclusion: The synchronous carcinomas detected in a gastric cancer patient are most frequently located in the colon and rectum, followed by the lung, esophagus and liver.

Considering that the incidence of synchronous cancers is increasing, the present study cautions the necessity for a detailed and accurate preoperative staging, imagistic investigations and intraoperative examination to identify multiple primary tumors, in order to ensure an appropriate care, management and surgical intervention for these patients.

Difficulties in diagnosis and treatment of cholangiocarcinoma

A.D. Comber, M.C. Chițu, A. Mărginean

Introduction: Cholangiocarcinoma or bile duct cancer is characterized by the presence of tumors that develop from the intrahepatic or extrahepatic biliary epithelium.

Method and materials: We report the case of a 78-years-old patient who has been admitted in our clinic for complaints of pain in the upper abdomen and mesogastrium for two weeks and was scheduled for surgery. The lab results showed altered values of certain parameters (white blood cells, ESR, billirubin, urea). Imaging revealed a dilated bile duct, a distended gallbladder and gallstones of about 2 cm. Cholangiocarcinoma was suspected but a proliferative process of the gallbladder with lymphadenopathy in hepatic hilum was not dismissed.

Results: The decision was decided intraoperatory, depending on local conditions. The tumor was invading the surrounding organs making it impossible to resect. Instead a cholecisto-gastroanastomosis was performed which showed a slow favorable postoperatory evolution.

Conclusions: The biliary tract tumors generally have a poor prognostic with a low survival rate, independent of their status at the detection.

A severe complication of perforated ulcer

C.A. Ciobanu, M.C. Chițu, Florina R. Badea, Ș. Bogdan

Introduction. Because of the success of medical therapy in the management of peptic ulcer disease (PUD), surgery currently plays only a very limited role, and elective peptic ulcer surgery has been virtually abandoned. The most common complications of PUD include bleeding, perforation, penetration and gastric outlet obstruction. However, the annual incidence of ulcer perforation is on the order of 4 to 14 cases per 100,000 individuals.

Therefore, nowadays the surgical treatment and the postoperative care of perforated ulcer represent a challenge for the young surgeons.

Case report. We report a case of a 40 year-old patient who presented febrile state and colicky pain on the right flank and hypochondrium, with irradiation in the right shoulder. The symptoms began after an emergency laparotomy for perforation of duodenal ulcer, 3 weeks before this event, but the diagnosis and postoperative care were poor. He was submitted to our hospital unit, where an abdominopelvic computer tomography revealed a secluded subphrenic hydroaeric formation, of a cranio-caudal diameter of 180 millimeters. Also, the liver tests were modified and the blood count indicated an infectious condition. The antibiogram was positive for streptococcus D group and fungi.

Results. The diagnosis was interhepatophrenic abscess and the surgical indication was median laparotomy with evacuation and drainage of the abscess and abundant lavage.

Postoperative evolution. The postoperative evolution was good, with normalization of liver tests and blood counts after 4 days and no fever after the antibiotic treatment.

Conclusions. The incidence of perforated ulcer is very low today. It must be diagnosed and treated early because of the risk of sepsis given by the acute generalized peritonitis. The rarity of this pathology entails the lack of experience of young surgeons. The lavage of the peritoneal cavity is extremely important for a favorable postoperative evolution. Among the complications of perforated ulcer, the intraperitoneal abscess has a severe evolutionary potential and surgery must be supplemented by antibiotic therapy.

Myxopapillary ependymoma – case presentation

Roxana F. Stefan, Madalina M. Iancu, R. Nica, Florina Vasilescu

The aim of this paper is to present the case of a 22 years old pacient, with a gluteal tumoral formation that was diagnosed as a myxopapillary ependymoma after the anatomopathological examination.

The particularity of the case consists in the absolutely atypical localization of the tumoral formation in the gluteal region, even though it is known that myxopapillary ependymoma is a benign tumor, with a slow growth, deriving from glial cells from CNS (gliom).

The difficulty of the diagnosis was represented by both the lack of symptoms, despite the active tumoral process, and also the differential diagnosis with a pylonidal cyst.

Myxopapillary ependymoma is a rare tumor, which has an incidence from 0.05 to 0.08 per 100.000 people per year, with a higher occurence in men.

The histopathological test reveals tisular fragments with tubular and bulgy structures, which contains positive tumoral cells for immunohistochemical tests for androgen receptor and S100 protein.

The presentation reveals suggestive pictures for the disease's states of evolution from the beginning to the post-operative status.

Notions of anatomy and embriology that underlie laryngeal nerves preservation techniques

A.G. Florescu, A. Tulin

This paper aims to present the most important anatomical and embryological landmarks which should be taken into account during a thyroidectomy, and also, their usefulness in the preservation of laryngeal nerves. Thorough knowledge of the embryological development of thyroid and laryngeal nerves explains some constant relationships between different anatomical elements (e.g., the relative position of recurrent laryngeal nerve and Zuckerkandl tuberculum) and a look at the anomalies of the aortic arches offers us an explanation for variations in the course of the laryngeal nerves which are important to be considered during operation (e.g., "disfagia lusoria" which consists of a non-recurrent inferior laryngeal nerve on the right side of the body). This information forces the surgeon to perform certain techniques during thyroidectomy in order to minimize the risks concerning the injury of the superior laryngeal nerves (identification and dissection of the nerve before the ligation of the superior vascular pedicle, ligation of the vascular branches right on the thyroid capsule) and inferior laryngeal nerves (capsular dissection, delicate dissection of the nerve).

Moreover, there will be done a brief description of the intraoperative neuromonitoring technique (IONM), used to identify and dissect the recurrent laryngeal nerve and to assess a possible injury of it.

Difficulties in treatment of hydrocephalus caused by perinatal meningitis

E.S. Mîinea, M.C. Chițu, S. Stoica

Introduction: Hydrocephalus (HCP) represents an abnormal accumulation of cerebrospinal fluid within the ventricles of the brain, displaying an estimated prevalence of 1-1.5%.

Hydrocephalus is caused by subnormal CSF reabsorption, or rarely, CSF overproduction. Statistically, it occurs in 20 % of the cases presenting subarachnoid hemorrhage and 1% of the cases presenting meningitis.

Subnormal CSF reabsorption comprises communicating hydrocephalus as well as obstructive hydrocephalus.

Case report: An 11 year old patient, previously diagnosed with perinatal meningitis, was presenting an obstructive hydrocephalus at the age of 3.

Following a surgical procedure in a neurosurgical service, namely a ventriculo-peritoneal drain, imagistic exams are periodically performed. During the examination, the 4th ventricle was notably isolated via an obstruction of aqueduct of Sylvius, the Magendie foramen, as well as a bilateral obstruction of the Luschka foramen.

An endoscopic procedure is performed to recover the flow through the cerebral aqueduct and for the perforation of the pellucidum septum.

Five months after the procedure, the patient had headaches, balance disorder and increased fatigue.

Imaging examination revealed tetraventricular hydrocephalus, for which a surgical procedure called ventriculo-cisternostomy was accomplished.

Postoperative evolution: After the procedure, a cerebral MRI showed a flow artifact at the floor of the 3rd ventricle and the cerebral aqueduct, which would account for the patients positive evolution.

Carpal tunnel syndrome

V.G. Mitrică, C. Năstase, M. Mitrică, M. Tudose

Definition: Compression of the median nerve at carpal tunnel level.

The treatment consists in the decompression of the median nerve of the carpal tunnel by a surgical intervention (section of the flexors retinaculum ligament).

The paper presents 12 cases of patients with this symptomatology who were hospitalized in neurosurgery department from SUUMC Bucharest between June 2016 –

June 2017. We will present information about the surgical procedure including accidents, complications, results and evolution of the patient.

The evaluation of certain physical parameters used to determine the volumic status of the critical patient

Maria C. Sanda

Introduction: In this paper we aim to evaluate the volemic repletion of patients with burns (over 20%) using a number of physiological parameters: mean blood pressure, hourly diuresis, diameter and inspirational collapse of the inferior vena cava, oxygen extraction fraction, arterio-venous difference of $PaCO_2$, lactate value.

Methods and materials: The study was made on patients who had skin burns on a surface area greater than 20%. Volemic repletion was performed according to the Parkland protocol (4 ml/kg/area burned). Mean blood pressure was invasively monitored, diuresis was monitored on a daily basis, diameter and inspirational collapse of the inferior vena cava, oxygen extraction fraction, arterio-venous difference of $PaCO_2$ and lactate value were monitored at 4 hours for 48 hours.

Results: After analysis of the information we discovered a good correlation between the hourly diuresis, diameter and collapse of inferior vena cava and arterio-venous difference of $PaCO_2$.

Conclusion: In the monitoring of volumic repletion of the patient, besides classical parameters, mean blood pressure and hourly diuresis, we consider that the diameter and the inspirational collapse of the inferior vena cava and the arterio-venous difference of PaCO2 can bring extremely useful information for performing a proper volumetric repletion of the patient burned.

OP session nursing 1

Mistakes in communications between nurs and patient

B. Silvesan

Communication is a key element, with a major and vital impact in achieving quality care. For a good communication between nurse and patient, honesty and sincerity are essential.

The problem of communication is that we do not always manage to convey the desired message The errors that may arise in the communication nurse-patient may be due to internal factors (individual characteristics, type of personality, level of understanding, prejudice, religion) and external factors (social context, family context, the involvement of other health professionals, previous experiences).

The communication between nurses and patients may be adversely affected by certain factors: patient mistrust, difficulties of understanding, lack of attention, fear, etc. The communication nurse-patient must coincide with his current state, with possibilities of understanding and associated with supporting elements to positively influence the evolution of his illness.

The relationship nurse - patient involves mutual acceptance, an attitude of respect, warmth and empathy for the patient, it should not be limited to applying the treatment, but also to establish a psychological communication with him to help express his inner feelings.

Detox Inc

Mihaela Andrev, Narciza Draghichi

Ladies' desire to look good involves many quests and exertions.

Sometimes internet is overcharged by information and it becomes a real "fashion" for a diet or a practice. A simple search on Google offers thousand and one bright things, from VIP or from sports coaches. Only few are from nutritionists and less from doctors. It is obvious that everybody is acquainted with the detox process. Any excess or pseudoscientific approach can cause great damage, the greater the appearances may be misleading.

Besides the common myth: "It is a plant, it does nothing wrong", there are many other statements that cannot be verified or sorted so as to provide the assuredness of some valid arguments: what can be harmful to drinking natural juices and eating "healthy"? So, the concept started here in 2000's has become the latest fashion in terms of health. Out of control, things can become serious for the health of those who experience.

That's why it's better to carefully check before trying on your own skin in order to avoid unpleasant or serious consequences. Correct and verified information from authorized sources, and not the "reliable" ones, is the one that keeps us safe for "unlimited liability company" from the point of view of assuming the consequences by those involved in this industry.

The management of radiation skin reaction

Mariana Iscru, N. Bocaniala, A. Toma, S. Vlad

Objectives: Radiotherapy is a treatment modality for many types of cancer and more than 75% of oncology patients will receive it as a component of their curative or palliative care.

The main goal of treatment is to irradiate tumor cells while minimizing damage to normal tissue, including skin. However, normal skin cells in the radiation field may also be damaged by radiation exposure. Typically, these are capable of self-repair, but a grade of side effects we usually find in our patients.

The manifestations of radiation skin reaction can range from erythema (redness, rash-like appearance), to dry desquamation (dryness, itching), and moist desquamation (tender, redness and exposure of the dermis) and necrosis.

Methods: We present few typical cases from our activity (breast cancer, head and neck cancer) focusing on clinical assessment, prophylaxis, general management guidelines and results of local treatment in acute radiation skin reactions.

Conclusions: The incidence of radiodermatitis with new technology (as 3D-conformal radiotherapy we use in our department) is lower than we observed with 2D technique (cobalt therapy), but 95% of patients have reported some degree of skin reaction to radiotherapy. The use of these guidelines for prophylaxis and treatment of radiodermatitis has effect on patient's quality of life.

OP session nursing 2

Tackling the problem of healthcare associated infections

Carole Hallam

Health care-associated infections are the most frequent adverse event in health-care delivery worldwide. Of every 100 hospitalized patients at any given time, 7 in developed and 10 in developing countries will acquire at least one health care-associated infection (World Health Organisation 2017). It is estimated that over 4 million patients are affected by at least one healthcare associated infection across Europe each year (ECDC 2013). With the increase of antimicrobial resistance micro-organisms world wide infection prevention and control has never been so important.

In 2005, the Department of Health in England set ambitious targets for the reduction of MRSA and additional targets were set in 2007 for the reduction of Clostridium difficile. This presentation will share some of the initiatives that were developed and implemented in English hospitals to reduce our infection rates.

The unseen face of the heart – right heart

Systemic consequences of right ventricular failure

Ana M. Vintila

Right ventricle failure can occur as an acute or chronic dysfunction in right heart or/and lung disease. It can develop as a spontaneous condition or can arise during an invasive or surgical maneuver. The acute decompensation of the right ventricle usually determines various degrees of hypotension, while the chronic decompensation generates a wider systemic response with lung, hepatic, renal involvement as well as inflammatory response.

The acute right heart decompensation is due to lack of compliance of both right ventricle and right atrium and the systemic venous response is immediate. In case of a chronic decompensation, the mechanism is more complex, with tricuspid annular distortion, right ventricle free wall hypertrophy and secondary tricuspid regurgitation.

Systemic consequences of right ventricle failure are usually represented by a wide spectrum of liver and renal failure syndromes as well as neurological vascular disturbances. An inflammatory response is noted and is associated not only to organ stasis, but also to bone marrow stasis.

A systemic response in case of right ventricle failure worsens the vital prognosis. Treatment of the underlying cause of ventricular failure might improve prognosis without annihilating the vital risk. One should consider liver and/or renal oriented therapies to alleviate this poor prognosis.

Pulmonary hypertension

Roxana Enache

Pulmonary hypertension (PH) is a complex syndrome that may complicate various cardiovascular, respiratory and systemic disorders.

Once considered an orphan disease, currently things have changed due to major progress recent years have seen towards the understanding of this complex and multidisciplinary disorder. PH is defined as an increase in mean pulmonary artery pressure (PAP) ≥25 mmHg at rest as measured invasively by right heart catheterization.

The term primary PH was used to define an idiopathic increase in pulmonary vascular pressure while secondary PH was employed for all the forms of PH in which an underlying cause could be identified. Nowadays, according to common pathophysiological and therapeutic characteristics, 5 clinical groups of PH are defined.

Pulmonary arterial hypertension (PAH) or group 1 of PH includes besides idiopathic and heritable PAH, different other forms of PAH associated with connective tissue disorders, congenital heart diseases, toxins and drugs, HIV infection and portal hypertension. The common feature of these forms is the involvement of the distal pulmonary arteries.

In PH, the right ventricle (RV) has to adapt to a dual pressure overload composed by the fixed pulmonary vascular resistance and the pulsatile pressure overload due to the pulmonary artery stiffness. The RV function is the main determinant of outcome in PH patients. Based on clinical suspicion or the screening of high risk population, the echocardiography establishes the probability of PH while the right heart catheterization is mandatory to confirm the diagnosis of PH, to assess the severity of haemodynamic impairment and to perform pulmonary vasoreactivity testing in selected patients PAH and to assess the response to PAH treatment or to confirm disease worsening.

Other diagnostic tests are useful to identify the specific forms of PAH and PH and to test the exercise capacity of these patients. Treatment of PAH has evolved considerably over the past three decades, in part due to the advances in knowledge of the disease and the availability of agents that target known pathways in the disease pathobiology.

Despite this real progress, PAH remains a chronic progressive disorder. Current therapeutic approaches are medical therapy, interventional and surgical procedures.

Right heart in the Cath lab

V. Vintila

There are 40 years since first PCI was performed on man. In the classical era of the Cath lab, the vast majority of procedures were coronary angiograms followed by vascular resistance studies. The modern era of echocardiography diminished the role of pressure exploration of the heart in establishing the correct diagnosis of vascular malformations, with a shift towards therapy. We will look into special tricuspid techniques which avoid surgical repair or replacement of the valve and also in special techniques used to close septal defects. Special tricuspid valve clips are currently under evaluation for patients with severe tricuspid regurgitation in which the cardiovascular surgical risk is severely elevated.

For children, closing techniques and devices for patent ductus arteriosus are widespread and avoid an unnecessary surgical closure and possible systemic complications.

Usually these special angiographic techniques request double vascular access, and because of the bulkiness of the delivery systems are still designed for femoral access.

Right ventricular dysfunction in cardiac surgery

O. Lazar

Right ventricular failure involves a great challenge due to the severity of this condition. In cardiac surgery RV failure is frequently associated with congenital disease and represent a high incidence among cardiac transplant patients when represent the main cause of graft failure.

Appropriate hemodynamic monitoring and advanced pharmacological and mechanical support can reduce perioperative mortality in RV perioperative failure patients.

Early postoperative care involves special measures to overcome compromised hemodynamics in RV failure cases.

Urosepsis

Internal medicine approach in urosepsis

Anca Ghiatau

Urosepsis is a severe infection of the urinary tract in females or/and genital tract in males. Almost a quarter of sepsis causes is the urinary tract. In order to reduce mortality the patient has to be carefully managed interdisciplinary between the infectious diseases specialist, the urologist, the intensivist specialist with the aid of the microbiology laboratory and the diagnostic imaging tests.

The first 2 definitions of sepsis – 1991, 2001 – sepsis implicated bacteremia or a clinic suspicion of sepsis and the addition of at least 2 of the SIRS (systemic inflammatory response syndrome) criteria. Sepsis was classified as severe when we add one of the MODS (multiple organ dysfunction syndrome) criteria, with the circulatory failure defining the septic shock.

In 2016 the definition of sepsis changed – Sepsis-3, excluding the SIRS criteria, due to the fact that it lacks specificity and sensitivity for diagnosis. Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection.

The Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score is used to asses organ dysfunction and the term severe sepsis is now out of use. Septic shock is that stage of sepsis associated with profound circulatory, cellular, and metabolic abnormalities and it can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia.

Sliding through this change in definition – the treating physician – must bear in mind that sepsis is life – threatening and a rapid diagnosis decreases mortality. This being said it is mainstay to search for a cause of persistent infection- proper and repeated imaging when necessary, careful clinical monitoring and prompt antibiotic therapy, urologic interventions, supportive and adjunctive treatment using the principles of early goal directed therapy.

Surgical management in urosepsis

D. Marcu, D. Mischianu, O. Bratu

Introduction: Urosepsis is one of the most frequent causes of death world-wide, accounting for approximately 25% of all causes of sepsis. There are numerous pathologies that favor urinary tract infection. According to literature the most common pathogens involved in the appearance of urosepsis are: E. coli, Proteus spp., Enterobacter spp., Klebsiella spp., Pseudomonas aeruginosa and Gram-positive pathogens like Enterococcus.

Objectives: The purpose of this paper is to underline the role of minimal invasive urological surgical techniques in the management of urosepsis, techniques that aim at eradicating the cause of the urosepsis.

Material and method: The control of the septic source is an important step in the management of this pathology, as well as volemic and electrolytes rebalancing and antibiotic treatment. A simple gesture like inserting a urinary catheter or suprapubic cystostomy may prove to have spectacular effects in patients with urinary retention. For the cases with ureteral obstacles and suspicion of urosepsis the insertion of a ureteral double j stent or percutaneous nephrostomy can significantly improve the patients general status. Ultrasound or CT guided puncture and drainage of a renal abscess should be preferred in patients with high operatory risks and general altered state.

Conclusions: Urosepsis is an important medical and surgical pathology, its management being a challenge for physicians and imposing a good collaboration between different medical specialties like Urology, Imagistics, Intensive Care and Infectious Disease specialty.

Vital support in urosepsis

Ioana S. Oprea, D. Corneci

Introduction: The urosepsis is a real health problem and occurs in 25% of sepsis cases.Therapeutic approach must be a multidisciplinary one, targeted both on the treatment of the cause but also to vital suport, simultaneously with decreasing the damage of other organs and systems.

Materials and methods: Most important is the direct and aggressive individualized care that each patient received, including early bacteriologic cultures of blood and urine, early and correct antibioprophilaxis, restoration of blood pressure, protective ventilation and obtain adequate organ system function and interrupt the progression to multiple organ dysfunction syndrome.

Results: After urological control of the infection source, we are using the volemic resuscitation therapy in order to normalize venous central pressure, blood pressure, diuresis and other biological markers. The patients are responding well at the treatment depending of initiation of volemic resuscitation, cardiovascular, respiratory and renal support, antibiotherapy etc.

Conclusions: Vital support in patients with urosepsis and urinary septic shock must be done in intensive care units and requires a good colaboration with urologist and infectionist.

The earlier you are treated for urosepsis, the better.

Antibiotic treatment of urosepsis: steps and criteria for monitoring treatment response

V. Gheorghita

Introduction: Sepsis is a critical life-threatening organ dysfunction induced by a complex and dysregulated inflammatory host response to a microbial aggression. Urosepsis accounts approximately 25% of all sepsis cases, and is in most cases due to complicated urinary tract infections. The prompt recognition of this condition and appropriate management are associated with lower mortality. In one study, each additional hour of delay of the antibiotic treatment in urosepis was found to decrease the survival rate by 7.6%.

Material and method: We did a review of the recent published data about management of urosepsis in order to describe the best treatment strategy of this condition, and to individualize therapy according to the epidemiological data reported in the Central Military University Emergency Hospital "Dr. Carol Davila" from Bucharest, Romania. There are defined four major objectives in the treatment of urosepsis: early diagnosis, early antimicrobials therapy, identification and control of the complicating factor in the urinary tract and specific sepsis therapy. The current antimicrobials treatment comprises two phases in the clinical practice. The empirical phase of the treatment extended for the first 72 hours which have to be fast and hit hard. The antimicrobials spectrum is tailored based on the individual characteristics of the patients: age, immune status, surgical history and antibiotic exposure in the last six months. In the second part of the treatment is recommended de-escalation or adjustment of antimicrobial spectrum according to susceptibility of isolated pathogen. Besides the traditional criteria involved in the treatment monitoring of septic patients, the procalcitonin-guided therapy is a better and safer algorithm in reducing the duration of antibiotic therapy with no effect on 28-day mortality.

In conclusion, the urosepsis is an emergent condition whose management requires an interdisciplinary approach, including urologist specialists, intensive care and infectious disease specialists, to individualize treatment and achieve the desired outcome.

Robotic surgery

Minimally invasive robotic surgery in benign pathology of the esogastric junction

F. Săvulescu, C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin

In the era of mini invasive surgery, the surgical approach of the esogastric junction occupies an important role, which regards both the results and the complete, long-term patient satisfaction. The main benign pathology of the esogastric pole includes hiatal hernia, gastroesophageal reflux disease, cardiospasm, oesophageal diverticula. At the moment, there is no room for doubt that minimally invasive surgery is the gold standard for treatment of achalasia and hiatal hernia. It is believed that robotically-assisted surgery's most notable contributions are reflected in its ability to extend the benefits of minimally invasive surgery to procedures not routinely performed using minimal access techniques. The additional advantages afforded by the use of minimally invasive surgical techniques, coupled with the desire to retain the natural ergonomics and visual advantages of open surgery, have propelled the development and progression of robot-assisted surgery which may allow surgeons to overcome many of the laparoscopy surgery difficulties: loss of depth perception, loss of natural hand eye co-ordination, loss of intuitive movement and loss of dexterity.

Conclusion: We believe that using the robot for those "difficult" cases, such as giant paraesophageal herniases, would be of most benefit from robotic surgery.

Minimally invasive robotic surgery in benign gynecologic disease

F. Săvulescu, C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin

Minimally invasive surgery has revolutionized the management of benign gynecologic disorders over the last 30 years. However, the most substantial improvements have come with the advent of robotic surgery. Initially, traditional laparoscopy afforded less invasive approaches to hysterectomies, myomectomies and adnexal surgery. However, not all surgeons are comfortable with the laparoscopic approach due to its steep and extended learning curve, nor are all patients and procedures amenable to traditional laparoscopy. In fact, the majority of advanced gynecologic surgeries are still being performed through an abdominal incision. Several studies concluded that robotic myomectomy and to hysterectomy for bening lesions is superior to laparoscopic and open procedures in terms of morbidity rates, esthetic results, adhesions, recovery, surgical accessibility (when compared to laparoscopic and not open surgery), and quality of sutures.

Conclusion: The use of robotics for benign gynecologic conditions increased in the past years. As this technology evolves and diffuses into practice, we should continue to examine the comparative effectiveness of robotic surgery in benign gynecologic disease.

Minimally invasive robotic surgery in malignant gynecologic disease

F. Săvulescu, C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin

Robotic surgery for the management of gynecologic cancers allows for minimally invasive surgical removal of cancer-bearing organs and tissues using sophisticated surgeon-manipulated, robotic surgical instrumentation. Now, robotic surgery represents an effective alternative to conventional laparoscopy. Since its widespread adoption, minimally invasive surgery has become an option not only for the morbidly obese but for women with gynecologic malignancy where conventional laparotomy has been associated with significant morbidity. When compared to the traditional radical hysterectomy performed via laparoscopy, the robotic approach allows for less blood loss and a shorter hospital stay at the cost of slightly increased procedure times.

Conclusion: Minimally invasive surgical techniques in gynecologic oncology have evolved greatly since the introduction of Da Vinci robot into the field. The advantages of robotic surgery over laparotomy, in the appropriately selected cancer patient, have proven benefits to the patient both intraoperatively and postoperatively, with similar outcomes. The fundamental aspects that are perhaps keeping this surgical approach from becoming more widespread are resources and surgeon skill and comfort level to complete extensive staging procedures. The advent of robotic-assisted surgery appears to offer the bridge between improved patient perioperative outcomes and surgeon ergonomics.

Minimally invasive robotic surgery in colon and rectal cancer

F. Săvulescu, C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin

Laparoscopic rectal surgery continues to be a challenging operation associated to a steep learning curve. The conventional laparoscopic approach to colon and rectal surgery has several limitations, and therefore many colorectal surgeons have great expectations for the robotic surgical system as an alternative modality in overcoming challenges of laparoscopic surgery and thus enhancing oncologic and functional outcomes. This presentation explores the possibility of robotic surgery as an alternative approach in laparoscopic surgery for colon and rectal cancer. The da Vinci® Surgical System was developed specifically to compensate for the technical limitations of laparoscopic instruments in rectal surgery. The robotic colorectal surgery is associated with comparable or better oncologic and pathologic outcomes, low morbidity and mortality, higher probability of autonomic nerve preservation and genitourinary function recovery. Furthermore, in very complex procedures such as intersphincteric dissections and transabdominal transections of the levator muscle, the robotic approach is associated with increased performance and safety compared to laparoscopic surgery.

Conclusion: The robotic surgery for rectal cancer is an advanced technique that may resolve the issues associated with laparoscopic surgery.

Single-site minimally invasive robotic surgery in cholelithiasis

F. Săvulescu, C. Cîrlan, C. Blăjuț, I. Budrugeac, R. Marin

Laparoscopic single-incision surgery is fraught with significant technical drawbacks but has witnessed increased growth mainly for its presumed aesthetic advantages. Recently, a single-site robotic platform has been introduced to alleviate some of the technical challenges with laparoscopic single-site surgery. While the cosmetic result of single-site laparoscopic surgery is self-evident, the technique suffers from loss of ergonomics. This is due to the lesser degree of freedom offered by laparoscopic instruments, compounded by working in a parallel setup with lack of first assistance and an unstable visual field. The robotic platform can help to address the limitations of the laparoscopic technique by providing a stable visual field, with movement of the arms switched by computerized inversion and instruments that offer a higher degree of freedom. The da Vinci Si system software automatically detects and reassociates the surgeon's hands with the instruments, giving the console surgeon the perception that the left hand is controlling instruments on the left side of the visual field while the right hand is controlling instruments on the right side of the field.12 Of all the available features, this is an undeniable advantage of the system that makes the approach simple, intuitive, and plausible even for a trainee.

Conclusion: The single-site robotic cholecystectomy is feasible and safe to perform in patients with acute or chronic cholecystitis, with good short-term perioperative outcomes. This technique can be used to train residents and surgical fellows, although long-term rates of incisional hernia and cost associated with the procedures still need to be analyzed. Continued use of this platform will also determine if it is possible to perform more complex cases with a single incision.

Initial experience with robotic-assisted laparoscopic radical prostatectomy in Central Military Hospital

M. Dinu, F. Săvulescu, A. Aungurenci, V. Botea, V. Mădan

Introduction: Robotic-assisted laparoscopic radical prostatectomy has gained popularity in the last two decades due to its superior 3-dimensional magnified vision and improved manual dexterity for surgeons. In this way robotic surgery facilitates the transition from standard open surgery to minimal-invasive surgery. We report our initial experience with the use of the da Vinci robot for the minimal invasive treatment of prostate cancer.

Materials and methods: Between February and June 2017 we have performed 10 robotic-assisted laparoscopic radical prostatectomies. All 10 patients were previously diagnosed with prostate cancer by biologic, imagistic and pathologic means. We have prospectively studied the perioperative parameters and early surgical outcome of our patients.

Results: The mean age at diagnosis was 65.5 ± 5.7 years. Based on the pathology examination of the biopsies, the Gleason score was 6 (n=5) or 7 (n=5) thus including the patients into low respectively intermediate risk prostate cancer according to d'Amico criteria. Mean preoperative prostate-specific antigen (PSA) level was 9.1 ± 2.33 ng/mL. The mean operative time was 238 ± 48.3 minutes. Perioperative blood loss was about 200 ± 73.7 mL but no patient required blood transfusion. We did not encountered intraoperative complications that required conversion to open surgery. Only one patient had a postoperative complication that required open surgery in the 3rd postoperative day. The mean hospital stay was 4 ± 1 days except for the patient that developed postoperative complication. The mean duration of bladder catheterization was 11.4 ± 2.7 days. Al patients received low molecular weight heparin for 21 days postoperative.

Conclusion: Robotic prostatectomy is a safe and well standardized procedure that confers the benefits of enhanced precision and dexterity for complex laparoscopic work in pelvis.

Obesity, interdisciplinary approach

Obesity – a leading cause in non-alcoholic fatty liver disease

Florentina Ionita Radu, Andrada L. Popescu, I.P. Nuta, Raluca S. Costache, Mariana Jinga, Sandica Bucurica, B. Macadon, M. Patrasescu, Maria M. Chereja, Gaudia V. Manescu Avram, A.I. Gavrila

Introduction: Worldwide obesity has more than doubled since 1980, with approximately 13% (600 million) of adults having BMI >30 kg/m2 as of 2014, making it one of the leading current health problems. One of obesity`s many complications, NAFLD (non-alcoholic fatty liver disease), can progress to more aggressive and potential fatal forms of liver injuries, so more attention should be given to this pathology by healthcare workers.

Materials and methods: Due to an imbalance between complex metabolic interactions in obese patients, excessive amounts of triglycerides get deposited in the hepatic tissue and steatosis (the hallmark feature of NAFLD) is born. In time these changes can lead to inflammation and hepatic cell damage, condition known as NASH (non-alcoholic steatohepatitis), which is a precursor state for liver cirrhosis, the ruling cause of hepatocellular carcinoma.

Although liver biopsy and histology is considered to be "golden-standard" in diagnosing steatosis, in recent years more accessible and easy to use methods for quantifying fatty liver deposits have become available, with transient elastography plus controlled attenuation parameter, FibroMax (SteatoTest, NashTest) and newer IMR techniques being in a constant battle to become the new standard.

It is very important to detect NAFLD and NASH in obese patients so that proper diet and treatment can be established, and so breaking the chain of events leading to severe liver afflictions.

Results and conclusion: Non-alcoholic fatty liver disease is emerging as a very important health problem in part due to an alarming rise in obesity incidence, meaning that a proper diet and weight loss can be the best solution.

Aorta from valve to bifurcation

Endovascular treatment of abdominal aortic aneurysms

I. Droc, M. Dumitrascu

Purpose: to assess the short and medium term safety and efficacy of endovascular abdominal aortic aneurysms repair (EVAR) in high-risk patients.

By helping patients avoid abdominal surgery, EVAR minimizes the perioperative impairment of cardiac, pulmonary, renal and gastrointestinal function. The greatest potential benefit is in high-risk patients who have large aneurysms and who are poorly suited to any of the current surgical alternatives.

Material and method: We report the results of 55 elective EVAR (endovascular abdominal aortic repair) procedures performed in two vascular surgical centers in Romania and Germany. The mean follow up was 18 months with CT-scan, duplex ultrasound and contrast-enhanced ultrasound

Results: The prosthesis used were 16 E-vita Abdominal XT, 12 Excluder, 8 Talent, 7 PowerLink, 3 Endurant and 9 custom made fenestrated or branched from Jotec. Primary-assisted technical success rate was 100%. There was 1 non AAA-related late death. Conversion to open repair was performed in 1 case, as a late conversion for a type III endoleak (at 15 months after EVAR) with aneurysm sac enlargement >8mm.

Conclusion: These results show that in the modern era of abdominal aortic aneurysm treatment EVAR is safe and effective in high-risk patients, at least during the short to intermediate term.

Hospital management of multiple casualties' incidents

Multiple trauma management in the Emergency Department

C.B. Teusdea, S. Dogaru, Ana I. Gheorghescu

The World Health Organization (WHO) has identified trauma as the major health care challenge of our century. Because trauma affects predominantly young population, it is responsible for more productive life years than any other disease. Treating trauma requires a combination of strategies consisting in prevention, intensive care and recovery. The patient's survival is dependent on the teamwork between different medical or surgical specialties all along their pathway care: the pre-hospital team, the in-hospital teams (emergency medicine, anesthesia, surgery, critical care) and finally the rehabilitation teams. For an efficient management there are four key areas which are closely linked and integrated by communication: teamwork, task management, situational awareness, decision making. In this paper we propose a review concerning the main stages of multiple trauma management, which represents the basis for investigation and diagnosis integrated of trauma patients.

Hospital management of mass casualty incident

C.B. Teusdea, F. Costea, M. Toma, S. Dogaru

Mass casualty incident with a large influx of victims are inevitable. A correct and careful risk assessment and an efficient planning of activities and resources will allow the impact of these situations to be reduced to the basic activity of the hospital and the quality of healthcare. Much of these situations can be managed using resources that work redundantly to mitigate the expected effects of the emergency.

Beyond the proper management of risks, the introduction of an alert and evaluation system is extremely important. These systems, together with precautionary measures related to unidentifiable risks, will help prevent traps that can occur after a crisis has occurred.

Communication systems in the event of disasters or in major emergencies may be affected. Under these circumstances, the White Plan sets out the main means of communication that must / can be used.

WHITE PLAN represents the response plan of bedside units in the event of a massive influx of patients following a collective accident, a calamity, epidemic or pandemic. The WHITE PLAN allows the management of emergencies outside the hospital, requiring additional human, logistical, financial, and appropriate training.

The course addresses to medical specialists and nurses and intend to make introduction to the White Plan's overall framework, explain what does the WHITE PLAN mean, present the crisis cell at the ED and at the hospital level, and organization of receiving multiple emergencies, explain the Triage of Patients and Triage Card, and finally present some concept regarding to decontamination in case of nuclear, chemical and biological accidents.

Management of traumatic brain injury algorithm in the Emergency Department

C.B. Teusdea, M. Toma, M. Salceanu

An enormous public health problem is representing by traumatic brain injury (TBI) even with modern medicine in the 21st century, that’s why a management algorithm is very important. Most patients with TBI (75-80%) have mild head injuries; the remaining injuries are divided equally between moderate and severe categories. Almost 100% of persons with severe head injury and as many as two thirds of those with moderate head injury will be permanently disabled in some fashion and will not return to their premorbid level of function. TBI may be divided into 2 broad categories, closed head injury and penetrating head injury, and treatment presents some important differences. Also, closed head injury treatment is divided further into the treatment of mild, moderate, and severe head injuries.

In this paper we propose a traumatic brain injury management algorithm to ease the diagnosis and treatment of this type of trauma.

Politrauma patient

Polytrauma management – the general surgeon’s approach

C. Musat, R. Nica, O. Albita, C. Dutu, T. Rogin, D. Orosan, C. Cirlan, Elena Starcu, A. Lica, A. Luchian, C. Blejut, Madalina Piron, Lavinia Raboj, R. Dragos, F. Savulescu

Polytrauma cases require almost always a multidisciplinary approach, after the patient’s admittance to the E.R. The team is usually conducted by the Trauma Surgeon, or the General Surgeon.

Various aspects of the specific pathology and treatment are discussed: BLS, ATLS and beyond, means of stabilisation, as well as primary, secondary and further surgical treatment, especially for abdominal trauma.

The surgical procedures, meant, in the early stages, to provide life support and stabilisation, are detailed.

The conclusions are devised towards the need for an accurate and highly aggressive surgical approach, as the mean to improve the outcome of polytrauma treatment, including the need for specific training in

Trauma Surgery and the development of Trauma Centers.

Melanoma

A clinical and dermatoscopical algorithm for the estimation of the Breslow index in primary cutaneous melanoma

A.M. Țilea, V. Trifu, Monica S. Dărmănescu, Mihaela Georgescu, Marcela Poenaru, Raluca I. Săftoiu, G.S. Țiplica

Introduction: The clinical and dermatoscopical estimation of the Breslow index (Bi) can be of paramount importance in the management of Primary Cutaneous Melanoma (PCM). Such estimation, if performed accurately, would greatly improve the management of PCM.

Material/methods: We have created an algorithm based on the clinical and dermatoscopical evaluation of the PCM. This algorithm consists of a first phase in which a highly accurate diagnosis of PCM is obtained. A second phase of the algorithm will estimate the Bi of being less than 2mm versus ≥2mm. This estimation led to the management of the PCM as follows: 1. tumours with estimated Bi of ≥ 2mm were excised with 2cm margins; 2. tumours with the estimated Bi of < 2mm were excised with 0,5cm margins; 3. when the final histopathological results arrived the tumours with Bi values between "in situ" and < 2mm were reexcised for curative margins according to the guidelines.

Results: Using this algorithm 69.7% of the cases were treated with curative margins from the first excisional biopsy. The rest of the 30.3% of the cases required reexcision according to the histopathological Bi per the guidelines.

Conclusions: These results highlight the importance of a preoperative algorithm in the management of PCM. In our study more than 2/3 of the patients were treated with curative margins from the first excisional biopsy. Only 1/3 of the patients required a second surgical intervention for curative margins.

Sentinel lymph node biopsy – an important prognostic indicator in patients with melanoma

B.M. Marinescu, C. Mazilu, D. Muraru, I. Guzganu

Sentinel lymph node represent the first regional lymph node that drains the lymph from the primary tumor. It is potentially the first node to receive the seeding of lymph-borne metastatic cells. Practical approach of SLNB include: lymphoscintigraphy, blue dye, gamma probe, +/- preoperative ultrasound and palpation.

SLNB represents a minimally invasive procedure, used to accurately stage nodal basins at risk for harboring occult metastases. It became a standard procedure performed to identify lymphatic metastases for melanomas greater than 1 mm in thickness, in an effort to better classify patients for prognosis and treatment regimens.

SLNB allows for a less extensive surgery to be performed, which has fewer side effects and a lower morbidity rate compared to more radical surgery (total lymphadenectomy).

By performing SLNB: it improved accuracy of staging; it can be optained one of the most important predictor of survival rate; it can provide limited specimen that can be examined in greater detail.

Patients with nodal metastases can be identified to undergo early therapeutic lymph node dissection and adjuvant therapy (patients with positive SLNBs are considered for adjuvant therapy with IFN or vaccines).

Radiotherapy in the management of melanoma

S. Vlad, G. Ricu, M. Matei, G. Balasa, M. Dumitrache, Alina Tanase, Irina Carnutu, A. Anghel

Historically, malignant melanoma was considered a relatively radioresistant tumour, but at present radiotherapy is recognized effective as palliative or locoregional therapy, part of the multidisciplinary management of the disease. Because of the developments in systemic and targeted therapy (preffered option in most clinical situation, in association with surgery) the use of radiation therapy is reserved for palliation or consolidation of other treatments.

We present the indication of radiotherapy as:

1. Palliative radiotherapy for metastatic disease (brain metastasis, bone metastasis associated with spinal cord compression, bone pain);
2. Regional treatment for nodal invasion (cervical, axillary, inguinal nodes);
3. Main treatment modality, replacing surgery or for local reccurences.

Radiosurgery (SRS) and Stereotactic Body Radiotherapy (SBRT) can be used in ablating oligometastatic disease in particular cases.

Response of melanoma to irradiation depends on initial tumor volume, total radiotherapy dose and fraction size (radiobiological considerations).

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EDITORIAL AND CONTENT CONSIDERATIONS

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, metaanalyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) accessible to the public at no charge;

(2) searchable by standard, electronic (Internet-based) methods;

(3) open to all prospective registrants free of charge or at minimal cost;

(4) validates registered information;

(5) identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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REVIEW

Non-alcoholic Steatohepatitis Diagnosis and Treatment – Current Concepts

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Abstract: NAFLD (Non-alcoholic fatty liver disease) is a condition of high importance due to its increased prevalence, estimated at 24% of the global population, and its potential progression to advanced liver disease, cirrhosis, and hepatocellular carcinoma. One-quarter of NAFLD patients have NASH (non-alcoholic steatohepatitis), which is histologically described as liver steatosis, inflammation, fibrosis, hepatocyte injury, and death. Metabolic disruptions, such as obesity, diabetes, and dyslipidemia, are considered to have a great impact on the development of NASH. Patients may have NAFLD for a long time before progressing into NASH, which highlights the necessity of periodic imaging and biochemical evaluation of the liver, and also presents an optimal window of therapeutic intervention. The challenge in developing treatment for NASH stems from the slow progression of the disease and the reliance on sequential biopsies as the diagnostic and staging tools. Several non-invasive tests have been developed as potential future alternatives to the current invasive gold standard. This review aims to assess the current available methods of diagnosis and treatment targeting inflammation.

Keywords: NAFLD; NASH; MASH; liver steatosis; liver fibrosis; metabolic syndrome; lanifibranor; obeticholic acid; semaglutide

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is an advanced form of non-alcoholic fatty liver disease (NAFLD), characterized by liver inflammation and hepatocyte injury, that is progressively evolving due to the accumulation of fat in the liver [1]. NAFLD results from an unhealthy diet and sedentary lifestyle, without high alcohol consumption, and is usually a silent disease before the advanced stages, including cirrhosis, liver failure, and hepatocellular carcinoma [2].

The highest rates of NAFLD were reported in the Middle East (32%) and South America (31%), followed by Asia (27%), USA (24%) and Europe (23%), whereas the lowest rates were in Africa (14%) [3-5]. It is estimated that 25% of the patients with NAFLD are thought to have NASH and a quarter of them are diagnosed with cirrhosis [3-5].

Recent studies show the association between metabolic disruptions and NASH, which is why a new medical terminology has emerged introducing the term MASH as metabolic-associated steatohepatitis. A bidirectional correlation exists between NAFLD and metabolic syndrome (obesity, insulin resistance, and dyslipidemia). Also, patients with type 2 diabetes mellitus (T2DM) have a greater risk of developing NAFLD, with a prevalence estimated at 57.80% (95%CI: 53.88% - 61.62%) [6]. NASH has been reported in 65.26% (95% CI: 51.73%-76.71%) of the patients with type 2 diabetes mellitus, who underwent liver biopsy [6]. In a meta-analysis of more than 8.5 million people from 22 countries, the results show that 80% of the patients with NASH are overweight or obese, 72% have dyslipidemia, and 44% are diagnosed with type 2 diabetes mellitus [3].

The evolution of NASH is non-linear. Some patients progress to cirrhosis, and most of them develop cardiovascular disease, but some patients, even those with fibrosis, may spontaneously regress. Cardiovascular mortality is the most common cause of death in patients with NASH. In contrast, in the U.S., due to decompensated liver disease, NASH is among the top three indications for liver transplantation [6,7].

In a study on patients with liver cirrhosis, secondary to HCV infection or NASH, over 4 years, it was estimated that the annual cumulative risk of HCC (hepatocellular carcinoma) was 2.6% per year in patients with NASH cirrhosis compared with 4.0%

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per year in those with HCV cirrhosis [8]. These figures suggest that NASH carries a risk of developing HCC that rivals the risk in patients with HCV-cirrhosis. As such, patients with NASH cirrhosis should be closely monitored for HCC. The decrease in HCV-related transplantation due to the significant effect of the HCV therapies placed NASH and ALD (alcohol-related liver disease) as the most common etiologies of liver disease among liver transplant waiting list registrants without HCC, and NASH is becoming a leading indication in patients with HCC [9].

MATERIALS AND METHODS

This review aims to assess the currently available methods of diagnosis and treatment targeting liver inflammation and fibrosis, named NAFLD, NASH, or similar.

We have selected over 200 English, French, and Romanian studies highlighting information on diagnosing and treating these hepatic diagnoses. For the development of this review, we further selected the articles based on our inclusion criteria, consisting of diagnosis and treatment-focused articles and recently published articles to ensure the current advancements in the field.

Databases such as PubMed, Up-to-date, Medline, and NCBI were used to perform the search using keywords: NAFLD, NASH, liver steatosis, liver fibrosis, and metabolic syndrome. Exclusion criteria were imposed to select the papers, such as the article publication date not exceeding 20 years old, and the publications to be in English, French, and Romanian, addressing the diagnosis and treatment concepts for NASH.

RESULTS

Diagnosis

Identifying a person with NASH can be difficult because neither the diagnosis nor the severity of the liver fibrosis can be predicted based on the risk factors (obesity, insulin resistance, or hepatic steatosis) alone. Accurate fibrosis staging, through biopsy series, is important for diagnosis and mortality assessment. All-cause and liver-specific mortality appear to increase once the F2 fibrosis stage has developed [10]. The risk of liver disease death rises by a factor of 50 to 80 for patients with NASH who have F3/F4 fibrosis, as compared with those who have little or no fibrosis [11,12]. People at risk for NASH, such as overweight or obese patients with metabolic syndrome, elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and negative noninvasive results for other causes of liver disease, should undergo further testing. Also, patients with type 2 diabetes mellitus over the age of 45 years are likely to have advanced fibrosis and bad liver outcomes [5].

Before advanced testing for NASH, patients should be thoroughly examined to exclude other causes of steatosis and liver damage and to determine the presence of commonly associated comorbidities (obesity, dyslipidemia, insulin resistance, diabetes, sleep apnea, hypothyroidism, and polycystic ovary syndrome). Excessive alcohol consumption, steatogenic medications, high serum ferritin with increased iron saturation, and viral or autoimmune hepatitis should be excluded [7].

The liver biopsy is an invasive procedure, currently considered the gold standard for diagnosing and staging NASH. Numerous non-invasive methods of diagnosis based on serum markers and imaging tests have been investigated, but their performance is not comparable with the biopsy (Table 1). The need for non-invasive tests has been enhanced to develop new treatments for NASH. Various emerging diagnostic tools are presented in Table 1 [7,13-25].

MRI-PDFF [7,13] (Proton density fat fraction MRI) is the most accurate method of detecting steatosis, whereas MRE [7,14] (Magnetic resonance elastography) is the most accurate tool of staging liver disease, by the "stiffness'' indirectly related to fibrosis. Although availability and costs are a major challenge for clinicians, combining these tests can provide a risk stratification in patients with NAFLD. Non-invasive serum markers include alanine aminotransferase levels, and circulating keratin18 fragment levels and there are also tests such as Fibrometer, microRNA panels, and lipidomic panels. In patients with ALT>70U/L, NASH ALT prediction testing was found to have a 50% sensitivity and 61% specificity [7,15]. Considering the prediction ability of the serum ALT, it should not be used for definitive diagnosis, staging, or evolution of the disease, but only to guide further testing. The NASH Test combines demographic characteristics (age, gender, and BMI), serum markers (ALT and lipids), and alpha-2 macroglobulin, ApoA1, and haptoglobin, with a sensitivity of 33% and a specificity of 94%, indicating that it has a good negative predictive value (NPV) [7,16]. The Fibrometer NAFLD (based on age, weight, fasting glucose, AST, ALT, ferritin, and platelet count) achieves reasonably good results for mild to moderate fibrosis. The positive predictive value (PPV) for correctly identifying F=1 was 84.9%, the NPV was 66.7% and the diagnostic accuracy was 80%. The PPV for F=3 was 74.5%, the NPV was 86.2% and the diagnostic accuracy was 82.1% [17-20]. Fibromax, a diagnostic tool composed of 4 non-invasive biomarker panels, can be used for the diagnosis of steatosis (estimated by the SteatoTest), necrosis and inflammation (evaluated by ActiTest and NashTest-2), and fibrosis (FibroTest). Although it is a well-validated and relatively frequently used test, it may not be accurate for patients with T2DM [21]. OWLiver (Overweight Liver) [16,18] is an alternative for overweight patients and PROC3 (Pro Collagen III) can be used as a differentiating test between steatosis and NASH [24]. NASH Index Score 4 (NIS4) [23] and Enhanced liver fibrosis (ELF) [25] are tools that exclude AST and ALT as parameters in the diagnosis process.

There are two main scoring systems for the biopsy results in NASH. The NAS (NAFLD Activity Score) sums the score of steatosis, lobular inflammation, and ballooning of the liver cells. The score (0-8) is not diagnostic. It was designed to evaluate the histological changes in seriate biopsies. It is usually interpreted by adding the fibrosis score (0-4) (Table 2) [7,26-27]. Another available score is SAF

(Steatosis, Activity, and Fibrosis). By evaluating 1000 biopsies, there was an excellent concordance between the two scores in determining the definite NASH, whereas 84% of the NAS borderline biopsies (3-4), were identified by SAF score as NASH [7,26-27].

Table 1: Non-invasive methods of diagnosis [7,13-25]

Test	Parameter measured	Observations
MRI-PDFF [7,13] (Proton density fat fraction magnetic resonance imaging)	steatosis	most accurate method of detecting steatosis
MRE [7,14] (Magnetic resonance elastography)	liver stiffness	most accurate modality of staging liver disease, by the ''stiffness'' indirectly related to fibrosis.
NASH Test [7,16]	age, sex, height, weight, serum triglycerides, cholesterol, a-macroglobulin, apolipoprotein A1, haptoglobin, gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin	sensitivity of 33% specificity of 94%
FibroMeter VCTE [17-20] (Vibration Controlled Transient Elastography)	Fibrosis, liver stiffness Combines TE (transient elastography) with biochemical markers (age, sex, AST, urea, platelets, prothrombin time, GGT, and alpha2-macroglobulin)	shows good results for mild to moderate fibrosis By combining biochemical markers with imagistic results it increases the accuracy
Fibromax [21]	Combines 4 non-invasive biomarker panels SteatoTest, ActiTest, NashTest-2, and FibroTest	affordable and simple score, but it may not be accurate for patients with T2DM
FNI [22] (The Fibrotic NASH Index)	fibrosis AST, high-density lipoprotein cholesterol, and hemoglobin A1c	accurate, affordable, and simple score for patients with fibrotic NASH
NIS4 [16,18,23] (NASH Index Score 4)	miR-34a-5p, alpha-2 macroglobulin, YKL-40, and glycated haemoglobin A1c	Identifies at-risk patients, defined as [NAS] ≥4 and fibrosis stage ≥2 on biopsy
OWLiver [16,18] (Overweight Liver)	extensive analyses of triglycerides panel by high-performance liquid chromatography and mass spectrometry	Used for individuals with BMI>25 kg/m^2
PROC3 [18,24] (Pro Collagen III)	Pro collagen III	Differentiates steatosis from NASH
ELF [18,25] (Enhanced liver fibrosis)	fibrosis hyaluronic acid, procollagen III, and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1)	stages liver fibrosis independently of AST, ALT elevations

Treatment

The main pathogenic processes identified in NASH are metabolic stress, inflammation, and fibrosis. Pharmacologic agents that target these key mechanisms are being investigated in clinical trials [2].

Due to the lack of pharmacological therapies for NASH, lifestyle modifications remain the main intervention for mitigating histological impairment. Weight loss appears to bring the highest benefit.

Usually, the recommendations include 5-10% weight reduction for obese and overweight patients, physical activity, and a Mediterranean diet. Ultra-processed foods and drinks, fructose-enriched beverages, and alcohol should be avoided. In addition, associated comorbidities (T2DM, dyslipidemia, POS) should be treated [1,26].

Vitamin E, pioglitazone, and statins are available options for patients with NASH. Studies have indicated the role of vitamin E in the reduction of steatosis and fibrosis [28,29]. Pioglitazone, a treatment used for type 2 diabetes mellitus, has been reported to significantly reduce fibrosis in NASH patients. In addition, statins have been tested on NAFLD/NASH patients, with substantial results in improving steatosis and fibrosis [30].

The PIVENS trial focused on NASH patients without diabetes, who were randomly assigned to one of three groups (pioglitazone 30 mg daily, vitamin E 800 IU daily, or placebo). The vitamin E group had a significantly higher rate of histology improvement than the placebo group 43% vs. 19%, but pioglitazone did not meet the primary outcome [31]. Based on several studies, there is a variability in treatment response for vitamin E supplementation. Genetic variations in haptoglobin and FADS1/FADS2 (fatty acid desaturase 1 and 2) have been suggested to interfere with the treatment response, determining whether a patient will show histological improvement or not [32].

Many new potential therapies for NASH are currently studied in phase III trials. The pan-PPAR agonist (Peroxisome proliferator-activated receptors), lanifibranor, has completed the phase IIb trial with 247 participants, the results show a reduction in the SAF activity score (> 2 points), with no increase in fibrosis, achieved by 49% of patients compared with 27% on placebo. The results were significantly higher among those who received 1200 mg of lanifibranor vs. the group with 800 mg dose. In the lanifibranor groups, there was an improvement in liver enzyme levels and also in the majority of lipid, inflammatory, and fibrosis biomarkers [33]. There is an ongoing phase 3 study conducted to evaluate the safety and efficacy of lanifibranor compared to placebo on 1000 participants

with NASH and liver fibrosis histological stage F2 or F3 [34].

The GLP-1 receptor agonist, semaglutide, has also completed a 72-week, phase 2 trial (NN9931-4296) with 320 participants, showing resolution of NASH and no worsening of fibrosis for 59% of patients, in the 0.4mg group, and 40% of patients, in the 0.1mg group, compared with 17% on placebo. The percentage of participants with an improvement in NAS score was 82.9% in the 0.4mg group compared with 43.8% on placebo [35]. A phase 3 study, ESSENCE (The Effect of Semaglutide in Subjects With Non-cirrhotic Non-alcoholic Steatohepatitis), was initiated in 2021 and the interim results are expected in 2024 [36].

Table 2: NAS score and fibrosis staging (adapted from [7,27])

Characteristic	Score
Steatosis	<5%=0 5-33%=1 (mild) 33%-66%=2 (moderate) >66%=3 (severe)
Lobular inflammation	No foci = 0 <2 foci per 200 field =1 2-4 foci per 200 field =2 >4 foci per 200 field = 3
Ballooning	None = 0 Few = 1 Many cells = 2
NAS Score	0-8
Fibrosis	None = 0 Mild = perisinusoidal or periportal (stage 1) Moderate = perisinusoidal and portal/periportal (stage 2) Severe = bridging fibrosis (stage 3) Cirrhosis = stage 4

For liraglutide, also a GLP-1 analog, a multicenter, double-blinded, randomized, placebo-controlled phase 2 trial (the LEAN trial) has demonstrated histological resolution of non-alcoholic steatohepatitis and a significant effect on liver fibrosis after 48 weeks of treatment. In addition, the comparison between pioglitazone and liraglutide showed better results for the latter [37-39].

Obeticholic acid, a selective FXR (farnesoid X receptor) agonist, approved by the FDA in 2016 for primary biliary cholangitis, previously completed a phase III trial (REGENERATE) on 1968 NASH participants with fibrosis F2–F3. The results show that the drug improved the fibrosis score by at least one stage, but did not meet the endpoint of NASH resolution [40]. The FDA rejected the approval of obeticholic acid for NASH in May 2023, based on the modest benefits that did not outweigh the risks.

A phase IIb randomized study of the efficacy and safety of Namodenoson, an A3 adenosine receptor (A3AR) agonist, in the treatment of NASH is ongoing. Previous trials on Namodenoson showed normalization in ALT and AST levels after 12 weeks of treatment [41].

Pegozafermin, a long-acting glycopegylated fibroblast growth factor 21 (FGF21) analog, showed improvements in fibrosis in a phase 2b trial (ENLIVEN). The fibrosis improvements occurred in 7% of the placebo group and between 22-27% in the pegozafermin groups (22% with 15 mg – pegozafermin, 26% on 30 mg dosage, and 27% on 44 mg dosage). The NASH resolution was 2% in the placebo group, 37% in the 15mg pegozafermin group, 23% in the 30 mg pegozafermin group, and 26% in the 44 mg pegozafermin group. The study completion date is estimated around September 2024, demonstrating favorable prospects for advancing to phase 3 [42,43].

Oxidative stress and the formation of reactive oxygen species (ROS) are often associated with the progression of NAFLD to NASH. Excessive ROS formation impairs insulin sensitivity and inflammatory responses. The release of pro-inflammatory molecules and uncontrolled ROS production in many cells is partially the result of TAGE (toxic advanced glycation end-products) and receptor for AGEs (RAGE) interactions [44,45]. Hyperglycemia contributes to the production of advanced glycation end-products (AGEs), especially AGEs formed from glyceraldehyde (GA), which are highly cytotoxic, damaging the hepatocytes. AGEs and TAGE were measured in a study of 66 patients with histologically confirmed NASH without cirrhosis, 10 with NAFL, and 30 control subjects [46]. The TAGE levels of NASH patients were significantly higher than those with NAFL. Ultra-processed foods with a high content of sugar (fructose-enriched or sugar-sweetened drinks) and dietary AGEs have been suggested to contribute to the development of obesity, metabolic syndrome, and the progression of NAFLD/NASH when consumed excessively [47].

Antioxidants such as pentoxifylline (PTX), silybin, resveratrol, and vitamins A, C, and E are used in clinical trials against NAFLD and NASH [47].

PTX treatment with three divided doses of 400 mg for one year improved hepatic histological activity. NAS ≥ 2 improved by (75%) in the PTX group and 30% in the placebo group, but there was no impact on fibrosis [48]. Also, another study on PTX (1200 mg/day) and fenofibrate showed a beneficial effect on indirect biochemical markers of hepatic fibrosis. After 24 weeks, the association of fenofibrate and PTX resulted in reduced liver fibrosis and stiffness, insulin resistance, and inflammation [49]. The combined administration of PTX (1200 mg/daily) and metformin (1500 mg/ daily) for six months reduced AST, and ALT levels and improved

insulin resistance in patients with NASH [50]. A meta-analysis based on 5 RCT on patients with NAFLD/NASH reported that PTX treatment could reduce glucose serum levels, liver transaminases, body weight, and TNF-α levels and also improve NAS and fibrosis scores [51]. Another study used combinational therapy (PTX+Vitamin E) and the results showed regression of fibrosis compared to vitamin E alone [52]. In a phase 2 study, pentoxifylline (PTX) alone significantly improved steatosis, lobular inflammation, and liver fibrosis and was well tolerated [53]. Therefore, PTX can be used along with other drugs as an adjuvant for metabolic diseases.

A phase 4 study conducted on 102 NASH patients divided into 3 intervention groups (PTX, vitamin E, and ursodeoxycholic acid) measured the levels of several serum markers (AST, ALT, IL6, and CCL2/ MPC-1, albumin, total bilirubin, direct bilirubin, total cholesterol, triglyceride, LDL, HDL) for 3 months [54].

In 2017 a study [55] based on an experimental rat model of NASH demonstrated for the first time that the combination of SAMe (S-Adenosyl-L-Methionine) and DLPC (Dilinoleoylphos-phatidylcholine), two physiologic antioxidants, significantly opposed liver injury generated by fat excess.

Numerous studies on hepatoprotective drugs, probiotics, and antioxidants, such as Siliphos—Selenium—Methionine—Alpha Lipoic Acid, omega-3 fatty acids, and vitamins A, C, and E, are currently ongoing [47,52,56-58].

CONCLUSION

The treatment for non-alcoholic steatohepatitis is, unfortunately, an unmet need. At present, there are no FDA-approved therapies for this condition, and the same holds for the EU. Considering the increasing prevalence and the risk for progression toward advanced liver disease, cirrhosis, and hepatocellular carcinoma, there is a major demand for reliable non-invasive biomarkers and specific therapy. In addition to healthy diet and exercise, many new pharmacological agents, such as lanifibranor, semaglutide, and obeticholic acid are being studied. Antioxidants as well are used in clinical trials against NAFLD and NASH.

Despite the clinical and epidemiological significance, the accurate diagnosis, monitoring of the diagnosed patients, and available licensed treatments remain the challenges of NASH.

Conflicts of interest and sources of funding

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Not applicable.

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REVIEW

Multinodular and Vacuolating Neuronal Tumor – Challenges and Perspectives

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Abstract: A multinodular and vacuolating neuronal tumor (MVNT) is a rare non-malign central nervous system tumor. The cerebral cortex of young to middle-aged adults is affected and seizures and focal neurological deficits could reveal it. Despite being considered a low-grade tumor, MVNT can be challenging to diagnose and manage due to its histopathological similarities to other neuronal tumors. The limited number of reported cases makes it difficult to establish definitive guidelines for treatment and follow-up. Given the relatively recent discovery of MVNT, there is a need for further research to better understand its pathogenesis, optimal treatment and long-term strategies. Furthermore, studying MVNT can contribute to the broader understanding of brain tumor biology and the intricacies of tumor heterogeneity. This paper explores the challenges and perspectives surrounding MVNT to improve clinical decision-making and patient outcomes. Based on the present narrative review, advancements in diagnostic technologies have shown promise in addressing the challenges associated with diagnosing MVNTs. Liquid biopsy is an innovative and non-invasive diagnostic tool that analyzes biomarkers to provide information about tumors, and radiomics is a method that extracts quantitative data from medical images to provide detailed insights into tumor characteristics. Such innovative diagnostic technologies are important because they have the potential to significantly improve the current diagnostic landscape for MVNTs, allowing for earlier detection, accurate classification, and personalized treatment strategies.

Keywords: multinodular and vacuolating neuronal tumor; radiomics; liquid biopsy; diagnostic methods; histological examination; FDG-PET/CT; immunohistochemistry

INTRODUCTION

First described in 2013, multinodular and vacuolating neuronal tumor (MVNT) was classified as a benign ganglion cell grade I tumor, potentially associated with seizures, with a specific molecular marker, as outlined in the WHO 2016 and 2021 classification [1-3]. The discovery of MVNT is often accidental, and it is frequently asymptomatic [1,2]. Studying MVNT is of utmost significance in neuro-oncology. Understanding the molecular mechanisms underlying the development and progression of MVNT can provide insights into potential therapeutic targets and treatment strategies.

Typically identified in adults, MVNTs seem to appear during neurogenesis and present as subcortical clusters of nodular lesions within the cortex and superficial subcortical white matter [4]. Structure abnormalities in the cortex may be present with it [4,5]. Initially classified as either neoplastic or malformation, recent research on MVNTs emphasizes their stable nature over time, suggesting that they are "leave me alone" lesions with minimal changes in volume, thus underscoring the potential for less frequent imaging surveillance [6,7]. Whether the nodules of vacuolar neurons are a definite neoplastic transformation or aberrant development is still up for debate [6]. Overall, these insights contribute to a nuanced understanding of MVNTs, highlighting the complexities and diagnostic considerations surrounding these distinct neuronal tumors. Moreover, the purpose of this research is to shed light on the challenges and perspectives surrounding MVNT.

MATERIALS AND METHODS

A narrative review was conducted through five electronic databases (PubMed, CINAHL, Google Scholar, Cochrane, Web of Science/Clarivate), using as keywords "multinodular and vacuolating neuronal tumor" AND "diagnosis" OR "pathogen*" OR "imaging" OR "treatment". All primary and secondary reports found from the inception of the respective databases up to July 2024 were explored for relevant data. Only papers that addressed explicitly the pathogenesis, diagnosis methods,

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and treatment strategies of MVNT were included in the secondary analysis**.**

RESULTS

Based on the literature search, 34 primary and secondary reports were reviewed in detail. The results were classified according to the three areas of interest, i.e., (1) pathogenesis and classification of MVNT, (2) imaging characteristics, and (3) diagnosis and treatment.

Classification and pathogenic characteristics of MVNT

Recent studies have highlighted the challenge of defining and classifying MVNT. Due to its rarity and unique histopathological features, there is ongoing debate within the scientific community regarding its precise definition and classification. Some researchers argue that MVNT should be considered a separate entity from other neuronal tumors due to its distinct morphological characteristics and clinical behavior. On the other hand, some propose classifying it under the umbrella of neuronal and mixed neuronal-glial tumors based on its neurocytic differentiation and immunohistochemical profile.

The purely neuronal tumors in the 2021 WHO classification include multinodular and vacuolating neuronal tumors, gangliocytoma, extraventricular neurocytoma, central neurocytoma, dysplastic cerebellar gangliocytoma, and cerebellar liponeurocytoma.

The mixed glioneuronal tumors include dysembryoplastic neuroepithelial tumor, diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters, ganglioglioma, desmoplastic infantile ganglioglioma/astrocytoma, papillary glioneuronal tumor, rosette-forming glioneuronal tumor, myxoid glioneuronal tumor, and diffuse leptomeningeal glioneuronal tumor [13].

There is a consensus on the need for standardized diagnostic criteria to identify and classify MVNT cases accurately. This would facilitate better communication among pathologists and clinicians, improving management strategies and outcomes for patients with MVNT.

The WHO 2016 classification defines MVNT as a benign ganglion cell tumor with specific pathologic hallmarks [2]. These nodular lesions are typically located in specific brain regions and can present differently across age groups, emphasizing the importance of histopathological examination for accurate diagnosis [1,14,15].

Histopathological analysis of MVNT reveals several distinctive features that aid in their diagnosis and differentiation from other neuronal tumors. One of the hallmark features of MVNT is the presence of numerous vacuoles within the tumor cells. This vacuolation can create a “floating” appearance of the cells and is a critical diagnostic criterion. MVNT typically exhibits a multinodular architecture. The tumor forms distinct nodules, which may vary in size, often surrounded by a gliotic background. Such detailed histopathological analyses are essential for elucidating the distinct features of MVNT and guiding effective clinical management strategies. Nevertheless, no histopathological evidence is available to confirm the diagnosis since only a small percentage of patients with suspected MVNT undergo surgery [10].

Immunohistochemical markers play a crucial role in the diagnosis and classification of MVNT. Various markers have been studied, differentiating MVNT from other tumors with similar histological features, such as dysembryoplastic neuroepithelial tumor (DNT) or ganglioglioma [16,17]. So, immunohistochemistry (IHC) is a crucial tool in the diagnosis and characterization of MVNT, establishing the neuronal nature of MVNT, as seen with positive staining for neuronal markers [18]. MVNT typically shows positivity for several neuronal differentiation markers: NeuN, a neuronal nuclear antigen typically expressed in mature neurons, β-III tubulin, which is highly expressed in neuronal cells and can help confirm the tumor’s neuronal lineage. Synaptophysin, a protein associated with synaptic vesicles, indicates neuroendocrine differentiation and is often used to demonstrate the presence of neurons. Although MVNT is primarily neuronal, some glial markers may also be expressed, reflecting maybe the mixed composition of the tumor. GFAP (Glial Fibrillary Acidic Protein) positivity can indicate a glial component in the tumor. Phenotypic analyses may also include checking for markers that indicate low proliferation rates, which can help distinguish MVNT from more aggressive tumors. Markers like Ki-67 are occasionally assessed but typically show low proliferation in MVNT. The absence of expression for markers associated with high-grade tumors, such as p53 or other markers linked to aggressive histology, reinforces its benign nature [18,19].

Understanding the molecular pathogenesis of Multinodular Vacuolating Neuronal Tumors (MVNT) presents a significant challenge due to the tumor’s unique histological features and clinical behavior. MVNTs are characterized by vacuolated tumor nodules with glial and neuronal differentiation, primarily affecting the cerebrum in adults. The pathognomonic radiological features, such as the soap-bubble morphology and lack of enhancement post-gadolinium injections, provide important clues for differential diagnosis and management strategies [4,11,20,21]. The molecular mechanisms driving its formation and growth remain elusive. Further research is warranted to elucidate the genetic alterations and signaling pathways involved in the development of MVNT. The MAP (Mitogen-Activated Protein) kinase signaling pathway is a critical cellular signaling pathway that regulates various cellular processes, such as growth, proliferation, differentiation, survival, and apoptosis. This pathway is activated by various extracellular signals, including growth factors, cytokines, and environmental stresses. MAP kinase signaling pathway possibly sheds light on targeted therapeutic approaches [22].

Research has identified specific genetic mutations that may contribute to the development of MVNTs. For instance, missense mutations in the BRAF gene or HRAS gene have been linked to the pathogenesis of tumors [22,23]. Vacuolization and neuronal differentiation in MVNTs are characterized by vacuolated neurons. This cellular morphology suggests that disruptions in normal

neuronal differentiation and cellular homeostasis may be due to genetic and epigenetic factors. Investigating the presence of other oncogenes and tumor suppressor genes may provide further insights. In exploring the molecular underpinnings of MVNT, a deeper understanding of its pathogenesis may pave the way for personalized treatment strategies and improved patient outcomes in the future. Like many tumors, MVNTs may exhibit altered vascularization patterns [24]. The tumor microenvironment, including the interaction between tumor cells and surrounding glial cells, immune cells, and extracellular matrix components, plays a crucial role in tumorigenesis [25,26]. Studying these interactions can show how the tumor ecosystem supports MVNT growth and survival.

Table 1: Multinodular and vacuolating neuronal tumor (MVNT) characteristics

Characteristics	Descriptors
Definition [2,3]	Low-grade tumors (grade I) comprising monomorphous neuronal elements in discrete and coalescent nodules, with vacuolar changes both in tumor cells and the neuropil, with a molecular pattern impaling MAPK pathway
Localization [4,9,10]	Parietal lobe, frontal lobe, temporal lobe, occipital lobe, spinal, rare cases of multiple localization, mainly subcortical white matter rare cases with cortical extension
Imaging features [4,11,12]	**Brain CT**- is usually normal or shows a non-enhancing/non-calcifying cortical/subcortical white-matter hypodense lesion, absence of mass effect and edema; **Brain MRI** – intra-axial multiple clustered nodules hyperintense on T2WI and FLAIR; T1WI: small hypointense, homogeneous, and well-defined nodules, some clustered tightly or with a small interface between them, located at the cortical-subcortical junctional side; Abnormal white matter signal surrounding the bubble-like or bunch of grape appearances lesions; No contrast enhancement; Restricted diffusion could be present with high ADC values rare **FDG-PET/CT** brain imaging showed tumor hypometabolism
Histological examination [4,8,12]	No evidence of mitosis, necrosis, or microvessel proliferation; Abnormal clusters and numerous vacuoles within the tumor cells, with different sizes, often surrounded by gliotic tissue; Glioneuronal hamartomas; Hypomyelinated plaque-like nodules extending into the deep cortex;
Immunohistochemical stains [4,8]	Many neuronal cells show weak immunoreactivity for synaptophysin and negative immunoreactivity for neuronal nuclear and neurofilament proteins; Variable background of positive dendritic staining for CD34; Negative for IDH1-R132H mutation antibody; Staining was normal for ATRX and p53; The Ki67 proliferation refraction was very low; GFAP highlighted the reactive astrocytes within the nodules; The vacuolated cells were immunoreactive for synaptophysin, while the fibrillary tumor matrix showed decreased synaptophysin expression compared to the adjacent cortex
Natural evolution [4]	Stable on time, „leave me alone” tumor

MAPK- Mitogen-Activated Protein Kinase; CT – computer tomography; MRI- magnetic resonance imaging; T2 FLAIR – space inversion recovery with fluid attenuation; FDG-PET/CT- fluorodeoxyglucose positron emission tomography/computed tomography; CD34 - transmembrane phosphoglycoprotein encoded by the CD34 gene; IDH1- isocitrate dehydrogenase; ATRX- chromatin remodeling · nucleosome assembly; p53- tumor suppressor gene; Ki67 – a nuclear nonhistone protein present in all active phases of cell cycle; GFAP – Glial fibrillary acidic protein responsible for astroglial cytoskeleton and is not found outside the CNS

Imaging Characteristics of MVNT

Imaging characteristics of MVNT reveal distinctive features that aid in their diagnosis and differentiation from other brain tumors. Described as low-grade lesions primarily found in the cerebrum, MVNT presents as clustered multinodular structures within the cortex and subcortical regions (Table 1). The brain MRI revealed multinodular T2-hyperintense lesions in both the deep cortex and superficial white matter of the frontal lobes or temporal lobes, exhibit a unique soap-bubble morphology, hyperintensity on T2 FLAIR sequences, and minimal or absent contrast enhancement [10,16,27]. Based on the signals from T1, T2, and T2 FLAIR, the nodules have nonhomogeneous signal intensity due to low T1, high T2, and low FLAIR vacuole aspects. Confluent T2/FLAIR hyperintensity overlying cortex was present in some cases [4]. The larger nodules have an inner core that is hypointense, a rim that is hyperintense, and areas with a high signal intensity that connect the nodules. The smaller nodules exhibit a signal intensity that is not uniform, with a central dot of low-intensity [11]. The nodules' periphery shows high signal intensity on FLAIR because of high protein content and neuronal demyelination [11].

Sometimes MR spectroscopy indicates an increase in the choline peak and a decrease in the N-acetyl aspartate peak, and tractography shows a partial disruption of the typical structure caused by the absence of lesional cortical layers [11,28]. No increase in perfusion was observed by perfusion-weighted imaging in another study, and abnormal peaks were not detected by MR spectroscopy [10]. Despite their benign nature, the pathologic hallmark of MVNT, questions their neoplastic or malformative origin and potential association with seizures [29]. Cases of a spinal cord lesion resembling cerebral MVNT of unknown significance were named MVNT-like spinal cord lesions. With intramedullary cystic ovoid nodules within the white matter, some immediately contiguous to the grey matter, this lesion was stable in a follow-up [9,30]. The presence of well-delineated intramedullary cystic ovoid nodules within the spinal cord raises questions about the underlying pathophysiology of MVNT [9]. A comprehensive MR protocol has been suggested to

increase the diagnostic confidence of MVNT [10].

Diagnostic and treatment of MVNT

Despite advancements in imaging and molecular techniques, MVNT can present with various clinical manifestations, posing diagnostic challenges. Usually, MVNT is revealed as an incidentaloma [31]. The clinical presentation is nonspecific, with symptoms such as seizures, headaches, or focal neurological deficits, often mimicking other more common brain tumors or epilepsy syndromes [1,14]. Additionally, the radiological features of MVNT on MRI can be subtle and easily overlooked, leading to misdiagnosis. The lack of specific diagnostic criteria for MVNT further complicates accurate identification, underscoring the need for histopathological examination for definitive diagnosis [16,32]. Biopsy remains the gold standard for confirming MVNT, highlighting the importance of integrating clinical, radiological, and pathological findings to navigate the diagnostic challenges posed by this rare entity in neuro-oncology. Additionally, the classification of MVNT as a benign neuronal tumor, possibly associated with seizures, further complicates the understanding of its neurological manifestations, especially in young patients [33].

Table 2: Immunohistochemical analysis [8,19]

Neuronal markers		Glial markers	
NeuN (Neuronal Nuclei)	A marker for neurons, specifically in post-mitotic neurons. It is widely used to identify neuronal populations in various brain regions.	GFAP (Glial Fibrillary Acidic Protein)	A specific marker for astrocytes. Increased expression is often seen in reactive astrocytes following injury
MAP2 (Microtubule-Associated Protein 2)	A neuron-specific protein that stabilizes microtubules and is important for dendritic structure.	Olig2	A transcription factor and marker for oligodendrocyte precursor cells and mature oligodendrocytes, essential for myelination in the central nervous system
βIII-Tubulin (Tuj1)	A marker commonly used to identify neurons, particularly in developmental studies.	S100	A protein found in astrocytes, used for identifying glial cells
Synaptophysin	A presynaptic vesicle protein, often used to identify synaptic structures and neuronal activity	CD68	A marker for microglia, reflecting macrophage activity in the central nervous system and often used in studies of neuroinflammation.

Distinguishing MVNT from entities such as dysembryoplastic neuroepithelial tumor (DNET), ganglioglioma, extraventricular neurocytoma, and low-grade glioma is sometimes challenging [34]. The bright diffusion sign and absence of cortical involvement could differentiate MVNT from DNET [35]. Moreover, incorporating genetic and molecular analyses can further aid in narrowing down the list of differential diagnoses and confirming the diagnosis of MVNT. Immunohistochemical analysis for neural and glial markers are also helpful (Table 2). Antibodies against neuronal protein HuC/HuD (HuC/HuD), pan-neurofilament (pan-NF), neurofilament 200 KD (NF200 KD), chromogranin, and antibodies against glial fibrillary acidic protein (GFAP), nestin, CD3, CD45, CD68, CD34, human leukocyte antigen-DR (HLA-DR), isocitrate dehydrogenase1 (IDH1), calbindin, parvalbumin, calretinin, Ankyrin-G, p62 and Ki67, BRAF(V600E) enhance diagnostic accuracy [8,19]. Immunopositivity for HuC/HuD and Olig2 proves that MVNT appears during neuronal development [4].

The latest WHO tumor brain classification has classified MVNTs as grade I, and the mitogen-activated protein kinase (MAPK) is considered a molecular marker [3,8]. The MAPK is a key signaling pathway that transmits signals from the cell surface to the nucleus and regulates various cellular processes, including growth, proliferation, differentiation, and survival. Further research in this area, particularly investigating specific diagnostic markers and biomarkers, is warranted to refine the differential diagnosis process for MVNT.

Factors such as tumor size, location, and the presence of associated neurologic deficits can influence treatment strategies and prognosis.

One of the foremost challenges in the preoperative diagnosis of MVNT is the difficulty in distinguishing them from other neoplastic or malformative lesions to avoid unnecessary surgical interventions [36]. The characteristic imaging features of MVNT, such as the subcortical nodular lesions with unique signal properties on MRI, can often be mistaken for more aggressive tumors due to their atypical presentation in younger patients. The rarity of MVNT in young individuals poses a diagnostic dilemma, questioning the

classification of these lesions as true neoplasms or malformative anomalies [22,31,21].

Imaging modalities and treatment strategies play pivotal roles in managing epileptic patients, especially those with drug-resistant epilepsy considered for neurosurgery. Utilizing advanced imaging techniques such as the Harmonized Neuroimaging of Epilepsy Structural Sequences (HARNESS-MRI) protocol and 7-Tesla MRI can aid in identifying previously occult epileptogenic lesions and improving surgical outcomes [37,38]. Additionally, the development of MRI postprocessing techniques and functional imaging modalities like EEG-functional MRI contribute to enhanced sensitivity in lesion detection and focus localization [39].

Surgery is often considered the primary treatment option for multinodular vacuolating neuronal tumors (MVNTs) under several circumstances. If the MVNT is causing neurological symptoms (such as seizures, headaches, or focal neurological deficits), surgical intervention may be necessary to alleviate these symptoms, when the tumor is accessible and can be safely resected without causing significant damage to surrounding brain tissue. Histological confirmation by surgical resection can provide a definitive diagnosis through the histopathological exam, which is crucial for confirming the nature of the tumor and ruling out other possible lesions. However, the precise location of the tumor within the brain can sometimes pose challenges in obtaining a biopsy sample. In such cases, advanced imaging techniques and neurosurgical expertise are indispensable in ensuring accurate sampling for an accurate diagnosis and subsequent management of MVNT. It is essential that surgical decisions are made in consultation with a team of specialists, including neurosurgeons and oncologists, who can evaluate the risks and benefits specific to each patient’s condition and tumor characteristics. Functional MRI is as accurate as WADA testing, for determining language dominance [40].

The management of asymptomatic MVNT often involves careful observation rather than immediate intervention. However, a strategy of active surveillance can be appropriate for asymptomatic or minimally symptomatic MVNT. This involves regular follow-up through serial neurological examinations and imaging studies (usually MRI) every 6-12 months.

DISCUSSION

Advancements in diagnostic technologies have shown promise in addressing the challenges associated with diagnosing MVNTs. One emerging technology that holds potential is liquid biopsy, an innovative and non-invasive diagnostic tool that analyzes biomarkers (circulating tumor cells and genetic material) from bodily fluids (such as blood, urine, or cerebrospinal fluid) to provide information about tumors [41-46]. Circulating Tumor DNA (ctDNA) from the blood allows for the detection of genetic mutations and alterations associated with MVNT. Liquid biopsy is less invasive than traditional tissue biopsy, making it a more feasible option for patients, especially in complex cases where surgical options may be limited. It allows for repeated sampling without the risks associated with multiple surgical procedures, enabling ongoing monitoring.

Additionally, the development of radiomics, a method that extracts quantitative data from medical images to provide detailed insights into tumor characteristics, could aid in distinguishing MVNTs from other brain tumors with similar radiographic features [44-46]. These innovative diagnostic technologies have the potential to revolutionize the current diagnostic landscape for MVNTs, allowing for earlier detection, accurate classification, and personalized treatment strategies. Furthermore, integrating these technologies into clinical practice could improve patient outcomes and enhance our understanding of MVNT biology.

More studies are needed to elucidate the genetic mutations and molecular pathways involved in the development of MVNT. This would not only provide valuable insights into the underlying mechanisms of this rare brain tumor but also potentially identify novel therapeutic targets. Additionally, clinical research focusing on the efficacy of different monitoring modalities is crucial for optimizing patient outcomes.

CONCLUSION

Based on the results of this narrative review, emerging technologies allow for a re-conceptualization of the MVNT diagnosis and treatment. Although data regarding the validity of the new imaging and molecular techniques are still gathering, the fact that WHO includes MAPK as a marker for MVNT indicates the need for further refinement of the differential diagnosis based on molecular explorations in this pathology. The references in the literature related to genetic mutations and alterations associated with MVNT suggest new research directions congruent with the paradigm of personalized medicine.

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Authors’ contribution

Conceptualization, M.M., O.V., and O.M.S.; methodology, O.V., A.P., and O.M.S.; software, M.M., A.P., and O.M.S.; validation, M.M., O.V., and A.P.; formal analysis, O.V.; investigation, M.M.; resources, A.P., and O.M.S.; data curation, O.V.; writing—original draft preparation, M.M., O.V., and O.M.S.; writing—review and editing, M.M., O.V., and O.M.S. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ARTICLE

Functional Evaluation and Neuro-Muscle-Skeletal Recovery in Lyme Disease: A Study on Motor Control Theories and Rehabilitation

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Abstract: Background: Patients with Lyme disease often suffer from neuro-motor sequelae following Borrelia infection, leading to impairments in balance, posture, and spinal musculoskeletal functionality. This study aims to evaluate the effectiveness of physical therapy in addressing these impairments. Methods: Initial assessments were conducted using the GPS Posture-test System to evaluate static bipodal balance and posture, and BACKFIX Technology for unipedal balance and spinal functionality. Disturbances in balance, postural asymmetries, and a forward-shifted center of gravity were observed. The patient then underwent two 10 sessions (each during two weeks) of computer-assisted physical therapy focused on neuro-muscular movement and balance control. Results: Post-therapy evaluations showed significant improvements in both bipodal and unipedal static balance, segmental and overall body posture, and spinal musculoskeletal functionality. The therapy effectively addressed the postural asymmetries and shifted the center of gravity closer to the optimal vertical posture point. Conclusion: Computer-assisted physical therapy with visual feedback significantly improved posture, balance, and spinal functionality in patients with Lyme disease-related neuro-motor sequelae. This approach facilitated the restoration of muscle coordination and enhanced motor control, aligning with findings from the existing literature.

Keywords: Lyme Disease; Global Postural System; unipedal static balance; BACKFIX; global posture; spine; physical therapy

INTRODUCTION

The specialized literature lacks scientific studies on the functional evaluation and neuromusculoskeletal recovery in Lyme Disease (LD). Approximately 80 articles from the past decade are available in databases, primarily focusing on early detection and antibiotic treatment. No studies provide information on potential methods for functional evaluation or physical therapy-based recovery for musculoskeletal dysfunctions caused by inflammation secondary to Borrelia infection.

LD is caused by the bite of a tick infected with Borrelia. The disease progresses through three stages and can result in multisystem complications. When the nervous system is affected, it is referred to as neuroborreliosis, which may manifest as meningitis, mononeuritis multiplex, or cranial neuritis, including inflammation of the vestibulocochlear nerve [1]. Exclusive involvement of the vestibular system can lead to dizziness and balance instability [2]. Musculoskeletal symptoms can appear at any stage, including early localized infection, often resulting in patients being misclassified into the incorrect stage [3].

We describe motor control and the potential effects of systemic inflammation caused by borreliosis, followed by the stages of assessment and recovery, and the conclusions drawn from these steps.

Although the exact mechanism by which LD causes small fiber neuropathy is unknown, several potential mechanisms of neuronal damage are considered. These include the direct neurotoxic effect of the spirochete, neuroinflammation, and increased autoimmunity affecting the peripheral nervous system (PNS) with subsequent impacts on muscles, joints, and specific post-infection pains. However, a clear causal link between neurological symptoms and Borrelia infection cannot be

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established for patients with polyneuropathy/polyneuritis who test positive for Borrelia [4].

Among the genospecies of Borrelia burgdorferi sensu lato: Borrelia burgdorferi sensu stricto (s.s.), Borrelia garinii and Borrelia afzelii, Borrelia burgdorferi s.s. has the strongest potential to damage joints [5]. It is the most common cause of LD in the United States [3, 6], while infections with this pathogen are much less common in Europe [7-9]. The pathogenesis of LD is primarily driven by a strong inflammatory reaction [5], resulting in a perivascular collection of lymphocytes, macrophages, and scattered mast cells in the interstitial tissue, as typically observed in histopathological examinations of severe LD cases [9].

Previous studies have shown that late manifestations affecting the nervous system or joints can appear months or years after the initial infection, presenting symptoms such as fatigue, difficulty walking, posture disorders, and muscle pain. These symptoms are also present in the subject of our study, secondary to the diagnosis of borreliosis [10].

The examination and treatment of patients with motor control disorders are based on theories of motor control that guide the understanding of movement mechanisms [11-14]. In our study, we utilize systems theory as a foundation for clinical applications. Systems theory posits that movement arises from the interaction of various processes, including perceptual, cognitive, and motor processes, as well as the interactions between the individual, the activity, and the environment.

The severity of symptoms observed in all the patients with LD that we have evaluated has led us to pay more attention to this condition. As a result, our study focuses on evaluating and recovering a patient with LD using methods and applications informed by previous scientific studies. We analyzed a patient with sequelae following LD, including postural, walking, and static and dynamic balance disorders, muscle fatigue, changes in balancing synergies, walking base, and the projection of the General Center of Gravity (GCG). We conducted initial and final tests after a series of 10 and 20 sessions of specific physical therapy exercises.

Pioneers in the study of motor control and movement, such as Bernstein and Latash [15-17], along with Charles Sherrington (1857 - 1952), Thomas Graham Brown (1882-1965), and Walter Hess (1881-1973), have significantly contributed to our understanding of joint freedom, redundancy, and muscle-articular synergies. Charles Sherrington contributed to the understanding of inhibition, spinal-muscle connectivity, and the sensory-motor cortex. Thomas Graham Brown researched central movement programming and pattern generation mechanisms. Walter Hess studied the central coordination of the autonomic system and anticipatory motor behavior. Bernstein, recognized posthumously by Western colleagues, demonstrated a central hierarchy of postural control mechanisms and distributed, parallel processing of movement.

Bernstein also participated in developing theories of motor programs, recognizing that understanding neural control of movement requires knowledge of the system's characteristics in response to external and internal stimuli [16]. His distributed model of motor control showed that central commands could produce different motions due to external forces and initial conditions. His student, Mark Latash, furthered this work by demonstrating that synergies evolve to ensure flexible and stable performance in motor activities [17].

Postural control for stability and body orientation in space requires a complex interaction between the musculoskeletal and neural systems. This includes joint amplitudes, intersegmental biomechanical relationships, muscle physiology, spinal flexibility, neuromotor processing, synergies, perceptual and sensory processes, and their central integration.

Research has concluded that postural control is not managed by a single system but depends on various intrinsic and extrinsic factors [18]. Initial studies highlighted the importance of maintaining the center of mass (COM) within certain limits for static posture. However, subsequent studies demonstrated that stability limits are not fixed and vary with individual characteristics or actions undertaken. When recovering balance, individuals tend to restore COM disturbances rather than segmental alignment disturbances, suggesting that COM is a key variable controlled by the central nervous system during postural control.

Further studies have shown that analyzing the relationship between the center of gravity (COG) and the center of pressure (COP) provides a better picture of stability [19-24]. Their interaction has been considered and used to measure and estimate posture control effectiveness in our study.

As actions have both stability and orientation components, and certain actions prioritize orientation over stability, research by Shumway-Cook & McCollum [25] and Horak & Macpherson [26] has demonstrated that stability and orientation vary with each action. The body can behave like an inverted pendulum, employing various strategies (ankle, hip) for both static and dynamic balance [21].

Bernstein's assertion that "Movement coordination is the process of mastering the redundant degrees of freedom of a mechanism in motion" [16] encapsulates the essence of motor control theories used in our study.

Equilibrium

While bipedal standing is characterized by minor postural imbalances, dynamic balance involves the integration of sensory information from the visual, vestibular, and proprioceptive systems in conditions of postural instability.

There are several theories about how sensory organization adapts to a changing environment, with two primary ones being: the intermodal theory of sensory organization by Stoffregen and Riccio [27], which posits that all sensory systems equally and continuously contribute to postural orientation; and the sensory weighting model, which suggests that the central nervous system adjusts the importance of each sensory system based on their accuracy for orientation and postural stabilization.

Torres and Oviedo [28] examined the structure and organization of muscle synergies for balance control, highlighting that a muscle can contribute to multiple synergies, each with a unique role. They demonstrated that muscle synergies can be continuously modulated by feedback signals to create postural adjustments, indicating that the central nervous system uses both pre-programmed synergies and continuous feedback control to regain stability.

Nashner et al. [29] investigated the selection of sensory information by the CNS for postural control by designing a dynamic platform with a moving visual wall to study sensory interactions in maintaining posture and balance. This approach allowed for the measurement and recording of posture and balance under various conditions.

Several studies on normative values using static platforms have examined the performance of normal subjects under varying sensory information conditions [29-33]. These studies have shown that adults and children over seven years old (the age of vestibular maturation) maintain balance well under all conditions of sensory disturbance.

The inability to maintain a stable static posture has significant functional consequences in neurological pathologies. It necessitates using the arms and hands for balance, detracting from daily activities [34-38]. Moreover, postural instability and the inability to stand on one leg for more than six seconds increase the risk of falls threefold.

Springer's 2007 study [39] showed that balance disorders are prevalent in patients with specific diseases or traumas, particularly head injuries, peripheral neuropathies, or acquired neurological conditions. Neurological pathologies can disrupt one of the postural control strategies (e.g., loss of pre-programmed synergies) while leaving the other (continuous feedback control) intact, necessitating complementary studies to distinguish between these aspects and propose specific solutions.

For the main types of static/dynamic balance (postural control/anticipatory and reactive postural control), numerous clinical tests measure performance, including the unipedal stance and computerized posturography. Our study used such tests to assess the patient's condition at the beginning and end of the recovery program.

Unipedal standing is a highly challenging situation for humans and allows researchers and clinicians to assess the effects of aging, disease, trauma, and the influence of multiple sensory systems. The single-leg stand test, which requires minimal equipment, measures balance aspects and shows abnormal values in conditions such as peripheral neuropathies and intermittent claudication. [35,36]

Various studies demonstrated the importance of maintaining unipedal posture and balance by examining corticomuscular coherence between the ankle joint and the sensorimotor cortex. This study showed that the sensorimotor cortex is directly involved in postural control during unipedal standing, compensating for high postural demands due to the low support base. Normative values in the literature indicate a significant decrease in unipedal stance time with age and pathology [40, 41].

Alain Berthoz et al. [20] demonstrated that during complex movements, the head is intermittently stabilized under gaze and fixation control, allowing it to act as an inertial guidance platform for controlling multisegmental movements. The otolithic organs, the saccule, and utricle, detect the linear accelerations of the head and provide a gravitational reference used not only to correct posture but also for perceptual actions requiring spatial orientation, including mental map rotation based on sensory information from all senses.

Merton's Servo-Control Hypothesis

The interaction between motor and sensory processes is bidirectional, and the importance of sensory information for motor control was established many years ago. The traditional feedforward and feedback control mechanisms could not fully explain the coactivation of the alpha-gamma muscle loops, necessitating alternative theories such as the equilibrium point hypothesis and the hierarchy of synergies to explain how correct posture is maintained during external destabilizations [42].

Merton proposed a model that adjusts the equilibrium point, where the CNS controls both speed and position during rapid segmental movements. Degenerative, infectious, and metabolic diseases have disrupted the transmission of sensory information from the periphery to the CNS, affecting the parameters and components of posture and movement control. Disorders of nerve conduction from proprioceptors to the brain, as seen in neurological or diabetic patients, result in significant balance issues, making it difficult for patients to stand upright with their eyes closed on a static plane [42].

Posture, a term introduced by André Bregegère in 1955 [43], is defined as a momentary attitude or body position [44]. It involves the synergistic and coordinated action of the musculoskeletal system and the central and peripheral nervous systems to maintain body stability, balance, and constant relations between body segments and the environment [45-47]. Correct posture involves the alignment of all joints, and any misalignment can lead to muscle-joint imbalances [48].

Maintaining postural stability requires the co-participation and complementarity of actions from all support components, followed by motor actions (balancing, distribution of motor control tasks), which can be synchronous or asynchronous, simultaneous, or alternating depending on the demands [44,49]. Optimal body alignment in an upright position involves:

Head: Maintained by neck muscles. Insufficiency or habitual forward tilt increases muscle demand.

Trunk: Tends to fall forward due to its weight and the head's weight, counteracted by vertebral muscles.

Shoulders: The scapular belt and upper limbs' weight cause the shoulders to fall and scapulae to separate from the spine, counteracted by shoulder girdle muscles.

Pelvis: Key to maintaining correct posture. Anteversion is balanced by gluteal and posterior thigh muscles, whose hypotonia causes anterior trunk tilt. Retroversion is balanced by rectus abdominis, obliques, and iliopsoas muscles, whose hypotonia causes posterior tilt.

Lower limbs: Stability is ensured by plantar flexor muscles and triceps surae, especially the soleus [45].

Postural defects can lead to fatigue, muscle strain, and pain in later stages, potentially affecting vital organs, particularly in the abdominal region. Our study integrates current scientific conclusions and hypotheses to address parameters affecting motor control and static and dynamic postural control in our subject, considering the literature review.

Motor control theories are inherently unfinished, always allowing room for new information from recent studies on normal and pathological brain and PNS functioning. Neuroscientists identify the scientific basis for movement disorders, while clinicians develop applications of this research, as theories are considered more or less useful based on their problem-solving efficacy for patients with movement disorders.

In this context, we present a study where a patient with Lyme disease sequelae is compared to literature findings regarding posture, balance, and neuromuscular control of movement. Previous works by Schor D, Schor V et al.[50-52] introduced the BACKFIX Method, used for spine functional impairments in preventive care. This study evaluates its application to a complex disease like LD, characterized by neurological symptoms affecting balance, walking patterns, general movement control, and muscle strength imbalance.

We demonstrate the patient's condition evolution using physical therapy with visual feedback on the rectangular force-amplitude model. Improvements in agonist-antagonist cooperation lead to enhanced muscle strength, functional muscle-joint balance of the spine, posture, and walking pattern. The patient exhibited muscle cramps in the calf and foot, pronounced thigh flexor fatigue during walking, insecurity in unipedal support, jerky walking with a wide support base, and reduced standing time in unipedal support.

MATERIALS AND METHODS

We performed the following measurements:

- Computerized somatoscopic examination
- Forces, amplitudes, and mechanical work on the supporting muscles of the trunk, enabling a computerized muscle-articular evaluation of the trunk
- Bipodal static balance - stabilometry
- Unipedal static balance

We utilized the GPS System (Global Postural System) for computerized somatoscopy and computerized static bipodal balance tests, provided by UNEFS Bucharest (University of Physical Education and Sport from Bucharest), and the Ergocontrol System for musculo-articular functionality tests of the spine using the Backfix Method, technology provided by Medisport Company.

An initial evaluation and two intermediate evaluations were conducted. For the static one-legged balance test, we measured the hold time while standing on one leg. An initial evaluation and two intermediate evaluations were conducted. For the static one-legged balance test, we measured the hold time while standing on one leg

RESULTS

Initial Assessment Results

Computerized Somatoscopy Test

Front:

- Right shoulder slightly lowered and rotated anteriorly
- Left shoulder slightly raised
- Right hip slightly lowered
- Left hip slightly raised

Profile:

- Abdominal hypotonia with increased lumbar curvature
- Projection of the GCG (general center of gravity) 11 cm anterior to the normal position

Back:

- Left shoulder blade normally positioned
- Right shoulder slightly lowered and rotated anteriorly
- Left shoulder slightly raised
- Right hip slightly lowered
- Left hip slightly raised

Evaluation of the Muscle-Articular Functionality of the Spine

The results of the computerized musculo-articular measurements regarding the muscular support of the spine are presented in Table 1. The movements were performed with difficulty and were jerky, which is characteristic of the neurological sequelae of the condition.

Table 1: Initial muscle-articular evaluation (3.02.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	5.5	26	14.47
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	9.31	49	45.45
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	8.47	39	32.85

Resources and methods used in recovery

The recovery protocol involved 20 sessions of exercises utilizing visual feedback on force-amplitude curves, performed on the Ergocontrol System. This protocol aimed to restore agonist-antagonist cooperation within muscle groups and chains responsible for global body posture while also focusing on strength enhancement through movement control exercises targeting deficient muscle groups and chains.

Each session included walking exercises designed to integrate the improvements from the neuro-muscular movement control exercises, to enhance the walking pattern.

Results After 20 Recovery Sessions

There was a noticeable improvement in overall body posture and a tendency towards optimizing the indices of musculo-articular functionality of the spine. The results from the intermediate evaluations demonstrate progressive improvements in all measured parameters, as detailed in Tables 2 and 3.

Table 2: Intermediate muscle-articular evaluation (27.02.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	15.6	41	64.93
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	181	61	110.29
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	179	58	104.37

Table 3: Intermediate muscle-articular evaluation (26.03.2023)

Test segment	F(daN)	L(cm)	Lm(J)
Direct – abdominal Indirect – lumbar	20.8	49	103.5
Direct- Scapular girdle ½ anterior, thorax anterior Indirect – anterior and posterior muscle groups of the trunk	21.5	66	140.7
Direct- Scapular girdle posterior ½ of the trunk Indirect – anterior and posterior muscle groups of the trunk	213	56	118.2

The evolution of the somatic functionality of the trunk

Spine Pathology and Accident Risk Scale:
- < 0.50 = High Risk
- 0.50-0.75 = Medium Risk
- 0.75-0.95 = Low Risk

Initial Personal Levels:
- Lumbar: High
- Antero-posterior Thoracic: Medium

After Two Series of 10 Physical Therapy Sessions:
- Lumbar: Medium
- Antero-posterior Thoracic: Low

Chart number 1 illustrates the progress in muscle-articular functionality indices of the spine, specifically Thoracic Antero-posterior and Thoraco-lumbar, as assessed by Test 2 and Test 3. These improvements are also evident in the overall body posture, with notable

enhancements in the static balance and the projection of the GCG (see Figure 1).

Table 4: Segmental and intersegmental muscle-articular functionality ratios of the spine

Segmental and inter-segmental ratio	Optimal values	Personal values 03.02.2023	Personal values 27.02.2023	Personal values 26.03.2023
Thoracic anterior - posterior	0.85 - 0.95	0.73	0.94	0.84
Thoraco-lumbar	0.85 – 0.95	0.31	0.59	0.73

Chart number 1: Indices of muscle-articular functionality of the spine - Thoracic antero-posterior and Thoraco-lumbar

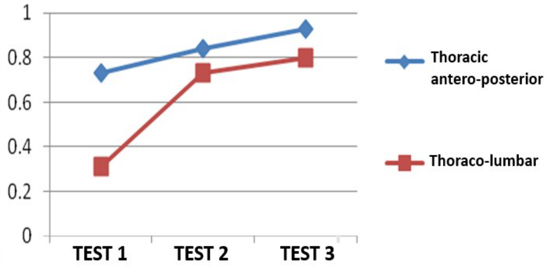

Figure 1: The improvements of the static ballance and the projection of the General Center of Gravity (CGG)

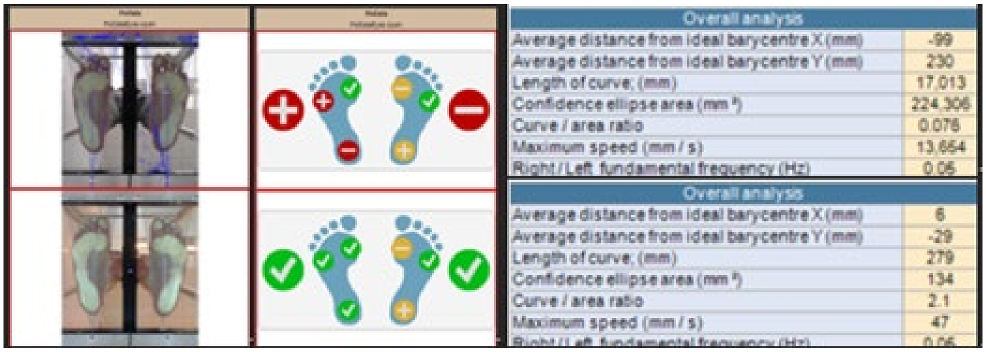

Overall analysis	
Average distance from ideal barycentre X (mm)	-99
Average distance from ideal barycentre Y (mm)	230
Length of curve; (mm)	17,013
Confidence ellipse area (mm ²)	224,306
Curve / area ratio	0.076
Maximum speed (mm / s)	13,664
Right / Left fundamental frequency (Hz)	0.06

Overall analysis	
Average distance from ideal barycentre X (mm)	6
Average distance from ideal barycentre Y (mm)	-29
Length of curve; (mm)	279
Confidence ellipse area (mm ²)	134
Curve / area ratio	2.1
Maximum speed (mm / s)	47
Right / Left fundamental frequency (Hz)	0.06

Unipedal Balance:
•Initially, unipedal support was impossible.
•After ten sessions, balance time increased to 21 seconds.
•After 20 sessions, the balance time reached 30 seconds.

General Center of Gravity (GCG)

Projection: Figures 2, 3, 4, and 5 depict the improvement in the migration of the General Center of Gravity in four positions: standing with eyes open, standing with eyes closed, facing left, and facing right.

Figure 2: The improvement of the migration of GCG in the position of stand-up face to the right side

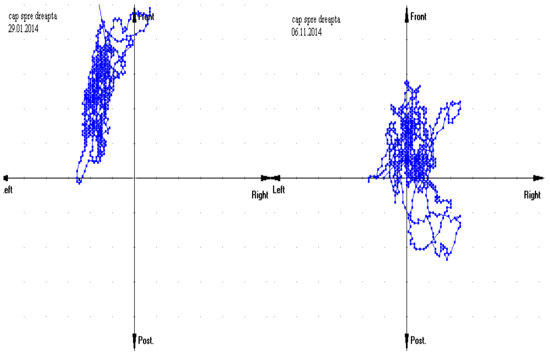

Figure 3: The improvement of the migration of GCG in the position of stand-up face to the left side

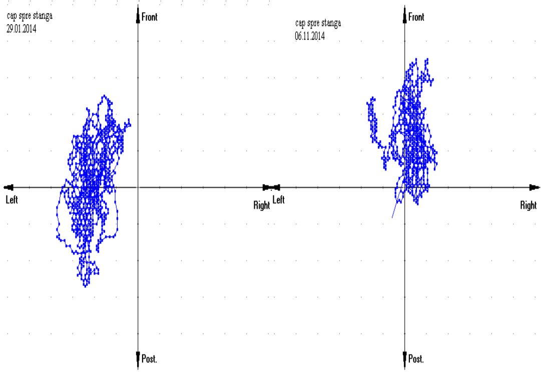

Figure 4: The improvement of the migration of GCG in the position of stand-up eyes closed

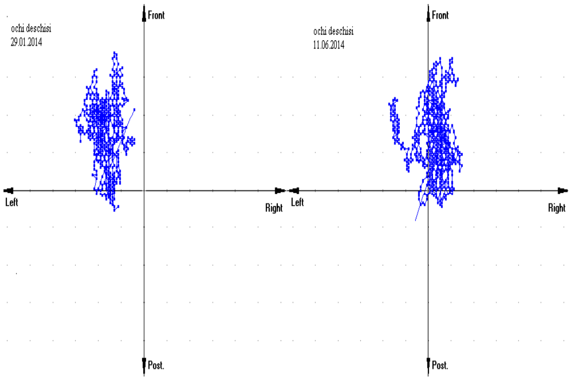

Figure 5: The migration of GCG improved in the position of standing up and eyes open

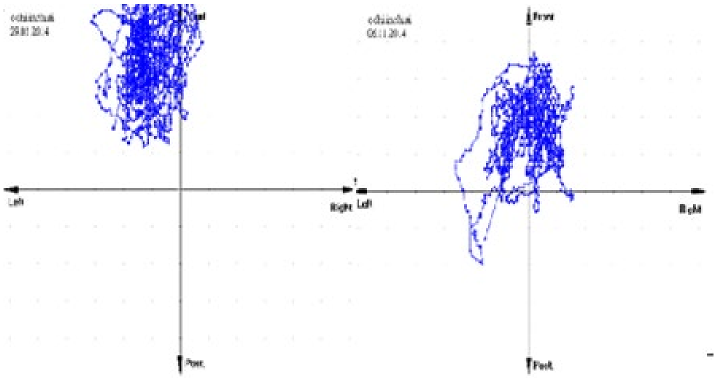

DISCUSSION

The key outcomes and benefits observed after completing two series of 10 physical therapy sessions in the study are presented in the following.

After Two Series of 10 Physical Therapy Sessions:
- Increased muscle strength and joint amplitude at the shoulder girdle.
- Continued need for abdominal muscle strength improvement to balance spinal muscle support.
- Enhanced muscle-articular functionality indices for the Thoracic Antero-Posterior and Thoraco-Lumbar regions, showing improvement from 0.73 to 0.84 and from 0.31 to 0.73, respectively.
- Balance time in the unipedal position with eyes open increased from 0 to 30 seconds.
- Improved gait from a jerky, insecure pattern to a smooth, balanced walk.
- During computerized somatoscopic evaluation, overall body posture improved, with the anterior projection of the CGG reducing from 11 cm to 5 cm from the normal projection.

The BACKFIX Method:
- Led to improved posture by optimizing muscle-articular functionality indices of the spine.

Visual Feedback on Force-Amplitude Patterns:
- Restored agonist-antagonist cooperation, positively affecting gait and balance motor control.

Optimized Functional Musculo-Articular Balance:
- Resulted in visible improvements in posture and walking pattern.

Risk Reduction:
- The risk of spinal pathology and accidents improved, moving from medium to low risk, with enhanced muscle chain synergies supporting proprioceptive and sensory system integration in unipedal stance.

The observed benefits from targeted physical exercises confirm previous studies on the uniqueness of bipodal and unipedal stance control, inter-segmental coordination, and the antigravity function of the leg extensors. Most cases of LD resolve after one course of therapy, although some patients experience gradual symptom relief even after treatment ends; therefore, follow-up assessments are recommended 3 months post-therapy. In some cases, symptoms persist despite antibiotic treatment, leading to the term "antibiotic-refractory LD", defined as persistent synovitis lasting more than 1 month after two four-week oral antibiotic courses or more than 2 months after intravenous ceftriaxone treatment [53].

Untreated arthritis may eventually resolve on its own, but it can persist for years, with some cases of LD lasting 7-8 years [54,55]. Potential treatments for patients with post-arthritis LD include non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), biologic agents, intra-articular steroids, and arthroscopic synovectomy [55,56].

The strengths of this study lie in its focus on the functional evaluation and rehabilitation of patients with Lyme disease-related neuro-muscular issues, an area with limited prior research. The use of computer-assisted physical therapy, employing technologies like the GPS Posture-test System and BACKFIX Technology, allowed for a precise and objective assessment of balance and posture, leading to significant improvements in the patients. These technologies facilitated a more thorough understanding of musculoskeletal impairments and offered targeted interventions that improved both static and dynamic balance, posture, and muscle coordination.

However, some limitations must be noted. The rehabilitation was conducted on one patient, and this limits the generalizability of the results. Additionally, the long-term sustainability of the therapy outcomes remains uncertain, and further follow-up studies are needed to verify the persistence of improvements beyond the immediate post-treatment period. Another limitation is the lack of control, which would have strengthened the validation of the study's findings.

Future research in this domain should focus on expanding the sample size and incorporating control groups for comparative analysis. Long-term studies are also crucial for understanding the chronic impact of rehabilitation techniques. Finally, integrating more advanced technologies, such as virtual reality or machine learning algorithms for personalized therapy adjustments, could further enhance patient outcomes in neuro-muscular recovery for Lyme disease.

CONCLUSION

This study demonstrates the effectiveness of computer-assisted physical therapy using technologies such as the GPS Posture-test System and BACKFIX Method in addressing the neuro-muscular and postural impairments associated with Lyme disease sequelae. Patients exhibited significant improvements in static and dynamic balance, posture, and musculo-articular functionality after completing two series of physical therapy sessions. The visual feedback provided during therapy sessions played a crucial role in restoring agonist-antagonist cooperation and enhancing motor control.

In conclusion, targeted physical therapy combined with advanced postural assessment technologies offers an effective means of improving functional recovery in Lyme disease sequelae patients, supporting their overall motor control and quality of life.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Acknowledgment

The current manuscript does not contain previously published materials or self-generated AI text.

Authors' contribution

Conceptualization D.S. and V.S.; methodology D.S.; software V.S.; validation D. S., L.G.E. and A.G.; formal analysis R.P.; investigation P.S.F.; resources S.S. and M.B; data curation R.R.G.; writing—original draft preparation D.S.; writing—review and editing L.G.E and A.G.; visualization R.R.G.; supervision L.G.E.; project administration D.S.; funding acquisition V.S. All authors have read and agreed to the published version of the manuscript elaboration.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of MEDISPORT Physical Therapy Clinic (11/30.03.2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

Navigating Cancer: Mental Adjustment as Predictor of Somatic Symptoms in Romanian Patients-A Cross-Sectional Study

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Abstract: Objective: This study aimed to examine the relationship between mental adjustment mechanisms and somatic symptoms in cancer survivors, a topic with limited research in Psycho-oncology. Methods: In this exploratory cross-sectional study, 88 cancer patients were surveyed using the Somatic Symptoms Scale (SSS-8), the Somatic Symptom Disorder Scale (SSD-12), and the Mini-Mental Adjustment to Cancer Scale (MINI-MAC). Additional questions were asked regarding cancer stage, treatment, and illness relapses. Data collection occurred over one month at a local oncology unit. Results: Several significant correlations were observed between the scales and subscales of the instruments applied during this study. The most important results showed that maladaptive adjustment mechanisms were significantly associated with somatic symptoms, while adaptive mechanisms were not. However, "fighting spirit," an adaptive mechanism, acted as a moderator between somatic symptoms and their psychological impact (effect size $t = 0.157$, $p < 0.05$). Lower "fighting spirit" scores correlated with higher SSS-8 and SSD-12 scores, indicating its potential protective role. Conclusion: A more specific assessment of somatic symptoms and their link to mental adaptation to cancer is needed. Psycho-oncological interventions should address both adaptive and maladaptive adjustment mechanisms due to their significant influence on patient outcomes.

Keywords: cancer, mental adjustment, somatic symptoms, adaptive mechanisms, maladaptive mechanisms, Psycho-oncology

INTRODUCTION

The prevalence of cancer is increasing worldwide, with almost 18.1 million cases in 2020 and a rise of 28 million cases in 2040 [1]. Romania has a lower incidence of cancer related to EU mean value, but the mortality of this disease is higher, which reflects the overall weakness in the cancer approach in our country, Romania having among the highest oncological mortality rates in Europe [2,3]. Approximately 100,000 people are diagnosed with cancer in Romania every year, a number that could be even higher. In response, Romania approved a National Plan for Beating Cancer in 2022 to be implemented during 2023-2026. Addressing the cancer burden will require sustained efforts to improve cancer prevention, early diagnosis, treatment access, and cancer surveillance systems [4,5,6]. Key measures included in the country`s National Plan for Beating Cancer are integrated and present a multidisciplinary approach, health innovation fund, cancer registry, and data collection, clinical guidelines and protocols, and oncological experts' boards. Among these last ones, a special place will be highlighted by the Psycho-oncological approach. Psycho-oncology is facing significant challenges, including major gaps in services and a critical shortage of specialized professionals, resulting in a nationwide deficit in Psycho-oncology care. However, the concepts of mental adjustment, psychological distress, and quality of life are very scarcely mentioned in local literature. Broader searches for Eastern Europe yielded limited similar results, indicating a general lack of research [7].

Patients with cancer may present more frequent anxiety, depressive symptoms, somatic symptoms, and overall psychological imbalance due to the perceived poor outcome and recurrence of the disease. Recent research has reinforced the link between psychological distress and an increased likelihood of mortality and worse survival outcomes [8]. Psychological distress in patients with cancer is defined as the psychological damage that impairs an individual's ability to operate. This illness comprises a range of emotional and behavioral features arising from the diagnosis and prognosis uncertainty [9]. Prior studies have emphasized the importance of depressive disorders and extreme emotional response patterns as crucial elements in diminishing the quality of life of these patients. It is important to acknowledge the

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interconnection between anxiety, sadness, and somatic symptoms in the context of cancer experience [10].

The cancer adjustment frame was developed by Watson and includes five adjustment mechanisms: fighting spirit, avoidance–denial, fatalism–stoic acceptance, helplessness–hopelessness, and anxiety [11]. Mental adjustment to cancer refers to the process of coping with emotional distress and restoring mental balance after a cancer diagnosis. It involves specific attitudes and strategies that patients use to adapt to the challenges of cancer and its treatment. Patients with a fighting spirit tend to have better quality of life and lower anxiety/depression compared to those with a helpless/hopeless attitude [12,13]. Maladaptive coping styles like helplessness/hopelessness are associated with a higher risk of mortality in some studies, although the evidence is not yet conclusive [12]. Factors like age, performance status, social support, and satisfaction with support can influence mental adjustment. Mental adjustment, economic status, perceived disease severity, and symptom severity directly affect the comprehensive quality of life outcome (CoQoLO) in terminally ill patients [12,13].

Somatic symptoms constitute about 50% of all general medical consultations, remaining prone to further investigations and bringing frustration to both patients and doctors in approximately one-third to one-half of cases. Both physiological and psychological variables seem to contribute to the reporting of somatic symptoms, especially in individuals with long-term medical conditions [14]. These symptoms are linked to significant limitations in functioning, disability, and utilization of healthcare services, even when considering other medical and mental conditions [14,15,16]. Research on the frequency of somatic symptoms in cancer has frequently concentrated on individuals with advanced stages of the disease or specific forms of cancer observed at specialized medical facilities [12,13,17,18]. Research revealed that a minimum of 30% of patients with cancer experience the following symptoms: fatigue and low energy, unspecified pain, loss of appetite, constipation, sleep disorders, and dizziness. The connection between psychological distress and reporting of somatic symptoms, also known as somatization, has only been examined in a limited number of small-scale research on cancer [15, 16,19,20]. This gap is significant since several studies in non-cancer medical populations have shown a high occurrence of somatic symptoms, even among symptoms that are regarded to be more specific to a particular disease. Moreover, the somatization concept refers to the expression of psychological distress through physical symptoms. Somatic symptoms, the term we chose to use throughout this paper, conversely denotes the way emotional stress can materialize as physical symptoms. A thorough comprehension of this matter is crucial to effectively address the psychological requirements of individuals with cancer, thereby enhancing their quality of life and their capacity to manage the illness [13,16,21]. Despite the recognized importance of mental adjustment on QoL and prognosis in cancer care [12], there is limited research focusing on the interplay between mental adjustment and somatic symptoms specifically.

This study aims to fill this gap by exploring the relationship between mental adjustment to cancer and somatic symptoms in patients with cancer. The findings could provide valuable insights for Psycho-oncology professionals enhancing supportive care strategies, ultimately improving patient outcomes and quality of life. The primary objectives of this study are to assess the levels of mental adjustment to cancer among Romanian patients, to measure the prevalence and severity of somatic symptoms, and to investigate their relationship with mental adjustment as being predicted by certain types of adaptive and maladaptive mechanisms. We hypothesize that maladaptive coping styles will predict somatic symptoms and their burden, while adaptive coping styles may represent protective factors in the onset and maintenance of somatic symptoms. This cross-sectional study involved a sample of Romanian patients, implementing validated psychological assessment tools to evaluate mental adjustment and somatic symptoms. Statistical analyses were conducted to explore correlations and identify significant predictors.

MATERIALS AND METHODS

Study design

This study employed a cross-sectional design to investigate the relationship between mental adjustment to cancer and somatic symptoms and their burden in Romanian cancer survivor outpatients.

Participants

A total of 88 patients with cancer were recruited from the cancer department of Elias University and Emergency Hospital in Bucharest, Romania, for one month, between May and June 2023. Inclusion criteria were: a confirmed diagnosis of cancer in the last three years, age 18 years or older, out-patients regularly monitored during their cancer treatment follow-up, and ability to understand and provide informed consent. Exclusion criteria included: severe cognitive impairment, DSM-5-TR diagnosis of a severe psychiatric disorder such as schizophrenia, depression or bipolar disorder currently or in the past based on information obtained from behavioral scales, a clinical psychiatric evaluation, and the Structured Clinical Interview for DSM Disorders [22]; diagnosis of neurological conditions (e.g., Parkinson's disease, multiple sclerosis) that could affect cognitive or emotional processing, other genetic diseases or syndromes that might affect mental adjustment mechanisms or cognitive function.

Ethical approval

The study was approved by the Ethics Committee of the Elias University and Emergency Hospital of Bucharest, Romania (nr. 9286/2023). All participants provided written informed consent before participation.

Data Collection

Data were collected through structured interviews and self-administered questionnaires. The following tools were used:

Socio-demographic data- this section included information such as age, gender, education level, employment status, marital status, residence, income level, number of children, cancer stage, cancer location, chemotherapy, hormonal, surgery, and radiotherapy history.

Mini-Mental Adjustment to Cancer (Mini-MAC) – The questionnaire, designed by Watson[11], used the Romanian-translated version [23]. The mini-MAC questionnaire consists of 29 items, assessing different ways of coping with cancer: Anxiety- refers to fear concerning the disease without prior control which is anxiety-causing; Fighting spirit- refers to framing cancer as a challenge that may prone the patient to take action and fight it; Helplessness–hopelessness- refers to feelings of passivity and fatalism towards cancer leading to submission; Positive revaluation- refers to framing the life already lived in the light of satisfaction. Coping with cancer can be made within a constructive framing including fighting spirit and positive revaluation while anxiety and helplessness may represent a destructive pattern of coping. The items are rated on a scale of 1 to 4 representing variations of responses from „definitely not" to „definitely yes" which may offer results for each of the coping strategies from 7 to 28 points. The higher the score the more frequent and intense each mental adjustment mechanism is represented.

Somatic Symptoms Scale-8 (SSS-8)- The 8-item was developed after a version of the Patient Health Questionnaire-15 (PHQ-15) [24] and validated in the general population and Romanian population [25] as well as inpatient and outpatient settings, with SSS-8 not yet validated in Romanian language [26] to detect somatic symptoms burden. The level of the experienced somatic symptoms is scored from 0 meaning „not at all" until 4 which represents „very strongly". The results are grouped into different categories with minimum, low, medium, and high groups containing symptoms such as pain in the arms, legs, or joints, gastric problems, insomnia, unspecified thoracal pain or trouble breathing, fatigue, or different ranges of headaches.

Somatic Symptom Disorder-12 (SSD-12)- this tool was first developed by Toussaint et al. [27] and focused on criterion B of the Somatic Symptom Disorder (SSD) diagnosis including subscales on cognition, affect, and behavior. 12 items are scored from 0 meaning „never" to 4 meaning „very often". The scale was already validated in outpatient settings [28], and the Romanian version used, was previously translated and validated [19,29]. 18 score was chosen to screen for potential cases by the creators of the scale with studies still being concerned with the validity of cut-off points [30].

Procedures

Participants were approached during their routine medical visits and invited to participate in the study. Those who consented were given the questionnaires to complete on-site, with instructions for returning them. Structured interviews were conducted on-site by trained researchers and clinical psychologists to ensure consistency and accuracy in data collection and that the data collection processes did not interfere with the regular cancer follow-up procedure.

Statistical Analysis

Data were analyzed using JASP software [31]. In line with the explorative nature of our study, we first described the demographics of the study participants. The internal consistency of psychometric scales was assessed using Cronbach's alpha coefficient, and all instruments had coefficients above 0.7, which is the minimum acceptable value for proper reliability. Descriptive statistics (means, standard deviations, frequencies) were used to summarize the demographic and clinical characteristics of the sample. Pearson correlation coefficients were calculated to examine the relationship between mental adjustment and somatization scores. Multiple regression analyses were conducted to identify significant predictors of somatic symptoms, controlling for potential confounders and considering standard p-value < 0.05

RESULTS

Psychometric scales reliability

All the psychometric tools used proved to be reliable. As confirmed in our analysis, a tool needs to have a Cronbach Alpha of at least 0.7 to be used. Moreover, SSS-8 presented values of 0.815, SSD-12 of 0.910, and Mini-MAC of 0.879, with all subscales also confirmed to be reliable.

Descriptive analysis

Socio-demographic data

Table 1 presents the socio-demographic characteristics of the participants. The most frequent age interval of the participants was 41-50 years with over 70% being in the age interval of 41-60 years. The sample included 86 females (97.7%). 69,3% were married, over 68% graduated from at least one university, and 75% declared a good financial status. Concerning cancer, 82,5% had local cancer, and 71% presented breast cancer with various periods since surgery, from less than one year (20,4%) to over three years (36.6%). 79% declared chemotherapy intervention, while 64% declared radiotherapy, with over 90% presenting surgery history.

Psychometric scales scores

The scores for SSS-8 indicated moderate somatic symptoms burden (mean= 10.214, SD=5.903), while SSD-12 presented a mean of 16.753, close to the cut-off point of 18, considered for SSD screening [30]. Also, the behavioral aspect of SSD-12 presented a mean of

6.110 (SD=3.256). Concerning Mini-MAC subscales, we identified anxious preoccupation (mean=17.352; SD=4.857) and helplessness (mean=14.082; SD=4.198) among the highest-scoring mechanisms checked.

Table 1: Socio-demographic characteristics

Variable	Category	Frequency	Percent	Valid Percent	Cumulative Percent
Age	20-30	3	3.409	3.409	3.409
	31-40	6	6.818	6.818	10.227
	41-50	36	40.909	40.909	51.136
	51-60	27	30.682	30.682	81.818
	>60	16	18.182	18.182	100.000
Gender	Feminine	86	97.727	97.727	97.727
	Masculine	2	2.273	2.273	100.000
Marital Status	Married	61	69.318	69.318	69.318
	Single	5	5.682	5.682	75.000
	Divorced	17	19.318	19.318	94.318
	Widowed	5	5.682	5.682	100.000
Education	Elementary	1	1.136	1.136	1.136
	Medium	25	28.409	28.409	29.545
	University	33	37.500	37.500	67.045
	Master, PhD	29	32.955	32.955	100.000
Financial Situation	Bad	16	18.182	18.182	18.182
	Good	66	75.000	75.000	93.182
	Very good	6	6.818	6.818	100.000
Cancer Stage	Local	73	82.955	83.908	83.908
	Advanced	14	15.909	16.092	100.000
Cancer Location	Breast	71	80.682	80.682	80.682
	Digestive	1	1.136	1.136	81.818
	Genital	15	17.045	17.045	98.864
	Respiratory	1	1.136	1.136	100.000
Time Since Surgery	<1 year	18	20.455	22.785	22.785
	< 2 years	14	15.909	17.722	40.506
	2-3 years	15	17.045	18.987	59.494
	>3 years	32	36.364	40.506	100.000
Chemotherapy	No	16	18.182	18.605	18.605
	Yes	70	79.545	81.395	100.000
Radiotherapy	No	30	34.091	34.483	34.483
	Yes	57	64.773	65.517	100.000
Hormone Therapy	No	35	39.773	39.773	39.773
	Yes	53	60.227	60.227	100.000
Surgical History	No	8	9.091	9.091	9.091
	Yes	80	90.909	90.909	100.000

Table 2: Psychometric scales scores characteristics

Variable	Valid	Missing	Mean	Std. Deviation	Minimum	Maximum
SSS-8	84	4	10.214	5.903	1.000	32.000
SSD-12	73	15	16.753	8.052	1.000	39.000
SSD--12_Cognitive	88	0	4.170	2.649	0.000	14.000
SSD12_Afective	88	0	5.784	3.758	0.000	16.000
SSD-12_Behavioral	73	15	6.110	3.256	1.000	15.000
MAC-Helplessness	85	3	14.082	4.198	8.000	28.000
MAC-Anxious preoccupation	85	3	17.353	4.857	8.000	29.000
MAC-Fighting Spirit	85	3	12.388	2.111	5.000	16.000
MAC-Cognitive avoidance	85	3	11.153	2.432	4.000	16.000
MAC-Fatalism	85	3	14.847	2.780	7.000	20.000

The missing data pertains specifically to the behavioral aspects subscale of the SSD-12, with approximately 17% of participants not completing this part of the scale. This indicates that the missing data is not spread across the entire SSD-12 scale but is confined to a particular subset. To address this, analyses related to the behavioral component were conducted on available data. This approach

minimizes data loss but may introduce bias if the missing data is systematic (e.g., patients with more severe symptoms avoiding completion). No imputation was used for the missing data, and as such, the findings should be interpreted with caution. Sensitivity analyses showed that the inclusion or exclusion of these cases did not substantially alter the overall findings of the study.

Correlation between all psychometric scales analysis

We found significant correlations between SSS-8 scores and overall SSD-12 score including affective and behavioral aspects subscales (r=0,63; r=0,57; p<.001), anxious preoccupation (r=0.328; p<.01), helplessness (r=0.354, p<.01) and significant negative correlations with fighting spirit (r=-0.299; p<.001). SSD-12 scores were significantly correlated with helplessness and anxious preoccupation (r=0.558, p<.001; r=0.578, p<.001) as seen in Table 3.

Table 3: Pearson correlation coefficients between different variables

Variable	SSS8	SSD12	SSD12_Cog	SSD12_Affect	SSD12_Beh	Helplessness	Anxious preoccupations	Fighting spirit	Cognitive avoidance	Fatalism
SSS8	—									
Pearson's r	—	0.537***	0.420***	0.630***	0.570***	0.354**	0.328**	-0.299**	-0.023	-0.171
p-value	—	1.615×10^-6	7.014×10^-5	1.318×10^-10	2.592×10^-7	0.001	0.003	0.007	0.837	0.126
SSD12	0.537***	—	0.796***	0.950***	0.919***	0.558***	0.578***	-0.195	0.257*	-0.004
p-value	1.615×10^-6	—	3.648×10^-17	1.515×10^-37	1.814×10^-30	3.581×10^-7	1.084×10^-7	0.101	0.030	0.971
SSD12_Cog	0.420***	0.796***	—	0.695***	0.562***	0.407***	0.469***	-0.111	0.104	0.110
p-value	7.014×10^-5	3.648×10^-17	—	5.610×10^-14	2.360×10^-7	1.124×10^-4	5.838×10^-6	0.312	0.344	0.315
SSD12_Afectv	0.630***	0.950***	0.695***	—	0.844***	0.520***	0.614***	-0.199	0.198	-0.029
p-value	1.318×10^-10	1.515×10^-37	5.610×10^-14	—	6.586×10^-21	3.423×10^-7	4.054×10^-10	0.068	0.069	0.790
SSD12_Beh	0.570***	0.919***	0.562***	0.844***	—	0.565***	0.544***	-0.165	0.241*	-0.013
p-value	2.592×10^-7	1.814×10^-30	2.360×10^-7	6.586×10^-21	—	2.278×10^-7	7.828×10^-7	0.167	0.043	0.914
Helplessness	0.354**	0.558***	0.407***	0.520***	0.565***	—	0.693***	-0.414***	0.057	-0.275*
p-value	0.001	3.581×10^-7	1.124×10^-4	3.423×10^-7	2.278×10^-7	—	2.733×10^-13	9.071×10^-5	0.606	0.012
Anxious preoccupations	0.328**	0.578***	0.469***	0.614***	0.544***	0.693***	—	-0.239*	0.152	-0.132
p-value	0.003	1.084×10^-7	5.838×10^-6	4.054×10^-10	7.828×10^-7	2.733×10^-13	—	0.028	0.170	0.233
Fighting spirit	-0.299**	-0.195	-0.111	-0.199	-0.165	-0.414***	-0.239*	—	0.311**	0.734***
p-value	0.007	0.101	0.312	0.068	0.167	9.071×10^-5	0.028	—	0.004	2.878×10^-15
Cognitive avoidance	-0.023	0.257*	0.104	0.198	0.241*	0.057	0.152	0.311**	—	0.425***
p-value	0.837	0.030	0.344	0.069	0.043	0.606	0.170	0.004	—	6.071×10^-5
Fatalism	-0.171	-0.004	0.110	-0.029	-0.013	-0.275*	-0.132	0.734***	0.425***	—
p-value	0.126	0.971	0.315	0.790	0.914	0.012	0.233	2.878×10^-15	6.071×10^-5	—

**p < 0.05, **p < 0.01, ***p < 0.001*

Tables 3 and 4 show the contribution and significance of each predictor of MAC mechanisms to somatization as follows. Helplessness and anxious avoidance negatively predict somatization (t=2.520, p<.01; t=2.462, p<.01) while fighting spirit is moderator of the relationship between somatization (SSS-8) and its burden (SSD-12) (t= 0.157, p<.01) with low and medium values having a positive correlation which disappears at highest levels.

Table 4: Multiple regression analysis

Model	Unstandardized Coefficient (B)	Standard Error	Standardized Coefficient (Beta)	t	p-value
H_0 (Intercept)	17.060	1.002	—	17.026	0.013
H_1 (Intercept)	-22.708	9.161	—	-2.479	0.016
Helplessness	0.744	0.295	0.385	2.520	0.014
Anxious preoccupations	0.583	0.237	0.342	2.462	0.017
Cognitive avoidance	0.701	0.377	0.191	1.858	0.068
Fatalism	0.601	0.479	0.174	1.254	0.215
Fighting spirit	0.108	0.685	0.025	0.157	0.876

DISCUSSION

Our study showed that mental adjustment to cancer and somatic symptoms are significantly interrelated in patients with cancer. Specifically, only negative mechanisms such as anxious preoccupation and helplessness predicted somatic symptoms burden and not the positive ones, while fighting spirit moderated the relationship between somatic symptoms burden and their overall impact on a cognitive, affective and behavioral level, suggesting a potential protective factor in the relationship between SSS-8 and SSD-12.

Previous research has proved that psychological distress can exacerbate somatic symptoms in patients with cancer [32]. Also, somatic symptoms were highlighted to have mediating effects between psychological distress and quality of life in patients with cancer, while screened-positive SSD patients represented a significant predictor of lower quality of life (QOL) ($B = -0.476$, $p < 0.00$) [33]. Also, previous studies reported somatic symptoms as being risk factors for cancer patients' prognosis using the same psychometric tool of SSD-12 but without an emphasis on mental adjustment mechanisms [34]. Moreover, research reported greater psychological adaptation and maturity in coping mechanisms are associated with lower levels of somatic symptoms and depression in patients with cancer [35]. Also, maladaptive emotional responses to cancer, characterized by strong associations between emotional and somatic symptoms, can lead to higher levels of somatization and poorer quality of life in patients with cancer [36]. To our knowledge, until now, no other study explored mental adjustment to cancer mechanisms in relationship with somatic symptoms and their burden.

Concerning specific mental adjustment to cancer mechanisms, previous research linked positive and negative affect to fatalism and fighting spirit with fighting spirit being negatively correlated to fatalism and negative affect [12,37], while another study highlighted anxious preoccupation as a predictor for significant decreases in all the domains of quality of life, with helplessness-hopelessness predictor for significant decreases in emotional and functional wellbeing domains [38].

We showed the strong association between helplessness and somatic symptoms suggesting that patients who feel more overwhelmed and powerless by their illness are more likely to report physical symptoms. Similarly, anxious preoccupation, characterized by excessive worry about the disease, may lead to heightened awareness and reporting of somatic symptoms. An interesting finding of our study relies on fighting spirit as an adaptive mechanism that moderates the relationship between somatic symptoms and overall impact on patients with low and medium values, having a positive correlation that disappears at the highest levels. This may explain fighting spirit as a protective factor in the relationship, an aspect worth considering in modern psycho-oncological approaches and in accordance with previous research on its link to positive affect [39]. The absence of significant effects for adaptive mechanisms in our sample may be attributed to the homogeneity of the sample, particularly regarding cancer type and stage, as well as potential measurement limitations of the Mini-MAC scale in capturing nuanced coping responses. These findings align with literature suggesting that adaptive mechanisms may exert more influence during acute or early-stage illness phases and less during chronic or long-term survivorship. However, our results contrast with studies reporting sustained benefits of fighting spirit over time. This discrepancy highlights the need for future research to explore these mechanisms in more diverse and heterogeneous patient populations and consider longitudinal designs to capture temporal changes in coping efficacy. Tailored interventions that account for individual patient contexts and preferences may be more beneficial than a one-size-fits-all approach focusing solely on adaptive mechanisms like fighting spirit [40].

Concerning somatic symptoms assessment, although the SSD-12 and SSS-8 have good reliability and validity, they cannot replace the diagnostic accuracy of a psychiatric interview. Our descriptive results obtained from this study for both SSD-8 and SSD-12 need to be interpreted appropriately. For many diagnoses, the DSM-5 requires that symptoms from SSD should not be the results of an underlying organic cause [41]. For patients with a medical condition such as cancer, this criterion is not realistic as symptoms may indeed be associated with both the underlying medical condition [42].

Somatic symptom identification in patients with cancer needs more attention to be paid to the psychological care of patients with physical symptoms. However, the observed relationship between mental adjustment mechanisms, such as fighting spirit, and somatic symptoms could be influenced by several confounding variables not fully controlled in our study. For instance, time since diagnosis may alter the effectiveness of coping strategies, with recently diagnosed individuals potentially exhibiting more distress and somatic symptomatology compared to long-term survivors who have developed adaptive coping mechanisms. Additionally, the type and intensity of treatment—particularly chemotherapy and radiotherapy—can exacerbate physical symptoms like fatigue and cognitive impairment complicating the assessment of psychological coping. Furthermore, the disease stage significantly impacts psychological well-being and symptom burden, with advanced stages often associated with higher levels of distress and lower fighting spirit. These

confounders could attenuate or amplify the observed relationships between mental adjustment and somatic symptoms, suggesting the need for future studies to incorporate these variables more comprehensively into their analytical models in order to disentangle their effects.

Therefore, it is necessary to conduct a comprehensive assessment of the patient`s psychological dimensions of the clinical presentation. Hence, we hope to present the phenomenon of somatic symptom prevalence as a consequence of poor mental adjustment mechanisms in patients with cancer, thus highlighting the need to consider this issue by both oncologists and psychiatrists [43,44].

Clinical Implications

Somatic symptoms can be as easily confusing as physical symptoms in this specific type of population, which results in both overmedication and insufficient psychosocial care. In our study, we demonstrated a significant correlation between somatic symptoms and maladaptive mental adjustment mechanisms, a finding that should warrant greater attention to this unique issue among patients with cancer. Identifying patients with high levels of helplessness and anxious preoccupation can help Psycho-oncologists target interventions to reduce it. Psycho-oncology programs focusing on enhancing coping skills and reducing anxiety may be beneficial in this regard for Romania and other countries as costs for invasive and time and financially resources consuming investigations may overwhelm an already limited and insufficiently developed multidisciplinary approach, which may lead to confusion and medical shopping among patients [3].

Limitations

Several limitations should be acknowledged. This was a cross-sectional study, which was unable to determine the causal relationship between mental adjustment mechanisms and somatic symptoms. We selected a single cancer department, including only one hospital from one area in the country, which may limit the generalizability of the findings considering the relatively small sample size. The patient population from this hospital may not be representative of the broader population of patients with cancer in other regions or countries considering factors such as regional healthcare practices, socioeconomic status, higher in Bucharest region, and cultural differences [2,45,46]. Also, the gender imbalance significantly limits the generalizability of the findings, especially for male cancer patients. Studies incorporating larger and more diverse cancer samples, as well as longitudinal studies, are recommended to further understand the relationship between previously described variables. In this study, a formal power analysis was not conducted prior to data collection. Given the complexity of the moderation analyses, particularly in examining the role of 'fighting spirit,' the current sample size of 88 participants may not provide sufficient power to detect small to medium interaction effects reliably. This limitation should be considered when interpreting the findings, as true moderating effects may not have been detected due to insufficient statistical power. Future research should aim to recruit larger samples and conduct a priori power analyses to ensure adequate power for detecting moderation effects. Many patients have more than one concomitant disease, especially advanced patients with cancer and those who have received or are undergoing conventional anticancer treatments. However, we collected information on all somatic symptoms but did not distinguish between symptoms derived from cancer, those derived from treatments and those that resulted from other illnesses. We would like to consider this issue in our future studies on somatic symptoms in cancer populations.

Future directions

Research on patients with cancer focusing on somatic symptoms is still scarce. Most of the studies remain in the stage of presenting descriptive outcomes. More research on the mechanism of somatic symptoms in cancer, whether it has a profound impact on quality of life or survivorship, how to identify it properly and in a timely manner, and which kind of psychosocial interventions would be more effective are necessary in the future. This is the first study to explore how mental adjustment to cancer may predict and moderate somatic symptoms and its burden in Romania and beyond. Future research should consider longitudinal designs to establish causal relationships between the described variables. Studies involving diverse cultural groups are also needed to explore the generalizability of these findings, considering the cultural aspect of somatic symptoms an important point to consider. Furthermore, investigating the effectiveness of psychological interventions in reducing maladaptive adjustment mechanisms would be a valuable contribution to the field of Psycho-Oncology**.**

CONCLUSION

In conclusion, this study highlights the significant relationship between mental adjustment mechanisms as predictors of somatic symptoms in patients with cancer. Considering the intricate nature of somatic symptoms and targeting through Psycho-oncologic interventions, helplessness, anxious avoidance, and fatalism while also exploring fighting spirit may help mitigate somatic symptoms and improve overall well-being in this population, leading to high-quality care for patients.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, M.L., C.G.I. and L.S.; methodology, L.S.; software, D.M.P.; validation, M.L.., O.V., and A.C.; formal analysis, D.M.P.; investigation, L.S.; resources, M.L.; data curation, C.G.I.; writing—original draft preparation, C.G.I.; writing—review and editing, M.L. and A.C.; visualization, O.V.; supervision, M.L.; project administration, L.S.; funding acquisition, M.L. All authors have read and agreed to the published version of the manuscript.

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Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Elias Emergency and University Hospital, Bucharest, Romania (nr. 9286/2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ARTICLE

The Neurocardiogenic Impact of Ischemic Stroke: Intricacies of Cardiac Enzymes and the Vegetative System

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Abstract: There is a potential lateralization of vegetative influences at the cerebral level. We sought to understand the impact of these influences in the setting of ischemic stroke. We conducted a prospective study on 110 who presented with acute ischemic stroke (symptom onset maximum 24 hours before admission). We investigated correlations between stroke subtype, insular location or lateralization, stroke severity, hospital mortality, and the dynamic of enzymes (Tn, TnT, CK, CK-MB). We demonstrated that a higher cardiac enzyme value is associated either with stroke severity or with a higher risk of death in the short term, this growth being a marker for a more severe prognosis of a large stroke rather than an independent cause of mortality. Cardiac monitoring in the acute phase of ischemic stroke can prevent cardiac morbidity and mortality, which is why it is important to identify patients at high risk of heart complications after stroke.

Keywords: stroke; vegetative system; cardiac enzymes

INTRODUCTION

The brain structures involved in cardiac autonomic activity regarding the modulation of the force of contraction, heart rate, and stress response are the anterior cingulate cortex, the parabrachial nucleus, the hypothalamus (the paraventricular nucleus, the dorsomedial nucleus, the lateral hypothalamic area), insula (especially its anterior portion), periaqueductal gray matter, and some parts of the medulla (ventrolateral rostral medulla, nucleus ambiguus, nucleus of the solitary tract) [1].

Right insular lesions lead to increased parasympathetic activity to the detriment of sympathetic activity, and the opposite occurs in left insular lesions [2]. Thus, it can be stated that there is a lateralization of vegetative influences at the cerebral level. This lateralization hypothesis applies to the intrinsic control of the cardiac vegetative system [3].

In a study of approximately 865,000 patients, it was observed that the incidence of acute myocardial infarction (MI) in patients with acute stroke reaches 1.6% (approximately 80% being non-STEMI and 20% STEMI). Advanced age is the strongest predictor of MI in stroke patients, along with ischemic heart disease, chronic kidney disease (CKD), and atrial fibrillation (AF) [4,5]. In the trial RANTTAS [6], it has been observed that in 6% of acute ischemic strokes, cardiac complications may occur, such as angina pectoris, acute MI, and cardiac ischemia. Cardiac monitoring is crucial after a stroke. Prolonged cardiac monitoring is a weak link in stroke care in Romania overall – evaluation past the first 24-48 hours is a rarity [7]. Acute MI after stroke may have the following etiopathogenic mechanisms: concomitant coronary artery occlusion or exacerbation of a pre-existing coronary artery disease secondary to excessive and abnormal catecholamine discharge in the acute phase of stroke [4,8,9]. Sympathetic hyperactivity in the acute phase of stroke leads to excessive stimulation of beta receptors, with the opening of Ca^{2+} channels with the intracellular flow of Ca^{2+}, contractile myocardial dysfunction, and ATP depletion, leading to mitochondrial dysfunction, and finally, cell death, translated into the paraclinical image of ECG changes and increased troponin (Tn) values [10].

In patients with acute ischemic stroke, myofibrillar degeneration, myocytolysis or the appearance of contraction band necrosis may occur, the death of myocardial

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cells occurring in a state of exaggerated contraction, followed by the rapid appearance of calcifications and infiltration of mononuclear cells. In contrast, in acute MI, the important occurrence of necrosis contraction bands is not identified, and the process of necrosis of myocardial cells takes hours - days, occurs in a state of relaxation, and calcifications are delayed. Thus, neurogenic myocytolysis involves the subendocardium with a high arrhythmogenic potential, different from the coagulative necrosis encountered in acute MI [11]. Scheiz et al. [12] proposed a method of classifying Tn growth in patients with acute stroke into two categories: patients with an acute Tn increase and patients with a chronic Tn increase. In those with an acute troponin (Tn) increase, after excluding acute MI, it was observed that, most likely, this increase is the consequence of neurogenic lesions.

MATERIALS AND METHODS

Patients selection

We conducted a prospective study on 110 patients hospitalized in the Neurovascular Emergency Unit who presented with acute ischemic stroke (symptom onset taking place a maximum of 24 hours before admission). We excluded patients with:

- Ischemic stroke with onset >24 hours before admission
- Hemorrhagic stroke
- The presence of simultaneous acute pulmonary edema
- Clinical and laboratory signs of simultaneous acute myocardial infarction
- History of MI in the last year
- Recent cardiac surgery (<1 year)
- History of severe CKD (terminal stage)
- History of lung disease with severe acute respiratory failure
- History of clinically relevant arrhythmias (especially atrial fibrillation)
- Advanced cardiac insufficiency (Stage III/IV NYHA)
- History of active malignancy

Objective

The study's objective was to highlight the correlation between enzymatic changes (their dynamic during the first five days after stroke onset) and insular involvement/insular lateralization, stroke etiology, severity of acute ischemic stroke (using the NIHSS score at admission and at seven days), and in-hospital mortality.

Data collection

Before inclusion in the study, patients were informed about the methodology and the clinical, paraclinical, imaging, and other explorations performed. Each patient (or caregiver if the patient has severe language disorder, impaired understanding, cognitive impairment, or impaired alertness) has signed informed consent, respecting the conditions of medical ethics. The approval of the Scientific Research Ethics Board of the University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania, was obtained for this study.

Etiological diagnosis of stroke

The etiological diagnosis of stroke was performed using the TOAST classification [13] after paraclinical tests (brain CT / MRI, Doppler ultrasound, standard ECG, 24-hour Holter monitoring, 5-day continuous ECG monitoring, and transthoracic echocardiogram).

Stroke severity

The clinical severity of ischemic stroke was calculated using the NIHSS (National Institutes of Health Stroke Scale) by a neurologist certified to use the NIHSS scale. According to the NIHSS scale, stroke patients were divided into 3 categories:

- mild stroke (NIHSS ≤8 points)
- moderate stroke (NIHSS = 9-15 points)
- severe stroke (NIHSS ≥16 points).

Statistical methods and statistical calculation tools

For both Tn and creatine-kinase MB (CK-MB) values, the Kolmogorov-Smirnov normality test (which determines to what extent the data have a normal/Gaussian distribution) was applied, which invalidated normality, which is why the Student t-test could not be applied. We resorted to a non-parametric test, Mann-Whitney (MW), which demonstrated the absence of a significant difference between troponin/CK-MB values on any 2 days (of the 5 days studied). The Mann-Whitney test was used to analyze the correlation

between stroke subtype, insular location/insular lateralization, stroke severity, hospital mortality, and enzymatic elevations (Tn, TnT, CK, CK-MB). The chi-squared test was applied to investigate the possible correlation between increased values of myocardial lesion indicators (choosing those with a higher cardiac specificity: TnT and CK-MB) and acute ischemic stroke.

RESULTS

Etiology, risk factors and demographic factors of patients

The mean age of the patients was 67.2+/-12.93 years, and the male-to-female ratio was 64.55%:35.55%. The prevalence of vascular risk factors and associated pathologies was the following – high blood pressure (HBP) 73.64%, obesity 31.82%, diabetes 23.64%, dyslipidemia 46.36%, active smoking 50%. Chronic alcohol usage was recorded in 27.27% of patients, stroke history in 21.82%, transient ischemic attack (TIA) in 1.82%, heart failure (HF) NYHA class 1 or 2)= in 5.45%, past MI in 8.18%, dementia in 10%, peripheral arteriopathy in 3.64%, chronic obstructive pulmonary disease in 1.82%, CKD (stage 1 or 2) in 0.91% of patients. Death during admission occurred in 14 of 110 patients (12.72%).

The etiological classification of stroke was performed based on the TOAST system criteria (Trial of Org 10172 in Acute Stroke Treatment) [13] in cardioembolic (29.09%), atherosclerosis of large vessels (50.90%) [including a small proportion atheroembolic (6.36%)], lacunar (3.64%), other causes (internal carotid artery dissection 1.81%) and cryptogenic (14.54%).

Insular localization of ischemic stroke and insular lateralization

Regarding the topographic location of stroke, out of the total of 110 patients included in the study, 63 patients (42.73%) had a stroke located in the insula. Of the 63 patients with insular localization, 23 patients (36.5%) had right insular localization and 40 patients (63.5%) had left insular localization.

The growth dynamic of cardiac enzymes was analyzed. There was no sustained increase in cardiac enzymes during the 5 days of follow-up. In all calculations concerning cardiac enzymes, the 3 patients who were found to have had acute MI after stroke onset were excluded.

Patients were divided into 2 categories according to Tn T levels: patients with normal values (≤0.02ng/ml) and patients with values above the upper limit (>0.02ng/ml). A similar division of patients was performed according to the CK-MB value: patients with normal values (≤25IU/L) and patients with superior values (>25IU/L). Following this analysis, it was observed that out of the total 110 patients, 60.86% had at least one elevated TnT value (in at least one of the 5 days of follow-up) (Figure 1), and 36.52% had at least one increased CK-MB value (in at least one of the 5 days of follow-up) (Figure 2).

For each patient, we took into consideration the highest value obtained in the 5 days of follow-up. The relatively high percentage of patients with elevated TnT is within the limits demonstrated in the literature, in a review [14] on increased Tn in patients with acute stroke (ischemic and hemorrhagic), this percentage difference is pending on various factors, such as the chosen day of reference, the number of patients and the arbitrary baseline. When applying the chi-squared test to analyze the correlation of TnT value above the normal limit of every 5 days with insular localization, on day 4 and 5, the TnT value correlation with insular involvement was statistically significant (Table 1). No statistically significant correlation with insular lateralization was observed. The increase in Tn (less specific for cardiac distress than TnT) did not correlate with insular involvement nor with left-right insular lateralization.

Figure 1: The distribution of TnT values in the studied group

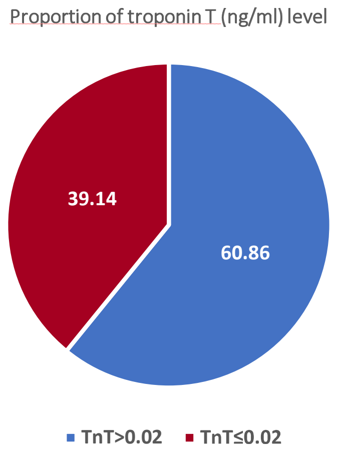

Figure 2: The distribution of CK-MB values in the studied group

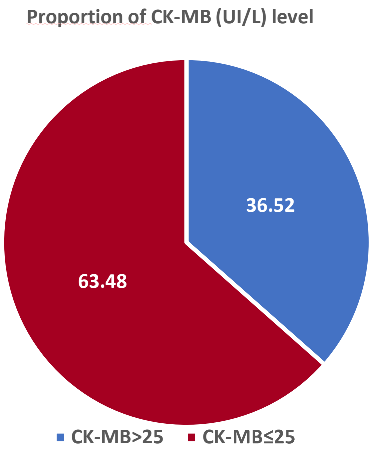

Table 1: TnT value and stroke location

Parameter	Insular	Non-insular	P-value
TnT day 4, [median (Q1-Q3)]	0.029(0.01-0.029)	0.01(0.01-0.029)	0,007 (Mann-Whitney)
TnT day 5 [median (Q1-Q3)]	0.029(0.01-0.029)	0.01(0.01-0.029)	0,02 (Mann-Whitney)

On average, CK-MB was significantly higher in patients with insular localization on each follow-up day (day 1 to day 5) (Table 2), but this was not statistically significant depending on insular lateralization. There was also no statistically significant correlation between insular location or insular lateralization and the CK value.

Table 2: CK-MB value related to stroke location

Parameters	Insular	Non-insular	P-value
CK-MB day 1, [median (Q1-Q3)]	17 (14-23)	14 (12-16)	0.006 (Mann-Whitney)
CK-MB day 2, [median (Q1-Q3)]	17 (13,21.5)	13 (11-15.25)	0,0004 (Mann-Whitney)
CK-MB day 3, [median (Q1-Q3)]	16.25 (13.25,20.75)	14 (11.25-17.75)	0,03 (Mann-Whitney)
CK-MB day 4, [median (Q1-Q3)]	19 (13.75-26.25)	13 (12-16)	0.0002 (Mann-Whitney)
CK-MB day 5, [median (Q1-Q3)]	16 (13-18.75)	13 (10.25-15)	0,003 (Mann-Whitney)

Stroke subtypes and cardiac enzymes

Regarding stroke etiology, a statistical correlation between stroke subtype and an increase in CK-MB or TnT during any of the 5 days of follow-up has not been demonstrated (neither by the Mann-Whitney test nor by the Chi-Square test).

Stroke severity and cardiac enzymes

When analyzing the correlation between stroke severity and cardiac enzyme elevations, the mean CK-MB on days 2, 3, 4, and 5 was statistically significantly higher in patients with severe stroke (NIHSS score ≥16) than in patients with moderate and mild stroke (NIHSS<16). CK (day 3 and 4) and Tn (day 4) were, on average, statistically significantly higher in patients with severe stroke than in patients with moderate to mild stroke (Table 3). TnT, much more sensitive for myocardial injury, did not record statistically significantly higher values in patients with severe stroke.

Table 3: Correlation between cardiac enzymes and stroke severity

Parameters	NIHSS<16 (moderate stroke)	NIHSS≥16 (severe stroke)	P-value
Tn day 4, [median (Q1-Q3)]	0.2 (0.2-0.2)	0.2 (0.2-0.26)	0.003 (Mann-Whitney)
CK day 3, [median (Q1-Q3)]	91 (51.9-139)	132 (82-254)	0.001 (Mann-Whitney)
CK day 4 (median (Q1-Q3))	100 (59-147)	115.5 (71.75-296)	0.004 (Mann-Whitney)
CK-MB day 2, [median (Q1-Q3)]	13 (12-16)	17 (13-20.75)	0.002 (Mann-Whitney)
CK-MB day 3, [median (Q1-Q3)]	14 (12-18)	17 (13-21)	0.002 (Mann-Whitney)
CK-MB day 4, [median (Q1-Q3)]	15 (12-19.5)	18.5 (13.75-26.25)	0.002 (Mann-Whitney)
CK-MB day 5, [median (Q1-Q3)]	13 (11-16)	15.5 (13-18.25)	0.002 (Mann-Whitney)

Cardiac enzymes and mortality

The correlation between enzyme increases and in-hospital mortality was analyzed. For Tn, a statistically significant correlation was observed between its increase on days 3, 4, 5 and hospital mortality. TnT on day 4 was, on average, significantly higher in patients who died during hospitalization than in those who survived. The increase in CK and CK-MB on days 4 and 5 correlated significantly with death during hospitalization (Table 4).

Table 4: Correlation between enzyme values and in-hospital death

Parameters	Death	No death	P-value
Tn day 3 [median (Q1-Q3)]	0.378 (0.2-1.129)	0.2 (0.2-0.2)	0,01 (MW)
Tn day 4 [median (Q1-Q3)]	0.506 (0.31-1.33)	0.2 (0.2-0.2)	0.002 (MW)
Tn day 5 [median (Q1-Q3)]	0.426 (0.26-0.81)	0.2 (0.2-0.2)	0,009 (MW)
TnT day 4 [median (Q1-Q3)]	0.049 (0.02-0.2)	0.01 (0.01-0.03)	0,008 (MW)
CK day 4, median (Q1, Q3)	326 (180.75-1621)	101 (61-177)	0,02 (MW)
CK day 5 [median (Q1-Q3)]	15 (188-4468)	288 (55.5-160)	0,01 (MW)
CK-MB day 4 [median (Q1-Q3)]	38 (21.5-55.75)	16 (12-20)	0.006 (MW)
CK-MB day 5 [median] (Q1-Q3)]	29 (19-63)	14 (12-17)	0,01 (MW)

MW=Mann-Whitney

DISCUSSION

With regard to the growth dynamics of cardiac enzymes, a time-based increase was not observed as in other studies [15]. No statistical correlation has been demonstrated (either by the Mann-Whitney test or by the chi-square test) between the stroke subtype and CK-MB or TnT elevation on any of the 5 follow-up days.

The increase in myocardial injury indicators has been interpreted differently in existing studies, with some authors considering it a marker of stroke severity and not an independent factor of poor prognosis [16] and others see it as an independent predictor of disability or mortality [17,18]. Apart from the current study, the literature has revealed only one study [19] that previously targeted the timed growth (days 1, 2, 3, 4, 5) of cardiac enzymes in stroke patients; other studies chose a variety of values of cardiac enzymes.

The possible pathophysiological mechanisms of increasing TnT and CK-MB in patients with acute stroke are multiple, but none of the studies existing so far in the literature could establish the exact cause. There is evidence of neurocardiogenic influences on myocytolysis in the acute phase of stroke, but there is great controversy regarding the cardiogenic increase in CK-MB. This increase may be possible in the case of a stroke location with a high potential for heart damage. Supporting the theory of a cardiac CK-MB increase secondary to neurogenic influences, in our study, 36.52% of patients had values above the normal limit of CK-MB, and the value of CK-MB (and TnT) correlated with insular involvement.

The current study offers different results than other literature references [20] - which concluded that TnT, unlike CK-MB, does not increase in patients with acute ischemic stroke. Also, the current study demonstrates contradicting conclusions to Barber et al. [21], which showed that in patients with acute ischemic stroke and elevated TnI, this increase did not correlate with insular location, one explanation being that in the cited study, only one TnI value was measured upon admission, whereas in the current study, statistically significant results were obtained on day 4 and 5 from stroke onset. Other examples from the literature [22] demonstrated a correlation between increased TnI and right hemisphere ischemia, but no insular involvement was specified, and no differences in insular lateralization were observed. One explanation could be that this increase is not related to other brain locations except insular ones, although further studies on other brain locations will need to be conducted to prove this claim. An argument against the theory mentioned above would be that the results obtained are controversial when it comes to the correlation of TnT alone with cardiac function, which makes it possible that CK-MB increase is not of cardiac origin and demonstrates that TnT is a much more reliable parameter to highlight the cardiac impact of chronic ischemia.

The statistical significance of elevated TnT and CK-MB values at days 4 and 5 with insular location may actually be due to a large ischemic stroke, and the increased TnT may reflect the systemic severity of stroke that may worsen the neurological status. A possible pathophysiological mechanism would be that in the case of large ischemic strokes, the growth curve of cerebral edema is maximum on days 4-5-6 from the onset of cerebral ischemia. Cerebral edema during these days may cause an exacerbation of sympathetic activity; this explanation is supported in a previous study [15], which revealed that CK-MB values are higher on day 5 of stroke onset. Obviously, this hypothesis will require further research in the future, by measuring catecholamines and enzymes simultaneously. Increased CK-MB value may originate elsewhere (muscle damage due to venous punctures, fluid restriction, and negative caloric

intake) [20]. In support of this argument comes the association of growth and CK.

In fact, it is well known that TnT is much more sensitive for assessing cardiac ischemia, either acute or chronic. The authors are of a similar opinion to the study conducted by Jensen et al. [18] that the increase in TnT is rather the consequence of HF. Another plausible explanation for the increase in TnT could be the existence of unstable atheroma plaques in the coronary arteries that could cause recurrent myocardial ischemia. It is probably necessary to dose other types of Tn (hsTnI) to correctly evaluate cardiac myocytolysis in patients with acute ischemic stroke and to redefine the concept of neurogenic cardiac ischemia.

Although this study cannot accurately state the pathophysiology of the increase in cardiac enzymes, we demonstrated that a higher value is associated either with stroke severity or with a higher risk of death in the short term, this growth being a marker for a more severe prognosis of a large stroke rather than an independent cause of mortality.

Looking at cardiac enzymes, the value of TnT and CK-MB was related to stroke severity, which indicates that in well-selected cases cardiac enzyme dosing may be useful for stratifying the risk of cardiac complications. It cannot be stated that the dosing of TnT and CK-MB should be performed routinely in all patients with acute stroke, being an expensive method and not providing additional net benefit**.**

CONCLUSION

Cardiac complications are common in patients with acute ischemic stroke, some of which may be potentiated by autonomic hyperactivity. Careful monitoring of patients at high risk of heart complications may change the short-term prognosis.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization and design, MMM, DD, ST; data analysis, MMM, DD; preparing the manuscript, MMM, IIE, ICS, MIG, OV, ST, DD, CV, CAS; writing, review and editing, MMM, DD.; visualization, MMM, DD; supervision, MMM. All authors have read and agreed to the published version of the manuscript.

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The current manuscript does not contain previously published materials or self-generated AI text.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of "CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY (protocol code PO-35-F-03 no. 83 and date of approval: 29.02.2016).

Patient consent for publication

Written informed consent has been obtained from the patient(s) to publish this paper.

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REVIEW

Radiotherapy and Reconstructive Surgery: Challenges, Implications and Optimized Therapeutic Approaches

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Abstract: Radiotherapy plays an indispensable role in cancer treatment, with its ability to target malignant cells and improve patient survival. However, its impact on healthy tissues poses significant challenges for reconstructive surgery. The altered tissue environment resulting from radiation, including fibrosis, vascular damage, and compromised wound healing, complicates reconstructive efforts and increases the risk of surgical complications. As cancer therapies advance, the approaches for managing radiation-induced complications must also adapt accordingly.

This article explores the complex interaction between radiotherapy and reconstructive surgery, particularly in the treatment of head and neck cancers, breast reconstruction following mastectomy, and sarcoma management. Special attention is given to the customization of surgical approaches based on cancer type, with the goal of optimizing both functional and aesthetic outcomes. A multidisciplinary approach, integrating advanced imaging techniques, modern radiation delivery methods, and innovative surgical techniques is essential for improving patient outcomes.

Keywords: radiotherapy, reconstructive surgery, head and neck cancer, breast reconstruction, sarcoma

INTRODUCTION

Radiotherapy is an essential part of therapeutic management, utilized in approximately 50-60% of all cancer patients during their treatment. It plays an important role in controlling tumor growth, reducing recurrence rates, and improving overall survival. However, despite its therapeutic efficacy, radiotherapy can have serious adverse effects on surrounding healthy tissues, leading to complications such as fibrosis, vascular damage, and impaired wound healing. These radiation-induced changes significantly complicate subsequent reconstructive surgery, particularly in areas where structural architecture and functional restoration are critical. [1-3]

The association between radiotherapy and reconstructive surgery presents unique challenges, especially in cases of head and neck cancers, breast cancer treatment, and sarcomas. This article aims to explore the impact of radiotherapy on reconstructive surgery, focusing on these three specific cancer types, the final goal being to optimize both functional and aesthetic outcomes for cancer survivors.

OVERVIEW OF PARTICULAR SITUATIONS REGARDING RADIOTHERAPY AND RECONSTRUCTIVE SURGERY

Head and neck cancer treatment

Head and neck cancers are frequently treated with radiotherapy, either as a primary modality or adjuvant therapy following surgery. While radiotherapy effectively targets malignant cells, it also damages surrounding healthy tissues, leading to fibrosis, vascular compromise, and wound healing complications. [4] Novel strategies for improving radiotherapy protocols are currently being explored. In the case of radioresistant tumors, for head and neck oncological cases, proton and carbon ion therapies are emerging as superior options. Proton therapy facilitates the delivery of high-dose radiation accurately, concurrently preserving adjacent healthy tissue, thereby rendering it appropriate for the eradication of microscopic disease.

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Carbon ion therapy, with its higher linear energy transfer, is more effective for larger tumors with significant burdens, offering enhanced biological effectiveness in tumor cell damage. [5]

For locoregional recurrences or second primary malignancies, reirradiation (RERT) with advanced techniques like intensity-modulated radiation therapy (IMRT) and stereotactic body radiotherapy (SBRT) is a viable option. These methods improve survival rates and reduce toxicity by precisely targeting the tumor while sparing normal tissues. [6]

Intraoperative Radiation Therapy (IORT) has been described to be efficient when utilized alongside surgical procedures. IORT provides a targeted radiation boost during surgery, potentially reducing the need for high doses of external beam radiation and minimizing exposure to normal tissues, particularly beneficial in recurrent cases where additional radiation is required. [7]

Radiotherapy in the head and neck region presents several unique challenges. One of the primary concerns is fibrosis and soft tissue contracture; radiation induces progressive fibrosis, which can result in trismus, dysphagia, and restricted neck mobility. Soft tissue contracture can further complicate flap elevation and wound closure. Compromised vascularity is another challenge, as radiotherapy damages the microvasculature, leading to poor perfusion and an increased risk of flap necrosis. Additionally, the complex anatomy of the head and neck region, which includes multiple vital structures such as the facial nerve and carotid artery, makes surgical navigation particularly challenging. [7-11]

To address these challenges, several surgical strategies are currently employed. Reconstructive surgery after head and neck cancer aims to restore function, appearance, and quality of life. Free tissue transfer, utilizing free flaps such as the anterolateral thigh (ALT) flap or radial forearm flap, remains a mainstay due to their robust vascularity and versatility. Preoperative imaging modalities like CT angiography or MR angiography are crucial for assessing recipient vessel patency and planning the anastomosis. In cases where free flap transfer is not feasible, pedicled flaps, such as the pectoralis major myocutaneous flap, provide reliable coverage. Additionally, functional reconstruction, including dynamic reconstruction of facial nerve function using gracilis muscle transfer and nerve grafts, is performed to restore facial symmetry and expression. [11-14]

Emerging techniques in this field show promise for improving outcomes. For mandibular reconstruction, the fibula flap remains the gold standard, followed by scapular flaps in selected cases; however, recent advancements in 3D printing technology have enabled the creation of custom-made implants, which can enhance functional outcomes. Despite their effectiveness, free flaps carry a higher risk of complications, such as pedicle thrombosis, especially when using irradiated vessels. [13,15-17]

Breast Reconstruction after breast cancer

Breast cancer is the most common malignancy among women and the leading cause of cancer-related mortality in females. [18,19] Surgical options for breast cancer treatment generally include breast-conserving therapy associated with radiotherapy or mastectomy, both of which offer comparable survival rates for patients with early-stage disease. [18,20] With the advent of magnetic resonance imaging (MRI), BRCA-1 and BRCA-2 genetic screening, and advancements in surgical techniques, mastectomy followed by reconstruction has become an increasingly preferred treatment option. [18] For patients undergoing mastectomy with reconstruction, a decision must be made between autologous tissue reconstruction and implant-based reconstruction. Both approaches can be performed either immediately following the mastectomy or at a later stage. Radiotherapy is recommended for high-risk cases, significantly increasing the complication rate associated with any type of reconstruction. [18,21-23]

Breast reconstruction after radiation therapy is a challenging process that aims to restore the breast's appearance while managing a series of adverse effects introduced by radiation. [24]

Deciding on the type of breast reconstruction involves a thorough discussion between the patient and her reconstructive surgeon, focusing on the expected outcomes and potential complications. Autologous reconstruction, which uses the patient’s own well-vascularized soft tissue, closely resembles natural breast tissue, can be easily shaped, and generally yields more natural-looking results. Moreover, autologous reconstruction is known to be more resilient to the effects of post-mastectomy radiation therapy (PMRT). [25,26]

A significant challenge in breast reconstruction, particularly for patients who have received or will receive radiation therapy, is determining the optimal timing for reconstruction. Radiation therapy, while crucial for reducing the risk of cancer recurrence, can complicate reconstruction efforts. Post-mastectomy radiotherapy is a common treatment that can reduce local recurrence rates and improve survival, especially in patients with lymph node-positive breast cancer. However, PMRT can negatively impact the outcome of flap reconstructions, leading to complications such as flap loss, wound contracture, and fat necrosis. [27-30]

Breast reconstruction using autologous tissue is favored because it results in a more natural-looking breast with a soft texture, consistent temperature, and a certain degree of ptosis that closely mimics the natural breast. Furthermore, autologous tissue is more resilient to the effects of radiation therapy, making it a suitable option for patients who have undergone or will undergo radiation. Autologous reconstruction is considered the gold standard by many plastic surgeons due to its superior aesthetic outcomes and the fact that it allows the reconstructed breast to age naturally alongside the other breast. It is also more customizable, enabling surgeons to tailor the reconstruction to the individual patient's needs. [31-34]

Multiple flap options are available for breast reconstruction, each tailored to meet specific patient anatomical considerations and reconstructive goals. The latissimus dorsi myocutaneous (LD) flap remains a versatile and reliable choice, particularly in cases where moderate tissue volume is required. Abdominally-based flaps, such as the transverse rectus abdominis myocutaneous (TRAM) flap,

deep inferior epigastric perforator (DIEP) flap, and superficial inferior epigastric artery (SIEA) flap, are considered the gold standard for autologous breast reconstruction due to their abundant soft tissue, robust vascularity, and the potential to achieve highly natural aesthetic outcomes. Thigh-based flaps, including the transverse upper gracilis (TUG), transverse myocutaneous gracilis (TMG), profunda artery perforator (PAP), and lateral thigh perforator (LTP) flaps, provide valuable alternatives for patients who are unsuitable candidates for abdominal donor sites, offering well-vascularized tissue with satisfactory volume and contour. Gluteal flaps, such as the superior gluteal artery perforator (SGAP) and inferior gluteal artery perforator (IGAP) flaps, are particularly beneficial in cases where other donor sites are not viable or in patients requiring a larger volume of tissue. The thoracodorsal artery perforator (TDAP) flap and lumbar artery perforator (LAP) flap represent additional options for autologous breast reconstruction, particularly in cases where more conventional donor sites are unsuitable. [35-46]

Two main strategies exist for combining flap reconstruction with radiotherapy: immediate reconstruction, where the reconstruction is performed immediately after the tumor is removed and before radiation therapy, and delayed reconstruction, where radiation is completed first, and reconstruction is performed 6-12 months later. Immediate reconstruction has the advantage of preserving the breast envelope, making the surgery easier and potentially resulting in better aesthetic outcomes. However, it also comes with significant risks. Radiation therapy can cause fibrosis, shrinkage, and fat necrosis in the reconstructed breast, leading to higher rates of complications compared to unirradiated reconstructions. Additionally, complications from immediate reconstruction can delay the start of radiotherapy, which could negatively affect the patient's cancer prognosis. [26,46,47]

Delayed reconstruction, on the other hand, allows the skin and tissues to recover from the effects of radiation before undergoing reconstruction. This approach may reduce the risk of complications but often results in less favorable cosmetic outcomes. The skin and tissues may become fibrotic and scarred, limiting the options for reconstruction and making it harder to achieve a natural breast shape. [48,49]

To minimize radiation injury to the flap while preserving the breast mound, a two-stage approach called delayed immediate autologous reconstruction (DIAR) can be considered. This technique, described by Kronowitz et al., allows for the preservation of the native breast skin and supports the patient’s psychological well-being. DIAR offers the advantages of skin-sparing mastectomy while reducing radiation-induced complications. However, the use of a tissue expander in this method is not without risks. It is debated whether deflating the tissue expander before radiotherapy is necessary. Historically, deflation was thought to give the radiotherapist better control, similar to treating a flat chest. However, more recent studies suggest that deflation is not essential, and keeping the expander inflated may actually reduce radiation-induced complications such as seroma formation and capsular contracture. [50-56]

While autologous reconstruction is often preferred for its aesthetic appeal and lower overall complication rates, prosthetic reconstruction is a practical alternative for some patients. Despite the well-documented increased risks of complications, infections, capsular contracture, the need for revision surgery, and overall reconstruction failure associated with prosthetic reconstruction in the setting of PMRT, it remains an option for a select group of patients. [57,58]

Prosthetic reconstruction, like autologous reconstruction, can be performed in either a single stage or two stages. Single-stage reconstruction, commonly known as direct-to-implant (DTI) reconstruction, involves placing the final implant during the initial surgery. The two-stage approach involves first placing a tissue expander, which is later replaced with a permanent implant. A key debate in prosthetic reconstruction is whether radiotherapy should be administered to the implant or the tissue expander. Recent systematic reviews indicate a significantly higher failure rate when PMRT is delivered to a tissue expander compared to an implant. Although the complication rates are generally lower when using implants rather than tissue expanders, these reviews often overlook the long-term results. Capsular contracture, which can negatively impact patient quality of life and satisfaction with the cosmetic outcome, often does not become apparent until years after the reconstruction. Patients who have previously undergone radiation therapy have the highest rates of reconstruction failure across all groups. Due to the significant morbidity associated with tissue expansion in irradiated skin, pure prosthetic delayed reconstruction is relatively contraindicated. [54,59-63]

Autologous fat grafting, or fat transfer, is frequently used in breast reconstruction, either as a complementary technique or a primary modality. This procedure implies the harvesting of adipose tissue from donor sites such as the abdomen, thighs, or flanks through liposuction, followed by purification and injection into the breast to augment volume, contour, and symmetry. Fat grafting improves the aesthetic results of both flap-based and implant-based reconstructions, addressing contour deformities and enhancing the overall cosmetic outcome. Moreover, fat grafting has demonstrated the capacity to improve the quality of irradiated skin by promoting neovascularization and decreasing radiation-induced fibrosis, providing not only simple volume restoration but also further therapeutic advantages. The grafted fat contains adipocyte-derived stem cells, whose regenerative capacity has been well-documented in various clinical contexts, contributing to improved tissue repair and functional recovery. Although long-term outcomes of fat grafting are still under investigation, current evidence supports its role as a versatile, minimally invasive technique that can significantly improve both the functional and aesthetic results of breast reconstruction. [64-69]

Reverse expansion is a technique used in breast reconstruction after nipple-sparing mastectomy, where a tissue expander is initially inflated to maintain the skin envelope of the breast and to provide adequate coverage for reconstruction. The expander is then gradually deflated, allowing the tissue to contract naturally, resulting in a more contoured and natural breast shape for the final reconstruction. In selected, reverse expansion can be combined with lipofilling to achieve very good outcomes in breast reconstruction, a technique described also as “intratissular expansion with serial deflation–lipofilling sessions”. While reverse expansion helps prepare the tissue and create the necessary space for reconstruction, lipofilling in multiple sessions is used to provide

volume, enhance the aesthetic result, smooth out any irregularities, and improve the quality of irradiated or compromised skin. [70-72]

Radiotherapy in the treatment of breast cancer also increases the risk of secondary lymphedema. A key area of focus is the prevention of lymphedema and the development of advanced therapeutic strategies. These approaches encompass not only conservative management but also cutting-edge super-microsurgical techniques, including the use of robotic microsurgery, to enhance treatment outcomes. [73-76]

These advanced reconstructive options enable the customization of surgical approaches to optimize both functional and aesthetic outcomes in breast reconstruction following mastectomy.

Sarcoma treatment

Sarcomas are highly aggressive tumors that pose a significant risk to patient survival, necessitating prompt and effective treatment. Comprehensive management strategies, including a combination of surgery, radiation therapy and chemotherapy in specific cases, are essential to optimize outcomes and mitigate the high potential for local recurrence and metastasis. Early diagnosis and a multidisciplinary approach are crucial to improving prognosis and reducing the substantial morbidity and mortality associated with these tumors. Radiation therapy plays a crucial role in the management of sarcomas, particularly in cases where surgical resection alone may not achieve optimal outcomes. Given the diverse histological subtypes and varying radiosensitivity of sarcomas, radiation therapy can be tailored to enhance local control, minimize recurrence, and improve overall survival rates. Preoperative radiation is often utilized to reduce tumor size, making surgical resection more feasible while preserving surrounding tissues and critical structures. Conversely, postoperative radiation may be employed to eradicate microscopic residual disease in cases where negative surgical margins are difficult to achieve. [77-79]

Concerns about negative microsurgical outcomes in patients receiving preoperative radiation therapy are justified. Irradiated wounds are described as challenging surgical environments, marked by dense fibrosis, tissue edema, and fragile recipient vessels that are prone to rupture. At the cellular level, in vitro studies have shown that radiation therapy induces cytokine upregulation in the endothelium and accumulation of inflammatory cells. [80,81]

Some studies suggested that a key strategy to successful free tissue transfer is selecting recipient vessels outside of the irradiated field. This is a more practical approach in head and neck reconstruction where there are a plethora of available recipient vessels. However, in limb reconstruction, the irradiated field often spans the entire limb circumference and getting out of the irradiated field may require the use of vein interposition grafts, which themselves can increase free flap failure rate. [82]

Surgical resection with wide margins, combined with perioperative radiation therapy, is the standard treatment for extremity soft tissue sarcomas. [83]

The timing of radiotherapy in the treatment of soft tissue sarcoma remains a topic of debate. Radiation protocols typically include either pre-operative or post-operative external beam radiotherapy, or brachytherapy. Pre-operative irradiation offers several benefits, including the delivery of a lower radiation dose to a smaller field, the potential for tumor downsizing before resection, and improved long-term functional outcomes. However, these advantages are offset by a higher incidence of wound healing complications compared to post-operative radiotherapy. [84]

Intraoperative radiotherapy (IORT) can be employed to enhance margin control by delivering a concentrated dose of radiation (typically 10-12 Gy) to the tumor bed during surgery. This approach is particularly useful when tumors are near vital neurovascular structures or when the resection margin is within several millimeters of the sarcoma. [85]

Regarding microsurgical outcomes, there are additional concerns about the effects of radiotherapy on the quality of recipient vessels, the recipient bed, and ultimately, the survival of the flap. When planning reconstruction for a soft tissue defect, key considerations include the size, location, and depth of the defect. Small-sized defects with a limited zone of injury can be effectively covered using local or perforator-based flaps. For medium-sized defects, axial pattern pedicled flaps are suitable, as they offer a greater reach while still utilizing local tissue.

Large defects, which cannot be adequately covered with local tissue, require distant or free flaps, as these are not constrained by local tissue availability. Locoregional flaps are often effective for reconstructing defects of moderate size. Among these, local fasciocutaneous flaps and pedicled muscle or myocutaneous flaps are viable options. The pedicled rectus abdominis flap, which can be oriented vertically (vertical rectus abdominis musculocutaneous, VRAM), obliquely, or transversely (TRAM), is versatile for reconstructing various defects.

Commonly used muscle flaps include the gracilis, rectus femoris, sartorius, and vastus lateralis. The anterolateral thigh (ALT) flap is the primary fasciocutaneous option, and keystone and freestyle propeller flaps can also be designed for a variety of thigh or truncal defects. For larger defects, the latissimus dorsi (LD) and rectus abdominis are commonly used free muscle flaps, both of which can be harvested with a skin paddle. Additionally, the subscapular and lateral femoral circumflex arterial systems (LFCA) allow for the harvesting of large chimeric flaps for composite tissue replacement when necessary. For reconstructing large defects, the ALT, TRAM, and latissimus dorsi free flaps are excellent choices. For smaller defects, options include the free gracilis, radial forearm, parascapular, lateral arm, and superficial circumflex iliac artery perforator (SCIP) flaps. [86-90]**.**

CONCLUSION

Radiotherapy, while essential for cancer treatment, introduces significant challenges to reconstructive surgery due to the damage it causes to healthy tissues. Advances in radiotherapy techniques, such as proton and carbon ion therapies, offer promising solutions for minimizing damage to surrounding tissues, thereby enhancing reconstructive outcomes. Surgical innovations, including the use of autologous flaps, 3D printing, and intraoperative radiotherapy, have significantly improved the ability to restore function and aesthetics in patients undergoing reconstruction after radiotherapy. However, the complexity of irradiated tissues requires a specific, multidisciplinary approach that takes into consideration the particular needs of each patient and cancer subtype. As reconstructive techniques continue to evolve, further research and collaboration between oncology, radiology, and surgery will be of utmost importance in the improvement of cancer survivors' outcomes.

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ORIGINAL ARTICLE

Multidrug-resistant ESKAPE Pathogens in a Romanian Secondary-Care Hospital during the COVID-19 Pandemic

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Abstract: The study aims to assess the frequency of ESKAPE pathogens and their antimicrobial resistance profile during the COVID-19 pandemic in a multidisciplinary hospital. Based on microbiological reports, the present study retrospectively evaluated the frequency and antimicrobial resistance (AMR) profile of ESKAPE microorganisms during 2020-2022 in a multidisciplinary hospital in Romania. There were reported 1117 bacterial isolates during the study and ESKAPE pathogens account for 33.5% of the strains. The specific AMR of ESKAPE pathogens cumulates 30.9%, while antimicrobial multidrug resistance characterizes 21% of all ESKAPE strains. The AMR profile of K. Pneumoniae, followed by S. aureus, and P. aeruginosa are alarming on ESKAPE surveillance. The most frequent bacterial isolates and antibiotic resistance reports were identified in the urology department. Based on the local AMR data we suggest the first-line antibiotic recommendations when ESKAPE pathogens are suspected. The directions of the near future antibiotic stewardship program focus on revising the clinical protocols of diagnosis and treatment in each department, increasing the bacteriological samples collection, improving the microbiological diagnostic techniques for AMR identification, and intensifying the epidemiological actions of surveillance, control, and education.

Keywords: ESKAPE; COVID-19; antimicrobial resistance; multidrug resistant organisms; antibiotic stewardship

INTRODUCTION

The World Health Organization (WHO) considers antimicrobial resistance (AMR) among the top 10 priorities for global public health. This phenomenon has developed and spread due to the selection pressure resulting from the excessive or incorrect use of antibiotics [1]. The number of deaths caused by antimicrobial resistance (AMR) could exceed 10 million annually by the year 2050 without active measures promoting the prudent use of antibiotics, improving hand hygiene in hospitals and environmental healthcare hygiene, as well as enhancing food biosecurity [2].

The emergence of multidrug-resistant (MDR) bacteria poses a concerning issue for public health due to the increased risk of healthcare-associated infections and limited treatment options. "ESKAPE" is an acronym for six bacteria of major interest in AMR surveillance systems, namely: (E) Enterococcus faecium, (S) Staphylococcus aureus, (K) Klebsiella pneumoniae, (A) Acinetobacter baumannii, (P) Pseudomonas aeruginosa, and (E) Enterobacter species [3, 4].

The "ESKAPE" pathogens are frequently implicated in severe infections, ranking high among the causes of morbidity and mortality worldwide. However, the WHO has developed a list of priorities within this group. Gram-negative bacilli are considered "critical": carbapenem-resistant CBR Acinetobacter baumannii (A. baumannii), CBR Pseudomonas aeruginosa (P. aeruginosa), third-generation cephalosporin-resistant

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(C3R) Klebsiella pneumoniae (K. pneumoniae), and C3R Enterobacter spp. In the second line of priority are Gram-positive cocci, namely vancomycin-resistant (VAN-R) Enterococcus faecium (E. faecium) and Methicillin-Resistant Staphylococcus aureus (MRSA) [5,6].

Antibiotics are frequently used to treat COVID-19-related bacterial infections. The excessive use of antibiotics during the COVID-19 pandemic can accelerate the emergence and spread of antibiotic-resistant bacteria [7].

In the first year of the COVID-19 pandemic, although many E.U. countries experienced a general decrease in antibiotic consumption both in the community and in healthcare facilities, certain antibiotics, such as azithromycin and ceftriaxone, saw an increase in usage. The reduction in prescriptions was induced by isolation, decreased mobility, and a decline in elective surgical interventions, but the impact of reduced antibiotic use on AMR, especially concerning the ESKAPE group, is insufficiently understood. In a survey conducted in 26 E.U. countries, monitoring antibiotic prescribing behaviors in healthcare facilities was halted in 9 countries, and antibiotic prescriber education programs were interrupted in 11 countries [8,9]. Understanding the link between COVID-19 and antibiotic use can contribute to the development of strategies to limit the impact of infections on vulnerable populations, thereby aiding the global effort to combat infectious diseases.

The study aims to assess the frequency of ESKAPE pathogens and their antimicrobial resistance profile during the COVID-19 pandemic (2020-2022) in a multidisciplinary Romanian hospital.

MATERIALS AND METHODS

A retrospective analysis of the isolated bacterial strains was conducted in the Microbiology Laboratory of the Emergency Military Hospital " Dr. Aristide Serfioti" Galati, derived from various biological samples obtained from hospitalized patients. The hospital is found in the Southeast region of Romania, on the eastern border of the European Union, and includes surgical departments (75 beds), medical departments (100 beds), and an intensive care unit (15 beds).

Escherichia coli and microorganisms in the ESKAPE group had their antibiotic susceptibility evaluated. Escherichia coli was included in the analysis due to its frequent association with horizontal gene transfer of resistance genes from other Enterobacterales, potentially reflecting the resistance profile of Klebsiella pneumoniae or Enterobacter spp, both of which are part of the ESKAPE pathogens [10].

The study was conducted from January 1, 2020, to December 31, 2022. This timeframe coincides with the hospital's focus on COVID-19 support, meaning that the hospital provided healthcare to COVID-19 patients, along with healthcare assistance to the general population, mostly in emergencies.

Microbiological Bacterial Identification Procedures

According to the current work procedures, bacterial isolation was performed by inoculating biological samples either "in sector" or "by exhaustion" on solid media, including Columbia blood agar, Chapman agar, and MacConkey agar (GRASO Biotech, Oxoid - Thermo Scientific, USA), followed by incubation for 24-48 hours at a temperature of 37°C. Biochemical identification of isolated bacterial strains was done through multi-testing and the automatic Vitek 2 Compact method (bioMérieux-l'Etoile).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using either the classic disk diffusion method or the minimum inhibitory concentration (MIC) method with the Vitek 2 Compact automated system, adhering to the current CLSI standards of the working period. The choice of the MIC testing method was contingent upon laboratory availability, the clinical severity of the patient, and the need for an urgent result, particularly in cases of multidrug-resistant bacteria or discrepancies found in the disk diffusion testing.

Depending on the isolated bacteria and the type of sample, specific sets of antibiotics were used for the antibiotic susceptibility testing, including Penicillin's (P – Penicillin - 10 U, A – Ampicillin - 10µg), β-lactam combination agents (AMC – Amoxicillin-clavulanate - 20/10, TZP – Piperacillin-tazobactam - 100/10 µg), cephems (CXM – Cefuroxime - 30 µg; FOX – Cefoxitin - 30 µg, CTX – Cefotaxime - 30 µg, CAZ – Ceftazidime, FEP – Cefepime - 30 µg), monobactams (AZM – Aztreonam - 30 µg), carbapenems (IMP – Imipenem - 10 µg, MEM – Meropenem - 10 µg, ERT – Ertapenem - 10 µg), fluoroquinolones (CIP – Ciprofloxacin - 5µg), macrolides (E – Erythromycin - 15 µg), TE – Tetracycline - 30 µg, lincosamides (DA – Clindamicyn); ansamycins (RIF – Rifampicine - 5 µg), sulfamides (SXT – Trimethoprim/suphamethoxazole - 25 µg), nitrofurans (NF – Nitrofurantoin - 300µg), aminoglycoside (CN – Gentamicin - 10 µg), glycopeptides (VAN – Vancomycin - 30µg, TEI – Teicoplanine - 30µg) and oxazolidinones (LNZ – Linezolid - 30 µg). The disk diffusion testing method used Oxoid Antimicrobial Susceptibility Disks from Thermo Scientific, USA, and Mast Group Ltd from the U.K. BioMérieux-l'Etoile products were employed for minimum inhibitory concentration (MIC) testing, defined as the lowest antibiotic concentration (µg/mL) that inhibits the bacterial growth. Only acquired antimicrobial resistance was taken into consideration, excluding intrinsic resistance. Results of susceptibility for each antibiotic were expressed as resistant, intermediate, or sensitive, and interpretation was made by the Clinical and Laboratory Standards Institute (CLSI) for the respective testing year [11,12,13]. Quality control procedures incorporated the use of reference strains for both identification and antibiotic resistance testing, including Staphylococcus aureus ATCC 29213, ATCC 25923; E. coli ATCC 25922; Pseudomonas aeruginosa ATCC 27853, Enterococcus casseliflavus ATCC 700327, Enterobacter hormaechei ATCC 700323, and Stenotrophomonas maltophilia ATCC 176666.

Data Collection, Interpretation, and Statistical Analysis

After removing duplicates, the antibiotic susceptibility test results were input into the electronic database of the laboratory. Analysis

of the antibiotic susceptibility profile facilitated the identification of significant resistance issues and risks posed by healthcare-associated infections in the hospital. Microorganisms that have minimum inhibitory concentrations (MIC) above the standard cut-off levels are resistant to antibiotics. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), a microorganism classified as sensitive (S) indicates therapeutic success with standard antibiotic doses, intermediate (I) responds to therapy after exposure to higher antibiotic concentrations, and resistant (R) exhibits therapeutic failure even after elevated antibiotic concentrations [14].

Multidrug resistance (MDR) was considered if non-susceptibility was found to be at least one antibiotic from two or more classes while maintaining susceptibility to at least one antibiotic from three or more categories15. Other patterns of AMR were also notified: extended-spectrum beta-lactamases (ESBL) in Enterobacterales, carbapenem resistance (CBR) in P. aeruginosa and A. baumannii, methicillin resistance in S. aureus (MRSA) and vancomycin resistance of enterococci (Van-R) [16,17,18].

Statistical analysis of the data used version 19 of the Statistical Package for Social Sciences (SPSS). Descriptive statistics tests were applied to determine frequency, incidence, and prevalence. The χ2 test was used for comparing categorical variables, considering a significance level of p<0.01.

RESULTS

Microbial Profile of the Isolates from Hospital

The microbiology laboratory obtained 1117 bacterial isolates, during the pandemic 2020-2022. The lowest annual frequency of positive cultures was recorded in 2020 (266 strains), when the specific activity of the hospital's departments was most restricted. The average length of hospitalization per year varied over the three years, ranging between 4.32 and 4.96 days.

Table 1: Distribution of the Bacterial Isolates in the Hospital's Departments

Hospital Department		Other surgery	ERT	General Surgery	Urology	ICU	Internal Medicine	Neurology	Pneumo	Other Medical	Total
GPC	*Enterococcus faecium*	-	-	-	1	3	1	1	-	-	6
	Non-faecium	1	1	5	65	12	6	7	3	1	102
	Staphylococcus aureus	1	39	18	3	20	2	2	3	3	91
	Co-Negative	-	-	2	13	13	3	5	1	-	37
	Partial sum	***1***	***40***	***25***	***82***	***48***	***12***	***15***	***7***	***2***	***236***
NF-GNR	*Pseudomonas aeruginosa*	1	18	2	20	18	1	-	7	4	71
	putida	-	-	-	1	-	-	-	-	-	1
	Acinetobacter baumanii	-	-	-	1	1	-	-	2	-	4
	Partial Sum	***2***	***18***	***2***	***22***	***19***	***1***		***9***	***4***	***76***
GNR	*Klebsiella Pneumoniae*	-	8	2	71	27	13	13	13	8	155
	Others	-	6	1	8	1	4	2	4	1	27
	Enterobacter spp	1	6	3	19	5	5	-	4	5	48
	E. coli	1	5	22	179	28	99	82	8	55	479
	Proteus spp	1	10	-	36	4	4	-	-	3	60
	Morganella spp	-	-	1	8	-	1	1	-	-	11
	Citrobacter spp	-	1	2	3	2	2	1	1	1	12
	Serratia marscenscens	-	1	1	4	1	-	1	-	-	8
	Providencia stuartii	-	-	1	1		-	-	1	-	3
	Hafnia alvei	-	-	-	-	1	-	-	-	-	1
	Salmonella spp	-	-	-	-		1	-	-	-	1
	Partial sum	***3***	***37***	***34***	***329***	***69***	***129***	***100***	***31***	***73***	***805***
Total		***6***	***95***	***61***	***433***	***136***	***142***	***115***	***47***	***79***	***1117***

The isolated bacteria were grouped into Gram-positive cocci (GPC), Gram-negative bacilli from the Enterobacterales order, and Gram-negative non-fermentative rods (NF-GNR). The highest frequency of GPC was recorded during the first pandemic year, 25.5% compared to 17% in 2021 and 21.1% in 2022, while NF-GNR was identified more frequently in the second pandemic year, representing 9.1% compared to 6% in 2020 and 5.6% in 2022.

Urine cultures were the most requested microbiological investigations, constituting 80% of bacterial cultures. Much fewer analyses were performed on other biological samples such as 5% purulent secretions, 5% respiratory secretions, 3% ear secretions, and 1% nasal and pharyngeal exudates. Blood cultures represented only 0.45% of the tested biological samples.

The urology department reported the highest number of isolates, representing 38.76% of the total bacterial strains. The Internal

Medicine department accounts for 12.71%, the Intensive Care Unit for 12.17%, and Neurology for 10.29%. Bacterial strains were obtained from between 4.9% and 2.68% of the identified isolates from the ENT, Pneumology, Rheumatology, Surgery, and Cardiology departments. Each other hospital's departments were associated with <1% of bacterial isolates (Table 1).

E. coli was isolated with the highest frequency, of 42.8% of species, potentially influencing antibiotic resistance in other Enterobacterales. At the same time, ESKAPE pathogens account for 33.5% of the bacterial strains.

Antimicrobial Resistance Profile of the ESKAPE Pathogens

Enterococcus spp.

Enterococci were identified in 108 isolates with the following distribution: 50.9% E. faecalis, 6% E. faecium, and 43% unspecified Enterococcus spp. E. faecium strains were notified in 6 cases, obtained from the urine cultures (3/6), blood cultures (2/6), and sputum (1/6), mostly on men (4/6), from the ICU (3/6). Each department of pulmonology, internal medicine, and urology reported a single strain. Among the six strains of E. faecium, one-third (2/6) were resistant to Vancomycin and Teicoplanin, and half of the strains (3/6) were multidrug-resistant (MDR). None of the strains were sensitive to Ciprofloxacin (Figure 1a).

Figure 1: Antibiotic susceptibility Profile of the ESKAPE group: a - E. faecium (n=6); b - S. aureus (n=128); c - K. Pneumoniae (n=155); d - A. baumannii (n=4); e - P. aeruginosa (n=71); f - Enterobacter spp. (n=48).

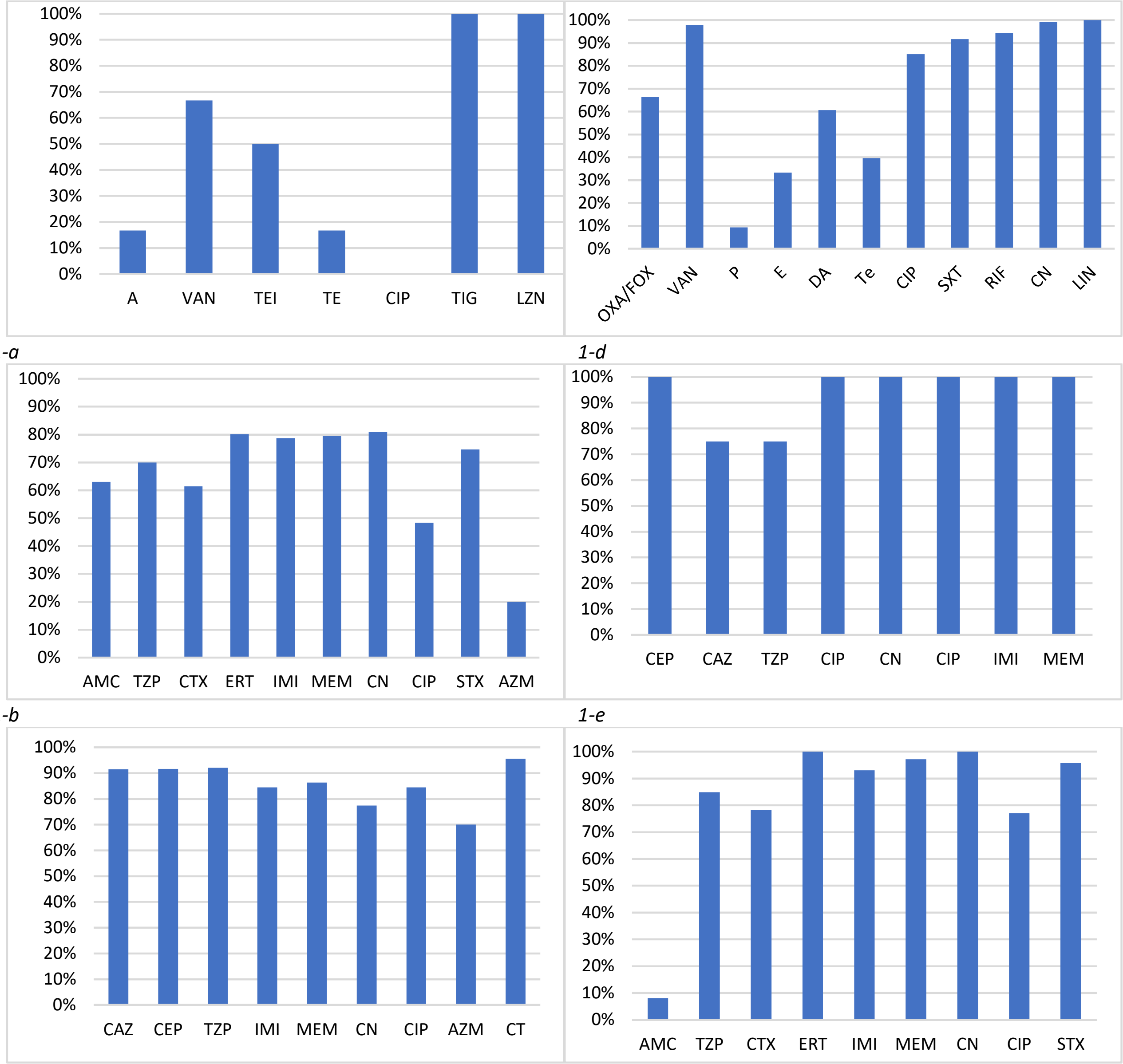

1-a *1-d*

1-b *1-e*

1-c *1-f*

Legend: P–penicillin; A–ampicillin; AMC-amoxicillin-clavulanate; TZP–piperacillin-tazobactam; CXM – cefuroxime; FOX – cefoxitin; CTX- cefotaxime; CAZ-ceftazidime; CEP-cefepime; CT: colistin; AZM-aztreonam; IMP-imipenem; MEM-meropenem; ERT-ertapenem; CIP- ciprofloxacin; E-erythromycin; TE-tetracycline; TIG- tigecycline; DA-clindamicyn; RIF-rifampicine; SXT– trimethoprim/suphamethoxazole; CN-gentamicin; VAN- vancomycin; TEI- teicoplanin; LNZ-linezolid.

Staphylococcus aureus

A total of 128 staphylococcal strains were isolated and identified as follows: 71% S. aureus and 29% Coagulase-Negative Staphylococcus (Co-NS), represented by S. epidermidis (23), S. warneri (4), S. haemolyticus (3), S. hominis (2), S. lugdunesis (1), S. saprophyticus (1), and unspecified Co-NS (3). Most S. aureus strains were identified in nasal or pharyngeal exudates (29.7%), purulent secretions (18.7%), and ear secretions (17.6%). Most samples were taken from patients in the Otorhinolaryngology (ENT) department, Intensive Care Unit, and Surgery departments (85.7%). The frequency of MRSA strains was 33.6%. The MDR rate for S. aureus was 44.5%. The lowest susceptibility of S. aureus was recorded for Penicillin at 9.4%, Erythromycin at 30%, Daptomycin at 60.1%, and Tetracycline at 39.6%. Susceptibility remained at 85% for CIP, 91.66% for SXT, 94.2% for RIF, and 99% for CN (Figure 1b).

Klebsiella pneumoniae

A total of 182 Klebsiella spp strains were isolated, including 85.16% K. pneumoniae, 5.5% K. oxytoca, and 9.3% unspecified species. Most of the cases (64.55%) were found in male patients, and most of the strains came from urine cultures (69%), sputum (9%), and purulent secretions (6%), mainly from the urology department (43.4%), intensive care (15.38%), pulmonology (9.3%), and internal medicine (9.3%). The antibiotic susceptibility profile highlighted AMC 69.8%, CTX 64.1%, TZP 75%, CN 80.9%, CIP 48.3%, STX 76.1%. Resistance to Aztreonam was found in 80% of antibiograms, noting that only 25 strains were tested. The resistance rate to carbapenems in K. pneumoniae strains was 21%, while MDR was found in 18.7% of the isolates (Figure 1c). There is no available data on the susceptibility of reserve antibiotics, as colistin, Ceftazidime + Avibactam. Meropenem + Vaborbactam.

Pseudomonas aeruginosa

Pseudomonas strains were identified in samples taken from 72 patients, of which 71 were P. aeruginosa and one was P. putida. 60.56% of the patients were males. The main products from which P. aeruginosa was isolated included urine (30.55%), ear secretion (23.6%), purulent secretions (15.27%), and sputum (6.9%), mainly from urology, ENT department, and intensive care units. The CBR rate is 15.5%, and the MDR rate is 6.8%. Among the 50 strains tested, resistance to Aztreonam (AZM) was 30%. Resistance issues were also identified for CN 22.5%, CIP 15.5%, but susceptibility was maintained for CAZ 91.55%, CEP 91.66%, TZP 92.1%, and CT 95.56% (Figure 1 e).

Enterobacter spp

Forty-eight strains of Enterobacter spp were isolated (identified) and distributed as follows: 16.66% E. aerogenes, 50% E. cloacae, and 33.33% unspecified strains. Most strains were isolated from male patients (34/48), in urine cultures (25/48), sputum (7/48), wound swabs (6/48), and pus (5/48). They originated predominantly from the urology department (39.85%), ENT department (12.5%), ICU (10.41%), internal medicine (10.41%), pneumology (8.33%), surgery (3/48), and cardiology (2/48). Isolates were also found in the orthopedics, rheumatology, and diabetology departments. ESBL frequency was found to be 14.58% among isolates, and CBR was identified in 2.85% of strains and MDR in 4.16%. The most concerning antibiotic resistance issues were observed with beta-lactams, influencing the following susceptibility profiles: AMC 8.1%, CTX 78.2%, TZP 84.85%, CIP 77%, SMX 95.75%, CN 100% (Figure 1f).

Antimicrobial Resistance Profile of E. coli

A total of 479 strains of E. coli were isolated, with 61.8% from female patients and 89.5% originating from urine cultures. E. coli strains were obtained from the following departments: urology 37.3%, internal medicine 20.6%, neurology 17.1%, ICU 5.8%, surgery 4.5%, and 14.7% from other departments. Identified antimicrobial resistance issues had frequencies of 15% MDR, 2.6% CBR, and 7.5% ESBL. The lowest susceptibility was found for AMP (37.5%), AZM (62%), CIP (64%), and SXT (72.4%). Better susceptibility was reported for AMC (86.7%), CTX (85.4%), TZP (95.5%), and CN (91.4%). Fosfomycin and Nitrofurantoin testing was limited to the E. coli strains isolated from urine culture, which proved to be susceptible in 100% and 98.25%, respectively (Figure 2).

Figure 2: Antibiotic Susceptibility Profile of E. coli (N=479)

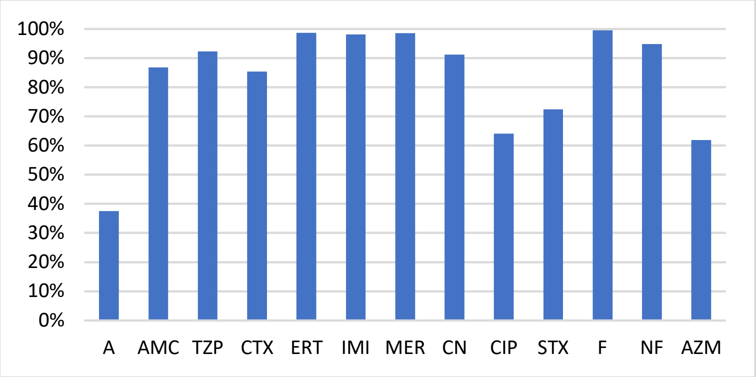

Legend: A – Ampicillin; AMC – amoxicillin-clavulanate; TZP – piperacillin-tazobactam; CXM – cefuroxime; CTX – cefotaxime; AZM – aztreonam; IMP – Imipenem; MEM – meropenem; ERT – ertapenem; CIP – ciprofloxacin; F – fosfomycine; SXT – trimethoprim/suphamethoxazole; CN – gentamicin.
Resistance of E. coli strains to AMC and CTX correlated with the resistance profile of K. pneumoniae (χ2 test: p<0.001) and Enterobacter spp (χ2 test: p<0.001).

Spread of ESKAPE Pathogen Resistance in Hospital Wards

Antibiotic resistance issues among ESKAPE pathogens have variable frequency and impact, both regarding the microorganisms surveyed and within each word, influenced by the characteristics of specific diseases to each specialty.

Acinetobacter baumannii is one of the most concerning public health problems worldwide but CBR has not been identified in any of the hospital's departments. All strains highlighted maintained sensitivity to carbapenems and other commonly tested antibiotics, but a single case evidenced an intermediary pattern, signaling decreasing susceptibility that requires vigilant epidemiological surveillance.

Pseudomonas aeruginosa - Carbapenem-resistant cases were found in isolated instances in the ICU, urology, and surgery departments without being considered a source for healthcare-associated infections. The urology department was the primary source for these isolates, but a sporadic case was also reported from the surgery department.

Enterobacter spp - Third-generation cephalosporin-resistant (C3R) was found occasionally, only in the urology department, having the slightest impact among "critical" Gram-negative bacteria on antibiotic stewardship.

The "critical" pathogen Klebsiella pneumoniae C3R is causing the most problems because it was most identified and is spreading the most in hospital wards. It mainly affects the urology department, then the intensive care unit, pneumology, internal medicine, and neurology departments. Epidemiological unrelated cases have also been reported rarely from the ENT, rheumatology, and psychiatry departments (Figure 3 a).

Figure 3: Distribution of ESKAPE Germs in Hospital Departments: a – by Specific AMR; b – by MDR

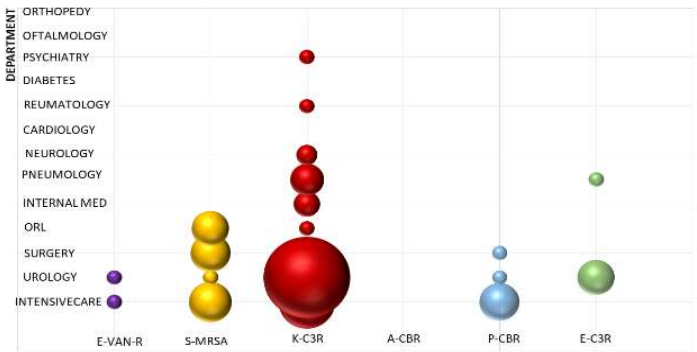

a: Specific AMR

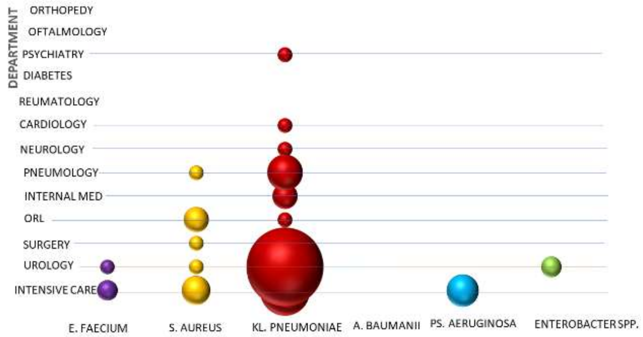

b. MDR

Among ESKAPE Gram-positive cocci, MRSA draws attention in the intensive care, surgery, and ENT departments, while only a single case was identified in urology. Vancomycin resistance in Enterococcus faecium is reported in isolated cases in the intensive care and urology departments (Figure 3 a).

Total ESKAPE pathogens cumulate specific AMRs (E. faecium Van-R, MRSA, P. aeruginosa CBR, A. baumannii CBR, K. pneumoniae, and Enterobacter spp- C3R) of 30.9%. In addition, MDR is superposing to 21% of these strains. The multidrug resistance of S. aureus, K. pneumoniae, and P. aeruginosa was associated with respect to Methicillin Resistance (χ^2 test: $p < 0.001$), C3R (χ^2 test: $p<0.001$) and CBR (χ^2 test: $p<0.001$). Concerning E. faecium and Enterobacter spp, we found a small number of isolated and MDR very few strains, that are not considered epidemiologically significant.

DISCUSSION

The reported bacterial burden is low, with a yearly average of 1.95 bacterial strains per hospital bed. This result could be explained by the hospital's multidisciplinary profile, mainly in short hospitalization, for patients from the community. The reorganization of COVID-19 support wards has disturbed the specific activities of specialized departments. Additionally, in COVID-19 wards there were

few recommendations for microbiological investigations and properly sampling bacteriological specimens was frequently difficult.

The major challenges of the COVID-19 pandemic for Antibiotic Stewardship, Infections Prevention, and Control were the increase in the number of critical cases, diminution of vigilance in infection control, short supply of specialized staff resources, and absence of guidelines for COVID-19 diagnostic and treatment at the beginning of the pandemic [1,19,20,21]. Moreover, redistribution of lab resources was required during the pandemic, by prioritizing virologic tests and reducing the bacteriological investigations1,19,20. Apart from these general respects, Romania is considered "in the red zone" of European antimicrobial resistance, since is the country with the second highest consumption of antibacterials in Europe and 11% of the population declared antibiotic prescription from the pharmacy, without medical consultation. Therefore, we could assume that patients hospitalized in most wards used antibiotics before hospital admission, so the results of this report should be taken with caution [22,23].

WHO report on antimicrobial resistance (AMR) surveillance in the European Region highlighted a decrease in E. coli isolates in 2020 compared to previous years, attributed to reduced non-COVID-19 care activities and a decline in AMR surveillance vigilance [20,24]. Unlike other countries in the European Region that reported an increase in the frequency of commonly associated healthcare pathogens, such as Acinetobacter baumannii and Enterococcus faecium, these issues were not observed in our study. However, monitoring VAN-R enterococci remains a priority for AMR surveillance in our hospital, given the elevated regional prevalence of Clostridioides difficile infections and the intensive use of oral vancomycin, exerting selective pressure on enteric bacteria [25,26].

Like other European countries, resistance to carbapenems in E. coli and Enterobacter spp. remains low, below 3%, while the rates of C3R in our study exceed 14%, consistent with the European report [1]. The complete susceptibility to Fosfomycin of urinal E. coli isolates justifies the local guideline recommendation of this antibiotic in the initial antibiotic therapy for uncomplicated infections, preserving the β-lactam class [27].

Our study's carbapenem-resistant Pseudomonas aeruginosa (CBR) rate was consistent with the European report (15.5% vs. 17.8%). However, it showed a higher resistance to aminoglycosides (22.5% vs. 9.4%) [1]. Surprisingly, susceptibility to cephalosporins of P. aerugionosa in our setting is above 90%, recommending piperacillin/tazobactam as a first-line therapeutic option for this presuming etiology of infections.

Klebsiella pneumoniae demonstrated the highest frequency among "critical" pathogens but also exhibited the most concerning antibiotic resistance. Resistance rates exceeded 20% for all antibiotic classes, including carbapenems, reaching over 50% for quinolones. It is necessary to systematically the antibiotic testing of K. pneumoniae strains for reserve antibiotics considering colimicin and combinations of ceftolozane–tazobactam, imipenem–cilastatin/relebactam, meropenem–vaborbactam or ceftazidime–avibactam [16]. The recommendation for the first-line treatment of suspected carbapenem-resistant strains should consider these reserve antibiotics. Other additional potent drugs for carbapenem-resistant K. pneumoniae such as plazomicin, eravacycline, cefiderocol or temocillin could become available by governmental regulations [28]. Furthermore, the emergence of K. pneumoniae hypervirulent strains, especially in association with CBR, is linked to antibiotic treatment failure and an elevated mortality rate. Monitoring these variants necessitates using advanced molecular techniques to identify virulence and resistance factors [29].

We have tested the susceptibility on Aztreonam, according to the standardized procedures, although Aztreonam has not been available in Romania for therapeutic use. We found over 30% resistance between the tested Gram-Negative rods, predicting low benefit as a first-choice antibiotic in clinical practice.

Staphylococcus aureus ranks second in the frequency of pathogens with antibiotic resistance risk. The MRSA rate is among the highest in Europe, meaning over 33% of strains. The notably high rate of multidrug-resistant (MDR) strains is mainly attributed to intensive community usage of penicillin, macrolides, and tetracyclines. Vancomycin and Linezolid are valuable options for first-line antibiotic treatment in severe invasive infections, while sulfamethoxazole could be used in non-severe infections. Although not within the study's scope, we observed a higher MDR rate in Coagulase-Negative Staphylococci (Co-N), which colonizes the skin, mucous membranes, and environmental surfaces in the community and the hospital. This may contribute to the transmission of antibiotic resistance to Staphylococcus aureus [30].

ESKAPE pathogens are found in the community, but more frequent in healthcare settings. The mechanisms of antibiotic resistance emergence and persistence of the ESKAPE pathogens are heterogeneous, but they share the ability to build biofilms related to biotic and abiotic surfaces [14]. Regarding the characteristics of our health care services, the source of the ESKAPE pathogens is more probable community than acquired in the hospital but could reflect the regional antimicrobial practices of the practitioners in family medicine and other specialists.

Monitoring and analyzing antimicrobial resistance in the hospital setting is essential for identifying the risks associated with the emergence of "critical" strains and healthcare-associated transmission. Specific resistances within the ESKAPE group, as well as MDR within this group, were more frequently identified in males compared to E. coli, which predominantly originated from females. Both Gram-negative ESKAPE pathogens and E. coli were predominantly associated with urinary tract infections in most cases. This observation could be considered for the risk evaluation in the first-line antibiotic treatment decision, in addition to the predictive clinical scores, such as CarbaSCORE, Tumbarello score, Duke score, or machine learning models [30,31,32,33].

This is the first surveillance report of AMR in the Emergency Military Hospital "Dr. Aristide Serfioti" from Galati, developed to understand the local impact of COVID-19 on bacterial charge, diversity, frequency, and antibiotic resistance, for updating the regional antibiotic stewardship strategy. Revising the clinical protocols for diagnosis and treatment in each department, intensifying the

bacteriological samples collection, and improving the microbiological diagnostic techniques for AMR identification, including high technologies of molecular investigations and point of care antimicrobial resistance tests are highly interests for the near future stewardship. Systematic epidemiological monitoring and control of the hospital environment, hand hygiene procedures stimulation, strict assurance of safe medical procedures, and continuing education are ongoing necessary components of the antibiotic stewardship program.

Limits of the Study

This is a retrospective study, based only on microbiological records. Consequently, the patient history and clinical data were not available. Furthermore, the community or healthcare-acquired source of infection was not analyzed. Hemocultures accounted for only 1% of the total positive samples and 3.18% of them are categorized in ESKAPE group. Therefore, comparing the antimicrobial resistance profile resulting from our study with reference data reported by representative laboratories that monitor antibiotic resistance in epidemiological surveillance networks for invasive bacterial strains, has limited significance [24]. The molecular investigation of antimicrobial resistance mechanisms was not affordable. The antibiotic consumption was not included in the present study but should be correlated with the antimicrobial resistance pattern of the hospital.

CONCLUSION

The COVID-19 pandemic impaired the hospital practice for infections and bacterial diagnostics and weakened the vigilance of antibiotic stewardship. ESKAPE pathogens are almost one-third of the total bacterial isolates, with 21% associated MDR.

The "red flag" for antibiotic stewardship between ESKAPE pathogens is Klebsiella pneumoniae. The directions of the near future antibiotic stewardship program focus on revising the clinical protocols of diagnostic and treatment in each department, to intensify the bacteriological samples collection, to improve the microbiological diagnostic techniques for AMR identification, and to intensify the epidemiological actions of surveillance, control, and education.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

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The current manuscript does not contain previously published materials or self-generated AI text.

Authors' contribution

Conceptualization, M.A., C-M.V.; methodology, A-V.I, , M.A., C-N.D.; software, I.D., C-S.S; validation, M.A., C-M. V. and A-V-I.; formal analysis, I.D., C-N.D., A.N.; investigation, C-M.V., I.D.; resources, C-M.V. and C-S.S.; data curation, A-V.I.; C-N.D.; writing—original draft preparation, C-M.V., C-S.S, A-V.I., I.D., C-N.D., A.N.; writing—review and editing, M.A.; visualization, C-S.S., A. N.; supervision, M.A.; project administration, C-M.V., A.N. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board Institutional Board of Emergency Military Hospital "Dr. Aristide Serfioti" from Galati (RCM 212/M1-544/2023) for studies involving humans.

Patient consent for publication

Not applicable. Our retrospective study used laboratory management which regularly collected data from the databases. The consent for the use of personal data was given by each patient as a routine procedure of the hospital health care provision.

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ORIGINAL ARTICLE

Impact of Physical Performance on Hemodynamic and Cognitive Status in Romanian Older Adults

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Abstract: Background: Frailty is a major challenge for the aging population, increasing the risk of falls, disability, hospitalization, and death. It's common with age, and often linked to reduced physical activity and poor diet, especially in those prone to sarcopenia. Gait speed tests are frequently used to assess mobility in older adults. Materials and methods: This six-month study at "Sfântul Luca" Chronic Disease Hospital involved 143 patients over 65 (mean age 72.85 ± 10.07), 113 of whom were women. The Tensiomed Arteriograph measured heart rate, pulse wave velocity, and central aortic blood pressure to assess acute vascular stiffness. Results: As patients aged, their physical circumstances changed: in the age group 75-84 years ("Very elderly") only 39% of the subjects achieved medium- and good-physical performance, while 56% of them were characterized by low-physical activity (Chi-square test, $p < 0.001$). The mean Mini-Mental State Examination (MMSE) values of individuals with inadequate physical activity were significantly lower (21.02 ± 4.68 points) than those with adequate physical activity (Student t-test, $p < 0.001$). Conclusion: Regular physical activity has been shown to improve hemodynamic factors and potentially slow down arterial aging, thus promoting healthy aging. It is imperative for healthcare professionals to promptly evaluate whether older, frail patients can benefit from rehabilitation, enabling swift recovery through multidisciplinary care.

Keywords: physical performance; hemodynamic status; older adults; cognitive status; personalized rehabilitation program

INTRODUCTION

The clinical state of frailty is one of the most difficult issues facing the senior citizen population. Frailty is linked to a higher risk of falls, disability, hospitalization, and mortality [1] and is thought to be common as people age. The onset of frailty in the elderly is often characterized by muscular weakness, reduced mobility, and constraints in performing routine physical tasks. [2]. Sarcopenia is characterized by the loss of muscle mass and can stem from chronic malnutrition, musculoskeletal changes related to aging, chronic illnesses, micro-inflammation, and related comorbidities. For elderly individuals, being admitted to the hospital is one of the most distressing experiences. The gait speed test is a widely used tool for assessing the mobility of elderly individuals [3]. The level of physical activity and adequate nutrition intake considerably declined among older adults at risk of developing sarcopenia [4]. Muscle weakness and reduced movement speed leading to disabilities characterize sarcopenia, a health condition associated with the aging process among the geriatric population. [5]. Physical activity and gait speed are protective factors for the occurrence of sarcopenia in older adults. The primary physical activity for seniors is walking. Clinical research and clinical objectives are increasingly relying on the evaluation of walking ability [6]. Evaluation of gait speed and its effects on hemodynamic parameters and cognitive status in older persons was the aim of this study.

MATERIALS AND METHODS

From the 160 patients hospitalized in the months of January-March who met the eligibility criteria: 8 patients refused to participate, 4 patients presented a new diagnosis of oncological disease in the middle of the hospitalization and 5 patients withdrew.

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Our study involved 143 patients, 113 of whom were women, and had a mean age of 72.85 ± 10.07 years. This six-month observational, clinical trial was conducted at the Geriatrics Clinic "Carol Davila" in the Chronic Disease Hospital "Sf. Luca." Enrolment was contingent upon the participants fulfilling the subsequent eligibility requirements: being 65 years of age or older; having no self-reported health conditions that would preclude physical examinations; being able to perform basic mobility tasks (such as walking without extreme pain, aching, or stiffness); and not being undergoing cancer treatment, knee or hip replacement, heart or spinal surgery, myocardial infarction within the previous six months, or experiencing chest pain while walking within the previous 30 days. A MMSE score < 20 points resulted in the exclusion of participants. Before participating, each participant gave written, informed consent. The Clock Drawing Test (CDT) is a simple tool used to check for early signs of dementia, scoring up to 10 points. We used the Short Physical Performance Battery (SPPB) to measure physical activity, which includes the tandem standing, 4-meter walk test, and Up-and-Go [7]. For functional capacity, we used the standardized scales Activity Daily Living (ADL) with ranges from 0 to 6 points and Instrumental Activity Daily Living (IADL) with ranges from 0 to 8 points, where a high score indicates high function [8, 9]. We measured hand grip strength using a hydraulic dynamometer and used the Tensiomed Arteriograph to evaluate arterial vascular stiffness: pulse wave velocity (PWVao), heart rate (HR), central aortic systolic blood pressure (SBPao), and central aortic pulse pressure (PPao) [10].

To illustrate the degree of comorbidity, we categorized the patients into three groups based on a comorbidity index: low comorbidity index, which represented patients with up to two chronic pathologies; moderate comorbidity index, which represented patients with three to five chronic pathologies; and high comorbidity index, which represented patients with more than five chronic pathologies.

The inclusion of patients with metabolic syndrome was achieved by evaluating those with 3 criteria (MS3) or with the presence of 4 of them (MS4) and all 5 criteria (MS5). In addition, we conducted a search through reference lists of pertinent publications, Google Scholar, EBSCOhost, and Medline in order to find published hand grip strength norms.

All determinations are performed according to the protocol and the results are analyzed according to standardized scales. This study was approved by the Ethics Commission (9/09.12.2023).

Statistical Analysis

All the data from this study were analyzed using IBM SPSS Statistics 25 and illustrated using Microsoft Office Excel/Word 2021 Stundent's t-test was used for parametric distributions. Qualitative variables were written as absolute frequencies with percentages and were tested between groups using Fisher's Exact Test.

RESULTS

The age caused changes in the physical status of patients. Thus, 56% of the subjects with low physical activity were in the age group 75-84 years, and only 39% of very elderly subjects were characterized by medium and good physical activity (Chi-square test, p < 0.001). Also, in the "very, very elderly" age group (over 85 years), we noticed the same statistically significant difference between the two groups (see Figure 1).

Figure 1. Distribution of physical activity by age

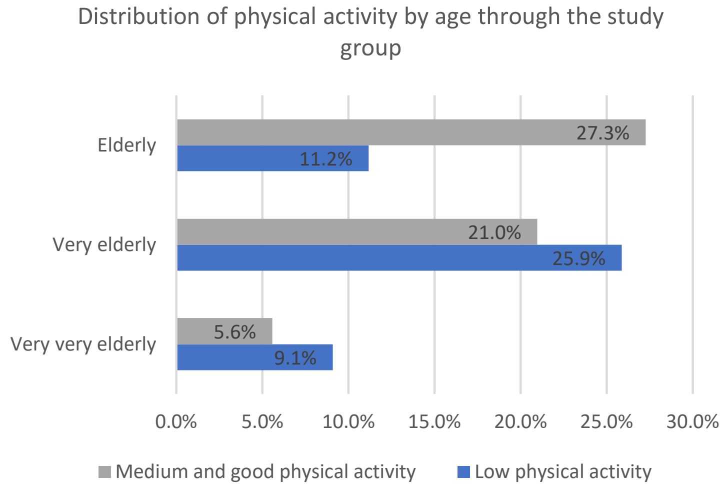

Within the group, there is no statistically significant difference in terms of physical performance and the number of associated diseases (expressed by the comorbidity index, student T-test) (see Table 1).

The functional capacity of the elderly in the study group was relatively normal, with no more than two activities affected, especially in the instrumental activities of living: the mean IADL is 6.66 ± 1.71 (out of a maximum of 8 points), and the mean ADL is 5.74 ± 0.92 (out of a maximum of 6 points). Quantitative independent variables were tested between groups using Mann–Whitney U tests (see

Table 2).

Table 1: Physical activity and comorbidity index

Characteristic	Low physical activity (N = 66)	Medium and good physical activity (N= 77)	p
Comorbidity index Low Moderate High	1 (2%) 52 (79%) 13 (19%)	2 (2%) 66 (86%) 9 (12%)	0.389
Metabolic syndrome criteria Without MS[1] MS3 MS4 and MS5	18 (27%) 23 (35%) 25 (38%)	14 (18%) 34 (44%) 29 (38%)	0.353
Diabetes Yes No	29 (44%) 37 (56%)	40 (52%) 37 (48%)	0.339

Table 2: Physical performance and functional capacity in the studied group

Characteristic	Low physical activity (N = 66)	Medium and good physical activity (N = 77)	p
ADL	5.50 ± 0.93	5.95 ± 0.87	0.004
IADL	6.06 ± 1.81	7.17 ± 1.43	< 0.001
Subjective fatigue Rare Sometimes Often	8 (12%) 9 (14%) 49 (74%)	17 (22%) 37 (48%) 23 (30%)	< 0.001
Hand grip strength	11.74 ± 2.9	17.08 ± 4.94	< 0.001
Walking speed	0.34 ± 0.1	0.69 ± 0.13	< 0.001
Statin treatment Yes Not	29 (44%) 37 (56%)	39 (51%) 38 (49%)	0.423

Physical performance and impact on daily activity scores show a direct relationship between the functionality and the SPPB values. A statistically significant difference was found in IADL scores within the group with low physical activity (6.06 ± 1.81 compared to 7.17 ± 1.43, Student's t-test, p < 0.001). Additionally, basic individual needs, such as eating and washing, are impacted in the group with reduced functionality, as measured by the ADL, like those with lower physical performance (Student's t-test, p = 0.004) (see Table 2).

Hand grip strength measured by a dynamometer, adjusted for age, gender, and height, revealed a significant difference in the group with low physical activity, with an average strength of just 11.74 ± 2.9 kg (Student's t-test, p < 0.001) (see Table 2).

In the context of long-term treatment of elderly patients with chronic cardiovascular disease, statins are often associated with the development of sarcopenia, chronic fatigue, and reduced physical activity. However, our study findings revealed no statistically significant correlation between the use of statins and the decline in hand grip strength or physical performance among the elderly patients in our study group. Remarkably, more than half (51%) of the elderly patients undergoing statin treatment maintained satisfactory levels of physical activity (see Table 2).

In our analysis of walking speed, we calculated patients' movement rates and correlated them with some hemodynamic parameters (HR, PWVao, PP) and functional capacity, through multiple regression analyses. We divided the patients into three groups: the first group (home movement) consisted of elderly individuals at heightened risk of disability who have limited mobility confined to their residence, with a walking speed of up to 0.4 m/s; the second group (limited community movement) included elderly people engage in movement within the community and are at moderate risk of losing their autonomy, with a walking speed between 0.4 - 0.8 m/s; the third group (full mobilization) comprised individuals who are not exposed to the risk of disability and are fully mobile with a walking speed exceeding 0.8 m/s (see Table 3).

The results showed a significant statistical difference on the pulse pressure (PP) (p = 0.001). This indicator could potentially serve as a valuable predictor for gauging cardiovascular risk and assessing disability risk, particularly in relation to walking speed.

Regarding the cognitive level there is a clear difference between the two groups. The mean MMSE values, which assess the cognitive status of the subjects, are significantly lower (21.02 ± 4.68 points) among those with low activity compared to those with good physical activity (Student t-test, p < 0.001) (see Table 4).

Table 3: Distribution of patients according to walking speed

	Group 1 = home movement (N = 48)	Group 2 = limited community movement (N = 71)	Group 3 = full mobilization (N = 24)	p
ADL	5.46 ± 0.84	5.80 ± 0.95	6.13 ± 0.85	0.011
IADL	6.19 ± 1.8	6.75 ± 1.71	7.33 ± 1.09	0.021
PWVao	10.37 ± 1.74	9.94 ± 1.93	9.95 ± 1.63	0.419
PP	**66.71 ± 14.23**	**65.17 ± 15.31**	**53.42 ± 12.75**	**0.001**
HR	68.81 ± 12.7	66.61 ± 11.36	65.33 ± 7.02	0.404

Table 4: Correlation of hemodynamic and cognitive parameters with walking speed

Characteristic correlation	Coefficient	p
PWVao and SPPB	- 0.141	0.092
HR and SPPB	- 0.166	0.047
Walking speed and SBPao	- 0.149	0.076
Walking speed and Body Mass Index (BMI)	0.125	0.138
Walking speed and MMSE	**0.339**	**< 0.001**
Right-Hand grip strength and SPPB	**0.422**	**< 0.001**
Left-Hand grip strength and SPPB	**0.440**	**< 0.001**
Fatigue and statin treatment	0.064	0.450

The presence of this difference comes as an important finding that supports the potential role of physical exercise in preventing cognitive impairments in the elderly. Given the limited effectiveness of current drug treatments for dementia, geriatric clinicians should place greater emphasis on incorporating physiotherapy programs for these elderly patients.

Another important finding is the difference between the two groups in the case of the Clock Drawing Test (CDT), which is easy to use, quick to administer, and can be implemented as a screening tool in primary care settings [11].

The group of those with low physical activity had an average CDT score of 6.63 ± 1.33 points (out of a max. 10 p), compared to an average score of 7.96 ± 1.72 points among those with good physical performance (Student t-test, $p < 0.001$). Our findings once again emphasize the importance of the age-old adage, "Mens sana in corpore sano".

DISCUSSION

Regular physical activity is not only crucial for maintaining autonomy but also for preserving cognitive function [12]. It is widely recognized that consistent aerobic exercise enhances cardiorespiratory function, reduces arterial stiffness [13], diminishes cardiovascular risk factors, and lowers cardiovascular mortality. Conversely, anaerobic exercises promote skeletal muscle strength and are instrumental in preventing age-related muscle atrophy [14]. Elderly patients can benefit from the high level of education of nurses, which influences the degree of clinical autonomy. The nurse needs to be aware of any barriers to learning, like loss of hearing or decline of vision and should tailor his or her education for that specific patient [15].

Physical activity is an effective strategy for protecting against sarcopenia and promoting healthy aging. Most studies on exercise in elderly people show that participants achieve positive results, although maintaining muscle strength appears to depend on the continued implementation of certain types of physical activities. One of the conclusions from a meta-analysis of 10 studies was that there was no consistency in the measurement of sarcopenia, indicating a need for further investigations [16].

For elderly people, regular exercise (at least one hour, at least twice a week) through personalized exercise programs can have beneficial cardiovascular effects, such as lowering blood pressure, improving skeletal muscle perfusion, and enhancing glucose utilization in myocytes. Additionally, regular exercise helps maintain muscle fiber quantity and supports skeletal muscle strength [17]. Numerous studies have demonstrated the significant impact of this factor on recovery rates in neurological system disorders and cardiovascular diseases, both of which are primary contributors to disability and diminished quality of life (QoL) [18-20].

The QoL significantly influences patients' adherence to treatment and their overall recovery, making it a pivotal endpoint in clinical research. Aspects such as pain management, emotional well-being, social interactions, and independence profoundly impact patient outcomes. Consequently, enhancing QoL becomes a primary objective from the point of diagnosis. While both patients and physicians acknowledge the importance of prolonging survival, maintaining an optimal quality of life throughout the treatment process is equally imperative [21]. We consider that physical activity plays a major role in enhancing QoL. Due to the Coronavirus (COVID-19) pandemic, many elderly individuals—already a high-risk group for severe illness and adverse SARS-CoV-2 outcomes—opted to remain at home out of fear of contracting the virus, regardless of their associated comorbidities. Consequently, it is hypothesized that a significant

number of older adults are now facing the repercussions of that period, notably sarcopenia, which is linked to a lack of physical activity. As a result, these individuals are now tasked with recovering from that phase, underscoring the need to increase awareness about the importance of physical exercise in this population [22]

In our study, the measurement of muscle strength through the dynamometer revealed a significant difference in the group of elderly patients with low physical activity (Student t-test, $p < 0.001$). Traumatic injuries accounted for the largest percentage of rehabilitation cases in Romania (73.17%), with musculoskeletal illnesses coming in second (70.06%). Reduced recovery was observed in cases of neurological system disorders (50.56%) and cardiovascular diseases (44.23%) [18].

Although other studies have debated the potential link between statin administration in the elderly and the development of sarcopenia, fatigue, and decreased physical activity [23], our group did not find a positive correlation supporting this relationship. Hence, it endorses the recommendations for the continued utilization of statins, even in advanced age, as they have exhibited protective advantages in averting cardiovascular incidents.

Analyzing and interpreting walking speed presents a challenge for clinicians, but recent specialist studies have provided new insights into survival and mortality predictors. Normal walking speed requires a relatively low level of energy consumption [24, 25]. Our study found statistically significant results related to pulse pressure (PP), which is considered a predictor for evaluating disability risk in the elderly population [26].

Cognitive status can be improved by exercise. Several prospective studies have demonstrated that maintenance of higher levels of physical fitness helps protect against cognitive deterioration, even at an advanced age [12,27,28]. Conversely, poor physical performance among older adults may accelerate cognitive decline and lead to early onset of dementia [29, 30]. Additionally, muscle strength has been linked to cognitive performance [31,32]. Promising trials utilizing AI and technology, which show significant improvements in autonomy and reduced reliance on human resources point to a bright future for the medical care and recovery of elderly patients with cognitive impairments [33,34,35].

Several limitations of the present study should be acknowledged. First, the study sample was predominantly female, which could potentially influence the results. Second, the study group was relatively small, and there was a lack of homogeneity, which may affect the comparability of the findings with the broader Romanian population.

CONCLUSION

Maintaining physical performance, especially in the elderly with associated chronic diseases, has an important role in maintaining autonomy and quality of life, especially in the very old and very, very old age groups that become frailer. Assessing hand grip strength can provide valuable insights into physical performance and guide lifestyle optimization and treatment recommendations for elderly patients. Exercise training has been shown to improve hemodynamic status (including SBP, PP, SBPao, PPao) and may help delay arterial aging, playing an important role in healthy aging. Muscle strength, balance, flexibility, endurance, and mobility are all aspects of whole-body functioning that are included in physical performance. When older adults perform poorly physically, they experience a decline in their quality of life, a loss of cognitive function, an increase in their risk of falling, poorer clinical outcomes, and a reduction in their independence when it comes to doing activities of daily living (ADL).

Early detection of rehabilitation potential in frail elderly patients is essential for initiating timely recovery interventions through specialized medical services and multidisciplinary teams.

From a practical and clinical perspective, developing a consistent program of adapted, personalized physical activities is necessary. Such programs can positively affect functional parameters and significantly enhance the quality of life for elderly individuals. In order to corroborate these findings and draw further conclusions, it is essential to conduct studies encompassing a more diverse and extensive demographic.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

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Authors' contribution

Conceptualization, J.A., S.M.A., A.Z. and C.O.; methodology, R.M., A.P..; software, S.M.G, C.D.G., A.G.P .; validation, J.A., S.M.A., A.Z. and C.O; formal analysis, J.A., A.Z., V.M.A.M., M.N; investigation, V.M.A.M., C.D.G., S.M.G.; resources, A.G.P., S.M.A., M.N., C.O.; data curation, S.M.G, C.D.G, A.G.P., M.N.; writing—original draft preparation, J.A, S.M.A, A.Z., C.O.; writing—review and editing, J.A., S.M.A., C.O.; visualization, A.Z., R.M., S.M.A.; supervision, S.M.A, J.A, C.O.; project administration, A.Z., J.A.; funding acquisition. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Commission of the HOSPITAL OF CHRONIC DISEASES "SF. LUCA" (protocol code 9, date of approval 09.12.2023).

Patient consent for publication

Written informed consent to participate in the research was obtained from each patient/subject..

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REVIEW

Case Management of Delirium in Patients with Major Neurocognitive Disorders

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Abstract: Delirium is associated with lower quality of life, increased healthcare costs, longer hospitalizations, and worse prognosis when detected in patients who are already confronted with a severe and invalidating disease, like the major neurocognitive disorder (MND). In order to identify the most evidence-based interventions that could be included in case management for patients with both delirium and MND, this narrative review explored three electronic databases (Pubmed, Cochrane, and Web of Science/Clarivate) for primary and secondary sources dedicated to this topic. All papers published in English between the inception of each database and September 2024 were screened for relevant data on pharmacological and non-pharmacological therapies targeting delirium. The results supported the use of a multicomponent approach as the first line of treatment, mitigating the precipitating factors, and only as a second line, the use of pharmacological instruments. There is a lack of validated therapies targeting specifically the delirium superimposed on MND, most of the recommendations being extrapolated from studies with delirium in the general population or in elderly patients with different organic diseases. Further studies are required to investigate the specifics of delirium in patients with MND and potential strategies focused on neurobiological mechanisms instead of just clinical manifestations.

Keywords: delirium; acute confusional states; dementia; major neurocognitive disorders; cognitive impairment; delirium assessment tools; antipsychotics

INTRODUCTION

Establishing adequate case management strategies for patients diagnosed with delirium is among the most challenging aspects of liaison psychiatry due to the complexity of the interactions between psychiatric phenomenology and organic pathology. Psychiatrists working in general hospitals are frequently called for consultation by colleagues with different medical specialties to „calm down” patients who suddenly become confused and agitated or, on the contrary, to „wake up” patients who abruptly become non-responsive to external stimuli. This psychiatric disorder is, however, far from being possible to approach by a single therapeutic method and requires an individualized case conceptualization and treatment. While in some cases, identification and correction of the causal factor(s) are simple and may be conducted promptly, such as in the case of severe dyselectrolytemia or hypoglycemia, in most cases, extensive investigations and multiple interventions are needed before the symptoms of delirium may be adequately managed. In this context, it would be necessary to mention that delirium is frequently deceptive because it can be the final manifestation of multiple organic comorbid conditions and a superficial investigation and treatment of the patient may make his or her prognosis worse. Therefore, psychiatrists should be aware of the intricate etiopathogenesis of delirium and of the necessity to investigate and treat such patients within a multidisciplinary framework. These considerations become more obvious when patients with severe chronic cognitive deterioration are evaluated since their anamnesis is reputedly difficult and the psychiatric exam also is frequently challenging.

Due to the changes in the names and definitions of neurocognitive disorders along the main classifications of psychiatric disorders, edited by the World Health Organization (WHO) and American Psychiatric Association (APA) [1-5], a trans-

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nosographic analysis is considered necessary to configure the framework of case management in patients affected by these conditions. Although there is a clear tendency to increase the concordance between the APA's Diagnostic and Statistical Manual of Mental Disorders (DSM) and the WHO's International Classification of Diseases (ICD) criteria for the diagnosis of psychiatric disorders, there are still important nosographic differences that need to be acknowledged by clinicians due to practical reasons.

Major neurocognitive disorders (MND) are defined, according to the latest editions of the DSM (DSM-5 and 5 TR, published in 2013 and 2022, respectively), by significant cognitive decline compared to the individual's previous level of performance in at least one cognitive domain; this cognitive impairment has to be supported by concerns reported by the patient, significant caregiver or clinician; also, a substantial deficit in cognitive performance, documented by a quantitative clinical assessment is required [1,2]. Also, significant interference of these cognitive impairments with the individual's ability to perform independent daily tasks is needed for the diagnosis, and the exclusion of other mental disorders is required as a differential diagnosis in these cases [2]. It is important not to consider the diagnosis of MND in patients who present cognitive impairment exclusively during the periods of delirium [2]. Once the diagnosis of MND is made, the presumed type of disease must be formulated, and the specifiers „with/without behavioral disturbance" and „severity" (mild/moderate/severe) are added [2].

The previous edition, DSM IV-TR, not only used different terminology for MNDs, which were called „dementias", but also the diagnosis criteria included neurological signs, i.e., aphasia, apraxia, agnosia, and the existence of disturbances in executive functioning, besides the memory impairment [3]. Regarding the ICD-10 and ICD-11, their perspective on the terminology is quite similar, and the term „dementia" is preserved in both editions, but in the latest version of ICD, there is an increased focus on the behavioral or psychological disturbances in dementia (BPDD), which received their own set of codes [5].

According to DSM-5 and 5 TR, to formulate a diagnosis of delirium, the clinician should confirm the existence of a disturbance in attention and awareness, accompanied by disturbances in cognition, i.e., memory, orientation, language, visuospatial abilities, or perception [1,2]. Additionally, this diagnosis requires an abrupt onset (e.g., hours to a few days), a change from the baseline level of attention/awareness, and a tendency towards fluctuation in severity during a single day [1,2]. The clinician must exclude the presence of a preexisting or evolving neurocognitive disorder and the presence of a coma or related conditions [1,2]. DSM-5 and 5 TR also state, as an essential criterion, the evidence supporting the direct causal-ship between a general medical condition, substance withdrawal/intoxication, exposure to a toxin/medication, or multiple etiologies and the symptomatology of delirium [1,2]. There are several specifiers for delirium, i.e., those related to the etiology, specifiers of severity (acute/persistent), and level of behavioral symptoms (hyperactive/hypoactive/mixed level of activity) [1,2].

When comparing the diagnostic criteria for delirium in the DSM-IV TR, DSM-5, and DSM-5 TR, several differences emerge [1-3]. The latest editions emphasize the need for disturbances in attention and awareness to represent a change from baseline, exclude coma from the category of delirium, and recognize the potential for multiple cognitive domains to be impaired by this disorder. Furthermore, both DSM-5 and DSM-5 TR provides a more comprehensive list of etiological types of delirium than does the DSM-IV TR [1-3].

The WHO's ICD-10 (the 2019 version) specifies that delirium is „an etiologically non-specific organic cerebral syndrome" that is characterized by disturbances of consciousness and attention, perception, thinking processes, psychomotor behavior, emotions, and the sleep-wake rhythm [4]. ICD-10 mentions that the duration of delirium is variable and the degree of severity may be different, from mild to severe [4]. Regarding the possible etiological considerations, ICD-10 includes different codes for delirium not induced by psychoactive substances, in cases where it is not superimposed on dementia, in cases of delirium superimposed on dementia, and contains codes for other types of delirium (e.g., mixed delirium, or post-surgery), and delirium-unspecified; delirium is also a complication of various withdrawal/intoxication states or somatic diseases (e.g., thyroid storm, kidney failure) [4].

The latest edition of the WHO Classification of Diseases (ICD-11) expands the definition of delirium (but preserves the core dimensions-phenomenological description, changes in functionality) and specifies the main differential diagnoses and potential etiologies, i.e., direct physiological effects of a medical condition, direct physiological effects of a substance or medication (including withdrawal), and unknown factors [5]. Residual categories, such as multiple etiological factors and other specific causes, are also coded [5].

The phenomenon of delirium in patients diagnosed with MND is very complex since the relationship between the two pathologies is far from being easy to define in each case. Individuals with MND have an increased risk of delirium onset when compared to the general population, but delirium in itself represents an independent risk factor for developing MND [6]. Also, delirium may be more difficult to diagnose in patients with MND because disentangling cognitive changes and sensorium clouding in a patient with an already deteriorated cognitive status, based on clinical evaluation, is challenging. Even more, confusional episodes appearing in patients with MND can worsen the cognitive decline and the overall functioning level [6]. It must also be mentioned that MNDs associate multiple well-defined psychiatric disorders (e.g., mood, anxiety, psychotic) as well as BPDDs, which creates supplementary difficulties in detecting new-onset cognitive decline due to delirium [7]. These convoluted interactions between delirium and MND are represented in Fig.1., but caution is recommended when interpreting these relationships due to the intricacy of organic and psychological factors (e.g., infectious diseases may trigger delirium, and delirium may, in patients with MND, worsen the psychological long-term evolution, or, a psychological trigger may be responsible for the clouding of sensorium in a patient with MND, which also may worsen the long-term biological evolution of the patient, if the trigger is not properly identified and corrected; also, psychosocial factors may inadvertently worsen a somatic disease that, in turn, may aggravate the preexisting MND).

Figure 1: The interaction between delirium and major neurocognitive disorders

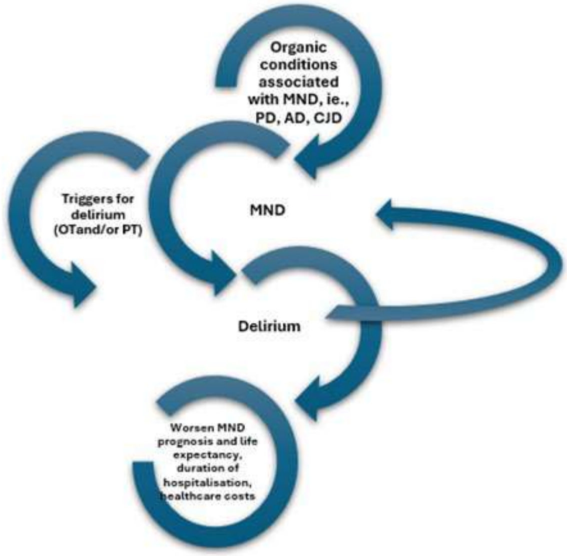
Organic conditions associated with MND, ie., PD, AD, CJD
Triggers for delirium (OTand/or PT)
MND
Delirium
Worsen MND prognosis and life expectancy, duration of hospitalisation, healthcare costs

AD= Alzheimer's disease; CJD= Creutzfeldt-Jakob disease; MND= major neurocognitive disorder; OT= organic triggers; PT= psychological triggers; PD= Parkinson's disease

Although the phenomenology of delirium encompasses prodromal and subsyndromal stages [8], no consensus about corresponding specific symptoms exists, making it difficult for clinicians to identify such forms of delirium in daily practice. Due to the complexity of the phenomenological presentation of delirium, the differential diagnosis is another important challenge for clinicians [8]. More exactly, delirium consists of core manifestations, which are expected to be present in all cases (e.g., awareness alterations), frequently met symptoms (e.g., motor activity dysfunctions), and associated, variable symptoms (e.g., hallucinations), which may reflect the etiological medical or toxic factors [8]. The evolution of delirium is difficult to predict because it is dependent on etiological factors, but it was reported that almost 57% of hospitalized patients may still have delirium after discharge from the hospital [9].

The reported prevalence in hospitalized patients varies across studies, depending on the criteria used, the type of department reporting the cases, the instruments applied for quantifying the severity of the symptoms, etc. The prevalence of delirium was reported between 1 and 31%, and the incidence between 3 and 25% [10-12]. For example, 39% of the patients admitted to intensive care units (ICUs) were diagnosed with delirium, according to a cross-sectional multicenter study (N=256 participants) that used a screening checklist as the core methodology of assessment [13]. In hospitalized patients aged ≥65, delirium was reported in the case of 55.4% of the 327 participants using the 3-Minute Diagnostic Confusion Assessment Method (3D-CAM) [9]. A surprisingly high rate of patients with delirium (35.4%) were not identified by the treating team, indicating the need for increased awareness about the existence of this neurocognitive disorder [9]. Also, it is important to explore the possibility of multiple etiologies in patients with delirium, including organic acute diseases, chronic illnesses that are acutely decompensated, new drugs recently introduced for the treatment of a physical or mental disorder, different environmental stressors, etc. [14-16].

The presence of delirium was independently associated with worse clinical prediction of survival, lower quality of life, and increased healthcare costs through longer hospitalizations have been reported in patients with delirium [17-19]. More specifically, an economic analysis of healthcare for older hospitalized patients with or without Alzheimer's disease and related dementias associated with delirium showed that in the first group, the adjusted mean difference in costs related to delirium was almost 35000 USD, and these costs increased progressively over one year vs. non-MND patients [20]. The same study revealed that patients with Alzheimer's disease-related MND were more likely to develop delirium (51% vs. 24%) and die within one year (38% vs. 21%), all values being statistically significant [20]. Because delirium is considered a modifiable factor for prognosis in patients with MND and, frequently, a preventable one, the necessity for a correct diagnosis and early treatment, whenever delirium is identified, could have both short-term and long-term substantial benefits for the MND population. According to a systematic review, the prevalence of delirium superimposed on dementia varies between 22% and 89% in hospitalized patients aged over 65 [21]. Such high prevalence indicates, once again, that there is an acute need to detect and treat delirium actively in vulnerable populations.

This narrative review aims to identify the most valid interventions that could be included in case management for patients with delirium associated with MND. Because it was anticipated that the sources for this specific population might be scarce, the secondary objective included finding case management recommendations for patients with delirium in clinical populations, regardless of the organic or psychiatric background; these supplementary data were explored to see if they allow for being extrapolated to MND + delirium cases.

MATERIALS AND METHODS

This review explored three electronic databases (Pubmed, Cochrane, and Web of Science/Clarivate) for primary and secondary sources dedicated to this topic, using as keywords „delirium", OR „confusional episodes" OR „confusion superimposed on dementia"

AND „major neurocognitive disorder” OR „dementia” AND „pharmacologic*” OR „drug” OR „non-pharmacologic*” OR „psychosocial”. All papers published in English between the inception of each database and September 2024 were screened for relevant data on pharmacological and non-pharmacological therapies targeting delirium. The results were grouped into two classes: (1) guidelines for patients with delirium in clinical populations and (2) case management for patients with delirium and neurocognitive disorders.

RESULTS

Based on the 36 sources reviewed in the first section, dedicated to delirium case management in clinical populations, and 15 sources (partially overlapping) focused on the evaluation and treatment of delirium superimposed on MND, the following results are presented.

Recommendations for case management in patients with delirium in the hospital settings

The papers reviewed and grouped in this section were distributed according to their main clinical use, i.e., (1) validated tools for screening and monitoring delirium and (2) therapeutic recommendations, since these two components were considered essential for clinicians to construct a case management plan (Fig.2).

Instruments for detection of delirium and its severity quantification in clinical population

There are many instruments for the diagnosis and monitoring of the presence of delirium, some of them being more widely used and largely validated than others. CAM was developed by an expert panel through a consensus process and has the clear advantage of its brevity and easiness of administration [22]. CAM may be applied in less than five minutes and consists of nine criteria derived from DSM III R, the main symptoms of delirium being acute onset and fluctuating course, lack of attention, disorganized thinking, and altered sensorium [22]. Different versions of this instrument emerged, such as CAM-ICU, which is dedicated to patients admitted to intensive care units and can be administered by non-specialists [23].

4-AT is another tool conceived for the screening of delirium symptoms in elderly patients and demonstrated good sensitivity and specificity for hospitalized subjects [24]. This instrument has four items, i.e., alertness, two items for cognitive function, and one for fluctuation in mental status [24].

The NEECHAM (Neelon and Champagne) Confusion Scale is also used for rapid assessment of delirium and has been proven to have good internal consistency and interrater reliability, and it also correlated well with the Mini-Mental State Examination [25]. This tool has nine scale items, is easy to use, and can be scored by nurses with minimal training. Also, NEECHAM may be administered initially and periodically to monitor mental status [25].

The Observational Scale of Level of Arousal (OSLA) can be used for the identification of abnormal levels of arousal associated with delirium and may be administered for screening and monitoring purposes [26]. The items refer to eye-opening, eye contact, posture, and movement, and the total score is between 0 and 15 [26].

Therapeutic and prophylactic recommendations in patients with delirium

Regarding the therapeutic approaches to delirium, after the diagnosis of an acute confusional state was formulated, the first step is to analyze the potential contributors to the onset of this disorder. Predisposing and precipitating factors must be evaluated, and modifiable etiological factors should be addressed [27]. The case management of patients with delirium has as the main objective the correction of the biological disequilibrium that fueled the confusional state, but the prevention of relapse is also of major interest since several causes may be chronic or recurrent. According to an expert consensus on delirium management, „early identification and intervention of subclinical delirium can reduce the risk of clinical delirium” [28]. Also, daily assessment of at-risk patients is important for early detection of delirium and adequate therapeutic interventions [28].

The most common precipitating factors that should be approached during the evaluation of a patient with delirium are infections, electrolyte imbalances, drugs (medications, drugs of abuse-intoxication/withdrawal, toxic substances), pain, organ failures, traumas, metabolic and nutritional imbalances, central nervous system (CNS) pathologies, environmental factors (e.g., sensorial deficits for patients with neurocognitive disorders, immobilization, sleep-wake cycle dysfunctions), but also psychiatric factors (e.g., anxiety, insomnia or depression in elderly patients with severe organic disease).

The ABCDE/ABCDEF protocol was suggested for managing delirium [29,30]. This acronym stands for (a) assessing, preventing, and managing pain, (b) both Spontaneous Awakening Trials and Spontaneous Breathing Trials (SAT and SBT, respectively), (c) choice of analgesia and sedation, (d) delirium - assessment, prevention, and management, (e) early mobility and exercise, and (f) family engagement and empowerment [31].

Another integrated approach algorithm for critically ill patients targeted the prevention of early deep sedation in the ICU as a risk factor for poor long-term outcomes, including mortality and neurocognitive disorders [32]. Flexible multimodal analgesia, with the minimal use of opioids, is the main concept of this algorithm, together with the use of the lowest doses of sedatives that are still efficient [32]. Promoting sleep, early mobilization, and improved communication of patients with medical personnel and caregivers are also included in the eCASH (early Comfort using Analgesia, minimal Sedatives, and maximal Human care) model as methods to avoid isolation, delirium, and a decreased quality of life [32].

Figure 2: Case management in patients with delirium in clinical populations

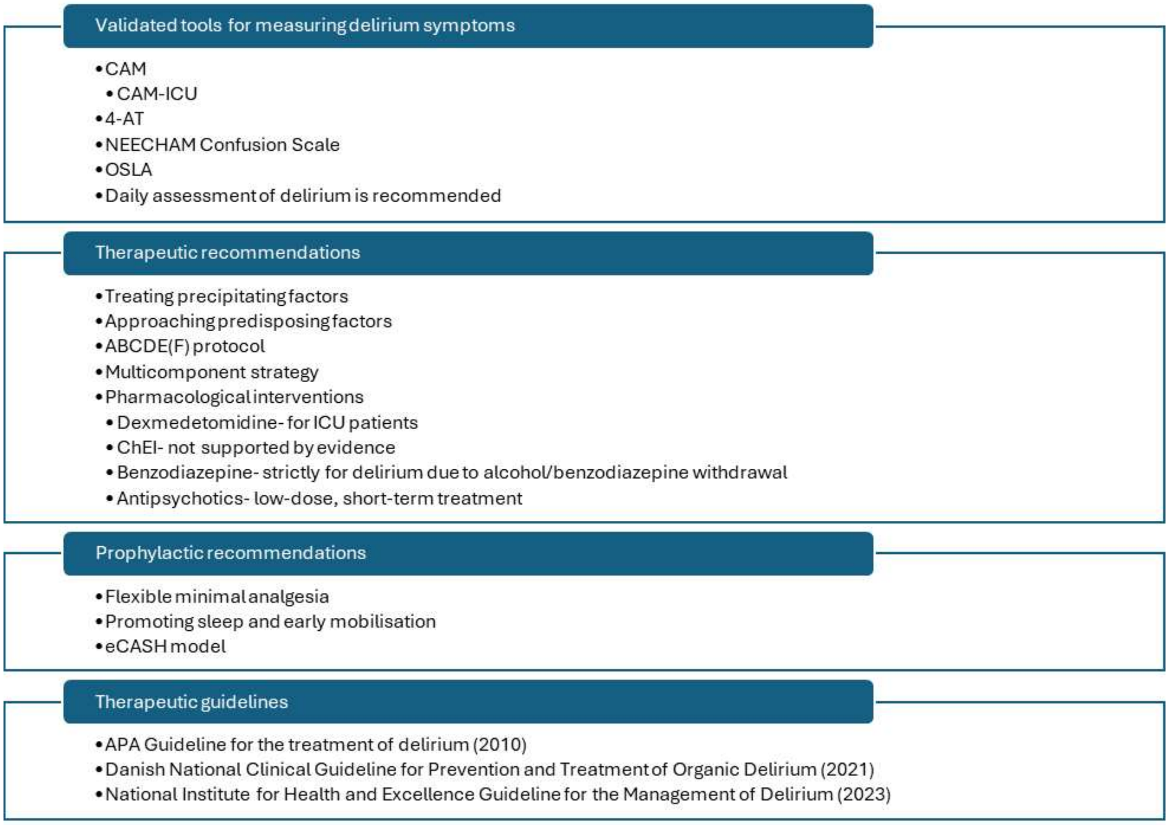

Validated tools for measuring delirium symptoms

- CAM
  - CAM-ICU
- 4-AT
- NEECHAM Confusion Scale
- OSLA
- Daily assessment of delirium is recommended

Therapeutic recommendations

- Treating precipitating factors
- Approaching predisposing factors
- ABCDE(F) protocol
- Multicomponent strategy
- Pharmacological interventions
  - Dexmedetomidine- for ICU patients
  - ChEI- not supported by evidence
  - Benzodiazepine- strictly for delirium due to alcohol/benzodiazepine withdrawal
  - Antipsychotics- low-dose, short-term treatment

Prophylactic recommendations

- Flexible minimal analgesia
- Promoting sleep and early mobilisation
- eCASH model

Therapeutic guidelines

- APA Guideline for the treatment of delirium (2010)
- Danish National Clinical Guideline for Prevention and Treatment of Organic Delirium (2021)
- National Institute for Health and Excellence Guideline for the Management of Delirium (2023)

APA= American Psychiatric Association, CAM= Confusion Assessment Method, ChEI= cholinesterase inhibitors, eCASH= early Comfort using Analgesia, minimal Sedatives and maximal Human care, ICU= Intensive Care Unit, NEECHAM= Neelon and Champagne, OSLA= Observational Scale of Level of Arousal

The multi-component strategy for delirium in the elderly includes six intervention directions, i.e., cognitive deficits, sleep deprivation, immobility, visual deficits, hearing impairments, and dehydration [33,34]. Early psychomotor and cognitive action may reduce the duration of delirium in critically ill patients [35].

Methods may be specific to the identified causal factors, such as the treatment of alcohol withdrawal, Wernicke encephalopathy, acute kidney injury, hepatic encephalopathy, diabetic ketoacidosis, etc. [36-40]. Exploring neurological disorders that may represent vulnerability factors for the onset of delirium is an important step in the prophylaxis of these patients [14,41,43]. In patients with neurocognitive disorders due to Alzheimer's disease, Parkinson's disease, or vascular diseases, the initiation of acetylcholinesterase inhibitors (AChEI) may help in reducing the risk of delirium onset/recurrence or its severity [44,45].

Dexmedetomidine was associated with an improved diagnosis for patients with delirium and mechanical ventilation admitted to ICUs [29]. Dexmedetomidine combined with analgesics may reduce the duration of delirium in patients with agitated forms of confusional states [29]. Dexmedetonimide is usually administered in the ICU for adult patients who require a level of sedation that allows for awakening as a response to verbal stimulation, meaning a Richmond Agitation and Sedation Scale (RASS) score between 0 and -3 [46]. Dexmedetomidine is administered by infusion and requires continuous monitoring of the blood pressure, cardiac rhythm, and respiratory functioning [46].

Impairment of cholinergic neurotransmission by high levels of circulating proinflammatory cytokines is considered a critical contributor to the onset of delirium; therefore, the administration of pharmacological agents that increase acetylcholine may be an adequate method to prevent or treat confusional episodes [47,48]. Following this line of reasoning, cholinesterase inhibitors (ChEI) were explored as symptomatic treatments for delirium, independent of the presumed pathophysiological substrate. However, a systematic review found only one study matching the inclusion/exclusion criteria (N=15 participants, out of which 7 have comorbid dementia) did not find a significant difference between rivastigmine and placebo regarding the duration of delirium, adverse events, use of rescue medication, mortality, and study discontinuation [49].

Benzodiazepines are used in cases of delirium due to withdrawal of substances in patients with substance use disorders, and where antipsychotics are contraindicated [29,50]. SAT, which is one of the core elements of the ABCDEF bundle previously mentioned, refers to the need to use a daily discontinuation strategy of sedatives or a continuous titration of these drugs in order to preserve the minimum level of sedation [31].

Antipsychotics are associated with variable results in this population. Based on the results of a meta-analysis and systematic review (n=5 randomized controlled trials, N=1750 patients), the use of antipsychotics in critically ill adults with delirium did not increase the

number of days without delirium or coma vs. placebo and did not show a significant effect on mortality, duration of mechanical ventilation, ICU stay or hospital length [51]. Another meta-analysis (n=19 studies) focused on the prevention and treatment of delirium by using antipsychotics showed these agents had no significant effect on delirium incidence, duration, severity, or ICU length of stay [52]. Yet another systematic review (n=14 sources) showed that uncontrolled studies reflect limited support for the use of low-dose, short-term treatment of delirium with several antipsychotics [53].

APA Guidelines for the Treatment of Delirium (2010) recommends (1) a close collaboration between the psychiatrist and other physicians to ensure appropriate care for these patients; (2) identify the precipitants; (3) initiate interventions for acute conditions; (4) provide disorder-specific treatment for co-occurring diseases; (5) monitor and ensure the safety of the patient; (6) assessment and monitoring of the psychiatric status; (7) assessment of individual and family psychosocial and social characteristics; (8) maintain the therapeutic alliance with the patient and his/her family; (9) education of the patient and family regarding the illness; (10) provide post-delirium management [54]. Environmental interventions, structuring and ensuring support for the patient, and support and education for the family are the most important non-pharmacological interventions, while antipsychotics (orally or intramuscularly, low doses, and for the shortest time needed), benzodiazepines (but there are only limited data, except for the treatment of delirium related to alcohol or benzodiazepine withdrawal), pro-cholinergic drugs (but there is a risk for cholinergic adverse events), vitamins (in specific cases, such as patients with hemodialysis, or alcohol use disorder), morphine or other opiates (in palliative care, and there is a risk of exacerbating the delirium) are considered the most important pharmacological strategies for the management of delirium [54].

The Danish National Clinical Guideline for Prevention and Treatment of Organic Delirium (2021) recommends non-pharmacological therapy to be initiated in all patients with a risk of delirium and in those with delirium (i.e., sensory optimization, orientation and cognitive stimulation, mobilization, nutrition, rehydration, supporting the circadian rhythm) [55]. Placing the delirious patient in a protected environment, actively involving relatives in the treatment of patients with delirium, and constantly reviewing medication for all these patients (i.e., adjusting, including stopping non-essential drugs), and avoiding routinely using antipsychotics for all delirious patients are also recommended by this guideline [55]. Benzodiazepines should not be used in this population except for palliative patients with agitation and terminal delirium or for patients in ICU where respiratory support and monitoring are accessible [55]. Melatonin is not considered by the Danish guidelines a useful intervention, but electroconvulsive therapy may be recommended in refractory cases where other therapeutic options have failed [55].

The National Institute for Health and Excellence (NICE) guideline for the management of delirium (updated January 2023) recommends, for the prevention of delirium, a multi-component approach conducted by a multidisciplinary team, addressing the cognitive impairment and/or disorientation by environmental measures, and address all potential triggers for confusion [56]. Treating delirium involves management of the underlying causes, ensuring communication and reorientation, de-escalation of the situation using verbal and non-verbal techniques, followed, if necessary, by short-term haloperidol, and, if there is still little or no improvement, re-evaluation of the potential precipitants and vulnerability factors, dementia included [56].

Case management for patients with delirium superimposed on a major neurocognitive disorder

The distribution of the sources retrieved during the literature search for the case management of patients with delirium overimposed on MND was similar to that mentioned in the previous section for delirium in clinical populations, and the results are synthetically represented in Figure 3.

Diagnostic aspects of delirium in patients with MND

Regarding the diagnosis aspects, an analysis of the potential biomarkers for delirium superimposed on MND showed that genetic, epigenetic, and pharmacogenetic variables might be used in the near future to construct personalized case management for these patients [57].

Gene-gene, protein-protein, gene-drug, and gene-miRNA interactions have been detected as potentially significant in this specific population, with several essential genes, like BDNF (brain-derived neurotrophic factor), APP (amyloid beta precursor protein), DNMT1 (DNA methyltransferase 1), APOE (apolipoprotein E), and TNF (tumor necrosis factor) presenting gene x drugs interactions also relevant for this pathology [57]. However, there is no single independent, validated biomarker for delirium, which leaves, for now, at least, the diagnosis to the clinicians [58].

A review dedicated to the best tools for identifying delirium in patients with MND concluded there is no concordance between validated diagnostic criteria, psychometric instruments, and therapeutic guidelines, which makes it difficult for clinicians to identify and treat this pathology appropriately [59]. Taking into account such limitations, CAM and CAM-ICU are the most recommended instruments for the diagnosis of delirium in dementia, based on the review of clinical studies [21,59]. Other instruments, such as 4-AT or 4-DSD (Delirium superimposed on dementia), have also been studied in this population, but there is less data to support their clinical use [59]. The 4-AT may be beneficial for the correct and timely detection of delirium in this population because it can help in the exclusion of cognitive impairments in emergency rooms and hospital admissions [33]. Therefore, as a screening tool, 4-AT is a helpful instrument that requires little time for administration.

The 4-DSD has four items and is derived from 4-AT, but the scoring algorithm is different, and several items have a changed content compared to the initial tool [59]. This instrument assesses the level of alertness, altered brain function, attention integrity, and acute change and fluctuation in mental status [59].

The use of the OSLA in combination with another very simple attention test (that requires the patient to identify a certain letter in a sequence) correctly identified more than 90% of the cases of delirium superimposed on dementia [33,60].

Figure 3: Case management of patients with delirium superimposed on major neurocognitive disorder

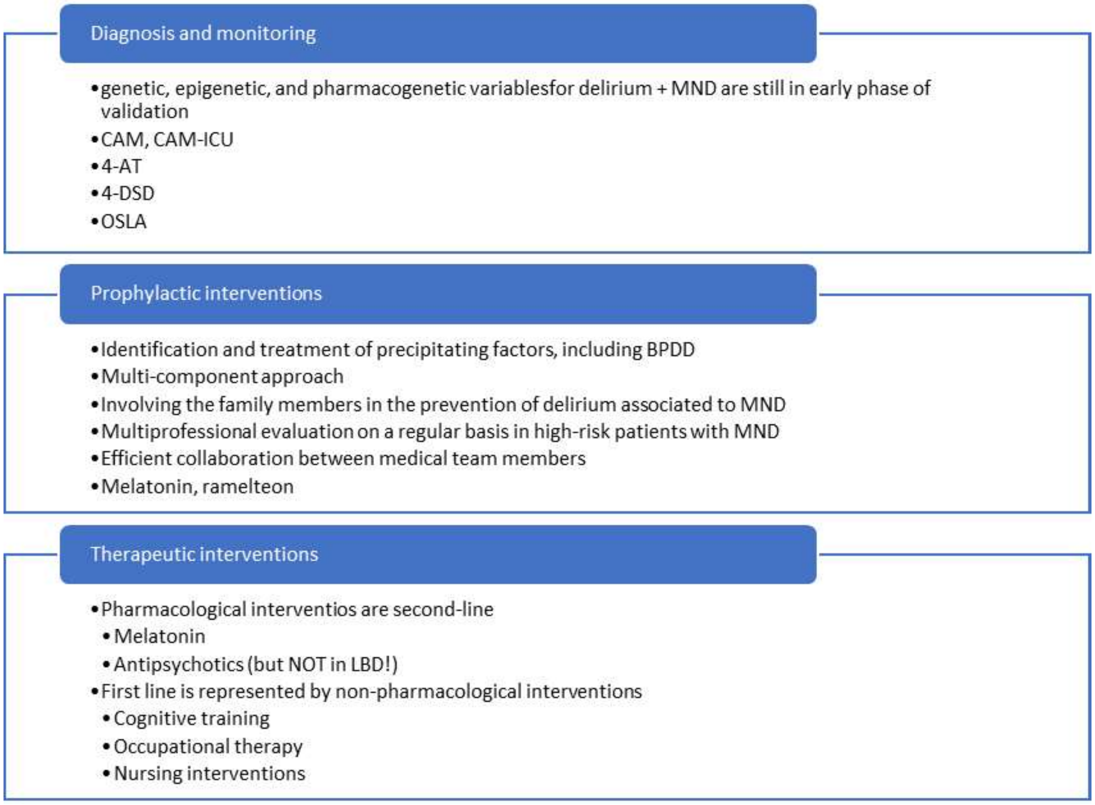

Diagnosis and monitoring

- genetic, epigenetic, and pharmacogenetic variablesfor delirium + MND are still in early phase of validation
- CAM, CAM-ICU
- 4-AT
- 4-DSD
- OSLA

Prophylactic interventions

- Identification and treatment of precipitating factors, including BPDD
- Multi-component approach
- Involving the family members in the prevention of delirium associated to MND
- Multiprofessional evaluation on a regular basis in high-risk patients with MND
- Efficient collaboration between medical team members
- Melatonin, ramelteon

Therapeutic interventions

- Pharmacological interventios are second-line
  - Melatonin
  - Antipsychotics (but NOT in LBD!)
- First line is represented by non-pharmacological interventions
  - Cognitive training
  - Occupational therapy
  - Nursing interventions

BPDD= behavioral and psychological disturbances in dementia, CAM= Confusion Assessment Method, DSD= Delirium Superimposed on Dementia, ICU= Intensive Care Unit, LBD+ Lewy body dementia, MND= major neurocognitive disorder, OSLA= Observational Scale of Level of Arousal

Preventative and treatment-focused recommendations in patients with delirium and MND

Mentioning that there is no „gold standard" for the diagnosis and treatment of delirium in patients with MND, a systematic review showed that a multi-professional evaluation regularly of high-risk patients with MND is recommended as a step toward the prevention of delirium [61]. An efficient collaboration between multiple members is also needed within the team, which is involved in case management [61]. Eliminating the risk factors for delirium in this vulnerable population is essential [61]. Screening for all the major causes of delirium in patients with MND is strongly recommended and could have significant benefits [33]. Constipation, medications with central nervous system adverse events, infections, vascular factors, electrolytic imbalance, low mobility, malnutrition, pain, incorrect sleep hygiene, and urinary retention should be screened for in patients with MND and sudden clouding sensorium [33].

A non-pharmacological approach is initially indicated in all individuals presenting delirium and MND, based on decreasing the number of medications used, maximization of family support, orienting techniques, adequate hydration, and correction of visual and hearing deficits, whenever possible [21]. However, the controlled studies did not show a consistent effect of environmental strategies in preventing delirium in patients with MND [21].

Pharmacological therapies are considered second-line interventions and applied only when non-pharmacological strategies have failed or are insufficient [61]. The use of melatonin and antipsychotics to treat delirium in MND is not supported enough by evidence [62].

As for the prevention of delirium, the identification of the precipitants and their treatment, initiation of multicomponent interventions, prevention of complications, and monitoring the resolution of the core symptoms are the main directions of case management in patients with delirium and MND [58,62]. The participation of the family members is strongly encouraged by the case managers because the evolution of the patients is often unpredictable even after hospital discharge, and caregivers may play an important role in re-orienting a confused individual by offering him or her a point of reference [58]. Re-assessment of precipitating and vulnerability factors is the first step, and only after that can non-pharmacological and pharmacological treatment be considered [62].

Special care should be given to patients with MND and BPDD, a sub-population that is more vulnerable to the onset of delirium than patients with dementia but without BPDD [63]. The recovery of delirium is slower in patients with both dementia and BPDD; therefore,

identifying early signs of delirium, like sleep impairments, mood lability, and psychomotor agitation, should trigger increased attention and more intensive monitoring [63].

Cognitive training was explored as a possible intervention for MND, to facilitate neuroplasticity in these patients [64]. Based on several similar cognitive features between dementia and MND, the use of recreational activities to enhance attentional abilities and offer cognitive stimulation was suggested to reduce delirium severity and duration, improving quality of life and reducing healthcare costs [64]. A pilot study dedicated to this intervention (N=16 participants) demonstrated a greater decrease in physical function and mental status during the 30 days of monitoring vs. the control group [65]. Delirium, but also the severity of delirium and attention had a trend toward significance in the study vs. control group [65].

Occupational therapy, based on preliminary evidence, can be another option for patients with delirium and MND [66]. A case series (N=6 patients with dual disorders) reported positive results of occupational therapy conducted twice daily for a total of 40 minutes until the delirium was remitted [66].

Nursing interventions for delirium over-imposed on MND were associated with good results [67]. A 10-step intervention was reported to help in the dissolution of delirium in a patient with Alzheimer's dementia: interdisciplinary communication with care providers, reduction of medications administered, communicating calmly and slowly with the patient, avoidance of physical restraints, re-hydration, regulation of the sleep-wake cycles, assess and prompt treatment of pain, education of the family and encouraging family members to participate in care, mobilize the patient as soon as possible, and regulation of the excretory function [67].

Melatonin and ramelteon may be recommended in older patients for the prevention of delirium when admitted to an acute geriatric facility [68,69]. According to the results of a multicenter, randomized study (N=67 patients admitted in ICUs), ramelteon administered nightly was associated with a lower risk of delirium, even after controlling the risk factors, vs. placebo [69].

Antipsychotics are frequently used in clinical practice for this population, but a trial with these medications should be recommended only for a short time, in low doses, and under careful supervision [33]. Special care should be taken when administering antipsychotics in patients with MND due to Parkinson's disease or Lewy Body Dementia [33].

DISCUSSION

The existing evidence reviewed in the previous chapter supports the use of validated instruments for (1) screening in vulnerable populations, patients with MND included; (2) supporting the clinical diagnosis of delirium in the absence of validated biomarkers; (3) monitoring the evolution of delirium during treatment, until the complete resolution of its symptoms [22-26, 33,61]. Also, preventative measures could be applied in vulnerable populations, which may lead to a reduction in the risk of delirium onset, i.e., treating risk factors, close assessment of the clinical status, multi-component interventions, and even limited data supporting pharmacological interventions for short periods of time [58,62,63]. Therapeutic measures that can be initiated in cases of delirium superimposed on MND are mainly non-pharmacological since, for the pharmacological agents, there is only limited data to support their use [29,33,46-50,53-56].

Limitations of this review are related to its narrative nature, and, as a result, not all significant sources may have been found and reviewed. Also, due to the narrative character of the review, no quality of data was assessed, and all sources were treated equally. Finally, there is a paucity of data derived from good-quality trials focused on delirium overimposed on MND, which limits the validity of the review's results, since most data are extrapolated from studies with patients presenting delirium in the clinical population, regardless of the causal factors.

The strengths of the present review are related to the enlarged focus on both pharmacological and non-pharmacological components of case management and the integration of structured assessment instruments into the clinical practice in vulnerable populations.

Future directions of research should include better-quality trials with homogenous populations, e.g., delirium with onset in patients with MND due to Alzheimer's disease, or the prevention of delirium in patients with MND due to Parkinson's disease, but also active comparators and placebo for the pharmacological trials, and a large number of participants with long-period of follow-up, in order to assess the risk of recurrence.

CONCLUSION

When formulating the case management plan for patients with delirium superimposed on MND, the clinician should take into account (1) the need for a multidisciplinary team since the precipitating and vulnerability factors may be complex and have intricate relationships; (2) regularly and frequently assess the patient using instruments like CAM, 4-AT, OSLA, or 4-DSD; (3) include the family in the monitoring of the patients, whenever possible; (4) non-pharmacological interventions, such as cognitive training and occupational therapy, nursing techniques, multi-component approaches, are first-line strategies; (5) pharmacological interventions are considered second-line, and they are administered only for short time and low doses; (6) prophylaxis of delirium in vulnerable populations, MND patients included, remains the single most important way to reduce the complications associated with this pathology.

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ORIGINAL ARTICLE

Gender-Dependent Associations Between Digit Ratio and Genetic Polymorphisms, BMI, and Reproductive Factors

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Abstract: The digit ratio (2D:4D) has been associated with prenatal hormonal influences and various traits and pathologies. This article explores the relationship between 2D:4D and a series of common polymorphisms and Torque Teno Viruses. In this study, 120 healthy participants were included. The IGF2 Apa I, ACE I/D, INS -23 Hph I, VDR Fok I, VDR Apa I, VDR Taq I, AT1R A1166C polymorphism were genotyped by PCR-RFLP technique, and the IL-6 -174 G/C polymorphism by tetra-primer ARMS-PCR. The presence of TTV was identified by a hemi-nested PCR technique. Haplotype analyses were performed using the SHEsis software. The average 2D:4D values were similar for men and women. Overweight men presented higher 2D4D ratios than normal-weight women ($p<0.05$). Lower 2D:4D values were recorded in women with pregnancy loss or one child or none ($p<0.001$). Men with lower 2D4D ratios reported a higher number of children ($p<0.001$). The IGF2 GG and ACE DD were associated with a higher digit ratio in all subjects and in the women's subset. A significant association was found in men between 2D:4D and the INS-23 Hph I – IGF2 Apa I T-G haplotype ($p<0.01$). The data obtained in this study indicate a sexual dimorphism for the digit ratio. The associations between 2D:4D and the genetic polymorphisms studied could be influenced by gender.

Keywords: 2D:4D, Insulin-IGF2 haplotype, ACE I/D, parity, BMI

INTRODUCTION

The ratio between the length of second and fourth digits (2D:4D) is determined prenatally, and remains stable throughout life, serving as a marker of early maternal hormonal influences [1,2]. This length is influenced by the ratio between testosterone and estrogen during the fetal period. A smaller digit ratio has been associated with a higher concentration of fetal testosterone and a lower concentration of fetal estrogen [3]. Additionally, the 2D4D ratio can be influenced by the density of receptors for testosterone and estrogen, which is greater for 4D than 2D [2], and also by the maternal environment [4]. Nevertheless, testosterone production during the first years of life could influence secondary sexual differences developing before puberty as 2D4D ratio [4]. Furthermore, the digit ratio was correlated with specific genetic particularities of the child such as a polymorphism in the androgen receptor gene [5]. Therefore, lower digit ratios are common in males whereas higher values are identified in females [6].

The study of the digit ratio offers a compelling gateway into understanding the intricate interplay of genetic, hormonal, and environmental factors from the intrauterine environment. These interactions also may shape an individual's predisposition for different physiological (e.g. height, weight, BMI, athletic performance, reproductive success) [7,8,9,10] or psychological traits (e.g. academic performance) [11]. In addition, the 2D:4D has been associated with various categories of pathologies, ranging from autoimmune diseases (e.g. vitiligo [12], thyroid disease [13]), metabolic disorders [14], and malignant tumors [15] to mental disorders such as autism, ADHD, and depression [16]. A series of polymorphisms such as Insulin, IGF2, angiotensin I-converting enzyme (ACE), angiotensin II type 1

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receptor (AT1R), vitamin D receptor (VDR), and IL-6 genes may imply fetal growth and development and have been associated with some of these pathologies [17,18,19].

Insulin-like growth factor two (IGF2) is a key protein regulating growth, secreted, mostly via the placenta, and after birth by hepatocytes [20]. INS-23Hhp influences IGF2 transcription in the human placenta and it may be implicated in insulin-regulated intrauterine growth and insulin secretion in adults [21].

ACE, a zinc metallopeptidase, plays a pivotal role in the renin-angiotensin-aldosterone system (RAAS), responsible for homeostasis, blood pressure regulation, and water balance in the body. ACE I/D polymorphism has been associated with different pathologies, including pregnancy complications and infertility [22]. Angiotensin II type 1 receptor (AT1R) is a well-reported biomarker for hypertension and AT1R A1166C was strongly associated with the risk of developing pregnancy-induced hypertension [23].

Vitamin D (VD) is a molecule involved in regulating various processes, including phosphate and calcium metabolism, bone mineral density, and immune response [24], but also plays an important role in fetal development. Low maternal VD levels during pregnancy are associated with various adverse obstetric outcomes, such as gestational diabetes mellitus [25]. Vitamin D, as cortisol and sex hormones are all derived from cholesterol, creating an intricate network of interactions. Cortisol and testosterone concentrations might be influenced by vitamin D [26] through the activity of the vitamin D receptor, which is present in Leydig cells [27] (that plays a role in testosterone synthesis) and regulates the activity of the adrenal gland [28] (to produce cortisol). Therefore, three of the most studied VDR polymorphisms may be of interest: VDR Fok I (C/T), VDR ApaI (A/C), and VDR Taq I (C/T). Additionally, a low cortisol reactivity was associated with a low digit ratio [29]. Nevertheless, no significant association between 2D:4D and vitamin D has been reported in the literature so far.

Having pro-inflammatory and anti-inflammatory properties, interleukin 6 (IL-6) plays a major contribution in modulating the immune response, being reported as a biomarker of inflammation and intraamniotic infection [30]. Moreover, IL-6 was associated with right 2D:4D in girls, suggesting that the gender of the subjects and the hand's position in the sagittal plane influence the correlation between IL-6 and digit ratio [31]. The synthesis of pro-inflammatory cytokines, including IL-6, may be influenced by Torque Teno viruses (TTV) [32]. TTV are non-enveloped viruses that belong to the Anelloviridae family that are widespread globally [33]. They have multiple transmission routes, including transplacental transmission, which may have implications in prenatal development, although studies are not consistent on this matter [34, 35]. TTV may represent a potential biomarker of immune function and for some diseases [36].

This article aims to investigate the associations between digit ratio, gender, certain characteristics (such as height, weight, BMI, and reproductive success), and a series of common polymorphisms.

MATERIALS AND METHODS

Selection of subjects.

This study included 120 healthy, unrelated, volunteers (61 women, 59 men), aged between 42 and 60 years (52.73 ± 3.6 years). All participants were informed about the purpose of the study. Selection criteria for the participants included alcohol intake and smoking. Alcohol intake consisted of no more than two standard drinks a day for men and one standard drink a day for women. A beverage is considered alcoholic if it contains up to 10 g of ethanol, according to the World Health Organization [37]. As for smoking, the threshold was 20 cigarettes per day, based on the participants' answers.

Methods and Protocols.

The length between the second digit and the fourth digit was measured by the caliper three times, therefore the value obtained is the average of these three consecutive measurements. It was measured on the ventral surface of the left hand, from a midpoint on the metacarpophalangeal flexion crease to the tip of the digit. The measurements were considered valid if the difference between the maximum and minimum values measured for a finger was less than 5 mm. If the difference was greater, the measurement process was repeated for that participant. The analysis was restricted to subjects with a digit ratio range of 0.96-1.005, with more extreme values excluded.

A peripheral blood sample (300 mL) was collected from each subject in an EDTA tube and genomic DNA was extracted using the Promega Wizard® Genomic DNA Purification Kit. The presence of TTV was identified using a hemi-nested PCR protocol [38]. The ACE I/D polymorphism (rs1799752) was identified by PCR with a primer set for insertion [39], while the PCR-RFLP technique was used for genotyping the polymorphisms IGF2 Apa I (820 G/A, rs680) [40], INS -23 Hph I (-23 A/T, rs689) [41], AT1R A1166C (rs5186) [42], VDR Fok I (C/T, rs2228570), VDR Apa I (A/C, rs7975232), VDR Taq I (C/T rs731236) [43, 44]. IL-6 -174 G/C (rs1800795) was genotyped by the tetra-primer ARMS-PCR technique [45]. Restriction products were resolved by electrophoresis.

Statistical Analysis.

The distribution of qualitative data in subgroups of subjects was analyzed with Chi-square or Fisher tests. The outlier values for 2D:4D were considered values that exceed the mean value ±3 standard deviations. The quantitative values were analyzed using ANOVA, one-way ANOVA with post-hoc Tukey HSD test. Simple linear regression analyses were used to assess the variation in anthropometric parameters with variation in 2D:4D. The statistical analyses were performed using the R Studio software, version 2023.12.1.+402. Haplotype analyses were performed using the SHEsis software [46]. Differences were considered statistically significant if $p<0.05$.

RESULTS

The average age (52.73 ± 3.6; 42-60), weight (71.71±5.06; 63-86), height (1.70±0.02; 1.65-1.75) and BMI (24.96±1.78; 21.89-29.41) and 2D/4D (0.9858 ±0.01; 0.97-1.01) of subjects were estimated. The characteristics of the investigated subjects are presented in Table 1.

We have identified no outliers for 2D:4D in the case of men or women investigated in this study. Men have a higher digit ratio than women, but this difference was not statistically significant (0.9871 vs 0.9845, p >0.05).

Table 1: Characteristics of subjects

Characteristics	Values
Male / Female	59 /61
Age: men vs women	52.89 vs 52.57
Weight: men vs women	74.10 vs 69.39[b]
Height: men vs women	1.70 vs 1.69
BMI: men vs women	25.72 vs 24.21[b]
Smokers: men vs women	24 vs 17
Alcohol drinkers: men vs women	12 vs 3[a]
2D/4D: men vs women	0.9871±0.007 (0.97-0.998) vs 0.9845 ±0.009 (0.97-1.005)
TTV positive samples: men vs women	45 vs 39
INS -23 Hph I: AA/AT/TT	49/66/5
IGF2 Apa: GG/GA/AA	55/57/8
VDR Fok I: CC/CT/TT	53/50/17
VDR Apa I: AA/AC/CC	41/53/26
VDR Taq I: TT/TC/CC	25/52/43
ACE I/D: II/ID/DD	35/52/33
AT1R A1166C: AA/AC/CC	57/48/15
IL-6 -174G/C: GG/GC/CC	75/38/7

[a] p<0.05; [b] p<0.0001

The IGF2 GG and ACE DD were associated with a higher digit ratio in all subjects and also in women (Table 2). No other genotype shows a significant association with the digit ratio.

Table 2: Characteristics associated with 2D:4D

Subgroup compared	2D: 4D average (interval)
All: IGF2 GG vs. (GA+AA)	0.9877±0.0074 (0.97-1.002) vs 0.9841±0.008 (0.97-1.005)[b]
Women: IGF2 GG vs (GA+AA)	0.9873±0.007 (0.97-1.002) vs 0.9823±0.009 (0.97-1.005)[a]
Men: IGF2 GG vs (GA+AA)	0.9881±0.006 (0.973-0.998) vs 0.9861±0.006 (0.97-0.997)
All: ACE DD vs. II	0.9893±0.007 (0.97-1.002) vs 0.9843±0.009 (0.97-1.005)[b]
Women: ACE DD vs. II	0.9899±0,008 (0.97-1,002) vs 0.9811±0.009 (0.97-1.005)[c]
Men: ACE DD vs. II	0.9885±0.005 (0.98-0.996) vs 0.9878±0.007 (0.97- 0.998)
All: ACE (DD+ID) vs II	0.9844±0.008 (0.97-1.005) 0.9843±0.009 (0.97-1.005)
Women: ACE (DD+ID) vs I	0.9823±0.008 (0.97-1.005) vs 0.9811±0.009 (0.97-1.005)
Men: ACE (DD+ID) vs ACE II	0.9866±0.007 (0.97-0.998) vs 0.9877±0.007 (0.973-0.998)

[a] p<0.05; [b] p<0.01; [c] p =0.006

The INS and IGF2 (11p15.5) polymorphisms or VDR (12q13.11) polymorphisms were mapped in close proximity. As a consequence, we evaluated the effects of Ins -23 Hph - IGF2 Apa I and VDR Fok I – Apa I – Taq I haplotypes on 2D:4D. The INS-23 Hph I – IGF2 Apa I T-G haplotype was found to be significantly associated with 2D:4D in men (p<0.01), but not in women (p>0.05). No statistical associations were identified between the digit ratio and VDR Fok I – Apa I – Taq I haplotypes (Table 3).

Twenty-four (39.34%) women reported at least a pregnancy loss. The 2D:4D value of these women was lower compared to those who did not report such an event (0.9799 vs 0.9876, p<0.001). The height in these subsets of women was similar (1.691 vs 1.697, p<0.05) (Figure 1). The average age at which they had their first child was similar between those who had one child and those who had multiple

children (22.93 vs 22.2, p>0.05).

The 2D:4D values were lower in women who had no children compared to those who had children (p <0.001), who had at least two children (p <0.001), or one child (0.9809 vs 0.9865, p < 0.3). In the case of men, the differences identified were significant between men who had at least two children and those who had one child (p<0.001) or who did not have children (p<0.02). In the subset of subjects who did not have children, the lowest average 2D:4D values were recorded in men compared to women (0.9883 vs 0.9809, p< 0.001). The height of men and women stratified by the number of children did not show any statistically significant differences (Figure 2).

Table 3: Association of INS-IGF2 and of VDR haplotypes with 2D:4D

Polymorphisms	Haplotypes	The total count in women's sublot	The total count in men's sublot
VDR Fok I – Apa I – Taq I	CAC	34	31
	TCT	20	7
	CCT	18	24
	TAC	15	22
	CAT	15	7
	CCC	13	14
	TCC	5	4
	TAT	-	9
INS -23Hph I – IGF2 Apa	AG	51	49
	TG	34	33[a]
	AA	35	29
	TA	-	7

(Haplotypes with frequency <0.03 were not taken into consideration; [a] p <0.01)

Figure 1: Analysis of 2D:4D ratio and number of children by gender

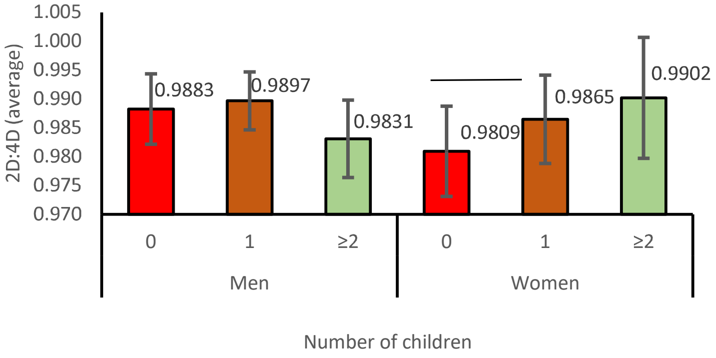

Figure 2: Analysis of height and number of children by gender

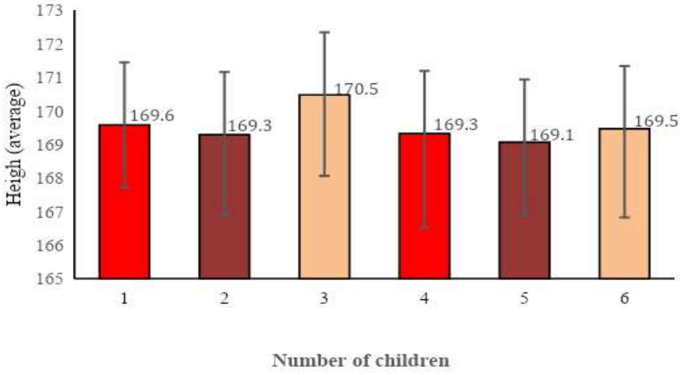

The 2D:4D values were higher in overweight subjects than in normal-weight participants (0.984 vs 0.988, p<0.008). The weight (74.1 vs 69.39 kg, p<0.0001) and BMI (25.72 vs 24.21 kg/m^2, p<0.0001), but not height (170 vs 169 cm, p>0.05), were significantly higher in men compared to women. Each gender was subsequently stratified according to BMI into normal-weight and overweight subgroups. There was only a significant difference between the 2D:4D values of overweight men and normal-weight women (0.9884 vs 0.9837,

p<0.05). No significant results were found when the male and female subsets were stratified according to height.

There appears to be a positive correlation between 2D:4D and BMI, regardless of gender (F=0.02) (Figure 3), and an inverse correlation between the 2D:4D and height of men (F = 0.03) (Figure 4). However, the proportion of 2D:4D variance that may be predicted based on BMI (R2=0.047) or height (R2 =0.08) appears to be weak.

The tested genetic polymorphisms were not associated with the height or BMI of men and women (p>0.05). These variables were similar in women stratified by parity.

Figure 3: Linear regression for 2D:4D and BMI (both genders)

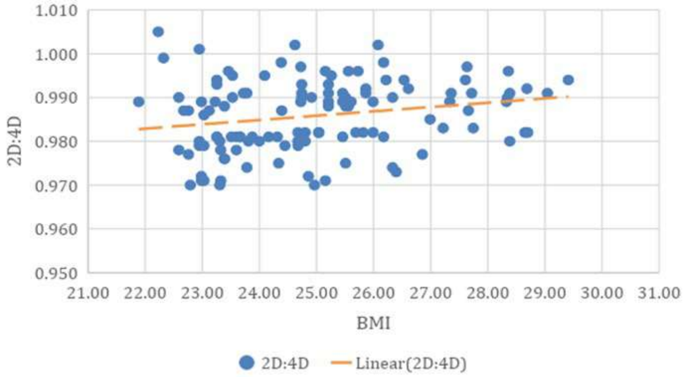

Figure 4: Linear regression for 2D:4D and height of men and women

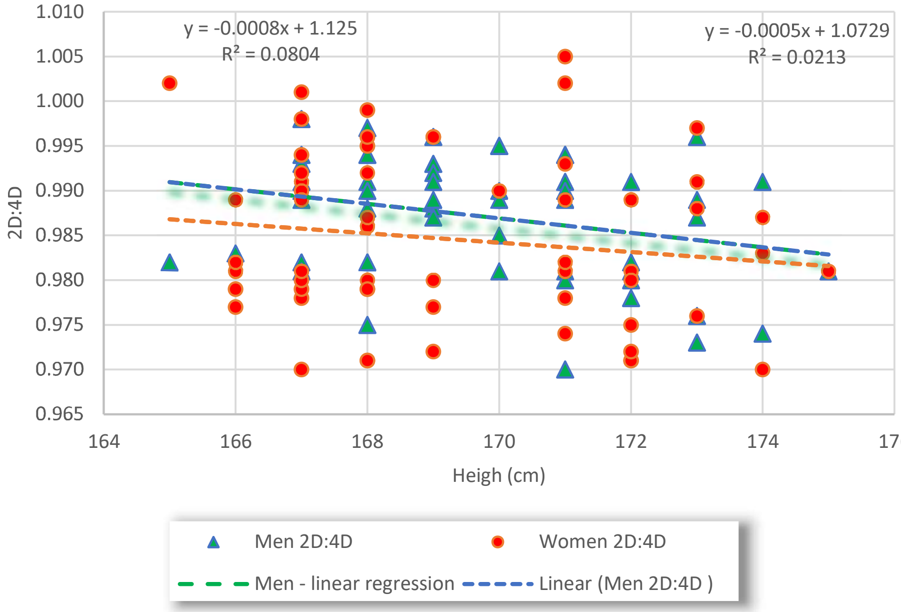

DISCUSSION

The digit ratio is determined during the prenatal period and in general remains stable throughout life [1]. A study that analyzed the 2D:4D ratio in 327 deceased fetuses (14 to 42 weeks old) concluded that the values were lower than those reported for children and adults in both sexes, suggesting 2D:4D could increase after birth [48]. In addition, a study has shown that this ratio can change, to a significantly reduced extent, during childhood, between the ages of one and five years [47]. Therefore, age may influence to some extent the length between the index and ring fingers. In the present study, the 2D:4D values were not correlated with age, neither at the level of the entire group of adult participants nor at the subgroup level by gender.

The difference between genders appears to be moderated by the type of finger length measurement and by the hand that was measured. Some studies suggest that there are greater differences between genders when measurements are taken on the right hand, which would better reflect prenatal hormone exposure [49,50]. In our sample, measurements were taken on the left hand, for which we obtained higher average values in men than in women, but the differences were not significant (0.9871 vs 0.9845, p >0.05). This result is contrary to the results reported by other studies [3,4]. In a meta-analysis that studied the representation of women in politics and was based on data collected in an online survey, the reported data for Romania indicated higher values in women than in

men for both the left hand (1 vs. 0.985) and the right hand (0.997 vs. 0.986) [50].

In contrast to the 2D:4D, height, weight, and BMI are characteristics that change postnatally, influenced by both genetic and environmental factors. Weak correlations between 2D:4D and these variables were identified in this study. Studies are not consistent on this matter. For example, 2D:4D was positively related to BMI in a very large sample of adults [51]. On the contrary, other studies have reported no significant correlations between these variables and 2D:4D [52,53].

The analysis of anthropometric data we performed showed that the values for weight and BMI were higher in men than in women. Moreover, we identified a significant difference between the 2D:4D values of overweight men and normal-weight women (0.9884 vs 0.9837, $p<0.05$). In the literature, significant positive correlations have been reported between higher (feminized) digit ratios and postnatal parameters (body weight, BMI, and waist and hip circumference) [54,55]. There were no statistically significant differences between height of men and women ($p>0.05$). The linear regression showed a negative correlation between 2D:4D and the height for males ($F = 0.03$), but not for females or both genders. One study reported that body height was negatively correlated with right- and left-hand digit ratios and the correlations were significant for the left-hand digit ratio in men and for the right-hand digit ratio in women [7].

Our data suggested a significant association between 2D:4D and reproductive success which is influenced by gender. Thus, lower average 2D:4D values were recorded in women who reported a pregnancy loss or who had no or one child ($p <0.001$). In the case of men lower 2D:4D values were associated with a higher number of children. Our results are in agreement with some results reported previously in the literature [9,10].

The minor allele frequencies for IGF2 Apa I (MAF A: 0.3 vs. 0.24) [56, 57] and ACE I/D (D: 0.49 vs 0.51) [58] in our lot are consistent with those previously reported in the Romanian population. Previous studies have reported that the IGF2 G allele may contribute to weight gain and eating disorders, and BMI [59]. Another study, which investigated multiple single nucleotide polymorphisms in the IGF2 gene region, concluded that IGF2 genetic variation is a significant determinant of body weight in middle-aged males [60]. The data we have obtained suggest that nearby genes, IGF2 or Insulin, could influence the digit ratio. The IGF2 GG genotype was associated with a higher digit ratio, especially in women ($p<0.05$) whereas the Insulin - IGF2 T-G haplotype was significantly associated with 2D:4D, but only in men. The closely linked IGF2 and Insulin genes have been previously associated with various conditions such as obesity, diabetes, metabolic syndrome, and coronary heart disease [61, 62]. Genetic imprinting influences IGF2 expression [63] and the association between the parent-of-origin allele and various phenotypes. The design of our study did not permit the investigation of IGF2 parent-of-origin.

ACE converts angiotensin I into angiotensin II and most of its functions are mediated by AT1R. ACE D was associated with a higher enzymatic activity of ACE [64]. Carriers of ACE DD have higher values of 2D:4D than ACE II in our lot of subjects ($p<0.01$) and especially in women ($p<0.01$). In a previous study, it was also reported that ACE DD individuals have higher 2D:4D than carriers of ACE II [65]. ACE I/D was also associated with various diseases, such as cardiovascular diseases and hypertension [66] or physical endurance or competitiveness [67]. The association may bias the results we have detected in our healthy subjects. Given the fact that ACE I/D polymorphism and 2D:4D ratio might play a role in cardiovascular pathology, our subjects (42-60 years old), although clinically healthy, may still have a predisposition to develop these diseases. This predisposition might influence the results obtained in this study, potentially causing a bias. The results could also be affected by the small number of subjects included in the study ($n=120$), as well as by measurement errors related to the length between the index and ring fingers.

Although no significant association was identified between the digit ratio and Torque Teno viruses, the high prevalence of TTV in the population was confirmed, being present in 70% of the subjects included in the study, in line with previously obtained results [32,68].

CONCLUSION

In our dataset, the digit ratio was similar in men and women. The data obtained in this study indicated that INS - IGF2 T-G haplotype, ACE I/D, pregnancy loss, number of children, and BMI are associated with digit ratio. These relations could be also influenced by the gender of the subject. Additional studies are needed to bring new data regarding the relationships between 2D:4D and the genetic polymorphisms of anthropometric characteristics to see if the results we obtained in this study could be replicated. This data could contribute to a broader understanding of how genetics can shape developmental outcomes through prenatal hormones.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. This study was funded by the Ministry of Research and Education, grant number PN 23 01 03 03.

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Authors' contribution

Conceptualization, N.S., R.S., R.M., and D.C.; methodology, O.C.; software, R.M. and M.T.; validation, M.I.N., M.L.C., and C.I.; formal analysis, M.T and M.I.N.; investigation, N.S., D.C., L.B., O.A.; resources, L.B., D.C.; data curation, S.R. and O.A..; writing—original draft preparation, N.S., E.S., R.M, O.C.; writing—review and editing, D.C., M.T, I.R., M.L.C.; visualization, M.T.; supervision, D.C. and O.C.; project administration, L.B, and D.C.; funding acquisition, L.B. and S.R.. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the National Research and Development Institute for Food Bioresources – IBA Bucharest (protocol code 285/20.03.2023).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Is The Preoperative C-Reactive Protein/Albumin Ratio Successful in Predicting Postoperative Pancreatic Fistula in Patients Undergoing Pancreatoduodenectomy? A Case-control Study

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Abstract: Pancreaticoduodenectomy (PD) is a complex procedure with a high complication rate. Postoperative pancreatic fistula (POPF) is a major complication. Therefore, preoperative determination of POPF risk is clinically important. The C-reactive protein-albumin ratio (CAR) is a parameter used for prognosis in some types of cancer. We hypothesized that CAR could serve as a predictive parameter of POPF. In this study, we investigated whether CAR can predict the risk of POPF after PD in patients with pancreatic cancer. Methods: A total of 83 patients were included in this study between March 2017 and December 2023. This retrospective evaluation included demographic characteristics, preoperative laboratory findings, preoperative clinic and hospital records, and the surgical and treatment conditions of patients undergoing PD for pancreatic cancer in our department. Results: POPF was observed in 41 patients. CAR value was significantly elevated in the POPF group ($P< 0.001$). According to the Receiver Operating Characteristic (ROC) analysis results, the optimal cut-off point determined for CAR was 0.445. Univariate and Multivariate Binary Logistic Regression analyses showed that CAR was reliable for predicting POPF (odds 57.2). Conclusion: Preoperative CAR is a reliable predictive marker for POPF following PD.

Keywords: C-reactive protein, albumin, postoperative pancreatic fistula

INTRODUCTION

Pancreaticoduodenectomy (PD) is a complex procedure performed in individuals with neoplastic or non-neoplastic tumors affecting the head of the pancreas and the periampullary region. Despite advancements in surgical techniques and perioperative management, the mortality rate is 1.31% based on a nationwide study from Japan; however, operative mortality rates are much higher in western pancreatic centers, reaching up to 5-8% according to nationwide western studies [1]. Postoperative pancreatic fistula (POPF) is a POTENTIALLY SEVERE complication of PD. POPF can lead to wound infections, abdominal abscesses, and intra-abdominal hemorrhages [2]. Therefore, precise and prompt prediction of POPF following PD is essential for mitigating the mortality associated with severe complications. Moreover, evidence indicates that POPF adversely affects the prognosis of patients with malignancies [3]. Although some studies have demonstrated that diverse surgical techniques and drugs can diminish the incidence of POPF [4,5], recent research has concluded that POPF cannot be predicted by any clinical variable [6].

Inflammatory responses at the systemic level are closely linked to surgical complications in various procedures. C-reactive protein (CRP), an established inflammatory marker, is a widely recognized positive acute-phase, non-specific reactant [7]. The C-reactive protein-albumin ratio (CAR), calculated by dividing serum CRP by albumin levels, serves as another inflammatory marker and has been correlated with unfavorable prognosis in individuals diagnosed with cancer [8]. CAR was originally developed as a prognostic factor for patients diagnosed with sepsis [9]. Based on these findings, we hypothesized that CAR could not only predict prognosis but also forecast the occurrence of POPF. Studies have indicated that CAR serves as a predictive factor for various types of cancer [10,11].

The management of POPF has undergone historical changes, transitioning from a previously adopted 'wait and see' approach to a strategy characterized by early and timely prevention [12]. In this study, we included only patients with pancreatic cancer to focus on a specific cohort and to avoid the heterogeneity that could arise from including patients with other types of periampullary tumors. We assessed CAR as a cost-effective and readily available parameter to predict POPF after PD. Therefore, in this study, we aimed to determine whether preoperative CAR has predictive value for POPF in patients undergoing PD for pancreatic cancer. The management of POPF has undergone historical changes, transitioning from a

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previously adopted 'wait and see' approach to a strategy characterized by early and timely prevention [12]. We assessed CAR as a cost-effective and readily available parameter to predict POPF after PD. Therefore, in this study, we aimed to determine whether preoperative CAR has predictive value for POPF in patients undergoing PD for pancreatic cancer.

MATERIALS AND METHODS

Study Design

Demographic characteristics, preoperative laboratory results, preoperative polyclinic and hospital application notes, surgery, and treatment conditions of patients who underwent PD for pancreatic cancer (n=83) in our department between March 2017 and December 2023 were evaluated retrospectively. Patients aged between 18 and 80 years who were diagnosed with pancreatic cancer were included. The exclusion criteria were as follows: patients who underwent total pancreatectomy, those outside the specified age range, and those whose data were not available. Data extracted from the medical records included variables such as sex, age, American Society of Anesthesiologists (ASA) classification, pancreatic tissue characteristics, duct size, length of hospital stay, POPF, CRP, and serum albumin levels.

Surgical Technique

All patients underwent subtotal stomach-preserving PD. PD reconstruction was performed using Child’s technique. Pancreatic anastomoses were performed in all patients using duct-mucosa and end-to-side pancreaticojejunostomy (modified Blumgart). Biliary drainage was provided by end-to-side hepaticojejunostomy. All surgeries were performed by the same team using the same procedure. POPF was determined according to the International Study Group On Pancreatic Surgery (ISGPS) classifications, and the severity of complications was graded using the Clavien-Dindo classification [13]. CAR was computed using preoperative CRP and serum albumin values using the following formula: CAR = CRP (mg/dL)/serum albumin (g/dL).

Amylase levels in the drainage fluid are routinely measured on postoperative days 3, 5, and 7. Two drainage tubes (one adjacent to the hepaticojejunostomy site and the other to the pancreaticojejunostomy site) were placed in the abdominal cavity of all the patients. The time interval for assessing the car was within 7 days before PD. Antibiotics were routinely administered. The drains were removed after full recovery from the pancreatic fistula. No data about postoperative sandostatin or other similar substance administration was provided in this study.

This study was conducted in accordance with the 1964 Declaration of Helsinki and its amendments. Consent was obtained from conscious patients and first-degree relatives of unconscious patients. Ethical approval was obtained from Hitit University Ethics Committee (Ref. Nr: 2023-48).

Statistical analysis

Statistical analysis was performed using the SPSS software (Version 22, SPSS Inc., Chicago, IL, USA). Descriptive statistics for categorical data are reported using frequencies (n) and percentages (%). For numerical data demonstrating a normal distribution, descriptive statistics were reported as mean ± standard deviation (SD), while non-normally distributed data were reported as median (min-max). The Chi-square test was used to compare the proportions between categorical variables. The Shapiro-Wilk test, histograms, and Q-Q plots were used to assess the normality of the numerical data. Levene’s test was conducted to evaluate the homogeneity of variances. When parametric test assumptions were met, the Student’s t-test was used to compare continuous data between two independent groups; in cases where these assumptions were not met, the Mann-Whitney U test was utilized. P values of less than 0.05 were accepted as statistically significant. Receiver Operating Characteristic (ROC) analysis was conducted to evaluate C-reactive protein to albumin ratio (CAR) values as predictive markers for postoperative pancreatic fistula (POPF). ROC curves and Area under the Curve (AUC) with 95% confidence intervals (CIs) were calculated. The AUC values were categorized as excellent (0.9-1), good (0.8-0.9), fair (0.7-0.8), weak (0.6-0.7), and poor (0.5-0.6). The Youden index was used to determine the optimal cut-off points in the ROC analysis, and their performance was evaluated using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive likelihood ratio (LR+). Univariate and multivariate binary logistic regression analyses were performed to assess the impact of the risk factors on POPF. Odds ratios (OR) were computed for each significant parameter in the univariate and multivariate models, with 95% confidence intervals (CIs). All risk factors with a p-value < 0.10 in the univariate analysis were included in multivariate logistic regression models to ensure that no potentially significant variables were excluded prematurely.

RESULTS

The study analyzed a total of 83 patient records; among these, postoperative pancreatic fistula (POPF) was present in 41 (49.4%) and not detected in 42 (50.6%) cases. Of the patients, 63.9% (n=53) were male and 36.1% (n=30) were female. The mean age of the patients was 69.3±10.9 (range: 41-89) years. The mean length of hospital stay for the patients was 29.1 ± 15.3 (8-81) days. Although patients who developed POPF might be expected to have longer hospital stays, the difference was not statistically significant in this study (p=0.078). This may be due to the high standardization of postoperative care and efficient management of POPF in our institution.

Statistical findings regarding the comparison of sociodemographic and clinical characteristics between the research groups are presented in Table 1. Sex distribution and ASA scores were similar between the groups (p=0.708 and p=0.520, respectively). The mean age and length of hospital stay did not significantly differ between the groups (P=0.079 and P=0.078, respectively). However, there were significant differences in the Fistula classification and Clavien-Dindo grades between the groups (P<0.001 and P<0.001, respectively). Patients who developed POPF had statistically higher CRP and CAR values compared to those who did not (P<0.001 and P<0.001, respectively), whereas their albumin values were significantly lower (P=0.001).

Factors such as co-morbidities, diabetes, chronic pancreatitis, jaundice, and preoperative biliary drainage were not included in the analysis of potential predictors of POPF. This decision was made due to the primary focus of the study on the relationship between car and POPF risk. However, it is acknowledged that these factors can influence the development of POPF and should be considered in future studies.

The soft tissue ratios of patients with POPF were significantly higher than those of patients without (P=0.001). Additionally, the canal sizes in the POPF group were significantly smaller than those in the non-POPF group (P=0.001). The occurrence rate of duct size ≤2 mm in the POPF group was significantly higher than that in the non-POPF group (P=0.001). The cut-off point for the Wirsung duct size at 2 mm was selected based on previous studies indicating that smaller duct diameters are associated with a higher risk of POPF [24,25].

Table 1: Statistical findings for the comparison of sociodemographic and clinical characteristics between postoperative pancreatic fistula (POPF) groups

		POPF		***p* values**
		No (n=42)	**Yes (n=41)**	
Gender (M/F)	Male Female	26 (61.9%) 16 (38.1%)	27 (65.9%) 14 (34.1%)	0.708^{a}
Age (years)		71.38±11.03	67.17±10.52	0.079^{c}
Hospital stay (days)		25 (8 - 43)	28 (12 - 81)	0.078^{d}
ASA	1 2 3	0 (0%) 7 (16.7%) 35 (83.3%)	1 (2.4%) 4 (9.8%) 36 (87.8%)	0.520^{b}
Fistula grade	None A B C	42 (100%) 0 (0%) 0 (0%) 0 (0%)	1 (2.4%) 8 (19.5%) 25 (61%) 7 (17.1%)	<0.001^{b}
Clavien-Dindo	None 2 3 4	42 (100%) 0 (0%) 0 (0%) 0 (0%)	1 (2.4%) 8 (19.5%) 21 (51.2%) 11 (26.8%)	<0.001^{b}
CRP		0.59 (0.1 - 2.86)	3.68 (0.39 - 20.7)	<0.001^{d}
ALB		3.4 (2.7 – 4.5)	3.1 (2.2 - 4.16)	0.001^{d}
CAR		0.18 (0.08 – 0.91)	1.2 (0.12 - 8.28)	<0.001^{d}
Tissue	Soft Hard	17 (40.5%) 25 (59.5%)	37 (90.2%) 4 (9.8%)	<0.001^{a}
Duct size (mm)		3.9 (1 – 12)	2 (1 - 6.5)	0.001^{d}
Duct size group	≤ 2 > 2	9 (21.4%) 33 (78.6%)	24 (58.5%) 17 (41.5%)	0.001^{a}

CRP: C-reactive protein, ALB: Albumin, CAR: C-reactive protein to albumin ratio, ^{a}Chi-square test with n (%), ^{b}Fisher exact test with n (%), ^{c}Student's t-test with mean±standard deviation (SD), ^{d}Mann Whitney U test with median (min-max).

Interestingly, in this cohort, there were no other major complications (such as delayed gastric emptying, hemorrhage, infections) in the group without POPF, which is unusual compared to other studies. This may be due to the strict inclusion criteria and exclusion of patients with incomplete data. However, this finding does limit the generalizability of the results, and future studies should include a broader range of complications to provide a more comprehensive analysis.

The ROC analysis findings, including sensitivity, specificity, positive and negative predictive values, and likelihood ratio (+) values computed using the cut-off values determined by ROC analysis to assess the predictive success of CAR in predicting POPF, are presented in Table 2. According to the ROC analysis results, CAR exhibited excellent predictive capability for POPF (AUC=0.934, 95% CI: 0.881-0.986) (Table 2). The calculated optimal cut-off point for CAR was 0.445, with a sensitivity of 90.2% (75.9 – 96.8) and specificity of 90.5% (76.5 – 96.9). The ROC curves for the CAR are shown in Figure 1a, while box plots illustrating the distribution of CAR values between groups and the optimal cut-off point are presented in Figure 1b.

The results of Univariate and Multivariate Binary Logistic Regression analyses conducted to determine the impact of the created CAR parameter using the cut-off points determined by ROC analysis, along with other risk factors, on predicting POPF, are presented in Table 3. In the Univariate model, sex showed no significant effect (p=0.708), whereas age had a significant effect p<0.10 level (p=0.082). Duct size, tissue, and CAR parameters exhibited significance (p=0.001, p<0.001, p<0.001, respectively). Sex, which did not show a significant effect on POPF in the univariate model, was not included in the multivariate model (p=0.708). Age, duct size, tissue, and CAR parameters, which were significant p<0.10 in the univariate model, rendered age (p=0.128) and duct size insignificant (p=0.183) in the multivariate model. In the final model considering tissue and CAR, tissue parameters were significant at p<0.10 level, while CAR parameters were significantly impactful at p<0.001 (p=0.083 and p<0.001, respectively, Table 3). According to the Multivariate model, the odds ratio for CAR was 57.2 (12.8 – 254.9). The likelihood of developing POPF in patients with a CAR value greater than 0.445 was 57.2 times higher compared to that in patients with a CAR value lower than 0.445.

Table 2: Findings of the ROC analysis showing the success of C-reactive protein to albumin ratio (CAR) values in predicting postoperative pancreatic fistula (POPF) along with sensitivity, specificity, positive-negative predictive values and positive likelihood ratio values

	CAR
AUC (95%CI)	0.934 (0.881-0.986)
p values	< 0.001
Cut-off	0.445
Sensitivity (95% CI)	90.2% (75.9 – 96.8)
Specificity (95% CI)	90.5% (76.5 – 96.9)
PPV (95% CI)	90.2% (75.9 – 96.8)
NPV (95% CI)	90.5% (76.5 – 96.9)
LR + (95% CI)	9.5 (3.7- 24.2)

CRP: C-reactive protein, ALB: Albumin, CAR: C-reactive protein to albumin ratio, ROC: Receiver Operating Characteristic, AUC: Area under curve, PPV: positive predictive value, NPV: negative predictive value, LR: Likelihood Ratio, CI: Confidence interval.

Table 3: The results of univariate and multivariate binary logistic regression analyses performed to determine the effect of risk factors in predicting postoperative pancreatic fistula (POPF)

	Univariate		**Multivariate**	
	***p* values**	**OR (CI 95%)**	***p* values**	**OR (CI 95%)**
Gender (M/F)	0.708	-	ni	-
Age (years)	0.082	0.96 (0.93 – 1.01)	ns	-
Duct size (mm) (≤ 2 & > 2)	0.001	5.18 (1.97 – 13.6)	ns	-
Tissue (Soft & Hard)	<0.001	13.6 (4.09 – 45.2)	0.083	4.46 (0.83 – 24.2)
CAR (≥ 0.445 & < 0.445)	<0.001	87.9 (20.5 – 377.6)	<0.001	57.2 (12.8 – 254.9)

Multivariate model: (Tissue + CAR) Nagelkerke R Square=0.727, Classification accuracy: 90.4%, Values below P < 0.1 were shown bold, ni: not included, ns: not significant (P>0.05), OR: Odds ratio, CI: Confidence interval, CAR: C-reactive protein to albumin ratio .

Figure 1: ROC Curves for C-reactive protein to albumin ratio (CAR) values (a.) and Box plot showing the distributions and optimal cut-off points of CAR values (b.) between postoperative pancreatic fistula (POPF) groups.

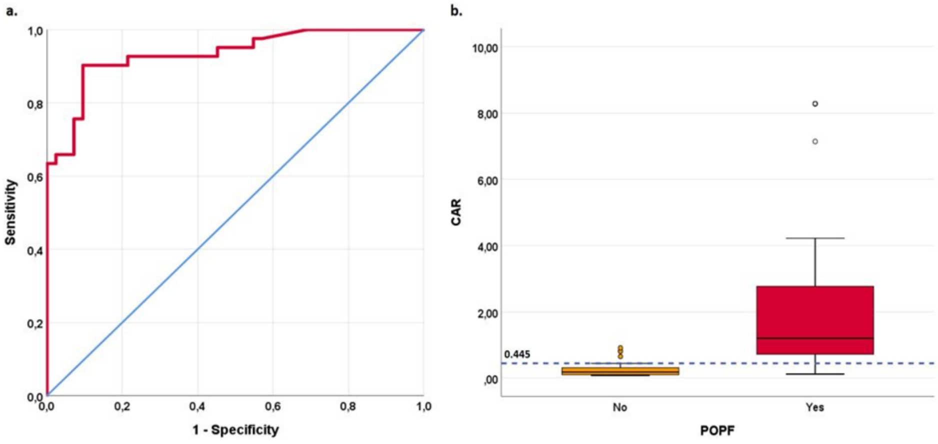

DISCUSSION

Despite advancements in surgical management, POPF continues to pose a significant complication following PD. Approximately 50% of the patients undergoing PD experience POPF [14]. Serious cases of POPF can lead to serious complications, including sepsis and intra-abdominal hemorrhage. This can result in prolonged hospitalization, escalated medical expenses, and mortality in extreme scenarios [2]. In addition, the poor prognosis of patients with malignant disease is exacerbated by the delayed initiation of adjuvant therapy resulting from POPF [15]. Several risk factors have been identified for the development of POPF after PD. These factors encompass pancreatic tissue characteristics, duct size, low albumin levels, gender, surgical technique, elevated CRP levels, and others [16]. As there are currently no definitive methods to entirely prevent POPF, despite numerous studies, there is a crucial need for accurate and reliable prediction of POPF in the early postoperative period after PD to prevent these complications. Many studies have investigated various parameters to predict POPF, but a definitive gold standard that can accurately predict POPF is yet to be established. Recent studies have shown that popular biochemical parameters can predict POPF [17]. In our study, we aimed to assess whether preoperative CAR is associated with the risk of POPF following PD.

CAR, a novel inflammatory marker, has been extensively used as a prognostic indicator for various cancers [18]. Sakamoto et al. [19] demonstrated in their study that the CAR value on the 3rd postoperative day proved to be a robust indicator for predicting POPF with statistical significance. However, it was not significant on the 1st postoperative day. The reason for this was thought to be that the expected postoperative CRP elevation may have affected the results and may have approached the preoperative values on the 3rd postoperative day. We thought that this parameter could be used in terms of POPF risk in the pre-operative period, and this became our main motivation for this study. In our study, 41 patients (49.4%) had POPF. In our study, a preoperative CAR value >0.445 was associated with an increased risk of POPF. Furthermore, this result indicates the patient's preoperative nutritional status. Improving the nutritional status may suggest a potential reduction in the risk of POPF. Studies have shown a parallel relationship between hypoalbuminemia and morbidity [20].

Our study showed that both soft pancreatic tissue and duct size have a significant relationship with the development of POPF. Following PD with soft pancreatic tissue, the high rates of POPF can be attributed to various factors. These may include an increase in the exocrine function of the soft glands and a limited suture-holding capacity [21,22]. Moreover, Xingjun et al. [23] indicated that a low degree of fibrosis is a risk factor for the occurrence of POPF after PD. Creating anastomosis in a narrow duct size is technically difficult. In the majority of studies, a cut-off point of ≤3 mm for duct size was stipulated [24,25]. However, in our study, duct sizes of ≤2 mm were found to be significantly higher in terms of POPF. Considering the factors that increase or decrease the risk of POPF, this value may not have been significant in our study for different reasons. Studies have shown that small duct diameter and soft pancreatic tissue are associated with an increased risk of POPF [16]. In a previous study, the relationship between CAR and POPF was found to be significant, however, the relationship between pancreatic duct diameter and tissue has not been investigated [17]. Therefore, we investigated the relationship between car, duct size, and pancreatic tissue characteristics. Our results indicated that car was more significantly associated with POPF risk in patients with small duct diameters and soft pancreatic tissue. This was the first study to be conducted, and it was the most important difference in our study.

Our findings can be used in clinical decision management for patients undergoing pd by incorporating car values into preoperative assessments. Identifying patients with elevated car values preoperatively can help in strategizing post-operative care, close monitoring, and interventions aimed at reducing POPF risk.

CONCLUSION

In conclusion, CAR is a reliable and easily calculable predictive indicator for POPF risk after PD. Further prospective studies with larger patient populations are required to conclusively evaluate the reliability of this parameter; our study could serve as an illuminating guide for prospective randomized controlled trials that can be planned for carefully selected patient groups.

Conflicts of interest and sources of funding

The authors declare that there are no conflicts of interest, financial or otherwise, related to the materials presented herein. The authors declare no conflicts of interest. The funder had no role in the design, data collaboration, data analysis, or reporting of this study

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The current manuscript does not contain previously published materials or self-generated AI text.

Authors' contribution

Conceptualization (BK, ITS); methodology (BK); software (ITS); validation (VBT); formal analysis (BK, VBT, EGA); investigation (EGA); resources (BK); data curation (ITS, VBT); statistical analysis (VBT, EGA); writing – original draft preparation (BK, VBT, EGA); writing – review and editing of manuscript (VBT, EGA); visualization (BK,VBT); supervision (EGA); project administration (BK). All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Hitit University. Approval No: 2023-48. Written informed consent was obtained from the patients included in this study prior to their participation. The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study. The study was obtained from clinical trials with study number NCT06300801.

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ORIGINAL ARTICLE

Ocular Manifestations in Psoriasis: The Importance of Ophthalmological Examination

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Abstract: Psoriasis is a chronic, inflammatory, immune-mediated skin disease affecting 1-3% of the adult population. It mainly involves the skin, nails, and joints. However, psoriasis can be associated with several comorbidities, including ocular complications. The most frequently reported pathologies are dry eyes syndrome, blepharitis, and uveitis. Being a systemic inflammatory disease, psoriasis also has the potential to directly affect the retina. It has been reported that ocular manifestations tend to appear much later than skin and joint involvement. Eye complications begin insidiously, and progress asymptomatically. This can lead to a decrease in the quality of life and even permanently compromised visual function if not treated properly. Thus, the present review aimed to present current pathological explanations between psoriasis and ocular manifestation, and to evaluate the prevalence of ocular changes in patients with psoriasis, in order to be diagnosed and managed appropriately.

Keywords: psoriasis, systemic disease, parakeratosis, ocular complications, uveitis, dry eye syndrome, retina, cornea, retina, cataract

INTRODUCTION

Psoriasis is a common chronic, inflammatory, immune-mediated skin disease, affecting both sexes equally, but men usually have more severe forms of the disease than women [1]. Skin manifestations are characterized by the presence of very well-circumscribed, erythematous papules or plaques with a grey or silvery-white, dry scale, over the surface of the skin with different morphology, distribution, and severity. The lesions are often distributed symmetrically on the elbows, knees, lumbosacral area, and scalp [2].

Psoriasis affects 1-3% of the adult population and negatively impacts the quality of life of the patients [2]. While it primarily affects the skin, psoriasis is associated with several comorbidities, such as psoriatic arthritis, Crohn’s disease, cardio and cerebrovascular disorders, and ocular diseases, which confirms that psoriasis is a systemic entity [3]. Ophthalmological manifestations in psoriasis have an occurrence rate of 10-12% involving different parts of the eye and presenting various symptoms [4]. Key ocular manifestations associated with psoriasis include uveitis, dry eye, retinal abnormalities, blepharitis, conjunctivitis, keratitis, iridocyclitis, and UV-induced cataracts [3]. In particular, patients with psoriatic arthritis show a higher risk of developing uveitis than psoriasis patients without joint involvement [3]. Moreover, psoriasis treatments may also lead to ocular complications, with a higher prevalence of ocular manifestations in patients treated with systemic treatments such as methotrexate, acitretin, and biologic drugs, and less frequent in cases treated with topical steroids and phototherapy [3]. However, ocular manifestations of psoriasis are often nonspecific or mildly symptomatic and may be often misdiagnosed [3].

PHYSIOPATHOLOGY OF PSORIASIS AND OCULAR COMORBIDITIES

Physiopathology of psoriasis

Psoriasis is a yet incompletely understood condition in which an unspecified stimulus triggers the activation of innate immune defense, with differentiation of proinflammatory M1-type macrophages. These in turn activate the stimulation of innate immune defense chains via IL23, which leads to the emergence of LyTh1 and LyTh17 cell clones with the release of IL17. At the tissue level, keratinocyte hyperproliferation, vascular hyperplasia, as well as the formation of tissue memory Ly Th and the reduction of regulatory Ly Th activity occur. Although there is now a

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wealth of data illustrating the etiopathogenic mechanism in psoriasis, the initial triggering of the immune system is unknown. It is hypothesized that environmental factors, a change in the composition of the skin microbiota, or other types of stressors may be at the origin, but in the absence of concrete data, we can only speculate.

From the present information, however, we are well aware of the close interrelationships between the cutaneous dendritic cell, neutrophils, macrophages, and the acquired immune system, with the triggering of immune relays leading both to the clinical manifestations of the disease and the formation of a local immunologic scar [5].

On exacerbation or onset of psoriasis, activated dendritic cells produce interleukin 12 (IL-12), IL-23, and TNF-alfa. IL-23 activates Th17 cells to produce IL-17, which stimulates keratinocytes to proliferate in an alternative pathway. Sustained inflammation leads to uncontrolled proliferation and abnormal differentiation of keratinocytes [6].

Potential physiopathology of ocular comorbidities

The exact mechanism by which psoriasis affects various parts of the eye is not fully known, but it is known that the skin and eye share a common origin from the ectoderm [4]. Thus, it is hypothesized that the ocular manifestations are closely associated with the cutaneous manifestations through an immune-mediated mechanism [4]. There are a few possible pathological mechanisms that can account for the ocular manifestations in psoriasis patients. The first explanation is the increased inflammatory reaction in psoriasis, which can trigger ocular inflammation [6]. The second is genetic predisposition, HLA B-27, which could lead to autoimmunity in both the skin and the eye [6].

MATERIALS AND METHODS

A structured literature search of titles in PubMed was performed in October 2024. The literature search was performed according to the updated preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [7]. The keywords used for finding relevant results were: “psoriasis”, “ocular manifestations”, “psoriatic”, “uveitis and psoriasis”, “ophthalmology” and all relevant synonyms, abbreviations, and combinations between these terms. The references of identified articles were manually checked to find potentially relevant studies. All studies, articles, and systematic reviews published before 2014 were excluded. All studies, articles, and systematic reviews concerning ocular side effects of the treatments used in psoriasis were excluded. The bibliography of these articles or reviews was analyzed by the authors and if relevant or complementary articles for this review were found, conducted to their integration in this study regardless of the year of publication. Studies were included for full-text review and qualitative analysis if they reported on well-known ocular manifestations of psoriasis, rare associations between psoriasis and ocular findings, as well as novel approaches of immunological links between psoriasis pathological mechanisms and subsequent clinical ocular affections. The authors acknowledge that this search string potentially may have unknowingly excluded important research papers.

RESULTS AND DISCUSSIONS

Being a chronic inflammatory disorder, psoriasis could manifest with various extra-cutaneous manifestations of which eye involvement is important. The ocular structures that could be directly or indirectly touched by the inflammatory imbalance induced by psoriasis are conjunctiva, Meibomian glands, eyelids, cornea, sclera, uvea, and retina [8]. From this enumeration, it is obvious that psoriasis could affect every ocular structure. Each of these ocular structures has a clinical impact on the patient and creates a broad spectrum of manifestations including relatively mild ocular disorders such as conjunctival hyperemia and severe ocular disorders such as corneal melting or retinal affections which could lead to vision loss [9].

Discussing ocular changes induced by a parakeratosis disease, we will structure the results depending on the presence of epithelium in the affected ocular structures. We classify the ocular structures into two categories: 1. External ocular structures- epithelium-containing structures, which could be exposed to the parakeratosis effect of psoriasis (conjunctiva, cornea, meibomian glands) and 2. Internal ocular structures -without epithelium exposed to parakeratosis effect (uvea, retina). This classification can define better the proportions of inflammatory status solely which subsequently affects the ocular structures and its combination with the epithelial damage.

External ocular structures

Conjunctival manifestations

The most frequent manifestation of psoriasis in conjunctiva is conjunctivitis [2]. Symptoms of conjunctivitis include conjunctival redness, tearing or thick yellow discharge, ocular pain, or the feeling of grittiness or foreign bodies in the eyes. The signs of conjunctivitis can include conjunctival hyperemia, demarcated, yellowish-red plaques on the palpebral conjunctiva, or areas of xerotic appearance on the bulbar conjunctiva [2]. After reviewing the literature, it was not possible to find a study that attests to the exact mechanism of the occurrence of conjunctivitis in psoriasis. Therefore, it is not known whether conjunctivitis appears as a result of the specific epithelial damage in psoriasis or as a result of systemic inflammation. Even though psoriasis commonly can cause conjunctivitis, it is important to remember that this condition is more commonly due to allergies, bacterial infection, or viral infection [10]. Regardless of the pathological mechanism of conjunctivitis in psoriasis, left untreated, conjunctivitis complicates itself with xerosis, symblepharon, or trichiasis which could lead to further complications involving the cornea [9].

Posterior Blepharitis

The cause of posterior blepharitis is the meibomian gland dysfunction (MGD). Meibomian glands (MG) are sebaceous glands located in the eyelids. They are responsible for secreting the lipid component of the eye tear film which plays an important role on the ocular surface by preventing the evaporation of tears [11]. Meibomian gland dysfunction is a chronic disease characterized by terminal duct obstruction and/or quantitative-qualitative changes in secretions [12]. The mechanism that links these two conditions is not known, but it was proposed that an increased epithelial turnover leads to high volumes of cell production and subsequent shedding that may ultimately lead to a mechanical block of the meibomian duct [2,13].

Regardless of the exact mechanism that links psoriasis and blepharitis, the epidemiological data suggest a prevalence rate of MGD in psoriasis patients even as high as 59.2% [14]. Using meibography, Kemeriz F. et al. [15] also found a statistically significant higher frequency of MGD and meibomian gland loss among psoriasis patients compared to the control group. Additionally, they found a significant relationship between MGD and psoriasis area severity index (PASI) scores. In a study by Santos da Cruz et al. [16] which included 43 psoriasis patients and 86 control subjects, it was found that patients with psoriasis had a statistically higher rate of blepharitis (16.28%) as compared to control subjects.

Posterior blepharitis remains a clinical diagnostic. It is important to recognize it because it further causes or exacerbates the dry eye syndrome through the tear evaporation mechanism. MGD is present in approximately two-thirds of patients with dry eye disease [17] [18]. This clinical entity is one of the most common ocular manifestations associated with psoriasis. The symptoms of blepharitis are eye itching, burning sensation, and foreign body sensation. The signs of blepharitis are hyperemia, inflammation, and edema at the free margin of the eyelids, with slight scaling [4]. Untreated, chronic blepharitis may lead to ectropion of the lower lacrimal point with epiphora, madarosis, trichiasis, and loss of lid tissue [19,20].

Dry Eye Syndrome

In patients with psoriasis dry eye syndrome (DES) occurs due to an obstructive dysfunction of the excretory ducts of the meibomian glands [4]. The DES may be independent of or may be the result of blepharitis or conjunctivitis [4]. Also, in patients with psoriasis, it was observed that a deficiency of L-arginine may be associated with the decrease of the tear film and DES [21].

DES is maybe the most common ocular manifestation in patients with psoriasis. Zohreh H. et al. [10]. suggest a prevalence rate of dry eye syndrome as high as 18.75%. Singh A. et al. [22] reported an incidence of 27% of dry eye among patients with psoriasis. Another reported incidence was 22% [15].

Beyond the already well-known higher incidence of DES among psoriasis patients, several studies suggested a direct correlation between PASI score and the severity of DES [14]. Aryanian Z. et al. [23] found a significant negative correlation between psoriasis severity based on PASI values and tear meniscus height for both right and left eyes. Also, MGD and MG loss were significantly correlated with PASI [15]. Chimenti M.S. et al. [24] reported even an incidence of DES as high as 75% among patients with psoriatic arthritis, and Allen T.S.R. et al. [25] had an incidence of 60%. Kharolia A. et al. [26] included in their study only patients with PASI scores of more than 10 and reported incidences of 19.1%, 10.2%, and 25% of mild, moderate, and severe dry eye, respectively.

Using a questionnaire to reveal misdiagnosed ocular involvement in psoriasis, Ruggiero A. et al. [3] showed that from a total of 372 patients with psoriasis enrolled in the study, ocular symptoms were detected in 39 patients (10.5%), and 37 patients were referred to ophthalmological examination which confirmed the presence of ocular manifestation in 30 patients. Among these 30 patients, there were 14 cases (46.6%) of dry eye syndrome. Also, given the symptoms of DES including burning, foreign-body sensations, or filmy vision, studies that measured the quality-of-life of psoriasis patients have shown that the impact of moderate to severe dry eye is similar to that of moderate to severe angina [27].

Untreated, this syndrome will complicate itself with keratoconjunctivitis sicca, which could manifest with corneal severe punctate erosions, filamentary keratitis, trichiasis, and even symblepharon or corneal ulceration [27]. Keratoconjunctivitis sicca has been reported at a prevalence rate of 2.7% of psoriatic arthritis patients. [2].

Cornea

Corneal changes in psoriasis are rare, but if present, may be sight-threatening [28]. It has been described peripheral corneal melting, which could lead to perforation [9,29], sterile corneal infiltrates [9], corneal abscess formation, stromal infiltration under Bowman's layer with superficial vascularization and punctuate epithelial keratitis [28,30],

Lee C-Y. et al [31] showed that psoriasis was associated with an increased risk of keratopathy in patients without the preexistent prominent corneal disease, and furthermore, this risk of incident keratopathy increases with exposure to psoriasis.

Starting from the parakeratosis effect of psoriasis, Güneș I.B et al. [32] evaluated the relationship between disease severity and central corneal epithelial thickness. They concluded that central corneal epithelial thickness could be an indicator of psoriasis severity.

Also, corneal hysteresis correlates negatively with disease activity and should be considered when determining intraocular pressure values in glaucoma patients and during corneal evaluation for keratoconus-suspected patients [33].

Furthermore, as shown earlier, it should be remembered that the cornea could be affected by dry eye syndrome which has a higher prevalence among psoriasis patients [10,15,22].

Internal ocular structures

Uveitis

Uveitis is the inflammation of the uveal tract composed of the iris, ciliary body, and choroid. The main classifications of uveitis are made according to the involved anatomic parts of the uvea (anterior, intermediate, or posterior) or according to etiology (infectious and non-infectious uveitis). Anterior uveitis involves the iris and/or the ciliary body, intermediate uveitis involves the vitreous and posterior uveitis involves the choroid [34].

Uveitis is a complication or a comorbidity of psoriasis [35]. Regularly, psoriasis causes an anterior uveitis [35]. The classic presentation of acute anterior uveitis is the sudden onset of pain, redness, and photophobia that can be associated with decreased vision [35]. Posterior segment involvement can also be present [35]. Uveitis associated with psoriasis tends to be anterior, bilateral, and chronic and tends to appear in older patients than those without psoriasis [36]. The incidence of uveitis tends to be higher in patients suffering from psoriasis and psoriatic arthritis (PsA) or PsA alone [37,38] and could reach 25% [35].

It is not known yet the exact pathological association between psoriasis and uveitis [36]. Possible explanations would be that a breakdown in the barrier between the blood and the aqueous humor could occur as a result of subclinical inflammation in patients with psoriasis, even when the patients do not complain about any ocular symptoms [39,40]. Subsequently, the activated neutrophils in peripheral blood may be responsible for the attacks of anterior uveitis [36]. Also, immune complexes or complement activations mediated by cell surface proteases associated with other chemotactic factors such as leukotriene B4 are found in high levels in the aqueous humor in cases of uveitis in experimental models [36]. Regardless of the mechanism that causes uveitis among psoriasis patients, the close relationship between uveitis and psoriasis has been shown by Chen Y-Y. et al. [41] in a revers manner. They aimed to evaluate whether the risk of subsequent psoriasis and psoriatic arthritis development is increased in patients with uveitis. They found that intermediate uveitis is an indicator of future development of psoriasis.

Activated dendritic cells migrate into draining lymph nodes and secrete tumor necrosis factor (TNF-α), IL-23, and IL-12 [42]. IL-23 and IL-12 will modulate the differentiation and proliferation of Th17 and Th1 cell subsets, respectively [42]. The activation of the adaptive immune response via the distinct T cell subsets drives the maintenance phase of psoriatic inflammation [43].

Regarding these main inflammatory signal cytokines found in the physiopathology of psoriasis, Weinstein et al. [44] studied the relationship between these cytokines and the occurrence of uveitis. In experimental autoimmune uveitis (EAU), both Th17 cells and interleukin (IL) 17 appear to have an important role in driving inflammation. In addition to driving inflammatory cytokine expression, IL-17 signaling also drives the expression of metalloproteases that can cause tissue injury and of chemokines that recruit neutrophils to the site of inflammation [44]. In the adoptive transfer model of EAU, interphotoreceptor retinoid-binding protein (IRBP) specific T cells that express IL-17 are strong effector cells to induce uveitis [45], and treatment with anti-IL-17 antibody is sufficient to block the development of disease [46].

Regarding TNF-α, in the experimental autoimmune uveitis model, neutralization of TNFα suppresses uveitis [47] and the mice with a deficit of TNF receptor 1 are resistant to the development of uveitis [48].

A remarkable feature in the clinical course of psoriasis patients is the presence of HLA-B27. Its importance is due to psoriatic arthritis which belongs to the group of spondyloarthropathies that includes some subgroups for which HLA-B27 is the genetic marker [36]. The relationship between psoriasis, uveitis, and HLA-B27 is not fully established [36]. Instead, there is a clear relationship between positivity for HLA-B27 in psoriasis patients and the severity and incidence of uveitis among them [36]. So, HLA-B27 is considered a risk factor associated with the presence and/or severity of uveitis [36].

The presence of HLA-B27 even has an impact on the cytokines involved in the inflammatory milieu of psoriasis and the intraocular milieu of uveitis. Elevated TNF⍰ levels were found in aqueous sampling in patients with HLA-B27 [49,50]. Hernandez Garfella ML. et al. [51] showed that aqueous levels do not appear to reflect the impact of disease activity or treatment with systemic adalimumab. Also, elevated serum IL-23 has been identified as a risk factor for developing uveitis in spondyloarthropathy patients [52]. Regarding data from intraocular studies, the results are mixed. One study did not detect elevated IL-23 in patients with HLA-B27 uveitis analyzing aqueous samples [53]. On the other hand, a proteomic study of vitreous samples from patients with posterior uveitis identified increased IL-23 [54].

Even if uveitis is more common among psoriasis patients with positivity for HLA-B27 and psoriatic arthritis, uveitis can occur in psoriasis without arthritic involvement [55,56]. These findings are supported by other studies [57,58].

Chi C-C et al. [59] concluded that the patients with severe psoriasis without psoriatic arthritis and the patients with mild psoriasis with psoriatic arthritis had an increased risk of incident uveitis. Also, they did not find that patients with mild psoriasis without psoriatic arthritis would have an increased risk for incident uveitis. The higher prevalence of uveitis in patients with psoriatic arthritis patients in comparison with those who suffer only from psoriasis was attributed to the higher frequency of the HLA-B27 seen in patients with PsA [60].

Uveitis is associated with a worse quality of life and greater functional disability [61]. The acute debut of ocular symptoms or signs which could indicate an acute uveitis in a psoriasis patient has to represent a clear indication for an ophthalmological consultation.

Left undiagnosed or untreated, uveitis can lead to posterior synechiae, trabeculitis, pupillary block, macular edema, disc edema [35] or complicated cataract. The risk factors for visual loss are male gender, the presence of posterior synechiae at onset, the use of corticosteroid-sparing therapy or periocular corticosteroid injections, poor control of ocular inflammation, and a chronic course of the disease [62].

Retinal involvement

Regarding retinal involvement of psoriasis, it has been shown that psoriasis patients had a significantly higher risk of developing retinal detachment, retinal vascular occlusion (both arterial and venous), and retinopathy than controls [63].

Psoriasis was suggested itself as a risk factor for retinal vein occlusion regardless of metabolic syndrome [64]. This association relies on two pathologies shared by psoriasis and retinal vein occlusion: metabolic syndrome, considered a risk factor for both psoriasis and retinal vein occlusion [65,66], and homocysteinemia, commonly observed in both psoriasis and retinal vein occlusion patients [67] [68]. Impairment of the retinal vasculature has been demonstrated in several systemic inflammatory diseases, even in the absence of clinical signs of ocular involvement [69,70]. This is why, Castellino N. et al. [71] assessed retinal vascular in psoriasis patients using optical coherence tomography angiography. They showed lower values of vascular density in both superficial and deep capillary plexuses in psoriasis patients compared to controls. Furthermore, another study showed evidence of retinal peripheral vascular leakage in patients with moderate to severe psoriasis [72].

Also, it has been described that birdshot retinopathy can occur in the course of psoriasis [30]. Within posterior uveitis, retina could be affected [35]. Regardless of the cause of posterior uveitis, including psoriasis, retinal involvement could embrace diverse clinical entities such as chorioretinitis, retinochoroiditis, or retinitis [35].

Retinal sensitivity can also be affected in psoriasis [73]. In a study it has been found a positive correlation between disease severity measured through the PASI score and the mean deviations in standard achromatic perimetry and short-wavelength automated perimetry and between disease severity and the corrected pattern standard deviation and pattern standard deviation of short wavelength automated perimetry [73].

As for the electrophysiology function of rods and cones, Shoeibi N. et al. [74] showed that the mean rod response b-wave, standard combined a- and b-waves, single-flash cone response b-wave, and the 30-Hz flicker amplitudes were significantly lower in psoriasis patients than the normal population.

Other ocular manifestations

Because of the inflammatory general status caused by psoriasis, episcleritis can occur in conjunction with psoriasis [2]. Another ocular manifestation described in the literature was a case of posterior scleritis in a patient with psoriasis masquerading as acute angle closure [75]. Also, a case of orbital myositis in a child with psoriasis and juvenile psoriatic arthritis was described by Ajitsaria et al. [76].

Regarding cataract formation, it is not yet known clearly if psoriasis per se causes cataracts, although there are several studies that reported that patients with psoriasis have a higher incidence of cataract development [20,77,78].

The process of cataract formation among patients with psoriasis may be triggered rather by the treatments used in psoriasis [20]. Since this study does not address the side effects of treatments used in psoriasis, we will mention two treatments widely used in psoriasis, which have a well-known link to cataract formation: corticosteroids and psoralen ultraviolet radiation therapy. The systemic use of corticosteroids is associated with the development of posterior subcapsular cataracts [20]. Secondly, psoralen ultraviolet radiation therapy may develop anterior cataracts, because light in the wavelength range of 300-400 nm is absorbed in the lens and may cause photochemical changes in the lens proteins [20].

CONCLUSION

Psoriasis is a complex, multifactorial disease, but most importantly, it is a systemic inflammatory disease, raising its potential to affect ocular structures. Although psoriasis can affect any ocular structure, the exact pathological mechanism of the onset of these conditions is not known. Apart from the postulation of an increased epithelial turnover of the epithelial cells within the meibomian ducts that would cause MGD, the other eye diseases such as uveitis, retinopathies, conjunctivitis, episcleritis still do not present an exact explanation of the increased incidence in psoriasis. On the other hand, although the eye diseases of psoriasis are diverse, more frequent, and more severe than in the general population, they often do not lead to visual disability and have a relatively good prognosis.

Apart from acute episodes of uveitis, retinal detachment, or occlusion of retinal vessels that require urgent ophthalmological care, the rest of the ocular manifestations of psoriasis have an insidious course but, left untreated, lead to a decrease in the quality of life and can become seriously complicated. Therefore, in this context, the ophthalmologist must be included in the multidisciplinary team in all cases of psoriasis patients.

Thus, psoriasis is defined as a disease with multiple associated morbidities, and along with concerns about the relationship with neoplasms [79] or with the adipose-hepatic axis [80], correlations with eye diseases must remain at the top of our concerns.

Conflicts of interest and sources of funding

The authors declare that there are no conflicts of interest, financial or otherwise, related to the materials presented herein. The authors declare no conflicts of interest.

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Authors' contribution

Conceptualization (MAV, SAA, DOC, LMM); methodology (MP, LMM, DOC); software (ACC); validation (DOC); formal analysis (ACC, LMM, DOC); investigation (MAV, SAA); resources (MAV, SAA, MP); data curation (MP, ACC, LMM, DOC); writing – original draft preparation (MAV, SAA); writing – review and editing of manuscript (MAV, SAA, ACC, DOC); visualization (MP, LMM); supervision (LMM, DOC); project administration (DOC). All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

The Role of Ultrasonography in Dermatology

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Abstract: Ultrasonography (US) is one of the most widely utilized imaging methods in contemporary medical practice. Unlike other imaging techniques primarily within the radiology domain, ultrasonography has been adopted by various medical and surgical specialties and tailored to the specific needs of each. This adaptability has contributed to ultrasonography's rapid diversification and remarkable evolution over recent decades. Ultrasonography was first employed in dermatology in 1979 when Alexander et al. used it to measure skin thickness. However, due to the limitations of conventional ultrasound devices at the time, which had resolutions and frequencies below 15 MHz, detailed elements of the skin could not be analyzed, tempering enthusiasm for the technique [2]. Modern dermatologic ultrasonography began with the development of fixed-frequency devices operating above 20 MHz, enabling detailed visualization of skin structures. Numerous studies on skin physiology and pathophysiology were conducted using this approach. Modern ultrasonography enables us to visualize skin structures, at times surpassing the detail provided by traditional anatomical dissections in educational settings. Given that patient safety and quality medical care remain fundamental priorities, ultrasonography, with its safety profile and versatility, represents the next step in modern dermatology. Ultrasonography represents a rapidly evolving diagnostic tool that holds the promise of significantly improving diagnostic accuracy and the quality of medical dermatology practice.

Keywords: skin, ultrasonography, high frequency

HISTORY

Ultrasonography (US) is one of the most widely utilized paraclinical methods in contemporary medical practice. Unlike other imaging techniques primarily within the radiology domain, ultrasonography has been adopted by various medical and surgical specialties and tailored to the specific needs of each. This adaptability has contributed to ultrasonography's rapid diversification and remarkable evolution over recent decades.

In its early days, the first ultrasound devices were massive machines requiring dedicated rooms, with images generated in A-mode (the earliest form of ultrasound imaging) as simple linear traces. Today, we use miniature devices, including wireless probes that connect to smartphones or tablets, delivering high-resolution images. Furthermore, numerous new ultrasound modalities have emerged, such as Doppler, pulsed Doppler, e-flow, power Doppler, and harmonic mode for contrast-enhanced ultrasonography. Simultaneously, many probes are available: linear, convex, cardiac, intraoperative, and hockey stick probes, among others [1–3].

Ultrasonography was first employed in dermatology in 1979 when Alexander et al. used it to measure skin thickness. However, due to the limitations of conventional ultrasound devices at the time, which had resolutions and frequencies below 15 MHz, detailed elements of the skin could not be analyzed, tempering enthusiasm for the technique [2].

Modern dermatologic ultrasonography began with the development of fixed-frequency devices operating above 20 MHz, enabling detailed visualization of skin structures. Numerous studies on skin physiology and pathophysiology were conducted using this approach. However, fixed-frequency devices had limited penetration depth and lacked capabilities such as color Doppler. Additionally, skin thickness varies based on anatomical location and pathology. For instance, the dermis on the dorsal trunk can reach up to 43 mm, which can hinder the detection of deeper lesions using fixed-frequency probes [2,3].

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Modern ultrasonography equipment with variable frequencies exceeding 15–22 MHz was developed to address these challenges. These devices offer features such as power Doppler, color Doppler, and small, easy-to-use probes designed for examining complex anatomical regions like nails, the face, or the ears [1].

ULTRASONOGRAPHY OF NORMAL SKIN

Ultrasound imaging of the skin results from the interaction between acoustic waves and the skin's layers, which reflect these waves differently.

Broadly, the epidermis appears as a hyperechoic line due to its high keratin content, while the dermis is less hyperechoic because of its high collagen concentration. The subcutaneous tissue consists of hypoechoic fat lobules separated by hyperechoic collagen septa [4].

Doppler ultrasound, including pulsed and power Doppler, assess vascular patency and organ perfusion. Based on vascular characteristics, it is possible to differentiate benign from malignant lesions [4]. Furthermore, blood supply can be monitored to assess the treatment response and inflammatory pattern. A limitation of Doppler ultrasonography is its reduced sensitivity in detecting blood flow in certain cases [2].

Contrast-enhanced ultrasonography allows visualization of microcirculation and neovascularization, aiding in cases where Doppler sensitivity is low. Parameters such as blood flow distribution, filling dynamics, and flow velocity assist in distinguishing between benign and malignant lesions [2].

Originally developed for evaluating hepatic fibrosis, shear wave elastography has recently shown promise in assessing fibrotic changes across various organs and pathological conditions. Its utility has extended to inflammatory skin diseases, such as hidradenitis suppurativa, as well as connective tissue disorders, where it enables differentiation between active inflammatory stages and chronic fibrotic phases, thereby enhancing diagnostic accuracy and disease staging [2].

Three-dimensional ultrasound, made possible through advanced imaging software, allows for the precise reconstruction of cutaneous structures in three dimensions. This technology provides a detailed visualization of both lateral and deep margins of lesions, enabling accurate mapping of their extent. By offering a comprehensive understanding of the spatial relationship between the lesion and surrounding tissues, it serves as a valuable tool for preoperative planning, ensuring well-defined excision margins and optimizing surgical outcomes [2].

There are two essential requirements for performing cutaneous ultrasonography: a color Doppler ultrasound device with a linear or compact linear probe of variable frequency, operating at ≥15 MHz, and an operator specialized in dermatological pathologies with expertise in ultrasonography [3].

As the emission frequency of an ultrasound probe increases, penetration depth decreases. Therefore, two probes with different frequency ranges may be necessary to visualize both the skin and deeper layers.

The axial spatial resolution of ultrasonography is superior to that of other imaging techniques, such as MRI or CT. For example, a 15 MHz probe has an axial resolution of 100 µm, while a 70 MHz probe has an axial resolution of 30 µm. By comparison, a 3.0 T MRI offers a resolution of 400 µm, and a 7.0 T MRI achieves a resolution of 100 µm. This parameter is crucial for studying the most superficial structures, as detecting small structures such as hair follicles is impossible with MRI or CT.

In addition to technical advantages, ultrasonography is cost-effective compared to MRI or CT, involves no radiation exposure, and can be repeated as frequently as necessary without hesitation or restrictions [3].

Ultrasound examination typically consists of a clinical evaluation followed by grayscale imaging, color or power Doppler ultrasonography, spectral curve analysis of vascular lesions (pulsed Doppler), elastography for detecting fibrosis, and contrast-enhanced ultrasonography for assessing the vascularization of cystic or tumoral formations [3].

CLINICAL APPLICATIONS OF CUTANEOUS ULTRASONOGRAPHY

Grounded in these principles, the clinical applications of this imaging modality have expanded to encompass the differentiation of benign from malignant cutaneous tumors, precise localization and detection of subcutaneous implants, evaluation of traumatic injuries and foreign bodies, and assessment of cutaneous vascular malformations. Furthermore, it plays a critical role in monitoring cutaneous changes in systemic diseases, guiding preoperative planning and intraoperative procedures, assessing treatment response, and reducing complications associated with aesthetic interventions [3].

Doppler ultrasonography is especially valuable for evaluating inflammatory processes, detecting neovascularization, and identifying vascular malformations [4].

Conventional B-mode imaging is employed to evaluate tumor morphology, including lateral and deep margins relative to surrounding tissues (muscle, cartilage, or bone), contours, echogenicity, and structure. Tumor thickness is measured along the axis of greatest depth, from just below the hyperechoic keratin band (epidermis) to the deepest invasion margin. Tumor thickness, particularly the Breslow index in melanomas, is a critical prognostic factor that informs therapeutic strategies. Studies have shown a correlation between ultrasound depth measurements and the Breslow index, underscoring ultrasonography's utility in preoperative planning and staging of malignancies [4,5].

In addition to delineating tumor structures, ultrasonography (US) provides evidence of their benign or malignant nature using Doppler mode and contrast-enhanced ultrasonography. Vascular findings suggestive of malignancy include hypervascularity, vascular disorganization, and irregular blood flow patterns with peripheral or mixed distribution, increased vascular velocity, and the presence of vascular pedicles. Furthermore, contrast-enhanced ultrasonography assesses tumor vascularization, starting with the arterial phase, followed by the venous phase, maximum blood supply, wash-up time, drainage time, and distribution patterns in the area of interest. According to published data, malignant tumors exhibit a heterogeneous filling pattern during contrast-enhanced ultrasonography, with significantly increased values during the wash-out phase [4,5].

Ultrasound can also be used to evaluate the responses to topical therapies in cases of actinic keratoses and superficial basal cell carcinomas.

In the case of hidradenitis suppurativa, ultrasonography has proven to be highly valuable, providing objective data for disease staging, enabling the calculation of severity scores, and monitoring disease activity with greater accuracy than clinical examination. Recent studies have also demonstrated its utility in therapeutic decision-making by tailoring treatments based on the degree of inflammation and disease activity visualized ultrasonographically, elements that are difficult to assess with the naked eye. Additionally, treatment responses can be more easily evaluated, providing quantifiable arguments for continuing or modifying therapy. Thus, ultrasonographic investigation is strongly oriented toward patient-centered care and improving quality of life.

Abscesses and cysts can be readily evaluated and diagnosed using ultrasonography. Moreover, ultrasonography guides therapeutic decisions by providing detailed imaging that helps determine the most appropriate approach, whether it involves performing drainage procedures or opting for conservative management [1].

Ultrasound evaluation of plantar wart extension and monitoring treatment response in HPV infections may appear trivial but can become a standard approach in selected cases.

Recently, the use of shear wave elastography has proven beneficial in examining patients with systemic sclerosis. In a study involving 59 patients and 104 plaques of morphea, Wortsman et al. demonstrated that ultrasonography has a sensitivity of 100% and a specificity of 98.4% in distinguishing between lesions in the inflammatory phase and those in the sclerotic phase. Subsequent efforts have been made to standardize results for monitoring treatment response [6].

In pediatric dermatology, ultrasonography is used to monitor treatment responses for infantile hemangiomas and vascular malformations in newborns, employing Doppler mode [7].

Evaluating subungual formations and diagnosing their nature is emerging as a valuable application, considering the limited alternatives for visualizing the nail bed and surrounding structures that are not directly observable [4].

The numerous clinical applications of ultrasonography and its ability to provide objective diagnostic information have increasingly captured the attention of dermatologists.

Although histopathological examination remains the gold standard for diagnosis, its invasive nature limits the ability to comprehensively and dynamically examine the skin [3]. Today, with the advancement of non-invasive skin evaluation techniques, we are entering a new era that promotes non-invasive diagnostic and therapeutic methods. For patients who refuse skin biopsy or for pediatric patients, ultrasonography is an accessible, cost-effective, and minimally invasive investigation that can guide diagnosis.

ADVANTAGES OF ULTRASONOGRAPHY IN DERMATOLOGY

Numerous advantages of ultrasonography have been defined in recent years, with the most relevant being:

1. Real-time and Safe Imaging. Ultrasonography can be performed safely at any time, even in children and pregnant women, allowing for repeated imaging to track lesions and vascularization without exposure to ionizing radiation.

2. High Resolution Across Layers. Modern ultrasonographic systems enable the identification of changes across both superficial and deep layers. The equipment also allows the examiner to adjust the depth of imaging depending on the clinical needs.

3. Comprehensive Cutaneous Visualization. Unlike methods such as dermoscopy or optical coherence tomography (OCT), ultrasonography allows examination of cutaneous structures at any depth. For example, dermoscopy provides a maximum penetration depth of 200–250 µm, while OCT reaches 1.5–2 mm. With OCT, structures beyond the penetration depth of light appear blurred or black, limiting its utility in evaluating the deeper margins of tumors.

4. Whole-Skin Examination. Unlike histopathological examination, which is restricted to a single tissue sample, ultrasonography facilitates the examination of the entire skin. This is particularly beneficial for diagnostic support, disease staging, and monitoring inflammatory disease activity.

5. Absence of Radiation Exposure. Ultrasonography uses sound waves, making it a radiation-free and safe imaging modality.

6. Accessibility for Patients with Implants. Unlike MRI, ultrasonography is safe for patients with pacemakers or metal implants.

7. Superior Axial Spatial Resolution. Ultrasonography's spatial resolution surpasses that of CT or MRI, making it an indispensable tool for evaluating fine superficial structures such as hair follicles.

8. Versatile Anatomical Access. Ultrasonographic probes can be applied to any anatomical region, regardless of shape or contour, enabling the visualization of otherwise inaccessible areas [3].

LIMITATIONS OF CURRENT TECHNOLOGY AND PRACTICE

While ultrasonography has significant advantages, it is not without limitations. Lesions smaller than 0.1 mm at 15 MHz or 0.03 mm at 70 MHz remain challenging to visualize. Furthermore, intraepidermal lesions like macules (e.g., lentigines or café-au-lait spots) and melanin are not easily detected [7,8].

Artifact interference, such as shadowing in hyperkeratotic lesions, can also limit diagnostic accuracy. Additionally, the efficacy of the method is heavily dependent on the operator's expertise. Evaluators must have advanced dermatological knowledge and specific ultrasonographic training to interpret findings accurately.

USE OF ULTRASONOGRAPHY IN AESTHETIC MEDICINE

According to statistics from the American Society of Plastic Surgeons, minimally invasive cosmetic procedures have increased by 174% over the past 20 years. Concurrently, there has been a global rise in reported complications following injectable and non-injectable aesthetic procedures.

As a result, there is a growing interest in utilizing high-frequency ultrasound (US) as the first-line imaging modality for assessing patient anatomy in the field of aesthetic medicine. Ultrasound enables a virtual dissection of skin layers, providing detailed visualization of the arterio-venous vascular network and its relationship with surrounding tissues. Additionally, most dermal fillers can be visualized using ultrasonography.

In cases of complications following aesthetic procedures, ultrasonography offers precise information about the nature of the complication, the skin layers involved, and the identification of previously injected fillers or materials. Moreover, it can guide the injection of hyaluronidase or the drainage of fluid collections.

Ultrasound is increasingly becoming an essential tool for managing aesthetic patients by offering high-resolution imaging and the ability to assess any cutaneous region. Furthermore, its global availability adds to its practicality. The literature in this field continues to advance, with a recently published book guiding aesthetic practitioners in this emerging domain [9].

ARTIFICIAL INTELLIGENCE IN CUTANEOUS ULTRASONOGRAPHY

With advancements in ultrasonography technology, it is now possible to obtain increasingly detailed information about the skin. Alongside other non-invasive tools such as dermoscopy, optical coherence tomography (OCT), and confocal microscopy, ultrasonography serves as a critical bridge between clinical examination and histopathological assessment.

Cutaneous ultrasonography remains a relatively new interdisciplinary field with vast potential for development. Despite its late introduction into dermatology, the rapid pace of current technological progress highlights the enormous potential for integrating artificial intelligence (AI) with this modality. The simultaneous development of AI tools and the training of dermatologists can transform AI into a reliable software solution with a high degree of accuracy, minimizing diagnostic errors.

The evolution of ultrasonography in skin exploration may allow dermatology to align with other specialties, as AI-driven advancements are adopted alongside medical personnel training.

TRAINING OPPORTUNITIES IN ULTRASONOGRAPHY

Over the past decade, several books have been published on cutaneous and aesthetic ultrasonography, providing foundational knowledge for practical training. In addition to these resources, the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) and the American Institute of Ultrasound in Medicine (AIUM) have developed specialized training courses for professionals in this field.

In this context, the new dermatology residency curriculum has introduced a module on cutaneous imaging, which holds significant potential for development in the coming years [3].

CONCLUSION

Modern ultrasonography enables us to visualize skin structures, at times surpassing the detail provided by traditional anatomical dissections in educational settings. Given that patient safety and quality medical care remain fundamental priorities, ultrasonography, with its safety profile and versatility, represents the next step in modern dermatology.

As the technique continues to mature, emphasis should be placed on:

- Standardization of techniques to ensure consistent and reliable imaging practices.

- Expanding professional training through residency programs and specialized courses.
- Clear classifications and develop AI integration to enhance diagnostic precision and reduce inter-operator variability

Ultrasonography represents a rapidly evolving diagnostic tool that holds the promise of significantly improving diagnostic accuracy and the quality of medical practice in dermatology.

Conflicts of interest and sources of funding

The authors declare that there are no conflicts of interest, financial or otherwise, related to the materials presented herein. The authors declare no conflicts of interest.

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Authors' contribution

Conceptualization (AMR, SAC); methodology (AMR); software (SAC, ACC); validation (DOC); formal analysis (AMR, SAC, DOC); investigation (AMR, SAC); resources (DOC, ACC); data curation (DOC); writing – original draft preparation (AMR, SAC); writing – review and editing of manuscript (AMR, DOC, ACC); supervision (DOC); project administration (DOC). All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

Correlation Between Rectal Cancer and AB0 Blood Groups - Myth or Reality?

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Abstract: AB0 blood group type has been linked with different types of cancer. For rectal cancer, there isn't enough data to assess whether such risk exists. We conducted a retrospective study to evaluate the association between ABO blood type and risk of susceptibility to development, progression, or protection against rectal cancer. We analyzed the medical records of 690 patients with rectal cancer from "Prof. Dr. Alexandru Trestioreanu" Oncological Institute of Bucharest during 8 years of follow-up. Data were scraped using Python. For analysis, we used the Chi-square test. The blood group count was A (287, 41.6%) followed by 0 (250, 36.2%), B (32, 4.6%), and AB (121, 17.5%). There are no differences in the female and male subgroups regarding blood type and the lack of evidence for the null hypothesis rejection was shown using the χ2 test statistic ($\chi^2 = 2.1$, d.f. 3, $p = 0.55$ for males and $\chi^2 = 2.9$, d.f. 3, $p = 0.4$ for females). These findings are consistent with the notion that even if AB0 blood type is a risk factor for many types of cancer, there is no specific association between rectal cancer and blood group type.

Keywords: AB0 blood group, rectal cancer, oncology, risk factors

INTRODUCTION

The rise of computing power and the development of machine learning algorithms allowed extensive data mining, which led to new possibilities for research from large unused data sources such as electronic medical records. This fact might provide some surprising interactions between patient's features. Along with this trend of high throughput data mining in medicine, new ethical issues appeared since there is a risk of data leakage, loss of anonymization, and in some cases, unscrupulous use of such information [1].

The AB0 blood group systems consist of four antigens (0, A, B, and AB), discovered by Karl Landsteiner [2,3]. The AB0 antigens were found in the protein and lipid membrane components of various cells (most importantly, erythrocytes) and soluble blood group substances [4]. The scientific literature shows important evidence of the involvement of the ABO blood group system in certain diseases, increasing the risk of developing bleeding or thrombotic events, influencing the susceptibility to different pathogens implicated in infectious diseases with different locations (digestive, urinary, etc.), or the association of a certain AB0 blood type with a certain malignancy [5–8]. The mechanisms by which the AB0 blood group plays a role in development and progression can be a dysregulation of the enzymatic activity of the AB0 glycosyltransferases or the association between polymorphisms at theAB0 gene locus and circulating levels of tumor necrosis factor-alpha, soluble intercellular adhesion molecule, E-selectin and P-selectin contributing to alterations in the host inflammatory system [9].

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Several studies describe the association between the individual`s blood group and the risk of developing certain malignancy risk, like leukemia, gastrointestinal cancer, pancreatic cancer, head, and neck cancer. Different blood groups are associated with different risks for head and neck cancer, and people with blood group A are at higher risk of developing head and neck cancer, followed by those with blood groups B, AB, and 0.

In patients with acute leukemia, and sometimes in aplastic anemia, A and B antigens commonly decrease until they are undetectable; as the patient's condition improves, the antigens increase again to their former levels, this loss of antigens may not be due to deficiency in transferase synthesis or activity, but instead may be due to an inhibitory factor related to antigen-antibody binding, or an abnormal distribution or density of antigen sites in the RBC membrane.

Patients with leukemia had significantly lower expression of A, B, or H antigens, between 17% and 37%, when compared to healthy controls; of A, B, or AB patients with myeloid malignancies, 55% had reduced expression of A or B antigens, and 21% of 0 patients had reduced H antigens when compared with healthy controls of the same AB0 genotype. The incidence of cancers of the gastrointestinal tract was statistically significantly different among different AB0 blood types. Compared with blood type A, individuals with non-A blood types were at an approximately 20% lower risk of cancer of the gastrointestinal tract [10–14].

Colorectal cancer ranks third in terms of incidence, and second in terms of mortality, linked to risk factors like obesity, diet, lack of physical activity, tobacco use, moderate-to-heavy alcohol use, and diabetes mellitus [15–17]. The cornerstones of therapy are surgery, neoadjuvant radiotherapy (for patients with rectal cancer), adjuvant chemotherapy (for non-metastatic rectal cancer), and a "wait and see" approach in case of complete remission after neoadjuvant therapy (inferior rectal cancer). 5-year relative survival ranges from greater than 90% in patients with stage I disease to slightly greater than 10% in patients with stage IV disease [18–20].

Blood transfusion in surgery for rectal cancer, along with other factors, can be associated with the risk of developing postoperative infectious complications and tumor recurrence. Few studies evaluate the risk of rectal cancer among different blood groups; therefore, we analyzed to check if there is a susceptibility of the AB0 blood group type that can be associated with the risk of developing, progression, or protection against rectal cancer [21–23].

MATERIALS AND METHODS

In a retrospective study, we analyzed electronic records of cancer patients admitted at "Prof. Dr. Alexandru Trestioreanu" Oncological Institute of Bucharest between 2013 and 2021.

The mining script searched all the entries in the hospital's electronic database using proprietary coding for each patient. These codes weren't saved in the final data set. We extracted blood group type, age, gender, and cancer diagnosis. From these records, we pooled rectal cancer patients and compared them with the general oncologic population for blood group differences. Statistical analysis was performed in Excel and R Studio ("ggplot2", "mosaic", and base libraries) using contingency tables, Chi-square test, and mosaic plots. For statistical significance, we chose p-value < 0.05.

RESULTS

We analyzed the records of 18 734 patients, 5152 males (27.5%) and 13582 females (72.5%), with a mean age of 65 (23-101), a mean age for males of 67 (23-100), and 65 (23-101) for females. Regarding blood type, the most frequent was type A (44.4%), followed by 0 (33.7%), B (17.2%), and AB (4.7%) for the entire cancer-analyzed population. We observed 690 rectal cancer patients (3.8%). In contingency Table 1, we summarize the frequencies for each blood type in relation to rectal cancer patients and other types of cancer. (Table 1).

Table 1: Contingency data for blood-type in rectal vs other cancer-type patients

Blood group	Other cancer type (%)	Rectal cancer (%)	Total (%)
A	8033 (44.5)	287 (41.6)	8320 (44.4)
AB	854 (4.7)	32 (4.6)	886 (4.7)
B	3101 (17.2)	121 (17.5)	3222 (17.2)
0	6056 (33.6)	250 (36.2)	6306 (33.7)
Total	18044	690	18734

The differences between the proportions of the two groups were not significant irrespective of blood type and the lack of evidence for the null hypothesis rejection was shown using the $\chi 2$ test statistic ($\chi 2=49.94$, d.f. 39, $p = 0.11$). The residuals are shown in Figure 1.

Similarly, no differences were found in the male and female subgroups ($\chi 2 = 2.1$, d.f. 3, $p = 0.55$ for males and $\chi 2 = 2.9$, d.f. 3, $p = 0.4$ for females). From Figure 2, we can see that the number of patients per group differs a lot, while the residuals are similar between the two groups.

Figure 1: Mosaic plot with the rectal and non-rectal patients grouped by blood type. Residuals are shown on the right

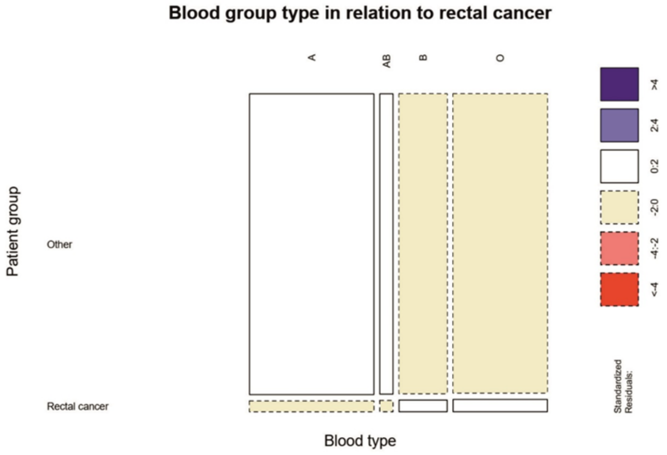

Figure 2: Mosaic plot showing (a) the male group and (b) the female group

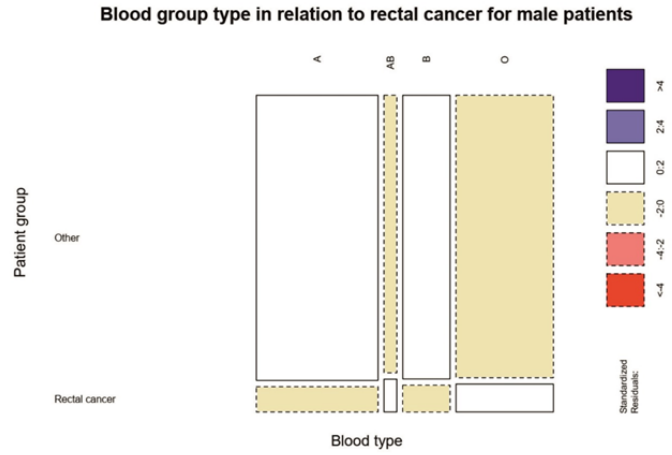

2a.

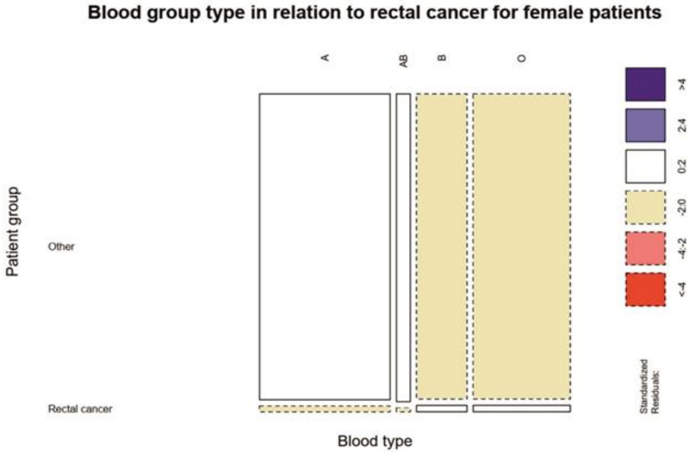

2b.

DISCUSSIONS

AB0 blood group type has been associated with the risk and survival for several malignancies. Regarding head and neck cancers, there is a relative risk of various HNSCCs amongst different blood groups [24,25].

A voluminous literature has accumulated on the role of blood type A and gastric cancer. Gastric cancer is one of the most common malignant tumors of the digestive system. Substance A in the saliva and gastric juices can act on the gastric mucosa, leading to an increased likelihood of a malignant change in the gastric mucosa. The individuals with blood group 0 showed a significantly reduced risk of gastric cancer compared with non-0 blood groups. The susceptibility of blood group A individuals to gastric cancer may be partially attributed to an increased risk of H. pylori infection. Impaired with the healthy population, the frequency distribution of gastric cancer patients with the A blood group was significantly increased, whereas the frequency distribution of gastric cancer patients with the AB blood group was significantly decreased. However, there was no significant difference in the distributions of the B blood group and 0 blood group. The risk of gastric cancer in people with the A blood group was higher, whereas the risk of gastric cancer in people with the AB blood group was lower. There was no significant difference in the risk of gastric cancer between type B and type 0 patients). The AB0 blood group was not related to pathological factors, including the size of the gastric tumor or the T stage or N stage of the disease. Univariate analysis results showed that the degree of differentiation, tumor size, T stage, lymph node metastasis, and type 0 blood were factors affecting the 5-year survival rate of gastric cancer patients. Multivariate analysis results showed that tumor size, T stage, lymph node metastasis, and 0 blood group were independent prognostic factors. The 5-year survival rate for gastric cancer was significantly better in patients with type 0 blood [26–29].

The prospective cohort study found that participants with blood groups A, AB, and B were more likely to develop pancreatic cancer compared with participants with blood group 0. A recent genome-wide association study (GWAS) among pancreatic cancer cases and controls (PanScan) found that several single nucleotide polymorphisms (SNPs) at the AB0 gene locus were among the most statistically significant associations with pancreatic cancer risk. AB0 blood group alleles might provide additional risk information and provide supportive evidence for the role of AB0 glycosyltransferase specificity in pancreatic tumorigenesis. AB0 blood group alleles represent a common, partially penetrant genetic determinant for pancreatic cancer. Chronic inflammation is a predisposing factor for pancreatic carcinogenesis; pancreatic cancer induces a strong desmoplastic reaction that acts as an abundant source of inflammatory mediators, supporting tumor growth and metastases. Interestingly, two recent GWAS suggest that AB0 blood group antigens may affect the systemic inflammatory state. NPs at the AB0 locus were associated with two serum markers of inflammation, tumor necrosis factor-alpha (TNF-α) and soluble intercellular adhesion molecule 1 (sICAM-1). TNF-α is a pro-inflammatory cytokine known to modulate rates of pancreatic ductal cell apoptosis, while plasma levels of sICAM-1 are associated with the risk of incident diabetes a known predisposing factor for pancreatic cancer. These results raise the possibility that blood group antigens may alter the systemic inflammatory state, thereby influencing the risk of developing pancreatic cancer [30].

Blood groups AB and B were associated with a borderline significant increased incidence of ovarian cancer, the magnitude of the association was similar for blood group AB and blood group B, suggesting that the B antigen may influence ovarian carcinogenesis. While the mechanism for an association between blood group and ovarian cancer risk is unknown, several possible explanations exist.

Blood group antigens are expressed even on the surface of gastrointestinal, bronchopulmonary, skin, and urogenital epithelial cells, including some cells of the ovarian surface epithelium and ovarian inclusion cysts. Alterations in glycosyltransferase specificity may be particularly important for ovarian cancer, supporting the role of the immune response in the association between blood group and ovarian cancer risk. Metabolic reprogramming represents an important characteristic of cancer cells that helps them survive in nutrient- and oxygen-deprived environments, proliferate, and metastasize in different body sites [31]. The observed interaction with BMI also suggests a possible immune-mediated mechanism, as the stronger positive associations with blood groups AB and B among overweight women may be related to higher levels of systemic inflammation in these women. It is possible that the AB0 gene may be in linkage disequilibrium with another gene that influences ovarian cancer risk [32]. Blood type B is associated with the risk of prostate and bladder cancer and could be evaluated as a determinant in the negative association with longevity [33].

Although blood group antigens can increase the risk of developing many forms of malignancies, this is not the case in breast, liver, or lung cancer. There are no significant associations between self-reported blood type and incidence of all (invasive plus in situ) cancers, invasive cancers, ductal cancers, and ER+/PR+ cancers, and there was no clear evidence of an association for the less common subtypes; in addition, there was no significant association for Rh factor or presence versus absence of the A or B antigen and incidence of all breast cancers. No significant differences were found among genotypes AA, B0, BB, and AB, or blood groups B and AB, used meta-analysis to explore the relationship between AB0 blood groups and the risk of liver cancer, the proportion of type 0 in patients with liver cancer was lower than that of healthy subjects, but no significant differences between patients with liver cancer and patients with hepatitis or cirrhosis were observed.

Smoking and male gender were significantly associated with lung cancer risk, whereas no significant association between AB0 blood type and lung cancer risk was observed, except for the known etiological and risk factors that are associated with lung cancer development, no association was observed between those that were examined [34–36]. Similar to other malignancies not associated with the blood type group, individuals with rectal cancer have no statistically significant relation with blood group type. The studies of Slater G. et al. and Khalili H.et al. conducted on consistent populations of patients diagnosed with rectal cancer demonstrate that there is no difference in the association between rectal and blood group type [37,38].

Some studies suggested the involvement of AB0 blood type antigens in the development and metastasis of colorectal cancer, cell surface H blood group of a highly tumorigenic clone contribute to tumor progression, and that the motility and proliferation of tumor cells are closely associated with antigen A expression, and so have importance in colorectal tumor malignancy. The molecular mechanism of deletion/reduction vs. continuous expression of A antigen in a tumor is a crucial determinant of human tumor malignancy. Deletion of A transcript in A- cells was not due to gene deletion, since Southern blot analysis showed an equal presence of genomic DNA regardless of A- vs. A+ (SW480 or HT29) or B+ (KATOIII) tumor cells. Two transcriptional control mechanisms leading to differences in A expression in SW480 cells are indicated. Luciferase assay in A- and A+ SW480 cells showed that promoter activities of segments of the 5' flanking sequence of the AB0 gene reflected transcript levels in these cell lines. The enhancing activity of a 43 bp tandem repeat unit located between -3899 and -3618 was reduced in A- compared to A+ cells. ii. Distinct differences in the pattern of CpG dinucleotide methylation were found in A- vs. A+ cells. Therefore, the methylation process of A promoter DNA may be another important factor controlling A activity in SW480 tumor cells. Since proliferation and motility of tumor cells are associated closely with

A expression, the transcription control mechanism for the expression of A transferase, as described above, may be of crucial importance in defining human tumor malignancy. The expression of carbohydrate ABH blood group antigens is regulated through oncologic progress, and their presence in tumor cells constitutes a prognostic factor. However, it is not clear whether they directly affect tumor behavior. Using a rat model of colon carcinoma, an association between the presence of H blood group antigens and tumorigenicity in syngeneic animals was observed. Through immunoprecipitation experiments was demonstrated that cell surface H blood group antigens of a highly tumorigenic clone (PROb) are essentially carried by splice variants of the CD44 molecule containing exon V6. PROb cells were then transfected with an antisense fragment of the gene coding for a rat alpha (1-2) fucosyltransferase. This enzyme allows the synthesis of H antigens from various beta-galactoside precursors. Transfected subclones of PROb cells were obtained, which had significantly decreased enzymatic activity and H antigenic cell surface levels. In contrast, no such changes were observed in control cells transfected with either the empty vector or with a sense fragment of the gene. Compared to controls, the antisense-transfected cells were far less tumorigenic in syngeneic animals. These results show that H blood group antigens at the surface of PROb colon carcinoma cells contribute to tumor progression. The presence of the fucosylated structures on CD44 could modulate the functions of this adhesion molecule [39,40].

Despite this rationale, in our large prospective study cohort of 18734 aged between 23 and 101, from which 690 cases are with rectal cancer, we did not find a consistent association between blood group type and overall risk of rectal cancer. Regardless of gender, blood type A is the most frequently common in patients with rectal cancer, followed by blood type 0, but comparing other cancers with rectal cancer, we noticed no differences in taking into account the blood group type. These findings are consistent with the notion that even if ABO blood type is a risk factor for many types of cancer, there is no specific association between rectal cancer and blood group type.

These findings contrast with our previous analysis of the AB0 blood group and risk of head and neck cancer using these same population cohorts in which we demonstrated that blood group B is associated with an increased incidence of hypopharyngeal cancer, whereas for the oral cavity was associated lower incidence and blood antigen A is associated with a higher risk of oral cavity cancer development, independent of B blood antigen.

Therefore, patients with blood group 0 diagnosed with laryngeal carcinoma had almost 10% shorter 3-, 5-, and 10-year OS rates compared to the median OS of the patient population [41]. Assessing the potential risks of the AB0 blood groups for the development of various head and neck cancers through odds ratio, it was found that blood group A had the highest potential of developing oral cancer. The probable reason behind this could be that in people belonging to A and B blood Groups, the precursor H antigen is converted to A and B antigen, increasing the risk for the development of oral cancer since H antigens are a protective factor for oral cancer. In 0 blood group individuals, it remains in its original form, and thus, people with blood group 0 have the highest amount of H antigen, which affords protection against oral cancer. Another genetic factor found consistently associated with oral cancers is the p56 gene mutation. The altered antigen pattern on the surface is a tumor-associated change resulting in malignancy. It is also possible that the observed associations are not due to the blood group antigens themselves but to the effects of genes closely associated with them. These results have been subsequently supported by findings from other head and neck cancer cohorts. The relative risk of various head and neck cancers among different blood groups: an analytical study [42,43]. Nonetheless, further research is needed, into a potential, more modest effect of AB0 blood group type on rectal cancer.

Most recent studies indicate a correlation between AB0 blood type and colorectal cancer risk [44–46]. Some large cohort studies have evaluated the influence of AB0 blood groups on the prognosis of colorectal cancer patients. The results indicate significant differences in survival rates based on blood type, highlighting its potential role in patient outcomes [45–47]. Many other recent studies have found links between cancer risk and the AB0 group. Bahardoust et al. demonstrated a significant association between blood group A and increased colorectal cancer risk [48]. Similarly, Al-Sawat et al. reported that individuals with blood group 0 had a lower risk of colorectal cancer [46]. Furthermore, insights from Rashid et al. regarding genetic predispositions linked to blood types underscore the biological mechanisms that may contribute to these observed risks [49]. Jodat et al. highlighted the impact of lifestyle factors, suggesting that environmental influences may further modify the risk associated with specific blood groups [50].

In the current study, we relied on patient-informed consent, which is the norm for our institution. However, this poses some risks because each entry from a patient file is now available in their electronic record. In an oncology setting, this can offer the possibility of having better follow-up and electronic graphs with trends for each blood parameter, type of treatment, dose, and so on. The cost is the risk of data leakage and loss of anonymity, and we believe that we should make new forms of consent in which we inform about these risks and offer the possibility to opt-out at any time from potential studies

CONCLUSION

The current literature data suggest that AB0 blood type is a risk factor for developing and has an impact on survival in many forms of cancers. However, few studies evaluate the relationship between the AB0 blood group and rectal cancer. The results of our study show that different blood groups, both in female and male populations, are not raising the risk of developing rectal cancer.

We propose that current informed consent for research be updated with information regarding the risk of using such data records in research with the possibility of opt-out.

Conflicts of interest and sources of funding

The authors declare that there are no conflicts of interest, financial or otherwise, related to the materials presented herein. The authors declare no conflicts of interest.

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Authors' contribution

Conceptualization, M.T.G. and A.D.M..; methodology, D.E.G. and S.S.I.I.; writing—original draft preparation, I.A.V..; writing—review and editing, A.G. and B.D.G..; supervision, B.S.A; correspondence, A.C. and A.M.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of "Bucharest Oncology Institute" number 5665/25.04.2021 for studies involving humans.

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ORIGINAL ARTICLE

Investigating the antimicrobial effect of bacteriocin and biosurfactant produced from Lactobacillus rhamnosus on the biofilm of Escherichia coli and Staphylococcus aureus

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Abstract: Bacterial resistance to antibiotics is considered the most important health problem in developed and developing countries. Studies on new pharmaceutical strategies, such as probiotics and their derivatives, have increased. The purpose of this study is to isolate and purify bacteriocin and biosurfactant from Lactobacillus rhamnosus and investigate its antibacterial and anti-biofilm activity. Bacteriocin and biosurfactant produced by Lactobacillus rhamnosus standard strain were isolated and purified. Biosurfactant screening tests, including oil expansion test and drop spreading test, were conducted. Bacteriocin was separated using two different methods, including separation with the help of 2-propanol and separation with the help of ammonium sulfate. The purification of bacteriocin was realized by dialysis, and the effect of biosurfactant and bacteriocin on Staphylococcus aureus and Escherichia coli was realized using the disc diffusion method. The antibacterial properties of these substances were determined by diffusion method from its anti-biofilm disk with the help of the ELISA reader. Statistical Program for Social Sciences (SPSS) software was utilized for data analysis. The diameter of the non-growth halo of E.coli in contact with the disc impregnated with bacteriocin in concentrations of 250, 500, and 1000mg/ml was 21 ± 1, 21.33 ± 2.3 and 34.66 ± 7.02 mm, respectively, and in Staphylococcus aureus was evaluated as 18.33 ± 3.78, 24.33 ± 4.72 and 43.66 ± 4.72 mm, respectively. The order was evaluated as 18.33 ± 2.88, 25 ± 5, 43.33 ± 5.77 mm. The biofilm inhibition percentage was measured for Escherichia coli 0.36, 2.15, and 71.75%, respectively, and for Staphylococcus aureus 1.07%, 5.96%, and 53.3%, respectively, at the mentioned concentrations. The results showed that bacteriocin and biosurfactant isolated from Lactobacillus rhamnosus have high antibacterial and anti-biofilm properties.

Keywords: Lactobacillus rhamnosus, Escherichia coli, Staphylococcus aureus, biofilm, bacteriocin, biosurfactant, bacteriocin

INTRODUCTION

In this era, bacterial resistance to antibiotics is considered the most important health problem in developed and developing countries. Antibiotic resistance is a phenomenon in which bacteria become resistant to the effects of antibiotics and make the treatment of infections caused by these bacteria difficult or impossible [1]. Antibiotic resistance can arise from a variety of mechanisms, including preventing drug access to the bacterial site of action, changes in the structure and protection of antibiotic receptors, and direct modification or inactivation of antibiotics. Excessive and incorrect use of antibiotics has led to antibiotic resistance, which is a growing global health crisis [2]. According to the currently available studies, antibiotic resistance in Staphylococcus aureus (and Escherichia coli strains has increased significantly in patients but also in preclinical populations. Today, the resistance of different strains of these bacteria against penicillin, gentamicin, clindamycin, tetracycline, and vancomycin has been reported in various studies [3,4]. Therefore, due to the increase in antibiotic resistance, as well as the high cost of producing new antibiotics, the lack of new antibiotics entering the market, and environmental problems, there is an urgent need to produce, discover, design, and increase the availability of alternative drugs for antibiotics around the world [5,6].

Probiotics and their derivatives are among the alternative medicines that have been studied recently. Until today, the antibacterial effects of these alternative agents have been studied on many antibiotic-resistant bacteria, including Escherichia coli, and the results of these studies have been very promising. It has also been found that these factors are very effective in treating various diseases, such as necrotizing enteritis, periodontitis, and diarrhea resulting from bacterial infection [7,8]. Also, the favorable effects of probiotics on the body, including improving immune system function, reducing inflammation, improving digestion, reducing the risk of bacterial diarrhea, and improving cardiovascular system function, and their significant positive effects in livestock and poultry have attracted scientists' attention to

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probiotics and their derivatives [9,10]. Lactobacillus is a genus of the Lactobacillaceae family. Lactobacilli are the most important food fermenters and are used in most cases as part of the natural flora or as starter cultures. This genus of bacteria produces lactic acid along with Leuconostoc, Pediococcus, Lactococcus, and Streptococcus, and for this reason, they belong to lactic acid bacteria or LAB. The size of lactobacilli varies in different species. Lactobacilli have different nutritional requirements for vitamins, amino acids, etc. Gram-positive bacilli are catalase-negative, lack cytochrome oxidase, their metabolism is fermentative, and they can survive in the presence of oxygen; because they have peroxidase activity to inactivate hydrogen peroxidase, they are therefore microaerophilic or facultative anaerobes. Most lactobacillus species are non-motile and have peripheral flagella when motile. They are present in the digestive system of healthy humans and reduce the acidity of the environment by converting sugar into lactic acid and are a small part of the intestinal flora [11]. Lactobacilli produce various substrates with antimicrobial properties, such as hydrogen peroxide, organic acids, inhibitory enzymes, antimicrobial compounds, bacteriocin, and biosurfactants [12]. Lactobacilli are also used in the pharmaceutical industry to produce pharmabiotics, which are probiotics used for therapeutic purposes. In addition, lactobacilli are used in the dairy industry to produce fermented foods such as cheese and yogurt [13,14]. They are also used as a biological preservative due to their strong antimicrobial activity against many microorganisms [12]. Lactobacillus rhamnosus is a probiotic bacterium of the Lactobacillus genus that has been studied for its potential health benefits. For example, Lactobacillus rhamnosus GG is a strain of Lactobacillus rhamnosus that has been shown to have good therapeutic properties and can be used as a spray in spray devices [13]. Lactobacillus rhamnosus is one of the four probiotics that have been studied to limit their growth in different environmental conditions. This study showed that Lactobacillus rhamnosus is able to tolerate a wide range of stressful conditions. This feature of heat tolerance is one of the prominent features of this bacterium [15]. Also, a controlled clinical trial showed that probiotic supplementation in early pregnancy with Lactobacillus rhamnosus HN001 may reduce the incidence of gestational diabetes [16]. The therapeutic properties of this bacterium do not end here, as Lactobacillus rhamnosus Lcr35 is a strain of Lactobacillus rhamnosus that is used to treat diarrhea and prevent vaginitis [17].

Bacteriocins are peptides or bacterial proteins that are synthesized in the ribosome and have antimicrobial properties [18]. Bacteriocins perform their antimicrobial action by binding to the surface receptors of the target bacteria, and their function depends on the strain. Destruction of target bacteria occurs as a result of metabolic, morphological, and biological changes caused by bacteriocins [19].

Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of cell membranes by various microorganisms. Commercially, biosurfactants are of special importance due to their use in various industries such as petroleum, petrochemical, food, pharmaceutical, cosmetic, agriculture, textile, papermaking, leathermaking, etc. For this reason, the microorganisms that produce these compounds are suitable candidates for the expansion of biosurfactant production due to their high surface-to-volume ratio and diverse biosynthetic capacity [1].

In medicine, swarming movement and biofilm formation are important actions in surface colonization by bacteria and increase the possibility of infections. Biosurfactants are produced to prevent pathogenic organisms from sticking to solid surfaces or to infection sites. Surfactin reduces the amount of biofilm formation. Accordingly, this study is conducted to isolate and purify bacteriocin and biosurfactant from Lactobacillus rhamnosus and investigating its antibacterial and anti-biofilm activity [2].

MATERIALS AND METHODS

The standard strains of Escherichia coli (ATCC25922), Staphylococcus aureus(ATCC25923), and Lactobacillus rhamnosus PTCC1607 (ATCC7469) were lyophilized from Iran's Fungi and Bacteria Collection Center, Iran Scientific and Industrial Research Organization.

Biosurfactant extraction method from Lactobacillus rhamnosus

Bacterial biosurfactant was prepared according to the method of Emmanuel et al. [19]. First, the bacteria were cultured on MRS agar medium and incubated for 48h at 37°C. Then, the single colonies created were transferred to MRS Broth medium and cultured. After 24h of incubation at 37°C, the tube containing bacteria was centrifuged at 8000 rpm at 4°C for 30 min. The supernatant was collected and analyzed for biosurfactant separation [20].

Biosurfactant screening tests

- Oil expansion test

In order to perform the oil spreading test, one milliliter of oil was added to the surface of 20 milliliters of distilled water. Then, 0.5 ml of bacterial supernatant was added to it. The formation of a clear area is a sign of the presence of biosurfactants in the supernatant [19].

- Drop spreading test

To test the droplet spreading, the first 25µl of biosurfactant was added as a drop on the parafilm, and the droplet of the desired substance was observed to spread on the parafilm. Similarly, 25µl of water was added to the parafilm, and changes were observed. Due to the low surface tension, the biosurfactant spreads and flattens on the parafilm while the water remains unchanged [19].

Production, extraction, and purification of biosurfactant

The supernatant obtained after centrifugation was collected, and its pH was adjusted to 2 using normal HCL 6. The supernatant was kept overnight at 4°C. An equal amount of mixture of chloroform: methanol (1:2) was added to the supernatant and mixed. Then, the resulting mixture was kept overnight for phase separation. Three layers were formed in the desired phase mixture - the lower layer is chloroform: methanol, the middle layer is biosurfactant, and the upper layer is the supernatant. The formed biosurfactant (middle layer) was carefully removed with the help of a micropipette and transferred to appropriate microtubes. Then 1 ml of distilled water was added to the microtubes containing biosurfactant and vortexed completely. This mixture was centrifuged at 7000 rpm for 30 minat 4°C. The supernatant was discarded and the obtained pellet was dried for 24h and separated from the supernatant as a precipitate [20].

Bacteriocin extraction method from Lactobacillus rhamnosus

Bacteriocin extraction was performed according to the protocol of Anjana et al. [21]. First, the desired strain was inoculated in an MRS Broth culture medium (with a pH of 4) and incubated at 37°C for 48h at 150 rpm in order to reduce the pH. The inhibitory activity of organic acids produced in the fermentation medium was neutralized by 4N sodium. To remove bacterial cells, it was centrifuged at 5000rpm for 15 min. The supernatant now contains bacteriocin, the activity of which was examined. [22].

Production, extraction, and purification of bacteriocins

In this study, the bacteriocin was separated using two different methods:

- Separation with the help of 2-propanol

In this method, first, the resulting supernatant was mixed with cold 2-propanol (isopropyl alcohol) 50% by volume in the vicinity of ice. Then, the resulting mixture was centrifuged at 4 degrees with 15000rpm for 40 min. Finally, the sediment obtained was dissolved in citrate phosphate buffer (final concentration 0.1 M and pH 4), and its bactericidal activity was investigated [22].

- Separation with the help of ammonium sulfate

In this method, first, the obtained supernatant was precipitated with the help of ammonium sulfate at 4 degrees Celsius. The resulting sediment was centrifuged with the previous conditions. Then, the precipitate was dissolved in potassium phosphate buffer (final concentration 0.06 M and pH 7), and finally, its bacteriocin activity was checked [22].

Purification of bacteriocin by dialysis method

The sample obtained from the previous step was dialyzed for 48h with potassium phosphate buffer (0.06 M and pH=7, at 4 degrees Celsius), and then using Nessler's reagent, the removal of ammonium ions from the solution was proven. Bacteriocin activity was evaluated against the biofilm of sensitive strains.

The effect of biosurfactant and bacteriocin on Staphylococcus aureus and Escherichia coli by disc diffusion method

For this purpose, first, 250, 500, and 1000mg of the obtained biosurfactant were dissolved by dimethyl sulfoxide with a concentration of 0.1% (1000 µl/liter), and the bacteriocin obtained was dissolved by potassium phosphate buffer (final concentration of 0.06 M and pH 7). Three different concentrations of 250, 500, and 1000 mg/ml were prepared from each of them. Then, uniform paper discs with a diameter of about 5 mm were prepared and then sterilized. Then 0.5 McFarland concentration was prepared from Staphylococcus aureus and Escherichia coli. With the help of the sterile swap and grass cultivation method, the desired pathogens were cultured on Müeller Hinton agar culture medium. After cultivating the desired bacteria on the Müeller Hinton agar culture medium, sterile paper discs impregnated with 50 µl of biosurfactant or bacteriocin were placed in certain places on the Müeller Hinton agar with the help of forceps. The desired plates were examined after incubation at 37 degrees Celsius for 24 hours in order to form a halo of non-growth around the paper discs [23].

The effect of biosurfactant and bacteriocin on the formation of biofilm of Staphylococcus aureus and Escherichia coli by 96-well microtiter plate method

The 96-well plate was used to perform this test. At first, the overnight culture of standard Staphylococcus aureus and Escherichia coli was prepared in TSA culture medium enriched with 0.2% glucose, and then the resulting single colonies were prepared in TSB culture medium enriched with 0.2% glucose to prepare a suspension with light absorption. 0.1 was used at 625 nm wavelength.

In the 96-well plate, one row was assigned to Escherichia coli and one row to Staphylococcus aureus. For each row, one column was assigned to the positive control (containing 200 µl of bacterial suspension) and one column to the negative control of 200 µl of TSA culture medium enriched with 0.2% glucose. Also, 100 µl of bacterial suspension was added to the column of each row and 100 µl of different concentrations of bacteriocin and biosurfactant (250, 500, and 1000 mg/ml) were added to each column. Then, the 96-well plate was transferred to the incubator and incubated for 24h at 37°C.

After the incubation period, the contents of the wells were aspirated with the help of a sampler, then re-aspiration of the wells was done first with the help of PBS buffer and then with sterile physiological serum. In the next step, 150 µl of pure methanol was added to the wells and aspirated after 10 min.

In the next step, 200 µl of 1% crystal violet was added to each well, and after 20 minutes, was aspirated, and the plates were washed with tap water. After drying the plate in the vicinity of free air, 150 µl of 33% glacial acetic acid was added to each well, and with the help of an ELISA reader, the optical absorption of the plate was measured at a wavelength of 625 nm.

In order to calculate the biofilm inhibition percentage by bacteriocin or biosurfactant, the formula ({100 × (optical absorbance of positive control well/optical absorbance of wells containing bacterial suspension and bacteriocin or biosurfactant)}-100 = inhibition percentage) was used (Figure 1) [24].

Figure 1: Preparation of 96-well plate to perform the anti-biofilm test of biosurfactant and bacteriocin produced by Lactobacillus rhamnosus

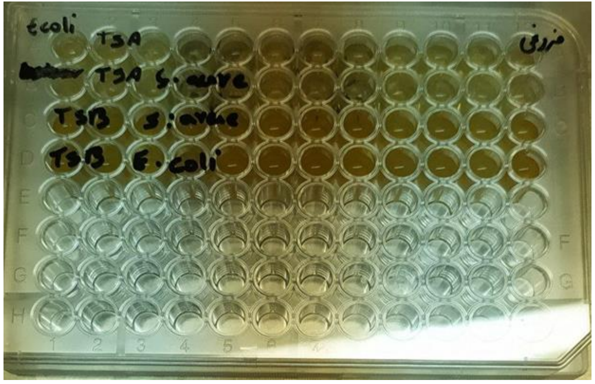

Statistical analysis

Statistical Program for Social Sciences (SPSS) software, version 17 (SPSS Inc., Chicago, IL, USA), was utilized for data analysis. All the data of this study were expressed as mean ± standard deviation. Also, one-way analysis of variance and Tukey's test were used for data analysis.

RESULTS

Oil spreading test results to prove the presence of biosurfactant

Based on the results of the oil spreading test, adding biosurfactant to the oil caused the oil to move and form a transparent area of about 2 cm in the oil (Figure 2).

Figure 2: Effect of biosurfactant on oil displacement

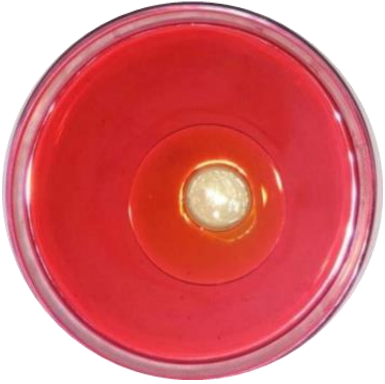

Drop spreading test results to prove the presence of biosurfactant

The results of this test showed that the biosurfactant was spread and smoothed, which indicated the low surface tension of the biosurfactant, while the water drop did not move (Figure 3). The results of this test together with the results of the previous test show the correct screening of biosurfactant.

The liquid containing 3 phases, according to Figure 4, was used to purify the biosurfactant. Biosurfactant pellets were also obtained based on the above method.

Antibacterial properties of biosurfactant on standard bacteria

In the present study, the antibacterial properties of biosurfactants extracted by the disk diffusion method were investigated. Based on the creation of a non-growth halo around the discs containing biosurfactant, it was concluded that this substance has antibacterial properties. The diameter of the halo of non-growth around the disks by the separation of Staphylococcus aureus and Escherichia coli (Figure 5) studied, and the series carried out is included in Tables 1, 2, and 3.

Figure 3: Drop spreading test results

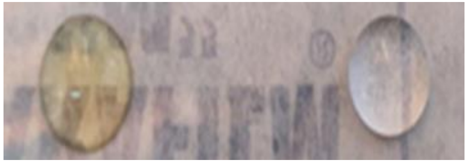

Figure 4: Three phases formed in order to purify biosurfactant

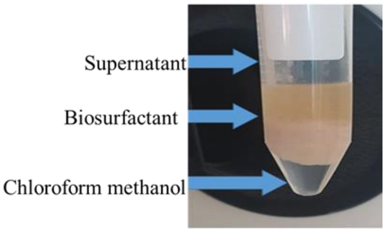

Figure 5: Diameter of non-growth halo in Staphylococcus aureus and Escherichia coli in contact with biosurfactant-coated discs

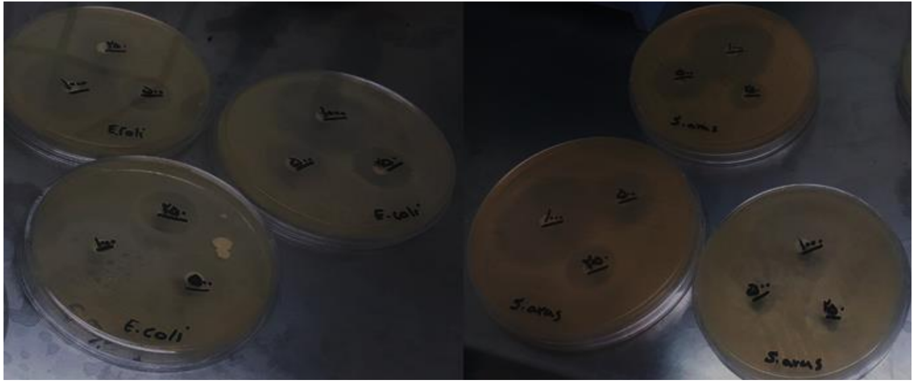

Table 1: The diameter of the halo of non-growth of Staphylococcus aureus due to contact with discs containing biosurfactant

The halo diameter in the third series	The halo diameter in the second series	The halo diameter in the first series	Biosurfactant concentration (mg/ml)
15 mm	20 mm	20 mm	**250**
20 mm	25 mm	30 mm	**500**
40 mm	40 mm	50 mm	**1000**

Table 2: The Diameter of the halo of non-growth of Escherichia coli due to contact with discs containing biosurfactant

The halo diameter in the third series	The halo diameter in the second series	The halo diameter in the first series	Biosurfactant concentration (mg/ml)
21 mm	20 mm	20 mm	**250**
20 mm	25 mm	20 mm	**500**
40 mm	35 mm	30 mm	**1000**

Table 3: The diameter of the halo of non-growth of Staphylococcus aureus due to contact with discs impregnated with bacteriocin

The halo diameter in the third series	The halo diameter in the second series	The halo diameter in the first series	Bacteriocin concentration (mg/ml)
14 mm	21 mm	20 mm	**250**
19 mm	26 mm	28 mm	**500**
38 mm	42 mm	49 mm	**1000**

Anti-biofilm properties of biosurfactant

The results of reading the 96-well plate and evaluating the inhibition percentage of biofilm formation showed that this substance has good anti-biofilm properties. The results of the reading of the ELISA device and the inhibition percentage of biofilm formation are included in Table 4.

Antibacterial activity of lactobacillus rhamnosus bacteriocin

The diameter of the non-growth halo around the discs impregnated with bacteriocin was measured as an indication of bacteriocin's antimicrobial activity. The diameter size of each halo of non-growth of Staphylococcus aureus and Escherichia coli is included in Table 3 and Table 5, respectively. Also, the images of the diffusion test results from the growth disk of Staphylococcus aureus and Escherichia coli can be seen in Figure 6, respectively.

Table 4: Percentage inhibition of biofilm formation by biosurfactant

Escherichia coli	Staphylococcus aureus	Biosurfactant concentration (mg/ml)
0.36	1.07	**250**
2.15	5.96	**500**
71.75	53.3	**1000**

Table 5: The diameter of the halo of non-growth of Escherichia coli due to contact with discs impregnated with bacteriocin

The halo diameter in the third series	The halo diameter in the second series	The halo diameter in the first series	Bacteriocin concentration (mg/ml)
22 mm	20 mm	21 mm	**250**
20 mm	24 mm	20 mm	**500**
42 mm	34 mm	28 mm	**1000**

Figure 6: Diameter of non-growth halo in Staphylococcus aureus and Escherichia coli in contact with bacteriocin-coated discs

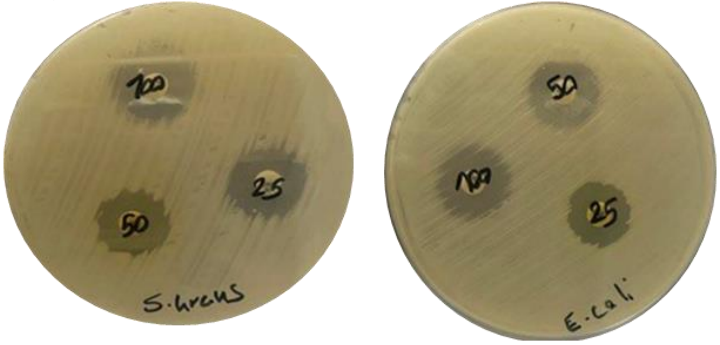

Anti-biofilm properties of bacteriocin

By examining the results obtained from the reading of the ELISA device, it was found that Lactobacillus rhamnosus bacteriocin has anti-biofilm properties; these results on biofilm inhibition by bacteriocin are included in Table 6.

Table 6: Biofilm inhibition percentage by different dilutions of bacteriocin

Escherichia coli	Staphylococcus aureus	Biosurfactant concentration (mg/ml)
5.06	7.74	**250**
46.32	16	**500**
76.65	58.9	**1000**

DISCUSSIONS

In this study, the biosurfactant and bacteriocin of Lactobacillus rhamnosus extract and their antibacterial and anti-biofilm properties were investigated in dilutions of 250 and 1000 mg/ml. The diameter of the halo of non-growth of Escherichia coli in contact with the disc impregnated with bacteriocin in concentrations of 250, 500, and 1000 mg/ml was 21 ± 1, 21.33 ± 2.3 and 34.66 ± 7.02 mm, respectively, and in Staphylococcus aureus was evaluated as 18.33 ± 3.78, 24.33 ± 4.72 and 43.66 ± 4.72 mm, respectively. The biofilm inhibition percentage of this material was for Escherichia coli in the three mentioned concentrations, respectively, 5.06, 46.32, and 76.65, and for Staphylococcus aureus in the three mentioned concentrations, respectively, 7.74, 16, and 58.9. Also, in this study, the diameter of the non-growth halo resulting from the diffusion test for biosurfactant around Escherichia coli in three different concentrations of 250, 500, and 1000 mg/ml was 20.33 ± 0.57, 66 ± 2.88, 21.21 and 35 ± 5 mm, respectively and in Staphylococcus aureus, it was evaluated as 18.33 ± 2.88, 25 ± 5, and 43.33 ± 5.77, respectively. Also, the percentage of biofilm inhibition was measured for Escherichia coli as 0.36, 2.15, and 71.75% in the three mentioned concentrations, and for Staphylococcus aureus, 1.07%, 5.96%, and 53.3%, respectively. Based on the obtained results, it was found that the biofilm inhibition effect of bacteriocin and biosurfactant at a concentration of 1000 mg/ml on Escherichia coli was higher than on Staphylococcus aureus. Therefore, bacteriocin and biosurfactant of the desired bacteria have antibacterial and anti-biofilm properties against standard bacteria Escherichia coli and Staphylococcus aureus. The anti-biofilm properties of biosurfactant and bacteriocin extracted in the present study are quite promising and remarkable based on the obtained results. These results are consistent with the results of different studies that have reported anti-biofilm properties.

The results of the study conducted by Leslie et al. in 2021 show that bacteriocin isolated from Lactobacillus plantarum has antibacterial properties against Staphylococcus aureus [25], which is consistent with the present study. Also, the antibacterial activity of class III bacteriocin from Lactobacillus acidophilus against Staphylococcus aureus and Escherichia coli was also reported by Meng et al. in 2021 [26]. Also, in the study of Mohapatra et al. in 2021, the anti-biofilm property of bacteriocin obtained from Lactobacillus plantarum bacteria was investigated on Staphylococcus aureus and Pseudomonas aeruginosa. The results of this study indicated that this bacteriocin has anti-biofilm properties against these bacteria [27]. Inhibition of Escherichia coli biofilm formation by bacteriocin BM1157 was evaluated by Luo et al. in 2021. Based on the findings of the mentioned study, this bacteriocin has the ability to inhibit the biofilm formation of this bacterium [28]. Also, according to recent studies, bacteriocin obtained from Lactobacillus bovis can also inhibit the formation of Escherichia coli biofilm [29]. Escherichia coli biofilm inhibition is not limited to bacteriocins derived from lactobacilli. According to the study conducted by Shanks et al. in 2012, the bacteriocin isolated from Citrobacter freundii also has the ability to inhibit the formation of Escherichia coli biofilm [30]. In addition to Staphylococcus aureus and Escherichia coli, the anti-biofilm properties of different bacteriocins have also been performed on other bacteria. In a study conducted by Menif et al. in 2013, the results proved that the biosurfactant produced by Bacillus subtilis SPB1 has antimicrobial activity and can be produced and optimized at an industrial level [31]. Also, the antibacterial activity of the biosurfactant produced by Streptomyces cellulase was recently reviewed by Panatola et al. In the mentioned study, the antibacterial property of Streptomyces cellulase probiotic biosurfactant against Escherichia coli and Staphylococcus aureus was reported [32].

The important point in the results of these reports is their agreement with the results of the present study, which shows that biosurfactants can be considered as a potential antibacterial substance. Also, it seems that in addition to Lactobacillus rhamnosus, the biosurfactant of other existing probiotics can also be studied and used.

The anti-biofilm property of this substance is one of the other important things mentioned in the studies. Considering the fact that the formation of biofilm is very effective in the rate of survival, pathogenicity and resistance to antibiotics, obtaining anti-biofilm substances is of double importance [33]. The results of similar studies show that biosurfactants have good anti-biofilm properties. A clear example of this is the study conducted by Ednan et al. in 2023, which shows that the biosurfactant extracted from Lactobacillus acidophilus has a high anti-biofilm effect against Gram-negative pathogenic bacteria such as Pseudomonas aeruginosa, Serratia marcescens, and Chromobacterium violaceum [34].

Meanwhile, Lactobacillus rhamnosus has been studied more than other Lactobacillus in terms of biosurfactant production and its various antibacterial properties. The high ability of biosurfactants obtained from this bacterium to destroy bacteria and its easy engineering for large-scale production has attracted the attention of scientists to this substance extracted from Lactobacillus rhamnosus [35]

The high anti-biofilm biosurfactant property of this bacterium has been investigated in various studies. For example, Lactobacillus rhamnosus glycolipid biosurfactant was extracted, and its anti-biofilm properties were investigated by Patel et al. In the mentioned study, this material had high anti-biofilm properties against Staphylococcus aureus, which is consistent with the present study [35]. In a similar case, the anti-biofilm properties of this substance extracted from Lactobacillus rhamnosus against Enterococcus faecalis were reported by Walried et al. [36]. In the study conducted by Samabantamoorthy et al. in 2014, the biofilm inhibition percentage of Lactobacillus rhamnosus biosurfactant against Escherichia coli and Staphylococcus aureus was reported to be 80 and 93% with a concentration of 25 mg/ml of this substance at a wavelength of 595 nm [37]. Although there is a general agreement between the present study and the study conducted by Samabanthamurthy in terms of understanding the anti-biofilm properties of biosurfactants, the inhibition percentage of these two studies is not the same. This mismatch can be due to the difference in light absorption, the difference in biosurfactant concentration, the type of origin strain, and the amount of biofilm formation by bacteria. The study conducted by Nataraj et al. in 2021 is another example of the effect of Lactobacillus rhamnosus biosurfactant on eliminating biofilm and bacteria.

In this study, the effect of different concentrations of this substance on the biofilm and bacteria of Staphylococcus aureus ATCC25923 (the standard bacterium examined in this study) and Staphylococcus aureus resistant and sensitive to methicillin was investigated. Although the anti-biofilm property of this study is consistent with the current study, the important point is the difference in the diameter of the non-growth state of the present study from the previously approached study, where the diameter of these states was found to be larger.

This difference can be due to reasons such as the difference in Lactobacillus rhamnosus strains (in the mentioned study, the GG strain was exclusively used). The different methods of evaluating the lack of growth halo (in the mentioned study, the diffusion method from the well was used instead of the diffusion from the disk) and the difference in biosurfactant concentration may explain the discrepancies**.**

CONCLUSION

The results of this study showed that bacteriocin and biosurfactant isolated from Lactobacillus rhamnosus have high antibacterial and anti-biofilm properties that can be used as an alternative drug to antibiotics. Due to the increasing antibiotic resistance, it is necessary to use alternative drugs to deal with this phenomenon, such as the one suggested by the results of this study. Also, in the future, these solutions can have potential applications in approaching environmental problems, solving challenges within the food industry, and enhancing the tehrapeutic armamentarium to fight diseases.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere.

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Authors' contribution

All authors participated in the presentation of the original idea and design, search for sources and review of articles, initial writing or revision of the article, and all agreed with the final version of the present article and took responsibility for the accuracy of the content.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

Antibacterial Susceptibility Patterns of Non-Fermentative Gram-Negative Bacilli among Patients at Tertiary Care Hospital, Jaipur

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Abstract: In recent years, infections frequently attributed to Pseudomonas aeruginosa and Acinetobacter baumannii have been seen not only in ICU settings but also in trauma patients following natural disasters, as well as in individuals with pre-existing conditions within the community. These non-fermenting bacteria are known to cause hospital-acquired bloodstream infections, especially in patients who are debilitated or immunocompromised. All non-fermenting Gram-negative bacteria (NFGNB) isolates from different clinical samples received in the clinical microbiology laboratory from outpatients and inpatients at Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, were included in the study. Routine microscopy of these samples was conducted. Gram staining was realized for all samples except for urine. All clinical samples were inoculated on blood agar and MacConkey agar and were incubated at 37°C for 18–24 hours. Colony characteristics were observed. All the organisms that produced pale or colorless colonies on MacConkey agar and also showed GNB on Gram staining of the colonies were considered to be NFGNB and further identified by the VITEK 2 compact system. Antimicrobial susceptibility testing of the isolated non-fermenting Gram-negative bacteria was conducted using the Kirby-Bauer disc diffusion method. In the study, it was reported that the majority out of 879 (100%) isolates of NFGNB were P. aeruginosa 415 (47.21%), followed by Ac. baumannii 380 (43.23%), and the majority of specimens were from endotracheal (ET) (30.60%), followed by pus swab (19.11%), blood (16.15%) and urine (10.01%) samples. The majority of isolates were the males in the age group of 61-70, i.e.121 (13.76%), followed by the age group 41-50, i.e., 113 (12.85%). P. aeruginosa was most commonly isolated from pus swabs (13.42%), while Ac. baumannii was isolated most commonly from ET (21.4%). Based on this study, it can be concluded that as antibiotic resistance rises, treating infections caused by NFGNB will become increasingly challenging unless proactive measures are implemented and new antibiotics are developed. To curb the spread of resistant strains of Pseudomonas aeruginosa and Acinetobacter, it is crucial to enforce stringent infection control practices, ensure collaboration between clinicians and laboratory personnel regarding antibiotic use, and adhere to strict hospital hygiene protocols.

Keywords: Non-fermenting Gram-negative bacteria, Antimicrobial susceptibility, Pseudomonas aeruginosa , Acinetobacter baumannii

INTRODUCTION

Non-fermentative Gram-Negative bacilli (NFGNB) represent a diverse group of aerobic, non-spore-forming bacteria that either do not use glucose as an energy source or metabolize it oxidatively. They account for approximately 20% of all Gram-Negative bacilli (GNB) [1]. These bacteria are typically found as saprophytes in natural environments like soil and water, and they also live as commensals in humans and other animals.

While they are often isolated incidentally, their role as pathogens has become more prominent due to the widespread use of antibiotics and the increasing number of patients with weakened immune systems.

Non-fermentative bacteria (NFs) are increasingly recognized as causes of opportunistic and sometimes severe infections, as well as nosocomial infections [2]. These bacteria are frequently isolated from patients with severe underlying conditions who have undergone extensive use of broad-spectrum antibiotics, prolonged surgical procedures, extended hospital stays, insufficient mechanical ventilation or tracheotomy, genitourinary procedures, or who are burn victims or low-birth-weight infants. Infections caused by these bacteria are commonly observed in vulnerable age groups, including neonates, children, and the elderly [3]. They are also commonly found in cases of septicemia, meningitis, pneumonia, urinary tract infections, and surgical wound infections [4].

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NFGNB can cause a variety of infections and account for approximately 15% of all Gram-negative bacilli isolated from clinical samples [5]. These infections are increasingly problematic in hospital environments, especially among immunocompromised patients [6]. Among the opportunistic pathogens affecting immunocompromised hosts, whether due to disease or treatment, Pseudomonas aeruginosa is especially significant.

Other important species include Acinetobacter baumannii, Pseudomonas fluorescens, Pseudomonas stutzeri, Stenotrophomonas maltophilia, Pseudomonas putida, and Burkholderia cepacia [7]. Rates of colonization by NFGNB increase in hospitalized patients, especially those who have been in the hospital for extended periods or have received broad-spectrum antimicrobial therapy or chemotherapy. These non-fermenters frequently cause nosocomial blood-stream infections, particularly in debilitated and immunocompromised individuals, and are often multidrug-resistant. According to data from the Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) study, approximately one-fourth of Gram-negative bacteremia were attributed to NFGNB [8].

Treating infections caused by these pathogens is challenging due to their multidrug resistance (MDR) and the rapid emergence of high-level MDR to various antibiotic classes, including β-lactams, aminoglycosides, and fluoroquinolones. This resistance complicates both treatment and infection control efforts [9]. Pseudomonas aeruginosa has a high potential for developing resistance to most antibiotics, as evidenced by its genome containing the largest resistance island with over 50 resistance genes. The mechanisms behind this antibiotic resistance include the production of enzymes that degrade or inactivate antibiotics, outer membrane proteins that expel antibiotics, and mutations that alter the antibiotic targets [10].

Production of antibiotic-degrading enzymes, such as extended-spectrum beta-lactamases, AmpC cephalosporinases, carbapenemases, and aminoglycoside-modifying enzymes, has been reported among non-fermentative Gram-negative bacilli [11]. NFGNBs exhibit resistance to many antibiotics and are known to produce extended-spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs) [12]. Non-fermentative Gram-negative bacteria have developed resistance to many commonly used antibiotics, including cephalosporins and carbapenems. This resistance complicates treatment, extends hospital stays, increases mortality rates, and drives up healthcare costs [13].

The aim of our study was to determine the antibacterial susceptibility patterns of non-fermentative Gram-negative bacilli isolated from clinical samples.

MATERIALS AND METHODS

A prospective study was carried out at Mahatma Gandhi Medical College and Hospital (MGMCH), focusing on patients exhibiting signs and symptoms of non-fermenter infections. The study period was conducted from June 2019 to May 2020. All NFGNB isolates were obtained from different clinical samples received in the clinical microbiology laboratory of Mahatma Gandhi Hospital (MGH), Sitapura, Jaipur, Rajasthan, included in the study.

Inclusion Criteria

Non-fermentative Gram-negative bacilli isolated from endotracheal (ET), pus swab, sputum, blood, urine, pus, and other body fluids, etc.

Exclusion Criteria

All Gram-negative bacilli except non-fermentative Gram-negative bacilli from different clinical samples.

Collection, transportation, and storage of specimen

Various types of samples were collected, including urine, blood, pus, discharge from the skin and soft tissue sites, sputum, ET, and other fluids such as Cerebrospinal fluid (CSF), Ascitic fluid, and Pleural fluid. Additionally, miscellaneous swabs (ear, throat, vaginal, wound, etc.), tissue samples, and central line tips were gathered. These samples were received in the clinical microbiology laboratory from both inpatients and outpatients at the hospital. The samples were collected using universal precautions and sterile techniques and were transported to the laboratory promptly under optimal conditions. Detailed patient histories were recorded, including age, sex, any use of indwelling medical devices, and duration of hospital or ICU stays.

After collection, each sample container was carefully labeled with the patient's name, ID number, and other relevant details. The specimens were promptly transported to the laboratory, typically within 1 hour of collection, and processed as quickly as possible. If processing was delayed, the samples were stored at 4°C until they could be analyzed.

Processing of Specimen

Routine microscopy was performed on all samples. Gram staining was carried out for all samples except urine. Wet microscopy was used to detect bacteria and pus cells in urine samples. Each clinical sample was inoculated onto Blood agar and MacConkey agar plates, which were incubated at 37°C for 18-24 hours. Colony characteristics were then observed. Organisms that produced pale or colorless colonies on MacConkey agar and appeared as gram-negative bacilli on gram staining were identified as NFGNB. Further identification was conducted using the VITEK-2 compact system [14]. All NFGNB isolates underwent antimicrobial susceptibility testing using the Kirby-Bauer method according to CLSI guidelines 2020 [15].

RESULTS

In our study, out of 10707 clinical samples, 879 NFGNB isolates were obtained, with a prevalence of 8.20%. Also, out of the 879 NFGNB isolates, 415 (47.21%) were Pseudomonas aeruginosa, followed by Acinetobacter baumannii 380 (43.23%), Burkholderia cepacia 29 (3.30%), Burkholderia pseudomallei 17 (1.93%), Stenotrophomonas maltophilia 16 (1.82%), Acinetobacter lwoffii 7 (0.80%), Pseudomonas putida 6 (0.68%), Acinetobacter coffee 2 (0.23%), Pseudomonas fluorescens 2 (0.23%), Pseudomonas stutzeri 2 (0.23%), A. junii 1 (0.11%), A. haemolyticus 1 (0.11%) and Pseudomonas luteola 1 (0.11%) as shown in Table 1. Out of the total 879 isolates of NFGNB, the majority of the specimens were from ET (30.60%), followed by pus swabs (19.11%), blood (16.15%), and urine (10.01%), as shown in Table 2. Out of the total positive NFGNB cases, the maximum number was from inpatients (IPD) 775 (88.17%), followed by outpatients (OPD) 104 (11.83%). Out of the total 879 NFGNB isolates, the majority of isolates were the males in the age group 61-70, i.e.121 (13.76%), followed by the age group 41-50, i.e.113 (12.85%), as shown in Table 3 and Figure 1.

Table 1: Total No. of isolates obtained from NFGNB

NFGNB spp.	Total No.	Percentage
Pseudomonas aeruginosa	415	47.21%
Acinetobacter baumannii	380	43.23%
Burkholderia cepacian	29	3.30%
Burkholderia pseudomallei	17	1.93%
Stenotrophomonas maltophilia	16	1.82%
Acinetobacter lwoffii	7	0.80%
Pseudomonas putida	6	0.68%
Acinetobacter coffee	2	0.23%
Pseudomonas fluorescens	2	0.23%
Pseudomonas stutzeri	2	0.23%
Acinetobacter junii	1	0.11%
Acinetobacter haemolyticus	1	0.11%
Pseudomonas luteola	1	0.11%
TOTAL	**879**	**100%**

Table 2: Various Clinical specimens included in the study

Sl. No.	Sample	No. of cases	Percentage
1	ET	269	30.60%
2	PUS SWAB	168	19.11%
3	BLOOD	142	16.15%
4	URINE	88	10.01%
5	SPUTUM	87	9.90%
6	CSF	44	5.01%
7	PUS	19	2.16%
8	DRAIN	16	1.82%
9	PLEURAL FLUID	11	1.25%
10	BAL	11	1.25%
11	TIPS	6	0.68%
12	TISSUE	5	0.57%
13	ASCITIC FLUID	4	0.46%
14	OTHER BODY FLUID	3	0.34%
15	SYNOVIAL FLUID	2	0.23%
16	SEMEN	2	0.23%
17	BILE	2	0.23%
	TOTAL	**879**	**100%**

ET: Endotracheal secretion, CSF: Cerebrospinal fluid, BAL: Bronchoalveolar lavage, TIPS: Transjugular Intrahepatic Portosystemic Shunt

Table 3: Distribution of Positive NFGNB isolates with respect to outpatients/inpatients (OPD/IPD)

Distribution of OPD/IPD	Number of Isolates	Percentage
OPD	104	11.83%
IPD	775	88.17%
Total	879	100%

Figure 1: Gender-wise Distribution of Patients

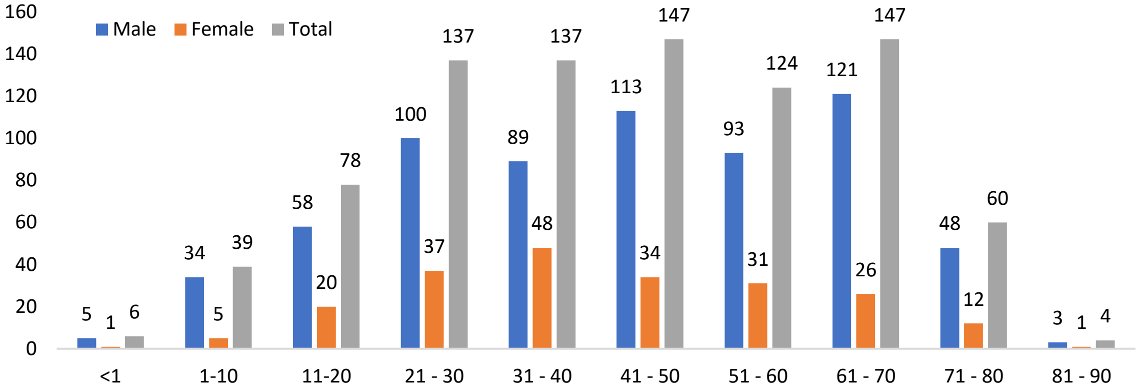

Table 4 shows the distribution of various bacterial isolates from different clinical samples. In this study, P. aeruginosa was most frequently isolated from pus swabs (13.42%), followed by endotracheal samples (8.19%), sputum (7.17%), urine (6.94%), and blood (4.44%). Ac. baumannii was predominantly isolated from ET samples (21.4%), followed by blood (7.85%), pus swabs (4.44%), CSF (3.53%), sputum (2.05%), and urine (1.82%). B. cepacia was most commonly found in blood samples (1.48%), with fewer instances in pus swabs (0.68%), sputum (0.34%), ET samples (0.23%), and other body fluids (0.23%) as shown in Table 4.

Table 4: Distribution of microorganisms on the basis of specimens

SAMPLE	P. AERUGINOSA		A. BAUMANNII		B. CEPACIA	
	N	%	N	%	N	%
ET (n=269)	72	8.19	190	21.6	2	0.23
PUS SWAB (n=168)	118	13.42	39	4.44	6	0.68
BLOOD (n=142)	39	4.44	69	7.85	13	1.48
URINE (n=88)	61	6.94	16	1.82	1	0.11
SPUTUM (n=87)	63	7.17	18	2.05	3	0.34
CSF (n=44)	12	1.37	31	3.53	0	0
PUS (n=19)	14	1.59	2	0.23	1	0.11
DRAIN (n=16)	12	1.37	3	0.34	0	0
PLEURAL FLUID (n=11)	5	0.57	4	0.46	0	0
BAL (n=11)	5	0.57	5	0.57	0	0
TIP (n=6)	5	0.57	0	0	1	0.11
TISSUE (n=5)	3	0.34	2	0.23	0	0
ASCITIC FLUID (n=4)	2	0.23	1	0.11	0	0
OTHER BODY FLUID (n=3)	0	0	0	0	2	0.23
SYNOVIAL FLUID (n=2)	0	0	0	0	0	0
SEMEN (n=2)	2	0.23	0	0	0	0
BILE (n=2)	2	0.23	0	0	0	0
TOTAL	415	47.21	380	43.23	29	3.3

As shown in Table 5, the present findings showed that the resistance ratio of various antibiotics was higher than that of sensitivity against NFGNB. The antimicrobial susceptibility testing conducted on all 879 non-fermenting Gram-negative bacteria revealed the following results: Cefoperazone/Sulbactam showed a sensitivity rate of 37.54%, making it the most effective among the tested agents.

This was followed by Meropenem at 35.26% & Ceftazidime at 34.47%. On the other hand, Ticarcillin/Clavulanic Acid exhibited the highest resistance rate at 90.78%. This was closely followed by Ciprofloxacin with 73.49% resistance, Levofloxacin at 72.81%, and Piperacillin/Tazobactam at 70.19%, as detailed in Table 5.

Table 5: Antibiotic Susceptibility Pattern of NFGNB

S. No.	Antibiotics	Sensitive		Resistance	
		No. of cases	%	No. of cases	%
1	Ticarcillin/Clavulanic Acid	81	9.21%	798	90.78%
2	Piperacillin/Tazobactam	262	29.80%	617	70.19%
3	Ceftazidime	303	34.47%	576	65.52%
4	Cefoperazone/Sulbactam	330	37.54%	549	62.45%
5	Cefepime	288	32.76%	591	67.23%
6	Imipenem	285	32.42%	594	67.57%
7	Meropenem	310	35.26%	569	64.73%
8	Ciprofloxacin	233	26.50%	646	73.49%
9	Levofloxacin	239	27.18%	640	72.81%

Among the 879 NFGNB isolates obtained, P. aeruginosa showed high sensitivity to several antibiotics. Cefepime was the most effective, with 258 out of 415 isolates (62.16%) demonstrating sensitivity. This was followed by Imipenem with 254 isolates (61.20%) and Ceftazidime with 250 isolates (60.24%). In contrast, P. aeruginosa exhibited high resistance rates to Ticarcillin/Clavulanic Acid, affecting 349 isolates (84.09%). The bacteria also showed considerable resistance to Levofloxacin (226 isolates or 54.45%) and Ciprofloxacin (208 isolates or 50.12%).

Out of the 380 total A. baumannii isolates, the highest sensitivity was observed for Cefoperazone/Sulbactam, with 70 isolates (18.42%) showing susceptibility. In contrast, A. baumannii exhibited very high resistance rates to Ticarcillin/Clavulanic Acid (366 isolates, 96.82%), and Ciprofloxacin (363 isolates, 96.03%). For B. cepacia isolates, which numbered 29 in total, the highest sensitivity was to Meropenem, with 19 isolates (65.51%) showing susceptibility. This was followed by Ceftazidime with 14 isolates (48.27%) and Levofloxacin with 11 isolates (37.93%). All 29 B. cepacia isolates were resistant to Ticarcillin/Clavulanic Acid, Piperacillin/Tazobactam, Cefepime, and Imipenem, as shown in Table 6.

Table 6: Antibiotic Susceptibility Pattern of isolated NFGNB Species

ANTIBIOTIC		*P. aeruginosa*		A. BAUMANNII		B. CEPACIA	
		(n=415)		(n=380)		(n=29)	
		N	%	N	%	N	%
Ticarcillin/Clavulanic Acid	R	349	84.09%	366	96.82%	29	100.00%
	S	66	15.90%	14	3.68 %	0	0.00%
Piperacillin/Tazobactam	R	185	44.57%	357	94.44%	29	100.00%
	S	230	55.42%	23	6.05%	0	0.00%
Ceftazidime	R	165	39.75%	363	96.03%	15	51.72%
	S	250	60.24%	17	4.47%	14	48.27%
Cefoperazone/Sulbactam	R	169	40.72%	310	82.01%	28	96.55%
	S	246	59.27%	70	18.42%	1	3.44%
Cefepime	R	157	37.83%	358	94.70%	29	100.00%
	S	258	62.16%	22	5.78%	0	0.00%
Imipenem	R	161	38.79%	358	94.70%	29	100.00%
	S	254	61.20%	22	5.78%	0	0.00%
Meropenem	R	167	40.24%	360	95.23%	10	34.48%
	S	248	59.75%	20	5.3%	19	65.51%
Ciprofloxacin	R	208	50.12%	363	96.03%	28	96.55%
	S	207	49.87%	17	4.47%	1	3.44%
Levofloxacin	R	226	54.45%	359	94.97%	18	62.06%
	S	189	45.54%	21	5.5%	11	37.93%

Resistance and Sensitivity patterns of various antibiotics from IPD & OPD Patients are shown in Table 7. Out of a total positive 879 NFGNB isolates, a maximum number of patients were from IPD 775 (88.17%), followed by OPD 104 (11.83%). The antimicrobial susceptibility pattern varied in both IPD & OPD patients, as results shown in Table 7.

The present results showed that IPD patients manifest high sensitivity to several antibiotics. Cefoperazone/Sulbactam showed a sensitivity rate (33.80%,) making it the most effective among the tested agents, followed by Meropenem (31.48%), and Ceftazidime (30.70%). On the other hand, Ticarcillin/Clavulanic Acid exhibited the highest resistance rate at 92.12%. This was similarly followed by Ciprofloxacin with 76.25% resistance, Levofloxacin (74.70%) & Piperacillin/Tazobactam (74.19%). Among the 104 OPD patients showed high sensitivity to Cefoperazone/Sulbactam with a sensitivity rate of 65.38%, followed by Meropenem (63.46%), Cefepime (63.46%), Ceftazidime (62.5%) against the tested agents and high resistance rates also observed against Ticarcillin/Clavulanic Acid (80.76%) followed by Levofloxacin (58.65%), Ciprofloxacin (52.88%).

Table 7: Antibiotic Susceptibility Pattern of NFGNB Based on IPD/OPD

S. No.	Antibiotics	IPD=775				OPD=104			
		Sensitive		Resistance		Sensitive		Resistance	
		No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
1	Ticarcillin/Clavulanic Acid	61	7.87 %	714	92.12%	20	19.23%	84	80.76%
2	Piperacillin/Tazobactam	200	25.80%	575	74.19%	62	59.61%	42	40.38%
3	Ceftazidime	238	30.70%	537	69.29%	65	62.5%	39	37.5%
4	Cefoperazone/Sulbactam	262	33.80%	513	66.19%	68	65.38%	36	34.61%
5	Cefepime	222	28.64%	553	71.35%	66	63.46%	38	36.53%
6	Imipenem	223	28.77%	552	71.22%	62	59.61%	42	40.38%
7	Meropenem	244	31.48%	531	68.51%	66	63.46%	38	36.53%
8	Ciprofloxacin	184	23.74%	591	76.25%	49	47.11%	55	52.88%
9	Levofloxacin	196	25.29%	579	74.70%	43	41.34%	61	58.65%

DISCUSSIONS

In the current study, a total number of 10707 specimens were received in the microbiology laboratory for culture. Out of these, 879 specimens yielded positive isolates for non-fermenting Gram-negative bacteria (NFGNB). The distribution of these isolates was as follows: Among them, P. aeruginosa, 415 (47.21%) was the most commonly isolated, followed by Ac. baumannii 380 (43.23%), B. cepacia 29 (3.30%), B. pseudomallei 17 (1.93%), S. maltophilia 16 (1.82%), Ac. lwoffii 7 (0.80%), P. putida 6 (0.68%), Ac. coffee 2 (0.23%), P. fluorescens 2 (0.23%), P. stutzeri 2 (0.23%), Ac. junii 1 (0.11%), Ac. haemolyticus 1 (0.11%) and P. luteola 1 (0.11%).

The current study's findings align with those reported by Simgamsetty et al., who isolated 260 NFGNB strains. Of these, 151 (58%) were identified as P. aeruginosa, 94 (36.1%) as Ac. baumannii, 1.92% as B. cepacia, 1.15% each as Ac. lwoffii and P. stutzeri, and 0.38% each as Achromonas xylosoxidans and Sphingomonas paucimobilis [16]. Similarly, other studies by Maniyan et al., Prashant et al., and Mahajan [17-19] reported comparable findings. In the present study, out of a total of 879 NFGNB isolates, the majority were from endotracheal (ET) specimens (30.60%), followed by pus swabs, blood, and urine samples.

The present study's results are similar to those reported by Wadhwa et al., who found that the majority of clinical specimens were from endotracheal (ET) samples (67.44%), followed by tracheal secretions (15.11%), sputum (9.30%), and bronchoalveolar lavage (BAL) (8.72%).

These findings are also consistent with studies conducted by Patel et al. [20,21]. In the present study, of the total 879 positive NFGNB isolates, 775 (88.17%) were from IPD, while 104 (11.83%) were from OPD. The present study is consistent with the findings of Nazir et al., who reported that among 120 NFGNB strains, 91 (75.8%) were isolated from IPD and 29 (24.16%) from OPD. These results align with those of Rashid et al. [22,23]. In the present study, the majority of isolates were from males in the age group of 61-70 years, accounting for 121 (13.76%) of the cases, followed by the age group of 41-50 years, with 113 (12.85%) of the isolates.

The present study's findings are similar to those reported by Mahajan et al., who observed that the majority of patients (35.13%) were in the age group of 40-60 years. Additionally, out of 342 NFGNB isolates, 59.79% were from males and 40.20% were from females. In the study conducted by Nazir et al., males were more commonly affected than females in the age group under 10 years (34.1%), followed by those over 60 years (32.5%) [19,22]. In the present study, P. aeruginosa was most commonly isolated from pus swabs, followed by endotracheal (ET) samples, sputum, urine, and blood. In the study conducted by Rashid et al., 10.5% of P. aeruginosa isolates were obtained from wound swab samples.

A similar study by Sarkar et al. reported that P. aeruginosa was most commonly isolated from pus (58%), followed by urine, stool, and other specimens. This finding is consistent with studies conducted by Mahajan and Madkey [23,24,19,25]. In the present study, Ac. baumannii was most commonly isolated from endotracheal (ET) samples (21.39%), followed by pus swabs, cerebrospinal fluid (CSF), sputum, and urine. These findings are similar to those reported by Madkey et al., who isolated 58.82% of Ac. baumannii from endotracheal (ET) samples, followed by pus, urine, blood, and sputum. This is consistent with other studies conducted by Mahajan et al. and Maniyan et al. [17,19,25]. In the present study, B. cepacia was isolated from blood (1.48%), followed by pus swabs, sputum, and endotracheal (ET) samples. These findings are consistent with reports from other studies, which indicated that 5.00% of B. cepacia isolates were obtained from blood, followed by sputum. Similar results were found in studies conducted by Maniyan, Madkey, and Kalidas [17,25,26].

The extensive use of antibiotics in the hospital environment has led to the selection of strains with resistance to multiple antibiotics. Increased antibiotic resistance of NFGNB associated with increased morbidity and mortality makes the infections they produce a major public health problem. In the present study, resistance to various antibiotics against NFGNB was noted. This finding was similar to Buzila [27], who also reported Acinetobacter spp. presented over 70% resistance to the majority of antibiotics. Three pan-drug-resistant P. aeruginosa strains were identified. The rate of colistin resistance was 2.91% for P. aeruginosa and 3.33% for Acinetobacter spp. Mechanical ventilation and hospitalization in intensive care units are known risk factors for contracting infections with microorganisms that have become resistant to multiple antibiotics. Increased antibiotic resistance of the strains isolated from tracheobronchial aspirate suggests that using such medical devices (endotracheal intubation tubes and urinary catheters) promotes infections with MDR strains. In the present study, among the 879 NFGNB isolates, the highest sensitivity was observed with Cefoperazone/Sulbactam (37.54%), followed by Meropenem (35.26%), Ceftazidime (34.47%). On the other hand, Ticarcillin/Clavulanic Acid exhibited the highest resistance rate at 90.78%. This was closely followed by Ciprofloxacin with 73.49% resistance, Levofloxacin at 72.81%, and Piperacillin/Tazobactam at 70.19%. This is similar to the findings of Bhargava et al. and Sarkar et al., who reported that Cefoperazone/Sulbactam was the most effective antibiotic against NFGNB [28,29].

In the present study, out of a total of 879 NFGNB isolates, P. aeruginosa showed high sensitivity to Cefepime, with 258 isolates (62.16%) being sensitive, followed by Imipenem with 254 isolates (61.20%), and Ceftazidime with 250 isolates (60.24%). This is similar to the study by Malini et al., which found sensitivity to Cefepime in 69 (38.33%) of isolates, followed by Imipenem in 156 (86.67%), and Ceftazidime in 61 (33.39%) of isolates. These findings coincide with those of earlier studies, which also reported that P. aeruginosa is highly sensitive to Cefepime, followed by Imipenem [30]. In the present study, out of 380 Ac. baumannii isolates, the highest sensitivity was observed to Cefoperazone/Sulbactam with 70 isolates (18.42%), In the present study, out of 29 B. cepacia isolates, the highest sensitivity was observed to Meropenem (19 isolates, 65.51%), followed by Ceftazidime (14 isolates, 48.27%), and Levofloxacin (11 isolates, 37.93%). These results are consistent with the findings of Sharma et al., Simgamsetty et al., and Kalidas et al. [31,16,26]. So, to prevent the spread of infections, it is essential to implement strict infection control measures. Clinicians should adhere to established hospital hygiene protocols and guidelines.

Advantages of Study

The study of Pseudomonas aeruginosa and Acinetobacter baumannii is crucial for improving patient outcomes in healthcare settings, especially in high-risk environments like ICUs and burn units. By advancing our understanding of their resistance mechanisms, persistence in the hospital environment, and diagnostic challenges, we can develop more effective strategies to manage infections and reduce the spread of antimicrobial resistance. Research in this area is pivotal for both the clinical management of infections and the developing of new antimicrobial therapies.

Limitations of Study

The study on Pseudomonas aeruginosa and Acinetobacter baumannii is subject to several limitations that may affect the accuracy. Addressing these limitations through broader geographic sampling, more detailed resistance profiling, better control of confounding factors, and more sophisticated study designs can enhance our understanding of these pathogens and improve strategies for managing infections in healthcare settings.

CONCLUSION

Pseudomonas aeruginosa and Acinetobacter baumannii are among the most frequently reported pathogens worldwide, posing significant challenges in healthcare settings. These organisms have become notable for their resistance to multiple antibiotics, making them particularly difficult to control and treat. They are especially problematic in patients admitted to burn units, intensive care units, and wards where central intravenous catheters and respiratory devices are used. These settings are prime environments for these hardy pathogens due to the increased risk of infection associated with invasive procedures and compromised patient conditions. Non-fermenters are significant pathogens responsible for both hospital- and community-acquired infections. NFGNBs are increasingly recognized as major contributors to various nosocomial infections. In our study, P. aeruginosa and Ac. baumannii were identified as the most common NFGNB isolates.

Accurate species-level identification of NFGNB, coupled with regular monitoring of their susceptibility patterns, is crucial for effective infection management. The high rates of isolation and antimicrobial resistance observed are concerning for healthcare professionals. These organisms can persist in hospital environments, underscoring the need for rigorous housekeeping practices, thorough equipment decontamination, and adherence to strict sterilization protocols. Continued research is essential to enhance our understanding of the evolving antimicrobial resistance patterns of these pathogens.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere.

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Authors' contribution

Author SK contributed to the design of experiments, review, discussion, and editing of the manuscript. Author VPM contributed to the manuscript review. Author RS contributed to editing and reviewing the manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee, dated 26/09/2019, MGMC&H/IEC/JPR/2019.

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ORIGINAL ARTICLE

Reliability and Validity Related to the Persian Version of the Trust in Physician Scale (TIPS) Questionnaire

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Abstract: Patients' trust in their physicians is reflected in their steadfast confidence, which encourages greater patient cooperation in treatment, thereby enhancing the prognosis of the disease. We aimed to evaluate the validity and reliability of the Trust in Physician Scale questionnaire (TIPS) by translating it into Persian. After obtaining the main author's permission, the forward-backward translation technique was employed to develop the Persian version. We conducted a cross-sectional study utilizing the TIPS questionnaire among adult patients who presented to the hospital emergency room. This survey involved 310 patients, of which 157 (50.6%) were male and 153 (49.4%) were female. During the study, the mean and standard deviation of patients' age were 56.6 and ± 20.6 years, respectively. The Cronbach's alpha coefficient for this scale was 0.875, demonstrating good internal consistency of the questionnaire. Using factor analysis, the scale items filled one subgroup. To evaluate the reliability of this test, the intra-class correlation coefficient (ICC) was determined to be 0.75, confirming its reliability. This study demonstrated acceptable validity and reliability of the TIPS questionnaire for evaluating patients' trust in their physicians in a Persian-speaking society.

Keywords: Trust in Physician scale, Persian, Questionnaires, prognosis, health services

INTRODUCTION

The efficacy of healthcare is closely linked to the patient's trust in their physicians [1], and there exists an apprehension that the constant modifications in the healthcare system may adversely impact this trust [2]. The expression of a patient's trust in a physician is characterized by a strong sense of belief shared by individuals and able to be relied upon [3]. Patients' trust in a physician may affect their satisfaction and also the outcomes of healthcare services [4]. This trust was directly related to the patient's recommendation of treatment to others, further follow-up of the patient during the treatment, and the patient's decision not to seek a second physician's opinion [3]. Good communication between doctor and patient plays an essential role in health care. It is the basis for the patients' improvement. "Trust" is also the most important factor of communication and has a positive effect on improving patients and increasing the impact of treatment. Trust will increase the patient's satisfaction with the treatment, better compliance with the doctor's orders, and continuity in health follow-up. In the past decades, the cost of medical services (due to their progress and diversity) has increased significantly, which is not only a serious and vital threat to the patient's trust in the doctor and the continuation of treatment but also causes a decrease in trust-building ethics, an increase in wrong treatments and Unnecessary treatments and therapeutic abuses [4].

Trust is one of the most significant aspects of the relationship between patients and physicians. Yet, despite its acknowledged significance, efforts to assess patients' trust in their physicians in Iran have been relatively insufficient [4]. In a study conducted in Iran [5], a self-administered written questionnaire, developed by the author, was used to assess patients' trust in their physicians. The result revealed a score of 3.76 (SD=0.574), which was significantly higher than the maximum score of 5 in another study [4]. Using the wake forest questionnaire developed by Hall, M.A [6], the score was 39.69 (SD=6.7) out of a maximum score of 50. The difference in the patients' trust in their physician's scores across diverse studies is primarily attributable to disparities in research methods, including various interviewers and questionnaires, and the absence of valid and reliable tools to assess this trust. To measure patients' trust in their physicians, the researchers have used various scales. Among these, the TIPS questionnaire is widely used in different studies and countries [7-10]. Considering the lack of valid and reliable measurement tools regarding patients'

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trust in their physicians in Iran, this study aims to estimate the patients' trust score in patients referred to the hospital emergency department of Kashan Beheshti Hospital while examining the validity and reliability of the Persian version of the TIPS questionnaire.

MATERIALS AND METHODS

We conducted a cross-sectional study to provide the Persian version of The TIPS questionnaire.

Translation and Society Adaptation

The owner of the questionnaire, David H Thom has granted written authorization to utilize it for research purposes and to provide a Persian version of the questionnaire in Iran. Then, the questionnaire was translated from English to Persian through forward-backward translation. Two experienced translators familiar with medical texts and questionnaires translated the original English version into Persian. Subsequently, they compared the translations and matched the questions regarding their meaning and concept. Finally, a Persian version of the TIPS questionnaire was prepared by choosing the optimal alternatives. To ensure that the Persian version and the original text corresponded, the Persian version was translated into English by two individuals who were proficient in English and had not previously seen the TIPS questionnaire. Respectively, no significant differences were observed between the two questionnaires.

Participants

As per the guidelines on validity and reliability, it is essential to obtain 10 samples for every query [11]. This study was conducted on a population of 310 individuals. A random sampling method was employed. There were 65 beds available in the emergency department and we accessed it on different days and times of the day. Our participants were randomly selected from a table of numbers. After obtaining our samples, patients were informed that the selection was randomized and the results of the questionnaire were ineffective in guiding the treatment process. Therefore, they were designated for university research, thereby avoiding the requirement to mention their names in the questionnaires. They were requested to read and respond to the questions carefully. Subsequently, questionnaires were gathered and inquired about the inclusion and exclusion criteria. The questionnaires that were not qualified were excluded from the study.

Inclusion criteria

Literacy to complete the questionnaire, consent to participate in the study, and an age range of more than 18 years.

Exclusion criteria

Patients who exhibit severe distress and emergency status, patients who have cognitive and dementia issues or mental illness, and those who fail to complete all questionnaire questions.

Instruments

Trust in Physician Scale Questionnaire (TIPS)

TIPS questionnaire is a self-report tool with 11 items. Each question was scored on a Likert scale ranging from 1=almost never to 5=almost always, and total scores ranged from a minimum of 11 to a maximum of 55. The Cronbach’s alpha in Anderson and Dedrick's study [12] is 0.90, while it is 0.89 in Thom's study for the total scale [13].

Additional measures

Public Trust of Health Care Providers Scale

This scale consists of 33 items and 6 subscales, including the patient-centered focus (6 questions), macro level policies (6 questions), professional expertise (4 questions), quality of care (9 questions), communication and information provision (6 questions), and quality of cooperation among providers (2 questions). Each component of the test is scored based on a Likert scale respectively. The Cronbach’s alpha is 0.8 in Straten GFM's [14] study, while for the Persian version of this questionnaire in Iran, it was 0.83 [15].

Ethical Considerations

The ethical committee of the University of Medical Sciences granted permission for this study. These results were reviewed and approved by the Research Ethics Committees of Kashan University of Medical Sciences with approval ID: IR.KAUMS.REC.1400.039. (2021- 9 - 15). The Declaration of Helsinki was followed to adhere to the ethical principles of the research. The data collection was conducted in a manner that ensured anonymity and confidentiality. The study participants were guaranteed that they would receive the study results upon request. The objective of the study was communicated to the participants, and they subsequently provided written consent.

Data Analysis

Reliability

We used Cronbach’s alpha coefficient to assess the reliability of the TIPS questionnaire. The intra-class correlation coefficient (ICC) was calculated using a test-retest method on a sample of 30 patients referred to the emergency room of Shahid Beheshti Hospital in

Kashan after 14 days. If the obtained ICC value is 0.7 or higher, it can be said that the tool has a reliable characteristic. Cronbach's alpha ranges from zero to one. According to Bland and Altman's theory, scales with a Cronbach's alpha greater than 0.7 are suitable for research purposes [16].

Content validity

To guarantee face validity among a group of five experts, and to identify the degree of difficulty, inconsistency, ambiguity of expressions, or inadequacy in the meanings of words, their opinions were incorporated as brief modifications in the questionnaire.

The questionnaire was evaluated for content validity by five experts (two psychiatrists, an epidemiologist, and two internal medicine specialists). Furthermore, the experts were provided with a checklist comprising four options. For each question, the experts selected one of the options based on their scores: a) No relation (with a score of 1), b) Slightly related (with a score of 2), c) Very relevant (with a score of 3), and d) Completely related (with a score of 4). The experts then explained the correlation between the question and measuring patients' trust in their physicians. Finally, the experts' opinions were asked about other items related to the patient's trust in their physicians that are not mentioned in this questionnaire. The checklists were collected and analyzed.

Construct validity

To examine construct validity, exploratory factor analysis (EFA), principal component analysis (PCA), and varimax rotation were used. Kaiser-Meyer-Olkin(KMO) and Bartlett's tests were used to perform the exploratory factor analysis. The 0.6 and higher scores of the KMO test, demonstrate the adequate size of the sample for EFA.

Statistical analysis

To assess the reliability of the scale, we used the test-retest method. Spearman rank correlation coefficient and the intraclass correlation coefficient (ICC) were calculated. In addition, to determine validity, an explanatory factor analysis was performed, and convergent validity was designed. To evaluate the correlation between the two scales, we used convergent validity. All of the analysis was performed using STATA 14 and SPSS 16 software.

RESULTS

325 questionnaires were distributed, and 310 of them were fully answered. There were 157 (50.61%) male participants, 153 (49.4%) female participants, and their average age was 56.6± 20.6 years. In this study, 110 (35.5%) were university graduates and 21 (6.8%) were illiterates. The number of patients with acute and chronic disorders was 178 (57.4%) and 132 (42.6%), respectively. The majority of physicians, 202 (65.2%), were male, while 108 (34.8%) were female, and their average age was 50.14 ± 10.8 years. The average work experience of physicians was 15.4± 8 years (Table 1).

Table 1: Demographic characteristics of the study participants

Variable		N (%)
Patients' sex	male	157(50.6)
	female	153(49.4)
Patients' age (year)($\bar{X}$±SD)		56.6± 20.6
Duration of disease	acute	178(57.4)
	chronic	132(42.6)
Patients' education	illiterate	21(6.8)
	primary	76(31.3)
	high school	103(33.3)
	university graduates	110(35.5)
Physicians' sex	male	202(65.2)
	female	108(34.8)
Physicians' age ($\bar{X}$±SD)		50.14± 10.8
Average work experience of physicians (Years)		15.4± 8

The mean and standard deviation of the overall score obtained from the TIPS questionnaire was 42.67 ± 7.53, whereas the mean and standard deviation of the overall score obtained from the general trust questionnaire was 95.59 ± 13.8. The Cronbach's alpha coefficient for the total scale in this study was 0.875, and despite the elimination of each item, no significant alteration in Cronbach's alpha was observed.

In the retest-test method conducted on 30 participants after 14 days, an ICC of 0.75 was obtained with a confidence interval (0.75-0.65), thereby confirming the repeatability of this test.

Content Validity

The content validity was estimated at 95, indicating that for experts, 95% of the questions were fully related to the topic.

Concurrent validity

In this study, the Pearson correlation coefficient was utilized to estimate the correlation coefficient between the TIPS questionnaire and the Public Trust of Health Care Providers Scale. The correlation coefficient obtained between the two scales (r= 0.749 and p=0.001), was significant at level p < 0.01.

Exploratory factor analysis

An exploratory factor analysis was conducted to evaluate the construct validity. Varimax rotation was used in an exploratory factor analysis (EFA) to evaluate the TIPS - construct. The factor was selected based on Eigenvalues above 1.00. The extracted factor included TIPS - objects with a factor loading of at least 0.40. The average variance extracted (AVE) was calculated for factor loadings. The average variance extracted measures the proportion of a variable's variance that can be attributed to a hidden variable, rather than measurement error [17].

To perform exploratory factor analysis, the sample size must be acceptable. The KMO value was estimated to be (0.933), and the KMO level between 0.8 and 1 indicates that sampling is sufficient to perform exploratory factor analysis. The 11 items of the TIPS questionnaire were loaded onto one factor, which accounted for 61% of the total variances (Tables 2, 3).

Table 2: Kaiser–Meyer–Olkin (KMO) and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.933
Bartlett's Test of Sphericity	Approx. Chi-Square	1606.204
	Df	55
	Sig.	0.000

Table 3: Component Matrix of TIPS

Question	Component	
	1	2
Q1	0.816	
Q2	0.795	
Q3	0.780	
Q4	0.770	
Q5	0.775	
Q6	0.740	
Q7	0.732	
Q8	0.718	
Q9	0.710	
Q10	0.702	
Q11		0.948

As can be observed in the scree plot diagram for the first factor, the diagram exhibits a steep gradient, followed by a constant and fixed pattern (Figure 1).

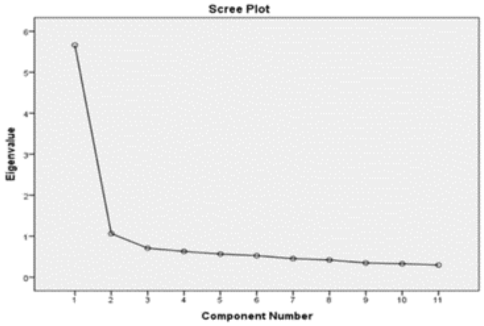

Figure 1: Scree Plot chart

DISCUSSIONS

This study aims to evaluate the validity and reliability of the Persian version of the TIPS questionnaire. This questionnaire is important for changing the policy and management of health services, as well as providing tools for other researchers. The findings have demonstrated that the TIPS questionnaire has high validity and reliability for conducting studies in the Persian-speaking community. Until now, there has been no study conducted for the TIPS questionnaire in Iran, and this is the first study. The present study employed the forward-backward translation method to create a Persian version of the TIPS questionnaire from the original version prepared by Dr. Thom, David H. In similar studies, including Aloba's study in Nigeria in 2014 [13] and Marcinowicz's in the Netherlands in 2017 [15], the same translation method was used for the Nigerian and the Dutch versions of the questionnaire, respectively.

The internal reliability of the questionnaire was assessed using Cronbach's alpha and it was found to be 0.9 and 0.89 in both the first study conducted by Anderson and Dedrick and the subsequent study conducted by Thom, respectively [13,18]. In our study, it was 0.87, which is close to the original version. This indicates the good internal reliability of the Persian version of the TIPS questionnaire.

Three experts evaluated and confirmed the face and content validity of the questionnaire. In fact, at this stage, it is necessary to use the opinion of experts rather than the scientific method of data collection. The original version employed this approach to ensure both face and content validity, which was deemed acceptable. The ICC was 0.75, which indicates that the questionnaire is reliable and desirable. There was no prior investigation conducted on the TIPS questionnaire to evaluate ICC.

The Pearson correlation coefficient was measured between the TIPS questionnaire and the Public Trust of Health Care Providers Scale. A linear correlation between two sets of data is measured by this coefficient, indicating that an increase or decrease in one variable affects the other one. In a study on cancer patients, Spearman's correlation was calculated and there was no relationship between the stage of cancer at the time of diagnosis and the usual provider continuity (UPC) score and TIPS [19]. Furthermore, in one study among Nigerian psychiatric patients, using Pearson's correlations, there was no significant correlation between the duration of disease and patients' trust in their physicians, but there was a positive relation between the period of illness and the patients' trust in their physicians [7]. In the exploratory factor analysis, it was found that the questions of this questionnaire are in one dimension, accounting for 61% of the total variance indicating the favorable internal validity of this questionnaire. However, the Nigerian study used a two-factor construct to explain the questionnaire, with doubt accounting for 28.82% of the total variance and trustworthiness accounting for 19.23% of the total variance [7]. Exploratory and confirmatory factor analysis yielded the same result for 1756 hospitalized patients in the United States [20].

It is suggested that in future studies, this tool should be used to measure patients' trust in their physicians because there are no tools to measure this important aspect of the therapeutic relationship in Iran. Furthermore, considering that patients will know more about their doctors and that the interpersonal trust will change, we suggest that in future studies the trust score should be tested on the first day of patient hospitalization and a few days later. More comprehensive population-based studies are required to verify this novel generic tool's predictive accuracy and consistency for application within the broader Iranian community.

One of the limitations of this study was that the patients who had recently been referred to the hospital were not familiar with their physician. Therefore, we recommend that in the future, the trust score should be assessed within the first day of patient hospitalization and a few days later. The study is limited by the small sample size. This research only included participants from the emergency department, which may have skewed the final outcome. As a result, additional research with a bigger group of participants is recommended.

CONCLUSION

The Persian version of the TIPS questionnaire exhibits high validity and reliability, making it a suitable choice for conducting research in the Persian-speaking community.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere.

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Authors' contribution

This study was based on Dr. Anita Zia's thesis under the guidance of Dr. Saber and Dr. Asgarian.

Ethics approval and consent to participate

This work was approved by the Research Ethics Committees of Kashan University of Medical Sciences with approval ID: IR.KAUMS.REC.1400.039.

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ORIGINAL ARTICLE

A Structured Approach to the Prevention of Psychoactive Substances Use in Vulnerable Populations and the Therapeutic Interventions in the Case of Patients Diagnosed with Substance Use Disorders – A Romanian Society-focused Perspective

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Abstract: The phenomenon of substance use disorders (SUDs) presents extremely complex etiopathogenesis with intermingled psychological, social, economic, and biological factors. Therefore, the prophylactic and therapeutic approach is multidimensional and should involve targeting all the aforementioned variables. However, it is frequently difficult to separate these factors and construct specific strategies to mitigate their direct negative effects, so caution is needed when formulating recommendations for this population, starting from guidelines for case management and finishing with optimization of healthcare policies. Based on the lack of good practices approved at the national level in Romania for the prevention and treatment of SUDs, this article suggests a draft for such an instrument that could help both clinicians and decisional factors with attribution in healthcare and drug abuse prevention. The methods used in this article are (1) a literature review of studies, guidelines, and other reviews focused on specific aspects of drug abuse/dependence and (2) expert opinions derived from clinical and administrative experience. The results are formulated as recommendations, scored according to the GRADE system, and grouped into three categories: (1) preventative measures for SUDs, (2) secondary prophylaxis, (3) therapeutic and other SUD-targeted interventions. In conclusion, the need for guidelines and policies focused on the prevention and treatment of SUDs, especially in vulnerable populations, is an unmet need in our country. The suggested algorithm in this paper tried to bridge the gap between the available literature dedicated to SUDs, practical experience, and future policies in the healthcare of patients with SUDs.

Keywords: substance use disorders, withdrawal, detoxification, post-detoxification, harm reduction, opioid agonists, opioid antagonists, disulfiram, acamprosate

INTRODUCTION

European Union Drugs Agency (EUDA) is the main EU institution with responsibilities in the domains of monitoring the regional drugs of abuse (DOAs) and formulating evidence-based practice guidelines, and its most recent published report, in 2024, showed the following data with epidemiological, social, and clinical implications: cannabis is the most commonly used illicit drug in Europe (approximately 8% in European adults used cannabis during the last year, and 1.3% are estimated to be daily or almost daily users of cannabis); cocaine is the second most frequently used illicit drug in Europe, but the prevalence and use patterns considerably vary across countries (2.5% of European adults used cocaine in the last year, and this drug is responsible for an important percentage of the deaths related to drugs use); synthetic stimulants (including synthetic cathinones), MDMA (ecstasy), heroin and other opioids, and new psychoactive substances (NPAs) are other important threats to the health of adult population (but also tending to incorporate younger adults and adolescents); although less known to the large public, hallucinogens, anaesthetics, dissociative and sedative agents are also use illicitly in EU (for example, lysergic acid diethyamide – LSD, ketamine, gamma hydroxybutyrate – GHB, nitrous oxide, or hallucinogenic mushrooms) and able to raise multiple difficulties both individual- and social-grounded [1].

The latest European Drug Report regarding drug-induced deaths, an indicator that refers to the total cases of drug overdose deaths, showed an increase in this indicator in the year 2022 in some EU countries, with a provisional estimate of 6400 drug-induced deaths [2]. Opioids, frequently in combination with other substances,

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remain the most reported cause of drug-induced deaths, and heroin was involved in approximately 1800 deaths in the EU [2]. However, synthetic opioids, e.g., fentanyl derivative carfentanil and nitazene opioids, are increasingly represented in these statistics [2]. Although a significant number of these deaths are accidental, in other cases, the intent (or lack of it) is difficult to ascertain; therefore, particularities among the EU countries regarding the methodology of evaluation of overdose intentionality should be noted when comparing the results. For example, in Hungary, Netherlands, Slovenia, and Sweden, more than 30% of the overdose deaths among women were considered triggered by suicidal intent, while in Bulgaria, Poland, and Finland, suicidal intent was recorded for over 25% of deaths among women in the year 2022 [2].

The threats related to substance use disorders (SUDs) in younger populations have been described worldwide. In the US, according to multiple reports, more than 40 million people aged 12 and older received a diagnosis of SUD in 2020, and the Center for Disease Control and Prevention (CDC) signaled overdose deaths in children aged 10 to 19 more than doubled from 2019 to 2021 [3]. Adolescents are especially vulnerable to the negative impact of SUDs in the long term at multiple levels, involving mental health problems, sub-standard academic performance, and family, social, and physical health complications [4]. For example, psychotic-like experiences are more frequent in young substance users than in non-users, and this may indicate a subclinical at-risk population for psychosis [5-8].

Special attention is needed in the post-COVID-19 pandemic era regarding the challenges related to patients with SUDs, especially because this population is considered more vulnerable to infections and somatic complications or comorbidities, and many countries still have outbreaks of this viral disease [9-12]. Therefore, this vulnerability should be considered when planning large-scale interventions or designing policies for patients with SUDs, and distinct resources should be allocated due to the risk of problematic evolution in those individuals with dual diagnoses.

According to the data presented in the Romanian "National Drug Strategy 2022-2026" [13], the three substances that produce the highest rates of requests for medical assistance in our country are (1) cannabis, (2) opioids, and (3) NPAs. The same document shows that the main aspects that require intensive monitoring in Romania are: (1) special care for populations at increased risk of addiction, mainly high school students, due to their high consumption of cannabis and NPAs; (2) infectious diseases associated with drug use, especially HIV, and type B and C hepatitis viruses, in medical personnel taking care of individuals who inject themselves DOAs; (3) the rate of deaths associated with drug use is on an increasing trend, against the background of methadone intoxications; (4) problematic use of opioids at the national level, with heroin being the main drug of consumption; (5) drugs use during incarceration -mainly opioids, but also cannabis and NPAs- a phenomenon that steady increased from 2013 to 2019; (6) drug crime and related problems, diversion of drug precursors (designer-precursors) and drug market dynamics (decreasing price of cocaine and increasing purity of the product, as well as increasing the availability for most types of DOAs) [13].

From the perspective of vulnerabilities, the same document mentions (1) economic aspects - shortage of specialized human resources, de-professionalization, underfunding, persistent dysfunctions in the organization of public service systems (health, education, social assistance), accentuated by the COVID-19 pandemic; (2) legislative and organizational aspects - collection of data on drugs, integrated assistance for these patients, monitoring of drug precursor operations; (3) aspects related to the international drug market - high availability at European level of synthetic and natural drugs, the emergence of new synthetic opioid substances, increased consumption of MDMA and cannabis, and the emergence of new cannabis derivatives; (4) access of organized crime groups to emerging technologies that increase the efficiency of their actions, a fact translated in the volume of drug trade that significantly increased [13].

The general objectives of the national drug strategy 2022-2026 are (1) strengthening the national prevention system, addressed to the general population, schoolchildren and vulnerable groups; (2) developing services and interventions within the national medical, psychological and social assistance system aimed at reducing the negative effects associated with drug use; (3) strengthening and developing specialized medical, psychological and social reintegration interventions and services; (4) developing an integrated approach to drug use in penitenciaries; (5) strengthening interventions to fight against organized crime entities with activities in the field of drug trafficking; (6) strengthening the capacity to detect large quantities of drugs, precursors and pre-precursors; (7) strengthening the mechanism for controlling the illicit circuit of precursors and narcotic drugs and fighting drug production; (8) intensifying the effective monitoring of the logistical and digital channels used for drug distribution and the confiscation of illicit substances; (9) international cooperation to reduce the demand for and supply of DOAs; (10) developing and strengthening an integrated system for collecting data on the drug abuse phenomenon; (11) ensuring a unified framework for action in the field of drugs and precursors, monitoring the implementation of national drug policies, efficient use of resources and maximizing the results of the interventions carried out [13].

Considering all the previously presented data regarding the main directions of the DOAs' risk analysis, the necessity of preventing these drugs’ use at the national level, and the objective of approaching therapeutically vulnerable populations, this paper aims to formulate recommendations targeting these essential aspects.

MATERIALS AND METHODS

Based on the existing data in the literature and the experts' opinions, directions of action for the core domains of DOA use prevention and treatment of patients diagnosed with SUDs are presented and grouped into distinct categories. The recommendations are hierarchized using a methodology based on the GRADE system (Grading the Recommendations Assessment, Development, and

Evaluation), which allows the scoring of strengths from very low (D) to low (C), moderate (B), and high (A) [14-16]. Each of the authors scored the strength of the recommendations in the first round of evaluation, while in the second, a consensus was searched. In cases where a consensus cannot be reached, the recommendation will include a comment referring to the disagreement and its reason.

RESULTS

All the recommendations have been distributed according to their main theme in (1) directions for the prevention of DOAs; (2) the role and directions of secondary prophylaxis; (3) educational and therapeutic interventions for SUDs.

According to the International Classification of Diseases, 11th edition [17], Table 1 represents the main classes of DOAs. The recommendations (and the literature reviews on which they are based) refer mainly to alcohol, cannabis and its synthetic derivatives, opioids, sedatives, and related drugs, cocaine and other psychostimulants, synthetic cathinone, hallucinogens, nicotine, and volatile inhalants.

Literature review and summary of recommendations

1. Directions for the prevention of the use of DOAs

Multiple-component programs have been associated with positive results because they incorporate school, family, and community resources and can be integrated into a psychoeducational framework, which is more effective than just delineating „forbidden” or „dangerous” zones from the „allowed” ones [18]. On the other hand, information in itself did not lead to sustained change, although some effects were observed regarding the modification of knowledge and attitudes [18]. Skills training-based programs are also useful [18]. According to the US National Institute of Drug Abuse [19] principles for prevention, the main directions that should be at the foundation of any guidelines dedicated to this objective include enhancing protective factors and neutralizing the risk factors; the interventions must be tailored to the target population; such interventions have to be long-term and repeated, in order to reinforce the original objectives [19]. Regarding alcohol abuse, the most effective prophylactic measures can be considered those limiting the availability of alcohol, establishing a minimum age for drinking, restrictions on hours and days of the sale, and different availability of alcohol-strength- drinks; regulations on drinking and driving also have been proved efficient; early treatment interventions, rules of promotion in mass media, as well as education and persuasion in schools and universities are also associated with favorable results [18]. Integrated policies for alcohol abuse prevention have been the most consistent interventions associated with good outcomes [18].

Table 1: Classification of the drugs of abuse according to the ICD-11 [17]

Alcohol
Cannabis
Synthetic cannabinoids
Opioids
Sedatives, hypnotics and anxiolytics
Cocaine
Stimulants (incl. amphetamines, methamphetamines, methcathinone)
Synthetic cathinones
Caffeine
Hallucinogens
Nicotine
Volatile inhalants
MDMA (3,4-methylenedioxymethamphetamine) or related drugs, including MDA (3,4-Methylendioxyamphetamine)
Dissociative drugs, including ketamine and phencyclidine (PCP)
Other specified psychoactive substances, including medications

Prevention of SUDs among adolescents according to the United Nations Office on Drugs and Crime (UNODC) World Health Organization (WHO) Informal Scientific Network should respect several recommendations, between them being promoting population-based and environmental prevention measures; enhancing the meaningful engagement of vulnerable groups in prevention initiatives and fighting stigmatization; deployment of effective, evidence-based methods in different settings and implementing these interventions along a continuum of care that includes screening, but also brief interventions and treatments (i.e., family therapy, contingency management, and cognitive behavioral therapies-CBT) [20].

The main barriers identified in delivering prevention-focused interventions are unstable funding, lack of coordination between systems, lack of workforce capacity, insufficient knowledge about evidence-based programs, and lack of information about how to adapt programs for certain settings [21,22]. A review that included 30 studies dedicated to the prevention and treatment of opioid use disorder (OUD) showed that stigma, limited access to treatment, inadequate healthcare infrastructure, regulatory barriers, and lack of clearly focused policies are the main challenges in adequately preventing and treating such disorders; several recommendations, formulated based on the available data are the development of culturally adapted prevention programs, assessment of the long-term effectiveness of potential treatment modalities, encouraging interventions focused on decreasing stigma and improving access to care by using the technology [23].

Socioecological strategies have been explored in the prevention of SUDs by focusing on the interaction between individual, interpersonal, and macro-level, i.e., community, social, and political factors, in the modeling of health outcomes and overall well-being [24]. A public health approach is encouraged when policy, research, and service delivery solutions targeting SUDs are considered [17]. Health equity is essential when applying the framework of socioecology to the prevention of SUDs; although this model is explored by the author of the cited report in the context of the US society, the core principles may be applied irrespective of the social context [24]. For example, public funds for the treatment of overdoses should cover individual, interpersonal, and macro-level investments in the primary, secondary, and tertiary prevention spectrum [24].

Prevention strategies targeting social risk factors modeled for children and adolescents can improve the outcome and decrease the risk of SUD onset [25]. Also, the treatment of SUDs is recommended to correspond to the „Chronic Care Model," with (a) adjustment of the intensity of intervention to the severity of the disorder and (b) the concomitant treatment of psychiatric and organic comorbidities [25]. Between the social risk factors that should be targeted by preventative interventions are, according to epidemiological studies, high intensity of stressors, poor social support, accessibility of drugs, and lack of opportunities for non-drug use behaviors; adverse social environmental exposures increase the risk for SUDs especially when appear in childhood and adolescence due to their impact on the brain development [25].

The National Institute for Health and Care Excellence (NICE) from the UK formulates the following recommendations for delivering drug misuse prevention activities to high-risk individuals for SUDs: health services- primary care services, community-based health services, mental health services, sexual and reproductive health services, drug and alcohol services, and school nursing and health visiting services; specialist services; community-based criminal justice services; accident and emergency services [26]. The initial discussion with the individuals at risk includes a complete assessment of physical and mental health, personal, social, educational and employment circumstances, and any drug use; based on this first assessment, the immediate safety of the person is to be considered [26]. Skills training for children and adolescents who are vulnerable to drug misuse is an important step, highlighted by the NICE guidelines, and this type of training has to help them develop listening, conflict resolution abilities, refusal, decision-making, identification and management of stressors, coping with criticism, dealing with a feeling of exclusion, and making healthy behavior choices [26]. Also, the families and important caregivers need to receive skills training, targeting communication, developing and maintaining healthy relationships, conflict resolution, and problem-solving [26]. In the case of the adult population, which is vulnerable to drug abuse, information about the risks of using such drugs should be offered, and also information about the local services where advice and support can be found; campaigns of providing information about drug use should be initiated in settings like nightclubs or festivals, gyms (for individuals who might abuse of steroids, for example), hostels, etc. [26].

Other authors mention the potential usefulness in children and adolescents of anti-substance use messaging, routine screening, clear pathways for referral to treatment in all at-risk settings, and mentoring programs [27]. Involvement of pediatric primary care is considered the most important step due to the possibility of incorporating preventive counseling and screening for substance use [27,28].

Based on the presented data and the particularities of the SUDs in our country, the following recommendations have been formulated:

1.1. Psychoeducational interventions need to target groups at high risk for abuse/harmful use/dependence/SUDs and should be tailored especially for children of school age, adolescents, patients with mental disorders, patients with somatic diseases requiring analgesic treatments, and persons deprived of liberty.

In order to implement a national prevention policy, the participation of the following categories of professionals is required:

a. representatives of primary health care services (GPs);

b. school psychologists and university campus-based psychologists;

c. psychiatrists and psychologists from the National Penitentiary Administration network;

d. psychiatrists and clinical psychologists from the national hospital network;

e. physicians with different specialties who prescribe drugs with abuse potential - oncologists, rheumatologists, specialists in palliative medicine, etc.

Also, integrating family members and other important caregivers in the preventative efforts could be of significant benefit.

The methodology of these interventions includes:

a. training programs for doctors in the primary healthcare network to recognize the specific symptoms of the use of DOAs, under the coordination of specialists trained through the programs mentioned in paragraph 3.1;

b. periodically organized workshops to update the scientific, legal, and practical knowledge of all physicians who approach high-risk population groups;

c. informative sessions and skills training programs for psychologists working in schools or other educational institutions;

d. educational sessions for educators, teachers, and professors;

e. organizing information groups for families of patients with SUDs or who have risk factors for developing a SUD;

f. establishing a hotline (phone and internet) for first aid dedicated to vulnerable individuals and their relatives;

g. informative leaflets and brochures that family doctors, school psychologists, and teachers may distribute to vulnerable populations.

Monitoring the implementation of these measures and determining their medico-social impact involves an integrated data processing system that can be accessed by all interested parties in order to maintain constant feedback between specialists in different cities and authorized trainers.

2. Secondary prophylaxis of individuals in the recovery phase after SUDs

This type of intervention is focused on early identification of an SUD, followed by approaches that tend to reduce/stop its progression [24]. Screening and referring patients to mental health specialists who can prescribe medications for addictive disorders is a crucial component of this stage of intervention [24]. An analysis of the risks vs. protective factors is also a part of secondary prevention because it can lead to further elaboration of local and national strategies. For example, lack of affordable or accessible treatment services, stigma against people with SUDs, lack of provider knowledge about this pathology, punitive drug policies, and high threshold treatment policies are risk factors approachable by secondary-stage interventions, while receipt of early diagnosis and referral to treatment, stable housing, economic stability, peer recovery support, and availability of quality treatment are protective factors [24]. Interventions used in this stage that were associated with good results in young people diagnosed with SUDs are, based on a systematic review, behavior therapy, Minnesota 12-step programs, residential care, and general drug treatment [29]. Also, involving parents and other significant caregivers may enhance the efficacy of these interventions [29,30]. Multiple levels for these interventions should be provided: college, family, community, peer-led, workplace-based, and technology-driven approaches must be planned and specific techniques must be chosen for each individual [29,30].

Community-based programs have been indicated by several authors as presenting the highest effectiveness if applied in the context of the general population but also in high-risk groups [31]. However, there is great heterogeneity in the interventions and techniques of secondary prevention [31]. Environmental methods have a positive trend because they take into account patients' needs in their natural environment, but cognitive-behavioral methods may also be recommended for this vulnerable population [31]. Experiential interventions in combination with cognitive-behavioral therapies may involve not only patients but also family members, teachers, and other community members and bodies [31].

Regarding the factors that contribute to the secondary prevention of SUDs, different aspects have been evoked, with various degrees of approachability, such as self-efficacy, hopefulness, and social support [31]. Skills training programs may still reinforce resilience factors and mitigate risk factors; therefore, such strategies are needed in this population. Primary care physicians may be involved in this type of prevention by applying screening tools, treating comorbidities, addressing patients to specialized counseling when social stressors are identified, early diagnosis or referral, and administration of brief behavioral change counseling [32].

More specialized interventions for patients with a defined SUD, for example, crack (cocaine) abuse/dependence, have been reviewed, and the results regarding their efficacy were mixed [33]. Behavioral and community-based prevention measures have led to heterogeneous results in the short-term and harm-reduction interventions (e.g., safer crack use kit distribution), but it is considered that they had modest efficacy in risk reduction [33]. Psycho-social therapies, including contingency management, showed some positive results but limited short-term efficacy [33]. Pharmacotherapy also did not produce convincing evidence [33]. Therefore, the authors of the respective review concluded that no „golden standard" prevention strategy exists for crack abuse [33].

As it can be inferred from these data, it is extremely difficult to find evidence-based recommendations for specific drugs, except for nicotine, alcohol, and opioid SUD, where multiple guidelines exist [34-36]. For example, when approaching recommendations for alcohol abuse/dependence, NICE guidelines recommend relapse prevention strategies such as psychological interventions (initiated ideally within two weeks after successful alcohol withdrawal for people with moderate or severe alcohol dependence)- cognitive behavioral therapies, behavioral therapies, behavioral couple therapies, social network and environmental based therapies; pharmacological interventions- acamprosate, oral naltrexone or disulfiram [34]. The American Psychiatric Association (APA) recommends, in its own guidelines, the monitoring of biological biomarkers for early identification of alcohol use disorder (AUD), identification and treatment of co-occurring conditions, the formulation of the therapeutic objectives in agreement with the patients, educational measures included in the therapy, initiation of naltrexone or acamprosate for patients with moderate to severe AUD, or disulfiram in those patients who want to achieve abstinence, prefer this drug, or are intolerant to naltrexone/acamprosate, and in those who are capable of understanding the risks of alcohol consumption while taking disulfiram, and have no contraindication to this drug; topiramate/gabapentin may be initiated in certain conditions [35]. Also, the US Department of Veterans Affairs/Department of Defense guidelines support similar recommendations, adding psychosocial interventions, such as group mutual help techniques (e.g., peer linkage, network support, 12-step facilitation) [35].

Regarding nicotine use disorder (NUD), extensive meta-analyses and clinical studies showed that behavioral treatments, relapse prevention psychotherapy, pharmacologic therapies, motivational enhancement, smoking reduction to quit, brief advice, alternative intervention modalities, self-help, and tailored treatments can be useful in preparing longer-term abstinence [37]. The European Network for Smoking and Tobacco Prevention (ENSP) guidelines highlight the need for persistent and repeated therapeutic interventions, as well as long-term follow-up visits [38]. According to these guidelines, educational measures and several pharmacological agents might be required after relapse, but no effective treatment has yet been identified to treat tobacco dependence in abstinent smokers [38].

In the domain of OUD, the American Society of Addiction Medicine (ASAM) guidelines showed that strategies directed at relapse prevention should be included in any case management plan for patients receiving active opioid treatment or ongoing monitoring of their health status [39]. Methadone should be reinitiated if relapse occurs after methadone discontinuation or when there is a high risk of relapse, and naltrexone can also be recommended as long-term treatment, with the possibility of its discontinuation if the risk of relapse becomes low [39].

Based on the available data and experts' opinion, the following recommendations are formulated:

2.1. Secondary prevention is facilitated by a comprehensive, bio-psycho-social approach to the patient diagnosed with SUD who is recovering and needs to be taken care of by specialized networking involving mental health workers, GPs, social workers, etc.

More specifically, the participants in this activity are psychiatrists, clinical psychologists, psychotherapists, general practitioners and social workers who have completed professional development programs. These specialists will apply the medical and psychological support techniques acquired during the aforementioned training and will collect epidemiological data necessary to estimate the specific needs of patients in all the regions of our country as a preliminary step for an adequate allocation of financial and human resources. The final goal of this approach is to ensure continuity of medical and psychological care after discharge from the hospital of patients treated for disorders related to DOAs.

Methods to efficiently organize specialized assistance services and to achieve their coordination:

a. the formation of a National Coordination Center for Medical Assistance Services for Patients with Substance Use Disorders would be required;

b. This center will ensure the continuity of healthcare services and related services (psychotherapy, clinical psychology) by integrating data from hospitals that admit individuals with SUDs, healthcare centers that provide methadone substitution therapy and polyclinics that keep records of such patients;

c. the necessary steps will be taken to establish post-detox treatment centers for patients who have a documented history of DOAs withdrawal therapy.

This activity will be monitored by analyzing data at the national level regarding the periodic checks of patients discharged with a diagnosis of SUD, such as relapse rate, duration until the first relapse, duration of medical and psychological supervision, the need for pharmacological resources, specialized equipment, and personnel, etc.

2.2. Guidelines for good practices in drug use prevention necessitate a working group that could be created with the participation of practicing physicians and academics who will oversee the drafting of this guide. Once finalized, the guideline will be submitted for approval by the Ministry of Health, in order to implement its recommendations at the national level.

The methodology includes a systematic review of the literature, exploring guidelines developed by EU societies, associations, and foundations, and an integration of epidemiological data at the national level.

Monitoring will be carried out through periodic reassessments of the level of recommendations for each SUD diagnosis, corresponding to epidemiological data at the national level and data from specific research at the international level.

3. Educational and therapeutic interventions focused on SUDs

The importance of specialized training for physicians involved in the assessment and management of SUDs has been acknowledged worldwide, and the lack of an adequately trained workforce remains a challenge in many countries [40,41]. According to a WHO survey on resources for the prevention and treatment of SUDs, which included 162 countries, deficiencies in the training programs for the workforce managing this pathology exist in almost one-third of the countries, and when low-income countries were analyzed separately, the percentage reached 60 [42]. The same survey concluded that counselors and community health workers benefitted from the lowest rate of specialized training (23% and 19%, respectively) [42].

Several key challenges in the training of specialists in addictive disorders are the lack of an agreed training pathway, gaps in addiction training for the main workforce groups, lack of identification of SUDs, development of new therapeutics that signal a rapidly changing therapeutic landscape, new diagnoses like behavioral addictions, and the necessity to create a common framework that fights stigma [43].

The content of the curricula is still a matter of debate, however, with interactive teaching methods and experiential and didactic components being considered the core of such programs [40]. Addiction specialists should be involved in promoting information about SUDs and refining the skills of medical students and residents, but also in enhancing knowledge on SUDs among practicing physicians, starting from primary-level physicians and up to tertiary center doctors [40]. Also, online programs are an option for the training of physicians due to decreasing time and other expenditures [40]. Attitudinal skills training refers to enhancing communication that should be patient-centered, focused on patient trust and autonomy, and involve empathy, respect, and compassion [44]. An evidence-based program integrating screening, brief intervention, and referral to treatment (SBIRT) has been created for clinicians confronted with challenges specific to SUDs and is considered time-efficient as a first-line intervention [44,45]. Another brief intervention is FRAMES, an acronym indicating feedback, responsibility, advice, menu for change, empathy, and enhancing self-efficacy [46]. Brief interventions are usually the first step, but they are associated with efficacy in reducing substance use and its harmful consequences, and they can be followed by referrals to appropriate treatment services in more complex SUD cases [44-47].

Long-term and multicomponent training of healthcare specialists dedicated to helping patients with SUDs is considered the most effective means to obtain self-efficacy in providers who may initially be reticent or uncomfortable with taking care of this particular population [48-50]. Several steps have been described in this direction, including institutional pairing (experienced team pairs with local leadership), „train the trainer" programs, telemedicine (virtual consultation technology for ensuring the quality of healthcare), booster sessions, and ongoing assessment of treatment delivery [48].

Closer integration of substance use-related services in the general health care system is a priority highlighted by the US Surgeon General in a report named „Facing Addiction in America" (2016) [51]. Such an integration is expected to address health disparities and costs for patients and caregivers and to improve general health outcomes [51]. The use of HealthIT is encouraged in the same report as a means to integrate different care systems better and to increase its collaborative nature but also to extend the workforce, improve care coordination, and reach individuals who are reluctant to participate in traditional treatment settings [51].

Increasing the psychoeducative approaches to the SUD phenomenon and targeting large populations, not only those most vulnerable, is expected to reduce the stigma associated with this pathology. In this direction, lack of knowledge about the causes of SUDs and the available treatments fuels stigmatization, and the patients' perceived disapproval by others contributes to the enhancement of self-stigmatization [48]. Fliers and brochures published by national and international authorities could reduce the phenomena of stigma and self-stigma [48,52].

Evidence-based interventions are grouped into behavioral treatments (mainly cognitive-behavioral therapy, motivational interviewing, and contingency-management approaches), including peer support interventions (also manualized techniques that encourage Alcoholics Anonymous – AA participation), applied in an individual/group format, but also technology-based treatment; pharmacological strategies for detoxification and relapse prevention (greatest efficacy demonstrated for OUD, NUD, and AUD); combined approaches [48]. The accent should be placed on multidisciplinary teams, which include psychiatrists, psychologists, GPs, teams focused on treating comorbid disorders, community-based health workers, and peer and lay counselors [48].

Harm reduction is defined as an attempt to decrease the negative consequences of drug use in patients who still continue to use such substances, and it appeared as a reaction to the excesses of the „zero tolerance approach" [53]. Although harm reduction has proven effective and its acceptance is increasing, the concept in itself is poorly defined [53]. According to a review dedicated to harm reduction for alcohol, tobacco, and illicit drug use (n=650 articles), there is insufficient evidence to support the extensive adoption of this intervention for any DOAs [54], but these results may be related to the low quality of the included studies. According to another systematic review/meta-analysis, the average effect for harm reduction interventions compared to treatment as usual was 0.03 SD (95% CI, -0.08, 0.14), based on the results of nine studies [55]. Still, in specific populations, such as incarcerated individuals, a meta-analysis showed that syringe service provision and opioid agonist treatment are effective in reducing risk behaviors and needle and syringe sharing (n=126 studies) [56]. Yet another systematic review supports the role of harm-reduction techniques, i.e., early screening for problematic alcohol use followed by brief and other interventions in first-contact medical healthcare facilities in reducing AUD onset [57].

Drug decriminalization is considered a variant of harm reduction and it was conceived that a disproportional exacerbation of drug-related harms and imposed short/long-term burdens on an already stigmatized and marginalized population due to the criminalization of drug use is counterproductive to general health [58]. This decriminalization involves policymakers and clear norms of drug prescriptions for pharmacists who must advocate actively for these policies, according to several authors [58].

Regarding the detox (withdrawal) and post-detox interventions for AUD, according to a systematic review (n=49 studies), medical/pharmacological interventions were less likely to demonstrate efficacy when the outcomes were treatment engagement and recovery, and psychological/psychosocial and technological interventions were more likely to be associated with success [59]. When OUD was considered in another review (n=64 articles), medical and combined interventions performed well, as did psychological interventions with one or multiple reinforcement components [60]. The availability of outpatient withdrawal and detox programs is limited when analyzed globally, and limitations are economical, stigma-related, unavailability of specific programs, and co-occurring mental health disorders [61]. As other authors have pointed out, there is a tendency to underutilize medication that can help in relapse prevention as fast as the detox phase has been overcome, which increases not only the risk of relapse but also of somatic or psychiatric complications [61]. Specific protocols for detox exist, depending on the DOAs involved [62-64]. At any rate, it is essential to consider the complexity of treatment in patients undergoing withdrawal but who are already diagnosed with severe somatic disorders, and hospitalization should always be recommended in these cases [65-68]. Also, the presence of psychiatric comorbidities needs further exploration and adequate treatment, concomitantly with the initiation of the withdrawal therapy [69-73].

The transition from detoxification to maintenance treatment is an important step for the insurance of long-term positive outcomes in patients with SUDs [74-77]. During this transitional phase, various facilitators or inhibitors may appear at the patient level, program level, or system level [74]. Continuity of care after detoxification heavily influences important outcomes like the rate of readmissions [75]. Patients with OUD who choose to be included in aftercare post-detoxification programs have significantly better outcomes when compared to those who do not have formal aftercare, up to nine months of follow-up [76].

Therapeutic communities for addictions are an evolving concept with very diverse theoretical and practical implications [78]. Such communities are a distinct psychosocial approach to the treatment of SUDs that emphasizes the role of the „community as a method", with the accent being placed on self-help and mutual help, social learning and finding the most appropriate motivation for change [78]. A review of therapeutic communities' effectiveness for addictions (n=16 reports) showed that two out of three studies reached significantly better results (substance use and legal outcomes), and five studies detected superior employment and psychological functioning [79].

The use of guidelines for SUDs in clinical practice is a subject of controversy in the literature. Several aspects that explain this controversial nature of the topic refer to the lack of clear, evidence-based recommendations for the choice of a certain pharmacological agent in the treatment of a special addiction, for example, between methadone, buprenorphine and naltrexone in OUD [80]; heterogeneity of the included studies, populations, methods of monitoring and interventions explored; the gap between the research and practice is derived from the fact that an individual with SUD or a family member will most likely not be offered a treatment drawn from the existing list of extensively studied variants [81]; different variables may interfere with the clinical recommendation of a certain therapy, such as costs, local availability, difficulties in its implementation, etc. [81].

3.1. Specialist training in addiction therapy is a prerequisite for any policy regarding this domain since there are no pre-qualified personnel based solely on faculty or college graduation.

The participants in this activity are physicians specialized in virtually any field of Clinical Medicine related to mental health or Psychology professionals who wish to obtain a certificate of complementary studies on the therapy of substance use disorders to assist patients with this pathology but also to continue acting as trainers of specialists in drug addiction therapy.

The proposed method is a complementary study program with a modular format, coordinated by the Ministry of Health in collaboration with the Romanian Academy - Section of Medical Sciences and the Romanian College of Physicians.

The curriculum of this training course will be realized based on the proposals of a working group of practicing doctors (psychiatrists, ICU specialists, toxicologists, clinical pharmacologists, pulmonologists, etc.) and clinical psychologists with experience in drug addiction and academic staff with teaching duties who are part of specialized departments.

The core themes of this complementary study program will be: (1) the neurobiology of substance use disorders, (2) clinical and diagnostic criteria for substance use disorders, (3) comorbidities and complications of substance use disorders, (4) psychopharmacology of substance use disorders, (5) counseling and psychotherapy techniques specific to substance use disorders, (6) case management in substance use disorders, (7) research methodology in substance use disorders, (8) clinical and psychological monitoring in substance use disorders, (9) methods for preventing relapse in substance use disorders.

The monitoring of this activity will be done periodically by representatives of the program coordinating institutions.

3.2. Intensification of harm-reduction interventions is considered an essential component of prevention and educational programs.

This activity involves non-governmental organizations, outreach teams, and interdisciplinary teams, which include volunteers, doctors, nurses, social workers, etc.

The proposed methods are:

a. training of specialists and volunteers in harm-reduction methods by graduates of the drug addiction therapy training program (according to section 3.1)

b. collection of data on the activity of these teams in order to establish the necessary resources

c. periodic supervision of volunteer teams

This activity will be monitored through an integrated system of periodic evaluation of the number of harm-reduction interventions by specialists trained in the courses in section 3.1.

An indirect parameter of monitoring is the number of HIV/HBV/HCV infections in patients using injectable ARVs and hospitalizations for somatic complications. This parameter is important for assessing the resources required and costs associated with outreach interventions.

3.3. Enhancing the healthcare system's capacity for ensuring adequate detox treatment is an objective that could increase the survival rate in the SUD population, as well as the overall health, quality of life, and general functionality.

The participants in this activity are primary care physicians for the purpose of early identification and referral to a specialist of patients with disorders related to the use of DOAs, but also specialist physicians from the national network who can refer patients with these disorders to hospital, or specialist physicians from the hospital network, responsible for the case management of these patients.

The proposed methods for ensuring superior detox therapy results are increasing awareness of the positive consequences of withdrawal therapy in the medium and long term at the level of vulnerable populations, developing/re-evaluating therapeutic guidelines for patients requiring detox therapy, and encouraging participation in continuous improvement programs for physicians providing detox treatment.

Monitoring is done by periodically evaluating the parameters related to the effectiveness of this therapy - the number of readmissions, the duration until the first relapse, the duration of treatment maintenance, etc.

3.4. Post-detox treatment is a complementary and necessary intervention to the previous step, and it must be observed that, in the absence of a clear continuity of care, the risk of relapse or acquiring short-term or long-term complications or comorbidities is very high.

This component involves all institutional bodies involved in the social, psychological, and medical assistance of patients who have undergone detoxification programs.

The proposed methods include the creation of the national center mentioned in section 2.1, capable of ensuring continuity of care and supervision of the patient with substance addiction after discharge from the hospital.

Monitoring treatment and patients' evolution is done by determining the effectiveness of post-detox therapy using specific effectiveness indicators, such as the number of readmissions, duration of treatment, and number of drug-related crimes committed after discharge.

3.5. Reducing patient access to DOAs is an objective with long-term beneficial consequences, which require the active and continuous participation of specialized institutions.

The participants in this section of care are representatives of the Ministry of Health and the National Anti-Drug Agency, but also other agencies with national representation. Accessibility is defined, in this context, as (1) access to illicit sources (an aspect controlled by law enforcement agencies) and (2) access resulting from the overprescription of drugs with abuse potential (by family physicians and specialist doctors).

The main method is to reduce the overprescription of drugs with abuse potential by doctors (benzodiazepines, barbiturates, opioids, methylphenidate, etc.) by the means of implementing real-time monitoring of these drugs' circuit (preventing the issuance of prescriptions for the same substance by several doctors to a single person). The center mentioned in section 2.1. will also have the objective of developing a program that alerts the prescribing doctor if a patient requests the issuance of drugs with abuse risk even though he/she has recently received another identical prescription.

Monitoring of this step involves the periodic assessment of the level of consumption of SPRA (legal and illegal) by specific means.

3.6. Many practitioners have urged the creation of a treatment guide for patients diagnosed with SUDs, yet there is no clear project for editing such an important work.

The participants in drafting this guide will be practicing physicians and academics with experience in drug addiction therapy. Once finalized, the guide can be submitted for approval by the Ministry of Health to implement its recommendations at the national level.

The methodology involves the systematic analysis of the literature dedicated to the efficacy and tolerability of pharmacological and psychosocial treatment methods in the case of patients with SUDs.

Monitoring will be done through periodic reassessments of the level of recommendations for each diagnosis, corresponding to epidemiological data at the national level and data from pharmaco-clinical research at the international level.

Table 2 presents the grading of recommendations formulated in the previous chapters, and specific observations have been mentioned whenever possible.

Table 2: Grading the recommendations for the prevention and treatment of SUDs

Recommendation	GRADE	Observations
1.1. Psychoeducational interventions	A	Such interventions should target not only vulnerable populations but also caregivers and other personnel involved in the healthcare/education of vulnerable groups
2.1. Secondary prevention	B	A comprehensive bio-psycho-social approach is strongly recommended
2.2. Guidelines for good practices in drug use prevention	C	Although there are multiple guidelines available worldwide, it was not demonstrated that guideline-focused interventions were significantly superior to other interventions

3.1. Training of specialists in the field of addiction therapy	B	All mental health specialists involved in the healthcare of patients with SUD should participate in such training
3.2. Intensification of harm-reduction interventions	C	The long-term efficiency of this intervention is still to be demonstrated
3.3. Enhancing the healthcare system's capacity for ensuring adequate detox treatment	B	National policy focused on funding and developing hospital resources for this intervention is needed
3.4. Post-detox treatment	B	Same observation as above
3.5. Reducing patient access to DOAs	B	National policies in this domain are needed
3.6. The need for a treatment guideline for patients diagnosed with SUDs	C	The guideline-focused interventions still need to be proven superior to other interventions

DISCUSSIONS

The recommendations formulated in the present paper may be considered a first draft for a framework that integrates multiple components to assist patients with SUDs. Although several therapeutic guidelines and good practices have been explored here, their recommendations and observations cannot be extrapolated to any country due to specific, regional aspects regarding the peculiarities of drug use, demographic data, local laws and healthcare policies, etc. This is an argument for constructing a more comprehensive national policy for the prevention of DOA use and treatment of individuals with SUDs through extending the Romanian "National Drug Strategy 2022-2026" [1] by adding more practical recommendations. The main directions of development in the domain of assisting vulnerable individuals to DOA use refer to psychoeducational measures in the general population, training professionals from different categories (professors, educators, psychologists, social workers, psychiatrists, GPs, etc.), promoting de-stigmatization and fighting against self-stigmatization, ensuring adequate protocols and funding for detoxification and post-detoxification protocols, and formulating general therapeutic recommendations and good practices for patients with SUDs.

This paper's limitations refer to the fact that only two authors scored the level of strength for each recommendation and to the paucity of available data regarding certain aspects of the DOAs' use in Romania, which reduces the ability to formulate clear, evidence-based recommendations on certain topics. Also, this was not a systematic review, so important primary and secondary reports may have been missed. Therefore, as a direction for further research, a comprehensive systematic literature review is expected to consolidate evidence-based recommendations for the prevention and treatment of patients with SUDs and to foster national policies in the domain of fighting against drug addictions.

CONCLUSION

Prophylaxis of DOA use in vulnerable populations, secondary prophylaxis and treatment in patients with SUDs, and educational measures for specialists who are involved in the healthcare of such patients are the pillars of a national policy dedicated to reducing the prevalence of SUDs and this pathology’s negative social, psychological, economic and medical consequences. In order to attain this purpose, multidisciplinary teams are needed to explore different components of the extremely complex phenomenon that is SUD, and to find the most appropriate measures to counter the risk factors and to enhance resilience traits. The anti-drug use policies should target all populational groups, starting from children of school age exposed to the use of NPAs, psychostimulants, or cannabis, to active adult individuals who may become dependent on virtually any DOAs, or patients with psychiatric disorders who may abuse their prescribed medications or might combine them with DOAs, and up to the elderly population who may abuse analgesics or sedatives.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere.

Authors’ contribution

Conceptualization, O.V., V.A.V; methodology, V.A.V., O.V.; software, O.V.; validation, O.V.; formal analysis, V.A.V., O.V.; investigation, V.A.V., O.V.; resources, O.V., V.A.V.; data curation, O.V.; writing—original draft preparation, O.V., V.A.V.; writing—review and editing, V.A.V., O.V.; visualization, O.V.; supervision, V.A.V., O.V.; project administration, V.A.V., O.V. Both authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

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REVIEW

Atypical Fibroxanthoma versus Hemosiderotic Dermatofibroma: Challenges in Differential Diagnosis - Literature Review and Case Report

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Abstract: Atypical fibroxanthoma and hemosiderotic dermatofibroma are both dermal tumors that can raise significant challenges for differential diagnosis. The definitive diagnosis is established based on histopathological appearance and immunohistochemistry. The aim of this paper is to present the differential diagnosis challenges encountered in the case of a 67-year-old male patient who developed within a month, following trauma to the lower third of the right thigh, a large asymptomatic tumor with an irregular outline.

Keywords: atypical fibroxanthoma, hemosiderotic dermatofibroma, cutaneous tumors

INTRODUCTION

Atypical fibroxanthoma, first described in the 1960s by EB Helwig, is a rare spindle-cell dermal tumor with histological features resembling a sarcoma, but with a predominantly benign evolution and an intermediate malignant potential, hence it is considered by some authors to be a form of undifferentiated pleomorphic sarcoma and it is also known as cutaneous pseudosarcoma or paradoxical fibrosarcoma [1-5]. This type of tumor usually occurs among elderly men, following exposure to ultraviolet radiation or radiotherapy, is mainly located at the cephalic extremity and has a rapid development over the course of several weeks or months [2-4].

Dermatofibroma represents a slowly progressing benign nodular dermal tumor associated with post-inflammatory tissue reaction and dermal fibrosis, with malignant potential, predominantly affecting young adult females [6-9]. The lesion is often asymptomatic, although some patients complain of pruritus, discomfort or pain and it is characteristically localized on the extremities – especially the lower limbs, although cases with osseous, periorbital, gastrointestinal, genitourinary or intracranial localization have also been reported [6,7].

The aim of this paper is to present the differential diagnosis challenges encountered in that category of cases, illustrating the disscussion with the case of a 67-year-old male patient who developed within a month, following trauma to the lower third of the right thigh, a rather large asymptomatic firm tumor with an irregular outline.

The diagnosis of both atypical fibroxanthoma and dermatofibroma requires going through several steps, starting with the information provided by the patient’s history and physical examination, and complementing those with imaging investigations, which provide additional information regarding the location and involvement of adjacent tissues. Nevertheless, the definitive laboratory examinations are

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histopathology and immunohistochemistry. In this case, histopathology showed changes suggestive for an atypical fibroxanthoma; the diagnosis was also supported by clinical criteria (macroscopic appearance, conditions of appearance, location, rapid progression to remarkable dimensions). Immunohistochemistry, however, established the diagnosis of a hemosiderotic dermatofibroma, thus changing the prognosis of the disease.

DISCUSSION

Definition

Atypical fibroxanthoma is defined as a dermal tumor with intermediate malignant potential, characteristically appearing among elderly men, following exposure to ultraviolet radiation (UV) or radiotherapy, predominantly located on the cephalic extremity, with rapid development over a period of several weeks or months [1-5]. However, cases have also been reported in young people without any associated risk factors [1,10].

Dermatofibroma, also known as superficial benign fibrous histiocytoma, represents a frequently asymptomatic, slowly progressing, benign, usually small nodular dermal tumor with malignant potential, predominantly affecting young adult females, with characteristic localization on the extremities - especially the lower limbs [6,7,8,11]. Although it most often appears as a hyperkeratotic solitary nodule with a diameter of approximately 0.3 - 1 cm, dermatofibroma is known for its polymorphous clinical and histopathological presentation, with various types of dermatofibromas having been described in specialized literature over the years [6,8].

Etiopathogenesis

In the case of atypical fibroxanthoma, there have been discovered UV-induced mutations of the p53 gene, which determine a decrease in the DNA ability to repair, but it has also been linked to trauma, radiotherapy, xeroderma pigmentosum and other diseases with defective DNA repair, as well as immunosuppressive disorders such as HIV and diabetes [1,2,3,12].

Regarding dermatofibroma, although its etiopathogenesis is incompletely elucidated, an association with trauma, especially with arthropod stings and tattoos, has been noted, although this hypothesis has been questioned following cytogenetic studies, which, along with the clinical evolution, recurrence frequency, and metastatic potential, represented arguments supporting the neoplastic origin of the lesion [6,7,13-15]. In the multiple eruptive variants, recorded in 0.3% of patients, immunological abnormalities are considered to play a role in the onset of the disease [6,7]. Thus, among patients with multiple lesions, a frequent association with HIV infection and systemic lupus erythematosus has been noted, or, more rarely, with immunosuppression following heart transplantation and conditions such as dermatomyositis, atopic dermatitis, myasthenia gravis, Hashimoto's thyroiditis, Basedow-Graves disease, Down syndrome, cutaneous T-cell lymphoma, leukemias, multiple myeloma, and myelodysplastic syndrome [6-8,16-23]. Additionally, antiretroviral agents and biologic therapy with monoclonal anti-CD11 antibodies have been implicated in the onset of the disease [7,8,24].

Clinical Presentation

Atypical fibroxanthoma presents as an erythematous nodular lesion, often ulcerated, with a moist appearance, located in sun-exposed regions, with erythematous, atrophic and telangiectatic skin [1,2,25]. Although the preferred location is on the head and neck region, cases with tumors located on the trunk and limbs have also been reported [1-4]. The tumor rarely exceeds 3 cm in diameter, and due to its rapid evolution patients often present within up to 6 months of onset [1-4]. In exceptional cases, association with xeroderma pigmentosum has been reported [1].

Dermatofibroma presents itself as a "button-like" papular lesion of firm, elastic consistency, with a diameter of 0.5-1 cm, surrounded by a discrete halo, covered with fine scales, non-adherent to deep planes, predominantly located on the limbs [6,7,8,11,26,27]. Dermatofibroma is covered with variously-colored teguments, often brown, although they can vary from pink-gray to black, its color being often attributed to the presence of hemosiderin, although recent studies have found an increased expression of stem cell factors and increased melanocyte tyrosinase at the lesion site [6,7,11,26,27]. Lateral compression reveals the presence of a central dimple, which is considered a clinically useful sign in differential diagnosis [6,7,28,29].

A series of clinical-pathological variants of dermatofibroma have been described (common, cellular, aneurysmal, hemosiderotic, epithelioid, lipidized, atrophic, clear cell, balloon cell, signet-ring cell, granular cell, atypical, palisading, keloidal, lichenoid, myxoid), among which the variants presented below in table 1 are included [6-9,23,26,30,31].

Upon dermatoscopy, atypical fibroxanthoma does not present specific features – Bugatti and Filosa described regions of white color, specific for fibrosis and healing in the middle and deep dermis, as well as an atypical polymorphic vascular network, with a punctate, linear, hairpin or arborescent vascular pattern diffusely spread over the tumor surface [1-4,32].

Dermatofibroma has a reticular-circular dermatoscopic appearance, with a regular pigmentary network, rare black dots and brown globules, with an imprecisely defined central white patch [6,7,8,33,34]. Occasionally, multiple white spots can be seen, resembling a galactic image according to some authors [6-9,23].

Histopathological examination

Atypical fibroxanthoma presents as an exophytic, non-encapsulated, relatively well-defined dermal tumor, surrounded by an epidermal collar, composed of large spindle cells, anaplastic or with a pleomorphic appearance similar to histiocytes, often appearing multinucleated, chaotically spread or occasionally organized in bundles, with an increased number of mitotic figures and cellular atypia [1-4,32]. Histiocyte-like cells often contain lipids and hemosiderin [1,3]. The lesion may extend to the adipose tissue, deforming adjacent structures, but without invasive characteristic [1-4]. Rarely, atypical granular or clear round-oval cell variants without mitotic figures have been described, features that, along with the presence of hemosiderin, may lead to difficulties in diagnosis, with atypical fibroxanthoma potentially being confused with a high-grade sarcoma or a malignant melanoma [1-4]. Electron microscopy demonstrates the fibrohistiocytic nature of the tumor [2,35].

Table 1: Clinical-histopathological variants of dermatofibroma [6,7,8,9,23,26,30,31].

Type of dermatofibroma	Characteristics
Cellular dermatofibroma	Less than 5% of cases More often among young males Cases have been reported in children Preferential localization on the limbs Present in other regions (head, neck, fingers) Of larger size (usually up to 2 cm, but lesions of more than 5 cm have been reported) Recurrence rate of circa 25% Potential for metastasis reported in a few cases
Aneurysmal dermatofibroma	Can be confused with a tumor of vascular origin Exhibits the particularity of rapid growth Reaches considerable dimensions Recurrence rate of circa 19%
Atypical dermatofibroma	More frequently among young males A papule, nodule or plaque of up to 1.5 cm Preferentially localized on the lower limbs Recurrence rate of about 14% Potential to metastasize
Epithelioid dermatofibroma	Found especially among young women Polypoid highly vascularized lesions Can resemble an ulcerated pyogenic granuloma Usually located on the limbs
Hemosiderotic dermatofibroma	Bluish or pink tumor Can be confused with melanoma or vascular tumors More frequent among young males Possibly an early variant of aneurysmal dermatofibroma Highly vascularized, small vessels Extravasated erythrocytes Intra/extracellular hemosiderin deposits

Dermatofibroma is a non-encapsulated tumor located in the mid dermis, composed of spindle cells organized in bundles surrounded by abundant collagenous deposits around which they seem to form a peripheral mold [6-9,23]. Moreover, the lesion is characterized by epidermal acanthosis, pseudoepitheliomatous hyperplasia, basaloid proliferation, rich pigmentation due to increased iron or melanin concentration, and the presence of a Grenz zone demarcating the normal papillary dermis from the overlying tumor [6-9,23,36]. Also, a proliferation of histiocyte-like or fibroblast-like cells associated with an abundant inflammatory infiltrate can be noted, as well as the presence of foamy macrophages, siderophages, and giant multinucleated cells [6-9,23,36]. Usually, neighboring structures are not affected, but in cases with storiform disposition, these may be invaded, suggesting a dermatofibrosarcoma protuberans [6,37].

Immunohistochemistry

Atypical fibroxanthoma is positive for vimentin, CD74, CD99, CD10, pro-collagen 1 and p53 and does not react with cytokeratin, MNF116 and AE1/AE3, S-100, desmin and smooth muscle actin, allowing differentiation from squamous cell carcinoma, malignant melanoma and leiomyosarcoma [1-4,38].

Dermatofibroma exhibits variable expression of factor XIIIa, CD34, stromelysin 3, transforming growth factor beta (TGF-β) I and II receptors, insulin-like growth factor-binding protein 7 (IGFBP7) binding protein 7, D2-40, cathepsin K and the CXCR4 cytokine receptor. Nestin is positive in only 13% of cases, and Cthrc1 (collagen triple helix repeat containing-1) is rarely present, aiding in differentiation from dermatofibrosarcoma protuberans [6-9,23,39-42].

The immunohistopathological features of various types of dermatofibroma are illustrated in Table 2, below [6-9,23,39-42].

The diagnosis of atypical fibroxanthoma is one of exclusion, primarily requiring differentiation from entities such as dermatofibrosarcoma protuberans, malignant melanoma and squamous cell carcinoma, which is mainly achieved through histopathological exam and immunohistochemistry [1-4].

In the case of dermatofibroma, the differential diagnosis is made with numerous conditions, including benign or malignant tumors (leiomyoma, lipoma, leiomyosarcoma, dermatofibrosarcoma protuberans, carcinomas, malignant melanoma, cutaneous T-cell lymphoma, keratoacanthoma, angiohistiocytoma with multinucleated cells, hemosiderotic hemangioma), cutaneous metastases, various forms of cutaneous nevi (Spitz, blue nevus, dysplastic nevus, melanocytic nevus), atypical fibroxanthoma, cutaneous manifestations of various conditions such as HIV infection, reticulohistiocytosis, mastocytosis, prurigo nodularis, etc [6-9,23,39]. Although the clinical appearance is suggestive in typical cases, the definitive diagnosis is established based on histopathological appearance and immunohistochemistry.

Table 2: Immunohistopathological features of dermatofibroma [6-9,23,39-42]

Type of dermatofibroma	Histopathology and immunohistochemistry
Cellular dermatofibroma	Epidermal hyperplasia Highly cellular Lower polymorphism Composed of spindle cells with eosinophilic cytoplasm and storiform disposition Variable mitotic index Necrosis in 12% of cases Frequent focal invasion of subcutaneous tissue, with a lacy pattern Often positive for CD34, factor XIIIa and actin
Aneurysmal dermatofibroma	Extensive hemorrhage Pseudovascular spaces similar to prominent caverns, without being lined with endothelial cells Mitotic index varies Often positive for CD34, factor XIIIa and actin
Atypical dermatofibroma	Mono- or multinucleated spindle-shaped or histiocyte-like cells Pleomorphic, with mitotic figures and cellular atypia
Epithelioid dermatofibroma	Cells with abundant eosinophilic cytoplasm and vesicular nuclei Often with a prominent vascular component Can be confused with Spitz nevus (unlike the latter, involvement of the dermo-epidermal junction cannot be observed) Positive for ALK (anaplastic lymphoma receptor tyrosine kinase) Negative for S-100
Hemosiderotic dermatofibroma	Abundant hemosiderotic deposits Often positive for factor XIIIa Variably positive for CD34, usually negative tumor cells and positive endothelial cells Negative for ALK

The **treatment** of choice is surgical for both conditions. In the case of atypical fibroxanthoma, Mohs micrographic technique can be used if the lesion is not well delimited, and given the risk of local recurrence and lymphatic dissemination, this procedure is increasingly recommended for macroscopically well-defined tumors [1-4,43-46].

In the case of dermatofibroma, special attention is given to cellular, atypical and aneurysmal variants, due to the risk of local recurrence, as well as metastasis in the first two variants [6-9,23,47,48]. Conventional excisional biopsy is the most commonly used technique, although cases successfully treated with cryosurgery, CO_2 or pulsed-dye lasers have been reported [6-9,49,50]. Intralesional corticosteroid injection has also been reported, but the results have been unsatisfactory [6-9,23,39].

Evolution and prognosis

Atypical fibroxanthoma mostly presents a benign course, but the risk of an unfavorable outcome requires careful patient monitoring [1-5]. Last but not least, sun exposure should be avoided, and it is recommended to use photoprotection [1-4].

For the same reasons as with atypical fibroxanthoma, surveillance of patients with dermatofibromas is recommended when there is suspicion of an aggressive clinical-histological variant. On average, the rate of local recurrences was estimated at about 20%, and in the specialized literature, metastases of over 5 cm in size with various locations, including pulmonary, have been reported in variants with increased malignant potential [6-9,23,51]. However, rare cases of spontaneous regression have also been reported, with lesions leaving behind lasting post-inflammatory hypopigmentation [6-9,23]. In the case of multiple eruptive variants, screening for possible associated autoimmune conditions is recommended [6-9,23,52].

CASE REPORT

We present the case of a 67-year-old patient of urban origin, with no significant hereditary or personal pathological history, who requested dermatological consultation after having developed a post-traumatic tumor in the lower third of the right thigh over the course of one month.

Upon clinical examination, a seemingly subcutaneous, round-oval mass with irregular contours, with a diameter of about 8/9 cm, was noted. The lesion was painless, firm and immobile upon palpation, with overlying violaceous-colored skin (Figure 1). No locoregional adenopathy was detected and there were no subjective complaints.

Figure 1: Hemosiderotic dermatofibroma, external aspect of the right thigh's distal third

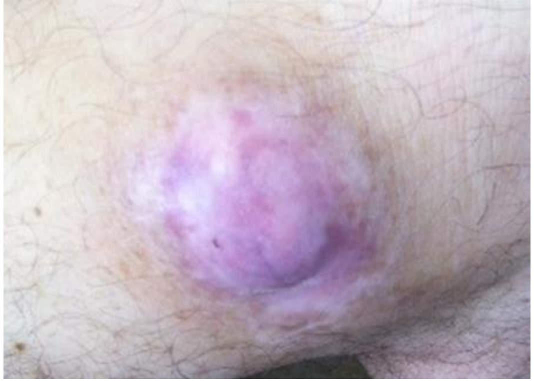

The patient was referred to a general surgery department, where imaging investigations were performed, including plain radiography (Figure 2) and an MRI examination (Figures 3-5), which showed a space-occupying process developed on the external aspect of the right thigh's distal third, in the subcutaneous cellular tissue, in contact with the vastus medialis tendon; it measured approximately 45/40/43 mm, showed heterogeneous signal, diffuse contours and intense and heterogeneous post-contrast enhancement. The lesion was surrounded by vascular tracts, deformed the affected region, was bordered by adipose tissue and exhibited infiltrative characteristic.

Figure 2: Plain radiography showing unaffected bone structures

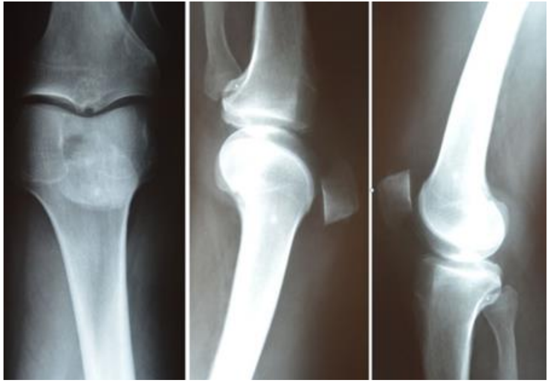

Figure 3: MRI aspect of a large hemosiderotic dermatofibroma of the thigh

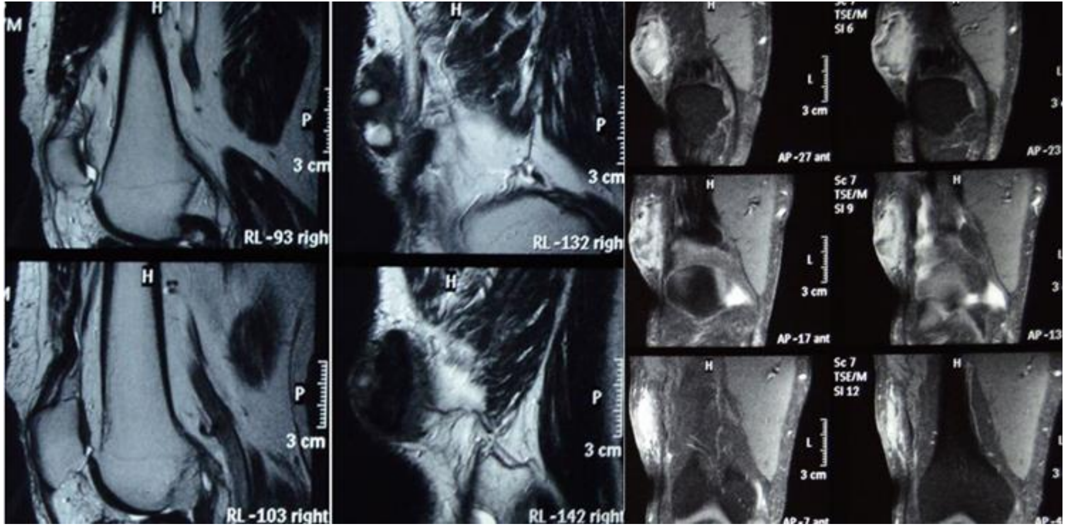

Regarding other paraclinical exams, the complete blood count and blood biochemistry were within normal limits upon admission. Electrocardiogram showed sinus rhythm and did not identify acute end-phase changes and the cardiopulmonary X-ray revealed no abnormalities.

The patient underwent surgical excision of the tumor formation, with complete macroscopic resection, and the post-operative evolution was favorable.

Figure 4: MRI aspect of a large hemosiderotic dermatofibroma of the thigh

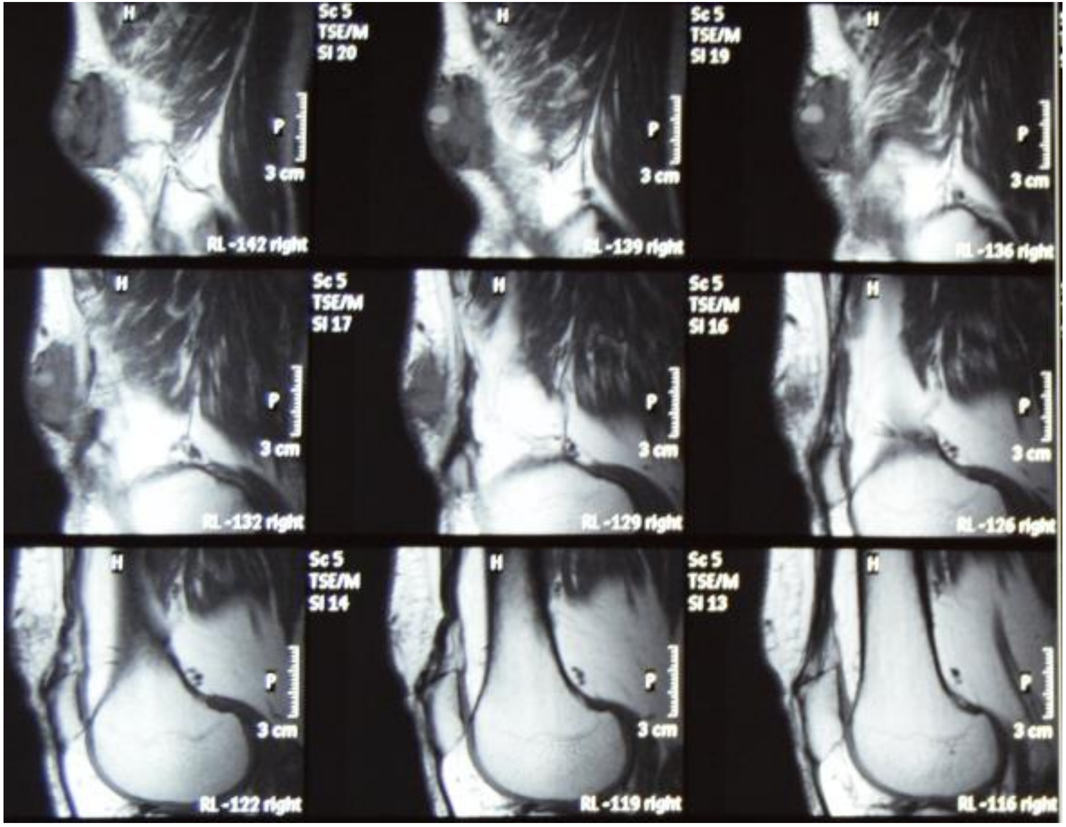

Figure 5: MRI aspect of large hemosiderotic dermatofibroma

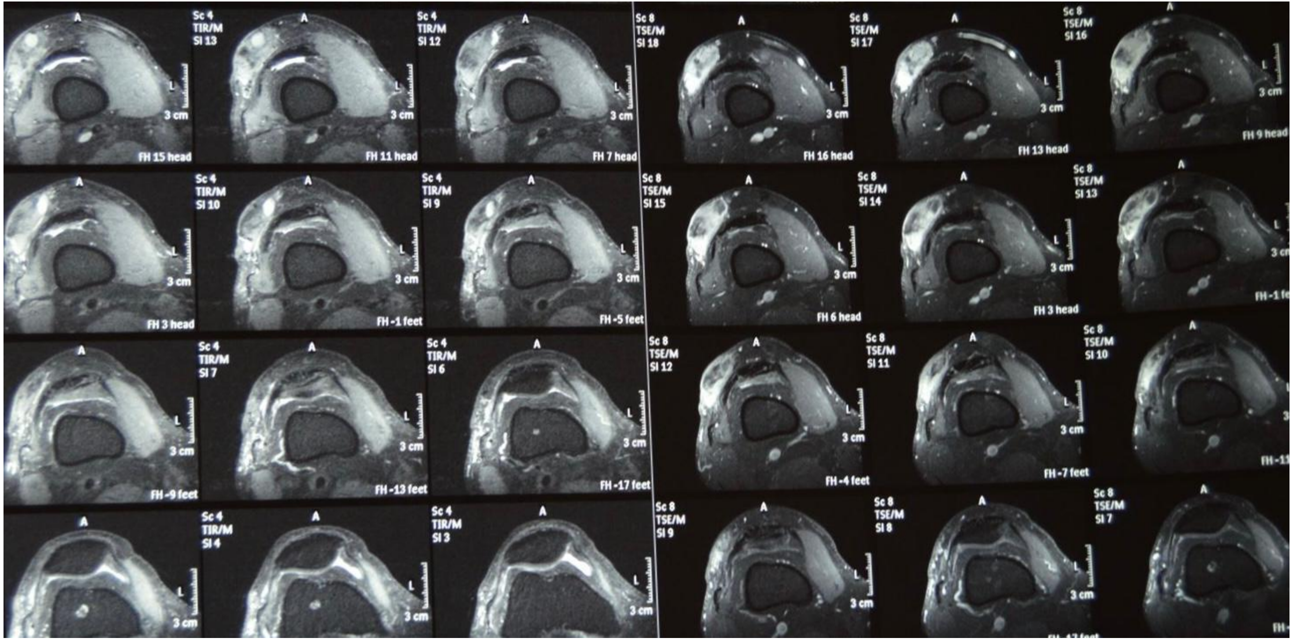

The specimen was sent for histopathological examination (Figures 6-9), which revealed a dense cellular tumor proliferation within the dermis, composed of pleomorphic round-oval cells with a vaguely storiform disposition, separated by a rich capillary network and numerous foamy macrophages, isolated and grouped, with a reduced mitotic index and dense hemosiderin deposits. The proliferation was separated from the overlying skin by a Grenz zone, with minimal interstitial lymphocytic infiltrate. The adipose tissue and examined muscle tissue showed no tumor invasion (Figures 6-9). The described histopathological features could be compatible with an atypical fibroxanthoma, but immunohistochemistry was recommended to confirm the diagnosis.

Figure 6: Hemosiderotic dermatofibroma - (a) hematoxylin-eosin and (b) reticulin (4x)

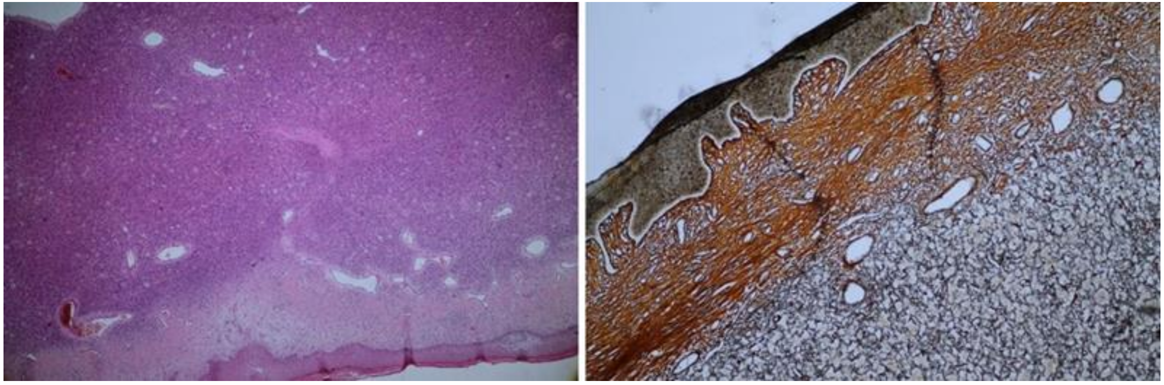

Figure 7: Hemosiderotic dermatofibroma - hematoxylin-eosin (20x and 40x)

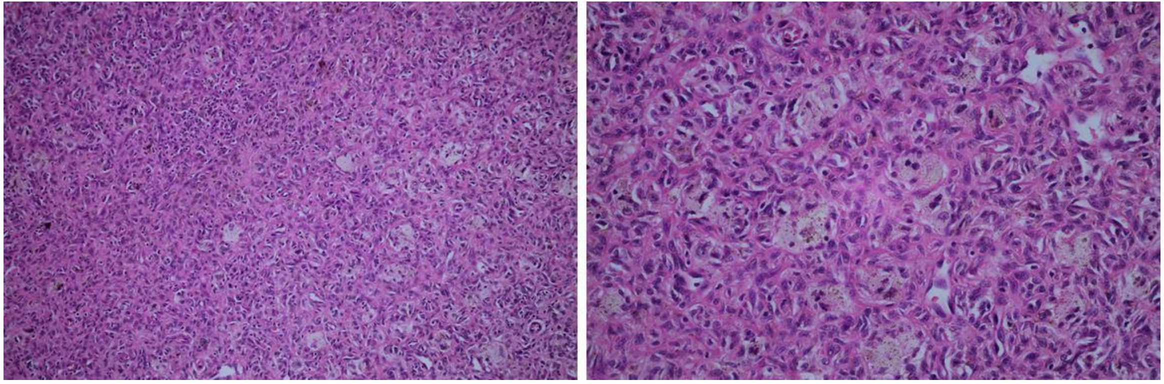

Figure 8: Hemosiderotic dermatofibroma - Van Gieson (4x and 20x)

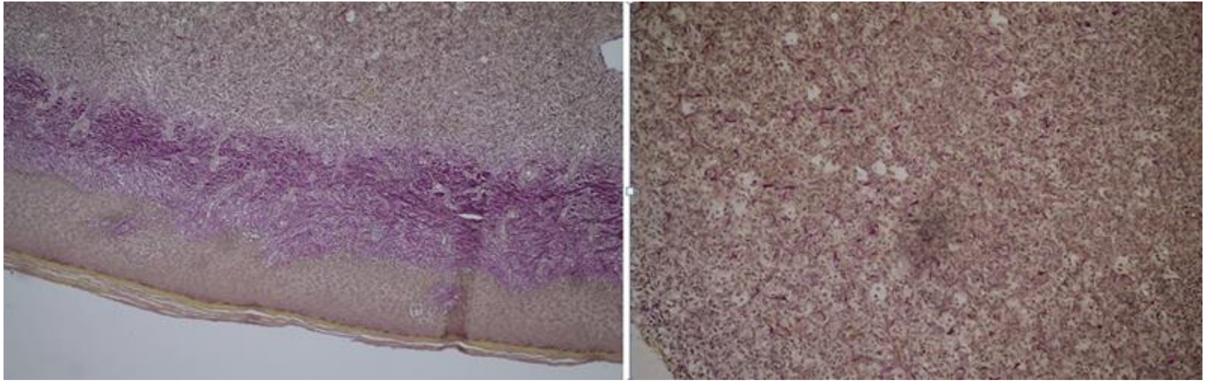

Figure 9: Hemosiderotic dermatofibroma - Pearls (10x and 20x)

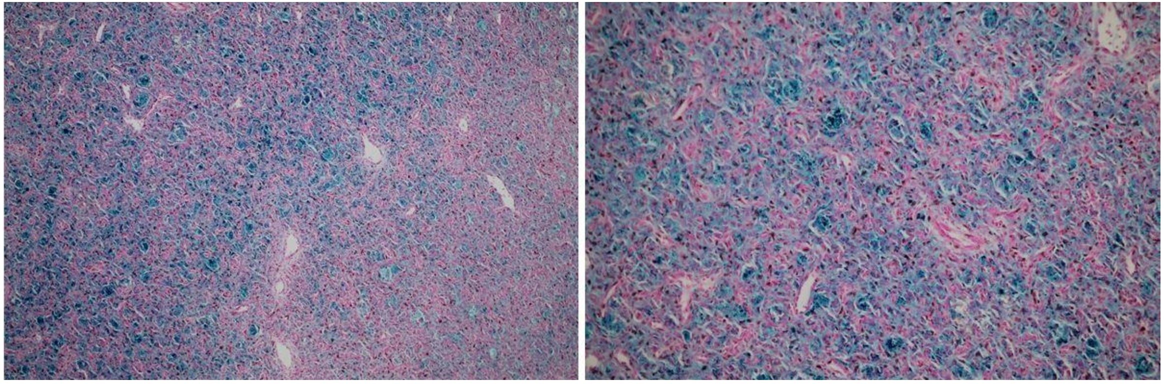

Immunohistochemical examination revealed diffuse positive staining for factor XIIIa in tumor cells, negative staining for CD34 in tumor cells and positive staining in vessels, positive staining for ACT in vascular walls, negative staining for CK 19 in tumor cells and positive staining for Ki67 in 3-5% of tumor cells. Thus, the immunohistochemical examination, in association with the histopathological aspect, supported the diagnosis of hemosiderotic dermatofibroma.

The evolution of the presented patient was favorable, with no signs of local or regional recurrence that could be observed during a 1-year follow-up period. Upon discharge, the patient received recommendations to avoid local trauma, exposure to harsh weather conditions and ultraviolet radiation, as well as careful monitoring, with the indication to seek specialized medical service in the event of any worrisome changes being noted.

CONCLUSION

Although establishing a clinical diagnosis of dermatofibroma is facile in typical cases, benign fibrous histiocytomas are tricky lesions, with plenty of surprising cases that raised differential diagnosis problems having been reported in specialized literature. The most reliable diagnosis is made by correlating histopathology with immunohistochemistry findings.

The particularity of the case consisted of the clinical appearance of the lesion, especially in terms of its size and rapidly progressive evolution, as well as the relatively rare clinicopathological variant. Also, given that the literature specifies a predominance of young adults, the age of the presented patient can be considered an atypical feature.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere.

Authors' contribution

Conceptualization, S.P. and A.B.; methodology, Ș.D. and Z.J.K.; software, S.P. and D.G.C.; validation, Ș.D. and Ș.B.; investigation, S.P., Ș.D. and Ș.B; resources, H.M. and D.G.C.; data curation Z.J.K. and M.A.P.; writing—original draft preparation, S.P.; writing—review and editing, M.A.P. and A.B.; visualization, H.M. and G.P.G; supervision G.P.G and S.P.; project administration, A.B. and G.P.G. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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ORIGINAL ARTICLE

Paneth Cells: A Comprehensive Review of Their Role, Prevalence and Molecular Mechanisms in Colorectal Neoplastic Lesions

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Abstract: Paneth cells (PCs) are specialized epithelial cells located at the base of the crypts of Lieberkühn in the small intestine and colon, that have been occasionally identified in colorectal adenomas and adenocarcinomas. Their presence in these lesions has raised questions regarding their role in colorectal neoplasia and their potential association with adenoma burden. To understand their potential role in carcinogenesis, we conducted an extensive review of the available literature on PubMed, focusing on the occurrence, molecular mechanisms, and implications of Paneth cells in neoplastic lesions of the colorectum. This review synthesizes current findings and discusses the potential of Paneth cells as diagnostic biomarkers and therapeutic targets in colorectal cancer.

Keywords: Paneth cells, colorectal adenoma, serrated, cancer, neoplasia

INTRODUCTION

Paneth cells are highly secretory cells with a lifespan of approximately two months [1], crucial in maintaining gut health and innate immunity [2]. The best-known function of PCs is controlling the microbiome composition, but they have much broader functions. These cells secrete antimicrobial peptides, such as defensins and lysozymes, which help protect the intestinal lining from pathogens. PCs also support intestinal stem cells by providing essential growth factors, ensuring continuous renewal of the gut lining. Some authors propose that Paneth cells serve as a niche for stem cells [3-5], due to their location and expression of factors that support the niche (Notch, Wnt, growth factors).

Paneth cells are typically found at the base of the crypts, along with enteroendocrine cells and stem cells, and have distinct histological features: they have a triangular shape, bright red cytoplasmic granules, containing antimicrobial peptides, and basally located nuclei (Figure 1).

In the small intestine, the abundance of PCs increases progressively from the proximal to the distal regions, aligning with heightened bactericidal activity [6]. In contrast, in the colon, their numbers decline from the proximal to the distal areas and are absent in the left colon and rectum under normal conditions. Paneth cells in the left colon could be a sign of chronic mucosal injury (Figure 2). Their dysfunction has been linked to various gastrointestinal disorders, including inflammatory bowel disease (Crohn's disease and ulcerative colitis) and graft-versus-host disease [7,8].

Human defensin 5 (HD-5) has been previously shown to be a specific marker of Paneth cells in the gastrointestinal tract and one of the target genes of the APC/β-catenin/Tcf pathway [9].

METHODOLOGY

A comprehensive search was conducted on PubMed using keywords such as "Paneth cells", "colonic adenomas", "serrated", and "colorectal cancer" (CRC). Relevant studies published from 1965 to 2024 were selected, including original studies and case reports. A total of 49 studies were found, out of which 14 articles were

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considered relevant to our review, focusing on the role of Paneth cells in early neoplastic lesions and their potential as biomarkers in colorectal carcinogenesis.

Figure 1: Paneth cells located at the base of a normal-looking small bowel mucosa (HE stain, 100x).

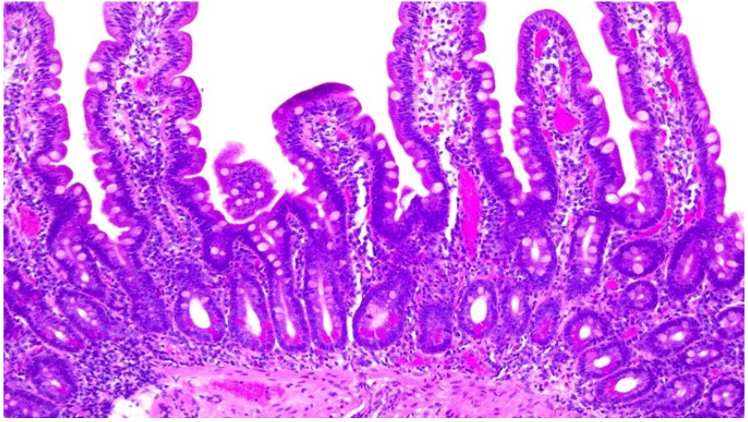

Figure 2: Paneth cell metaplasia in the left colonic inflamed mucosa of a patient with ulcerative colitis (HE stain, 400x).

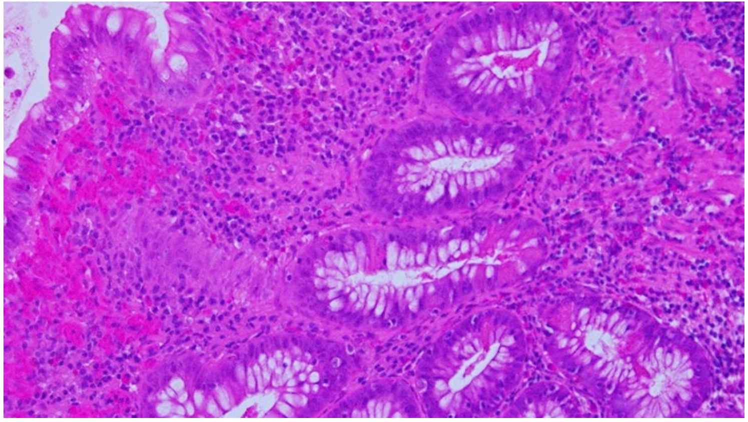

RESULTS

A summary of our research is presented in Table 1, which includes the main findings regarding the presence of PCs in neoplastic lesions of the colorectum, based on original studies.

PCs have been described in colonic epithelial neoplasms, particularly in adenomas (Figures 3A and 3B). Based on the studies we reviewed from PubMed, Paneth cells were encountered in colorectal adenomas. As one would expect, due to their normal distribution in the colon, PC differentiation is more frequently observed in adenomas located in the proximal colon and in patients with familial adenomatous polyposis [9-11]. The reported prevalence of PC differentiation in adenomas ranges widely from 0.2% to 50% in our research [9,12-19].

Figure 3 (A, B): Presence of Paneth cells in a tubulo-villous adenoma with low-grade dysplasia (HE stain, 100x and 400x)

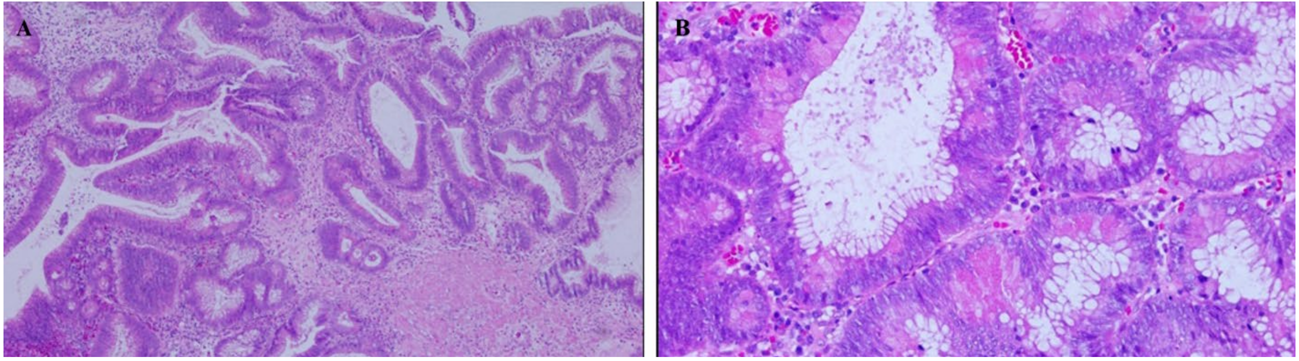

In 1984, Bansal and Fenoglio identified PCs (described as “Paneth cell metaplasia”) in 0.2% of colonic adenomas among 3,215 patients, which was the largest number of patients included in the studies that we identified. They proposed that PCs in colonic adenomas represent the terminal differentiation of neoplastic cells. However, since their study, limited research has been conducted to evaluate the presence of PCs in relation to colonic neoplasms. As a result, it remains unclear whether PC differentiation is associated with specific types of colonic tumors.

Table 1: Main findings regarding the presence of PCs in neoplastic lesions of colorectum, based on original studies

First Author/Publication year/Reference number	Main findings, including the number of colorectal polyps examined/percent of lesions containing Paneth cells
Gibbs/1967 /[11]	• 69 solitary adenomas/ 27% PCs • 23 adenomas from 11 cases of polyposis coli/34% PCs • 140 CRC/3% PCs
Subbuswamy/1973/[12]	• 5 adenomas and 26 CRC • Describes immature Paneth cells and intermediate cells (with intermediate features between Paneth cells and goblet cells)
Bansal/1984/[13]	• 5770 adenomas/0,2% PCs • Presence of PCs is associated with a tubulovillous architecture • Paneth cell, squamous cell, and melanocyte differentiation can be seen in adenomas when epithelial cells undergo neoplastic alterations
Iwashita/1989/[14]	• 212 adenomas/25% PCs
Rubio/2003 /[15]	• Intense lysozyme expression was found in 9.5% of the hyperplastic polyps, in about 90 % of conventional adenomas and serrated adenomas and in 76.9% of the CRC
Mee Joo/2009/[9]	• 39 adenomas/38,5% PCs; 40 CRC/1% PCs • None of the serrated polyps presented PCs • Coexpression of HD-5 and nuclear β-catenin staining was identified in most conventional adenomas (74.4%, P < .01) • Paneth cell differentiation was more frequent in right-sided adenomas
Pai/2013/[16]	• 152 adenomas/17,1% PCs • Presence of Paneth cells is associated with male sex and adenoma burden
Mahon/2016/[17]	• 1900 adenomas/18,8% PCs • PCs-containing adenomas at the distal colorectum were inversely associated with the presence of synchronous advanced adenomas or CRC
López-Arribillaga/2021/[18]	• 38 adenomas/ about 50% of PCs • Accumulation of PCs in early colorectal adenomas is associated with Beta-Catenin signalling and poor patient prognosis

Lysozyme immunohistochemical positive reaction, a sensitive PCs marker, was identified by Rubio, in 2003, in most colorectal adenomas, in many CRCs and in 9,5% of hyperplastic polyps that he examined, raising the question if intense lysozyme production might herald a possible dysplastic evolution in some hyperplastic polyps [15]. Later it was proven that lysozyme expression is less specific than HD-5 in gastrointestinal tissues [19].

In their study, Mee Joo et al [9] found histologic evidence of PCs differentiation in 38.5% of colonic adenomas and in 1 adenocarcinoma (2.5%) but not in any of the other types of polyps in this study. They also investigate the role of HD-5 and β-catenin in this process, as HD-5 has been previously shown to be a specific marker of Paneth cells [19-21] and is also one of the targeted genes of the APC/β-catenin/Tcf pathway of colon carcinogenesis. APC (adenomatous polyposis coli) is a key tumor suppressor gene, which helps control the Wnt signaling pathway. Its mutation plays a crucial role in the progression of colorectal cancer through the adenoma-carcinoma sequence, both in hereditary conditions like familial adenomatous polyposis and in sporadic cases. The loss of APC function causes β-catenin to accumulate in the cell, which promotes uncontrolled cell growth and the formation of conventional adenomas [22].

HD-5 was expressed in their study in neoplastic tissues, in cells with and without histologic evidence of Paneth cell differentiation, meanwhile in normal colonic mucosa HD-5 was expressed exclusively in normal and metaplastic Paneth cells [9]. They found a significant correlation between nuclear β-catenin and HD-5 immunohistochemical expression in adenomatous and cancerous areas since all neoplastic cells with histologic evidence of Paneth cell differentiation showed nuclear accumulation of β-catenin. This suggests that the activation of APC/β- catenin/Tcf pathway may play a role in Paneth cell differentiation in human colonic neoplasms [9].

Furthermore, in a more recent study, the authors indicated that Paneth cells contribute to the initial steps of cancer progression, and their accumulation in early colorectal adenoma is associated with β-catenin signaling and poor patient outcome [18]. Their immunohistochemical research proved also that PCs in adenomas show positive synaptophysin and membranous c-kit expression compared to non-dysplastic cells in the adjacent colonic mucosa.

Also, the presence of PCs containing adenomas had a strong association with male sex and adenoma burden [16]. No association was identified with the patient’s age, adenoma size, villous architecture, or grade of dysplasia. In their study, Mahon et al showed that there is a strong inverse association between PCs-containing adenomas in the distal colon and the presence of synchronous advanced adenomas or CRC [17].

Concerning colonic serrated polyps, Paneth cells have not been reported in this type of lesion, although available literature on the topic is very limited [9,23]. The absence of Paneth cells in all types of serrated polyps [9] supports the fact that these lesions are biologically heterogenous, characterized by aberrant cell proliferation and differentiation, leading to cancer through a distinct pathway, characterized by BRAF and KRAS mutations [24].

Although Paneth cell differentiation is relatively frequent in adenomas, it is rarely reported in adenocarcinomas of the gastrointestinal system [9,12,25,26].

Dysplasia with increased Paneth cell differentiation is a type of nonconventional dysplasia described in patients with inflammatory bowel disease. Also, Paneth cell differentiation was observed at a lower extent in goblet cell deficient dysplasia [27]. Changes in Paneth cell function have been shown to influence the gut microbiota, which in turn may impact the progression of colorectal cancer. Dysbiosis could exacerbate Paneth cell dysfunction, creating a feedback loop that drives tumor progression.

CONCLUSION

The presence of Paneth cells in the colon is often associated with pathological states such as inflammation and neoplasia. The appearance of Paneth cells in colonic neoplastic lesions reflects alterations in epithelial differentiation and could serve as a marker of early neoplastic transformation.

Molecular mechanisms governing Paneth cell differentiation, including the Wnt/β-catenin signaling pathway, significantly contribute to their presence, especially in colonic adenomas. Further research is needed to better understand the mechanisms underlying Paneth cell plasticity and their role in tumor progression. Given their potential to serve as early biomarkers for neoplastic changes, Paneth cells may offer significant clinical utility in the early detection, diagnosis, and monitoring of precancerous lesions and colorectal cancer.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration of being published elsewhere.

Authors' contribution

Conceptualization M.A.F and L.G.E.; methodology M.A.F.; software D.E..; validation G.B. and M.C.; formal analysis M.A.F.; investigation M.A.F.; resources L.G.E. and D.E.; data curation M.C.; writing—original draft preparation M.A.F.; writing—review and editing L.G.E.; visualization G.B.; supervision M.C.; project administration L.G.E.; funding acquisition M.A.F. All authors have read and agreed to the published version of the manuscript elaboration.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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ORIGINAL ARTICLE

The Impact of Total Synovectomy on Blood Loss and Knee Function. A Prospective Randomized Study

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Abstract: *Synovial proliferation is a common intraoperative finding during total knee arthroplasty (TKA) and many studies have proposed synovectomy for the reduction of postoperative pain. We included 180 patients that were split into two groups, one which received a total synovectomy (TS) and the other with partial synovectomy (PS). We measured the amount of intra- and post-operative bleeding as well as perceived pain (using the Visual Analogue Scale) and knee function (using the Knee Society Knee Score) at 4 and 12 weeks. The blood loss during the surgical procedure was 367.77 ± 115.71 mL for TS, while the other group recorded 295.55 ± 106.17 mL ($p < 0.05$). Regarding postoperative bleeding, the TS group aspirated 533.77 ± 281.65 mL, which was significantly higher than the PS group (404.44 ± 211.55 mL, $p < 0.05$). No significant differences were recorded between the TS and PS groups regarding pain and knee function at 12 weeks. Total synovectomy demonstrated significantly higher blood loss and lower postoperative hemoglobin levels, even though knee function and pain level did not show improvements. We consider that the decision of performing synovectomy should rely on the clinical indication and, if conditions allow for it, a limited resection should be attempted*.

Keywords: *synovectomy; total knee arthroplasty; osteoarthritis; therapeutic method; pathophysiology; surgical technique; joint replacement*

INTRODUCTION

Osteoarthritis is common in the elderly population and is the most frequent condition affecting the synovial joints, especially the knee. Restricted range of motion, discomfort, and local pain are the main symptoms deriving from progressive cartilage deterioration of the articular surface of the knee joint [1,2]. A decisive role in cartilage degrading is played by chronic inflammation of the synovial membrane, known as synovitis [3,4]. Furthermore, residual synovial tissue can be associated with postoperative impingement and decreased range of motion [4,5].

Total knee arthroplasty (TKA) is regarded as a radical effective treatment for patients with end-stage knee osteoarthritis [6,7]. This intervention is typically reserved for individuals who experience severe, persistent pain and mobility issues that cannot be adequately addressed through more conservative interventions such as anti-inflammatory medications or physical therapy. In advanced osteoarthritis, patients experience continuous pain that is unresponsive to typical anti-inflammatory medications, and radiographic imaging shows a narrowing of the joint space and the formation of bony growths known as osteophytes [8].

During total knee arthroplasty, surgeons often identify synovial proliferation in patients with osteoarthritis or other inflammatory conditions [3,9-11]. This proliferation of the synovial membrane can contribute to inflammation and cartilage degradation, leading to decreased joint function and increased pain. Surgical synovectomy involves excising the proliferated synovial membrane and was shown to improve joint function and reduce pain in patients with osteoarthritis [3,9,10]. However, while this procedure can be effective in reducing pain and inflammation, it also carries risks and potential complications that need to be carefully evaluated on a case-by-case basis.

Usually, the surgeon opts for the type of synovectomy depending on intraoperative assessment or personal preferences. Therefore, some surgeons perform total synovectomy as a mandatory step in the surgical technique of total knee arthroplasty, while other surgeons rather avoid performing total synovectomy because they consider it a substantial factor in postoperative bleeding [12-14]. Some researchers suggested that total synovectomy influences postoperative blood loss and operative time without being beneficial in terms of postoperative pain and knee function [12,15].

Decreasing postoperative discomfort and pain is essential to achieve a fast and efficient recovery and to improve the patient's living quality [16,17]. The primary objective of this study is to assess the influence of synovectomy performed during total knee arthroplasty for knee osteoarthritis on blood loss, postoperative pain, knee range of motion, and function.

MATERIALS AND METHODS

1. Study design

We conducted a prospective single-center randomized controlled trial between January 2022 and June 2022. A total of 180 patients were included and split into two even groups, one which received a total synovectomy (TS) and another with partial synovectomy during total knee replacement (PS). The randomization was performed by an independent doctor using sealed envelopes. The envelopes were stored in a box. The envelopes were drawn before surgery and contained instructions regarding the surgical technique.

The inclusion criteria were patients with late-stage knee arthritis. We excluded from our study patients with revisions and cases requiring a more significant constraint than a posterior stabilized TKA (constrained or hinged knee). We also excluded patients with acute coronary diseases, neurological diseases, infectious conditions, coagulation disorders, and a history of deep vein thrombosis or pulmonary embolism.

This paper was approved by the "Foisor" Orthopaedics Clinical Hospital Ethics Committee of Bucharest, Romania, approval number 6384/2021. Written informed consent was obtained from all patients included in this study. The procedures were conducted by international ethical standards and the 1964 Helsinki Declaration.

A radiological assessment was performed using plain X-rays, including an anteroposterior, lateral, and full weight-bearing lower limb, to develop proper preoperative digital planning.

2. Surgical Technique

All TKA procedures were performed according to a standard protocol by a single highly experienced orthopedic surgeon in our institution, specialized in TKA. The implant of choice was the Zimmer Biomet NexGen implant system. Preoperatively, 1 g of tranexamic acid was administered to all cases, and an additional 1 g after the procedure. All patients received spinal anesthetic with a femoral nerve block. A tourniquet was installed after three minutes of elevating the lower limb and was inflated up to 320 mm Hg before the incision and deflated after the implantation to perform hemostasis. We used a midvastus approach, an extramedullary tibial guide, an intramedullary femoral guide, and a measured resection technique. Wound closure was performed in a layered fashion and all patients received a deep drain. The drain was clamped for three hours and then released and removed 24 hours after surgery. The rehabilitation protocol was similar for both groups and began on the first day after surgery. Physical therapy was continued either at home or in a specialized clinic. All cases received Heparin for deep vein thrombosis prophylaxis for 30 days.

We recorded preoperative data, which included age, gender, knee range of motion, hemoglobin values, bleeding, and comorbidities. Knee function was measured using the Knee Society Knee Score (KSKS). The amount of bleeding was assessed by measuring the postoperative hemoglobin and bleeding during and after the surgery. Intraoperatively we measured the amount of blood aspiration by taking into account the amount of physiological serum used for lavage and the operative time. After the surgical procedure, we measured the amount of blood aspirated through the drains and hemoglobin. All complications were recorded throughout the study. For the functional evaluation, we measured flexion, extension, the Visual Analogue Scale (VAS), and KSKS score after 4 and 12 weeks. A blind independent observer evaluated all the measurements.

3. Outcome measures

The Visual Analogue Scale is a measure used to evaluate the patient's perspective of pain level. It may be dependent on the patient's mental status but is very frequently used to correlate with knee function evaluation [17,18].

The KSKS contains questions in 2 sections: knee score (pain, range of motion, stability) and function (walking distance, ability to climb stairs, walking aids) [19]. Both measures were provided to patients for answering in the hospital and during follow-up visits.

4. Statistical analysis

All values were calculated as mean values ± standard deviation. The statistical analysis was developed using the Student t-test and Mann-Whitney U test for comparison between groups. A p-value lower than 0.05 was considered statistically significant. The data was analyzed for Windows OS using SPSS version 28.0 (SPSS Inc., Chicago, IL).

RESULTS

We recorded 180 cases split into two even groups. The patient characteristics are displayed in Table 1.

The blood loss measured during the surgical procedure was 367.77 ± 115.71 mL for the total synovectomy, while the second group recorded 295.55 ± 106.17 mL of blood loss ($p < 0.05$). Regarding postoperative bleeding, the TS group aspirated 533.77 ± 281.65 mL, which was significantly higher than the PS group, where we recorded an aspiration of 404.44 ± 211.55 mL (Table 2).

Table 1: Baseline characteristics of the study groups

	Total synovectomy	Partial synovectomy	p-value
Age (years)	70.41±7.25	70.8±7.23	0.71
Gender (M/F)	41/49	44/46	0.77
Preoperative hemoglobin (g/dL)	13.24±1.22	13.22±1.18	0.90

Table 2: Postoperative parameters of the study groups

	Total synovectomy	Partial synovectomy	p-value
Intraoperative bleeding (mL)	367.77±115.71	295.55±106.17	<0.05
Postoperative bleeding (mL)	533.77±281.65	404.44±211.55	<0.05
Postoperative hemoglobin (g/dL)	11.43±1.26	11.88±1.22	<0.05
VAS[1] score (4 weeks)	3.39±1.50	3.79±1.32	0.058
VAS score (12 weeks)	1.3±1.01	1.41±0.89	0.43
KSKS[2] score (4 weeks)	82.28±4.09	82.42±4.46	0.83
KSKS score (12 weeks)	87.9±3.7	88.33±4.11	0.47
Operating Room time (min.)	96.5±7.93	92.2±6.37	<0.05

[1] VAS=Visual Analogue Scale; [2] KSKS=Knee Society Knee Score

The mean preoperative hemoglobin was 13.24 ± 1.22 g/dL for the TS group and 13.22 ± 1.18 g/dL for the PS group ($p = 0.90$). Postoperatively, a statistically significant decrease was recorded in the two groups, the hemoglobin dropping to 11.43 ± 1.26 g/dL and 11.88 ± 1.22 g/dL, respectively, ($p < 0.05$). However, after four weeks, the VAS score was 3.39 ± 1.50 in the TS group and 3.79 ± 1.32 in the PS group, while KSKS was 82.28 ± 4.09 and 82.42 ± 4.46, respectively. Both scores' differences between groups were not significant. At the three months follow-up, both parameters showed improvements for both groups. VAS score was 1.3 ± 1.01 for the TS group, while PS patients had a score of 1.41 ± 0.89. ($p = 0.43$) KSKS score showed no statistically significant differences after three months, the results being 87.9 ± 3.7 and 88.33 ± 4.11, respectively ($p = 0.47$). However, we recorded significant differences regarding the operative room time between groups: 96.5 ± 7.93 minutes for the TS group and 92.2 ± 6.37 minutes for the PS group, respectively ($p < 0.05$).

Regarding complications, we recorded 7 cases of deep vein thrombosis in the total synovectomy group and 5 cases in the partial synovectomy group.

We recorded wound complications in three cases in the first group and two patients in the second group. Also, we noticed hemarthrosis following drain removal in 20 cases that underwent total synovectomy and 5 cases with partial synovectomy.

DISCUSSION

The synovial tissue is regarded as a critical component in the locomotor function [20,21]. The synovial tissue of the knee joint plays multiple roles, including the secretion of synovial fluid that lubricates the joint, providing nutrients to the articular cartilage, protection against infection, and removal of waste products from the joint. The aforementioned functions are responsible for the maintenance of the health and proper functioning of the knee joint. On the other hand, progressive joint degeneration, which leads to osteoarthritis, is caused by the involvement of bone, cartilage, and synovial pathological processes [21,22]. For this reason, synovectomy is performed to relieve pain or to attempt the prevention of cartilage and/or bone destruction and to improve knee function [23,24].

During a total knee arthroplasty, total synovectomy may be performed for different reasons. One reason is to remove any inflamed or diseased synovial tissue that may be present in the joint, with the aim of improving joint function and reducing pain and inflammation. Another reason is to prevent the formation of a pseudo capsule, a thick layer of scar tissue that can form around the artificial joint and cause complications. However, total synovectomy may also have drawbacks, such as an increased risk of infection and reduced lubrication and nutrition of the articular cartilage [21,22]. The decision to perform a total synovectomy during a total knee arthroplasty should be taken individually, for each patient, by weighing the potential benefits and risks. Furthermore, proper planning of the procedure is essential to ensure shorter operating times and faster recovery; this includes digital radiological planning, choosing the ideal implant type and model, and controlling the patient risk factors [25-27].

Our study aimed to evaluate the influence of synovectomy on postoperative results and blood loss following total knee replacement. The intraoperative and postoperative blood loss was found to be significantly higher after total synovectomy compared to the partial synovectomy group. We found variable results regarding average blood loss in the literature, which ranged between 300 and 2500 mL [12,28-30]. This variability may be explained by the different surgical techniques, thromboprophylaxis treatments, or implant types that were applied. In our paper, the total synovectomy group had a blood loss from drains of 533.77 ± 281.65 mL for the total synovectomy group compared to the second group, which had 404.44 ± 211.55 mL blood loss. We consider this to be one of the main reasons behind the preference of some surgeons to avoid total synovectomy.

Our study also revealed a significantly lower postoperative hemoglobin level after synovectomy. This result was also recorded in other papers [12,21,31]. However, some studies concluded that even though the bleeding rates are higher, the blood transfusion threshold is not reached when total synovectomy is performed, therefore the two surgical techniques do not yield statistically significant differences in this regard [15,21].

Another significant result of this study was that total synovectomy demonstrated some postoperative improvements but these were not statistically significant compared to the other group and did not show any differences regarding pain relief, despite the considerable blood loss difference. It is well known that the synovium, as well as other articular structures like muscles, joint capsules, or ligaments, is well-innervated and could be a source of pain in osteoarthritis [32,33]. Even though synovectomy reduces knee pain, especially in cases with pathological synovial tissues, the VAS score in our study did not show any significant differences during the follow-ups. Also, the KSKS score showed no significant differences after 4 weeks or after 12 weeks. This result is also reported by other authors who also recorded increased concealed bleeding, higher drainage, and longer operative time in patients that underwent total synovectomy [12]. Moreover, similar studies evaluating the quality of life and complications recorded no significant improvements regarding these parameters [34].

Literature data have indicated that total synovectomy during total knee arthroplasty may be linked to an increased incidence of certain complications such as deep vein thrombosis, hemarthrosis, and wound complications [12,21,33-37]. However, it is essential to note that the available data is currently limited. Therefore, additional investigations with a larger sample size are required to establish a more robust statistical correlation on this subject. Despite this observation, it should be noted that individual patient characteristics and other factors may also contribute to the occurrence of these complications.

One major limitation of our study is that it was conducted in a single center, which may limit the generalization of our results to other study populations. Additionally, we did not evaluate hematocrit levels or blood transfusion requirements, which are important factors that could reveal further associations between total synovectomy and blood loss. Furthermore, we acknowledge that more extended follow-up periods could be beneficial in the thorough evaluation of the functional outcomes and risk of synovial recurrences associated with total synovectomy during total knee arthroplasty. Additionally, it is important to note that our study did not assess the risk of other potential complications that could arise from this procedure. Therefore, while our study provides important insights into the potential risks and benefits of total synovectomy during total knee arthroplasty, additional research with a larger sample size and more extended follow-up periods are needed to fully understand the implications of this procedure for patients undergoing knee arthroplasty.

CONCLUSIONS

Patients with total synovectomy associated with total knee arthroplasty experienced a notably more significant amount of blood loss and lower postoperative hemoglobin levels than the control group. However, no statistically significant differences were observed between the two groups in terms of knee function and pain level. Further multicenter studies with longer follow-up times are required to more comprehensively assess the potential complication rate and risk of synovial recurrence associated with total synovectomy during total knee arthroplasty. Ultimately, more comprehensive research is required to determine the optimal approach to synovectomy and to provide the most beneficial outcomes for patients undergoing total knee arthroplasty.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

M.-A.P. and L.L.P. planned the clinical study and contributed to the concept and design of the study. M.E.G., D.C.C., and A.L. contributed to the data analysis and interpretation as well as to the translation and the critical revision of important intellectual content. C.S. and R.C. contributed to the writing of the manuscript and performed revision, language, and textual editing of the final version. All authors contributed to the original draft preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

Ethics approval and consent to participate

All procedures performed in this paper were by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and according to the national ethical standards. The study received approval from the "Foisor" Orthopaedics Clinical Hospital Ethics Committee of Bucharest, Romania, approval number 6384/2021 (date of approval 10th of December 2021). Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Evaluation of Serum Magnesium Levels in Patients with Hypertension: A Case-control Study

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Abstract: *There are conflicting data on the effects of magnesium on hypertension in the literature. Therefore we aim this study to investigate whether there is a relationship between serum magnesium levels and hypertension in newly diagnosed. This is a case-control study. The patients were selected from the records of patients who applied to the family medicine outpatient clinic between June 2016 and May 2017. A total of 276 patients, 68 patients with newly diagnosed hypertension in the case group and 208 patients without any disease in the control group were included in the study. Binary logistic regression analysis was performed between the case and control groups. The odds ratio for magnesium was 0.1 (0.012-0.810). When the value of the magnesium variable increases by 1mg/dl, the risk of hypertension decreases by 90%. However, in the presence of magnesium as an independent variable, the predictability rate of our model was expected to increase but decreased. The odds ratio for age was 1.14 (1.092-1.186). The odds ratio for exercise was 0.18 (0.03-0.99). The odds ratio for glucose was 1.03 (1.001-1.065). In our study that magnesium deficiency causes an increased risk of hypertension, the predictability rate of our model was expected to increase instead of decrease. These findings show that a cause-effect approach or incidental association cannot explain the relationship between magnesium and hypertension*.

Keywords: *hypertension; magnesium; age; exercise; glucose*

INTRODUCTION

The prevalence of hypertension continues to increase globally [1]. The prevalence of hypertension in the adult population in the world is 31% [2]. For over forty years, the causes of most hypertensive diseases have been unknown and are defined as primary or essential hypertension. Secondary causes are accepted as 5-10% [3].

Recently, risk factors such as age, sex, race, family history, genetic composition, obesity, excessive salt intake, inactivity or lack of exercise, high-fat diet, tobacco use, and alcohol consumption have been proven to lead to the development of hypertension [4].

Magnesium (Mg) can be found in the fourth sequence in the human body and the second after potassium in the intracellular area [5]. Magnesium is an essential cation playing a crucial role in many physiological functions. It is critical in energy-requiring metabolic processes, protein synthesis, membrane integrity, nervous tissue conduction, neuromuscular excitability, muscle contraction, and hormone secretion [6]. Given the role of magnesium in calcium and potassium transport, cell signaling, energy metabolism, genome stability, DNA repair, and replication, it should be expected that hypomagnesemia is associated with many diseases, including neurological disorders, osteoporosis, diabetes, coronary heart disease, and hypertension [7].

Magnesium levels were lower in white hypertensives than in normotensives, while no significant difference was found in black hypertensives compared to normotensives [8]. It has been shown that those with essential hypertension have a significantly higher intra-erythrocytic magnesium level than healthy individuals [9]. A systemic review and meta-analysis study suggested no relationship between serum magnesium and hypertension but that dietary magnesium supplementation reduces blood pressure [10]. Magnesium deficiency was found to be responsible for calcium and magnesium disorders in those with pregnancy-induced hypertension, and magnesium sulfate therapy was effective in these patients [11]. In a meta-analysis of twelve studies, a reduction in diastolic blood pressure was observed, while a decrease in systolic blood pressure was not observed [12]. Again, a meta-analysis of 20 studies found a reduction in systolic blood pressure while no change in diastolic blood pressure [13]. A meta-analysis of 22 studies noted a reduction in both systolic and diastolic blood pressure [14].

The fact that there are conflicting data on the effects of magnesium on hypertension in the literature shows a need for research on this subject. For this purpose, we wanted to evaluate magnesium levels in patients with newly diagnosed hypertension. This study aims to investigate whether there is a relationship between serum magnesium levels and hypertension.

MATERIALS AND METHODS

Study Design

This study is a case-control study. The sample size was determined for logistic regression analysis, considering the number of seven independent variables, such as age, gender, body mass index (BMI), smoking, alcohol consumption, exercise status, and magnesium. For each group, using the power analysis method, the minimum sample size of at least 48 which is needed to detect a significant difference when taken into account at 0.05 type-I error (Alpha), 0.35 effect size, 0,80 power (1-beta).

Population selection

The patients included in the study were selected from those who applied to the family medicine outpatient clinic between June 2016 and May 2017 from the hospital information system and patient records. Sixty-eight people with hypertension constituted the case group. Two hundred-eight people without hypertension formed the control group. The control group was selected considering demographic characteristics such as age and gender.

Inclusion and exclusion criteria

Among the patients tested for magnesium at the time of admission, patients with hypertension newly diagnosed were included in the case group, and those with no chronic disease and no medication were included in the control group. The study did not include those with any illness and drug use before the application, those with a previous hypertension diagnosis and treatment, and those without magnesium checked at the time of application.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (V25). A P-value below 0.05 was considered statistically significant. In the data expression, categorical data, numbers and percentages, and numerical data were expressed with averages and standard deviation. The distribution of demographic data was analyzed by frequency tests, comparison of categorical data by chi-square test, and comparison of numerical data by independent sample t-test. The stepwise enter model was used in the binary logistic regression test to evaluate the effect of magnesium on hypertension. Skewness and kurtosis analyses were used to conform the data to the normal distribution.

Ethical Considerations

Ethical approval was obtained from the İstanbul Training Research Hospital Clinical Research Ethics Committee (Decision no. 14.10.2022, 318).

RESULTS

A total of 276 people, including 68 cases and 208 control groups, were included in the study. There was no found difference between those with hypertension and those without it in terms of sex, smoking, and alcohol consumption. However, the proportion of those without exercise was found to be lower in the hypertension group (Table 1).

Table 1: Comparison of the case and control groups in terms of gender, smoking, alcohol consumption, and exercise status

Characteristics of participants		Hypertension N (%)			
		No	Yes	Total	p
Gender	Women	149 (71.6)	52 (76.5)	201 (72.8)	0.530
	Men	59 (28.4)	16 (23.5)	75 (27.2)	
Smoking	No	167 (80.3)	58 (85.3)	225 (81.5)	0.472
	Yes	41 (19.7)	10 (14.7)	51 (18.5)	
Alcohol	No	204 (98.1)	68 (100.0)	272 (98.6)	0.575
	Yes	4 (1.9)	0 (0.0)	4 (1.4)	
Exercise	No	168 (80.8)	66 (97.1)	234 (84.8)	< 0.001*
	Yes	40 (19.2)	2 (2.9)	42 (15.2)	

Chi-Square Tests: The statistically significant difference was accepted as $p < 0.05$

The average age and BMI were found to be higher in the group with hypertension. Additionally, glucose, total cholesterol, triglycerides, and LDL- cholesterol (LDL-C) were found to be increased in the case group. Magnesium levels were found to be higher in the control group than in the case group (Table 2).

Table 2: Comparison of case and control groups in terms of age, body mass, and biochemical parameters

	Hypertension			
	No	Yes		
	Mean ± SD		t.	p
Glucose (mg/dl)	95.25 ± 11.04	107.79 ± 15.96	-6.02	< 0.001*
Cholesterol (mg/dl)	206.55 ± 44.32	229.43 ± 35.59	-3.86	< 0.001*
Triglycerides (mg/dl)	111.96 ± 61.58	154.07 ± 60.27	-4.92	< 0.001*
HDL-C (mg/dl)	54.55 ± 12.66	52.68 ± 7.40	1.49	0.137
LDL-C (mg/dl)	129.35 ± 37.07	143.49 ± 29.66	-3.19	0.002*
Calcium (mg/dl)	9.47 ± 0.46	9.54 ± 0.43	-1.11	0.266
Magnesium (mg/dl)	1.99 ± 0.19	1.92 ± 0.15	2.82	0.005*
Sodium (mmol/L)	139.14 ± 1.99	145.24 ± 32.97	-1.52	0.133
TSH (mIU/L)	1.91 ± 1.29	1.90 ± 1.20	0.06	0.950
Vitamin B12 (ng/L)	231.69 ± 127.26	207.32 ± 90.38	1.73	0.085
Vitamin D (ng/ml)	20.08 ± 10.69	19.32 ± 9.60	0.52	0.602
Age (years)	39.69 ± 12.80	59.71 ± 11.65	-11.42	< 0.001*
BMI	25.83 ± 4.48	29.50 ± 6.25	-4.47	< 0.001*

*SD: Standard Deviation; BMI: Body Mass Index; *Independent sample t-test is significant at the 0.05 level (2-tailed)*

Binary logistic regression analysis was performed by considering the different parameters between the case and control groups. In addition to the magnesium, age, exercise, BMI, glucose, triglyceride, and LDL-C variables which were different between the case and control groups, gender and sodium variables were also included in the analysis according to the literature [4]. Total cholesterol was not included in the study because it is affected by LDL-C and triglyceride.

In the analysis in which only magnesium as an independent variable was added, the model is insignificant because the Cox-Snell R2 and Nagelkerke R2 values are smaller than the 0.2 value. However, the model is significant when other independent variables are added. Except for the step in which triglyceride was added, the model's goodness of fit is acceptable since the p-values are bigger than 0.05 in the Hosmer-Lemeshow test. Changes in -2 Log Likelihood values (Chi-square, $p < 0.05$) are significant in magnesium, age, exercise, and glucose steps.

These results show that the logistic regression analysis is generally valid (Table 3).

Table 3: Distribution of data showing the validity of the logistic regression analysis

Metod=Enter: stepwise	-2 Log Likelihood	Chi-square	P	Cox and Snell R Square	Nagelkerke R Square	Hosmer and Lemeshow Test	Predicted percentage
Beginning	308.192						75.4
+ Magnesium	299.999	8.193	0.004*	0.029	0.043	0.150	74.6
+ Age	194.958	105.041	< 0.001*	0.337	0.500	0.493	82.6
+ Exercise	186.608	8.350	0.004*	0.356	0.530	0.642	81.9
+ Gender	186.341	0.267	0.606	0.357	0.531	0.592	81.9
+ BMI	183.822	2.519	0.112	0.363	0.539	0.695	83.3
+ Glucose	179.281	4.541	0.033*	0.373	0.555	0.398	84.1
+ Triglycerides	179.265	0.015	0.901	0.373	0.555	0.044*	83.3
+ LDL-C	178.064	1.201	0.273	0.376	0.559	0.744	82.6
+ Sodium	178.059	0.005	0.944	0.376	0.559	0.746	82.6

**The statistically significant difference was accepted as $p < 0.05$*

The odds ratio for magnesium was found to be 0.1 (0.012-0.810). When the value of the magnesium variable increases by 1mg/dl, the risk of hypertension decreases by 90% (Table 4). However, in the presence of magnesium as an independent variable, the predictability rate of our model was expected to increase but decreased (Table 3). The odds ratio for age was found to be 1.14 (1.092-1.186). When the value of the age variable increases by one unit, the risk of hypertension increases by 1.14 times. The odds ratio for exercise was found to be 0.18 (0.03-0.99). When the reference group is taken as those with non-exercise, the risk of hypertension was found to be 82% less in those with exercise. The odds ratio for glucose was found to be 1.03 (1.001-1.065). When the value of the glucose variable increases by 1 mg/dl, the risk of hypertension increases by 1.03 times (Table 4).between vitamin D and total cholesterol and LDL-C in the young age group. However, serum vitamin D had no significant correlation with serum glucose, triglyceride, HDL-C, calcium, magnesium, sodium, TSH levels, and BMI scores (Table 4).

Table 4: Logistic regression analysis showing the relationship between hypertension and variables of magnesium, age, exercise, gender, BMI, glucose, triglyceride, LDL-C, and sodium

Hypertension[a]	B	SE	Wald	P	Risk (Odds) coefficient	95% C.I.for (Exp B)	
						Lower	Upper
Magnesium (mg/dl)	-2.325	1.079	4.645	0.031*	0.1	0.012	0.810
Age (years)	0.129	0.021	37.532	< 0.001*	1.14	1.092	1.186
Exercise (1: -/+)	-1.723	0.874	3.882	0.049*	0.18	0.032	0.991
Gender (1: Women/Men)	-0.628	0.456	1.898	0.168	0.53	0.219	1.304
BMI	0.031	0.040	0.621	0.431	1.03	0.955	1.115
Glucose (mg/dl)	0.032	0.016	4.140	0.042*	1.03	1.001	1.065
Triglycerides (mg/dl)	0.001	0.003	0.019	0.890	1.00	0.994	1.007
LDL-C (mg/dl)	-0.006	0.006	1.172	0.279	0.99	0.983	1.005
Sodium (mmol/L)	0.002	0.034	1.897	0.947	1.00	0.938	1.071

*[a]Dependent Variable: Hypertension; BMI: Body Mass Index; B: Estimated Coefficients; CI: Confidence Interval; SE: Standard Error; *Logistic regression analysis is significant at the p < 0.05 level (2-tailed)*

DISCUSSION

The fact that the age and BMI values, glucose, total cholesterol, triglycerides, and LDL-C levels are high in those with hypertension and the low rate of exercisers shows that the study groups are compatible with the general population.

In our study, magnesium levels were lower in those with hypertension. When the value of the magnesium variable increases by 1mg/dl, the risk of hypertension decreases by 90%. However, in the presence of magnesium as an independent variable, the predictability rate of our model for hypertensive patients decreased, while it was expected to increase [2]. In two meta-analysis studies involving seven and 11 studies, it has been suggested that magnesium supplementation provides a significant, albeit small, decrease in blood pressure [15,16]. In a meta-analysis in which thirty-four studies were evaluated, it was found that there was a decrease in both systolic and diastolic blood pressure [17]. A recent meta-analysis study claimed that oral magnesium supplementation does not affect blood pressure in normotensives and controlled hypertensives but reduces blood pressure in uncontrolled hypertensives [18]. In addition, there was no difference in magnesium levels between those with hypertensive crisis and the control group [19]. However, in light of these contradictory findings, considering that the number of participants in meta-analysis studies is high, it is a point that should not be overlooked that statistical significance will be high, even if there is no significance in reality.

In our study, it was found that when the value of the age variable increased by one unit, the risk of hypertension increased by 1.14 times. In our working model, the probability of predicting hypertensive patients in the presence of magnesium and age is relatively high. This result indicates that the age variable is an important risk factor. In the literature, age is recognized as a risk factor for hypertension [4]. Studies have been reported showing that magnesium decreases with age [20,21]. It has been suggested that this relationship may be related to decreased absorption with age and slower kidney reabsorption [22,23]. In addition, recent reports indicate that many Americans, especially older people, do not consume the recommended daily amount of magnesium [24]. In countries with high incomes, the rate of hypertension is lower than in poor countries [2]. Again, it is stated that an unhealthy diet causes hypertension [25]. All these findings and literature information suggest that the relationship between magnesium and hypertension may be an incidental association.

Our study found that the risk of hypertension decreased by 82% in those who exercised. In the literature, it is accepted that inactivity or lack of exercise is a risk factor for hypertension [4]. It is stated that regular exercise causes a decrease in blood pressure of 5 mmHg [26]. In addition, emphasis has been placed on the curative effect of both aerobic and moderate-weight exercise on hypertension [27]. Aerobic exercise is assumed to positively affect hypertension by reducing oxidative stress [28]. A recent meta-analysis indicates that aerobic exercise is an effective adjunctive treatment method for lowering ambulatory blood pressure in hypertension patients receiving drug therapy [29].

Our study found no relationship between body mass index and hypertension. However, when the value of the glucose variable increased by one unit, the risk of hypertension increased 1.03 times, albeit very slightly. Obesity is accepted as a risk factor for hypertension in the literature [4,30]. Mechanisms of obesity causing hypertension are explained by the activation of the sympathetic nervous system activated by hormones such as leptin, insulin, melanocortin, and the activation of the renin-angiotensinogen system due to oxidative effects caused by proinflammatory cytokines released as a result of dyslipidemia [31]. Many pathophysiological mechanisms explain the relationship between diabetes and hypertension. These mechanisms are mainly hyperinsulinemia-mediated sympathetic activation, hyperglycemia-induced sodium-fluid retention, the stimulatory effect of hyperglycemia on the renin-angiotensin-aldosterone system, and increased insulin resistance as a result of oxidative stress [32,33].

An increased risk of type-2 diabetes and increased LDL-C levels were found in low magnesium [34]. It has been suggested that the positive effects of magnesium on diabetes occur by inhibiting oxidative stress [35]. It is also stated that the primary pathology in gestational hypertension in which magnesium sulfate is used in the treatment is oxidative stress [36]. The positive effects of regular and aerobic exercise on oxidative stress and its curative effects on diabetes and obesity have been emphasized [37]. Both magnesium deficiency and oxidative stress have been identified as pathogenic factors in aging and age-related diseases. Additionally, severe magnesium deficiency has been shown to increase oxidative stress in experimental animals [38]. In light of these findings, it seems complicated to define the relationship of magnesium with hypertension without fully elucidating the complex effects of oxidative stress on hypertension.

CONCLUSIONS

As a result, it was found in our study that magnesium deficiency causes an increased risk of hypertension. However, in the presence of magnesium as an independent variable, the predictability rate of our model was expected to increase but decreased. These findings show that the relationship between magnesium and hypertension cannot be explained only by a cause-effect connection or incidental association. This may explain the reason for the conflicting results in the literature. More detailed analyzes are needed in this regard.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. This research received no external funding.

Authors' contribution

All authors made substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of data for the work. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Because of the retrospective study, patient consent was not obtained.

Ethics approval and consent to participate

Ethical approval was obtained from the İstanbul Training Research Hospital Clinical Research Ethics Committee (Decision no. 14.10.2022, 318).

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REVIEW

Combat Stress in Military Personnel: Theory, Genesis, Prevention, and Control

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Abstract: *On February 24, 2022, the Russian-Ukrainian war began, in which hundreds of thousands of military personnel are participating. Almost all military personnel experience combat stress. In our opinion, the most fully reflecting the occurrence of stress in humans, including combat stress in military personnel, is the conservation of resource theory proposed by S. Hobfoll. According to this theory, stress occurs when: central or key resources (health, well-being, family, self-esteem, and a sense of purpose and meaning in life) are threatened with loss, are lost, or cannot be retrieved following significant effort. Combat stress in military personnel can manifest itself in the form of negative manifestations of the psychological, physical, psychophysiological, and behavioral register. The most effective system for the prevention and control of combat stress among military personnel was developed in the US Army. Such a program should contain medical and psychological work activities carried out in three stages: preparatory (before performing combat missions), the stage of direct performance of tasks in the combat zone, and the final stage (after completing tasks upon returning to permanent deployment points).*

Keywords: *stress, combat stress, military personnel, fighting, COSC*

INTRODUCTION

There are few areas of contemporary psychology that receive more attention than stress [1,2]. This literature reflects researchers' belief that stress is a major factor affecting people's lives, is intimately tied to mental health, and is very possibly linked with many problems of physical health [1]. The interest in stress has also caught the attention of the popular press, illustrating that stress is of concern to the lay public as well as the academic community [3]. However, despite the breadth of interest in the problem of stress, there is little research to substantiate the relevant theories [2]. But in psychology, as in other sciences, it is difficult to create a sincere body of knowledge without a clear theoretical foundation, because there are no definite limits to the theory that can be denied [4].

An analysis of scientific sources on the subject of combat stress has shown that a lot of applied research has been carried out to solve this rather complex problem. This is because the last fifty years have been characterized by a significant increase in local and regional armed conflicts in the world [5-7]. The localization, time, nature, goals, the pace of hostilities, and military operations varied greatly during this period. But, as a rule, the majority of military personnel in their military career took part only in a series of combat deployments [8].

However, in the 21st century, not a single country, except Ukraine, has faced the armed aggression of the Russian Federation, and a large-scale war has been going on for more than a year. Hundreds of thousands of troops from both sides are taking part in the fighting. Almost all Ukrainian servicemen experience combat stress [9-11]. These circumstances, as well as the duration and complexity of staying in extreme (combat) conditions, put forward high demands on the mental health, army values and motivation of military personnel, as well as on their psychological qualities and the adequacy of behavior for this activity [12-18]. Despite the high motivation to conduct combat operations, the improvement of military professional training and psychological preparation, some military personnel have problems with mental health, manifested by the development of acute stress reactions, post-traumatic stress disorders (PTSD), as well as addictive and delinquent behavior, an increase in suicidal manifestations [19-25].

Similar mental, physical, and behavioral health problems have occurred in US and coalition forces during and after combat deployments in Iraq and Afghanistan [5-7,26-30]. In combatants, these disorders manifested in persistent sleep disturbances, headaches, fatigue, and other symptoms associated with the influence of various stress factors in the combat environment, as well as the occurrence of more complex and severe disorders such as PTSD, traumatic brain injury and depression [31-33].

The conducted studies revealed that the significance of the influence on the psyche of military personnel and the development of the consequences of experiencing combat stress were determined by: factors of the combat situation; individual psychological characteristics of a person; the level of professional and psychological preparation for the conduct of hostilities; the nature and level of motivation of military personnel; the influence of combat stress factors on the further life of military personnel, etc. [9,20,28,34,35].

The objective of this study

The purpose of the article was to systematize scientific research on the problem of stress, combat stress, to study the modern system of mental health care for military personnel of the leading NATO partner countries, and to determine the main directions for improving the Ukrainian system for the prevention and control of combat stress among military personnel.

BASIC STRESS THEORIES

All theories of stress, in particular, combat stress, are based mainly on medical, biological, and psychological studies that reflect the processes of a distinct reaction of the human body to stress. In general, the term “stress” was borrowed by researchers from physics: it was previously believed that people are in some sense similar to physical objects, such as metals, resisting moderate external forces, but losing their stability at some point of higher pressure [1]. Walter Cannon was probably the first researcher to apply the concept of “stress” to humans in these kinds of terms [36]. He concluded that a person can resist initial stressors of moderate strength or lower-level negative factors that cause stress. However, prolonged or more severe stressors will lead to disruption of the body’s biological systems [36].

W. Cannon’s research on stress was continued and developed by Hans Selye. He described stress as a reaction (organized defense) of the body to non-specific harmful agents and called it the “general adaptation syndrome” [37]. According to the author’s definition, the general adaptation syndrome is the body’s response to severe damage by non-specific harmful agents (for example, exposure to cold, surgical shock, excessive physical exertion, or intoxication with sublethal doses of various drugs) and it does not depend on the nature of the damaging agent [37]. Thus, the protective role of the general adaptation syndrome consisted in the formation of nonspecific resistance of the organism to pathogenic agents, regardless of their nature. The researcher believed that there is a response of the body to external factors, which go through three stages in succession: anxiety (anxious response), resistance, and exhaustion [38].

Another direction in the study of the mechanisms of development of stress has already taken into account not only responses to stressors but also directly stressful events, as well as individual differences in their assessment. Spielberger suggested that certain events are stressful if they are thought to be threats to the physical self or the phenomenological self [39]. He proved that although individuals with different personalities responded some-what uniformly to physical threats, people’s responses to ego-threats were related to personality traits. In particular, Spielberger noted that people with high trait anxiety tended to react with state elevations in anxiety to ego-threat, whereas those who were low in trait anxiety tended to be comparatively impervious to ego-threats. Thus, the researcher concluded that it is not the stimulus and not the assessment that is important, but their specific interaction. This three-step approach was a conceptual leap for stress researchers but rarely followed up in further research. Instead, many researchers have reverted to models that only assessed stress, almost completely ignoring actual environmental or personality traits; others reflected only personality characteristics, while others remained tied only to the model’s stimuli.

In modern studies of the problem of stress, the homeostatic and transactional models of stress are most often used. The homeostatic model is presented in detail by McGrath [40] but is based in large part on the work of Lazarus [41]. According to research results, McGrath proposed the definition of stress as “a significant imbalance between the needs of the environment and the ability to respond to the body” [40, p. 17].

In 1966, the concept of cognitive appraisal was proposed by Richard Lazarus [41]. According to this theory, stress is perceived as the imbalance between the demands placed on the individual and the individual's resources to cope [2]. The transactional model of stress and coping contended that a person's capacity to cope and adjust to challenges and problems is a consequence of transactions (or interactions) that occur between a person and their environment. The authors defined stress as "a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being" [2, p. 19].

However, according to Hobfoll, these models and their definitions have a number of shortcomings [1]. First, the author notes, these definitions state that stress is not the product of an imbalance between objective demands and the ability to respond, but is the result of the perception of these factors. Secondly, the consequences of failure should be perceived as very important for a person. Third, McGrath suggested that imbalances might manifest as underutilization rather than overload, i.e. too little need for the ability to cope with stress [1].

In our opinion, one of the leading modern paradigms of the problem of stress, which is used by both scientists and practical psychologists in their work, is the conservation of resource theory proposed by Stephen Hobfoll. According to this theory, stress arises from motivation that encourages people to both maintain their current psychological resources and seek new opportunities [1]. Its main principle is that individuals strive to obtain, retain, foster, and protect those things they centrally value. The main postulates of this theory are understanding that stress occurs when: (a) central or key resources are threatened with loss, (b) central or key resources are lost, or (c) there is a failure to gain central or key resources following significant effort [1].

Essentially, the conservation of resource theory is a motivational theory that explains much of human behavior in terms of the evolutionary need to obtain and maintain resources for survival, which is central to the genesis of human behavior [42]. Resources are defined as those objects, personal characteristics, conditions, or energies that are valued by the individual or that serve as a means for the attainment of these objects, personal characteristics, conditions, or energies. According to the authors, people must acquire and maintain both strong personal characteristics and social ties. Thus, people must use key resources not only to respond to stress but also to create a certain amount of supportive resources in case of future needs. Moreover, the acquisition and maintenance of personal, social, and material resources create in people, families, and organizations the feeling that they are able to cope with stressful situations.

According to Hobfoll, a critical tenet conservation of resource theory is that individual value is secondary to what is most valued and universal to people. Typically, these valuable resources are health, well-being, family, self-esteem, and a sense of purpose and meaning in life [42]. How these evaluations are expressed may vary across cultural backgrounds but always reflects the same basic elements. The conservation of resource theory, in general, explains the mechanisms of development and transformation of the psychological safety of personality in military personnel during activities under different conditions [43].

DEFINITION OF COMBAT STRESS

An analysis of scientific research has shown that there is no generally accepted definition of the "combat stress" construct: depending on the field of its study (medicine, biology, psychology, sociology, other social sciences), there are many definitions of this category. Specialists who study stress in military personnel during combat operations suggest using such constructs as combat mental trauma, combat stress, combat mental pathology, post-traumatic stress disorders, and others definitions.

The official website of the US Department of Defense provides the definition of combat stress, also known as battle fatigue, which is a common response to the mental and emotional strain that can result from dangerous and traumatic experiences. It is a natural reaction to the wear and tear of the body and mind after extended and demanding operations [44]. Combat stress reaction (CSR) is a term used within the military to describe acute behavioral disorganization seen by personnel as a direct result of the trauma of war. CSR is an acute reaction that includes a range of behaviors resulting from the stress of battle that decrease the combatant's fighting efficiency. The most common symptoms are fatigue, slower reaction times, indecision, disconnection from one's surroundings, and the inability to prioritize. The combat stress reaction is generally short-term. The US Army uses the term/acronym COSR (Combat Stress Reaction) in official medical reports. This term can be applied to any stress reaction in the military unit environment. Many reactions look like symptoms of mental illness (such as panic, extreme anxiety, depression, and hallucinations), but they are only transient reactions to the traumatic stress of combat and the cumulative stresses of military operations [45].

Blinov defines combat stress as a reaction of the psyche to a threat to life and health in the form of a process of experiencing mental states at the stages of adaptation using the resource potential [9].

Snedkov makes a distinction between combat stress, combat psychic trauma, and combat psychic pathology [46]. Under combat stress, the author understands “a multilevel process of adaptive activity of the human body in a combat situation, accompanied by a tension in the mechanisms of reactive self-regulation and the consolidation of specific adaptive psychophysiological changes. Combat mental trauma is a pathological condition of the central nervous system, which is formed as a result of combat stress that exceeds the adaptive potential of a particular individual and forms the 'patos' of combat mental pathology. Combat mental pathology is a syndromic and nosologically structured clinical manifestation of combat mental trauma, the occurrence of which is due to the failure of compensation and the generalization of the pathogenetic mechanism” [46, pp. 28-29].

The authors of this article define the term “combat stress” as the process of the influence of combat situation factors on a soldier, accompanied by a decrease in the level of psychological safety of personality and the appearance of non-specific preclinical psychological manifestations, which together are presented by the concept of “combat psychological trauma of personality” or reach specific nosological symptoms of mental disorders, united in the concept of “combat mental trauma”. Combat stress in military personnel can manifest itself in the form of negative manifestations of the psychological, physical, psychophysiological, and behavioral register.

According to American mental health experts, combat and operational stress include all types of psychological and emotional stress that arise as a result of the dangers and requirements of performing tasks in warfare and other military operations [47]. The development of combat stress in military personnel, according to the authors, is directly influenced by: the intensity and duration of a traumatic event or situation; the level of psychological resilience and endurance of the individual; self-efficacy of a person (the ability to take certain actions to achieve a specific goal); collective effectiveness (general belief in the collective power to achieve the desired results); neurobiological factors (heredity, differences in the activity of the sympathetic nervous system, features of cortisol release in response to psychological stress, serotonin metabolism, etc.).

The effects of combat stress are mediated by a complex interaction between changing factors specific to each person and situation, as well as the dynamic interaction between them. Thus, managed moderately mild stressors can have a “hardening” or stress-modifying effect, when the body becomes less reactive to future stressors. Researchers have found that attention and arousal are essential for survival [30,48]. However, when they go beyond the maximum range, this is fraught with detrimental biological consequences: excessive and prolonged nervous excitement after an injury can increase the likelihood of developing PTSD [49].

EFFECTS OF COMBAT STRESS ON MILITARY PERSONNEL

American researchers have proposed a more detailed classification of stressors associated with external and internal factors [47]. They determined that combat-related external stressors included: self-injury; the killing of combatants; monitoring the death of people; death of colleagues; injury resulting in the loss of a limb. External stressors associated with military operations include prolonged exposure to extreme environmental conditions; reduced quality of life and communication resources for a long time; prolonged separation from important support systems (e.g., family); overseeing significant casualties across many missions (for example, overseeing the deaths of several unit comrades during combat operations). External physical stressors include exogenous (heat, cold, moisture, dust; vibration, noise, explosions); harmful odors (smoke, poison, chemical compounds); ionizing radiation; infected environment; physical work; poor visibility (bright light, darkness); difficult terrain; being at height. Physiological stressors that can cause combat stress include sleep deprivation; dehydration; malnutrition; poor hygiene; muscle and hypoxic fatigue; illness or injury; sexual frustration; the use of psychoactive substances (caffeine, alcohol, drugs); obesity; poor physical condition [47].

Mental (internal) stressors include cognitive; requirements or expectations, difficult decisions (combat rules); organizational dynamics and change; difficult choices or lack of it; work that goes beyond their own competence; preliminary failures. Emotional (internal) stressors are new to the unit; isolation, loneliness; fear and threats that cause anxiety (death, injury, failure, or loss); losses that cause grief (mourning); indignation, aggression, and frustrations, causing anger and a feeling of sadness; conflict (ambiguous) motives; inactivity that causes a feeling of sadness; religious confrontation or temptation to lose faith; interpersonal conflicts (in a unit or with comrades); family problems, nostalgia for home; victimization, sexual claims; observation of the battle, dead bodies; murder [47].

During deployment, military personnel is influenced by combat stressors (seeing dead human bodies, being attacked or ambushed, knowing someone who was injured or killed, and handling dead bodies) as well as operational stressors: long deployments, uncertain redeployment date, separation from families and lack of privacy [49,50,51]. Potentially morally compromising war circumstances, as noted by Wortmann et al., could involve prescribed roles (e.g. maiming and killing), proscribed behaviors (e.g. the use of excessive force or cruelty), bearing witness to the aftermath of violence or terror (e.g. handling or uncovering human remains) or being a victim of others' failures to uphold a moral code [52]. As a consequence, many service members suffer moral injury, characterized by guilt, shame, anger, social withdrawal, feelings of worthlessness, self-blame, and spiritual distress [33,52].

Effects of combat stress can also cause psychological trauma in military personnel, which can manifest itself both during the performance of combat missions and in the long-term period after their completion [7,30]. However, according to Born and Zamorski, deployment-related factors and exposure to potentially traumatic deployment experiences have the strongest and most consistent association with post-deployment mental health problems [53]. They may have short-term transient signs of the pre-nosological registry or more complex and severe somatic, psychiatric disorders, which should be well documented [27].

Recovery after exposure to combat stress can be facilitated by: a person's desire to open up and social support; the feeling that the social environment will accept the person's reactions with understanding and will encourage his openness; perception of the veteran as a hero or victim, but not as a victim; a sense of connection with God, a higher power, or a philosophical teaching; trauma-focused treatment to rethink negative reactions, clear intrusive memories, and help distinguish past from present threats [47].

Post-traumatic social support and relatively rare post-traumatic negative events may also serve as protective factors that will aid recovery from trauma [54].

COMBAT AND OPERATIONAL STRESS CONTROL (COSC) PROGRAMS IN MILITARY PERSONNEL

In our opinion, programs for the prevention and control of combat stress among military personnel are more effectively implemented in the Armed Forces of the United States, Great Britain, and Israel; other countries are developing similar activities based on these programs.

Trauma Risk Management (TRiM) is a peer-support program that aims to promote help-seeking military personnel in the aftermath of traumatic events, developed by the UK Royal Navy [55]. This program reflects a peer-led, occupational mental health support process that aims to identify and assist U.K. military personnel with persistent mental ill health related to potentially traumatic events [56]. TRiM seeks to modify attitudes about PTSD, stress, and help-seeking and trains military personnel to identify at-risk individuals and refer them for early intervention.

The use of TRiM may assist in increasing the psychological resilience of military personnel through the facilitation of social support; this may have particular utility during operational deployments [57].

The Israel Defense Forces developed YaHaLOM training to teach service members how to manage ASRs in team members [58] and then adapted into ICOVER by the U.S. Army. YaHaLOM is a novel, rapid, peer-based intervention specifically designed for use amid a high-stress event.

The analysis of scientific sources made it possible to determine that the most effective system for the prevention and control of combat stress among military personnel exists in the US Army. Let's consider this system in more detail. There is a specific terminological apparatus for defining terms related to combat stress that is used by mental health professionals in the US military. They are set out in various legal documents that regulate the professional activities of these specialists: COSC programs are set out in Field Manual 4-02.51 "Combat and Operational Stress Control" and 6-22.5 "Combat and Operational Stress Control Manual for Leaders and Soldiers" [45,59].

Thus, the control of combat and operational stress in the US Army is defined as the developed programs and activities of military leaders aimed at preventing, identifying, and managing dangerous behavior and combat stress reactions that may occur in military personnel [59]. The objectives of the COSC program are optimization of task performance; maintaining combat power; preventing the harmful effects of combat stress reactions on the physical, psychological, intellectual, and social health of military personnel or minimizing these effects; prompt return of soldiers to duty [60].

Also, one of the main tasks of the COSC is to increase unit cohesion and combat capability in a highly stressful military environment and to maximize post-traumatic growth [48]. COSC includes such activities as in-depth medical examination (screening) and psychodiagnostic of recruits; long-term observation of the passage of service, especially before, during, and after combat deployment; ongoing monitoring and consultation of mental health personnel from the US home base to the combat deployment area [61].

In the past, when US military personnel participated in operations in Korea and Vietnam, the consequences of the negative impact of combat stress led to almost half of the psychogenic losses on the battlefield, depending on the complexity of the conditions [47]. Although the level of acute (combat) stress reactions remained high in the wars of the 21st century, losses were significantly reduced. This was due to the introduction of COSC programs into the functioning of military units. According to American mental health professionals, in modern wars, military leaders can expect 95% of military personnel with ASRs should return to combat missions [47]. COSC is effective when it comes not only to military personnel but also to their training and support systems, including meaningful interpersonal relationships, families, and external resources. COSC aims to identify members of the armed forces and their families, as well as US Army civilians who may need assistance due to challenges associated with participating in combat deployments, and to ensure that they are better prepared, stable, and reliable at all stages accomplishment of combat missions. If we consider the personal level of a soldier, then the goal of COSC is to improve the readiness of each soldier and the unit as a whole to perform a combat mission. This can be achieved by: improving adaptive stress responses and preventing unproductive coping strategies; helping military personnel control combat stress responses; helping soldiers with behavioral problems [6].

Overall, the US Army's COSC is one of 10 medical systems active during pre-combat, on the battlefield, and at the end of a mission. Five professional disciplines in mental health and two additional ones are directed to the implementation of COSC. Professional disciplines include social work, clinical psychology, psychiatry, occupational therapy, and psychiatric nursing. Additional disciplines include mental health and vocational rehabilitation.

An analysis of the COSC programs of different armies of NATO partner countries in the field of international security assistance showed that they are similar in terms of organizational principles, main goals, and tasks. However, these programs differ structurally from the US Army COSC and have their own characteristics depending on the conditions of the missions in which the military personnel take part and the performance of combat missions. Such a comparison of COSC programs was made during a mission in Afghanistan among five NATO partner countries: the armed forces of Australia, Canada, the UK, the Netherlands, and the USA [8]. This allowed the authors to identify and compare organizational structures and key practices in providing mental health care and support to military personnel, identify issues, and illustrate new developments in this area.

The main comparative themes of the COSC programs were: education, prevention, early detection, intervention (care), and follow-up (support) in the field of mental health. The main method of organizing support for the mental health of servicemen was training (psycho-prophylaxis) and monitoring their mental state. The educational goal was to enhance the mental stability of individual military personnel while providing a range of mental health services. All mental health officials from these five countries have waived the need for a standard psychological debriefing after critical incidents (military deaths and large numbers of wounded). Instead, these specialists monitored the mental state of military personnel and their commanders during mandatory post-combat checkups and interviews [35].

To organize psychological support for military personnel, specialized mental health units were deployed near the line of combat, the main purpose of which was the early identification, assistance, and early return to normal activities of such military personnel in the units [62]. All countries have deployed mental health support teams made up of a variety of professionals, including psychiatrists, psychologists, social workers, nurses, and priests [63]. There was no consensus on the distribution of mental health disciplines in the war zone.

All countries, except the United States, organized psychological decompression for personnel in temporary deployment elsewhere (outside the combat zone, but in areas of temporary deployment of units) within a few days after the completion of combat deployment. Its main goal was to recognize faces with signs of combat stress reactions, to provide them with assistance, psychological support, and preparation for sending them to permanent deployment points (home). The US Army conducted intra-garrison decompression or reintegration training directly in the US [8]. But psychological decompression had a fairly comparable infrastructure in the field of psychiatric and psychological care.

Common concerns across countries included perceived stigma (unwillingness or fear of military personnel to seek help from mental health professionals), barriers to mental health care, and the need to increase awareness and acceptance of mental health issues among military personnel [64].

In addition to psychological support, modern preventive psychiatric treatment was included in all COSC programs, including a positive approach to strengthening mental stability. There has also been an emphasis on building self-regulatory skills, empowering military personnel, and several other innovations well integrated into the military context [63]. An important innovation in the operational mental health support of military personnel was the recognition of the role of social leadership and the need to increase support from colleagues.

However, not all researchers are positive about the introduction of COSC programs to prevent the development of mental disorders in military personnel that occur during and after combat deployment. Maglione et al. believe that although COSC interventions may play a valuable role in decreasing stress, decreasing absenteeism, and enabling a return to duty, a systematic review of studies that included a control/comparison group found little evidence that COSC is effective in preventing PTSD or decreasing PTSD symptom scores in military personnel [65].

CONCLUSIONS

One of the most important directions in the development of military psychology is the study of the problem of stress. The conservation of resource theory by S. Hobfoll, in our opinion, most fully and reasonably explains the genesis of combat stress that occurs in military personnel during activities in extreme conditions. The main postulate of this theory is that stress occurs when a person's central or key resources (health, well-being, family, self-esteem, and a sense of purpose and meaning in life) are threatened with loss, lost, or there is a failure to gain key resources following significant effort. At its core, the conservation of resource theory is a motivational theory that explains much of human behavior in terms of the evolutionary need to obtain and conserve resources in order to survive.

It was revealed that during the fighting, almost all servicemen experience combat stress. Combat stress is the process of the influence of combat situation factors on a soldier, accompanied by a decrease in the level of psychological safety of personality and the appearance of non-specific preclinical psychological manifestations, which together are presented by the concept of "combat psychological trauma of personality" or reach specific nosological symptoms of mental disorders, united in the concept of "combat mental trauma".

To prevent the occurrence and development of combat stress, it is necessary to develop and implement a system for the prevention and control of combat stress in military personnel. Such a program should contain psychological work activities carried out at three stages: preparatory (before performing combat missions), the stage of direct performance of tasks in the combat zone, and the final stage (after completing tasks upon returning to permanent deployment points).

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Authors' contribution

Conceptualization and project management, I.P.; research, YaM., O.K and S.V.; methodology, V.V.; formal analysis, N.V.; software, L.B.; writing – original draft preparation, V.K. and S.M. All authors read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

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ORIGINAL ARTICLE

A Survey on Mental Health Status in West of Iran: A Spatial Analysis

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Abstract: *Mental disorders clearly are among the major challenges of global health causing many concerns for health systems, health experts, and health policy makers. The present study was conducted to evaluate the mental health program in comprehensive health centers and health houses under the coverage of the Ilam University of Medical Sciences in the West of Iran. The present study was conducted based on the reports obtained from the mental health program in 2019. The data were recorded into registration and evaluation forms of mental disorders with the codes 102-19-03 and 102-19-01 and then entered into the Excel software. The prevalence per 100 and 100,000 people, as well as their 95% confidence intervals, were calculated using Formula 1 ROUND (Number of cases*100000/population) and Formula 2 ROUND (Prevalence ±1.96*SQRT (Prevalence *(1-Prevalence)/population). The results showed that the prevalence of depression was 19,100 [95% CI: 18999-19201] 19100), and the prevalence of anxiety was 304,66 [95% CI: 20348-30584] per 100,000 population. The highest prevalence among mental disorders was related to depression and anxiety. Considering the high average score of mental health status in people over 65 years old, it is necessary to implement measures and interventions to improve these individuals' mental conditions and upgrade their mental health.*

Keywords: *Spatial analysis; Ilam province; Mental health; Iran*

INTRODUCTION

According to the definition provided by the World Health Organization (WHO), mental health is "a health status that turns one's potential talents into reality, enabling people to cope with routine life tensions, accomplish valuable work, and upgrade their social participation" [1]. Mental health is an important component for evaluating the health status of a population and can be evaluated using various indicators [2]. In other words, the concept of mental health falls under the general concept of health and refers to all the methods and measures used to prevent people from developing mental disorders, as well as for the treatment and rehabilitation of affected individuals. Psychologists believe that mental health can be affected by individual characteristics, mental and environmental burdens, economic, social, and familial status, failure to achieve life goals, and lack of motivation [3]. Industrialization of countries, the growth of urbanization, migration to cities, etc. are phenomena that are associated with numerous troubles, including psychological and social problems. For this reason, mental health and mental disorders are considered top priorities in today's world. The high prevalence of mental disorders and their associated morbidities render them an immediate health priority [4].

Excessive attention to physical health problems (such as infectious and contagious diseases) has caused mental health to receive less priority in many countries around the world, including its social and spiritual aspects, which have been overlooked in most cases [5]. Statistics on the global burden of mental illnesses highlight the need to pay attention to these aspects more than ever. In parallel with the estimation of the WHO, Harvard University and the World Bank declared that mental disorders were responsible for about 13% of the total burden of diseases in 2006, which was predicted to reach 15% by 2020. The rate of morbidities caused by these disorders has been reported beyond 33% [6]. In Iran, mental health disorders account for 14.5% of the total burden of diseases, and after traffic accidents, they share the second rank with cardiovascular diseases. In women, depression claims the first place of disease burden, while in men, addiction is considered the third cause of disease burden[7]. According to studies in Iran on individuals older than 15 years old, the prevalence of mental disorders has been 25.9% in women and 14.9% in men, and it was reported that overall, 21% of people suffered from some sort of mental problems. Some studies have assessed the prevalence of mental disorders in Ilam province{8]. In a population-based study by Visani et al. [9], the prevalence of DSM-IV mental disorders in both genders was investigated in the urban areas of Ilam province. Out of 763 participants aged 15 years and older in the recent study, 25.8% showed symptoms of mental distress, and the prevalence of mental disorders was reported to be 20.9% in men and 29.8% in women. Also, the prevalence of anxiety, major depressive disorder (MDD), and obsessive-compulsive disorder was higher in women than in men (23.6% vs. 18.2%; 22.7% vs. 17.4%; 13.9% vs. 10%, respectively). In women, the prevalence of phobia was reported as 10.4%.

Studying mental health status can provide managers and decision-makers of organizations with valuable information for planning educational, therapeutic, and preventive measures to reduce the incidence of these diseases in society. Such information can be obtained by monitoring the mental health status of people in different societies [5].

Assessment and monitoring of mental disorders in the country and in Ilam province can help better understand the condition of mental health and public health in society, which is an important and influential factor affecting social productivity and wealth creation. Therefore, researchers here aimed to investigate the mental health status of the urban population of Ilam using the data recorded in the registration and assessment forms designed for mental disorders. Furthermore, we evaluated the mental health program performed in comprehensive health centers and health houses covered by the Ilam University of Medical Sciences in the West of Iran.

MATERIALS AND METHODS

The present cross-sectional study was conducted on the basis of the reports derived from the mental health program in Ilam province in 2020. According to the statistics announced in 2021, Ilam province harbors a population of over 630,000 people living in ten cities: Ilam, Ivan, Chardavl, Sirvan, Malekshahi, Mehran, Abdanan, Dehloran, Badreh, and Darrehshahr (Figure 1).

Figure 1: The geographical location of Ilam province

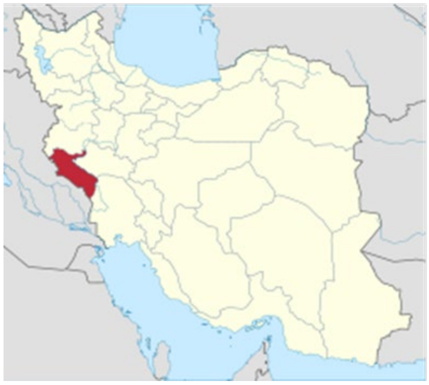

This study was conducted to assess the mental health program implemented in the comprehensive health centers and health houses affiliated with the Ilam University of Medical Sciences in 2019. The study was approved by the institutional ethics committee under the code IR.MEDILAM.REC.1399.266. The data were gathered by reviewing registration and evaluation forms for mental disorders with the codes 102-19-03 and 102-19-01 in all ten cities of Ilam province. Then based on the population of each city in 2021, the prevalence of mental disorders was calculated in 100 and 100,000 people.

Analytic Method

After collection, the data of each city were entered into Microsoft Excel software, and formulas 1 and 2 were used to calculate the prevalence of mental disorders (with 95% CIs) in 100 and 100,000 people.

Formula 1: ROUND (Number of cases*100000/population)

Formula 2: ROUND Prevalence ±1.96*SQRT (Prevalence *(1-Prevalence)/population)

Also, to determine the geographical distribution of mental disorders in the cities of Ilam province, the commands of spatial analysis in the STATA 11 software (Stata Corp. LLC College Station, Texas, USA) were used.

RESULTS

The results showed that in Ilam province, the prevalence of depression was 19100 (95% CI: 18999-19201), and the prevalence of anxiety was 30466 (95% CI: 20348-30584) per 100,000 people. According to the results, the highest prevalence among mental disorders was related to depression and anxiety.

The highest rate of depression belonged to Ilam (20300, 95% CI: 20137-20463) and Malekshahi (21836, 95% I: 21297-22375) cities, while the lowest rate of depression was reported in

Sirvan (16292, 95% CI: 15691-16897) and Badreh (17299, 95% CI: 16706-17892).

Also, the highest rates of anxiety were recorded in the cities of Abdanan (22298, 95% CI: 21922-22674), Ivan (22559, 95% CI: 22228-22970), and Ilam (21200, 95% CI: 210135-21365). On the other hand, the cities of Sirvan (16769, 95% CI: 16184-17404), Chardavl (16499, 95% CI: 16228-16770), and Darrehshahr (19099, 95% CI: 17790-18408) had the lowest rate of anxiety (Table 1).

Table 1: The prevalence of depression and anxiety in the population of Ilam province per 100,000 people in separate cities

Location	**Population**	**Major Depressive Disorder**		**Anxiety Disorder**	
		Prevalence		**Prevalence**	
		Number of cases	**Rate per 100,000 (CI 95%)**	**Number of cases**	**Rate per 100,000 (CI 95%)**
Ilam	235144	47734	21200(210135-21365)	49850	20300(20137-20463)
Abdanan	46977	9630	22298(21922-22674)	10475	20499(20134-20463)
Ivan	48833	10236	22559(22228-22970)	11036	20961(20600-21322)
Mehran	27506	5281	22402(21909-22895)	6162	19199(18734-19664)
Badreh	15614	2701	17299(16706-17892)	2701	17299(16706-17892)
Dehloran	66339	13135	18900(18602-19198)	12538	19800(19497-20103)
Darrehshahr	59551	10838	18099(17790-18408)	10778	18200(17890-18510)
Shirvan	14404	2347	16796(16184-17404)	2419	1294(15691-16897)
Chardavl	72167	12412	16499(16228-16770)	11907	17199(16924-17474)
Malekshahi	22587	4932	17196(16704-17668)	3884	21836(21297-22375)
Total	580148	110808	30466(20348-30584)	176750	19100(18999-19201)

Regarding age distribution, the results showed that the highest prevalence of depression (29%), anxiety (35.5%), and psychotic disorder (22.6%) was observed in people over 65 years of age. The psychological disorders of mania (6.3%) and obsessive-compulsive disorder (13.3%) were more commonly observed in the age group of 15-24 years compared to other age groups.

Regarding the gender distribution of mental disorders, it was noticed that depression (20.3% vs. 22.7%), mania (2.6% vs. 4.7%), anxiety (23.6% vs. 32.18%), obsessive-compulsive disorder (10% vs. 13.79%), phobia (10.4% vs. 5%), and psychotic disorder (6.17% vs. 2.4%) were more frequent in women than in men (Table 2).

Table 2: The prevalence of mental disorders in Ilam province in various age groups and genders

Age groups/gender	**Depression**	**Mania**	**Anxiety**	**OCD***	**Phobia**	**Psychotic**
	Prevalence (CI95%)	**Prevalence (CI95%)**	**Prevalence (CI95%)**	**Prevalence (CI95%)**	**Prevalence (CI95%)**	**Prevalence (CI95%)**
15-24 years	14.0 (7.08-15.1)	6.3 (5.9-7.3)	14.7 (12.1-16.8)	13.3 (9.04-15.15)	8.4 (6.9-11.1)	0.7 (0.01-5.9)
25-44 years	20.6 (18.2-22.3)	4.0 (2.6-9.1)	21.2 (16.2-18.2)	11.3 (9.13-14.2)	7.5 (6.9-11.1)	4.8 (3.6-6.3)
55-64 years	22.7 (20.2-24.3)	2.3 (1.4-2.9)	23.6 (20.3-25.2)	13.4 (8.1-16.1)	8.3 (7.9-11.3)	3.7 (1.2-5.7)
>65 years	23.6 (20.3-25.2)	0.4 (0.1-1.1)	35.5 (32.3-37.8)	9.7 (7.1-11.2)	9.7 (7.1-12.2)	22.6 (20.2-27.6)
Male	17.4 (15.1-19.6)	2.6 (1.3-8.7)	18.2 (15.2-21.1)	10.0 (8.1-12.4)	5.0 (3.7-8.2)	2.4 (1.4-4.1)

**OCD – Obsessive-compulsive disorder*

The results of the spatial analysis regarding the geographical distribution of mental disorders in Ilam province showed that depression, anxiety, and mania were more commonly observed in the northern compared to southern regions of the province, showing the highest prevalence in Ivan City. Also, panic disorder was more prevalent in southern cities of the province (Abdanan and Dehloran) than in other cities (Figure 2).

Figure 2: The results of spatial analysis. Geographical distribution of the most common mental disorders in Ilam province

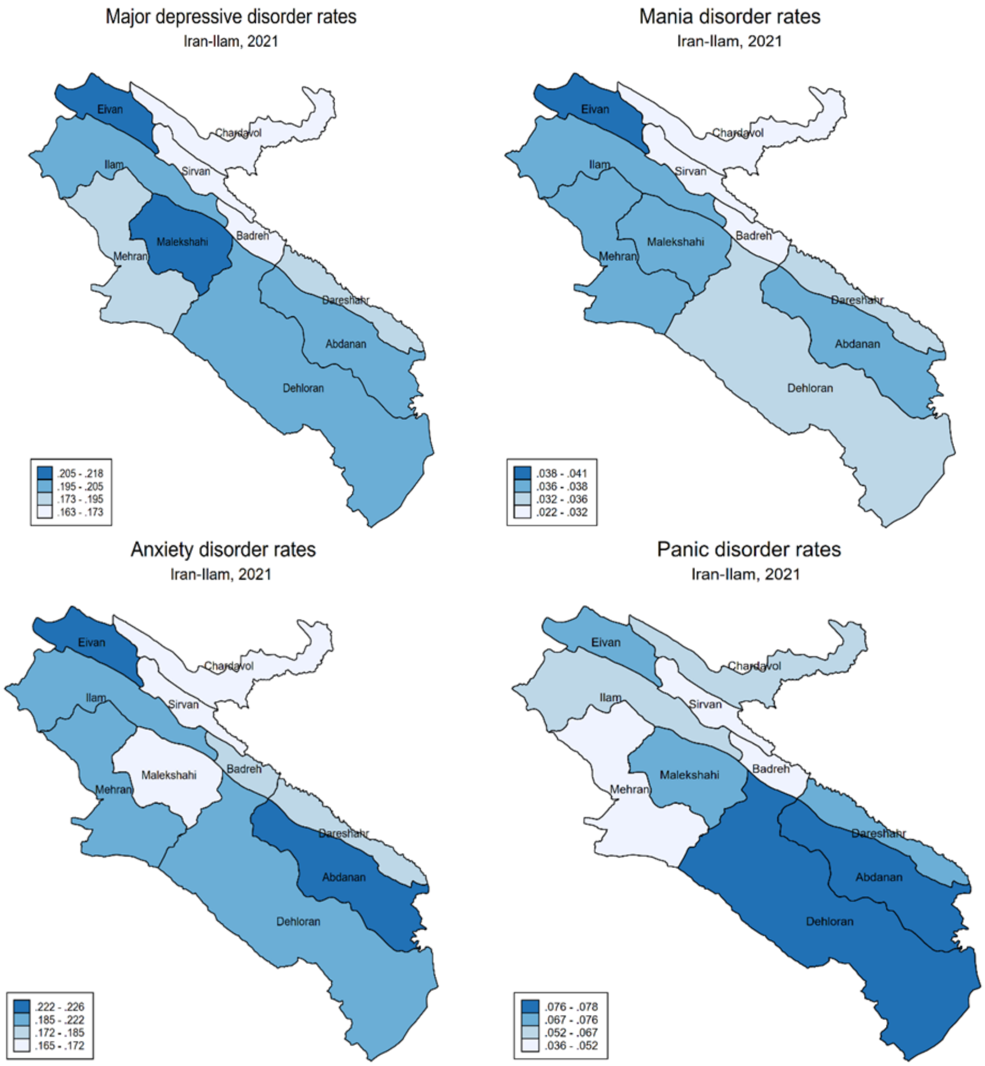

Major depressive disorder rates
Iran-Ilam, 2021

Mania disorder rates
Iran-Ilam, 2021

Anxiety disorder rates
Iran-Ilam, 2021

Panic disorder rates
Iran-Ilam, 2021

DISCUSSION

The results of the present study showed that the highest prevalence of depression, anxiety, and psychotic disorders was observed in people over 65 years old. In this study, age showed a direct and significant correlation with having unsatisfactory mental health, indicating the importance of advancing age in the development of mental health problems such as depression, anxiety, and psychotic disorders. In other words, the incidence of mental health disorders increased with age, contradicting the findings of Heidari et al. [10] and Jafari et al. [11]. In recent studies, higher age was reported to be significantly associated with more favorable mental health. Other studies; however, have reported a significant relationship between mental health and advancing age, including a report by Visani et al. [12]. In another study, Noorbala et al. [13] noted that with increasing age, the likelihood of the development of mental disorders increased so the highest prevalence (49.9%) was related to people over the age of 65 years. Among the reasons for the higher prevalence of mental health disorders in older people can be retirement, menopause, loneliness-associated anxiety, and biological alterations. The results of these studies are generally consistent with our findings; nevertheless, some discrepancies can be explained by differences in the assessment tools employed and the age spectra of the studied populations.

The results of this study showed that the highest prevalence of panic disorder and obsessive-compulsive disorder was related to the age group of 15-24 years. Mental disorders in teenagers and young adults exacerbate the predicted future burden of diseases and adversely affect numerous life aspects, including educational performance, employment, income, personal relationships, and social participation, as well as workforce [14]. In a study in Brazil conducted on individuals aged 18 years and older, it was shown that younger people were more vulnerable to mental problems [15]. In another study in Iran, Shirzadi et al. [16] declared a higher prevalence of these disorders in people aged 41 years or older compared to 18-40-year-old individuals.

Also, the results of the present study revealed a lower prevalence of depression and anxiety in women than in men. In a study, Falahzadeh and Momayezi [17], who investigated the prevalence of depression, anxiety, and stress in Yazd city of Iran, noted that depression, anxiety, and stress were more prevalent in women than in men, and this difference was statistically significant for anxiety (P=0.01) and stress (P=0.02), which was not consistent with our observation. We also noticed a higher prevalence of obsessive-compulsive disorders, phobia, and psychotic disorders in women compared to men, which was in agreement with the reports of Pendrez et al. [18], Kesler et al. [19], and Chang et al. [20]. The higher prevalence of mental disorders in women compared to men can be attributed to biological factors, gender-related differences, environmental and occupational stress, as well as limited sources of satisfaction and social participation of females.

Although in the present study, a significant portion of the urban population declared that they had unsatisfactory mental health conditions, this may not exactly match the clinical definition of adverse mental health. Therefore, the results of such subjective studies should not be regarded as a basis for judgment about the true condition of the mental health of respondents. Nevertheless, one should admit that a ratio of people, especially among women, suffer from some degree of mental health disorders. In this regard, the key measures that can be taken may be to initially achieve a correct understanding of the issue and then to implement comprehensive interventions for monitoring populations as large as possible, especially among vulnerable groups. Numerous viable strategies are available to maintain and improve public health. For example, unemployment has been pointed out as an important factor contributing to the development of mental health disorders [21]. So, an important intervention that governments can employ is to create more jobs, especially for youth, and to improve people's livelihoods as much as possible. Also, another effective measure for improving mental health is to remove the sources of distress, psychological pressure, and unnecessary tensions, which can be achieved with the help of mass media by nurturing a comfortable atmosphere. Moreover, the establishment of psychological counseling clinics can be among low-cost and beneficial measures to cover a large population, including young people, middle-aged, and elderly people, so that they can amend and improve their mental health.

CONCLUSIONS

The results of this study affirmed a relatively high prevalence of suspected mental health problems among people over 65 years of age, which is an alarming call, requiring authorities and researchers to pay more attention to this issue and to identify and modify the factors affecting the mental health of these people. A unique observation in this study was the lower prevalence of anxiety and depression in women than in men. Also, we noticed that panic disorder and obsessive-compulsive disorder were most prevalent in the age group of 15-24 years. A reduction in the prevalence of mental disorders can enhance social liveliness. In this regard, increasing people's awareness of ways to reduce mental disorders, expanding psychological counseling centers across the city, the establishment of counseling phone lines, residing counselors and psychologists in public places such as mosques, expansion of parks and enjoyable environments, equipping parks with sports devices, and finally, encouraging people to use these services can help reduce depression and anxiety in society.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, E.N. and Y.V.; methodology, E.N.; formal analysis, H.S; investigation, Y.V.; data curation, F.M. and A.S; writing—original draft preparation, S.A.; and M.Kh.; writing—review and editing, S.A; F.M. and M.Kh. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Investigating the Predictive Power of the Theory of Planned Behavior on the Behavioral Intention of Healthy Eating in Adolescents

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Abstract: *Background: Adolescence is a complex and sensitive period, and learning nutritional concepts during this period is very important. The purpose of the present study is to determine the predictive power of the theory of planned behavior on the behavioral intention of healthy eating in adolescents. Methods: 400 first-grade female students of Bandar Anzali participated in this descriptive-correlational study. Among the first secondary schools of this city, two schools were randomly selected and sampled by census method. The data collection tool was the healthy eating behavior questionnaire based on the theory of planned behavior, which included two sections of demographic information and the constructs of the theory of planned behavior, which were checked and confirmed with the content validity ratio, content validity index, and alpha coefficient. Data were analyzed using SPSS version 22 software. Results: Linear regression analysis showed that a total of 34% of the variance of healthy eating behavior intention is predicted by the constructs of perceived behavioral control and attitude. Conclusion: Considering the high predictive power of the theory of planned behavior in the field of healthy eating behavior, educational interventions based on it and centered on predictive structures are suggested.*

Keywords: *theory of planned behavior, healthy eating, adolescents*

INTRODUCTION

Non-communicable diseases (such as cardiovascular diseases, diabetes, cancer, high blood pressure, obesity, etc.) are the main cause of death worldwide [1]. Due to the increase in the death rate caused by these diseases, especially in developing countries, the World Health Organization (WHO) declared non-communicable diseases among the health priorities in developing countries two decades ago [2]. Iran, as an example of a middle-income country, accounted for 83.5% of all deaths and 78.1% of the total disease burden in 2019 due to non-communicable diseases [3-5].

The role of nutrition as the main cause of these diseases has been proven correctly [6]. In this regard, especially, the unhealthy eating behavior of teenagers is worrying [7]. Rapid growth, and changes in body composition, physiology, and endocrine system accelerate nutritional needs and increase the risk of malnutrition in this group [8]. Adolescents have more decision-making power and independence than children and are heavily influenced by friends, people around them, the environment, and various advertisements, and they turn to foods with an attractive and seductive appearance but lacking in nutritional value, many ready-made foods and snacks are among these materials [9]. Most of them do not consume enough fruits and vegetables, instead, they consume high-fat, energy-rich, and nutrient-poor foods [10].

According to the 2019 GSHS (Global School-based Student Health Survey), almost half of the countries reported that 10-30% of students aged 13-15 did not eat any fruit, a quarter reported that 10-30% of students did not eat any vegetables, and almost 70% of countries reported that at least half of their students eat junk food on a weekly basis and all countries have found that one in five students consumes carbonated drinks at least once a day [11].

Among Iranian teenagers, high consumption of junk foods and unhealthy snacks, not eating breakfast, and low consumption of fruits, vegetables, whole grains, and dairy products have been commonly reported [12] and this will cause them to develop chronic diseases in the future, especially girls who are considered as the future mothers and their eating behavior and learning nutritional concepts affect their own health and that of their children and families [13,14].

Modifying eating habits and behaviors during childhood and adolescence can prevent the occurrence of many non-communicable diseases in adulthood [15]. On the other hand, the future of any country depends on how it takes care of its children and teenagers. If action is taken to meet their health needs, the possibility that the adults of that society will have a healthy and valuable life pattern in the future will increase [16]. On the other hand, the future of any country depends on how it takes care of its children and teenagers. If action is taken to meet their health needs, the possibility that the adults of that society will have a healthy and valuable life pattern in the future will increase [17].

Studies show that the most effective educational programs are based on theory-based approaches that are rooted in behavior change patterns, and the "theory of planned behavior" is one of the well-known ones [18]. According to this theory, attitude, subjective norms, and perceived behavioral control affect the intention to perform a behavior [19]. A person's attitude is a favorable or unfavorable evaluation of behavior formed through past experiences.

Subjective norms refer to the social pressures perceived by the person who agrees or disagrees with the behavior and the media, family, and friends play a role in it. Finally, perceived behavioral control is a person's belief about how easy or difficult it is to perform a certain behavior [20]. According to the explanations provided, the present study was conducted with the aim of determining the predictive power of the theory of planned behavior on the behavioral intention of healthy eating in adolescents.

MATERIALS AND METHODS

This is a descriptive-correlational study, which was conducted in 2022. The research population was 400 first-grade high school girls in Bandar Anzali. From among the first high school girls in this city, two schools were randomly selected and sampled by census method. Inclusion criteria included: students' willingness to participate in research, lack of a special diet and not suffering from chronic diseases, and exclusion criteria were unwillingness to participate and continued cooperation by students.

The study was initiated with the permission of the ethics committee of Iran University of Medical Sciences and the necessary coordination with the education organization of Bandar Anzali city, the principals and officials of the relevant schools, and the informed consent of the students. The data collection tool was the healthy eating behavior questionnaire based on the theory of planned behavior [21].

This questionnaire includes two sections of demographic information and constructs of the theory of planned behavior. The first part of this questionnaire included 8 items related to demographic characteristics, including student age, height, weight, parent’s education, parent's occupation, and household size. The second part or constructs of the theory of planned behavior consists of 32 items, and the third part, or food behavior part includes 7 items, based on the 5-point Likert scale each item, as (strongly disagree, disagree, no idea, agree, strongly agree) with a minimum and maximum score of 1 to 5. The second part of the questionnaire includes attitudes toward behavior with 12 items with an achievable score range of 12 to 60, 13 items for subjective norm with an achievable score range of 13 to 65, perceived behavioral control with 7 items with an achievable score range of 7 to 35, behavioral intention with 6 items with an achievable score range of 6 to 30. In the present study, due to the limitation of answer options for items in the questionnaire, which were 4-point Likert, the questionnaire was re-validated and reliable.

To assess the validity, content validity was used, so that the questionnaire was given to 10 health and nutrition experts, and its validity was confirmed with a content validity ratio (CVR) of 0.81 and a content validity index (CVI) of 0.8. To assess the reliability, Cronbach's alpha test method was used. For this purpose, a questionnaire was given to 20 female high school students. Cronbach's alpha coefficient was 0.85 for the attitude construct, 0.75 for the subjective norm construct, and 0.71 for the perceived behavioral control construct, 0.76 for the behavioral intention construct. Data analysis was done by SPSS software version 24.

RESULTS

In this study, the mean age of students was 14.9 ± 0.78 years. Their mean height and weight were 159.7 ±10.7 and 54.7± 1.4, respectively, and the household size was 4.1 ± 0.8. In terms of employment, the highest frequency was for fathers (45%) as self-employment and 86% of mothers were housewives. In terms of education, 42% of fathers and 45% of mothers were at the level of high school and high school diploma, each of which with the highest percentage of frequency. The mean, standard deviation, and range of obtainable scores for each construct of the theory of planned behavior in the studied students can be seen in Table 1.

Table 1: Mean, standard deviation, and range of obtainable scores for each of the constructs of the theory of planned behavior in the studied students

Variable	Mean ± SD‣	Rang of attainable score
attitude	40 ± 6.21	12-60
subjective norms	38.9 ± 6.44	13-65
perceived behavioral control	24.06 ± 6.44	7-35
behavioral intention	25.47 ± 9.21	6-30

‣ Standard Deviation

Table 2 shows the results of Pearson correlation between the constructs of the theory of planned behavior that there is a direct and significant correlation between all constructs (attitude, subjective norm, and perceived behavioral control) with the intention of eating behavior (P<0.001). The construct of perceived behavioral control has the highest correlation with the intention of eating behavior (r=0.302).

The linear regression analysis in Table 3 showed that a total of 34% of the variance of the intention of healthy eating behavior in the studied students is predicted by the constructs of perceived behavioral control and attitude, and perceived behavioral control is the strongest construct related to the intention of eating behavior in this study.

Table 2: Correlation between the constructs of the theory of planned behavior

Variable	Attitude	Subjective norms	Perceived behavioral control	Behavioral intention
attitude	1			
subjective norms	0.191	1		
perceived behavioral control	0.276	0.145	1	
behavioral intention	0.182	0.255	0.302	1

Table 3: Predicting the behavioral intention of healthy eating based on the theory of planned behavior in the studied students

Model		B	Std. Error	Beta	sig
1	(Constant)	5.231	3.424	_	0.127
	attitude	0.157	0.070	0.113	0.024
	subjective norms	0.119	0.085	0.088	0.163
	perceived behavioral control	0.371	0.103	0.230	0.000

a. Dependent Variable: Intention of food behavior; b. R2 = 0.34

DISCUSSION

The present study was conducted with the aim of determining the predictive power of the theory of planned behavior on the behavioral intention of healthy eating in adolescents. The result of the study shows that a total of 34% of the variance of healthy eating behavior intention in the studied students is predicted by the constructs of perceived behavioral control and attitude, and perceived behavioral control is the first and strongest construct related to behavioral intention, which is consistent with studies predicting healthy eating in Danish adolescents [22] and the results of the study by Seo et al in Seoul, which implies a significant relationship between behavioral intention and perceived behavioral control construct [23]. It is also consistent with the findings by Yarmohammadi et al. [24] and Dunn et al. [25] on junk food consumption based on the theory of planned behavior. However, it is not consistent with the findings of Ickes et al. [26] on beverage consumption and the findings of Gholami et al. [27] on fruit and vegetable consumption. Perceived behavioral control may not predict behavior when a person has little information or the resources available to the person change and when new elements such as eating habits are added to the situation [28, 29].

The second construct predicting the intention of healthy eating behavior in this study is the attitude, which is consistent with the results of the study by Mousavi et al. [30] concerning beverage consumption and the study by Gronhoj et al. [22] regarding the prediction of healthy eating behavior and the study by Mamun et al. [31] on the determinants of healthy eating. Attitude shows an individual’s general feeling about the desirability or undesirability of something, and sometimes people's attitude during life as well as sufficient awareness and knowledge, which is a prerequisite for attitude change, leads to the intention of performing the desired behavior in the future [27]. Therefore, the attitude that originates from feelings and internal factors can affect his eating behavior by increasing his information and experiences concerning nutrition.

In this study, the subjective norm is not a predictor of eating behavior intention, which is similar to the finding by Ismawati et al. [32] on the application of the theory of planned behavior in the intention of healthy eating in Malaysia and the study of Brouwer et al. [33] related to healthy eating behavior prediction and finding by Dehdari et al. [34] regarding the consumption of unhealthy foods. However, it is inconsistent with the findings of Khorasani et al. [35] on unhealthy food consumption and White et al. [36] regarding intention to consume low-fat food. The reason for this inconsistency can be the difference in the gender of the participants, in the study of Dehdari et al. [34], as in the present study, the target group is girls, and in the study of Khorasani et al. [35], boys are the target group. On the other hand, diverse food cultures and customs in Iran and other countries of the world can be the reason for the discrepancy of these studies. The diversity and complexities of health behaviors in different groups and in different conditions can lead to the necessity of planning to use the appropriate theories and structures to achieve success in interventions in health education and health promotion.

CONCLUSIONS

Perceived behavioral control and attitude are among the factors predicting on the behavioral intention of healthy eating in the studied students. The research limitation in the present study was the use of self-reporting, and it is necessary to carry out educational interventions based on the "theory of planned behavior" with emphasis on the two predictive constructs mentioned.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research. The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

Authors' contribution

All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

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Ethics approval and consent to participate

This article is taken from the approved research project of Iran University of Medical Sciences with ethics code number: 1401.886 IR.IUMS.REC. Informed consent was obtained from all individual participants included in the study before conducting.

Consent to publish

All participants consented verbally to the publication of the interview data.

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ORIGINAL ARTICLE

A Conceptual Analysis of Post-psychotic Depression

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Abstract: *The conundrum of post-psychotic depression (PPD) is still present in the psychiatric literature, for more than a century since it was first described by Mayer-Gross. After a short existence in the DSM-IV and ICD-10, this nosological construct was excluded from newer versions of the mental disorders classifications. Therefore, an exploratory analysis of the PPD concept was considered useful both from clinical and theoretical perspectives. There is a high prevalence of depressive symptoms in patients with schizophrenia spectrum disorders (SSD) and, although not all of them represent cases of PPD, they are considered risk factors for suicide. The arguments and contra-arguments for/against PPD were reviewed, and the potential pharmacological interventions for this disorder were analyzed. The explored evidence indicates that PPD is worth further investigation, and in order to differentiate it from schizoaffective disorder, negative symptoms of SSD, or depressive manifestations within the acute psychotic episodes, clearly defined criteria should be found. The use of validated scales, like the Calgary Depression Scale for Schizophrenia and the InterSePT Scale for Suicidal Thinking, is recommended for the initial assessment of depression and suicidal risk in patients with SSD, but also for their monitoring during the acute and maintenance phases. Besides the administration of combined, antidepressant and antipsychotic treatment, the use of clozapine, and the recommendation to initiate treatment for SSD with atypical antipsychotics whenever possible, there is a dearth of studies exploring specific interventions for PPD. Future studies are expected to address the validity of the nosological construct of PPD and the most adequate therapeutic and prophylactic interventions in patients with SSD.*

Keywords: *post-psychotic depression, schizophrenia spectrum disorders, atypical antipsychotics, clozapine, typical anti-psychotics, antidepressants*

INTRODUCTION

Depression that may appear in the context of schizophrenia is considered a complex and heterogeneous group of disorders [1,2]. Mayer-Gross was the first author who described in 1920 the reaction to a psychotic experience that manifests itself as a clinical version of depression [3]. He considered "despair" as the main clinical characteristic of this depression that follows a full-blown psychotic episode, and considered this manifestation to be determined by the patient's increased awareness about the severity of his/her illness [3,4]. A few decades later, Semrad also explored the psychodynamic mechanisms for post-psychotic depression (PPD), emphasizing the denial, projection, and distortion of reality after the remission of acute psychotic episodes [5]. PPD was described by several authors as "retarded depression, with strong neurasthenic and schizoid components" emerging after the patient is discharged from the hospital, and often ignored by mental health specialists [6]. Depressive manifestations in patients with SSD have been conceptualized as a psychological reaction to acute psychosis, as previously mentioned, but also as a comorbidity of two distinct pathologies or a proof for the existence of a continuum between schizophrenia and mood disorders [2]. According to psychoanalysts, PPD is a reaction to psychosis, which appears after the remission of the psychotic episode, more specifically, after the Ego regained a significant proportion of its strengths [7,8].

Some authors, however, reported, based on the analysis of the temporal relationship between the onset of depressive and psychotic symptoms in 27 patients, that the first reports on mood manifestations were frequently at the same time same as the onset of psychosis [9]. Therefore, PPD is a controversial concept, as no specific pattern for its onset could be detected, leading to the assumption that depressive manifestations may be only associated with acute psychotic manifestations.

However, a series of studies focused on PPD have tried to delineate the risk factors, clinical features, and prognosis of this nosological entity, as a distinctive pathology from depressive symptoms belonging to acute schizophrenia, mood episodes within a schizoaffective disorder, or adverse events of the antipsychotics or other drugs. For example, a comparison of patients with schizophrenia presenting or not with PPD (N=30 participants in each group) showed that belonging to elementary (or nuclear) families, longer duration of psychotic phase, more frequent hospitalizations, and more sadness and anxiety-somatization during the acute phase of the disease were associated with PPD [10]. A past history of depression was also more frequently reported in the study group vs. controls [10]. Age of onset, sadness during the acute psychosis phase, premorbid adjustment, social support, and life events were significant determinants of severity for PPD, according to the multiple regression analysis [10].

A cohort study with a 10-year follow-up compared the evolution of 29 patients with schizophrenia and PPD vs. only schizophrenia [11]. Patients with PPD presented at an older age with the onset of schizophrenia and more auditory hallucinations; also, they were admitted more often, but their hospitalizations were of shorter duration [11].

A study that explored the main clinical differences between primary depression (N1= 98 patients) and PPD (N2= 71 patients) concluded that depressed mood, reduced energy, and pathological guilt were more frequently detected in patients with primary depression, while sleep disturbance and ideas of reference with a theme of guilt were more consistently associated with PPD [1].

The duration of PPD was estimated by different authors as varying between several days to several months [12], or, more specifically, between 2 and 26 weeks [8].

Depressive symptoms during a psychotic episode may be as frequent as 50%, and they may correlate with the frequency of relapses, defined by re-admission rate and total length of inpatient treatment [13]. By comparison, the prevalence of PPD reported in the literature was around 30-36% [14,15]. Higher rates of PPD were reported in patients with a first episode of psychosis (FEP), up to 50% [3,15]. Older age, a higher familial load of psychoses, a longer duration of untreated psychosis, the presence of an FEP, and a higher number of stressful life events were associated with a higher risk of PPD [14]. Depression was reported by patients with FEP as being linked to the psychotic experience and to the process of recovering from this experience [16]. Feelings of shame, doubt, and embarrassment, but also loss and social isolation, were associated with the presence of the psychotic experience [16]. Also, the onset of PPD has been correlated to the existence of positive symptoms during acute schizophrenia, especially delusions and hallucinations [17]. A study that enrolled 44 patients (aged 10-40 years) with schizophrenia, all of them during their first six months of the illness, explored the risk of developing PPD one year after discharge from the hospital [18]. Four patients were diagnosed with depression in this interval, according to a Hamilton Depression Rating Scale (HAMD) score ≥17 [18]. Only one case recovered from PPD completely during the follow-up [18]. All patients in this trial were undergoing phenothiazine treatment throughout the monitoring period [18].

In a longitudinal study, 17 patients with schizophrenia were monitored for 6 months, using the 18-item Profile for Rating Depressive and Schizophrenic Behavior (PRDSB) [19]. After recovering from acute psychosis, only 2 participants developed major depressive syndrome [19]. However, over 40% of these patients were interviewed only by telephone after their discharge, so a potential bias regarding the sensitivity of the assessment method can not be excluded [19]. Also, the ethnic homogeneity of the study group could be construed as another potential bias that limits the generalizability of the results.

A longitudinal, observational study (N=105 patients with schizophrenia) reported during 12 months of monitoring that depression accompanied acute psychosis in 70% of cases, and remitted together with psychosis, while PPD appeared in 36% of these patients, without a concomitant increase in psychotic symptoms [20].

Focusing the analysis of the PPD concept on the most renowned classifications of mental disorders, this pathology was mentioned for the first time in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), published in 1994 [21]. PPD, according to the cited source, involves the presence of “a Major Depressive Episode that is superimposed on and occurs only during the residual phase of schizophrenia” [21]. The definition of PPD required in the DSM-IV the presence of depressed mood, as a core symptom, and excluded the direct consequences of drug abuse, medication use, or a general medical condition [21]. However, in the fifth edition of the DSM, published in 2013, this nosological entity no longer appears [22].

The tenth edition of the International Classification of Diseases, edited by the World Health Organisation (ICD-10) in 1993, grouped PPD (named "post-schizophrenic depression") together with different types of schizophrenia, in the same chapter, coded F20 [23]. The definition of PPD was "a depressive episode, which may be prolonged, arising in the aftermath of a schizophrenic illness" [29]. It is important, according to the ICD-10, that residual schizophrenic symptoms still be present, although they cannot dominate the clinical status [23]. Newer versions of ICD-10 placed PPD in the category of "other specified depressive episodes" [24]. The latest edition of ICD (ICD-11), the first version published in 2019, did not reference PPD [25].

The convoluted trajectory of the PPD concept from its genesis to the present day is rather difficult to explain, but several important factors contributing to this conundrum represent the main objective of the current review (Figure 1).

Figure 1: Rationale for exploring post-psychotic depression

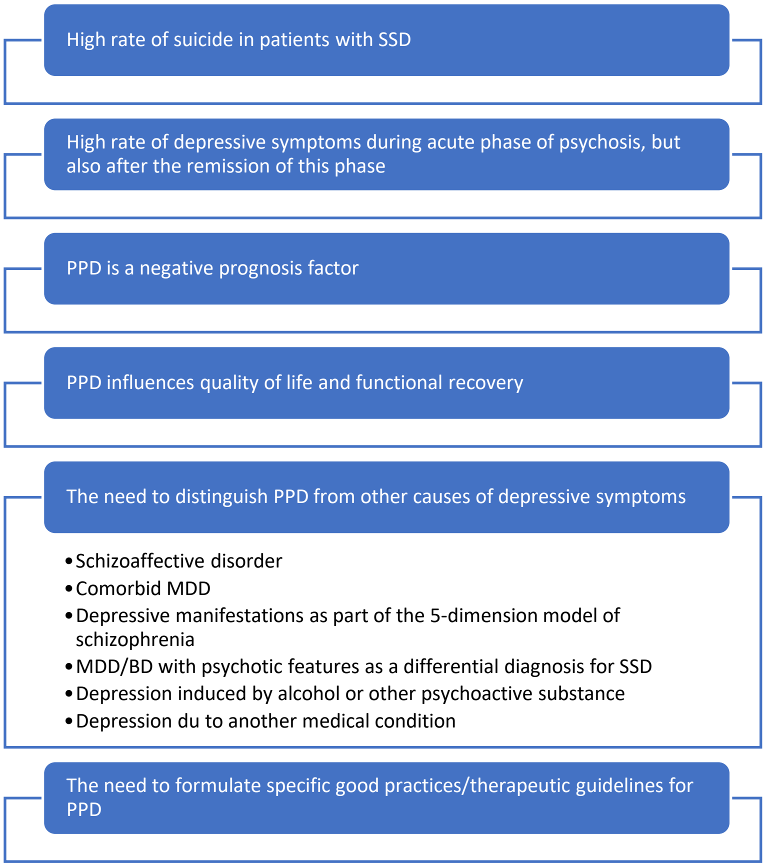
High rate of suicide in patients with SSD
High rate of depressive symptoms during acute phase of psychosis, but also after the remission of this phase
PPD is a negative prognosis factor
PPD influences quality of life and functional recovery
The need to distinguish PPD from other causes of depressive symptoms
•Schizoaffective disorder
•Comorbid MDD
•Depressive manifestations as part of the 5-dimension model of schizophrenia
•MDD/BD with psychotic features as a differential diagnosis for SSD
•Depression induced by alcohol or other psychoactive substance
•Depression du to another medical condition
The need to formulate specific good practices/therapeutic guidelines for PPD

PPD= postpsychotic depression, SSD= schizophrenia spectrum disorders, MDD= major depressive disorder

The rationale for conducting this review derives from several important clinical and theoretical aspects. First, it is essential to obtain adequate control over depressive symptoms in patients with SSD, because of the high rates of suicide reported in this population [26]. PPD seems to be a factor for negative prognosis in the long-term for patients with schizophrenia, especially because of the higher risk for suicide, but also due to its significant impact on the quality of life and general well-being [17]. Second, the need to delineate clinically PPD from other causes of depression in patients with SSD, in order to find the most adequate therapeutic strategies. To support this, the data presented suggest that PPD is a distinct nosological entity from antipsychotic-induced adverse events, negative symptoms of schizophrenia, depression with psychotic symptoms, or schizoaffective disorders [14,17]. This means that good clinical practices in patients with SSD would involve differentiating between PPD and other frequently reported confounding factors.

THE MULTIFACETED CONSTRUCT OF PPD

Although the history of PPD has been over a century, there is a dearth of good-quality studies exploring this pathology's clinical and epidemiological aspects. However, an analysis of the main dimensions of the PPD construct can be done, based on the available evidence (Table 1).

Table 1: Pros and cons in the analysis of the PPD construct

Pros	Cons
PPD was associated with any type of antipsychotic treatment, therefore it is not an adverse event to the first generation antipsychotics	PPD does not exist because it consists of adverse effects of older antipsychotics
There are longitudinal studies that confirm the persistence of depressive symptoms after the remission of acute psychosis and cases where depression followed a period of psychosis remission	The construct should not be treated as a distinct entity, it only accompanies acute schizophrenia
Negative symptoms and depression do not overlap to the point they are indistinguishable	Negative symptoms cover the presence of depression, therefore depression may be observed only after the remission of those symptoms
There are specific scales for measuring depression in patients with SSD, as well as their suicide risk	PPD can not have its own diagnosis criteria and its severity can not be determined, due to the fact that it is secondary to other symptoms

To begin with the presentation of the reasons why PPD is not yet a fully recognized diagnosis, it must be specified that PPD was correlated by different authors with the effects of first-generation antipsychotics, and they called this phenomenon “pharmacological depression” [27-29]. In a multicenter study (N=211 patients with schizophrenia), depression (quantified with HAMD) was detected in 25% of the cases, all of them treated with fluphenazine (either hydrochloride or decanoate) [30]. This study also showed that depression was associated with a more chronic psychiatric history and a higher rate of relapse [30]. Also, older drugs used to treat SSD, such as chlorpromazine or reserpine, have been associated with lethargy, psychomotor retardation, and anergy, which are acknowledged adverse events of these agents [30].

Secondly, the negative symptoms of schizophrenia may cover the depressive symptoms, making them more difficult to detect [14,17]. Anhedonia, defined as a reduced ability to experience pleasant emotions, is considered both a negative symptom in SSD, and a central manifestation in major depressive disorder (MDD) [31-34]. Therefore, it is considered “a transdiagnostic symptom”, and its explained by dysfunctions of the reward and aversion pathways in the central nervous system (CNS) [31]. Furthermore, negative symptoms like alogia and avolition may prevent the detection of depressive symptoms, because they interfere with both the interview and the observation of the patients.

The third aspect refers to a contradiction: although current diagnostic classifications do not include mood symptoms in the core criteria for schizophrenia, these are frequently considered part of the clinical picture, as additional manifestations [35]. According to the five-dimension model of schizophrenia, depression is a core clinical component, together with the positive, negative, agitation-excitement, and disorganization symptoms [36]. However, as mentioned in the introductory chapter, there is no reference to PPD in the latest versions of the ICD or DSM [22,25].

The fourth facet of the PPD controversy is represented by the fact that although differentiating between schizophrenia, schizoaffective disorder, and depressive/bipolar disorder with psychotic features is quintessential, in clinical practice it is frequently difficult to make the right diagnosis. Factorial analysis using validated instruments for psychosis and mood symptoms measurement (e.g., Positive and Negative Syndrome Scale – PANSS, Young Mania Rating Scale – YMRS, Montgomery Depression Rating Scale – MADRS, and Calgary Depression Scale – CDSS) confirmed the existence of very complex structure of the clinical manifestations in patients with schizophrenia [37]. Subclinical mood symptoms have been confirmed by this type of analysis, structured within up to 15 factors – e.g., positive, negative, somatic, anxiety, neurocognitive, disorganization and manic, depressive, psychomotor agitation, hostility/violence [37]. These results support the overlapping model of phenotypes between psychotic mood disorders and SSD, and the possibility of misdiagnosing a patient with a mood disorder and associated psychotic features as having an SSD [37].

However, each of these confounding factors may be addressed. The first argument is precarious because even SSD patients treated with newer antipsychotics may develop depressive symptoms and the effect of these pharmacological agents on depressive symptoms is only small to medium [38]. According to a Cochrane review, based on five reports about patients diagnosed with both schizophrenia and depression, there was no significant difference in efficacy (evaluated by PANSS scores) between quetiapine and haloperidol, sulpiride was significantly superior to chlorpromazine (reflected in the severity of depressive symptoms), and clozapine was superior to the association of another antipsychotic and an antidepressant (moclobemide/mianserin) [39]. A significant reduction of depressive symptoms in patients with schizophrenia was reported only if a CDSS score at baseline was ≥5, unlike the positive, negative, and general symptoms (scored with PANSS), in a naturalistic study that enrolled adult patients with ≥2 years of treatment with clozapine (N=23) or other atypical antipsychotics (N=23) [40]. At the follow-up visit, patients who received other antipsychotics led o superior results when compared to clozapine on depressive symptoms, but only when the analysis was limited to those with clinically relevant depression [40]. It can easily be deduced that the results of studies exploring the impact of antipsychotics on depressive symptoms in SSD are quite heterogenous, varying from no difference in outcomes between typicals and atypicals, to superiority of clozapine vs. other antipsychotics combined with antidepressants, and to superiority of other atypicals to clozapine, but only in certain subpopulations.

The second argument is even more difficult to support because authors exploring PPD showed that this syndrome "is usually stable phenomenologically, often lengthy, and may be resistant to all treatment modalities" [6]. Even considering the last part as an exaggeration determined by the lack of adequate therapeutic resources when that statement was formulated, there is still enough ground to back up an independent syndrome, with pharmacological and clinical peculiarities. There are authors that contradict the third argument, stating that depression is "a frequent comorbidity in the course of schizophrenia" [7], and not just a cluster of symptoms intrinsic to SSD.

The fourth argument can also be easily disqualified because there are validated instruments, currently in clinical use, that allow for the monitoring of depressive symptoms in patients with SSD. CDSS is an instrument created by Addington et al., first published in 1990, for the purpose of measuring depression in SSD, starting from selected items within HAMD and Present State Examination (PSE) [41]. This instrument was validated on 50 patients with acute psychosis, and it has 11 items, with a Cronbach's alpha of 0.84-0.89 [41,42]. The validity of CDSS in evaluating mood symptoms in patients with SSD was confirmed by subsequent studies and reviews, and this scale was significantly correlated with HAMD and MADRS [43,44]. CDSS differentiated depressive symptoms from other clinical manifestations of SSD and also had good predictive validity [44]. Another study comparing CDSS and HAMD (N=119 in-patients with acute schizophrenia) concluded that CDSS had a higher sensitivity than HAMD in assessing mild or severe depression, while the specificity was comparable high for both instruments [45].

The InterSePT Scale for Suicidal Thinking (ISST) has 12 items and was created to assess suicidal ideation in SSD patients [46]. Two validation studies (N=22 and 980 patients with SSD) confirmed that ISST is reliable and valid if used to assess ongoing suicidal ideation in patients with SSD [46]. Both ISST and CDSS can be used to provide information necessary in the case management of patients with SSD and suicidal risk [47].

Based on its properties, CDSS is considered a first-line instrument to determine the severity of depressive symptoms in patients with SSD, and a review that compared 6 different instruments targeting the same symptoms formulated this recommendation [44]. A study that explored the diagnosis of major depressive episodes in 84 patients with schizophrenia found that CDSS was superior to the Beck Depression Inventory (BDI), HAMD, and PANSS-D when evaluating the Receiver Operating Characteristics (ROC) curves [48].

However, the possibility that PPD symptoms may be, in fact, the continuation of depressive symptoms with onset in the acute psychotic phase, can not be completely excluded [7]. On the other hand, if PPD is detected after a period of psychotic symptoms remission, then it may be considered part of a new episode of schizophrenia [7].

PPD may be confounded with negative symptoms of SSD, as previously mentioned. The discussion about primary and secondary negative symptoms is a very complex topic, that clearly exceeds the objective of this paper. As a general definition, primary negative symptoms are intrinsic to schizophrenia, a specific clinical dimension, while secondary negative symptoms derive from the presence of positive symptoms, depression, antipsychotics' pharmacodynamical properties, substance use disorders (SUDs), and social withdrawal [49-51]. After the remission of psychotic symptoms, the depressive symptoms were still present, although their rate decreased from 60% during the acute psychotic episode, to 40% after 5 years follow-up [52].

In conclusion, a correct diagnosis of PPD should include a clinical and psychometric evaluation of psychotic and depressive symptoms, a thoroughly-conducted screening for comorbidities (organic, toxic, or psychiatric) [53-55], an analysis of the risk factors for the persistence of depressive symptoms [56,57], a clear collection of personal medical history (in order to establish the temporal succession of psychotic and depressive manifestations), and an accurate pharmacological anamnesis.

THERAPEUTIC INTERVENTIONS FOR PPD

Adapting the type and dosage of antipsychotic agents in patients with SSD was signaled by different authors as a method to decrease the risk of PPD [2]. Re-evaluation of the main drugs administered during the acute phase of psychosis or the prescription of adjuvant antidepressants or ECT has also been suggested as a case management strategy in PPD [2]. Psychotherapeutic, social, and institutional support are useful options for patients with PPD [2].

A review (n=20 articles) exploring the results of available pharmacological and psychosocial treatments of PPD after the first episode of psychosis did not find support for one intervention in favor of another [58]. Online psychotherapy and treatment with ω-3 polyunsaturated fatty acids (PUFA) have been associated with some favorable results [58].

Olanzapine (5-15 mg/day) was compared to risperidone (4-8 mg/day) in the treatment of PPD (according to the DSM IV criteria) in patients with schizophrenia (N=76), and a clinically significant decrease in MADRS scores (-4 points vs. baseline) was reported after 8 weeks of treatment with either antipsychotic [59]. This study used a randomized, double-blind, parallel-group design. The comparative analysis of the two interventions did not detect any significant MADRS differences at the end-point [59]. A high response rate (≥20% reduction in MADRS scores) was observed during antipsychotic treatment [59]. In order to control for possible confounders of PPD, patients with akinetic symptoms were excluded from this trial [59]. The same team reported on another randomized, placebo-controlled trial, with 24 patients diagnosed with schizophrenia or schizoaffective disorder and PPD or negative symptoms, who continued their six-month adjunctive imipramine to fluphenazine decanoate/ benztropine mesylate treatment or were tapered to placebo + fluphenazine decanoate/benztropine during one year [60]. More patients receiving a placebo relapsed into PPD, at a statistically significant level; also, patients who received placebo experienced relapses into psychoses [60]. Yet another trial conducted by the same team showed that double-blind, controlled discontinuation of imipramine after stabilization (N=14 participants with schizophrenia undergoing treatment with fluphenazine + benztropine) led to relapse into PPD in all patients with placebo vs. only two out of eight in the antidepressant-receiving group (significant difference, p=0.009) [61].

A controlled trial investigated the effects of imipramine hydrochloride for PPD for six weeks, in a double-blind manner [62]. All patients (N=33) were diagnosed with schizophrenia or schizoaffective disorder and received a stable dosage of fluphenazine decanoate and one week-trial with benztropine mesylate was initiated to rule out akinesia; all participants were either non-psychotic or only residually psychotic at baseline [62]. The PPD diagnosis was based on current major or minor depression for at least four weeks, and a HAMD score ≥12 for at least three consecutive weeks [62]. The depressive symptoms (according to HAMD and SADS, Schedule for Affective Disorders and Schizophrenia scores) were controlled better with imipramine vs. placebo and their global clinical status (according to the CGI scores) also improved more consistently in patients receiving the active drug [62]. The psychotic symptoms and tolerability profile did not change significantly between groups [62]. The use of adjunctive imipramine to fluphenazine was further explored by the same team, in a larger and more heterogenous study group [63]. The positive effects of the combined treatment were still detected in the total sample, but the significance of the results was lower [63]. These differences were explained in a post-hoc analysis by the higher level of psychotic symptoms severity, and by "more refractoriness of depression" due to failed previous trials of antidepressants [63].

In a continuation trial, four out of five patients previously stabilized on imipramine + fluphenazine decanoate + benztropine for PPD relapsed into depression six months after the tapering of the antidepressant [64]. The control group, consisting of four patients who did not discontinue imipramine, did not present depressive relapses [64]. The same research team reported on ten PPD patients with good responses to imipramine who relapsed six months after discontinuation of the antidepressant, that was added to fluphenazine decanoate and benztropine [65].

A 24-week double-blind trial compared haloperidol (mean dose 17.5 mg/day) and thiothixene (mean dose 31.8 mg/day) in 46 outpatients with schizophrenia, from their efficacy and safety properties [66]. One of the outcomes was the incidence of treatment-emergent depression, assessed by the Hamilton Depression Rating Scale (HAMD) and Zung Self-Rating Depression Scale (ZSRS) [66]. Haloperidol was superior to thiothixene on the "cognitive disturbance" HAMD factor [66]. Clinically significant symptoms were reported in five patients treated with haloperidol and three receiving thiothixene [66]. The impact on the psychotic symptoms was also more important for haloperidol, according to the Brief Psychiatric Rating Scale (BPRS) scores [66].

In a trial that compared the antidepressant effects of fluphenazine decanoate (mean dose 32.8 mg fortnightly) and flupenthixol decanoate (mean dose 36.9 fortnightly) administered two-weekly in a group of 48 out- and inpatients with schizophrenia during 15 weeks [67]. No significant differences could be found between groups regarding the number and severity of mood impairments and depression

[67]. Nineteen patients developed depression, and the addition of clomipramine was necessary for five participants, with a mean duration of four weeks, and an average dose of 75 mg/day [67]. The onset of depressive symptoms was reported between days 14 and 70 for outpatients and between days 7 and 77 for inpatients [67]. The onset of depression was unrelated to the antipsychotic dose [67]. The instruments used to assess depression in this trial were HAMD, von Zeersen's self-evaluation scale, and AMP-system [67].

According to a review of 7 studies focused on the use of antidepressants in patients with SSD, tricyclic antidepressants administered in combination with first-generation antipsychotics are a safe choice after the acute psychotic phase is controlled [68]. The risk of exacerbating residual psychotic symptoms is considered minimal by the authors of that review [68]. Still, these antidepressants were ineffective against anergy, considered a core symptom of PPD [68]. This is a controversial conclusion because "anergy" can be easily confounded with several negative signs of schizophrenia (such as avolition, apathy, or even anhedonia) [13,49,50,52,69,70]. The incidence of negative symptoms in patients with SSD is reported to be high, with more than 57% presenting with at least one such symptom, and 41% with at least two negative symptoms [69]. Other authors report a prevalence of negative symptoms that may reach 90% for at least one such manifestation, and up to 60% for at least one depressive symptom in FEP [70]. There is a risk of anticholinergic adverse events because of the pharmacokinetic interactions between the two classes of pharmacological agents [68].

Clozapine is considered a good therapeutic intervention in SSD associating mood disorders [71]. Risperidone was superior to haloperidol in decreasing anxiety and depression scores (at 8 mg/day and 10 mg/day, respectively), and the same atypical antipsychotic was more effective than haloperidol and levomepromazine on the Psychotic Anxiety Scale (at 8.6 mg/day, 9.2 mg/day, and 125 mg/day, respectively) [71]. Another review (n=33 studies focused on treatments for SSD with mood symptoms) concluded that combined treatments are as effective as antipsychotics used in monotherapy for acute psychotic exacerbations with depressive manifestations, although "some evidence" for superiority was detected in the case of antipsychotics [72]. For patients developing a major depressive syndrome after remission of acute psychosis, there has been found evidence to support the administration of antidepressants, as add-ons to antipsychotics [72]. The results regarding therapeutic response to antidepressants in cases of subsyndromal depression were mixed [72].

Figure 2: Potential therapeutic interventions for post-psychotic depression

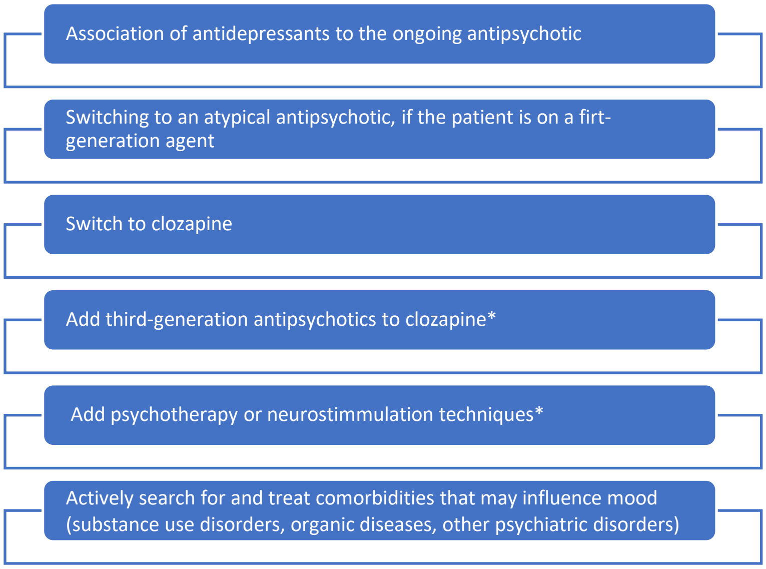

Association of antidepressants to the ongoing antipsychotic

Switching to an atypical antipsychotic, if the patient is on a firt-generation agent

Switch to clozapine

Add third-generation antipsychotics to clozapine*

Add psychotherapy or neurostimmulation techniques*

Actively search for and treat comorbidities that may influence mood (substance use disorders, organic diseases, other psychiatric disorders)

**= extrapolated recommendations from studies with other populations than patients with post-psychotic depression; not yet enough evidence to support their use*

Newer antidepressants have been launched in clinical use, but their efficacy profile in patients with PPD still remains unexplored [73-77]. Also, older antidepressants may be of interest for this topic, if added to atypical antipsychotics, because, as previously reported, the rate of residual depressive symptoms in this population is quite high [52,78]. Other interventions, besides antidepressants and atypical antipsychotics, are waiting to be investigated in clinical trials for depressive symptoms or major depressive episodes associated with SSD [79-81]. Adding third-generation antipsychotics to clozapine, in cases of ultra-resistance to treatment SSD cases, would also have to be considered a viable option for controlling depressive and negative symptoms [82,83]. Exploring organic, toxic, and psychiatric risk factors for treatment resistance is important when analyzing the most adequate treatment plan for patients with depression, PPD included [84-85].

CONCLUSIONS

The controversy surrounding the diagnosis of PPD is still under scrutiny, because (1) SSDs associate a high rate of suicide, even after the introduction of new generation antipsychotics, (2) there is a significant impact of depression on the quality of life and functional recovery in SSD patients, and (3) the case management of PPD may differ from the interventions recommended in patients with schizoaffective disorder, major depression with psychotic features, adverse events of different drugs with CNS depressogenic properties, and other conditions that may present themselves with depressive symptoms. The data about efficient interventions for PPD derives from older studies, therefore new trials exploring new generations of antidepressants are needed for this population. Based on the reviewed data, there are elements referring to risk factors, clinical characteristics, epidemiology, and even treatment for PPD, but they need to be validated by prospective, good-quality trials.

Conflicts of interest and sources of funding

None to declare.

Authors' contribution

OV conceived the idea of the review. OV, AGM, BMP, CAC, CT and CFP designed the study. OV, CFP, AGM, CAC, CFP, CT, DGV, CN and CAS screened the studies during the primary search. OV, AGM, CAC, CFP and CAS drafted the first version of the review. OV, CAC, CFP, DGV, CN and CAS revised the manuscript and elaborated the review's conclusions. All authors had full access to the data contained in this review and decided to submit the manuscript for publication.

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ORIGINAL ARTICLE

Evaluation of Adsorption Therapy as an Extracorporeal Blood Purification Technique in Critically Ill Covid-19 Patients

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Abstract: *We aimed to evaluate the efficacy of adsorption therapy as an extracorporeal blood purification technique in critically ill patients with Coronavirus disease 2019 (COVID-19). A retrospective analysis was performed on 17 adult patients with severe COVID-19, who underwent adsorption therapy using an HA330 cartridge, at Ankara City Hospital. Each adsorption therapy was administered for three consecutive days, and each therapy session lasted three hours. Several parameters, including cytokine levels, blood count, biochemistry panel, clinical status, and mortality rate were assessed before and after each therapy session. The results obtained from this study revealed that adsorption therapy reduced IL-6 levels and improved oxygenation in the short term. However, heart rate, mean arterial pressure and SOFA score did not demonstrate significant changes. Notably, the dose of norepinephrine increased after the third session. Regrettably, only 4 out of 17 patients (23.5%) survived. Consequently, adsorption therapy appears to be effective in reducing IL-6 levels in severe COVID-19 patients. Nonetheless, further investigation is warranted to evaluate its impact on clinical outcomes.*

Keywords: *Adsorption therapy; coronavirus disease 2019; cytokine storm; extracorporeal blood purification*

INTRODUCTION

COVID-19 infects the airway epithelium, leading to widespread damage, severe inflammation, and pneumonia with a cytopathic effect. A cytokine storm, capillary leak syndrome, lung injury, and Acute Respiratory Distress Syndrome (ARDS) are all brought on by the local and circulating cytokines that are produced at this time [1-4]. In patients with severe COVID-19, several inflammatory mediators are involved, and the cytokine storm has a deleterious impact on the disease's progression [5].

Additionally, COVID-19 exhibits immunological dysfunction. Techniques for extracorporeal blood purification have previously been utilized as supportive therapy to manage immunological dysregulation in the treatment of sepsis [6]. Despite the use of a variety of treatments, critically ill COVID-19 patients have consistently significant mortality rates. As a result, the use of cytokine filters as a therapy option for these individuals is now being given further thought.

Patients with COVID-19 have considerably higher levels of proinflammatory cytokines and chemokines like tumor necrosis factor-α, interleukin 1 and 6, granulocyte-colony stimulating factor, monocyte chemoattractant protein-1, interferon gamma-induced protein-10, and macrophage inflammatory proteins 1-α [7]. In individuals with severe COVID-19, these proinflammatory cytokines and chemokines lead to a cytokine storm. The extracorporeal removal of these cytokines from COVID-19 patients' blood circulation using an adsorption therapy is hypothesized to minimize hyperinflammation and calm the cytokine storm. Extracorporeal blood purification systems do not only remove pathogenic toxins and proinflammatory mediators; they also remove regulating cytokines. As a result, their process is non-selective. These regulatory cytokines are also advantageous because they restrain the immune system from overreacting to inflammation [8]. Therefore, while the application of extracorporeal blood purification procedures may have short-term advantages, its possible adverse effects on long-term outcomes have not yet been thoroughly assessed and understood.

An adsorption therapy, which is an extracorporeal blood purification technique can be used as a cytokine filter in severe COVID-19 patients. For this aim, an extracorporeal filter which is a cartridge with a sorbent inside is used for blood purification by combining convection and diffusion for solute removal [9]. Additionally, through ionic attraction, hydrophobic interactions, hydrogen bonds, and van der Waals interactions, the sorbent in the cartridge comes into direct contact with blood to adsorb solutes [10,11]. The sorbent in the cartridge also targets TNF, IL-1, IL-6, IL-10, IL-18, and other cytokines [11]. Thus, cytokines and solutes in the bloodstream bind to the sorbent material. Therefore, adsorption therapy can also be used for patients with autoimmune diseases, chronic uremic symptoms, intoxications, and inflammatory conditions with cytokine storms such as sepsis and pancreatitis [9,12].

For adsorption therapy in COVID-19 patients, the Jafron HA330 cartridge can be used [13]. The neutro-macroporous resin-adsorbing beads in the HA330 cartridge are constructed of the styrene-divinylbenzene copolymer. The resin beads' average diameter ranges from 0.60 to 1.18 mm and is 0.8 mm. The resin's pore sizes range from 500 D to 60 kD, whereas the HA330 cartridge removes molecular weights from 10 to 60 kDa (kiloDalton; Dalton [9,12]. Multiple pore sizes of the sorbent material allow the removal of several molecules with different molecular weights such as free hemoglobin, cytokines, complements, drugs, uremic toxins, and protein-bound or hydrophobic substances. The recommended treatment duration with an HA330 cartridge is 2-2.5 hours, while the sorbent material can be saturated if it is used for a longer period which causes a decrease in clearance efficiency [9,12].

In this study, we wanted to assess the efficacy of adsorption therapy in patients with severe COVID-19. To understand the effects of adsorption therapy using HA330 cartridges in severe COVID-19 patients in the intensive care unit (ICU), we evaluated the changes in the clinical status of patients, cytokine levels, complete blood count, and biochemistry panel before and after the administration of the therapy

MATERIALS AND METHODS

A retrospective analysis was performed on 14 male (82.3%) and 3 female (17.6%) adult severe COVID-19 patients who received adsorption therapy (HA330 cartridge, Jafron) in the ICU at Ankara City Hospital between April 2020 and October 2021 (Table 1). Before and after adsorption therapy with HA330, the patients' laboratory results and clinical outcomes were assessed. Ankara City Hospital (E2-21-983) granted permission for this study with the approval of the local ethics committee.

Table 1: Characteristics of severe COVID-19 patients treated with HA330 adsorption cartridge

Characteristics of COVID-19 patients	
Demographics	
Age (years) (median, IQR)	53.5 (45-62.5)
Gender, male (n, %) female (n, %)	14 (82.3) 3 (17.6%)
Treatment	
Methylprednisolone 40 mg (n, %)	17 (100)
IVIG (n, %)	1 (5.8)
Anakinra (n, %)	9 (52.9)
Tocilizumab (n, %)	1 (5.8)
Outcomes	
Length of ICU stay (days) (median, IQR)	22 (34)
Mechanical ventilator duration (days) (median, IQR)	20 (30)
Survived (n, %)	4 (23.5)
Died (n, %)	13 (76.5)

1 IQR: interquartile range. IVIG: intravenous immunoglobulin. ICU: intensive care unit. Anakinra: An interleukin-1 receptor antagonist. Tocilizumab: A monoclonal antibody against the IL-6 receptor. In this study, 14 male (82.3%) and 3 female (17.6%) adult patients with severe COVID-19 were treated with adsorption therapy (HA330 cartridge, Jafron©) in the ICU. The median age of the patients was 53.5 (IQR: 45-62.5). All 17 patients received a ten-day course of iv methylprednisolone 40 mg once daily. Of the patients, nine were treated with anakinra, one with tocilizumab, and one received IVIG treatment without tocilizumab or anakinra. Four patients died after the first adsorption therapy session, and another four died after the second. Nine patients completed three sessions of adsorption therapy, but five of them died due to sepsis and ARDS-related complications in the ICU. Overall, 4 out of 17 critically ill COVID-19 patients survived after adsorption therapy with an HA330 cartridge (23.5%).

Respiratory distress with peripheral capillary oxygen saturation (SpO$_2$) 93% in room air, dyspnea with a respiratory rate of at least 30 breaths per minute, a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO$_2$/FiO$_2$) less than 300 mmHg, and more than 50% infiltration of the lung fields are all considered to be severe COVID-19 in adult patients [14]. Moreover, cytokine release syndrome was defined as having one or more of C-reactive protein >100 (or >50 mg/L but doubled in the past 48 hours), lymphocyte count <0.6 × 109/L, serum interleukin-6 (IL-6) level higher than 3 times of the upper normal limit, ferritin >300 ug/L with doubling within 24 hours, ferritin >600 ug/L at presentation and LDH >250 U/L, and elevated D-dimer (>1 mcg/mL) [15]. These 17 patients had confirmed COVID-19 diagnoses based on real-time reverse transcription polymerase chain reaction (RT-PCR) test results and ground glass appearance with pneumonia on thorax computed tomography before admission to the ICU. They also had previously defined severe COVID-19 disease and cytokine release syndrome features. The exclusion criteria for this study included being under the age of 18, having severe heart failure with a left ventricular ejection fraction of less than 35%, having coagulation issues, and being pregnant.

All 17 patients received methylprednisolone 40 mg iv once daily for ten days. 9 patients were treated with anakinra, whereas 1 patient was treated with tocilizumab. 1 patient received immunoglobulin (IVIG) treatment without tocilizumab or anakinra.

Adsorption therapy was initially planned for 3 hours daily with an HA330 cartridge for 3 consecutive days. However, adsorption therapy could be applied to 17 patients once, 13 patients twice, and 9 patients three times. Thus, creatinine, glomerular filtration rate, urea, albumin, alanine transaminase (ALT), aspartate transferase (AST), total bilirubin, lactate dehydrogenase (LDH), troponin, fibrinogen, D-dimer, lactate, ferritin, erythrocyte sedimentation rate, C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6), hemoglobin, neutrophil/lymphocyte ratio (NLR), number of leukocytes, neutrophils, lymphocytes, and platelets were noted before and after adsorption therapy sessions. Additionally, the patients' vital signs, requirements for vasopressor medications, partial pressure of oxygen to fraction of inspired oxygen (PaO$_2$/FiO$_2$), and Sequential Organ Failure Assessment (SOFA) scores were recorded from their medical records before and after adsorption therapy sessions.

Statistical evaluation was performed using the Statistical Packages for the Social Sciences (v17.0, SPSS 24 Inc., Chicago, IL) software. Categorical data were presented as numbers and percentages, while quantitative data were presented as mean standard deviation or median and interquartile range (IQR), as appropriate. The differences between the two groups were evaluated with the Mann-Whitney U test and the Wilcoxon test. The statistical significance level was accepted as p<0.05.

RESULTS

Adsorption therapy with a Jafron HA330 cartridge was applied once to 17 patients, twice to 13 patients, and three times to 9 patients. The median age of the patients was 53.5 (IQR: 45-62.5). The median ICU length of stay was 22 (IQR:34) days. The median mechanical ventilator duration was 20 days (IQR:30). The median APACHE II score was 18 (IQR:10). The median length of stay in the ICU before the initiation of adsorption therapy was 4 days (IQR:2-6) (Table 1).

Table 2: Clinical status of patients before and after 1st, 2nd and 3rd sessions of adsorption therapy with HA330 cartridge

Clinical parameters	Before adsorption therapy (IQR)	After 1st session (IQR)	*P**	After 2nd session (IQR)	*P**	After 3rd session (IQR)	*P**
PaO$_2$/FiO$_2$ (mmHg) (n=12)	0.59 (0.46-0.92)	0.69 (0.47-0.95)	*0.12*	0.54 (0.46-0.69)	*0.44*	0.62 (0.51-0.71)	*1.0*
HR (bpm/min) (n=13)	87.0 (66.5-100.0)	80.0 (69.5-102.5)	*0.72*	77.0 (67.5-120.0)	*0.86*	90.0 (72.8-113.8)	*0.74*
MAP (mmHg) (n=14)	80.0 (73.8-102.5)	80.0 (68.8-97.5)	*0.44*	75.0 (65.0-92.5)	*0.23*	71.5 (61.2-96.0)	*0.53*
Dose of NE (μg/kg/min) (n=9)	0.12 (0.04-0.43)	0.15 (0.03-0.67)	*0.46*	0.04 (0.12-0.17)	*0.89*	0.25 (0.07-0.19)	*0.11*

*[2] Numerical data were given as a median and interquartile range (IQR). PaO$_2$/FiO$_2$: partial pressure of arterial oxygen/the fraction of inspired oxygen. HR: Heart rate. MAP: Mean arterial pressure. NE: norepinephrine. SOFA: Sequential Organ Failure Assessment. *Wilcoxon test was used.*

The first, second, and third sessions of adsorption therapy using the HA330 cartridge are shown in Table 2 along with the patients' clinical status before and after each session. For each clinical parameter, including PaO$_2$/FiO$_2$, heart rate (HR), mean arterial pressure (MAP), norepinephrine dose (NE), and Sequential Organ Failure Assessment (SOFA) score, the median and interquartile range (IQR) are shown in the table. According to these findings, the first adsorption therapy session led to a statistically significant increase in PaO$_2$/FiO$_2$ levels (p=0.03), whereas the second and third sessions did not. None of the three sessions of adsorption therapy resulted in statistically significant changes in heart rate, mean arterial pressure, or SOFA score.

The dose of NE showed a statistically significant increase after the third session of adsorption therapy (p<0.11). Overall, these data imply that the use of an HA330 cartridge in adsorption therapy may improve oxygenation in patients with severe COVID-19, but larger sample sizes and additional research are required to confirm these results.

Table 3 shows the variations in cytokine levels, complete blood counts, and biochemistry panels in severely ill COVID-19 patients receiving first, second, and third adsorption therapy sessions using HA330 cartridges. A statistically significant decrease in IL-6 levels was observed after the first, second, and third adsorption therapy sessions (361, 86.5, 28, 20.3, p<0.03, p<0.03, p<0.007, respectively). There was a statistically significant increase in leukocyte count after the first adsorption therapy compared to the leukocyte count before adsorption therapy (12.2 to 14.6, p=0.02). However, the leukocyte count decreased from 14.6 to 12.9 after the 2nd session and increased to 13.5 after the 3rd therapy session, neither of which was statistically significant (p=0.42, p=0.12, respectively). When compared to the neutrophil count prior to the first adsorption therapy (10.9 to 13.6, p=0.03), there was a statistically significant rise in neutrophil count.

However, the neutrophil count climbed to 12.1 after the third therapy session but declined to 11.6 after the second (p=0.46 p=0.19). Neither change was statistically significant (Table 3).

Table 3: Statistical analysis of laboratory parameters of severe COVID-19 patients before and after first, second, and third sessions of adsorption therapy with HA330 cartridge

Laboratory parameters	Before adsorption therapy (n=17) (IQR)	After 1st session (n=17) (IQR)	*P**	After 2nd session (n=13) (IQR)	*P**	After 3rd session (n=9) (IQR)	*P**
Hemoglobin (g/dL)	11.6 (9.1-12.7)	11.0 (9.4-12.9)	0.64	11.0 (10.1-12.4)	0.48	11.0 (9.1-11.6)	0.22
Leukocyte Count x10^9/L	12.2 (9.1-14.3)	14.6 (9.7-21.0)	**0.02**	12.9 (8.8-20.2)	0.42	13.5 (8.9-14.6)	0.12
Neutrophil Count x10^9/L	10.9 (7.8-13.4)	13.6 (8.2-19.3)	**0.03**	11.6 (7.1-19.2)	0.46	12.1 (7.7-13.0)	0.19
Lymphocyte Count x10^9/L	0.64 (0.39-0.77)	0.62 (0.42-0.80)	0.96	0.55 (0.43-0.70)	0.92	0.48 (0.38-0.78)	0.82
Platelet count x10^9/L	262.0 (200.0-303.0)	274.0 (162.0-328.0)	0.47	266.0 (195.0-350.0)	0.75	213.0 (175.0-307.0)	0.60
NLR	20.2 (15.9-32.8)	28.8 (17.9-42.1)	**0.03**	24.3 (22.1-49.7)	0.20	23.4 (18.7-29.3)	0.80
Glucose mg/dL	189.5 (114.3-236.8)	211.0 (146.5-224.0)	0.51	153.0 (128.0-205.5)	0.38	151.0 (118.5-210.5)	0.53
Creatinine mg/dL	0.93 (0.56-1.93)	0.86 (0.64-2.0)	0.64	1.0 (0.73-2.40)	0.03	1.09 (0.62-1.30)	0.26
Estimated GFR ml/min/m^2	88.0 (41.0-118.0)	94.0 (43.5-112.5)	0.60	78.0 (30.0-99.5)	0.11	83.5 (59.0-108.0)	0.25
Urea mg/dL	62.0 (42.8-98.0)	65.5 (50.3-132.8)	0.06	84.5 (52.3-161.3)	0.07	73.0 (64.0-105.0)	0.24
Albumin g/L	29.0 (26.0-32.0)	28.0 (22.5-31.5)	**0.04**	30.0 (28.3-31.8)	0.20	28.0 (26.3-30.8)	0.49
ALT U/L	46.5 (27.8-126.8)	59.0 (30.0-200.0)	0.55	51.0 (30.0-225.5)	0.64	66.0 (26.0-100.5)	0.99
AST U/L	37.0 (27.3-81.0)	59.0 (32.5-228.0)	0.84	58.0 (22.5-180.5)	0.51	45.0 (21.0-63.0)	0.30
Total bilirubin mg/dL	0.9 (0.5-1.2)	0.8 (0.5-1.2)	0.84	0.6 (0.5-0.8)	0.18	0.75 (0.4-1.3)	0.21
Troponin Ng/L	49.0 (8.8-423.1)	114.4 (24.0-647.0)	0.74	186.0 (22.8-360.7)	0.07	-	-
INR	1.4 (1.2-1.7)	-	-	1.3 (1.2-1.48)	0.60	-	-
Fibrinogen g/L	6.4 (3.7-7.2)	6.5 (3.8-7.3)	0.51	5.7 (3.4-7.8)	0.65	5.0 (1.6-7.0)	0.64
D-dimer mg/L	5.8 (1.9-13.6)	4.9 (2.4-7.3)	0.35	8.6 (1.8-17.7)	0.44	7.90 (4.4-22.0)	0.98
Lactate	2.3 (1.6-3.2)	1.9 (1.3-2.9)	0.07	1.8 (1.4-2.4)	0.21	-	-
LDH U/L	600.5 (516.8-766.5)	659.0 (543.0-963.5)	0.65	671.0 (468.5-889.0)	0.97	578.5 (488.8-674.5)	0.39
Ferritin ml/ng	961.0 (393.5-1540.0)	1500.5 (880.3-5546.0)	0.06	1461.0 (1025.5-3667.0)	0.58	1236.5 (377.5-1611.5)	0.12
ESR mm/h	56.0 (21.8-108.3)	78.0 (15.0-125.0)	0.07	65.0 (22.5-103.0)	0.72	-	-
CRP mg/L	0.16 (0.13-0.218)	0.15 (0.13-0.25)	0.51	0.15 (0.09-0.17)	0.10	0.10 (0.06-0.14)	0.09
Procalcitonin µg/L	1.01 (0.17-11.3)	4.2 (0.28-11.6)	0.44	0.53 (0.22-8.84)	0.35	0.19 (0.11-0.58)	0.06
IL-6 pg/mL	361.0 (68.2-739.8)	86.5 (10.9-167.7)	**0.03**	28.0 (20.8-76.9)	**0.03**	20.3 (7.6-32.4)	**0.007**

*[3] Numerical data are given as a median and interquartile range (IQR). NLR: neutrophil-lymphocyte ratio, GFR: glomerular filtration rate, ALT: Alanine transaminase, AST: Aspartate transferase LDH: lactate dehydrogenase, INR: international normalized ratio, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, IL-6: interleukin-6. (-): data could not be retrieved. There was a statistically significant decrease in IL-6 levels after the first, second, and third adsorption therapy sessions (361, 86.5, 28, 20.3, p <0.03, p<0.03, p<0.007, respectively). *Wilcoxon test was used*

There was a statistically significant increase in the neutrophil-lymphocyte ratio (NLR) after the first adsorption therapy compared to the NLR before adsorption therapy (20.2 to 28.8, p=0.03). Although the NLR dropped from 28.8 to 24.3 after the second session and then further to 23.4 after the third (p=0.20 and p=0.80, respectively), neither of these decreases was statistically significant. When compared to the albumin level before the initial adsorption therapy, there was a statistically significant drop in albumin (29.0 to 28.0, p=0.04). However, albumin increased from 28.0 to 30.0 after the second session and reduced to 28.0 after the third (p=0.20 and p=0.49, respectively), neither of which was statistically significant.

No statistically significant changes were observed in creatinine, glomerular filtration rate, urea, alanine transaminase (ALT), aspartate transferase (AST), total bilirubin, lactate dehydrogenase (LDH), troponin, fibrinogen, D-dimer, lactate, ferritin, erythrocyte sedimentation rate, CRP, procalcitonin, hemoglobin, glucose, lymphocyte, or platelet count after the first, second, and third sessions of adsorption therapy with HA330 cartridges. Four patients died after the first, and another four patients died after the second adsorption therapy session. Nine patients completed three sessions of adsorption therapy. However, five of these nine patients died due to sepsis and ARDS-related complications in the ICU. Only 4 out of 17 critically ill COVID-19 patients survived after adsorption therapy with an HA330 cartridge (23.5%).

DISCUSSION

There are a limited number of publications in the English medical literature related to the use of extracorporeal blood purification techniques with the United States Food and Drug Administration (FDA) approved filters such as oXiris filter (Baxter, Deerfield, IL), CytoSorb filter (CytoSorbents, Monmouth Junction, NJ), Seraph 100 Microbind blood affinity filter (ExThera Medical, Martinez, CA) and the Spectra Optia Apheresis System with the Depuro D2000 Adsorption Cartridge (Terumo BCT, Lakewood, CO) for the treatment of COVID-19 patients [8]. According to Padala et al., using an oXiris filter improved clinical outcomes in two out of three patients and reduced levels of interleukin-6, erythrocyte sedimentation rate, and CRP [16]. According to Zhang et al., CRP, IL-6, IL-8, IL-10, APACHE II, and Sequential Organ Failure Assessment (SOFA) scores all significantly decreased after oXiris filter treatment, while IL-4, IL-8, and procalcitonin showed a nonstatistically significant downward trend. Unfortunately, two of five patients passed away despite treatment [2]. Ugurov et al. reported using oXiris in ten patients with COVID-19. Two of the ten patients passed away related to complications of ARDS. According to Ugurov et al., the number of white blood cells and thrombocytes increased to the normal range whereas IL-6, CRP, and fibrinogen levels decreased [17]. According to Villa et al., treatment with oXiris resulted in a significant drop in IL-6 levels as well as improvements in hemodynamic stability, multiorgan dysfunction, and oxygenation index in 37 patients [18].

Case studies of COVID-19 patients who received treatment with a CytoSorb filter were described by Rizvi et al., Melegari et al., and Berlot et al. [1, 19, 20]. All three patients survived with an improvement in CRP, IL-6, and oxygenation. Rieder et al. treated 4 patients under venovenous extracorporeal membrane oxygenation (vv-ECMO) support with cytokine adsorption with CytoSorb filter, which decreased IL-6 in COVID-19 patients [21]. According to Alharthy et al., continuous renal replacement therapy (CRRT) sessions using a CytoSorb cartridge were administered to 50 patients. Thirty-five patients who survived after CytoSorb therapy had decreased ferritin, lactate dehydrogenase, CRP, IL-6, and D-dimer levels [15].

According to Olson et al., the Seraph-100 filter was utilized in two COVID-19 patients, and as a result, post-treatment vasopressor dose, IL-6, and CRP levels decreased [22]. The use of a Seraph-100 filter on a patient with COVID-19 significantly decreased D-dimer level, according to Pape et al. [23]. Four hemodialysis patients with severe COVID-19 were treated using a Seraph-100 haemoperfusion device, according to Sandoval et al. Despite findings from other research, Sandoval et al. failed to find any appreciable drop in IL-6, D-dimer, or ferritin levels following the treatment [24]. According to Schmidt et al., 78 COVID-19 patients who received treatment with a Seraph-100 microbind affinity blood filter were evaluated. According to Schmidt et al, patients who received Seraph-100 treatment within 60 hours of ICU admission had a reduced mortality rate [25].

Reverse Takotsubo cardiomyopathy associated with COVID-19 was treated with therapeutic plasma exchange utilizing a Spectra Optia apheresis system outfitted with a Depuro D2000 adsorption cartridge, according to Faqihi et al. Following treatment, both lactate levels and left ventricular function returned to normal [26].

Koc et al. published a review of hemadsorption therapy in severe COVID-19 patients [13]. 86 patients with severe COVID-19 were included in 16 trials that were examined by Koc et al. CytoSorb®, oXiris®, Biosky filter, SeaStar® CLR filter, HA280, HA330 Jafron, and resin-directed hemadsorption cartridges were used in hemoperfusion therapy. According to Koc et al., the mortality rate, mean intubation time, and length of stay in the ICU and hospital were, respectively, 29%, 14.93 days, 17.21 days, and 31.7 days. Following hemoperfusion sessions, the mean levels of CRP and interleukin-6 reduced (131.7 to 66.0 and 527.5 to 334.7, respectively). According to Koc et al., hemoperfusion and cytokine adsorption reduced cytokine levels, CPR, and IL-6 relative to pre-therapy, decreased mortality to 29%, and improved clinical conditions. However, these outcomes lacked statistical significance. Hemadsorption therapy was described by Koc et al. as an alternate salvage therapy for COVID-19 patients who were critically unwell. Moradi et. al reported a 73-year-old male patient was treated with hemoperfusion (HA230 cartridge, Jafron) due to a cytokine storm for 4.5 hours [27]. Moradi et al. claimed that the symptoms and need for oxygen support were alleviated during hemoperfusion treatment.

Theoretically, blocking the formation of proinflammatory cytokines can reduce hyperinflammation. The clinical outcome of severely ill COVID-19 patients can be improved by reducing hyperinflammation. Thus, anti-interleukin-6R monoclonal antibodies and corticosteroids were recommended to reduce the inflammatory response. However, IL-6 promotes neutrophil-mediated viral clearance by initiating a preliminary response against viral infections [7]. Moreover, Dienz et al. reported that IL-6 or IL-6 receptor-deficient mice could not resist Influenza A infection, which caused pulmonary damage leading to death [28]. Despite its theoretical benefit, Clark et al. reported that hemoperfusion was an experimental treatment because of its limited evidence of efficacy [29]. However, Kang et al. reported that in patients with severe COVID-19 and non-acute kidney injury, continuous renal replacement therapy using an oXiris filter might not be able to successfully reduce cytokine release syndrome [30].

Hemadsorption therapy has several additional drawbacks. Arrhythmia, coagulation issues, thrombocytopenia, and bleeding were listed as the most typical short-term hemoperfusion consequences in patients with COVID-19 by Darban et al. [31]. However, the majority of the difficulties emerged on the second and third days following hemoperfusion, not during hemoperfusion itself.

A highly biocompatible sorbent covered with a surface that inhibits platelet adherence and clotting activation is necessary for hemoperfusion. The various sorbent types utilized determine how hemoperfusion removes substances, with the effective surface area being a key factor. The sorbent material within the hemoperfusion cartridge can both absorb and lead to the loss of antibiotics and nutrients from the bloodstream [9, 10, 32].

The Jafron HA330 cartridge was the only available single-use hemoperfusion cartridge at Ankara City Hospital. Thus, we performed adsorption therapy on severe COVID-19 patients in the ICU with single-use Jafron HA330 cartridges. We used adsorption therapy as a salvage therapy when the patients did not respond to other treatment options such as antibiotics, corticosteroids, hydroxychloroquine, immunoglobulin, convalescent plasma, tocilizumab or anakinra [33].

There is still no single laboratory parameter with a cut-off value identified to initiate adsorption therapy. Moreover, the duration of therapy and the parameters to monitor the patient’s response to the treatment are still under investigation [6]. Unfortunately, there is no universally accepted standardized adsorption therapy protocol. We preferentially used adsorption therapy to treat severely ill COVID-19 patients with low procalcitonin but high CRP and IL-6 levels.

Except for a decline in IL-6, we were unable to find a statistically significant link between the quantity of adsorption therapy sessions and laboratory values. The majority of the research previously cited showed that adsorption treatment reduced IL-6 levels. However, anakinra and tocilizumab might be responsible for the IL-6 decline in COVID-19 patients [34].

Moreover, our study has certain drawbacks. First of all, the sample size was quite small. Secondly, during the pandemic, we mostly served severe COVID-19 patients in our intensive care unit, which restricts the applicability of our findings to other patient populations. Finally, because a control group was not included in our investigation, additional variables, such as medication modifications and illness progression, might have an impact on the observed changes in laboratory values. Hopefully, our study contributes to the growing body of research on the use of adsorption therapy in COVID-19 patients despite these limitations.

CONCLUSION

After examining this study's data, it can be concluded that in patients with severe COVID-19, the use of an HA330 cartridge during adsorption therapy was connected to a statistically significant drop in IL-6 levels after the first, second, and third therapy sessions. Except for a rise in the leukocyte and neutrophil counts following the first session, there were no statistically significant associations between the quantity of adsorption therapy sessions and other laboratory findings.

To draw a definitive conclusion about the effect of adsorption therapy with HA330 cartridges on COVID-19 patients, further studies with larger and more diverse patient populations are needed. These studies should also consider other potential confounding factors, such as the administration of other treatments and underlying medical conditions. Nevertheless, the findings of this study point to the possibility that adsorption therapy could be a potential supplementary treatment option for individuals with severe COVID-19.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, M.E.Y, N.H.S., C.M.A., S.I.; methodology, M.E.Y., S.I.; software, M.E.Y.; validation, N.H.S., C.M.A., M.E.Y., S.I.; formal analysis, M.E.Y.; investigation, M.E.Y.; resources, N.H.S., C.M.A., M.E.Y., S.I.; data curation, N.H.S, C.M.A, M.E.Y., S.I.; writing-original draft preparation, M.E.Y.; writing-review and editing, N.H.S., C.M.A., M.E.Y., S.I.; visualiza-tion, M.E.Y.; supervision, S.I.; project administration, S.I. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Ankara City Hospital (E2-21-983).

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ORIGINAL ARTICLE

Psychometric Properties of the Method "Evaluation of Negative Mental Reactions and States of Combatants" and Experience of its Application in Short-term Psychological Recovery

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Abstract: *The article shows the development of the method of psychological evaluation called "Evaluation of Negative Mental Reactions and States of Combatants". In the study participated 1300 male servicemen (29.84% from junior lieutenant to colonel and servicemen under contract and demobilized, and 70.16% from private to senior warrant officer). The age of participants varied from 20 to 55 years. The system of evaluation developed consisted of 16 instruments that could help to determine the presence of negative psychological symptoms of servicemen related to their participation in hostilities. The results indicated that the evaluation method developed is a tool that allows determining the presence of negative psychological symptoms related to participation in combat. Likewise, it is a useful and fast method to assess the effectiveness of short-term psychological recovery programs. Unlike existing methods of diagnosing negative mental reactions and states of an individual, which arose after their participation in hostilities, the developed psychodiagnostic toolkit could consider the physical and mental fatigue of the respondents, their deterioration, and other cognitive dysfunctions*.

Keywords: *combat activity, psychological rehabilitation, psychological recovery, post-traumatic stress disorder, evaluation system*

INTRODUCTION

Long participation in hostilities depleted the psychological adaptation resources of servicemen, sharply posing the question concerning the need for psychological recovery [1,2]. This issue was complicated and comprehensive; it related both to the definition of rotation of servicemen and to the determination of the duration and development of effective programs of psychological recovery for all participants of hostilities [3] as well as to the determination of indications for medical and psychological rehabilitation of the servicemen, whose mental health and ability to readaptation was lost [4]. The aim of psychological recovery was to restore the physical and mental resources of servicemen to prolong their ability to perform tasks under the conditions of combat stress factors action [5].

Nowadays, the psychological decompression measures were directed not only to ensuring the adequacy of switching from military service to peaceful life [6], including traditional stress relief and help in civilian life realization, taking into account the acquired military experience, accepting this experience to reduce the probability of post-traumatic stress disorders (PTSD) formation [7,8], but also for the psychological restoration of mental health of servicemen who participated in hostilities to relieve the accumulated stress prolonging the possibility of adequate functioning of servicemen under combat conditions when it was impossible to realize the massive rotations to keep their resistance – psychological stability, ability to resist negative influence from the side of combat stress factors [2]. To the institutions of psychological recovery were sent the servicemen with expressed signs of maladjustment [9], acute stressful reactions [7], significant exhaustion, devastation, and fatigue [10], and servicemen, who had difficulties in adaptation [11] while getting back in line after injuries and concussion and after their stay in hospitals.

Institutions that implemented psychological recovery programs were located in the third echelon of defense in safe places in order to form in the minds of servicemen the idea of the need to return to the performance of combat missions, and military duty, which was a way of posing the direction of recovery – the restoration of combat capacity.

Realization of activities of psychological rehabilitation and implementation of existing psychological programs of decompression on the basis of their personal professional experience and world experience of rehabilitation of servicemen was imposed on the military psychologists of the Armed Forces and the National Guard of Ukraine, as well as on the volunteer psychologists from NGOs who had appropriate training and experience. Usually, the implementation of psychological recovery programs was a combination of procedures of medical treatment orientation with the use of appropriate material and technical bases and the involvement of appropriate specialists.

Even though the system of implementation of the psychological restoration of combat servicemen was well-established and proved its effectiveness in practice, there arose a question on the development of effective psychodiagnostic tools of the mental state control of servicemen before and after a complex of actions by the program of psychological rehabilitation for individual servicemen and correction of the recovery program if required. There were distinguished the following requirements for the psychodiagnostic method:

1) convenient while conducting both individual and group psycho-diagnosis of servicemen, typical for a survey with a large number of people (100-200 people), capable of taking into account the physical and mental fatigue of the respondents, deterioration of their attention, and other cognitive dysfunctions related to stressful experience;

2) suitable for the diagnosis of negative mental reactions and conditions that occur after participation in hostilities;

3) reliable for re-diagnosis due to a minor period of time, excluding the ability to remember accurately their past answers;

4) able to clearly demonstrate changes in psychological state that occurred during their participation in the psychological recovery program;

5) convenient for summarizing data and receiving the psychologist's feedback on the effectiveness of the introduction of new actions into the programs of psychological recovery;

6) useful for obtaining information related to the effectiveness of individual consulting, providing a psychologist with a reflection on his/her professional activity. With existing pressure on psychologists, there occurred a need for supervision and their rotation in institutions that dealt with psychological recovery.

The objective of the study was the development of a psychological evaluation system of the consequences of participation in combat activities for military personnel participating in hostilities. Unlike the existing systems to diagnose the psychological consequences of combat participation, it is intended to develop a set of psychodiagnostic tools that assess the physical and mental fatigue of combatants, their deterioration, and other cognitive dysfunctions. And also, that it is capable of clearly showing the psychological changes achieved by participation in psychological recovery programs.

MATERIALS AND METHODS

Participants and Procedure

The study was conducted in 2022. The study included 1300 servicemen who underwent a psychological recovery program after taking part in a long combat mission. The tested group consisted of officers – 29.84% (from junior lieutenant to the colonel) and servicemen under the contract and mobilized – 70.16% (from private to senior warrant officer). The age of the study participants varied from 20 to 55 years.

While developing the method "Evaluation of negative mental reactions and states of combatants", there were combined the simplest procedures of conditions evaluation which were typical for the method such as "Profile of mood states" (POMS) [12,13], "Self-esteem of psychophysiological state" (Kokun) [14], "Measurement of states" used to express evaluation of emotional states of sportsmen [15]. Also, there was conducted analysis of negative mental reactions and states that often occurred in servicemen while their participation in hostilities, including those who described the ability to perform tasks and the ability to interact with others in the performance of command work [1,16]. Thus, the list of negative mental reactions and states (experience) was quite wide and contained 16 points: irritability; anxiety; aggressiveness;

anger; inattention; self-doubt; devastation; apathy; concern; sense of guilt; sense of powerlessness; lack of concentration; unwillingness to communicate; lack of trust in comrades in the service; lack of trust in commanders; inability to perform the assigned tasks. It was assumed that the use of factor analysis would give the possibility to group these points into the scale.

It was necessary to point out that the simplicity of the instructions and of the testing procedure itself was an important circumstance for effective diagnostic work with servicemen who had been experiencing the influence of combat stress factors for a long time and who had difficulty in the redistribution of attention.

However, a number of points (16) of the evaluation of negative mental states but the evaluation scale itself (from 0 to 10 points) was quite large (exceeding the amount of short-term memory 7±2 elements), which complicated their memorization and accuracy reproduction during re-testing.

The determination of psychometric indicators of the method “Evaluation of negative mental reactions and states of combatants” and its testing was carried out during the participation in a psychological recovery program by servicemen. 1300 servicemen participated at this stage.

The servicemen filled out the form of the method at the beginning of the psychological recovery program and after it. The results obtained in accordance with the method “Evaluation of negative mental reactions and states of combatants” were compared with the results of methods that were conducted with servicemen to control their condition before and after their participation in the psychological recovery program. Before participating in a psychological recovery program, servicemen were surveyed with the following methods: “Maladjustment” (Prykhodko, Matsegora, Kolesnichenko, and Baida) [17], “Mississippi Scale for Combat-Related PTSD” (Keane, Caddell, and Taylor) [18], “Diagnostics of psychological safety of an individual” (Prykhodko, 2012) [19], “Evaluation of traumatism of combat experience of servicemen” (Kolesnichenko) [20]. After participating in the psychological recovery program, servicemen were surveyed in accordance with the following psychodiagnostic methods – “Resistance to psychological traumatism of combat experience” (Kolesnichenko) [21].

All procedures in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants consent for their data to be used in this research.

Instruments and Measures

To determine the constructive validity of the method "Evaluation of negative mental reactions and states of combatants" there were used methods which were standardized on the Ukrainian selection.

Method "Maladjustment" (Prykhodko, Matsegora, Kolesnichenko, and Baida) [17] was an express version of the method "Adaptability" (Maklakov) [22] which had a long tradition of use in the Armed Forces of Ukraine. The "Adaptability" method was concluded by military psychologist A.H. Maklakov [22] to determine the adaptation potential of servicemen based on the individual scales of the method "Minnesota Multiphasic Personality Inventory" (McKinley, Hathaway) [23]. The developer reported satisfaction with the psychometric characteristics of the formed methodology [22]. Since the beginning of hostilities in eastern Ukraine among military psychologists of Ukraine, there was spread a modification of the methodology proposed by Maklakov "Adaptability 200", carried out by Chermianin and Kondratiev [24]. Unlike the previous method, it was supplemented with a number of issues that allowed us to distinguish "military and professional orientation", "tendency to deviant behaviors", and "suicidal risk" and to calculate "the level of resistance to combat stress". This method included 200 questions; thus, there was a need to form a simpler and more reliable toolkit, which was the basis for the development of an express version of the method. The developed method "Maladjustment" (Prykhodko, Matsegora, Kolesnichenko, and Baida) [17] was aimed at the identification of specific violations of adaptation potential which could have a big influence on the ability to adequately perform the tasks. Thus, the method had 4 scales, each with 10 questions – "Violation of behavioral regulation", "Probability of committing suicidal attempts", "Violation of moral normativity" and "Loss of communicative potential". There was highlighted the calculation of the general indicator of "Maladjustment". This calculation was simple and convenient and could be carried out under field conditions without any additional tools. Although working with the method involved the presence of trust in psychologists, the method had a scale of sincerity. The developers provided data about the satisfactory psychometric indicators of the method.

The study used the "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor), which was restored for the Ukrainian selection [18]. Nowadays, it was one of the most common methods used to measure the signs of PTSD. The scale included 35 statements, each of which can be estimated on a five-point scale. The total indicator which was made up in accordance with this method gave the possibility to estimate the degree of influence of the traumatic experience transferred by an individual.

"Diagnosis of Psychological Safety of an Individual" (Prykhodko), which was developed on the basis of the concept of psychological safety of an individual of specialists of extreme activities [19]. According to this concept, psychological safety was not only a lack of danger but also an opportunity for development and self-realization. Psychological safety had a level structure. Each level in the method met a scale with a similar name. The first level was moral and communicative, it meant that knowledge and use of the rules of communication and norms of morality made interaction with the social environment controlled and predictable, formed the ability to escape potentially conflict situations, and involved external resources to overcome problematic situations. The motivational and volitional levels suggested that the developed goal setting, intensity in goal setting, and flexibility in their corrections could ensure the self-realization of an individual in difficult conditions of life. It was assumed that the ability to flexible restructuring of relations with the environment gave the possibility to be leading in these relationships, to control them. The valuable and semantic level was considered as an ability to conscious attitude to one's own life, awareness of one's own influence on the world, and responsibility for its changes. The developer believed that this conscious attitude gave the possibility for an individual to tolerate life difficulties and sufferings that arose in the process of self-realization. Moreover, due to the developer, the hierarchy of the semantic sphere gave the possibility to depreciate the experience in frustration as unimportant and to consolidate efforts to achieve important aims. The level of internal comfort was the factor that provided a change in the direction of activity. Thus, the evaluation of life as safe, and the condition as comfortable contributed to the decision to continue normal regulation. The decisions related to the changes were made in case of non-compliance of the environment with the needs and capabilities of an individual. There was highlighted the calculation of the general indicator of psychological safety of an individual, its height indicated the ability to set new goals of self-realization. When the indicators were reduced the thing was in a supportive reproduction of usual goals of life or in the arrest of development, and further impossibility of self-realization. The developed method had satisfactory psychometric indicators. The National Guard of Ukraine had been using this method for more than 10 years.

The method of "Evaluation of traumatism of combat experience of servicemen" [20] was based on the American method "Combat Exposure Scale" [25]. In the process of its development, there were considered the traumatic events and their intensity which were typical for military actions in Ukraine with the beginning of the Russian invasion in 2014. Unlike the "Combat Exposure Scale" method, the method of "Evaluating of traumatic experience of servicemen" (Kolesnichenko) had been developed; it included a list of phenomena that gave the possibility to reduce the trauma of combat stress factors influence. The method had 5 scales that met 3 groups of combat stress factors and 2 factors that described internal and external resources used to counteract stress factors. In the process of standardizing the method, there was used the procedure of regressive analysis which gave the possibility to determine the contribution of each scale to acute stress disorder which occurred due to the action of combat stress factors. It was reported that the method had satisfactory psychometric characteristics.

The method "Resistance to psychological traumatism of combat experience" (Kolesnichenko) [21] was developed on the basis of the author's concept of combat psychological traumatism of servicemen [1]. The method reflected the given in this concept three levels (stages) of realization of resistance to the action of combat stress factors, which 1) were related to the preparation for activity under the conditions of combat stress factors and under the existing expectations concerning the stress factors’ activity and their ability to overcome them, 2) had the direct ability to overcome stressful influence and used adequate situational copings, and 3) had the ability to accept and realize the acquired experience under the stressful conditions. There was provided the calculations of general resistance to combat psychological traumatism. It was reported that this indicator had a reverse correlation with the "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18], r = -0.60, p≤0.01. Moreover, the method of the scale of the second order – "helplessness", "conscious attitude to professional training", "ability to acquire experience", "cognitive dysfunction", and "attitude to military duties". The method had satisfactory psychometric indicators.

Data Analysis

To represent the data, there was used the main descriptive statistics (arithmetic mean M and standard deviation SD). To distinguish the correlational rate between the variables there was used the correlation analysis. To determine the internal coherence of the method "Evaluation of negative mental reactions and states of combatants" there were used calculations based on Cronbach's alpha. To determine the specific negative mental reactions and states which were experienced by servicemen due to their participation in hostilities there was used regression analysis. To determine the structure of the method "Evaluation of negative mental reactions and states of combatants" there was used factor analysis with the help of the method "Principal components". Mathematical data was processed with the help of SPSS 17.0.

RESULTS

We have developed the following form of psychodiagnostic method "Assessment of negative mental reactions and states in combatants" (Table 1). The instructions are as follows:

"Please assess your psychological state at the moment on a scale from 0 - slightly expressed, 10 - strongly expressed". The internal consistency of the method was evaluated using the α-Cronbach indicator, which was 0.944 having the selection of 1300 people for 16 indicators.

Table 1: Form of the methodology "Evaluation of negative mental reactions and states of combatants"

Mental reactions and states	Evaluation of reactions and states										
Irritability	0	1	2	3	4	5	6	7	8	9	10
Anxiety	0	1	2	3	4	5	6	7	8	9	10
Aggressiveness	0	1	2	3	4	5	6	7	8	9	10
Anger	0	1	2	3	4	5	6	7	8	9	10
Inattention	0	1	2	3	4	5	6	7	8	9	10
Self -doubt	0	1	2	3	4	5	6	7	8	9	10
Devastation	0	1	2	3	4	5	6	7	8	9	10
Apathy	0	1	2	3	4	5	6	7	8	9	10
Concern	0	1	2	3	4	5	6	7	8	9	10
Sense of guilt	0	1	2	3	4	5	6	7	8	9	10
Sense of powerlessness	0	1	2	3	4	5	6	7	8	9	10
Lack of concentration	0	1	2	3	4	5	6	7	8	9	10
Unwillingness to communicate	0	1	2	3	4	5	6	7	8	9	10
Lack of trust in comrades in the service	0	1	2	3	4	5	6	7	8	9	10
Lack of trust in commanders	0	1	2	3	4	5	6	7	8	9	10
Inability to perform the assigned tasks	0	1	2	3	4	5	6	7	8	9	10

Despite the fact that the developed method included negative mental reactions and states for evaluation of PTSD, and maladjustment, and testified both negative experiences and inability to manage their behavior, its purposefulness and inability to maintain interaction and consistency in teamwork, factor analysis which was conducted in accordance with "Principal Components" presented the presence of only one factor that included all 16 variables with different factors of factor evaluation: "irritability" (0.743), "anxiety" (0.796), "aggressiveness" (0.692), "anger" (0.722), "inattention" (0.744), "self-doubt" (0.744), "devastation" (0.808), "apathy" (0.733), "concern" (0.808), "sense of guilt" (0,793), "lack of concentration " (0.801), "unwillingness to communicate" (0.714), "lack of trust in comrades in the service" (0.640), "lack of trust in commanders" (0.675), "inability to perform the assigned tasks" (0.746).

Taking into account the received data, the method included the calculations of general indicators. Experience using the method had shown that the most convenient indicator was the calculation of the average arithmetic value for all 16 indicators. Thus, the general indicator had a range from 0 to 10 points, which was convenient to perceive general results by the method and did not require memorization of additional information for manual data processing.

It was necessary to point out that the selection of a single-factor structure of the method was a certain confirmation of less regularity of negative mental reactions and states, a reflection of greater integration of mental processes while operating under stressful conditions.

The determination of the validity of the method was conducted in two stages. In the first stage, to determine the constructive validity, the data of the method "Evaluation of negative mental reactions and states of combatants" was compared with the data of methods which gave the possibility to diagnose the peculiarities of negative mental reactions and states of servicemen, proving that they were related to their participation in hostilities. First of all, the talk was about the method "Maladjustment" which gave the possibility to evaluate the violation of "behavioral regulation", "the probability of committing suicidal attempts", "violation of moral normativity" and "loss of communicative potential" by servicemen [17], and "Mississippi Scale for Combat-Related PPSD" (Keane, Caddell, and Taylor) [18], which gave the possibility to diagnose PTSD symptoms of those who had participated in hostilities and left the battlefield [24]. Moreover, there was used the method of "Evaluation of traumatic experience of servicemen" (Kolesnichenko) [20] and "Resistance to psychological traumatism" (Kolesnichenko) [21], which in this study gave the possibility to connect the experience of negative mental reactions and states with the experience of participation in hostilities, as well as with their peculiarities and preparation for them.

Correlational relations with the method of "Diagnosis of psychological safety" (Prykhodko) [19] indicated the depth of violation of personality structures, which ensured its safety and ability to develop, which was an important factor for making a prognosis for the restoration of an individual in the process of psychological decompression and rehabilitation. The results of correlational analysis, which were obtained at this stage of validity determination were given in Table 2. Considering that the selection of the study at this stage included 1300 people, then all the correlational indicators given reached the level of statistical significance and did not require additional indicators.

Dense correlation relations of the method "Evaluation of negative mental reactions and states of combatants" and methods such as "Maladjustment" (Prykhodko et al.) [17] and "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18] pointed out that according to the data provided by the developed method, it was really possible to consider negative experience, which was connected with adaptation disorders and PTSD symptoms. The data provided by the method "Evaluation of traumatic experience of servicemen" [20] proved that negative experience was mostly related to the force of negative effects than to the existing external support.

However, the formed personality structure (according to the method "Diagnosis of Psychological Safety” [19] including the process of professional and professional-psychological training, due to the method "Evaluation of traumatism of combat experience of servicemen" [20] was able to restrain the development of negative states, providing adequate functioning of an individual under combat conditions. But at this stage, it was impossible to monitor the accumulated influence of traumatic events on the development of negative mental states while exceeding the recommended time for rotation (more than 6 months).

The second stage of determination of the method’s validity was developed to determine its ability to be a tool for tracking the dynamics of changes during psychological recovery. The methods which were used at the first stage had satisfactory indicators of reliability, and, therefore, could be insensitive to the changes which occurred during the week (this was a usual term of the program of psychological recovery of servicemen). The method of "evaluation of negative mental reactions and states of combatants" was developed specifically for evaluating the control of negative states’ changes before and after participation in the psychological recovery program, including the possibility to evaluate its effectiveness.

Considering such purpose, the data obtained by the method "evaluation of negative mental reactions and states of combatants" before and after the participation in the recovery program was compared to the data obtained by Lüsher's color test including the indicator of anxiety. This indicator pointed out the availability of frustrated needs and inadequacy of compensation and, unlike other indicators by this projective method, could be confused with indicators by other methods. The indicator of anxiety in accordance with Lüsher's color test was used as an integrated state evaluation since the main determinants of mental states were the ratio: firstly, conscious and unconscious needs, desires, and aspirations of an individual; secondly, the capabilities of an individual (obvious abilities and hidden potentials); thirdly, environmental conditions (objective influence and subjective perception of the current situation) [25].

40 servicemen participated at this stage of the study. Thus, the general indicator by the method "evaluation of negative mental reactions and states of combatants" correlated with anxiety before the beginning of the decompression program at a moderate level – r = 0.41, p≤ 0.01; after the decompression program the data was – r = 0.47, p≤ 0.01. These data proved that the developed method was sensitive to the changes in states and could be used to evaluate the dynamics of states during the programs of psychological recovery which were developed for short-term (up to a week).

It was figured out that the general indicator (see Table 2) had a slightly higher density than individual indicators by the method "evaluation of negative mental reactions and states of combatants" with general indicators of maladjustment, PTSD, psychological safety of an individual, traumatism of combat experience, and resistance to psychological traumatism. It was another piece of evidence that the method would be more reliable if it was used as a single scale.

Then there was used the procedure of regression analysis to determine specific negative mental reactions and states experienced in these psychological categories; thus, there were formed relevant regression equations:

Maladjustment = 3.804 + 0.216*Irritability + 0.307* Aggressiveness + 0.259*Self-doubt + 0.249*Devastation + 0,245*Concern + 0.386*Reluctance to communicate + 0.229* Lack of trust in comrades in the service + 0.350*Lack of trust in commanders +0.264* Inability to perform the assigned tasks + 0,529.

Post-traumatic stress disorder = 54.66 + 1,070*Irritability + 0.719*Aggressiveness + 0.572*Inattention + 0.557* Devastation + 1.230*Apativity + 0.794*Concern + 0.613* Unwillingness to communicate + 0.602*Lack of trust in commanders +0.941* Inability to perform the assigned tasks +0.506.

Table 2: The indicators of correlative relations between the scales of method "Evaluation of negative mental reactions and states of combatants" and the scales of other psychodiagnostics methods which were used to estimate its validity (in points).

Indicators	"Estimation of negative mental reactions and states of combatants"																
	Irritability	Anxiety	Aggressiveness	Anger	Inattention	Self-doubt	Devastation	Apathy	Concern	Sense of guilt	Sense of powerlessness	Lack of concentration	Unwillingness to communicate	Lack of trust in comrades in the service	Lack of trust in commanders	Inability to perform the assigned tasks	General indicators due to the method
"Maladjustment" (Prykhodko, Matsegora, Kolesnichenko, and Baida) [17]																	
Violation of behavioral regulation	0.50	0.56	0.46	0.44	0.49	0.51	0.52	0.51	0.54	0.41	0.54	0.52	0.49	0.42	0.49	0.55	0.68
Probability of committing suicidal attempts	0.41	0.52	0.36	0.39	0.47	0.48	0.54	0.48	0.49	0.41	0.53	0.49	0.48	0.38	0.42	0.51	0.62
Violation of moral normativity	0.34	0.24	0.37	0.33	0.20	0.16	0.25	0.18	0.23	0.14	0.22	0.23	0.29	0.28	0.36	0.19	0.34
Loss of communicative potential	0.36	0.37	0.39	0.38	0.35	0.35	0.38	0.34	0.37	0.27	0.35	0.35	0.43	0.41	0.40	0.36	0.50
General indicators of maladjustment	0.50	0.52	0.49	0.47	0.47	0.47	0.52	0.47	0.50	0.38	0.51	0.49	0.52	0.46	0.52	0.50	0.66
"Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18]																	
PTSD	0.54	0.56	0.51	0.48	0.50	0.50	0.57	0.53	0.54	0.44	0.54	0.53	0.50	0.44	0.48	0.55	0.69
"Diagnostics of psychological safety of an individual" (Prykhodko) [19]																	
Trust	-0.29	-0.34	-0.25	-0.29	-0.29	-0.32	-0.38	-0.30	-0.39	-0.35	-0.39	-0.35	-0.35	-0.33	-0.30	-0.35	-0.44
Respect	-0.25	-0.25	-0.26	-0.29	-0.22	-0.18	-0.28	-0.20	-0.30	-0.24	-0.26	-0.26	-0.32	-0.29	-0.24	-0.21	-0.34
Moral and communicative	-0.30	-0.33	-0.28	-0.32	-0.29	-0.28	-0.37	-0.28	-0.38	-0.33	-0.36	-0.35	-0.37	-0.34	-0.31	-0.32	-0.44
Motivational and volitional	-0.31	-0.38	-0.27	-0.31	-0.34	-0.36	-0.38	-0.33	-0.39	-0.36	-0.40	-0.41	-0.36	-0.28	-0.25	-0.34	-0.46
Valuable and semantic	-0.28	-0.30	-0.27	-0.28	-0.28	-0.28	-0.35	-0.29	-0.32	-0.32	-0.36	-0.33	-0.36	-0.30	-0.25	-0.29	-0.41
Internal comfort	-0.24	-0.30	-0.22	-0.25	-0.30	-0.31	-0.39	-0.33	-0.31	-0.32	-0.37	-0.34	-0.33	-0.27	-0.25	-0.29	-0.40
General indicators of psychological safety of an individual	-0.30	-0.35	-0.28	-0.31	-0.32	-0.32	-0.39	-0.33	-0.37	-0.35	-0.39	-0.38	-0.38	-0.32	-0.28	-0.33	-0.45
"Evaluation of traumatism of combat experience of servicemen" (Kolesnichenko) [20]																	
Unfavorable social conditions of performing service combat tasks	0.47	0.49	0.41	0.37	0.37	0.39	0.42	0.41	0.44	0.33	0.42	0.42	0.34	0.41	0.50	0.45	0.58
Specific traumatic combat conditions	0.27	0.29	0.23	0.22	0.13	0.17	0.14	0.21	0.23	0.21	0.14	0.18	0.11	0.22	0.29	0.15	0.28
Unfavorable social situation of participation under combat conditions	0.21	0.23	0.25	0.26	0.08	0.09	0.09	0.13	0.18	0.06	0.11	0.13	0.15	0.14	0.23	0.09	0.21
Internal resources of resistance	-0.12	-0.14	-0.03	0.00	-0.23	-0.20	-0.24	-0.27	-0.12	-0.10	-0.24	-0.22	-0.10	-0.15	-0.07	-0.27	-0.21
External resources of resistance	-0.11	-0.14	-0.08	-0.10	-0.16	-0.17	-0.20	-0.13	-0.17	-0.12	-0.16	-0.08	-0.07	-0.09	-0.01	-0.14	-0.16
General indicators of traumatism of combat experience	0.32	0.37	0.25	0.24	0.32	0.32	0.38	0.37	0.34	0.23	0.36	0.31	0.17	0.28	0.24	0.36	0.42
"Resistance to psychological traumatism of combat experience" (Kolesnichenko) [21]																	
Expectations of participation in hostilities	-0.32	-0.37	-0.25	-0.15	-0.40	-0.37	-0.39	-0.38	-0.34	-0.31	-0.43	-0.39	-0.26	-0.27	-0.34	-0.46	-0.48
Overcoming a stressful situation	-0.38	-0.38	-0.29	-0.16	-0.40	-0.41	-0.38	-0.31	-0.30	-0.27	-0.40	-0.41	-0.28	-0.27	-0.26	-0.46	-0.46
Implementation of the received experience	-0.36	-0.34	-0.31	-0.19	-0.39	-0.30	-0.44	-0.38	-0.30	-0.25	-0.41	-0.40	-0.30	-0.29	-0.30	-0.42	-0.47
General indicators of resistance to traumatism	-0.40	-0.41	-0.32	-0.18	-0.45	-0.41	-0.45	-0.40	-0.36	-0.31	-0.46	-0.45	-0.32	-0.31	-0.34	-0.50	-0.53
Helplessness	0.28	0.32	0.23	0.16	0.34	0.38	0.30	0.28	0.29	0.26	0.35	0.33	0.27	0.25	0.27	0.39	0.41
Conscious attitude towards professional training	-0.12	-0.07	-0.10	0.01	-0.16	-0.08	-0.17	-0.16	-0.09	-0.07	-0.18	-0.18	-0.07	-0.09	-0.10	-0.17	-0.15
The ability to get experience	-0.35	-0.36	-0.25	-0.17	-0.34	-0.28	-0.33	-0.25	-0.22	-0.23	-0.30	-0.39	-0.19	-0.27	-0.24	-0.34	-0.38
Cognitive dysfunction	0.40	0.38	0.29	0.13	0.37	0.36	0.38	0.32	0.28	0.26	0.39	0.39	0.27	0.23	0.27	0.42	0.45
Conscious attitude towards military duties	-0.21	-0.27	-0.20	-0.09	-0.28	-0.22	-0.34	-0.32	-0.22	-0.18	-0.35	-0.28	-0.18	-0.21	-0.25	-0.38	-0.35

Psychological safety of an individual = 5.325 – 0.072*Irritability – 0.064*Devastation – 0,077*Sense of guilt – 0,095*Sense of powerlessness – 0.071* Reluctance to communicate + 0.775.

Traumatism of combat experience = -17.672 + 0.426*Anxiety + 0.393*Apathy + 0.502*Inability to perform the assigned tasks + 0.789.

Resistance to combat psychological traumatization = 167.165 – 2.514*Anxiety + 2.869*Anger – 1.621*Apathy – 1,510* Lack of trust in comrades in the service – 4.812*Inability to perform the assigned tasks + 0.599.

The procedure of regression analysis had to give the possibility to reduce the dimension of measurements for each state, but as it could be seen, the error of evaluation in the concluded regression equations was quite high, especially in the process of predicting traumatism of combat experience (e = 0.789) and psychological safety of an individual (e = 0.775) that made it inappropriate to use them for calculations. However, the information obtained through regression analysis gave the possibility to better understand the experience of servicemen who had different negative symptoms due to their participation in hostilities.

Moreover, a long-last period of testing and a large selection of the study had shown that the calculations made with the help of regression analysis by individual scales were less accurate than the normalized general indicator by the method (that indicated one more time the importance of using the method as a single scale). It was the regulation for different negative symptoms that prevailed in determining the ways of processing data by the method.

Thus, the obtained data showed that if the general indicator by the method "Evaluation of negative mental reactions and states of combatants" exceeded 3.5 points, then 65.45% of servicemen-participants of the psychological recovery program were likely to be symptoms of maladjustment by the same method. If the average points according to the developed method were 5 or more, then more than 85% of servicemen with this indicator were diagnosed with expressed symptoms of maladjustment.

The comparison of the general indicator by the method "evaluation of negative mental reactions and states of combatants" with the method "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18] showed that while exceeding 3.5 points according to the scale of the developed method more than 90% of servicemen with this indicator had expressed PTSD symptoms. When the average quantity of points was 5.5% – 100% of servicemen who participated in psychological recovery activities had an indicator that exceeded the norm by the "Mississippi Scale for Combat-Related PTSD" (Caddell and Taylor) [18].

The comparison with the data provided by the method "Resistance to psychological traumatism of combat experience" showed that with general points 3 or more in accordance with the method "Evaluation of negative mental reactions and conditions of combatants" 75.59% of servicemen with such quantity of points were diagnosed with an unsatisfactory indicator of resistance (from 0 to 143 points).

If the general indicator was exceeded in accordance with the method "Evaluation of negative mental reactions and states of combatants" the score of 5 points meant that low resistance to combat psychological traumatism was diagnosed in almost 86% of servicemen who participated in psychological recovery activities.

The comparison with the express method "Psychological Safety of an Individual" (Prykhodko) [19] and "Evaluation of traumatism of combat experience of servicemen" (Kolesnichenko) [20] pointed out that if the points were less than 3 by the general scale of the method "Evaluation of negative mental reactions and states of combatants" 89% of servicemen retained satisfactory indicators of psychological safety of an individual, and 85.41% were diagnosed with low trauma of combat experience.

More reliable for these two methods was a forecast concerning psychological well-being than negative symptoms.

Thus, the given data showed that for the general indicator by the method "Evaluation of negative mental reactions and states of combatants" it was normal to have a high probability of negative symptoms' absence (PTSD, maladjustment, instability to combat psychological traumatization, and psychological safety of an individual) and it also showed that high ability to recover was possible when an indicator was less than 3 points.

It was necessary to point out that the results obtained with the help of the method, both individual and group ones, were convenient to present in the form of column histograms. As an example, there was proposed the histogram of the dynamics of negative mental reactions and conditions of servicemen with signs of maladjustment (in particular, violation of behavioral regulation) in the process of one of the decompression courses (Figure 1).

Figure 1: Dynamics of negative mental reactions and conditions while participating in psychological recovery in a group of servicemen with violation of behavioral regulation (in points)

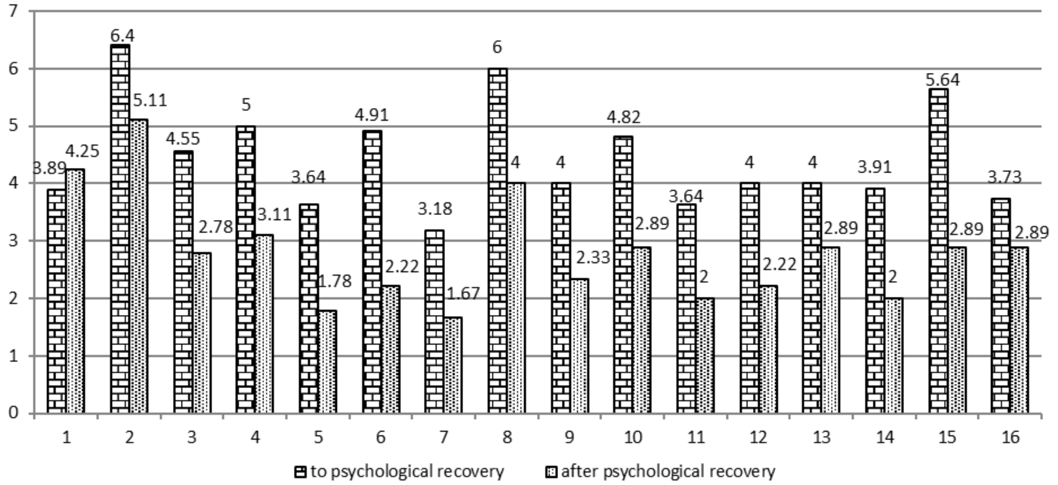

Notes: 1 – inability to perform the assigned tasks; 2 – lack of trust in commanders; 3 – lack of trust in comrades in the service; 4 – unwillingness to communicate; 5 – lack of focus; 6 – sense of impotence; 7 – sense of guilt; 8 – concern; 9 – apathy; 10 – devastation; 11 – self-doubt; 12 – inattention; 13 – anger; 14 – aggressiveness; 15 – anxiety; 16 – irritability

DISCUSSION

According to Ukrainian [14] and foreign specialists [26,27], early diagnosis of negative mental reactions and states [28], post-stress states, and determination of a group of increased psychological attention among personnel gave the possibility to identify servicemen with low personal adaptive potential and high probability of early and severe manifestations of combat psychological traumas [29] and its consequences [30]; also gave the possibility to create individual tactics and methods of prevention and restoration of each surveyed serviceman. To solve the problem of diagnosis of negative mental reactions and states of servicemen, it was suggested to use the following psychodiagnostic tools: "The Hospital Anxiety and Depression Scale (HADS) ", "Brief Scale of Anxiety, Depression, and PTSD", "The Montgomery-Asberd Depression Rating Scale (MADRS)” [31], "Beck Depression Inventory (BDI)" [32], "Primary Screening for Post-Traumatic Stress Disorder (PTSD)", "Mississippi Scale for Combat-Related PTSD" (Keane, Caddell, and Taylor) [18], "Impact of Event Scale–Revised (IES-R)" [33], "Patient Health Questionnaire (PHQ)-9" [34], "Clinical-administered PTSD Scale" [35], "Neurotization and Psychopathization (LNP) questionnaire" [14] and others. As an additional diagnostic tool, at the request of a psychologist, there was used the method "The Suicidal Behaviors Questionnaire-Revised (SBQ-R)" [36] to diagnose the suicidal inclinations of servicemen. However, this psychological study could have been placed under the conditions of medical and psychological rehabilitation which lasted not less than two weeks, but it was quite difficult to realize this tool under the conditions of psychological recovery, which lasted not more than one week. Traditionally, in clinical practice, there had been used for a long time the projective method such as Lusher's color test, in particular after psychotherapy and training activities for self-regulation and restoration of adaptive capacity, the selection of colors could be compared with the so-called autogenic norm by Wallnöfer [37].

World tendencies in psychological studies showed that the diagnosis of mood, mental reactions, and conditions was relevant not only for the needs of the clinic but also in the workplace. The study of workers’ moods and states was an important component of the psychological well-being (health) of workers. These studies were extremely large-scale and attracted thousands of working people as participants [38]. Such interest in the problem of studying the state of a working person, as an indicator of his/her psychological well-being, was quite clear since the psychological state was a holistic, systematic, integral formation of human psychological activity. The psychological state of an individual could be internally and externally observed, it acted as a regulatory function of adaptation to the external situation and environment [16]. According to the definition by Kirilenko, the psychological state was the formation of psychological activity, which reflected the moment of stability and specificity of psychological processes’ duration, as a form of response, and reflected the attitude of an individual toward his/her own psychological phenomena at a certain point in time under certain conditions [39]. Varii pointed out that the psychological state was a temporary functional level of the psyche, which reflected the interaction of the influence of the internal environment of the body or external factors and determined the orientation of psychological processes' duration at a certain moment and manifestation of psychological properties of an individual [40]. The author defined the following areas in the studies of mental states: 1) mental states were an integral characteristic of the psyche at a particular moment; 2) psychological states were the background on the bases of which operated the psychological activity and orientation of the psychological activity of an individual; 3) it was a systemic reaction of the human psyche to changes of the conditions.

Volzhentseva highlighted that the leading functions of mental states were the regulation and integration into functional units, which were formed in the hierarchy of a single, holistic set of psychological processes and properties [25]. According to the author, the adaptive function of the psychological state was to establish the correspondence between the current needs and capabilities of an individual, considering the external conditions, features of activity, and behavior.

It was likely that the integration and integrity of psychological states became the basis for reducing the dimensions of the methods which were intended for their research. Thus, in foreign scientific sources, it was reported that to determine the moods and conditions, which were important indicators of short-term effects of intervention, was also used in clinical practice, "The Profile of Mood States (POMS)" [12,41]. This scale was developed by American experts, it had the form of a self-report and included 65 elements that related to 7 different scales: depression, anxiety, fatigue, energy, irritability, tension, and confusion.

The answer scale was divided into 5 categories from "not at all" to "completely yes". However, German psychologists in the re-standardization of POMS [12,13] created a shortened version of the method that included only 35 points which belonged to the scale "Depression/anxiety", "Fatigue", "Energy" and "Irritability" [38]. Another study was intended to reduce the dimension of POMS [41]. For this purpose, the POMS scale [12,13] with the help of regression analysis correlated with "The Eight State Questionnaire" (8SQ) [42], which was formed to measure anxiety, stress, depression, regression, fatigue, guilt, extraversion, and excitement. Researchers found that the excessiveness of measurement between these two methods mainly referred to negative emotional states.

CONCLUSIONS

Thus, the developed method "evaluation of negative mental reactions and states of combatants" was a tool that gave the possibility to determine the presence of negative psychological symptoms of servicemen related to their participation in hostilities and was a convenient tool for estimation of the dynamics of the state under the conditions of short-term programs of psychological recovery without the use of complex evaluation procedures and calculation of data.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. No financial support was received from any governmental institution, economic or non-governmental organization.

Authors' contribution

Conceptualization, O.K. and YaM.; managed the project, I.P.; conducted the research, V.K. and V.P.; contributed to the final draft of the manuscript, A.P., S.S. and N.P.; substantiated the methodology, S.L. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Informed consent was obtained from all subjects for inclusion in the study while maintaining their confidentiality. Written informed consent has been obtained from all subjects to publish this paper.

Ethics approval and consent to participate

All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

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ORIGINAL ARTICLE

Associations Between Medical Students' Opinions on Usage of Internet Services and Digital Teaching Tools

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Abstract: *Currently, the educational process must integrate new tools, to keep pace with technological progress. Our paper aims to investigate the extent to which the preferences of medical students for the autonomous use of multimedia resources in the learning process are correlated with their opinions towards the Internet and the intensity of using it in their daily activities. Methods: We investigated 395 medical students from 4 medical universities in Romania, 75.9% females, and 88.8% in the 1st and 2nd years of study. Students were asked to express on Likert-scales their agreement with a list of statements about Internet services, as well as whether they like learning using multimedia tools and how intensively they use Internet services. Results: The students agree to a medium to large extent with the favorable statements about Internet services but are also aware of the negative influences that indiscriminate use of such technology can have. The intensity of Internet usage is high, although not exaggerated. With small exceptions, the students who enjoy using multimedia resources during the learning process have also favorable opinions about Internet services, and use them constantly, mainly for information and instant messaging on social networks*.

Keywords: *multimedia-based learning; blended learning; Internet services; social networks; medical students*

INTRODUCTION

Currently, digital technology has become increasingly used in everyday activities, and its intensive use in most fields of activity has attracted changes in the structure of society, and even in the psychology of individuals, who gradually change their conceptions about life, work, and education, about the ideal way of spending leisure time and about the features of an optimal work climate. In this general context, it is obvious that the educational process must integrate new tools, in an attempt to keep pace with technological progress. The protagonists of this process, students as well as teachers, are people with a fundamentally different lifestyle than 20 years ago, with different expectations and conceptions.

The most obvious changes are noticed in the case of the current generation of students. This is the so-called "Generation Z" (children born after 1997), also known as iGen, post-millennial, or digitally native. These are children who have been exposed to technology from the beginning of their lives, so using computers and gadgets has become second nature to them, as have the activities online and on social networks. Consequently, these children prefer to study independently, at their own pace, using visual or kinaesthetic didactic tools, which give them immediate feedback. It is important for them to respect the right to privacy and personal space; they are pragmatic, entrepreneurial, and generally reluctant to take risks (because they faced periods of economic recession in childhood); although they generally enjoy interaction with the teacher, Generation Z children have fewer social skills associated with face-to-face communication and the art of conversation, compensated instead by intense online activity [1].

There are currently relatively few studies in the literature concerning Generation Z issues, which usually focus on describing its characteristics [2,3], effective strategies for recruitment in the public sector [4], and individuals' attitudes towards work [5], but without focusing on the specific problems of medical students of this generation, with their own needs, preferences and learning styles.

Obviously, the learning style of Generation Z students has features that differentiate it from the learning styles preferred by other generations of students. The most preferred method is active learning (through discussion and involvement in practical projects), based on sensory stimuli (visual - images, graphics, auditory and kinetic). Knowledge is achieved through observation, practice, and quick and targeted access to the necessary information through the use of technology and online resources: e.g. YouTube for video tutorials, Facebook (for chatting with classmates and sharing projects and teaching materials), or Google (for documenting and searching for scientific references). Students prefer self-study, at their own pace, with a flexible schedule and teaching materials that intensively use multimedia resources: explanatory or demonstrative video sequences, PowerPoint presentations with audio support, simulations, collaborative projects, discussion forums, online questionnaires, and case studies in the classical environment (seminar room or practical works). In this context, the portrait of the ideal teacher has also changed significantly: firstly, he must be a good communicator, close and friendly, transparent, enthusiastic, passionate and competent in his field, able to propose realistic goals and to provide immediate and constructive feedback on their achievement, willing to provide professional and emotional support to his students, both academically and individually, to prepare them for their future careers.

In this context, the concept of blended education, based on the intensive integration of digital resources into the teaching process, is becoming increasingly popular among teaching tools.

Such education programs are usually developed in mixed regimes, online and onsite, combining as much as possible in an optimal manner the advantages of both methods. Blended courses are a current trend that provides a new and effective instructional experience to the students they address, and have several major advantages:

- Flexibility in the teaching schedule, which simplifies students' access to the course, regardless of their physical location and allows them to organize their time and pace of learning according to their own needs and availability;

- More effective teaching: Most scientific studies show that blended teaching is more effective than traditional courses, but also than full online courses. A 2009 report from the United States examined 51 studies comparing online and traditional methods and concluded that students enrolled in blended learning programs score on average better on exams than those enrolled in full-onsite programs [6]; on the other hand, blended teaching programs also perform better than fully online ones as well [7], as they still give to the students the opportunity to interact effectively with the teacher when needed.

- Clearer organization of content: It is the natural result of a well-designed blended course, which must provide students with clear and detailed indications of using the proposed resources, activities, and tests, helping them to concentrate better and proposing an optimal pace of learning, in the same way that the teacher guides students onsite in going through the exposed materials;

- Stimulation of active learning, which emphasizes the student’s autonomy in effective learning communities;

- Lower costs: Reducing the number of onsite meetings frees up the teaching spaces of the institution and reduces some utility expenses. The teachers and the students will gain lower transport costs and time savings, although logistics expenses can be slightly increased (it is necessary to purchase adequate personal computers and involves additional costs for electricity consumption and telephone subscription).

Our paper aims to investigate the extent to which the preferences of students at medical faculties for the autonomous use of multimedia resources in the learning process are correlated with their opinions towards the online environment and the Internet and the intensity with which they use such resources in their daily activities, not necessarily related to the learning process.

MATERIALS AND METHODS

We conducted a case study that enrolled 395 medical students from 4 medical universities in Romania. The general features of the sample are shown in Table 1. Students are 3 quarters females (75.9%); most students are in the 1st and 2nd years of study (88.8%), aged between 18-20 years (74.4%), with an average age of 21.78 ± 3,736 years and a range of variation between 18-48 years.

Also, most students come from faculties of Dental Medicine (34.4%) or General Medicine (47.6%), mainly from the "Grigore T. Popa" University of Medicine and Pharmacy in Iasi, Romania (50.9%); 27.1% of the participating students are from University of Medicine and Pharmacy in Craiova, and 20.3% from University of Medicine and Pharmacy "Victor Babeș" in Timisoara.

Students were invited to fill in an online questionnaire in order to express their agreement degree with a list of statements about Internet services (favorable – 7 items and unfavorable – 3 items). In addition, they were asked whether they like learning using multimedia tools and resources and how intensively they use different Internet services (7 items).

The agreement degree with the proposed statements was quantified using a 5-step Likert scale, ranging from 1 – very small agreement to 5 – very large agreement.

Table 1: General features of the study sample

		n	%
Gender	masculine	95	24.1
	feminine	300	75.9
Age group	18-20 years	294	74.4
	21-24 years	78	19.7
	over 25 years	23	5.8
Year of study	1	228	57.7
	2	123	31.1
	3	44	11.1
Specialization	Dentistry	136	34.4
	General medicine	188	47.6
	Dental technique	70	17.7
	Prophylaxis assistance	1	0.3
University	UMPh Iasi	201	50.9
	UMPh Craiova	107	27.1
	UMPh Timișoara	80	20.3
	UMPh Cluj Napoca	7	1.8
Total		395	100.0

The questionnaire was presented and explained individually to each study participant.

Participation in the study was voluntary. Participants were informed about the study and the overall objectives of the research and, at the beginning of the questionnaire, were invited to give their informed consent. The questionnaires were completed anonymously.

The study was approved by the Research Ethics Commission of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (decision no. 21/16.11.2020).

Statistical analysis

Data collected from students were recorded and processed in SPSS 29.0 (SPSS Inc., Chicago, IL) for Windows.

The responses to each item were characterized by frequency distributions and contingency tables. The numerical variables were characterized by descriptive statistics (mean, standard deviation, standard error, range of variation, and median). Comparisons between samples were made using the Kruskal-Wallis test for quantitative data, after having previously verified that they comply with the normal distribution law, using the Shapiro-Wilks normality tests. We considered the p-value ≤ 0.05 to be statistically significant (*) and the p-value ≤ 0.01 to be statistically highly significant (**).

RESULTS

The 7 favorable statements about the Internet were as follows:
- Item 1: The main advantage of the Internet is that it provides information fast;
- Item 2: The main advantage of the Internet is that it provides correct information;
- Item 3: Freedom of expression should not be restricted on the Internet;
- Item 4: Most people would not be able to cope if they no longer had access to the Internet;
- Item 5: I can't imagine my life without Internet access;
- Item 6: People who socialize on the Internet are happier than others;
- Item 7: If I didn't socialize on the Internet I would be sadder.

88.6% of students evaluate the Internet as a fast source of information (item 1) but are more reserved about its correctness (only 30.6% of them think that information on the Internet is much or very much correct – item 2).

68.9% of students believe that most people would not be able to cope if they no longer had access to the Internet (item 4) and 60.7% believe that they themselves would not be able to cope if there is no Internet (item 5). 53.9% of students believe that freedom of expression should not be limited to the Internet (item 3).

On the other hand, only a small percentage of students believe that socializing in the virtual environment ensures a comfortable psychological status, making them happier (item 6 – 10.9%, respectively item 7 – 21.5%) – Table 2.

The 3 unfavorable statements about the Internet were as follows:
- Item 8: Internet is addictive;
- Item 9: Parents should limit their children's access to Internet to at most one hour;
- Item 10: People's privacy is at risk due to social media.

A large part of students believe that the Internet can be addictive (item 8 – 76.2%). Although they generally do not consider it necessary for parents to limit their children's access to the Internet (item 9 – only 41.0% agree to a large or very large extent), they still believe that people's privacy is at risk due to social networks (item 10 – 59.5%) – Table 3.

Table 2: Favourable opinions about Internet services – students' responses

Favorable opinions about Internet services	Agreement:									
	1 – to a very small extent		2 – to a small extent		3 – in average measure		4 – to a large extent		5 – to a very large extent	
	n	%	n	%	n	%	n	%	n	%
Item 1	1	0.3	4	1.0	40	10.1	125	31.6	225	57.0
Item 2	8	2.0	49	12.4	217	54.9	93	23.5	28	7.1
Item 3	9	2.3	16	4.1	157	39.7	107	27.1	106	26.8
Item 4	7	1.8	25	6.3	91	23.0	143	36.2	129	32.7
Item 5	21	5.3	35	8.9	99	25.1	115	29.1	125	31.6
Item 6	81	20.5	121	30.6	150	38.0	34	8.6	9	2.3
Item 7	75	19.0	118	29.9	117	29.6	46	11.6	39	9.9

Table 3: Unfavourable opinions about Internet services – students' responses

Unfavorable opinions about Internet services	Agreement:									
	1 – to a very small extent		2 – to a small extent		3 – in average measure		4 – to a large extent		5 – to a very large extent	
	n	%	n	%	n	%	n	%	n	%
Item 8	3	0.8	17	4.3	74	18.7	139	35.2	162	41.0
Item 9	43	10.9	56	14.2	134	33.9	94	23.8	68	17.2
Item 10	10	2.5	44	11.1	106	26.8	118	29.9	117	29.6

The consumer of Internet services profile was outlined through another 7 questions, regarding the way, intensity, and main purposes for which students use Internet services. The recorded responses are shown in Table 4.

Most students (97.5%) say they use the Internet daily, and 70.9% of them use the Internet at least 3 hours a day. Almost 1/3 of students (29.4%) believe that they could not give up the Internet without problems even for a day - which indirectly means acknowledging an addiction to this practice. On the other hand, half of students (47.6%) indicate the one-week period as the maximum limit for opting out of the Internet, which is also a limited time frame. Most students access the Internet using their mobile phones (84.6%).

Although they use online services quite intensively, the main objective of students is not written communication – thus, almost 3 quarters of them (71.6%) say they receive/send between 1 - 10 emails per day, and 9.4% say they do not use e-mail at all. Half of students (52.4%) are active on social networks, having over 100 friends; however, there is also a significant percentage of students (20.8%) who have fewer than 10 friends online. In contrast, 74.2% of students post on social media only a few times a year or a few times a month. Therefore, students use social networks in a relatively moderate regime, mainly for instant communication and less for permanent posts.

Finally, the students were asked whether they liked learning, even autonomously, using multimedia tools and resources (Item 18). The answers to this question are generally favorable: 41.5% of students say they like much or very much to learn using multimedia tools, 38.0% of students agree on average with this statement, and only 20.5% of students show a total or partial disagreement (Table 5).

In order to express more precisely the obtained results, we quantified them as numerical scores, calculated as arithmetic averages of the responses. We calculated 2 scores, one for favorable opinions about Internet services (INTF) and one for unfavorable opinions (INTNF), with a range of variation between 1 – 5, which we interpreted as follows:

- Values between 1.00 – 1.49 = agreement to a very small extent;
- Values between 1.50 – 2.49 = agreement to a small extent;
- Values between 2.50 – 3.49 = agreement in average measure;
- Values between 3.50 – 4.49 = agreement to a large extent;
- Values between 4.50 – 5.00 = agreement to a very large extent.

Using the same procedure, we calculated also a third score, to characterize the intensity in using Internet services (INTS) – with a range of variation between 1 – 4 and interpreted accordingly:

- Values between 1.00 – 1.49 = very low intensity;
- Values between 1.50 – 2.49 = low intensity;
- Values between 2.50 – 3.49 = high intensity;
- Values between 3.50 – 4.00 = very high intensity.

The analysis of these scores reveals that students agree to a medium to large extent with the favorable statements about Internet services (INTF = 3.44 ± 0.590), but also with the unfavorable statements about these services (INTNF = 3.69 ±

0.817), being therefore aware of the negative influences that indiscriminate use of such technology can have. The intensity of Internet usage is high, although not exaggerated (INTS = 2.85 ± 0.350) – Table 6.

Table 4: Intensity and main purposes in using Internet services – students' responses

		n	%
Item 11. On average, I access the Internet:	several times a month	3	0.8
	several times a week	7	1.8
	daily	385	97.5
Item 12. On average, the amount of time I spend on the Internet during a day is:	less than 1 hour	7	1.8
	1 - 3 hours	108	27.3
	3 - 6 hours	145	36.7
	over 6 hours	135	34.2
Item 13. I could quit the Internet without problems for a time frame of:	1 year	25	6.3
	1 month	66	16.7
	1 week	188	47.6
	not even a day	116	29.4
Item 14. Generally, I access the Internet using:	a desktop	13	3.3
	a laptop	47	11.9
	a tablet	1	0.3
	a mobile phone	334	84.6
Item 15. On average, the number of emails I receive/send daily is:	none	37	9.4
	1 to 10	283	71.6
	11 to 30	58	14.7
	31 to 50	12	3.0
	over 50	5	1.3
Item 16. The number of friends (contacts) I have on the most frequently used social network is:	under 10	82	20.8
	between 11 and 50	68	17.2
	between 51 and 100	38	9.6
	between 101 and 500	109	27.6
	over 500	98	24.8
Item 17. On average, the frequency with which I post new information on my social media profile is:	none	35	8.9
	several times a year	152	38.5
	several times a month	141	35.7
	several times a week	55	13.9
	several times a day	12	3.0

Table 5: General opinion about the use of multimedia resources during the learning process – students' responses

General opinion	**Agreement:**									
	1 – to a very small extent		**2 – to a small extent**		**3 – in average measure**		**4 – to a large extent**		**5 – to a very large extent**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Item 18: I like to learn using multimedia tools and resources	19	4.8	62	15.7	150	38.0	81	20.5	83	21.0

Table 6: Internet services usage scores - descriptive statistics parameters

	n	**Mean (M)**	**Standard deviation (SD)**	**Standard error of the mean (SEM)**	**Min**	**Max**	**Median**
Favourable opinions about Internet usage (INTF)	395	3.44	0.590	0.030	1.57	5.00	3.43
Unfavourable opinions about Internet usage (INTNF)	395	3.69	0.817	0.041	1.00	5.00	3.67
Internet usage intensity (INTS)	395	2.85	0.350	0.018	1.71	3.71	2.86

We compared these scores according to the students' degree of agreement with the usage of multimedia resources during the learning process, to identify the possible links between these two elements. We started with the hypothesis that the students who enjoy using digital technologies for entertainment in leisure time should also enjoy using them professionally, respectively during the learning process.

The obtained results are presented in Table 7 and actually validate this research hypothesis.

Table 7: Internet services usage scores – compared according to the degree of agreement with the use of multimedia resources during the learning process

	Agreement:					**Kruskal-Wallis test**
	1 – to a very small extent	**2 – to a small extent**	**3 – in average measure**	**4 – to a large extent**	**5 – to a very large extent**	
	M ± SD	**M ± SD**	**M ± SD**	**M ± SD**	**M ± SD**	
Favorable opinions about Internet usage (INTF)	3.02 ± 0.705	3.43 ± 0.612	3.32 ± 0.545	3.53 ± 0.522	3.67 ± 0.593	H = 23.634 p < 0.001**
Unfavorable opinions about Internet usage (INTNF)	4.02 ± 0.842	3.68 ± 0.692	3.74 ± 0.807	3.58 ± 0.733	3.63 ± 0.973	H = 5.414 p = 0.247
Internet usage intensity (INTS)	2.75 ± 0.415	2.97 ± 0.325	2.81 ± 0.350	2.88 ± 0.324	2.80 ± 0.358	H = 12.310 p = 0.015*

There are statistically significant differences between the scores of favorable opinions about Internet usage, determined by the degree of agreement with the use of multimedia resources during the learning process. The lowest scores are found in students who agree to a very small extent with the use of multimedia resources (3.02 ± 0.705), and the highest in students who agree to a very large extent with the use of multimedia resources (3.67 ± 0.593), so these 2 elements are in clear correlation.

The situation is different for the scores of unfavorable opinions: the highest score of unfavorable opinions about Internet usage is found in students who strongly disagree with the use of multimedia resources in the learning process (4.02 ± 0.842). As the degree of agreement with the use of multimedia resources increases, the score of unfavorable opinions decreases, but with random fluctuations, so the observed differences are not statistically significant.

The lowest score of Internet usage intensity is also found in students who strongly disagree with the use of multimedia resources in the learning process (2.75 ± 0.415) – therefore they are fundamentally against digital technologies. On the other hand, the highest score of Internet usage intensity (2.97 ± 0.325) is observed in students with little agreement for the use of multimedia resources in the learning process – so they are obviously not against digital technologies but probably prefer to use them exclusively for leisure. Students who agree to a medium or large extent with the use of multimedia resources in the learning process have scores of Internet usage intensity between the 2 categories mentioned above, so they enjoy using digital technologies both professionally and for leisure. The differences between the investigated categories of students are statistically significant.

DISCUSSION

The data from the literature prove that multimedia resources are an effective tool for learning because they are able to stimulate the students' interest [8] and improve their cognitive skills [9], also contributing to the development of supplementary skills – like empathy [10,11] or the academic writing [12]. Such tools are preferred by medical students because they tend to be kinaesthetic learners, who use multiple ways to present a topic for a better understanding [13,14,15] – observations also confirmed by our study.

Medical students are advanced users of smartphones and laptops and easily use such devices during their daily activities and learning [15,16] – a fact also confirmed by our results. The students investigated by us are generally interested in digital technologies and their applicability during the learning process [15].

It is thus natural that our students have generally positive opinions about Internet services, without thinking that they are addicted to them. Moreover, they are even aware of the presence of such a risk, which proves realistic and mature thinking. Most students use the Internet for information, although they are also aware of the risk of not obtaining accurate data – a fact confirmed by other literature data as well [17]. We must admit that the media and entertainment tools familiar to previous generations (TV, radio, written press) have now been joined by new, equally important, or even more important partners – the smart devices, which seem to have become the most convenient ways to access virtual environments.

Almost all students use the Internet daily, from their mobile phones, and most of them for at least 3 hours – which shows a certain degree of dependence on this technology, although it is not recognized as such. The most frequently used Internet services are, according to some authors, WhatsApp (as a fast communication solution) [18,19,20], YouTube, and Blackboard [21].

It is also interesting to note that socializing in cyberspace is not very important for our students and does not replace real socialization. Only half of the students have many friends on social media, but even they don't post very frequently (only a few times a year or a few times a month, which is a moderate pace). Moreover, almost 60% of students believe that people's privacy is at risk due to social networks – a result that is questionable, because not the social networks pose a danger, but the people using them inappropriately – an aspect that seems to have been omitted by students. Literature data also confirms that the students, particularly the medical students, are not very well informed about the ethical issues regarding the use of social media resources [21].

There is an obvious and positive association between the degree of agreement expressed by students towards the use of multimedia resources during the learning process and their favorable opinions towards the use of Internet services. This means that the general openness to modern technologies is reflected by integrating them into personal life, but also professionally. On the other hand, unfavorable opinions towards the use of Internet services are associated sometimes with the general avoidance of modern technologies. We also identified a separate category of students who, although they do not like to use multimedia resources in the learning process, are instead very active on the Internet; they are especially interested in activities on social networks, without being very concerned about the other advantages provided by digital technologies. Anyway, the students who enjoy using multimedia resources during the learning process are also constant users of Internet services, which they use mainly for information, but also for activities on social networks (mainly instant messaging) – a fact also confirmed by previous studies in the literature [21,22,23].

CONCLUSIONS

Technology is only a tool that can improve the teaching process, but not only by itself. Those who are responsible for change are the teachers, who can improve the students' outcomes by using technology to convey knowledge, streamlining their methods, and perfecting themselves [24]. The concept of blended education facilitates such approaches and can trigger positive changes, satisfying for students, who will benefit from flexibility and comfort, and, consequently, also for teachers, who will have the satisfaction that their work achieves its purpose and that the information they transmit is properly heard and acquired.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, C.G.D. and V.L.P.; methodology, C.G.D.; software, C.G.D. and R.S.C.; validation, R.S.C.; formal analysis, C.G.D.; investigation, M.E.A. and R.S.C.; resources, V.L.P.; data curation, C.G.D.; writing—original draft preparation, C.G.D. and M.E.A.; writing—review and editing, C.G.D. and V.L.P.; supervision, V.L.P.; project administration, C.G.D. and M.E.A. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethical Committee of "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Romania (decision no. 21/16.11.2020).

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ORIGINAL ARTICLE

The Impact of Physical Therapy on Psychomotor Functions in a Patient with Systemic Lupus Erythematosus and Diabetes Mellitus – Case Illustrated Literature Review

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Abstract: *The scientific and medical world is making permanent efforts to study, understand, and cure the complicated pathology of Systemic Lupus Erythematosus (SLE) – the disease with 100 faces, due to the diversity and complexity of the symptoms. SLE has a devastating impact on the patient's life, causing multiple metabolic imbalances produced by both the disease itself and the necessary medication. This case report addresses the therapeutic aspects of SLE and diabetes mellitus, on a 47-year-old male with a sensitive multi-pathology and aggressive evolution, medication, and approaching psychomotor rehabilitation therapy, up to now, when SLE treatment has ended. Also, this case study report highlights the importance of flexibility in the psychomotor rehabilitation strategy, in the context of a large range of factors that influence its definition.*

Keywords: *SLE - systemic lupus erythematosus, diabetes mellitus, medication, recovery therapy, psychomotor*

INTRODUCTION

The scientific and medical staff are making permanent efforts to study, understand, and cure the complicated pathology of Systemic Lupus erythematosus (SLE) [1-3]. SLE, an inflammatory autoimmune disease, also named the disease with 100 faces due to the diversity and complexity of the symptoms, has a devastating impact on the patient's life due to the multiple metabolic imbalances produced by the disease itself and the necessary medication [4].

The etiology of SLE is unknown, as are the possible disorders (but not necessarily) – hematological, nephrological, neurological, skin, and ocular, because of cellular and tissue damage [5-7]. There is no chronological sequence of SLE manifestations, even in the same patient. It is problematic to diagnose, hard to treat (due to the various symptomatology from minimum to severe), and difficult to continuously adapt the strategy of preserving psychomotor capacity, sometimes necessary to change in an abrupt way.

Diabetes Mellitus in the case of SLE is a particular situation with the need for continuous monitoring because it can be influenced by this long-term disease and its complex medication treatment [8,9]. Administration of insulin units and/or gliclazide must be dosed by the treating physician according to the particularities of the case [9,10]. It is well known that diabetes can be controlled with careful diet and weight monitoring, and physical exercise as a supplement to medical treatment. That is why we consider the good collaboration between family, patient, doctor, and physical therapist, alongside the regular medical checkouts for blood variables of vital importance. The medical approach therapy is individualized and customized to fit each patient's need depending on the type of disease and each symptom.

Reflection of the theme in specialized literature and conceptual boundaries

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with multisystem involvement. It is triggered by deregulation of the immune system which, due to the autoantibodies produced, attacks the body's healthy cells and tissues. The clinical picture is very rich, it can affect several organs, it has an undulating evolution with periods of exacerbation and remission, and the forms of presentation are from clinical manifestations without life risk (affects the skin, and joints) to potentially severe forms (affects the patient at neurological, renal, cardiovascular level).

There are 4 types of lupus:

- Systemic lupus erythematous – is the most common form of lupus. The manifestation of the disease differs depending on the affected structures.

- Cutaneous lupus – manifests itself in the skin.

- Lupus induced by drugs – iatrogenic lupus occurs as a reaction to the administration of certain drugs.

- Neonatal lupus – is a particular form of the condition and occurs due to the transplacental transmission of anti-Ro antibodies from mother to fetus during pregnancy and can have skin, cardiac (the most serious being atrioventricular block), hematological, and neurological manifestations.

Symptoms can range from mild to very severe, and although they can be chronic, they usually come and go, with the disease having an undulating course.

The most common complaints are fatigue, altered general condition, fever, lymphadenopathy (inflammation of the lymph nodes), and joint and muscle pain. Almost any organ can be affected.

Skin-mucosal damage

It is present in approximately 70% of patients. The classic manifestation of acute lesions is the heliotrope rash, erythema in "butterfly wings" present at the level of the nasal pyramid and the cheeks (redness that extends from the nose to the cheeks, below the eye area), without affecting the nasolabial folds. Erythema can also spread to other areas exposed to UV radiation – forehead, chin, décolleté. Photosensitivity is another characteristic of lupus patients. Subacute lupus may initially present with erythematous lesions that, over time, become papulosquamous, mimicking psoriasis or lichen planus. Discoid lupus erythematosus can appear on the face, scalp, ears, arms, hands, and back, the erythema being followed by hyperkeratosis or atrophy. Discoid lupus can be a strictly dermatological lesion, without being accompanied by systemic manifestations [29].

There may also be alopecia, skin purpura, erythema, small red dots, urticarial eruptions, livedo reticularis, Raynaud's syndrome, and ulcerations of the fingers pulp. Mucous membrane lesions consist of frequently painless petechial and ulceration of the nasal, oral, and, less commonly, genital mucosa.

Musculoskeletal damage

About 90% of patients diagnosed with lupus will have joint pain, arthritis, or muscle pain at some point. The joints of the hands, fists, and then the large joints (knees, ankles, shoulders, elbows) are most frequently affected. They can be painful, swollen, or red, the damage is usually symmetrical. Unlike rheumatoid arthritis, lupus arthritis rarely causes severe joint deformities and is not erosive.

Hematological impairment

Anemia, thrombocytopenia, leukopenia with neutropenia or lymphopenia may occur within the disease or secondary to pharmacological treatment.

Cardiovascular damage

Pericarditis, myocarditis, and endocarditis can occur in this disease.

Lupus endocarditis, called Libman-Sacks endocarditis, has no infectious cause. Atherosclerosis occurs more frequently and develops more rapidly than in the general population.

Lupus patients can also associate antiphospholipid syndrome (SAFL), in which antiphospholipid antibodies are present (anti-beta 2 glycoprotein 1 antibodies, anti-cardiolipin antibodies, lupus anticoagulant).

The presence of SAFL causes thrombosis), and spontaneous abortions.

Lung damage

It can take the following forms: pleurisy, inflammation of the pleura, pleurisy, pneumonitis, interstitial lung disease, pulmonary hypertension, and pulmonary thromboembolism. These can cause coughing, dyspnea (choking hazard), chest pain, and hemoptysis.

Kidney damage (lupus nephritis)

Renal damage determines the severity of the evolution of lupus patients. One-third of patients have kidney damage, suggested by proteinuria or hematuria, serum creatinine is above the upper limit of the laboratory, and creatinine clearance is low.

Neuropsychological impairment

It is the least understood manifestation of systemic lupus erythematosus and one of the most severe. Among the neurological manifestations, we mention convulsions, severe headaches, peripheral neuropathies, cranial nerve damage, cerebral vascular accidents, acute meningitis, and chorea. Psychiatric manifestations include psychoses, cognitive dysfunction, anxiety syndrome, and mood disorders.

Gastrointestinal symptoms

The most common gastrointestinal symptoms are anorexia, nausea, vomiting, and abdominal pain, and may be manifestations of mesenteric vasculitis, peritonitis, pancreatitis, or secondary to the administered medication.

Liver damage is found in up to 60% of patients – hepatomegaly, increased liver enzymes, and hepatic steatosis. Up to 8% of patients develop retinal artery inflammation with thrombosis at this level. The conjunctiva and cornea can be affected by secondary Sjogren's syndrome [30].

Specific immunological abnormalities

The presence of autoantibodies that target their own structures. Antinuclear antibodies (ANA) occur in approximately 95% of patients but have low specificity and may be associated with other conditions (rheumatoid polyarthritis, autoimmune thyroiditis, autoimmune liver diseases, infections, neoplasms, or in healthy individuals). The most frequent antibodies encountered in SLE are the antibodies: anti-double-chain DNA (SLE specific, associated with disease activity), anti-Sm (SLE specific), anti-U1 RNP, anti-Ro, anti-La, anti-ribosomal protein P (SLE specific, appear in neurological, hepatic damage), anti-histones (occur in classical and drug-induced lupus), anti-phospholipids (characteristics of SAFL), rheumatoid factor (non-specific, occurs in many other autoimmune conditions, infections). Decreased serum complement fractions (C3, C4, CH50) are associated with disease activity.

As is the case with many chronic conditions, systemic lupus erythematosus (SLE) negatively affects the quality of life, due to factors such as depression, pain, cognitive dysfunction, and sleep disorders.

Despite a little-understood etiology, fatigue is associated with SLE in approximately 80% of cases, which leads to a decrease in the level of physical activity necessary to carry out usual daily activities and thus dramatically decreases the quality of life.

Physical medicine and recovery in Systemic Lupus Erythematous may involve physical therapy, occupational therapy, speech therapy, recreational therapy, or combinations thereof. The specialist doctor should coordinate the patient's care and be attentive to subtle changes in symptoms that may indicate periods of disease activity [32].

Ultrasound is a frequently used procedure to provide heat in the depth of the affected joint, but it should not be used in the presence of inflammation; locally applied ice is the preferred method for inflamed joints. When ultrasound is used to improve joint mobility, the application of ultrasound and joint movement should be simultaneous.

Hydrokinetic therapy is recommended for the mobilization of inflamed joints, due to the buoyancy that ensures the unloading of the joint and the soothing action of the warm water.

The cardiovascular and pulmonary systems can also be affected by lupus. We try to improve aerobic capacity through physical exercises and specific breathing exercises [31]. These exercises will also contribute to maintaining a strong bone system, as well as strengthening ligaments and joints. Regular practice of sports activities is particularly recommended for patients with lupus.

Occupational therapy has the role of helping the patient regain as much of his functional independence as possible, despite the problems caused by the disease. Activities of daily living are encouraged and may require training with special equipment, techniques, and procedures. These activities include feeding, dressing, bathing, grooming, grooming, and cooking in the home.

A safety assessment at the patient's home may be indicated. Occupational therapy can provide recommendations for equipment (for example bathtub bench, elevated toilet seat, grab bar) to increase the patient's independence and safety at home.

Patients who have neuropsychiatric complications due to lupus may have cognitive dysfunctions, with deficits in attention, language, and memory. The speech therapist can be helpful when a patient with systemic lupus erythematous has slurred speech, difficulty understanding speech, or difficulty speaking properly.

Because systemic lupus erythematosus is a condition that manifests itself episodically, through bouts of activity alternating with episodes of remission, it is important that the patient does nothing without consulting the rheumatologist who deals with his disease – thus, it will reduce the risk of triggering a set of activities.

Unfortunately, there is still no treatment that can cure lupus, but there are treatments that can improve the symptoms while prolonging the episodes of remission of the disease. Adopting a healthy lifestyle also contributes to increasing the quality of life of the lupus patient.

Other things that the lupus patient can do to improve their condition:

- To give up smoking, but also the consumption of caffeine or alcohol.
- To adopt a healthy, balanced diet, based on as many natural foods as possible.
- To rest enough.
- Follow exactly the treatment recommended by the doctor, even during periods of remission of the disease.
- To avoid exposure to the sun because ultraviolet radiation

increases the risk of triggering a flare-up of systemic lupus erythematous.

It is recommended to reduce exposure to UV rays (using creams with a minimum sun protection factor of 50, avoiding exposure to the sun), a diet adapted to the patient undergoing corticosteroid therapy (to prevent its adverse effects – diabetes, hypertension, dyslipidemia), quitting smoking.

To prescribe a drug treatment, it is essential to identify the type of organ damage and its severity.

Non-life-threatening damage (skin-mucous, musculoskeletal, serositis) is indicated for the administration of non-steroidal anti-inflammatory drugs, low-dose systemic cortico-therapy, and synthetic antimalarials.

Potentially vital damage (cardio-pulmonary, hepatic, renal, hematological, central nervous system) requires cortico-therapy in moderate or high doses, associated depending on the affected organ, with immunosuppressants, anticoagulants, anticonvulsants, or even biological therapy.

Systemic lupus erythematosus is a chronic condition with multisystem involvement that presents severe evolutionary potential. Currently, thanks to advances in the knowledge of mechanisms, diagnosis, and treatment, the 10-year survival rate of lupus patients is almost 90% [30].

MATERIALS AND METHODS

This review started with a case study and addresses the therapeutic aspects of SLE and Diabetes Mellitus of a 47-year-old male with a delicate multi-pathology and aggressive evolution, caused by both the diagnostic and the difficult approach of the psychomotor rehabilitation therapy when SLE treatment has ended. In this case study report, we approach the importance of the management of the disease combining not only the multiple therapeutic actions but also the proper medication and nutrition and the psychomotor rehabilitation [11]. The study includes stages of life-threatening biological depreciation, and treatment, until the present when the therapy is positively affecting the patient's quality of life.

We present the short version of the patient's clinical history:

- At 27 years old, he underwent surgery for Non-Hodgkin's Lymphoma, associated with polychemotherapy and radiotherapy, with good response, in remission and without any specific medication from 2003.

- No forms of SLE manifestation until 2017.

- The aggressive form of SLE debuted in September 2017, caused by an unstable lifestyle associated with many detrimental factors accumulated in the last 15 years: unbalanced alimentation (almost exclusively processed meat products/minced meats and semi-prepared products, and carbonated beverages), smoking, stress, lack of sleep, improper self-medication for diabetes mellitus, sustained effort.

- The SLE treatment itself begins in February 2018, the patient’s previous treatment included only medication for his symptoms - edema, hypertension, cytopenia, etc.

- In his delicate condition, with the complex ongoing treatment, the patient beneficiated from the support of his family, nutritionist, priest, and doctors which improved his mental state of being.

- The period 2018-2019 was an equilibrated one when the patient was under observation, good nutritional plan, and 3 weekly sessions of physical therapy.

- In February 2022 the patient gets infected with SARS-CoV-2 Covid 19 Omicron Type which lead to a 10 days hospitalization in the nephrology unit medical care.

- The clinical picture of the subject presented above starts from the aggressive debut in the autumn of 2017 until 2021. We mention that multi-pathology and all associated diagnoses are not included in this presentation.

We also mention that during this period of multiple hospitalizations, there were diagnosed multiple associated pathology such as Diabetes mellitus type 2 with established Diabetic Nephropathy, Hypertensive Nephropathy with Renal Impairment, Hyperuricemia without signs of Inflammatory Arthritis or Tophaceous Disease, Vulgar Ichthyosis, Glomerular disorders in systemic connective tissue disorders, other water and electrolyte disturbances not elsewhere classified, Anemia in other chronic diseases classified elsewhere, Non-inflammatory Pericardial infusion, Medication-induced Specific Polyneuropathies, Secondary hypertension of other kidney disorders, Ascites, Hypo-osmolality and hyponatremia, Chronic nephritis syndrome, etc.

Also, here are some additional landmarks: the dietary regimen consisted of predominant vegetarian and hypoglycemic food, water management with 500 ml more than the urinary removal, medical plant tea indication, measurement of food in carbs no more than 150 per day (50/50/50 for each meal) associated with the prescribed medication; glycemic test each day before meals with calculated insulin administration (rapid and long-acting insulin) and/or gliclazide.

The multidisciplinary team of doctors (diabetologists, nephrologists, cardiologists, and Physiotherapists) [12-17] followed the patient during all the periods.

Treatment of SLE with the administration of combined diuretic treatment: high-ceiling diuretics (furosemide), Vasopressin antagonists (tolvaptan), Diuretic thiazide and potassium-sparing (amiloride hydrochloride and hydrochlorothiazide), thiazide diuretics (hydrochlorothiazide) and immune-suppressive combination (cyclophosphamide, mycophenolate mofetil), cyclosporine and corticosteroids (prednisolone).

Evaluation methods

To carry out this research from the Physiotherapists point of view, we chose the following measurements and evaluation tests:

1. to assess the level of the peripheral inflammatory stage at the level of the lower limbs as well as that of the muscle mass of the subject, we performed the anthropometric-perimetry method for the ankle and calf.

2. to evaluate the degree of mobility of the lower limb necessary for functionality, we used the goniometric method for the ankle and hip joints.

3. to assess static balance, we used the simple bipedal balance test with eyes open with several penalties for imbalance for 5 minutes.

4. to assess dynamic balance we asked the patient to walk on a drawled line of 5 meters distance, eyes open, without imbalance, dizziness, or lateral support.

5. to evaluate the subject's effort capacity, we used the ergometric bike without resistance with heart rate measurement.

Description of the used methods

1. The patient is in the supine position, and the measuring tape is applied at the level of the ankle joint including the most inflamed area, measuring an average of the right/left ankles before the physical therapy program.

2. The patient lying on his back, and the goniometer is applied according to the goniometric measurement and evaluation standards of the ankle and hip joint for the flexion movement, before the physical therapy program.

3. The patient in the standing position with bare feet on the floor for better plantar kinesthesia, eyes open, is timed for how many minutes/seconds he can stand without imbalances, dizziness, or lateral support. The evaluation was carried out at the beginning of the meeting for static balance.

4. For dynamic balance, a 5m line was marked on the floor, and the patient was asked to follow the line walking, eyes open, marking any sign of imbalance, dizziness, or lateral support, evaluated in how many meters the patient succeed to go on, at the end of the session.

5. The patient on the ergometric bicycle without lumbar support, with a distance between the hip and the pedals so that the knee is slightly flexed, without load, he pedals freely at a slow pace, following the heart rate so that it does not exceed 120 beats/min, it is marked how many minutes he can pedal up to the indicated maximum cardiac effort, or any other sign of imbalance, dizziness, numbness or pain. The test was held at the end of the physical therapy session.

Evaluation Instruments

The used instruments were a tape measure, goniometer, stopwatch, liner, ergometric bicycle, and pulse tester.

Research methods used

The research methods used in the paper were:

- the theoretical documentation method.

- pedagogical observation.

- the experimental method.

- the method of tests and evaluation samples.

- the investigation method.

- the method of recording and statistical-mathematical data processing.

- the graphic method.

Applied intervention

To streamline and synthesize the process of describing exercises applied, they are presented below (Tables 1 and 2).

The tables show the means we worked with, the exercise program proposed for every period, and their dosage.

Teaching materials used to fulfill the improvement program of motor functional skills and of balance and effort capacity were the stick, a small hand weight 1 kg, a terra band, the balance board, the inflatable balance disc, the gym ball, the small spikes ball for prehension, the bicycle, marker.

The physical therapy program no. 1 consisted of this period in: specific exercises that do not involve risks factors for strokes, bruises, or bleeding; exercises that do not present risk factors for a patient with high blood pressure; exercises and techniques for lymphatic drainage; specific techniques for tone muscle and equilibrium; specifically adapted methods for connective flexibility and joints mobility; programs for developing effort capacity; outdoor adapted program; exercises that consider the electrolyte imbalance (already existing due to the disease); breathing exercises.

Table 1: The process of describing initial exercises applied – the first program

Type of method	Exercise	Series	Reps or seconds/minutes	Pause (sec)
Mobilization	From the lying position - slow passive mobilization of the ankle in all directions	3	30 reps	30 sec
	From the lying position - slow passive mobilization of the knee for flexion and extension	3	15 reps	30 sec
	From lying position - Slow passive mobilization of the hip in all ranges of motions from simple to complex	3	15 reps	60 sec
	From lying position and sitting - Active mobilization of the upper limbs with a stick in all ranges of motions	3	15 reps	60 sec
	From lying position - Free active mobilization of the upper limb in all ranges of motions	3	15 reps	30 sec
	From lying position - Free active mobilization of lower limbs in all ranges of motion (bicycle, shears, bypasses)	3	15 reps	60 sec
	Quadrupedal position, cat forward and backward	3	10	120 sec
Tone muscle	Lying position - all range motion mobility with 1-kg hand weights	3	15	60 sec
	Sitting position - all range motion mobility with 1-kg hand weights	3	15	60 sec
	Sitting position - terra band extension between arms in abduction and extension	3	10	120 sec
	Sitting position - leg extension with a terra band resistance	3	10	120 sec
	Lying position - knee flexion and extension with terra band resistance	3	10 reps	120 sec
Static balance	Sitting position - imbalance from the shoulders in all directions with the requirement to maintain the posture from the abdomen without the help of the hands	3	10 reps	60 sec
	Standing position - Keep abduction of the hands	3	10-60 seconds	120 sec
	Standing position - maintain the balance without lateral support or movements	3	10-60 seconds	120 sec
Dynamic balance	Standing position - Raise 1 leg on a support ball	3	10-60 seconds	60 sec
	Standing position - walking with a plastic cup with water in the hand	3	10 m	60 sec
	Standing position - walking on a drawled line on the floor without lateral support or lateral deviations	3	10 m	120 sec
	Standing position - with lateral or face hand support - on a balance platform or ball - back straight, crunches	3	10 reps	120 sec
	Standing position - lateral support - kick a ball	3	10 reps	60 sec
Effort capacity	Bicycle ride - level 0	1	5 min	-
	Bicycle ride - level 0	1	15 min	-
	Claiming stairs from 1 to 3 floors	1	15-30 min	5 min
Outdoor training	Walking outdoors on a parc with support (frame, cane or crutch, or human lateral support)	1	15-30 min	-
Breathing techniques	Lying position - costal and abdominal breathing techniques, slowly and following the dizziness or pain	1	5- 10 min	-
	Sitting position - gently inhale with the extension of the trunk and arms, and exhale slowly and long with the return and withdrawal of the navel towards the spine.	1	5-10 min	-
Manual techniques	Manual lymphatic drainage and pump exercises	1	15 – 30 min	-
	Manual therapy for connective tissues	1	15-30 min	-

All exercises and techniques were adapted to the daily dynamic state of functionality of the patient from very simple basic to the maximum possible in his case. The programs were carried out on an outpatient basis at the patient's home, with a frequency of 3 sessions per week (Monday, Wednesday, Friday), the duration of the session being between 30 and 120 minutes depending on the daily state of the patient and the applied methods.

Table 2: The process of describing initial exercises applied – the second program

Type of method	Exercise	Series	Reps or seconds/minutes	Pause (sec)
Mobilization	From the lying position - slow active mobilization of the ankle in all directions	3	30 reps	30 sec
	From the lying position - slow active mobilization of the knee for flexion and extension	3	15 reps	30 sec
	From lying position - Slow active mobilization of the hip in all ranges of motions from simple to complex	3	15 reps	60 sec
	From lying position and sitting - Active mobilization of the upper limbs with a stick in all ranges of motions	3	15 reps	60 sec
	From lying position - Free active mobilization of the upper limb in all range of motions with 1 kg hand weights	3	15 reps	30 sec
	From lying position - Free active mobilization of lower limbs in all ranges of motion with 1 kg leg weights (bicycle, shears, bypasses)	3	15 reps	60 sec
	Quadrupedal position, cat forward and backward	3	10	120 sec
Tone muscle	Face lying position - all range motion mobility with terra band	3	15	60 sec
	Sitting position - all range motion mobility with 1 kg hand weights	3	15	60 sec
	Sitting position - terra band extension between arms in abduction and extension	3	10	120 sec
	Sitting position - leg extension with a terra band resistance or 1 kg leg weights	3	10	120 sec
	Lying position - knee flexion and extension with terra band resistance or 1 kg leg weights	3	10 reps	120 sec
Static balance	Sitting position - imbalance from the shoulders in all directions with the requirement to maintain the posture from the abdomen without the help of the hands or with a ball or a weight of 1 kg in the hands	3	10 reps	60 sec
	Standing position - Keep abduction from 90° - 180° of the hands	3	10-60 seconds	120 sec
	Standing position - maintain the balance without lateral support or movements and keep a 1 kg weight on the hands	3	10-60 seconds	120 sec
Dynamic balance	Standing position - Raise 1 leg on a support ball with 1 kg leg weight	3	10-60 seconds	60 sec
	Standing position - walking with a 1 kg weight on the hand	3	10 m	60 sec
	Standing position - walking on a drawled line on the floor without lateral support or lateral deviations with a cup of plastic water in the hand or on the head	3	10 m	120 sec
	Standing position - with lateral or face hand support- on a balance platform or ball - back straight, squats or lunges	3	10 reps	120 sec
	Standing position - lateral support - kick a ball or roll a 1 kg leg weight	3	10 reps	60 sec
Effort capacity	Bicycle ride - level 1	1	5 min	-
	Bicycle ride - level 1	1	15 min	-
	Claiming stairs from 1 to 6 floors	1	15-30 min	5 min
Outdoor training	Walking outdoors on a parc with or without support (frame, cane or crutch or human lateral support)	1	15-30 min	-
Breathing techniques	Standing position - costal and abdominal breathing techniques, slowly and following the dizziness or pain	1	5- 10 min	-
	Standing or quadrupedal position - gently inhale with the extension of the trunk, leg, and arm, and exhale slowly and long with the return and withdrawal of the navel towards the spine	1	5-10 min	-
Manual techniques	Manual lymphatic drainage and pump exercises	1	15 – 30 min	-
	Manual therapy for connective tissues	1	15-30 min	-

The program contains breathing exercises from decubitus and sitting, easy mobilization exercises for upper limbs with the stick and weights no more than 1 kg, slow exercises for lower limbs at the bedside level, lymphatic drainage, progressive passive mobilizations, progressive active spine mobilizations, static and dynamic equilibrium methods, core and leg muscle tone exercises, home bicycle program from 0 weight, outdoor walking sessions. Monday and Wednesday were addressed to the indoor program and Friday evening (due to the ocular and skin problems in SLE) if the weather was proper and the state of the patient was a good one, was addressed to the outdoor program.

Objectives

The 1 physical therapy program followed the objectives of each period of the evolution of the patient's state.

The initial objectives of the treatment were those of:
- preserving life
- following developments in functionality and evasion of complications.

The main objectives were:
- Improve the quality of life
- Decrease in inflammation and lymph retention
- Preserve and gain flexibility
- Develop tone muscle
- Develop a functional effort capacity
- Obtain a good static and dynamic stability
- Maintain a good psychological state of mind and optimism with the patient and family.

The functional state of the patient translates into functional disability to service and personal care, unable to have an orthostatic position, seated posture, and displacement.

The physical therapy program no. 2 consisted of the same exercises as in the first period but improved and adapted to the new daily possibilities of the patient. Such as upper and lower limb mobilization in a full range motion and with resistance, static and dynamic balance improved techniques for outdoor walking without the support and on uneven terrain, effort capacity development on the bicycle with resistance, maneuverability, and coordination exercises.

The program contains breathing exercises from sitting and standing positions, mobilization, and tonus exercises for upper limbs with the stick and 1 kg weight, exercises for lower limbs at the bedside level and from standing position with terra band, abdominal and diaphragmatic exercises, equilibrium exercises with or without lateral support, progressive active mobilizations, prehension exercises, lymphatic drainage, and manual therapy, bicycle training, outdoor walking sessions.

All exercises and techniques were adapted to the daily dynamic state of functionality of the patient following the rule of "primum non nocere" [26].

Objectives

The 2 physical therapy programs followed the objectives of each period of the evolution of the patient's state.

The initial objectives of the treatment were those of:
- preserving the quality of life and existent functionality
- following developments in functionality and evasion of complications.

The main objectives were:
- Improve the quality of life
- Preserve and gain flexibility and connective tissue elasticity
- Develop tone muscle and regain muscle mass
- Gain a functional effort capacity for outdoor functionality
- Obtain good static and dynamic stability together with coordination skills
- Maintain an optimistic continuous psychological state of mind and optimism with the patient and family.

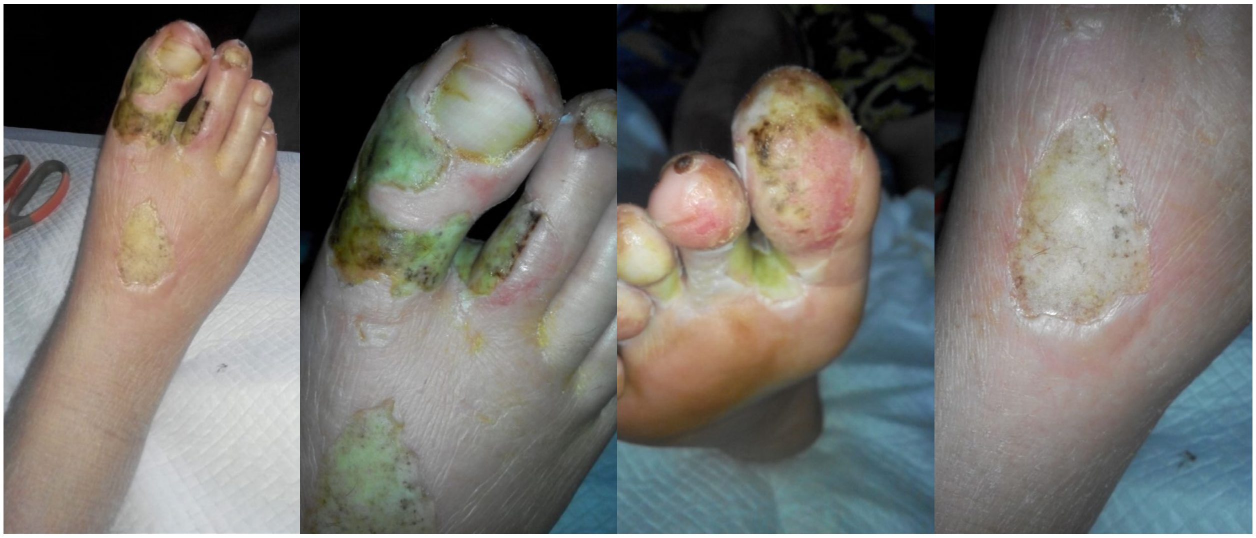

Figure 1: Representative images of the foot edema and neuropathy skin lesions

The recovery protocol consisted of tracking the functionality of each step according to the patient's evolution, following the food and drug indications and the degree of muscle and joint recovery, and also considering the equilibrium function that has been greatly affected.

During these months of complex treatment, the patient had positive or stagnant recovery moments, being affected by atmospheric pressure temperature, and climate change.

The purpose was to perform standing posture and walking with a moderate balance and state of equilibrium.

The functional state of the patient in March 2021 was that of functional and personal care integrity, the ability to perform standing posture, and walking with a moderate balance and state of equilibrium (Figure 3).

The patient continued the prescribed programs on his own at home during the pandemic period and the functional state of the patient in June 2022 was that of functional and personal care integrity, the ability to perform standing posture and walking with a moderate balance, and a state of equilibrium. (Figure 4).

RESULTS

The results concerning our patient were very promising (Table 3).

The perimetric measurements showed a constant decrease between the initial, intermediate, and final evaluations of the dimensions by 6 centimeters for the ankle joint and by 8 centimeters for the calf, relevant to a good drainage process both through the function of the kidney and through the lymphatic system, due to both effective medication and the sustained kinetic program.

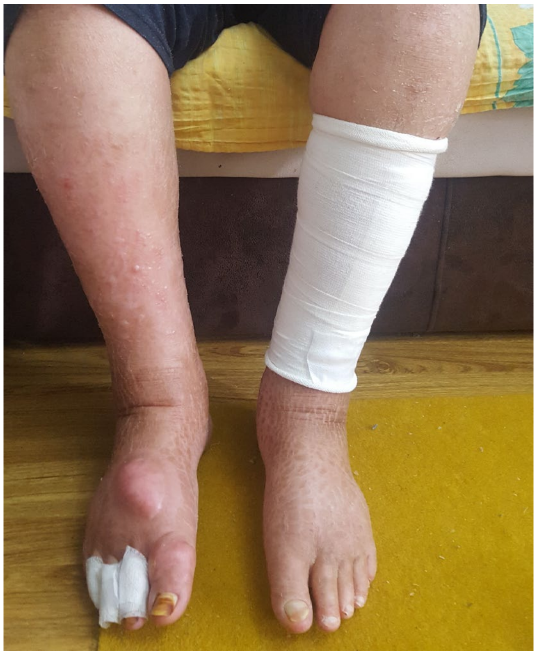

Figure 2: Representative images of the foot edema and neuropathy skin lesions

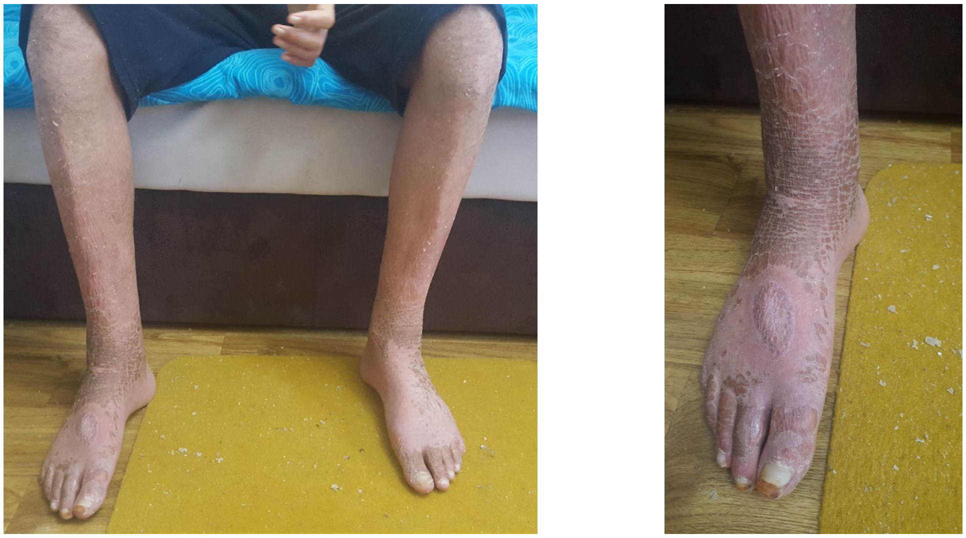

Figure 3: Representative images of the foot from March 2021.

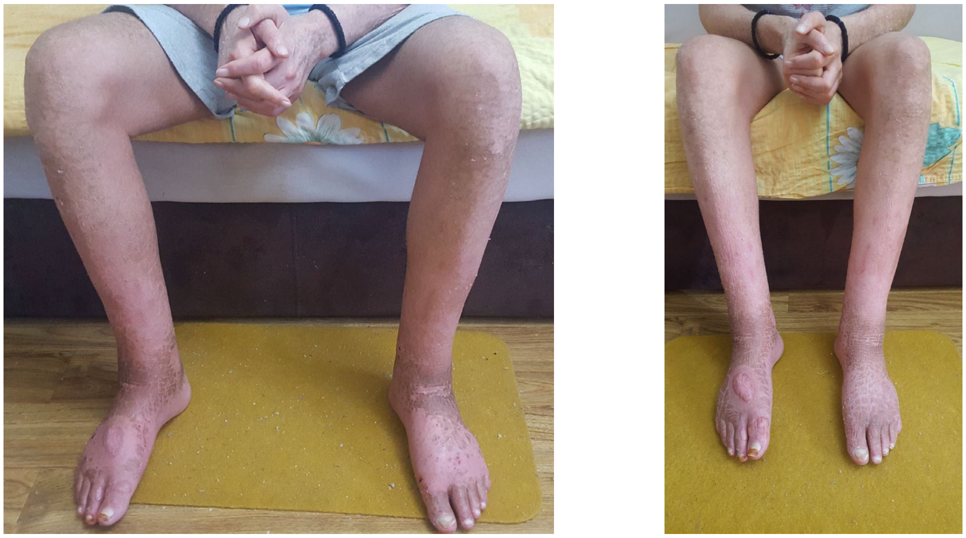

Figure 4: Representative images of the foot from June 2022.

The goniometric measurements for ankle and hip flexion had values of increased joint mobility, thus the ankle gained 10 degrees and the hip 55 degrees in a range of motion, which allowed the patient better mobility and dynamic functionality.

Table 3: Results of evaluation tests

Measurements	Initial testing Feb 2017	Intermediary testing Feb 2018	Final testing March 2019	Observations
Ankle perimeter	30 cm	27 cm	24 cm	Decreased by 6 cm
Calf perimeter	45 cm	43 cm	37 cm	Decreased by 8 cm
Ankle flexion mobility	5°	10°	15°	Improved with 10°
Hip flexion mobility	35°	85°	90°	Improved with 55°
Static stability	0	5 min	10 min	Improved 10 min
Dynamic stability	0	10 meters	30 meters	Improved 30 meters
Effort capacity	0	10 min	15 min	Improved 15 min

The evaluation of static stability recorded beneficial increases for the patient's life from the value of 0 minutes to 10 minutes without imbalance. The dynamic stability tests increased from 0 meters to 30 meters of walking without imbalance and support. Thus, the static stability gained 10 minutes without imbalances, and the dynamic one from value 0 reached a displacement of 30m without imbalances and support, which allowed the patient to go outside the house.

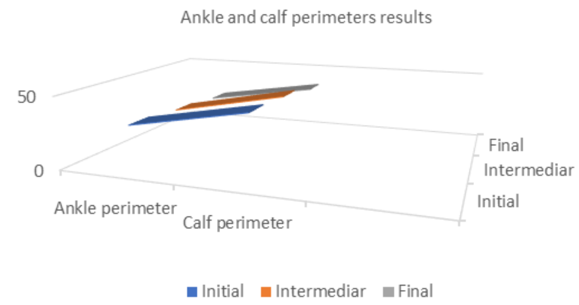

Figure 5: Ankle and calf perimeters measurements results

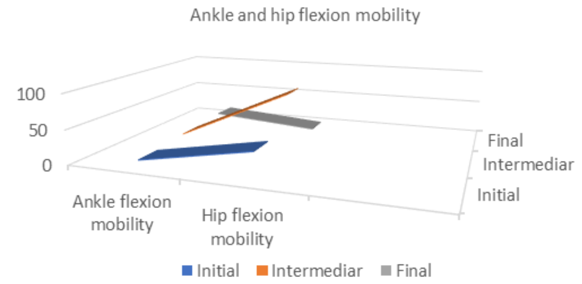

Figure 6: Ankle and hip flexion mobility results

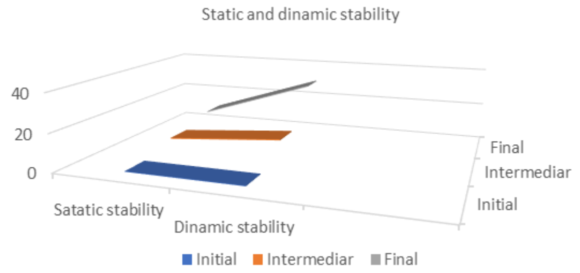

Figure 7: Static and dynamic stability evaluation results

The evaluation of the effort capacity from 0 initial value has reached 10 minutes without effort disorders so that later on the final evaluation on the best day of the patient will be 15 minutes without effort disorders. We mention that every day the patient's dynamics undergo changes from one hour to the next and the time of day matters a lot.

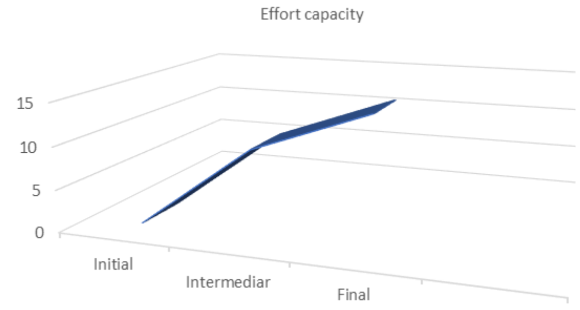

Figure 8: Effort capacity results.

DISCUSSION

The recommended strategy for addressing such a complex SLE case, for recovery of motor function, requires monitoring of at least three lines of observation:

1 Always be aware of disease progression (more in the context of risk of relapse of disease) and medication applied:

- Consulting physician recommendations, the results of medical tests, imaging investigations, etc.

- Check the treatment schedule, especially in the case of a disease involving a long-term treatment scheme with the adjustment of the substances and doses used (side effects of drugs can often have an impact on the patient's condition).

2 Use in an efficient manner the pluses of a positive psychic state (a calm, orderly mind with clear targets) and cultivate the will of the patient.

3 Permanent assessment of patient evolution, in points where difficulties were found: posture, coordination, and motricity.

Following the lines of this strategy, the physical therapist will be able:

- To work together with the patient, in a flexible system, the recovery exercises - maximum 10-30 minutes.

- To recommend to the patient a set of exercises to perform at home (immunosuppressive regimens do not recommend the presence of the SLE patient in the community over long periods of time, even more in the presence of other patients with transmissible diseases).

- And additionally, where appropriate, to apply therapeutic manual therapy and drainage sessions, as well as breathing techniques.

The recommended strategy for addressing the rehabilitation of motor function in such a complex case requires monitoring of at least these five levels of observation:

- Always be aware of the disease progression (even more in the context of risk of relapse) and medication administration, constantly consult physician recommendations, the results of medical tests, imagistic investigations, and so on. In this case, multiple impairments of the organ systems have led to chronic or acute manifestations of imbalances in the body [21-25,33, 34]. Permanently check the treatment schedule, especially in the case of a long-term treatment plan disease with the adjustment of the substances and doses used (side effects of drugs can often have an impact on the patient's condition).

- Permanent assessment of the patient’s evolution, in points where difficulties were found posture, coordination, and motricity, cause many of the associated diseases, both in the kidneys and other organs, make it difficult to choose the correct and balanced diet and medication.

- An important path leading to complete rehabilitation in this case, as in many others, was the combination of sustained social - psychological - spiritual support through faith and management with attention focused on the patient. The psychological support received from the medical staff and family, largely based on the patient’s trust in them, was even more necessary as the treatment with corticosteroids can cause changes in behavior (periods of extreme energy and activity, combined with periods of insomnia and irritability). We identified at least three main engines of the patient’s desire to recover – confidence in the attending physician and successful completion of the treatment, the desire to return to a normal life and sacred therapy.

The multi-pathology, as well as the delicate situation of the patient, have greatly raised the stakes of the entire medical staff in choosing the most suitable treatment management, which is still in progress daily.

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ORIGINAL ARTICLE

The Effect of an Empowerment Program on the Spiritual Care Competence of Nursing Students: A Randomized Clinical Trial Study

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Abstract: *Spiritual care is an integrated section of nursing. Proper implementation of it to patients requires that care providers have the necessary competencies in this regard. Therefore, this study was performed to investigate the effect of an empowering program on spiritual care competence (SCC) in nursing students. This study was a randomized controlled trial that was performed on 60 nursing students at Kashan University of Medical Sciences, Iran. Participants were randomly assigned to two groups. The intervention group was under the spiritual empowering plan in seven sessions. The control group was traditionally trained. Data were collected by a spiritual care competence scale and analyzed using Fisher, independent and paired t-test in SPSS 16v. Baseline characteristics were similar in the two groups. The mean score of SCC was significantly increased post-empowerment for the intervention group. The differences between the two groups were statistically significant in the SCC scores ($p<0.037$). A significant difference was observed between the mean of the SCC score of the intervention group before and after the empowering ($p < 0.0001$). While, no significant changes were observed in the control group, before and after the study ($p= 0.44$). Empowering spiritual care skills in nursing students develops the quality of care in patients, which requires more attention in the educational curriculum.*

Keywords: *holistic care; spiritual care; competence; empowerment program; nursing students*

INTRODUCTION

A holistic attitude is a dynamic way of educating an individual with a holistic view, and the nursing profession is always focused on caring for a person as a whole and covering the physical, mental, social, and spiritual dimensions of a person [1]. Along with the physical and mental dimensions, the spiritual dimension is considered an important factor [2] and it is the basis that all principles, ideas, thoughts, beliefs, choices, actions, and final goals of a person are focused on it [3]. Considering this important human dimension and providing comprehensive care will promote the recovery and well-being of patients [4]. Hence, spiritual care is an integrated section of the nursing process [5]. Spiritual care contains religious and non-religious skills [6]. The religious skills contain such things as patient accompaniment in worship and provision of the necessary requisites for religious ritual, talking with him about God, clarifying the patient's relationship with God, applying religious books, and referring to religious institutions. The non-religious skills also contain relationship skills, empathy, respect, counseling, emotional support of people, and encouraging the client to express feelings and avoid negative thoughts [7]. The correct implementation of spiritual care requires having the necessary skills in this field [8]. This competence is very important for nurses and nursing students because the patients' spiritual needs become apparent when disease or crisis occurs [9]. Spiritual competence empowers nurses and increases their knowledge and skills to nurture hope, meaning, and purpose in patients [2]. During the course of the disease, nursing students are admitted to the private domain of patients, along with health workers, so they should be able to deal with their spiritual issues [10]. Students have different cultural and spiritual views, beliefs, and values than current nurses [11], and institutionalization of ethical principles among students and preparing them for future professional life is an important part of university responsibility. The task of universities is to organize the educational environment in accordance with the culture and religion of the country and the needs of the community [12]. Studies show that the spiritual competence of nurses and nursing students is not in good condition [11, 13-15]. A study showed that only 42% of nurses in the clinic pay attention to the patient’s spiritual needs [13]. Also, Ross et al. (2014) reported that only 25% of nursing students are qualified to perform spiritual care [16], and most nursing students in clinical practice ignore spiritual care [17]. Although nurses are motivated to become qualified in spiritual care [18] and nursing students value it [11], they do not implement spiritual care, and lack of attention to spirituality in undergraduate programs is stated as the predominant obstacle to the lack of competence in implementation spiritual care [19-21]. The results of the studies show that the nursing curriculum needs to integrate spirituality and spiritual care more than ever [22, 23] so that nurses become more aware of the patients’ spiritual needs [2]. Culatto and Summerton (2015) reported that spirituality is an important part of the curriculum before and after a clinical period, and 64.2% of educational institutions considered spirituality a valuable and significant part of education, and only 14.3% considered this part worthless [24]. Nursing literature also recognizes the need for nurses' empowerment in the field of spiritual care [25-27]. However, so far this issue has not been formally and explicitly included in nursing education programs [16]. Spiritual empowerment can improve students' attitudes and awareness about the spirituality of patients, assess spiritual needs, respond differently to their needs, the ability to refer patients, and their communication skills [28,29]. It was also found in another qualitative study that spiritual care education had a positive effect on their personal, academic, and professional lives [30]. In spite of the benefits of spirituality education, a review study by Cooper et al. (2013) noted its negative effects, such as the concern and the sense of coercion in changing personal opinions about spirituality [31], and in another study, little effect of spiritual care education was found on students' attitude towards spirituality and communication skills [32]. Few studies have been done on the form and manner of empowering spirituality [33]. Therefore, it is necessary to conduct numerous studies to evaluate the benefits of empowering methods in the field of spirituality [34]. The purpose of this study was to investigate the effect of an empowering plan on the competence of implementation of spiritual care in nursing students.

MATERIALS AND METHODS

The present study is a randomized control clinical trial that was performed on senior nursing students of the Faculty of Nursing and Midwifery of Kashan University of Medical Sciences.

Participants and data sampling

The sample size was estimated to be 30 in each group based on the mean and standard deviation of the previous study [28, 29] at a 95% confidence level and 80% test capacity ($\alpha = 0.95$, $\beta = 0.2$, $s1 = 10$ and $s2 = 10$). Sampling in the first stage was simply random so the samples were selected from the list of senior nursing students of the faculty using the random number table. Then, students were randomly assigned to two intervention (30) and control (30 people) groups by block randomization (four blocks). Inclusion criteria included students who were studying in the seventh and eighth semesters, were not guests and free from academic probation or transfer, had no known physical and psychological problems, and were satisfied with the research. Students who were reluctant to continue to cooperate at each stage of the research, had severe crises (like the death of a close family member, divorce, separation, and quarrel with the spouse), and had two sessions or more absences in the classes, were excluded from the study.

Procedure

The researchers gave information on the objectives, type, advantages, and confidentiality of the study to the students. If they were willing to collaborate in the study, written informed consent was completed and the study instrument was self-reported by them. The samples were then assigned to intervention and control groups. The intervention group was under the empowering plan. The intervention method was such that the students participated in a group and in a single month in seven 60-minute sessions. The control group was trained under the former and routine curriculum of the university. Educational pamphlets about care were also provided. In the final of the study (one month after starting the study), the study tool was completed by the samples again.

Intervention

The empowerment content of the present study is taken from past studies [29,33,35-37] and consists of spiritual subjects such as the description of spirituality and spiritual care, assessment and implementation of spiritual care, communication skills, respect for human dignity, individual support, counseling, hope therapy, and betterment the quality of life, which was explained and taught in the form of lectures and small group discussions (Box 1). This content was confirmed by 10 experts and faculty members who had the awareness and experience in this background.

Data Collection

The study instrument consists of two sections of individual social information inventory including 9 items containing age, gender, marital status, semester, accommodation status, interest in the subject of spirituality, passing the course of spiritual care, participation in the ethics seminar or workshop, and the degree of personal religiosity and the scale spiritual competence.

Box 1: Subjects of the training plan

Session 1:
- The description of spiritual health and its dimensions
- Acceptance of life and relationship with God, self, society, and the environment
- The expression of the integrity of the biological, psychological, and social components
- Essential characteristics of spirituality
- Differences and similarities between spirituality and religion
- Explain spirituality and spiritual care
- Religious and non-religious care

Session 2:
- Types of spiritual care (personal, procedural, cultural, and evangelical)
- Essential skills for performing its
- Spiritual distress, the influence of disease on individual life
- Detect meaning and goal of life
- The role of hope in patient spirituality
- Facilitate coping and adaptation strategies used by patients during illness

Session 3:
- Dimension of professionalism and betterment of the quality of life
- Obstacles to the implementation of its
- Strategies to improve its

Session 4:
- Assessment and implementation of its
- The patients’ spiritual needs during illness and how to assess them
- How to provide spiritual care by nurses
- The nursing process in spirituality
- Communication skills

Session 5:
- Communication techniques, trust building, and patient acceptance
- Active listening
- How to express feelings

Session 6:
- Importance of human dignity
- Efficacy of observance of privacy in patient’s spirituality
- Paying attention to spiritual care in the charter of patient rights
- Personal support
- Counseling and referral

Session 7:
- Attention and support of religious rituals
- Religious and spiritual referral
- Protective presence

The Spiritual Care Competence Scale (SCCS) was designed by Van Leeuwen & Cusveller (2009). This scale has 6 dimensions, the first dimension is "assessment and implementation of spiritual care" (Questions 1-6), the second dimension "professionalism and betterment of the quality of spiritual care" (Questions 7-12), the third dimension "personal support and patient counseling" (Questions 13-18), the fourth dimension "referral" (Questions 19-21), the fifth dimension "attitude towards patients' spirituality" (Questions 22-25) and finally the sixth dimension is "communication" (Questions 26-27) that the responses were ranked according to the 5-point Likert scale (fully agree, to fully disagree). The total score of this scale is between 27 and 135 and it has three grades. A score of 27 to 62 indicates a low level, a score of 63 to 98 indicates an average level and a score of more than 98 indicates a high level of spiritual competence. The validity and reliability of this instrument were investigated by Van Leeuwen et al. In 2009, the Cronbach's alpha coefficient for the sub-scales was between 0.56 to 0.82 and the mean inter-item correlation ((MIIC ≥ 0.25) was reported as an acceptable level of internal consistency or reliability [38]. The validity and reliability of this scale have been confirmed in Iran. In the study of Nasehi et al. (2013), the Cronbach's alpha coefficient was 0.78 and in the other study, the Cronbach's alpha coefficient for the whole instrument was 0.77, and for the subcategories have been reported between 0.65 and 0.85 [8,10].

Data Analysis

Data analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine the normal distribution of the data. Descriptive statistics (i.e., mean, standard deviation, frequency, and percentage) were calculated. Fisher and t-tests were used to compare demographic variables in the two groups. The independent-sample t-test was also used to examine the differences between the competence of spiritual care mean scores, and the subscale mean scores within the two groups at the beginning and the final of the study. In addition, a paired-sample t-test was used to compare the changes in the competence of spiritual care mean scores of the individual groups at the beginning and the final of the study. The significance level was considered less than 0.05 in all tests.

RESULTS

Out of 60 participants in this study, eight persons in the intervention group and five persons in the control group were excluded from the analysis due to irregular attendance at training sessions and incomplete answers in the post-test instrument, respectively. Finally, the data of 47 samples were analyzed (Figure 1).

The mean age of participants was 22.43 ± 0.85. About 25.5% of the participants in the intervention group and 36.2% in the control group were female.

Figure 1: CONSORT flow diagram

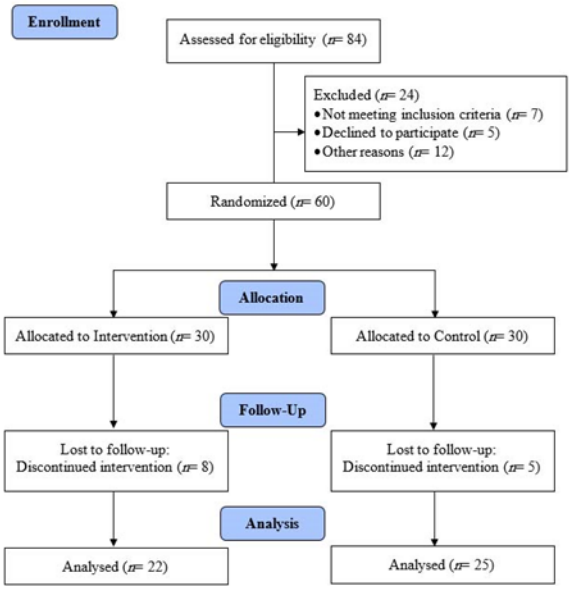
Enrollment
Assessed for eligibility (n= 84)
Excluded (n= 24)
•Not meeting inclusion criteria (n= 7)
•Declined to participate (n= 5)
•Other reasons (n= 12)
Randomized (n= 60)
Allocation
Allocated to Intervention (n= 30)
Allocated to Control (n= 30)
Follow-Up
Lost to follow-up: Discontinued intervention (n= 8)
Lost to follow-up: Discontinued intervention (n= 5)
Analysis
Analysed (n= 22)
Analysed (n= 25)

The results showed that there was no significant difference between the two groups' demographic variables, including age, semester, sex, marital status, accommodation status, interest in the subject of spirituality, and attending spiritual care of patient course (p > 0.05) (Table 1). The results obtained from conducting independent sample t-tests revealed that there was no significant difference between the mean scores of spiritual care competence and its subscales for the two groups at the beginning of the study (p > 0.05) (Table 2). However, the mean score of spiritual care competence (104.9 ± 9.7 for the intervention group, and 97.4 ± 14 for the control group), was significantly increased post-intervention for the intervention group. The differences between the two groups were statistically significant in both the overall spiritual care competence scores (p < 0.037) and across two of the subscales, including "assessment and implementation of spiritual care" and "professionalism and betterment of the quality of spiritual care" (p < 0.05) (Table 3). Using paired t-tests, we found that spiritual care competence and its subscales, including "assessment and implementation of spiritual care", "professionalism and betterment of the quality of spiritual care", "personal support and patient counseling" and "referral" in the intervention group were significantly different before and after the spiritual education (p < 0.0001). There was no significant difference between the mean scores of the spiritual care competence and its subscales in the control group at the beginning and the final of the study (p > 0.05) (Table 4).

Table 1: Demographic information of samples

Variables	Intervention group *(n= 22)*	Control group *(n= 25)*	*p*-value
Age, year†	22.27 ± 0.76	22.56 ± 0.91	0.25*
Semester, number†	7.55 ± 51	7.52 ± 51	0.86*
Gender††			0.38**
Male	10 (21.3)	8 (17)	
Female	12 (25.5)	17 (36.2)	
Marital status††			0.17**
Single	19 (40.4)	17 (36.2)	
Married	3 (6.4)	8 (17)	
Accommodation status††			0.21**
Personal house	17 (77.3)	14 (56)	
Dormitory	5 (22.7)	11 (44)	
Interest in the subject of spirituality††			0.21**
Low	17 (77.3)	14 (56)	
High	5 (22.7)	11 (44)	
Passing the course of spiritual care††			0.4**
Yes	3 (13.6)	6 (24)	
No	19 (86.4)	19 (76)	

† Mean ± Standard Deviation
†† No. (%)
** Obtained from independent t-test*
*** Obtained from Fisher test*

Table 2: Comparison of spiritual care competence at the beginning of the study between the intervention and control group

Variables	Intervention group† (*n*= 22)	Control group† (*n*= 25)	*p*-value*
Assessment and implementation of spiritual care	18.9 ± 3.8	20.08 ± 3.17	0.26
Professionalism and betterment of the quality of spiritual care	17.81 ± 3.6	19.28 ± 3.28	0.15
Personal support and patient counseling	20 ± 3.3	21.08 ± 3.87	0.31
Referral	9.77 ± 1.6	10.2 ± 2.04	0.43
Attitude toward patients' spirituality	15.68 ± 2.57	16.56 ± 2.32	0.22
Communication	7.45 ± 1.53	8.2 ± 1.63	0.11
Spiritual care competence	89.63 ± 10.57	95.33 ± 10.82	0.07

† Mean ± Standard Deviation

** Obtained from independent t-test*

Table 3: Comparison of spiritual care competence at the final of the study between the intervention and control group

Variables	Intervention group† (*n*= 22)	Control group† (*n*= 25)	*p*-value*
Assessment and implementation of spiritual care	23.54 ± 2.08	21.28 ± 3.72	0.01
Professionalism and betterment of the quality of spiritual care	22.5 ± 3.01	20.36 ± 3.86	0.03
Personal support and patient counseling	23.13 ± 2.73	21.8 ± 3.3	0.14
Referral	11.5 ± 1.76	10.76 ± 2	0.18
Attitude toward patients' spirituality	16.54 ± 1.81	15.44 ± 2.61	0.1
Communication	7.68 ± 1.61	7.76 ± 1.76	0.8
Spiritual care competence	104.9 ± 9.7	97.4 ± 14	0.03

† Mean ± Standard Deviation

** Obtained from independent t-test*

Table 4: Comparison of spiritual care competence pretest-posttest between the intervention and control group

Variables	Intervention group (*n*= 22)			Control group (*n*= 25)		
	Baseline†	After intervention†	*p*-value*	Baseline†	Final of study†	*p*-value*
Assessment and implementation of spiritual care	18.9 ± 3.8	23.54 ± 2.08	0.00	20.08 ± 3.17	21.28 ± 3.72	1.37
Professionalism and betterment of the quality of spiritual care	17.81 ± 3.6	22.5 ± 3.01	0.00	19.28 ± 3.28	20.36 ± 3.86	0.26
Personal support and patient counseling	20 ± 3.3	23.13 ± 2.73	0.00	21.08 ± 3.87	21.8 ± 3.3	0.53
Referral	9.77 ± 1.6	11.5 ± 1.76	0.00	10.2 ± 2.04	10.76 ± 2	0.22
Attitude toward patients' spirituality	15.68 ± 2.57	16.54 ± 1.81	0.19	16.56 ± 2.32	15.44 ± 2.61	0.05
Communication	7.45 ± 1.53	7.68 ± 1.61	0.59	8.2 ± 1.63	7.76 ± 1.76	0.19
Spiritual care competence	89.63 ± 10.57	104.9 ± 9.7	0.00	95.33 ± 10.82	97.4 ± 14	0.44

† Mean ± Standard Deviation

** Obtained from independent t-test*

DISCUSSION

The purpose of this study was to investigate the efficacy of empowerment programs on the competence of implementation of spiritual care in nursing students it showed that spiritual empowerment generally increases the competence of implementation of nursing students' spiritual care, but its impact on the dimensions of "assessment and implementation of spiritual care", "professionalism and betterment of the quality of spiritual care", "personal support and patient counseling" and "referral" are more than the dimensions of "attitude toward patients' spirituality" and "communication skills". The findings of this study are consistent with the findings of studies with the aim of determining the efficacy of the empowering plan in betterment of the competence of providing spiritual care by nursing students [32], the effectiveness of an educational intervention in the ability to perform spiritual care and spiritual well-being of undergraduate and postgraduate students [39], the effectiveness of simulated-spiritualized training in implementation of spiritual care for nursing students [28], and the changes in the spiritual experiences of medical and nursing students before and after a period of clinical and spiritual care [40]. Also, in a qualitative study, the survey of experiences of nursing students after the course of spiritual care showed that spirituality had a positive effect on their personal, academic, and professional lives, and led to increased self-awareness, individual development, knowledge about spirituality and spiritual care, sensitivity to the patient's spiritual needs, familiarity with adaptation methods, and providing holistic care [33]. In fact, students who are trained in spirituality, realize the concept of spirituality and give more notice to the patient's spiritual needs [41]. Increasing their moral sensitivity and ethical decision-making ability in clinical settings [29], in addition, they do it in their daily routines. Concerning the cause of the effect of the empowerment plan, the following may be mentioned: since spiritual awareness is prior to the spiritual assessment of patients [41], spirituality empower-ing by increasing awareness of students about patients' spiritual needs and improving their attitude toward spirituality, provides the required competencies in assessing spiritual needs, responding differently to needs, referring patients and communication skills [28]. Empowering also leads to an increase in understanding of the value of spirituality [40] and the ability to provide spiritual care to nursing students in the future.

The method of spiritual empowerment has also been effective in the efficacy of education. According to Taylor, the best method of spiritual care education is to induce a positive attitude and compassionate skills in order to create a sensitivity to spiritual care, which is not achieved except through student discussion [42]. In this study, students used the ideas of each other and recognized their and other's religious views to understand and develop spiritual self-awareness. In addition, inspirational stories were also assisted.

The reason for the low impact of the plan on some aspects of the competence of providing spiritual care, including the attitude toward spirituality and communication, is probably lack of work experience because spirituality is not only rooted in one's beliefs, but also rooted in his experience [4] and factors such as individual spirituality, degree of beliefs, experience, and age of individuals are associated with their attitude to spiritual care [43]. Of course, there is another point to consider that communication is more effective in responding to this topic and is also a kind of research limitation, and it is a self-report of students. Hence, the use of other instruments in assessing these skills is suggested. Considering the influence of the group discussion method, it is suggested that other active educational methods be evaluated and compared in subsequent studies to better spiritual care education and to choose the best em-powering method for spirituality and spiritual care.

CONCLUSION

Based on the results, the empowerment plan is effective and useful in the field of spiritual care competence of nursing students. Therefore, authorities should pay more attention to the importance of empowering spiritual care to nursing students, because the more nursing students have knowledge of spiritual care is greater, the higher the quality of care provided to the patient. Nursing students need to be trained in a wider range of spiritual care skills and it is best to pay more notice to this topic in the nursing educational curriculum. Valuing spiritual care and proper nurse-patient spiritual communication enriches the nursing profession increases nurses' job satisfaction and provides opportunities for better performance.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization and supervision: KS and MA; Data collection: ZS, MH; Data analysis: MA; Writing-original draft and Writing-review & editing: ZS and MA; Final approval: All authors.

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Ethics approval and consent to participate

This study was confirmed by the institutional review board and the ethics committee of the University (IR.KAUMS.NUHEPM.REC.1396.41). The objectives of the study were stated for all nursing students and informed written consent was obtained from them. All student's participation in this study was voluntary and they had the right to withdraw from the study at any time and stage of the study. Also, they were reassured that their personal information would remain confidential. The current research was registered with registration code IRCT20130721014086N11.

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ORIGINAL ARTICLE

The Effect of COVID-19 and Vaccination against COVID-19 on Patients with Psoriasis

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Abstract: *COVID-19 has not been adequately investigated in patients with psoriasis. We wanted to evaluate the effect of COVID-19 and COVID-19 vaccines on disease course in patients with psoriasis. We retrospectively reviewed the medical records of patients who were admitted for the treatment of psoriasis between March 2020 and May 2022. Afterward, patients were contacted by telephone call to collect data on COVID-19. This study included 257 patients, 118 females, and 139 males. 238 (92.6%) patients were vaccinated against COVID-19. The mean age of the vaccinated and unvaccinated patients was 49.23±14.60 and 40.37±11.66 years, respectively (p=0.010). Exacerbation of psoriasis after COVID-19 vaccination was detected in 32 (13.4%) patients. 114 (44.4%) patients had been diagnosed with COVID-19 and 18.4% of them stated exacerbation of psoriasis after COVID-19. No association was detected between COVID-19 and having an accompanying disease and psoriasis treatment (p=0.843, p=0.845, respectively). Hospitalization was required in 12 (10.5%) patients. Unvaccinated patients with psoriasis were significantly younger than those who were vaccinated. Exacerbation of psoriasis was reported in 13.4% of vaccinated patients and in 18.4% of patients after COVID-19. Nevertheless, accompanying disease and conventional or biological agent treatment were associated neither with COVID-19 nor COVID-19-related hospitalization.*

Keywords: *COVID-19; psoriasis; vaccine*

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease that affects 2-3% of the general population [1]. Psoriasis may be triggered by infections and various vaccines such as influenza and tetanus-diphtheria vaccines [2]. Recently, exacerbation and onset of psoriasis have been reported after Coronavirus disease 2019 (COVID-19) and vaccination against COVID-19 [3,4]. Common inflammatory cytokine production has been reported both in patients with psoriasis and in patients with COVID-19 or vaccinated against COVID-19 [5,6]. However, the exact mechanism of exacerbation of psoriasis after vaccination against COVID-19 has not been clearly understood yet. Increased Th17 response in psoriasis has also been detected in patients with severe COVID-19. Therefore, mRNA COVID-19 vaccines may lead to exacerbation of psoriasis by inducing Th17 cells [7].

In addition, the course of COVID-19 in patients with psoriasis remains controversial. It has been suggested that psoriasis might be a risk factor for severe COVID-19 since psoriasis comorbidities such as hypertension, diabetes, and cardiovascular disease have been associated with poor prognosis of COVID-19 [8]. Increased serum level of angiotensin-converting enzyme (ACE) has been reported in psoriasis patients with comorbidities such as cardiovascular disease and atherosclerosis. Therefore, hyperactivity of ACE in COVID-19 may lead to susceptibility to cardiovascular events in psoriasis patients with COVID-19 [9].

Moreover, data on the association between immune-suppressive treatment and increased risk for COVID-19 are inadequate and it is a matter of debate that cessation of immunosuppressive treatment and biological agents in psoriasis patients with COVID-19 is required or not [6,8]. On the other hand, vaccination against COVID-19 is of utmost importance in patients with psoriasis, especially in those under immunosuppressive treatment. Nevertheless, vaccination against COVID-19 in patients with psoriasis has not been adequately investigated [10]. Within this study, COVID-19 vaccination status and the effect of COVID-19 and Pfizer-BioNTech and Sinovac-CoronaVac COVID-19 vaccines on disease course were evaluated in patients with psoriasis.

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained before this study (approval number: 2022-700). We retrospectively reviewed the medical records of patients who were admitted for the treatment of psoriasis between March 2020 and May 2022. Patients aged 18 years and over with complete data were included in the study. The exclusion criteria were pregnancy, lactation, and receiving any vaccines other than COVID-19 such as the flu shot. Data on age, gender, psoriasis type, psoriasis duration, psoriatic arthritis, past medical history, and psoriasis treatment were evaluated. Afterward, patients were contacted by telephone call to collect the data on COVID-19 vaccination status, vaccine type, vaccine dose, effect of COVID-19 vaccines on psoriasis, time to initiation of exacerbation of psoriasis after vaccination, other cutaneous side effects of vaccines, diagnosis of COVID-19, effect of COVID-19 on psoriasis, time to initiation of exacerbation of psoriasis after COVID-19, other cutaneous effects of COVID-19 and requirement of hospitalization. Exacerbation of psoriasis was regarded as an increase in the size and number of the psoriasis lesions.

Statistical analysis was performed using SPSS version 20.0. Data were presented as mean ± standard deviation or median (interquartile range) for quantitative variables, counts, and percentages for categorical variables. Differences between the groups were evaluated by using the chi-square test and Mann-Whitney U test. P values less than 0.05 were considered statistically significant.

RESULTS

This study included 257 patients, 118 (45.9%) females and 139 (54.1%) males. The mean age of the patients was 48.57±14.57 years (range: 18-84 years). 233 (90.7%) patients had psoriasis vulgaris, 14 (5.4%) had palmoplantar pustular psoriasis, 7 (2.7%) had generalized pustular psoriasis, 1 (0.4%) had inverse psoriasis, 1 (0.4%) had nail psoriasis and 1 (0.4%) had guttate psoriasis. The mean psoriasis duration was 17.56±12.36 years (range: 1-54 years). Psoriatic arthritis was observed in 70 (27.2%) patients. The past medical history was unremarkable in 140 (54.5%) patients. However, hypertension was detected in 60 (23.3%) patients, diabetes in 36 (14%), chronic hepatitis B virus infection in 20 (7.8%), coronary artery disease in 16 (6.2%), hypothyroidism in 11 (4.3%), hyperlipidemia in 11 (4.3%), chronic obstructive pulmonary disease in 5 (1.9%), chronic renal disease in 5 (1.9%) and asthma in 5 (1.9%) patients. Malignancies such as thyroid, bladder, non-small cell lung, and breast cancers were detected in 5 (1.9%) patients, rheumatic diseases such as rheumatoid arthritis, ankylosing spondylitis, gout, and Sjögren's syndrome in 4 (1.6%) patients, benign prostatic hyperplasia in 4 (1.6%), chronic venous insufficiency in 2 (0.8%) and migraine in 2 (0.8%) patients. 129 (50.2%) patients were treated with biological agents. 52 (20.2%) patients received ustekinumab, 35 (13.6%) received interleukin-17 (IL-17) inhibitors such as secukinumab and ixekizumab, 34 (13.2%) received tumor necrosis factor-α (TNF-α) inhibitors such as adalimumab, etanercept, infliximab and certolizumab pegol, 8 (3.1%) patients received IL-23 inhibitors such as guselkumab and risankizumab. In addition, 93 (36.2%) patients were treated with methotrexate, 27 (10.5%) with acitretin, 5 (1.9%) with cyclosporine, and 3 (1.2%) with narrowband ultraviolet B therapy.

238 (92.6%) patients were vaccinated against COVID-19 whereas, 19 (7.4%) patients were unvaccinated (Table 1). The mean age of the vaccinated and unvaccinated patients was 49.23±14.60 and 40.37±11.66 years, respectively (p=0.010). 114 (44.4%) patients received Pfizer-BioNTech, 77 (30%) patients received both Pfizer-BioNTech and Sinovac-CoronaVac, 47 (18.3%) patients received Sinovac-CoronaVac COVID-19 vaccines. The mean age of the patients vaccinated with only Pfizer-BioNTech and only Sinovac-CoronaVac COVID-19 vaccine was 44.12±12.72 and 49.49±15.19 years, respectively (p=0.023). The mean psoriasis duration of the patients vaccinated with only Pfizer-BioNTech and only Sinovac-CoronaVac COVID-19 vaccine was 14.75±11.29 and 19.72±13.23 years, respectively (p=0.017). 29 (61.7%) patients vaccinated with only Sinovac-CoronaVac had at least one accompanying disease whereas, 39 (34.2%) patients vaccinated with only Pfizer-BioNTech had at least one accompanying disease (p=0.002). 8 (17%) patients vaccinated with only Sinovac-CoronaVac had diabetes whereas, 5 (4.4%) patients vaccinated with only Pfizer-BioNTech had diabetes (p=0.007). Among 191 (74.3%) patients who received Pfizer-BioNTech COVID-19 vaccine, 93 (36.2%) received 2 doses, 68 (26.5%) received 3 doses, 29 (11.3%) received 1 dose and 1 (0.4%) received 4 doses of vaccine. Among 124 (48.2%) patients who received Sinovac-CoronaVac COVID-19 vaccine, 88 (34.2%) received 2 doses, 16 (6.2%) received 4 doses, 14 (5.4%) received 3 doses, 4 (1.6%) received 1 dose and 2 (0.8%) received 5 doses of vaccine. No accompanying disease has been detected in 22 (73.3%) patients who were unvaccinated or received only a single dose of vaccine and in 118 (52%) patients who received two or more doses of vaccine (p=0.027). 206 (86.6%) patients reported no effect of COVID-19 vaccines on psoriasis, however, 32 (13.4%) patients reported exacerbation of psoriasis after COVID-19 vaccines. Among them, 24 (75%) received Pfizer-BioNTech, 7 (21.9%) received Sinovac-CoronaVac and 1 (3.1%) received both Pfizer-

BioNTech and Sinovac-CoronaVac COVID-19 vaccines. Exacerbation of psoriasis lesions was reported in 28 (87.5%) patients, generalized psoriasis lesions in 3 (9.4%) patients, and exacerbation of nail involvement in 1 (3.1%) patient (Table 2). The median time to initiation of symptoms after COVID-19 vaccination was 15 (25.25) days. Moreover, 1 (0.4%) patient had urticaria, 1 (0.4%) had papules and pustules on soles and 1 (0.4%) had COVID arm following Pfizer-BioNTech COVID-19 vaccination.

Table 1: Vaccination status of patients with psoriasis against COVID-19

	Vaccinated	**Unvaccinated**	**P value**
Patients, n (%)	238 (92.6%)	19 (7.4%)	
Mean age ± SD (years)	49.23±14.60	40.37±11.66	0.01
Administered vaccine, n (%)	Pfizer-BioNTech, 114 (44.4%) Pfizer-BioNTech and Sinovac-CoronaVac, 77 (30%) Sinovac-CoronaVac, 47 (18.3%)		
	Pfizer-BioNTech	**Sinovac-CoronaVac**	
Mean age ± SD (year)	44.12±12.72	49.49±15.19	0.023
Mean psoriasis duration ± SD (year)	14.75±11.29	19.72±13.23	0.017
Accompanying disease, n (%)	39 (34.2%)	29 (61.7%)	0.002
Diabetes, n (%)	5 (4.4%)	8 (17%)	0.007

COVID-19: Coronavirus disease 2019, SD: Standard deviation. Vaccination with Pfizer-BioNTech was more common in younger patients and patients with shorter psoriasis duration compared to vaccination with Sinovac-CoronaVac COVID-19 vaccine (p=0.023, p=0.017, respectively)

Table 2: The effect of COVID-19 and Pfizer-BioNTech and Sinovac-CoronaVac COVID-19 vaccines on patients with psoriasis

Exacerbation of psoriasis after vaccines, n (%)	32 (13.4%)
Vaccine type, n (%)	Pfizer-BioNTech, 24 (75%) Sinovac-CoronaVac, 7 (21.9%) Pfizer-BioNTech and Sinovac-CoronaVac, 1 (3.1%)
Effect of vaccines on psoriasis, n (%)	Exacerbation of the lesions, 28 (87.5%) Generalized lesions, 3 (9.4%) Exacerbation of nail involvement, 1 (3.1%)
Initiation of the symptoms after vaccination, median time (IQR)	15 (25.25) days
Cutaneous effects of Pfizer-BioNTech vaccine, n (%)	Urticaria, 1 (0.4%) Papules and pustules on soles, 1 (0.4%) COVID arm, 1 (0.4%)
Exacerbation of psoriasis after COVID-19, n (%)	21 (18.4%)
Time to onset of COVID-19 and exacerbation of psoriasis, (mean ± SD)	11.83±9.59 days
COVID-19 related hospitalization, n (%)	12 (10.5%)

COVID-19: Coronavirus disease 2019, IQR: Interquartile range, SD: Standard deviation. Exacerbation of psoriasis after vaccines was reported in 32 (13.4%) patients. Among them, 24 (75%) patients received the Pfizer-BioNTech COVID-19 vaccine

114 (44.4%) patients had been diagnosed with COVID-19 whereas, 143 (55.6%) patients never had the infection. No statistically significant difference was observed between patients who had been diagnosed with COVID-19 and those who never had COVID-19 in age, gender, psoriasis duration, having an accompanying disease, psoriatic arthritis, hypertension, and diabetes (p=0.660, p=0.154, p=0.254, p=0.843, p=0.093, p=0.194, p=0.726, respectively). 21 (18.4%) patients stated exacerbation of psoriasis after COVID-19, however, 93 (81.6%) patients stated no effect of COVID-19 on psoriasis. The mean time between the onset of COVID-19 and exacerbation of psoriasis was 11.83±9.59 days (range: 1-30 days). Exacerbation of psoriasis after COVID-19 was detected in 6 (37.5%) patients who reported exacerbation of psoriasis after COVID-19 vaccines and in 11 (12.5%) patients who reported no effect of vaccines on psoriasis (p=0.013). Besides psoriasis, none of the patients developed any skin lesions which might be associated with COVID-19. No statistically significant difference was observed between patients who had been diagnosed with COVID-19 and those who never had COVID-19 in psoriasis treatment such as biological agents and conventional medication, in biological agents received such as anti-TNF-α, anti-IL-17, anti-IL-12/23 and anti-IL-23 agents and in vaccination status or vaccine type (p=0.845, p=0.274, p=0.452, p=0.694, respectively). 12 (10.5%) patients were hospitalized for COVID-19. None of the patients required

treatment in an intensive care unit. No statistically significant difference was observed between patients who were hospitalized and did not require hospitalization for COVID-19 in age, gender, psoriasis duration, having an accompanying disease, hypertension, diabetes, psoriatic arthritis, treatment with conventional and biological agents, and vaccination status (p=0.462, p=0.587, p=0.972, p=0.309, p=0.615, p=0.705, p=0.473, p=0.249, p=0.309, respectively).

DISCUSSION

Vaccination of patients with psoriasis is crucial in the fight against COVID-19 since immunosuppressive treatment leads to predisposition to infections and psoriasis patients with comorbidities such as metabolic syndrome may be more vulnerable to severe COVID-19 [11]. Therefore, vaccination against COVID-19 is recommended for all psoriasis patients without vaccine allergies, especially for those who receive immunosuppressive treatment [12]. However, it has been suggested that safety concerns might lead to decreased vaccination rates in patients with psoriasis [13,14]. In the United States, Gondo et al. reported that 35.9% of patients with psoriasis were unlikely to get the COVID-19 vaccine. Gondo et al. reported no association between age, gender, having comorbid disease, and being likely to get the COVID-19 vaccine [15]. Sotiriou et al. stated that younger patients with psoriasis, females, patients under biological agent treatment, patients with diabetes, chronic obstructive pulmonary disease, and malignancy had positive beliefs towards COVID-19 vaccines. Concerns about the safety and effectiveness of vaccines and the risk of the exacerbation of psoriasis led to unwillingness to vaccination among patients with psoriasis [16]. Wang et al. reported that 68.9% of patients with psoriasis were vaccinated against COVID-19 in China. Young age, anti-IL-17 treatment, psoriatic arthritis, pustular psoriasis, erythrodermic psoriasis, anxiety, and depression were related to unvaccinated patients [13]. Within this study, 92.6% of the patients with psoriasis were vaccinated. Unvaccinated patients were younger than those who were vaccinated (p=0.010). In addition, most (73.3%) of the psoriasis patients who were unvaccinated or received only a single dose of vaccine had no accompanying systemic disease. Vaccination with Pfizer-BioNTech was more common in younger patients and patients with shorter psoriasis duration compared to vaccination with Sinovac-CoronaVac COVID-19 vaccine (p=0.023, p=0.017, respectively). However, vaccination with Sinovac-CoronaVac was more common in patients with diabetes or at least one accompanying disease compared to vaccination with the Pfizer-BioNTech COVID-19 vaccine (p=0.007, p=0.002, respectively).

Despite the importance of vaccination, data on the safety of COVID-19 vaccines in patients with psoriasis are scarce [17]. New onset and exacerbation of psoriasis have been reported following the administration of COVID-19 vaccines [18]. COVID-19 vaccines have been associated with plaque, guttate, pustular, nail, and erythrodermic psoriasis [5,18-21]. However, the effect of COVID-19 vaccines on psoriasis has not been clearly identified [11]. Wang et al. reported deterioration of psoriasis in 21% of patients and rash and itching in 4% of patients after COVID-19 vaccination [13]. El-Qushayri et al. reviewed the published data on patients who experienced flare-ups of psoriasis due to COVID-19 vaccines. The flare-up of psoriasis was reported in 51 patients, 55% of whom were female after Pfizer-BioNTech, Oxford-AstraZeneca, Moderna, Sinovac-CoronaVac and Covaxin COVID-19 vaccines [17]. Wu et al. reviewed the data on 279 patients with flare-ups of psoriasis following COVID-19 vaccines. Wu et al. reported slight female predominance and plaque psoriasis as the most commonly observed psoriasis type. The time to onset of the flare was 1 to 90 days and the Pfizer-BioNTech COVID-19 vaccine was the most commonly implicated one [22]. Within this study, 32 (13.4%) patients reported exacerbation of psoriasis after COVID-19 vaccines. Exacerbation of skin lesions was the most common finding (87.5%) followed by generalized skin lesions (9.4%) and exacerbation of nail involvement (3.1%). Pfizer-BioNTech COVID-19 vaccine was implicated in most of the cases and the median time to onset of symptoms after COVID-19 vaccination was 15 (25.25) days. Urticaria, papules, and pustules on soles and COVID arm were the other cutaneous side effects detected after the administration of the Pfizer-BioNTech COVID-19 vaccine.

Moreover, COVID-19 has been implicated in the exacerbation and onset of psoriasis [23]. It has been suggested that the synthesis of cytokines such as TNF-α, IL-17, and IL-23 due to activation of toll-like receptor 3 in patients with COVID-19 might trigger psoriasis [23]. For instance, Rouai et al. reported a patient with guttate psoriasis which was started 5 days after recovery from COVID-19 [23]. Shah et al. reported exacerbation of psoriasis following COVID-19 in 3 patients who were well-controlled under treatment with ustekinumab, apremilast, and risankizumab [4]. Erythroderma has also been reported in patients with psoriasis due to COVID-19 [24-26]. Furthermore, concerns have been raised on whether immunosuppressive treatment in patients with psoriasis might negatively affect the course of COVID-19, especially in those with comorbidities [27]. Kridin et al. reported no difference in the risk for COVID-19 and hospitalization and mortality due to COVID-19 in patients with psoriasis treated with IL-17 inhibitors compared to patients who received methotrexate or non-systemic treatments [27]. Yan et al. reported COVID-19 in 33.5% of 173 patients with psoriasis and psoriatic arthritis. Requirement of hospitalization and intensive care stay was detected in 19% and 10% of patients, respectively. Yan et al. suggested that hypertension was associated with severe COVID-19 whereas, treatment with IL-12/23 and IL-23 inhibitors led predisposition to COVID-19. However, methotrexate, apremilast, TNF-α, and IL-17 inhibitor treatment did not affect COVID-19 outcomes [28]. Nevertheless, a meticulous approach is recommended in psoriasis patients treated with biological agents [29]. Within this study, 114 (44.4%) patients had been diagnosed with COVID-19. No association was detected between COVID-19 and age, gender, having an accompanying disease, psoriasis treatment such as biological agents and conventional medications, COVID-19 vaccination status, and vaccine type (p=0.660, p=0.154, p=0.843, p=0.845, p=0.452, p=0.694, respectively). 21 (18.4%) patients stated that COVID-19 exacerbated their psoriasis and the mean time to onset of symptoms was 11.83±9.59 days. Exacerbation of psoriasis after COVID-19 was more frequent in patients who reported exacerbation after COVID-19 vaccines. This result may indicate a common mechanism of action of both COVID-19 and COVID-19 vaccines on psoriasis flare. Hospitalization for COVID-19 was required in 12 (10.5%) patients. No association was detected between the requirement of hospitalization and age, having an accompanying disease, treatment with conventional or biological agents, and vaccination status (p=0.462, p=0.309, p=0.249, p=0.309, respectively).

CONCLUSION

The rate of vaccination against COVID-19 in patients with psoriasis was 92.6%. Besides the high vaccination rate, unvaccinated patients with psoriasis were at a younger age. Most (44.4%) of the patients were vaccinated with the Pfizer-BioNTech COVID-19 vaccine. However, age, psoriasis duration, having an accompanying disease and diabetes affected the preferred vaccine type. 13.4% of the patients stated exacerbation of psoriasis after vaccination which more commonly occurred after the administration of the Pfizer-BioNTech COVID-19 vaccine. COVID-19 exacerbated psoriasis in 18.4% of the patients. Nevertheless, having an accompanying disease and treatment with conventional medications or biological agents were associated neither with COVID-19 nor hospitalization due to COVID-19. Our study is noteworthy in that it included psoriasis patients vaccinated with both Pfizer-BioNTech and Sinovac-CoronaVac COVID-19 vaccines. The results will hopefully contribute to the literature on the effect of COVID-19 and COVID-19 vaccines on patients with psoriasis, which has not been clearly elucidated yet.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.
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Authors' contribution
Conceptualization, F.T., Y.A., A.B.A.; methodology, F.T.; software, F.T.; validation, F.T., Y.A., A.B.A.; formal analysis, F.T.; investigation, F.T., Y.A.; resources, F.T., Y.A., A.B.A.; data curation, F.T., Y.A., A.B.A.; writing—original draft preparation, F.T.; writing—review and editing, F.T., Y.A., A.B.A.; visualization, F.T.; supervision, A.B.A.; project administration, F.T., Y.A., A.B.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2022-700).

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Effectiveness of Pre-procedural Psycho-education on Pain Perception and Anxiety in Women Undergoing Colposcopy

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Abstract: *Cervical cancer is a major cause of morbidity and mortality among women. It is the fourth most commonly diagnosed cancer in females. Screening strategies such as colposcopy can cause anxiety, discomfort, and pain in patients during this procedure and poor quality of life. The aim of this study was to evaluate the effect of psychoeducation on pain perception and anxiety in women undergoing colposcopy. This randomized clinical trial included 60 women, candidates for colposcopy referred to the surgical clinic of Kashan, Iran. The patients were randomly assigned into two groups: the experimental group, which received 60 minutes of psychoeducation, and the control group. Data were collected with the Spielberger anxiety inventory and visual analog score of pain and were analyzed using independent and dependent t-tests, Chi-square, and co-variance performed with SPSS version 16. The means of anxiety scores were significantly decreased post-psychoeducation in the intervention group, and the differences between the two groups were statistically significant in the anxiety scores ($P < 0.0001$). A significant difference was observed between the mean anxiety scores of the intervention group prior to and after the psychoeducational program (PEP) intervention (99.50 ± 5.28 vs. 84.89 ± 8.80) ($P < 0.0001$). No significant changes were observed in the control group pre- and post-psychoeducation ($P> 0.05$). There was no statistically significant difference between the mean pain score between the study and the control group at the end of the study ($P= 0.17$). Psychoeducation resulted in reduced anxiety in women undergoing colposcopy. Therefore, it is recommended to consider this intervention as a suitable and effective complementary, non-invasive, low-cost method to reduce anxiety in these patients.*

Keywords: *anxiety; colposcopy; pain; psychoeducation, woman*

INTRODUCTION

Cervical cancer is a major cause of morbidity and mortality among women. It is the fourth most commonly diagnosed cancer in females [1]. According to global estimates from 2008, there are approximately 530,000 new cases of cervical cancer and it is responsible for 275,000 deaths annually [2]. Screening strategies such as colposcopy have successfully decreased cervical cancer incidence and mortality [3]. Colposcopy is the visual inspection of the cervix under magnification and is one of the first steps in the diagnostic workup of women with abnormal cervical cytology [4]. Like any other medical intervention, colposcopy can cause anxiety, discomfort, and pain in patients during this procedure. High anxiety during colposcopy can lead to a more difficult procedure for the doctor and the patient, as well as a lack of follow-up for the next steps [5].

Studies have reported long-term anxiety in the majority of women after colposcopy [6]. Although in these patients increased anxiety can result in impairment of health-related quality of life [5].

Anxiety is defined as a non-specific, unpleasant feeling of fear and discomfort, associated with a perceived danger, feelings of powerlessness, and prolonged tension in preparation for the expected danger/event [7]. General anxiety caused by the possibility of having cancer can be troublesome for some patients and high levels of anxiety before colposcopy can have adverse consequences, including pain and discomfort during the procedure and a high rate of loss to follow-up various interventions such as group educational counseling, and information leaflets. Listening to music during colposcopy and video colposcopy have been used to reduce anxiety during colposcopy examinations [7-11].

A majority of the studies on PEPs have been conducted on patients with psychiatric disorders while being limitedly implemented in patients with medical disorders [12]. This study aimed to examine the effects of a PEP intervention on anxiety and pain in women undergoing colposcopy.

MATERIALS AND METHODS

This randomized controlled trial was conducted on 60 women candidates for colposcopy who were referred to the surgical clinic of Kashan, Iran, in 2019. According to previous studies, the sample size was determined to be 30 patients in each group. The patients were recruited into the study consecutively. The subjects who met the inclusion criteria were randomly allocated into experimental and control groups through a randomized block sampling method. Blocking is used to ensure that comparison groups will be generated according to a predetermined ratio, 1:1. Blocking be used to ensure a close balance of the numbers in each group for every block of eight participants, for example, four would be allocated to each arm of the trial

The inclusion criteria were the age of 20- 50 years, anxiety scores higher than 30, no history of colposcopy, absence of psychological disorders, and recent use of anti-anxiety drugs. The exclusion criteria were the occurrence of any acute or urgent medical or psychological upheaval, and the patient's decision to leave the study. A written informed consent was obtained from each participant.

Procedure

In addition to routine medical care, the experimental group received PEP intervention, which included discussions and training materials on skills for coping with anxiety and pain, with an emphasis on muscular relaxation techniques, as presented in Box 1. The STAI inventory was assessed before and after the PEP session in the intervention group.

Patients in the control group only received routine medical care, plus a training pamphlet from the patient care and health info (colposcopy) containing general information about the colposcopy. These patients answered the STAI inventory at the beginning of the recruitment and again after studying the pamphlet.

Both the intervention and control group's pain was assessed using the VAS scale after colposcopy.

Box 1: Content of the Psychoeducation session

Timing
5 to 10 minutes
Introducing the participants to each other, presenting the objectives of the procedure, and listening to the participants' feelings and problems.
10 minutes
Providing general information about the pap-smear, colposcopy, and its importance in cervical cancer prevention
5 minutes
Introducing the medical staff and providing information about the way the procedure is done by using educational photos, slides, and videos.
30 to 40 minutes
emotional support, through empathy, congruence, and unconditional positive acceptance and trying to encourage the patients in times of stress and improve their tendency to accept the importance of the procedure to decrease anxiety, Relaxation training and pain reduction techniques
10 minutes
informing the patients about possible side effects of the procedure

Instruments

A three-part instrument was used in this study. The first part contained questions about socio-demographic characteristics (the patient's age, marital status, family history of cancer, and delivery history). The second part of the instrument included a section on sociodemographics (such as educational level, employment status, parity, and smoking), State-Trait Anxiety Inventory (STAI), and VAS.

State-Trait Anxiety Inventory (STAI)

The State-Trait Anxiety Inventory (STAI) measures how a person feels in a certain situation at a certain period. It consists of 40 statements, and subjects are asked to indicate how they felt "at the moment", using a 4-point scale ranging from "not at all" to "very much so". The test is split into the State Anxiety Scale which is considered a subjective and temporary strain and the Trait Anxiety Scale (T-Anxiety) which evaluates relatively stable aspects of anxiety, including general states of calmness, confidence, and security. Total scores for each score obtained from the STAI range from 20 to 80. In this study, we classified the score into three categories: less than 40, low anxiety; between 40 and 60, average anxiety; and greater than 60, high anxiety class [13]. In the study, the reliability of the Persian version of STAI was determined from Cronbach's alpha which was 0.94 for the overall score [14]. The third part of the instrument was the VAS scale.

The VAS is a scale that measures the intensity of acute pain. It is a trustable, valid, and useable asset of measurement of pain in repeated measurements. The VAS is a scale of about 10 cm in length, and it can be utilized in a horizontal or vertical fashion. It starts with the "no pain" and ends with the "unendurable pain" definitions. The patient is asked to point out the choice of his/her perception of the intensity of his/her pain between two endpoints. The distance between the marked point and the outset of the line (0: no pain) is measured in centimeters and the result indicates the intensity of pain in a numeric fashion [15].

Ethical Considerations

Permission for this study was obtained from the ethics committee of the University of Medical Sciences. Ethical issues in this study involved the assurance of confidentiality and the anonymity of the participants. All participants were informed of the purpose and design of the study and understood that their participation was voluntary. Participants signed a written informed consent form before their participation.

Data Analysis

Data analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The normal distribution of the study data was tested with Shapiro-Wilk. The Student's t-test was performed for the analysis of the normally distributed permanent variants. The mean values were expressed using central tendency indicators: mean, median, standard deviation, 95% confidence interval of the mean, and range. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 60 patients participated in the study. Eighty-nine percent of the women in the intervention group and 93% of the women in control group were married. The mean participant age was 39.19 ± 6.28. The majority of patients had a history of childbirth and had children and had a normal vaginal delivery (67.9% in the intervention group and 53.6% in the control group). The mean age in the intervention group was 39.07 years, the majority of whom had a diploma and university education (42.9%), while the mean age in the control group was 39.31 years, and the majority (44.8%) had a secondary and high school education. Twenty-five percent of the intervention group and 34.5 % of the control group reported a history of cancer in family members and friends. There were no significant differences between the intervention and control groups in age, marital status, education, family history, or parity.

The findings of the present study showed that PEP reduces anxiety levels in women undergoing colposcopy. Of the total participants, two in the intervention and one in the control group were excluded from the analysis due to incomplete answers on the post-test questionnaire. In total, the data from 57 patients were analyzed (Figure 1).

Figure 1: Study's data

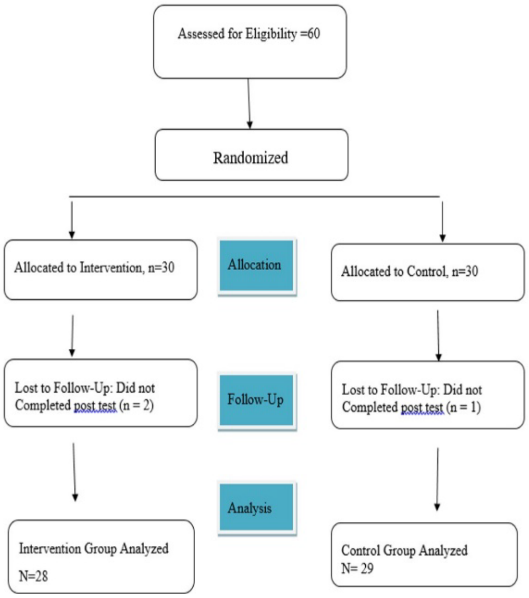
Assessed for Eligibility =60
Randomized
Allocated to Intervention, n=30
Allocation
Allocated to Control, n=30
Lost to Follow-Up: Did not Completed post test (n = 2)
Follow-Up
Lost to Follow-Up: Did not Completed post test (n = 1)
Analysis
Intervention Group Analyzed N=28
Control Group Analyzed N= 29

Table 1: Demographic composition of the two randomly assigned groups

	Control	Intervention
Age*	39.31±6.27	39.07±6.51
Marital status		
Married	27 (93.1)**	25 (89.3)
Divorced/widow	2 (6.9)	3 (10.7)
Education		
Primary	7 (24.1)	8 (26.8)
Secondary	13 (44.8)	8 (26.8)
Graduated	9 (31)	12 (42.9)
Family history		
Yes	10 (34.5)	7 (25)
No	19 (65.5)	21 (75)
Children		
Yes	28 (96.6)	28 (100)
No	1 (3.4)	0 (0)
Delivery		
Yes	28 (96.6)	28 (100)
No	1 (3.4)	0 (0)
Type of delivery		
Vaginal	15 (53.6)	19 (67.9)
Cesarean section	8 (28.6)	5 (17.9)
Both	5 (17.9)	4 (14.3)

** Mean ± Standard Deviation ** No. (%)*

No significant differences were found between the two groups in terms of socio-demographic variables (Table 1).

Analysis of covariance showed the means of overall anxiety and its attributes (state, trait) in the intervention group was significantly less than the control group at the end of the study (P<0.0001). Moreover, the t-dependent test results showed a significant decrease in overall anxiety score post-intervention (P<0.0001) while no significant decrease was seen in anxiety score in the control group at the end of the study (P>0.05). Using the independent t-test no significant differences were observed between the intervention and control groups in terms of the means of VAS score at the end of the study (Tables 2, 3).

Table 2: Comparison of anxiety scores between and within two groups

Anxiety		Intervention group	Control group	p-value*
State	Before	50.86 ± 3.99	49/79 ± 3.56	0.0001
	After	43.78 ± 3.94	48.24 ± 3.58	
	p-value**	0.0001	0.0001	
Trait	Before	48.64 ± 3.58	49.79 ± 2.66	0.0001
	After	41.11 ± 4.05	48.93 ± 3.68	
	p-value**	0.0001	0.3	
Total	Before	99.5 ± 5.28	99.57 ± 5.38	0.0001
	After	84.89 ± 6.37	97.17 ± 5.56	
	p-value**	0.0001	0.11	

Table 3: Comparison of pain score at the end of the study in two groups

	Control group		Intervention group			
	mean	SD	mean	SD	t	P value
Pain	3.60	2.05	2.75	2.36	-1.45	0.15

DISCUSSION

This randomized trial showed that psychoeducation based on providing information and emotional support in a psychotherapeutic atmosphere and relaxation techniques before a colposcopy reduced anxiety among the participants. This finding is consistent with the results of Lungulescu, who studied the effects of PEP intervention on anxiety in women undergoing their initial breast cancer screening mammography [13]. Azogh et al. studied the effect of Psychoeducation on anxiety in subsequent pregnancy following stillbirth [16]. Ozturk et al. studied the effect of psychoeducation on anxiety and pain in patients with mastalgia [15]. Sancak et al have also reported that providing verbal and written information reduces anxiety in patients during third molar extraction [17]. PEPs combine methods of relaxation with psychological training, such as trying to convince patients to accept the importance of the procedure and muscle relaxation and pain relief techniques. Several studies have used single methods, such as anger management or relaxation, and reported positive effects. For instance, a systematic review study conducted confirmed the positive effect of relaxation techniques on reducing anxiety and depression among older adults [18]. Some studies [19, 20] used relaxation and distraction in patients with cardiac disorders and post-cardiac surgery and reported that these methods were effective in reducing patients' anxiety. However, some studies that used PEPs did not find any positive significant effects. This could be attributed to differences in the content and techniques used or to the methods. For example, Howells et al. provided information leaflets before colposcopy and Hartford et al. implemented psychological training methods through telephone contact [10,21]. It appears that PEP is more effective if it is applied in a face-to-face approach.

In general, it seems that PEP can reduce patient's anxiety by providing general information about the screening program, teaching relaxation techniques, emotional support with empathy and unconditional positive acceptance, and trying to encourage the patients in times of stress and improve their tendency to accept the importance of the procedure. It was shown with the results of this study that psychoeducation is not effective in reducing the patient's pain during the colposcopy.

Various studies have been performed on the effect of PEP on pain caused by therapeutic interventions, especially after surgery. For example, Horn et al. studied preemptive and preventive pain psychoeducation and its potential application as a multimodal perioperative pain control option [22]. Ozturk et al. confirmed the positive effects of psychoeducation on anxiety and pain in patients complaining of mastalgia [15]. Jochum et al. studied the effect of psychoeducation and physiotherapy on the reduction of chronic myofascial pain [23]. These studies showed the positive effect of psychological training on pain reduction, which contradicted the results of our study.

It seems that PEP can reduce pain and the need for analgesics after surgery by raising awareness, reducing anxiety, changing attitudes toward pain, and providing information about expected pain and how to deal with it [22,24,25].

The inconsistency of the results of the present study with the previous research may be due to differences in the nature, and severity of pain, the acuity and chronicity, and the nature of disease. Since women who are candidates for colposcopy do not experience pain before colposcopy and the procedure itself does not cause severe pain, this pain is not clinically notable.

CONCLUSION

Based on the results, it seems that PEP is a useful, effective, and economic method that can reduce anxiety. Also, considering the psychological needs of women and the positive effect of this intervention on women's psychological condition, it is recommended that PEP interventions be added to medical and nursing education curricula, and it is also recommended to study the effect of this intervention on other aspects of women's mental health as well as other medical interventions.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization and supervision: Z.V and M.A; Data collection, M.J; Data analysis: F.S.A; Writing-original draft and Writing-review & editing: M.A and F.S. A; Final approval: All authors.

Ethics approval and consent to participate

This study was registered in the Iranian Registry for Clinical Trials (IRCT) with registration code 20130721014086N12.

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REVIEW

Ethical Challenges for Healthcare Providers in War, an Integrative Review

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Abstract: *Background: Healthcare providers should care for everyone without discrimination based on gender, race, religion, ethnic background, and social and economic status. Therefore, considering the importance of ethical challenges in war, this study was conducted with the aim of investigating the ethical challenges of healthcare providers in war. Method: This integrative review study was conducted in the field of ethical challenges for healthcare providers in war. Using the keywords of ethics, challenge, war, and healthcare providers that were selected based on the words of the medical subject heading (Mesh); a targeted search was conducted under the title and abstract in Google Scholar, ProQuest, SID, Web of Science, PubMed, Elsevier, and Scopus from 2000 to November 2022 and in Persian and English languages. Findings: twelve articles of 1160 were reviewed. Findings showed that healthcare providers face ethical challenges in war, including discrimination in care, unprofessional work, cultural and gender care, ethics versus security, and ethical decisions. Conclusion: Recognizing ethical challenges and providing solutions helps to provide better care and save people's lives in war. Developing programs for the preparation of health service providers, using existing experiences, emphasizing religious and humanitarian teachings, and holding periodical missions, can prepare health service providers to face these ethical challenges.*

Keywords: *challenge, ethics, morality, war, healthcare providers*

INTRODUCTION

Patient care is based on ethics [1]. In addition, compliance with the principles of professional ethics is the right of recipients of health care [2]. Crises and natural disasters are exceptional situations that make healthcare providers face many ethical challenges along the way in providing care. The professional competence of health care providers depends on adherence to the principles of professional ethics. The issue of professional ethics becomes more important, especially in difficult situations and natural disasters. On the other hand, healthcare providers, who play the main role in disaster-based relief, when they are in critical situations, face many ethical challenges [3].

Ethical performance is one of the main components in providing comprehensive and high-quality care to patients, especially in critical situations [4]. It is necessary to pay attention to the issues of professional ethics, especially in times of crisis and natural disasters, because the provision of health care in a normal situation is different from a crisis, and it causes more ethical responsibilities [5].

Wartime care is an undeniable reality in current clinical practice. Being at war situation can happen to anyone, including care providers [6]. The review and comparison of various studies regarding the experiences of healthcare workers during the war show that most of the results of these studies indicate the continuous and self-sacrificing efforts of this group in providing medical services to the wounded and injured despite the enormous pain and suffering they endure [7]. Health service providers are obliged to take care of everyone without discrimination based on gender, sexual orientation, race, religion, ethnic background, and social and economic status. This standard is true in all the countries of the world and is expressed in rituals such as the code of ethics for nurses of the International Council of Nurses [8].

According to the declaration of the World Medical Association (WMA) published in 2012, medical ethics in wartime is the same as medical ethics in peacetime, and adherence to medical ethics standards in wartime and peacetime is essential [9]. Since healthcare personnel are also present in the war zone along with military personnel, and they work in different environments such as battlefield field hospitals and air transport and evacuation, they face very important ethical challenges [10]. Studies show that healthcare providers suffer many problems and hardships during the war, including moral challenges, (for example: discrimination in care, care of enemies, and safety) which have many positive and negative effects on them [11]. During the Iran-Iraq war, healthcare providers faced ethical dilemmas caused by challenges such as insufficient personnel and facilities to deal with critically ill patients when referring large numbers of wounded soldiers and insufficient information on the care of victims of chemical attacks [12]. The experience of moral challenges in the war zones of Iraq made health service providers face psychological distress [13].

The Syrian civil war has created an environment of violence and instability in which humanitarian health workers have repeatedly faced ethical challenges that have resulted in ongoing threats to personal safety and lasting psychological trauma. Challenges include disruption of services during an attack, relocation or reconstruction of health facilities, limited access to resources, equipment, or medical supplies, lack of qualified personnel, dealing with armed groups, working with local communities, gaining organizational support for decisions difficult, deciding to stay despite the risks, facing discrimination based on gender and experiencing distress and internal conflict [14]. In the Lebanon war in 2006, ethical challenges were experienced including the protection of employees, the continuation of normal treatment of patients, and the protection of patients by health care providers [15]. In the war in Bosnia and Serbia, the health care providers of The Netherlands, who were stationed in the conflict centers, faced ethical challenges in providing care to the wounded, such as discrimination in care and conflict with military duties [16].

There are certainly cases of terrible or tragic behavior during war that many ordinary citizens, and most military members, do not remember with pride or approval. The My Lai massacre during the US war in Vietnam, prisoner abuse at the Abu Ghraib prison in Iraq, “saturation” or “terror bombing” of German cities by Allied forces toward the end of World War II, the use of nuclear weapons against civilian targets in Japan, the killing or severe mistreatment of prisoners of war by all sides in the Atlantic and Pacific theaters during World War II. The list is not a short one. The preparation of healthcare providers to face moral challenges in unusual conditions and to use appropriate strategies during war is very important [10].

Therefore, due to the importance of ethical challenges in war zones for healthcare providers and Less attention is paid to this issue, in this study, we decided to investigate the ethical challenges of healthcare providers in war.

It should be noted that today we are facing wars in different parts of the world and healthcare providers in the context of these wars are facing many ethical challenges and our information about them is very limited. Since there is an information gap in the healthcare provider's ethical challenges in war, this study was conducted with the aim of investigating the ethical challenges of healthcare providers in war. The question of this study: What are the ethical challenges that healthcare providers face in war?

MATERIALS AND METHODS

Because the methodology of the reviewed studies was different and unsuitable for statistical analysis, an integrated review method was used. This integrated study was carried out with the help of the guidelines provided in Hanna Snyder's article [17]. An integrative review is a method that collects and synthesizes the knowledge generated through the analysis of results extracted from selected studies. This study was carried out in the following steps: Setting the research question. Sampling or selection of studies for review; Introduction of reviewed studies; Analysis of selected studies; Interpretation of results and final report [18].

The research question was: "What ethical challenges do health service providers face in war?" In this regard, the researchers searched native and international databases such as SID.ir, Iranmedex, PubMed, Web of Science, Scopus, and Google Scholar, with the keywords War, Healthcare Providers, Challenge, morality, and ethics in the years 2000 to 2022. For Iranian databases, keywords were searched in Farsi and English. In this way, related articles in this field with quantitative and qualitative approaches are extracted. Gray sources, such as unpublished results, dissertations, and articles published in sources with low credibility, were excluded due to their insufficient credibility (Table 1).

The criteria for entering the study were: relevance and continuity in the subject of study, publication of the article in Persian and English language, and the availability of the full text of the article; The criterion for selecting the article after the search was the relevance to the article context for the question from the study. At first, 1160 articles were extracted, and after evaluation by the research group based on the inclusion criteria, 12 articles were studied and analyzed by removing irrelevant and duplicate articles. Figure 1 describes the PRISMA method in extracting the target articles.

Table 1: Example of the search strategy in the PUBMED database

Query	Sort By	Filters	Search Details	Results
((((health care provider) AND (war)) AND (morality)) AND (ethics)) AND (challenge)	Publication Date	from 2000 - 2022	(("health personnel"[MeSH Terms] OR ("health"[All Fields] AND "personnel"[All Fields]) OR "health personnel"[All Fields] OR ("health"[All Fields] AND "care"[All Fields] AND "provider"[All Fields]) OR "health care provider"[All Fields]) AND ("armed conflicts"[MeSH Terms] OR ("armed"[All Fields] AND "conflicts"[All Fields]) OR "armed conflicts"[All Fields] OR "war"[All Fields]) AND ("morale"[MeSH Terms] OR "morale"[All Fields] OR "morales"[All Fields] OR "moralism"[All Fields] OR "moralities"[All Fields] OR "moralization"[All Fields] OR "moralize"[All Fields] OR "moralized"[All Fields] OR "moralizing"[All Fields] OR "morally"[All Fields] OR "morals"[MeSH Terms] OR "morals"[All Fields] OR "moral"[All Fields] "morality"[All Fields]) AND ("ethic s"[All Fields] OR "ethicality"[All Fields] OR "ethically"[All Fields] OR "ethics"[MeSH Terms] OR "ethics"[All Fields] OR "ethic"[All Fields] OR "ethics"[MeSH Subheading] OR "morals"[MeSH Terms] OR "morals"[All Fields] OR "ethical"[All Fields]) AND ("challenge"[All Fields] OR "challenged"[All Fields] OR "challenges"[All Fields] OR "challenging"[All Fields])) AND (2000:2022[pdat])	34
(((health care provider) AND (war)) AND (ethics)) AND (morality)	Publication Date	from 2000 - 2022	(("health personnel"[MeSH Terms] OR ("health"[All Fields] AND "personnel"[All Fields]) OR "health personnel"[All Fields] OR ("health"[All Fields] AND "care"[All Fields] AND "provider"[All Fields]) OR "health care provider"[All Fields]) AND ("armed conflicts"[MeSH Terms] OR ("armed"[All Fields] AND "conflicts"[All Fields]) OR "armed conflicts"[All Fields] OR "war"[All Fields]) AND ("ethic s"[All Fields] OR "ethicality"[All Fields] OR "ethically"[All Fields] OR "ethics"[MeSH Terms] OR "ethics"[All Fields] OR "ethic"[All Fields] OR "ethics"[MeSH Subheading] OR "morals"[MeSH Terms] OR "morals"[All Fields] OR "ethical"[All Fields]) AND ("morale"[MeSH Terms] OR "morale"[All Fields] OR "morales"[All Fields] OR "moralism"[All Fields] OR "moralities"[All Fields] OR "moralization"[All Fields] OR "moralize"[All Fields] OR "moralized"[All Fields] OR "moralizing"[All Fields] OR "morally"[All Fields] OR "morals"[MeSH Terms] OR "morals"[All Fields] OR "moral"[All Fields] OR "morality"[All Fields])) AND (2000:2022[pdat])	232

Figure 1: PRISMA diagram in extracting articles

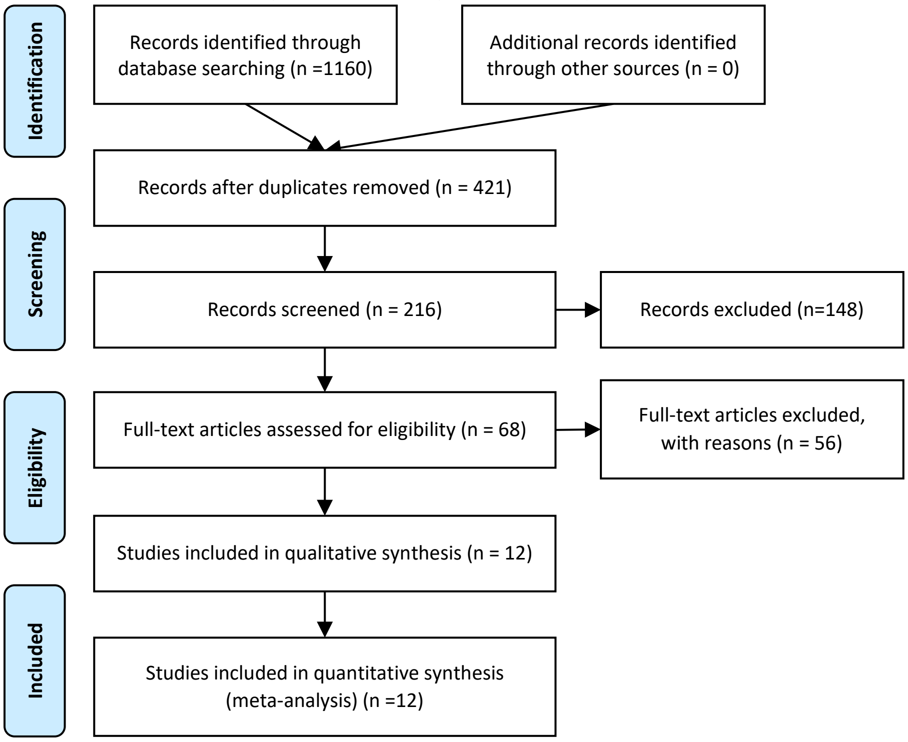
Identification
Records identified through database searching (n =1160)
Additional records identified through other sources (n = 0)
Records after duplicates removed (n = 421)
Screening
Records screened (n = 216)
Records excluded (n=148)
Eligibility
Full-text articles assessed for eligibility (n = 68)
Full-text articles excluded, with reasons (n = 56)
Studies included in qualitative synthesis (n = 12)
Included
Studies included in quantitative synthesis (meta-analysis) (n =12)

RESULTS

From 1160 articles that obtained in the first stage by searching the key words mentioned, after removing the articles not related to the subject of the study and complying with the inclusion and exclusion criteria, finally 12 articles that related to the purpose of the study examined. The findings of this study showed the ethical challenges of health service providers in war zones in terms of: Discrimination in care, Unprofessional work, Cultural and Gender care, Ethics versus Security and Ethical Decisions. A summary and complete description of the reviewed studies showed in the Table 2. This review summary includes authors, year of publication, methodology and results (Table 2).

Table 2: Final articles used in the study

Title	Authors	Journal	Year	Method	Ethical Challenges
Army nursing practice challenges in humanitarian and wartime missions [19]	Janice Agazio	International Journal of Nursing Practice	2010	Descriptive exploratory design	Use and/or disposal of medications while caring for local nationals The dilemma of taking care of coalition forces or local wounded
Making the hard decisions: Ethical care decisions in wartime nursing practice [8]	Janice Agazio, Petra Goodman	Nursing outlook	2017	Secondary analysis	Management of ethical situations in war Use of recognized ethical codes Ethical decision making
Military Nursing Care of Iraqi Patients [13]	COL Petra Goodman, et al	Military Medicine	2013	Focus groups interview	Bias in providing care to Iraqi patients. lack of trust in translators, Discomfort from not being able to guarantee
The challenges and psychological impact of delivering nursing care within a war zone [20]	Alan Finnegan, William Lauder, Hugh McKenna	Nursing outlook	2016	Constructivist grounded theory	The challenge of caring for prisoners of war or the wounded in the army
Lived experiences of military nurses from war - a mini-review [10]	Fateme Teymoury, Zahra Farsi	Journal of Military Medicine	2019	Mini-review	Discrimination in providing care for the injured. Ethical challenges in facing the native culture and cultural differences
Military physicians' ethical experience and professional identity: a Canadian perspective [21]	Christiane Rochon	JRAMC	2018	Miles and Huberman's mixed approach	Ethical challenge in caring for indigenous people in war-torn areas The challenge of using limited facilities in dealing with the injured Safety of injured or employees
Experiences of military nurses in Iraq and Afghanistan [22]	Louisa Jane Kenward. Gary Kenward	Nursing standard	2015	Review	The challenge of choosing the injured due to limited facilities. The challenge of caring for a wounded enemy
Women's Health and Hygiene Experiences During Deployment to the Iraq and Afghanistan Wars, 2003 through 2010 [23]	Mary Ellen Doherty, Elizabeth Scannell-Desch,	Journal of Midwifery & Women's Health	2011	Phenomeno-logical	Providing cultural and gender care Provision of support for female employees and privacy protection Moral protection of employees
Ethical Issues Encountered by Military Nurses During Wartime [24]	Janice Agazio, et al	Annual review of nursing research	2016	Review	Feelings of confusion, uncertainty, frustration, anger, discouragement and anxiety in caring for captives Ethical conflicts in care
Nursing Ethics and the 21st-Century Armed Conflict: The Example of Ciudad Juárez [25]	Kathleen O'Connor	Journal of Transcultural Nursing	2015	Review	Personal safety and time to evacuate a situation Caring for both sides without bias, Witnessing suffering and intervening in torture. Support and defend the rights of others. Fixing conditions that harm others, Helping the disabled and saving people in danger.

'We will never give up': a qualitative study of ethical challenges Syrian health workers face in situations of extreme violence [14]	Namrita S. Singh et al	Disasters	2002	Qualitative	Service disruption during attack. Moving or renovating health centers. Limited access to medical resources, equipment, or supplies. Lack of qualified personnel, dealing with armed groups. Cooperation with local communities. Obtain organizational support for difficult decisions. Decided to stay despite the risks. Facing discrimination based on gender, and experiencing distress and internal conflict.
Legal framework versus moral framework: military physicians and nurses coping with practical and ethical dilemmas [16]	Francesca Baukje Hooft	J R Army Med Corps	2019	Personal view	Discrimination in providing care when facilities are lacking Care in unsafe conditions Taking care of the wounded enemy Facing anger, rage and shame.

DISCUSSION

The purpose of this study was to consolidate the results of studies related to the ethical challenges of healthcare providers in war. Ethical challenges of healthcare providers in war mentioned in the areas of discrimination in care, unprofessional work, cultural and gender care, ethics versus security and ethical decisions.

Discrimination in care

Discrimination, in the non-moralized sense, is ubiquitous. Discrimination is a surprisingly slippery concept, and much of the attention it has received in recent years has focused on constructing a precise definition of the term. At least in part, this may be because the concept appears to cover such a wide variety of disparate cases crossing traditional boundaries in applied ethics and political theory, from distributive justice, medical ethics, and criminal justice to multiculturalism, liberal rights, and feminism [26].

Everyone is entitled to human rights without distinction of any kind, such as nationality, race, religion, age, gender, sexual orientation, economic background, or disability. Even if humanitarian workers are trying to do things well, they usually cannot do everything that is needed. In most situations, humanitarian organizations are faced with rationing problems difficult choices about exactly who they can help and what they can do. With limited resources and typically widespread needs, organizations have to focus. To focus responsibly means finding a need that one's agency is good at meeting—this may be health, food, or protection—and maximizing their programs in some way so that as many people benefit as possible [27].

When supplies are short, who is first in line? Health service providers at war environment always faced by a lack of medical facilities due to the special conditions prevailing in war. Using these limited facilities to save the lives of many people who injured in the war environment is one of the ethical challenges of health service providers. These challenges will be more difficult when caring for enemies wounded, prisoners of war and indigenous people. In addition, the decision to save a person in a critical situation, which may be accompanied by neglecting to save sicker or dying people, is a challenging situation for health service providers [13,19, 20,22].

The evidence indicates that the health service providers faced by a series of moral dilemmas, including discrimination in providing care to patients, and due to political beliefs and culture, they tried to have less contact with the enemy wounded. In this regard, the results of Desch-Scannell and Doherty's study showed that nurses did not want to provide care to the enemy wounded. In addition, the results of Lal and Spence's study showed that due to the limitations of medical equipment, human and technical resources, nurses had to perform differently than the injured and make difficult decisions about providing care to people. Limitation of resources in providing care knew as one of the ethical challenges of health service providers in war [10,14,16,21]. The decision to save the seriously injured for whom there is no hope for a healthy life or the wounded who are at risk of death and their care in war conditions lead to a waste of limited resources is one of the ethical challenges of health service providers [8,14,21].

Discrimination is sometimes the product of what the discriminator takes to be a fundamental distinction between classes of people. For example, placing a lesser value in war on the life of an enemy than on a country's own citizens is typically premised on a belief in a basic distinction between friend and foe, or between citizens and non-citizens. When prisoners captured they should receive treatment as prisoners of war, unless they are known to have committed criminal acts on the battlefield in contravention of the laws of war. They have a right to self-defense against other combatants, unlike terrorists, and their use of deadly force against their enemy is not considered murder [28]. International laws, say they should not, for example, be tortured during questioning. Nor can they be beaten, starved or somehow medically experimented on. They cannot be used as shields between oneself and the opposing side; the understanding is that captured enemy soldiers are to be incarcerated far away from the front lines. Moreover, very basic medical and hygienic treatment is supposed to be offered: things like aspirin, soap, water, and toothbrushes [29].

Thus, caring of prisoners under torture and cooperation of healthcare providers with investigators causes many ethical challenges. Here, hard ethical problems arise when doctors, nurses, and medics are called upon to assist in interrogation or force-feed hunger-striking inmates [30]. The use of medical staff to determine the amount of torture that a person endures, the use of psychological methods to obtain information, keeping captives alive to receive information, and the use of therapeutic tools for torture are among these challenges. In these situations, emotional responses such as anger, fear, resentment, hate and prejudice are in conflict with moral requirements [24,31,32].

Unprofessional work

The quality of humanitarian action is a constant concern in humanitarian ethics. The great majority of humanitarian workers recognize that it is not enough simply to try to help people. One must help them professionally and well. As a result, there has always been a considerable effort put into standard-setting, professional guidelines, training, and competence [27]. One of the ethical challenges of health service providers in the war is the employment of people in non-specialist positions in the conditions of shortage of specialist personnel. Although in many cases this issue has caused more growth and training in health service providers, it faces people with ethical challenges in providing care that they do not have enough skills. For example, health service providers were faced with the challenge of caring for children injured in war that they may harm them during care [19,20, 24]. Furthermore, healthcare providers realized that they have not fully trained to treat a wide range of injured patients, including severe traumas, extensive bleeding and critical care, health problems, care of infants, women, children, and the elderly [13]. Because of staff shortages, participants emphasized the difficulty of taking on new roles for which they not trained. They feel forced to play multiple roles or provide care beyond their skill level. In addition, volunteers who came to hospitals without educational qualifications will cause ethical challenges [14].

Cultural considerations

Today, official declarations abound avowing the indispensability of gender inclusiveness and diversity for military effectiveness in the twenty-first-century operating environment. Today's "militaries rely more and more on women and members of visible minority and Aboriginal groups to fill their ranks, rendering the recruitment, retention, and optimum employment of these members important to the success of the organization from the perspectives of both operations and public accountability. In some units and services, women and members of nonwhite ethnic, non-Judeo-Christian religious, or non-heterosexual minorities have been integrated effectively. However, equal opportunity and diversity policies have not been uniformly successful. Oftentimes, our inability to understand other's culture is a direct cause of needless strife and deprivation. The past decade of armed conflict speaks emphatically to the increased pain and suffering that follow from our violating the cultural norms of countries that have the misfortune of playing host to militaries [30].

Taking care of an injured patient of the opposite gender

The presence of women in military teams as well as their use as health service providers in the war environment is facing issues such as moral challenges. Providing security, meeting the needs of women, maintaining privacy, preventing sexual assaults, and providing health care to injured women can be among the ethical challenges of health service providers in war. Furthermore, not paying attention to local culture in the place of war, especially in relation to women, can cause health service providers to face ethical challenges [23].

Difference in values

Another ethical dilemma in the field of cultural difference between nurses and injured people was the difference in values, especially regarding gender. Some patients believed that women's bodies should not be exposed to men, even if that man is a health service provider [10,13]. Cultural differences identified between health care providers and patients lead to ethical dilemmas. Findings indicated that health care providers had difficulty confronting cultural norms that conflicted with care delivery. Gender issues can lead to differences in care and expectations regarding female patients. Based on the male dominant role, men were the main decision-makers and could dictate the provision or care of female members or children of the family. Cultural customs in terms of social, political and economic factors are effective on ethical challenges when providing care [8,13,24]. Furthermore, women's health service providers face ethical dilemmas while taking care of men who avoid contacts with women [14].

Ethics versus security

One area of humanitarian ethics that is often uppermost in the minds of humanitarian workers in armed conflict is the level of risk they are prepared to accept for themselves and their teams as they try to help others at risk. Humanitarian workers like soldiers, policemen, and firefighters may genuinely expect to suffer and die in pursuit of their duty. Humanitarian workers have often died while doing their jobs—most usually from infectious diseases or traffic accidents. Armed actors of various kinds also deliberately kill them as humanitarian workers. The ethics of personal risk in humanitarian action is not discussed publicly very much in order to maintain the moral and legal taboo on killing humanitarian workers. However, in reality, many humanitarian workers decide to put their lives at risk every day. To do so responsibly means balancing risks against results how important is it to deliver this convoy today? It also means working with a strong duty of care towards one's staff and with their full and informed consent when it comes to engaging in risky operations [27].

In addition, one of the ethical dilemmas of healthcare providers at war environment is maintaining the safety of employees and recognizing when to leave the war environment. Sometimes health care providers have to make a difficult choice between saving someone's life and their own safety and health, because laws regarding the immunity of health care providers in war not always respected. When the order to leave an environment announced, healthcare providers may face with a lack of facilities to evacuate the injured. In this situation, they should act between staying and protecting the injured or leaving the situation according to the order [14,25].

Sometimes leaving a wounded person alone for a few minutes, for example, during the warning of an imminent attack, may endanger his life. In such a situation, the decision to go in to the shelter or stay with the injured will create a significant challenge [15,16]. Facing organizational needs in opposition to the needs of the injured, such as applying pressure from the commanders to provide service to an injured person, or releasing some of the injured, or transferring the injured who are not ready to be transferred, puts healthcare providers in a situation of ethical challenges [21].

The intervention or pressure of armed groups to leave the triage process and accepted clinical practice was one of the ethical challenges of the treatment staff. This included verbal threats, harassment, humiliation, acts of violence, and requests for preferential treatment for some patients, such as members of armed groups or their family members. Obligation to comply with laws contrary to medical requirements, requests for participation in decision-making, and intervention in medical affairs, under threat from the military, are among the ethical challenges of health service providers in war [14].

Ethical decisions

“Working to enhance national security,” writes the BMA, “may not always be compatible with the fundamental tenets of medical ethics.” This is one of the central dilemmas of military medical ethics: To whom do medical practitioners—doctors, nurses, and medics—owe their loyalty? Are they caregivers or soldiers first? Here is a dual-loyalty dilemma in the most quotidian terms: the patient’s interests or those of the military? The mission of the Army Medical Department is to conserve the fighting strength. Combat health support maximizes the system’s ability to maintain presence with the supported soldier, to return injured, sick, and wounded soldiers to duty, and to clear the battlefield of soldiers who cannot return to duty [30].

The most controversial issue was that military medical ethics based more on the expediency of returning soldiers to battle than treating the wounded, meaning that the priority was returning soldiers to duty rather than providing care to the seriously wounded. The moral challenge for many was about returning to war, including soldiers who wanted to return to combating but were judged unsuitable and those who did not want to return but were deemed fit. Health service providers considered this as a great moral responsibility both towards the patients and towards the mission [21,24].

The most troubling ethical issues cited related to conflict with physicians in treatment decisions, working with incompetent or unprofessional colleagues, and staffing patterns. Among the resources that helped nurses in dealing with ethical issues, role models, staff meetings, and physician, supervisor, and peer consultations more used. However, nurses also used professional sources (journal articles and books) and personal sources (church) [24].

CONCLUSION

The findings from this study showed that health service providers face many ethical challenges in providing health services while in war zones. Understanding these challenges in order to provide solutions to face them helps to provide better health services and save people's lives in the battle scene. One of the applications of this study for health service providers is that it informs them about the ethical challenges in the battlefield so that they can make the right decision in the event of war. Decision-making training in critical situations seems essential for healthcare providers. Healthcare providers must have adequate military training to protect themselves and the injured. Developing programs for the preparation of health service providers, using existing experiences, emphasizing religious and humanitarian teachings, and holding training maneuvers can be effective in preparing them to face these ethical challenges. Holding classes to be acquainted with the local culture of the war zone and the values governing the society before deployment is effective in reducing moral problems. Managers should consider providing mental health services to people who provided care in war zones and faced moral challenges.

Conflicts of interest and sources of funding

The authors declare that there is no conflict of interest in the present study.

Authors' contribution

GDM conceived of the presented idea. KhM, VZ contributed to documentation on the topic of interest, work conception and revised the manuscript. GDM, KhM, VZ performed the final revision of the manuscript. All authors discussed the results and contributed to the final manuscript. KhM and VZ gave final approval. All authors have read and agreed to the published version of the manuscript. PH, HS, GDM had equal contributions.

Ethical considerations

In the present study, all ethical considerations, including the condition of trust and honesty, have been taken into consideration. The present study was written in line with the results of the doctoral dissertation in nursing approved by Baqiyatullah University of Medical Sciences with the ethics code IR.MBSU.REC.13990442.

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REVIEW

Current Trends, Challenges for Research, and Therapeutic Perspectives in Nutritional Psychiatry

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Abstract: *Nutritional interventions in the field of psychiatry have been explored more intensively in the last two decades, but definitive conclusions based on the retrieved data in the literature about the efficacy of this therapeutic approach are difficult to formulate. This observation has multiple explanations, e.g., these therapeutic interventions are very heterogenous, cohort studies usually enroll different psychiatric populations or healthy subjects with minor psychiatric symptoms, nutritional supplements or special diets are commonly recommended as add-ons to the pharmacological interventions (thus making it difficult to differentiate the impact of each therapy on the outcomes), placebo-controlled randomized studies are still very few, etc. Therefore, a narrative review focused on the main challenges reported during the research in the field of nutritional psychiatry (NP) may be useful for clinicians interested in strategies to augment the efficacy of pharmacological treatments or to find new solutions with low risk of adverse events in patients with organic comorbidities or multiple concomitant medications. Data about the efficacy and tolerability of nutritional interventions explored for psychiatric disorders were searched in four electronic databases (PubMed, Cochrane, Clarivate/Web of Science, and EMBASE). Omega-3 polyunsaturated fatty acids supplementation, healthy diet interventions, folate and vitamin B12, S-adenosyl-L-methionine, N-acetyl cysteine, vitamin B1 and B6, vitamin D, minerals, psychobiotics, inositol, and herbal products were the main interventions identified, and their demonstrated efficacy was quite heterogenous, while their tolerability and safety were good. Therefore, NP is a domain of research that may benefit from more good-quality and longer-duration trials, in order to verify the effectiveness of such augmentation strategies to the already validated treatments.*

Keywords: *prebiotics, probiotics, synbiotics, vitamin D, folate, B vitamins, S-adenosyl-L-methionine, N-acetyl-cysteine, inositol, micronutrients*

INTRODUCTION

Nutritional psychiatry (NP) explores the impact of nutrient-based interventions on clinical symptoms and behavioral models of mental disorders. The number of published research in this field has continually increased during the past decades [1]. Dietary recommendations and the use of nutraceutical supplements sparked interest for psychiatrists in the management of psychiatric disorders or in the prophylactic strategies targeting the same type of disorders [1]. Nutrition has been considered able to modulate different pathophysiological mechanisms of psychiatric disorders, especially inflammation, oxidative stress, the structure of the gut microbiome, epigenetic influences, and neuroplasticity [2]. Associations between variables related to diet quality and mental health have been supported by epidemiological research, and they do not seem influenced significantly by lifestyle characteristics, demographic factors, or reverse causality [2]. There is accumulating evidence supporting the relationship between diet quality and/or feeding imbalances, on one side, and mental health, on the other [3]. Such evidence already exists for other medical specialties, such as endocrinology, cardiology, and gastroenterology [3]. Nutrient-based supplements and augmenting strategies could be of major interest to psychiatry, if proven efficient and well tolerated, because of their lack of significant pharmacokinetic and pharmacodynamic interactions with the drugs, and due to their more socially-accepted, non-stigmatizing, image as therapeutic interventions in patients with mental disorders.

The founding in the year 2013 of the International Society for Nutritional Psychiatry Research (ISNPR) reflects the importance gained by this domain in the last decade [4]. According to a position statement of this institution, diet is part of an integrative psychiatric model, and evidence-based nutraceuticals should be considered elements of case management in psychiatry [4]. The education of both clinicians and patients about evidence-based nutrition or nutraceuticals is also a key element of the ISNPR position statement [4].

Another domain of intensive exploration for nutritional psychiatry is represented by the connection between gut microbiota (GM) and the pathogenesis of mental disorders. This aspect was explored through the lens of the bidirectional signaling that was observed between the intestinal flora and the brain functioning, i.e., the microbiome-gut-axis [5]. The possibility of changing the composition of GM through probiotics, prebiotics, synbiotics, or fecal material transplantation (FMT) was considered important as an approach to psychiatric disorders [6,7]. Also, the impact of early-life nutrition and maternal diet has been investigated in connection with the onset of psychiatric disorders in offspring [1].

The results of studies investigating the efficacy of nutraceuticals in the treatment of psychiatric disorders are still far from being convincing [1]. Heterogeneity, residual confounding, measurement errors, and difficulties in quantifying and ensuring adherence to a certain diet have been considered challenges for experimental research in NP [8]. A high rate of placebo effect could be one of the factors that mitigate the difference between study groups and hinder the development of nutraceutical-oriented therapies in psychiatry [1]. Therefore, methodological aspects should be considered when interpreting the results of such trials, and the use of biomarkers, e.g., specific nutrient deficiencies, allelic variants, and metabolomic variables, should be encouraged [1]. Also, taking into consideration individual dietary patterns and nutrient necessities is important when constructing a therapeutic strategy for patients with mental disorders from the perspective of NP [1]. However, the effects of nutraceuticals are also actively investigated in different categories of psychiatric disorders and advances have been recorded in the field of mood-modifying and cognitive-improving agents in clinical populations and healthy individuals [9]. Systemic and central vascular function, inflammation, metabolism, and neuroprotection may explain the positive impact of nutraceuticals [9]. A synthesis of the methodological difficulties in finding answers to the main questions in NP is represented in Figure 1.

Figure 1: Challenges identified in the research of nutritional interventions in patients with psychiatric disorders *(SSD= schizophrenia spectrum disorders, MDD= major depressive disorder, GM= gut microbiota)*

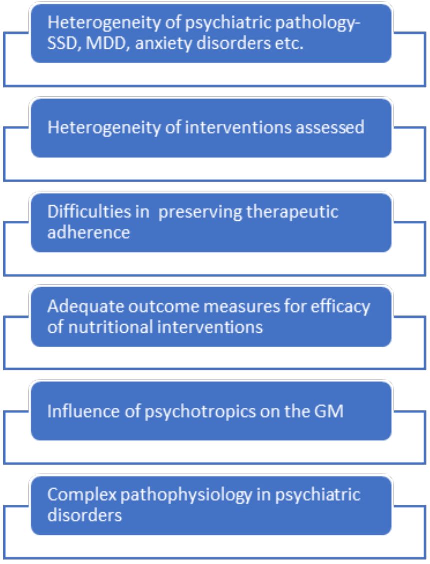
Heterogeneity of psychiatric pathology- SSD, MDD, anxiety disorders etc.
Heterogeneity of interventions assessed
Difficulties in preserving therapeutic adherence
Adequate outcome measures for efficacy of nutritional interventions
Influence of psychotropics on the GM
Complex pathophysiology in psychiatric disorders

Psychotropics may exert different effects on the GM, thus influencing the gut-brain axis [21]. Antipsychotics have been associated with anti-commensal activity, which is difficult to explain since these agents are targeting monoaminergic neurotransmission in the central nervous system (CNS) [21]. According to a complex study that observed the influence of various non-antibiotic drugs on the growth of 40 representative bacterial isolates, which can be found in the human GM, almost all antipsychotic categories present anti-commensal activity [21]. There are suppositions that direct bacterial inhibition of antipsychotics might not be an adverse event, but a potential mechanism of action attributed to these agents [21]. However, the negative effects of this antimicrobial activity have been reported, although the significance of this effect was not yet clearly demonstrated. For example, an alteration of GM by antipsychotics may result in body weight gain and metabolic dysfunctions [22].

Antidepressants, like monoamine oxidase inhibitors (MAOIs), serotonin selective reuptake inhibitors (SSRIs) and tricyclic antidepressants also have antimicrobial properties by different mechanisms, e.g., inhibition of DNA replication, blocking of the bacterial efflux pumps, etc. [23,24]. Dysbiosis induced by this type of pharmacological agents has been considered a possible contributor to the effectiveness but also to the treatment resistance in patients with major depressive disorder (MDD) [23].

OBJECTIVE AND METHODOLOGY

Because the methodology of the reviewed studies was different and unsuitable for statistical analysis, an integrated review method was used. This integrated study was carried out with the help of the guidelines provided in Hanna Snyder's article [17]. An integrative review is a method that collects and synthesizes the knowledge generated through the analysis of results extracted from selected studies. This study was carried out in the following steps: Setting the research question. Sampling or selection of studies for review; Introduction of reviewed studies; Analysis of selected studies; Interpretation of results and final report [18].

SPECIFIC INTERVENTIONS IN NUTRITIONAL PSYCHIATRY

Data supporting the influence of nutrition, as a category of interventions, on mental health are derived from meta-analyses and systematic reviews, based on randomized controlled trials and cohort studies [25]. The main nutritional interventions found during the literature review are represented in Figure 2.

Figure 2: Main nutritional interventions explored for the management of psychiatric disorders *(PUFA= polyunsaturated fatty acids, SAME= S-adenosyl L-methionine)*

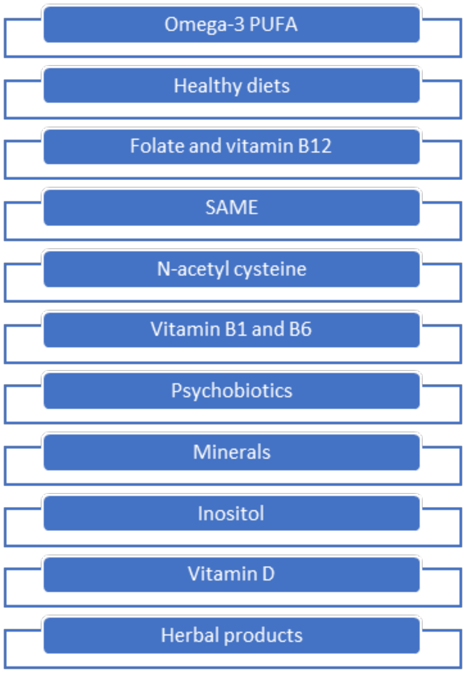

For example, a meta-review of the meta-analyses focused on nutritional interventions in the field of psychiatry (N=10951 individuals) concluded that the strongest evidence supports the use of PUFAs, especially eicosapentaenoic acid (EPA), as an adjunctive treatment for MDD, and the therapeutic potential of these interventions for ADHD; folate supplements, and especially high-dose methyl folate regimens, were associated with positive results in MDD; emergent evidence for N-acetylcysteine as an adjunctive for mood disorders and schizophrenia were reported [26]. All nutritional interventions assessed had good safety profiles, without evidence of serious adverse effects or contraindications in psychiatric disorders [26]. Another review (n=69 trials) reported the statistically significant or clinically important changes observed in the majority of the research conducted (n=48 trials) when nutritional interventions were used as supplementation for psychiatric disorders; however, the heterogeneity of the psychiatric diagnoses, clinical outcomes, and treatments assessed preclude the extraction of definitive conclusions [27].

Omega-3 polyunsaturated fatty acids supplementation

EPA and docosahexaenoic acid (DHA) are the two ω-3 PUFAs explored in the treatment of psychiatric disorders based on their anti-inflammatory role, involvement in preserving neurons' stability, and possible importance for the proper functioning of neurotransmission [28]. The American Psychiatric Association practice guideline for depression recommends ω-3 PUFAs as adjunctive therapy in patients diagnosed with MDD [29].

In a placebo-controlled study, 2.4 g/day ω-3 PUFAs were added to venlafaxine in a group of 72 patients presenting their first episode of depression [30]. After 12 weeks, both groups (active and placebo) had improvements in their clinical status vs. baseline, but the rate of responders for anxiety symptoms was significantly higher in patients who received PUFA at week 4 [30]. These results support a possible faster response in patients who received venlafaxine + PUFA regarding their associated anxiety symptoms, although the final scores did not differentiate between the two groups [30]. Also, childhood trauma and lack of social support have been associated with poor prognosis in patients with first episodes of depression in this study [30].

A double-blind, placebo-controlled trial evaluated the effects of ω-3 PUFAs (3.4 g/day) as monotherapy vs. placebo in pregnant women (N=36) with MDD during eight weeks [31]. Patients in the active intervention group had significantly lower HAMD (Hamilton Depression Rating Scale) scores at week 6 and at the endpoint, higher response and remission rates [31]. Also, the changes in the secondary outcome measures (EPDS- Edinburgh Postnatal Depression Scale and BDI- Beck Depression Inventory) supported the significant efficacy of PUFAs vs. placebo [31]. The tolerability of PUFAs was good, without adverse effects reported by either mothers or newborns [31].

A meta-analysis (n=19 trials, N=2240 participants) supported the existence of a beneficial effect of ω-3 PUFAs vs. control interventions over anxiety symptoms [32]. The subgroup analyses showed that patients with well-defined psychiatric conditions responded better to adding ω-3 PUFAs than individuals without clinical diagnoses [32]. The anxiolytic effect of ω-3 PUFAs was superior to controls in subgroups with a higher dosage (≥2 g/day) vs. a lower-dose regimen [32].

Epidemiological studies reported a correlation between reduced levels of ω-3 PUFAs or low intake of fish products and the increased risk for age-related cognitive decline or AD [33]. Also, increased dietary consumption or blood levels of DHA are protective against AD and other dementia in epidemiological studies, with a possible negative interference from the apoE4 genotype [33].

Possible mechanisms explaining the protective effects of DHA against cognitive decline and AD are the following: some of its metabolites have neuroprotective effects, there is a decrease in the production of arachidonic acid metabolites, the transduction for trophic factors is increased, and the inhibition of signal transduction pathways induced by β-amyloid is more intense [33].

The addition of ω-3 PUFAs in patients with ADHD has been explored starting from the potential benefits of these agents on oxidative stress (which can be increased in this population), inflammation (by competitive inhibition of the ω-6 PUFAs, which are pro-inflammatory), and increasing levels of dopamine and serotonin neurotransmission [34]. Meta-analyses report small to insignificant effect sizes on ADHD behavioral symptoms of ω-3 PUFAs when added to ongoing medication [35-38]. The overall tolerability of ω-3 PUFAs was good, and no severe adverse were reported in these meta-analyses [35-38]. Dyspepsia and transient nosebleeds have been the most frequently reported adverse events [34-38]. In patients with ADHD and developmental disorders or other comorbidities, the add-on of ω-3 PUFAs may lead to superior results when compared to other groups [35].

Schizophrenia has been associated with alterations in lipid metabolism and antipsychotic treatment supplementation with ω-3 PUFAs has been explored as a potential way to improve these patients' prognosis [39]. A recent systematic review (n=26 clinical studies) found that the results of ω-3 PUFAs supplementation may significantly improve positive and negative symptoms during the prodromal phase, ameliorates the negative symptoms during the first episode of psychosis (FEP), and has a favorable, although partial, effect in patients with chronic schizophrenia [39]. The effects of ω-3 PUFAs in this population are modulated by demographic variables (e.g., age), clinical variables (e.g., duration of untreated psychosis), and the status of antioxidant processes in individuals with psychosis [39].

Healthy diet interventions

According to a review dedicated to dietary interventions for the prevention of depression, five recommendations are supported by evidence: "traditional" diets (e.g., Mediterranean, Norwegian, or Japanese diet); increased consumption of fruits, vegetables, legumes, wholegrain cereals, nuts, and seeds; high use of foods rich in ω-3 PUFAs; replacement of unhealthy foods with wholesome, nutritious foods; reduction of processed foods, " fast-foods", sweets, etc. [40]. Low-carbohydrate high-fat diets may be useful in improving the outcome of anxiety disorders and ADHD [28,41]. This type of ketogenic diet has anti-inflammatory effects due to the lower production of reactive oxygen species and free radicals [28].

A multicenter, randomized study focused on preventing cardiovascular disease evaluated the effects of the Mediterranean diet supplemented with nuts in community-dwelling men (age 55-80 years) and women (age 60-80 years) at high risk of cardiovascular disease vs. the control group (low-fat diet) [42]. A number of 224 new cases of depression were identified at follow-up (after three years), and an inverse association with depression was reported for patients with the Mediterranean diet vs. the control group, but this was not significant [42]. When only patients with type 2 diabetes mellitus were included in the analysis, the magnitude of the effect determined by the dietary intervention did reach statistical significance [42].

Dietary interventions as adjuvants to the treatment of moderate to severe MDD were evaluated by comparison with social support group, in a single-blind, randomized controlled trial for 12 weeks, with 67 participants [43]. The nutritional intervention consisted of personalized dietary advice and counseling conducted by a clinical dietician who respected the "ModiMedDiet" program [43]. Whole grains, vegetables, fruit, legumes, low-fat and unsweetened dairy foods, raw and unsalted nuts, fish, lean red meats, chicken, eggs, and olive oil were the core ingredients of this dietary intervention (18% protein, 40% fat 37% carbohydrates, 2% alcohol, and fiber/other 3% of the total energy) [43]. After 12 weeks, the results of MADRS supported the superiority of the active intervention vs. social support (control) at a significant level [43]. Remission (MADRS score <10) was reported in 32.2% of the active intervention group vs. only 8% of the control group, a statistically significant difference [43].

In a prospective, randomized trial, adults (N=152) who had depressive symptoms received MedDiet cooking workshops and food hampers for 12 weeks, plus fish oil supplements for six months vs. participation in social groups for three months [44]. The MedDiet group used more vegetables, fruit, nuts, legumes, whole grains, and vegetable diversity, less unhealthy snacks and red meat/chicken [44]. At the endpoint, the MedDiet group presented a lower severity of depression and improved quality of life scores; at six months improved diet and mental health were sustained in this group [44]. Reduced depression correlated with increased MedDiet score, use of nuts and vegetable diversity [44].

The relationship between adherence to the Mediterranean dietary pattern and the prevalence of psychological disorders in adults was investigated in a cross-sectional study (N=3172 participants), using the Hospital Anxiety and Depression Scale (HADS) and General Health Questionnaire (GHQ) as outcome measures for mental health [45]. A high intake of fruits and vegetables was associated with a lower risk of depression, anxiety, and psychological stress [45]. On the contrary, a high intake of grains was more frequently detected in individuals who presented depression, anxiety, and psychological distress [45].

A cross-sectional study (N=5371 individuals from the general population) reported a significant correlation between a healthy diet (vegetables, salads, fruits, rice, pasta, cereals, fish, wine, non-processed meats) quality score (based on Food Frequency Questionnaire, FFQ) and a lower risk for depression and anxiety in women and a similarly lower risk for depression in men (evaluated in HADS) [46]. A traditional Norwegian diet (fish, shellfish, potatoes, fruits, vegetables, butter, margarine, milk and yogurt, bread, pasta, rice, meat spreads, legumes, and eggs) was associated with a reduced risk of depression in women and anxiety in men, while a western-type diet (meat, liver, processed meats, pizza, salty snacks, chocolates, sugar and sweets, soft drinks, margarine, mayonnaise, French fries, beer, coffee, cake, and ice cream) correlated with higher anxiety in men and women [46].

Also, the “healthy Japanese” diet (high intake of vegetables, mushrooms, seaweed, soybean products, potatoes, fish, shellfish and fruits) was inversely correlated with depressive symptoms severity during an exposure-response association study 47]. It is hypothesized that this type of diet exerts its beneficial effect (as opposed to “sweets-fat” and “ seafood-alcohol” patterns) through an anti-inflammatory diathesis, which might act as a factor for decreasing the risk for mood and anxiety disorders [47].

Folate and vitamin B12

Preclinic evidence to support the direct influence of folate and cobalamin on CNS functioning has been gathered, and the relationship between the deficits of these substances and the onset or worsening of different psychiatric disorders has also been explored with convincing results [48]. Folate deficiency has been associated with neurocognitive disorders and MDD, while the cobalamin deficit can be connected to psychosis [48]. Both folate and vitamin B12 are essential for the process of homocysteine methylation and for the synthesis of S-adenosyl-L-methionine (SAMe) [49]. A defect in the methylation process has been hypothesized as the key to the pathophysiological basis of many neuropsychiatric disorders [49]. The toxic effects of homocysteine can also be part of the negative effects of folate and vitamin B12 deficiency [49]. The causal relationship is, however, still unclear, and multiple variables are modulating the effects of vitamin B12 deficiency [48]. Also, it is important to note that normal hematological indices do not automatically exclude vitamin deficiencies [48].

According to a meta-analysis (n=43 studies) that explored the relationship between levels of folate and depression, a small but statistically significant effect size was found (low levels of folate correlated with a higher risk of depression) [50]. This meta-analysis had a high heterogeneity (over 84%), which could not be explained by the folate measurement methods or study population [50]. Regarding the relationship between cognition and vitamin B12 deficiency, the context of its investigation is more complex. According to a meta-analysis (n=21 observational studies, adults ≥45 years old), higher levels of vitamin B12 and folate were associated with better cognitive functioning in cross-sectional research, but not in prospective studies [51]. Yet, another meta-analysis (n=95 studies, N=46175 participants) concluded that B vitamins have a positive impact on cognitive functioning (assessed by Mini-Mental State Examination, MMSE) [52]. B vitamins can slow cognitive decline in prospective studies, especially on long-term administration (over 12 months) [52]. In individuals without dementia, B vitamin supplementation slowed cognitive decline vs. placebo, but this effect was not observed in patients with neurocognitive disorders [52]. Lower folate levels, but not lower B12 or B6 vitamins level, and higher homocysteine levels were significantly correlated with a higher risk of dementia [52]. Also, a higher intake of dietary folate, but not of B12 or B6, was associated with a lower risk for dementia in the old age population [52].

Adjunctive folate for psychiatric disorders can be an efficient intervention, but the results of good-quality clinical trials differ in terms of results. According to a systematic review (n=14 studies), adjunctive folate did not improve the total psychopathology, positive, negative, or general symptoms more than placebo in patients with schizophrenia, but it was superior in patients with bipolar or unipolar depression, in terms of decreasing the severity of the depressive symptoms [53]. Also, in patients with bipolar disorder, adding folate to the conventional treatment of acute mania had a favorable impact in terms of efficacy [53]. The overall tolerability of folate was good, in terms of discontinuation due to adverse events [53].

Another review (n=23 studies) concluded that oral levomefolic acid or 5-methyl folate was the most frequent nutritional factor associated with improvements in clinical status in patients with psychiatric disorders, mainly MDD, including postpartum and post-menopausal depression, schizophrenia, autism spectrum disorders (ASD), ADHD, and bipolar disorder, with a variable magnitude of effect [54]. The tolerability of folate supplements was reported as good [54].

Vitamin B1 and B6

Thiamine (vitamin B1) is a cofactor for many enzymes involved in energy metabolism, biosynthesis of nucleic acids, and anti-oxidative stress protective mechanisms [55]. Because of the importance this vitamin has in ATP mitochondrial production, thiamine deficiency can be associated with neurologic, cardiovascular, and psychiatric dysfunctions manifested as confusion, memory deficits, sleep problems, encephalopathy, ataxia, or congestive heart failure, but it can also lead to death, in severe cases [55]. Wernicke encephalopathy is the result of an acute thiamine deficiency and usually appears as a consequence of alcohol abuse [55]. This disease has as its main clinical features ataxia, loss of coordination, memory deficits, confusion, and ophthalmoplegia, and it could result in death in 17% of the cases, in the absence of appropriate treatment [55]. Korsakoff's syndrome may result from a thiamine deficit and has a chronic evolution, with short-term memory deficiencies and hallucinations [55]. Also, depression in adults has been associated with low levels of thiamine, and more severe mood symptoms have been correlated with lower levels of thiamine in the erythrocytes [56].

Pyridoxine (vitamin B6) is part of the synthesis processes of certain neurotransmitters and it has been invoked as a potential contributor to the pathogenesis of AD, ASD, ADHD, anxiety disorders, depression, and psychosis [57]. Patients with mood disorders had low pyridoxine and riboflavin levels in a study with 172 participants [58].

A cross-sectional study evaluated the correlation between dietary intake of vitamin B6 and the risk of depression and anxiety in 3362 adults [59]. The supplementation of the ongoing antipsychotic treatment with vitamin B6 (up to 400 mg/day) in a 9-week, double-blind, placebo-controlled, cross-over study in 15 patients with SSD did not lead to differences between active and placebo groups, according to the Positive and Negative Syndrome Scale (PANSS) [57]. However, a subgroup of patients with schizophrenia and comorbid minor depression may benefit from pyridoxine adjuvant treatment, as reported during an open-label study (N=9 patients) [60].

Vitamin D

A favorable impact of vitamin D was suggested for the preservation of mental health because this substance may interfere with complex homeostatic, trophic, and immunomodulatory mechanisms that can modulate different processes in the CNS [61]. A systematic review of the studies (n=14) which included various doses of vitamin D supplementation explored the effects of this intervention on mental status in healthy adults [62]. The majority of studies do not support a favorable impact of vitamin D supplementation, but data on depression outcomes were conflicting [62]. Vitamin D combined with physical activity could be more efficient than vitamin D in these patients, and there is evidence to recommend vitamin D from food sources instead of vitamin supplementation [62].

Another review (n=61 articles) concluded that a relationship between vitamin D and depression is possible, but its causality is uncertain [61]. A more important effect of this supplementation was observed in patients with MDD and vitamin D deficiency [61]. Serum vitamin D levels inversely correlate with the clinical severity of depression, but the results of clinical trials are not consistent enough to recommend a regular supplementation of the diet with this vitamin in patients with MDD [61]. A meta-analysis demonstrated that vitamin D supplements administered in patients with psychiatric disorders (N=9 trials) can reduce the BDI scores, improves the quality of sleep on Pittsburgh Sleep Quality Index (PSQI), and increase the glutathione and total antioxidant capacity while decreasing the level of C-reactive protein [63].

Neonatal vitamin D deficiency is correlated with an increased risk of schizophrenia, and preclinical studies support the alteration of brain development by vitamin D deficiency leading to subtle neurochemical and cognitive changes that may represent vulnerability factors for schizophrenia [64]. Also, patients with schizophrenia or even first-episode of psychosis may have an increased risk of vitamin D deficiency vs. healthy controls [64].

A cohort study (N=152 participants with various psychiatric diagnostics) assessed the correlation between the Brief Psychiatric Rating Scale (BPRS) and the plasma levels of 25-HO-vitamin D and parathyroid hormone (PTH), as well as the calcium serum levels [65].

The study concluded that a negative relationship may exist between serum levels of 25-OH-vitamin D and BPRS total and items scores, and inpatients had lower PTH and 25-OH-vitamin D levels [65]. This trend toward vitamin D deficiency in hospitalized patients was not dependent on the specific diagnosis or diagnosis category [65].

S-adenosyl-L-methionine

SAMe is a compound with essential roles in multiple biochemical reactions, which impact the production of molecules involved in the regulation of monoaminergic transmission [66]. Therefore, SAMe can modulate the pathophysiological mechanisms of MDD, and there are studies suggesting the antidepressant efficacy of this compound [66]. SAMe in monotherapy has been proven superior to placebo and tricyclic antidepressants, while other studies demonstrated its efficacy in patients with SSRIs- and serotonin and norepinephrine reuptake inhibitors (SNRIs)-non-responsive MDD [66]. These data are derived from open-label trials and placebo-controlled randomized trials, which investigated the effects of oral and parenteral SAMe, in doses varying from 200 mg/day (i.v.) to 1600 mg/day (p.o), and durations of treatment between 2 and 21 weeks [66-69].

According to a meta-analysis (n=13 uncontrolled trials and 25 controlled studies), the response to SAMe was superior to the placebo, and the global effect size ranged between 17% and 38% [70]. The antidepressant effect was comparable to that of tricyclic antidepressants in this meta-analysis [70].

The results of a Cochrane systematic review (n=8 studies comparing SAMe with placebo, imipramine, desipramine, or escitalopram, N=934 adults with MDD) showed that there are no differences in the depressive symptoms severity scores change between endpoint and baseline in patients treated with SAMe or placebo as monotherapy [71]. Low evidence quality supported a favorable effect of SAMe similar to imipramine when administered as monotherapy [71]. There was low-quality evidence that SAMe was superior to placebo as an add-on to SSRIs in the change of depressive symptoms severity from baseline to endpoint [71].

N-acetyl cysteine

This compound influences CNS activity through multiple mechanisms, i.e., it contributes to the production of glutathione and oxidative stress, and dysfunctions at this level have been frequently reported in many psychiatric disorders; also, it attenuates dopamine and glutamate dysfunctions in vitro, and it may be a useful adjuvant in the modulation of inflammatory pathways [72-74]. N-acetyl cysteine (NAC) is the acetylated form of cysteine and it is able to cross the blood-brain barrier [72]. The preclinical evidence in animal models of schizophrenia, bipolar disorder, MDD, anxiety disorders, and obsessive-compulsive disorders (OCD) spectrum disorders support the potential benefits of NAC administration [75].

Clinical evidence supports the use of NAC as an add-on treatment for patients with schizophrenia, with a favorable effect being reported on total and negative symptoms of psychosis [72]. A positive impact of NAC was observed in decreasing the craving and risk of relapse in patients with substance use disorders (SUDs), but proof of a clear effect of NAC on OCD spectrum disorders, ASD, and mood disorders is still lacking [72]. Orally administered NAC is considered safe and no severe tolerability problems have been detected in the clinical trials when recommended dosage (2000-2400 mg/day) was used [72].

In 24 patients with cannabis use disorders (CUD), NAC 1200 mg BID was administered for four weeks in an open-label design [76]. The treatment was well tolerated and had a favorable impact on the addictive pathology, decreasing the self-reported measures of marijuana use and craving [76]. The results of a mixed-models study (preclinical and clinical) indicated that NAC reduced the number of cigarettes smoked in patients with nicotine use disorder (NUD) [77]. In a group of treatment-refractory OCD patients (N=48), NAC (up to 2400 mg/day) was compared to a placebo for 12 weeks, and the results supported the efficacy of the active intervention, according to the Yale-Brown Obsessive Compulsive Scale (Y-BOCS); 52.6% of the NAC-receiving patients were full responders at the endpoint vs. only 15% in the placebo group [78]. NAC (1200-2400 mg/day) was also evaluated in a group of 50 patients with trichotillomania during a placebo-controlled randomized trial with a 12 weeks duration; NAC reduced significantly more than placebo the Massachusetts General Hospital Hair Pulling Scale (MGHPS) and the Psychiatric Institute Trichotillomania Scale (PITS) scores; 56% of the patients on NAC were “much” or “very much improved” vs. 16% on placebo [79]. A randomized, double-blind trial enrolled 66 participants diagnosed with excoriation (skin-picking) disorder who received NAC (1200-3000 mg/day) or placebo for 12 weeks; NAC was associated with significant improvements on the modified Y-BOCS vs. placebo at the endpoint; the overall tolerability of NAC was good [80].

Most studies evaluating NAC were underpowered and of short duration, which are important methodological limitations [75]. Therefore, although NAC demonstrated positive effects on animal models of psychiatric disorders, the clinical efficacy of this compound has not been fully explored [81].

Psychobiotics

Prebiotics, probiotics, and synbiotics have been explored in the treatment of psychiatric disorders based on their presumed properties of gut microbiota (GM) modulation. Potential mechanisms underlying their therapeutic effect in psychiatric disorders are very complex and include the participation of the immune system, vagus nerve, various neurotransmitters, and metabolic or endocrine factors [82].

Probiotics are defined as living organisms without pathogenic properties that can produce beneficial effects in hosts, and in this category are included a large variety of lactobacilli, bifidobacteria, strains of Escherichia coli, certain enterococci, Saccharomyces boulardii, etc. [83]. The probiotic products available on the market are usually containing a combination of strains, which makes quite challenging the interpretation of results in clinical trials exploring this intervention (i.e., high heterogeneity of the active product) [83]. Prebiotics are mainly fibers, non-digestible ingredients of the diet, that may have a positive impact on the host's health through different mechanisms, e.g., selective enhancement of the activity and/or growth of some GM genera [83]. Prebiotics are, for example, inulin and pectin, which have been associated with increased bioavailability and uptake of minerals, lower risk of cardiovascular disease, and promoted satiety and weight loss [83]. Synbiotics represent mixtures of pre and probiotics, which presumably improve the survival and implantation of live microbial dietary supplements in the gastrointestinal tract [83].

A meta-analysis (n=34 trials) concluded that prebiotics did not differ from placebo for depression or anxiety, while probiotics had small but significant effects for depression and anxiety [84]. Larger effects were reported for probiotics in clinical samples of depressed patients, than in community samples, but the pooled effects were low, due to a paucity of trials focused on mental health status-related outcomes [84]. According to another systematic review (n=54 randomized placebo-controlled trials) and meta-analysis (n=30 trials), the impact of most probiotics on mood, anxiety, depression, and psychiatric distress was not relevant vs. placebo at a qualitative level, but only at a quantitative level [85]. The same source concluded that depression severity and psychiatric distress registered but minor improvements in the probiotic group [85].

A systematic review (n=11 randomized controlled trials) showed that probiotics can be beneficial in MDD and AD, with potentially favorable effects in mania, by reducing the rehospitalization rate in these patients [86]. In the case of ASD, the results of the trials were mixed, but early-in-life administration of probiotics may have a preventive role [86]. The tolerability was good in most of the reviewed studies [86].

A randomized, double-blind, placebo-controlled trial enrolled 48 healthy adults with a body mass index (BMI) > 25 kg/m2 and investigated the impact of 21 g oligofructose/day or placebo (maltodextrin) for 12 weeks on food intake and related variables [87]. Body weight decreased by 0.45+/-0.31 kg during three months, and the self-reported caloric intake was correlated with a lower area under the curve (AUC) for ghrelin and a higher AUC for peptide YY in the oligofructose-receiving group [87]. Insulin concentrations mirrored the evolution of glucose, which decreased in the oligofructose group and increased in the control group at the endpoint vs. baseline [87]. The overall tolerability of oligofructose was good [87].

Another double-blind, randomized clinical trial explored the effect of supplementation with synbiotics and probiotics on mental health, quality of life, and anemia in patients undergoing hemodialysis for 12 weeks, and the results showed a significant decrease in BDI and Beck Anxiety Inventory (BAI) scores vs. placebo [88]. Health-related quality of life was not significantly improved by probiotics/synbiotics vs. placebo [88].

Minerals: Zinc, magnesium, copper, selenium, and iron

Micronutrients have been linked with the status of mental health and potential neuroprotective effects [89]. Selenium is important for the construction of selenoproteins, which are involved in the protection against oxidative stress in the CNS, and low selenium levels have been associated with cognitive loss and neurotransmitter turnover rate changes [89]. Zinc is involved in numerous cellular processes, potentially significant for the pathophysiology of depression and neurocognitive disorders; probable antidepressant effects of zinc may be mediated by its glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonistic properties [89]. Copper homeostasis is important, and an excessive accumulation of this micronutrient leads to Wilson disease, which includes depressive and anxiety manifestations; copper could be involved in the neuroplasticity mediated by BDNF (brain-derived neurotrophic factor) and NGF (nerve growth factor); immune function could be affected by copper imbalances, and may influence the pathogenesis of depression [89]. Magnesium participates in the enhancement of DNA replication, transcription and translation, and it is a coenzyme or activator for many enzymatic systems; also, it is an NMDA glutamate receptor antagonist and is involved in memory functioning [89].

Micronutrient supplementation in healthy individuals has a limited favorable impact on mood and anxiety manifestations, but minor general improvements may still be reported [90]. In adults with physical or psychiatric conditions, the effect can be more favorable, although this consideration is limited by small samples and nutrient heterogeneity in the administered products [90]. Broad-spectrum nutrient products are preferable to a few preselected micronutrients [90].

A study exploring the long-term safety of micronutrients (N=17, average length of monitoring= 2.66 years) reported good preliminary tolerability and long-term safety [91]. The overall evaluation concluded that substantial psychiatric benefits outweigh the minimal risks reported in these patients

[91]. However, screening for potential medical diseases is recommended before the initiation of treatment with micronutrients, and long-term monitoring is also considered useful [91].

There are preliminary data about the potential benefits of micronutrients in autism, depressed mood, stress-related conditions, and elderly populations, but less significant benefits in individuals presenting with bipolar disorder, ADHD, and SUDs [92].

Inositol

This is a simple precursor included in the phosphatidylinositol cycle, a secondary messenger system important for CNS activity [93,94]. In patients with MDD, a low level of inositol in the cerebrospinal fluid (CSF) has been reported [93]. This compound was investigated in patients diagnosed with MDD, panic disorder, and OCD with positive results, while in cases of SSD, AD, ADHD, and ASD the results were negative [93]. In preclinical models, chronic administration of inositol reduced anxiety-like and depression-like behaviors in rats but did not lead to significant effects on amphetamine-induced hyperactivity, apomorphine-induced stereotypy, or on memory functioning in monkeys [94].

A meta-analysis of the trials focused on the effects of inositol in patients with depression and anxiety disorders (n=11 and 4 reports, respectively, reaching a total of 242 and 70 participants) did not detect statistically significant results vs. placebo on depressive, anxiety, and obsessive-compulsive symptoms [95]. The discontinuation rate reported in these trials was not significantly influenced by inositol, either [95]. The percentage of responders was marginally higher in the patients with depression treated with inositol vs. placebo, and patients with premenstrual dysphoric disorder had a trend towards a better evolution if they received inositol [95]. Gastrointestinal discomfort was reported marginally more frequently in patients treated with inositol compared to placebo [95].

Herbal products

St. John’s wort, kava, Ginkgo biloba extract, and valerian are the most explored herbal supplements in patients with psychiatric disorders [96], but their efficacy is controversial due to many potential confounders: lack of adequate, good quality trials; the complexity of the standardized products investigated; difficulties in differentiating the effects of a herbal add-on from the effect of the concomitantly administered psychotropics, etc.

According to a review (n=9 controlled trials), while several studies reported herbs’ superiority vs. placebo, others did not find differences between herbs and antidepressants in terms of effectiveness [96]. Kava has been associated with anxiolytic properties in studies with short duration; clinically significant improvements were detected during Ginkgo biloba administration in domains like memory impairments, concentration, fatigue, anxiety, and depressed mood; valerian may decrease sleep latency and nocturnal awakenings, but the effect was not consistently reported across studies as superior to placebo; St. John’s wort was associated with positive results when administered for patients with depressive symptoms, but there are several negative studies, also [96].

Another review of studies exploring 27 herbal medicines and formulas in the treatment of psychiatric disorders reported that kava and St. John’s wort benefit from good quality evidence in the treatment of anxiety and depression, respectively; other phytotherapies (saffron, passionflower, etc.) are not, at least for now, supported by enough data derived from well-designed trials [97].

Continuous research on the efficacy and safety of herbal products is necessary [98]. Although this type of therapeutic intervention has been increasingly explored and is an attractive alternative due to its potential superior tolerability and acceptability for patients with psychiatric disorders, clinicians must be aware of the limitations associated with the generally low-quality evidence on which such products are used [98].

DISCUSSION

The interest of clinicians and researchers in NP is increasing, and a continuously growing body of literature is published regarding the potential benefits of different nutritional interventions in patients with psychiatric disorders. This may be a consequence of the inherent limits of the pharmacological and psychotherapeutic interventions in this population, reflected in the high rate of non-response or partial response to conventional approaches. For example, more than one-third of all patients diagnosed with MDD have treatment resistance [99-101], and almost a quarter of individuals with FEP will become treatment-resistant in their early stages [102,103].

Omega-3 PUFAs were explored in clinical and epidemiological studies, meta-analyses, and systematic reviews, and the available evidence supports a potential benefit of this intervention in patients with MDD, ADHD, FEP, anxiety disorders, and AD [30,31,33-39]. The main positive effect involved in the PUFAs' favorable consequences, when used as an add-on to current psychiatric treatment, was supposed to be their influence on oxidative stress and lipid metabolism [34,39]. However, the number of reviewed sources was small, and the duration of the studies was too short to allow for definitive conclusions.

Healthy diets, especially Mediterranean, Norwegian, and Japanese, were explored in prospective clinical trials and literature reviews, and the main mechanism of action supposed for these interventions was an antiinflammatory activity [8,40-47]. The results of these interventions were positive in lowering the severity of MDD, ADHD, anxiety symptoms, depressive symptoms, and psychological stress, but also in increasing the quality of life in patients with depression [40-47]. The methodology of these sources was of moderate and high quality, but the high degree of heterogeneity of these diets prevents the extraction of clearly-cut recommendations.

Vitamins added to the diet as a therapeutic strategy (vitamins B1, B6, B12, D, and folate) were also explored in clinical and preclinical studies, reviews, and meta-analyses, with mixed results [48-53,55-64]. Except for some well-established recommendations, e.g., vitamin B1 for Wernicke encephalopathy and Korsakoff syndrome, other psychiatric disorders, like MDD, bipolar disorder, ASD, anxiety disorders, and SSD, are still explored as potential targets for vitamin therapy. The potential beneficial effect of B vitamins in slowing cognitive decline is also a subject of interest for researchers [52]. The main mechanism of action of vitamin therapy is patients with psychiatric disorders is supposed to be this intervention's positive effects on multiple metabolic processes within the CNS.

Preclinical studies, clinical trials, reviews, and meta-analyses regarding the effects of SAMe and NAC were analyzed [66-81]. While SAMe benefits from good-quality evidence to be recommended in patients with MDD, due to its efficacy comparable to that of certain antidepressants, the main recommendation for NAC regards the patients with SUD (especially NUD and CUD) [66,70,71,76-78]. The importance of NAC and SAMe on the normal function of neurotransmission has been supported by preclinical models [66,81], but the positive results obtained on preclinical models for a diversity of psychiatric disorders were not yet confirmed in clinical trials [75].

Psychobiotics interfere with the microbiome-gut-brain axis and data about prebiotics and synbiotics are encouraging in MDD, ASD, and anxiety disorders, but not so much for AD, according to the primary and secondary sources reviewed [84-88]. Micronutrients lead to minor general improvements in mood and anxiety symptoms, and preliminary data about their usefulness in ASD, stress-related conditions, and elderly populations also exist [90,92]. The number of reviewed sources for this intervention was low; therefore it could be expected that further research may change its level of recommendation. Inositol was associated with good results in MDD, panic disorder, and OCD, and negative results in SSD, AD, ADHD, and ASD, according to limited sources [93-95]. Herbal products are largely used nowadays, but the evidence to support their evidence is scarce; according to the reviews analyzed here, kava may be useful for the treatment of anxiety symptoms (on short duration) and St.John's wort for depressive symptoms [96,97]. It is important to note that NP interventions have been associated with good tolerability, and no significant adverse events were reported in the reviewed reports.

These conclusions about mixed results of NP interventions support the results of other available secondary reports. For example, a meta-analysis concluded that the response to treatment in the case of adding ω-3 PUFAs and zinc in patients with MDD and antidepressant treatment was similar [103]. Folic acid was not supported by studies in terms of efficacy; the preliminary data for inositol was negative, and the evidence for vitamin D was considered scarce [104]. Mixed results also were reported for vitamin B12 and SAMe [104]. Another review of 356 sources concluded that the highest therapeutic potential in the prevention or treatment of psychiatric disorders can be attributed to the rational diet, physical activity, use of psychobiotics, and consumption of antioxidants [11].

There are a large number of ongoing studies that approach the subject of psychobiotics augmentation in patients with psychiatric disorders. For example, the augmentation of antipsychotic treatment with probiotics (bifidobacteria, lactobacillus, and maltodextrin) or prebiotics (inulin + maltodextrin) in patients with schizophrenia is intended to be explored in a 26-week prospective, randomized, controlled multicenter trial [108]. Dietary supplements (microbiotherapy, GynMDD) are explored as add-ons to venlafaxine in patients with MDD in a 12-week randomized trial, after failure of a first-line antidepressant [109]. The exploration of a potentially favorable impact of prebiotics (Akkermanisa muciniphila, through enteric-coated orally-administered acetate) on the most problematic side effects of psychotropics, i.e., weight gain and metabolic syndrome, is the objective of a 5-month trial, which is expected to enroll 16-25-year old patients undergoing psychiatric treatment [110]. In children, the effects of probiotics, more specifically a combination of BB-12 and LGG (Lacticaseibacillus rhamnosus GG and Bifidobacterium animalis subsp. lactis) are explored in a randomized, placebo-controlled trial with an expected target of enrollment of 70 patients (4-16-year old) with confirmed ASD, who will be monitored for 84 days [111].

It is expected that further research in the field of nutritional interventions for psychiatric disorders would include long-term, double-blind, controlled design, in order to obtain high-quality data. Also, decreasing the heterogeneity of the study population would be beneficial for detecting a signal in efficacy studies. This objective can be attained by different means, such as using structured interviews, excluding different confounders (like concomitant use of antibiotics for studies with psychobiotics), and applying cut-off values at baseline for certain psychological instruments used to quantify the severity of core psychiatric symptoms.

Limitations of this review refer to its narrative design, due to which certain important sources may be missed. However, based on the reviewed material, a systematic review of the nutritional interventions, and even an umbrella review, may be granted. Also, due to its design, there was no structured assessment of the quality of evidence; therefore reports of different design and methodological quality have been treated equally. There are some data about the utility of fecal material transplantation in patients with psychiatric disorders, and about the potential benefits of cannabinoids [6,105-107], but these aspects were not approached because it was appreciated that they would increase too much the heterogeneity of the explored interventions.

As strengths of this review, it is important to mention its aim, i.e., to explore the available evidence for the nutritional interventions both at clinical and preclinical levels. Also, the inclusion of a large variety of psychiatric disorders undergoing intense exploration from the perspective of NP may be considered another strength. Last but not least, the awareness of available nutritional interventions is still precarious in mental health specialists, therefore, a presentation of the current trends, challenges, and perspectives in the field of NP was considered beneficial for the case managers responsible for treating patients with psychiatric disorders

CONCLUSION

NP is still a young science and the systematic exploration of its core interventions requires intensive investigation in good-quality, large-scale trials, before they can be included in the therapeutical guidelines. Based on this review of the literature, preliminary data to support the use of omega-3 PUFAs, healthy diets, vitamin therapy, SAMe, NAC, psychobiotics, micronutrients, inositol, and herbal products exist, and most of the evidence refers to potential benefits of these augmentative strategies in patients with anxiety and depressive symptoms. The translation of data from preclinical models to clinical populations is difficult, and a difference between the results in animal studies and those in human trials has been reported. The main advantages of NP interventions refer to their good tolerability, their superior acceptability (due to lower stigma), and their flexibility, which allows to use them as add-ons to other treatments.

Conflicts of interest and sources of funding
None to declare.

Authors' contribution
OV conceived the idea of the review. OV, AGM, BMP and CFP designed the study. OV, CFP, AGM, CFP, DU, MM, CF, AID, CN and CAS screened the studies during the primary search. OV, AGM and CAS drafted the first version of the review. OV, CFP, DU, MM, CF, CN and CAS revised the manuscript and elaborated the conclusions of the review. All authors had full access to the data contained in this review and decided to submit the manuscript for publication.

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ORIGINAL ARTICLE

A Survey on Mental Health Status in West of Iran: A Spatial Analysis

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Abstract: *Mental disorders clearly are among the major challenges of global health causing many concerns for health systems, health experts, and health policy makers. The present study was conducted to evaluate the mental health program in comprehensive health centers and health houses under the coverage of Ilam University of Medical Sciences in the West of Iran. The present study was conducted based on the reports obtained from the mental health program in 2019. The data were recorded into registration and evaluation forms of mental disorders with the codes 102-19-03 and 102-19-01 and then entered into the Excel software. The prevalence per 100 and 100,000 people, as well as their 95% confidence intervals were calculated using Formula 1 ROUND (Number of cases*100000/population) and Formula 2 ROUND (Prevalence ±1.96*SQRT (Prevalence *(1-Prevalence)/population). The results showed that the prevalence of depression was 19,100 [95% CI: 18999-19201] 19100), and the prevalence of anxiety was 304,66 [95% CI: 20348-30584] per 100,000 population. The highest prevalence among mental disorders was related to depression and anxiety. Considering the high average score of mental health status in people over 65 years old, it is necessary to implement measures and interventions to improve these individuals' mental condition and upgrade their mental health.*

Keywords: *Spatial analysis; Ilam province; Mental health; Iran*

INTRODUCTION

According to the definition provided by the World Health Organization (WHO), mental health is "a health status that turns one's potential talents into reality, enabling people to cope with routine life tensions, accomplish valuable work, and upgrade their social participation" [1]. Mental health is an important component for evaluating the health status of a population and can be evaluated using various indicators [2]. In other words, the concept of mental health falls under the general concept of health and refers to all the methods and measures used to prevent people from developing mental disorders, as well as for the treatment and rehabilitation of affected individuals. Psychologists believe that mental health can be affected by individual characteristics, mental and environmental burdens, economic, social, and familial status, failure to achieve life goals, and lack of motivation [3]. Industrialization of countries, the growth of urbanization, migration to cities, etc. are phenomena that are associated with numerous troubles, including psychological and social problems. For this reason, mental health and mental disorders are considered top priorities in today's world. The high prevalence of mental disorders and their associated morbidities render them an immediate health priority [4].

Excessive attention to physical health problems (such as infectious and contagious diseases) has caused mental health to receive less priority in many countries around the world, including its social and spiritual aspects, which have been overlooked in most cases [5]. Statistics on the global burden of mental illnesses highlight the need to pay attention to these aspects more than ever. In parallel with the estimation of the WHO, Harvard University and the World Bank declared that mental disorders were responsible for about 13% of the total burden of diseases in 2006, which was predicted to reach 15% by 2020. The rate of morbidities caused by these disorders has been reported beyond 33% [6]. In Iran, mental health disorders account for 14.5% of the total burden of diseases, and after traffic accidents, they share the second rank with cardiovascular diseases. In women, depression claims the first place of disease burden, while in men, addiction is considered the third cause of disease burden [7]. According to studies in Iran on individuals older than 15 years old, the prevalence of mental disorders has been 25.9% in women and 14.9% in men, and it was reported that overall, 21% of people suffered from some sort of mental problems. Some studies have assessed the prevalence of mental disorders in Ilam province{8]. In a population-based study by Visani et al. [9], the prevalence of DSM-IV mental disorders in both genders was investigated in the urban areas of Ilam province. Out of 763 participants aged 15 years and older in the recent study, 25.8% showed symptoms of mental distress, and the prevalence of mental disorders was reported to be 20.9% in men and 29.8% in women. Also, the prevalence of anxiety, major depressive disorder (MDD), and obsessive-compulsive disorder was higher in women than in men (23.6% vs. 18.2%; 22.7% vs. 17.4%; 13.9% vs. 10%, respectively). In women, the prevalence of phobia was reported as 10.4%.

Studying mental health status can provide managers and decision-makers of organizations with valuable information for planning educational, therapeutic, and preventive measures to reduce the incidence of these diseases in society. Such information can be obtained by monitoring the mental health status of people in different societies [5].

Assessment and monitoring of mental disorders in the country and in Ilam province can help better understand the condition of mental health and public health in society, which is an important and influential factor affecting social productivity and wealth creation. Therefore, researchers here aimed to investigate the mental health status of the urban population of Ilam using the data recorded in the registration and assessment forms designed for mental disorders. Furthermore, we evaluated the mental health program performed in comprehensive health centers and health houses covered by the Ilam University of Medical Sciences in the West of Iran.

MATERIALS AND METHODS

The present cross-sectional study was conducted on the basis of the reports derived from the mental health program in Ilam province in 2020. According to the statistics announced in 2021, Ilam province harbors a population of over 630,000 people living in ten cities: Ilam, Ivan, Chardavl, Sirvan, Malekshahi, Mehran, Abdanan, Dehloran, Badreh, and Darrehshahr (Figure 1).

Figure 1: The geographical location of Ilam province

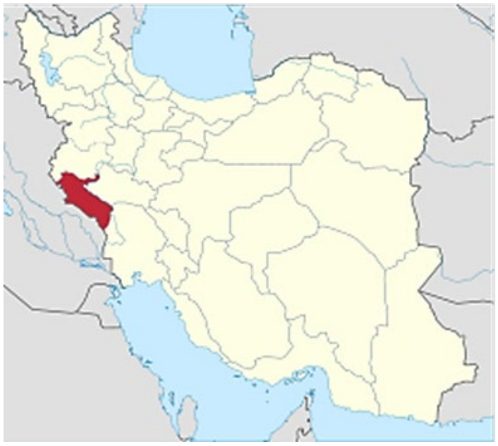

This study was conducted to assess the mental health program implemented in the comprehensive health centers and health houses affiliated with the Ilam University of Medical Sciences in 2019. The study was approved by the institutional ethics committee under the code IR.MEDILAM.REC.1399.266. The data were gathered by reviewing registration and evaluation forms for mental disorders with the codes 102-19-03 and 102-19-01 in all ten cities of Ilam province. Then based on the population of each city in 2021, the prevalence of mental disorders was calculated in 100 and 100,000 people.

Analytic Method

After collection, the data of each city were entered into Microsoft Excel software, and formulas 1 and 2 were used to calculate the prevalence of mental disorders (with 95% CIs) in 100 and 100,000 people.

Formula 1: ROUND (Number of cases*100000/population)

Formula 2: ROUND Prevalence ±1.96*SQRT (Prevalence *(1-Prevalence)/population)

Also, to determine the geographical distribution of mental disorders in the cities of Ilam province, the commands of spatial analysis in the STATA 11 software (Stata Corp. LLC College Station, Texas, USA) were used.

RESULTS

The results showed that in Ilam province, the prevalence of depression was 19100 (95% CI: 18999-19201), and the prevalence of anxiety was 30466 (95% CI: 20348-30584) per 100,000 people. According to the results, the highest prevalence among mental disorders was related to depression and anxiety. The highest rate of depression belonged to Ilam (20300, 95% CI: 20137-20463) and Malekshahi (21836, 95% I: 21297-22375) cities, while the lowest rate of depression was reported in Sirvan (16292, 95% CI: 15691-16897) and Badreh

(17299, 95% CI: 16706-17892). Also, the highest rates of anxiety were recorded in the cities of Abdanan (22298, 95% CI: 21922-22674), Ivan (22559, 95% CI: 22228-22970), and Ilam (21200, 95% CI: 210135-21365). On the other hand, the cities of Sirvan (16769, 95% CI: 16184-17404), Chardavl (16499, 95% CI: 16228-16770), and Darrehshahr (19099, 95% CI: 17790-18408) had the lowest rate of anxiety (Table 1).

Table 1: The prevalence of depression and anxiety in the population of Ilam province per 100,000 people in separate cities

Location	Population	Major Depressive Disorder Prevalence		Anxiety Disorder Prevalence	
		Number of cases	Rate per 100,000 (CI95%)	Number of cases	Rate per 100,000 (CI95%)
Ilam	235144	47734	20300 (20137-20463)	49850	21200 (210135-21365)
Abdanan	46977	9630	20499 (20134-20463)	10475	22298 (21922-22674)
Ivan	48833	10236	20961 (20600-21322)	11036	22559 (22228-22970)
Mehran	27506	5281	19199 (18734-19664)	6162	22402 (21909-22895)
Badreh	15614	2701	17299 (16706-17892)	2701	17299 (16706-17892)
Dehloran	66339	13135	19800 (19497-20103)	12538	18900 (18602-19198)
Darrehshahr	59551	10838	18200 (17890-18510)	10778	18099 (17790-18408)
Shirvan	14404	2347	1294 (15691-16897)	2419	16796 (16184-17404)
Chardavl	72167	12412	17199 (16924-17474)	11907	16499 (16228-16770)
Malekshahi	22587	4932	21836 (21297-22375)	3884	17196 (16704-17668)
Total	580148	110808	19100 (18999-19201)	176750	30466 (20348-30584)

Regarding age distribution, the results showed that the highest prevalence of depression (29%), anxiety (35.5%), and psychotic disorder (22.6%) was observed in people over 65 years of age. The psychological disorders of mania (6.3%) and obsessive-compulsive disorder (13.3%) were more commonly observed in the age group of 15-24 years compared to other age groups. Regarding the gender distribution of mental disorders, it was noticed that depression (20.3% vs. 22.7%), mania (2.6% vs. 4.7%), anxiety (23.6% vs. 32.18%), obsessive-compulsive disorder (10% vs. 13.79%), phobia (10.4% vs. 5%), and psychotic disorder (6.17% vs. 2.4%) were more frequent in women than in men (Table 2).

Table 2: The prevalence of mental disorders in Ilam province in various age groups and genders

Age groups/ gender	Depression	Mania	Anxiety	Obsessive-compulsive disorder	Phobia	Psychotic
	Prevalence (CI 95%)	Prevalence (CI 95%)	Prevalence (CI 95%)	Prevalence (CI 95%)	Prevalence (CI 95%)	Prevalence (CI 95%)
15-24 years	14.0 (7.08-15.1)	6.3 (5.9-7.3)	14.7 (12.1-16.8)	13.3 (9.04-15.15)	8.4 (6.9-11.1)	0.7 (0.01-5.9)
25-44 years	20.6 (18.2-22.3)	4.0 (2.6-9.1)	21.2 (16.2-18.2)	11.3 (9.13-14.2)	7.5 (6.9-11.1)	4.8 (3.6-6.3)
55-64 years	22.7 (20.2-24.3)	2.3 (1.4-2.9)	23.6 (20.3-25.2)	13.4 (8.1-16.1)	8.3 (7.9-11.3)	3.7 (1.2-5.7)
> 65 years	23.6 (20.3-25.2)	0.4 (0.1-1.1)	35.5 (32.3-37.8)	9.7 (7.1-11.2)	9.7 (7.1-12.2)	22.6 (20.2-27.6)
Male	17.4 (15.1-19.6)	2.6 (1.3-8.7)	18.2 (15.2-21.1)	10.0 (8.1-12.4)	5.0 (3.7-8.2)	2.4 (1.4-4.1)

The results of spatial analysis regarding the geographical distribution of mental disorders in Ilam province showed that depression, anxiety, and mania were more commonly observed in the northern compared to southern regions of the province, showing the highest prevalence in Ivan City. Also, panic disorder was more prevalent in southern cities of the province (Abdanan and Dehloran) than in other cities (Figure 2).

DISCUSSION

Sciences. The results of the present study showed that the highest prevalence of depression, anxiety, and psychotic disorders was observed in people over 65 years old. In this study, age showed a direct and significant correlation with having unsatisfactory mental health, indicating the importance of advancing age in the development of mental health problems such as depression, anxiety, and psychotic disorders. In other words, the incidence of mental health disorders increased with age, contradicting the findings of Heidari et al. [10] and Jafari et al. [11]. In recent studies, a higher age was reported to be significantly associated with more favorable mental health. Other studies; however, have reported a significant relationship between mental health and advancing age, including a report by Visani et al. [12]. In another study, Noorbala et al. [13] noted that with increasing age, the likelihood of the development of mental disorders increased so the highest prevalence (49.9%) was related to people over the age of 65 years. Among the reasons for the higher prevalence of mental health disorders in older people are retirement, menopause, loneliness-associated anxiety, and biological alterations. The results of these studies are generally consistent with our findings; nevertheless, some discrepancies can be explained by differences in the assessment tools employed and the age spectra of the studied populations.

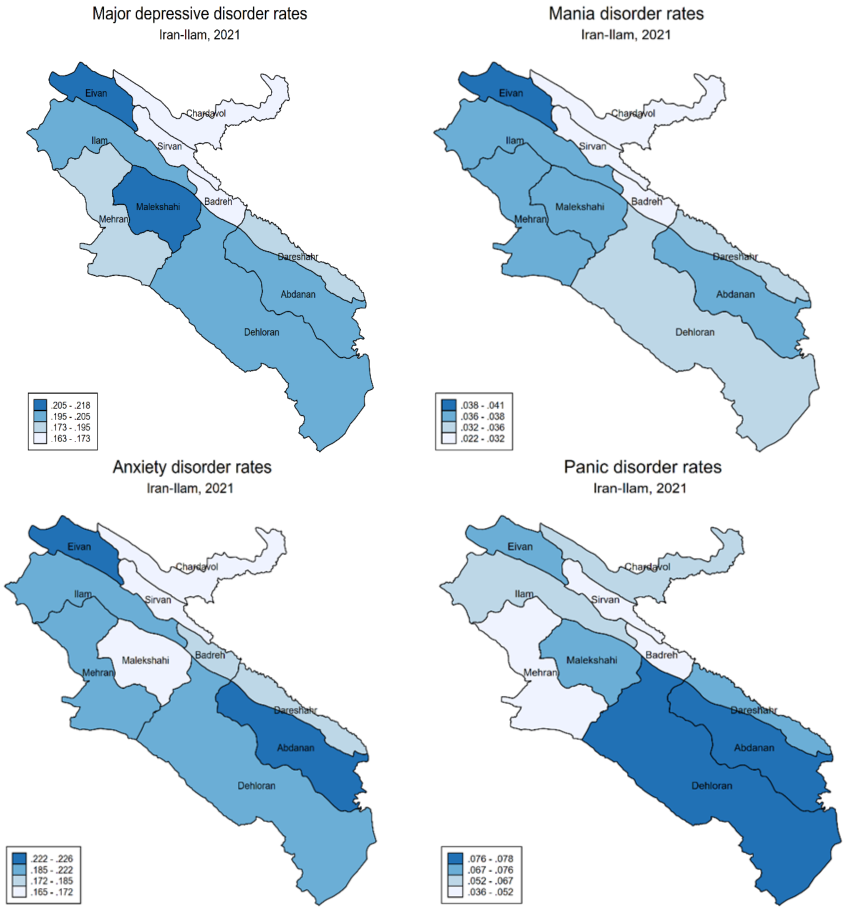
Major depressive disorder rates
Iran-Ilam, 2021
Eivan
Chardavol
Ilam
Sirvan
Malekshahi
Badreh
Mehran
Dareshahr
Abdanan
Dehloran
.205 - .218
.195 - .205
.173 - .195
.163 - .173
Mania disorder rates
Iran-Ilam, 2021
.038 - .041
.036 - .038
.032 - .036
.022 - .032
Anxiety disorder rates
Iran-Ilam, 2021
.222 - .226
.185 - .222
.172 - .185
.165 - .172
Panic disorder rates
Iran-Ilam, 2021
.076 - .078
.067 - .076
.052 - .067
.036 - .052

Figure 2: The results of spatial analysis. Geographical distribution of the most common mental disorders in Ilam province

The results of this study showed that the highest prevalence of panic disorder and obsessive-compulsive disorder was related to the age group of 15-24 years. Mental disorders in teenagers and young adults exacerbate the predicted future burden of diseases and adversely affect numerous life aspects, including educational performance, employment, income, personal relationships, and social participation, as well as the workforce [14]. In a study in Brazil conducted on individuals aged 18 years and older, it was shown that younger people were more vulnerable to mental problems [15]. In another study in Iran, Shirzadi et al. [16] declared a higher prevalence of these disorders in people aged 41 years or older compared to 18-40-year-old individuals.

Also, the results of the present study revealed a lower prevalence of depression and anxiety in women than in men. In a study, Falahzadeh and Momayezi [17], who investigated the prevalence of depression, anxiety, and stress in Yazd City, Iran, noted that depression, anxiety, and stress were more prevalent in women than in men, and this difference was statistically significant for anxiety (P=0.01) and stress (P=0.02), which was not consistent with our observation.

We also noticed a higher prevalence of obsessive-compulsive disorders, phobia, and psychotic disorders in women compared to men, which was in agreement with the reports of Pendrez et al. [18], Kesler et al. [19], and Chang et al. [20]. The higher prevalence of mental disorders in women compared to men can be attributed to biological factors, gender-related differences, environmental and occupational stress, as well as limited sources of satisfaction and social participation of females.

Although in the present study, a significant portion of the urban population declared that they had unsatisfactory mental health conditions, this may not exactly match the clinical definition of adverse mental health. Therefore, the results of such subjective studies should not be regarded as a basis for judgment about the true condition of the mental health of respondents. Nevertheless, one should admit that a ratio of people, especially among women, suffer from some degree of mental health disorders. In this regard, the key measures that can be taken may be to initially achieve a correct understanding of the issue and then to implement comprehensive interventions for monitoring populations as large as possible, especially among vulnerable groups.

Numerous viable strategies are available to maintain and improve public health. For example, unemployment has been pointed out as an important factor contributing to the development of mental health disorders [21]. So, an important intervention that governments can employ is to create more jobs, especially for youth, and to improve people's livelihoods as much as possible.

Also, another effective measure for improving mental health is to remove the sources of distress, psychological pressure, and unnecessary tensions, which can be achieved with the help of mass media by nurturing a comfortable atmosphere. Moreover, the establishment of psychological counseling clinics can be among low-cost and beneficial measures to cover a large population, including young people, middle-aged, and elderly people, so that they can amend and improve their mental health**.**

CONCLUSION

The results of this study affirmed a relatively high prevalence of suspected mental health problems among people over 65 years of age, which is an alarming call, requiring authorities and researchers to pay more attention to this issue and to identify and modify the factors affecting the mental health of these people. A unique observation in this study was the lower prevalence of anxiety and depression in women than in men. Also, we noticed that panic disorder and obsessive-compulsive disorder were most prevalent in the age group of 15-24 years. A reduction in the prevalence of mental disorders can enhance social liveliness. In this regard, increasing people's awareness of ways to reduce mental disorders, expanding psychological counseling centers across the city, establishing counseling phone lines, residing counselors and psychologists in public places such as mosques, expansion of parks and enjoyable environments, equipping parks with sports devices, and finally, encouraging people to use these services can help reduce depression and anxiety in society.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The source of the funding code is the code of IR.MEDILAM.REC.1399.266.

Authors' contribution

Conceptualization, E.N., and Y.V.; methodology, E.N.; formal analysis, H.S; investigation, Y.V.; data curation, F.M., and A.S; writing—original draft preparation, S.A.; and M.Kh.; writing—review and editing, S.A; F.M. and M.Kh. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Dermatologists’ Views on the Use of Teledermatology

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Abstract: *Boosted by the COVID-19 pandemic, the adoption of technology has shifted people’s lifestyles, impacting social interactions and leading to an increase in the use of telemedicine, including teledermatology (TD). Social media platforms facilitate communication between patients and physicians, between primary care physicians and specialists, and between dermatologists. The study aimed to assess the attitudes, views, and usage of dermatologists regarding TD consultations. A cross-sectional study was performed with the use of an anonymous, web-based questionnaire intended to be completed by dermatologists. A total number of 73 physicians, both resident and board-certified dermatologists completed the survey. Almost everyone (98.6%) used some form of teledermatology, the most frequently used platforms being WhatsApp, followed by classical telephone calls/messages, email, and other forms of TD. TD is considered to be especially useful in communicating with peer colleagues (94.4%), with physicians of other specialties (90.0%), with family doctors (72.9%) and with patients (69.9%). Since TD can facilitate the treatment-related follow-up of adverse effects, while also being useful in the triage of patients, its use will likely increase in the future. The main concerns were related to the accuracy of the diagnosis through TD, a less developed doctor-patient relationship, a less informative consultation, an increased workload as well and a diminished financial reimbursement. Dermatologists currently use TD in addition to the classical consultations of patients, as well as to communicate with family physicians and peer colleagues to better manage complex cases. Furthermore, TD proves to be an important tool in the education of young dermatologists.*

Keywords: *teledermatology, consultations, education*

INTRODUCTION

Skin diseases are frequent pathologies encountered in the general population that require standard or specialized care, depending on disease rarity or difficulty. The Global Burden Disease study that was performed over 10 years in 187 countries revealed that skin conditions ranged from the 2nd to the 11th leading cause of years lived with disability, and, at a global level, cutaneous conditions were the 4th cause of nonfatal disease burden [1]. Such a high prevalence of skin disorders is met with a significant effort on the part of the medical personnel and the medical system, requiring new solutions to optimize medical care. Teledermatology (TD) developed in recent years as a response to this need. Its use, boosted by the COVID-19 pandemic [2], is expected to increase soon. Technology facilitates professional communication between patients and healthcare providers, as well as between healthcare professionals of identical or different specialties.

Several social media platforms facilitate communication between patients and healthcare providers, allowing individuals to contact specialized personnel more quickly. During the past years, departments already offering asynchronous TD created new systems to support the direct interaction between specialists and patients [3].

As expected, the use of TD increased along with other branches of Telemedicine, such as Tele-echography[4], women's health telemedicine, family medicine telemedicine, telecardiology, and others. Taking into consideration these changes in technology and social interactions, we aimed to evaluate the attitudes, views, and usage patterns of dermatologists.

MATERIALS AND METHODS

A cross-sectional study was performed between February and July 2022 among specialist and resident dermatologists using a web-based survey. Contact details were retrieved from the database of the Dermatology Department at the University of Medicine and Pharmacy "I. Hatieganu" Cluj Napoca, Romania. Both email and phone messages were used to distribute the questionnaire to dermatologists, explaining that the completion of the survey was anonymous and voluntary.

The survey was developed by the authors based on previously published data and was sent via Google Forms (Google LLC, Mountain View, CA, USA) to the participants. The questionnaire was divided into several sections: Initially, the participants were invited to provide certain anonymous personal data such as age, gender, area of dwelling, workplace, and amount of time spent using appliances such as telephone, tablet, etc. This introductory part was followed by a second section assessing the current use of TD, a section focusing on the accuracy of medical consultation via TD, the advantages and disadvantages of TD, and future perspectives.

Statistical analysis

Data was collected and further analyzed using SPSS, version 12, SPSS Inc., Chicago, IL, USA. Spearman correlation was used between ordinal variables and between ordinal and numeric variables. The biserial correlation coefficient was used between ordinal and nominal variables. A p-value under 0.05 was considered statistically significant.

RESULTS

A total of 73 participants completed the online questionnaire, of which 25 (34.2%) were during residency and 48 (65.8%) were board-certified dermatologists (Table 1). The female gender predominated with 65 answers (89%), with the following age distribution: 20 participants (27.4%) in the 20–30-year age group, 27 (37%) in the 30–40-year age group, 16 (21.9%) in the 40-50 year age group and 10 (13.6%) above 50 years.

Most responders worked under contract with the national health care system, while 12 (16.4%) participants only worked in private settings. 72 (98.6%) out of 73 responders use some form of TD, the most frequently utilized platform being WhatsApp with 69 users (95.8%), followed by classical telephone call/message in 52 participants (76.5%) and email-based TD in 35 participants (57.4%) (Figure 1). TD is being used for the diagnosis and treatment of dermatological disorders daily by 23 (31.5%) participants and every few days by 30 (41.1%) participants.

Table 1: Participant characteristics

Total no of responders	**73**	
	No	**%**
Gender		
Female	65	89%
Male	8	11%
Area of dwelling		
Urban	73	100%
Years in practice		
0-10 years	24	32.9%
11-20	11	15.1%
21-30	5	6.8%
31+	8	11%
Current resident	25	34.2%
Setting		
Out-patient clinic public	11	15.1%
Out-patient clinic private	10	13.7%
Inpatient public	23	31.5%
Both public and private settings	27	37%
Inpatient private	2	2.7%

TD is being used as a tool to professionally discuss cases between peer colleagues daily by 21 (29.2%), every few days by 27 (37.5%), once a week by 10 (13.9%), and less than once a week by 14 (19.4%) of participants.

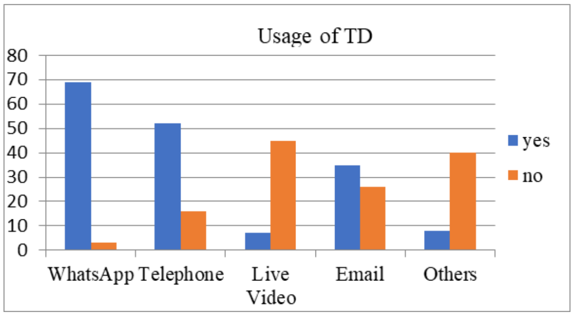

Figure 1: Usage of TD among dermatologists

TD is considered to be most useful as tertiary TD, in communicating with peer dermatologists, with 68 (94.4%) affirmative responses; it may be useful to some extent to communicate with physicians of other specialties 63 (90.0%), with family doctors 51 (72.9%) and with patients 51 (69.9%). Only 4 responders (5.5%) consider that TD did not increase workload, while 19 physicians (26.0%) consider that the workload mostly increased during their free time and 48 (65.8%) realized that TD-related tasks surged both during working hours and leisure period (Figure 2).

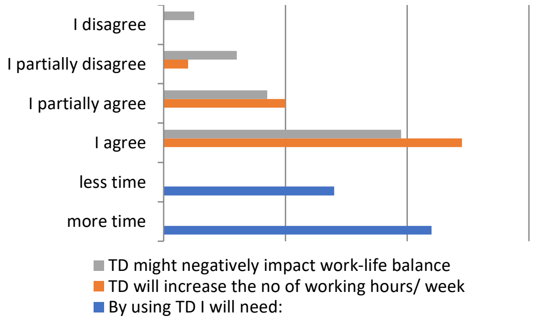

Figure 2: TD and work-life Balance

58 (79.5%) participants agreed to some extent that the relationship between a patient and the physician may be less developed in a TD setting and 67 (91.8%) responders contend that it may be less informative compared to classical consultations. Most replies, 55 (75.3%), were not reimbursed for the service of TD consultations; for 7 (9.6%) it was expected from the work environment, while another 7 (9.65) were partially or fully reimbursed 4 (5.5%).

41 (56.2%) dermatologists agree that consultations performed via TD might have a lower accuracy compared to classical consultations, while 29 (39.7%) partially agreed with this statement; clear images, complete anamnesis, and communication with the patient were considered key factors that might enhance diagnostic accuracy. Both forms of TD, synchronous and asynchronous TD, were considered as having advantages and disadvantages. Participants were asked the most common pathology they were contacted for during the last week through TD and the most frequent skin disorders were allergic reactions with 25 (34.7%) answers, acne with 10 answers (13.9%), bacterial infections with 7 (9.7%), followed by viral infections and malignant lesions with 6 (8.3%) answers each. Most dermatologists 61 (83.6%) felt uncomfortable offering medical information regarding malignant cutaneous pathology through TD. Physicians partially agree 38 (52.1%) and agree 22 (30.1%) that TD may facilitate the follow-up of treatment related to adverse effects. Regarding the statement that TD will increase the number of working hours/week, 49 (67.1%) agreed and 20 (27.4%) partially agreed. A large number of responders, 45 (61.6%) agreed and 24 (32.9%) partially agreed that TD might negatively impact the work-life balance. Participants agreed in 53.4% (39 answers) and partially agreed in 23.3% (17 answers) that by using TD many patients trivialize the dermatological consultation. Also, dermatologists feel that TD should be financially sustained in 76.4% (55) of answers or partially agree with the statement in 20.8% (15). In our cohort, 51 (69.8%) physicians believe that they will use more TD in the future. 41 (56.2%) agree and 28 (38.4%) partially agree that this rise will be due to patients request. Most health care professionals agree 29 (39.7%) and partially agree 31 (42.5%) that TD could be employed as a useful method in the triage of patients.

Upon statistical analysis, age was inversely correlated with the custom of discussing certain cases with colleagues ($p=0.009$), with the utility of TD with the family doctor ($p=0.018$) and peer dermatologists ($p=0.02$), with the future usage of TD ($p=0.015$) and the utility of TD in the triage of patients ($p=0.015$). The usefulness of TD in communicating with the patient and with general practitioners was correlated with the usefulness of TD as a tool in the triage of patients ($p<0.1$ and $p=0.01$). Future use of TD was inversely correlated with a less developed doctor-patient relationship ($p<0.01$), with a lower diagnostic accuracy ($p<0.01$), with the trivialization of consultation ($p<0.01$) and longer working hours ($p<0.01$) and positively related to better monitoring of adverse effects ($p=0.0$). Lack of information through TD was related to more time invested by the physician ($p<0.01$) and inversely correlated with monitoring of adverse effects ($p=0.02$). The lack of accuracy of TD was also related to more time invested by the physician ($p<0.01$) and longer working hours ($p<0.01$). Disturbance of work-life balance was related to more time required by TD ($p=0.05$) and consult trivialization ($p<0.01$). Finding a financial solution for TD was related to better monitoring of adverse effects through TD ($p<0.01$).

DISCUSSION

A relatively high usage of TD was noticed in our cohort, with 53 (72.6%) participants employing it a few times a week. Taking into account that 60 (82.2%) participants consider that TD could be a useful method for selecting patients in the future, we believe that some of these consultations are aimed at prioritizing patients according to pathology. Indeed, TD might be useful in the triage of patients, its' cost- effectiveness increasing when patients need to travel farther distances to benefit from the dermatological service [5].

Tertiary dermatology, or a “second opinion” among specialists, is a valuable tool for reinforcing or modulating the physician’s perspective on certain pathology but also as a learning tool for residents/young specialists. In our study, TD was used for the means of tertiary dermatology by 48 (66.7%) participants daily or every few days and once or less than once a week by 24 (23.3%) participants. The vast majority of our responders, 68 (94.4%), considered TD as being useful in communicating with peer colleagues while younger age was also correlated with the use of TD in communicating with peer colleagues, family, or specialist physicians. A recent systematic review that focused on TD education both in medical students and postgraduate trainees indicated high satisfaction rates and improvement in educational outcomes among medical students, residents, and other trainees, some residents mentioning that TD provided a low-stress learning environment and some students considering that eConsult-based training enhanced their education [6,7].

Secondary to tertiary-care teleconsultation is applied less frequently, however, a study performed in the Netherlands that included 39 dermatologists showed that it had important advantages such as fast response time, formalized records, and data and privacy security(6). However, physicians also prefer to use means of communication they are accustomed to because of ease of use and direct personal network[8,9]. A study evaluating practice models in TD in Spain indicated that the main advantages of TD were prioritization in cancer screening, rapid emergency care, training of and communication with primary care physicians, and screening for trivial conditions while also reducing in-person visits [10]. A TD service between primary healthcare centers and the National Skin Centre in Singapore using "store and forward TD" indicated satisfaction for the TD service (by both primary and specialist doctors), more convenience for the patients but also an educational value for family physicians, while considering challenging the quality of the images, lack of personal assessment of the skin pathology and of the patient as a whole [11,12].

In our study, 56 (76.7%) agreed (fully or to a certain extent) that patients trivialize dermatological consultations when performed as TD. This usually implies a lack of complete information regarding the skin problem but also carelessness regarding compensation, leading to incomplete medical advice and frustration.

Financial aspects will directly influence the usage of TD. A cross-sectional study performed in the US involving 591 dermatologists documented low reimbursement (69.8%) as one of the most common barriers to the implementation of TD while the majority of respondents (85.2%) felt reimbursement for store-and-forward TD was too low [13]. A global survey developed by WHO in 2010 indicated that TD is more acknowledged in high-income countries, difficulties in implementing were legal issues concerning the privacy of patients, limited reimbursement for practitioners as well and competing health system priorities. High costs are also an issue in developing countries, alongside resource, infrastructure, and technical limitations [14,15]. Therefore, we may observe that financial issues do influence in different manners the implementation and use of TD. In certain areas, specialists do not receive extra compensation for addressing professional questions from primary care physicians but time slots in the weekly schedule were allocated for such tasks, integrating TD alongside other tasks [16].

In our cohort, the use of TD has increased workload both during working hours and in free time, physicians expect a further increase in the future while 69 (94.5%) dermatologists partially/completely agree to the fact that TD might negatively impact the work-life balance. A cross-sectional study evaluating usage patterns on WhatsApp TD (WhatsApp being the most frequently used platform in our study as well) underlined the fact that the added workload both during and after work hours was the biggest disadvantage of WhatsApp, potentially leading to burnout. Both primary health care physicians, as well as specialists, reported being consulted during working hours but also after 20:00, during weekends, holidays, or when abroad [16].

The most common pathologies dermatologists were contacted for were allergic reactions, acne, bacterial and viral infections as well as malignant lesions. A study performed in pediatric patients described acne, atopic dermatitis, benign melanocytic nevi, and verruca as being the most common diseases treated by TD in the adolescent age group. In contrast, atopic dermatitis, verruca, and molluscum were the most frequent diseases in the 3-12 year age group [17]. Most dermatologists who participated in the survey considered that TD might have a lower accuracy compared to classical consultations. This concern has previously been reported by US-based pediatric dermatologists who, even though positive about TD's present and prospects, considered TD as being inferior to in-person care regarding accuracy and management plans [9,18]. This may pose the specialist in an uncomfortable position, having to give a diagnosis and offer a treatment plan while not being fully confident of the diagnosis. However, this situation is avoided when there is a face-to-face meeting for the initial diagnosis and the monitoring is performed by TD. A randomized clinical trial including patients with atopic dermatitis, where the initial visit was in-person while the follow-up was through TD versus personal consultation for a year, showed similar improvement in the disease severity of patients [19]. A similarly structured- study demonstrated that the quality of life of patients with atopic dermatitis improved both when followed up through TD or in person [20]. Very good results for monitoring were also obtained for store-and-forward TD in case of pathologies like acne and psoriasis [21].

When asked about which pathologies they felt uncomfortable offering consultation through TD, 59 (80.8%) answered malignant tumoral lesions. Indeed, the accuracy of TD in skin neoplasms was inconsistent between studies, with a moderate accuracy reported by Giavina-Bianchi et al [22] and a high accuracy reported by Moreno-Ramirez et al [23] and Kroemer et al [24]. One should take into consideration that diagnostic precision increases when both clinical and dermoscopic images are available, but, also the cautiousness of dermatologists of not missing a melanoma and, while a severe dysplastic nevus would still be in a "benign" category, the excision of such a lesion would be in the benefit of the patient. Another aspect to take into consideration is that dermatological consultation of patients referred through TD detected "unobserved" malignant lesions both on exposed and covered skin [25], highlighting the usefulness of intertwining both methods of consultation.

The future perspectives of TD look promising. Most of our participants 51 (69.8%) agreed or partially agreed to the statement that they will be using TD in the future and 69 (94.6%) to the statement that it will requested by patients in the future. A cross-sectional study performed in the US aiming at dermatologists also showed that 70.9% of the physicians foresee that TD could become an ongoing component of their practice, provided that reimbursement for video visits remains similar to that of in-person visits [9].

Among the limits our study has, we would like to mention that participants were selected from the database of the clinic, therefore, we believe most answers were from the Transylvanian part of the country. Number of participants can virtually always be increased to obtain more accurate results. Still, we believe the data we obtained is relevant, especially because the official implementation of TD differs more between employers and the type of work setting compared to a certain region in the country. Rapid development of social media platforms and artificial intelligence quickly alters the work environment for dermatologists, leading to changes in perception and usage that only be captured by repetitive assessments performed at different time-frames**.**

CONCLUSION

In conclusion, dermatologists currently use TD in addition to classical consultations for patients, to manage cases from family physicians but also to discuss and better manage complex cases. TD proves to be an important tool in the education of young dermatologists. The swift evolution of technology brought us this tool that is under continuous development but that is here to stay.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, CE, MNM, AB; methodology, CE, MNM, SD.; software, PAI; validation, all authors; investigation, CE, MNM, F.S.; data curation, SD, PAI., A.N.; writing—CE, X.X., F.S., M.C.D.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted following the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Medicine and Pharmacy "I. Hatieganu" Cluj Napoca number 172.

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Supplementary material – Dermatologists' views on the use of teledermatology

Within the first section, anonymous personal data was collected through several questions. Participants had the option of selecting gender, area of the dwelling, and age (by selecting between age groups, namely "20-30","30-40","40-50", "50-60", "60-70" and ">70"), the setting they provide dermatological health care ("public ambulatory", "private ambulatory", "public hospital", "private hospital" and "public & private ambulatory/hospital") and the years of clinical experience ("Resident", "Specialist for 0-10 years", "Specialist for 10-20 years", "Specialist for 20-30 years", "Specialist for 30-40 years" and finally "Specialist for >40 years"). A question assessing the time of current use of computer/tablet/phone/television was included in the survey but due to its lack of statistical significance, it was not reported in the manuscript. The second section of the questionnaire focused on the current use of Teledermatology. Participants were first asked to express the method they currently employ for TD by choosing from the following possible answers: "WhatsApp messenger", "Telephone", "Email", "Live Video conferencing" and "other devices". To assess the frequency of TD in skin disease diagnosis/treatment and the frequency of using TD in the management of cases, four possible answers were offered to the two questions: "never", "daily", "every couple of days", "once per week", "less than once per week". To evaluate the workload, the statement "The use of TD increased my workload during:" was followed by four possible answers "working hours", "free time", "both working hours and free time" and "it did not increase my workload". The following question referred specifically to the impact of COVID-19 on TD, asking "Usage of TD increased:" with the following answers: "during the COVID-19 pandemic", "after the COVID-19 pandemic" and "COVID-19 did not influence the usage". Regarding compensation, participants were asked whether they received any benefits for TD consultations and also whether they believed they were supposed to; responders had the option of selecting the following: "Yes", "No", "Partially" and "not applicable (part of the contract)".

To answer the questions of whether through TD the doctor-patient relationship is less developed and the TD-consultation is less informative, four choices were given: "I don't agree, I don't agree, I partially agree, I agree". Participants were given the option of selecting "yes, no, partially agree" to whether TD is more useful in communicating with patients, family physicians, dermatologists, and other specialists.

The next section of the questionnaire was directed towards how physicians feel about their diagnostic accuracy through TD. To the question of whether TD- followed diagnosis accuracy could be lower compared to in-person consultations and whether accuracy increases when collaborating with family physicians, the answer options were: I don't agree", "I rather not agree", "I agree partially" and "I agree". To improve the diagnostic accuracy in TD, the following options were given: "Clear images with optimal resolution", "Complete information from anamnesis", "Prior appointment for the TD consultation", "the presence of a dialogue with the patient". In the following question, dermatologists could select which type of TD is more useful, what was the cutaneous pathology they were most frequently contacted for during the last week and what is the pathology for which they don't feel comfortable offering TD-consultations.

To evaluate the workload, participants were asked whether they would be more or less time-efficient by using TD and whether the use of TD would increase the number of working hours/week. The agreement of participants regarding the trivialization of consultation, regarding ease in the follow-up of treatment-related adverse effects was also assessed.

Future perspectives and usage trends of TD were assessed by asking physicians whether they believe they will use more TD in the future, whether they believe patients will request more TD in the future, whether TD could be an option for triage, whether TD would affect their work-life balance and whether the future of TD would depend on finding a financial reimbursement, with the following scale of agreement: "I don't agree", "I rather not agree", "I agree partially" and "I agree".

Nevertheless, throughout the study, all participants had the chance to add personal ideas after each section, if they wanted to express answers that were not displayed.

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REVIEW

Monkeypox: Pathogenesis, Prevention, Threats, Challenges and Immune Response Against It

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Abstract: *A rare zoonotic disease, monkeypox is brought on by the Monkeypox virus, which is a member of the Orthopoxvirus genus, Chordopoxvirinae subfamily, and Poxviridae family. Smallpox-like symptoms are brought on by the monkeypox disease, which is closely linked to the variola virus (smallpox virus). In the past, it has been demonstrated that smallpox vaccination with the vaccinia virus (another orthopoxvirus) is 85% protective against monkeypox. The two most likely forms of monkeypox transmission are animal-human transmission and human-human transmission. Transmission between people has been connected to respiratory droplets, coming into contact with bodily fluids, contaminated patient things or surroundings, and skin lesions on infected people. Numerous strategies or tactics have been developed by monkeypox to subvert or exacerbate the host's immune reaction to infection. A crucial element of innate immunity, natural killer [NK] cells employ cytokines to kill virus-infected cells and interfere with the operation of other cell types like T cells and dendritic cells. Studies have shown that the smallpox vaccine provides cross-protection against other Orthopoxvirus species, including monkeypox. This study was out to provide a thorough overview of the pathogenesis, prevention, risks, immune response problems, and analysis of possible concerns associated with the monkeypox virus.*

Keywords: *Monkeypox, pathogenesis, prevention, threats, challenges, immune response*

INTRODUCTION

Numerous levels of worry, terror, mistrust, and rumors have been circulated among the general public as a result of the quick spread of new diseases. Rapid scientific knowledge dissemination is a useful strategy for reducing the fear and worry that emerge with new outbreaks. This won't be possible unless epidemiologists and statistical modelers offer in-the-moment studies to forecast future conditions and potential treatment effects [1]. In order to solve the health issue as soon as is practical, it should be underlined that real-time analysis is the analysis of fresh available data utilizing logic and mathematics as soon as the data is created. In Wuhan, China, instances of pneumonia with no known cause were first reported in late December 2019. A unique form of coronavirus was later found to be the source of those pneumonia cases in January 2020 [2]. The World Health Organization (WHO) officially named the ailment COVID-19 (short for coronavirus illness 2019), and the virus was given the moniker SARSCOV-2 (severe acute respiratory syndrome coronavirus 2). Iran and other nations across the world made major steps in this direction despite the COVID-19 epidemic being alarming and it being crucial to design and implement appropriate regulations [3]. In Wuhan, China, instances of pneumonia with no known cause were first reported in late December 2019. A unique form of coronavirus was later found to be the source of those pneumonia cases in January 2020. The World Health Organization (WHO) officially named the ailment COVID-19 (short for coronavirus illness 2019), and the virus was given the moniker SARSCOV-2 (severe acute respiratory syndrome coronavirus 2). Iran and other nations across the world made major steps in this direction despite the COVID-19 epidemic being alarming and it being crucial to design and implement appropriate regulations [4]. Since the late 1970s, there have been no confirmed cases of smallpox. As a result of their disease, up to 30% of people passed away. Toximemia and systemic shock, a condition that affects the entire body, were the causes of death (toxins in the blood). This study set out to provide a thorough overview of the pathogenesis of the monkeypox virus, its hazards, obstacles to the immune system's response, and an analysis of potential threats.

SMALLPOX

In a second outbreak, up to 80% of household contacts could contract the highly contagious disease known as smallpox. Those who survived the illness typically experienced long-term effects like blindness and significant scarring [5]. In order to continue studying vaccines and treatments, researchers saved some samples of the smallpox-causing variola virus. Only two locations on Earth contain these virus samples. They are secure at the State Research Center of Virology and Biotechnology in Russia and the Centers for Disease Control and Prevention (CDC) in Atlanta (VECTOR Institute). Public health officials are only concerned about smallpox spreading as a result of biological warfare because it is no longer present in nature [6]. There hasn't been any recent evidence of terrorists using smallpox as a weapon. On the other side, scientists are prepared to react if smallpox is employed as a weapon. The CDC has enough smallpox vaccine to protect everyone living in the United States in the event that the virus reappears. The variola virus is what causes smallpox. In the past, prolonged, direct face-to-face contact with people was the main method of spreading smallpox. When they coughed or sneezed, respiratory particles would travel through the air. If other people inhaled these large drops, they would catch the infection [7]. A less frequent way for people to contract an infection is by direct contact with the rash or crust material from a swab. People can pass the virus to one another by sharing linens, towels, and clothing. The condition is most contagious when throat and mouth ulcers initially appear. On the other hand, smallpox remains contagious for a number of weeks after the initial lesions manifest. Since smallpox no longer occurs naturally, smallpox diagnoses are extremely uncommon today. Any symptoms you have are almost always a sign of another illness or condition [8]. Prior to its elimination, smallpox was easy to distinguish from a few other common illnesses, such as severe chickenpox. If other people breathed in these large drops, they would catch the infection. A less frequent way for people to contract an infection is by direct contact with the rash or crust material from a swab. People can pass the virus to one another by sharing linens, towels, and clothing. The condition is most contagious when throat and mouth ulcers initially appear. Contrarily, smallpox is infectious for several weeks following the onset of the first lesions [9]. Since smallpox no longer occurs naturally, smallpox diagnoses are extremely uncommon today. Any symptoms you experience are almost certainly a sign of another illness. Prior to its elimination, smallpox was easy to distinguish from a few other common illnesses, such as severe chickenpox. Human monkeypox was discovered in 1970 after regional smallpox elimination revealed isolated cases of a comparable illness in remote areas of the DRC. Since then, extensive research has been done on the epidemiological and clinical features of the condition, but no specific medical countermeasures have been created [10]. It could take anywhere between 5 and 21 days from the moment of exposure to the onset of symptoms. On average, symptoms endure between two and four weeks. Although it is unknown to what extent it can happen without symptoms, there may be very few indicators.

MONKEYPOX

There is currently no known animal reservoir host for the zoonosis monkeypox (Figure 1), making preventative strategies challenging. In addition to the DRC (CAR), cases have recently been reported from Nigeria, the Republic of the Congo (ROC), Sierra Leone, Liberia, Cameroon, and the Central African Republic [11].

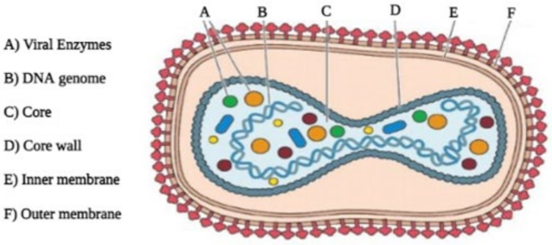

Figure 1: Structure of Monkeypox

In several of these nations, there had been no reports of human monkeypox for decades. The cessation of routine smallpox immunization, which led to a decline in orthopoxvirus immunity, has been blamed for the rise in cases of monkeypox. Since May 13, 2022, cases of monkeypox have been reported to WHO from 12 Member States that are not endemic for the virus and span three WHO regions. Epidemiological research is ongoing, but to date, there have been no established links to travel to endemic regions [12]. According to the information that is currently available, occurrences have mainly — but not exclusively — been found among men who have sex with men (MSM) who visit general care and sexual health clinics for treatment. This Disease Outbreak News aims to raise awareness, offer technical support for urgently advised measures, and provide information to support readiness and response operations. The situation is evolving, and according to WHO, additional cases of monkeypox will be discovered as surveillance in non-endemic countries increases [13]. In order to stop the disease from spreading further, the first step will be to accurately notify those who are most at risk of contracting monkeypox. The most vulnerable individuals, according to the available studies, are those who have had close personal contact with a monkeypox patient. The WHO is also creating policies to safeguard frontline healthcare providers and other vulnerable healthcare employees, such as housekeepers. The WHO will offer more technical recommendations over the coming days [14].

Monkeypox signs and symptoms

Early signs of monkeypox frequently include headaches, muscle pains, fever, and fatigue, thus they may initially resemble the flu (Figure 2). Swollen glands are a symptom of the illness, which might mimic chickenpox, measles, or smallpox. These generally show up behind the ear, below the jaw, in the neck, or in the groin before the rash does. After a few days of the fever, lesions often start on the face before spreading centrifugally to the palms of the hands and soles of the feet [15].

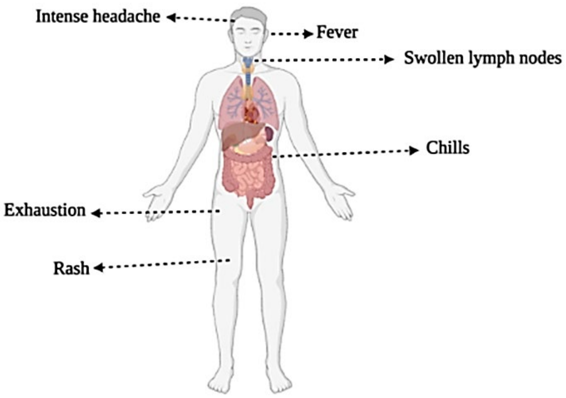

Figure 2: Symptoms of monkeypox disease (illness lasts for 2-3 weeks)

Patients with HIV may have a number of symptoms. Several people in the 2022 monkeypox outbreak also experienced fever, swollen lymph nodes, and difficulties swallowing in addition to vaginal and perianal lesions. One in five infected people have lesions in their eyes, more than two-thirds have lesions in their mouths, more than two-thirds have lesions in their genital area, and more than two-thirds have lesions on their palms and soles. They begin as little flat patches, then transform into tiny lumps, filling with clear, then yellow fluid, finally bursting and scarring [16]. There may be a few or many thousand lesions, which occasionally combine to form larger lesions. Every area of the body that is afflicted by lesions develops at the same stage. It resembles a smallpox rash in appearance. The rash often lasts for ten days or less. Affected people may feel under the weather for two to four weeks. After the lesions have healed, they may leave behind pale marks before leaving behind black scars. Only a small amount of infection from person to person has been observed in Africa's disease-endemic regions [17].

Pathogenesis of monkeypox

With the exception of the fact that viral entry from a wildlife source most frequently occurs through small lesions on the skin or oral mucous membranes, the pathogenesis of human monkeypox is substantially the same as that of smallpox. Viral entry can also happen through the respiratory tract in rare instances of person-to-person transmission. Although the monkeypox virus replicates in lymphoid tissue similarly to the smallpox virus, it results in greater lymphadenopathy [18]. The virus initially manifests in mononuclear phagocytic cells, moves through the bloodstream, and eventually resurfaces in skin cells. The virus swiftly infects nearby lymph nodes after an infection. On the third or fourth day following infection, the virus enters the bloodstream and moves to the bone marrow, sleep, and other lymph nodes. Once the virus has been in the blood for 8 to 12 days, fever and toxemia set in [19]. The virus has now been localized in the oral mucosa and dermal blood vessels, resulting in the initial enanthem and exanthem. By day 14, the host is contagious, and high viral titers are found in the kidneys, liver, spleen, lymph nodes, and bone marrow. Skin lesions develop as a result of endothelial cell vessel walls and dermal capillaries dilating [20]. When these lesions reach the epidermis, swelling cells eventually grow so enormous that their membranes rupture. Pus forms after vesicular lesions as a result of the concentration of polymorph nuclear cells inside the vesicle. The legions scramble when the pus progressively vanishes. The most frequent cause of mortality is overwhelming toxemia, which is most likely brought on by circulating immune complexes. The long-term repercussions of secondary infections include encephalitis, blindness, and arthritis [21].

Epidemiology of the disease

While clinically less severe than smallpox, monkeypox is a viral zoonosis (a virus that spreads from animals to people) with symptoms that are strikingly similar to those experienced by smallpox patients in the past. It is brought on by the orthopoxvirus genus monkey poxvirus, which is a member of the Poxviridae family. The West African and Congo Basin (Central African) clades of the monkeypox virus are separated. Monkeypox is the name given to the virus because it was originally identified in primates in a Danish laboratory in 1958. [22]. The first human case was found in a child in the Democratic Republic of the Congo in 1970. The monkeypox virus can be transferred from one person to another by close contact with lesions, bodily fluids, respiratory droplets, and infected surfaces like beds. Monkeypox can incubate for anywhere from 5 and 21 days, but it typically takes 6 to 13 days. Numerous animal species have been identified to be susceptible to the monkeypox virus's contagiousness [23]. Further investigation is required to identify the precise reservoir(s) and how virus circulation is maintained in nature because the natural history of the monkeypox virus is still unknown. Eating raw meat and other animal products from diseased animals may pose a risk [24]. Although it can be severe in some people, such as children, expectant mothers, or those whose immune systems have been weakened by other illnesses, monkeypox is typically self-limiting. With a case fatality rate of 3.6% compared to 10.6% for the Congo Basin clade, the West African clade appears to induce less severe disease in humans [25].

Geographical distribution and high-risk areas

With 106 persons confirmed to have the disease as of May 26, 2022, the UK has the highest number of cases in a non-endemic country, according to the WHO, followed by 49 people in Portugal and 26 in Canada (Figure 3).

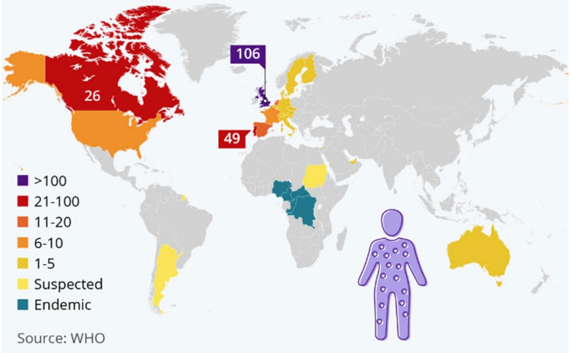

Figure 3: Number of recorded monkeypox cases per country (May 13-26, 2022) and where the disease is endemic (Source WHO).

No fatalities have been connected to the incident as of yet. In the instances that have been recorded so far, there have been no confirmed travel connections to an endemic region [26]. According to the information that is currently available, occurrences have mainly — but not exclusively — been found among men who have sex with men (MSM) who visit general care and sexual health clinics for treatment. All cases whose samples had the West African lineage of the virus confirmed by PCR to date have been identified. A genomic sequencing from a swab sample from a confirmed case in Portugal showed a close match to the monkeypox virus driving the current outbreak to cases shipped from Nigeria to the United Kingdom, Israel, and Singapore in 2018 and 2019. [27]. Finding confirmed and probable cases of monkeypox without any known travel history to an endemic region is unusual. Until recently, surveillance in non-endemic areas was minimal, but it is currently being increased. According to the WHO, more instances are anticipated to be reported in non-endemic regions. The research suggests that those who come into contact with symptomatic instances directly on a bodily level are where human-to-human transmission happens [28]. Aside from this new epidemic, WHO continues to receive data through established surveillance techniques on the status of ongoing cases of monkeypox in endemic countries (Integrated Disease Surveillance and Response). Monkeypox is endemic in Benin, Cameroon, Central African Republic, Gabon, Ghana (only in animals), Ivory Coast, Liberia, Nigeria, Republic of the Congo, Sierra Leone, South Sudan, and the Central African Republic [29].

WHO risk assessment

Only West and Central Africa often experience the endemic monkeypox disease. There is an urgent need to increase awareness of monkeypox and carry out thorough case finding and isolation [along with supportive care], contact tracing, and supportive care to prevent further onward transmission because it is unusual to find confirmed and suspected cases of monkeypox in multiple countries without a history of travel to an endemic area [30]. Cross-protective immunity from smallpox immunization will be restricted to the elderly since populations under the age of 40 or 50 no longer benefit from the protection offered by prior smallpox vaccination programs. Younger individuals have only a limited level of immunity to monkeypox because the virus has not been seen in non-endemic regions. In the past, it has been discovered that the smallpox vaccine can prevent monkeypox [31]. Despite the licensing of one vaccine (MVA-BN) and one specific treatment (Tecovirimat) for monkeypox in 2019 and 2022, respectively, these preventative interventions are still not generally accessible. According to the information that is currently available, occurrences have mainly — but not exclusively — been found among men who have sex with men (MSM) who visit general care and sexual health clinics for treatment. No fatalities have been noted to date [32]. The extent of local transmission is currently uncertain, though, because of the scant observation. There is a good likelihood that other cases, including in different population subgroups, with unidentified transmission networks, will be found. Cases of monkeypox have been recorded in several countries spanning several WHO regions, and it is very likely that cases will be found in more nations [33]. The situation is evolving, and according to WHO, additional cases of monkeypox will be discovered as surveillance in non-endemic nations grows. So far, there have been no fatalities connected to this outbreak. The first efforts should concentrate on informing those most at risk of monkeypox infection accurately, stopping the disease's spread, and safeguarding first responders [34].

Host Immune Responses to Monkeypox

Poxviruses have developed a variety of methods or tactics to circumvent or worsen the host's immune reaction to infection. Although much study has been done on the pathology and pathogenesis of monkeypox, the innate and adaptive immune responses to monkeypox infection are still poorly understood due to a paucity of data. A crucial element of innate immunity, natural killer (NK) cells employ cytokines to kill virus-infected cells and interfere with the function of other cell types like T cells and dendritic cells [35]. Activating or inhibitory NK cell receptors engage with their ligands, such as major histocompatibility complex 1 (MHC-1) molecules, to cause activation or inhibition of NK cells. Granule secretion, which includes perforin and granzyme, and cell-cell contacts are the mechanisms by which NK cells carry out their killing activity. IFN- and TNF-, which are generated by NK cells in the early stages of infection, regulate dendritic cells to polarize T-helper type 1 (Th1) cells and mediate inflammatory responses in inflamed tissues [36]. Infections with human monkeypox have not been associated with T cell or antibody responses in Nigeria. Additionally, there are no data or reports on the immunological reactions that occur after experimental infections in animal models. An immunologically naive population that is very susceptible to monkeypox infection has been caused by the lack of cross-protection produced by the smallpox vaccination, as well as the probable weakening of herd immunity and immunosuppression as a result of HIV/ AIDS. This may be the cause of the current global resurgence of monkeypox (Figure 4) [37].

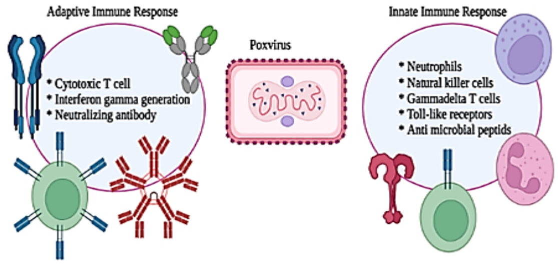

Figure 4: Host Immune Responses to Monkeypox

Prevention and Vaccination

It is expected that smallpox vaccination will protect against human monkeypox infection because the two viruses are closely related and the vaccine shields animals from lethal monkeypox challenges in experiments. This has not been properly demonstrated in individuals because systematic smallpox immunization was stopped when smallpox was eradicated. The smallpox vaccine has been demonstrated to reduce the risk of monkeypox in previously immunized individuals in Africa. A reduction in poxvirus immunity in communities that have been exposed affects the occurrence of monkeypox [38]. It is attributed to a rising proportion of unvaccinated people as well as a decline in cross-protective immunity among those who received an immunization before the cessation of mass smallpox vaccinations in 1980. The US Centers for Disease Control and Prevention (CDC) advises being vaccinated against smallpox in order to protect themselves from the disease if they are going to be handling sick people or animals or looking into outbreaks of monkeypox. Anyone who has had close or direct contact with people or animals that have monkeypox infection is also advised to get vaccinated [39]. The CDC advises against pre-exposure immunization for veterinary professionals, veterinary assistants, and animal control officers unless they are taking part in field investigations. The CDC advises healthcare professionals to put on a full set of personal protective equipment before treating an infected person (PPE). Included are a gown, mask, pair of goggles, and a disposable filtering respirator (such as an N95). An infected person should be isolated in a negative air pressure chamber or, at the very least, a private exam room to prevent others from coming into touch with them [40]. In the United States and the European Union, Tecovirimat has a license to treat a variety of poxviruses, including monkeypox. BMJ Best Practice recommends tecovirimat or the smallpox drug Brincidofovir, together with supportive care, if antiviral treatment is required [including antipyretic, fluid balance, and oxygenation]. Aciclovir or empirical antibiotic therapy may be used if a subsequent bacterial or varicella zoster infection is suspected [41].

AN ANALYSIS OF POTENTIAL THREATS

While the COVID-19 epidemic is still going strong, other viral diseases are starting to show warning symptoms in various nations around the world. Since the disease's initial identification in 1958, small, recurring outbreaks of monkeypox have been noted. However, with 100 confirmed cases and almost 300 suspected cases recorded in more than 16 countries, we are now witnessing a larger spread of the virus [42]. 13 solitary cases of monkeypox have been documented in recent years, the majority in a single nation. The virus can be found in Central and West Africa, where it is believed to be present. Three cases of monkeypox were reported in Britain in May and June 2021; each case was linked to travel to West Africa. The World Health Organization claims that the current outbreak is different and that there are far more cases, more cases of human-to-human transmission, and more cases of infection outside of Africa [43]. Although there is limited human-to-human transmission, monkeypox is typically spread from diseased animals to humans in Central and West Africa. Before skin irritation manifests as blisters on the skin, mouth, genitals, eyes, or soles of the feet, smallpox first causes fever, headache, exhaustion, and enlarged lymph nodes. It's probable that the short-term increase in instances of monkeypox transmission has resulted from the resumed travel and big social gatherings after the coronary limitations were loosened [44]. Although current statistics indicate that this strain of the virus is quite similar to the West African variant that has previously caused modest outbreaks, it is also likely that the virus has been changed or modified to allow for wider transmission. However, because of prior coronary infections, this outbreak could develop differently [45]. Our immune systems can be affected by infections from the COVID-19 virus, and current research from Australian experts reveals that this condition may last for weeks to months after the initial infection. According to scientists, it's also conceivable that this immunological dysfunction may modify clinical illness, accelerate viral transmission, or raise vulnerability to infection [46]. Despite the peculiarity of the present monkeypox epidemic, the US Centers for Disease Control and Prevention predict that there will certainly be more in the following weeks. It takes some time to determine the entire scope of an epidemic since symptoms can appear up to three weeks after exposure and the virus can spread for up to a month. Given that the primary way that monkeypox is transmitted is via intimate contact with someone who has a rash or blister, it is unlikely that the illness will provide a fresh hazard at this time [47].

CONCLUSION

Considering what has been said so far, healthcare executives ought to provide staff members with the proper training in four areas: potential threats, prevention, detection, and care, as well as reporting.

Potential threats: Monkeypox is most commonly transferred by intimate contact with someone who has a rash or blister, thus under the current circumstances it is unlikely that the illness would pose a new threat.

Prevention: Direct contact with an infected person, especially sexual contact, puts one at risk for contracting monkeypox. Self-protection measures include avoiding skin-to-skin or face-to-face contact with people who are exhibiting symptoms, engaging in safer sex, washing your hands often with soap and water or an alcohol-based hand rub, and following respiratory etiquette.

Detection and care: People should visit their doctor and get tested for monkeypox if they get a rash that is accompanied by a fever, a feeling of discomfort or sickness, or both. If you have monkeypox, you should stay home until the scabs come off and avoid intercourse, especially oral sex. Patients can get supportive care to ease their monkeypox symptoms throughout this period. Anyone providing care for someone who has monkeypox should take the necessary measures, such as donning a mask and wiping down all objects and surfaces that have been touched.

Reporting: As soon as possible after returning home, anybody with a rash-like illness should consult a doctor and provide information about all recent travel, sexual history, and smallpox vaccinations. Avoid interacting with ill animals including rats, marsupials, and nonhuman primates (alive or dead), as well as eating or handling wild game, if you reside in or visit a country where monkeypox is prevalent (bush meat).

Conflicts of interest and sources of funding

The authors declare that there were no confict of interest regarding this paper.

Authors' contribution

Conceptualization, H.E.G.G. and M.F.; methodology, S.Y.S.; validation, T.B., and M.J.; formal analysis, M.M.; investigation, A.R.; resources, M.M.; data curation H.E.G.G.; writing original draft, A.R.; writing, review and editing, A.R.; visualization, H.E.G.G.; supervision, A.R. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

PET-CT with 18F-FDG in Gynecological Malignancies. Prognostic Significance of the Standardized Uptake Value

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Abstract: *Gynecologic cancers are among the most common cancers in women and are a significant cause of mortality in women worldwide. FDG-PET images play a critical role in several areas of oncology and are an essential tool for the diagnosis and staging of gynecologic cancer, providing information about tumor metabolism. Aim: The aim of this study was to investigate the relationship between tumor differentiation, type of malignancy, and glucose metabolism in patients with confirmed gynecologic cancer. Materials and Methods We performed a retrospective review of 60 consecutive patients (ages 31-76) with histopathologically confirmed gynecologic cancer who underwent PET-CT in our department between January 2021 and March 2022. Quantitative data on glucose metabolism were collected using the maximum standardized uptake value normalized by lean body mass (SULmax). We reviewed a total of 60 patients with a mean age of 60±9 years, and a median of 62. Results: 14 patients were diagnosed with cervical cancer, 20 with endometrial cancer, and 26 with ovarian cancer; 13 patients had well-differentiated cancer, 11 had moderately differentiated cancer, and 36 had poorly differentiated cancer. A positive correlation was observed between the measured SULmax and the degree of tumor differentiation, indicating higher glucose uptake in poorly differentiated cancer. When examining the relationship between the type of cancer and SULmax, we found that endometrial cancer exhibited the highest mean SULmax (8.36±6.75), followed by ovarian cancer and cervical cancer. Additionally, we noted that glucose uptake was higher in patients aged over 62 years. Regardless of the tumor differentiation, the findings in endometrial cancer showed the highest mean SUVmax values in all grades of tumor differentiation. Conclusion: Our study showed that glucose consumption correlates not only with the degree of differentiation but also with age and type of tumor, with the highest values in patients older than 62 years with endometrial cancer. Given the correlation between glucose consumption and tumor aggressiveness, this finding may be important for prognostic evaluation of patients with gynecologic malignancies.*

Keywords: *18F-FDG, PET-CT, Gynecologic cancer, glucose consumption, cancer differentiation*

BACKGROUND

Gynecologic cancers are among the most common cancers in women and are a major cause of mortality in women worldwide. There are five main types of gynecologic cancers: cervical cancer, ovarian cancer, uterine cancer, vaginal and vulvar cancer.

Despite advances in diagnosis, gynecologic cancers remain a major health problem worldwide, with a large number of cases already in a locally advanced stage of the disease at presentation.

Of all gynecologic cancers, only cervical cancer has screening tests that can detect it at an early stage when treatment is most effective. However, despite existing screening programs, cervical cancer is the third most common cancer in women worldwide [1]. With the exception of cervical cancer, there is no simple and reliable method for early detection of gynecologic cancers, making it especially important to recognize the warning signs.

Based on histologic criteria, gynecologic cancers are classified into three grades. Grading is a measure of cell differentiation in the tumor, based on the similarity of the tumor to the tissue of origin. The grade describes the appearance of the cancer cells as follows: Grade 1 (G1; well differentiated) and Grade 2 (G2; moderately differentiated) are the most common categories, while Grade 3 (G3; poorly differentiated) indicates a very aggressive tumor [2].

Positron emission tomography (PET) with fluorine-18-fluorodeoxyglucose (FDG) is an important tool for the diagnosis and staging of gynecologic cancer, providing information about tumor metabolism [3].

FDG-PET images play a critical role in several areas of oncology, primarily because of its ability to detect metabolic changes that may occur earlier and provide more meaningful insights into the effects of therapy compared with morphologic changes alone [4]. As detected by FDG-PET, tumor metabolic activity can provide a faster and more accurate assessment of treatment response. This allows physicians to assess the effectiveness of therapies earlier and adjust treatment plans in a timely manner if needed. By providing valuable metabolic information, FDG-PET contributes to more precise and personalized cancer management that can support treatment decision-making and improve patient outcomes [5].

PET is used in conjunction with computed tomography (CT) to precisely localize regions of increased focal uptake and to compensate for the attenuation effect of incident photons (attenuation correction). With the introduction of integrated PET/CT scanners, the limitations associated with inadequate anatomical definition of functional examinations have been overcome and image quality has been significantly improved [6,7].

In routine clinical practice, PET lesions are analyzed using the standardized uptake value (SUV), a semiquantitative parameter that allows assessment of metabolic activity but is also useful for quantifying the post-therapeutic metabolic response of the tumor. Since 1999, the European Organization for Research and Treatment of Cancer (EORTC) has introduced metabolic criteria that use the standardized uptake value (SUV) in positron emission tomography (PET) to evaluate the response to therapy. These criteria were developed to assess tumor metabolic activity and monitor changes in SUV levels before and after treatment. By analyzing SUV values, these criteria are expected to provide valuable information about the efficacy of cancer treatments and support clinical decision-making [8].

Response to therapy can also be assessed using CT imaging according to the Response Evaluation Criteria in Solid Tumors, but these criteria do not reflect metabolic changes. Tumors detected by morphologic imaging may contain nonviable necrotic tissue. Therefore, measurements from PET may provide more accurate information than a purely anatomic evaluation such as CT [9,10].

The aim of this retrospective study was to investigate the relationship between glucose metabolism, characterized by the uptake of 18F-FDG, measured by the maximum standardized uptake value normalized to lean body mass (SULmax), tumor differentiation, and the type of gynecologic cancer. The study was performed on the 3 major types of gynecologic malignancies – cervical, endometrial, and ovarian cancer.

MATERIALS AND METHODS

Patients

We retrospectively reviewed 60 patients with a type of gynecologic cancer aged 31 to 76 years who underwent FDG-PET/CT between January 2021 and March 2022. PET/CT images were evaluated for primary tumors, locoregional spread, and distant metastases. Quantitative data were collected using the maximum standardized uptake value normalized to lean body mass (SULmax) calculated in the pathologic regions of interest.

PET-CT imaging

PET/CT examinations were performed at the Central Emergency Military University Hospital in Bucharest, Romania, using a dedicated scanner. The weight and blood glucose level of each patient were measured before the examination. The actual injected and residual radioactive doses were also measured.

Before the scan, patients fasted for 6 hours, and only patients with a serum glucose level of < 200mg/dl were injected intravenously with 18F-FDG. Image acquisition started 50-60 minutes after injection. Patients were positioned with their arms raised above their heads. All examinations were performed according to a standard protocol, scanning from tentorium to mid-thigh.

PET/CT data analysis

Maximum SUV normalized to lean body mass (SULmax), was determined from FDG-PET studies and was measured in primary tumors (when possible) and in secondary lesions.

Statistical data analysis

The mean SUV was calculated for each type of cancer and for each of the three grades of differentiation.

RESULTS

From January 2021 to March 2022, a total of 60 patients with a mean age of 60±9 years, and a median of 62, were enrolled in the study. Of these, 14 patients were diagnosed with cervical cancer, 20 with endometrial cancer, and 26 with ovarian cancer. After analyzing the grade of tumor differentiation, we found that 13 patients were diagnosed with well-differentiated cancer, 11 patients with moderately differentiated cancer, and 36 patients with poorly differentiated cancer (Figure 1).

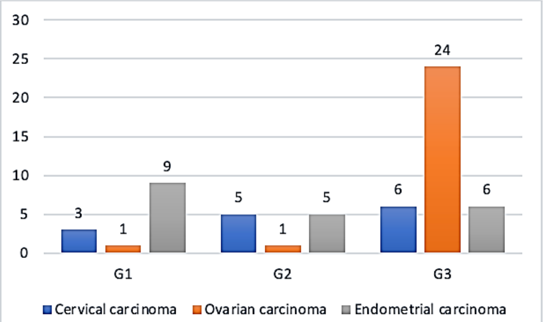

Figure 1: The relationship between gynecologic cancer type and tumor differentiation

The patients' and tumors' characteristics are listed in Table 1.

Table 1: Patient and tumor characteristics

	Age, year
Mean	60+/-9
Range	31-76
Type of cancer, nr.	
Cervical cancer	14
Ovarian cancer	26
Endometrial cancer	20
Tumor differentiation, nr.	
Well-differentiated cancer	13
Moderate differentiated cancer	11
Poorly differentiated cancer	36

The mean SULmax value for the entire group of patients was calculated as 7.83±5.73, with a mean SULmax value of 4.20±3.42 in the group of patients with well-differentiated cancer; 7.96±3.74 in the group with moderately differentiated cancer, and 8.40±6.28 in poorly differentiated cancer. There was a positive correlation between the measured SULmax, tumor differentiation, with higher glucose uptake in poorly differentiated cancer (Figure 2).

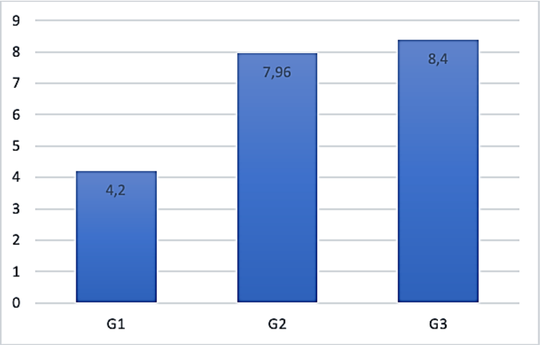

Figure 2: The relationship between glucose metabolism and tumor differentiation

When we analyzed the relationship between cancer type and SULmax, we found that endometrial cancer had the highest mean SULmax (8.36±6.75), followed by ovarian and cervical cancer.

Regardless of differentiation grade, endometrial cancer had the highest mean SULmax values in all differentiation grades. Among moderately differentiated tumors, cervical carcinoma showed higher values than ovarian carcinoma and lower values than endometrial carcinoma, and among poorly differentiated tumors, ovarian carcinoma showed higher values than cervical carcinoma and lower values than endometrial carcinoma (Figure 3).

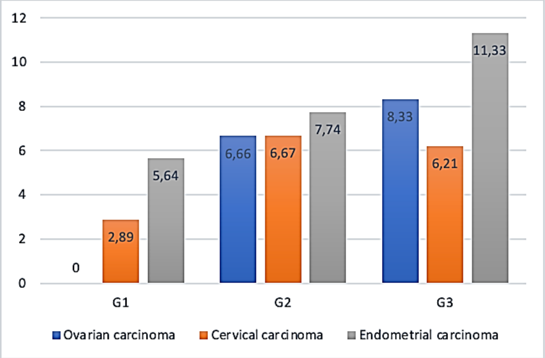

Figure 3: The relationship between glucose metabolism, type of malignancy, and tumor differentiation

The degree of tumor differentiation positively correlated with age, with patients diagnosed with G1 tumors having a mean age of 57±10 years, while patients with G3 tumors have a mean age of 62.7 ±9 years. We also found that glucose uptake was higher in patients older than 62 years.

DISCUSSION

In this study, we found that FDG uptake in primary tumor and secondary lesions varied according to cancer type and degree of differentiation. The SULmax value correlated with the degree of tumor differentiation in all gynecologic cancers.

Endometrial cancer had a significantly higher SULmax value than ovarian and cervical cancer for all tumor differentiation grades. Cervical cancer had higher values than ovarian cancer for moderately differentiated tumors, and for poorly differentiated tumors, the SULmax value was higher for ovarian cancer than for cervical cancer.

The current study also found that poorly differentiated tumors had a higher SULmax value than well- or moderately-differentiated tumors, with a significant difference in SULmax value for well- and poorly-differentiated tumors.

Similar results were obtained by Takagi et al. on a group of patients with endometrial carcinoma. They discovered that the level of glucose uptake significantly differs in patients with different grades of differentiation, being higher in those with G2 and G3. They also found that the degree of glucose uptake correlates with tumor size [11].

Another study that refers to the prognostic value of glucose uptake and its relationship with the degree of differentiation was conducted by Walentowicz-Sadlecka et al. The results demonstrated a progressive increase in glucose uptake as the degree of tumor differentiation advanced. Specifically, patients categorized as G1 exhibited lower uptake, while those in G2 and G3 displayed higher values. However, in their study, statistically significant differences between the three grades of differentiation were observed only in the case of patients classified in the G1 group compared to those in the G2 and G3 groups [12].

Kidd et al. show that tumor differentiation affects 18F-FDG uptake in cervical cancer. They found the same results regarding the correlation between FDG uptake and tumor differentiation as we did in our study. They also found that higher glucose uptake was correlated with an increased risk of lymph node metastasis in patients with cervical cancer [13,14].

More recently in patients with recurrent ovarian cancer, Jiang et al demonstrated that the level of glucose uptake in peritoneal lesions was a significant independent prognostic element [15].

On the other hand, in the study of patients with cervical cancer conducted by Mocciaro V et al, no correlation was found between the SULmax value and the degree of tumor differentiation [16].

The presence of conflicting results indicates that this is a complex area of research that requires prospective studies with a larger number of patients to evaluate the correlation between the parameters of PET and the histopathological features of gynecologic tumors.

Glucose uptake has also been associated with tumor aggressiveness in other cancers. Sachs S et al. reached similar results in a study of patients with lung cancer, showing that higher glucose uptake in the primary lesion was predictive of lymph node involvement and more aggressive tumor behavior [17]. Some authors also suggested that the correlation between FDG uptake in non-small cell lung cancer (NSCLC) and tumor proliferation may allow FDG-PET to serve as a non-invasive method for tumor grading. This is the case in the work published by Vesselle et al. in which tumor differentiation in NSCLC was found to correlate exactly with glucose uptake, with well-differentiated tumors having lower FDG uptake. [18].

As for the relationship between the age of patients at diagnosis and the aggressiveness of the cancer, expressed by the degree of tumor differentiation and SUVmax values, the results of our study suggest a positive correlation. These findings are also supported by studies conducted by Koual et al. and Gonzales-Rodilla et al. who described similar results when studying patients with endometrial cancer [19,20].

The current study provides some new insights into the relationship between gynecologic cancer, tumor differentiation, and glucose metabolism. We believe that the relationship between glucose uptake and the degree of tumor differentiation in gynecologic cancer could serve as a valuable prognostic tool and add to the existing clinical knowledge in this field.

Our study has some limitations – the relatively small number of patients and also the histologic type of malignancies, which included only carcinomas. As mentioned earlier, larger prospective studies are needed to validate glucose uptake, expressed as SUL values, as a prognostic marker in gynecologic malignancies.

CONCLUSIONS

In conclusion, the current study provides valuable insight into gynecologic cancer by correlating glucose uptake and the degree of tumor differentiation. The study also shows that glucose metabolism is highest in endometrial cancer, followed by ovarian and cervical cancer. In addition, the results show that glucose metabolism tends to increase with age. These findings contribute to our understanding of gynecologic cancer and highlight the importance of considering SULmax values and glucose metabolism as potential factors in disease evaluation and treatment.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

R.M, D.C and P.M.S. planned the clinical study and contributed to the concept and design of the study. C.S, C.M., D.C., M. J and F.I. contributed to the data analysis and interpretation as well as to the translation and the critical revision of important intellectual content. T.M. and L.M. contributed to the writing of the manuscript and performed revision, language, and textual editing of the final version.

All authors contributed to the original draft preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

Ethics approval and consent to participate

All procedures performed in this paper were by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and according to the national ethical standards.

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ORIGINAL ARTICLE

The Psychiatric, Psychological, and Psychotherapeutic Approach to Erectile Dysfunction – Between Good Practices and Clinical Challenges

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Abstract: *Erectile dysfunction (ED) has an intricate pathogenesis, with organic and psychosocial factors contributing to the shaping of its clinical manifestations and functional impairment. ED disrupts not only an individual’s sexual life but may also contribute to impairments of self-esteem, social functioning, quality of life, overall well-being, mood, etc. The assessment process and therapeutic interventions should be adequate to the specific profile of each patient, therefore an interdisciplinary approach is usually recommended, in order to find the vulnerability factors, recent triggers, psychiatric and organic comorbidities or causes, and elements that contribute to maintaining the sexual dysfunction, such as lifestyle factors, interpersonal conflicts, or inadequate sexual education. This narrative review explores the evidence-based approaches to structured psychiatric, psychological, and therapeutical assessment in patients with ED, and the most validated psychosocial treatments available. The results support the use of recognized diagnostic criteria within DSM-5TR and ICD-11, together with structured instruments (scales, questionnaires, and inventories), in a multidisciplinary approach. The cognitive model and cognitive-emotional model of ED support the initiation of cognitive-behavioral therapy in these patients. Other interventions, like the PLISSIT model, sexual therapy, couple therapy, and sexual-focused psychoeducation are also supported by evidence. In conclusion, ED requires a combined, psychiatric, psychologic, psychotherapeutic, and organic-oriented assessment, with the purpose of tailoring the treatment to the patient’s particularities.*

Keywords: *major depressive disorder, erectile dysfunction, antidepressants, antipsychotics, schizophrenia spectrum disorders, psychotherapy, psychoeducation*

INTRODUCTION

Erectile dysfunction (ED) is one of the most frequently reported sexual pathologies, with an estimated global prevalence varying from 3% to 76.5% [1]. The large variations in the reviewed reports regarding the prevalence of ED are explained by the method chosen during studies to define and quantify the manifestations of this disorder. When structured, validated clinical scales, questionnaires, or inventories were used, the prevalence of ED in young men was high [1]. Non-validated instruments have lower sensitivity, therefore the prevalence obtained using such tools is expected to be less accurate [1,2].

It is also important to highlight that ED was positively correlated with cardiovascular diseases, a high risk of all-cause mortality, mortality due to cardiovascular pathology, and a higher risk of developing dementia [1]. Further exploring this topic, ED is considered a “sentinel marker” for cardiovascular disease and could indicate a chronic and undetected coronary artery disease [2]. This correlation was supposed to be more significant in men with diabetes mellitus [2]. ED demonstrated significant value as an indicator of general health in men, irrespective of the cardiovascular risk factors [3].

Therefore, the importance of exploring the most adequate ways to diagnose and treat ED derives not only from the relevance of this disorder on the quality of life and well-being of the patients but also from the potential comorbid conditions this diagnosis may reveal during the multidisciplinary assessment.

OBJECTIVES AND METHODOLOGY

The main objective of this review was to find the most evidence-based assessment methods for patients with ED from the perspective of mental health professionals. Secondary objectives were founding data on (a) the available psychosocial approaches to ED; (b) ways to integrate psychological, psychiatric, and psychotherapeutic evaluations for patients with ED; (c) finding the key aspects that can help the training of mental health specialists in the evaluation and treatment of patients with ED.

The identification of relevant articles involved searching three electronic databases (PubMed, Cochrane, and Clarivate/ Web of Science) as well as their list of references. There was no inferior time limit for the search, while the superior limit was June 2023.

All primary and secondary reports were allowed into the analysis. No language restrictions were applied.

The paradigm used for retrieving sources was “erectile dysfunction” OR “male sexual dysfunctions” AND “assessment” OR “psychotherapy” OR “counseling” OR “diagnosis” OR “cultural aspects” OR “skills training” OR “mental health specialists”.

RESULTS

The results of the literature search were distributed in three sections, according to their relevance for each category of mental health specialists involved in the assistance of patients with ED. The accent is placed on the need to conduct a multidisciplinary exploration of the ED (Figures 1 and 2) and to formulate a therapeutic plan that takes into account the main vulnerability factors identified during the initial assessment of the patient.

The psychiatric approach

The diagnosis of ED implies, according to the 5th edition (revised) of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5TR), significant difficulty in reaching or maintaining an erection, or a marked decrease in the penile rigidity during erection, that appears on almost all occasions of sexual activity (75-100%) [4]. There is also the requirement of a minimal duration of 6 months for these symptoms, and they should cause clinical distress, in order to complete the diagnosis criteria [4]. Like in the case of all sexual dysfunctions, ED should be distinguished from organic, toxic, stress-induced pathology, or other mental disorders [4]. ED may be lifelong (if the patient did not have any period of normal sexual functioning) or acquired (if the dysfunctions started after a period of normal sexual functioning) [4]. ED can also be mild, moderate, or severe (depending on the level of symptoms-induced distress), or it may be generalized or situational (depending on the types of stimulation, situations, or partners that may or may not trigger the symptoms) [4].

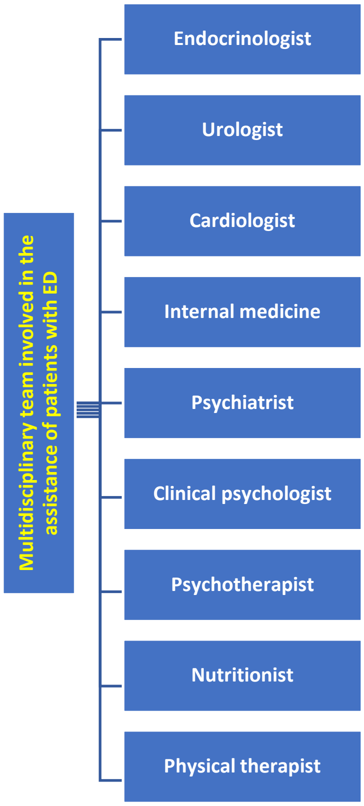

Figure 1: Multidisciplinary assessment of patients with ED

The diagnostic criteria in the DSM-5 and DSM-5TR changed from the previous versions (DSM-IV and DSM-IV TR) [4-7]. In these early editions, only two clinical criteria were mentioned, without any reference to the decrease of penile rigidity, as an independent criterion; also, no frequency of failed sexual acts was mentioned; no severity specifiers existed, but ED could have been classified as exclusively psychogenic or with mixed causation [6,7]. Further back, the third edition (revised) of DSM (DSM-III(R)) included the “male erectile disorder”, defined as a partial or complete, persistent and recurrent, incapacity to obtain and maintain the erection until the end of a sexual act, or recurrent/persistent lack of sexual excitation and pleasure during the sexual activity [8]. ED is included in the category of “male sexual dysfunctions”, together with delayed ejaculation, premature ejaculation, and male hypo-sexual desire disorder (Figure 3) [4-7].

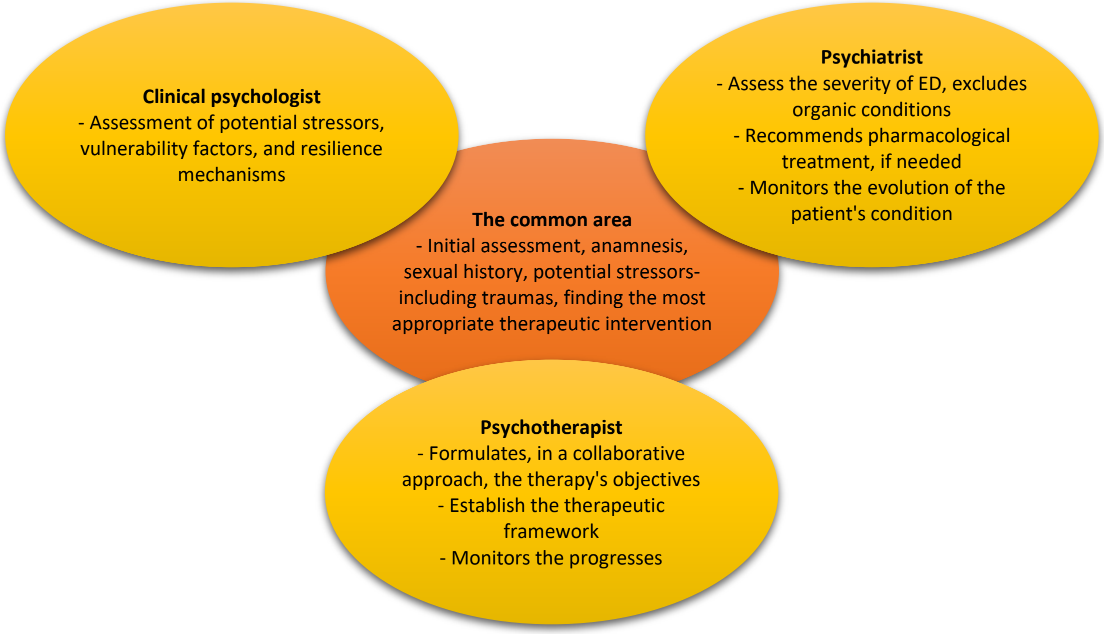
Clinical psychologist
- Assessment of potential stressors, vulnerability factors, and resilience mechanisms
Psychiatrist
- Assess the severity of ED, excludes organic conditions
- Recommends pharmacological treatment, if needed
- Monitors the evolution of the patient's condition
The common area
- Initial assessment, anamnesis, sexual history, potential stressors-including traumas, finding the most appropriate therapeutic intervention
Psychotherapist
- Formulates, in a collaborative approach, the therapy's objectives
- Establish the therapeutic framework
- Monitors the progresses

Figure 2: The roles of the clinical psychologist, psychiatrist, and psychotherapist in the assistance of patients with ED

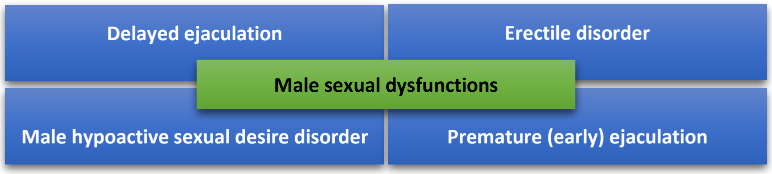
Delayed ejaculation
Erectile disorder
Male sexual dysfunctions
Male hypoactive sexual desire disorder
Premature (early) ejaculation

Figure 3: Male sexual dysfunctions [4-7]

The World Health Organisation's Classification of Diseases (ICD) mentioned in its latest edition (ICD-11), that ED is the "inability or marked reduction in the ability in men to attain or sustain a penile erection of sufficient duration or rigidity to allow for sexual activity" [9]. Also, ICD-11 specifies that ED appears although the libido and sexual stimulation exist, and its manifestations may be episodic or persistent (several months), and associated with significant distress at the clinical level [9]. In the previous edition, ICD-10 (the version for clinical research), ED had as its main clinical characteristics (1) the impossibility of participating in sexual activity according to the desire of the individual, (2) the dysfunction appears frequently, but may not be observed on every occasion, (3) the manifestations have a history of at least 6 months [10]. The ED may manifest itself as: a complete erection that may be obtained in the initial stage of sexual activity, but then disappears before ejaculation (if this appears); erection may be detected, but only in situations where the sexual activity is unprepared; partial erection, insufficient for sexual activity; or complete absence of erection [10].

The psychiatric assessment of patients with ED is recommended to be carried out from both the clinician's perspective (focusing on signs and symptoms) and the patient's (focusing on the consequences of dysfunction). There may be significant differences between the two perspectives, depending on the criteria for defining ED, for example, the duration of the manifestations, the frequency of their occurrence, and the functional impact. Sometimes the patient can admit the presence of erectile dysfunction and the associated discomfort, but at other times he avoids mentioning any aspect related to sexual functioning, presenting only general symptoms - "weakness", "permanent fatigue", "lack of initiative", etc.

Sexual dysfunction can be an isolated symptom or it can be integrated into a complex context, as it may appear together with multiple psychiatric symptoms (such as anxiety, insomnia, depression, psychotic manifestations, and substance addiction) or organic manifestations (like urinary hesitancy, high blood pressure, pelvic pain, etc.).

Among the main differential diagnoses in ED, major depressive disorder (MDD) is important to be considered, because a decreased libido has been detected in these patients during the acute episodes, and some of the adverse events of antidepressants involve sexual functioning [11-17]. According to a systematic review and meta-analysis (n=49 reports), there is a high comorbidity rate of ED in patients with MDD (OR=1.39) and vice versa (OR=2.92), but a causal relationship can not be determined [11]. The incidence of sexual dysfunctions in patients undergoing treatment for MDD is difficult to be determined, but decreased sexual desire, decreased sexual excitement, reduced ability to reach orgasm, delayed orgasm, ED, delayed ejaculation, priapism, painful ejaculation, penile anesthesia were all reported in this population [12]. Therefore, sexual dysfunction may be a common MDD symptom, but also a frequent adverse effect of antidepressant treatment [14]. Due to this lastly-mentioned phenomenon, premature drug discontinuation in patients with MDD is not an uncommon event [14,18]. Nefazodone and bupropion have been associated with a lower incidence of sexual adverse events, due to their pharmacodynamic properties, unlike the selective serotonin reuptake inhibitors (SSRIs), which are among the most frequent drugs associated with such negative events [18]. At any rate, monitoring sexual adverse events during antidepressant treatment, as well as monitoring sexual functioning in these patients as part of the MDD clinical picture, are very important for preserving therapeutic adherence and quality of life [18-20].

Psychosocial stressors can trigger both major depression and erectile dysfunction [11]. Depression itself can reduce sexual functioning through multiple mechanisms (decreased initiative, fatigue, decreased self-esteem, ideas of uselessness and inability, etc.) [11-13]. Persistent erectile dysfunction can lead to reduced self-esteem, rumination about personal incapacity, and depression. In conclusion, patients with ED should be evaluated for signs of major depression and vice versa [11].

Psychotic disorders involve the presence of delusional ideas (some of which may have a sexual theme), hallucinations (some may be proprio- or interoceptive), thought and language disorganization, behavioral disorganization, negative symptoms (avolition, apathy, flat affect, anhedonia, alogia, etc.). Patients with psychotic disorders frequently associate sexual dysfunctions as a result of negative symptoms, antipsychotic medication, associated depression, etc [21-29]. In patients with psychotic disorders who had never received psychotropic treatment, 17% still accused the presence of sexual dysfunction [22]. Therefore, it is clear that not all sexual dysfunctions reported in this population are secondary to pharmacologic aspects, although a false causal relationship may be construed by patients, relatives, or even treating physicians. It must be mentioned that most mental health professionals do not ask their patients with psychosis about sexual dysfunctions, in clinical settings [23]. The sexual functioning in outpatients with schizophrenia spectrum disorders (SSD) has been associated with the level of subjective quality of life [24]. Also, a correlation between sexual dysfunctions in patients with SSD and their therapeutic adherence has been demonstrated, even when the dose, number, and duration of antipsychotics were not associated with the presence of sexual dysfunctions [25].

In an interesting study, patients with schizophrenia who were drug-naïve (N=20) were compared with patients who received long-acting first-generation antipsychotics (N=51) and with healthy controls (N=51), from the perspective of their sexual functioning [28]. Untreated patients with schizophrenia reported decreased sexual desire, while those who were receiving antipsychotics reported a restoration of the libido, although presented ED, orgasmic and sexual satisfaction problems [28]. Therefore, the structured assessment of sexual function is recommended to be conducted by mental health specialists in all patients diagnosed with SSD.

Anxiety disorders are included in the differential diagnosis of ED, but they may also be comorbid conditions with, or predisposing factors to sexual dysfunctions. While fear is the response to real or perceived imminent danger, anxiety is conceptualized as anticipating an unknown threat or an internal stressor [30]. Both anxiety and fear are associated with an activation of the sympathetic nervous system when anticipating an imminent danger, which prepares the individual for a fight or flight response [30-32].

One of the topics of interest for the current review is the presence of some specific phobias, like genophobia or erotophobia (fear of intimacy and sex), heterophobia (fear of the opposite sex), nosophobia (fear of microbes), coitophobia (fear of sexual intercourse), and haptophobia (fear of being touched by another person) [33-34]. These disorders may negatively affect the quality of sexual intercourse and even lead to avoidant behaviors.

Social anxiety disorder is characterized by fear of being evaluated or criticized by others when an individual is facing social situations, with severe negative consequences over all areas of functioning [35]. Unlike shyness, social anxiety disorder (or social phobia) has a severe impact on the quality of life in affected individuals [35]. According to a study (N=40 participants), men with social phobia report more frequent sexual dysfunctions and are more inclined to pay for sex [36]. The same study found moderate impairment in arousal, orgasm, sexual enjoyment, and subjective satisfaction with sexual activity in men with social phobia [36].

Panic disorder is defined by recurrent and spontaneous panic attacks, with significant negative functional impact [4-10]. Patients with panic disorder have a higher tendency to develop sexual dysfunctions vs. the general population [37]. According to a review (n=6 reports), panic disorder was associated with a high prevalence of sexual aversion and sexual infrequency, while untreated patients with panic disorder may have a higher risk of ED vs. controls [37]. Patients with panic disorder can perceive the similarity between the neurovegetative activation during sexual intercourse and the one with onset during a panic attack [38]. Therefore, this symptomatic overlap maintains a negative conditioning that leads to the continuation of ED [38].

Agoraphobia is characterized by the fear of enclosed spaces, although the focus of this disorder is not limited to such triggers (i.e., newer classifications also include the fear of open spaces, being alone outside the home or being in a crowd, and using the public transportation) [4-10]. The exposure to feared situations can trigger discomfort, anxiety, or panic attacks [4-10]. The exploration of this disorder during the psychiatric evaluation of patients with ED is important because of the significant impact this type of phobia has on the overall functioning and quality of social relationships.

Generalized anxiety disorder has as its core criterion excessive anxiety or worries related to various daily activities, occurring for at least 6 months, and this disorder has been frequently associated with sexual dysfunctions in men [4-10,39]. In a study that evaluated 64 men with ED or premature ejaculation, 23.4% presented with anxiety disorders, and generalized anxiety disorder was the most frequently detected [39]. Men with preexisting anxiety disorders had a higher probability of presenting performance anxiety related to sex and of being diagnosed with premature ejaculation [39].

The prevalence of anxiety disorders in patients with ED ranges from 2.5% to 37%, followed closely by depressive disorders (18-35%) [40,41]. This association was most frequently reported in patients aged between 45 and 54 years [42]. Anxiety, as a non-specific symptom, can impair both sexual functioning and interpersonal behaviors. But the relationship between ED and anxiety is bidirectional because sexual dysfunction can induce anxiety, and consequently lead to a decrease in self-esteem, performance anxiety, and social avoidance.

Obsessive-compulsive disorders (OCDs) are currently conceived as a spectrum, with core symptoms represented by obsessions, compulsions, or both, that occur over a significant period of time or cause discomfort or significant dysfunctions to the patient [4-10]. Individuals with OCDs often present problems with sexual functioning, and 6% of them report the presence of ED, while 39% are dissatisfied with their sexual functioning [43,44]. The specific treatment for OCDs may also play an important role in the onset of ED, starting from tricyclics to SSRIs, especially because this disorder may require higher doses of drugs than MDD or anxiety disorders [44,45].

Body dysmorphic disorder (BDD) is considered the result of a preoccupation with self-perceived defects in physical appearance, which are not observable by others, or are considered minor when evaluated objectively [4-10]. ED was more commonly reported in men with BDD vs. the control group, although libido, frequency of intercourse, and masturbation were similar in the two groups [46]. Patients with BDD had more concerns about their penis size and tried to use different methods to increase it [46].

Post-traumatic stress disorder (PTSD) occurs following exposure (direct or indirect) to life-threatening or severe physical trauma or sexual violence, which translates into intrusion symptoms, avoidance of trauma-related stimuli, negative alterations in cognitions and mood, and alterations in arousal and affectivity [4-10]. The risk of erectile dysfunction in PTSD patients was significantly higher than in the general population [47]. PTSD was associated with overall sexual functioning, sexual desire and satisfaction, and sexual distress [47]. Avoidance and negative alterations in cognition/mood were associated with a higher risk for sexual dysfunctions in this population [47]. Psychotropic medication for patients with PTSD was not significantly associated with the risk of erectile dysfunction [48].

The presence of a defined sexual dysfunction does not exclude the existence of comorbid disorders from the same category. This observation is supported by the results of a study in which 86% of the respondents to an online questionnaire who had a sexual dysfunction also reported the presence of another sexual condition [49]. Men with one severe sexual dysfunction were significantly more likely to have such comorbidities [49]. ED and premature ejaculation were comorbid in 23-29% of cases explored in this study [49].

Substance use disorders may frequently cause the onset of sexual dysfunctions. Alcohol, opioid derivates, cannabis, and ecstasy were associated with higher scores on tests of sexual dysfunction vs. controls in a clinical study (N=101 vs. 43 participants) [50]. The association was not significant for alcohol and cannabis ($p>0.05$), but opioid and ecstasy users had significantly higher scores on the International Index of Erectile Function (IIEF) vs. controls [50]. Patients with alcohol dependence have a 72% rate of being diagnosed with one or more sexual dysfunctions, the most common being ED, reduced libido, and premature ejaculation, according to another study that enrolled 100 male subjects admitted to a detox center [51]. Additionally, illicit substances are sometimes used as aphrodisiacs to enhance sexual performance or pleasure. However, existing data show that most drugs have negative effects on erection, libido, and ejaculation latency [50,51]. The data on cannabis use and its relationship with sexual functioning is controversial, because both men and women perceived this compound may increase their sexual functioning and satisfaction, especially desire and orgasm intensity [52]. Cannabinoids also have been associated with positive effects in a large variety of neurological disorders that may alter sexual functioning [53].

Pornography addiction or excessive use of online pornographic material represents a type of behavioral addiction with a potential negative impact on sexual functioning. There are several studies that have shown a high association between erectile dysfunction and excessive use of pornography, while other studies do not support this association [54-56]. Almost 60% of the patients with sexual dysfunction manifested when having intercourse with their partner, did not report this problem when using pornography [54]. However, a causal relationship between pornography and sexual dysfunctions does not exist, due to the lack of controlled studies and difficulties in designing such studies: sample biases, lack of clear diagnostic criteria, lack of validated instruments, the possibility that pornography abuse may be only a dimension of the more complex entity of sex addiction [54].

Cybersex is an excessive and uncontrolled use of online sexual activities leading to negative functional consequences [57]. Difficulties in forming intimate relationships have been associated with cybersex and pornography craving [58]. Men had higher scores for frequency of pornography online use than women and higher scores for craving the same materials [58].

Chronic organic conditions, such as diabetes mellitus (DM), blood hypertension, dyslipidemias, renal or hepatic failure, and endocrine pathologies (e.g., hypogonadism, hyperprolactinemia, hypothyroidism/hyperthyroidism) may all have an impact on sexual functioning [59]. Also, lifestyle factors such as smoking, chronic alcohol consumption, and excessive use of caffeine may be considered potential nocive stimuli on sexual functioning [59]. Neurological causes of ED that should be considered in the differential diagnosis are multiple sclerosis, spinal cord injuries, Parkinson’s diseases, cerebrovascular accidents, etc., and the most common urological causes of ED are priapism, Peyronie's disease, and anatomical abnormalities [59]. The use of drugs like thiazides, spironolactone, clonidine, alpha-blockers, beta-blockers, antipsychotics, antidepressants, digoxin, ketoconazole, phenobarbital, etc. should be included among the potential triggers of ED [59]. Pelvic surgery and radiation therapy in the same area are other possible ED causes [59].

The pathogenetic factors of ED are traditionally split between psychological and organic, but this separation is artificial and, usually, there is mixed pathogenesis of this disorder in clinical practice (Figure 4). According to the results of a study focused on this distinction, in men ≤40 years old, approximately 85% had psychological causes for their ED and only 15% organic factors, while in men >40 years old, the rapport was 60/40 in favor of the organic causes [60]. Anxiety, depression, and relational conflicts were both causes and results of ED [60].

Predominantly organic ED	Predominantly psychogenic ED
- Gradual onset	- Abrupt onset
- Progressive evolution	- Intermitent evolution
- Constantly diminished response to sexual stimuli	- Loss of the ability to maintain erection
- Better quality of erection while standing	- Nocturnal penile tumescence is preserved
- Elderly patients	- Very good response to PDE5 inhibitors

Figure 4: Indicators of psychogenic nature vs. organic causes of erectile dysfunction

Still, the dichotomous model of organic vs. psychological causes in ED is outdated, and the currently suggested multidimensional model, which includes vulnerability factors and precipitating factors, is preferred [61]. This model involves the participation of (1) immediate factors, such as performance anxiety, (2) personal history variables, like recent traumatic events/stressors, and (3) vulnerability factors in childhood and adolescence [61]. Additionally, there are some important elements that should be integrated into the initial evaluation of patients with ED, related to vulnerability: rigid pattern of education in childhood, dysfunctional intrafamilial relationships, uncertainty about gender roles during early phases of development, lack of sexual education, personality disorders [62-64]. Between the most commonly reported factors that contribute to the onset of ED, in combination with vulnerability factors, are a large variety of interpersonal stressors (e.g., dysfunctional marital relationships, extra-marital relationships), psychological reactions to organic diseases, recent physical or mental traumas, occasional failure of erection [61-63]. There are also a variety of elements that maintain sexual dysfunctions, such as performance anxiety, guilt, habituation, fear of intimacy, an untreated sexual dysfunction in the partner, low self-esteem, etc. [61-63].

Sensitivity to sociocultural aspects is needed when patients are evaluated for any sexual dysfunction. Perception of sexuality varies from one population to another, and the impact of social or professional stressors may vary across different cultures [64,65]. Also, attitudes toward sex and the role of sexual activity are variables dependent on cultural context [65]. Gender roles are influenced by social and cultural factors, and they must be taken into account by practitioners when assessing patients with ED who are coming from another culture [64,65].

Studies exploring differences in sexual function and rates of sexual dysfunctions in homosexual and heterosexual populations are extremely few and their results are inconclusive [66]. ED in homosexual men was reported 1.5 times more frequently than in heterosexual individuals, according to a meta-analysis (n=4 studies) [66]. Homosexual men presented, in another study, a 45% higher risk of difficulties in obtaining an erection vs. heterosexual men [67]. Of these, 71% had little difficulty, 22% mild-moderate, 6% moderate, and 2% severe (according to IIEF-5 scores) [67]. Independent predictors for the presence of erectile dysfunction were age, a stable relationship, frequency of sexual activity with the partner, versatile sexual role, libido problems, ejaculation problems, and anodyspareunia [67]. Administration of phosphodiesterase-5 (PDE5) inhibitors was reported in 10% of homosexual men with ED, and 83% of them were satisfied with the effects of the medication [67]. Also, 43% used "legal drugs" to improve erectile dysfunction [67].

To conclude, the psychiatric evaluation of patients with ED involves the following steps: (1) formulating the diagnosis according to DSM-5 or ICD-11 criteria, based on an accurate collection of data on current mental and physical health status, personal medical history, and an assessment of vulnerability factors, triggers, and factors that maintain the sexual dysfunction; (2) verifying if there are concomitant sexual dysfunctions, besides ED; (3) exclusion of organic diseases, by recommending the adequate interdisciplinary and laboratory assessments; (4) formulating a therapeutic strategy, depending on the specific clinical aspects of the ED: psychotherapy, pharmacotherapy, or combined therapy. If non-psychogenic causes of ED or organic comorbidities have been identified, a multidisciplinary approach is recommended.

The psychological perspective

When evaluating a patient with possible ED, it is important to invite his partner to the initial interview, when possible, and to observe the dynamics of the couple's relationship [68]. The following aspects should be investigated during initial visits: fear of rejection, lack of satisfaction in the relationship, inappropriate sexual techniques, and sexual dysfunctions in the partner [68]. If one or more of the problems listed above are detected, the partner is invited to participate in psychotherapy [68].

However, there are also advantages to interviewing the patient alone: this way, it is possible to obtain a higher level of confidentiality, thus self-disclosure would be favored; various fantasies associated with guilt regarding his partner may be verbalized; aggressive sexual drives or traumatic personal experiences can be more easily revealed in the absence of the partner [68].

An example of structure for the first interview is the following: the couple can be interviewed for 10 minutes, to collect relevant historical data and establish the objectives of the assessment sessions; then each partner is interviewed separately, for 20 minutes; then the couple is brought together to formulate the conclusions [68]. Another assessment model considers the couple as the patient, and consequently, all assessments are made in the presence of both partners; the quality of the couple's relationship is a determining factor in the occurrence of erectile dysfunction and its resolution, therefore interviewing the couple is of paramount importance [68].

Forming an alliance with the patient is essential to a successful psychological interview, and to attain this result, the atmosphere must be safe, open, and non-judgmental; a strict agenda is not advisable, and flexibility is preferable [68]. The psychological assessment framework requires unconditional respect toward patients and an empathic, calm, and semantically adequate way to communicate with them [68,69]. The patient’s perspective on his own sexual dysfunction should be assessed and de-structured if based on myths or wrongfully understood scientific information [68,69]. The patient's personality needs to be assessed, as well as his core values, and attitudes towards life and others, his major existential goals, and the importance of relationships for the patient [68,69].

More specifically, the psychologist needs to assess the educational and cultural background of the patients, in order to formulate the questions and conclusions in an adequate manner; the main mechanisms of defense should be assessed, as well as his main coping strategies; gender identity disorders, sexual preferences, paraphilias, other sexual dysfunctions, childhood trauma (i.e., incest, rape, sexual or physical abuse) are essential elements to be evaluated; current level of functioning in all major areas is important to determine- actual relationships, professional performances, social support, relationships with nuclear family, hobbies; stressors in his current environment- bullying, mobbing, sexual harassment, discrimination, conflicts with peers; detailed analysis of the current sexual relationship; which are the main reasons for lack of satisfaction or failure in any functional domain?; dysfunctions in the main stages of the sexual cycle; factors that are associated with the onset and maintenance of the ED; how does it work a typical sexual intercourse for the evaluated individual?; which are the possible factors that negatively interfere with the stages of sexual cycle?; personal sexual history and possible relationship with the current problems [69].

The structured assessment of ED symptoms and functional consequences is done using validated psychological instruments. Arizona Scale for Sexual Experiences (ASEX) was developed by the Department of Psychiatry and Psychology at the University of Arizona and the Department of Psychiatry and Behavioral Sciences at Stanford University [70]. This instrument is self-administered, but it can also be completed during an interview, by a mental health specialist [70]. It measures the following dimensions: sexual desire, sexual arousal, penile erection/vaginal lubrication, ability to achieve orgasm, and orgasmic satisfaction [70]. There are two versions, one for women and one for men [70]. Each item is scored on a six-point Likert scale, with higher scores indicating greater severity of the respective sexual dysfunctions [70].

The Brief Sexual Function Inventory (BSFI) is intended for the evaluation of sexual dysfunction for men only [71]. This inventory has 11 items scored on a Likert scale, which assesses 4 dimensions - sexual desire, the ability to achieve or maintain an erection, ejaculatory function, and satisfaction with one's own sexual activity [71]. Low scores on this scale indicate high severity of sexual dysfunction [71]. For erectile dysfunction there are 3 items, scored from 0 (no dysfunction) to 5 (severe/ persistent dysfunction): frequency of partial/complete erections in the presence of sexual stimulation in the last 30 days; the presence of erections consistent enough to allow sexual intercourse in the last month; difficulty getting an erection in the last 30 days [71]. The scale is self-administered, although it was originally created to be administered by the clinician [71-73].

The UKU scale (Udvalg for kliníske Undersogelser) is an instrument for the assessment of adverse effects of psychotropic medication, sexual adverse events included [74]. It has 48 items scored from 0 to 4 on a Likert scale included in its first section; the second section comprises the assessment of the functional impact of the reported adverse effects; the third part refers to the management of the respective adverse effects [74]. The items represent symptoms and clinical signs distributed by categories: mental (difficulties concentrating, drowsiness, depression, etc.), neurological (dystonia, stiffness, tremors, etc.), neurovegetative (diarrhea, constipation, palpitations, etc.), and other (pruritus, increased/weight loss, photosensitivity, etc.) [74].

The scale also includes 6 items dedicated to sexual functioning: increase/decrease in sexual desire, erectile dysfunction, ejaculatory dysfunction, orgasmic dysfunction, and lack of vaginal lubrication [74]. The scale enables global assessment of the functional impact these symptoms may have [74]. The causal relationship between the medication and the adverse effect is also evaluated as being improbable, possible, or probable [74]. Low scores on this scale mean reduced severity of the respective manifestation [74]. It is administered by a clinician, but there is also a simpler self-administered form (UKU-SERS-Pat) [74,75]. All items can be applied or only relevant ones can be selected; the full version is recommended for clinical trials [75].

International Index for Erectile Dysfunction (IIEF) is a multidimensional tool for assessing sexual dysfunction and comprises 5 factors - erectile function, orgasmic function, sexual desire, sexual satisfaction, and overall satisfaction [76]. These factors result from 15 items formulated as questions, referring to erection frequency, erection firmness, penetration capacity, erection maintenance frequency, sexual satisfaction, etc. [76]. Items are scored from 0-5 and refer to the manifestations detected in the last few weeks; the instrument is self-administered [76]. The scores were used as indicators of the possibility of initiating sildenafil administration (scores below 14 out of a maximum of 30 in the "erectile dysfunction" domain) or psychosexual counseling (scores between 14 and 25 in the same domain) [76]. IIEF version 1 is only administrable to heterosexual men, but IIEF version 2 is applicable to both heterosexual and homosexual men [76,77]. A shortened 5-item version was also in use (IIEF-5), with a total score between 0 and 30; a high severity of dysfunction is present at scores 0–10; scores below 26 indicate the presence of some degree of erectile dysfunction [78].

Erectile Firmness Scale (EHS) contains only one item and is a self-administered instrument [79]. It rates the firmness of the erection from (0) (absent) to 5 (complete) and it is used in clinical trials to determine response to medication [79].

Still, the most important tool for the psychological assessment of patients with sexual dysfunctions remains a detailed history focusing on the history of sexual development. Aspects related to the partner's perception of sex life are essential for building an accurate picture of sexual dysfunction. All the scales previously mentioned are in no way meant to be a substitute for an accurate collection of personal history and psychological examination.

However, these instruments help the psychologist in detecting ED symptoms when patients are embarrassed to talk about the subject, and they are also useful for the monitoring process (i.e., effectiveness or tolerability of a drug or the efficacy of psychotherapy). They may also be needed for screening purposes in vulnerable populations. The main limitations of the scales are the lack of cross-cultural validation and possible differences in symptom reporting based on patient age [80].

The psychotherapeutic approach

According to the results of a review [60], the most frequently therapeutic intervention for ED is sexual therapy (50%), followed by cognitive-behavioral sex therapy (33%), while other types of psychosocial approaches have an equal representation (16%) – cognitive-behavioral therapy, group therapy, and couple therapy.

The psychotherapies dedicated to ED are derived from various interpretative models of this disorder. For example, the cognitive model of ED states that explicit/implicit requests for sexual performance lead to an expectation of performance (e.g., messages received from the partner create the expectation of an erection) [81]. In normal conditions, the attention of the individuals is focused on erotic stimuli, which increases the vegetative excitation, and favors the onset of erection (positive feedback); on the contrary, negative feedback involves catastrophic expectations, lack of perceived control over own body, focus of attention on partner's opinion about a potential failure or on other, non-sexual, stimuli, which increases the vegetative activation and focus on negative consequences, thus leading to ED [81].

The cognitive-emotional model of ED is based on a presumed occasional failure to obtain an adequate erection (no triggers are identified); following this random event, the individual activates cognitive schemata focused on personal incompetence and the misconceptions about the "macho" role; ruminations about future failures and an internal pressure to perform better next time is the following step; possibly as a defense mechanism that tries to protect the individual against a future failure, thoughts and images related to sexual activity disappear, therefore the stimulation decreases; the final stage is the "natural" consequence of the previous steps, and consists in ED's onset [82].

The main questions that help the therapist to formulate a treatment plan are: "What kind of phantasies and related emotions are associated with the current partner?", "Who has the initiative or how does the engagement in sexual activity appear?", "Is achieving sexual arousal within the current relationship and in general an easy process or a difficult one?", "How does the patient consider he can satisfy his partner and how does the partner perceive the satisfaction she receives?" [83]. Also, the therapist should explore the ability of an individual to achieve orgasm and under what conditions is it achieved; to verify what happens when one of the partners does not reach orgasm, and if this becomes a source of relational conflict; which is the definition of satisfactory sexual activity the same for both partners; to determine the level of satisfaction with sex life and with life in general in both partners [83].

According to other therapists specialized in sex-marital therapy, the specialist must keep detailed reports on the patient's status at each visit and of the suggested techniques; the therapist offers operationalized definitions of the problems he/she wants to elaborate on; the therapist evaluates the global functioning of the patient, not only of his sexual function; the therapist schedules monitoring visits at pre-determined intervals [84].

The PLISSIT model comprises "permission", i.e., the patients want to be considered "normal people", not "perverted", "deviant", "sick" or "abnormal"; often they are not disturbed by a certain behavior, but by the thought that they might do something bad or wrong; therefore, reassurance of normality is important for fantasies, thoughts, and dreams (e.g., those related to other people, or homosexual phantasies), for anxiety that can be associated with sexual stimulation (e.g., the appearance of spontaneous erections in inappropriate conditions); there are, of course, limits to validation, like fantasies with extreme sadism, pedophilic drives, etc. [85]. "Limited Information" is another core concept of this model that refers to the fact that clinicians provide limited information on relevant aspects of sexual concerns presented by the patient; the information is adjusted to the particularities of the patient, avoiding unnecessary scientific terminology and over-generalization; it is considered helpful in combating sexual myths [85]. "Specific Suggestions" refer to the fact that the therapist should formulate her/his suggestions according to the specific information obtained from the patient; these are direct attempts to change problematic behavior; these suggestions take place within the theoretical framework of short therapy [85]. "Intensive Therapy" includes specific techniques of sexual therapy [85]. During psychotherapy, the patient is encouraged to elaborate on the topic of his sex life [85]. The psychotherapist's attitude is one of empathetic listening and guiding the interview toward areas of interest in a discreet, non-confrontational manner [85]. The therapist is non-critical, normalizes, and helps validate the patient's emotions [85].

A frequently used technique in sex therapy is sensation focus [86]. These exercises progress from non-genital to genital tactile stimulation, with the instruction for the patient to refrain from vaginal penetration; thus, the patient's anxiety related to the sexual act will be reduced (desensitization), and the partner can be stimulated even in the absence of focusing on the genital area; after the patient has regained the confidence in himself, by managing to focus his attention on bodily sensations, penetration can be re-introduced into the therapy plan, gradually; it is recommended to formulate as objectives sensory focus sessions, 2 times a week, 10 minutes each; by changing roles between partners, each of them being active and passive, the patient gains experience in giving and receiving sexual stimulation [86].

Cognitive therapy and sexual fantasy training involve masturbation using guided imagery to regain control over erection, coupled with cognitive restructuring; homework includes scoring the satisfaction obtained by the patient and his partner [86].

Psychoeducation targets the increase of patients' level of information on the phases of the sexual cycle and encourages open discussions about sexuality [87]. This psychoeducational approach may have as an objective the modification of sexual scripts, which largely depend on social myths and misperceptions about sexual functioning [87].

CONCLUSIONS

The etiology of ED is usually mixed and, as a result, a multidisciplinary approach to both its diagnosis and treatment is recommended. Therefore, this review supports the conclusions of currently available guidelines for sexual dysfunctions, that a multidisciplinary, pharmacological, and psychosocial treatment is superior to monotherapy [86-88]. It is important for mental health specialists to approach the subject of ED in an empathic, non-conflictual, and non-judgmental manner, in order to obtain the information needed not only for the diagnosis but also for the formulation of the therapeutic objectives.

The main factors to be approached during the initial interview are the circumstances of the ED onset, vulnerability factors and triggers, sexual orientation of the patients, gender-related problems, and personal history of trauma [86]. Questionnaires and structured interviews are useful both initially and during the therapeutic process, as a way to document the evolution of ED symptoms. These assessment methods are selected according to the specifics of the case, but a combination of unstructured, structured interviews and scoring techniques using a validated clinical tool is preferred.

There are multiple psychometric methods for evaluating the severity of erectile dysfunction, quality of life, general functionality, etc., and the mental health specialist is the one who selects the most appropriate tools for each individual patient based on objective (severity of symptoms, functional impact), but also on subjective factors (personal experience). The stages of evaluating a patient with ED are flexible but generally involve a stage of constructing the psychiatrist/ psychologist/psychotherapist-patient relationship, describing the objectives and methods used for the evaluation, then a stage of investigating the sexual history, and a phase summarizing the collected data, which paves the way to a therapeutic solution. The desired outcomes of the therapy should be framed around satisfaction, pleasure, and quality, rather than quantity [86]. Directive and non-directive techniques can be combined depending on the particularities of the case. For an experienced mental health specialist, cultural, social, or psychological "barriers" can not represent major obstacles in the relationship with the patient. These aspects are discussed and re-interpreted through the lens of the objectives established by mutual agreement with the patient. The clinician is attentive to cultural, social, and educational details and integrates the patient's values into his/ her discourse. Active listening is essential to obtain "sensitive" information and to create an appropriate therapeutic framework.

Conflicts of interest and sources of funding

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Authors' contribution

OV conceived the idea of the review. OV, AGM, BMP, CAC, CFP and DU designed the study. OV, CFP, AGM, CAC, CFP, DU, MD, DGV, CN and CAS screened the studies during the primary search. OV, AGM, BPM and CAS drafted the first version of the review. OV, CAC, CFP, DU, MD, DGV, CN and CAS revised the manuscript and elaborated the conclusions of the review. All authors had full access to all data contained in the review and decided to submit the manuscript for publication.

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ORIGINAL ARTICLE

Management of Urinary Dysfunction in Patients with Multiple Sclerosis

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Abstract: *Besides the motor and sensory dysfunction that characterizes multiple sclerosis, during the disease evolution and with the accumulation of new demyelinating lesions in specific areas of the central nervous system, autonomic dysfunction be-comes more prevalent among patients suffering from multiple sclerosis. Although not usually addressed, the complex spectrum of autonomic dysfunction manifestations has the potential of a negative impact on the daily activities of multiple sclerosis patients, needing specific investigations and therapeutic measures destined to alleviate symptoms and increase the quality of life. Since urinary dysfunction is evident from the early stages of multiple sclerosis on urodynamic studies even in patients without urinary complaints, it is indicated to thoroughly screen for urinary tract dysfunction as well as other autonomic dysfunctions in all the multiple sclerosis patients.*

Keywords: *autonomic dysfunction, urinary dysfunction, multiple sclerosis*

INTRODUCTION

Multiple sclerosis (MS) is one of the most common causes of disability in young patients. Clinically it does manifest uniquely for every patient, but depending on the site of the demyelinating lesions, some symptoms have a higher frequency in different combinations, particularly sensory symptoms, motor weakness, and bladder dysfunction. Most commonly, patients will present with numbness, tingling, diplopia, Lhermitte sign, and motor weakness, that last for at least 24 hours and may or may not resolve completely. Many patients may have residual symptoms, heat sensitivity with worsening of symptoms in case of increased body temperature, known as Uhthoff's symptom [1–3].

AUTONOMIC DYSFUNCTION IN MULTIPLE SCLEROSIS

As the disease follows its course over the years, inevitably patients will also experience damage to the autonomic nervous system with the involvement of both sympathetic and parasympathetic parts in different proportions. The promoters of the autonomic dysfunction appear to be lesions located in the nuclei of the periventricular regions of the fourth ventricle, medulla, but it was also seen in patients who presented cervical spinal cord atrophy with or without demyelinating lesions, suggesting that autonomic dysfunction may also result from axonal depletion [4].

Although severely overlooked, autonomic dysfunction has a great impact on the quality of life of patients suffering from multiple sclerosis, the most commonly addressed issues being bowel and bladder disturbances, which tend to be more bothersome in the case of longer disease, greater disability, or recent relapses [5].

Approximately 50% of patients experience orthostatic intolerance manifested by dizziness, loss of postural tonus, and tachycardia while transitioning from a supine to an upright position or in case of prolonged standing. The impaired sympathetic vasoconstrictor reflex responsible for maintaining normal blood pressure leads to an increased frequency of orthostatic hypotension among those patients[4].

Sleep disturbances in patients with multiple sclerosis are not very well studied, the most common symptom being difficulty initiating and maintaining sleep. There are several factors like muscle spasms, trigeminal neuralgia, restless legs syndrome, anxiety, depression, and side effects of medication used to treat fatigue, that contribute to insomnia, which can be searched for and addressed [2,4].

Another common but less addressed pathological aspect of multiple sclerosis is the sexual dysfunction that can be a consequence of multiple lesions including spinal cord lesions of the motor and sensory pathways and can be aggravated by the urinary incontinence and the spasticity accompanying upper motor lesions. About 50% of MS patients tend to become sexually inactive during the disease, with consequences like decreased libido, impotence, and infertility [2,5,6].

Bowel dysfunction is present in up to 50% of MS patients, about 43 % of them will complain of constipation partially due to disease exacerbations, and partially to decreased ambulation and certain medications that may alter bowel movement. A great proportion of patients with bowel dysfunction will present fecal incontinence as a consequence of impaired external anal sphincter function [2,4,7].

UROLOGICAL DYSFUNCTIONS

The most frequent autonomic dysfunction present in patients suffering from multiple sclerosis is bladder dysfunction, the prevalence of neurologic lower urinary tract dysfunction varies between 65 to 92% [8]. Approximately 75% of patients report bladder dysfunction secondary to a storage phase dysfunction leading to incontinence, or the voiding phase dysfunction leading to urinary retention and incomplete bladder emptying [2,4]. Bladder control requires complex coordinated functions of the cerebral cortex, pontine and sacral micturition centers, and the integrity of the peripheral nervous system [6]. The demyelinating lesions localized at the spinal cord level disrupt the fiber connections from the pontine micturition center to the parasympathetic sacral micturition center causing the silent C fibers, usually insensitive to bladder filling in normal conditions, to be hypersensitive leading to detrusor hyperactivity [9,10]. Spinal cord centers activate storage reflexes in the filling phase of the bladder, while the brain centers of micturition are responsible for the act of urine output [4,11]. Detrusor overactivity, which translates in clinical terms to symptoms like urgency to void that occurs at a lower bladder volume than normal, frequency or urge and incontinence are the leading complaints. On the opposite side lies detrusor sphincter dyssynergia described as detrusor contraction without urethral sphincter relaxation, which is responsible for the impossibility of bladder emptying causing symptoms like hesitancy, or interrupted stream. While the former urologic abnormality is caused by demyelinating lesions of the frontal cortex, the second one is a consequence of lesions of the pontine centers of micturition or lesions above the sacral parasympathetic centers [2,6,12]. Unlike spinal cord lesions due to multiple sclerosis, spinal cord injuries, even in patients with relatively preserved neurological function, are characterized by universal bladder dysfunction, meaning that there is an impairment of both sensation of bladder fullness as well as the motor control of bladder and sphincter [6,11].

For many patients, urological symptoms arise only in the later stages of disease progression with a proportion of over 50% of patients with severe MS presenting severe bladder symptoms, whereas only 5-10% of MS patients have mild bladder abnormalities at the beginning. Nevertheless, a retrospective study conducted in Ankara, Turkey demonstrated that 52% of their multiple sclerosis patients without urological complaints had altered urodynamic results which could be explained by a subclinical impairment [13,14].

Patients with overactive bladder syndrome usually complain of urinary urgency, increased daytime frequency or nocturia, and sometimes incontinence in the absence of urinary tract infections or other associated pathology that could explain the symptoms. Obstructive symptoms are also an important point of complaint especially when leading to urinary tract infections. These manifestations can be associated and can have a huge impact on patients' quality of life in terms of decreased quality of sleep, decreased work productivity, and depressive symptoms [15,16].

Urine retention is a consequence either of neurogenic underactive bladder or of detrusor sphincter dyssynergia causing bladder obstruction of urine flow, leading to frequent lower urinary tract infections that can be another cause of disease relapse through the autoimmune process which it sets off [9,17,18]. Regarding the complications of the upper urinary tract there have also been mentioned vesica-urethral reflux, hydronephrosis, and end-stage kidney disease have as consequences of the morphological changes in the bladder wall caused by high intravesical pressures [16,17,19–21].

EVALUATION OF LOWER URINARY TRACT SYMPTOMS

Urological evaluation should be specifically made for every patient considering their disease type and duration, the level of disability on the EDSS scale, comorbidities, and current medications especially opiates, antipsychotics, and antidepressants, starting with a complete history taking. The clinical dialogue should be directed towards describing urinary symptoms like frequency, urgency, hesitancy, retention, incontinence, and nocturia and the impact these symptoms have on the quality of the patient's life [16,22]. A bladder diary can provide additional information about the patient's habits of fluid ingestion and the exact frequency of bladder emptying [9].

Regarding physical examination, besides local inspection and examination of pelvic and genital organs including sacral cord mediated reflexes (bulbocavernosus, anal reflex), the neurologist should look for pontine, cerebellar as well as pyramidal signs which are indicative of lower urinary tract symptom severity [16,22]. For an initial evaluation of patients with multiple sclerosis, a bladder and renal ultrasound and a questionnaire for assessing overactive bladder and voiding dysfunction are in order, while for patients with red flags (recurring urinary tract infections, immunosuppressive treatment, EDSS >6, male patients, age >55) should be referred to a neuro-urology clinic or specialist [23].

Furthermore, some investigations can help us better understand the nature of the urological dysfunction. Firstly, all patients complaining of urinary symptoms should undergo a urinalysis and urine culture to assess the presence of a possible urinary tract infection as well as serum creatinine. Ultrasonography can allow us to measure the post-void residual volume, and it can be done at different times a day for better quantification of the bladder emptying pattern. Patients with a known high risk of upper urinary tract disease should benefit from periodical ultrasonography examinations for early detection of upper urinary tract dilation. Since a high post-void residual volume is not enough to distinguish between detrusor contractility problems and obstruction, urodynamic studies are required. Urodynamic studies represent a combination of cystometry and pressure flow study, useful in evaluating the outlet function and compliance of the detrusor and bladder. It can be used as a prognostic tool as well as for guiding the best treatment approach. In the case of hematuria, a urethrocystoscopy evaluation is required to rule out bladder tumors, bladder stones, and urethral stricture [16,20].

TREATMENT APPROACHES IN MULTIPLE SCLEROSIS-ASSOCIATED URINARY DYSFUNCTION

Physical therapy

One of the areas worth addressing is physical therapy, which in this case refers to physical exercises like pelvic floor exercises alone or combined with intravaginal electrical stimulation. Cognitive behavioral therapies may be helpful in some patients in trying to develop a bladder voiding schedule according to each patient's natural voiding pattern which combined with restricted water intake before bedtime can improve sleep quality and reduce complaints like fatigue [16].

Medical treatment

Regarding the drugs efficient for urinary dysfunction, there are several options depending on the main cause of dysfunction, that can be assessed by urodynamic studies. The first treatment line for neurogenic bladder is antimuscarinic drugs (oxybutin, tolterodine). Anticholinergics can alleviate complaints of neurogenic bladder as well as phosphodiesterase inhibitors and desmopressin which can be used as an alternative to antimuscarinics or as an adjuvant [6,20]. In an observational prospective study on the bladder and urodynamic alterations in patients with multiple sclerosis, the degree of urinary symptom severity decreased following symptomatic urological medication by lowering the bladder pressure, improving muscle spasms, and reducing pain caused by urine storage [24].

In case of bladder outlet resistance, autonomic dysreflexia, and postvoid residual volume, alpha-blockers (prazosin, terazosin) can help sphincter relaxation by lowering bladder pressure during contraction, but administered with caution because they can aggravate hypotension [6,20,25].

In patients with hypotonic bladders, cholinergic medications (bethanechol) may help empty the bladder, while in patients with pathologic relaxation of the vesical sphincter, alpha-adrenergic medication may increase bladder storage [6,17].

Minimally invasive treatment

Catheterization, self-catheterization, or third-party catheterization is the preferred method for patients who cannot otherwise empty their bladder. Patient medical education in managing a sterile intermittent catheterization can help reduce complications like urinary tract infections or bacteriuria. The habit of keeping a bladder diary can also help choose the frequency of catheterization so that the volume of the bladder at catheterization does not exceed 400-500 ml. As short-term methods, patients can dispose of condom catheters and adult diapers.

Botulinum toxin injections in the detrusor cause reversible chemical denervation with effects lasting for approximately 9 months, but also with known side effects like urinary tract infections, elevated postvoid residual volume, and increasing need for intermittent catheterization. In the case of bladder neck and urethral obstruction, to reduce the outlet resistance and to protect the upper urinary tract, patients may benefit from chemical denervation of the detrusor sphincter using botulinum toxin [6,17,20].

Surgical treatment

Sphincterotomy for reducing the bladder outlet resistance without completely losing the closure of the urethra comports the risk of secondary narrowing of the bladder neck and needs to be repeated at regular intervals. Bladder augmentation is indicated in patients with overactive or low compliance bladder when conservative methods have not been beneficial [20]. Urethral stents, sphincterotomy, and electrical implantation devices can be taken into account in selected cases, but with limited evidence of efficacy [6,26].

CONCLUSIONS

Multiple sclerosis is a central nervous system inflammatory demyelinating disease with multiple clinical manifestations, including autonomic dysfunctions. The most common and the most addressed is bladder dysfunction, but it is only a small part of the many bothersome effects of multiple sclerosis lesions, hence the need to evaluate and treat accordingly to ensure a higher quality of life for patients.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, C.G.L., C.A.S. and D.M.; methodology, V.I.V., R.A.A., F.A.A. and A.M.R..; resources, D.M., C.A.S., and F.A.A..; data curation, C.G.L., V.I.V., and A.M.R.; writing—original draft preparation, C.G.L., C.A.S., F.A.A., and A.M.R..; writing—review and editing, V.I.V., and R.A.A. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Chronic Pulmonary Sequels on CT Scans of Patients with COVID-19

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Abstract: *Background: Lung tissue changes following COVID-19 may persist for a long time after discharge and recovery, but not enough information is available about these chronic pulmonary sequels. We addressed to evaluation of chronic pulmonary sequels on CT scans of patients with COVID-19. Methods: This cross-sectional study was conducted on 94 patients with COVID-19 who were evaluated by CT scan at the time of discharge and also underwent continuous evaluation by CT scan of the lungs for at least three months. Through reviewing the hospital archive files, patients' backgrounds and three-month follow-up information were collected. Results: Chronic pulmonary involvement during the follow-up of patients was found in 83%. Peripheral and diffuse involvements were revealed in 79.8% and 3.2% respectively. Also, single-lobar and multi-lobar involvements were found in 69.1%% and 13.8% respectively. In terms of abnormal lung findings on CT scan, ground-glass opacity was found in 20.2%, Crazy-paving pattern in 2.1%, reticulation in 3.2%, parenchymal band in 12.8%, consolidation in 1.1% and bronchiectasis in 3.2%. The mean CT score was also 4.21 ± 3.45. The appearance of findings in CT patients was strongly influenced by the patients 'age, body mass index, underlying comorbidities, and also, the severity of initial COVID-19. Conclusion: Within at least three months of disease recovery in hospitalized patients with COVID-19, evidence of pulmonary involvement in 83% in the form of chronic pulmonary sequels can be expected. The most common findings related to pulmonary involvement included GOO in 20.2% and parenchymal band in 12.8% of cases.*

Keywords: *Chronic pulmonary sequels, CT scan, Covid-19*

INTRODUCTION

Although several effective vaccines with high immunity have been developed to prevent the spread of COVID-19, preventing its transmission is now believed to be the only way to combat the disease. In recent months, much effort has been published to explain the epidemiology, clinical manifestations, and clinical features and imaging of COVID-19 [1-4]. In this regard, the occurrence of fever and dry cough are the first and most common symptoms of the disease [5]. However, these symptoms are similar to those of other infections, such as the flu A and B, making it difficult to diagnose. Also, laboratory evaluation in patients is often time-consuming and its diagnostic kit may not be available for homogeneity. On the other hand, laboratory tests are sometimes associated with high levels of false negatives. In this regard, imaging plays a key role in the diagnosis and management of COVID-19-induced pneumonia. CT scan is the first diagnostic modality in patients with suspected disease and also a desirable protocol in following the effect and treatment process in these patients [6]. Especially in patients with a negative RT-PCR result, CT scan evaluation can be very useful [7,8]. Various studies have evaluated and described radiographic changes in the acute phase of COVID-19 disease and have correlated these changes with the severity of clinical manifestations [9-11]. Recent studies have also shown that about 94% of hospitalized patients still experience pulmonary parenchymal changes at discharge [12, 13]. In addition, some have shown that pulmonary opacities persisted in 53% of patients with mild COVID-19 without developing negative and complicating scaffolds for up to three weeks after discharge and clinical recovery [14]. Therefore, it seems that lung tissue changes, even the occurrence of pulmonary fibrosis, may persist for a long time after discharge and recovery of patients [15-17]. But not enough information is available about these chronic pulmonary sequels. What we addressed in the present study was the evaluation of chronic pulmonary sequels on CT scan of patients with Covid-19.

MATERIALS AND METHODS

This cross-sectional study was conducted on patients with COVID-19 who were admitted to our medical center during 2020 and 2021 and were evaluated by CT scan at the time of discharge and also underwent continuous evaluation by CT scan of the lungs for at least three months after treatment and recovery. Initially, through reviewing the hospital archive files, patients' background information including sex, age, history of underlying diseases, body mass index (BMI), and clinical characteristics of patients were collected. Also, in the initial CT scan of patients (during hospitalization) and also at the time of follow-up of patients (at least three months after discharge) and by an experienced radiologist, a complete interpretation of pulmonary involvement patterns was provided in terms of form and extent of involvement and CT score and classified according to the underlying characteristics of the patients. The severity of COVID-19 was stratified into four types mild, moderate, severe, and critical on the basis of patients' symptoms, laboratory results, and imaging findings at admission as previously described [18]. Pulmonary involvement was also assessed based on the type of lesion (peripheral or diffuse), the pattern of lobar involvement (single-lobar or multi-lobar), and the type of pulmonary lesions. To determine the CT score, each of the five lung lobes was visually scored on a scale of 0 to 5, with 0 indicating no involvement and 5 indicating more than 75% involvement. The total CT score was determined as the sum of lung involvement, ranging from 0 (no involvement) to 25 (maximum involvement) [19].

For statistical analysis, results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using the t-test or Mann-Whitney test whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. The categorical variables were compared using the Chi-Square test or Fisher's exact test if required. P values of ≤ 0.05 were considered statistically significant. For the statistical analysis, the statistical software SPSS version 23.0 for Windows (IBM, Armonk, New York) was used. Interventional studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

RESULTS

A total of 94 patients were evaluated and followed up for at least three months after discharge. The average age of patients was 53.02±4.86 years ranged 40 to 62 years and 59.6% were male. The mean BMI was also 25.41±1.91 kg/m^2 and 5.3% were obese. Regarding comorbidity, 22.3% were hypertensive, 13.8% were diabetics, and 6.4% had a history of ischemic heart disease. With respect to disease severity, 4.3% were classified as mild, 86.2% as moderate, 6.4% as severe, and 3.2% as critical.

First, there was no evidence of pulmonary involvement in CT scans in 17.0% and therefore the frequency of chronic pulmonary involvement during the follow-up of patients was 83%. In terms of how the lesions were spread, 79.8% of the involvement was peripheral, and only 3.2% was diffuse. Also, single-lobar and multi-lobar involvements were revealed in 69.1%% and 13.8% respectively. In terms of abnormal lung findings on CT scan, GOO was found in 20.2%, Crazy-paving pattern in 2.1%, reticulation in 3.2%, parenchymal band in 12.8%, consolidation in 1.1% and bronchiectasis in 3.2%. The mean CT score was also 4.21 ± 3.45.

As shown in Table 1, the patterns of chronic pulmonary sequels in patients suffering from COVID-19 were independent of patients' gender. However, the pattern and distribution of pulmonary involvements were different in the younger and the elderly, and the diffuse and multi-lobar patterns were more found in older adults. Also, abnormal lung lesions were more prevalent in older patients. Totally, the mean CT score was significantly higher in older adults than in the younger. Similar patterns were revealed in obese as compared to non-obese patients and thus obesity was found to be another factor predicting more severity of chronic sequels on CT. As shown in Table 1, the presence of comorbidities was in line with more severe pulmonary expansion of abnormal lesions. Also, as indicated in Table 2, we showed diffuse and multi-lobar lesions as well as a higher rate of pulmonary lesions in those with more severe initial COVID-19 disease. There was also a significant association between CT score and disease severity (Figure 1).

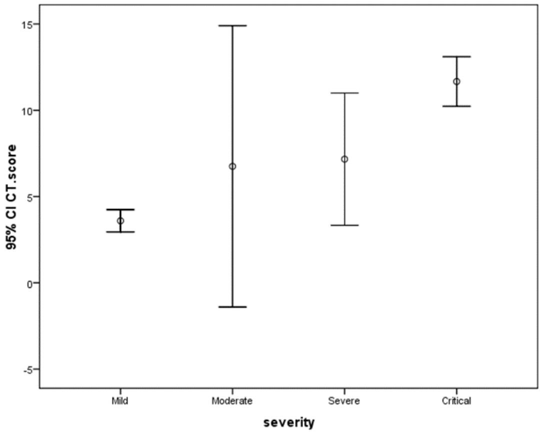

Figure 1: The correlation between CT score and severity of primary Covid-19

Table 1: Status and pattern of pulmonary involvement according to underlying factors

Item	Gender		Age		Obesity		Morbidity	
	Male	Female	≤50	>50	Obese	Non-obese	Present	Absent
Distribution								
Peripheral	76.8	84.2	63.4	92.5*	50.0	81.1*	78.3	80.3*
Diffuse	3.6	2.6	0.0	5.7	50.0	1.1	13.0	0.0
Pattern								
Single-lobar	64.3	16.1	53.7	81.1*	50.0	70.0*	52.2	74.6*
Multi-lobar	76.3	10.5	9.8	17.0	50.0	12.2	39.1	5.6
GOO	17.9	23.7	17.1	22.6	50.0	18.9*	43.5	12.7*
Crazy-paving	3.6	0.0	0.0	3.8	25.0	1.1*	8.7	0.0*
Reticulation	3.6	2.6	0.0	5.7	50.0	1.1*	13.0	0.0*
Parenchymal band	14.3	10.5	9.8	15.1	0.0	13.3	26.1	8.5*
Consolidation	1.8	0.0	0.0	1.9	0.0	1.1	4.3	0.0
Bronchiectasis	3.6	2.6	0.0	5.7	0.0	3.3	13.0	0.0*
Mean CT score	4.62±3.49	3.61±3.33	3.37±3.47	4.78±3.32*	7.25±4.92	4.08±3.34*	5.83±3.97	3.69±3.11*

Table 2: Status and pattern of pulmonary involvement according to disease severity

Item	Mild	Moderate	Severe	Critical	P value
Distribution					0.001
Peripheral	100	80.2	100	0	
Diffuse	0	0	0	100	
Pattern					0.001
Single-lobar	77.8	25.0	16.7	0	0.001
Multi-lobar	2.5	75.0	83.3	100	0.001
GOO	9.9	75.0	83.3	100	0.001
Crazy-paving	0	0	0	66.7	0.001
Reticulation	0	0	0	100	0.001
Parenchymal band	50.0	7.4	50.0	33.3	0.201
Consolidation	0	0	0	33.3	0.001
Bronchiectasis	0	1.2	16.7	33.3	0.004
Mean CT score	3.59±2.92	6.75±5.12	7.17±3.65	11.67±0.57	0.001

DISCUSSION

In the acute phase of Covid-19, different patterns of pulmonary involvement are examined in depth and the severity and extent of the disease are classified accordingly. But the question remains, how long will these involvements last after recovery from the disease, and in other words, will there still be evidence of lung involvement due to the disease months after the symptoms have improved? Obviously, the presence of these abnormal findings in the form of chronic scoliosis in CT scans of patients, weeks and months after discharge from the hospital can indicate the continuation of clinical manifestation of the disease and sometimes even exacerbation of these symptoms. What we did in the present study was the evaluation of chronic pulmonary edema on CT scans of patients with COVID-19 and related underlying factors. First, the study showed that for at least three months after treatment and recovery from COVID-19 disease, evidence of pulmonary involvement would still be expected in 83% of patients even after improvement in clinical symptoms. The most common findings of CT as chronic disease sequels included GOO patterns in 20.2% and parenchymal band in 12.8%. Other findings included reticulation in 3.2%, bronchiectasis in 3.2%, Crazy-paving pattern in 2.1%, and consolidation in 1.1% of patients. Therefore, even after about three months of the disease and the relative recovery, the occurrence of some evidence in CT patients with COVID-19, especially GOO, is still predictable. Additionally, in terms of the pattern of involvements, the lesions are mainly in the form of peripheral, and in most cases, they will be single-lobar involvements rather than multi-lobar or diffuse ones. As a second finding, we found that the appearance of these findings in CT patients during the months following recovery from COVID-19 was strongly influenced by the patients 'age, BMI, underlying comorbidities (such as hypertension, diabetes, and history of ischemic heart disease) and also, the severity of initial Covid-19. In other words, the incidence of chronic pulmonary sequels in CT is more predictable in the months following recovery from COVID-19 at older ages, in obese individuals with underlying comorbidities, and in individuals with severe underlying COVID-19.

Various studies have reported the persistence of chronic pulmonary lesions in the weeks and months after discharge from the hospital, although due to differences in patients' underlying characteristics, different follow-up periods, and different intensities of underlying COVID-19 disease, CT scans were quite different during patient follow-up. In a study by Caruso et al and at 6-month follow-up, 72% of patients showed fibrosis-like changes and 42% of patients showed evidence of GOO [20]. In a study by Han et al., fibrotic-like changes were observed in 35% of cases, while 65% of cases either had completely clean lungs or had grand glass opacity or increased interstitial thickness. Age over 50, heart rate above 100 at admission, length of hospital stay over 17 days, respiratory distress syndrome, non-invasive mechanical ventilation, and CT scan score above 18 as factors associated with fibrotic-like changes over six months after discharge [21]. In another study by Dehan Liu et al during the first to third weeks, the GOO opacity and pulmonary fibrosis remained constant [22]. In a study by Safont et al., during a 180-day follow-up with a CT scan, 66% had chronic pulmonary lesions. Among this evidence, 68.3% had GOO and 38.4% had parenchymal bands [23]. In the study by Blanco et al., 19 patients with COVID-19 were evaluated by CT scan during a six-month follow-up period, in which only 48% had a normal CT scan [24]. Also, in the study of Hossein et al., 42.3% had pulmonary sequels in CT scan follow-up. The most common residual patterns in CT scans included GOO at 54.5% followed by grand glass mix at 31.8% and parenchymal bands at 13.7% [25]. Therefore, a wide range of findings related to COVID-19 can be seen in the weeks and months after recovery, sometimes without symptoms and sometimes with signs and symptoms associated with COVID-19. It seems that follow-up of these patients and simultaneous and continuous evaluation of lung CT scan findings along with clinical manifestations in patients will be necessary even after partial recovery and hospital discharge.

CONCLUSIONS

As a final conclusion, within at least three months of disease recovery in hospitalized patients with COVID-19, evidence of pulmonary involvement in 83% in the form of chronic pulmonary sequels can be expected. The most common findings related to pulmonary involvement included GOO in 20.2% and parenchymal band in 12.8% of cases. The presence of chronic pulmonary lesions in COVID-19 patients is strongly influenced by old age, obesity, the presence of underlying comorbidities as well and the severity of early COVID-19 disease.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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Authors’ contribution
Design and concepts: TFL, SK, MH, KO; Data gathering and analysis: TFL, SK, MH, KO; Preparing the manuscript: TFL, SK, MH, KO.

Ethics approval and consent to participate
The ethical committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran confirmed the proposal of this study with IR.SBMU.MSP.REC.1400.253 code.

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Investigation of Mutations in H19, IGF2, CDKN1C, KCNQ1, and NSD1 Genes in Iranian Children Suspected of Silver-Russell Syndrome (SRS) and Beckwith-Wiedemann Syndrome (BWS) with MS-MLPA

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Abstract: *Background: Silver-Russell Syndrome (SRS) and Beckwith-Wiedemann Syndrome (BWS) are two syndromes that are poorly diagnosed in many affected people due to mild and subtle symptoms, genetic complexity, and lack of familiarity with the hallmarks. Objective: The present study was conducted with the aim of determining mutations in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes in Iranian children suspected of SRS and BWS by the MS-MLPA method. Methods: In this case series study, which was conducted in 2022 in Pars Genome Laboratory, Karaj, Iran, 10 children suspected of SRS or BWS syndrome were included. These 10 Iranian children were referred by pediatric endocrinologists. 5 ml of peripheral blood was taken per patient for DNA extraction. MS-MLPA method was undertaken for possible mutations (methylation and deletion) in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes. Results: The interpretation of MS-MLPA results showed that out of 10 children (4 boys and 6 girls) suspected of having SRS or BWS syndrome (based on the pediatric endocrinologist's diagnosis), only 3 children were definitively diagnosed with SRS or BWS syndrome. Based on this, methylation changes in the promoter of ICR1 and ICR2, which are related to the genes H19, IGF2, CDKN1C, KCNQ1, and NSD1, lead to the development of SRS or BWS syndrome. Conclusion: The present findings showed that methylation changes in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes are associated with the occurrence of SRS or BWS syndrome. In this study, we show that MS-MLPA can serve as a rapid, inexpensive, and reliable method for the molecular diagnosis of these syndromes.*

Keywords: *Silver-Russell Syndrome (SRS), Beckwith-Wiedemann Syndrome (BWS), Methylation, MS-MLPA*

INTRODUCTION

Silver-Russell Syndrome (SRS) is a rare disorder characterized by intrauterine growth restriction, poor postnatal growth, macrocephaly, a triangular facial appearance, prominent forehead, body asymmetry, and significant feeding difficulties [1]. Patients can have a wide range of clinical findings. The majority of people with SRS have normal intelligence but have motor or speech delays. SRS is genetically heterogeneous, which means that different genetic abnormalities cause this disorder [2]. In around 60% of people with SRS, the disease is related to mutations in chromosome 7 or 11. Genetic defects that may be responsible for this syndrome include uniparental disomy of maternal chromosome 11P15.5, deletion of chromosome 7, or duplication of chromosome 7 or mutation of the KCNQ1, H19 (located on 11p15) and NSD1 (located on 5p35). However, in about 40% of patients with SRS, the cause is unknown. In general, the diagnosis of SRS is based on clinical findings. Since many symptoms are subtle and nonspecific, SRS remains difficult to diagnose [3]. Consensus guidelines for the evaluation and diagnosis of SRS have recently been published based on the Netchine-Harbison clinical scoring system for SRS [4]. Testing for known genetic causes of SRS (chromosomes 7 and 11) can confirm the clinical diagnosis in up to 60% of individuals. Knowing the main genetic cause can also help to select the appropriate treatment approach [5]. Treatment of this syndrome focuses on managing the symptoms [6,7]. SRS is present at birth and many symptoms improve as the patient grows [8].

Beckwith-Wiedemann Syndrome (BWS) is an overgrowth syndrome that affects certain chromosomes of the fetus. People with this syndrome may experience mild to severe signs and symptoms. One out of every 11,000 infants is affected by BWS, which is equally common in both boys and girls. This syndrome causes excessive growth of some body parts. BWS affects many areas of the body and makes the child appear significantly larger than their peers. Patients show different signs and symptoms depending on the affected organs [9,10], including macrosomia, hemihyperplasia, macroglossia, hypoglycemia in infancy, and sometimes long-term hypoglycemia (due to high insulin). The management of this syndrome focuses on its common symptoms and complications.

Physicians monitor and check affected children for low blood sugar and increase the frequency of feeding to obtain optimal blood glucose levels. There are several genetic causes of BWS, usually resulting in changes in one or more genes in a region of chromosome 11 known as 11p15 [11,12]. In most cases, there is genetic mosaicism. Other genetic changes in this syndrome include methylation defects in the region of 11p15, uniparental disomy (UPD) in 11p15, or mutation of the CDKN1C and IGF2. The diagnosis of BWS is clinical and it is often confirmed by genetic testing. To assay these abnormalities, a blood sample or other tissue samples should be taken [13,14].

In addition to organ growth defects, BWS patients are susceptible to other diseases, including cancer (such as Wilms' kidney tumor, hepatoblastoma, and neuroblastoma), heart disease (pericarditis, cardiomyopathy, etc.), hypoglycemia, hemihyperplasia, and digestive disorders [15]. Conducting investigations of H19, IGF2, CDKN1C, KCNQ1 (located 11p15), and NSD1 (located 5p35) genes in these syndromes and increasing information in this field can lead to a more accurate and precise diagnosis; preventing secondary diseases including cancer; and also be effective in preventing the transfer of these genes to the fetus before and during pregnancy [3,13,14]. Considering that no study in this field has been done in Iran, the present research aims to investigate mutations in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes in children suspected of SRS and BWS with Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA) method.

MATERIALS AND METHODS

Study procedures

In this case series study that was conducted in 2022 at Pars Genome Laboratory in Karaj, Iran, 10 children suspected of SRS or BWS were included. These 10 Iranian children were referred to us by a pediatric endocrinologist for final confirmation of the diagnosis. 5 ml of peripheral blood was taken for DNA extraction from these patients. MS-MLPA method was undertaken to identify mutations (methylation and deletion) in H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes [16].

Inclusion criteria

The pediatric endocrinologists referred children under 12 years of age and clinically suspicious for the diagnosis of SRS or BWS. Written informed consent from parents and guardians of the children was documented.

Exclusion criteria

Children with multiple comorbidities

DNA extraction and purification

5 ml of peripheral blood was taken from each patient. DNA extraction from peripheral blood leukocytes was performed for all children using a DNA extraction kit (DENA Zist Asia, Cot.No:S-1031-1, made in Iran). Then, the quantity of extracted DNA was determined and confirmed with the nanodrop device, and the quality of the DNA samples was confirmed by using 1% agarose gel.

MS-MLPA

MS-MLPA probe mixture (ME030BWS/SRS) was purchased from the manufacturer (MRC-Holland, Amsterdam/NL).

This mix contains 42 probes, 26 of which are specific for the BWS/SRS region in 11p15. Ten of the 11p15 probes are methylation-specific since they contain a HhaI recognition site. Two probes for NSD1 and 13 reference probes (without HhaI recognition site) and one digestion control probe.

We used a total of 200 ng of genomic DNA extracted from peripheral blood by the salting-out procedure. 10 Mmol/l Tris-EDTA (pH 8.0) was added to DNA if dilution was necessary. After 16 h of hybridization at 60C, samples were equally split into two aliquots. The first aliquot underwent ligation only, whereas the second one underwent ligation followed by enzymatic digestion with HhaI [a restriction enzyme recognizing only unmethylated DNA] and PCR amplification using universal primers.

In the latter case, amplification products were obtained and detected by capillary electrophoresis only if the CpG included in the HhaI site was methylated. Ligation, enzymatic digestion, and PCR amplification were performed according to the manufacturer’s instructions.

All samples were run in duplicate and at least two normal samples were analyzed in each MS-MLPA experiment, as controls. PCR products (1 ml) were mixed with 0.4 ml of internal size standard (500LIZ Genescan; Applied Biosystems) and 9 ml of deionized formamide and injected into an ABI-3130 genetic analyzer (Applied Biosystems) equipped with a 36 cm, four capillaries array using POP7 as polymer. The samples were injected for 20 s at 1.5 kV and run for a total time of 1200 s at 15 kV. Genemarker v1.95 software is used for analysis, which performs all steps of data normalization and sloping data correction.

Ethical considerations

This study was approved by the ethics committee of Azad University of Tehran Medical Sciences (code: IR.IAU.PS.REC.1401.062). Throughout the study, researchers adhered to the Declaration of Helsinki. Written informed consent was obtained from the patients in this research. All information related to children was kept confidential. This study did not interfere with the process of providing medical services and care by physicians in the clinic. This research did not impose any additional costs on the patient.

RESULTS

The interpretation of MS-MLPA findings demonstrated that out of 10 children (4 boys and 6 girls) suspected of SRS or BWS (based on the diagnosis of a pediatric endocrinologist), only 3 were definitively diagnosed with these syndromes. None of the children were the result of a consanguine marriage. No deletion or addition changes were observed in any of the children, especially in the NSD1 gene.

The first patient

A newborn baby girl was diagnosed by an endocrinologist with suspicion of BWS. DNA was extracted from the blood sample and molecular analysis was done with MS MLPA genetic test. Copy number analysis showed that no deletions or duplications occurred in the chromosomal segments and therefore deemed normal (Figure 1A). Methylation analysis showed that hypomethylation of IC2 occurred in the 11p15 section [Figure 1B]. Therefore, this infant indeed had BWS.

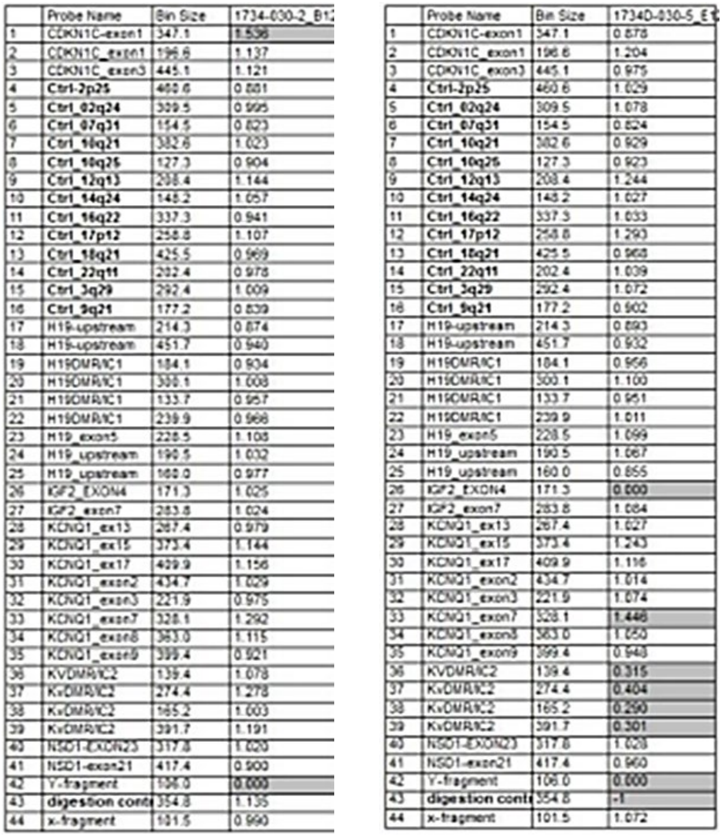

	Probe Name	Bin Size	1734-030-2_B1
1	CDKN1C-exon1	347.1	1.536
2	CDKN1C_exon1	196.6	1.137
3	CDKN1C_exon3	445.1	1.121
4	**Ctrl-2p25**	460.6	0.881
5	**Ctrl_02q24**	309.5	0.995
6	**Ctrl_07q31**	154.5	0.823
7	**Ctrl_10q21**	382.6	1.023
8	**Ctrl_10q25**	127.3	0.904
9	**Ctrl_12q13**	208.4	1.144
10	**Ctrl_14q24**	148.2	1.057
11	**Ctrl_16q22**	337.3	0.941
12	**Ctrl_17p12**	258.8	1.107
13	**Ctrl_18q21**	425.5	0.969
14	**Ctrl_22q11**	202.4	0.978
15	**Ctrl_3q29**	292.4	1.009
16	**Ctrl_9q21**	177.2	0.839
17	H19-upstream	214.3	0.874
18	H19-upstream	451.7	0.940
19	H19DMR/IC1	184.1	0.904
20	H19DMR/IC1	300.1	1.008
21	H19DMR/IC1	133.7	0.957
22	H19DMR/IC1	239.9	0.968
23	H19_exon5	228.5	1.108
24	H19_upstream	190.5	1.032
25	H19_upstream	160.0	0.977
26	IGF2_EXON4	171.3	1.025
27	IGF2_exon7	283.8	1.024
28	KCNQ1_ex13	267.4	0.979
29	KCNQ1_ex15	373.4	1.144
30	KCNQ1_ex17	409.9	1.156
31	KCNQ1_exon2	434.7	1.029
32	KCNQ1_exon3	221.9	0.975
33	KCNQ1_exon7	328.1	1.292
34	KCNQ1_exon8	363.0	1.115
35	KCNQ1_exon9	399.4	0.921
36	KVDMR/IC2	139.4	1.078
37	KvDMR/IC2	274.4	1.278
38	KvDMR/IC2	165.2	1.003
39	KvDMR/IC2	391.7	1.191
40	NSD1-EXON23	317.8	1.020
41	NSD1-exon21	417.4	0.900
42	Y-fragment	106.0	0.000
43	**digestion cont**	354.8	1.135
44	x-fragment	101.5	0.990

A. Copy number analysis

	Probe Name	Bin Size	1734D-030-5_E
1	CDKN1C-exon1	347.1	0.878
2	CDKN1C_exon1	196.6	1.204
3	CDKN1C_exon3	445.1	0.975
4	**Ctrl-2p25**	460.6	1.029
5	**Ctrl_02q24**	309.5	1.078
6	**Ctrl_07q31**	154.5	0.824
7	**Ctrl_10q21**	382.6	0.929
8	**Ctrl_10q25**	127.3	0.923
9	**Ctrl_12q13**	208.4	1.244
10	**Ctrl_14q24**	148.2	1.027
11	**Ctrl_16q22**	337.3	1.033
12	**Ctrl_17p12**	258.8	1.293
13	**Ctrl_18q21**	425.5	0.968
14	**Ctrl_22q11**	202.4	1.039
15	**Ctrl_3q29**	292.4	1.072
16	**Ctrl_9q21**	177.2	0.902
17	H19-upstream	214.3	0.893
18	H19-upstream	451.7	0.932
19	H19DMR/IC1	184.1	0.956
20	H19DMR/IC1	300.1	1.100
21	H19DMR/IC1	133.7	0.951
22	H19DMR/IC1	239.9	1.011
23	H19_exon5	228.5	1.099
24	H19_upstream	190.5	1.067
25	H19_upstream	160.0	0.855
26	IGF2_EXON4	171.3	0.000
27	IGF2_exon7	283.8	1.084
28	KCNQ1_ex13	267.4	1.027
29	KCNQ1_ex15	373.4	1.243
30	KCNQ1_ex17	409.9	1.116
31	KCNQ1_exon2	434.7	1.014
32	KCNQ1_exon3	221.9	1.074
33	KCNQ1_exon7	328.1	1.446
34	KCNQ1_exon8	363.0	1.050
35	KCNQ1_exon9	399.4	0.948
36	KVDMR/IC2	139.4	0.315
37	KvDMR/IC2	274.4	0.404
38	KvDMR/IC2	165.2	0.290
39	KvDMR/IC2	391.7	0.301
40	NSD1-EXON23	317.8	1.028
41	NSD1-exon21	417.4	0.960
42	Y-fragment	106.0	0.000
43	**digestion cont**	354.8	-1
44	x-fragment	101.5	1.072

B. Methylation analysis

Figure 1: MLPA genetic test findings related to the first patient

The second patient

A 21-month-old girl, with the examination of an endocrinologist, was diagnosed with suspected SRS. DNA was extracted from the blood sample and molecular analysis was done with MS-MLPA genetic test. Copy number analysis showed that no deletions or duplications occurred in the chromosomal segments, hence a normal finding (Figure 2A). Methylation analysis showed that hypomethylation of IC1 occurred in the 11p15 segment. This hypomethylation occurred in probes 19 to 22 (Figure 2B). Therefore, this baby definitely had SRS.

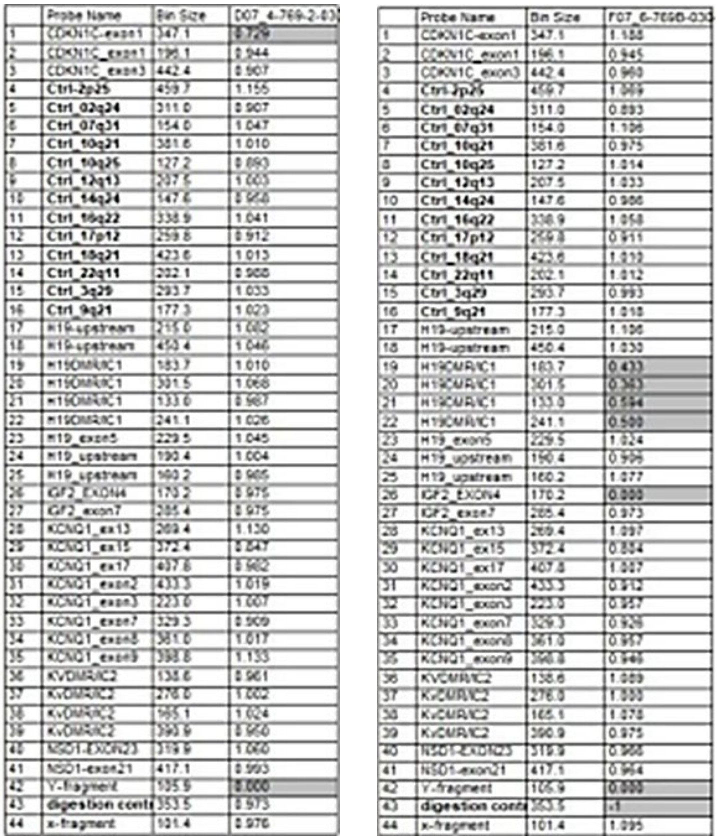

	Probe Name	Bin Size	D07_4-769-2-03
1	CDKN1C-exon1	347.1	0.729
2	CDKN1C_exon1	196.1	0.944
3	CDKN1C_exon3	442.4	0.907
4	**Ctrl-2p25**	459.7	1.155
5	**Ctrl_02q24**	311.0	0.907
6	**Ctrl_07q31**	154.0	1.047
7	**Ctrl_10q21**	381.6	1.010
8	**Ctrl_10q26**	127.2	0.890
9	**Ctrl_12q13**	207.5	1.003
10	**Ctrl_14q24**	147.6	0.958
11	**Ctrl_16q22**	338.9	1.041
12	**Ctrl_17p12**	259.8	0.912
13	**Ctrl_18q21**	423.6	1.013
14	**Ctrl_22q11**	202.1	0.988
15	**Ctrl_3q29**	293.7	1.033
16	**Ctrl_9q21**	177.3	1.023
17	H19-upstream	215.0	1.082
18	H19-upstream	450.4	1.046
19	H19DMR/IC1	183.7	1.010
20	H19DMR/IC1	301.5	1.068
21	H19DMR/IC1	133.0	0.987
22	H19DMR/IC1	241.1	1.026
23	H19_exon5	229.5	1.045
24	H19_upstream	190.4	1.004
25	H19_upstream	160.2	0.965
26	IGF2_EXON4	170.2	0.975
27	IGF2_exon7	285.4	0.975
28	KCNQ1_ex13	269.4	1.130
29	KCNQ1_ex15	372.4	0.847
30	KCNQ1_ex17	407.8	0.982
31	KCNQ1_exon2	433.3	1.019
32	KCNQ1_exon3	223.0	1.007
33	KCNQ1_exon7	329.3	0.909
34	KCNQ1_exon8	361.0	1.017
35	KCNQ1_exon9	398.8	1.133
36	KVDMR/IC2	138.6	0.961
37	KvDMR/IC2	276.0	1.002
38	KvDMR/IC2	165.1	1.024
39	KvDMR/IC2	390.9	0.960
40	NSD1-EXON23	319.9	1.060
41	NSD1-exon21	417.1	0.993
42	Y-fragment	105.9	0.000
43	**digestion cont**	353.5	0.973
44	x-fragment	101.4	0.976

	Probe Name	Bin Size	F07_6-7698-03
1	CDKN1C-exon1	347.1	1.188
2	CDKN1C_exon1	196.1	0.945
3	CDKN1C_exon3	442.4	0.960
4	**Ctrl-2p25**	459.7	1.069
5	**Ctrl_02q24**	311.0	0.893
6	**Ctrl_07q31**	154.0	1.106
7	**Ctrl_10q21**	381.6	0.975
8	**Ctrl_10q26**	127.2	1.014
9	**Ctrl_12q13**	207.5	1.033
10	**Ctrl_14q24**	147.6	0.986
11	**Ctrl_16q22**	338.9	1.058
12	**Ctrl_17p12**	259.8	0.911
13	**Ctrl_18q21**	423.6	1.010
14	**Ctrl_22q11**	202.1	1.012
15	**Ctrl_3q29**	293.7	0.993
16	**Ctrl_9q21**	177.3	1.018
17	H19-upstream	215.0	1.106
18	H19-upstream	450.4	1.030
19	H19DMR/IC1	183.7	0.433
20	H19DMR/IC1	301.5	0.383
21	H19DMR/IC1	133.0	0.594
22	H19DMR/IC1	241.1	0.500
23	H19_exon5	229.5	1.024
24	H19_upstream	190.4	0.906
25	H19_upstream	160.2	1.077
26	IGF2_EXON4	170.2	0.000
27	IGF2_exon7	285.4	0.973
28	KCNQ1_ex13	269.4	1.097
29	KCNQ1_ex15	372.4	0.884
30	KCNQ1_ex17	407.8	1.087
31	KCNQ1_exon2	433.3	0.912
32	KCNQ1_exon3	223.0	0.957
33	KCNQ1_exon7	329.3	0.926
34	KCNQ1_exon8	361.0	0.957
35	KCNQ1_exon9	398.8	0.946
36	KVDMR/IC2	138.6	1.089
37	KvDMR/IC2	276.0	1.000
38	KvDMR/IC2	165.1	1.078
39	KvDMR/IC2	390.9	0.975
40	NSD1-EXON23	319.9	0.988
41	NSD1-exon21	417.1	0.964
42	Y-fragment	105.9	0.000
43	**digestion cont**	353.5	<1
44	x-fragment	101.4	1.095

A. Copy number analysis B. Methylation analysis

Figure 2: MLPA genetic test findings related to the second patient

The third patient

A 2-year-old boy was suspected of having BWS according to the examination of an endocrinologist. DNA was extracted from the blood sample and molecular analysis was done with MS-MLPA genetic test. Copy number analysis showed that no deletions or duplications occurred in the chromosomal segments and therefore, it was deemed normal [Figure 3A]. Methylation analysis showed that hypermethylation of IC1 occurred in the 11p15 segment. This hypomethylation occurred in probes 36, 38, and 39. IC2 hypomethylation was detected in the 11p15 segment. This hypomethylation occurred in probes 19 to 22 (Figure 3B). Therefore, this boy definitely had BWS. Uniparental disomy occurred in this patient and he inherited both chromosomes from his father.

The fourth patient

A 9-year-old boy was suspected of SRS according to the examination of an endocrinologist. DNA was extracted from the blood sample and molecular analysis was done with MS-MLPA genetic test. Copy number analysis showed that no deletions or duplications occurred in the chromosomal segments and therefore, it was deemed normal (Figure 4A). Methylation analysis showed a normal pattern in the IC1 and IC2 (Figure 4B). Therefore, this child did not suffer from either SRS or BWS.

Fifth to tenth patients

The fifth to seventh patients were a 5-year-old girl, an 8-year-old girl, and a 12-year-old girl, respectively, suspected of SRS. The eighth patient was a 4-year-old boy, the ninth patient was a 6-year-old girl, and the tenth patient was a 7-year-old boy who was suspected of having BWS; according to MS-MLPA, it was found that none had either SRS or BWS.

DISCUSSION

The main reason for investigating mutations and methylation changes in H19, IGF2, CDKN1C, and KCNQ1 (located on 11p15) and NSD1 (located on 5q35.3) genes lies in their function. Alterations in these genes affect function and can give rise to the phenotypes associated with SRS or BWS. The gene CDKN1C (Cyclin Dependent Kinase Inhibitor 1C) is a tumor suppressor and a strong inhibitor and a negative regulator of cell proliferation.

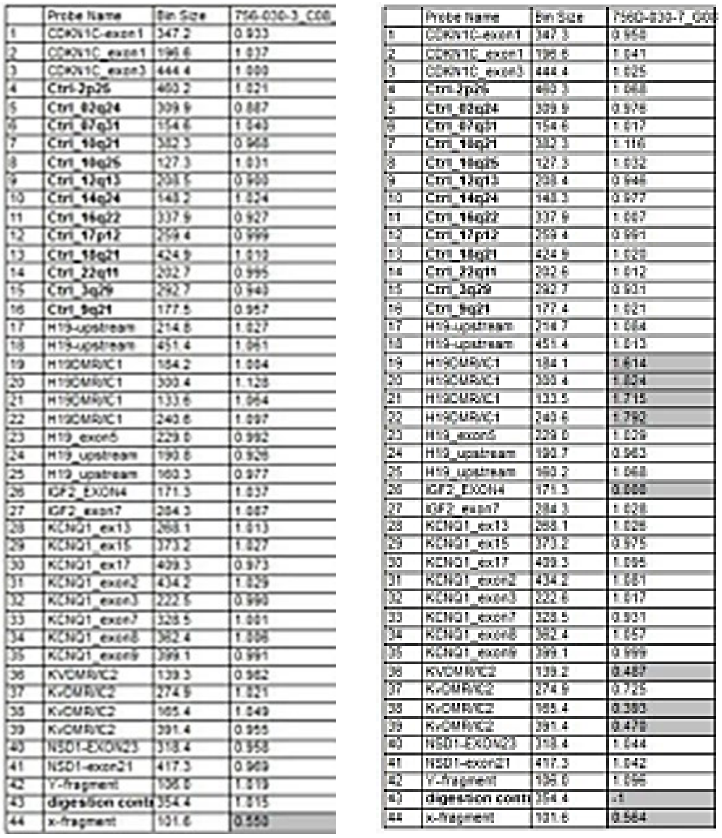

	Probe Name	Bin Size	756-030-3_C08_
1	CDKN1C-exon1	347.2	0.933
2	CDKN1C_exon1	196.6	1.037
3	CDKN1C_exon3	444.4	1.000
4	**Ctrl-2p25**	403.2	1.021
5	**Ctrl_02q24**	309.9	0.887
6	**Ctrl_07q31**	154.6	1.043
7	**Ctrl_10q21**	382.3	0.968
8	**Ctrl_10q26**	127.3	1.031
9	**Ctrl_12q13**	208.5	0.900
10	**Ctrl_14q24**	143.2	1.024
11	**Ctrl_16q22**	337.9	0.927
12	**Ctrl_17p12**	259.4	0.999
13	**Ctrl_18q21**	424.9	1.012
14	**Ctrl_22q11**	202.7	0.995
15	**Ctrl_3q29**	292.7	0.943
16	**Ctrl_9q21**	177.5	0.957
17	H19-upstream	214.8	1.027
18	H19-upstream	451.4	1.061
19	H19DMR/IC1	184.2	1.004
20	H19DMR/IC1	300.4	1.128
21	H19DMR/IC1	133.6	1.064
22	H19DMR/IC1	240.6	1.097
23	H19_exon5	229.0	0.992
24	H19_upstream	190.8	0.926
25	H19_upstream	160.3	0.977
26	IGF2_EXON4	171.3	1.037
27	IGF2_exon7	284.3	1.087
28	KCNQ1_ex13	268.1	1.013
29	KCNQ1_ex15	373.2	1.027
30	KCNQ1_ex17	409.3	0.973
31	KCNQ1_exon2	434.2	1.029
32	KCNQ1_exon3	222.5	0.990
33	KCNQ1_exon7	328.5	1.001
34	KCNQ1_exon8	382.4	1.006
35	KCNQ1_exon9	399.1	0.991
36	KvDMR/IC2	139.3	0.962
37	KvDMR/IC2	274.9	1.021
38	KvDMR/IC2	165.4	1.049
39	KvDMR/IC2	391.4	0.955
40	NSD1-EXON23	318.4	0.958
41	NSD1-exon21	417.3	0.969
42	Y-fragment	106.8	1.019
43	**digestion contr**	354.4	1.015
44	x-fragment	101.6	0.553

A. Copy number analysis

	Probe Name	Bin Size	7560-030-7_G0
1	CDKN1C-exon1	347.3	0.958
2	CDKN1C_exon1	196.6	1.041
3	CDKN1C_exon3	444.4	1.025
4	**Ctrl-2p25**	403.3	1.068
5	**Ctrl_02q24**	309.9	0.976
6	**Ctrl_07q31**	154.6	1.017
7	**Ctrl_10q21**	382.3	1.116
8	**Ctrl_10q26**	127.3	1.032
9	**Ctrl_12q13**	208.4	0.946
10	**Ctrl_14q24**	143.3	0.977
11	**Ctrl_16q22**	337.9	1.067
12	**Ctrl_17p12**	259.4	0.991
13	**Ctrl_18q21**	424.9	1.028
14	**Ctrl_22q11**	202.6	1.012
15	**Ctrl_3q29**	292.7	0.931
16	**Ctrl_9q21**	177.4	1.021
17	H19-upstream	214.7	1.084
18	H19-upstream	451.4	1.013
19	H19DMR/IC1	184.1	1.614
20	H19DMR/IC1	300.4	1.824
21	H19DMR/IC1	133.5	1.715
22	H19DMR/IC1	240.6	1.792
23	H19_exon5	229.0	1.039
24	H19_upstream	190.7	0.963
25	H19_upstream	160.2	1.068
26	IGF2_EXON4	171.3	0.888
27	IGF2_exon7	284.3	1.028
28	KCNQ1_ex13	268.1	1.026
29	KCNQ1_ex15	373.2	0.975
30	KCNQ1_ex17	409.3	1.095
31	KCNQ1_exon2	434.2	1.081
32	KCNQ1_exon3	222.6	1.017
33	KCNQ1_exon7	328.5	0.931
34	KCNQ1_exon8	382.4	1.057
35	KCNQ1_exon9	399.1	0.999
36	KvDMR/IC2	139.2	0.487
37	KvDMR/IC2	274.9	0.725
38	KvDMR/IC2	165.4	0.393
39	KvDMR/IC2	391.4	0.478
40	NSD1-EXON23	318.4	1.044
41	NSD1-exon21	417.3	1.042
42	Y-fragment	106.0	1.096
43	**digestion contr**	354.4	<1
44	x-fragment	101.6	0.564

B. Methylation analysis

Figure 3: MLPA genetic test findings related to the third patient

Mutation in this gene can cause abnormal height and abnormal bladder structure and function. It also provides instructions for making proteins that help growth regulation [17]. NSD1 (Nuclear Receptor Binding SET Domain Protein 1) acts as a basic transcription factor and a regulator in transcription. Mutation in this gene can cause childhood acute myeloid leukemia and abnormal body height [18]. KCNQ1 [potassium voltage-gated channel subfamily Q member 1] gene belongs to a large family of genes responsible for making potassium channels [19]. The IGF2 gene (insulin-like growth factor 2) plays an essential role in the growth and development of the fetus before birth, and studies also showed that IGF2 causes the growth and division (multiplication) of cells in many different tissues [20]. The H19 gene is located near the IGF2 gene and provides instructions for making a non-coding RNA. Despite many genes, the H19 gene does not contain instructions for making a protein. The function of this non-coding RNA produced from this gene is unknown, but researchers believe it may act as a tumor suppressor. The H19 gene is highly active before birth in various tissues and appears to play an important role in early development [21].

The molecular defects of BWS and SRS are very heterogeneous. Many of these abnormalities involve epigenetic or copy number changes within the 11p15.5 gene cluster. Identification of these defects usually requires the use of multiple assays. Molecular diagnosis of SRS and BWS is currently possible only in one-third of patients [22] The aim of this study was to evaluate the methylation status and copy number of genes H19, IGF2, CDKN1C, KCNQ1, and NSD1 in blood samples of 10 Iranian children suspected of SRS or BWS.

Analysis of methylation, deletion, and addition in the mentioned genes in 10 children suspected of SRS or BWS, led to the diagnosis of SRS in one child and BWS in 2 children. In line with the present study, Gaston et al. showed that analysis of KvDMR1 methylation in leukocyte DNA alone identified BWS in 60% of all patients (64% of patients with severe form and 53% of patients with incomplete form of BWS). The analysis of the H19 gene methylation identified 13 other BWS patients with H19 hypermethylation, which confirms the effect of methylation in the occurrence of BWS in the two mentioned genes in line with our study [23].

Therefore, KCNQ1OT and H19 gene analysis is useful in routine evaluation and leads to the diagnosis of BWS or SRS in children suspected of these syndromes [24].

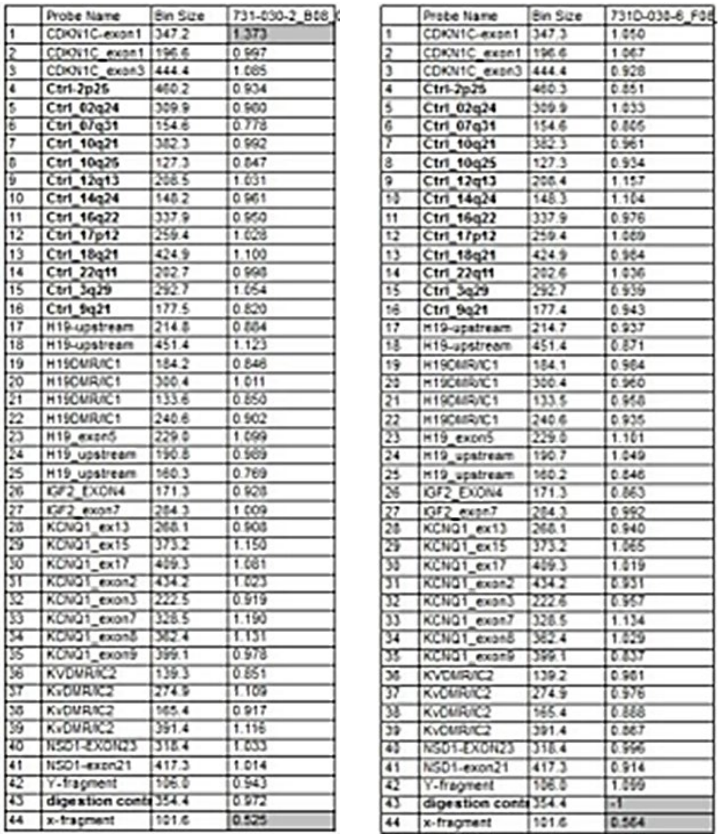

	Probe Name	Bin Size	731-030-2_B08
1	CDKN1C-exon1	347.2	1.373
2	CDKN1C_exon1	196.6	0.997
3	CDKN1C_exon3	444.4	1.085
4	**Ctrl-2p25**	460.2	0.934
5	**Ctrl_02q24**	309.9	0.900
6	**Ctrl_07q31**	154.6	0.778
7	**Ctrl_10q21**	382.3	0.992
8	**Ctrl_10q25**	127.3	0.847
9	**Ctrl_12q13**	208.5	1.031
10	**Ctrl_14q24**	148.2	0.961
11	**Ctrl_16q22**	337.9	0.950
12	**Ctrl_17p12**	259.4	1.028
13	**Ctrl_18q21**	424.9	1.100
14	**Ctrl_22q11**	202.7	0.998
15	**Ctrl_3q29**	292.7	1.054
16	**Ctrl_9q21**	177.5	0.820
17	H19-upstream	214.8	0.884
18	H19-upstream	451.4	1.123
19	H19DMR/IC1	184.2	0.846
20	H19DMR/IC1	300.4	1.011
21	H19DMR/IC1	133.6	0.850
22	H19DMR/IC1	240.6	0.902
23	H19_exon5	229.0	1.099
24	H19_upstream	190.8	0.989
25	H19_upstream	160.3	0.769
26	IGF2_EXON4	171.3	0.928
27	IGF2_exon7	284.3	1.009
28	KCNQ1_ex13	268.1	0.908
29	KCNQ1_ex15	373.2	1.150
30	KCNQ1_ex17	409.3	1.081
31	KCNQ1_exon2	434.2	1.023
32	KCNQ1_exon3	222.5	0.919
33	KCNQ1_exon7	328.5	1.190
34	KCNQ1_exon8	382.4	1.131
35	KCNQ1_exon9	399.1	0.978
36	KVDMR/IC2	139.3	0.851
37	KvDMR/IC2	274.9	1.109
38	KvDMR/IC2	165.4	0.917
39	KvDMR/IC2	391.4	1.116
40	NSD1-EXON23	318.4	1.033
41	NSD1-exon21	417.3	1.014
42	Y-fragment	106.0	0.943
43	**digestion conti**	354.4	0.972
44	x-fragment	101.6	0.525

A. Copy number analysis

	Probe Name	Bin Size	731D-030-6_F0
1	CDKN1C-exon1	347.3	1.050
2	CDKN1C_exon1	196.6	1.067
3	CDKN1C_exon3	444.4	0.928
4	**Ctrl-2p25**	460.3	0.851
5	**Ctrl_02q24**	309.9	1.033
6	**Ctrl_07q31**	154.6	0.805
7	**Ctrl_10q21**	382.3	0.961
8	**Ctrl_10q25**	127.3	0.934
9	**Ctrl_12q13**	208.4	1.157
10	**Ctrl_14q24**	148.3	1.104
11	**Ctrl_16q22**	337.9	0.976
12	**Ctrl_17p12**	259.4	1.089
13	**Ctrl_18q21**	424.9	0.984
14	**Ctrl_22q11**	202.6	1.036
15	**Ctrl_3q29**	292.7	0.939
16	**Ctrl_9q21**	177.4	0.943
17	H19-upstream	214.7	0.937
18	H19-upstream	451.4	0.871
19	H19DMR/IC1	184.1	0.984
20	H19DMR/IC1	300.4	0.960
21	H19DMR/IC1	133.5	0.958
22	H19DMR/IC1	240.6	0.935
23	H19_exon5	229.0	1.101
24	H19_upstream	190.7	1.049
25	H19_upstream	160.2	0.846
26	IGF2_EXON4	171.3	0.863
27	IGF2_exon7	284.3	0.992
28	KCNQ1_ex13	268.1	0.940
29	KCNQ1_ex15	373.2	1.065
30	KCNQ1_ex17	409.3	1.019
31	KCNQ1_exon2	434.2	0.931
32	KCNQ1_exon3	222.6	0.957
33	KCNQ1_exon7	328.5	1.134
34	KCNQ1_exon8	382.4	1.029
35	KCNQ1_exon9	399.1	0.837
36	KVDMR/IC2	139.2	0.981
37	KvDMR/IC2	274.9	0.976
38	KvDMR/IC2	165.4	0.888
39	KvDMR/IC2	391.4	0.867
40	NSD1-EXON23	318.4	0.996
41	NSD1-exon21	417.3	0.914
42	Y-fragment	106.0	1.099
43	**digestion conti**	354.4	<1
44	x-fragment	101.6	0.564

B. Methylation analysis

Figure 4: MLPA genetic test findings related to the fourth patient

Gaston et al. showed complete or nearly complete demethylation (KvDMR1 MI >90%) in 70% of BWS patients, but about one-third showed partial demethylation, suggesting that this abnormality can occur with mosaicism. These findings were supported by the analysis of other pathological tissues that show different demethylation patterns from blood cells. For example, KvDMR1 is incompletely demethylated in the tongue tissue samples of patients while it is fully demethylated in blood cells, indicating that the analysis of blood samples was sufficient to diagnose BWS [23]. Gaston et al., observed that demethylation of the KCNQ1OT gene was never associated with abnormal methylation of the H19 gene [23] except in patients with UPD 11p15, indicating at least two imprinting centers at 11p15.5. These findings were consistent with a recent study by Engel et al [12].

In the present study, the analysis of H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes was normal in 7 out of 10 children. In line with these findings, Gaston et al reported that the analysis of KCNQ1OT and H19 genes was normal for 26 patients (26.8%). CDKN1C gene sequencing was performed in 10 of these patients [5 sporadic forms and 5 patients from families]. No abnormalities were found in sporadic cases. CDKN1C mutations were found in the families of 2 patients, and both patients had exomphalos [23]. Other unknown 11p15 abnormalities may account for other cases. Various mechanisms may cause over-growth syndrome by inducing the activity of growth factors such as IGF2. Several transgenic mouse models have provided evidence for this by showing increased levels of bioactive IGF2 induced by inhibition of the IGF type 2 receptor [IGF2R] or glypican 3, two agents known to bind and downregulate IGF2 expression, making it less accessible to the IGF1R [25]. Further studies on 11p15 genes and IGF2-related regulators in overgrowth syndromes should be conducted to determine the association between genotype and phenotype and to elucidate functional mechanisms.

In a study by Jurkiewicz et al., the MS-MLPA screening test identified the triplication of the IGF2/H19 domain for all probes of these genes in the ME030 kit, although this result was contrary to the results of our study because we did not find any duplication or deletion in individuals. They also showed that the H19 (ICR1) region had increased methylation and also showed normal methylation patterns for the IGF2R KCNQ1OT1 genes, which are in line with the results of our study [26].

In the present study, MS-MLPA was used for the molecular diagnosis of BWS and SRS. The results showed that MS-MLPA is a sensitive and reliable tool for further identification of molecular defects of BWS and SRS. These findings are in line with the study of Priolo et al., which was conducted in Italy [27]. Most epigenetic defects of BWS and SRS occur sporadically and exist in the mosaic form in individuals [28,29]. Therefore, it is important to use a sensitive diagnostic method to identify all mosaic cases. MS-MLPA method for the detection of 11p15.5 defects is very accurate and sensitive [27].

The great advantage of MS-MLPA is that this method simultaneously determines the copy number and methylation level of the loci. This is clearly shown in the third patient who had both hypomethylation and hypermethylation. While the ligation-only reaction revealed an increase in copy number, the ligation-digestion reaction provided information about the parental origin of the amplified region. Another study also confirmed that MS-MLPA can be considered a robust assay for the analysis of chromosome 11p5.5 defects [30]. In conclusion, our experience with these 10 suspected children showed that MS-MLPA is an easy, low-cost, and reliable method for the molecular diagnosis of BWS/SRS cases with im-printed and repetitive defects on chromosome 11p15. However, due to the small sample size as one limitation in this study, these results need to be confirmed in a larger series of patients. Also, for 7 children who were not confirmed to be suffering from BWS and RSS syndromes in this study, we suggest performing a WES test for them because the doctor may have made a mistake in diagnosing their symptoms and they may be suffering from another disease.

CONCLUSIONS

This was the first study to document these two syndromes in Iran. The present findings showed that methylation changes in the control regions of ICR1 and ICR2, which are related to H19, IGF2, CDKN1C, KCNQ1, and NSD1 genes, lead to the development of SRS or BWS. A diagnosis of the two syndromes is not accurate solely based solely on clinical symptoms and without the use of cellular and molecular genetic testing. MS-MLPA is suggested as a low-cost, reliable, and rapid method for the molecular diagnosis of BWS/SRS with imprinting and duplication defects on chromosome 11P15.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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Authors' contribution

MAE, ME, OM, NS, and SS were responsible for the study concept and design. MAE, ME, OM, NS, and SS led data collection. MAE, ME, OM, NS, and SS were responsible for the analysis and interpretation of data. MAE, ME, OM, NS, and SS wrote the draft.

Ethics approval and consent to participate

This study was approved by the ethics committee of Azaz University of Medical Sciences [code: IR.IAU.PS.REC.1401.062]. Throughout the study, researchers adhered to the Declaration of Helsinki. Written informed consent was obtained from the children's parents in this research.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Body Composition Differences in Army Paratroopers: Effect of Speciality, Age, and Gender

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Abstract: *Body composition has been associated with better performance in military populations. However, there is no consensus about the most suitable body composition values for army paratroopers. To analyze body composition differences among the type of specialty, age group, and gender of army paratroopers, for a better improvement of their operability. 104 paratroopers of the Spanish Army were analyzed. Anthropometric and morphological data were analysed throw an In-Body720. There was a significant effect of age on intracellular water. Older soldiers had lower values of intracellular water, protein, and skeletal muscle mass than middle-aged soldiers. No significant differences (P ≤ 0.05) for the effect of the kind of work and age together or taken separately. Men had significantly higher values than women for height, weight, extracellular water, protein, fat-free mass, and skeletal muscle mass, and significantly lower values for the percentage of body fat, while no differences were found for body fat mass and body mass index. We conclude that paratroopers' body composition depends on age in skeletal muscle mass, intracellular water, and protein. For better unit readiness, soldiers must be monitored to analyze the effect of training and nutrition habits on their body composition.*

Keywords: *military; body fat mass; lean mass; body water*

INTRODUCTION

Physical training is a key factor for soldiers, who are expected to perform efficiently in physical tasks and maneuvers, i.e. lifting and carrying weights, deployments, long displacements, or fighting [26]. Traditionally, Body Composition (BC) has been associated with better performance in military populations [13], like in load-carriage jobs, in which lean mass (together with aerobic power) has been identified as more important than speed of movement [21]. Despite these facts, there is still a tendency towards considering perceived assumptions, like appearance, rather than empirical data to evaluate the capabilities of each soldier, and weight plus Body Max Index (BMI) by themselves are not enough to assess military training since many other factors, like lean mass gain, are positive aspects of training that need to be measured [22,24].

Although some standards have been used and considered in the Army to encourage soldiers to keep fit and with good nutritional habits, there is no consensus about the most suitable BC for all the healthy ranges of weight, soft lean mass, or body fat percentage. This attempt becomes more complicated if we consider the wide range of duties required by the different units of the Army and the different soldiers´ ages [14,28]. The incorporation of women in the Army further complicates this issue, because there is a need to define gender-appropriate standards to maximize female soldiers' health and performance [14]. Taking sports, as a reference, different specializations would require different standards of body composition [2,37], like different jobs would require different BC in the Army, as it was reported for the Army [16,17,38] and Air Forces [4]. Moreover, BC is a variable that could be easily managed by any motivated soldier both with good fitness and nutrition habits, with the aim, not to predict the performance, but to obtain health benefits, resistance to disease or injuries [18,27], and better general capabilities, both mental and physical due to a balanced lifestyle [14,25].

Paratroopers are considered an elite unit of the Army since their deployments are highly operational and involve a parachute jump, which has been reported to increase heart rate and decrease fine motor skills [10,34]. Apart from the physical readiness required for a quick setup, prior to entering the battlefield, they face additional hazards related to high altitudes, like hypoxia, disorientation, or night flights [3,5,6]. The principal tactical jumps are automatic para-chute jumps, in which the soldier has no control of the parachute opening, and manual parachute jumps, in which the subject has control of the parachute in all the maneuvers. Previous research found that both jumps produced a large stress response increasing cortisol, blood lactate, and sympathetic activation [8,10,11]. The health and performance of these kinds of units, which are under extreme psychological and physiological environments [7], can be undermined and there is a need to monitor their body composition [36], especially during their deployment in crisis management operations [29].

MATERIALS AND METHODS

Experimental approach to the problem

To implement the body composition assessment, we followed previous procedures [4,9,30] in which the participants stood upright on foot electrodes on the instrument platform, with arms not touching the torso. They were barefooted and without excess clothing. Four-foot electrodes were used, two of which were oval-shaped and two heel-shaped, and prior to testing the skin was cleaned and dried. Participants were asked to grip the palm and thumb electrodes. The system was calibrated before the testing session and the contacting surface of the electrodes was cleaned with alcohol before each measurement.

Participants abstained from alcohol consumption and vigorous exercise for 24h before the measurement and were measured in the morning after overnight fasting to control hydration status, as in previous research with bioelectrical impedance.

Subjects

A cross-sectional experimental design was conducted to analyze body composition differences of paratroopers, depending on their specialty, group age, and gender. 7 female paratroopers (specialty: automatic parachute; age: M = 32.1, SD = 8.79), and 97 male paratroopers were analyzed and classified into groups of specialty (43 automatic, age: M = 33.3, SD = 9.75; 54 manual, age: M = 34.8, SD = 7.58) and age (21 younger, under 29 years; 48 middle-aged, between 29 and 38 years; and 28 older, above 38 years) of the Spanish Army with a qualification of “fit” according to the periodic medical examination as recorded in the ministerial order 23/2011. Besides, during the research, they were carrying out the periodic aeromedical training included in the ministerial order 23/2011 and the STANAG 3114 “Aeromedical Training of Flight Personnel” (NATO regulations). Before starting the research, the experimental procedures were explained to all the participants, who gave their voluntary written informed consent under the Declaration of Helsinki. The procedures conducted in the present research were designed and approved by the Medical Service of the Aerospace Medicine Instruction Centre of Spanish Air Forces and the University Ethic Committee (CIPI/18/093).

Instruments

A bioimpedance analyzer (InBody 720, Biospace Co. Ltd., Seoul, South Korea) was used for body mass measurement (to the nearest 0.1 kg) and body height (to the nearest 0.1 cm). InBody 720 is a multifrequency impedance body composition analyzer, which uses an eight-point tactile electrode method to take readings from the body. It measures the resistance at five specific frequencies (1 kHz, 50 kHz, 250 kHz, 500 kHz, and 1 MHz) and reactance at three specific frequencies (5 kHz, 50 kHz, and 250 kHz) on each of five segments (right arm, left arm, trunk, right leg, and left leg). Bioelectrical-impedance analysis (BIA) is one of the methods available for measuring body composition in healthy populations. Its simplicity, portability, cost, and subject acceptance make it a very desirable technique. The reliability of BIA compared to the gold standard body composition measurement (DXA) has been successfully demonstrated for a wide range of normal and overweight populations [15,35] and military samples [1].

Data were electronically imported to Excel using Lookin’Body 3.0 software. The following parameters were analyzed: i. Body mass (kg), ii. Height (cm), iii. Body Mass Index (BMI) (kg/m^2), iv. Total Body Water (TBW) (kg) (including left and right arms, trunk, and left and right legs), v. Intracellular Water (ICW) (l) (including left and right arms, trunk, and left and right legs), vi. Extracellular Water (ECW) (l) (including left and right arms, trunk, and left and right legs), vii. Proteins (kg), viii. Minerals (kg), ix. Body Fat Mass (BFM) (kg) (including left and right arms, trunk, and left and right legs, in kg and %), x. Percentage of Body Fat (PBF) (%), xi. Soft Lean Mass (SLM) (kg), xii. Fat-Free Mass (FFM) (kg) (including left and right arms, trunk, and left and right legs, in kg and %), xiii. Skeletal Muscle Mass (kg).

Statistical analysis

The SPSS statistical package (version 21.0; SPSS, Inc. Chicago, Illinois) was used to analyze the data. Normality assumptions were checked with a Shapiro-Wilk test for all the subgroups and a Kolmogorov-Smirnov test for the whole group. Gender differences were analyzed for the same specialty (automatic parachute) with a T-Student test for parametric samples and a Mann-Whitney test for no parametric samples. Homoscedasticity was tested with the Levene test. A factorial ANOVA test was used to compare the type of work, the group of age, and the interaction between the type of work and the group of age on each parameter together with a Bonferroni post hoc test to analyze pairwise comparisons. The level of significance for all the comparisons was set at P ≤ 0.05.

RESULTS

The results are reported with their mean and standard deviation. Table 1 shows the results of body composition. No significant differences were found for the effect of type of work and age together, but there was a significant effect of age [F(2, 91)=3.172, p = 0.047, η2 = 0.06], in ICW. Older soldiers (M = 28.3, SD = 2.8) had lower values (p = 0.041) of ICW than middle-aged (M = 30.3, SD = 3.7). There was a significant effect of age [F(2, 91)=3.108, p = 0.049, η2 = 0.06], in protein. Older soldiers (M = 12.2, SD = 1.2) had lower values (p = 0.043) of protein than middle-aged (M = 13.0, SD = 1.6). There was a significant effect of age [F(2, 91)=3.154, p = 0.047, η2 = 0.07], in SMM. Older soldiers (M = 34.9, SD = 3.6) had lower values (p = 0.041) of SMM than middle-aged (M = 37.5, SD = 4.9).

Table 1: Results of body composition variables by specialty and age groups

	Age < 28				28 < Age < 38				Age > 38				Age and specialty effect		
	Automatic		Manual		Automatic		Manual		Automatic		Manual				
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	F	p	η^2
Height (cm)	178.8	7.5	177.5	7.0	177.3	6.2	175.2	5.9	174.3	5.8	175.0	5.7	0.47	0.66	0.01
Body Mass (kg)	75.3	7.1	79.4	14.1	83.4	15.4	78.9	10.6	73.6	9.4	76.5	9.2	1.40	0.25	0.03
BMI (kg/m^2)	23.6	2.6	25.1	3.6	26.3	3.5	25.6	2.8	24.2	3.0	24.9	2.6	1.03	0.35	0.02
TBW (l)	46.4	3.1	47.6	5.5	49.0	7.8	47.5	4.8	45.5	4.5	45.3	4.4	0.47	0.62	0.01
ICW (l)	29.1	1.9	29.9	3.6	30.7	4.9	30.0	3.0	28.4	2.9	28.3	2.8	0.36	0.69	0.01
ECW (l)	17.2	1.2	17.7	2.0	18.3	2.9	17.5	1.8	17.0	1.5	17.0	1.6	0.72	0.48	0.02
Proteins (kg)	12.6	0.8	12.9	1.5	13.2	2.1	12.9	1.3	12.2	1.2	12.2	1.2	0.33	0.71	0.00
Minerals (kg)	4.2	0.4	4.5	0.7	4.7	0.8	4.4	0.5	4.3	0.4	4.2	0.5	1.17	0.31	0.03
BFM (kg)	11.9	4.1	14.3	10.1	16.3	7.4	13.9	6.4	11.5	6.5	14.6	5.1	1.86	0.16	0.04
PBF (%)	15.6	4.4	16.9	9.1	19.0	6.3	17.1	5.7	15.1	6.8	18.8	5.3	1.83	0.17	0.04
SLM (kg)	59.8	4.0	61.3	7.2	63.2	10.0	61.3	6.2	58.5	5.8	58.3	5.6	0.45	0.64	0.01
FFM (kg)	63.3	4.3	65.1	7.7	67.0	10.8	64.9	6.6	62.1	6.1	61.8	6.0	0.48	0.61	0.01
SMM (kg)	36.0	2.5	37.1	4.7	38.1	6.4	37.1	3.9	35.0	3.8	34.9	3.6	0.38	0.68	0.01

Note. M = Mean; SD = Standard Deviation; F = Fisher-Snedecor test; η2 = Partial eta squared; BMI = Body Mass Index; TBW = Total Body Water; ICW = Intracellular Water; ECW = Extracellular Water; BFM = Body Fat Mass; PBF = Percentage of Body Fat; SLM = Soft Lean Mass; FFM = Fat Free Mass; SMM = Skeletal Muscle Mass

Table 2 shows the results of FFM and BFM by body segments. No significant differences were found for the effect of kind of work and age together or taken separately.

Table 2: Results of Fat-Free Mass and Body Fat Mass by body segments

	Age < 28				28 < Age < 38				Age > 38				Age and specialty effect		
	Automatic		Manual		Automatic		Manual		Automatic		Manual				
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	F	p	η2
FFM Right Arm (kg)	3.65	0.31	3.76	0.52	3.93	0.76	3.79	0.49	3.56	0.55	3.58	0.47	0.39	0.67	0.01
FFM Left Arm (kg)	3.61	0.30	3.72	0.54	3.89	0.78	3.78	0.51	3.50	0.59	3.78	0.51	0.34	0.71	0.01
FFM Trunk (kg)	28.1	1.79	28.7	3.20	29.6	4.44	28.9	2.93	27.2	3.43	27.6	2.88	0.39	0.67	0.01
FFM Right Leg (kg)	10.0	1.01	9.94	1.09	10.1	1.49	9.71	1.02	9.32	0.91	9.37	1.00	0.42	0.65	0.01
FFM Left Leg (kg)	9.92	1.02	9.89	1.13	10.0	1.52	9.64	1.02	9.32	0.80	9.32	0.97	0.37	0.69	0.01
BFM Right Arm (kg)	0.52	0.26	0.75	0.86	0.86	0.56	0.68	0.56	0.55	0.51	0.75	0.35	1.67	0.19	0.04
BFM Left Arm (kg)	0.55	0.28	0.78	0.90	0.90	0.58	0.67	0.56	0.55	0.49	0.76	0.36	1.97	0.14	0.04
BFM Trunk (kg)	6.16	2.59	7.45	5.70	8.80	4.34	7.36	3.74	5.83	3.81	7.78	3.16	1.93	0.15	0.04
BFM Right Leg (kg)	1.82	0.47	2.07	1.22	2.30	0.90	1.98	0.77	1.74	0.82	2.10	0.57	1.87	0.16	0.04
BFM Left Leg (kg)	1.79	0.46	2.02	1.25	2.27	0.89	1.98	0.76	1.74	0.82	2.08	0.59	1.60	0.20	0.03

Note. M = Mean; SD = Standard Deviation; F = Fisher-Snedecor test; η2 = Partial eta squared; FFM = Fat-Free Mass; BFM = Body Fat Mass

Table 3 shows the results of body water (total, intracellular, and extracellular) by body segments. No significant differences were found for the effect of type of work and age together, but there was a significant effect of age [F(2, 91)=3.314, p = 0.041, η2 = 0.06], in the ICW of the trunk. Older soldiers (M = 13.3, SD = 1.51) had lower values (p = 0.035) in the ICW of the trunk than middle-aged (M = 14.2, SD = 1.71). Men had significant higher values than women for height (p=0.000; M = 176.9, SD = 6.6 vs M = 164.1, SD = 5.9), weight (p=0.000; M = 78.0, SD = 12.2 vs M = 60.0, SD = 6.8), TBW (M = 47.2, SD = 5.8 vs M = 34.0, SD = 2.1), ECW (p=0.000; M = 17.6, SD = 2.1 vs M = 12.8, SD = 0.73), ICW (p=0.000; M = 29.5, SD = 3.6 vs M = 21.2, SD = 1.3), Protein (p=0.000; M = 12.7, SD = 1.5 vs M = 9.2, SD = 0.6), Minerals (p=0.000; M = 4.4, SD = 0.62 vs M = 3.27, SD = 0.27), FFM (p=0.000; M = 64.4, SD = 8.0 vs M = 46.5, SD = 2.96), SLM (p=0.000; M = 60.7, SD = 7.5 vs M = 43.8, SD = 2.76), SMM (p=0.000; M = 36.5, SD = 4.8 vs M = 25.7, SD = 1.83), and significant lower values for PBF (p=0.034; M = 16.8, SD = 6.0 vs M = 22.0, SD = 4.3). No significant differences were found for the effect of gender between men and women in BFM (p=0.955; M = 13.5, SD = 6.5 vs M = 13.4, SD = 4.3) and BMI (p=0.065; M = 24.8, SD = 3.29 vs M = 22.34, SD = 2.9).

Table 3: Results of Body Water by Body Segments

	Age < 28				28 < Age < 38				Age > 38				Age and specialty effect		
	Automatic		Manual		Automatic		Manual		Automatic		Manual				
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	F	p	η^2
TBW Right Arm (l)	2.84	0.23	2.92	0.40	3.05	0.59	2.94	0.38	2.77	0.42	2.78	0.37	0.39	0.67	0.01
TBW Left Arm (l)	2.80	0.23	2.89	0.42	3.02	0.61	2.93	0.40	2.72	0.46	2.77	0.38	0.37	0.69	0.01
TBW Trunk (l)	21.8	1.42	22.3	2.46	23.0	3.45	22.4	2.28	21.1	2.66	21.4	2.20	0.44	0.64	0.01
TBW Right Leg (l)	7.78	0.80	7.71	0.85	7.86	1.15	7.52	0.79	7.23	0.70	7.28	0.77	0.47	0.62	0.01
TBW Left Leg (l)	7.71	0.80	7.68	0.89	7.81	1.18	7.48	0.80	7.24	0.62	7.25	0.76	0.39	0.67	0.01
ICW Right Arm (l)	1.77	0.14	1.83	0.25	1.91	0.36	1.84	0.23	1.73	0.27	1.73	0.23	0.37	0.68	0.01
ICW Left Arm (l)	1.75	0.14	1.81	0.26	1.89	0.37	1.84	0.24	1.70	0.29	1.73	0.24	0.31	0.72	0.01
ICW Trunk (l)	13.7	0.85	14.0	1.60	14.4	2.17	14.1	1.43	13.2	1.71	13.3	1.40	0.32	0.72	0.01
ICW Right Leg (l)	4.90	0.47	4.88	0.53	4.95	0.73	4.77	0.49	4.55	0.46	4.55	0.49	0.29	0.74	0.01
ICW Left Leg (l)	4.83	0.48	4.82	0.54	4.90	0.74	4.72	0.49	4.53	0.39	4.52	0.47	0.27	0.76	0.01
ECW Right Arm (l)	1.06	0.09	1.08	0.14	1.14	0.22	1.09	1.14	1.04	0.15	1.04	0.14	0.43	0.64	0.01
ECW Left Arm (l)	1.05	0.09	1.08	0.15	1.13	0.23	1.09	1.15	1.02	0.16	1.04	0.14	0.47	0.62	0.01
ECW Trunk (l)	8.15	0.59	8.28	0.88	8.61	1.29	8.27	0.85	7.95	0.95	8.07	0.81	0.68	0.50	0.02
ECW Right Leg (l)	2.88	0.32	2.83	0.33	2.91	0.43	2.74	0.30	2.68	0.24	2.72	0.28	0.86	0.42	0.02
ECW Left Leg (l)	2.87	0.33	2.85	0.35	2.91	0.45	2.75	0.31	2.17	0.22	2.72	0.29	0.63	0.53	0.01

Note. M = Mean; SD = Standard Deviation; F = Fisher-Snedecor test; η2 = Partial eta squared; TBW = Total Body Water; ICW = Intracellular Water; ECW = Extracellular Water

DISCUSSION

This study aimed to analyze body composition differences between the type of specialty, age group, and gender of paratroopers.

The first hypothesis was fulfilled since there were no differences in body composition between specialties. The second hypothesis was partially fulfilled since there were differences in intracellular water, protein, and skeletal muscle mass between middle-aged and older soldiers. The third hypothesis was not fulfilled since there were no significant differences in body composition between male and female paratroopers.

The main parachute jumps conducted by paratroopers' units are automatic or manual. The characteristics of both jumps are different since in automatic parachute jumps there is a lack of parachute control during the flight and especially during the landing (with high impacts that directly affect the muscle and bone structures of jumpers).

On the contrary, in manual jumps the flight and landing phases allow for greater control by the jumpers, leading to a less traumatic landing. But independently of these differences, we did not find differences between jump specialties, a fact probably due to the similar training, instruction, and nutritional regimen of both groups. The only difference was the tactical maneuvers after both jumps, but the rest of the preparation and military context were similar in both groups.

Differences between ages were found in intracellular water, protein, and skeletal muscle mass. The middle-aged group had a better mean than the older group in all those variables. Since the skeletal muscle is mainly built upon protein and water, these results show a tendency towards skeletal muscle loss with age.

The differences found could be related to the fact that middle-aged soldiers have complete growth, they are in their most operational phase in the unit, where they perform a higher number of jumps and the training is more intense, facts that could explain the higher intracellular water, protein, and skeletal muscle mass. This fact would join, with the investigations that support that resistance training promotes an increase in body water, principally by intracellular content [32]. On the other hand, older and younger participants perform a lower number of jumps: older paratroopers begin with the natural muscle mass loss process associated with age [33] and the younger paratroopers have not yet reached their growth zenith and their training in the unit is not yet maximum.

It is important to note that body fat mass, percentage of body fat, and soft lean mass, which have been usually reported as crucial for the performance of the units [13,21] were not different between groups, supporting the idea of the adequate habits of these troops during their service. Also, data analysis for body segments, reinforces the idea of the correct BC maintenance of these units, throughout their military service, despite their age, since no significant differences were found between groups of work and age.

Besides, if we compare the values of body mass index (24.12 kg/m^2 vs 25.88 kg/m^2 for age <28, 25.89 kg/m^2 vs 26.94 kg/m^2 for age between 28 and 38, and 24.64 kg/m^2 vs 26.86 kg/m^2 for age > 38); and body fat percentage (16.0% vs 16.6% for age < 28, 17.8% vs 18.8% for age between 28 and 38, and 17.2% vs 19.7% for age > 38) with some US Army standards, they are better for all the age groups [20]. Nevertheless, we should bear in mind that these groups are considered "elite" inside the Spanish army, and we are comparing them with a combination of soldiers from the combat support, combat arms, and combat service support units. However, results of body mass index and body fat percentage were higher than those reported for soldiers after 6 months of deployment in a crisis management operation (25.89 kg/m^2 vs 24.50 kg/m^2 and 17.8% vs 13.7%), a fact that highlights the need to monitor body composition during field activities, and especially on those with higher length, intensity or more stressful type [36]. Not only fat mass but fat-free mass and protein could be undermined during operational training or deployment [23,24].

If we compare these results with other Army units, body mass index and percentage of body fat are quite similar to airplane and helicopter pilots, but lower to other aircrew support members (25.49 kg/m^2 and 23.79% respectively) [4]. If we take body segments, there is a tendency towards having more fat-free mass in the right arm, which is consistent with previous research in the Air Forces [4], light forces [12], and even in females [39], which could be due to the prominence of right-handedness.

Regarding our third hypothesis about differences in BC according to sex, most of the BC male parameters in absolute terms were higher, while BFM and BMI did not show significant differences. Further investigation is recommended on this issue since the female sample size does not allow the values to be extrapolated and generalized.

Finally, regarding body water composition, there are no significant differences between age groups and manual or automatic parachute jumps in any of the variables analyzed, total, intracellular, and extracellular water. These values suggest an optimal balance of hydration among the healthy population and are correlated with a lower and healthier BMI [19], consequent with the normal-weight distribution among age and type of jump. Indeed, similar values of intracellular and extracellular values have been found in professional athletes depending on their composition whether it is an athlete or not [31].

The main limitation of this study was the small sample size of women. Secondly, we did not have permission to gather other demographic data (like nutrition, economic, education, and physical activity levels in leisure time) that could have helped us to better understand the differences between groups. These background variables could be studied further to design longitudinal research to understand the mechanisms of BC changes.

CONCLUSIONS

Paratroopers' Body Composition is not affected by their type of parachute specialty (automatic or manual) but depends on age for skeletal muscle mass, intracellular water, and protein. Older soldiers had lower values of ICW than middle-aged. Older soldiers had lower values of protein than middle-aged. Older soldiers had lower values of SMM than middle-aged. This data suggests good nutrition and training habits among the paratroopers. When comparing these data with previous research, it suggests that the body fat percentage of paratroopers is similar to airplane pilots, lower than aircrew personnel and combat and service soldiers, but higher than soldiers deployed. Body composition should be monitored to analyze the effect of training and nutrition habits during service, especially during stressful deployments. These facts must be considered to better prepare each unit.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, V.J.C.S. and A.B.S.; methodology, V.J.C.S. and A.B.S.; formal analysis, V.J.C.S. and A.B.S.; investigation, V.J.C.S. and A.B.S..; resources, V.J.C.S. and A.B.S.; data curation, A.B.S.; writing—original draft preparation, A.B.S.; writing—review and editing, V.J.C.S. and A.B.S..; supervision, V.J.C.S.; project administration, V.J.C.S.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of UNIVERSIDAD EUROPEA DE MADRID (protocol code CIPI/18/093).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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ORIGINAL ARTICLE

Functional and Dysfunctional Coping Strategies in Patients Diagnosed with Cancer – From Initial Assessment to Therapeutic Interventions

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Abstract: *The assessment of coping mechanisms in patients diagnosed with oncological diseases is essential for mental health specialists, who have to design the most appropriate case management strategy for comorbid mood disorders, anxiety disorders, adjustment disorders, and other psychiatric conditions that may be detected in this vulnerable population. The adequate treatment of these disorders is important for the preservation of mental health status, quality of life, and overall functionality in patients diagnosed with cancer. Coping mechanisms modulate the vulnerability toward psychiatric disorders, but they also have an impact on treatment adherence, which is an important factor correlating with prognosis. Appraisal-focused, problem-focused, emotion-focused, and occupation-focused coping represent the most well-defined strategies patients use when confronted with a stressful life situation, like a diagnosis with potentially vital consequences. Maladaptive coping strategies may also be identified in these patients, e.g., withdrawal from reality, including complete or partial denial of the disease, substance abuse, behavioral addictions, refusal of the recommended treatment, etc. The psychotherapeutic approach in patients with oncological diseases should include an initial evaluation of the coping strategies used either currently or in the past stressful conditions, an assessment of all the psychosocial resources the patients have (e.,g., support group, professional insertion, hobbies), and screening for mood and anxiety disorders that may have been triggered by the cancer diagnosis. Consequently, within the psychotherapeutic framework, a trained specialist can enhance the role of adaptive coping strategies and highlight the disadvantages of dysfunctional ones. This process may possess a favorable impact on treatment adherence, mental health status, and quality of life in patients with cancer.*

Keywords: *coping mechanisms, treatment adherence, quality of life, cognitive behavioral therapy, executive functions*

INTRODUCTION

Coping mechanisms are defined as thoughts and behaviors activated when finding a solution to either internal or external stressful situations is required [1]. These triggers may vary from different feared situations, a perceived lack of control over own impulses, or difficult life decisions (when internal events are taken into consideration), to physically traumatic stimuli, imposed changes in lifestyle, or interpersonal stressors (when external events are the focus of attention). Combined, internal and external triggers, may exist, as in severe physical conditions, such as oncological diseases, when the diagnosis, the treatment, the stigma, and the overall functionality (personal, familial, social, and professional) are all affected.

A traditional difference exists between the „coping mechanisms", which are conscious and voluntary, and the „defense mechanisms", which are psychodynamically-based constructs, and by their definition unconscious. Coping mechanisms represent sets of structured, stable responses that have a well-defined adaptive function [1]. Defense mechanisms, on the other hand, have as their purpose to disguise internal impulses, in order to protect the Ego; therefore they operate outside the sphere of the conscious mind [2-5]. However, not all authors agree with the assumption that all defense mechanisms are unconscious, because of examples like suppression or humor, which involves, at least partially, a participation of the Ego [6-8]. At any rate, it is considered that defense mechanisms act as „conscious suppression”, and this may be observed in interpersonal relationships [2].

There are many classifications of coping mechanisms because these psychological processes are numerous and quite diverse, therefore different authors group them according to more or less arbitrary criteria [9-15]. One example of such a classification has as its core criterion the targeted stressor(s), and includes (1) reactive coping – the individual responds to a certain stress factor; people who activate this type of coping have presumably better adaptive skills in an unstable environment with many hard-to-predict stressors; (2) proactive coping – the individual activates behaviors/thoughts aimed at neutralizing the effects of anticipated stressors; people who activate this coping perform better in a stable environment with routine tasks [1,16].

Another classification of coping mechanisms includes (a) problem-focused strategies – address the origin of the discomfort, and these may be manifested as active coping, planning, restrictive coping (not acting prematurely, holding back until a good opportunity to act appears), and inhibition of concurrent activities; (b) emotion-focused strategies – targeting the decrease of negative emotions correlated with the ongoing stressor, e.g., positive reframing, acceptance, recourse to religion, and humor; (c) meaning-based strategies, i.e., extraction and management of the meaning the stressful situation has for an individual in a certain moment; (d) social or support-based strategies, which refer to the ability of stress reduction by seeking emotional or behavioral support from the group to which that person belongs [1,17].

The coping mechanisms mentioned above are „adaptive” processes, but there is also a category of „maladaptive” coping strategies [18]. It is supposed that problematic coping may develop in the early stages of conscious life when individuals have to face overwhelming stressors like maltreatment, abuse, or neglect [18]. For example, the excessive activation of primitive coping strategies, lack of modeling for healthy coping alternatives, and repeated exposure to intense stressors in the immediate environment are potential factors that may increase the individual's vulnerability to the development of maladaptive coping strategies [18]. These coping mechanisms are associated with a negative evolution of mental health problems and more severe levels of psychopathological manifestations [1]. Examples of maladaptive or dysfunctional coping are represented by disengagement (defined by efforts to move away from the stress triggers), emotional suppression (i.e., inhibition of emotional reactions), cognitive avoidance (manifested by distraction, worry, and thought suppression), and denial (i.e., the unwillingness to accept reality) [19-21].

The role of maladaptive coping mechanisms in psychopathology has been explored intensively, and conditions like posttraumatic stress disorder, anxiety disorders, depressive disorders, psychosomatic disorders, and somatoform disorders have all been correlated with avoidance-based coping or other maladaptive strategies [1]. The situation of treatment or diagnostic refusal in patients with psychiatric disorders, but also in those presenting with different organic illnesses, including cancer, has been associated with maladaptive coping (i.e., denial) [21].

The rationale for exploring coping mechanisms in patients with oncological and other severe diseases is the need to understand better the psychological strategies activated by patients in stressful situations and to work with these strategies during psychotherapy. One aspect of this process is to allow for the formation of a more solid therapeutic relationship, by exploring the adaptive strategies. Other objectives that may involve coping mechanisms are the training of functional coping skills and helping patients to deconstruct the dysfunctional ones.

There is an additional aspect for exploring these mechanisms in patients with cancer, and it refers to the risk of psychiatric comorbidities. As can be anticipated, this diagnosis, as well as the associated treatment and prognosis, may lead to the onset of adjustment disorders, depressive disorders, stress-related pathology, etc. Many authors are highlighting the need for identifying and treating depression in this specific population because this would have a favorable impact on the quality of life and mortality [22-24]. Cases of major depressive disorders (MDD) have frequently been detected in patients with cancer, reaching prevalences of 2-43.5% [22-24]. The importance of coping mechanisms derives from their impact on the evolution of clinical manifestations and therapeutic responses in patients with MDD [25]. A study exploring this impact concluded that maladaptive mechanisms, mainly ruminating, catastrophizing, and self-blaming were the most frequently used by patients with depression [25]. Accepting the current situation, positive refocusing and re-evaluation are functional coping mechanisms, with a protective role in these patients, and these strategies can be used during the psychotherapeutic process [25].

In another study, the importance of coping strategies for resilience was evaluated in patients with panic disorder [26]. The severity of clinical manifestations and maladaptive coping skills decreased during cognitive-behavioral therapy (CBT) administered in a group format [26]. CBT also had beneficial effects on increasing resilience and quality of life in patients with panic disorder, thus highlighting the role of psychotherapy in modulating the influence of coping mechanisms on daily functioning, besides its positive clinical impact [26].

Adjustment disorders illustrate the failure of coping mechanisms' functionality in preserving a safe distance toward stressful stimuli’ negative impact [27]. Also, acute stress reactions and posttraumatic stress disorder are consequences of coping strategies failure, but in these cases, the stressors represent vital threats [27]. Psychotherapy in these cases targets enhancing coping abilities with the specific stressor and increasing the adaptive responses [28]. This is usually done by identifying negative thoughts and replacing them with more adaptive thoughts (i.e., cognitive restructuring), monitoring the experiences aimed at increasing self-efficacy, and behavioral activation [28-30].

FROM CONCEPTUAL ANALYSIS TO CLINICAL SITUATIONS – AN ANALYSIS OF HOW THE COPING MECHANISMS FUNCTION IN SPECIFIC SITUATIONS

As mentioned in the introductory chapter, coping mechanisms are usually activated in stressful situations, and configuring the individual profile of these mechanisms allows the therapist to represent the specific vulnerability features and the resilience factors to stressful situations, like a vital threat raised by a cancer diagnosis. The presentation of several theoretical situations is considered helpful for a better understanding of how the coping mechanisms may work in clinical settings. For example, when a patient finds out that she has been diagnosed with early-stage breast cancer, she may engage in seeking information about this disease and/or making an appointment for further investigations (which implies active, problem-focused coping); also, she may decide to join a self-help group for patients with the same diagnosis (i.e., a coping based on social support), or she may look for spiritual support from a priest (defining a coping based on recourse to religion). However, continuously looking for information that may invalidate the oncological diagnosis, by exploring different online resources, or by repeated medical consultations in the hope of finding a doctor willing to offer another diagnosis, is a maladaptive coping strategy based on denial. In the case of a patient with prostate cancer who has already been investigated by collecting all the necessary laboratory analyses (prostate biopsy included) and by undergoing all imagistic investigations required, the delay of treatment initiation is caused by his lack of trust in the results of these investigations. Due to this maladaptive coping strategy, the treatment initiation is delayed, and the prognosis may worsen.

Another example is represented by a woman with breast cancer who has undergone a total mastectomy and developed a depressive disorder because she refuses to accept her new body image. As a manifestation of her depression, she does not want to consult a mental health specialist, thus activating a coping strategy based on denial and avoidance.

Yet another typical situation is when a person who goes through a divorce caused by health and financial problems ends up consuming alcohol excessively, to suppress his/her negative emotions. This way of facing the specific life situation is characterized by disengagement and avoiding confrontation with own emotions, i.e., two types of maladaptive coping.

On the other end of the coping mechanisms spectrum, a person who has to retire prematurely due to an oncological disease (now in remission, but with several negative functional consequences) may go initially through an adjustment disorder caused by the recent change of the daily activity regime, but then she/he resignifies the situation and considers that she/he has more free time to spend with own family. This is an example of active coping, planning, and coping based on significance because the individual perceives her/himself as a survivor of the disease and focuses on future prospects.

Of particular importance for understanding how coping mechanisms work, is the exploration of the strategies activated and the solutions each patient has found when confronted previously with difficult situations, such as interpersonal conflicts, social isolation, work conflicts, economic difficulties, and severe illnesses [31].

NEUROBIOLOGICAL FOUNDATION OF COPING MECHANISMS AND THE STRUCTURED APPROACH TO THEIR CLINICAL ASSESSMENT

The prefrontal cortex, responsible for executive functioning (i.e., planning, analysis and synthesis, abstracting, problem-solving, verbal fluency, etc.), and the nucleus accumbens, which is part of the reward system or the mesocorticolimbic circuit, have been associated with the functioning of the coping mechanisms [32,33]. These central nervous system (CNS) structures are sensitive to chronic stress, and disruptions in the functioning of the prefrontal cortex have negative consequences on the ability to perform complex cognitive tasks [32,33]. Executive functions, including cognitive flexibility, planning, inhibitory control, error detection, working memory, and verbal fluency, are largely dependent on the morphological integrity and functionality of the prefrontal cortex [34-39]. These functions are essential for constructing coping mechanisms, their preservation or inhibition, and mediating the relationship between coping strategies and different psychiatric disorders, such as MDD [34,35]. It was even demonstrated that interventions targeting the enhancement of executive functions and/or coping mechanisms are useful in decreasing the risk of MDD recurrence [34]. Executive functions have a role in increasing the risk of suicidality by mediating the relationship between high levels of stress and avoidant coping, therefore enhancing executive functioning through computerized training and cognitive rehabilitation may reduce the impact of stressors on individuals [35]. Also, executive dysfunction and mnestic impairments were associated with lower use of active coping mechanisms in patients with schizophrenia, highlighting the important correlations existing between these variables [39].

The activity of the hypothalamic-pituitary-adrenocortical axis (HPAA) and the plasma level of cortisol have no confirmed direct effect on coping mechanisms, but they may influence intermediate variables with impact on, or associated with, these mechanisms [33,40,41]. Such mediators are depressive mood, anxiety, insomnia, psychomotor restlessness, and attentional and memory deficits [33,40,41]. For example, in a study (N=30 participants), the presence of MDD was correlated with a higher level of morning serum cortisol vs. healthy individuals, and an inverse correlation between these levels and the frequency of activated prosocial coping mechanisms was observed [42].

Serotonergic and dopaminergic pathways in the CNS are responsible for modulating mood, strengthening/decreasing certain behaviors, and influencing memory and attention [43-48]. The prefrontal cortex, hippocampus, and corpus striatum, which are involved in cognition, are densely populated by dopaminergic and serotonergic neurons originating within the raphe complex, mesocortical, and nigrostriatal systems [43,49]. Processes like learning and memory depend on the modulation of serotonergic and dopaminergic activity and imply short- and long-lasting synaptic modifications [43]. Modulation of serotonergic and dopaminergic pathways using pharmacological agents, as is the case with MDD and schizophrenia spectrum disorders, can influence not only the core clinical symptoms of these disorders but, indirectly, may modulate the coping mechanisms [50-53].

The impact of vasopressin and oxytocin on psychological mechanisms that are important for the modulation of coping strategies was also explored [54,55]. These pituitary neuropeptides are responsible for shaping social behavior, aggression, and social memory, according to preclinical studies [54,55].

Data about the importance of vasopressin and oxytocin in humans also emerged recently, and functional neuroimaging studies showed that impaired operation of these neuropeptides appears in mental disorders that imply an early attachment disruption or difficulties in forming social relationships, such as autism spectrum disorders (ASD), social anxiety disorder, borderline personality disorder, and schizophrenia spectrum disorders (SSD) [55].

In conclusion, the biological foundation of cognition is extremely complex, and the adequate functioning of the cognitive processes (as a prerequisite for the operational coping mechanisms) depends heavily on the integrity of multiple neurotransmission and neurohormonal pathways.

The assessment of coping mechanisms is conducted by mental health specialists trained in using validated instruments. Besides the general evaluation, based on a psychological interview targeting current mental status and personal history, the specialists may use various scales, inventories, and questionnaires constructed and validated for this purpose.

Brief-COPE is a 28-item, self-administered instrument that quantifies functional and dysfunctional coping methods in stressful situations [56]. It is often used in clinical settings to assess how patients cope with stressors such as cancer diagnosis, heart failure, trauma, and mental and behavioral disorders [56]. Based on interpreting this questionnaire's results, psychological counseling strategies may be formulated [56]. Brief-COPE evaluates three types of coping: (1) problem-focused – includes the following dimensions: active coping, planning, suppression of competing activities, restraint coping, and seeking instrumental social support; a high score indicates coping strategies aimed at changing stressful situations, psychological stability, a pragmatic approach and is a positive prognostic factor; (2) focused on emotions – includes seeking social support, positive reinterpretation, acceptance, denial, and appeal to religion; high scores indicate strategies aimed at regulating stress-related emotions; (3) avoidant – includees emotional venting, behavioral disengagement, mental disengagement; a high score suggests physical or cognitive disengagement efforts, low scores suggest adaptive coping [56-61]. The original COPE inventory included 15 dimensions, each with 4 items [57,62]. However, this form was criticized because the number of factors differed across studies, varying from 3 to 5, it was considered too long, and the distinction between its dimensions was not supported by further analyses [57].

The most frequently used tools for evaluating coping mechanisms, besides the COPE and Brief-COPE, according to a meta-analysis, are the Ways of Coping Questionnaire, Coping Strategies Questionnaire, Coping Response Inventory, Coping Strategies Inventory, Adolescent Coping Scale, Pain Coping Questionnaire, Religious COPE, and Utrecht Coping List [63]. The same source mentioned that coping scales are often used for the structured assessment of patients with various health problems, such as illnesses, pain, and medical diagnoses [63].

COPING MECHANISMS IN PATIENTS WITH ONCOLOGIC DISEASES

Coping mechanisms influence the decision-making process regarding participation in treatment and implicitly shape the evolution and prognosis of the condition. The same mechanisms impact the patient’s desire to be informed about their own illness, their perception of self-efficacy, their relationship with others during the illness, etc. The main coping mechanisms identified in a study (N=603 patients with cancer) were (1) seeking or using social support, (2) focusing on the positive aspects of life, (3) distancing, (4) cognitive avoidance/cognitive abandonment, and (5) behavioral avoidance/behavioral abandonment [64]. This study concluded that the type of oncological disease, the time elapsed since the diagnosis of the disease, and the presence of treatment correlated non-significantly with the type of coping [64]. The stress perception associated with the oncological condition correlated significantly with the activation of coping based on social support, but also with cognitive and behavioral disengagement [64]. Coping based on social support, focusing on the positive, and distancing were all associated with less emotional distress, while cognitive/behavioral withdrawal was associated with more emotional distress [64].

The relationship between coping strategies, quality of life, and mood in patients with incurable oncological conditions was investigated in a randomized trial of palliative care, that enrolled patients with advanced stages of lung or gastrointestinal cancer (N=350), who were in the first 8 weeks after the diagnosis was confirmed [65]. All main outcomes were assessed in a structured way, and the most frequently detected coping mechanism was emotional support (77%), followed by acceptance (49%), self-blame (38%), and denial (28%) [65]. Coping based on emotional support was significantly associated with better quality of life and less severe depressed mood [65]. In the same study, acceptance correlated significantly with better quality of life and inversely with depression and anxiety, denial and self-blame correlated significantly inversely with quality of life and non-significantly with depression and anxiety [65]. The study's authors concluded that interventions aimed at improving the quality of life and mood of these patients should include increasing the use of adaptive coping strategies [65]. It also may be stated that patients with incurable forms employ different coping mechanisms than those with oncological conditions presenting a favorable prognosis [65]. The explanation is that the emotional burden and severity of physical symptoms create a different setting for activating coping mechanisms in the two population subgroups [65].

Types of coping activated by patients with breast cancer predict long-term psychological adjustment, according to the results of a study that explored the strategies for facing difficulties related to this diagnosis in a group of 55 patients [66]. Coping mechanisms, decisional control, expression of frustration, and psychological adaptation in the first 6 months after finding out the diagnosis were assessed initially and after 3 years [66]. Patients who were initially depressed and who responded to finding out the diagnosis with cognitive avoidance (acceptance/resignation) showed significantly less chance of psychological adjustment 3 years later [66]. The poor psychological adjustment was significantly associated with cognitive avoidance and minimal activation of proximity/ contact-based coping [66].

The relationship between quality of life and coping mechanisms was evaluated in women extensively treated for advanced-stage genital cancer (N=64) [67]. A control group included women with genital cancer in the early stages, who consequently received less aggressive treatment [67]. The main stressors in the study group were the adverse effects of chemotherapy, poor physical condition, and poor prognosis [67]. Using the COPE inventory to assess coping mechanisms, it was observed that avoidance and engagement were the dominant strategies in patients with severe forms of cancer [67]. Also, these patients presented more frequently both types of coping (commitment/avoidance) vs. patients in the control group [67]. Avoidant coping strategies correlated with more negative mood and poorer overall well-being, while avoidance and seeking instrumental support were associated with worse prognosis in patients with severe forms of cancer, but not in those with early forms [67]. Thus, coping mechanisms have a stronger impact on prognosis in patients with severe forms of genital cancer [67]. Avoidant coping strategies in particular may adversely affect the quality of life and mood in this population [67].

The predictive value of coping mechanisms in patients who were diagnosed with stage I or II breast cancer no more than 12 months ago was the objective of a longitudinal study that included 70 patients [68]. This study explored the abilities of these patients to activate situational-specific coping mechanisms and hope (considered a more stable factor), as predictors of adaptation after finding out their diagnosis [68]. Active acceptance-based coping at the moment of diagnosis predicted better adjustment over time in this population, while avoidance-based coping correlated with greater fear of cancer recurrence, independent of the patient's initial medical condition and age [68]. Religion-based coping proved more effective for women who had a lower initial level of hope [68]. The conclusion of the study was that protective and risk factors for adaptation in first-year breast cancer survivors can be identified even before surgery [68]. Mood characteristics, such as hope, as well as situation-specific coping strategies, must be considered by the case manager [68].

Optimism was explored as a variable mediated by coping strategies in patients with breast cancer in the early stages, in a study with 59 participants [69]. The level of optimism was determined one day before surgery, 10 days after it, and 3-, 6-, and 12 months post-surgery [69]. Acceptance-based coping, positive reframing, and religion were the most frequently activated strategies, and optimism correlated inversely with the level of distress at each of the study visits [69]. Denial and behavioral disengagement were the least frequently activated coping strategies in this population [69]. In conclusion, optimism and pessimism are mediated by the activation of different coping mechanisms in the adaptive process and they have an important role in favoring or hindering this process [69].

The correlation between coping strategies and psychological distress was investigated in patients with advanced stages of cancer [70]. In such a study, that enrolled 132 patients diagnosed with breast, ovarian, lung, colorectal, and other types of cancer, a correlation between distancing, cognitive avoidance, and behavioral abandonment, on the one hand, and psychological distress, on the other, was demonstrated [70]. Distancing coping correlated negatively with psychological distress, while cognitive and behavioral avoidance correlated positively with the same distress [70]. Overall, coping methods explained 36% of the variation in the level of psychological distress [70]. The most important coping mechanism contributing to psychological distress was behavioral avoidance, followed by cognitive avoidance [70].

Emotional expressive coping has predictive value for physical and mental adjustment to breast cancer diagnosis, according to a prospective, 3-month study (N=92 participants) [71]. Active processing of emotions is a coping mechanism based on promoting emotional contact, that favors adaptation and health status [71]. Women who reported emotional expression-type of coping had fewer visits to the doctor for cancer-related symptoms, had better physical health and energy levels, and had lower levels of distress over the next 3 months compared to patients who had less emotional expression [71]. Quality of life was also better in patients with emotionally expressive coping [71].

MDD, personality disorders, and coping strategies were studied as moderators of the quality of life in patients with non-metastatic breast cancer [72]. A cross-sectional study included 120 patients diagnosed with breast cancer 7 months ago, and the main outcomes were quality of life, coping strategies, biological and physiological variables, presence of MDD and personality disorders, and pain severity [72]. Poorer physical quality of life was associated with MDD, younger age, more severe initial tumor stage, and behavioral disengagement coping [72]. A lower emotional quality of life was associated with MDD, the presence of a personality disorder, more severe pain, a high level of self-blame, and a lower use of acceptance-based coping [72]. Also, lower global quality of life was associated with MDD, the presence of a personality disorder, more intense pain, high levels of self-blame, less use of reframing coping strategies, and the absence of hormone therapy [72]. In conclusion, lower quality of life was associated with premorbid patient characteristics (personality disorder) and coping variables (depression, personality, and specific strategies), less so with cancer-related variables (type of treatment, disease severity) [72].

TRAINING OF THE COPING MECHANISMS IN ONCOLOGICAL PATIENTS

Psychotherapy and psychological counseling in patients diagnosed with severe organic illnesses have, between their objectives, the enhancement of functional coping mechanisms and the reduction of maladaptive ones, in order to enhance resilience to stressful situations (e.g., pain, invalidity, changes in lifestyle, adverse events of drugs). Coping skills training programs have been associated with improving some biological and psychosocial parameters in patients with chronic somatic conditions [73]. For example, a decrease in HbA1c levels and an increase in self-efficacy have been observed in patients with diabetes after applying a coping skills training program [73]. The same programs led to increased self-care capacity in diabetic patients, decreased the severity of depressive symptoms, improved the prognosis of chronic obstructive pulmonary disease (COPD), and ameliorated the quality of life in individuals with various somatic conditions [73]. The training of these skills involves lectures, Q&A sessions, group discussions, role-plays, etc., under the direction of a clinical psychologist/psychotherapist; nurses trained for this purpose can also lead such support groups [73]. The topics addressed are the principles of effective self-care, stress management, communication skills training, cognitive-behavioral restructuring techniques, and problem-solving techniques [73].

A brief coping skills training program for oncology patients was designed to increase the patient's feelings of self-efficacy, personal control, and resilience to stress [74]. Applied in the perioperative framework, this program was created for nurses, and they were trained on how to enhance the patient's trust in their own abilities to manage dysfunctional cognitions and behaviors [74]. This training was used to prepare the patients for solving situations or events that may occur before or after surgery [74]. Providing information, education, emotional support, and specific interventions – adapted to the objectives of each individual case, were part of this brief training [74]. Relaxation based on breathing, cognitive restructuring, mindfulness, goal setting, and motivational interviewing may be part of more comprehensive training [74].

Encouraging caregivers of oncology patients to participate in the training of coping mechanisms is considered an important aspect of case management. A study that included 233 patients with lung cancer and their relatives compared two interventions – 14 telephone sessions of coping skills training assisted by a relative vs. education/support with the help of a relative [75]. Patients in both groups showed improvements in anxiety and self-efficacy during the 4-month follow-up [75]. However, family-assisted coping skills training has been associated with better outcomes in patients with stage II or III cancer [75]. Caregiver-assisted education/psychological support was more effective in patients with stage I lung cancer [75].

The effects of coping skills training on caregivers of oncological patients were also explored. Such a study enrolled 70 participants and looked at the effects of coping skills training on the quality of life in daughters of patients with breast cancer [76]. The intervention consisted of an educational program, with workshop-type lectures, followed by group discussions [76]. Relatives' quality of life was considerably improved by this intervention, according to Pediatric Quality of Life Inventory scores [76]. Physical, and emotional functioning and participation in school activities were improved vs. the control group [76].

Particular aspects of adaptive strategies training depend on the specific elements of oncological conditions. The approach in a psychotherapeutic setting to patients with cancer must include the training of coping skills, depending on the particularities of each case. It is essential for the therapist to work with the patient in identifying the stress factors that activate coping mechanisms: self-image changing (e.g. mastectomy, limb amputation), reduced mobility, adverse effects of chemotherapy and radiotherapy (e.g. alopecia, persistent vomiting, swallowing disorders, sexual dysfunctions), lifestyle changes (eg, colostomy, indwelling urinary catheter), physical pain, psychiatric symptoms/ disorders – depression, anxiety, restlessness, insomnia, apathy, anhedonia, etc.

Therapeutic interventions may be focused on the activation of functional coping mechanisms, based on action and social support, e.g. looking for a support group for patients who have undergone similar surgical interventions; planning activities based on the spared skills; analyzing the pros and cons of correcting deficits (prosthetics, plastic surgery); re-signification of the situation, e.g. post-operative scars are an indicator of the battle with the disease and of the fact that the person has overcome it, assuming the state of "survivor" or "victor"; creating an optimal framework for expressing emotions, concerns, fears related to the new situation in which the person finds him/herself, dysfunctions of the social image, self-image, and relationships with those close to the patient, decreased professional functioning, etc.; encouraging acceptance of the present situation and redirecting resources towards functional, healthy aspects of own life; acceptance does not mean resignation; adding non-pharmacological methods to drug pain therapy – mindfulness, progressive muscle relaxation, diaphragmatic breathing, guided imagery, planning hobby activities; specific techniques for augmenting the effect of medication in depressive, anxiety, adaptation disorders, insomnia, etc., e.g., cognitive restructuring in depression and anxiety, aversive therapy or relaxation techniques in insomnia, guided imagery and activity planning for isolated affective/anxiety symptoms, etc.

CONCLUSIONS

Coping mechanisms in patients diagnosed with oncological diseases have an important role in preserving mental health, quality of life, and general functionality. Coping mechanisms modulate the vulnerability towards mood and anxiety disorders in patients diagnosed with cancer, but these mechanisms also have an impact on therapeutic adherence, thus representing important factors that correlate with prognosis. The best-defined functional coping strategies are those centered on realistic assessment, problem-solving, emotion-expression, and occupational activation, therefore it is recommended that the therapist train these healthy mechanisms in patients with severe organic diseases.

Coping resources activated in stressful life circumstances include the participation of family or friends, recourse to religious rituals, at least partial maintenance of professional roles whenever possible, and self-help therapy (online sessions, organized groups, etc.).

In oncology patients, maladaptive coping strategies can also be identified, for example, withdrawal from reality, including complete or partial denial of the disease, substance abuse, behavioral addictions, treatment refusal, requesting the administration of insufficiently validated treatments not recommended by a specialist, etc.

The psychotherapeutic approach to patients with cancer should include an initial assessment of coping strategies used both now and in the past under stressful conditions, an evaluation of the psychosocial resources that patients have previously activated in life-threatening situations, and a screening for mood, stress-related, and anxiety disorders that may have been triggered since the onset of the current illness (Figure 1).

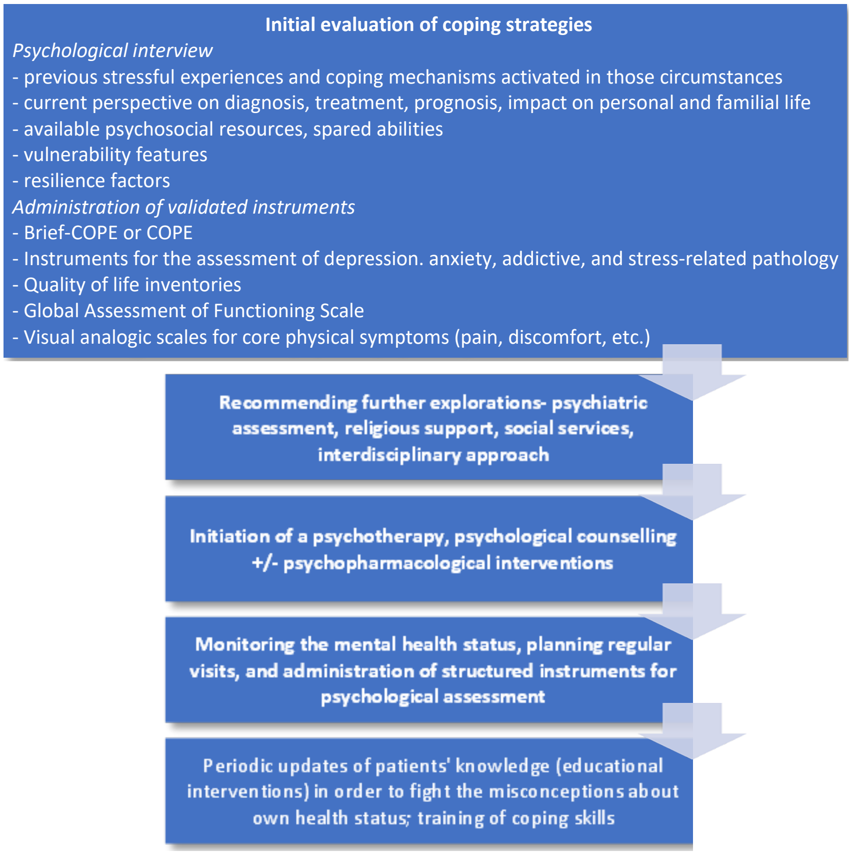

Initial evaluation of coping strategies

Psychological interview

- previous stressful experiences and coping mechanisms activated in those circumstances
- current perspective on diagnosis, treatment, prognosis, impact on personal and familial life
- available psychosocial resources, spared abilities
- vulnerability features
- resilience factors

Administration of validated instruments

- Brief-COPE or COPE
- Instruments for the assessment of depression. anxiety, addictive, and stress-related pathology
- Quality of life inventories
- Global Assessment of Functioning Scale
- Visual analogic scales for core physical symptoms (pain, discomfort, etc.)

Recommending further explorations- psychiatric assessment, religious support, social services, interdisciplinary approach

Initiation of a psychotherapy, psychological counselling +/- psychopharmacological interventions

Monitoring the mental health status, planning regular visits, and administration of structured instruments for psychological assessment

Periodic updates of patients' knowledge (educational interventions) in order to fight the misconceptions about own health status; training of coping skills

Figure 1: The algorithm for assessing and training coping mechanisms in patients with cancer

After this stage, a psychiatric consultation may be recommended, if necessary (acute psychiatric pathology, the need for pharmacological treatment). The initiation of psychological counseling, psychotherapy, and, in specific cases, psychopharmacological treatment may be prescribed, depending on the results of the initial evaluation. Monitoring the mental health status by using standardized tools, and planning follow-up visits to update patients' knowledge and coping skills learned is necessary. The psychotherapeutic intervention should be focused on increasing adaptive coping through systematic training and highlighting the disadvantages of dysfunctional coping mechanisms. This therapeutic process can have a significant favorable impact on treatment adherence, mental health status, and quality of life in patients with cancer

Further research on this topic should investigate the impact of structured coping skills training programs on more homogenous populations, such as patients with a certain type of oncological disease and in a specified stage of the disease's evolution [77-79]. Of major interest would be the exploration of combined therapeutic interventions in this population, and also the assessment of the impact different types of surgical interventions may have on mental health and quality of life [77-83]. As a limitation of this review, it should be mentioned that it was not designed as a systematic approach to this topic, its immediate purpose is to enhance the awareness of mental health specialists about the importance of assessing and addressing during therapy the important aspect of coping strategies.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

OV conceived the idea of the review. OV, AGM, BMP, CAC, CFP and REBB designed the study. OV, CFP, REBB, AFF, MCP, CAS and CN screened the studies and abstracted the primary sources. OV, AGM, BPM and CAS drafted the first version of the review. OV, CAC, CFP, AFA, MCP and CAS revised the manuscript and elaborated the conclusions of the review. All authors had full access to all data contained in the review and decided to submit the manuscript for publication.

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ORIGINAL ARTICLE

Confidentiality in the Pharmaceutical Managed Entry Agreements in Romania

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Abstract: *Background and Aim: Health technology assessment for drugs is a necessary step in developing health policies that are focused on patients and getting the best value for scarce resources. One important feature of health technology assessment is transparency. In many countries, health technology assessment is followed by negotiations between pharmaceutical companies and health authorities to determine whether a drug will be publicly funded. These negotiations often result in Managed Entry Agreements, which typically include confidentiality clauses covering the final price of the drug. Methods: We reviewed Romanian legislation starting in 2014 to assess the level of confidentiality and transparency in drug pricing and reimbursement. Results: We found that for drugs with Managed Entry Agreements, the level of discounts is confidential, the public does not know how much the government is paying for each of these drugs, the volumes (units) of drugs are not transparently published by the payer, the outcomes, in terms of patients treated by therapeutic area, therapeutic success, and resource utilization, are confidential. Conclusions: We consider that too much confidentiality can prevent the public from knowing if the government is getting good value for money, but too little confidentiality can lead to higher drug prices or a lack of drugs from the market.*

Keywords: *managed entry agreements, health technology, confidentiality, pricing, reimbursement*

INTRODUCTION

Every year, the pharmaceutical industry introduces new drugs on the market, constantly developing new solutions for known illnesses. The licensing mechanisms of new drugs are already fully regulated, and the beneficial effects and potential risks have been proven through numerous preclinical and clinical studies [1].

One of the main concerns of each country in establishing its policy on medicines is the permanent increase in the price of new medicines, which generates a growing pressure on the budgets allocated to health [2]. Once they enter the market, the big challenge for a new drug is to be accepted for financing from public funds (or through the health care mechanisms specific to each country). For this, in most of the EU countries, each drug is passed through the very fine sieve of the Health Technology Assessment (HTA) process, the ultimate goal of which is to provide health decision-makers with sufficient relevant information based on which to establish priorities in the field of care, such as the reasonable cost that they afford, and the level of financing, their efficiency, respect for legal and ethical values, and equity.

To develop patient-focused health policies that seek to achieve the best value for scarce resources, one characteristic of the HTA is the transparency of the process [3]. Together or after the HTA process, in numerous countries, there is a practice of negotiations between the Marketing Authorization Holder (MAH) and health authorities to get access to a drug to public financing using Managed Entry Agreements (MEA). In 1993, first-time conventions, lately called MEAs, were introduced between producers and third-party payers, targeting discounts and rebates for innovative drugs [4,5].

The need for these contracts was born from the producers' desire to make price reductions only in the conditions in which they obtain guarantees regarding the avoidance of parallel import and the downward spiral of the price as a result of the fact that the visible price decrease in one country attracts a decrease in all other countries and finally even in the respective country as a result of the use of reference prices. From this perspective, MEAs have a common denominator, respectively, the confidentiality covering the final price of the drug generally as a means to avoid price decreases in other countries.

On the other hand, the third-party payer is interested in keeping costs within a predictable and bearable margin, obtaining superior outcomes for patients, and spending the available resources as efficiently as possible [4]. MEAs can be divided into financial agreements that directly affect the budget (confidential discount or rebate, price-volume agreements, volume or expenditure cap, free initial treatment, etc.) and performance-based agreements that evaluate the treatment's performance (coverage with evidence development, payment by result, conditional treatment continuation, etc.) [4,6,7].

In Romania, a scorecard model called HTA was introduced in 2014. This is based primarily on the results of the assessments or reimbursement status for the respective drugs in EU countries. The health authorities decided in 2015 to increase the financial sustainability of the new drugs through the transfer of financial risk to MAHs via Managed Entry Agreements (known locally as Cost-Volume contracts, which are financial-based agreements, and Cost-Volume-Results contracts, which are performance-based agreements) [8]. The introduction of MEAs allowed a thawing of the introduction of innovative products on the reimbursement list for medicine (the List), after many years of impasse.

To conclude a functional MEA for a drug, there are several mandatory steps in Romania, some of which are confidential and some of which are transparent. These steps are (1) publishing of the List prices in the Official Gazette; (2) submitting the HTA dossier, obtaining the HTA body's approval and publishing the HTA Report; (3) negotiating the MEA, if applicable; and (4) adding the drug on the List.

METHODS

We performed a critical review of the Romanian legislation and rules, from the last 10 years, looking for the confidentiality and transparency aspects covering the pricing and reimbursement of drugs with MEA.

In our research, we worked to identify the elements of the lack of transparency in the price negotiation process for medicines or related to it that could lead to a discrepancy between the benefits offered to patients and the actual price paid.

RESULTS

The Romanian regulations do not stipulate the confidentiality of the final purchase prices. The joint order from the Minister of Health and the President of the National Health Insurance House (NHIH) requires that any confidential information obtained during the execution of the MEA contract (Cost-Volume contract) be used only to fulfill the MEA's purpose and not be disclosed or utilized for other reasons, including the final price of the medication in question [9].

Our analysis revealed that drug reimbursement in Romania through MEAs involves a blend of public and confidential data.

In Romania, there are two List Price catalogs: (1) one used for public reimbursement (Canamed) [10] and (2) one used only by pharmacies or health care providers not having a public reimbursement (Public Catalogue) [11]. Both catalogs are publicly available in the Official Gazette and on the Ministry of Health website [12], while Canamed can be seen also with the reimbursement price on the local payer website [13] (last update on July 1st, 2021). For the International Reference Pricing process done by other countries, the Ministry of Health recommends the use of the Public Catalogue (with higher List prices than Canamed).

The submission of the HTA dossier is a highly confidential process, with most of the steps not transparent: the content of the HTA dossier, the members of the evaluation team from the HTA body, the deadline for the evaluation, and the involvement or not of the Specialty Commissions from the Ministry of Health. Every month, the HTA body (National Agency for Drugs and Medical Devices – ANMDM) publishes a list of the drugs received for HTA evaluation. Next, the HTA report for each drug and the decision and the meeting minutes of the Appeal Committee (only for the drugs where the MAH was not satisfied with the results of the HTA report) are also published.

The HTA methodology allows the MAH to offer (part of the HTA dossier) a "confidential discount" to the payer (NHIH), one key aspect of confidentiality, resulting in a lower price compared to its competitor. This "confidential discount" should be part of a Protocol between MAH and the payer to include the obligation of the MAH to lower the net price (not visible) once the HTA report is positive and then the drug is included in the Reimbursement List 2020 [14].

The negotiation of a MEA is mainly a confidential process. The decision to apply for an MEA relies on the MAH level and also the proposal to be addressed to the Negotiation Committee. The negotiation is contingent upon the number of patients under contract and the eligible population. Ministry of Health (MoH) Specialty Commissions establish the eligible population and publish it on the payer website. Often, these Commissions are not transparent about the data they use to define and establish the eligible population. The number of contracted patients is strictly confidential because the ratio of contracted patients to eligible patients determines the discounts to be paid by MAH as part of MEA (between 25-75%). The table with these discounts is publicly available and published in the Official Gazette (Table 1) [9,15]. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Table 1: Discounts for MEA depending on the market share covered

Percent (%) applied to quarterly consumption (single drug)	Percent (%) applied to quarterly consumption (multiple winners for substitutable drugs)	Percent (%) no. of contracted patients from the no. of total eligible patients
25%	30%	<5%
30%	35%	5-15%
35%	40%	15-25%
40%	45%	25-35%
45%	50%	35-45%
50%	55%	45-55%
55%	60%	55-65%
60%	65%	65-75%
70%	75%	>=75%

The term for these MEAs is one year and is publicly available, their starting point is the first month after the inclusion of the drug in the List.

The renegotiation process involves a simpler approach, with a publication of the moment for negotiation and a clear request for the Specialty Commissions to provide arguments regarding the change in the eligible population.

Other optional aspects of the negotiation, like the coverage by the MAH of some testing or other services, are usually available to the public on the websites of the local affiliates of the payer, due to the need to inform the providers of these aspects [9].

After the conclusion and the execution of the MEA, the MAH will pay the final discount to the payer according to the effective patient share, irrespective of the contracted patient share and discount. The respective data is provided by the payer only to the MAH and nobody else could access this information.

The Parliament approves the payer budget for MEA negotiation and contracting through the State Budget Law. The process of determining this MEA budget is not transparent to the public [16]. Meanwhile, the payer discloses the remaining budget after every round of MEA negotiations. The payer typically reserves a certain amount of its drug budget for drugs that require a new yearly MEA contract to ensure the continued operation of existing MEAs. If the contracting budget is over, no new MEA is signed and for the existing MEAs, there is no renegotiation; only the included patients can continue their treatment and there are no new inclusions of patients.

The budget for drugs with MEA is not available to the public on a monthly or quarterly basis, but at the end of each year, the authorities issue a note detailing the total budget used. MEA's budget information is also included in the Payer Yearly Report, published in the second half of the following year.

The decision to include the drug on the List is highly political, as it requires budget allocation for its introduction. The process for the calculation of budget needs for new drugs is not transparent. The information is only publicly available once the draft of the List is published for public comment.

DISCUSSION

Some authors have pointed out that the classically invoked reasons, namely that the prices of new drugs are very high because most of the income is reinvested in research, are no longer in line with the reality of the time. Drug manufacturers often carry out a series of additional purely financial operations, such as mergers and acquisitions and stock transactions, and play an active role in the capital market, etc. [9,17]. In recent years, concern has grown regarding the confidentiality of the final purchase prices of medicines; in 2019 WHO recommended to member states that they take measures in this regard [18].

The MEA process's opacity creates advantages and disadvantages for the MAH and NHIH regarding the confidentiality of final prices paid by the public payers. The opaque process of setting new budgets extends also for the case when MEAs and the random addition of expenses every time a new product is conditionally approved creates confusion among drug beneficiaries and competing MAHs. Other beneficiaries of the health-care system who require additional funds (except for new drugs) frequently raise concerns.

The legislation for the MEA budget grants access to new drugs on a first-come, first-served basis. Priority criteria are also considered and include the unmet need, the drugs with EU emergency approval, and the public health reasons [15].

The payer does not transparently publish the volume (units) of drugs, including those with MEAs. The only publicly available data on the payer's website is at the ATC3 level and the INN level for the top 10 drugs in terms of value reimbursed in open pharmacies for each category (A, B, C, D, and E) of the reimbursement List [19]. Contract monitoring is challenging because currently there is no way to access treatment outcome data or resource utilization for patients receiving MEA treatments; the payer does not conduct cost-effectiveness analyses or publish data; and manufacturers do not have access to confidential patient outcomes.

The confidentiality stipulated in the MEA prevents not only price comparisons but also the comparisons of the treatment outcomes, making it impossible for any researcher to develop any pharmacoeconomic analysis or to monitor the physician's performance.

There are no transparent rules on the term to publish the updated List (and include the newly evaluated drugs), as the legislation only specifies an update once a year. The practice has shown that shorter terms are needed.

The payer agrees to keep the final prices confidential to avoid the risk of the producer choosing not to sell in Romania than to affect the prices in other countries and decrease the access to new technologies for the population, which would be discriminatory.

The confidentiality of the final price could also be viewed as means to respond to limited resources available in the healthcare sector and is linked with delayed access to innovative technology which could address also the quality of care and patient safety, but even with more resources these aspects will be removed only through a better understanding from the health care professionals [20].

Before 2017, payers and manufacturers would sign collaboration protocols, which included confidential discounts. Protocols aimed to decrease prices in Romania's public healthcare system without affecting the visible drug price or international reference price. Due to unclear legislation, these protocols were abolished and replaced with a new type of protocol where the MAH could support some of the financing burden of the payer [21].

The Payer determines the reimbursement prices (reference price) based on a specific methodology, but its application is not transparent [22]. Anyone can find prices on NHIH's website, and pharmacies will charge co-payments if drug prices are higher than the reimbursement price.

As other authors have already pointed out, the various confidentiality elements of the MAH process prevent other possible cost-containment policies from being used. Meanwhile, in Romania, some of these policies are also used (biosimilars/generic stimulation for prescription, reference pricing, protocols and guidelines for prescriptions, some open tenders for exclusivity) while others are waiting to be implemented (price ceilings, value-based pricing, pay for performance, etc.) [23]

Looking at the future development of MEA in Romania, there are plans to expand from simple cost-volume contracts to other types of MEA, including the payment for performance mechanisms. For this aspect, there will be a need for merging patient-level data with drug-patient data, yet not generally available in Romania at the health system level, but only in some of the hospitals, and the definition and the confidentiality of these patient outcomes will be a new issue during the implementation [24,25].

The future of MEA in Romania will be linked with the new EU Directive on HTA, which will become effective in 2025, and some local projects funded by the MOH in regarding pricing, reimbursement, and HTA. The utilization of a classic HTA model with cost-effectiveness in place could replace the actual scorecard HTA model. This will create the case for the confidentiality of prices used in the cost-effectiveness analysis but will benefit from the public availability of outcomes measures like the local values for Quality Adjusted Life Years (QALY) and will catalyze the efforts to determine the cost of QALY in Romania [26,27,28].

CONCLUSIONS

The negotiation process for the MEA in Romania results in a confidential final price, making it difficult to compare with other EU countries.

Confidential price rises question the legitimacy of some decisions and, in particular, the impossibility of checking over time the cost-effectiveness of drugs in the absence of the knowledge of the cost effectively paid. The solution is finding proper ways to provide more transparency in the process, especially regarding budget allocation. This can be obtained using guidelines as a result of cooperation between all interested actors.

From this perspective, the entire process of delivering drugs to the public domain is a new paradigm that requires new means of increasing the equity of financial allocations within the healthcare system while ensuring profitability for the MAH and accessibility for the patients. The key solution is better collaboration and communication between stakeholders keeping in mind to meet patients' needs and expectations.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, M.S.P. and C.P.R. methodology, M.S.P. and C.P.R., validation, M.S.P. and C.P.R., formal analysis, M.S.P., C.P.R., and B.C.P. resources, M.S.P., C.P.R., B.C.P., data curation, M.S.P., writing—original draft preparation, M.S.P., writing—review and editing, C.P.R., and B.C.P. visualization, B.C.P.; supervision, M.S.P. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable

Patient consent for publication

Not applicable.

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ORIGINAL ARTICLE

Sentinel Node Biopsy Positivity: Is Axillary Dissection Always Necessary? – A Retrospective Analysis

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Abstract: *Breast cancer remains a significant health concern, with predictions indicating a rise in global incidence. While the primary focus is on oncological radicality, the aesthetic and psychological impacts of surgical interventions, such as radical mastectomy, cannot be ignored. This study, conducted over 8 years, aimed to identify factors that can prevent unnecessary lymphadenectomy. The research analyzed various parameters, including age, BMI, tumor size, and immunohistochemistry, to determine their correlation with axillary lymph node invasion. The results highlighted the importance of tumor size and estrogen receptor status in predicting axillary lymph node metastasis. The study underscores the value of sentinel lymph node biopsy in early breast cancer treatment, emphasizing its benefits over complete axillary lymph node dissection.*

Keywords: *breast cancer, lymphadenectomy, sentinel node biopsy*

INTRODUCTION

According to the American Cancer Society, one out of eight women in the United States will develop breast cancer during their lifetime [1]. Predictions regarding the global incidence of breast cancer in women outline the idea that the incidence will reach approximately 3.2 million new cases per year by 2050.

These numbers reflect the magnitude of breast cancer incidence, its impact on women worldwide, and the ongoing and urgent need to take measures towards prevention and proper therapeutic management [2].

The goal of oncological radicality remains essential and obligatory, but breast neoplasm surgery must also consider the aesthetic component due to the psychological impact that a major surgical intervention, such as radical mastectomy, has on patients. In the early stages, breast cancer can be treated without such a procedure being necessary.

Therefore, in today's times, the gold standard of breast cancer surgery is represented by conservative treatment. It involves the resection of the primary tumor with oncological safe margins and sentinel lymph node biopsy, followed by adjuvant radiotherapy [2,3].

The surgical management of the axilla has always been a controversial topic. For a very long time, it was believed that achieving good loco-regional control involves a complete axillary lymph node dissection, with this method being the gold standard of that era.

In recent years, due to the observed increase in morbidity and decrease in the quality of life for these patients, the method has been abandoned and replaced by sentinel lymph node biopsy.

The technique of identifying the sentinel lymph node (SLN) is based on the theory of the lymphatic spread of cancers, with the neoplastic invasion occurring in a lymph node that drains lymph from the site of the tumor [4,5] .

The prime objective of this study is the identification of factors that can prevent unnecessary lymphadenectomy.

MATERIALS AND METHODS

Based on the purpose and objectives of the research, the research design and methodology have been defined, with its main characteristics being: retrospective - spanning over an 8-year interval - January 1, 2014 - December 31, 2021, single-center, descriptive and correlational, non-interventional.

The eligibility criteria were as follows:

- Diagnosis of breast cancer
- The presence of axillary management after a positive sentinel node biopsy
- Female sex
- Stage IIA-IIIB:
  - o IIA (T2N0M0, T1N1M0)
  - o IIB (T2N1M0, T3N0M0)
  - o IIIA (T3N1M0)
  - o IIIB (T4N1M0)
- Single tumor, excluding multifocal and bilateral cases
- The presence of all required data
- Age > 18 years
- M0
- Any Histopathological type

RESULTS

The working hypothesis considers patients who underwent the sentinel lymph node technique, and the result was positive. In this situation, studies have shown that up to 1/3 of cases present a negative result of the axillary lymphadenectomy. To assess the risk factors for a positive axillar biopsy, we made a comparison between group E – a positive result of axillary lymphadenectomy (34 cases), and group D – a negative result of axillary lymphadenectomy (15 cases) (Table 1).

Table 1: Comparative data of groups (E vs. D)

Study lot	D	E
SNB	✓	✓
SNB results	+	+
ALND	✓	✓
ALND results	-	+
Case numbers	15	34

Age

There was no statistical correlation between the age of the patients and the presence of axillary invasion in patients with a positive sentinel lymph node, even though there was a mean difference of 5.85 years - p=0.175 (Table 2 and Figure 1).

Table 2: Comparative statistical data regarding age (E vs. D)

	Age	
	E	D
Valid	34	15
Missing	0	0
Mean	63.324	57.467
Std. Deviation	14.367	12.118
Minimum	28.000	29.000
Maximum	89.000	78.000

	t	df	p	Mean difference	SE difference
Age	1.376	47	0.175	5.857	4.258

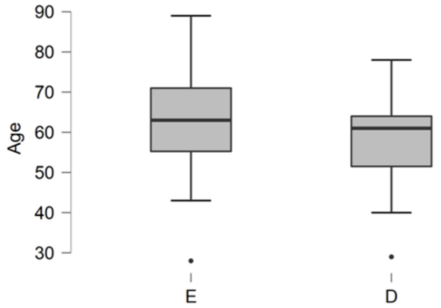

Figure 1: Comparative statistical data regarding age (E vs. D)

BMI

The Body Mass Index (BMI) value calculated for the two groups showed a higher average in the case of patients with axillary invasion, with a difference of 3.58 and moderate statistical significance - p=0.05 (Table 3 and Figure 2).

Table 3: Comparative statistical data regarding BMI (E vs. D)

	BMI	
	E	D
Valid	34	15
Missing	0	0
Mean	28.696	25.107
Median	28.100	24.900
Std. Deviation	6.187	4.608
Minimum	18.970	19.760
Maximum	45.650	33.650

	t	df	p	Mean difference	SE difference
BMI	2.009	47	0.050	3.589	1.786

Bio-clinical analysis

Comparative assessment of routine laboratory test values collected upon admission highlights relatively small differences between the groups discussed in this hypothesis.

The only parameter that demonstrated moderate statistical significance was the white blood cell count - with a mean difference of 1.44 and p=0.065 (Table 4 and Figure 3). Tumor markers did not show statistically significant differences.

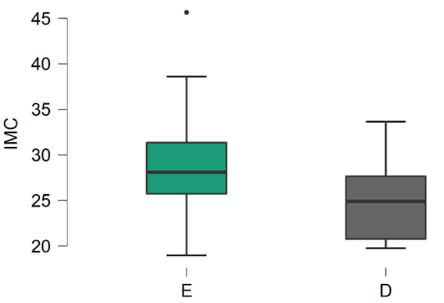

Figure 2: Comparative statistical data regarding BMI (E vs. D)

Table 4: Comparative statistical data regarding bio-clinical analysis (E vs. D)

	Hb		Ht		WBC		ESR		INR		Fibrinogen	
	E	D	E	D	E	D	E	D	E	D	E	D
Valid	34	15	34	15	34	15	34	15	34	15	34	15
Mean	12.515	12.647	40.529	40.067	8.726	7.280	31.971	29.800	1.189	1.130	434.029	378.600
Std. Deviation	1.259	1.148	3.518	4.559	2.550	2.253	18.909	16.618	0.313	0.215	186.974	174.482
Minimum	8.50	9.90	35.00	31.00	5.00	3.90	8.00	3.00	0.830	0.840	201.00	205.00
Maximum	15.30	13.80	48.00	47.00	14.70	10.90	101.00	57.00	1.940	1.60	835.00	823.00

Quadrant

The location of the tumor did not correlate with the extent of the axillary invasion, even though there were differences in the distribution of cases concerning this parameter, the statistical significance was not relevant (Tables 5 and 6).

Table 4: Comparative statistical data regarding bio-clinical analysis (E vs. D) – cont.

	t	df	p	Mean difference	SE difference
Hb	-0.347	47	0.730	-0.132	0.380
Ht	0.387	47	0.701	0.463	1.196
WBC	1.893	47	0.065	1.446	0.764
ESR	0.384	47	0.703	2.171	5.659
INR	0.657	47	0.514	0.059	0.089
Fibrinogen	0.975	47	0.334	55.429	56.830

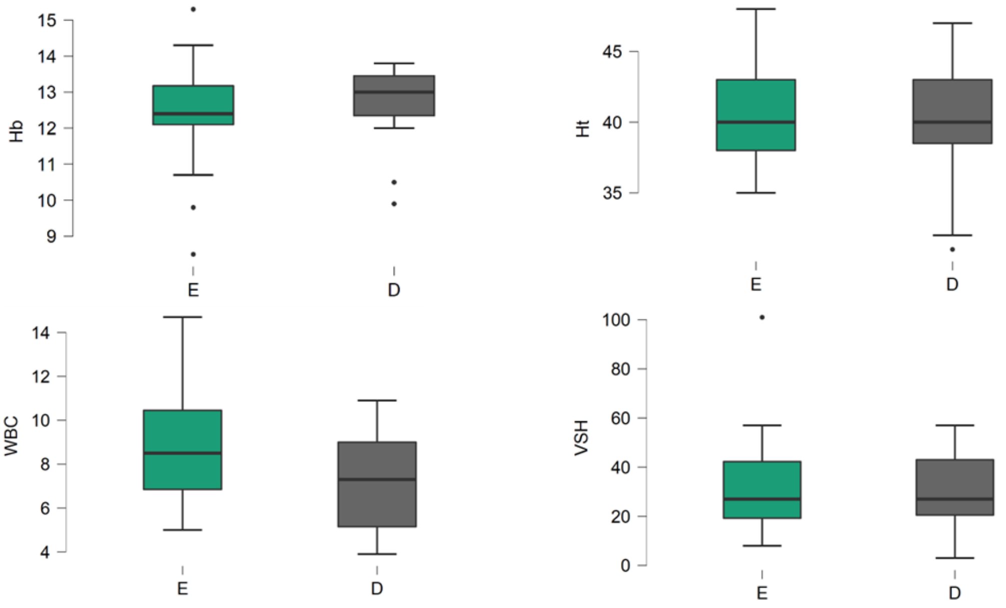

Figure 3: Comparative statistical data regarding bio-clinical analysis (E vs. D)

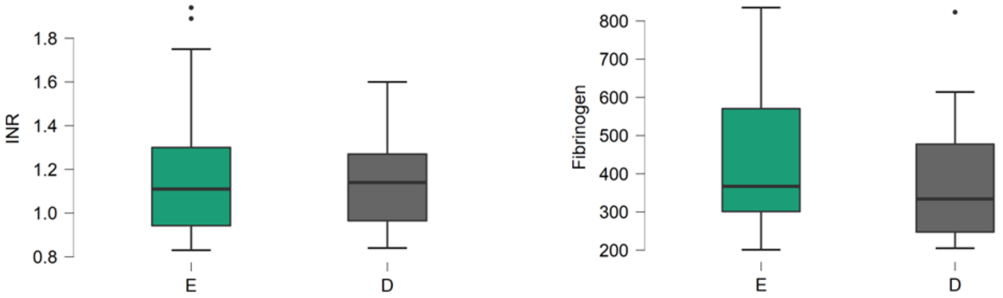

Figure 3: Comparative statistical data regarding bio-clinical analysis (E vs. D) – cont.

Table 5: Comparative statistical data regarding the tumor location (E vs. D)

Frequencies for Quadrant					
Hypothesis 2	**Quadrant**	**Frequency**	**Percent**	**Valid Percent**	**Cumulative Percent**
E	C	3	8.824	8.824	8.824
	SE	20	58.824	58.824	67.647
	SI	3	8.824	8.824	76.471
	IE	7	20.588	20.588	97.059
	II	1	2.941	2.941	100.000
	Missing	0	0.000		
	Total	34	100.000		
D	C	2	13.333	13.333	13.333
	SE	12	80.000	80.000	93.333
	SI	0	0.000	0.000	93.333
	IE	1	6.667	6.667	100.000
	II	0	0.000	0.000	100.000
	Missing	0	0.000		
	Total	15	100.000		

Chi-Squared Tests			
	Value	**df**	**p**
X^2	3.922	4	0.417
N	49		

Tumor size

The distribution of tumor sizes highlights higher values within group E - with axillary lymph node invasion, with a mean difference of 8.7 mm and a statistical significance of p=0.04 (Table 7 and Figures 4 and 5).

Immunohistochemistry

From the perspective of evaluating the presence of hormone receptors in the immunohistochemical assessment of breast resection specimens, only estrogen receptors have been identified as a risk factor for axillary invasion beyond the sentinel lymph node. The calculated risk is 7.33 times with a statistical significance of p=0.005 (Tables 8 - 10).

Subtype

From the perspective of molecular subtypes, the division into Luminal A, Luminal B, and Triple Negative did not show a statistically significant correlation with the presence of axillary lymph node invasion in patients with a positive sentinel lymph node. In the case of the HER2 sub-type, the risk of axillary lymph node invasion was nearly 10 times higher - p=0.084 (Tables 11-14).

Table 6: Distribution of cases concerning the localization in the interior of the exterior quadrant of the tumor (E vs D)

Ext 1 vs Int 0 quadrant		**Hypothesis 2**		
		0	**1**	**Total**
0	Count	7.000	2.000	9.000
	% within row	77.778 %	22.222 %	100.000 %
	% within column	20.588 %	13.333 %	18.367 %
1	Count	27.000	13.000	40.000
	% within row	67.500 %	32.500 %	100.000 %
	% within column	79.412 %	86.667 %	81.633 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

Table 6: Distribution of cases concerning the localization in the interior of the exterior quadrant of the tumor (E vs D) – cont.

	Log Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	0.522	-1.183	2.227	
Fisher's exact test	0.512	-1.331	2.926	0.702

Table 7: Comparative statistical data regarding the tumor size (E vs. D)

	Size	
	E	D
Valid	34	15
Mean	21.235	12.533
Std. Deviation	14.078	11.154
Minimum	4.000	1.000
Maximum	58.000	47.000

	t	df	p	Mean Difference	SE Difference
Size	2.115	47	0.040	8.702	4.115

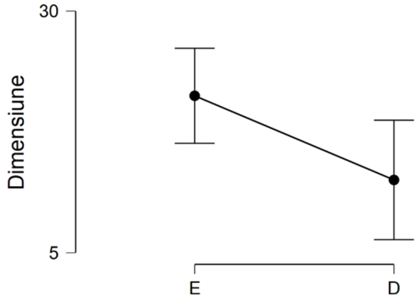

Figure 4: Comparative statistical data regarding tumor size (E vs. D)

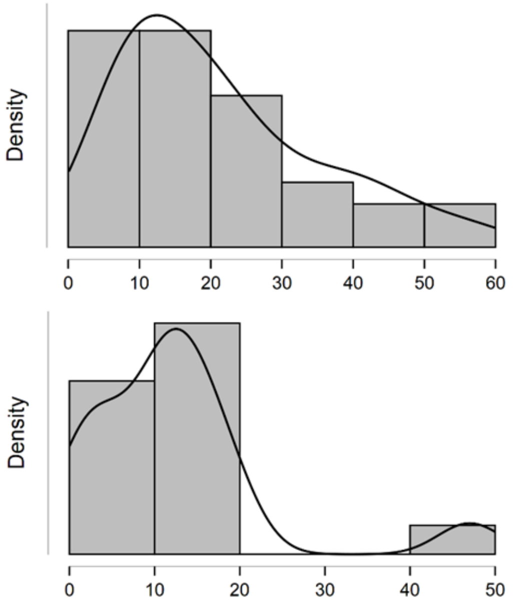

Figure 5: Distribution of cases concerning the tumor size (E left vs. D right)

Table 8: Distribution of cases concerning the presence of ER receptors (E vs D)

ER+		Hypothesis 2		Total
		0	1	
0	Count	22.000	3.000	25.000
	% within row	88.000 %	12.000 %	100.000 %
	% within column	64.706 %	20.000 %	51.020 %
1	Count	12.000	12.000	24.000
	% within row	50.000 %	50.000 %	100.000 %
	% within column	35.294 %	80.000 %	48.980 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	7.333	1.724	31.186	
Fisher's exact test	7.019	1.498	46.390	0.005

Table 9: Distribution of cases with the presence of PR receptors (E vs D)

PR+		Hypothesis 2		Total
		0	1	
0	% within row	72.000 %	28.000 %	100.000 %
	% within column	52.941 %	46.667 %	51.020 %
1	Count	16.000	8.000	24.000
	% within row	66.667 %	33.333 %	100.000 %
	% within column	47.059 %	53.333 %	48.980 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	1.286	0.381	4.344	
Fisher's exact test	1.279	0.321	5.206	0.762

Table 10: Distribution of cases with the presence of PR receptors (E vs D)

HER2+		Hypothesis 2		Total
		0	1	
0	Count	22.000	10.000	32.000
	% within row	68.750 %	31.250 %	100.000 %
	% within column	64.706 %	66.667 %	65.306 %
1	Count	12.000	5.000	17.000
	% within row	70.588 %	29.412 %	100.000 %
	% within column	35.294 %	33.333 %	34.694 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	0.917	0.254	3.308	
Fisher's exact test	0.918	0.198	3.851	1.000

Table 11: The distribution of cases concerning the Luminal A subtype (E vs D)

Luminal A		Hypothesis 2		Total
		0	1	
0	Count	21.000	7.000	28.000
	% within row	75.000 %	25.000 %	100.000 %
	% within column	61.765 %	46.667 %	57.143 %
1	Count	13.000	8.000	21.000
	% within row	61.905 %	38.095 %	100.000 %
	% within column	38.235 %	53.333 %	42.857 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	1.846	0.541	6.302	
Fisher's exact test	1.823	0.455	7.538	0.363

DISCUSSION

The literature describes four parameters as independent factors of lymph node metastasis, namely lymphovascular invasion, primary tumor size, multiple foci, and tumor localization.

An independent prognostic factor for disease-free survival (DFS) that has been recently analyzed in literature for patients with breast carcinoma is the degree of lymphovascular invasion [6].

Additionally, lymphovascular invasion is associated with a higher frequency of axillary lymph node metastases [7]. To use this data as a tool in determining the indication for sentinel lymph node biopsy (SLNB) or axillary lymphadenectomy, more extensive studies are needed to specifically determine the degree of invasion and the cutoff value that necessitates radical intervention.

Table 12: The distribution of cases concerning the Luminal B subtype (E vs D)

Luminal B		Hypothesis 2		Total
		0	1	
0	Count	26.000	10.000	36.000
	% within row	72.222 %	27.778 %	100.000 %
	% within column	76.471 %	66.667 %	73.469 %
1	Count	8.000	5.000	13.000
	% within row	61.538 %	38.462 %	100.000 %
	% within column	23.529 %	33.333 %	26.531 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	1.625	0.428	6.171	
Fisher's exact test	1.608	0.331	7.329	0.500

Table 13: The distribution of cases concerning the Luminal B subtype (E vs D)

Her2		Hypothesis 2		Total
		0	1	
0	Count	27.000	15.000	42.000
	% within row	64.286 %	35.714 %	100.000 %
	% within column	79.412 %	100.000 %	85.714 %
1	Count	7.000	0.000	7.000
	% within row	100.000 %	0.000 %	100.000 %
	% within column	20.588 %	0.000 %	14.286 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	0.118	0.006	2.214	
Fisher's exact test	0.000	0.000	1.466	0.084

A series of studies have demonstrated that younger age is a risk factor for positive axillary lymph nodes (ALN) [8–11]. This has been attributed to more biologically aggressive tumors in this younger age group. The average age of menopause is around 50 years, so most literature establishes 50 years as a cutoff point in exploring the relationship between age and clinicopathological factors in invasive breast cancer [8,12]. The current study did not show correlations between the risk of axillary lymph node spread and age, BMI, and the biological status of breast cancer patients, with results similar to those described in the studies.

Table 14: The distribution of cases concerning the Triple-negative subtype (E vs D)

Triple negative		Hypothesis 2		Total
		0	1	
0	Count	28.000	13.000	41.000
	% within row	68.293 %	31.707 %	100.000 %
	% within column	82.353 %	86.667 %	83.673 %
1	Count	6.000	2.000	8.000
	% within row	75.000 %	25.000 %	100.000 %
	% within column	17.647 %	13.333 %	16.327 %
Total	Count	34.000	15.000	49.000
	% within row	69.388 %	30.612 %	100.000 %
	% within column	100.000 %	100.000 %	100.000 %

	Odds Ratio	95% Confidence Intervals		p
		Lower	Upper	
Odds ratio	0.718	0.127	4.051	
Fisher's exact test	0.723	0.063	4.803	1.000

Histologic grade and tumor size (often over 5 centimeters in size) are also important predictors of lymph node metastasis. The probability is high if a patient has a large tumor and a high histologic grade. Our presented data show that patients with larger tumor sizes and a high histologic grade are more likely to have axillary invasion, a finding that aligns with those from previous studies [1,6,13–15]. Patients with small invasive breast cancer might benefit from SLNB.

The inclusion of extranodal extension in the evaluation protocol is necessary to achieve precise oncologic staging. Although attempts have been made to establish cut-off values for the circumferential and perpendicular diameters of invasion extension, these have not yielded significant predictive values. Therefore, more extensive studies are needed to specifically determine the dimensions of invasion from which axillary lymph node metastatic burden can be predicted, subsequently guiding the appropriate therapeutic approach [16,17].

Estrogen receptor status was another factor that showed a significant correlation with axillary lymph node involvement, with a calculated risk of axillary invasion beyond the sentinel lymph node being 7.33 times higher in HER+ patients. Various studies have evaluated the role of hormonal receptor status in predicting axillary lymph node metastasis (ALNM) with contradictory results. Capdet et al. [18,19], in their study of 1416 patients with early breast cancer, found no association between hormonal status and lymph node involvement. In contrast, Lee et al. [20] reported a high incidence of ALNM regardless of tumor size in the Korean population with T1-2 breast carcinoma. Finally, in a recent study conducted by Holm-Rasmussen et al. [21], patients with ER-negative tumors had a reduced risk of ALN involvement at the time of diagnosis compared to other patients.

Large integrative analyses are necessary to explain why ER-negative tumors have a low rate of axillary metastases and a high rate of distant metastases. Conversely, HER2-positive tumors have an axillary metastasis rate closely linked to tumor size and a high rate of distant metastases [22,23].

Among the limitations of the study, the retrospective nature of its design, the small number of tracked patients, and the lack of information regarding the use of chemotherapy, radiotherapy, or hormone therapy are identified.

Systemic therapies, including hormonal therapy, cytotoxic chemotherapy, and/or radiation therapy, remain critically important, especially in the treatment of breast cancer with positive lymph nodes [24–28]. Therefore, the results of this study will contribute to decision-making regarding the addition of axillary lymph node dissection (ALND), systemic therapy, and/or radiotherapy in the case of SLN metastases efforts to determine the cost of QALY in Romania [26,27,28].

CONCLUSIONS

Regional lymph node staging is necessary for tumor staging and surgical planning. Previous studies have identified several important factors in predicting axillary lymph node metastasis (ALNM), but the predictive power has not been satisfactory.

With advancements in surgical techniques, sentinel lymph node biopsy (SLNB) is now widely used in early breast cancer and has become a standard procedure in clinically node-negative patients. The benefits of SLNB include fewer surgical complications than complete axillary lymph node dissection (ALND) and effectiveness and accuracy in long-term follow-up.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, R.A-M. and D.N.P.; methodology, O.A.; software, S.N.; validation, A.B., F.S. and M.C.D.; formal analysis, D.I.; investigation, R.A-M.; resources, D.N.P.; data curation, O.A.; writing—original draft preparation, S.N.; writing—review and editing, A.B.; visualization, F.S.; supervision, M.C.D.; project administration, D.I.; funding acquisition, R.A-M. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted following the Declaration of Helsinki, and approved by the Institutional Review of University Emergency Hospital of Bucharest.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Hepatic Steatosis is the Most Common Finding in Abdominal Ultrasonography Performed in Patients with Psoriasis before Biological Agent Treatment

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Abstract: *Psoriasis has been associated with metabolic syndrome and nonalcoholic fatty liver disease. We wanted to investigate the abdominal ultrasonography results of psoriasis patients before biological agent treatment. Between April 2019 and February 2022, abdominal ultrasonography results of psoriasis patients which were performed before biological agent treatment were reviewed retrospectively. This study included 90 patients, 45 females, and 45 males with a mean age of 48.91±13.43 years. The most common ultrasonography finding was increased echogenicity of the liver parenchyma due to steatosis (58.9%) which was followed by an increase in liver size (25.6%), kidney cysts (17.8%), gallstones (8.9%) and increase in spleen size (7.8%). Hepatic steatosis was more frequent in older patients (p<0.001), patients with diabetes (p=0.007), and patients who were previously treated with biological agents (p=0.007). 70.4% of the patients with moderate or severe hepatic steatosis were female. Herein, hepatic steatosis was detected in 53 (58.9%) patients. Nonalcoholic fatty liver disease which presents as hepatic steatosis in early stages has an asymptomatic course and poor prognosis in psoriasis patients. We suggest that abdominal ultrasonography should be performed in psoriasis patients before biological agent treatment, especially in females, patients with advanced age, patients who were previously treated with biological agents, and patients with diabetes.*

Keywords: *abdomen; biological agents; hepatic steatosis; psoriasis; ultrasonography*

INTRODUCTION

Psoriasis is a systemic inflammatory disorder that primarily affects the skin and may present with various comorbidities such as psoriatic arthritis, nonalcoholic fatty liver disease, diabetes, metabolic syndrome, and inflammatory bowel disease [1]. Tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17), and IL-23 are the cytokines mainly responsible for the inflammation in psoriasis [2]. Chronic inflammation has been implicated in the development of accompanying comorbidities. On the other hand, non-alcoholic fatty liver disease which is an important comorbidity of psoriasis due to its risk of progressing to cirrhosis has been associated with the development of severe psoriasis since it may lead to inflammatory cytokine production from the liver [3]. Biological agents affect specific pathways of the immune system that are implicated in the etiopathogenesis of psoriasis. Several biological agents have been developed and successfully used for the treatment of psoriasis. It has been suggested that biological agents were more effective and safer than conventional systemic treatments of psoriasis which lead to a broad immunosuppressive activity [4]. However, some safety concerns have been raised due to the widespread and long-term use of biological agents. Hepatotoxicity, reactivation of hepatitis B and latent tuberculosis infection, exacerbation of inflammatory bowel disease, and invasive fungal infections have been reported in psoriasis patients treated with biological agents. Side effects vary on each biological agent received [5]. For instance, administration of TNF-α inhibitors for long periods may lead to weight gain which has a negative impact on nonalcoholic fatty liver disease in patients with psoriasis [6].

Furthermore, it is controversial whether biological agent treatment increases the risk of malignancy in patients with psoriasis or not. Fiorentino et al. reported that the risk of malignancy might be increased in patients with psoriasis treated with TNF-α inhibitors for longer than a year [7]. On the other hand, Mastorino et al. stated that the use of biological agents affecting TNF-α, IL-17, IL-12, and IL-23 for the treatment of psoriasis was safe even in patients with a past medical history of malignancy [8]. It has been suggested that abdominal ultrasonography might be performed in patients with psoriasis before the initiation of treatment with biological agents to exclude intraabdominal malignancy such as lymphoma and it might be repeated during the treatment [9]. However, abdominal ultrasonography is not included as a pretreatment test to be performed in psoriasis patients before the initiation of biological agents in major guidelines such as Joint American Academy of Dermatology – National Psoriasis Foundation (AAD-NPF) guidelines of care for the management and treatment of psoriasis with biologics and EuroGuiDerm guideline on the systemic treatment of psoriasis vulgaris [10, 11].

Within this study, we aimed to evaluate abdominal ultrasonography results of patients with psoriasis which were performed before the initiation of biological agent treatment.

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained before this study (approval number: 2022-264). Between April 2019 and February 2022, medical records of patients with psoriasis who underwent abdominal ultrasonography before biological agent treatment were evaluated retrospectively. Psoriasis type, disease duration, psoriasis treatment, accompanying psoriatic arthritis, and systemic diseases were reviewed. All patients with psoriasis aged 18 years and over with complete data have been included in the study.

Statistical analysis was performed using SPSS version 20.0. Quantitative variables were represented as mean ± standard deviation or median and interquartile range, categorical variables were represented as counts and percentages. Kolmogorov-Smirnov test was used to determine whether variables were normally distributed or not. Differences in variables between the two groups were evaluated by chi-square test, independent sample t-test, and Mann-Whitney U test. P values less than 0.05 were considered statistically significant.

RESULTS

This study included 90 patients, 45 females and 45 males. The mean age of the patients was 48.91±13.43 years (range: 19-75 years). 79 (87.8%) patients had psoriasis vulgaris, 8 (8.9%) patients had generalized pustular psoriasis, and 3 (3.3%) patients had palmoplantar psoriasis. The mean disease duration was 19.35±11.93 years (range: 2-53 years). The median Psoriasis Area and Severity Index (PASI) was 7.2 (11.4) [12]. Psoriatic arthritis was detected in 34 (37.8%) patients. 34 (37.8%) patients were treated with ustekinumab, 29 (32.2%) patients were treated with IL-17 inhibitors such as secukinumab and ixekizumab, 23 (25.6%) patients were treated with TNF-α inhibitors such as adalimumab, infliximab and certolizumab pegol, 4 (4.4%) patients were treated with IL-23 inhibitors such as risankizumab and guselkumab. All patients previously received conventional treatments such as acitretin, cyclosporine, and methotrexate. 15 (16.7%) patients were previously treated with phototherapy. 37 (41.1%) patients were previously treated with biological agents whereas 53 (58.9%) patients were biologic-naive. None of the patients stated excessive alcohol consumption. Past medical history was unremarkable in 48 (53.3%) patients. However, 23 (25.6%) patients had hypertension, 17 (18.9%) patients had diabetes, 4 (4.4%) patients had chronic obstructive pulmonary disease, 4 (4.4%) patients had asthma, 4 (4.4%) patients had hypothyroidism, 4 (4.4%) patients had chronic hepatitis B virus infection, 3 (3.3%) patients had chronic kidney disease, 3 (3.3%) patients had coronary artery disease, 1 (1.1%) patient had nephrolithiasis, 1 (1.1%) patient had villous adenoma of the colon. In addition, 1 (1.1%) patient had a history of testicular seminoma, 1 (1.1%) patient had cervical cancer, and 1 (1.1%) patient had urothelial carcinoma.

Abdominal ultrasonography revealed an increase in liver size in 23 (25.6%) patients (the mean craniocaudal length in the midclavicular line: 16.69±0.96 mm, range: 15.5-20 mm). Among the patients with increased liver size, 17 (73.9%) were female and 6 (26.1%) were male (p=0.013). Increased liver size was observed in 14 (60.9%) patients with psoriatic arthritis and in 9 (39.1%) patients without arthritis (p=0.008) (Table 1). Increased liver size was more common in patients with hypertension (10, 43.5%) compared to patients without hypertension (13, 19.4%) (p=0.023). Increased liver size was observed in 6 (35.3%) patients with diabetes and 17 (23.3%) patients without diabetes (p=0.309).

The mean psoriasis duration was 22.15±12.47 years in patients with increased liver size and 18.39±11.68 years in patients with normal liver size (p=0.185). Among the patients with increased liver size, the mean craniocaudal length was 17.03±1.10 mm in those who were biologic-naive and 16.25±0.48 mm in those who received biological agents previously (p=0.037). All patients with increased liver size showed increased echogenicity of the liver parenchyma due to steatosis. An increase in echogenicity of the liver parenchyma due to steatosis was reported in 53 (58.9%) patients [mild: 26 (28.9%), moderate: 14 (15.6%), severe: 13 (14.4%)]. The mean age of the patients with and without increased echogenicity of the liver parenchyma due to steatosis was 53.62±11.71 years and 42.16±12.99 years, respectively (p<0.001).

Table 1: Patients with increased liver size and increased echogenicity of the liver parenchyma

	Increased liver size		**P value**
Patient characteristics (n, %)	Female (17, 73.9)	Male (6, 26.1)	0.013
	With psoriatic arthritis (14, 60.9)	Without psoriatic arthritis (9, 39.1)	0.008
	With hypertension (10, 43.5)	Without hypertension (13, 19.4)	0.023
Craniocaudal length (mm, mean±SD)	Biologic-naïve (17.03±1.10)	Previous biologic (16.25±0.48)	0.037
	Increased echogenicity of the liver parenchyma due to steatosis		
Patient characteristics (n, %)	Previous biologic (28, 75.7)	Biologic-naïve (25, 47.2)	0.007
	With diabetes (15, 88.2)	Without diabetes (38, 52.1)	0.007
Moderate/severe (n, %)	Female (19, 70.4)	Male (8, 29.6)	0.021

SD: Standard deviation. Increased liver size was more common in patients with psoriatic arthritis and hypertension. Moderate or severe increase in the echogenicity of the liver parenchyma due to steatosis was more common in female patients.

Among the patients with increased echogenicity of the liver parenchyma due to steatosis, 28 (75.7%) received biological agents previously and 25 (47.2%) were biologic-naive (p=0.007). Increased echogenicity of the liver parenchyma due to steatosis was detected in 15 (88.2%) patients with diabetes and in 38 (52.1%) patients without diabetes (p=0.007). Among the patients with a moderate or severe increase in the echogenicity of the liver parenchyma due to steatosis, 19 (70.4%) were female and 8 (29.6%) were male (p=0.021). In addition, heterogeneous liver parenchyma was reported in 5 (5.6%) patients, hypoechoic areas in the liver which might be compatible with focal fatty sparing in 4 (4.4%) patients, liver cyst in 3 (3.3%) patients, hyperechoic liver lesions which might be compatible with hemangioma in 3 (3.3%) patients and Chilaiditi syndrome was reported in 1 (1.1%) patient.

Moreover, gallstones were detected in 8 (8.9%) patients (the mean diameter: 15±7.05 mm, range: 3-20 mm) and gallbladder polyps in 3 (3.3%) patients (the mean diameter: 4.5±0.5 mm, range: 4-5 mm). Among the patients with gallstones and gallbladder polyps, 7 (63.6%) were female and 4 (36.4%) were male (p=0.141). The mean age was 53.73±13.32 years in patients with gallstones and gallbladder polyps and 48.24±13.40 years in those who had neither (p=0.094). Gallbladder wall thickening (4 mm) and echogenicities in the gallbladder wall (cholesterolosis) were detected in 1 (1.1%) patient. An increase in the transverse diameter of the gallbladder (42 mm) and accompanying gallbladder wall thickening (6 mm) were detected in 1 (1.1%) patient. Furthermore, kidney cysts were detected in 16 (17.8%) patients (the mean diameter: 26.5±15.8 mm, range: 8-50 mm). Increased echogenicity of kidney parenchyma was detected in 5 (5.5%) patients. The mean ages of the patients with and without kidney cysts were 56.81±7.54 years and 47.20±13.85 years, respectively (p=0.008). Kidney stones were detected in 4 (4.4%) patients (the mean diameter: 7.5±5.8 mm, range: 3-16 mm). Lobulated kidney contour was detected in 4 (4.4%) patients. Dilated renal collecting system was detected in 3 (3.3%) patients, focal caliectasis was detected in 1 (1.1%) patient, increase in kidney size was detected in 1 (1.1%) patient, decrease in kidney size was detected in 1 (1.1%) patient and a renal angiomyolipoma was detected in 1 (1.1%) patient. Abdominal ultrasonography also revealed increase in spleen size in 7 (7.8%) patients (the mean craniocaudal length: 13.87±1.05 mm, range: 13-16 mm), accessory spleen in 2 (2.2%) patients, splenic cyst with a diameter of 7 mm in 1 (1.1%) patient, situs inversus totalis in 1 (1.1%) patient, calcifications in prostatic parenchyma in 1 (1.1%) patient, heterogeneous prostatic parenchyma in 1 (1.1%) patient, an ovarian cyst measured 33x27 mm in size in 1 (1.1%) patient, uterine myoma with a diameter of 11 mm in 1 (1.1%) patient, increased bladder wall thickness (6 mm) in 1 (1.1%) patient, echogenic debris within the bladder which might be compatible with urinary tract infection in 1 (1.1%) patient (Table 2).

Based on ultrasonography findings, abdominal computed tomography (CT) was performed in 3 (3.3%) patients for further evaluation of kidney cysts and abdominal magnetic resonance imaging (MRI) in 2 (2.2%) patients for hypoechoic liver lesions and liver cysts. Renal scintigraphy was performed in 1 (1.1%) patient for the dilation of the renal collecting system.

DISCUSSION

Within this study, the most common abdominal ultrasonography finding in patients with psoriasis was increased echogenicity of the liver parenchyma due to steatosis (58.9%). Hepatic steatosis was more frequent in older patients, patients with diabetes, and patients who were previously treated with biological agents (p<0.001, p=0.007, p=0.007, respectively). These results may be related to the fact that patients previously treated with biologic agents were older than biologic-naive ones (p=0.021). In addition, increased echogenicity of the liver parenchyma due to steatosis was moderate or severe in 50.9% of the patients and most of these patients (70.4%) were female. An increase in liver size (25.6%) was the other common abdominal ultrasonography finding followed by kidney cysts (17.8%), gallstones (8.9%), and an increase in spleen size (7.8%). The increased liver size was statistically significantly more prevalent in female patients, and patients with psoriatic arthritis and hypertension (p=0.013, p=0.008, p=0.023, respectively).

Table 2: Abdominal ultrasonography findings in patients with psoriasis before biological agent treatment

	Patients n (%)	Mean size±SD (mm)	Range (mm)
Increased liver parenchyma echogenicity	53 (58.9)		
Increased liver size	23 (25.6)	16.69±0.96	15.5-20
Kidney cysts	16 (17.8)	26.5±15.8	8-50
Gallstones	8 (8.9)	15±7.05	3-20
Increased spleen size	7 (7.8)	13.87±1.05	13-16
Increased kidney parenchyma echogenicity	5 (5.6)		
Heterogeneous liver parenchyma	5 (5.6)		
Kidney stones	4 (4.4)	7.5±5.8	3-16
Lobulated kidney contour	4 (4.4)		
Hypoechoic areas in the liver	4 (4.4)		
Liver cyst	3 (3.3)		
Liver hemangioma	3 (3.3)		
Gallbladder polyps	3 (3.3)	4.5±0.5	4-5
Dilated renal collecting system	3 (3.3)		
Accessory spleen	2 (2.2)		
Gallbladder wall thickening	2 (2.2)		
Echogenicities in the gallbladder wall	1 (1.1)		
Focal caliectasis	1 (1.1)		
Echogenic debris within the bladder	1 (1.1)		
Increased kidney size	1 (1.1)		
Decreased kidney size	1 (1.1)		
Renal angiomyolipoma	1 (1.1)		
Increased bladder wall thickness	1 (1.1)		
Splenic cyst	1 (1.1)		
Uterine myoma	1 (1.1)		
Ovarian cyst	1 (1.1)		
Heterogeneous prostatic parenchyma	1 (1.1)		
Calcifications in the prostatic parenchyma	1 (1.1)		
Chilaiditi syndrome	1 (1.1)		
Situs inversus totalis	1 (1.1)		

SD: Standard deviation. An increase in echogenicity of the liver parenchyma due to steatosis was the most common abdominal ultrasonography finding in patients with psoriasis.

Data on abdominal ultrasonography findings in patients with psoriasis treated with biological agents are scarce. Raudonis et al. reported that hepatic and pancreatic steatosis, hepatomegaly, kidney cyst, splenomegaly, gallbladder polyp, and pancreatic stiffness were commonly detected findings in abdominal ultrasonography which were annually performed in patients with psoriasis treated with infliximab, etanercept, and ustekinumab. Among them, hepatic steatosis (12.7%) and pancreatic steatosis (12.7%) were the most common [13].

Nonalcoholic fatty liver disease is a metabolic disorder that may present with steatosis, and nonalcoholic steatohepatitis and may result in cirrhosis or hepatocellular carcinoma [14-16]. Advanced age and diabetes are the risk factors for the development of nonalcoholic fatty liver disease, whereas the effect of gender on disease remains controversial [17]. In addition, nonalcoholic fatty liver disease is more common in psoriasis patients with a prevalence of up to 65% compared to the general population [15]. Furthermore, poor prognosis has been reported in patients with nonalcoholic fatty liver disease accompanied by psoriasis [14,15]. Dermatologists are recommended to perform abdominal ultrasonography for screening fatty liver in patients with psoriasis, particularly in those who have metabolic syndrome even without elevated liver enzyme levels [6]. Dermatologists must determine accompanying nonalcoholic fatty liver disease in patients with psoriasis to choose appropriate treatment options. Dermatologists should also encourage patients for lifestyle changes or refer them to a gastroenterologist if needed [16]. For instance, Campanati et al. suggested that weight gain during anti-TNF-α treatment might increase the risk of hepatic fibrosis in psoriasis patients with nonalcoholic fatty liver disease [18]. Medications that may contribute to steatosis should be omitted in these patients. However, no specific treatment has been reported or primarily recommended for the treatment of psoriasis patients with nonalcoholic fatty liver disease [16]. On the other hand, TNF-α inhibitors such as adalimumab, etanercept, and infliximab may lead to hepatotoxicity. Among them, infliximab was the most commonly implicated one in the development of adverse effects on the liver such as jaundice, hepatitis, acute liver failure, cholestasis, and autoimmune hepatitis [19]. Even mild and rare, hepatotoxicity has been detected in patients who received ustekinumab [20].

Abdominal ultrasonography may be useful in patients with psoriasis since psoriasis has been associated with nonalcoholic fatty liver disease or intraabdominal malignancies such as hepatocellular carcinoma, gastric cancer, bladder cancer, lymphoma of the stomach and colon adenocarcinoma [21,22]. Intraabdominal malignancy and infections have also been reported in patients who received biological agents for the treatment of psoriasis. For instance, Nosotti et al. reported a 59-year-old male patient with psoriasis treated with adalimumab and diagnosed with B cell lymphoma with gastric, splenic, hepatic, abdominal lymph node, and vertebral involvement. Nosotti et al. suggested that the risk for the development of abdominal lymphoma should be kept in mind in patients with psoriasis under biological agent treatment. Nosotti et al. recommended performing abdominal ultrasonography before and during biological agent treatment for psoriasis [9]. Fortaleza et al. reported a 43-year-old male patient with psoriasis who developed splenic tuberculosis under infliximab treatment. Fortaleza et al. stated that close follow-up of patients treated with TNF-α inhibitors on the risk for extrapulmonary tuberculosis and disseminated tuberculosis was crucial [23]. Bud et al. reported peritoneal tuberculosis in a 32-year-old patient who received adalimumab for the treatment of psoriasis vulgaris. The patient had multiple splenic lesions on computed tomography of the abdomen and underwent surgery due to an initial diagnosis of a possible malignancy. Bud et al. suggested that Quantiferon test, abdominal ultrasonography, and computed tomography might be performed to exclude intestinal tuberculosis in patients with gastrointestinal symptoms treated with TNF-α inhibitors [24].

CONCLUSIONS

We suggest that patients with psoriasis should undergo abdominal ultrasonography before biological agent treatment not only to exclude intraabdominal malignancy and infections but also to determine hepatic steatosis. It should be kept in mind that nonalcoholic fatty liver disease has an asymptomatic course and poor prognosis in patients with psoriasis. Since lifestyle modification and weight loss can reverse early stages of nonalcoholic fatty liver disease such as steatosis [25], early detection of hepatic steatosis is crucial in the treatment of patients with psoriasis. Therefore, we suggest that abdominal ultrasonography should be performed in patients with psoriasis before biological agent treatment, especially in females, patients with advanced age, patients who were previously treated with biological agents, and patients with comorbidities such as diabetes. In addition, considering the high frequency of nonalcoholic fatty liver disease in patients with psoriasis, the most appropriate biological agent treatment should be determined in this population and stated in psoriasis treatment guidelines.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, F.T., O.A., M.P., E.A.; methodology, F.T.; software, F.T.; validation, F.T., O.A., M.P., E.A.; formal analysis, F.T.; investigation, F.T., O.A.; resources, F.T., O.A., M.P., E.A.; data curation, F.T., O.A., M.P., E.A.; writing-original draft preparation, F.T.; writing-review and editing, M.P., E.A.; visualization, F.T.; supervision, M.P., E.A.; project administration, F.T., O.A., M.P., E.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2022-264).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Essential Oils and their Components in Combating Fungal Pathogens of Human Skin-lipophilic Yeast Malassezia furfur

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Abstract: *The most common problem of modern medicine is the development of increasing antifungal drug resistance nowadays including fungal infections of the skin and its appendages caused by dermatomycetes, yeast-like fungi, and molds so, there will be a need for alternative antifungal treatment. Natural substances with high antifungal properties like essential oils and their components show potential in this regard. Citrus lemon and Citrus sinensis essential oils were screened against Malassezia furfur by disc diffusion method and Minimum Inhibitory Concentration was determined by Modified Microdilution method. Citrus lemon (lemon) and Citrus sinensis (orange) show excellent inhibitory effects on the lipophilic, yeast-like fungus Malassezia furfur which causes Pityriasis versicolor, a chronic superficial fungal disease of the skin. The diameter of the inhibition zone was found to be 70 mm and 60 mm respectively. Malassezia furfur was found to be resistant against Clotrimazole & Ketoconazole. The minimum inhibitory concentration (MIC) of lemon and orange oil against Malassezia furfur was found to be 0.6 µl/ml and 0.8 µl/ml. These findings concluded that the use of Citrus lemon (lemon) and Citrus sinensis (orange) oil showed remarkable and excellent antifungal activity, and can be used as a traditional herbal medicine for the control of Pityriasis versicolor infection of the skin after undergoing clinical trials.*

Keywords: *Pityriasis versicolor, skin, Malassezia furfur, Citrus lemon and Citrus sinensis oils*

INTRODUCTION

Pityriasis versicolor (PV) is a superficial mycosis, that affects the superficial layer of stratum corneum [1]. The causative organism is Malassezia furfur, a yeast-like lipophilic fungus. Previously the mycelial form was called either Pityrosporum ovale or Pityrosporum orbiculare [2]. In 1951, Gordon isolated the organism M.furfur and renamed it P.orbiculare. It was recognized that M.furfur is the correct name and that P.orbiculare and P.ovale are synonyms [3]. The disease is most prevalent in early adulthood and small children are rarely affected [4-5]. PV is common in the post-pubertal age where sebaceous glands are active and in individuals who sweat more [6]. The prevalence in colder climates is less than 1% [7]. M. furfur is a component of normal skin flora in more than 90% of adults living in tropical areas [8]. Infectious diseases account for a high proportion of health problems in developing countries including India. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created [9]. The resistance of the organisms increased due to the indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases. This situation forced the researchers to search for new antimicrobial substances from various sources including medicinal plants [10-11]. A survey of the literature reveals that many essential oils possess antifungal activity. Therefore, we need to search for plant-derived antifungal drugs which are safe and without side effects. Herbal medicines may be in the form of powders, liquids, or mixtures, which may be raw or boiled, ointments, liniments, and incisions [12]. Essential oils have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [13]. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties

[14-15]. Numerous essential oils have been tested for both in vitro as well as in vivo antifungal activity and some pose much potential as antifungal agents. Studies on the antimicrobial, especially antibacterial and antifungal activity of lemon grass oil and its components were reported [16-18]. Orange oil was composed principally of d-limonene, at 94%, and myrcene at about 3%. The essential oils of lemon and orange exhibited antifungal activity against common molds Aspergillus niger, A.flavus, Penicillium chrysogenum, and P.verrucosum. In the present study, the antifungal activities of lemon and orange oils were investigated to discover the medicinal potential of these oils for future application as an antifungal agent for Pityriasis versicolor disease.

MATERIALS AND METHODS

Extraction and Preparation of Oil

In the summer season, extraction of oil from the peels of lemon (Citrus lemon) and orange (Citrus sinensis) was carried out by standard hydrodistillation method, Clevenger's apparatus, and all operations were carried out at room temperature. The fresh peels of lemon and orange were washed to remove soil and sliced. Sliced pieces of fresh peels of lemon and orange (250 g) were placed in a separate flask, together with distilled water (1 L). After 5-6 hours, oil was collected from the apparatus, anhydrous with sodium sulfate for removal of water traces, then this 100% pure essential oil was dispensed into dark bottles and stored at 4°C until used. Essential oil was ready to use for disc diffusion and determination of MIC.

Screening of essential oil using Disc Diffusion method

Oil was screened for its antifungal activity against M.furfur by disc diffusion method [19]. Standard size Whatman No.1 filter paper discs, 6.0 mm in diameter, sterilized by dry heat at 140°C in an oven for one hour were used to determine antifungal activity. SDA medium for the disc diffusion test was prepared. After sterilization, it was poured into sterilized Petri plates and allowed to solidify. Then one day old, fresh culture of yeast will be used for inoculum preparation. A suspension that was just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending yeast in 0.9% NaCl solution and the homogeneous suspension was used for inoculation. Using a sterile cotton swab, yeast cultures were swabbed on the surface of sterile Sabourauds dextrose agar plates. Sterilized filter paper discs were soaked in neat, undiluted (100%) concentration of oils. Using ethanol-dipped and flamed forceps, oil-saturated discs of 100% concentration were aseptically placed over Sabourauds dextrose agar plates seeded with the respective test microorganism. The antibiotic discs of Clotrimazole and Ketoconazole (10 mcg/disc) were also aseptically placed over the seeded Sabouraud's dextrose agar plates as a standard drug for comparison of antifungal activity of lemon and orange oils. The plates were incubated at 37ºC for 24 hours. The diameter of the inhibition zones was measured in mm. Three replicates were kept in each case and average values were calculated. The activity of oils was measured by the following formula:

AI (Activity index) = Inhibition zone of sample/Inhibition zone of standard

Determination of Minimum inhibitory concentration using Microdilution method

The modified microdilution method by Provine and Hadley (2000) was followed to determine MIC [20]. Media used for MIC was semisolid agar media (Brain Heart Infusion Agar) aliquots of semisolid agar media (Bacto Agar; Difco Laboratories) at a pH of approximately 7.4 were poured into a 16-by 125-mm glass tube and autoclaved. A suspension that was just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending the selected fungi in 0.9% NaCl solution, vortexing, and homogeneous suspension was used for inoculation. Different concentrations of lemon and orange oils were added in media containing test tubes, afterwards a standard platinum loopful (~0.001 ml, Himedia, Flexiloop) of the inoculum suspension was inserted deep into each tube of medium containing a different concentration of oils, as well as an oil-free control, by a centered down-up motion to form a two-dimensional inoculum. The tubes were then incubated at 30OC for 48 hours to determine the MIC. MIC was read to be the lowest concentration at which there was no visible growth of the organism. Then, by visual inspection, good growth of the respective fungi in an oil-free medium as a control was detected (48 hrs for yeasts). Afterward, the growth in all tubes at different concentrations of lemon and orange oil was compared with that of the oil-free control to determine inhibition.

RESULTS

In our present work, M.furfur was found main etiological agent of Pityriasis versicolor disease hence antimycotic studies were carried out on M.furfur (Figure 1). The conventional treatment of fungal disease is limited, and part of the reason is due to the limited spectrum of the current antifungal drugs, and the expensive treatment, particularly due to the need for prolonged therapy. Thus, new drugs and alternative therapies are necessary, including natural products. We report here the antimycotic study of lemon and orange oil against M.furfur in vitro (Figure 2). The results of the present work on the antifungal activity of lemon and orange oil against M. furfur studied by two methods i.e Disc diffusion and Microdilution method are presented in Tables 1, 2, and 3.

Table 1: Antifungal activity of lemon and orange oil against Malassezia furfur

Essential Oil	Test Strain	IZ of Essential Oil
C. limon	*Molassezia furfur*	70 mm
C. sinensis	*Molassezia furfur*	60 mm

Table 2: MIC of C. limon oil against Malassezia furfur

Test Strain	Different concentrations of lemon Oil used in µl/ml	Growth visually inspected in different concentrations of oil
Malassezia furfur	0.1	+4
	0.2	+3
	0.3	+3
	0.4	+2
	0.5	+1
	0.6	0
	0.7	0
	0.8	0
	0.9	0
	1	0
	Control without oil	100% growth

Growth was scored in the following manner: *4+, growth comparable to that of the oil free control; 3+, growth approximately 75% that of the control; 2+, growth approximately 50% that of the control; 1+, growth 25% or less that of the control; and 0, no visible growth*

Table 3: MIC of C. sinensis oil against Malassezia furfur

Test Strain	Different concentrations of lemon Oil used in µl/ml	Growth visually inspected in different concentrations of oil
Malassezia furfur	0.1	+4
	0.2	+3
	0.3	+2
	0.4	+2
	0.5	+1
	0.6	+1
	0.7	+1
	0.8	0
	0.9	0
	1	0
	Control without oil	100% growth

Growth was scored in the following manner: *4+, growth comparable to that of the oil free control; 3+, growth approximately 75% that of the control; 2+, growth approximately 50% that of the control; 1+, growth 25% or less that of the control; and 0, no visible growth*

In our study, lemon and orange oils presented higher diameters of inhibition zones. The diameter of the inhibition zone obtained against lemon and orange oil at 100% concentration of pure oil was 70 mm and 60 mm respectively by disc diffusion method (Figure 3). According to our study by comparing with the reference drugs, lemon and orange oil was found to be more effective in inhibiting the growth of M. furfur. Clotrimazole and ketoconazole were found to be resistant to Malassezia furfur (Figure 4). Results of MIC of lemon and orange oils against M. furfur. are summarized in Tables 2 and 3. The results show that the lemon and orange oil exhibited inhibitory action at 0.6 µl/ml and 0.8 µl/ml concentrations against M. furfur. Both oils can be used for the development of natural antifungal agents against Pityriasis versicolor infections.

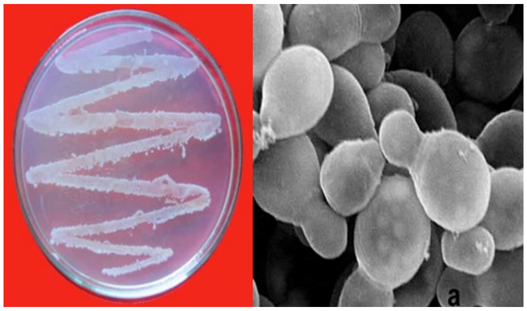

Figure 1: M. furfur on SDA media with olive oil & Bottle shape yeast cells under microscopic examination

Figure 2: Fruit of Citrus lemon (lemon) & Citrus sinensis (sweet orange)

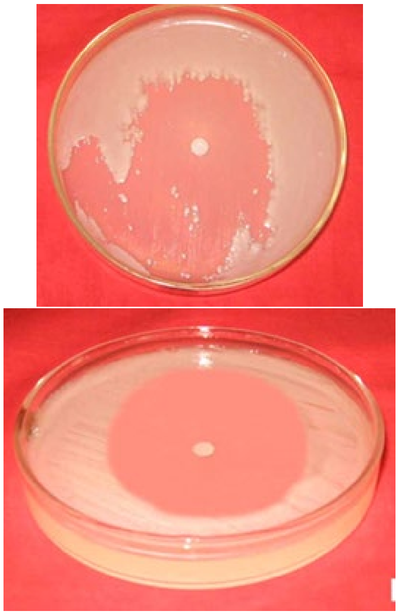

Figure 3: Antifungal activity of C. lemon & C. sinesis against Malassezia furfur

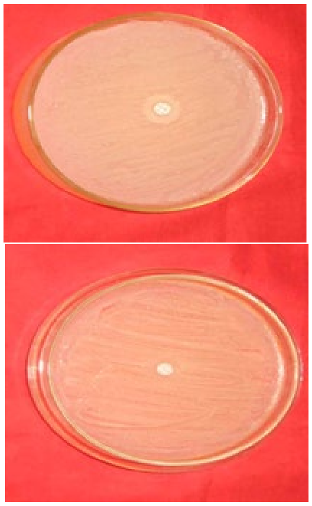

Figure 4: Resistance of Clotrimazole & Ketoconazole against Malassezia furfur

Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage and cell death; blocking the membrane synthesis; and inhibition the spore germination, fungal proliferation, and cellular respiration [22]. Because of the high volatility and lipophilicity of the essential oils, they are readily attached to penetrate the cell membrane to exert their biological effect [23]. The results showed the efficacy of lemon oil on animal skin infections caused by Trichophyton rubrum and Microsporum gypseum. In our study, lemon oil exhibited strong antimycotic activity against M. furfur. In the screening of lemon oil, the diameter of the inhibition zone by the disc-diffusion method was found to be 50 mm. MIC of oil obtained by microdilution method was 0.8µl/ml. Our work is in agreement with the observations of Silvia et al in which screening of lemon grass oil against Candida species was studied and it was found as an effective antifungal agent [24]. Our work also coincides with the previous findings of Ezzat et al which revealed that the essential oil of Citrus lemon at 200 µl concentration showed a diameter of inhibition zone 37 mm against Candida albicans 10261 [25]. According to Rusenova and Parvano Citrus lemon does not show antimicrobial activity by disc-diffusion method against both yeasts, Candida albicans and Malassezia pachydermatis [26]. These results are not in agreement with our studies which showed that antifungal activity by disc-diffusion and minimum inhibitory concentration by microdilution method, lemon oil was found to be effective in inhibiting the growth of M. furfur at a low concentration of oil. Lemon oil is also preferentially more active against Gram-positive bacteria as compared to Gram-negative bacteria. The antimicrobial activity of lemon oil is due to the presence of terpenoids-a-pinene, camphene, b-pinene, sabinene, myrcene, terpinene, b-bisabolene, limonene, trans-a-bergamotene, nerol and neral [27]. Essential oils from citrus offer the potential for all-natural antimicrobials for use in improving the safety of organic or all-natural foods [28]. Shahi et al in 2003 found that the MIC of oil of Citrus sinensis were in the range of 0.7-1 µl/ml against Candida albicans, Aspergillus flavus, A. fumigatus, A. niger, A. ustus, Epidermophyton floccosum, Microporum audouinii, M. canis, M. gypseum, M. nanum, Rhizopus nigricans, Trichophyton tonsurans, T. rubrum, T. mentagrophytes and T. violaceum [29]. The present investigation suggests that essential oils exhibit a narrow or wide range of antifungal activity, which may prove useful in the development of effective antifungal substances. Souza et al (2005) reported MIC of lemon oil at 0.5% concentration against mold strains i.e. Fusarium spp, Penicillium spp, Aspergillus niger, A. flavus, and Rhizopus spp [30]. In our findings, MIC of lemon oil against M. furfur was 0.8 µl/ml i.e. very close to that concentration. The small differences may be due to local environmental and climatic conditions and variations in the choice of microorganism tested.

Our results of orange (Citrus sinensis) and lemon (Citrus lemon) are also similar to the work of Viuda-Martos et al which suggests that both oils were effective in inhibiting the growth of Aspergillus niger, A.flavus, Penicillium chrysogenum and P. verrucosum [18]. The hydrodistilled oil of lemon and orange was also found to be more sensitive against Penicillium digitatum [31]. Antimicrobial activity of Rutaceae family species has been observed on dermatophytes [32] and on some molds [33]. Essential oils of some plants have recently been proven to be successful eco-friendly, bio-control agents [34]. Many authors have reported antimicrobial, antifungal, antioxidant, and radical-scavenging properties of essential oils [35-36].

DISCUSSION

The present results suggest that lemon and orange oils exhibit strong antifungal activity. This is in agreement with the findings of Valero and Frances who reported narrow and wide ranges of antifungal activites. Essential oils derived from many plants are known to possess antifungal activities [21].

CONCLUSIONS

The present study suggests that the oils extracted from the waste product (peels) of C. sinensis and C. lemon hold good promise as an antifungal agent, which could be used in therapeutic remedy against human pathogenic fungi on account of its various antifungal properties, viz. strong fungicidal action, long-shelf life, withstand heavy inoculum density, thermostable, broad range of antifungal activity and absence of any adverse effects. The waste product of C.

sinensis and C. lemon can be used for the development of a potential source of effective and economically viable herbal antifungal against Pityriasis versicolor (fungal skin infection) after undergoing successful clinical trials.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

RS contributed to design of experiments, review, discussion, and editing of the manuscript. GS contributed to the review and writing of the manuscript. RS contributed to all editing of the manuscript. ER contributed to the review of the manuscript. MS contributed to the editing and review of the manuscript.

Ethics approval and consent to participate

This article does not contain any studies with human and animal participants performed by any of the authors,

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ORIGINAL ARTICLE

Correlations between Etiopathogenic Factors and Persistence on Anti-IL-17 Therapies in Patients with Psoriasis Vulgaris

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Abstract: *With recent advances in the understanding of psoriatic disease, it is increasingly considered a systemic inflammatory condition rather than limited to the skin and joints. A variety of biologics are available today for the treatment of psoriasis, but with them, characteristics such as rapidity of onset, long-term efficacy, safety profile, and effects on comorbidities differ. We designed an observational, non-interventional, retrospective study of patients with severe psoriasis receiving biologic treatment with IL-17 inhibitors and aimed to investigate the correlations between etiopathogenic factors and the efficacy and persistence of these therapies in a group of psoriasis patients from Romania. Study results show that patients treated with ixekizumab had better persistence and high adherence compared to those treated with secukinumab. In this study, ixekizumab demonstrated lower risks of non-persistence, discontinuation, and switching and a higher likelihood of high adherence compared with secukinumab. This study contributes to the understanding of the persistence of anti-IL17 biologic therapies in psoriasis and the factors that may influence it.*

Keywords: *psoriasis vulgaris, comorbidities, anti-IL-17 biological therapies, persistence*

INTRODUCTION

Plaque psoriasis is a common chronic immunological condition with a worldwide prevalence ranging from 1.0 to 8.5% [1,2]. With recent advances in the understanding of psoriatic disease, it is increasingly considered a systemic inflammatory condition rather than limited to the skin and joints. These extracutaneous disorders that have been linked to psoriasis include psoriatic arthritis, metabolic syndrome, obesity, cardiovascular disease, various malignancies, autoimmune diseases, chronic kidney disease, psychiatric disorders, smoking or alcohol abuse. Despite evidence supporting an increased prevalence of these risk factors among patients with psoriasis, data suggest that patients are under-screened and under-treated [3-7].

With the advent of biological therapies, the treatment of psoriasis has changed dramatically due to its high efficacy and safety. A variety of biologic agents are available for the treatment of psoriasis nowadays, however, characteristics such as rapidity of onset, long-term efficacy, safety profile, and effects on comorbidities differ. A better understanding of these characteristics leads to the correct choice of treatment for patients, leading to greater persistence, greater patient satisfaction, and minimizing the impact of psoriatic disease. Meta-analyses of randomized clinical trials in psoriasis have suggested that newer biologic therapies targeting interleukin (IL)-17 and IL-23 generally have greater efficacy than other biologic therapies [8]. However, the response to therapy and the safety of biological treatment for psoriasis in clinical trials do not fully reflect the usual clinical setting [9,10]. Therefore, in this study, we aimed to investigate both the efficacy and persistence of ixekizumab and secukinumab therapies, as well as the correlations between etiopathogenic factors and, respectively, psoriasis-associated diseases, with these biological therapies in a group of psoriasis patients from Romania.

MATERIALS AND METHODS

We designed an observational, non-interventional, retrospective study of patients with moderate to severe psoriasis receiving biologic treatment with IL-17 inhibitors (secukinumab and ixekizumab). The patients signed the informed consent to participate in this study, and it received the approval of the Ethics Committee no. 3122/04.02.2022. For this study, we used data from patients from the IInd Department of Dermatology of the Colentina Clinic Hospital in Bucharest, Romania, and data from patients belonging to dermatologists from private clinics in Bucharest. Initially, the analyzed group included 311 patients with moderate-severe psoriasis, from the National Registry of Dermato-Venereal Diseases, who at the time of the study are being treated or have had ixekizumab (IXE) and secukinumab (SEC) therapies in the past. Data were collected using questionnaires completed by treated patients and dermatologists at regular office visits at 3-month intervals for initiations and 6 months for periodic evaluations, respectively.

In the first phase of the study, we divided the group of patients into two groups determined by the two therapies, IXE and SEC, and analyzed the patients from the perspective of the main characteristics of sex, age, duration of the disease, time of onset of the therapy, body mass index, and associated comorbidities. In this analysis, we calculated the main descriptive indicators and then used the z, Student, and χ2 tests to evaluate the differences between groups. To ensure the comparability of the two groups defined above, from the initial sample of patients, in accordance with the literature, we extracted a subgroup of 255 patients who had or are still on the two biological therapies. For this subsample, we presented the main characteristics of the two lots. In the second phase of the study, we continued with the evaluation of the persistence of the patient's treatment, using the Kaplan-Meier curves. The impact of comorbidities on persistence in therapy is also analyzed. The Log-rank test was used to statistically assess potential differences between groups [11]. The difference is considered statistically significant if the p-risk associated with a test is below the 0.05 threshold.

RESULTS

Analyzing the two groups of patients, it is observed that in the case of IXE therapy, the gender ratio is 1.9 in favor of men, while in the case of SEC, it is 1.04 in favor of women. Consequently, it can be stated that between the two groups, there are statistically significant differences (p=0.028) from the perspective of the gender structure.

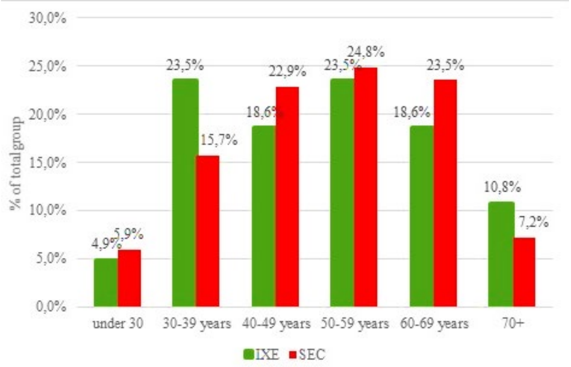

Figure 1: Distribution of patients by age group for each therapy

The distribution of patients by age groups does not determine significant differences between the groups as a whole, in the sense of the χ2 test (p=0.46), even if in the case of IXE therapy, the share of patients under 40 is slightly higher than in the case of SEC. Calculating the average age, we obtain 50.4 years in the case of IXE and 51.2 in the case of SEC, so the difference between the groups is statistically insignificant in the sense of the t-Student test (p=0.77).

The most common risk identified in patients with psoriasis is that related to smoking. The results (Figure 2) show that 42.2% of patients with IXE therapy and 34.0% of patients with SEC are smokers, proportions that do not indicate significant differences between the two groups (p=0.23). The second most frequent comorbidity among patients with psoriasis treated with IXE or SEC is the presence of cardiovascular disease (CVD). About a quarter of the patients in the study, 25% of the SEC group and 20.6% of the IXE group, had an associated CVD.

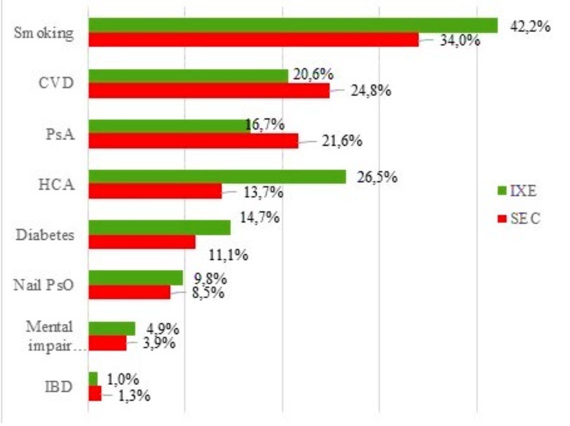

Figure 2: The presence of risks and comorbidities in comparison for IXE and SEC

Heredo-collateral antecedents (HCA) are identified to a lesser extent in patients under SEC therapy. On this branch, only 13.7% of patients had a family history of the disease. Among patients with IXE, the number is almost double, and the difference between groups becomes statistically significant (p=0.032). Without identifying a statistically significant difference (p=0.40) between groups, psoriatic arthritis (PsA) is the third most common comorbidity. This is present in 21.6% of patients in the SEC branch and 16.7% of patients with IXE. Other comorbidities, such as inflammatory bowel disease (IBD) or mental impairment, occur less frequently in patients with psoriasis. Obesity is also an important risk factor for psoriasis. To highlight the differences between the groups in this perspective, a structure is presented in Figure 3.

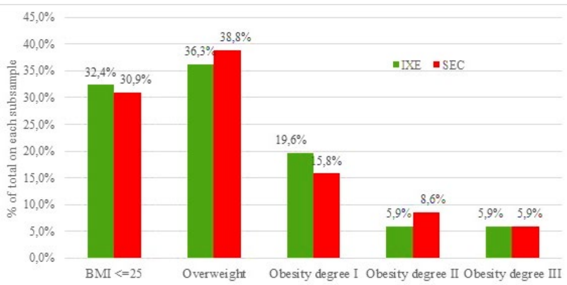

Figure 3: Distribution of patients according to BMI and biological therapy

The largest proportion of patients undergoing psoriasis treatment have an above-normal BMI. The data presented synthetically in Figure 3 shows that only one-third of patients have a BMI within normal limits. Applying the χ2 test, we did not identify a statistically significant difference between the two groups in terms of the distribution of body mass index classes.

Table 1: The main treatment-related characteristics of the two groups, IXE vs. SEC

Treatment	IXE, n (%) n1=102 (100%)	SEC, n (%) n2=153 (100%)	Testing the difference between groups
Switch	13,7%	27,5%	p=0,032[*1]
Methotrexate	96,9%	97,1%	p=0,888
Neotigason	7,4%	5,1%	p=0,577
UVB	4,9%	5,9%	p=0,724
PUVA	35,6%	24,2%	p=0,095

No significant differences were found between the two groups in the analysis of previous treatments. As can be seen in Table 1, 97% of patients followed methotrexate therapy, 5% with UVB. Further, approx. 6% of patients in the study had prior treatment with neotigason. It can also be pointed out that a third of the patients now under IXE treatment had undergone PUVA treatment and a quarter of the SEC patients had previously had this treatment. From the perspective of PUVA, a statistically significant difference between groups can only be considered if an error of p=0.095 is accepted.

It should be noted that during the analyzed period, January 31, 2018 - December 18, 2021, 13.7% of patients with IXE and 27.5% of patients with SEC, respectively, changed therapy due to an adverse reaction or due to treatment inefficiency. In particular, there are 6 patients who during the analyzed period switched from SEC to IXE and 4 patients from IXE to SEC. Following the two curves, it can be seen that 2 years (24 months) after the initiation of biological therapy, 88% of patients with IXE are still on treatment, while only 75% of patients with SEC still benefit from this treatment (Figure 4). Following patients on both IXE and SEC trajectories, at four years, 80% of IXE patients and 67% of SEC patients were still adherent to treatment.

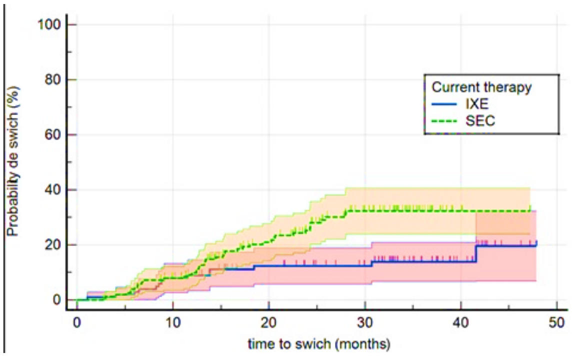

Figure 4: Probability of switching patients on the two therapies

Overall, the median persistence for IXE is 43 months, and for SEC is 37 months. Thus, a statistically significant advantage in favor of IXE is observed. In total, the average duration of treatment is approximately 40 months.

Next, we will analyze the possible impact of determinants such as sex, age, and comorbidities on treatment persistence in the two biological therapies IXE and SEC, respectively. The age group is a significant factor when analyzing treatment persistence in patients on biologic therapy with IXE. Thus, people over 50 years tend to have a lower persistence, respectively a higher switching probability, compared to the younger group (p=0.038), in the case of IXE therapy. It should be noted that the differences by age groups, for IXE therapy, as can be seen in Figure 5, are achieved quite quickly over time, generally after the first 12 months.

In the case of SEC therapy, a significant difference (p=0.046) is observed between the two age groups analyzed, but the difference between the two arms appears later, after 24 months of treatment.

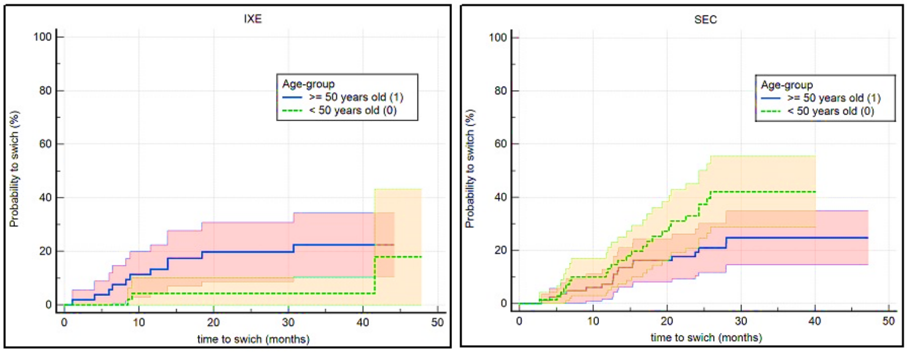

Figure 5: Probability of switching current biological therapy according to age group

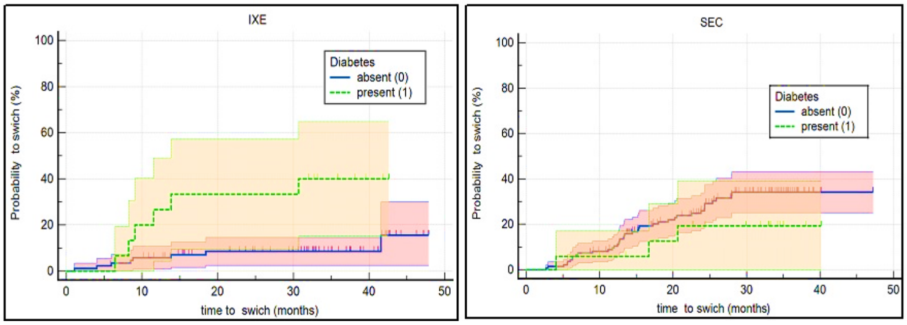

Figure 6: Probability of changing current biological therapy depending on the presence of diabetes

Patients with diabetes mellitus (p<0.01) on IXE therapy tend to have a longer duration of treatment. At 36 months after initiation of therapy, 40% of patients with diabetes switch to IXE therapy. At the same time, only 10% of non-diabetic patients switch from IXE. In the case of SEC therapy, no statistically significant difference was observed between the two subgroups (p=0.30) (Figure 6).

The average duration of persistence for people with a BMI below 29 is 45.9 months, and patients with a BMI above 29 have an average duration of treatment of 41.7 months. Accepting a p=0.023 error, it can be stated that there is a statistically significant difference between the subgroups defined by BMI, on IXE therapy. Regarding SEC therapy, we found no significant difference between subgroups (Figure 7).

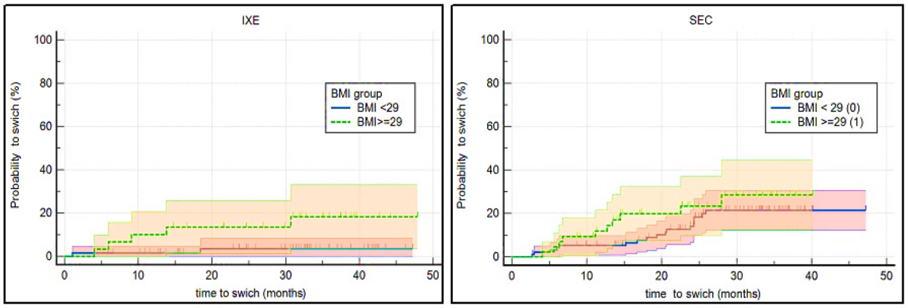

Figure 7: Probability of changing current biologic therapy by BMI class

It was observed that patients with PsA do not have a higher switch probability from IXE therapy than patients without PsA (p=0.76). This result is also similar to SEC therapy (Figure 8).

The presence of nail involvement in psoriasis does not statistically significantly influence the persistence of treatment on any of the therapies studied. In both cases, the first-generation risk associated with the LR Test is greater than 0.5 (Figure 9).

In the case of patients under IXE therapy, there is a significant difference (p<0.01) between patient subgroups determined by the age of the condition. Thus, patients with the condition over 13 years have a higher probability of change. For this category, four years after starting IXE therapy, 40% of patients change or stop biological therapy. For patients who discovered the disease more recently, the probability of passing to 48 months is below 10% (Figure 10).

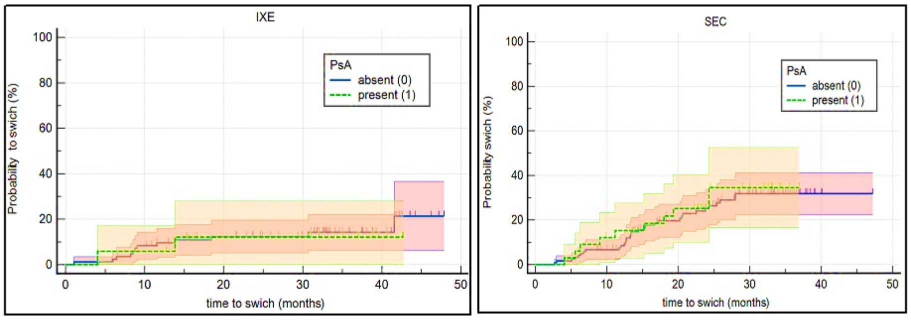

Figure 8: Probability of changing current biological therapy based on the presence of PsA

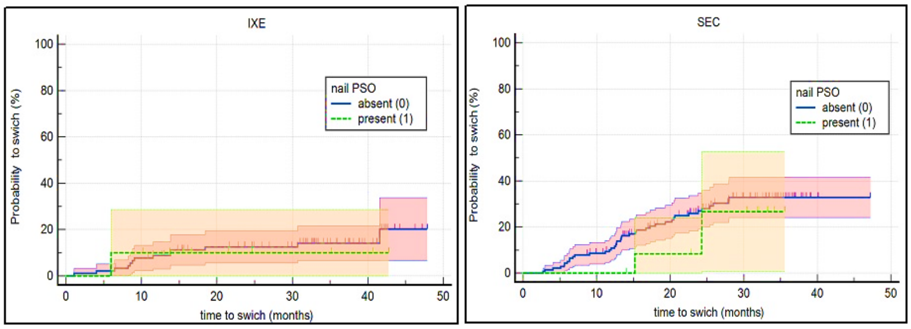

Figure 9: Persistence on biological therapies concerning the presence of nail psoriasis

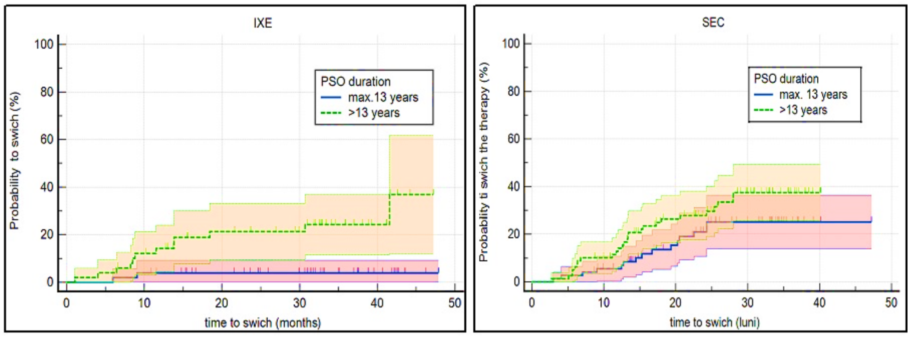

Figure 10: The psoriasis duration and the probability of switching

For the subgroups with SEC therapy, we did not identify a statistically significant difference, so at 48 months, 35-40% of patients on SEC therapy will switch this treatment regardless of the age of the underlying condition.

Other factors such as HCA, CVD, IBD, or previous systemic conventional therapy (PUVA or UVB) were not significant for any of the therapies studied.

DISCUSSION

Evidence from "real life" is of undeniable importance because it assesses efficacy and safety in the everyday context, which may be different (multiple comorbidities, hard-to-treat areas, etc.) than that represented by clinical trials [12]. Unfortunately, few studies are comparing IXE and SEC for patients with psoriasis. Based on the results of this study, it can be seen that patients treated with IXE had better persistence and high adherence compared to those treated with SEC. Several studies have shown a lower efficacy of SEC in real life compared to clinical trials, and the present study reveals similar results consistent with these [13–17]. These observations are also supported by the fact that 2 years after initiation of biologic therapy, a higher percentage (88%) of patients with IXE are still on treatment compared to a lower percentage (75%) of patients with SEC who continue this treatment. Moreover, we can observe that after 4 years of therapy, the results do not differ much. Thus, 80% of patients treated with IXE are still on therapy 48 months after onset, compared to only 67% of patients treated with SEC, showing a statistically significant advantage in favor of IXE.

Previous studies in the literature have already identified female gender as an independent risk factor for discontinuation of biological treatment in psoriasis [18-27]. In contrast, our study concluded that there was no statistically significant difference between the sexes in persistence on therapies for any of the therapies studied.

Regarding age group, there is very little data in the literature to reveal a correlation between it and the persistence on biologic therapies with IXE or SEC. The present study showed that patients older than 50 years treated with IXE tend to have lower persistence compared to the younger group. It is worth noting that differences by age groups are realized quite quickly in the case of IXE, after the first 12 months, in contrast to SEC, after 24 months.

Significant associations have been observed between psoriasis and obesity or being overweight. The relationship is hypothesized to be bidirectional, with obesity predisposing patients to psoriasis and psoriasis increasing the risk of obesity. About 70% of patients treated with both therapies have a BMI above normal. Taking into account the fact that in the Romanian population, the overweight rate was 52.5% [28], the present study indicates that the distribution of patients with psoriasis is inclined toward people with BMI above normal (Figure 3).

On the other hand, obesity may influence the therapeutic approach and clinical response to systemic treatment in psoriasis or psoriatic arthritis. Indeed, adipose tissue can alter the volume of distribution of the drug and thus limit its efficacy [29]. Because biologic therapies are usually given in a fixed dose, a weight-adjusted dose is likely necessary for overweight/obese patients. It was reported that in 33 patients with moderate to severe psoriasis on biologic therapy, only 7 patients who lost weight achieved the greatest improvement in mean PASI score at the end of the study [30]. Evidence was also provided that in obese patients with psoriasis on biological therapies following an 8-week nutritional program with a low-calorie diet (≤1000 kcal per day), body weight reduction was associated with increased therapy efficacy [31].

Supporting the correlation between obesity and treatment impairment in patients with psoriasis [32], the present study showed that individuals with a BMI above 29 have a higher risk of discontinuation/switching concerning IXE therapy. Regarding SEC therapy, no significant difference was observed between subgroups.

The study showed that psoriatic arthritis did not significantly influence the likelihood of switching treatment for either therapy, in contrast to an Austrian retrospective observational study, where they observed a slight increase in the number of patients with psoriatic arthritis who discontinued biologic therapy [33], or, on the contrary, another study in which we can observe the fact that the simultaneous existence of psoriatic arthritis with skin damage was correlated with the persistence of biological therapy [34].

After analyzing the existence of the diabetes factor concerning the persistence of therapies, patients with diabetes under IXE therapy tend to have a longer duration of treatment, more than 36 months after the start of therapy, a fact that contradicts a recent American study [35]. In the case of SEC, we found no statistically significant difference between patient subgroups. Biologic agents are also known to counteract insulin resistance and demonstrate an even greater protective effect against the development of diabetes in patients with psoriasis [36].

Finally, the results of the analysis of etiopathogenic factors, such as HCA, CVD, IBD, or the presence of conventional systemic therapy in the past (PUVA or UVB), were not found to be significant for any of the studied therapies.

This study has some limitations, such as the relatively small number of patients and the short follow-up period. Indeed, the small number of patients may limit the interpretation of whether the differences in performance between the two therapies were significant and clinically relevant. Over time, as larger samples with longer follow-ups are created, they may allow such analysis.

CONCLUSIONS

In this study, we explored the persistence of anti-IL17 biologic therapies and the association between these therapies and various comorbidities in patients with severe psoriasis to determine the optimal systemic management of psoriasis through early screening and intervention for these comorbidities. Our results provide an unbiased and real-world analysis of the persistence of anti-IL-17A therapies in a cohort of patients with severe psoriasis. Thus, it is found that IXE presented a significantly higher persistence than SEC in patients with psoriasis in the studied groups, these data being consistent with those of other studies in the literature [37,38]. Differences in their IL-17 binding affinity could explain these findings, and stronger binding affinity is usually related to less antibody dissociation [39]. At the same time, it is not clear whether the frequency of administration should be increased following insufficient responses to standard dose SEC therapy. Although the initial loading dose may have a rapid response, over time the maintenance dose may be too low, leading to a possible relapse at a certain threshold [40,41].

Following the association of psoriasis with multiple comorbidities, the need for comprehensive screening and treatment must be recognized and addressed. Biologic therapies prescribed in psoriasis may exacerbate comorbidities, which in turn may influence psoriatic disease.

Therefore, the appropriate management of psoriasis must involve an integrated approach [42,43].

Conflicts of interest and sources of funding

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Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

Authors' contribution

For the research of this article, the authors contributed equally.

Ethics approval and consent to participate

For this study, we received the approval of the Ethics Committee no. 3122/04.02.2022.

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ORIGINAL ARTICLE

The Benefits of Phytotherapy in Chronic Pelvic Pain Syndrome

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Abstract: *Chronic pelvic pain syndrome (CPPS) presents a diagnostic challenge due to its multifactorial nature, often involving urological, gynecological, and psychological components. This study focuses on the effectiveness of prophylactic phytotherapy in preventing CPPS recurrence, aiming to mitigate symptoms and psychosocial impact. The study includes 74 patients following a six-month treatment regimen with Graminex, Seronoa Repens, Curcuma Longa, and Acediboswellic ke-to-acetyl-11. Symptom assessment employs the IPSS and NIH-CPSI questionnaires, conducted at 3, 6, 8, 9, and 12 months. Patients exhibit significant improvements in IPSS scores, with maintenance of treatment impact observed at 3, 6, and 9 months. Interruption of therapy leads to symptom recurrence, but upon resumption, responses are more effective. The third group, under observation for three months, reports positive outcomes. Both IPSS and NIH-CPSI scores show a consistent impact on urinary symptoms and pelvic pain, indicating the therapy's effectiveness. The study demonstrates a concordance in the positive effects of phytotherapy across different age groups, emphasizing its efficacy in alleviating urinary symptoms and pelvic pain in CPPS patients. Symptom remission or improvement in quality of life is reported, with a notable tropism for symptom reduction in the cold season. Patient adherence to treatment is high, and even after treatment interruption, resumed therapy yields significant improvements. The observed benefits include the absence of pelvic pain, dysuria, and enhanced sexual competence, contributing to an overall improvement in the quality of life for patients with CPPS.*

Keywords: *CPPS, phytotherapy, IPSS, NIH-CPSI, dysuria*

INTRODUCTION

Recent reports have highlighted more and more accusations from patients regarding chronic pelvic pain of apparently idiopathic causes, symptoms that are often accompanied by urinary tract dysfunctions, as well as sexual dysfunctions. [1]

Chronic pelvic pain can in many cases be the result of affecting several systems, devices, and organs, this fact determining over time a multidisciplinary collaboration between medical and surgical specialties such as urology, gynecology, gastroenterology, neurology, psychiatry, and general surgery.[2]

A subdivision of the concept of Chronic Pelvic Pain is known as Chronic Pelvic Pain Syndrome (formerly known as chronic prostatitis), the diagnosis of the syndrome is, according to the European Asociation of Urology (EAU), established only when the presence of an infection has been ruled out, or of an inflammation or a local pathology of any other nature.[3]

Thus, chronic pelvic pain syndrome becomes a diagnosis of exclusion. [4]

Along with the organic considerations related to the pathology, the psychological implications must also be taken into account, such as depression, anxiety, and social disturbances, at the workplace as well as at the couple level, all of which will negatively influence the patient's quality of life. [5]

Pain mechanisms include infection, central nervous system modulation, as well as emotional, cognitive, behavioral, and sexual mechanisms. [6]

The treatment of this condition consists (according to the European urology guidelines) of the administration of anti-inflammatory drugs, alpha-blockers, antibiotics, 5-alpha-reductase inhibitors, phytotherapy, pregabalin, pentosan polysulfate, muscle relaxants, botulinum toxin, leukotriene antagonists, allopurinol and analgesics. [7]

The main objective of this study is to observe the effectiveness of prophylactic phytotherapy in terms of preventing the recurrence of pelvic pain syndrome in patients known to have this pathology.

In contrast to the pharmacological therapy used in the classic treatment schemes of this pathology, phytotherapy associates significantly lower risks of side or adverse reactions, a fact that can ensure the patient's long-term treatment adherence superior to the classical treatment schemes, with the prevention of both of the recurrence of symptoms, as well as of the psychosocial effects associated with this condition.

The therapeutic scheme proposed for the patients included in this study consists of the synchronous administration of:
- Graminex – contains Cernitlon T60 and Cernitin GBX, pollen-based compounds with a proven anti-inflammatory effect.
- SeronoaRepens – is an extract of dwarf palm, with anti-androgenic and anti-inflammatory effects.
- CurcumaLonga – is represented by the extract from the perennial plant of the same name, of Asian origin, with antimicrobial, anti-inflammatory, antioxidant, and hypoglycemic effects.
- Acediboswellic keto-acetyl-11 – extract from Boswellia Serrata, which is a variety of incense of Asian origin, with anti-inflammatory action.

Among the objectives of this study are:
- Retrospective evaluation of the working group and the results obtained to create a unitary diagnostic and treatment protocol.
- Creating questionnaires or improving the existing ones regarding this pathology, taking into account the already existing protocols.
- Professional training of the team of specialists in the field to achieve the desired object.

MATERIALS AND METHODS

Up to this moment, we have a number of 74 patients who followed the previously mentioned treatment scheme over 6 months (52 from the initial batch 22 from the second batch, and 20 from the third batch). The distribution of patients by groups of age is in Table 1.

Table 1: The distribution of patients by groups of age

Age	21-25	26-30	31-35	36-40	41-45	46-50	50-60
Number	5	13	11	16	18	16	15

The symptomatology assessment was done using two internationally validated questionnaires: IPSS and NIH-CPSI.

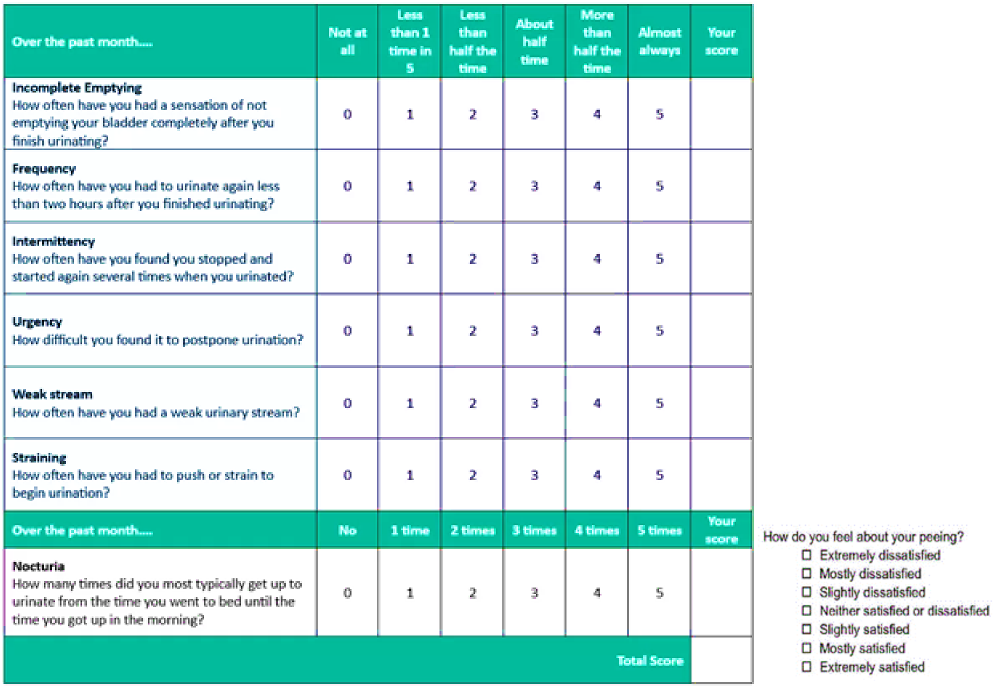

Over the past month....	Not at all	Less than 1 time in 5	Less than half the time	About half time	More than half the time	Almost always	Your score
Incomplete Emptying How often have you had a sensation of not emptying your bladder completely after you finish urinating?	0	1	2	3	4	5	
Frequency How often have you had to urinate again less than two hours after you finished urinating?	0	1	2	3	4	5	
Intermittency How often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
Urgency How difficult you found it to postpone urination?	0	1	2	3	4	5	
Weak stream How often have you had a weak urinary stream?	0	1	2	3	4	5	
Straining How often have you had to push or strain to begin urination?	0	1	2	3	4	5	
Over the past month....	No	1 time	2 times	3 times	4 times	5 times	Your score
Nocturia How many times did you most typically get up to urinate from the time you went to bed until the time you got up in the morning?	0	1	2	3	4	5	
						Total Score	

How do you feel about your peeing?
- ☐ Extremely dissatisfied
- ☐ Mostly dissatisfied
- ☐ Slightly dissatisfied
- ☐ Neither satisfied or dissatisfied
- ☐ Slightly satisfied
- ☐ Mostly satisfied
- ☐ Extremely satisfied

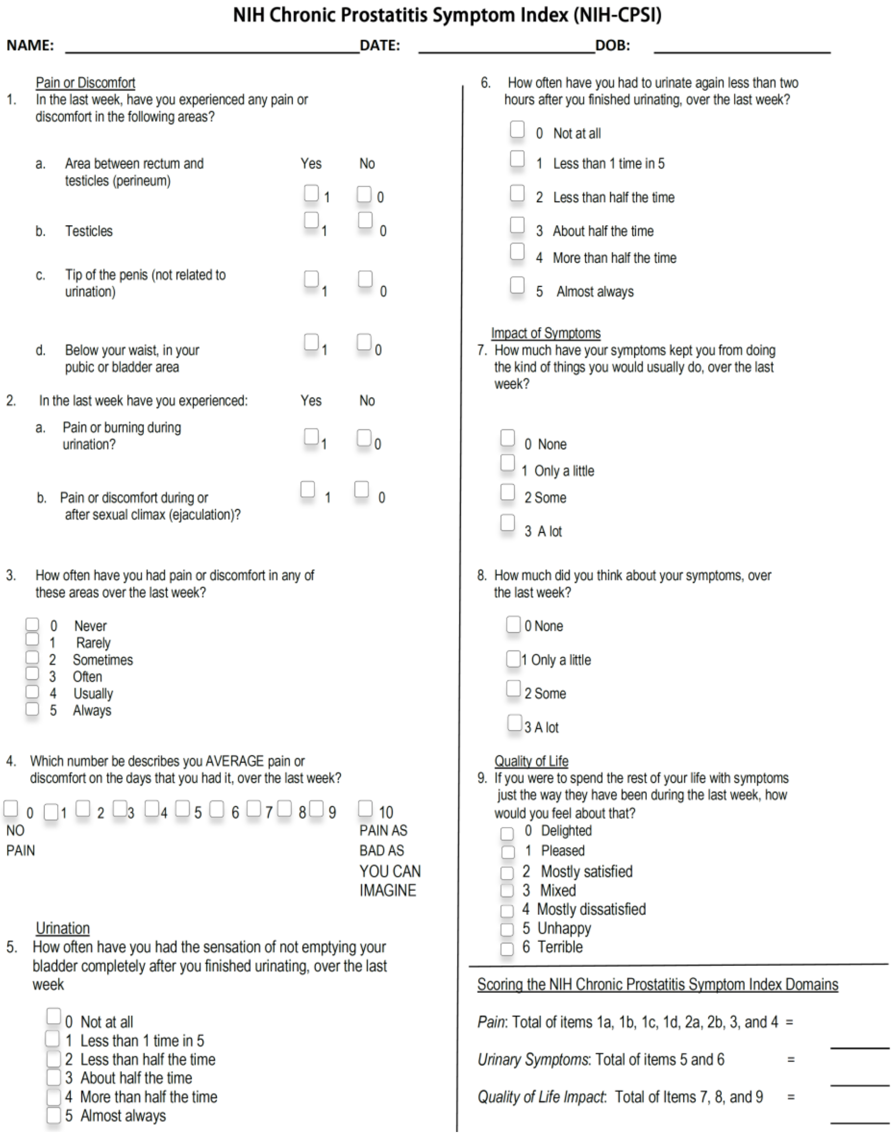

NIH Chronic Prostatitis Symptom Index (NIH-CPSI)

NAME: ________________ **DATE:** ________________ **DOB:** ________________

Pain or Discomfort

1. In the last week, have you experienced any pain or discomfort in the following areas?

		Yes	No
a.	Area between rectum and testicles (perineum)	☐ 1	☐ 0
b.	Testicles	☐ 1	☐ 0
c.	Tip of the penis (not related to urination)	☐ 1	☐ 0
d.	Below your waist, in your pubic or bladder area	☐ 1	☐ 0

2.	In the last week have you experienced:	Yes	No
a.	Pain or burning during urination?	☐ 1	☐ 0
b.	Pain or discomfort during or after sexual climax (ejaculation)?	☐ 1	☐ 0

3. How often have you had pain or discomfort in any of these areas over the last week?

☐ 0 Never
☐ 1 Rarely
☐ 2 Sometimes
☐ 3 Often
☐ 4 Usually
☐ 5 Always

4. Which number be describes you AVERAGE pain or discomfort on the days that you had it, over the last week?

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

NO PAIN

PAIN AS BAD AS YOU CAN IMAGINE

Urination

5. How often have you had the sensation of not emptying your bladder completely after you finished urinating, over the last week

☐ 0 Not at all
☐ 1 Less than 1 time in 5
☐ 2 Less than half the time
☐ 3 About half the time
☐ 4 More than half the time
☐ 5 Almost always

6. How often have you had to urinate again less than two hours after you finished urinating, over the last week?

☐ 0 Not at all
☐ 1 Less than 1 time in 5
☐ 2 Less than half the time
☐ 3 About half the time
☐ 4 More than half the time
☐ 5 Almost always

Impact of Symptoms

7. How much have your symptoms kept you from doing the kind of things you would usually do, over the last week?

☐ 0 None
☐ 1 Only a little
☐ 2 Some
☐ 3 A lot

8. How much did you think about your symptoms, over the last week?

☐ 0 None
☐ 1 Only a little
☐ 2 Some
☐ 3 A lot

Quality of Life

9. If you were to spend the rest of your life with symptoms just the way they have been during the last week, how would you feel about that?

☐ 0 Delighted
☐ 1 Pleased
☐ 2 Mostly satisfied
☐ 3 Mixed
☐ 4 Mostly dissatisfied
☐ 5 Unhappy
☐ 6 Terrible

Scoring the NIH Chronic Prostatitis Symptom Index Domains

Pain: Total of items 1a, 1b, 1c, 1d, 2a, 2b, 3, and 4 = ______

Urinary Symptoms: Total of items 5 and 6 = ______

Quality of Life Impact: Total of Items 7, 8, and 9 = ______

The IPSS score is part of all prostate adenoma diagnostic guidelines, but its interpretation is not absolute, as the answers to the questionnaire questions have a great variability from one patient to another. Although any patient can complete such a questionnaire by himself, it is at least useful for the interpretation of the results to be done by a doctor.

It should be noted that mild and average (moderate) symptoms do not require, most of the time, a therapeutic attitude. However, this score can hide early serious conditions (such as prostate cancer), and to exclude it, a PSA test and possibly a urological consultation is necessary. Severe symptomatology requires a therapeutic attitude represented by drug treatment or surgical intervention.

Classification according to severity (according to the American Urological Association):

- Mild (gr. I): IPSS = 0-7; Quality of life = 0-2. Absence of prostate adenoma complications.
- Medium (gr. II): IPSS = 8-19; Quality of life = 3-4. Possibly, the presence of chronic complications of the prostate adenoma; The patient with IPSS > 7 or quality of life > 2 is referred to a urologist for evaluation of prosta-te adenoma.
- Severe (gr. III): IPSS = 20-35; Quality of life = 5-6. The presence of chronic complications of prostate adenoma. Possibly, the presence of acute complications.

The NIH-Chronic Prostatitis Symptom Index (NIH-CPSI) is a commonly used 13-item questionnaire for the assessment of symptom severity in men with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). For each item, score ranges are 0–1 (6 items), 0–3 (2 items), 0–5 (3 items), 0–6 (1 item), and 0–10 (1 item).

We have gathered the results from these people that have started the study, at 3, 6, 8, 9, and 12 months. During this time, at 6 months, the patients have stopped taking medication and have reinitiated it after 3 months of break.

RESULTS

Compared to the first data, the maintenance of the impact of the treatment on pelvic pain, urinary symptoms, and quality of life is observed among the first group, with the second having the same effect at 3, 6, and 9 months as the previous group.

It has been observed that when the therapy is interrupted, the symptoms return, and when it is resumed, the response is even more effective compared to the first treatment.

The outcomes at 12 months by baseline batch IPSS score are described in Table 1, and Figure 1. The outcomes at 9 months by baseline batch IPSS score are described in Table 2, and Figure 2.

The last group of patients included in the study followed the treatment for a period of approximately 3 months, with the IPSS score values being as in Table 3, and Figure 3.

Table 1: Outcomes at 12 months by baseline batch IPSS score

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial IPSS	7	11.5	15.2	18.7	20	26.1	22.3
IPSS after 3 months	2	4.2	5.4	8	10.5	12	7
IPSS after 6 months	2.5	4.6	5.5	8	11	12.2	7.5
IPSS after 8 months	4	6	9	12.2	16.4	14.8	15
IPSS after 12 months	2	4.1	5.2	7.5	10	11.5	6.8

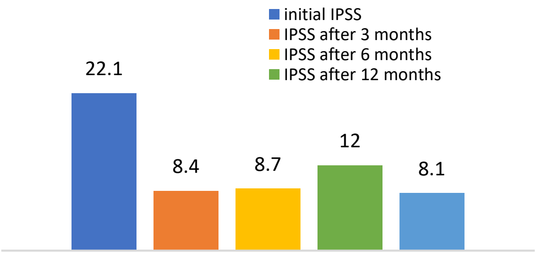

Figure 1: Mean IPSS score at the start of treatment and at 3, 6, 8, and 12 months later

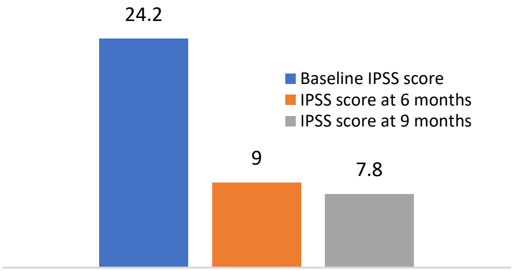

Figure 2: Mean IPSS score at the initiation of treatment of the second group at 9 months

Table 2: Outcomes at 9 months according to the IPSS score of the second batch

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial IPSS	9	13.6	14.8	15.5	18	29.4	23.8
IPSS after 3 months	3	5.4	6	7.5	11.2	14.6	11.2
IPSS after 9 months	2.5	5	5.8	7	10.6	13.6	10.5

Table 3: Mean IPSS score at treatment initiation of the third group over 3 months

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial IPSS	8	14.6	15.8	15.5	17.2	21.4	19.8
IPSS after 3 months	3	5.4	6	7.5	11.2	14.6	11.2

The outcomes of NIH-CPSI are illustrated in Tables 4 and 5 and in Figures 4 and 5.

DISCUSSION

A concordance between the two evaluations can be observed. In both cases, there is a significant positive impact regarding the urinary symptoms and also the pelvic pain, these findings being observed in all 3 groups of patients, thus revealing the beneficial effects of the therapy, as well as the tropism for the cold season of this pathology.

Another important aspect that we have noticed in those patients who have interrupted the therapy, the symptomatology tends to re-establish itself, but when the treatment is resumed is that the patients respond very well, even better compared to the moment of its initiation.

Table 4: Outcomes at 12 months by NIH-CPSI score of the first cohort

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial NIH-CPSI	11.5	14.4	17.6	20.5	27.4	28.8	25.6
NIH-CPSI after 3 months	4	5.5	7	11.6	14.3	18	16.2
NIH-CPSI after 6 months	4.5	6	9.5	10.3	15.5	18.5	15.2
NIH-CPSI after 8 months	8	9.6	13.2	15.6	20.2	19.8	18.8
NIH-CPSI after 12 months	4	5	6.8	10	12	17	14.6

Table 5: Outcomes at 12 months by NIH-CPSI score of the second cohort

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial NIH-CPSI	13	15.4	17	21	26.5	29.2	24.5
NIH-CPSI after 3 months	4.5	6.5	9	9.8	14.6	19.5	14.4
NIH-CPSI after 9 months	4	6	8	8.2	12.5	17	12.4

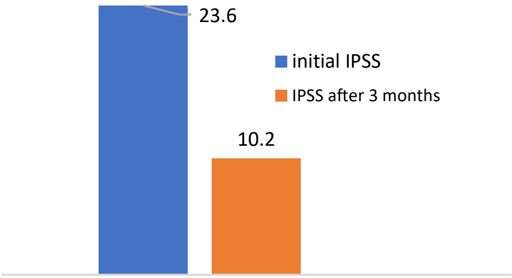

Figure 3: Mean IPSS score at treatment initiation of the third group over 3 months

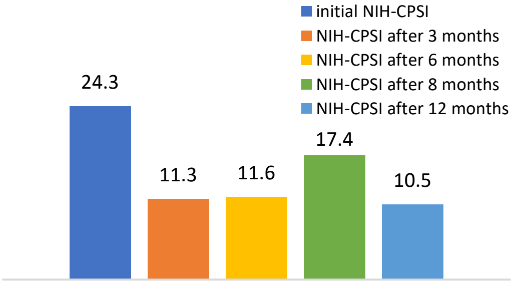

Figure 4: Mean baseline NIH-CPSI score of the first group and at 3, 6, 8, and 12 months

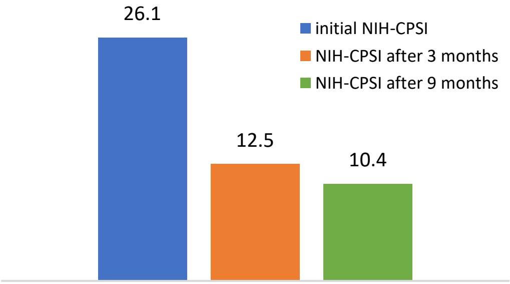

Figure 5: Mean NIH-CPSI score at treatment initiation of treatment of the second group at 3 and 9 months

In the case of 44 of the 52 patients from the initial group, compared to the 3-month results, the symptomatology remained constant, with the patients reporting a significant improvement in the quality of life. It was evident that compared to the cold season, the symptoms decreased, responding more effectively to the treatment.

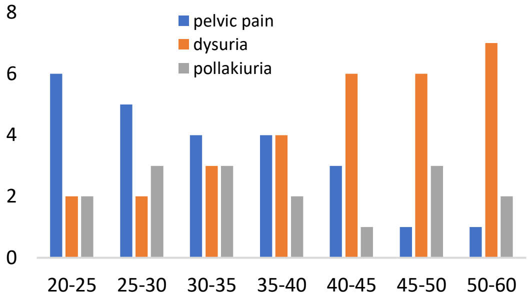

Figure 6: Initial symptomatology

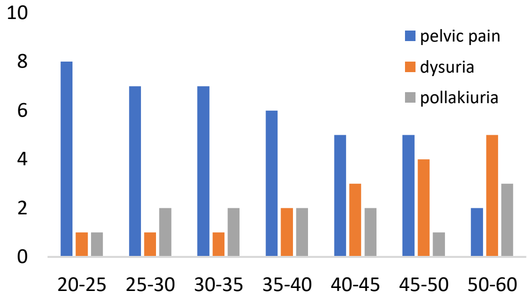

Figure 7: Symptomatology at 3 months

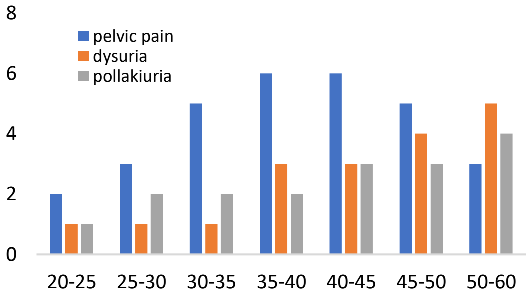

Figure 8: Symptomatology at 6 months

From the group of 22 patients introduced between 3 and 12 months into the study, remission of symptoms was observed in the case of 19, with the other 3 presenting a significant improvement in the quality of life and having a high adherence to the treatment. Again, the impact of chronic pelvic pain is highlighted, more pronounced in the cold season.

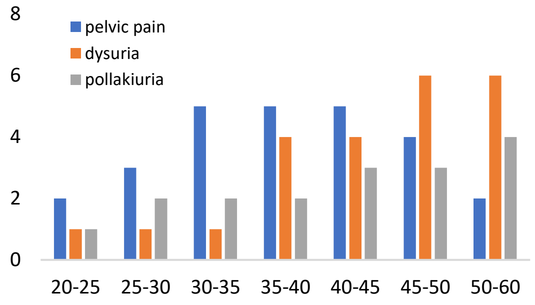

Figure 9: Symptomatology at 12 months

In the case of 44 of the 52 patients from the initial group, compared to the 3-month results, the symptomatology remained constant, with the patients reporting a significant improvement in the quality of life. It was evident that compared to the cold season, the symptoms decreased, responding more effectively to the treatment.

From the group of 22 patients introduced between 3 and 12 months into the study, remission of symptoms was observed in the case of 19, with the other 3 presenting a significant improvement in the quality of life and having a high adherence to the treatment. Again, the impact of chronic pelvic pain is highlighted, more pronounced in the cold season.

The main benefits reported by patients are represented by the absence of pelvic pain, dysuria, and improvement of sexual competence. Young people continue to report complete or almost complete remission of symptoms, and people over 50 years of age, in addition to a significant increase in quality of life, report an improvement in urinary frequency and the complete or almost complete disappearance of dysuria.

Furthermore, for the 54 patients in the initial group, the treatment was interrupted for 3 months, with its resumption, reporting significant improvement after the resumption of treatment.

The 22 patients in the second group followed the same therapeutic regimen as the first group, reporting the same benefits, and then stopped treatment for the first time.

The last batch of patients are still under observation, but all report improvements in both urinary and sexual performance, significantly increasing their quality of life**.**

CONCLUSIONS

The study demonstrates a concordance in the positive effects of phytotherapy across different age groups, emphasizing its efficacy in alleviating urinary symptoms and pelvic pain in CPPS patients.

Symptom remission or improvement in quality of life is reported, with a notable tropism for symptom reduction in the cold season. Patient adherence to treatment is high, and even after treatment interruption, resumed therapy yields significant improvements [8].

The observed benefits include the absence of pelvic pain, dysuria, and enhanced sexual competence, contributing to an overall improvement in the quality of life for patients with CPPS.

Conflicts of interest and sources of funding
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Authors' contribution
Conceptualization, A.A. and D.S.; methodology, D.S.; software, D.M.; validation, E.C., D.M. and D.G.; formal analysis, D.M.; investigation, A.A.; resources, D.M., T.N.; data curation, A.A.; writing—original draft preparation, A.A.; writing—review and editing, A.A.; visualization, D.M.; supervision, D.S.; project administration, D.O.C.; funding acquisition, D.M. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate
The study was conducted following the Decla-ration of Helsinki, and approved by the Institutional Review Board.

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

The Role of Diffusion-weighted Magnetic Resonance as a Potential Biomarker in the Characterization of Ovarian Tumor Lesions and the Assessment of Post-treatment Evolution

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Abstract: *This paper focuses on the role of diffusion-weighted MRI of ovarian tumors as a potential biomarker to detect and characterize malignancy, and to improve the accuracy of preoperative diagnosis and postoperative follow-up to optimize therapeutic management. This is an original retrospective study of 76 patients with ovarian masses who underwent surgery between May 2014 and March 2016 at the "Dr Carol Davila" Central Military University Gynaecology Department. Patients underwent preoperative, 6-month, and 12-month postoperative evaluation of ovarian tumors using diffusion-weighted MRI. Due to the accuracy of this method using specific sequences in tissue characterization, magnetic resonance imaging can be used in the diagnostic work-up of complex adnexal masses.*

Keywords: *ADC, MRI, ovarian tumor, postoperative follow-up, borderline ovarian tumor, biomarkers*

INTRODUCTION

This paper is a retrospective study of 76 patients with ovarian cancer undergoing surgery between May 2014 and March 2016 in the Gynaecology Department of the Central Military University Emergency Hospital, who were assessed by preoperative MRI scans.

Following postoperative pathological/immunohistochemical diagnosis for each surgical patient, the role of diffusion-weighted MRI in preoperative ovarian mass profiling and 6- and 12-month postoperative follow-up were evaluated.

Magnetic Resonance Imaging (MRI) is a high-performance examination offering increased sensitivity in assessing and characterizing tissue structures.[1-4]

Diffusion-weighted imaging is a functional technique recently perfected on all MRI machines with powerful gradients and advanced software.[4-8] By tracking intracellular water diffusion, the technique provides data on biological tissue structure.[9-12] Changes in tissue cellularity and cell membrane integrity can affect the free movement of water in the interstitial and intracellular space, resulting in altered signals.[13-15] Measurement of the Apparent Diffusion Coefficient (ADC) provides a quantitative and qualitative assessment of water movement at the cellular level while providing functional information about tissues.[16-19]

In oncological imaging, areas with low ADC, for example, increased diffusion restriction, represent structures with increased cellularity, suggesting tumor recurrence.[1,20] Areas with increased ADC (increased diffusivity) are most likely to indicate favorable disease progression with no recurrence or residual tumor. [21-25]

Most malignancies have low diffusion due to increased cell density, but other factors such as extracellular fibrosis may be involved.[26-30]

MATERIALS AND METHODS

In the study group, 38 patients were diagnosed with benign ovarian tumors, 32 were diagnosed with malignant ovarian tumors, and 6 were diagnosed with borderline ovarian tumors.

Of the 32 malignancy patients, 24 were monitored post-operatively at 6 and 12 months by MRI, mainly by diffusion weight imaging.

Inclusion criteria consisted of having peritoneal metastases at the time of surgery, previously identified by MRI.

Descriptive statistics, testing for possible correlations, and univariate analysis (T-test and ANOVA) were used to organize the data from the patient's MRI scans. Logistic modeling and the calculation of the ROC curve (Receiver Operator Curve) were used.

The ROC curve is a graphical representation that illustrates the diagnostic ability of a binary classification system, in this study referring to the benign or malignant nature of the tumor according to the ADC value. A significance threshold of $p<0.05$ was used for correlative and univariate analysis. Calculations were performed in EXCEL and Matlab R2015.

RESULTS

Analysis of the information provided by the ADC value determined during the diagnostic MRI examination.

Descriptive statistics, analysis of variance (ANOVA), and logistic regression were used to assess the ability of the ADC index to discriminate between malignant, benign, and borderline tumors as finally classified by histopathological examination.

Final histopathological tumor classification defined 3 categories: malignant, benign, and borderline. Of the 76 patients who underwent MRI, 32 were diagnosed as malignant, 38 as benign, and 6 as borderline.

ADC index values measured on MRI were classified into these three groups. Descriptive statistics were used to calculate the mean value, the mean standard deviation, and the range of variation of the ADC value (Table 1).

Table 1: Characteristics of the distribution of ADC values (mm^2/s) by type of tumor at the final assessment

	Malignant	Benign	Borderline
Patient nr	32	38	6
Minimal value	0.8×10^{-3}	0.65×10^{-3}	0.98×10^{-3}
Maximal value	1.22×10^{-3}	1.52×10^{-3}	1.34×10^{-3}
Median	1.038×10^{-3}	1.308×10^{-3}	1.188×10^{-3}
Standard deviation	0.083×10^{-3}	0.11×10^{-3}	0.14×10^{-3}

The graphical representation of the data distribution has been made in the Box-Wisckers plot (Figure 1), which highlights some unexpected values in the data sets corresponding to benign tumors, called "outliers".

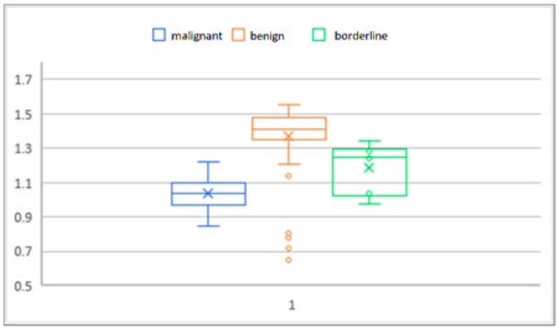

Figure 1: Box and whisker plot for ADC values

These are values that deviate greatly from the range of other measurements. Such data may be due to measurement errors, or they may represent special cases. In this study, the four very low values correspond to patient numbers 39, 125, 138, and 140 (ADC index values = 0.65×10^{-3} mm^2/s, 0.78×10^{-3} mm^2/s, 0.81×10^{-3} mm^2/s, 0.72×10^{-3} mm^2/s respectively). The final diagnosis for these patients was ovarian fibroids. The lowest ADC value for benign tumors excluding these 4 outliers is 1.14 $\times 10^{-3}$ mm^2/sec.

Analysis of variance (ANOVA) was used to test the variation in ADC measured on MRI by tumor type by comparing the three data sets. This is a method of testing whether there are significant differences between the mean values of the populations for which the data are being analyzed. According to the ANOVA performed in EXCEL (Table 2), at a 5% significance level, there is a difference between the three samples ($p=1.6 \times 10^{-8}$).

According to the Tukey-Kramer test (Table 3), only the difference between the malignant and benign groups is significant ($9.61 > 3.74$). The borderline group is not significantly different from any of the other groups.

According to the Box and Wiskers plot, the borderline group is very close to the malignant tumor group, but due to the presence of those very low ADC values corresponding to benign fibroids, the mean ADC of the benign group cannot be clearly distinguished from that of the borderline group.

Table 2: The ANOVA study

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	1.31E-06	2	6.56E-07	23.12192	1.66E-08	3.122103
Within Groups	2.07E-06	73	2.84E-08			
Total	3.38E-06	75				

Table 3: The Tukey Kramer Test

Group 1	Group 2	Difference between environments	n1	n2	SE	Q
Malignant	Benign	2.75E-04	32	38	2.857E-05	9.617
Benign	Borderline	1.25E-04	38	6	5.231E-05	2.381
Borderline	Malignant	1.50E-04	6	32	5.297E-05	2.835

When only two groups (malignant/benign) are considered after the histopathological examination (with borderline tumors being included in the malignant category), their differentiation in terms of ADC value can be tested using the Student's t-test at a significance level of $\alpha=0.05$.

Since the dispersions of the two groups are not the same, a t-test was carried out to test for the equality of the means in the variance of the unequal dispersions in the groups. The null hypothesis of equal means was rejected, and the alternative hypothesis of unequal means was accepted ($p=0.04<0.05$). The value of the ADC index is significantly different for tumors that are benign or malignant. Using a one-tailed test with the alternative hypothesis "mean ADC of malignant group < mean ADC of the benign group", the result of the test is to reject the null hypothesis (equality of means) and accept the alternative hypothesis. The p-value = 0.02 confirms that the mean ADC values in the malignant tumors are significantly lower than the mean ADC values in the benign tumors.

Analysis of the distribution of ADC values according to tumor type

Of the 38 benign tumors for which an MRI scan was performed, 13 tumors were germinal, 8 were stromal and 17 were epithelial. The majority of malignant and borderline tumors were epithelial tumors (35). Only 2 malignant tumors were germinal, and one was a stromal tumor. Peritoneal diffusion-restricted areas were also measured in malignancies. Figure 2 compares the distribution of intratumoral and peritoneal diffusion-restricted (ADC) values for these tumor types.

A clear differentiation of ADC values according to tumor type and nature can be seen in the figure. In benign epithelial and germinal tumors, the mean ADC is 1.4 x 10^{-3} mm^{2}/s, and the lowest values are above 1.2 x 10^{-3} mm^{2}/s. For malignant epithelial tumors, the mean value is 1.05 x 10^{-3} mm^{2}/s, and the highest values are slightly higher than 1.2 x 10^{-3} mm^{2}/s. The mean ADC in peritoneal lesions is also close to 1 x 10^{-3} mm^{2}/s. The distribution of values is similar to that of malignant tumors.

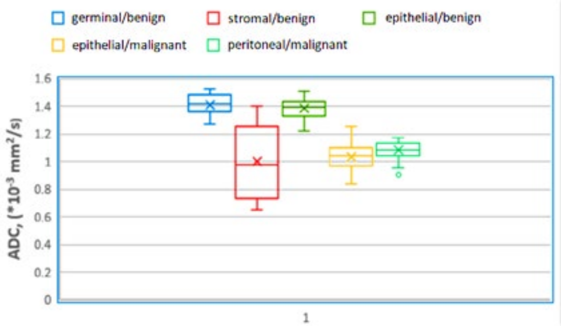

Figure 2: Distribution of intratumoral and peritoneal ADC values by tumor type.

The benign stromal tumors have a special feature: half of the values are less than 1 x 10^{-3} mm^{2}/s. Of the 8 benign tumors of this type, 4 are uterine fibroids, whose ADC is very low, below 1 x 10^{-3} mm^{2}/s as mentioned above.

Malignant tumors of the stromal and germinal types could not be statistically analyzed due to their low number.

Analysis of parameter values recorded at 6 and 12 months post-operatively

Twenty-four patients with malignant tumors were re-examined 6 and 12 months postoperatively.

Figure 3 shows the distribution of ADCs at 6 and 12 months postoperatively. The increase in mean values can be seen, as well as the increase in minimum values during the follow-up period.

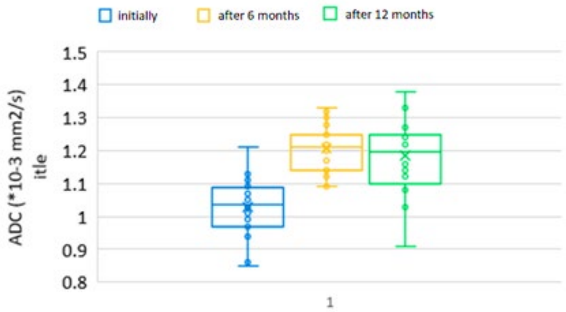

Figure 3: Change in distribution of intratumoral (baseline) and peritoneal ADC during postoperative follow-up

Comparison of the ADC values measured at 6 and 12 months with the peritoneal ADC values measured at the time of diagnosis shows a clear improvement in these values at 6 months. This improvement was maintained at 12 months in all but less than 20% of cases (Figure 4).

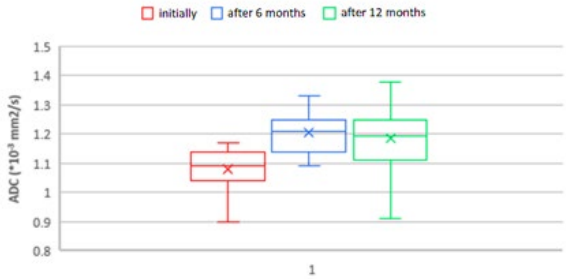

Figure 4: Peritoneum ADC values at diagnosis and follow-up.

The mean and median values of ADC induction from peritoneal lesions measured at 6 and 12 months after the intervention increased significantly from baseline values, as shown in Figure 4. The distribution of ADC values at 12 months shows that there are several ADC values lower than those recorded at 6 months, indicating that these cases may not have had a favorable postoperative course at 6-12 months.

The paired t-test at a significance level of $p=1 \times 10^{-8}$ also confirms the significant increase in ADC in the first 6 months.

For the period from 6 to 12 months, the increase was no longer significant ($p= 0.16>0.05$).

Logistic regression was used to correlate the probability of identifying a malignant tumor as a function of ADC alone, and the receiver operator characteristic (ROC) curve was constructed, which is a probability curve plotting the true positive rate (TPR) versus the false positive rate (FPR) at different cut-off values of the variable (ADC).

The area under the curve (AUC) is used to measure the ability of the classifier to discriminate between classes and is used to conclude the ROC. In the present study, AUC=0.89, which corresponds to a good classifying model. The closer the AUC is to 1, the higher the value of the classification method (high accuracy).

The ROC curve (Figure 5) shows how the sensitivity and specificity can vary for different cut-off values for the ADC. In the analysis, the case was considered positive with the qualifier "malignant".

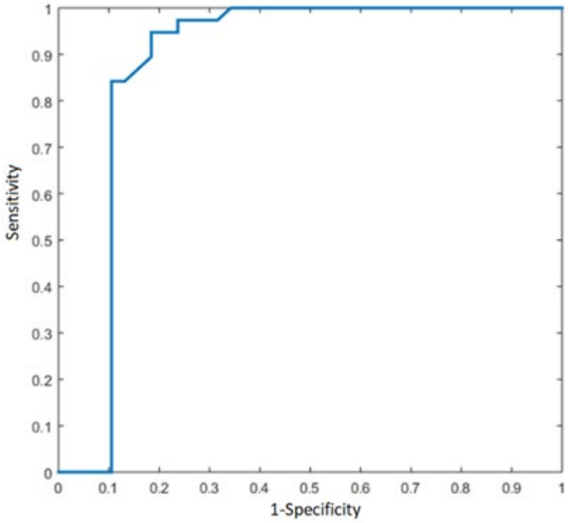

Figure 5: ROC curve for ADC performance in diagnosis

DISCUSSION

Analysis of the information provided by the ADC value at the level of solid components of ovarian tumors assessed by MRI

The role of the diffusion MRI sequence was analyzed by evaluating the ADC index within the intratumoral solid component and the macroscopic peritoneal determinations.

Patients with malignant and benign ovarian disease showed significant differences in ADC scores. The group of patients with a borderline tumor did not differ significantly from the other two groups of patients. The ADC values recorded in the borderline ovarian lesion group were close to those in the malignant lesion group, but such low ADC values were also found in benign fibromatous tumors, with the mean ADC values in the benign lesion group not distinguishable from the ADC values in borderline lesions. Due to the very low number of germinal and stromal tumors in the study group, analysis of ADC values for malignant tumors could only be performed for epithelial tumors.

The mean ADC value for the epithelial malignancies was 1.05×10^{-3} mm^2/s, which was also close to the mean value for peritoneal lesions (1×10^{-3} mm^2/s).

For benign epithelial and germinal lesions, the mean ADC value was 1.4×10^{-3} mm^2/s, which is significantly different from that found in malignant lesions.

ADC analysis confirmed the peculiarity of benign stromal tumors, which have a decreased ADC index, with half of their ADC values less than 1x10^{-3} mm^2/sec.

Assessment of postoperative evolution by ADC index analysis at the level of residual peritoneal deposits

Among the patients with a diagnosis of ovarian malignancy, MRI was also used in 24 patients to monitor the follow-up after treatment at 6 months and 12 months, respectively. Biochemistry and ADC levels were analyzed compared to baseline (pre-intervention).

ADC values showed a significant increase in the first 6 months (mean 0.18x10^{-3} mm^2/s), but not significant in the following 6 months. The change in ADC in controls at 6- and 12-months post-intervention was also analyzed concerning patient age, without showing a statistically significant correlation.

Comparison of the ADC values measured at 6 and 12 months with the ADC values in the peritoneal deposits measured at the time of diagnosis showed a clear improvement in these values at 6 months. This improvement was also maintained in the controls at 12 months in about 80% of the cases. The remaining 20% of patients had lower ADC scores compared to the previous year, indicating a poor postoperative outcome in the 6- to 12-month follow-up.

Magnetic resonance imaging (MRI) was found to be a powerful examination in the study due to the multiple sequences available to characterize tissue structures to differentiate the components of tumor formations with increased specificity in the assessment of malignancy

The multiple sequences that can be used to analyze and differentiate tissue structures, with an optimal assessment of lipomatous, cystic, hematogenous, or components in different stages of hemoglobin degradation, combined with the value of diffusion sequences, can be a powerful alternative for patients with allergies or impaired renal function when the administration of contrast agents for tumor assessment is contraindicated.

The possibility of multiplanar imaging provides a superior evaluation of tumor masses with a good appreciation of organ of origin and anatomical relations, and diffusion sequences and characterization of post-contrast load curves have provided a significant increase in sensitivity in the determination of malignancy, of particular importance for the choice of optimal therapeutic approach.

Areas of increased signal in diffusion sequences and low absolute diffusion index (ADC) represent structures with increased cellularity, indicating tumorigenicity.

Increased ADC values after therapy in residual tumor masses indicate a favorable course of the disease and can be considered as a biomarker for the dynamic assessment of therapeutic response in oncological disease. Increased ADC correlated in most cases with decreased serological marker titers.[31]

At the same time, the low ADC index value had an increased sensitivity in the assessment of lymph node hypertrophy that was suspicious for metastatic disease, regardless of size.

Some low ADC values did not always correlate with malignancy, as other factors such as extracellular fibrosis, shape and size of intercellular spaces could influence the ADC value, and some formations, in particular fibromatous formations, despite being benign, showed a decreased ADC value. In these cases, other imaging characteristics (in particular the type of contrast agent uptake) and some clinical and biological parameters were used to guide the diagnosis.[32]

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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None

Authors' contribution
Conceptualization, IAN, and MFN; methodology, EDSNM and FCA; software, FN and EDSNM; validation, IAN, FCA, and MFN; formal analysis, MFN; investigation, IAN, IAC, MAN, and PCA; resources, EDSNM; data curation, MFN; writing—original draft preparation, IAN; writing—review and editing, IAN, MFN and PCA; visualization, IAN, MAN, IAC; supervision, IAN and MFN; project administration, IAN. All authors have read and agreed to the published version of the manuscript.

Ethics approval
The present study was approved by the hospital's ethics committee Nr. 601/ 24.05.2023 and is performed under the ethical standards laid down by the Declaration of Helsinki

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ORIGINAL ARTICLE

Couvelaire Syndrome: A Case Report and Literature Review

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Abstract: *Introduction. Couvelaire syndrome is a rare complication of pregnancy involving the placenta and the uterine wall. It is caused by placental abruption, preeclampsia, or other disorders that compromise the blood supply to the placenta. Diagnosis is typically made through history, clinical evaluation, and ultrasound. This study aims to report a grave case of Couvelaire syndrome complemented by presenting a review of the relevant literature, to aid the meaning of a clinical path, as well as early and adequate diagnosis and treatment of Couvelaire syndrome, to prevent feto-maternal consequences. The treatment of Couvelaire syndrome depends on the severity of the condition and the well-being of both the mother and the fetus. If the condition is severe and poses a risk to the mother or fetus, immediate delivery may be recommended, mainly with urgent Cesarean section in general endotracheal anesthesia. If there is significant blood loss leading to a decrease in hemoglobin levels, blood transfusions may be necessary to restore normal blood volume and oxygen-carrying capacity. Part of the general treatment plan should also be the management of the underlying cause of placental abruption, for example, in cases of preeclampsia, medications are prescribed to control blood pressure and prevent seizures.*

Keywords: *ADC, MRI, ovarian tumor, postoperative follow-up, borderline ovarian tumor, biomarkers*

INTRODUCTION

The placenta is an essential organ that develops during pregnancy and supplies the fetus with blood and, through it, with oxygen and nutrients.

Couvelaire syndrome, also known as uteroplacental apoplexy or placental apoplexy, is a rare and severe complication of placental abruption that can occur during pregnancy, especially during the third trimester of pregnancy. This condition involves the infiltration of blood into the uterine myometrium (the muscular layer of the uterus) due to a ruptured placental vessel. The blood collects in the space between the uterine muscle fibers, creating a hematoma. The incidence of Couvelaire syndrome is relatively low, and it is considered a rare complication of placental abruption. The incidence of placental abruption itself happens in approximately 1% of all pregnancies [1]. Even within the cases of placental abruption, Couvelaire syndrome is even less frequent.

The main cause of Couvelaire syndrome is placental abruption, where the placenta can partially or completely separate from the uterine wall before delivery [2,3].

Risk factors that lead to abruption of the placenta and consequently to Couvelaire syndrome are eclampsia, preeclampsia, chronic hypertension and pregnancy-induced hypertension, trauma or injury (car accident), previous placental abruption, uterine abnormalities, fibroids or a history of uterine surgery, multiple pregnancies (twins, triplets), cigarette smoking, drug use (including cocaine and methamphetamines), polyhydramnios, short umbilical cord, and women who are younger than 20 or older than 35 years. All these factors above can increase the risk of placental abruption [4-6].

Couvelaire uterus is typically a result of severe placental abruption, where there is bleeding between the placenta and the uterine wall. This bleeding can imbibe and extend into the myometrium.

The bleeding leads to the formation of blood clots within the uterine muscle, causing a hematoma. This hematoma can compromise the blood supply to the uterine muscles. The uterus may take on a characteristic blue or purple hue due to the presence of blood within the myometrium (the image of the tiger) [7-10]. This accumulation of blood between the muscular fibers of the uterine wall can disrupt the contractile function of the muscular ultrastructure, leading to uterine atony and increasing the risk of complications, including postpartum hemorrhage and disseminated intravascular coagulation (DIC), a serious disorder that affects blood clotting [6]. Early detection and appropriate management are crucial and can significantly improve outcomes for both the maternal and the fetus.

CASE PRESENTATION

Clinical history and treatment

A 27-year-old multipara in the 34th week of pregnancy was admitted to the Department of Obstetrics and Gynecology in October 2023, with vaginal bleeding, abdominal pain, back pain, uterine rigidity, and uterine contraction. The vital parameters were; a low blood pressure of 100/60 mmHg, pulse of 97 minutes, and SpO_2 was 95%. The patient had a previous birth, at term, and without any apparent complications. Our patient is atypical because she does not have a history of hypertension, pregnancy-induced hypertension, diabetes, trauma or injury (such as a car accident), no polyhydramnios, no chorioamnionitis, no history of drug use, no uterine abnormalities, fibroids, or a history of uterine surgery. She was a daily smoker, for over five years and over 20 cigarettes a day.

A general physical examination confirms uterine rigidity, continuous uterine contraction, and abdominal pain. The vaginal examination showed vaginal bleeding and a dilation of the cervix for 1.5 cm.

An ultrasound examination revealed a retroplacental hematoma with dimensions of about 5 cm, (Figure 1). A cardiotocography (CTG) examination revealed various abnormalities such as pseudo-sinusoidal pattern of fetal heart rate and late decelerations, (Figure 2).

All emergency examinations suggested central placental abruption and indicated urgent Cesarean section in general endotracheal anesthesia. Following the Pfannenstiel-Kerr incision laparotomy, massive uteroplacental apoplexy or Couvelaire syndrome (Figure 3).

A female hypotrophic neonate was born at 1700 gram/45 cm and with Apgar scores of 6/7. Laboratory findings showed moderate anemia, with a level of hemoglobin concentration of 8.7 g/dL, mild thrombocytopenia, and with intact coagulogram.

During hysterorrhaphy, 20 UI intravenous oxytocin and afterward 2 gram intravenous tranexamic acid, were administered because of the hypotonic and non-contractible uterus, simultaneously continuous pressure of the uterus was performed with wet compresses with a warm physiological solution, resulting in complete success in preserving the uterus. After surgery, the patient was stable in terms of hemodynamic parameters.

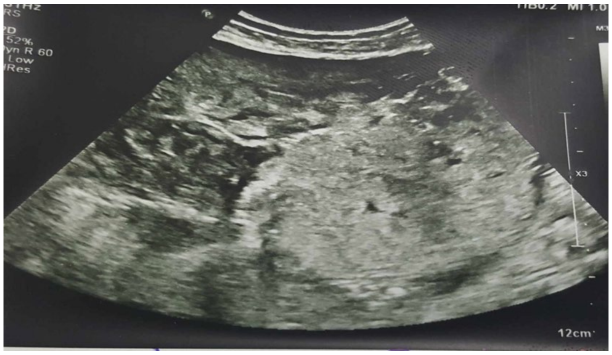

Figure 1: Retroplacental hematoma. With ultrasonography, a hematoma between the placenta and the wall of the uterus, measuring about 5 cm, is found in a patient with placental abruption

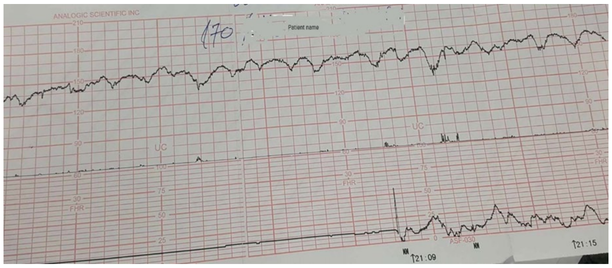

Figure 2: Pseudosinusoidal pattern. External fetal heart rate monitoring at 1 cm/min. In patients with placental abruption has been found pseudo sinusoidal fetal heart rate patterns and late decelerations

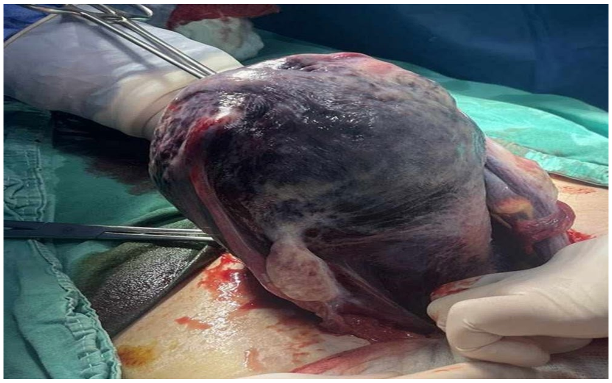

Figure 3: Couvelaire syndrome or uteroplacental apoplexy. It is found massive infiltration of blood into the uterine myometrium (the muscular layer of the uterus) due to a ruptured placental vessel

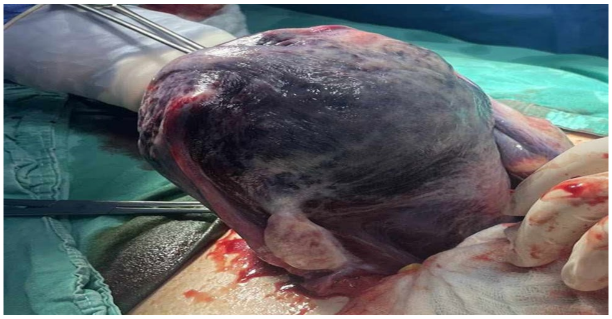

Figure 4: Infiltration of adnexal. It is found even massive infiltration of blood into the uterine adnexa

DISCUSSION AND LITERATURE REVIEW

For the literature review utilized the Items from databases such as Medline (PubMed), EMBASE, Scopus, and Google Scholar. For all items about 'Couvelaire Syndrome' abbreviations are used *OR Couvelaire Syndrome* OR Couvelaire uterus* OR Uteroplacental apoplexy* OR Placental abruption* OR Placental abruption complications* OR Uteroplacental apoplexy treatment* OR Couvelaire uterus causes* Couvelaire uterus symptoms* OR Couvelaire uterus complications, etc.

The patient had a previous birth, at term, and without any apparent complications. Our patient did not have any risk factors for placental abruption, except that she was a smoker for five years and used more than 20 cigarettes a day. It is now known that smoking is associated with a significant risk of placental abruption and a possible complication in uteroplacental apoplexy or Couvelaire Syndrome. Cigarette smoking during pregnancy is associated with numerous adverse outcomes for both the maternal and the developing fetus. One of the potential complications linked to smoking during pregnancy is placental abruption. Placental abruption occurs when the placenta separates from the inner wall of the uterus before delivery. This separation can compromise the flow of oxygen and nutrients to the fetus and may lead to serious complications for both the mother and fetus.

The physiopathological mechanism of how cigarette smoking can cause placental abruption follows several steps:

- Reduced Oxygen Supply: Smoking cigarettes can lead to the constriction of blood vessels, including those in the placenta. This constriction reduces the amount of oxygen that the fetus receives, potentially leading to stress on the placenta.

- Increased Risk of Blood Clotting: Smoking is known to increase the risk of blood clotting, and this can contribute to the detachment of the placenta from the uterine wall.

- Hypertension and Vasoconstriction: Smoking is a risk factor for hypertension (high blood pressure), and elevated blood pressure can contribute to the detachment of the placenta. Additionally, the vasoconstrictive effects of smoking can reduce blood flow to the placenta, further increasing the risk of complications.

- Placental Insufficiency: Smoking has been linked to placental insufficiency, where the placenta is not able to provide sufficient nutrients and oxygen to the developing fetus. This can increase the risk of placental abruption.

- Inflammatory Response: Cigarette smoke triggers an inflammatory response in the body. Chronic inflammation can contribute to the breakdown of the placental tissue and increase the risk of placental abruption.

The placenta is an organ that provides oxygen and nutrients to the developing fetus. Placental abruption is a severe pregnancy complication where the placenta partially or completely separates from the inner wall of the uterus before the delivery of the fetus [11]. This separation can lead to bleeding and directly endanger the life of the fetus and maternal. Prevalence of placental abruption occurs in about 0.2%-1% of pregnancies [12,13]. Placental abruption can be classified into partial or complete, depending on the extent of detachment. Partial abruption involves only a portion of the placenta separating, while complete abruption involves the entire placenta detaching from the uterine wall. Couvelaire uterus is a rare complication that happens due to severe placental abruption. One systematic review found that 16.5% of patients with placental abruption were complicated in the Couvelaire uterus, and they needed emergency hysterectomy [14]. This rare complication occurs when vascular damage within the placenta causes hemorrhaging that progresses to and infiltrates the wall of the uterus.

Definition, classification, and characteristics of Couvelaire syndrome

Definition

Couvelaire uterus can be defined as a histopathological entity caused by severe forms of placental abruption.

This histopathological entity was first described by Couvelaire at the beginning of the 20th century and is now often referred to as Couvelaire uterus or Couvelaire syndrome [15].

Initially, due to various risk factors, occurs pathologic vascular damage within the placenta itself, causing hemorrhage and hematoma formation, blood from hematoma seeps into the deciduas basalis, eventually causing a separation of the placentas [16,17].

The hemorrhage often progresses, in all structures of the uterus and adnexa including the walls of the uterus, beneath the tubal serosa, into the connective tissue of the broad ligaments, in the ovaries, as well as in the peritoneal cavity [18, 19].

Macroscopically, the uterus assumes the appearance of a purplish or copper-colored ecchymotic. Histologically, blood is found between muscle bundles, in perivascular tissue, and in the subserosa. The decidual spiral arterioles may show acute atheromatous processes, with foamy macrophages present. Physiologically, indurated the uterus often loses its contractile power.

Since the diagnosis of Couvelaire uterus is made only with direct visualization of the uterus after laparotomy or biopsy, it is almost impossible to know the precise incidence of Couvelaire uterus. However, the incidence of Couvelaire uterus behaves between 5%-20% of all cases with placental abruption [15,20].

Classification

Depending on the degree of blood infiltration through the walls of the uterus, mild, moderate, and severe forms of Couvelaire uterus can develop. In mild and moderate forms of the Couvelaire uterus, blood infiltration penetrates until the serous layer of the uterus, while in severe forms of the Couvelaire uterus, in addition to the uterine wall, blood also infiltrates other structures such as the adnexa, peritoneal cavity, and surrounding structures.

Clinically, the Couvelaire uterus can develop in a mild, moderate, and severe form, depending on the degree of blood loss and the impact on the hemodynamic state.

Characteristics

The condition is characterized by the infiltration of blood into the uterine muscle, creating a hematoma. This bleeding into the myometrium can compromise the ability of the uterus to contract effectively.

The presence of blood in the uterine muscle may result in a "bruised" appearance of the uterus, giving it a bluish or purple discoloration.

Couvelaire uterus can lead to uterine atony, a condition where the uterus loses its ability to contract properly, which can result in excessive bleeding after delivery (postpartum hemorrhage) [21,22].

Maternal and fetal outcomes can be compromised due to the potential for severe bleeding, reduced oxygen supply to the fetus, and other complications associated with placental abruption.

Aetiology

The cause of Couvelaire uterus is placental abruption, especially its heavy form [14]. All the factors that directly or indirectly affect the development of placental abruption are also risk factors event for the Couvelaire uterus [4,5,24,25]. The exact cause of placental abruption is not always clear, but several factors and risk factors have been associated with an increased likelihood of its occurrence. These include:

- Trauma or Injury: A sudden impact or trauma to the abdomen, such as might occur in a car accident or a fall, can increase the risk of placental abruption.

- High Blood Pressure: Chronic hypertension or pregnancy-induced hypertension (preeclampsia, eclampsia) is a significant risk factor for placental abruption. High blood pressure can lead to damage to the blood vessels in the uterus.

- Advanced Maternal Age: Older women, particularly those over the age of 35, may have a higher risk of placental abruption.

- Multiple Pregnancies: The risk of placental abruption is higher in pregnancies involving twins, triplets, or other multiple births.

- Cigarette Smoking: Smoking during pregnancy is associated with an increased risk of placental abruption, likely due to the effects of smoking on blood vessels.

- Drug Use: The use of certain drugs, such as cocaine and methamphetamine, is linked to an elevated risk of placental abruption.

- Previous History: Women who have had a previous placental abruption are at a higher risk of experiencing it again in subsequent pregnancies.

- Uterine Conditions: Conditions that affect the uterus, such as fibroids or an abnormally shaped uterus may increase the risk of placental abruption.

- Rupture of Membranes: The premature rupture of the amniotic sac (bag of water) can increase the risk of placental abruption.

- Abdominal Surgery: Women who have had previous abdominal surgeries, especially uterine surgeries, may have an increased risk.

- Blood Clotting Disorders: Certain blood clotting disorders, such as disseminated intravascular coagulation (DIC), can contribute to placental abruption.

It's important to note that some cases of placental abruption occur without any identifiable risk factors. Additionally, the severity of placental abruption can vary, and its management depends on factors such as the extent of detachment and the gestational age of the fetus.

Clinical findings

Couvelaire uterus, also known as Couvelaire syndrome, refers to a condition where blood from a placental abruption infiltrates into the myometrium (the muscular wall) of the uterus. This condition is associated with placental abruption, which is the premature separation of the placenta from the uterine wall. Clinical findings of Couvelaire's uterus may include:

- Bluish or Purple Uterus: Blood infiltration into the myometrium can give the uterus a bluish or purple discoloration. This is due to the presence of blood within the uterine muscle.

- Bruised Appearance: The uterus may have a "bruised" appearance, visually resembling a contusion or hematoma. This appearance is a result of the blood infiltrating the uterine tissues.

- Abdominal pain: Women with Couvelaire uterus may experience abdominal pain. The severity of pain can vary depending on the extent of the placental abruption and the involvement of the uterine musculature.

- Uterine Rigidity or tetanic uterine contraction: The uterine wall may become rigid or firm due to the infiltration of blood into the myometrium. This rigidity can contribute to uterine dysfunction and increase the risk of complications.

- Compromised Uterine Contractility: The infiltration of blood into the uterine muscle can compromise the ability of the uterus to contract effectively. This may lead to uterine atony, a condition where the uterus loses its normal tone and may not contract adequately.

The clinical findings of Couvelaire uterus are often part of a larger clinical picture associated with placental abruption [23]. Placental abruption itself can present with symptoms such as vaginal bleeding, abdominal pain, back pain, and signs of fetal distress.

Diagnosis

The diagnosis of Couvelaire uterus is typically made in the context of diagnosing placental abruption [23-25]. The steps and methods involved in the diagnosis are:

- Clinical Evaluation: One must perform a thorough clinical evaluation, taking into consideration the patient's symptoms, medical history, and risk factors.

- Symptoms and Signs: Couvelaire uterus is often associated with symptoms of placental abruption, including vaginal bleeding, abdominal pain, tetanic uterine contractions, and signs of fetal distress.

- Physical Examination: A physical examination may reveal vaginal bleeding and abdominal pain or palpation sensitivity. The uterus may also feel rigid or firm.

- Ultrasound Imaging: Ultrasound imaging is a valuable tool for assessing the condition of the placenta, the amount of amniotic fluid, and the overall well-being of the fetus. While ultrasound may not directly visualize the blood within the uterine muscle, it can provide information about the placental abruption, her position, and possible hematoma (not always).

- Laboratory Tests: Laboratory tests, such as blood tests, may be performed to assess the maternal overall health and detect signs of complications, such as anemia or coagulation disorders.

- Fetal Monitoring: Continuous fetal monitoring may be employed to assess the well-being of the fetus. This can include monitoring the fetal heart rate and assessing for signs of distress.

- Clinical Judgment: Diagnosis may involve the clinical judgment of healthcare professionals based on the overall presentation of symptoms, physical findings, and diagnostic tests.

Treatment and Prognosis

The treatment and prognosis of Couvelaire uterus are generally addressed within the broader context of managing placental abruption. The key aspects of treatment and prognosis:

Treatment:

- Stabilization: The initial focus is on stabilizing the maternal. This may involve addressing any immediate life-threatening complications, such as managing severe bleeding or addressing signs of shock.

- Monitoring: Continuous monitoring of the maternal and the fetus is essential. This includes monitoring vital signs, assessing the extent of the placental abruption, and evaluating fetal well-being through methods like fetal heart rate monitoring.

- Blood Transfusion: If there is significant blood loss, blood transfusions may be required to replace lost blood and address any resulting anemia.

- Delivery: The decision to deliver the baby depends on various factors, including the severity of the placental abruption, the gestational age of the fetus, and the overall condition of the maternal and fetal. In some cases, emergency delivery, often via cesarean section, may be necessary to improve outcomes.

- Uterine Massage: Uterine massage may be performed to help the uterus contract and reduce bleeding.

- Medical Management: Medications may be administered to address complications such as hypertension, coagulation disorders, or other medical conditions that could exacerbate the situation.

Prognosis:

- Varied Outcomes: The prognosis for Couvelaire uterus and placental abruption can vary widely depending on the severity of the condition, the gestational age of the fetus, and the effectiveness of medical interventions.

- Maternal Complications: Potential maternal complications include excessive bleeding, disseminated intravascular coagulation (DIC), and the need for surgical interventions. Severe cases can lead to maternal mortality.

- Fetal Complications: Fetal outcomes depend on factors such as gestational age, the degree of placental abruption, and the speed with which medical intervention is provided. Preterm birth, low birth weight, and neonatal complications are possible.

- Long-Term Effects: Long-term effects on both the maternal and the fetus may include consequences related to prematurity, low birth weight, and any complications resulting from the extent of the placental abruption.

It's crucial to note that the Couvelaire uterus is a relatively rare and severe complication, and its management requires prompt and comprehensive medical care. The prognosis can be influenced by the rapidity of diagnosis, the effectiveness of interventions, and the overall health of both the maternal and the fetus. Pregnant women experiencing symptoms suggestive of placental abruption, such as vaginal bleeding and abdominal pain, should seek immediate medical attention for timely evaluation and intervention.

CONCLUSION

It's significant to know that the management of Couvelaire syndrome requires an individualized approach based on the specific circumstances of each case. The treatment of Couvelaire syndrome depends on the severity of the condition and the well-being of both the maternal and the fetus. If the condition is severe and poses a risk to the maternal or fetus, immediate delivery may be recommended, mainly with urgent Cesarean section in general endotracheal anesthesia. If there is significant blood loss, blood transfusions may be necessary to restore normal blood volume and oxygen-carrying capacity. Part of the general treatment plan should also be the management of the underlying cause of placental abruption, for example, in cases of preeclampsia, medications are prescribed to control blood pressure and prevent seizures [26].

The guidelines development for diagnosis and treatment of Couvelaire uterus as a complication of placental abruption would aid in the early detection and proper management of the disease, thus reducing the rates of morbidity, recurrence, and mortality feto-maternal due to placental abruption and complications in Couvelaire uterus. To date, treatment options are mostly empirical, they are not evidence-based practices, and therefore multicenter clinical collaborative research is required to provide useful guidance for the precise diagnosis and treatment of Couvelaire uterus.

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ORIGINAL ARTICLE

Dry Eye Disease in Systemic Lupus Erythematosus

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Abstract: *Systemic lupus erythematosus is a multi-organ autoimmune disease of unknown etiology characterized by widespread inflammation and significant morbidity and mortality. Organ affection includes joint and cutaneous involvement, pulmonary, neurological, and cardiac problems, renal and hematological involvement as well as ocular comorbidities, both posterior but mostly anterior ocular involvement. Therefore, we aimed to evaluate dry eye involvement in lupus patients. A cross-sectional study was performed with the aid of a web-based, anonymous questionnaire that was distributed to both patients diagnosed with lupus erythematosus and to a control group. A total number of 123 patients diagnosed with lupus and 200 responders in the control group completed the questionnaire. Dry eye disease has already been diagnosed in 25 lupus patients (28.9%) compared to 44 (21.9%) individuals in the control group. Dry eye-related symptomatology was reported in 43 (34.60%) patients compared to 57 (28.35%) healthy responders. Upon statistical analysis, more symptoms were reported in the lupus group (p=0.01), in responders diagnosed with dry eye syndrome (p<0.01), and in responders using lubrication (p<0.01) but no association was obtained between the incidence of dry eye symptoms and the presence of the disease. The number of episodes of lupus reactivation since diagnosis was correlated with dry eye syndrome (p=0.15) and the use of lubricating tear drops (p<0.01). Dry eye symptoms and disease are more frequent in lupus patients and further research should be performed to understand the connection between these two disease entities.*

Keywords: *systemic lupus erythematosus, dry eye disease*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease, the most commonly affected organs being the joints, and the skin but also having hematologic, renal, central nervous system, and cardiac involvement. Even though eye involvement has not been included in the diagnostic criteria of SLE, possibly also because eye involvement occurs in several systemic autoimmune diseases, thus making it less specific in diagnosing certain pathologies, ocular manifestations in SLE have increasingly gained importance. Ophthalmic manifestations may occur independently or may be a reflection of systemic inflammation. In the course of the disease, several anatomic structures may be involved, such as the periorbita, adnexa, eye, and optic nerve [1]. Some of the most frequent conditions found in SLE patients are keratoconjunctivitis sicca or dry eye disease, iritis and ciliary body inflammation, vascular changes of the retina and occlusive vasculitis as well as optic neuritis [2]. Retinopathy is highly suggestive of disease activity and may precede other organ involvement, thus being a marker of not only poor visual outcome but for survival prognosis [1]. On the other hand, the anterior part of the eye is responsible for the most frequent ocular manifestations such as keratoconjunctivitis sicca and Sjogren's syndrome. The various degrees of severity of dry eye syndrome may range from stinging, and burning sensation to light sensitivity but also to corneal ulceration, scleritis, uveitis, and optic neuritis, having a significant impact on the visual function. Early treatment may prevent the appearance of complications, therefore, prompt recognition in slightly symptomatic or asymptomatic patients would be advisable. We aimed to evaluate the prevalence of dry eye disease and of associated symptoms in a group of patients diagnosed with systemic lupus erythematosus**.**

MATERIALS AND METHODS

A cross-sectional study using a web-based questionnaire was performed. The survey was developed by the authors based on previously published data and was sent via Google Forms (Google LLC, Mountain View, CA, USA) to participants. The completion of the survey was anonymous and voluntary. The data regarding lupus patients was retrieved from a study evaluating photoprotection habits in patients with lupus erythematosus (manuscript submitted). Participants in the control group were randomly selected and no responder received any financial benefits from completing the questions.

Initially, participants had the opportunity to provide anonymous personal data such as age, gender, area of dwelling, and education. Dry eye disease was assessed with the following questions: the first item asked whether they were diagnosed with dry eye disease (with two answer options: “yes”, “no”), the second item evaluated whether subjects used lubricating tear drops (with four answer options “never”, “rarely”, “often”, “daily”) and the third item was a visual Likert-scale with answer possibilities ranging from 1-2 (“my symptoms are not a problem”) to 9-10 (my symptoms are severe and I need immediate medical treatment”). A question regarding time spent using appliances such as telephone, television, tablet, and laptop was also included (with answers ranging from not at all to 5 hours a day). Subjects were asked a free-answer question for them to include relevant medical history related to dry eye disease and a free-answer question to add personal ideas or information. Lupus patients were asked how many disease reactivations were experienced from the onset of the disease and whether they had been diagnosed with Sjogren syndrome.

Statistical analysis

Data was collected and further analyzed using SPSS, version 12, SPSS Inc., Chicago, IL, USA. The biserial correlation coefficient was used between ordinal and nominal variables, the Pearson correlation between numeric variables, and the chi-square test between nominal variables. A p-value under 0.05 was considered statistically significant.

RESULTS

A total number of 123 patients diagnosed with lupus and 200 responders in the control group completed the questionnaire, with a predominance of female participants living in the urban area (Table 1). 25 patients (28.9%) among the participants with lupus erythematosus have already been diagnosed with dry eye syndrome, compared to 44 (21.9%) responders from the control group. 22 (18.2%) patients use lubricating tear drops daily, 8 (6.6%) frequently, 30 (24.8%) rarely, and 61 (50.4%) never use them. In the control group, 13 (6.5%) use lubricating tear drops daily, 17 (8.5%) frequently use them, 85 (42.3%) never and 89 (44.3%) rarely use them. Symptoms were further assessed with the aid of a Likert scale, and, in the lupus group, 43 (34.60%) patients reported having eye discomfort while, in the control group, 57 (28.35%) disclosed similar uneasiness. 23 (18.6%) lupus patients had already been diagnosed with Sjogren syndrome. Upon statistical analysis, a correlation was obtained between the dry eye diagnosis and the use of lubricating tear drops (p<0.01) but no statistically significant relation was noted between the incidence of dry eye symptoms and the presence/absence of lupus. However, more symptoms were reported in the lupus group (p=0.01), in responders diagnosed with dry eye syndrome (p<0.01), and in responders using lubrication (p<0.01). The number of episodes of lupus reactivation was correlated with dry eye syndrome (p=0.15) (Figure 1) and the use of lubricating tear drops (p<0.01). The association of lupus with other conditions was also related to dry eye syndrome (p<0.05).

Table 1: Participant characteristics

	Lupus Group	Control Group
Gender	118 (95.9%) female	184 (92%) female
	5 (4.1%) male	16 (8%) male
Area of dwelling	32 (26.2%) rural area	21 (10.5%) rural
	90 (73.8%) urban area	180 (90%) urban
Education	7 (5.69%) gymnasium	0 gymnasium
	41 (33.33%) high school	15 (7.5%) high school
	15 (12.19%) college	6 (3%) college
	38 (30.89%) university	110 (55%) university
	22 (17.88%) master/PhD	69 (34.5%) master/PhD

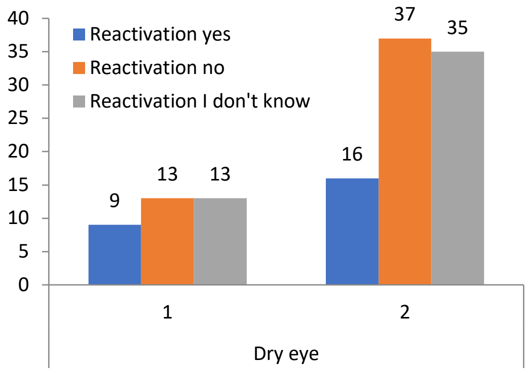

Figure 1: The relationship between dry eye and disease reactivation

A relationship was observed between television viewing in the lupus group compared to the control group (p<0.01), an inverse relationship between the habit of phone watching in the same group (p<0.01) and a relationship between the use of lubricating tear drops and tablet watching (p=0.03).

DISCUSSION

In our cohort, individuals with lupus were more frequently diagnosed with dry eye syndrome and presented more symptoms compared to the control group, even though the differences did not reach statistical significance. A literature search aiming to assess the relation between dry eye disease and several autoimmune diseases concluded that the association between the two entities is frequent but the diagnosis is commonly delayed or missed, leading to complications that affect the visual function and the quality of life of patients [3]. Lupus patients do not represent an exception to this finding. Significant modifications in dry eye parameters were displayed by a prospective consecutive case series of SLE patients. It was demonstrated that tear production and stability were pathological in patients, ocular surface disease index was higher and central density of Langerhans cells was more prominent in this group compared to controls [4]. This data is valid not only in adults but also in the younger population. A prospective study including children with systemic lupus erythematosus performed a subjective and objective dry eye analysis. Even though no difference was found in the symptomatology, corneal staining was more prevalent in children with lupus compared to healthy children, also having higher mean corneal fluorescein staining scores [5]. This data suggests the benefit of active screening in patients with lupus, both in asymptomatic patients and in those presenting subjective complaints.

Overall, the prevalence of dry eye disease was reported to be 16% in patients diagnosed with lupus erythematosus, in a study that performed a literature search of over 18.000 participants. When focusing on patient symptoms or abnormal tests, the prevalence of dry eye symptoms was 26% and the modified Schirmer test in 24% of individuals. The studies that offered enough data for statistical analysis revealed a significant association between dry eye and systemic lupus erythematosus, with lupus patients being 4 times more likely to get DED than healthy controls (OR: 4.26)[6]. The connection between DED and other systemic diseases was revealed through a study performed at a tertiary eye center involving more than 200 consecutive patients with a primary diagnosis of DED. Data showed that 108 patients (47.4%) were in evidence with a systemic inflammatory disease. Of the remaining patients, a significant number underwent further examinations and 25 patients with an existing systemic disease were identified [7].

While posterior eye involvement was traditionally considered to be related to disease activity or treatment-related adverse effects, dry eye disorder may also reflect underlying systemic inflammation. Research focusing on SLE patients with dry eye but without Sjogren syndrome revealed that the severity of keratoconjunctivitis sicca was correlated with anti-dsDNA and C3 titers but not with ESR, C4 nor with ANA [8]. In our study we did not include biomarker data as evidence of disease status, however, a correlation was found between dry eye disorder and the number of disease flare-ups since diagnosis. Reactivation in autoimmune diseases has been widely studied. However, some elements comprising the pathogenetic cascade remain incompletely understood [9]. Certain auto-immune disorders enhance immune cell infiltration and inflammatory cytokine expression in various ocular structures, amplifying inflammation and tissue dysfunction with failure of normal tear production [10]. In light of this data, we believe that LES patients with an increased number of flare-ups would benefit from dry eye disease screening.

As expected, in our study, a correlation was found between the dry eye diagnosis and the use of lubricating tear drops, more symptoms were reported in responders using lubrication and the episodes of disease reactivation were related to the use of tear drops. Achieving control of DED in patients with systemic autoimmune diseases is demanding and typically requires a systemic control of the underlying disease, with a long-term therapeutic strategy [3] that is directed toward several areas of the body [11]. Accurate identification of ocular surface inflammation, of evaporative or aqueous deficient dry eye is important for providing the most appropriate treatment for this disorder.

Therapy includes non-pharmacological interventions such as lifestyle modifications and pharmacological treatments such as topical lubricants, corticosteroids, lifitegrast and cyclosporine A and systemic agents that comprise antibiotics, poly-unsaturated omega-3 fatty acids and antioxidants, or varenicline nasal spray [10]. Procedural therapies may be complementary to the above-mentioned options and consist of punctual occlusion, meibomian gland thermal pulsation and expression, intense pulsed light and low-level light therapy, and microblepharoexfoliation [10].

Our study is limited by the nature of questionnaires and could be completed in the future by objective ophthalmological dry eye assessments that could provide further data related to this condition. Also, the inclusion of further information related to treatment options could highlight new associations.

CONCLUSION

In conclusion, dry eye disease and dry eye-related symptoms are more frequent in SLE patients. Asymptomatic patients or patients with mild symptoms could also benefit from ophthalmological consultations.

Conflicts of interest and sources of funding
The authors have no conflict of interest to declare.

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Authors' contribution
Conceptualization, EC and ML; methodology EC, CP, LP, AN; software, AB, SR; validation, all authors; investigation EC, CP, LP; data curation, LP; writing—EC, CP; writing—review and editing AB, SR, AN; visualization, ML; supervision, ML. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The research was approved by the Ethics Committee of UMF 'I. Hatieganu', Cluj Napoca.

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ORIGINAL ARTICLE

Effect of Experience and Professional Role on Psychophysiological Stress Response in an Underwater Evacuation Training

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Abstract: *Background: This research aimed to analyze the effect of experience and professional role on psychophysiological stress response in underwater evacuation training. Methods: We analyzed 36 participants (39.06±9.01 years), divided into two different groups; 17 crew members (38.6±7.2 years) and 19 medical members (39.5±10.5 years). modifications in the rating of perceived exertion, subjective stress perception, heart rate, blood oxygen saturation, cortical arousal, heart rate variability, spirometry, isometric hand strength, and short-term memory before and after underwater evacuation training were analyzed. Results: The maneuver produced a significant increase in SSP, RPE, IHS, FVC, and SatO$_2$ at different moments of the intervention, being higher in crew members. Conclusions: We found that the underwater evacuation training produced an anticipatory anxiety response, and an increase in autonomous sympathetic nervous system modulation not affecting strength capacities, cortical arousal, and memory independently of the aircraft role (medical or crew).*

Keywords: *stress; underwater; military; aircrew; experience; role; cortical arousal; heart rate variability*

INTRODUCTION

Previous studies have shown how military combat situations are one of the most stressful events a human can cope with, due to their unpredictable, dangerousness, and highly demanding context [1]. Independently of the situation, military interventions produce the activation of the autonomic nervous system (ANS), initiating the flight-fight system involved in survival behaviors [2]. The stress produced by these extreme situations negatively impacts cognitive processes like memory, cortical arousal, decision-making, and information processing, reducing their normal functioning and even promoting the appearance of pathologies such as post-traumatic disorder (PTSD) [3-5].

Several types of research have been carried out in the military population, especially in ground military units, and the results show how an acute stress exposition can lead the organism to a downregulation in cortical arousal and high activation of the Sympathetic Nervous System (SNS), and finally modifying the blood pressure (BP), heart rate (HR), cortisol hormones, cortical arousal (CFFT), skin temperature, subjective stress perception (SSP), and heart rate variability (HRV) among others [6-10]. In this line, aircrews and paratroopers researchers have shown an increase in the physiological response by the increase in cortisol hormones, an increase in sympathetic modulation, and a decrease in cortical arousal, which is a symptom of Central Nervous System (CNS) fatigue [1,5,6,11-15].

Another factor that can negatively affect stress response is the possibility of having an aircraft accident [16]. The survival of the aircrew depends extremely on their knowledge of underwater evacuation training and stress management, and little is known about the physical and mental requirements of this specific and demanding situation, but as some researchers have demonstrated previously, the personal experience and professional training could modulate the stress experience and reduce the negative impact on the organism [2,4,11,13,15,17-18]. For this reason, we conducted the present research to analyze and compare the psychophysiological stress response of crew members (4 crew members; 4 jump chiefs; 9 paratroopers) and medical members (15 flight nurses and 4 doctors) in an underwater evacuation training. We hypothesized that 1) the underwater evacuation training would increase the sympathetic autonomic modulation of the crew members, and 2) crew members would present a lower stress response.

MATERIALS AND METHODS

In the present work were analyzed 36 participants (39.06±9.01 years), divided into two different groups; 17 crew members (38.6±7.2 years; 174.9±8.6 cm; 82.1±11.2 kg; 18.1±8.7 years of service; 14.9±13.4 months in mission; 357.9±366.9 flying hours) and 19 medical members (39.5±10.5 years; 173.6±6.14 cm; 71.4±10.5 kg; 15.5±9.99 years of service; 11.5±15.3 months in mission; 25.1±73.8 flying hours). The entire research procedure was conducted by soldiers with the standard flying suit and boots, portable oxygen cylinder, life vest, helmets, and earphones to simulate the equivalent operating equipment for this type of action. None of the participants had previous or recent experience in these underwater simulations.

A pre-intervention-post intervention was conducted, analyzing the following psychological and physiological variables previous to and immediately after an underwater evacuation training.

1. Rating of perceived exertion (RPE). Using Borg 6–20 scale [19];

2. Stress subjective perception (SSP) on a 1–100 scale [15];

3. Blood oxygen saturation ($SatO_2$) and heart rate (HR) by a finger pulse-oximeter system (PO 30 Beurer Medical) [6];

4. Cortical arousal and fatigue of the central nervous system (CNS) were measured by the Lafayette Instrument critical flicker fusion threshold (CFFT) (Model 12.021) by the average of five incremental tests (20 to 100 Hz) according to previous authors [5,16];

5. Isometric handgrip strength (IHS) by a grip dynamometer using the dominant hand and taking the better result of two attempts (Takei Kiki Koyo, Japan);

6. Spirometry: forced vital capacity (FVC), volume exhale at the end of the first second of forced expiration (FEV1) and the peak expiratory flow (PEF) using a QM-SP100 (Quirumed, Spain) spirometer in a maximum inhale-exhale cycle [5,16];

7. Short-term memory (STM). A three-digit number was shown to the participants for one second. After five seconds, they were asked to remember the number and to say it backward [16];

Finally, the heart rate (HR) and heart rate variability (HRV), which refers to the autonomic modulation, were recorded with a Polar V800 heart rate monitor with RR measurement function (POLAR. Finland) and posteriorly analyzed by the Kubios HRV standard 3.2.0 software (University of Kuopio, Kuopio, Finland). The parameters analyzed were:

1. Temporal domain: mean HR; minimum HR (Min HR); maximum HR (Max HR); the square root of the mean value of all sums of square differences of all R-Rs following intervals (RMSSD); the percentage of differences between normal adjacent R-R intervals greater than 50 ms (pNN50);

2. Frequency domain: low-frequency band (LF); high-frequency band (HF); A ratio of low frequency to high frequency (Ratio LF/HF);

3. Nonlinear domain: sensitivity of the short-term variability (SD1); sensitivity of the long-term variability (SD2).

The underwater evacuation training was performed in a swimming pool in the following conditions: 4 × 4 × 3 swimming pool size (m); water characteristic: chlorine 2 (ppm); pH 7.5; turbidity values 0.13 (NTU); temperature: 26°C. The complete training was performed in the presence of two instructors and two water assistants. It was composed of four different stages, with a duration of 30 minutes each one, where the participants had to: 1) use a portable oxygen cylinder and carry out apnea training in several positions; 2) evacuate the submerged aircraft accident through the window; 3) rescue training of a raft and rescue training from a helicopter; and finally 4) underwater evacuation during the turning of a ship.

Statistical analysis was performed with the SPSS 24.0 statistical program. The descriptive statistics used to report the results were the mean (M) ± standard deviation (SD). The normality of the sample was determined with the Kolmogorov-Smirnov test, and all the variables presented a parametric distribution. The repeated measures ANOVA was conducted to test differences in the evaluation moments analyzed. A T-test for independent variables was used to analyze differences between the two groups analyzed (crew and medical). The Effect Size was calculated by Cohen's D. The significance level was $P \leq 0.05$.

RESULTS

The results were reported as M ± SD. SSP showed a significant increase in post-values of both crew and medical members concerning the pre-values, being higher in crew members, but there were no significant differences in pre- or post-values between groups. RPE presented a significant difference in post values, being higher in crew members, and also showed a significant increase in post values of both groups in comparison with pre values. IHS showed a significant difference in pre-values between crew and medical responses, being higher in crew members. FVC showed only a significant increase in post values of crew members in comparison with pre-values of crew members. $SatO_2$ showed a significant difference in post values of crew and medical members, being higher in crew members (Table 1).

Table 1: Role comparison in pre-post variables

Variables	Role	Pre Intervention				Post Intervention			
		M±SD	Bilateral signification	95% confidence interval		M±SD	Bilateral signification	95% confidence interval	
			P	Inferior	Superior		P	Inferior	Superior
SSP	Crew member	16.2±20.9	0.142	-26.71	4.01	60.6±27.2 (0.000)	0.358	-9.70	26.14
	Medical member	27.5±24.1				52.4±25.2 (0.001)			
RPE	Crew member	6.7±1.5	0.075	-0.07	1.38	13.7±2.9 (0.000)	0.023	0.32	4.13
	Medical member	6.1±0.2				11.4±2.7 (0.000)			
IHS (N)	Crew member	50.9±11.4	0.026	1.17	17.24	50.4±10.7	0.084	-1.02	15.53
	Medical member	41.7±12.3				43.2±13.4			
FVC (ml)	Crew member	4.4±0.8	0.412	-0.48	1.13	4.8±0.9 (0.047)	0.374	-0.36	0.94
	Medical member	4.1±1.4				4.5±1.0			
FEV1 (ml)	Crew member	3.7±0.7	0.464	-0.47	1.00	3.8±0.7	0.956	-0.52	0.55
	Medical member	3.4±1.4				3.8±0.8			
PEF (ml)	Crew member	9.8±1.8	0.548	-1.31	2.42	9.8±1.9	0.705	-1.27	1.86
	Medical member	9.3±3.4				9.5±2.6			
$SatO_2$ (%)	Crew member	95.9±1.9	0.186	-2.01	0.41	97.1±2.4	0.040	0.08	3.30
	Medical member	96.7±1.7				95.4±2.4			
HR (bpm)	Crew member	81.5±14.2	0.701	-8.27	12.17	81.4±18.8	0.401	-7.15	17.44
	Medical member	79.6±15.8				76.3±17.5			
STM	Crew member	1.0±0.0	—	—	—	1.0±0.0	—	—	—
	Medical member	1.0±0.0				1.0±0.0			
CFFT (Hz)	Crew member	39.9±3.9	0.365	-1.34	3.55	38.1±3.5	0.840	-2.29	2.80
	Medical member	38.8±3.4				37.8±3.9			

1(M) mean; (SD) standard deviation; (SSP) stress subjective perception; (RPE) rating of perceived exertion; (IHS) isometric hand strength; (FVC; FEV1; PEF) spirometry; (SatO2) blood oxygen saturation; (HR) heart rate; (STM) short term memory; (CFFT) cortical arousal.

The mean HR of medical members presented the lowest value during the intervention and increased post-intervention, being higher than in crew members. Min HR presented similar values in both groups in pre- and during moments, but crew members' values were higher in post-intervention. Max HR of medical members was higher than crew members in all moments, during the intervention the highest. RMSSD presented similar values in both groups in the pre-moment; medical member values were higher during the intervention and decreased in post-intervention in comparison with crew members.

Variable pNN50 presented in both groups an increase during the intervention, being higher in medical members than in crew members, and decreasing in post-intervention in both groups. LF showed high values in all moments in medical members, being higher in pre-moment than post-intervention, and decreasing during the intervention. HF presented higher values in crew members than in medical members pre- and during the intervention, being the highest value during the intervention, and decreasing post-intervention in both groups. LF/HF Ratio showed higher values in medical members than in crew members in pre- and during the intervention, being the highest value in the pre-moment, while crew members presented higher values in post-intervention. SD1 presented similar values in pre-intervention in both groups, being higher during intervention in medical members than in crew members and decreasing in post-intervention in comparison with crew members.

SD2 presented higher values in all moments in medical members than in crew members, being the highest during the intervention and decreasing in both groups in post-intervention in comparison with pre-momentum (Table 2).

Table 2: Role comparison in pre-, during, and post-HRV variables

Variables	Role	Pre-intervention (1)				During intervention (2)				Post-intervention (3)				F	IC
		M±SD	BS	95 % confidence interval		M±SD	BS	95 % confidence interval		M±SD	BS	95 % confidence interval			
			P	Inferior	Superior		P	Inferior	Superior		P	Inferior	Superior		
Mean HR (bpm)	Crew member	84.9±10.6	0.179	-16.49	3.19	90.8±7.7	0.837	-8.08	6.59	97.4±13.8	0.919	-9.66	8.73	9.942	1<3;2<3;3>1<2
	Medical member	91.6±17.3				54.3±18.1				97.9±13.4				5.711	2<3; 3>2
Min HR (bpm)	Crew member	67.1±10.8	0.983	-8.67	8.48	54.9±13.9	0.906	-10.38	11.68	71.4±9.9	0.265	-3.45	12.15	13.639	1>2; 2<1<3; 3>2
	Medical member	67.2±14.1				54.3±18.1				67.1±12.8				5.637	1>2; 2<1>3; 3>2
Max HR (bpm)	Crew member	116.0±18.1	0.111	-36.68	3.95	165.2±25.1	0.351	-53.56	19.54	131.7±23.9	0.162	-42.48	7.41	24.432	1<2; 2>1<3; 3<2
	Medical member	132.4±37.4				182.2±70.1				149.2±45.2				4.710	1<2; 2>1
RMSSD (ms)	Crew member	33.1±19.2	0.989	-12.98	12.80	41.7±19.8	0.523	-15.34	7.95	36.8±24.2	0.743	-10.89	15.13	3.948	—
	Medical member	33.2±18.9				45.4±14.4				34.7±13.1				10.764	1<2; 2>1<3; 3<2
pNN50 (%)	Crew member	7.8±7.9	0.768	-4.27	5.74	8.1±5.3	0.051	-7.29	0.02	6.5±6.1	0.790	-4.07	3.12	1.102	—
	Medical member	7.1±6.9				11.7±5.5				6.9±4.4				15.047	1<2; 2>1>3; 3<2
LF (n.u)	Crew member	77.4±13.8	0.408	-11.19	4.65	72.6±11.1	0.756	-7.22	5.29	76.1±11.2	0.992	-6.98	6.91	1.081	—
	Medical member	80.6±9.5				73.5±7.2				76.2±9.3				4.296	1>2; 2<1
HF (n.u)	Crew member	22.6±13.7	0.409	-4.64	11.14	27.3±11.0	0.762	-5.28	7.15	23.7±11.1	0.990	-6.94	6.85	1.085	—
	Medical member	19.3±9.4				26.4±7.1				23.8±9.3				4.286	1<2; 2>1
LF/HF Ratio (ms^2)	Crew member	5.2±3.9	0.942	-2.32	2.16	3.4±2.4	0.888	-2.21	1.92	4.8±4.3	0.521	-1.61	3.11	3.028	—
	Medical member	5.3±2.7				3.6±3.5				4.0±2.5				2.730	—
SD1 (ms)	Crew member	23.4±13.6	0.969	-9.44	9.08	29.5±14.0	0.516	-10.96	5.61	26.1±17.2	0.751	-7.82	10.73	3.912	—
	Medical member	23.6±13.7				32.2±10.4				24.6±9.5				10.780	1<2; 2>1<3; 3<2
SD2 (ms)	Crew member	54.8±17.8	0.507	-20.23	10.19	58.5±13.1	0.138	-19.55	2.83	51.6±17.8	0.276	-19.56	5.76	1.768	—
	Medical member	59.8±25.9				66.9±19.0				58.5±19.4				5.168	2>3; 3<2

2(M) mean; (SD) standard deviation; (BS) bilateral signification; (IC) intermoment comparison; (Mean HR) mean heart rate; (Min HR) minimum heart rate; (Max HR) maximum heart rate; (RMSSD) square root of the mean of the sum of the squared differences between adjacent normal R-R intervals; (pNN50) percentage of differences between normal adjacent R-R intervals greater than 50 ms; (LF) low frequency; (HF) high frequency; (Ratio LF/HF) A ratio of low frequency to high frequency; (SD1; standards deviations of the scattergram 1; (SD2) standards deviations of the scattergram 2; (n.u) normalized unit.

DISCUSSION

This study aimed to analyze and compare the psychophysiological stress response of crew members (4 crew members, 4 jump chiefs, 9 paratroopers) and medical members (15 flight nurses and 4 doctors) in an Underwater Evacuation Training. Hypothesis 1) was confirmed since underwater evacuation training increased the sympathetic autonomic modulation of crew members. Hypothesis 2) was not confirmed, since the stress response was similar in both crew member and medical member groups, independently of the professional role.

The underwater evacuation training includes apnea periods, in which aircrew members have to deal with a lack of oxygen that produces a lower oxygen partial pressure in the alveolus and affects the oxygen that the arterial blood can deliver to the brain [20]. In both hypoxia and underwater training, the lack of oxygen impacts the RPE, although in underwater training SSP and SatO2 values are generally higher than those in hypoxia [3,21-24]. These results suggest that for underwater training, the exertion mechanism is produced by short acute periods of apnea instead of larger periods with progressively less oxygen partial pressure if compared to hypoxia training. IHS has also been previously studied in military aircrew with different results depending on the previous training and roles [3], which coincides with the pre-results of this study with aircrew members having more initial strength capacity than medical personnel. If we analyze the breath capacity, PEF, and FEV1 have traditionally been the most affected variables in contexts of sustained hypoxia as specific training in these conditions, special operation selection course, and maneuvers, or real flight conditions [1,3,14,15,26]. Whilst FVC was the most activated variable in underwater training: a constant need to store oxygen in the alveolus before an acute period of apnea activates the lunges to store more air as a physiological adaptation. Autonomic modulation has been previously evaluated in military aircrews by the HRV in contexts of hypoxia, finding an increased sympathetic autonomic modulation as expected, since the lack of oxygen acts as a stressor of the central nervous system [21,24,26,27]. Hypoxia symptoms have been previously studied in military crews through cortical arousal with different results: the decrease of oxygen saturation had a negative [27] or neutral [3] impact in cortex performance, which are similar results to those found in this study.

The underwater evacuation training simulates an extreme situation in which aircrew and medical members first try to survive. This fact could be the reason the post measures were not different for each group, since the responses in these extremely hazardous situations tend to be maximal and could equal the response in both groups. This large stress response independently of the role was also measured in different military units in combat maneuvers where, contrary to the present study, there were changes in respiratory musculature, memory, and cortical activation [2,28-30]. Medical members had less RPE and SatO2 after the training, maybe because of shorter periods of physical activity during some maneuvers. The most expected difference was found in the measures before the training, where the aircrew members were able to develop more strength capacity due to their previous training, more oriented to physical fitness.

Regarding the HRV analysis, we observed an increase in vagal tone (RMSSD, HF, HF/LF ratio, and SD2) of participants, a result probably due to the 2h training in the water and the possible induced hypothermia [31]. Increases in the HF values under these hypothermia conditions may reflect a reduced delay in baroreflex response, enabling lower resonant frequency in blood pressure and RR interval, which may be a consequence of temperature-mediated increase in peripheral sympathetic activity and myogenic reactivity due to increased vascular tone and transmural pressure caused by peripheral vasoconstriction to prevent heat dissipation [32]. LF/HF ratio is a controversial marker of sympathovagal balance, and is difficult to interpret due to the complexity of the origin of LF oscillations, along with the effects of varying breathing pattern on HF oscillations, which may present low accuracy and physiological confidence. However, it presents the same trend as RMSSD which is a stronger marker of cardiac vagal activity, independent of the breathing pattern, consequent with SD2 values which reflect the long-term fluctuations of RR intervals, an inverse indicator of sympathetic activity. This autonomic response was similar to crew or medical members in the underwater evacuation training.

The cardiovascular response measured was higher compared to the effort developed, mean HR among training is likely produced due to the shifts of blood volume from the periphery to the central circulation due to peripheral vasoconstriction and major cardiovascular output, to reduce heat loss and defend core body temperature by increasing the thickness of the insulative layer between the ambient air and perfused blood vessels [33]. The fact that HR response increased despite a likely augmented venous return highlights the fact that significant thermogenesis was occurring, requiring an increased HR to support the metabolic activity.

When basal HRV values analyzed are compared to reference basal values [34], it suggests an anticipatory anxiety response, which may be explained as the training was an evaluative test/training for soldier's professional careers, in addition to the risk, demands, and specific implications of the training. This response is consequent with the pre-SSP values evaluated, showing the large stress of this eliciting training, in line with other military operations such as underground combat, parachute jumps, urban combat, or even air combat jet maneuvers and disorientation training [2,6,13,14,31,35, 36]. In addition, a reminiscence of the sympathetic activation produced by the training was found in the decrease of RMSSD or the increases in HR parameters, highlighting the psychophysiological demands of the task.

Limitations of the study and future research lines

The small number of subjects analyzed has been one of the main limitations of the present work. A greater number of participants would provide more reliable results. However, the difficulty of accomplishing these types of actions due to this complexity, hazard, and economic and human resources needed were a limiting factor. Another limitation was the use of indirect measurement instruments such as the fusion flicker system. Logistics and economic difficulties have limited the use of more modern systems, but the fact that the evaluations were conducted in a very limiting environment, made nearly impossible the use of other instruments such as electroencephalography. Finally, a lack of control of hormones such as alpha-amylase and cortisol to control the hormonal stress response. Future research might seek to address these issues. Given the importance of operational and specific training to allow better survival in air accidents in open water areas, further research should try to design an optimal stimulus and protocol to improve the habituation process.

Practical applications

The present study highlights the psychophysiological response of both crew and medical members undergoing underwater rescue training. This information leads us to a better understanding of the physiological and psychological stress response to this specific and demanding situation in which there is a life risk. The application of this information could contribute to a better design of underwater evacuation training and for both civil and military air personnel preparation.

CONCLUSION

We found that underwater evacuation training produced an anticipatory anxiety response, and an increase in autonomous sympathetic nervous system modulation not affecting strength capacities, cortical arousal, and memory independently of the aircraft role (medical or crew).

Conflicts of interest and sources of funding
The authors declare no conflict of interest. This research received no external funding.

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Authors' contribution
Conceptualization, Marta Vicente-Rodríguez, Álvaro Bustamante-Sanchez, and Jose Alberto Parraca.; methodology, Marta Vicente Rodríguez, and Jose Tornero-Aguilera.; software, Vicente Javier Clemente-Suárez.; validation, Vicente Javier Clemente-Suárez, and Jose Tornero-Aguilera.; formal analysis, Jose Tornero-Aguilera, and Álvaro Bustamante-Sanchez.; investigation, Marta Vicente-Rodríguez, and Jose Alberto Parraca.; resources, Vicente Javier Clemente-Suárez.; data curation, Marta Vicente-Rodríguez, and Vicente Javier Clemente-Suárez.; writing—original draft preparation, Marta Vicente-Rodríguez and Jose Alberto Parraca.; writing—review and editing, Marta Vicente-Rodríguez.; visualization, Jose Tornero-Aguilera.; supervision, Vicente Javier Clemente-Suárez.; project administration, Álvaro Bustamante-Sanchez and Vicente Javier Clemente-Suárez.; funding acquisition, Vicente Javier Clemente-Suárez. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The study was conducted following the Declaration of Helsinki, and approved by the Ethics Committee of European University of Madrid (CIPI/18/093). Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Sedation Techniques for Drug-induced Sleep Endoscopy in Adult Patients with Sleep-disordered Breathing

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Abstract: *Drug-induced sleep endoscopy emerged and developed as an essential alternative diagnosis tool in sleep-disordered breathing patients. Accurate identification and dynamic characteristics of upper respiratory airway obstruction are paramount in establishing an adequate therapeutic plan and selecting for particular surgical or non-surgical strategies. This article reviews the current aspects of intravenous sedation for drug-induced sleep endoscopy (DISE) in adults, emphasizing its implications for patients with sleep apnea. The ideal sedative agent for DISE should replicate natural sleep patterns while maintaining upper airway dynamics. The clinical practice exhibits a significant variability in drug selection, with midazolam and propofol often being preferred. The review presents emerging trends and advancements in DISE, emphasizing its role in optimizing surgical indications, predicting outcomes, and screening patients for alternative therapeutic strategies for OSA. Future research is needed to understand better the impact of specific sedative drugs on DISE findings, contributing to the development of standardized protocols and the evolution of new sedative drugs with improved effects and optimized techniques for delivering sedation.*

Keywords: *drug-induced sleep endoscopy, sedation, target-controlled infusion, obstructive sleep apnea*

INTRODUCTION

Obstructive sleep apnea (OSA) represents one of the most encountered forms of sleep-disordered breathing. OSA is characterized by iterative complete or partial collapse of the upper respiratory airway (UA). Number of obstruction events registered during one hour of sleep, using polysomnography (PSG), quantifies clinical severity. This number of hourly events, known as the apnea-hypopnea index (AHI), has paramount utility in the diagnosis and grading of the severity of OSA.

The prevalence of OSA, determined from extensive population studies, varies largely, between 9% and 38% in the general adult population [1]. Clinical consequences of OSA are increased cardiovascular risk, arterial hypertension, cognitive decline, obesity, and elevated risk of being part of a vehicle accident.

Diagnosis and classification of sleep-disordered breathing are accomplished using nocturnal polysomnography, which represents the gold standard investigation. Evaluation of the upper respiratory airway using drug-induced sleep endoscopy (DISE) emerged as an alternative diagnosis tool with significant benefits in some OSA patients.

DISE represents a complementary diagnosis technique helpful in offering additional data about upper airway (UA) dynamics and conformation. The principle of DISE resides in real-time dynamic and three-dimensional fiberoptic airway evaluation during pharmacologically induced profound sedation aimed to reproduce as similar as possible natural sleep.

Concerning the therapeutic perspective, the first-line accepted treatment for OSA is continuous positive airway pressure (CPAP). However, this therapeutic approach has some limitations, including low adherence and difficulty in long-term acceptance [2]. To date, expert opinion divergences persist concerning the routine DISE evaluation in all OSA patients or performing it only in selected patients in whom intolerance to CPAP is encountered or the clinician intends to offer treatments alternative to CPAP, such as oral appliance therapy

(OAT), upper airway surgery (UAS), positional therapy (PT), or some combination of different modalities [3].

The purpose of this article is to provide a review of current aspects of the intravenous sedation approach for performing DISE in adults. Pediatric DISE is beyond the scope of this review; a comprehensive expert consensus statement on this topic was published in 2021 [4], aiming to clarify some controversies on this topic in the pediatric population

CONCEPT OF DISE

Croft and Pringle [5] delineated the principle of DISE for the first time in 1991. The authors described the use of midazolam sedation in sleep-disordered breathing patients concomitantly performing endoscopic airway evaluation of obstruction sites. The developments of the technique over the last four decades have established a definitive role in the clinical approach of OSA patients.

Compared to awake examination of the upper respiratory airway, DISE was described in one study as a potential tool able to change the surgical approach in OSA patients candidates for surgery, interfering consequently with operative indication in approximately 50% of patients [6]. In contrast, in a prospective multicentric trial addressing the impact of DISE on surgical outcome, Pang et al enrolled 326 patients [7] and demonstrated that performing preoperative accurate DISE may not significantly impact the surgical success outcome in OSA patients.

Multiple controversies persist in the literature concerning other aspects of the DISE technique: feasibility for selecting a particular patient for surgery or non-surgical treatment, standardization in sedation technique, monitoring depth of sedation, scoring and classification systems for anatomic levels, and configuration of obstruction pattern. Although DISE evolution encompassed a long period and the topic of performing sedation similar to natural sleep evolved and developed on the strength of new technical acquisitions (BIS monitoring, use of propofol with target-controlled infusion devices, new sedative drugs), its role in OSA patients needs more clarifications.

SEDATIVE DRUGS FOR DISE

There is a large variability in the sedation techniques, drugs, and protocols described in the literature regarding DISE. Conflicting data exist regarding available drugs for sedation, technique of administration, combination of drugs, and dosage; accordingly, a standard, unanimously accepted sedation protocol has not been established and available so far. Beyond the selection of the specific sedative drug or combination, avoiding oversedation is another essential topic to be considered when performing the technique.

Sedative drugs most widely used are midazolam, propofol, dexmedetomidine, and, more recently, remimazolam [8]. The ideal sedative agent for DISE is debated; this is intended to have some neurophysiologic characteristics in reproducing normal sleep patterns and insignificant relevance on UA dynamics during sedation. Neurophysiologic characteristics of an ideal sedative drug for DISE are summarized in Table 1 [9].

Table 1: Neurophysiologic characteristics of ideal agents for DISE

Reproduction of
- Stage NREM 1, NREM 2, and/or REM sleep
Preservation of
- Mechanoreceptor input to the brainstem - Chemoreceptor input to the brainstem - Respiratory rhythmicity by pre-Botzinger complex - Lower cranial nerve output to pharyngolarynx

NREM, nonrapid eye movement; REM, rapid eye movement

Intravenous anesthetic drugs most frequently used are midazolam and propofol [9]. These drugs present some pharmacologic properties that confer advantages in achieving the desired level of sedation. Therefore, the possibility of attaining the critical closing pressure of the airway is similar to natural sleep [10]. Critical closing pressure (Pcrit) represents a measure of UA collapsibility, an essential parameter variable from a healthy patient (low collapsibility) to an OSA patient (high collapsibility). Some studies [10,11] have demonstrated that sedation reproduces natural sleep in non-REM sleep; consequently, DISE represents a valid tool for examining respiratory airways in OSA patients. The REM sleep phase is difficult to reproduce in studies. Therefore, in clinical practice, for patients who manifest apneas only in REM sleep, DISE is not an option for diagnosis.

One another important topic in UA evaluation during DISE is adequately targeting the clinical depth of sedation. A very deep plane of sedation may be inaccurate as it induces obstruction at different levels, not reproducing it similarly to natural patient sleep. Consequently, monitoring the depth of sedation, and the objective measurement of sleep depth represents an important tool in technique validity. This can be accomplished by using EEG-derived index monitoring techniques, such as bispectral index monitoring (BIS), adopted in advanced clinical practice, and replacing simple clinical evaluation (patient's reaction to verbal or tactile stimuli). A recent meta-analysis [12] demonstrated a statistically significant correlation between BIS sedation level and the area of obstruction on the VOTE score (a grading score of UA collapsibility that quantifies obstruction at vellum, oropharynx, tongue, and epiglottis level).

Comparing different sedative drugs for DISE, in one study, Viana and Zhao [13] enrolled 52 patients sedated with propofol, midazolam, and dexmedetomidine for performing airway evaluation. The selection of the drug was proved to have a relevant influence on evaluation findings. Midazolam and propofol presented an increased incidence of tongue base collapse compared with dexmedetomidine.

The clinical practice is characterized by a significant variability in drugs used for DISE. As mentioned before, midazolam or/ and propofol remain the first option, but infrequently, sedative drugs like ketamine, dexmedetomidine, and remifentanil are also used. Recently, remimazolam demonstrated feasibility and a better safety profile than propofol [14]. Some authors described a combination of different drugs.

The European position paper on drug-induced sleep endoscopy (DISE) [15], published in 2014, and the update [16] from 2017 offers suggestions regarding sedative drugs and techniques, a recommendation as the first option consisting of using midazolam and propofol, single or in combination. Since 2017, new studies have been published relevant to new sedative agents or new aspects of airway obstruction, correlated to sedation depth and mechanism. The crucial aspect reiterated in these papers involves the precautious and careful execution of technique, because overdosing may induce UA collapse and misinterpretation of investigation results. Table 2 presents the advantages and disadvantages of sedative drugs used in DISE and their combination.

Table 2: Sedative drugs for DISE – advantages and disadvantages

Sedative agents	Advantages	Disadvantages
Propofol	Rapid onset Predictable effect Less muscular relaxation Inactive metabolites	Technique Advanced expertise in TCI (target controlled infusion) Increased collapsibility when inadvertently profound sedation
Midazolam	The examination window is longer Antagonist available	Slower onset Very careful titration is necessary Prolonged recovery and longer facility stay
Dexmedetomidine	Sedation pattern similar to natural sleep	Prolonged recovery Slower onset Precaution in unstable cardiac patients Some protective effects for airway collapse
Combination propofol plus midazolam	Faster onset	More frequent sneezing than propofol alone
Remimazolam	Better profile than propofol Less hypoxemia Very fast recovery	Less studied in DISE
Propofol plus remifentanil	Enable a reduced target concentration of propofol No respiratory depression	Only a few studies
Dexmedetomidine plus ketamine	Good safety profile in children Ketamine has minimal effect on airway patency	DISE in the pediatric population Not universally used

Propofol

Propofol is one of the most widely used intravenous anesthetics in current medical practice. The mechanism of action of propofol remains unknown, possibly consisting of positive modulation of the inhibitory function of the neurotransmitter gamma-aminobutyric acid (GABA) through GABAA receptors [17]. Concerning the differences and similarities between natural sleep and sedation induced with propofol, studies with high-density encephalography (hd-EEG) have revealed that propofol sedation is a sleep-like state and the large slow waves induced by propofol anesthesia were similar in appearance, topography, negative-going slope, and positive-going slope to the slow waves seen during NREM sleep [18].

Propofol pharmacokinetics (PK) is characterized by hepatic elimination as the primary route and inactive metabolites with kidney excretion. The onset of effect is fast, depending on the administered dose, rate of administration, and patient's response.

Administration and dosage of propofol

The DISE technique can be accomplished in three different ways: iterative bolus technique, manual controlled infusion (MCI), and target-controlled infusion (TCI).

Manually controlled infusion involves delivering a dose at 50-100 ml/h and adjusting according to clinical response [15]. The bolus technique comprises different administration protocols, depending on clinician preference, with a starting dose of 30-50 mg and repeated 10 mg every 2 min or initially 1 mg/kg and afterward 20 mg every 2 min.

Target-controlled infusion systems perform sedative drug infusion using software-controlled infusion pumps to achieve a desired target concentration of drug in the blood or brain (effect compartment). This method offers the possibility of rapid achievement of concentration in the target compartment and maintenance of the intended effect based on incorporated software algorithms. Several studies have confirmed the superiority of TCI technology in delivering propofol for sedation in DISE, consequently, this approach is currently recommended as a standard approach [15,16]. The main models conventionally in use are those described by Marsh [19] and Schnider [20]. The Schnider model is an established pharmacokinetic model that has been widely used in clinical practice. It is an extension of the Marsh model and is designed for TCI systems. Recently, a new pharmacokinetic-pharmacodynamic (PK-PD) model for propofol was described by Eleveld for a broad population range [21]. It was developed to address limitations in previous models and improve accuracy in predicting drug concentrations. This general-purpose model presents multiple advantages in reducing the risk of incorrect selection of a specific model for a particular patient, extrapolation, and unfamiliarity of clinician anesthesiologists with preexisting models [22].

European consensus update from 2017 [16] recommends the TCI technique as the first option in administering propofol sedation for DISE; the starting effect-site concentration should be 2.0-2.5 µg/ml, with gradually increasing dose by 0.2-0.5 µg/ml until reaching the target state of sedation.

The cited consensus does not recommend a specific PK-PD model for propofol administration. Marsh or Schnider models are equally utilized in different studies, and up to the present, to our knowledge, the Eleveld model has not been tested for DISE.

Midazolam

The literature recommends only the bolus technique, with initially 0.05 mg/kg, observing the effect for 2-5 minutes, and readministering 0.03 mg/kg if the patient is not asleep. Repeated doses of 0.015 mg/kg may be necessary for supplementing sedation depth, with careful observing after every administration for 5 minutes [16].

Combination of propofol plus midazolam

Midazolam influences the pharmacokinetics of propofol by interfering with metabolic clearance and increasing the plasma concentration of propofol [23]. The purpose of this combination is to decrease patient anxiety with midazolam 0.05 mg/kg initially and after 2 minutes proceed with sedation using propofol delivered with TCI pump in effect-site concentration 1.5-3 µg/ml, and subsequent concentration increased by 0.2-0.5 µg/ml, until achieving the desired level of sedation [16].

Dexmedetomidine

European consensus on DISE [4] does not recommend sedation with dexmedetomidine as the first choice, although this drug presents some advantages, including a lower risk of hypoxemia and respiratory depression. The disadvantages of using dexmedetomidine consist of prolonged evaluation time and a protective effect on airway collapse (tongue base and hypopharyngeal structures [24]. Loading dose should be 1-1.5 µg/kg in 10 minutes, continuing with 1-2 µg/kg/h, with careful clinical observation of sedation level.

Remifentanil

Some authors recommend against using fentanyl, remifentanil, and ketamine as adjunctive drugs to propofol or midazolam, due to the increased risk of oxygen desaturation [24]. Recent research suggests that remifentanil may offer benefits as an adjunctive sedative drug, decreasing cough reflex, shortening the time of the procedure, and decreasing effect site concentration intended for propofol sedation [25,26]. The effect-site concentrations of remifentanil administered in two studies are 1.5 ng/ml and 0.5 ng/ml respectively using the Minto PK-PD model [25,26].

Ketamine

Ketamine is primarily used in pediatric DISE in association with dexmedetomidine [27]. It is administrated in doses 1-2 mg/kg, simultaneously with a loading dose of dexmedetomidine (3 µg/kg in 10 minutes followed by continuous infusion of 2 µg/kg/h). This combination is preferred due to lower risk (upper airway obstruction, respiratory depression) and the preservation of non-rapid eye movement sleep [28].

PATIENT SAFETY

Most studies do not describe significant complications associated with the DISE procedure. Standard monitoring is mandatory, and avoiding oversedation and muscular UA relaxation is vitally important in performing a safe and accurate investigation. Most frequently cited incidents consist of central apneas [29] and respiratory depression, especially when the depth of sedation is not carefully titrated. Patients should be thoroughly evaluated for any contraindications before undergoing DISE, and the procedure should be conducted with careful monitoring and adherence to safety protocols. The incidence of complications with DISE is generally low, and the procedure is considered safe when performed by trained and experienced healthcare professionals in a controlled environment. The Guidelines of the French Society of ENT on DISE [30] emphasize the lack of data in the literature regarding the contribution of capnography to safety during DISE, as well as routine oxygen administration to prevent procedure-induced hypoxemic complications.

EMERGING TRENDS AND ADVANCEMENT

It has been demonstrated in multiple studies that DISE could help the surgeon in formulating the optimal operative indication and could predict outcomes after UA surgery [31]. Concerning the therapy with CPAP, the role of DISE consists mainly of identifying the anatomic basis of CPAP failure and obstruction pattern with this therapeutic indication. New developments have placed DISE as a useful instrument in screening patients for alternative therapeutic strategies for OSA: oral appliance therapy, trans-oral robotic surgery, hypoglossal nerve stimulation, and maxillomandibular advancement surgery [20]. Some limitations of the DISE technique impose awareness in the therapeutic approach of OSA patients. Although the technique is complementary to polysomnography, the clinical findings from DISE do not represent the entire night's sleep but only a limited fraction of it. Standardization of sedation techniques and establishing protocols will improve observed obstruction patterns in OSA heterogeneous groups of patients.

CONCLUSION

Nowadays, it is unanimously accepted that we need a better understanding of the impact of using a specific sedative drug on DISE findings. Future prospective randomized trials will provide insight into clarifying the controversies on the impact of sedative drug selection on DISE findings and on the influence on sleep architecture and stages, contributing to a better sedation protocol. The development of new sedative drugs with more predictive effects and rapid elimination, along with optimizing the administration technique with the aid of a new general-purpose PK-PD model incorporated in TCI devices could offer progress in the future.

Conflicts of interest and sources of funding

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ORIGINAL ARTICLE

Effects of Military Equipment on the Psychophysiological Stress Response during a HIIT Workout

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Abstract: *Background: Army operations produce various psychophysiological responses of military personnel. Traditionally, to prepare soldiers for these requirements, military workouts have been based on long-distance and low-intensity training. Recent researches suggest that high-intensity interval training (HIIT) may be more effective due to the characteristics of actual theaters of operation. there is a lack of research on the actual effects of military equipment in the context of combat or HIIT training. This study aimed to analyze the effects of military equipment on psychophysiological responses during a HIIT session. Methods: 20 soldiers of the Spanish Army (aged 27.3 ± 3.4 years; height 176.6 ± 4.7 m; weight 76.8 ± 7.4 kg) were evaluated after and before a traditional running HIIT which consisted of 12 repetitions of 30 seconds at maximum intensity, with a 30-second rest between exercises. Blood lactate, rate of perceived exertion and stress, upper and lower limbs, and respiratory muscle strength, skin temperature, blood oxygen saturation, heart rate, cortical arousal were measured before and after the training. Results: Results are reported with their mean and standard deviation. Significant differences were found in RPE, SSP, BOS, and Lactate in both groups in pre and post-training with the two outfits analyzed. Also, it was observed significant differences in HJ, UMS, and PEF values when the group was equipped with military equipment. Significant differences were found in RPE pre-training values and RPE, Stress perception, BOS, HR, and lactate post-training values. Conclusions: The use of military equipment during a high-intensity interval training session produces higher stress perception and perceived exertion than the civil outfit. The mechanical difficulty and the lack of operability in movements could be a reason for this psychophysiological response.*

Keywords: *stress, military equipment, performance, High-intensity interval training*

INTRODUCTION

Combat stress produces various modifications in the physiological responses of military personnel, such as an increase in anaerobic lactic metabolism, activation of the sympathetic nervous system, higher cardiovascular activity, activation of muscle strength, and a decrease in cortical activation [1,2]. Additionally, these physiological responses can be accompanied by cognitive effects related to a distortion of subjective perception of effort [3] and negative effects on memory [4-6]. When these responses are prolonged over time, they can lead to psychological disorders related to stress and anxiety [7].

This exacerbated organic response is due to the activation of the fight-flight mechanism, a primitive survival mechanism that develops an acute adaptive response and provides the organism with the capacity to anticipate risks [1]. The manifestation of this mechanism has been observed in paratroopers and military personnel during simulated operational maneuvers [8-10], helicopter pilots during combat maneuvers [2] aircraft personnel during operational flights [3], and tactical parachute jump [4]. In all of these cases, the soldiers' organisms adapt to face risk situations, and the psychophysiological stress response is modulated by the soldiers' experience [5–7].

In addition to the conditions of the military environment and the psychophysiological requirements, military equipment can also affect operative performance, decreasing speed of movements, increasing heart rate, body temperature, somatic anxiety, and subjective perception of effort [8]. Traditionally, to prepare soldiers for these requirements, military workouts have been based on long-distance and low-intensity training, contrary to the psychophysiological requirements evidenced by actual scenarios [11]. Previous studies suggest that high-intensity training may be more effective due to the characteristics of actual theaters of operation [9,12]. Also, researchers offer high-intensity training protocols as valid methods for improving sol-dier performance [9,10].

Previous studies have observed that high-intensity interval training (HIIT) produces a significant increase in the psychophysiological response of soldiers, like those observed in operative scenarios [11]. Additionally, a 6-week periodization of HIIT-based integral operative training has been shown to significantly improve combat performance [12]. In the same way, the benefits of HIIT training programs have been seen in other population groups where there is a highly stressful condition such as firefighters or police officers [13,14].

However, there is a lack of research on the actual effects of military equipment in the context of combat or HIIT training. Therefore, this study aimed to analyze the effects of military equipment on psychophysiological responses during a HIIT session to adjust future military training protocols. The initial hypothesis is that military equipment will produce a greater psychophysiological response in soldiers during the HIIT session.

MATERIALS AND METHODS

Participants

We analyzed 20 soldiers of the Spanish Army (aged 27.3 ± 3.4 years; height 176.6 ± 4.7 m; weight 76.8 ± 7.4 kg). All procedures were approved by the headquarters of the unit and explained to all volunteers before intervention. Volunteers provided voluntary written informed consent in accordance with the Declaration of Helsinki.

Procedure

All subjects performed a traditional running HIIT consisting of 12 repetitions of 30 seconds at maximum intensity, with a 30-second rest between exercises. The subjects completed the workout in two different conditions:

i. Without military equipment, wear a T-shirt, shorts, and sports shoes.

ii. Wearing full army gear, the army gear included boots, a helmet, a military uniform, and a combat backpack, resulting in a total weight of 20 kg carried by the military personnel during the intervention.

The participants were randomly divided into two groups, one group did the training under condition i and the other group under condition ii. After 48 hours, they repeated the training, and each group performed the training they did not do on the first day.

The psychophysiological response of participants was measured before and immediately after training intervention as previous researchers in the military population [15–17]. We analyzed the following parameters in this order before and after the HIIT:

1. Blood lactate by a 5µl blood sample in a finger in the Lactate Pro (Arkay, Inc. system, Kyoto, Japan) [18].

2. Rating of perceived exertion (RPE), 6–20 scale [19].

3. Stress subjective perception (SSP) 0 – 100 scale [20].

4. Legs strength by horizontal jump (HJ) test.

5. Upper muscular strength (UMS) by the maximum isometric contraction in the dominant hand in a dynamometer.

6. Skin temperature with a digital infrared thermometer (Temp Touch; Xilas Medical, San Antonio, TX).

7. Blood Oxygen saturation (BOS) and HR by a pulse oximeter (PO 30 Beurer Medical).

8. FVC manifestations by a digital spirometer QM-SP100 (Quirumed, Spain) [21].

9. Cortical activation with the Critical Flicker Fusion Threshold (CFFT), analyzed in a viewing chamber Lafayette Instrument Flicker Fusion Control Unit (Model 12,021), by the mean of five incremental flickering light perceptions from 20 to 100 Hz. Increases in CFFT are linked with a decrease in information processing ability and central nervous system fatigue [21,22].

The SPSS statistical package (version 21.0; SPSS, Inc. Chica-go, Illinois) was used to analyze the data. Descriptive statistics, including mean and standard deviation, were calculated for each variable. An independent samples T-test was used to analyze differences between pre- and post-session and between groups in each workout. The level of significance for all comparisons was set at $p \leq 0.05$.

RESULTS

Results are reported with their mean and standard deviation. Table 1 shows the psychophysiological values of military personnel pre and post-training with the two outfits analyzed. Significant differences were found in RPE, SSP, BOS, and Lactate in both groups.

Table 1: Psychophysiological response of military personnel with civil task and military task previous and post HIIT operative training

	Civil outfit						Military outfit					
	pre		post		T	P	pre		post		T	P
	M	SD	M	SD			M	SD	M	SD		
CFFT (Hz)	35.49	2.20	35.32	3.62	0.28	0.78	34.34	3.65	35.44	4.42	-1.70	0.11
SSP (0-100 scale)	14.05	16.12	41.79	32.25	-3.31	0.00	9.60	8.17	77.50	12.82	-22.43	0.00
RPE (6-20 scale)	6.00	0.00	14.00	3.45	10.11	0.00	6.80	0.83	17.15	1.46	-27.83	0.00
HJ (m)	1.69	0.19	1.71	0.15	-1.34	0.20	1.58	0.24	1.65	0.22	-3.43	0.00
IHS (N)	46.95	5.93	47.89	6.32	-1.30	0.21	46.60	7.01	50.55	6.39	-6.07	0.00
Temperature (ºC)	36.73	0.39	36.49	0.42	1.51	0.15	36.84	0.32	36.56	0.72	1.83	0.08
BOS (%)	97.37	1.01	96.47	1.54	2.45	0.03	97.00	1.12	94.65	2.28	3.98	0.00
HR (bmp)	71.53	15.97	146.84	11.43	15.81	0.00	62.75	12.74	155.65	9.87	-29.01	0.00
FEV1	3.94	0.61	4.07	0.43	-1.27	0.22	4.08	0.55	3.99	0.62	1.09	0.29
PEF(l/min)	11.04	1.59	11.24	1.87	-0.86	0.40	11.19	1.81	10.61	1.85	2.39	0.03
FVC (l)	4.68	0.64	4.65	0.70	0.21	0.84	4.88	0.74	4.81	0.76	0.82	0.42
Lactate (mmol/L)	3.79	2.78	19.41	5.45	10.35	0.00	3.70	1.50	12.95	5.68	-7.39	0.00

CFFT (Critical Flicker Fusion Threshold), Stress subjective perception (SSP), RPE (Rating of Perceived Exertion); BOS (Blood oxygen saturation): Horizontal Jump (HJ); IHS (Isometric hand strength); FVC (Forced Vital Capacity); FEV1 (Forced Expiratory Volume in 1 Second); PEF1 (Peak Expiratory Flow)

Also, it was observed significant differences in HJ, UMS, and PEF values when the group was equipped with military equipment.

Table 2 shows the intergroup comparison before and after training for each of the evaluated values. Significant differences were found in RPE pre-training values and RPE, Stress perception, BOS, HR, and lactate post-training values.

DISCUSSION

The objective of this study was to analyze the effect of military equipment on the psychophysiological response of the military during a traditional running HIIT. The initial hypothesis was fulfilled since military equipment produced a higher psychophysiological response during the HIIT session.

The cognitive system may be impacted during a HIIT session due to its high intensity, HIIT training results in high levels of perceived effort and stress [23]. Consistent with this, the data collected in this study revealed an increase in RPE and perceived stress levels before the intervention in both groups. Moreover, the values were greater in the group wearing military equipment. The RPE values of the military outfit group after training were higher than those observed in soldiers before an operative HIIT session [11]. Furthermore, the RPE values of the military outfit group were higher than those recorded by soldiers before the resistance and endurance HIIT [24], and higher than those observed by soldiers before a combat simulation [25]. This higher perception of effort could be due to the lack of experience and the higher physical requirements of this kind of training with military equipment. This effort in the cognitive system also is reflected by a high level of perception of stress. These values are higher than those observed in soldiers in rescue maneuvers, before underwater evacuation training [26], or during combat simulation maneuvers [27]. Therefore, it is emphasized that the absence of the experience produces a great cognitive impact.

The findings of the study indicate that the cognitive impact of HIIT training is considerably more intense when soldiers wear military equipment. This suggests that the additional physical and psychological stress of wearing the equipment during training can further challenge the cognitive system and potentially impact the soldier's performance and ability to respond effectively in actual combat situations. Therefore, it is crucial to consider these findings when designing training protocols for military personnel, to better prepare them for the demands of real-life situations [24,28,29].

Additionally, cardiovascular effects were observed due to the physiological demands of HIIT. Regarding HR values, both groups showed an increase after the training session, but HR levels were higher in the military outfit group. These HR values were even higher than those observed in ultra-endurance races [30] and were higher than those observed by soldiers before operative HIIT training [11]. However, the HR values were lower than those shown by the military group before a resistance and endurance HIIT [24]. Therefore, HR values were lower than those observed by the military during military asymmetrical and symmetrical combat maneuvers [29] probably due to the cognitive, psychological, and physical requirements.

Table 2: Comparison of the psychophysiological results between groups

	CIVIL OUTFIT		**MILITARY OUTFIT**				**95% confidence interval for the difference**	
	M	**SD**	**M**	**SD**	**t**	**p**	**inf**	**sup**
CCFT(Hz) pre	35.49	2.20	34.34	3.65	1.18	0.25	-0.82	3.11
CFFT (Hz) post	35.32	3.62	35.44	4.42	-0.10	0.92	-2.75	2.50
ssp pre	14.05	16.12	9.60	8.17	1.10	0.28	-3.77	12.68
SSP post	41.79	32.25	77.50	12.82	-4.59	0.00	-51.48	-19.94
RPE pre	6.00	0.00	6.80	0.83	-4.18	0.00	-1.19	-0.41
RPE post	14.00	3.45	17.15	1.46	-3.75	0.00	-4.85	-1.45
HJ (m) pre	1.69	0.19	1.58	0.24	1.58	0.12	-0.03	0.25
HJ (m) post	1.71	0.15	1.65	0.22	1.07	0.29	-0.06	0.19
IHS (N) pre	46.95	5.93	46.60	7.01	0.17	0.87	-3.88	4.57
IHS (N) post	47.89	6.32	50.55	6.39	-1.30	0.20	-6.78	1.47
Temperature (ºc) pre	36.73	0.39	36.84	0.32	-0.95	0.35	-0.34	0.12
Temperature (ºC) post	36.49	0.42	36.56	0.72	-0.34	0.73	-0.45	0.32
BOS (%) pre	97.37	1.01	97.00	1.12	1.07	0.29	-0.33	1.06
BOS (%) post	96.47	1.54	94.65	2.28	2.91	0.01	0.56	3.09
HR (bpm) pre	71.53	15.97	62.75	12.74	1.90	0.07	-0.57	18.13
HR (bpm) post	146.84	11.43	155.65	9.87	-2.58	0.01	-15.73	-1.89
FEV1 pre	3.94	0.61	4.07	0.43	-0.76	0.45	-0.52	0.23
FEV1 post	4.08	0.55	3.99	0.62	0.44	0.66	-0.27	0.43
PEF (l/min) pre	11.04	1.59	11.19	1.81	-0.27	0.79	-1.26	0.96
PEF (l/min) post	11.24	1.87	10.61	1.85	1.06	0.30	-0.58	1.84
FVC (l) pre	4.68	0.64	4.88	0.74	-0.89	0.38	-0.65	0.25
FVC (l) post	4.65	0.70	4.81	0.76	-0.68	0.50	-0.64	0.32
Lactate (mmol/L) pre	3.79	2.78	3.70	1.50	0.13	0.89	-1.34	1.53
Lactate (mmol/L) post	19.41	5.45	12.95	5.68	3.62	0.00	2.85	10.08

CFFT (Critical Flicker Fusion Threshold), Stress subjective perception (SSP), RPE (Rating of Perceived Exertion); BOS (Blood oxygen saturation): Horizontal Jump (HJ); IHS (Isometric hand strength); FVC (Forced Vital Capacity); FEV1 (Forced Expiratory Volume in 1 Second); PEF1 (Peak Expiratory Flow)

In line with this, the training resulted in a decrease in BOS values for both groups. However, the military outfit group showed a significant decrease in BOS values after the training. These values decreased to levels lower than those observed by soldiers before an operative HIIT [11]. The cardiorespiratory response is a consequence of the training-induced respiratory distress, and it was more pronounced when soldiers carried a heavier weight during the session. This respiratory distress is manifested through the PEF values where the military outfit group showed a significant decrease in PEF before training. These values were higher than those presented by soldiers before a HIIT resisted training [11]. However, no significant differences were observed when compared to the civilian outfit group.

Likewise, significant differences were observed between the groups at the metabolic level. Previous research has demonstrated that HIIT sessions lead to an increase in lactate production due to metabolic demands. Similarly, both groups exhibited an elevation in lactate levels immediately after completing the HIIT training. However, the group wearing military outfits had significantly lower lactate levels compared to the group wearing civilian outfits. The lactate levels in the military outfit group were comparable to those of soldiers before engaging in operational HIIT [9] and similar to those observed during firefighting operations [25]. In contrast, the civilian outfit group exhibited higher lactate values indicating a greater degree of metabolic acidosis following exercise. These values were similar to those observed in athletes before the HIIT session [31], therefore a good performance and physical level of the soldiers were observed.

The difference in lactate production between the two groups may be attributed to the mechanical effects caused by wearing military gear. The added mechanical load on the soldier hampers their ability to perform exercises at a higher speed.

This mechanical condition is also evident in the effects of HIIT on upper and lower body strength levels. The military outfit group demonstrated a significant increase in upper body strength levels post-training. This improvement is likely due to the muscular activation generated by the metabolic system. These values were similar to those exhibited by soldiers before engaging in resisted HIIT [20] and before a crane rescue maneuver performed by a helicopter crew [2]. Although these values were higher than those of the civilian outfit group, which could be attributed to the physical requirements influenced by military equipment, there were no significant differences between the groups.

In contrast, a significant decrease in lower body strength levels (HJ values) was observed in the military outfit group after the training. These values were lower than those observed by soldiers before resistance and endurance HIIT [20], lower than those observed by soldiers before operational HIIT [11], and lower than those shown by pilots after 30 and 35-minute jet maneuvers [20]. The results highlight a direct impact of wearing military gear on lower body performance. However, similar to the upper body findings, no significant differences were observed when compared to the civilian outfit group.

CONCLUSION

The use of military equipment during a high-intensity interval training session produces higher stress perception and perceived exertion than the civil outfit. Physiological response, heart rate, blood oxygen saturation, and lactate values were significantly higher with military equipment although the level of lactate was lower than observed with the civil outfit. The mechanical difficulty and the lack of operability in movements could be a reason for this psychophysiological response.

Practical application

The results showed the direct influence of military equipment on the soldier when performing submaximal performance. This will open new lines of research for designing training protocols with and without military equipment.

Limitations of the study and future research lines

The main limitation of this study is the low sample of participants. Also, it could be recommended the use of ECG to compare values as in previous research. In future research, it is suggested to validate PPG protocols with longer durations.

Conflicts of interest and sources of funding
The authors declare no conflict of interest. This research received no external funding.

Authors' contribution
Conceptualization, A.C.-R., J.F.L and A.B.-S.; methodology and formal analysis A.C.-R., and V.J.C.-S investigation, A.C.-R., J.F.L and V.J.C.-S.; data curation, V.J.C.-S.; Writing—Original draft preparation, , A.C.-R., A.B.-S and V.J.C.-S.; Writing—Review and Editing, A.C.-R., J.F.L and V.J.C.-S.; visualization, , A.C.-R., Aand V.J.C.-S.; supervision and funding acquisition, V.J.C.-S.

Ethics approval and consent to participate
Procedures and protocols were explained to all the subjects who gave the voluntary written information in accordance with the Declaration of Helsinki and approved by the Euro-Pean University Ethics Committee (CIPI/18/093).

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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REVIEW ARTICLE

Pathophysiological Association Between Helicobacter Pylori Infection and Colorectal Cancer - An Analysis of the Latest Studies

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Abstract: *Colorectal cancer is the third most common cancer worldwide and represents a significant major public health issue. Therefore, the interest in detecting the multiple factors implied in the development of cancer has increased recently. One of the factors could be the Helicobacter pylori infection, but a direct causal connection is missing. This gram-negative bacterium infects approximately 4 billion individuals globally and various conditions such as gastroduodenal ulcers or gastric adenocarcinomas can be induced by it. Some of the reported carcinogenic effects of Helicobacter pylori bacterium are hypergastrinemia, chronic inflammation, dysbiosis, and toxin production. Each of these elements involved in the pathogenesis has various mechanisms of action. Dysbiosis can occur due to various treatments for the eradication of the Helicobacter pylori infection. This bacterium also has a proinflammatory effect at the gastric level which can predispose to the development of dysplasia or even neoplasia. The toxins produced by this bacterium, such as the vacuolating cytotoxin A, promote inflammation. In this review, we discuss the possible connections between the Helicobacter pylori infection, through its diverse pathogenic mechanisms, and colorectal neoplasm. Prospective studies are necessary to determine whether there certainly is a direct link between the two conditions.*

Keywords: *colorectal cancer, Helicobacter pylori, dysbiosis, neoplasia, inflammation*

INTRODUCTION

Colorectal cancer (CRC) is the third most common type of cancer in the world with an increasing incidence and mortality rate, especially in developed countries [1].

Since the 1970s, infection has been considered a possible etiopathogenic factor of CRC. Numerous studies have been conducted on the intestinal microbiota and on the various bacterial species comprising it, which can become pathogenic. These studies have shown the role of inflammation and cytokines in the development of CRC, but without finding a significant association between CRC and the bacterial species analyzed [2-5].

After the 1990s, when Helicobacter pylori (H. pylori) was identified, numerous researches focused on its effects were carried out. H. pylori, a Gram-negative bacterium, is already known to be a risk factor for gastric cancer, but its role in CRC remains uncertain. Numerous studies have shown a significant association between the two, but the pathophysiologic mechanisms underlying the development of colorectal cancer still remain inaccurate [6].

The purpose of this article is to provide an overview of the latest studies on the evidence supporting the link between Helicobacter pylori infection and colorectal cancer.

MATERIALS AND METHODS

Here, we review the epidemiological evidence, potential biological mechanisms, and clinical implications related to this association.

A detailed search in the literature was conducted using PubMed.com and Elsevier.com platforms to identify relevant articles published in peer-reviewed logs. Search terms included "Helicobacter pylori", "colorectal cancer", "gastro-intestinal neoplasms", "epidemiology", "pathogenesis" and "mechanisms". We included original research articles, reviews, and meta-analyses that investigated the association between H. pylori infection and CRC. The selection criteria were based on the design of the study, relevance to the working hypothesis, and methodological quality, with only articles written in English being selected (Table 1).

DISCUSSIONS AND RESULTS

Helicobacter pylori is a bacterium that settles in the mucus layer lining the gastric epithelium, being present in about 50% of the population [7]. Infection is associated with several gastrointestinal conditions, such as chronic gastritis, peptic ulcers, or gastric cancer. Recent studies have suggested a possible link between H. pylori infection and the development of colorectal neoplasm, however, the mechanisms at the base remain uncertain.

Numerous epidemiological studies support the increased risk of developing CRC in the presence of the bacterium.

Table 1: Summary of scientific resources regarding the subject of the article

Author, year	Number of subjects	Significant results	Conclusions
Zumkeller N. et al - 2006 apr [8]	meta-analysis on 11 studies (1991-2002)		Summary odds ratio of 1.4 for the association between CRC and H. pylori infection
Ryoo SK. et al - 2020 [9]	3753		H. pylori infection has been associated with a high risk of advanced CRC development
Teimoorian F. et al - 2018 [10]	50	H. pylori infection (IgA > 20 U/mL, IgG > 10 U/mL) was significantly more prevalent among patients with RCC and adenomatous polyps	High risk of CRC
Shmuely H. et al - 2013 [11]	273	112 patients diagnosed with cancerous lesions (75% presented anti-H. pylori antibodies) 161 without cancerous lesions (48% presented antibodies) There was no association in patients who were CagA positive.	Uncertain association CagA+ seropositivity is associated with an increased risk of gastric and colorectal cancer
Zhao YS. et al [12]	meta-analysis on 13 studies - 2008		Possible involvement of H. pylori infection in the development of CRC

There are various hypotheses according to which Helicobacter pylori infection contributes to colorectal carcinogenesis, primarily altering the intestinal microbiota. A study conducted in 2018 showed that the bacteria that make up the intestinal microbiota in patients infected with H. pylori do not differ from those without infection, but differ in those who have gastric damage caused by this bacterium (intestinal metaplasia or gastritis) [13].

In the etiopathogenesis of CRC, several mechanisms have been proposed: hypergastrinemia, changes in the microbiota, chronic inflammation, and production of toxins by H. pylori.

Hipergastrinemia

Prospective studies have researched the potential connection between hypergastrinemia (increased serum gastrin levels) and the development of colorectal cancer. Gastrin is a peptide hormone secreted by G-cells in the gastric and duodenal antral mucosa and has an essential role in regulating acid secretion and gastric motility [14,15]. Increased levels of this hormone influence cell growth and differentiation, including cells of the colon mucosa [16,17]. Gastrin also promotes angiogenesis, a key factor in tumor growth and metastasis, amplifying the production of vascular endothelial growth factor in colon tumor cells [18,19,20]. Hypergastrinemia has also been associated with an increase in the number of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and the α factor of tumor necrosis (TNF-α), which can contribute to the development of CRC [21].

At the same time, it has been shown that epithelial cells of the gastric mucosa infected with H. pylori secrete TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), as well as other pro-inflammatory chemokines and modulating the immune response [20]. The synthesis of TNF-α by the host organism's cells is mediated by TNF-α inducing protein (Tipα). Tipα is a virulence factor secreted by H. pylori, which causes the synthesis of TNF-α and other proinflammatory cytokines [20], without having any counterpart among the other virulence factors of H. pylori.

Epithelial-mesenchymal transition (TEM) is a biological process that plays a significant role in tumor progression and metastasis. Gastrin has been shown to promote the TEM process of colorectal cancer cells by activating the TGF-β/Smad signaling pathway. This effect is associated with increased migration, invasion, and metastatic potential of colorectal cancer cells [22].

Modulation of the microbiota

The hydrochloric acid secreted by the parietal cells of the gastric mucosa has numerous digestive roles, but it also has an important bactericidal role, destroying the bacteria ingested with food, so that most of the small intestine is a sterile environment. With the hyposecretion of hydrochloric acid, the small intestine is colonized with bacteria and the colonic microbiota changes. Hyposecretion of parietal cells can be determined by atrophy of the oxyntic glands, as it happens in H. pylori-induced atrophic gastritis, as well as prolonged treatment with proton pump inhibitors as a therapeutic indication for the eradication of H. pylori infection [23]. The gut microbiome consists of 10^{13} to 10^{14} bacteria, fungi, protozoa, and viruses, as well as their genetic material. It is characteristic of each individual, depending on its exposure after birth to a wide variety of microorganisms. The microbiome has three important categories of roles: structural, protective, and metabolic [24,25]. Thus, it has an important role in absorbing some nutrients and minerals, synthesizing vitamins, amino acids, and enzymes and producing short-chain fatty acids. Also, the microbiota has an essential role in the processes of fermentation and putrefaction, with the production of acetate, propionate, and butyrate, essential for the health of the colon: it provides the necessary energy source for intestinal mucosal cells, increases the integrity of the mucous barrier, contributes to immunomodulation and protection against pathogens [24,26].

The change in the composition of the microbiome is called dysbiosis and can be caused by a multitude of dietary factors and changes in the environment. Dysbiosis is associated with the pathogenesis of a variety of neurological, gastrointestinal, and metabolic conditions, including colorectal cancer [27].

In a review published in 2012, Tatishchev SF et al suggest that modulation of the intestinal microbiota, increased cytokine production and increased secretion of gastrin induced by H. pylori infection are possibly involved in the occurrence of CRC.

The etiopathogenic role of H. pylori in colorectal carcinogenesis remains controversial [28,29].

Characteristics of microbial virulence factors may be included in routine sequencing for tumor mutation testing [30]. This approach could be useful both in the diagnosis and treatment of patients with solid colorectal tumors [30].

Chronic inflammation

Chronic inflammation of the mucosa is considered to be a predisposing factor for the development of CRC, similar to the mechanism of inflammatory bowel disease. Given the well-established proinflammatory and carcinogenic effect of H. pylori at the gastric level, a sequence of the type "chronic inflammation → dysplasia → neoplasia", similar to that for inflammatory bowel disease, can also occur in the colon, initiated by direct colonization of H. pylori [31].

Recently, the association between gastric infection with H. pylori and its presence in the intestine, with the development of colon adenomas, has been demonstrated by the mechanism of up-regulation of the expression of the TLR5 mucosal protein [32].

Cytokines are critical mediators of the immune response for maintaining health, but they have been shown to have important roles both anti-inflammatory and pro-inflammatory, and intervene in intestinal carcinogenesis. These include the cytokine families TNF, TGF-β, IL-6, IL-10, IL-17, IL-21, IL-22, IL-23. These cytokines modulate the intestinal microbiota, both in terms of their composition and secretions. Inflammatory cytokines also act on altered colon epithelial cells regulating their proliferation and vitality, thus acting as a connecting factor between the microbiota and CRC [33].

Toxin production

Some studies reported a significant association between the seropositivity of H. pylori and an increased risk of CRC [34]. H. pylori infection is based on the production of virulence factors, including the pathogenicity island CagA (cytotoxin-associated gene A), which encodes a type 4 secretory system (T4SS), VacA, and urease. They interact with the cells of the epithelium of the gastric mucosa, allowing colonization and pathogenicity of this bacterium [28].

CagA has been shown to induce proinflammatory responses, alter cell adhesion, and promote cell proliferation, which can contribute to the development of CRC [35]. Similarly, it has been suggested that vacuolating cytotoxin A (VacA) could play a role in the pathogenesis of CRC by inducing cell vacuolation, destroying tight junctions, and determining apoptosis [36].

In 2013, Shmuely H. et al published the results of a study in 273 patients. In patients infected with H. pylori, CagA+ seropositivity is associated with an increased risk of gastric and colorectal cancer [37].

Fujimori S. also shows that the risk of developing CRC is increased in individuals carrying vacuolytic cytotoxin A antibodies [38].

The potential association between H. pylori infection and CRC risk has important implications for the prevention of CRC and its early detection.

If a causal relationship is established, the eradication of H. pylori could be considered a preventive measure against the CRC.

In addition, screening among people prone to H. pylori infection may be beneficial for identifying the population at high risk for CRC.

CONCLUSIONS

The association between H. pylori infection and colorectal cancer remains an ongoing topic of research. While some studies have reported a significant association, others have shown conflicting results. Additional epidemiological and well-designed studies are needed to clarify the relationship and establish a causal link between H. pylori infection and CRC.

The pathophysiologic mechanisms of the possible association between H. pylori infection and colorectal cancer are complex. They are based on infection-induced hypergastrinemia and prolonged treatment with PPIs necessary for its eradication, cytotoxin production, dysbiosis, and associated chronic inflammation.

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ORIGINAL ARTICLE

The Effect of Mental Health Conditions on Dental Implants

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Abstract: *The use of dental implants as a treatment for replacing missing teeth has been increasingly prevalent. Historical evidence suggests that the Mayans were among the first to create implants around 600 AD. Over time, this technique has evolved, offering numerous benefits to humanity. Regardless of the causes of tooth loss and the patient's age, it significantly impacts various aspects of an individual's life, including nutrition, psycho-emotional well-being, and social interactions. One of the primary consequences is the negative effect on self-esteem due to the aesthetic concerns associated with missing teeth. Additionally, pronunciation difficulties can lead to communication challenges, reducing the desire and ability to engage with others. Extensive research in the specialized literature explores the psychological impact of dental implant usage. It is widely acknowledged that the dental implant technique is complex and carries inherent risks and potential adverse effects. An illustrative case serves to underscore the significance of psychological considerations in dental implant treatment. In this instance, a 55-year-old woman attempted suicide by throwing herself down the stairs of her home after receiving dental implants in both sections of her oral cavity. The distress she experienced stemmed from the inability to consume anything beyond fluids and semisolid food for an extended period of eight months. This alarming incident underscores the importance of recognizing and addressing the psychological impact that dental implant procedures can have on vulnerable individuals.*

Keywords: *dental implants, anxiety, depression, suicide*

INTRODUCTION

Oral health and mental health are closely linked, and there is evidence to suggest that individuals with dental pathologies may be at increased risk for developing psychiatric conditions [1,2,3].

The dental implant is a technique that is increasingly used as a treatment for replacing missing teeth [4,5]. There is evidence that the first implants were made by the Mayans around 600 AD, later this technique developed over time considering the benefits brought to humanity. It is even though the edentation and the age of the patient [6,7] bring with it, beyond the negative impact regarding the act of nutrition, a multitude of psycho-emotional problems, among which in the foreground is the decrease in self-esteem concerning the aesthetic aspect of the person and the decrease in the desire and ability to relate to other people due to the complications of correctly pronouncing words and thus the individual's ability to communicate. Regarding the impact of using the dental implant technique on the psyche, the specialized literature offers numerous studies on this topic [8.9]. The complexity of the dental implant technique is well-known, as well as its risks and adverse effects [10,11]. Weighing its benefits, in most situations the final decision is in favor of its use, important in this sense being the choice of the type of implant [12]. One of the major inconveniences refers to the long period in which patients are forced to resort to a liquid or semi-solid diet. This stage, which the patient is forced to go through, can trigger the appearance of mental disorders leading to anxiety and depression [13,14] and sometimes, in extreme cases but fortunately with reduced frequency, even to suicidal acts. Another aspect that constitutes a challenge for dentistry is represented by the phobia per se manifested by patients regarding the use of dental equipment [15,16] all the more so since the period of realization of such techniques stretches over several months. In this context, it is also worth mentioning the patient's personality type and, of course, his motivation, an aspect related to other individual and social variables (age, social status, sex, profession, etc.) or pre-existing psychiatric comorbidities [17,18].

Dental pathologies can have a significant impact on an individual's quality of life, including their physical health, social relationships, and self-esteem. The two-way association between oral and mental health is discussed, including how dental treatment can cause anxiety and phobia, and how psychiatric disorders can lead to dental disease. For example, individuals with untreated dental pain may experience feelings of anxiety and depression, which can affect their quality of life and overall mental well-being. About one-half of all dental patients experience some anxiety about their dental visits, and in some cases, this leads to dental phobia, a form of specific phobia [19]. Dental problems are causing stress to the patient and this can be intensified by the duration of the clinical intervention, an aspect that is significant in the case of the dental implant. A current study discusses the impact of mood states and psychological flexibility in stressful situations, in this context the dental implant, recognizing their influence on the patient's clinical satisfaction [20].

Also, some studies approved that adults have problems in eating solid food and not only, have pain and difficulties in their relationships because of oral disorders and there is a strong association between poor self-perceived oral health and quality of life [21,22]. Additionally, individuals with dental pathologies may be less likely to engage in social activities or seek treatment for their dental issues, which can increase their risk of developing psychiatric conditions and can be a real challenge for dentists [23]. A study performed in Britain found a significant correlation between dental anxiety and oral health-related quality of life, and individuals with high levels of dental anxiety were twice as likely to experience poor oral health-related quality of life. The study suggests that dental anxiety is an important factor in oral health-related quality of life and should be considered in clinical practice [24,25]. Dental anxiety is a public health concern that can lead to oral health problems due to delayed dental visits. More precise data is needed on the prevalence of dental fear-related problems to identify groups at risk. A multidisciplinary approach involving mental health and community dentistry professionals is necessary to break the vicious cycle of fear and anxiety. Research needs to focus on the antecedents of dental anxiety and on children, as dental fear usually starts in childhood and extends to adulthood. The Cognitive Vulnerability Model offers a promising framework to analyze the interplay between cognitive and psychosocial determinants of dental fear [26].

MATERIALS AND METHODS

Accessing the PubMed database, we identified a number of 116 articles published between 2012-2023 that studied the issue of mental health, the technique of dental implantology, as well as a series of approaches to the connection between the two subjects. The articles included in the study were original studies, case studies, clinical studies, or therapeutic trials that addressed the subject of the consequences of oral implantology on the mental health of individuals. Of these, we analyzed a number of 8 review articles.

We also present the case of a 55- years old woman who tried to commit suicide by throwing herself on the stairs of her house after having dental implants applied on both parts of her oral cavity she could not resist eating only fluids and semisolid food for 8 months. the patient's first presentation to the psychiatric service (3 months before suicide attempts) was due to a sad mood and increased anxiety following a dental treatment with the application of dental implants started approximately 8 months ago. The patient needs complex oral rehabilitation, presenting poor oral health. She had no elements of a psychiatric nature, at the first consultation in the dental office and she had partial bimaxillary edentation, partially restored with bridges. The patient was advised to eat pureed and as much liquid as possible until the healing of the oral lesions that occurred after the initiation of the dental treatment and the insertion of the dental implants. We mention that the patient had a history of 2 psychiatric consultations and 2 admissions for 7 days each We mention that the patient had a history of 2 psychiatric consultations and 2 admissions for 7 days each. After the insertion of the implants, no temporary prosthesis was used; this fact can be considered as one of the triggering factors of the pathology psychiatric. The evolution was favorable and he was discharged with the diagnosis of a moderate depressive episode, with an indication for treatment and to return for a periodical consultation. After the consultation with the dentist, the patient returned 3 weeks after the last hospitalization, to the emergency room, with a suicide attempt by throwing herself down the stairs of the attic because the dentist recommended an extension of the period to eat liquid and semi-solid, a fact that demoralized her completely. When admitted to the emergency room, the patient presented with aphasia, difficulty cooperating, and a possible fracture of the mandible, which was not confirmed by the oral surgeon. A right iliac fracture was diagnosed on the abdominal and pelvic computer tomography. During the hospitalization, the patient presented a stationary evolution in the first 14 days, the patient being uncooperative, with the absence of will and the refusal to accept oral medication, for which a nasogastric tube and a urinary tube were applied. After the partial remission of the symptoms in the second stage, the patient presented with ideas of persecution and guilt, but without autolytic ideation. The patient received psychiatric treatment with therapeutic benefit and resumed dental treatment after discharge.

RESULTS

Concerning the clinical case, the patient is now in a harmonious relationship with her husband, and her son, she continued her hobby related to swimming, winning numerous prizes in her age category, and from a dental viewpoint, the work was completed. Concerning the generic link between mental health and dental implants: there is a direct link between the favorable results of the use of the dental implant and the improvement of the mental status, especially in the elderly.

Concerning suicide in people who resort to implants for education, the studies took into account the implications related to the situations in which the failure of the implant technique took place. The aspect of the quality of life and the implications on the mental status during the implementation of the implant have not been studied. For this reason, the current approach presents an aspect of interest considering the large number of people who resort to the dental implant technique and the same extent, the large number of people with a history of psychiatric disorder in whom this period can represent a trigger in the psychiatric pathology.

We have chosen to present the case of a 55-year-old patient who presented herself in the emergency psychiatric service for a suicide attempt by throwing herself down the stairs from the attic of her house. The gesture occurred because of non-adaptation to the mandatory liquid and semi-solid diet established following the application of the dental implant; the procedure and lifestyle started 8 months before the event.

Heredo-collateral antecedents: father former ethanol user now deceased, a mother with hypertension, and an ischemic stroke in the antecedents.

Personal physiological antecedents: the patient has a 28-year-old son, and abortions = 10 up to the current age. Menopause from 51 years.

Personal pathological antecedents: autoimmune thyroiditis not treated with medication, appendectomy at 7 years old, tonsillectomy at 10 years old, 2 psychiatric consultations, and 2 admissions for 7 days each.

Living and working conditions: the patient has a higher education, is married, is in a harmonious relationship with her husband and son, has a satisfactory social life, and is a top swimmer in her age category with awards won.

She does not currently smoke but has smoked approximately 1 pack of cigarettes/day for 20 years, and does not consume alcohol or psychostimulant substances.

Treatments administered before admission: multiple antidepressants, anxiolytics, and hypnotics.

History of the disease: the patient's first presentation to the psychiatric service (3 months before suicide attempts) was due to a sad mood and increased anxiety following a dental treatment with the application of dental implants started approximately 8 months ago.

At the first consultation in the dental office, the patient presented for complex oral rehabilitation, presenting poor oral health, but without elements of a psychiatric nature. The patient had partial bimaxillary edentation, partially restored with bridges (Figures 1 and 2).

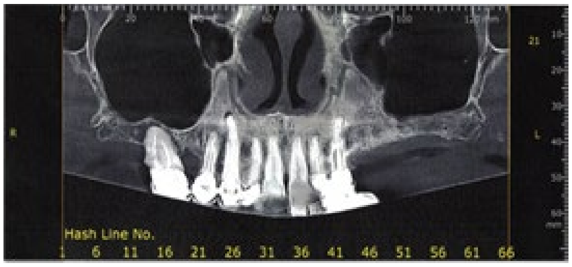

Figure 1: Upper arch before implant placement

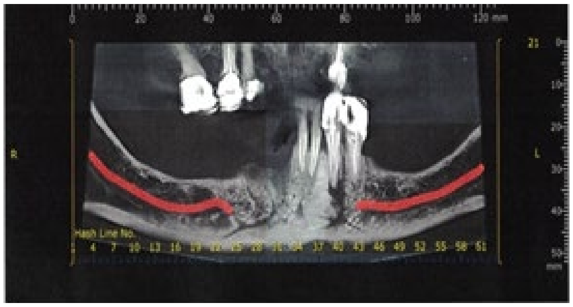

Figure 2: Lower arch before implant placement

The patient was advised to eat pureed and as much liquid as possible until the healing of the oral lesions that occurred after the initiation of the dental treatment and the insertion of the dental implants. Through the treatment plan for complete and complex oral rehabilitation, it was decided to insert implants on both arches, for the upper arch in quadrant 2 and for the lower arch in quadrants 3 and 4, all being inserted at a distance of 2 weeks between, the first being inserted on the upper arch. Bone augmentation was also necessary for the lower arch, following the marked atrophy of the ridge that remained edentulous a long time before. Prior to the procedure of applying the dental implants, the remaining teeth were extracted from the lower arch and during the ablation of the crowns of the teeth restored with bridges from quadrant 2. After the insertion of the implants, no temporary prosthesis was used; this fact can be considered as one of the triggering factors of the pathology psychiatric (Figure 3).

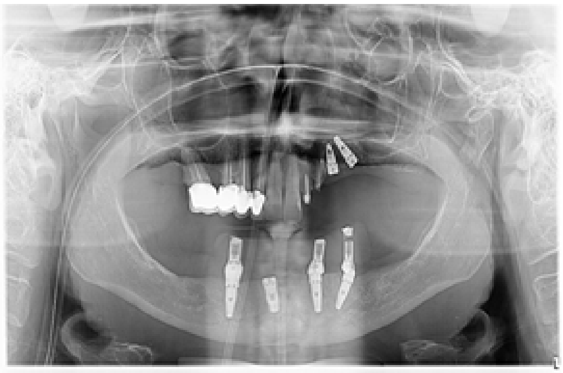

Figure 3: Xray control after implants' placement

The second day after presenting to the first psychiatric consultation, she requested admission to the hospital's psychiatric department. She is given specialized treatment (Sertralinum 50 mg, Bromazepamum 3 mg) and is discharged after 7 days. After another 4 days, she returns to the emergency room for insomnia and is recommended Zopiclonum 10 mg. The second hospitalization is carried out 3 weeks after the last consultation and the patient's treatment is changed to double therapy with an antidepressant (paroxetine-mirtazapine), anxiolytic (lorazepam), and hypnoinducer (cinolazepam). The evolution was favorable and he was discharged with the diagnosis of a moderate depressive episode, with an indication for treatment and to return for a periodical consultation. After the consultation with the dentist, the patient returned 3 weeks after the last hospitalization, to the emergency room, with a suicide attempt by throwing herself down the stairs of the attic because the dentist recommended an extension of the period to eat liquid and semi-solid, a fact that demoralized her completely. When admitted to the emergency room, the patient presented with aphasia, difficulty cooperating, and a possible fracture of the mandible, which was not confirmed by the oral surgeon. A right iliac fracture was diagnosed on the abdominal and pelvic computer tomography, for which he was administered Nadroparinum 3800 UI/0.4ml IM and physical therapy was performed.

During the hospitalization, the patient presented a stationary evolution in the first 14 days, the patient being uncooperative, with the absence of will and the refusal to accept oral medication, for which a nasogastric tube and a urinary tube were applied. After the partial remission of the symptoms in the second stage, the patient presented with ideas of persecution and guilt, but without autolytic ideation.

At the psychological examination carried out 14 days after admission, the patient was calm, present in the environment, cooperative, flow, and slow talking rhythm with ideas of incapacity and major concerns regarding the existing organic affections. Severe depressive mood with mixed hypnotic disorders, food refusal, apathy, delusional ideation of helplessness, and social withdrawal. Voluntary hypoprosexia, constricted affect. Reduced efficiency. Accentuated low level of obsessive-compulsive personality, HRSD=38, GAFS=50.

At the psychological examination performed one month after the first one, the patient was cooperative, optimistic, and reflective regarding the "accident" suffered a few weeks ago. Clear and flexible communication, sufficiently logical thinking, has realistic goals, show slight concerns regarding dental treatment. Depressive-anxious configuration (light intensity). HRSD=11, HRSA=10, GAFS=75.

During hospitalization, the patient initially received hydrating, vitaminizing, hepatoprotective, and psychiatric infusion treatment with atypical neuroleptic (aripiprazole), antidepressant (mirtazapine), anxiolytic (lorazepam), hypnoinductor (cinolazepam) and trihexyphenidyl.

After the psychiatric improvement and discharge, the patient returned to the dental office to complete the dental treatments. The end of the dental treatment consisted of a bridge in quadrant 1 on the teeth still present in the arch, in quadrant 2, a crown on the lateral incisor and a bridge on the 2 implants, and for the lower arch, there was a bridge on implants having a distal extension in quadrant 3 and two distal extensions in quadrant 4.

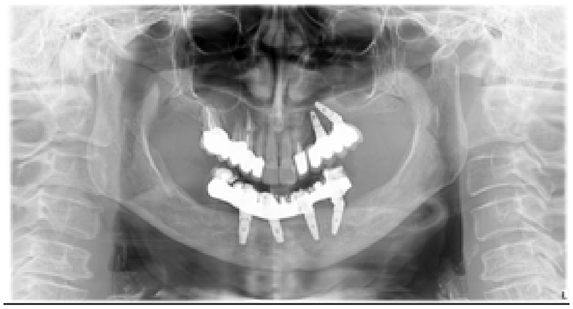

Figure 4: Xray control after finishing the full mouth rehabilitation process

From a psychiatric perspective, the patient was followed clinically with consultation for a period of approximately 2 years, initially with monthly then with quarterly consultations. The treatment was not changed during the plateau period, there were no relapses, and at the time of complete treatment elimination, the patient did not return to the psychiatric service. Currently, the patient is in a harmonious relationship with her husband, and her son, she continued her hobby related to swimming, winning numerous prizes in her age category, and from a dental viewpoint, the work was successfully completed.

While dental implants can improve one's self-esteem and confidence, some individuals may experience feelings of depression related to the surgical procedure or recovery process. This can be caused by a variety of factors, such as physical pain, swelling, and the length of time required for recovery. Additionally, the cost of dental implants can also be a financial burden, which can lead to stress and depression.

Anxiety can have a significant impact on the success of dental implants. Anxious individuals may have difficulty following the necessary steps for successful implantation, such as proper oral hygiene and regular dental visits. They may also experience difficulty in following a healthy diet, which can impact the healing process and overall success of the dental implants.

Depression can have a significant impact on the success of dental implants. Individuals who are depressed may have difficulty following the necessary steps for successful implantation, such as proper oral hygiene and regular dental visits. They may also experience difficulty in following a healthy diet, which can impact the healing process and overall success of the dental implants.

A proof in this sense is represented by the fact that a mechanism involved in the triggering of depression, namely, the recapture of serotonin is also at the basis of the failure of the oral implant through the anabolic effect on the bone reducing the bone turnover, a fact that can explain the reduced production of the alveolar bone.

From the perspective of psychiatric conditions with strict reference to the behavioral factor, we mention the role of factors such as dental hygiene or certain practices such as smoking, which have an impact on dental health.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of “Elisabeta Doamna” Clinical Psychiatry Hospital (02/18.05.2021) for studies involving humans.

DISCUSSION

In some cases, depression can be a pre-existing condition that is exacerbated by the stress and anxiety of undergoing a dental implant procedure. This is why individuals must have a thorough evaluation of their mental and emotional health before undergoing any dental procedure because it is a great challenge for a dentist to treat a patient with psychiatric problems [23,27]. Some studies show that psychiatric and genetic diseases do not represent an impediment to dental implant application, but multiple complicating factors can reduce implant survival [28,29]. Studies on phobic patients are totally satisfied with implant rehabilitation under general anesthesia, which means that this treatment can be considered a treatment of choice giving these patients the same opportunity as others to improve their oral health and well-being [27,30] but there are no studies about the clinical psychological impact that dental implants have on one’s mental health.

A study in Hong Kong investigated the relationship between dental anxiety, oral health status, and oral health-related quality of life (OHQoL) in 1,000 predominantly Chinese residents aged 25-64. Results showed that those with high dental anxiety had a higher risk of poor dental and periodontal status and lower OHQoL. The study suggests that dental anxiety is a significant risk indicator and should be considered in oral health assessments [31]. The study of Kanaffa-Kilijanska et al. assessed oral health and hygiene habits in relation to dental anxiety among 117 adult patients. The results showed that higher levels of dental anxiety were associated with a higher calculus index and a lower number of filled teeth. There was no significant correlation between dental anxiety and age, gender, or education level, but smokers had a significantly higher anxiety level (our patient smoked for about 20 years). The study concluded that dental anxiety has a negative impact on oral health and can lower the quality of life of the patient [32], data that were also confirmed by a 2015 national Swedish cross-sectional survey [33].

Furthermore, individuals who experience significant anxiety related to dental procedures may be less likely to seek dental care, which can lead to further dental problems and a lower quality of life, and patients suffering from mental illnesses are more vulnerable to dental neglect and poor health [34]. Not only in Lalabonova’s studies, there are strong correlation between the level of anxiety and dental implants proving that only those with low levels of dental anxiety decide to proceed with such a treatment [35,36,37].

Dental fear can cause a "vicious cycle dynamic" of avoiding treatment, which worsens oral health. Research on the antecedents of dental anxiety could help design community programs to prevent dental fear. Public mental health and community dentistry can benefit from a multidisciplinary approach to dental fear and oral health [26]. Clinicians should integrate interdisciplinary knowledge, and carefully evaluate the risks and benefits of dental implant treatment [38]. The association between markers of dental health and depression at the population level was observed in a 2014 study and the researchers found a clear association between poor dental health and depression, independent of inflammatory markers. Although inflammation was not found to influence the relationship, other factors such as nutritional deficits, lifestyle, comorbidities [39], and certain antidepressant medications could explain the association. This study recommends that prospective cohort studies should be conducted to determine the direction of the relationship between dental health and depression and explore its underlying physiology. Such studies could identify modifiable risk factors for depression.

A key limitation was that most data used were derived from self-report instruments over different recall periods. Future studies investigating this relationship should include an objective measure of dental health and diagnostic interviews to assess depression. The findings suggest that oral health could be a potential key determinant of population health and that educational programs designed to improve oral health could be considered a primary prevention strategy [40,41,42].

In a short communication published in 2016, Ehrenthal et al conducted a pilot study on 310 patients with periodontitis and determined that the number of teeth at the beginning of treatment was predictive of depressive symptoms 13 years later. The fewer teeth a patient had, the higher their level of depression. The study also found that the number of comorbid somatic diseases was related to depression.

Tooth loss may impact depression through psychological, social, and biological pathways, and low self-esteem is a predictor of depression. While no association was found between the predefined variables and current levels of anxiety, depression may be more harmful to treatment success than anxiety, but anxiety and dental fear can be a factor for not having long-term dental procedures [43]. In De Gomez et al., study the correlation between state anxiety and depression, and objective and subjective pain at day 7 was not statistically significant. A strong correlation was found between objective and subjective pain during the immediate postoperative period. In the postoperative period immediately following dental implant insertion, a strong correlation exists between subjective and objective pain [44].

Moreover, informing patients about the anticipated surgical procedure of implant placement and the postsurgical pain intensity can reduce their anxiety level and subsequent postsurgical pain and discomfort. Patients can be informed that, in general, implant placement surgical experience is less unpleasant compared to tooth extraction with less postsurgical pain and limitation of daily activities. However, some factors can increase the pain intensity and discomfort level on an individual basis [45], statements that are contrary to the symptomatology of our patient who came to the psychiatry service after 8 months of psychical „pain" because there was no physical pain that she endured, there was all about the consistency of the food. During the dental implant process, there are some modifications concerning the alimentation. There are some eating troubles because patients cannot always adapt to the new consistency of the food so there also may exist some alimentary deficits that produce some deficits in our brain synthesis of tryptophane and serum serotonin that lead to depression, anxiety, and insomnia [46,47,48].

Also, Oral Health-Related Quality of Life should be a key consideration for global oral health programs, especially with the projected shortage of dentists, underserved populations, and the importance of interdisciplinary collaborations [49]. The same problems can be found in people with different chronic comorbidities such as diabetes [39]. Oral health-related quality-of-life tools may be socio-dental indicators, global self-ratings of oral health, and multiple-item questionnaires as Baiju et al., 2017 suggest [50]. Recent medical research indicated that oral health significantly impacts the quality of life. Studies on total prosthesis users show that those with implants have better quality of life and satisfaction. Higher satisfaction with oral health correlated with a better quality of life [51]. There is limited evidence to suggest a link between dental implants and suicide. While some individuals may experience a boost in self-esteem and confidence after getting dental implants, others may experience temporary feelings of depression related to the surgical procedure or recovery process. However, suicide is a complex issue that is typically caused by a combination of genetic, environmental, and psychological factors. Dental implants are unlikely to be the sole cause of suicide in any individual.

Comparison with literature

Oral health and mental health are closely linked, and there is evidence to suggest that individuals with dental pathologies may be at increased risk for developing psychiatric conditions.

Dental pathologies can have a significant impact on an individual's quality of life, including their physical health, social relationships, and self-esteem. The two-way association between oral and mental health is discussed, including how dental treatment can cause anxiety and phobia, and how psychiatric disorders can lead to dental disease.

Analyzing the literature and reviewing the articles published in the period 2012-2023, having as a topic of study the link between mental health and dental implantology technique, with different approaches we observed: if initially the topic was focused on technical problems of implantology [52,53,54, 55,56] the articles from the last years of the mentioned period for which I made the review, bring into discussion subtle physiopathological aspects of the relationship between mental disorders and oral implants [57,58,59]. Regarding the importance of the subject from a practical point of view, we can say that there is a relatively small number of articles related to this subject. This is the reason for writing this article. Thus, this subject is approached from the perspective of the holobiome, taking into account the fact that currently, on the one hand, the etiopathogenesis of psychiatric disorders is explained through the connection between the intestinal microbiome and the central nervous system (gut-brain axis), and on the other hand, it is sought evidence about the involvement of the oral microbiome, part of the microbiome in the digestive tract, in oral pathology. The link between mental pathology and oral pathology can be explained for now by the behavioral changes of people with psychiatric disorders [60] but there is evidence that demonstrates the existence of complex mechanisms at the level of chemical mediators [61,62].

Regarding the relationship between the oral implant and psychiatric pathology, a series of factors related to the type of personality or individual reactivity can be discussed on one hand, and on the other, factors directly related to the technique per se [13,63] (e.g. the long period of time required by the technique, interference with the patient's feeding possibilities, the need to attend a large number of consultations in the dental office, complications) [64,65] as well as related aspects related to costs high that can represent an obstacle for patients and implicitly a stress factor as well as a generator of psychiatric disorders. Worldwide, people search for improvement in the physical aspect not only in the dental field; non-surgical lifting has gained more ground because it requires little recovery time and the results are very positive [66].

Discussions regarding the connection between the implant logical technique sometimes related to certain particular individual anatomical peculiarities [67,68] and pre-existing psychiatric disorders or triggered by the stress of the procedure [69] are highlighted both by the articles that were the subject of the mentioned review and by the study carried out by us. In this context, it is important to emphasize the aspect of choosing the procedure in the field of implantology so that the duration of its realization is as short as possible, as well as the aspects related to the knowledge of the patient's medical history, especially that of the existence of psychiatric conditions [70] but also of behavioral habits, of financial resources and tolerance to dental interventions. The patient's motivation regarding the need for intervention from the perspective of functional [71] and aesthetic aspects related to self-esteem and social interactions represents the nucleus around which the intervention strategy in the dental sphere is built and the intervention plan regarding mental disorders. The need for studies in this field is obvious, considering the frequency of both categories of diseases as well as the implications in the economy of health systems due to the costs and consequences in the immediate and distant term such as causes of dental implant failure and the means of prevention these causes [72]. Also, anxiety disorders present a significant medical and societal concern, demanding thorough assessment and timely intervention. The COVID-19 pandemic exacerbated chronic dental disorders due to the enforced isolation and heightened fear of contracting the disease [73, 74]. Additionally, individuals with pre-existing dental pathology may experience a compounding effect on their anxiety levels, as oral health concerns contribute to overall well-being and can exacerbate existing mental health conditions [48,73].

CONCLUSIONS

By understanding the relationship between dental implants and anxiety, we can help individuals receive the dental care they need and ensure successful outcomes for those undergoing dental implant procedures.

In conclusion, while dental implants can improve one's self-esteem and confidence, it is important to recognize the potential for temporary feelings of anxiety and depression related to the surgical procedure or recovery process. Individuals who are experiencing symptoms of depression should speak with a mental health professional. By understanding the connection between dental implants and depression, we can help ensure successful outcomes for those undergoing dental implant procedures.

The relationship between dental implants and suicide is still not well understood, and more research is needed to fully understand the potential causes and impacts.

Oral health and mental health are closely linked, and there is evidence to suggest that individuals with dental pathologies may be at increased risk for developing psychiatric conditions. The relationship between dental pathologies and psychiatric pathologies is complex and multi-faceted, and it is important to address both oral and mental health in order to improve overall well-being.

Highlights:

1. Anxiety and depression scales should be applied to all patients before applying the implants

2. A study should be expanded regarding potential psychiatric pathologies triggered by pain and the new diet established for patients requiring dental implants.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, M.M. and L.A.M.; methodology, M.M and L.A.M.; software, C.C..; validation, M.M, and H.R; formal analysis, C.B.; investigation, M.M and L.A.M; data curation, M.N.M.; writing—original draft preparation, L.A.M and C.C.; writing—review and editing, visualization, R.H.; supervision, M.N.M and R.H. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of "Elisabeta Doamna" Clinical Psychiatry Hospital (02/18.05.2021) for studies involving humans, this study being part of the doctoral study of dr Moroianu Marius.

Patient consent for publication

Written informed consent has been obtained from the patient(s) to publish this paper.

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Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

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Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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The journal uses US spelling and authors should, therefore, follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

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Acknowledgments

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ORIGINAL ARTICLE

Correlations between Etiopathogenic Factors and Persistence on Anti-IL-17 Therapies in Patients with Psoriasis Vulgaris

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Abstract: *With recent advances in the understanding of psoriatic disease, it is increasingly considered a systemic inflammatory condition rather than limited to the skin and joints. A variety of biologics are available today for the treatment of psoriasis, but with them, characteristics such as rapidity of onset, long-term efficacy, safety profile, and effects on comorbidities differ. We designed an observational, non-interventional, retrospective study of patients with severe psoriasis receiving biologic treatment with IL-17 inhibitors and aimed to investigate the correlations between etiopathogenic factors and the efficacy and persistence of these therapies in a group of psoriasis patients from Romania. Study results show that patients treated with ixekizumab had better persistence and high adherence compared to those treated with secukinumab. In this study, ixekizumab demonstrated lower risks of non-persistence, discontinuation, and switching and a higher likelihood of high adherence compared with secukinumab. This study contributes to the understanding of the persistence of anti-IL17 biologic therapies in psoriasis and the factors that may influence it.*

Keywords: *psoriasis vulgaris, comorbidities, anti-IL-17 biological therapies, persistence*

INTRODUCTION

Plaque psoriasis is a common chronic immunological condition with a worldwide prevalence ranging from 1.0 to 8.5% [1,2]. With recent advances in the understanding of psoriatic disease, it is increasingly considered a systemic inflammatory condition rather than limited to the skin and joints. These extracutaneous disorders that have been linked to psoriasis include psoriatic arthritis, metabolic syndrome, obesity, cardiovascular disease, various malignancies, autoimmune diseases, chronic kidney disease, psychiatric disorders, smoking or alcohol abuse. Despite evidence supporting an increased prevalence of these risk factors among patients with psoriasis, data suggest that patients are under-screened and under-treated [3-7].

With the advent of biological therapies, the treatment of psoriasis has changed dramatically due to its high efficacy and safety. A variety of biologic agents are available for the treatment of psoriasis nowadays, however, characteristics such as rapidity of onset, long-term efficacy, safety profile, and effects on comorbidities differ. A better understanding of these characteristics leads to the correct choice of treatment for patients, leading to greater persistence, greater patient satisfaction, and minimizing the impact of psoriatic disease. Meta-analyses of randomized clinical trials in psoriasis have suggested that newer biologic therapies targeting interleukin (IL)-17 and IL-23 generally have greater efficacy than other biologic therapies [8]. However, the response to therapy and the safety of biological treatment for psoriasis in clinical trials do not fully reflect the usual clinical setting [9,10]. Therefore, in this study, we aimed to investigate both the efficacy and persistence of ixekizumab and secukinumab therapies, as well as the correlations between etiopathogenic factors and, respectively, psoriasis-associated diseases, with these biological therapies in a group of psoriasis patients from Romania.

MATERIALS AND METHODS

We designed an observational, non-interventional, retrospective study of patients with moderate to severe psoriasis receiving biologic treatment with IL-17 inhibitors (secukinumab and ixekizumab). The patients signed the informed consent to participate in this study, and it received the approval of the Ethics Committee no. 3122/04.02.2022. For this study, we used data from patients from the IInd Department of Dermatology of the Colentina Clinic Hospital in Bucharest, Romania, and data from patients belonging to dermatologists from private clinics in Bucharest. Initially, the analyzed group included 311 patients with moderate-severe psoriasis, from the National Registry of Dermato-Venereal Diseases, who at the time of the study are being treated or have had ixekizumab (IXE) and secukinumab (SEC) therapies in the past. Data were collected using questionnaires completed by treated patients and dermatologists at regular office visits at 3-month intervals for initiations and 6 months for periodic evaluations, respectively.

In the first phase of the study, we divided the group of patients into two groups determined by the two therapies, IXE and SEC, and analyzed the patients from the perspective of the main characteristics of sex, age, duration of the disease, time of onset of the therapy, body mass index, and associated comorbidities. In this analysis, we calculated the main descriptive indicators and then used the z, Student, and χ2 tests to evaluate the differences between groups. To ensure the comparability of the two groups defined above, from the initial sample of patients, in accordance with the literature, we extracted a subgroup of 255 patients who had or are still on the two biological therapies. For this subsample, we presented the main characteristics of the two lots. In the second phase of the study, we continued with the evaluation of the persistence of the patient's treatment, using the Kaplan-Meier curves. The impact of comorbidities on persistence in therapy is also analyzed. The Log-rank test was used to statistically assess potential differences between groups [11]. The difference is considered statistically significant if the p-risk associated with a test is below the 0.05 threshold.

RESULTS

Analyzing the two groups of patients, it is observed that in the case of IXE therapy, the gender ratio is 1.9 in favor of men, while in the case of SEC, it is 1.04 in favor of women. Consequently, it can be stated that between the two groups, there are statistically significant differences (p=0.028) from the perspective of the gender structure.

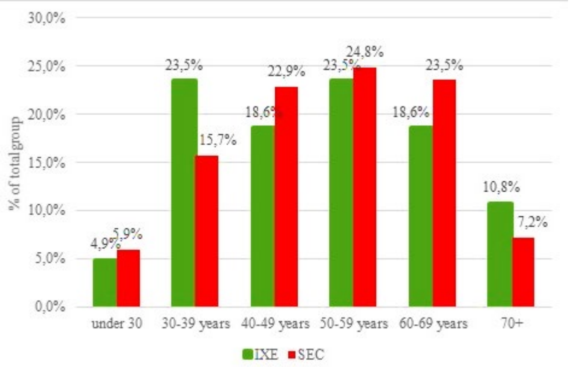

Figure 1: Distribution of patients by age group for each therapy

The distribution of patients by age groups does not determine significant differences between the groups as a whole, in the sense of the χ2 test (p=0.46), even if in the case of IXE therapy, the share of patients under 40 is slightly higher than in the case of SEC. Calculating the average age, we obtain 50.4 years in the case of IXE and 51.2 in the case of SEC, so the difference between the groups is statistically insignificant in the sense of the t-Student test (p=0.77).

The most common risk identified in patients with psoriasis is that related to smoking. The results (Figure 2) show that 42.2% of patients with IXE therapy and 34.0% of patients with SEC are smokers, proportions that do not indicate significant differences between the two groups (p=0.23). The second most frequent comorbidity among patients with psoriasis treated with IXE or SEC is the presence of cardiovascular disease (CVD). About a quarter of the patients in the study, 25% of the SEC group and 20.6% of the IXE group, had an associated CVD.

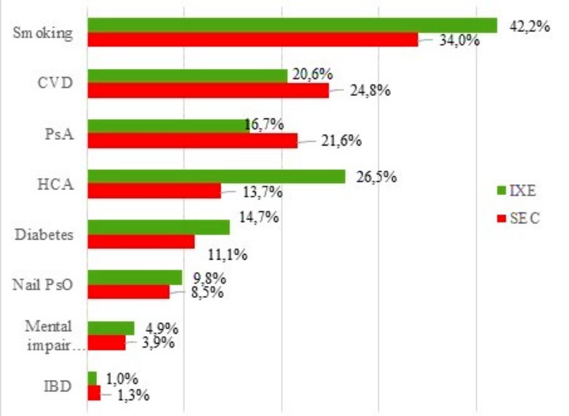

Figure 2: The presence of risks and comorbidities in comparison for IXE and SEC

Heredo-collateral antecedents (HCA) are identified to a lesser extent in patients under SEC therapy. On this branch, only 13.7% of patients had a family history of the disease. Among patients with IXE, the number is almost double, and the difference between groups becomes statistically significant (p=0.032). Without identifying a statistically significant difference (p=0.40) between groups, psoriatic arthritis (PsA) is the third most common comorbidity. This is present in 21.6% of patients in the SEC branch and 16.7% of patients with IXE. Other comorbidities, such as inflammatory bowel disease (IBD) or mental impairment, occur less frequently in patients with psoriasis. Obesity is also an important risk factor for psoriasis. To highlight the differences between the groups in this perspective, a structure is presented in Figure 3.

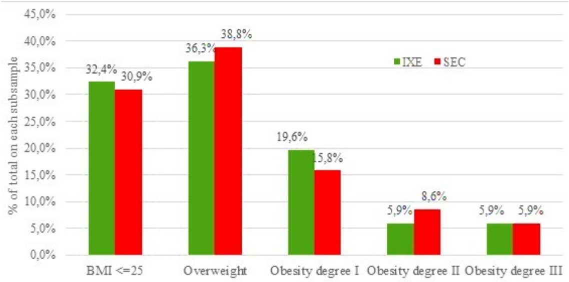

Figure 3: Distribution of patients according to BMI and biological therapy

The largest proportion of patients undergoing psoriasis treatment have an above-normal BMI. The data presented synthetically in Figure 3 shows that only one-third of patients have a BMI within normal limits. Applying the χ2 test, we did not identify a statistically significant difference between the two groups in terms of the distribution of body mass index classes.

Table 1: The main treatment-related characteristics of the two groups, IXE vs. SEC

Treatment	IXE, n (%) n1=102 (100%)	SEC, n (%) n2=153 (100%)	Testing the difference between groups
Switch	13,7%	27,5%	p=0,032^{*1}
Methotrexate	96,9%	97,1%	p=0,888
Neotigason	7,4%	5,1%	p=0,577
UVB	4,9%	5,9%	p=0,724
PUVA	35,6%	24,2%	p=0,095

No significant differences were found between the two groups in the analysis of previous treatments. As can be seen in Table 1, 97% of patients followed methotrexate therapy, 5% with UVB. Further, approx. 6% of patients in the study had prior treatment with neotigason. It can also be pointed out that a third of the patients now under IXE treatment had undergone PUVA treatment and a quarter of the SEC patients had previously had this treatment. From the perspective of PUVA, a statistically significant difference between groups can only be considered if an error of p=0.095 is accepted.

It should be noted that during the analyzed period, January 31, 2018 - December 18, 2021, 13.7% of patients with IXE and 27.5% of patients with SEC, respectively, changed therapy due to an adverse reaction or due to treatment inefficiency. In particular, there are 6 patients who during the analyzed period switched from SEC to IXE and 4 patients from IXE to SEC. Following the two curves, it can be seen that 2 years (24 months) after the initiation of biological therapy, 88% of patients with IXE are still on treatment, while only 75% of patients with SEC still benefit from this treatment (Figure 4). Following patients on both IXE and SEC trajectories, at four years, 80% of IXE patients and 67% of SEC patients were still adherent to treatment.

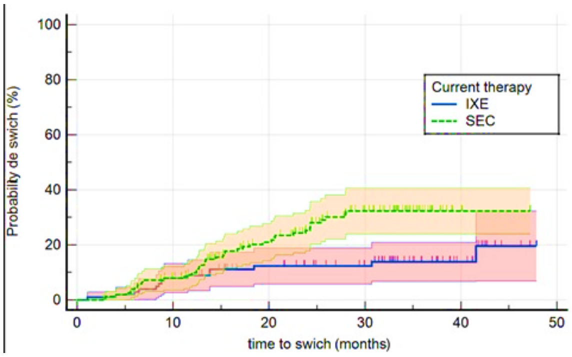

Figure 4: Probability of switching patients on the two therapies

Overall, the median persistence for IXE is 43 months, and for SEC is 37 months. Thus, a statistically significant advantage in favor of IXE is observed. In total, the average duration of treatment is approximately 40 months.

Next, we will analyze the possible impact of determinants such as sex, age, and comorbidities on treatment persistence in the two biological therapies IXE and SEC, respectively. The age group is a significant factor when analyzing treatment persistence in patients on biologic therapy with IXE. Thus, people over 50 years tend to have a lower persistence, respectively a higher switching probability, compared to the younger group (p=0.038), in the case of IXE therapy. It should be noted that the differences by age groups, for IXE therapy, as can be seen in Figure 5, are achieved quite quickly over time, generally after the first 12 months.

In the case of SEC therapy, a significant difference (p=0.046) is observed between the two age groups analyzed, but the difference between the two arms appears later, after 24 months of treatment.

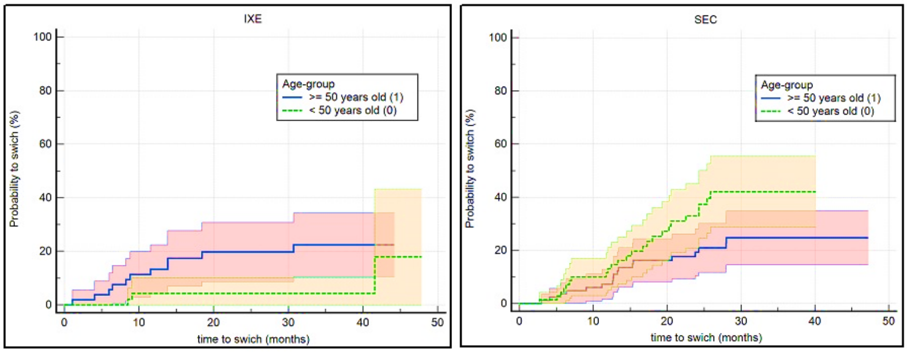

Figure 5: Probability of switching current biological therapy according to age group

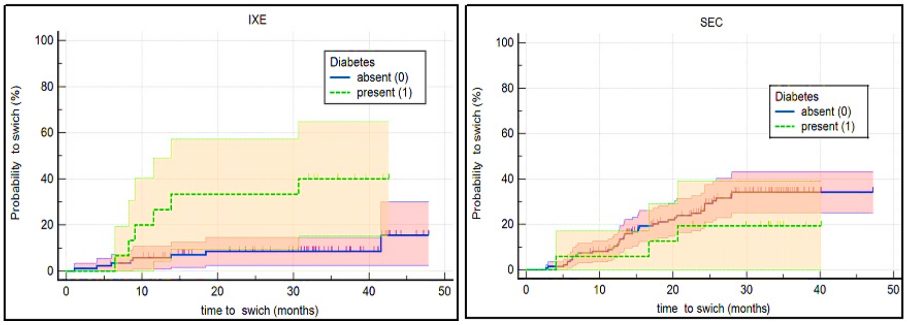

Figure 6: Probability of changing current biological therapy depending on the presence of diabetes

Patients with diabetes mellitus (p<0.01) on IXE therapy tend to have a longer duration of treatment. At 36 months after initiation of therapy, 40% of patients with diabetes switch to IXE therapy. At the same time, only 10% of non-diabetic patients switch from IXE. In the case of SEC therapy, no statistically significant difference was observed between the two subgroups (p=0.30) (Figure 6).

The average duration of persistence for people with a BMI below 29 is 45.9 months, and patients with a BMI above 29 have an average duration of treatment of 41.7 months. Accepting a p=0.023 error, it can be stated that there is a statistically significant difference between the subgroups defined by BMI, on IXE therapy. Regarding SEC therapy, we found no significant difference between subgroups (Figure 7).

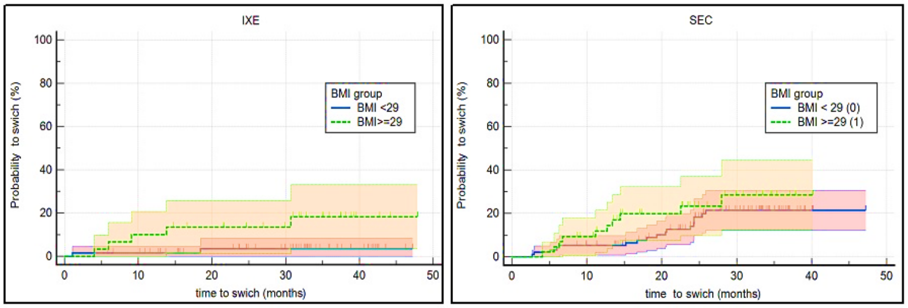

Figure 7: Probability of changing current biologic therapy by BMI class

It was observed that patients with PsA do not have a higher switch probability from IXE therapy than patients without PsA (p=0.76). This result is also similar to SEC therapy (Figure 8).

The presence of nail involvement in psoriasis does not statistically significantly influence the persistence of treatment on any of the therapies studied. In both cases, the first-generation risk associated with the LR Test is greater than 0.5 (Figure 9).

In the case of patients under IXE therapy, there is a significant difference (p<0.01) between patient subgroups determined by the age of the condition. Thus, patients with the condition over 13 years have a higher probability of change. For this category, four years after starting IXE therapy, 40% of patients change or stop biological therapy. For patients who discovered the disease more recently, the probability of passing to 48 months is below 10% (Figure 10).

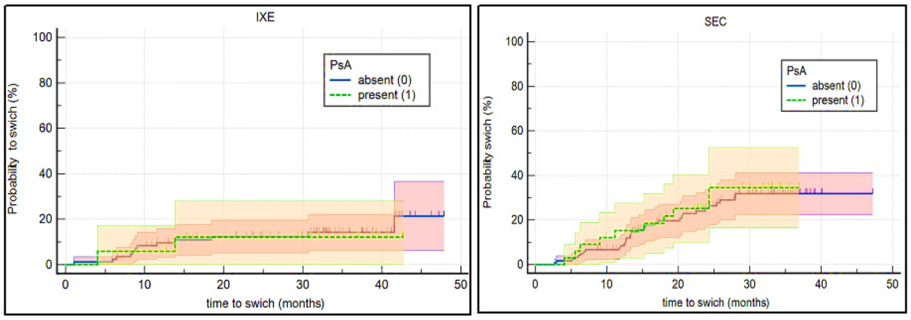

Figure 8: Probability of changing current biological therapy based on the presence of PsA

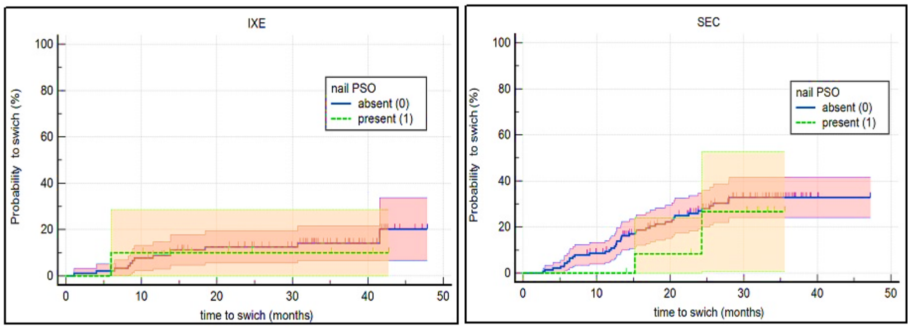

Figure 9: Persistence on biological therapies concerning the presence of nail psoriasis

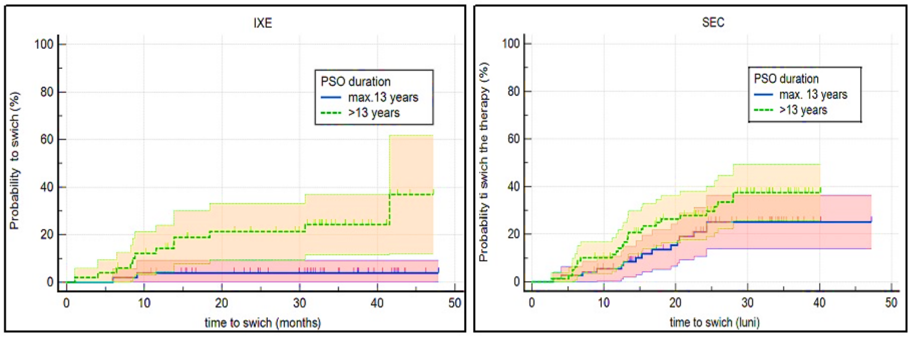

Figure 10: The psoriasis duration and the probability of switching

For the subgroups with SEC therapy, we did not identify a statistically significant difference, so at 48 months, 35-40% of patients on SEC therapy will switch this treatment regardless of the age of the underlying condition.

Other factors such as HCA, CVD, IBD, or previous systemic conventional therapy (PUVA or UVB) were not significant for any of the therapies studied.

DISCUSSION

Evidence from "real life" is of undeniable importance because it assesses efficacy and safety in the everyday context, which may be different (multiple comorbidities, hard-to-treat areas, etc.) than that represented by clinical trials [12]. Unfortunately, few studies are comparing IXE and SEC for patients with psoriasis. Based on the results of this study, it can be seen that patients treated with IXE had better persistence and high adherence compared to those treated with SEC. Several studies have shown a lower efficacy of SEC in real life compared to clinical trials, and the present study reveals similar results consistent with these [13–17]. These observations are also supported by the fact that 2 years after initiation of biologic therapy, a higher percentage (88%) of patients with IXE are still on treatment compared to a lower percentage (75%) of patients with SEC who continue this treatment. Moreover, we can observe that after 4 years of therapy, the results do not differ much. Thus, 80% of patients treated with IXE are still on therapy 48 months after onset, compared to only 67% of patients treated with SEC, showing a statistically significant advantage in favor of IXE.

Previous studies in the literature have already identified female gender as an independent risk factor for discontinuation of biological treatment in psoriasis [18-27]. In contrast, our study concluded that there was no statistically significant difference between the sexes in persistence on therapies for any of the therapies studied.

Regarding age group, there is very little data in the literature to reveal a correlation between it and the persistence on biologic therapies with IXE or SEC. The present study showed that patients older than 50 years treated with IXE tend to have lower persistence compared to the younger group. It is worth noting that differences by age groups are realized quite quickly in the case of IXE, after the first 12 months, in contrast to SEC, after 24 months.

Significant associations have been observed between psoriasis and obesity or being overweight. The relationship is hypothesized to be bidirectional, with obesity predisposing patients to psoriasis and psoriasis increasing the risk of obesity. About 70% of patients treated with both therapies have a BMI above normal. Taking into account the fact that in the Romanian population, the overweight rate was 52.5% [28], the present study indicates that the distribution of patients with psoriasis is inclined toward people with BMI above normal (Figure 3).

On the other hand, obesity may influence the therapeutic approach and clinical response to systemic treatment in psoriasis or psoriatic arthritis. Indeed, adipose tissue can alter the volume of distribution of the drug and thus limit its efficacy [29]. Because biologic therapies are usually given in a fixed dose, a weight-adjusted dose is likely necessary for overweight/obese patients. It was reported that in 33 patients with moderate to severe psoriasis on biologic therapy, only 7 patients who lost weight achieved the greatest improvement in mean PASI score at the end of the study [30]. Evidence was also provided that in obese patients with psoriasis on biological therapies following an 8-week nutritional program with a low-calorie diet (≤1000 kcal per day), body weight reduction was associated with increased therapy efficacy [31].

Supporting the correlation between obesity and treatment impairment in patients with psoriasis [32], the present study showed that individuals with a BMI above 29 have a higher risk of discontinuation/switching concerning IXE therapy. Regarding SEC therapy, no significant difference was observed between subgroups.

The study showed that psoriatic arthritis did not significantly influence the likelihood of switching treatment for either therapy, in contrast to an Austrian retrospective observational study, where they observed a slight increase in the number of patients with psoriatic arthritis who discontinued biologic therapy [33], or, on the contrary, another study in which we can observe the fact that the simultaneous existence of psoriatic arthritis with skin damage was correlated with the persistence of biological therapy [34].

After analyzing the existence of the diabetes factor concerning the persistence of therapies, patients with diabetes under IXE therapy tend to have a longer duration of treatment, more than 36 months after the start of therapy, a fact that contradicts a recent American study [35]. In the case of SEC, we found no statistically significant difference between patient subgroups. Biologic agents are also known to counteract insulin resistance and demonstrate an even greater protective effect against the development of diabetes in patients with psoriasis [36].

Finally, the results of the analysis of etiopathogenic factors, such as HCA, CVD, IBD, or the presence of conventional systemic therapy in the past (PUVA or UVB), were not found to be significant for any of the studied therapies.

This study has some limitations, such as the relatively small number of patients and the short follow-up period. Indeed, the small number of patients may limit the interpretation of whether the differences in performance between the two therapies were significant and clinically relevant. Over time, as larger samples with longer follow-ups are created, they may allow such analysis.

CONCLUSIONS

In this study, we explored the persistence of anti-IL17 biologic therapies and the association between these therapies and various comorbidities in patients with severe psoriasis to determine the optimal systemic management of psoriasis through early screening and intervention for these comorbidities. Our results provide an unbiased and real-world analysis of the persistence of anti-IL-17A therapies in a cohort of patients with severe psoriasis. Thus, it is found that IXE presented a significantly higher persistence than SEC in patients with psoriasis in the studied groups, these data being consistent with those of other studies in the literature [37,38]. Differences in their IL-17 binding affinity could explain these findings, and stronger binding affinity is usually related to less antibody dissociation [39]. At the same time, it is not clear whether the frequency of administration should be increased following insufficient responses to standard dose SEC therapy. Although the initial loading dose may have a rapid response, over time the maintenance dose may be too low, leading to a possible relapse at a certain threshold [40,41].

Following the association of psoriasis with multiple comorbidities, the need for comprehensive screening and treatment must be recognized and addressed. Biologic therapies prescribed in psoriasis may exacerbate comorbidities, which in turn may influence psoriatic disease.

Therefore, the appropriate management of psoriasis must involve an integrated approach [42,43].

Conflicts of interest and sources of funding

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Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

Authors' contribution

For the research of this article, the authors contributed equally.

Ethics approval and consent to participate

For this study, we received the approval of the Ethics Committee no. 3122/04.02.2022.

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ORIGINAL ARTICLE

The Benefits of Phytotherapy in Chronic Pelvic Pain Syndrome

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Abstract: *Chronic pelvic pain syndrome (CPPS) presents a diagnostic challenge due to its multifactorial nature, often involving urological, gynecological, and psychological components. This study focuses on the effectiveness of prophylactic phytotherapy in preventing CPPS recurrence, aiming to mitigate symptoms and psychosocial impact. The study includes 74 patients following a six-month treatment regimen with Graminex, Seronoa Repens, Curcuma Longa, and Acediboswellic ke-to-acetyl-11. Symptom assessment employs the IPSS and NIH-CPSI questionnaires, conducted at 3, 6, 8, 9, and 12 months. Patients exhibit significant improvements in IPSS scores, with maintenance of treatment impact observed at 3, 6, and 9 months. Interruption of therapy leads to symptom recurrence, but upon resumption, responses are more effective. The third group, under observation for three months, reports positive outcomes. Both IPSS and NIH-CPSI scores show a consistent impact on urinary symptoms and pelvic pain, indicating the therapy's effectiveness. The study demonstrates a concordance in the positive effects of phytotherapy across different age groups, emphasizing its efficacy in alleviating urinary symptoms and pelvic pain in CPPS patients. Symptom remission or improvement in quality of life is reported, with a notable tropism for symptom reduction in the cold season. Patient adherence to treatment is high, and even after treatment interruption, resumed therapy yields significant improvements. The observed benefits include the absence of pelvic pain, dysuria, and enhanced sexual competence, contributing to an overall improvement in the quality of life for patients with CPPS.*

Keywords: *CPPS, phytotherapy, IPSS, NIH-CPSI, dysuria*

INTRODUCTION

Recent reports have highlighted more and more accusations from patients regarding chronic pelvic pain of apparently idiopathic causes, symptoms that are often accompanied by urinary tract dysfunctions, as well as sexual dysfunctions. [1]

Chronic pelvic pain can in many cases be the result of affecting several systems, devices, and organs, this fact determining over time a multidisciplinary collaboration between medical and surgical specialties such as urology, gynecology, gastroenterology, neurology, psychiatry, and general surgery.[2]

A subdivision of the concept of Chronic Pelvic Pain is known as Chronic Pelvic Pain Syndrome (formerly known as chronic prostatitis), the diagnosis of the syndrome is, according to the European Asociation of Urology (EAU), established only when the presence of an infection has been ruled out, or of an inflammation or a local pathology of any other nature.[3]

Thus, chronic pelvic pain syndrome becomes a diagnosis of exclusion. [4]

Along with the organic considerations related to the pathology, the psychological implications must also be taken into account, such as depression, anxiety, and social disturbances, at the workplace as well as at the couple level, all of which will negatively influence the patient's quality of life. [5]

Pain mechanisms include infection, central nervous system modulation, as well as emotional, cognitive, behavioral, and sexual mechanisms. [6]

The treatment of this condition consists (according to the European urology guidelines) of the administration of anti-inflammatory drugs, alpha-blockers, antibiotics, 5-alpha-reductase inhibitors, phytotherapy, pregabalin, pentosan polysulfate, muscle relaxants, botulinum toxin, leukotriene antagonists, allopurinol and analgesics. [7]

The main objective of this study is to observe the effectiveness of prophylactic phytotherapy in terms of preventing the recurrence of pelvic pain syndrome in patients known to have this pathology.

In contrast to the pharmacological therapy used in the classic treatment schemes of this pathology, phytotherapy associates significantly lower risks of side or adverse reactions, a fact that can ensure the patient's long-term treatment adherence superior to the classical treatment schemes, with the prevention of both of the recurrence of symptoms, as well as of the psychosocial effects associated with this condition.

The therapeutic scheme proposed for the patients included in this study consists of the synchronous administration of:
- Graminex – contains Cernitlon T60 and Cernitin GBX, pollen-based compounds with a proven anti-inflammatory effect.
- SeronoaRepens – is an extract of dwarf palm, with anti-androgenic and anti-inflammatory effects.
- CurcumaLonga – is represented by the extract from the perennial plant of the same name, of Asian origin, with antimicrobial, anti-inflammatory, antioxidant, and hypoglycemic effects.
- Acediboswellic keto-acetyl-11 – extract from Boswellia Serrata, which is a variety of incense of Asian origin, with anti-inflammatory action.

Among the objectives of this study are:
- Retrospective evaluation of the working group and the results obtained to create a unitary diagnostic and treatment protocol.
- Creating questionnaires or improving the existing ones regarding this pathology, taking into account the already existing protocols.
- Professional training of the team of specialists in the field to achieve the desired object.

MATERIALS AND METHODS

Up to this moment, we have a number of 74 patients who followed the previously mentioned treatment scheme over 6 months (52 from the initial batch 22 from the second batch, and 20 from the third batch). The distribution of patients by groups of age is in Table 1.

Table 1: The distribution of patients by groups of age

Age	21-25	26-30	31-35	36-40	41-45	46-50	50-60
Number	5	13	11	16	18	16	15

The symptomatology assessment was done using two internationally validated questionnaires: IPSS and NIH-CPSI.

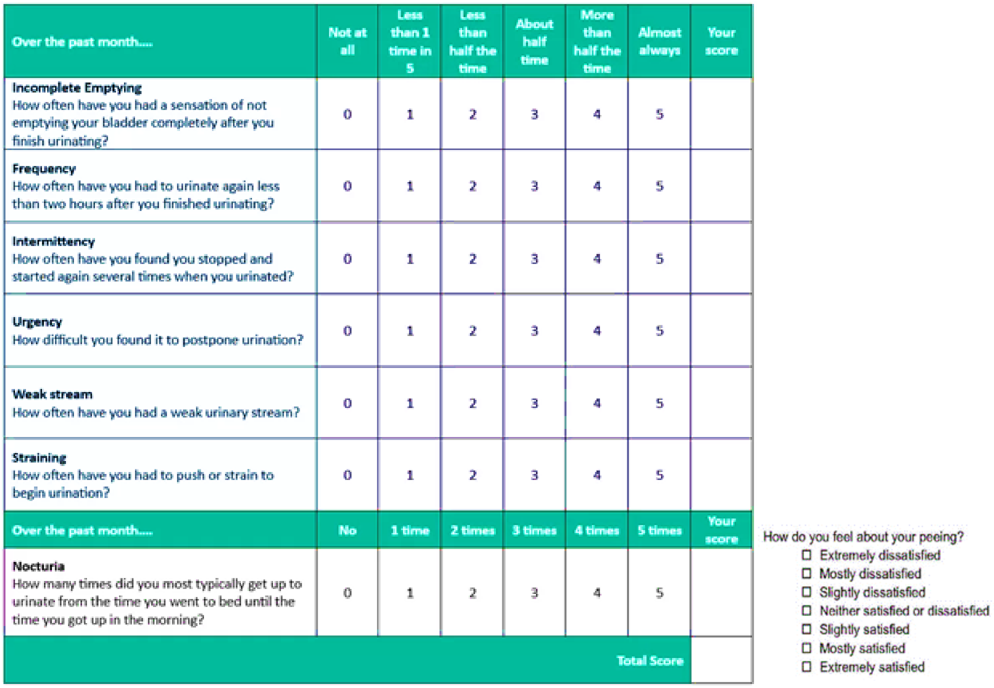

Over the past month....	Not at all	Less than 1 time in 5	Less than half the time	About half time	More than half the time	Almost always	Your score
Incomplete Emptying How often have you had a sensation of not emptying your bladder completely after you finish urinating?	0	1	2	3	4	5	
Frequency How often have you had to urinate again less than two hours after you finished urinating?	0	1	2	3	4	5	
Intermittency How often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
Urgency How difficult you found it to postpone urination?	0	1	2	3	4	5	
Weak stream How often have you had a weak urinary stream?	0	1	2	3	4	5	
Straining How often have you had to push or strain to begin urination?	0	1	2	3	4	5	
Over the past month....	No	1 time	2 times	3 times	4 times	5 times	Your score
Nocturia How many times did you most typically get up to urinate from the time you went to bed until the time you got up in the morning?	0	1	2	3	4	5	
						Total Score	

How do you feel about your peeing?
☐ Extremely dissatisfied
☐ Mostly dissatisfied
☐ Slightly dissatisfied
☐ Neither satisfied or dissatisfied
☐ Slightly satisfied
☐ Mostly satisfied
☐ Extremely satisfied

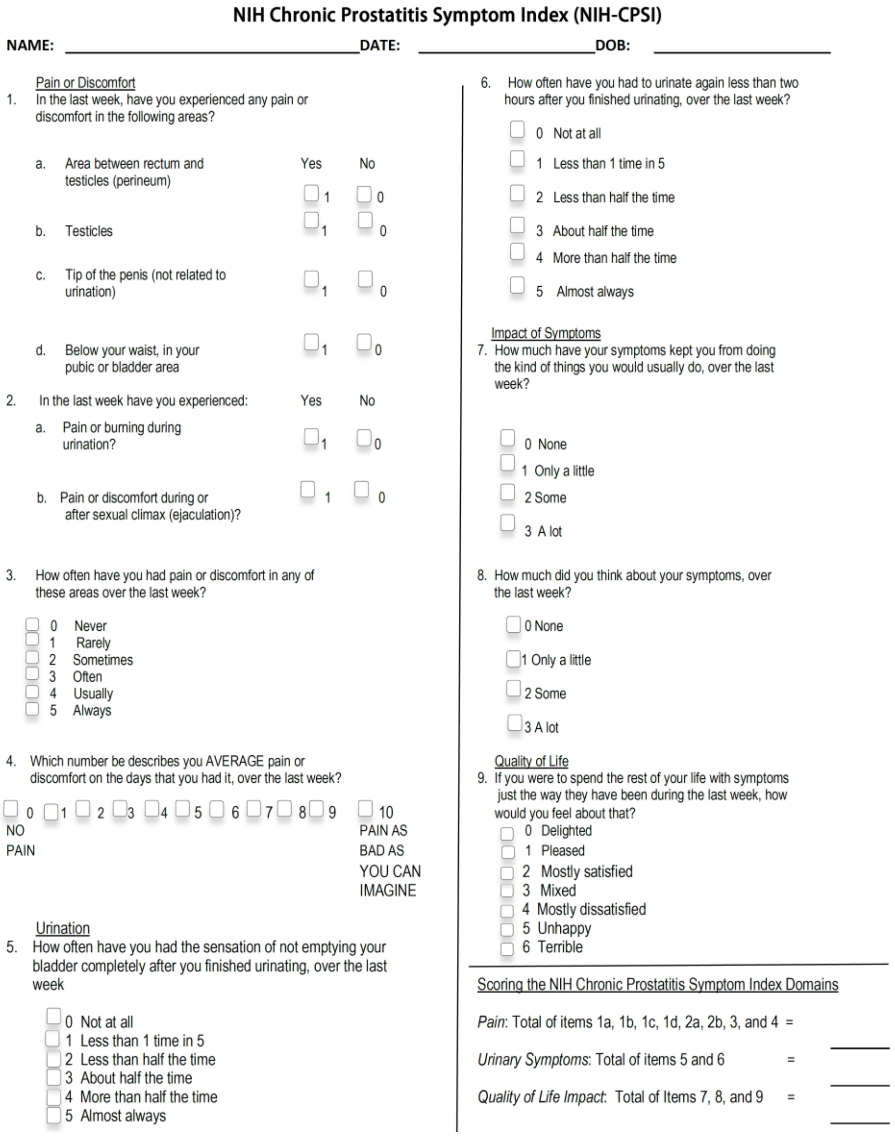

NIH Chronic Prostatitis Symptom Index (NIH-CPSI)

NAME: ______________________ **DATE:** ______________ **DOB:** ______________

Pain or Discomfort

1. In the last week, have you experienced any pain or discomfort in the following areas?

		Yes	No
a.	Area between rectum and testicles (perineum)	☐ 1	☐ 0
b.	Testicles	☐ 1	☐ 0
c.	Tip of the penis (not related to urination)	☐ 1	☐ 0
d.	Below your waist, in your pubic or bladder area	☐ 1	☐ 0

2. In the last week have you experienced:

		Yes	No
a.	Pain or burning during urination?	☐ 1	☐ 0
b.	Pain or discomfort during or after sexual climax (ejaculation)?	☐ 1	☐ 0

3. How often have you had pain or discomfort in any of these areas over the last week?

☐ 0 Never
☐ 1 Rarely
☐ 2 Sometimes
☐ 3 Often
☐ 4 Usually
☐ 5 Always

4. Which number be describes you AVERAGE pain or discomfort on the days that you had it, over the last week?

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

NO PAIN — PAIN AS BAD AS YOU CAN IMAGINE

Urination

5. How often have you had the sensation of not emptying your bladder completely after you finished urinating, over the last week

☐ 0 Not at all
☐ 1 Less than 1 time in 5
☐ 2 Less than half the time
☐ 3 About half the time
☐ 4 More than half the time
☐ 5 Almost always

6. How often have you had to urinate again less than two hours after you finished urinating, over the last week?

☐ 0 Not at all
☐ 1 Less than 1 time in 5
☐ 2 Less than half the time
☐ 3 About half the time
☐ 4 More than half the time
☐ 5 Almost always

Impact of Symptoms

7. How much have your symptoms kept you from doing the kind of things you would usually do, over the last week?

☐ 0 None
☐ 1 Only a little
☐ 2 Some
☐ 3 A lot

8. How much did you think about your symptoms, over the last week?

☐ 0 None
☐ 1 Only a little
☐ 2 Some
☐ 3 A lot

Quality of Life

9. If you were to spend the rest of your life with symptoms just the way they have been during the last week, how would you feel about that?

☐ 0 Delighted
☐ 1 Pleased
☐ 2 Mostly satisfied
☐ 3 Mixed
☐ 4 Mostly dissatisfied
☐ 5 Unhappy
☐ 6 Terrible

Scoring the NIH Chronic Prostatitis Symptom Index Domains

Pain: Total of items 1a, 1b, 1c, 1d, 2a, 2b, 3, and 4 = ______
Urinary Symptoms: Total of items 5 and 6 = ______
Quality of Life Impact: Total of Items 7, 8, and 9 = ______

The IPSS score is part of all prostate adenoma diagnostic guidelines, but its interpretation is not absolute, as the answers to the questionnaire questions have a great variability from one patient to another. Although any patient can complete such a questionnaire by himself, it is at least useful for the interpretation of the results to be done by a doctor.

It should be noted that mild and average (moderate) symptoms do not require, most of the time, a therapeutic attitude. However, this score can hide early serious conditions (such as prostate cancer), and to exclude it, a PSA test and possibly a urological consultation is necessary. Severe symptomatology requires a therapeutic attitude represented by drug treatment or surgical intervention.

Classification according to severity (according to the American Urological Association):

- Mild (gr. I): IPSS = 0-7; Quality of life = 0-2. Absence of prostate adenoma complications.
- Medium (gr. II): IPSS = 8-19; Quality of life = 3-4. Possibly, the presence of chronic complications of the prostate adenoma; The patient with IPSS > 7 or quality of life > 2 is referred to a urologist for evaluation of prosta-te adenoma.
- Severe (gr. III): IPSS = 20-35; Quality of life = 5-6. The presence of chronic complications of prostate adenoma. Possibly, the presence of acute complications.

The NIH-Chronic Prostatitis Symptom Index (NIH-CPSI) is a commonly used 13-item questionnaire for the assessment of symptom severity in men with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). For each item, score ranges are 0–1 (6 items), 0–3 (2 items), 0–5 (3 items), 0–6 (1 item), and 0–10 (1 item).

We have gathered the results from these people that have started the study, at 3, 6, 8, 9, and 12 months. During this time, at 6 months, the patients have stopped taking medication and have reinitiated it after 3 months of break.

RESULTS

Compared to the first data, the maintenance of the impact of the treatment on pelvic pain, urinary symptoms, and quality of life is observed among the first group, with the second having the same effect at 3, 6, and 9 months as the previous group.

It has been observed that when the therapy is interrupted, the symptoms return, and when it is resumed, the response is even more effective compared to the first treatment.

The outcomes at 12 months by baseline batch IPSS score are described in Table 1, and Figure 1. The outcomes at 9 months by baseline batch IPSS score are described in Table 2, and Figure 2.

The last group of patients included in the study followed the treatment for a period of approximately 3 months, with the IPSS score values being as in Table 3, and Figure 3.

Table 1: Outcomes at 12 months by baseline batch IPSS score

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial IPSS	7	11.5	15.2	18.7	20	26.1	22.3
IPSS after 3 months	2	4.2	5.4	8	10.5	12	7
IPSS after 6 months	2.5	4.6	5.5	8	11	12.2	7.5
IPSS after 8 months	4	6	9	12.2	16.4	14.8	15
IPSS after 12 months	2	4.1	5.2	7.5	10	11.5	6.8

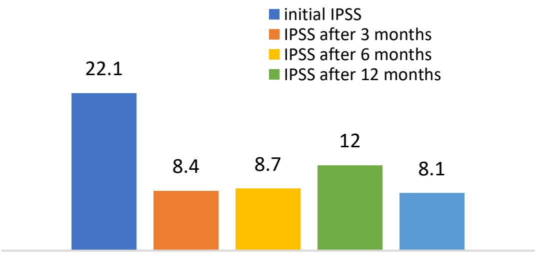

Figure 1: Mean IPSS score at the start of treatment and at 3, 6, 8, and 12 months later

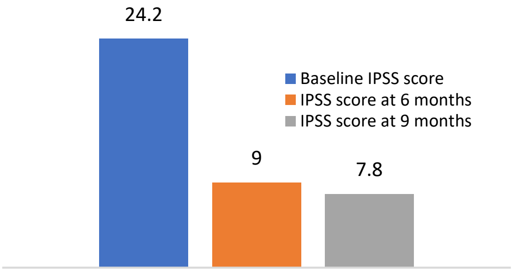

Figure 2: Mean IPSS score at the initiation of treatment of the second group at 9 months

Table 2: Outcomes at 9 months according to the IPSS score of the second batch

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial IPSS	9	13.6	14.8	15.5	18	29.4	23.8
IPSS after 3 months	3	5.4	6	7.5	11.2	14.6	11.2
IPSS after 9 months	2.5	5	5.8	7	10.6	13.6	10.5

Table 3: Mean IPSS score at treatment initiation of the third group over 3 months

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial IPSS	8	14.6	15.8	15.5	17.2	21.4	19.8
IPSS after 3 months	3	5.4	6	7.5	11.2	14.6	11.2

The outcomes of NIH-CPSI are illustrated in Tables 4 and 5 and in Figures 4 and 5.

DISCUSSION

A concordance between the two evaluations can be observed. In both cases, there is a significant positive impact regarding the urinary symptoms and also the pelvic pain, these findings being observed in all 3 groups of patients, thus revealing the beneficial effects of the therapy, as well as the tropism for the cold season of this pathology.

Another important aspect that we have noticed in those patients who have interrupted the therapy, the symptomatology tends to re-establish itself, but when the treatment is resumed is that the patients respond very well, even better compared to the moment of its initiation.

Table 4: Outcomes at 12 months by NIH-CPSI score of the first cohort

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial NIH-CPSI	11.5	14.4	17.6	20.5	27.4	28.8	25.6
NIH-CPSI after 3 months	4	5.5	7	11.6	14.3	18	16.2
NIH-CPSI after 6 months	4.5	6	9.5	10.3	15.5	18.5	15.2
NIH-CPSI after 8 months	8	9.6	13.2	15.6	20.2	19.8	18.8
NIH-CPSI after 12 months	4	5	6.8	10	12	17	14.6

Table 5: Outcomes at 12 months by NIH-CPSI score of the second cohort

Age	20-25	25-30	30-35	35-40	40-45	45-50	50-60
Initial NIH-CPSI	13	15.4	17	21	26.5	29.2	24.5
NIH-CPSI after 3 months	4.5	6.5	9	9.8	14.6	19.5	14.4
NIH-CPSI after 9 months	4	6	8	8.2	12.5	17	12.4

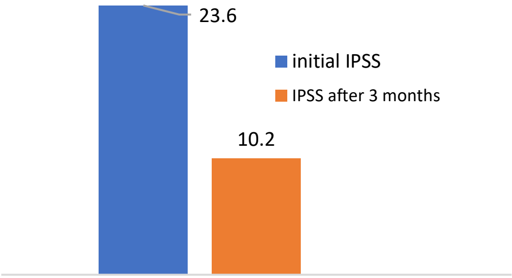

Figure 3: Mean IPSS score at treatment initiation of the third group over 3 months

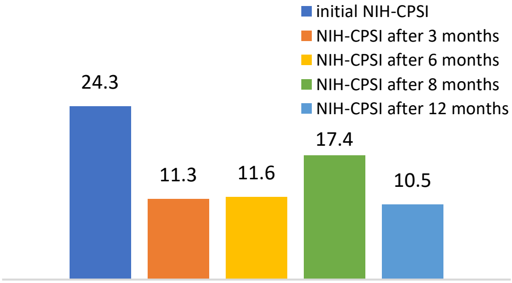

Figure 4: Mean baseline NIH-CPSI score of the first group and at 3, 6, 8, and 12 months

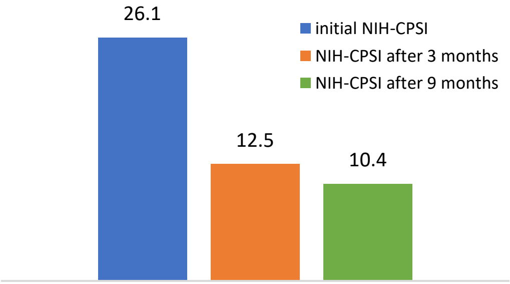

Figure 5: Mean NIH-CPSI score at treatment initiation of treatment of the second group at 3 and 9 months

In the case of 44 of the 52 patients from the initial group, compared to the 3-month results, the symptomatology remained constant, with the patients reporting a significant improvement in the quality of life. It was evident that compared to the cold season, the symptoms decreased, responding more effectively to the treatment.

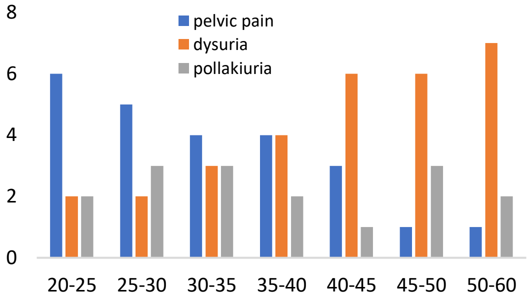

Figure 6: Initial symptomatology

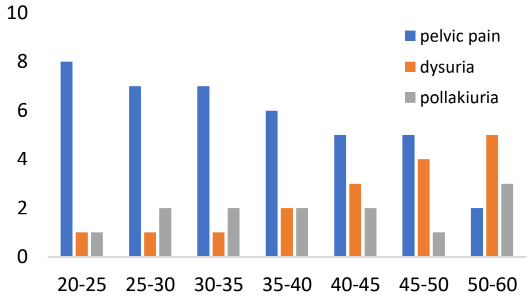

Figure 7: Symptomatology at 3 months

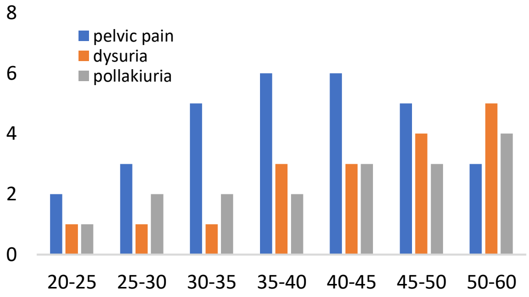

Figure 8: Symptomatology at 6 months

From the group of 22 patients introduced between 3 and 12 months into the study, remission of symptoms was observed in the case of 19, with the other 3 presenting a significant improvement in the quality of life and having a high adherence to the treatment. Again, the impact of chronic pelvic pain is highlighted, more pronounced in the cold season.

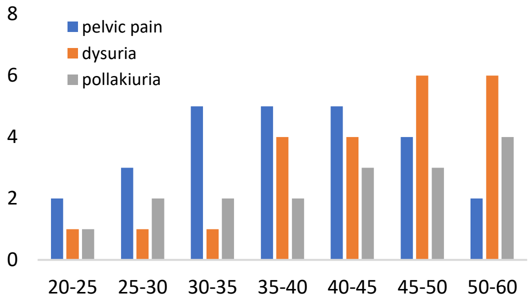

Figure 9: Symptomatology at 12 months

In the case of 44 of the 52 patients from the initial group, compared to the 3-month results, the symptomatology remained constant, with the patients reporting a significant improvement in the quality of life. It was evident that compared to the cold season, the symptoms decreased, responding more effectively to the treatment.

From the group of 22 patients introduced between 3 and 12 months into the study, remission of symptoms was observed in the case of 19, with the other 3 presenting a significant improvement in the quality of life and having a high adherence to the treatment. Again, the impact of chronic pelvic pain is highlighted, more pronounced in the cold season.

The main benefits reported by patients are represented by the absence of pelvic pain, dysuria, and improvement of sexual competence. Young people continue to report complete or almost complete remission of symptoms, and people over 50 years of age, in addition to a significant increase in quality of life, report an improvement in urinary frequency and the complete or almost complete disappearance of dysuria.

Furthermore, for the 54 patients in the initial group, the treatment was interrupted for 3 months, with its resumption, reporting significant improvement after the resumption of treatment.

The 22 patients in the second group followed the same therapeutic regimen as the first group, reporting the same benefits, and then stopped treatment for the first time.

The last batch of patients are still under observation, but all report improvements in both urinary and sexual performance, significantly increasing their quality of life**.**

CONCLUSIONS

The study demonstrates a concordance in the positive effects of phytotherapy across different age groups, emphasizing its efficacy in alleviating urinary symptoms and pelvic pain in CPPS patients.

Symptom remission or improvement in quality of life is reported, with a notable tropism for symptom reduction in the cold season. Patient adherence to treatment is high, and even after treatment interruption, resumed therapy yields significant improvements [8].

The observed benefits include the absence of pelvic pain, dysuria, and enhanced sexual competence, contributing to an overall improvement in the quality of life for patients with CPPS.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, A.A. and D.S.; methodology, D.S.; software, D.M.; validation, E.C., D.M. and D.G.; formal analysis, D.M.; investigation, A.A.; resources, D.M., T.N.; data curation, A.A.; writing—original draft preparation, A.A.; writing—review and editing, A.A.; visualization, D.M.; supervision, D.S.; project administration, D.O.C.; funding acquisition, D.M. All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was conducted following the Decla-ration of Helsinki, and approved by the Institutional Review Board.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

The Role of Diffusion-weighted Magnetic Resonance as a Potential Biomarker in the Characterization of Ovarian Tumor Lesions and the Assessment of Post-treatment Evolution

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Abstract: *This paper focuses on the role of diffusion-weighted MRI of ovarian tumors as a potential biomarker to detect and characterize malignancy, and to improve the accuracy of preoperative diagnosis and postoperative follow-up to optimize therapeutic management. This is an original retrospective study of 76 patients with ovarian masses who underwent surgery between May 2014 and March 2016 at the "Dr Carol Davila" Central Military University Gynaecology Department. Patients underwent preoperative, 6-month, and 12-month postoperative evaluation of ovarian tumors using diffusion-weighted MRI. Due to the accuracy of this method using specific sequences in tissue characterization, magnetic resonance imaging can be used in the diagnostic work-up of complex adnexal masses.*

Keywords: *ADC, MRI, ovarian tumor, postoperative follow-up, borderline ovarian tumor, biomarkers*

INTRODUCTION

This paper is a retrospective study of 76 patients with ovarian cancer undergoing surgery between May 2014 and March 2016 in the Gynaecology Department of the Central Military University Emergency Hospital, who were assessed by preoperative MRI scans.

Following postoperative pathological/immunohistochemical diagnosis for each surgical patient, the role of diffusion-weighted MRI in preoperative ovarian mass profiling and 6- and 12-month postoperative follow-up were evaluated.

Magnetic Resonance Imaging (MRI) is a high-performance examination offering increased sensitivity in assessing and characterizing tissue structures.[1-4]

Diffusion-weighted imaging is a functional technique recently perfected on all MRI machines with powerful gradients and advanced software.[4-8] By tracking intracellular water diffusion, the technique provides data on biological tissue structure.[9-12] Changes in tissue cellularity and cell membrane integrity can affect the free movement of water in the interstitial and intracellular space, resulting in altered signals.[13-15] Measurement of the Apparent Diffusion Coefficient (ADC) provides a quantitative and qualitative assessment of water movement at the cellular level while providing functional information about tissues.[16-19]

In oncological imaging, areas with low ADC, for example, increased diffusion restriction, represent structures with increased cellularity, suggesting tumor recurrence.[1,20] Areas with increased ADC (increased diffusivity) are most likely to indicate favorable disease progression with no recurrence or residual tumor. [21-25]

Most malignancies have low diffusion due to increased cell density, but other factors such as extracellular fibrosis may be involved.[26-30]

MATERIALS AND METHODS

In the study group, 38 patients were diagnosed with benign ovarian tumors, 32 were diagnosed with malignant ovarian tumors, and 6 were diagnosed with borderline ovarian tumors.

Of the 32 malignancy patients, 24 were monitored post-operatively at 6 and 12 months by MRI, mainly by diffusion weight imaging.

Inclusion criteria consisted of having peritoneal metastases at the time of surgery, previously identified by MRI.

Descriptive statistics, testing for possible correlations, and univariate analysis (T-test and ANOVA) were used to organize the data from the patient's MRI scans. Logistic modeling and the calculation of the ROC curve (Receiver Operator Curve) were used.

The ROC curve is a graphical representation that illustrates the diagnostic ability of a binary classification system, in this study referring to the benign or malignant nature of the tumor according to the ADC value. A significance threshold of $p<0.05$ was used for correlative and univariate analysis. Calculations were performed in EXCEL and Matlab R2015.

RESULTS

Analysis of the information provided by the ADC value determined during the diagnostic MRI examination.

Descriptive statistics, analysis of variance (ANOVA), and logistic regression were used to assess the ability of the ADC index to discriminate between malignant, benign, and borderline tumors as finally classified by histopathological examination.

Final histopathological tumor classification defined 3 categories: malignant, benign, and borderline. Of the 76 patients who underwent MRI, 32 were diagnosed as malignant, 38 as benign, and 6 as borderline.

ADC index values measured on MRI were classified into these three groups. Descriptive statistics were used to calculate the mean value, the mean standard deviation, and the range of variation of the ADC value (Table 1).

Table 1: Characteristics of the distribution of ADC values (mm^2/s) by type of tumor at the final assessment

	Malignant	Benign	Borderline
Patient nr	32	38	6
Minimal value	0.8×10^{-3}	0.65×10^{-3}	0.98×10^{-3}
Maximal value	1.22×10^{-3}	1.52×10^{-3}	1.34×10^{-3}
Median	1.038×10^{-3}	1.308×10^{-3}	1.188×10^{-3}
Standard deviation	0.083×10^{-3}	0.11×10^{-3}	0.14×10^{-3}

The graphical representation of the data distribution has been made in the Box-Wisckers plot (Figure 1), which highlights some unexpected values in the data sets corresponding to benign tumors, called "outliers".

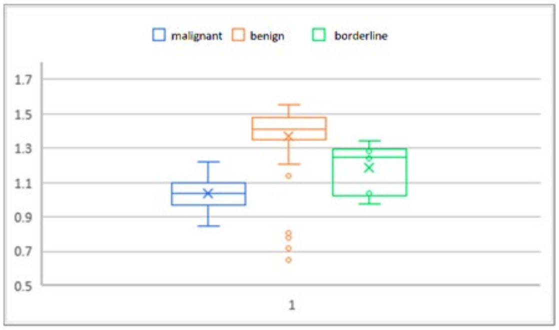

Figure 1: Box and whisker plot for ADC values

These are values that deviate greatly from the range of other measurements. Such data may be due to measurement errors, or they may represent special cases. In this study, the four very low values correspond to patient numbers 39, 125, 138, and 140 (ADC index values = 0.65×10^{-3} mm^2/s, 0.78×10^{-3} mm^2/s, 0.81×10^{-3} mm^2/s, 0.72×10^{-3} mm^2/s respectively). The final diagnosis for these patients was ovarian fibroids. The lowest ADC value for benign tumors excluding these 4 outliers is 1.14 x 10^{-3} mm^2/sec.

Analysis of variance (ANOVA) was used to test the variation in ADC measured on MRI by tumor type by comparing the three data sets. This is a method of testing whether there are significant differences between the mean values of the populations for which the data are being analyzed. According to the ANOVA performed in EXCEL (Table 2), at a 5% significance level, there is a difference between the three samples ($p=1.6 \times 10^{-8}$).

According to the Tukey-Kramer test (Table 3), only the difference between the malignant and benign groups is significant (9.61 > 3.74). The borderline group is not significantly different from any of the other groups.

According to the Box and Wiskers plot, the borderline group is very close to the malignant tumor group, but due to the presence of those very low ADC values corresponding to benign fibroids, the mean ADC of the benign group cannot be clearly distinguished from that of the borderline group.

Table 2: The ANOVA study

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	1.31E-06	2	6.56E-07	23.12192	1.66E-08	3.122103
Within Groups	2.07E-06	73	2.84E-08			
Total	3.38E-06	75				

Table 3: The Tukey Kramer Test

Group 1	Group 2	Difference between environments	n1	n2	SE	Q
Malignant	Benign	2.75E-04	32	38	2.857E-05	9.617
Benign	Borderline	1.25E-04	38	6	5.231E-05	2.381
Borderline	Malignant	1.50E-04	6	32	5.297E-05	2.835

When only two groups (malignant/benign) are considered after the histopathological examination (with borderline tumors being included in the malignant category), their differentiation in terms of ADC value can be tested using the Student's t-test at a significance level of α=0.05.

Since the dispersions of the two groups are not the same, a t-test was carried out to test for the equality of the means in the variance of the unequal dispersions in the groups. The null hypothesis of equal means was rejected, and the alternative hypothesis of unequal means was accepted ($p=0.04<0.05$). The value of the ADC index is significantly different for tumors that are benign or malignant. Using a one-tailed test with the alternative hypothesis "mean ADC of malignant group < mean ADC of the benign group", the result of the test is to reject the null hypothesis (equality of means) and accept the alternative hypothesis. The p-value = 0.02 confirms that the mean ADC values in the malignant tumors are significantly lower than the mean ADC values in the benign tumors.

Analysis of the distribution of ADC values according to tumor type

Of the 38 benign tumors for which an MRI scan was performed, 13 tumors were germinal, 8 were stromal and 17 were epithelial. The majority of malignant and borderline tumors were epithelial tumors (35). Only 2 malignant tumors were germinal, and one was a stromal tumor. Peritoneal diffusion-restricted areas were also measured in malignancies. Figure 2 compares the distribution of intratumoral and peritoneal diffusion-restricted (ADC) values for these tumor types.

A clear differentiation of ADC values according to tumor type and nature can be seen in the figure. In benign epithelial and germinal tumors, the mean ADC is 1.4 x 10^{-3} mm^2/s, and the lowest values are above 1.2 x 10^{-3} mm^2/s. For malignant epithelial tumors, the mean value is 1.05 x 10^{-3} mm^2/s, and the highest values are slightly higher than 1.2 x 10^{-3} mm^2/s. The mean ADC in peritoneal lesions is also close to 1 x 10^{-3} mm^2/s. The distribution of values is similar to that of malignant tumors.

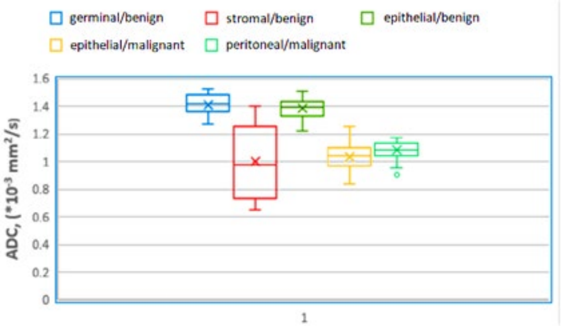

Figure 2: Distribution of intratumoral and peritoneal ADC values by tumor type.

The benign stromal tumors have a special feature: half of the values are less than 1 x 10^{-3} mm^2/s. Of the 8 benign tumors of this type, 4 are uterine fibroids, whose ADC is very low, below 1 x 10^{-3} mm^2/s as mentioned above.

Malignant tumors of the stromal and germinal types could not be statistically analyzed due to their low number.

Analysis of parameter values recorded at 6 and 12 months post-operatively

Twenty-four patients with malignant tumors were re-examined 6 and 12 months postoperatively.

Figure 3 shows the distribution of ADCs at 6 and 12 months postoperatively. The increase in mean values can be seen, as well as the increase in minimum values during the follow-up period.

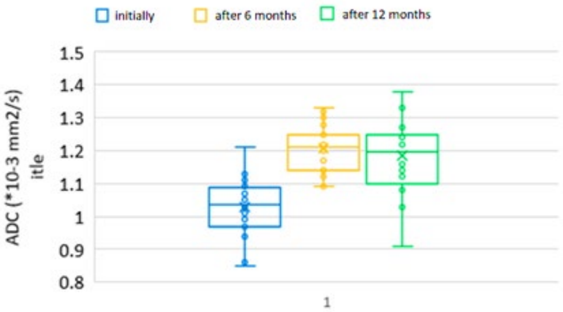

Figure 3: Change in distribution of intratumoral (baseline) and peritoneal ADC during postoperative follow-up

Comparison of the ADC values measured at 6 and 12 months with the peritoneal ADC values measured at the time of diagnosis shows a clear improvement in these values at 6 months. This improvement was maintained at 12 months in all but less than 20% of cases (Figure 4).

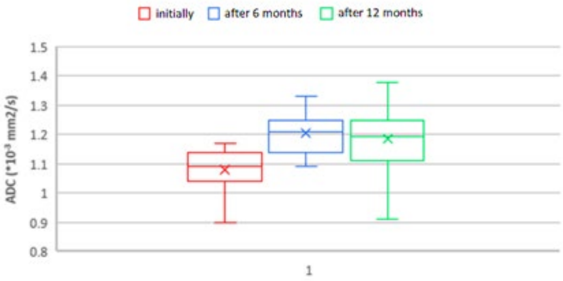

Figure 4: Peritoneum ADC values at diagnosis and follow-up.

The mean and median values of ADC induction from peritoneal lesions measured at 6 and 12 months after the intervention increased significantly from baseline values, as shown in Figure 4. The distribution of ADC values at 12 months shows that there are several ADC values lower than those recorded at 6 months, indicating that these cases may not have had a favorable postoperative course at 6-12 months.

The paired t-test at a significance level of $p=1 \times 10^{-8}$ also confirms the significant increase in ADC in the first 6 months.

For the period from 6 to 12 months, the increase was no longer significant ($p= 0.16>0.05$).

Logistic regression was used to correlate the probability of identifying a malignant tumor as a function of ADC alone, and the receiver operator characteristic (ROC) curve was constructed, which is a probability curve plotting the true positive rate (TPR) versus the false positive rate (FPR) at different cut-off values of the variable (ADC).

The area under the curve (AUC) is used to measure the ability of the classifier to discriminate between classes and is used to conclude the ROC. In the present study, AUC=0.89, which corresponds to a good classifying model. The closer the AUC is to 1, the higher the value of the classification method (high accuracy).

The ROC curve (Figure 5) shows how the sensitivity and specificity can vary for different cut-off values for the ADC. In the analysis, the case was considered positive with the qualifier "malignant".

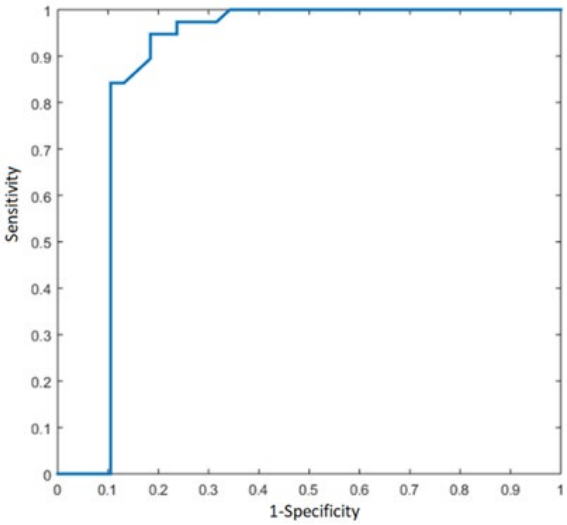

Figure 5: ROC curve for ADC performance in diagnosis

DISCUSSION

Analysis of the information provided by the ADC value at the level of solid components of ovarian tumors assessed by MRI

The role of the diffusion MRI sequence was analyzed by evaluating the ADC index within the intratumoral solid component and the macroscopic peritoneal determinations.

Patients with malignant and benign ovarian disease showed significant differences in ADC scores. The group of patients with a borderline tumor did not differ significantly from the other two groups of patients. The ADC values recorded in the borderline ovarian lesion group were close to those in the malignant lesion group, but such low ADC values were also found in benign fibromatous tumors, with the mean ADC values in the benign lesion group not distinguishable from the ADC values in borderline lesions. Due to the very low number of germinal and stromal tumors in the study group, analysis of ADC values for malignant tumors could only be performed for epithelial tumors.

The mean ADC value for the epithelial malignancies was 1.05×10^{-3} mm^2/s, which was also close to the mean value for peritoneal lesions (1×10^{-3} mm^2/s).

For benign epithelial and germinal lesions, the mean ADC value was 1.4×10^{-3} mm^2/s, which is significantly different from that found in malignant lesions.

ADC analysis confirmed the peculiarity of benign stromal tumors, which have a decreased ADC index, with half of their ADC values less than 1×10^{-3} mm^2/sec.

Assessment of postoperative evolution by ADC index analysis at the level of residual peritoneal deposits

Among the patients with a diagnosis of ovarian malignancy, MRI was also used in 24 patients to monitor the follow-up after treatment at 6 months and 12 months, respectively. Biochemistry and ADC levels were analyzed compared to baseline (pre-intervention).

ADC values showed a significant increase in the first 6 months (mean 0.18×10^{-3} mm^2/s), but not significant in the following 6 months. The change in ADC in controls at 6- and 12-months post-intervention was also analyzed concerning patient age, without showing a statistically significant correlation.

Comparison of the ADC values measured at 6 and 12 months with the ADC values in the peritoneal deposits measured at the time of diagnosis showed a clear improvement in these values at 6 months. This improvement was also maintained in the controls at 12 months in about 80% of the cases. The remaining 20% of patients had lower ADC scores compared to the previous year, indicating a poor postoperative outcome in the 6- to 12-month follow-up.

Magnetic resonance imaging (MRI) was found to be a powerful examination in the study due to the multiple sequences available to characterize tissue structures to differentiate the components of tumor formations with increased specificity in the assessment of malignancy

The multiple sequences that can be used to analyze and differentiate tissue structures, with an optimal assessment of lipomatous, cystic, hematogenous, or components in different stages of hemoglobin degradation, combined with the value of diffusion sequences, can be a powerful alternative for patients with allergies or impaired renal function when the administration of contrast agents for tumor assessment is contraindicated.

The possibility of multiplanar imaging provides a superior evaluation of tumor masses with a good appreciation of organ of origin and anatomical relations, and diffusion sequences and characterization of post-contrast load curves have provided a significant increase in sensitivity in the determination of malignancy, of particular importance for the choice of optimal therapeutic approach.

Areas of increased signal in diffusion sequences and low absolute diffusion index (ADC) represent structures with increased cellularity, indicating tumorigenicity.

Increased ADC values after therapy in residual tumor masses indicate a favorable course of the disease and can be considered as a biomarker for the dynamic assessment of therapeutic response in oncological disease. Increased ADC correlated in most cases with decreased serological marker titers.[31]

At the same time, the low ADC index value had an increased sensitivity in the assessment of lymph node hypertrophy that was suspicious for metastatic disease, regardless of size.

Some low ADC values did not always correlate with malignancy, as other factors such as extracellular fibrosis, shape and size of intercellular spaces could influence the ADC value, and some formations, in particular fibromatous formations, despite being benign, showed a decreased ADC value. In these cases, other imaging characteristics (in particular the type of contrast agent uptake) and some clinical and biological parameters were used to guide the diagnosis.[32]

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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Authors' contribution
Conceptualization, IAN, and MFN; methodology, EDSNM and FCA; software, FN and EDSNM; validation, IAN, FCA, and MFN; formal analysis, MFN; investigation, IAN, IAC, MAN, and PCA; resources, EDSNM; data curation, MFN; writing—original draft preparation, IAN; writing—review and editing, IAN, MFN and PCA; visualization, IAN, MAN, IAC; supervision, IAN and MFN; project administration, IAN. All authors have read and agreed to the published version of the manuscript.

Ethics approval
The present study was approved by the hospital's ethics committee Nr. 601/ 24.05.2023 and is performed under the ethical standards laid down by the Declaration of Helsinki

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ORIGINAL ARTICLE

Couvelaire Syndrome: A Case Report and Literature Review

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Abstract: *Introduction. Couvelaire syndrome is a rare complication of pregnancy involving the placenta and the uterine wall. It is caused by placental abruption, preeclampsia, or other disorders that compromise the blood supply to the placenta. Diagnosis is typically made through history, clinical evaluation, and ultrasound. This study aims to report a grave case of Couvelaire syndrome complemented by presenting a review of the relevant literature, to aid the meaning of a clinical path, as well as early and adequate diagnosis and treatment of Couvelaire syndrome, to prevent feto-maternal consequences. The treatment of Couvelaire syndrome depends on the severity of the condition and the well-being of both the mother and the fetus. If the condition is severe and poses a risk to the mother or fetus, immediate delivery may be recommended, mainly with urgent Cesarean section in general endotracheal anesthesia. If there is significant blood loss leading to a decrease in hemoglobin levels, blood transfusions may be necessary to restore normal blood volume and oxygen-carrying capacity. Part of the general treatment plan should also be the management of the underlying cause of placental abruption, for example, in cases of preeclampsia, medications are prescribed to control blood pressure and prevent seizures.*

Keywords: *ADC, MRI, ovarian tumor, postoperative follow-up, borderline ovarian tumor, biomarkers*

INTRODUCTION

The placenta is an essential organ that develops during pregnancy and supplies the fetus with blood and, through it, with oxygen and nutrients.

Couvelaire syndrome, also known as uteroplacental apoplexy or placental apoplexy, is a rare and severe complication of placental abruption that can occur during pregnancy, especially during the third trimester of pregnancy. This condition involves the infiltration of blood into the uterine myometrium (the muscular layer of the uterus) due to a ruptured placental vessel. The blood collects in the space between the uterine muscle fibers, creating a hematoma. The incidence of Couvelaire syndrome is relatively low, and it is considered a rare complication of placental abruption. The incidence of placental abruption itself happens in approximately 1% of all pregnancies [1]. Even within the cases of placental abruption, Couvelaire syndrome is even less frequent.

The main cause of Couvelaire syndrome is placental abruption, where the placenta can partially or completely separate from the uterine wall before delivery [2,3].

Risk factors that lead to abruption of the placenta and consequently to Couvelaire syndrome are eclampsia, preeclampsia, chronic hypertension and pregnancy-induced hypertension, trauma or injury (car accident), previous placental abruption, uterine abnormalities, fibroids or a history of uterine surgery, multiple pregnancies (twins, triplets), cigarette smoking, drug use (including cocaine and methamphetamines), polyhydramnios, short umbilical cord, and women who are younger than 20 or older than 35 years. All these factors above can increase the risk of placental abruption [4-6].

Couvelaire uterus is typically a result of severe placental abruption, where there is bleeding between the placenta and the uterine wall. This bleeding can imbibe and extend into the myometrium.

The bleeding leads to the formation of blood clots within the uterine muscle, causing a hematoma. This hematoma can compromise the blood supply to the uterine muscles. The uterus may take on a characteristic blue or purple hue due to the presence of blood within the myometrium (the image of the tiger) [7-10]. This accumulation of blood between the muscular fibers of the uterine wall can disrupt the contractile function of the muscular ultrastructure, leading to uterine atony and increasing the risk of complications, including postpartum hemorrhage and disseminated intravascular coagulation (DIC), a serious disorder that affects blood clotting [6]. Early detection and appropriate management are crucial and can significantly improve outcomes for both the maternal and the fetus.

CASE PRESENTATION

Clinical history and treatment

A 27-year-old multipara in the 34th week of pregnancy was admitted to the Department of Obstetrics and Gynecology in October 2023, with vaginal bleeding, abdominal pain, back pain, uterine rigidity, and uterine contraction. The vital parameters were; a low blood pressure of 100/60 mmHg, pulse of 97 minutes, and SpO_2 was 95%. The patient had a previous birth, at term, and without any apparent complications. Our patient is atypical because she does not have a history of hypertension, pregnancy-induced hypertension, diabetes, trauma or injury (such as a car accident), no polyhydramnios, no chorioamnionitis, no history of drug use, no uterine abnormalities, fibroids, or a history of uterine surgery. She was a daily smoker, for over five years and over 20 cigarettes a day.

A general physical examination confirms uterine rigidity, continuous uterine contraction, and abdominal pain. The vaginal examination showed vaginal bleeding and a dilation of the cervix for 1.5 cm.

An ultrasound examination revealed a retroplacental hematoma with dimensions of about 5 cm, (Figure 1). A cardiotocography (CTG) examination revealed various abnormalities such as pseudo-sinusoidal pattern of fetal heart rate and late decelerations, (Figure 2).

All emergency examinations suggested central placental abruption and indicated urgent Cesarean section in general endotracheal anesthesia. Following the Pfannenstiel-Kerr incision laparotomy, massive uteroplacental apoplexy or Couvelaire syndrome (Figure 3).

A female hypotrophic neonate was born at 1700 gram/45 cm and with Apgar scores of 6/7. Laboratory findings showed moderate anemia, with a level of hemoglobin concentration of 8.7 g/dL, mild thrombocytopenia, and with intact coagulogram.

During hysterorrhaphy, 20 UI intravenous oxytocin and afterward 2 gram intravenous tranexamic acid, were administered because of the hypotonic and non-contractible uterus, simultaneously continuous pressure of the uterus was performed with wet compresses with a warm physiological solution, resulting in complete success in preserving the uterus. After surgery, the patient was stable in terms of hemodynamic parameters.

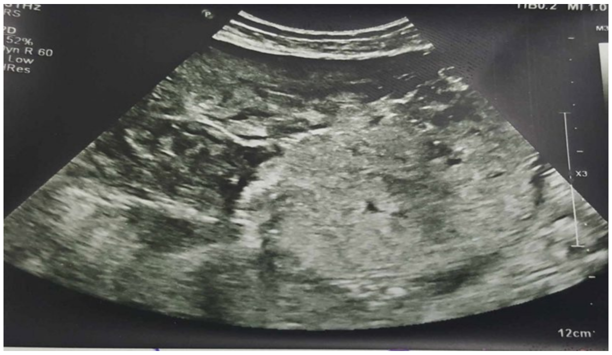

Figure 1: Retroplacental hematoma. With ultrasonography, a hematoma between the placenta and the wall of the uterus, measuring about 5 cm, is found in a patient with placental abruption

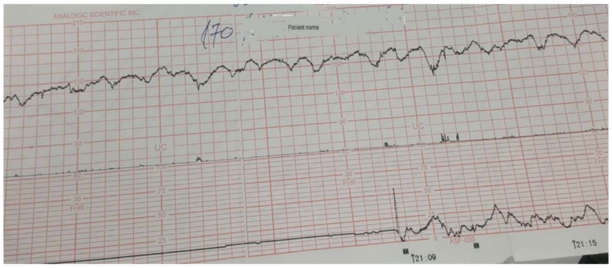

Figure 2: Pseudosinusoidal pattern. External fetal heart rate monitoring at 1 cm/min. In patients with placental abruption has been found pseudo sinusoidal fetal heart rate patterns and late decelerations

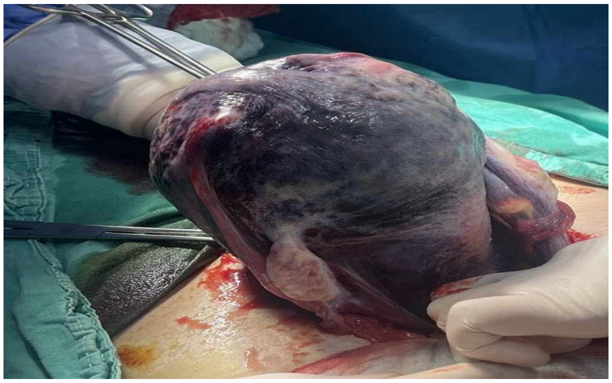

Figure 3: Couvelaire syndrome or uteroplacental apoplexy. It is found massive infiltration of blood into the uterine myometrium (the muscular layer of the uterus) due to a ruptured placental vessel

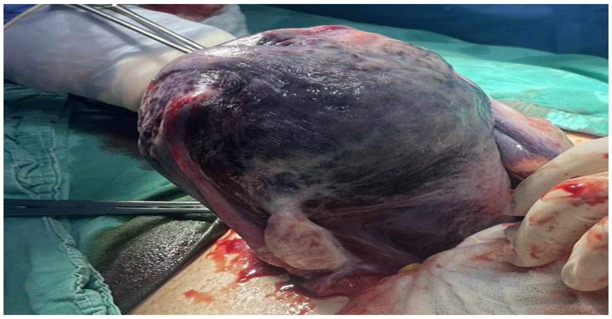

Figure 4: Infiltration of adnexal. It is found even massive infiltration of blood into the uterine adnexa

DISCUSSION AND LITERATURE REVIEW

For the literature review utilized the Items from databases such as Medline (PubMed), EMBASE, Scopus, and Google Scholar. For all items about 'Couvelaire Syndrome' abbreviations are used *OR Couvelaire Syndrome* OR Couvelaire uterus* OR Uteroplacental apoplexy* OR Placental abruption* OR Placental abruption complications* OR Uteroplacental apoplexy treatment* OR Couvelaire uterus causes* Couvelaire uterus symptoms* OR Couvelaire uterus complications, etc.

The patient had a previous birth, at term, and without any apparent complications. Our patient did not have any risk factors for placental abruption, except that she was a smoker for five years and used more than 20 cigarettes a day. It is now known that smoking is associated with a significant risk of placental abruption and a possible complication in uteroplacental apoplexy or Couvelaire Syndrome. Cigarette smoking during pregnancy is associated with numerous adverse outcomes for both the maternal and the developing fetus. One of the potential complications linked to smoking during pregnancy is placental abruption. Placental abruption occurs when the placenta separates from the inner wall of the uterus before delivery. This separation can compromise the flow of oxygen and nutrients to the fetus and may lead to serious complications for both the mother and fetus.

The physiopathological mechanism of how cigarette smoking can cause placental abruption follows several steps:

- Reduced Oxygen Supply: Smoking cigarettes can lead to the constriction of blood vessels, including those in the placenta. This constriction reduces the amount of oxygen that the fetus receives, potentially leading to stress on the placenta.

- Increased Risk of Blood Clotting: Smoking is known to increase the risk of blood clotting, and this can contribute to the detachment of the placenta from the uterine wall.

- Hypertension and Vasoconstriction: Smoking is a risk factor for hypertension (high blood pressure), and elevated blood pressure can contribute to the detachment of the placenta. Additionally, the vasoconstrictive effects of smoking can reduce blood flow to the placenta, further increasing the risk of complications.

- Placental Insufficiency: Smoking has been linked to placental insufficiency, where the placenta is not able to provide sufficient nutrients and oxygen to the developing fetus. This can increase the risk of placental abruption.

- Inflammatory Response: Cigarette smoke triggers an inflammatory response in the body. Chronic inflammation can contribute to the breakdown of the placental tissue and increase the risk of placental abruption.

The placenta is an organ that provides oxygen and nutrients to the developing fetus. Placental abruption is a severe pregnancy complication where the placenta partially or completely separates from the inner wall of the uterus before the delivery of the fetus [11]. This separation can lead to bleeding and directly endanger the life of the fetus and maternal. Prevalence of placental abruption occurs in about 0.2%-1% of pregnancies [12,13]. Placental abruption can be classified into partial or complete, depending on the extent of detachment. Partial abruption involves only a portion of the placenta separating, while complete abruption involves the entire placenta detaching from the uterine wall. Couvelaire uterus is a rare complication that happens due to severe placental abruption. One systematic review found that 16.5% of patients with placental abruption were complicated in the Couvelaire uterus, and they needed emergency hysterectomy [14]. This rare complication occurs when vascular damage within the placenta causes hemorrhaging that progresses to and infiltrates the wall of the uterus.

Definition, classification, and characteristics of Couvelaire syndrome

Definition

Couvelaire uterus can be defined as a histopathological entity caused by severe forms of placental abruption.

This histopathological entity was first described by Couvelaire at the beginning of the 20th century and is now often referred to as Couvelaire uterus or Couvelaire syndrome [15].

Initially, due to various risk factors, occurs pathologic vascular damage within the placenta itself, causing hemorrhage and hematoma formation, blood from hematoma seeps into the deciduas basalis, eventually causing a separation of the placentas [16,17].

The hemorrhage often progresses, in all structures of the uterus and adnexa including the walls of the uterus, beneath the tubal serosa, into the connective tissue of the broad ligaments, in the ovaries, as well as in the peritoneal cavity [18, 19].

Macroscopically, the uterus assumes the appearance of a purplish or copper-colored ecchymotic. Histologically, blood is found between muscle bundles, in perivascular tissue, and in the subserosa. The decidual spiral arterioles may show acute atheromatous processes, with foamy macrophages present. Physiologically, indurated the uterus often loses its contractile power.

Since the diagnosis of Couvelaire uterus is made only with direct visualization of the uterus after laparotomy or biopsy, it is almost impossible to know the precise incidence of Couvelaire uterus. However, the incidence of Couvelaire uterus behaves between 5%-20% of all cases with placental abruption [15,20].

Classification

Depending on the degree of blood infiltration through the walls of the uterus, mild, moderate, and severe forms of Couvelaire uterus can develop. In mild and moderate forms of the Couvelaire uterus, blood infiltration penetrates until the serous layer of the uterus, while in severe forms of the Couvelaire uterus, in addition to the uterine wall, blood also infiltrates other structures such as the adnexa, peritoneal cavity, and surrounding structures.

Clinically, the Couvelaire uterus can develop in a mild, moderate, and severe form, depending on the degree of blood loss and the impact on the hemodynamic state.

Characteristics

The condition is characterized by the infiltration of blood into the uterine muscle, creating a hematoma. This bleeding into the myometrium can compromise the ability of the uterus to contract effectively.

The presence of blood in the uterine muscle may result in a "bruised" appearance of the uterus, giving it a bluish or purple discoloration.

Couvelaire uterus can lead to uterine atony, a condition where the uterus loses its ability to contract properly, which can result in excessive bleeding after delivery (postpartum hemorrhage) [21,22].

Maternal and fetal outcomes can be compromised due to the potential for severe bleeding, reduced oxygen supply to the fetus, and other complications associated with placental abruption.

Aetiology

The cause of Couvelaire uterus is placental abruption, especially its heavy form [14]. All the factors that directly or indirectly affect the development of placental abruption are also risk factors event for the Couvelaire uterus [4,5,24,25]. The exact cause of placental abruption is not always clear, but several factors and risk factors have been associated with an increased likelihood of its occurrence. These include:

- Trauma or Injury: A sudden impact or trauma to the abdomen, such as might occur in a car accident or a fall, can increase the risk of placental abruption.

- High Blood Pressure: Chronic hypertension or pregnancy-induced hypertension (preeclampsia, eclampsia) is a significant risk factor for placental abruption. High blood pressure can lead to damage to the blood vessels in the uterus.

- Advanced Maternal Age: Older women, particularly those over the age of 35, may have a higher risk of placental abruption.

- Multiple Pregnancies: The risk of placental abruption is higher in pregnancies involving twins, triplets, or other multiple births.

- Cigarette Smoking: Smoking during pregnancy is associated with an increased risk of placental abruption, likely due to the effects of smoking on blood vessels.

- Drug Use: The use of certain drugs, such as cocaine and methamphetamine, is linked to an elevated risk of placental abruption.

- Previous History: Women who have had a previous placental abruption are at a higher risk of experiencing it again in subsequent pregnancies.

- Uterine Conditions: Conditions that affect the uterus, such as fibroids or an abnormally shaped uterus may increase the risk of placental abruption.

- Rupture of Membranes: The premature rupture of the amniotic sac (bag of water) can increase the risk of placental abruption.

- Abdominal Surgery: Women who have had previous abdominal surgeries, especially uterine surgeries, may have an increased risk.

- Blood Clotting Disorders: Certain blood clotting disorders, such as disseminated intravascular coagulation (DIC), can contribute to placental abruption.

It's important to note that some cases of placental abruption occur without any identifiable risk factors. Additionally, the severity of placental abruption can vary, and its management depends on factors such as the extent of detachment and the gestational age of the fetus.

Clinical findings

Couvelaire uterus, also known as Couvelaire syndrome, refers to a condition where blood from a placental abruption infiltrates into the myometrium (the muscular wall) of the uterus. This condition is associated with placental abruption, which is the premature separation of the placenta from the uterine wall. Clinical findings of Couvelaire's uterus may include:

- Bluish or Purple Uterus: Blood infiltration into the myometrium can give the uterus a bluish or purple discoloration. This is due to the presence of blood within the uterine muscle.

- Bruised Appearance: The uterus may have a "bruised" appearance, visually resembling a contusion or hematoma. This appearance is a result of the blood infiltrating the uterine tissues.

- Abdominal pain: Women with Couvelaire uterus may experience abdominal pain. The severity of pain can vary depending on the extent of the placental abruption and the involvement of the uterine musculature.

- Uterine Rigidity or tetanic uterine contraction: The uterine wall may become rigid or firm due to the infiltration of blood into the myometrium. This rigidity can contribute to uterine dysfunction and increase the risk of complications.

- Compromised Uterine Contractility: The infiltration of blood into the uterine muscle can compromise the ability of the uterus to contract effectively. This may lead to uterine atony, a condition where the uterus loses its normal tone and may not contract adequately.

The clinical findings of Couvelaire uterus are often part of a larger clinical picture associated with placental abruption [23]. Placental abruption itself can present with symptoms such as vaginal bleeding, abdominal pain, back pain, and signs of fetal distress.

Diagnosis

The diagnosis of Couvelaire uterus is typically made in the context of diagnosing placental abruption [23-25]. The steps and methods involved in the diagnosis are:

- Clinical Evaluation: One must perform a thorough clinical evaluation, taking into consideration the patient's symptoms, medical history, and risk factors.

- Symptoms and Signs: Couvelaire uterus is often associated with symptoms of placental abruption, including vaginal bleeding, abdominal pain, tetanic uterine contractions, and signs of fetal distress.

- Physical Examination: A physical examination may reveal vaginal bleeding and abdominal pain or palpation sensitivity. The uterus may also feel rigid or firm.

- Ultrasound Imaging: Ultrasound imaging is a valuable tool for assessing the condition of the placenta, the amount of amniotic fluid, and the overall well-being of the fetus. While ultrasound may not directly visualize the blood within the uterine muscle, it can provide information about the placental abruption, her position, and possible hematoma (not always).

- Laboratory Tests: Laboratory tests, such as blood tests, may be performed to assess the maternal overall health and detect signs of complications, such as anemia or coagulation disorders.

- Fetal Monitoring: Continuous fetal monitoring may be employed to assess the well-being of the fetus. This can include monitoring the fetal heart rate and assessing for signs of distress.

- Clinical Judgment: Diagnosis may involve the clinical judgment of healthcare professionals based on the overall presentation of symptoms, physical findings, and diagnostic tests.

Treatment and Prognosis

The treatment and prognosis of Couvelaire uterus are generally addressed within the broader context of managing placental abruption. The key aspects of treatment and prognosis:

Treatment:

- Stabilization: The initial focus is on stabilizing the maternal. This may involve addressing any immediate life-threatening complications, such as managing severe bleeding or addressing signs of shock.

- Monitoring: Continuous monitoring of the maternal and the fetus is essential. This includes monitoring vital signs, assessing the extent of the placental abruption, and evaluating fetal well-being through methods like fetal heart rate monitoring.

- Blood Transfusion: If there is significant blood loss, blood transfusions may be required to replace lost blood and address any resulting anemia.

- Delivery: The decision to deliver the baby depends on various factors, including the severity of the placental abruption, the gestational age of the fetus, and the overall condition of the maternal and fetal. In some cases, emergency delivery, often via cesarean section, may be necessary to improve outcomes.

- Uterine Massage: Uterine massage may be performed to help the uterus contract and reduce bleeding.

- Medical Management: Medications may be administered to address complications such as hypertension, coagulation disorders, or other medical conditions that could exacerbate the situation.

Prognosis:

- Varied Outcomes: The prognosis for Couvelaire uterus and placental abruption can vary widely depending on the severity of the condition, the gestational age of the fetus, and the effectiveness of medical interventions.

- Maternal Complications: Potential maternal complications include excessive bleeding, disseminated intravascular coagulation (DIC), and the need for surgical interventions. Severe cases can lead to maternal mortality.

- Fetal Complications: Fetal outcomes depend on factors such as gestational age, the degree of placental abruption, and the speed with which medical intervention is provided. Preterm birth, low birth weight, and neonatal complications are possible.

- Long-Term Effects: Long-term effects on both the maternal and the fetus may include consequences related to prematurity, low birth weight, and any complications resulting from the extent of the placental abruption.

It's crucial to note that the Couvelaire uterus is a relatively rare and severe complication, and its management requires prompt and comprehensive medical care. The prognosis can be influenced by the rapidity of diagnosis, the effectiveness of interventions, and the overall health of both the maternal and the fetus. Pregnant women experiencing symptoms suggestive of placental abruption, such as vaginal bleeding and abdominal pain, should seek immediate medical attention for timely evaluation and intervention.

CONCLUSION

It's significant to know that the management of Couvelaire syndrome requires an individualized approach based on the specific circumstances of each case. The treatment of Couvelaire syndrome depends on the severity of the condition and the well-being of both the maternal and the fetus. If the condition is severe and poses a risk to the maternal or fetus, immediate delivery may be recommended, mainly with urgent Cesarean section in general endotracheal anesthesia. If there is significant blood loss, blood transfusions may be necessary to restore normal blood volume and oxygen-carrying capacity. Part of the general treatment plan should also be the management of the underlying cause of placental abruption, for example, in cases of preeclampsia, medications are prescribed to control blood pressure and prevent seizures [26].

The guidelines development for diagnosis and treatment of Couvelaire uterus as a complication of placental abruption would aid in the early detection and proper management of the disease, thus reducing the rates of morbidity, recurrence, and mortality feto-maternal due to placental abruption and complications in Couvelaire uterus. To date, treatment options are mostly empirical, they are not evidence-based practices, and therefore multicenter clinical collaborative research is required to provide useful guidance for the precise diagnosis and treatment of Couvelaire uterus.

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ORIGINAL ARTICLE

Dry Eye Disease in Systemic Lupus Erythematosus

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Abstract: *Systemic lupus erythematosus is a multi-organ autoimmune disease of unknown etiology characterized by widespread inflammation and significant morbidity and mortality. Organ affection includes joint and cutaneous involvement, pulmonary, neurological, and cardiac problems, renal and hematological involvement as well as ocular comorbidities, both posterior but mostly anterior ocular involvement. Therefore, we aimed to evaluate dry eye involvement in lupus patients. A cross-sectional study was performed with the aid of a web-based, anonymous questionnaire that was distributed to both patients diagnosed with lupus erythematosus and to a control group. A total number of 123 patients diagnosed with lupus and 200 responders in the control group completed the questionnaire. Dry eye disease has already been diagnosed in 25 lupus patients (28.9%) compared to 44 (21.9%) individuals in the control group. Dry eye-related symptomatology was reported in 43 (34.60%) patients compared to 57 (28.35%) healthy responders. Upon statistical analysis, more symptoms were reported in the lupus group (p=0.01), in responders diagnosed with dry eye syndrome (p<0.01), and in responders using lubrication (p<0.01) but no association was obtained between the incidence of dry eye symptoms and the presence of the disease. The number of episodes of lupus reactivation since diagnosis was correlated with dry eye syndrome (p=0.15) and the use of lubricating tear drops (p<0.01). Dry eye symptoms and disease are more frequent in lupus patients and further research should be performed to understand the connection between these two disease entities.*

Keywords: *systemic lupus erythematosus, dry eye disease*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease, the most commonly affected organs being the joints, and the skin but also having hematologic, renal, central nervous system, and cardiac involvement. Even though eye involvement has not been included in the diagnostic criteria of SLE, possibly also because eye involvement occurs in several systemic autoimmune diseases, thus making it less specific in diagnosing certain pathologies, ocular manifestations in SLE have increasingly gained importance. Ophthalmic manifestations may occur independently or may be a reflection of systemic inflammation. In the course of the disease, several anatomic structures may be involved, such as the periorbita, adnexa, eye, and optic nerve [1]. Some of the most frequent conditions found in SLE patients are keratoconjunctivitis sicca or dry eye disease, iritis and ciliary body inflammation, vascular changes of the retina and occlusive vasculitis as well as optic neuritis [2]. Retinopathy is highly suggestive of disease activity and may precede other organ involvement, thus being a marker of not only poor visual outcome but for survival prognosis [1]. On the other hand, the anterior part of the eye is responsible for the most frequent ocular manifestations such as keratoconjunctivitis sicca and Sjogren’s syndrome. The various degrees of severity of dry eye syndrome may range from stinging, and burning sensation to light sensitivity but also to corneal ulceration, scleritis, uveitis, and optic neuritis, having a significant impact on the visual function. Early treatment may prevent the appearance of complications, therefore, prompt recognition in slightly symptomatic or asymptomatic patients would be advisable. We aimed to evaluate the prevalence of dry eye disease and of associated symptoms in a group of patients diagnosed with systemic lupus erythematosus**.**

MATERIALS AND METHODS

A cross-sectional study using a web-based questionnaire was performed. The survey was developed by the authors based on previously published data and was sent via Google Forms (Google LLC, Mountain View, CA, USA) to participants. The completion of the survey was anonymous and voluntary. The data regarding lupus patients was retrieved from a study evaluating photoprotection habits in patients with lupus erythematosus (manuscript submitted). Participants in the control group were randomly selected and no responder received any financial benefits from completing the questions.

Initially, participants had the opportunity to provide anonymous personal data such as age, gender, area of dwelling, and education. Dry eye disease was assessed with the following questions: the first item asked whether they were diagnosed with dry eye disease (with two answer options: “yes”, “no”), the second item evaluated whether subjects used lubricating tear drops (with four answer options “never”, “rarely”, “often”, “daily”) and the third item was a visual Likert-scale with answer possibilities ranging from 1-2 (“my symptoms are not a problem”) to 9-10 (my symptoms are severe and I need immediate medical treatment”). A question regarding time spent using appliances such as telephone, television, tablet, and laptop was also included (with answers ranging from not at all to 5 hours a day). Subjects were asked a free-answer question for them to include relevant medical history related to dry eye disease and a free-answer question to add personal ideas or information. Lupus patients were asked how many disease reactivations were experienced from the onset of the disease and whether they had been diagnosed with Sjogren syndrome.

Statistical analysis

Data was collected and further analyzed using SPSS, version 12, SPSS Inc., Chicago, IL, USA. The biserial correlation coefficient was used between ordinal and nominal variables, the Pearson correlation between numeric variables, and the chi-square test between nominal variables. A p-value under 0.05 was considered statistically significant.

RESULTS

A total number of 123 patients diagnosed with lupus and 200 responders in the control group completed the questionnaire, with a predominance of female participants living in the urban area (Table 1). 25 patients (28.9%) among the participants with lupus erythematosus have already been diagnosed with dry eye syndrome, compared to 44 (21.9%) responders from the control group. 22 (18.2%) patients use lubricating tear drops daily, 8 (6.6%) frequently, 30 (24.8%) rarely, and 61 (50.4%) never use them. In the control group, 13 (6.5%) use lubricating tear drops daily, 17 (8.5%) frequently use them, 85 (42.3%) never and 89 (44.3%) rarely use them. Symptoms were further assessed with the aid of a Likert scale, and, in the lupus group, 43 (34.60%) patients reported having eye discomfort while, in the control group, 57 (28.35%) disclosed similar uneasiness. 23 (18.6%) lupus patients had already been diagnosed with Sjogren syndrome. Upon statistical analysis, a correlation was obtained between the dry eye diagnosis and the use of lubricating tear drops ($p<0.01$) but no statistically significant relation was noted between the incidence of dry eye symptoms and the presence/absence of lupus. However, more symptoms were reported in the lupus group ($p=0.01$), in responders diagnosed with dry eye syndrome ($p<0.01$), and in responders using lubrication ($p<0.01$). The number of episodes of lupus reactivation was correlated with dry eye syndrome ($p=0.15$) (Figure 1) and the use of lubricating tear drops ($p<0.01$). The association of lupus with other conditions was also related to dry eye syndrome ($p<0.05$).

Table 1: Participant characteristics

	Lupus Group	Control Group
Gender	118 (95.9%) female	184 (92%) female
	5 (4.1%) male	16 (8%) male
Area of dwelling	32 (26.2%) rural area	21 (10.5%) rural
	90 (73.8%) urban area	180 (90%) urban
Education	7 (5.69%) gymnasium	0 gymnasium
	41 (33.33%) high school	15 (7.5%) high school
	15 (12.19%) college	6 (3%) college
	38 (30.89%) university	110 (55%) university
	22 (17.88%) master/PhD	69 (34.5%) master/PhD

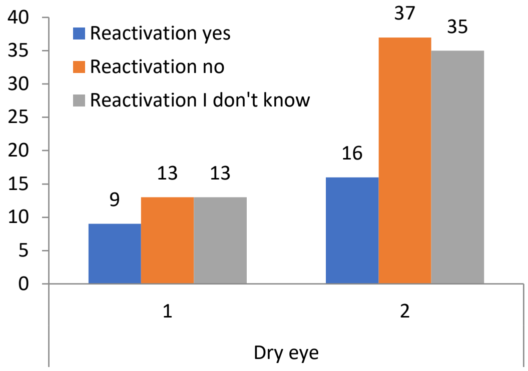

Figure 1: The relationship between dry eye and disease reactivation

A relationship was observed between television viewing in the lupus group compared to the control group ($p<0.01$), an inverse relationship between the habit of phone watching in the same group (p<0.01) and a relationship between the use of lubricating tear drops and tablet watching (p=0.03).

DISCUSSION

In our cohort, individuals with lupus were more frequently diagnosed with dry eye syndrome and presented more symptoms compared to the control group, even though the differences did not reach statistical significance. A literature search aiming to assess the relation between dry eye disease and several autoimmune diseases concluded that the association between the two entities is frequent but the diagnosis is commonly delayed or missed, leading to complications that affect the visual function and the quality of life of patients [3]. Lupus patients do not represent an exception to this finding. Significant modifications in dry eye parameters were displayed by a prospective consecutive case series of SLE patients. It was demonstrated that tear production and stability were pathological in patients, ocular surface disease index was higher and central density of Langerhans cells was more prominent in this group compared to controls [4]. This data is valid not only in adults but also in the younger population. A prospective study including children with systemic lupus erythematosus performed a subjective and objective dry eye analysis. Even though no difference was found in the symptomatology, corneal staining was more prevalent in children with lupus compared to healthy children, also having higher mean corneal fluorescein staining scores [5]. This data suggests the benefit of active screening in patients with lupus, both in asymptomatic patients and in those presenting subjective complaints.

Overall, the prevalence of dry eye disease was reported to be 16% in patients diagnosed with lupus erythematosus, in a study that performed a literature search of over 18.000 participants. When focusing on patient symptoms or abnormal tests, the prevalence of dry eye symptoms was 26% and the modified Schirmer test in 24% of individuals. The studies that offered enough data for statistical analysis revealed a significant association between dry eye and systemic lupus erythematosus, with lupus patients being 4 times more likely to get DED than healthy controls (OR: 4.26)[6]. The connection between DED and other systemic diseases was revealed through a study performed at a tertiary eye center involving more than 200 consecutive patients with a primary diagnosis of DED. Data showed that 108 patients (47.4%) were in evidence with a systemic inflammatory disease. Of the remaining patients, a significant number underwent further examinations and 25 patients with an existing systemic disease were identified [7].

While posterior eye involvement was traditionally considered to be related to disease activity or treatment-related adverse effects, dry eye disorder may also reflect underlying systemic inflammation. Research focusing on SLE patients with dry eye but without Sjogren syndrome revealed that the severity of keratoconjunctivitis sicca was correlated with anti-dsDNA and C3 titers but not with ESR, C4 nor with ANA [8]. In our study we did not include biomarker data as evidence of disease status, however, a correlation was found between dry eye disorder and the number of disease flare-ups since diagnosis. Reactivation in autoimmune diseases has been widely studied. However, some elements comprising the pathogenetic cascade remain incompletely understood [9]. Certain auto-immune disorders enhance immune cell infiltration and inflammatory cytokine expression in various ocular structures, amplifying inflammation and tissue dysfunction with failure of normal tear production [10]. In light of this data, we believe that LES patients with an increased number of flare-ups would benefit from dry eye disease screening.

As expected, in our study, a correlation was found between the dry eye diagnosis and the use of lubricating tear drops, more symptoms were reported in responders using lubrication and the episodes of disease reactivation were related to the use of tear drops. Achieving control of DED in patients with systemic autoimmune diseases is demanding and typically requires a systemic control of the underlying disease, with a long-term therapeutic strategy [3] that is directed toward several areas of the body [11]. Accurate identification of ocular surface inflammation, of evaporative or aqueous deficient dry eye is important for providing the most appropriate treatment for this disorder.

Therapy includes non-pharmacological interventions such as lifestyle modifications and pharmacological treatments such as topical lubricants, corticosteroids, lifitegrast and cyclosporine A and systemic agents that comprise antibiotics, poly-unsaturated omega-3 fatty acids and antioxidants, or varenicline nasal spray [10]. Procedural therapies may be complementary to the above-mentioned options and consist of punctual occlusion, meibomian gland thermal pulsation and expression, intense pulsed light and low-level light therapy, and microblepharoexfoliation [10].

Our study is limited by the nature of questionnaires and could be completed in the future by objective ophthalmological dry eye assessments that could provide further data related to this condition. Also, the inclusion of further information related to treatment options could highlight new associations.

CONCLUSION

In conclusion, dry eye disease and dry eye-related symptoms are more frequent in SLE patients. Asymptomatic patients or patients with mild symptoms could also benefit from ophthalmological consultations.

Conflicts of interest and sources of funding
The authors have no conflict of interest to declare.

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Authors' contribution
Conceptualization, EC and ML; methodology EC, CP, LP, AN; software, AB, SR; validation, all authors; investigation EC, CP, LP; data curation, LP; writing—EC, CP; writing—review and editing AB, SR, AN; visualization, ML; supervision, ML. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The research was approved by the Ethics Committee of UMF 'I. Hatieganu', Cluj Napoca.

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ORIGINAL ARTICLE

Effect of Experience and Professional Role on Psychophysiological Stress Response in an Underwater Evacuation Training

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Abstract: *Background: This research aimed to analyze the effect of experience and professional role on psychophysiological stress response in underwater evacuation training. Methods: We analyzed 36 participants (39.06±9.01 years), divided into two different groups; 17 crew members (38.6±7.2 years) and 19 medical members (39.5±10.5 years). modifications in the rating of perceived exertion, subjective stress perception, heart rate, blood oxygen saturation, cortical arousal, heart rate variability, spirometry, isometric hand strength, and short-term memory before and after underwater evacuation training were analyzed. Results: The maneuver produced a significant increase in SSP, RPE, IHS, FVC, and SatO*$_2$ *at different moments of the intervention, being higher in crew members. Conclusions: We found that the underwater evacuation training produced an anticipatory anxiety response, and an increase in autonomous sympathetic nervous system modulation not affecting strength capacities, cortical arousal, and memory independently of the aircraft role (medical or crew).*

Keywords: *stress; underwater; military; aircrew; experience; role; cortical arousal; heart rate variability*

INTRODUCTION

Previous studies have shown how military combat situations are one of the most stressful events a human can cope with, due to their unpredictable, dangerousness, and highly demanding context [1]. Independently of the situation, military interventions produce the activation of the autonomic nervous system (ANS), initiating the flight-fight system involved in survival behaviors [2]. The stress produced by these extreme situations negatively impacts cognitive processes like memory, cortical arousal, decision-making, and information processing, reducing their normal functioning and even promoting the appearance of pathologies such as post-traumatic disorder (PTSD) [3-5].

Several types of research have been carried out in the military population, especially in ground military units, and the results show how an acute stress exposition can lead the organism to a downregulation in cortical arousal and high activation of the Sympathetic Nervous System (SNS), and finally modifying the blood pressure (BP), heart rate (HR), cortisol hormones, cortical arousal (CFFT), skin temperature, subjective stress perception (SSP), and heart rate variability (HRV) among others [6-10]. In this line, aircrews and paratroopers researchers have shown an increase in the physiological response by the increase in cortisol hormones, an increase in sympathetic modulation, and a decrease in cortical arousal, which is a symptom of Central Nervous System (CNS) fatigue [1,5,6,11-15].

Another factor that can negatively affect stress response is the possibility of having an aircraft accident [16]. The survival of the aircrew depends extremely on their knowledge of underwater evacuation training and stress management, and little is known about the physical and mental requirements of this specific and demanding situation, but as some researchers have demonstrated previously, the personal experience and professional training could modulate the stress experience and reduce the negative impact on the organism [2,4,11,13,15,17-18]. For this reason, we conducted the present research to analyze and compare the psychophysiological stress response of crew members (4 crew members; 4 jump chiefs; 9 paratroopers) and medical members (15 flight nurses and 4 doctors) in an underwater evacuation training. We hypothesized that 1) the underwater evacuation training would increase the sympathetic autonomic modulation of the crew members, and 2) crew members would present a lower stress response.

MATERIALS AND METHODS

In the present work were analyzed 36 participants (39.06±9.01 years), divided into two different groups; 17 crew members (38.6±7.2 years; 174.9±8.6 cm; 82.1±11.2 kg; 18.1±8.7 years of service; 14.9±13.4 months in mission; 357.9±366.9 flying hours) and 19 medical members (39.5±10.5 years; 173.6±6.14 cm; 71.4±10.5 kg; 15.5±9.99 years of service; 11.5±15.3 months in mission; 25.1±73.8 flying hours). The entire research procedure was conducted by soldiers with the standard flying suit and boots, portable oxygen cylinder, life vest, helmets, and earphones to simulate the equivalent operating equipment for this type of action. None of the participants had previous or recent experience in these underwater simulations.

A pre-intervention-post intervention was conducted, analyzing the following psychological and physiological variables previous to and immediately after an underwater evacuation training.

1. Rating of perceived exertion (RPE). Using Borg 6–20 scale [19];

2. Stress subjective perception (SSP) on a 1–100 scale [15];

3. Blood oxygen saturation ($SatO_2$) and heart rate (HR) by a finger pulse-oximeter system (PO 30 Beurer Medical) [6];

4. Cortical arousal and fatigue of the central nervous system (CNS) were measured by the Lafayette Instrument critical flicker fusion threshold (CFFT) (Model 12.021) by the average of five incremental tests (20 to 100 Hz) according to previous authors [5,16];

5. Isometric handgrip strength (IHS) by a grip dynamometer using the dominant hand and taking the better result of two attempts (Takei Kiki Koyo, Japan);

6. Spirometry: forced vital capacity (FVC), volume exhale at the end of the first second of forced expiration (FEV1) and the peak expiratory flow (PEF) using a QM-SP100 (Quirumed, Spain) spirometer in a maximum inhale-exhale cycle [5,16];

7. Short-term memory (STM). A three-digit number was shown to the participants for one second. After five seconds, they were asked to remember the number and to say it backward [16];

Finally, the heart rate (HR) and heart rate variability (HRV), which refers to the autonomic modulation, were recorded with a Polar V800 heart rate monitor with RR measurement function (POLAR. Finland) and posteriorly analyzed by the Kubios HRV standard 3.2.0 software (University of Kuopio, Kuopio, Finland). The parameters analyzed were:

1. Temporal domain: mean HR; minimum HR (Min HR); maximum HR (Max HR); the square root of the mean value of all sums of square differences of all R-Rs following intervals (RMSSD); the percentage of differences between normal adjacent R-R intervals greater than 50 ms (pNN50);

2. Frequency domain: low-frequency band (LF); high-frequency band (HF); A ratio of low frequency to high frequency (Ratio LF/HF);

3. Nonlinear domain: sensitivity of the short-term variability (SD1); sensitivity of the long-term variability (SD2).

The underwater evacuation training was performed in a swimming pool in the following conditions: 4 × 4 × 3 swimming pool size (m); water characteristic: chlorine 2 (ppm); pH 7.5; turbidity values 0.13 (NTU); temperature: 26°C. The complete training was performed in the presence of two instructors and two water assistants. It was composed of four different stages, with a duration of 30 minutes each one, where the participants had to: 1) use a portable oxygen cylinder and carry out apnea training in several positions; 2) evacuate the submerged aircraft accident through the window; 3) rescue training of a raft and rescue training from a helicopter; and finally 4) underwater evacuation during the turning of a ship.

Statistical analysis was performed with the SPSS 24.0 statistical program. The descriptive statistics used to report the results were the mean (M) ± standard deviation (SD). The normality of the sample was determined with the Kolmogorov-Smirnov test, and all the variables presented a parametric distribution. The repeated measures ANOVA was conducted to test differences in the evaluation moments analyzed. A T-test for independent variables was used to analyze differences between the two groups analyzed (crew and medical). The Effect Size was calculated by Cohen's D. The significance level was $P \leq 0.05$.

RESULTS

The results were reported as M ± SD. SSP showed a significant increase in post-values of both crew and medical members concerning the pre-values, being higher in crew members, but there were no significant differences in pre- or post-values between groups. RPE presented a significant difference in post values, being higher in crew members, and also showed a significant increase in post values of both groups in comparison with pre values. IHS showed a significant difference in pre-values between crew and medical responses, being higher in crew members. FVC showed only a significant increase in post values of crew members in comparison with pre-values of crew members. $SatO_2$ showed a significant difference in post values of crew and medical members, being higher in crew members (Table 1).

Table 1: Role comparison in pre-post variables

Variables	Role	Pre Intervention				Post Intervention			
		M±SD	Bilateral signification	95% confidence interval		M±SD	Bilateral signification	95% confidence interval	
			P	Inferior	Superior		P	Inferior	Superior
SSP	Crew member	16.2±20.9	0.142	-26.71	4.01	60.6±27.2 (0.000)	0.358	-9.70	26.14
	Medical member	27.5±24.1				52.4±25.2 (0.001)			
RPE	Crew member	6.7±1.5	0.075	-0.07	1.38	13.7±2.9 (0.000)	0.023	0.32	4.13
	Medical member	6.1±0.2				11.4±2.7 (0.000)			
IHS (N)	Crew member	50.9±11.4	0.026	1.17	17.24	50.4±10.7	0.084	-1.02	15.53
	Medical member	41.7±12.3				43.2±13.4			
FVC (ml)	Crew member	4.4±0.8	0.412	-0.48	1.13	4.8±0.9 (0.047)	0.374	-0.36	0.94
	Medical member	4.1±1.4				4.5±1.0			
FEV1 (ml)	Crew member	3.7±0.7	0.464	-0.47	1.00	3.8±0.7	0.956	-0.52	0.55
	Medical member	3.4±1.4				3.8±0.8			
PEF (ml)	Crew member	9.8±1.8	0.548	-1.31	2.42	9.8±1.9	0.705	-1.27	1.86
	Medical member	9.3±3.4				9.5±2.6			
$SatO_2$ (%)	Crew member	95.9±1.9	0.186	-2.01	0.41	97.1±2.4	0.040	0.08	3.30
	Medical member	96.7±1.7				95.4±2.4			
HR (bpm)	Crew member	81.5±14.2	0.701	-8.27	12.17	81.4±18.8	0.401	-7.15	17.44
	Medical member	79.6±15.8				76.3±17.5			
STM	Crew member	1.0±0.0	—	—	—	1.0±0.0	—	—	—
	Medical member	1.0±0.0				1.0±0.0			
CFFT (Hz)	Crew member	39.9±3.9	0.365	-1.34	3.55	38.1±3.5	0.840	-2.29	2.80
	Medical member	38.8±3.4				37.8±3.9			

1(M) mean; (SD) standard deviation; (SSP) stress subjective perception; (RPE) rating of perceived exertion; (IHS) isometric hand strength; (FVC; FEV1; PEF) spirometry; (SatO2) blood oxygen saturation; (HR) heart rate; (STM) short term memory; (CFFT) cortical arousal.

The mean HR of medical members presented the lowest value during the intervention and increased post-intervention, being higher than in crew members. Min HR presented similar values in both groups in pre- and during moments, but crew members' values were higher in post-intervention. Max HR of medical members was higher than crew members in all moments, during the intervention the highest. RMSSD presented similar values in both groups in the pre-moment; medical member values were higher during the intervention and decreased in post-intervention in comparison with crew members.

Variable pNN50 presented in both groups an increase during the intervention, being higher in medical members than in crew members, and decreasing in post-intervention in both groups. LF showed high values in all moments in medical members, being higher in pre-moment than post-intervention, and decreasing during the intervention. HF presented higher values in crew members than in medical members pre- and during the intervention, being the highest value during the intervention, and decreasing post-intervention in both groups. LF/HF Ratio showed higher values in medical members than in crew members in pre- and during the intervention, being the highest value in the pre-moment, while crew members presented higher values in post-intervention. SD1 presented similar values in pre-intervention in both groups, being higher during intervention in medical members than in crew members and decreasing in post-intervention in comparison with crew members.

SD2 presented higher values in all moments in medical members than in crew members, being the highest during the intervention and decreasing in both groups in post-intervention in comparison with pre-momentum (Table 2).

Table 2: Role comparison in pre-, during, and post-HRV variables

Variables	Role	Pre-intervention (1)				During intervention (2)				Post-intervention (3)				F	IC
		M±SD	BS	95 % confidence interval		M±SD	BS	95 % confidence interval		M±SD	BS	95 % confidence interval			
			P	Inferior	Superior		P	Inferior	Superior		P	Inferior	Superior		
Mean HR (bpm)	Crew member	84.9±10.6	0.179	-16.49	3.19	90.8±7.7	0.837	-8.08	6.59	97.4±13.8	0.919	-9.66	8.73	9.942	1<3;2<3;3>1<2
	Medical member	91.6±17.3				54.3±18.1				97.9±13.4				5.711	2<3; 3>2
Min HR (bpm)	Crew member	67.1±10.8	0.983	-8.67	8.48	54.9±13.9	0.906	-10.38	11.68	71.4±9.9	0.265	-3.45	12.15	13.639	1>2; 2<1<3; 3>2
	Medical member	67.2±14.1				54.3±18.1				67.1±12.8				5.637	1>2; 2<1>3; 3>2
Max HR (bpm)	Crew member	116.0±18.1	0.111	-36.68	3.95	165.2±25.1	0.351	-53.56	19.54	131.7±23.9	0.162	-42.48	7.41	24.432	1<2; 2>1<3; 3<2
	Medical member	132.4±37.4				182.2±70.1				149.2±45.2				4.710	1<2; 2>1
RMSSD (ms)	Crew member	33.1±19.2	0.989	-12.98	12.80	41.7±19.8	0.523	-15.34	7.95	36.8±24.2	0.743	-10.89	15.13	3.948	—
	Medical member	33.2±18.9				45.4±14.4				34.7±13.1				10.764	1<2; 2>1<3; 3<2
pNN50 (%)	Crew member	7.8±7.9	0.768	-4.27	5.74	8.1±5.3	0.051	-7.29	0.02	6.5±6.1	0.790	-4.07	3.12	1.102	—
	Medical member	7.1±6.9				11.7±5.5				6.9±4.4				15.047	1<2; 2>1>3; 3<2
LF (n.u)	Crew member	77.4±13.8	0.408	-11.19	4.65	72.6±11.1	0.756	-7.22	5.29	76.1±11.2	0.992	-6.98	6.91	1.081	—
	Medical member	80.6±9.5				73.5±7.2				76.2±9.3				4.296	1>2; 2<1
HF (n.u)	Crew member	22.6±13.7	0.409	-4.64	11.14	27.3±11.0	0.762	-5.28	7.15	23.7±11.1	0.990	-6.94	6.85	1.085	—
	Medical member	19.3±9.4				26.4±7.1				23.8±9.3				4.286	1<2; 2>1
LF/HF Ratio (ms^2)	Crew member	5.2±3.9	0.942	-2.32	2.16	3.4±2.4	0.888	-2.21	1.92	4.8±4.3	0.521	-1.61	3.11	3.028	—
	Medical member	5.3±2.7				3.6±3.5				4.0±2.5				2.730	—
SD1 (ms)	Crew member	23.4±13.6	0.969	-9.44	9.08	29.5±14.0	0.516	-10.96	5.61	26.1±17.2	0.751	-7.82	10.73	3.912	—
	Medical member	23.6±13.7				32.2±10.4				24.6±9.5				10.780	1<2; 2>1<3; 3<2
SD2 (ms)	Crew member	54.8±17.8	0.507	-20.23	10.19	58.5±13.1	0.138	-19.55	2.83	51.6±17.8	0.276	-19.56	5.76	1.768	—
	Medical member	59.8±25.9				66.9±19.0				58.5±19.4				5.168	2>3; 3<2

2(M) mean; (SD) standard deviation; (BS) bilateral signification; (IC) intermoment comparison; (Mean HR) mean heart rate; (Min HR) minimum heart rate; (Max HR) maximum heart rate; (RMSSD) square root of the mean of the sum of the squared differences between adjacent normal R-R intervals; (pNN50) percentage of differences between normal adjacent R-R intervals greater than 50 ms; (LF) low frequency; (HF) high frequency; (Ratio LF/HF) A ratio of low frequency to high frequency; (SD1; standards deviations of the scattergram 1; (SD2) standards deviations of the scattergram 2; (n.u) normalized unit.

DISCUSSION

This study aimed to analyze and compare the psychophysiological stress response of crew members (4 crew members, 4 jump chiefs, 9 paratroopers) and medical members (15 flight nurses and 4 doctors) in an Underwater Evacuation Training. Hypothesis 1) was confirmed since underwater evacuation training increased the sympathetic autonomic modulation of crew members. Hypothesis 2) was not confirmed, since the stress response was similar in both crew member and medical member groups, independently of the professional role.

The underwater evacuation training includes apnea periods, in which aircrew members have to deal with a lack of oxygen that produces a lower oxygen partial pressure in the alveolus and affects the oxygen that the arterial blood can deliver to the brain [20]. In both hypoxia and underwater training, the lack of oxygen impacts the RPE, although in underwater training SSP and SatO2 values are generally higher than those in hypoxia [3,21-24]. These results suggest that for underwater training, the exertion mechanism is produced by short acute periods of apnea instead of larger periods with progressively less oxygen partial pressure if compared to hypoxia training. IHS has also been previously studied in military aircrew with different results depending on the previous training and roles [3], which coincides with the pre-results of this study with aircrew members having more initial strength capacity than medical personnel. If we analyze the breath capacity, PEF, and FEV1 have traditionally been the most affected variables in contexts of sustained hypoxia as specific training in these conditions, special operation selection course, and maneuvers, or real flight conditions [1,3,14,15,26]. Whilst FVC was the most activated variable in underwater training: a constant need to store oxygen in the alveolus before an acute period of apnea activates the lunges to store more air as a physiological adaptation. Autonomic modulation has been previously evaluated in military aircrews by the HRV in contexts of hypoxia, finding an increased sympathetic autonomic modulation as expected, since the lack of oxygen acts as a stressor of the central nervous system [21,24,26,27]. Hypoxia symptoms have been previously studied in military crews through cortical arousal with different results: the decrease of oxygen saturation had a negative [27] or neutral [3] impact in cortex performance, which are similar results to those found in this study.

The underwater evacuation training simulates an extreme situation in which aircrew and medical members first try to survive. This fact could be the reason the post measures were not different for each group, since the responses in these extremely hazardous situations tend to be maximal and could equal the response in both groups. This large stress response independently of the role was also measured in different military units in combat maneuvers where, contrary to the present study, there were changes in respiratory musculature, memory, and cortical activation [2,28-30]. Medical members had less RPE and SatO2 after the training, maybe because of shorter periods of physical activity during some maneuvers. The most expected difference was found in the measures before the training, where the aircrew members were able to develop more strength capacity due to their previous training, more oriented to physical fitness.

Regarding the HRV analysis, we observed an increase in vagal tone (RMSSD, HF, HF/LF ratio, and SD2) of participants, a result probably due to the 2h training in the water and the possible induced hypothermia [31]. Increases in the HF values under these hypothermia conditions may reflect a reduced delay in baroreflex response, enabling lower resonant frequency in blood pressure and RR interval, which may be a consequence of temperature-mediated increase in peripheral sympathetic activity and myogenic reactivity due to increased vascular tone and transmural pressure caused by peripheral vasoconstriction to prevent heat dissipation [32]. LF/HF ratio is a controversial marker of sympathovagal balance, and is difficult to interpret due to the complexity of the origin of LF oscillations, along with the effects of varying breathing pattern on HF oscillations, which may present low accuracy and physiological confidence. However, it presents the same trend as RMSSD which is a stronger marker of cardiac vagal activity, independent of the breathing pattern, consequent with SD2 values which reflect the long-term fluctuations of RR intervals, an inverse indicator of sympathetic activity. This autonomic response was similar to crew or medical members in the underwater evacuation training.

The cardiovascular response measured was higher compared to the effort developed, mean HR among training is likely produced due to the shifts of blood volume from the periphery to the central circulation due to peripheral vasoconstriction and major cardiovascular output, to reduce heat loss and defend core body temperature by increasing the thickness of the insulative layer between the ambient air and perfused blood vessels [33]. The fact that HR response increased despite a likely augmented venous return highlights the fact that significant thermogenesis was occurring, requiring an increased HR to support the metabolic activity.

When basal HRV values analyzed are compared to reference basal values [34], it suggests an anticipatory anxiety response, which may be explained as the training was an evaluative test/training for soldier's professional careers, in addition to the risk, demands, and specific implications of the training. This response is consequent with the pre-SSP values evaluated, showing the large stress of this eliciting training, in line with other military operations such as underground combat, parachute jumps, urban combat, or even air combat jet maneuvers and disorientation training [2,6,13,14,31,35, 36]. In addition, a reminiscence of the sympathetic activation produced by the training was found in the decrease of RMSSD or the increases in HR parameters, highlighting the psychophysiological demands of the task.

Limitations of the study and future research lines

The small number of subjects analyzed has been one of the main limitations of the present work. A greater number of participants would provide more reliable results. However, the difficulty of accomplishing these types of actions due to this complexity, hazard, and economic and human resources needed were a limiting factor. Another limitation was the use of indirect measurement instruments such as the fusion flicker system. Logistics and economic difficulties have limited the use of more modern systems, but the fact that the evaluations were conducted in a very limiting environment, made nearly impossible the use of other instruments such as electroencephalography. Finally, a lack of control of hormones such as alpha-amylase and cortisol to control the hormonal stress response. Future research might seek to address these issues. Given the importance of operational and specific training to allow better survival in air accidents in open water areas, further research should try to design an optimal stimulus and protocol to improve the habituation process.

Practical applications

The present study highlights the psychophysiological response of both crew and medical members undergoing underwater rescue training. This information leads us to a better understanding of the physiological and psychological stress response to this specific and demanding situation in which there is a life risk. The application of this information could contribute to a better design of underwater evacuation training and for both civil and military air personnel preparation.

CONCLUSION

We found that underwater evacuation training produced an anticipatory anxiety response, and an increase in autonomous sympathetic nervous system modulation not affecting strength capacities, cortical arousal, and memory independently of the aircraft role (medical or crew).

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Authors' contribution
Conceptualization, Marta Vicente-Rodríguez, Álvaro Bustamante-Sanchez, and Jose Alberto Parraca.; methodology, Marta Vicente Rodríguez, and Jose Tornero-Aguilera.; software, Vicente Javier Clemente-Suárez.; validation, Vicente Javier Clemente-Suárez, and Jose Tornero-Aguilera.; formal analysis, Jose Tornero-Aguilera, and Álvaro Bustamante-Sanchez.; investigation, Marta Vicente-Rodríguez, and Jose Alberto Parraca.; resources, Vicente Javier Clemente-Suárez.; data curation, Marta Vicente-Rodríguez, and Vicente Javier Clemente-Suárez.; writing—original draft preparation, Marta Vicente-Rodríguez and Jose Alberto Parraca.; writing—review and editing, Marta Vicente-Rodríguez.; visualization, Jose Tornero-Aguilera.; supervision, Vicente Javier Clemente-Suárez.; project administration, Álvaro Bustamante-Sanchez and Vicente Javier Clemente-Suárez.; funding acquisition, Vicente Javier Clemente-Suárez. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The study was conducted following the Declaration of Helsinki, and approved by the Ethics Committee of European University of Madrid (CIPI/18/093). Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Sedation Techniques for Drug-induced Sleep Endoscopy in Adult Patients with Sleep-disordered Breathing

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Abstract: *Drug-induced sleep endoscopy emerged and developed as an essential alternative diagnosis tool in sleep-disordered breathing patients. Accurate identification and dynamic characteristics of upper respiratory airway obstruction are paramount in establishing an adequate therapeutic plan and selecting for particular surgical or non-surgical strategies. This article reviews the current aspects of intravenous sedation for drug-induced sleep endoscopy (DISE) in adults, emphasizing its implications for patients with sleep apnea. The ideal sedative agent for DISE should replicate natural sleep patterns while maintaining upper airway dynamics. The clinical practice exhibits a significant variability in drug selection, with midazolam and propofol often being preferred. The review presents emerging trends and advancements in DISE, emphasizing its role in optimizing surgical indications, predicting outcomes, and screening patients for alternative therapeutic strategies for OSA. Future research is needed to understand better the impact of specific sedative drugs on DISE findings, contributing to the development of standardized protocols and the evolution of new sedative drugs with improved effects and optimized techniques for delivering sedation.*

Keywords: *drug-induced sleep endoscopy, sedation, target-controlled infusion, obstructive sleep apnea*

INTRODUCTION

Obstructive sleep apnea (OSA) represents one of the most encountered forms of sleep-disordered breathing. OSA is characterized by iterative complete or partial collapse of the upper respiratory airway (UA). Number of obstruction events registered during one hour of sleep, using polysomnography (PSG), quantifies clinical severity. This number of hourly events, known as the apnea-hypopnea index (AHI), has paramount utility in the diagnosis and grading of the severity of OSA.

The prevalence of OSA, determined from extensive population studies, varies largely, between 9% and 38% in the general adult population [1]. Clinical consequences of OSA are increased cardiovascular risk, arterial hypertension, cognitive decline, obesity, and elevated risk of being part of a vehicle accident.

Diagnosis and classification of sleep-disordered breathing are accomplished using nocturnal polysomnography, which represents the gold standard investigation. Evaluation of the upper respiratory airway using drug-induced sleep endoscopy (DISE) emerged as an alternative diagnosis tool with significant benefits in some OSA patients.

DISE represents a complementary diagnosis technique helpful in offering additional data about upper airway (UA) dynamics and conformation. The principle of DISE resides in real-time dynamic and three-dimensional fiberoptic airway evaluation during pharmacologically induced profound sedation aimed to reproduce as similar as possible natural sleep.

Concerning the therapeutic perspective, the first-line accepted treatment for OSA is continuous positive airway pressure (CPAP). However, this therapeutic approach has some limitations, including low adherence and difficulty in long-term acceptance [2]. To date, expert opinion divergences persist concerning the routine DISE evaluation in all OSA patients or performing it only in selected patients in whom intolerance to CPAP is encountered or the clinician intends to offer treatments alternative to CPAP, such as oral appliance therapy

(OAT), upper airway surgery (UAS), positional therapy (PT), or some combination of different modalities [3].

The purpose of this article is to provide a review of current aspects of the intravenous sedation approach for performing DISE in adults. Pediatric DISE is beyond the scope of this review; a comprehensive expert consensus statement on this topic was published in 2021 [4], aiming to clarify some controversies on this topic in the pediatric population

CONCEPT OF DISE

Croft and Pringle [5] delineated the principle of DISE for the first time in 1991. The authors described the use of midazolam sedation in sleep-disordered breathing patients concomitantly performing endoscopic airway evaluation of obstruction sites. The developments of the technique over the last four decades have established a definitive role in the clinical approach of OSA patients.

Compared to awake examination of the upper respiratory airway, DISE was described in one study as a potential tool able to change the surgical approach in OSA patients candidates for surgery, interfering consequently with operative indication in approximately 50% of patients [6]. In contrast, in a prospective multicentric trial addressing the impact of DISE on surgical outcome, Pang et al enrolled 326 patients [7] and demonstrated that performing preoperative accurate DISE may not significantly impact the surgical success outcome in OSA patients.

Multiple controversies persist in the literature concerning other aspects of the DISE technique: feasibility for selecting a particular patient for surgery or non-surgical treatment, standardization in sedation technique, monitoring depth of sedation, scoring and classification systems for anatomic levels, and configuration of obstruction pattern. Although DISE evolution encompassed a long period and the topic of performing sedation similar to natural sleep evolved and developed on the strength of new technical acquisitions (BIS monitoring, use of propofol with target-controlled infusion devices, new sedative drugs), its role in OSA patients needs more clarifications.

SEDATIVE DRUGS FOR DISE

There is a large variability in the sedation techniques, drugs, and protocols described in the literature regarding DISE. Conflicting data exist regarding available drugs for sedation, technique of administration, combination of drugs, and dosage; accordingly, a standard, unanimously accepted sedation protocol has not been established and available so far. Beyond the selection of the specific sedative drug or combination, avoiding oversedation is another essential topic to be considered when performing the technique.

Sedative drugs most widely used are midazolam, propofol, dexmedetomidine, and, more recently, remimazolam [8]. The ideal sedative agent for DISE is debated; this is intended to have some neurophysiologic characteristics in reproducing normal sleep patterns and insignificant relevance on UA dynamics during sedation. Neurophysiologic characteristics of an ideal sedative drug for DISE are summarized in Table 1 [9].

Table 1: Neurophysiologic characteristics of ideal agents for DISE

Reproduction of
- Stage NREM 1, NREM 2, and/or REM sleep
Preservation of
- Mechanoreceptor input to the brainstem - Chemoreceptor input to the brainstem - Respiratory rhythmicity by pre-Botzinger complex - Lower cranial nerve output to pharyngolarynx

NREM, nonrapid eye movement; REM, rapid eye movement

Intravenous anesthetic drugs most frequently used are midazolam and propofol [9]. These drugs present some pharmacologic properties that confer advantages in achieving the desired level of sedation. Therefore, the possibility of attaining the critical closing pressure of the airway is similar to natural sleep [10]. Critical closing pressure (Pcrit) represents a measure of UA collapsibility, an essential parameter variable from a healthy patient (low collapsibility) to an OSA patient (high collapsibility). Some studies [10,11] have demonstrated that sedation reproduces natural sleep in non-REM sleep; consequently, DISE represents a valid tool for examining respiratory airways in OSA patients. The REM sleep phase is difficult to reproduce in studies. Therefore, in clinical practice, for patients who manifest apneas only in REM sleep, DISE is not an option for diagnosis.

One another important topic in UA evaluation during DISE is adequately targeting the clinical depth of sedation. A very deep plane of sedation may be inaccurate as it induces obstruction at different levels, not reproducing it similarly to natural patient sleep. Consequently, monitoring the depth of sedation, and the objective measurement of sleep depth represents an important tool in technique validity. This can be accomplished by using EEG-derived index monitoring techniques, such as bispectral index monitoring (BIS), adopted in advanced clinical practice, and replacing simple clinical evaluation (patient's reaction to verbal or tactile stimuli). A recent meta-analysis [12] demonstrated a statistically significant correlation between BIS sedation level and the area of obstruction on the VOTE score (a grading score of UA collapsibility that quantifies obstruction at vellum, oropharynx, tongue, and epiglottis level).

Comparing different sedative drugs for DISE, in one study, Viana and Zhao [13] enrolled 52 patients sedated with propofol, midazolam, and dexmedetomidine for performing airway evaluation. The selection of the drug was proved to have a relevant influence on evaluation findings. Midazolam and propofol presented an increased incidence of tongue base collapse compared with dexmedetomidine.

The clinical practice is characterized by a significant variability in drugs used for DISE. As mentioned before, midazolam or/ and propofol remain the first option, but infrequently, sedative drugs like ketamine, dexmedetomidine, and remifentanil are also used. Recently, remimazolam demonstrated feasibility and a better safety profile than propofol [14]. Some authors described a combination of different drugs.

The European position paper on drug-induced sleep endoscopy (DISE) [15], published in 2014, and the update [16] from 2017 offers suggestions regarding sedative drugs and techniques, a recommendation as the first option consisting of using midazolam and propofol, single or in combination. Since 2017, new studies have been published relevant to new sedative agents or new aspects of airway obstruction, correlated to sedation depth and mechanism. The crucial aspect reiterated in these papers involves the precautious and careful execution of technique, because overdosing may induce UA collapse and misinterpretation of investigation results. Table 2 presents the advantages and disadvantages of sedative drugs used in DISE and their combination.

Table 2: Sedative drugs for DISE – advantages and disadvantages

Sedative agents	Advantages	Disadvantages
Propofol	Rapid onset Predictable effect Less muscular relaxation Inactive metabolites	Technique Advanced expertise in TCI (target controlled infusion) Increased collapsibility when inadvertently profound sedation
Midazolam	The examination window is longer Antagonist available	Slower onset Very careful titration is necessary Prolonged recovery and longer facility stay
Dexmedetomidine	Sedation pattern similar to natural sleep	Prolonged recovery Slower onset Precaution in unstable cardiac patients Some protective effects for airway collapse
Combination propofol plus midazolam	Faster onset	More frequent sneezing than propofol alone
Remimazolam	Better profile than propofol Less hypoxemia Very fast recovery	Less studied in DISE
Propofol plus remifentanil	Enable a reduced target concentration of propofol No respiratory depression	Only a few studies
Dexmedetomidine plus ketamine	Good safety profile in children Ketamine has minimal effect on airway patency	DISE in the pediatric population Not universally used

Propofol

Propofol is one of the most widely used intravenous anesthetics in current medical practice. The mechanism of action of propofol remains unknown, possibly consisting of positive modulation of the inhibitory function of the neurotransmitter gamma-aminobutyric acid (GABA) through GABAA receptors [17]. Concerning the differences and similarities between natural sleep and sedation induced with propofol, studies with high-density encephalography (hd-EEG) have revealed that propofol sedation is a sleep-like state and the large slow waves induced by propofol anesthesia were similar in appearance, topography, negative-going slope, and positive-going slope to the slow waves seen during NREM sleep [18].

Propofol pharmacokinetics (PK) is characterized by hepatic elimination as the primary route and inactive metabolites with kidney excretion. The onset of effect is fast, depending on the administered dose, rate of administration, and patient's response.

Administration and dosage of propofol

The DISE technique can be accomplished in three different ways: iterative bolus technique, manual controlled infusion (MCI), and target-controlled infusion (TCI).

Manually controlled infusion involves delivering a dose at 50-100 ml/h and adjusting according to clinical response [15]. The bolus technique comprises different administration protocols, depending on clinician preference, with a starting dose of 30-50 mg and repeated 10 mg every 2 min or initially 1 mg/kg and afterward 20 mg every 2 min.

Target-controlled infusion systems perform sedative drug infusion using software-controlled infusion pumps to achieve a desired target concentration of drug in the blood or brain (effect compartment). This method offers the possibility of rapid achievement of concentration in the target compartment and maintenance of the intended effect based on incorporated software algorithms. Several studies have confirmed the superiority of TCI technology in delivering propofol for sedation in DISE, consequently, this approach is currently recommended as a standard approach [15,16]. The main models conventionally in use are those described by Marsh [19] and Schnider [20]. The Schnider model is an established pharmacokinetic model that has been widely used in clinical practice. It is an extension of the Marsh model and is designed for TCI systems. Recently, a new pharmacokinetic-pharmacodynamic (PK-PD) model for propofol was described by Eleveld for a broad population range [21]. It was developed to address limitations in previous models and improve accuracy in predicting drug concentrations. This general-purpose model presents multiple advantages in reducing the risk of incorrect selection of a specific model for a particular patient, extrapolation, and unfamiliarity of clinician anesthesiologists with preexisting models [22].

European consensus update from 2017 [16] recommends the TCI technique as the first option in administering propofol sedation for DISE; the starting effect-site concentration should be 2.0-2.5 µg/ml, with gradually increasing dose by 0.2-0.5 µg/ml until reaching the target state of sedation.

The cited consensus does not recommend a specific PK-PD model for propofol administration. Marsh or Schnider models are equally utilized in different studies, and up to the present, to our knowledge, the Eleveld model has not been tested for DISE.

Midazolam

The literature recommends only the bolus technique, with initially 0.05 mg/kg, observing the effect for 2-5 minutes, and readministering 0.03 mg/kg if the patient is not asleep. Repeated doses of 0.015 mg/kg may be necessary for supplementing sedation depth, with careful observing after every administration for 5 minutes [16].

Combination of propofol plus midazolam

Midazolam influences the pharmacokinetics of propofol by interfering with metabolic clearance and increasing the plasma concentration of propofol [23]. The purpose of this combination is to decrease patient anxiety with midazolam 0.05 mg/kg initially and after 2 minutes proceed with sedation using propofol delivered with TCI pump in effect-site concentration 1.5-3 µg/ml, and subsequent concentration increased by 0.2-0.5 µg/ml, until achieving the desired level of sedation [16].

Dexmedetomidine

European consensus on DISE [4] does not recommend sedation with dexmedetomidine as the first choice, although this drug presents some advantages, including a lower risk of hypoxemia and respiratory depression. The disadvantages of using dexmedetomidine consist of prolonged evaluation time and a protective effect on airway collapse (tongue base and hypopharyngeal structures [24]. Loading dose should be 1-1.5 µg/kg in 10 minutes, continuing with 1-2 µg/kg/h, with careful clinical observation of sedation level.

Remifentanil

Some authors recommend against using fentanyl, remifentanil, and ketamine as adjunctive drugs to propofol or midazolam, due to the increased risk of oxygen desaturation [24]. Recent research suggests that remifentanil may offer benefits as an adjunctive sedative drug, decreasing cough reflex, shortening the time of the procedure, and decreasing effect site concentration intended for propofol sedation [25,26]. The effect-site concentrations of remifentanil administered in two studies are 1.5 ng/ml and 0.5 ng/ml respectively using the Minto PK-PD model [25,26].

Ketamine

Ketamine is primarily used in pediatric DISE in association with dexmedetomidine [27]. It is administrated in doses 1-2 mg/kg, simultaneously with a loading dose of dexmedetomidine (3 µg/kg in 10 minutes followed by continuous infusion of 2 µg/kg/h). This combination is preferred due to lower risk (upper airway obstruction, respiratory depression) and the preservation of non-rapid eye movement sleep [28].

PATIENT SAFETY

Most studies do not describe significant complications associated with the DISE procedure. Standard monitoring is mandatory, and avoiding oversedation and muscular UA relaxation is vitally important in performing a safe and accurate investigation. Most frequently cited incidents consist of central apneas [29] and respiratory depression, especially when the depth of sedation is not carefully titrated. Patients should be thoroughly evaluated for any contraindications before undergoing DISE, and the procedure should be conducted with careful monitoring and adherence to safety protocols. The incidence of complications with DISE is generally low, and the procedure is considered safe when performed by trained and experienced healthcare professionals in a controlled environment. The Guidelines of the French Society of ENT on DISE [30] emphasize the lack of data in the literature regarding the contribution of capnography to safety during DISE, as well as routine oxygen administration to prevent procedure-induced hypoxemic complications.

EMERGING TRENDS AND ADVANCEMENT

It has been demonstrated in multiple studies that DISE could help the surgeon in formulating the optimal operative indication and could predict outcomes after UA surgery [31]. Concerning the therapy with CPAP, the role of DISE consists mainly of identifying the anatomic basis of CPAP failure and obstruction pattern with this therapeutic indication. New developments have placed DISE as a useful instrument in screening patients for alternative therapeutic strategies for OSA: oral appliance therapy, trans-oral robotic surgery, hypoglossal nerve stimulation, and maxillomandibular advancement surgery [20]. Some limitations of the DISE technique impose awareness in the therapeutic approach of OSA patients. Although the technique is complementary to polysomnography, the clinical findings from DISE do not represent the entire night's sleep but only a limited fraction of it. Standardization of sedation techniques and establishing protocols will improve observed obstruction patterns in OSA heterogeneous groups of patients.

CONCLUSION

Nowadays, it is unanimously accepted that we need a better understanding of the impact of using a specific sedative drug on DISE findings. Future prospective randomized trials will provide insight into clarifying the controversies on the impact of sedative drug selection on DISE findings and on the influence on sleep architecture and stages, contributing to a better sedation protocol. The development of new sedative drugs with more predictive effects and rapid elimination, along with optimizing the administration technique with the aid of a new general-purpose PK-PD model incorporated in TCI devices could offer progress in the future.

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ORIGINAL ARTICLE

Effects of Military Equipment on the Psychophysiological Stress Response during a HIIT Workout

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Abstract: *Background: Army operations produce various psychophysiological responses of military personnel. Traditionally, to prepare soldiers for these requirements, military workouts have been based on long-distance and low-intensity training. Recent researches suggest that high-intensity interval training (HIIT) may be more effective due to the characteristics of actual theaters of operation. there is a lack of research on the actual effects of military equipment in the context of combat or HIIT training. This study aimed to analyze the effects of military equipment on psychophysiological responses during a HIIT session. Methods: 20 soldiers of the Spanish Army (aged 27.3 ± 3.4 years; height 176.6 ± 4.7 m; weight 76.8 ± 7.4 kg) were evaluated after and before a traditional running HIIT which consisted of 12 repetitions of 30 seconds at maximum intensity, with a 30-second rest between exercises. Blood lactate, rate of perceived exertion and stress, upper and lower limbs, and respiratory muscle strength, skin temperature, blood oxygen saturation, heart rate, cortical arousal were measured before and after the training. Results: Results are reported with their mean and standard deviation. Significant differences were found in RPE, SSP, BOS, and Lactate in both groups in pre and post-training with the two outfits analyzed. Also, it was observed significant differences in HJ, UMS, and PEF values when the group was equipped with military equipment. Significant differences were found in RPE pre-training values and RPE, Stress perception, BOS, HR, and lactate post-training values. Conclusions: The use of military equipment during a high-intensity interval training session produces higher stress perception and perceived exertion than the civil outfit. The mechanical difficulty and the lack of operability in movements could be a reason for this psychophysiological response.*

Keywords: *stress, military equipment, performance, High-intensity interval training*

INTRODUCTION

Combat stress produces various modifications in the physiological responses of military personnel, such as an increase in anaerobic lactic metabolism, activation of the sympathetic nervous system, higher cardiovascular activity, activation of muscle strength, and a decrease in cortical activation [1,2]. Additionally, these physiological responses can be accompanied by cognitive effects related to a distortion of subjective perception of effort [3] and negative effects on memory [4-6]. When these responses are prolonged over time, they can lead to psychological disorders related to stress and anxiety [7].

This exacerbated organic response is due to the activation of the fight-flight mechanism, a primitive survival mechanism that develops an acute adaptive response and provides the organism with the capacity to anticipate risks [1]. The manifestation of this mechanism has been observed in paratroopers and military personnel during simulated operational maneuvers [8-10], helicopter pilots during combat maneuvers [2] aircraft personnel during operational flights [3], and tactical parachute jump [4]. In all of these cases, the soldiers' organisms adapt to face risk situations, and the psychophysiological stress response is modulated by the soldiers' experience [5–7].

In addition to the conditions of the military environment and the psychophysiological requirements, military equipment can also affect operative performance, decreasing speed of movements, increasing heart rate, body temperature, somatic anxiety, and subjective perception of effort [8]. Traditionally, to prepare soldiers for these requirements, military workouts have been based on long-distance and low-intensity training, contrary to the psychophysiological requirements evidenced by actual scenarios [11]. Previous studies suggest that high-intensity training may be more effective due to the characteristics of actual theaters of operation [9,12]. Also, researchers offer high-intensity training protocols as valid methods for improving sol-dier performance [9,10].

Previous studies have observed that high-intensity interval training (HIIT) produces a significant increase in the psychophysiological response of soldiers, like those observed in operative scenarios [11]. Additionally, a 6-week periodization of HIIT-based integral operative training has been shown to significantly improve combat performance [12]. In the same way, the benefits of HIIT training programs have been seen in other population groups where there is a highly stressful condition such as firefighters or police officers [13,14].

However, there is a lack of research on the actual effects of military equipment in the context of combat or HIIT training. Therefore, this study aimed to analyze the effects of military equipment on psychophysiological responses during a HIIT session to adjust future military training protocols. The initial hypothesis is that military equipment will produce a greater psychophysiological response in soldiers during the HIIT session.

MATERIALS AND METHODS

Participants

We analyzed 20 soldiers of the Spanish Army (aged 27.3 ± 3.4 years; height 176.6 ± 4.7 m; weight 76.8 ± 7.4 kg). All procedures were approved by the headquarters of the unit and explained to all volunteers before intervention. Volunteers provided voluntary written informed consent in accordance with the Declaration of Helsinki.

Procedure

All subjects performed a traditional running HIIT consisting of 12 repetitions of 30 seconds at maximum intensity, with a 30-second rest between exercises. The subjects completed the workout in two different conditions:

i. Without military equipment, wear a T-shirt, shorts, and sports shoes.

ii. Wearing full army gear, the army gear included boots, a helmet, a military uniform, and a combat backpack, resulting in a total weight of 20 kg carried by the military personnel during the intervention.

The participants were randomly divided into two groups, one group did the training under condition i and the other group under condition ii. After 48 hours, they repeated the training, and each group performed the training they did not do on the first day.

The psychophysiological response of participants was measured before and immediately after training intervention as previous researchers in the military population [15–17]. We analyzed the following parameters in this order before and after the HIIT:

1. Blood lactate by a 5µl blood sample in a finger in the Lactate Pro (Arkay, Inc. system, Kyoto, Japan) [18].

2. Rating of perceived exertion (RPE), 6–20 scale [19].

3. Stress subjective perception (SSP) 0 – 100 scale [20].

4. Legs strength by horizontal jump (HJ) test.

5. Upper muscular strength (UMS) by the maximum isometric contraction in the dominant hand in a dynamometer.

6. Skin temperature with a digital infrared thermometer (Temp Touch; Xilas Medical, San Antonio, TX).

7. Blood Oxygen saturation (BOS) and HR by a pulse oximeter (PO 30 Beurer Medical).

8. FVC manifestations by a digital spirometer QM-SP100 (Quirumed, Spain) [21].

9. Cortical activation with the Critical Flicker Fusion Threshold (CFFT), analyzed in a viewing chamber Lafayette Instrument Flicker Fusion Control Unit (Model 12,021), by the mean of five incremental flickering light perceptions from 20 to 100 Hz. Increases in CFFT are linked with a decrease in information processing ability and central nervous system fatigue [21,22].

The SPSS statistical package (version 21.0; SPSS, Inc. Chica-go, Illinois) was used to analyze the data. Descriptive statistics, including mean and standard deviation, were calculated for each variable. An independent samples T-test was used to analyze differences between pre- and post-session and between groups in each workout. The level of significance for all comparisons was set at $p \leq 0.05$.

RESULTS

Results are reported with their mean and standard deviation. Table 1 shows the psychophysiological values of military personnel pre and post-training with the two outfits analyzed. Significant differences were found in RPE, SSP, BOS, and Lactate in both groups.

Table 1: Psychophysiological response of military personnel with civil task and military task previous and post HIIT operative training

	Civil outfit						Military outfit					
	pre		post		T	P	pre		post		T	P
	M	SD	M	SD			M	SD	M	SD		
CFFT (Hz)	35.49	2.20	35.32	3.62	0.28	0.78	34.34	3.65	35.44	4.42	-1.70	0.11
SSP (0-100 scale)	14.05	16.12	41.79	32.25	-3.31	0.00	9.60	8.17	77.50	12.82	-22.43	0.00
RPE (6-20 scale)	6.00	0.00	14.00	3.45	10.11	0.00	6.80	0.83	17.15	1.46	-27.83	0.00
HJ (m)	1.69	0.19	1.71	0.15	-1.34	0.20	1.58	0.24	1.65	0.22	-3.43	0.00
IHS (N)	46.95	5.93	47.89	6.32	-1.30	0.21	46.60	7.01	50.55	6.39	-6.07	0.00
Temperature (ºC)	36.73	0.39	36.49	0.42	1.51	0.15	36.84	0.32	36.56	0.72	1.83	0.08
BOS (%)	97.37	1.01	96.47	1.54	2.45	0.03	97.00	1.12	94.65	2.28	3.98	0.00
HR (bmp)	71.53	15.97	146.84	11.43	15.81	0.00	62.75	12.74	155.65	9.87	-29.01	0.00
FEV1	3.94	0.61	4.07	0.43	-1.27	0.22	4.08	0.55	3.99	0.62	1.09	0.29
PEF(l/min)	11.04	1.59	11.24	1.87	-0.86	0.40	11.19	1.81	10.61	1.85	2.39	0.03
FVC (l)	4.68	0.64	4.65	0.70	0.21	0.84	4.88	0.74	4.81	0.76	0.82	0.42
Lactate (mmol/L)	3.79	2.78	19.41	5.45	10.35	0.00	3.70	1.50	12.95	5.68	-7.39	0.00

CFFT (Critical Flicker Fusion Threshold), Stress subjective perception (SSP), RPE (Rating of Perceived Exertion); BOS (Blood oxygen saturation): Horizontal Jump (HJ); IHS (Isometric hand strength); FVC (Forced Vital Capacity); FEV1 (Forced Expiratory Volume in 1 Second); PEF1 (Peak Expiratory Flow)

Also, it was observed significant differences in HJ, UMS, and PEF values when the group was equipped with military equipment.

Table 2 shows the intergroup comparison before and after training for each of the evaluated values. Significant differences were found in RPE pre-training values and RPE, Stress perception, BOS, HR, and lactate post-training values.

DISCUSSION

The objective of this study was to analyze the effect of military equipment on the psychophysiological response of the military during a traditional running HIIT. The initial hypothesis was fulfilled since military equipment produced a higher psychophysiological response during the HIIT session.

The cognitive system may be impacted during a HIIT session due to its high intensity, HIIT training results in high levels of perceived effort and stress [23]. Consistent with this, the data collected in this study revealed an increase in RPE and perceived stress levels before the intervention in both groups. Moreover, the values were greater in the group wearing military equipment. The RPE values of the military outfit group after training were higher than those observed in soldiers before an operative HIIT session [11]. Furthermore, the RPE values of the military outfit group were higher than those recorded by soldiers before the resistance and endurance HIIT [24], and higher than those observed by soldiers before a combat simulation [25]. This higher perception of effort could be due to the lack of experience and the higher physical requirements of this kind of training with military equipment. This effort in the cognitive system also is reflected by a high level of perception of stress. These values are higher than those observed in soldiers in rescue maneuvers, before underwater evacuation training [26], or during combat simulation maneuvers [27]. Therefore, it is emphasized that the absence of the experience produces a great cognitive impact.

The findings of the study indicate that the cognitive impact of HIIT training is considerably more intense when soldiers wear military equipment. This suggests that the additional physical and psychological stress of wearing the equipment during training can further challenge the cognitive system and potentially impact the soldier's performance and ability to respond effectively in actual combat situations. Therefore, it is crucial to consider these findings when designing training protocols for military personnel, to better prepare them for the demands of real-life situations [24,28,29].

Additionally, cardiovascular effects were observed due to the physiological demands of HIIT. Regarding HR values, both groups showed an increase after the training session, but HR levels were higher in the military outfit group. These HR values were even higher than those observed in ultra-endurance races [30] and were higher than those observed by soldiers before operative HIIT training [11]. However, the HR values were lower than those shown by the military group before a resistance and endurance HIIT [24]. Therefore, HR values were lower than those observed by the military during military asymmetrical and symmetrical combat maneuvers [29] probably due to the cognitive, psychological, and physical requirements.

Table 2: Comparison of the psychophysiological results between groups

	CIVIL OUTFIT		MILITARY OUTFIT				95% confidence interval for the difference	
	M	SD	M	SD	t	p	inf	sup
CCFT(Hz) pre	35.49	2.20	34.34	3.65	1.18	0.25	-0.82	3.11
CFFT (Hz) post	35.32	3.62	35.44	4.42	-0.10	0.92	-2.75	2.50
ssp pre	14.05	16.12	9.60	8.17	1.10	0.28	-3.77	12.68
SSP post	41.79	32.25	77.50	12.82	-4.59	0.00	-51.48	-19.94
RPE pre	6.00	0.00	6.80	0.83	-4.18	0.00	-1.19	-0.41
RPE post	14.00	3.45	17.15	1.46	-3.75	0.00	-4.85	-1.45
HJ (m) pre	1.69	0.19	1.58	0.24	1.58	0.12	-0.03	0.25
HJ (m) post	1.71	0.15	1.65	0.22	1.07	0.29	-0.06	0.19
IHS (N) pre	46.95	5.93	46.60	7.01	0.17	0.87	-3.88	4.57
IHS (N) post	47.89	6.32	50.55	6.39	-1.30	0.20	-6.78	1.47
Temperature (ºc) pre	36.73	0.39	36.84	0.32	-0.95	0.35	-0.34	0.12
Temperature (ºC) post	36.49	0.42	36.56	0.72	-0.34	0.73	-0.45	0.32
BOS (%) pre	97.37	1.01	97.00	1.12	1.07	0.29	-0.33	1.06
BOS (%) post	96.47	1.54	94.65	2.28	2.91	0.01	0.56	3.09
HR (bpm) pre	71.53	15.97	62.75	12.74	1.90	0.07	-0.57	18.13
HR (bpm) post	146.84	11.43	155.65	9.87	-2.58	0.01	-15.73	-1.89
FEV1 pre	3.94	0.61	4.07	0.43	-0.76	0.45	-0.52	0.23
FEV1 post	4.08	0.55	3.99	0.62	0.44	0.66	-0.27	0.43
PEF (l/min) pre	11.04	1.59	11.19	1.81	-0.27	0.79	-1.26	0.96
PEF (l/min) post	11.24	1.87	10.61	1.85	1.06	0.30	-0.58	1.84
FVC (l) pre	4.68	0.64	4.88	0.74	-0.89	0.38	-0.65	0.25
FVC (l) post	4.65	0.70	4.81	0.76	-0.68	0.50	-0.64	0.32
Lactate (mmol/L) pre	3.79	2.78	3.70	1.50	0.13	0.89	-1.34	1.53
Lactate (mmol/L) post	19.41	5.45	12.95	5.68	3.62	0.00	2.85	10.08

CFFT (Critical Flicker Fusion Threshold), Stress subjective perception (SSP), RPE (Rating of Perceived Exertion); BOS (Blood oxygen saturation): Horizontal Jump (HJ); IHS (Isometric hand strength); FVC (Forced Vital Capacity); FEV1 (Forced Expiratory Volume in 1 Second); PEF1 (Peak Expiratory Flow)

In line with this, the training resulted in a decrease in BOS values for both groups. However, the military outfit group showed a significant decrease in BOS values after the training. These values decreased to levels lower than those observed by soldiers before an operative HIIT [11]. The cardiorespiratory response is a consequence of the training-induced respiratory distress, and it was more pronounced when soldiers carried a heavier weight during the session. This respiratory distress is manifested through the PEF values where the military outfit group showed a significant decrease in PEF before training. These values were higher than those presented by soldiers before a HIIT resisted training [11]. However, no significant differences were observed when compared to the civilian outfit group.

Likewise, significant differences were observed between the groups at the metabolic level. Previous research has demonstrated that HIIT sessions lead to an increase in lactate production due to metabolic demands. Similarly, both groups exhibited an elevation in lactate levels immediately after completing the HIIT training. However, the group wearing military outfits had significantly lower lactate levels compared to the group wearing civilian outfits. The lactate levels in the military outfit group were comparable to those of soldiers before engaging in operational HIIT [9] and similar to those observed during firefighting operations [25]. In contrast, the civilian outfit group exhibited higher lactate values indicating a greater degree of metabolic acidosis following exercise. These values were similar to those observed in athletes before the HIIT session [31], therefore a good performance and physical level of the soldiers were observed.

The difference in lactate production between the two groups may be attributed to the mechanical effects caused by wearing military gear. The added mechanical load on the soldier hampers their ability to perform exercises at a higher speed.

This mechanical condition is also evident in the effects of HIIT on upper and lower body strength levels. The military outfit group demonstrated a significant increase in upper body strength levels post-training. This improvement is likely due to the muscular activation generated by the metabolic system. These values were similar to those exhibited by soldiers before engaging in resisted HIIT [20] and before a crane rescue maneuver performed by a helicopter crew [2]. Although these values were higher than those of the civilian outfit group, which could be attributed to the physical requirements influenced by military equipment, there were no significant differences between the groups.

In contrast, a significant decrease in lower body strength levels (HJ values) was observed in the military outfit group after the training. These values were lower than those observed by soldiers before resistance and endurance HIIT [20], lower than those observed by soldiers before operational HIIT [11], and lower than those shown by pilots after 30 and 35-minute jet maneuvers [20]. The results highlight a direct impact of wearing military gear on lower body performance. However, similar to the upper body findings, no significant differences were observed when compared to the civilian outfit group.

CONCLUSION

The use of military equipment during a high-intensity interval training session produces higher stress perception and perceived exertion than the civil outfit. Physiological response, heart rate, blood oxygen saturation, and lactate values were significantly higher with military equipment although the level of lactate was lower than observed with the civil outfit. The mechanical difficulty and the lack of operability in movements could be a reason for this psychophysiological response.

Practical application

The results showed the direct influence of military equipment on the soldier when performing submaximal performance. This will open new lines of research for designing training protocols with and without military equipment.

Limitations of the study and future research lines

The main limitation of this study is the low sample of participants. Also, it could be recommended the use of ECG to compare values as in previous research. In future research, it is suggested to validate PPG protocols with longer durations.

Conflicts of interest and sources of funding
The authors declare no conflict of interest. This research received no external funding.

Authors' contribution
Conceptualization, A.C.-R., J.F.L and A.B.-S.; methodology and formal analysis A.C.-R., and V.J.C.-S investigation, A.C.-R., J.F.L and V.J.C.-S.; data curation, V.J.C.-S.; Writing—Original draft preparation, , A.C.-R., A.B.-S and V.J.C.-S.; Writing—Review and Editing, A.C.-R., J.F.L and V.J.C.-S.; visualization, , A.C.-R., Aand V.J.C.-S.; supervision and funding acquisition, V.J.C.-S.

Ethics approval and consent to participate
Procedures and protocols were explained to all the subjects who gave the voluntary written information in accordance with the Declaration of Helsinki and approved by the Euro-Pean University Ethics Committee (CIPI/18/093).

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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REVIEW ARTICLE

Pathophysiological Association Between Helicobacter Pylori Infection and Colorectal Cancer - An Analysis of the Latest Studies

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Abstract: *Colorectal cancer is the third most common cancer worldwide and represents a significant major public health issue. Therefore, the interest in detecting the multiple factors implied in the development of cancer has increased recently. One of the factors could be the Helicobacter pylori infection, but a direct causal connection is missing. This gram-negative bacterium infects approximately 4 billion individuals globally and various conditions such as gastroduodenal ulcers or gastric adenocarcinomas can be induced by it. Some of the reported carcinogenic effects of Helicobacter pylori bacterium are hypergastrinemia, chronic inflammation, dysbiosis, and toxin production. Each of these elements involved in the pathogenesis has various mechanisms of action. Dysbiosis can occur due to various treatments for the eradication of the Helicobacter pylori infection. This bacterium also has a proinflammatory effect at the gastric level which can predispose to the development of dysplasia or even neoplasia. The toxins produced by this bacterium, such as the vacuolating cytotoxin A, promote inflammation. In this review, we discuss the possible connections between the Helicobacter pylori infection, through its diverse pathogenic mechanisms, and colorectal neoplasm. Prospective studies are necessary to determine whether there certainly is a direct link between the two conditions.*

Keywords: *colorectal cancer, Helicobacter pylori, dysbiosis, neoplasia, inflammation*

INTRODUCTION

Colorectal cancer (CRC) is the third most common type of cancer in the world with an increasing incidence and mortality rate, especially in developed countries [1].

Since the 1970s, infection has been considered a possible etiopathogenic factor of CRC. Numerous studies have been conducted on the intestinal microbiota and on the various bacterial species comprising it, which can become pathogenic. These studies have shown the role of inflammation and cytokines in the development of CRC, but without finding a significant association between CRC and the bacterial species analyzed [2-5].

After the 1990s, when Helicobacter pylori (H. pylori) was identified, numerous researches focused on its effects were carried out. H. pylori, a Gram-negative bacterium, is already known to be a risk factor for gastric cancer, but its role in CRC remains uncertain. Numerous studies have shown a significant association between the two, but the pathophysiologic mechanisms underlying the development of colorectal cancer still remain inaccurate [6].

The purpose of this article is to provide an overview of the latest studies on the evidence supporting the link between Helicobacter pylori infection and colorectal cancer.

MATERIALS AND METHODS

Here, we review the epidemiological evidence, potential biological mechanisms, and clinical implications related to this association.

A detailed search in the literature was conducted using PubMed.com and Elsevier.com platforms to identify relevant articles published in peer-reviewed logs. Search terms included "Helicobacter pylori", "colorectal cancer", "gastro-intestinal neoplasms", "epidemiology", "pathogenesis" and "mechanisms". We included original research articles, reviews, and meta-analyses that investigated the association between H. pylori infection and CRC. The selection criteria were based on the design of the study, relevance to the working hypothesis, and methodological quality, with only articles written in English being selected (Table 1).

DISCUSSIONS AND RESULTS

Helicobacter pylori is a bacterium that settles in the mucus layer lining the gastric epithelium, being present in about 50% of the population [7]. Infection is associated with several gastrointestinal conditions, such as chronic gastritis, peptic ulcers, or gastric cancer. Recent studies have suggested a possible link between H. pylori infection and the development of colorectal neoplasm, however, the mechanisms at the base remain uncertain.

Numerous epidemiological studies support the increased risk of developing CRC in the presence of the bacterium.

Table 1: Summary of scientific resources regarding the subject of the article

Author, year	Number of subjects	Significant results	Conclusions
Zumkeller N. et al - 2006 apr [8]	meta-analysis on 11 studies (1991-2002)		Summary odds ratio of 1.4 for the association between CRC and H. pylori infection
Ryoo SK. et al - 2020 [9]	3753		H. pylori infection has been associated with a high risk of advanced CRC development
Teimoorian F. et al - 2018 [10]	50	H. pylori infection (IgA > 20 U/mL, IgG > 10 U/mL) was significantly more prevalent among patients with RCC and adenomatous polyps	High risk of CRC
Shmuely H. et al - 2013 [11]	273	112 patients diagnosed with cancerous lesions (75% presented anti-H. pylori antibodies) 161 without cancerous lesions (48% presented antibodies) There was no association in patients who were CagA positive.	Uncertain association CagA+ seropositivity is associated with an increased risk of gastric and colorectal cancer
Zhao YS. et al [12]	meta-analysis on 13 studies - 2008		Possible involvement of H. pylori infection in the development of CRC

There are various hypotheses according to which Helicobacter pylori infection contributes to colorectal carcinogenesis, primarily altering the intestinal microbiota. A study conducted in 2018 showed that the bacteria that make up the intestinal microbiota in patients infected with H. pylori do not differ from those without infection, but differ in those who have gastric damage caused by this bacterium (intestinal metaplasia or gastritis) [13].

In the etiopathogenesis of CRC, several mechanisms have been proposed: hypergastrinemia, changes in the microbiota, chronic inflammation, and production of toxins by H. pylori.

Hipergastrinemia

Prospective studies have researched the potential connection between hypergastrinemia (increased serum gastrin levels) and the development of colorectal cancer. Gastrin is a peptide hormone secreted by G-cells in the gastric and duodenal antral mucosa and has an essential role in regulating acid secretion and gastric motility [14,15]. Increased levels of this hormone influence cell growth and differentiation, including cells of the colon mucosa [16,17]. Gastrin also promotes angiogenesis, a key factor in tumor growth and metastasis, amplifying the production of vascular endothelial growth factor in colon tumor cells [18,19,20]. Hypergastrinemia has also been associated with an increase in the number of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and the α factor of tumor necrosis (TNF-α), which can contribute to the development of CRC [21].

At the same time, it has been shown that epithelial cells of the gastric mucosa infected with H. pylori secrete TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), as well as other pro-inflammatory chemokines and modulating the immune response [20]. The synthesis of TNF-α by the host organism's cells is mediated by TNF-α inducing protein (Tipα). Tipα is a virulence factor secreted by H. pylori, which causes the synthesis of TNF-α and other proinflammatory cytokines [20], without having any counterpart among the other virulence factors of H. pylori.

Epithelial-mesenchymal transition (TEM) is a biological process that plays a significant role in tumor progression and metastasis. Gastrin has been shown to promote the TEM process of colorectal cancer cells by activating the TGF-β/Smad signaling pathway. This effect is associated with increased migration, invasion, and metastatic potential of colorectal cancer cells [22].

Modulation of the microbiota

The hydrochloric acid secreted by the parietal cells of the gastric mucosa has numerous digestive roles, but it also has an important bactericidal role, destroying the bacteria ingested with food, so that most of the small intestine is a sterile environment. With the hyposecretion of hydrochloric acid, the small intestine is colonized with bacteria and the colonic microbiota changes. Hyposecretion of parietal cells can be determined by atrophy of the oxyntic glands, as it happens in H. pylori-induced atrophic gastritis, as well as prolonged treatment with proton pump inhibitors as a therapeutic indication for the eradication of H. pylori infection [23]. The gut microbiome consists of 10^{13} to 10^{14} bacteria, fungi, protozoa, and viruses, as well as their genetic material. It is characteristic of each individual, depending on its exposure after birth to a wide variety of microorganisms. The microbiome has three important categories of roles: structural, protective, and metabolic [24,25]. Thus, it has an important role in absorbing some nutrients and minerals, synthesizing vitamins, amino acids, and enzymes and producing short-chain fatty acids. Also, the microbiota has an essential role in the processes of fermentation and putrefaction, with the production of acetate, propionate, and butyrate, essential for the health of the colon: it provides the necessary energy source for intestinal mucosal cells, increases the integrity of the mucous barrier, contributes to immunomodulation and protection against pathogens [24,26].

The change in the composition of the microbiome is called dysbiosis and can be caused by a multitude of dietary factors and changes in the environment. Dysbiosis is associated with the pathogenesis of a variety of neurological, gastrointestinal, and metabolic conditions, including colorectal cancer [27].

In a review published in 2012, Tatishchev SF et al suggest that modulation of the intestinal microbiota, increased cytokine production and increased secretion of gastrin induced by H. pylori infection are possibly involved in the occurrence of CRC.

The etiopathogenic role of H. pylori in colorectal carcinogenesis remains controversial [28,29].

Characteristics of microbial virulence factors may be included in routine sequencing for tumor mutation testing [30]. This approach could be useful both in the diagnosis and treatment of patients with solid colorectal tumors [30].

Chronic inflammation

Chronic inflammation of the mucosa is considered to be a predisposing factor for the development of CRC, similar to the mechanism of inflammatory bowel disease. Given the well-established proinflammatory and carcinogenic effect of H. pylori at the gastric level, a sequence of the type "chronic inflammation → dysplasia → neoplasia", similar to that for inflammatory bowel disease, can also occur in the colon, initiated by direct colonization of H. pylori [31].

Recently, the association between gastric infection with H. pylori and its presence in the intestine, with the development of colon adenomas, has been demonstrated by the mechanism of up-regulation of the expression of the TLR5 mucosal protein [32].

Cytokines are critical mediators of the immune response for maintaining health, but they have been shown to have important roles both anti-inflammatory and pro-inflammatory, and intervene in intestinal carcinogenesis. These include the cytokine families TNF, TGF-β, IL-6, IL-10, IL-17, IL-21, IL-22, IL-23. These cytokines modulate the intestinal microbiota, both in terms of their composition and secretions. Inflammatory cytokines also act on altered colon epithelial cells regulating their proliferation and vitality, thus acting as a connecting factor between the microbiota and CRC [33].

Toxin production

Some studies reported a significant association between the seropositivity of H. pylori and an increased risk of CRC [34]. H. pylori infection is based on the production of virulence factors, including the pathogenicity island CagA (cytotoxin-associated gene A), which encodes a type 4 secretory system (T4SS), VacA, and urease. They interact with the cells of the epithelium of the gastric mucosa, allowing colonization and pathogenicity of this bacterium [28].

CagA has been shown to induce proinflammatory responses, alter cell adhesion, and promote cell proliferation, which can contribute to the development of CRC [35]. Similarly, it has been suggested that vacuolating cytotoxin A (VacA) could play a role in the pathogenesis of CRC by inducing cell vacuolation, destroying tight junctions, and determining apoptosis [36].

In 2013, Shmuely H. et al published the results of a study in 273 patients. In patients infected with H. pylori, CagA+ seropositivity is associated with an increased risk of gastric and colorectal cancer [37].

Fujimori S. also shows that the risk of developing CRC is increased in individuals carrying vacuolytic cytotoxin A antibodies [38].

The potential association between H. pylori infection and CRC risk has important implications for the prevention of CRC and its early detection.

If a causal relationship is established, the eradication of H. pylori could be considered a preventive measure against the CRC.

In addition, screening among people prone to H. pylori infection may be beneficial for identifying the population at high risk for CRC.

CONCLUSIONS

The association between H. pylori infection and colorectal cancer remains an ongoing topic of research. While some studies have reported a significant association, others have shown conflicting results. Additional epidemiological and well-designed studies are needed to clarify the relationship and establish a causal link between H. pylori infection and CRC.

The pathophysiologic mechanisms of the possible association between H. pylori infection and colorectal cancer are complex. They are based on infection-induced hypergastrinemia and prolonged treatment with PPIs necessary for its eradication, cytotoxin production, dysbiosis, and associated chronic inflammation.

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ORIGINAL ARTICLE

The Effect of Mental Health Conditions on Dental Implants

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Abstract: *The use of dental implants as a treatment for replacing missing teeth has been increasingly prevalent. Historical evidence suggests that the Mayans were among the first to create implants around 600 AD. Over time, this technique has evolved, offering numerous benefits to humanity. Regardless of the causes of tooth loss and the patient's age, it significantly impacts various aspects of an individual's life, including nutrition, psycho-emotional well-being, and social interactions. One of the primary consequences is the negative effect on self-esteem due to the aesthetic concerns associated with missing teeth. Additionally, pronunciation difficulties can lead to communication challenges, reducing the desire and ability to engage with others. Extensive research in the specialized literature explores the psychological impact of dental implant usage. It is widely acknowledged that the dental implant technique is complex and carries inherent risks and potential adverse effects. An illustrative case serves to underscore the significance of psychological considerations in dental implant treatment. In this instance, a 55-year-old woman attempted suicide by throwing herself down the stairs of her home after receiving dental implants in both sections of her oral cavity. The distress she experienced stemmed from the inability to consume anything beyond fluids and semisolid food for an extended period of eight months. This alarming incident underscores the importance of recognizing and addressing the psychological impact that dental implant procedures can have on vulnerable individuals.*

Keywords: *dental implants, anxiety, depression, suicide*

INTRODUCTION

Oral health and mental health are closely linked, and there is evidence to suggest that individuals with dental pathologies may be at increased risk for developing psychiatric conditions [1,2,3].

The dental implant is a technique that is increasingly used as a treatment for replacing missing teeth [4,5]. There is evidence that the first implants were made by the Mayans around 600 AD, later this technique developed over time considering the benefits brought to humanity. It is even though the edentation and the age of the patient [6,7] bring with it, beyond the negative impact regarding the act of nutrition, a multitude of psycho-emotional problems, among which in the foreground is the decrease in self-esteem concerning the aesthetic aspect of the person and the decrease in the desire and ability to relate to other people due to the complications of correctly pronouncing words and thus the individual's ability to communicate. Regarding the impact of using the dental implant technique on the psyche, the specialized literature offers numerous studies on this topic [8.9]. The complexity of the dental implant technique is well-known, as well as its risks and adverse effects [10,11]. Weighing its benefits, in most situations the final decision is in favor of its use, important in this sense being the choice of the type of implant [12]. One of the major inconveniences refers to the long period in which patients are forced to resort to a liquid or semi-solid diet. This stage, which the patient is forced to go through, can trigger the appearance of mental disorders leading to anxiety and depression [13,14] and sometimes, in extreme cases but fortunately with reduced frequency, even to suicidal acts. Another aspect that constitutes a challenge for dentistry is represented by the phobia per se manifested by patients regarding the use of dental equipment [15,16] all the more so since the period of realization of such techniques stretches over several months. In this context, it is also worth mentioning the patient's personality type and, of course, his motivation, an aspect related to other individual and social variables (age, social status, sex, profession, etc.) or pre-existing psychiatric comorbidities [17,18].

Dental pathologies can have a significant impact on an individual's quality of life, including their physical health, social relationships, and self-esteem. The two-way association between oral and mental health is discussed, including how dental treatment can cause anxiety and phobia, and how psychiatric disorders can lead to dental disease. For example, individuals with untreated dental pain may experience feelings of anxiety and depression, which can affect their quality of life and overall mental well-being. About one-half of all dental patients experience some anxiety about their dental visits, and in some cases, this leads to dental phobia, a form of specific phobia [19]. Dental problems are causing stress to the patient and this can be intensified by the duration of the clinical intervention, an aspect that is significant in the case of the dental implant. A current study discusses the impact of mood states and psychological flexibility in stressful situations, in this context the dental implant, recognizing their influence on the patient's clinical satisfaction [20].

Also, some studies approved that adults have problems in eating solid food and not only, have pain and difficulties in their relationships because of oral disorders and there is a strong association between poor self-perceived oral health and quality of life [21,22]. Additionally, individuals with dental pathologies may be less likely to engage in social activities or seek treatment for their dental issues, which can increase their risk of developing psychiatric conditions and can be a real challenge for dentists [23]. A study performed in Britain found a significant correlation between dental anxiety and oral health-related quality of life, and individuals with high levels of dental anxiety were twice as likely to experience poor oral health-related quality of life. The study suggests that dental anxiety is an important factor in oral health-related quality of life and should be considered in clinical practice [24,25]. Dental anxiety is a public health concern that can lead to oral health problems due to delayed dental visits. More precise data is needed on the prevalence of dental fear-related problems to identify groups at risk. A multidisciplinary approach involving mental health and community dentistry professionals is necessary to break the vicious cycle of fear and anxiety. Research needs to focus on the antecedents of dental anxiety and on children, as dental fear usually starts in childhood and extends to adulthood. The Cognitive Vulnerability Model offers a promising framework to analyze the interplay between cognitive and psychosocial determinants of dental fear [26].

MATERIALS AND METHODS

Accessing the PubMed database, we identified a number of 116 articles published between 2012-2023 that studied the issue of mental health, the technique of dental implantology, as well as a series of approaches to the connection between the two subjects. The articles included in the study were original studies, case studies, clinical studies, or therapeutic trials that addressed the subject of the consequences of oral implantology on the mental health of individuals. Of these, we analyzed a number of 8 review articles.

We also present the case of a 55- years old woman who tried to commit suicide by throwing herself on the stairs of her house after having dental implants applied on both parts of her oral cavity she could not resist eating only fluids and semisolid food for 8 months. the patient's first presentation to the psychiatric service (3 months before suicide attempts) was due to a sad mood and increased anxiety following a dental treatment with the application of dental implants started approximately 8 months ago. The patient needs complex oral rehabilitation, presenting poor oral health. She had no elements of a psychiatric nature, at the first consultation in the dental office and she had partial bimaxillary edentation, partially restored with bridges. The patient was advised to eat pureed and as much liquid as possible until the healing of the oral lesions that occurred after the initiation of the dental treatment and the insertion of the dental implants. We mention that the patient had a history of 2 psychiatric consultations and 2 admissions for 7 days each We mention that the patient had a history of 2 psychiatric consultations and 2 admissions for 7 days each. After the insertion of the implants, no temporary prosthesis was used; this fact can be considered as one of the triggering factors of the pathology psychiatric. The evolution was favorable and he was discharged with the diagnosis of a moderate depressive episode, with an indication for treatment and to return for a periodical consultation. After the consultation with the dentist, the patient returned 3 weeks after the last hospitalization, to the emergency room, with a suicide attempt by throwing herself down the stairs of the attic because the dentist recommended an extension of the period to eat liquid and semi-solid, a fact that demoralized her completely. When admitted to the emergency room, the patient presented with aphasia, difficulty cooperating, and a possible fracture of the mandible, which was not confirmed by the oral surgeon. A right iliac fracture was diagnosed on the abdominal and pelvic computer tomography. During the hospitalization, the patient presented a stationary evolution in the first 14 days, the patient being uncooperative, with the absence of will and the refusal to accept oral medication, for which a nasogastric tube and a urinary tube were applied. After the partial remission of the symptoms in the second stage, the patient presented with ideas of persecution and guilt, but without autolytic ideation. The patient received psychiatric treatment with therapeutic benefit and resumed dental treatment after discharge.

RESULTS

Concerning the clinical case, the patient is now in a harmonious relationship with her husband, and her son, she continued her hobby related to swimming, winning numerous prizes in her age category, and from a dental viewpoint, the work was completed. Concerning the generic link between mental health and dental implants: there is a direct link between the favorable results of the use of the dental implant and the improvement of the mental status, especially in the elderly.

Concerning suicide in people who resort to implants for education, the studies took into account the implications related to the situations in which the failure of the implant technique took place. The aspect of the quality of life and the implications on the mental status during the implementation of the implant have not been studied. For this reason, the current approach presents an aspect of interest considering the large number of people who resort to the dental implant technique and the same extent, the large number of people with a history of psychiatric disorder in whom this period can represent a trigger in the psychiatric pathology.

We have chosen to present the case of a 55-year-old patient who presented herself in the emergency psychiatric service for a suicide attempt by throwing herself down the stairs from the attic of her house. The gesture occurred because of non-adaptation to the mandatory liquid and semi-solid diet established following the application of the dental implant; the procedure and lifestyle started 8 months before the event.

Heredo-collateral antecedents: father former ethanol user now deceased, a mother with hypertension, and an ischemic stroke in the antecedents.

Personal physiological antecedents: the patient has a 28-year-old son, and abortions = 10 up to the current age. Menopause from 51 years.

Personal pathological antecedents: autoimmune thyroiditis not treated with medication, appendectomy at 7 years old, tonsillectomy at 10 years old, 2 psychiatric consultations, and 2 admissions for 7 days each.

Living and working conditions: the patient has a higher education, is married, is in a harmonious relationship with her husband and son, has a satisfactory social life, and is a top swimmer in her age category with awards won.

She does not currently smoke but has smoked approximately 1 pack of cigarettes/day for 20 years, and does not consume alcohol or psychostimulant substances.

Treatments administered before admission: multiple antidepressants, anxiolytics, and hypnotics.

History of the disease: the patient's first presentation to the psychiatric service (3 months before suicide attempts) was due to a sad mood and increased anxiety following a dental treatment with the application of dental implants started approximately 8 months ago.

At the first consultation in the dental office, the patient presented for complex oral rehabilitation, presenting poor oral health, but without elements of a psychiatric nature. The patient had partial bimaxillary edentation, partially restored with bridges (Figures 1 and 2).

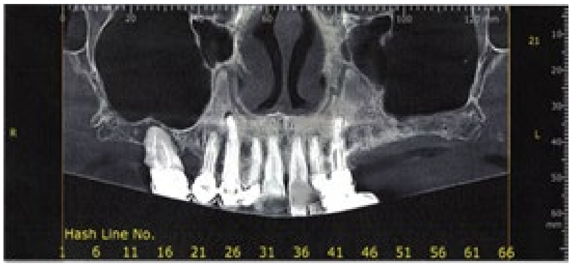

Figure 1: Upper arch before implant placement

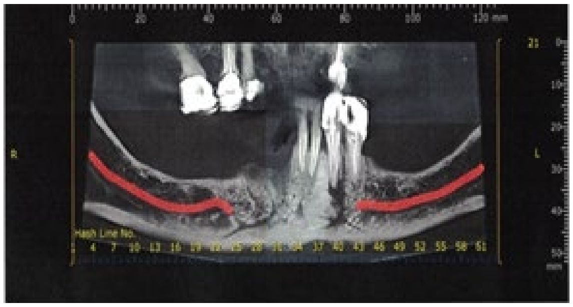

Figure 2: Lower arch before implant placement

The patient was advised to eat pureed and as much liquid as possible until the healing of the oral lesions that occurred after the initiation of the dental treatment and the insertion of the dental implants. Through the treatment plan for complete and complex oral rehabilitation, it was decided to insert implants on both arches, for the upper arch in quadrant 2 and for the lower arch in quadrants 3 and 4, all being inserted at a distance of 2 weeks between, the first being inserted on the upper arch. Bone augmentation was also necessary for the lower arch, following the marked atrophy of the ridge that remained edentulous a long time before. Prior to the procedure of applying the dental implants, the remaining teeth were extracted from the lower arch and during the ablation of the crowns of the teeth restored with bridges from quadrant 2. After the insertion of the implants, no temporary prosthesis was used; this fact can be considered as one of the triggering factors of the pathology psychiatric (Figure 3).

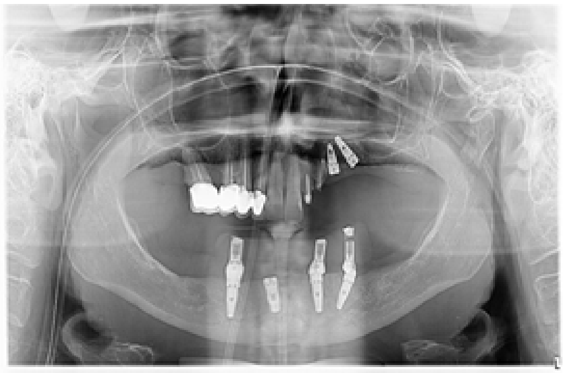

Figure 3: Xray control after implants' placement

The second day after presenting to the first psychiatric consultation, she requested admission to the hospital's psychiatric department. She is given specialized treatment (Sertralinum 50 mg, Bromazepamum 3 mg) and is discharged after 7 days. After another 4 days, she returns to the emergency room for insomnia and is recommended Zopiclonum 10 mg. The second hospitalization is carried out 3 weeks after the last consultation and the patient's treatment is changed to double therapy with an antidepressant (paroxetine-mirtazapine), anxiolytic (lorazepam), and hypnoinducer (cinolazepam). The evolution was favorable and he was discharged with the diagnosis of a moderate depressive episode, with an indication for treatment and to return for a periodical consultation. After the consultation with the dentist, the patient returned 3 weeks after the last hospitalization, to the emergency room, with a suicide attempt by throwing herself down the stairs of the attic because the dentist recommended an extension of the period to eat liquid and semi-solid, a fact that demoralized her completely. When admitted to the emergency room, the patient presented with aphasia, difficulty cooperating, and a possible fracture of the mandible, which was not confirmed by the oral surgeon. A right iliac fracture was diagnosed on the abdominal and pelvic computer tomography, for which he was administered Nadroparinum 3800 UI/0.4ml IM and physical therapy was performed.

During the hospitalization, the patient presented a stationary evolution in the first 14 days, the patient being uncooperative, with the absence of will and the refusal to accept oral medication, for which a nasogastric tube and a urinary tube were applied. After the partial remission of the symptoms in the second stage, the patient presented with ideas of persecution and guilt, but without autolytic ideation.

At the psychological examination carried out 14 days after admission, the patient was calm, present in the environment, cooperative, flow, and slow talking rhythm with ideas of incapacity and major concerns regarding the existing organic affections. Severe depressive mood with mixed hypnotic disorders, food refusal, apathy, delusional ideation of helplessness, and social withdrawal. Voluntary hypoprosexia, constricted affect. Reduced efficiency. Accentuated low level of obsessive-compulsive personality, HRSD=38, GAFS=50.

At the psychological examination performed one month after the first one, the patient was cooperative, optimistic, and reflective regarding the "accident" suffered a few weeks ago. Clear and flexible communication, sufficiently logical thinking, has realistic goals, show slight concerns regarding dental treatment. Depressive-anxious configuration (light intensity). HRSD=11, HRSA=10, GAFS=75.

During hospitalization, the patient initially received hydrating, vitaminizing, hepatoprotective, and psychiatric infusion treatment with atypical neuroleptic (aripiprazole), antidepressant (mirtazapine), anxiolytic (lorazepam), hypnoinductor (cinolazepam) and trihexyphenidyl.

After the psychiatric improvement and discharge, the patient returned to the dental office to complete the dental treatments. The end of the dental treatment consisted of a bridge in quadrant 1 on the teeth still present in the arch, in quadrant 2, a crown on the lateral incisor and a bridge on the 2 implants, and for the lower arch, there was a bridge on implants having a distal extension in quadrant 3 and two distal extensions in quadrant 4.

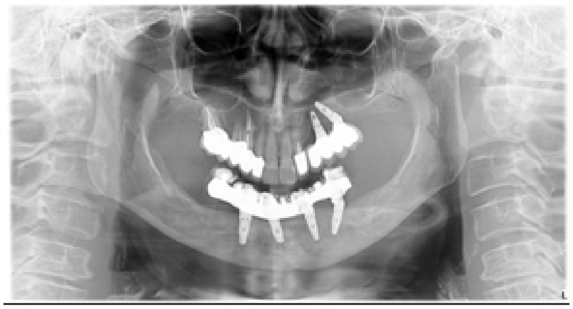

Figure 4: Xray control after finishing the full mouth rehabilitation process

From a psychiatric perspective, the patient was followed clinically with consultation for a period of approximately 2 years, initially with monthly then with quarterly consultations. The treatment was not changed during the plateau period, there were no relapses, and at the time of complete treatment elimination, the patient did not return to the psychiatric service. Currently, the patient is in a harmonious relationship with her husband, and her son, she continued her hobby related to swimming, winning numerous prizes in her age category, and from a dental viewpoint, the work was successfully completed.

While dental implants can improve one's self-esteem and confidence, some individuals may experience feelings of depression related to the surgical procedure or recovery process. This can be caused by a variety of factors, such as physical pain, swelling, and the length of time required for recovery. Additionally, the cost of dental implants can also be a financial burden, which can lead to stress and depression.

Anxiety can have a significant impact on the success of dental implants. Anxious individuals may have difficulty following the necessary steps for successful implantation, such as proper oral hygiene and regular dental visits. They may also experience difficulty in following a healthy diet, which can impact the healing process and overall success of the dental implants.

Depression can have a significant impact on the success of dental implants. Individuals who are depressed may have difficulty following the necessary steps for successful implantation, such as proper oral hygiene and regular dental visits. They may also experience difficulty in following a healthy diet, which can impact the healing process and overall success of the dental implants.

A proof in this sense is represented by the fact that a mechanism involved in the triggering of depression, namely, the recapture of serotonin is also at the basis of the failure of the oral implant through the anabolic effect on the bone reducing the bone turnover, a fact that can explain the reduced production of the alveolar bone.

From the perspective of psychiatric conditions with strict reference to the behavioral factor, we mention the role of factors such as dental hygiene or certain practices such as smoking, which have an impact on dental health.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of “Elisabeta Doamna” Clinical Psychiatry Hospital (02/18.05.2021) for studies involving humans.

DISCUSSION

In some cases, depression can be a pre-existing condition that is exacerbated by the stress and anxiety of undergoing a dental implant procedure. This is why individuals must have a thorough evaluation of their mental and emotional health before undergoing any dental procedure because it is a great challenge for a dentist to treat a patient with psychiatric problems [23,27]. Some studies show that psychiatric and genetic diseases do not represent an impediment to dental implant application, but multiple complicating factors can reduce implant survival [28,29]. Studies on phobic patients are totally satisfied with implant rehabilitation under general anesthesia, which means that this treatment can be considered a treatment of choice giving these patients the same opportunity as others to improve their oral health and well-being [27,30] but there are no studies about the clinical psychological impact that dental implants have on one’s mental health.

A study in Hong Kong investigated the relationship between dental anxiety, oral health status, and oral health-related quality of life (OHQoL) in 1,000 predominantly Chinese residents aged 25-64. Results showed that those with high dental anxiety had a higher risk of poor dental and periodontal status and lower OHQoL. The study suggests that dental anxiety is a significant risk indicator and should be considered in oral health assessments [31]. The study of Kanaffa-Kilijanska et al. assessed oral health and hygiene habits in relation to dental anxiety among 117 adult patients. The results showed that higher levels of dental anxiety were associated with a higher calculus index and a lower number of filled teeth. There was no significant correlation between dental anxiety and age, gender, or education level, but smokers had a significantly higher anxiety level (our patient smoked for about 20 years). The study concluded that dental anxiety has a negative impact on oral health and can lower the quality of life of the patient [32], data that were also confirmed by a 2015 national Swedish cross-sectional survey [33].

Furthermore, individuals who experience significant anxiety related to dental procedures may be less likely to seek dental care, which can lead to further dental problems and a lower quality of life, and patients suffering from mental illnesses are more vulnerable to dental neglect and poor health [34]. Not only in Lalabonova’s studies, there are strong correlation between the level of anxiety and dental implants proving that only those with low levels of dental anxiety decide to proceed with such a treatment [35,36,37].

Dental fear can cause a "vicious cycle dynamic" of avoiding treatment, which worsens oral health. Research on the antecedents of dental anxiety could help design community programs to prevent dental fear. Public mental health and community dentistry can benefit from a multidisciplinary approach to dental fear and oral health [26]. Clinicians should integrate interdisciplinary knowledge, and carefully evaluate the risks and benefits of dental implant treatment [38]. The association between markers of dental health and depression at the population level was observed in a 2014 study and the researchers found a clear association between poor dental health and depression, independent of inflammatory markers. Although inflammation was not found to influence the relationship, other factors such as nutritional deficits, lifestyle, comorbidities [39], and certain antidepressant medications could explain the association. This study recommends that prospective cohort studies should be conducted to determine the direction of the relationship between dental health and depression and explore its underlying physiology. Such studies could identify modifiable risk factors for depression.

A key limitation was that most data used were derived from self-report instruments over different recall periods. Future studies investigating this relationship should include an objective measure of dental health and diagnostic interviews to assess depression. The findings suggest that oral health could be a potential key determinant of population health and that educational programs designed to improve oral health could be considered a primary prevention strategy [40,41,42].

In a short communication published in 2016, Ehrenthal et al conducted a pilot study on 310 patients with periodontitis and determined that the number of teeth at the beginning of treatment was predictive of depressive symptoms 13 years later. The fewer teeth a patient had, the higher their level of depression. The study also found that the number of comorbid somatic diseases was related to depression.

Tooth loss may impact depression through psychological, social, and biological pathways, and low self-esteem is a predictor of depression. While no association was found between the predefined variables and current levels of anxiety, depression may be more harmful to treatment success than anxiety, but anxiety and dental fear can be a factor for not having long-term dental procedures [43]. In De Gomez et al., study the correlation between state anxiety and depression, and objective and subjective pain at day 7 was not statistically significant. A strong correlation was found between objective and subjective pain during the immediate postoperative period. In the postoperative period immediately following dental implant insertion, a strong correlation exists between subjective and objective pain [44].

Moreover, informing patients about the anticipated surgical procedure of implant placement and the postsurgical pain intensity can reduce their anxiety level and subsequent postsurgical pain and discomfort. Patients can be informed that, in general, implant placement surgical experience is less unpleasant compared to tooth extraction with less postsurgical pain and limitation of daily activities. However, some factors can increase the pain intensity and discomfort level on an individual basis [45], statements that are contrary to the symptomatology of our patient who came to the psychiatry service after 8 months of psychical „pain" because there was no physical pain that she endured, there was all about the consistency of the food. During the dental implant process, there are some modifications concerning the alimentation. There are some eating troubles because patients cannot always adapt to the new consistency of the food so there also may exist some alimentary deficits that produce some deficits in our brain synthesis of tryptophane and serum serotonin that lead to depression, anxiety, and insomnia [46,47,48].

Also, Oral Health-Related Quality of Life should be a key consideration for global oral health programs, especially with the projected shortage of dentists, underserved populations, and the importance of interdisciplinary collaborations [49]. The same problems can be found in people with different chronic comorbidities such as diabetes [39]. Oral health-related quality-of-life tools may be socio-dental indicators, global self-ratings of oral health, and multiple-item questionnaires as Baiju et al., 2017 suggest [50]. Recent medical research indicated that oral health significantly impacts the quality of life. Studies on total prosthesis users show that those with implants have better quality of life and satisfaction. Higher satisfaction with oral health correlated with a better quality of life [51]. There is limited evidence to suggest a link between dental implants and suicide. While some individuals may experience a boost in self-esteem and confidence after getting dental implants, others may experience temporary feelings of depression related to the surgical procedure or recovery process. However, suicide is a complex issue that is typically caused by a combination of genetic, environmental, and psychological factors. Dental implants are unlikely to be the sole cause of suicide in any individual.

Comparison with literature

Oral health and mental health are closely linked, and there is evidence to suggest that individuals with dental pathologies may be at increased risk for developing psychiatric conditions.

Dental pathologies can have a significant impact on an individual's quality of life, including their physical health, social relationships, and self-esteem. The two-way association between oral and mental health is discussed, including how dental treatment can cause anxiety and phobia, and how psychiatric disorders can lead to dental disease.

Analyzing the literature and reviewing the articles published in the period 2012-2023, having as a topic of study the link between mental health and dental implantology technique, with different approaches we observed: if initially the topic was focused on technical problems of implantology [52,53,54, 55,56] the articles from the last years of the mentioned period for which I made the review, bring into discussion subtle physiopathological aspects of the relationship between mental disorders and oral implants [57,58,59]. Regarding the importance of the subject from a practical point of view, we can say that there is a relatively small number of articles related to this subject. This is the reason for writing this article. Thus, this subject is approached from the perspective of the holobiome, taking into account the fact that currently, on the one hand, the etiopathogenesis of psychiatric disorders is explained through the connection between the intestinal microbiome and the central nervous system (gut-brain axis), and on the other hand, it is sought evidence about the involvement of the oral microbiome, part of the microbiome in the digestive tract, in oral pathology. The link between mental pathology and oral pathology can be explained for now by the behavioral changes of people with psychiatric disorders [60] but there is evidence that demonstrates the existence of complex mechanisms at the level of chemical mediators [61,62].

Regarding the relationship between the oral implant and psychiatric pathology, a series of factors related to the type of personality or individual reactivity can be discussed on one hand, and on the other, factors directly related to the technique per se [13,63] (e.g. the long period of time required by the technique, interference with the patient's feeding possibilities, the need to attend a large number of consultations in the dental office, complications) [64,65] as well as related aspects related to costs high that can represent an obstacle for patients and implicitly a stress factor as well as a generator of psychiatric disorders. Worldwide, people search for improvement in the physical aspect not only in the dental field; non-surgical lifting has gained more ground because it requires little recovery time and the results are very positive [66].

Discussions regarding the connection between the implant logical technique sometimes related to certain particular individual anatomical peculiarities [67,68] and pre-existing psychiatric disorders or triggered by the stress of the procedure [69] are highlighted both by the articles that were the subject of the mentioned review and by the study carried out by us. In this context, it is important to emphasize the aspect of choosing the procedure in the field of implantology so that the duration of its realization is as short as possible, as well as the aspects related to the knowledge of the patient's medical history, especially that of the existence of psychiatric conditions [70] but also of behavioral habits, of financial resources and tolerance to dental interventions. The patient's motivation regarding the need for intervention from the perspective of functional [71] and aesthetic aspects related to self-esteem and social interactions represents the nucleus around which the intervention strategy in the dental sphere is built and the intervention plan regarding mental disorders. The need for studies in this field is obvious, considering the frequency of both categories of diseases as well as the implications in the economy of health systems due to the costs and consequences in the immediate and distant term such as causes of dental implant failure and the means of prevention these causes [72]. Also, anxiety disorders present a significant medical and societal concern, demanding thorough assessment and timely intervention. The COVID-19 pandemic exacerbated chronic dental disorders due to the enforced isolation and heightened fear of contracting the disease [73, 74]. Additionally, individuals with pre-existing dental pathology may experience a compounding effect on their anxiety levels, as oral health concerns contribute to overall well-being and can exacerbate existing mental health conditions [48,73].

CONCLUSIONS

By understanding the relationship between dental implants and anxiety, we can help individuals receive the dental care they need and ensure successful outcomes for those undergoing dental implant procedures.

In conclusion, while dental implants can improve one's self-esteem and confidence, it is important to recognize the potential for temporary feelings of anxiety and depression related to the surgical procedure or recovery process. Individuals who are experiencing symptoms of depression should speak with a mental health professional. By understanding the connection between dental implants and depression, we can help ensure successful outcomes for those undergoing dental implant procedures.

The relationship between dental implants and suicide is still not well understood, and more research is needed to fully understand the potential causes and impacts.

Oral health and mental health are closely linked, and there is evidence to suggest that individuals with dental pathologies may be at increased risk for developing psychiatric conditions. The relationship between dental pathologies and psychiatric pathologies is complex and multi-faceted, and it is important to address both oral and mental health in order to improve overall well-being.

Highlights:

1. Anxiety and depression scales should be applied to all patients before applying the implants

2. A study should be expanded regarding potential psychiatric pathologies triggered by pain and the new diet established for patients requiring dental implants.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, M.M. and L.A.M.; methodology, M.M and L.A.M.; software, C.C..; validation, M.M, and H.R; formal analysis, C.B.; investigation, M.M and L.A.M; data curation, M.N.M.; writing—original draft preparation, L.A.M and C.C.; writing—review and editing, visualization, R.H.; supervision, M.N.M and R.H. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of "Elisabeta Doamna" Clinical Psychiatry Hospital (02/18.05.2021) for studies involving humans, this study being part of the doctoral study of dr Moroianu Marius.

Patient consent for publication

Written informed consent has been obtained from the patient(s) to publish this paper.

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ORIGINAL ARTICLE

Influence of Combat Experience on Psychophysiological and Fine Motor Skill Responses in Air Force Warfighters under Acute Stress

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Abstract: *This study investigates the influence of experience on stress responses and fine motor skills among professional male military parachutists. We analyzed 132 paratroopers, divided into a non-experienced group (NEG; ≤6 parachute jumps) and an experienced group (EG; >50 jumps). Baseline samples were collected 2.5 hours before participants performed a 500 m jump, with post-jump evaluations conducted immediately after landing. Results indicated that experience significantly influences fine motor skills and stress responses. Non-experienced jumpers showed greater degradation in fine motor skills and higher cortical arousal pre-jump levels, suggesting an increased anticipatory state. In contrast, experienced parachutists maintained more consistent fine motor skills and exhibited a ceiling effect in stress response. Leg strength was higher in non-experienced jumpers, reflecting youth and greater muscle mass, with no significant difference in hand strength. These findings highlight the role of experience in managing stress and maintaining motor skills under pressure. The study suggests further exploration into psychological aspects like motivation and resilience, along with the long-term effects of stress exposure. The importance of experience in high-stress military operations emphasizes the need for targeted training interventions to enhance stress-coping mechanisms in less experienced individuals.*

Keywords: *military parachutists; experience; stress response; fine motor skills; psychophysiological analysis*

INTRODUCTION

Fine motor skills are critical for various professionals, including athletes, and are notably influenced by an array of stressors, both psychological and physical [1]. Specifically, acute fatigue resulting from physical exertion can significantly impair these skills [1]. The impact of fatigue is modulated by several factors such as exhaustion, intensity, duration, and type of exercise, as well as the individual's experience level [2]. Traditionally, physiological explanations for the decline in fine motor skills due to fatigue have been focused on biochemical changes, such as the accumulation of hydrogen ions, inorganic phosphate, or ammonia [3]. These alterations adversely affect muscle contractility, hindering ATP-producing enzymes, disrupting the reuptake of calcium ions (Ca^{2+}), and leading to increased sarcoplasmic calcium concentrations [4]. Consequently, these physiological shifts can detrimentally affect the quality of neural connections and electrical activity, ultimately impairing fine motor coordination [3].

Supporting this concept, prior research has revealed notable impacts of physical exertion on motor and cognitive functions [5]. For example, soccer players exhibited a decrease in passing accuracy following a session of intense lower-body resistance training [3]. Similarly, cognitive functions like attention and reaction time were significantly impaired in individuals cycling at 70-100% of their maximum heart rate on an ergometer [6]. These findings underscore the complex interplay between physical activity, physiological stress, and fine motor skill performance, further elucidating the importance of understanding how different variables like exercise type, intensity, and individual training background influence these outcomes [7].

Further on, stress is a pivotal factor that can significantly affect fine motor skills, particularly in high-pressure environments such as the military [8]. The intense demands of military tasks often lead to brain overstimulation as soldiers must allocate extensive attentional resources to their surroundings and activities [9]. This overstimulation can result in concentration difficulties and distractions, shifting focus away from primary tasks and disrupting automatic motor execution [10]. Additionally, anxiety, a common response to the high-stress situations often encountered in military settings, can lead to reduced blood flow to the prefrontal cortex [2]. This reduction means less oxygen and nutrients reach the brain, thereby diminishing the effectiveness of executive functions [11]. Such impairments can negatively influence various brain regions, including the hippocampus, leading to issues with concentration, memory, decision-making, working memory, and ultimately, fine motor skills [11].

Moreover, in a military context, where decisions and actions often have critical consequences, the impact of stress and anxiety on fine motor skills becomes even more significant. Therefore, authors have noted that extreme social pressure, such as pre-competitive anxiety in military exercises or operations, exacerbates these effects [2,12-17]. On this line, both somatic and cognitive anxiety, often heightened in combat or high-intensity training scenarios, can impair motor skills. Furthermore, environmental factors typical in military settings, like exposure to loud noises exceeding 89 dB, can also contribute to the decline in fine motor skills [18]. These factors, combined with the need for precise and coordinated movements in various military operations, underline the importance of understanding and mitigating the effects of stress and anxiety on motor performance in military personnel [18]. Addressing these challenges through training, mental health support, and adaptive strategies can enhance the overall effectiveness and safety of soldiers in demanding operational environments [19].

In addition, the psychophysiological stress response and its modulation by experience is a critical area of study, particularly relevant in high-stakes environments such as medicine, the military, and sports. It has been demonstrated that experience plays a pivotal role in this modulation [20]. For example, studies show that the stress response of novice paratroopers after a low-altitude jump is significantly more pronounced compared to their experienced counterparts [21]. This difference highlights how experience directly influences the psychophysiological reaction to stress, an essential consideration for learning and performing under pressure [21-23]. The presence of stress hormones like cortisol, glucocorticoids, and corticotropin can impair the central nervous system and higher cognitive processes, affecting overall performance [22,23]. Skilled and experienced individuals, therefore, might exhibit optimal motor performance due to a more selectively tuned stress response, coupled with potentially enhanced cognitive automation and executive function efficiency under stress [21,24,25].

However, the intricate relationship between experience in stressful contexts and fine motor skills remains not fully elucidated. This uncertainty underscores the objective of our current research, which is to analyze the impact of experience on the psychophysiological response and fine motor skills under intense stress. We hypothesize that: i) non-experienced participants will show a more substantial negative effect on fine motor skills compared to their experienced counterparts, and ii) higher cortical arousal states and self-confidence, along with lower levels of cognitive and somatic anxiety, will be positively correlated with enhanced fine motor skills. This investigation aims to provide a deeper insight into how experience shapes our physiological and psychological responses in stress-filled scenarios, offering critical implications for training and performance optimization in various high-pressure fields.

MATERIALS AND METHODS

Participants

In this study, we conducted a comprehensive analysis of 132 male professional paratroopers, meticulously categorized into two distinct cohorts: i) the Non-Experienced Group (NEG), comprising younger members (mean age: 23.2 ± 2.3 years, ≤ 6 parachute jumps) primarily recruited from the first-year cohort of the air academy; and ii) the Experienced Group (EG), consisting of significantly seasoned practitioners (mean age: 34.8 ± 4.0 years, > 50 parachute jumps), each boasting over five years of dedicated service within the unit. We applied stringent exclusion criteria to both groups, disqualifying individuals presenting any medical or physical conditions potentially hindering participation or firearms usage, such as cardiovascular diseases, as well as those currently using dietary supplements, stimulants, or any other ergogenic aids.

Participants from both groups were uniformly outfitted in standard-issue military attire, which included boots, helmets, tactical fighting load carriers, body armor, and an HK G-36 rifle. Additionally, each individual was equipped with regulatory emergency parachutes for the execution of the jump. The total gear weight amounted to approximately 39.3 kilograms. Before their involvement, detailed explanations of the experimental procedures were provided to all participants, from whom voluntary written informed consent was subsequently obtained.

It is crucial to note that the entire study adhered rigorously to the ethical standards outlined in the Helsinki Declaration (as revised in Brazil, 2013). The research protocol received the necessary approval from the University's ethical committee, ensuring the highest standards of research integrity and participant welfare.

Procedure

To comprehensively address the objectives of our study, we conducted a detailed investigation into the physiological and psychological responses of both non-experienced and experienced paratroopers before and after performing a parachute jump.

Baseline data were meticulously collected 2 hours and 30 minutes before the embarkation, ensuring a robust understanding of the participants' initial state.

The participants boarded a Hercules T-21 aircraft for the jump, which was executed from an altitude of 500 meters. In this structured activity, the parachute deployment was automatic, triggered immediately upon exiting the aircraft. This process ensured a standardized jumping experience for all participants, crucial for the reliability of our data.

Following the descent and immediate landing, participants were swiftly escorted to a specially equipped field laboratory situated near the landing site. This arrangement was critical to capture the immediate post-jump physiological and psychological responses with minimal delay or external influence, thereby preserving the integrity and immediacy of the data.

In the field laboratory, a comprehensive post-jump evaluation was conducted, encompassing a series of assessments designed to measure the effects of the jump on various fine motor skills, cognitive functions, and stress responses. This timely assessment was vital to understanding the acute impact of parachuting on these key variables in both inexperienced and seasoned military personnel.

Materials

Before and after the automatic parachute jump the following parameters were analyzed according to previous authors [21]:

Psychophysiological parameters

Lower body muscular strength manifestation using a vertical jump test. We used the Sensorize FreePowerJump system (SANRO Electromedicina, Madrid. Spain) which recorded flight time (s) and jump height (cm) to evaluate the Squat Jump (SJ). Three attempts were recorded, and only the best attempt was considered.

Isometric hand-grip strength

It was measured using a TKK 5402 dynamometer (Takei Scientific Instruments CO. LTD)

Each participant's grip strength was measured on the shooting hand while standing with 0 degrees of shoulder flexion, 90 degrees of elbow flexion, and the forearm in neutral. Idem, was done pre-post and among series.

Cortical arousal by the critical flicker fusion threshold (CFFT)

An increase in CFFT suggests an increase in cortical arousal and information process, by contrast, when the values fall below the baseline, it suggests a reduction in the efficiency of processing information and fatigue of the central nervous system [26]. Subjects were seated in front of a viewing chamber (Lafayette Instrument Flicker Fusion Control Unit Model 12021), which was constructed to control extraneous factors that might distort CFFT values. Two light-emitting diodes (58 cd/m^2) were presented simultaneously in the viewing chamber, one for the left eye and one for the right eye. The stimuli were separated by 2.75 cm (center to center) with a stimulus-to-eye distance of 15 cm and a viewing angle of 1.9°. The inside of the viewing chamber is painted flat black to minimize reflection. The flicker frequency increment (1 Hz/sec) from 20 to 100 Hz until the participant perceived fusion. After a fovea binocular fixation, participants were required to respond by pressing a button upon identifying the visual fusion thresholds. Before the experiment, they performed as many practice trials as needed to become familiarized with the exigencies of the CFF test. Then, five trials were performed with an interval of 5 seconds. In each one of the tests, time was quantified as the amount of time that a subject took to detect the changes in the lights from the beginning of the test until the moment of pressing a button. The average of these times was calculated to determine the CFFT [15,27,28]

Cognitive Anxiety (CA), Self Confidence (SC), and Somatic Anxiety (SA)

Those parameters were evaluated using the CSAI-2R [29]. In this questionnaire, 17 items are evaluated: 5 items for CA, 7 items for SA, and 5 items for SC, respectively. The response scale evaluated the intensity of each symptom on a scale from 1 (not at all) to 4 (very much). Higher scores on cognitive and somatic anxiety indicated a higher level of anxiety, as well as higher scores on the self-confidence scale, indicated a higher level of self-confidence.

Fine motor skills were evaluated through the time of re-loading a 15 bullet of 9 mm caliber into a Beretta pistol magazine since previous authors used the same protocol to analyze specific fine motor skills for the population used in the present research [30–32].

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Ill., USA). Means and SDs were calculated using traditional statistical techniques. Normality was tested with the Kolmogorov-Smirnov test. A one-factor ANOVA for intergroup comparisons (i.e. pre and post-test differences; change score (Δ) differences) and a Student's T-test for intragroup comparisons were conducted. The Effect Size was tested by Cohen's D. Pearson correlation coefficient was calculated, and separate multiple regression analysis using the Forward method was performed to determine the presence of predictor variables on the cortical arousal and the fine motor performance. In all these analyses, the significance level was set at p≤0.05.

RESULTS

The analysis of psychophysiological and fine motor skills in non-experienced and experienced paratroopers before and after a jump revealed some key insights (Table 1). Both groups demonstrated significant improvements in Squat Jump performance, indicating enhanced lower-body muscular strength, likely influenced by the acute stress or adrenaline rush associated with the jump. Notably, this improvement was more pronounced in non-experienced jumpers. In terms of Isometric Hand Strength, there was a stable performance in the non-experienced group, while the experienced group showed a slight but significant decrease post-jump. This outcome might suggest a more nuanced impact of experience on the regulation of grip strength under stress. Critical Flicker Fusion Threshold results indicated an increase in both groups, with the experienced group showing a significant improvement. This finding implies an enhanced capacity for cortical arousal and information processing, especially among the experienced paratroopers.

Regarding fine motor skills, measured through Pistol Magazine Reload Time, there was a significant improvement in the non-experienced group but no change in the experienced group. This difference highlights the possible influence of prior experience on maintaining consistent performance under stress.

Cognitive and Somatic Anxiety levels decreased in both groups, with a notable reduction in physical symptoms of anxiety post-jump across both groups. Interestingly, while Self-Confidence remained unchanged in non-experienced paratroopers, it significantly increased among the experienced ones after the jump, suggesting a boost in self-assurance likely stemming from their familiarity and proficiency in such high-stress scenarios.

Overall, these results underscore the significant effects of experience in shaping the psychophysiological and fine motor skills responses of paratroopers to stress. While certain responses like improvements in muscular strength and reductions in anxiety seem universal to both groups, aspects like grip strength regulation, cortical arousal, fine motor skills, and self-confidence exhibit distinct patterns, emphasizing the nuanced impact of experience in high-stress military operations.

Table 1: Modifications of study variables in non-experienced and experienced participants

Variable	Non-experienced					Experienced				
	Pre	Post	t	p	Effect Size	Pre	Post	t	p	Effect Size
SJ (m)	35.7±1.5 (0.070)	37.7±2.9 (0.021)	-5.686	0.000	0.67	33.4±4.1	35.6±4.6	-7.976	0.000	0.54
IHS (N)	48.2±7.7	48.0±7.3	0.255	0.800	0.03	51.2±7.3 (0.027)	49.8±8.1 (0.039)	2.310	0.024	0.19
CFFT (Hz)	39.3±3.3 (0.000)	39.8±2.0 (0.030)	-1.316	0.194	0.15	37.1±3.2	38.5±3.6	-4.544	0.000	0.44
PMTR (s)	75.1±17.8 (0.000)	59.5±14.9 (0.050)	6.562	0.000	0.56	41.1±11.5	40.7±12.1	0.515	0.608	0.03
CA	8.9±4.2 (0.003)	7.8±4.7 (0.009)	1.947	0.057	0.26	2.8±3.2	2.4±2.9	1.547	0.126	0.13
SA	12.2±5.9 (0.000)	6.0±4.9 (0.002)	5.991	0.000	1.05	5.8±3.8	1.7±1.7	9.097	0.000	1.08
SC	15.2±2.6	15.6±5.1	-0.697	0.489	0.15	18.6±1.7 (0.037)	19.3±1.2 (0.020)	-4.155	0.000	0.41

Between parenthesis p values lower than 0.05 between novel and veterans' paratroopers. Squat Jump (SJ), Isometric Hand Strength (IHS), Critical Flicker Fusion Threshold (CFFT), Pistol Magazine Reload Time (PMRT), Cognitive Anxiety (CA), Somatic Anxiety (SA) and Self-Confidence (SC).

In Table 2, the results of a one-factor ANOVA analysis on various psychophysiological and performance variables are presented, shedding light on the impact of an unstated factor (likely experience or a specific training intervention) on these variables. Data suggests that whatever factor was being analyzed (experience, training, or another intervention) had a pronounced and often significant effect on physical abilities (like SJ and PMTR), cognitive function (as in CFFT), and psychological states (CA, SA, and SC). The lack of significant changes in some deltas might indicate that while the factor made a significant initial impact, its effect on the rate of change over time was not always strong, possibly due to a ceiling effect, adaptation over time, or other intervening variables (Table 3 and Table 4). The strong influence of age also suggests that demographic factors can be crucial in how individuals respond to training, experience, or other interventions in these domains.

Table 2: Results of the variables analyzed in the one-factor ANOVA

Variable	F	P	Variable	F	P
Age	373.128	0.000	PMTR Post	65.687	0.000
SJ Pre	9.185	0.003	Δ PMTR	26.330	0.000
SJ Post	8.831	0.004	CA Pre	88.429	0.000
Δ SJ	0.003	0.960	CA Post	66.286	0.000
IHS Pre	4.992	0.027	Δ CA	3.643	0.058
IHS Post	1.650	0.201	SA Pre	57.671	0.000
Δ IHS	2.536	0.114	SA Post	50.266	0.000
CFFT Pre	13.882	0.000	Δ SA	0.083	0.774
CFFT Post	5.404	0.022	SC Pre	79.531	0.000
Δ CFFT	2.986	0.086	SC Post	38.843	0.000
PMTR Pre	93.494	0.000	Δ SC	0.512	0.476

Squat Jump, Isometric Hand Strength (IHS), Critical Flicker Fusion Threshold (CFFT), Pistol Magazine Reload Time (PMRT), Cognitive Anxiety (CA), Somatic Anxiety (SA) and Self-Confidence (SC)

Table 3: Correlations between the Δ cortical arousal

		Cortical arousal pre-test	**Cortical arousal post-test**	**Vertical jump post-test**	**Vertical jump (Δ)**	**Fine motor performance pre-test**	**Fine motor performance post-test**	**Fine motor performance (Δ)**	**Self-confidence pre-test**	**Self-confidence (Δ)**
Cortical arousal (Δ)	Pearson correlation coefficient	-0.527**	0.303**	-0.222*	-0.174*	0.127	0.080	-0.149	0.251**	-0.378**
	Sig. (bilateral)	0.000	0.000	0.011	0.046	0.146	0.364	0.088	0.004	0.000
	N	132	132	132	132	132	132	132	132	132

**p<0,05; **p<0,01*

Table 4: Correlations between the fine motor performance (Δ) and psychophysiological parameters

		Age	**Records of jumps**	**Vertical jump pre-test**	**Fine motor performance pre-test**	**Fine motor performance post-test**	**Cognitive anxiety pre-test**	**Self-confidence pre-test**	**Cognitive anxiety post-test**	**Somatic anxiety post-test**	**Self-confidence post-test**
Fine motor performance (Δ)	Pearson correlation coefficient	0.355**	0.410**	-0.213*	-0.598**	-0.172*	-0.246**	0.284**	-0.341**	-0.340**	0.202*
	Sig. (bilateral)	0.000	0.000	0.014	0.000	0.048	0.005	0.001	0.000	0.000	0.020
	N	132	132	132	132	132	132	132	132	132	132

**p<0,05; **p<0,01*

Table 4 shows the correlations between changes in fine motor performance and various psychophysiological parameters. Significant findings include positive correlations of changes in fine motor performance with age and records of jumps, suggesting that improvements in fine motor skills are associated with older age and more jumping records. A negative correlation is observed with pre-test vertical jump performance, indicating that better initial physical performance might relate to lesser improvements in motor skills. Furthermore, there is a strong negative correlation with pre-test fine motor performance, suggesting that higher initial skill levels might see smaller subsequent improvements. Furthermore, negative correlations of changes in fine motor performance with cognitive and somatic anxiety post-test, and a positive correlation with self-confidence pre-test were also seen.

DISCUSSION

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and the working hypotheses. The findings and their implications should be discussed in the broadest context possible.

Future research directions may also be highlighted. The present research aimed to analyze the effect of experience on the psychophysiological response and fine motor skills in a highly stressful situation. Hypothesis i. was partially supported, as non-experienced participants demonstrated a more pronounced decline in fine motor skills compared to their experienced counterparts. This outcome underscores the impact of experience in managing the physiological and psychological stressors affecting motor skill performance. For hypothesis ii., the findings revealed a nuanced relationship between psychophysiological responses and fine motor skills. While greater cortical arousal and self-confidence, as well as lower levels of cognitive and somatic anxiety, generally correlated with enhanced fine motor skills, these associations were more complex than initially hypothesized. Experienced participants displayed better stress management and resilience, leading to superior fine motor skill execution under pressure. However, this relationship was not strictly linear and suggests the influence of additional moderating factors, such as individual stress tolerance, coping mechanisms, and task-specific training. These results highlight the multifaceted nature of psychophysiological responses in high-stress scenarios and their differential impact on fine motor skill performance based on an individual's experience level.

On this line, the data analysis revealed an intriguing pattern: experienced participants exhibited an increase in Critical Flicker Fusion Threshold (CFFT) following their jumps, suggesting heightened cortical demands imposed by this complex and perilous task. Notably, before the jump, non-experienced participants displayed significantly higher CFFT compared to their experienced counterparts. This unexpected finding could be attributed to an increased level of cortical hyperactivation. While previous studies have identified sympathoadrenal hyperactivation in similar contexts [2,20,33], our results indicate a concurrent elevation in cortical arousal. This heightened state in non-experienced participants may be a preparatory response to the novel and challenging situation, potentially facilitating mental anticipation of potential hazards and the formulation of strategies to address them. This phenomenon also includes a thorough mental review of the jump procedures. Similar patterns of elevated cortical arousal in novice participants have been reported in military personnel engaged in ground operations [2,34], lending support to our observations. Conversely, the lower cortical arousal observed in experienced paratroopers may indicate a tendency to rely on ingrained standard operating procedures, honed through extensive training and requiring lesser cognitive exertion. This strategy, as suggested by prior research, likely optimizes performance by conserving cognitive resources, allowing for a more efficient and focused execution of well-practiced tasks.

Following the parachute jump, participants exhibited an increase in leg strength, a phenomenon previously linked to enhanced sympathetic modulation triggered by the body's fight-or-flight response system [30,35]. However, in this instance, the significant muscular demand on the leg muscles, due to the impact of landing, may also explain this strength augmentation. Conversely, no significant changes were observed in isometric hand strength post-jump, possibly reflecting the less intense muscular engagement required by these muscles during an automatic parachute jump. Yet, notable differences emerged between experienced and non-experienced participants: non-experienced paratroopers showed higher leg strength, but lower hand strength compared to their experienced counterparts. This variation in major muscle groups like the legs might be attributable to the generally greater muscle mass in the younger, non-experienced group [36], though this factor appears less relevant in smaller muscle groups such as the hands.

From a psychological standpoint, experienced paratroopers reported lower levels of anxiety compared to their non-experienced peers, aligning with findings from prior studies involving similar demographic groups [37]. Interestingly, we noted a reduction in somatic anxiety among non-experienced participants post-jump, a shift potentially linked to the alleviation of immediate stress following the completion of the parachute jump. This differential response highlights the nuanced interplay between physical exertion, psychological stress, and experience in high-stress environments like military parachute jumping [31].

Furthermore, the established negative impact of anxiety on fine motor skills has been well documented in previous research [38]. In this context, it was anticipated that the anxiety reduction observed in non-experienced jumpers, despite their significant anticipatory anxiety response, would enhance their performance in fine motor skills tasks. Correspondingly, improvements were seen in Pistol Maga-zine

Reload Time (PMTR) among non-experienced jumpers, which might also be linked to the sustained physiological arousal following the jump [32]. In contrast, experienced paratroopers exhibited no notable differences in PMTR before and after the jump, a phenomenon potentially explained by a performance ceiling effect. In such a highly skilled group, PMTR appears to be minimally affected by the stress and excitement inherent in a parachute jump. This outcome suggests an optimal level of coping in challenging scenarios, a trait similarly observed in seasoned military parachute jumpers [39]. Indeed, a strong correlation has been established between Isometric Hand Strength (IHS) and better performance in both PMTR and shooting tasks [40]. This finding is consistent with the observations that experienced paratroopers displayed superior IHS and PMTR at both pre-jump and post-jump measurements. Their advanced skills and training may facilitate a more consistent and effective engagement in tasks requiring fine motor control, underscoring the significant advantage conferred by experience and expertise in military operations.

Finally, regarding the correlational analysis, data suggest that the interplay between psychophysiological states and fine motor skills is complex, particularly under high-stress conditions. Significant correlations were found between fine motor skills and various psychophysiological parameters, including arousal and anxiety, suggesting a relationship that is more intricate than the traditional perspectives on stress and performance. Indeed, there seems to be a nuanced connection between the level of initial cortical arousal, its changes over time, and motor skill performance. In this vein, studies have supported this assertion, indicating that higher levels of initial cortical arousal correlated positively with enhanced performance in precision tasks under pressure. For instance, in archery, an optimal level of initial arousal was found conducive to increased focus and steadier hand movements, thereby improving accuracy [41]. Similarly, studies on musicians revealed that those with lower initial levels of stress and anxiety, and who exhibited smaller physiological arousal changes during performances, tended to maintain better motor control, thus delivering superior performance. These findings corroborate our data and underscore the idea that managing arousal levels can be crucial in fine motor skill activities where precision and control are paramount [42]. Nonetheless, it has been proposed that the straightforward interpretation of the arousal-performance relationship must also consider factors such as task complexity, individual stress tolerance, and prior training [43].

Furthermore, research indicates that variables like age and self-confidence significantly influence fine motor performance under stress. These insights are in line with studies demonstrating that older individuals often excel in high-pressure medical scenarios, likely benefiting from their life experiences and heightened self-confidence [44]. Similarly, police officers with greater self-confidence have been observed to exhibit superior performance in stress-induced precision tasks [45].

Therefore, acknowledging these multifaceted influences underscores the necessity for bespoke training and stress management strategies. Such recognition heralds a pivotal shift towards more personalized approaches in performance training and preparation for high-stress environments. This paradigm shift not only reflects an evolving understanding of stress and performance dynamics but also emphasizes the importance of individual differences, advocating for more nuanced and effective training methods that cater to these variances.

Limitations of the study

One limitation of this study is its focus solely on male professional parachutists, restricting the generalizability of findings to other populations, including females and amateur or non-military parachutists. The specific stressor — military parachuting — may elicit unique psychophysiological responses, different from those induced by other stressors in various professional or athletic settings. Additionally, the measurement of fine motor skills and stress responses was confined to specific methods and tools, which might not capture all nuances of these complex phenomena. Future studies should consider a broader, more diverse participant base and a wider range of stressors and assessment techniques.

Practical applications

Our research on the effects of experience on psychophysiological responses and fine motor skills, with a focus on military parachutists, reveals critical insights with broad practical applications, particularly for high-stress professions. We discern that targeted interventions could markedly improve stress management and fine motor skills in less experienced individuals. In this line, simulation-based training stands out as an influential tool, offering a realistic, yet controlled, platform for developing crucial skills and adapting to stress. The use of VR and AR in simulations could provide immersive, risk-free environments for practitioners to build experience and confidence. This technique not only enhances skill acquisition but also allows individuals to mentally prepare for the high-stress scenarios they will face. Moreover, mindfulness and cognitive training programs, targeting attention control, emotional regulation, and mental resilience, present another viable strategy. These programs could help mitigate stress effects, enhance decision-making, and maintain fine motor control during high-pressure situations. Particularly, mindfulness exercises can reduce the psychophysiological impacts of stress, leading to improved performance.

Furthermore, physical conditioning specific to the professional demands also plays a key role. Appropriate fitness training can alleviate physiological stress responses, indirectly benefiting cognitive functions and fine motor skills. Regular psychological assessments can identify individual stress and anxiety responses, enabling more personalized training approaches. Building resilience, managing anxiety, and learning effective stress coping mechanisms are crucial in these trainings. In addition, neurofeedback and biofeedback techniques offer innovative methods for monitoring and controlling physiological stress responses. For example, controlling heart rate variability might be an effective way to manage anxiety and bolster focus in tense environments. Also, training that emphasizes team dynamics and communication is particularly valuable where teamwork is crucial under stress. Effective team interactions can significantly lessen individual stress levels and elevate collective performance.

Therefore, implementing these strategies not only aims to boost individual performance in stressful settings but also contributes to long-term well-being, professional durability, and the efficiency of teams in such environments. For less experienced individuals, these interventions can be crucial in bridging the experience gap, enhancing their capacity to manage stress, and improving their fine motor skills, ultimately leading to better overall performance and adaptability in their respective high-stress professions.

Future research lines

Considering the intricate connections between psycho-physiological responses and fine motor skills as observed in military parachutists, future research avenues appear both extensive and varied. Investigating these dynamics across different high-stress professions and sports could offer a broader understanding of how distinct stress levels influence fine motor skills in contexts such as surgery, law enforcement, or competitive disciplines like archery. Longitudinal studies focusing on the long-term impact of chronic stress exposure could unveil critical insights into the effects on mental health, well-being, and the maintenance of fine motor skills, highlighting the need for proactive intervention strategies. Further exploration into psychological factors beyond anxiety, including resilience, motivation, and risk perception, would enrich our comprehension of their roles in performance under stress. There's also a pressing need to develop and evaluate intervention strategies and training programs, including mindfulness, cognitive-behavioral approaches, and simulated environments, aimed particularly at enhancing the skills and stress management capabilities of the less experienced. Assessing the efficacy of simulator training in preparing individuals for high-stress scenarios, understanding the influence of physical fitness on stress response, and integrating neuroimaging and physiological monitoring to decrypt the brain-body interactions during stress, could provide transformative insights. Furthermore, exploring genetic and biochemical markers like cortisol levels might lead to more tailored training and development protocols. Lastly, cross-cultural studies would offer a valuable perspective on how cultural variations impact stress perception and coping mechanisms, potentially guiding global strategies in stress management and skill enhancement. This wide-ranging research agenda underscores the complexity of stress and performance, especially in high-stakes, high-stress environments, promising not only to deepen our understanding but also to significantly improve training and support for professionals in such challenging roles.

CONCLUSIONS

This study's exploration into the effects of experience on psychophysiological responses and fine motor skills in military parachutists underscores the intricate relationship between stress, experience, and motor performance. Notably, experienced parachutists displayed superior motor skills and lower stress responses, likely attributed to their habitual exposure to high-stress environments and refined coping mechanisms. Contrarily, non-experienced individuals showed heightened stress markers and impacted motor skills, although some improvement in performance post-exposure suggests potential adaptability. These findings illuminate the importance of experience in moderating stress and enhancing motor proficiency. They also highlight the need for tailored training programs, especially for novices, focusing on stress management, skill acquisition, and psychological resilience to bridge the experience gap. This research contributes valuable insights into stress-induced motor skill impairment, suggesting that experience plays a crucial role in optimally managing high-stress situations essential in military and other high-risk professions.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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Authors' contribution
All authors contributed equally to the development of this manuscript. All

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Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Investigating EGFR, BRAF, and RAS Mutations in Oral and Cutaneous Squamous Cell Neoplasms: A Preliminary Report on Romanian Patients

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Abstract: *Background: Head and neck cancers, and particularly, oral cancers have a complex pathogenesis that includes genetic mutations and epigenetic alterations which interfere with cellular signaling and can trigger tumor development. The purpose of this study was to reveal whether low-frequency hotspot mutations may be detected in a study lot with histopathological evidence of squamous cell carcinoma (SCC) of the oral mucosa and skin of the head and neck. Methods: Tumor biopsies from treatment-naïve patients were tested for BRAF V600, NRAS G12/G13, NRAS Q61, KRAS Q61 mutations, and EGFR exon 19 deletions (Ex19Del) using droplet digital PCR (ddPCR). The tumors were also analyzed for EGFR T790M mutations by RT-PCR, using a CE-IVD validated kit, with a limit of detection of 0.05%. Results: None of the examined cases exhibited NRAS G12/G13, NRAS Q61, KRAS Q61, BRAF V600, or EGFR T790M mutations, indicating that these alterations are rare events in SCC pathogenesis. Interestingly, among the 12 specimens tested by ddPCR for EGFR Ex19Del, an HPV-negative cSCC tumor occurring in the parotid region tested positive for this drug-sensitizing mutation, offering unexplored therapeutic perspectives to the patient from whom it was collected. Conclusions: Our study highlights the important clinical implications of detecting low-frequency hotspot mutations in tumor biopsies by ddPCR. We believe that the ddPCR-assisted analysis of these mutations in larger SCC cohorts may provide us with mechanistic insights regarding their role in SCC pathogenesis and guide the development of novel therapeutic strategies for this problematic disease.*

Keywords: *head and neck squamous cell carcinoma; droplet digital PCR (ddPCR); targeted therapy, immunotherapy*

INTRODUCTION

Squamous cell carcinomas (SCCs) are one of the most unpredictable and widespread neoplasms globally, presenting a significant socio-economic burden on healthcare systems worldwide [1]. SCC development has been linked to the dysregulation of several molecules involved in different stages of squamous lineage commitment and keratinocyte terminal differentiation [2]. However, the exact identity and the precise order of the genetic and epigenetic alterations facilitating keratinocyte malignant transformation remain elusive. Several research groups postulated that epidermal clones with mutated p53 may be at the origin of SCC, as they lead to squamous dysplasia and in situ SCC, which subsequently progress to metastatic SCC [3]. At the same time, emerging evidence suggests that the disruption of the mitogen-activated protein kinase (MAPK) signaling pathway, activated by epidermal growth factor receptor (EGFR), facilitates the development and progression of various malignancies, including head and neck squamous cell carcinoma (HNSCC) and cutaneous SCC (cSCC) [3,4]. The MAPK pathway may be depicted as a complex regulatory network that links the tumor microenvironment with the cell nucleus, converting the extracellular signals into intracellular responses such as cell proliferation, differentiation, or apoptosis [4,5]. RAFs (A-/B-/C-RAF), RASs (N-/H-/K-RAS), MEKs, and MAPKs kinases are the most notorious constituents of the MAPK signaling pathway, behaving as powerful oncogenes in various human cancers. Thus, any alteration within those signaling components may lead to apoptosis resistance and uncontrolled cellular growth [4]. Beyond the critical role of EGFR/MAPK signaling in HNSCC development, the various nodal points within the signaling pathway can be converted into therapeutic vulnerabilities in these neoplasms. For instance, BRAF V600E-mutant ameloblastomas may be targeted with BRAF inhibitors such as vemurafenib and dabrafenib, whereas HRAS-mutant HNSCC can be approached with farnesyltransferase inhibitors (FTis) like tipifarnib [4,6]. Despite these recent discoveries, the main therapeutic approach for HNSCC and cSCC combines surgery with radiation therapy and systemic chemotherapy, with ancillary reconstructions depending on case complexity and structures involved [7–9]. Surgical excision of the tumor mass remains the cornerstone of treatment for resectable SCC, followed by adjuvant radiotherapy, with or without chemotherapy [10,11]. The tumoral resection may carry additional risks when considering local infiltration, anatomical variants, or surgical approach [12–14].

The stratified epithelium from which SCC originates can be found distributed on the surface of the outer integument or covering organs and cavities such as the oral cavity, sinonasal cavity, oropharynx, larynx, esophagus, lung, and anogenital region [15]. SCCs are currently classified and examined taking into account the anatomical divisions of the human body; therefore, the SCCs of the head and neck are treated differently from those of the lung [2]. Here, we will focus exclusively on SCCs of the skin and of the mucosal epithelium of the oral cavity and lips (OSCC). Although both tumor types arise from the disruption of the same differentiation programs, cSCC and OSCC are considered distinct entities with different risk factors [16,17]. The main etiological factors for OSCC include tobacco and alcohol usage, and human papillomavirus (HPV) infection [15,18,19]. For cSCC and lower lip SCC, the most prominent risk factor remains increased exposure to ultraviolet radiation (UVR), followed by a personal history of skin cancer and HPV infection [19,20].

cSCC is the second most common cancer worldwide, and its incidence is steadily increasing in the Caucasian population. As cSCC is often invasive, inaesthetic, and locally destructive, such diagnosis usually involves complex skin surgeries and facial reconstructions [21]. While the majority of cSCC patients present with a very good prognosis, 1.5-5.2% of cases may develop life-threatening metastases [22,23]. Moreover, owing to the high incidence rates, in the United States, the mortality rate associated with cSCC is comparable to that reported for more aggressive tumors such as leukemia and melanoma [22]. The number of cSCC-associated deaths is hence expected to surpass that of melanoma in the next 5 years, mainly due to the rise in HPV infections [24]. HNSCC, the sixth most common type of cancer globally, represented mainly by OSCC, also exhibits a poor overall survival (OS) rate. Despite advancements in diagnostic and treatment methods, the HNSCC 5-year OS rate remains approximately 50% [25], suggesting that more intensive scientific efforts should be devoted to understanding the complex biology of HNSCC tumors. Molecular studies to identify the key factors driving cSCC and OSCC development are ongoing, aiming to identify the most promising diagnostic biomarkers and therapeutic targets for these problematic diseases [5,26,27].

The latest advancements in omics technologies facilitated the identification of several hotspot mutations in the EGFR/MAPK signaling pathway which have been harnessed for targeted pharmacological interventions in cancer patients. For instance, the observation that 80% of HNSCCs exhibit EGFR overexpression led to the development and subsequent approval by the Food and Drug Administration (FDA) of Cetuximab, an anti-EGFR monoclonal antibody, to be used in combination with radiotherapy in locally advanced HNSCC [28]. Yet, the dissection of the mutational spectrum of the EGFR gene served as a foundation for the development and implementation of novel EGFR-targeted therapies in clinical settings. For instance, genetic alterations such as exon 19 deletions (Ex19Del) and exon 21 substitutions (e.g., L858R), representing the most common EGFR mutations in non-small cell lung cancer (NSCLC), confer sensitivity to first (gefitinib and erlotinib) and second-generation (nilotinib, dasatinib) tyrosine kinase inhibitors (TKIs) [29]. In addition, exon 20 substitutions which include EGFR T790M mutations and are generally associated with NSCLC resistance to first-generation TKIs, may be also matched with targeted therapy in cancer patients, particularly with third-generation TKIs such as osimertinib and alflutinib [29]. In HNSCC/cSCC clinical cohorts, single-target TKIs, such as gefitinib and erlotinib were shown to improve modestly patient survival rates when given as monotherapy [30]. However, promising results have been reported when small TKIs were combined with other agents such as pembrolizumab, cisplatin, or docetaxel, in recurrent and metastatic settings [31]. This suggests that EGFR mutations may be exploited for targeted therapeutic interventions in the future, once the clinicians validate the most beneficial drug combinations. Alternative strategies for tailored therapy in HNSCC include a) FTIs, such as tipifarnib, for HRAS-mutant HNSCC (recently FDA-approved); b) cetuximab for SCC presenting with KRAS germline variants; c) EGFR inhibitors for HNSCC tumors harboring MAPK1 mutations; d) BRAFi and

MEKi for BRAF V600E-mutant ameloblastomas, combined or not with immunotherapies [4].

As we described earlier, the genetic assessment of BRAF, a serine/threonine protein kinase mutated in 60% of cutaneous melanomas and more than 15% of human cancers, may also expand the anti-cancer armamentarium in cSCC/HNSCC [32]. Despite the low prevalence of BRAF mutations in SCC of the head and neck (2%), HNSCC patients carrying these alterations present with improved clinical outcomes after BRAF and MEK inhibition [33]. RAS is another key oncogene in the pathogenesis of human cancers. KRAS mutations were identified in 2.89% of HNSCC cases, most frequently in tumors with sinonasal localization, whereas NRAS alterations were reported in 2.2% of cases, especially in tumors of the nasopharynx [34]. A recent meta-analysis highlights that oncogenic KRAS may be associated with significantly enhanced immune checkpoint molecule (ICM) expression and immune evasion programs within the tumor microenvironment, making KRAS-mutant tumors promising candidates for immune checkpoint inhibition (ICI) in the clinical setting [35]. However, several recent reports highlight that the MAPK-mutant status may serve as an indicator of a favorable response to immunotherapy in HNSCC, mainly due to the increased CD8+ T cell and dendritic cell infiltrations reported within these tumors, representative of an active cytolytic CD8+T-inflamed status [36].

The present study aims to explore the role and prevalence of KRAS, NRAS, BRAF, and EGFR mutations in a pilot group of patients diagnosed with SCC of the oral mucosa and skin.

MATERIALS AND METHODS

Patients and samples

Twelve patients diagnosed with squamous cell neoplasms of the head and neck between February 2017 and October 2019 were included in the present study. Three patients presented with cSCC, whereas nine had OSCC. Archived formalin-fixed paraffin-embedded (FFPE) tissues from these 12 subjects were obtained from the Department of Oral and Maxillofacial Surgery of the Carol Davila Military Emergency Hospital in Bucharest, Romania. All participants provided written informed consent to take part in this study. Ethical approval for this retrospective analysis was obtained from the Ethics Committee of the Carol Davila Central Military Emergency Hospital. The study including all procedures and handling of patients' samples was carried out in accordance with the guidelines of the Declaration of Helsinki and national as well as institutional guidelines for research ethics. The clinical and histopathological characteristics of the SCC tumors were analyzed following the latest version of the international standard American Joint Committee on Cancer (AJCC) tumor/node/metastasis (TNM) system for the classification and staging of tumors. Presence of the EGFR T790M mutation was analyzed with the gb ONCO EGFR (T790M) Real-Time PCR assay (PN: 3245-048, Generi Biotech, Czech Republic); additional mutations in the EGFR-RAS-RAF mutations were tested using the droplet digital PCR (ddPCR) Screening assays for BRAF V600 (PN: 12001037), NRAS G12/G13 (PN: 12001627), NRAS Q61 (PN: 12001006), KRAS Q61 (PN: 12001626) mutations and EGFR exon 19 deletions (PN: 12002392) (all BioRad, Hercules, USA); details of these techniques are outlined below.

DNA extraction

The total genomic DNA was extracted from FFPE tissues (3 x 15-μM-thick sections) using the ReliaPrep™ FFPE gDNA Miniprep kit (PN: A2352, Promega). The protocol includes a proprietary deparaffinization method that does not rely on the use of xylene or any other hazardous solvents, ensuring at the same time the extraction of high-purity nucleic acids from FFPE samples. Following the deparaffinization of the FFPE tissue sections, the extraction protocol involves the lysis of the tissues. Nucleic acids released from the cells are further bound on the surface of a spin filter membrane and eluted in 50 μL of elution buffer after several rounds of washing. The purified genomic DNA was kept on ice and quantified using a Nanodrop spectrophotometer.

Droplet digital PCR

Before carrying out the ddPCR reaction, the genomic DNA samples were subjected to uracil-DNA-glycosylase treatment (UDG; PN: 1B1634, VWR) following the manufacturer's instructions. This step aimed to eliminate potential C>T transitions that could arise from tissue fixation, therefore reducing the risk of false-positive ddPCR reactions. All gDNA samples were analyzed in triplicate.

The ddPCR mixture requires the addition of 10 μL of 2x ddPCR Supermix for Probes (no dUTP) per reaction. The 2x ddPCR Supermix is a proprietary reagent containing an optimized reaction mix for primers and probes. Additionally, 1 μL of 20x BRAF (NRAS/KRAS/EGFR) primer and 3 μL of UDG-treated gDNA (7 ng/μL) were included in each ddPCR reaction, and the final reaction volume was adjusted to 20 μL by adding an appropriate volume of RNase/DNase-free water. Each analytical run included negative (wild-type control, WT) and positive controls at the same concentration as the analyzed gDNA samples. The positive controls were provided by Horizon Discovery (PN: HD701) and contained recurrent mutations in BRAF, KRAS, NRAS, cKIT, EGFR, and PIK3CA genes, at allelic frequencies ranging from 1% to 24.5%. The negative controls were WT genomic DNA samples acquired from Promega

Corporation (PN: G1471).

In the droplet generation step, individual reaction mixtures were loaded into disposable DG8 cartridges (Bio-Rad) along with 70 µL of droplet generation oil and passed through the microfluidic channels of a droplet generator. The resulting droplets from each well were transferred into a 96-well PCR plate, heat-sealed, and subjected to the following PCR conditions: 95°C for 10 min, followed by 40 cycles of 94°C for 30 s, 60°C for 1 min, and 98°C for 10 min. Post-PCR, the droplets from each well were analyzed using a QX200 droplet reader (Bio-Rad) and quantified based on target DNA fluorescence. The obtained data were analyzed using the QuantaSoftTM Analysis Pro software, version 1.0.596 (Bio-Rad). Additional details were reviewed recently [37].

Real-Time PCR

The EGFR T790M mutational status was evaluated using the CE-IVD validated gb ONCO EGFR (T790M) Real-Time (RT-) PCR kit (PN: 3245-048, Generi Biotech, Czech Republic). All reagents included in the kit were prepared for use according to the manufacturer's instructions. For each sample, we set two separate RT-PCR reactions. One reaction contained the Assay qPCR EGFR Control T790M and the other, the Assay qPCR EGFR MUT T790M, both in a total volume of 20 µL. 5 µL of the same gDNA sample (diluted to a final concentration of 50 ng/µL) was added to each of the two reactions. Thermal cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 50 cycles with denaturation at 95°C for 10 s, and annealing/elongation at 60°C for 20 s. Fluorescence acquisition was set on the FAM/SYBR channel. All gDNA samples were analyzed in triplicate for the detection of EGFR T790M mutation. Each run contained two types of positive controls according to manufacturer instructions. We included one EGFR wild-type (WT) control and one EGFR T790M positive control, with 5 µL of the provided kit standards pipetted to each. Finally, to check the purity and quality of the PCR reagents, we introduced a third assay control, the so-called 'negative control'. In this case, RNase/DNase-free water was used to replace the corresponding volume of gDNA. The qRT-PCR assays were run on the Rotor-Gene Q 5plex HRM equipment (Qiagen, Germany) and the results were analyzed using the Rotor-Gene Q Series Software (version 2.3.1) (Qiagen, Germany). For each sample, the assessment of the mutational status was based on calculating fold change (FC) values from the Ct values obtained in the two separate reactions, as per the manufacturer's specifications.

Table 1: Demographic and clinicopathological data of SCC patients

Patient #	Gender	Age (years)	Area	Tobacco usage	Alcohol use	The primary site of the tumor	HPV infection	TNM	T stage	Lymph node metastases	Outcome
1	M	80	rural	no	no	lower lip	no	I	1	0	alive
2	M	67	urban	yes	no	tongue	no	IV A	3	2	dead due to disease progression
3	M	50	urban	yes	yes	tongue	no	IV A	2	3	alive
4	M	65	rural	no	yes	lower lip	no	I	1	0	alive
5	M	45	urban	yes	no	lower lip	no	III	3	1	alive
6	F	54	urban	yes	no	tongue	no	II	2	0	alive
7	M	60	rural	no	no	retromolar trigone	no	IV A	4	5	dead due to disease progression
8	M	69	urban	yes	yes	buccal mucosa	no	IV A	3	1	alive
9	F	85	rural	no	no	lower lip	no	II	2	0	alive
10	M	88	urban	no	no	cSCC of the parotid region	no	III	3	1	alive
11	M	51	rural	yes	yes	cSCC of the nasal region	no	IV A	4	2	dead due to disease progression
12	F	86	urban	no	no	cSCC of the genian region	no	I	1	0	alive

The limit of detection (LoD) declared for this assay is 5 copies per 25 µL reaction. This corresponds to a minimum detectable mutant allelic fraction of 0.05% in a background of 100,000 WT copies.

Statistical analysis

Data analysis was performed using The GraphPad Prism software (Boston, Massachusetts USA, www.graphpad.com) version 10.0.0. Patients' demographic and clinicopathological data were analyzed by descriptive statistics.

RESULTS

Patients' general characteristics

Table 1 describes the demographic and clinicopathological characteristics of the SCC patients. The clinical cohort consisted of 12 patients diagnosed with SCC of the head and neck region, encompassing 9 men (75%) and 3 women (25%). Among them, nine patients presented with OSCC (Patients #1-#9), while three subjects had cSCC (Patients #10-#12). The median age of the patients was 66 years, ranging from 45 to 88 years.

Fifty-nine percent (n=7) of the analyzed patients are from an urban environment, and 50% (n=6) of them are active smokers. Additionally, 33% (n=4) of the patients declared that they frequently consume alcohol, and 58% (n=7) presented with pathologically confirmed lymph node metastasis. Five patients were diagnosed with TNM stage IVA disease, three with stage I, and two patients each with stages II and III. All patients tested negative for the presence of high-risk HPV strains.

DNA extraction

The average concentration of DNA extracted from the archived FFPE tissues was 255.7 ng/µL (ranging from 70.2 to 484.3 ng/µL), and the average A260/A280 value was 1.83.

The A260/A280 values obtained for gDNA from FFPE tissues are within the optimal range (~1.8), indicating that the extracted gDNA is free from protein contamination. In addition, all A260/A230 values fall within the recommended range (2.0-2.2), indicating that gDNA has minimal contamination by polysaccharides. These observations confirm the obtained gDNA's suitability for subsequent molecular manipulations.

ddPCR results

The ddPCR analysis demonstrated that BRAF V600, KRAS Q61, NRAS G12/G13, and NRAS Q61 mutations were not present in the examined samples, emphasizing the rarity of these genetic alterations in the pathogenesis of squamous cell cancers. Figure 1 illustrates the absolute quantification of the NRAS Q61 mutation in FFPE samples using the ddPCR system. In addition, by exploiting the ultrahigh sensitivity of ddPCR technology, we successfully identified the presence of the EGFR Ex19Del mutation in a cSCC tumor developing in an 88-year-old male diagnosed with stage III disease (Figure 2). None of the remaining 11 SCC patients within the clinical cohort exhibited this particular mutation.

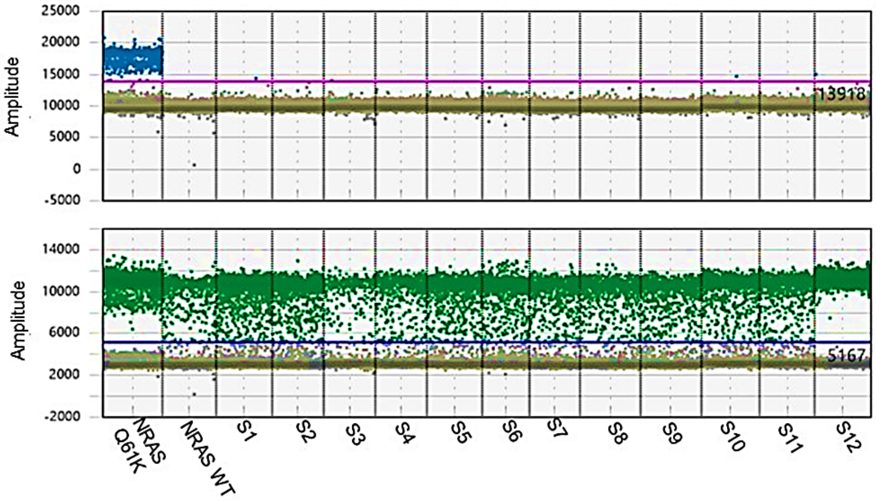

Figure 1: One-dimensional (1D) droplet fluorescence plot for NRAS Q61 mutation analysis. Blue dots represent the FAM-labeled droplets containing the NRAS Q61 -mutant allele, while green dots depict the HEX-labeled fluorescence probes associated with the NRAS WT allele. The pink line represents the threshold between positive and negative signals of the droplets. This determination enables precise NRAS Q61 mutation quantification in FFPE samples (S1-S12- SCC samples; NRAS Q61K- positive control; NRAS WT- negative control).

Real-Time PCR results

The 12 SCC samples were additionally subjected to qRT-PCR analysis to detect the EGFR T790M mutation. All SCC samples included in the study tested negative for the presence of the EGFR T790M mutation.

According to the manufacturer's recommendations, each analytical run included a positive control (containing the investigated mutation at an allelic frequency of 1%), a WT control (where only the WT allele was amplified), and a negative control (without gDNA), to investigate the purity and the quality of the reagents used. Figure 3 illustrates the positive control of the EGFR T790M RT-PCR assay, the negative control along with a sample negative for this mutation (EGFR wild-type; in blue).

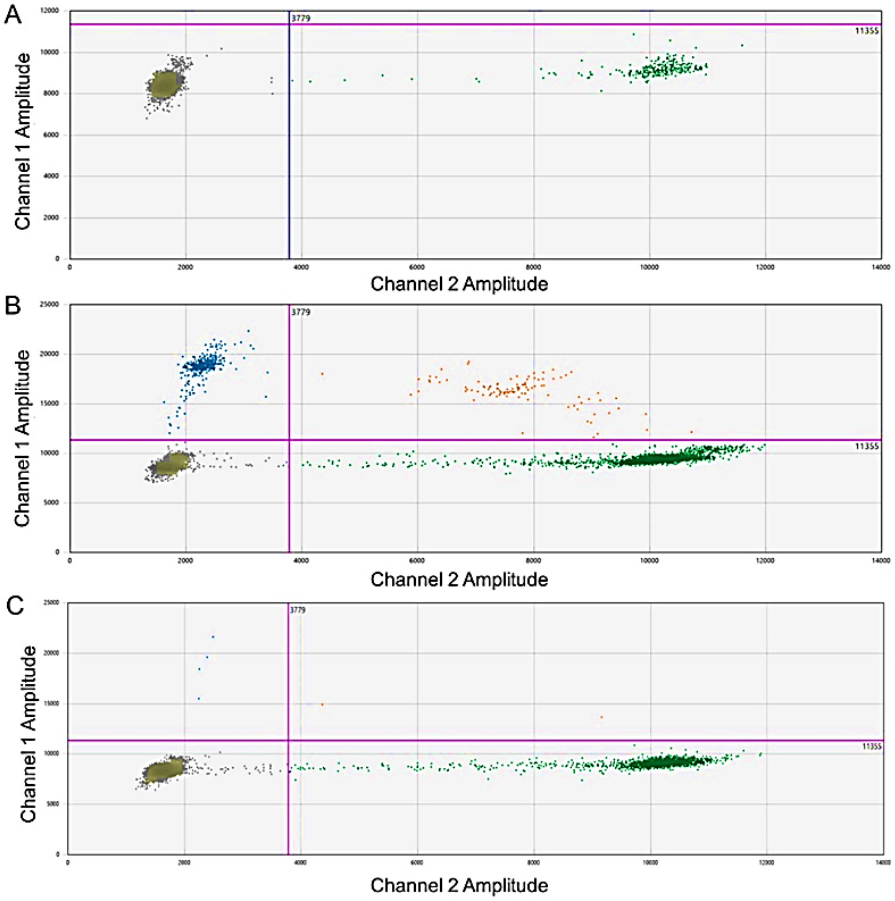

Figure 2: Two-dimensional (2D) ddPCR plots of EGFR Ex19Del mutation detection in FFPE samples. (A) ddPCR results for an EGFR wild-type DNA sample (negative control). The 2D plot illustrates that no droplets are positive for EGFR Ex19Del mutant alleles on channel 1 (FAM labeled, in blue) while EGFR Ex19 wild-type alleles are abundantly detected on channel 2 (HEX labeled, in green). Empty droplets (in grey) do not contain the EGFR Ex19Del target amplicon. (B) ddPCR results for the positive control (presenting the EGFR Ex19Del mutation). Mutant alleles are detectable on channel 1 (FAM labeled, blue). Double-positive droplets (orange) contain both mutant and wild-type EGFR Ex19 alleles. (C) ddPCR results for the cSCC tumor tested positive for EGFR Ex19Del.

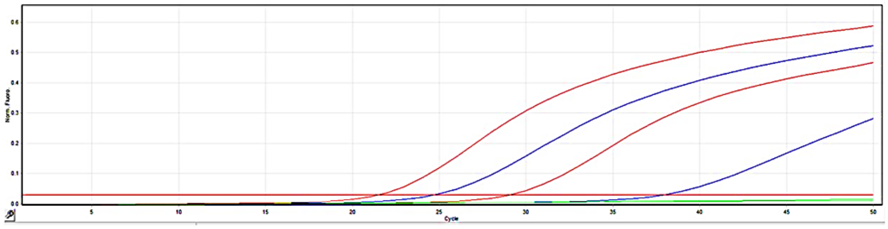

Figure 3: Determination of the EGFR T790M mutational status by RT-PCR. The positive control of the qRT-PCR analysis is marked in red, whereas the negative control is in green. The amplification curve of the EGFR WT SCC sample is depicted in blue. The threshold for the entire run was set at 0.03. A sample can be considered mutated when its FC value is lower than the cut-off FC value and the difference between the CtM of a sample and the CtC of the WT standard is lower than 13. If the latter condition is not met, or the FC value of a sample is higher than the cut-off FC, the sample is evaluated as WT.

DISCUSSION

SCC neoplasms of the head and neck are one of the most heterogeneous and aggressive tumors ever described [10]. Despite recent advances in SCC diagnosis and therapy, the prognosis of advanced patients remains poor, highlighting the need for a more comprehensive evaluation of these tumors [38–40]. From the beginning of modern medicine until now, SCCs have always been approached separately, depending on the tissue from which they originate (e.g., cutaneous SCC, oral SCC) or the anatomical region where they occur. However, in the present study, we wanted to deliver a unifying approach regarding cSCCs and HNSCCs, by analyzing the shared molecular mechanisms involved in their pathogenesis. Our point of view is also supported by the fact that the cSCC and HNSCC pathogenesis converge toward the same risk factors and genetic alterations [2,41]. Additionally, we believe this approach may facilitate a comparative examination of intrinsic tumor cell heterogeneity, stromal composition, and therapeutic efficacy across SCCs occurring in different anatomical sites, an idea that is attracting considerable research interest from the international community as well [2].

Here, we embarked on a preliminary analysis of the frequency and role of hotspot mutations in the EGFR-RAS-RAF signaling components within a clinical cohort encompassing 12 Romanian patients diagnosed with SCCs (3 cSCCs and 9 OSCC cases). We employed the third-generation PCR technology, also known as ddPCR, to screen for mutations such as BRAF V600, NRAS G12/G13, NRAS Q61K, KRAS Q61, and EGFR Ex19Del, while the mutational status of EGFR T790M was assessed using RT-PCR. None of the examined tumor specimens exhibited mutations in the BRAF, KRAS, and NRAS genes. Our findings are consistent with previous observations in the field, supporting the assertion that these genes are rarely mutated in cSCCs and HNSCCs [34,42].

The analysis of the demographic data of the subjects enrolled in the study revealed that 75% of them are men (n=9) and half (n=6) are smokers. We also observed that 58% of the subjects (n-=7) have metastases with lymph node involvement and all SCC tumors are HPV-negative (n=12).

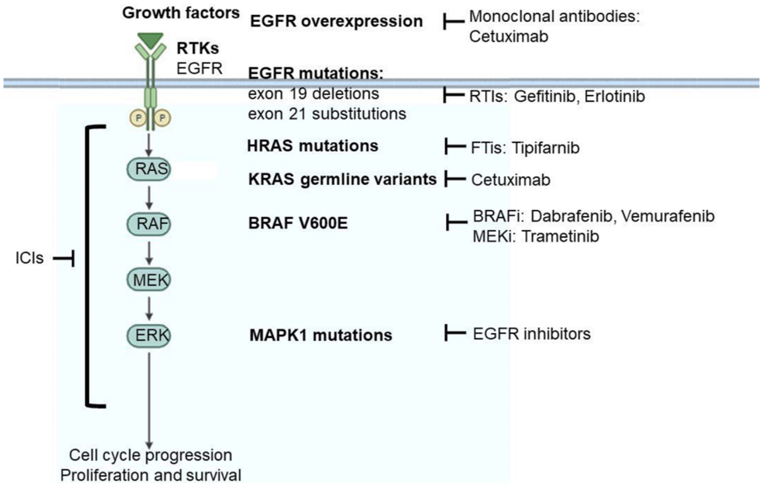

Figure 4: Precision treatment strategies for EGFR/MAPK pathway-mutated head and neck squamous cell neoplasms. ICIs- Immune checkpoint inhibitors; RTKs- Receptor tyrosine kinases; RTis- Receptor tyrosine kinase inhibitors; FTis- Farnesyl transferase inhibitors; BRAFi- BRAF inhibitors; MEKi- MEK inhibitors

However, among the 12 FFPE samples analyzed for EGFR Ex19Del, one HPV-negative cSCC tumor identified in the parotid region of a male tested positive for this mutation. Interestingly, the patient presenting with this tumor is an 88-year-old man from an urban environment who neither smokes nor drinks alcohol. Nevertheless, current literature emphasizes the role of HPV infection status as a valuable prognostic indicator for HNSCC/cSCC patients. Usually, HPV-negative SCCs display alterations in the tumor suppressors such as p53 and cell cycle regulators and present with an unfavorable prognosis compared to HPV-positive tumors, which show less genomic complexity [43]. Several research groups have examined the prevalence of EGFR Ex19Del mutations in head and neck SCCs, reporting a 1% occurrence in the Caucasian population [44] and 7.3% in the Asian population [45]. Despite providing tumor cells with different adaptive advantages via downstream hyperactivated pathways (ERK/MAPK, PI3K/Akt, and JAK/STAT), these mutations also confer sensitivity to TKIs [3]. Several ongoing clinical trials are investigating TKIs' clinical safety and efficacy in cSCC/HNSCC. Single-target TKIs gefitinib and erlotinib given as monotherapy have not demonstrated clinical efficacy in

HNSCC and cSCCs [46,47]. However, remarkable benefits have been reported when small-molecule TKIs were administered in conjunction with other therapies such as cisplatin, docetaxel, or immunotherapy [31,48]. Therefore, these mutations might be exploited for targeted pharmacological interventions in the future, once the most effective drug combinations are identified.

The evaluation of the mutational status of the MAPK signaling pathway is another hot topic in the field of SCC bio-medical research. Several recent studies potentiated that mutations in different components of the MAPK signaling pathway, such as BRAF and RAS, may serve not only as important therapeutic targets but also as valuable indicators of prognosis in SCC, indicating those patients who are most likely to respond to immunotherapy (Figure 4) [36].

This is why we focused our research on investigating these mutations in a small group of patients diagnosed with SCC of the skin and oral mucosa, the present study being also the first one of this type conducted in Romania. Recent studies have shown that even in p53-mutated HNSCC tumors, notorious for their unpredictable evolution, MAPK pathway mutations may help identify those patients with superior outcomes [36]. It is also worth mentioning that the positive prognostic value of these mutations in HNSCC has been reported independently of HPV status [36].

Bioinformatic analysis of HNSCC patient data retrieved from The Cancer Genome Atlas (TCGA) revealed that MAPK-mutant HNSCC tumors present with an increased infiltration of CD8+ T cells and dendritic cells and immune-active cytolytic and interferon-gamma signatures in situ, data that were also reported by our group [36,38,49].

Those features are not observable in HNSCC tumors with mutations in the PI3K, WNT, NOTCH, JAK/STAT, NF-kB, or TGF-beta signaling pathways [36]. Moreover, independent of tumor mutational burden (TMB), MAPK pathway mutations alone predicted those HNSCC patients who benefited the most from clinical anti-PD1/PD-L1 immunotherapy [36].

Similar prognostic roles of MAPK pathway mutations were reported in pan-cancer immunotherapy studies, suggesting that the mutational analysis of this signaling pathway might be essential to improve SCC patient risk stratification.

CONCLUSIONS

Oral and cutaneous squamous cell neoplasms (SCCs) remain a public health challenge and a topic of current scientific interest. Genetic analysis is essential to understand the biology and the clinical behavior of SCCs, unraveling potential biomarkers and therapeutic targets with practical applications in this challenging disease. Therefore, to align with the precision medicine initiatives in the field, we initiated a clinical study to explore the prevalence and significance of hotspot mutations in the EGFR-RAS-RAF signaling pathway within a small clinical cohort of Romanian patients diagnosed with squamous cell neoplasms of the head and neck. Taking into account the shortcomings reported with traditional chemotherapies, administered on a ‘one size fits all’ basis and resulting in poor efficacy, limited selectivity, and increased toxicity in HNSCCs/cSCCs, we are actively supporting the transition towards the adoption of more individualized anticancer agents in SCC clinical practice.

The present retrospective study emphasizes the absence of mutations in the NRAS, BRAF, and KRAS genes within a small clinical cohort of cSCC and HNSCC Romanian patients, marking the first study of this type conducted in Romania. In addition, our research underlines the remarkable sensitivity of the third-generation PCR technology, the so-called ‘droplet digital PCR’ (ddPCR), which may be instrumental for detecting low-frequency actionable mutations in cSCC/OSCC tissue biopsies.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research was funded by grants from the Ministry of Research, Innovation and Digitization, CCCDI-UEFISCDI, project number [PN-III-P4-PCE-2021-0549] and [PN-III-P2-2.1-PED-2021-2243], and within PNCDI III by the Core Program, implemented with the support of NASR, project [PN 23.16.01.03].

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Authors’ contribution

Conceptualization, M.N., and EG.D.; methodology, EG.D.; software, A.C, and C.Constantin.; validation, A.N.M., C.Constantin., M.S. and C.S.; formal analysis, C.Caruntu.; investigation, A.C.; resources, M.N. and C.Carruntu.; data curation, EG.D.; writing—original draft preparation, EG.D., A.C., A.N.M., M.S., C.Constantin, C.Caruntu, C.S., M.N.; writing—review and editing, M.N. and C.Caruntu.; funding acquisition, M.N., and C.Caruntu. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee from Carol Davila Central Military Emergency Hospital, Bucharest (No. 333/28.03.2019).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Should Serum Levels of Creatine kinase be Evaluated in Patients with Acne Vulgaris Treated with Systemic Isotretinoin?

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Abstract: *The laboratory tests that should be performed and their frequency during isotretinoin treatment are controversial. We wanted to investigate muscle and joint pain and laboratory tests in acne patients who received isotretinoin. Between April 2019 and February 2023, serum CK, AST, ALT, and GGT levels were retrospectively evaluated in acne patients before and three months after systemic isotretinoin treatment. This study included 410 patients. The median serum AST, ALT, and GGT levels significantly increased, whereas serum CK levels were similar in all patients three months after treatment. 23 (5.6%) patients revealed muscle or joint pain after treatment. No significant difference was detected in patients with or without pain in the median serum CK, AST, ALT, and GGT levels after treatment. Isotretinoin treatment at a dose of 10 mg/day was more common in patients with pain than in those without pain, whereas none of the patients who received isotretinoin 40 mg/day developed muscle or joint pain. We suggest that routine evaluation of serum CK levels is unnecessary in acne patients treated with isotretinoin. However, muscle and joint pain may develop even at doses as low as 10 mg/day. Furthermore, monitoring AST, ALT, and GGT levels may be beneficial in acne patients treated with isotretinoin.*

Keywords: *acne vulgaris; creatine kinase; isotretinoin; joint pain; muscle pain; treatment*

INTRODUCTION

Acne vulgaris is a common and chronic inflammatory skin disease that usually occurs during adolescence. Acne vulgaris may also affect adults or even initiate in adulthood. The etiopathogenesis of acne includes seborrhea, follicular hyperkeratinization, Cutibacterium acnes, and inflammation. Lesions mainly appear on the face and may lead to scarring and pigmentation changes. Therefore, acne has been associated with psychological complications such as depression and anxiety. Prompt and appropriate treatment is crucial both to improve lesions and to prevent the development of hyperpigmentation and scars [1].

Oral isotretinoin is a systemic retinoid used to treat patients with moderate to severe acne. Oral isotretinoin inhibits the function of sebaceous glands and shows anti-inflammatory effects [2]. Cheilitis is the most frequent dose-dependent side effect of isotretinoin, observed in approximately 90% of patients [3]. However, various side effects such as xerosis, pruritus, hair loss and photosensitivity have been reported after systemic isotretinoin treatment. In addition, musculoskeletal system disorders such as tendinitis and muscle and joint pain can also develop [2,3].

It has been suggested that about 50% of patients under isotretinoin treatment might develop increased serum creatine kinase (CK) levels, which indicate muscle damage [4]. Slight elevations in creatine kinase are usually reversible, while significant elevations may show rhabdomyolysis. Half of the patients with rhabdomyolysis are asymptomatic, and a minority of patients may develop fatigue and myalgia. Therefore, it has been proposed that it might be useful to evaluate serum CK levels before the initiation of isotretinoin treatment and regularly or when necessary during treatment [5].

In addition, patients with acne are usually monitored monthly, performing laboratory tests for potential side effects such as deterioration of the lipid profile and liver enzymes or complete blood count abnormalities such as leukopenia [6]. However, no consensus exists on the tests that should be performed in patients treated with isotretinoin. For instance, routine evaluation of complete blood count has not been recommended by Hansen et al. [6,7]. Moreover, it is controversial whether frequent laboratory testing is necessary during systemic isotretinoin treatment. It has been reported that laboratory tests might be checked before and two months after treatment, and in case the results are within normal limits, there is no need for further evaluation [6,8].

Herein, we investigated muscle and joint pain and serum levels of CK, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) in patients with acne vulgaris treated with systemic isotretinoin.

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained before this study (approval number: 2023-210). Between April 2019 and February 2023, medical records of patients with acne vulgaris treated with systemic isotretinoin were reviewed retrospectively. Age, sex, past medical history, chronic medication use, duration of acne, the age of onset of acne, and the severity of acne were evaluated. The severity of acne was classified as papulopustular and nodular acne. Isotretinoin dose, muscle or joint pain developed after isotretinoin treatment, localization of the pain, and time to onset of pain after treatment were also noted. In addition, serum levels of CK, AST, ALT, and GGT before and three months after the initiation of systemic isotretinoin treatment were evaluated by the biochemistry panel. The reference range was (46-171 U/L) for CK, (<34 U/L) for AST, (<49 U/L) for ALT and (<73 U/L) for GGT. Exclusion criteria were having liver and kidney diseases, using medications that may affect liver or muscle enzymes and intense exercise.

Statistical analysis was performed using SPSS version 25.0. Quantitative variables were stated as mean ± standard deviation or median (interquartile range). Categorical variables were stated as counts and percentages. The normality of the distribution of the data was evaluated by Kolmogorov-Smirnov test. Wilcoxon signed ranks test, chi-square test, and Mann-Whitney U test were used to determine the differences between the groups. P value less than 0.05 was considered statistically significant.

RESULTS

This study included 410 patients, 324 (79%) females and 86 (21%) males. The median age of the patients was 21 (6) years (range: 14-34 years). The past medical history was unremarkable in 391 (95.4%) patients. The characteristics of the patients are stated in Table 1. None of the patients reported regular use of a medication or excessive alcohol consumption. The patients were treated with oral isotretinoin at a dose of 10 to 40 mg daily. Among females, 220 (67.9%) had papulopustular, 104 (32.1%) had nodular acne, whereas among males, 44 (51.2%) had papulopustular and 42 (48.8%) had nodular acne (p=0.005). The median acne duration was 5 (5) years, (range: 1-20 years) in patients with papulopustular acne and 5 (4) years, (range: 1-20 years) in nodular acne (p=0.174). The median age of onset of disease was 15 (4) years, in patients with both papulopustular and nodular acne (p=0.693).

Table 1: Characteristics of patients with acne vulgaris

	Patients with acne vulgaris (n = 410)
Sex, n (%)	Females: 324 (79) Males: 86 (21)
Age Median (IQR), years Range, years	 21 (6) 14-34
Medical history, n (%)	Unremarkable, 391 (95.4) Seborrheic dermatitis, 3 (0.7) Asthma, 3 (0.7) Depression, 2 (0.5) Hashimoto's thyroiditis, 2 (0.5) Insulin resistance, 2 (0.5) Thalassaemia carrier, 2 (0.5) Allergic rhinitis, 1 (0.2) Urticaria, 1 (0.2) Familial Mediterranean fever, 1 (0.2) ITP, 1 (0.2) Mitral valve insufficiency, 1 (0.2)
Acne duration Median (IQR), years Range, years	 5 (5) 1-20
Age of onset of acne Median (IQR), years Range, years	 15 (4) 10-22
Severity of acne, n (%)	Papulopustular, 264 (64.4) Nodular, 146 (35.6)

IQR: Interquartile range. ITP: Idiopathic thrombocytopenic purpura. This study included 324 females and 86 males with acne between the ages of 14 and 34 years

The median serum levels of AST (p<0.001), ALT (p=0.024), and GGT (p<0.001) statistically significantly increased in all patients three months after systemic isotretinoin treatment. However, no statistically significant difference was ob-served in serum CK levels in all patients three months after systemic isotretinoin treatment (p=0.991) (Table 2). On the other hand, high serum GGT levels were significantly more frequent after isotretinoin treatment than those before treatment (p=0.012) (Table 3).

23 (5.6%) patients revealed muscle or joint pain during systemic isotretinoin treatment, while 387 (94.4%) patients did not have any musculoskeletal complaints. Low back pain was the most common and detected in 16 (3.9%) patients. In

addition, widespread pain was observed in 5 (1.2%) patients and leg pain in 2 (0.5%) patients (Table 4).

Table 2: Laboratory test results of patients with acne vulgaris before and three months after isotretinoin treatment

	Before treatment	After treatment	P value
CK, median (IQR) U/L Range: U/L	74 (43) 30-756	75 (40) 32-717	0.991
AST, median (IQR) U/L Range: U/L	18 (5) 9-215	21 (6) 8-853	<0.001
ALT, median (IQR) U/L Range: U/L	13 (9) 3-95	14 (9) 4-250	0.024
GGT, median (IQR) U/L Range: U/L	13 (6) 5-60	16 (7) 6-110	<0.001

ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CK: Creatine kinase. GGT: Gamma-glutamyl transferase. IQR: Interquartile range. A statistically significant increase was observed in serum levels of AST, ALT, and GGT, whereas no significant difference was observed in serum CK levels three months after systemic isotretinoin treatment.

Table 3: The frequency of high enzyme levels in patients with acne vulgaris before and three months after isotretinoin treatment

	Before treatment n (%)	After treatment n (%)	P value
CK	Normal: 379 (92.4) High: 31 (7.6)	Normal: 377 (92) High: 33 (8)	0.739
AST	Normal: 403 (98.3) High: 7 (1.7)	Normal: 398 (97.1) High: 12 (2.9)	0.251
ALT	Normal: 398 (97.1) High: 12 (2.9)	Normal: 395 (96.3) High: 15 (3.7)	0.532
GGT	Normal: 404 (98.5) High: 6 (1.5)	Normal: 393 (95.9) High: 17 (4.1)	0.012

ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CK: Creatine kinase. GGT: Gamma-glutamyl transferase. High serum GGT levels were significantly more frequent after isotretinoin treatment

Table 4: Patients with acne vulgaris who revealed muscle or joint pain after systemic isotretinoin treatment

	Patients n (%)
Muscle or joint pain	Yes: 23 (5.6) No: 387 (94.4)
Localization of the pain	Low back: 16 (3.9) Widespread: 5 (1.2) Leg: 2 (0.5)
Time to onset of pain after treatment	Within the first month: 5 (1.2) Within the second month: 9 (2.2) Within the third month: 9 (2.2)
Isotretinoin dose	10 mg/day: 6 (26.1) 20 mg/day: 10 (43.5) 30 mg/day: 7 (30.4)

Muscle or joint pain was detected in 23 (5.6%) patients with acne vulgaris after systemic isotretinoin treatment. Among them, low back pain was the most common

When patients were evaluated according to whether muscle and joint pain developed or not, no difference was observed in age (p=0.640), gender (p=0.436), duration of acne (p=0.919), the age of onset of acne (p=0.898) and the severity of acne (p=0.660). Nevertheless, among patients who developed muscle or joint pain, 6 (26.1%) received oral isotretinoin 10 mg/day, 10 (43.5%) received 20 mg/day, and 7 (30.4%) received 30 mg/day, whereas, among patients without pain, 13 (3.4%) received oral isotretinoin 10 mg/day, 177 (45.7%) received 20 mg/day, 174 (45%) received 30 mg/day and 23 (5.9%) received 40 mg/day (p=0.001). Furthermore, no statistically significant difference was detected in patients with or without pain in the median serum levels of CK, AST, ALT and GGT before and after systemic isotretinoin treatment (Table 5).

DISCUSSION

Musculoskeletal symptoms have been reported in about 25% to 87% of patients under isotretinoin treatment [9,10]. Various adverse effects have been described in patients with acne following systemic isotretinoin treatment such as myalgia, arthralgia, gout, spondyloarthritis, low back pain, muscle weakness, muscle stiffness, and myopathy [9,11,12]. Among them, temporary arthralgia or myalgia is the most common [13]. Musculoskeletal symptoms have been described even with low-dose regimens [9]. Drozd et al. revealed that low back pain was the most frequent musculoskeletal complaint in patients with skin diseases who received isotretinoin and that symptoms initiated within the first month of the treatment in 32.9% of patients, whereas between the second and the sixth month in 64.7% of patients. A dose reduction or temporary discontinuation of isotretinoin was recommended in patients with muscle pain or high CK levels [10].

On the contrary, De Francesco et al. stated that arthritis did not have a dose-dependent effect that it was unnecessary to discontinue the drug or reduce the dose, and that isotretinoin treatment could be continued at the same amount without complications [13]. Furthermore, Feily reported patients with acne aged 18 to 29 years who developed musculoskeletal symptoms such as back and leg pain within two months after the initiation of oral isotretinoin treatment and responded to folic acid and vitamin B12 supply [14].

Table 5: Characteristics and laboratory test results of patients with and without muscle or joint pain after systemic isotretinoin treatment

	Patients with pain	Patients without pain	P value
Sex, n (%)	Female: 20 (87) Male: 3 (13)	Female: 304 (78.6) Male: 83 (21.4)	0.436
Age, median (IQR) years Range, years	21 (4) 16-31	21 (6) 14-34	0.640
Acne duration, median (IQR) years Range, years	5 (2) 1-14	5 (5) 1-20	0.919
Age of onset of acne, median (IQR) years Range, years	15 (3) 10-22	15 (4) 10-22	0.898
Acne severity, n (%)	Papulopustular: 16 (69.6) Nodular: 7 (30.4)	Papulopustular: 248 (64.1) Nodular: 139 (35.9)	0.660
Patients received different isotretinoin doses, n (%)	10 mg/day: 6 (26.1) 20 mg/day: 10 (43.5) 30 mg/day: 7 (30.4)	10 mg/day: 13 (3.4) 20 mg/day: 177 (45.7) 30 mg/day: 174 (45) 40 mg/day: 23 (5.9)	0.001
Before treatment CK, median (IQR) U/L	70 (29)	75 (45)	0.494
n (%)	Normal: 22 (95.7) High: 1 (4.3)	Normal: 357 (92.2) High: 30 (7.8)	0.549
AST, median (IQR) U/L	17 (3)	18 (5)	0.389
n (%)	Normal: 22 (95.7) High: 1 (4.3)	Normal: 381 (98.4) High: 6 (1.6)	0.334
ALT, median (IQR) U/L	14 (7)	13 (9)	0.620
n (%)	Normal: 23 (100) High: 0	Normal: 375 (96.9) High: 12 (3.1)	0.392
GGT, median (IQR) U/L	13 (5)	13 (6)	0.299
n (%)	Normal: 23 (100) High: 0	Normal: 381 (98.4) High: 6 (1.6)	0.548
After treatment CK, median (IQR) U/L	69 (39)	75 (41)	0.147
n (%)	Normal: 22 (95.7) High: 1 (4.3)	Normal: 355 (91.7) High: 32 (8.3)	0.502
AST, median (IQR) U/L	21 (8)	21 (5)	0.649
n (%)	Normal: 23 (100) High: 0	Normal: 375 (96.9) High: 12 (3.1)	0.392
ALT, median (IQR) U/L	15 (14)	14 (9)	0.832
n (%)	Normal: 21 (91.3) High: 2 (8.7)	Normal: 374 (96.6) High: 13 (3.4)	0.203
GGT, median (IQR) U/L	16 (9)	16 (7)	0.285
n (%)	Normal: 23 (100) High: 0	Normal: 370 (95.6) High: 17 (4.4)	0.613

Muscle damage due to isotretinoin treatment is generally mild and presents with well-tolerated pain or tenderness of muscles and increased serum CK levels [15,16]. However, isotretinoin can lead to severe muscle damage such as rhabdomyolysis which may result in renal insufficiency and even mortality [5,16]. Evaluation of CK has been recommended in patients treated with oral isotretinoin, especially in those who exercise regularly [5,15]. Furthermore, during follow-up, muscle and joint pain should be questioned [16]. Besides CK, laboratory tests performed to evaluate possible side effects in patients treated with systemic isotretinoin include serum levels of AST, ALT, lipid panel, and complete blood count [17]. The American Academy of Dermatology guidelines of care for the management of acne vulgaris recommend monitoring liver function tests and serum lipids until treatment response is achieved [18]. Zane et al. stated that among patients who used isotretinoin, 11% developed increased serum levels of AST or ALT which was usually mild and reversible, and that they principally evaluated ALT levels since ALT was more specifically indicated liver damage than AST [19]. On the other hand, Webster et al. stated that although AST and ALT were used to detect liver damage, they were not specific to the liver, they were also found in muscles, whereas GGT was a liver-specific and CK was a muscle-specific enzyme. In addition, Webster et al. suggested that monitoring serum GGT level was more beneficial than AST and ALT to detect liver damage in patients treated with isotretinoin for acne and that the increase in AST originated from the muscle rather than the liver [17].

Within this study, the median serum levels of CK, AST, ALT, and GGT were increased three months after oral isotretinoin treatment, however, a statistically significant increase was detected in serum AST ($p<0.001$), ALT ($p=0.024$) and GGT ($p<0.001$) levels. The number of patients with high serum CK, AST, ALT, and GGT levels increased after treatment. Nevertheless, a statistically significant increase was observed only in the number of patients with high GGT levels ($p=0.012$).

Muscle or joint pain occurred in 23 (5.6%) patients, among them, low back pain was the most common. There was no significant difference in age, gender, acne severity, acne duration, age of onset of acne, and serum CK, AST, ALT, and GGT levels between patients who developed and did not develop pain after isotretinoin treatment. On the other hand, isotretinoin treatment dose differed significantly in patients with and without pain ($p=0.001$). Treatment with isotretinoin at a dose of 10 mg/day was more common in patients with muscle or joint pain than in those without pain, whereas none of the patients who received isotretinoin 40 mg/day developed muscle or joint pain.

CONCLUSIONS

In conclusion, since no significant change was detected in serum CK levels with isotretinoin treatment, as well as no association was observed between serum CK levels and muscle or joint pain, we suggest that routine evaluation of serum CK levels is not necessary in patients with acne vulgar-is treated with isotretinoin. Although muscle or joint pain was observed in 5.6% of patients with acne vulgaris used isotretinoin, it should be kept in mind that muscle and joint pain may develop regardless of the isotretinoin dose, even at doses as low as 10 mg daily. Our results revealed a significant increase in serum AST, ALT, and GGT levels due to isotretinoin which indicated that monitoring serum AST, ALT, and GGT levels might be beneficial in patients with acne vulgaris treated with systemic isotretinoin.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, F.T., L.F., E.A.; methodology, F.T., L.F.; software, F.T.; validation, F.T., L.F., E.A.; formal analysis, F.T.; investigation, F.T., L.F.; resources, F.T., L.F., E.A.; data curation, F.T., L.F., E.A.; writing-original draft preparation, F.T.; writing-review and editing, E.A.; visualization, F.T.; supervision, E.A.; project administration, F.T., L.F., E.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2023-210).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

A Comprehensive Review of the Factors Affecting the Redefinition of Therapeutic Use in Metropolitan Cities during the Emerging and Re-emerging Pandemics

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Abstract: *Resilience refers to the means and ability to deal with adverse conditions. Resilience is defined as the ability of the system to recover easily after disturbances and changes in the system and the ability of the organization to respond quickly to them. The resilient system can withstand environmental pressures so that it can provide optimal performance in critical situations. Resilience in the use of healthcare services is related to reducing vulnerability to shocks caused by natural disasters and increasing adaptive capacity due to improved measures and opportunities. Considering the importance of evaluation, monitoring, and planning to improve user resilience against accidents, spatial evaluation and redefinition of the use of health and medical services to prepare a comprehensive model for measuring the resilience of hospitals against accidents and calamities Naturally, at the top of which is the coronavirus epidemic, it is necessary. The aim of the current study is a comprehensive review of the factors influencing the redefinition of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a pandemic disease with a resilience approach. It is expected that the managers and decision-makers of the country's health field can use the results of the present research for better planning.*

Keywords: *Resilience, Economic factors, Metropolis, Healthcare services, Coronavirus*

INTRODUCTION

The accelerated and unbalanced development of physical space in some countries in the last few decades has brought adverse social, economic, and physical effects and consequences [1].

The exorbitant costs of urban transportation and services, energy waste, wasting material and social capital in the city, intensifying social segregation, environmental destruction, lack of beauty and cohesion of the city environment, social identitylessness, and instability are among the most important problems of cities in the discussion [2]. Undesirable development of physical space and low density of cities are considered. We are now living in the era of urbanization and urban development, where the ecology and morphology of cities have been increasingly affected by human and natural activities, with the development of urbanization and the desire of people to live in cities, as well as the occurrence of such diseases [3]. In recent years, the existing healthcare spaces in big cities have been seriously challenged. So during the peak of the epidemic, it reaches its peak time and the lack of these uses (hospitals and other medical centers) is felt in urban communities. For this reason, in recent years, many cities have implemented measures to create such spaces (sanitary-therapeutic) during the crisis of pandemic diseases [4].

In Iran, especially since the 1950s, the need to compile different levels of the health network cycle of the country has been felt and based on specific studies of culture, geographical environment, the population of each region, and various types of treatment facilities, from health centers to hospitals. This important thing has been specified and defined. Of course, the country's goal of these divisions is to create the possibility of greater efficiency of financial, equipment, construction, human resources, etc. [5]. In the shortest possible time and with the highest quality of treatment and health, especially when pandemic diseases (coronavirus) occur. But the format of city managers looks for other things beyond these necessities [6]. Improving access to medical services as an indicator of quality of life and social welfare plays an important role. Equal access to medical services for different sections of society in a fair and balanced distribution of these services is one of the important foundations of social welfare [7]. Treatment spaces can be designed in 2 formats: 1) Health spaces and 2) Health-treatment spaces The basis of work in the health management system of countries is the discussion of the care and survival system of health centers and the chain of health spaces. Along with and parallel to the completion of this chain, the treatment system is proposed and then the treatment spaces are defined [8]. Although in most countries, the health care system is necessarily more complete than the treatment chain, but the possibility of full adaptation of these two chains to provide health services in cities is one of the ideals of every country. Today, the growth and development of urbanization have led to the creation of many facilities, but it should be kept in mind that with the growth of urbanization, the factors that because crises have also increased [9]. The issue of access to urban services is one of the important issues that affect the city from various dimensions. In some cases, the lack of proper access to some uses puts people's health at risk. And suitable for it is necessary for all members of the society to access services from two dimensions: the access of the target space is not the space that can be examined [10]. Access is one of the key components affecting the important quality of the urban environment, among the important ideals in this field is homogeneous access with the highest degree related to urban facilities [11]. The sustainability of cities and even megacities when pandemic diseases start, depends on the function and ability and more optimal use of health and treatment spaces, especially urban hospitals, therefore, based on the announced policies of the urban planning and treatment system of each country, it is effective for the life of that city [12]. It will be useful, however, when such crises (pandemic disease) occur, in such a way that it shows the best reaction and response in the shortest possible time during the accident. This will require the review, definition, and re-review of the uses of health and treatment spaces during pandemic crises (after the start of the coronavirus) [13]. Natural and man-made disasters inflict various types of damages, injuries, and death on the affected communities. Such disasters can disrupt various infrastructures and facilities, including hospitals, schools, transportation systems, and emergency services [14]. Non-attendance or lack of preparation in the continuity of services in emergencies caused by accidents has destructive and unfavorable effects on the responsiveness of hospitals. Recent disasters around the world have resulted in irreparable damage to hospitals and disruption of medical services [15]. One of the calamities that has spread all over the world today and has affected all the countries of the world is the coronavirus. In December 2019, the first case of coronavirus was reported in Wuhan, China, and due to the high death rate caused by it, it caused global panic. The rapid transmission of this virus has led to the diagnosis of more than a few million patients over the past two years [16]. A significant increase in the number of infected patients has made the work of medical and health centers difficult in dealing with this disease. Disruption and inconsistency in the provision of medical and health services can lead to irreparable losses and damages [17]. Therefore, it is very important to encourage researchers and decision-makers in order to prepare strategies for improving resilience in health and treatment centers [18]. The aim of the current study is a comprehensive review of the factors influencing the redefinition of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a pandemic disease with a resilience approach.

METHODS

This research is a scoping review that has been conducted by searching for systematic studies to identify the factors influencing the redefining of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a new and re-emerging pandemic disease with a resilience approach using the Cochrane protocol in 1402. accepted. The study inclusion criteria were defined as follows:

Publication date: Due to the comprehensiveness and breadth of both keywords of the research, preparedness against emerging and re-emerging pandemic diseases and the spatial pattern of therapeutic uses of megacities were searched with a resilience approach. In terms of time, only researches that were published in the last decade, from 2010 to 2023 AD and from 1390 to 1402 AD, were examined.

Language: Due to time, financial, and manpower limitations, only the research published in English or Farsi was examined. On the other hand, to evaluate the quality of the studies and use them in the review, only the researchers whose texts are accessible to the researcher have been used.

Place of publication: Relevant sources published in reputable scientific journals, conferences, and theses were used.

Type of studies: Quantitative, qualitative, mixed, or review studies were examined.

The extracted sources were examined by two researchers independently. In this way, the mentioned keywords were first searched in reliable Persian and English scientific databases, including Direct Science, Scopus, PubMed, Google Scholar, and SID in the period from 2010 to 2023. The reason for choosing these databases was their validity and comprehensiveness including a large number of authoritative English-language publications in the field of health and environmental studies, as well as the availability of access to most of the articles and books of these databases for the researcher. In the next step, articles whose full text could not be accessed were excluded from the study. Then, the articles that appeared more than once in the search results were removed using Endnote software, and according to the title of the article, articles not related to the topic were also removed. In the next step, the abstracts of these articles were studied more deeply, and according to the proposed limitations, at this stage, the articles that did not meet the mentioned criteria were removed. Finally, to evaluate the quality of the articles, the standard evaluation checklist (Critical Appraisal Skills Program: CASP) of qualitative studies was used, and the studies that received a score of 25 or higher were included in the study. Due to the inconsistency of included studies and the impossibility of meta-analysis, the meta-synthesis method was used to analyze the data. Out of 2583 reviewed articles, after considering the entry and exit criteria, 37 articles were included in the final review.

RESULTS AND DISCUSSION

The topic of social justice was first introduced to the science of geography by David Harvey in the book Social Justice and the City in 1976. In this book, Harvey has examined social justice with a radical Marxist perspective. In general, many articles and theses have been presented in the discussion of social justice in Iran, the most important of which are mentioned in the following. English et al. (2021) in the research they conducted in the city of Seoul, South Korea, have concluded that in a city, despite having enough services per capita (such as a park), due to improper distribution, they may be available to everyone.

Therefore, the residents have evaluated the distribution of parks concerning the population and other uses of the city and the proposed density of the areas. The results of their findings show that some of the residential areas in Seoul are deprived of access to city parks [19]. Esser et al. (2019) conducted research titled Capacity in Education: A Step Towards Social Justice, which was carried out from a 3-year collaborative activity of 8 primary schools in Darussalam and the coastal region of Powani in Tanzania, and concluded that there are countless obstacles to capacity (Education) In the world, communities and school levels exist, and in Tanzania some progress has been made in terms of (education) capacity, but equity and quality of education still have a long way to go [20].

In their article, Yasobant et al. (2019) express the idea of locating hospital centers in cities, in general, there is no long history in the field of locating medical and health service centers, and it goes back to the 1970s, since 1979, the department Social security health in England paid attention to the strategic development of medical and health service centers in cities, and it was followed in Austria in 1980 and 1982 [21].

Manenti et al. (2020) in an article on urban health addressed the evidence, challenges and guidelines. As a result, we note that although in this review we highlight challenges, this is due to the potential power of urban health research and the importance of studying health. Although the study of urban health encompasses considerable complexity, research with specific research questions and appropriate study designs can improve our understanding of the relationship between specific characteristics of urban nature and health in both specific cities and as generalizable to National cities and recent methodological advances, especially the widespread adoption of multilevel methods in public health research, have made it possible to test hypotheses about urban characteristics and their association with specific health outcomes. Finally, newer methods may contribute to a better understanding of competing influences on the health of urban populations over time. Such research can inform local interventions and policies in urban areas. We hope that efforts such as this review aim to build Our thinking about cities and health will help stimulate both empirical and theoretical developments that can lead to improved health in cities around the world [22].

Schäfer et al. (2021) in research have examined the spatial access of primary care, including methods, challenges, and concepts, and finally concluded that almost all healthcare centers to date, whether based on simple or complex measures, are limited to social inequality in access to health services and to investigate how much primary care centers affect people's health [23]. Sevá et al. (2017) have conducted a research regarding the access and use of health services in Ghana, in which the aim is to investigating the impact of access and socioeconomic factors on the use of health services in developing countries (specifically Ghana). Utilization of health services in Ghana distribution shows a significant difference between rural and urban areas in all variables, except health status and age. Even though the metropolis uses relatively more health services than the villages, the difference in the health status (need) of the two districts is not significant. The coefficient between the two districts is insignificant. Therefore, there is a difference between the need and use between the districts. Success is part of a local understanding of the dimensions and determinants of access to health services, as well as concrete efforts to improve services for the poor. Part of the innovations in health care financing and regulation of care promises to improve access for the poor in any case.

The challenge remains to find ways to ensure that vulnerable populations have a say in how strategies are developed, implemented, and formulated, and to ensure that information and incentives are channeled in ways that can lead to improvements in access for the poor. It is expressed whether or not it can be given [24].

Morris et al. (2021) researched regarding the regulation of access and use of health services. The results showed, first, descriptively, how the countries are using different doctors to refer to a specialist. Second, a multilevel analysis shows how regulatory indicators affect each quantity of care consumed in a country, and finally, they examine how institutional structure affects differences in service utilization among groups with different incomes, education levels, and Health statuses [25].

Lingam et al. (2021) studied the determinants and pattern of health service consumption among graduate students and the result of this research showed that the healthcare system in Pakistan includes public and private health centers. In Pakistan, the majority of doctors are in the regions. They live in the city and attract the educated classes than the private sector. The use of public healthcare facilities is not the main priority of the educated class of the country, and government officials should pay more attention to making it more common for the people [26].

Gatto et al. (2022) studied poverty and access to health care in developing countries. The result of this research showed that despite the progress in access to health care in developing countries, a significant number of their population has limited access. Poor countries have a higher burden of disease but usually have less access to health care. Whether based on geographical access, accessibility, physiological access, acceptance or quality of care. However, recent studies show that this exodus is inevitable [27].

Peng (2020) discussed the optimal location for the construction of a hospital. In this study, the AHP method was used as one of the methods of multi-criteria analysis and GIS. The result of the study showed the use of the hierarchical analysis process in location. The hospital has been very efficient in the study area [28].

Lewis et al. (2021) investigated the improvement of access to health services in urban and regional areas for indigenous people. The results of this literature study provide information about barriers to access to health and mental health services for local people living in urban and regional communities. These barriers can be classified as barriers of accessibility (physical accessibility), affordability, appropriateness, and cultural acceptance [29].

Sousa et al. (2021) analyzed the equity and use of health services in Uganda in a research that focused on several factors (supply and demand) that affect the access and use of health services in Uganda, such as Education, age, household expenses, use of the outpatient department, hospital capacity and the distance from medical services, this study has analyzed a number of measures that are used in the implementation of policies, especially in the main fields. Key policies such as malaria, tuberculosis, and HIV/AIDS have been created. The analysis shows, however, that despite efforts to improve health by improving health outcomes for people, mountainous challenges still remain. For example, living near essential health services, although they work as an incentive to use care, does not necessarily ensure that health services are actually used. Secondly, the elimination of the usage fees of all government centers will increase the utilization of new healthcare facilities, especially fixed and fee-based service providers with catastrophic costs [30].

Joshi et al. (2022) in a study investigated the access and use of health care among rural households in Nigeria. The results showed that the average age of the respondents was 46 years and the average household was 8 people. The majority of the respondents, 43.5%, do not have formal education and the main occupations are agricultural officials. 58% of the respondents have access to medical services, while only 42.5% use these services. Most of the respondents, 40.5%, are 5-9 kilometers away from accessing medical centers. Accessibility indicators show unequal access to modern health facilities in the study area, so this study recommends that rural development policies should create an organizational environment to increase participation and access to justice in the provision of modern health care throughout rural areas. strengthen in the country [31].

McKenzie 2017, in an article on the access and use of health services in Massachusetts, the United States, conducted a qualitative study of the views and experiences of immigrant women in Brazil. The findings of the research show that illegal immigrant women in Brazil are more likely to access health services in countries that have health services. They are satisfied with the global level. Health care is critical to quality of life, but many immigrants and ethnic minorities often lack adequate access to health care, increasing health risks. This study adds to the current literature on healthcare access and use by immigrants living in the United States and also provides new information on Brazilian immigrant women's perspectives and experiences of healthcare access and use in Massachusetts, USA to give While identifying the opportunities to improve the services and health of the entire immigrant population [32].

Allam et al. (2022) examined the relationship between transportation networks and healthcare services and their impact on urban sprawl. The findings of this study show that there are more dynamic relationships between the content of these urban components and access to health care [33].

Das et al. (2021) investigated the design of decision support systems for access to space services. The results of the research showed that improving access to health care can help reduce health costs, increase healthcare equity, and improve health outcomes. Through our research, we have designed a new application-based framework for measuring access to health care and a practical example of SDSS that is rooted in the theoretical concepts of BM and existing FCA methods. Our unique method to obtain the weight of the population characteristics is provided by the use of predictive analysis using real data to evaluate the need to integrate non-spatial factors into existing algorithms in the creation of SPAI. The findings should encourage future research to further examine how demographic characteristics may shape the demand for health care gives the hardness by considering the actual manufacturer's needs [34].

Cotta et al., 2020, reviewed the approaches to measuring potential spatial access to urban health services in a research where they examined the types of distances and their related issues [35].

Berendes et al. (2018) examined the urban status and health competence of critical issues and exercises, and it was concluded that if the global community is serious about the sustainable development goals of SDGs, more attention should be paid to understanding and taking action to improve urban conditions to have a life and social and economic conditions that can promote health competence. The result of the research showed that healthy and fair urban places are very important to achieve health for all the population, however, strict emphasis is placed on built environments or risk factors. A single person does not address the numerous and often cumulative social and environmental obstacles that exist in cities today and does not help the inequalities that burden poor urban communities. To collaborate or help the urban poor to analyze and solve problems. We realize that this will not be easy and the evidence base for how to do this effectively is still emerging. Our challenge is great, but in an urban planet public health Environment is urban health and health justice should be our goal [36].

Pak et al. (2018) investigated network and spatial analysis to evaluate and guide decision-making about equal access and health services in the general patient care system in Ederdamora (Spain). The simultaneous use of access and coverage maps to show the relative distribution of resources in a population, while showing the coverage levels according to the time of access to physical and professional facilities with and without administrative restrictions, can help decision-makers to improve the optimal level of stocks in a region. Large and low-density geography is very valuable [37].

Coccia et al. 2020 studied the issue of access to health care by urban refugees and the host population around Uganda. The results of this research showed that health services were available every five kilometers for both refugees and the host population. Refugees are generally unable to pay for access to health services, including transportation, research, essential drugs, and other informal costs. Refugees' perception of public health services is weaker compared to general citizens. Primarily due to long-term expectations of a small number of inadequate essential drug services, perceived discrimination and the existence of unofficial fees [38].

Paris et al. (2020) in a study to measure the resilience of traditional societies against natural disasters have concluded that traditional societies face natural disasters using four dimensions: nature of risk, level of resilience, cultural structure and policies. Managers can show resilience in the face of disasters [39].

Finn (2022) conducted a research as a place-based model to understand the resilience of local communities against natural disasters. This study presents a new framework of the place of urban resilience in order to improve the method of assessing resilience against disasters at the local and regional level, and a set of variables have been considered in this study as the first step in the target research. The final output of this research is presented in the form of environmental, social, economic, institutional, infrastructure, and competence of society [40].

Park et al (2022) in an article titled "Towards resilient regions: regional development policy" use a descriptive-analytical method and emphasizes the economic dimension of resilience and analyzing indicators of employment rates, large-scale economic centers, income and production gross and knowledge mobility introduced the process of making the regions resilient [41].

Plastina et al (2019) worked on an article entitled Regional planning and the future of resilience in the Ning ladder coral coastal region of Australia in 2019, which was the indicator of resilience and the amount of tourist attraction, which finally reached the correlation between resilience and tourist attraction [42].

Teng et al (2022) in an article titled "Tissue-based identification of old urban neighborhoods in Hyderabad, India using remote sensing data with a renovation approach, concluded that the location map of worn-out tissue is an efficient tool in identifying areas." The densely populated city can be used as a reliable source for vulnerability and resilience assessment in the next step. The presented method allows for quick analysis and comparison of multi-temporal data and can be used in many densely developing cities around the world [43].

Acharya et al. (2017) collected the articles and opinions of different people in one collection and investigated the reconstruction of built environments after disasters in order to increase resilience and found that resilience should be considered among the necessities of reconstruction [44].

Hasson et al. (2022) categorized urban resilience indicators from a landscape ecologist perspective and the result of this article introduces five urban design and planning indicators to create urban resilience, which include redundancy, multi-functional (biodiversity) Being an urban system, urban ecological networks and connection with connections, adaptive design or adapting to changes, he still emphasizes the combination of ecological principles and urban planning to achieve urban resilience [45].

Ferdowsi and Arkhodi (2016) in an article entitled "Evaluation of health and medical resilience of Semnan province cities against natural disasters" stated that due to the high importance of health and medical resources in order to improve the resilience of settlements against natural disasters Naturally, managers and planners should have a special approach to providing healthcare facilities and infrastructure. The purpose of this research is to evaluate the cities of Semnan province based on health-medical indicators effective in resilience. In this research, the desired indicators have been evaluated using the Vikor model. According to the results, the cities of Shahrood, Damghan, Garmsar, Aradan and Sorkheh have a more efficient performance in providing services during the occurrence of natural disasters by having proper medical facilities and infrastructures, and in other words, they have favorable health-medical resilience in the face of natural disasters. They will have accidents. On the other hand, the cities of Mahdishahr, Miami and Semnan, due to the lack of appropriate proportion between the population and medical facilities and infrastructure, will have low resilience when faced with accidents compared to other parts of the province. In general, by evaluating and analyzing this process in Semnan province and resilience strategies, managers and planners can lead the cities of the province to strengthen resilience against natural hazards, considering the existing capacities and competitive advantages [46].

Saffari Darberazi et al. (2019) in an article entitled "Designing a Comprehensive Hospital Resilience Model in the Face of Corona Disease" stated that healthcare centers should be able to quickly adapt to catastrophic events such as natural and human disasters. One of the ways to deal with various disasters in healthcare centers is to increase resilience.

The current research aimed to design a comprehensive hospital resilience model by identifying the factors affecting hospital resilience, and the way these factors are related in the face of the corona disease. At first, by using the background study of the research, the effective factors on hospital resilience were identified, and then by using the interpretive structural modeling technique, the communication model between the identified factors was obtained. The conceptual model obtained from interpretive structural modeling was fitted using Smart PLS software version three. For this purpose, the questionnaire containing 33 questions was provided to 80 managers, experts, and employees of Yazd teaching hospitals. The research results indicate the identification of eight general factors affecting hospital resilience. The eight factors identified in the present study were structured in four general levels by interpretive structural modeling. The starting level of the model is the factors of "sustainability" and "information technology and communication system". Also, the model-fitting results confirm the relationships formed by interpretive structural modeling. Based on the results of the present research, the country's health and treatment managers can use to make the country's hospitals resilient in the face of natural disasters and unforeseen events [47].

Ebrahimzadeh et al (2009) in an article entitled "Spatial-spatial planning and organization of health and medical services using GIS case: Zanjan city" found that in most cities there is a lack of appropriate allocation of space and optimal placement of service and physical elements. The city, especially the medical centers (hospitals) and the number of effective factors in the location of these centers, has followed and continues to increase the problems of the city and its citizens. Therefore, this has revealed the necessity of using effective methods and computer systems in the optimal location of these centers. The current location of most of the medical centers and hospitals in Zanjan city does not match the scientific criteria of the needs of this land use, at the same time, the findings of this analysis show that the current need of medical centers in Zanjan city is at least 7 hospitals according to the city's per capita, which the city officials They can use 11 points extracted from gis to solve this deficiency [48].

Rahnama and Amirfakhrian (2010) analyzed spatial access to healthcare services in the city of Mashhad. They stated that access planning, as a method of integrated planning of users and transportation networks, has two spatial and non-spatial dimensions. The availability and availability of services is one of the dimensions of spatial access, and this study examines medical services in Mashhad. The study method is of descriptive-analytical type, based on which, after forming a spatial data bank of therapeutic spaces in ARC-GIS software (including 6 major uses with 4528 frequency items) and using accessibility models. two-stage floating basin", the condition of Mashhad's districts' access index to each of the treatment spaces was determined. The advantage of using this method, compared to other methods, is the simultaneous use of indicators such as distance, functional radius, and number of facilities. The result of this analysis shows the mismatch between the spatial distribution of the population and the distribution of medical services. In other words, the central areas of the city have the best access and the peripheral areas have the weakest access to medical services. In the following, while using SPSS software and the "two-stage cluster analysis" model, 1266 population zones of Mashhad city were classified in terms of access to health care services. In this section, the results of the findings showed that the city of Mashhad can be classified into two clusters, including the first cluster with an average accessibility index of 3.24, including 2.3% of urban areas (areas with excellent access conditions) and the cluster The second one with an average accessibility index of 0.061 includes 97.7% of the domains, which does not show a good situation compared to the first cluster. The graphs prepared from the evaluation of this model showed that the most important indicator regarding the difference between the first cluster and the second cluster is the index of access to pharmacies. On the other hand, the index of access to emergency bases shows the least role in this classification [49].

Shojaiyan and Rahimpour (2017) in an article entitled "Evaluation of land suitability of health-treatment centers using CF analysis (case study: regions one and eight of Ahvaz city)" stated that the assessment of urban land suitability is the basis of urban planning. Especially in the field of proper use of land. This important issue directs the existing uses in the city in such a way as to prevent incorrect and incompatible adjacencies and ultimately lead to the establishment of land suitability. The present research was carried out with a descriptive-analytical approach in order to evaluate the land suitability of health-therapeutic centers in areas one and eight of Ahvaz city. ArcGIS software and CF relationship were used to achieve the mentioned goal and analyze the obtained information. Finally, the spatial distribution of land and the suitability percentage of each of the selected uses were determined with health-therapeutic use. The results of the research showed that among the uses studied, industrial use with 16.17%, residential use with 8.17%, and then fire stations with 7.53% had the highest incompatibility, and main roads with 0.71% had the lowest incompatibility. Is. In the end, 41.09% of the total area of the studied area is occupied by incompatible areas, 18.66% by neutral areas, and 13.02% by compatible areas. According to the conducted studies, the suitability of the land and the proximity of the selected uses in relation to the health-therapeutic use in the studied area have not been proportionately observed [50].

Motamedi (2019) in a research article entitled "Investigation and locating the access of the citizens of Shirvan city" to therapeutic uses. In this article, one of the basic and important tasks of urban and regional planners is to allocate land to various urban uses while paying attention to the role and function of the city, the economy of the city, and also the effect of mutual factors of uses on each other. The results showed that in terms of location, the studied clinics are concentrated in the eastern side of the city, and the central and western parts of the city are limited in terms of access to the clinics, and only 28,136 people out of the city's population of 83,000 have access to the standard radius range of the clinics; In other words, it can be said that about 75% of the city's population is outside the standard radius of the clinics, and the access radius of the clinics is not suitable for the citizens of the city, and there is a need for a well-equipped hospital in the west of Shirvan [51].

Hataminejad et al (2017) in a research entitled "Analysis of access to urban services in Esfrain" which was conducted using a questionnaire and unbalanced Morris index, concluded that the upper socio-economic classes in the city of Esfrain, in terms of having better financial conditions, they have more favorable uses, and the distribution pattern of service uses is in favor of more prosperous groups so that it is not consistent with the concepts of social justice [52].

Rostami (2010) in the research "Investigation and analysis of urban public service distribution from the perspective of spatial justice, case study: Yasouj city" the degree of access and need of residents to public services using the access network analysis model, hierarchical analysis model and analysis model local spatial autocorrelation has been investigated and analyzed. He also used the Gini coefficient to analyze the inequality of access to urban services and the Moran coefficient to analyze the pattern of distribution of injustices among urban blocks and finally concluded that urban public services are based on accessibility, public benefit, efficiency Population distribution and residents' needs for public services have not been fair and facilities are concentrated in certain areas [53].

Rahnama and Zabihi (2011) analyzed the distribution of public facilities in line with spatial justice with the integrated model of access in Mashhad in 2013 and in this research they concluded that the integrated access analysis of services with the help of geographic information system and spatial autocorrelation technique This indicates that the spatial distribution of public facilities based on Moran's index is clustered and based on the general index, it is a cluster with low concentration. The final result is that the spatial distribution of public facilities is not fair based on the integrated model [54].

CONCLUSIONS

The range of topics and topics of strategic urban research is very wide. For example, studying sports, recreational, therapeutic, educational spaces, police stations, fire stations, etc. All of them are topics that the researcher can investigate. To scientifically and accurately examine urban issues, it is necessary to carefully examine the components and influencing factors according to their type and degree of importance. In addition, due to the rapid growth and expansion of cities, the existence of a multitude of variables affecting them, as well as the incredible amount of information, the use of traditional methods in the analysis of problems is not very reasonable and expressive, and it is misleading. Factors such as population growth, migration and its consequences, and the fair and optimal distribution of services in the city environment and especially the metropolises are associated with obstacles and problems, therefore, adopting efficient and appropriate policies in relation to the provision of services and optimal allocation of space for the population living in different sectors of the city (outskirts, center, etc.) is one of the important goals of urban planning for quick and low-cost access to citizens. In this chapter, theories and models such as spatial allocation models, access models, attraction models, therapeutic approaches, sustainable development theory, and spatial theories were discussed.

Therefore, considering the problems stated above and the large spatial scale and analysis of a large amount of mechano-descriptive and statistical information, the researcher was motivated to solve various problems by having analytical functions (especially network analysis facilities, spatial allocation models and modeling in general) including analysis of accesses, determination of sphere of influence, location, and allocation of resources, sustainable development, development of social justice and so on.

With the help of these tools and statistical analysis, reach scientific results. Biological accidents and threats, re-emerging, and emerging diseases are increasing, and in these diseases, accidents, and threats, the first place to get involved is the Ministry of Health, especially hospitals. Hospitals must have the necessary preparation to face these incidents and threats so that in case of such incidents, they have the least vulnerability reduce casualties, and can provide the best and safest care to clients. One of the ways to create readiness is to conduct exercises and maneuvers, therefore, for this purpose, hospitals must write and practice several scenarios regarding various types of accidents and biological threats.

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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REVIEW

Immunomodulatory Effects of Statins in the Treatment and Prophylaxis of Influenza Viruses

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Abstract: *Background and aim: Influenza, commonly referred to as "the flu," is an infectious illness caused by influenza viruses (IV). Despite the availability of efficient antiviral drugs, the IV still significantly increases mortality. Antiviral drugs swiftly remove these viruses from pulmonary secretions, suggesting that a prolonged inflammatory response may be to blame for the poor result. Therefore, using immunomodulatory medications looks advantageous. Numerous anti-inflammatory effects are produced by statins. Numerous retrospective studies recommended that statins be considered for IV therapy. This study set out to comprehensively examine the immunomodulatory potential of statins in IV prophylaxis and therapy. Methods: From the beginning until 25 March 2022, a number of electronic databases (Scopus, EMBASE, PubMed, Web of Science, ProQuest, OVID, EBSCO, and CINAHL) were searched for cohort studies and randomized trials assessing the association between outcomes or risk of infections and statin therapy. Data were gathered on the investigated characteristics, measuring statin usage, quality evaluation, and results (set for potential confounders). Results: Finding additional therapeutic agents for influenza prevention and management is crucial since there are uncertain public health issues regarding the clinical efficacy of conventional medications. Pro-inflammatory cytokines are released as a result of influenza. According to the results, by using anti-inflammatory medications, severe influenza infections can be prevented along with pleiotropic effects. As a result, statins, which are cardioprotective medications with immunomodulatory and anti-inflammatory properties, may be beneficial for individuals with IV. Conclusion: Based on the positive immunomodulatory effects of statin administration, our systematic review's findings suggested that IVs may be prevented and treated.*

Keywords: *immunomodulation, antiviral, anti-inflammation, statin, prophylaxis, Influenza viruses*

INTRODUCTION

Despite the Influenza virus (IV) being recognized as a serious pathogen during the past century, influenza continues to cause a sizable number of ailments. According to estimates, only in the US in 2017–2018. (Table 1) [1].

Universally, The worldwide WHO estimates that each year, influenza epidemics cause 3,000,000–5,000,000 serious illnesses, with 290,000–650,000 recorded fatalities due to the severity of IV [2]. Although yearly influenza vaccinations are advised and antiviral drugs are available, both have a number of limitations. Following influenza infection, immunological dysregulation, which is determined to release the pro-inflammatory cytokines generating major risk factors for mortality and morbidity, is the cause of the severity of the disease and clinical presentations [3]. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are the most significant implicated cytokines, generating innate immune responses and inflammation [4]. The antigenic changes over time (requiring a precise strain match annually), vaccine strain egg-adapted changes, slow manufacturing processing, the absence of cross-reactivity, brief duration of protection, and poor immunogenicity in certain populations (for example, the elderly) all play a significant role in the effectiveness of the seasonal influenza vaccine [5,6]. If given at the right moment during the illness, antiviral medications such as neuraminidase inhibitors might have a profound effect; otherwise, they have only a subtle impact on clinical symptoms [7]. Prophylaxis is defined as a preventative measure against disease.

A preventative measure has immediate effects against response-triggering and can reduce the systemic reaction to physiological stress or illness. This development might greatly reduce the detrimental disease-related circumstances [8]. The most often given medications in the world are statins, which decrease cholesterol. Statins block the enzyme 3-hydroxy-3-methyl glutaryl coenzyme-A reductase, which catalyzes the rate-restrictive step of cholesterol production. Statins have been shown to have positive cardiovascular benefits (Figure 2). [9].

Table 1: It was extremely well-established how viral respiratory tract illnesses compare epidemiologically

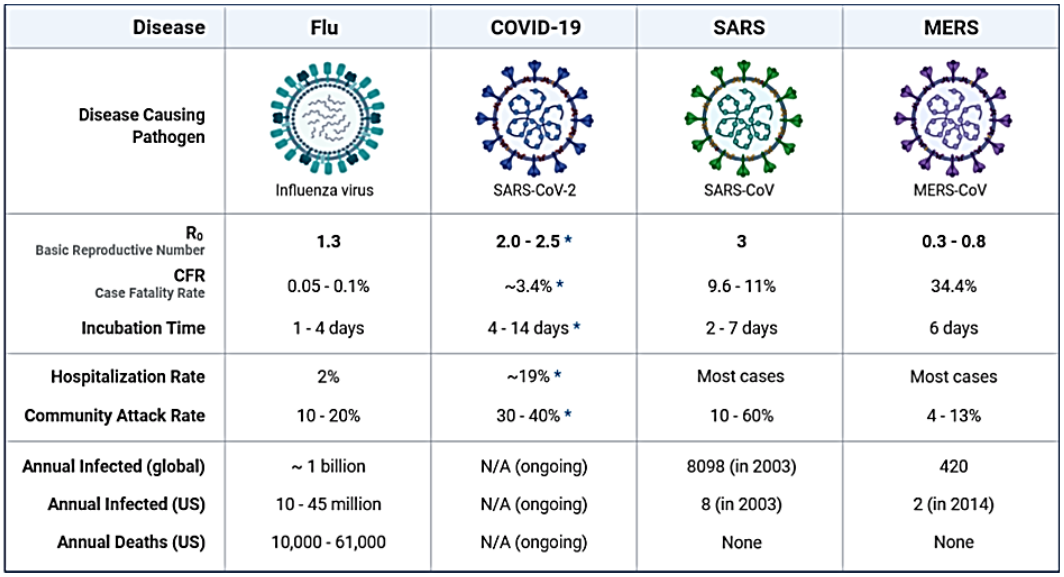

Disease	Flu	COVID-19	SARS	MERS
Disease Causing Pathogen	Influenza virus	SARS-CoV-2	SARS-CoV	MERS-CoV
R_0 Basic Reproductive Number	1.3	2.0 - 2.5 *	3	0.3 - 0.8
CFR Case Fatality Rate	0.05 - 0.1%	~3.4% *	9.6 - 11%	34.4%
Incubation Time	1 - 4 days	4 - 14 days *	2 - 7 days	6 days
Hospitalization Rate	2%	~19% *	Most cases	Most cases
Community Attack Rate	10 - 20%	30 - 40% *	10 - 60%	4 - 13%
Annual Infected (global)	~ 1 billion	N/A (ongoing)	8098 (in 2003)	420
Annual Infected (US)	10 - 45 million	N/A (ongoing)	8 (in 2003)	2 (in 2014)
Annual Deaths (US)	10,000 - 61,000	N/A (ongoing)	None	None

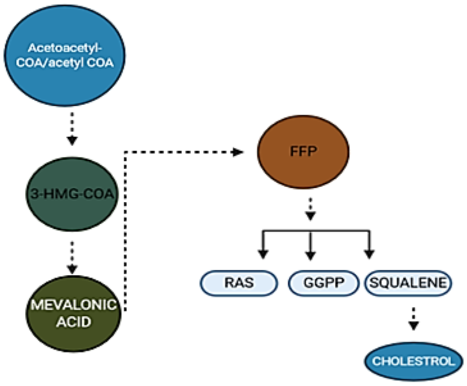

Figure 1: Statins' advantageous cardiovascular effects

Regardless of its effects on cholesterol levels, statins have been shown in clinical and experimental studies to decrease inflammation, improve endothelial function, and stabilize atherosclerotic plaques through a direct mechanism [10]. Statins can also alter cell shape, which is necessary for viral propagation [11]. Despite conflicting scientific findings, several retrospective observational analyses identified a link between statin use and decreased IV morbidity effects in people. However, these evaluations are constrained by the significant variation in medication delivery timing and duration across contributors or the participation of pneumonia morbidity in the absence of laboratory-approved IV infection [12]. A systematic review of statins' possible immunomodulatory properties in the treatment and prevention of IVs was the goal of this assessment.

METHODS

From the beginning until 25 March 2020, a number of electronic databases (Scopus, PubMed, EMBASE, Web of Science, ProQuest, OVID, EBSCO, and CINAHL) were searched for cohort studies and randomized trials exploring the connection between statin prescription and the result or risk of infections. Data on trial characteristics, statin consumption measurement, quality analysis, and outcomes were retrieved (set for potential confounders).

Influenza and the immune system

IVs are single-stranded RNAs that belong to the Orthomyxoviridae family and are divided into 4 categories based on their negative sense, segmented, and ssRNA genomes [12]. (classes of A, B, C, and D). In the meantime, many animal species might contract the influenza A virus (IAV) [13]. The IAV genome is almost 13,500 bases long and has 8 ribonucleoproteins (RNP) units, which together may encode at least 17 different proteins, including the recently identified

NS3, M42, PA-N155, and PA-N182 [14].

The genetic characteristics and molecular makeup of the neuraminidase (NA) and hemagglutinin (HA) proteins allow for further classification of IAVs. However, 9 NA and 16 HA subtypes were shown to be present in both human and animal bodies [15]. There have been reports linking many important IAV-encoded proteins to the host immune response and virus pathogenesis to the viral infection. According to research, changes in viral proteins' amino acid composition are linked to greater immune evasion and illness severity in humans and birds caused by IAVs [16]. In order for B cells to start producing neutralizing antibodies, HA contains epitopes. Therefore, the primary factors influencing recombination processes and viral mutation are the HA epitopes [17]. An innate immune response is the initial line of defense for the body against viral infection. IAV replicates itself by concentrating on respiratory tract epithelial cells. Understanding the innate immune systems defending these cells against IAV infection is crucial. Pathogen-associated molecular patterns (PAMPs), which are present in airway epithelial cells, are identified by receptors such Toll-like receptor (TLR) 3, melanoma differentiation-associated protein 5 (MDA5), and retinoic acid-inducible gene I (RIGI) [18]. Different autocrine signaling pathways can trigger the production of class 1 and 3 interferons (IFNs) and other cytokines instead of locating PAMP [19]. IFN lambda receptor 1 (IFNLR1-IL10R2) and IFN alpha/beta receptor (IFNAR1-IFNAR2), which are the receptors for class 1 and class 3 IFNs, respectively, can result in the paracrine response, which in turn activates a number of ISGs with antiviral activity and, as a result, induces the defensive activity of neighboring cells against viral infection [20]. The majority of adaptive immunity against IAV infection comes from B and T cells. T lymphocytes are mostly recognized as CD8+ and CD4+. In order to create cytokines and other powerful chemicals that can stop viral replication and kill virus-infected cells, CD8+ T cells are developed into cytotoxic T lymphocytes (CTLs). T cells are therefore essential to limit viral infection [21]. The naive CD8+ T cells are stimulated by the DCs to go from the lungs to the draining lymph nodes' T-cell zone during IAV infection, where they differentiate and multiply into CTLs [22]. Additionally, IL-12, IL-2, class 1 IFN-, and IFNs help to differentiate CD8+ T cells into CTLs [23]. It has also been demonstrated that IFN-increases T-cell proliferation during IV [24]. The workings of CTL have been extensively studied. The CTLs create cytotoxic granules that contain chemicals like granzymes and perforin while the virus targets the cells [25]. Additionally, CTLs can activate death receptors in IAV-infected cells by secreting cytokines including TNF, TRAIL, and FASL [26]. Following infection, lymphoid organs, blood, and the infection site are circulated with virus-specific DCs and CTLs [27]. The differentiation and activation process during the first infection increases the memory CTL cells' effectiveness and proficiency in secondary infection, and the memory CTL cells respond swiftly to subsequent IAV infection [28]. Although neutralizing antibodies are protected from subsequent infection with the same IAV serotype, CTLs are epitope-specific in conserved IAV proteins including NP, PA, and M1. As a result, the CTL response is heterosubtypic [29]. CD4+ T cells are a crucial subset of immune cells that are involved in adaptive immunity against IAV infection. In mouse models, CD4+ T lymphocytes may promote MHC type 2 expression in epithelial cells and target IAV-infected epithelial cells [20]. The CD40 ligand (CD40L) is notable among the several co-stimulatory ligands produced by CD4+ T cells that are important in antibody production and B cell activation [21]. TNF, IL-2, and IFN- are examples of antiviral cytokines generated by Th1 effector CD4+ T cells that activate alveolar macrophages [22]. IFN- and IL-2 released by Th1 cells control CD8+ T-cell differentiation to clear viral infection [23]. Additionally, CD4+ T cells can develop into killer, Th17, Th2, follicular helper, regulatory, and rarely Th2 cells [24]. Antigen-offering cells attach to MHC type II-related peptides produced from viruses, and Th2 cells produce IL-13 and IL-4 primarily to encourage B cell responses [25]. It was discovered that the control of cellular immunity against IAV infection includes the Treg and Th17 cells.

Immunopathogenesis of Influenza

The primary targets of IV for infection are respiratory epithelial cells, which are also amplified cytokines by the choreographers [26]. Following first exposure, the offspring viruses that have multiplied inside of these cells can spread to other cell types, including alveolar macrophages. The inflammatory reactions start when infected cells die through necrosis or apoptosis [27]; acute inflammation is thought to be the body's main defense mechanism against damaging stimuli. In order for leukocytes and plasma to reach the site of extra-vascular injury, blood flow must be increased. Acute inflammation is identified by an increase in local temperatures and discomfort [3]. Acute inflammation is indicated by the activation of pro-inflammatory chemokines or cytokines [28]. Inflammatory cell recruitment may happen as a result of these pro-inflammatory chemokines or cytokines. Then, there is extensive tissue damage, immune cell infiltration, and a gradual expression of antiviral, inflammatory, and apoptotic genes [29]. Both the repair of the damage and the regeneration processes begin at the same time. Most of the time, this repair technique can fully restore the function [30].

The formation of hyaline membranes, diffuse alveolar damage, fibrotic healing, and fibrin exudate (Figure 3), which are thought to be signs of severe capillary damage, persistent organ dysfunction, and immunopathologic injury, are among the more pathologically severe alterations found for severe inflammation related to cytokine storm. Additionally, the release of strong inflammatory chemokines and cytokines into the bloodstream can result in systemic cytokine storms and multiple organ failure [32].

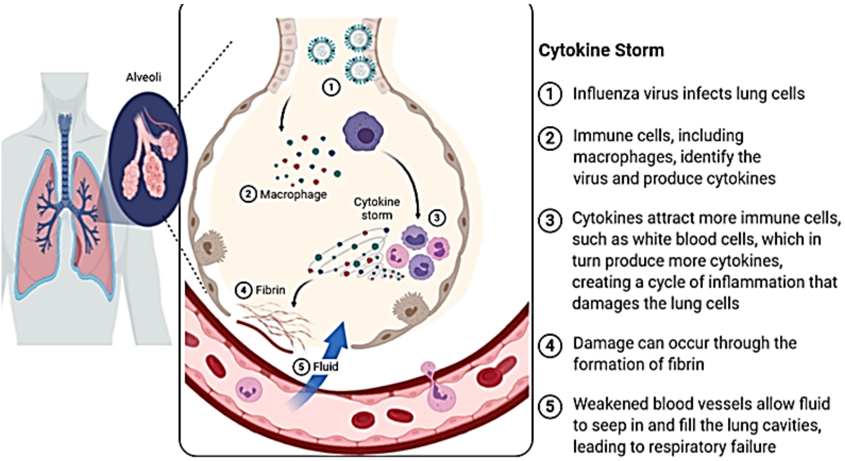
Alveoli
①
② Macrophage
Cytokine storm
③
④ Fibrin
⑤ Fluid
Cytokine Storm
① Influenza virus infects lung cells
② Immune cells, including macrophages, identify the virus and produce cytokines
③ Cytokines attract more immune cells, such as white blood cells, which in turn produce more cytokines, creating a cycle of inflammation that damages the lung cells
④ Damage can occur through the formation of fibrin
⑤ Weakened blood vessels allow fluid to seep in and fill the lung cavities, leading to respiratory failure

Figure 2: Severe inflammation related to cytokine storm

Because viral virulence and host resistance both have a role in the severity of influenza outcomes, it is imperative to use immunomodulatory treatment in addition to traditional antiviral medication. Indeed, several studies have shown that administering immunomodulatory medicines, with or without antiviral medications, can reduce organ damage and death from severe influenza. Additionally, the majority of these medications are not costly and are made simply, making them potentially useful in large-scale influenza pandemics. Here, the characteristics of statins and some instances of these were discussed.

Statins as immunomodulatory agents

The enzyme hydroxyl methylglutaryl-coenzyme A (HMG-CoA) reductase is inhibited by statins. By catalyzing the reduction of HMG-CoA for mevalonate during cholesterol synthesis, this enzyme can gauge the pace of the early step of the cholesterol biosynthesis pathway [33]. In addition to their ability to lower cholesterol levels, statins are pleiotropic cardioprotective medications that are gradually becoming recognized as direct cellular effect mediators. Statins inhibit downstream molecules, which are essential for viral contagiousness [34]. They reduce or prevent the production of downstream isoprenoid intermediates such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGPP) (FPP). These isoprenoid intermediates interact with membranes when post-translational proteins are modified. Statins regulate GDP and GTP-bound states by controlling their isoprenylation and preventing them from switching from the cytosol to the membrane when they are inactive [35].

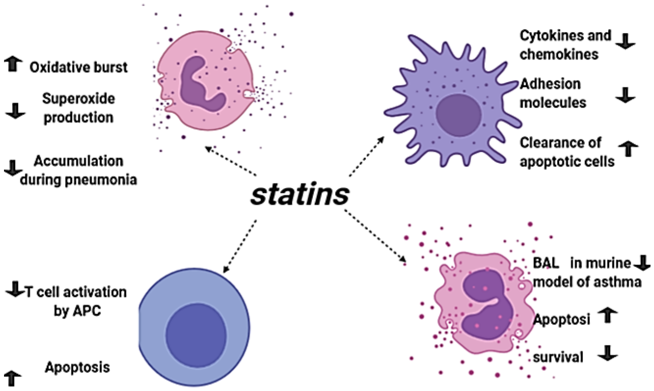
↑ Oxidative burst
↓ Superoxide production
↓ Accumulation during pneumonia
statins
Cytokines and chemokines ↓
Adhesion molecules ↓
Clearance of apoptotic cells ↑
↓ T cell activation by APC
↑ Apoptosis
BAL in murine model of asthma ↓
Apoptosi ↑
survival ↓

Figure 3: Some functions of statins

As a result, statins stop the activity and membrane translocation of isoprenoid intermediates and reduce their production. It is thought that lowering the isoprenylation of several proteins mediates the pleiotropic effects of statins.

This is best shown by the Rho GTPase family, which includes RhoA, Cdc42, and Rac, and which performs distinct roles dependent on the shape, secretion, motility, control, and proliferation of gene expression in cells [36]. To apply their activities and membrane interactions, members of the Ras family must add a farnesyl group to processes such as platelet activation, cell division, differentiation, intracellular vesicle transport, and the production of oxygen radicals [37]. These effects of statins include immunomodulatory actions such as the reduction of T-cell activation, the inhibition of inflammatory responses in neutrophils and macrophages, and the inhibition of a number of pro-inflammatory cytokines like TNF, IL6, and IL8. Furthermore, it has been demonstrated that blocking MAPK pathways such p38 MAPK, JNK, and ERK1/2 prevented the expression of RANKL, the production of cytokines and MMPs, and the degeneration of cartilage in different cells from RA patients (Figure 3). [38-42]

Use of immunomodulatory features of statins on IV treatment

According to three categories, including in vitro, human, and animal investigations, the effects of statin-related immune-modulation were examined to treat influenza in a number of studies. The following sections were used to review particular research from each category.

In vitro study

In the US Vaccine Effectiveness Network trial across 6 influenza courses (2011-2012 to 2016-2017), Havers et al. evaluated the influenza VE in statin users and nonusers with age higher than 45. All individuals with acute respiratory illnesses were screened for influenza at outpatient clinics. According to the data, out of 11692 eligible contributors, 3359 (30%) used statins, and 2806 (24%) tested positive for IV illness. Meanwhile, only 60% of nonusers and 78% of statin users obtained influenza vaccination. Setting for possible confounders came in at 39 percent (95 percent CI, 32 percent -45 percent) among nonusers and 22 percent (95 percent confidence interval [CI], 22 percent -47 percent) among statin users, with influenza VE coming in at 36 percent. They discovered no appreciable VE alteration as a result of statin use. Between nonusers and statin users, VE against influenza A(H3N2), A(H1N1) pdm09, and B viruses were comparable. When they analyzed the data by type and subtype, they discovered that the use of statin administration did not change the effect of vaccination on influenza [43].

According to research by Gower et al., lovastatin can stop virally induced cell-to-cell fusion and RSV replication. The experimental research showed that isoprenylation inhibition is a potential new antiviral strategy for treating or preventing severe RSV infection [44]. Statins, a widely used and affordable pharmacological medication, may be effective in preventing current and future influenza epidemics, particularly in less developed and underdeveloped nations, according to Mehrbod et al. By lowering cytokine over-expression and modulating strong inflammatory responses, statin therapy may protect the body from inflammatory reactions in the pulmonary system and other inflammatory reactions connected to severe influenza sickness. The availability of statins, which might be used as preventative or supplemental drugs to lessen the severity of the disease, and possible preventive medical therapy for unanticipated pandemics, may help to diminish the inflammatory repercussions of influenza that are connected with it [45]. Simvastatin and H1N1 elevated membrane localization and LC3 protein lipidation, according to Mehrbod et al. Simvastatin, however, has a direct influence on LC3 lipidation through a reduction in cholesterol, while H1N1's contribution to autophagy is more convoluted due to its effects on the activation of several autophagy-related genes to boost the autophagic flux. Their results were in line with those of an earlier study on the primary human lung mesenchymal cells, highlighting the relationship between the relative amount of LC3-II and the dynamic turnover of LC3-II through lysosome activity [46].

Animal study

Despite the ambiguous results, several animal studies showed that alternative statin medications may be protective against acute lung damage. In C57BL/6 mice infected by IAV, Haidari et al. observed that atorvastatin (40 mg/kg/day) prescription decreased the lung viral titers and mortality. Statins work directly against influenza by inhibiting the Rho/Rho-kinase pathway. The statins' protection against influenza might be viewed as a component of their cardiovascular event prevention benefits [47]. According to Lee et al., the treatment of simvastatin plus the neutralizing RANTES antibody effectively inhibited the growth of neutrophils caused by poly I:C in the lungs. Additionally, they said that simvastatin inhibits neutrophilia in poly I:C-induced pneumonia by inhibiting STAT3-mediated RANTES production. Understanding the etiology of viral pneumonia is crucial in order to develop effective anti-inflammatory or antiviral medications for certain inflammatory conditions. Simvastatin should be considered as a potential therapeutic option for viral pneumonia, particularly in neutrophilic inflammation due to a viral component, they further said. Simvastatin can reduce airway inflammation, but its exact mechanism of action has to be understood [48]. Simvastatin's efficacy against influenza A/PR/8/34 virus infection in the mouse model was evaluated by Glück et al. The first trial used oral administration of simvastatin. In a subsequent investigation, intraperitoneal administration was used to achieve high plasma levels. Bodyweight loss, survival rate, histologic lung damage, and viral titers in the lungs and trachea were evaluated. Surprisingly, simvastatin treatment caused the mice to lose more body mass and have lower survival rates than the virus-infected control animals. Simvastatin also did not affect histological lesions, tracheas, or lung viral loads. In general, they demonstrated that simvastatin did not shield the mice from IV infection [49]. According to Radigan et al., rosuvastatin treatment did not successfully treat influenza A illness. Inflammation of the lungs, lung damage, and the degree of weight loss were comparable in rosuvastatin- and control-treated mice. Rosuvastatin treatment had no impact on mortality in influenza A-infected mice (A/WSN/33) Statins did not affect influenza A replication in vitro or its removal from the lung in vivo. Statins did not affect influenza A-related mortality and did not lessen the severity of the lung damage caused by the virus. According to them, there is no connection between HMG CoA reductase inhibitors and improved outcomes for patients with sepsis and pneumonia related to their effects on influenza A infection [50].

Human study

In critically ill patients with severe sepsis, Kruger et al. investigated the clinical and biochemical effects of atorvastatin treatment. Before using statins, the site stratified a phase II, prospective, multicenter, double-blind, randomized, and placebo-controlled experiment. A matching placebo or atorvastatin was given to 250 individuals in a cohort trial who had severe sepsis and acute infection (127 received placebos, 123 received statins) (20 mg daily). The results showed no interactions between treatment groups, showing that there were distinct behaviors across the groups throughout time (P = 0.26), and no variations in IL-6 concentrations (initial endpoint) between the placebo and atorvastatin groups (P = 0.76). The baseline IL-6 plasma titer was decreased (129 [87-191] vs. 244 [187-317] pg/ml; P = 0.01) in those who had previously taken statins. There was no difference between the two groups in terms of the length of hospital stays, hospital discharges at 28 or 90 days (15% vs. 19%), changes in Sequential Organ Failure Assessment scores, death at intensive care unit release, or negative effects. Patients on atorvastatin had reduced cholesterol (2.6 [0.06] vs. 2.4 [0.07] mmol/L; P = 0.006). Greater than 28-day mortality was detected for those randomly assigned to the placebo (28 percent vs. 5 percent; P = 0.01) than for those getting atorvastatin in the selected group of 77 past statin users. At 90 days, there were no statistically significant differences (11 percent vs. 28 percent; P = 0.06). A therapy with atorvastatin for severe sepsis did not appear to have an impact on IL-6 levels. A lower baseline IL-6 concentration in this population was associated with improved survival, while prior statin usage was associated with the continuation of atorvastatin [51].

According to Papazian et al., simvastatin (60 mg) prescriptions for supplementary VAP therapy did not lower day-28 mortality. Simvastatin did not substantially worsen any side effects. Although the superiority of a placebo to simvastatin was not tested in our investigation, it is important to note that day-28 mortality in the simvastatin group increased by approximately 6% absolute and by almost 8% absolute in the statin-naive category. It would be pertinent to look at the potential supremacy of a placebo over a statin as normal practice does not involve de novo statin administration in ICU patients with infection. They thus investigated the claim that simvastatin is preferable to a placebo. The results did not support the use of supplementary statin medication in ICU patients with VAP. Therefore, this result may be extended to ICU patients who have any type of nosocomial infection [52].

With the aid of connected administrative databases, Kwong et al. conducted a population-based cohort analysis across 10 influenza seasons (1996–2006) in Ontario, Canada. Before the start of the influenza season, they identified all persons over the age of 65 who had had an influenza vaccination, and they also separated those who had received prescription statins (23 percent) from those who had not (77 percent). A final cohort of 2,240,638 patients—exactly half of those taking statins—was obtained by matching based on propensity, which took into consideration each patient's likelihood of receiving a statin. Statins had poor protective effects against pneumonia hospitalization (odds ratio [OR] 0.92; 95 percent confidence interval [CI] 0.89-0.95), all-cause mortality (0.87; 95 percent CI 0.84-0.89), and pneumonia mortality within 30 days (0.84; 95 percent CI 0.77-0.91). The significantly decreased with time, varied between risk groups and propensity score quintiles, and remained unaltered across post-influenza season periods after multivariate regulation and while omitting multiple clarifications for each individual. They suggested that statin use could have a statistically significant, but little, protective effect on influenza morbidity, which was merely attributable to public health authorities and residual confounding. Additionally, doctors must focus on minimizing mortality and morbidity from the ensuing influenza epidemic [53].

CONCLUSIONS

The immunomodulatory effects of statins are mediated by a number of mechanisms, including direct activation of heme oxygenase and inhibition of major histocompatibility type II complexes, which interfere with the interaction between leukocytes and endothelium. The most crucial mechanism is intracellular inflammatory signaling, however. GTP-binding proteins serve as molecular on/off switches for various protein kinases, which makes them essential for intracellular inflammatory signaling. Both the cycle's inactive and active stages require isoprenylation for the route to be activated. By blocking 3-hydroxy-3-methylglutaryl-CoA reductase, statins lessen but do not completely abolish protein isoprenylation. The response strength of the impacted signaling pathway is decreased by this drop in prenylated protein concentration. As a result, it could balance out the "cytokine storm" associated with IVs. Based on the aforementioned effects, statins were found to have direct antiviral effects in some cases when used at physiologically appropriate concentrations. This suggests that they may prove to be an effective adjuvant treatment in the fight against pandemic and seasonal influenza, particularly in health systems that cannot afford expensive vaccines or antiviral medications. Despite conflicting results from several research, statins are affordable and easily available medicines in underdeveloped nations. By controlling immune system cytokine overexpression and modulating severe inflammatory reactions, they may be used as preventative medication or therapy for influenza sickness.

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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ORIGINAL ARTICLE

Pyloric-preserving Pancreaticoduodenectomy with Duct to Mucosa Pancreatico-gastrostomy Reconstruction: Long-term Follow-up in a Tertiary Center

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Abstract: *Background: Duct to mucosa Pancreatico-gastrostomy (DMPG) is a method for reconstruction of pancreatic mass following pancreaticoduodenectomy (PD). There are still controversies about the benefits of this surgical technique compared to the old ones. Objectives: This study aimed to evaluate the long-term outcomes of DMPG reconstruction following PD operation. Method: Through a prospective observational setting, 164 patients, undergoing PD surgery, with diagnosed pancreatic head or peri-ampullary cancer, were enrolled. A pylorus-preserving PD and DMPG were done for all patients to reconstruct the pancreatic mass. Patients were followed up to a mean of 21.06±14.12 months. Demographic data, risk factors, tumoral staging, and postoperative follow-up data were collected for the analytic study. Results: The mean survival of the patients was 21.06±14.23 months after surgery. DGE (delayed gastric emptying) (34.75%), pancreatic fistula (21.34%), bile duct fistula (7.31%), and hemorrhage (5.5%) were recorded as the most important complication in patients. Some post-operative complications were significantly observed in patients with diabetes mellitus (pancreatic fistula, increased amylase, and DGE), Smokers (bile duct fistula, hemorrhage, and increased Amylase), and the elders (bile duct fistula) (p-value <0.05). Conclusions: Although our results indicate that DMPG reconstruction is a safe technique, our findings did not confirm the priority of this method over the older techniques.*

Keywords: *pancreatic neoplasms, gastrostomy, jejunostomy, pancreaticoduodenectomy*

INTRODUCTION

Pancreaticoduodenectomy (PD) is the main surgical treatment option for ampullary and peri-ampullary cancers, and some modifications have been described for this surgical operation. Therefore, these surgical modifications, complications, and outcomes need to be evaluated.

Despite the surgical techniques and management improvements, there are still many concerns about its high incidence of post-operative complications and morbidity rate (30-50%) [1,2]. Pancreatic fistula is considered the most common postoperative complication followed by bile duct fistula, thromboembolism, intra-abdominal hemorrhage, intra-abdominal abscess, wound infection, and gastro-intestinal bleeding as other important complications [2].

Reconstruction with pancreaticojejunostomy (PJ) is the preferred anastomosis technique following PD surgery. While this method has been commonly used for several decades, its high prevalence of complications (40-50%) prompts surgeons to come up with new approaches [3-5].

Duct to mucosa pancreaticogastrostomy (DMPG) is a method of reconstruction following PD surgery. There are still controversies about its benefits compared to previous ones. Few studies are available evaluating DMPG's outcomes, and complications [6-8]. This study aimed to evaluate the long-term outcomes of DMPG reconstruction following PD operation.

MATERIALS AND METHODS

Patients

In this prospective observational study, patients with the diagnosis of pancreatic head or peri-ampullary tumors who underwent PD surgery at Baqiyatallah Hospital from January 2007 to February 2016 were enrolled. Informed consent was taken from patients. All operations were performed by the same surgeon. Patients were excluded from the study if failed to follow or their data were incomplete or inaccessible. Altogether 191 patients were included. During the study, 27 patients were excluded, (12, due to changes in their address, and 15, because of incomplete data and/or failure to follow). The study was approved by the ethics committee of Baqiyatallah University of Medical Sciences (national approval number: IR.BMSU.REC.1393.87).

Surgical Procedure

In all operations, a right subcostal incision was made and a pylorus-preserving PD and DMPG (using 4-0 polydioxanone sutures) were performed for the reconstruction. The Head of the pancreas was transected with at least a 2 cm macroscopic surgical margin from the mass, usually at the margin of the head and neck of the pancreas. The proximal duodenum was transected about 1.5-2 cm distal to the pylorus.

During the DMPG reconstruction, a small piece of the feeding tube was inserted into the DMPG anastomosis, like a stent, to secure the patency of the anastomosis within the first few days. The tube was not fixed with sutures. An end-to-side duodeno-jejunostomy hand-sewn anastomosis was made using 3-0 vicryl sutures. The coledoco-jejunostomy anastomosis was created about 50 cm distal to duodeno-jejunostomy using 3-0 Vicryl sutures. Tumor Lymphadenectomy was performed in all patients with palpable regional lymph nodes. A Penrose drain was inserted in the bed of the surgical site, and a closed-suction drain was placed in the Morison pouch.

Data Collection

Demographic information including age, sex, risk factors for the disease and surgery, history and laboratory, data essential to calculate the American Society of Anesthesiology scoring (ASA) class, and clinical staging of the tumor were collected and recorded into a checklist before surgery.

Duration of the operation, length of stay (LOS) in the hospital, post-operative complications, short- and long-term outcomes, pathologic result of the sample, morbidity, and mortality were recorded into the checklist during close follow-up. The patients were followed up to a maximum of 11 years or up to the date of death. The post-operative complications include pancreatic fistula, bile fistula, thromboembolic events, intra-abdominal fluid collection such as hemorrhage and abscess, wound infection, gastrointestinal bleeding, and gastroparesis. International Study Group on Pancreatic Fistulas (ISGPF) criteria was used to estimate pancreatic fistula [9]. Delayed gastric emptying (DGE) was graded according to the validated criteria of the ISGPF classification [10]. Pre-operative ASA classification was also assessed according to the ASA classification of physical status [11].

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows (version 21, IBM Corp, NY). Averages were expressed as mean ± SD. Kolmogorov-Smirnov test was used to assess the normal distribution of data. Possible statistical relationships between the risk factors and complications after the surgery were calculated using student t-test, ANOVA, and Chi-Square tests. The p-values equal to or less than 0.05 were defined as statistical significance levels.

RESULTS

In this study, a total of 164 patients with a mean age of 62.21± 9.34 years (including 93 males and 71 females) were included. The demographic information, risk factors, and also the number of cases in each ASA class are demonstrated in Table 1. Most of the patients were in class II of ASA classification (45.73%). According to the exclusion criteria, the patients with class V of ASA classification were excluded from the study.

Table 1: Demographic information, risk factors, and ASA class of patients

Variable (N=164)		
Age, (Mean± SD)	62.21 ± 9.34	
Sex, n (%)	Male	93 (56.70)
	Female	71 (43.29)
Smoking, n (%)	58 (35.36)	
DM, n (%)	48 (29.24)	
IHD, n (%)	42 (25.60)	
ASA Class n, (%)	I	15 (9.14)
	II	75 (45.73)
	III	64 (39.02)
	IV	10 (6.09)
	V	0 (0.00)

DM: Diabetes Mellitus, IHD: Ischemic Heart Disease, ASA: American Society of Anesthesiology scoring

Adenocarcinoma was the most prevalent tumoral type in this study (41.46%). Less common pathologies were cholangiocarcinoma, ampullary carcinoma, high-grade dysplasia, neuro-endocrine carcinoma, and intraductal papillary mucinous neoplasm (IPMN). The mean pancreatic duct size was 4±1 millimeters. According to the TNM tumoral staging, 55 (33.5%), 70 (42.7%), and 39 (23.8%) of tumors were classified as T1, T2, and T3, respectively.

Post-operative data such as complications, mortality rate, and their relationship with the risk factors are illustrated in Table 2. In brief, DGE (delayed gastric emptying) (34.75%), pancreatic fistula (21.34%), bile duct fistula (7.31%), and hemorrhage (5.5%) were recorded as the most important post-operative complications in patients. Some post-operative complications were significantly observed in patients with diabetes mellitus [pancreatic fistula (p-value 0.038), increased amylase (p-value 0.010) and DGE (p-value 0.024)], smokers [bile duct fistula (p-value 0.027), hemorrhage (p-value 0.026) and increased amylase (p-value 0.045)] and the elders [bile duct fistula (p-value 0.006)]. No patient reported a history of alcohol consumption as a risk factor for pancreatic cancers.

Table 2: Post-operative data; and their relationship with the risk factors

Complications		Descriptive, n(%)	Age (P)	Sex (P)	Smoking (P)	DM (P)	IHD (P)	HTN (P)
Pancreatic fistula	0	129 (78.65)	0.690	0.607	0.293	0.038*	0.777	0.919
	A	6 (3.65)						
	B	17 (10.36)						
	C	12 (7.31)						
Bile duct fistula		12 (7.31)	0.006*	0.626	0.027*	0.99	0.506	0.134
PGIB		9 (5.48)	0.075	1.00	0.722	0.722	1.00	0.733
IAAC		23 (14.02)	0.174	0.176	0.062	0.272	0.9701	0.120
TE		18 (10.97)	0.503	0.266	0.740	0.134	1.00	0.375
SSC		23 (14.02)	0.117	0.664	0.950	0.392	0.955	0.493
Amylase[a]		34 (20.7)	0.943	0.619	0.045*	0.010*	0.755	0.170
DGE		57 (34.75)	0.611	0.791	0.117	0.024*	0.418	0.949
Hemorrhage		9 (5.5)	0.409	0.459	0.026*	0.199	0.838	0.896
Reoperation		18 (10.97)	0.332	0.366	0.169	0.213	0.567	0.908
R-resection[b]		30 (18.29)	0.503	0.223	0.869	0.429	0.883	0.909
Survival (month)		21.06±14.23	0.282	0.915	0.665	0.924	0.616	0.291
LOS (day)		13.12±7.15	0.115	0.880	0.800	0.580	0.700	0.241

PGIB=Post-operative-Gastrointestinal Bleeding, IAAC= Intra-abdominal abscess collection, TE= Thromboembolism, SSC= Surgical Site Complication, DGE= Delayed gastric emptying, LOS= Long of stay, P= p-value

[a]: *Significant increasing amylase = triple time level for more than 3 days*

[b]: *Surgical resection margins were not restricted to malignant tumors, and the surgeon had to conduct more resection to reach a free margin*

*The character * in the above P values indicates that there is a significant statistical relationship between the two methods of surgery*

The mean follow-up time of the patients was about 21.06±14.12 months. The mean survival of the patients was 21.06±14.23 months after surgery. There were 5 early post-operative mortality; one case due to massive lung embolism, three because of post-operative intra-abdominal hemorrhage, and one patient due to myocardial infarction. The survival rate of patients who were diagnosed with adenocarcinoma was 19.93±15.02 months. Survival rates for other groups are shown in Table 3. Also, the mean survival years in each pathologic type of tumor are shown in Figure 1. The longest survival is seen in non-malignant masses and the shortest survival is seen in PMN tumors.

DISCUSSION

Pancreaticoduodenectomy is an accepted surgical treatment for pancreatic head and peri-ampullary cancers. However, its complications have made surgeons find a better surgical technique in recent years [12].

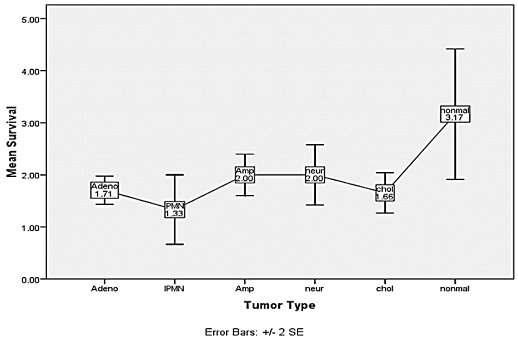

Figure 1: Mean survival year of the patients for each tumor type (*Adeno: Adenocarcinoma, IPMN: Intraductal papillary mucinous neoplasm, Amp: Ampullary Carcinomacarcinoma, Neur: Neuroendocrine tumor, Chol: Cholangiocarcinoma*)

PJ reconstruction is one of the accepted and common methods of anastomosis after the excision of the pancreatic head and duodenum. However, the current method has many challenges such as a high rate of postoperative pancreatic fistula (POPF) and abdominal collections that are reported to be decreased in PG reconstruction in comparison to PJ reconstructions in different studies [13].

Table 3: The patients' survival rate (±SE); according to the pathologic type of tumor

Tumor Survival	Ade (%)	IPMN (%)	Amp-Car (%)	Neur (%)	Chol (%)	Non-mal (%)	Total (%)
1-year	94±3	83±17	97±4	100	90±6	100	93.9±1.8
2-year	51±5	50±22	65±10	67±17	48±10	75±13	54.8±3.8
3-year	15±4	0	67±17	33±17	24±8	50±15	23.1±3.3
4-year	4±2	0	33±17	0	3±3	33±14	4.8±1.6
5-year	1±1	0	0	0	0	25±13	2.4±1.2
6-year	1±1	0	0	0	0	17±11	1.8±1.0
7-year	1±1	0	0	0	0	17±11	1.8±1.0
8-year	1±1	0	0	0	0	0	0.6±0.6
9-year	1±1	0	0	0	0	0	0.6±0.6
10-year	1±1	0	0	0	0	0	0.6±0.6

Ade: Adenocarcinoma, IPMN: Intra-ductal papillary mucinous neoplasm, Amp-Car: Ampullary Carcinomacarcinoma, Neur: Neuroendocrine tumor, Chol: Cholangiocarcinoma

Table 4: The Difference of Overall POPF Complication Rate in Recent Studies between PG and PJ Anastomosis after PD Surgery

Study	Year of Publication	PG		PJ		P value*
		N (%)	Total	N (%)	Total	
Wang et al. (DMPG) [7]	2014	2 (2.7)	75	-	-	-
Figueras et al. [4]	2013	10 (15.38)	65	20 (34.48)	58	0.006*
Topal et al. [14]	2013	13 (8.02)	162	33 (19.76)	167	0.002*
Wellner et al. (grade B/C) [20]	2012	6 (10.16)	59	7 (12.28)	57	0.775
Current study (DMPG)	2018	35 (21.32)	164	-	-	-

PG= Pancreatico-gastrostomy, PJ= Pancreatico-jejunostomy, DMPG= Duct to mucosa Pancreatico-gastrostomy
*The character of * in the above P values indicates that there is a significant statistical relationship between the two methods of surgery*

Table 5: Comparison of the various complication rates after PG Anastomosis in the previous studies

Complications/Study (Year, N)	Wang et al. (2014, 75) [7]	Figueras et al. (2013, 65) [19]	Topal et al. (2012, 162) [14]	Wellner et al. (2012, 59) [20]	Current Study (2018, 164)
Bile duct Fistula, n (%)	1 (1.3)	1 (2)	-	-	12 (7.31)
IAAC, n (%)	3 (4.0)	5 (8)	9 (6)	7 (12)	23 (14.02)
SSC, n (%)	3 (4.0)	-	65 (40)	_	23 (14.02)
DGE, N (%)	13 (17.3)	19 (29)	25 (15)	14 (27)	57 (34.75)
Hemorrhage, n (%)	2 (2.7)	13 (20)	21 (13)	6 (10)	9 (5.5)
Reoperation, n (%)	1 (1.3)	4 (6)	14 (9)	7 (12)	18 (10.97)
R-resection[a], n (%)	-	-	22 (10)	-	30 (18.29)
LOS (day, min-max)	19 (12-39)	12 (1-52)	19 (14-25)	15 (7-135)	14 (7-40)

PGIB=Post-GI Bleeding, IAAC= Intra-abdominal abscess collection, SSC= Surgical Site Complication, DGE= Delayed gastric emptying, LOS= Long of stay
a: Surgical resection margins were not restricted to malignant tumors, and the surgeon had to conduct more resection to reach a free margin

Duct to mucosa pancreatico-gastrostomy anastomosis is a method of anastomosis following PD surgery. There are few studies to show the distribution of complication rates after the DMPG surgical technique [7].

There are many accepted risk factors for POPF. Many risk factors are related to the pancreas itself and are different among patients; these include the size of the duct, histologic features, and the blood supply of the tail. However, many risk factors are related to the techniques of surgery such as anastomosis type after PD, stenting of the anastomosis, and blood loss during surgery [5,7,14].

In this study, there was also a significant relationship between the history of diabetes mellitus and the development of POPF (P=0.038). In a previous study, the risk of POPF was significantly higher in patients with diabetes mellitus (P=0.03). There was no significant relationship between age and POPF (P=0.69). Our finding in this regard is different from other studies [15]. Amylase levels and delayed gastric emptying (DGE) in diabetic patients were higher than in other patients.

In most studies, the male gender has a strong risk factor for pancreatic cancers. In our study, 56.7% of patients were male. However, gender was not an important risk factor for complications after surgery, the rate of bile duct fistula was higher in the elders significantly (P=0.006).

Smoking is another major risk factor for pancreatic cancer. The actual mechanism of this process is unknown. However, cigarettes contain thousands of carcinogenic compounds that can cause cancers [16]. Smoking is also an important risk factor for complications after surgery as seen in our study smokers developed higher rates of bile duct fistula and hemorrhage after the surgery. In these patients, the increase in amylase level was also higher than in other patients (Table 2).

In the current study, the prevalence of POPF was higher than the similar study on DMPG (21.32% vs. 2.7%), and also higher compared with PG (pancreatico-gastrostomy) reconstruction [17]. Although have no justification for that, it seems that DMPG doesn't have any priority in this regard. There are few reports on the comparison between POPF rates following DMPG and PJ anastomosis (21.32% vs. 12.28 and 34.48%) [18, 19]. In other studies, some complication rates such as hemorrhage, bile duct fistula, DGE, and also LOS are higher in PG in comparison to PJ anastomosis [17,19].

In this study, the prevalence of DGE was 34.75% which is higher compared to another study [5]. It might be because of the pylorus-preserving surgery done for the patient; however, this finding is not in favor of the priority of DMPG (Table 4). The prevalence of hemorrhage as a life-threatening post-operation complication was lower in this study (5.5%). On the other hand, the prevalence of bile duct fistula after PG was higher than in other studies. (7.31%, Table 5).

We calculated the DMPG survival rate for the first time according to a recommendation for future researchers by Topal et al [17]. The Survival rate after a mean follow-up of 21.06±12 months was 21.06±14.23 months in our study [17]. Survival rates for all pathologic types were similar and ranged from 1.33-2 years except for non-malignant tumors which resulted in a higher mean survival of 3.17 years (Figure 1).

Although our results indicate that DMPG reconstruction is a safe technique following PD, the claimed lower rate of post-operative complications and lower hospital stay is still in question and needs further investigation. Compared to other studies and the prevalence of this condition, the number of patients in this study is acceptable, however, it had some limitations. We did not perform subgroup analysis based on duct diameter. Also, pancreatic remnant consistency was not documented after our operations. Results might also be influenced by the surgical technique used for PG here. Also, we did not compare outcomes after DMPG with PJ anastomosis as a control group.

CONCLUSIONS

This study with a rather large number of patients and long-term follow-up after DMPG showed a lower rate of many post-operative life-threatening complications such as hemorrhage. On the other hand, the prevalence of bile duct fistula after PG was higher than in other studies. Though our results indicate that

Duct-to-mucosa pancreatico-gastrostomy reconstruction is a safe technique following PD however, does not confirm the priority of this method over older techniques. However, this contradiction calls for more studies including clinical trials and metaanalysis to clear all obscure dimensions.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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None

Authors' contribution

SNS, JAM, HG, ARK, MH, MR, SM were responsible for the study concept and design. SNS, JAM, HG, ARK, MH, MR, SM led data collection. SNS, JAM, HG, ARK, MH, MR, SM were responsible for the analysis and interpretation of data. SNS, JAM, HG, ARK, MH, MR, SM wrote the draft.

Ethics approval and consent to participate

This study was approved by the ethics committee of Baqiyatallah University of Medical Sciences. Throughout the study, researchers adhered to the Declaration of Helsinki. Written informed consent was obtained from the participants in this research.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Primary Headaches and Their Most Important Imagistic Features in Clinical Practice

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Abstract: *Background. Primary headaches are comprised of three big categories: migraines (with or without aura), the third most common and the 6th most disabling disease worldwide, tension-headaches, and trigeminal autonomic cephalalgias, each with different, complex, and not completely understood pathophysiological, clinical, and neuroimagistic characteristics. Structures like the brain stem, hippocampus, or hypothalamus have been depicted as being the main triggers of these types of headaches, which have a significant impact on the quality of life. Methods. We performed a broad literature review on primary headaches from January 2016 to June 2023, analyzing the latest scientific updates and emphasizing the pathophysiological and neuroimagistic characteristics. Results. We provide a concise review of the main neuroimaging studies that help in differential diagnosis between several types of primary headaches. Conclusions. It is difficult to approach the pathophysiology of these three primary headache categories. Keeping in mind that neuroimagistic methods are rapidly developing (especially in the MRI sphere), the most important endpoint of future studies will be to establish a reliable distinction system for all these types of cephalalgias, thus updating the protocols and guidelines. Currently, imaging studies are indicated only in certain circumstances.*

Keywords: *migraine; tension-type headache; pathophysiology; trigeminal autonomic cephalalgias; imaging studies; cluster headache; SUNCT; MRI; fMRI; SUNA*

INTRODUCTION

Headache is a recurrent and upsetting symptom in the neurological sphere, also being one of the most frequent ones, patients often present to the physician for it. Most of the studies suggested that more than 90% of the population has suffered from headaches at least once in their lives [1]. The diagnostic standard for headaches of any cause has been offered over the past two decades by ICHD since 2004 and today reaches the updated ICHD-3 [2]. This accounts for approximately 3% of emergency department admissions, but some authors have identified percentages as high as 8% [3,4]. It is important to emphasize that the repeated presentation of a patient to ED for the same symptoms significantly increases the costs associated with medical care. These recurrent presentations occur in approximately 10% of headache patients [3]. When a severe headache begins, patients present to the ED frightened by the thought of a serious condition. However, one study showed that of 3026 imaging studies of patients presenting to the emergency department, pathology was objectified by imaging in less than 3% of them [5].

Given the high prevalence of this type of neurologic symptomatology, clinicians must carefully consider the indication for imaging studies because they are expensive and usually do not provide the desired information to guide subsequent treatment. In addition, headaches can lead to absenteeism from school or work, increasing the indirect costs associated with pathology. In a recent study, workers with headaches were observed to have low productivity or, more rarely, to be absent from work for up to 7.5 days/month [6].

Headaches of a primary cause are set apart by the presence of headache without any underlying pathology. These are far more common than secondary headaches. For example, the prevalence of TTH is as high as 70% during a person's lifetime [7]. In the past, categorization of these pathologies has been difficult because symptoms vary from person to person, but also in the same individual over time [8]. Standardization of primary cause headaches has been achieved by the ICHD-3 classification, which divides primary headaches into migraine, both with aura or without it, tension-type headache (TTH), and cephalalgias which belong to the trigeminal autonomic sphere [2,9]. The mechanism of action of these pathologies is not yet known, but research is ongoing. In this paper, we propose to present the situations in which imaging studies are indicated.

MATERIALS AND METHODS

We reviewed the literature related to primary cause headaches by searching the Google Scholar and Pubmed English databases between 2000 and 2023 with keywords such as "headache", "cephalalgia", "imaging", "MRI", "primary", "migraine", "tension-type", "cluster", "trigeminal", "autonomic", or any logical combination of these terms. In the first stage, the retrieved studies were filtered by a title check to exclude duplicates and assess their relevance. After this first step, the outstanding studies were distributed evenly and randomly to the authors of this study, in the first phase by reviewing the abstract to determine relevance and later by studying the entire text. We have extracted relevant, basic information from these articles, but also important news that may change the paradigm of primary headaches.

RESULTS

Migraine

Migraine is a disabling disease, being the most prevalent type of primary headache, with 12-15% of the general population suffering from it [10,11]. Prevalence is different between males and females, and also it varies by the age group: interestingly, in children, puberty, this pathology is more common in boys. In young adults, it differs, being more usual in females, probably due to the hormonal changes they undergo [12]. Throughout life, the prevalence is 12-17% for women and 4-6% for men [13]. It manifests as moderate to severe headache episodes, usually unilateral, with mild to moderate cognitive deficit and cortical hyper-excitability manifestations such as phonophobia or photophobia, nausea, and vomiting. Two phases of migraine are described: the ictal phase, which occurs during a migraine attack, and the interictal phase, which occurs between two migraine attacks.

Up to 30% of patients with headaches accuse migraine attacks, and their frequency also influences cognitive deficits [14,15]. The ratio of men to women is 1:3. According to the ICHD-3, the main types of migraine are those without aura, those with aura, chronic-type migraine, complications of migraine, probable migraine, and episodic syndromes associated with migraine [16]. Migraine appears as a result of electrical abnormalities occurring in the brainstem, and vascular abnormalities in the cerebrum, and when we talk about migraine with aura, the result of cortical spreading depolarization [17].

The aura manifests most often as a visual aura (80-90% of cases), characterized by flashes of light in the simplest situations to scotomas or complex hallucinations [18].

Four phases of migraine have been described: the prodromal phase, in which the first warning symptoms appear (depression, fatigue, hunger, food cravings), which are most likely associated with dopaminergic activation and precede the headache by 2-48 hours [19]; the aura (which occurs in one-third of migraine patients) represents reversible neurological symptomatology that usually lasts about one hour and it usually manifests as sensory, verbal or motor symptoms. However, the visual ones are the most common. These changes come before the start of the cephalalgia [16,20]; headache is commonly on only one side of the head, having a progressively aggravating pattern at the beginning, being linked with autonomic symptoms like photophobia or phonophobia, or with symptoms like nausea, sickness-like condition or vomiting [20]; the postdromal phase, which is associated with a localized headache that is aggravated by movement, fatigue, or dizziness.

Migraine can be clinically assessed by structural MR imaging. For example, we now know that both aura and headache are mainly associated with vasoconstrictive changes in cerebral arteries and veins. Hypersensitivity in the region innervated primarily by cranial nerve V, but also in extracranial regions, causes allodynia when a normally painless stimulus becomes painful, such as wearing glasses or a light touch to the face.

MRI examinations are not routinely recommended in migraine patients without aura when preventive treatment is not necessary, and some authors recommend imaging studies when three lines of preventive treatment fail [21]. Another recent study emphasizes that imaging examinations are not needed in migraine patients without red flags [22]. In migraine patients with aura, no examinations are recommended in typical cases, and in the remaining ones, MRI or MRA of the brain is recommended to rule out vascular anomalies [21]. Cerebral MRI is also recommended for vascular comorbidities or headache onset after 50 years of age. MRI is not recommended for chronic migraine, especially if the history shows progressive worsening of headache with typical symptoms [21]. In patients with persistent aura, hospitalization, and diffusion-type imaging are mandatory to rule out cerebral ischemia.

Migraineurs are sensitive to olfactory stimuli. Up to 40% of them complain of headache exacerbation when exposed to strong, irritating, or sensitizing odors, even in the interictal phase, with studies demonstrating abnormal activation of the amygdala and insular cortex in the case of certain crises caused by odor stimuli [23,24]. On the other hand, other studies determining thermal stimulation showed unequal activation of certain areas between migraine patients and the control population, such as in the lentiform nucleus, parahippocampal gyrus, subthalamic nucleus, prefrontal cortex, or temporal lobe [25,26]. 40% of migraineurs report that their migraines are activated by strong light stimuli in the interictal phase, and 90% of them report that they suffer from photophobia in the ictal phase. Some studies demonstrate visual-nociceptive integration at the brainstem level [27].

Usually, imaging studies are not required in patients with a normal clinical examination who present with typical migraine symptoms according to the ICHD-3 definition [20,28]. To distinguish between patients who do not require additional imaging studies and patients with warning signs who do require imaging studies, the SNOOP algorithm is used successfully, as follows: S for systemic disease (e.g., infections, HIV), N for neurologic assessment (confusion, seizures, focal neurologic signs), O for onset (sudden onset, first episode after age of 50), O from other associated features (medications, toxins, trauma, association of headache with Valsalva maneuver), and P from previous episodes (or headache progression). The American College of Emergency Physicians 2019 (ACEP 2019) and American Academy of Neurology (AAN) guidelines recommend imaging studies only for warning signs that are not indicative of a common headache [29,30]. The American College of Radiology (ACR) emphasizes that clinical physicians should not perform repeated imaging studies when the headache is not complicated [31].

However, in migraine, deep white matter lesions (Figure 1A, T2, and FLAIR hyperintensities) are seen in 20-30% of MRI examinations, regardless of age or periventricular white matter lesions. Their exact pathophysiology is not known, but two theories have been mainly discussed: the theory of ischemia due to circulatory disturbances in the small perforating vessels together with inflammatory changes with consequent hypoperfusion, evidenced in MRI imaging by the appearance of small focal ischemic spots, mainly at the level of the frontal lobe (Figure 1B), but also in the corpus callosum, and the theory of neuroinflammation [32,33]. The latter emerged after the CAMERA study, which highlighted in females that deep WML takes place without a connection with ischemic lesions [32]. It is noteworthy that numerous subsequent studies have highlighted the fact that in migraine, the deep supratentorial white matter lesions are important but these do not correlate with the development of the disease, whereas the infratentorial lesions do not affect migraine attacks [30]. In addition, migraine patients appear to have a higher risk for cerebrovascular disease, but also doubling the risk of ischemic stroke [34,35].

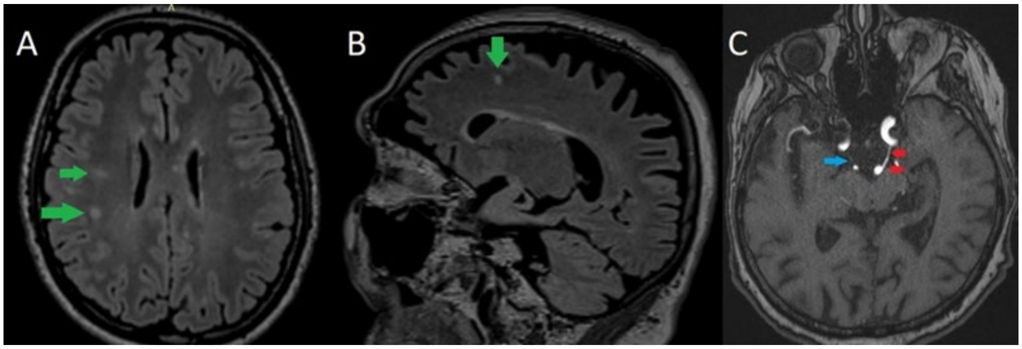

Figure 1: A: T2-weighted and FLAIR hyperintense lesions, in the centrum semiovale on the right side and deep white matter in a patient accusing repeated migraine attacks (green arrows). **B**: T2-weighted and FLAIR hyperintense lesion in the superior frontal gyrus (green arrow), the usual location for demyelinating lesions in migraine patients. **C**: asymmetry of the circle of Willis, showing a filiform right posterior communicating artery (blue arrow) compared to the contralateral one that shows significant blood flow (red arrows).

In migraine with aura, there may be transient restricted diffusion in the affected areas during the aura, in many studies taking place, especially in the corpus callosum [36].

Following some recent studies using MRI angiography (MRA), a new theory arises, advocating that there are asymmetries pointed out in the circle of Willis in a bigger population of migraine patients, particularly with aura (Figure 1C) [37,38].

In summary, in migraine patients without aura, imaging studies are not recommended for usual symptomatology that does not require treatment. For treatment-emergent migraine episodes, some authors recommend cerebral MRI or MRA when three lines of therapy have failed. In migraine patients with aura, an MRI of the brain is almost always recommended, or MRA if posterior fossa symptoms are present. In chronic migraine patients, additional imaging studies are not recommended as long as the symptomatology is not progressing, as long as the symptoms do not progress, but fundoscopy should be an integral part of the clinical examination in all patients with headaches [2,21].

Tension-type headache (TTH)

Tension-type headache represents a mild to moderate cephalalgia with a pressure-like character and usually occurs bilaterally, sometimes with anterior or posterior topography and sometimes it is longitudinal, lasting from minutes to days or even weeks. In contrast to migraine, it does not go along with autonomic manifestations or nausea and vomiting, and the presence of the above features precludes the diagnosis of tension-type headache. ICHD-3 classified TTH into four broad categories: infrequent epi-sodic TTH lasting < 1 day/month, frequent episodic TTH lasting 1-14 days/month throughout> 3 months, chronic TTH with a duration of > 14 days over > 3 months, and probable TTH [16]. The best diagnostic tool for tension-type headaches is the creation of a diary in which the patient records the frequency of painful episodes, their intensity, and their characteristics [39]. Considering the lower pain intensity, a lower proportion of patients with TTH consult a physician compared with migraine patients. One study has shown that the number of physician visits for TTH is 3 times lower than for migraine patients [40].

TTH is the second leading cause of chronic disease worldwide and is a socioeconomic burden, with 820 days missed from work or school per 1000 patients [41,42]. The most common differential diagnosis is migraine, as migraine patients often overlap with tension-type headaches.

The reported prevalence is 70% of the population, although 59% of patients report only episodic TTH [7,43]. Prevalence peaks in the 30-to-39-year-old age group and then decreases slightly [44]. Several studies have observed that sleep deprivation in combination with a disorganized lifestyle contributes to the occurrence of TTH [42,45].

Central factors are particularly important in tension-type headaches. While the pain threshold is normal in patients with eTTH, this threshold is somehow lower in tension-type headache patients with a chronic form [46,47]. Negative states, like depression or anxiety, and also chronic stress in TTH patients can increase the risk of headaches by lowering the nociceptive threshold [48,49].

In a study from Taiwan, it was suggested that the risk factors for TTH were more than 7 days of headache and female gender [50].

To date, although we use more and more advanced imaging techniques, the TTH pathophysiology is still not completely elucidated. Myofascial factors are incriminated to have a crucial role in episodic TTH along with pericranial muscle contractures, whereas sensitization of nociceptive sensory pathways by repetitive headaches leads to chronic TTH [51-53].

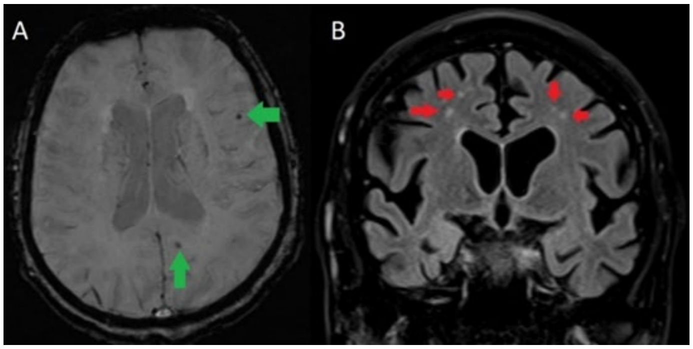

Figure 2: A. Magnetic susceptibility sequence in the axial plane. Changes in the magnetic susceptibility sequence with a suggestive appearance for microhemorrhages in the deep white matter and cortico-subcortical junction. **B**. Lesions in T2 and FLAIR hypersig-nal at the level of the bilateral corona radiata, in TTH patients without being specifically associated with some deep localizations such as in migraineurs.

As we already found out in the migraines chapter, imaging studies should not be carried out with typical-ly-described symptoms. More specifically, in patients with infrequent tension-type headaches, imaging is not recommended. In patients with frequent or chronic TTH, similar to migraine, some authors recommend an imaging examination when three lines of preventive treatment have failed [21]. Patients with chronic tension-type headaches should undergo one single imaging examination, either MR or CT, at the initial admission in the ED to exclude other differential diagnosis pathologies. The same applies to the TTH patients. The latter ones will generally have small foci of hemorrhage defined as hypodensities on susceptibility-weighted sequences deep in the white matter (Figure 2A) and deep WML, having the same physiopathological substrate as the migraineurs, increasing the risk of dementia or stroke later in life [54-56]. TTH and migraine patients generally have a similar number and amplitude of deep WML, but contrary to migraineurs, the TTH-associated WML does not show a preferred topography (Figure 2B). However, studies have shown that imaging examinations are not recommended because they do not improve the diagnosis and treatment, but they can cause and increase anxiety related to health status [57,58]. Acetaminophen and aspirin are thought to be efficient in cutting down acute symptomatology of hypertensive headaches. Opioids or triptans should be avoided. Ibuprofen and ketoprofen are the most effective NSAIDs for pain relief. Most patients benefit from lifestyle changes that promote a healthy lifestyle by quitting smoking and eating foods rich in antioxidants, and nonpharmacologic and behavioral therapies such as relaxation, biofeedback, or acupuncture can be helpful in most cases.

Trigeminal autonomic cephalalgias (TAC)

Cluster headache

Cluster headache is not a common headache, but is the most prevalent one in the group of TAC cephalalgias, having a prevalence of about 0,1% in the general population, affecting males and females differently, in a 6:1 manner. Many of the patients affected are older than 20 years of age [14,59]. Its most characteristics are severe pain with spontaneous remission, recurrent (usually circadian), unilateral, lasting 15-180 minutes, associated with autonomic ipsilateral cranial clinical signs such as lacrimation, conjunctival injection, or nasal congestion, the latter often leading to the misdiagnosis of sinus pain, with a completely different treatment from cluster headache [16,60]. In rare cases, cluster headaches may occur without autonomic symptoms [61].

80-90% of patients have episodic cluster headaches that occur apart from circadian development related to season (especially spring and autumn), number of daily light hours, and sleep stage, being more frequent during REM sleep [62]. Moreover, the relationship between sleep and cluster headache is very close. Many studies have shown that approximately 80% of patients who have manifestations of cluster headache will present cephalalgia attacks in the middle of the night, disturbing their sleep, but only 30% of them will show cephalalgias when they are napping during the day [63]. However, most of the patients complain of disturbed sleep quality, whether it occurs during or outside the headache period, and as a compensatory measure, some patients forget nightly sleep to avoid developing cephalalgic episodes [64]. An association between cluster headaches and sleep apnea has also been noted [63]. 10-20% of patients suffer from chronic cluster headaches, which have a very short period of remission, generally of the order of days.

The physiopathologic mechanism of this pain is not fully known. Both neurovascular causes and central mechanisms are suspected [65,66]. Given the predominant development with circadian recurrences, it has been suggested that the hypothalamus is of central importance for the development of pain, and also the trigeminovascular system and the parasympathetic systems being incriminated for starting and maintaining the symptomatology, those being involved in the autonomic trigeminal reflex [67]. Even though the V1 division of the 5th cranial nerve plays an important role in the development of algic symptoms, the autonomic ones are developed by the parasympathetic fibers of the facial nerve [68]. Since the 1990s, but also in more recent research, a genetic substrate is suspected, as there is a familial predisposition [69-71].

An association between cluster headaches and smoking has been confirmed. A recent study showed that 88% of the participating patients were active smokers or had smoked in the past [72]. When partial Horner syndrome occurs, cerebral MRI scans and targeted imaging studies at the level of the lung apex must be performed to identify lesions of the recurrent laryngeal nerve. Polysomnography may also be performed in cluster headache patients, as it is often associated with restless legs syndrome and helps to identify and treat this pathology [21].

In all types of TAC, cerebral MRI with high-resolution sequences of the cranial nerves is required at baseline to evaluate the parasellar structures. Similar to migraine, MRA is recommended if the first three lines of preventive treatment fail [21]. Changes in some brain structures were observed on imaging, particularly at the level of the thalamus, nucleus cinguli, prefrontal, parietal, and insular cortex [73,74]. Some older studies showed structural changes in the hypothalamus by MRI scans when headache was active, whereas other studies did not find these changes [75-77]. Some authors suggested that a decrease in grey matter volume in the visual cortex can occur, but it is non-specific [78,79].

Treatment of cluster headaches is divided into acute episode treatment and prophylactic treatment [80]. It includes drug treatment as well as patient education and lifestyle modification. As lifestyle changes, we can mention the avoidance of alcohol consumption, daytime sleep, or excessive physical activity. Treatment of the acute episode is initially by administering 100% oxygen by mask, up to 10 L/minute, for 15-20 minutes, which can relieve the cephalalgic episode in up to 15 minutes. Other therapeutic options include sumatriptan, a serotonin receptor agonist known to be the most effective therapeutic option and effective within 15 minutes when administered intravenously; dihydroergotamine, effective within 10 minutes when administered intravenously; zolmitriptan, effective within 30 minutes when administered orally; and local anesthetics [81]. Prophylactic treatment consists mainly of prednisone, dexamethasone, and ergotamine derivatives.

SUNCT and SUNA

SUNCT and SUNA are the other two types of TAC. Together, they are referred to as SUNHA [82]. The etiology and pathophysiology are not fully known. The association between these pathologies and posterior fossa lesions is well known, and recently the association between pituitary adenomas and these headaches has been discussed [83]. The pain generally occurs in the trigeminal area, more specifically in the V1 division and there is a high difficulty in differentiating between SUNHA and trigeminal neuralgia. These headaches have a sharp or burning character, last for a minimum of 5 seconds to a maximum of 10 minutes, and have a "sawtooth" development. A minimum of 20 pain attacks correlating with autonomic symptoms is required for clinical diagnosis, criteria defined by ICHD-3 [16,84]. The incidence is 0.1 per 10,000 population and the prevalence is 0.6 per 10,000 population [85].

The physiopathologic mechanism incriminated is represented by the activation of the trigeminal reflex and, similar to cluster headache, by triggering the hypothalamus [86]. Some recent studies have shown that trigeminal neurovascular conflict is important in the etiology of SUNCT and probably SUNA, with neuroimaging highlighting the similarity of changes in both pathologies, even assuming that the distinction between these two entities can be discarded [87,88]. High-resolution MRI imaging revealed neurovascular conflicts in more than 85% of patients with SUNCT or SUNA [87]. In addition, a recent study demonstrated ischemic changes in the left ventricular septal defect in approximately 15% of patients, leukoaraiosis in 5% of patients, and brainstem infarction in 5%. 88% of all patients had a vasculonervous conflict between the trigeminal nerve and certain vascular branches [85].

Therapeutically, the administration of many drugs used in other primary headaches has been tried, but with rather poor results. In this respect, SUNCT and SUNA differ from the other primary headaches. They respond mainly to the treatments used to treat neuropathic pain. Lamotrigine is effective in a large percentage of patients with the first pathology and less effective in the latter and is administrated at a dose of 100 to 300 mg daily. Topiramate isn't as effective as lamotrigine, and a smaller percentage of patients report symptomatology improvement. Carbamazepine is extensively studied and given in the treatment of these two pathologies, although with some poor results for the improvement of symptomatology in the SUNA group of patients. It is generally administrated in the standard dose of 200-1600 mg. Lidocaine is administered in an intravenous infusion of 1-4 mg/kg/hour to relieve severe headache attacks. Attempts may also be made to damage the trigeminal nerve or Gasser's ganglion by stereotactic surgery, radiofrequency stimulation, glycerol gangliolysis, or mechanical compression [89]. It is important to note that SUNCT and SUNA may also occur secondary to lesions at the level of the posterior fossa: damage to the cerebellum or brainstem by ischemic, tumoral, or vascular mechanisms. It is important to note that high-resolution MRI imaging studies can provide details about neurovascular compression, but these are not essential [2,21].

Hemicrania continua (HC)

Hemicrania continua is another primary headache that presents as a unilateral headache of moderate intensity with development over several months and belongs to the group of TACs according to ICHD-3 [2]. As the name implies, the pain is continuous and constant, but sometimes exacerbations associated with autonomic symptoms may occur. The response to indomethacin is pathognomonic [90]. It is estimated that the incidence of pathology is 1.0% and that continuous hemicrania accounts for approximately 1.5% of all primary headache cases [90]. Cases of continuous bilateral hemicrania were rarely presented [91]. According to ICHD-3, a clinical diagnosis requires a unilateral headache with pain duration of more than 3 months, ipsilateral autonomic symptoms, restlessness associated with the symptoms, and response to indomethacin treatment. Exacerbations may occur as a result of stress, alcohol consumption, or, in women, menstruation [90]. If HC is clinically suspected, cerebral MRI is recommended in all patients, and MRA or CT angiography of the head and neck should be indicated if an aneurysm or vascular pathology is suspected. Response to treatment with indomethacin is pathognomonic. Start with a dose of 25 mg three times daily, and if the patient does not respond to this treatment within 2-3 days, the dose is gradually increased up to a maximum of 300 mg per day [92].

Other primary headaches

Primary cough headache (PCH) represents a rare disorder in which bilateral cephalgia starts suddenly during coughing and may last up to two hours. According to ICHD3, the clinical diagnosis of primary-type headache is made by at least two episodes of pain that occur only after coughing or the Valsalva maneuver and last between one second and two hours, with no other associated intracranial pathology. However, up to 50% of patients hospitalized for this type of pain may have underlying lesions, such as Chiari malformation type 1, obstructive hydrocephalus, or tumors. Therefore, it is strongly recommended that imaging studies of the brain be performed, such as MRI or MRA [2,21,93]. Indomethacin is the treatment of choice for this type of headache [2].

Primary exercise headache (PEH) is a rare pathology similar to PCH, characterized by a bilateral pulsating headache that occurs only in the context of prolonged physical activity, lasting 45 minutes or less [2]. According to ICHD-3, when the patient first presents to the hospital with symptoms, it is mandatory to perform imaging studies to rule out "subarachnoid hemorrhage, arterial dissection, or vasoconstriction syndrome." Indomethacin is the treatment of choice for this pathology, in a dose of 25-150 mg before starting physical activity [94].

Primary headache associated with sexual activity is a diffuse bilateral headache that begins during sexual activity and lasts from 1 minute to 24 hours, accompanied by nausea or vomiting but without autonomic symptoms. These may present as progressive or sudden headaches, most often beginning at the moment of climax. Although it is generally not a serious medical problem, brain imaging is recommended to rule out other pathologies, such as subarachnoid hemorrhage or aneurysm [95]. It is difficult to quantify the epidemiology of this pathology because patients are reluctant to go to the doctor to be examined due to shame or ethical reasons. Some authors recommend prophylaxis with beta-blockers and symptomatic treatment with indomethacin [96,101].

Because of the rarity of these pathologies, there are no large, homogeneous studies quantifying the imagistic changes specific to each of these three pathologies, but rather small studies and case reports. For this reason, it is important to undergo a CT scan, especially in cases of sudden onset headache, to rule out secondary causes such as rupture of an aneurysm or subarachnoid hemorrhage, and subsequent vascular studies such as CT angiography or MRA are recommended in specific cases.

DISCUSSION

Despite the use of increasingly advanced imaging examination methods that have improved the understanding of the pathophysiology and mechanisms of development of primary headaches, the medical community still cannot fully understand and fully, optimally treat primary headaches. Structural MR imaging has demonstrated the presence of T2 and FLAIR hypersignal lesions that can be correlated with different pathologies that mimic the primary types of headache, although those appear especially with aura migraine. MR Perfusion imaging has shown several abnormalities in blood circulation and neuronal activity during cephalalgia exacerbations in every type of primary headache, demonstrating a lowering of pain threshold and, compared with the general population, deficient activation of many different areas in the brain responsible for interpreting pain, and in the case of migraineurs in particular, there is both a supernumerary activation of the areas as well as a more important or intense activation of them. Many authors have demonstrated that the autonomic nervous system takes part in the mechanism of headache development, due to age-related development and the decrease in headache amplitude in the case of noninvasive stimulation of the vagal endings for cluster headache prevention. These suspicions, especially in migraine, are not confirmed by imaging studies, either structural or functional. The use of imaging studies, regardless of their type, to diagnose migraine or tension-type headaches is not recommended because of the lack of specificity of the studies. Because these are debilitating diseases that affect both individual lives and society, sufferers search for answers and go from one physician to another in search of them. Repeated imaging studies in these cases represent a significant waste of resources, and each pseudonormal imaging result leads to even greater concern and anxiety about health status. The correlation of the new, recently discovered data with the clinical evolution of the patient can improve the selection and treatment of patients at risk for primary headache development, sometimes even from childhood. Today we know that primary headaches begin around the age of 5 years and the highest prevalence is found in the age group between 20 and 40 years, decreasing with age. The phenotypes and treatments of each pathology are different, even if they belong to the same class of pathologies. Currently, we can summarize that there are ischemic lesions, circulatory disturbances, vascular conflicts, 5th cranial nerve activation, and a lower pain threshold, all having their role in the development of these pathologies. Current studies include far too small and inhomogeneous groups of patients to be able to present convincing results regarding structural changes in the brain parenchyma or the various neuronal circuits.

CONCLUSIONS

In recent years, significant progress has been made in understanding the pathophysiologic mechanisms, both central and peripheral, of the primary cause of headaches, by using highly advanced technologies like structural high-resolution

MR, both of these imaging studies of cerebral structures allow the detection of cerebral parenchyma lesions with greater precision.

Pharmacologic interventions may help alleviate headaches, and recently several minimally invasive interventions have been tried, mainly targeting the physiopathologic mechanism that occurs via the cranial V nerve, obtaining promising results. However, there are still physiopathologic and imaging ambiguities related to these primary pathologies.

There is still an unmet need for studies in all fields, in large groups of homogeneous patients, to answer these questions.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, RID, FAA, AMR, and CAS; methodology, AFN, FAA, AMS, and CAP; software, RID, AFN, OV, and AMR; validation, AMR, OV, CAS, and CAP; formal analysis, RID, AFN, AMR and FAA; investigation, RID, AMR, OV, AMS, and CAS; resources, AFN, FAA, and OV; data curation, RID, AMR, AMS, CAS, and CAP; writing—original draft preparation, RID, AMR and CAP; writing—review and editing, AFN and AMS; visualization, RID; FAA, AMR, and CAS; project administration, FAA. RID and AMR contributed equally to this paper. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

E-cigarettes and Associated Medical Burden: For Better and For Worse

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Abstract: *The harmful effects of smoking cigarettes on human health are well documented; thus various smoking cessation methods have been assessed and new options are currently under development to provide a "better' alternative to the "worse" one, namely smoking. However, no ideal method of ending or replacing conventional smoking has been developed so far, and actually, none of them has been proven entirely effective or safe. The laxity, or rather the absence of regulations in the first decade of existence of electronic nicotine delivery systems (ENDS) allowed their use with prohibited substances instead of nicotine or other substances with detrimental effect, with the emergence of a severe syndrome - e-cigarette and vaping-associated lung injury (EVALI), whose treatment required even lung transplantation in young people without any prior underlying lung disease. This narrative review aims to provide a brief overview of concerns about medical issues associated with e-cigarette use, particularly cardiovascular and respiratory panels. We organized the data in several micro-sections varying from practical aspects of understanding ENDS to clinical issues. To combat tobacco addiction, electronic cigarettes are increasingly widely accepted. A global regulatory framework is required to prevent the emergence of an illicit e-cigarette business with detrimental impacts on health.*

Keywords: *e-cigarettes, EVALI, smoking, lung, respiratory, cardiovascular, pulmonary, electronic cigarette, ENDS*

INTRODUCTION

The harmful effects of smoking cigarettes on human health are well documented – from a simple addiction to a significant risk factor for lung cancer. Recent data places smoking as the leading risk factor for lung cancer, the most common type of cancer in the world, which is responsible for more deaths annually. The detrimental effects on cardiovascular, pulmonary, and reproductive systems are also considerable. Therefore, various smoking cessation methods have been assessed – behavioral, pharmacological, and combined methods and new options are currently under development to provide a "better' alternative to the "worse" one, namely smoking. However, no ideal method of ending or replacing conventional smoking has been developed so far, and actually, none of them has been proven entirely effective or safe [1-4].

Therefore, that's why there has been a continuous struggle to find smoking methods that do not involve burning nicotine, with heat-not-burn devices being the best, safest alternative, and an efficient smoking cessation tool. However, in the last decade, considerable evidence has been presented showing an induction of addiction in adolescents and smoking initiation in those who have never smoked both adolescents and adults. On the other hand, the laxity, or rather the absence of regulations in the first decade of existence of electronic nicotine delivery systems (ENDS) allowed their use with prohibited substances instead of nicotine or other substances with detrimental effects, with the emergence of a severe syndrome – e-cigarette and vaping-associated lung injury (EVALI), whose treatment required even lung transplantation in young people without any underlying lung disease. After the emergence of EVALI, which unfortunately has been overshadowed by the coronavirus disease-19 (COVID-19) pandemic, an increasing number of countries around the world have imposed regulations on the use of ENDS, which are now considered medical devices in some countries and are banned in others. Since it has been determined that the numerous flavors available to users have led to an increase in their use by adolescents, current legislation in most countries that have regulations limits the number and type of flavors available [5-8].

Aim

We aim to provide a brief overview of concern for medical issues associated with e-cigarette use.

METHODS

This is a narrative review of literature with no timeline restriction of research. We organized the data in several micro-sections varying from practical aspects of understanding ENDS to clinical issues.

ENDS – electronic nicotine delivery systems

ENDS include various devices, such as electronic cigarettes, e-hookahs, advanced personal vaporizers, and cig-a-likes. Currently, there are two main types of heat-not-burn products – tobacco heating devices and electronic cigarettes (four generations). From its first release in China in 2003 until today, the e-cigarette has undergone numerous improvements, with the current generation allowing a complex control of the device with significantly improved user safety. Electronic cigarettes are composed of an energy source (a rechargeable battery available from the second generation), a heating device - an atomizer, responsible for the generation of aerosols, a flow generator, a refillable (available from second generation) cartridge for e-liquid (water, propylene glycol, nicotine, flavors, and glycerol) and a mouthpiece. The third generation of e-cigarettes allows resistance and power adjustment while the fourth generation offers the possibility of temperature control (generally temperatures range from 40-65 Celsius), allowing for the modulation of the oropharyngeal experience [9-13].

It has been observed that by increasing the heating temperature, the aerosol generation rate also increases, and the aerosol particle size is modified as well (staying in the 100-600 nm range), with a potential impact on aerosol toxicity. Tobacco heating devices consist of a holder, an accumulator, and a nicotine heating unit (blade or induction heat technology) with aerosol generation. The differences between tobacco heat devices and e-cigarettes consist of user experience, the first one having a different operating time (6 minutes and a maximum of 14 puffs) and the temperature at which it operates (conventional smoking requires 200-600 Celsius). Nicotine is present in the tobacco that is used in the device. Also, not all e-cigarette devices use e-liquid with nicotine [14-19].

ENDS chemical compounds

E-liquid compounds include solvents (humectants or stabilizing agents), nicotine, cannabinoids, metals (in both the nicotine extract and the device constituents – nickel, manganese, copper, iron, arsenic, and lead), and flavors. The harmful effects of smoking are due to the inhalation of toxic products in cigarette smoke, generated by the combustion and pyrolysis of nicotine. Smoke from regular cigarettes contains more than 7000 different substances, including at least 70 carcinogens [20-24].

Unfiltered cigarette smoke is an aerosol with more than 400-500 gaseous components (nitrogen, oxygen, carbon monoxide and carbon dioxide, water, hydrogen, ammonia, argon, nitric oxide, hydrogen cyanide, hydrogen sulfide, methane, volatile alkenes, volatile alkenes, aromatic hydrocarbons - benzene, toluene, styrene, acids - formic acid, acetic acid, propionic acid, aldehyde - formaldehyde, acetaldehyde, acrolein, acetone, methanol, furan, pyridine, methylamine, etc.) as well as over 3500 other particulate constituents (nicotine, nornicotine, anatabine, anabazine, non-volatile hydrocarbons, carcinogenic polynuclear aromatic hydrocarbons, phenols, phthalates, polyphenols, acids – palmitic, stearic, oleic, linoleic, linolenic, lactic, indoles, quinolones, aromatic amines, nitrosamines and glycerol). In addition to these, various flavorings, pesticides, and metals are also present. Cigarette smoke contains 72 carcinogens and at least six compounds that harm reproduction [25-29].

Because they inhale both mainstream and sidestream smoke, passive smokers are more exposed to these dangerous substances than regular smokers. The amount of nicotine delivered by the e-cig is the result of several factors – smoking pattern (experienced versus inexperienced), aerosol characteristics (pH, particle size), pharmacokinetic and pharmacodynamics features, the site of absorption (oral or pulmonary), and of course the nicotine level in the e-liquid. The new generations of e-cigarettes deliver more aerosols and consequently higher amounts of nicotine, as demonstrated by their serum levels. The amount of nicotine in traditional cigarettes ranges between 10-15 mg nicotine/cigarette, of which 1-2 mg nicotine/cigarette is systemically absorbed, compared to 0-36 mg/mL liquid in electronic cigarettes [30-35].

E-liquid includes propylene glycol, vegetable glycerine, nicotine, and flavors. The e-cig structure includes absorbent materials in the atomizer (silicon, organic cotton, rayon fibers, stainless steel mesh, bamboo yar), as well as heating coils manufactured from different iron, chrome, aluminum, or nickel alloys. The usual heating temperature in early generation ENDS ranged from 45 to 60 degrees, but in new generations that allow the user to adjust the temperature, it can reach over 200 Celsius, resulting in the release of potentially harmful carbonyl products, such as acrolein in increased quantities at voltages above 3.3 V [36-39].

The most commonly encountered flavorings in e-cigarettes are diacetyl, acetoin (buttery flavor), benzaldehyde (almond/ cherry aroma), cinnamaldehyde (cinnamon aroma), cresol (leather aroma), eugenol (clove aroma) or isoamyl acetate (banana flavor). The products resulting from the conversion of liquid into aerosol can vary, the temperature obtained by heating in e-cigs not being uniform and depending on several factors – the volatilization rate, the effectiveness of aerosol absorption, product design, and battery voltage. Thus, by converting solvents, formaldehyde, acetaldehyde, acrolein, methyl glyoxal, and glyoxal are obtained, and from vitamin E, ketene, benzene, and alkene are generated [33-39].

Cardiovascular effects

As a result of nicotine absorption, there is a release of catecholamines with a secondary increase in heart rate, blood pressure, and heart contractility, concomitant with reduced coronary vascular flow and increased musculoskeletal flow. The decreased risk of cardiovascular disease has been demonstrated in both in vitro and in vivo studies. It has been shown a reduction in monocyte cell adhesion to endothelial cells, a reduction in platelet activation markers (thromboxane B2), oxidative stress markers (prostaglandin F2 alpha), and C-reactive protein and cholesterol [37-40].

The currently available studies have assessed both short-term and medium to long-term effects (years). Heart rate and blood pressure abnormalities have been shown to vary according to the smoking status of e-cigarette or former conventional tobacco smokers. Thus, in short-term use of e-cigarettes with or without nicotine, an increase in heart rate and blood pressure was observed, whereas in chronic smokers the data are not conclusive. In e-cig smokers, an increase in arterial pressure was found only for short-term users. In contrast to conventional cigarette smokers converted to e-cigarettes, the switch to ENDS reportedly promotes a reduction in cardiovascular risk and BP [41-45].

Respiratory system effects

Studies have consistently identified a significant association between using electronic cigarettes and respiratory disorders. At the pulmonary level nicotine acts by activating nicotinic receptors for acetylcholine resulting in bronchoconstriction by action on bronchial smooth muscle. Epigenetic changes resulting in reduced PPAR-gamma (mesenchymal peroxisome proliferator-activated receptor gamma) receptor expression results in an asthmatic phenotype. Lung nicotine levels are more than 200 times the systemic peak in both conventional smokers and vapers [46-48].

Propylene glycol and vegetable glycerine are used as humectants, but repeated inhalation is not associated with significant changes in lung function if the exposure is limited. On the other hand, the new generations of devices, which can reach temperatures above 300 Celsius, can generate toxic carbonyl volatile compounds – formaldehyde and acetaldehyde (at temperatures above 215 Celsius) and acrolein at temperatures above 270 Celsius, two compounds that are associated with exacerbation of bronchial asthma. Diacetyl (2,3 butanedione) a butter flavoring is known to induce irreversible acute bronchiolitis obliterans (long popcorn). Other flavorings such as cinnamaldehyde are known irritants for asthma and benzaldehyde is an irritant for the mucous membranes of the respiratory tract. Yet, benzaldehyde higher levels provided by newer generation ENDS were proven to suppress allergic inflammation in a murine model by decreasing the number of inflammatory cells in lung and nasal tissues [49-53].

Destructive effects on respiratory epithelial cells have been demonstrated in several in vitro studies, as well as on nasal epithelial cells [49-53]. In vivo research has shown reduced lung cancer risk in ENDS users [54-56]. Increased oxidative stress and mitochondrial dysfunction have also been demonstrated in in vivo studies [57-60].

Shortly before the COVID-19 pandemic, a new outbreak occurred in ENDS users- EVALI, with more than 2500 cases reported in the US over 5 months [61-72]. The predominant symptomatology was respiratory – dyspnea, cough, and chest pain, associated also digestive symptoms and fever. Most cases were caused by exposure to tetrahydrocannabinol while the remainder by exposure to vitamin E acetate which was used as a thickening agent. The latter is thought to alter the structure of the surfactant and by heating may generate keten, a toxic compound [73-77]. The introduction of regulations on ENDS status has led to a dramatic decrease in EVALI cases.

DISCUSSION

Apart from the mentioned inflammatory issues and the cardiovascular/respiratory associated entities related to the use of e-cigarettes, other medical consequences have been identified, yet, with various levels of statistical significance and some require further exploration to adequately assess their clinical impact.

For instance, while most studies have focused on pulmonary and cardiovascular effects, smoking is a well-known risk factor for oral cancer.

Current data shows a similar effect of e-cigarettes when compared to traditional tobacco smoking in terms of oxidative stress, wound healing arrest, promotion of a pro-inflammatory environment by increased pro-inflammatory cytokines, and incidence of periodontal disease, caries, and edentations.

However, the few available data on the carcinogenic effect of e-cigarette use have not led to any relevant practical conclusions, as they did not have a well-defined duration of exposure that enabled an objective risk assessment [78-84]. In addition to the direct effects on the user's health, environmental effects are described, which are attributed to the combination of compounds in mainstream and sidestream smoke.

Finally, as mentioned the most dramatic consequence in relationship with e-cigarette use is EVALI which particularly affects young individuals. While severe COVID-19 forms of infection might mimic an episode of EVALI, during pandemic years a growing number of hospitalizations for this syndrome had been registered.

The medical community increased the level of awareness concerning EVALI and recently various public health resources and scientific papers have been released to address this particular aspect [85-88].

CONCLUSIONS

Although ENDS are promoted as a safe alternative to smoking tobacco, it has been shown that adolescents who use them are more likely to start smoking tobacco again or start smoking it for the first time. Smoking is a significant global public health issue. The methods for quitting smoking have changed over time, but none have achieved perfect success. To combat tobacco addiction, electronic cigarettes are increasingly widely accepted. A global regulatory framework is required to prevent the emergence of an illicit e-cigarette business with detrimental impacts on health.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; methodology, C.N., A.-P.C., A.C., A.-I.T, A.-M.G., and M.C.; software, C.N., A.-P.C., O.-C.S., A.-M.G., and M.C.; validation, C.N., A.-P.C., O.-C.S., A.C., A.-M.G., and M.C.; formal analysis, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; investigation, C.N., A.-P.C., A.-M.G., and M.C.; resources, C.N., A.-P.C., O.-C.S., A.C., and M.C.; data curation, C.N., A.-P.C., and M.C.; writing—original draft preparation, C.N., and A.-P.C.; writing—review and editing, C.N., A.-P.C., and M.C.; visualization, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; supervision, C.N., A.-P.C., and M.C.; project administration, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; funding acquisition, C.N, and M.C.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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REVIEW

The Spectrum of Neurological Complications after Bariatric Surgery – Review of the Literature

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Abstract: *Bariatric surgery represents one of the most efficient treatments addressing obesity and its associated complications with an increasing trend. Even if the medical benefits for patients are huge, due to weight loss and despite technical improvements in bariatric procedures, it is still associated with diverse types of complications. Most of the complications appear due to nutrient deficiency, as absorption is compromised after surgical anatomy modification. Neurological complications after bariatric surgery are very diverse with an occurrence within days to years after surgery, most frequently in patients with gastrointestinal or surgical complications. Any segment of the nervous system can be affected with distinct types of manifestations ranging from peripheral axonal neuropathy, nerve palsy secondary to compartment syndrome, encephalopathy, and myelopathy, to psychiatric and demyelinating central nervous system diseases. Identifying patients at risk and systematic screening for nutrient deficiency with proper supplementation is essential for preventing neurological complications and lifelong sequelae. In this paper, we present the spectrum of neurological complications reported after bariatric surgery.*

Keywords: *bariatric surgery; nutrient deficiency; neurological complications*

INTRODUCTION

Bariatric surgery still represents one of the most efficient treatments addressing obesity and because the incidence of severe obesity is rising worldwide, more and more bariatric procedures are done annually in specialized medical centers. Even if the medical benefits for the patients are huge due to weight loss and despite the technical improvements for bariatric procedures, it is still associated with different types of complications.

Most of the complications are due to the modified anatomy which leads to impaired absorption of the nutrient, besides the inflammatory state and mechanical stress induced by surgical procedure. The most common complications are gastrointestinal and surgical complications and include but are not limited to reflux disease, vomiting, dumping syndrome, leaks with high mortality getting up to 15%, stenosis, twists and kinks causing dysphagia, bleeding, enteric perforations, internal hernias, bowel obstruction, ulcerations, and gallstones disease. Other complications seen following bariatric surgery are weight regain, pulmonary thromboembolism, metabolic and hematological disorders, such as anemia, leucopenia, and neuropsychiatric complications. [1]

MATERIALS AND METHODS

The purpose of our paper was to review the existing literature data regarding the neurological complications that occurred after bariatric surgery and the presence of nutrient impairment.

We performed a scoping review of the published literature, and we included original studies, reviews, and a series of cases by searching the Medline, PubMed, and Google Scholar databases. We included articles written in the English language.

We searched for the terms: “bariatric surgery”, “neurological complications and bariatric surgery”, “nutrient deficit and bariatric surgery”, “vitamin deficit and bariatric surgery”, “gastric sleeve”, “gastric banding”, “gastric bypass”, “vertical gastroplasty”, “obesity surgical treatment”.

We excluded papers with respect to bariatric surgery-related complications and pre-operative nutritional deficiencies.

NEUROLOGICAL COMPLICATIONS

Neurological complications after bariatric surgery were reported in 3-16% of cases and usually affected more than one component of the nervous system [2,3].

Neurological disorders are various and affect mainly the peripheral nervous system and less common central nervous system, but both are associated with a poorer quality of life and substantial morbidity. Neurological complications of these patients might occur days to years after surgery and include peripheral neuropathy, compartment syndrome, plexopathy, mononeuropathy, encephalopathy, myelopathy, ataxia, myopathy, myotonia, behavioral and psychiatric disorders [2,3,4].

The physiopathology of neurological impairment comprises nutritional deficiency, rapid weight loss, and compartment syndrome. The vitamin B12 shortage, thiamine deficit, and decreased levels of copper are the most commonly deficient nutrients responsible for neurological conditions development after bariatric surgery. Deficiency of riboflavin, pyridoxine, folate, vitamin D, E, phosphorus, calcium, selenium, zinc, and iodine can also contribute to neurologic dysfunction [3,5].

Impact Of Vitamin and Micronutrient Deficiency After Bariatric Surgery and Neurological Complications

Nutritional impairment after bariatric surgery is quite common and manifests a large spectrum of vitamin and micro-nutrient deficiencies affecting both, the water-soluble vitamins (thiamine, riboflavin, pyridoxine, folate, cyanocobalamin, vitamin C), and the liposoluble ones (A, D, E, K), alongside the fat malabsorption, loss of proteins, and minerals deficiencies (iron, zinc, copper, selenium). All these associate different clinical manifestations within a variable time frame of appearance and usually are overlooked by clinicians. Some bariatric procedures, such as Roux-en-Y gastric bypass and post-biliopancreatic diversion/duodenal switch put the patient at higher risk of developing multiple nutritional deficiencies since the absorptive surface is reduced or bypassed [5,6].

The most common deficiencies reported after bariatric surgery are iron deficiency (which was found in more than 30% of patients) [5], cobalamin and folate deficiency in up to 35%, respectively 39% after five years [6], and thiamine deficiency in 49% of patients [7].

Reports from a French cohort study showed a high rate of overall vitamin B deficiency (74%), with thiamine deficit in more than 47% of the cases [8]. A significant number of patients, up to 30%, already had micronutrient deficiency before bariatric surgery which increased the risk of new complications [9]. Hypovitaminosis D after bariatric surgery was reported in 25-70% of the patients, with calcium deficiency in 10%, but it should be considered that obesity is associated with suboptimal 25-hydroxy-vitamin D and 30% of obese patients could have vitamin D deficiency [10,11]. Vitamin E deficiency was reported up to 65.7% in a recent systematic review, and vitamin A in 5-69% in 2-4 years following surgery. Clinical manifestations of vitamin E deficiency are usually seen after Roux-en-Y gastric bypass, being mainly neurological (ataxia, peripheral neuropathy), but also hemolytic anemia, impaired immune response, ophthalmological disorders, infertility, and nystagmus can occur [12]. Although rarely clinically manifested, deficiency of vitamin C and vitamin K was reported in almost half of the patients [6,12].

Low zinc level after bariatric surgery was associated with high inflammatory status. There are reports of necrolytic migratory erythema with severe bacteremia and optic neuropathy due to zinc depletion [13,14]. The zinc repletion therapy should be done cautiously because it can induce copper deficiency. The copper deficiency occurs mostly after gastric bypass as its absorption occurs in the stomach and duodenum. Many cases of copper deficiency had been reported up till 9 years after bariatric surgery with capricious clinical expression, the majority mimicking vitamin B12 deficiency, consisting of myelopathy, ataxia, peripheral neuropathy, optic neuropathy, blindness, muscle weakness, anemia, and neutropenia [15,16, 17,18].

The diagnosis of copper deficiency usually requires the measurement of serum levels associated with the ceruloplasmin level under 20 mg/dl and C reactive protein because ceruloplasmin is an acute phase protein and levels could be misinterpreted in inflammatory states. MRI study with T2 hyperintensity in the posterior dorsal column of the spinal cord can suggest the diagnosis [19,20].

Most of the neurological complications are often seen in patients with surgical complications, longer hospitalization time, or those with gastrointestinal symptoms, especially protracted vomiting. Moreover, these patients underwent a surgical reintervention in 8-32% of the cases [8,21].

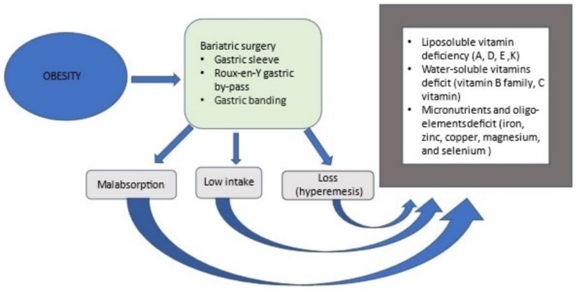
OBESITY
Bariatric surgery
Gastric sleeve
Roux-en-Y gastric by-pass
Gastric banding
Liposoluble vitamin deficiency (A, D, E ,K)
Water-soluble vitamins deficit (vitamin B family, C vitamin)
Micronutrients and oligo-elementsdeficit (iron, zinc, copper, magnesium, and selenium)
Malabsorption
Low intake
Loss (hyperemesis)

Figure 1: Pathophysiology mechanisms of neurological complications after bariatric surgery and types of nutrient deficiencies

Peripheral nervous system complications

Neuropathy is the most frequent neurological complication described after bariatric surgery and has been reported in 16% of patients after this type of procedure [22]. Its occurrence is induced by vitamin deficiency, especially thiamine, and cobalamin, which are involved in myelin synthesis, but also riboflavin, pyridoxine, and copper deficiency [21].

Another mechanism of occurrence is attributed to entrapment and mechanical compression. Rapid weight loss with subcutaneous protective fat tissue disappearance and inaccurate leg postures can cause compression neuropathy, frequently unilateral. Involvement is usually sensory predominant with late motor progression, chronic and symmetrical, mostly affecting distal limbs, but also small fiber neuropathies and bilateral neuropathies were several times described, contrary to plexopathy which was rarely seen [23,24].

The median nerve compression, the peroneal nerve palsy, and the ulnar nerve palsy were the commonest nerves affected after bariatric surgery. Peroneal nerve palsy is usually unilateral, but cases with bilateral affection were described.

Rapid weight loss with a mean of 45-50 kg reduction in several months is the main risk factor identified for peroneal nerve injury as the nerve's superficial anatomic localization makes it susceptible to impingement [24]. One case of meralgia paresthetica after bariatric surgery was described in an Asiatic report [25]. Rare cases of dry beriberi disease characterized initially by nausea, vomiting, constipation, severe weakness and then progressing to feet tingling and burning were described in the literature with late diagnosis, which was only suggested after performing electromyography [26].

A few cases of acute axonal polyneuropathy resembling Guillain Barre syndrome, rapidly progressive were described in the medical literature after bariatric surgery with good response to vitamin B1 and B12 high dose replacement [27]. There are also recently reported cases of mixed polyneuropathy after bariatric surgery both axonal and demyelinating after five to 24 months post-surgery and some case reports of Guillain Barre syndrome, raising the suspicion that bariatric surgery could induce immunological changes which predispose to autoimmunity [14,28]. Cases of plexopathies affecting the lumbar plexus were reported, manifesting with unilateral severe pain with sensory loss in the hip and thigh region, muscle wasting, patellar reflex loss, and extensive denervation on electromyography study [25].

Several other risk factors for predicting neuropathy development, despite rapid weight loss, were previously reported, such as low albumin and transferrin levels, gastrointestinal complications, surgical complications with long hospitalization, jejunoileal bypass, and lack of nutritional assessment after bariatric surgery [22]. Symptoms of neuropathies include pain, paresthesia, burning feet in small fiber neuropathy, usually progressive weakness, and rarely acute, foot drop, slapping gait, loss of reflection, and objective sensory loss. The diagnosis is confirmed by electromyography studies indicating distal axonal neuropathies. Biopsies of the affected nerves show axonal degeneration and peri-vascular inflammation [24,29].

Treatment includes a high dose of vitamin B12 dose of 1000 ug intramuscular once daily for a week and then weekly, vitamin B1 500 mg daily, intravenous for three days, and afterward 100 mg oral route and acid folic supplementation 5 mg per day, with good response and resolution of symptoms. Vitamin supplementation should be done for long periods and started as soon as possible when diagnosing neuropathy in post-bariatric surgery patients without waiting for confirmation of vitamin deficiency tests since vitamin levels can be normal. Nerve decompression was cited as the most efficient therapy for peroneal nerve entrapment after bariatric surgery [23]. Intravenous immunoglobulin is the treatment of choice for Guillain Barre syndrome, with a good response. Physical therapy can be used as a complementary treatment to vitamin replacement when treating neuropathy [24,29].

Functional impairment is usually mild, but it can be debilitating when the neuropathy becomes chronic or when the patient experiences weakness and foot drop. Also, small fiber neuropathy can evolve into distal neuropathic chronic pain syndrome in all extremities, deteriorating life quality [24,29].

Central nervous system complications (CNC)

The most reported CNS complications after bariatric surgery are encephalopathy, myelopathy, neuropsychiatric disorders, and optic neuropathy [30].

Encephalopathy

The most common type of encephalopathy seen after bariatric surgery is Wernicke encephalopathy secondary to thiamin deficiency, but also different types of encephalopathy are being described, such as hyperammonemia encephalopathy due to high plasmatic levels of glutamine and ammonia, zinc deficiency and urea cycle disorders. Episodic encephalopathy associated with D lactic acidosis is another type of encephalopathy encountered after bariatric surgery, usually precipitated by high carbohydrate intake, and hardly diagnosed by clinicians [31,32].

Clinical manifestation of Wernicke encephalopathy consists of acute confusion, ataxia, nystagmus, sometimes associated with cranial nerve palsies (third and sixth), seizures, dysarthria, myoclonus, asterixis, papilledema, vestibular symptoms, blurred vision, and polyneuropathy. Almost 80% of patients surviving encephalopathy develop Korsakov syndrome defined by amnesia (retrograde and anterograde), confabulation, and loss of insight, being more common in alcohol consumers. Most cases of Wernicke encephalopathy occur between 14 days to 1-2 years, mostly in women, and are usually precipitated by several factors. Vomit-ing is the most common gastrointestinal symptom precipitating Wernicke encephalopathy, usually preceding thiamine (Gloy VL) deficiency manifestations [8,12,33]. Other factors include rapid weight loss, food avoidance, poor appetite, and lack of vitamin supplementation, as thiamine reserves are depleted in less than 3 weeks without supplementation. In the absence of rapid diagnosis and appropriate treatment, the disease is fatal. Diagnosis of Wernicke encephalopathy is usually clinical, supported by laboratory and IRM studies. Tissue thiamine levels are accurately determined by red blood assays of specific enzymes (erythrocyte transketolase activity coefficient since blood and urine levels are not reliable [34].

IRM changes in Wernicke encephalopathy include symmetrical increased signal on T2/FLAIR phase in dorsomedial thalami, mamillary bodies, tectum plate, periaqueductal area, and around the third ventricle. Treatment of Wernicke encephalopathy consists of intravenous/intramuscular of thiamine 100 mg for 3-5 days minimum, followed by 30 mg twice orally for several months and correction of hypo-magnesemia since magnesium is a cofactor in thiamine-dependent metabolism. Prevention is mandatory and patients at risk should get thiamine supplementation of 100 mg intramuscularly followed by 50 orally once daily. Recovery in Wernicke encephalopathy induced by bariatric surgery after optimum thiamine supplementation is seen in more than half of the cases, the others remaining with cognitive deficiencies, gait disturbances, nystagmus, psychosis, and rarely death [15,30,33].

Hyperammonemia encephalopathy represents a particular type of encephalopathy recognized in the past years in patients who have undergone Roux-en-Y gastric bypass with a high mortality rate, characterized by a high level of ammonia in the absence of liver disease [31]. In a review from Andrew Z. Fenves et all in 2015 a mortality rate of 50% was reported in these patients with a predominance of women as the conditions were most often seen in this category of patients [35].

Laboratory findings include mild to moderate increased levels of ammonia, high levels of plasma glutamine, low albumin and zinc levels, essential amino acids deficiency, and reactive hypoglycemia [36].

The precise mechanism of onset is not yet understood, but several factors were until now described in the hyperammonemia state after bariatric surgery. Most of the lethal cases of hyperammonemia encephalopathy showed genetic predisposition caused by urea cycle abnormalities with a partial ornithine transcarbamylase deficiency or a mild carbamyl phosphate synthetase (CPS) deficiency [32].

One of the predisposing factors for hyperammonemia is the presence of huge nitrogenous residues in the liver. Intake reduction triggers a high catabolic state with protein breakdown. Ammonia level is also increased by altered gut microbiota that produces more urea lithic strains because of small bowel pouches created by bariatric surgery [31,36]. Case reports from patients with this neurological complication after bariatric surgery reported lower citrulline levels in the intestine affecting ureagenic capacity. Moreover, other predisposing conditions as the presence of a splenorenal shunt should be suspected and ruled out in patients developing hyperammonemia [32,35].

Therapeutic options include protein intake reduction, zinc and amino acid repletion, parenteral glucose, lipid supplementation, and oral lactulose. In refractory cases, hemodialysis and setting back of surgical procedures could be an option [37].

D-lactic acidosis encephalopathy is a rare condition reported mostly in short bowel syndrome, but also described years after bariatric surgery, especially after jejunum-ileostomy, and should be taken into consideration in a patient with anatomic changes of the digestive tract who develops confusion syndrome [38]. Confusion episode is associated with behavioral changes, dysarthria, ataxia, lethargy, and weakness, and is usually triggered by high carbohydrate intake. D lactate serum elevation is a result of carbohydrate fermentation in the small bowel or colon and is hardly diagnosed as serum and urine measurement of D lactate is not commonly done, L lactate being the only form that is most frequently checked when assessing lactic acidosis. A level over 3 mmol/l is diagnostic for this pathology and is often associated with a high serum anion gap [38]. Treatment involves metabolic acidosis correction with intravenous bicarbonate, fasting reducing carbohydrate levels, and intravenous amino acids, but also modulating intestinal microbiota with antibiotics which improve neurological symptomatology [39].

Myelopathy is usually seen years after bariatric surgery being one of the most disabling complications. It commonly affects posterior columns with the dorsal ganglia root and can be also associated with neuropathy (myeloneuropathy) or optic neuropathy. Manifestations consist of vibration and proprioception loss, gait ataxia, hyperreflexia, and Babinsky sign, sometimes accompanied by sphincter dysfunction and limb weakness [40].

When neuropathy occurs sensory changes in glove and stocking patterns are also seen in these patients after bariatric surgery. This complication is caused primarily by cobalamin deficiency but is also attributed to other deficient nutrients such as copper, vitamin E, and pyridoxine. Correction of these micronutrients initially intravenously and then long-term orally or intramuscularly for vitamin B12 improves neurologic symptomatology but without full recovery in some patients. For optimum management copper levels should be regularly checked and supplemented as quickly as needed initially intravenous and then oral administration [41].

Optic neuropathy affecting one or both side nerves is associated with bariatric procedures because of nutritional deficiencies in vitamin B12 and vitamin A, copper, and rarely zinc, causing malfunction of nerve fibers. The onset can vary during a large period and can be acute or progressive causing blindness. As optic neuropathy pathogenesis is linked to demyelination, permanent blindness may not be overcome [22,42].

Central nervous system demyelination presenting as multiple sclerosis which fulfilled the McDonald criteria for diagnosis was reported in a series of cases after various types of bariatric surgery with relapsing-remitting forms [4,43,44], without understanding the mechanism of appearance or the exact relationship between the events, but it might be attributed to rapid and substantial weight loss. Other previous case reports also described multiple sclerosis onset and myasthenia gravis after rapid weight loss induced by bariatric surgery or highly restrictive diets [45]. Two cases of monophasic central nervous system demyelination were also reported by Falah et al in a series of patients with neurological complications after bariatric surgery, and in a Brazilian study [44].

NEUROPSYCHIATRIC DISORDERS

Neuropsychiatric disorders are seen after bariatric surgery, usually consisting of eating disorders (anorexia and bulimia nervosa, binge eating, avoidance eating (post-surgical avoidance eating disorder), reports of acute mania [46], and higher risk of suicide. These disorders frequently appear in preexisting psychiatric conditions, making psychiatric evaluation before surgery mandatory to find patients at risk who need to be followed. Several cases of cognitive complaints were described following bariatric surgery in Roux-en-Y Gastric Bypass induced by focal brain atrophy in the bilateral posterior thalamus [47] but need to be further studied.

Other conditions described after bariatric surgery with higher incidence are stroke, seizures [48,49], spontaneous intracerebral hypotension linked to vitamin A, and D deficiency, and spontaneous leakage of cerebrospinal fluid, but the exact relationship needs to be established in future studies [30].

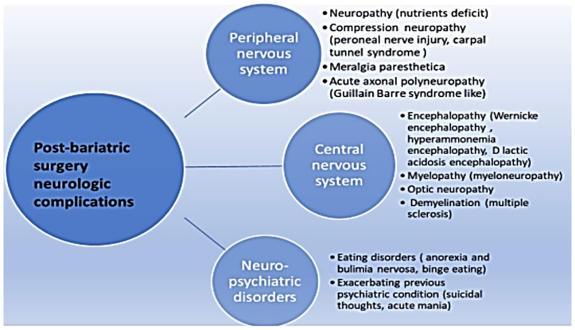
Post-bariatric surgery neurologic complications
Peripheral nervous system
• Neuropathy (nutrients deficit)
• Compression neuropathy (peroneal nerve injury, carpal tunnel syndrome)
• Meralgia paresthetica
• Acute axonal polyneuropathy (Guillain Barre syndrome like)
Central nervous system
• Encephalopathy (Wernicke encephalopathy , hyperammonemia encephalopathy, D lactic acidosis encephalopathy)
• Myelopathy (myeloneuropathy)
• Optic neuropathy
• Demyelination (multiple sclerosis)
Neuro-psychiatric disorders
• Eating disorders (anorexia and bulimia nervosa, binge eating)
• Exacerbating previous psychiatric condition (suicidal thoughts, acute mania)

Figure 2: The spectrum of neurological complications described after bariatric surgery. From peripheral to central nervous complications, to neuropsychiatric disorders.

CONCLUSIONS

Complications after bariatric surgery have a broad spectrum of manifestations involving both the peripheral and central nervous system and should be suspected and diagnosed as soon as possible to prevent long-term sequelae. The risk of developing central nervous system demyelination or stroke events after bariatric surgery should be further evaluated in future studies. Patients with rapid weight loss and those who develop gastrointestinal symptoms and surgical complications are considered at high risk for developing neurological complications, which is why nutritional deficiency assessment and supplementation should be done as early as possible.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization: FIR; Methodology: MJ, RSC, PN; Writing - original draft preparation: MC; writing-review and editing SB, MC; Supervising: FIR. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

Roadmap of Photoplethysmography Technology in Advanced Cardiovascular Assessment

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Abstract: *This review explores the trajectory of photoplethysmography (PPG) technology from its inception in 1934 to its integration into smart devices in 2013. While PPG has proven effective in estimating left ventricular ejection time (LVET) and distinguishing between hypertensive and normotensive patients, challenges persist. The need for a robust mathematical model to explain physiological behaviors, address calibration protocols, and handle waveform variability is emphasized. Despite limitations, PPG is on the cusp of achieving clinical-grade confidence, particularly in estimating blood pressure and tracking vasomotor states. Integration into wearable devices is a prominent trend, with major companies exploring applications for improved health monitoring. Open-source databases and the availability of physiological data aim to enhance understanding, paving the way for universally accepted protocols. As PPG advances, it holds promise for personalized healthcare, revolutionizing treatment evaluations and preventive measures. However, potential disagreements over protocols may impede progress. Nevertheless, the technology's potential to remotely monitor cardiovascular markers could reduce physicians' workload for routine tasks. PPG stands as a beacon for the future of noninvasive cardiovascular assessment.*

Keywords: *photoplethysmography roadmap; cardiovascular assessment; cardiovascular disorders; left ventricular ejection time; blood pressure estimation*

INTRODUCTION

Cardiovascular diseases are, according to WHO, the most common cause of death globally. There are made continuous efforts to reduce the incidence, morbidity, and mortality of these conditions. All over the world, the physicians involved in preventing, detecting, and treating cardiovascular diseases are insufficient and subjected to an overwhelming workload: theoretically, after a certain age, virtually all individuals have a certain risk of developing cardiovascular conditions and these patients, after diagnosis, need periodical evaluation for monitoring, assessment of treatment efficacy and, also, management of complications. Therefore, in the context of nowadays technology capabilities, it has raised the question if there could be developed some methods to monitor these patients over a distance, using suitable medical technologies to non-invasively measure some biomarkers.

At present, it is available to remotely measure, using various wearable devices, pulse, SpO_2 and to record an ECG. However, in detecting or monitoring the risk of adverse events, it would be useful to be able to remotely monitor arterial blood pressure, hypertension being the most prevalent cardiovascular risk factor, or, more advanced, to monitor various parameters of heart performance.

Photoplethysmography (PPG) technology has already demonstrated promising efficiency in distinguishing between normotensive and hypertensive patients or in estimating left ventricular ejection time. In our opinion, with more research in this field and with better mathematical models that can explain all physiological behaviors, PPG could help healthcare professionals offer personalized care regarding the prevention, treatment, and follow-up of cardiovascular diseases**.**

PHOTOPLETHYSMOGRAPHY APPLICATIONS

Short History

The founding concept of the PPG technique is older than a century when German physicist M.R. Bonnsman invented a photocell device in 1934 intended for peripheral blood volume evaluation [1]. This novel method was taken over by Alrick Hertzman [2,3] for further investigations in the physiological domain. Based on numerous experimental investigations, but limited by the technology capabilities at that time, he proposed blood volume variation as the origin of the PPG signal. No new progress in this technique has been made in the next years since the associated sensor was more of a tool in circulation research.

A valuable clinical application emerged in the form of blood oxygen saturation ratio (SpO_2) [4], pioneered by Takuo Aoyagi in the 1970s [5]. The device, now known as a pulse oximeter, was introduced into U.S. clinical monitoring a few years later, confirming its utility. Following this significant advancement, there was no immediate surge in research within the PPG technology domain; instead, efforts were concentrated on enhancing the accuracy of SpO_2 measurements. The pulse oximeter device became commercially accessible in the 2000s, owing to technological advancements and its availability in the open market [6].

Technology nowadays

The subsequent period that drew attention to PPG was marked by the integration of this technology into the first smartwatches in 2013 [7]. This significant development raised awareness among both academic and industry researchers regarding the potential for cardiovascular monitoring through wearable devices. While the well-established SpO_2 measurement and heart rate determination are valuable for assessing heart performance during resting periods or intense activity [8,9], they do not constitute a comprehensive tool for physicians to remotely monitor a patient in terms of cardiovascular disease (CVD) prevention or tracking.

Therefore, intense research has commenced in the PPG domain over the last decade, exploring various capabilities as depicted in Figure 1. One sought-after application is noninvasive arterial blood pressure (ABP) monitoring, considered a crucial hemodynamic marker in the evaluation of cardiovascular diseases (CVD) [10,11]. By incorporating this targeted assessment into smart devices such as watches, bracelets, and rings, personalized healthcare becomes accessible for end-users. The confidence in this feature has prompted major companies like Samsung [12] to integrate it into their devices. Start-ups such as Valencell [13] and Aktiia [14] assert their ability to provide this measurement using Machine Learning (ML) techniques. Additionally, numerous individual research entities report significant progress in this direction, leveraging artificial intelligence (AI) networks [15].

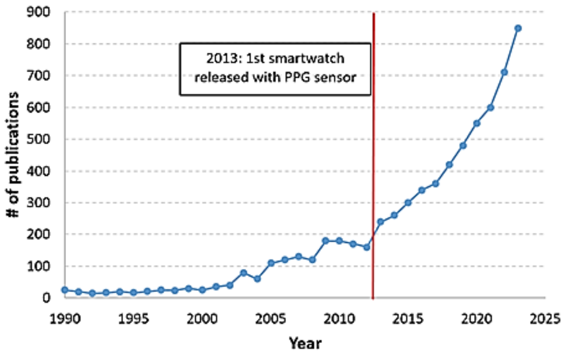

Figure 1: A timeline of PPG-related publications every year from 1990 to 2023. Data obtained from Google Academic, last accessed on 15th December 2023

The key factors contributing to the popularity of PPG encompass the mitigation of motion artifacts, the precision of SpO_2 readings and heart rate measurements, initiatives for extracting arterial compliance, and the estimation of blood pressure. Furthermore, a novel approach, remote photoplethysmography (rPPG) [16], has emerged in recent years, focusing on extracting hemodynamic information through facial recognition. The advancement of information technology, notably the internet and easy access to information, has played a significant role in augmenting the popularity of PPG.

However, as depicted in Figure 1, the upward trend in the number of publications does not align with the maturity of the technology. Regrettably, numerous topics are repetitive, merely contributing to the validation of specific assessments rather than yielding outstanding discoveries or breakthrough research. As will be presented in the next section, a strategy focused solely on PPG signal interpretation is not sustainable. Reflective sensors are commonly employed for wearable applications, as illustrated in Figure 2, in contrast to the transmissive ones more prevalent in clinical settings.

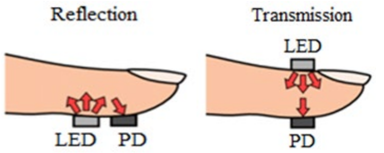

Figure 2: Two types of PPG modes: reflective and transmissive. In wearable devices, the first mode is prevalent, while in clinical settings the second one is present.

Eligible models must be developed to comprehensively explain all observed physiological behavior, and the correlation or causation with arterial blood pressure needs to be elucidated. Without such a focused direction, the repetition of topics will persist without yielding new and significant discoveries.

PPG as a standalone biomarker

As a standalone biomarker, information extracted from PPG waveform contour analysis becomes crucial. Following this approach, significant features such as the tidal wave (TDW) [17] and the dicrotic wave (DCW) [18] can be associated with the vasomotor state. The latter waveform component proves to have a strong correlation in estimating vasomotor tone, as observed in Figure 3, where the dicrotic notch sharpens proportionately with the level of vasodilation [19]. When combined with other waveform markers, such as the ratio between systolic upstroke and downstroke, and aided by Machine Learning (ML) models, the hypertensive state can be reliably detected [20].

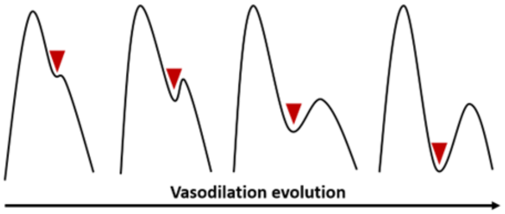

Figure 3: Evolution of PPG waveform during vasodilation progress, by highlighting incisura or DCW feature. Reconstructed after the findings on [19]

Another application involves the myocardial performance index (MPI or TEI) [21], as the PPG waveform encompasses information about isovolumetric contraction time (IVCT) [22], isovolumetric relaxation time (IVRT), and left ventricular ejection time (LVET) [23]. Despite the potential for overestimation and underestimation of measurements due to the hemodynamic state, these values closely align with the golden standard method of Doppler echography.

PPG in sensor fusion

One popular technology in this category is represented by the pulse pressure transit time (PTT) method [24], developed for arterial compliance and blood pressure assessment. The current literature protocol [25] guides the measurement of this marker from the electrocardiogram (ECG) R peak to the PPG systolic point, as depicted in Figure 4.

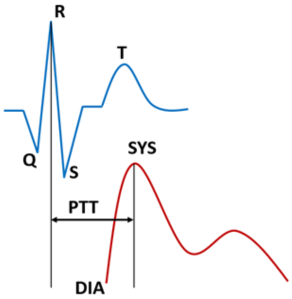

Figure 4: PTT protocol based on guidelines [25]

Based on the physical equation developed by Moens–Korteweg [26], which describes the traveling behavior of mechanical pulse pressure, blood pressure estimation can be extracted. Thus, a stiffer system will promote rapid traveling of the pulse wave, resulting in a shorter PPT time. Conversely, an elastic system, due to its ability to absorb mechanical energy, will slow down the traveling mode, leading to a longer measured time.

Another application that combines PPG capabilities involves fusion with a medical cuff. During cuff inflation, PPG acts as a monitor of blood supply at the targeted site. After a certain point, the externally induced pressure will trigger blood vessel occlusion, which is reflected by a missing PPG waveform. This measurement approach was embedded in the Omron smartwatch [27] to enhance the accuracy of blood pressure assessment.

A recent application involves remote photoplethysmography [16], which targets face recognition in combination with pulsatile detection of facial skin. By selecting two separate regions of interest (ROI), such as the chin and forehead, equivalent approaches to the PPT technique are employed to compute and estimate blood pressure, as depicted in Figure 5.

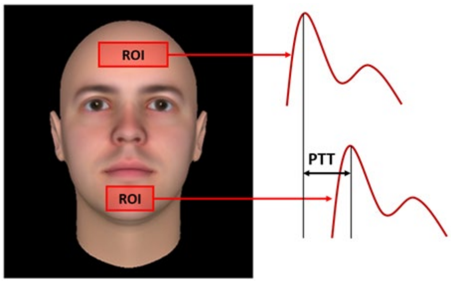

Figure 5: Principle of rPPG. Two locations of skin pulsation generate PPG waveform but with a time delay in between. This delay is exploited by a similar to the PPT method to extract blood pressure estimation

PHOTOPLETHYSMOGRAPHY LIMITATIONS

Physiological model

Despite the important and useful applications of the PPG technique, either standalone or in conjunction with other methods, the technology has demonstrated some limitations.

The first significant limitation pertains to the proper understanding of PPG origin, particularly in relation to pulse formation. While Alrick Hertzman proposed blood volume variation as a working model in the 1930s, recent investigations have revealed some drawbacks. One common limitation arises during reduced SpO_2 readings [28], which actually represent the ratio between oxygenated and deoxygenated blood volume. Additionally, the hand position concerning the heart level [29] modulates the PPG waveform amplitude according to specific rules, contrary to the initially proposed volume variation model. Therefore, significant progress in the hemodynamic domain cannot be achieved without a suitable PPG model that can explain and predict different physiological states. At this juncture, two mathematical models are required: one to elucidate the optical nature of the recorded signal and another to describe the mechanical nature that provides a specific waveform morphology. Training Machine Learning (ML) or Artificial Intelligence (AI) systems with a large set of hemodynamic data will not unveil the true relationship between these two physical quantities. Instead, it serves as an interim step to understand which waveform components are predominant in blood pressure assessment.

A recently developed model proposes the red blood cell deformation effect as the origin of PPG formation [30]. The mere presence of erythrocytes in the measurement area modulates the direct component or baseline of the recorded waveform. Interestingly, as cells move under a defined velocity field, they deform in the capillary regime. Thus, targeting red blood cells with light results in the absorption of radiant flux proportional to the deformation level. Given that many publications attempt to correlate arterial blood pressure (ABP) waveform with PPG, it is observable that the physiological stimuli contributing to PPG formation are the blood flow velocity at the capillary level, ranging from 50 µm/s to 2000 µm/s [31]. As a result, as depicted in Figure 6, the PPG waveform is exclusively modulated by the velocity values through the capillary network, reflected in the final waveform by red blood cell deformation.

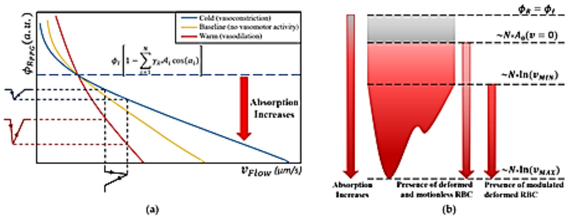
Cold (vasoconstriction)
Baseline (no vasomotor activity)
Warm (vasodilation)
$\phi_{R_{PPG}}(a.u.)$
$\phi_I\left[1-\sum_{i=1}^{N}\gamma_R A_i\cos(\alpha_i)\right]$
Absorption Increases
v_{Flow} (µm/s)
(a)
$\phi_R = \phi_I$
$\sim N \cdot A_0(v=0)$
$\sim N \cdot \ln(v_{MIN})$
$\sim N \cdot \ln(v_{MAX})$
Absorption Increases
Presence of deformed and motionless RBC
Presence of modulated deformed RBC
(b)

Figure 6: RBCM MODEL. Reused from [30]

It is important to note that both the mechanical blood pressure pulse and red blood cell deformation—precursors for PPG—are driven by blood flow velocity. Therefore, this physical quantity serves as the same input stimulus, the causal factor, for both associated waveforms. In this context, a correlation rather than direct causation exists between arterial blood pressure (ABP) and PPG information. From this standpoint, estimations of hemodynamic markers can be extracted with the aid of wearable technologies. However, due to the logarithmic nature of PPG origin, as depicted in Figure 6, the hemodynamic information will undergo modifications that could lead to wrongly interpreted results.

Finally, without a proper optical model, the improvement of pulse oximeter sensors becomes challenging. The most critical design considerations are related to mechanical features, such as the frequency of the LED used, initial light flux power, and the arrangement of the photodiode. In terms of signal processing, important design inputs include signal amplification, sampling frequency, and filtering stages.

Measurement protocols

All the aforementioned PPG measurement capabilities require a universally agreed-upon protocol to ensure that different devices provide consistent results for the same user or patient. This requirement is crucial for accurate measurements to be eligible for clinical applications. Moreover, regardless of the device's manufacturer, doctors need to rely on the measured data to provide correct treatment or formulate an observational plan.

While the PPT method has demonstrated high-confidence results in estimating blood pressure with the defined protocol, there is still an applicability problem on the user end. Measuring PPT involves two sensor readings simultaneously: EKG and PPG, as discussed earlier. Even though the latter doesn't require user intervention, the former necessitates the user to close the electrical circuit of their body to perform an EKG. Consequently, the measurement cannot be performed frequently without user intervention, for example, during sleep or physical exercise. In essence, it shares the same measurement limitations as a traditional oscillometric blood pressure device [32].

For assessments that involve standalone PPG sensors, a major limitation is the calibration protocol process. Given the optical nature of the recorded quantity, the readings need to be calibrated with a mechanical reference, especially for applications intended for blood pressure estimation. In the assessment of vasomotor state, where absolute values aren't critical, a protocol should outline which PPG waveform components, besides DCW, need to be evaluated for high-confidence results. This is necessary due to the various waveform morphologies that appear in the real world, apart from the idealized perfect shape.

The same approach is necessary for measurements involving time values, such as left ventricular ejection time (LVET) determination. While the association with the TDW feature yields good results, a common agreement on the onset and ending points is essential. Given that the TDW component comprises a finite disturbance event in combination with different morphologies, a protocol is also required for consistency in interpretation.

Finally, from a numerical standpoint, all the measured hemodynamic markers should be evaluated by a specialist within the proper context. The obtained data must be assessed to determine if the measurements were intended for prevention, treatment evaluation, or cardiovascular disease monitoring.

PHOTOPLETHYSMOGRAPHY FUTURE

An important stride in pulse oximeter technology has already been taken by integrating it into wearable devices. The increased computational power per integrated circuit in these devices holds the potential to enhance the signal processing part of the technology.

The availability of open-source databases containing hemodynamic and physiological data intended for researchers [33,34] will contribute to a proper understanding of PPG technology. In the final analysis, having such resources will help reduce, mitigate, or avoid the discussed drawbacks with a supportive tool.

The existing technological capabilities are poised to elevate PPG technology to clinical grade, enabling it to provide high-confidence measurements. Consequently, cardiovascular markers such as LVET and MPI can be remotely monitored, reducing the need for unnecessary physician office visits. This advancement holds potential benefits for both patients and doctors.

The capability to monitor patients' markers with a higher frequency rate would enhance the evaluation of the efficiency of the treatment plan. Additionally, cardiovascular diseases (CVDs) could be prevented or tracked more effectively, enabling better control and management.

An important assumption pertains to future protocols. The competition to embed hemodynamic markers into wearable devices will likely lead the first entity with proven and credible tracking capabilities to establish standalone protocols. Consequently, the community researching in the respective domain will likely follow the defined rules. However, a potential drawback is that this could trigger disagreements over a common, acceptable protocol, which may delay the accessibility of personalized healthcare [35].

CONCLUSIONS

The age-old noninvasive method, photoplethysmography, holds immense potential for enhancing cardiovascular assessment. However, realizing this potential requires the development of a proper mathematical model that can link and explain all physiological behaviors.

While the interest in PPG surged with the advent of the first smart devices in 2013, no disruptive progress has been achieved since then. Instead, some recurrent topics have been validated rather than proposing novel waveform markers for association with clinical applications. Blood pressure estimation and vasomotor state tracking are currently receiving the most attention, with popular companies piloting projects to integrate them into wearable devices.

Despite current limitations, PPG has shown validity, especially in estimating LVET and in binary detection between hypertensive and normotensive patients. Given the current context of technological advancement, there is optimism that this technology will achieve clinical-grade confidence. Consequently, it is anticipated to become an integral part of human lives by providing easy accessibility to personalized healthcare. This, in turn, will unlock improved health monitoring and contribute to a reduced workload for physicians in handling less critical tasks.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, L.E. and L.D; methodology, L.E.; software, L.E.; validation, L.E., A.F and L.D.; formal analysis, L.E; investigation, L.E.; re-sources, L.D.; data curation, A.F.; writing—original draft preparation, L.E.; writing—review and editing, A.F; visualization, L.E.; supervision, A.F.; project administration, L.D.; All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Ethics approval and consent to participate

Not applicable.

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REVIEW

From Controversy to Consensus: Neurogenesis and Neuroplasticity

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Abstract: *More and more research in recent years has focused on neurogenesis and neuroplasticity. The hippocampus is a key location of adult neurogenesis. Because this part of the brain is regulated by environmental variables, it must be mentioned the therapeutic potential for neurodegenerative illnesses and brain injuries. Furthermore, this information challenges the historical concept of a static brain and tries to demonstrate its flexibility, adaptability, and possibility for regeneration. Those mechanisms which are found in a variety of mammalian species, including humans, are important when it comes to adaptive learning and memory, thus providing insights into cognitive well-being and mental health. The discovery of the brain's dynamic nature represented by new neurons, marks a shift in the field of neurology with far-reaching potentials. This paper highlights the significant potential of adult neurogenesis when it comes to clinical applications such as Alz-heimer's disease.*

Keywords: *adult neurogenesis; neurodegeneration; neuroplasticity; therapeutical approaches; Alzheimer's disease; hippocampus; ethanol exposure*

INTRODUCTION

From a historical perspective, the brain was frequently defined as a static organ post-development. That means that most of its structures and also its functions are established during the early years of life [1]. This belief was held due to the absence of knowledge regarding neurogenesis, defined as the development of new neurons. The way we understand brain function, its plasticity, and the potential for recovery after an injury has been impacted by this perspective [1].

During the 1960s, remarkable contributions by Altman and Das provided convincing support for the occurrence of neurogenesis in certain regions of the adult rat brain, particularly in the hippocampus [2]. Although these statements led to various debates in the field of neurology, they also initiated a significant change in the way neurosciences is understood.

Based on the latest research, the hippocampus is deeply involved in not only the process of memory formation but also in spatial awareness processes [3-5]. The study of this process at the level of the dentate gyrus has yielded even more insights regarding the brain's innate possibility of self-repair. Neurogenesis also influences cognitive processes such as learning, memory, and mood regulation [6-9].

Advanced techniques ranging from neural imaging to molecular biology were used. The primary objective of these investigations was to ascertain the specific circumstances that facilitate neurogenesis. It is also essential to comprehend the role played by the newly formed neurons and explore the external factors that impact neurogenesis. Researchers have analyzed the effect of stimulating environments and physical activity on this process.

The more robust the evidence supporting adult neurogenesis was discovered, the more researchers became aware of its potential consequences for human health. The inquiry arises as to whether neurogenesis can be utilized for therapeutic purposes, i.e., "What are the possible effects on conditions characterized by neuronal degeneration, such as Alzheimer's disease or traumatic brain injuries?" The scientific community is enthusiastic and motivated about the potential use of adult neurogenesis for treating diverse neurological and psychological disorders [10].

MATERIALS AND METHODS

A rigorous methodology was employed in this narrative review to gather, analyze, and synthesize scholarly literature on adult neurogenesis. Due to the abundance of available research, we employed a systematic approach to thoroughly examine the most relevant and up-to-date studies.

Our analysis involved articles that explored the concept of neurogenesis in adult mammals, their intricacies, implications, and potential therapeutic applications. We methodically sourced diverse academic articles, primarily from PubMed, Scopus, and Google Scholar, ensuring a comprehensive exploration. We searched for various aspects of neurogenesis using both broad and specific search terms, and we prioritized recent research while recognizing foundational studies.

FROM NEUROGENESIS TO NEUROPLASTICITY

Adult neurogenesis is a process involving the formation of new neurons. The process takes place in the mature brain. It has been a topic of intense examination and debate in neuroscience [4,11]. While it is accepted that mammals, particularly rodents, exhibit this phenomenon in their hippocampus, the occurrence of such a process in humans has been controversial [1,12].

Historically, the mature mammalian Central Nervous System (CNS) was believed to possess limited regenerative potential. However, emerging evidence challenges this perspective, proposing that the mature CNS, including that of humans, might harbor a latent regenerative ability influenced by environmental factors [13,14]. Moreover, the adult hippocampal neurogenesis (AHN) observed in rodents is not merely an isolated phenomenon. It manifests neural plasticity prevalent across many mammalian species, including humans. Based on all this information, it is worth mentioning the fact that plasticity which is essential for both memory processes and also adaptive learning, is now starting to shift our understanding of the capabilities of the human brain [12,15-18].

Due to that, further researchers in the field of adult neurogenesis are perceived as being essential. Some of the potential consequences that are highlighted in the literature include an enhancement of cognitive well-being together with the potential of using it as a form of treatment strategy. As this topic becomes more and more prevalent in scientific literature, the current aim is to recognize neurogenesis complexities across different species and try to apply these notions to human brain health and the potential pathologies that might arise at this level.

Based on the results of the current literature review, the most relevant themes referring to neurogenesis and neuroplasticity were clustered into 12 categories (Figure 1).

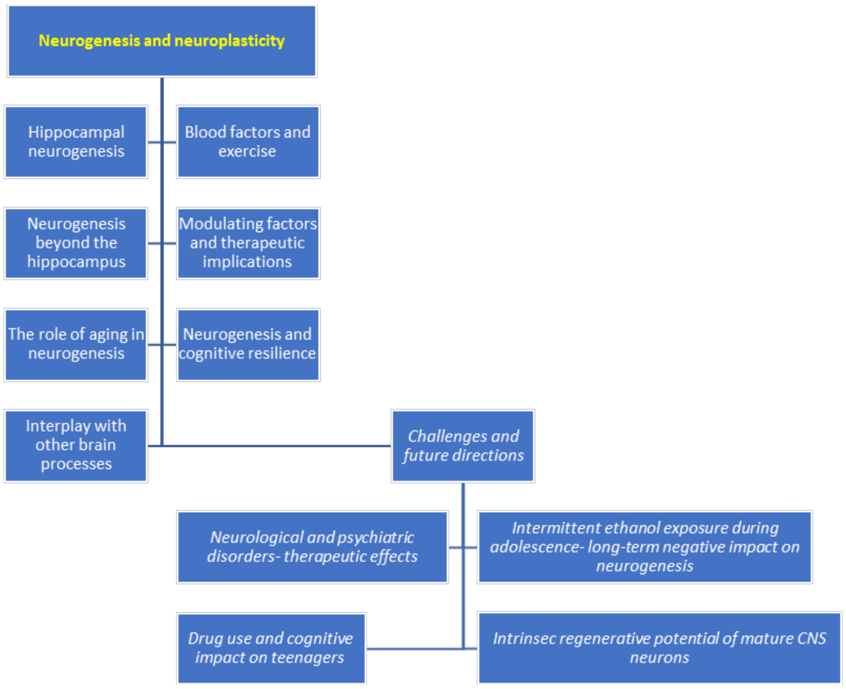
Neurogenesis and neuroplasticity
Hippocampal neurogenesis
Blood factors and exercise
Neurogenesis beyond the hippocampus
Modulating factors and therapeutic implications
The role of aging in neurogenesis
Neurogenesis and cognitive resilience
Interplay with other brain processes
Challenges and future directions
Neurological and psychiatric disorders- therapeutic effects
Intermittent ethanol exposure during adolescence- long-term negative impact on neurogenesis
Drug use and cognitive impact on teenagers
Intrinsec regenerative potential of mature CNS neurons

Figure 1: Neurogenesis and neuroplasticity – paradigm shifters in understanding the brain's functionality

Hippocampal Neurogenesis

Regarding the concept of neurogenesis, the primary focus was on the function of the hippocampus, particularly the area known as the dentate gyrus [7,8,19-22]. This is because, from a historical perspective, this region was frequently mentioned as being associated with various important cognitive functions. It is essential in memory formation, spatial awareness, and emotional control. Discovering neurogenesis in this region has reevaluated our understanding of its importance [2,7].

The incorporation of new neurons into existing hippocampal networks boost plasticity. This could also lead to enhanced cognitive functions. For instance, studies on rodents have shown a positive link between increased neurogenesis and better performance in spatial memory tasks [3,4,5,20,23-26]. Additionally, evidence points to a relationship between disruptions in neurogenesis and mood disorders. This claim suggests a potential link between creating new neurons and emotional regulation [9].

Blood Factors and Exercise

The influence of the environment on neurogenesis is becoming a significant research focus [27]. Evidence suggests that elements present in the bloodstream can affect the brain's ability to regenerate [28,29]. Notably, a study by Horowitz et al. [23] found that plasma from trained animals could transfer the beneficial effects of physical activity on neurogenesis to older individuals. This indicates that a systemic environment shaped by exercise can strengthen the brain's regenerative potential [23, 27].

Such discoveries are of great significance, particularly for the elderly. As concerns about cognitive decline with age intensify, it becomes crucial to grasp how systemic elements can enhance brain health [29]. These blood-derived factors might stimulate neurogenesis in those who can't partake in physical exercise [23].

Neurogenesis Beyond the Hippocampus

While the hippocampus remains a focal point, recent research has expanded the exploration of neurogenesis to other brain areas. Current scientific research has presented persuasive evidence for neurogenesis in various brain regions: hypothalamus, striatum, substantia nigra, cortex, olfactory bulb, and amygdala [28,30-32].

The potential functional impacts of neurogenesis in these areas are still under study. For instance, the striatum, involved in motor functions and reward-driven behaviors, might witness improved plasticity by new neurons. Similarly, neurogenesis in the amygdala, a key center for emotion processing, could shape how emotional memories and knowledge are acquired and stored [3-5,9,20,30].

Modulating Factors and Therapeutic Implications

Numerous intrinsic and extrinsic elements have been identified as influencers of neurogenesis. Neurotrophic factors, signaling molecules that aid neuron growth and differentiation, are known to boost neurogenesis [10,27,33-36]. For example, the Brain-derived neurotrophic factor (BDNF) plays a fundamental role in supporting the survival of new neurons by activating the tropomyosin receptor kinase B (TrKB) receptor, which then triggers pathways related to cell survival and movement [31,36].

Environmental elements, like being in stimulating surroundings or participating in cognitive activities, are associated with heightened neurogenesis [30,37]. Additionally, the development of new neurons in specific brain parts, remarkably in the context of brain injury (such as stroke) and neurodegenerative conditions, can be shaped by particular processes [38]. The importance of understanding neurogenesis is crucial for considering therapeutic approaches [10].

Furthermore, pharmacological interventions targeting specific signaling pathways involved in neuron differentiation offer promising possibilities. As research progresses, more targeted drug interventions might be elaborated to fine-tune the neurogenesis process, providing benefits in conditions where neural regeneration is needed [10,39].

Emerging research also implies that stem cell therapies could be employed to promote neurogenesis. Researchers aim to enhance the brain's regenerative capability, particularly in conditions marked by substantial neuron reduction. This could be achieved by introducing pluripotent stem cells that can differentiate into neurons. The potential of stem cell therapies lies in their ability to replace lost neurons and provide a supportive environment that promotes the growth of new neurons [14,40].

The Role of Aging in Neurogenesis

The implications for aging are one of the most compelling aspects of research on adult neurogenesis [23,24,26,29,41-44]. As individuals age, an inherent decline is observed in multiple cognitive domains. This is accompanied by a reduced brain ability to recuperate from injuries or diseases [45]. Identifying adult neurogenesis, particularly in older mammals, prompts inquiry into the potential harnessing or augmentation of regenerative capabilities in the aging brain [14].

Research has indicated a multifaceted association between the aging process and the generation of new neurons. Although there is an inherent decrease in the rate of neurogenesis with age, it is essential to note that this process does not come to a complete cessation [28]. The population of dormant stem cells in the hippocampal dentate gyri diminishes with advancing age. This happens while the number of actively dividing progenitor cells and immature neurons remains consistent over aging [7,8,28]. This observation implies that the brain maintains its regenerative capacity even during advanced stages of life. Furthermore, while neuroplasticity decreases in older individuals, the volume of granule cells (GCs), glia, and dentate gyrus remains unaltered [28,45].

Significantly, interventions such as exercise have demonstrated the capacity to counteract the process of brain aging [27]. Previous studies have observed that specific blood components present in the plasma of mice subjected to exercise can transmit the advantageous impacts of exercise on adult neurogenesis and cognitive function to mice that had a sedentary lifestyle [33]. The hepatic enzyme glycosylphosphatidylinositol (GPI)-specific phospholipase D1 (Gpld1) exhibited elevated levels following physical exertion. This molecule was associated with enhanced cognitive performance in elderly mice. In a promising manner, high levels of Gpld1 were likewise detected in the bloodstream of physically active and robust older individuals. According to Horowitz et al. [23], when administered systemically in aged mice, it can alleviate age-related impairments in regeneration and cognition.

Neurogenesis and Cognitive Resilience

An emerging area of interest is the role of neurogenesis in cognitive resilience. Cognitive resilience refers to the brain's ability to resist or recover from potential cognitive decline, often attributed to aging, injury, or disease [7,38,46-48]. Generating new neurons and their successful integration into existing neural networks could strengthen the brain's resilience. This adaptation allows the brain to maintain or enhance cognitive functions in the face of adversities [7].

This has implications for age-related disorders and conditions like traumatic brain injuries, stroke, or even chronic stress [38]. If neurogenesis can be stimulated and directed appropriately, it might offer a therapeutic strategy to counteract the cognitive deficits.

Interplay with Other Brain Processes

Neurogenesis is not isolated; it's intricately linked with other brain processes [37,49,50]. Synaptic plasticity, the capability of synapses to toughen or deteriorate over time, is believed to play a role in learning and memory [4]. Introducing new neurons into neural circuits could enhance synaptic plasticity, facilitating better learning and memory retention. Moreover, processes like long-term potentiation, critical for memory formation, might be influenced by new neurons, further emphasizing neurogenesis's role in cognitive functions [49].

Challenges and Future Directions

While the findings on adult neurogenesis are promising, challenges remain (Figures 1, and 2). The exact mechanisms regulating neurogenesis, the factors determining the survival and integration of new neurons, and the functional implications of these neurons are areas of active research [26,29]. Additionally, translating findings from animal models to humans presents its own set of challenges [52]. However, with continued research and technological advancements, the potential to harness neurogenesis for therapeutic benefits appears promising [10].

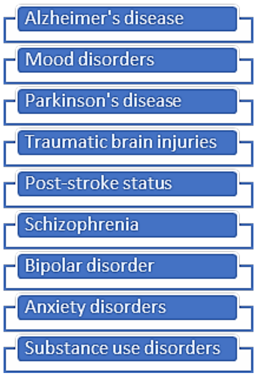

Figure 2: Potential targets of the neurogenic therapies

Implications for Neurological and Psychiatric Disorders

Identifying adult neurogenesis carries significant implications for neurological and psychiatric disorders [38,47,53-61]. Neurodegenerative disorders, such as Alzheimer's disease, are characterized by the loss of neurons. These may benefit from a deeper comprehension of the processes underlying neurogenesis [41,47]. Similarly, mood disorders like depression have been linked to decreased hippocampal volume and disrupted neurogenesis. These could also benefit from interventions aimed at promoting the generation of new neurons [4].

Furthermore, conditions such as Parkinson's disease and Huntington's disease, characterized by specific neuronal cell loss, could be targeted with neurogenic therapies. The interventions should replace the lost neurons or protect the remaining ones. Understanding the factors influencing intrinsic or extrinsic neurogenesis can pave the way for novel therapeutic strategies [57,62-66].

Traumatic brain injuries and strokes resulting in acute neuronal damage might also benefit from enhanced neurogenesis [44]. Strengthening the brain's inherent ability to generate new neurons could speed up recovery, improve cognitive function, and potentially restore lost skills [1].

Moreover, the implications of neurogenesis aren't limited to just the diseases that involve neuronal loss. The balance and health of neurogenesis might influence conditions like schizophrenia, bipolar disorder, and anxiety disorders—these disorders have an unclear etiology that is still under investigation. Disruptions in the process could contribute to the pathophysiology of these disorders [54].

Understanding how lifestyle choices, physical activity, the way we manage stress, and the approach dietary habits might impact the process of neurogenesis are also perceived as being essential to enhancing our understanding of this topic [51].

The impact of intermittent ethanol exposure during adolescence on the process of neurogenesis in the hippocampus

The adolescence period is a key point in neurology due to the high rate of both neuroplasticity and neurogenesis which characterizes it [67-73]. Despite this, during this maturational phase, many young people tend to engage in various risky behaviors such as consuming large amounts of alcohol and binge drinking [29,36,72]. Such behaviors, while transient for many, can have persistent consequences for brain health and function [36].

Recent studies that approached preclinical rodent models have shed light on the possible effects of adolescent intermittent ethanol (AIE) exposure [67]. These models have revealed an alarming pattern, especially because AIE was linked to a significant decrease in the formation of new neurons at the level of the hippocampus [33,36,67-73]. Notably, this decline doesn't merely correspond with the period of ethanol exposure. Instead, the detrimental effects persist into adulthood, even when ethanol exposure has ceased. This indicates a long-lasting impact on the brain's ability to produce new neurons [33].

Recent discoveries have pointed towards neuroimmune and epigenetic pathways. Increased neuroimmune signaling post-AIE exposure suggests an inflammatory response. This might inhibit the normal process of neuron generation [33]. Simultaneously, there's evidence of de-creased neurotrophic support. This implies that the usual signals promoting neuron growth and differentiation are compromised. Epigenetic mechanisms, characterized by modifications in gene expression that do not involve modifications to the underlying DNA sequence, have a crucial role in facilitating these effects. These mechanisms could alter the gene expression for neurogenesis, thereby perpetuating the decline in neuron generation [33,36].

Drug Use and Cognitive Impact on Teenagers

The brain, in particular, undergoes profound developmental processes that give rise to intricate behavioral and mental faculties. However, the trajectory of this development is not solely determined by biological factors; it is also susceptible to environmental influences. Among these, drug exposure is a particularly concerning factor that can derail neurobiological maturation [74-77].

Historically, the health implications of drug use during adolescence have been a subject of considerable concern [75,76]. The predominant focus has been understanding how adolescent drug use might predicate or correlate with drug-using behaviors in adulthood. However, recent scientific inquiries have expanded this focus, revealing that the ramifications of teenage drug use are not confined to future drug-related behaviors. Other behavioral domains, notably learning and memory, have been scrutinized [52].

Emerging evidence suggests that learning and memory are particularly vulnerable to the effects of drugs during adolescence [75]. More alarmingly, deficits in these cognitive domains, once established due to drug exposure, appear to persist into adulthood. These findings indicate a deep-seated interaction between drug exposure and the natural developmental processes of the adolescent brain. This interaction yields consequences that endure well beyond the cessation of drug use, emphasizing the gravity of the issue [74-76].

In light of these revelations, it becomes imperative to understand the exact nature and extent of the cognitive impairments caused by adolescent drug exposure. While the research landscape is vast, some patterns are discernible. Adolescents, due to societal norms and legal frameworks, are more inclined towards drugs that are legally accessible to adults. As such, alcohol, nicotine, and marijuana emerge as substances of primary concern [77,78].

Intrinsic Regenerative Potential of Mature CNS Neurons

For years, the broader field of neuroscience has operated under a prevailing assumption: that the neurons in the adult mammalian CNS possess limited if any, capacity for regeneration following injury. This perspective contrasts our understanding of peripheral nerves, which have consistently demonstrated a robust ability to regenerate after various forms of trauma [14].

Delving into the reasons behind this perceived inability of CNS neurons to regenerate, researchers have pinpointed a few key factors. Paramount among these is the presence of specific extracellular signaling factors in the CNS environment [28]. These factors inhibit axonal growth, blocking the natural regenerative processes that might otherwise occur. Furthermore, there's the notion that mature central neurons, as they age, may lack the adaptive responsiveness required to counteract and recover from damage. This idea aligns with a broader dogma in neuroscience, which suggests that as central neurons mature, they inherently lose their structural plasticity [14].

However, recent discoveries have started to challenge this traditional viewpoint. Emerging evidence suggests that the mature CNS might not be as rigid and unresponsive. More exactly, some of the neurons might be defined by a latent regenerative ability. This capacity might be masked by various environmental factors, but they continue to exist. If this capability can be better understood, it could reform the methodology of treating CNS injuries and neurodegenerative conditions [14].

CONCLUSIONS

The discovery of adult neurogenesis has enhanced our understanding of the concepts of brain plasticity and regeneration. Although we previously believed that the fully developed mammalian brain remained unchanged, this static paradigm was proven wrong based on new discoveries in the field of neuroplasticity and neuroregeneration.

However, the latest academic research in the field of neurosciences highlights that this is incorrect, as our brain is still able to produce new neurons and also can adjust itself based on various internal and external variables. This significant advancement has had a profound impact on various medical fields, as is the case of neurosciences and therapeutic practices regarding the brain.

The phenomenon of adult neurogenesis provides information that contradicts traditional beliefs as it highlights the ever-changing nature of the brain. The process is not limited to only the synaptic plasticity, as completely new neurons can develop. This implies a remarkable durability that provides optimism when it comes to treating patients with neurotrauma or neurodegenerative pathologies.

Therapies that promote neurogenesis might potentially be beneficial for diseases such as Alzheimer's and depression, both of them being conditions that are frequently associated with a significant loss of viable neurons. The understanding of the novel neuron generation process and how this could be modified and incorporated holds significant therapeutical possibilities.

It is also important to mention that the process of neurogenesis can be influenced by external factors such as physical activity. Based on this observation, structured physical exercises must also be considered when trying to facilitate neurogenesis in the case of the elderly population who are diagnosed with dementia and a cognitive decline [79-81]

The neural development of our brains may be significantly influenced not only by our previous experiences but also by the environment in which we live and the decisions we make, often to a greater extent than we are currently aware of. The entire concept of adult neurogenesis research still encounters persistent controversies. This is mostly due to the lack of understanding of this process which is frequently believed to be too complex at this stage or inadequately studied.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, CAS, IGI, OV, MM and CAP; methodology, CAS, MM and CAP; validation, CAS, IGI, MM, and CAP; resources, IGI, OV and CAP; data curation, CAS, OV, and MM; writing—original draft preparation, CAS, IGI, MM and CAP; writing—review and editing, OV and CAP; visualization, CAS, IGI, OV, MM, and CAP; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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REVIEW

Eggshell Calcifications at Thyroid Ultrasound: A Sample-focused Analysis of Cytological Findings and Post-thyroidectomy Pathological Correlates

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Abstract: *Eggshell calcifications at thyroid ultrasound are connected to the challenging field of thyroid nodules and/or malignancy from a multidisciplinary perspective. Calcifications may be found in malignant and benign tumors; typically, micro-calcifications are identified in the papillary type of cancer, being equivalent to psammoma bodies in the pathological report. The risk of macro-calcifications, however, is more nuanced; recent data suggest a potential connection with a differentiated malignancy as well. Eggshell calcifications, a distinct type of macro-calcification, are generally considered benign findings, especially uninterrupted forms. Yet, the real incidence is not clear, which is why the topic remains open. In this narrative review, we analyzed eggshell calcifications in relationship with cytological diagnosis upon fine needle aspiration and post-thyroidectomy histological report. A PubMed-based search of the English-language papers using several keywords identified 171 articles. We only included original studies (no timeline restriction). After removing duplicates and applying the inclusion and exclusion criteria, 14 original studies were analyzed. The number of subjects per study varied (N=61 to 2678) so did the number of analyzed nodules per study (a maximum of 2902). Some studies found connections with benign features; others revealed a higher risk of malignancy at fine needle aspiration. Data remain heterogeneous, and oftentimes, studies investigated it only as a secondary endpoint. A high TI-RADS score is important in malignancy prediction, while eggshell calcification might add to the positive predictive value, yet, this trait is difficult to classify as a standalone element. Nevertheless, an interrupted pattern does not exclude thyroid cancer.*

Keywords: *thyroid, ultrasound, calcification, thyroidectomy, surgery, thyroid nodule, thyroid*

INTRODUCTION

Eggshell calcifications amid thyroid ultrasound are connected to the challenging field of thyroid nodules and/or thyroid malignancy. Recently, an important rise in thyroid nodule detection was brought about by the widespread use of ultrasonography, with detection rates reaching 60% of the general population (noting that the general prevalence of a thyroid nodule is 5%) [1,2]. As a consequence, there was also an apparent increase in over-diagnosis of differentiated thyroid cancer [3]. The increased costs, risks associated with surgical cures such as hypoparathyroidism and dysphonia, and the fact that most thyroid carcinomas are indolent, led current guidelines to lean towards a more conservative and individualized approach to thyroid nodules [4-7]. However, even though the mortality rates of thyroid carcinoma remained stable despite an increasing incidence, this is in opposition to decreasing rates of other solid malignancies [8]. The need for adequate risk assessment of thyroid nodules, therefore, remains clear. The importance of approaching this type of ultrasound lesion comes from providing additional clues and even risk factors for suspected malignancies to assess these patients particularly or to start meticulous screening protocols. Moreover, nowadays, thyroid ultrasound is largely available to a multitude of specialists coming from different backgrounds thus the importance of identifying the risk (if any) behind such lesions [4-8].

Currently, there are several risk stratification systems for thyroid nodules, including the American College of Radiology

Thyroid Imaging Reporting and Data System (ACR TI-RADS) [9], the American Thyroid Association (ATA) guidelines [10], and the European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults EUTIRADS [11]. All of them use similar sonographic characteristics to assess the risk of a thyroid nodule being benign or malignant.

Overall, calcifications may be found both in malignant and benign tumors. Findings such as spongiform or cystic nodules, with regular borders suggest benign nodules, while echogenic foci or micro-calcifications plead for malignancy [12,13]. Typically, micro-calcifications are found in malignant tumors and are the ultrasonographic equivalent of psammoma bodies (that are described in pathological reports) [14,15]. The risk of macro-calcifications, however, is more nuanced, but recent data suggest a possible connection with papillary carcinoma [16,17].

One particular type of peripheral macro-calcification, rim calcifications, which surround at least 120º of the nodule were found to be associated with malignancy [18,19]. Another type of macro-calcification is so-called "eggshell calcification", a peripheral thin rim-like calcification surrounding the nodule. Typically, they are considered benign findings, especially when they are uninterrupted [20]. They may, however, be found in papillary carcinoma as well, but the real incidence is not yet clear, which is why the topic represents a challenging multidisciplinary approach in the modern era [21].

We aim to identify what type of lesions may present with eggshell calcifications on thyroid ultrasound, what risk of malignancy nodules with eggshell calcification have, and what other sonographic features help in elucidating diagnosis.

MATERIALS AND METHODS

This was a narrative review. A PubMed-based analysis of the English-language medical literature was conducted by using the following keywords: "eggshell", "peripheral calcification", "annular calcification", and "curvilinear calcification" in association with "thyroid".

The initial search resulted in 171 articles. We only included original studies (no timeline restriction).

Articles were included if they provided data regarding nodules with eggshell calcifications and were selected based on clinical relevance. Case reports and studies exclusively focusing on different types of peripheral calcifications or rim calcifications were excluded.

After removing duplicates and applying the inclusion and exclusion criteria, 14 original studies were analyzed.

RESULTS

Sample-focused analysis

Data regarding eggshell calcifications was heterogeneous, and, oftentimes, studies investigated the relationship between this distinct form of calcification as a secondary objective. The number of subjects varied largely, with the smallest study including 61 subjects and 64 nodules [22]; the largest had 2678 subjects and a total of 2902 nodules [23]. While some studies found connections with benign character [24,25], (others found a higher risk for suspicion of malignancy at fine needle aspiration (FNA) [26]. Some studies, however, did not find statistical significance [22,27-29].

Prevalence of eggshell calcifications amid thyroid ultrasound followed by thyroidectomy

The largest study was performed by Kobayashi et al. [23] and included 2678 subjects who underwent thyroid surgery and a total of 2902 nodules. 26% of nodules had calcifications. This study provided the prevalence of eggshell calcifications in different types of thyroid malignancies. The highest prevalence of eggshell calcifications, 43.90% (N=18/41), was found in undifferentiated carcinomas. In the papillary type of carcinoma, the prevalence was 4.78% (N=45/941), while in follicular carcinoma it reached 11.86% (N=40/337). In some malignancies, such as cyst-forming variants of papillary carcinoma, medullary carcinoma, and primary thyroid lymphoma, no eggshell calcifications were found [23]. The second largest study was conducted by Zayadeen et al. [30] and included 1466 patients and, respectively, 2002 nodules; eggshell calcifications were found in 45 benign nodules and in 11 malignant ones [30].

Eggshell calcifications and thyroid malignancy risk

Overall, no statistically significant association between eggshell calcifications and malignancy was found. However, a link between calcification subtype, namely discontinuous eggshell calcifications, and malignant tumors was reported [31]. Yin et al. [31] conducted a retrospective study on 543 subjects with single nodules who underwent thyroid surgery. Pathology results revealed that 61.32% (N=333) of them were malignant. The relationship between the presence of malignancy and various calcification types, including eggshell calcification, was analyzed. In terms of eggshell sub-type, eggshell in a row (continuous) forms were more frequent in benign nodules (1.7% versus 0.6%, $p<0.01$), while discontinuous eggshell calcifications were more frequent in malignant nodules (2.2% versus 0.6%, $p<0.01$). The distribution of calcification types among nodules prompts further studies investigating possible relationships between calcification types and different types of benign and malignant lesions. Of note, in benign nodules, all eggshell calcifications were found in adenomas, while in malignant nodules, all in-a-row calcifications were in medullary carcinoma (N=3/3), while discontinuous calcifications were found in papillary carcinoma (N=12, 3.84% of papillary carcinomas) [31]. Ha et al. [25] also found that nodules with eggshell calcifications are more frequently benign (66.7% benign versus 33.3% malignant, p=0.002) [25].

Another study investigating the connection between eggshell calcifications and malignancy risk was conducted by Arpaci et al. [26]. The study included 907 nodules of 269 subjects. Most were investigated via FNA (N=907). Even though benign nodules were less frequent in cases with eggshell calcifications compared with those without macro-calcifications, and suspicion for malignancy rates were higher in nodules with eggshell calcifications, no statistical significance was found for malignancy or diagnostic rates, based on cytology. Lack of statistical significance in terms of eggshell calcification presence in benign versus malignant nodules was also shown in patients who underwent surgery and had pathologic diagnoses [26]. Another study that failed to find statistical significance between the presence of eggshell calcifications and benign or malignant nodules was that of Lee et al. [22], which included 103 nodules in 92 patients. The diagnosis was made based on FNA and follow-up [22]. Kim et al. [27] did not find a statistically significant association either. However, the study described a prevalence of eggshell calcifications of 2.4% in malignant nodules and 5% in benign nodules, in a population of 1078 subjects with 1432 nodules who underwent thyroidectomy for nodular disease [27].

Yoon et al. [29] did not find a statistically significant difference between benign and malignant groups in terms of the presence and type of peripheral calcifications in a retrospective study on 65 subjects, most of whom were diagnosed by FNA [29]. Regarding differentiated papillary carcinoma in particular, Wang et al. [24] found that eggshell calcifications decreased the risk of papillary carcinoma (OR=0.208, CI=0.049-0.886, p=0.034). Moreover, they were not an independent risk factor for aggressive behavior [24].

Hashimoto's chronic autoimmune thyroiditis

Sonographic features of nodules in patients with Hashimoto's thyroiditis were analyzed by Anderson et al. [32] in 61 subjects with 64 nodules. Overall, eggshell calcifications represented 23% of all calcifications, appeared in 4.7% (N=3) of nodules, and were more frequent in subjects with normal parenchyma [32]. Another study by Anderson et al. [28] extended the search to 82 nodules of 78 subjects with Hashimoto's thyroiditis who underwent FNA or surgery. 84% (N=69) of nodules were benign, while 16% (N=13) were malignant, most of which were papillary carcinoma (N=12). One primary thyroid lymphoma was also found. Even though eggshell calcifications were present in 15% of malignant nodules and 1% of benign nodules, the difference was not statistically significant [28].

Neuroendocrine neoplasia

While eggshells in a row were present in medullary carcinoma in Yin et al.'s study [31], Kobayashi et al. [23] did not find any eggshell calcifications in the medullary type of malignancy [23]. The largest study analyzing eggshell calcification in patients with a form of thyroid cancer was performed by Cai et al. [33] on 27 cases and 50 controls. Even though macro-calcifications, in general, were more frequent in patients with medullary thyroid carcinoma, none of the 27 cases had eggshell calcifications. In controls, however, eggshell calcifications were found in 4 subjects [33].

Potential pitfalls of assessment: eggshell calcifications and non-diagnostic FNA

Anderson et al. [34] investigated ultrasound features in nodules with non-diagnostic FNA results in 393 subjects with single nodules. In terms of eggshell calcifications, they appeared in 9 lesions, but no association with malignancy was found [34].

Factors influencing malignancy risk assessment concerning eggshell calcifications

The retrospective study of Kim et al. [35] focused on eggshell calcifications and included 92 subjects with 93 nodules with eggshell calcifications who underwent thyroid surgery. Sonographic features that had a high sensitivity for malignancy in these patients were the presence of the halo (sensitivity of 62.7%) and disrupted calcification rim (sensitivity of 76.3%) [35]. Regarding malignancy risk detection, Ha et al. [25] found that the positive predictive value of peripheral or eggshell calcification was increased by being combined with high suspicion TIRADS from 33.3% to 66.7-80% [25].

DISCUSSION

Eggshell calcification in a thyroid nodule was described as early as 1976 in a patient with mixed papillary and follicular carcinoma [36], while the first case of a thyroid nodule with eggshell calcification on ultrasonography was reported in 1978 by Gooding [37]. Since then, thyroid ultrasound progressed, but data regarding eggshell calcifications remains scarce. The number of studies providing information regarding eggshell calcifications of thyroid nodules is relatively small when compared to the large volume of data that is published in the specific fields of thyroid ultrasound, FNA, and post-operatory pathological reports. Concerning the specific histological profile amid thyroid malignancies, we discuss the following highlights.

Table 1: Main findings regarding thyroid nodules with eggshell calcifications according to our methods of research, including the PICO analysis [22-35]

First Author/ Publication year/ Reference number **Study design** **Studied population** ***P (patients)***	**Positive diagnosis method** ***I (intervention)***	**Nodules** ***C (comparison)***	**Main findings** ***O (outcome)***
Wang/ 2022/[24] Retrospective analysis N=950 subjects (208 males and 742 females) Mean age: 47.0±12 y N=527 patients with papillary carcinoma	surgery	N=1113 Mean size=13.8±9.9 mm Benign: N=486 Malignant: N=627, out of which 527 had aggressive behavior	Eggshell calcifications were a protective factor for papillary carcinoma (OR=0.208, CI=0.049–0.886, p=0.034) Eggshell calcifications were not independent risk factors for aggressive behavior in papillary carcinoma
Yin/2020/[31] Retrospective N=543 Mean age: 49.3±13.2 y	surgery	N=543 Benign: N1=210 (38.67%), out of which 177 adenomas Malignant: N2=333 (61.32%) Macro-calcifications in 204 (37.6%)	Eggshell-in-a-row calcifications were more frequent in benign nodules (1.7% vs. 0.6%, p<0.01) Eggshell discontinuous calcifications were more frequent in malignant nodules (2.2% vs. 0.6%, p<0.01) In benign nodules (N=210): eggshell in a row calcifications N=9 (only adenomas - 7.69%) eggshell discontinuous calcifications N=3 (only adenomas - 2.56%) In malignant nodules: eggshell in a row calcifications, in medullary carcinoma N=3/3 eggshell discontinuous calcifications, in papillary carcinoma N=12/312 (3.84%)
Ha/2019/[25] Retrospective N=954 Mean age: 50.8 y (range: 13–86 y)	surgery CNB FNA	N=1112 nodules (≥ 5 mm) Nodules with echogenic foci N=390 Benign: N=136 Malignant: N=254 Nodules with eggshell calcifications N=21	Nodules with eggshell calcifications (N=21) were more frequently benign (66.7% benign vs. 33.3% malignant, p=0.002 In malignant nodules: surgery (N=413) or CNB (N=4) In benign nodules: surgery (N=65), repeated FNA or CNB (N=41), FNA or CNB with no change in size >12 months (N=589)
Kobayashi/2018/[23] Retrospective N=2678 subjects	surgery	N=2902 Nodules with calcifications=747 (26%) Among patients who underwent surgery: papillary carcinoma N=941 diffuse sclerosing variant of papillary carcinoma N=18 cyst-forming type of papillary carcinoma N=32 metastatic lymph nodes from papillary carcinoma 161 nodes follicular carcinoma N=337 undifferentiated carcinoma N=41 medullary carcinoma N=137 primary thyroid lymphoma N=173	Eggshell calcifications were found in: papillary carcinoma N=45/941 (4.78%) metastatic lymph nodes from papillary carcinoma N=2/161 (1.24%) follicular carcinoma N=40/337 (11.86%) undifferentiated carcinoma N=18/41 (43.90%) No eggshell calcifications were found in diffuse sclerosing and cyst-forming variants of papillary carcinoma, medullary carcinoma and primary thyroid lymphoma.

Zayadeen/2016/[30] Retrospective N=1466 patients (1201 female and 265 male, female-to-male ratio=4.75:1 Mean age: 56 y (range: 11–96 y)	FNA (N=1991) and/or surgery (N=376)	N=2002 nodules 348 nodules in male patients (17%) and 1654 nodules (83%) in female patients Malignant: N=148 (7.4%)	Eggshell calcifications in 45 benign lesions (from 1854 confirmed benign nodules = 2.42%) and 11 malignant lesions (from 148 malignant nodules = 7.43%)
Anderson/ 2014/[34] Retrospective N=393 (328 females, 65 males) subjects with single nodules with non-diagnostic FNA Median age: 57 y (range: 12-94 y)	Following non-diagnostic FNA: Repeated FNA and/or surgery and/or US follow-up	N=393 Nodules with eggshell calcifications N=9/393 (2.3%, 95% CI: 1.1%, 4.3%)	Nodules with eggshell calcifications did not associate with malignancy Increases in volume, anteroposterior diameter, mean or minimum diameter were associated with increases in odds of malignancy
Arpaci/2014/[26] Retrospective N=269 subjects (79.9% female) Mean age: 56.9±13.1 y	FNA (N=907) Surgery (N=43)	N=907 Mean diameters: with macro-calcification: 23.92 ± 14.15 mm without macro-calcification: 15.72 ± 7.53 mm Nodules with macro-calcifications were larger ($p < 0.001$)	Benign nodules rates were higher in nodules without eggshell calcifications compared with nodules with eggshell calcifications (80.3% vs. 67.9%, $p=0.036$) Suspicion for malignancy rates was higher in nodules with eggshell calcifications (7.5% vs. 1.9%, $p=0.010$) In nodules with vs. without eggshell calcifications, no statistical significance was found for: malignant and non-diagnostic rates, pathology results in patients who underwent surgery
Lee/2013/[22] Retrospective N=92 (63 females, 29 males)	FNA (N=103)	N=103 After FNA: Benign N=31 Malignant N=69 Non-diagnostic N=3 After histological confirmation/follow-up: Benign N=34 Malignant N=69	No statistically significant association between eggshell calcification and malignancy
Kim/2013/[27] Retrospective N=1078 subjects (171 males and 907 females) who underwent thyroidectomy for nodular disease Mean age: 47.2 ± 11.1 y (range: 16-82 y)	surgery	N=1432 Benign: N=126 Malignant: N=1305	There was no statistically significant association between annular calcifications and malignant or benign nodules Prevalence of annular calcifications in malignant nodules=31/1305 (2.4%) Prevalence of annular calcifications in benign nodules=5/126 (5%)
Cai/2010/[33] Case-control N=27 cases with medullary carcinoma (13 males and 14 females) Mean age: 45.7±12.8 y N=50 controls (5 males and 45 females) Mean age: 47.2±11.6 y	MTC - surgery controls - NA	N=35 Sizes: 2.39±1.22 cm in the medullary cancer group and 2.42±1.00 in controls - a difference not statistically significant	Eggshell calcifications were found only in controls (N=4) Macro-calcifications were more frequent in patients with medullary carcinoma
Anderson/2010/[28] Retrospective N=61 subjects with nodular Hashimoto's thyroiditis (54 females and 7 males) Median age: 49 y (range: 28–87 y)	FNA	N=64 average mean diameter=15.3 ± 7.33 mm average maximum diameter=19.5 ± 10.39 mm average minimum diameter=11.5 ± 5.63 mm	20% (13/64) nodules had calcifications 4.7% (3/64) nodules had eggshell calcifications 23% (3/13) calcifications were eggshell Eggshell calcifications - more frequent in subjects with normal parenchyma

Anderson/2010/[32] Retrospective N=78 subjects with Hashimoto thyroiditis and nodules Subjects with benign nodules: 85% females Median age: 50 y (range: 30–89 y) Subjects with malignant nodules: 92% females Median age: 54 y (range: 35–89 y)	FNA or surgery	N=82 Benign: N1=69 (84%) Malignant: N2=13 (16%) (12 papillary carcinoma and 1 lymphoma)	Eggshell calcifications were present in: 15% of malignant nodules and 1% of benign nodules - the difference was not statistically significant
Kim/2008/[35] Retrospective N=92	surgery	N=93 nodules and eggshell calcification who underwent surgery Benign: N=34 Malignant: N=59 (papillary carcinoma) Sizes from 4 to 50 mm (mean=16 mm), with no statistically significant difference between groups	In nodules with eggshell calcification, the highest sensitivity for malignancy was found in halo (62.7%) and disrupted calcification rim (76.3%)
Yoon/2007/[29] Retrospective N=65 (57 females) no statistically significant difference in age between benign (54.3 ± 12.7 y) and malignant (54.3 ± 12.7 y) groups	FNA (N=44) surgery (N=3) both (N=18)	mean sizes ± SD: benign nodules (N=53): 13 ± 8 mm (range=3–37 mm) malignant nodules (N=9): 14.4 ± 6.8 mm (range=5–28 mm)	No statistically significant difference between benign and malignant groups in terms of the presence and type of peripheral calcifications

Abbreviations: CI=confidence interval; CNB=core needle biopsy; FNA=fine needle aspiration; N=number of patients; OR=odds ratio; vs.=versus; US=ultrasound; y=years

Papillary thyroid carcinoma

Papillary carcinoma accounts for the majority of thyroid cancer cases and has increased in prevalence in recent years, mainly through the increased use of diagnosis techniques, including ultrasonography and FNA. Even though its prognostic is usually good, properly identifying cases remains an important link in the management of these patients [38-40]. While the presence of micro-calcifications is a highly suggestive feature and may even predict cervical lymph node metastasis [41], the implications of macro-calcifications such as eggshell calcifications are not so clear until the present time thus their importance for daily practitioners in the field of ultrasound of any type, endocrinology or endocrine surgery as well as primary health care. None of the studies linked eggshell calcifications to a higher risk of malignancy. However, many of them did not reach statistical significance for tested hypotheses [22,26,27,29].

One of the most important findings was provided by Wang et al. [24], who found that eggshell calcifications decreased the risk of papillary carcinoma and did not promote aggressive tumor behavior [24]. Even though data does not link eggshell calcifications with malignancy, they have been reported in patients with papillary carcinoma [23,28]. When assessing a nodule with eggshell calcifications, other characteristics should also be taken into consideration, and the risk of papillary carcinoma, even though small, should not be completely overlooked [42].

Medullary thyroid carcinoma

Medullary type of carcinoma involves ultrasound as the most important preoperative imaging technique, as stated by the ATA 2015 guidelines [43], aiding not only in identifying the solitary nodule but also in investigating lymph node metastases [44]. Usually, nodules appear hypoechoic, with a patchy structure, vascularized, and may have micro- or macro-calcifications [24,45-47].

When analyzing data regarding eggshell calcifications and medullary carcinoma, evidence was scarce, and three studies were included [23,31,33]. Even though the largest one did not find any eggshell calcifications in patients with medullary carcinoma, the study of Yin et al. [31] found these calcifications in all three patients with medullary carcinoma. One important difference between these studies is the fact that Yin et al. [31] included only patients with calcifications, while Cai et al. [33] focused on ultrasound features of medullary carcinoma in general. Even though the study of Cai et al. [33] included more patients with medullary carcinoma (N=27), overall, the study of Yin et al. [31] identified the three medullary carcinoma patients from a larger pool of patients with thyroid nodules and calcifications (N=543) [31,33]. Despite the rarity, it is important to keep in mind that there have been cases of eggshell calcifications in patients with medullary carcinoma so that the diagnosis is not overlooked solely based on this characteristic. Further research is needed to understand the significance of egg-shell calcification in relation to the medullary type of carcinoma.

Follicular thyroid carcinoma

Kobayashi et al. [23] found eggshell calcifications in 11.86% of patients with follicular carcinoma and nodules with calcification [23]. Apart from this large study, eggshell calcifications have also been mentioned in case reports of patients with follicular carcinoma. Cai et al. [48] reported the case of a 67-year-old female who presented with a solitary kidney tumor diagnosed by pathology as follicular thyroid carcinoma. Thyroid ultrasound identified two different foci in the same lobe, and one of the nodules presented eggshell calcification with an incomplete halo [48].

Three more cases of follicular thyroid carcinoma were reported by Lee et al. [49]. Two of the three cases involved 75-year-old females who were diagnosed with bone metastases caused by a follicular type of thyroid cancer. Both patients had single nodules with interrupted eggshell calcifications on ultrasonography. A third case involved a 54-year-old female with hyperthyroidism and a thyroid nodule with interrupted eggshell calcifications classified as benign by FNA two years prior. A second FNA classified the nodule as indeterminate, and following thyroidectomy, the patient was diagnosed with follicular carcinoma [49].

It is important to emphasize that interrupted eggshell calcifications may be a sign of malignancy, as stated by the ETA 2017 guidelines [50]. Moreover, a high TI-RADS score remains important in malignancy detection in patients with eggshell calcifications as well, increasing positive predictive value [25].

Thyroid autoimmune disease

Hashimoto's chronic thyroiditis is the most common autoimmune thyroid disease and the main cause of hypothyroidism in the modern era [51]. It may lead to the development of nodules [52] and it is another thyroid condition where eggshell calcifications can be found with a low prevalence of less than 5% [32]. It is unclear why these calcifications are more frequent in nodular thyroiditis with otherwise normal gland parenchyma.

Another aspect worth mentioning is the association of Hashimoto's thyroiditis with thyroid cancers, including primary thyroid lymphoma and papillary carcinoma [53]. The connection with papillary thyroid cancer, however, is still debated, with some studies finding an increased risk while other studies finding thyroiditis as a protective factor [54-56]. This raises the question of how the presence of calcifications in general and eggshell calcifications in particular should be interpreted in patients confirmed with Hashimoto's thyroiditis based on serum thy-roid antibodies and/or cytological reports. The lack of statistically significant data regarding the prevalence of eggshell calcifications in benign versus malignant nodules requires larger studies to confirm or infirm the hypothesis that in patients with Hashimoto's thyroiditis, eggshell calcifications are more frequent in malignant nodules [28,32].

Further researches

For some thyroid pathologies, evidence regarding eggshell calcifications remains at the case report level. Malignancies previously found in patients with nodules with eggshell calcifications include oncocytic thyroid cancer [57], and primary squamous cell carcinoma [58], a rare tumor with similar behavior to anaplastic carcinoma [59]. Other benign conditions reported include follicular thyroid adenoma [60] and Hürthle cell adenoma [61]. Hürthle cell adenomas and carcinomas are classified as subtypes of follicular neoplasms, despite their distinct molecular pathogenesis [62]. Recently, Hürthle cells have been renamed to oncocytic cells [63]. (Figure 1).

Of note, apart from thyroid disease, when faced with eggshell calcifications, clinicians should also be mindful of diseases in surrounding organs. One such example is the case reported by Ahn et al. of a 36-year-old female who underwent a thyroid ultrasound due to a complaint of a cervical mass [64]. The ultrasound revealed a thyroid mass with eggshell calcification. However, surgery revealed that the mass was an oesophageal tumor, and pathology diagnosed it as a schwannoma [64].

Overall, the identification of calcifications at thyroid ultrasound should be assimilated to the larger frame of malignancy criteria according to the current guidelines in the thyroid field [65-67].

On a larger scale, we mention the fact that calcium deposits represent incidental findings in many medical areas outside the specific thyroid exploration, including other malignancies (at the neck, thorax, and mediastinum), infectious diseases, inflammatory, and autoimmune conditions [68-72].

Figure 1: This is a prior unreported unusual case of eggshell calcifications on an 80-year-old male admitted for enlargement of a prior goiter (that he refused to investigate for more than one decade); due to compressive neck symptoms, total thyroidectomy was done with a good post-surgery outcome; the pathological report showed a follicular adenoma with necrosis and hemorrhage

1A. Pre-operatory interrupted eggshell calcification at thyroid ultrasound

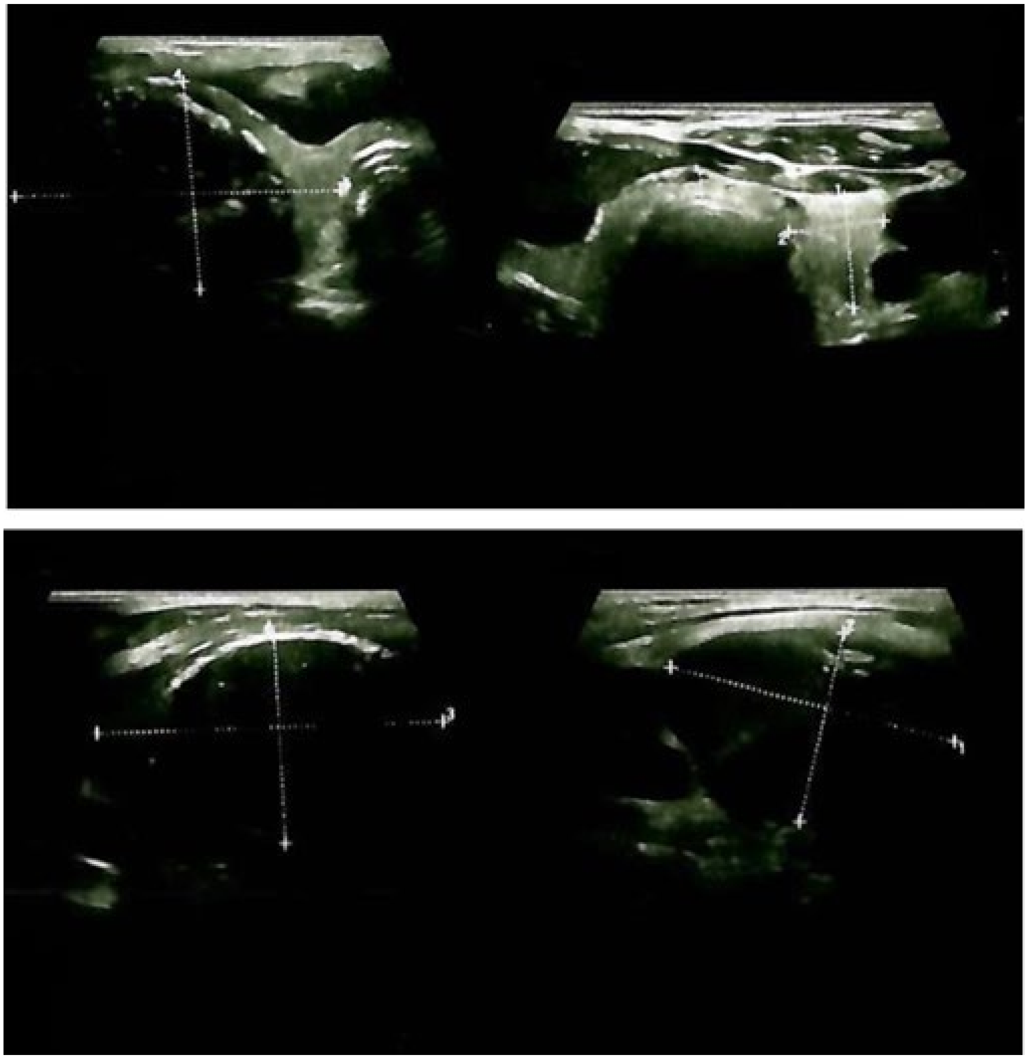

1B. Contrast computed tomography (blue signs show eggshell calcifications)

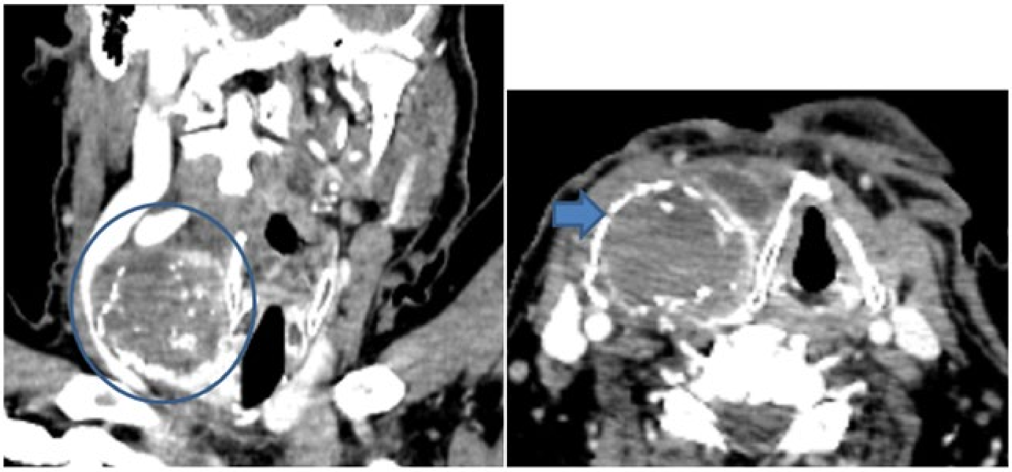

1C. Intra-operatory aspects: right recurrent laryngeal nerve (yellow arrow); trachea (blue arrow); right external jugular vein (green arrow); right common carotid artery (pink arrow); right superior thyroid pedicle (white arrow); right thyroid lobe (black arrow)

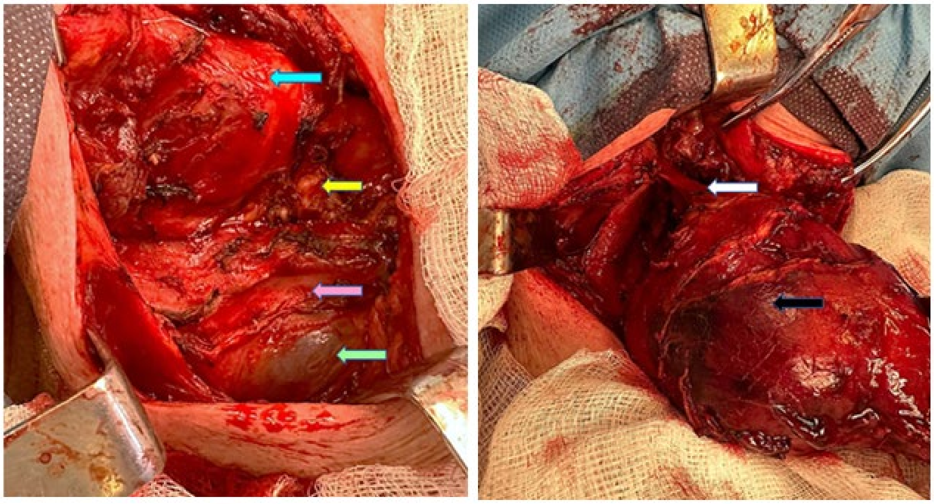

1D. Post-operatory macroscopic specimen (yellow arrow pointing at left lobe)

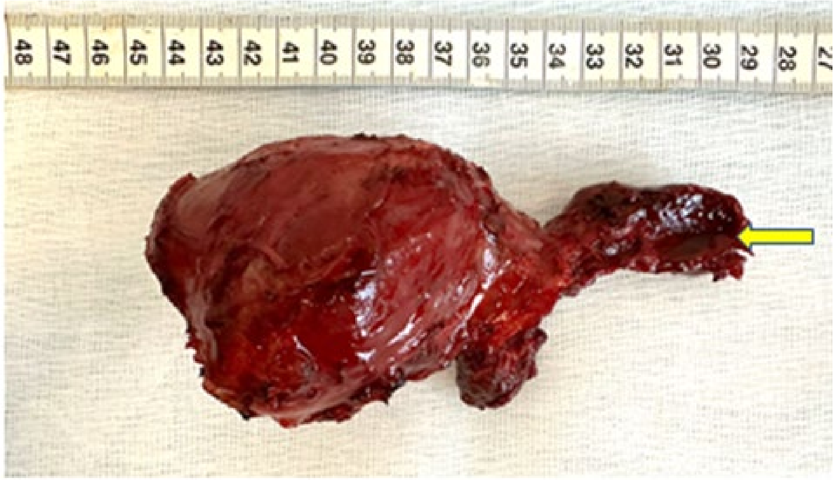
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1E. 1-month post-operatory neck ultrasound check-up shows no thyroid remnants

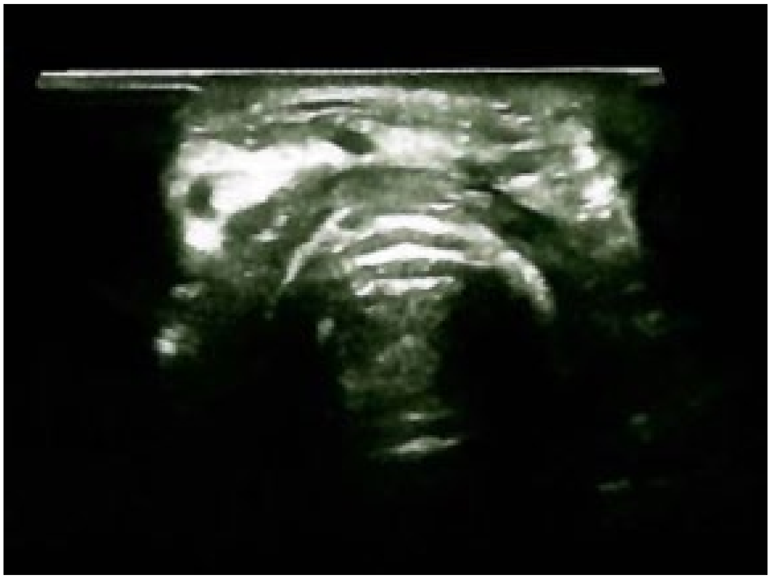

The most common clinical entities associated with eggshell calcification in these mentioned anatomic areas may involve lymphomas, amyloidosis, sarcoidosis, tuberculosis, scleroderma, etc. [73,74]. Another pathogenic trait of calcifications includes calcium and phosphorus anomalies of signal transduction pathways as seen in primary hyperparathyroidism, but, mostly, in chronic renal disease-related tertiary (or secondary) hyperparathyroidism (especially in patients undergoing different types of dialysis) and hypoparathyroidism underlying dysfunction of parathormone and even 25-hydroxyvitamin D regulation [75-77]. Arterial (and generally endothelial) calcifications may be a sign of increased cardiovascular disease in addition to the mentioned anomalies. This might add to a globally increased disease burden leading to a poor outcome [78-82].

CONCLUSIONS

A high TI-RADS score remains important in malignancy prediction while eggshell calcifications might add to the positive predictive value, yet, as a single input this trait is difficult to be classified as a standalone contributor element. Based on these findings, it is safe to say that when faced with eggshell calcifications on thyroid ultrasound, especially if they appear interrupted, clinicians should not rule out the possibility of thyroid carcinoma, as pointed out by our research (Table 1). Despite the clinical and epidemiological significance, the accurate diagnosis, monitoring of the diagnosed patients, and available licensed treatments remain the challenges of NASH.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, F.I.R., A.-M.G., O.-C.S., M.C., and C.N.; methodology, F.I.R., A.-M.G., M.C. and C.N.; software, F.I.R., A.-M.G., M.C., and C.N.; validation, F.I.R., A.-M.G., O.-C.S., and C.N.; formal analysis, F.I.R., A.-M.G., O.-C.S., M.C., and C.N.; investigation, F.I.R., A.-M.G., O.-C.S., M.C., and C.N.; resources, F.I.R., M.C., and C.N.; data curation, F.I.R., A.-M.G., O.-C.S., M.C. and C.N.; writing—original draft preparation, A.-M.G.; writing—review and editing, F.I.R., A.-M.G., M.C. and C.N.; visualization, F.I.R., A.-M.G., O.-C.S., M.C. and C.N.; supervision, F.I.R., M.C. and C.N.; project administration, F.I.R; funding acquisition, F.I.R. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The Local Committee of Ethics of Dr. Carol Davila Central Military Emergency University Hospital, Bucharest, Romania approved the release of these data (number 608/2023).

Patient consent for publication

The patient signed informed consent during hospitalization and gave us permission to use his medical and surgical records.

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SYSTEMATIC REVIEW

A Systematic Review of the Influence of Aquatic Exercises on the Physical and Psychological Health of Children with Autism Spectrum Disorder

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Abstract: *Background: Children with Autism Spectrum Disorder (ASD) show significant motor deficits and behavioral dysfunctions, as part of their clinical manifestations. Also, these children are facing important difficulties in social learning, which negatively impacts their adjustment to society's requirements. Apart from other modes of exercise, aquatic exercises may be more attractive to children. Aquatic exercises have been shown to have a beneficial effect on gross motor skills and executive functions in children with ASD. Aim: To study the influence of aquatic exercises on the physical and psychological health of children with ASD. Methods: Researchers used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting systematic reviews and meta-analyses. Researchers looked for articles on PubMed, Google Scholar, and by manual search. Searched articles were screened for relevancy. Potential articles were selected using inclusion and exclusion criteria. Results: Out of the 231 articles, four studies were included in the current systematic review. The age of participants ranged from 3−15 years. Overall, included studies found improvements in various aspects of gross motor and executive functions by aquatic exercise in children with ASD. Conclusion: Aquatic exercises can provide health benefits to children with ASD. Aquatic exercises can be useful as a part of therapy in children with ASD. There is wide scope for further studies to evaluate the beneficial effects of aquatic exercises.*

Keywords: *aquatic exercise, autism spectrum disorder, motor skills, cognitive functions*

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by repetitive behaviors, and deficiencies in social and communication skills [1,2,3]. Because of motor deficits, persons with ASD are not involved in social learning experiences to acquire sensory information the same way as others [3,4,5]. Children with ASD often show problems of behavior that negatively impact involvement in the community [6]. Significantly more aggression, inattention, and withdrawal behaviors are shown in children with ASD [6,7]. Self-injurious and stereotypical behaviors are also seen in children with ASD [6].

As movement disorders are seen in ASD [3,4], the goal of physical activity is to control the neural mechanisms that support the acquisition and retention of new skills [3,8]. Exercise programs are useful for improving motor and cognitive skills in children with ASD [3,9,10]. Apart from the other modes of physical exercise, the use of aquatic exercise in children with disabilities is appealing as they are usually not interested in regular physical exercises that may not be attractive to them [3,11,12,13,14,15].

This systematic review evaluates the influence of aquatic exercises on the physical and psychological health of children with ASD. Aquatic exercise's valuable response may provide good hope for therapies in children with ASD.

MATERIALS AND METHODS

Researchers used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting systematic reviews and meta-analyses (Figure 1) [17].

Search Strategy

Relevant studies were identified by a literature search of the papers published only in English, in electronic databases of Google Scholar and PubMed Central. Words used in the search strategy were “therapeutic effect”, “autism spectrum disorder”, “aquatic exercise”, “motor skill”, “cognitive functions”, “social activities”, and “psychological health”. The search string used for PubMed search is (autism spectrum disorder) AND (aquatic exercises OR aquatic therapy) AND (cognitive functions) AND (motor functions) AND (social activities) AND (psychological health). For the search interval, the start date restriction was not applied, and the end date was kept till the end of 2023. A literature search was also done directly online through Google search. A reference list of filtered studies was also searched to get relevant articles, while the grey literature was not searched.

Figure 1: Flow chart article search and study selection

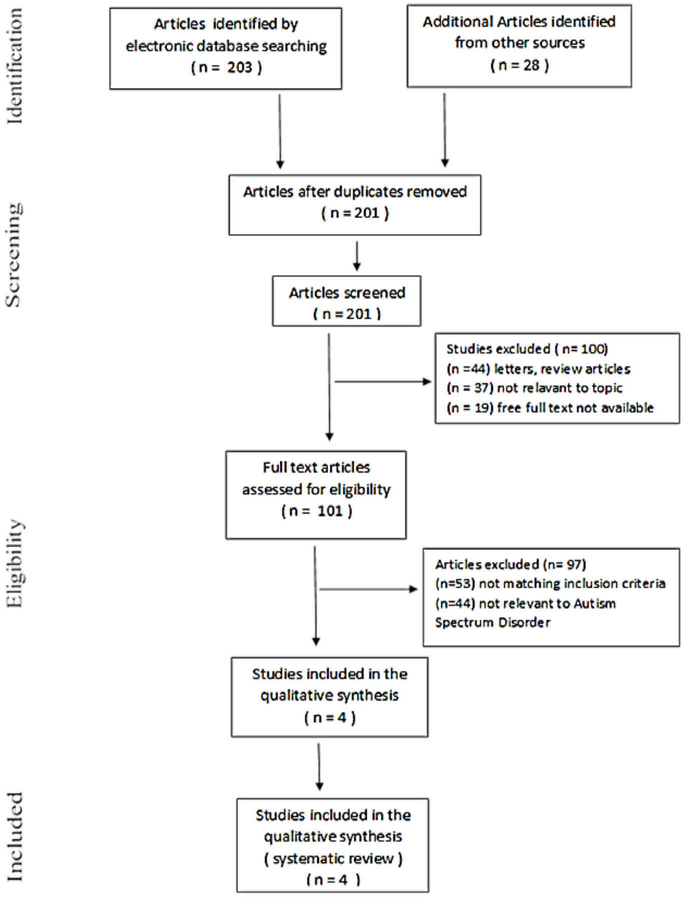
Identification
Articles identified by electronic database searching (n = 203)
Additional Articles identified from other sources (n = 28)
Screening
Articles after duplicates removed (n = 201)
Articles screened (n = 201)
Studies excluded (n= 100)
(n =44) letters, review articles
(n = 37) not relavant to topic
(n = 19) free full text not available
Eligibility
Full text articles assessed for eligibility (n = 101)
Articles excluded (n= 97)
(n=53) not matching inclusion criteria
(n=44) not relevant to Autism Spectrum Disorder
Studies included in the qualitative synthesis (n = 4)
Included
Studies included in the qualitative synthesis (systematic review) (n = 4)

Studies Selection Criteria

Studies filtered in the primary search were assessed for the following inclusion and exclusion criteria. PICO (an acronym for Population, Intervention, Comparison, Outcome) criteria [18,19,20,21], as described in Table 1, was used to retrieve relevant studies.

Table 1: Search strategy (PICO criteria)

	Definition
Population	Children with ASD
Intervention	Aquatic exercises
Comparison	N/A
Outcome	Assessing physical and psychological health through questionnaires or measurement

Abbreviations: ASD - autism spectrum disorder; N/A- not available; PICO - Population, Intervention, Comparison and Outcome

Inclusion criteria:

- Studies including aquatic exercise
- Studies having participants with ASD
- Studies include the evaluation of the aquatic exercise effects

on participants

Exclusion criteria:
- Articles not relevant to ASD
- Free full-text articles not available of studies
- Type of articles as letters and review articles

Study Quality

Reviewers assessed studies independently for inclusion and exclusion criteria mentioned in the study selection criteria. During the detailed reading of studies, queries regarding inclusion and exclusion were sorted out and finally decided by discussion between the authors.

Data Extraction

Reviewers analyzed selected studies under the following headings: name of the first author with initials, year of publication, brief detail of study population, sample size, age, gender, brief detail of aquatic exercise, and brief detail of outcome measure. Reviewers verified the data, and any discrepancies were addressed by discussion and resolved by consensus between the authors.

Data analysis

We extracted aquatic exercise activities and outcome measures in individual articles. Aquatic exercise activities and outcomes in each individual study were analyzed, and collected data was compared.

RESULTS

Literature search

We found 231 eligible articles by combined electronic database search and manual search. After the removal of 30 duplicated studies, the remaining 201 studies were screened by titles and abstracts. In the screening process, a total of 100 studies were excluded for reasons such as 37 studies were not relevant to the topic, 44 studies were letters or review articles, and a free full article was not available for 19 studies. Full-text articles were assessed for eligibility in the remaining 101 studies after the screening process. In this assessment, a total of 97 studies were excluded because 53 studies did not match the inclusion criteria, and 44 studies were not relevant to ASD. Finally, four articles were identified and included in the qualitative analysis. The flow diagram for the study selection process and the reason for exclusion is displayed in Figure 1.

Description of included studies

A brief description of each eligible four studies [3,6,12,22] is detailed in Table 2. The sample size of eligible 4 studies ranged from 7 to 40 participants. All eligible studies were published between 2016 and 2023. Out of the four eligible included studies, one study [3] has not provided information on participants' gender. All four eligible studies [3,6,12,22] included participants with ASD. The ages of participants ranged from 3 to 15 years. Out of four eligible included studies, one study [22] has not provided information on the inclusion and exclusion criteria of participants.

Table 2: Summary of Characteristics of the included studies

First Author, Year	Brief detail of the study population	Sample Size	Age group (Year)	Gender	Brief detail of aquatic exercise	Brief detail of outcome measure
Alaniz LM [12] (2017)	Participants with ASD were recruited. A swim lesson history was gathered. Inclusion and exclusion criteria are described in the study.	7	3 to 7	Both	The intervention was implemented over 3 phases, with eight 1-hour sessions in each phase. Participants attended 8, 16, or 24 hours of group intervention provided in 1-hour increments, once a week. Aquatic occupational therapy intervention is described in the study.	Single-group pre-test and post-test designs were used. Social skills were calculated using a standardized questionnaire, as described in the study, completed by the caregiver before session 1 and again after session 8, session 16, and session 24.
Adin E [22] (2023)	15 individuals with autism, 8 of which were in the experimental group and 7 in the control group, participated in the study	15	10 to12	Both	The experimental group was subjected to swimming exercise for 1 hour, 3 days a week, for 6 weeks. The control group was not included in this exercise.	The respiratory functions and respiratory muscle strength measurements of the subjects were taken 1 day before and 1 day after the 6-week training program. The same pretest–post-test period was applied to the control group.
Faraji S [3] (2023)	40 participants with ASD were recruited and then randomly assigned to a response-oriented aquatic exercise group	40	7 to 10	-----	The response-oriented aquatic group participated in an aquatic program for 8 weeks, three times weekly, for 45 minutes each session at an indoor pool. The control group participants were requested	The pre-and post-test designs were used. Motor skills and executive function were assessed in both groups as described in the

	(n=20) or a control group (n=20). Inclusion and exclusion criteria are described in the study.				not to alter their physical activity patterns for the duration of the study. The control group maintained their daily activities and did not change their physical activities during the intervention.	study, before and after the intervention.
Kanupka JW [6] (2016)	Children with ASD were selected. Inclusion and exclusion criteria are described in the study.	13	3 to 15	Both	The aquatic exercise program was conducted for 1 hour, 1 day per week for 6 weeks. The exercise program consisted of a 10-minute warm-up, a 10-minute strengthening program, 15 minutes of games, a 15-minute free swim, and a 10-minute cool-down, which were all completed in the pool.	Outcome measures as described in the study assessed before and after completion of the aquatic exercise program.

ASD – Autism Spectrum Disorder

In the study by Alaniz LM [12], “social skills” were calculated using the Social Skills Improvement System (SSIS) [23], a 79-item standardized questionnaire completed by the caregiver before session 1 and again after session 8, 16, and 24. The SSIS [23] assesses social skills like cooperation, communication, assertion, empathy, responsibility, engagement, and self-control and problem behaviors like bullying, externalizing, internalizing, hyperactivity/ inattention, and autism spectrum symptoms [12].

In the study by Adin E [22], forced vital capacity measurement, Vital capacity measurement, Breath inspiratory flow, and peak expiratory flow calculation were used.

In the study by Faraji S [3], The Bruininks-Oseretsky Test of Motor Proficiency – Second Edition (BOTMP-2) Short Form [24] was used. This instrument uses 8 subscales, including 14 separate parts, to comprehensively measure motor skills. Four subtests measure fine motor competence: 1) fine motor precision (FMP), 2) fine motor integration (FMI), 3) manual dexterity (MD), and 4) bilateral coordination (BC), and four subtests assess gross motor competence: 1) balance, 2) running speed and agility (RSA), 3) upper-limb coordination (ULC), and 4) strength [3,10,24]. In the study by Faraji S [3], to assess executive function, the computer version of the Wisconsin Card Sorting Test (WCST) was used [25]. This comprises 4 stimulus cards and 128 response cards [3,25].

In the study by Kanupka JW [6], outcome measures included The Pervasive Developmental Disorder Behaviour Inventory (PDDBI) [26] and the Intervention Rating Profile (IRP-15) [27,28,29,30]. The PDDBI is a rating scale designed to assess responsiveness to intervention in children diagnosed with one of the Pervasive Developmental Disorders (PDD) [6,26]. PDDBI subdomains are Sensory/Perceptual Approach Behaviours, Ritualism/Resistance to Change, Social Pragmatic Problems, Semantic/Pragmatic Problems, Arousal Regulation Problems, Specific Fears, Aggressiveness, Social Approach Behaviours, Expressive Language, Learning, Memory, and Receptive language [6,26]. The IRP-15 [6,27,28,29,30] was used to assess the social validity of aquatic exercise as an intervention to improve behavior in children with ASD [6]. One parent of each of the 13 participants reported on the social validity of aquatic exercise as an intervention for their children using a modified version of the IRP – 15 [6,27,28,29,30]. Parents in the study by Kanupka JW [6] rated their opinions of the effectiveness of the aquatic exercise program on a 15-question 6-point scale ranging from 1 (strongly disagree) to 6 (strongly agree), with total scores ranging from 15 to 90 and higher scores indicating greater acceptability [27,28,29,30]. A total score on the IRP - 15 greater than 52.5 indicates that the intervention is acceptable [6,27,28,29,30].

Outcome

All included studies used pre and post-test designs. Alaniz LM [12] observed significant improvement in the overall swim skills score. When examining individual swim skills, the participants advanced most consistently with breath control [12]. At all three time points, the participants, on average, significantly increased their breath control score compared to that of the pre-evaluation [12]. The changing position skill demonstrated significant improvement at 16 and 24 hours [12]. Propulsion skills improved after 24 hours of intervention only [12]. Navigation, exiting the pool, and back float skills did not improve significantly as measured on the Aquatic Skills Checklist (ASC). [12]. Alaniz LM [12] reported analysis of the Social Skills Improvement System (SSIS) [23] scores from baseline to 8 hours of intervention revealed no significant difference in social skills. There was a high rate of noncompliance for completion of the SSIS [23] after 16 and 24 hours of intervention, which prevented statistical analysis of the data [12].

Adin E [22] observed a significant difference between the pre- and post-test in the forced vital capacity (FVC) and forced expiratory volume (FEV1) values of the experimental group. There was no significant difference between the pre-test and post-test in the FVC and FEV1 values of the control group.

The results of the study by Faraji S [3] demonstrated improvements in gross motor skills and executive function following 8 weeks of response-oriented aquatic exercise. Faraji S [3] showed significant differences between re-sponse-oriented aquatic exercise and control groups in receiving, throwing, static balance, dynamic balance, correct responses, conceptual responses, and perseverative errors. A significant difference was observed in the study [3] between response-oriented aquatic exercise groups and control groups from pre- to post-test in bilateral coordination (BC).

In the study by Kanupka JW [6], there was a statistically significant difference in the scores for pre-arousal and post-arousal while there were no other statistically significant differences for pre and post-behavioral subdomains of the PDDBI [6,26]. In the study by Kanupka JW [6], the ratings for all 15 questions on the IRP - 15 ranged from 62 – 90, these scores were all above 52.5, indicating that aquatic exercise is an acceptable intervention for children with ASD [27,28,29,30]. In the study by Kanupka JW,[6], all of the parents agreed the aquatic exercise intervention had a positive effect on their child’s behavior (92.3% strongly agree/agree, 7.7% slightly agree), and parents also all indicated that they would be willing to use the intervention again in the future (100% strongly agree) [27,28,29,30].

DISCUSSION

Studies included in the systematic review investigated the effect of aquatic exercises on the physical and psychological health of children with ASD. Overall, included studies found improvements in various aspects of gross motor and executive functions by aquatic exercise in children with ASD.

Studies [3,31,32,33] in the past have shown an improvement in gross motor skills and executive functions with aquatic exercise programs in individuals with ASD. Aquatic exercise may improve arousal levels in children with ASD. [6]. Improved arousal may help children with ASD to learn more efficiently and interact in the aquatic environment [6,16]. A substantial increase in paying attention, tolerating touch, and maintaining eye contact is observed by therapists who have worked with children with ASD in aquatic environments [6,16].

Aquatic therapy may cause complex adaptations of the neuromuscular system and cause improvement in the transmission of neural impulses [3,34]. Aquatic therapy may increase muscle strength by a resistive medium without much pressure on the weight-bearing joints [3,35]. These improvements in executive function in children with ASD could be because of the improvement in activation of the neuron tract and transfer of messages from the brain to the body to perform movement in children with ASD [3,36]. The aquatic environment provides individuals with three-dimensional movements for continuity of movement, which gives more strength to coordination, temporal-spatial knowledge, kinaesthetic perception, and balance [37].

Limitations

In this review, the effect of aquatic exercise on ASD is the primary focus. However, the schedule and duration of the aquatic exercise program module were different among the selected studies. Parameters to measure outcomes were also different among selected studies. As a similar module of the aquatic exercise program is not used in the different studies explored and a similar outcome parameter is not used, it is difficult to generalize the interpretation of the individual studies. Other health problems were not screened for in the study selection criteria. The sample size was also comparatively small in selected studies. No paid tool or utility was used in the assessment of the quality of studies at any stage, which may increase the risk of bias. Another limitation of the search strategy was the English language, and articles that were not freely available also decreased the number of reviewed articles. The selection of articles was done by using only a pre-defined set of electronic databases. Therefore, studies of other electronic databases, which were not included in the search strategy, may contain relevant articles for the topic of this review.

Recommendations for future research

For a more specific evaluation of the positive effects, future research should focus on specific health benefits of aquatic exercise in individuals with ASD. Research should be done using a comparatively large sample size, proper methodology, and standardized outcome measures.

CONCLUSIONS

The present review focused on the effect of aquatic exercises on the health of individuals with ASD. Overall, included studies found beneficial effects of aquatic exercise in individuals with ASD. Further research is required to explore the long-term effects of aquatic exercise and to explore effects with optimal frequency and duration of sessions. However, the role of aquatic exercise seems promising in ASD, but before utilizing it in practice and treatment, properly designed studies are required to examine these effects in more detail, with adequate specificity and accuracy.

Conflicts of interest and sources of funding
The authors declare no conflict of interest. This research received no external funding.

Authors’ contribution
Dholakiya JR contributed to the manuscript preparation and assembly of data. Ghuntla TP contributed to the interpretation of results and literature review. All

authors agree with the content of the manuscript.

Ethics approval and consent to participate
Not applicable.

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ORIGINAL ARTICLE

Extracting Data from Medical Records for Monitoring Diseases and Generating Medical Alerts

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Abstract: *Background: Automated data processing is creating and implementing technology that automatically processes data. This computer tool is recommended for doctors because it supports their everyday work, assists in medical diagnosis, and enhances patient care. The aim of this paper is to propose an informatic tool that can extract the values of some parameters of interest from blood test sheets in order to get medical alerts and to monitor the chronic disease. Methods: An application, written in Python, was developed Results: The computer tool extracted automatically the values of glucose, triglycerides, HDL-cholesterol, total cholesterol, and LDL-cholesterol from medical sheets (text-based file or graphic file, respectively), and saved them in a database, accessed and represented in a graphic form the most recent values of these parameters; alerts according to metabolic syndrome criteria and Framingham risk score were generated. Conclusions: This tool contributes to the management of the medical process, saving precious time and helping the doctor in detecting current or future health problems.*

Keywords: *process automation in medicine; Python; medical alerts; metabolic syndrome; Framingham risk score*

INTRODUCTION

Process automation is defined as using software and technologies to automate business processes and functions to accomplish well-defined organizational goals. Automated data processing is creating and implementing technology that automatically processes data [1].

Automated systems, meant to emulate human cognitive capabilities, deploy supervised applications to perform repetitive tasks more accurately and efficiently, saving time and effort for high-volume workloads. In medicine, artificial intelligence has become a valuable tool for improving patient outcomes, particularly in diagnostics, by extracting information from image and text-based files [2].

Chronic patients are followed for years and the disease is monitored according to the clinical status and certain biological parameters. Therefore the medical data about them, saved and quickly accessed, can be of real use in the medical act.

Physicians consult many patients daily, being busy, apart from the actual medical act, with other tasks (electronic filling in of various data, issuing prescriptions, administrative work). Due to the relatively limited time allocated to a consultation, doctors can benefit, using this IT tool, from real support in their work.

Although the computer tool we propose was originally intended for family doctors, specialist doctors can successfully use it (by activating medical alerts, depending on the specialty), especially for chronic patients who return for regular consultations.

The aim of our application is the automation of the medical act, as follows:

- extracting (reading) automatically the values of some parameters of interest from blood test sheets (text-based pdf file or graphic file format);

- saving the blood values and anthropometric parameters for a patient in a database;

- activating medical alerts;

- monitoring the disease by displaying, in a graphic format (chart), the most recent values of the interest parameters.

MATERIALS AND METHODS

In this version, the application generates alerts according to metabolic syndrome and Framingham risk score.

Nowadays, the well-established term 'metabolic syndrome' (MetS) remains the most useful and widely accepted description of this cluster of metabolically related cardiovascular risk factors, which also predict a high risk of developing diabetes [3].

Many expert groups have attempted to develop a unifying definition for the MetS. We focus, in this paper, on the NCEP ATPIII and IDF criteria.

The NCEP ATPIII (National Cholesterol Education Program - Adult Treatment Panel III) criterion establishes that meta-bolic syndrome is present if three or more of the following five risk factors [3,4]:

• raised triglycerides level: ≥ 1.7 mmol/l (150 mg/ dl);

• reduced HDL-cholesterol: < 1.03 mmol/l (40 mg/dl) in males and < 1.29 mmol/l (50 mg/dl) in females (or specific treatment for these lipid abnormalities);

• raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg) (or treatment of previously diagnosed hypertension);

• raised fasting plasma glucose ≥ 5.6 mmol/l (100 mg/dl);

• raised waist circumference >102 cm for men and >88 cm for women.

The new definition given by the International Diabetes Federation (IDF) says that for a person to be defined as having the MetS, he/she must have central obesity plus any two of four additional factors [3]:

• raised triglycerides level: ≥ 1.7 mmol/l (150 mg/ dl);

• reduced HDL-cholesterol: < 1.03 mmol/l (40 mg/dl) in males and < 1.29 mmol/l (50 mg/dl) in females (or specific treatment for these lipid abnormalities);

• raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg) (or treatment of previously diagnosed hypertension);

• raised fasting plasma glucose ≥ 5.6 mmol/l (100 mg/dl)] (or previously diagnosed type 2 diabetes).

Figure 1: Use-case diagram made in UML

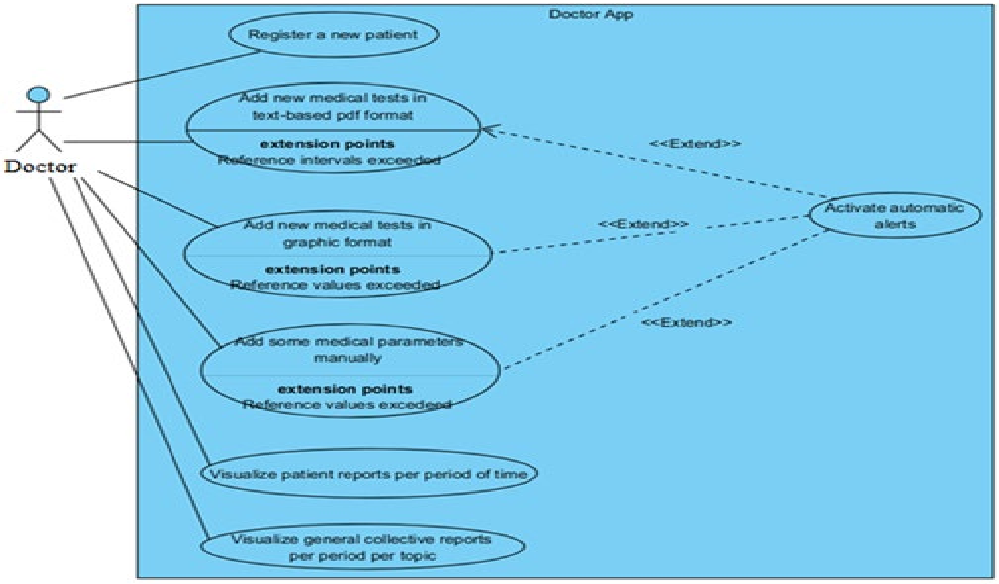
Doctor App
Register a new patient
Doctor
Add new medical tests in text-based pdf format
extension points
Reference intervals exceeded
<<Extend>>
Activate automatic alerts
Add new medical tests in graphic format
extension points
Reference values exceeded
<<Extend>>
Add some medical parameters manually
extension points
Reference values exceded
<<Extend>>
Visualize patient reports per period of time
Visualize general collective reports per period per topic

Central obesity is most easily measured by waist circumference with cut-points that are gender and ethnic-group specific. For example, an abnormal waist circumference for European males is ≥ 94 cm and for European females is ≥ 80 cm. The cut-points for central obesity adopted in the USA by the National Institutes of Health clinical guidelines for obesity were 102 cm for men and 88 cm for women. An abnormal waist circumference for South Asians and Chinese males is ≥ 94 cm and for South Asians and Chinese females is ≥ 80 cm [3,4].

Framingham Risk Score (FRS) and estimation of 10-year Cardiovascular Disease (CVD) Risk is an algorithm adapted from Canadian Cardiovascular Society/Canadian guidelines [5-7].

The PREDATORR (PREvalence of DiAbeTes mellitus, prediabetes, overweight, Obesity, dyslipidemia, hyperuricemia, and chronic kidney disease in Romania) study shows that the prevalence of diabetes mellitus was 11.6% and of prediabetes was 16.5% in the Romanian population aged 20-79 years [8].

Figure 2: Activity diagram made in UML

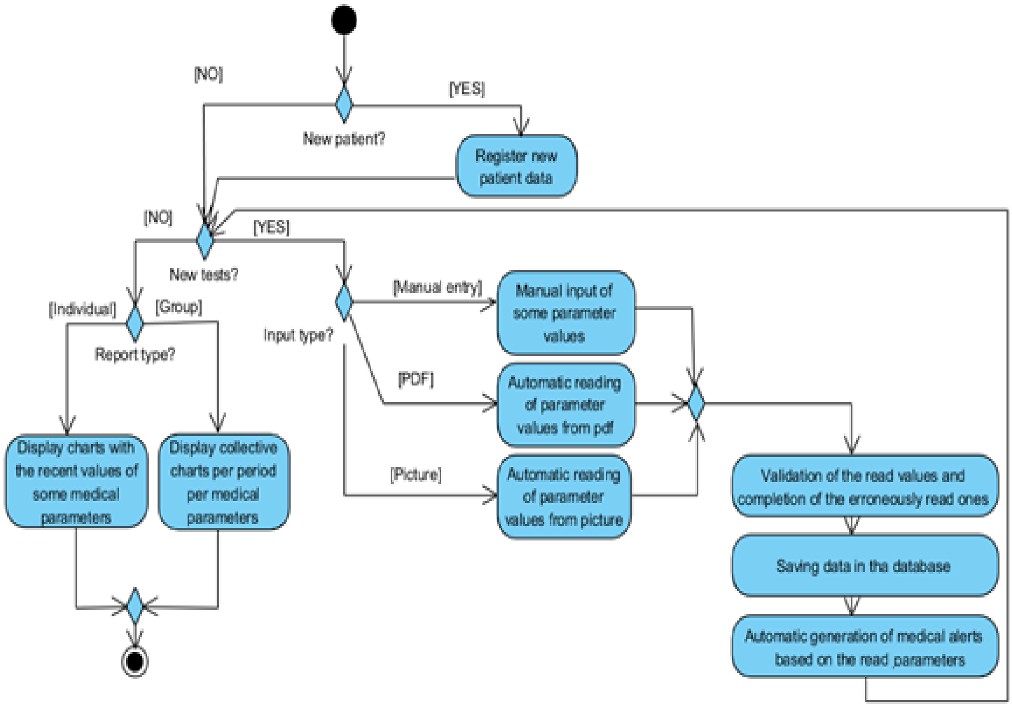
[NO]
[YES]
New patient?
Register new patient data
[NO]
[YES]
New tests?
[Individual]
[Group]
Report type?
Input type?
[Manual entry]
Manual input of some parameter values
[PDF]
Automatic reading of parameter values from pdf
[Picture]
Automatic reading of parameter values from picture
Display charts with the recent values of some medical parameters
Display collective charts per period per medical parameters
Validation of the read values and completion of the erroneously read ones
Saving data in tha database
Automatic generation of medical alerts based on the read parameters

The increased risk for cardiovascular disease in prediabetes is multifactorial, with etiologies including insulin resistance, hyperglycemia, dyslipidemia, hypertension, systemic inflammation, and oxidative stress. The preferred treatment is intensive lifestyle management and aggressive pharmacologic therapies directed toward individual coronary heart disease risk factors [9].

The early diagnosis and screening of prediabetes are essential steps toward the prevention of diabetes and associated complications. The American Diabetes Association (ADA) recommends screening adults over 45 years old without known diabetes every 3 years [10].

The Unified Modeling Language (UML) is a general-purpose visual modeling language that is used to specify, visualize, construct, and document the artifacts of a software system. It captures decisions and understanding about systems that must be constructed [11].

Because of its high level of functionality, Python language is used for web development, data science and data analysis, machine learning, startups, and the finance industry, among others [12].

Our application, written in Python, can be schematically described in Unified Modeling Language, using a use-case diagram (Figure 1), which explains the system's static structure, and an activity diagram (Figure 2), which captures information about the system's dynamic behavior.

As one can see in the above diagrams, the application allows, in a successful scenario:

- automatic reading (extracting) (details are given in Result section) from a text-based pdf file, the values of the parameters required for the metabolic syndrome algorithms (ATP III and IDF), respectively, the Framingham score calculation algorithm;
- automatic reading, from a graphic file (tif) (details are given in the “Results” section), of the values of the parameters required for the metabolic syndrome algorithms (ATP III and IDF), respectively, the Framingham score calculation algorithm;
- generating medical alerts;
- saving the values of parameters in the database;
- displaying charts showing the evolution (the last 3 or less, if the patient hasn’t 3 medical tests yet) of the parameters, each graph showing one parameter.

Suppose the patient does not present himself/herself with any analysis sheet. In that case, the application does not read any parameters and displays the chart showing the evolution of the medical parameters from the previous blood tests. The application also highlights the parameters from the most recent test, which were not within normal limits.

In the current version of the application, the database, created in SQLite 3, contains a single table, called Patient, which has the following structure:

- ID (INT, PRIMARY KEY, NOT NULL) – as the primary key;
- CNP (CHAR(13), NOT NULL) – as personal identification number;
- NAME (TEXT, NOT NULL);
- DT (TIMESTAMP) – as the date and time of analysis collection
- G (REAL) – as fasting glucose (or glycemia), expressed in mg/dl
- TG (REAL) as triglycerides, expressed in mg/dl
- HDL (REAL) – as HDL-Cholesterol, expressed in mg/dl
- WC (INT) - as waist circumference, expressed in cm
- COL (REAL) – as total cholesterol, expressed in mg/dl
- LDL (REAL) – as LDL-Cholesterol, expressed in mg/dl;
- SBP (INT) – as Systolic Blood Pressure, expressed in mm Hg;
- DBP(INT) – as Diastolic Blood Pressure, expressed in mm Hg.

There are situations where the scenario is unsuccessful due to automatic data reading errors (or the absence of some parameters from the analysis sheet), but a message will be displayed in this case. Also, the application uses mg/dl, not mmol/l, as the default measurement unit for triglycerides, plasma glucose, HDL-cholesterol, LDL-cholesterol, and total cholesterol, a shortcoming that will be fixed in future versions of the application.

RESULTS

Launching the application

The doctor can launch the application by double-clicking on the link when consulting a patient. According to the structure of the application presented in the UML diagrams, there can be several use cases of the application, discussed below.

The patient has no new medical tests

The identification of the patient using the personal numerical code is performed in all cases. The medic is asked to complete this information. The personal numeric code is programmatically validated (text should contain 13 digits and no other character):

Figure 3: Identify the patient

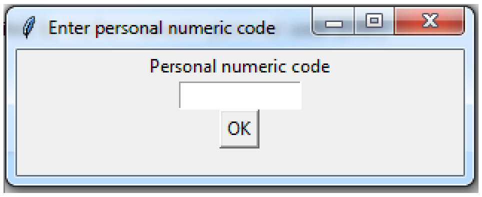

The program will display a chart with the three most recent values for each medical parameter involved in the metabolic syndrome (for example, HDL is displayed in Figure 4), so the doctor can remember the patient's condition. The application automatically retrieves and displays the parameters and their values for the last test made by the patient and programmatically highlights the abnormal values (Figure 5).

Figure 4: Evolution of HDL (the three most recent values) for a patient. The red line indicates the threshold value of the parameter (in this case, all values below 40, being a man, are abnormal)

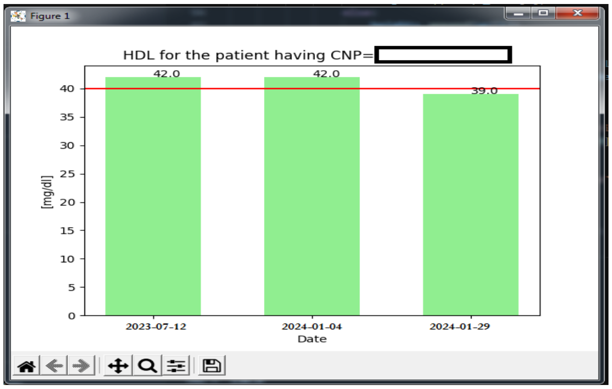

Figure 5: The values from the most recent test

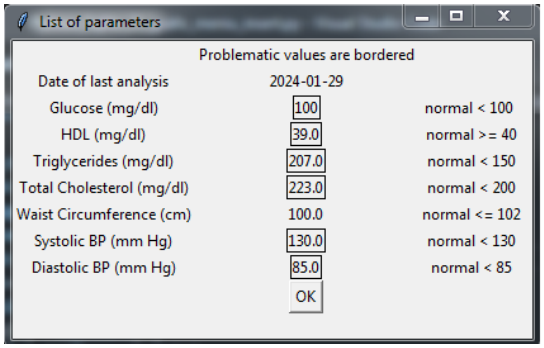

Problematic values are bordered

Date of last analysis	2024-01-29	
Glucose (mg/dl)	100	normal < 100
HDL (mg/dl)	39.0	normal >= 40
Triglycerides (mg/dl)	207.0	normal < 150
Total Cholesterol (mg/dl)	223.0	normal < 200
Waist Circumference (cm)	100.0	normal <= 102
Systolic BP (mm Hg)	130.0	normal < 130
Diastolic BP (mm Hg)	85.0	normal < 85

The patient has a new pdf text-based medical test

The program automatically reads (extracts) values of glucose level, triglycerides, HDL, total cholesterol, and LDL cholesterol from a medical test containing more than 20 parameters. We imported the <PyPDF2> module [13], which allows dividing the document into pages. The requested parameters and their corresponding values were searched on each page, using regular expression facilities (after importing the <re> module [14]).

The doctor will validate the automatically read values (Glucose level, Triglycerides, HDL, Total cholesterol, LDL cholesterol) and fill in other values (Systolic blood pressure, blood pressure treatment, Waist circumference, Smoker status, Diabetes history, and presence of Arterial fibrillation) which appear in the window with default values (shown in Figure 6).

Figure 6: The window for validating the read values and for introducing the other requested values. The first six top parameters have default values, the bottom five parameters have the values programmatically extract from the docu-ment

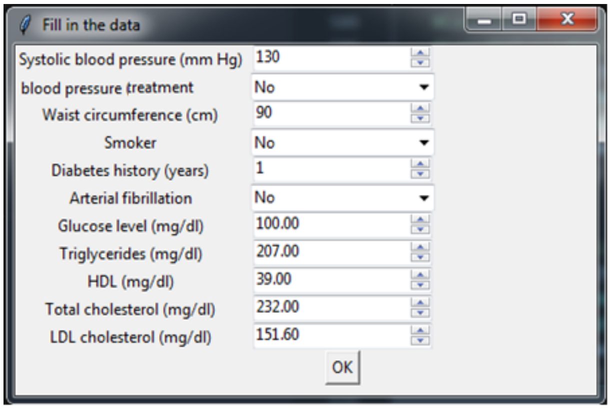

Systolic blood pressure (mm Hg)	130
blood pressure treatment	No
Waist circumference (cm)	90
Smoker	No
Diabetes history (years)	1
Arterial fibrillation	No
Glucose level (mg/dl)	100.00
Triglycerides (mg/dl)	207.00
HDL (mg/dl)	39.00
Total cholesterol (mg/dl)	232.00
LDL cholesterol (mg/dl)	151.60

After validating and completing the data, the values corresponding to the columns in the database are saved as a new record in the database, considering the analysis date as the current date, obtained from the system.

An alert regarding the presence or absence of prediabetes is displayed, as in Figure 7 (Prediabetes according to NCEP-ATP III criterion) and Figure 8 (Prediabetes according to IDF criterion). We observe the differences between the two criteria.

The Framingham score is displayed in Figure 9.

In the final step, charts are displayed with the most three recent values of each parameter, including the one from the control day. For example, in Figure 10, the evolution of glucose level is shown (the red line being the threshold value).

Figure 7: Prediabetes alert according to NCEP ATP III criterion

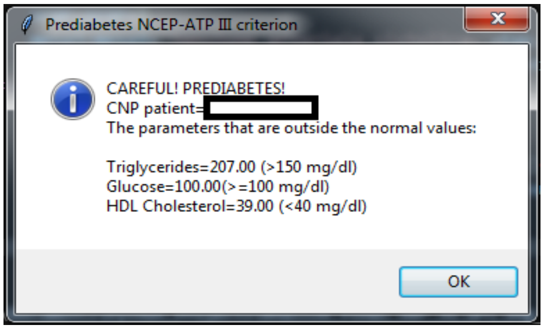

CAREFUL! PREDIABETES!
CNP patient=[redacted]
The parameters that are outside the normal values:

Triglycerides=207.00 (>150 mg/dl)
Glucose=100.00(>=100 mg/dl)
HDL Cholesterol=39.00 (<40 mg/dl)

Figure 8: Prediabetes alert according to IDF criterion

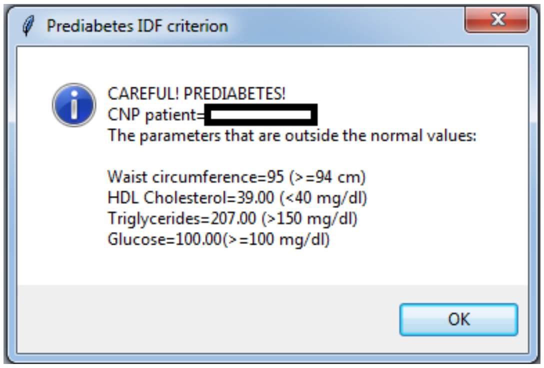

CAREFUL! PREDIABETES!
CNP patient=[redacted]
The parameters that are outside the normal values:

Waist circumference=95 (>=94 cm)
HDL Cholesterol=39.00 (<40 mg/dl)
Triglycerides=207.00 (>150 mg/dl)
Glucose=100.00(>=100 mg/dl)

Figure 9: Cardiovascular disease risk alert according to Framingham score

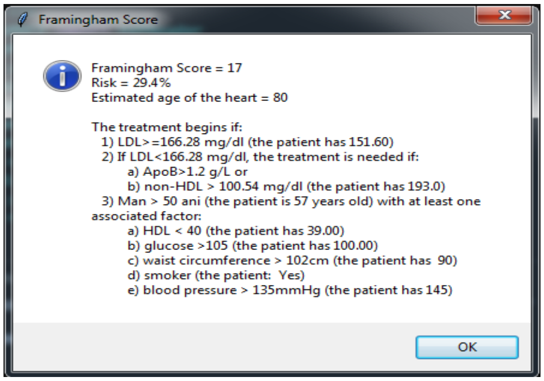

```
Framingham Score = 17
Risk = 29.4%
Estimated age of the heart = 80

The treatment begins if:
  1) LDL>=166.28 mg/dl (the patient has 151.60)
  2) If LDL<166.28 mg/dl, the treatment is needed if:
       a) ApoB>1.2 g/L or
       b) non-HDL > 100.54 mg/dl (the patient has 193.0)
  3) Man > 50 ani (the patient is 57 years old) with at least one
associated factor:
       a) HDL < 40 (the patient has 39.00)
       b) glucose >105 (the patient has 100.00)
       c) waist circumference > 102cm (the patient has  90)
       d) smoker (the patient:  Yes)
       e) blood pressure > 135mmHg (the patient has 145)
```

The patient has a new medical test in graphic format

The program automatically reads values of plasma glucose, triglycerides, HDL-cholesterol, total cholesterol and LDL-cholesterol. We use pytesseract [15], PIL [16] and cv2 [17,18, 19] modules for corrections and extracting requested values. As shown in the activity diagram, the following steps, in this case, coincide with those from the previous subsection, therefore, everything presented above and illustrated by Figures 7, 8, 9, 10 is also valid here.

Situations in which the application does not offer a successful scenario

If a parameter is missing or an error occurs during the automatic reading, a window indicating the missing parameter is displayed (Figure 11), and the medical alert cannot be generated correctly (for example, for the lack of HDL, the prediabetes alert cannot be generated – Figure 12).

Figure 10: Glucose values for the three most recent blood tests

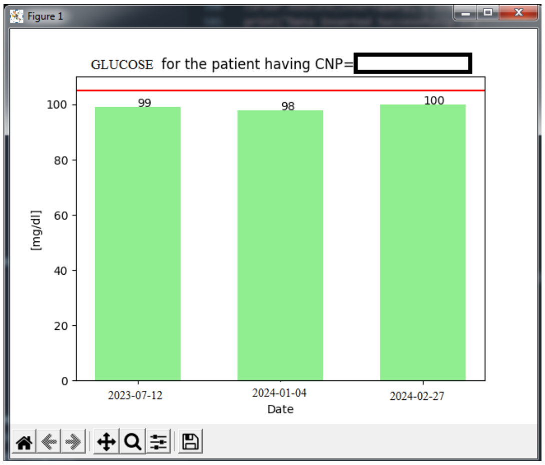

Figure 11: The window indicating a missing parameter

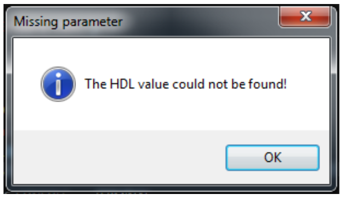

The HDL value could not be found!

Figure 12: The window indicating that the alert could not be generated

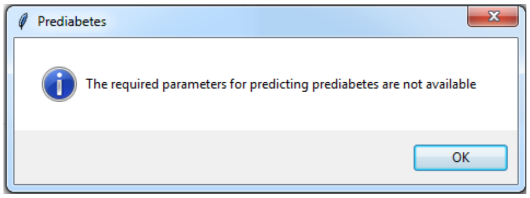

The required parameters for predicting prediabetes are not available

DISCUSSION

In this version, the application automatically generates medical alerts according to metabolic syndrome criteria and the Framingham risk score.

Because risk scores, such as the Framingham Risk Score, indicate the patient's cardiovascular risk for the next ten years, they are very useful. To apply an algorithm classically, the doctor manually uploads the patient's data in the fields related to the algorithm and this procedure takes time. Also, the dynamic verification of the biological values requires a search of the old documents and this is another task that consumes time. This application resolves these medical objectives at once. Therefore, an advantage of the application is that it saves precious time by automatically reading the values of some blood parameters (Figure 6). The values that are not correctly read by the application will be filled in by the doctor.

In Figure 4 and Figure 10, the charts for monitoring the serum HDLc and glucose values are shown. This IT tool can be adapted (extended) depending on the medical specialty. For example, in rheumatology, monitoring can be done by inflammatory markers (C reactive protein, fibrinogen; in endocrinology, the thyroid function can be monitored by TSH values, and in diabetes, the monitoring can be based on HbA1c values. The examples can go on. Therefore, the application is versatile because it can extract any parameter of interest, depending on the medical specialty.

Medical alerts are generated (Figures 7, 8, 9), indicating, in this case, current (metabolic syndrome) or future problems (Framingham risk score).

If the application, in future versions, will access only blood parameters in order to generate medical alerts, the doctor will not have to fill in the window (Figure 6) any other values (such as, in the present case, systolic blood pressure, waist circumference, etc.) than those automatically extracted, thus saving more time.

The limits of the application refer to errors in the automatic process of reading the values, and to the fact that the application uses as measurement unit mg/dl, not mmol/l (for plasma glucose, triglycerides, HDL-cholesterol, LDL-cholesterol, and total cholesterol). The application also presumes that the patients are European (when taking into account waist circumference). These shortcomings will be fixed in a future version of the application, in which the measurement units will be automatically read from the sheets and the application will take into account the patient's race (including a field in the database).

The current version of the application uses classical algorithms, based on medical guides, but it can also use machine learning and deep learning algorithms for prediction.

CONCLUSIONS

This application, despite some problems that could appear when reading the data automatically, can improve the medical activity of a doctor, making his/her work more efficient and saving valuable time through the automation processes it proposes.

The application offers quick access to the parameters involved in a disease, displaying them in charts that illustrate the evolution of the patient's state of health; in this way, the doctor does not rely on information about what the patient remembers from previous visits or on the papers that the doctor has to look for, but on data stored in the database.

The application automatically activates alerts that can help the doctor in diagnosis. Disease prevention measures can also be recommended by the doctor, based on these alerts.

The application is original and can be easily installed. It is scalable because new facilities can be added. However, the number of medical alerts raised by the application must not be excessively high because instead of helping the doctor's work, it can make it more difficult.

This application is intended to be a support tool and not a substitute for the doctor's work. The collaboration between doctors and computer scientists opens new ways of approaching the medical act.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, O.V., A.R.B. and R.S.C.; methodology, A.R.B., A.C.C. and B.V.; software, O.V and A.R.B.; validation, R.S.C and B.V.; formal analysis, B.V.; investigation, O.V. and A.C.C.; resources, R.S.C. and B.V.; data curation, O.V.; writing—original draft preparation, O.V.; writ-ing—review and editing, O.V. and A.R.B.; visualization, O.V.; supervision, B.V., R.S.C. and A.R.B.; project administration, O.V. and A.C.C.; funding acquisition, R.S.C. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Body Composition Analysis in the Air Force: A Comparative Study of Medical Personnel and Mechanics

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Abstract: *Given the rigorous demands of aerial operations on the human body, this study aims to investigate the body composition differences among aircrew specialties, specifically between medical personnel and mechanics within the Air Force. By examining key body composition metrics such as fat mass, lean mass, and body water, the study seeks to uncover how these variables correlate with aircrew members' distinct occupational duties and overall health. Initial findings indicate subtle but significant body composition variations between groups, suggesting occupational roles may influence physical attributes. There were small differences in body composition between mechanics and medical personnel, primarily based on a greater body mass index, weight, body water, skeletal muscle mass, soft lean mass, and fat-free mass of the mechanics. Body composition analysis by segments suggest a better muscle activation during their duties, but worse nutrition habits in mechanics when compared to medical personnel. This underscores the importance of designing occupation-specific training and nutrition programs to optimize the health and operational performance of aircrew members across specialties.*

Keywords: *aircrew; body composition; nutrition; training; health; performance*

INTRODUCTION

Accurate body composition assessment in military settings is paramount for operational readiness, physical performance, and health outcomes. It emphasized the need for reliable measurement methods within military populations, particularly among Air Force Reserve Officers' Training Corps (ROTC) cadets, highlighting a significant aspect of military fitness assessments, underscoring the necessity of employing accurate and reliable body composition measurement techniques to serve the needs of military personnel better [24]. Other researchers point out the potential adverse effects of unhealthy weight control behaviors, such as weight cycling and disordered eating, prevalent among military personnel [8]. In this line, it was highlighted how body composition is basic to reach operative cardiorespiratory fitness in infantry maneuvers [14]. Additionally, a systematic review and meta-analysis conducted to assess changes in physical performance, body composition, and physical training during military operations revealed significant findings. This study highlighted that deployments could lead to a decrease in endurance performance, among other changes, suggesting the critical role of continuous monitoring and adjustment of training and nutritional strategies to mitigate negative impacts on soldiers' physical conditioning and body composition during deployments [28].

For flight crew members, managing optimal body composition (BC) is crucial due to the unique and stressful nature of their work environment, which includes long durations at high altitudes and irregular working hours that can disrupt sleep patterns and dietary habits [7,13,37]. It is of vital importance to address fatigue among flight crews, especially under extra augmented crew (EAC) schedules implemented during the COVID-19 pandemic, which increases fatigue levels, underscoring the necessity of continuous monitoring and management of crew fatigue to ensure safety and health, suggesting that BC plays a role in maintaining operational efficiency and reducing the risk of fatigue-related errors [19]. It was also found that optimal body composition is critical for pilots, impacting their ability to handle high G-forces and hypoxia at altitude [5,6]. In this line previous authors presented that air force cadets with higher muscle mass and lower fat performed better in G-tests, suggesting that muscle mass improves G-force resistance and cognitive function during flight by enhancing blood flow to the brain [36]. Another important aspect is dysmetria, fact that could negatively impact flight maneuvers, potentially leading to injuries among pilots, showing the necessity to develop specialized training programs aimed at correcting this issue is advisable [10].

Flight crew roles, including pilots, medical staff, and mechanics, are diverse yet integral to ensuring flight safety and efficiency. Pilots helm the aircraft, navigating through various weather conditions and regulatory environments to guarantee a secure journey from start to finish. They communicate with air traffic control and handle emergencies with precision. Medical personnel aboard, such as flight nurses and paramedics, are pivotal in aeromedical transport missions, offering crucial care during emergencies, patient transfers, and providing specialty care mid-flight. Their expertise is indispensable for medical repatriation and in managing healthcare in the unique and stressful conditions of flight. Mechanics and aviation engineers ensure that aircraft are safe and airworthy. Their work involves regular maintenance, pre-flight checks, and addressing any mechanical issues that arise. They are essential for the aircraft's reliability, working behind the scenes to prevent technical failures and ensure that all systems function correctly throughout the flight [20,21].

Understanding body composition standards serves as a foundational step towards enhancing nutritional habits and tailoring training systems to fit the specific realities of individuals, especially those in physically demanding roles such as military personnel. Previous studies emphasize the multifaceted relationship between body composition and military performance, highlighting the diverse implications of BC across various aspects of physical readiness and performance [16]. By recognizing BC benchmarks, individuals and organizations can better design nutritional and training programs that are not only effective but also aligned with the unique demands and realities faced by the individuals. Tailored approaches that consider the specific BC requirements can significantly contribute to improved overall health, fitness, and performance outcomes. This understanding underscores the necessity of integrating comprehensive BC knowledge into the development of personalized nutrition and exercise regimens [7,11]. Therefore, this study aimed to analyze body composition differences between the types of specialty of aircrews, to provide data of their standards, and to improve their preparation for the extra risks they need to face when they are transported in an aircraft. We hypothesized that: i) there would be differences in body composition between specialties, ii) mechanics would have higher values of lean mass and lower values of fat mass than the medical personnel, iii) mechanics would have higher values of fat-free mass in their arms than the medical personnel**.**

MATERIALS AND METHODS

1. Experimental approach to the problem

To implement the body composition assessment, we followed previous procedures [4,9,29] in which the participants stood upright on foot electrodes on the instrument platform, with arms not touching the torso. They were barefooted and without excess clothing. Four-foot electrodes were used, two of which were oval-shaped and two heel-shaped, and prior to testing the skin was cleaned and dried. Participants were asked to grip the palm and thumb electrodes. The system was calibrated before the testing session and the contacting surface of the electrodes was cleaned with alcohol before each measurement. Participants abstained from alcohol consumption and vigorous exercise for 24 hours before the measurement and were measured in the morning after overnight fasting to control hydration status, as in previous research with bioelectrical impedance.

2. Subjects

A randomized cross-sectional experimental study was conducted to analyze body composition differences of aircrews, depending on their specialty. 54 male aircrews were analyzed and classified according to their specialty (mechanics, N = 27, age: M = 43.6, SD = 7.1; medical personnel, N = 27, age: M = 42.2, SD = 11.8) of the Spanish Army with a qualification of “fit” according to the periodic medical examination as recorded in the ministerial order 23/2011. Besides, during the research, they were carrying out the periodic aeromedical training included in the ministerial order 23/2011 and the STANAG 3114 “Aeromedical Training of Flight Personnel” (NATO regulations). Before starting the research, the experimental procedures were explained to all the participants, who gave their voluntary written informed consent under the Declaration of Helsinki. The procedures conducted in the present research were designed and approved by the Medical Service of the Aerospace Medicine Instruction Centre of Spanish Air Forces and the University Ethic Committee (CIPI/18/093).

3. Instruments

A bioimpedance analyzer (InBody 720, Biospace Co. Ltd., Seoul, South Korea) was used for body mass measurement (to the nearest 0.1 kg) and body height (to the nearest 0.1 cm). InBody 720 is a multifrequency impedance body composition analyzer, which uses an eight-point tactile electrode method to take readings from the body. It measures the resistance at five specific frequencies (1 kHz, 50 kHz, 250 kHz, 500 kHz, and 1 MHz) and reactance at three specific frequencies (5 kHz, 50 kHz, and 250 kHz) on each of five segments (right arm, left arm, trunk, right leg, and left leg). Bioelectrical-impedance analysis (BIA) is one of the methods available for measuring body composition in healthy populations. Its simplicity, portability, cost, and subject acceptance make it a very desirable technique. The reliability of BIA compared to the gold standard body composition measurement (Dual-energy X-ray absorptiometry, DXA) has been successfully demonstrated for a wide range of normal and overweight populations [12,33] and military samples [1].

Data were electronically imported to Excel using Lookin'Body 3.0 software. The following parameters were analyzed: i. Body mass (kg), ii. Height (cm), iii. Body Mass Index (BMI) (kg/m2), iv. Total Body Water (TBW) (kg) (including left and right arms, trunk, and left and right legs), v. Intracellular Water (ICW) (l) (including left and right arms, trunk, and left and right legs), vi. Extracellular Water (ECW) (l) (including left and right arms, trunk, and left and right legs), vii. Proteins (kg), viii. Minerals (kg), ix. Body Fat Mass (BFM) (kg) (including left and right arms, trunk, and left and right legs, in kg and %), x. Percentage of Body Fat (PBF) (%), xi. Soft Lean Mass (SLM) (kg), xii. Fat-Free Mass (FFM) (kg) (including left and right arms, trunk, and left and right legs, in kg and %), xiii. Skeletal Muscle Mass (kg).

4. Statistical analysis

The SPSS statistical package (version 21.0; SPSS, Inc. Chicago, Illinois) was used to analyze the data. Normality assumptions were checked with a Shapiro-Wilk test for all the subgroups. Specialty differences were analyzed with a T-Student test for parametric samples and a Mann-Whitney test for no parametric samples. Homoscedasticity was tested with the Levene test. The level of significance for all the comparisons was set at $P \leq 0.05$.

RESULTS

The results are reported with their mean and standard deviation. Table 1 shows the results of body composition. No significant differences were found for the effect of type of work. There was a small effect of the specialty (mechanics had higher values than the medical personnel) in body mass (84.3 kg vs 81.1 kg), BMI (27.1 kg/m^2 vs 25.8 kg/m^2), TBW (46.9 l vs 45.7 l), ICW (29.4 l vs 28.6 l), proteins (12.7 kg vs 12.3 kg), SLM (60.4 kg vs 58.8 kg), SMM (36.4 kg vs 35.2 kg), FFM (64.1 kg vs 62.5 kg).

Table 1: Results of body composition variables by specialty groups

	Medical Personnel		**Mechanics**		**Specialty effect**		
	M	**SD**	**M**	**SD**	**t**	**p**	**Cohen's d**
Height (cm)	177.3	6.54	176.1	6.46	0.703	0.485	0.191
Body Mass (kg)	81.1	14.7	84.3	84.3	-0.848	0.400	-0.231
BMI (kg/m^2)	25.8	4.68	27.1	3.49	-1.175	0.245	-0.320
TBW (l)	45.7	5.41	46.9	5.51	-0.797	0.429	-0.217
ICW (l)	28.6	3.42	29.4	3.39	-0.906	0.369	-0.247
ECW (l)	17.1	2.01	17.5	2.14	-0.608	0.546	-0.166
Proteins (kg)	12.3	1.48	12.7	1.45	-0.860	0.394	-0.234
Minerals (kg)	4.36	0.49	4.44	0.55	-0.609	0.545	-0.166
BFM (kg)	18.6	11.0	20.1	7.74	-0.612	0.543	-0.167
PBF (%)	21.7	9.27	23.3	6.28	-0.701	0.486	-0.191
SLM (kg)	58.8	6.97	60.4	7.06	-0.810	0.421	-0.221
FFM (kg)	62.5	7.37	64.1	7.50	-0.803	0.426	-0.219
SMM (kg)	35.2	4.46	36.4	4.44	-0.920	0.362	-0.250

Note. M = Mean; SD = Standard Deviation; t = t-test; BMI = Body Mass Index; TBW = Total Body Water; ICW = Intracellular Water; ECW = Extracellular Water; BFM = Body Fat Mass; PBF = Percentage of Body Fat; SLM = Soft Lean Mass; FFM = Fat Free Mass; SMM = Skeletal Muscle Mass

Table 2 shows the results of FFM and BFM by body segments. No significant differences were found for the effect of specialty. There was a small effect of the specialty (mechanics had higher values than the medical personnel) in the fat-free mass of the right arm (3.75 kg vs 3.58 kg), the fat-free mass of the left arm (3.69 kg vs 3.53 kg), the fat-free mass of the trunk (28.6 kg vs 27.7 kg), and the body fat mass of the trunk (10.9 kg vs 9.75 kg).

Table 3 shows the results of body water (total, intracellular and extracellular) by body segments. No significant differences were found for the effect of type of work. There was a small effect of the specialty (mechanics had higher values than the medical personnel) in the total body water of the right arm (2.92 l vs 2.78 l), the left arm (2.87 l vs 2.84 l), and the trunk (22.2 l vs 21.5 l). There was a small effect of the specialty (mechanics had higher values than the medical personnel) in the intracellular water of the right arm (1.82 l vs 1.74 l), the left arm (1.79 l vs 1.71 l), and the trunk (13.9 l vs 13.4 l). There was a small effect of the specialty (mechanics had higher values than the medical personnel) in the extra-cellular water of the right arm (1.09 l vs 1.04 l), the left arm (1.08 l vs 1.03 l), and the trunk (8.31 l vs 8.09 l).

Table 2: Results of body composition variables by specialty groups

	Medical personnel		Mechanics		Specialty effect		
	M	SD	M	SD	t	p	Cohen's d
FFM Right Arm (kg)	3.58	0.61	3.75	0.57	-1.052	0.298	-0.286
FFM Left Arm (kg)	3.53	0.61	3.69	0.59	-0.985	0.329	-0.268
FFM Trunk (kg)	27.7	3.62	28.6	3.43	-0.953	0.345	-0.259
FFM Right Leg (kg)	9.64	1.12	9.64	1.21	-0.065	0.948	-0.018
FFM Left Leg (kg)	9.61	1.14	9.61	1.23	0.057	0.489	0.016
BFM Right Arm (kg)	1.20	1.24	1.22	0.71	-0.067	0.947	-0.018
BFM Left Arm (kg)	1.23	1.24	1.25	0.68	-0.081	0.936	-0.022
BFM Trunk (kg)	9.75	5.90	10.9	4.46	-0.834	0.408	-0.227
BFM Right Leg (kg)	2.59	1.26	2.73	0.86	-0.477	0.635	-0.130
BFM Left Leg (kg)	2.56	1.24	2.71	0.86	-0.495	0.623	-0.135

Note. M = Mean; SD = Standard Deviation; t = t-test; FFM = Fat-Free Mass; BFM = Body Fat Mass

Table 3: Results of Body Water by Body Segments

	Medical personnel		Mechanics		Specialty effect		
	M	SD	M	SD	t	p	Cohen's d
TBW Right Arm (l)	2.78	0.48	2.92	0.44	-1.054	0.297	-0.287
TBW Left Arm (l)	2.74	0.47	2.87	0.45	-0.994	0.325	-0.270
TBW Trunk (l)	21.5	2.80	22.2	2.66	-0.931	0.356	-0.253
TBW Right Leg (l)	7.49	0.87	7.49	0.95	-0.031	0.975	-0.009
TBW Left Leg (l)	7.47	0.89	7.45	0.96	0.094	0.926	0.025
ICW Right Arm (l)	1.74	0.30	1.82	0.27	-1.060	0.294	-0.289
ICW Left Arm (l)	1.71	0.29	1.79	0.28	-1.016	0.314	-0.276
ICW Trunk (l)	13.4	1.77	13.9	1.64	-1.026	0.309	-0.279
ICW Right Leg (l)	4.69	0.54	4.72	0.58	-0.183	0.856	-0.050
ICW Left Leg (l)	4.66	0.55	4.67	0.59	-0.067	0.947	-0.018
ECW Right Arm (l)	1.04	0.18	1.09	0.17	-1.038	0.304	-0.283
ECW Left Arm (l)	1.03	0.18	1.08	0.17	-0.952	0.346	-0.259
ECW Trunk (l)	8.09	1.04	8.31	1.02	-0.762	0.450	-0.207
ECW Right Leg (l)	2.79	0.33	2.77	0.37	0.213	0.832	0.058
ECW Left Leg (l)	2.81	0.34	2.77	0.38	0.344	0.732	0.094

Note. M = Mean; SD = Standard Deviation; t = t- test; TBW = Total Body Water; ICW = Intracellular Water; ECW = Extracellular Water

DISCUSSION

This study aimed to analyze body composition differences between mechanics and medical personnel of the Air Force. The first hypothesis was partially fulfilled since there were small differences in body composition between specialties. The second hypothesis was partially fulfilled since the effect of the specialty in the results was small. The third hypothesis was partially fulfilled since there were small differences in the fat-free mass between specialties.

Height, body mass and body mass index measures have been traditionally used as a first basic approach to study body composition [2,11]. Although the differences found were small, it is important to note a greater weight and body mass index in the mechanics group. The morphological distribution of the body mass can be more easily identified through the soft lean mass, the skeletal muscle mass, and the fat-free mass [17], which were greater in mechanics. These differences suggest predominant values of lean mass, rather than body fat, in the body mass values of mechanics. Although mechanics are not as responsible as pilots in the control of aircraft, their performance is crucial to control and maintain the correct functioning of aircraft devices [23]. Mechanics need to use tools in flight time, and to cope with the vibrations, accelerations, and changes of directions of the aircraft, while they maintain their stance [3,15,25]. These aspects could be crucial in the development of muscle mass groups, especially segmental stabilizers. Muscles are made of water and proteins, and the small differences that were found between specialties (greater in mechanics than in medical personnel) are coherent with the previous results and previous research [4].

The analysis of body composition by segments can provide better insights of which are anatomical regions better involved in the morphological differences between specialties. The fat-free mass was higher in the upper extremities for the mechanics' group, and this aspect reinforces the previous assumptions and descriptions about the different jobs of each specialty. Mechanics usually work with tools that need to be controlled by their hands, involving the muscles of their arms, applying different kind of forces and torques to adjust the devices. Many muscle groups in the trunk are responsible of the stability of the core, that is crucial to maintain the stance in flight time [15,18,38]. It could be perceived that the most part of the aircrew are sitting down in the cockpit, as happens with the pilots. However, several factors that happens in an aircraft, like vibrations, accelerations, and 3-axes movements, keep the muscles active to react and stabilize against forces [31], while muscle contractions are even harder when the air-crew stand up to perform their task [3]. Despite the mechanics having higher values for the fat-free mass of the trunk, they also had greater fat mass in this anatomical region when compared to medical personnel. Fat exceedances are normally stored in the abdominal region of the trunk, especially in men [26,32]. If we analyze these two factors together, the results suggest a better activation of the core muscles in the mechanics, but worse nutrition habits than the medical personnel. Healthcare professionals probably have a deeper knowledge of good nutrition habits that could help them to maintain healthier levels of fat, and that could balance their least muscle activation in flight [34]. In any case, it is important to notice that both the aerobic and anaerobic metabolism are activated in an aircraft, as was reported in real flight research [3], and it would be beneficial for these populations to maintain physical training that could help them to perform well in their job environments.

Body water results reinforced the previous assumptions about a greater muscle mass in mechanics than in the medical personnel. Muscle mass is mainly built of water, and there is a coherent relationship between the fat-free mass of the upper extremities and the trunk segments, and higher values of body water [30]. Both body water and fat-free mass, were higher in the right arm than in the left arm for each specialty, which suggests a predominance of the right hand (usually the dominant hand) in the tasks that both job groups must accomplish. If we compare their values with previous research, both mechanics and medical personnel had worse body composition values than pilots [4], and other transported units that usually required to perform high levels of physical activity. Even though there are similarities between transport aircrews, there are still subtle differences related to their occupation needs, and there is a need to monitor and provide body composition standards that gather these gaps [11,27]. Good nutrition habits [35] and training preparation [22] would be beneficial in these units to help them face the hazards that military aircrews need to cope with, both in flight time and when they are deployed.

Practical application

The practical application of this study emphasizes the necessity for air forces to implement specialized training and nutrition programs tailored to the unique demands of different aircrew specialties. Recognizing the specific body composition needs of medical personnel and mechanics can lead to more effective health and performance outcomes, contributing to enhanced operational readiness and safety in aerial missions. This approach supports the development of targeted interventions that promote optimal physical conditioning and well-being among aircrew members, ultimately improving their capacity to handle the physical challenges of flight duties.

Limitations of the study and future research lines

The study's limitations include its narrow focus on specific aircrew specialties within a single air force, potentially limiting the generalizability of the findings to broader populations or different military contexts. Additionally, the cross-sectional design precludes causal inferences between body composition and occupational roles. Lastly, we did not register the years of work experience in this sample, which may help to develop longitudinal studies to assess the evolution of body composition with and without dietary and training interventions. Future research should expand to longitudinal studies across diverse military settings to explore the dynamic changes in body composition over time and their impact on aircrew performance. Investigating the effectiveness of tailored training and nutrition interventions in enhancing operational efficiency and reducing injury risks in varied aircrew roles will also be valuable.

CONCLUSIONS

There are small differences in body composition between aircrew specialties (mechanics and medical personnel) primarily based on a greater body mass index, weight, body water, skeletal muscle mass, soft lean mass, and fat-free mass of the mechanics. Body composition analysis by segments, suggests better muscle activation during their duties, but worse nutrition habits in mechanics when compared to medical personnel. The performance requirements of these units are different from those that are principally responsible for the aircraft (pilots) or those who are transported to be deployed in a conflict area. Perceived assumptions about the tasks that each unit must accomplish need to be clarified by an adequate body composition assessment.

This information could help to understand better each unit requirements and to develop training and nutritional habits that are crucial to performing well in the stressful job contexts of the aircrews.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, V.J.C.S. and A.B.S.; methodology, V.J.C.S. and A.B.S.; formal analysis, V.J.C.S. and A.B.S.; investigation, V.J.C.S. and A.B.S..; resources, V.J.C.S. and A.B.S.; data curation, A.B.S.; writing—original draft preparation, A.B.S.; writing—review and editing, V.J.C.S. and A.B.S..; supervision, V.J.C.S.; project administration, V.J.C.S.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of UNIVERSIDAD EUROPEA DE MADRID (protocol code CIPI/18/093).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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LITERATURE REVIEW

Sculpting Hope: Advanced MEN 2 – Associated Tumors. A Narrative Review Starting from a Case Report

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Abstract: *Multiple endocrine neoplasias (MEN) represent a rare and intricate group of disorders with diverse endocrine and extra-endocrine manifestations. Among the five identified types of MEN syndromes, MEN 2 stands out due to its complexity, featuring medullary thyroid carcinoma (MTC), pheochromocytoma, and primary hyperparathyroidism (PHPT). MEN 2 is inherited in an autosomal dominant manner, with nearly a 100% lifetime risk of developing MTC, which can be fatal if not detected early. Comprehensive diagnostic protocols and timely interventions significantly mitigate morbidity. To guide surgical decisions, management strategies include regular imaging and biomarker monitoring (calcitonin, CEA, metanephrines/normetanephrines, and parathyroid hormone). Genetic screening of at-risk families and prophylactic thyroidectomy remain cornerstone preventive measures. Although tyrosine kinase inhibitors are generally reserved for specific cases of MTC and are not first-line therapies for MEN2-associated tumors, they hold the potential for mitigating tumor progression and recurrence despite the risk of endocrine dysfunction. This paper presents a narrative review focusing on multiple endocrine neoplasia type 2 (MEN2) syndrome, encompassing its genetic underpinnings, clinical presentations, diagnostic modalities, and therapeutic approaches. A total of 48 articles were included in this review.*

Keywords: *multiple endocrine neoplasia, medullary thyroid carcinoma, pheochromocytoma, primary hyperparathyroidism*

INTRODUCTION

Multiple endocrine neoplasias (MEN) are rare hereditary syndromes characterized by the co-occurrence of multiple endocrine tumors in the same individual or related individuals of the same family; these tumors usually secrete hormones that give rise to distinct clinical syndromes [1,2]. Currently, five distinct types of MEN have been identified: MEN1, MEN2 (previously known as MEN2A), MEN3 (formerly known as MEN2B) and the recently recognized MEN4 and MEN5 (Table 1) [3–11].

MEN 2 is a complex autosomal dominant inherited syndrome characterized by medullary thyroid carcinoma (MTC), pheochromocytoma, and primary hyperparathyroidism (PHPT)[12,13].

The estimated prevalence of MEN2 is approximately 1 in 35,000 [3]. More than 95% of MEN2 patients develop MTC, and about 50% have pheochromocytomas. HPT occurs in 20–30% of MEN2 patients, generally without evident symptoms, and is found by biochemical and imaging assessments [14].

The majority of pathogenic variants associated with Multiple Endocrine Neoplasia type 2 (MEN2) follow an autosomal dominant inheritance pattern and are situated within the extracellular cysteine-rich domain of the RET proto-oncogene's exon 10 (specifically at codons 609, 611, 618, or 620) or exon 11 (at codon 634). Pathogenic variants at codon 634 are responsible for at least 50% of diagnosed cases [13-20]. Approximately 25% of these cases are hereditary due to activating mutations in the REarranged during Transfection (RET) proto-oncogene. The course of MTC is indolent, and survival rates depend on the tumor stage at diagnosis. Initial management involves total thyroidectomy with bilateral dissection of the central node compartment [22]. Serum calcitonin and carcinoembryonic antigen should be performed pre-operatively. These can be followed over time and used as markers of disease recurrence as their doubling times have been shown to correlate with disease progression and survival [8]. Neck metastases appear early during MTC. Distant metastases are detected in 7-17% of all cases at the time of diagnosis and frequently appear in multiple sites. The most frequent sites of distant metastasis are the liver (49%), bones (45%), and lungs (35%) [23].

Table 1: Update in the classification of MEN syndromes [8–11]

	MEN1 (Wermer Syndrome)	**MEN2 (Sipple syndrome)**	**MEN 3 (Gorlin-Vickers syndrome)**	**MEN4 (MEN X syndrome)**	**MEN5**
Gene alteration	Loss-of-function of the tumor suppressor gene MEN1 (menin)	Germline variants in the *RE*arranged during *T*ransfection proto-oncogene (specially codon 634)	Germline variants in the *RE*arranged during *T*ransfection proto-oncogene (specially codon 918)	Inactivating germline mutation of tumor suppressor gene CDKN1B	Heterozygous missense variant in MAX (only 2 families described in the literature)
Inheritance	Autosomal-dominant	Autosomal-dominant	Autosomal-dominant	Autosomal-dominant	Autosomal-dominant
Dominant endocrine pathology	Primary hyperparathyroidism 85% - usually four-gland hyperplasia	Medullary thyroid carcinoma 95-100%	Medullary thyroid carcinoma 95-100%	Primary hyperparathyroidism 75%	Pheochromocytoma
Associated endocrine disorders	Enteropancreatic tumor: gastrinoma, insulinoma, non-functioning, glucagonoma, VIPoma Pituitary neuroendocrine tumors Bronchopulmonary neuroendocrine tumors, Thymic neuroendocrine tumors Adrenal cortical tumors (usually non-functioning)	Pheochromocytoma (50%) Primary hyperparathyroidism (20-30%): four-gland hyperplasia or single adenoma	Pheochromocytoma (50%)	pNET: non-functional, gastrinoma	Pituitary neuroendocrine tumors Parathyroid adenomas
Associated non-endocrine tumors or disorders	Breast cancer lipomas, angiofibromas, collagenomas, meningiomas	Cutaneous lichen amyloidosis (or) Hirschsprung's disease	Ganglioneuroma of the lips, tongue, or gastrointestinal tract + Marfanoid habitus (95%)		Ganglioneuromas, Neuroblastomas, Chondrosarcoma, Lung adenocarcinoma

The American Thyroid Association (ATA) stratified patients with MEN2 from moderate to the highest-risk groups based on the identified pathogenic variant in RET proto-oncogene and the severity of the clinical course of MTC [24]. According to the 2015 ATA guidelines, the highest-risk pathogenic variant includes MEN3 patients with RET mutations in codon p.Met918Thr [8,25]. High-risk MEN2 pathogenic variants are RET codon p.Cys634 and p.Ala883Phe pathogenic variants. Moderate-risk pathogenic variants include identified RET codon pathogenic variants other than those noted to be at "high" or "highest risk"[26,27].

In metastatic MTC, the focus is on local and systemic treatment modalities. Surgery remains the first recommendation for recurrent cases, dependent on factors like size, extent, and concurrent distant metastases. External beam radiation therapy (EBRT) may be considered when surgical resection is not feasible. Focal treatments such as surgery, embolization, radiofrequency ablation, and EBRT aim at relieving local symptoms and reducing the risk of complications. Embolization or chemoembolization for liver metastases may reduce tumor masses and alleviate symptoms. Multikinase and selective kinase inhibitors represent the systemic treatment for MTC with identified RET mutation [28]. Multikinase inhibitors with anti-RET activity, such as Vandetanib and Cabozantinib, are approved for MTC patients ineligible for surgery with symptomatic or progressive disease [29]. Despite benefits, these inhibitors have limitations, including toxicity impacting quality of life, unclear overall survival benefits, and the potential for tumor cell resistance [28].

Two new highly selective RET inhibitors — Selpercatinib and Pralsetinib — have shown promising results in MTC patients with a RET mutation, regardless of mutation or the number or type of prior systemic therapies received. Selpercatinib exhibited marked antitumor activity in RET-mutant MTC patients, even in those previously treated with Vandetanib or Cabozantinib [30]. Pralsetinib demonstrated increased RET potency and selectivity, with a positive response in RET-mutant MTC patients [31].

Pheochromocytomas are the first manifestation of MEN2 in 13-27% of cases [32]. The clinical picture is similar to that of the sporadic type (hypertension, headache, increased sweating, and tachycardia). However, MEN2-related pheochromocytomas are more likely to be bilateral when compared with sporadic ones. Primary hyperparathyroidism (PHPT) is typically mild and manifests concurrently or after MTC [1].

The objective of this paper is to provide a comprehensive review of multiple endocrine neoplasia type 2 syndrome, focusing on its genetic basis, clinical manifestations, diagnostic approach, and management strategies.

MATERIALS AND METHODS

Search Strategy

A comprehensive literature search was conducted to identify relevant studies on MEN2 syndrome, focusing on treatment strategies and outcomes. The search strategy encompassed electronic databases and academic repositories from January 1995 to February 2024. The following databases were utilized: PubMed/MEDLINE, Scopus, Web of Science, and Embase.

The search strategy combined Medical Subject Headings (MeSH) terms and free-text keywords, including but not limited to: "Multiple Endocrine Neoplasia Type 2", "MEN2 syndrome", "Medullary Thyroid Carcinoma", "Pheochromocytoma", "Hyperparathyroidism", "RET proto-oncogene", "Genetic testing", "Tyrosine kinase inhibitors", "Thyroidectomy", "Treatment outcomes", "Prophylactic surgery", "Patient outcomes", "Targeted therapy", "Clinical trials".

The search paradigm included combinations of Boolean operators (AND, OR) to refine the search results. Example search strings used were: "Multiple Endocrine Neoplasia Type 2" OR "MEN2 syndrome" AND "treatment" OR "selective RET inhibitor", "Medullary Thyroid Carcinoma" OR "MTC" AND "genetic testing" OR "prophylactic thyroidectomy".

Inclusion criteria encompassed peer-reviewed articles, clinical trials, reviews, and observational studies published in English. Exclusion criteria included non-English articles, case reports, letters, and studies not directly related to MEN2 syndrome treatment or outcomes.

Two independent reviewers screened titles and abstracts for relevance. Full-text articles meeting inclusion criteria underwent detailed review. No third reviewer was involved. Data extraction included study design, participant characteristics, interventions, outcomes, and key findings.

A Journey from Personal Medical Cases to Broader Horizons

We presented the clinical case of a 37-year-old patient diagnosed with MEN2 syndrome, characterized by bilateral benign pheochromocytomas, medullary thyroid carcinoma with recurrent disease, and liver metastasis (Figure 1). In her 20s (in 2009), following a severe hypertensive episode and palpitations, abdominal ultrasonography raised suspicion of an adrenal gland tumor.

Figure 1: The evolution of a patient with MEN2 syndrome over 25 years

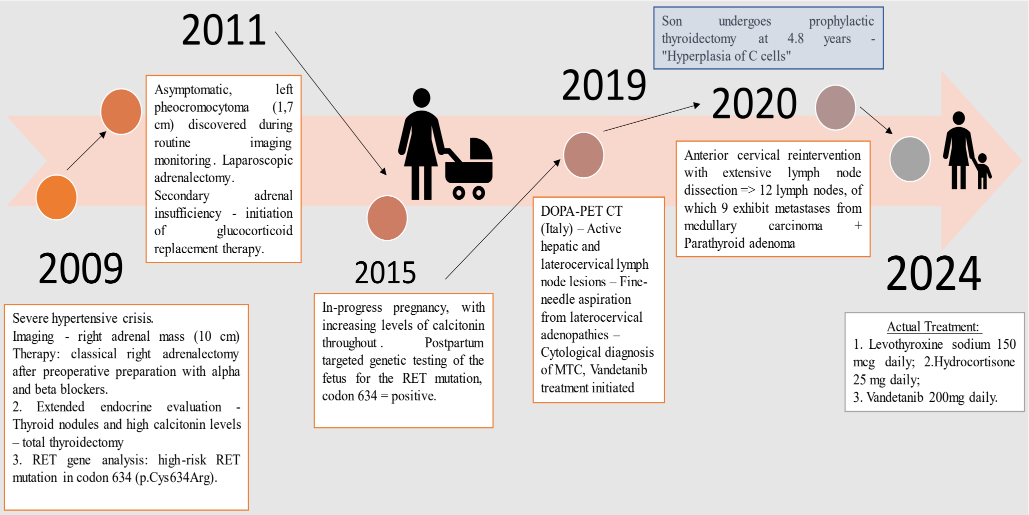

Severe hypertensive crisis.
Imaging - right adrenal mass (10 cm) Therapy: classical right adrenalectomy after preoperative preparation with alpha and beta blockers.
2. Extended endocrine evaluation - Thyroid nodules and high calcitonin levels – total thyroidectomy
3. RET gene analysis: high-risk RET mutation in codon 634 (p.Cys634Arg).

Asymptomatic, left pheocromocytoma (1,7 cm) discovered during routine imaging monitoring. Laparoscopic adrenalectomy.
Secondary adrenal insufficiency - initiation of glucocorticoid replacement therapy.

In-progress pregnancy, with increasing levels of calcitonin throughout. Postpartum targeted genetic testing of the fetus for the RET mutation, codon 634 = positive.

DOPA-PET CT (Italy) – Active hepatic and laterocervical lymph node lesions – Fine-needle aspiration from laterocervical adenopathies – Cytological diagnosis of MTC, Vandetanib treatment initiated

Anterior cervical reintervention with extensive lymph node dissection => 12 lymph nodes, of which 9 exhibit metastases from medullary carcinoma + Parathyroid adenoma

Son undergoes prophylactic thyroidectomy at 4.8 years - "Hyperplasia of C cells"

Actual Treatment:
1. Levothyroxine sodium 150 mcg daily; 2.Hydrocortisone 25 mg daily;
3. Vandetanib 200mg daily.

The diagnosis was confirmed by biochemical tests (increased plasma and urinary metanephrines and normetanephrines levels) and MRI, which visualized an ovoid tumor with a diameter of about 10 cm. Therapy with alpha and beta blockers was initiated for preoperative preparation, and later, a classical right adrenalectomy was performed. Examination of the surgically removed adrenal gland revealed multicentric, predominantly alveolar pheochromocytoma (Figure 2).

Figure 2: Macroscopic appearance of the resected adrenal gland including the pheochromocytoma tumor

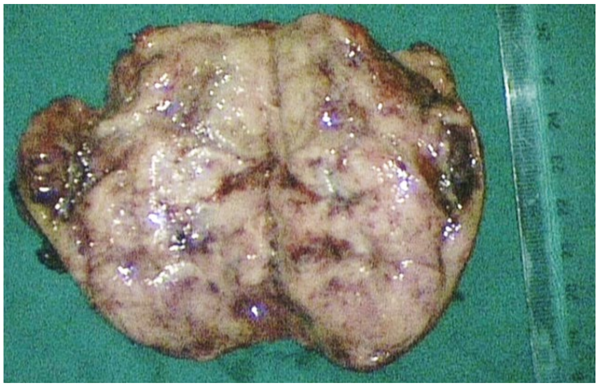

After the discovery of pheochromocytoma, an extensive endocrine evaluation was conducted, revealing high levels of calcitonin (>2000 pg/ml) and multiple heterogeneous thyroid nodules. A neck ultrasound revealed bilateral thyroid nodules. One month following her right adrenalectomy, the patient underwent total thyroidectomy with bilateral dissection of central and lateral lymph nodes of the neck. The thyroid lobes were submitted separately and were enlarged. Well-circumscribed nodules were seen with nests of round cells among fibrous stroma. The histopathological result of the thyroid indicated the presence of thyroid medullary carcinoma.

Given the young age of disease onset and pathological confirmation of the diagnosis of 2 neuroendocrine tumors, suspicion of MEN2 syndrome was raised, and genetic testing to identify RET gene mutations was performed. When the high-risk RET mutation on codon 634 was found, and considering the patient's young age, the potential risks of the condition being passed on to children were discussed with her. While awaiting genetic screening results, biochemical, hormonal, and imaging tests were initiated for all family members. Calcitonin, parathyroid hormone, calcemia, normetanephrine and metanephrine values in plasma and urine were normal, and no thyroid nodules were detected by USG screening among her first-degree relatives. During the years 2009–2011, following the surgical interventions, the patient's condition improved. In 2011, with no characteristic signs, during biannual monitoring, a CT of the abdomen detected a tumor (1.7 cm) on the left adrenal. Laparoscopic left adrenalectomy was performed, and glucocorticoid substitution treatment was initiated.

Between 2012 and 2014, the patient stopped attending endocrinological consultations, and in 2015 she returned for follow-up when she declared she was pregnant and an increase in calcitonin levels was observed during pregnancy. After childbirth, although the calcitonin level did not normalize, it significantly decreased compared to the values at the end of pregnancy. Following birth, the child underwent targeted testing for a mutation in the RET proto-oncogene at codon 634, yielding a positive result (Figure 3). Prophylactic thyroidectomy was performed at the age of 4.8 years. The histopathological result revealed the presence of C-cell hyperplasia.

Figure 3: Targeted testing for RET proto-oncogene indicated the mutation on the level of codon 634-p.Cys634Arg

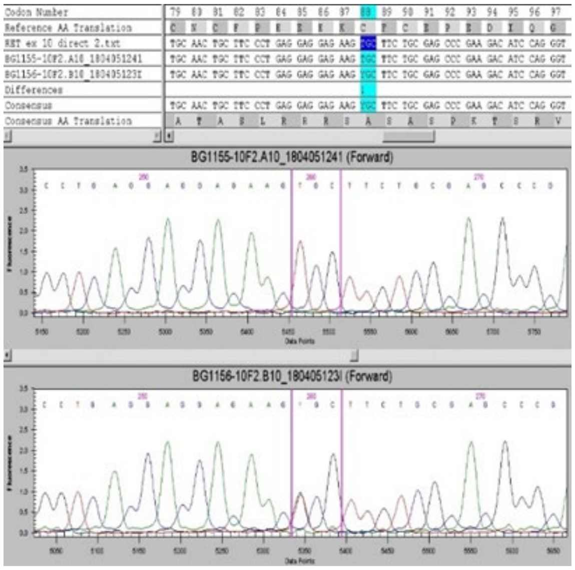
Codon Number
Reference AA Translation
RET ex 10 direct 2.txt
BG1155-10F2.A10_1804051241
BG1156-10F2.B10_1804051231
Differences
Consensus
Consensus AA Translation
BG1155-10F2.A10_1804051241 (Forward)
BG1156-10F2.B10_1804051231 (Forward)
Data Points

Annual reassessments of our patient revealed persistently elevated calcitonin levels. In 2019, due to increasing calcitonin levels, an 18F-DOPA-PET CT scan was conducted in Italy. The results described in the cervical and hepatic regions were suspicious of neoplastic pathology (recurrence) with high absorption of the dopamine tracer. Upon returning to Romania, after performing a fine-needle aspiration from the pointed nodule, the diagnosis of recurrent disease was established.

Recurrent central, bilateral tracheal, and left laterocervical lymphadenectomy was performed in January 2020. Although only one parathyroid gland was removed, the findings suggested parathyroid adenoma. The histopathological diagnosis confirmed the presence of twelve lymph nodes, nine of which exhibited metastases of medullary carcinoma with amyloid stroma.

Following surgical intervention, due to consistently increasing levels of calcitonin and concurrent secondary hepatic determinations, Vandetanib treatment was initiated in October 2020. In subsequent biannual follow-ups, calcitonin levels did not normalize, but there was a significant decrease compared to the values before the initiation of tyrosine kinase inhibitor therapy. Figure 4 presents the evolution of calcitonin levels from 2009 to 2023.

Figure 4: The evolution of the calcitonin level and the main events in the evolution of the patient

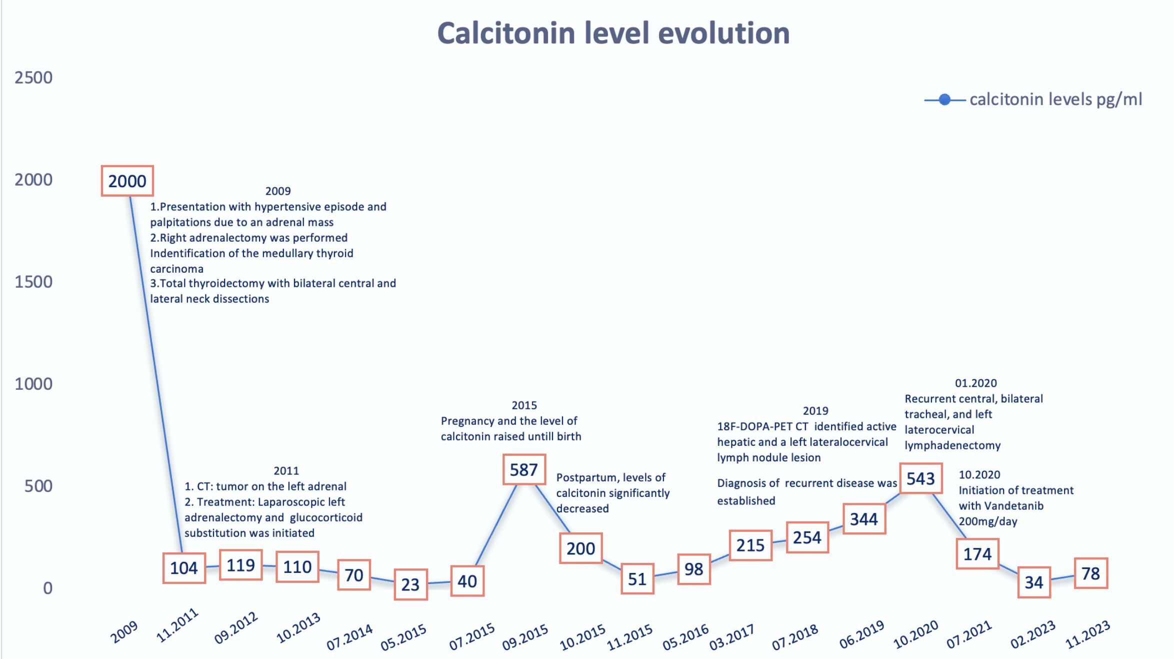

Levels of plasma and urinary metanephrines/ normetanephrines, serum calcium, and parathyroid hormone (PTH) remained stable and within normal ranges throughout the monitoring period after the second adrenal surgical intervention.

The serum PTH level was also normal, including the assessment preceding the surgical re-intervention for the local recurrence of medullary thyroid carcinoma (CMT), during which a parathyroid adenoma was also detected.

Recent laboratory tests have shown stable values, indicating a favorable therapeutic response to the tyrosine kinase inhibitor.

At present, the patient is receiving thyroid and glucocorticoid substitution: 25 mg of cortisone daily and L-thyroxine 150 mcg daily. Additionally, she has been undergoing Vandetanib 200 mg daily treatment since October 2020, for approximately 3.5 years. Calcitonin and CEA values remained stable under this therapy. However, consideration is being given to switching to Selpercatinib, a highly selective RET inhibitor, in the future.

Results of the Narrative Review

In our review, we examined 34 papers to assess their relevance to MEN2 syndrome, and 26 of these papers met our inclusion criteria. Here, we present a summary of the findings from these studies.

The MEN2 syndrome encompasses several neuroendocrine tumors, including medullary thyroid carcinoma (MTC), pheochromocytoma (PCC), and primary hyperparathyroidism (PHPT) [33]. MTC, being the most prevalent and potentially life-threatening component, typically manifests first within the syndrome [31].

The development and severity of tumors in MEN2 syndrome are significantly influenced by specific mutations in the RET gene [34,35]. Prophylactic thyroidectomy is strongly recommended for individuals harboring these mutations, ideally before the onset of MTC [34,35].

Clinical presentations vary, with MTC often presenting as thyroid nodules or symptoms associated with elevated calcitonin levels, while PCC may manifest with symptoms such as palpitations, headache, and diaphoresis. Hyperparathyroidism in MEN2 is frequently asymptomatic and is commonly diagnosed alongside MTC [8].

Surgical removal of MTC and any associated metastases is essential for achieving a favorable outcome [36]. Prognosis in MEN2-related MTC largely hinges on the disease stage at diagnosis [14,37].

Compared to sporadic cases, hereditary MTC typically appears at a younger age and displays distinct pathological features [38–41].

Post-surgery, patients with persistently elevated calcitonin levels require vigilant monitoring using biochemical markers to detect recurrence and assess disease progression effectively [27,42,43].

This narrative review underscores the complex nature of MEN2 syndrome, highlighting its genetic basis, clinical manifestations, therapeutic strategies, and the critical role of ongoing surveillance in managing patient outcomes effectively.

DISCUSSION

MEN2 syndrome consists of several neuroendocrine origin abnormalities: MTC, PCC, and PHPT. Diagnosis of any of these tumors implies correlative analyses and undertaking further investigations for the detection of the other possible associated neoplasia [33]. The case emphasizes the importance of the radical approach to MEN 2 syndrome from both a therapeutic and surgical point of view. The key to the MEN2 phenotype is that the frequency and severity of specific tumors depend on the specific causative mutation (genotype). MEN2 patients with RET mutations require a prophylactic thyroidectomy, that is, ideally, before MTC has developed [34,35]. The timing of this is based on the risk stratification of the RET mutation described by the American Thyroid Association [26].

MTC may present with a thyroid lump or symptoms of calcitonin excess, like diarrhea and flushing. Pheochromocytomas may present with the classic triad of palpitations, headache, and diaphoresis, but can be asymptomatic upon diagnosis. They often exhibit bilateral involvement, and unilateral presentations frequently lead to the development of a contralateral pheochromocytoma within 10 years. Pheochromocytomas in MEN2 syndrome are seldom malignant and the prognosis in MEN2 is primarily influenced by MTC, the most life-threatening component. Hyperparathyroidism in MEN2 is generally without symptoms and is diagnosed concurrently with MTC [8].

One dermatological manifestation associated with MEN2 syndrome is cutaneous lichen amyloidosis (CLA), frequently co-existing with pheochromocytomas. Additionally, reported cutaneous conditions linked to MEN2 comprise dermal hyperplasia and multiple sclerosing fibromas [14].

MTC develops in almost all MEN 2 cases and is usually the first manifestation of the syndrome. Before MTC develops, it is preceded by thyroid C-cell hyperplasia. MEN 2 MTC is typically multifocal and often bilateral [31]. Only a few MEN 2 patients are symptomatic at MTC diagnosis. A recent nationwide population-based study reported that only 15% of the MEN 2 cases with MTC had clinically suggestive characteristics at diagnosis, while the remaining 85% were diagnosed by screening [44]. MTC can result in fatal outcomes within the spectrum of MEN2 when managed ineffectively or remains unrecognized. Because MTC frequently involves the cervical lymph nodes, lymph node dissection, in addition to total thyroidectomy is required [36]. Complete surgical resection of the thyroid tumor and any locoregional metastases is the only curative option for locoregional MTC. Therefore, early diagnosis and detection of MTC are very important for prognosis [38].

There are several key differences in the presentation of hereditary compared to sporadic MTC [39]. Hereditary MTC presents at a significantly younger age, even after excluding patients identified via genetic screening. It is usually diagnosed in the 2nd - 3rd decade [45]. Depending on the specific RET variant, the mean age at diagnosis of normal thyroid/C cell hyperplasia ranges from 5 to 11 years, while the mean age at diagnosis of node-negative MTC ranges from 19 to 33 years [46][40]. In a large cohort of patients with MTC, Saltiki et al. found that compared to sporadic MTC, hereditary MTC patients had significantly more microscopic disease, multifocality, C-cell hyperplasia, and smaller tumor size [41]. Sporadic MTC patients had significantly more capsular invasion, soft tissue invasion, and distant metastases than hereditary MTC patients [42]. According to research, there is a tendency towards a more unfavorable progression in the case of sporadic MTC, and the presented case supports this notion, highlighting the hepatic metastases 10 years after the initial diagnosis of MTC.

In patients with persistently high levels of calcitonin after surgery, biochemical markers with calculated doubling times have been used to monitor MTC recurrence, progression, and survival. Barbet et al. (2005) found in multivariate analysis that calcitonin and carcinoembryonic antigen (CEA) doubling times are independent risk factors for survival. When calcitonin doubling time was 2 years, 100% of patients were alive at 10 years [43]. Some limitations of calcitonin include inter-assay variability, a concentration-dependent half-life, and rapid degradation at room temperature or refrigeration. With the development of increasingly reliable assays, investigation into other biomarkers continues [42].

Survival for patients with MEN2-related MTC treated surgically is dependent upon the disease stage at diagnosis [47]. Post-resection, all patients with MEN2 should be followed with surveillance for recurrent disease. In addition, any patients with MTC, or a constellation of two MEN2 tumors, should undergo RET genetic testing. Children of MEN2 patients should have genetic testing performed within the first 6 months of life [48,49]. Calcitonin and CEA are markers used to assess initial disease burden and followed post-resection to assess disease progression in patients with metastatic MTC [27].

Distance metastasis of MTC depends on tumor stage and calcitonin level. Liver metastases are observed in 13% of MTC with a serum Calcitonin level of more than 400 pg/ml. Liver metastases are generally hypervascular and exhibit variable atypical imaging appearances, therefore, the differentiation between them and other vascular hepatic lesions, especially hemangioma, is challenging. State-of-the-art 68GaDOTA-somatostatin analogue-PET/CT improves specificity in detecting the metastases of NET, especially in liver and lymph nodes; nevertheless, this imaging technique is not yet a routine protocol [50,51].

Before thyroidectomy, the presence of pheochromocytoma should be ruled out by appropriate biochemical analysis in all MEN 2 and MEN 3 patients [52]. If a pheochromocytoma is detected, adrenalectomy should be performed before thyroidectomy or other surgery to avoid severe intraoperative complications. Laparoscopic adrenalectomy is the gold standard in the treatment of pheochromocytoma. Limitations only arise because of technical difficulties or tumor size. Pheochromocytoma is an infrequent initial presentation of MEN2 and is typically recognized during screening evaluations in MEN2 patients [53]. It typically becomes apparent approximately a decade following the onset of MTC or C cell hyperplasia [37]. In our case, the patient presented initially with unilateral, benign left pheochromocytoma, whose symptoms manifested before the diagnosis of medullary thyroid carcinoma. Due to the large dimensions of the adrenal adenoma (10 cm), surgery was performed through the classical approach. When contralateral adrenal involvement was detected 2 years later, the tumor dimensions (1.7 cm) allowed for a laparoscopic approach.

According to the literature, pheochromocytoma has a variable penetrance in MEN2A patients, depending on the mutation of RET [54]. The highest frequency is found in patients with a mutation of codon 634 [55]. It is frequently bilateral, multicentric, and not always synchronous. Patients require alpha blockade, with or without beta-blockade, before and during surgery.

Finally, in MEN2 patients with parathyroid adenomas, open neck exploration is recommended with the removal of enlarged parathyroid glands only, rather than the more aggressive approach adopted for parathyroid tumors in MEN1 patients [56]. Recurrence is uncommon [8]. The clinical manifestations of MEN2-related PHPT are similar to sporadic cases, including nephrolithiasis, osteopenia or osteoporosis. Although parathyroid hyperplasia is more common in MEN syndromes, in the presented case, the discovery of a single parathyroid adenoma was made incidentally, intraoperatively, in the context of the surgical intervention for the removal of metastatic lateral cervical lymph nodes.

What draws our attention and raises questions in this case, are the markedly elevated levels of calcitonin in the last trimester of pregnancy, with a sudden decrease in the last 2 weeks of pregnancy and postpartum. Studies in the literature regarding changes in calcitonin levels throughout pregnancy among patients with a personal history of MEN2 syndrome are limited.

Considering the rare cases described in the literature of an association between MEN2 and pregnancy, we can take this as a limitation of the current study. We can also add the lack of specific guidelines to help manage such a case where elevated calcitonin values are recorded during pregnancy.

As directions for future research, we will consider the continued development of generations of MTTs, which have minimal toxicity and greater specificity for mutated RET and whose purpose is to either cure patients with advanced MTC or change their progressive illness into a chronic, long-lasting.

That is why, in the future, we will be committed to forming and monitoring further studies with larger sample sizes and long-term follow-up to draw definitive conclusions.

CONCLUSIONS

MEN2 is a rare hereditary disorder distinguished by MTC, either unilateral or bilateral pheochromocytoma and primary parathyroid hyperplasia. The risk of developing MTC over one's lifetime is almost 100%, with varying expressions of other symptoms. Neglected or undetected, MTC within the context of MEN2 can lead to fatal outcomes.

Yet, timely identification and thorough diagnostic protocols enable prompt interventions, thereby reducing the associated morbidity and mortality linked to MEN2.

Imaging check-ups in combination with annual monitoring of calcitonin, CEA, metanephrines/normetanephrines, and parathyroid hormone in a patient with MEN 2 syndrome are practical ways to supervise the case, make timely decisions about surgical intervention, and prevent complications.

Genetic testing of MEN2 families and prophylactic thyroidectomy are mandatory. In cases where there is a family history of the RET mutation gene, a proactive approach to performing thyroidectomy significantly reduces the risk of complications and death, as evidenced by the positive outcome observed in our patient's son.

Even in the face of repeated endocrine challenges and recurring illnesses, the potential for utilizing tyrosine kinase inhibitor therapy stands as a robust choice in managing tumors associated with MEN2 syndrome.

By intertwining our clinical observations with established literature, we navigate the complexities of MEN 2 syndrome management. This blend of personalized care and evidence-based medicine sheds light on the nuanced treatment of MEN 2 syndrome. Over 25 years of patient follow-up, we witness sustained health and improved quality of life. As we evolve alongside advancements in targeted systemic therapies, our approach remains grounded in comprehensive understanding and patient-centered care.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

This research received no external funding. All the images are original, not previously published and not collected from other sources.

Authors' contribution

Conceptualization, D.T., and D.P.; methodology, D.T., and D.P.; software, D.T., and A.M.; validation, A.M., and I.S; formal analysis, D.P.; investigation, D.T.; resources, A.A.; data curation, S.P.; writing—original draft preparation, D.T.; writing—review and editing, D.T.; visualization, A.M..; supervision, M.G.; project administration, I.S..

Patient consent for publication

The patient has given written informed consent to publish this paper, including permission for the details/images to be available on the Internet and viewable by the general public.

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NARRATIVE REVIEW

Structural and Functional Imaging in Epilepsy – A Narrative Review

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Abstract: *The primary objective of this narrative review is to evaluate the most frequently recommended structural and functional imaging methods in managing epilepsy cases as effectively as possible from a neurologist's perspective. This review examines the background of current practice, from the beginnings of neuroimaging by computer tomography to the newly introduced methods and their combination, which helps diagnose and monitor these patients. The neuroimaging investigations required for the presurgical evaluation of patients with epilepsy are presented, and recommendations for specific MRI protocols are provided. The clinical use of functional imaging methods is also considered, and correlations between clinical aspects and specific imaging methods are explored, based on the retrieved data in the literature. In conclusion, this review emphasizes using functional and structural imaging methods, guided by clinical evidence, for better therapeutic management in patients with epilepsy.*

Keywords: *Positron Emission Tomography, Single-photon-emission computed tomography, medically refractory epilepsy, HARNESS protocol, MRI*

INTRODUCTION

Epilepsy is one of the most common neurological pathologies globally, with an incidence of 1-2% of the population [1]. According to the 2017 International League Against Epilepsy (ILAE) classification, seizures are stratified into focal, generalized, or unknown onset [2].

Differentiating focal onset from generalized onset seizures is sometimes very difficult because patients may present with bilateral tonic-clonic seizures without a clear description of the initial symptomatology and with inconclusive electro-encephalography (EEG) data. Neuroimaging is crucial because many etiologies can be diagnosed with its help. An epileptogenic area discovered by Magnetic Resonance Imaging (MRI) can support the focal onset of seizures and help for better therapeutic management, either surgical or pharmacological (Figure 1). Unfortunately, about one-third of epilepsy patients become drug-resistant [3]. In this situation, it is necessary to delimitate the epileptogenic area and surgically remove it [4]. The epileptogenic zone is defined by Lüders et al. as "the minimum amount of cortex that must be resected (inactivated or completely disconnected) to produce seizure freedom" [5].

Figure 1: Some non-invasive methods

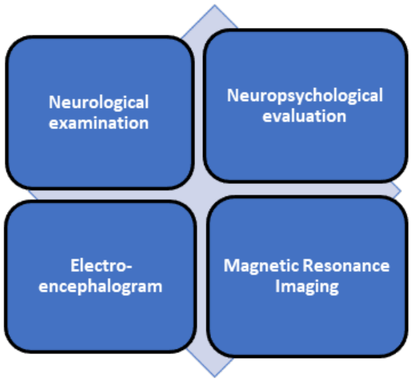

A presurgical evaluation is necessary to decide which candidates are suitable. All patients with pharmacoresistant epilepsy should be evaluated for epilepsy surgery. This may cause or increase the risk of sudden unexpected death in epilepsy (SUDEP), which is why patients should be encouraged to have better therapeutic compliance [6]. This is also helped by good communication between doctor and patient; the better informed the patient is, the more chances there are to provide appropriate therapeutic management [7].

Regrettably, quite a significant number of patients are not considered or cannot access such a program. This happens mainly in underdeveloped or developing countries due to the need for more specialized medical personnel in this field [8].

In many situations, more than these are needed, and more advanced investigations are required (Figure 2) [9].

Figure 2: Advanced investigations

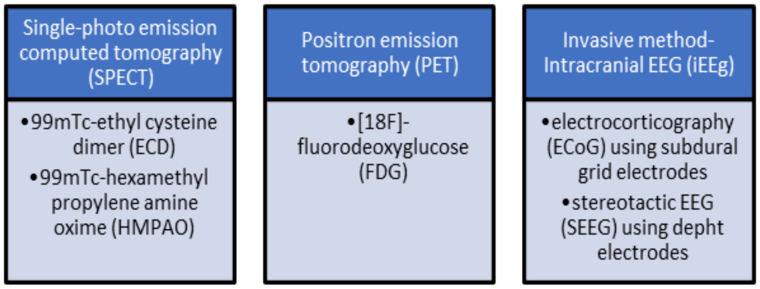

Single-photo emission computed tomography (SPECT)	Positron emission tomography (PET)	Invasive method- Intracranial EEG (iEEg)
•99mTc-ethyl cysteine dimer (ECD) •99mTc-hexamethyl propylene amine oxime (HMPAO)	•[18F]-fluorodeoxyglucose (FDG)	•electrocorticography (ECoG) using subdural grid electrodes •stereotactic EEG (SEEG) using depht electrodes

These investigations aim to determine the patient's chances of remaining seizure-free postoperatively and, of course, to minimize the post-surgery risks and functional dysfunction. After these steps have been taken, the next one would be to establish a few essential things: the symptomatic area, the irritative area, the ictal area, the epileptogenic area, and the eloquent area [10].

OBJECTIVE

This article aims to summarize the role of structural and functional imaging of the brain by use of CT, MRI, SPECT, FDG PET, and non-FDG PET radio-tracers in epilepsy from a multidisciplinary perspective. Our search strategy on PubMed included terms related to epilepsy, neuroimaging in epilepsy including PET and SPECT, and HARNESS protocol MRI, in studies published from 1995 until January 2024. Only papers published in English were selected.

RESULTS

A number of 44 primary and secondary reports have been selected to be included in the review. These sources explored both structural brain imaging techniques and functional neuroimaging investigations focused on diagnosing and monitoring epilepsy.

STRUCTURAL BRAIN IMAGING

Magnetic Resonance Imaging (MRI)

According to ILAE publications, a brain MRI should be performed after the first seizure to determine therapeutic attitude (Figure 3). It has a high specificity for detecting possible cortical malformations, including cortical dysplasia, mesiotemporal sclerosis, and other structural changes that have an increased epileptogenic effect and may also increase the risk of treatment resistance. As for epileptic syndromes, there is little evidence to recommend advanced imaging, but if abnormal neurological development, cognitive decline, treatment resistance, or focal interictal spikes occur, it should be considered and performed [11].

The ILAE Neuroimaging Task Force has proposed a set of acquisition sequences known as the optimal protocol for epilepsy, the "HARNESS"-MRI protocol (Bernasconi et al., 2019 – Figure 4) [11]. The optional sequences are Gadolinium-enhanced MR and Susceptibility-weighted imaging (SWI) [12].

The role of structural MRI in detecting subtle structural abnormalities

Focal cortical dysplasia (FCD) is one of the most frequent structural changes requiring advanced imaging evaluation and the third most common etiology of medically intractable seizures in adults [13]. There are three types; the first type is associated with a late onset, in adulthood, with changes in the temporal lobe. Clinical symptoms are more severe in type 2, especially in children with predominantly frontal lobe changes. FCD type 2 correlates with other tumors like hippocampal sclerosis, tumor, vascular malformation, or attained pathology during early life [13]. FCD type 3 refers to malformation connected with different cortical lamination abnormalities with a main lesion within the same area/lobe. In a significant number of cases, especially FCD type 1, it may remain undetected.

Additional curvilinear reformatting and morphometric analysis of T1 and T2-FLAIR sequences may be helpful in this situation. For detecting these abnormalities, a satisfactory specificity of 90%, accompanied by a sensitivity of 50%, was observed only by FLAIR sequencing [14-17]. To avoid these limitations, an excellent therapeutic approach is to combine MRI with functional imaging, PET, or SPECT [18].

Another standard structural change (Figure 5) is temporal lobe epilepsy, particularly mesiotemporal sclerosis (MTLE). It refers to damage to the mesial or inner portion of the temporal lobe, with a focus on the hippocampus. This can result from various reasons, including infection, head trauma, or other causes [19]. Symptomatology is polymorphic but may include altered consciousness, memory disturbances, and emotional changes [20]. Patients usually have an increased risk of developing drug resistance, so it is important to refer them to a specialized center. Brain MRI is a central and essential method of diagnosis and assessment. It can show a certain level of atrophy in this area, increased T2 signal, and loss of hippocampal architecture [21,22].

Figure 3: Indications for magnetic resonance imaging (MRI) in patients with epilepsy [22]

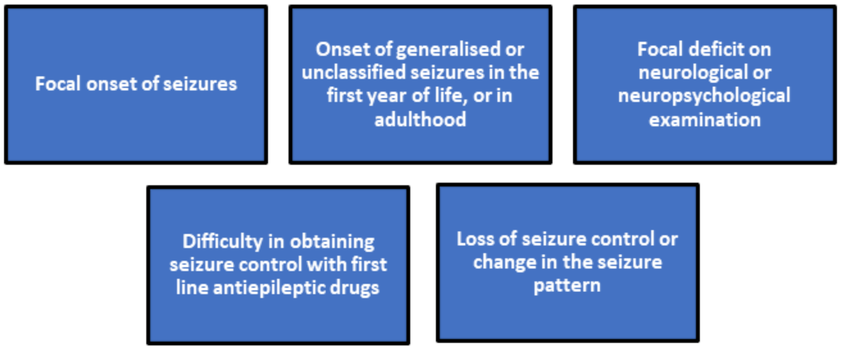
Focal onset of seizures
Onset of generalised or unclassified seizures in the first year of life, or in adulthood
Focal deficit on neurological or neuropsychological examination
Difficulty in obtaining seizure control with first line antiepileptic drugs
Loss of seizure control or change in the seizure pattern

Figure 4: Required sequences of the HARNESS-MRI protocol [11]

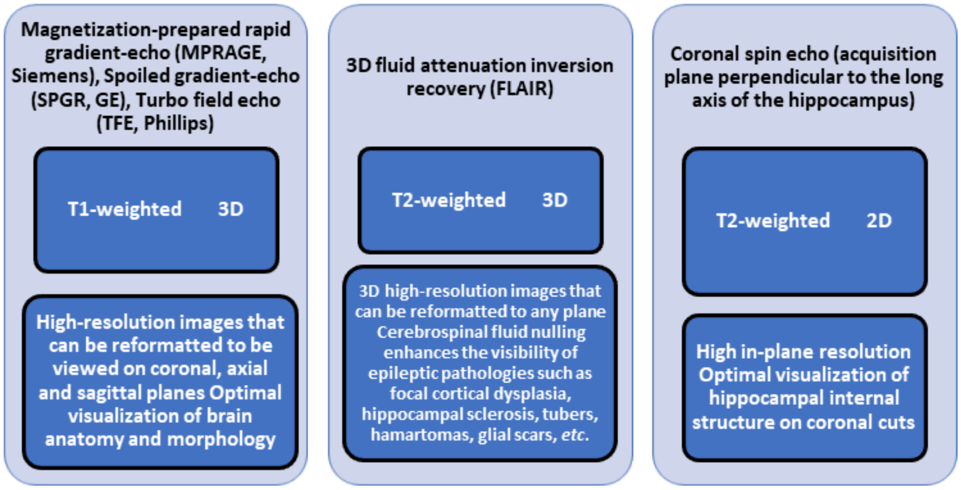
Magnetization-prepared rapid gradient-echo (MPRAGE, Siemens), Spoiled gradient-echo (SPGR, GE), Turbo field echo (TFE, Phillips)
T1-weighted 3D
High-resolution images that can be reformatted to be viewed on coronal, axial and sagittal planes Optimal visualization of brain anatomy and morphology
3D fluid attenuation inversion recovery (FLAIR)
T2-weighted 3D
3D high-resolution images that can be reformatted to any plane Cerebrospinal fluid nulling enhances the visibility of epileptic pathologies such as focal cortical dysplasia, hippocampal sclerosis, tubers, hamartomas, glial scars, etc.
Coronal spin echo (acquisition plane perpendicular to the long axis of the hippocampus)
T2-weighted 2D
High in-plane resolution Optimal visualization of hippocampal internal structure on coronal cuts

Figure 5: Other common epileptic pathologies

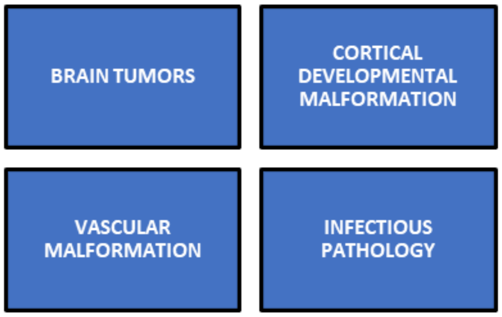
BRAIN TUMORS
CORTICAL DEVELOPMENTAL MALFORMATION
VASCULAR MALFORMATION
INFECTIOUS PATHOLOGY

Computed tomography (CT)

CT can play an essential role in the assessment of patients with epilepsy in certain situations. However, in general, CT is not used as the first option for diagnosing epilepsy but rather to rule out other causes of seizures or to identify possible brain damage. According to the American Academy of Neurology (A.A.N.), this investigation should be performed and recommended to all patients presenting with a first epileptic seizure, especially if they have an abnormal neurological examination or focal onset of the seizure [23]. The role of CT in epilepsy may refer to:

- Exclusion of other causes: CT may be performed to exclude brain lesions or abnormalities that could cause seizures. This may include tumors, vascular malformations, or traumatic injuries.

- Post-traumatic assessment: if the patient has suffered a severe head injury, CT may be used to assess any brain injury or bleeding that could be involved in triggering seizures.

- Preoperative guidance: in cases where epilepsy is resistant to treatment and surgery is considered an option, CT can guide the surgeon in pinpointing the exact brain area responsible for the seizures.

However, for a more detailed evaluation of the brain in epilepsy cases, other imaging techniques, such as MRIs, are usually preferred [24].

FUNCTIONAL IMAGING

Functional imaging (Figure 6) can help us when there are situations where structural imaging is not sufficient or there are negative results (negative MRI) to identify and demarcate the epileptogenic focus, or when multiple epileptogenic foci are detected [25].

A large proportion of patients with drug-resistant epilepsy, almost 50%, have no evident lesion found on brain MRI. In this situation, we can speak of a negative MRI. However, there are cases in which a second interpretation of the same investigation, accompanied by additional information, revealed the lesion [26]. In this case, it should be checked if the HARNESS protocol was used or if there are artifacts that require the imaging to be repeated.

Functional imaging is remarkable in that epileptogenic brain tissue has a different metabolism and electrophysiology than normal tissue. These regions of difference are then visually expressed and projected into existing structural images, usually MRI [27].

Figure 6: The most widely used functional imaging in epilepsy

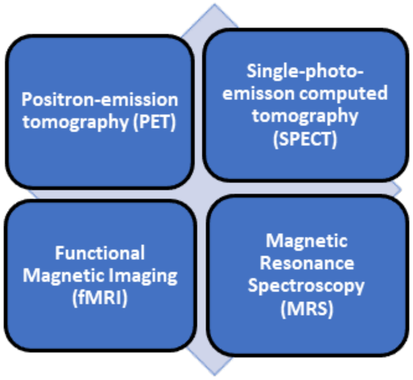
Positron-emission tomography (PET)
Single-photo-emisson computed tomography (SPECT)
Functional Magnetic Imaging (fMRI)
Magnetic Resonance Spectroscopy (MRS)

Functional MRIs can be helpful when the patient is asked to do consecutive, usually simple, tasks. During this time, there may be changes in T2 sequences. This is why this investigation is recommended in some presurgical evaluations to detect hemispheric language dominance, especially when a resection of the dominant temporal lobe is planned [12]. Other indications include motor, somatosensory, or visual cortex mapping. It is beneficial in situations where there is the proximity of tumors or FCD to eloquent areas [28].

In general, areas with changes in contrast-dependent blood oxygenation levels are secondary changes in the ratio of deoxy- to oxyhemoglobin that occur during the performance of cognitive, motor, or other tasks, which permits us to map the neural pathways implicated in the delivery of these tasks. One of the most critical applications of this investigation is the localization and lateralization of cognitive function. A crucial step before surgery is to reduce the risk of causing a significant impairment. This is why a cooperative patient who can smoothly execute the indications and tasks is needed. This investigation is not recommended for patients who are claustrophobic, who have metal devices, or who are not able to perform tasks. For preoperative fMRI, it is advisable to choose appropriate procedures depending on the area of the brain to be resected [29].

In recent years, there has been a growing association between simultaneous electroencephalography monitoring and fMRI [30]. This method makes use of EEG changes, in particular, interictal spikes and discharges that may be associated with hemodynamic changes. This can have a significant impact on the therapeutic decision and case management [31].

Positron Emission Tomography

PET examination of the brain is used to observe a variety of images and biological phenomena such as blood flow, metabolism, protein and DNA synthesis, and more. This happens after injecting a radiolabelled compound.

Radiotracers are imprinted with a positron-emitting isotope manufactured by a cyclotron. After administration, they spread to different areas of the body, even to the brain, crossing the blood-brain barrier and gathering in the tissues to reveal the biological impact of the radiotracer. The mechanism of positron detection is via the coincidence system, which allows PET to have spatial resolution in the mm range. This also differentiates it from SPECT, which has a weaker resolution. Even though there are several PET Radiotracers, the most commonly used for epilepsy is FDG (18F-fluorodeoxyglucose) [32].

FDG-PET imaging

As mentioned above, when MRI and video EEG monitoring are not relevant, we can use PET, and the most common tracer in epilepsy is FDG-PET. This measures glucose metabolism linked to the synaptic and neuronal activity of the cerebral tissue. Even if there is not enough evidence for a precise time window, the indicated postictal period differs depending on the type of seizure. For focal aware seizures, interictal FDG-PET should be performed at least after 24h and 48h after focal impaired awareness and focal to bilateral tonic-clonic seizures. This is important because it can complicate the outcome of investigations. This can be misinterpreted, leading to erroneous lateralization because it should be interictal, and the basis of the interpretation is finding the ratio of hypometabolism to the contralateral side [33]. Another factor that can have an impact by lowering the rate of glucose metabolism is the use of some drugs, for example, barbiturates, valproic acid, phenytoin, and carbamazepine [34].

Some patients need sedation, which can be done 10 minutes before the examination. Several studies have shown that propofol sedation can reduce the rate of glucose metabolism and the rate of cerebral blood flow, which need to be taken into account when these procedures are required [35].

Usually, in interictal periods, FDG-PET shows hypometabolism in epileptogenic areas. This is directly related to seizure duration, frequency, and severity. An increased frequency also leads to increased hypometabolism area and vice versa [36]. There are also some particularities, such as in some cases of focal epilepsy, frontal or temporal, hypometabolism may be present in the ipsilateral thalamus, or contralateral cerebellar hypometabolism may be found especially in frontal lobe epilepsy [36].

In order to avoid false results that could mislead us by hypo or hypermetabolism, a proper strategy is to register the EEG during the 30-40-minute period of FDG capture. If this is not feasible, an epileptologist can be called in to follow the patient. EEG monitoring can be stopped once the patient enters the scanner.

In the case of temporal lobe epilepsies, the specificity and sensitivity are over 80%, but a unique contribution is made in situations where there are dual-pathology cases (coexistence of medial temporal and neocortical seizure foci) [37]. The localization of epileptogenic areas is diminished when dealing with frontal lobe epilepsy especially in some cases of occipital epilepsy [38].

Another essential feature is that FDG-PET can help assess the functionality of the remaining unresected brain tissue when an epileptogenic lesion has been taken into account for surgical resection. The post-surgical neurocognitive status is essential and also depends on the integrity and functionality of the remaining brain tissue.

Also, this type of patient can be associated with other comorbidities, and among the most common are cognitive disorders and depression that contribute to a decrease in quality of life. It has been shown that those with depression and epilepsy have a predisposition to ipsilateral or bifrontal hypometabolism [39]. PET studies in patients with temporal lobe epilepsy using a serotonin transporter ligand as a receptor have shown a decrease in its activity. This supports the hypothesis of using medication with selective serotonin reuptake blockers in patients with epilepsy and depression [40,41]. Memory deficits are among the frequent complaints of these patients. In those with occipital lobe seizures, deficits in verbal memory and executive function have been correlated with left temporal lobe hypometabolism. Patients with postictal amnesia were associated with disturbances in the left mid-temporal metabolism [42]. Under specific circumstances, other radio transmitters can be used, including [^{11}C] flumazenil, which has been shown to have a higher sensitivity to temporal lobe epilepsy, particularly hippocampal sclerosis [43].

SPECT

This type of imaging is used to measure cerebral blood flow in vivo. Although brain perfusion and metabolism are not the same type of process, they are connected in most situations [44,45]. The difference between FDG-PET and SPECT is the lower spatial but higher temporal resolution, leading to the detection of the seizure onset zone. Cerebral blood flow is strongly correlated with neuronal activity, so during the ictal phase, they increase concomitantly. Therefore, for an accurate interpretation of the results, it is necessary to precisely measure the dissimilarity between ictal and interictal SPECT results (which are interrelated with CT or MRI) [46]. The areas of hyperperfusion in the ictal phase are considered to have the most significant potential for delimiting the epileptogenic zone.

In general, interictal SPECT has a lower sensitivity compared to ictal SPECT. In the interictal period, hypoperfusion or normal perfusion may be observed in epileptogenic areas. Even when hypoperfusion is present, it can be of a relatively low intensity, making it quite hard to distinguish from normal tissue. Its primary role is to support the ictal assessment using SISCOM (subtraction ictal SPECT connected to MR imaging; that is, the interictal SPECT images are retrieved from the ictal photos, and the results are displayed on cosigned MR images), by furnishing a baseline blood flow [47].

However, logistics is not an advantage. The patient must be under constant medical supervision, with EEG monitoring and possibly tapering of antiepileptic medication to increase the possibility of a new seizure recurrence. The radiotracer should be placed at the patient's bedside for faster administration. So that immediately at the onset of a seizure, either announced by the patient or by clinical or EEG changes, the radiotracer can be injected. This is important because focal seizures, as we know, bilateralize quite quickly, and a late injection may miss the initial focus. Nevertheless, SPECT remains a crucial investigation in MRI-negative patients [48].

Magnetic resonance spectroscopy (MRS)

This investigation can measure different metabolites. Among those that are detectable are N-acetyl aspartate (NAA), choline, creatine, lactate, glutamate, and gamma-aminobutyric acid (GABA) [49].

Numerous studies examining various brain pathologies have found that NAA, which is predominantly found in neuronal mitochondria, is the most significant informative measure of neuronal function. On the other hand, creatine is found as well in neurons as in astrocytes, but the highest concentration is in the astrocytes [50]. Therefore, when considering the integrity of neuronal function, the NAA/creatine ratio is used. In epileptic patients with cortical anomalies, it has been shown that NAA values are specific to the epileptogenic area and have average values in patients with therapeutically controlled seizures. Even in those patients who have recurrent seizures but with non-epileptogenic structural abnormalities, there's a high likelihood of changing NAA levels [50].

Unlike other investigations, proton MRS does not typically include the whole brain in measurements, and sometimes, a few significant voxels are sampled with proton MRS. A relatively low signal-to-noise ratio is calculated, and this, together with the extended time taken to obtain the spectra, makes the technique of somewhat limited clinical use.

MRS may play a significant role in temporal lobe epilepsy because it is better defined clinically and anatomically. However, the same cannot be said for neocortical or extratemporal epilepsies. In this case, we have a much larger brain volume with the potential to trigger seizures with different propagation paths [51]. Other trials targeting MTLE groups have shown that bilateral metabolic alterations in these patients have predictive value for postoperative outcomes. So, those with significant bilateral or contralateral lesion metabolic changes had a much worse postoperative outcome through poor seizure control [52,53].

Results were compared with EEG localization and surgical results, showing that reduced NAA signal intensity can lateralize the epileptogenic area and those with focal epilepsy, especially MTLE. Nevertheless, these changes are often bilateral in patients with MTLE. After successful surgery, the NAA concentration can normalize [54].

CONCLUSIONS

Neuroimaging plays a vital role in the assessment and management of patients with epileptic seizures. Magnetic resonance imaging can lead to the detection of the etiology of focal seizures and the demonstration of anatomical changes associated with epileptic seizures. Even if its increased efficiency has been demonstrated in some situations, such as in the case of negative MRI or pharmacoresistant epilepsies, other investigations are helpful. One of them is PET, a functional interictal technique that can help us identify the onset of these seizures due to metabolic changes. SPECT is another functional but ictal investigation with preoperative guidance.

SPECT and PET imaging modalities play a vital role in the presurgical evaluation of patients with epilepsy, especially those with negative MRIs or multiple foci on EEG monitoring. Another essential feature is the assessment of the integrity of the rest of the brain tissue, which provides information about the possible pathogenesis of neurocognitive or behavioral disorders. It is important to note and underline that these are not diagnostic tools and should not be used to support a diagnosis of epilepsy.

The aim of these new imaging techniques, together with a multidisciplinary perspective on the presurgical assessment of patients with epilepsy, is to have increasingly better outcomes, with reduced post-surgical neurological deficits and improved quality of life.

The combination of structural and functional methods has been used in multimodal learning models to discover the seizure onset zone and assimilate the more accurate mechanisms involved in structural and functional abnormalities in a very often encountered pathology, i.e., epilepsy.

Authors' contribution

Conceptualization, A.M.M., C.A.S., and R.G.; methodology, O.V., R.I.D., M.M., and O.M.S.; validation, A.M.M., C.A.S., M.M., and R.G.; resources, O.V., R.I.D., and O.M.S.; data curation, A.M.M., C.A.S., M.M., and R.G.; writing—original draft preparation, A.M.M., C.A.S., and R.G.; writ-ing—review and editing, O.V., R.I.D., M.M., and O.M.S.; visualization, O.V., R.I.D., and O.M.S.; supervision, A.M.M., C.A.S., and R.G.; project administration, C.A.S. All authors have read and agreed to the published version of the manuscript reported.

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REVIEW

Comprehensive Review and Update on Multiple Organ Dysfunction Syndrome in Burn Patients: Pathophysiology, Risk Factors, and Management Strategies

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Abstract: *Burn injuries are among the most severe forms of trauma, often leading to multiple organ dysfunction syndrome (MODS), a major contributor to morbidity and mortality in burn patients. This review examines the pathophysiological mechanisms, clinical manifestations, and management strategies associated with MODS in burn patients. Following a severe burn injury, the systemic inflammatory response and subsequent release of pro-inflammatory cytokines precipitate dysfunction across various organ systems. Early recognition and aggressive management of MODS, including fluid resuscitation, infection control, and organ support, are crucial for improving survival outcomes. A comprehensive understanding of the multifactorial etiology and interrelated nature of organ dysfunction in burn patients is essential for developing targeted therapies and optimizing patient care. This review provides a current overview of the topic. In the coming years, new insights into the pathophysiological mechanisms are expected to enhance our comprehension of the subject further.*

Keywords: *burn injury, systemic inflammatory response, multiple organ dysfunction syndrome, cardiac dysfunction, respiratory dysfunction, acute renal failure, hepatic dysfunction, encephalopathy, coagulopathy, sepsis*

INTRODUCTION

Burn injuries are a major threat in military environments, historically present for over 5,000 years. Modern weapons have heightened the risk, causing significant casualties among soldiers and civilians. Advanced weapons, such as napalm, white phosphorus, anti-armor weapons, and fuel-air explosives, pose a high risk of causing severe burns. While protective gear like Kevlar vests has increased survival rates by protecting vital areas, exposed body parts still suffer from serious burns and shrapnel injuries. Advances in battlefield medical care have reduced the fatality rate of combat injuries, but severe burns remain complicated and require long-term treatment and rehabilitation. [1]

In the best circumstances, optimal treatment of war-related burn injuries can salvage about 50% of patients with burns covering 60-70% of their total body surface area (TBSA). Limited resources necessitate prioritizing care for those who will benefit the most, focusing on patients with burns covering 20-70% TBSA. In battlefield triage with resource constraints, patients with burns over 80% TBSA may receive palliative care, while those with burns of 20% or less and who are stable may have care delayed. Triage adjustments should consider coexisting inhalation or mechanical injuries, which reduce the treatment threshold by 10%. Burns on the hands, face, feet, and perineum may require increased care despite a lower TBSA burn percentage. [2]

Multiple organ dysfunction syndrome (MODS) is a severe complication in burn patients, particularly those with extensive burns. Severe burns trigger a systemic inflammatory response syndrome (SIRS), leading to widespread inflammation and potential organ failure. Extensive fluid loss from burn wounds can cause hypovolemic shock, impairing organ perfusion and function. Burn wounds are highly susceptible to infection, which can lead to sepsis and subsequent organ dysfunction. MODS significantly increases morbidity and mortality in burn patients, necessitating a multidisciplinary approach to manage and support affected organ systems effectively. [3-5]

In the early 1970s, Baue characterized the syndrome known as progressive or sequential multiple organ failure [6]. The term "multiple organ dysfunction syndrome" was introduced in 1991 during the Consensus Conference of the American College of Chest Physicians and the Society of Critical Care Medicine and it was defined as the presence of compromised organ function necessitating therapeutic interventions for maintaining homeostasis. The conference also addressed the systemic inflammatory response syndrome (SIRS) to delineate the clinical and paraclinical manifestations of systemic inflammation [7]. Later on, several scoring systems were devised to assess multiple organ dysfunction, including the MOD (Multiple Organ Dysfunction) score [8], the SOFA (Sequential Organ Failure Assessment) score [9], and the LODS (Logistic Organ Dysfunction) score [10], all based on the assessment of six parameters: respiratory, cardiovascular, hepatic, renal, neurological, and coagulation systems.

As the quality of medical care has improved, multiple organ dysfunction has become the leading cause of mortality associated with trauma in patients who survive the initial injury and are admitted to intensive care. The presence of multiple organ dysfunction is associated with increased morbidity and mortality in patients who have survived the first few hours after trauma [11].

PATHOPHYSIOLOGY OF MODS

The pathophysiology of MODS is multifactorial, complex, and still not fully understood. In the 1990s, multiple organ dysfunction was described as a bimodal phenomenon: the one-hit model and the two-hit model [12].

Currently, three models have been proposed to explain the occurrence of MODS:

- One-hit/one-event model: A massive trauma induces an intense SIRS associated with the onset of organ dysfunction.
- Two-hit/two-event model: Describes an initial injury followed by a second, seemingly minor injury that promotes inflammation and immunologic dysfunction. Thus, in the presence of moderate SIRS, a secondary activating event (such as surgical interventions, infections, embolism, transfusions) occurring during the compensatory anti-inflammatory response syndrome (CARS) will lead to late MODS
- Sustained injury model: Describes a continuous injury that acts both as the initial injury and maintains dysfunction [12-13].

Modern theories propose a system in which aggression simultaneously triggers two opposing responses: a pro-inflammatory response (SIRS) and an anti-inflammatory response (SARS, previously called compensatory, CARS), whose intensity and predominance correlate with the occurrence of MODS [14-15]. Early-onset multiple organ dysfunction is induced by the pro-inflammatory status with the activation of the immune system within SIRS, while late-onset multiple organ dysfunction is due to the exacerbation of anti-inflammatory responses within SARS, leading to immunosuppression, delayed healing, and increased susceptibility to infections [16].

There are several mechanisms implicated in the development of MODS, including mitochondrial dysfunction, alterations in immune system regulation, tissue or cellular hypoxia, activation of cellular apoptosis, and bacterial translocation from the gastrointestinal tract [17]. The factors involved in the development of MODS include:

- Persistent thrombocytopenia: It has been demonstrated that the interaction between platelets and neutrophils is involved in the development of acute lung injury (ALI), and antiplatelet therapies have been associated with a reduced risk of ALI, MODS, and decreased mortality in patients with adult respiratory distress syndrome (ARDS) [18].
- Polymorphonuclear leukocytes (PMNs): They act through local cytotoxic effects produced by PMN degranulation and the release of reactive oxygen species, nitric oxide, and pro-inflammatory mediators such as IL-6, IL-8, and TNF-α [19].
- Tissue macrophages and circulating monocytes [19].
- Pro-inflammatory cytokines (TNF-α, IL-1, IL-2, IL-6, IL-8, IL-17, MIP, GM-CSF, IFN-γ) and anti-inflammatory cytokines (IL-4, IL-10, IL-13) [20].
- The complement system: It is activated with the production of pro-inflammatory molecules C3a, C3b, and C5a, and the formation of the C5b-C9 complex, which leads to the lysis of the target cell. The activation of the complement system results in the production of reactive oxygen species, arachidonic acid metabolites, and cytokines [21-22].

In burn patients, it is considered that multiple organ dysfunction syndrome (MODS) is triggered by an inflammatory status, often associated, but not always, with sepsis. The onset of MODS can be caused either by extensive infection or by multiple infections with a smaller systemic impact, but which stimulate inflammation [23].

Other possible causes for MODS include:

- Gastrointestinal barrier impairment: involves bacterial translocation into the portal and lymphatic circulation, promoting the inflammatory response, or causing sepsis [24].

- Presence of uncovered burn wounds: leads to persistent inflammation and elevated levels of IL-6, IL-8, and TNF, contributing to a hypermetabolic and inflammatory status [25].

- Tissue hypoxia associated with hypoperfusion: causes cellular dysfunction through inadequate oxidative mechanisms and produces oxygen radicals [4].

In severely burned patients, MODS is the leading cause of death, with reported mortality rates in studies ranging from 29% to 86% [26-29]. The severity of MODS and mortality in burned patients correlate with the burned body surface area, the presence of deep burns, and the patient's age. The onset of MODS leads to prolonged hospitalization in intensive care, increased duration of ventilatory support, and longer hospital stays [26].

Burns over 30% of the body surface area induce a systemic inflammatory response associated with fluid loss through burn wounds and fluid extravasation into the interstitial space. This results in hypovolemia with hemodynamic changes similar to those seen in hemorrhagic shock, including decreased plasma volume, cardiac output, and urine output, as well as increased peripheral vascular resistance with decreased peripheral blood flow. These manifestations characterize post-burn shock, a particularly severe type of shock in severely burned patients, occurring primarily within the first 24 hours, peaking at 6-8 hours post-burn [31-32].

Inflammatory mediators, both local and systemic, involved in the pathogenesis of post-burn shock include histamine, TNF, interleukins, products of the arachidonic acid cascade (prostaglandins and thromboxanes), serotonin, bradykinin, nitric oxide, and oxygen radicals. Additionally, levels of certain hormones and cardiovascular function mediators are frequently elevated: epinephrine, norepinephrine, vasopressin, angiotensin II, and neuropeptide Y [33].

Similar to the treatment of other forms of hypovolemic shock, the main therapeutic objective is the prompt restoration of intravascular volume to preserve tissue perfusion. However, volume resuscitation in burns is complicated by the presence of edema not only in burned tissues but also in unburned tissues.

Consequently, large volumes of fluids are necessary to maintain intravascular volume in the initial hours following extensive burns [32]. Studies have shown that 50% of the edema fluid volume is found in unburned tissues [34].

RESPIRATORY DYSFUNCTION

Post-burn respiratory dysfunction is multifactorial, with the most common causes being the presence of inhalation injuries and the development of acute respiratory distress syndrome (ARDS) [35]. However, any pulmonary injury can be exacerbated by damage to the small airways (<2mm) and pulmonary alveoli induced by mechanical ventilation [36]. The evolution of respiratory dysfunction is also contributed to by the onset of pneumonia, which has an increased incidence, especially in patients with inhalation burns [37]. The presence of inhalation burns is a negative prognostic factor for morbidity and mortality, as established in the literature [38-39]. The severity of inhalation burns correlates with an increased need for tracheostomy, the number of days of mechanical ventilation, and a longer duration of hospitalization in intensive care [40]. In association with cutaneous burns, the presence of inhalation injuries increases the volume requirements for fluid resuscitation of burn patients and leads to a higher incidence of pulmonary complications [41].

From the perspective of affected structures and the etiopathogenic mechanism, inhalation injuries are divided into three categories:

1. Thermal injury to the upper airways: This results directly from elevated temperatures and consists of edema in the supraglottic structures and obstruction of the upper airways within the first few hours post-burn.

2. Chemical irritation of the lower airways: This is caused by the presence of toxic chemical compounds (such as sulfur dioxide, nitrogen dioxide, ammonia, cyanides, and aldehydes) in smoke, that cause epithelial and capillary endothelial damage to the respiratory tract, with histological features similar to tracheobronchitis. Physiopathological changes include increased local blood flow in the bronchi, local exudate and bronchiolar edema, necrotizing bronchitis, impaired mucociliary clearance with decreased bacterial removal, alveolar collapse, and atelectasis due to surfactant loss, activation of local macrophages with inflammatory response, bronchospasm, and increased capillary permeability leading to enhanced pulmonary edema. After smoke exposure, respiratory failure may occur due to airway obstruction from epithelial detritus, fibrin clots, and mucus within a variable timeframe, from 12 hours to 2 days post-burn.

3. Systemic injury: This occurs through the inhalation of carbon monoxide (CO) or cyanides, which causes both pulmonary effects, such as hypoxia and hypercapnia, and systemic effects mediated by pro-inflammatory cytokines. [41-44].

Acute respiratory distress syndrome (ARDS) is characterized by severe hypoxemia in the context of pulmonary alveolar damage, progressing to acute pulmonary edema and acute respiratory failure [45]. In burn patients, ARDS can occur either in association with airway burns or in their absence due to the systemic inflammatory response associated with burns during the post-burn shock period or septic complications of burns following the post-burn shock period. Inhalation injuries are an independent risk factor for the development of ARDS, with earlier onset and greater severity compared to ARDS induced by cutaneous burns [46]. The severe form of ARDS with refractory respiratory failure is a significant cause of death in severely burned patients [47].

CARDIAC DYSFUNCTION

Cardiac dysfunction leads to organ hypoperfusion, peripheral microcirculation damage, burn wound expansion, and decreased resistance of burned wounds to bacterial infection [48-49]. Cardiac failure is one of the main causes of mortality in severely burned patients within the first two days post-burn [50]

In the first 24-48 hours after a burn, corresponding to the ebb phase, there is a decrease in myocardial contraction and cardiac output [51]. The reduction in cardiac contractility has been associated with cardiomyocyte apoptosis within the first 48 hours post-burn, particularly affecting cells in the subendocardial tissue of the left ventricle [52]. Increased capillary permeability leads to a reduction in circulating plasma volume and an increase in peripheral vascular resistance. Some authors have considered hypovolemia as the primary factor responsible for reduced cardiac output in the early post-burn period [53]. Others have suggested that elevated levels of serum catecholamines, vasopressin, angiotensin II, and neuropeptide Y are responsible for inducing cardiovascular dysfunction, even in the absence of hypovolemia [49].

Data from the literature showed that hypovolemia can persist despite aggressive fluid resuscitation and in the absence of routinely monitored signs of hypoperfusion, such as decreased urine output and decreased mean arterial pressure [51]. Studies have demonstrated that serum lactate levels or the base deficit (the sum of lactate and other acidic metabolites released in the presence of tissue hypoxia) correlate better with the severity of hypoperfusion and remain elevated during fluid resuscitation, even when urine output and blood pressure are adequate [49,54]

From the third day post-burn, corresponding to the flow phase, myocardial contractility improves, and tachycardia and increased metabolic demands with increased catabolism appear [55]. The association of inhalation injuries can further cause right ventricular dysfunction through increased resistance in the pulmonary arteries and pulmonary hypertension, increasing the afterload of the right ventricle [56]. Consequently, left ventricular dysfunction can occur either as global heart failure or due to the displacement of the interventricular septum towards the left ventricle due to increased right ventricular filling [49,57].

Cardiac dysfunction is associated with increased mortality during hospitalization and persistent elevated serum catecholamine levels, tachycardia, and increased oxygen consumption continuing up to two years post-burn [49,58]. Studies in the literature have concluded that sepsis contributes to already present cardiac dysfunction in burn cases [59].

RENAL DYSFUNCTION

Another significant complication in the progression of burn patients is acute kidney injury (AKI), which may manifest either early after the burn injury or later, concurrent with the development of sepsis and in conjunction with other organ dysfunctions [60]. AKI is characterized by a sudden reduction in glomerular filtration with the kidneys' inability to maintain fluid and electrolyte balance, with the first clinical and paraclinical signs being decreased urine output and a subsequent increase in serum creatinine and urea [61]. The incidence of acute renal failure in burn patients has decreased following advancements in the treatment of cardiovascular dysfunction [60].

The causes of early acute renal failure are multiple:

- Myocardial depression: causes decreased cardiac output and renal hypoperfusion [63]
- Hypovolemia and renal hypoperfusion due to underestimation of fluid requirements during fluid resuscitation or delayed fluid resuscitation [62]. Renal hypoperfusion is followed by renal tubular necrosis [63]
- Hemolysis: free hemoglobin can precipitate in renal tubules or cause damage to the tubular epithelium [64]
- Rhabdomyolysis: increased serum levels of myoglobin in extensive deep burns, electrocutions, and compartment syndromes lead to myoglobinuria with myoglobin precipitation in renal tubules, vasoconstriction of afferent arterioles, and production of oxygen free radicals [65]
- Renal hypoperfusion: through increased levels of catecholamines, angiotensin II, aldosterone, and vasopressin, and reduced atrial natriuretic peptide [66]
- Inflammatory mediators such as TNF, IL1, prostaglandins, leukotrienes, thromboxane, and platelet-activating factor: released early post-burn, causing renal tissue damage and

microthrombi in renal glomerular capillaries [66]

- Use of nephrotoxic medications, such as aminoglycoside antibiotics, nonsteroidal anti-inflammatory drugs, barbiturates, and hydroxocobalamin [67]

- Respiratory dysfunction: through reduced oxygen supply to the kidneys [68]

Early acute renal failure is usually reversible [252]. Late renal injury typically occurs within the syndrome of multiple organ dysfunction, often associated with severe sepsis. The etiology of acute renal failure in sepsis is also multifactorial, caused by renal arteriole vasoconstriction (prerenal cause), a procoagulant status that induces disseminated intravascular coagulation (DIC), and micro-thrombosis in renal glomeruli and the endothelial damage [67]

Prevention of renal failure is based on monitoring renal function, correcting hypovolemia, hypoxia, and acidosis, limiting nephrotoxic medications, administering thromboprophylaxis in the context of procoagulant status, and early excision-grafting of post-burn lesions to prevent wound infection and sepsis [69].

HEPATIC DYSFUNCTION

Liver injury following burns occurs via multiple mechanisms such as decreased hepatic blood flow, portal hypertension due to the action of endotoxins, release of hepatotoxic products from burn wounds, intrahepatic cholestasis, programmed cell death of hepatocytes through ischemia-reperfusion mechanisms, decreased hepatic blood flow, and the action of proinflammatory cytokines IL1 and TNF-α and hepatic dysfunction due to sepsis from pathogenic microorganisms, toxins, and inflammatory mediators [70-72].

Major burns lead to transient increases in levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, in up to 50% of cases, which typically resolve spontaneously without significant clinical impact [73]. Less frequently, there may be an increase in serum bilirubin, which, however, implies more severe consequences. Severe and persistent increases in liver enzymes, especially bilirubin, often indicate burn-associated complications such as sepsis and are associated with a poor prognosis [74].

During the acute phase response in severely burned patients, there is an increase in the production of acute phase proteins (α1-acid glycoprotein, C-reactive protein, haptoglobin, and α1-antitrypsin) along with a decrease in the levels of constitutive hepatic proteins (prealbumin, transferrin, retinol-binding protein). [75] In the context of meeting the nutritional needs of patients, the decrease in constitutive hepatic proteins is due more to decreased hepatic synthesis and expression. Proinflammatory cytokines such as IL-1β, IL-6, TNF-α, IL-8, and IL-10 act as mediators of the acute phase response and increase immediately post-burn, with their levels remaining elevated for a long time after injury. The uncontrolled and prolonged action of proinflammatory cytokines and acute phase proteins can lead to hypermetabolism and catabolism, which result in multiple organ failure, significantly increasing both morbidity and mortality rates [75-77].

Liver damage has been associated with the development of hepatic edema, with the liver significantly increasing in weight within 2 to 7 days post-burn and reaching a maximum size at 2 weeks post-injury. At 12 months, liver weight can still be increased by 40 to 50% compared to predicted weight [75,78].

Manifestations of liver dysfunction in burns include:

- Hypoalbuminemia due to decreased albumin synthesis; hypoalbuminemia leads to worsening edema and delayed wound healing

- Coagulopathy: due to impaired synthesis of coagulation factors in the liver, increasing the risk of bleeding

- Hyperbilirubinemia

- Glucose metabolism disorders due to impaired hepatic gluconeogenesis and glycogen storage; hyperglycemia is often observed due to deficient glycogenesis and insulin resistance, while hypoglycemia can occur in severe liver failure cases where gluconeogenesis is profoundly affected

- Hepatic steatosis due to altered lipid metabolism

- Sepsis: impaired liver function influences the immune response, making burn patients more susceptible to infections and sepsis [73,75,79].

NEUROLOGIC DYSFUNCTION

Post-burn encephalopathy is defined as acute neurological dysfunction following burns, independent of iatrogenic factors. Patients with post-burn encephalopathy exhibit seizures, agitation, hallucinations, delirium, and other neurological disturbances [80].

Morphopathological studies have demonstrated significant pathological changes in the brain, including massive edema leading to herniation, diffuse intraparenchymal hemorrhage, cerebral infarcts, metabolic encephalopathies, pontine demyelination, infectious complications such as meningitis, cerebral microabscesses, and septic emboli [81-82].

Cerebral ischemia, often underestimated, is a frequent cause of post-burn encephalopathy and is primarily due to hypovolemia associated with severe burns. Hemorrhagic cerebral complications are less common than ischemic ones and present as multiple subarachnoid hemorrhages in both the brain and spinal cord. In patients without hypoxia and hemodynamic disturbances, hemorrhages are more frequently associated with inhalation injuries than with cutaneous burns [83-84].

Proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, along with reactive nitrogen species and oxygen-free radicals released from burned tissues, can alter the integrity of the blood-brain barrier. These molecules, along with neutrophils, can reach the brain, causing microglial cell activation and neuronal apoptosis. The activation of microglial cells in the brain and spinal cord leads to a local inflammatory response and neuronal apoptosis. Activated neutrophils, once they reach the central nervous system, release proteases, oxygen free radicals, reactive nitrogen species, and cytokines, further amplifying the inflammatory process [85].

HEMATOLOGIC DYSFUNCTION

Causes of burn-associated coagulopathy include a systemic inflammatory response with massive cytokine release that interferes with normal coagulation, tissue damage causing direct blood vessel injury and tissue factor release, activating the extrinsic coagulation pathway, impaired normal platelet function, hypoperfusion leading to hypoxia and acidosis, consumption of coagulation factors through clot formation, increased fibrinolytic activity in response to clots and iatrogenic factors (fluid resuscitation and associated hemodilution, as well as blood losses from surgical excision and grafting) [86-87]. Disseminated intravascular coagulation (DIC) is also part of the spectrum of coagulopathy in burn patients. DIC is a clinical-biological syndrome characterized by uncontrolled systemic activation of coagulation, with risks of micro- and macrovascular thromboses and progressive consumptive coagulopathy, increasing the risk of bleeding. [88].

In burn patients, sepsis-induced coagulopathy may also occur, ranging from subclinical coagulopathy to fulminant DIC. [89] Septic patients exhibit a prothrombotic status due to activation of the extrinsic coagulation pathway, amplification of the cytokine-induced coagulation cascade, reduced fibrinolysis, and suppression of natural anticoagulant pathways [90].

Hypercoagulability in burn patients is associated with an increased risk of thromboembolic complications (deep vein thrombosis and pulmonary embolism), with incidence rates in studies ranging from 0.2% to 25% [91]. Contributing factors include patient-specific predispositions (genetic thrombophilia or comorbidities such as cancer history, atherosclerosis, heart failure, chronic kidney disease, previous thromboembolic episodes), iatrogenic factors (presence of central or peripheral venous catheters, repeated venipunctures, certain medications), and the lack of mobilization, serial surgical interventions, and long operative times [92]

Preventive and management measures for coagulopathy in burn patients include general supportive measures such as avoiding dilutional coagulopathy and preventing hypothermia, as well as targeted therapeutic measures like blood product transfusion or antithrombin administration [93].

INFECTIONS IN BURN PATIENTS

The destruction of the skin barrier, as well as the decrease in local cellular and humoral immune responses, predisposes patients with severe burns to an increased risk of infectious complications. Infections can progress to sepsis, septic shock, and multiple organ dysfunction syndrome, and are one of the main causes of morbidity and mortality, accounting for 42-65% of deaths in severely burned patients. [94-95]

The burned wound in deep partial-thickness burns and full-thickness burns is a protein-rich and avascular environment, representing an ideal area for the colonization and proliferation of germs. The lack of vascularization of the post-burn eschar opposes the migration of immune cells to this area and decreases the local availability of systemically administered antimicrobial therapy, while the toxic substances released from the eschar decrease the local immune response. [96] The burned wound is susceptible to colonization with exogenous or endogenous opportunistic microorganisms. Depending on factors such as the patient's age, the burned surface area and burn depth, the type and number of microorganisms, and the production of enzymes and toxins, invasive infections of the burned wound may occur. [97]

Burn patients can be colonized with multiple germs, often resistant to antibiotics, without showing perilesional erythema or cellulitis. In contrast, invasive infections are characterized by the presence of cellulitis in the surrounding tissues, deepening of the burn wounds, debridement of the eschar, or the appearance of necrotic areas. Patients may become febrile or exhibit elevated inflammatory markers, but these clinical and paraclinical signs are difficult to differentiate from those associated with the inflammatory response in severe burns. [94]

According to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. For practical application, organ dysfunction is represented by an increase of 2 or more points in the SOFA score, which is associated with in-hospital mortality greater than 10%. Septic shock is defined as a subset of sepsis in which underlying circulatory, metabolic, and cellular abnormalities are profound enough to substantially increase mortality compared to sepsis without shock. The diagnosis of septic shock corresponds to the need for vasopressor support to maintain a mean arterial pressure above 65 mmHg and an increase in serum lactate levels above 2 mmol/L, in the absence of hypovolemia. [98]

Burn patients exhibit the clinical and paraclinical signs of SIRS even in the absence of sepsis, which has led to the necessity of identifying other diagnostic methods for sepsis. At a consensus conference of the American Burn Association, an attempt was made to establish specific criteria for the diagnosis of sepsis in burn patients. [99] Sepsis should be suspected in the presence of a documented infection and clinical and paraclinical criteria, which are described in Table I.

Table 1: American Burn Association sepsis in burns criteria

At least 3 of the following criteria:	Documented infection:
Temperature >39°C or <36.5°C	Positive culture results
Heart rate >110 bpm	Identification of the pathogen through tissue biopsy
Respiratory rate >25/min in spontaneously breathing adults or Minute respiratory volume >12 L/min in mechanically ventilated adults	Clinical response to antimicrobial treatment
Thrombocytopenia <100,000/µl (considered after more than 3 days from the onset of initial resuscitation)	
Hyperglycemia >200 mg/dl in the absence of pre-existing diabetes mellitus or Insulin resistance is defined as an insulin requirement of more than 7 IU/hour continuous infusion or an increase of more than 25% in insulin requirement over 24 hours	
Inability to administer enteral nutrition for more than 24 hours due to: - Intolerance to enteral nutrition (gastric residual volume twice the volume of administered nutrition) - Uncontrolled diarrhea (more than 2500 ml/day in adults) - Abdominal distension	

In a meeting of experts, Dupuy and colleagues described the characteristics of the ideal biomarker, which should fulfill multiple roles: identifying high-risk patients, stratifying patients based on individual risk factors, and guiding therapeutic management. Thus, an ideal biomarker should be inexpensive, rapid, non-invasive, easily accepted by the patient, routinely available, and should combine diagnostic characteristics (including differentiating between non-infectious SIRS and sepsis, and between bacterial and viral infections) with prognostic characteristics (including mortality prediction) and the ability to evaluate treatment efficacy. [100]

This ideal biomarker has not been established, but inflammatory markers analyzed in studies for identifying sepsis in burn patients include C-reactive protein, procalcitonin, erythrocyte sedimentation rate, presepsin, serum TNFα, interleukins 6, 8, and 10, serum leukocyte levels, serum platelet levels, and serum lactate levels. [101-102]

With the development of burn centers, advances in the fluid resuscitation of patients with severe burns, and the promotion of early excision and grafting of burn wounds, the past trend of early death due to post-burn shock has now shifted to mortality due to infections and sepsis following prolonged hospitalizations. Preventing sepsis is a primary objective in severely burned patients. Several measures have been proposed to reduce the risk of infectious complications. Early excision of burn wounds and coverage with skin grafts reduce bacterial colonization of burn wounds, decrease the release of inflammatory mediators and the incidence of SIRS, and reduce the risk of sepsis and multiple organ dysfunction. [103-104]

In severely burned patients, some succumb early due to the severity of their burns, while others die after prolonged hospitalization complicated by the development of numerous complications. Multiple organ dysfunction syndrome (MODS) associated with sepsis is the leading cause of death in patients who survive early post-burn shock. [27-28]

The risk factors for the development of sepsis in severely burned patients include:

- Total body surface area (TBSA) burned
- Extreme ages
- Pre-existing conditions accompanied by immune-suppression: diabetes, renal or hepatic pathologies, AIDS, malignancies
- Presence of uncovered burn wounds

- Use of urinary catheters, venous and arterial catheters, or mechanical ventilation [105-106]

A particularly concerning group of bacteria in the context of major burns due to their notorious ability to evade standard antibiotic treatments and develop resistance to last-line antibiotics are the ESKAPE pathogens, comprising Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. [107]

In patients suspected of having sepsis, a thorough search for a probable source should be conducted; clinical symptoms and signs, appropriate microbiological cultures, and relevant imaging techniques should be used to try to determine the infectious source. Antimicrobial medication should be administered early and not delayed until culture data are obtained. Broad-spectrum antibiotic therapy, preferably a combination of antimicrobials, should be administered to cover all suspected germs based on the probable source of infection, local microbiological flora, and specific intensive care flora. After obtaining culture results, antibiotic therapy should be reassessed and de-escalated, narrowing the spectrum to optimize treatment efficacy, limit toxicity, prevent the emergence of multidrug-resistant germs, and reduce treatment costs. [108-109]

CONCLUSIONS

Understanding these mechanisms and pathological findings is crucial for managing and preventing complications in burn patients. Early intervention and vigilant monitoring are essential for mitigating the risks associated with severe burns and reducing the incidence of multiple organ dysfunction syndrome. By recognizing the systemic inflammatory responses, fluid loss, and potential for infection that accompany severe burn injuries, healthcare providers can implement targeted strategies to support organ function, prevent sepsis, and optimize patient outcomes. Continuous advancements in burn care and precise triage and treatment approaches are vital for improving survival rates and ensuring better long-term recovery for burn patients. This is a current overview of the subject. In the coming years, new information on the pathophysiological mechanisms will likely emerge to complete the level of understanding on the subject.

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ARTICLE

Investigation of Factors Influencing Soldiers' Disabilities in China: A Cross-Sectional Study

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Abstract: *Due to the frequent occurrence of international military conflicts, there has been an increasing number of soldiers who have suffered injuries and disabilities. The soldiers' disability is a crucial factor influencing combat effectiveness. Objective: This study aims to investigate the current situation of military disability and its influencing factors to provide a basis for reducing the rate of military disability. A total of 430 Chinese military disability assessment materials from 2021 were collected retrospectively between April 2023 and September 2023. The data on injuries, illnesses, and disabilities of soldiers were subjected to statistical analysis with informed consent, and personal information was anonymized to ensure privacy. The occurrence of disability was analyzed based on gender, rank, and different disability systems using unifactor analysis and multiple linear regression analysis. Statistical differences (P<0.05) were found in the degree of disability among soldiers of different ages, status categories, nature of disabilities, disability systems, and causes of disabilities. Age, status category, nature of disabilities, disability systems, and causes were identified as the main factors affecting the degree of soldiers' disabilities, with a total explanation rate of 58.3%. Injuries to the motor system during training were identified as the primary cause leading to soldier disabilities. In conclusion, this study shows that it is essential to prioritize protection against training injuries to minimize soldier disabilities.*

Keywords: *soldier; disabilities; influencing factors; investigation*

INTRODUCTION

Soldiers are the primary component of the national defense force, responsible for safeguarding national security, protecting and defending national borders, maintaining social stability, and other related duties. The physical and mental well-being of soldiers is directly linked to their combat effectiveness, with disability being a major factor affecting this during peacetime operations [1]. The effects of military activities on soldiers include not only physical aspects but also psychological aspects [2].

Previous research has indicated that the impact of military operations on soldiers is irreversible, with some individuals experiencing lasting effects such as physical disabilities [3,4,5,6], pain [7], hearing loss [8], blindness [9], post-traumatic stress disorder (PTSD) [10], traumatic brain injury (TBI) [11], and fractures [12,13]. Data from the US Army, Navy, and Air Force disability agencies [14] show that 10 to 30 out of every 1000 soldiers are disabled each year. However, only 30% of these cases are due to pre-existing conditions; the majority result from injuries sustained during military operations.

The American Veterans Benefits Administration offers disability benefits to individuals and service members who previously served full-time active military duty and were discharged generally or honorably [15]. These benefits can be categorized into three types based on the causes of disabilities: (1) Service-connected disability, which refers to a disability resulting from direct injury or disease while on active duty; (2) Nonservice-connected disability, which is attributed to an injury not acquired during active duty; (3) Presumptive service connection, which encompasses various chronic conditions that appear within 1 year after discharge from active duty.

In China, military training is a significant contributing factor to physical disabilities among soldiers [16]. Research has indicated that the incidence of military training injuries in the new recruit population in China is 21.04%, suggesting that one out of every five individuals will experience a military training injury [17]. Therefore, this study conducts a cross-sectional investigation to analyze the occurrence and influencing factors of disability among Chinese military personnel, to enhance the effectiveness of prevention and treatment of military training injuries.

MATERIALS AND METHODS

Research objects

A total of 430 Chinese military disability assessment materials from 2021 were collected retrospectively between April 2023 and September 2023. The data on injuries, illnesses, and disabilities of soldiers underwent statistical analysis with consent, and personal information was anonymized to ensure privacy. The disability assessment materials included 400 males and 30 females, with an average age of 30.75 ± 7.38 years.

Research methods

According to the "Military Disability Rating Standards"[17], injuries, illnesses, and disabilities of officers and soldiers were graded on a scale from one to ten. A lower rating indicated a more severe injury or disability.

The classification of the causes of disability includes two aspects: (1) Disability due to service, which refers to disabilities directly caused by participating in work or military activities during active duty; (2) Non-service related disability, which refers to disabilities caused by illness during active duty due to physical reasons. The data from disability assessment materials were entered into the computer and checked by two individuals to ensure accuracy.

Inclusion criteria: the disability condition meets the Chinese Military Disability Assessment Standards and is eligible for disability assessment [17], disability assessment standards are mainly based on the site of injury, severity and sequelae to determine the level of disability. Other conditions were excluded from this study, such as cases where recovery after injury was good, and there were no obvious functional abnormalities.

Classifying and analyzing the levels of disability in different groups based on the disability assessment materials. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of The Strategic Support Force Military Center (LS202203, Approved:2022-10-27).

Statistical Methods

Statistical analysis of the data was conducted using SPSS 22.0. Descriptive statistics were used to represent continuous variables as mean and standard deviation and categorical variables as rates or proportions. The data was categorized based on patient medical records, and chi-square tests and multiple linear regression analysis were performed with a significance level set at P<0.05.

RESULTS

The basic situation of disability assessment of military personnel in 2021

In 2021, a total of 430 disability assessment materials submitted to a certain Ministry met the military standards for disability assessment.There were 2 cases (0.5%) of Level 1, 1 case (0.2%) of Level 2, 2 cases (0.5%) of Level 3, 2 cases (0.5%) of Level 5, 31 cases (7.2%) of Level 6, 4 cases (0.9%) of Level 7, 109 cases (25.3%) of Level 8, 175 cases (40.7%) of Level 9, and 104 cases (24.2%) of Level 10.

Statistical differences were observed among different grades of disability in different age groups, status categories, and the nature of disability, as shown in Table 1.

Table 1: Comparison of disability among soldiers with different demographic variables

Divisions	Disability Grade									χ2	P
	Level 1	Level 2	Level 3	Level 5	Level 6	Level 7	Level 8	Level 9	Level 10		
Gender											
Male	2 (100%)	1 (100%)	2 (100%)	2 (100%)	30 (96.8%)	4 (100%)	104 (95.4%)	161 (92%)	94 (90.4%)	3.854	0.870
Female	0	0	0	0	1 (3.2%)	0	5 (4.6%)	14 (8%)	10 (9.6%)		
Age group (years)											
≦29	2 (100%)	1 (100%)	1 (50%)	1 (50%)	29 (93.5%)	2 (50%)	46 (42.2%)	85 (48.6%)	50 (48.1%)	37.593	0.002
30~≦39	0	0	1 (50%)	0	2 (13.4%)	1 (25%)	54 (49.5%)	68 (38.9%)	44 (42.3%)		
≧40	0	0	0	1 (50%)	0	1 (25%)	9 (8.3%)	22 (12.6%)	10 (9.6%)		
Identity category											
Conscripts	1 (50%)	0	0	0	14 (45.2%)	1 (25%)	9 (8.3%)	28 (16%)	21 (20.2%)	38.999	0.001
NCO	1 (50%)	1 (100%)	2 (100%)	1 (50%)	17 (54.8%)	1 (25%)	56 (51.4%)	91 (52%)	51 (49.0%)		
Officers	0	0	0	1 (50%)	0	2 (50%)	44 (40.4%)	56 (32%)	32 (30.8%)		
Disabling Character											

Non-service	2 (100%)	1 (100%)	2 (100%)	1 (50%)	31 (100%)	0	0	0	0	423.64	0.001
Service	0	0	0	1 (50%)	0	4 (100%)	109 (100%)	175 (100%)	104 (100%)		
Total	2 (0.5%)	1 (0.2%)	2 (0.5%)	2 (0.5%)	31 (7.2%)	4 (0.9%)	109 (25.3%)	175 (40.7%)	104 (24.2%)		

χ2 for chi-square tests.
NCO – Non-commissioned Officers

Grade distribution of disability in different disability systems

In 2021, the majority of cases (90.9%) were related to motor system disability, followed by 4.9% of neurological disability cases and 1.4% of urinary disability cases, and were statistically differentiated by examination (P<0.01). See Table 2 for more details.

Table 2: Comparison of disability among soldiers with different physiological systems

Disability systems	Disability Grade									Total
	Level 1	Level 2	Level 3	Level 5	Level 6	Level 7	Level 8	Level 9	Level 10	
Respiratory System	0	0	0	0	0	0	0	0	1 (0.2%)	1 (0.2%)
Urinary System	0	0	0	0	3 (0.7%)	0	2 (0.5%)	0	1 (0.2%)	6 (1.4%)
Endocrine System	0	0	1 (0.2%)	0	4 (0.9%)	0	0	0	0	5 (1.2%)
Nervous System	2 (0.5%)	1 (0.2%)	0	1 (0.2%)	7 (1.6%)	0	4 (0.9%)	5 (1.2%)	1 (0.2%)	21 (4.9%)
Digestive System	0	0	0	0	1 (0.2%)	0	0	0	0	1 (0.2%)
Circulatory System	0	0	1 (0.2%)	1 (0.2%)	2 (0.5%)	0	0	0	0	4 (0.9%)
Motor System	0	0	0	0	13 (3%)	4 (0.9%)	103 (24%)	170 (39.5%)	101 (23.5%)	391 (90.9%)
Blood System	0	0	0	0	1 (0.2%)	0	0	0	0	1 (0.2%)
Total	2 (0.5%)	1 (0.2%)	2 (0.5%)	2 (0.5%)	31 (7.2%)	4 (0.9%)	109 (25.3%)	175 (40.7%)	104 (24.2%)	430 (100%)

χ2 =351.838, P<0.01, χ2 for chi-square tests

Table 3: Comparison of disability among soldiers with different reasons

Causes of Disability	Disability Grade									Total
	Level 1	Level 2	Level 3	Level 5	Level 6	Level 7	Level 8	Level 9	Level 10	
Illness	1 (0.2%)	1 (0.2%)	1 (0.2%)	1 (0.2%)	29 (6.7%)	0	0	1 (0.2%)	0	34 (7.9%)
Traffic Accident	0	0	0	0	0	0	1 (0.2%)	4 (0.9%)	1 (0.2%)	6 (1.4%)
Physical Activity	0	0	0	0	0	0	1 (0.2%)	1 (0.2%)	2 (0.5%)	4 (0.9%)
Daily Training	1 (0.2%)	0	0	1 (0.2%)	1 (0.2%)	3 (0.7%)	89 (20.7%)	136 (31.6%)	78 (18.1%)	309 (71.9%)
Exercise and Training Tasks	0	0	0	0	1 (0.2%)	1 (0.2%)	9 (2.1%)	28 (6.5%)	13 (3%)	52 (12.1%)
Other	0	0	1 (0.2%)	0	0	0	9 (2.1%)	5 (1.2%)	10 (2.3%)	25 (5.8%)
Total	2 (0.5%)	1 (0.2%)	2 (0.5%)	2 (0.5%)	31 (7.2%)	4 (0.9%)	109 (25.3%)	175 (40.7%)	104 (24.2%)	430 (100%)

χ2=391.947, P<0.01, χ2 for chi-square tests.

Grade distribution of disability caused by different causes

In 2021, the majority of injuries (71.9%) occurred during daily training, followed by 12.1% during exercise and training, and 7.9% due to illness, and were statistically differentiated by examination (P<0.01). See Table 3 for more details.

Multi-factor analysis of the influencing factors of a soldier's assessment of disability

Multiple linear regression analysis was conducted to examine the relationship between disability rating and various independent variables that showed statistical significance in univariate analysis (see Table 4 for assigned values). The findings revealed that the nature of disability, identity category, age, disability system, and cause of disability were the primary factors influencing the degree of disability (F=121.185, P<0.01). The overall explanatory power of these variables was 58.3%, as presented in Table 5.

Table 4: Argument assignment method

Variables	Assignment method
Gender	Male = 1, Female = 2.
Age	Original value input.
Identity category	Conscript = 1, Noncommissioned Officer = 2, Officer = 3.
Disabling Character	Non-service = 1, Service = 2.
Disabling System	Respiratory System = 1, Urinary System = 2, Endocrine System = 3, Nervous System = 4, Digestive System = 5, Circulatory System = 6, Motor System = 7, Blood System = 8.
Causes of Disability	Illness = 1, Traffic Accident = 2, Physical Activity = 3, Daily Training = 4, Exercise and Training Tasks= 5, Other = 6.

Table 5: Multivariate analysis of influencing factors of disability among soldiers

Variables	Beta	SE	Beta '	t	P
Constant	1.210	0.379		3.191	0.002
Age	0.015	0.007	0.086	2.070	0.039
Identity Category	-0.245	0.081	-0.127	3.031	0.003
Disabling Character	3.640	0.211	0.780	17.257	0.001
Disabling System	0.097	0.047	0.074	2.044	0.042
Cause of Disability	-0.129	0.064	-0.081	-1.988	0.047

[Note] R2=0.588, Adjust R2=0.583, F=121.185, P<0.01.

DISCUSSION

In 2021, the majority of soldiers' disabilities were concentrated in grades 8 to 10 (90.2%). This finding is consistent with the research results of Wang [19] and Li [20], indicating that most soldiers injured during normal work experience moderate to low levels of disability, with fewer cases of severe disability. This may be attributed to a greater emphasis on safety measures during peacetime work, more effective preventive measures against major safety accidents, and successful implementation of the safety form analysis system [21,22,23].

In the analysis of the disability system, it is evident that soldiers' disabilities are primarily related to sports activities, with a concentration of disabilities falling within the 8 to 10 level. The causes of these disabilities can be attributed to daily training, and factors such as the nature of the disability, identity category, and age have an impact on the severity of the disability. This pattern is similar to that observed in Chinese Air Force soldiers [24]. In 2020, certain departments under the Central Military Commission made adjustments to the "Military Physical Training Outline", resulting in an increase in training-related injuries due to heightened difficulty levels [25]. Furthermore, young soldiers' competitive spirit often leads them to neglect their physical well-being during training sessions, making them susceptible to injuries or fatigue. Disabilities resulting from training missions are typically linked to officers and soldiers having lax attitudes towards prevention measures and non-standard equipment operation due to psychological tension. Pang [26] also argues that military training missions' high physical load and tension are primary contributors leading to bodily harm, physiological fatigue, and potentially severe consequences.

Methods to Improve the Condition of Injured Soldiers

1. Comprehensive Preventive Measures

In recent years, training organizations at all levels have increasingly prioritized the prevention of various types of training injuries. Despite these efforts, training injuries continue to be the leading cause of military disability. Therefore, it is crucial to strengthen protection in the following areas.

1.1. Pre-training preparation

Proper pre-training preparation is crucial in reducing the occurrence of training injuries. Engaging in warm-up activities can enhance the body's flexibility, adaptability, and malleability, thereby improving its ability to withstand high-intensity training. Furthermore, it can increase blood flow redistribution and velocity in the body, thus reducing the risk of injury to the musculoskeletal system. Additionally, providing comprehensive instruction in operational or action skills is essential for skilled athletes to prevent individual injury.

1.2. Moderation in training is essential

Moderate training plays a key role in preventing physical injuries. It is important for individuals to recognize their own limitations and for training organizations at all levels to consider each individual's body capacity when designing training programs. Gradually increasing the intensity of training can also help avoid injuries caused by overexertion during cross-training activities.

1.3. Post-training recovery

The post-training recovery stage is a critical factor in individual energy and power storage. Following high-intensity training, soldiers often experience exhaustion. Effective recovery can facilitate the body's recuperation and growth, ultimately enhancing soldiers' resilience to future training sessions.

2. Enhance organizational structure and management

Scientific training and program-based organization are fundamental principles for conducting military training. Training and exercise departments at all levels should enhance the planning of various types of training, organize tasks rationally and scientifically, and continuously improve soldiers' physical fitness while enhancing combat effectiveness. It is essential to maintain existing combat effectiveness standards while improving overall combat readiness. When implementing operational actions, organizers should consider operational processes, standards, and proficiency skills to avoid inexperienced individuals operating alone, which could lead to security risks.

Rigorously enforce the annual physical examination system

A well-organized arrangement of officers and soldiers participating in the annual military physical examination task can lead to early detection, diagnosis, and treatment of asymptomatic diseases. This proactive approach helps prevent permanent functional loss such as dysfunction, significantly reducing the disability rate among soldiers.

Limitations and Generalizability

This study is based on a sample of 430 disability assessment materials, which were the result of collaborative efforts from all our researchers. However, it should be noted that these materials may not fully represent all disability assessment outcomes in 2021, thus limiting the generalizability of our results. Additionally, there was a skewed gender ratio in our study with only 30 females compared to 400 males, even though the number of males in the military far exceeds that of females. This imbalance may have impacted the accuracy of our findings regarding gender differences.

Nevertheless, it is important to highlight that this study was able to capture some aspects of disability assessment among Chinese soldiers in 2021. By analyzing the causes of disability among 430 soldiers, we are still able to provide valuable recommendations for preventing disabilities within this population.

CONCLUSIONS

This study adopts the method of cross-sectional investigation to investigate the factors affecting the disabled status of Chinese soldiers. We found that age, rank category, nature of disability, involvement system and cause of disability were the main factors affecting the degree of disability in military personnel. Injury to the musculoskeletal system during training is the main cause of military disability. The prevention and treatment of training injuries is a very important research topic that can provide better health protection for soldiers. Therefore, coaches and managers at all levels need to pay attention to injury prevention in the training process, which can minimize the occurrence of military disabilities.

Conflicts of interest and sources of funding

The authors declare no conflict of interest, and this research has received no external funding.

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Authors' contribution

The initial draft was prepared and revised by LS, while FR and SX contributed by proposing hypotheses and preparing the dataset. QS further refined the analysis and provided crucial support for the manuscript. DNL, XG, SX, and YW conducted data collection in various units and offered theoretical support. LS and QS supervised all aspects of the study, including hypothesis formulation, design of data analysis plan, oversight of data analysis process, verification of results, and development of the manuscript based on the initial draft. All authors participated in reviewing and finalizing the manuscript which has been read and approved by everyone involved.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Strategic Support Force Military Center (LS202203,2022.01.01).

Patient consent for publication

Written informed consent has been obtained from the patient (s) to publish this paper

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REVIEW

Gestational Diabetes: From Mechanism to Therapy – A Short Review

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Abstract: *Gestational diabetes (GDM) is characterized by the initial onset of elevated glucose levels while pregnant throughout the 24th week up until the 28th week of gestation affecting around 21.1 million live births in 2021. GDM is related to significant prolonged health risks for the mother and the unborn baby. A comprehensive research of the PubMed database was conducted between April and June 2024. As a response to IR, maternal beta cells increase in number and ramp up insulin production and release to sustain glucose balance. Physiological IR during pregnancy is driven by hormonal and inflammatory changes. Multiple risk factors contribute to the occurrence of GDM. Glycemic targets during pregnancy are more stringent than those for nonpregnant women. GDM can lead to several short-term or long-term complications during pregnancy for both the mother and the unborn baby. The first step in managing GDM involves lifestyle modifications. The next step in managing GDM involves the use of medication and insulin is the preferred pharmacological treatment. However, there are still many steps to be taken towards establishing an international prevention strategy and accurately determining the mechanisms involved in the development of GDM.*

Keywords: *gestational diabetes; pregnancy; placental hormones; gestational treatment*

INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized as the initial onset of elevated glucose levels while pregnant, typically throughout the 24th week up until the 28th week of gestation. GDM has appeared among many prevalent pregnancy complications, with the rate of occurrence increasing in recent years [1]. The International Diabetes Federation (IDF) specifies that GDM affected around 21.1 million live births in 2021, with a higher prevalence noticed in low- and middle-income countries, where hyperglycemia during pregnancy is more common [2]. More recently, the term “early” gestational diabetes (early GD) has been used to define elevated glucose levels occurring before twenty weeks of pregnancy, and its prevalence is expected to be between 0.7% and 36.8% [3]. The term “late” gestational diabetes (late GD) is defined as elevated glucose levels between 24 to 28 weeks of gestation [4].

The history of diabetes in pregnancy dates back to 1824 when it was first described by Bennewitz [5]. The initial diagnostic criteria for this condition were proposed by Williams in 1909 [6]. Over time international organizations, including the World Health Organization (WHO), the International Association of Diabetes and Pregnancy Study Group (IADPSG), the Canadian Diabetes Association (CDA), and the American Diabetes Association (ADA) have developed guidelines outlining the diagnosis and screening for GDM [7].

GDM is related to significant prolonged health risks for the mother and the unborn baby. Pregnant women diagnosed with GDM face about 8-fold high risk regarding the possibility of developing type 2 diabetes (T2D), especially in the first 5-10 years postpartum [8]. Additionally, GDM can result in a variety of pregnancy complications, including fetal anomalies, spontaneous abortion, fetal demise, preeclampsia, macrosomia, neonatal hyperbilirubinemia, and hypoglycemia [9].

METHODS

A comprehensive research of the PubMed database was conducted between April and June 2024. Keywords used in the search included "gestational diabetes", "prevalence", "hyperglycemia", "risk factors", “diagnosis”, "placental hormones", "mechanism", and “treatment” as well as various combinations of these terms. The research was restricted to studies published in English. Exclusion criteria included studies that identified GDM diagnosis solely by codes, studies focusing on specific populations (e.g., by age, species, or race), and publications in the form of abstracts, letters, or case reports.

DISCUSSIONS

Etiopathogenesis

Insulin is secreted by pancreatic beta cells and plays a vital role in promoting glucose utilization by peripheral cells, decreasing glucose synthesis within the liver, and regulating lipid release from fat stores. When regular levels of insulin fail to obtain the desired response from insulin receptors, insulin resistance (IR) can develop. To compensate, the pancreatic beta cells must release more insulin to ensure glucose levels remain normal, leading to IR [10]. This process is a natural aspect of a healthy pregnancy, induced through placental hormones to guarantee that the unborn baby obtains sufficient essential nutrients for optimal development. As a response to IR, maternal beta cells increase in number and ramp up insulin production and release to sustain glucose balance [11].

GDM’s underlying mechanisms remain unclear, but it is believed that it results from the maternal pancreas's inability to adapt to the increase of insulin demand during pregnancy, leading to hyperglycemia [12,13]. The placenta seems to play a crucial role in GDM formation, as several studies have explored the physiological mechanisms involved.

Physiological IR during pregnancy is driven by hormonal and inflammatory changes [14]. Metabolic changes also occur, with IR typically emerging during the first half of pregnancy and then decreasing. This phenomenon is influenced by raised levels of prolactin (PRL), human placental lactogen (hPL), progesterone, and estrogen, which contribute to the hyperplasia and hypertrophy of maternal pancreatic beta cells. These beta cell modifications are part of the body's physiological adaptation to hPL, a polypeptide hormone secreted by the placenta that peaks around 30 weeks of gestation [15]. This timing may explain why blood glucose levels tend to stabilize after 30-32 weeks of pregnancy. hPL decreases activation of insulin receptor substrate 1 (IRS1) through phosphorylation, thereby inducing IR [16]. PRL, another polypeptide hormone produced by the anterior pituitary gland, also affects insulin sensitivity and lipid metabolism. PRL can induce IR both during and outside the pregnancy period, working alongside hPL to contribute to IR [17]. Prolactin receptors, which are expressed in pancreatic beta cells, play a fundamental role in pregnancy metabolism as well as IR. These receptors regulate pro-proliferative and anti-apoptotic pathways through the mitogen-activated protein kinase (MAPK), protein kinase B (AKT), phosphatidylinositol 3-kinase (PI3K), the Janus kinase 2-signal transducer and activator of transcription 5 (JAK2/STAT5) pathway. After childbirth, beta-cell mass is reduced through apoptosis, cell size reduction, or other mechanisms [18]. Estradiol induces IR by activating the JNK pathway through membrane estrogen receptor (ER)-mediated actions and serine phosphorylation of IRS-1. Progesterone contributes to IR by inhibiting the PI3-kinase pathway and reducing IRS1 expression [19]. Placental growth hormone (PGH) is found in the syncytiotrophoblast and extravillous cytotrophoblast layers of the human placenta [20]. It promotes gluconeogenesis, lipolysis, and anabolism in maternal tissues. PGH plays an important role in maternal adaptation to pregnancy, ensuring adequate fetal growth and development by inducing physiological IR [21,22]. An overproduction of PGH has been observed in GDM, but this phenomenon is not fully understood and deserves further studies [18].

Recent research has emphasized the impact of inflammatory markers in the development of GDM. One mechanism that could induce physiological IR in pregnancy is the reduction in adiponectin synthesis, a protein hormone with more properties such as insulin-sensitizing and anti-inflammatory. Adiponectin inhibits the insulin intracellular signaling pathway, contributing to IR [18]. In contrast, leptin produced by adipocytes, the ovaries, and the placenta inhibits insulin action [23]. GDM is characterized by elevated oxidative stress exceeding levels that are typically seen in normal pregnancies along with an inflammatory state. Elevated oxidative stress leads to increased production of inflammatory cytokines and stimulates the appearance of free radicals. In GDM, reactive oxygen species (ROS) levels are elevated while total antioxidant capacity (TAC) is reduced. Pancreatic beta cells are particularly sensitive to ROS, and oxidative stress can induce beta cell dysfunction through apoptotic events. Additionally, oxidative stress reduces cellular glucose uptake and contributes to IR [24]. The pro-inflammatory environment in GDM may be explained by an increase in platelet-based growth factor and the subsequent formation of other pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and chemokines (CXCL1, CXCL5, CXCL8, CCL2). During pregnancy, there is an elevated secretion of pro-inflammatory cytokines, which reduce insulin release from beta cells, impair insulin signaling, and contribute to IR [25,26].

It is crucial to detect hyperglycemia early in pregnancy because it can have harmful impacts on both the mother and the fetus, over both short and long periods**.**

Risk factors of GDM

Multiple factors contribute to the occurrence of GDM, including financial status, diet, lifestyle, physical activity, and history of diabetes in pregnant women's family or personal history of GDM [27]. The advanced age of the mother represents a significant risk factor. Women over 45 years old have a higher risk for hyperglycemia during pregnancy and associated complications [28]. It appears that women with a history of GDM may develop T2D within 5 to 10 years after childbirth, though not every woman with a history of GDM will progress to diabetes mellitus [2,29]. Excess weight and obesity, are also major contributing factors for GDM. Pregnant women with a higher body mass index (BMI≥ 29 kg/m²) are at heightened chance of developing GDM and experiencing pregnancy-related complications [30]. Excessive body weight has an important role in the deterioration of pancreatic beta cells. After a diagnosis of GDM, the most effective strategy for preventing T2DM is through lifestyle modifications or medications that improve biological outcomes and reduce IR [31]. Diet is another important factor: intake of fat-rich foods, sugary products, as well as red meat increases the likelihood of GDM, while a fiber-rich diet, micronutrients, and polyunsaturated fatty acids can reduce the risk. Additionally, a history of PCOS and multiparity significantly increases the likelihood of GDM and related metabolic complications [32]. Having a family history of diabetes or GDM during a previous pregnancy also raises the chance of developing GDM [2].

Pregnant women with at least one risk factor for diabetes should be early tested (<20 weeks) to establish or not the diagnosis of early GD. Once the diagnosis is established, the treatment must be initiated quickly [33].

Screening and diagnosis of GDM

Over time, the methods for diagnosing GDM have evolved from targeted screening solely on risk factors to the utilization of the oral glucose tolerance test (OGTT) for universal screening [34]. Advanced maternal age (over 30 years) is a major risk factor associated with GDM [28]. Women who have previously given birth to a baby weighing over 4 kilograms or had GDM in a prior pregnancy or are overweight or with a family medical previous occurrence of T2D are considered at higher likelihood for developing GDM. Additionally, women with polycystic ovary syndrome (PCOS) and those with pluriparity are at greater risk [35].

Glycemic targets during pregnancy are more stringent than those for nonpregnant women. According to ADA the screening for GDM is recommended between 24 and 28 weeks of gestational period among pregnant women who have not been before diagnosed with diabetes. Pregnant women with diabetes must consume consistent carbohydrates to establish their insulin doses and avoid both low and high glucose levels, as these conditions can adversely affect the fetus [36]. In 2010, the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study introduced other targets for OGTT, prompting a reassessment of the diagnostic criteria for GDM identified between 24 and 28 weeks of pregnancy. These thresholds have been endorsed by the WHO and Croatian Society for Gynecology-Obstetrics [37].

1. The One-Step Strategy (derived from the IADPSG criteria) involves a 75-gram OGTT. It is performed in the morning, after at least 8 hours of fasting. Blood glucose levels are evaluated at fasting, 1 hour, and 2 hours in individuals who have not been formally diagnosed with diabetes (Table 1). A positive assessment is established if glucose levels are achieved or excelled [36].

Table 1: The One-Step Strategy (adapted from [36])

Fasting	≥ 92 mg/dL (5.1 mmol/L)
1 hour	≥ 180 mg/dL (10.0 mmol/L)
2 hours	≥ 153 mg/dL (8.5 mmol/L)

2. The Two-Step Strategy involves an initial 50-gram OGTT (non-fasting) and followed by a 100-gram OGTT if the first test is positive according to Carpenter-Coustan criteria. The American College of Obstetricians and Gynecologists (ACOG) and the National Institutes of Health (NIH) recommend this strategy for diagnosing GDM [36].

Step 1: A 50-gram OGTT (non-fasting) is administered, and glucose level is measured an hour later. If the plasma glucose value is ≥ 130 mg/dL (7.2 mmol/L), 135mg/dL (7.5 mmol/L), or 140 mg/dL (7.8 mmol/L), the patient proceeds to Step 2.

Step 2: A 100-gram OGTT (fasting) is performed, with glucose levels assessed at fasting, 1 hour, 2 hours, and 3 hours. If two or more of the following glucose thresholds are reached, GDM is confirmed (Table 2).

Table 2: The Two-Step Strategy (adapted from [36])

Fasting	≥ 95 mg/dL (5.3 mmol/L)
1 hour	≥ 180 mg/dL (10.0 mmol/L)
2 hours	≥ 155 mg/dL (8.6 mmol/L)
3 hours	≥ 140 mg/dL (7.8 mmol/L)

The assessment of GDM is based on these standards adapted from [36], [38], [39].

Postpartum evaluation for women experienced GDM is recommended from 4 to 12 weeks after childbirth. This screening should use a 75-gram OGTT, and the diagnosis should be based on non-pregnancy criteria [36].

Complications while pregnant: for mother as well as newborn

Effects on the mother

Short-term complications. GDM can lead to several short-term complications during pregnancy. These include high blood pressure (hypertension), pre-eclampsia, spontaneous abortion, the need for a cesarean section, and difficulties during childbirth [9]. If the fetus is macrosomic, vaginal delivery becomes riskier. Instrumental delivery, using forceps or a vacuum, may be necessary, or an unexpected or emergency cesarean section might be required. Large babies increase the likelihood of perineal tears, vaginal lacerations, and other birth injuries [40]. Additionally, macrosomic births carry risks such as uterine atony, heavy bleeding, postpartum hemorrhage, and genital tract injuries. These risks are further heightened if the mother has previously had a cesarean section [13].

Long-term complications. In chronic complications, mothers experiencing GDM have high risk of recurrence in future pregnancies and a greater likelihood of onset of T2D in the future [2]. The HAPO (Hyperglycemia and Adverse Pregnancy Outcomes) research found that maternal elevated blood sugar during pregnancy was associated with a higher risk of early childbirth, cesarean delivery, macrosomia, congenital malformations, neonatal intensive care unit admission, hyperbilirubinemia, and neonatal hypoglycemia [41]. Generally, GDM resolves after childbirth.

Effects on the newborn

Fetal complications such as premature birth, hypoglycemia at birth, shoulder dystocia, Erb's palsy, congenital anomalies, and fetal macrosomia are common in pregnancy with GDM. Neonatal respiratory distress syndrome and low Apgar scores are also potential issues. This respiratory distress can be linked to alterations in the integrity and composition of fetal pulmonary surfactant, as GDM can delay the secretion of essential surfactant components, such as phosphatidylglycerol [42]. Infants born to mothers with GDM have a higher likelihood of evolving overweight and T2D later in life [14].

Fetal macrosomia is the most common difficulty associated with GDM. It is characterized by a birth weight of 4 kilograms or more. This condition is marked by an increase in the abdominal diameter and scapular area of the fetus [43]. Excess glucose, which crosses the placenta, serves as the primary energy substrate for fetal growth. Elevated maternal glucose levels lead to increased glycogen storage in the fetus, resulting in fetal hyperinsulinemia and an increase in adipose tissue [44,45]. Macrosomic newborns often exhibit asymmetrical growth, characterized by central deposition of subcutaneous fat around the abdomen and back, increased shoulder circumference, and more skin folds [46].

Treatment of late GD brings benefits to both the mother and unborn baby, but more recent studies highlight that the treatment started even earlier (in early GD) brings even more benefits, both in the long term and in the short term [47]. The Treatment of Booking Gestational Diabetes Mellitus (TOBOGM) clinical trial was the first study to evaluate the effectiveness of immediate initiation of treatment in patients diagnosed with early GD in terms of pregnancy complications. There was a significant reduction in the risk of neonatal adverse events in the group in which the treatment was initiated immediately [33].

Treatment (blood glucose monitoring, lifestyle changes, pharmacotherapy)

The main goal of treating GDM is to reach and sustain the optimal blood glucose range. Mothers must understand the condition and aim to manage their blood sugar levels effectively [9,13].

Blood glucose monitoring in pregnancy

In managing GDM daily monitoring of blood sugar at home is very important. Expectant mothers with diabetes should regularly check their glucose levels, including fasting and postprandial (after meal) readings. For those using basal-bolus therapy or insulin pumps, preprandial (before meal) testing is also recommended. ADA sets target values for blood sugar levels as follows: fasting - must be below 95 mg/dL (<5.3 mmol/L), 1-hour postprandial glucose should be below 140 mg/dL (<7.8 mmol/L), and 2 hours postprandial glucose should be below 120 mg/dL (<6.7 mmol/L). For managing hypoglycemia, thresholds are set at less than 70 mg/dL (<3.9 mmol/L) for blood sugar and less than 63 mg/dL (<3.5 mmol/L) for sensor glucose. Glycated hemoglobin (A1C) is used as an additional measure of glycemic control rather than for diagnosing GDM, as A1C levels can be influenced by changes in red blood cell exchange during pregnancy. Consequently, A1C may need to be monitored more frequently, such as monthly [9]. Factors like increased vitamin C intake accelerated red blood cell renewal, and higher iron demands during pregnancy can lower A1C levels [48,49]. Continuous glucose monitoring (CGM) is another valuable tool, providing real-time tracking of glucose levels throughout the day and night. This method helps in monitoring glucose fluctuations and offers data that can improve both patient and clinician management of GDM, enhancing patient compliance and reducing the discomfort associated with frequent finger pricks

[49].

Lifestyle changes

The first step in managing GDM involves lifestyle modifications, including nutritional support therapy, regular active lifestyle, and body mass control based on weight before pregnancy [9,13]. For many pregnant women, these changes may be sufficient to control GDM.

Nutrition. The dietary plan should ensure sufficient calorie intake to aid both the mother and the unborn baby, although the ideal calorie amount during pregnancy for women with GDM has not been established. The nutrition plan should be aligned with the recommendations of the National Academy of Medicine. It is suggested that the dietary reference intake for all expecting women includes a starting amount of 175 grams of carbohydrates (thirty-five percent of 2,000 calories per day), at least 71 grams of protein, and 28 grams of fiber [9, 50]. Emphasizing superior quality, carbohydrates rich in nutrients help to maintain regulated pre-meal and post-meal glucose levels, whereas simple carbohydrates can lead to higher post-meal glucose excursions [9]. A diet rich in whole foods, such as fresh produce, veggies, fruits, beans, whole cereals, and healthy fats (including omega-3 fatty acids from nut products, seeds, as well as fish) is highly recommended. Processed foods, sweetened foods, beverages, and consumption of fatty red meats should be reduced. An expert panel on nutrition advises a balanced intake of primary nutrients and cautions against very restrictive diets, trans fats, ketogenic diets lacking carbohydrates, dairy-restricted diets, and those high in saturated fats [9,51].

Physical activity can improve blood glucose control and decrease the need for insulin. Recommended exercises include aerobic activities, resistance training, or a combination of both. The duration should last between 20 to 50 minutes daily, 2 to 7 days a week, at medium force [9,52].

Pharmacotherapy

The next step in managing GDM involves the use of medication. Up to 30 percent of women with GDM will require pharmacotherapy [53]. Insulin is the preferred pharmacological treatment for GDM when lifestyle modifications (diet and exercise) fail to achieve blood glucose targets. Due to its large molecular size, insulin does not cross the placenta, being safe for the fetus [9,54]. The insulin doses must be calculated according to glycemic values and the amount of carbohydrates consumed. Long-acting insulin (detemir or glargine) may be used for basal glucose control. Rapid (lispro or aspart) or short (regular) insulin is used to control elevated post-prandial blood sugars [55,56,57]. In addition, short-acting insulin analogs have a lower risk of hypoglycemia and offer greater flexibility in administration compared to human insulin [58]. For pregnant women with both higher fasting and postprandial blood sugar basal-bolus is used: 50% of the total daily dose of long-acting insulin and 50% of the total daily dose of short or rapid-acting insulin. In the first trimester of pregnancy, insulin is initiated with 0.7 U/Kg/day and can reach up to 1U/kg/day until birth. In some cases of pregnancy, intermediate-acting insulin (neutral protamine Hagedorn (NPH)) can also be used: 2/3 of total daily insulin before breakfast and 1/3 of TDD before dinner [55,56, 57]. Recently ultra rapid-acting insulin aspart (Fiasp) and lispro (Lyumjev) have received approval for use in pregnancy [58]. Emerging yet limited evidence suggests that insulin degludec may be suitable for use in pregnancy [59]. Also, the data regarding the effectiveness and risks of glulisine and glargine U-300 insulin administration during pregnancy is still limited [4]. Insulin pumps are not yet recommended for women with GDM because the clinical data is still limited [58].

Oral medications that have been researched include metformin and glyburide. These oral antidiabetic drugs do pass through the placental membrane and should not be used as first-line treatments. Sulfonylureas, such as glyburide, have been linked with elevated risks of neonatal hypoglycemia, macrosomia, and a larger abdominal circumference [9,54]. Fetuses exposed to metformin have shown smaller head and chest circumferences, reduced birth size, and decreased lean mass [60]. These oral antidiabetic drugs can impact fetal development and growth.

CONCLUSIONS

GDM is an increasingly common complication with negative outcomes for both the mother and the unborn child. It is described by the body's lack to compensate for the physiological IR that occurs during pregnancy. Although many factors of risk contribute to the development of GDM. IR looks to be the primary factor which in turn triggers other mechanisms. The placenta also plays an important role in the onset of GDM. Inflammatory markers, along with placental hormones, are involved in this process which is not yet fully understood. These changes impact fetal development and growth, making early diagnosis and prompt treatment of GDM crucial to reducing the risk of complications.

Additionally, early postnatal preventive approaches should be considered in reducing the development from GDM to T2D, thereby increasing awareness of this condition during pregnancy. However, there are still many steps to be taken towards establishing an international prevention strategy and accurately determining the mechanisms involved in the development of GDM.

Conflicts of interest and sources of funding

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Ethics approval and consent to participate

Not applicable.

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REVIEW

The Amazing History of Methotrexate – 75 Years Later from its Discovery, Still the "Golden Standard" Therapy

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Abstract: *More than 75 years after its accidental discovery, methotrexate remains an important treatment option for many diseases. Whether it is various hematological and non-hematological neoplasms, rheumatological, dermatological, or other conditions, methotrexate remains in scope. For rheumatoid arthritis, current clinical guidelines (EULAR, ACR) recommend methotrexate as the first therapeutic option. This article aims to highlight important moments in the history of this remarkable drug, to review the literature on its mechanisms of action and the arguments for which after more than half a century, methotrexate remains the gold standard in the treatment of rheumatoid arthritis but also an important option for treatment of psoriasis vulgaris (PV) and psoriatic arthritis (PsA).*

Keywords: *methotrexate; treatment; rheumatoid arthritis; psoriasis; history*

THE HISTORY OF METHOTREXATE

Most people link methotrexate (MTX) history with researcher names like Farber or Gubner. The history of methotrexate begins a few years earlier, in 1945 when American researcher of Indian origin, Subbarow (forgotten or too little known) first isolated folic acid from the liver, in the laboratory of the pharmaceutical company that would later become Lederlee [1].

In the same year, the researcher and his team also discovered a microbial source from which they subsequently synthesized folic acid. Administration of "this preparation of folic acid" to laboratory mice with tumors was followed by tumor regression. This finding was opposite to the hypothesis that deprivation of tumor cells of folic acid leads to suppression of tumor growth. But at the time, they did not know that what they had injected into the mice was in effect a slightly different compound – a folic acid antagonist that led to the deprivation of folic acid in tumor cells, disrupting their DNA synthesis and causing tumor regression.

Encouraged by the laboratory results, Farber fast organizes a clinical study for patients with leukemia, to whom he administers... folic acid. As you can imagine, the results were devastating due to accelerated tumor growth. But this accidentally wrong lead, helped Farber confirm the hypothesis that folic acid deprivation of tumor cells leads to the suppression of their growth.

Farber returns to Subbarow and emboldens him to continue his research and Subbarrow ended up synthesizing two new molecules, aminopterin, and amethopterin, which competitively inhibit folic acid synthesis.

Farber successfully treats acute leukemia in children with these two molecules and publishes the results in his 1948 paper [2].

Aminopterin molecule proves to be quite unstable and difficult to synthesize. Therefore, an amethopterin analog becomes the star that is later called METHOTREXATE. Concerned about the potential side effects, Farber considered it wise to add, in the name of the substance, to met = metabolism and an "x" that was usually found, at the time, on poison bottles [1].

The first report of the use of methotrexate in rheumatoid arthritis appeared in 1951 when Gubner (who was a cardiologist) et al. found that 86% of rheumatoid arthritis patients treated with methotrexate and receiving a clinical response, relapsed after its discontinuation.

Gubner and colleagues have shown that aminopterin mimics the actions of cortisone with the effect of an anti-metabolite of folic acid and with anti-inflammatory and anti-proliferative responses. This was considered to be the likely explanation for the beneficial effects of these two drugs in a spectrum of unrelated clinical and experimental conditions in which the common denominator was an inflammatory and proliferative connective tissue response [3].

At this point, it should be said that Gubner's 1951 paper on the use of methotrexate in rheumatoid arthritis was received at that time with the question "Why use such a toxic anti-cancer drug to treat a benign condition such as rheumatoid arthritis?" The reason behind this statement was that despite the fact they were using low doses of methotrexate for the treatment of rheumatoid arthritis and psoriasis, the frequency of administration was daily or several times a week, which increased MTX toxicity [1].

In 1973, a group of Scandinavian dermatologists described several major side effects of methotrexate use, including liver cirrhosis in patients diagnosed with psoriasis receiving methotrexate several times a week- without pointing out that those patients had unrestricted access to alcohol and were previously exposed to arsenic (which was still being used to treat psoriasis at the time).

Despite all of these, methotrexate received FDA approval for psoriasis in 1972 and rheumatoid arthritis in 1988.

MECHANISMS OF ACTION

Methotrexate (MTX, amethopterin, Figure 1) is a synthetic organic compound, part of an anti-folate therapeutic group.

Figure 1: Adapted from Koźmiński P, Halik PK, Chesori R, Gniazdowska E. (with permission) [5]

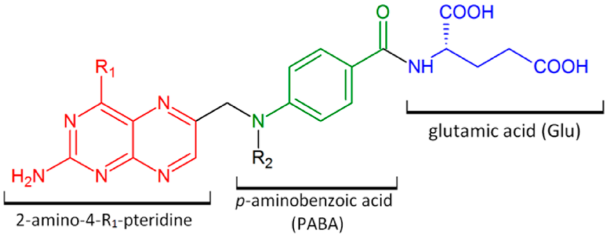

Folic acid	R_1= OH	R_2= H
Aminopterin	R_1= NH_2	R_2= H
Methotrexate	R_1= NH_2	R_2= CH_3

Methotrexate mechanisms of action are not yet completely understood after all these years. It is clear now that given in low doses (mg) methotrexate is used for the treatment of autoimmune and inflammatory diseases (such as rheumatoid arthritis, psoriasis, myasthenia gravis, Crohn's disease, multiple sclerosis, polyarticular juvenile idiopathic arthritis) [4]. On the other hand, high doses of methotrexate (up to grams) are commonly used for the treatment of various neoplasms (acute lymphoblastic leukemia, acute myeloid leukemia, meningeal leukemia and lymphoma, osteosarcomas, non-Hodgkin's lymphoma, also breast, bladder, and several other cancers).

Screening the available literature, we chose an overview published in 2020 by Koźmiński P. et al [5] explaining the dual action of methotrexate (MTX). According to this paper, MTX indirectly inhibits cell division through the blockage of folate-related enzymes, mainly DHFR, that catalyzes the conversion of dihydrofolate to tetrahydrofolate (THF). THF serves as a significant coenzyme in several transmethylation reactions in pyrimidine and purine nucleotide synthesis pathways, essential in the synthesis, repair, or replication of DNA strands [6,7]. The methyl-THF acts as a proximal methyl donor in numerous methylation reactions of DNAs, RNAs, proteins, phospholipids, and amino acids syntheses. Inhibition of intracellular THF production by MTX results in the disruption of cell proliferation and its metabolic imbalance.

MTX crosses the biological barriers very poorly, being highly ionized and generally hydrophilic. The bioavailability and biodistribution of the drug are determined by an active transport system. Intestinal tissue adsorption of MTX occurs by the proton-coupled folate transporters (PCFTs), which are a solute carrier transporter, while a cellular drug penetration is followed mainly by the reduced folate carrier 1 (RFC1), an APT-binding cassette transporter. To a small extent, MTX also uses receptor-mediated endocytosis via folate receptors (FRs), the glycosyl-phosphatidyl-inositol (GPI)-anchored membrane proteins that may internalize bound folates and folate conjugates [8,9]. Intracellularly, MTX is metabolized by folylpolyglutamyl synthase (FPGS) to polyglutamate derivatives (MTXGlu), which show significantly increased cell residence time and bioactivity in comparison to initial MTX form [10,11,12] (Figure 2). This is a key pharmacokinetic step that determines the attributed effect of this drug, defining MTX as a representative type 1 prodrug, that undergoes bioactivation inside the cell. Polyglutamated MTX is a superior anti-folate agent to MTX, capable of highly potent DHFR inhibition. Moreover, it also induces inhibition of other enzymes like thymidylate synthase (TYMS), 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICART) [13, 14], and amido-phosphoribosyltransferase [15,16], participating in de novo biosynthesis of purine and pyrimidine nucleotides. Consequently, it is MTXGlu that deprives a cell of precursors for the synthesis of DNA and RNA necessary for cell proliferation (Figure 2), leading to DNA synthesis disturbances and subsequent cell apoptosis.

Figure 2: Adapted from Koźmiński P, Halik PK, Chesori R, Gniazdowska E. (with permission) [5]

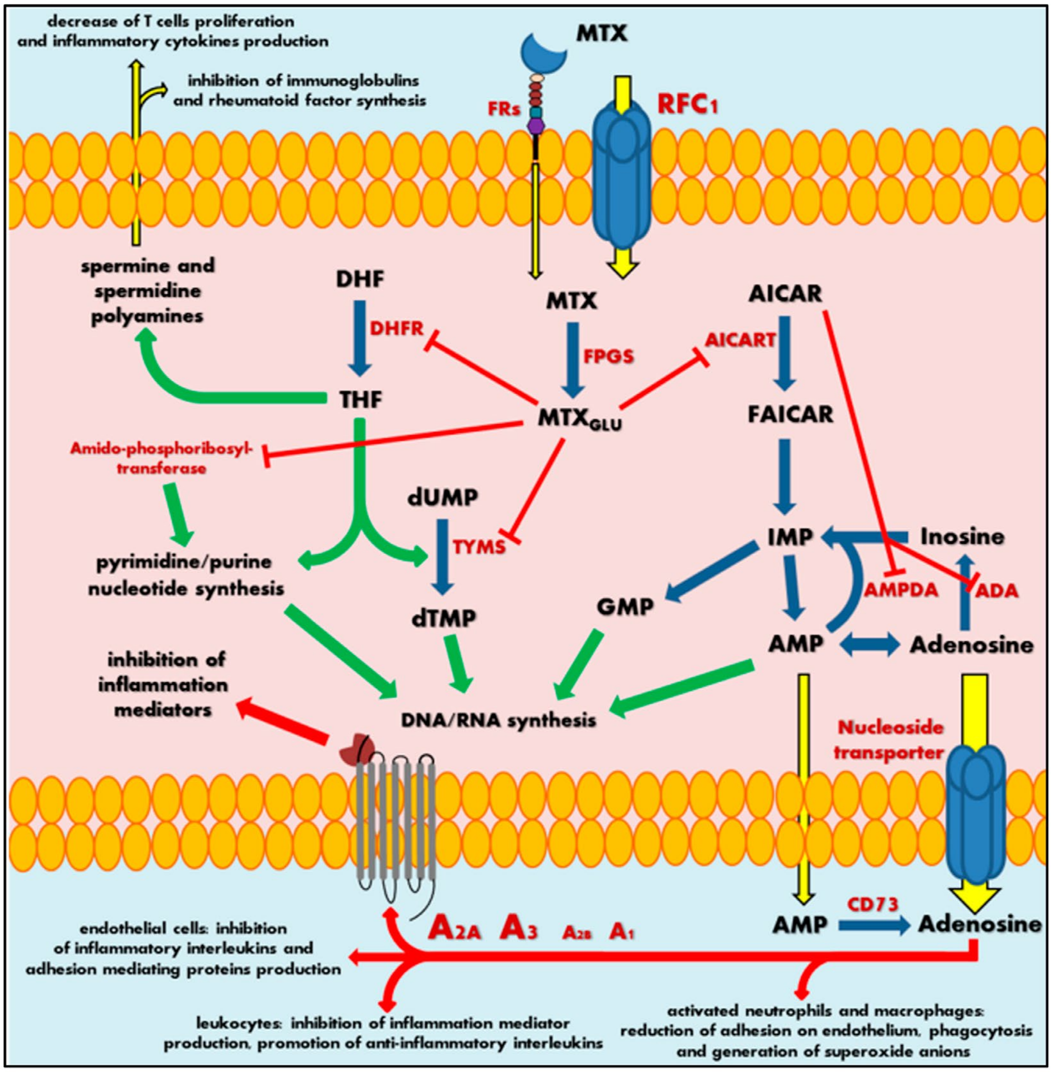
decrease of T cells proliferation
and inflammatory cytokines production
inhibition of immunoglobulins
and rheumatoid factor synthesis
MTX
FRs
RFC1
spermine and
spermidine
polyamines
DHF
DHFR
THF
MTX
FPGS
MTXGLU
AICAR
AICART
FAICAR
Amido-phosphoribosyl-
transferase
dUMP
TYMS
dTMP
pyrimidine/purine
nucleotide synthesis
IMP
Inosine
AMPDA
ADA
GMP
AMP
Adenosine
inhibition of
inflammation
mediators
DNA/RNA synthesis
Nucleoside
transporter
A2A
A3
A2B
A1
AMP
CD73
Adenosine
endothelial cells: inhibition
of inflammatory interleukins and
adhesion mediating proteins production
leukocytes: inhibition of inflammation mediator
production, promotion of anti-inflammatory interleukins
activated neutrophils and macrophages:
reduction of adhesion on endothelium, phagocytosis
and generation of superoxide anions

MTX activity is most visible in actively dividing cells, mainly in the S phase of the cell cycle, and in fact, highly proliferating cancer cells are the most susceptible to the cytotoxic effect of this drug, indicating that antagonism of folate is related to the anti-tumor activity of MTX.

At the same time, the continuous action of MTX polyglutamates in cellular biochemistry results in intracellular accumulation of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) by AICART inhibition. AICAR can diminish the activity of adenosine deaminase (ADA) and adenosine monophosphate (AMP) deaminase (AMPDA) as well [16] (Figure 2). An MTX-mediated excess of AICAR promotes the AMP and adenosine increase and subsequent release of these adenine derivatives outside the cell. Extracellularly, AMP follows easily dephosphorylation to adenosine via ecto-5'-nucleotidase, also known as the CD73 enzyme. Adenosine, a significant signaling agent that modulates diverse physiological functions, acts as the major mediator of anti-inflammatory action associated with MTX. In the local extracellular space, adenosine might interact with its specific receptors (A1, A2A, A2B, or A3) present on the surface of the original tissue, but also on immune system cells (Figure 2). Adenosine receptors belong to the seven-transmembrane receptor family, mediating signals into the cell by coupled specific G proteins depending on the receptor subtype. In RA patients, adenosine-specific receptors on immune and synovial cells are elevated, especially A2A and A3 that highly mediate adenosine regulation of immune response and inflammation [17,18,19,20]. In psoriasis, there is a correlation between A2B and A3, mast cells, and inflammation especially mediated by TNF-alpha, and through A2A influences Treg activity [21]. The adenosine action on A2A and A2B stimulates intracellular production of cyclic adenosine monophosphate (cAMP), a significant cellular second messenger, while stimulation of A3 induces phospholipases C and D or inhibits adenylate cyclase producing cAMP. As a result, adenosine in activated neutrophils and macrophages reduces their ability to adhesion to endothelium and phagocytosis as well as generation of superoxide anions and further reactive oxygen species [22,23]. Moreover, leukocytes decrease the production of tumor necrosis factor α (TNF-α) and interleukin (IL)-12—the mediators of inflammation—and promote anti-inflammatory IL-4 and IL-10. Additionally, it is observed that inhibition of IL-1 action and suppression of TNF-α excretion in T cells and macrophages inhibit the prostaglandins and leukotrienes syntheses [24,25,26]. At the same time, adenosine disrupts the cytokine-mediated inflammation in endothelial cells by the decrease in the production of IL-6 and IL-8 and hindering leucocytes' adhesion [27]. Thus, an adenosine-mediated effect seems to be a key mechanism in MTX anti-inflammatory action. Nevertheless, the MTXGlu also has some other impact on immune chemotaxis and reduction of the occurrence of inflammation mediators. A sustained decrease of THF-mediated methylation downregulates an accumulation of spermine and spermidine polyamines in the extracellular fluids and lymphocytes of patients with RA [28,29]. These polyamines are essential cellular growth factors among others in lymphocytes. MTX action inhibits T cell proliferation and synthesis of immunoglobulins or rheumatoid factor in RA patients [30,31], followed by a local reduction in lymphocytes interferon (IFN)-γ and IL-2 production, decrease of inflammation, convergent to adenosine receptor-mediated effects.

All of the above indicates that MTX has a dual mechanism of action. Potent anti-metabolic and anti-proliferating actions are related to the therapeutic use of MTX as an anticancer agent. The action leading to a local increase of the adenosine concentration at the site of inflammation and also the specific immunosuppressive effect of MTX play an important role in the treatment of severe inflammation and autoimmune diseases (arthritis, psoriasis, and myasthenia gravis).

THE USE OF METHOTREXATE IN RHEUMATOLOGY

There are strong arguments for which methotrexate is still the golden standard in the treatment of rheumatoid arthritis:

1. According to EULAR recommendations for the management of rheumatoid arthritis, methotrexate (MTX) should be part of the first treatment strategy. MTX remains the anchor drug in RA; not only is it an efficacious csDMARD by itself but it is also the basis for combination therapies, either with GC or with other csDMARDs, bDMARDs, or tsDMARDs. MTX (whether administered orally or subcutaneously) should be escalated to a weekly dose of about 0.3 mg/kg and this escalation should be done within 4–6 weeks [32].

2. There are nowadays available, hundreds of clinical trials proving the clinical efficacy and safety profile of methotrexate, used in monotherapy or combination therapy. The vast majority of bDMARDs clinical trials are done in fact with the combination treatment MTX + bDMARDs.

3. In Romania, the therapeutical protocol for rheumatoid arthritis stipulates that methotrexate is the conventional synthetic remission medication of the first choice, except in cases where there are major contraindications, in the usual maintenance dose: 20 mg/week (depending on tolerance), usually orally. To increase tolerance, the combination of folate is usually recommended, and injection (subcutaneous or intramuscular) of methotrexate should be considered to increase bioavailability and reduced the risk of digestive side effects (along with domperidone and antiemetics: ondansetron or granisetron). Depending on the particularities of the case treated and the degree of activity of the disease, the attending physician formulates the treatment and indicates these remissive preparations, which can be used alone or in combination, and the combination should usually include methotrexate. Initiation of bDMARDs can be done in cases of rheumatoid arthritis that did not respond to conventional synthetic remission therapy, administered correctly (both doses and durations of therapy), respectively after the use of at least 2 conventional synthetic remission therapies, with a duration of at least 12 weeks each, one of which is usually methotrexate (except cases with a major contraindication to this preparation or cases which do not tolerate this treatment, as appropriate documented). As a rule, any targeted biological therapy (bDMARDs) and synthetic therapy (tsDMARDs) is recommended to be administered in combination with a conventional synthetic remission (usually only one, most commonly used being methotrexate, for which a minimum dose of 10 mg/week is recommended), which is maintained even after the initiation of bDMARDs or tsDMARDs

[33].

4. MTX is available in the form of tablets with increments of 2.5, 5, or 10 mg/tablet, and as prefilled syringes or auto-injectors with various doses per injection, allowing titrating weekly doses to pay attention to the patient's tolerability. Oral and (SC) are the preferred routes used in rheumatology patients. Oral MTX at a dose of 7.5 mg/week has 100 percent bioavailability. However, bioavailability is reduced by 30% starting at just 15 mg/week due to gut absorption limitations [34,35]. By contrast, the bioavailability of SC MTX is greater than that of oral MTX and does not depend on the MTX dose with a linear increase in systemic exposure for increasing doses. RA patients with an inadequate clinical response to orally administered MTX may benefit from the higher drug exposure offered when switched to the SC formulation [36]. Initiating the MTX regimen with SC administration has been associated with better clinical outcomes at the same dosages [37].

5. There is enough evidence that the association of MTX to bDMARDs is followed by bDMARDs reduced immunogenicity [38].

6. Treatment with MTX is highly cost-efficient.

Of course, treatment with MTX is not free of challenges. The major ones are related to MTX toxicities which can be prevented by folic acid supplementation. The good news is that MTX toxicities are well-known and therefore manageable. One other challenge related to MTX treatment is transforming the patient into a physician's partner. Setting for the patients the right expectations on treatment benefits, mostly related to time-course of the clinical benefits, will improve patient compliance and adherence to therapy and monitoring, and eventually avoid unnecessary premature discontinuation of MTX. The EULAR guidelines emphasize that the best outcomes with the use of MTX are achieved when a shared decision-making approach to treatment is used in which the patient is an active participant. Patients need to understand and take responsibility for following all physicians' recommendations.

THE USE OF METHOTREXATE IN DERMATOLOGY

The prevalence of psoriasis in Romania is 4.9% according to the prevalence study carried out a few years ago [39,40]. In Romania, the therapeutical protocol for psoriasis stipulates that methotrexate is the conventional synthetic remission medication of the first choice, except in cases where there are major contraindications or high risk. Only non-responders, after at least three to six months of treatment, are allowed to switch to biological therapy [41]. We use the usual maintenance dose: 15-25 mg/week orally or through subcutaneous injection. We prefer the injection because it has increased bioavailability and reduced the risk of digestive side effects [36,37]. To increase tolerance, the combination of folate is usually recommended in a single dose of 5 mg/week, one day after taking methotrexate. In the case of biological therapies, methotrexate can be associated with but is not a current practice. It is possibly associated in the event of the appearance of neutralizing antibodies to increase the persistence and effectiveness of biological therapy [42]. These are less common situations when we combined the two therapies for synergistic and complementary action.

Inflammation in psoriasis very often develops or worsens associated diseases [43,44,45]. For these reasons, we recommend the early start of systemic treatment. According to data from Romania, 31% of patients with psoriasis have associated hypertension, 22% dyslipidemia, 17% obesity, and 10% diabetes. By reducing inflammation, methotrexate therapy may decrease the risk of associated cardiovascular disease, although not to the same extent as it does with biological therapies [46,47,48,49]. Methotrexate is a solution for controlling patients with psoriasis and metabolic comorbidities, but additional precautions are needed at least for overweight, obese or diabetic patients [50,51]. For this reason, in a holistic approach to the patient, we must consider additional monitoring for these patients.

CONCLUSION

In conclusion, MTX is a remarkable drug with a unique place in the pharmacotherapeutic management of RA. There is now enough clinical experience to aim to achieve the best outcomes from MTX use.

MTX has a large dose titratable range and can be administered as a once-weekly dose regimen by either oral or subcutaneous routes. Parenteral administration of MTX has the advantages of maximizing bioavailability, reducing gastrointestinal intolerance, and potentially enhancing compliance and adherence.

For the treatment of rheumatoid arthritis, MTX is recommended as the first-line csDMARD treatment and can also be used in combination with other csDMARDs, bDMARDs, or tsDMARDs. In the case of concomitant use with targeted therapies, MTX facilitates optimum achievable efficacy and also has the advantage, when used with bDMARDs, of reducing immunogenicity. For the treatment of psoriasis, it is a first-line option (the most frequently used) but also an alternative in association with biological treatment. MTX is also highly cost-effective and has a favorable benefit/risk profile overall.

Conflicts of interest and sources of funding

The author declares no conflict of interest.

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REVIEW

Advances in Squamous Cell Carcinoma Antigen and Cervix Cancer Relationship

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Abstract: *The squamous cell carcinoma antigen is a tumor marker that is receiving more interest due to its biological properties and importance in both pathological and normal physiological processes. Numerous clinical studies have been conducted to determine the potential function of squamous cell carcinoma antigen and its isoform complexes in clinical practice. These studies have been prompted by the fact that not only squamous cell carcinoma antigen but also circulating immune complexes of it and immunoglobulin M are involved in normal physiological and pathological processes. The therapeutic use of squamous cell carcinoma antigen as a tumor marker for either squamous carcinoma diagnosis or for monitoring the response to radiation or chemotherapy, tumor recurrence, and treatment failure are supported by prior investigations. The diagnostic or prognostic utility of squamous cell carcinoma antigen is debatable, nevertheless, as these investigations provide conflicting findings. A uniform detection method, scoring system, and cutoff level must be devised to limit clinical variability between studies and to provide a more accurate and trustworthy comparison of data. Additionally, even if the effectiveness of several approaches is equivalent, only one method should be used for the dynamic monitoring of tumor marker kinetics.*

Keywords: *uterine cervix disorders, squamous cell carcinoma, markers, diagnosis, treatment*

INTRODUCTION

Worldwide, cervical cancer (CC) is the third most frequent malignancy in women. Approximately 520,000 women are diagnosed with CC each year, and 260,000 of those women pass away from the illness. The majority of CC-prone nations are developing nations, where access to healthcare is poor and resources are scarce. On the other hand, industrialized nations often employ health programs that call for immunization, screening, and treatment. As a result, the incidence of and death rates for CC are lower in these nations. The presence of the squamous cell carcinoma antigen (SCCA) tumor marker was examined in the serum of 103 individuals who had been diagnosed with squamous cell cervical cancer based on histology. The majority of cases (75%) dropped below 7.9 ng/ml even though values varied from 0.2-109.7 ng/ml. Clinical stage and SCCA readings have no association with one another. Its use as a diagnostic screen would be prohibited by the significant false negative rate (29%) of this marker in individuals with the established illness. The cellular lining of the cervix contains CC. Transformation zones, which are the areas where the endocervix and exocervix intersect, are where precancerous lesions usually first appear.

Squamous cell carcinoma (SCC; roughly 80%) and adenocarcinoma (ADC; around 5–20%) are the two subtypes that make up CC [1-3]. The earliest age at first sexual contact or first pregnancy, smoking status [4,5,6] long-term oral contraceptive usage [7], and a weakened immune system [8] are only a few of the risk factors that contribute to cervical carcinogenesis. Additionally, a person's vulnerability to CC is influenced by their family history and genetic makeup [9,10]. However, human papillomavirus (HPV) is recognized as the primary cause of CC and is found in almost all tumors [11,12].

Patients with CC in its early stages typically don't exhibit any symptoms.

Patients who have advanced cancer may experience one or more of the symptoms listed below, including vaginal bleeding, pelvic discomfort, atypical vaginal discharge, and pain during sexual activity. The number of people who will develop invasive CC may thus be reduced by effective screening and diagnostic techniques. Previously, it has been demonstrated that epigenetic modifications such as DNA methylation, histone modification, and microRNAs (miRNAs) have a role in the emergence of cancer [13]. Overexpressed miRNAs that are associated with cancer may act as oncogenes. MiRNAs that are active in normal tissue as tumor suppressors may be downregulated in cancer [14,15]. MiRNA expression patterns and tissue and cell lineage specificity have shed light on pathognomonic information [16]. Individual susceptibility to cancer is influenced not only by miRNAs but also by miRNA-associated single nucleotide polymorphisms (miRSNPs). The most promising route for developing CC-markers was highlighted in this review by our attention to changes in miRNA expression in CC.

TUMOR MARKERS FOR CC

Clinically helpful tumor indicators include soluble glycoproteins, which are substances that may be discovered in blood, urine, or other bodily fluids and are linked to certain cancer types. The advantages of tumor markers are shown in the screening of individuals with high-risk factors, the diagnosis and prognosis of certain malignancies, the assessment of therapy outcomes, and the identification of recurring illnesses [28]. We categorize tumor markers for CC screening into 2 types, including tumor indicators that are readily available and utilized frequently in laboratories and new proteins that are related to CC as tumor markers under research.

SCCA MEASUREMENT

Due to the critical role that SCCA plays in the diagnosis and prognosis of SCCs and other disorders, the demand for routine clinical SCCA testing has been rising quickly. The advancement of biotechnology has also led to improvements in detection techniques. Because biotechnological products have a limited level of biochemical variety, it has been possible to develop monoclonal antibodies that are specific for either SCCA1 or SCCA2, which allows for individual assessments of each rather than total SCCA. There are several popular techniques for determining the concentrations of SCCA in blood and tissue, including radioimmunoassay, ELISA, Western blot (WB), immunohistochemistry (IHC), and immune-luminometric assay. Radioimmunoassay was one of the earliest techniques to identify SCCA [17-18]. Radioimmunoassay is a sensitive and trustworthy method, but due to its various drawbacks, such as radiation exposure and low reagent stability, it is increasingly losing popularity.

The most popular technique for identifying SCCA was the ELISA test, which uses an antigen-antibody interaction and a color shift to determine protein levels. However, ELISA also has drawbacks, such as poor reproducibility, a constrained linear range, poor detection efficiency, and time-consuming experimental methods; as a result, it is insufficient to fulfill clinical demand. In order to assess protein expression, the basic idea behind WB is to use particular antibodies to target proteins in biological tissues or cells during gel electrophoresis. The molecular size of the targeted proteins is then compared to a reference ladder. The application of WB for large-batch testing in the clinic has also been constrained by the intricate experimental processes and high criteria for it. However, WB continues to be a method of choice in research investigations due to its high specificity and accuracy. IHC, on the other hand, is a technique for in situ qualitative or quantitative component identification of cells or tissues using the concepts of immunology and histochemistry. Immunohistochemistry and WB are both only appropriate for tiny samples.

SCCA levels have recently been measured using automated non-radioimmunoassay techniques. The Abbott ARCHITECT i2000 system and the Roche Cobas e602 system are two typical automated systems used in clinical laboratories for chemiluminescent microparticle immunoassays (ECLIAs) and ECLIAs, respectively. The measured SCCA levels are allegedly greater with the Roche system than with the Abbott system, despite the two systems having a significant positive correlation (r = 0.9658) [19,20]. These variations might be explained by the varied heterophilic antibodies and serum proteins utilized [21,22,23] as well as by the many manufacturer-specific variations in the reactivities of the individual antibodies towards the antigens. According to reports, the Roche system can identify SCCA1 and SCCA2 in an equimolar way [24], but the Abbott system is unable to establish the relative proportions of SCCA1 and SCCA2 [25] or can only detect SCCA1 but not SCCA2 [26]. In terms of SCCA detection, automated ECLIA and manual ELISA have both demonstrated equivalent capabilities [27]. The dynamic monitoring of tumor marker kinetics should be carried out using a single approach notwithstanding equivalent performances across several methods.

LEVELS OF SCCA IN CANCER AND INFLAMMATION

Numerous studies have been conducted on the biological roles and relevance of SCCA in both healthy physiological and pathological processes. Studies examining the possible function of SCCA, SCCA isoforms, and SCCA-Immunoglobulin M (IgM) complexes in clinical practice have been prompted by the discovery of circulating immunological complexes of SCCA and IgM in addition to SCCA that have been observed in typical physiological and pathological processes [29].

A new investigation on the connections between SCCA and 39 other clinically defined disorders demonstrates that SCCA is a clinically utilized biomarker not just for SCC but also for other human diseases [30].

CERVICAL CANCER

In the clinical diagnosis and treatment of cervical cancer, imaging techniques such as computed tomography (CT) and positron emission tomography (PET)/CT have become more widely used; nevertheless, these techniques have drawbacks, such as the potential for false-negative and false-positive outcomes [31,32].

However, the combined PET/CT and serum SCCA approach has greater diagnostic adequacy in the early detection of recurrent cervical cancer than either PET/CT or serum SCCA methods alone [33]. The identification of early postoperative metastases and recurrence in cervical cancer is therefore clinically benefited by this combination approach.

According to Ryu HK et al., SCCA levels > 1.86 and > 0.9 ng/mL were the ideal threshold values for predicting recurrence before and after therapy, respectively, and were strongly related to poor disease-free survival (DFS). Therefore, following full remission therapy, closer observation is required due to the increased chance of cancer recurrence [34]. Another study found that the optimal cutoff values for SCCA at pretreatment, treatment, and recurrence were 4, 1.5, and 4 ng/mL.

The recurrence and survival predictive values of SCCA at post-treatment and recurrence are only significant when pretreatment SCCA levels are 4 ng/mL, according to this study [35]. According to multivariate analysis, SCCA levels below 2.75 ng/mL can be employed as a possible marker to predict LNM in early-stage cervical cancer before surgery, especially for surgical treatment of early-stage cervical SCC (stages IB-IIA) [36].

COMBINATION OF SCCA WITH OTHER MARKERS

Many studies have assessed the efficacy of combining the detection of SCCA with other markers such as CRP, albumin, and noncoding RNAs for clinical application, in addition to the combination of SCCA and AFP in HCC. According to one research, individuals with cervical cancer who experienced recurrence had considerably higher blood levels of SCCA, hypersensitive C-reactive protein (hs-CRP), and CA125 than those who did not. There was also a statistically significant positive connection between SCCA and hs-CRP as well as SCCA and CA125 in the recurrence group of patients [37]. The DFS and OS of oral SCC patients are negatively impacted by preoperatively elevated CRP and SCCA levels [38]. As independent indicators for LNM, advanced tumor stage, and disease-specific survival in patients with penile SCC, preoperative CRP and SCCA levels were also discovered [39].

Patients with high SCCA and low albumin levels may not survive as well after surgery for esophageal SCC with stage T13N0M0, according to preoperative blood SCCA and albumin levels [40]. Both miR-215 and SCCA-IgM are more effective in diagnosing HCC than AFP, especially when it comes to separating cirrhotic patients from those with HCC with AFP levels below 200 ng/mL and small focal lesions [41].

Furthermore, coupled pretreatment serum circulating tumor cells (CTC) and pretreatment SCCA show greater predictive accuracy compared to FIGO stage and serum CTC or SCCA alone [42].

CLINICAL USE OF TUMOR MARKER

The serum marker for SCCs now used in clinical settings is called squamous cell carcinoma antigen (SCC-Ag). In between 20% and 60% of individuals with early-stage CC, it is present in high quantities. In 64% of patients with SCC and 25% of patients with ADC, abnormal levels of SCC-Ag are seen [43,44].

The tumor diameter and illness stage are related to the level of SCC-Ag before therapy [45]. The blood level of SCC-Ag after therapy indicates how well the treatment worked. Additionally, SCC-Ag rises in people who have tumor recurrence [46,47]. According to Ryu et al. [49], a post-treatment SCC-Ag value of more than 0.9 ng per mL is the ideal cut-off value for predicting recurrence in patients with pretreatment SCC-Ag levels larger than 1.86 ng per mL. This marker has a sensitivity range of 56% to 86% and a specificity range of 83% to 100% for CC recurrence [48]. An indication of a gynecological malignancy is the cancer antigen 125 (CA-125). The preferred tumor marker is now used to follow women receiving ovarian cancer treatment on a regular basis and to calculate patient survival rates [49,50]. Additionally, changes in CA-125 levels are a strong indicator of how advanced endometrial cancer will turn out [51,52]. Precancerous lesions can be identified using elevated levels in serum and cervical or vaginal secretions [53]. Ngan et al. [54] discovered that higher illness stages for CC were associated with greater levels of CA-125. In patients with ADC and SCC, abnormal CA-125 levels were found in 42.6% and 18.9% of patients, respectively.

CONCLUSION

Although there have been some discrepancies in the results due to different distributions of SCCA isoforms, previous studies have shown the clinical utility of the tumor marker SCCA for diagnosis or monitoring of the response to radiation or chemotherapy, tumor relapse, and treatment failure. As a result, there is still debate concerning SCCA's diagnostic and prognostic capabilities. A uniform detection technique, scoring system, and cutoff level are required to lessen clinical variability between studies and to enable more accurate and trustworthy comparisons. Despite similar performances of various approaches, the dynamic observation of marker kinetics should always be carried out using the same method.

Disclosure statement

This statement is to certify that all Authors have seen and approved the manuscript being submitted. The authors have no relevant financial interest in this article.

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All authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the Romanian Journal of Military Medicine.

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ORIGINAL ARTICLE

Dichotomisation of Rotator Cuff Tendinopathy in Shoulder MRIs Reveals the Need for Further Diagnostic Improvements: A Cohort Study

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Abstract: *Background: Rotator cuff tendinopathy is most often described as a continuum between the normal cuff and rotator cuff tears with calcific tendinitis having its place along this continuum. Although many studies have focused on the role of magnetic resonance imagining (MRI) in diagnosing the extent of rotator cuff tears and their associated findings with good interobserver reliability, the same cannot be stated about MRI tendinopathy findings. Because of this discrepancy in diagnostic reliability, tendinopathy tends to be overtreated with injections when associated with symptoms, thus potentially increasing the risk of calcific tendinitis and progression toward rotator cuff tears. This study aims to assess whether diagnosing shoulder MRI tendinopathy patterns through dichotomization can accelerate clinical progress toward consensus. Methods: This study is a large retrospective cohort of 184 patients that underwent a 1.5T shoulder MRI for shoulder pain. Inclusion criteria were acromioclavicular arthrosis diagnosed in patients of any age. Exclusion criteria were partial or complete rotator cuff tears. Tendinopathy was considered the dependent variable and registered as a dichotomous variable while acromioclavicular joint arthrosis together with gender was categorical and age was the continuous variable. An attempt was made to generate a clinically significant binary logistic regression to assess the odds ratio of diagnosing tendinopathy based on age, gender, and acromioclavicular joint arthrosis status. Results: An overwhelming proportion of patients was positive for tendinopathy findings (95.11%). 64.12% of patients were within the active age group with patients within the 50-59 group being diagnosed the most with rotator cuff tendinopathy. Conclusions: Due to the high variability of MRI findings that can be considered positive for rotator cuff tendinopathy, an overwhelming skew toward a positive diagnosis was observed, thus dichotomizing tendinopathy diagnosis is not appropriate for clinically relevant conclusion-making.*

Keywords: *rotator cuff, tendinopathy, magnetic resonance imaging, biostatistics*

INTRODUCTION

Rotator cuff tendinopathy has become increasingly relevant to diagnosis as its involvement in the shoulder's rotator cuff pathology continuum is dominating [1-5]. While the progression rate to full-thickness rotator cuff tears has not yet been elucidated early management of tendinopathy might decrease the progression rate and improve patient-reported outcomes [4,6]. Special attention is advised when managing the early stage, symptomatic, rotator cuff tendinopathy with corticoid injections as they might lead to an increased rate of calcific tendinopathy incidence that has a stronger progression towards full-thickness rotator cuff tears, thus decreasing the patient's quality of life in the long run [7,8]. In regards to early diagnosis, some early contributions have been made toward increasing the diagnostic accuracy of rotator cuff tendinopathy, although most studies are focused on diagnosing rotator cuff tears [9-12]. There is still a lack of consensus on the contribution of associated imagistic findings in tendinopathy and their link towards full-thickness rotator cuff tears; most known associated findings are bone cysts adjacent to tendon insertions, bone marrow edema adjacent to tendon insertions, subacromial and subdeltoid signal increase on fat-saturated T2 weighted magnetic resonance imaging (MRI), bursal or articular sided fraying and fibrillation of the rotator cuff tendons and articular effusions [2-4,10,13-22].

It is worth mentioning that further difficulty is encountered when attempting to differentiate rotator cuff tendinopathy and partial articular-sided, bursal-sided, or instar-substance supraspinatus tendon tears [12,13,23-28]. This study aimed to assess the feasibility of using dichotomization of rotator cuff tendinopathy to fast-track clinical research as categorical or continuous data require significantly more time that the clinician has to invest in data gathering.

MATERIALS AND METHODS

This study is conducted according to the ICMJE Recommendations on Uniform Requirements for Manuscripts (URMs) [29].

Our research question was Is dichotomization of shoulder MRI findings in patients diagnosed with rotator cuff tendinopathy a useful method for logistic regression?

Before the study inception, a preliminary search has been conducted in MEDLINE via Pubmed using the search terms (("Rotator Cuff Injuries"[Mesh]) OR ("Rotator Cuff"[Mesh])) AND (("Tendinopathy"[Mesh]) OR ("Tendon Injuries"[Mesh])) AND ("Magnetic Resonance Imaging"[Mesh]) AND (y_5[Filter]) to properly interpret our findings within the context of the current state-of-the-art on shoulder rotator cuff tendinopathy MRI diagnostic.

Considered for inclusion were studies assessing the role of MRI and its findings in the context of rotator cuff tendinopathy. Exclusion criteria were all studies focusing on pre- or postoperative MRI assessment, studies on full-thickness rotator cuff tears, biomechanical, cadaver, simulation, or any other study that does not assess tendinopathy patterns. We have identified 551 results that we have imported into Zotero for reference management. Two authors, G.M.A. and A.S. have selected relevant articles in three successive steps, excluding articles based on title, abstract and full text. After the title and abstract assessment, 68 studies were included for full-text evaluation against the proposed inclusion and exclusion criteria. After reading the remaining 68 articles, 50 articles met the inclusion criteria and were included in this study.

The present study is a retrospective large cohort study that assesses the reliability of MRI diagnosis in rotator cuff tendinopathy. We have included 184 patients that underwent shoulder MRI for any reason between January 2019 and January 2022. Coronal proton density (PD) and fat-suppressed T2 (T2-FS) sequences have been assessed for each patient and tendinopathy presence has been documented by two radiologists M.H. and D.C. together with a senior orthopedic surgeon, S.M. Dichotomous values were used to record the dependent variable's, tendinopathy, presence against the independent variable, acromioclavicular arthrosis, which was recorded as a categorical variable according to the Shubin-Stein classification [30] together with patients age and gender. All variables were imported and processed in SPSS version 26 (IBM Corp., Armonk, N.Y., USA) to conduct descriptive statistics and perform binary logistic regression on the relationship between the dependent and independent variables. Before conducting binary logistic regression, a Box-Tidwell test has been performed to assess the relationship between the continuous independent variable, age, and its logit. Also, an Eta-squared ($\eta2$) test was performed to investigate how much of the variability in acromioclavicular arthrosis was accounted for by age and the result of the $\eta2$ was verified using the F ratio.

RESULTS

Descriptive statistics show an overwhelming skew towards positive rotator cuff tendinopathy diagnosis by using dichotomization of the dependent variable. Thus, comparisons within the group were not conducted due to the low statistical power. Patients' mean age was 55 years (S.D. 14). Results show that 95.11% of the included patients (Figure 2) were positive for rotator cuff tendinopathy. Surprisingly, tendinopathy does not seem to be correlated with acromioclavicular joint arthrosis status (Figure 3) and neither age seems to have a significant impact on acromioclavicular arthrosis ($\eta2=0.238$), F (50, 133)=0.831, p=0.770. Most patients diagnosed with tendinopathy were within the 50-59 age group (Figure 4), but 64.12% of all patients diagnosed with tendinopathy were within the active, employed, group (Figure 5). Binary logistic regression has been performed to assess the effects of age, gender, and acromioclavicular joint arthrosis status on the likelihood that patients are diagnosed with tendinopathy. The logistic regression model was not significant, $\chi2 = 2.622$, p = 0.454. The explained variation in the dependent variable based on our model ranges from 1.4% to 4.4%, depending on whether we reference the Cox & Snell R2 or Nagelkerke R2, respectively, and correctly classified 100% of the case. The odds of being diagnosed with tendinopathy were determined to be 1.769 times higher when having concomitant acromioclavicular arthrosis (p=0.206, 95% CI [0.730, 4.287]), but this result was not significant. Similarly, age and gender were not significant factors in tendinopathy diagnosis (p=0.397, 95% CI [0.972, 1.075]) and (p=0.493, 95% CI [0.155, 2.457]), respectively.

Figure 1: Tendinopathy pattern according to Sein et. al[3]. (a) Image A, shows no increase in signal intensity, corresponding to tendinopathy grade 0, and B, C, and D show a gradual increase in signal intensity at the level of the supraspinatus tendon corresponding to tendinopathy grades 1, 2, and 3 respectively.

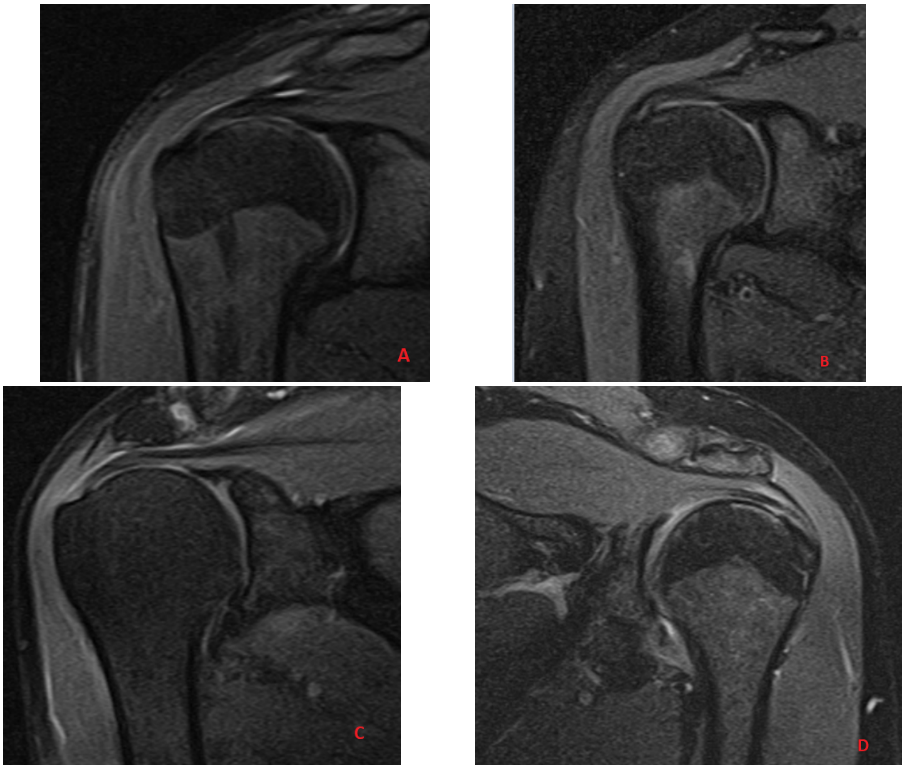

Figure 2: More than 95% of the included patients presented with rotator cuff tendinopathy

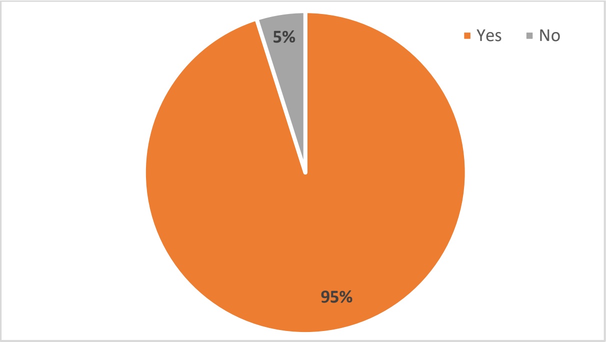

Figure 3: Tendinopathy diagnostic based on acromioclavicular joint status

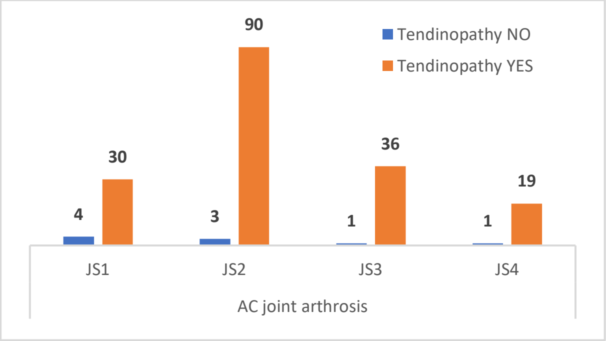

Figure 4: Tendinopathy distribution between age groups

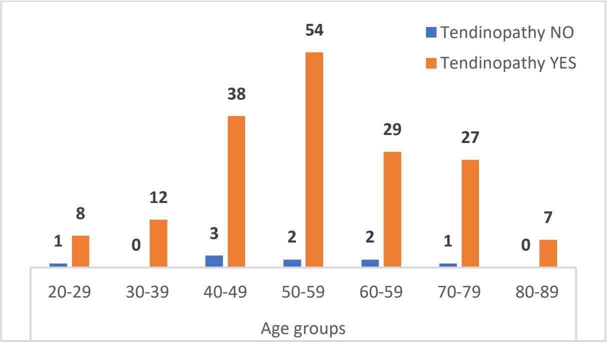

Figure 5: Tendinopathy distribution within age groups

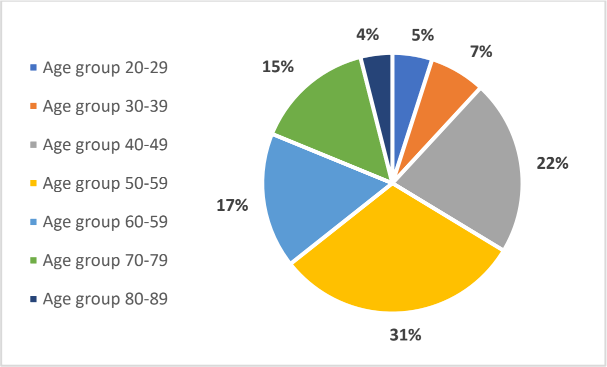

DISCUSSION

We would like to mention that the results obtained by performing binary logistic regression show that by using dichotomization when categorizing tendinopathy researchers will unbalance their results because of the high prevalence of rotator cuff tendinopathy in the general population.

It is worth noticing that tendinopathy was prevalent in all stages of acromioclavicular arthrosis, but no increase in tendinopathy rate was seen with the increasing acromioclavicular arthrosis stage. The patients with Shubin-Stein stage 2 acromioclavicular arthrosis presented rotator cuff tendinopathy most frequently (Figure 3). This result is in disagreement with our hypothesis that the tendinopathy rate would be expected to increase with increasing acromioclavicular arthrosis.

The most important result was that tendinopathy is widely distributed within the more active age groups. This aspect underlines the need for identifying efficient treatment measures that are aimed at alleviating symptoms and delaying tendon wear. Most patients, 80.97% (Figure 5), that were within their most active years, presented tendinopathy, with an overall increased incidence in the 50-59 age group. This is a relevant observation since the socio-economic impact of rotator cuff tendinopathy can be anticipated as patients require a home to rest, medication, and physiotherapy.

After a thorough literature search, we have identified several studies focusing on investigating rotator cuff status after arthroscopic repair but very few studies on rotator cuff tendinopathy patterns [1-6,18,31-37]. Two relevant studies used a comprehensive approach to classifying tendinopathy from normal to severely tendinopathic rotator cuff using categorical data [3,15,38].

We have tested the usefulness of classifying tendinopathy as a dichotomous dependent variable and tested its relation to acromioclavicular joint arthrosis, gender, and age. We were not able to conclude any clinically relevant information due to the previously unrecognized high prevalence of tendinopathy in the general population. We would also like to mention that healthy patients usually do not undergo a shoulder MRI, thus the prevalence of tendinopathy in our study cohort might very probably be overestimated.

Our approach was an attempt to simplify these previous efforts to accelerate clinical research toward reaching a consensus on rotator cuff pathology management. It is worth mentioning that, based on our experience, subacromial corticoid injections have yielded mixed results, most often than not offering only short-term pain relief. Thus, studies questioning the usefulness of corticoid injections and their suspected involvement in calcific tendinitis should be considered when deciding long-term patient benefits [7,8,39-44]. We would also like to point out that in comparison to rotator cuff tears, tendinopathy seems to be much more difficult to accurately diagnose because of the mixed patient presentation, its association with calcific tendinitis, partial supraspinatus tears, and subacromial or subdeltoid increased signal in T2-FS sequences [4,9,10,13,17,23,24,32,39,40,42,45,46]. We consider that differential diagnosis between tendinopathy and partial supraspinatus tendon tears, the contribution of bone cysts to tendinopathy severity, and the possible accelerated progression of calcific tendinitis to rotator cuff tear are the 3 most important aspects that should be taken into consideration when suspecting tendinopathy in a patient suffering from shoulder pain.

Several limitations need to be mentioned. Data gathering was performed only once, thus no intra- and inter-observer reliability testing was performed. No intra-class or inter-class correlation coefficients were calculated to determine associations among and within groups. The most severe limitation was that tendinopathy patterns were recorded as dichotomized data instead of ordinal data, thus underestimating the high prevalence of rotator cuff tendinopathy in patients that require a shoulder MRI due to pain and/or function deficit.

CONCLUSION

Due to the high variability of MRI findings that can be considered positive for rotator cuff tendinopathy, an overwhelming skew towards a positive diagnosis was observed, thus dichotomizing tendinopathy diagnosis is not appropriate for clinically relevant conclusion-making. We recommend the use of tendinopathy gradings like the one reported by Sein et al. At the same time, clinicians should be aware of the increased prevalence of rotator cuff tendinopathy, especially within the active age groups, and should aim for efficient treatment methods that offer fast recovery so patients can return to work and sports in a timely fashion.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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Authors' contribution
Conceptualization G.M.A, E.M.C; methodology G.M.A; formal analysis G.M.A., F.A.S.; investigation G.M.A., S.M., M.H., D.C.; data curation G.M.A., A.S., M.H.,

D.C.; writing – original draft preparation G.M.A., F.A.S.; writing – review and editing, G.M.A., S.M., E.M.C., M.H., D.C.; visualization, G.M.A., S.T., S.L.; supervision, E.M.C., F.A.S., S.M., D.C.; project administration G.M.A., E.M.C., S.M., D.C.

Ethics approval and consent to participate

The present study was approved by the hospital's ethics committee, number 521/05/05/2022, and is performed under the ethical standards laid down by the Declaration of Helsinki.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

The Endocrine Approach of Melanoma: The Puzzle of Estrogen Receptors Expression

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Abstract: *Melanoma outcome seems different between females and males, with a potential protective role of estrogen (E) through estrogen receptors (ER) expression into the tumor. In the study of ERs, both alfa (ERα) and beta (ERβ) is a well-known endocrine elements in non-melanoma tumors, like mammary and endometrial cancer. Immunohistochemistry (IHC) assessment of melanoma concerning ERs represents a path to explore the tumor profile to provide useful information concerning the prognostic and potential adjuvant treatment. Currently, this is not a routine practice, nor a mandatory step for deciding the medical therapy. Typically, IHCs are based on usual kits for mammary tumors regarding ERs configuration. Prior/concomitant use of oral contraceptives and hormonal replacement therapy is not correlated with a better prognostic in melanoma; neither have they represented a contraindication for survivors of melanoma; a subset of tumors might present a higher ER expression which is potentially targeted by the hormone-based treatment as SERMs (Selective Estrogen Receptors Modulator), for instance, tamoxifen. Experimental studies on melanoma cell lines confirmed the anti-tumor activity of ERβ which might function as a prognostic marker. G-protein-coupled estrogen receptors in melanocytes and keratinocytes might be involved, too. Additional crosstalk of TGF-β (Transforming Growth Factor β), respective IGF1 (Insulin-like Growth Factor), and ERα expression are involved in tumorigenic pathways. Recent preclinical studies showed the potential benefits of diarylpropionitrile, a selective agonist of ERβ; pyrazole derivates 21-23 can block ERs. Murine melanoma models showed the interference of anti-estrogenic medication (like molecule fulvestrant) to enhance immune checkpoint blockade, a modern approach to solid cancers. The proliferation of melanoma might be partially explained by ERs; whether this is generally applicable or there is a subgroup of tumors particularly related to E status is still debatable. The subject of E status in melanoma is far from clear at this point and further studies are necessary concerning this particular issue to implement it as a practical approach in the daily management of a disease that still has a very severe prognostic nowadays.*

Keywords: the *estrogen receptor, melanoma, endocrine, dermatology, estrogen, progesterone, prognostic*

INTRODUCTION

Melanoma outcome seems different between females and males, with a potential protective role of estrogens (E) through estrogen receptors (ER) expression into the tumor [1-10]. However, the subject is far from clear at this point and further studies are necessary concerning this particular issue, and also they are necessary to take the next step to implement it as a practical approach in the daily management of an otherwise very severe disease [1-10]

The study of ERs, both alfa (ERα) and beta (ERβ) is a well-known endocrine element in non-melanoma tumors; while positive ER status is essential to be assessed in gynecological cancers including breast and uterine malignancies; as well as other endocrine conditions like atypical endometriosis implants, etc. [11-20]. Physiologically, ERs at the level of the endometrium (which is also responsive to progesterone receptors and androgen receptors) cause mucosal proliferation (proliferation phase) and enhance the expression of progesterone receptors which become strong players during the secretory phase [21]. Moreover, ERs are mediators of the pro-angiogenetic status under normal and pathological circumstances [22].

Estrogenic status has been reported in relationship with different autoimmune conditions, including in dermatology [23-26]. An interplay via CD8+ T cells has been identified in both melanoma and vitiligo [27,28]. ERs have been revealed as biomarkers in complex heterogeneous tumors like neuroendocrine neoplasia, or in adenocarcinomas with a neuroendocrine component, as mostly described in mammary malignancy [29-35]. Moreover, prenatal exposure to E has been associated in murine experiments with different behaviors of somatotroph and lactotroph cells at the level of the pituitary gland; however; the daily clinical approach of somatomamotroph cell-derivate tumors is not particularly different between females and males unless pregnancy and lactation status [36-38]. One of the most interesting topics addressing the various roles of ERs concerns E status in COVID-19 infection [39-44]. It was suggested that generally, females tend to present less severe coronavirus–associated clinical manifestations due to immune-mediated E protection [39] It seems that menopausal replacement treatment might reduce inflammation due to E actions and, also, modulate ACE2 (angiotensin-converting enzyme) as the receptor of the virus [39]. A recent phase 2, multicenter, randomized trial studied the efficacy and safety of raloxifene, a SERM (Selective Estrogen Receptor Modulator) traditionally used in gynecological endocrinology, which might be recommended to improve some aspects of SARS-CoV-2 (severe acute respiratory syndrome) presentation [40].

AIM

Our purpose is to focus on melanoma in relationship with E and ERs status concerning the current level of knowledge and future lines of addressing this topic in daily practice.

METHOD

This is a narrative mini-review. We revised the full-length papers published in English literature through a search on the PubMed database. We only included articles published between 2022 and 2019, with no restriction concerning the level of statistical evidence.

IMMUNOHISTOCHEMISTRY AND GENETIC STUDIES

One controlled study from 2022 addressed ERs configuration in 4 female cases diagnosed with melanomas; they were mammary cancer survivals; the ER expression was compared to a group of women who had an ovarian stimulation performed versus age-matched groups [45]. ER expression (nuclear and cytoplasmic) was analyzed based on the percent of ER-positive cells (score of 0, 1, and 2, for ≤20%, 21-50%, respective ≥50% positive cells) [45]. Nuclear ERβ was detected in females with previous breast cancer; the expression in melanoma is more frequent if the patients were previously treated with anti-estrogen medication [45]. Cytoplasmic ERα expression was lower in the control group, as well as in subjects with prior ovarian stimulation when compared to the patients with mammary malignancy [45].

Immunohistochemistry assessment of melanoma concerning ERs represents a path to explore to provide potentially useful information [45]. However, in everyday practice, we do not have a particular protocol to asses ERs in melanomas.

Immunohistochemistry studies using the usual kit for mammary tumors regarding ER have been done, including on uveal melanoma, a very aggressive form. We mention one study from 2021 showing that more than half of these tumors are ER positive; while elevated nuclear expression correlates with a most severe outcome [46].

This raises the question if uveal melanoma is a hormonal sensitive cancer [47]. A systematic review from 2021 confirmed that prior/concomitant use of oral contraceptives and hormonal replacement therapy is not correlated with a better melanoma prognostic; neither have they represented a contraindication for survivors of melanoma; a subset of tumors might present a higher ER expression which is potentially targeted by hormone-based treatment as SERMs (Selective Estrogen Receptor Modulator), for instance, tamoxifen [47]. One study on melanoma B16F10 cells showed the potential benefit of atorvastatin (a statin for hypercholesterolemia) in addition to tamoxifen [48].

One retrospective study on 448 subjects with melanoma analyzed the expression of the ERα gene that was correlated with an increased overall survival rate (p-value of 0.018); ERβ gene expression was not associated with melanoma progression [49]. The use of an ERβ-selective modulator like NO-SERM 4d which is a nitric oxide-releasing selective ER modulator 4d has a potential benefit [49]. The interaction of E, respective SERMs, with nitric oxide release might represent the clue for the antiproliferative effect [50].

A cross-sectional study on 73 melanocytic lesions analyzed the status of ERα, ERβ, and G-protein coupled estrogen receptor (GPER) in melanocytes and keratinocytes; ERβ expression was higher in dysplastic versus common nevi (melanocytes); ERα distribution was similar among the three types of dermatological lesions; GPER expression was increased in margin tissue melanocytes versus melanoma melanocytes [51]. Also, nuclear receptor estrogen-related receptor γ (ERRγ) might be a contributor to an immune defense [52].

GPER is involved in different systems a part of the immune system, like cardio-metabolic and reproductive systems [53]. The interaction with ERα modifies the immune response, as well as the proliferative potential [53]. Moreover, melanoma cell growth is promoted through TGF-β (Transforming Growth Factor β) and ERα expression is a contributor to this pathway [54].

ERs cross-talk with IGF1 (Insulin-like Growth Factor) pathways at several levels [55]. A single nucleotide polymorphism (SNP) study on melanoma–associated ER/IGF1 signaling showed that IGF1-GG phenotype (recessive model for rs1520220) associates a higher risk of this tumor in males (p=0.006), but the lowest risk for females (p=0.045), respective IGF1R-AA genotype (recessive model for rs2229765) correlates with protection for men (p=0.006), and it does not represent a protective/risk factor for women [55].

Another study that included immunohistochemistry and nuclear/cytoplasmic analysis of ERß expression on 86 subjects with different types of melanocytic lesions showed that the expression of ERß is more intense in males with Spitz and Reed nevi versus melanoma and common nevi, while Breslow thickness, as a prognostic marker in melanoma, is higher in cases with low immune reactivity versus high immunoreactivity of ERβ [56].

BREAST CANCER AND MELANOMA

One study between 2005 and 2015 included 238 subjects with metastatic non-hematopoietic mammary disease and 42% of them had melanoma as a primary site [57]. Another study on 64 patients identified 13 melanomas among them as the primary site, suggesting a potential common loop with the E system [58].

Some cancers associate a genetic susceptibility; the correlation with estrogen status might be a missing piece of the melanoma puzzle. One study on UK Biobank data included 106,278 patients with breast cancer and a control group of similar size [59]. The authors showed that the overall risk of mammary malignant tumors according to a score called polygenetic risk score based on genome-wide association studies is positively correlated with melanoma, as well as with other cancers like ovarian, gastric, and colorectal [59].

Another common hypothesis involves syndecan-4 which is a transmembrane proteoglycan mediating cell adhesion and proliferation of different cancer cells including mammary neoplasia and melanoma, with potential interference over E – related pathways [60]. For instance, zoledronic acid, a bisphosphonate drug used for osteoporosis and bone metastasis, is associated with additional anti-proliferative effects on invasive mammary cancer by increasing the expression of syndecan-4 [60].

NEW POTENTIAL TARGETS OF THERAPY AGAINST MELANOMA

ERβ might seem like a new potential line of management in severe cases of melanoma that require multi-leveled management [61]. Experimental studies on melanoma cell lines confirmed the anti-tumor activity of ERβ which might function as a prognostic marker [62]. For instance, diarylpropionitrile (DPN), a selective agonist of ERβ, activates signaling pathways in cultures of the brain and mammary cells; it seems that the inhibitory influence on melanogenesis is exerted also independently from ERβ via protein kinase A(PKA)/cAMP-response element-binding protein (CREB) signal transduction pathways [63].

Other experiments involve pyrazole derivates 1-29 with antiproliferative effects. Cell lines of melanoma are inhibited by derivatives 21-23 being able to block ERs [64]. Murine melanoma models showed the interference of anti-estrogenic medication (like molecule fulvestrant) to enhance immune checkpoint blockade, knowing that immune checkpoint inhibitors represent a modern approach to solid cancers [65].

Also, rat melanoma models showed the effect of ERα activation as the promotor of cell growth via an immune suppressive status causing abnormal CD8+ T cells function which might explain poor response to this new class of anti-tumor drugs [66]. Whether this combination will be clinically applicable for patients with melanoma, as well, is still an open issue.

As mentioned, GPER has been studied in relationship with proliferative effects and potential involvement in hormonal therapy resistance of different cancers, including in some malignancies without a clear expression of ER [67]. The use of GPER – targeted medication in combination with immune checkpoint inhibitors is under evaluation in persons diagnosed with melanoma [68]. Immunotherapy represents a major line of therapy in modern oncology, and its adequate placement in addition to ERs modulation might bring benefits in cases with melanoma [69].

As mentioned, uveal melanoma represents one of the most aggressive forms of melanoma. An alternative therapy that is under evaluation is the combination of MEK inhibitors with CDK4/6 selective inhibitors (approved for patients diagnosed with mammary cancer with positive estrogen receptors in association with ER antagonists or aromatase inhibitors)

[70,71]. In melanoma, the regulation of the PRMT5-MDM4 pathway plays a major role in the response to CDK4/6 inhibitors' effects [72].

Cyanidin-3-o-glucoside, a phytoestrogen compound with anti-proliferative effects concerning melanoma, has been studied in vitro and in vivo, and it was identified to promote apoptosis of melanoma cells via ERβ which is rather paradoxical [73].

Some controversies around the concept of ER as a future target of therapy in melanoma relate to the observation that traditional ER and androgen receptors are not identified in all melanoma studies; also, some data concern testosterone as an activator of melanoma growth through ZIP9 which is a zinc transporter, thus inhibitors of androgen receptors might act as anti-tumorigenic in melanoma, as a collateral unexpected effect [74].

O mice study with acteoside, an inhibitor of tumor growth in different cancer lines, showed that the molecule inhibited melanoma growth by reducing inflammation levels, as well as reactive oxygen species, and by promoting a higher rate of apoptosis by regulating ERβ [75].

An alternative to ER and E approach has been proposed for the modulation of progesterone receptors by using selective progesterone receptor modulators (SPRM) like asoprisnil, which seem to act on melanogenesis, as well [76].

DISCUSSION

The proliferation of melanoma might be explained by ER; whether this is generally applicable or there is a subgroup of tumors particularly related to E status is still debatable [77]. If melanoma represents to some extent a steroid hormone–dependent cancer is difficult to say at this moment [78]. Genetic and hormonal elements might have a place among risk factors, but it is difficult to explain the higher mortality in male patients versus females based on clinical and preclinical data we have so far [79]. Melanoma is currently related to several prognostic biomarkers [80-83]. We mention V600E mutation of the BRAF gene [80,81]. Also, local invasion and clinic-pathological characteristics are important [82-85]**.**

CONCLUSION

Multiple factors simultaneously act on cell growth and therapeutic intervention should be multi-leveled. On the other hand, systematically using immunohistochemistry and genetic analysis of ER will identify different subgroups concerning E status thus different prognostic profiles among patients with melanoma. We need to find new management solutions for this condition, knowing that melanoma is still a very aggressive disorder nowadays, with an alarming increasing incidence.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Investigating the Operational Management Challenges of a Reference Military Hospital in the COVID-19 Pandemic

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Abstract: *Introduction: Following the crisis of coronavirus outbreak in numerous regions of the world, this epidemic enhanced promptly in most countries, including Iran, and sent a host of patients in need of medical services to hospitals. This study aimed to investigate the challenges of operational management of a reference military hospital during the pandemic COVID-19. Methods: This research is a qualitative study with the content analysis method with a deductive and guided approach. Data collection was conducted through a semi-structured interview method and utilizing a questionnaire designed based on the management functions of the Hospital Incident Command System (HICS). The targeted sampling method and participants in this study included senior-middle managers working in organizational positions of the reference hospital in 2020 employed during the organizational position in Covid-19 Pandemic. Classifications and coding were performed employing MAXQDA 2018 software. Results: The findings of this study were explored in the form of strengths, weaknesses, and challenges. Weaknesses included taking surprise, early and non-missionary entry into the COVID-19 crisis, lack of appropriate depot, instant management, deficiencies in the training of patients and staff, poor functioning of the crisis management system, deficiencies in monitoring, and poor provision of psychological support. The strengths were the devoted and altruistic presence of the medical staff and managers, the financial support of the headquarters, the effective field research of the management team of the organization, and the proper management of the volunteer forces. The most significant challenge was the poor coordination with the relevant organizations, including the headquarters, the Defense Organization, and the Ministry of Health. Conclusions: The findings of this study demonstrate that in the peak period of the COVID-19 pandemic to promote the operational management of the hospital, sticking to the general policies of the organization, structured coordination with internal and external organizations, organized management of equipment and human resources, generating a robust documentation structure and decision-making based on the evidence, effective and practical use of the crisis management system, systematic training of staff and patients, prioritizing the safety of patients and staff, organized dissemination of information and systematic monitoring as well as evaluation are the most significant points to deal with the epidemic crisis.*

Keywords: *management, hospital, pandemic, COVID-19*

INTRODUCTION

On January 30, 2020, the World Health Organization issued a statement declaring the new coronavirus an outbreak of a public health emergency that menaced the entire world, and not just China [1].

This epidemic extended immediately in most countries, including Iran, and sent a host of patients to hospitals in need of medical care [2,3].

Hospitals are the most important provider of healthcare services in any pervasive disease, involving COVID-19. Owing to the critical peak of COVID-19 in a short time and the abrupt enhancement of load in hospitals, the efficiency, and effectiveness of the hospital depend to a considerable extent on the management and effective use of resources [4,5]. Nowadays, it is believed that without dynamic management and mastery of modern management methods, the intended efficiency and effectiveness in the healthcare network and hospital system cannot be attained [6,7] and poor performance of hospital managers is a paramount factor in boosting costs and wastage of human and financial resources in crises [8,9]; however, hospital managers face many challenges and issues to manage the crisis in this significant and critical organization [10]. This study aims to investigate the operational management challenges of a reference military hospital in the COVID-19 disease crisis.

METHOD

This study is a qualitative research based on the content analysis method with a deductive and guided approach. Data collection was conducted through a semi-structured interview method and utilizing a questionnaire planned based on the management functions of the Hospital Incident Command System (HICS). In this study, a purposive sampling method was employed to interview 40 senior and middle managers in organized positions whose field of work was directly or indirectly involved in responding to the COVID-19 crisis.

In the beginning, intending to perform a semi-structured interview, we designed a questionnaire based on the management functions of the Hospital Incident Command System (HICS) and reviewed similar studies in eight areas; support, decision-making, incidents command system, health and Safety of patients and staff, information dissemination management, monitoring and evaluation, patients management and human resources. Afterward, to determine the validity and ensure the importance of the questions from the perspective of the respondents, five experts in the field were interviewed, and depending on their views, if necessary, the required corrections were made to address the shortcomings. The emphasis of the interview questions was on the positives and improvements of the hospital management, management challenges, and providing solutions to address these challenges.

Then, the research team coordination sessions were held to perform the same interview. The samples were selected purposefully by selecting managers who had a significant operational role in the corona pandemic peaks. Among the most significant inclusion criteria were being experienced in chosen specialized areas, experiencing active participation in the COVID-19 crisis in the hospital, the capability to express oneself well, and having time for interviews. In the meantime, people who did not have the time or desire to participate in the study and interview were excluded from the study. Participants in the interview were selected based on the purposive and snowballing methods. In this regard, the people who had the most information about the subject were selected and each person was asked to introduce other participants and people who met the inclusion criteria (at least two people).

The interviews were performed by making an appointment and selecting the place of the interview from the participant's point of view for their convenience. During the interview, the interviewer began with open-ended questions for the respondent to feel comfortable and freely express his feelings, thoughts, and views. The questioner then proceeded with the interview based on the designed questionnaire. This study was performed in the form of attitudes of hospital operational management and the views of all stakeholders, including patients, were not considered. The analytical points of this study were based on summarizing the findings of interviews with middle operational managers in the hospital and evaluating as well as investigating the acquired content in expert meetings with the presence of the research team. Data analysis was performed employing MAXQDA 2018 software. Considering the deductive approach of this study, the initial coding was conducted in the form of HICS structure classifications and ultimately, the final classification was performed utilizing the software. Afterward, the obtained results were reviewed and finalized in a two-hour absentee panel with senior policymakers. To study, the code of ethics was obtained from the ethics committee in biomedical research. In the meantime, the principles of information confidentiality were considered. The results of this study were finally announced to senior management and managers of the studied units to help make managerial decisions.

RESULTS

The findings of the current study were assessed in the form of examining the strengths, improvements, and challenges of operational management in the hospital in the face of the issues caused by the corona pandemic.

Shortcomings are those that, if completed and improved, the system shall be better prepared for future crises, and via solving the problems of these cases, better services can be offered in the future. In the study areas, the highest frequently repeated point in the interviews was the surprise and unpreparedness for the epidemic crisis, despite global and regional warnings. Exploring the points for improvement in the eight areas studied led to the following key points (Table 1).

Strengths are those points that have been administered well and the organization has been capable of managing them properly, effectively, and promptly. In the study areas, the key point repeated in most interviews and all domains was the devoted and altruistic presence of medical staff and managers. Examining the strengths in the eight studied areas led to the following key points (Table 2).

Table 1: The points for improvement in the eight key elements

Supports	Deficiency of creating a suitable depot despite knowing the outbreak of the disease in the world and the existence of a suitable opportunity Presence of process problems in the field of logistics and non-separation of duties of the drug unit and support unit Shortcomings in the existence of standard warehouses for the storage of masks and disinfectants and non-compliance with standard principles in the storage area can be catastrophic.
Health and Safety of patients and staff	Severe shortcomings in education and information in the field of health and safety of patients Lack of particular program and discipline in supplying personal equipment to staff and long and excessive use of equipment and non-compliance with standards Lack of appropriate preparation despite being aware of the spread of the disease in the world, like infection control training and the use of personal protective equipment
External organizational decision making	Entering the COVID-19 crisis without a mission and a formal request
Internal decision making	Parallel work and lack of coordination between managers and waste of resources, particularly over the first weeks of the crisis Deficiency in targeting decision-making sessions
Incident management system	Deficiency of a written program in the system to isolate suspicious patients Defects in evaluating system performance Deficiency in explaining and comprehending the job description of system members
Information dissemination management	Lack of planning and taking operational measures to alleviate staff anxiety Failure to form a specialized working group for Information management with the presence of related experts
Monitoring and evaluation	Deficiency in monitoring the performance of non-therapeutic units The non-use of the results of evaluations and repeating past mistakes Defects in planning to solve the problems listed in the evaluations
Patient management	Non-segregation of patients from any section of coronavirus cases particularly in the emergency ward Defects in the forming of the psychological support team Deficiency in composing a systematic mechanism of follow-up of patients and training during discharge Defects in the process of disinfection of imaging sections Defects in educational methods and content of education about COVID-19 Defects in the targeting of preventing staff infection Lack of planning to remodel for other use and create a biological emergency
Human resources	Lack of planning and the requirement to train human resources after recruiting volunteers Deficiency in managing the organization of human resources, especially over the first weeks of the disease outbreak

Table 2: The points for strengths in the eight key elements

Supports	Financial support on behalf of headquarters
Health and Safety of patients and staff	Daily disinfection of environments (at the beginning of a pandemic) Promoting personnel safety by improving facilities Active participation of the infection control ward in training
External organizational decision making	Presence and continuous inspection of both the management teams of the organization and the Ministry of Health from the medical departments
Internal decision making	The flexibility of managers along with their continuous and responsible follow-up
Incident management system	Activating and holding daily meetings of the Hospital Incident and Disaster Risk Committee
Information dissemination management	Monitoring hospital news in the media continuously
Monitoring and evaluation	Daily and active inspection by the hospital management team, especially the continuous presence of the hospital chief
Patient management	Prominent, devoted, and altruistic presence of medical staff and managers
Human resources	Summoning and managing devoted and volunteer forces

Challenges are problems that appear as an obstacle to effective performance and will be solved with the efforts of the upper echelons of the organization. In the current study, the most important challenge was the poor coordination with the relevant organizations, including the headquarters, the Ministry of Health, and the Defense Organization, and other important challenges, consisting of the following (Table 3).

Table 3: The points for challenges in the eight key elements

Supports	Providing and supporting timely finance
Health and Safety of patients and staff	Lack of facilities for the presence of patients' companions for more appropriate care of coronavirus cases Being taken unawares over the first two weeks of the outbreak
External organizational decision making	Frequent alterations in treatment and prescription of medication (multiple protocols)
Internal decision making	Perpetual changes in notifications
Incident management system	Unpredictable number of patients and the time of crisis
Information dissemination management	Lack of trust of people and patients in news and information
Monitoring and evaluation	Failure to appoint the main trustee of supervision Lack of appropriate monitoring tools in infectious disease conditions such as specific checklists
Patient management	Deficiency in coordination and solidarity between the orders of the Ministry of Health and the headquarters
Human resources	Lack of support in the human resource supply chain on behalf of the headquarters (as an upper echelon of the organization)

DISCUSSION

The present study scrutinizes the operational management challenges of a reference hospital over the peak (first peak) of the COVID-19 pandemic. There are numerous experiences of dealing with pandemics, but the Covid-19 pandemic due to the extent and spread of the disease and in the light of treatment management and patients care is unique in the world. Numerous mutations and changes in the symptoms of the disease and many other unexpected conditions caused countries to face multiple peaks of the disease in their region and experience the crisis in every sense. Different definitions and some aspects of crisis have been mentioned in the relevant texts which are confirmed based on the findings of this study. For instance, Coombs has considered a crisis as an unpredictable incident and defines it as dealing with an unpredictable incident that menaces the stakeholders' expectations that can influence the function of an organization [11]. In this study, most managers pointed to the unpredictability of subsequent incidents at the peak of the COVID-19 pandemic, which caused a kind of surprise and confusion among managers. This issue was especially evident in the first stage of the peak (first peak). Of course, the point made by all participants in this study was the disregard for global warnings, which unfortunately dealt with the crisis of previous warnings like a completely unannounced situation!

Incident Management System

A robust crisis management system can address multiple potential problems in a crisis. The major problems in this section were delays in the call and poor response to the call in the early days, delays in notifications through the system, lack of transparency in the description of tasks in the system, and lack of evaluation of system performance, which made the system fail to respond to the crisis. The most significant solution to eliminate the deficiencies and problems listed in the hospital crisis management system involves compiling and communicating detailed job descriptions in the system, holding operational maneuvers based on predetermined scenarios in the field of infectious diseases, updating system processes and protocols, especially how to call human resources and their replacement as well as determining the evaluation team and compiling evaluation checklists based on existing scenarios [15].

Human Resources

One of the principal fields examined was the challenges relevant to human resources. There were two critical shortages in the field of human resources, firstly, the lack of effective human resources during the peak of the disease, and secondly, the lack of trained human resources, which was one of the main challenges in this field. Laschinger et al.'s study over 1996-2001 indicates that the lack of caregiver forces is one of the structural variables which by pressuring staff, causes them to feel dissatisfied and incompetent.

In Germany, the government summoned newly graduated medical students to work at hospitals that did not have enough staff [14]. In Italy, which at one time had the highest rate of COVID-19 in the world, the government asked new retirees to return to work [15]. Shortening shifts and enhancing the number of shifts, boosting salaries to augment motivation, maintaining the physical and mental health of staff, and preventing workforce erosion are other strategies [16]. The shortage of trained human resources reserved for use in crisis periods is another problem relevant to human resources in the present study. In other studies, the presence of crisis maneuvers is the most important way to get experience for such situations, and it sounds like in designing training maneuvers, staff should be provided with more realistic conditions and better training [17].

Equipment

The next serious challenge was the equipment shortage during the critical peak of COVID-19. The lack of equipment in crises has always been discussed by experts.

In other studies, another perspective of the crisis concept is the requirement for more resources. Harrison and Ferguson, for instance. have emphasized the need for more resources, arguing that the crisis is a baffling enigma that requires the use of more resources and immediate attention to maintain services [18]. In other studies, it seems that creating new resources and attracting sufficient resources can help solve the problem of lack of equipment and facilities [19].

If ample resources are not provided, equipment distribution based on prioritization is the main solution to this challenge. Clear and consistent criteria must be defined to prioritize allocation. At the same time, resource allocation policies need to receive up-to-date information at regular intervals from specific information sources [20].

Decision-making and accountability

Another significant challenge at the height of the corona was the kind of decision-making and crisis response. Taking unawares and, by its nature, non-missionary entry, without formal request and foresight, lead to the depletion of strategic reserves in a critical, treatment-oriented military organization.

On the other hand, postponement in management decisions has direct and indirect negative consequences on all organizational processes, including the duties and responsibilities of staff and the consequences of patients. Decision-makers and policymakers must improve timely and appropriate accountability in the organization by using optimization techniques such as maneuvers [21]. Studies have demonstrated that overcrowding of patients on the one hand owing to lack of facilities and on the other hand, due to increased stress, disrupts the decision-making process of managers [22]. This indicates the importance of timely response to organizational needs in such situations.

The general policies of an organization, even at crisis height, should by no means be ignored or prioritized. Having a pre-determined plan to deal with epidemic crises is the most remarkable way to prevent taking unawares and haste in decisions. In the meantime, constructing a robust documentation structure as a decision-supporting system helps managers to utilize past experiences and decline decision-making errors.

Health and Safety of patients and staff

The next critical challenge striking in this crisis was the safety challenge, both for the patients and the medical staff. Considering the safety of patients, the most significant threats were lack of companionship and hardship in meeting the basic needs of patients, such as nutritional requirements and personal hygiene. Meanwhile, educating patients about how to deal with COVID-19 disease during hospitalization and after discharge in cases such as the duration of quarantine, duration of taking drugs and indication for discontinuation of drugs, type and method of nutrition in different stages of the disease, etc. had not been sufficient. The important point is that this training should be performed with a precise mechanism with specific protocols and should be taught professionally; however, this has not been achieved.

Concerning the safety of staff, lack of training on how to utilize personal protective equipment, long shifts and long-term use of PPE, nutritional status during shifts, and emergency visits to infected patients without full protection are among the problems that threaten the safety of staff which in some cases, has transmitted the disease to them.

Information dissemination management

At the onset of the crisis, the spread of exaggerated information and rumors from unofficial sources caused fear, irrational attitude, or withdrawal of some medical staff from dealing with patients. It appears that under the warning system of the hospital crisis management system, the formation of a specialized working group for news management and the design of an integrated, continuous, and regular information system that covers all hospital staff, along with staff training in distinguishing news and disseminating reliable news is of great importance.

Monitoring and Evaluation

The next recognized challenge for the hospital in this crisis was the lack of systematic, planned, regular, and constant evaluation. Evaluation and monitoring are the main basis of planning and determining the next steps in dealing with crises. Determining particular indicators or designing an integrated checklist for monitoring and evaluation can make evaluation effective [26], and the strategic information provided by these indicators can help decision-makers at the hospital level, or even at the national level, to have a logical and evidence-based process for crisis management. Such indicators and checklists in times of crisis should either be pre-planned or designed as soon as possible so that time is not lost.

Patient management

The crowd of patients in the hospital can have a couple of consequences. On the other hand, it enhances the incidence of medical errors, escalates mortality, and waiting time for patients and prolongs the length of hospital stay, and leads to disruptions in receiving treatment and care, and ultimately patient dissatisfaction. On the other hand, a high workload causes fatigue and burnout for the staff.

Another critical issue was the lack of a psychological support team for staff and patients, so over the first weeks when the various dimensions of the disease were still unknown, both the medical staff and most of the patients went through stressful and scary days. The need to go through such difficult days requires the formation and support of professional teams in the field of psychological support, whose vacancy at the height of the COVID-19 crisis was quite felt in the hospital. No such team has been formed so far, while psychological support interventions in dealing with crises are an integral part of crisis response worldwide [24].

In China, on January 27, the National Health Commission issued its first comprehensive guideline for psychological intervention in people with COVID-19, highlighting the need to provide mental health support to patients and caregivers. In Singapore, psychological support is also one of the five components of patients' treatment strategies that focus on psychiatrists [25].

To conclude, this study generally has two kinds of influences. On the one hand, the spectrum of crisis outcomes involves the beneficial and effective effects such as empowerment, participation, and self-sacrifice of staff, and on the other hand, it involves the negative and at the same time strong effects such as lack of equipment and medicine and dissatisfaction with services for patients and lack of equipment and high workload as well as fatigue for the staff. The consequences of the crisis have also been mentioned in relevant texts and as James and Gillland believe, the crisis is both a danger and an opportunity to experience [26]. Crisis experience helps individuals to show appropriate interventions in managing critical situations that lead to growth and increase motivation for change in the future.

CONCLUSION

This qualitative study illustrates the experience of managers and officials of a reference hospital in the challenges and consequences of the Covid-19 pandemic. Robust documentation and evidence-based decision-making, structured coordination of internal and external organizations, effective and practical use of crisis management systems, basic training of staff and patients, prioritizing patient and staff safety, organized dissemination of information, and systematic monitoring and evaluation are the significant tips for dealing with a crisis such as the Covid-19 pandemic.

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Ethical approval

The ethics code of this research (IR.BMSU.BAQ.REC.1400.016) has been officially received from the National Ethics in Biomedical Research system of the Islamic Republic of Iran Ministry of Health and Medical Education. All stages of this research were performed under national ethical standards.

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ORIGINAL ARTICLE

The Relationship Between the Tolerance for Uncertainty and Psychophysiological Qualities of Servicemen

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Abstract: *The purpose of this study was to establish the relationship between the tolerance for uncertainty level and several psychophysiological and psychological qualities of military specialists for determining additional approaches to occupational selection. The study was conducted on a group of servicemen who performed special tasks and were capable of responding to terrorism – 49 men (main group) and 19 military doctors (control group) – men aged 25-34 years. Tolerance for uncertainty was studied based on the modified S. Badnder's method. The level of manipulation score (MAC) was assessed by the adapted I.A. Romanova and O.O. Zhdanov questionnaire (estimated by the so-called "MacScale"). Psycho-physiological parameters were determined using a special computer program. Statistical analysis of data was performed by descriptive and nonparametric statistics, as well as a cluster, stepwise discriminant, and correlation analysis. A personality trait "tolerance for uncertainty" was valuable for the occupational activity of servicemen performing special tasks. The such feature had 63.3% of servicemen in the studied group. The specificity of the occupational activity of military doctors was the quality of manipulation of people. Moreover, those who were the most tolerant of uncertainty had an inverse relationship with Machiavellianism, which indicates the existence of compensatory psychological mechanisms balancing and harmonizing service and personal relationships among micro-teams of servicemen. Servicemen with a lower level of tolerance for uncertainty had positive relationships with several psychophysiological functions (strength and mobility of nervous processes, quality of dynamic memorization), which confirms the idea of psychophysiological mechanisms to enhance tolerance for uncertainty by increasing the working capacity of these people. Such personal quality as tolerance for uncertainty is a valuable occupationally important feature that allows performing the selection of servicemen who are more efficient and reliable in the performance of official duties tasks.*

Keywords: *servicemen, psychophysiological and psychological qualities, tolerance for uncertainty*

INTRODUCTION

The work of a military specialist is always associated with the in-depth complexity and dynamic variability of performed tasks, which causes an increased risk of obtaining a positive result, and increases the emotional stress of the specialist. The ability to adequately respond to extreme factors has a positive effect on the reliability of occupational activities [1].

Several psycho-emotional stress factors in the combat zone have been identified [2]. One of them was the uncertainty of the enemy's behavior, which did not allow to assess his strength; the formation of distrust in the command of combat operation, which affected the understanding of the implementation of certain maneuvers; failure to conduct timely rotation of servicemen from the active hostilities zone, which caused their mental and physical overload; the beginning of positional warfare, the monotony of which is exhausting for the human being; command policy on the ceasefire regime when the enemy continues to fire, etc.

The presence of such respectable reasons determines the existence of strict requirements for the psychophysiological and psychological qualities of servicemen, in particular, resistance to the unpredictable influence of various extreme factors, as well as the quality of being tolerant of uncertainty

[3,4,5]. According to [6] the concept of resilience to uncertainty is associated with the existence of a self-regulation mechanism in the form of compensation for the uncertainty of choice based on the use of all possibilities of their intellectual and personal potential.

If tolerance for uncertainty can be perceived as a willingness to accept the event with a certain tolerance and endurance, to be satisfied with partial and perhaps not entirely irrational problem solutions, then, to some extent, the opposite term - intolerance reflects the process of active "conservation" and negative attitudes to existing differences in situations where new events have been formed [3]. The authors showed that a more tolerant attitude towards uncertain situations in servicemen evokes a calm, positive attitude towards these situations, which helps them to control and regulate their emotions, reduce stress and show self-control in problematic situations. It was also shown that in the conditions of testing the possibility of using prompts, persons tolerant of uncertainty used the opportunity to raise their awareness, and intolerant – ignored this possibility [7].

Tolerance for uncertainty is one of the most important factors in improving the reliability of occupational activities, which affects the combat effectiveness of servicemen and other professionals in extreme jobs [8]. Therefore, the analysis of this characteristic in the military is relevant and will have a significant effect taking into account such properties as tolerance for uncertainty in the occupational selection and other measures for improving the human capital of the Armed Forces of Ukraine.

The objective of this study

The purpose of this study was to establish the relationship between the tolerance for uncertainty level and several psychophysiological and psychological qualities of military specialists for determining additional approaches to occupational selection.

MATERIALS AND METHODS

The study was conducted on a group of servicemen who performed special tasks and were capable of responding to terrorism – 49 men (main group) and 19 military doctors (control group) – men aged 25-34 years. Tolerance for uncertainty was studied based on the modified S. Badnder's method (Tolerance Ambiguity Scale) [9,10] using scales: novelty, complexity, insolubility, and total uncertainty. According to the literature, the scales of the questionnaire S. Badner are independent of gender and age [11].

In addition, tolerance for uncertainty was associated with high levels of interpersonal intelligence and self-efficacy, and intolerance was associated with decreased verbal intelligence. Therefore, these parameters can be used to diagnose tolerance for uncertainty for occupational selection.

The level of manipulation score (MAC) was assessed by the adapted I.A. Romanova and O.O. Zhdanov questionnaire (estimated by the so-called "McScale") [12,13,14], consisting of twenty statements.

Psychophysiological parameters were determined using a special computer program [15,16,17]. The quality of dynamic memorization (QDM), indicators of strength, and functional mobility of nervous processes (SNP and FMNP, respectively) were determined.

All examinations were performed under the ethical standards of the responsible committee and the Helsinki Declaration and have been approved by the Bioethics Commission of the Ukrainian military medical academy.

Statistical analysis of data was performed by descriptive and nonparametric statistics, as well as a cluster, stepwise discriminant, and correlation analysis using the software package STATISTICA 13.3, license AXA905I924220FAACD-N.

RESULTS

First of all, it should be noted that the data on the components of tolerance for uncertainty among the main group in the previous analysis were heterogeneous. Therefore, it was decided to divide this group into two homogeneous subgroups based on these parameters. This procedure was performed using cluster analysis (method k – means). The obtained data, as well as data on other psychophysiological and psychological parameters inherent in these subgroups, are given in Table 1.

Consideration of the data in the table shows that all four signs of tolerance for uncertainty were significantly different in subgroups 1 and 2. According to the classification in [9], people of the study group on the "novelty" scale were on the average level of expression, on the scale of "complexity" were on the average level of expression, on the scale of insolubility were on the average level of expression for subgroup 1 and high level of expression, as well as a general indicator – was at the average level of severity. However, the levels of indicators in subgroup 2 were significantly higher (marked by the sign "↑") than in subgroup 1. Therefore, representatives of subgroup 2 can be considered more tolerant of uncertainty. This was evidenced by the increase in the values of tolerance for uncertainty characteristics in subgroup 2, specifically: on the scale of "novelty" by 18.5%, on the scale of "complexity" by 19.2%, on the scale of "insolubility " by 26.3% and the scale of "total uncertainty" – by 20.8%.

Table 1: Characteristics of psychological and psychophysiological parameters of subgroups 1, 2, and control group

Parameters	Subgroups		100 × (M_x-M_y)/ M_y, %	Control group (n=19)
	1 (n=18)	2 (n= 1)		
Novelty, *c.u.*	13.0 ± 0.5 **	↑ 15.4 ± 0.7	18.5	13.9 ± 0.6
Complexity, *c.u.*	29.1 ± 0.6 ***	↑ 34.7 ± 0.8	19.2	33.8 ± 0.9 ^^^
Insolubility, *c.u.*	13.7 ± 0.3 ***	↑ 17.3 ± 0.5	26.3	13.4 ± 0.8ººº
Total uncertainty, *c.u.*	55.8 ± 0.7 ***	↑ 67.4 ± 1.1	20.8	61.1 ± 1.3 ^^^ ººº
Quality of dynamic memorization, *s*	0.61 ± 0.05	0.64 ± 0.07	-	-
The strength of nervous processes, *ms*	359.4 ± 8.4 *	↑ 320.4 ± 14.6	12.2	-
Functional mobility of nervous processes, *ms*	286.0 ± 7.1 * *	↑ 253.4 ± 11.9	12.7	-
Level of manipulation, *mac*	67.6 ± 3.4	67.6 ± 4.8	-	↑ 75.8 ± 2.4 ^^^ ººº

*Note: *, **, *** significant difference of means between subgroups 1 and 2, by Student's criterion, corresponds to the level p <0.05, p <0.01, p <0.001. ^^^ - a significant difference of means between subgroup 1 and the control group by Student's criterion corresponds to the level p <0,001. ººº – a significant difference of means between subgroup 2 and the control group by Student's criterion corresponds to the level p <0,001. Mx, My – respectively larger and smaller values of the indicator (reflects the degree of a better expression of psychological and psychophysiological qualities in subgroups 1 and 2). ↑ – a sign of improved performance.*

According to C. Arbona and co-authors [18] people with low values of the variable "novelty" find it difficult to perceive incomprehensible dual situations, the unusualness of the situation causes them some actions in professional activities and ignorance of prospects, they perceive new tasks as unsolvable, individuality and independence cause anxiety and discomfort in new problematic situations among such professionals, and stability – as a necessary condition favorable for personal and professional life. These authors believe that the higher the value of the variable "complexity", the more such individuals have the opportunity to solve complex and intractable difficulties in professional activities, they are ready to compete, overcome obstacles, and gain new knowledge, they need to gain positive value for others, do good deeds for people, etc. Such specialists are ready to solve complex professional issues within which experience has already been acquired. Those who are more tolerant of uncertainty will compete more actively and strive to win professionally. They prefer to overcome difficulties and try to solve them creatively in a situation of uncertainty, using a variety of approaches.

Thus, subgroup 2 servicemen, who compound 63% of those whose professional duties were focused on special tasks and were more tolerant of uncertainty, have better adaptability to perform their duties. As V. Ryutin emphasized [19] content of high tolerance for uncertainty is the ability of servicemen in problematic and crisis situations to actively interact with the environment and constantly restore their mental balance, help prevent confrontation and develop positive relationships with themselves and others. It should also be emphasized that the psychophysiological indicators of the working capacity of servicemen of subgroup 2 (according to the SNP and FMNP) have a significantly better level. This indicates better endurance and the ability to perform operations faster in the ever-changing environment of these individuals.

Correlation analysis (Spearman's correlation coefficient, p <0.05) was used to further consider the peculiarities of the formation of behavioral reactions among representatives of subgroups 1 and 2. The results of this analysis are shown in Figure 1. In this figure, the representatives of subgroup 1 were conditionally defined as intolerant, and the representatives of subgroup 2 – as tolerant. This separation was based on the assumption that the levels of all tolerance indicators of subgroup 2 were significantly higher than those of subgroup 1.

Figure 1: Relationship between psychological and psychophysiological functions with indicators of tolerance to uncertainty in servicemen of subgroups 1 and 2. T – tolerance for uncertainty (in servicemen of subgroup 2); IT – intolerance for uncertainty (in servicemen of subgroup 1). Continuous line – direct correlation; dashed line – negative (inverse) correlation

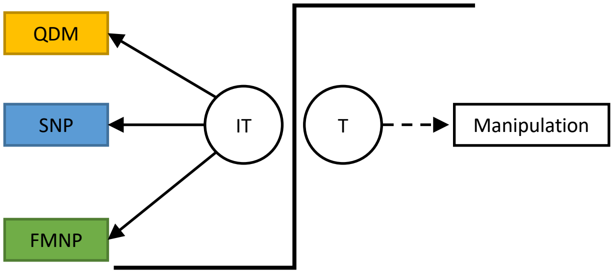

The figure shows that the indicators interacted differently in subgroups 1 and 2. Subgroup 1 was characterized by the relationship of indicators of intolerance with a set of psychophysiological parameters that reflect the correlates of performance. From this fact we can conclude that people with a reduced tolerance for uncertainty were related to the level of efficiency of subgroup 1. All applied psychophysiological techniques were characterized by the unpredictability of each stimulus in terms of short-term or long-term processing of information. Another special feature of the registered responses of representatives of subgroup 1 was the independence of using short-term memory when performing tasks, because when testing the strength and mobility of nervous processes, this memory is used in minimal amount, and when testing dynamic memorizing, this feature is present at all times.

With a higher tolerance for uncertainty, a different picture was observed only with the level of manipulation (subgroup 2). Moreover, there was an inverse correlation. In this case, the increase in tolerance for uncertainty is accompanied by a decrease in the level of manipulation. As is known [13], the presence of more pronounced manifestations of morality and humanity is more common in the military, compared to civilians, who are significantly dominated by selfishness and the use of various tricks in management. Based on the data obtained in the present study, it can be assumed that high tolerance for uncertainty significantly unloads human capabilities, which increases the potential for morality and humanity.

The formulated conclusion is confirmed by the data of other authors. As M. Kovaleva et al. [20] had confidence that people with a high tolerance for uncertainty will demonstrate mental resilience and professional reliability, and people with a low tolerance for extreme conditions will have low levels of these qualities. According to [6], this psyche feature affects the ability of self-regulation of the person to overcome the uncertainty of choice based on the use of all possibilities of their intellectual and personal potential.

The characteristics of the control group for all indicators of tolerance for uncertainty were between the values of identical characteristics of subgroups 1 and 2. But the rate of manipulation of individuals in this group was much higher than that of subgroups 1 and 2. Since the control group consisted of military doctors that habits of manipulation were inherent in their profession, where communication with the patient requires certain specific skills to affect his mental sphere. In this case, there was no correlation between the Machiavellianism (level of manipulation) of the control group and subgroups 1 and 2. This result may indicate that high levels of Machiavellianism mask a tolerant attitude towards the patient.

DISCUSSION

The analysis of the obtained data testifies to the value of the uncertainty tolerance indicator for determining the professional suitability of a serviceman, especially those contingents that perform complex special tasks. This thesis is confirmed by many other authors. Thus, M. Kovaleva et al. data in the study of tolerance for uncertainty in officers showed the dependence of their mental adaptability on the conditions of service, which allowed us to conclude the need to determine the professional suitability of officers when transferring them to a military unit [20]. Barko V. et al. [9] stressed that tolerance for uncertainty was an occupationally important feature of a police officer in the selection process, and during professional training, this quality must be diagnosed, taken into account, strengthened, and developed in every possible way. Principally such personal quality, such as tolerance for uncertainty, will increase the efficiency of task performance, as well as allow better endurance of unforeseen situations that constantly accompany official activities; open opportunities for creative thinking of complex professional situations, search for alternative solutions; increase stress resistance and strengthen productive mechanisms of psychological protection of the individual (rationalization, sublimation, displacement); strengthen the psychological readiness and reliability of the individual to act in special and extreme conditions; will help preserve officers' mental health".

Thus, as a result of this study, it was found that the vast majority of tested servicemen (63.3%) who perform complex special tasks had a fairly high level of tolerance for uncertainty. This allows us to make a reasonable assumption that tolerance for uncertainty is a valuable professionally important quality, which allows us to separate those servicemen who are more efficient and reliable in the performance of official duties.

CONCLUSION

1. A personality trait "tolerance for uncertainty" was valuable for the occupational activity of servicemen performing special tasks. Such feature had 63.3% of servicemen in the studied group.

2. The specificity of the occupational activity of military doctors was the quality of manipulation of people. Moreover, those who were the most tolerant of uncertainty had an inverse relationship with Machiavellianism, which indicates the existence of compensatory psychological mechanisms balancing and harmonizing service and personal relationships among micro-teams of servicemen.

3. Servicemen with a lower level of tolerance for uncertainty had positive relationships with several psychophysiological functions (strength and mobility of nervous processes, quality of dynamic memorization), which confirms the idea of psychophysiological mechanisms to enhance tolerance for uncertainty by increasing the working capacity of these people.

4. Such personal quality as tolerance for uncertainty is a valuable occupationally important feature that allows

performing the selection of servicemen who are more efficient and reliable in the performance of official duties tasks.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.
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Authors' contribution

Conceptualization, Kalnysh, Shvets; methodology, Kalnysh, Shvets; software, Kalnysh; validation Shvets; formal analysis, Shvets, Maltsev, Nagovitsyn; investigation, Shvets, Maltsev, Nagovitsyn; resources Shvets, Maltsev, Nagovitsyn; data curation, Shvets, Maltsev; writing—original draft preparation, Shvets, Maltsev; writing—review and editing Shvets; visualization, Maltsev, Nagovitsyn; supervision, Shvets; project administration, Kalnysh, Shvets; funding acquisition, Kalnysh. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the Ukrainian military medical academy (protocol 122, 12/09/2019) for studies involving humans.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.
Written informed consent has been obtained from the patient(s) to publish this paper.

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ORIGINAL ARTICLE

The Effect of Reciprocal Imitation Training on Increasing Expressive Vocabulary of Children with Autism Spectrum Disorders

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Abstract: *Objective: One of the areas of impairment in childhood autism spectrum disorders is delayed imitation. Due to the defect in the social function of imitation in these children and its negative consequences on lexical and verbal skills, Researchers in the field of educating autistic children consider increasing imitation skills to be a very important therapeutic goal. Previous studies have shown the effects of improving imitation on increasing the social, linguistic, and verbal skills of these children. In the present study, the effect of reciprocal imitation training on increasing the vocabulary of autistic children was investigated. Materials and Methods: This was an interventional study with a pretest-posttest design. 16 autistic children were trained in cross-border imitation based on expressive vocabulary in 96 sessions. After performing post-test and data collection in data analysis from the McNemar test to determine the changes of each word before and after the intervention and the Wilcoxon Marked Test was used to determine changes in vocabulary categories before and after the intervention and the level of statistical significance was considered (P> 0.05). Results: The results of data analysis show that the average number of responses "expresses" in each of the words after the intervention increased significantly in all vocabulary groups (P> 0.05). It seems that the intervention resulted in significant changes in participants. Conclusion: The results of this study show that teaching the method of reciprocal imitation has a significant effect on increasing the number of expressive words in autistic children.*

Keywords: *autism spectrum disorder; reciprocal imitation; expressive vocabulary*

INTRODUCTION

Childhood autism spectrum disorders are characterized by stereotyped and repetitive behaviors, impaired verbal and non-verbal communication, and impaired social behaviors. The prevalence of autism in 2014 in Iran was reported to be 95.2 per 10,000 children [1,2]. The skill of imitating others is one of the essential abilities for newborns [3]. Various studies have shown an association between imitation and social communication skills such as language, play, and attention. Some researchers believe that imitation in children has two important functions: 1- Imitation of sounds, and facial expressions to communicate and social interaction with others; 2- Enabling the child in the first half of life to be aware of activities and the intention of the other person and the subsequent formation of social learning in the child [4].

However, research has shown that children with autism spectrum disorders are deficient in the use of the social function of imitation [5]. From the first study of the nature of imitation in children with autism spectrum disorders by Damier (1972) to recent research on the fact that children with autism spectrum disorders develop the ability to imitate normal children and other disabilities. They are weaker, there is agreement [6]. Sigman and Angers (1984) reported that these children delayed voice imitation and body movement imitation compared with normal children and children with developmental delays [7]. Due to the impairment in the social function of imitation, which may have many negative consequences, numerous researchers have paid special attention to the treatment of imitation impairment in these children [8].

Researchers in the field of education of children with autism spectrum disorders now consider the increase of imitation skills as a very important therapeutic goal and believe that the improvement of imitation skills will lead to an increase in social communication skills [9]. In general, in the field of social interaction skills training, two therapeutic models of classical structured behavioral interventions can be included, including the applied analysis method, Lavas, and Pax, and in contrast, the evolutionary model that imitates spontaneous imitation skills during natural interaction. It has a special effect on increasing flexibility, social imitation, and other social communication skills, and includes natural fluoride behavioral interventions, comprehensive interaction methods, and cross-imitation training [10]. The method of mutual imitation teaching is a kind of natural intervention program and its main emphasis is on the social role of imitation in improving speech and non-verbal skills [11].

Unlike structural imitation instruction, in reciprocal imitation instruction, the therapist imitates all of the child's gestures, words, and actions using toys, and the child's level of interest as well as imitation of the child's speech, proverbial pronunciations, and use. Language and speech are spontaneously considered by the therapist [12]. Enjoyment of communication, perception of people's faces, cooperation, understanding of non-verbal concepts and signs, ability to pay attention to another person, perception of meaning in sounds, ability to choose, perform subtle movements, spatial awareness, the pleasure of being in a group, from the most important therapeutic goals in the teaching method are mutual imitation [10].

Studies on the method of mutual imitation training by Ingersell (2003) show that this method increases social communication skills such as social interaction, language, pretend play, and the use of body movements [10]. For example, a longitudinal study of children with autism showed that there was a relationship between mimicry of body movements and the development of expressive language in the next six months [13]. Wallen, Schreibman, and Ingersell (2006) showed that educating children with autism spectrum disorders using cross-imitation leads to an increase in behaviors related to social communication skills in imitation, play, and spontaneous speech [14]. In another study, Ingersell and Laland (2004) examined the effect of object and gesture imitation training on the speech and language of 4 children with autism spectrum disorders. The results of this study showed that the method of mutual imitation education leads to an increase in a speech in children with autism spectrum disorders [15].

Also, in another study, Pusheneh, Zadeh Mohammadi, Siampour (2012) examined the effect of cross-imitation training on the ability to imitate children with autism spectrum disorders. The results showed that cross-imitation training has increased the ability of these children to imitate in the fields of action-related imitation, gesture imitation, and facial expressions [16]. Given that the ability to imitate is the most important in the field of early childhood education, this has created the need for this research. Concerning this issue, the present study investigates the effectiveness of cross-mimicry training interventions on increasing the vocabulary of children with autism spectrum disorders.

ANALYSIS METHOD

The research method was an intervention with a pretest-posttest design without a control group. The statistical population included children aged 3 to 7 years with autism spectrum disorders in two rehabilitation centers with autism spectrum disorders in Lorestan province, Khorramabad-Iran, and Boroujerd-Iran, which were selected by convenience sampling method. Based on the available files of the clients in the rehabilitation center and by the diagnosis of the center psychiatrist and clinical observation and diagnostic tests in the files including Garz and the autism assessment checklist of diagnostic criteria (DSM-V-TR) of all clients of the center. At that date, they had been diagnosed with Autism Spectrum Disorders, and due to the comprehensiveness of the assessments, they were not given a separate diagnostic test.

The sample size in this study is 16 people (4 girls and 12 boys) with a mean age of 55.88 months with a standard deviation of 17.02. The age range of participants is 36 to 83 months (Table 1). 9 of these 16 people were trained in the Rehabilitation Center for Children with Autism Spectrum Disorders in Khorramabad and 7 others were trained in the Rehabilitation Center for Children with Autism Spectrum Disorders in Boroujerd.

Table 1: Frequency distribution of age

	Number	Average (Mean)	Standard deviation	At least (Min)	Maximum (Max)
Age	16	55.88	17.02	36	83

The severity of autism symptoms in these children was based on DSM-V levels 1 and 2 and level 3 children were excluded from the study due to the high severity of symptoms. The inclusion and exclusion criteria in this study were as follows: (1) being in the range of 3 to 7 years; (2) diagnosis by the psychiatrist of autism and tests by the center to classify children with autism spectrum disorders; (3) having a normal hearing threshold and healthy vision based on medical records and clinical observation; (4) No seizures and no delay in the development of motor skills by reviewing the medical file and the developmental and personal information form of the children, which was filled in by the parents and educators of the center. According to the same criteria, 4 children were excluded from the study.

A pre-test was performed before the intervention. The list used included 49 words that were placed in 7 lexical categories. These 7 categories were: body parts, verbs, clothing, fruits, persons, objects, and foods. For each category, 7 words were selected from the most used words used by normal children.

A pre-test was performed for each child individually using pictures and objects related to each word. The child's answer is marked in two columns/does not express was marked. After conducting the pre-test for children, a cross-imitation training intervention program was implemented for 12 months in 96 sessions. Each child was trained 2 days a week for 30 minutes each time by a speech and language pathologist trained at the center. During the training, the therapist first imitates the children's play with toys in the treatment room of the center, to attract the child's attention by imitating body movements and spoken sounds, and to prepare him for imitation. The therapist begins the training by identifying the activities and toys that the child is familiar with. For example, throwing a ball that the child is already familiar with and throwing. Cards or objects and gestures related to that word were then used to motivate the child to imitate and express the word. The therapist encouraged and strengthened any efforts and voices of the children to imitate the movement and sounds of the word.

At the end of the training sessions, the children were tested based on the same list of 7 lexical categories, and the children's responses to the pictures or objects related to each of the words in the list are expressed in two columns. Slow registration and data were collected. In this study, number and percentage indices for qualitative variables and mean and standard deviation indices for quantitative variables were used to determine frequency tables. In data analysis, McNeamar test was used to determine the changes in each word before and after the intervention, and Wilcoxon Signed Rank test was used to determine the changes in vocabulary categories before and after the intervention.

All analyzes were performed by SPSS software version 21 and the level of statistical significance was considered 0.05.

RESULTS

To obtain a more accurate measure of the effect of the intervention on each group of words, according to Table 2, the number of items related to the column "Expresses" in each category was counted and a score was set for before and after the intervention. For this purpose, the number of "expressed" responses in the table below was evaluated as a score.

Table 2: The score analysis "expresses" the number of answers in each group of words

		Average (Mean)	**Standard deviation**	**At least (Min)**	**Maximum (Max)**	**Significance***
Organ	Before	0.50	0.97	0	3	0.003
	After	3.69	2.96	0	7	
Verbs	Before	0.56	0.89	0	2	0.003
	After	2.69	2.47	0	7	
Clothing	Before	0.25	1.00	0	4	0.011
	After	2.13	2.47	0	7	
Fruits	Before	0.94	1.29	0	4	0.003
	After	3.19	2.79	0	7	
People	Before	1.00	1.03	0	3	0.003
	After	2.75	2.29	0	7	
Equipment	Before	1.19	1.17	0	3	0.007
	After	2.81	2.66	0	7	
Foods	Before	0.13	0.34	0	1	0.005
	After	2.38	2.60	0	7	

** Wilcoxon Signed Rank Test.*

The results show that the average number of "expresses" responses in each of the words after the intervention increased significantly in all vocabulary groups ($P < 0.05$). In other words, in general, the intervention was effective in all vocabulary groups. Among the 7 lexical categories, the group related to body parts shows significant changes for each of the 7 words, while the clothing category with a significant level for only one word shows the least effective intervention method among the classes. The results of this study according to Table 3 show that the expression of all words of the body class has significantly improved after the intervention ($P < 0.05$). In other words, the intervention was effective in all vocabulary related to the organ class. Among the 7 words in this category, three words (eyes, ears, and tongue) were not uttered by any of the children studied according to the pre-test design. But after performing the method, words (eyes and ears) were expressed by 7 people and words (language) by 5 autistic children in the study.

Table 3: Summary of vocabulary analysis related to body class

		Before		After		Significance*
		Number	%	Number	%	
Hair	Does not express	13	81.3	5	31.3	0.008
	Express	3	18.8	11	68.8	
Hand	Does not express	14	87.5	6	37.5	0.007
	Express	2	12.5	10	625	
Leg	Does not express	14	87.5	6	37.5	0.008
	Express	2	12.5	10	62.5	
The eye	Does not express	16	100.0	9	56.3	0.016
	Express	0	0.0	7	43.8	
Ear	Does not express	16	100.0	7	43.8	0.004
	Express	0	0.0	9	56.3	
Nose	Does not express	15	93.8	9	56.3	0.031
	Express	1	6.3	7	43.8	
Tongue	Does not express	16	100.0	11	68.8	0.031
	Express	0	0.0	5	31.3	

** McNemar Test.*

The results of this study according to Table 4 show that the vocabulary expression of the verb class (give, come, fall, not, and go) has significantly improved after the intervention (P <0.05). In other words, the intervention has been effective on these words. But as can be seen in Table 5, the other two words (coming and kissing) do not show significant changes. Vocabulary expression for clothing (shoes) improved significantly after the intervention (P <0.05). In other words, the intervention has been effective in this term. But the expression of other words of this class (pants, hat, socks, clothes, blouse, and slippers) do not show significant changes after the intervention (Table 5). The expression of 3 words of fruit class (apple, banana, and pomegranate) significantly improved after the intervention (P <0.05). But other words (orange, cucumber, berry, plum) do not show significant changes. In this category, the word orange was not uttered by any of the children according to the pre-test design, but after the intervention and post-test, 2 children uttered the word (Table 5). Among the 7 words in the category of persons, after the intervention, the expression of two words (father and uncle) has significantly improved (P <0.05). But other words (mom, uncle, aunt, grandfather, and grandmother) do not show significant changes. Vocabulary (grandparents, aunts) was not spoken by any of the children before the intervention. After performing the post-test, the word grandfather was uttered by 2 people, the word grandmother was uttered by 1 person and the word aunt was uttered by 2 children. However, these changes do not show significant levels (Table 5). The expression of two items of equipment class (dolls and cars) significantly improved after the intervention (P <0.05). In other words, the intervention has been effective on these words. But other words (spoon, mobile, plate, knife, glass) do not show significant changes. In these two classes, the words glass and knife were not uttered by any of the 16 people at the beginning of treatment, while after the intervention, 31.3% of children (5 people) had the word glass, and 18.8% of children (3 people) uttered the word knife. After the intervention, the expression of words (soup, bread, and food) from the food category significantly improved (P <0.05). But the words (milk, meat, rice, and water) that at first none of the 16 people in the study in the pre-test design were able to express in the post-test design of two words milk and rice by 25% (4 people and word meat were expressed by 31% (n = 5) and the word water was expressed by 18.8 (n = 3) of children but did not show significant changes.

DISCUSSION

This study investigated the effect of reciprocal imitation training on increasing the vocabulary of children with autism spectrum disorders aged 3-7 years. The findings of this study showed that cross-curricular training improves the vocabulary of children with autism spectrum disorders and the average number of "expresses" responses in each vocabulary after the intervention is significant in all vocabulary groups increased (P <0.05). In explaining the results, it can be stated that it is possible that the method of mutual imitation training, which involves the child engaging in fun activities by taking over the treatment space, focuses attention on communication and stereotyped behaviors during this process.

Table 4: Summary of vocabulary analysis results related to 7-word words of vocabulary that show significant differences

Vocabulary		Before		After		Significance*
		Number	%	Number	%	
Give	Does not express	11	68.8	5	31.3	0.014
	Express	5	31.3	11	68.8	
Come on	Does not express	12	75.0	6	37.5	0.014
	Express	4	25.0	10	62.5	
To fall	Does not express	16	100.0	12	75.0	0.046
	Express	0	0.0	4	25.0	
Is not	Does not express	16	100.0	8	50.0	0.005
	Express	0	0.0	8	50.0	
To go	Does not express	16	100.0	11	68.8	0.025
	Express	0	0.0	5	31.3	
Shoe	Does not express	15	93.8	7	43.8	0.008
	Express	1	6.3	9	56.3	
Apple	Does not express	12	75.0	5	31.3	0.016
	Express	4	25.0	11	68.8	
The banana	Does not express	13	81.3	6	37.5	0.016
	Express	3	18.8	10	62.5	
Pomegranate	Does not express	15	93.8	9	56.3	0.031
	Express	1	6.3	7	43.8	
Dad	Does not express	16	100.0	7	43.8	0.004
	Express	0	0.0	9	56.3	
Uncle	Does not express	15	93.8	7	43.8	0.008
	Express	1	6.3	9	56.3	
Dolls	Does not express	15	93.8	9	56.3	0.031
	Express	1	6.3	7	43.8	
Machine	Does not express	16	100.0	9	56.3	0.016
	Express	0	0.0	7	43.8	
Ash	Does not express	16	100.0	10	62.5	0.031
	Express	0	0.0	6	37.5	
Bread	Does not express	14	87.5	7	43.8	0.016
	Express	2	12.5	9	56.3	
Food	Does not express	16	100.0	9	56.3	0.016
	Express	0	0.0	7	43.8	

** McNemar Test.*

The child is guided and guided during play and the child acts more calmly and confidently. This increase in attention will be effective in communicating to increase children's ability to imitate and express words. Findings from the test of this hypothesis are consistent with the results of the research of Ingersell and Schreibman (2006), which was conducted to investigate the training of reciprocal imitation in children with autism spectrum disorders using a natural behavioral approach and its effect on language, pretend play and focused attention. Has [9]. It is also consistent with the results of research conducted by Ingersell and Laland (2010) on the effect of object imitation teaching and body movements on the language and speech of children with autism spectrum disorders using the cross-educational method [15].

Table 5: Summary of the results of vocabulary analysis related to 7-word words of vocabulary that do not show a significant difference

Vocabulary		Before		After		Significance*
		Number	%	Number	%	
Coming	Does not express	16	100.0	13	81.3	0.083
	Express	0	0.0	3	18.8	
Kissing	Does not express	16	100.0	14	87.5	0.157
	Express	0	0.0	2	12.5	
Pants	Does not express	15	93.8	11	68.8	0.219
	Express	1	6.3	5	31.3	
Hat	Does not express	15	93.8	10	62.5	0.125
	Express	1	6.3	6	37.5	
Socks	Does not express	15	93.8	12	75.0	0.375
	Express	1	6.3	4	25.0	
Dress	Does not express	16	100.0	12	75.0	0.125
	Express	0	0.0	4	25.0	
Blouse	Does not express	16	100.0	13	81.3	0.250
	Express	0	0.0	3	18.8	
Slippers	Does not express	16	100.0	13	81.3	0.250
	Express	0	0.0	3	18.8	
Orange	Does not express	16	100.0	14	87.5	0.501
	Express	0	0.0	2	12.5	
Cucumber	Does not express	15	93.8	11	68.8	0.125
	Express	1	6.3	5	31.3	
Berry	Does not express	13	81.3	8	50.0	0.063
	Express	3	18.8	8	50.0	
Plum	Does not express	13	81.3	8	50.0	0.063
	Express	3	18.8	8	50.0	
Mom	Does not express	10	62.5	6	37.5	0.125
	Express	6	37.5	10	62.5	
Uncle	Does not express	7	43.8	5	31.3	0.500
	Express	9	56.3	11	68.8	
Grandpa	Does not express	16	100.0	14	87.5	0.500
	Express	0	0.0	2	12.5	
Aunt	Does not express	16	100.0	14	87.5	0.500
	Express	0	0.0	2	12.5	
Spoon	Does not express	9	56.3	7	43.8	0.625
	Express	7	43.8	9	56.7	
Mobile	Does not express	7	43.8	7	43.8	0.999
	Express	9	56.3	9	56.3	
Plate	Does not express	14	87.5	11	68.8	0.375
	Express	2	12.5	5	31.3	
Glass	Does not express	16	100.0	11	68.8	0.063
	Express	0	0.0	5	31.3	
Knife	Does not express	16	100.0	13	81.3	0.250
	Express	0	0.0	3	18.8	

Milk	Does not express	16	100.0	12	75.0	0.125
	Express	0	0.0	4	25.0	
Water	Does not express	16	100.0	13	81.3	0.250
	Express	0	0.0	3	18.8	
Meat	Does not express	16	100.0	11	68.8	0.063
	Express	0	0.0	5	31.3	
Rice	Does not express	16	100.0	9	56.3	0.125
	Express	0	0.0	7	43.8	

** McNemar Test.*

In these two studies, the effects of reciprocal imitation training on increasing imitative communication skills have been pointed out and it has been stated that by engaging in this method through games and reciprocal imitation activities, the child enters into social interaction with the other party and thus Exchanges create opportunities that both parties can use during communication, and this is an important and effective strategy in expanding children's vocabulary and can increase communication skills.

The results of this study are also consistent with the findings of Ingersell (2008), who in an article examined the role of cross-imitation training on imitation skills in social communication conditions. Imitation motivates the development of social communication skills and interactions and social interaction, followed by improved social communication skills including language, play, shared attention, and eye communication [17].

All of this research demonstrates the positive impact of cross-curricular teaching on improving communication skills, including speech and language skills, in children with autism spectrum disorders.

A look at the basics of the basic components of a reciprocal imitation training program, including sitting face to face, imitating play with toys, imitating gestures and body movements, using speech imitation, and the activities and games that take place within this framework. It will help to understand why the subjects of this study have shown significant changes in improving their vocabulary. One of the most important limitations of this study was the limitation in the number of subjects.

To generalize the results of this study, it is better to study with a larger number of subjects. On the other hand, it is suggested that the words of other classes be used to evaluate the effectiveness of this method.

Future researchers are encouraged to study the effect of reciprocal imitation therapy in comparison with other therapies on improving the vocabulary of children with autism spectrum disorders.

CONCLUSION

The cross-mimicry training program improves the social and speech skills of children with autism spectrum disorders. This program can be implemented in the educational and rehabilitation environments of these children. The inclusion of such programs as one of the main themes of educational programs for children with autism spectrum disorders before primary school education can be facilitated in its application and implementation by medical and rehabilitation centers. Specialized services to these children, take an effective step towards teaching the social, speech, and vocabulary skills needed by children in educational settings.

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ORIGINAL ARTICLE

Nursing Journal Club as a Tool to Facilitate Evidence-Based Practice: Participant's Feedback

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Abstract: *Background and aim: Nursing journal clubs have a proven value in nursing education and continuing education, which has expanded widely in the promotion of evidence-based clinical care in recent years. This study aimed to evaluate the clinical nursing journal clubs from the viewpoints of nurses. Methods: This descriptive study was conducted from July 2019- October 2020. This study was conducted in two phases, consisting of designing the format and flowchart and implementing nursing journal clubs and the second phase was evaluation. Results: Format and executive flowchart prepared by the research team and reviewed and approved in the group. In terms of evaluation, minimum (33) and maximum (165) scores with a mean± SD total score of 123.49 ± 19.91 calculated for nursing journal clubs indicate an optimum quality. Conclusions: The qualities of nursing journal clubs were evaluated to be favorable. Accordingly, authorities and planners should consider appropriate actions to facilitate Nursing journal clubs, such as credit points in rank promotion for faculty members, and inclusive in continuing education privileges for nurses, to adapt these activities to the roles of the clinical faculty and the benefits of this educational method in nursing.*

Keywords: *journal club, nursing, evidence-based practice, evidence-based nursing*

INTRODUCTION

As the nursing profession is expanding, the nursing education system should educate nurses who can function effectively and efficiently in therapeutic settings that are constantly changing and subject to huge volumes of information [1,2]. Nowadays, the establishment of a bridge between theory and clinic has become one of the major challenges [3]. A pivotal action to achieve this goal is the ability to transfer and apply research in clinical settings [1,4].

Moreover, learning and updating methods of knowledge and skills have changed in the present world and learning has shifted from individual guidance to the group learning process [5]. Meanwhile, the journal club has a proven value in both nursing and continuing education and has expanded widely in the promotion of evidence-based clinical care (EBCC) and clinical collaboration in recent years [6].

To become familiar with and promote evidence-based research and practice, nurses need to know and apply new health-related texts. Over the past 25 years, substantial advances have been made in the models and frameworks of Evidence-Based Nursing (EBN) to replace processes in the health system, and the results of the research are appropriately used in clinical settings to maximize patient safety [3].

Nurses should be prepared to apply evidence-based practice in decision-making about the clinical care of patients [7,8]. EBN is generally the process of systematic diagnosis, meticulous and accurate evaluation, and subsequent dissemination of using research findings to influence clinical practice. It is continued through changes in standards of care that can lead to positive changes in clinical interventions [9].

Journal club facilitates the learning process in the context of clinical problem-solving and enhances the skills of criticism [5].

This strategy is based on the principles of adult education, active participation in learning and peer learning, using the concept of intimate class, providing the opportunity to shape informal discussions, learning to read and review nursing literature, and encouraging open participation in journal clubs [7]. The time to use journal clubs varies more widely in the nursing profession [10].

Nursing journal clubs are usual in Iranian schools, but in the clinical setting have launched for the first experience in line with the development plan on the roles of clinical faculty members at Tabriz University of Medical Sciences. Therefore, this study was conducted to evaluate the clinical NJCs at Tabriz University of Medical Sciences during 2019-2020 and evaluated the clinical nursing journal clubs from the viewpoints of nurses.

METHOD

This descriptive study was conducted from July 2019 to October 2020 at the School of Nursing and Midwifery and two affiliated hospitals of Tabriz University of Medical Sciences, Tabriz, Iran. Inclusion criteria were willingness to attend nursing journal clubs. Exclusion criteria were voluntary withdrawal from the study and do not answer more than 20% of the questions in the questionnaire. One month before the implementation of nursing journal clubs, the relevant hospitals were informed about the meeting time. Attendance was open to all nurses.

For data collection, the NJC evaluation tool was designed by considering factors influencing the success of NJC according to Kirkpatrick's model [2,11]. NJC quality assessment questionnaire consisted of 33 questions in four domains of satisfaction (18 items), change in knowledge (7 items), change in behavior (4 items), and organizational outcome (4 items). The tool was scored on a 5-point Likert scale from strongly agree to strongly disagree (scoring from 5 to 1).

The Kirkpatrick model is an accepted and widely used framework for the evaluation of educational programs. This model was introduced by Kirkpatrick (1950) and has since been modified by many people, but its four-step structure has remained unchanged.

The model has been described as a comprehensive, simple, and practical model for most educational situations and is recognized by the majority of experts in this field. Kirkpatrick defines evaluation as the effectiveness of a curriculum and divides the evaluation process into four levels (feedback, learning, behavior, and results) [12].

Ten nursing faculty members reviewed the questionnaire for face and content validity. Their comments were applied. CVR of all items was above 0.65 and CVI was above 0.8. The reliability of the tool was calculated and confirmed by Cronbach's alpha (0.95).

This study was conducted in two phases. The first step was to attain the format and flowchart of clinical NJC implementation. Initially, a committee was formed consisting of 3 faculty members and 2 doctoral students. A related comprehensive review of articles published from 2000 to 2018 on databases (SID, Iran Doc, Google Scholar, PubMed, Medline, Scopus, Cochrane Library, Science Direct, and CINHAL) was done. Also, 5 cases of NJC PowerPoints on the Internet were repeatedly assessed and analyzed precisely. Thus, three duties (a - identifying instructions, b - designing the flowchart, and c - determining template content of PowerPoint for NJC) were carried out in the committee for clinical NJC teaching events. Then, the same committee held one-day workshops to inform all members of different departments and all Ph.D. students at Tabriz school of Nursing and Midwifery (n= 40 participants).

Through the workshop NJC fully (history of JC, definition, aim and objectives, outcomes and benefits, types of JC, etc.) was explained and then discussed in the group. Received comments were applied and clinical NJC instructions, flowchart [13], and template PowerPoint were approved at the end of the workshop. Necessary coordination was first made with the hospital authorities, and then NJC sessions were held in two educational centers' of Shahid Madani and Sinai in Tabriz based on an agreement. NJC sessions were set up in a conference hall in hospitals affiliated with Tabriz University of Medical Sciences during 2019-2020.

In the second phase, the viewpoints of faculty members, nurses, charge nurses, nursing supervisors, nurse managers, and nursing students on these sessions were evaluated using a questionnaire, at the final ten minutes of JC. The above two hospitals were selected as research environments because there were clinical faculty members therein and NJC was implemented as their related task.

The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran, approved this study (code: IR.TBZMED.REC. 1397.643.). The study aim was explained to participants and they were ensured of the confidentiality of the data, the voluntariness of participation in the study, and their freedom to withdraw from the study at will.

The data were analyzed using the SPSS software v. 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics (mean, and standard deviation) were used for presenting scores for each domain and the whole domains of the questionnaire. $P < 0.05$ was considered statistically significant.

RESULTS

In the first phase, implementation format and flowchart were attained for clinical NJC as shown in Table 1.

In the second phase, several NJC sessions were evaluated in the two teaching hospitals. NJC topics were “contamination of blood sample cultures (two articles)”, “best practice in wound examination (three articles)”, and “delirium nursing protocol after coronary artery bypass graft and cardiac surgery (three articles)”. The frequency of NJC sessions by a faculty member scheduled at least once in the trimester, with repeated program twice a week (an observer is mandatory for the first time), and 1-2 hours per subject. The results of the evaluation of the face-to-face NJCs held at Tabriz University of Medical Sciences are presented in Table 2.

Minimum and maximum scores for the questionnaire can be 33-165, with a mid-range of 99. A total mean (SD) score of 123.49 (19.91) was reported for clinical NJCs in the current study. The quality of all sections was desirable in the participants’ views, and a desirable quality was evaluated for the NJC implementation.

Table 1: Flowchart of clinical NJC sessions at Tabriz School of Nursing and Midwifery

No.	Activity
1	Problem-finding through focus group sessions, virtual sessions, and PDPs
2	In the event of several issues, prioritizing them through focus group sessions or by Delphi technique
3	Selection of lecturer and assistants for holding NJC
4	Searching and selecting articles related to the topic from those of the last 5 years, systematic reviews, meta-analyses, randomized clinical trials, etc. indexed in valid databases of ISI, PubMed, Cochrane, and Scopus
Holding a meeting of attending knowledge committee, auditing lecturers’ reports on the prior steps, and deciding on steps 5 and 6	
5	Critical evaluation of articles in focus group sessions at the Faculty Determination of articles and journal club type (content or methodological evidence-based practice) Determination of core members (at least five people), and the day, time, venue, and registration
	Designation of a leader and supervisor for monitoring NJC sessions
	Determining a coordinator of NJC meetings with clinical employees
	Regular holding of NJC meetings (face-to-face or webinar sessions)
	Attendants at NJC session: Undergraduate, postgraduate, and doctoral nursing students, experienced and novice clinical nurses, head nurses, clinical and educational supervisors, and if necessary statistics counselor
6	Reserving venue and then notifying Mr. … at least one week in advance to record film, photos, and news of the meetings
Preparing a schedule planned for a one-hour presentation: A review and a one-time exercise by the lecturer and assistant, review of articles by lecturers (25 minutes) + review of articles by core individuals (total 20 minutes for five people) + commentary, and exchange of participants’ thoughts with lecturers (totally 15 minutes up to five people)	
7	Implementation of NJC meeting: - Presentation, evaluation, and discussion of articles and general conclusions; - Preparing report (minutes of the meeting: MoM) - Notification of summary to meeting participants and related audiences
8*	Preparing evidence-based guidelines and protocols or revising existing guidelines based on literature review and focus group sessions at the faculty and hospital
9*	Presenting developed/revised guidelines, protocols, and standards to the secretariat of the operating council for final consensus and approval, then, if necessary, approval by the vice-chancellor of the university
10*	Implementation of evidence-based guidelines and protocols in nursing practice
11*	Evaluation of results

** Steps 8-11 apply to 10% of NJCs.*

Table 2: Mean (standard deviation) of clinical NJCs quality held by nursing faculty members

Domain(range)	Mean (SD)
Satisfaction (18-90)	10.15 (67-75)
Change in knowledge (7-35)	27.15 (5-47)
Change in behavior (4-20)	16.24 (3-22)
Organizational outcome (4-20)	11.69 (2-66)

DISCUSSION

EBN is the use of best research evidence together with clinical expertise and skills by considering the patient's needs and status. In general, four components are involved in EBN decision-making, namely research evidence, patient values, clinical skills, and resource availability [14].

Nowadays, evidence-based education is also gaining ground with the aim of serious, explicit, and rational use of the best evidence to make decisions about nursing education. There is also an emphasis on the inclusion of evidence-based curricula in education [15]. Nursing curricula have included journal clubs, case presentations, group discussions, and rounds in the latest edition of nursing educational techniques revision [16]. Unfortunately, no clinical application of these widely used educational techniques facilitating evidence-based learning and practice is available in the present clinical setting of Iran.

Various studies demonstrate that journal club is accepted as a mechanism the recommendations of which are reflected in evidence-based practice. Journal clubs are not only useful in academic settings but are also valuable in clinical settings.

The findings of this study revealed a desirable evaluation of total quality scores for NJC implemented by the participants in meetings. The repetitive features of NJC implementation were considered to be successful in many respects. However, little is known about the best guidance and implementation to achieve the greatest educational benefit [3]. Besides, there is no standardized method of running journal clubs and evaluating the knowledge and outcomes achieved by the participants, nor a method of using that knowledge in practice [15].

The use of a questionnaire to determine the interest and personnel involvement in planning can help motivate participation. Our questionnaire also contained a question, in the end, asking participants to determine and comment on future topics for the implementation of NJC.

There were also some limitations, such as the lack of U-shaped space to make eye contact in NJC, the lack of reception, and no presentation of summaries to departments for employees not present at the meeting, which reduced the overall implementation quality.

Selection of journal club members from the same discipline or with interests and commonalities in a particular clinical area, long-term journal club goal setting with periodical revisions, setting goals tailored to the articles under review, acquisition skills, and selection of case base clinical articles of interest by all participants are all the factors that can help motivate participation and benefit participants. A journal club should be specified with frequency at predetermined dates, preferably monthly, at an hour appropriate to all members. Motivational factors should be applied to participants. Promotional strategies, such as the use of technology or the presentation of a brief discussion, are also helpful for all staff to access journal club achievement [1].

Limitation

Our study included a small sample size. However, we did identify a statistically significant change in each of the domains assessed.

CONCLUSION

Evidence-Based Nursing (EBN) is now one of the main goals and concerns of nursing, and one of the main objectives of Nursing Journal Clubs (NJCs) is to improve EBN practice. Nursing schools should prepare students for entry to the clinic to be able to identify clinical care challenges for nurses, and critically evaluate nursing studies. For rich clinical education in nursing, NJCs should be considered as one of the essential parts of the nursing curricula before graduation.

In this study, the authors attained a format and flowchart for the implementation of NJC by clinical nursing faculty members. Based on defined goals, NJC was organized and evaluated with the related tool. If necessary, deficiencies and shortcomings will be improved in the continuous and extensive assessments throughout the country.

NJC is a favorable method of education in the healthcare professions that can improve searching evidence for practice and the use of evidence in clinical practice. Therefore, this approach should also be used more extensively in managing nursing students' educational programs and in continuing education for the training of employed nurses.

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Disclosure statement
The authors declare they have no competing interests.

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ORIGINAL ARTICLE

Investigating the Role of Adenovirus, Herpes Simplex 1, 2, and Varicella Zoster Virus in Incidence of Conjunctivitis in Tehran, Iran

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Abstract: *Conjunctivitis is one of the most common ocular diseases across the world. In this study, the prevalence of viral Conjunctivitis in patients who were referred to the Eye specialized Hospitals was evaluated. Some of the most important viruses that cause conjunctivitis are Human Adenoviruses (HAdV), Herpes Simplex 1, 2 (HSV-1, HSV-2), and Varicella Zoster Virus (VZV). The clinical diagnosis of eye viral infections is difficult and often controversial. Thus, research to experiment with these causative factors is of pivotal importance. In this cross-sectional study, 200 conjunctival swab samples were collected from symptomatic patients with signs of conjunctivitis who had been referred to two hospitals. After DNA extraction, Multiplex Real-Time PCR was carried out in two separate reactions for each sample. Among 200 collected samples, 34 (17%) and 8 (4%) were positive for Adenovirus and HSV-1 DNA, respectively. There were no HSV-2 and VZV conjunctivitis. No significant correlation was identified between gender and conjunctivitis (P-value: 0.845); however, there was a close correlation between age and conjunctivitis (P-value: 0.05). In this study, 66.3% of Adenoviral conjunctivitis occurs in the summer compared to 33.7% in spring. No significant seasonal variation was observed for HSV-1. Our results showed that adenovirus has a key role in causing viral conjunctivitis.*

Keywords: *adenoviruses; conjunctivitis; Herpes Simplex; Multiplex Real-Time PCR; Varicella Zoster Virus*

INTRODUCTION

Conjunctivitis is one of the most common eye diseases around the world [1]. Several factors may cause conjunctivitis including viral, bacterial, and parasitic infections, trauma, allergies, and foods. Among these risk factors, viral pathogens can play a crucial role in the incidence of these infections [2, 3]. Likewise, among viral agents, Human Adenoviruses (HAdV), Herpes simplex 1, and 2 (HSV-1, HSV-2), and Varicella Zoster Virus (VZV) are the well-recognized causes of conjunctivitis [4, 5]. Viral factors can infect different parts of the eye such as eyelids, conjunctiva, cornea, retina, and optic nerve vascular tissue. Sometimes infections caused by these viruses cannot be distinguished from each other, but the outcome may be different [3]. As a result, in the absence of a timely diagnosis, major complications may occur.

Herpes simplex 1, and 2 can affect the eye and its tissues widely. Conjunctivitis and Keratitis are the most common eye diseases that occur after infection by these viruses [6]. Likewise, HSV has considered a cause of endemic eye infection in most communities. Approximately, 300,000 cases of ocular HSV infections are diagnosed annually in the United States. HSV infection is the second cause of corneal blindness in this country, as well. Transmission of this type of virus is variable from sporadic to epidemic. Its incidence relates to virus serotype and vulnerable age groups (children or old ages) [6, 7].

Adenoviruses have been widely distributed in many species. Adenoviral infection is often asymptomatic but may be associated with some respiratory, gastrointestinal, and ocular diseases. Sporadic and epidemic outbreak forms of Adenoviral conjunctivitis occur almost in every society as well as every age [3]. Furthermore, the major epidemic form which is hemorrhagic conjunctivitis often spreads in hospitals, schools, military garrison, and offices.

Varicella Zoster Virus (VZV) is an Alpha human herpes virus that causes varicella (chickenpox). It may also stay latent in the nerve ganglia following an early infection [8]. Varicella-zoster reactivation, in the trigeminal nerve, may develop conjunctivitis, keratitis, uveitis, and ocular cranial-nerve palsies [9]. Traditional methods for the detection of eye viral infections include cytological investigation, viral culture, antigen detection, and serology [10]. Choosing the best method for the detection of viral conjunctivitis depends on the efficiency, speed, and cost of the test. The old conventional tests lack these features.

With the advent of PCR, the sensitivity and speed of diagnosis of a wide range of diseases were increased. Therefore, it was considered a proper method for clinical diagnosis [3,10]. Inherent specificity and sensitivity of nucleic acid amplification processes triggered as the best tool to identify adenoviruses and herpes simplex viruses in clinical samples of conjunctiva swabs [11-14]. By adding entry PCR Time-Real techniques to the field of molecular diagnosis, the main step for quick and easy diagnosis of viral infections was taken [7,15,16]. Multiplex Real-Time PCR has been introduced recently which provides analysis of the quality, quantity, and genotype of several pathogens at the same time in one test tube [15,17].

Knowledge of the incidence and prevalence of different types of viruses that may lead to conjunctivitis may help clinicians to consider the more possible causative viruses, which in turn may change the clinical and laboratory approach and treatment management. In this study, we investigated the prevalence of HSV, Adenovirus, and VZV in swab specimens from patients with conjunctivitis who were referred to Eye-specialized Hospitals in Tehran, by multiplex real-time PCR method.

MATERIALS AND METHODS

Participants

In our cross-sectional study, conjunctival swabs were collected from patients with signs of conjunctivitis during the spring and summer of 2015, who had been referred to Eye specialized Hospitals in the South and North of Tehran, respectively. During 6 months Conjunctival swabs were preserved in 1.2 ml of Viral Transport Media and stored at proper condition (-80°C). A questionnaire was designed to find out the relationship between factors such as age, sex, number of household members, and occupation as well as types of material exposed to the eyes with conjunctivitis.

Quantitative Real-time PCR

Nucleic acid was extracted from 200 µL of the clinical specimen by using a QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer's manual with slight modifications in time and the speed of the centrifuge. The quality of extracted DNA was determined using Nanodrop spectrophotometry. The multiplex real-time PCR assay was performed in a total reaction mixture (25 µL) containing 5 µL of DNA extracts, 12.5 µL of 2µL multiplex PCR master mix, each forward and reverse primer, and each probe Amplification and real-time fluorescence detection were performed with a model Mx3000P real-time PCR system (Stratagene, La Jolla, CA) using the following protocol: first enzyme activation at 95 °C for 1 min followed by 40 cycles of denaturation at 94 °C for 10 s, and annealing/extension at 60 °C for 40 s. At the end of the amplification cycles, melting temperature analysis was set by a slow increase in temperature (0.3 °C/s) from 60 up to 95 °C. Furthermore for diagnostic assay, Multiplex TaqMan Real-Time PCR Kit (FTD eye, Fast-track Diagnostics Luxembourg S.a.r.l) was used. Two tube multiplex Real-Time PCR reactions for detection of HSV-1, and 2, varicella-zoster virus, and adenovirus, including internal control (IC), were done. All runs included negative and positive controls. The presence of Specific viral sequences in the reaction was indicated by an increase in the fluorescence from the relevant dual-labeled probes and was reported as a cycle threshold value (Ct) by the real-time thermocycler. StatView J 4.02 (Abacus Concepts Inc., Berkeley, CA) was used to perform the data analysis. To discern PCR inhibitors that are responsible for false-negative results, murine cytomegalovirus (mCMV) as an IC that amplified with the target DNA was added to the samples. It was introduced into the lysis buffer at the extraction stage of each sample and the positive and negative control.

Statistical analysis

Pearson χ2, Mann-Whitney, and Spearman's rho were used for Statistical analysis. To find out the relationship between factors such as age, sex, number of household members, and occupation, we benefited from SPSS 16.0 software. P-value<0.05 was considered to be statistically significant.

RESULTS

The prevalence of HSV-1 and 2, Varicella-Zoster virus, and Adenovirus was assessed in these patients During 6 months, spring and summer, a total of 200 patients (78 females [39%] and 122 males [61%]) with signs of conjunctivitis were referred to a couple of Eye specialized Hospitals. The patient's age range was from 3 to 55 years with a mean age of 31.00±12.41 years. A high proportion of conjunctivitis swaps 158 patients were negative for expected viruses in this study (79%) (Figure 1).

Adenovirus DNA was detected in 34 (17%) patients. HSV-1 was found in 8 cases (4%). There was no patient with positive results for HSV-2 and VZV (Figure 2). Moreover, Adenovirus was more frequently distinguished during the summer compared to spring, whereas no significant difference was observed in the distribution of HSV-1 in spring and summer. (Figure 3) The burden of conjunctivitis was lower among women to compare men; however, this difference was not statistically significant (P value = 0.850). Furthermore, statistical analysis showed a close correlation between age and conjunctivitis which was statistically significant (p-value <0.05). There was not any significant relationship between social status or location (North versus South) and conjunctivitis.

Figure 1: Patients infected with adenoviral 34/200, and 8/200 to HSV-1. In the 158 patients studied, there was no clue for the presence of VZV and HSV-2 viruses.

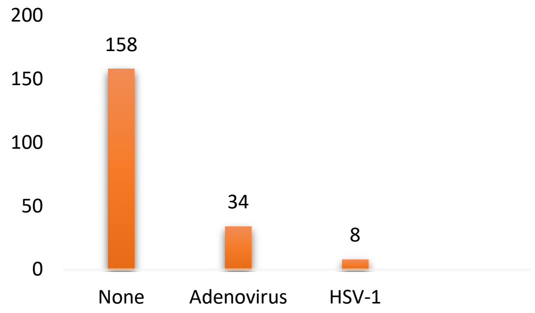

Figure 2: The amplification curve of adenovirus and HSV-1 genome in Multiplex Real time-PCR

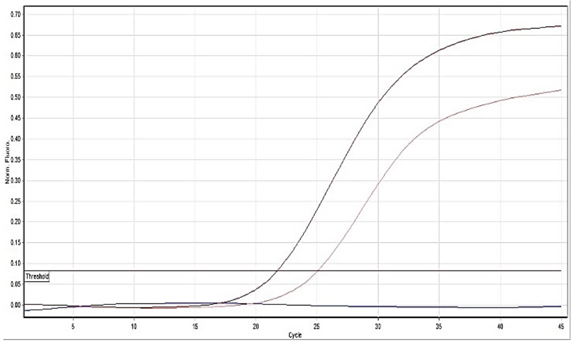

Figure 3: 66.3% (22 of 34) of adenoviral cases were seen in summer. The seasonal fluctuation was not observed for HSV-1

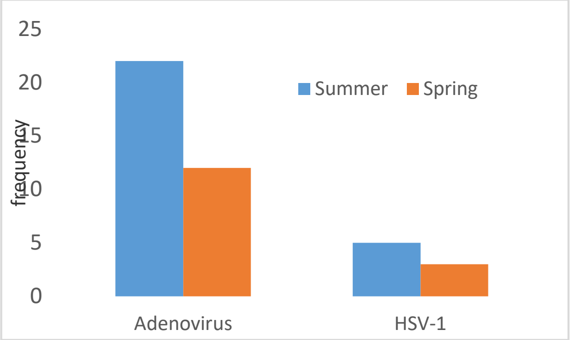

DISCUSSION

Conjunctivitis is one of the most important human eye diseases around the world. Several biological factors can contribute to the frequency of eye infections including viral agents [1,2]. In this cross-sectional study Multiplex, Real-Time PCR was applied to investigate the prevalence of HSV-1, 2, VZV, and Adenovirus in a total of 200 patients with signs of conjunctivitis that were referred to two Eye specialized Hospitals in North and South of Tehran during spring and summer of 2015. In all of the patients, 34 (17%) patients with Adenovirus DNA and 8 (4%) with HSV-1, there were no patients with positive results for HSV-2 and VZV. Failure to diagnose adenovirus eye diseases can lead to outbreaks of keratoconjunctivitis [18]. In other words, rapid diagnostic methods may help us to detect this contagious conjunctivitis early enough to avoid the spreading of conjunctivitis and to prevent complications.

Several methods are available for the diagnosis of a viral infection such as antigen detection, viral culture, and serology. Although the diagnostic accuracy of some tests has dramatically improved, they still lack the required diagnostic criteria for optimum tests. Serological methods cannot be used during the acute phase of the disease due to cross-reactions with other related organisms. Thus, they may raise the frequency of false positive results [19]. The techniques based on antigen detection do not require living organisms, however, false-negative and false-positive results may still be obtained. Separation of adenovirus in the ELISA method is time-consuming (virtually 4 weeks for Adenovirus and 3 days for herpes simplex virus) [3]. In this study, the PCR technique was used due to its high sensitivity and rapidity for the diagnosis of viral infections.

It has been shown that DNA identification of a virus is much easier, faster, and more sensitive than isolating the virus from clinical samples [11]. PCR method can detect very small amounts of DNA with high specificity, and would potentially revolutionize the diagnosis [10]. The first use of this method for eye diseases was reported in 1990 and to date, it has largely been developed in ophthalmology [12]. Several studies have been performed to evaluate the use of PCR as a diagnostic tool to identify adenovirus, and herpes simplex virus in swabs conjunctivitis [14,20]. Kensington et al published a study showing a 97.3% specificity of PCR for the detection of adenoviruses in eye swabs [3,14]. Although the PCR method is very sensitive and specific, some post-amplification processes can be time-consuming [20]. For instance, electrophoretic analysis of nucleic acid in the presence of ethidium bromide and ultraviolet light bands [21], the southern blotting method for detection of labeled oligonucleotides, and the target sequence are prolonged and time-consuming. In addition, it requires several PCR products that increase the possibility of contamination [22]. ELISA - PCR, despite using biotinylated probes and increase specificity, needs a lot of time that could potentially lead to some errors in the results [16].

Nowadays, the advantages of Real-Time PCR are not hidden from any laboratory specialist. Monitoring the accumulation of amplified products, by labeled probes or primers is possible and is superior to the use of radioactive materials. Moreover, the need for post-amplification (because of using fluorogenic for labeling primer and probe) is compensated by the higher speed (due to the short-time cycle and short length of the fragment) of the technique [15]. Different studies have suggested that Adenovirus is the main viral agent in conjunctivitis with a frequency that varies between 14 to 70 % around the world [23-25]. Adriana et al showed that the frequency of adenoviral conjunctivitis was 60% in the population of Rio de Janeiro, Brazil in a study between 2004 to May 2007 [26]. In another investigation conducted in 2010 in Turkey, Yagci et al studied eye swabs from 34 patients with acute conjunctivitis. The PCR results showed that 26.5 % (9 out of 34) of patients were infected by different adenoviral serotypes (HAdV-8 was reported as the predominant serotype) [27]. Shamsi–Shahrabadi et al have reported that the prevalence of adenoviral conjunctivitis in Tehran is 16% in 2009 [28]. This was similar to the result of the study by Goudarzi et al showing a frequency of 15.7% Adenovirus conjunctivitis in 2006 [29]. Despite using different methods, the results of those studies are similar. The same is true for our study indicating conjunctivitis epidemiological pattern has not changed in Iran in recent years.

The frequency of HSV-1 conjunctivitis in Iran is much less common than the reported frequency of this kind of infection in other countries. In a study on 1,964 samples collected from conjunctivitis between 1987 and 2001, Marangon et al reported that 514 samples were positive for HSV-1. HSV-1 was the most frequent (56%) viral agent followed by adenovirus (31%). Adenovirus and HSV1 were the most important cause of conjunctivitis infections (66%) and corneal infections (88%), respectively [30]. This was previously suggested in another study conducted in India in 2003, showing that from 140 samples 33.6% were infected with HSV. The pattern is slightly different in other countries [31]. In 2003, a similar study in Japan showed that 19 out of 33 samples of eye infection were related to viral infection including 4.68 % HSV-1 and 31.6% VZV. In this study, they didn't look at the frequency of adenovirus [8].

According to our investigation, the incidence of HSV-1 was 4%. On the other hand, there was no positive test for HSV-2 and VZV which can be concluded that these viruses are not endemic in Iran. Our study has some limitations. The samples have been obtained from subjects who live in the capital city of Iran which may not necessarily represent the frequency of viral infection in other rural areas in the country. Tehran, on the other hand, is considered a reference city with a high population, and the selected hospitals are considered the main eye-specialized hospitals which receive the majority of eye diseases in the city. Therefore we recommend more research for the recognition of the main viruses involved in conjunctivitis' pathogenesis in other areas of Iran and nearby countries. Finally, it is noteworthy that in this project, all of the enrolled patients were suffering from conjunctivitis but only 21% of them were infected with Adenovirus or Herpes 1.

As a result, the role of other viral factors such as Chlamydia trachomatis and Picornaviruses remains to be unclear.

CONCLUSION

In conclusion, we suggest that adenovirus is the main responsible viral agent for conjunctivitis in Tehran. The frequency of Adenoviral conjunctivitis was affected by seasonal variation. The seasonal variation didn't affect the frequency of HSV-1 infection.

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ORIGINAL ARTICLE

Relationship between Moral Intelligence and Clinical Competency of Nurses in Ilam Educational Hospitals

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Abstract: *In this descriptive-analytical study, 110 nurses working in educational hospitals of the Ilam University of Medical Sciences were included. Participants were selected using a random sampling method. The data collection tools were the demographic information form, the moral intelligence questionnaire of Lenik and Kiel (2005), and the standard clinical competency assessment questionnaire (CIRN). Data were analyzed using SPSS software, version 21. Descriptive and inferential statistical tests were used to analyze the data. The mean ± SD score of nurses' moral intelligence and clinical competency was estimated at 157.59 ± 26.60 and 136.65 ± 46.08, respectively. Fifty-one percent of participants had low clinical competency, and 46% had high clinical competency. There was a significant association between moral intelligence and clinical competency ($p<0.001$, $r=0.517$). The moral intelligence and clinical competency of nurses were significantly associated with age, education status, work experience, employment status, and rank/position ($P<0.05$). A negative association was found between working unit/ ward and clinical competency ($P<0.05$).*

Keywords: *moral intelligence, clinical competency, nurses*

INTRODUCTION

As the largest group of care providers, nurses face many ethical challenges due to their working professional nature [1]. Since moral practice in nursing plays an important role in the quality of care provided, the improvement of patients, and the achievement of health goals, paying attention to the dimension of moral intelligence in nurses as a moral guide for their performance is highly important [2]. Borba first introduced moral intelligence in psychology. He defined moral intelligence as the capacity to understand right from wrong, have strong moral beliefs, and practice them to provide the right behavior [3].

Moral intelligence includes seven essential principles: empathy, conscience, self-control, respect, kindness, tolerance, and fairness [4]. Adopting appropriate strategies such as improving the quality of nursing services can increase the moral intelligence of nurses [2]. Several factors, such as the rapid change in the health monitoring system, providing safe and affordable services, increasing the awareness of the community in health-related areas, and the expectation of receiving high-quality services, along with the willingness of health service providers to use skilled personnel cause clinical competency to be of great interest [5].

Clinical competency in the nursing profession comprises different dimensions and areas. Knowledge, attitude, skill, and communication are the main dimensions. Other dimensions that are emphasized in different literature include professional practice (principles of nursing care, instructions, and interventions), ethics, decision-making, cooperation, work development, education, clinical judgment, problem-solving ability and critical thinking, management, leadership and motivation, culture, spirituality, research, information technology and the ability to work with advanced devices [6]. Ensuring the care of patients to ensure the clinical competency of nurses in hospitals and other medical service settings is a major concern and is of great importance for managers and health service provider systems [7]. Since the clinical competency of the working nurses is considered the main concern of the care provider systems, it deserves more attention. Other nurses with appropriate moral intelligence can guarantee their position by keeping healthy, optimizing hospital performance, and communicating effectively with colleagues and patients. This study, therefore, aimed to examine the association between moral intelligence and clinical competency among nurses working in educational hospitals affiliated with the Ilam Univesity of Medical Sciences.

MATERIALS AND METHODS

Population of study

This was a cross-sectional, descriptive-analytical study. A total of 110 nurses working in educational hospitals (Mostafa Khomeini Hospital, Imam Khomeini Hospital, and Taleqani Hospital) affiliated with the Ilam University of Medical Sciences were included. Sampling was conducted using a random sampling method from 23 September to 21 December 2021.

Inclusion and Exclusion Criteria

In our study, inclusion criteria were: having a bachelor's degree in nursing, having at least one year of work experience in nursing, not having work experience in other fields of medical sciences, and giving informed consent to participate in the study. The exclusion criteria were: incomplete questionnaires and unwillingness to participate in the study.

Demographic Variables

The first section was the demographic characteristics questionnaire. This questionnaire included eight items: age, gender, marital status, education status, work experience, employment status, the working unit/ward, and rank/position.

Questionnaires

Our study’s data collection tools consisted of a demographic characteristic questionnaire, Lennick and Kiel’s (2005) moral intelligence questionnaire, and Competency Inventory for Registered Nurses (CIRN) questionnaire. The first section was the demographic characteristics questionnaire.

The second section was the moral intelligence questionnaire, designed by Lennick and Kiel (2005). This questionnaire consists of 40 questions encompassing four components: honesty, responsibility, compassion, and forgiveness. The items are rated on a 5-point Likert scale, with scores ranging from 1 (never) to 5 (always). The minimum and maximum scores are 40 and 200, respectively, divided into two to convert to a maximum score of 100. In this study, items were scored as follows: 40-59 (poor), 60-79 (good), 80-89 (very good), and 90-100 (excellent). The reliability and validity of the questionnaire have already been confirmed in the study of Mohammadi et al., with a Cronbach alpha of 0.87 [8]. In our study, the Cronbach alpha was 0.96.

The third section was Competency Inventory for Registered Nurses (CIRN) questionnaire, designed by Liu et al. in 2007. This questionnaire consists of 55 questions encompassing 7 dimensions: clinical care (10 questions), leadership (9 questions), interpersonal relation (8 questions), legal/ethical practice (8 questions), professional development (6 questions), teaching-coaching (6 questions), critical thinking and research aptitude (8 questions). The items are rated on a 5-point Likert scale, with scores ranging from 0 to 4. In our study, the questions were scored as follows: less than 100 (low competence), 110-165 (moderate competence), and 165-220 (high competence). The reliability of the questionnaire has already been confirmed in the study of Ghasemi et al., with a Cronbach alpha of 0.97 with the range for each dimension between 0.68 and 0.87 [9]. In this study, the Cronbach alpha was 0.98.

Statistical Analysis

Data were analyzed using SPSS software version 26. Descriptive statistics (e.g. percentage, mean, and standard deviation (SD) and analytical statistics (e.g., Pearson correlation coefficient and Analysis of variance (ANOVA) were used to analyze the data.

RESULTS

A total of 110 nurses were included in this study.

Nearly 55% of participants were women (60 Female, 50 Male), and 87.3% of them had a bachelor's degree(96 Bachelor, 14 Master). Most participants (61.8 %) were formally employed with a work experience of 7.17 years. 38.2% (43 Nurses) and 33.6 % (37 Nurses) of nurses were employed in the Intensive Care Unit (ICU) and specialized wards, respectively (Table 1).

As shown in Table 2, the mean ± SD of moral intelligence and clinical competency in nurses was estimated at 157.59 ± 26.60 and 136.65 ± 46.08, respectively. 46.4% of nurses had low clinical competency, 11.8% had moderate clinical competency, and 41/8% had high clinical competency.

Figure 1 shows a significant correlation between moral intelligence and clinical competency in nurses ($r=0.517$, $p < 0.001$). With an increase in moral intelligence scores, clinical competency in nurses would increase. Moral intelligence and clinical competency in nurses were significantly and positively associated with age, education status, work experience, employment status, and rank/position ($P < 0.05$). On the contrary, there was a negative and significant association between the working unit/ward and clinical competency ($P > 0.05$) (Table 3).

Figure 1: The association between moral intelligence and clinical competency.

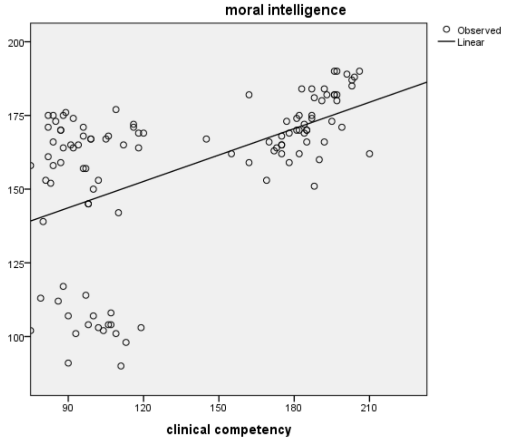

Table 1: The demographic characteristics of the nurses

Variable		Frequency (%)
Gender	Male	5(45.5)
	Female	60 (54.5)
Marital status	Single	55 (50)
	Marries	55 (50)
Education status	Bachelor	96 (87.3)
	Master	14 (12.7)
	Ph.D.	0 (0)
Employment status	Company	42 (38.2)
	Contractual	0 (0)
	Formal	68 (61.18)
Working unit/ward	Intensive Care Unit	43 (38.2)
	Specialized ward	37 (33.6)
	General ward	31 (28.2)
Rank/position	Staff nurse	96 (87.3)
	nursing managers	14 (12.7)
Working experience in nursing	1-5 years	55 (50)
	6-10 years	30 (27.3)
	11-15 years	10 (9.1)
	≥16 years	15 (13.6)

Table 2: The mean ± SD of moral intelligence and clinical competency in the nurses

	Minimum	Maximum	Mean± SD
Moral intelligence	90	190	157.59± 26.609
Clinical competency	75	210	136.65± 46.084
The level of clinical competency	**The range of score**		**Frequency (%)**
Low	1-110		51 (46.4)
Moderate	110-165		13 (11.8)
High	165-220		46 (41.8)

Table 3: The association between demographic characteristics and moral intelligence, clinical competency

Variable	Moral intelligence		Clinical competency	
	Correlation	P-Value	Correlation	P-Value
Age	0.31	<0.001	0.42	<0.001
Gender	-0.06	0.51	0.002	0.98
Marital status	-0.09	0.32	-0.01	0.89
Education status	0.32	<0.001	0.39	<0.001
Work experience	0.31	<0.001	0.44	<0.001
Employment status	0.37	<0.001	0.38	<0.001
Working unit/ward	-0.09	0.32	-0.19	0.04
Rank/position	0.24	0.01	0.34	<0.001

DISCUSSION

This study aimed to examine the association between moral intelligence and clinical competency and its relevant factors in nurses working in the educational centers of Ilam University of Medical Sciences in Iran. Our results showed that nurses had good moral intelligence, which is in line with the previous study conducted in Iran and Turkey [10,11], indicating the importance of acquiring moral values and moral practice by nurses in clinical settings indirectly. In contrast, a study in Iran demonstrated that 62% of nurses had moderate moral intelligence [12]. This difference can be attributed to study methodology, clinical setting, and statistical population.

In this study, 46.1% and 41.8% of nurses had low and high clinical competency, respectively. Our results are consistent with the previous studies in Iran [13,14], highlighting the high clinical competency level among nurses in the country. In Iran, every hospital has a quality improvement and in-service training ward responsible for holding training courses, assessing nurses' clinical competency, and encouraging excellent nurses. Therefore, this high score can be related to regular training courses and periodic evaluations of nurses' clinical competency.

Previous studies in other countries also demonstrated good and excellent clinical competency among nurses working in different hospital wards [15,16]. That has meant the importance of clinical competency-related issues has gained attention from the nursing management of hospitals and nurses themselves, and measures have been taken to improve nurses' knowledge levels.

We found that nurses' clinical competency increased by increasing the mean scores of moral intelligence, which is in line with previous studies [17,18]. Also, previous research demonstrated that the high moral intelligence of nurses improves the provision of care and treatment services to patients [19], and similar results were reported in our study.

In the present study, those with higher age, higher educational level, more work experience, formal employment, and those with a better rank/position had higher moral intelligence. A study by Dehghani et al. showed that moral intelligence in nurses is associated with their age and work experience [1]. Some studies suggested that younger nurses are influenced by their emotions and cannot properly use moral reasoning in moral challenges created in the clinical setting [20]. Older nurses are more likely to be in different clinical and social situations and face more moral challenges. Hence, they have more ability to promote their moral intelligence. Nurses with more work experience have more ability to cope with difficult moral situations.

Regarding the higher score of moral intelligence in nurses with higher educational levels, it can be attributed to the importance of nursing moral training due to the change in the educational content of the curriculum related to a master's degree in nursing. Moreover, the higher score of moral intelligence in nurses working in wards compared to nursing managers can be due to a longer period of clinical training, more clinical shifts in the hospital, continuous communication with patients, and being or participating in decision-making situations for patients. In this study, there was no significant association between gender and moral intelligence.

Moreover, we found that those with older age, higher educational level, formal employment, and a better rank/position, and those working in ICU wards had greater clinical competency. Our results are inconsistent with the study of Farajai et al [13]. This difference could be due to variations in participants' sociodemographic characteristics and clinical settings. The different clinical setting due to the different needs of care required for patients and differences in various clinical experiences among nurses causes a different understanding and perspective of clinical competency among nurses. This study was also inconsistent with previous research that showed female nurses were more sensitive in reasoning and clinical decision-making regarding patient care [14]. Also, some studies found that the mean score of clinical competency was higher among nurses aged 30 years and over than those with lower age [16,21]. Since older nurses have more experience taking care of patients, they are more likely to provide better service quality and face lower challenging situations [22]. Consistent with the present results, previous studies showed that nurses with higher educational levels had more clinical competency [21,23]. Those with higher educational levels are more likely to receive the necessary training to understand care better.

Furthermore, in this study, those with more work experience had more clinical competency, which is in line with the results of previous research [16,21]. With the increase in work experience, nurses would benefit from more clinical experience, job skills, and knowledge, resulting in them becoming more sensitive to patient care issues [22].

CONCLUSION

The results of the present study showed that the clinical competency of half of the nurses was high, and their moral intelligence was favorable. There was a significant association between moral intelligence and clinical competency in nurses. Moral intelligence and clinical competency were associated with age, gender and educational level, job factors, clinical activity history, employment status, and nurses' work roles. Considering the importance of clinical competency and the role of moral intelligence in nurses' clinical practice, hospital nursing managers must adopt measures to improve nurses' moral intelligence and clinical competency using new educational approaches such as electronic education and continuous training. In addition, periodically evaluating the abovementioned cases among nurses with standard tools can be very beneficial.

Conflicts of interest and sources of funding
Declare no conflicts of interest.

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Ilam University of Medical Sciences.

Ethics approval and consent to participate
REC,1400,123

Informed written consent
was obtained from all participants included in the study. All participants were informed about the purpose of the study and assured of confidentiality and anonymity. The respondents were informed of their freedom to withdraw from the study at any time.

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ORIGINAL ARTICLE

Risk Factors for Musculoskeletal Disorders in Nursing Personnel and Prevention Strategies

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Abstract: *Objectives: Health professions such as nursing are at high risk of work-related musculoskeletal disorders (WRMDs). This study aims to present the most common WRMSDs that occur in nursing personnel, investigate the risk factors associated with their occurrence, and identify preventive and therapeutic strategies. Material and Methods: A comprehensive literature search in PubMed, Scopus, and Google Scholar was performed using keywords, until May 2021, while the results reported by various authors were summarized. Results: Low back discomfort (30% to 60%), neck disorders (30% to 48%), and shoulder disorders (43 to 53%) are the most common WRMSDs in nursing personnel. The major risk factors are classified as demographics and job characteristics, physical, psychosocial, and workplace organizational factors, which often co-exist and act together. WRMSDs have serious consequences such as absenteeism, reduced productivity, increased morbidity, health costs as well as lower quality of life. To reduce the incidence of WRMSDs, it is crucial to implement prevention strategies, such as ergonomic measures and training programs in high-risk nursing departments as well as immediate therapeutic approaches, where required. Organizational interventions are also required to improve the working environment in terms of assigning tasks among nursing staff, setting working hours, keeping a positive attitude, and conducting medical examinations. Conclusion: Early identification of risk factors concerning WRMSDs among nursing personnel and immediate treatment is needed to maintain their productivity as well as to provide quality nursing care, followed by evidence-based ergonomic and organizational intervention strategies for their safety and health protection.*

Keywords: *work-related musculoskeletal disorders, nursing personnel, risk factor for musculoskeletal disorders, ergonomic measures, prevention, low back discomfort*

INTRODUCTION

Work-related musculoskeletal disorders (WRMSDs) are a worldwide major concern in occupational health. They are an umbrella term for musculoskeletal symptoms caused or worsened during work. These disorders may be defined as discomfort, impairment, disability, or persistent pain in the locomotor system [1]. The upper body such as the spine and upper extremities are the most sensitive structures for the appearance of WRMSDs [2]. Moreover, WRMSDs are the main cause of lost working time, production downtime, increased labor costs, decreased quality of life, turnovers, job changes, and injuries that can lead to temporary or permanent disability [3].

WRMSDS are usually due to genetic, morphological, psychosocial, and biomechanical factors [4]. They can be triggered or intensified by work, as well as by other activities such as housework and sports [5]. An increased risk of their development has been found in occupations with intensely repetitive work tasks, excessive load bearing, intense efforts, prolonged improper work postures, and weight lifting, as well as in demanding psychosocial working environments [6].

A survey found that 25% of workers in Europe reported back pain and 23% of cases were work-related. The differences between the countries were large, with workers in Greece reporting work-related back pain at 47% and muscle pain at 46%, while in the United Kingdom the corresponding rates were 11% and 9% [7]. Musculoskeletal pain has been reported in computer users, office workers, ophthalmologists, professional musicians, and marines [8]. In 2014-2015 in the UK, around 9.5 million working days were lost due to WRMSDs, accounting for 40% of the lost working days due to bad health [8].

The Military Health System and military health workers are responsible for the provision of health services for the active military, retirees, as well as their families [9,10]. The military health system has two main goals: to properly prepare the health team so that the team may provide health services during military operations and to ensure that every active military is perfectly prepared to deliver maximum performance on the battlefield. All military health workers, including nurses, have a crucial role in delivering those purposes [9].

In health professions such as nursing, WRMSDs have a high prevalence due to intense physical straining from prolonged standing, frequent weight lifting (e.g., patient transfer), as well as systematic bending or twisting of the torso during nursing skills [11-14]. It is estimated that almost one-third of all cases of sick leave among health professionals are related to WRMSDs [15]. This occupational health concern is a quite common issue in both civilian and military health personnel [8, 16,17]. Consequently, prevalence, symptomatology and required interventions for WRMSDs should be taken into consideration for both military and civilian health workers.

MATERIALS AND METHODS

The scope of this non-systematic literature review was to identify the most common WRSDs that occur in nursing staff and to investigate the risk factors associated with their occurrence. In addition, the most important strategies regarding the prevention and treatment of these disorders are reported. The inclusion criteria for the selection of the articles were concerning nursing staff, musculoskeletal disorders, English language, published until May 2021, and access to the full text. The PubMed, Scopus, and Google Scholar databases were searched with the terms “work-related musculoskeletal disorders”, “nurse” or “nursing personnel”, “musculoskeletal pain” and “risk factors for musculoskeletal disorders”. Both review articles and original research articles were used to synthesize this review with a focus on contemporary and seminal papers.

PREVALENCE OF MUSCULOSKELETAL DISORDERS IN NURSING PERSONNEL

The incidence of WRMSDs in nursing personnel varies according to numerous studies. Low back discomfort (30% to 60%), neck disorders (30% to 48%), and shoulder disorders (43 to 53%) are the most common [18,19].

Studies in Nigeria, Turkey, Australia, Estonia, and Japan have found that the rate of WRMSDs in nursing personnel is about 84%, with low back discomfort being the most common [8]. Furthermore, WRMSDs in knees were associated with standing and low levels of satisfaction from their work [20]. Increased prevalence of WRMSDs in nursing personnel was reported in specific working settings such as in intensive care nurses and those working in a department of surgery and anesthesia [20]. These settings have increased demands for caring for patients that were more dependent and immobilize and require nurses to be in a prolonged standing situation and other painful postures [6,21,22].

RISK FACTORS FOR MUSCULOSKELETAL DISORDERS IN NURSING PERSONNEL

The major risk factors of WRMDs are classified into four groups: demographics and job characteristics, physical, psychosocial, and workplace organizational factors (Table 1) [23-25]. They often co-exist and may directly or indirectly act together.

Table 1: Risk factors for the occurrence of musculoskeletal disorders in nursing personnel

Demographic and Job Characteristics related factors	Age, gender, education, hospital type where they worked, marital status, job title, years of employment in high-risk departments (e.g., ICU), level of exercise
Physical Factors	Physical workloads such as force, posture, and repetition
Psychosocial factors	Job stress, effort-reward imbalance
Workplace organizational factors	Management support, supplies, equipment, barriers and conflicts, peer support, feedback, and training

Demographic and Job Characteristics related factors

Female nurses were more likely to suffer from WRMSDs, especially in the upper extremities and torso. Female nurses face significant burdens due to the combination of family and work responsibilities including parental responsibilities, domestic load, lack of exercise, and insufficient rest time [21, 26]. Similar problems were observed among married nurses, due to the double burden of different types of tasks (family and professional) that increase the psychological and physical requirements and reduce the recovery time required for the working muscle groups [19,27].

The incidence of WRMSDs also increases with age and length of service [28]. Age-related changes, usually above the age of 40, include reduced strength and mobility due to loss of muscle mass, muscle capacity, dynamics of intervertebral discs and degeneration of musculoskeletal structures, reduced time reaction, and acuity of senses [2,29,30]. Nursing personnel above the age of 45 years old need more time to recover after work and those who work with shifts have more severe health problems than nursing personnel below the age of 40 [31,32].

Age is often associated with years of work. That is, nurses with longer working experience have cumulative effects from long-term exposure to injuries in their work environment, possibly due to non-compliance with the principles of safe work [7,33, 34]. It is reported that nurses with more than 20 years of clinical work experience were approximately 4 times more likely to develop WRMSDs than nurses with 11-20 years of work experience [35].

Socioeconomic status is considered to be a risk factor for WRMSDs development and affects the ability of workers to deal with their health problems, especially those with a lower educational level [36]. Compared to nurses, nursing assistants who are supposed to have lower socioeconomic status and a lower level of education, have a higher chance of developing WRMSDs, especially in the lumbar spine [19].

WRMSDs are also associated with factors related to lifestyle and human behavior, such as high body mass index and lack of exercise [21,37]. Nursing personnel with poor physical health status are six times more likely to face problems with their legs and ankles, limiting their activity [38]. The possibility of nurses gaining weight due to their busy schedules and the stressful nature of their profession, which in turn leads to sleep and eating disorders, makes it necessary that nurses adopt rules of healthy behavior [39]. Moreover, a predisposing risk factor for WRMSDs is the poor physical condition of nurses, especially when combined with poor transport techniques [40,41].

Physical Factors

Nurses' daily tasks include frequent weight lifting and excessive manual labor in bad body postures at high frequency and repetition. Nurses often have to lift patients, the weight of which, at times, is greater than their body weight to help or to put them to bed. It is estimated that during a typical 8-hour shift a nurse may lift a cumulative weight of 1.8 tons [42]. The frequency and severity of exposure to these risk factors vary depending on the type of health care [42].

Activities related to the immediate care of patients and their transport have an impact on the musculoskeletal system, especially on the spine, because compression and shear loads in the lumbar spine during patient handling are quite high [19, 43]. These WRMSDs are affected by the mean age of employees, years at work, patient characteristics, and health status, as well as by the technology available for patient transportation [19].

Nurses are often involved in lifting and pushing manipulations in everyday clinical practice, which cause muscle fatigue, being an important risk factor for lower back pain [44,45]. Frequent standing, persistent anterior projection of the head, and frequent transport of hospital equipment are also common [44,45]. Transferring patients is complicated and unpredictable action due to their weight, the possibility of confusion and tripping, and the lack of body handles. Additionally, patients may not be cooperative during transport, like some cognitively impaired advanced-aged patients, adding more burden to the nursing personnel [42, 45]. Professional tasks involving twisting and lifting increase the risk of lumbar injury by 4.84 times [46].

Physical risk factors are more common in nursing assistants because they lift and transfer patients more often, as part of their professional duties as well as they have reduced rest and recovery time between these nursing activities [47]. The incidence of low back pain in nursing assistants is 3.3 times higher than in other nursing staff [46].

Psychosocial factors

Psychosocial risk factors associated with WRMSDs include work intensity, repetitive tasks, working hours, high physical and psychological demands, fatigue, stress, and employees' perception regarding work organization [48,49]. Additional psychosocial risk factors may be the high perceived workload, time pressure, low levels of work control, work satisfaction, and social support [19,50]. Frequent unplanned work as well as workload difficulties in reduced working capacity conditions are some factors associated with low work control by nurses that tend to be strongly associated with low back pain [51].

Nursing personnel with increased long-term high stress, often have difficulty responding to workload, due to understaffing and time pressure, leading to rapid accelerations or poor postures [52,53]. As a result, they are more likely to suffer from WRMSDs, especially in the neck and shoulders, compared to those who do not report facing high levels of stress [52,53].

Workload and stress have been linked not only to pain but also to injuries. Clarke et al. found that a high workload was associated with a 50 to 200% increase in injury possibility as well as other accidents among hospital nurses [54]. Subsequent studies in Estonia have shown a high degree of risk between WRMSDs and mental health problems including symptoms of anxiety, exhaustion, and physical stress among hospital nursing staff [18]. The occurrence of WRMSDs in nurses is associated with physical workload, emotional exhaustion, and symptoms of physical stress [18]. Another study also identified stress and the level of anxiety in everyday life as a related causative factor for WRMSDs [55].

In addition, work-family conflict may be a major stressor for nurses, increasing the impact of WRMSDs on the lower back, shoulder, and cervical spine. The problem is exacerbated due to the rolling work schedules, which is a common situation in nurses' typical life expectancy [55].

Workplace Organizational Factors

Nurses usually have considerable burdens due to staff shortages. They may also become frustrated by the hard-working hours and schedule, resulting in mental and physical exhaustion, consequent emotional distress, increased risk of WRMSDs, and affecting their intention to leave [21,52,56-58].

Numerous studies have examined the relationship between staffing levels in nursing personnel and injuries/illnesses in the workplace [59,60]. Inadequate staffing has been associated with back injuries among nurses. It has been found that when nursing positions in hospitals had decreased by 9%, the number of WRMSDs increased by 65% [60].

Rotating shifts create unusual sleep patterns and higher levels of unhealthy behavior that can reduce work efficiency [61,62]. Nursing staff also experience, among other things, higher rates of accidents and WRMSDs as well as reduced levels of physical and mental health (e.g., fatigue, lack of energy and weakened immune system), compared to nurses working in morning shifts. These findings are also related to the short rest period between shifts [63]. In addition, in a study of Japanese nurses, it was found that after a restful sleep break for every two-night shifts, WRMSDs in the upper and lower extremities were significantly decreased [64]. According to another research, the risk of low back pain increases among nursing assistants working the night shift and is also associated with weight lifting and moderate work requirements [65].

IMPACT OF WRMSDS ON NURSING PERSONNEL

WRMSDs are the main cause of nurses' lost work time as well as increased morbidity, increased health costs, and lower quality of life [21]. WRMSDs may have a serious impact on the quality of life of nursing staff, resulting in higher absences from work or even the desire to change jobs [21].

WRMSDs are characterized as multifactorial and responsible for morbidity in many working populations. It is also reported that WRMSDs cause absenteeism or lost work time and increase work restriction, with serious economic consequences on individuals, organizations as well as society. However, even in developed countries, these disorders seem to be underestimated among health professionals (Table 2) [66].

It seems that the largest percentage of participants who encountered WRMSDs continued to work. In a study conducted in New Zealand, the prevalence of absenteeism due to WRMSDs ranged from 6.2% to 18%, and less than 10% of nurses were absent from work due to the occurrence of WRMSDs [67]. Probable reasons for this reaction may be the tendency to ignore the pain or to continue working, avoiding certain activities until the pain subsides [57]. In addition, any occupational insecurity of the participants may act as a disincentive in the absence due to illness (sick leave) [68].

Table 2: Effects of musculoskeletal disorders on nursing personnel

Absenteeism	Lower quality of life
Reduced productivity	Transfer to another job
Work constraints	Disability
Increased healthy costs	Decrease in nurse efficiency
Financial implications for the individual	Mistakes/Safety problems of patients

Another study found that 61.3% of nurses resorted to self-medication for pain or discomfort and 24.7% of participants had also consulted a physiotherapist [69]. In another study, less than 20% of nurses visited healthcare professionals for WRMSDs rehabilitation with a similar finding, reflecting the degree of information and awareness of participant nurses and suggesting a common practice among nurses, possibly due to their prior knowledge [67]. Such disorders when they are not treated properly with effective treatment and sick leave or when nurses take self-prescribed analgesic drugs on their initiative may not recover quickly or even worsen. At the same time, WRMSDs lead nursing personnel to low work performance which is another type of indirect cost associated with WRMSDs, quite difficult to be measured [70,71].

PREVENTIVE AND THERAPEUTIC STRATEGIES FOR WRMSDS

Undoubtedly, nursing personnel must maintain their ability to work while remaining healthy until retirement. To reduce the incidence of WRMSDs in their work, it is crucial that prevention strategies be followed, such as the implementation of ergonomic measures and training programs in high-risk nursing departments and immediate therapeutic approaches, where required [21,72]. Prevention of WRMSDs presupposes the analysis of the clinical environment where nursing actions are offered and the identification of risk factors, as their mitigation requires targeted interventions [48,73].

Ergonomic recommendations

The ability to reduce injuries in direct care nurses is improved by ensuring easy access to ergonomic mechanical devices to lift and transfer patients, reducing the number of hasty body positions involving muscle tension combined with educational interventions [42,74]. Other ergonomic interventions are the ergonomic patient evaluation, the practice of zero weight lifting, the establishment of groups that handle the lifting of patients, the adaptation of the clinical environment, and the application of specific procedures (e.g., guidelines for a safe task execution such as lifting or pushing a heavy object) [75].

The goal of interventions regarding WRMSDs rate reduction is to implement good work practices that prevent physical workload, better information about potential work hazards that are responsible for injuries, as well an explanation of the proper operation of the necessary equipment [6,42].

A recent review emphasizes that preventive measures for these risks are still insufficient and this fact has an impact on both nurses' professional performance and their desire to stay at work [21]. In another study, nursing personnel with WRMSDs reported that their workplaces did not provide training on how to reduce injuries and that their prior training was insufficient or incomplete regarding WRMSDs prevention [76]. Ergonomic training reduces the tension of nurses' muscles during a working day [48]. Nursing personnel training should be a priority for hospital administrations even if the effectiveness of the training interventions as well as the handling of assistive devices is not satisfactory [77].

Adequate rest is a protective factor for nurses’ WRMSDs. Rest contributes to muscle relaxation by reducing muscle fatigue [3]. A study by Wang et al., revealed that a rest time of 15 min could restore in the normal range the erector muscle of the spine as well as the heart rate [78]. Decompression and relaxation after a long workday may often improve staff satisfaction, as the provision of relaxation activities has been proven to reduce stress levels in employees [79].

Physical exercise may also be beneficial, reducing the prevalence of WRMSDs in nursing personnel [61]. Exercise improves muscle sensitivity and coordination, response speed, and psychosocial behavior by delaying the decrease in muscle strength due to age [80]. It also has a protective effect on low back and neck pain in workers with heavy and sedentary activities [81,82].

Organizational interventions

Reducing the excessive stress of nursing personnel over time while adapting nurses’ workload appropriately to reduce the significant physical and psychosocial burden and exposure to WRMSDs requires organizational involvement and support [68]. The prevention of WRMSDs is enhanced by the assignment of specific patients to nurses without known health problems, who may be more capable of providing heavy physical care [83]. In addition, adjustments to work organization should be made including adequate rest time between work shifts and an acceptable work schedule [83].

Because the majority of people with anxiety fail to recognize the underlying conditions, mental health should be monitored as well as specialist care will be needed, especially for nurses working in high-stress nursing departments [55,84]. At the same time, organizational interventions are required to improve the working environment in terms of assigning tasks among nursing staff, setting working hours, keeping a positive attitude, and conducting medical examinations for the prevention or early detection of WRMSDs [62]. Other actions include the implementation of healthy lifestyle choices (engagement in physical activity, tobacco and alcohol limitation, eating a balanced diet) as well as education on body posture, especially when lifting and transporting loads or patients [84]. Preventing WRMSDs in nursing personnel is vital to providing quality patient care, improving nursing morale, and increasing productivity by reducing time loss and other absences [47].

CONCLUSION

Cost-effective high-quality health care requires, among other actions, an adequate and productive nursing staff. Maintaining the competence of employees presupposes the identification of risk factors that contribute to the development of WRMSDs, early recognition, and documentation of health problems related to WRMSDs, as well as policy strategies for modifying working conditions such as the implementation of ergonomic measures.

Evidence-based intervention strategies are necessary for the safety and productivity of nursing staff and are likely to lead to significant cost savings. The modern challenges in high-quality nursing care provision in hospitals require not only the knowledge but also the attitudes and skills of well-trained nurses. The recognition of the nursing profession as a cornerstone of the health service automatically implies policies that allow the maximum performance of the nursing personnel.

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ORIGINAL ARTICLE

An Overview of Eating Disorders in Iran: Frequency and Some Related Factors

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Abstract: *Background and Aim: Eating disorders are one of the most important issues in mental health that leading to many serious psychic and somatic problems and even death. These disorders were known for years as a disease of the western societies. Recent studies showed that they are also spreading in eastern countries. Limited studies are in Iran. The purpose of this study is to review the Iranian researches. The results of this study in Iran as a developing Asian country will helpful for other societies, especially similar Asian communities. Methods: The authors searched PubMed, ProQuest, Cochrane, Scopus, Web of Sciences, and SID and Magiran (In Persian) to find the relevant articles. The Joanna Briggs Institute Critical Appraisal Checklist was used to assess the quality of research. Results: Twenty- nine studies reported the prevalence or occurrence of eating disorders among the Iranian population. The samples consisted of 40 to 30,532 subjects. Forty papers examined eating disorders among females. Twenty-seven studies were in both genders, and only one was in males. Most eating disorders have significantly varied depending on the different studies and tools used. Most of these articles studied young people. This study breaks the determinants into three parts: demographic, psychosocial, and miscellaneous factors. The incidence of eating disorders in Iran is on the rise. Conclusions: It should be considered a significant health problem. Moreover, despite a clear cultural difference, eating disorders' factors were similar to those in western communities. This matter may be an essential health issue. In addition, despite noticeable cultural differences, the factors affecting eating disorders were similar to those of western communities.*

Keywords: *eating disorders; prevalence; contributing factors; review; Iran*

INTRODUCTION

The main issue in eating disorders is a distortion of body perception. People with eating disorders use weight loss methods such as diet or compensatory behavior (intensive exercise, vomiting) to obtain the ideal appearance [1]. Although eating disorders are less prevalent as compared to some psychiatric disorders such as depression and anxiety [2,3], these are one the severe health issues.

The complications of these problems include 1. Psychological like low self-esteem and depression, substance use and even suicidal attempts, or 2. physical conditions such as malnutrition, severe imbalance of water and electrolytes, digestive problems, and even death [4].

Various biological, psychological, and social factors influence eating disorders [3-5]. These disorders are known as Western cultural disorders [6]. However, various studies have shown that the disease is widespread in non-Western crops, both developed and developing economies such as Brazil and Asia [7]. According to some researchers, these disorders are culture-influenced rather than culture-related phenomena [8]. Most Asian studies on eating disorders are in East Asia. Western Asian countries, particularly the Middle East, have a different culture from East Asia. Therefore, it may be helpful to study these conditions in this region [9].

A review of Iranian articles on bodily perception has shown that body dissatisfaction is high in Iran. Regarding globalization and the increased influence of Western culture, eating disorders are a health problem among Iranian adolescents and youth [10,11].

The current study sought to collect scattered information on the prevalence and contributing factors of eating disorders in

Iran. The results of this study conducted in Iran as an Asian country will benefit other similar Asian companies. In addition, the findings may assist health researchers and planners in the management of disorders.

MATERIALS AND METHODS

This study was carried out according to the The Joanna Briggs Institute Critical Appraisal Checklist [12]. We used that directive at all stages of the study. The authors searched The databases of PubMed, ProQuest, Cochrane, Scopus, Web of Sciences, and SID and Magiran (In Persian) to find the related articles to 2021 with no limitation at publication time.

The search strategy was designed to cover all articles related to eating disorders in Iranian people. A combination of Medical Subject Headings (MeSH) and keywords (Persian and English) was searched for related articles. These keywords included: "Eating disorders "Binge-Eating Disorder" OR "Anorexia Nervosa" OR Bulimia OR "Binge Eating" OR "Bulimia Nervosa" OR "Compulsive Eating" OR “Eating and Feeding Disorders"

Inclusion Criteria

All original papers on eating disorders among Iranians were examined. The papers have been divided into three types: Reporting the prevalence of eating disorders, Effective factors on eating disorders, Treatment of these disorders.This study reviewed articles about the prevalence and influential factors of eating disorders. Letters to publishers, reviews, and comments have been excluded.

Collecting data

The related articles were selected according to the title, abstract, and keywords.

Data Extracting

Two researchers separately extracted information such as the year of publication, location of study, sample size, age, type of study, gender, and other variables presented in Table 1. They recorded the findings in the data extraction form. Any disagreement between the researchers discussed and the outcome nearest the opinions of the two researchers chosen. In the conflict between the two researchers, the advice of a third expert on the topic was used.

Evaluate the research quality

The Joanna Briggs Institute Critical Appraisal Checklist evaluated the quality of the research [12]. Each article was examined based on methodological criteria such as the relevance of the participants, the sufficient size of the sample, the validity of the research method, and the use of appropriate statistical methods. Each article was investigated based on the methodology criteria such as suitability of participants, sufficient sample size, the validity of the research method, and use of appropriate statistical methods.

Figure 1: Flow diagram of the literature search and study selection process

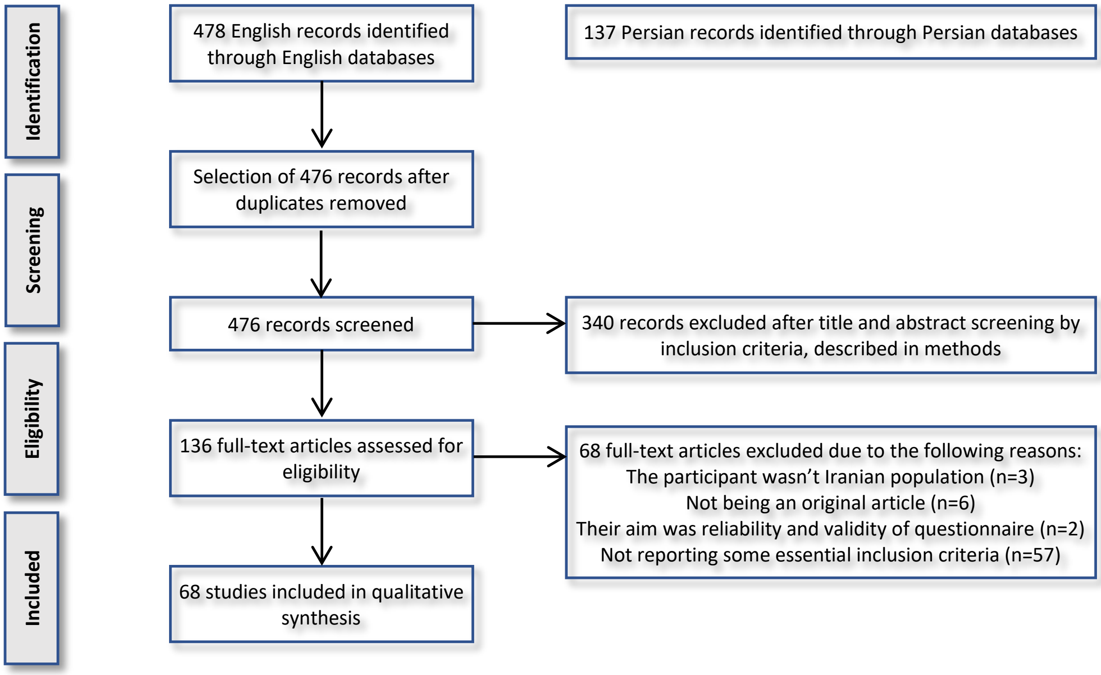
Identification
Screening
Eligibility
Included
478 English records identified through English databases
137 Persian records identified through Persian databases
Selection of 476 records after duplicates removed
476 records screened
340 records excluded after title and abstract screening by inclusion criteria, described in methods
136 full-text articles assessed for eligibility
68 full-text articles excluded due to the following reasons:
The participant wasn’t Iranian population (n=3)
Not being an original article (n=6)
Their aim was reliability and validity of questionnaire (n=2)
Not reporting some essential inclusion criteria (n=57)
68 studies included in qualitative synthesis

RESULTS

The authors found 615 articles, including 478 English and 137 Persian articles. About 476 articles were approved according to their titles, abstracts, and keywords. Only 68 articles met the inclusion criteria, including 27 English and 41 Persian articles (Figure 1).

Twenty-nine studies reported the prevalence of eating disorders in Iranian populations(8, 13, 14, 16, 19, 21-23, 25, 29-31, 33, 36, 38, 44, 46-49, 51, 52, 55-57, 59, 60, 62, 68, 70).

The prevalence of these disorders has reported very different from 0.2% to 80% (Table 1).

The articles` characteristics are presented in Table 1. These studies were conducted between 2000 and 2021. Among these studies, 40 articles were conducted on females, 27 articles on both genders, and only one article on males(56). The number of subjects ranged between 40 and 30532.

Most studies have investigated the characteristics of participants in a descriptive method and two studies used Ex-post and Facto method [37,53]. Eighteen studies showed relation between BMI and eating disorders. Sixteen studies investigated the relationship between body satisfaction and eating disorders. Nine studies have shown the relationship between parental characteristics and eating disorders. In the most articles participants' age was less than 35 years old. Four studies confirmed the relation between eating disorders and media use, especially Western media. Six studies investigated Depression, Anxiety and Stress, Obsessive-Compulsion Psychological signs, Poor psychosocial domains of HRQOL. Related factors and the articles that refer to them are shown in Table 2.

DISCUSSION

Prevalence

The prevalence of eating disorders varies according to the research. A study looked at eating disorders among Iranian students living in Iran and the United States. She found that Iranian women living in Iran are more affected by these troubles than women living in America [13]. Abdollahi & Mann have shown that women living in Iran have a higher prevalence of eating disorders [14]. Studies around the world have shown that prevalence rates are not the same. A large national study conducted in the US found that AN, BN, and BED lifetime prevalence estimates were 0.80%, 0.28%, and 0.85%, respectively [81]. The widespread prevalence of eating disorders in Asian countries has been reported [7]. As noted earlier, most studies are limited to eastern and southeastern Asia. However, limited studies in the Western Asian and Eastern Mediterranean regions have reported relatively high rates of such disturbances. The prevalence of eating disorders in West Asian countries like Pakistan has shown that eating disorders have increased from 10.3% to approximately 21.6% since 1992 – 2014 [9]. Various statistics have been reported in the Arab countries (Eastern Mediterranean): 11.5% of women in Egypt and 49% in the UAE. This rate in men varied from 6% in Saudi Arabia to 36% in Oman [82]. The prevalence of these conditions decreased from 0.2% to 80%. Based on a national study, the total prevalence of eating disorders among children and adolescents was 0.89 (0.81-1.10) [70]. This difference can be due to conducting studies at various ages, groups such as athletes, and diagnostic tools. For the most part, self-administered screening questionnaires were used. The clinical interview makes it possible to better estimate the prevalence of eating disorders.

Factors contributing to eating disorders

Over the last few years, many studies have focused on the factors contributing to eating disorders' development and persistence. The papers examined biological, social, interpersonal, internalizing the ideal body announced by the media, low self-esteem, cognitive pattern, and family dynamics. It may be challenging to differentiate their direct effects due to their interaction. For example, people with low self-esteem have a deformed perception of the body and higher dissatisfaction they are more influenced by social pressures and the media. In this section, we focus on contributing factors in three sub-categories.

Demographic variables

Age

Limited studies have investigated the effect of age on eating disorders [16,25,30,51,59,62-64,70,83].

Because most studies were in the narrow age range, it is impossible to discuss the age effect.

BMI

Many studies have defined contributing effects of BMI through affecting body image distortion. Some researchers believe self-devaluation, feeling incompetent, worries regarding weight gain disproportionate to the advertised ideal body are the consequences of high BMI that have been directly or indirectly affected by eating disorders [2,84].

BMI was studied in many articles [8,10,16,19,21,25,30,35,46,48,49,52,57,58,62,64]. Body mass index had a positive relation to eating disorders and body dissatisfaction. In these researches, such as Western studies, BMI was considered a significant contributing factor.

Table 1: Main characteristic of studies

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
1	Nobakht,2000, Tehran (13)	3100	15-18	Prevalence of Eating Disorders	Cross Sectional	Female	Boy dissatisfaction levels of education of the mother, father profession	Eating Disorder Diagnostic Inventory	ANOVA	The prevalence of eating disorders was more than other non-Western societies, and comparable with Western societies	Lifetime prevalence of 0.9% for Anorexia Nervosa, 3.2% for Bulimia Nervosa, and 6.6% for the partial syndrome.
2	Abdollahi,2001, Tehran (14)	59	NA	Comparisons between Iranian women in Iran and in America	Cross Sectional	Female	Exposure to Western media, acculturation	Eating Disorder Examination-Questionnaire and the Figure Rating Scale		Neither exposure to Western media, nor acculturation to Western norms appeared to be affected by disordered eating.	The prevalence of eating disorders in students that lived in Iran was as much as who lived in America
3	Jaeger, 2002 (15)	1751	19-23	Risk factors for bulimia nervosa in 12 countries	Cross Sectional	Female-Male	Body dissatisfaction self-esteem’	Silhouettes Dieting’ scale of the Eating Attitudes Test (EAT), Ineffectiveness scale of the Eating Disorders Inventory	Functional model, structural equation methodology	That body dissatisfaction seems independent from the actual BMI, could be affected Iranian population	
4	Zarghami (8)	1200	13-18	Abnormal attitudes frequency towards eating	Cross Sectional	Female-Male	BMI	Attitude Test (EAT-26) Silberberg Anxiety test Beck Depression	Logistic regression	Individuals with these abnormal attitudes, are more at the risk of developing psychological disorders,	Their prevalence has been estimated to be about 10.5%
5	Jahanfar, 2005, Tehran (16)	250 (twins)	15-45	Relation between Eating Disorder, Polycystic Ovary Syndrome	Cross Sectional	Female	BMI, Presence of Acne, Amenorrhea, Number of periods per year	Bulimia investigation test (Edinburgh)	Student's t-test and Chi-square test Fisher test	The genetic property of bulimia nervosa was not found in this twin study. Bulimia score was found to be positively correlated with BMI, acne, amenorrhea. Number of periods per year. No significant correlation was found between bulimia score and age, age of menarche, hirsutism. serum leptin level was correlated significantly with bulimia score	Only 8% of subjects were diagnosed subclinical eating disorder

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
6	Yousefi Asl, 2007, Tehran (17)	420	14-18	Data processing and selective attention in Eating disorders	Cross Sectional	Female	Task sensitivity	EAT-26 , Dot Probe Task	Student's t-test and Chi-square test	Attention bias with avoidance from body-shape stimuli was more in who had high score of Eating Attitudes Test.	
7	Momeni, 2008, Isfahan (18)	53	14-17	Relationship Between Parents' Child Rearing Styles and Eating disorders	Cross Sectional	Female	Parenting style	Minnesota Eating Behavior Survey (MEBS,Child rearing questionnaire of Baum Rind	Pearson correlation test ,logistic regression	There was significant positive relationship between democratic style of parents and anorexia nervosa and negative relation between authorities' style of parents and anorexia nervosa. Among female adolescents.	
8	Pourghassem Gargari, 2008, Tabriz (19)	1877	14-19	Prevalence of eating disorders in Female students	Cross Sectional	Female	Overweight perception, Desire for loss of weight Cigarette smoking, Age of menarche	Eating Attitude Test 26	ANOVA, Chi-square test	About half of the participants were unhappy with their body weight Mean disordered eating attitude was higher in them. They were intended to weight loss, were smoker, and who had age of menarche less than 11 years.	About 16.7% of students had eating disorders. This prevalence was lower than Tehran (capital of Iran) and some of Arabian countries.
9	Asgari, 2009, Ahvaz (20)	145	NA	Emotion regulation, Mental stress and Body image in Eating disorders	Cross Sectional	Female	Emotion Regulation, Mental Stresses	Emotion Regulation Questionnaire", Mental Stress Questionnaire, Eating Disorder Questionnaire ,Body Image Questionnaire	Regression	Mental stresses and eating disorders were significantly related to each other. Body image and eating disorders had negative relation	
10	Safavi, 2009, Tehran (21)	400	NA	Body image perception and eating disorders	Cross Sectional	Female	BMI, Parents characteristics	Multidimensional Body Self Relation Questionnaire-Eating Attitude Test 26	Chi-square test	BMI and father`s job had positive relation with eating disorders.	About 21.5% had eating disorders that included 1.8%Anorexia Nervosa and 7.8% Bulimia Nervosa

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
11	Gargari, 2010, Tabriz (22)	250	14-51	Eating attitudes, self-esteem and social physique anxiety in Iranian fitness females	Cross Sectional	Female	Weight, Height, Body Mass Index (BMI)	Eating attitude test-26 (EAT-26), Rosenberg's self-esteem scale (RSES), and Social Physique Anxiety Scale (SPAS)	ANOVA, Pearson Correlation Test	Disordered eating attitude was correlated negatively with Self steam and positively with body weight, BMI, and SPAS	28.4% of subjects were disordered eating attitudes (EAT-26220)
12	Mahmoodi, 2010, (23)	195	21-25	Relationship between social pressure and body image with eating disorders	Cross Sectional	Female	Exercise	The Multidimensional Body-Self Relations Questionnaire (MBSRQ), Perceived Socio-cultural Pressure Scale (PSPS), EAT 26	Student's t-test, Pearson Correlation	The was positive relation between eating disorders with social pressure. Positive relation was demonstrated between eating disorders and body image.	About 27% of athletes and 19% of non-athletes had eating disorders
13	Moloodi, 2010, Tehran (24)	151	20-50	Comparison of early maladaptive schema in eating disorders	Cross Sectional	Female	Obesity-BMI	Binge Eating Scale, Young Schema Questionnaire	MANOVA	Binge eating disorder had significant relation with higher scores of abandonment/ instability, emotional deprivation and insufficient self-control/ self-discipline	
14	Shamsaddin Saeed, 2010, Kerman (25)	650	13-22	Eating disorders and Body image	Cross Sectional	Female	Age- Parents Education	Eating Disorder Diagnostic Scale Body Image questionnaire	Student's t-test, linear and Logistic Regression	BMI was predictive factor for eating disorders	About 6.3% of students had eating disorder, consisting of 1.7% anorexia nervosa, 1.7% bulimia nervosa and 2.9% eating disorder not otherwise specified.
15	Shayeghian, 2010, Tehran (26)	403	16-17	Maladaptive Core Beliefs and Eating Disorder	Cross Sectional	Female	Parental Bonding	Parental Bonding Inventory- Young Schema Questionnaire- Eating Disorder University Belief Questionnaire- Eating Disorder Inventory	Pearson Correlation Test	Eating disorder beliefs and Maladaptive core beliefs respectively play a contributing role between parental bonding and eating disorder symptoms	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
16	Behrad, 2011, Yazd (27)	220	NA	Thought control strategies and eating disorders	Cross Sectional	Female	Self-punishment and Worry	Thought Control Questionnaire (TCQ) - Eating Attitude Test 26	Pearson correlation-regression analysis	Self-punishment and Worry both were the best predictors of eating disorder	
17	Khosravian, 2011, Tabriz (28)	140 (70 with bulimia and 70 without bulimia)	NA	Age-Gender , Education	Cross Sectional	Female - Male	Perfectionism, Negative Affect, Brain-Behavioral Systems	Hewitt and Flett multidimensional perfectionism scale, Hed depression and anxiety scale and Carver and White’s brain-behavioral systems questionnaires	MANOVA	Results showed Bulimia nervosa was associated with higher levels of perfectionism, negative affect and BAS and lower BIS	
18	Mozaffari-Khosravi, 2011, Yazd (29)	1400	NA	Eating Disorders High School Girl Students	Cross Sectional	Female	Age, Parents education, Menstrual status, Birth order, Family size	Eat Eating Attitude Test 26	Chi-square, Fisher’s exact test and Pearson correlation	Between BMI status and risk of eating disorders a significant relationship was showed. Family sizehad relation with eating disorders	About 12% of students were at the risk of eating disorders. 0.5% had Anorexia Nervosa and 5.3% were diagnosed with Bulimia Nervosa
19	Valizade, 2011, Ahvaz (30)	181	NA	Eating Disorders and Psychological Signs among women with Sport Activities	Cross Sectional	Female	Marriage Status, Education Psychological status, Depression, Anxiety	Ahvaz Eating Disorder Scale - Psychological Signs Questionnaire	Pearson correlation, Multivariate regression	Eating disorder signs, anorexia nervosa signs and bulimia nervosa signs were related to psychological signs, Depression and Anxiety.	16.5% percent of athletes have eating disorder signs, 14.3% anorexia nervosa, and 3.3% bulimia nervosa
20	Mashhadi, 2012, Tehran (31)	99	NA	Dieting and the Development of Eating Disorders among Iranian Women	Cross Sectional	Female	BMI, Weight satisfaction, Self-esteam Age	Eating Attitude Test (EAT-26),the Sick, Control, One stone, Fat and Food (SCOFF) questionnaire,	Chi-square, Student's t-test	Iranian women that lived in Iran with a low BMI (below 18), high higher tendency toward eating disorders.	About 17% of participants had eating disorders. The Iranian women in Iran had equal or even higher eating disorders than Iranian woman in Netherland.

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
21	Abbaszadeh, 2013, Tabriz (32)	400	Mean 20	Eating disorders and emotional intelligence	Cross Sectional	Female	Social awareness	Siberia Shearing Questionnaire-Eating Disorder Diagnostic Scale	ANOVA, logistic regression	Multiple aspects of emotional intelligence had negative relation with eating disorders. Social awareness was predictive factor in eating disorders	
22	Garrusi, 2013, Kerman (33)	1204	14-55	Eating Disorders and Their Associated Risk Factors	Cross Sectional	Female - Male	Body Mass Index (BMI), Media, Body dissatisfaction, Self-esteem	Eating Disorder Diagnostic Scale-Body-Esteem Scale for Adolescents and Adults-Perceived Socio-Cultural Pressure Scale-Rosenberg Self-Esteem Scale	Two-sample t - Chi-square tests- logistic regression	Symptoms of bulimic syndrome were greater in males. BMI, Social pressure, Body dissatisfaction, and Western TV were only significant in the male. Educational level, Self-steam, were significant in the females	The prevalence of eating disorders was 11.5% that included 0.8% anorexia nervosa, 6.2% full threshold bulimia nervosa, 1.4% sub threshold anorexia nervosa and 30% sub threshold binge eating disorder
23	Hossein Zadeh, 2013, Mazandaran, (34)	525	NA	Maternal Bonding and Vulnerability related to Eating: The Mediational Role of Eating Disorder Beliefs and Alexithymia	Cross Sectional	215 females, 210 males	Alexithymia	Eating Disorder Inventory, Alexithymia Scale, Eating Disorder Belief Questionnaire, Parental Bonding Instrument	ANCOVA	The findings demonstrated that maternal bonding can play an important role in preventing eating disorders	
24	Mohamadzadeh 2013 Tabriz, (35)	280	Mean 21.8± 2.49	Eating disorders physical dissatisfyaction and physical activity behavior change	Cross Sectional	Female	Physical activity behavior change	EAT-26, Body dissatisfaction - Physical activity staging questionnaires	Pearson correlation, Spearman correlation and one way ANOVA test	There was positive relationship between eating disorders and body dissatisfaction. There was significant relationship between eating disorders and stages of change for physical activity.	
25	Mohebi, 2013, Kermanshah (36)	578	21-24	Eating disorders prevalence	Cross Sectional	Female	Demographic variable Amenorrhea	Eating Disorder Diagnostic Scale	Student's t-test	BMI was significant relation with Anorexia Nervosa and Eating Disorders NOS	Results showed frequency of Anorexia Nervosa and Eating Disorders NOS were 0.2% and 2.4 respectively

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
26	Narimani, 2013, Ardebil (37)	104 normal students and 104 bright students	NA	Eating disorders in normal and bright students	Ex post facto research	Female-Male	Parenting styles	Baumrind parenting style and Coker and Roger eating habits questionnaires	MANOVA	There was no difference in eating attitude between normal and bright students	
27	Kordi,2014, Mashhad (38)	190	Mean 25.14 ±4.4	Eating disorder symptoms in nulliparous women	Cross Sectional	Female	Social anxiety symptoms	Eating Disorder Assessment	Pearson correlation tests. Linear regression	Social anxiety had positive correlation with eating disorders symptoms	About 8% of pregnant woman had eating disorders symptoms
28	Mohamadzadeh , 2014, (39)	1008	Mean 24 years	Factor Structure of disordered eating attitudes	Cross Sectional	Female-Male	Gender	Eating Attitude Test (EAT)	Explanatory factor analysis of variance - independent samples t-test	There is more internal constancy between Dieting, Bulimia plus Food Preoccupation, & Oral control with eating disorders attitude. Women have higher mean scores on the eating attitudes	
29	Narimani, 2014, (40)	53 Student with binge eating disorder, 64 Normal	Mean 17 years	The role of psychological factors in predicting binge eating disorder	Cross Sectional	Female-Male	Demographic variable	Composite Scale of Morningness (CSM), Lynda's Depression Anxiety Stress Scales (DASS), Weight efficacy Life Style Questionnaire and coping styles questionnaire. Discriminant analysis	Compare Means	Analysis showed that the depression, food availability and negative emotions have a significant effect on the predicting binge eating disorder.	
30	Roustaee, 2014, Tehran (41)	515 (231 sample with eating disorder)	NA	Major Dietary Patterns and Their Relation with Eating Disorders	Cross Sectional	Female	NA	Body Shape Satisfaction Scale (BSSS) and semi-quantitative food frequency questionnaires	Logistic regression	High fiber and low fatty food had relation with eating disorders None of the demographic characteristics, weight, or body satisfaction showed any significant relationship with eating disorders	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
31	Poursharifi,2014 (42)	142	14-18	Social physique anxiety and eating attitudes	Cross Sectional	Female	BMI - Social Pressure	Social Physique Anxiety (SPAS), Eating Attitudes (EAT-26,), Socio-cultural Influence on Weight loss and Body change	Multivariate two-factor ANOVA	High sociocultural pressure had a significant role in the social physique anxiety and eating attitudes	
32	Bahri, 2015, Gonbad Kavoos (43)	250	NA	Eating Disorders in children and Its Relationships With Their Mothers' General Health And Nutrition Attitude	Cross Sectional	Female	Age of menarche?	General Health Questionnaire (GHQ-28), the Eating Attitudes Test (EAT-26), Eating Disorder Inventory Questionnaire	Multivariate regression, Pearson correlation tests	Significant correlation was between aspects of mothers' general health, nutrition attitude and female children's overeating, desire for slimness, body dissatisfaction	
33	Eftekhari, 2015, Shiraz (44)	594	14-17	Eating disorders and anthropometric indices in female adolescent students	Cross Sectional	Female	Socioeconomic variables, BMI	Nutritional attitude questionnaire	independent t- test and Chi-square	BMI had relation with eating disorders	Results showed that 80% of the studied population are at risk of eating disorders
34	Foladvand, 2015, Tehran (45)	300 (100 patients with eating disorders, 100 patients with high depression, and 100 normal subjects	NA	The Contribution of Self-Concept, Impulse Control and Dysmorphic on Eating Disorders	Cross Sectional	Female-Male	Depression, Anxiety, Stress	Rogers Self-concept scale, The Body Dysmorphic Disorder Questionnaire, Bar-On Emotional Quotient Inventory, Depression Anxiety Stress Scales	Multiple regression analysis	Self-concept, impulse control and body dissatisfaction had significant relation with eating disorders .Depression, anxiety and stress had relation with eating disorders	
35	Jalali-Farahani, 2015. Tehran (46)	465	14-17	Disordered Eating, Health-Related Quality of Life Among Adolescents	Cross Sectional	Female	Overweigh	Eating Attitudes Test-26 (EAT-26) and Pediatric Quality of Life Inventory (PedsQLTM4.0)	T test, Chi square	Disordered eating was associated with overweight in girls and poor psychosocial domains of health-related quality of life	Disordered eating was prevalent in 18.9 % of adolescents

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
36	Mohamadirizi, 2015, Mashhad (47)	213	24.14 ±4.4 years	Eating disorder symptoms and Obsessive Compulsive Disorder in Prime- gravid women	Cross Sectional	Female	BMI, Gestational age	Eating Disorder Examination Questionnaire and Maudsley Obsessive Compulsive Questionnaire (30Q)	Pearson correlation coefficient, t test, one way ANOVA and linear regression	There was a poor positive correlation between the rate of Eating Disorder Symptoms and Obsessive Compulsive	About 18% of pregnant women had Eating Disorder Symptoms.
37	Rauof, 2015, Urmia and Tabriz (48)	1990	15.8 ±1.24	Prevalence of eating disorders among adolescents	Cross Sectional	Female-Male	BMI-Income	Eating attitudes test (EAT-26) and DSM-IV criteria	Linear and logistic regression and Fisher’ s exact test	BMI was related to eating disorders.	24.2% of students were at risk of eating disorder .About 0.25%had eating disorder.
38	Rezaei, 2015, Shiraz (49)	302	15-35	Prevalence of eating disorders	Cross Sectional	Female	Age- Education - BMI	Eating attitudes test (EAT-26)	Descriptive statistics section, the central and dispersion indices		Participants had 0.7% Bulimia Nervosa and 0.3% had binge eating Disorders.
39	Rezaei, 2015, Shiraz (50)	302	15-35	Study of the Perfectionism Role and Body Mass Index in Prediction of Eating Disorders among Female Students of University	Cross Sectional	Female	Age- Education, BMI, Body dissatisfaction	Perfectionism Ahvaz Scale and Body Shape Questionnaire, Eating attitudes test (EAT-26)	Descriptive statistics section, the central and dispersion indices	The perfectionism and BMI were good precedents and body dissatisfaction was good mediator for eating disorder in female students.	
40	Rouzitalab, 2015, Tabriz (51)	105 males and 105 females	18-25	The relationship of disordered eating attitudes with body composition and anthropometric indices	Cross Sectional	Female-Male	Weight, height	Eating attitude test (EAT-26)	Mann-Whitney U test, Kruskal-Wallis test and Spearman correlation test	In females, weight and body mass index (BMI) were significantly different between disordered eating and healthy subjects.	About 10% of the studied subject had disturbed eating attitudes.

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
41	Safarzade, 2015, Gonabad (52)	750 males and 750 females	13-18	Eating Disorders, Adolescents	Cross Sectional	Female-Male	Age, Education level	Eating attitude test (EAT-26)	Chi-square test	There was a significant association in prevalence of this disorder among these opposite sexes, with different ages and grads	About 15.7% of students had distorted eating. Prevalence of Anorexia Nervosa and Bulimia Nervosa were about 1.1 % and 3.2% respectively
42	Javanmard, 2016, Tabriz (53)	20 bulimic, 20 normal	NA	Explicit Memory (Recognition and Recall Memory) and Bulimia	Cross Sectional	Female	NA	Bulimia Questionnaire , Explicit Memory Inventory	MANOVA	There was a significant difference in recognition memory in bulimic group	
43	Bodaghi, 2016, Arak (54)	32 bulimia, 32drug addiction and 32 normal	NA	Activity of Brainal Behavioral Systems, eating disorders	Cross Sectional		NA	Gurmally overeating scales ,Jackson' s five-factor Inventory	MANOVA and Tukey	Activation system and freezing are have critical roles in the creation and persistence of the eating disorder	
44	Farid, 2016, Karaj (55)	537	14-18	Eating disorder Adolescence	Cross Sectional	Female	Weight, Height	Eating Attitudes Test (EAT-26)	Chi-square, ANOVA and t-test	The attitude toward eating disorder was related with BMI of and their self-concept	The prevalence of eating disorders was estimated to be 23.6%.
45	Garrusi, 2016, Kerman (56)	433	14-18	Eating disorders Male adolescents	Cross Sectional	Male	Educational level, Economic status, Social pressures, Self-esteem, Body satisfaction Media	Eating disorder diagnostic scale, Body Satisfaction Instrument, Socio-Cultural Pressure Instrument, Self-Esteem Questionnaire	Two-sample T-test and Chi-square, logistic regression	There were significant differences between the two groups (with and without eating disorders) in terms of social pressure, body dissatisfaction and body mass index (BMI). Body dissatisfaction and BMI were identified as predictive factors in eating disorders	About 15% of male adolescents had eating disorders. 7.4%) subjects had diagnostic criteria for full threshold BN, and 6.5%) individuals had sub-threshold BED. Only (0.7%) adolescents had sub-threshold BN.
46	Hosseini, 2016, Hamadan (57)	231	18-32	Fitness intention Eating attitudes	Cross Sectional	Female	Age-Residency status, Marriage status	TPB variables scale	T-test, ANOVA, Bivariate correlations, and linear regression	Results showed a positive correlation between fitness intention and eating attitude.	Nearly 21.6% of the participants had abnormal eating attitudes

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
47	Kachooei, 2016,Tehran (58)	104 (52 women with BED, 52 women without BED)	With BED 30.63, without BED 28.63	Impulsivity and Difficulties in Emotion Regulation among Overweight Women With and Without Binge Eating Disorder	Cross Sectional	Female	Age, BMI	Eating Disorders Diagnostic Scale (EDDS), the Barratt Impulsivity Scale (BIS-11) and the Difficulties in Emotion Regulation Scale (DERS)	MANOVA	Binge eating women had more impulsivity, difficulties in emotion regulation, attentional impulsivity	
48	Khajrnoori, 2016, Shiraz (59)	600	15-29	Body image and Eating disorders	Cross Sectional	Female	Parental variables	Self-discrepancies for physical characteristics, Body Areas Satisfaction Scale, Parental Attitudes Regarding Body, Peer Attitudes Regarding Body	Correlation test	The results showed parents' attitudes toward body, BRSC, and ICTs use can affect both body image and eating disorders, on the other hand body image can affect eating disorders	15% of subjects had Anorexia Nervosa, 17.7% and 10% had Bulimia and BED respectively.
49	Miri, 2016, Qazvin (60)	226 athlete students and 350 non-athlete	Mean age 21.92	Eating disorders Body image in athletes and non-athlete students	Cross Sectional	Female-Male	Age, BMI	Eating Attitudes Test (EAT-26), and Multidimensional Body-Self Relations Questionnaire (MBSRQ)	T-test and Chi-square test	Anorexia nervosa was found to be more prevalent than bulimia nervosa in both groups. .	The frequency of eating disorders was 11.5% among the athlete students and 11.2% among the non-athlete students.
50	Mohamadzadeh , 2016, (61)	790 (455 Female, 335 Male)	18-52	Obsessive Compulsive Features , Pathological Eating Attitudes in Non-clinical Samples	Cross Sectional	Female-Male	Age	Obsessive Compulsive Inventory-Revised (OCI-R), and Eating Attitudes Test (EAT-26) questionnaires	Multivariate regression analysis	The results showed that obsessive-compulsive features can predict 15% of pathological eating attitudes There were a correlation between obsessive-compulsive features and pathological eating attitudes.	
51	FarsadNaeimi, 2016, Tabriz (62)	430 (296 Female, 134 Male)	Mean 21.09	Eating disorders risk Self-esteem and Body image	Cross Sectional	Female-Male	Age, BMI, Self-Steam	EAT-26, Multidimensional Body-Self Relations Questionnaire (MBSRQ) and Rosenberg Self-Esteem Questionnaires	Chi-square, Independent-samples T test or Mann–Whitney U test	Self-esteem and mother' s education level were predictors of eating disorders risk.	The overall eating disorders risk was 9.5 %.

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
52	Asghari, 2017, Rasht (63)	370	15-18	Perceived stress ,Coping styles on Eating disorders	Cross Sectional	Female-Male	Age-Gender	Eating disorders' scale, Perceived stress scale Coping styles questionnaire	Pearson correlation coefficient ,Stepwise regression	Negative perceive stress, Emotional- focused coping, and Problem focused coping could significantly predict of anorexia and bulimia	
53	Mofradnejad, 2017, Behbahan (64)	200	16.9 ±4.21	Evening-ness, Sleep quality, Eating disorder	Cross Sectional	Female	Oral control	Composite Scale of Morning-ness, Pittsburgh Sleep Quality Inventory and Eating Attitudes Test	Pearson correlation ,Regression tests	There was a positive correlation between evening-ness and negative correlation between sleep quality and eating disorder	
54	Mohagheghi, 2017, Hamedan (65)	245	NA	Self-compassion ,Eating disorder behaviors, Perfectionism, Body dissatisfaction	Cross Sectional	Female	NA	Self- compassion scale-short form, The Almost Perfect Scale, Body dissatisfaction scale, Disorder eating behavior questionnaire	Structural Equation Model	The self-compassion variable is related with eating behaviors through perfectionism and body dissatisfaction. Body dissatisfaction is directly related to eating behaviors.	
55	Hosseini, 2017 (66)		15-35	Testing a Tripartite Model In Body Image and Eating Problems	Cross Sectional	Female	BMI – Body Dissatisfaction – Media	Eating Attitudes Test, The Perception of Teasing Scale (POTS), Multidimensional Body- Self Relations Questionnaire, and Socio-cultural Attitudes towards Appearance Questionnaire	Path analysis	Family factor and social comparison directly affect overeating, whereas inhibition of eating has an indirect effect on overeating. Media through comparison and inhibition of eating indirectly affected overeating. Body image dissatisfaction does not play a mediating role	
56	Radfar, 2018 (67)	264	15-17	Association between eating behaviors with BMI	Cross Sectional	Female	Parents education	Dutch Eating Behavior Questionnaire DEBQ,	Spearman correlation.	Negative relationship existed between eating external stimuli and body mass index. BMI had positive relation with dietary restraint.	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
57	Ebrahimzadeh, 2018, Tehran (68)	400	About 17 years	Eating disorder, Body dysmorphic disorder symptoms	Cross Sectional	Female-Male	NA	Yale-Brown Practical Obsessive-compulsive Scale (BMD) for Body Dysmorphic disorders (YBOCSBDD), Eat-Inventory Questionnaire (EAT-26), and Short-Term Short Term Syndrome Questionnaire (BSI)	Pearson correlation, t-test, Liner regression	The correlation between eating disorder and body dysmorphic disorder was significant	About 67.6% of girls and 55% of boys had eating disorders
58	Kehtari Harzangh, 2019, Tabriz (69)	60	18-65	Comparison of Impulsivity and Experiential Avoidance in Obese Individuals With and Without Binge Eating Disorder	Cross Sectional	Female-Male	Education level	Gurmally Binge Eating Scale (BES), Barratt Impulsiveness Scale (BIS), and Bond Acceptance and Action Questionnaire (AAQ)	descriptive statistics section, the central and dispersion indices	Among components in two groups of with and without binge eating disorder, the components of non-planning impulsivity , and emotional avoidance and control were found significant	
59	Mohammadi, 2020, national (70)	30,532	6-18	Prevalence, correlates and comorbidities of feeding and eating disorders	Cross Sectional	Female-Male	Place of residence	Persian version of K-SADS-PL and DSM diagnostic criteria	multivariate binary logistic regression	Older age, female gender and living in an urban area are predisposing factors. in feeding and eating disorders (in binge-eating disorder, the male gender is positive correlate	The total prevalence offeeding and eating disorders among children and adolescents was 0.89 (0.81–1.10). In all types of feeding and eating disorders
60	Amirfakhraei (2019) (71)	210	NA	Alexithymia, Coping Styles and Cognitive Emotion Regulation	Cross Sectional	Female-Male	Education level	Toronto Alexithymia, Scale, Endler & Parker coping scale of stressful situations	Pearson correlation and multiple regression with enter model methods	Positive cognitive emotion regulation strategies, negative cognitive emotion regulation strategies and problem-focused coping style significantly could predict 45.5 percent of variance eating disorder	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
61	Sahlan (2020) (72)	637	College	Eating-disordered behavior and college students	Cross Sectional	Female-Male	Binge eating, self-induced vomiting, laxative misuse, extreme dietary restriction and excessive exercise	EDE-Q	Pearson and Spearman test	The occurrence of other EDBs did not differ by gender and binge eating was the most common EDB in both gender	
62	Sahlan (2020) (73)	1105	12-19	Eating-disordered behavior and adolescents	Cross Sectional	Female-Male	Binge eating, self-induced vomiting, laxative misuse, extreme dietary restric-	EDE-Q	Pearson and Spearman test	ED symptoms appear to be relatively common among Iranian adolescent boys and girls.	
63	Daeie Farshbaf (2021) (74)	725	14-18	Age at menarche, eating disorders Individuals With and Without Binge Eating Disorder	Cross Sectional	Female	BMI, waist circumference ,socioeconomic status (SES)	Eating Disorders Diagnostic Scale (EDDS) (AAQ)	Analyses of Variance (ANOVA) and Tukey' s post hoc	A significant positive correlation was found between eating disorders and Age of M enarch	
64	Farsimadan (2021) (75)	392/52	14-18	Emotional distress, eating self-efficacy, morningness and coping styles students in binge eating disorder	Cross Sectional	Female-Male	Coping styles, morningness, self-efficacy in eating, stress, anxiety and depression	Interview Composite Scale of Morningness (CSM), Lynda's Depression Anxiety Stress Scales (DASS), Weight efficacy Life Style Questionnaire, Binge Eating Scale and Coping Styles Questionnaire uations	multivariate analysis of covariance.	Persons with binge eating disorder had more anxiety , depression , less self-control , more emotion-focused coping strategies and eveningness tendencies	
65	Mahmoudi (2021)(76)	567	13-18	Emotion regulation and family structure	Cross Sectional	Female-Male		EAT , Difficulties in Emotion Regulation Scale , Family Adaptability & Cohesion Evaluation scale	Structural equation modeling	Indirect effect of unbalanced family functioning to eating disorders through difficulty in regulating emotion is significant	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
66	Sahlan (2021)(77)	730	9-13	Eating disorder and social anxiety symptoms (ED/SAD)	Cross Sectional	Female-Male	Gender	Children Eating Attitudes Test-20, Social Anxiety Scale for Children	Network models (ED and ED/SAD networks	Concern over being judged was central in networks including both ED and SAD symptoms. Additionally, concern over being judged was the strongest bridge symptoms.	
67	Sahlan (2021)(78)	1748	Adolesent; Young adult	Self-esteem, and depression symptoms in eating disorders	Cross Sectional	Female-Male	Gender	Eating Disorder Examination-Questionnaire (EDE-Q), Rosenberg Self-Esteem Scale (RSES) and Beck Depression Inventory, Second Edition (BDI-II)	Six Glasso networks	Central disordered eating nodes in most models were a desire to lose weight and discomfort when seeing one's own body	
68	Sahlan (2021)(79)	564	College	Cross-cultural comparisons in eating disorders	Cross Sectional	Female	Nationality	Body, Eating, and Exercise Comparison Orientation Measure (BEECOM) and the Eating Disorder Examination-Questionnaire (EDE-Q)	One-way analyses of covariance ,partial correlations	Eating disorder-related social comparison is a recommended clinical target in both Eastern and Western cultures.	

Table 2: Summary of (some of) topics that are mentioned in articles

	Variables	**Articles (*n*)**	**References**
Gender	Female	40	13, 14, 16-27, 29-32, 35, 36, 38, 41-44, 46, 47, 49, 50, 53, 55, 57-59, 64-67, 74, 79
	Male/Female	27	8, 15, 28, 33, 34, 37, 39, 40, 45, 48, 51, 52, 54, 60-63, 68-73, 75, 76, 78, 79
	Male	1	56
Language	English	27	13-16, 22, 31, 33, 42, 43, 46-48, 51, 55-57, 59, 62, 69, 70, 72-74, 77-80
	Persian	41	17-21, 23-30, 32, 34-41, 44, 45, 49, 50, 52-54, 58, 60, 61, 63-68, 71, 75, 76
Study type	Cross-sectional	66	8, 13-36, 38-52, 54-79
	Ex-Post facto method	2	37, 53
	BMI	18	8, 16, 21, 22, 25, 30, 33, 35, 44, 46, 48, 50, 52, 57, 58, 62, 64, 74
	Social Pressure	5	23, 35, 58, 61, 77
	Self-esteem	7	23, 35, 58, 64, 66, 75, 78
	16	13	13, 15, 19, 23, 35, 37, 38, 40, 44, 47, 57-60, 66, 79
	Education	2	35, 54
	PCO-Genetic Tendency	1	16
	Menstruation history (Age of menarche, Amenorrhea, Number of periods per year)	5	16, 19, 30, 44, 74
	Western media	4	14, 35, 58, 66
	Parents Characteristics (levels of Parents Education, Parent's job)	9	13, 19, 21, 22, 26, 30, 35, 58, 64
	Activity of Brain Behavioral System, Emotion Regulation, Maladaptive core beliefs, Self-punishment and Worry, Impulsivity, Emotional coping	11	20, 24, 26, 27, 43, 48, 56, 58, 69, 75, 76
	Age	10	16, 25, 30, 51, 54, 59, 62-64, 70
	Depression, Anxiety and Stress, Obsessive-Compulsion Psychological signs, Poor psychosocial domains of HRQOL	6	8, 31, 43, 47, 63, 77
	Mothers' general health and nutrition, attitude	1	45

Gender

There have been several studies that have indicated a higher prevalence of eating disorders among women. However, the increased prevalence in men is observed and other worldwide studies [7]. The current study found that the prevalence of eating disorders is higher among women than men. However, new studies suggest that the prevalence of these disorders is also rising among men [33,46,72]. Prevalence among men ranged from 7.5% to 18.2% [48,56,83]. One study indicated a very high prevalence (55%) [24]. According to male roles in eastern societies, increasing eating disorders among men is necessary for further research.

Socio-economic variables

There are conflicting findings of the effect of family socioeconomic status, parental education, or individual education on eating disorders [1,16,79]. We found little research on the socioeconomic status of the family. Some of these studies have considered the contributing role of father's occupation [13,15-18,20,64,82,85] and mother's education in these disorders [16,83]. However, some of the papers reviewed found no relationship between parental education and eating disorders in children [25,30,56]. Birth order, family size, income, and marital status had no relationship to eating disorders. Two studies have investigated the relationship between anorexia nervosa and bulimia nervosa and participant education [30,48]. Iranian studies' lack of association between socioeconomic status and eating disorders may be due to indirect mediating factors such as media effects and virtual social networks.

Psychosocial variables

Body Image – Body dissatisfaction

Literature review indicates that people with eating disorders suffer from excessive attention to the body, weight, or distorted body image [84,86]. The present study mentions the limited studies on the effect of body dissatisfaction or body image on eating disorders [13,15,23,35,37,38,40,47,57-60,66, 78]. These studies have shown that eating disorders are the most critical predictors of incorrect weight and height perception, body image distortion, and body dissatisfaction. However, two studies revealed no effect of body satisfaction on eating disorders [38,58]. This finding may be due to the more potent effects of other variables.

Social pressures

Body image is a function of social and cultural factors. The perception of body image is strongly influenced by the messages received regarding what's beautiful or not. Intense sociocultural pressure to lose weight and body change increases eating disorders [9,84]. Media, families, and peer groups have the most pressure on individuals to change bodies [11,79].

Media

The media affect body satisfaction by advertising the ideal body. The stated beauty and fitness norms are introduced as the principal means of success and social acceptance. Internalization of norms can result in body dissatisfaction and eating disorders [9]. Iranian studies have not focused much attention on the media in eating disorders. Few studies have been conducted in this field [14,35,58,66]. investigated contributing effect of media, especially Western media. A study also showed that media limiting food consumption had an indirect role [66]. The findings were contradictory; the media effects appear indirect [87].

Peer groups

The opinions of peer groups have a significant effect on body satisfaction and body change behaviors. Peers can exert this influence through encouragement or criticism. Despite the excellent studies on adolescents and youth, only a few studies have studied the effects of peer groups. Although the great studies have in adolescents and youth, only limited studies have investigated peer group's influences. Except one [39], all these studies thought that peer groups directly impacted eating disorders.

Family

The role of the family is linked to their messages about the body, and its management behaviors, family structure, parenting style, and relationship with parents may impact eating disorders [84]. Pre-defined studies have studied this question [17,25,30,36,39,45,57,83]. Another family factor that determines social and personal behavior is parenting styles. Two studies examined this issue [18,39].

Some studies have revealed that the relationships between mother and child can affect eating disorders [30,36,88]. This study was consistent with previous studies on the significant positive effect of maternal connections [89]. Another study showed that parental relationships influenced eating attitudes through inadequate core beliefs [88].

Psychological factors

Emotionally regulated is associated with eating disorders. Studies have shown that people with eating disorders have higher emotional intensity and emotional regulation problems, less acceptance of emotional responses, and low emotional clarity and high. Dynamic control strategies are more dysfunctional than ordinary people [89].

People who are not managing their emotions have more symptoms of eating disorders than others [90,91]. Limited Ian studies focus on this task [17,71,76].

Impulsivity is a multifactor construct. Some components of this structure are associated with eating disorders [92,93]. The current study mentioned a few studies in this regard. One study found that women with hyperphagia had higher impulsivity scores [60].

Self-esteem

In this field, the auto-team is a matter of interest. Some research has shown that low self-esteem is a risk factor for eating disorders [5]. Review of literature on the relationship between self-esteem and eating disorders [23,39,58,64,66]. However, conflicting studies have shown that low self-esteem does not predict eating disorders [94]. It seems that self-team, through body satisfaction, is effective on eating disorders.

Personality

Various relationships have been established between personality traits and eating disorders [5]. One study found an association between experiential openness and emotional instability with eating disorders among students. The results of predicting overeating behavior and mental occupation by food showed that emotional instability or neuroticism could be a positive predictor factor. None of the five personality factors could predict the oral restraint behavior of female students [94].

Over the past decade, the role of perfectionism and the behavioral system in eating disorders has been emphasized [95]. Some studies have found a connection between perfectionism and eating disorders [52,66].

Other variables

An article looked at emotional intelligence. One of the components of Emotional Intelligence is Emotional Awareness. It also plays an essential role ineffective regulation.

The results of Abbasszade's study showed that emotional intelligence and its components such as self-awareness, self-control, social awareness, social skills have a negative and significant relationship with an eating disorder [32]. Another psychological variable is perceived stress and coping styles which may play a role in predisposing or perpetuating many illnesses.

Previous studies have shown a significant relationship between stress and eating disorders, especially during adolescence [3]. One study found a significant negative relationship between perceived positive stress with anorexia nervosa and bulimia nervosa. These articles also revealed a crucial negative relationship between the problem-based coping style and an essential positive relationship between emotional and eating disorders [18,71,75,78].

The association between memory disorders and eating disorders has been studied in several studies. The distortion of verbal, visual, working, executive, and memory bias is found in eating disorders [96].

Studies have been on this issue in Iran [20,24]. One study showed that bulimia nervosa had significantly lower recognition memory performance than ordinary people. Women with bulimia nervosa perform more poorly in certain aspects of memory than normal people [97].

One of the topics covered by a limited number of articles is cognition. studies have shown that women with eating disorders have a more inadequate pattern for themselves, the world, and others [98].

The present study showed that the schema of instability, emotional deprivation, inadequate self-control, and self-discipline are different between obese people with binge eating disorder and those without this disorder [33,57].

Also, another study showed a positive, significant relationship between the overall factors of thought control and all thought control strategies (self-punishment, worry, re-evaluation, and distraction except social control) and eating disorder [27].

Mental Disorders

Eating disorders are associated with psychological disorders such as anxiety, depression, obsessive-compulsive disorder, social isolation, suicidal ideation, hypersensitivity, and guilt feelings [99].

There have been numerous studies in Iran. One study showed that anxiety among university students with and without eating disabilities was significantly different. However, the difference between depression, physical symptoms, social dysfunction, and the overall mental health score was not meaningful between the two groups [8,73].

The relationship between depression and adverse emotions associated with eating disorders has been demonstrated in other studies. However, the difference in depression, physical symptoms, social dysfunction, and overall mental health score was not significant between the two groups. There was also a substantial positive relationship between anxiety and depression and eating disorders. The relationship between depression and negative emotions with eating disorders has been proved in other studies.

One study showed a weak positive relationship between OCD and eating disorders. A significant positive relationship between anxiety and depression to eating disorders was also found. In contrast, another study found that obsessive-compulsive traits can predict 15% of changes in morbid dietary attitudes.

One study showed a weak positive relationship between obsessive-compulsive disorder and an eating disorder. There is a negative relationship between eating disorders and quality of life, which is increasingly reflected in the psychological perception of quality of life [35].

Another study showed that obsessive-compulsive traits could predict 15% of changes in morbid eating attitudes [63]. There is a negative relationship between eating disorders and quality of life, reflected more in the psychological aspect of life quality [35].

Miscellaneous variables

Smoking

Furthermore, the high link between eating disorders and impulse control behaviors, such as substance use, is one of the factors that may affect smoking among people with eating disorders [100]. Only one study looked at smoking on eating disorders in the current study [19]. That study confirmed the connection between those two issues. However, it was a minimal study [22,74].

Menstruation

Early-onset of puberty due to rapid mood changes may be associated with increased eating disorders. Few studies have looked at this question in this study. Some studies have reported that menstruation is lower in eating disorders than in average persons [22,74]. Other studies have revealed no link between these two questions [16,30,44].

Sleep

Many studies have concentrated on the relationship between sleep and diet disorders [101,102]. These studies are very limited in Iran. One study revealed that both qualities of sleep and being an eveningness and morningness are associated with eating disorders [75,83]. Only one study of twin disorders exists. This study showed no differences regarding eating disorders between the two groups [64]. While familial studies of twins have shown that anorexia nervosa, bulimia nervosa, and binge eating disorder are complex genetic diseases, and their transmission rate is 50% to 83% [28].

DISCUSSIONS AND CONCLUSIONS

Eating disorders have received attention in recent years, and it seems that further research is needed to identify the contributing factors to the development, persistence, and treatment of these disorders. The studies mentioned above reveal several notes: The prevalence of eating disorders is rising in the Iranian population and should be considered a severe health problem. Although eating disorders are considered culture-bound disorders, but the factors affecting these disorders are similar to those in other societies.

The emphasis of most studies on women, specific age groups, use of "self-report" tools " are the limitations of the most recent studies, which can affect the generalization. The prevalence of overweight is high in Iran. Association between overweight and eating disorders, it is suggested that future studies regarding epidemiological aspects. These studies can be performed with a longitudinal design, larger sample size, varied population, and clinical assessment.

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Conflict of interest

The authors declare that they have no competing interests.

Consent to participate

Informed consent was obtained from all individual participants included in the study before conducting.

Ethics approval and consent to participate

This study has been approved by the Neuroscience Research Center of Kerman University of Medical Sciences. Regarding the type of article, all licensed full text articles in the databases mentioned in the manuscript have been used.

Consent to publish

All participants consented verbally to the publication of the interview data.

Availability of data and materials

The datasets using in the study are available from the corresponding author on reasonable request.

Authors' contribution

All authors were responsible for the study. HRSH, BG were accountable for the study Conceptualization and led the writing of the paper. ZB conducted the Literature review and assisted in writing the paper. FM and AA assisted in interpreting the data and writing the paper. AA and BG assisted with the interpretation of the results and drafting programmatic Implications. SRSH and BG were responsible for data collection and coordination of the study. SD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript.

All the authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

Modeling and Forecasting the Death Trend Due to Respiratory System Diseases using the Lee-Carter model in Northern Iran

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Abstract: *Background and Aim: Respiratory system diseases (RSDs) represent the major burden of disease, including death worldwide. The main purpose of this study was to model and forecast the death rate due to diseases of the respiratory system Based on Lee Carter's model during the years 2011-2028. Methods: The type of study was a secondary analysis based on available data. The statistical population included all deaths registered in the health department of Golestan University of Medical Sciences during the years 2011-2018. Data analysis was performed using Demography 18.1 and StMoMo packages in R3.6.2 software. Results: The death rate due to RSDs in 2011 in the total population was 0.22, in women 0.19, and in men 0.25 per 1000 population. The death rate due to these diseases shows an increasing trend in 2018 to 0.33 in the total population, 0.29 in women, and 0.36 in men per 1000 population. In general, the death rate due to these diseases increased by approximately 0.10 per 1000 population from the years 2011 to 2018. It is predicted that the increasing trend of deaths due to RSDs will continue and in 2028 it will reach 4.88 in the total population, 3.56 in women, and 2.27 in men per 1000 population. Conclusions: Our findings show a significant increase in respiratory disease mortality over the past eight years. A combination of prevention and treatment strategies through urban planning, including the control of environmental pollutants and environmental health programs, is essential for the effective control of respiratory diseases.*

Keywords: *respiratory system, mortality, forecasting, trends, Iran*

INTRODUCTION

Respiratory system diseases (RSDs) represent the major burden of disease, including death worldwide [1,2]. As major public health issues worldwide, chronic respiratory diseases (CRDs), including chronic obstructive pulmonary disease (COPD), pneumoconiosis, asthma, interstitial lung disease, and pulmonary sarcoidosis, impose significant socioeconomic burdens on individuals and communities and compared to other non-communicable diseases such as cardiovascular disease, cancer, and diabetes, RSDs are severely ignored [3-5]. Therefore, RSDs are of particular importance to public health and are also a major focus of WHO monitoring and prevention strategies [6,7]. Although COVID-19 has recently focused on the respiratory system, RSDs were a major cause of death and disability before the new infection emerged. RSDs represent five of the 30 most common causes of death worldwide [8,9]. Annually, more than 9.5 million deaths are attributed to these diseases worldwide [10] and four million people die prematurely due to chronic respiratory diseases [8].

In the current context of the International System of Classification of Diseases (Tenth edition of the International Statistical Classification of Diseases and Related Health Problems: ICD-10), this group of diseases covers a wide range of acute and chronic respiratory disorders (J00-J99), including acute infections, upper respiratory tract, influenza, and pneumonia, other acute lower respiratory tract infections, other upper respiratory tract diseases, chronic lower respiratory tract diseases, pulmonary diseases caused by external factors, other lung diseases that mainly affect the interstitial tissue, pus-secreting conditions, and death tissue (necrosis) of the lower respiratory tract, other diseases of the pleura and other diseases of the respiratory system [11].

The mortality rate for lung diseases is staggering. Hundreds of millions of people are under chronic respiratory conditions; Every year, four million people die prematurely due to chronic respiratory diseases [12]. Respiratory infections are a major cause of death in developing countries. Although the 2020 Global Burden of Disease Study showed lower mortality rates from lower respiratory tract infections less than two decades ago, many are still dying. Infants and young children are especially vulnerable. Approximately 3 million children, mostly under the age of 5, died of pneumonia and lower respiratory tract infections [13]. In Iran, the mortality rate due to respiratory system diseases (MRRSDs) was 9.8% of total deaths in 2019 [14], with the level as high as 14% in the capital city Tehran. This means that 5000 people in Tehran die of RSDs every year, making it one of the most common causes of death in the city from 2008 to 2018 [15].

Mortality rates and their age pattern are indicators of development and different societies try to reach high levels of growth and excellence by reducing mortality by implementing various health and development programs. The study of mortality and the factors affecting it plays an important role in understanding the patterns of mortality and how to intervene in this matter to reduce it to researchers, planners, and policymakers. It also helps to model and identify past mortality changes in the future and to predict mortality rates, the possible effects of development programs, and their success in the field of mortality [16].

Considering that the most important risk factors for respiratory diseases include smoking, exposure to internal and external pollutants, allergens, occupational exposure, unhealthy diet, obesity, physical inactivity, and other factors [17] and to Due to rapid population growth and exposure to these risk factors, RSD is becoming a more prominent problem for all regions of the world, so more comprehensive research is needed to model and predict the death rate due to respiratory diseases throughout Iran. A deep and expert attitude is essential for the future health planning of the country. The main purpose of this study was to model and predict the mortality rate in Golestan province during the years 2011-2028.

MATERIALS AND METHODS

The type of study was a secondary analysis based on available data. The statistical population included all deaths registered in the health department of Golestan University of Medical Sciences during the years 2011-2018. Mortality information was obtained from the Statistics and Performance Analysis Unit at Golestan University of Medical Sciences in the form of an Excel file. In this file, in addition to the cause of death, information about the age and sex of the deceased is also provided.

The disease classification system is based on ICD10. Causes of death are determined and coded according to the International Classification of Diseases. Then, to qualitatively evaluate the death cause data, the data are examined and modified in terms of the impossible cause of death codes in terms of gender and age, the probable cause of death codes in terms of lethal causes, and the cause of death codes in poorly defined or absurd cases. To correct the codes of impossible causes of death in terms of age and sex and the codes of possible causes of death in terms of fatality, the framework for studying the global burden of diseases for 2010 and 2013 was used [18-20].

After qualitative correction of the data, the study population, i.e. the total number of deaths recorded during 2011-2018, was extracted and the death rates and trends were calculated. Then, the mortality rate was modeled and predicted during the years 2019-2028. The population of Golestan province in the general census of 2011 and 2016 was 1777014 and 1868819 people, respectively. It should be noted that for the denominator of the quotas, the census data of 2011 and 2016, as well as demographic estimates between the two censuses, the use of population growth rate and population information by sex and age group, and location, were used as demographic estimates. For demographic estimates in this paper, the mathematical method according to equation one was used. Where P (t + n) population in the second census, P(t) population in the first census, n is the distance between the two censuses, and r is the annual population growth rate that is the basis of the calculations (equation 1):

$$P\_(t+n\ )= P\_(t\ )\ (1+r)\ \ n$$

All steps of Lee Carter model analysis and fitting were performed using Demography 1.18 and StMoMo packages in R3/2.6 software.

RESULTS

The total number of deaths due to respiratory system diseases during the years 2011 to 2018 had an upward trend, increasing from 394 cases in 2011 to 643 cases in 2018. The death rate due to RSDs in 2011 in the total population was 0.22, in women 0.19, and in men 0.25 per 1000 population. The death rate due to these diseases, shows an increasing trend in 2018 to 0.33 in the total population, 0.29 in women, and 0.36 in men per 1000 population. In general, the death rate due to these diseases has increased by approximately 0.10 per 1000 population during the years 2011 to 2018 (Table 1).

The results of predicting the mortality trend due to RSDs in Golestan province during the years 2011-2028 in terms of age groups and in general and by sex are presented in Tables 2-4, respectively. According to the results of Tables 1, 2, and 3, it is expected that the trend of deaths due to RSDs in all age and sex groups during the years 2019 to 2028 will have an upward trend.

Table 1: Death rate (per 1000 people) due to RSDs during the years 2011-2018

Years	2011	2012	2013	2014	2015	2016	2017	2018
Total	0.22	0.12	0.19	0.22	0.21	0.28	0.30	0.33
Male	0.25	0.13	0.21	0.25	0.24	0.33	0.35	0.36
Female	0.19	0.12	0.12	0.18	0.19	0.27	0.25	0.29

Table 2: Prediction of mortality rate (per 1000 people) due to RSDs during the years 2019-2028

Number	Age groups	Years									
		2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
1	0-4	0.24	0.25	0.27	0.28	0.30	0.32	0.34	0.36	0.39	0.41
2	5-9	0.06	0.06	0.07	0.08	0.08	0.09	0.10	0.11	0.12	0.14
3	10-14	0.03	0.03	0.03	0.04	0.04	0.04	0.05	0.05	0.06	0.06
4	15-19	0.03	0.03	0.04	0.05	0.05	0.06	0.07	0.08	0.09	0.10
5	20-24	0.09	0.10	0.12	0.13	0.16	0.18	0.21	0.24	0.28	0.33
6	25-29	0.05	0.06	0.07	0.08	0.09	0.10	0.12	0.13	0.15	0.17
7	30-34	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08
8	35-39	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.08	0.08	0.08
9	40-44	0.12	0.13	0.14	0.16	0.18	0.20	0.22	0.25	0.28	0.31
10	45-49	0.15	0.17	0.19	0.22	0.25	0.29	0.33	0.38	0.43	0.49
11	50-54	0.26	0.29	0.32	0.36	0.41	0.45	0.51	0.57	0.63	0.71
12	55-59	0.51	0.56	0.61	0.67	0.73	0.80	0.87	0.95	1.04	1.14
13	60-64	0.79	0.72	0.66	0.60	0.55	0.50	0.45	0.41	0.38	0.34
14	65-69	1.77	1.99	2.24	2.51	2.83	3.18	3.57	4.02	4.52	5.08
15	70-74	3.36	3.83	4.37	4.99	5.70	6.50	7.42	8.47	9.66	11.02
16	75-79	4.75	5.52	6.42	7.47	8.69	10.10	11.74	13.64	15.84	18.39
17	>80	12.21	14.10	16.28	18.80	21.69	25.02	28.84	33.24	38.27	44.04

Table 3: Prediction of mortality rate (per 1000 people) due to RSDs in the male population during the years 2019-2028

Number	Age groups	Years									
		2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
1	0-4	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29
2	5-9	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09	0.09
3	10-14	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
4	15-19	0.04	0.05	0.06	0.07	0.09	0.11	0.13	0.15	0.18	0.21
5	20-24	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05
6	25-29	0.06	0.06	0.07	0.07	0.08	0.09	0.10	0.11	0.12	0.13
7	30-34	0.06	0.07	0.08	0.10	0.11	0.13	0.15	0.17	0.20	0.23
8	35-39	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
9	40-44	0.11	0.12	0.13	0.13	0.14	0.15	0.16	0.17	0.18	0.19
10	45-49	0.16	0.21	0.23	0.25	0.27	0.30	0.33	0.36	0.39	0.43
11	50-54	0.30	0.34	0.38	0.43	0.49	0.55	0.62	0.70	0.79	0.89
12	55-59	0.60	0.66	0.72	0.78	0.85	0.92	1.00	1.09	1.19	1.30
13	60-64	0.84	0.78	0.72	0.66	0.62	0.57	0.53	0.49	0.45	0.42
14	65-69	1.97	2.14	2.33	2.53	2.75	3.00	3.26	3.55	3.86	4.20
15	70-74	3.82	4.10	4.40	4.71	5.05	5.41	5.80	6.22	6.67	7.15
16	75-79	5.08	5.76	6.52	7.38	8.35	9.46	10.70	12.11	13.70	15.49
17	>80	11.08	12.35	13.77	15.36	17.12	19.08	21.26	23.68	26.37	29.36

Table 4: Prediction of mortality rate (per 1000 people) due to RSDs in the female population during the years 2019-2028

Number	Age groups	years									
		2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
1	0-4	0.25	0.26	0.28	0.30	0.32	0.34	0.36	0.38	0.41	0.43
2	5-9	0.04	0.04	0.05	0.06	0.07	0.07	0.09	0.10	0.11	0.13
3	10-14	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03
4	15-19	0.02	0.02	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.05
5	20-24	0.12	0.14	0.17	0.21	0.25	0.29	0.35	0.42	0.50	0.59
6	25-29	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.08	0.08
7	30-34	0.07	0.10	0.13	0.17	0.22	0.29	0.38	0.50	0.66	0.87
8	35-39	0.04	0.04	0.05	0.05	0.06	0.07	0.07	0.08	0.09	0.11
9	40-44	0.09	0.09	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.12
10	45-49	0.08	0.08	0.09	0.09	0.10	0.11	0.12	0.12	0.13	0.14
11	50-54	0.22	0.28	0.35	0.43	0.54	0.67	0.83	1.04	1.30	1.62
12	55-59	0.30	0.32	0.33	0.35	0.37	0.39	0.41	0.43	0.45	0.47
13	60-64	0.59	0.53	0.48	0.44	0.40	0.36	0.32	0.29	0.27	0.24
14	65-69	1.16	1.22	1.27	1.33	1.39	1.45	1.52	1.59	1.66	1.74
15	70-74	2.26	2.42	2.59	2.77	2.96	3.17	3.39	3.62	3.87	4.14
16	75-79	4.44	4.81	5.22	5.66	6.14	6.66	7.22	7.82	8.48	9.20
17	>80	10.07	10.78	11.54	12.35	13.23	14.16	15.16	16.22	17.36	18.58

In both men and women, the highest increase in deaths due to RSDs is in the age group over 50 years, and the age group above 80 years has the highest increase. It is predicted that in 2028, in the age group over 80 years, it will reach 44.04 in the total population, 29.36 in men, and 18.58 in women (per 1000 population).

DISCUSSION

This study aimed to model the death process due to respiratory diseases. For this purpose, the first census data and death data have been evaluated and after modeling, mortality changes between 2011 and 2018 and recognizing the death pattern, mortality rates up to 2028 have been predicted.

Based on the results of this study, during the years 2011-2018, 60082 deaths were recorded in the system, of which 3487 deaths were due to deaths due to RSD (about 6% of all deaths). In the study of Pishgar et al., during ten years (2008-2008), 519,312 people died in Tehran, of which 43,177 (about 8% of all deaths) were due to respiratory diseases, which is equal to 831.1 people in there were 10,000 deaths and 0.5 per 100,000 population [21]. The results show that the mortality rate for chronic respiratory diseases increased by 29.7% between 1980 and 2014 in the United States, which agrees with the results of this study [22]. In the study by Khademi et al, 17% of the participants reported opium use, with a mean duration of 12.7 years, and during the follow-up period, 2145 deaths were reported. The strongest associations of Opium consumption were seen with deaths from asthma, tuberculosis, and chronic obstructive pulmonary disease (11.0, 6.22, and 5.44, respectively) [23]. Perhaps this issue is one of the reasons justifying the high death rate due to respiratory system diseases in this region.

In Iran, the death rate due to RSDs was 9.8% of the total deaths and also a rate higher than 14% in Tehran in 2019 [24]. This means that 5,000 people die every year in Tehran due to RSD and this is one of the most common causes of death in this city from 2008 to 2018 [25].

The results of the study showed that the death rate due to respiratory diseases in the entire population of Golestan province during the years 2019-2028 and at all ages, has been upward. So that the death rate due to these diseases during the years 2011 to 2018 has increased by approximately 0.10 per 1000 population. This finding is related to the results of other researchers. In the probe study from 2008 to 2018, a significant increase in the trend of RSDs was observed. Also, in the study of Kermani et al. during the years 2005 to 2014 in Tehran, the total number of deaths due to SO_2 exposure in the last decade was 8836 cases and the total number of deaths due to RSDs was 1915 cases and the total number of chronic obstructive pulmonary diseases in the last decade has been 1806 [21].

The results showed that the increasing trend of deaths due to RSDs continued and in 2028 reached 4.88 in the total population, 2.27 in men, and 3.56 in women per 1000 population. In general, deaths from these diseases are higher in men than women. Which was similar to the findings of other studies [26,27]. In recent years, most studies on RSDs have attempted to examine the association between these diseases and genetic predispositions with physical activity and the economic and social status of urban environments. They also confirmed the importance of individual-level variables in the prevalence of COPD, especially comorbidities, age, sex, and smoking status [28,29].

According to the results, in both sexes, men and women, the highest increase in deaths due to RSDs will be in the age group over 50 years, and the age group over 80 years will have the highest increase. Other studies have reported the highest rate of death from RSD at older ages [30-32]. Given that the future of the world, including Iran, is a future with an increase in the elderly population, we need to accept this phenomenon in the first place, and the health system in Iran should have the potential to change its policy goals to meet the needs of the elderly population [33].

One limitation of the research is that the prediction models do not include the results of perceived changes such as medical advances in improving life expectancy, and the prevalence of some infectious diseases such as Covid-19 may interfere with the model's prediction accuracy. This study was conducted before the onset of the Covid-19 disease epidemic and according to demographic changes and mortality information that may have occurred in 2019 in Golestan province, studies to identify the main causes of death and the trend of the result are recommended during this epidemic. Information about all years without taking into account stillbirths is considered.

CONCLUSION

Our findings show a significant increase in mortality due to respiratory diseases in Golestan province during the last eight years. A combination of prevention and treatment strategies through urban planning, including the control of environmental pollutants and environmental health programs, is essential for the effective control of respiratory diseases. This should be considered by health policymakers when faced with an increased risk of death from environmental factors.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Authors' contribution

Supervision: AH; Conceptualization: AH, MJK and ZK; Methodology: ZK; Project administration: AH; Data curation: MG, NJ, and MRH; Formal analysis: ZK; Writing - original draft: MJK, AH, ZK, NJ, MRH, MG; Writing - review and editing: ZK and AH.

Ethics approval and consent to participate

Ethical approval for this study was obtained from Iran National Committee for Ethics in Biomedical Research (IR.GOUMS.REC.1399.270).

Patient consent for publication

Verbal informed and Written informed consent were not obtained because recorded and library data were used.

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REVIEW

Medial Pivot Knee in Total Knee Arthroplasty

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Abstract: *In the context in which life expectancy increases and the population becomes more active, the number of people who are affected by gonarthrosis symptoms increases proportionally. By the year 2030, in the United States of America, one in three adults is expected to suffer from gonarthrosis, this prediction will be the beginning of an epidemic. Total knee arthroplasty has been shown to relieve pain and improve joint function; however, studies have shown that active young patients still have limitations in performing high-level activities such as dancing, golfing, skiing, and gardening. Currently, modern TKA implants are designed to reproduce the normal biomechanics of the knee joint, mimicking the physiological "medial pivot" pattern with greater compliance on the medial compartment between the tibial insert and femoral condyle and less congruence on the lateral side.*

Keywords: *total knee arthroplasty, medial pivot, biomechanics*

INTRODUCTION

In the context in which life expectancy increases and the population becomes more active, the number of people who are affected by gonarthrosis symptoms increases proportionally. By the year 2030, in the United States of America, one in three adults is expected to suffer from gonarthrosis, this prediction will be the beginning of an epidemic [1].

Total knee arthroplasty (TKA) should provide functional movement and stability for all desired daily activities. It should also reproduce normal knee kinematics and function [2,3,4].

Total knee arthroplasty is a successful intervention, which aims to reduce pain and restore knee function [5,6]. Numerous studies have shown that the satisfaction rate of patients with total knee arthroplasty is between 80% and 95%, and the survival rate of implants 10 years after the surgery is approximately 90%. However, major malfunctions after total knee arthroplasty occur in 15-20% of patients, often requiring revision surgery [7].

Currently, modern TKA implants are designed to reproduce the normal biomechanics of the knee joint, mimicking the physiological "medial pivot" pattern with greater compliance on the medial compartment between the tibial insert and femoral condyle and less congruence on the lateral side [2,4,5,8].

The implants in total knee arthroplasty can be cemented or not. Cemented fixation is the gold standard in TKA. In medical applications, the cement is represented by Polymethyl-methacrylate (PMMA). This is an acrylic polymer formed by mixing two sterile components: a liquid methacrylate (MMA) monomer and a powered MMA-styrene copolymer. Heat is generated during the process due to the exothermic reaction. [9,10].

Numerous studies investigating knee kinematics using magnetic resonance imaging have confirmed a physiological medial pivot pattern in the normal knee [11].

EVOLUTION OF TKA

Swedish surgeon Börje Walldius, MD, designed the first TKA implant in 1950. It was a system that sacrificed the collateral and cruciate ligaments for implantation and it functioned in a single plane of motion. More sophisticated designs of total knee systems have been developed since 1970 [12].

Over the past few years, the total knee system has undergone significant changes.

The medial-pivot system was developed to produce normal knee kinematics and better patient satisfaction than traditional prostheses. The MP prosthesis includes a ball and socket articulation on the medial side with more significant restriction and relatively unconstrained mobility on the lateral side. The asymmetric polyethylene insert is designed to allow normal function and motion by replicating the native structure of the knee [13]. The system avoids paradoxical anterior movement and may bring about superior patient satisfaction [14].

In the following, we will present arguments for and against the role of the medially stabilized knee

THE ROLE OF THE MEDIALLY STABILIZED KNEE: AN EMERGING SOLUTION FOR TKA – PRO ARGUMENTS

Total knee arthroplasty has been shown to relieve pain and improve joint function; however, studies have shown that active young patients still have limitations in performing high-level activities such as dancing, golfing, skiing, and gardening. Functional outcomes are compromised in approximately half of TKA patients. This loss may be caused by the abnormal kinematics and inherent instability of many contemporary implants.

In the anatomical knee, the femur externally rotates and rolls backward over the tibial plateau during mid-flexion. In full flexion, there is more translation of the lateral femoral condyle. In contrast, the conventional total knee begins in an unrotated and posteriorly subluxated position because the ligament insertion points are located posteriorly. Paradoxical anterior slip and insufficient axial rotation usually characterize femoral movement after conventional TKA. This instability and malrotation significantly reduce the effectiveness of the muscles involved in the extension and flexion of the knee, which negatively modifies the kinematics and joint movement patterns of the anatomical knee [15].

Medially stabilized knee prostheses have an adequate medial articular surface, which promotes natural rotational motion and increases anteroposterior implant stability [16]. There are several approaches to medially stabilized knee implants, which retain, sacrifice, or replace the posterior cruciate ligament. The classic design of medially stabilized prostheses sacrifices the posterior cruciate ligament and has increased medial joint stability combined with a less stable lateral joint [17]. In this design, the function of the posterior cruciate ligament is replaced by the high anterior edge of the polyethylene insert.

Some newer generation medially stabilized models have the same degree of stability in the medial joint or even less and allow for both sacrificial and retention of the cruciate. Another medial-stabilized design, bicruciate stabilized, incorporates a compliant medial joint and an anatomically shaped but less contented lateral compartment to promote rotation.

Restoring stability and rotation/kinematics as close as possible to normal is thought to improve functional outcomes and patient satisfaction [18]. In vivo fluoroscopic analysis revealed improved kinematics with medially stabilized designs [19]. Furthermore, improvements in clinical outcomes and patient satisfaction have been reported for these designs [3, 20, 21].

In conclusion, these medially stabilized TKA models restore anatomical rotational motion and have favorable outcomes (Figure 1, Figure 2).

THE ROLE OF THE MEDIALLY STABILIZED KNEE: AN EMERGING SOLUTION FOR TKA – CONTRA ARGUMENTS

The medial pivot (medially stabilized) knee prosthesis is designed to reproduce the kinematics of the knee, reduce polyethylene wear, and improve the functional outcome.

A prospective randomized study on a group of patients who received bilateral knee arthroplasty in the same surgical procedure and in whom a total knee prosthesis with a medial pivot (Advance, Wright Medical) was implanted in one knee and the other knee a total prosthesis with a movable plate, with preservation of the posterior cruciate ligament (PFC Sigma, DePuy Synthes) [22]. One hundred eighty-two (182) patients were enrolled, with no differences in preoperative demographics, range of motion, or knee scores. In the post-operative follow-up after a minimum of 11 years, medially pivoted knees had significantly worse results, with lower Knee Society Score scores (95 vs. 90 points), lower WOMAC index score (18 vs. 25 points), and a reduced mobility degree (117 degrees vs. 128 degrees). Patients were more satisfied with the PFC Sigma (93%) than the medial pivot (75%).

There was no difference in survival. The authors of this study hypothesized that excessive constraint imposed by the medial tibiofemoral joint may not restore normal knee kinematics and may inhibit lateral condyle translation during flexion.

Warth et al. studied a group of 141 TKA patients implanted with the same type of prosthesis (Triathlon, Stryker) not specifically classified as a medial pivot design and used an intraoperative sensor (VERASENSE, Orthosensor) to classify the kinematics pattern in terms of the type of rotation meaning with medial pivot or without medial pivot [23]. Forty percent (40%) of the knees were classified as having a medial pivot pattern during flexion-extension motions. There were no differences between the two groups regarding the Knee Society Score or the UCLA score.

Figure 1: Preoperative x-rays (orthostatic)

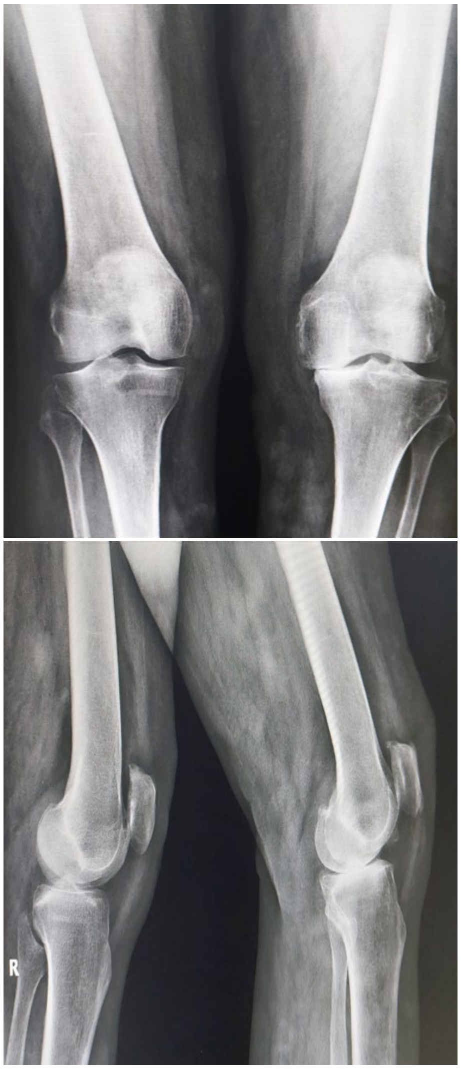

In another prospective randomized study, Benjamin (BJJ, 2018) compared a medial pivot TKA with a conventionally designed TKA and there were no differences related to the Knee Society Score, Oxford Knee scores, or the gait analysis [24]. Also, another prospective, randomized study by Nishitani et al. (Knee, Dec 2018) compared TKA with a medial pivot to TKA with a conventional tibial insert and found no differences related to the degree of mobility or Knee Society Scores [25]. Finally, a comparative analysis that included 153 medially pivoted TKAs (Advance, Wright Medical) and the same number of posteriorly stabilized TKAs (DePuy Synthes) revealed no difference for Knee Society Scores, WOMAC score, or degree of mobility between groups [26].

Another widely used type of TKA popular worldwide is the posterior stabilized (PS) implant. The PS knee has a cam and post mechanism that induces femoral roll-back, which has been shown in multiple studies to provide improved motion compared to a cross-retaining TKA. By preserving the translational movement of the femur on the tibia, the chance of impingement between the posterior part of the femur and the polyethylene insert is reduced.

Figure 2: X-rays after TKA with primary medial-pivot knee system

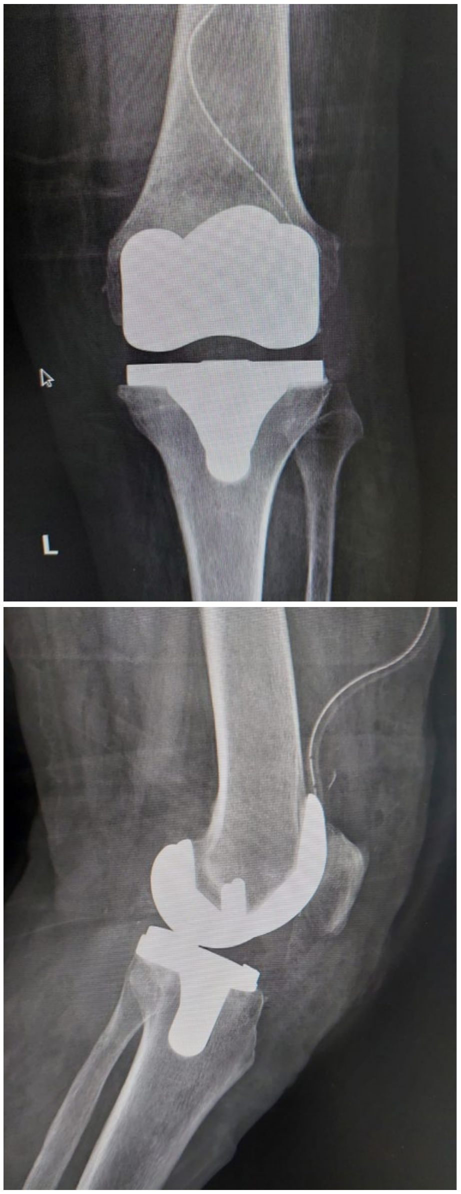

The cam and post mechanism induces posterior sliding and is closer to native knee kinematics compared to the cruciate ligament-preserving knee, which may be subject to "paradoxical motion," the forward sliding of the femur on the tibia during knee flexion.

Disadvantages of the posterior-stabilized knee include the presence of sounds produced during flex-ion/extension movements and patellar crackles, inter-condylar fracture of the femur, early decimation, and significant bone loss due to specific cutting blocks. The spaces resulting from the bone cuts, in flexion or extension, must be well balanced to avoid the appearance of an enlarged flexion space, which can lead to loss of contact between the posterior cam and the pin of the tibial plateau.

With improved implant design and materials, each of these

complications have been reduced, but orthopedists need to recognize the occurrence of these complications to identify them when they occur so that the incidence can decrease. Improvements in trochlear biomechanics have significantly reduced patellofemoral complications, a common problem for patients with posterior-stabilized implants.

CONCLUSIONS

Preserving knee biomechanics after total arthroplasty could be the solution to improve functional outcomes, increase satisfaction rates and decrease complication rates. This can be done through a series of intraoperative maneuvers, by using some implants designed for this function.

The benefits of medial pivot implant total knee arthroplasty are high component compliance, anteroposterior stability, and a large contact surface with minimal contact stress.

The existing literature has provided evidence supporting a better clinical effect, anatomic rotational movement with favorable outcomes, and a lower complication rate of the medial pivot knee prosthesis compared to the posterior-stabilized knee prosthesis. The obtained results provide a reference for doctors when choosing a certain type of prosthesis.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

The Relationship between Clinical Competence and Ethical Reasoning and Factors Affecting it in Nurses Working in Therapeutic Educational Centers of Ilam

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Abstract: *Clinical competence means a person's ability to use techniques on time, to communicate effectively with others, and to benefit from the knowledge and clinical reasoning ability when responding to different clinical situations in such a way that a person can use these skills at all times. The aim of the study is the relationship between clinical competence and ethical reasoning and the factors affecting it in nurses working in therapeutic educational centers of Ilam. Methods: In this descriptive-analytical study, 110 nurses working in three therapeutic educational centers in Ilam city were randomly selected. Demographic information forms, standard nursing clinical competence questionnaire (NCCQ), and nursing dilemma test (NDT) were used to collect data. The results also showed a positive and significant relationship between age, level of education, years of work experience, employment status, and occupational role with clinical competence and ethical reasoning ($P<0.05$). On the other hand, there was an inverse and significant relationship between the workplace and clinical competence and ethical reasoning ($P<0.05$). Data analysis was carried out using Descriptive statistics (frequency, percentage, mean and standard deviation) and analytical tests (Pearson correlation tests and analysis of variance) in SPSS. P-value< 0.05 was considered as the significance level. Using the results of the present study, nursing education planners and managers can periodically organize workshops on nursing ethics and nursing problems in hospitals and universities. Moreover, clinical competence and ethical reasoning should be taken into account when training nursing personnel in university classrooms and in-service courses.*

Keywords: *moral reasoning, clinical competence, nurses, therapeutic training center*

INTRODUCTION

Nurses provide at least 50% of healthcare services, and this figure even reaches 80% in some countries [1]. Nursing is now moving towards professionalization, and one of the most important pillars of the nursing profession is ethical issues that must be taken into consideration [2]. The ultimate and main goal of the nursing profession is to ensure the recovery of humans, and this can only be achieved through scientific and ethical care [3]. The nursing practice has a human and ethical nature and adherence to ethical principles has a great impact on improving the quality of nursing care [4]. Factors such as the advancement of medical technology, increasing treatment costs, attention to the individual rights of people, and changes in the roles of nurses can lead to ethical conflicts that require nurses to recognize problems and make appropriate ethical reasoning [5]. Ethical reasoning is the ability of a person to choose a solution from among several solutions to an ethical dilemma and to provide appropriate reasons according to his/her knowledge and conscience [6]. Lack of ethical reasoning can lead to wrong decisions in complex clinical situations and, subsequent inappropriate action, which in turn affect the patient, the patient's family, and even the nurse [7].

From the management point of view, one of the effective factors in guaranteeing quality-of-care services and obtaining patient satisfaction is clinical competence [8]. Clinical competence means a person's ability to use techniques promptly, to communicate effectively with others, and to benefit from the knowledge and clinical reasoning ability when responding to different clinical situations in such a way that a person can use these skills at all times [9]. The British Nursing

Association defines competence as a set of knowledge, skills, and attitudes required to work safely and effectively without a direct supervisor [10]. Several factors such as the rapid change in the health monitoring system, the need to provide safe and cost-effective services, increasing public awareness in health-related areas, and increasing the expectation of receiving services of appropriate quality, along with the willingness of healthcare organizations to employ skilled personnel, have led researchers to pay more attention to clinical competence [11]. Since the clinical competence of nurses is considered the main concern of healthcare provider systems and their main focus, it is one of the areas that need improvement [10]. Therefore, considering the importance of knowing the ethical reasoning status of nurses and the role of clinical competence in providing high-quality nursing care, the relationship between clinical competence and the ethical reasoning of nurses was investigated in the present study.

MATERIALS AND METHODS

In this descriptive-analytical study, 110 nurses working in three therapeutic educational centers of Ilam city (Imam Khomeini, Shahid Mostafa Khomeini, and Ayatollah Taleghani) were randomly selected. Inclusion criteria included informed consent to participate in the research, a bachelor's degree in nursing, no transfer or acceptance from practical nursing and other medical sciences, and having at least one year of clinical work experience. Exclusion criteria included unwillingness to continue cooperation in the study and incomplete or incorrect completion of questionnaires. Demographic information forms, standard nursing clinical competence questionnaire (NCCQ), and nursing dilemma test (NDT) were used to collect data. The demographic information form included questions on age, gender, marital status, level of education, work experience, employment status, workplace, and occupational role.

The standard nursing clinical competence questionnaire (NCCQ) (Liu et al., 2007) consists of 55 questions in seven dimensions of clinical care, leadership, interpersonal relationships, ethical/legal performance, professional development, willingness to research, and critical thinking. NCCQ is scored based on a five-point Likert scale ranging from 0 to 4. Scores less than 110, 110-165, and 165-220 indicate low, moderate, and high competence, respectively [12]. The reliability of this questionnaire was confirmed in Ghasemi et al.'s study ($\alpha = 0.97$) [13] and the present study ($\alpha = 0.98$).

The nursing dilemma test (NDT) was developed by Cherisham (1981) [14]. This questionnaire was developed based on Kohlberg's theory of moral development and contains 6 scenarios about the problems of nursing care of the client, as follows: a baby with an organ defect, wrong prescription, medication error, a patient's request to end his/her life, a new nurse entering practice and end of life care. Following each scenario, two questions are asked. The first question asks the person to choose what action he would take if he were in this scenario. The options for this question are Yes, No, and Can't decide the format. The answers given to this part are mostly dependent on the religious and cultural background of the nurses, and whatever option is chosen does not affect the level of their ethical reasoning. The second question consists of six options in which the nurse's ethical reasoning is measured. This part of the questionnaire is designed based on stages 1 to 6 of Kohlberg's ethical development. In other words, two options for this question show the nurse's ethical reasoning. If the respondent chooses these two options in his/her priorities, (s)he will obtain a score of 6 in one item and 5 in the other. To determine the ethical reasoning score, the scores of stages 5 and 6 of Kohlberg's moral development (codes 5 and 6) are added together. Therefore, the highest and the lowest scores in each scenario are 11 and 3, respectively, and the total possible score range of six scenarios is 18 and 66 [15]. This questionnaire was translated by Zirak et al. (2009). It was then reviewed by 10 faculty members of Tabriz University of Medical Sciences in terms of content validity and the necessary changes were made to the questionnaire. Its reliability was calculated by the test-retest method and the correlation coefficient was 0.95, and its match with the culture of Iranian society was also confirmed [16]. In the present research, its reliability was calculated $\alpha=0.97$ using Cronbach's alpha method.

Data analysis was carried out using Descriptive statistics (frequency, percentage, mean and standard deviation) and analytical tests (Pearson correlation tests and analysis of variance) in SPSS. P-value <0.05 was considered as the significance level.

RESULTS

Out of a total of 110 samples, the majority were women (54.5%), had a bachelor's degree in nursing (87.3%), were permanent nurses (61.8%), and had 7.17 years of work experience on average. The number of single and married people was equal. The mean ± SD of clinical competence and ethical reasoning scores in nurses was 136.65 ± 46.08 and 36.51 ± 16.17, respectively (Table 1). Results showed low, moderate, and high clinical competence in 46.4%, 11.8%, and 41.8% of cases, respectively.

Figure 1 shows a significant correlation between clinical competence and ethical reasoning of nurses ($p<0.001$, $r=0.802$). In other words, ethical reasoning in nurses has increased with increasing clinical competence scores.

Table 1: The demographic characteristics of the nurses (average score and standard deviation of nurses' clinical competence and moral reasoning)

Variable	SD	Mean	Maximum score	Minimum score
clinical competence	46/084	136/65	210	75
moral reasoning	16/178	36/51	60	19
level of clinical competence	%	N	Average score	
low clinical competence	46/4	51	1-110	
moderate clinical competence	11/8	13	110-165	
high clinical competence	41/8	46	165-220	

Table 2: Relationship between nurses' personal-occupational characteristics with clinical competence and moral reasoning

Variable	Clinical competence		Moral reasoning	
	P-Value	Coefficient correlation	P-Value	Coefficient correlation
age	<0/001	0/42	<0/001	**0/45**
gender	0/98	0/002	0/38	**0/08**
marital status	0/89	-0/01	0/47	**0/06**
level of education	<0/001	0/39	<0/001	**0/38**
work experience	<0/001	0/44	<0/001	**0/47**
employment status	<0/001	0/38	0/01	**0/24**
workplace section	0/04	-0/19	<0/001	**-0/34**
work role	<0/001	0/34	<0/001	**0/37**

The results also showed a positive and significant relationship between age, level of education, years of work experience, employment status, and occupational role with clinical competence and ethical reasoning (Table 2) (P<0.05). On the other hand, there was an inverse and significant relationship between the workplace and clinical competence and ethical reasoning (P<0.05).

Figure 1: The relationship between clinical competence and ethical reasoning

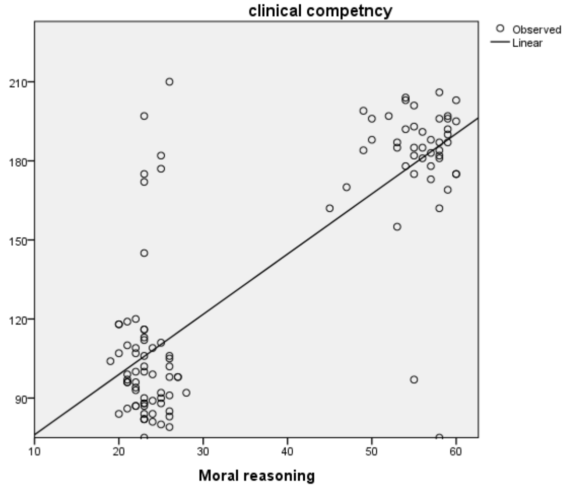

DISCUSSION

The studied nurses had low and high clinical competence in 46.4% and 41.8% of cases, respectively. The results of a study by Faraji et al. (2019) indicated an excellent and good level of clinical competence among nurses, which is not consistent with the results of the present study [17]. The high clinical competence score of nurses can be related to many factors such as holding regular training courses and periodical clinical competence assessments. It is possible to provide appropriate conditions for improving the clinical competence of nurses with low clinical competence scores by holding training courses [17]. The results of the previous relevant foreign studies also indicated a good and excellent level of clinical competence among nurses working in different hospital wards [18,19]. A high level of clinical competence among nurses can be expected considering the expertise and ability of nurses based on the importance of the issue of clinical competence and recruitment of nurses based on strict clinical criteria in recent years.

The studied nurses had moderate ethical reasoning. According to the results of the present study and the definition of ethical reasoning in nurses, it can be stated that the nurses of the present study can choose one solution from among several solutions to an ethical dilemma in nursing to some extent, and performed moderately when presenting appropriate reasons according to their knowledge and conscience. Since nurses have the most interaction and closeness with patients compared to other medical personnel, they need more ethical reasoning knowledge [20]. Ethical dilemmas are one of the most important causes of distress and discomfort in clinical environments; therefore, they need proper treatment [21]. Since ethical reasoning is one of the most important criteria for achieving an ethical performance [22]. The training of nurses in this field can increase the readiness of nurses to deal with ethical issues and dilemmas in their professional role and thus can improve their performance in ethical decision-making.

In a study of the ethical reasoning status of nurses, Behbodhi et al. (2018) showed that the ethical reasoning of nurses was at a low level in 75% of nurses [23], which is not consistent with the results of the present study. This discrepancy may be due to the use of different questionnaires, as well as factors such as cultural diversity and differences in the values and opinions of nurses in different parts of Iran. It is stated that factors such as; the views and experiences of other nurses, physicians, and nurses' families can also affect nurses' ethical reasoning ability, which warrants further research on the factors related to ethical reasoning in nurses.

The results of another study on 360 nurses working in different wards of educational hospitals affiliated with Shahid Sadougi University of Medical Sciences in Yazd, showed that these nurses had average ethical reasoning ability in the face of ethical dilemmas [24], which is consistent with the results of the present study, which may be due to use of a similar questionnaire to evaluate the ethical dilemma. It can be stated that it is necessary to plan and apply appropriate strategies to improve the reasoning ability of nurses working in hospitals in the face of ethical dilemmas.

The results of some foreign studies, including a study in the United States, showed the high ethical reasoning of nurses [25], which is not consistent with the results of the present study. The nurses in this study mostly attributed their high level of reasoning to organizational factors related to ethical dilemmas, the most important of which was effective physician-nurse communication.

In the present study, the ethical reasoning ability increased with increasing clinical competence scores. According to the results of the present study, clinical competence played an important role in the ethical reasoning ability of nurses. According to the concepts and theories of the field of ethics, the ethical reasoning ability is not a stable issue because the type of ethical distress depends on whether the person does the ethically right thing or not or whether the person refrains from doing an action, which is prohibited from fulfilling that obligation, in which case ethical distress occurs [26]. If the main cause of this distress is eliminated, this stress and psychological distress will certainly decrease, which will lead to an improvement in the ethical reasoning ability of nurses. The studied nurses had the necessary ethical reasoning ability considering their average clinical competence level.

The results of the Escolar-Chua et al. study showed nurses with poor ethical reasoning had poorer patient decision-making for ICU patients [27]. According to these nurses, the critical and bad conditions of the ICU patients had caused an ethical challenge when providing care to this group of patients, the consequence of which was the increase in the ethical distress of the nurses. Moreover, these persistent challenges in turn reduced the ethical reasoning ability of the nurses. In the present study, older age, higher education level and work experience, permanent employment, being a nurse, and working in ICUs ensured a higher mean clinical competence score, which was inconsistent with the results of the study by Faraji et al. (2019) [17]. The discrepancy between the results of these two studies is due to the personal-occupational characteristics of nurses and the research environment. The difference in clinical environments, the distinct care needs of patients, and the difference in the types of clinical experiences of nurses lead to a different understanding and a different view of the nurse's clinical competence. The studies by Fotoohi et al. (2019) [28] and Salehi et al. (2015) [29] showed that female nurses are more sensitive in reasoning and clinical decision-making. Considering, there are more sensitive and precise ethical e in patient care than men, women recognize and understand challenging care situations. The results of the study by Adib Haj. Bagheri et al. and Kim & Choi (2019) showed that the average clinical competence of nurses at the age of 30 and above was higher than other age groups [18,30]. Clinical experiences and repeated experiences with increasing age led to an improvement in the level of patient care. Nurses provide high-quality care services by using their experiences and are involved in the challenges of clinical and ethical decision-making less frequently [31,32]. The results of studies by Mirlashari and Loui et al. (2020) were consistent with the results of the present study and showed that nurses with higher educational levels have more clinical competence [33, 34]. In postgraduate education, nurses receive further training to achieve a better understanding of patient care, which can be effective. The results of several other studies, like the results of the present study, showed the relationship between work experience and the clinical competence of nurses [18,35]. Nurses with higher work experience have higher-level clinical experience and are more sensitive to patient care. Nurses' sensitivity to patient care increases by improving their job skills and awareness of their supportive and effective role in this process [32].

The results of the present study showed that the ethical reasoning ability was better in nurses with higher age, MA education, more work experience, permanent employment, and nurses working in ICU, specialized, and general wards. Also, ICU nurses reported higher ethical reasoning. The lack of library-focused and research skills, and mistrust in the accuracy of online sources are among the factors contributing to the inappropriate use of information sources to improve the ethical reasoning ability of nurses with a bachelor's degree, which in turn causes a decrease in the use of scientific resources and evidence-based articles for making clinical decisions under challenging patient care situations [36]. While MA nurses have a higher ethical reasoning ability by learning how to use valid and updated scientific resources for clinical decision-making under ethical dilemmas.

With increasing years of work experience and experiencing more clinical ethical distress and ethical dilemma, nurses will be abler to make independent ethical decisions and to identify facilitating and prohibiting factors for clinical decision-making, which happens with increasing age. These experiences provide appropriate conditions for promoting ethical decision-making and thus, improving the ethical-reasoning ability of nurses [37].

Inconsistent with the results of the current study, Amoroso et al. (2019) reported no statistically significant relationship between the demographic indicators of American ICU nurses (age, gender, years of clinical experience, and employment status) with ethical reasoning [38]. Also, consistent with the present study, the results of the study by Mousavi Nesab et al. indicated no significant relationship between years of work experience, level of education, age, and gender of nurses working in different hospital wards with the ethical reasoning ability [39]. It seems that the difference between the results of the present study and the aforementioned studies is mainly due to the difference between the individual-social variables of the studied populations.

According to the results of the present study and other studies, various internal or external factors affect the nurses' ethical reasoning ability in different clinical wards. Internal factors include variables such as mental and physical condition, personal characteristics, values, past experiences, inclinations, knowledge, and attitudes of nurses. External factors also include environmental conditions and time [37]. Since correct and timely ethical decisions have facilitated and increased the correct use of human resources, materials, and equipment and improved the quality of care, therefore, it is necessary to take all measures and interventions to improve the ethical-reasoning ability of nurses, taking into account the afore-mentioned internal and external factors to guarantee the highest level of efficiency in this field.

CONCLUSIONS

The results of the present study showed that half of the nurses had poor clinical competence and the majority of the studied nurses had moderate clinical reasoning. There was a significant relationship between ethical reasoning and nurses' clinical competence. Also, the individual factors of age and education level, occupational factors of clinical work experience, employment status, and occupational role of nurses were effective on the clinical competence and ethical reasoning of nurses. Using the results of the present study, nursing education planners and managers can periodically organize workshops on nursing ethics and nursing problems in hospitals and universities. Moreover, clinical competence and ethical reasoning should be taken into account when training nursing personnel in university classrooms and in-service courses.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgments

Ilam University of Medical Sciences

Ethics approval and consent to participate

IR-MEDILAM-REC-1400.123

Informed written consent

was obtained from all participants included in the study. All participants were informed about the purpose of the study and assured of confidentiality and anonymity. The respondents were informed of their freedom to withdraw from the study at any time.

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EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

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– Identifies trials with a unique number; and

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The journal uses US spelling and authors should, therefore, follow the latest edition of the Merriam-Webster’s Collegiate Dictionary.

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Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors’ industrial links and affiliations. In the case of clinical trials or an article describing the use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

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Original articles must have a structured abstract that states in 200 words or less the purpose, basic procedures, main findings, and principal conclusions of the study. If appropriate, divide the abstract with the headings: Background and Aim, Methods, Results, and Conclusions. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to ten keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine’s Medical Subject Headings (MeSH) browser – http://www.nlm.nih.gov/mesh/meshhhome.html.

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Use Index Medicus as the style guide for references and other journal abbreviations.

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REVIEW

The Amazing History of Methotrexate – 75 Years Later from its Discovery, Still the “Golden Standard” Therapy

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Abstract: *More than 75 years after its accidental discovery, methotrexate remains an important treatment option for many diseases. Whether it is various hematological and non-hematological neoplasms, rheumatological, dermatological, or other conditions, methotrexate remains in scope. For rheumatoid arthritis, current clinical guidelines (EULAR, ACR) recommend methotrexate as the first therapeutic option. This article aims to highlight important moments in the history of this remarkable drug, to review the literature on its mechanisms of action and the arguments for which after more than half a century, methotrexate remains the gold standard in the treatment of rheumatoid arthritis but also an important option for treatment of psoriasis vulgaris (PV) and psoriatic arthritis (PsA).*

Keywords: *methotrexate; treatment; rheumatoid arthritis; psoriasis; history*

THE HISTORY OF METHOTREXATE

Most people link methotrexate (MTX) history with researcher names like Farber or Gubner. The history of methotrexate begins a few years earlier, in 1945 when American researcher of Indian origin, Subbarow (forgotten or too little known) first isolated folic acid from the liver, in the laboratory of the pharmaceutical company that would later become Lederlee [1].

In the same year, the researcher and his team also discovered a microbial source from which they subsequently synthesized folic acid. Administration of "this preparation of folic acid" to laboratory mice with tumors was followed by tumor regression. This finding was opposite to the hypothesis that deprivation of tumor cells of folic acid leads to suppression of tumor growth. But at the time, they did not know that what they had injected into the mice was in effect a slightly different compound – a folic acid antagonist that led to the deprivation of folic acid in tumor cells, disrupting their DNA synthesis and causing tumor regression.

Encouraged by the laboratory results, Farber fast organizes a clinical study for patients with leukemia, to whom he administers... folic acid. As you can imagine, the results were devastating due to accelerated tumor growth. But this accidentally wrong lead, helped Farber confirm the hypothesis that folic acid deprivation of tumor cells leads to the suppression of their growth.

Farber returns to Subbarow and emboldens him to continue his research and Subbarrow ended up synthesizing two new molecules, aminopterin, and amethopterin, which competitively inhibit folic acid synthesis.

Farber successfully treats acute leukemia in children with these two molecules and publishes the results in his 1948 paper [2].

Aminopterin molecule proves to be quite unstable and difficult to synthesize. Therefore, an amethopterin analog becomes the star that is later called METHOTREXATE. Concerned about the potential side effects, Farber considered it wise to add, in the name of the substance, to met = metabolism and an "x" that was usually found, at the time, on poison bottles [1].

The first report of the use of methotrexate in rheumatoid arthritis appeared in 1951 when Gubner (who was a cardiologist) et al. found that 86% of rheumatoid arthritis patients treated with methotrexate and receiving a clinical response, relapsed after its discontinuation.

Gubner and colleagues have shown that aminopterin mimics the actions of cortisone with the effect of an anti-metabolite of folic acid and with anti-inflammatory and anti-proliferative responses. This was considered to be the likely explanation for the beneficial effects of these two drugs in a spectrum of unrelated clinical and experimental conditions in which the common denominator was an inflammatory and proliferative connective tissue response [3].

At this point, it should be said that Gubner's 1951 paper on the use of methotrexate in rheumatoid arthritis was received at that time with the question "Why use such a toxic anti-cancer drug to treat a benign condition such as rheumatoid arthritis?" The reason behind this statement was that despite the fact they were using low doses of methotrexate for the treatment of rheumatoid arthritis and psoriasis, the frequency of administration was daily or several times a week, which increased MTX toxicity [1].

In 1973, a group of Scandinavian dermatologists described several major side effects of methotrexate use, including liver cirrhosis in patients diagnosed with psoriasis receiving methotrexate several times a week- without pointing out that those patients had unrestricted access to alcohol and were previously exposed to arsenic (which was still being used to treat psoriasis at the time).

Despite all of these, methotrexate received FDA approval for psoriasis in 1972 and rheumatoid arthritis in 1988.

MECHANISMS OF ACTION

Methotrexate (MTX, amethopterin, Figure 1) is a synthetic organic compound, part of an anti-folate therapeutic group.

Figure 1: Adapted from Koźmiński P, Halik PK, Chesori R, Gniazdowska E. (with permission) [5]

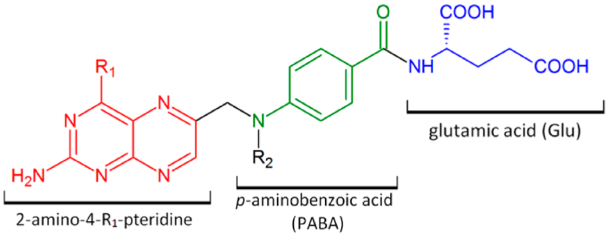

Folic acid	R_1= OH	R_2= H
Aminopterin	R_1= NH_2	R_2= H
Methotrexate	R_1= NH_2	R_2= CH_3

Methotrexate mechanisms of action are not yet completely understood after all these years. It is clear now that given in low doses (mg) methotrexate is used for the treatment of autoimmune and inflammatory diseases (such as rheumatoid arthritis, psoriasis, myasthenia gravis, Crohn's disease, multiple sclerosis, polyarticular juvenile idiopathic arthritis) [4]. On the other hand, high doses of methotrexate (up to grams) are commonly used for the treatment of various neoplasms (acute lymphoblastic leukemia, acute myeloid leukemia, meningeal leukemia and lymphoma, osteosarcomas, non-Hodgkin's lymphoma, also breast, bladder, and several other cancers).

Screening the available literature, we chose an overview published in 2020 by Koźmiński P. et al [5] explaining the dual action of methotrexate (MTX). According to this paper, MTX indirectly inhibits cell division through the blockage of folate-related enzymes, mainly DHFR, that catalyzes the conversion of dihydrofolate to tetrahydrofolate (THF). THF serves as a significant coenzyme in several transmethylation reactions in pyrimidine and purine nucleotide synthesis pathways, essential in the synthesis, repair, or replication of DNA strands [6,7]. The methyl-THF acts as a proximal methyl donor in numerous methylation reactions of DNAs, RNAs, proteins, phospholipids, and amino acids syntheses. Inhibition of intracellular THF production by MTX results in the disruption of cell proliferation and its metabolic imbalance.

MTX crosses the biological barriers very poorly, being highly ionized and generally hydrophilic. The bioavailability and biodistribution of the drug are determined by an active transport system. Intestinal tissue adsorption of MTX occurs by the proton-coupled folate transporters (PCFTs), which are a solute carrier transporter, while a cellular drug penetration is followed mainly by the reduced folate carrier 1 (RFC1), an APT-binding cassette transporter. To a small extent, MTX also uses receptor-mediated endocytosis via folate receptors (FRs), the glycosyl-phosphatidyl-inositol (GPI)-anchored membrane proteins that may internalize bound folates and folate conjugates [8,9]. Intracellularly, MTX is metabolized by folylpolyglutamyl synthase (FPGS) to polyglutamate derivatives (MTXGlu), which show significantly increased cell residence time and bioactivity in comparison to initial MTX form [10,11,12] (Figure 2). This is a key pharmacokinetic step that determines the attributed effect of this drug, defining MTX as a representative type 1 prodrug, that undergoes bioactivation inside the cell. Polyglutamated MTX is a superior anti-folate agent to MTX, capable of highly potent DHFR inhibition. Moreover, it also induces inhibition of other enzymes like thymidylate synthase (TYMS), 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICART) [13, 14], and amido-phosphoribosyltransferase [15,16], participating in de novo biosynthesis of purine and pyrimidine nucleotides. Consequently, it is MTXGlu that deprives a cell of precursors for the synthesis of DNA and RNA necessary for cell proliferation (Figure 2), leading to DNA synthesis disturbances and subsequent cell apoptosis.

Figure 2: Adapted from Koźmiński P, Halik PK, Chesori R, Gniazdowska E. (with permission) [5]

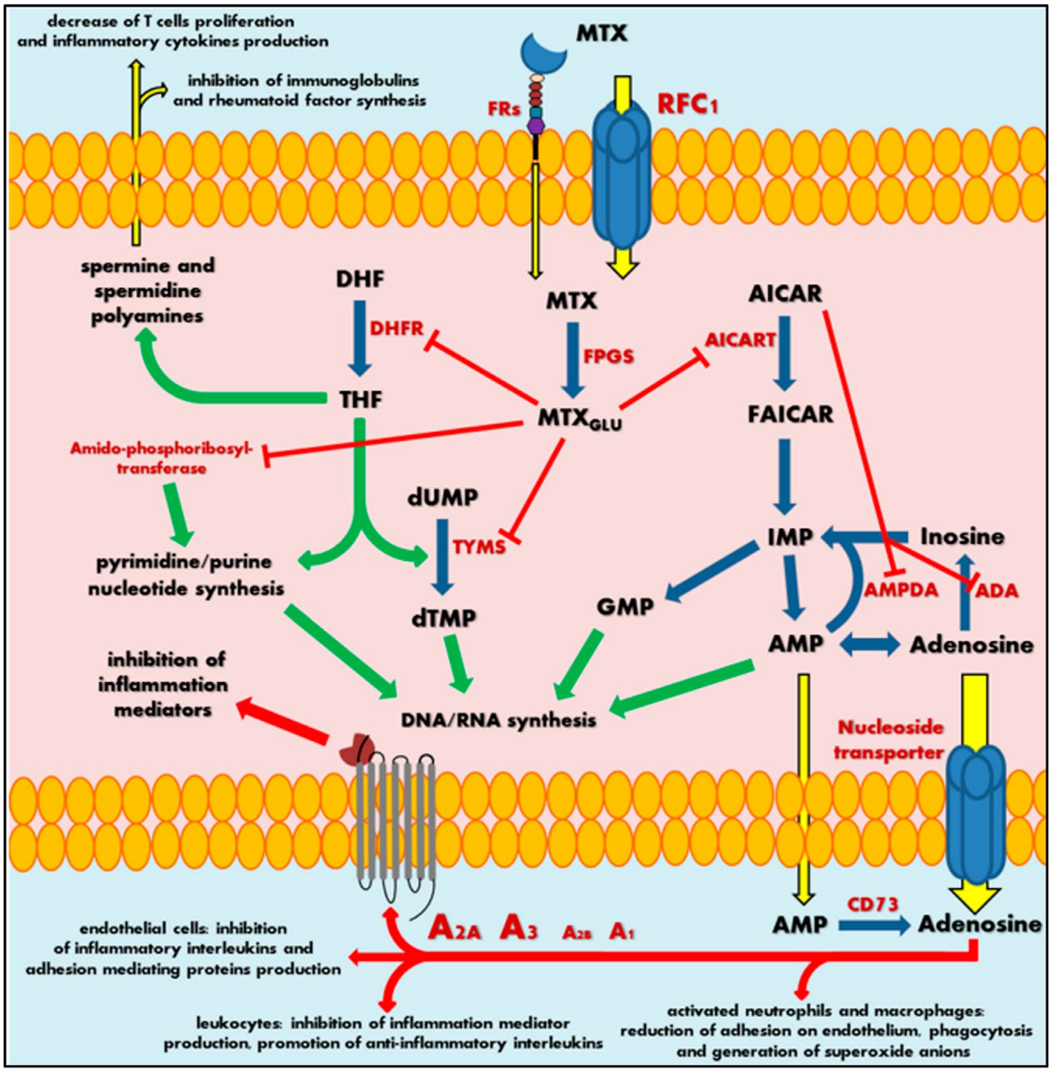
decrease of T cells proliferation
and inflammatory cytokines production
inhibition of immunoglobulins
and rheumatoid factor synthesis
MTX
FRs
RFC1
spermine and
spermidine
polyamines
DHF
DHFR
THF
MTX
FPGS
MTX_{GLU}
AICAR
AICART
FAICAR
Amido-phosphoribosyl-
transferase
dUMP
TYMS
dTMP
pyrimidine/purine
nucleotide synthesis
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Inosine
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inhibition of
inflammation
mediators
DNA/RNA synthesis
Nucleoside
transporter
AMP
CD73
Adenosine
A_{2A} A_3 A_{2B} A_1
endothelial cells: inhibition
of inflammatory interleukins and
adhesion mediating proteins production
leukocytes: inhibition of inflammation mediator
production, promotion of anti-inflammatory interleukins
activated neutrophils and macrophages:
reduction of adhesion on endothelium, phagocytosis
and generation of superoxide anions

MTX activity is most visible in actively dividing cells, mainly in the S phase of the cell cycle, and in fact, highly proliferating cancer cells are the most susceptible to the cytotoxic effect of this drug, indicating that antagonism of folate is related to the anti-tumor activity of MTX.

At the same time, the continuous action of MTX polyglutamates in cellular biochemistry results in intracellular accumulation of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) by AICART inhibition. AICAR can diminish the activity of adenosine deaminase (ADA) and adenosine monophosphate (AMP) deaminase (AMPDA) as well [16] (Figure 2). An MTX-mediated excess of AICAR promotes the AMP and adenosine increase and subsequent release of these adenine derivatives outside the cell. Extracellularly, AMP follows easily dephosphorylation to adenosine via ecto-5’-nucleotidase, also known as the CD73 enzyme. Adenosine, a significant signaling agent that modulates diverse physiological functions, acts as the major mediator of anti-inflammatory action associated with MTX. In the local extracellular space, adenosine might interact with its specific receptors (A1, A2A, A2B, or A3) present on the surface of the original tissue, but also on immune system cells (Figure 2). Adenosine receptors belong to the seven-transmembrane receptor family, mediating signals into the cell by coupled specific G proteins depending on the receptor subtype. In RA patients, adenosine-specific receptors on immune and synovial cells are elevated, especially A2A and A3 that highly mediate adenosine regulation of immune response and inflammation [17,18,19,20]. In psoriasis, there is a correlation between A2B and A3, mast cells, and inflammation especially mediated by TNF-alpha, and through A2A influences Treg activity [21]. The adenosine action on A2A and A2B stimulates intracellular production of cyclic adenosine monophosphate (cAMP), a significant cellular second messenger, while stimulation of A3 induces phospholipases C and D or inhibits adenylate cyclase producing cAMP. As a result, adenosine in activated neutrophils and macrophages reduces their ability to adhesion to endothelium and phagocytosis as well as generation of superoxide anions and further reactive oxygen species [22,23]. Moreover, leukocytes decrease the production of tumor necrosis factor α (TNF-α) and interleukin (IL)-12—the mediators of inflammation—and promote anti-inflammatory IL-4 and IL-10. Additionally, it is observed that inhibition of IL-1 action and suppression of TNF-α excretion in T cells and macrophages inhibit the prostaglandins and leukotrienes syntheses [24,25,26]. At the same time, adenosine disrupts the cytokine-mediated inflammation in endothelial cells by the decrease in the production of IL-6 and IL-8 and hindering leucocytes' adhesion [27]. Thus, an adenosine-mediated effect seems to be a key mechanism in MTX anti-inflammatory action. Nevertheless, the MTXGlu also has some other impact on immune chemotaxis and reduction of the occurrence of inflammation mediators. A sustained decrease of THF-mediated methylation downregulates an accumulation of spermine and spermidine polyamines in the extracellular fluids and lymphocytes of patients with RA [28,29]. These polyamines are essential cellular growth factors among others in lymphocytes. MTX action inhibits T cell proliferation and synthesis of immunoglobulins or rheumatoid factor in RA patients [30,31], followed by a local reduction in lymphocytes interferon (IFN)-γ and IL-2 production, decrease of inflammation, convergent to adenosine receptor-mediated effects.

All of the above indicates that MTX has a dual mechanism of action. Potent anti-metabolic and anti-proliferating actions are related to the therapeutic use of MTX as an anticancer agent. The action leading to a local increase of the adenosine concentration at the site of inflammation and also the specific immunosuppressive effect of MTX play an important role in the treatment of severe inflammation and autoimmune diseases (arthritis, psoriasis, and myasthenia gravis).

THE USE OF METHOTREXATE IN RHEUMATOLOGY

There are strong arguments for which methotrexate is still the golden standard in the treatment of rheumatoid arthritis:

1. According to EULAR recommendations for the management of rheumatoid arthritis, methotrexate (MTX) should be part of the first treatment strategy. MTX remains the anchor drug in RA; not only is it an efficacious csDMARD by itself but it is also the basis for combination therapies, either with GC or with other csDMARDs, bDMARDs, or tsDMARDs. MTX (whether administered orally or subcutaneously) should be escalated to a weekly dose of about 0.3 mg/kg and this escalation should be done within 4–6 weeks [32].

2. There are nowadays available, hundreds of clinical trials proving the clinical efficacy and safety profile of methotrexate, used in monotherapy or combination therapy. The vast majority of bDMARDs clinical trials are done in fact with the combination treatment MTX + bDMARDs.

3. In Romania, the therapeutical protocol for rheumatoid arthritis stipulates that methotrexate is the conventional synthetic remission medication of the first choice, except in cases where there are major contraindications, in the usual maintenance dose: 20 mg/week (depending on tolerance), usually orally. To increase tolerance, the combination of folate is usually recommended, and injection (subcutaneous or intramuscular) of methotrexate should be considered to increase bioavailability and reduced the risk of digestive side effects (along with domperidone and antiemetics: ondansetron or granisetron). Depending on the particularities of the case treated and the degree of activity of the disease, the attending physician formulates the treatment and indicates these remissive preparations, which can be used alone or in combination, and the combination should usually include methotrexate. Initiation of bDMARDs can be done in cases of rheumatoid arthritis that did not respond to conventional synthetic remission therapy, administered correctly (both doses and durations of therapy), respectively after the use of at least 2 conventional synthetic remission therapies, with a duration of at least 12 weeks each, one of which is usually methotrexate (except cases with a major contraindication to this preparation or cases which do not tolerate this treatment, as appropriate documented). As a rule, any targeted biological therapy (bDMARDs) and synthetic therapy (tsDMARDs) is recommended to be administered in combination with a conventional synthetic remission (usually only one, most commonly used being methotrexate, for which a minimum dose of 10 mg/week is recommended), which is maintained even after the initiation of bDMARDs or tsDMARDs

[33].

4. MTX is available in the form of tablets with increments of 2.5, 5, or 10 mg/tablet, and as prefilled syringes or auto-injectors with various doses per injection, allowing titrating weekly doses to pay attention to the patient's tolerability. Oral and (SC) are the preferred routes used in rheumatology patients. Oral MTX at a dose of 7.5 mg/week has 100 percent bioavailability. However, bioavailability is reduced by 30% starting at just 15 mg/week due to gut absorption limitations [34,35]. By contrast, the bioavailability of SC MTX is greater than that of oral MTX and does not depend on the MTX dose with a linear increase in systemic exposure for increasing doses. RA patients with an inadequate clinical response to orally administered MTX may benefit from the higher drug exposure offered when switched to the SC formulation [36]. Initiating the MTX regimen with SC administration has been associated with better clinical outcomes at the same dosages [37].

5. There is enough evidence that the association of MTX to bDMARDs is followed by bDMARDs reduced immunogenicity [38].

6. Treatment with MTX is highly cost-efficient.

Of course, treatment with MTX is not free of challenges. The major ones are related to MTX toxicities which can be prevented by folic acid supplementation. The good news is that MTX toxicities are well-known and therefore manageable. One other challenge related to MTX treatment is transforming the patient into a physician's partner. Setting for the patients the right expectations on treatment benefits, mostly related to time-course of the clinical benefits, will improve patient compliance and adherence to therapy and monitoring, and eventually avoid unnecessary premature discontinuation of MTX. The EULAR guidelines emphasize that the best outcomes with the use of MTX are achieved when a shared decision-making approach to treatment is used in which the patient is an active participant. Patients need to understand and take responsibility for following all physicians' recommendations.

THE USE OF METHOTREXATE IN DERMATOLOGY

The prevalence of psoriasis in Romania is 4.9% according to the prevalence study carried out a few years ago [39,40]. In Romania, the therapeutical protocol for psoriasis stipulates that methotrexate is the conventional synthetic remission medication of the first choice, except in cases where there are major contraindications or high risk. Only non-responders, after at least three to six months of treatment, are allowed to switch to biological therapy [41]. We use the usual maintenance dose: 15-25 mg/week orally or through subcutaneous injection. We prefer the injection because it has increased bioavailability and reduced the risk of digestive side effects [36,37]. To increase tolerance, the combination of folate is usually recommended in a single dose of 5 mg/week, one day after taking methotrexate. In the case of biological therapies, methotrexate can be associated with but is not a current practice. It is possibly associated in the event of the appearance of neutralizing antibodies to increase the persistence and effectiveness of biological therapy [42]. These are less common situations when we combined the two therapies for synergistic and complementary action.

Inflammation in psoriasis very often develops or worsens associated diseases [43,44,45]. For these reasons, we recommend the early start of systemic treatment. According to data from Romania, 31% of patients with psoriasis have associated hypertension, 22% dyslipidemia, 17% obesity, and 10% diabetes. By reducing inflammation, methotrexate therapy may decrease the risk of associated cardiovascular disease, although not to the same extent as it does with biological therapies [46,47,48,49]. Methotrexate is a solution for controlling patients with psoriasis and metabolic comorbidities, but additional precautions are needed at least for overweight, obese or diabetic patients [50,51]. For this reason, in a holistic approach to the patient, we must consider additional monitoring for these patients.

CONCLUSION

In conclusion, MTX is a remarkable drug with a unique place in the pharmacotherapeutic management of RA. There is now enough clinical experience to aim to achieve the best outcomes from MTX use.

MTX has a large dose titratable range and can be administered as a once-weekly dose regimen by either oral or subcutaneous routes. Parenteral administration of MTX has the advantages of maximizing bioavailability, reducing gastrointestinal intolerance, and potentially enhancing compliance and adherence.

For the treatment of rheumatoid arthritis, MTX is recommended as the first-line csDMARD treatment and can also be used in combination with other csDMARDs, bDMARDs, or tsDMARDs. In the case of concomitant use with targeted therapies, MTX facilitates optimum achievable efficacy and also has the advantage, when used with bDMARDs, of reducing immunogenicity. For the treatment of psoriasis, it is a first-line option (the most frequently used) but also an alternative in association with biological treatment. MTX is also highly cost-effective and has a favorable benefit/risk profile overall.

Conflicts of interest and sources of funding
The author declares no conflict of interest.

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REVIEW

Advances in Squamous Cell Carcinoma Antigen and Cervix Cancer Relationship

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Abstract: *The squamous cell carcinoma antigen is a tumor marker that is receiving more interest due to its biological properties and importance in both pathological and normal physiological processes. Numerous clinical studies have been conducted to determine the potential function of squamous cell carcinoma antigen and its isoform complexes in clinical practice. These studies have been prompted by the fact that not only squamous cell carcinoma antigen but also circulating immune complexes of it and immunoglobulin M are involved in normal physiological and pathological processes. The therapeutic use of squamous cell carcinoma antigen as a tumor marker for either squamous carcinoma diagnosis or for monitoring the response to radiation or chemotherapy, tumor recurrence, and treatment failure are supported by prior investigations. The diagnostic or prognostic utility of squamous cell carcinoma antigen is debatable, nevertheless, as these investigations provide conflicting findings. A uniform detection method, scoring system, and cutoff level must be devised to limit clinical variability between studies and to provide a more accurate and trustworthy comparison of data. Additionally, even if the effectiveness of several approaches is equivalent, only one method should be used for the dynamic monitoring of tumor marker kinetics.*

Keywords: *uterine cervix disorders, squamous cell carcinoma, markers, diagnosis, treatment*

INTRODUCTION

Worldwide, cervical cancer (CC) is the third most frequent malignancy in women. Approximately 520,000 women are diagnosed with CC each year, and 260,000 of those women pass away from the illness. The majority of CC-prone nations are developing nations, where access to healthcare is poor and resources are scarce. On the other hand, industrialized nations often employ health programs that call for immunization, screening, and treatment. As a result, the incidence of and death rates for CC are lower in these nations. The presence of the squamous cell carcinoma antigen (SCCA) tumor marker was examined in the serum of 103 individuals who had been diagnosed with squamous cell cervical cancer based on histology. The majority of cases (75%) dropped below 7.9 ng/ml even though values varied from 0.2-109.7 ng/ml. Clinical stage and SCCA readings have no association with one another. Its use as a diagnostic screen would be prohibited by the significant false negative rate (29%) of this marker in individuals with the established illness. The cellular lining of the cervix contains CC. Transformation zones, which are the areas where the endocervix and exocervix intersect, are where precancerous lesions usually first appear.

Squamous cell carcinoma (SCC; roughly 80%) and adenocarcinoma (ADC; around 5–20%) are the two subtypes that make up CC [1-3]. The earliest age at first sexual contact or first pregnancy, smoking status [4,5,6] long-term oral contraceptive usage [7], and a weakened immune system [8] are only a few of the risk factors that contribute to cervical carcinogenesis. Additionally, a person's vulnerability to CC is influenced by their family history and genetic makeup [9,10]. However, human papillomavirus (HPV) is recognized as the primary cause of CC and is found in almost all tumors [11,12].

Patients with CC in its early stages typically don't exhibit any symptoms.

Patients who have advanced cancer may experience one or more of the symptoms listed below, including vaginal bleeding, pelvic discomfort, atypical vaginal discharge, and pain during sexual activity. The number of people who will develop invasive CC may thus be reduced by effective screening and diagnostic techniques. Previously, it has been demonstrated that epigenetic modifications such as DNA methylation, histone modification, and microRNAs (miRNAs) have a role in the emergence of cancer [13]. Overexpressed miRNAs that are associated with cancer may act as oncogenes. MiRNAs that are active in normal tissue as tumor suppressors may be downregulated in cancer [14,15]. MiRNA expression patterns and tissue and cell lineage specificity have shed light on pathognomonic information [16]. Individual susceptibility to cancer is influenced not only by miRNAs but also by miRNA-associated single nucleotide polymorphisms (miRSNPs). The most promising route for developing CC-markers was highlighted in this review by our attention to changes in miRNA expression in CC.

TUMOR MARKERS FOR CC

Clinically helpful tumor indicators include soluble glycoproteins, which are substances that may be discovered in blood, urine, or other bodily fluids and are linked to certain cancer types. The advantages of tumor markers are shown in the screening of individuals with high-risk factors, the diagnosis and prognosis of certain malignancies, the assessment of therapy outcomes, and the identification of recurring illnesses [28]. We categorize tumor markers for CC screening into 2 types, including tumor indicators that are readily available and utilized frequently in laboratories and new proteins that are related to CC as tumor markers under research.

SCCA MEASUREMENT

Due to the critical role that SCCA plays in the diagnosis and prognosis of SCCs and other disorders, the demand for routine clinical SCCA testing has been rising quickly. The advancement of biotechnology has also led to improvements in detection techniques. Because biotechnological products have a limited level of biochemical variety, it has been possible to develop monoclonal antibodies that are specific for either SCCA1 or SCCA2, which allows for individual assessments of each rather than total SCCA. There are several popular techniques for determining the concentrations of SCCA in blood and tissue, including radioimmunoassay, ELISA, Western blot (WB), immunohistochemistry (IHC), and immune-luminometric assay. Radioimmunoassay was one of the earliest techniques to identify SCCA [17-18]. Radioimmunoassay is a sensitive and trustworthy method, but due to its various drawbacks, such as radiation exposure and low reagent stability, it is increasingly losing popularity.

The most popular technique for identifying SCCA was the ELISA test, which uses an antigen-antibody interaction and a color shift to determine protein levels. However, ELISA also has drawbacks, such as poor reproducibility, a constrained linear range, poor detection efficiency, and time-consuming experimental methods; as a result, it is insufficient to fulfill clinical demand. In order to assess protein expression, the basic idea behind WB is to use particular antibodies to target proteins in biological tissues or cells during gel electrophoresis. The molecular size of the targeted proteins is then compared to a reference ladder. The application of WB for large-batch testing in the clinic has also been constrained by the intricate experimental processes and high criteria for it. However, WB continues to be a method of choice in research investigations due to its high specificity and accuracy. IHC, on the other hand, is a technique for in situ qualitative or quantitative component identification of cells or tissues using the concepts of immunology and histochemistry. Immunohistochemistry and WB are both only appropriate for tiny samples.

SCCA levels have recently been measured using automated non-radioimmunoassay techniques. The Abbott ARCHITECT i2000 system and the Roche Cobas e602 system are two typical automated systems used in clinical laboratories for chemiluminescent microparticle immunoassays (ECLIAs) and ECLIAs, respectively. The measured SCCA levels are allegedly greater with the Roche system than with the Abbott system, despite the two systems having a significant positive correlation (r = 0.9658) [19,20]. These variations might be explained by the varied heterophilic antibodies and serum proteins utilized [21,22,23] as well as by the many manufacturer-specific variations in the reactivities of the individual antibodies towards the antigens. According to reports, the Roche system can identify SCCA1 and SCCA2 in an equimolar way [24], but the Abbott system is unable to establish the relative proportions of SCCA1 and SCCA2 [25] or can only detect SCCA1 but not SCCA2 [26]. In terms of SCCA detection, automated ECLIA and manual ELISA have both demonstrated equivalent capabilities [27]. The dynamic monitoring of tumor marker kinetics should be carried out using a single approach notwithstanding equivalent performances across several methods.

LEVELS OF SCCA IN CANCER AND INFLAMMATION

Numerous studies have been conducted on the biological roles and relevance of SCCA in both healthy physiological and pathological processes. Studies examining the possible function of SCCA, SCCA isoforms, and SCCA-Immunoglobulin M (IgM) complexes in clinical practice have been prompted by the discovery of circulating immunological complexes of SCCA and IgM in addition to SCCA that have been observed in typical physiological and pathological processes [29].

A new investigation on the connections between SCCA and 39 other clinically defined disorders demonstrates that SCCA is a clinically utilized biomarker not just for SCC but also for other human diseases [30].

CERVICAL CANCER

In the clinical diagnosis and treatment of cervical cancer, imaging techniques such as computed tomography (CT) and positron emission tomography (PET)/CT have become more widely used; nevertheless, these techniques have drawbacks, such as the potential for false-negative and false-positive outcomes [31,32].

However, the combined PET/CT and serum SCCA approach has greater diagnostic adequacy in the early detection of recurrent cervical cancer than either PET/CT or serum SCCA methods alone [33]. The identification of early postoperative metastases and recurrence in cervical cancer is therefore clinically benefited by this combination approach.

According to Ryu HK et al., SCCA levels > 1.86 and > 0.9 ng/mL were the ideal threshold values for predicting recurrence before and after therapy, respectively, and were strongly related to poor disease-free survival (DFS). Therefore, following full remission therapy, closer observation is required due to the increased chance of cancer recurrence [34]. Another study found that the optimal cutoff values for SCCA at pretreatment, treatment, and recurrence were 4, 1.5, and 4 ng/mL.

The recurrence and survival predictive values of SCCA at post-treatment and recurrence are only significant when pretreatment SCCA levels are 4 ng/mL, according to this study [35]. According to multivariate analysis, SCCA levels below 2.75 ng/mL can be employed as a possible marker to predict LNM in early-stage cervical cancer before surgery, especially for surgical treatment of early-stage cervical SCC (stages IB-IIA) [36].

COMBINATION OF SCCA WITH OTHER MARKERS

Many studies have assessed the efficacy of combining the detection of SCCA with other markers such as CRP, albumin, and noncoding RNAs for clinical application, in addition to the combination of SCCA and AFP in HCC. According to one research, individuals with cervical cancer who experienced recurrence had considerably higher blood levels of SCCA, hypersensitive C-reactive protein (hs-CRP), and CA125 than those who did not. There was also a statistically significant positive connection between SCCA and hs-CRP as well as SCCA and CA125 in the recurrence group of patients [37]. The DFS and OS of oral SCC patients are negatively impacted by preoperatively elevated CRP and SCCA levels [38]. As independent indicators for LNM, advanced tumor stage, and disease-specific survival in patients with penile SCC, preoperative CRP and SCCA levels were also discovered [39].

Patients with high SCCA and low albumin levels may not survive as well after surgery for esophageal SCC with stage T13N0M0, according to preoperative blood SCCA and albumin levels [40]. Both miR-215 and SCCA-IgM are more effective in diagnosing HCC than AFP, especially when it comes to separating cirrhotic patients from those with HCC with AFP levels below 200 ng/mL and small focal lesions [41].

Furthermore, coupled pretreatment serum circulating tumor cells (CTC) and pretreatment SCCA show greater predictive accuracy compared to FIGO stage and serum CTC or SCCA alone [42].

CLINICAL USE OF TUMOR MARKER

The serum marker for SCCs now used in clinical settings is called squamous cell carcinoma antigen (SCC-Ag). In between 20% and 60% of individuals with early-stage CC, it is present in high quantities. In 64% of patients with SCC and 25% of patients with ADC, abnormal levels of SCC-Ag are seen [43,44].

The tumor diameter and illness stage are related to the level of SCC-Ag before therapy [45]. The blood level of SCC-Ag after therapy indicates how well the treatment worked. Additionally, SCC-Ag rises in people who have tumor recurrence [46,47]. According to Ryu et al. [49], a post-treatment SCC-Ag value of more than 0.9 ng per mL is the ideal cut-off value for predicting recurrence in patients with pretreatment SCC-Ag levels larger than 1.86 ng per mL. This marker has a sensitivity range of 56% to 86% and a specificity range of 83% to 100% for CC recurrence [48]. An indication of a gynecological malignancy is the cancer antigen 125 (CA-125). The preferred tumor marker is now used to follow women receiving ovarian cancer treatment on a regular basis and to calculate patient survival rates [49,50]. Additionally, changes in CA-125 levels are a strong indicator of how advanced endometrial cancer will turn out [51,52]. Precancerous lesions can be identified using elevated levels in serum and cervical or vaginal secretions [53]. Ngan et al. [54] discovered that higher illness stages for CC were associated with greater levels of CA-125. In patients with ADC and SCC, abnormal CA-125 levels were found in 42.6% and 18.9% of patients, respectively.

CONCLUSION

Although there have been some discrepancies in the results due to different distributions of SCCA isoforms, previous studies have shown the clinical utility of the tumor marker SCCA for diagnosis or monitoring of the response to radiation or chemotherapy, tumor relapse, and treatment failure. As a result, there is still debate concerning SCCA's diagnostic and prognostic capabilities. A uniform detection technique, scoring system, and cutoff level are required to lessen clinical variability between studies and to enable more accurate and trustworthy comparisons. Despite similar performances of various approaches, the dynamic observation of marker kinetics should always be carried out using the same method.

Disclosure statement

This statement is to certify that all Authors have seen and approved the manuscript being submitted. The authors have no relevant financial interest in this article.

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All authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the Romanian Journal of Military Medicine.

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ORIGINAL ARTICLE

Dichotomisation of Rotator Cuff Tendinopathy in Shoulder MRIs Reveals the Need for Further Diagnostic Improvements: A Cohort Study

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Abstract: *Background: Rotator cuff tendinopathy is most often described as a continuum between the normal cuff and rotator cuff tears with calcific tendinitis having its place along this continuum. Although many studies have focused on the role of magnetic resonance imagining (MRI) in diagnosing the extent of rotator cuff tears and their associated findings with good interobserver reliability, the same cannot be stated about MRI tendinopathy findings. Because of this discrepancy in diagnostic reliability, tendinopathy tends to be overtreated with injections when associated with symptoms, thus potentially increasing the risk of calcific tendinitis and progression toward rotator cuff tears. This study aims to assess whether diagnosing shoulder MRI tendinopathy patterns through dichotomization can accelerate clinical progress toward consensus. Methods: This study is a large retrospective cohort of 184 patients that underwent a 1.5T shoulder MRI for shoulder pain. Inclusion criteria were acromioclavicular arthrosis diagnosed in patients of any age. Exclusion criteria were partial or complete rotator cuff tears. Tendinopathy was considered the dependent variable and registered as a dichotomous variable while acromioclavicular joint arthrosis together with gender was categorical and age was the continuous variable. An attempt was made to generate a clinically significant binary logistic regression to assess the odds ratio of diagnosing tendinopathy based on age, gender, and acromioclavicular joint arthrosis status. Results: An overwhelming proportion of patients was positive for tendinopathy findings (95.11%). 64.12% of patients were within the active age group with patients within the 50-59 group being diagnosed the most with rotator cuff tendinopathy. Conclusions: Due to the high variability of MRI findings that can be considered positive for rotator cuff tendinopathy, an overwhelming skew toward a positive diagnosis was observed, thus dichotomizing tendinopathy diagnosis is not appropriate for clinically relevant conclusion-making.*

Keywords: *rotator cuff, tendinopathy, magnetic resonance imaging, biostatistics*

INTRODUCTION

Rotator cuff tendinopathy has become increasingly relevant to diagnosis as its involvement in the shoulder's rotator cuff pathology continuum is dominating [1-5]. While the progression rate to full-thickness rotator cuff tears has not yet been elucidated early management of tendinopathy might decrease the progression rate and improve patient-reported outcomes [4,6]. Special attention is advised when managing the early stage, symptomatic, rotator cuff tendinopathy with corticoid injections as they might lead to an increased rate of calcific tendinopathy incidence that has a stronger progression towards full-thickness rotator cuff tears, thus decreasing the patient's quality of life in the long run [7,8]. In regards to early diagnosis, some early contributions have been made toward increasing the diagnostic accuracy of rotator cuff tendinopathy, although most studies are focused on diagnosing rotator cuff tears [9-12]. There is still a lack of consensus on the contribution of associated imagistic findings in tendinopathy and their link towards full-thickness rotator cuff tears; most known associated findings are bone cysts adjacent to tendon insertions, bone marrow edema adjacent to tendon insertions, subacromial and subdeltoid signal increase on fat-saturated T2 weighted magnetic resonance imaging (MRI), bursal or articular sided fraying and fibrillation of the rotator cuff tendons and articular effusions [2-4,10,13-22].

It is worth mentioning that further difficulty is encountered when attempting to differentiate rotator cuff tendinopathy and partial articular-sided, bursal-sided, or instar-substance supraspinatus tendon tears [12,13,23-28]. This study aimed to assess the feasibility of using dichotomization of rotator cuff tendinopathy to fast-track clinical research as categorical or continuous data require significantly more time that the clinician has to invest in data gathering.

MATERIALS AND METHODS

This study is conducted according to the ICMJE Recommendations on Uniform Requirements for Manuscripts (URMs) [29].

Our research question was Is dichotomization of shoulder MRI findings in patients diagnosed with rotator cuff tendinopathy a useful method for logistic regression?

Before the study inception, a preliminary search has been conducted in MEDLINE via Pubmed using the search terms (("Rotator Cuff Injuries"[Mesh]) OR ("Rotator Cuff"[Mesh])) AND (("Tendinopathy"[Mesh]) OR ("Tendon Injuries"[Mesh])) AND ("Magnetic Resonance Imaging"[Mesh]) AND (y_5[Filter]) to properly interpret our findings within the context of the current state-of-the-art on shoulder rotator cuff tendinopathy MRI diagnostic.

Considered for inclusion were studies assessing the role of MRI and its findings in the context of rotator cuff tendinopathy. Exclusion criteria were all studies focusing on pre- or postoperative MRI assessment, studies on full-thickness rotator cuff tears, biomechanical, cadaver, simulation, or any other study that does not assess tendinopathy patterns. We have identified 551 results that we have imported into Zotero for reference management. Two authors, G.M.A. and A.S. have selected relevant articles in three successive steps, excluding articles based on title, abstract and full text. After the title and abstract assessment, 68 studies were included for full-text evaluation against the proposed inclusion and exclusion criteria. After reading the remaining 68 articles, 50 articles met the inclusion criteria and were included in this study.

The present study is a retrospective large cohort study that assesses the reliability of MRI diagnosis in rotator cuff tendinopathy. We have included 184 patients that underwent shoulder MRI for any reason between January 2019 and January 2022. Coronal proton density (PD) and fat-suppressed T2 (T2-FS) sequences have been assessed for each patient and tendinopathy presence has been documented by two radiologists M.H. and D.C. together with a senior orthopedic surgeon, S.M. Dichotomous values were used to record the dependent variable's, tendinopathy, presence against the independent variable, acromioclavicular arthrosis, which was recorded as a categorical variable according to the Shubin-Stein classification [30] together with patients age and gender. All variables were imported and processed in SPSS version 26 (IBM Corp., Armonk, N.Y., USA) to conduct descriptive statistics and perform binary logistic regression on the relationship between the dependent and independent variables. Before conducting binary logistic regression, a Box-Tidwell test has been performed to assess the relationship between the continuous independent variable, age, and its logit. Also, an Eta-squared ($\eta2$) test was performed to investigate how much of the variability in acromioclavicular arthrosis was accounted for by age and the result of the $\eta2$ was verified using the F ratio.

RESULTS

Descriptive statistics show an overwhelming skew towards positive rotator cuff tendinopathy diagnosis by using dichotomization of the dependent variable. Thus, comparisons within the group were not conducted due to the low statistical power. Patients' mean age was 55 years (S.D. 14). Results show that 95.11% of the included patients (Figure 2) were positive for rotator cuff tendinopathy. Surprisingly, tendinopathy does not seem to be correlated with acromioclavicular joint arthrosis status (Figure 3) and neither age seems to have a significant impact on acromioclavicular arthrosis ($\eta2=0.238$), $F(50, 133)=0.831$, $p=0.770$. Most patients diagnosed with tendinopathy were within the 50-59 age group (Figure 4), but 64.12% of all patients diagnosed with tendinopathy were within the active, employed, group (Figure 5). Binary logistic regression has been performed to assess the effects of age, gender, and acromioclavicular joint arthrosis status on the likelihood that patients are diagnosed with tendinopathy. The logistic regression model was not significant, $\chi2 = 2.622$, $p = 0.454$. The explained variation in the dependent variable based on our model ranges from 1.4% to 4.4%, depending on whether we reference the Cox & Snell R2 or Nagelkerke R2, respectively, and correctly classified 100% of the case. The odds of being diagnosed with tendinopathy were determined to be 1.769 times higher when having concomitant acromioclavicular arthrosis ($p=0.206$, 95% CI [0.730, 4.287]), but this result was not significant. Similarly, age and gender were not significant factors in tendinopathy diagnosis ($p=0.397$, 95% CI [0.972, 1.075]) and ($p=0.493$, 95% CI [0.155, 2.457]), respectively.

Figure 1: Tendinopathy pattern according to Sein et. al[3]. (a) Image A, shows no increase in signal intensity, corresponding to tendinopathy grade 0, and B, C, and D show a gradual increase in signal intensity at the level of the supraspinatus tendon corresponding to tendinopathy grades 1, 2, and 3 respectively.

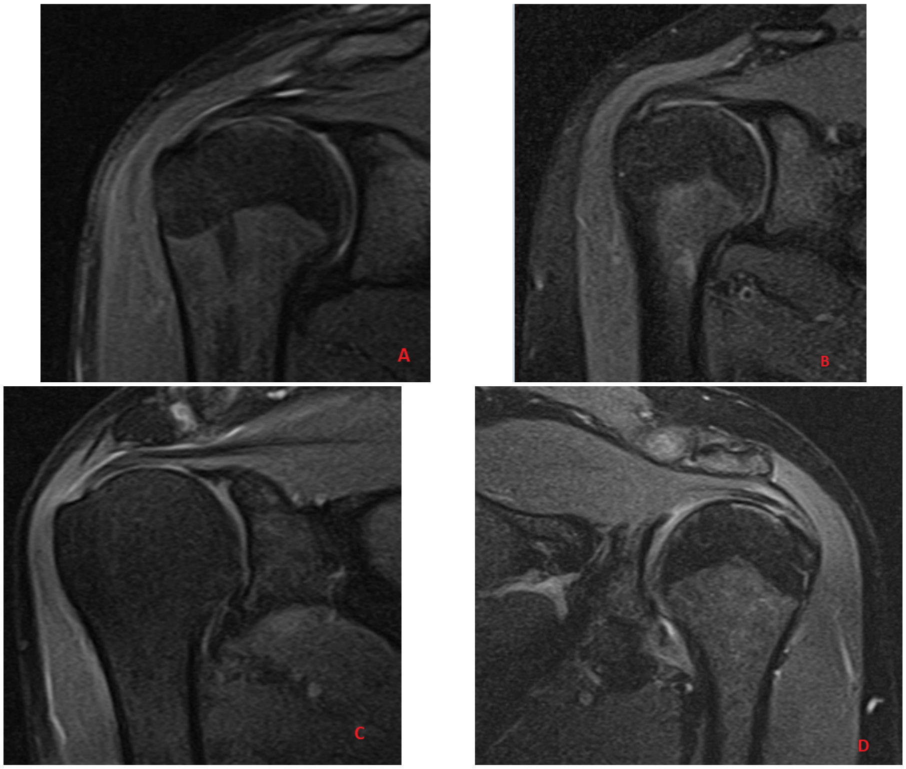

Figure 2: More than 95% of the included patients presented with rotator cuff tendinopathy

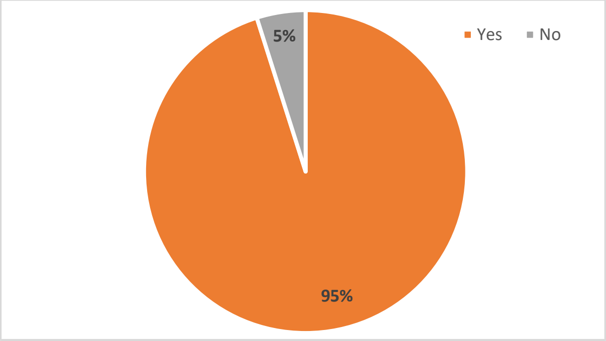

Figure 3: Tendinopathy diagnostic based on acromioclavicular joint status

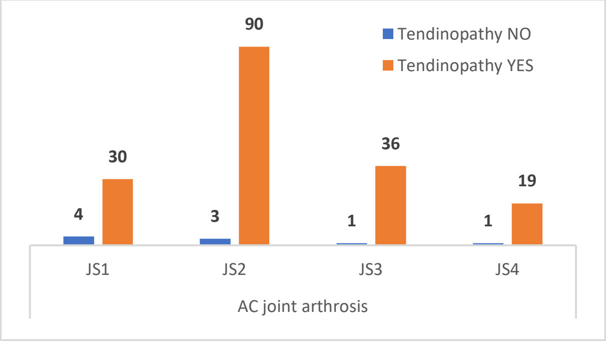

Figure 4: Tendinopathy distribution between age groups

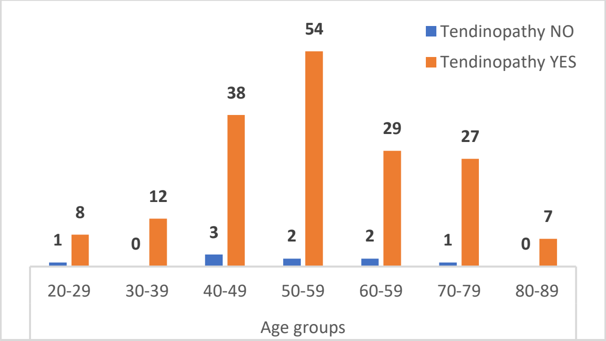

Figure 5: Tendinopathy distribution within age groups

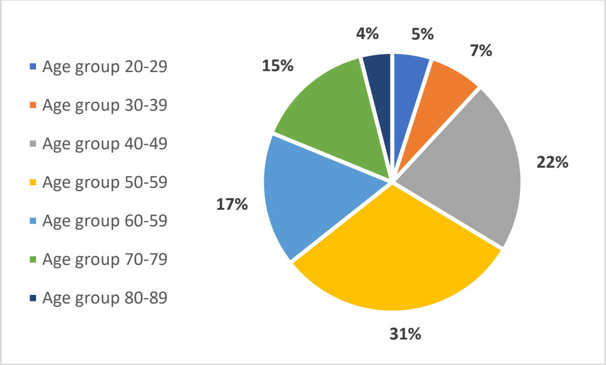

DISCUSSION

We would like to mention that the results obtained by performing binary logistic regression show that by using dichotomization when categorizing tendinopathy researchers will unbalance their results because of the high prevalence of rotator cuff tendinopathy in the general population.

It is worth noticing that tendinopathy was prevalent in all stages of acromioclavicular arthrosis, but no increase in tendinopathy rate was seen with the increasing acromioclavicular arthrosis stage. The patients with Shubin-Stein stage 2 acromioclavicular arthrosis presented rotator cuff tendinopathy most frequently (Figure 3). This result is in disagreement with our hypothesis that the tendinopathy rate would be expected to increase with increasing acromioclavicular arthrosis.

The most important result was that tendinopathy is widely distributed within the more active age groups. This aspect underlines the need for identifying efficient treatment measures that are aimed at alleviating symptoms and delaying tendon wear. Most patients, 80.97% (Figure 5), that were within their most active years, presented tendinopathy, with an overall increased incidence in the 50-59 age group. This is a relevant observation since the socio-economic impact of rotator cuff tendinopathy can be anticipated as patients require a home to rest, medication, and physiotherapy.

After a thorough literature search, we have identified several studies focusing on investigating rotator cuff status after arthroscopic repair but very few studies on rotator cuff tendinopathy patterns [1-6,18,31-37]. Two relevant studies used a comprehensive approach to classifying tendinopathy from normal to severely tendinopathic rotator cuff using categorical data [3,15,38].

We have tested the usefulness of classifying tendinopathy as a dichotomous dependent variable and tested its relation to acromioclavicular joint arthrosis, gender, and age. We were not able to conclude any clinically relevant information due to the previously unrecognized high prevalence of tendinopathy in the general population. We would also like to mention that healthy patients usually do not undergo a shoulder MRI, thus the prevalence of tendinopathy in our study cohort might very probably be overestimated.

Our approach was an attempt to simplify these previous efforts to accelerate clinical research toward reaching a consensus on rotator cuff pathology management. It is worth mentioning that, based on our experience, subacromial corticoid injections have yielded mixed results, most often than not offering only short-term pain relief. Thus, studies questioning the usefulness of corticoid injections and their suspected involvement in calcific tendinitis should be considered when deciding long-term patient benefits [7,8,39-44]. We would also like to point out that in comparison to rotator cuff tears, tendinopathy seems to be much more difficult to accurately diagnose because of the mixed patient presentation, its association with calcific tendinitis, partial supraspinatus tears, and subacromial or subdeltoid increased signal in T2-FS sequences [4,9,10,13,17,23,24,32,39,40,42,45,46]. We consider that differential diagnosis between tendinopathy and partial supraspinatus tendon tears, the contribution of bone cysts to tendinopathy severity, and the possible accelerated progression of calcific tendinitis to rotator cuff tear are the 3 most important aspects that should be taken into consideration when suspecting tendinopathy in a patient suffering from shoulder pain.

Several limitations need to be mentioned. Data gathering was performed only once, thus no intra- and inter-observer reliability testing was performed. No intra-class or inter-class correlation coefficients were calculated to determine associations among and within groups. The most severe limitation was that tendinopathy patterns were recorded as dichotomized data instead of ordinal data, thus underestimating the high prevalence of rotator cuff tendinopathy in patients that require a shoulder MRI due to pain and/or function deficit.

CONCLUSION

Due to the high variability of MRI findings that can be considered positive for rotator cuff tendinopathy, an overwhelming skew towards a positive diagnosis was observed, thus dichotomizing tendinopathy diagnosis is not appropriate for clinically relevant conclusion-making. We recommend the use of tendinopathy gradings like the one reported by Sein et al. At the same time, clinicians should be aware of the increased prevalence of rotator cuff tendinopathy, especially within the active age groups, and should aim for efficient treatment methods that offer fast recovery so patients can return to work and sports in a timely fashion.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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Authors’ contribution
Conceptualization G.M.A, E.M.C; methodology G.M.A; formal analysis G.M.A., F.A.S.; investigation G.M.A., S.M., M.H., D.C.; data curation G.M.A., A.S., M.H.,

D.C.; writing – original draft preparation G.M.A., F.A.S.; writing – review and editing, G.M.A., S.M., E.M.C., M.H., D.C.; visualization, G.M.A., S.T., S.L.; supervision, E.M.C., F.A.S., S.M., D.C.; project administration G.M.A., E.M.C., S.M., D.C.

Ethics approval and consent to participate

The present study was approved by the hospital's ethics committee, number 521/05/05/2022, and is performed under the ethical standards laid down by the Declaration of Helsinki.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

The Endocrine Approach of Melanoma: The Puzzle of Estrogen Receptors Expression

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Abstract: *Melanoma outcome seems different between females and males, with a potential protective role of estrogen (E) through estrogen receptors (ER) expression into the tumor. In the study of ERs, both alfa (ERα) and beta (ERβ) is a well-known endocrine elements in non-melanoma tumors, like mammary and endometrial cancer. Immunohistochemistry (IHC) assessment of melanoma concerning ERs represents a path to explore the tumor profile to provide useful information concerning the prognostic and potential adjuvant treatment. Currently, this is not a routine practice, nor a mandatory step for deciding the medical therapy. Typically, IHCs are based on usual kits for mammary tumors regarding ERs configuration. Prior/concomitant use of oral contraceptives and hormonal replacement therapy is not correlated with a better prognostic in melanoma; neither have they represented a contraindication for survivors of melanoma; a subset of tumors might present a higher ER expression which is potentially targeted by the hormone-based treatment as SERMs (Selective Estrogen Receptors Modulator), for instance, tamoxifen. Experimental studies on melanoma cell lines confirmed the anti-tumor activity of ERβ which might function as a prognostic marker. G-protein-coupled estrogen receptors in melanocytes and keratinocytes might be involved, too. Additional crosstalk of TGF-β (Transforming Growth Factor β), respective IGF1 (Insulin-like Growth Factor), and ERα expression are involved in tumorigenic pathways. Recent preclinical studies showed the potential benefits of diarylpropionitrile, a selective agonist of ERβ; pyrazole derivates 21-23 can block ERs. Murine melanoma models showed the interference of anti-estrogenic medication (like molecule fulvestrant) to enhance immune checkpoint blockade, a modern approach to solid cancers. The proliferation of melanoma might be partially explained by ERs; whether this is generally applicable or there is a subgroup of tumors particularly related to E status is still debatable. The subject of E status in melanoma is far from clear at this point and further studies are necessary concerning this particular issue to implement it as a practical approach in the daily management of a disease that still has a very severe prognostic nowadays.*

Keywords: the *estrogen receptor, melanoma, endocrine, dermatology, estrogen, progesterone, prognostic*

INTRODUCTION

Melanoma outcome seems different between females and males, with a potential protective role of estrogens (E) through estrogen receptors (ER) expression into the tumor [1-10]. However, the subject is far from clear at this point and further studies are necessary concerning this particular issue, and also they are necessary to take the next step to implement it as a practical approach in the daily management of an otherwise very severe disease [1-10]

The study of ERs, both alfa (ERα) and beta (ERβ) is a well-known endocrine element in non-melanoma tumors; while positive ER status is essential to be assessed in gynecological cancers including breast and uterine malignancies; as well as other endocrine conditions like atypical endometriosis implants, etc. [11-20]. Physiologically, ERs at the level of the endometrium (which is also responsive to progesterone receptors and androgen receptors) cause mucosal proliferation (proliferation phase) and enhance the expression of progesterone receptors which become strong players during the secretory phase [21]. Moreover, ERs are mediators of the pro-angiogenetic status under normal and pathological circumstances [22].

Estrogenic status has been reported in relationship with different autoimmune conditions, including in dermatology [23-26]. An interplay via CD8+ T cells has been identified in both melanoma and vitiligo [27,28]. ERs have been revealed as biomarkers in complex heterogeneous tumors like neuroendocrine neoplasia, or in adenocarcinomas with a neuroendocrine component, as mostly described in mammary malignancy [29-35]. Moreover, prenatal exposure to E has been associated in murine experiments with different behaviors of somatotroph and lactotroph cells at the level of the pituitary gland; however; the daily clinical approach of somatomamotroph cell-derivate tumors is not particularly different between females and males unless pregnancy and lactation status [36-38]. One of the most interesting topics addressing the various roles of ERs concerns E status in COVID-19 infection [39-44]. It was suggested that generally, females tend to present less severe coronavirus–associated clinical manifestations due to immune-mediated E protection [39] It seems that menopausal replacement treatment might reduce inflammation due to E actions and, also, modulate ACE2 (angiotensin-converting enzyme) as the receptor of the virus [39]. A recent phase 2, multicenter, randomized trial studied the efficacy and safety of raloxifene, a SERM (Selective Estrogen Receptor Modulator) traditionally used in gynecological endocrinology, which might be recommended to improve some aspects of SARS-CoV-2 (severe acute respiratory syndrome) presentation [40].

AIM

Our purpose is to focus on melanoma in relationship with E and ERs status concerning the current level of knowledge and future lines of addressing this topic in daily practice.

METHOD

This is a narrative mini-review. We revised the full-length papers published in English literature through a search on the PubMed database. We only included articles published between 2022 and 2019, with no restriction concerning the level of statistical evidence.

IMMUNOHISTOCHEMISTRY AND GENETIC STUDIES

One controlled study from 2022 addressed ERs configuration in 4 female cases diagnosed with melanomas; they were mammary cancer survivals; the ER expression was compared to a group of women who had an ovarian stimulation performed versus age-matched groups [45]. ER expression (nuclear and cytoplasmic) was analyzed based on the percent of ER-positive cells (score of 0, 1, and 2, for ≤20%, 21-50%, respective ≥50% positive cells) [45]. Nuclear ERβ was detected in females with previous breast cancer; the expression in melanoma is more frequent if the patients were previously treated with anti-estrogen medication [45]. Cytoplasmic ERα expression was lower in the control group, as well as in subjects with prior ovarian stimulation when compared to the patients with mammary malignancy [45].

Immunohistochemistry assessment of melanoma concerning ERs represents a path to explore to provide potentially useful information [45]. However, in everyday practice, we do not have a particular protocol to asses ERs in melanomas.

Immunohistochemistry studies using the usual kit for mammary tumors regarding ER have been done, including on uveal melanoma, a very aggressive form. We mention one study from 2021 showing that more than half of these tumors are ER positive; while elevated nuclear expression correlates with a most severe outcome [46].

This raises the question if uveal melanoma is a hormonal sensitive cancer [47]. A systematic review from 2021 confirmed that prior/concomitant use of oral contraceptives and hormonal replacement therapy is not correlated with a better melanoma prognostic; neither have they represented a contraindication for survivors of melanoma; a subset of tumors might present a higher ER expression which is potentially targeted by hormone-based treatment as SERMs (Selective Estrogen Receptor Modulator), for instance, tamoxifen [47]. One study on melanoma B16F10 cells showed the potential benefit of atorvastatin (a statin for hypercholesterolemia) in addition to tamoxifen [48].

One retrospective study on 448 subjects with melanoma analyzed the expression of the ERα gene that was correlated with an increased overall survival rate (p-value of 0.018); ERβ gene expression was not associated with melanoma progression [49]. The use of an ERβ-selective modulator like NO-SERM 4d which is a nitric oxide-releasing selective ER modulator 4d has a potential benefit [49]. The interaction of E, respective SERMs, with nitric oxide release might represent the clue for the antiproliferative effect [50].

A cross-sectional study on 73 melanocytic lesions analyzed the status of ERα, ERβ, and G-protein coupled estrogen receptor (GPER) in melanocytes and keratinocytes; ERβ expression was higher in dysplastic versus common nevi (melanocytes); ERα distribution was similar among the three types of dermatological lesions; GPER expression was increased in margin tissue melanocytes versus melanoma melanocytes [51]. Also, nuclear receptor estrogen-related receptor γ (ERRγ) might be a contributor to an immune defense [52].

GPER is involved in different systems a part of the immune system, like cardio-metabolic and reproductive systems [53]. The interaction with ERα modifies the immune response, as well as the proliferative potential [53]. Moreover, melanoma cell growth is promoted through TGF-β (Transforming Growth Factor β) and ERα expression is a contributor to this pathway [54].

ERs cross-talk with IGF1 (Insulin-like Growth Factor) pathways at several levels [55]. A single nucleotide polymorphism (SNP) study on melanoma–associated ER/IGF1 signaling showed that IGF1-GG phenotype (recessive model for rs1520220) associates a higher risk of this tumor in males ($p=0.006$), but the lowest risk for females ($p=0.045$), respective IGF1R-AA genotype (recessive model for rs2229765) correlates with protection for men ($p=0.006$), and it does not represent a protective/risk factor for women [55].

Another study that included immunohistochemistry and nuclear/cytoplasmic analysis of ERß expression on 86 subjects with different types of melanocytic lesions showed that the expression of ERß is more intense in males with Spitz and Reed nevi versus melanoma and common nevi, while Breslow thickness, as a prognostic marker in melanoma, is higher in cases with low immune reactivity versus high immunoreactivity of ERβ [56].

BREAST CANCER AND MELANOMA

One study between 2005 and 2015 included 238 subjects with metastatic non-hematopoietic mammary disease and 42% of them had melanoma as a primary site [57]. Another study on 64 patients identified 13 melanomas among them as the primary site, suggesting a potential common loop with the E system [58].

Some cancers associate a genetic susceptibility; the correlation with estrogen status might be a missing piece of the melanoma puzzle. One study on UK Biobank data included 106,278 patients with breast cancer and a control group of similar size [59]. The authors showed that the overall risk of mammary malignant tumors according to a score called polygenetic risk score based on genome-wide association studies is positively correlated with melanoma, as well as with other cancers like ovarian, gastric, and colorectal [59].

Another common hypothesis involves syndecan-4 which is a transmembrane proteoglycan mediating cell adhesion and proliferation of different cancer cells including mammary neoplasia and melanoma, with potential interference over E – related pathways [60]. For instance, zoledronic acid, a bisphosphonate drug used for osteoporosis and bone metastasis, is associated with additional anti-proliferative effects on invasive mammary cancer by increasing the expression of syndecan-4 [60].

NEW POTENTIAL TARGETS OF THERAPY AGAINST MELANOMA

ERβ might seem like a new potential line of management in severe cases of melanoma that require multi-leveled management [61]. Experimental studies on melanoma cell lines confirmed the anti-tumor activity of ERβ which might function as a prognostic marker [62]. For instance, diarylpropionitrile (DPN), a selective agonist of ERβ, activates signaling pathways in cultures of the brain and mammary cells; it seems that the inhibitory influence on melanogenesis is exerted also independently from ERβ via protein kinase A(PKA)/cAMP-response element-binding protein (CREB) signal transduction pathways [63].

Other experiments involve pyrazole derivates 1-29 with antiproliferative effects. Cell lines of melanoma are inhibited by derivatives 21-23 being able to block ERs [64]. Murine melanoma models showed the interference of anti-estrogenic medication (like molecule fulvestrant) to enhance immune checkpoint blockade, knowing that immune checkpoint inhibitors represent a modern approach to solid cancers [65].

Also, rat melanoma models showed the effect of ERα activation as the promotor of cell growth via an immune suppressive status causing abnormal CD8+ T cells function which might explain poor response to this new class of anti-tumor drugs [66]. Whether this combination will be clinically applicable for patients with melanoma, as well, is still an open issue.

As mentioned, GPER has been studied in relationship with proliferative effects and potential involvement in hormonal therapy resistance of different cancers, including in some malignancies without a clear expression of ER [67]. The use of GPER – targeted medication in combination with immune checkpoint inhibitors is under evaluation in persons diagnosed with melanoma [68]. Immunotherapy represents a major line of therapy in modern oncology, and its adequate placement in addition to ERs modulation might bring benefits in cases with melanoma [69].

As mentioned, uveal melanoma represents one of the most aggressive forms of melanoma. An alternative therapy that is under evaluation is the combination of MEK inhibitors with CDK4/6 selective inhibitors (approved for patients diagnosed with mammary cancer with positive estrogen receptors in association with ER antagonists or aromatase inhibitors)

[70,71]. In melanoma, the regulation of the PRMT5-MDM4 pathway plays a major role in the response to CDK4/6 inhibitors' effects [72].

Cyanidin-3-o-glucoside, a phytoestrogen compound with anti-proliferative effects concerning melanoma, has been studied in vitro and in vivo, and it was identified to promote apoptosis of melanoma cells via ERβ which is rather paradoxical [73].

Some controversies around the concept of ER as a future target of therapy in melanoma relate to the observation that traditional ER and androgen receptors are not identified in all melanoma studies; also, some data concern testosterone as an activator of melanoma growth through ZIP9 which is a zinc transporter, thus inhibitors of androgen receptors might act as anti-tumorigenic in melanoma, as a collateral unexpected effect [74].

O mice study with acteoside, an inhibitor of tumor growth in different cancer lines, showed that the molecule inhibited melanoma growth by reducing inflammation levels, as well as reactive oxygen species, and by promoting a higher rate of apoptosis by regulating ERβ [75].

An alternative to ER and E approach has been proposed for the modulation of progesterone receptors by using selective progesterone receptor modulators (SPRM) like asoprisnil, which seem to act on melanogenesis, as well [76].

DISCUSSION

The proliferation of melanoma might be explained by ER; whether this is generally applicable or there is a subgroup of tumors particularly related to E status is still debatable [77]. If melanoma represents to some extent a steroid hormone–dependent cancer is difficult to say at this moment [78]. Genetic and hormonal elements might have a place among risk factors, but it is difficult to explain the higher mortality in male patients versus females based on clinical and preclinical data we have so far [79]. Melanoma is currently related to several prognostic biomarkers [80-83]. We mention V600E mutation of the BRAF gene [80,81]. Also, local invasion and clinic-pathological characteristics are important [82-85].

CONCLUSION

Multiple factors simultaneously act on cell growth and therapeutic intervention should be multi-leveled. On the other hand, systematically using immunohistochemistry and genetic analysis of ER will identify different subgroups concerning E status thus different prognostic profiles among patients with melanoma. We need to find new management solutions for this condition, knowing that melanoma is still a very aggressive disorder nowadays, with an alarming increasing incidence.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Investigating the Operational Management Challenges of a Reference Military Hospital in the COVID-19 Pandemic

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Abstract: *Introduction: Following the crisis of coronavirus outbreak in numerous regions of the world, this epidemic enhanced promptly in most countries, including Iran, and sent a host of patients in need of medical services to hospitals. This study aimed to investigate the challenges of operational management of a reference military hospital during the pandemic COVID-19. Methods: This research is a qualitative study with the content analysis method with a deductive and guided approach. Data collection was conducted through a semi-structured interview method and utilizing a questionnaire designed based on the management functions of the Hospital Incident Command System (HICS). The targeted sampling method and participants in this study included senior-middle managers working in organizational positions of the reference hospital in 2020 employed during the organizational position in Covid-19 Pandemic. Classifications and coding were performed employing MAXQDA 2018 software. Results: The findings of this study were explored in the form of strengths, weaknesses, and challenges. Weaknesses included taking surprise, early and non-missionary entry into the COVID-19 crisis, lack of appropriate depot, instant management, deficiencies in the training of patients and staff, poor functioning of the crisis management system, deficiencies in monitoring, and poor provision of psychological support. The strengths were the devoted and altruistic presence of the medical staff and managers, the financial support of the headquarters, the effective field research of the management team of the organization, and the proper management of the volunteer forces. The most significant challenge was the poor coordination with the relevant organizations, including the headquarters, the Defense Organization, and the Ministry of Health. Conclusions: The findings of this study demonstrate that in the peak period of the COVID-19 pandemic to promote the operational management of the hospital, sticking to the general policies of the organization, structured coordination with internal and external organizations, organized management of equipment and human resources, generating a robust documentation structure and decision-making based on the evidence, effective and practical use of the crisis management system, systematic training of staff and patients, prioritizing the safety of patients and staff, organized dissemination of information and systematic monitoring as well as evaluation are the most significant points to deal with the epidemic crisis.*

Keywords: *management, hospital, pandemic, COVID-19*

INTRODUCTION

On January 30, 2020, the World Health Organization issued a statement declaring the new coronavirus an outbreak of a public health emergency that menaced the entire world, and not just China [1].

This epidemic extended immediately in most countries, including Iran, and sent a host of patients to hospitals in need of medical care [2,3].

Hospitals are the most important provider of healthcare services in any pervasive disease, involving COVID-19. Owing to the critical peak of COVID-19 in a short time and the abrupt enhancement of load in hospitals, the efficiency, and effectiveness of the hospital depend to a considerable extent on the management and effective use of resources [4,5]. Nowadays, it is believed that without dynamic management and mastery of modern management methods, the intended efficiency and effectiveness in the healthcare network and hospital system cannot be attained [6,7] and poor performance of hospital managers is a paramount factor in boosting costs and wastage of human and financial resources in crises [8,9]; however, hospital managers face many challenges and issues to manage the crisis in this significant and critical organization [10]. This study aims to investigate the operational management challenges of a reference military hospital in the COVID-19 disease crisis.

METHOD

This study is a qualitative research based on the content analysis method with a deductive and guided approach. Data collection was conducted through a semi-structured interview method and utilizing a questionnaire planned based on the management functions of the Hospital Incident Command System (HICS). In this study, a purposive sampling method was employed to interview 40 senior and middle managers in organized positions whose field of work was directly or indirectly involved in responding to the COVID-19 crisis.

In the beginning, intending to perform a semi-structured interview, we designed a questionnaire based on the management functions of the Hospital Incident Command System (HICS) and reviewed similar studies in eight areas; support, decision-making, incidents command system, health and Safety of patients and staff, information dissemination management, monitoring and evaluation, patients management and human resources. Afterward, to determine the validity and ensure the importance of the questions from the perspective of the respondents, five experts in the field were interviewed, and depending on their views, if necessary, the required corrections were made to address the shortcomings. The emphasis of the interview questions was on the positives and improvements of the hospital management, management challenges, and providing solutions to address these challenges.

Then, the research team coordination sessions were held to perform the same interview. The samples were selected purposefully by selecting managers who had a significant operational role in the corona pandemic peaks. Among the most significant inclusion criteria were being experienced in chosen specialized areas, experiencing active participation in the COVID-19 crisis in the hospital, the capability to express oneself well, and having time for interviews. In the meantime, people who did not have the time or desire to participate in the study and interview were excluded from the study. Participants in the interview were selected based on the purposive and snowballing methods. In this regard, the people who had the most information about the subject were selected and each person was asked to introduce other participants and people who met the inclusion criteria (at least two people).

The interviews were performed by making an appointment and selecting the place of the interview from the participant's point of view for their convenience. During the interview, the interviewer began with open-ended questions for the respondent to feel comfortable and freely express his feelings, thoughts, and views. The questioner then proceeded with the interview based on the designed questionnaire. This study was performed in the form of attitudes of hospital operational management and the views of all stakeholders, including patients, were not considered. The analytical points of this study were based on summarizing the findings of interviews with middle operational managers in the hospital and evaluating as well as investigating the acquired content in expert meetings with the presence of the research team. Data analysis was performed employing MAXQDA 2018 software. Considering the deductive approach of this study, the initial coding was conducted in the form of HICS structure classifications and ultimately, the final classification was performed utilizing the software. Afterward, the obtained results were reviewed and finalized in a two-hour absentee panel with senior policymakers. To study, the code of ethics was obtained from the ethics committee in biomedical research. In the meantime, the principles of information confidentiality were considered. The results of this study were finally announced to senior management and managers of the studied units to help make managerial decisions.

RESULTS

The findings of the current study were assessed in the form of examining the strengths, improvements, and challenges of operational management in the hospital in the face of the issues caused by the corona pandemic.

Shortcomings are those that, if completed and improved, the system shall be better prepared for future crises, and via solving the problems of these cases, better services can be offered in the future. In the study areas, the highest frequently repeated point in the interviews was the surprise and unpreparedness for the epidemic crisis, despite global and regional warnings. Exploring the points for improvement in the eight areas studied led to the following key points (Table 1).

Strengths are those points that have been administered well and the organization has been capable of managing them properly, effectively, and promptly. In the study areas, the key point repeated in most interviews and all domains was the devoted and altruistic presence of medical staff and managers. Examining the strengths in the eight studied areas led to the following key points (Table 2).

Table 1: The points for improvement in the eight key elements

Supports	Deficiency of creating a suitable depot despite knowing the outbreak of the disease in the world and the existence of a suitable opportunity Presence of process problems in the field of logistics and non-separation of duties of the drug unit and support unit Shortcomings in the existence of standard warehouses for the storage of masks and disinfectants and non-compliance with standard principles in the storage area can be catastrophic.
Health and Safety of patients and staff	Severe shortcomings in education and information in the field of health and safety of patients Lack of particular program and discipline in supplying personal equipment to staff and long and excessive use of equipment and non-compliance with standards Lack of appropriate preparation despite being aware of the spread of the disease in the world, like infection control training and the use of personal protective equipment
External organizational decision making	Entering the COVID-19 crisis without a mission and a formal request
Internal decision making	Parallel work and lack of coordination between managers and waste of resources, particularly over the first weeks of the crisis Deficiency in targeting decision-making sessions
Incident management system	Deficiency of a written program in the system to isolate suspicious patients Defects in evaluating system performance Deficiency in explaining and comprehending the job description of system members
Information dissemination management	Lack of planning and taking operational measures to alleviate staff anxiety Failure to form a specialized working group for Information management with the presence of related experts
Monitoring and evaluation	Deficiency in monitoring the performance of non-therapeutic units The non-use of the results of evaluations and repeating past mistakes Defects in planning to solve the problems listed in the evaluations
Patient management	Non-segregation of patients from any section of coronavirus cases particularly in the emergency ward Defects in the forming of the psychological support team Deficiency in composing a systematic mechanism of follow-up of patients and training during discharge Defects in the process of disinfection of imaging sections Defects in educational methods and content of education about COVID-19 Defects in the targeting of preventing staff infection Lack of planning to remodel for other use and create a biological emergency
Human resources	Lack of planning and the requirement to train human resources after recruiting volunteers Deficiency in managing the organization of human resources, especially over the first weeks of the disease outbreak

Table 2: The points for strengths in the eight key elements

Supports	Financial support on behalf of headquarters
Health and Safety of patients and staff	Daily disinfection of environments (at the beginning of a pandemic) Promoting personnel safety by improving facilities Active participation of the infection control ward in training
External organizational decision making	Presence and continuous inspection of both the management teams of the organization and the Ministry of Health from the medical departments
Internal decision making	The flexibility of managers along with their continuous and responsible follow-up
Incident management system	Activating and holding daily meetings of the Hospital Incident and Disaster Risk Committee
Information dissemination management	Monitoring hospital news in the media continuously
Monitoring and evaluation	Daily and active inspection by the hospital management team, especially the continuous presence of the hospital chief
Patient management	Prominent, devoted, and altruistic presence of medical staff and managers
Human resources	Summoning and managing devoted and volunteer forces

Challenges are problems that appear as an obstacle to effective performance and will be solved with the efforts of the upper echelons of the organization. In the current study, the most important challenge was the poor coordination with the relevant organizations, including the headquarters, the Ministry of Health, and the Defense Organization, and other important challenges, consisting of the following (Table 3).

Table 3: The points for challenges in the eight key elements

Supports	Providing and supporting timely finance
Health and Safety of patients and staff	Lack of facilities for the presence of patients' companions for more appropriate care of coronavirus cases Being taken unawares over the first two weeks of the outbreak
External organizational decision making	Frequent alterations in treatment and prescription of medication (multiple protocols)
Internal decision making	Perpetual changes in notifications
Incident management system	Unpredictable number of patients and the time of crisis
Information dissemination management	Lack of trust of people and patients in news and information
Monitoring and evaluation	Failure to appoint the main trustee of supervision Lack of appropriate monitoring tools in infectious disease conditions such as specific checklists
Patient management	Deficiency in coordination and solidarity between the orders of the Ministry of Health and the headquarters
Human resources	Lack of support in the human resource supply chain on behalf of the headquarters (as an upper echelon of the organization)

DISCUSSION

The present study scrutinizes the operational management challenges of a reference hospital over the peak (first peak) of the COVID-19 pandemic. There are numerous experiences of dealing with pandemics, but the Covid-19 pandemic due to the extent and spread of the disease and in the light of treatment management and patients care is unique in the world. Numerous mutations and changes in the symptoms of the disease and many other unexpected conditions caused countries to face multiple peaks of the disease in their region and experience the crisis in every sense. Different definitions and some aspects of crisis have been mentioned in the relevant texts which are confirmed based on the findings of this study. For instance, Coombs has considered a crisis as an unpredictable incident and defines it as dealing with an unpredictable incident that menaces the stakeholders' expectations that can influence the function of an organization [11]. In this study, most managers pointed to the unpredictability of subsequent incidents at the peak of the COVID-19 pandemic, which caused a kind of surprise and confusion among managers. This issue was especially evident in the first stage of the peak (first peak). Of course, the point made by all participants in this study was the disregard for global warnings, which unfortunately dealt with the crisis of previous warnings like a completely unannounced situation!

Incident Management System

A robust crisis management system can address multiple potential problems in a crisis. The major problems in this section were delays in the call and poor response to the call in the early days, delays in notifications through the system, lack of transparency in the description of tasks in the system, and lack of evaluation of system performance, which made the system fail to respond to the crisis. The most significant solution to eliminate the deficiencies and problems listed in the hospital crisis management system involves compiling and communicating detailed job descriptions in the system, holding operational maneuvers based on predetermined scenarios in the field of infectious diseases, updating system processes and protocols, especially how to call human resources and their replacement as well as determining the evaluation team and compiling evaluation checklists based on existing scenarios [15].

Human Resources

One of the principal fields examined was the challenges relevant to human resources. There were two critical shortages in the field of human resources, firstly, the lack of effective human resources during the peak of the disease, and secondly, the lack of trained human resources, which was one of the main challenges in this field. Laschinger et al.'s study over 1996-2001 indicates that the lack of caregiver forces is one of the structural variables which by pressuring staff, causes them to feel dissatisfied and incompetent.

In Germany, the government summoned newly graduated medical students to work at hospitals that did not have enough staff [14]. In Italy, which at one time had the highest rate of COVID-19 in the world, the government asked new retirees to return to work [15]. Shortening shifts and enhancing the number of shifts, boosting salaries to augment motivation, maintaining the physical and mental health of staff, and preventing workforce erosion are other strategies [16]. The shortage of trained human resources reserved for use in crisis periods is another problem relevant to human resources in the present study. In other studies, the presence of crisis maneuvers is the most important way to get experience for such situations, and it sounds like in designing training maneuvers, staff should be provided with more realistic conditions and better training [17].

Equipment

The next serious challenge was the equipment shortage during the critical peak of COVID-19. The lack of equipment in crises has always been discussed by experts.

In other studies, another perspective of the crisis concept is the requirement for more resources. Harrison and Ferguson, for instance. have emphasized the need for more resources, arguing that the crisis is a baffling enigma that requires the use of more resources and immediate attention to maintain services [18]. In other studies, it seems that creating new resources and attracting sufficient resources can help solve the problem of lack of equipment and facilities [19].

If ample resources are not provided, equipment distribution based on prioritization is the main solution to this challenge. Clear and consistent criteria must be defined to prioritize allocation. At the same time, resource allocation policies need to receive up-to-date information at regular intervals from specific information sources [20].

Decision-making and accountability

Another significant challenge at the height of the corona was the kind of decision-making and crisis response. Taking unawares and, by its nature, non-missionary entry, without formal request and foresight, lead to the depletion of strategic reserves in a critical, treatment-oriented military organization.

On the other hand, postponement in management decisions has direct and indirect negative consequences on all organizational processes, including the duties and responsibilities of staff and the consequences of patients. Decision-makers and policymakers must improve timely and appropriate accountability in the organization by using optimization techniques such as maneuvers [21]. Studies have demonstrated that overcrowding of patients on the one hand owing to lack of facilities and on the other hand, due to increased stress, disrupts the decision-making process of managers [22]. This indicates the importance of timely response to organizational needs in such situations.

The general policies of an organization, even at crisis height, should by no means be ignored or prioritized. Having a pre-determined plan to deal with epidemic crises is the most remarkable way to prevent taking unawares and haste in decisions. In the meantime, constructing a robust documentation structure as a decision-supporting system helps managers to utilize past experiences and decline decision-making errors.

Health and Safety of patients and staff

The next critical challenge striking in this crisis was the safety challenge, both for the patients and the medical staff. Considering the safety of patients, the most significant threats were lack of companionship and hardship in meeting the basic needs of patients, such as nutritional requirements and personal hygiene. Meanwhile, educating patients about how to deal with COVID-19 disease during hospitalization and after discharge in cases such as the duration of quarantine, duration of taking drugs and indication for discontinuation of drugs, type and method of nutrition in different stages of the disease, etc. had not been sufficient. The important point is that this training should be performed with a precise mechanism with specific protocols and should be taught professionally; however, this has not been achieved.

Concerning the safety of staff, lack of training on how to utilize personal protective equipment, long shifts and long-term use of PPE, nutritional status during shifts, and emergency visits to infected patients without full protection are among the problems that threaten the safety of staff which in some cases, has transmitted the disease to them.

Information dissemination management

At the onset of the crisis, the spread of exaggerated information and rumors from unofficial sources caused fear, irrational attitude, or withdrawal of some medical staff from dealing with patients. It appears that under the warning system of the hospital crisis management system, the formation of a specialized working group for news management and the design of an integrated, continuous, and regular information system that covers all hospital staff, along with staff training in distinguishing news and disseminating reliable news is of great importance.

Monitoring and Evaluation

The next recognized challenge for the hospital in this crisis was the lack of systematic, planned, regular, and constant evaluation. Evaluation and monitoring are the main basis of planning and determining the next steps in dealing with crises. Determining particular indicators or designing an integrated checklist for monitoring and evaluation can make evaluation effective [26], and the strategic information provided by these indicators can help decision-makers at the hospital level, or even at the national level, to have a logical and evidence-based process for crisis management. Such indicators and checklists in times of crisis should either be pre-planned or designed as soon as possible so that time is not lost.

Patient management

The crowd of patients in the hospital can have a couple of consequences. On the other hand, it enhances the incidence of medical errors, escalates mortality, and waiting time for patients and prolongs the length of hospital stay, and leads to disruptions in receiving treatment and care, and ultimately patient dissatisfaction. On the other hand, a high workload causes fatigue and burnout for the staff.

Another critical issue was the lack of a psychological support team for staff and patients, so over the first weeks when the various dimensions of the disease were still unknown, both the medical staff and most of the patients went through stressful and scary days. The need to go through such difficult days requires the formation and support of professional teams in the field of psychological support, whose vacancy at the height of the COVID-19 crisis was quite felt in the hospital. No such team has been formed so far, while psychological support interventions in dealing with crises are an integral part of crisis response worldwide [24].

In China, on January 27, the National Health Commission issued its first comprehensive guideline for psychological intervention in people with COVID-19, highlighting the need to provide mental health support to patients and caregivers. In Singapore, psychological support is also one of the five components of patients' treatment strategies that focus on psychiatrists [25].

To conclude, this study generally has two kinds of influences. On the one hand, the spectrum of crisis outcomes involves the beneficial and effective effects such as empowerment, participation, and self-sacrifice of staff, and on the other hand, it involves the negative and at the same time strong effects such as lack of equipment and medicine and dissatisfaction with services for patients and lack of equipment and high workload as well as fatigue for the staff. The consequences of the crisis have also been mentioned in relevant texts and as James and Gillland believe, the crisis is both a danger and an opportunity to experience [26]. Crisis experience helps individuals to show appropriate interventions in managing critical situations that lead to growth and increase motivation for change in the future.

CONCLUSION

This qualitative study illustrates the experience of managers and officials of a reference hospital in the challenges and consequences of the Covid-19 pandemic. Robust documentation and evidence-based decision-making, structured coordination of internal and external organizations, effective and practical use of crisis management systems, basic training of staff and patients, prioritizing patient and staff safety, organized dissemination of information, and systematic monitoring and evaluation are the significant tips for dealing with a crisis such as the Covid-19 pandemic.

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Ethical approval

The ethics code of this research (IR.BMSU.BAQ.REC.1400.016) has been officially received from the National Ethics in Biomedical Research system of the Islamic Republic of Iran Ministry of Health and Medical Education. All stages of this research were performed under national ethical standards.

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ORIGINAL ARTICLE

The Relationship Between the Tolerance for Uncertainty and Psychophysiological Qualities of Servicemen

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Abstract: *The purpose of this study was to establish the relationship between the tolerance for uncertainty level and several psychophysiological and psychological qualities of military specialists for determining additional approaches to occupational selection. The study was conducted on a group of servicemen who performed special tasks and were capable of responding to terrorism – 49 men (main group) and 19 military doctors (control group) – men aged 25-34 years. Tolerance for uncertainty was studied based on the modified S. Badnder's method. The level of manipulation score (MAC) was assessed by the adapted I.A. Romanova and O.O. Zhdanov questionnaire (estimated by the so-called "MacScale"). Psycho-physiological parameters were determined using a special computer program. Statistical analysis of data was performed by descriptive and nonparametric statistics, as well as a cluster, stepwise discriminant, and correlation analysis. A personality trait "tolerance for uncertainty" was valuable for the occupational activity of servicemen performing special tasks. The such feature had 63.3% of servicemen in the studied group. The specificity of the occupational activity of military doctors was the quality of manipulation of people. Moreover, those who were the most tolerant of uncertainty had an inverse relationship with Machiavellianism, which indicates the existence of compensatory psychological mechanisms balancing and harmonizing service and personal relationships among micro-teams of servicemen. Servicemen with a lower level of tolerance for uncertainty had positive relationships with several psychophysiological functions (strength and mobility of nervous processes, quality of dynamic memorization), which confirms the idea of psychophysiological mechanisms to enhance tolerance for uncertainty by increasing the working capacity of these people. Such personal quality as tolerance for uncertainty is a valuable occupationally important feature that allows performing the selection of servicemen who are more efficient and reliable in the performance of official duties tasks.*

Keywords: *servicemen, psychophysiological and psychological qualities, tolerance for uncertainty*

INTRODUCTION

The work of a military specialist is always associated with the in-depth complexity and dynamic variability of performed tasks, which causes an increased risk of obtaining a positive result, and increases the emotional stress of the specialist. The ability to adequately respond to extreme factors has a positive effect on the reliability of occupational activities [1].

Several psycho-emotional stress factors in the combat zone have been identified [2]. One of them was the uncertainty of the enemy's behavior, which did not allow to assess his strength; the formation of distrust in the command of combat operation, which affected the understanding of the implementation of certain maneuvers; failure to conduct timely rotation of servicemen from the active hostilities zone, which caused their mental and physical overload; the beginning of positional warfare, the monotony of which is exhausting for the human being; command policy on the ceasefire regime when the enemy continues to fire, etc.

The presence of such respectable reasons determines the existence of strict requirements for the psychophysiological and psychological qualities of servicemen, in particular, resistance to the unpredictable influence of various extreme factors, as well as the quality of being tolerant of uncertainty

[3,4,5]. According to [6] the concept of resilience to uncertainty is associated with the existence of a self-regulation mechanism in the form of compensation for the uncertainty of choice based on the use of all possibilities of their intellectual and personal potential.

If tolerance for uncertainty can be perceived as a willingness to accept the event with a certain tolerance and endurance, to be satisfied with partial and perhaps not entirely irrational problem solutions, then, to some extent, the opposite term - intolerance reflects the process of active "conservation" and negative attitudes to existing differences in situations where new events have been formed [3]. The authors showed that a more tolerant attitude towards uncertain situations in servicemen evokes a calm, positive attitude towards these situations, which helps them to control and regulate their emotions, reduce stress and show self-control in problematic situations. It was also shown that in the conditions of testing the possibility of using prompts, persons tolerant of uncertainty used the opportunity to raise their awareness, and intolerant – ignored this possibility [7].

Tolerance for uncertainty is one of the most important factors in improving the reliability of occupational activities, which affects the combat effectiveness of servicemen and other professionals in extreme jobs [8]. Therefore, the analysis of this characteristic in the military is relevant and will have a significant effect taking into account such properties as tolerance for uncertainty in the occupational selection and other measures for improving the human capital of the Armed Forces of Ukraine.

The objective of this study

The purpose of this study was to establish the relationship between the tolerance for uncertainty level and several psychophysiological and psychological qualities of military specialists for determining additional approaches to occupational selection.

MATERIALS AND METHODS

The study was conducted on a group of servicemen who performed special tasks and were capable of responding to terrorism – 49 men (main group) and 19 military doctors (control group) – men aged 25-34 years. Tolerance for uncertainty was studied based on the modified S. Badnder's method (Tolerance Ambiguity Scale) [9,10] using scales: novelty, complexity, insolubility, and total uncertainty. According to the literature, the scales of the questionnaire S. Badner are independent of gender and age [11].

In addition, tolerance for uncertainty was associated with high levels of interpersonal intelligence and self-efficacy, and intolerance was associated with decreased verbal intelligence. Therefore, these parameters can be used to diagnose tolerance for uncertainty for occupational selection.

The level of manipulation score (MAC) was assessed by the adapted I.A. Romanova and O.O. Zhdanov questionnaire (estimated by the so-called "McScale") [12,13,14], consisting of twenty statements.

Psychophysiological parameters were determined using a special computer program [15,16,17]. The quality of dynamic memorization (QDM), indicators of strength, and functional mobility of nervous processes (SNP and FMNP, respectively) were determined.

All examinations were performed under the ethical standards of the responsible committee and the Helsinki Declaration and have been approved by the Bioethics Commission of the Ukrainian military medical academy.

Statistical analysis of data was performed by descriptive and nonparametric statistics, as well as a cluster, stepwise discriminant, and correlation analysis using the software package STATISTICA 13.3, license AXA905I924220FAACD-N.

RESULTS

First of all, it should be noted that the data on the components of tolerance for uncertainty among the main group in the previous analysis were heterogeneous. Therefore, it was decided to divide this group into two homogeneous subgroups based on these parameters. This procedure was performed using cluster analysis (method k – means). The obtained data, as well as data on other psychophysiological and psychological parameters inherent in these subgroups, are given in Table 1.

Consideration of the data in the table shows that all four signs of tolerance for uncertainty were significantly different in subgroups 1 and 2. According to the classification in [9], people of the study group on the "novelty" scale were on the average level of expression, on the scale of "complexity" were on the average level of expression, on the scale of insolubility were on the average level of expression for subgroup 1 and high level of expression, as well as a general indicator – was at the average level of severity. However, the levels of indicators in subgroup 2 were significantly higher (marked by the sign "↑") than in subgroup 1. Therefore, representatives of subgroup 2 can be considered more tolerant of uncertainty. This was evidenced by the increase in the values of tolerance for uncertainty characteristics in subgroup 2, specifically: on the scale of "novelty" by 18.5%, on the scale of "complexity" by 19.2%, on the scale of "insolubility " by 26.3% and the scale of "total uncertainty" – by 20.8%.

Table 1: Characteristics of psychological and psychophysiological parameters of subgroups 1, 2, and control group

Parameters	Subgroups		$100 \times (M_x - M_y)/M_y$, %	Control group (n=19)
	1 (n=18)	2 (n= 1)		
Novelty, *c.u.*	13.0 ± 0.5 **	↑ 15.4 ± 0.7	18.5	13.9 ± 0.6
Complexity, *c.u.*	29.1 ± 0.6 ***	↑ 34.7 ± 0.8	19.2	33.8 ± 0.9 ^^^
Insolubility, *c.u.*	13.7 ± 0.3 ***	↑ 17.3 ± 0.5	26.3	13.4 ± 0.8ººº
Total uncertainty, *c.u.*	55.8 ± 0.7 ***	↑ 67.4 ± 1.1	20.8	61.1 ± 1.3 ^^^ ººº
Quality of dynamic memorization, *s*	0.61 ± 0.05	0.64 ± 0.07	-	-
The strength of nervous processes, *ms*	359.4 ± 8.4 *	↑ 320.4 ± 14.6	12.2	-
Functional mobility of nervous processes, *ms*	286.0 ± 7.1 * *	↑ 253.4 ± 11.9	12.7	-
Level of manipulation, *mac*	67.6 ± 3.4	67.6 ± 4.8	-	↑ 75.8 ± 2.4 ^^^ ººº

*Note: *, **, *** significant difference of means between subgroups 1 and 2, by Student's criterion, corresponds to the level p <0.05, p <0.01, p <0.001. ^^^ - a significant difference of means between subgroup 1 and the control group by Student's criterion corresponds to the level p <0,001. ººº – a significant difference of means between subgroup 2 and the control group by Student's criterion corresponds to the level p <0,001. Mx, My – respectively larger and smaller values of the indicator (reflects the degree of a better expression of psychological and psychophysiological qualities in subgroups 1 and 2). ↑ – a sign of improved performance.*

According to C. Arbona and co-authors [18] people with low values of the variable "novelty" find it difficult to perceive incomprehensible dual situations, the unusualness of the situation causes them some actions in professional activities and ignorance of prospects, they perceive new tasks as unsolvable, individuality and independence cause anxiety and discomfort in new problematic situations among such professionals, and stability – as a necessary condition favorable for personal and professional life. These authors believe that the higher the value of the variable "complexity", the more such individuals have the opportunity to solve complex and intractable difficulties in professional activities, they are ready to compete, overcome obstacles, and gain new knowledge, they need to gain positive value for others, do good deeds for people, etc. Such specialists are ready to solve complex professional issues within which experience has already been acquired. Those who are more tolerant of uncertainty will compete more actively and strive to win professionally. They prefer to overcome difficulties and try to solve them creatively in a situation of uncertainty, using a variety of approaches.

Thus, subgroup 2 servicemen, who compound 63% of those whose professional duties were focused on special tasks and were more tolerant of uncertainty, have better adaptability to perform their duties. As V. Ryutin emphasized [19] content of high tolerance for uncertainty is the ability of servicemen in problematic and crisis situations to actively interact with the environment and constantly restore their mental balance, help prevent confrontation and develop positive relationships with themselves and others. It should also be emphasized that the psychophysiological indicators of the working capacity of servicemen of subgroup 2 (according to the SNP and FMNP) have a significantly better level. This indicates better endurance and the ability to perform operations faster in the ever-changing environment of these individuals.

Correlation analysis (Spearman's correlation coefficient, p <0.05) was used to further consider the peculiarities of the formation of behavioral reactions among representatives of subgroups 1 and 2. The results of this analysis are shown in Figure 1. In this figure, the representatives of subgroup 1 were conditionally defined as intolerant, and the representatives of subgroup 2 – as tolerant. This separation was based on the assumption that the levels of all tolerance indicators of subgroup 2 were significantly higher than those of subgroup 1.

Figure 1: Relationship between psychological and psychophysiological functions with indicators of tolerance to uncertainty in servicemen of subgroups 1 and 2. T – tolerance for uncertainty (in servicemen of subgroup 2); IT – intolerance for uncertainty (in servicemen of subgroup 1). Continuous line – direct correlation; dashed line – negative (inverse) correlation

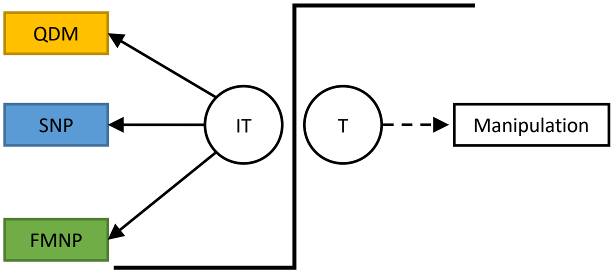

The figure shows that the indicators interacted differently in subgroups 1 and 2. Subgroup 1 was characterized by the relationship of indicators of intolerance with a set of psychophysiological parameters that reflect the correlates of performance. From this fact we can conclude that people with a reduced tolerance for uncertainty were related to the level of efficiency of subgroup 1. All applied psychophysiological techniques were characterized by the unpredictability of each stimulus in terms of short-term or long-term processing of information. Another special feature of the registered responses of representatives of subgroup 1 was the independence of using short-term memory when performing tasks, because when testing the strength and mobility of nervous processes, this memory is used in minimal amount, and when testing dynamic memorizing, this feature is present at all times.

With a higher tolerance for uncertainty, a different picture was observed only with the level of manipulation (subgroup 2). Moreover, there was an inverse correlation. In this case, the increase in tolerance for uncertainty is accompanied by a decrease in the level of manipulation. As is known [13], the presence of more pronounced manifestations of morality and humanity is more common in the military, compared to civilians, who are significantly dominated by selfishness and the use of various tricks in management. Based on the data obtained in the present study, it can be assumed that high tolerance for uncertainty significantly unloads human capabilities, which increases the potential for morality and humanity.

The formulated conclusion is confirmed by the data of other authors. As M. Kovaleva et al. [20] had confidence that people with a high tolerance for uncertainty will demonstrate mental resilience and professional reliability, and people with a low tolerance for extreme conditions will have low levels of these qualities. According to [6], this psyche feature affects the ability of self-regulation of the person to overcome the uncertainty of choice based on the use of all possibilities of their intellectual and personal potential.

The characteristics of the control group for all indicators of tolerance for uncertainty were between the values of identical characteristics of subgroups 1 and 2. But the rate of manipulation of individuals in this group was much higher than that of subgroups 1 and 2. Since the control group consisted of military doctors that habits of manipulation were inherent in their profession, where communication with the patient requires certain specific skills to affect his mental sphere. In this case, there was no correlation between the Machiavellianism (level of manipulation) of the control group and subgroups 1 and 2. This result may indicate that high levels of Machiavellianism mask a tolerant attitude towards the patient.

DISCUSSION

The analysis of the obtained data testifies to the value of the uncertainty tolerance indicator for determining the professional suitability of a serviceman, especially those contingents that perform complex special tasks. This thesis is confirmed by many other authors. Thus, M. Kovaleva et al. data in the study of tolerance for uncertainty in officers showed the dependence of their mental adaptability on the conditions of service, which allowed us to conclude the need to determine the professional suitability of officers when transferring them to a military unit [20]. Barko V. et al. [9] stressed that tolerance for uncertainty was an occupationally important feature of a police officer in the selection process, and during professional training, this quality must be diagnosed, taken into account, strengthened, and developed in every possible way. Principally such personal quality, such as tolerance for uncertainty, will increase the efficiency of task performance, as well as allow better endurance of unforeseen situations that constantly accompany official activities; open opportunities for creative thinking of complex professional situations, search for alternative solutions; increase stress resistance and strengthen productive mechanisms of psychological protection of the individual (rationalization, sublimation, displacement); strengthen the psychological readiness and reliability of the individual to act in special and extreme conditions; will help preserve officers’ mental health".

Thus, as a result of this study, it was found that the vast majority of tested servicemen (63.3%) who perform complex special tasks had a fairly high level of tolerance for uncertainty. This allows us to make a reasonable assumption that tolerance for uncertainty is a valuable professionally important quality, which allows us to separate those servicemen who are more efficient and reliable in the performance of official duties.

CONCLUSION

1. A personality trait “tolerance for uncertainty” was valuable for the occupational activity of servicemen performing special tasks. Such feature had 63.3% of servicemen in the studied group.

2. The specificity of the occupational activity of military doctors was the quality of manipulation of people. Moreover, those who were the most tolerant of uncertainty had an inverse relationship with Machiavellianism, which indicates the existence of compensatory psychological mechanisms balancing and harmonizing service and personal relationships among micro-teams of servicemen.

3. Servicemen with a lower level of tolerance for uncertainty had positive relationships with several psychophysiological functions (strength and mobility of nervous processes, quality of dynamic memorization), which confirms the idea of psychophysiological mechanisms to enhance tolerance for uncertainty by increasing the working capacity of these people.

4. Such personal quality as tolerance for uncertainty is a valuable occupationally important feature that allows

performing the selection of servicemen who are more efficient and reliable in the performance of official duties tasks.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Authors' contribution

Conceptualization, Kalnysh, Shvets; methodology, Kalnysh, Shvets; software, Kalnysh; validation Shvets; formal analysis, Shvets, Maltsev, Nagovitsyn; investigation, Shvets, Maltsev, Nagovitsyn; resources Shvets, Maltsev, Nagovitsyn; data curation, Shvets, Maltsev; writing—original draft preparation, Shvets, Maltsev; writing—review and editing Shvets; visualization, Maltsev, Nagovitsyn; supervision, Shvets; project administration, Kalnysh, Shvets; funding acquisition, Kalnysh. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the Ukrainian military medical academy (protocol 122, 12/09/2019) for studies involving humans.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

Written informed consent has been obtained from the patient(s) to publish this paper.

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ORIGINAL ARTICLE

The Effect of Reciprocal Imitation Training on Increasing Expressive Vocabulary of Children with Autism Spectrum Disorders

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Abstract: *Objective: One of the areas of impairment in childhood autism spectrum disorders is delayed imitation. Due to the defect in the social function of imitation in these children and its negative consequences on lexical and verbal skills, Researchers in the field of educating autistic children consider increasing imitation skills to be a very important therapeutic goal. Previous studies have shown the effects of improving imitation on increasing the social, linguistic, and verbal skills of these children. In the present study, the effect of reciprocal imitation training on increasing the vocabulary of autistic children was investigated. Materials and Methods: This was an interventional study with a pretest-posttest design. 16 autistic children were trained in cross-border imitation based on expressive vocabulary in 96 sessions. After performing post-test and data collection in data analysis from the McNemar test to determine the changes of each word before and after the intervention and the Wilcoxon Marked Test was used to determine changes in vocabulary categories before and after the intervention and the level of statistical significance was considered (P> 0.05). Results: The results of data analysis show that the average number of responses "expresses" in each of the words after the intervention increased significantly in all vocabulary groups (P> 0.05). It seems that the intervention resulted in significant changes in participants. Conclusion: The results of this study show that teaching the method of reciprocal imitation has a significant effect on increasing the number of expressive words in autistic children.*

Keywords: *autism spectrum disorder; reciprocal imitation; expressive vocabulary*

INTRODUCTION

Childhood autism spectrum disorders are characterized by stereotyped and repetitive behaviors, impaired verbal and non-verbal communication, and impaired social behaviors. The prevalence of autism in 2014 in Iran was reported to be 95.2 per 10,000 children [1,2]. The skill of imitating others is one of the essential abilities for newborns [3]. Various studies have shown an association between imitation and social communication skills such as language, play, and attention. Some researchers believe that imitation in children has two important functions: 1- Imitation of sounds, and facial expressions to communicate and social interaction with others; 2- Enabling the child in the first half of life to be aware of activities and the intention of the other person and the subsequent formation of social learning in the child [4].

However, research has shown that children with autism spectrum disorders are deficient in the use of the social function of imitation [5]. From the first study of the nature of imitation in children with autism spectrum disorders by Damier (1972) to recent research on the fact that children with autism spectrum disorders develop the ability to imitate normal children and other disabilities. They are weaker, there is agreement [6]. Sigman and Angers (1984) reported that these children delayed voice imitation and body movement imitation compared with normal children and children with developmental delays [7]. Due to the impairment in the social function of imitation, which may have many negative consequences, numerous researchers have paid special attention to the treatment of imitation impairment in these children [8].

Researchers in the field of education of children with autism spectrum disorders now consider the increase of imitation skills as a very important therapeutic goal and believe that the improvement of imitation skills will lead to an increase in social communication skills [9]. In general, in the field of social interaction skills training, two therapeutic models of classical structured behavioral interventions can be included, including the applied analysis method, Lavas, and Pax, and in contrast, the evolutionary model that imitates spontaneous imitation skills during natural interaction. It has a special effect on increasing flexibility, social imitation, and other social communication skills, and includes natural fluoride behavioral interventions, comprehensive interaction methods, and cross-imitation training [10]. The method of mutual imitation teaching is a kind of natural intervention program and its main emphasis is on the social role of imitation in improving speech and non-verbal skills [11].

Unlike structural imitation instruction, in reciprocal imitation instruction, the therapist imitates all of the child's gestures, words, and actions using toys, and the child's level of interest as well as imitation of the child's speech, proverbial pronunciations, and use. Language and speech are spontaneously considered by the therapist [12]. Enjoyment of communication, perception of people's faces, cooperation, understanding of non-verbal concepts and signs, ability to pay attention to another person, perception of meaning in sounds, ability to choose, perform subtle movements, spatial awareness, the pleasure of being in a group, from the most important therapeutic goals in the teaching method are mutual imitation [10].

Studies on the method of mutual imitation training by Ingersell (2003) show that this method increases social communication skills such as social interaction, language, pretend play, and the use of body movements [10]. For example, a longitudinal study of children with autism showed that there was a relationship between mimicry of body movements and the development of expressive language in the next six months [13]. Wallen, Schreibman, and Ingersell (2006) showed that educating children with autism spectrum disorders using cross-imitation leads to an increase in behaviors related to social communication skills in imitation, play, and spontaneous speech [14]. In another study, Ingersell and Laland (2004) examined the effect of object and gesture imitation training on the speech and language of 4 children with autism spectrum disorders. The results of this study showed that the method of mutual imitation education leads to an increase in a speech in children with autism spectrum disorders [15].

Also, in another study, Pusheneh, Zadeh Mohammadi, Siampour (2012) examined the effect of cross-imitation training on the ability to imitate children with autism spectrum disorders. The results showed that cross-imitation training has increased the ability of these children to imitate in the fields of action-related imitation, gesture imitation, and facial expressions [16]. Given that the ability to imitate is the most important in the field of early childhood education, this has created the need for this research. Concerning this issue, the present study investigates the effectiveness of cross-mimicry training interventions on increasing the vocabulary of children with autism spectrum disorders.

ANALYSIS METHOD

The research method was an intervention with a pretest-posttest design without a control group. The statistical population included children aged 3 to 7 years with autism spectrum disorders in two rehabilitation centers with autism spectrum disorders in Lorestan province, Khorramabad-Iran, and Boroujerd-Iran, which were selected by convenience sampling method. Based on the available files of the clients in the rehabilitation center and by the diagnosis of the center psychiatrist and clinical observation and diagnostic tests in the files including Garz and the autism assessment checklist of diagnostic criteria (DSM-V-TR) of all clients of the center. At that date, they had been diagnosed with Autism Spectrum Disorders, and due to the comprehensiveness of the assessments, they were not given a separate diagnostic test.

The sample size in this study is 16 people (4 girls and 12 boys) with a mean age of 55.88 months with a standard deviation of 17.02. The age range of participants is 36 to 83 months (Table 1). 9 of these 16 people were trained in the Rehabilitation Center for Children with Autism Spectrum Disorders in Khorramabad and 7 others were trained in the Rehabilitation Center for Children with Autism Spectrum Disorders in Boroujerd.

Table 1: Frequency distribution of age

	Number	Average (Mean)	Standard deviation	At least (Min)	Maximum (Max)
Age	16	55.88	17.02	36	83

The severity of autism symptoms in these children was based on DSM-V levels 1 and 2 and level 3 children were excluded from the study due to the high severity of symptoms. The inclusion and exclusion criteria in this study were as follows: (1) being in the range of 3 to 7 years; (2) diagnosis by the psychiatrist of autism and tests by the center to classify children with autism spectrum disorders; (3) having a normal hearing threshold and healthy vision based on medical records and clinical observation; (4) No seizures and no delay in the development of motor skills by reviewing the medical file and the developmental and personal information form of the children, which was filled in by the parents and educators of the center. According to the same criteria, 4 children were excluded from the study.

A pre-test was performed before the intervention. The list used included 49 words that were placed in 7 lexical categories. These 7 categories were: body parts, verbs, clothing, fruits, persons, objects, and foods. For each category, 7 words were selected from the most used words used by normal children.

A pre-test was performed for each child individually using pictures and objects related to each word. The child's answer is marked in two columns/does not express was marked. After conducting the pre-test for children, a cross-imitation training intervention program was implemented for 12 months in 96 sessions. Each child was trained 2 days a week for 30 minutes each time by a speech and language pathologist trained at the center. During the training, the therapist first imitates the children's play with toys in the treatment room of the center, to attract the child's attention by imitating body movements and spoken sounds, and to prepare him for imitation. The therapist begins the training by identifying the activities and toys that the child is familiar with. For example, throwing a ball that the child is already familiar with and throwing. Cards or objects and gestures related to that word were then used to motivate the child to imitate and express the word. The therapist encouraged and strengthened any efforts and voices of the children to imitate the movement and sounds of the word.

At the end of the training sessions, the children were tested based on the same list of 7 lexical categories, and the children's responses to the pictures or objects related to each of the words in the list are expressed in two columns. Slow registration and data were collected. In this study, number and percentage indices for qualitative variables and mean and standard deviation indices for quantitative variables were used to determine frequency tables. In data analysis, McNeamar test was used to determine the changes in each word before and after the intervention, and Wilcoxon Signed Rank test was used to determine the changes in vocabulary categories before and after the intervention.

All analyzes were performed by SPSS software version 21 and the level of statistical significance was considered 0.05.

RESULTS

To obtain a more accurate measure of the effect of the intervention on each group of words, according to Table 2, the number of items related to the column "Expresses" in each category was counted and a score was set for before and after the intervention. For this purpose, the number of "expressed" responses in the table below was evaluated as a score.

Table 2: The score analysis "expresses" the number of answers in each group of words

		Average (Mean)	Standard deviation	At least (Min)	Maximum (Max)	Significance*
Organ	Before	0.50	0.97	0	3	0.003
	After	3.69	2.96	0	7	
Verbs	Before	0.56	0.89	0	2	0.003
	After	2.69	2.47	0	7	
Clothing	Before	0.25	1.00	0	4	0.011
	After	2.13	2.47	0	7	
Fruits	Before	0.94	1.29	0	4	0.003
	After	3.19	2.79	0	7	
People	Before	1.00	1.03	0	3	0.003
	After	2.75	2.29	0	7	
Equipment	Before	1.19	1.17	0	3	0.007
	After	2.81	2.66	0	7	
Foods	Before	0.13	0.34	0	1	0.005
	After	2.38	2.60	0	7	

** Wilcoxon Signed Rank Test.*

The results show that the average number of "expresses" responses in each of the words after the intervention increased significantly in all vocabulary groups (P <0.05). In other words, in general, the intervention was effective in all vocabulary groups. Among the 7 lexical categories, the group related to body parts shows significant changes for each of the 7 words, while the clothing category with a significant level for only one word shows the least effective intervention method among the classes. The results of this study according to Table 3 show that the expression of all words of the body class has significantly improved after the intervention (P <0.05). In other words, the intervention was effective in all vocabulary related to the organ class. Among the 7 words in this category, three words (eyes, ears, and tongue) were not uttered by any of the children studied according to the pre-test design. But after performing the method, words (eyes and ears) were expressed by 7 people and words (language) by 5 autistic children in the study.

Table 3: Summary of vocabulary analysis related to body class

		Before		After		Significance*
		Number	%	Number	%	
Hair	Does not express	13	81.3	5	31.3	0.008
	Express	3	18.8	11	68.8	
Hand	Does not express	14	87.5	6	37.5	0.007
	Express	2	12.5	10	625	
Leg	Does not express	14	87.5	6	37.5	0.008
	Express	2	12.5	10	62.5	
The eye	Does not express	16	100.0	9	56.3	0.016
	Express	0	0.0	7	43.8	
Ear	Does not express	16	100.0	7	43.8	0.004
	Express	0	0.0	9	56.3	
Nose	Does not express	15	93.8	9	56.3	0.031
	Express	1	6.3	7	43.8	
Tongue	Does not express	16	100.0	11	68.8	0.031
	Express	0	0.0	5	31.3	

** McNemar Test.*

The results of this study according to Table 4 show that the vocabulary expression of the verb class (give, come, fall, not, and go) has significantly improved after the intervention ($P < 0.05$). In other words, the intervention has been effective on these words. But as can be seen in Table 5, the other two words (coming and kissing) do not show significant changes. Vocabulary expression for clothing (shoes) improved significantly after the intervention ($P < 0.05$). In other words, the intervention has been effective in this term. But the expression of other words of this class (pants, hat, socks, clothes, blouse, and slippers) do not show significant changes after the intervention (Table 5). The expression of 3 words of fruit class (apple, banana, and pomegranate) significantly improved after the intervention ($P < 0.05$). But other words (orange, cucumber, berry, plum) do not show significant changes. In this category, the word orange was not uttered by any of the children according to the pre-test design, but after the intervention and post-test, 2 children uttered the word (Table 5). Among the 7 words in the category of persons, after the intervention, the expression of two words (father and uncle) has significantly improved ($P < 0.05$). But other words (mom, uncle, aunt, grandfather, and grandmother) do not show significant changes. Vocabulary (grandparents, aunts) was not spoken by any of the children before the intervention. After performing the post-test, the word grandfather was uttered by 2 people, the word grandmother was uttered by 1 person and the word aunt was uttered by 2 children. However, these changes do not show significant levels (Table 5). The expression of two items of equipment class (dolls and cars) significantly improved after the intervention ($P < 0.05$). In other words, the intervention has been effective on these words. But other words (spoon, mobile, plate, knife, glass) do not show significant changes. In these two classes, the words glass and knife were not uttered by any of the 16 people at the beginning of treatment, while after the intervention, 31.3% of children (5 people) had the word glass, and 18.8% of children (3 people) uttered the word knife. After the intervention, the expression of words (soup, bread, and food) from the food category significantly improved ($P < 0.05$). But the words (milk, meat, rice, and water) that at first none of the 16 people in the study in the pre-test design were able to express in the post-test design of two words milk and rice by 25% (4 people and word meat were expressed by 31% ($n = 5$) and the word water was expressed by 18.8 ($n = 3$) of children but did not show significant changes.

DISCUSSION

This study investigated the effect of reciprocal imitation training on increasing the vocabulary of children with autism spectrum disorders aged 3-7 years. The findings of this study showed that cross-curricular training improves the vocabulary of children with autism spectrum disorders and the average number of "expresses" responses in each vocabulary after the intervention is significant in all vocabulary groups increased ($P < 0.05$). In explaining the results, it can be stated that it is possible that the method of mutual imitation training, which involves the child engaging in fun activities by taking over the treatment space, focuses attention on communication and stereotyped behaviors during this process.

Table 4: Summary of vocabulary analysis results related to 7-word words of vocabulary that show significant differences

Vocabulary		Before		After		Significance*
		Number	%	Number	%	
Give	Does not express	11	68.8	5	31.3	0.014
	Express	5	31.3	11	68.8	
Come on	Does not express	12	75.0	6	37.5	0.014
	Express	4	25.0	10	62.5	
To fall	Does not express	16	100.0	12	75.0	0.046
	Express	0	0.0	4	25.0	
Is not	Does not express	16	100.0	8	50.0	0.005
	Express	0	0.0	8	50.0	
To go	Does not express	16	100.0	11	68.8	0.025
	Express	0	0.0	5	31.3	
Shoe	Does not express	15	93.8	7	43.8	0.008
	Express	1	6.3	9	56.3	
Apple	Does not express	12	75.0	5	31.3	0.016
	Express	4	25.0	11	68.8	
The banana	Does not express	13	81.3	6	37.5	0.016
	Express	3	18.8	10	62.5	
Pomegranate	Does not express	15	93.8	9	56.3	0.031
	Express	1	6.3	7	43.8	
Dad	Does not express	16	100.0	7	43.8	0.004
	Express	0	0.0	9	56.3	
Uncle	Does not express	15	93.8	7	43.8	0.008
	Express	1	6.3	9	56.3	
Dolls	Does not express	15	93.8	9	56.3	0.031
	Express	1	6.3	7	43.8	
Machine	Does not express	16	100.0	9	56.3	0.016
	Express	0	0.0	7	43.8	
Ash	Does not express	16	100.0	10	62.5	0.031
	Express	0	0.0	6	37.5	
Bread	Does not express	14	87.5	7	43.8	0.016
	Express	2	12.5	9	56.3	
Food	Does not express	16	100.0	9	56.3	0.016
	Express	0	0.0	7	43.8	

** McNemar Test.*

The child is guided and guided during play and the child acts more calmly and confidently. This increase in attention will be effective in communicating to increase children's ability to imitate and express words. Findings from the test of this hypothesis are consistent with the results of the research of Ingersell and Schreibman (2006), which was conducted to investigate the training of reciprocal imitation in children with autism spectrum disorders using a natural behavioral approach and its effect on language, pretend play and focused attention. Has [9]. It is also consistent with the results of research conducted by Ingersell and Laland (2010) on the effect of object imitation teaching and body movements on the language and speech of children with autism spectrum disorders using the cross-educational method [15].

Table 5: Summary of the results of vocabulary analysis related to 7-word words of vocabulary that do not show a significant difference

Vocabulary		Before		After		Significance*
		Number	%	Number	%	
Coming	Does not express	16	100.0	13	81.3	0.083
	Express	0	0.0	3	18.8	
Kissing	Does not express	16	100.0	14	87.5	0.157
	Express	0	0.0	2	12.5	
Pants	Does not express	15	93.8	11	68.8	0.219
	Express	1	6.3	5	31.3	
Hat	Does not express	15	93.8	10	62.5	0.125
	Express	1	6.3	6	37.5	
Socks	Does not express	15	93.8	12	75.0	0.375
	Express	1	6.3	4	25.0	
Dress	Does not express	16	100.0	12	75.0	0.125
	Express	0	0.0	4	25.0	
Blouse	Does not express	16	100.0	13	81.3	0.250
	Express	0	0.0	3	18.8	
Slippers	Does not express	16	100.0	13	81.3	0.250
	Express	0	0.0	3	18.8	
Orange	Does not express	16	100.0	14	87.5	0.501
	Express	0	0.0	2	12.5	
Cucumber	Does not express	15	93.8	11	68.8	0.125
	Express	1	6.3	5	31.3	
Berry	Does not express	13	81.3	8	50.0	0.063
	Express	3	18.8	8	50.0	
Plum	Does not express	13	81.3	8	50.0	0.063
	Express	3	18.8	8	50.0	
Mom	Does not express	10	62.5	6	37.5	0.125
	Express	6	37.5	10	62.5	
Uncle	Does not express	7	43.8	5	31.3	0.500
	Express	9	56.3	11	68.8	
Grandpa	Does not express	16	100.0	14	87.5	0.500
	Express	0	0.0	2	12.5	
Aunt	Does not express	16	100.0	14	87.5	0.500
	Express	0	0.0	2	12.5	
Spoon	Does not express	9	56.3	7	43.8	0.625
	Express	7	43.8	9	56.7	
Mobile	Does not express	7	43.8	7	43.8	0.999
	Express	9	56.3	9	56.3	
Plate	Does not express	14	87.5	11	68.8	0.375
	Express	2	12.5	5	31.3	
Glass	Does not express	16	100.0	11	68.8	0.063
	Express	0	0.0	5	31.3	
Knife	Does not express	16	100.0	13	81.3	0.250
	Express	0	0.0	3	18.8	

Milk	Does not express	16	100.0	12	75.0	0.125
	Express	0	0.0	4	25.0	
Water	Does not express	16	100.0	13	81.3	0.250
	Express	0	0.0	3	18.8	
Meat	Does not express	16	100.0	11	68.8	0.063
	Express	0	0.0	5	31.3	
Rice	Does not express	16	100.0	9	56.3	0.125
	Express	0	0.0	7	43.8	

** McNemar Test.*

In these two studies, the effects of reciprocal imitation training on increasing imitative communication skills have been pointed out and it has been stated that by engaging in this method through games and reciprocal imitation activities, the child enters into social interaction with the other party and thus Exchanges create opportunities that both parties can use during communication, and this is an important and effective strategy in expanding children's vocabulary and can increase communication skills.

The results of this study are also consistent with the findings of Ingersell (2008), who in an article examined the role of cross-imitation training on imitation skills in social communication conditions. Imitation motivates the development of social communication skills and interactions and social interaction, followed by improved social communication skills including language, play, shared attention, and eye communication [17].

All of this research demonstrates the positive impact of cross-curricular teaching on improving communication skills, including speech and language skills, in children with autism spectrum disorders.

A look at the basics of the basic components of a reciprocal imitation training program, including sitting face to face, imitating play with toys, imitating gestures and body movements, using speech imitation, and the activities and games that take place within this framework. It will help to understand why the subjects of this study have shown significant changes in improving their vocabulary. One of the most important limitations of this study was the limitation in the number of subjects.

To generalize the results of this study, it is better to study with a larger number of subjects. On the other hand, it is suggested that the words of other classes be used to evaluate the effectiveness of this method.

Future researchers are encouraged to study the effect of reciprocal imitation therapy in comparison with other therapies on improving the vocabulary of children with autism spectrum disorders.

CONCLUSION

The cross-mimicry training program improves the social and speech skills of children with autism spectrum disorders. This program can be implemented in the educational and rehabilitation environments of these children. The inclusion of such programs as one of the main themes of educational programs for children with autism spectrum disorders before primary school education can be facilitated in its application and implementation by medical and rehabilitation centers. Specialized services to these children, take an effective step towards teaching the social, speech, and vocabulary skills needed by children in educational settings.

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ORIGINAL ARTICLE

Nursing Journal Club as a Tool to Facilitate Evidence-Based Practice: Participant's Feedback

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Abstract: *Background and aim: Nursing journal clubs have a proven value in nursing education and continuing education, which has expanded widely in the promotion of evidence-based clinical care in recent years. This study aimed to evaluate the clinical nursing journal clubs from the viewpoints of nurses. Methods: This descriptive study was conducted from July 2019- October 2020. This study was conducted in two phases, consisting of designing the format and flowchart and implementing nursing journal clubs and the second phase was evaluation. Results: Format and executive flowchart prepared by the research team and reviewed and approved in the group. In terms of evaluation, minimum (33) and maximum (165) scores with a mean± SD total score of 123.49 ± 19.91 calculated for nursing journal clubs indicate an optimum quality. Conclusions: The qualities of nursing journal clubs were evaluated to be favorable. Accordingly, authorities and planners should consider appropriate actions to facilitate Nursing journal clubs, such as credit points in rank promotion for faculty members, and inclusive in continuing education privileges for nurses, to adapt these activities to the roles of the clinical faculty and the benefits of this educational method in nursing.*

Keywords: *journal club, nursing, evidence-based practice, evidence-based nursing*

INTRODUCTION

As the nursing profession is expanding, the nursing education system should educate nurses who can function effectively and efficiently in therapeutic settings that are constantly changing and subject to huge volumes of information [1,2]. Nowadays, the establishment of a bridge between theory and clinic has become one of the major challenges [3]. A pivotal action to achieve this goal is the ability to transfer and apply research in clinical settings [1,4].

Moreover, learning and updating methods of knowledge and skills have changed in the present world and learning has shifted from individual guidance to the group learning process [5]. Meanwhile, the journal club has a proven value in both nursing and continuing education and has expanded widely in the promotion of evidence-based clinical care (EBCC) and clinical collaboration in recent years [6].

To become familiar with and promote evidence-based research and practice, nurses need to know and apply new health-related texts. Over the past 25 years, substantial advances have been made in the models and frameworks of Evidence-Based Nursing (EBN) to replace processes in the health system, and the results of the research are appropriately used in clinical settings to maximize patient safety [3].

Nurses should be prepared to apply evidence-based practice in decision-making about the clinical care of patients [7,8]. EBN is generally the process of systematic diagnosis, meticulous and accurate evaluation, and subsequent dissemination of using research findings to influence clinical practice. It is continued through changes in standards of care that can lead to positive changes in clinical interventions [9].

Journal club facilitates the learning process in the context of clinical problem-solving and enhances the skills of criticism [5].

This strategy is based on the principles of adult education, active participation in learning and peer learning, using the concept of intimate class, providing the opportunity to shape informal discussions, learning to read and review nursing literature, and encouraging open participation in journal clubs [7]. The time to use journal clubs varies more widely in the nursing profession [10].

Nursing journal clubs are usual in Iranian schools, but in the clinical setting have launched for the first experience in line with the development plan on the roles of clinical faculty members at Tabriz University of Medical Sciences. Therefore, this study was conducted to evaluate the clinical NJCs at Tabriz University of Medical Sciences during 2019-2020 and evaluated the clinical nursing journal clubs from the viewpoints of nurses.

METHOD

This descriptive study was conducted from July 2019 to October 2020 at the School of Nursing and Midwifery and two affiliated hospitals of Tabriz University of Medical Sciences, Tabriz, Iran. Inclusion criteria were willingness to attend nursing journal clubs. Exclusion criteria were voluntary withdrawal from the study and do not answer more than 20% of the questions in the questionnaire. One month before the implementation of nursing journal clubs, the relevant hospitals were informed about the meeting time. Attendance was open to all nurses.

For data collection, the NJC evaluation tool was designed by considering factors influencing the success of NJC according to Kirkpatrick's model [2,11]. NJC quality assessment questionnaire consisted of 33 questions in four domains of satisfaction (18 items), change in knowledge (7 items), change in behavior (4 items), and organizational outcome (4 items). The tool was scored on a 5-point Likert scale from strongly agree to strongly disagree (scoring from 5 to 1).

The Kirkpatrick model is an accepted and widely used framework for the evaluation of educational programs. This model was introduced by Kirkpatrick (1950) and has since been modified by many people, but its four-step structure has remained unchanged.

The model has been described as a comprehensive, simple, and practical model for most educational situations and is recognized by the majority of experts in this field. Kirkpatrick defines evaluation as the effectiveness of a curriculum and divides the evaluation process into four levels (feedback, learning, behavior, and results) [12].

Ten nursing faculty members reviewed the questionnaire for face and content validity. Their comments were applied. CVR of all items was above 0.65 and CVI was above 0.8. The reliability of the tool was calculated and confirmed by Cronbach's alpha (0.95).

This study was conducted in two phases. The first step was to attain the format and flowchart of clinical NJC implementation. Initially, a committee was formed consisting of 3 faculty members and 2 doctoral students. A related comprehensive review of articles published from 2000 to 2018 on databases (SID, Iran Doc, Google Scholar, PubMed, Medline, Scopus, Cochrane Library, Science Direct, and CINHAL) was done. Also, 5 cases of NJC PowerPoints on the Internet were repeatedly assessed and analyzed precisely. Thus, three duties (a - identifying instructions, b - designing the flowchart, and c - determining template content of PowerPoint for NJC) were carried out in the committee for clinical NJC teaching events. Then, the same committee held one-day workshops to inform all members of different departments and all Ph.D. students at Tabriz school of Nursing and Midwifery (n= 40 participants).

Through the workshop NJC fully (history of JC, definition, aim and objectives, outcomes and benefits, types of JC, etc.) was explained and then discussed in the group. Received comments were applied and clinical NJC instructions, flowchart [13], and template PowerPoint were approved at the end of the workshop. Necessary coordination was first made with the hospital authorities, and then NJC sessions were held in two educational centers' of Shahid Madani and Sinai in Tabriz based on an agreement. NJC sessions were set up in a conference hall in hospitals affiliated with Tabriz University of Medical Sciences during 2019-2020.

In the second phase, the viewpoints of faculty members, nurses, charge nurses, nursing supervisors, nurse managers, and nursing students on these sessions were evaluated using a questionnaire, at the final ten minutes of JC. The above two hospitals were selected as research environments because there were clinical faculty members therein and NJC was implemented as their related task.

The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran, approved this study (code: IR.TBZMED.REC. 1397.643.). The study aim was explained to participants and they were ensured of the confidentiality of the data, the voluntariness of participation in the study, and their freedom to withdraw from the study at will.

The data were analyzed using the SPSS software v. 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics (mean, and standard deviation) were used for presenting scores for each domain and the whole domains of the questionnaire. $P < 0.05$ was considered statistically significant.

RESULTS

In the first phase, implementation format and flowchart were attained for clinical NJC as shown in Table 1.

In the second phase, several NJC sessions were evaluated in the two teaching hospitals. NJC topics were “contamination of blood sample cultures (two articles)”, “best practice in wound examination (three articles)”, and “delirium nursing protocol after coronary artery bypass graft and cardiac surgery (three articles)”. The frequency of NJC sessions by a faculty member scheduled at least once in the trimester, with repeated program twice a week (an observer is mandatory for the first time), and 1-2 hours per subject. The results of the evaluation of the face-to-face NJCs held at Tabriz University of Medical Sciences are presented in Table 2.

Minimum and maximum scores for the questionnaire can be 33-165, with a mid-range of 99. A total mean (SD) score of 123.49 (19.91) was reported for clinical NJCs in the current study. The quality of all sections was desirable in the participants’ views, and a desirable quality was evaluated for the NJC implementation.

Table 1: Flowchart of clinical NJC sessions at Tabriz School of Nursing and Midwifery

No.	Activity
1	Problem-finding through focus group sessions, virtual sessions, and PDPs
2	In the event of several issues, prioritizing them through focus group sessions or by Delphi technique
3	Selection of lecturer and assistants for holding NJC
4	Searching and selecting articles related to the topic from those of the last 5 years, systematic reviews, meta-analyses, randomized clinical trials, etc. indexed in valid databases of ISI, PubMed, Cochrane, and Scopus
Holding a meeting of attending knowledge committee, auditing lecturers’ reports on the prior steps, and deciding on steps 5 and 6	
5	Critical evaluation of articles in focus group sessions at the Faculty Determination of articles and journal club type (content or methodological evidence-based practice) Determination of core members (at least five people), and the day, time, venue, and registration
	Designation of a leader and supervisor for monitoring NJC sessions
	Determining a coordinator of NJC meetings with clinical employees
	Regular holding of NJC meetings (face-to-face or webinar sessions)
	Attendants at NJC session: Undergraduate, postgraduate, and doctoral nursing students, experienced and novice clinical nurses, head nurses, clinical and educational supervisors, and if necessary statistics counselor
6	Reserving venue and then notifying Mr. … at least one week in advance to record film, photos, and news of the meetings
Preparing a schedule planned for a one-hour presentation: A review and a one-time exercise by the lecturer and assistant, review of articles by lecturers (25 minutes) + review of articles by core individuals (total 20 minutes for five people) + commentary, and exchange of participants’ thoughts with lecturers (totally 15 minutes up to five people)	
7	Implementation of NJC meeting: - Presentation, evaluation, and discussion of articles and general conclusions; - Preparing report (minutes of the meeting: MoM) - Notification of summary to meeting participants and related audiences
8*	Preparing evidence-based guidelines and protocols or revising existing guidelines based on literature review and focus group sessions at the faculty and hospital
9*	Presenting developed/revised guidelines, protocols, and standards to the secretariat of the operating council for final consensus and approval, then, if necessary, approval by the vice-chancellor of the university
10*	Implementation of evidence-based guidelines and protocols in nursing practice
11*	Evaluation of results

** Steps 8-11 apply to 10% of NJCs.*

Table 2: Mean (standard deviation) of clinical NJCs quality held by nursing faculty members

Domain(range)	Mean (SD)
Satisfaction (18-90)	10.15 (67-75)
Change in knowledge (7-35)	27.15 (5-47)
Change in behavior (4-20)	16.24 (3-22)
Organizational outcome (4-20)	11.69 (2-66)

DISCUSSION

EBN is the use of best research evidence together with clinical expertise and skills by considering the patient's needs and status. In general, four components are involved in EBN decision-making, namely research evidence, patient values, clinical skills, and resource availability [14].

Nowadays, evidence-based education is also gaining ground with the aim of serious, explicit, and rational use of the best evidence to make decisions about nursing education. There is also an emphasis on the inclusion of evidence-based curricula in education [15]. Nursing curricula have included journal clubs, case presentations, group discussions, and rounds in the latest edition of nursing educational techniques revision [16]. Unfortunately, no clinical application of these widely used educational techniques facilitating evidence-based learning and practice is available in the present clinical setting of Iran.

Various studies demonstrate that journal club is accepted as a mechanism the recommendations of which are reflected in evidence-based practice. Journal clubs are not only useful in academic settings but are also valuable in clinical settings.

The findings of this study revealed a desirable evaluation of total quality scores for NJC implemented by the participants in meetings. The repetitive features of NJC implementation were considered to be successful in many respects. However, little is known about the best guidance and implementation to achieve the greatest educational benefit [3]. Besides, there is no standardized method of running journal clubs and evaluating the knowledge and outcomes achieved by the participants, nor a method of using that knowledge in practice [15].

The use of a questionnaire to determine the interest and personnel involvement in planning can help motivate participation. Our questionnaire also contained a question, in the end, asking participants to determine and comment on future topics for the implementation of NJC.

There were also some limitations, such as the lack of U-shaped space to make eye contact in NJC, the lack of reception, and no presentation of summaries to departments for employees not present at the meeting, which reduced the overall implementation quality.

Selection of journal club members from the same discipline or with interests and commonalities in a particular clinical area, long-term journal club goal setting with periodical revisions, setting goals tailored to the articles under review, acquisition skills, and selection of case base clinical articles of interest by all participants are all the factors that can help motivate participation and benefit participants. A journal club should be specified with frequency at predetermined dates, preferably monthly, at an hour appropriate to all members. Motivational factors should be applied to participants. Promotional strategies, such as the use of technology or the presentation of a brief discussion, are also helpful for all staff to access journal club achievement [1].

Limitation

Our study included a small sample size. However, we did identify a statistically significant change in each of the domains assessed.

CONCLUSION

Evidence-Based Nursing (EBN) is now one of the main goals and concerns of nursing, and one of the main objectives of Nursing Journal Clubs (NJCs) is to improve EBN practice. Nursing schools should prepare students for entry to the clinic to be able to identify clinical care challenges for nurses, and critically evaluate nursing studies. For rich clinical education in nursing, NJCs should be considered as one of the essential parts of the nursing curricula before graduation.

In this study, the authors attained a format and flowchart for the implementation of NJC by clinical nursing faculty members. Based on defined goals, NJC was organized and evaluated with the related tool. If necessary, deficiencies and shortcomings will be improved in the continuous and extensive assessments throughout the country.

NJC is a favorable method of education in the healthcare professions that can improve searching evidence for practice and the use of evidence in clinical practice. Therefore, this approach should also be used more extensively in managing nursing students' educational programs and in continuing education for the training of employed nurses.

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Disclosure statement
The authors declare they have no competing interests.

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ORIGINAL ARTICLE

Investigating the Role of Adenovirus, Herpes Simplex 1, 2, and Varicella Zoster Virus in Incidence of Conjunctivitis in Tehran, Iran

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Abstract: *Conjunctivitis is one of the most common ocular diseases across the world. In this study, the prevalence of viral Conjunctivitis in patients who were referred to the Eye specialized Hospitals was evaluated. Some of the most important viruses that cause conjunctivitis are Human Adenoviruses (HAdV), Herpes Simplex 1, 2 (HSV-1, HSV-2), and Varicella Zoster Virus (VZV). The clinical diagnosis of eye viral infections is difficult and often controversial. Thus, research to experiment with these causative factors is of pivotal importance. In this cross-sectional study, 200 conjunctival swab samples were collected from symptomatic patients with signs of conjunctivitis who had been referred to two hospitals. After DNA extraction, Multiplex Real-Time PCR was carried out in two separate reactions for each sample. Among 200 collected samples, 34 (17%) and 8 (4%) were positive for Adenovirus and HSV-1 DNA, respectively. There were no HSV-2 and VZV conjunctivitis. No significant correlation was identified between gender and conjunctivitis (P-value: 0.845); however, there was a close correlation between age and conjunctivitis (P-value: 0.05). In this study, 66.3% of Adenoviral conjunctivitis occurs in the summer compared to 33.7% in spring. No significant seasonal variation was observed for HSV-1. Our results showed that adenovirus has a key role in causing viral conjunctivitis.*

Keywords: *adenoviruses; conjunctivitis; Herpes Simplex; Multiplex Real-Time PCR; Varicella Zoster Virus*

INTRODUCTION

Conjunctivitis is one of the most common eye diseases around the world [1]. Several factors may cause conjunctivitis including viral, bacterial, and parasitic infections, trauma, allergies, and foods. Among these risk factors, viral pathogens can play a crucial role in the incidence of these infections [2, 3]. Likewise, among viral agents, Human Adenoviruses (HAdV), Herpes simplex 1, and 2 (HSV-1, HSV-2), and Varicella Zoster Virus (VZV) are the well-recognized causes of conjunctivitis [4, 5]. Viral factors can infect different parts of the eye such as eyelids, conjunctiva, cornea, retina, and optic nerve vascular tissue. Sometimes infections caused by these viruses cannot be distinguished from each other, but the outcome may be different [3]. As a result, in the absence of a timely diagnosis, major complications may occur.

Herpes simplex 1, and 2 can affect the eye and its tissues widely. Conjunctivitis and Keratitis are the most common eye diseases that occur after infection by these viruses [6]. Likewise, HSV has considered a cause of endemic eye infection in most communities. Approximately, 300,000 cases of ocular HSV infections are diagnosed annually in the United States. HSV infection is the second cause of corneal blindness in this country, as well. Transmission of this type of virus is variable from sporadic to epidemic. Its incidence relates to virus serotype and vulnerable age groups (children or old ages) [6, 7].

Adenoviruses have been widely distributed in many species. Adenoviral infection is often asymptomatic but may be associated with some respiratory, gastrointestinal, and ocular diseases. Sporadic and epidemic outbreak forms of Adenoviral conjunctivitis occur almost in every society as well as every age [3]. Furthermore, the major epidemic form which is hemorrhagic conjunctivitis often spreads in hospitals, schools, military garrison, and offices.

Varicella Zoster Virus (VZV) is an Alpha human herpes virus that causes varicella (chickenpox). It may also stay latent in the nerve ganglia following an early infection [8]. Varicella-zoster reactivation, in the trigeminal nerve, may develop conjunctivitis, keratitis, uveitis, and ocular cranial-nerve palsies [9]. Traditional methods for the detection of eye viral infections include cytological investigation, viral culture, antigen detection, and serology [10]. Choosing the best method for the detection of viral conjunctivitis depends on the efficiency, speed, and cost of the test. The old conventional tests lack these features.

With the advent of PCR, the sensitivity and speed of diagnosis of a wide range of diseases were increased. Therefore, it was considered a proper method for clinical diagnosis [3,10]. Inherent specificity and sensitivity of nucleic acid amplification processes triggered as the best tool to identify adenoviruses and herpes simplex viruses in clinical samples of conjunctiva swabs [11-14]. By adding entry PCR Time-Real techniques to the field of molecular diagnosis, the main step for quick and easy diagnosis of viral infections was taken [7,15,16]. Multiplex Real-Time PCR has been introduced recently which provides analysis of the quality, quantity, and genotype of several pathogens at the same time in one test tube [15,17].

Knowledge of the incidence and prevalence of different types of viruses that may lead to conjunctivitis may help clinicians to consider the more possible causative viruses, which in turn may change the clinical and laboratory approach and treatment management. In this study, we investigated the prevalence of HSV, Adenovirus, and VZV in swab specimens from patients with conjunctivitis who were referred to Eye-specialized Hospitals in Tehran, by multiplex real-time PCR method.

MATERIALS AND METHODS

Participants

In our cross-sectional study, conjunctival swabs were collected from patients with signs of conjunctivitis during the spring and summer of 2015, who had been referred to Eye specialized Hospitals in the South and North of Tehran, respectively. During 6 months Conjunctival swabs were preserved in 1.2 ml of Viral Transport Media and stored at proper condition (-80°C). A questionnaire was designed to find out the relationship between factors such as age, sex, number of household members, and occupation as well as types of material exposed to the eyes with conjunctivitis.

Quantitative Real-time PCR

Nucleic acid was extracted from 200 µL of the clinical specimen by using a QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer's manual with slight modifications in time and the speed of the centrifuge. The quality of extracted DNA was determined using Nanodrop spectrophotometry. The multiplex real-time PCR assay was performed in a total reaction mixture (25 µL) containing 5 µL of DNA extracts, 12.5 µL of 2µL multiplex PCR master mix, each forward and reverse primer, and each probe Amplification and real-time fluorescence detection were performed with a model Mx3000P real-time PCR system (Stratagene, La Jolla, CA) using the following protocol: first enzyme activation at 95 °C for 1 min followed by 40 cycles of denaturation at 94 °C for 10 s, and annealing/extension at 60 °C for 40 s. At the end of the amplification cycles, melting temperature analysis was set by a slow increase in temperature (0.3 °C/s) from 60 up to 95 °C. Furthermore for diagnostic assay, Multiplex TaqMan Real-Time PCR Kit (FTD eye, Fast-track Diagnostics Luxembourg S.a.r.l) was used. Two tube multiplex Real-Time PCR reactions for detection of HSV-1, and 2, varicella-zoster virus, and adenovirus, including internal control (IC), were done. All runs included negative and positive controls. The presence of Specific viral sequences in the reaction was indicated by an increase in the fluorescence from the relevant dual-labeled probes and was reported as a cycle threshold value (Ct) by the real-time thermocycler. StatView J 4.02 (Abacus Concepts Inc., Berkeley, CA) was used to perform the data analysis. To discern PCR inhibitors that are responsible for false-negative results, murine cytomegalovirus (mCMV) as an IC that amplified with the target DNA was added to the samples. It was introduced into the lysis buffer at the extraction stage of each sample and the positive and negative control.

Statistical analysis

Pearson χ2, Mann-Whitney, and Spearman's rho were used for Statistical analysis. To find out the relationship between factors such as age, sex, number of household members, and occupation, we benefited from SPSS 16.0 software. P-value<0.05 was considered to be statistically significant.

RESULTS

The prevalence of HSV-1 and 2, Varicella-Zoster virus, and Adenovirus was assessed in these patients During 6 months, spring and summer, a total of 200 patients (78 females [39%] and 122 males [61%]) with signs of conjunctivitis were referred to a couple of Eye specialized Hospitals. The patient's age range was from 3 to 55 years with a mean age of 31.00±12.41 years. A high proportion of conjunctivitis swaps 158 patients were negative for expected viruses in this study (79%) (Figure 1).

Adenovirus DNA was detected in 34 (17%) patients. HSV-1 was found in 8 cases (4%). There was no patient with positive results for HSV-2 and VZV (Figure 2). Moreover, Adenovirus was more frequently distinguished during the summer compared to spring, whereas no significant difference was observed in the distribution of HSV-1 in spring and summer. (Figure 3) The burden of conjunctivitis was lower among women to compare men; however, this difference was not statistically significant (P value = 0.850). Furthermore, statistical analysis showed a close correlation between age and conjunctivitis which was statistically significant (p-value <0.05). There was not any significant relationship between social status or location (North versus South) and conjunctivitis.

Figure 1: Patients infected with adenoviral 34/200, and 8/200 to HSV-1. In the 158 patients studied, there was no clue for the presence of VZV and HSV-2 viruses.

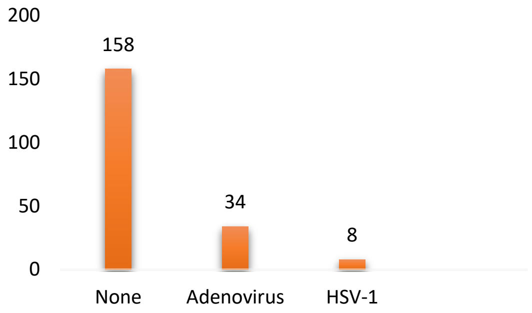

Figure 2: The amplification curve of adenovirus and HSV-1 genome in Multiplex Real time-PCR

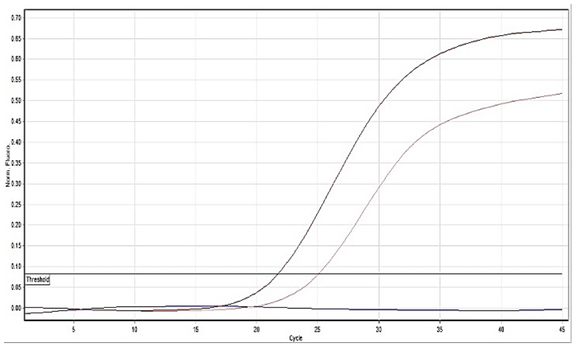

Figure 3: 66.3% (22 of 34) of adenoviral cases were seen in summer. The seasonal fluctuation was not observed for HSV-1

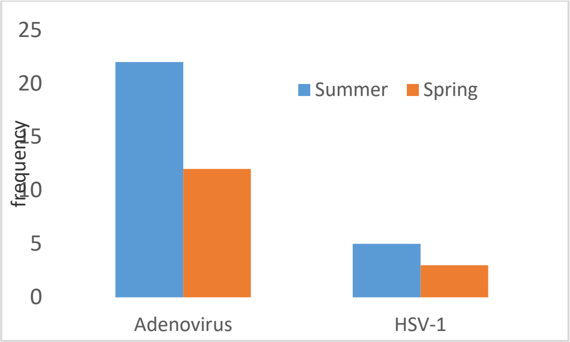

DISCUSSION

Conjunctivitis is one of the most important human eye diseases around the world. Several biological factors can contribute to the frequency of eye infections including viral agents [1,2]. In this cross-sectional study Multiplex, Real-Time PCR was applied to investigate the prevalence of HSV-1, 2, VZV, and Adenovirus in a total of 200 patients with signs of conjunctivitis that were referred to two Eye specialized Hospitals in North and South of Tehran during spring and summer of 2015. In all of the patients, 34 (17%) patients with Adenovirus DNA and 8 (4%) with HSV-1, there were no patients with positive results for HSV-2 and VZV. Failure to diagnose adenovirus eye diseases can lead to outbreaks of keratoconjunctivitis [18]. In other words, rapid diagnostic methods may help us to detect this contagious conjunctivitis early enough to avoid the spreading of conjunctivitis and to prevent complications.

Several methods are available for the diagnosis of a viral infection such as antigen detection, viral culture, and serology. Although the diagnostic accuracy of some tests has dramatically improved, they still lack the required diagnostic criteria for optimum tests. Serological methods cannot be used during the acute phase of the disease due to cross-reactions with other related organisms. Thus, they may raise the frequency of false positive results [19]. The techniques based on antigen detection do not require living organisms, however, false-negative and false-positive results may still be obtained. Separation of adenovirus in the ELISA method is time-consuming (virtually 4 weeks for Adenovirus and 3 days for herpes simplex virus) [3]. In this study, the PCR technique was used due to its high sensitivity and rapidity for the diagnosis of viral infections.

It has been shown that DNA identification of a virus is much easier, faster, and more sensitive than isolating the virus from clinical samples [11]. PCR method can detect very small amounts of DNA with high specificity, and would potentially revolutionize the diagnosis [10]. The first use of this method for eye diseases was reported in 1990 and to date, it has largely been developed in ophthalmology [12]. Several studies have been performed to evaluate the use of PCR as a diagnostic tool to identify adenovirus, and herpes simplex virus in swabs conjunctivitis [14,20]. Kensington et al published a study showing a 97.3% specificity of PCR for the detection of adenoviruses in eye swabs [3,14]. Although the PCR method is very sensitive and specific, some post-amplification processes can be time-consuming [20]. For instance, electrophoretic analysis of nucleic acid in the presence of ethidium bromide and ultraviolet light bands [21], the southern blotting method for detection of labeled oligonucleotides, and the target sequence are prolonged and time-consuming. In addition, it requires several PCR products that increase the possibility of contamination [22]. ELISA - PCR, despite using biotinylated probes and increase specificity, needs a lot of time that could potentially lead to some errors in the results [16].

Nowadays, the advantages of Real-Time PCR are not hidden from any laboratory specialist. Monitoring the accumulation of amplified products, by labeled probes or primers is possible and is superior to the use of radioactive materials. Moreover, the need for post-amplification (because of using fluorogenic for labeling primer and probe) is compensated by the higher speed (due to the short-time cycle and short length of the fragment) of the technique [15]. Different studies have suggested that Adenovirus is the main viral agent in conjunctivitis with a frequency that varies between 14 to 70 % around the world [23-25]. Adriana et al showed that the frequency of adenoviral conjunctivitis was 60% in the population of Rio de Janeiro, Brazil in a study between 2004 to May 2007 [26]. In another investigation conducted in 2010 in Turkey, Yagci et al studied eye swabs from 34 patients with acute conjunctivitis. The PCR results showed that 26.5 % (9 out of 34) of patients were infected by different adenoviral serotypes (HAdV-8 was reported as the predominant serotype) [27]. Shamsi–Shahrabadi et al have reported that the prevalence of adenoviral conjunctivitis in Tehran is 16% in 2009 [28]. This was similar to the result of the study by Goudarzi et al showing a frequency of 15.7% Adenovirus conjunctivitis in 2006 [29]. Despite using different methods, the results of those studies are similar. The same is true for our study indicating conjunctivitis epidemiological pattern has not changed in Iran in recent years.

The frequency of HSV-1 conjunctivitis in Iran is much less common than the reported frequency of this kind of infection in other countries. In a study on 1,964 samples collected from conjunctivitis between 1987 and 2001, Marangon et al reported that 514 samples were positive for HSV-1. HSV-1 was the most frequent (56%) viral agent followed by adenovirus (31%). Adenovirus and HSV1 were the most important cause of conjunctivitis infections (66%) and corneal infections (88%), respectively [30]. This was previously suggested in another study conducted in India in 2003, showing that from 140 samples 33.6% were infected with HSV. The pattern is slightly different in other countries [31]. In 2003, a similar study in Japan showed that 19 out of 33 samples of eye infection were related to viral infection including 4.68 % HSV-1 and 31.6% VZV. In this study, they didn't look at the frequency of adenovirus [8].

According to our investigation, the incidence of HSV-1 was 4%. On the other hand, there was no positive test for HSV-2 and VZV which can be concluded that these viruses are not endemic in Iran. Our study has some limitations. The samples have been obtained from subjects who live in the capital city of Iran which may not necessarily represent the frequency of viral infection in other rural areas in the country. Tehran, on the other hand, is considered a reference city with a high population, and the selected hospitals are considered the main eye-specialized hospitals which receive the majority of eye diseases in the city. Therefore we recommend more research for the recognition of the main viruses involved in conjunctivitis' pathogenesis in other areas of Iran and nearby countries. Finally, it is noteworthy that in this project, all of the enrolled patients were suffering from conjunctivitis but only 21% of them were infected with Adenovirus or Herpes 1.

As a result, the role of other viral factors such as Chlamydia trachomatis and Picornaviruses remains to be unclear.

CONCLUSION

In conclusion, we suggest that adenovirus is the main responsible viral agent for conjunctivitis in Tehran. The frequency of Adenoviral conjunctivitis was affected by seasonal variation. The seasonal variation didn't affect the frequency of HSV-1 infection.

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ORIGINAL ARTICLE

Relationship between Moral Intelligence and Clinical Competency of Nurses in Ilam Educational Hospitals

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Abstract: *In this descriptive-analytical study, 110 nurses working in educational hospitals of the Ilam University of Medical Sciences were included. Participants were selected using a random sampling method. The data collection tools were the demographic information form, the moral intelligence questionnaire of Lenik and Kiel (2005), and the standard clinical competency assessment questionnaire (CIRN). Data were analyzed using SPSS software, version 21. Descriptive and inferential statistical tests were used to analyze the data. The mean ± SD score of nurses' moral intelligence and clinical competency was estimated at 157.59 ± 26.60 and 136.65 ± 46.08, respectively. Fifty-one percent of participants had low clinical competency, and 46% had high clinical competency. There was a significant association between moral intelligence and clinical competency ($p<0.001$, $r=0.517$). The moral intelligence and clinical competency of nurses were significantly associated with age, education status, work experience, employment status, and rank/position ($P<0.05$). A negative association was found between working unit/ ward and clinical competency ($P<0.05$).*

Keywords: *moral intelligence, clinical competency, nurses*

INTRODUCTION

As the largest group of care providers, nurses face many ethical challenges due to their working professional nature [1]. Since moral practice in nursing plays an important role in the quality of care provided, the improvement of patients, and the achievement of health goals, paying attention to the dimension of moral intelligence in nurses as a moral guide for their performance is highly important [2]. Borba first introduced moral intelligence in psychology. He defined moral intelligence as the capacity to understand right from wrong, have strong moral beliefs, and practice them to provide the right behavior [3].

Moral intelligence includes seven essential principles: empathy, conscience, self-control, respect, kindness, tolerance, and fairness [4]. Adopting appropriate strategies such as improving the quality of nursing services can increase the moral intelligence of nurses [2]. Several factors, such as the rapid change in the health monitoring system, providing safe and affordable services, increasing the awareness of the community in health-related areas, and the expectation of receiving high-quality services, along with the willingness of health service providers to use skilled personnel cause clinical competency to be of great interest [5].

Clinical competency in the nursing profession comprises different dimensions and areas. Knowledge, attitude, skill, and communication are the main dimensions. Other dimensions that are emphasized in different literature include professional practice (principles of nursing care, instructions, and interventions), ethics, decision-making, cooperation, work development, education, clinical judgment, problem-solving ability and critical thinking, management, leadership and motivation, culture, spirituality, research, information technology and the ability to work with advanced devices [6]. Ensuring the care of patients to ensure the clinical competency of nurses in hospitals and other medical service settings is a major concern and is of great importance for managers and health service provider systems [7]. Since the clinical competency of the working nurses is considered the main concern of the care provider systems, it deserves more attention. Other nurses with appropriate moral intelligence can guarantee their position by keeping healthy, optimizing hospital performance, and communicating effectively with colleagues and patients. This study, therefore, aimed to examine the association between moral intelligence and clinical competency among nurses working in educational hospitals affiliated with the Ilam Univesity of Medical Sciences.

MATERIALS AND METHODS

Population of study

This was a cross-sectional, descriptive-analytical study. A total of 110 nurses working in educational hospitals (Mostafa Khomeini Hospital, Imam Khomeini Hospital, and Taleqani Hospital) affiliated with the Ilam University of Medical Sciences were included. Sampling was conducted using a random sampling method from 23 September to 21 December 2021.

Inclusion and Exclusion Criteria

In our study, inclusion criteria were: having a bachelor's degree in nursing, having at least one year of work experience in nursing, not having work experience in other fields of medical sciences, and giving informed consent to participate in the study. The exclusion criteria were: incomplete questionnaires and unwillingness to participate in the study.

Demographic Variables

The first section was the demographic characteristics questionnaire. This questionnaire included eight items: age, gender, marital status, education status, work experience, employment status, the working unit/ward, and rank/position.

Questionnaires

Our study's data collection tools consisted of a demographic characteristic questionnaire, Lennick and Kiel's (2005) moral intelligence questionnaire, and Competency Inventory for Registered Nurses (CIRN) questionnaire. The first section was the demographic characteristics questionnaire.

The second section was the moral intelligence questionnaire, designed by Lennick and Kiel (2005). This questionnaire consists of 40 questions encompassing four components: honesty, responsibility, compassion, and forgiveness. The items are rated on a 5-point Likert scale, with scores ranging from 1 (never) to 5 (always). The minimum and maximum scores are 40 and 200, respectively, divided into two to convert to a maximum score of 100. In this study, items were scored as follows: 40-59 (poor), 60-79 (good), 80-89 (very good), and 90-100 (excellent). The reliability and validity of the questionnaire have already been confirmed in the study of Mohammadi et al., with a Cronbach alpha of 0.87 [8]. In our study, the Cronbach alpha was 0.96.

The third section was Competency Inventory for Registered Nurses (CIRN) questionnaire, designed by Liu et al. in 2007. This questionnaire consists of 55 questions encompassing 7 dimensions: clinical care (10 questions), leadership (9 questions), interpersonal relation (8 questions), legal/ethical practice (8 questions), professional development (6 questions), teaching-coaching (6 questions), critical thinking and research aptitude (8 questions). The items are rated on a 5-point Likert scale, with scores ranging from 0 to 4. In our study, the questions were scored as follows: less than 100 (low competence), 110-165 (moderate competence), and 165-220 (high competence). The reliability of the questionnaire has already been confirmed in the study of Ghasemi et al., with a Cronbach alpha of 0.97 with the range for each dimension between 0.68 and 0.87 [9]. In this study, the Cronbach alpha was 0.98.

Statistical Analysis

Data were analyzed using SPSS software version 26. Descriptive statistics (e.g. percentage, mean, and standard deviation (SD) and analytical statistics (e.g., Pearson correlation coefficient and Analysis of variance (ANOVA) were used to analyze the data.

RESULTS

A total of 110 nurses were included in this study.

Nearly 55% of participants were women (60 Female, 50 Male), and 87.3% of them had a bachelor's degree(96 Bachelor, 14 Master). Most participants (61.8 %) were formally employed with a work experience of 7.17 years. 38.2% (43 Nurses) and 33.6 % (37 Nurses) of nurses were employed in the Intensive Care Unit (ICU) and specialized wards, respectively (Table 1).

As shown in Table 2, the mean ± SD of moral intelligence and clinical competency in nurses was estimated at 157.59 ± 26.60 and 136.65 ± 46.08, respectively. 46.4% of nurses had low clinical competency, 11.8% had moderate clinical competency, and 41/8% had high clinical competency.

Figure 1 shows a significant correlation between moral intelligence and clinical competency in nurses ($r=0.517$, $p < 0.001$). With an increase in moral intelligence scores, clinical competency in nurses would increase. Moral intelligence and clinical competency in nurses were significantly and positively associated with age, education status, work experience, employment status, and rank/position ($P < 0.05$). On the contrary, there was a negative and significant association between the working unit/ward and clinical competency ($P > 0.05$) (Table 3).

Figure 1: The association between moral intelligence and clinical competency.

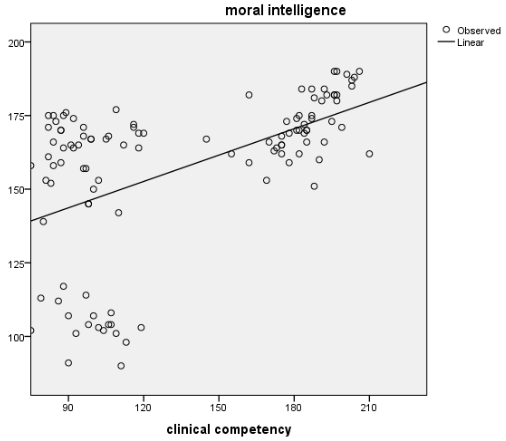

Table 1: The demographic characteristics of the nurses

Variable		Frequency (%)
Gender	Male	5(45.5)
	Female	60 (54.5)
Marital status	Single	55 (50)
	Marries	55 (50)
Education status	Bachelor	96 (87.3)
	Master	14 (12.7)
	Ph.D.	0 (0)
Employment status	Company	42 (38.2)
	Contractual	0 (0)
	Formal	68 (61.18)
Working unit/ward	Intensive Care Unit	43 (38.2)
	Specialized ward	37 (33.6)
	General ward	31 (28.2)
Rank/position	Staff nurse	96 (87.3)
	nursing managers	14 (12.7)
Working experience in nursing	1-5 years	55 (50)
	6-10 years	30 (27.3)
	11-15 years	10 (9.1)
	≥16 years	15 (13.6)

Table 2: The mean ± SD of moral intelligence and clinical competency in the nurses

	Minimum	Maximum	Mean± SD
Moral intelligence	90	190	157.59± 26.609
Clinical competency	75	210	136.65± 46.084
The level of clinical competency	**The range of score**		**Frequency (%)**
Low	1-110		51 (46.4)
Moderate	110-165		13 (11.8)
High	165-220		46 (41.8)

Table 3: The association between demographic characteristics and moral intelligence, clinical competency

Variable	Moral intelligence		Clinical competency	
	Correlation	P-Value	Correlation	P-Value
Age	0.31	<0.001	0.42	<0.001
Gender	-0.06	0.51	0.002	0.98
Marital status	-0.09	0.32	-0.01	0.89
Education status	0.32	<0.001	0.39	<0.001
Work experience	0.31	<0.001	0.44	<0.001
Employment status	0.37	<0.001	0.38	<0.001
Working unit/ward	-0.09	0.32	-0.19	0.04
Rank/position	0.24	0.01	0.34	<0.001

DISCUSSION

This study aimed to examine the association between moral intelligence and clinical competency and its relevant factors in nurses working in the educational centers of Ilam University of Medical Sciences in Iran. Our results showed that nurses had good moral intelligence, which is in line with the previous study conducted in Iran and Turkey [10,11], indicating the importance of acquiring moral values and moral practice by nurses in clinical settings indirectly. In contrast, a study in Iran demonstrated that 62% of nurses had moderate moral intelligence [12]. This difference can be attributed to study methodology, clinical setting, and statistical population.

In this study, 46.1% and 41.8% of nurses had low and high clinical competency, respectively. Our results are consistent with the previous studies in Iran [13,14], highlighting the high clinical competency level among nurses in the country. In Iran, every hospital has a quality improvement and in-service training ward responsible for holding training courses, assessing nurses' clinical competency, and encouraging excellent nurses. Therefore, this high score can be related to regular training courses and periodic evaluations of nurses' clinical competency.

Previous studies in other countries also demonstrated good and excellent clinical competency among nurses working in different hospital wards [15,16]. That has meant the importance of clinical competency-related issues has gained attention from the nursing management of hospitals and nurses themselves, and measures have been taken to improve nurses' knowledge levels.

We found that nurses' clinical competency increased by increasing the mean scores of moral intelligence, which is in line with previous studies [17,18]. Also, previous research demonstrated that the high moral intelligence of nurses improves the provision of care and treatment services to patients [19], and similar results were reported in our study.

In the present study, those with higher age, higher educational level, more work experience, formal employment, and those with a better rank/position had higher moral intelligence. A study by Dehghani et al. showed that moral intelligence in nurses is associated with their age and work experience [1]. Some studies suggested that younger nurses are influenced by their emotions and cannot properly use moral reasoning in moral challenges created in the clinical setting [20]. Older nurses are more likely to be in different clinical and social situations and face more moral challenges. Hence, they have more ability to promote their moral intelligence. Nurses with more work experience have more ability to cope with difficult moral situations.

Regarding the higher score of moral intelligence in nurses with higher educational levels, it can be attributed to the importance of nursing moral training due to the change in the educational content of the curriculum related to a master's degree in nursing. Moreover, the higher score of moral intelligence in nurses working in wards compared to nursing managers can be due to a longer period of clinical training, more clinical shifts in the hospital, continuous communication with patients, and being or participating in decision-making situations for patients. In this study, there was no significant association between gender and moral intelligence.

Moreover, we found that those with older age, higher educational level, formal employment, and a better rank/position, and those working in ICU wards had greater clinical competency. Our results are inconsistent with the study of Farajai et al [13]. This difference could be due to variations in participants' sociodemographic characteristics and clinical settings. The different clinical setting due to the different needs of care required for patients and differences in various clinical experiences among nurses causes a different understanding and perspective of clinical competency among nurses. This study was also inconsistent with previous research that showed female nurses were more sensitive in reasoning and clinical decision-making regarding patient care [14]. Also, some studies found that the mean score of clinical competency was higher among nurses aged 30 years and over than those with lower age [16,21]. Since older nurses have more experience taking care of patients, they are more likely to provide better service quality and face lower challenging situations [22]. Consistent with the present results, previous studies showed that nurses with higher educational levels had more clinical competency [21,23]. Those with higher educational levels are more likely to receive the necessary training to understand care better.

Furthermore, in this study, those with more work experience had more clinical competency, which is in line with the results of previous research [16,21]. With the increase in work experience, nurses would benefit from more clinical experience, job skills, and knowledge, resulting in them becoming more sensitive to patient care issues [22].

CONCLUSION

The results of the present study showed that the clinical competency of half of the nurses was high, and their moral intelligence was favorable. There was a significant association between moral intelligence and clinical competency in nurses. Moral intelligence and clinical competency were associated with age, gender and educational level, job factors, clinical activity history, employment status, and nurses' work roles. Considering the importance of clinical competency and the role of moral intelligence in nurses' clinical practice, hospital nursing managers must adopt measures to improve nurses' moral intelligence and clinical competency using new educational approaches such as electronic education and continuous training. In addition, periodically evaluating the abovementioned cases among nurses with standard tools can be very beneficial.

Conflicts of interest and sources of funding
Declare no conflicts of interest.

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Ilam University of Medical Sciences.

Ethics approval and consent to participate
REC,1400,123

Informed written consent
was obtained from all participants included in the study. All participants were informed about the purpose of the study and assured of confidentiality and anonymity. The respondents were informed of their freedom to withdraw from the study at any time.

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ORIGINAL ARTICLE

Risk Factors for Musculoskeletal Disorders in Nursing Personnel and Prevention Strategies

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Abstract: *Objectives: Health professions such as nursing are at high risk of work-related musculoskeletal disorders (WRMDs). This study aims to present the most common WRMSDs that occur in nursing personnel, investigate the risk factors associated with their occurrence, and identify preventive and therapeutic strategies. Material and Methods: A comprehensive literature search in PubMed, Scopus, and Google Scholar was performed using keywords, until May 2021, while the results reported by various authors were summarized. Results: Low back discomfort (30% to 60%), neck disorders (30% to 48%), and shoulder disorders (43 to 53%) are the most common WRMSDs in nursing personnel. The major risk factors are classified as demographics and job characteristics, physical, psychosocial, and workplace organizational factors, which often co-exist and act together. WRMSDs have serious consequences such as absenteeism, reduced productivity, increased morbidity, health costs as well as lower quality of life. To reduce the incidence of WRMSDs, it is crucial to implement prevention strategies, such as ergonomic measures and training programs in high-risk nursing departments as well as immediate therapeutic approaches, where required. Organizational interventions are also required to improve the working environment in terms of assigning tasks among nursing staff, setting working hours, keeping a positive attitude, and conducting medical examinations. Conclusion: Early identification of risk factors concerning WRMSDs among nursing personnel and immediate treatment is needed to maintain their productivity as well as to provide quality nursing care, followed by evidence-based ergonomic and organizational intervention strategies for their safety and health protection.*

Keywords: *work-related musculoskeletal disorders, nursing personnel, risk factor for musculoskeletal disorders, ergonomic measures, prevention, low back discomfort*

INTRODUCTION

Work-related musculoskeletal disorders (WRMSDs) are a worldwide major concern in occupational health. They are an umbrella term for musculoskeletal symptoms caused or worsened during work. These disorders may be defined as discomfort, impairment, disability, or persistent pain in the locomotor system [1]. The upper body such as the spine and upper extremities are the most sensitive structures for the appearance of WRMSDs [2]. Moreover, WRMSDs are the main cause of lost working time, production downtime, increased labor costs, decreased quality of life, turnovers, job changes, and injuries that can lead to temporary or permanent disability [3].

WRMSDS are usually due to genetic, morphological, psychosocial, and biomechanical factors [4]. They can be triggered or intensified by work, as well as by other activities such as housework and sports [5]. An increased risk of their development has been found in occupations with intensely repetitive work tasks, excessive load bearing, intense efforts, prolonged improper work postures, and weight lifting, as well as in demanding psychosocial working environments [6].

A survey found that 25% of workers in Europe reported back pain and 23% of cases were work-related. The differences between the countries were large, with workers in Greece reporting work-related back pain at 47% and muscle pain at 46%, while in the United Kingdom the corresponding rates were 11% and 9% [7]. Musculoskeletal pain has been reported in computer users, office workers, ophthalmologists, professional musicians, and marines [8]. In 2014-2015 in the UK, around 9.5 million working days were lost due to WRMSDs, accounting for 40% of the lost working days due to bad health [8].

The Military Health System and military health workers are responsible for the provision of health services for the active military, retirees, as well as their families [9,10]. The military health system has two main goals: to properly prepare the health team so that the team may provide health services during military operations and to ensure that every active military is perfectly prepared to deliver maximum performance on the battlefield. All military health workers, including nurses, have a crucial role in delivering those purposes [9].

In health professions such as nursing, WRMSDs have a high prevalence due to intense physical straining from prolonged standing, frequent weight lifting (e.g., patient transfer), as well as systematic bending or twisting of the torso during nursing skills [11-14]. It is estimated that almost one-third of all cases of sick leave among health professionals are related to WRMSDs [15]. This occupational health concern is a quite common issue in both civilian and military health personnel [8, 16,17]. Consequently, prevalence, symptomatology and required interventions for WRMSDs should be taken into consideration for both military and civilian health workers.

MATERIALS AND METHODS

The scope of this non-systematic literature review was to identify the most common WRSDs that occur in nursing staff and to investigate the risk factors associated with their occurrence. In addition, the most important strategies regarding the prevention and treatment of these disorders are reported. The inclusion criteria for the selection of the articles were concerning nursing staff, musculoskeletal disorders, English language, published until May 2021, and access to the full text. The PubMed, Scopus, and Google Scholar databases were searched with the terms “work-related musculoskeletal disorders”, “nurse” or “nursing personnel”, “musculoskeletal pain” and “risk factors for musculoskeletal disorders”. Both review articles and original research articles were used to synthesize this review with a focus on contemporary and seminal papers.

PREVALENCE OF MUSCULOSKELETAL DISORDERS IN NURSING PERSONNEL

The incidence of WRMSDs in nursing personnel varies according to numerous studies. Low back discomfort (30% to 60%), neck disorders (30% to 48%), and shoulder disorders (43 to 53%) are the most common [18,19].

Studies in Nigeria, Turkey, Australia, Estonia, and Japan have found that the rate of WRMSDs in nursing personnel is about 84%, with low back discomfort being the most common [8]. Furthermore, WRMSDs in knees were associated with standing and low levels of satisfaction from their work [20]. Increased prevalence of WRMSDs in nursing personnel was reported in specific working settings such as in intensive care nurses and those working in a department of surgery and anesthesia [20]. These settings have increased demands for caring for patients that were more dependent and immobilize and require nurses to be in a prolonged standing situation and other painful postures [6,21,22].

RISK FACTORS FOR MUSCULOSKELETAL DISORDERS IN NURSING PERSONNEL

The major risk factors of WRMDs are classified into four groups: demographics and job characteristics, physical, psychosocial, and workplace organizational factors (Table 1) [23-25]. They often co-exist and may directly or indirectly act together.

Table 1: Risk factors for the occurrence of musculoskeletal disorders in nursing personnel

Demographic and Job Characteristics related factors	Age, gender, education, hospital type where they worked, marital status, job title, years of employment in high-risk departments (e.g., ICU), level of exercise
Physical Factors	Physical workloads such as force, posture, and repetition
Psychosocial factors	Job stress, effort-reward imbalance
Workplace organizational factors	Management support, supplies, equipment, barriers and conflicts, peer support, feedback, and training

Demographic and Job Characteristics related factors

Female nurses were more likely to suffer from WRMSDs, especially in the upper extremities and torso. Female nurses face significant burdens due to the combination of family and work responsibilities including parental responsibilities, domestic load, lack of exercise, and insufficient rest time [21, 26]. Similar problems were observed among married nurses, due to the double burden of different types of tasks (family and professional) that increase the psychological and physical requirements and reduce the recovery time required for the working muscle groups [19,27].

The incidence of WRMSDs also increases with age and length of service [28]. Age-related changes, usually above the age of 40, include reduced strength and mobility due to loss of muscle mass, muscle capacity, dynamics of intervertebral discs and degeneration of musculoskeletal structures, reduced time reaction, and acuity of senses [2,29,30]. Nursing personnel above the age of 45 years old need more time to recover after work and those who work with shifts have more severe health problems than nursing personnel below the age of 40 [31,32].

Age is often associated with years of work. That is, nurses with longer working experience have cumulative effects from long-term exposure to injuries in their work environment, possibly due to non-compliance with the principles of safe work [7,33, 34]. It is reported that nurses with more than 20 years of clinical work experience were approximately 4 times more likely to develop WRMSDs than nurses with 11-20 years of work experience [35].

Socioeconomic status is considered to be a risk factor for WRMSDs development and affects the ability of workers to deal with their health problems, especially those with a lower educational level [36]. Compared to nurses, nursing assistants who are supposed to have lower socioeconomic status and a lower level of education, have a higher chance of developing WRMSDs, especially in the lumbar spine [19].

WRMSDs are also associated with factors related to lifestyle and human behavior, such as high body mass index and lack of exercise [21,37]. Nursing personnel with poor physical health status are six times more likely to face problems with their legs and ankles, limiting their activity [38]. The possibility of nurses gaining weight due to their busy schedules and the stressful nature of their profession, which in turn leads to sleep and eating disorders, makes it necessary that nurses adopt rules of healthy behavior [39]. Moreover, a predisposing risk factor for WRMSDs is the poor physical condition of nurses, especially when combined with poor transport techniques [40,41].

Physical Factors

Nurses' daily tasks include frequent weight lifting and excessive manual labor in bad body postures at high frequency and repetition. Nurses often have to lift patients, the weight of which, at times, is greater than their body weight to help or to put them to bed. It is estimated that during a typical 8-hour shift a nurse may lift a cumulative weight of 1.8 tons [42]. The frequency and severity of exposure to these risk factors vary depending on the type of health care [42].

Activities related to the immediate care of patients and their transport have an impact on the musculoskeletal system, especially on the spine, because compression and shear loads in the lumbar spine during patient handling are quite high [19, 43]. These WRMSDs are affected by the mean age of employees, years at work, patient characteristics, and health status, as well as by the technology available for patient transportation [19].

Nurses are often involved in lifting and pushing manipulations in everyday clinical practice, which cause muscle fatigue, being an important risk factor for lower back pain [44,45]. Frequent standing, persistent anterior projection of the head, and frequent transport of hospital equipment are also common [44,45]. Transferring patients is complicated and unpredictable action due to their weight, the possibility of confusion and tripping, and the lack of body handles. Additionally, patients may not be cooperative during transport, like some cognitively impaired advanced-aged patients, adding more burden to the nursing personnel [42, 45]. Professional tasks involving twisting and lifting increase the risk of lumbar injury by 4.84 times [46].

Physical risk factors are more common in nursing assistants because they lift and transfer patients more often, as part of their professional duties as well as they have reduced rest and recovery time between these nursing activities [47]. The incidence of low back pain in nursing assistants is 3.3 times higher than in other nursing staff [46].

Psychosocial factors

Psychosocial risk factors associated with WRMSDs include work intensity, repetitive tasks, working hours, high physical and psychological demands, fatigue, stress, and employees' perception regarding work organization [48,49]. Additional psychosocial risk factors may be the high perceived workload, time pressure, low levels of work control, work satisfaction, and social support [19,50]. Frequent unplanned work as well as workload difficulties in reduced working capacity conditions are some factors associated with low work control by nurses that tend to be strongly associated with low back pain [51].

Nursing personnel with increased long-term high stress, often have difficulty responding to workload, due to understaffing and time pressure, leading to rapid accelerations or poor postures [52,53]. As a result, they are more likely to suffer from WRMSDs, especially in the neck and shoulders, compared to those who do not report facing high levels of stress [52,53].

Workload and stress have been linked not only to pain but also to injuries. Clarke et al. found that a high workload was associated with a 50 to 200% increase in injury possibility as well as other accidents among hospital nurses [54]. Subsequent studies in Estonia have shown a high degree of risk between WRMSDs and mental health problems including symptoms of anxiety, exhaustion, and physical stress among hospital nursing staff [18]. The occurrence of WRMSDs in nurses is associated with physical workload, emotional exhaustion, and symptoms of physical stress [18]. Another study also identified stress and the level of anxiety in everyday life as a related causative factor for WRMSDs [55].

In addition, work-family conflict may be a major stressor for nurses, increasing the impact of WRMSDs on the lower back, shoulder, and cervical spine. The problem is exacerbated due to the rolling work schedules, which is a common situation in nurses' typical life expectancy [55].

Workplace Organizational Factors

Nurses usually have considerable burdens due to staff shortages. They may also become frustrated by the hard-working hours and schedule, resulting in mental and physical exhaustion, consequent emotional distress, increased risk of WRMSDs, and affecting their intention to leave [21,52,56-58].

Numerous studies have examined the relationship between staffing levels in nursing personnel and injuries/illnesses in the workplace [59,60]. Inadequate staffing has been associated with back injuries among nurses. It has been found that when nursing positions in hospitals had decreased by 9%, the number of WRMSDs increased by 65% [60].

Rotating shifts create unusual sleep patterns and higher levels of unhealthy behavior that can reduce work efficiency [61,62]. Nursing staff also experience, among other things, higher rates of accidents and WRMSDs as well as reduced levels of physical and mental health (e.g., fatigue, lack of energy and weakened immune system), compared to nurses working in morning shifts. These findings are also related to the short rest period between shifts [63]. In addition, in a study of Japanese nurses, it was found that after a restful sleep break for every two-night shifts, WRMSDs in the upper and lower extremities were significantly decreased [64]. According to another research, the risk of low back pain increases among nursing assistants working the night shift and is also associated with weight lifting and moderate work requirements [65].

IMPACT OF WRMSDS ON NURSING PERSONNEL

WRMSDs are the main cause of nurses' lost work time as well as increased morbidity, increased health costs, and lower quality of life [21]. WRMSDs may have a serious impact on the quality of life of nursing staff, resulting in higher absences from work or even the desire to change jobs [21].

WRMSDs are characterized as multifactorial and responsible for morbidity in many working populations. It is also reported that WRMSDs cause absenteeism or lost work time and increase work restriction, with serious economic consequences on individuals, organizations as well as society. However, even in developed countries, these disorders seem to be underestimated among health professionals (Table 2) [66].

It seems that the largest percentage of participants who encountered WRMSDs continued to work. In a study conducted in New Zealand, the prevalence of absenteeism due to WRMSDs ranged from 6.2% to 18%, and less than 10% of nurses were absent from work due to the occurrence of WRMSDs [67]. Probable reasons for this reaction may be the tendency to ignore the pain or to continue working, avoiding certain activities until the pain subsides [57]. In addition, any occupational insecurity of the participants may act as a disincentive in the absence due to illness (sick leave) [68].

Table 2: Effects of musculoskeletal disorders on nursing personnel

Absenteeism	Lower quality of life
Reduced productivity	Transfer to another job
Work constraints	Disability
Increased healthy costs	Decrease in nurse efficiency
Financial implications for the individual	Mistakes/Safety problems of patients

Another study found that 61.3% of nurses resorted to self-medication for pain or discomfort and 24.7% of participants had also consulted a physiotherapist [69]. In another study, less than 20% of nurses visited healthcare professionals for WRMSDs rehabilitation with a similar finding, reflecting the degree of information and awareness of participant nurses and suggesting a common practice among nurses, possibly due to their prior knowledge [67]. Such disorders when they are not treated properly with effective treatment and sick leave or when nurses take self-prescribed analgesic drugs on their initiative may not recover quickly or even worsen. At the same time, WRMSDs lead nursing personnel to low work performance which is another type of indirect cost associated with WRMSDs, quite difficult to be measured [70,71].

PREVENTIVE AND THERAPEUTIC STRATEGIES FOR WRMSDS

Undoubtedly, nursing personnel must maintain their ability to work while remaining healthy until retirement. To reduce the incidence of WRMSDs in their work, it is crucial that prevention strategies be followed, such as the implementation of ergonomic measures and training programs in high-risk nursing departments and immediate therapeutic approaches, where required [21,72]. Prevention of WRMSDs presupposes the analysis of the clinical environment where nursing actions are offered and the identification of risk factors, as their mitigation requires targeted interventions [48,73].

Ergonomic recommendations

The ability to reduce injuries in direct care nurses is improved by ensuring easy access to ergonomic mechanical devices to lift and transfer patients, reducing the number of hasty body positions involving muscle tension combined with educational interventions [42,74]. Other ergonomic interventions are the ergonomic patient evaluation, the practice of zero weight lifting, the establishment of groups that handle the lifting of patients, the adaptation of the clinical environment, and the application of specific procedures (e.g., guidelines for a safe task execution such as lifting or pushing a heavy object) [75].

The goal of interventions regarding WRMSDs rate reduction is to implement good work practices that prevent physical workload, better information about potential work hazards that are responsible for injuries, as well an explanation of the proper operation of the necessary equipment [6,42].

A recent review emphasizes that preventive measures for these risks are still insufficient and this fact has an impact on both nurses' professional performance and their desire to stay at work [21]. In another study, nursing personnel with WRMSDs reported that their workplaces did not provide training on how to reduce injuries and that their prior training was insufficient or incomplete regarding WRMSDs prevention [76]. Ergonomic training reduces the tension of nurses' muscles during a working day [48]. Nursing personnel training should be a priority for hospital administrations even if the effectiveness of the training interventions as well as the handling of assistive devices is not satisfactory [77].

Adequate rest is a protective factor for nurses' WRMSDs. Rest contributes to muscle relaxation by reducing muscle fatigue [3]. A study by Wang et al., revealed that a rest time of 15 min could restore in the normal range the erector muscle of the spine as well as the heart rate [78]. Decompression and relaxation after a long workday may often improve staff satisfaction, as the provision of relaxation activities has been proven to reduce stress levels in employees [79].

Physical exercise may also be beneficial, reducing the prevalence of WRMSDs in nursing personnel [61]. Exercise improves muscle sensitivity and coordination, response speed, and psychosocial behavior by delaying the decrease in muscle strength due to age [80]. It also has a protective effect on low back and neck pain in workers with heavy and sedentary activities [81,82].

Organizational interventions

Reducing the excessive stress of nursing personnel over time while adapting nurses' workload appropriately to reduce the significant physical and psychosocial burden and exposure to WRMSDs requires organizational involvement and support [68]. The prevention of WRMSDs is enhanced by the assignment of specific patients to nurses without known health problems, who may be more capable of providing heavy physical care [83]. In addition, adjustments to work organization should be made including adequate rest time between work shifts and an acceptable work schedule [83].

Because the majority of people with anxiety fail to recognize the underlying conditions, mental health should be monitored as well as specialist care will be needed, especially for nurses working in high-stress nursing departments [55,84]. At the same time, organizational interventions are required to improve the working environment in terms of assigning tasks among nursing staff, setting working hours, keeping a positive attitude, and conducting medical examinations for the prevention or early detection of WRMSDs [62]. Other actions include the implementation of healthy lifestyle choices (engagement in physical activity, tobacco and alcohol limitation, eating a balanced diet) as well as education on body posture, especially when lifting and transporting loads or patients [84]. Preventing WRMSDs in nursing personnel is vital to providing quality patient care, improving nursing morale, and increasing productivity by reducing time loss and other absences [47].

CONCLUSION

Cost-effective high-quality health care requires, among other actions, an adequate and productive nursing staff. Maintaining the competence of employees presupposes the identification of risk factors that contribute to the development of WRMSDs, early recognition, and documentation of health problems related to WRMSDs, as well as policy strategies for modifying working conditions such as the implementation of ergonomic measures.

Evidence-based intervention strategies are necessary for the safety and productivity of nursing staff and are likely to lead to significant cost savings. The modern challenges in high-quality nursing care provision in hospitals require not only the knowledge but also the attitudes and skills of well-trained nurses. The recognition of the nursing profession as a cornerstone of the health service automatically implies policies that allow the maximum performance of the nursing personnel.

Conflicts of interest and sources of funding
None.

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ORIGINAL ARTICLE

An Overview of Eating Disorders in Iran: Frequency and Some Related Factors

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Abstract: *Background and Aim: Eating disorders are one of the most important issues in mental health that leading to many serious psychic and somatic problems and even death. These disorders were known for years as a disease of the western societies. Recent studies showed that they are also spreading in eastern countries. Limited studies are in Iran. The purpose of this study is to review the Iranian researches. The results of this study in Iran as a developing Asian country will helpful for other societies, especially similar Asian communities. Methods: The authors searched PubMed, ProQuest, Cochrane, Scopus, Web of Sciences, and SID and Magiran (In Persian) to find the relevant articles. The Joanna Briggs Institute Critical Appraisal Checklist was used to assess the quality of research. Results: Twenty- nine studies reported the prevalence or occurrence of eating disorders among the Iranian population. The samples consisted of 40 to 30,532 subjects. Forty papers examined eating disorders among females. Twenty-seven studies were in both genders, and only one was in males. Most eating disorders have significantly varied depending on the different studies and tools used. Most of these articles studied young people. This study breaks the determinants into three parts: demographic, psychosocial, and miscellaneous factors. The incidence of eating disorders in Iran is on the rise. Conclusions: It should be considered a significant health problem. Moreover, despite a clear cultural difference, eating disorders' factors were similar to those in western communities. This matter may be an essential health issue. In addition, despite noticeable cultural differences, the factors affecting eating disorders were similar to those of western communities.*

Keywords: *eating disorders; prevalence; contributing factors; review; Iran*

INTRODUCTION

The main issue in eating disorders is a distortion of body perception. People with eating disorders use weight loss methods such as diet or compensatory behavior (intensive exercise, vomiting) to obtain the ideal appearance [1]. Although eating disorders are less prevalent as compared to some psychiatric disorders such as depression and anxiety [2,3], these are one the severe health issues.

The complications of these problems include 1. Psychological like low self-esteem and depression, substance use and even suicidal attempts, or 2. physical conditions such as malnutrition, severe imbalance of water and electrolytes, digestive problems, and even death [4].

Various biological, psychological, and social factors influence eating disorders [3-5]. These disorders are known as Western cultural disorders [6]. However, various studies have shown that the disease is widespread in non-Western crops, both developed and developing economies such as Brazil and Asia [7]. According to some researchers, these disorders are culture-influenced rather than culture-related phenomena [8]. Most Asian studies on eating disorders are in East Asia. Western Asian countries, particularly the Middle East, have a different culture from East Asia. Therefore, it may be helpful to study these conditions in this region [9].

A review of Iranian articles on bodily perception has shown that body dissatisfaction is high in Iran. Regarding globalization and the increased influence of Western culture, eating disorders are a health problem among Iranian adolescents and youth [10,11].

The current study sought to collect scattered information on the prevalence and contributing factors of eating disorders in

Iran. The results of this study conducted in Iran as an Asian country will benefit other similar Asian companies. In addition, the findings may assist health researchers and planners in the management of disorders.

MATERIALS AND METHODS

This study was carried out according to the The Joanna Briggs Institute Critical Appraisal Checklist [12]. We used that directive at all stages of the study. The authors searched The databases of PubMed, ProQuest, Cochrane, Scopus, Web of Sciences, and SID and Magiran (In Persian) to find the related articles to 2021 with no limitation at publication time.

The search strategy was designed to cover all articles related to eating disorders in Iranian people. A combination of Medical Subject Headings (MeSH) and keywords (Persian and English) was searched for related articles. These keywords included: "Eating disorders "Binge-Eating Disorder" OR "Anorexia Nervosa" OR Bulimia OR "Binge Eating" OR "Bulimia Nervosa" OR "Compulsive Eating" OR “Eating and Feeding Disorders"

Inclusion Criteria

All original papers on eating disorders among Iranians were examined. The papers have been divided into three types: Reporting the prevalence of eating disorders, Effective factors on eating disorders, Treatment of these disorders.This study reviewed articles about the prevalence and influential factors of eating disorders. Letters to publishers, reviews, and comments have been excluded.

Collecting data

The related articles were selected according to the title, abstract, and keywords.

Data Extracting

Two researchers separately extracted information such as the year of publication, location of study, sample size, age, type of study, gender, and other variables presented in Table 1. They recorded the findings in the data extraction form. Any disagreement between the researchers discussed and the outcome nearest the opinions of the two researchers chosen. In the conflict between the two researchers, the advice of a third expert on the topic was used.

Evaluate the research quality

The Joanna Briggs Institute Critical Appraisal Checklist evaluated the quality of the research [12]. Each article was examined based on methodological criteria such as the relevance of the participants, the sufficient size of the sample, the validity of the research method, and the use of appropriate statistical methods. Each article was investigated based on the methodology criteria such as suitability of participants, sufficient sample size, the validity of the research method, and use of appropriate statistical methods.

Figure 1: Flow diagram of the literature search and study selection process

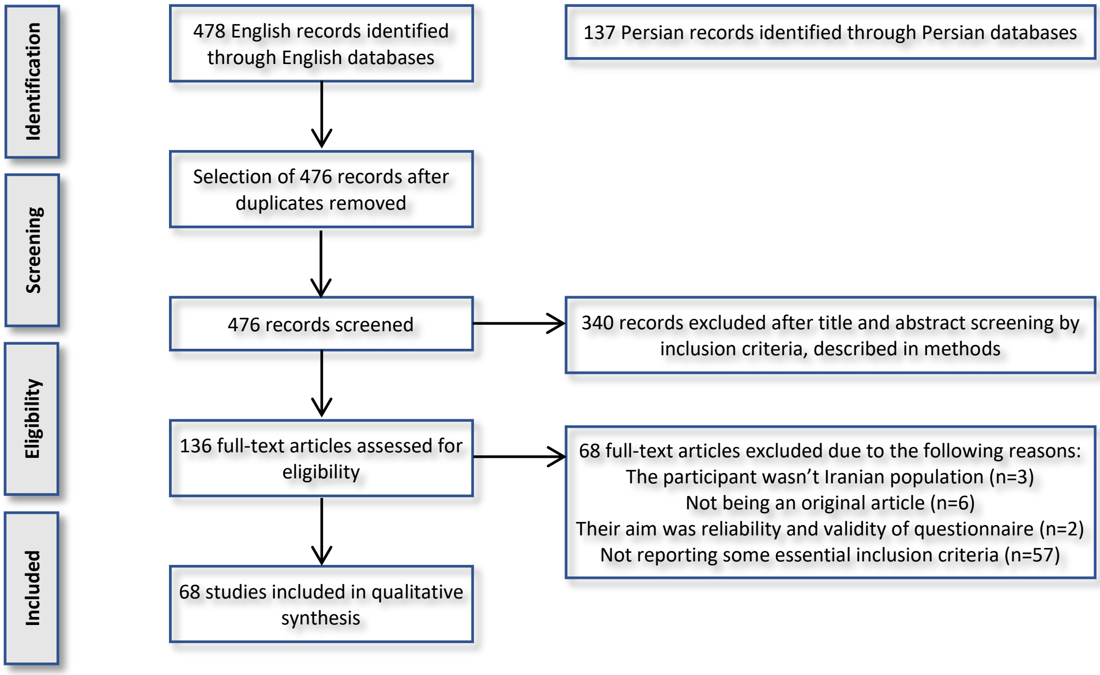
Identification
Screening
Eligibility
Included
478 English records identified through English databases
137 Persian records identified through Persian databases
Selection of 476 records after duplicates removed
476 records screened
340 records excluded after title and abstract screening by inclusion criteria, described in methods
136 full-text articles assessed for eligibility
68 full-text articles excluded due to the following reasons:
The participant wasn’t Iranian population (n=3)
Not being an original article (n=6)
Their aim was reliability and validity of questionnaire (n=2)
Not reporting some essential inclusion criteria (n=57)
68 studies included in qualitative synthesis

RESULTS

The authors found 615 articles, including 478 English and 137 Persian articles. About 476 articles were approved according to their titles, abstracts, and keywords. Only 68 articles met the inclusion criteria, including 27 English and 41 Persian articles (Figure 1).

Twenty-nine studies reported the prevalence of eating disorders in Iranian populations(8, 13, 14, 16, 19, 21-23, 25, 29-31, 33, 36, 38, 44, 46-49, 51, 52, 55-57, 59, 60, 62, 68, 70).

The prevalence of these disorders has reported very different from 0.2% to 80% (Table 1).

The articles` characteristics are presented in Table 1. These studies were conducted between 2000 and 2021. Among these studies, 40 articles were conducted on females, 27 articles on both genders, and only one article on males(56). The number of subjects ranged between 40 and 30532.

Most studies have investigated the characteristics of participants in a descriptive method and two studies used Ex-post and Facto method [37,53]. Eighteen studies showed relation between BMI and eating disorders. Sixteen studies investigated the relationship between body satisfaction and eating disorders. Nine studies have shown the relationship between parental characteristics and eating disorders. In the most articles participants' age was less than 35 years old. Four studies confirmed the relation between eating disorders and media use, especially Western media. Six studies investigated Depression, Anxiety and Stress, Obsessive-Compulsion Psychological signs, Poor psychosocial domains of HRQOL. Related factors and the articles that refer to them are shown in Table 2.

DISCUSSION

Prevalence

The prevalence of eating disorders varies according to the research. A study looked at eating disorders among Iranian students living in Iran and the United States. She found that Iranian women living in Iran are more affected by these troubles than women living in America [13]. Abdollahi & Mann have shown that women living in Iran have a higher prevalence of eating disorders [14]. Studies around the world have shown that prevalence rates are not the same. A large national study conducted in the US found that AN, BN, and BED lifetime prevalence estimates were 0.80%, 0.28%, and 0.85%, respectively [81]. The widespread prevalence of eating disorders in Asian countries has been reported [7]. As noted earlier, most studies are limited to eastern and southeastern Asia. However, limited studies in the Western Asian and Eastern Mediterranean regions have reported relatively high rates of such disturbances. The prevalence of eating disorders in West Asian countries like Pakistan has shown that eating disorders have increased from 10.3% to approximately 21.6% since 1992 – 2014 [9]. Various statistics have been reported in the Arab countries (Eastern Mediterranean): 11.5% of women in Egypt and 49% in the UAE. This rate in men varied from 6% in Saudi Arabia to 36% in Oman [82]. The prevalence of these conditions decreased from 0.2% to 80%. Based on a national study, the total prevalence of eating disorders among children and adolescents was 0.89 (0.81-1.10) [70]. This difference can be due to conducting studies at various ages, groups such as athletes, and diagnostic tools. For the most part, self-administered screening questionnaires were used. The clinical interview makes it possible to better estimate the prevalence of eating disorders.

Factors contributing to eating disorders

Over the last few years, many studies have focused on the factors contributing to eating disorders' development and persistence. The papers examined biological, social, interpersonal, internalizing the ideal body announced by the media, low self-esteem, cognitive pattern, and family dynamics. It may be challenging to differentiate their direct effects due to their interaction. For example, people with low self-esteem have a deformed perception of the body and higher dissatisfaction they are more influenced by social pressures and the media. In this section, we focus on contributing factors in three sub-categories.

Demographic variables

Age

Limited studies have investigated the effect of age on eating disorders [16,25,30,51,59,62-64,70,83].

Because most studies were in the narrow age range, it is impossible to discuss the age effect.

BMI

Many studies have defined contributing effects of BMI through affecting body image distortion. Some researchers believe self-devaluation, feeling incompetent, worries regarding weight gain disproportionate to the advertised ideal body are the consequences of high BMI that have been directly or indirectly affected by eating disorders [2,84].

BMI was studied in many articles [8,10,16,19,21,25,30,35,46,48,49,52,57,58,62,64]. Body mass index had a positive relation to eating disorders and body dissatisfaction. In these researches, such as Western studies, BMI was considered a significant contributing factor.

Table 1: Main characteristic of studies

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
1	Nobakht,2000, Tehran (13)	3100	15-18	Prevalence of Eating Disorders	Cross Sectional	Female	Boy dissatisfaction levels of education of the mother, father profession	Eating Disorder Diagnostic Inventory	ANOVA	The prevalence of eating disorders was more than other non-Western societies, and comparable with Western societies	Lifetime prevalence of 0.9% for Anorexia Nervosa, 3.2% for Bulimia Nervosa, and 6.6% for the partial syndrome.
2	Abdollahi,2001, Tehran (14)	59	NA	Comparisons between Iranian women in Iran and in America	Cross Sectional	Female	Exposure to Western media, acculturation	Eating Disorder Examination-Questionnaire and the Figure Rating Scale		Neither exposure to Western media, nor acculturation to Western norms appeared to be affected by disordered eating.	The prevalence of eating disorders in students that lived in Iran was as much as who lived in America
3	Jaeger, 2002 (15)	1751	19-23	Risk factors for bulimia nervosa in 12 countries	Cross Sectional	Female-Male	Body dissatisfaction self-esteem'	Silhouettes Dieting' scale of the Eating Attitudes Test (EAT), Ineffectiveness scale of the Eating Disorders Inventory	Functional model, structural equation methodology	That body dissatisfaction seems independent from the actual BMI, could be affected Iranian population	
4	Zarghami (8)	1200	13-18	Abnormal attitudes frequency towards eating	Cross Sectional	Female-Male	BMI	Attitude Test (EAT-26) Silberberg Anxiety test Beck Depression	Logistic regression	Individuals with these abnormal attitudes, are more at the risk of developing psychological disorders,	Their prevalence has been estimated to be about 10.5%
5	Jahanfar, 2005, Tehran (16)	250 (twins)	15-45	Relation between Eating Disorder, Polycystic Ovary Syndrome	Cross Sectional	Female	BMI, Presence of Acne, Amenorrhea, Number of periods per year	Bulimia investigation test (Edinburgh)	Student's t-test and Chi-square test Fisher test	The genetic property of bulimia nervosa was not found in this twin study. Bulimia score was found to be positively correlated with BMI, acne, amenorrhea. Number of periods per year. No significant correlation was found between bulimia score and age, age of menarche, hirsutism. serum leptin level was correlated significantly with bulimia score	Only 8% of subjects were diagnosed subclinical eating disorder

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
6	Yousefi Asl, 2007, Tehran (17)	420	14-18	Data processing and selective attention in Eating disorders	Cross Sectional	Female	Task sensitivity	EAT-26 , Dot Probe Task	Student's t-test and Chi-square test	Attention bias with avoidance from body-shape stimuli was more in who had high score of Eating Attitudes Test.	
7	Momeni, 2008, Isfahan (18)	53	14-17	Relationship Between Parents' Child Rearing Styles and Eating disorders	Cross Sectional	Female	Parenting style	Minnesota Eating Behavior Survey (MEBS,Child rearing questionnaire of Baum Rind	Pearson correlation test ,logistic regression	There was significant positive relationship between democratic style of parents and anorexia nervosa and negative relation between authorities' style of parents and anorexia nervosa. Among female adolescents.	
8	Pourghassem Gargari, 2008, Tabriz (19)	1877	14-19	Prevalence of eating disorders in Female students	Cross Sectional	Female	Overweight perception, Desire for loss of weight Cigarette smoking, Age of menarche	Eating Attitude Test 26	ANOVA, Chi-square test	About half of the participants were unhappy with their body weight Mean disordered eating attitude was higher in them. They were intended to weight loss, were smoker, and who had age of menarche less than 11 years.	About 16.7% of students had eating disorders. This prevalence was lower than Tehran (capital of Iran) and some of Arabian countries.
9	Asgari, 2009, Ahvaz (20)	145	NA	Emotion regulation, Mental stress and Body image in Eating disorders	Cross Sectional	Female	Emotion Regulation, Mental Stresses	Emotion Regulation Questionnaire", Mental Stress Questionnaire, Eating Disorder Questionnaire ,Body Image Questionnaire	Regression	Mental stresses and eating disorders were significantly related to each other. Body image and eating disorders had negative relation	
10	Safavi, 2009, Tehran (21)	400	NA	Body image perception and eating disorders	Cross Sectional	Female	BMI, Parents characteristics	Multidimensional Body Self Relation Questionnaire-Eating Attitude Test 26	Chi-square test	BMI and father`s job had positive relation with eating disorders.	About 21.5% had eating disorders that included 1.8%Anorexia Nervosa and 7.8% Bulimia Nervosa

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
11	Gargari, 2010, Tabriz (22)	250	14-51	Eating attitudes, self-esteem and social physique anxiety in Iranian fitness females	Cross Sectional	Female	Weight, Height, Body Mass Index (BMI)	Eating attitude test-26 (EAT-26), Rosenberg's self-esteem scale (RSES), and Social Physique Anxiety Scale (SPAS)	ANOVA, Pearson Correlation Test	Disordered eating attitude was correlated negatively with Self steam and positively with body weight, BMI, and SPAS	28.4% of subjects were disordered eating attitudes (EAT-26220)
12	Mahmoodi, 2010, (23)	195	21-25	Relationship between social pressure and body image with eating disorders	Cross Sectional	Female	Exercise	The Multidimensional Body-Self Relations Questionnaire (MBSRQ), Perceived Sociocultural Pressure Scale (PSPS), EAT 26	Student's t-test, Pearson Correlation	The was positive relation between eating disorders with social pressure. Positive relation was demonstrated between eating disorders and body image.	About 27% of athletes and 19% of non-athletes had eating disorders
13	Moloodi, 2010, Tehran (24)	151	20-50	Comparison of early maladaptive schema in eating disorders	Cross Sectional	Female	Obesity-BMI	Binge Eating Scale, Young Schema Questionnaire	MANOVA	Binge eating disorder had significant relation with higher scores of abandonment/ instability, emotional deprivation and insufficient self-control/ self-discipline	
14	Shamsaddin Saeed, 2010, Kerman (25)	650	13-22	Eating disorders and Body image	Cross Sectional	Female	Age- Parents Education	Eating Disorder Diagnostic Scale Body Image questionnaire	Student's t-test, linear and Logistic Regression	BMI was predictive factor for eating disorders	About 6.3% of students had eating disorder, consisting of 1.7% anorexia nervosa, 1.7% bulimia nervosa and 2.9% eating disorder not otherwise specified.
15	Shayeghian, 2010, Tehran (26)	403	16-17	Maladaptive Core Beliefs and Eating Disorder	Cross Sectional	Female	Parental Bonding	Parental Bonding Inventory- Young Schema Questionnaire- Eating Disorder University Belief Questionnaire- Eating Disorder Inventory	Pearson Correlation Test	Eating disorder beliefs and Maladaptive core beliefs respectively play a contributing role between parental bonding and eating disorder symptoms	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
16	Behrad, 2011, Yazd (27)	220	NA	Thought control strategies and eating disorders	Cross Sectional	Female	Self-punishment and Worry	Thought Control Questionnaire (TCQ) - Eating Attitude Test 26	Pearson correlation-regression analysis	Self-punishment and Worry both were the best predictors of eating disorder	
17	Khosravian, 2011, Tabriz (28)	140 (70 with bulimia and 70 without bulimia)	NA	Age-Gender , Education	Cross Sectional	Female - Male	Perfectionism, Negative Affect, Brain-Behavioral Systems	Hewitt and Flett multidimensional perfectionism scale, Hed depression and anxiety scale and Carver and White' s brain-behavioral systems questionnaires	MANOVA	Results showed Bulimia nervosa was associated with higher levels of perfectionism, negative affect and BAS and lower BIS	
18	Mozaffari-Khosravi, 2011, Yazd (29)	1400	NA	Eating Disorders High School Girl Students	Cross Sectional	Female	Age, Parents education, Menstrual status, Birth order, Family size	Eat Eating Attitude Test 26	Chi-square, Fisher' s exact test and Pearson correlation	Between BMI status and risk of eating disorders a significant relationship was showed. Family sizehad relation with eating disorders	About 12% of students were at the risk of eating disorders. 0.5% had Anorexia Nervosa and 5.3% were diagnosed with Bulimia Nervosa
19	Valizade, 2011, Ahvaz (30)	181	NA	Eating Disorders and Psychological Signs among women with Sport Activities	Cross Sectional	Female	Marriage Status, Education Psychological status, Depression, Anxiety	Ahvaz Eating Disorder Scale - Psychological Signs Questionnaire	Pearson correlation, Multivariate regression	Eating disorder signs, anorexia nervosa signs and bulimia nervosa signs were related to psychological signs, Depression and Anxiety.	16.5% percent of athletes have eating disorder signs, 14.3% anorexia nervosa, and 3.3% bulimia nervosa
20	Mashhadi, 2012, Tehran (31)	99	NA	Dieting and the Development of Eating Disorders among Iranian Women	Cross Sectional	Female	BMI, Weight satisfaction, Self-esteam Age	Eating Attitude Test (EAT-26),the Sick, Control, One stone, Fat and Food (SCOFF) questionnaire,	Chi-square, Student's t-test	Iranian women that lived in Iran with a low BMI (below 18), high higher tendency toward eating disorders.	About 17% of participants had eating disorders. The Iranian women in Iran had equal or even higher eating disorders than Iranian woman in Netherland.

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
21	Abbaszadeh, 2013, Tabriz (32)	400	Mean 20	Eating disorders and emotional intelligence	Cross Sectional	Female	Social awareness	Siberia Shearing Questionnaire-Eating Disorder Diagnostic Scale	ANOVA, logistic regression	Multiple aspects of emotional intelligence had negative relation with eating disorders. Social awareness was predictive factor in eating disorders	
22	Garrusi, 2013, Kerman (33)	1204	14-55	Eating Disorders and Their Associated Risk Factors	Cross Sectional	Female - Male	Body Mass Index (BMI), Media, Body dissatisfaction, Self-esteem	Eating Disorder Diagnostic Scale-Body-Esteem Scale for Adolescents and Adults-Perceived Socio-Cultural Pressure Scale-Rosenberg Self-Esteem Scale	Two-sample t - Chi-square tests- logistic regression	Symptoms of bulimic syndrome were greater in males. BMI, Social pressure, Body dissatisfaction, and Western TV were only significant in the male. Educational level, Self-steam, were significant in the females	The prevalence of eating disorders was 11.5% that included 0.8% anorexia nervosa, 6.2% full threshold bulimia nervosa, 1.4% sub threshold anorexia nervosa and 30% sub threshold binge eating disorder
23	Hossein Zadeh, 2013, Mazandaran, (34)	525	NA	Maternal Bonding and Vulnerability related to Eating: The Mediational Role of Eating Disorder Beliefs and Alexithymia	Cross Sectional	215 females, 210 males	Alexithymia	Eating Disorder Inventory, Alexithymia Scale, Eating Disorder Belief Questionnaire, Parental Bonding Instrument	ANCOVA	The findings demonstrated that maternal bonding can play an important role in preventing eating disorders	
24	Mohamadzadeh 2013 Tabriz, (35)	280	Mean 21.8± 2.49	Eating disorders physical dissatisfyaction and physical activity behavior change	Cross Sectional	Female	Physical activity behavior change	EAT-26, Body dissatisfaction - Physical activity staging questionnaires	Pearson correlation, Spearman correlation and one way ANOVA test	There was positive relationship between eating disorders and body dissatisfaction. There was significant relationship between eating disorders and stages of change for physical activity.	
25	Mohebi, 2013, Kermanshah (36)	578	21-24	Eating disorders prevalence	Cross Sectional	Female	Demographic variable Amenorrhea	Eating Disorder Diagnostic Scale	Student's t-test	BMI was significant relation with Anorexia Nervosa and Eating Disorders NOS	Results showed frequency of Anorexia Nervosa and Eating Disorders NOS were 0.2% and 2.4 respectively

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
26	Narimani, 2013, Ardebil (37)	104 normal students and 104 bright students	NA	Eating disorders in normal and bright students	Ex post facto research	Female-Male	Parenting styles	Baumrind parenting style and Coker and Roger eating habits questionnaires	MANOVA	There was no difference in eating attitude between normal and bright students	
27	Kordi,2014, Mashhad (38)	190	Mean 25.14 ±4.4	Eating disorder symptoms in nulliparous women	Cross Sectional	Female	Social anxiety symptoms	Eating Disorder Assessment	Pearson correlation tests. Linear regression	Social anxiety had positive correlation with eating disorders symptoms	About 8% of pregnant woman had eating disorders symptoms
28	Mohamadzadeh, 2014, (39)	1008	Mean 24 years	Factor Structure of disordered eating attitudes	Cross Sectional	Female-Male	Gender	Eating Attitude Test (EAT)	Explanatory factor analysis of variance - independent samples t-test	There is more internal constancy between Dieting, Bulimia plus Food Preoccupation, & Oral control with eating disorders attitude. Women have higher mean scores on the eating attitudes	
29	Narimani, 2014, (40)	53 Student with binge eating disorder, 64 Normal	Mean 17 years	The role of psychological factors in predicting binge eating disorder	Cross Sectional	Female-Male	Demographic variable	Composite Scale of Morningness (CSM), Lynda's Depression Anxiety Stress Scales (DASS), Weight efficacy Life Style Questionnaire and coping styles questionnaire. Discriminant analysis	Compare Means	Analysis showed that the depression, food availability and negative emotions have a significant effect on the predicting binge eating disorder.	
30	Roustaee, 2014, Tehran (41)	515 (231 sample with eating disorder)	NA	Major Dietary Patterns and Their Relation with Eating Disorders	Cross Sectional	Female	NA	Body Shape Satisfaction Scale (BSSS) and semi-quantitative food frequency questionnaires	Logistic regression	High fiber and low fatty food had relation with eating disorders None of the demographic characteristics, weight, or body satisfaction showed any significant relationship with eating disorders	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
31	Poursharifi,2014 (42)	142	14-18	Social physique anxiety and eating attitudes	Cross Sectional	Female	BMI - Social Pressure	Social Physique Anxiety (SPAS), Eating Attitudes (EAT-26,), Socio-cultural Influence on Weight loss and Body change	Multivariate two-factor ANOVA	High sociocultural pressure had a significant role in the social physique anxiety and eating attitudes	
32	Bahri, 2015, Gonbad Kavoos (43)	250	NA	Eating Disorders in children and Its Relationships With Their Mothers' General Health And Nutrition Attitude	Cross Sectional	Female	Age of menarche?	General Health Questionnaire (GHQ-28), the Eating Attitudes Test (EAT-26), Eating Disorder Inventory Questionnaire	Multivariate regression, Pearson correlation tests	Significant correlation was between aspects of mothers' general health, nutrition attitude and female children's overeating, desire for slimness, body dissatisfaction	
33	Eftekhari, 2015, Shiraz (44)	594	14-17	Eating disorders and anthropometric indices in female adolescent students	Cross Sectional	Female	Socioeconomic variables, BMI	Nutritional attitude questionnaire	independent t- test and Chi-square	BMI had relation with eating disorders	Results showed that 80% of the studied population are at risk of eating disorders
34	Foladvand, 2015, Tehran (45)	300 (100 patients with eating disorders, 100 patients with high depression, and 100 normal subjects	NA	The Contribution of Self-Concept, Impulse Control and Dysmorphic on Eating Disorders	Cross Sectional	Female-Male	Depression, Anxiety, Stress	Rogers Self-concept scale, The Body Dysmorphic Disorder Questionnaire, Bar-On Emotional Quotient Inventory, Depression Anxiety Stress Scales	Multiple regression analysis	Self-concept, impulse control and body dissatisfaction had significant relation with eating disorders .Depression, anxiety and stress had relation with eating disorders	
35	Jalali-Farahani, 2015. Tehran (46)	465	14-17	Disordered Eating, Health-Related Quality of Life Among Adolescents	Cross Sectional	Female	Overweigh	Eating Attitudes Test-26 (EAT-26) and Pediatric Quality of Life Inventory (PedsQLTM4.0)	T test, Chi square	Disordered eating was associated with overweight in girls and poor psychosocial domains of health-related quality of life	Disordered eating was prevalent in 18.9 % of adolescents

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
36	Mohamadirizi, 2015, Mashhad (47)	213	24.14 ±4.4 years	Eating disorder symptoms and Obsessive Compulsive Disorder in Prime- gravid women	Cross Sectional	Female	BMI, Gestational age	Eating Disorder Examination Questionnaire and Maudsley Obsessive Compulsive Questionnaire (30Q)	Pearson correlation coefficient, t test, one way ANOVA and linear regression	There was a poor positive correlation between the rate of Eating Disorder Symptoms and Obsessive Compulsive	About 18% of pregnant women had Eating Disorder Symptoms.
37	Rauof, 2015, Urmia and Tabriz (48)	1990	15.8 ±1.24	Prevalence of eating disorders among adolescents	Cross Sectional	Female- Male	BMI-Income	Eating attitudes test (EAT-26) and DSM-IV criteria	Linear and logistic regression and Fisher' s exact test	BMI was related to eating disorders.	24.2% of students were at risk of eating disorder .About 0.25%had eating disorder.
38	Rezaei, 2015, Shiraz (49)	302	15-35	Prevalence of eating disorders	Cross Sectional	Female	Age- Education - BMI	Eating attitudes test (EAT-26)	Descriptive statistics section, the central and dispersion indices		Participants had 0.7% Bulimia Nervosa and 0.3% had binge eating Disorders.
39	Rezaei, 2015, Shiraz (50)	302	15-35	Study of the Perfectionism Role and Body Mass Index in Prediction of Eating Disorders among Female Students of University	Cross Sectional	Female	Age- Education, BMI, Body dissatisfaction	Perfectionism Ahvaz Scale and Body Shape Questionnaire, Eating attitudes test (EAT-26)	Descriptive statistics section, the central and dispersion indices	The perfectionism and BMI were good precedents and body dissatisfaction was good mediator for eating disorder in female students.	
40	Rouzitalab, 2015, Tabriz (51)	105 males and 105 females	18-25	The relationship of disordered eating attitudes with body composition and anthropometric indices	Cross Sectional	Female- Male	Weight, height	Eating attitude test (EAT-26)	Mann-Whitney U test, Kruskal-Wallis test and Spearman correlation test	In females, weight and body mass index (BMI) were significantly different between disordered eating and healthy subjects.	About 10% of the studied subject had disturbed eating attitudes.

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
41	Safarzade, 2015, Gonabad (52)	750 males and 750 females	13-18	Eating Disorders, Adolescents	Cross Sectional	Female-Male	Age, Education level	Eating attitude test (EAT-26)	Chi-square test	There was a significant association in prevalence of this disorder among these opposite sexes, with different ages and grads	About 15.7% of students had distorted eating. Prevalence of Anorexia Nervosa and Bulimia Nervosa were about 1.1 % and 3.2% respectively
42	Javanmard, 2016, Tabriz (53)	20 bulimic, 20 normal	NA	Explicit Memory (Recognition and Recall Memory) and Bulimia	Cross Sectional	Female	NA	Bulimia Questionnaire , Explicit Memory Inventory	MANOVA	There was a significant difference in recognition memory in bulimic group	
43	Bodaghi, 2016, Arak (54)	32 bulimia, 32drug addiction and 32 normal	NA	Activity of Brainal Behavioral Systems, eating disorders	Cross Sectional		NA	Gurmally overeating scales ,Jackson’ s five-factor Inventory	MANOVA and Tukey	Activation system and freezing are have critical roles in the creation and persistence of the eating disorder	
44	Farid, 2016, Karaj (55)	537	14-18	Eating disorder Adolescence	Cross Sectional	Female	Weight, Height	Eating Attitudes Test (EAT-26)	Chi-square, ANOVA and t-test	The attitude toward eating disorder was related with BMI of and their self-concept	The prevalence of eating disorders was estimated to be 23.6%.
45	Garrusi, 2016, Kerman (56)	433	14-18	Eating disorders Male adolescents	Cross Sectional	Male	Educational level, Economic status, Social pressures, Self-esteem, Body satisfaction Media	Eating disorder diagnostic scale, Body Satisfaction Instrument, Socio-Cultural Pressure Instrument, Self-Esteem Questionnaire	Two-sample T-test and Chi-square, logistic regression	There were significant differences between the two groups (with and without eating disorders) in terms of social pressure, body dissatisfaction and body mass index (BMI). Body dissatisfaction and BMI were identified as predictive factors in eating disorders	About 15% of male adolescents had eating disorders. 7.4%) subjects had diagnostic criteria for full threshold BN, and 6.5%) individuals had sub-threshold BED. Only (0.7%) adolescents had sub-threshold BN.
46	Hosseini, 2016, Hamadan (57)	231	18-32	Fitness intention Eating attitudes	Cross Sectional	Female	Age-Residency status, Marriage status	TPB variables scale	T-test, ANOVA, Bivariate correlations, and linear regression	Results showed a positive correlation between fitness intention and eating attitude.	Nearly 21.6% of the participants had abnormal eating attitudes

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
47	Kachooei, 2016,Tehran (58)	104 (52 women with BED, 52 women without BED)	With BED 30.63, without BED 28.63	Impulsivity and Difficulties in Emotion Regulation among Overweight Women With and Without Binge Eating Disorder	Cross Sectional	Female	Age, BMI	Eating Disorders Diagnostic Scale (EDDS), the Barratt Impulsivity Scale (BIS-11) and the Difficulties in Emotion Regulation Scale (DERS)	MANOVA	Binge eating women had more impulsivity, difficulties in emotion regulation, attentional impulsivity	
48	Khajrnoori, 2016, Shiraz (59)	600	15-29	Body image and Eating disorders	Cross Sectional	Female	Parental variables	Self-discrepancies for physical characteristics, Body Areas Satisfaction Scale, Parental Attitudes Regarding Body, Peer Attitudes Regarding Body	Correlation test	The results showed parents' attitudes toward body, BRSC, and ICTs use can affect both body image and eating disorders, on the other hand body image can affect eating disorders	15% of subjects had Anorexia Nervosa, 17.7% and 10% had Bulimia and BED respectively.
49	Miri, 2016, Qazvin (60)	226 athlete students and 350 non-athlete	Mean age 21.92	Eating disorders Body image in athletes and non-athlete students	Cross Sectional	Female-Male	Age, BMI	Eating Attitudes Test (EAT-26), and Multidimensional Body-Self Relations Questionnaire (MBSRQ)	T-test and Chi-square test	Anorexia nervosa was found to be more prevalent than bulimia nervosa in both groups. .	The frequency of eating disorders was 11.5% among the athlete students and 11.2% among the non-athlete students.
50	Mohamadzadeh , 2016, (61)	790 (455 Female, 335 Male)	18-52	Obsessive Compulsive Features , Pathological Eating Attitudes in Non-clinical Samples	Cross Sectional	Female-Male	Age	Obsessive Compulsive Inventory-Revised (OCI-R), and Eating Attitudes Test (EAT-26) questionnaires	Multivariate regression analysis	The results showed that obsessive-compulsive features can predict 15% of pathological eating attitudes There were a correlation between obsessive-compulsive features and pathological eating attitudes.	
51	FarsadNaeimi, 2016, Tabriz (62)	430 (296 Female, 134 Male)	Mean 21.09	Eating disorders risk Self-esteem and Body image	Cross Sectional	Female-Male	Age, BMI, Self-Steam	EAT-26, Multidimensional Body-Self Relations Questionnaire (MBSRQ) and Rosenberg Self-Esteem Questionnaires	Chi-square, Independent-samples T test or Mann–Whitney U test	Self-esteem and mother' s education level were predictors of eating disorders risk.	The overall eating disorders risk was 9.5 %.

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
52	Asghari, 2017, Rasht (63)	370	15-18	Perceived stress ,Coping styles on Eating disorders	Cross Sectional	Female-Male	Age-Gender	Eating disorders' scale, Perceived stress scale Coping styles questionnaire	Pearson correlation coefficient ,Stepwise regression	Negative perceive stress, Emotional- focused coping, and Problem focused coping could significantly predict of anorexia and bulimia	
53	Mofradnejad, 2017, Behbahan (64)	200	16.9 ±4.21	Evening-ness, Sleep quality, Eating disorder	Cross Sectional	Female	Oral control	Composite Scale of Morning-ness, Pittsburgh Sleep Quality Inventory and Eating Attitudes Test	Pearson correlation ,Regression tests	There was a positive correlation between evening-ness and negative correlation between sleep quality and eating disorder	
54	Mohagheghi, 2017, Hamedan (65)	245	NA	Self-compassion ,Eating disorder behaviors, Perfectionism, Body dissatisfaction	Cross Sectional	Female	NA	Self- compassion scale-short form, The Almost Perfect Scale, Body dissatisfaction scale, Disorder eating behavior questionnaire	Structural Equation Model	The self-compassion variable is related with eating behaviors through perfectionism and body dissatisfaction. Body dissatisfaction is directly related to eating behaviors.	
55	Hosseini, 2017 (66)		15-35	Testing a Tripartite Model In Body Image and Eating Problems	Cross Sectional	Female	BMI – Body Dissatisfaction – Media	Eating Attitudes Test, The Perception of Teasing Scale (POTS), Multidimensional Body- Self Relations Questionnaire, and Socio-cultural Attitudes towards Appearance Questionnaire	Path analysis	Family factor and social comparison directly affect overeating, whereas inhibition of eating has an indirect effect on overeating. Media through comparison and inhibition of eating indirectly affected overeating. Body image dissatisfaction does not play a mediating role	
56	Radfar, 2018 (67)	264	15-17	Association between eating behaviors with BMI	Cross Sectional	Female	Parents education	Dutch Eating Behavior Questionnaire DEBQ,	Spearman correlation.	Negative relationship existed between eating external stimuli and body mass index. BMI had positive relation with dietary restraint.	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
57	Ebrahimzadeh, 2018, Tehran (68)	400	About 17 years	Eating disorder, Body dysmorphic disorder symptoms	Cross Sectional	Female-Male	NA	Yale-Brown Practical Obsessive-compulsive Scale (BMD) for Body Dysmorphic disorders (YBOCSBDD), Eat-Inventory Questionnaire (EAT-26), and Short-Term Short Term Syndrome Questionnaire (BSI)	Pearson correlation, t-test, Liner regression	The correlation between eating disorder and body dysmorphic disorder was significant	About 67.6% of girls and 55% of boys had eating disorders
58	Kehtari Harzangh, 2019, Tabriz (69)	60	18-65	Comparison of Impulsivity and Experiential Avoidance in Obese Individuals With and Without Binge Eating Disorder	Cross Sectional	Female-Male	Education level	Gurmally Binge Eating Scale (BES), Barratt Impulsiveness Scale (BIS), and Bond Acceptance and Action Questionnaire (AAQ)	descriptive statistics section, the central and dispersion indices	Among components in two groups of with and without binge eating disorder, the components of non-planning impulsivity , and emotional avoidance and control were found significant	
59	Mohammadi, 2020, national (70)	30,532	6-18	Prevalence, correlates and comorbidities of feeding and eating disorders	Cross Sectional	Female-Male	Place of residence	Persian version of K-SADS-PL and DSM diagnostic criteria	multivariate binary logistic regression	Older age, female gender and living in an urban area are predisposing factors. in feeding and eating disorders (in binge-eating disorder, the male gender is positive correlate	The total prevalence offeeding and eating disorders among children and adolescents was 0.89 (0.81–1.10). In all types of feeding and eating disorders
60	Amirfakhraei (2019) (71)	210	NA	Alexithymia, Coping Styles and Cognitive Emotion Regulation	Cross Sectional	Female-Male	Education level	Toronto Alexithymia, Scale, Endler & Parker coping scale of stressful situations	Pearson correlation and multiple regression with enter model methods	Positive cognitive emotion regulation strategies, negative cognitive emotion regulation strategies and problem-focused coping style significantly could predict 45.5 percent of variance eating disorder	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
61	Sahlan (2020) (72)	637	College	Eating-disordered behavior and college students	Cross Sectional	Female-Male	Binge eating, self-induced vomiting, laxative misuse, extreme dietary restriction and excessive exercise	EDE-Q	Pearson and Spearman test	The occurrence of other EDBs did not differ by gender and binge eating was the most common EDB in both gender	
62	Sahlan (2020) (73)	1105	12-19	Eating-disordered behavior and adolescents	Cross Sectional	Female-Male	Binge eating, self-induced vomiting, laxative misuse, extreme dietary restric-	EDE-Q	Pearson and Spearman test	ED symptoms appear to be relatively common among Iranian adolescent boys and girls.	
63	Daeie Farshbaf (2021) (74)	725	14-18	Age at menarche, eating disorders Individuals With and Without Binge Eating Disorder	Cross Sectional	Female	BMI, waist circumference ,socioeconomic status (SES)	Eating Disorders Diagnostic Scale (EDDS) (AAQ)	Analyses of Variance (ANOVA) and Tukey' s post hoc	A significant positive correlation was found between eating disorders and Age of M enarch	
64	Farsimadan (2021) (75)	392/52	14-18	Emotional distress, eating self-efficacy, morningness and coping styles students in binge eating disorder	Cross Sectional	Female-Male	Coping styles, morningness, self-efficacy in eating, stress, anxiety and depression	Interview Composite Scale of Morningness (CSM), Lynda's Depression Anxiety Stress Scales (DASS), Weight efficacy Life Style Questionnaire, Binge Eating Scale and Coping Styles Questionnaire uations	multivariate analysis of covariance.	Persons with binge eating disorder had more anxiety , depression , less self-control , more emotion-focused coping strategies and eveningness tendencies	
65	Mahmoudi (2021)(76)	567	13-18	Emotion regulation and family structure	Cross Sectional	Female-Male		EAT , Difficulties in Emotion Regulation Scale , Family Adaptability & Cohesion Evaluation scale	Structural equation modeling	Indirect effect of unbalanced family functioning to eating disorders through difficulty in regulating emotion is significant	

Table 1: Main characteristic of studies – cont.

ID	Authors, Year, location	Population size	Ages	Mean topic	Study type	Sex	Main contributing Factors	Study`s instrument	Statistical analysis	Main results	Prevalence
66	Sahlan (2021)(77)	730	9-13	Eating disorder and social anxiety symptoms (ED/SAD)	Cross Sectional	Female-Male	Gender	Children Eating Attitudes Test-20, Social Anxiety Scale for Children	Network models (ED and ED/SAD networks	Concern over being judged was central in networks including both ED and SAD symptoms. Additionally, concern over being judged was the strongest bridge symptoms.	
67	Sahlan (2021)(78)	1748	Adolesent; Young adult	Self-esteem, and depression symptoms in eating disorders	Cross Sectional	Female-Male	Gender	Eating Disorder Examination-Questionnaire (EDE-Q), Rosenberg Self-Esteem Scale (RSES) and Beck Depression Inventory, Second Edition (BDI-II)	Six Glasso networks	Central disordered eating nodes in most models were a desire to lose weight and discomfort when seeing one's own body	
68	Sahlan (2021)(79)	564	College	Cross-cultural comparisons in eating disorders	Cross Sectional	Female	Nationality	Body, Eating, and Exercise Comparison Orientation Measure (BEECOM) and the Eating Disorder Examination-Questionnaire (EDE-Q)	One-way analyses of covariance ,partial correlations	Eating disorder-related social comparison is a recommended clinical target in both Eastern and Western cultures.	

Table 2: Summary of (some of) topics that are mentioned in articles

	Variables	**Articles (*n*)**	**References**
Gender	Female	40	13, 14, 16-27, 29-32, 35, 36, 38, 41-44, 46, 47, 49, 50, 53, 55, 57-59, 64-67, 74, 79
	Male/Female	27	8, 15, 28, 33, 34, 37, 39, 40, 45, 48, 51, 52, 54, 60-63, 68-73, 75, 76, 78, 79
	Male	1	56
Language	English	27	13-16, 22, 31, 33, 42, 43, 46-48, 51, 55-57, 59, 62, 69, 70, 72-74, 77-80
	Persian	41	17-21, 23-30, 32, 34-41, 44, 45, 49, 50, 52-54, 58, 60, 61, 63-68, 71, 75, 76
Study type	Cross-sectional	66	8, 13-36, 38-52, 54-79
	Ex-Post facto method	2	37, 53
	BMI	18	8, 16, 21, 22, 25, 30, 33, 35, 44, 46, 48, 50, 52, 57, 58, 62, 64, 74
	Social Pressure	5	23, 35, 58, 61, 77
	Self-esteem	7	23, 35, 58, 64, 66, 75, 78
	16	13	13, 15, 19, 23, 35, 37, 38, 40, 44, 47, 57-60, 66, 79
	Education	2	35, 54
	PCO-Genetic Tendency	1	16
	Menstruation history (Age of menarche, Amenorrhea, Number of periods per year)	5	16, 19, 30, 44, 74
	Western media	4	14, 35, 58, 66
	Parents Characteristics (levels of Parents Education, Parent's job)	9	13, 19, 21, 22, 26, 30, 35, 58, 64
	Activity of Brain Behavioral System, Emotion Regulation, Maladaptive core beliefs, Self-punishment and Worry, Impulsivity, Emotional coping	11	20, 24, 26, 27, 43, 48, 56, 58, 69, 75, 76
	Age	10	16, 25, 30, 51, 54, 59, 62-64, 70
	Depression, Anxiety and Stress, Obsessive-Compulsion Psychological signs, Poor psychosocial domains of HRQOL	6	8, 31, 43, 47, 63, 77
	Mothers' general health and nutrition, attitude	1	45

Gender

There have been several studies that have indicated a higher prevalence of eating disorders among women. However, the increased prevalence in men is observed and other worldwide studies [7]. The current study found that the prevalence of eating disorders is higher among women than men. However, new studies suggest that the prevalence of these disorders is also rising among men [33,46,72]. Prevalence among men ranged from 7.5% to 18.2% [48,56,83]. One study indicated a very high prevalence (55%) [24]. According to male roles in eastern societies, increasing eating disorders among men is necessary for further research.

Socio-economic variables

There are conflicting findings of the effect of family socioeconomic status, parental education, or individual education on eating disorders [1,16,79]. We found little research on the socioeconomic status of the family. Some of these studies have considered the contributing role of father's occupation [13,15-18,20,64,82,85] and mother's education in these disorders [16,83]. However, some of the papers reviewed found no relationship between parental education and eating disorders in children [25,30,56]. Birth order, family size, income, and marital status had no relationship to eating disorders. Two studies have investigated the relationship between anorexia nervosa and bulimia nervosa and participant education [30,48]. Iranian studies' lack of association between socioeconomic status and eating disorders may be due to indirect mediating factors such as media effects and virtual social networks.

Psychosocial variables

Body Image – Body dissatisfaction

Literature review indicates that people with eating disorders suffer from excessive attention to the body, weight, or distorted body image [84,86]. The present study mentions the limited studies on the effect of body dissatisfaction or body image on eating disorders [13,15,23,35,37,38,40,47,57-60,66, 78]. These studies have shown that eating disorders are the most critical predictors of incorrect weight and height perception, body image distortion, and body dissatisfaction. However, two studies revealed no effect of body satisfaction on eating disorders [38,58]. This finding may be due to the more potent effects of other variables.

Social pressures

Body image is a function of social and cultural factors. The perception of body image is strongly influenced by the messages received regarding what's beautiful or not. Intense sociocultural pressure to lose weight and body change increases eating disorders [9,84]. Media, families, and peer groups have the most pressure on individuals to change bodies [11,79].

Media

The media affect body satisfaction by advertising the ideal body. The stated beauty and fitness norms are introduced as the principal means of success and social acceptance. Internalization of norms can result in body dissatisfaction and eating disorders [9]. Iranian studies have not focused much attention on the media in eating disorders. Few studies have been conducted in this field [14,35,58,66]. investigated contributing effect of media, especially Western media. A study also showed that media limiting food consumption had an indirect role [66]. The findings were contradictory; the media effects appear indirect [87].

Peer groups

The opinions of peer groups have a significant effect on body satisfaction and body change behaviors. Peers can exert this influence through encouragement or criticism. Despite the excellent studies on adolescents and youth, only a few studies have studied the effects of peer groups. Although the great studies have in adolescents and youth, only limited studies have investigated peer group's influences. Except one [39], all these studies thought that peer groups directly impacted eating disorders.

Family

The role of the family is linked to their messages about the body, and its management behaviors, family structure, parenting style, and relationship with parents may impact eating disorders [84]. Pre-defined studies have studied this question [17,25,30,36,39,45,57,83]. Another family factor that determines social and personal behavior is parenting styles. Two studies examined this issue [18,39].

Some studies have revealed that the relationships between mother and child can affect eating disorders [30,36,88]. This study was consistent with previous studies on the significant positive effect of maternal connections [89]. Another study showed that parental relationships influenced eating attitudes through inadequate core beliefs [88].

Psychological factors

Emotionally regulated is associated with eating disorders. Studies have shown that people with eating disorders have higher emotional intensity and emotional regulation problems, less acceptance of emotional responses, and low emotional clarity and high. Dynamic control strategies are more dysfunctional than ordinary people [89].

People who are not managing their emotions have more symptoms of eating disorders than others [90,91]. Limited Ian studies focus on this task [17,71,76].

Impulsivity is a multifactor construct. Some components of this structure are associated with eating disorders [92,93]. The current study mentioned a few studies in this regard. One study found that women with hyperphagia had higher impulsivity scores [60].

Self-esteem

In this field, the auto-team is a matter of interest. Some research has shown that low self-esteem is a risk factor for eating disorders [5]. Review of literature on the relationship between self-esteem and eating disorders [23,39,58,64,66]. However, conflicting studies have shown that low self-esteem does not predict eating disorders [94]. It seems that self-team, through body satisfaction, is effective on eating disorders.

Personality

Various relationships have been established between personality traits and eating disorders [5]. One study found an association between experiential openness and emotional instability with eating disorders among students. The results of predicting overeating behavior and mental occupation by food showed that emotional instability or neuroticism could be a positive predictor factor. None of the five personality factors could predict the oral restraint behavior of female students [94].

Over the past decade, the role of perfectionism and the behavioral system in eating disorders has been emphasized [95]. Some studies have found a connection between perfectionism and eating disorders [52,66].

Other variables

An article looked at emotional intelligence. One of the components of Emotional Intelligence is Emotional Awareness. It also plays an essential role ineffective regulation.

The results of Abbasszade's study showed that emotional intelligence and its components such as self-awareness, self-control, social awareness, social skills have a negative and significant relationship with an eating disorder [32]. Another psychological variable is perceived stress and coping styles which may play a role in predisposing or perpetuating many illnesses.

Previous studies have shown a significant relationship between stress and eating disorders, especially during adolescence [3]. One study found a significant negative relationship between perceived positive stress with anorexia nervosa and bulimia nervosa. These articles also revealed a crucial negative relationship between the problem-based coping style and an essential positive relationship between emotional and eating disorders [18,71,75,78].

The association between memory disorders and eating disorders has been studied in several studies. The distortion of verbal, visual, working, executive, and memory bias is found in eating disorders [96].

Studies have been on this issue in Iran [20,24]. One study showed that bulimia nervosa had significantly lower recognition memory performance than ordinary people. Women with bulimia nervosa perform more poorly in certain aspects of memory than normal people [97].

One of the topics covered by a limited number of articles is cognition. studies have shown that women with eating disorders have a more inadequate pattern for themselves, the world, and others [98].

The present study showed that the schema of instability, emotional deprivation, inadequate self-control, and self-discipline are different between obese people with binge eating disorder and those without this disorder [33,57].

Also, another study showed a positive, significant relationship between the overall factors of thought control and all thought control strategies (self-punishment, worry, re-evaluation, and distraction except social control) and eating disorder [27].

Mental Disorders

Eating disorders are associated with psychological disorders such as anxiety, depression, obsessive-compulsive disorder, social isolation, suicidal ideation, hypersensitivity, and guilt feelings [99].

There have been numerous studies in Iran. One study showed that anxiety among university students with and without eating disabilities was significantly different. However, the difference between depression, physical symptoms, social dysfunction, and the overall mental health score was not meaningful between the two groups [8,73].

The relationship between depression and adverse emotions associated with eating disorders has been demonstrated in other studies. However, the difference in depression, physical symptoms, social dysfunction, and overall mental health score was not significant between the two groups. There was also a substantial positive relationship between anxiety and depression and eating disorders. The relationship between depression and negative emotions with eating disorders has been proved in other studies.

One study showed a weak positive relationship between OCD and eating disorders. A significant positive relationship between anxiety and depression to eating disorders was also found. In contrast, another study found that obsessive-compulsive traits can predict 15% of changes in morbid dietary attitudes.

One study showed a weak positive relationship between obsessive-compulsive disorder and an eating disorder. There is a negative relationship between eating disorders and quality of life, which is increasingly reflected in the psychological perception of quality of life [35].

Another study showed that obsessive-compulsive traits could predict 15% of changes in morbid eating attitudes [63]. There is a negative relationship between eating disorders and quality of life, reflected more in the psychological aspect of life quality [35].

Miscellaneous variables

Smoking

Furthermore, the high link between eating disorders and impulse control behaviors, such as substance use, is one of the factors that may affect smoking among people with eating disorders [100]. Only one study looked at smoking on eating disorders in the current study [19]. That study confirmed the connection between those two issues. However, it was a minimal study [22,74].

Menstruation

Early-onset of puberty due to rapid mood changes may be associated with increased eating disorders. Few studies have looked at this question in this study. Some studies have reported that menstruation is lower in eating disorders than in average persons [22,74]. Other studies have revealed no link between these two questions [16,30,44].

Sleep

Many studies have concentrated on the relationship between sleep and diet disorders [101,102]. These studies are very limited in Iran. One study revealed that both qualities of sleep and being an eveningness and morningness are associated with eating disorders [75,83]. Only one study of twin disorders exists. This study showed no differences regarding eating disorders between the two groups [64]. While familial studies of twins have shown that anorexia nervosa, bulimia nervosa, and binge eating disorder are complex genetic diseases, and their transmission rate is 50% to 83% [28].

DISCUSSIONS AND CONCLUSIONS

Eating disorders have received attention in recent years, and it seems that further research is needed to identify the contributing factors to the development, persistence, and treatment of these disorders. The studies mentioned above reveal several notes: The prevalence of eating disorders is rising in the Iranian population and should be considered a severe health problem. Although eating disorders are considered culture-bound disorders, but the factors affecting these disorders are similar to those in other societies.

The emphasis of most studies on women, specific age groups, use of "self-report" tools " are the limitations of the most recent studies, which can affect the generalization. The prevalence of overweight is high in Iran. Association between overweight and eating disorders, it is suggested that future studies regarding epidemiological aspects. These studies can be performed with a longitudinal design, larger sample size, varied population, and clinical assessment.

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Conflict of interest

The authors declare that they have no competing interests.

Consent to participate

Informed consent was obtained from all individual participants included in the study before conducting.

Ethics approval and consent to participate

This study has been approved by the Neuroscience Research Center of Kerman University of Medical Sciences. Regarding the type of article, all licensed full text articles in the databases mentioned in the manuscript have been used.

Consent to publish

All participants consented verbally to the publication of the interview data.

Availability of data and materials

The datasets using in the study are available from the corresponding author on reasonable request.

Authors' contribution

All authors were responsible for the study. HRSH, BG were accountable for the study Conceptualization and led the writing of the paper. ZB conducted the Literature review and assisted in writing the paper. FM and AA assisted in interpreting the data and writing the paper. AA and BG assisted with the interpretation of the results and drafting programmatic Implications. SRSH and BG were responsible for data collection and coordination of the study. SD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript.

All the authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

Modeling and Forecasting the Death Trend Due to Respiratory System Diseases using the Lee-Carter model in Northern Iran

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Abstract: *Background and Aim: Respiratory system diseases (RSDs) represent the major burden of disease, including death worldwide. The main purpose of this study was to model and forecast the death rate due to diseases of the respiratory system Based on Lee Carter's model during the years 2011-2028. Methods: The type of study was a secondary analysis based on available data. The statistical population included all deaths registered in the health department of Golestan University of Medical Sciences during the years 2011-2018. Data analysis was performed using Demography 18.1 and StMoMo packages in R3.6.2 software. Results: The death rate due to RSDs in 2011 in the total population was 0.22, in women 0.19, and in men 0.25 per 1000 population. The death rate due to these diseases shows an increasing trend in 2018 to 0.33 in the total population, 0.29 in women, and 0.36 in men per 1000 population. In general, the death rate due to these diseases increased by approximately 0.10 per 1000 population from the years 2011 to 2018. It is predicted that the increasing trend of deaths due to RSDs will continue and in 2028 it will reach 4.88 in the total population, 3.56 in women, and 2.27 in men per 1000 population. Conclusions: Our findings show a significant increase in respiratory disease mortality over the past eight years. A combination of prevention and treatment strategies through urban planning, including the control of environmental pollutants and environmental health programs, is essential for the effective control of respiratory diseases.*

Keywords: *respiratory system, mortality, forecasting, trends, Iran*

INTRODUCTION

Respiratory system diseases (RSDs) represent the major burden of disease, including death worldwide [1,2]. As major public health issues worldwide, chronic respiratory diseases (CRDs), including chronic obstructive pulmonary disease (COPD), pneumoconiosis, asthma, interstitial lung disease, and pulmonary sarcoidosis, impose significant socioeconomic burdens on individuals and communities and compared to other non-communicable diseases such as cardiovascular disease, cancer, and diabetes, RSDs are severely ignored [3-5]. Therefore, RSDs are of particular importance to public health and are also a major focus of WHO monitoring and prevention strategies [6,7]. Although COVID-19 has recently focused on the respiratory system, RSDs were a major cause of death and disability before the new infection emerged. RSDs represent five of the 30 most common causes of death worldwide [8,9]. Annually, more than 9.5 million deaths are attributed to these diseases worldwide [10] and four million people die prematurely due to chronic respiratory diseases [8].

In the current context of the International System of Classification of Diseases (Tenth edition of the International Statistical Classification of Diseases and Related Health Problems: ICD-10), this group of diseases covers a wide range of acute and chronic respiratory disorders (J00-J99), including acute infections, upper respiratory tract, influenza, and pneumonia, other acute lower respiratory tract infections, other upper respiratory tract diseases, chronic lower respiratory tract diseases, pulmonary diseases caused by external factors, other lung diseases that mainly affect the interstitial tissue, pus-secreting conditions, and death tissue (necrosis) of the lower respiratory tract, other diseases of the pleura and other diseases of the respiratory system [11].

The mortality rate for lung diseases is staggering. Hundreds of millions of people are under chronic respiratory conditions; Every year, four million people die prematurely due to chronic respiratory diseases [12]. Respiratory infections are a major cause of death in developing countries. Although the 2020 Global Burden of Disease Study showed lower mortality rates from lower respiratory tract infections less than two decades ago, many are still dying. Infants and young children are especially vulnerable. Approximately 3 million children, mostly under the age of 5, died of pneumonia and lower respiratory tract infections [13]. In Iran, the mortality rate due to respiratory system diseases (MRRSDs) was 9.8% of total deaths in 2019 [14], with the level as high as 14% in the capital city Tehran. This means that 5000 people in Tehran die of RSDs every year, making it one of the most common causes of death in the city from 2008 to 2018 [15].

Mortality rates and their age pattern are indicators of development and different societies try to reach high levels of growth and excellence by reducing mortality by implementing various health and development programs. The study of mortality and the factors affecting it plays an important role in understanding the patterns of mortality and how to intervene in this matter to reduce it to researchers, planners, and policymakers. It also helps to model and identify past mortality changes in the future and to predict mortality rates, the possible effects of development programs, and their success in the field of mortality [16].

Considering that the most important risk factors for respiratory diseases include smoking, exposure to internal and external pollutants, allergens, occupational exposure, unhealthy diet, obesity, physical inactivity, and other factors [17] and to Due to rapid population growth and exposure to these risk factors, RSD is becoming a more prominent problem for all regions of the world, so more comprehensive research is needed to model and predict the death rate due to respiratory diseases throughout Iran. A deep and expert attitude is essential for the future health planning of the country. The main purpose of this study was to model and predict the mortality rate in Golestan province during the years 2011-2028.

MATERIALS AND METHODS

The type of study was a secondary analysis based on available data. The statistical population included all deaths registered in the health department of Golestan University of Medical Sciences during the years 2011-2018. Mortality information was obtained from the Statistics and Performance Analysis Unit at Golestan University of Medical Sciences in the form of an Excel file. In this file, in addition to the cause of death, information about the age and sex of the deceased is also provided.

The disease classification system is based on ICD10. Causes of death are determined and coded according to the International Classification of Diseases. Then, to qualitatively evaluate the death cause data, the data are examined and modified in terms of the impossible cause of death codes in terms of gender and age, the probable cause of death codes in terms of lethal causes, and the cause of death codes in poorly defined or absurd cases. To correct the codes of impossible causes of death in terms of age and sex and the codes of possible causes of death in terms of fatality, the framework for studying the global burden of diseases for 2010 and 2013 was used [18-20].

After qualitative correction of the data, the study population, i.e. the total number of deaths recorded during 2011-2018, was extracted and the death rates and trends were calculated. Then, the mortality rate was modeled and predicted during the years 2019-2028. The population of Golestan province in the general census of 2011 and 2016 was 1777014 and 1868819 people, respectively. It should be noted that for the denominator of the quotas, the census data of 2011 and 2016, as well as demographic estimates between the two censuses, the use of population growth rate and population information by sex and age group, and location, were used as demographic estimates. For demographic estimates in this paper, the mathematical method according to equation one was used. Where P (t + n) population in the second census, P(t) population in the first census, n is the distance between the two censuses, and r is the annual population growth rate that is the basis of the calculations (equation 1):

$$P_{(t+n)}= P_{(t)}\ (1+r)\ \ n$$

All steps of Lee Carter model analysis and fitting were performed using Demography 1.18 and StMoMo packages in R3/2.6 software.

RESULTS

The total number of deaths due to respiratory system diseases during the years 2011 to 2018 had an upward trend, increasing from 394 cases in 2011 to 643 cases in 2018. The death rate due to RSDs in 2011 in the total population was 0.22, in women 0.19, and in men 0.25 per 1000 population. The death rate due to these diseases, shows an increasing trend in 2018 to 0.33 in the total population, 0.29 in women, and 0.36 in men per 1000 population. In general, the death rate due to these diseases has increased by approximately 0.10 per 1000 population during the years 2011 to 2018 (Table 1).

The results of predicting the mortality trend due to RSDs in Golestan province during the years 2011-2028 in terms of age groups and in general and by sex are presented in Tables 2-4, respectively. According to the results of Tables 1, 2, and 3, it is expected that the trend of deaths due to RSDs in all age and sex groups during the years 2019 to 2028 will have an upward trend.

Table 1: Death rate (per 1000 people) due to RSDs during the years 2011-2018

Years	2011	2012	2013	2014	2015	2016	2017	2018
Total	0.22	0.12	0.19	0.22	0.21	0.28	0.30	0.33
Male	0.25	0.13	0.21	0.25	0.24	0.33	0.35	0.36
Female	0.19	0.12	0.12	0.18	0.19	0.27	0.25	0.29

Table 2: Prediction of mortality rate (per 1000 people) due to RSDs during the years 2019-2028

Number	Age groups	Years									
		2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
1	0-4	0.24	0.25	0.27	0.28	0.30	0.32	0.34	0.36	0.39	0.41
2	5-9	0.06	0.06	0.07	0.08	0.08	0.09	0.10	0.11	0.12	0.14
3	10-14	0.03	0.03	0.03	0.04	0.04	0.04	0.05	0.05	0.06	0.06
4	15-19	0.03	0.03	0.04	0.05	0.05	0.06	0.07	0.08	0.09	0.10
5	20-24	0.09	0.10	0.12	0.13	0.16	0.18	0.21	0.24	0.28	0.33
6	25-29	0.05	0.06	0.07	0.08	0.09	0.10	0.12	0.13	0.15	0.17
7	30-34	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08
8	35-39	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.08	0.08	0.08
9	40-44	0.12	0.13	0.14	0.16	0.18	0.20	0.22	0.25	0.28	0.31
10	45-49	0.15	0.17	0.19	0.22	0.25	0.29	0.33	0.38	0.43	0.49
11	50-54	0.26	0.29	0.32	0.36	0.41	0.45	0.51	0.57	0.63	0.71
12	55-59	0.51	0.56	0.61	0.67	0.73	0.80	0.87	0.95	1.04	1.14
13	60-64	0.79	0.72	0.66	0.60	0.55	0.50	0.45	0.41	0.38	0.34
14	65-69	1.77	1.99	2.24	2.51	2.83	3.18	3.57	4.02	4.52	5.08
15	70-74	3.36	3.83	4.37	4.99	5.70	6.50	7.42	8.47	9.66	11.02
16	75-79	4.75	5.52	6.42	7.47	8.69	10.10	11.74	13.64	15.84	18.39
17	>80	12.21	14.10	16.28	18.80	21.69	25.02	28.84	33.24	38.27	44.04

Table 3: Prediction of mortality rate (per 1000 people) due to RSDs in the male population during the years 2019-2028

Number	Age groups	Years									
		2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
1	0-4	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29
2	5-9	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09	0.09
3	10-14	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
4	15-19	0.04	0.05	0.06	0.07	0.09	0.11	0.13	0.15	0.18	0.21
5	20-24	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05
6	25-29	0.06	0.06	0.07	0.07	0.08	0.09	0.10	0.11	0.12	0.13
7	30-34	0.06	0.07	0.08	0.10	0.11	0.13	0.15	0.17	0.20	0.23
8	35-39	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
9	40-44	0.11	0.12	0.13	0.13	0.14	0.15	0.16	0.17	0.18	0.19
10	45-49	0.16	0.21	0.23	0.25	0.27	0.30	0.33	0.36	0.39	0.43
11	50-54	0.30	0.34	0.38	0.43	0.49	0.55	0.62	0.70	0.79	0.89
12	55-59	0.60	0.66	0.72	0.78	0.85	0.92	1.00	1.09	1.19	1.30
13	60-64	0.84	0.78	0.72	0.66	0.62	0.57	0.53	0.49	0.45	0.42
14	65-69	1.97	2.14	2.33	2.53	2.75	3.00	3.26	3.55	3.86	4.20
15	70-74	3.82	4.10	4.40	4.71	5.05	5.41	5.80	6.22	6.67	7.15
16	75-79	5.08	5.76	6.52	7.38	8.35	9.46	10.70	12.11	13.70	15.49
17	>80	11.08	12.35	13.77	15.36	17.12	19.08	21.26	23.68	26.37	29.36

Table 4: Prediction of mortality rate (per 1000 people) due to RSDs in the female population during the years 2019-2028

Number	Age groups	years									
		2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
1	0-4	0.25	0.26	0.28	0.30	0.32	0.34	0.36	0.38	0.41	0.43
2	5-9	0.04	0.04	0.05	0.06	0.07	0.07	0.09	0.10	0.11	0.13
3	10-14	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03
4	15-19	0.02	0.02	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.05
5	20-24	0.12	0.14	0.17	0.21	0.25	0.29	0.35	0.42	0.50	0.59
6	25-29	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.08	0.08
7	30-34	0.07	0.10	0.13	0.17	0.22	0.29	0.38	0.50	0.66	0.87
8	35-39	0.04	0.04	0.05	0.05	0.06	0.07	0.07	0.08	0.09	0.11
9	40-44	0.09	0.09	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.12
10	45-49	0.08	0.08	0.09	0.09	0.10	0.11	0.12	0.12	0.13	0.14
11	50-54	0.22	0.28	0.35	0.43	0.54	0.67	0.83	1.04	1.30	1.62
12	55-59	0.30	0.32	0.33	0.35	0.37	0.39	0.41	0.43	0.45	0.47
13	60-64	0.59	0.53	0.48	0.44	0.40	0.36	0.32	0.29	0.27	0.24
14	65-69	1.16	1.22	1.27	1.33	1.39	1.45	1.52	1.59	1.66	1.74
15	70-74	2.26	2.42	2.59	2.77	2.96	3.17	3.39	3.62	3.87	4.14
16	75-79	4.44	4.81	5.22	5.66	6.14	6.66	7.22	7.82	8.48	9.20
17	>80	10.07	10.78	11.54	12.35	13.23	14.16	15.16	16.22	17.36	18.58

In both men and women, the highest increase in deaths due to RSDs is in the age group over 50 years, and the age group above 80 years has the highest increase. It is predicted that in 2028, in the age group over 80 years, it will reach 44.04 in the total population, 29.36 in men, and 18.58 in women (per 1000 population).

DISCUSSION

This study aimed to model the death process due to respiratory diseases. For this purpose, the first census data and death data have been evaluated and after modeling, mortality changes between 2011 and 2018 and recognizing the death pattern, mortality rates up to 2028 have been predicted.

Based on the results of this study, during the years 2011-2018, 60082 deaths were recorded in the system, of which 3487 deaths were due to deaths due to RSD (about 6% of all deaths). In the study of Pishgar et al., during ten years (2008-2008), 519,312 people died in Tehran, of which 43,177 (about 8% of all deaths) were due to respiratory diseases, which is equal to 831.1 people in there were 10,000 deaths and 0.5 per 100,000 population [21]. The results show that the mortality rate for chronic respiratory diseases increased by 29.7% between 1980 and 2014 in the United States, which agrees with the results of this study [22]. In the study by Khademi et al, 17% of the participants reported opium use, with a mean duration of 12.7 years, and during the follow-up period, 2145 deaths were reported. The strongest associations of Opium consumption were seen with deaths from asthma, tuberculosis, and chronic obstructive pulmonary disease (11.0, 6.22, and 5.44, respectively) [23]. Perhaps this issue is one of the reasons justifying the high death rate due to respiratory system diseases in this region.

In Iran, the death rate due to RSDs was 9.8% of the total deaths and also a rate higher than 14% in Tehran in 2019 [24]. This means that 5,000 people die every year in Tehran due to RSD and this is one of the most common causes of death in this city from 2008 to 2018 [25].

The results of the study showed that the death rate due to respiratory diseases in the entire population of Golestan province during the years 2019-2028 and at all ages, has been upward. So that the death rate due to these diseases during the years 2011 to 2018 has increased by approximately 0.10 per 1000 population. This finding is related to the results of other researchers. In the probe study from 2008 to 2018, a significant increase in the trend of RSDs was observed. Also, in the study of Kermani et al. during the years 2005 to 2014 in Tehran, the total number of deaths due to SO_2 exposure in the last decade was 8836 cases and the total number of deaths due to RSDs was 1915 cases and the total number of chronic obstructive pulmonary diseases in the last decade has been 1806 [21].

The results showed that the increasing trend of deaths due to RSDs continued and in 2028 reached 4.88 in the total population, 2.27 in men, and 3.56 in women per 1000 population. In general, deaths from these diseases are higher in men than women. Which was similar to the findings of other studies [26,27]. In recent years, most studies on RSDs have attempted to examine the association between these diseases and genetic predispositions with physical activity and the economic and social status of urban environments. They also confirmed the importance of individual-level variables in the prevalence of COPD, especially comorbidities, age, sex, and smoking status [28,29].

According to the results, in both sexes, men and women, the highest increase in deaths due to RSDs will be in the age group over 50 years, and the age group over 80 years will have the highest increase. Other studies have reported the highest rate of death from RSD at older ages [30-32]. Given that the future of the world, including Iran, is a future with an increase in the elderly population, we need to accept this phenomenon in the first place, and the health system in Iran should have the potential to change its policy goals to meet the needs of the elderly population [33].

One limitation of the research is that the prediction models do not include the results of perceived changes such as medical advances in improving life expectancy, and the prevalence of some infectious diseases such as Covid-19 may interfere with the model's prediction accuracy. This study was conducted before the onset of the Covid-19 disease epidemic and according to demographic changes and mortality information that may have occurred in 2019 in Golestan province, studies to identify the main causes of death and the trend of the result are recommended during this epidemic. Information about all years without taking into account stillbirths is considered.

CONCLUSION

Our findings show a significant increase in mortality due to respiratory diseases in Golestan province during the last eight years. A combination of prevention and treatment strategies through urban planning, including the control of environmental pollutants and environmental health programs, is essential for the effective control of respiratory diseases. This should be considered by health policymakers when faced with an increased risk of death from environmental factors.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Authors' contribution

Supervision: AH; Conceptualization: AH, MJK and ZK; Methodology: ZK; Project administration: AH; Data curation: MG, NJ, and MRH; Formal analysis: ZK; Writing - original draft: MJK, AH, ZK, NJ, MRH, MG; Writing - review and editing: ZK and AH.

Ethics approval and consent to participate

Ethical approval for this study was obtained from Iran National Committee for Ethics in Biomedical Research (IR.GOUMS.REC.1399.270).

Patient consent for publication

Verbal informed and Written informed consent were not obtained because recorded and library data were used.

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REVIEW

Medial Pivot Knee in Total Knee Arthroplasty

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Abstract: *In the context in which life expectancy increases and the population becomes more active, the number of people who are affected by gonarthrosis symptoms increases proportionally. By the year 2030, in the United States of America, one in three adults is expected to suffer from gonarthrosis, this prediction will be the beginning of an epidemic. Total knee arthroplasty has been shown to relieve pain and improve joint function; however, studies have shown that active young patients still have limitations in performing high-level activities such as dancing, golfing, skiing, and gardening. Currently, modern TKA implants are designed to reproduce the normal biomechanics of the knee joint, mimicking the physiological "medial pivot" pattern with greater compliance on the medial compartment between the tibial insert and femoral condyle and less congruence on the lateral side.*

Keywords: *total knee arthroplasty, medial pivot, biomechanics*

INTRODUCTION

In the context in which life expectancy increases and the population becomes more active, the number of people who are affected by gonarthrosis symptoms increases proportionally. By the year 2030, in the United States of America, one in three adults is expected to suffer from gonarthrosis, this prediction will be the beginning of an epidemic [1].

Total knee arthroplasty (TKA) should provide functional movement and stability for all desired daily activities. It should also reproduce normal knee kinematics and function [2,3,4].

Total knee arthroplasty is a successful intervention, which aims to reduce pain and restore knee function [5,6]. Numerous studies have shown that the satisfaction rate of patients with total knee arthroplasty is between 80% and 95%, and the survival rate of implants 10 years after the surgery is approximately 90%. However, major malfunctions after total knee arthroplasty occur in 15-20% of patients, often requiring revision surgery [7].

Currently, modern TKA implants are designed to reproduce the normal biomechanics of the knee joint, mimicking the physiological "medial pivot" pattern with greater compliance on the medial compartment between the tibial insert and femoral condyle and less congruence on the lateral side [2,4,5,8].

The implants in total knee arthroplasty can be cemented or not. Cemented fixation is the gold standard in TKA. In medical applications, the cement is represented by Polymethyl-methacrylate (PMMA). This is an acrylic polymer formed by mixing two sterile components: a liquid methacrylate (MMA) monomer and a powered MMA-styrene copolymer. Heat is generated during the process due to the exothermic reaction. [9,10].

Numerous studies investigating knee kinematics using magnetic resonance imaging have confirmed a physiological medial pivot pattern in the normal knee [11].

EVOLUTION OF TKA

Swedish surgeon Börje Walldius, MD, designed the first TKA implant in 1950. It was a system that sacrificed the collateral and cruciate ligaments for implantation and it functioned in a single plane of motion. More sophisticated designs of total knee systems have been developed since 1970 [12].

Over the past few years, the total knee system has undergone significant changes.

The medial-pivot system was developed to produce normal knee kinematics and better patient satisfaction than traditional prostheses. The MP prosthesis includes a ball and socket articulation on the medial side with more significant restriction and relatively unconstrained mobility on the lateral side. The asymmetric polyethylene insert is designed to allow normal function and motion by replicating the native structure of the knee [13]. The system avoids paradoxical anterior movement and may bring about superior patient satisfaction [14].

In the following, we will present arguments for and against the role of the medially stabilized knee

THE ROLE OF THE MEDIALLY STABILIZED KNEE: AN EMERGING SOLUTION FOR TKA – PRO ARGUMENTS

Total knee arthroplasty has been shown to relieve pain and improve joint function; however, studies have shown that active young patients still have limitations in performing high-level activities such as dancing, golfing, skiing, and gardening. Functional outcomes are compromised in approximately half of TKA patients. This loss may be caused by the abnormal kinematics and inherent instability of many contemporary implants.

In the anatomical knee, the femur externally rotates and rolls backward over the tibial plateau during mid-flexion. In full flexion, there is more translation of the lateral femoral condyle. In contrast, the conventional total knee begins in an unrotated and posteriorly subluxated position because the ligament insertion points are

located posteriorly. Paradoxical anterior slip and insufficient axial rotation usually characterize femoral movement after conventional TKA. This instability and malrotation significantly reduce the effectiveness of the muscles involved in the extension and flexion of the knee, which negatively modifies the kinematics and joint movement patterns of the anatomical knee [15].

Medially stabilized knee prostheses have an adequate medial articular surface, which promotes natural rotational motion

and increases anteroposterior implant stability [16]. There are several approaches to medially stabilized knee implants, which retain, sacrifice, or replace the posterior cruciate ligament. The classic design of medially stabilized prostheses sacrifices the posterior cruciate ligament and has increased medial joint stability combined with a less stable lateral joint [17]. In this design, the function of the posterior cruciate ligament is replaced by the high anterior edge of the polyethylene insert.

Some newer generation medially stabilized models have the same degree of stability in the medial joint or even less and allow for both sacrificial and retention of the cruciate. Another medial-stabilized design, bicruciate stabilized, incorporates a compliant medial joint and an anatomically shaped but less contented lateral compartment to promote rotation.

Restoring stability and rotation/kinematics as close as possible to normal is thought to improve functional outcomes and patient satisfaction [18]. In vivo fluoroscopic analysis revealed improved kinematics with medially stabilized designs [19]. Furthermore, improvements in clinical outcomes and patient satisfaction have been reported for these designs [3, 20, 21].

In conclusion, these medially stabilized TKA models restore anatomical rotational motion and have favorable outcomes (Figure 1, Figure 2).

THE ROLE OF THE MEDIALLY STABILIZED KNEE: AN EMERGING SOLUTION FOR TKA – CONTRA ARGUMENTS

The medial pivot (medially stabilized) knee prosthesis is designed to reproduce the kinematics of the knee, reduce polyethylene wear, and improve the functional outcome.

A prospective randomized study on a group of patients who received bilateral knee arthroplasty in the same surgical procedure and in whom a total knee prosthesis with a medial pivot (Advance, Wright Medical) was implanted in one knee and the other knee a total prosthesis with a movable plate, with preservation of the posterior cruciate ligament (PFC Sigma, DePuy Synthes) [22]. One hundred eighty-two (182) patients were enrolled, with no differences in preoperative demographics, range of motion, or knee scores. In the post-operative follow-up after a minimum of 11 years, medially pivoted knees had significantly worse results, with lower Knee Society Score scores (95 vs. 90 points), lower WOMAC index score (18 vs. 25 points), and a reduced mobility degree (117 degrees vs. 128 degrees). Patients were more satisfied with the PFC Sigma (93%) than the medial pivot (75%).

There was no difference in survival. The authors of this study hypothesized that excessive constraint imposed by the medial tibiofemoral joint may not restore normal knee kinematics and may inhibit lateral condyle translation during flexion.

Warth et al. studied a group of 141 TKA patients implanted with the same type of prosthesis (Triathlon, Stryker) not specifically classified as a medial pivot design and used an intraoperative sensor (VERASENSE, Orthosensor) to classify the kinematics pattern in terms of the type of rotation meaning with medial pivot or without medial pivot [23]. Forty percent (40%) of the knees were classified as having a medial pivot pattern during flexion-extension motions. There were no differences between the two groups regarding the Knee Society Score or the UCLA score.

Figure 1: Preoperative x-rays (orthostatic)

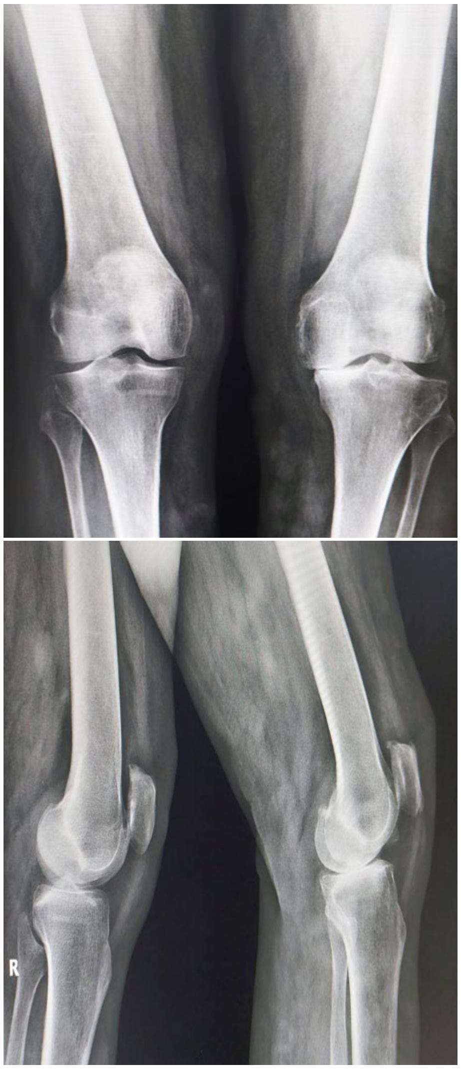

In another prospective randomized study, Benjamin (BJJ, 2018) compared a medial pivot TKA with a conventionally designed TKA and there were no differences related to the Knee Society Score, Oxford Knee scores, or the gait analysis [24]. Also, another prospective, randomized study by Nishitani et al. (Knee, Dec 2018) compared TKA with a medial pivot to TKA with a conventional tibial insert and found no differences related to the degree of mobility or Knee Society Scores [25]. Finally, a comparative analysis that included 153 medially pivoted TKAs (Advance, Wright Medical) and the same number of posteriorly stabilized TKAs (DePuy Synthes) revealed no difference for Knee Society Scores, WOMAC score, or degree of mobility between groups [26].

Another widely used type of TKA popular worldwide is the posterior stabilized (PS) implant. The PS knee has a cam and post mechanism that induces femoral roll-back, which has been shown in multiple studies to provide improved motion compared to a cross-retaining TKA. By preserving the translational movement of the femur on the tibia, the chance of impingement between the posterior part of the femur and the polyethylene insert is reduced.

Figure 2: X-rays after TKA with primary medial-pivot knee system

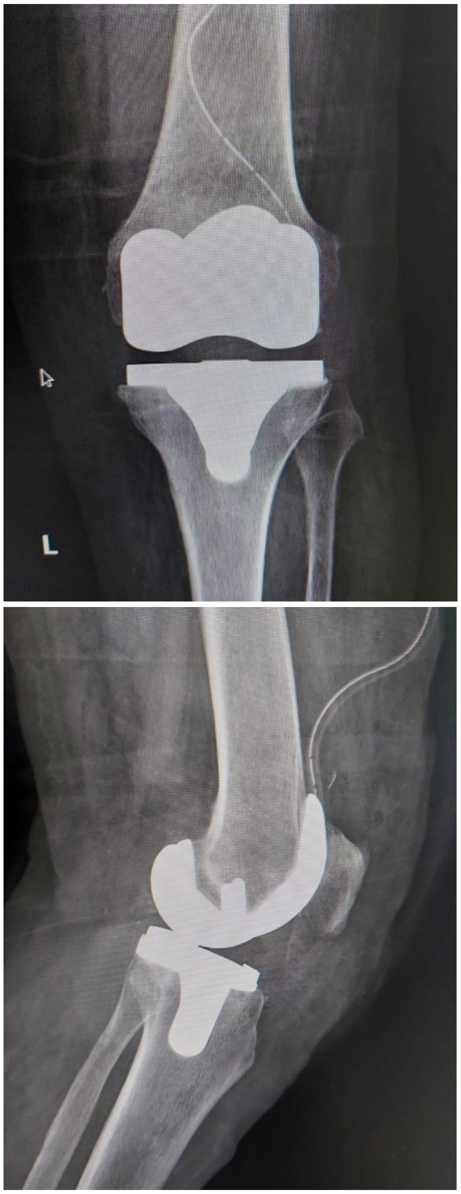

The cam and post mechanism induces posterior sliding and is closer to native knee kinematics compared to the cruciate ligament-preserving knee, which may be subject to "paradoxical motion," the forward sliding of the femur on the tibia during knee flexion.

Disadvantages of the posterior-stabilized knee include the presence of sounds produced during flex-ion/extension movements and patellar crackles, inter-condylar fracture of the femur, early decimation, and significant bone loss due to specific cutting blocks. The spaces resulting from the bone cuts, in flexion or extension, must be well balanced to avoid the appearance of an enlarged flexion space, which can lead to loss of contact between the posterior cam and the pin of the tibial plateau.

With improved implant design and materials, each of these

complications have been reduced, but orthopedists need to recognize the occurrence of these complications to identify them when they occur so that the incidence can decrease. Improvements in trochlear biomechanics have significantly reduced patellofemoral complications, a common problem for patients with posterior-stabilized implants.

CONCLUSIONS

Preserving knee biomechanics after total arthroplasty could be the solution to improve functional outcomes, increase satisfaction rates and decrease complication rates. This can be done through a series of intraoperative maneuvers, by using some implants designed for this function.

The benefits of medial pivot implant total knee arthroplasty are high component compliance, anteroposterior stability, and a large contact surface with minimal contact stress.

The existing literature has provided evidence supporting a better clinical effect, anatomic rotational movement with favorable outcomes, and a lower complication rate of the medial pivot knee prosthesis compared to the posterior-stabilized knee prosthesis. The obtained results provide a reference for doctors when choosing a certain type of prosthesis.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

The Relationship between Clinical Competence and Ethical Reasoning and Factors Affecting it in Nurses Working in Therapeutic Educational Centers of Ilam

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Abstract: *Clinical competence means a person's ability to use techniques on time, to communicate effectively with others, and to benefit from the knowledge and clinical reasoning ability when responding to different clinical situations in such a way that a person can use these skills at all times. The aim of the study is the relationship between clinical competence and ethical reasoning and the factors affecting it in nurses working in therapeutic educational centers of Ilam. Methods: In this descriptive-analytical study, 110 nurses working in three therapeutic educational centers in Ilam city were randomly selected. Demographic information forms, standard nursing clinical competence questionnaire (NCCQ), and nursing dilemma test (NDT) were used to collect data. The results also showed a positive and significant relationship between age, level of education, years of work experience, employment status, and occupational role with clinical competence and ethical reasoning ($P<0.05$). On the other hand, there was an inverse and significant relationship between the workplace and clinical competence and ethical reasoning ($P<0.05$). Data analysis was carried out using Descriptive statistics (frequency, percentage, mean and standard deviation) and analytical tests (Pearson correlation tests and analysis of variance) in SPSS. P-value< 0.05 was considered as the significance level. Using the results of the present study, nursing education planners and managers can periodically organize workshops on nursing ethics and nursing problems in hospitals and universities. Moreover, clinical competence and ethical reasoning should be taken into account when training nursing personnel in university classrooms and in-service courses.*

Keywords: *moral reasoning, clinical competence, nurses, therapeutic training center*

INTRODUCTION

Nurses provide at least 50% of healthcare services, and this figure even reaches 80% in some countries [1]. Nursing is now moving towards professionalization, and one of the most important pillars of the nursing profession is ethical issues that must be taken into consideration [2]. The ultimate and main goal of the nursing profession is to ensure the recovery of humans, and this can only be achieved through scientific and ethical care [3]. The nursing practice has a human and ethical nature and adherence to ethical principles has a great impact on improving the quality of nursing care [4]. Factors such as the advancement of medical technology, increasing treatment costs, attention to the individual rights of people, and changes in the roles of nurses can lead to ethical conflicts that require nurses to recognize problems and make appropriate ethical reasoning [5]. Ethical reasoning is the ability of a person to choose a solution from among several solutions to an ethical dilemma and to provide appropriate reasons according to his/her knowledge and conscience [6]. Lack of ethical reasoning can lead to wrong decisions in complex clinical situations and, subsequent inappropriate action, which in turn affect the patient, the patient's family, and even the nurse [7].

From the management point of view, one of the effective factors in guaranteeing quality-of-care services and obtaining patient satisfaction is clinical competence [8]. Clinical competence means a person's ability to use techniques promptly, to communicate effectively with others, and to benefit from the knowledge and clinical reasoning ability when responding to different clinical situations in such a way that a person can use these skills at all times [9]. The British Nursing

Association defines competence as a set of knowledge, skills, and attitudes required to work safely and effectively without a direct supervisor [10]. Several factors such as the rapid change in the health monitoring system, the need to provide safe and cost-effective services, increasing public awareness in health-related areas, and increasing the expectation of receiving services of appropriate quality, along with the willingness of healthcare organizations to employ skilled personnel, have led researchers to pay more attention to clinical competence [11]. Since the clinical competence of nurses is considered the main concern of healthcare provider systems and their main focus, it is one of the areas that need improvement [10]. Therefore, considering the importance of knowing the ethical reasoning status of nurses and the role of clinical competence in providing high-quality nursing care, the relationship between clinical competence and the ethical reasoning of nurses was investigated in the present study.

MATERIALS AND METHODS

In this descriptive-analytical study, 110 nurses working in three therapeutic educational centers of Ilam city (Imam Khomeini, Shahid Mostafa Khomeini, and Ayatollah Taleghani) were randomly selected. Inclusion criteria included informed consent to participate in the research, a bachelor's degree in nursing, no transfer or acceptance from practical nursing and other medical sciences, and having at least one year of clinical work experience. Exclusion criteria included unwillingness to continue cooperation in the study and incomplete or incorrect completion of questionnaires. Demographic information forms, standard nursing clinical competence questionnaire (NCCQ), and nursing dilemma test (NDT) were used to collect data. The demographic information form included questions on age, gender, marital status, level of education, work experience, employment status, workplace, and occupational role.

The standard nursing clinical competence questionnaire (NCCQ) (Liu et al., 2007) consists of 55 questions in seven dimensions of clinical care, leadership, interpersonal relationships, ethical/legal performance, professional development, willingness to research, and critical thinking. NCCQ is scored based on a five-point Likert scale ranging from 0 to 4. Scores less than 110, 110-165, and 165-220 indicate low, moderate, and high competence, respectively [12]. The reliability of this questionnaire was confirmed in Ghasemi et al.'s study (α =0.97) [13] and the present study (α =0.98).

The nursing dilemma test (NDT) was developed by Cherisham (1981) [14]. This questionnaire was developed based on Kohlberg's theory of moral development and contains 6 scenarios about the problems of nursing care of the client, as follows: a baby with an organ defect, wrong prescription, medication error, a patient's request to end his/her life, a new nurse entering practice and end of life care. Following each scenario, two questions are asked. The first question asks the person to choose what action he would take if he were in this scenario. The options for this question are Yes, No, and Can't decide the format. The answers given to this part are mostly dependent on the religious and cultural background of the nurses, and whatever option is chosen does not affect the level of their ethical reasoning. The second question consists of six options in which the nurse's ethical reasoning is measured. This part of the questionnaire is designed based on stages 1 to 6 of Kohlberg's ethical development. In other words, two options for this question show the nurse's ethical reasoning. If the respondent chooses these two options in his/her priorities, (s)he will obtain a score of 6 in one item and 5 in the other. To determine the ethical reasoning score, the scores of stages 5 and 6 of Kohlberg's moral development (codes 5 and 6) are added together. Therefore, the highest and the lowest scores in each scenario are 11 and 3, respectively, and the total possible score range of six scenarios is 18 and 66 [15]. This questionnaire was translated by Zirak et al. (2009). It was then reviewed by 10 faculty members of Tabriz University of Medical Sciences in terms of content validity and the necessary changes were made to the questionnaire. Its reliability was calculated by the test-retest method and the correlation coefficient was 0.95, and its match with the culture of Iranian society was also confirmed [16]. In the present research, its reliability was calculated α=0.97 using Cronbach's alpha method.

Data analysis was carried out using Descriptive statistics (frequency, percentage, mean and standard deviation) and analytical tests (Pearson correlation tests and analysis of variance) in SPSS. P-value <0.05 was considered as the significance level.

RESULTS

Out of a total of 110 samples, the majority were women (54.5%), had a bachelor's degree in nursing (87.3%), were permanent nurses (61.8%), and had 7.17 years of work experience on average. The number of single and married people was equal. The mean ± SD of clinical competence and ethical reasoning scores in nurses was 136.65 ± 46.08 and 36.51 ± 16.17, respectively (Table 1). Results showed low, moderate, and high clinical competence in 46.4%, 11.8%, and 41.8% of cases, respectively.

Figure 1 shows a significant correlation between clinical competence and ethical reasoning of nurses (p<0.001, r=0.802). In other words, ethical reasoning in nurses has increased with increasing clinical competence scores.

Table 1: The demographic characteristics of the nurses (average score and standard deviation of nurses' clinical competence and moral reasoning)

Variable	SD	Mean	Maximum score	Minimum score
clinical competence	46/084	136/65	210	75
moral reasoning	16/178	36/51	60	19
level of clinical competence	%	N	Average score	
low clinical competence	46/4	51	1-110	
moderate clinical competence	11/8	13	110-165	
high clinical competence	41/8	46	165-220	

Table 2: Relationship between nurses' personal-occupational characteristics with clinical competence and moral reasoning

Variable	Clinical competence		Moral reasoning	
	P-Value	Coefficient correlation	P-Value	Coefficient correlation
age	<0/001	0/42	<0/001	**0/45**
gender	0/98	0/002	0/38	**0/08**
marital status	0/89	-0/01	0/47	**0/06**
level of education	<0/001	0/39	<0/001	**0/38**
work experience	<0/001	0/44	<0/001	**0/47**
employment status	<0/001	0/38	0/01	**0/24**
workplace section	0/04	-0/19	<0/001	**-0/34**
work role	<0/001	0/34	<0/001	**0/37**

The results also showed a positive and significant relationship between age, level of education, years of work experience, employment status, and occupational role with clinical competence and ethical reasoning (Table 2) (P<0.05). On the other hand, there was an inverse and significant relationship between the workplace and clinical competence and ethical reasoning (P<0.05).

Figure 1: The relationship between clinical competence and ethical reasoning

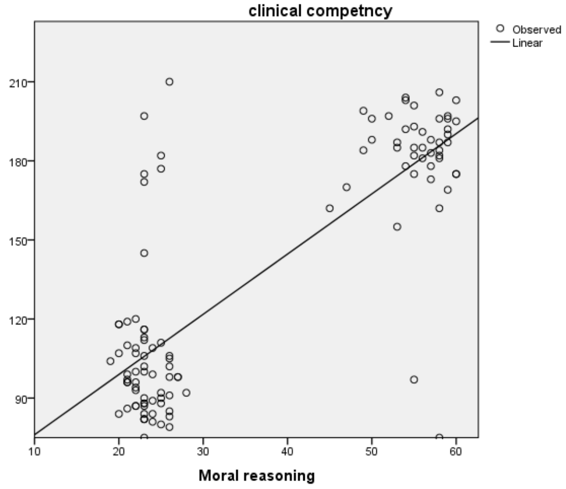

DISCUSSION

The studied nurses had low and high clinical competence in 46.4% and 41.8% of cases, respectively. The results of a study by Faraji et al. (2019) indicated an excellent and good level of clinical competence among nurses, which is not consistent with the results of the present study [17]. The high clinical competence score of nurses can be related to many factors such as holding regular training courses and periodical clinical competence assessments. It is possible to provide appropriate conditions for improving the clinical competence of nurses with low clinical competence scores by holding training courses [17]. The results of the previous relevant foreign studies also indicated a good and excellent level of clinical competence among nurses working in different hospital wards [18,19]. A high level of clinical competence among nurses can be expected considering the expertise and ability of nurses based on the importance of the issue of clinical competence and recruitment of nurses based on strict clinical criteria in recent years.

The studied nurses had moderate ethical reasoning. According to the results of the present study and the definition of ethical reasoning in nurses, it can be stated that the nurses of the present study can choose one solution from among several solutions to an ethical dilemma in nursing to some extent, and performed moderately when presenting appropriate reasons according to their knowledge and conscience. Since nurses have the most interaction and closeness with patients compared to other medical personnel, they need more ethical reasoning knowledge [20]. Ethical dilemmas are one of the most important causes of distress and discomfort in clinical environments; therefore, they need proper treatment [21]. Since ethical reasoning is one of the most important criteria for achieving an ethical performance [22]. The training of nurses in this field can increase the readiness of nurses to deal with ethical issues and dilemmas in their professional role and thus can improve their performance in ethical decision-making.

In a study of the ethical reasoning status of nurses, Behbodhi et al. (2018) showed that the ethical reasoning of nurses was at a low level in 75% of nurses [23], which is not consistent with the results of the present study. This discrepancy may be due to the use of different questionnaires, as well as factors such as cultural diversity and differences in the values and opinions of nurses in different parts of Iran. It is stated that factors such as; the views and experiences of other nurses, physicians, and nurses' families can also affect nurses' ethical reasoning ability, which warrants further research on the factors related to ethical reasoning in nurses.

The results of another study on 360 nurses working in different wards of educational hospitals affiliated with Shahid Sadougi University of Medical Sciences in Yazd, showed that these nurses had average ethical reasoning ability in the face of ethical dilemmas [24], which is consistent with the results of the present study, which may be due to use of a similar questionnaire to evaluate the ethical dilemma. It can be stated that it is necessary to plan and apply appropriate strategies to improve the reasoning ability of nurses working in hospitals in the face of ethical dilemmas.

The results of some foreign studies, including a study in the United States, showed the high ethical reasoning of nurses [25], which is not consistent with the results of the present study. The nurses in this study mostly attributed their high level of reasoning to organizational factors related to ethical dilemmas, the most important of which was effective physician-nurse communication.

In the present study, the ethical reasoning ability increased with increasing clinical competence scores. According to the results of the present study, clinical competence played an important role in the ethical reasoning ability of nurses. According to the concepts and theories of the field of ethics, the ethical reasoning ability is not a stable issue because the type of ethical distress depends on whether the person does the ethically right thing or not or whether the person refrains from doing an action, which is prohibited from fulfilling that obligation, in which case ethical distress occurs [26]. If the main cause of this distress is eliminated, this stress and psychological distress will certainly decrease, which will lead to an improvement in the ethical reasoning ability of nurses. The studied nurses had the necessary ethical reasoning ability considering their average clinical competence level.

The results of the Escolar-Chua et al. study showed nurses with poor ethical reasoning had poorer patient decision-making for ICU patients [27]. According to these nurses, the critical and bad conditions of the ICU patients had caused an ethical challenge when providing care to this group of patients, the consequence of which was the increase in the ethical distress of the nurses. Moreover, these persistent challenges in turn reduced the ethical reasoning ability of the nurses. In the present study, older age, higher education level and work experience, permanent employment, being a nurse, and working in ICUs ensured a higher mean clinical competence score, which was inconsistent with the results of the study by Faraji et al. (2019) [17]. The discrepancy between the results of these two studies is due to the personal-occupational characteristics of nurses and the research environment. The difference in clinical environments, the distinct care needs of patients, and the difference in the types of clinical experiences of nurses lead to a different understanding and a different view of the nurse's clinical competence. The studies by Fotoohi et al. (2019) [28] and Salehi et al. (2015) [29] showed that female nurses are more sensitive in reasoning and clinical decision-making. Considering, there are more sensitive and precise ethical e in patient care than men, women recognize and understand challenging care situations. The results of the study by Adib Haj. Bagheri et al. and Kim & Choi (2019) showed that the average clinical competence of nurses at the age of 30 and above was higher than other age groups [18,30]. Clinical experiences and repeated experiences with increasing age led to an improvement in the level of patient care. Nurses provide high-quality care services by using their experiences and are involved in the challenges of clinical and ethical decision-making less frequently [31,32]. The results of studies by Mirlashari and Loui et al. (2020) were consistent with the results of the present study and showed that nurses with higher educational levels have more clinical competence [33, 34]. In postgraduate education, nurses receive further training to achieve a better understanding of patient care, which can be effective. The results of several other studies, like the results of the present study, showed the relationship between work experience and the clinical competence of nurses [18,35]. Nurses with higher work experience have higher-level clinical experience and are more sensitive to patient care. Nurses' sensitivity to patient care increases by improving their job skills and awareness of their supportive and effective role in this process [32].

The results of the present study showed that the ethical reasoning ability was better in nurses with higher age, MA education, more work experience, permanent employment, and nurses working in ICU, specialized, and general wards. Also, ICU nurses reported higher ethical reasoning. The lack of library-focused and research skills, and mistrust in the accuracy of online sources are among the factors contributing to the inappropriate use of information sources to improve the ethical reasoning ability of nurses with a bachelor's degree, which in turn causes a decrease in the use of scientific resources and evidence-based articles for making clinical decisions under challenging patient care situations [36]. While MA nurses have a higher ethical reasoning ability by learning how to use valid and updated scientific resources for clinical decision-making under ethical dilemmas.

With increasing years of work experience and experiencing more clinical ethical distress and ethical dilemma, nurses will be abler to make independent ethical decisions and to identify facilitating and prohibiting factors for clinical decision-making, which happens with increasing age. These experiences provide appropriate conditions for promoting ethical decision-making and thus, improving the ethical-reasoning ability of nurses [37].

Inconsistent with the results of the current study, Amoroso et al. (2019) reported no statistically significant relationship between the demographic indicators of American ICU nurses (age, gender, years of clinical experience, and employment status) with ethical reasoning [38]. Also, consistent with the present study, the results of the study by Mousavi Nesab et al. indicated no significant relationship between years of work experience, level of education, age, and gender of nurses working in different hospital wards with the ethical reasoning ability [39]. It seems that the difference between the results of the present study and the aforementioned studies is mainly due to the difference between the individual-social variables of the studied populations.

According to the results of the present study and other studies, various internal or external factors affect the nurses' ethical reasoning ability in different clinical wards. Internal factors include variables such as mental and physical condition, personal characteristics, values, past experiences, inclinations, knowledge, and attitudes of nurses. External factors also include environmental conditions and time [37]. Since correct and timely ethical decisions have facilitated and increased the correct use of human resources, materials, and equipment and improved the quality of care, therefore, it is necessary to take all measures and interventions to improve the ethical-reasoning ability of nurses, taking into account the afore-mentioned internal and external factors to guarantee the highest level of efficiency in this field.

CONCLUSIONS

The results of the present study showed that half of the nurses had poor clinical competence and the majority of the studied nurses had moderate clinical reasoning. There was a significant relationship between ethical reasoning and nurses' clinical competence. Also, the individual factors of age and education level, occupational factors of clinical work experience, employment status, and occupational role of nurses were effective on the clinical competence and ethical reasoning of nurses. Using the results of the present study, nursing education planners and managers can periodically organize workshops on nursing ethics and nursing problems in hospitals and universities. Moreover, clinical competence and ethical reasoning should be taken into account when training nursing personnel in university classrooms and in-service courses.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgments

Ilam University of Medical Sciences

Ethics approval and consent to participate

IR-MEDILAM-REC-1400.123

Informed written consent

was obtained from all participants included in the study. All participants were informed about the purpose of the study and assured of confidentiality and anonymity. The respondents were informed of their freedom to withdraw from the study at any time.

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REVIEW

Concise Review on Modulation Mechanism of Matrix Metalloproteinase via Oncoviruses in Aggressiveness of Malignancies

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Abstract: *Matrix metalloproteinases (MMPs) are a member of the protein family intimately associated with zinc-containing endopeptidases, which are the main proteases implicated in extracellular matrix (ECM) degradation. The high ability of MMPs in the degradation of extracellular proteins is an important event in many normal biological as well as pathologic processes, including tumor progression, tissue repair, wound healing, developmental morphogenesis, and inflammatory diseases. This review is focused on the description of the modulation mechanism of MMPs via oncoviruses. Up to now, a large number of oncogenic viruses have been detected and recognized as etiologic factors of multiple human cancers. Actually, by expression of several viral oncoproteins of tumorigenic viruses with the simple genetic system, we are able to deregulate or modulate the pathways of cell transformation procedure in malignancy. In brief, upregulated expression of MMPs genes by direct (through DNA binding) , as well as indirect interactions (by activating PI-3K/AKT, ERK/MAPK, IFN/JAK/STAT, JNK, NF-κB signaling pathways and/or immune response) with tumorigenic viruses, have a noticeable role in the proliferation of cells, as well as angiogenesis, mobility, EMT, invasiveness, and metastatic features. In fact, the growth of tumor-related researchers offers a future perspective for the efficiency of targeted, molecular-based therapies and its anticancer effects via diverse mechanisms such as the suppression of viral oncoproteins.*

Keywords: *Matrix Metalloproteinases (MMPs), Tumor Viruses, Tumor Progression*

INTRODUCTION

Matrix metalloproteinases (MMPs), form a family of zinc-dependent extracellular matrix (ECM) remodeling endopeptidase enzymes that play a role in a number of physiological processes, like the healing of wounds, organismal development, and effectors of immune responses, as well as in pathological conditions, like pulmonary dysfunctions, autoimmune diseases, and cancer [1,2]. To date, almost 25 various vertebrate MMPs have been recognized, including 23 members in humans [3]. Based on sequence homology and specificity for substrate, the MMPs family divides into six categories of stromelysins, collagenases, matrilysins, gelatinases, membrane-type as well as other non-classified MMPs [4]. Each of these groups has its subunits that perform unique functions [5]. Chemokines, cytokines, as well as growth factors, cell-to-to-cell adhesion molecules, and binding proteins, can be mentioned as other potential targets for MMP substrates [6]. The classification of MMPs is indicated in Table 1 shown [7-11]. Matrix metalloproteinase (MMPs) activity might be regulated at three transcription levels, production of enzymes like zymogens, which require activation, as well as co-expression of tissue inhibitors of metalloproteinases (TIMPs) [12,13]. MMPs transcription is perfectly controlled, and their expression level of them is low. TIMPs are endogenous suppressors of metalloproteinases that can inhibit the MMM's catalytic activity, therefore maintaining ECM homeostasis [14]. MMPs are produced in their inactive zymogenic forms (proenzymes or pro-MMPs), which are subsequently proteolytically activated by removing propeptides by other proteases [15]. The majority of MMPs are activated in the extracellular space via plasma, tissues, and microbial proteases. The rest of the activated MMPs like MT-MMPs, MMP-11, 23, and 28 possess a furin-dependent cleavage motif RXK/RR in the propeptide sequence, which are intracellularly activated via furin-like proteases of Golgi [16]. Any disruption in the regulation of MMPs modulatory mechanisms may associate with the progression of the disease in pathological conditions [12].

Table 1: Classification of the main members of the MMP family by chromosomal location and the group of substrates

MMP number	MMP subclass/ Family/Group	MMP name	Substrates	Chromosome
MMP-1	Collagenases	Interstitial collagenase	Collagens (I,II,III,VIII a X); gelatin; aggrecan; L-selectin; IL-1β proteoglycans, entactin; ovostatin; MMP-2; MMP-9	11q22-q23
MMP-2	Gelatinases	Gelatinase A	Collagens (I,IV,V,VII,X,XI a XIV); gelatin; elastin; fibronectin; aggrecan; MBP; osteonectin; laminin-1; MMP-1; MMP-9; MMP-13	16q13
MMP-3	Stromelysins	Stromelysin 1	Collagens (III,IV.V, a IX); gelatin; aggrecan; perlecan; decorin; laminin; elastin; casein; osteonectin; ovostatin; antactin; plasminogen; MBP; IL-1β; MMP-2/TIMP-2; MMP-7; MMP8; MMP-9; MMP-13	11q23
MMP-7	Matrilysins	Matrilysins	Collagens (IV a X); gelatin; aggrecan; decorin; fibronectin; laminin; entactin; elastin; casein; transferrin; plasminogen; MBP; β4-integrin; MMP-1; MMP-2; MMP-9; MMP-9/TIMP-1	11q21-q22
MMP-8	Collagenases	Neutrophil collagenase	Collagens (I,II,III,V,VII,VIII a X); gelatin; aggrecan; fibronectin	11q21-q22
MMP-9	Gelatinases	Gelatinase B	Collagens (IV,V,VII,X a XIV); gelatin; entactin; aggrecan; elastin; fibronectin; osteonectin; plasminogen; MBP; IL-1β	20q11.2-q13.1
MMP-10	Stromelysins	Stromelysin 2	Collagens (III-V); gelatin; casein; aggrecan; elastin; MMP-1; MMP-8	11q22.3-q23
MMP-11	Stromelysins	Stromelysin 3	Unknown (casein)	22q11.2
MMP-12	Elastase	Metalloelastase	Collagen IV; gelatin; elastin; casein; fibronectin; vitronectin; laminin; entactin; MBP; fibrinogen; fibrin; plasminogen	11q22.2-q22.3
MMP-13	Collagenases	Collagenase-3	Collagens (I,II,III,IV,IX,X,a XIV); gelatin; plasminogen; aggrecan; perlecan; fibronectin; osteonectin; MMP-9	11q22.3
MMP-14	Membrane-MMP	MT1-MMP	Collagens (I-III); gelatin; casein; fibronectin; laminin; vitronectin; entactin; proteoglycans; MMP-2; MMP-13	14q11-q12
MMP-15	Membrane-MMP	MT2-MMP	Fibronectin; entactin; laminin; perlecan; MMP-2	16q12.2-q21
MMP-16	Membrane-MMP	MT3-MMP	Collagen III; gelatin; casein; fibronectin; MMP-2	8q21
MMP-17	Membrane-MMP	MT4-MMP	Gelatin	12q24
MMP-18	Collagenases	Collagenase-4	Unknown	Unknown
MMP-19	Other	RASI-1	Gelatin; tenascin; fibronectin; collagen IV; laminin; entactin; fibrin/fibrinogen; aggrecan	12q14
MMP-20	Other	Enamelysin	Amelogrenein; aggrecan; COMP	11q22
MMP-23	Other	Unnamed	Amelogenin	11q22
MMP-24	Membrane-MMP	MT5-MMP	Pro-MMP 2	20q11.2
MMP-25	Membrane-MMP	MT6-MMP	Pro-gelatinase A; fibrin; fibronectin; collagen IV; gelatin	16p/3.3
MMP-26	Matrilysins	Endometase, matrilysin-2	Gelatin Iα; P1; fibrinogen; fibronectin; vitronectin; β-casein	Unknown

MMPs AND TUMOR PROGRESSION

Cancer is the second factor of death globally, which is estimated to account for 9.6 million deaths in 2018 [17]. Recent two decades of biomedical research have led to large amounts of information about the molecular events which occur during carcinogenesis as well as signaling pathways involved in the progression of cancer. Complex molecular mechanisms play different roles in the tumor cells and the tumor environment associated with this process [18]. Several interactions of tumor cells with the involved environment of the tumor are crucial for the pathological changes observed during the transformation of cells and carcinogenesis in the extracellular matrix (ECM), as well as cytokines, growth factors associated with ECM, along with neighboring cells (neutrophils, endothelial cells, mast cells, macrophages, fat cells, and pericytes) [19-21]. Different steps of cancer consisting of migration, attack, metastasis, and angiogenesis are in interaction with the neighboring microenvironment [22]. MMPs are the main molecules in these processes, and since they break down numerous cell adhesion molecules, indicating their roles in the regulation of cell-to-cell, and cell-ECM interactions [23,24]. Previous researchers have identified that MMPs play an essential role in all phases of tumor maintenance and growth, including invasiveness, migration of tumor cells, evasion from apoptosis as well as immune surveillance, angiogenesis, and metastasis, so that all these major stages may be modulated by MMPs [25].

Interferon, interleukins, growth, factors, and extracellular MMP inducers secreted from tumor cells in a paracrine, manner stimulating adjacent host cells to generate the required MMPs. MMPs produced by normal cells can be bound and used on the surface of tumor cells [26]. MMPs gene expression and its activity increase in many types of cancers in humans. And this increased expression causes an increase in tumor size, invasion, and decreased survival [27]. Thus, MMPs are used as a novel and capable therapeutic target in different types and cancer phases [28]. MMPs use their proteolytic function to remove physical barriers, developing angiogenesis and tumor metastasis [29]. Tumor development and angiogenesis depend on the facilitated accessibility of signaling molecules. For example, cytokines and growth factors by MMPs enhance by making them available for tumor cells and their microenvironment [18]. This process is performed by releasing these factors from the ECM (VEGF, IGF, and bFGF) or via secreting them from the surface of the cell (HB-EGF, EGF, TGF-a) [4]. The release of endogenous angiogenesis suppressers can regulate angiogenesis perfectly, for instance, angiogenesis inhibitors like tumstatin, endostatin, angiostatin, and endorepellin [30]. Disruptions in the regulation of cell-cell and cell-ECM interactions by MMPs during the processing of E-cadherin and integrins, respectively, affect both cell phenotype (EMT) as well as increasing cell migration [31,32]. Almost all members of the MMP family have been reported to be dysregulated in human tumors, although MMPs such as MMP-1, -2, -7, -9, -13, and 14 are more important [33]. We have demonstrated the most noticeable MMPs, which are related to the phases of tumor growth, biological effects, and their consequences in Table 2.

Table 2: Key MMPs in relation to the stages of cancer progression, biological effect, and consequences

Function	MMP	Biological effect	Consequence	Reference
Cancer cell invasion	MT1-MMP, MMP-2 and MMP-9	Proteolytic	Breakdown physical barriers	[144]
Cancer cell proliferation	MMP-1, -2, -3, -7, -9, -11, -19	Cleavage of IGF binding proteins, Shedding of membrane, anchored ligands of EGFR, including HB-EGF, TGF-a and amphiregulin, Activation of TGF-β, Shedding of HB-EGF	Proliferation	[145-147] [148] [149] [150]
Cancer cell apoptosis	MMP-7 Several MMPs	Cleaving the Fas ligand Indirect activation of the serine/serine/threonine Akt ⁄ protein kinase B through the signaling cascades of EGFR and IGFR	Anti-apoptotic	[151] [147, 152]
Tumor angiogenesis	MMP-2, -9 MMP-3, -10, -11 MMP-1, -8, -13	Degradation of COL-IV, perlecan; release of VEGF and bFGF, respectively Degradation of COL-IV, COL-XVIII, perlecan; generation of tumstatin, endostatin, angiostatin and endorepellin, respectively	Upregulation of angiogenesis Downregulation of angiogenesis	[153, 154] [4, 155]
Cell adhesion, migration, and EMT	MMP-2 MT1-MMP MMPs MMP-2, -3, -9, -13, -14 MMP-1 and -7 MMP-28	Degradation of COL-IV; generation of cryptic peptides Degradation of laminin-5; generation of cryptic peptides Integrins as substrates Overexpression this MMPs associated with EMT, a highly conserved and fundamental process of morphological transition By cleavage E-cadherincontribute to morphological transition Proteolytic activation of TGF-β	Promote migration Induction of EMT; cell migration Powerful inducer of EMT, leading to cell migration	[156, 157] [158] [159] [160] [161]
Immune surveillance	MMP-9 MMP-9, -2, -14 MMP-7, -11, -1, -8, -3 MMP-7, -8	Cleavage of IL-2R alpha Release active TGF- β Release of a1-proteinase inhibitor Cleave several members of the CC (β-chemokine) and CXC (α-chemokine) chemokine subfamilies or to regulate their mobilization	Repress T-lymphocyte proliferation Abolish T-lymphocyte reaction against cancer cells Decrease cancer cell sensitivity to NK cells Affecting leukocyte infiltration and migration	[162] [163] [164] [165, 166]

ONCOVIRUSES

Approximately 20% of all human malignancies worldwide are associated with viruses [34]. Seven viruses have been implicated as oncogenic, including HPV, EBV, HCV, HBV, Kaposi's sarcoma-associated herpesvirus (KSHV), HTLV-1, and Merkel cell polyomavirus (MCV), which have been reported to be etiologically implicated in the maintenance and progression of human cancers [35]. However, viral oncogenesis processes are a complex process, with induction of chronic inflammation conditions, disruption of cellular DNA repair mechanisms, interrupting host genetic and epigenetic integrity, and homeostasis, viral infection may result in genome variation and cell cycle deregulation. Viral 'oncoproteins' are capable of modulating or deregulating cellular signaling pathways, making changes in the expression of genes and microRNAs in either transcription or post-transcription, and disrupting tumor suppressor factors and proteins, which regulate cell polarity, immune response, signal transduction, and cell apoptosis. Genetic and epigenetic modifications caused via oncoviruses may be resulting in the morphology and variation of cancer stem cells, which are essential for the primary initiation, development, migration, invasiveness, and chemotherapy resistance of tumors. These molecular events underlying the interactions between oncoviruses that use specific cellular genes and cellular signaling pathways are important issues to research efforts [36-38]. MMPs have several specific biological actions in every cancer phase, from the beginning to the extension of pertinent metastases. Accordingly, this review's focus is on characterizing the mechanism of modulation or deregulation of MMPs by oncoviruses.

INTERACTION BETWEEN ONCOVIRUSES AND MMPs

Epstein-Barr virus

Epstein-Barr virus (EBV) is a linear dsDNA virus that belongs to the human gamma-herpesvirus family, playing a role in different B-cells lymphomas, including Hodgkin's lymphoma, Burkitt's lymphoma, post-transplant lymphoproliferative disorders, as well as other tumors like T-cell non-Hodgkin lymphomas and nasopharyngeal carcinoma [39]. In tumors associated with EBV, the virus leads to a latent infection expressing only a subset of 12 latent viral transcripts, two early RNAs (EBERs), three latent membrane proteins (LMPs), six nuclear antigens (EBNAs), and BARF transcripts. Out of these genes, EBNA1, EBNA-2, EBNA-3A, EBNA-3C, EBNA-LP, and LMP1 enable the virus to control a number of cellular signaling pathways associated with oncogenesis [40,41]. EBV LMP1 increases MMP-9 expression by recruitment of Sp-1, AP-1, and NF-kappa B transcription factors on MMP-9 promoter that is related to invasiveness and metastasis in EBV-associated NPC [42]. Previous research demonstrated that C-terminal activation regions 1 and 2 (CTAR-1 and CTAR-2), two crucial signaling domains within the carboxy terminus of LMP1, might activate the MMP-9 promoter and stimulate MMP-9 activity [43]. TANG Jian showed EBV-induced MMP-9 upregulation correlates with lymph node metastasis and clinical stage in NPC tumors [44]. Lee demonstrated that upregulation of MMP1 and MMP3 expression in LMP1-positive NPC tumors led to the enhancement of invasiveness in NPC cells [45]. BZLF1 (also known as Zta) is one of the critical inducers of EBV lytic cycle alteration and has been reported to trigger metastasis in epithelial cells [46, 47]. In vitro studies demonstrated that Zta stimulates MMP3 and MMP9 expression in epithelial cells derived from NPC tumors. Zta can activate the MMP3 promoter by AP-1 elements, and its DNA-binding domain is necessary for the process of promoter binding and MMP3 stimulation. Zta-enhanced cell migration requires MMP3 but not MMP9. Zta-elevated cell migration needs MMP3, but not MMP9. Therefore, the expression of both MMP3 and MMP9 is important for the co-expression of the two MMPs (MMP3 and MMP9) induced by Zta, which simultaneously promotes cell invasiveness of epithelial cells differentiated from NPC tumor [48]. Moreover, Zta protein via binding AP-1 site of its promoter increases TIMP-1 expression in EBV-infected B-cells as well as EBV-immortalized lymphoblastoid cell lines (LCLs). Upregulation of TIMP-1 expression through Zta enhances the maintenance of EBV-infected B-cells and promotes the progression of EBV-associated cancer [49]. EBNA3C regulates the transcription of numerous viral as well as cellular genes important for the process of immortalization by EBV in primary B-lymphocytes [50]. Nm23-H1 is a well-known metastasis inhibitor, and its low expression is correlated with metastatic tumor capability in different types of human carcinoma [51]. In vitro research showed the interaction of EBNA3C with Nm23-H1 leads to the translocation of Nm23-H1 to the nucleus and inhibits the antimotility effect. However, studies have shown that this interaction also can increase transcriptional activity on a related promoter of the target gene [52]. Interaction between EBNA3C and Nm23-H1 upregulates the expression of MMP-9 by binding the transcription factors AP-1 and NF-kB on its promoter in B-cells that were isolated from Burkitt's lymphoma [53]. LMP2A promotes cell transformation, migration, and invasion by affecting host cellular signaling pathways, mainly by its N-terminus, intracellular domain, which consists of an immunoreceptor tyrosine-based activation motif (ITAM), a YEEA motif, and two PPPPY (PY) motif [54,55]. LMP2A promotes invasion of NPC cells by the PY motif via stimulation of extracellular signal-regulated kinase (ERK)-1/2 as well as the downstream transcription factor Fra-1, therefore leading to upregulation of MMP9 expression [56]. WW domain-containing oxidoreductase WOX1, also called WWOX or FOR, is a candidate tumor inhibitor encoded by the human fragile WWOX gene, related to several signaling pathways modulating embryonic development, metabolism, cell proliferation, and apoptosis [53]. The function of WOX1 in cancer suppression is commonly used by stimulating apoptosis and sequestering some oncoproteins [57,58]. According to a previous study, the interaction of the tyrosine residue 33 of WOX1 and the PY motif of LMP2A is needed for the LMP2A-induced ERK1/2-Fra-1-MMP9 pathway in NPC cells (Figure 1) [59]. Two SNPs (rs8113877T>G or rs17576A>G) of MMP-9 in ab_EBV positive individuals were associated with more possibility of progression to chronic lymphocytic leukemia in contrast to those individuals who were negative [60]. In summary, the upregulation of MMPs by EBV plays a significant role in EBV-triggered metastasis.

Figure 1: Schematic model of deregulation of MMPs by EBV virus

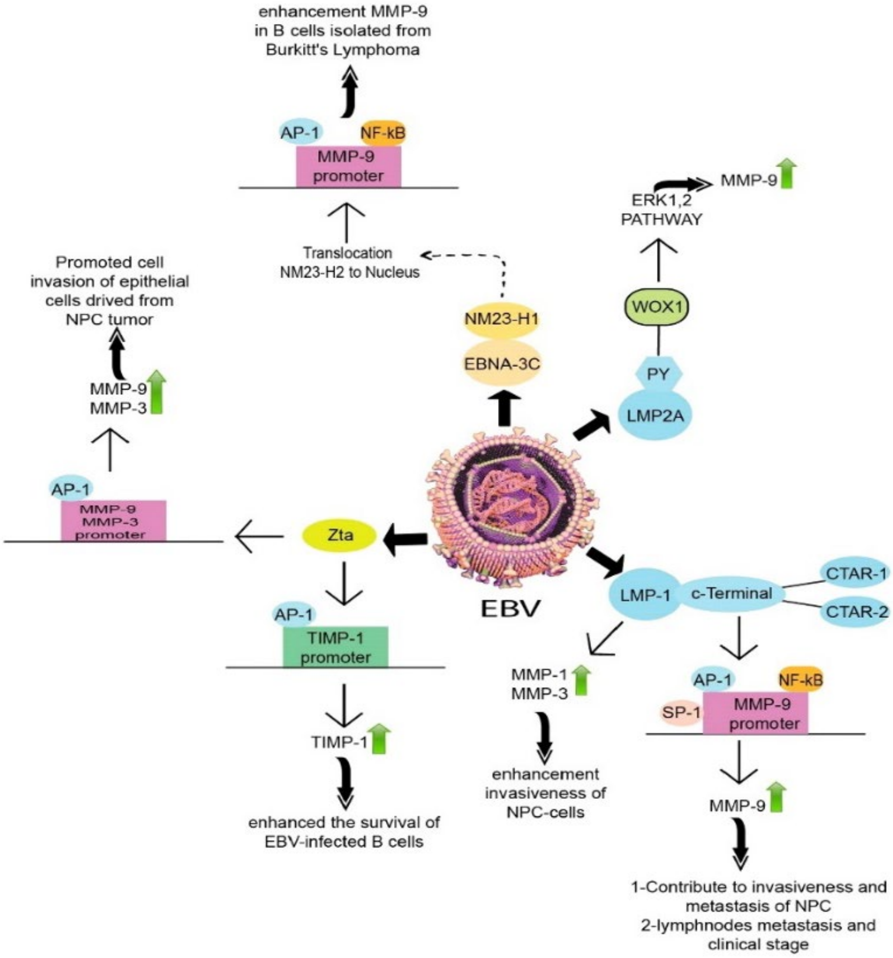
enhancement MMP-9 in B cells isolated from Burkitt's Lymphoma
AP-1
NF-kB
MMP-9 promoter
MMP-9
ERK1,2 PATHWAY
Translocation NM23-H2 to Nucleus
Promoted cell invasion of epithelial cells drived from NPC tumor
WOX1
NM23-H1
EBNA-3C
PY
MMP-9
MMP-3
LMP2A
AP-1
MMP-9 MMP-3 promoter
Zta
EBV
CTAR-1
LMP-1
c-Terminal
CTAR-2
AP-1
TIMP-1 promoter
MMP-1
MMP-3
AP-1
NF-kB
SP-1
MMP-9 promoter
TIMP-1
enhancement invasiveness of NPC-cells
MMP-9
enhanced the survival of EBV-infected B cells
1-Contribute to invasiveness and metastasis of NPC
2-lymphnodes metastasis and clinical stage

Human papillomavirus

Human papillomaviruses (HPVs), namely double-stranded DNA viruses, are recognized as one of the main agents that relate to the progression of numerous cancers such as head and neck, cervix, vagina, vulva, anal and penile carcinomas [61]. Continuing infection with high-risk human papillomavirus (HPV) types, especially HPV type 16 or 18, is essential for the development of a cancer precursor lesion and invasive cervical cancer, which is identified through deregulated and high-level expression of HPV early genes (E6 and E7) [62]. The high-risk HPV E6 and E7 oncoproteins result in transformation by a disruption in the activity of cellular tumor suppressor proteins p53 and RB, respectively, and several cellular proteins [63,64]. Induction of MT1-MMP expression in HaCaT cells, an immortalized human keratinocyte line, by the E7 protein of high-risk HPV types 16 and 8 contributes to cellular proliferation, angiogenesis, and, malignant progression, for the invasion of HPV-positive tumor cells [65]. In human keratinocytes and organotypic skin cultures, HPV8 E2 protein overexpresses the expression of MMP-9 by direct activation of its promoter, resulting in increased squamous fragility involved in facilitating viral escape [66]. A recent study demonstrated that HPV-16 E6/E7 oncoproteins cause upregulation of MT1-MMP, MMP-2, and MMP-9 to promote cervical cancer cells [67]. Previous studies reported that HPV 16 infection might lead to lung tumor cells through increasing in interleukin-17 (IL-17) expression. IL-17 along with its downstream signaling mediator, interleukin-8 (IL-8), play an efficient role in tumor angiogenesis and metastasis by modulation of various pro-angiogenic factors [68]. Induction of IL-8 by HPV upregulates the expression of MMP-2 and MMP-9 in lung adenocarcinoma that correlated with progressive mechanisms of growth, and metastatic and angiogenic characteristics [69]. HPV-16 E6/E7 overexpressed the expression of MT1-MMP and MMP-2, which are correlated with increased cell invasion in cervical cancer [70]. According to recent research (Haukioja), low salivary levels of MMP-8 are due to continued oral HPV infection, which appears like a failure of the anti-inflammatory defense of the oral mucosa to allow an incident oral HPV infection to become a chronic infection [71]. Common skin extract immunoblots and zymographic analysis revealed increased expression of MMP-9, MMP-13, and MT1-MMP in HPV8-positive mice rather than HPV8-negative mice, which are involved in the development of HPV8-induced cutaneous tumors (Figure 2) [72]. Singh et al. have shown that SNPs -1306CC (rs243865: C>T) in the promoter of the MMP2 gene, enhanced transcription and enzymatic activity of the MMP-2 gene is associated with the incidence possibility of cervical cancer. A significant relationship between the −1306CC genotype and HPV infection in increasing the risk of cervical cancer was observed [73].

Figure 2: Schematic model of deregulation of MMPs by HPV virus

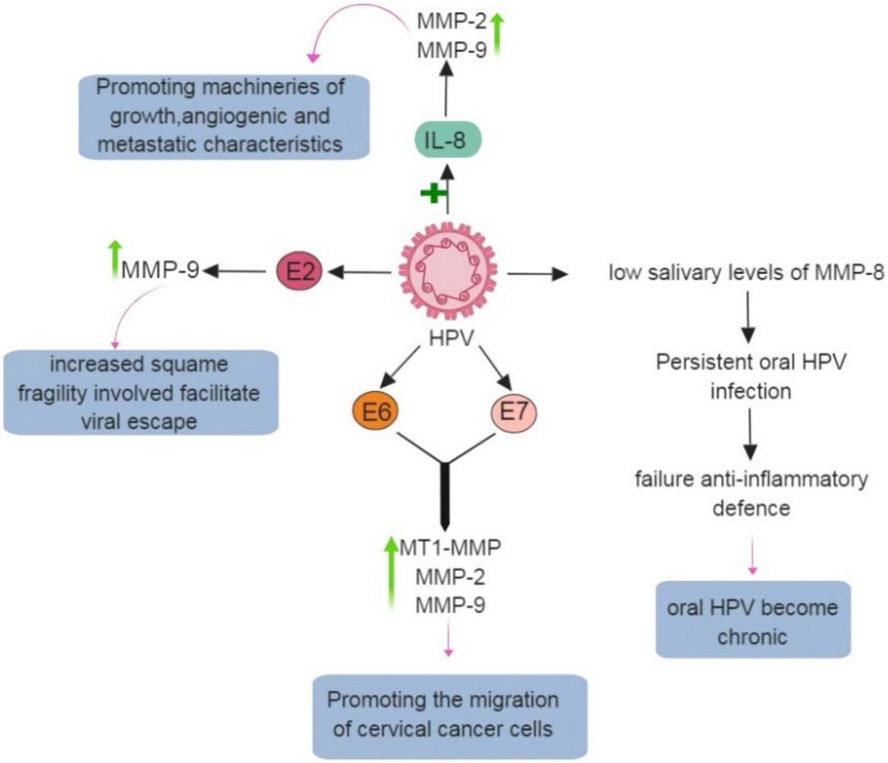
MMP-2
MMP-9
Promoting machineries of growth,angiogenic and metastatic characteristics
IL-8
MMP-9
E2
HPV
low salivary levels of MMP-8
increased squame fragility involved facilitate viral escape
E6
E7
Persistent oral HPV infection
failure anti-inflammatory defence
MT1-MMP
MMP-2
MMP-9
oral HPV become chronic
Promoting the migration of cervical cancer cells

HBV

Chronic hepatitis B (CHB), an important agent responsible for liver cirrhosis and hepatocellular carcinoma, is among the primary health problems of the world [74,75]. HBV transactivator protein (HBx) enables the invasive capacity of the virus, which is mediated by increased the expression of membrane-type one matrix metalloproteinase (MT1-MMP) that subsequently activates matrix metalloproteinase-2. HBx is able to induce both MT1-MMP expression and cell invasion through dependence on cyclooxygenase-2 (COX-2) activity. Furthermore, HBx increases COX-2 expression through the activation of COX-2 gene promoter transcription in a nuclear factor of an activated T-cell-dependent (NF-AT–dependent) manner. These data indicated the capacity of HBx to augment tumor cell invasion by a mechanism consisting of MT1-MMP and COX-2 upregulation and led to new insights into HBx protein mechanism of action, its involvement in tumor metastasis and hepatocellular carcinoma resulting from intrahepatic metastasis to HBx protein expression following establishment of tumor [76]. HBx protein plays a crucial role in the progression of hepatocellular carcinoma (HCC). HBx is often the only HBV protein that would be detected in hepatic tumor cells, which enhances tumor cell invasion and metastasis. HBx, by inducing the PI-3K/AKT and ERK pathways except for the P38 MAPK pathway, leads to the activation of NF-kB and AP-1 transcription factors. HBx triggers the translocation of NF-kB and AP-1 into the nucleus, leading to increasing MMP-9 expression. Thus, HBx modulates the transcriptional function of MMP-9 that is essential to the incursion and metastasis of tumors through PI-3 K/AKT and

ERK pathways. These findings suggest that HBx could induce MMP-9 and, in turn, in the invasion and metastasis of cancer, by activating ERK and PI-3K signaling pathways (Figure 3) [77].

Figure 3: Molecular mechanism of HBX-activated MMP-9 expression. HBx induces PI-3K/AKT and ERK pathways but not the p38 MAPK pathway. This process leads to the stimulation of NF-kB and AP-1 transcription factors. Translocation of NF-kB and AP-1 activated by HBx into the nucleus leads to an increase of MMP-9 expression. Therefore, HBx via regulation of transcriptional activation of MMP-9, plays an important role in the invasion and metastasis of tumors via PI-3K/AKT and ERK pathways

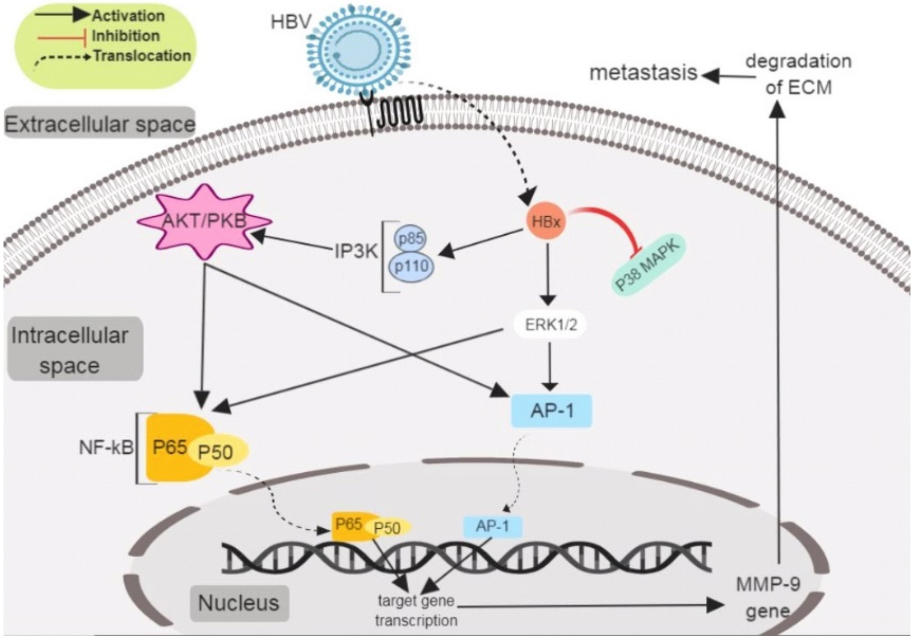

Genes that were defined to be modulated in the presence of HBx include those genes involved in signal transduction, cell cycle, cytokine production, metabolism, apoptosis, cell adhesion, and oncogenesis. The functions of other less-defined genes also were affected. Transactivation by HBx upregulates the translation, transcription, and release of matrix metalloproteinase-3 (MMP-3), which manifests as a cell migratory phenotype. This effect of HBx was suppressed in the presence of an MMP-3-specific peptide inhibitor. HBx applies selective control of transcription in hepatoma cells as well as inducing cell migration via activation of MMP-3. Therefore, by upregulation of MMPs, HBx increases the invasion of tumor cells and the destruction of the extracellular matrix that is reported to increased cell migration [78]. The progression of tumors tightly depends on a permissive microenvironment that is highly correlated with the destiny of tumor cells. The normal structure of ECM is of high importance in the modulation of cell growth. For invasiveness, cancer cells must overwhelm the mechanical barriers caused by the ECM. It has been demonstrated which conformational changes in the ECM by MMPs have a noticeable role in the mobility and spread of tumor cells, resulting in invasion and metastasis. The activation of MMP induced by HBx signifies a role of HBx protein in the development of HCC metastasis. However, HBx is demonstrated to relieve transcriptional stimulation activity, it is not defined in what way HBx induces the expression of MT1-MMP [79,80]. HBV replication is assisted by MMP-9 through repression of IFN/JAK/STAT signaling as well as IFNAR1 function. Thus, these outcomes have discovered a novel role for MMP-9 in viral replication, revealing a new mechanism by which HBV escapes host immunity to maintain persistent infection. Indeed, there is a positive feedback loop between the injection of HBV and the production of MMP-9. Firstly, HBV activates MMP-9 production in PBMCs and macrophages. Subsequently, HBV replication would be facilitated by MMP-9 through repressing IFN/JAK/STAT signaling, interacting with IFNAR1 to prevent its binding to IFN-α and promoting the ubiquitination phosphorylation, degradation of IFNAR1 and ISRE, subcellular distribution, and interferon-stimulated response element. Thus, MMP-9 would benefit HBV by blocking innate immunity to launch or maintain chronic infection (Figure 4) [81].

In hepatitis B infection, apoptosis of liver cells that is induced by varying degrees of inflammation plays an essential role in the outcome of CHB [82]. The MMP-9 expression level is directly related to TNF-α and IL-6. Because IL-6 and TNF-α can regulate MMP-9 activity in a concentration- and time-dependent mode, overexpression of MMP-9 can degrade ECM to destroy the basement membrane and lead to disorganized and loose tissue structures, leading to further penetration of TNF-α and IL-6. These factors can interact with each other and create a vicious cycle, resulting in pathological changes in the liver. In conclusion, the MMP-9 level is increased remarkably in patients with varying degrees of CHB, it would be important in the pathological progress of the liver, and it has a close correlation with inflammation, which can provide a theoretical basis for clinical treatment [83].

Figure 4: MMP-9 assists HBV replication by suppressing IFN/JAK/STAT signaling and IFNAR1. Indeed, HBV induces MMP-9 in infected patients and in HBV-stimulated leukocytes. Then, MMP-9 comforts HBV replication through repressing IFN/JAK/STAT signaling, interacting with IFNAR1 to block its binding to IFN-α, and promoting the phosphorylation, ubiquitination, subcellular distribution, and degradation of IFNAR1

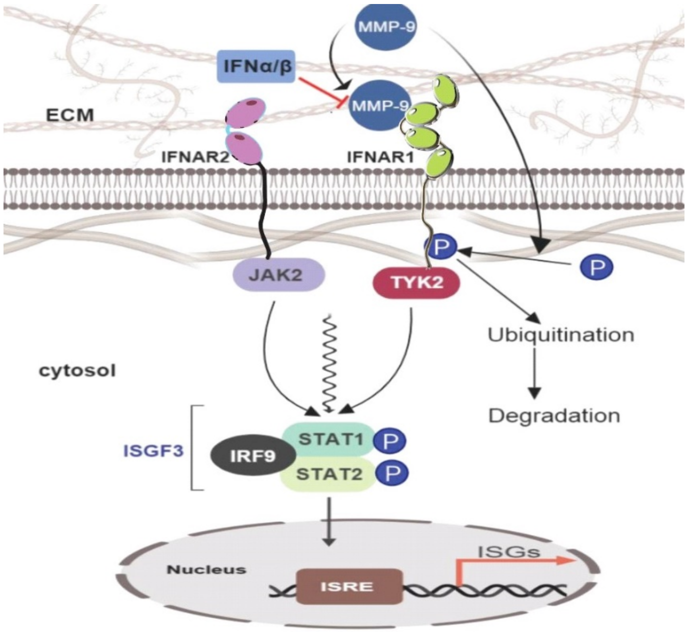

HCV

Hepatic inflammation, cirrhosis, developing liver fibrosis, and hepatocellular carcinoma (HCC) are common problems in HCV patients [84,85]. In the liver, the main reason for fibrogenesis is pathological repletion of the extracellular matrix (ECM), which leads to an imbalance in the rate of enhanced matrix synthesis to reduce the degradation of connective tissue proteins that can be controlled by matrix metalloproteinases (MMPs). Recent studies showed that increasing levels of hepatic gelatinases, including MMP-9, and MMP-2, are related to the progression of HCC and the fibrotic index of chronic HCV patients [86,87]. The disparity between matrix metalloproteinases (MMPs) and tissue suppressors of matrix metalloproteinases (TIMPs) is remarked to be a significant index of degradation of the extracellular matrix. MMPs are members of gene families that can degrade collagen and play an essential role in the natural mechanism of tissue repair in pathological states, including liver fibrosis. Thus, an increase of the MMP-1/TIMP-1 ratio by IFN treatment can relieve liver fibrosis of chronic hepatitis C that demonstrates the anti-fibro genetic effect of IFN on the factors modulating hepatic fibrosis [88]. During chronic liver harm, activated hepatic stellate cells (HSC) turned backward into myofibroblast-like cells and proliferated. This stage is an important factor in the creation of fibrosis and subsequent cirrhosis [89]. HSCs are considered important sources of fibrillar as well as nonfibrillar collagens of the liver, matrix-degrading proteases (MMPs), and their specific blockers (TIMPs). Briefly, recent studies on patients with chronic hepatitis C reported important variations in hepatic TIMP and MMP expression. It is likely that TIMP expression was affected via the inflammatory conditions of the disease, which can reflect the effects of the disease. Promoted fibrosis enhances the expression of MMP-7 and MMP-2 and provides a condition for the destruction assessment of connective tissue [86]. The primary activity in the biosynthesis of different prostaglandins (PG) and thromboxanes catalyzes via cyclooxygenases [90,91]. There are two cyclooxygenases (COX) isoforms: COX-1 is basically expressed in several cell types and is the main factor in the homeostatic activity of PG, while COX-2 is triggered by different proinflammatory stimuli, including lipopolysaccharide (LPS) and cytokines [92-94]. COX-2 is involved in fibrogenesis, inflammation, and carcinogenesis [95-97]. Therefore, different researchers reported that increased COX-2 expression and PG production might be associated with liver dysfunction and tumorigenesis in various animal models [98-100], in addition to fibrosis and hepatocellular carcinoma (HCC) among humans [101,102]. HCV core and NS5A proteins, both alone and in association with the impact of endotoxin and proinflammatory cytokines, are capable of regulating the expression of COX-2 and MMP-9 genes through chronic HCV infection. Therefore, this mechanism may be considered evidence for this process that chronic HCV infection may result in liver fibrosis. Thus, it seems reasonable that much PGs generated in hepatocytes via the correlated effect of HCV proteins in addition to inflammatory factors may affect neighboring liver cells by autocrine and/or paracrine pathways, therefore participating in hepatocarcinogenesis [103]. The E2 envelope glycoprotein of the hepatitis C virus (HCV) is an important modulator of HCV interaction with cell surface receptors. E2 interacts with an extracellular loop of human CD81; it's a receptor expressed on various cell types such as hepatocytes and B-cells. CD81 on the cell surface is expressed in human hepatic stellate cells (HSC) at a high level, Matrix metalloproteinase-2 (MMP-2; gelatinase A), an important enzyme responsible for the degradation of the normal hepatic extracellular matrix, upregulates subsequently after binding E2 toCD81. Furthermore, E2/CD81 interaction in human HSC causes an increasing amount of MMP-2 via increasing the binding activity of activator protein-2/DNA via ERK/MAPK phosphorylation. Increased degradation of the hepatic extracellular matrix in HCV-concentrated regions may be associated with inflammatory as well as parenchymal damage [104]. HCV replication may be regulated by signal cascades, cellular factors, and viral proteins [105-108]. The HCV nonstructural protein 3 (NS3) has a serine protease required for the synthesis of viral protein and helicase activity needed for viral replication [109,110]. NS3 also is a major protein in modulating cell proliferation, indicating its role in carcinogenesis [111,112]. HCV NS3 protein facilitates tumor cell invasion in two ways. Firstly, NS3 induces several signaling pathways like protein kinase D2 (PKD2), JNK, and ERK1/2 to modulate the transcription factor NF-κB, and subsequently, it regulates the expression of COX-2 [113], and COX-2, in turn, activates MMP-9 expression. Secondly, NS3 induces the ERK1/2–p38 signal pathway to modulate the activity of NF-κB, which triggers the translocation of NF-κB to the nucleus and binding of NF-κB to MMP-9 promoter, which consequently upregulates MMP-9 expression. MMP-9 (an important enzyme in degrading the extracellular matrix), and COX-2 (the main enzyme in prostaglandin E2 production) exert key roles in tumor invasion and tumorigenesis. Therefore, NS3 enhanced tumor cell metastasis by MMP-9 and COX-2, leading to tumorigenesis (Figure 5) [114]. The MMP2, MMP9, and TNF-α levels have an effective increase in chronic HCV patients. However, no considerable correlation has been reported in the levels of MMPs and TNF-α between fibrotic and cirrhotic cases. In consequence, MMP2, MMP9, and TNF-α revealed high reproduction capacity in distinct patients afflicted with liver fibrosis (F1–F4) compared to the control group. In contrast, MMP9, MMP2, and TNF-α were not distinguishable factors for liver fibrosis in patients with chronic HCV. Therefore it is likely that they would be used as prognostic markers for liver fibrosis [115].

Figure 5: The mechanism of hepatoma cell invasion by HCV NS3 protein. First, NS3 activates multiple signaling components such as protein kinase D2 (PKD2), JNK, and ERK1/2 to regulate the activity of transcription factor NF-κB, which in turn regulates the expression of COX-2, and then COX-2 activates MMP-9 expression. Second, NS3 activates ERK1/2–p38 signaling pathway to regulate NF-κB activity, stimulates the translocation of NF-κB from the cytosol to the nucleus and the binding of NF-κB to the MMP-9 promoter, and thus activate MMP-9 expression.Thus, NS3 enhances tumor cell migration through COX-2 and MMP-9, resulting in oncogenesis or tumorigenesis

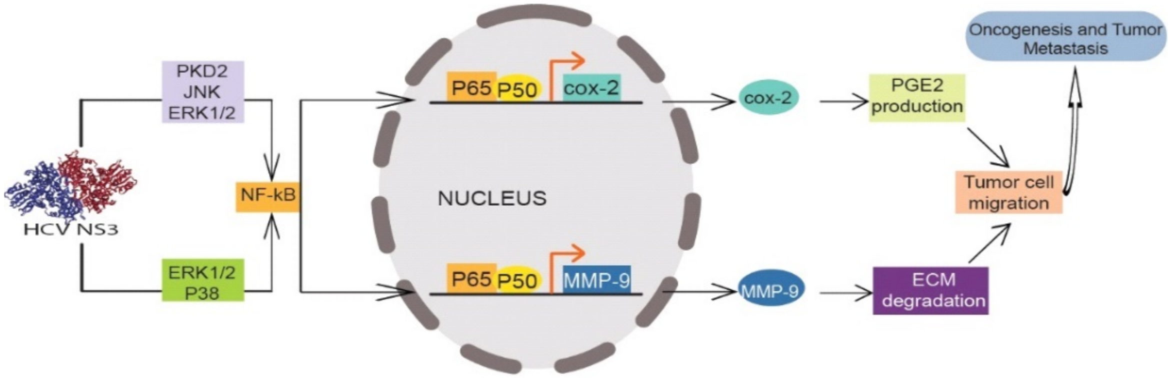

KSHV

Human herpesvirus-8 (HHV-8) or Kaposi sarcoma-associated herpesvirus (HHV-8 /KSHV) is a ds DNA virus with 165 kb in length. The HHV-8 life cycle consists of two states, lytic replication, and a latent phase. After primary infection, HHV-8 uses the cellular machinery of the host for replication and is subsequently observed in a latent phase as an episome. Like all herpesviruses, the B lymphocyte is the primary source of HHV-8 latency. The second sites for viral latency are endothelial cells, but these cells are the first infected cells in Kaposi sarcoma (KS). During latency, HHV-8 can regulate host cellular signaling pathways by different cellular mechanisms. Latent proteins, which include viral Cyclin a (v-Cyclin), LANA, kaposin, viral FLICE inhibitory protein (v-FLIP), and several miRNAs contribute to the pathogenesis of malignancies associated with HHV-8 [116]. HHV-8 has been identified in a Kaposi sarcoma lesion in an AIDS patient in 1994. This virus was reported to be associated with many B cell lymphoproliferative disorders (LPDs) such as posttransplant and germinotropic LPDs, multicentric Castleman disease (MCD), as well as primary effusion lymphoma (PEL) [117]. MMPs possibly associate with the mechanism of invasive KS growth and can be a proper target for effective therapeutic measures [118]. Developing KS lesional cells express gelatinases (MMP-2, MMP-9), stromelysin-1 (MMP-3), matrilysin (MMP-7), and collagenase (MMP-1, MMP-13) [119]. Cheng F. et al. demonstrated KSHV induces endothelial-to-mesenchymal transition (EMT). EMT was triggered by the viral proteins vGPCR and vFLIP via activation of the Notch pathway, which leads to a gain of membrane-type-1 matrix metalloproteinase (MT1-MMP) (Figure 6) [120]. The transcription factor PROX1 expression was recently investigated in KS. PROX1 binds the MMP14 promoter to repress its transcription. PROX1 negatively regulates MMP14 in normal tissues. Silvia Gramolelli et al. revealed that infection of lymphatic endothelial cells (LECs) with KSHV decreases PROX1 expression [121].

Figure 6: Schematic model of deregulation of MMPs by KSHV virus

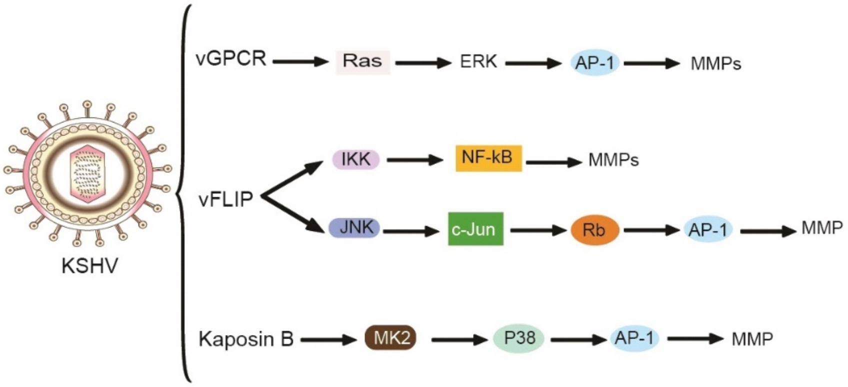

Human T-cell lymphotropic virus type I (HTLV-I)

HTLV1 is a member of the retroviridae family, which is an etiologic factor of Adult T-cell Leukemia (ATL).ATL is a malignancy responsible for the extremely aggressive proliferation of CD4+ cells with no known cure [122]. ATL commonly originates from an infection that is acquired in early life [123]. Also, besides ATL, other manifestations of infiltrated T-cells, matrix metalloproteinases (MMPs), and cytokines of viral infection are Sjogren syndrome with an inflammatory appearance and HAM/TSP associated with neurological symptoms and incomplete paralysis of limbs [124-126]. The 3’ end of the genome (pX region) encodes some specific genes, including Rex, Tax, p12, p13, p30, and HTLV-1 bZIP factor (HBZ) [127,128]. HTLV-1 encodes two important genes, basic leucine zipper factor (HBZ), and tax, that are recognized to exert key roles in the infectivity of HTLV-1, survival, and growth of leukemic cells. Unlike HTLV-1 tax, HBZ escapes the antiviral response and is not detectable via the immune system. HTLV-1 provirus pX region encodes HBZ protein and in contrast to Tax, expressed in 100% of ATLL cells [123,129,130]. Although the proliferation of T cells is increased by HBZ, its function is interestingly antagonist to many tumorigenic activities of tax, such as activation of classical signaling pathways such as NFAT, AP-1, CREB, and NF-κB. Tax Repression through HBZ provides a condition for HTLV-1 to develop ATL by evading the immune system [123,131]. Tax may interact with cellular factors including AP-1, CBP/p300, CREB/ATF, and NF-κB. Both HTLV-1-replication and T-cell proliferation are generally enhanced by activating of IκB kinase (IKK)-NF-κB that is stimulated by viral Tax oncoproteins. Viral transactivator Tax is a presumed E3 ubiquitin ligase that is unitedly related with UbcH7, UhcH5c, and UbcH2 and is responsible for direct activation of IKK via binding to the NEMO subunit [132,133]. Bazarbachi et al. proved the interaction of HTLV-I-transformed cells in relation to endothelial cells triggers the gelatinase activity of MMP-9 and MMP-2 in endothelial cells and decreases the tissue blocker of MMP. In a study by Szymocha et al. on infected astrocytes (T cells) with HTLV-1, contact with T cells is the factor that the astrocyte obtains a typical phenotype of gliosis and causes the release of proinflammatory cytokines (i.e. IL-1 α, IL-6, TNF-α) and matrix metalloproteinases (MMP-3, MMP-9). The high expression of MMPs and their endogenous suppressors (TIMP-3 and TIMP-1) cause MMP/TIMP imbalance. It changes the extracellular matrix [134]. Giraudon et al. showed cytokines secreted by infected glial cells with HTLV-1 are responsible for increasing the expression of TIMP-3, and MMP-3, MMP-9, while MMP-2, TIMP-1, and TIMP-2 remained stable (Figure 7) [135].

Figure 7: The demographic characteristics of the nurses (average score and standard deviation of nurses' clinical competence and moral reasoning)

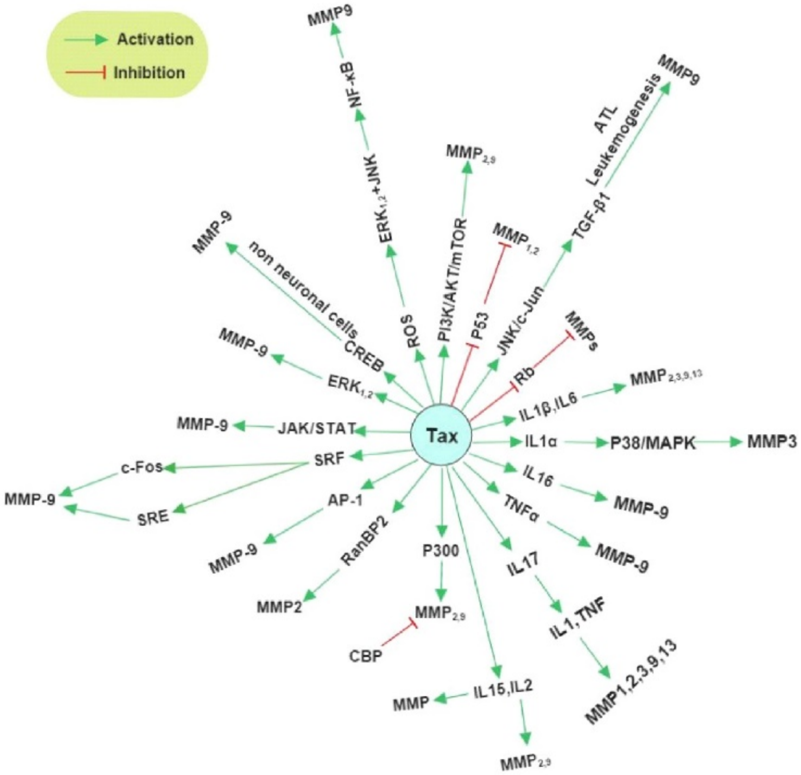
Activation
Inhibition
MMP9
NF-κB
ERK$_{1,2}$+JNK
MMP$_{2,9}$
PI3K/AKT/mTOR
MMP$_{1,2}$
P53
ATL
Leukemogenesis
MMP9
TGF-β1
JNK/c-Jun
MMPs
Rb
MMP-9
non neuronal cells
CREB
ROS
MMP-9
ERK$_{1,2}$
MMP$_{2,3,9,13}$
IL1β,IL6
MMP-9
JAK/STAT
Tax
IL1α
P38/MAPK
MMP3
SRF
c-Fos
MMP-9
SRE
AP-1
IL16
MMP-9
TNFα
MMP-9
MMP-9
RanBP2
P300
IL17
MMP2
MMP$_{2,9}$
CBP
IL1,TNF
MMP1,2,3,9,13
MMP
IL15,IL2
MMP$_{2,9}$

MATRIX METALLOPROTEINASE INHIBITORS

Nowadays, MMPS targeting suppression of the activity of MMPs in the extracellular matrix as a novel anticancer strategy could be reconsidered. TIMPs, which are natural inhibitors of MMPs, at first, were the compounds to be developed for medical research. Hypothetically, TIMPs with potent inhibitory activity specifically can suppress the function of some MMPs which could lead to a useful therapeutic effect. However, their clinical application is still hampered by the absence of an efficient gene delivery style that has restricted the clinical use of this method [136]. Nevertheless, the progress of several types of natural synthetic suppressors of MMPs has been completely adopted and broadly investigated in clinical trials and researched in various cancers during the past decade (Table 3). Generally, synthetic suppressors of MMPs divide into three distinct pharmacologic groups including collagen non-peptidomimetics and peptidomimetics, tetracycline derivatives, and bisphosphonates [137].

THE RELATION BETWEEN INHIBITING OF MAJOR SIGNALING PATHWAYS BY MMP INHIBITORS AND ONCOVIRUSES

As previously mentioned, in HBV infection, HBx controls the transcription induction of MMP-9 which plays a key role in migration as well as metastasis of the tumor via PI-3 K/AKT and ERK pathways. Therefore, these data show that HBx plays a major role in inducing MMP-9 and, in turn, in the invasion and metastasis of cancer via activating ERK and PI-3K signaling pathways [77]. On the other hand, all the findings of one study already implied that 3-azidoWA suppresses 3-azidoWA-mediated MMP-2 expression through the attenuation of internal phospho-ERK as well as phospho-Akt expression in a dose-dependent condition that may have a significant role in the prevention of mouse angiogenesis as a natural inhibitor [138].

Table 3: An overview of the mechanisms deregulation MMPs by oncoviruses

Virus	Viral Factor	Number MMP	Mechanism/Effect	References
EBV	LMP1	MMP-9	Enhanced MMP-9 expression through induction of the MMP9 promoter by recruitment transcription factors AP-1, Sp-1, and NF-kappa B on MMP-9 promoter	[42]
	LMP1	MMP-1, MMP3	Upregulated MMP-1 and MMP-3 expression in NPC tumors	[45]
		MMP-9	Upregulated MMP-9 expression correlation with lymph nodes metastasis and clinical stage	[44]
	Zta	MMP-9, MMP-3	Overexpressed MMP-9 and MMP-3 expression in epithelial cells derived from NPC tumor	[48]
	EBNA3C	MMP-9	The interaction between EBNA3C and Nm23-H1 upregulated expression of MMP-9	[53]
	LMP2A	MMP-9	Upregulated MMP-9 expression by activation of extracellular signal-regulated kinase (ERK)-1/2 and a downstream transcription factor Fra-1	[56]
	Zta		Zta protein also up-regulated TIMP-1 gene expression in EBV-infected B cells and EBV-immortalized LCLs	[49]
HPV	E7	MT-1 MMP	E7 protein of the high-risk HPV types 16 and 8 lead to the induction of MT-1 MMP in in HaCaT cells	[65]
	E2	MMP-9	Increases the expression of MMP-9 in human keratinocytes and organotypic skin cultures	[66]
	E6/E7	MT1-MMP, MMP-2 and MMP-9	Upregulated expression of MT1-MMP, MMP-2 and MMP-9 in cervical cancer	[67]
		MMP-2, MMP-9	Up-Regulates MMP-2 and MMP-9 Expression and Activity by Inducing Interleukin-8 in Lung Adenocarcinomas	[69]
		MMP-8	Persistent oral HPV infection was associated with a low salivary MMP-8 concentration	[71]
		MMP-2, MMP-9, MMP-13 and MT1-MMP	Increased expression of MMP-9, MMP-13 and MT1-MMP in HPV8-positive mice than HPV8-negative animals	[72]
		MMP-2	A significant relationship was observed between the −1306C>T functional polymorphism in MMP-2 gene and HPV infection in increasing the cervical cancer risk	[73]
HBV	HBX	MT1-MMP, MMP-2	Up-regulated MT1-MMP expression by cyclooxygenase-2 (COX-2) activity and activates MMP-2	[76]
	HBX	MMP-9	Increased expression and regulate the transcriptional activaton of MMP-9 by activation of ERK and PI-3K signal pathways	[77]
	HBX	MMP-3	Up-regulated thetranscription,translationandsecretionofMMP-3	[78]
HCV	CORE AND NS5A	MMP-9	Up-regulated expression of MMP-9 with the synergistic effect of endotoxin and proinflammatory cytokines during chronic HCV infection	[103]
	E2	MMP-2	Up-regulation of MMP-2 by interaction between E2 and CD81and E2/CD81 interaction induced the up-regulation of MMP-2 by increasing activator protein-2/DNA binding activity via ERK/MAPK phosphorylation	[104]
	NS3	MMP-9	Activates MMP-9 expression by activates PKD2, JNK, and ERK1/2 to regulate NF-κB which regulates COX-2 expression , finally cox-2 activates mmp-9 expression	[114]
			Activates ERK1/2–p38 signal pathway to regulate NF-κB activity, stimulates the translocation of NF-κB from cytosol to the nucleus and the binding of NF-κB to the MMP-9 promoter	
KSHV	vGPCR and vFLIP	MT1-MMP	Increases of MT-MMP by induced EMT through activation of Notch pathway, leading to gain of MT1-MMP	[120]
		MMP-14	KSHV reduced prox-1 expression(Prox-1 binds to MMP14 promoter and represses its transcription),therefore prevents the repression of MMP-14 by prox-1 and induces mmp-14 expression	[121]
HTLV-1	TAX	MMP-2 MMP-9	Induces the gelatinase activity of MMP-9 and MMP-2 in endothelial cells and downregulates the tissue inhibitor of MMP	[134]
		MMP-9 MMP-3	Increases expression of MMP-9 and MMP-3 bysecreted proinflammatory cytokines (IL-1alpha, IL-6, TNF-alpha) by infected glial cells with HTLV-1	[134, 135]

Moreover, the results of a study by LU et al. provide molecules for the molecular signaling basis of the effects of newly synthesized quinazolinone MJ-29 on the inhibition of metastasis and invasion that could be beneficial as a therapeutic strategy to treat human oral cancer [139]. Accordingly, it can be concluded that 3-azidoWA and MJ-29 as natural MMP-9 inhibitors may have a more effective inhibitory ability on HBV, and HCV-related cancers by attenuating the internal phospho-ERK and phospho-Akt protein expression, which led to the repression of ERK and PI-3K signal pathways.

Other natural compounds such as curcumol via activation of JNK1/2, Akt, and nuclear translocation of NF-κB p65 [140], as well as magnolol through the inhibition of TNF-α by suppressing NF-κB binding activity [141], both caused the reduction of MMP-9 expression in MDA-MB-231 cells, breast cancer cell metastasis, and human urinary bladder cancer 5637 cells, respectively. Meanwhile, there is a possibility that these natural inhibitors can also modulate MMP-9 protein expression through multiple signaling pathways such as JNK, Akt, and TNF-α in HCV-related cancers. Antcin-H, a pure compound that is isolated from A.cinnamomea, can downregulate MMPs through inhibition of the FAK-ERK-C/EBP-/β/c-Fos-MMP-7 pathway, especially MMP-7 expression in renal cancer cell [142] (Table 4).

Table 4: Natural and synthetic inhibitors of MMPs in inhibiting targets of major signaling pathways in various types of cancer

Inhibitor	Class	Selectivity	Type of cancer studied	Inhibiting targets of major signaling pathways	Reference
Batimastat	Peptidomimetic	Broad spectrum	malignant ascites and malignant pleural effusion		[33]
Marimastat	Peptidomimetic	Broad spectrum	Breast cancer, small cell lung cancer, on-small cell lung cancer		[33]
BB-2516	Peptidomimetic	Broad spectrum	Head and neck squamous cell carcinoma (HNSCC)	through interference with autocrine loops in EGFR/c-erbB signaling pathways	[143]
BAY 12-9566	Nonpeptidomimetic inhibitor	MMP-2, MMP -3	advanced ovarian cancer, Breast cancer		[167]
AG3340	Nonpeptidomimetic inhibitor	MMP-2, MMP -3	non-small cell lung cancer		[168]
BMS-275291	Nonpeptidomimetic inhibitor	MMP-2, MMP -9	Non-small-cell lung cancer, prostate cancer, colorectal cancer, Breast cancer		[169, 170]
CGS 27023A	Small molecule	Broad spectrum	Advanced solid cancer		[33]
Col-3 (metastat)	Chemically modified tetracycline	MMP-2, MMP -9	Soft-tissue sarcoma, AIDS-related Kaposi's sarcoma, high-grade gliomas		[171, 172]
Prinomastat	Small molecule	Broad spectrum	Non-small cell lung cancer, , prostate cancer		[136]
Tanomastat	Small molecule	MMP-2, MMP-3, MMP-9	Ovarian cancer, adenocarcinoma of the pancreas, non-small cell lung cancer		[136]
Doxycycline	Tetracycline derivative	Broad spectrum	polycystic ovarian cancer		[136]
SB-3CT	Small molecule	MMP-2, MMP-9	T-cell lymphoma and prostate cancer models		[173]
ND-322	Small molecule	MMP-2/MT1-MMP	melanoma		[174]
curcumol	Major active components in the essential oil of RhizomaCurcumae	MMP-9	Breast cancer cell metastasis	via JNK1/2 and Akt(Ser473)-dependent NFκB signaling pathways	[140]
3 -azidoWA	derivative of Withaferin A	MMP-2	human cervical HeLa and prostate PC-3 cells	concomitant marked reduction in pAkt and pERK signaling by modulating extracellular par-4	[138]
MJ-29	2-phenyl6-pyrrolidinyl-4-quinazolinone derivatives	MMP-2/9	Oral squamous cell carcinoma	Down-regulation of MAPK and AKT Signaling	[139]
antcin-H	pure compound isolated from A. cinnamomea	MMP-7	Renal Cancer Cell	Partly through Inactivation of FAK-ERK-C/EBP-/β/c-Fos-MMP-7 Pathways	[142]
magnolol	the main constituent of Magnoliaceae	MMP-9	human urinary bladder cancer 5637 cells	Inhibition TNF-α-induced MMP-9 expression by suppressing NF-κB binding activities	[141]

The presence of MMP-inhibitory effects in virus-related cancers is still unknown. It has been determined that BB-2516, among all synthetic inhibitors, decreases the protein expression of a broad spectrum of MMPs by interfering with autocrine loops of EGFR/c-erbB signaling pathway in head and neck squamous cell carcinoma (HNSCC) [143]. However, more studies are necessary to understand the connections between MMP inhibitors and these signaling pathways in oncovirus-related cancers, making it especially important to examine how the relationships are.

CONCLUSIONS

In summary, matrix metalloproteinases as cellular proteases play the main role in biological processes as well as pathological conditions, including tissue repair, wound healing, developmental morphogenesis, inflammatory diseases, and tumor progression. Oncoviruses, in many cases, promote tumor progression through multiple mechanisms that affect the expression of these cell proteases. As oncoviruses upregulate the expression of MMPs genes through direct (via DNA binding) as well as indirect interactions (via activating PI-3K/AKT, ERK/MAPK, IFN/JAK/STAT, JNK, NF-κB signaling pathways or immune response), causing escape from the immune system and play a key role in cell proliferation, migration, angiogenesis, EMT, invasion, metastatic features, and subsequent tumor progression. Today, several compounds have been studied as matrix metalloproteinase inhibitors. Various studies have shown that these inhibitors activate the immune system and suppress tumor progression by inhibiting signaling pathways activated by viruses. With these interpretations, these cell proteases may be used as biomarkers to detect different stages of the development of oncovirus-related tumors, soon followed by inhibitors of these proteases in the treatment of viral tumors.

Conflict of interest

The authors report no conflicts of interest.

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Ethics approval and consent to participate

The study protocol was reviewed and approved by the ethics committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1399.520).

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REVIEW

Vascular Changes in Hypertension: Is There Cell Death Involved? – A Narrative Review

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Abstract: *Background: Cardiovascular disease can be triggered and accelerated by hypertension and the underlying changes in the structure and function of arteries. The purpose of this review is to explore some vascular changes that occur in hypertension as a consequence of the imbalance between cell proliferation and cell death, processes that play an important role in stabilizing the thickness of the arterial wall during vascular remodeling. Methods: The authors conducted research through PubMed and Google Scholar databases using the following search formula: (hypertensive*) AND ((vascular modifications)) OR (vascular changes)) AND (cell death). Results: From 40 articles, only 17 publications were included in this study, taking into account four processes that can be preceded or followed by inflammation and depend on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli: Cell proliferation and growth; Cell migration; Cell death; Degradation or reorganization of extracellular matrix. Conclusions: To summarize, maladaptive vascular changes in hypertension can represent a major argument for prompt and maximal therapeutic intervention in patients with high cardiovascular risk. Moreover, they can represent an important step in discovering new markers of cardiovascular risk and in the development of new targeted therapies for different pathways of cellular signaling through which the reversibility of abnormal vascular changes could be obtained.*

Keywords: *cell death; inflammation; cellular metabolism alteration; vascular remodeling; hypertension; pathophysiology*

INTRODUCTION

Cardiovascular disease, one of the leading causes of death worldwide, can be triggered and accelerated by hypertension and the underlying changes in the structure and function of arteries. Knowing the pathophysiological mechanisms that are responsible for the maladaptive changes occurring at the vascular level in the context of hypertension, can represent a first step in the development of new targeted therapies for different pathways through which the reversibility of the abnormal vascular changes can be obtained.

Some studies have shown that hypertension linked to the imbalance between cell death and cell proliferation could explain vascular remodeling: accelerates endothelial apoptosis, promotes cell migration, and accelerates the proliferation of vascular smooth muscle cells [1] resulting in the thickening of the middle tunic of the arteries [1,2]. The role of cell death in blood pressure charge is still unknown [3]. The prevalence of hypertension increases with age and the proposed pathophysiological mechanisms are represented by hemodynamic changes, arterial stiffness (due to elastic lamina disruption and intimal hyperplasia), and neurohormonal and renal dysfunction [4]. In the elderly, vascular injuries can heal through the formation of vascular scars due to the increase in the proliferation of smooth muscle cells and the reduction of their apoptosis and decrease in sensitivity to apoptotic stimuli in fibroblasts and myofibroblasts, with secondary fibrosis [5,6].

In terms of the cell death process, some major pathways have been described (apoptosis, necrosis, and autophagy), depending on the injury and the type of cell [7]. Programmed cell death and necrotic cell death sometimes have mechanisms that can be overlapped [5]. The initiation of apoptosis is the result of death signaling pathways, intrinsic (mechanisms triggered by cellular stress through hypoxia, oxidative stress, and ADN damage) or extrinsic (mediated by transmembrane receptors of the TNF receptors family). In apoptosis, an essential process in maintaining tissue homeostasis, as a result of caspase cascade activation and imbalance between pro and antiapoptotic factors, the cell is broken for-ming membrane-bound vesicles and apoptotic bodies (after the shrinkage of the cell, fragmentation of nucleus and chromatin and plasma membrane blebbing [7]) responsible for clearance of dead cells and also for intercellular communication [8]. Necrosis is an accidental form of cell death but in certain instances is initiated or modulated under programmed control mechanisms. Unlike apoptosis, which requires energy consumption, necrosis is a process in which cells die without energy consumption; in the necrotic cell death process, as a result of the breaking of the cell membrane and the elimination of the cell content in the extra-cellular space, inflammation occurs. It has been described as a form of programmed necrosis (necroptosis), caspase-independent, with mitochondrial changes involvement [5]. Another type of cell death, autophagy, is the process through a staving or deprivation of growth factors cell generate energy and metabolites by digesting its organelles or macromolecules. There were also described multiple sub-types of cell death, depending on caspase involvement or the presence of some of the necrosis criteria: aponecrosis, paraptosis, anoikis, cornification, etc [5].

Recent studies examine how certain factors affect endothelial apoptosis in patients with hypertension and other comorbidities [1,4,9]. Endothelial cell apoptosis was found in patients with hypertension and peripheral arterial disease by studying endothelial cell cultures [9]. Vascular cell re-modeling, an active process, involves changes in at least 4 cellular processes: cell growth and cell migration, inflammation, cell death, and degradation or reorganization of the extracellular matrix. It is dependent on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli [10].

Although the in vitro studies [11] demonstrated a relationship between the level of some plasma membrane receptors involved in apoptosis induction (named death receptors) [12] and the phenomenon of apoptosis, the causal link between cell death and vascular changes was not proved yet [11].

Considering that the phenomenon of cell death and cardiovascular outcomes are linked through metabolic risk factors intervention and exogenous causes of cellular stress that lead to cell destruction via different pathways and demonstrating to what extent the alteration of cellular signaling, the alteration of the extracellular environment and the triggering of cell death mechanisms are responsible for the abnormal remodeling of the arterial wall in the hypertensive patients, the research could be directed to discover new markers of cardiovascular risk and new therapies that can be targeted in patients with cardiovascular risk factors. The benefit could be major, the measurement of death receptors levels related to cell death phenomena that can predict cardiovascular outcomes and death [11], in patients with high cardiovascular risk, became a major argument for prompt and maximal therapeutic intervention in this specific populational group.

The purpose of this review is to explore some vascular changes that occur in hypertension as a consequence of an imbalance between cell proliferation and cell death, processes that play an important role in stabilizing the thickness of the arterial wall during vascular remodeling.

MATERIALS AND METHODS

A comprehensive search through PubMed and Google Scholar using the following search formula: (hypertension*) AND ((vascular modifications)) OR (vascular changes)) AND (cell death) was performed. Only full-text freely available articles published in English between 2000 and 2021 were used. Articles that were most relevant to the subject of this review were included after thorough consideration. Inclusion criteria were published in English, publication within the mentioned period, and inclusion of hypertensive models or animal models, or cell cultures studies. From 40 articles, only 17 publications were included in this study. All the selected papers were reviewed by an independent reviewer to check that the interpretations were correct.

Hypertension has a great impact on the cardiovascular system; the thickness of the arterial wall during the remodeling process could be the result of cell death mechanisms triggered by factors potentially involved in the etiology of arterial hypertension. Considering that the connection between cell proliferation and cell death is responsible by a vascular re-modeling process in hypertension, we take into account for our study four processes that can be preceded or followed by inflammation and depend on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli:
- Cell proliferation and growth
- Cell migration
- Cell death
- Degradation or reorganization of extracellular matrix

RESULTS

The search strategy identified 40 articles of which 17 were deemed to be eligible for inclusion in this systematic review. Of these, 12 studies addressed markers of cell prolifera-tion/growth, 7 studies addressed cell migration, 12 studies addressed cell death processes/markers, 7 studies focused on the degradation of extracellular matrix, and 13 studies addressed inflammation markers (shown in Table 1).

Table 1: Type of processes involved in vascular remodeling

Reference	First author, year of publishing	Study type	Pathophysiological mechanisms, Type of process					
			Arterial remodelling	**Inflammation**	**Cell proliferation/ cell growth**	**Cell migration**	**Cell death**	**Reorganization/ degradation of extracellular matrix**
1	Brown, I.A.M *et al,* 2018	Review	X	X	VSMC hypertrophy and hyperplasia	X	X	
2	Xu, C. *et al,* 2001	Experimental study	X		X		X	
16	Martinez-Quinones, P. *et al,* 2018	Review	X	X	VSMC hypertrophy and hyperplasia	X		X
10	Renna, N.F. *et al,* 2013	Review	X	X	X	X	X	X
21	Schiffrin, E.L. 2012	Review	X	X	VSMC hypertrophy and hyperplasia		X (apoptosis; anoikis – in endotelial cells)	X
19	Virdis, A. *et al*, 2014	Review	X	X	X	X		
15	Sun, H.J *et al,* 2020	Review	X			X		X
18	Munoz-Durango, N. *et al,* 2016	Review	X	X	X	X	X	X
17	Bruno, R.M. *et al*, 2017	Research article	X	X	X		X (apoptosis)	
3	Abdelbary, M. *et al*, 2019	Comparative study		X	X		X (necrosis, apoptosis)	
22	Intengan, H.D.; Schiffrin, E.L. , 2001	Review	X	X	X		X (apoptosis)	X
9	Gardner, A.W. *et al*, 2019	Randomized Controlled Trial		X			X (apoptosis)	
26	Krishna, S. M. *et al*, 2021	Randomized Controlled Trial	X	X			X (apoptosis)	X
27	Fekete, A. A. *et al*, 2016	Randomized Controlled Trial	X	X				
28	Li, X. *et al*, 2022	Review	X	X	X	X	X (apoptosis)	X
32	Xu, W.;Erzurum, S.C., 2011	Review	X		X		X (apoptosis)	
33	Levy, M. *et al*, 2007	Clinical research	X	X	X		X (apoptosis)	

In hypertension, the connection between cell proliferation and cell death could be critical in determining the thickness of the arterial wall during the remodeling process. There are 2 types of criteria for classifying cell remodeling:

- according to the size of the middle tunic: hypertrophic, eutrophic, and hypotrophic
- according to the size of the lumen: internal and external remodeling.

The consequences of cell remodeling are:

- high pulse pressure,
- stiffening of the arterial wall
- changes in the extracellular matrix.

All consequences of cell remodeling are determined by the release of growth factors and cytokines. The immune system, namely T lymphocytes, plays a key role in remodeling, more precisely based on a T-reg/T-effector imbalance.

Because of its ability to modify the vascular tone, vascular endothelium plays a significant role in hypertension. Endothelial dysfunction is caused by a reduction in nitric oxide (NO) availability, which causes endothelial dysfunction. The main cause of the decline in NO is the excessive production of reactive oxygen species (ROS)[13].

Blood pressure is affected by the phenomenon of cell death that can occur at different levels of the arterial wall, however, in hypertension the role of different types of vascular cell death is unclear. Researchers discovered that vascular smooth muscle cell growth is increased by cell death [1]. The cell death process is thought to play a key part in remodeling, but it is unclear whether it is the main process or a growth-related compensatory mechanism, being influenced by reactive oxygen species, NO, angiotensin type 2 (AT-II), and endothelins.

Due to vascular damage, elevated blood pressure values may alter the integrity of the endothelium and may also represent a risk factor for the onset and progression of vascular atherosclerosis changes leading to the destabilization of atheroma plaques [14]. Atherosclerosis favors the processes of inflammation and necrosis of the blood vessel and plaque can become unstable without proper treatment and cause even more complications.

DISCUSSION

In our study, we collected a lot of information about vascular modifications in hypertension. From the 17 selected articles, in 15 we found information about vascular remodeling (88.23%), in 14 articles we found arguments for the presence of the inflammatory phenomenon (82.35%), 13 articles (77.47%) argued for the presence of cell death and also 13 described cell proliferation, 7 articles (41.17%) described the phenomenon of cell migration, and 8 articles (47.05%) described reorganization of the extracellular matrix.

Starting from the information provided by the articles included in our study, our hypothesis related to the involvement of cell death at the vascular level in arterial hypertension seems to be valid. Maintaining a balance between the processes of cell proliferation, cell migration, cell death, and degradation of the extracellular matrix could ensure adequate vascular elasticity and delay the installation of specific hypertensive vascular changes. It seems that cells from all layers of the vascular wall are involved and can be affected by different types of death, the damage to each cell type leading to cellular metabolic changes and changes in the extracellular environment.

Vascular tone and vascular remodeling

The endothelial cells which form the tunica interna of the arterial wall, are essential for regulating hemostasis, vascular tone, and host defense [15]. Secretion, protection as a barrier, and local metabolism are the endothelium's three primary activities, each of which is altered by hypertension. The endothelial cells produce vasoactive substances such as nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H2S)[15], prostacyclin (PGI2), endothelin 1 (ET-1), AT-II, prostaglandins. Normally, there is an equilibrium between these substances to maintain proper blood flow. In hypertension, at the vascular level is an increased production of vasoconstrictor factors (ET-1, AT-II, prostaglandins) which determine endothelium dysfunction (increased permeability, induce inflammation, and edema). It was discovered that the substances and structures that are released from a cell when it dies can affect the endothelial function in an autocrine, paracrine or endocrine model [16].

The endothelium is important for both short-term vascular tone modulation and long-term vascular remodeling. Nitric oxide is the most significant endothelium-derived vasodilator molecule which, under healthy conditions, is capable of inhibiting the primary fundamental pathways driving the formation of atherosclerosis and thereby improving vascular health. Reduced NO availability and endothelial dysfunction are caused by NO breakdown by reactive oxygen species (ROS) in physiological aging and many pathological diseases, including arterial hypertension.

Essential hypertension patients (HT) had a lower endothelial response to acetylcholine (Ach) than normotensive people (NT) in each age group, but it was proved that the vascular response to Ach in both HT and NT patients decreases as they become older [17]. In the pathogenesis of endothelial dysfunction and the cardiovascular consequences related to endothelial inflammation [18], apart from the effects of nitric oxide and carbon monoxide, the role of hydrogen sulfide has been also described. H2S formation was detected in smooth muscle cells, vascular endothelial cells, and cardiomyocytes and it was shown that H2S could protect endothelial cells against apoptosis by attenuating the overproduction of ROS and inhibiting mitochondrial apoptosis [15].

The vascular smooth muscle cells (VSMCs) that form tunica media are responsible for vasoconstriction, vasodilatation, and the process of remodeling [19]. The hyperplasia and hypertrophy of VSMCs are the processes that underlie vascular remodeling. The most frequent type of remodeling in hypertension is the inward type causing the narrowing of the arterial lumen. The outward type of remodeling occurs during anti-hypertensive treatment and is also caused by increased blood flow[16]. Some studies have revealed that a marked cell death influences and accelerates the proliferation of vascular smooth muscle cells, resulting in the thickening of the middle tunic of the arteries [2]. As a result of cell death through necrosis and the breakdown of plasma membrane integrity, the contents of necrotic cells are released, triggering a proinflammatory response [3].

Fibroblasts that form the tunica externa secrete chemokines, cytokines, and growth factors that contribute to normal vascular function and participate in the degradation of the extracellular matrix components. They play an important role in remodeling as a response to injury. However, fibroblasts suffer structural changes that are accompanied by proliferation and migration into the tunica media during hypertension [16].

Cell remodeling

Cell remodeling involves cell growth and cell migration, cell death, and degradation of the extracellular matrix and is dependent on multiple factors [10]. The media-to-lumen ratio (M/L), modifying the vessel wall width for increasing muscle mass, the reorganization of cellular and noncellular elements and the lumen dimensions are the most prominent manifestations of these adaptations [20]. As a result, remodeling can be classified based on the degree of change of the medium arterial tunic in hypertrophic, which is the most common, hypotrophic, or eutrophic remodeling, as well as the size of the lumen in internal or exterior remodeling [21]. With age, large arteries undergo outward hypertrophic remodeling and stiffening [4], and in hypertension, this process may be accelerated, resulting in higher pulse pressure [22-24]. The elastic laminae, on the other hand, develop multiplication and fragmentation in severe hypertension, with increased deposition of collagen and fetal fibronectin, leading to greater stiffness [23]. Small artery remodeling has typically been associated with increased media thickness in hypertension, but new studies have shown that there are two forms of remodeling, inward eutrophic and inward hypertrophic, depending on whether the media cross-sectional area is enlarged [23]. The discovery that inward eutrophic remodeling may be prominent in some kinds of hypertension has raised the prospect that countervailing mechanisms in hypertension, including apoptosis, may compensate for or modify growth [23,25]. The outer and lumen diameters are decreased, the media cross-sectional area is unchanged and the M/L is increased without stiffening in inward eutrophic remodeling [25]. Inward eutrophic remodeling reduces the outer diameter of the vessel through a mix of growth and apoptosis, with apoptosis concentrated to the periphery, whilst inward growth decreases lumen diameter, which may explain the preservation of media volume. It is uncertain whether apoptosis is a growth-related compensatory mechanism or a primary process[25]. Mechanical forces exerted on the arterial wall also play a significant role in remodeling. Changes in the extracellular matrix, the cellular release of growth factors and cytokines, and vascular sensitivity to circulating humoral factors are all caused by them. Force duration, load, and force properties all have an impact on all of these factors [1].

Low-grade inflammation

In hypertension and cardiovascular disease, low-grade inflammation plays a critical pathogenic function [26-28]. Innate and adaptive immune systems, specifically T cells, are implicated, according to a significant body of research. T-lymphocytes play a key role in the development of hyper-tension-related vascular remodeling. The balance between T-effector and Treg lymphocytes is a critical regulatory system that, when disrupted, favors blood pressure increase and the development of organ damage [21]. Treg lymphocytes have potent anti-inflammatory characteristics, ensuring vascular homeostasis and protecting the arterial wall against the onset of atherosclerosis [21,29]. In humans, the majority of data establishing essential hypertension as a chronic low-grade inflammatory state found a clear and independent link between c-reactive protein (CRP), tumor necrosis factor (TNF), Interleukin 1 beta (IL-1 beta)[30], Interleukin-6 (IL-6), and adhesion molecules and vascular alterations in essential hypertensive patients [21].

An important role in the process of vascular remodeling is played by the renin-angiotensin-aldosterone system (RAAS)[29,31] because of its role in VSMC migration and inflammation [24,32]. This system acts through 2 types of angiotensin II receptors AT1-R and AT2-R, but the AT1-R is thought to be the primary receptor that causes Ang II's proliferative and profibrotic actions [20,33]. The AT2-Rs are thought to cause opposite effects, preventing vascular deterioration. But there are times when AT2-R acts similarly to AT1-R (for example, in renovascular hypertension it causes tonic renal medullary vasoconstriction) [32,34]. Recent studies showed that there exists an AT1-R autoantibody (AT1-AA) which is an agonist of AT1-R. AT1-AA raises AT2-R expression and encourages cell migration through cytoskeletal remodeling [34]. The RAAS is implicated in physiological and pathological actions when it comes to vascular changes. It reduces ROS generation and modifies vascular tone via vasoconstriction and vasodilatation. During hypertension, this RAA system induces fibroblast proliferation, collagen deposition, and vascular hypertrophy [20].

Cell death

Apoptosis is a type of gene-controlled cell death that was first discovered in development as a mechanism for fine-tuning growth. The vascular system has a large number of apoptosis modulators. Reactive oxygen species, NO, angiotensin type 2 receptors, and the endothelin system are all possible candidates. Reactive oxygen species play a key role in the pathogenesis of hypertension and vascular cell apoptosis [35], with O_2^- inducing proliferation and H_2O_2 inducing apoptosis through a protein kinase C–dependent pathway, respectively. Angiotensin II is also a potential apoptotic trigger [25,31,36].

Recent studies examine how certain factors affect endothelial apoptosis in patients with hypertension and other comorbidities. Endothelial cell apoptosis was found in patients with hypertension and peripheral arterial disease (PAD) by studying endothelial cell cultures. They demonstrated that physical activity can lower this type of cell death[9]. PAD patients present increased cardiovascular stress, inflammation, and oxidative stress. Inflammation and oxidative stress speed up the progression of myopathy and increase the risk of apoptosis and sarcopenia. In older persons, exercise decreases oxidative stress and improves reactivity to oxidative processes [9].

Inflammation, VSMC apoptosis, and oxidative stress in patients with hypertension [1,18,19] can also play an important role in the pathology of abdominal aortic aneurysm AAA [37,38]. According to some studies [39] a member of the seric proteinase inhibitor superfamily, Human Kallistatin, acts as a protective factor by blocking the pathway that stimulates AAA generation [39], and even whey-protein supplementation has a beneficial effect, improving vascular function by inhibiting the serum angiotensin-converting enzyme [40].

Lesions that appear on the arterial wall and the dysfunction of endothelial cells determined by high blood pressure values are factors that contribute to atherosclerosis. In the injured artery intima, extra low-density lipoprotein (LDL) accumulated suffer processes of oxidation and can attract monocytes and macrophages, forming the foam cells. The local inflammatory reaction is severe [41] and it triggers a cascade of events [38,42]. Foam cell secretion, deposition, and apoptosis [43] are crucial steps in the occurrence of atheroma plaque [44]. In advanced stages, the macrophage function of phagocytosis is destabilized, the atheromatous plaque continues to grow and, in the end, it will become unstable, promoting local inflammation and necrosis without the proper treatment [41]. Studies have revealed that at the cellular level microRNA plays a role in the control of atherosclerosis [41,45], acting on the TGF-β signaling pathway, inducing proliferation, migration, and apoptosis of endothelial cells, VSMCs, and macrophages [37,43,46].

Sometimes, associated with long-term uncontrolled hypertension and left ventricular dysfunction (or other conditions such as left heart disease, thromboembolic pulmonary condition, lung disease, liver disease, hematologic disease, metabolic disease, connective tissue disease, human immunodeficiency virus infection, thyroid diseases, etc.), pulmonary hypertension may occur [47,48]. In this situation, through the lungs arteries that have a reduced lumen and thickened walls, blood will flow slowly. Pulmonary arterial hypertension (PAH) is defined by mean pulmonary arterial pressure (mPAP)>20 mmHg at rest assessed by right heart catheterization [47-49]. Histopathological changes at the pulmonary level are neointima formation in pulmonary arteries, wall thickness, and plexiform lesions formed by the proliferation of endothelial cells. There is an increased level of ROS that contributes to oxidative stress and vascular remodeling [50], similar to the changes found in the arteries of hypertensive patients. It has been discovered that there is a deficient activity of NO-synthase, the level of NO is low and so are the vasodilatory effects. The role of proapoptotic and antiapoptotic proteins has been incriminated in the initiation of the pathogenesis of PAH and the proliferation of apoptosis-resistant vascular cells [51,52]. Both reversible and irreversible PAH express the apoptotic markers (caspase-3 and p-53) [51,53], while irreversible PAH expresses BCL-2 only in severely wounded endothelial cells [51]. Apoptosis plays an important role in pulmonary hypertension among the proliferation of VCMCs and plexiform lesions [51,52].

In our study we highlighted some evidence in favor of the hypothesis that cell death is involved and has consequences on vascular changes in people with arterial hypertension; the vascular changes occurring in the hypertensive patient may be due to the alteration of the cellular metabolism, which may represent a triggering factor of cell death processes by activating abnormal cellular signaling pathways. However, there are insufficient data to show the cell death types involved and the types of cells in the vascular walls involved in the process of vascular remodeling, therefore we consider that further studies are necessary to elucidate these aspects

CONCLUSIONS

Cell death and cardiovascular outcomes seem to be linked through metabolic risk factors intervention and exogenous causes of cellular stress. Demonstrating that alteration of cellular signaling, the alteration of the extracellular environment, and abnormal cell death mechanisms are responsible for the abnormal remodeling of the arterial wall in hypertensive patients could direct future research toward the identification of new molecules as plasma membrane receptors involved in apoptosis and new therapies that can be targeted in patients with cardiovascular risk factors. Once the vascular impact of imbalances between cell proliferation and cell death will be demonstrated, its early detection could become an argument for maximum therapeutic intervention in this population group.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution
EB conceived of the presented idea. AMD, EB contributed to documentation on the topic of interest, work conception and revised the manuscript. EB, ŞSB, OA performed the final revision of the manuscript. All authors discussed the results and contributed to the final manuscript. ŞSB and OA gave final approval. All authors have read and agreed to the published version of the manuscript. EB, ŞSB, OA had equal contributions.

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ORIGINAL ARTICLE

Is Still Neurology Desirable as a Specialty for Aspiring Physicians?

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Abstract: *This article aims to highlight the burnout burden among neurologists and provide information on some of its causes and consequences. We report an original article that reviews our current understanding of burnout matter in the neurology field. The main search engine through which we performed extensive literature research was PubMed, Scopus, and Google Scholar. The following information was collected: triggers of burnout and factors that perpetuate it, major complaints, values, and goals that dictate how coaching with it. Physician burnout is an umbrella term characterized by symptoms determined by exposure to chronic workplace stressors. Simply put, it encompasses many dimensions: emotional exhaustion, feelings of cynism, detachment (depersonalization), and a sense of ineffectiveness at work (low personal accomplishment). Burnout is common among all medical specialties, but neurology ranks at the top of the list. Several identified factors continue to fuel this problem. Properly addressing them, rethinking practice, and implementing wise, modern strategies can make significant contributions to reducing it. Aside from the chronic harm to the physician, burnout puts at risk the healthcare community at many levels including patients, other professionals, finances, etc. This paper wants to emphasize that burnout in neurology should be regarded as a global crisis. Thus, some strategies are proposed.*

Keywords: *neurology, burnout, work-life, emotional exhaustion, stress, fatigue, depersonalization, second-victim phenomenon, complaints, functional neurology*

INTRODUCTION

Physician burnout is an umbrella term characterized by symptoms in response to prolonged exposure to chronic stressors related to the workplace. This topic attracted interest from researchers since it exhibits a concerning prevalence. In the field of medicine, we can consider burnout a pandemic that has a negative impact both on doctors and their patients, but most of the time this reaches far beyond that, to families and the overall healthcare system. All medical specialties are affected, but some of them carry a higher percentage of burnout. Over 40% of general physicians experienced at least one symptom of burnout, neurology being ranked the second specialty affected, with more than 60% of neurologists signaling symptoms [1,2]. Medscape 2022 report on Physician Burnout and Depression showed that 47% of neurology specialists are experiencing mental health challenges, which represents a substantial increase in its prevalence compared with the previous year's reports when it was 42% [3]. The purpose of this article is to highlight the burnout burden among neurologists and provide information on some of its causes and consequences.

BURNOUT AND NEUROLOGY

To date, many research studies have contributed to a comprehensive overview and understanding of the root causes that fuel physician burnout.

The prevalence of neurological disorders increased in the last two centuries and the increased burden that comes along can be attributed to explosive population growth, people reaching older ages, unhealthy lifestyles, and unawareness of the importance of prophylactic measures [4].

In opposition to this growth, the global shortage of neurologists led to inequalities in the availability of patient care with those already existing clinicians being overwhelmed. The Association of American Medical Colleges biannually investigates the number of patients per active physician. Reports published two years ago indicated that the number was higher in neurology (23.429) than in ophthalmology (17.162), cardiovascular diseases (14.717), radiology (11.896), psychiatry (8.544), emergency medicine (7.332), internal medicine (2.758), and is approaching specialties with the highest burnout rates such us urology (32.490) [5]. Thus, the low number of practicing neurologists per patient increases the amount of work, compromises patient care, and exacerbates burnout.

Switching to professional working hours per week, an interesting study concluded that neurologists worked 57.1 hours per week, with 42.3 hours being spent performing inpatient activities. In our neurology department, the average number of professional working hours per week is 59, with a peak of 100 on rare occasions (absenteeism of other colleagues because of medical conditions/vacations or lack of staff) [6].

From a holistic point of view, burnout can be regarded as an indicator of quality and duration of life, given that it has a direct damaging effect on health: overnight shifts, exposure to all kinds of infective agents, irregular and exaggerated effort, lack or inadequate physical exercise, emotional detachment, interrupted meals or maladaptive coping strategies such as excessive caffeine intake or other power drinks, alcohol, and drug abuse. Johann Ludwig Casper, a prominent name in forensic medicine stated that there is no profession with a comparable physical and mental force demand as the medical one, an observation comparable with recent studies [7]. It is also worth mentioning that being on call requires working very fast or accomplishing more tasks being short on time. Very similar ideas are presented in a meta-analysis of 17 studies that in people with high demands, the risk of mortality is 20% higher than in the reference group [8]. Similar opinions are found in a study involving 1.671 American neurologists, where 60.1% of the responders had at least one symptom of burnout [2].

According to the World Health Organisation and International Classification of Diseases, burnout has become 2019 a legitimate medical disorder and has been given its ICD-10 code (Z73.0 - Burn-out state of vital exhaustion). Burnout is a state of chronic stress, that occurs after the body system is repeatedly triggered. It can exacerbate mental conditions like anxiety disorders, depression, and sleep problems. Some authors focused on the cortisol secretion parameters and concluded burnout is a mental syndrome with unique pathophysiology [9]. Moreover, as it often happens, the presence of a disease leads to a higher chance for another one to develop. Burnout influences not only subjective well-being but other biological processes as well. In a large study of nearly 9000 employed adults, was found that burnout is a significant risk factor for developing coronary heart disease and finally, heart attacks [10]. These findings support the idea that physicians with high demands as neurologists are exposed to challenges regarding their health and are more vulnerable to stress and burnout.

There is an excessive workload and clerical duties, including finishing notes, phone calls, ordering tests and reviewing results, responding to phone calls and patient requests, and prescribing medication. Assessing indicators related to burnout in a national survey on 9561 neurology residents and fellows after studying 354 responses to the survey the following was revealed: regarding the amount of time spent on clerical tasks directly related to patient care" only 26.2% of residents and 29.7% of fellows agreed that it is reasonable [2]. Another particular challenge is finding meaning in work and this often translates to helping others. Some neurological disorders do not come with treatments or cures or are associated with a significant burden of disability. In the same study, in the matter of, satisfaction with the job" only 68.9% of residents and 63.1% of fellows were more likely to be satisfied with their career choice and in line with, work being meaningful", 78.8 % of residents and 85.6% of fellows had positive feelings towards their job [11]. In comparison to attending physicians, an important number of neurology residents experience burnout due to extra workload and the challenges that come with a new environment, 73% of residents mentioned at least one symptom from the constellation of burnout. Furthermore, both residents and practicing physicians are affected by the second-victim phenomenon, which refers to feelings of guilt caused by medical errors which result in poor patient outcomes [12,13].

Some authors focused instead on sources and outcomes of burnout, on potential interventions to treat it or to prevent the onset. It must be mentioned that burnout is a consequence of systemic deficiencies rather than a result of poor uncoping skills. Increasing trainee resilience and cutting clerical tasks through hiring administrative workers or through using user-friendly technology [14], positive reinforcement and support during residency, and implementing regular burnout evaluations such as Maslach Burnout Inventory. The last one mentioned is the most widely accepted evaluation of burnout and outlines three key aspects of this response: the state of feeling emotionally worn out, feelings of cynicism toward work, and a personal sense of inefficiency in the workplace [15].

The supply-and-demand issue that perpetuates burnout and challenges patient care in neurology could be overcome by recruiting other medical staff. For example, nurse practitioners could get in charge of some medical tasks, without actually exceeding their abilities, as the healthcare oath foremost promotes abstaining from all intentional wrong-doing and harm. This topic with potential usefulness has already been addressed in an article published in the JAMA journal, but it aroused a lot of controversies [16]. Most complaints came from those in need of help, like physicians, stating that to figure out the pathology, prove competency, and be able to give medical assistance one has to go for over a decade in training. This amount of time is spent in school only by doctors, to fully become licensed, and no other alternative shorter programs can replace this training. But maybe they can approach it. An answer to this problem could be by enhancing nonphysician practitioners in medical student courses or by attending residents, giving them templates and free access to educational programs. In conjunction with this, in the UK for example, specialist nurses are known for their crucial role. They can choose and qualify for various neurological conditions (multiple sclerosis, Parkinson's disease, and epilepsy). Sometimes they can order tests, administer basic treatments, and in some states even prescribe medications.

Another indicator of burnout and shortage of neurologists is also projected by the difficulty in making an appointment for new patients and by lengthy waiting lists. Data from 626.000 first appointments scheduled in 2016 with 1.044 providers concluded that wait times for new patients are longer for high-demand specialties, with neurology patients waiting approximately 32.3 days for first appointments [17]. This is particularly relevant since waiting for more than a month often determines patients to cancel their appointments, and never return to the clinic with consequences resulting from delayed diagnosis and treatment. Moreover, significant information was revealed by 2021 research on NHS data conducted by Neurological Alliance – a coalition with over 80 organizations including the MS Society, Parkinson's UK, and MND Association. Thus, they showed that by the end of March 2021, over 150.000 people were waiting for an assessment by a specialist and over 10.000 of these have been waiting for over a year [18]. These numbers indicate how overwhelmed are the neurology services, putting a lot of pressure on existing physicians, and prolonging a state of excessive stress and fatigue that is burnout.

As a consequence of different workplace stressors and poor coping mechanisms, it was estimated that 10-15% of doctors will develop a problem with substance addiction at some point in their careers [19]. Regarding the neurology field, in a published survey among all specialties, answers from 470 chronic pain/neurology subjects as well, it was shown that in this category of physicians there is a prevalence of 3.7% marijuana use. The issue is not limited to illegal drugs, pain medicine, and alcohol count as well. The anonymous questionnaire indicated a prevalence of 88.6% of alcohol use in physicians from the category of chronic pain/neurology, also being the group with the highest prevalence for benzodiazepine of 15.5%. In addition to this, they found 16.3% prevalence for minor opiates and 1.9% for major opiates, hence being ranked the third specialty. Neurologists frequently come across chronic pain patients in need of benzodiazepines and opiates and somehow this exposes them to these drugs. They ranked second only to psychiatrists in the matter of the risk of using benzodiazepines [20].

Many neurologic diseases require interdisciplinary care and this sometimes creates the setting for conflict between providers offering healthcare services [21]. To reduce the burnout burden, neurologists should try to understand and develop the concept of teamwork. Literature describing the issue of individual vs. teamwork in neurology is scarce, but limited evidence suggests there are more benefits from the adoption of a teamwork approach, where feasible. By avoiding professional isolation, rethinking professional roles, and abandoning territorial behavior.

Receiving complaints is another issue that lies at the heart of burnout in neurology as well. Many of these complaints arise from the limits that go along with disabling or cureless illnesses, giving a false feeling that the healthcare workers did not do anything possible to resolve the case. This situation can be overcome by improving the informative part of the medical act with better communication skills with the patient and through offering honest expectations for patients and their families. Moreover, it is not uncommon that patients to have a low level of medical education and not understand the limits of medicine, priorities, or medical procedures, so this is another important factor that promotes complaints [22,23].

We propose some strategies for the prevention of burnout on neurology specialists (Table 1). There are three categories of factors: related to patient education, related to the workplace, and the healthcare system, and factors related to patient education. An important contributor that fuels burnout is the offering of patients by medical professionals who come across neurological diseases in their practice and whose residency programs do not provide minimum knowledge. Many of these patients have functional neurological disorders which do not always require neurologist interventions. The same problem is with incidental findings on brain imaging such as minimal white matter changes with the improbability of multiple sclerosis or other significant CNS diseases. Poor medical knowledge, not understanding the limits of medicine, medical priorities, or investigations are factors of complaints between relatives. Time-consuming work, the complexity of neurological diseases, communicating a difficult diagnosis, or spending more time doing bureaucratic tasks than caring for patients, all of these are frustrating. Adequate working conditions, generous salaries, creation of functional interdisciplinary teams, filling vacancies, and introduction of telemedicine and artificial intelligence in the field of neurology, would be some solutions for attracting young doctors to this specialty.

Table 1: Significant factors contributing to burnout among neurologists and strategies for prevention

Main factors related to burnout	Proposed solutions
Factors related to the patient education	
Many referrals to neurology that do not need neurology intervention Eg: Functional neurology (often, patients with functional conditions tend to show more dissatisfaction) [22].	Educational initiatives/ online courses targeting other medical professionals that send patients and have minimal to no knowledge of common neurological diseases. Early neuroscience curricula in medical school.
The pandemic of cardiovascular risk factors: high blood pressure and cholesterol, obesity, smoking, and poor diet - put a person at high risk for neurological diseases as well.	Preventive medicine. Screening programs and campaigns to raise awareness.
Factors related to the workplace and healthcare system	
Complaints from patients/families arise from: - the poor medical knowledge - not understand the limits of medicine, medical priorities, or investigations (23)	Legal assistance for the doctors involved.
Shortage of neurologists because of not choosing the specialty [24].	Programs in medical school promote and value neurology. Adequate salary.
Neurologists choose urban over rural settings [25].	Improving rural health settings. Providing medical tools/gadgets/making technology available so the rural residents have equal opportunities to receive medical treatment.
The complexity of neurological diseases creates a job mismatch.	Development of team-based neurologic care Increased need for subspecialty medical care Eg: In Romania, there are no specialists in movement disorders or neuro-ophthalmology
Responsibility fatigue at the workplace.	The schedule of work must be dependent on the complexity of the tasks.
The neutral attitude of the hospital management board [26].	Providing support, understanding human needs, expand vacation policies.
Many rescheduling of appointments decreases physician and healthcare system efficiency overall.	Telemedicine and e-consults increase the capacity to respond to the demand for care and help patients with difficulties in coming to their clinic visits [27].
Mounds of paperwork - spending more time doing bureaucratic tasks than caring for patients. Lack of access to medical records - especially in older patients with many investigations.	Digitalization and artificial intelligence are the future of healthcare. Enabling tracking system for ordered tests/ follow-ups.
Time-consuming tasks. Eg: documenting a history of falls in Parkinson	Artificial intelligence Eg: smartwatches or other wearables that quantify falls or periods of inactivity and already provide a pattern or a conclusion.
Factors related to the physician	
Communicating a difficult diagnosis (often the first interaction occurs already at the time of critical illness).	Programs/Training courses that will help doctors improve their medical communication with patients.
The emotional burden of the specialty itself.	Providing necessary psychological assistance to guide neurologists facing mental health problems like depression or anxiety.

CONCLUSIONS

Overall, burnout can be regarded as a matter of specialty, and it has built up slowly over time, creating a vicious cycle, perpetuated by workforce dropout or choosing other medical specialties with a better lifestyle. Identification of burnout effects in neurology is policy relevant for some reasons: it affects the overall healthcare system with all its components, work-life integration (family), emotional and physical well-being, and social engagement. To conclude, it must be regarded as a worldwide issue.

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REVIEW

Prognostic Value of Serum Biomarkers in Secondary Peritonitis - A Literature Review

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Abstract: *Background: Infection and sepsis represent major complications after abdominal surgery for secondary peritonitis. Finding new tools that can help identify patients at risk for developing postoperative complications is of the utmost importance for clinicians. We tried to evaluate the use of different biomarkers in evaluating the postoperative prognosis of patients with secondary peritonitis. Methods: We searched the available literature on the usefulness of serum biomarkers in evaluating prognosis in patients with secondary peritonitis. Results: Elevated postoperative lactate levels and high procalcitonin (PCT) levels were associated with negative outcomes. C-reactive protein values increase rapidly in response to the surgical insult but have no value in assessing the overall prognosis. Conclusion: Procalcitonin (PCT) is superior to other biomarkers in predicting severe septic complications and overall mortality in patients with secondary peritonitis.*

Keywords: *biomarkers, peritonitis, C-reactive protein, lactate, procalcitonin, presepsin*

INTRODUCTION

Secondary peritonitis is a condition caused by the spread of bacteria and contents of intraabdominal organs into the peritoneal cavity [1] and is the second leading cause of sepsis in intensive care units worldwide [2]. Overall mortality is 6%, but mortality rises to 35% in patients who develop severe sepsis [2]. Secondary peritonitis requires immediate surgical intervention but after the initial procedure, persisting or new-onset abdominal sepsis continues to be a major problem in the postoperative course [3].

Using clinical symptoms such as fever, tachycardia, tachypnea, non-specific laboratory findings, and scoring systems are valuable tools to assess overall severity and prognosis but will not allow a differentiation between an infectious and non-infectious etiology of a systemic inflammatory response. The clinical assessment of sepsis biomarkers has gained considerable interest in identifying patients at risk for severe complications following surgical intervention for secondary peritonitis [1].

This review aimed to identify the most important biomarkers evaluated in patients with secondary peritonitis and their association with prognosis.

MATERIALS AND METHODS

We searched in three databases using the keywords „peritonitis“ and „prognosis“ in the title or abstract AND specific biomarkers by full name and abbreviated form in full text: lactate, C-reactive protein (CRP), procalcitonin (PCT), presepsin.

Due to the scarcity of the results, a broader search was conducted using the keywords „peritonitis“ AND „biomarker“ to ensure that all relevant literature is covered. The search was last updated on 24/06/2022. A total of 361 publications were primarily retrieved in PubMed, Embase, and Web of Science. No articles were added after manually searching major scientific journals and other references. After excluding 80 duplicates, 281 titles were screened for eligibility and 43 articles were assessed for full-text evaluation. Only English-written human studies were accepted and most articles were excluded due to irrelevancy. We focused only on circulating biomarkers for prognosis in secondary peritonitis. No cut-off point was set for the date of publishing due to the paucity of articles. At the end of the process, seven articles were included in the review. The search strategy is detailed in Figure 1.

Figure 1: Search strategy – identification of studies via databases and registers

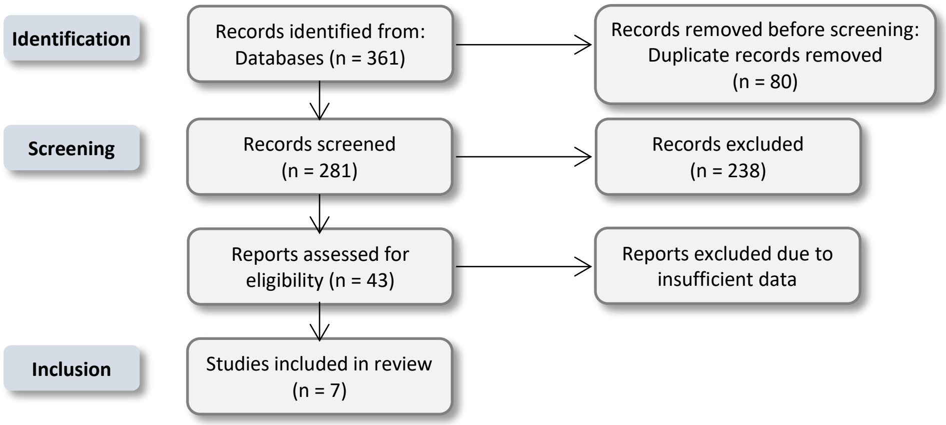
Identification
Records identified from: Databases (n = 361)
Records removed before screening: Duplicate records removed (n = 80)
Screening
Records screened (n = 281)
Records excluded (n = 238)
Reports assessed for eligibility (n = 43)
Reports excluded due to insufficient data
Inclusion
Studies included in review (n = 7)

OVERVIEW OF THE SELECTED ARTICLES

Seven articles were included in the current review. Four studies were prospective and three were retrospective, and one study included a control group. Biomarkers of interest were lactate, procalcitonin, and C-reactive protein. No study evaluating presepsin was identified. All studies focused on mortality and severity of septic shock induced by secondary peritonitis.

Further details on the selected studies are summarized in Table 1.

SERUM BIOMARKERS CORRELATED WITH PROGNOSIS IN SECONDARY PERITONITIS

Lactate

Lactate is the metabolic end product of anaerobic glycolysis. Approximately 1500 mmol of lactate is produced daily in all tissues, but mostly in skeletal muscle, brain, intestine, and red blood cells [6,9]. The concentration of arterial lactate is a reflection of the delicate balance between net production and clearance. Disturbances in lactate production, clearance, or both lead to hyperlactatemia and it is seen in a variety of conditions such as sepsis, shock, cardiac arrest, tissue hypoxia, burns, or after some pharmacological agents [6].

The prognostic value of lactate in patients with secondary peritonitis was evaluated in four studies. Negi et al. conducted a prospective study to evaluate the utility of arterial lactate measured on admission (AL1) and 24h after surgery (AL24) as well as lactate clearance as prognostic indicators for mortality in patients with secondary peritonitis. The study concluded that mean values of initial lactate, postoperative lactate, and percentage lactate clearance correlated significantly with higher morbidity and mortality [6]. Of these three lactate indices, postoperative lactate (AL24) was the most accurate in predicting morbidity and mortality [6]. Elevated lactate levels persisting postoperatively probably indicate irreversible pathophysiological changes and global tissue hypoperfusion, indicative of poor lactate clearance and higher morbidity and mortality. Andreevich et al., after conducting a retrospective study, concluded that increased lactate levels both before and 72h post-operatively were associated with a negative prognostic[8]. In the study conducted by Suarez-de-la-Rica et al., 24h postoperative lactate concentrations predicted mortality in the Surgical Critical Care Units, but overall mortality was predicted better by concurrent high procalcitonin and lactate peak concentrations [7].

Jung et al. conducted a retrospective study that aimed to compare the performance of the qSOFA score alone with a combined score derived from qSOFA and serum lactate levels for predicting in-hospital mortality for patients with secondary peritonitis. The study results showed that the qSOFA score alone has poor sensitivity and a modest predictive performance in patients with secondary peritonitis [5]. The addition of one point for hyperlactatemia (plasma lactate level > 2.0 mmol/L) resulted in improved sensitivity (5). The use of the qSOFA + lactate score can be useful to assist clinicians in immediately assessing the risk of mortality in patients with secondary peritonitis [5].

Most studies agreed that elevated post-operative lactate levels correlate better with negative outcomes than preoperative lactate levels.

Procalcitonin

Procalcitonin (PCT) is the 116-amino acid pro-peptide of the biologically active hormone calcitonin [10]. The clinical value of PCT assessment in infection and sepsis has been investigated in almost every medical discipline so far. Most studies have shown that, in general, only in the presence of systemic host response to bacterial or fungal stimuli, does PCT concentration rise above the reference range of 0.5 ng/mL. However, new data demonstrated an early production of PCT related to tissue damage after trauma and the magnitude of the hypovolemic challenge, yet unrelated to infection (4, 10). We analyzed four studies on the usefulness of PCT measurements in predicting negative outcomes in patients with secondary peritonitis. In 246 patients with intraoperatively proven secondary peritonitis of various origins, Reith et al. showed that PCT is a sensitive parameter for the prediction of severe septic complications. Moreover, the study demonstrated that PCT reflects the effectiveness of the surgical procedure in eliminating the septic intra-abdominal focus in the postoperative course (4, 10). PCT was of excellent prognostic value and achieved a sensitivity of 84% and a specificity of 91% in discriminating survivors from non-survivors within the first four postoperative days [4]. Similar observations have been reported in a study conducted by Rau et al. on 82 patients with intraoperatively proven secondary peritonitis. Pro-calcitonin values were elevated in patients who developed septic multiple organ dysfunction syndromes (MODS) with peak levels on the third and fourth days after the onset of symptoms. Comparing the preoperative and postoperative courses, PCT concentrations were significantly elevated in patients with septic MODS during the immediate postoperative course. A persistent procalcitonin elevation beyond the first week after the onset of symptoms was evident in non-survivors, whereas normal concentrations were measured in survivors [3].

Table 1: Studies focused on mortality and severity of septic shock induced by secondary peritonitis

Author	Study design	Biomarker	No. of patients	Correlation with prognosis
Akcay et al (2014)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	84	Serum PCT levels follow a falling trend after surgery as long as the postoperative course remains uneventful. PCT was a better marker than CRP in following postoperative complications and overall prognosis.
Reith et al (2000)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	246	A reduction in the PCT concentration correlated with improvement in the infection and sepsis and was found in all surviving patients. Compared with the values for CRP, PCT showed significant differences between survivors and patients who died, while CRP did not [4].
Rau et al (2007)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	82	The cumulative analysis of all PCT and CRP values measured in the first, second, and third to fourth weeks identified persisting PCT levels greater than 1 ng/mL as an excellent means to assess the overall outcome. Irrespective of the interval, PCT was significantly better than CRP in assessing overall prognosis [3].
Jung et al (2018)	retrospective	lactate	457	The qSOFA score alone has poor sensitivity and a modest predictive performance in patients with secondary peritonitis. The addition of one point for hyperlactatemia (plasma lactate level > 2.0 mmol/L) resulted in improved sensitivity. The use of the qSOFA + lactate score will assist clinicians in immediately assessing the risk of mortality in patients with secondary peritonitis [5].
Negi et al (2020)	prospective	lactate	224	Mean values of initial lactate, postoperative lactate, and percentage lactate clearance were found to correlate significantly with higher morbidity and mortality in patients with secondary peritonitis. Of these three lactate indices, postoperative lactate was the most accurate in predicting morbidity and mortality [6].
Suarez-de-la-Rica et al (2014)	retrospective	lactate, C-reactive protein (CRP), procalcitonin (PCT)	221	Both PCT and lactate provided early prognostic information, more accurate than clinical scores, for overall mortality [7].
Andreevich et al (2021)	retrospective	lactate, C-reactive protein (CRP)	136	Increased lactate and CRP values both before and 72 h after surgery were associated with negative prognostic. PCT value before surgery was insignificant and only an increase in PCT 72h after surgery was associated with a negative outcome [8].

In a prospective study conducted on 84 patients, Akcay et al. were able to demonstrate that serum PCT levels follow a falling trend after surgery as long as the postoperative course remains uneventful. In addition, they showed that after the surgical intervention PCT was a better marker than CRP in following postoperative complications and overall prognosis in patients with secondary peritonitis [1].

Suarez-de-la-Rica et al. compared the prognostic value of lactate, procalcitonin, and c-reactive protein. Peak lactate (>1,8 mmol/L) and PCT (>100 ng/mL) concentrations were associated with mortality, but peak PCT level was the best biomarker for overall mortality. In this study, concomitant high peak lactate and peak PCT concentrations considered as a single dummy variable were highly predictive of overall mortality [7].

C-Reactive Protein

C-reactive protein (CRP), named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae, was the first acute-phase protein to be described [11]. CRP is an evolutionarily conserved protein. From arthropods to humans, CRP has been found in every organism where its presence has been sought [12].

C-reactive protein is a useful marker of systemic inflammation without discriminating infection from other inflammatory processes [7]. When it comes to the value of CRP in evaluating prognosis in patients with secondary peritonitis, after searching the available literature, we found five studies that included this biomarker. All studies agreed that measured CRP values, which increase rapidly in response to the surgical insult, had no value in assessing overall prognosis. C-reactive protein did not reveal any differences between patients with or without septic MODS or in non-survivors and survivors in the perioperative course [3].

CONCLUSIONS

Although a multitude of biomarkers is elevated in patients with secondary peritonitis in the perioperative course, only a few are reliable parameters for assessing the course and prognosis in this category of patients. Many studies have clearly shown that procalcitonin (PCT) is superior to other biomarkers in predicting severe septic complications and overall mortality. In combination with careful clinical patient assessment, PCT offers distinct advantages for the evaluation of response to treatment and prediction of outcome in patients with secondary peritonitis. In regards to the fact that procalcitonin is a well-known „sepsis“ parameter, this may not embrace the real properties of PCT, which remain to be defined.

Further studies must be conducted to obtain more knowledge about the importance of serum biomarkers in patients with secondary peritonitis and their correlation with prognosis.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Etiology of Chronic Kidney Disease and Comorbidities in Chronic Hemodialysis Patients

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Abstract: *Introduction: As general population tends to have increasing life expectancy, the risk associated with developing chronic kidney disease (CKD) with multiple incapacitating concequences, also increases. Method: For the present study, we registered the data from the observation files of 37 patients diagnosed with CKD undergoing treatment by chronic hemodialysis and noted the CKD associated diagnoses included in the notion of comorbidities. We monitored their statistical incidence both in the whole group and separately, in women and men using TTEST and CORREL. Results: The median age of the subjects was 55.86 (± 12.00) years. The study population mean weight was 74.90 (± 14.44) kg, with a mean weight of 69.33 kg for female subjects, and 77.92 kg for males, respectively. Diabetes was identified in 35.13% of patients, whilst heart failure was present in 16.21% of patients. Conclusions: Following the analysis of the information about the patients with CKD in the dialysis program, which we included in the study group, we observed the existence of variations that occur with age, significant correlations between age and weight and between albuminemia and weight. The most common comorbidity is high blood pressure followed by anemia.*

Keywords: *chronic kidney disease; chronic hemodialysis patients; comorbidities; plasma albumin*

INTRODUCTION

Chronic kidney disease (CKD) can develop due to a multitude of pathologies that all have in common the evolution to kidney failure, with gradual or rapid loss of the main function of the kidneys, that is to remove waste products and excess fluid from the body [1]. The last stage of the development of CKD, especially in patients who are treated by peritoneal dialysis or hemodialysis, is characterized by multiple organs and systems damage [2].

Not only CKD, but also the treatment of its various manifestations, mainly affect the body tissues and systems, at all levels: nervous, cardiovascular, respiratory, endocrine, hematopoietic, digestive, renourinary [2].

The tendency to increase life expectancy of the population is also associated with an increase of the incidence and prevalence of CKD, considering the already known relationship between aging and decreased kidney function, which is seen as a consequence of the physiological aging of the kidneys [2,3]. An even steeper increase in the number of patients with CKD is expected in the coming years. In this context, the medical goal becomes to slow down the progression to terminal stages, achievable primarily by diagnosing the condition as early as possible [1].

Compared to conservative treatment, extrarenal treatment methods provided clear advantages for patients with chronic end-stage renal disease, the duration and quality of life being considerably superior. There are several methods of extrarenal purrification: peritoneal dialysis, hemodialysis, hemodiafiltration. The decision for each type of treatment is based primarily on medical considerations, depending on the underlying disease and the comorbidities present [4]. Diabetic patients are usually at higher risk of developing more severe forms of chronic diseases (e.g. cancer) [5,6,7]. Peritoneal dialysis is preferred for diabetic patients with CKD as this method of treatment ensures the maintenance of a residual diuresis for a longer time, the prevention of complications due to the present vascular damage, the possibility of intraperitoneal administration of insulin and a better hemodynamic stability than chronic hemodialysis. In cases where the patient has a poor state of hygiene or if peritoneal dialysis does not allow a satisfactory volume control to be maintained, chronic hemodialysis is preferred [4].

MATERIALS AND METHODS

For the study we performed, we registered the data from the observation files of 37 patients diagnosed with CKD undergoing treatment by chronic hemodialysis, in the Nephrology Department of the Bucharest University Emergency Hospital.

We noted their general data and the presence of associated comorbidities, as well as the determined values of plasma albumin concentration.

The statistical data processing regarding the incidence of comorbidities revealed important information for understanding the clinical and biochemical context of this particular type of patient in the hemodialysis department.

Among the tests performed on the patients that had presented for chronic dialysis, we noted the value of plasma albumin concentration, because it is a parameter that provides information about the loss of protein in the urine and the nutritional status of renal patients. We determined the statistical differences between the values from women and men, as well as the correlations between this parameter and age, respectively weight.

We performed the statistical processing using the Student test (t-test) to determine the statistical significance of the differences in the values of the parameters determined in women and men and the Correl test to determine whether there are correlations with statistical significance between the parameters we tracked.

We noted the diagnoses associated with CKD, included in the notion of comorbidities and we monitored their statistical incidence both in the whole group and separately, in women and men.

RESULTS

In terms of gender distribution, of the 37 patients, 13 (35.13%) were women and 24 (64.86%) were men.

The median age of the subjects was 55.86 (± 12.00) years. For the women, the median age was 56.46 (± 10.86) years, whereas for the men it 55.54 (± 12.78) years. Although there is a slight gender difference regarding age, this failed to be statistically significant (p> 0.05).

Regarding weight, as presented in Table 1, the study population mean weight was 74.90 (± 14.44) kg, with a mean weight of 69.33 kg for female subjects, and 77.92 kg for males, respectively. As presented in Table 1, this difference was found to be statistically significant, p = 0.01.

Table 1: Average weight and the statistical difference between men and women

	Average weight in women (kg)	Average weight in men (kg)
Average value	56.46	52.27
Standard Deviation	10.87	17.16
p (t-test) 0.43		

Applying the Correl test, we observed that age and weight are two parameters that correlate statistically significantly (p = 0.04) (Figure 1).

Figure 1: Significant correlation between the values of weight and age

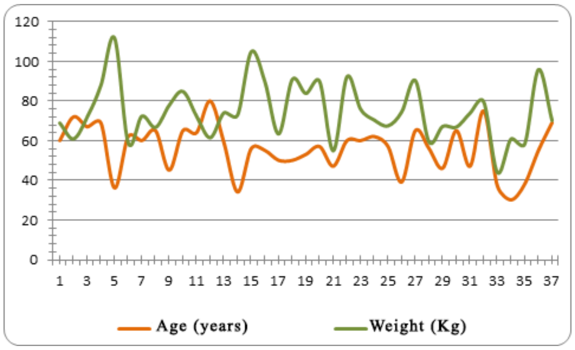

For the 37 patients, we noted the value of the plasma albumin concentration and determined the difference in values according to the gender of the patients and the statistical significance of this difference, using the Student test (t-test) (Table 2).

It is observed that the albuminemia values that were determined in these patients are not outside the normal laboratory limits of 3.5-5.2 g/dl (Table 2). By applying the

Correl test, we calculated the statistical significance of the correlations of albuminemia with age and weight (Table 3).

Table 2: Statistical significance of differences in plasma albumin concentrations in patients in the group by sex

Albuminemia(g/dl)		
	Women	Men
Average value	4.10	4.25
Standard deviation	0.24	0.27
t-test	p = 0.41	

Table 3: Statistical significance of correlations between plasma albumin concentrations with age and weight in patients in the group and by sex

Significance of the correlation between plasma albumin concentration and		
	Age	**Weight**
Women	p = 0.1	p = -0.2
Men	p = -0.37	p = 0.1
Total	p = -0.24	p = 0.02

Figure 2: Correlation weight-albuminemia

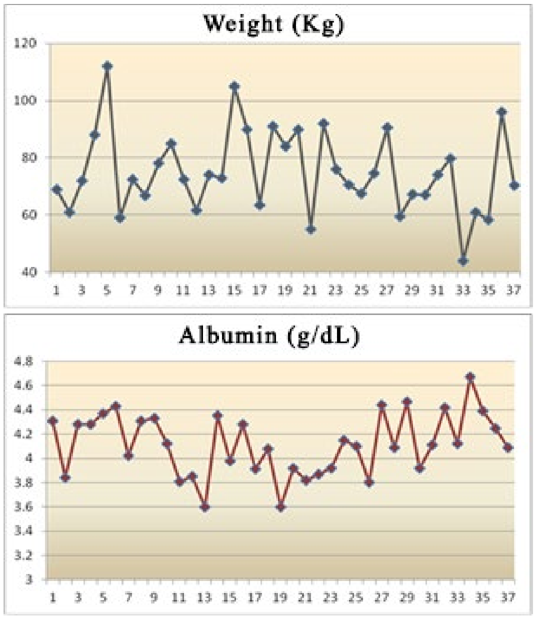

We observed that separately, in women and men, the plasma concentrations of albumin do not correlate significantly with age or weight, but, after using the Correl test for the whole study group, a significant correlation between albuminemia and weight has been identified.

We made this determination, knowing that the albumin dosage is a test to assess the general state of nutrition, especially in elderly patients with various conditions, in this category falling most of the patients with CKD in the lot we studied.

COMORBIDITIES DIAGNOSED IN THE STUDIED GROUP

Diabetes mellitus, dyslipidemia, atherosclerosis, hypertension, arteriopathy obliterans of the lower limbs

Diabetes is present in 35.13% of patients, 3 of women and 10 of men, which means 23.07% and 41.66%, respectively. Dyslipidemia occurs in a number of 3 male patients, which means a percentage of 8.1% of the total group. Atherosclerosis is noted in a female patient with kidney disease and in 9 male patients, the incidence rate being 27.02% in the whole group. High blood pressure is a more common comorbidity in patients in the study group: 11 out of 13 women are hypertensive and 23 out of 24 men with CKD are hypertensive. Lower limb arteriopathy is present in only 2 of the male patients, which means 5.4% of the group. Both patients also have diabetes and high blood pressure.

Heart failure, cardiomyopathy, arrithmia, valvulopathy, chronic coronary heart disease

Heart failure is present in 5 of the women and only 1 of the men, this comorbidity is therefore found in 16.21% of patients. Cardiomyopathy is rarer, 1 female patient and 2 of the men in the group, ie 8.1% also have this diagnosis. Arrhythmias are more common in women, 5 out of 14, ie 35.7%, presenting this manifestation in the form of atrial fibrillation with medium or large ventricular allure or in the form of supraventricular tachycardia. Among men, only 2.33% have atrial fibrillation with a medium ventricular allure. Thus, 18.91% of the total group have a form of arrhythmia. Valvular damage is identified in 4 patients out of a total of 37, ie 10.81%, more frequently in women 3 out of 14 and only in 1 male patient. Coronary ischemic disease is diagnosed in 3 women and 6 men, the incidence being 24.32%.

Diabetic/hypertensive/mixed nephropathy, chronic tubule-interstitial nephropathy, autosomal dominant polycystic kidney disease, single kidney, chronic pyelonephritis, chronic glomerulonephritis

Chronic tubulointerstitial nephropathy is diagnosed in a single male patient. The incidence at group level is 2.7%. Out of 13 women in the group, hypertensive nephropathy is present in 2 and diabetic nephropathy in 3 of them, a total of 38.46% with nephropathy. Of the 24 men in the group, 6 have diabetic nephropathy, 4 have hypertensive nephropathy and 2 have mixed nephropathy, a total of 50% of patients with nephropathy. A number of 4 of the 13 women, 30.76%, are also diagnosed with autosomal dominant polycystic kidney disease. Of the 24 men, 4 also have this diagnosis, representing

16.66%. At the level of the whole group, the percentage is 21.62%. Only one male patient has a single kidney, the cause being congenital. The incidence at group level is 2.7%. And pyelonephritis rarely occurs, only one patient, a woman, has this diagnosis. The incidence at group level is 2.7%. More common is chronic glomerulonephritis, which is present in 6 of the men, respectively 25%, and in 2 of the women, respectively 15.38%, with an incidence in the whole group of 21.62%.

Kidney stones, parathyroidectomy, secondary hyperparathyroidism, anemia, chronic viral hepatitis

Kidney stones are present in 2 women and 3 men in the group, which means an incidence of 13.51%. Secondary hyperparathyroidism is diagnosed in only 2 patients, a man and a woman, the incidence is 5.4% in the whole group. Surgical removal of the parathyroid glands was performed in 3 women and 1 man in the group, the frequency being 10.81%. Anemia is more common, 7 of the 13 women, ie 53.8% and 15 of the 24 men, ie 62.5%, have pathologically low hemoglobin. Out of the whole lot 59.45% of patients have anemia. Chronic hepatitis B infection is uncommon as viral hepatitis B is present in 2 women, 1 man in the group is infected with hepatitis B virus and 1 is coinfected with hepatitis B + C viruses. The frequency of chronic viral hepatitis in the group is 10.81%.

DISCUSSIONS

Gender and age are factors that influence some characteristics related to CKD in patients with type 1 or type 2 diabetes, and certain particular aspects of pathogenesis, diagnosis and treatment management have been identified, but the mechanisms are incompletely known and more studies are needed to clarify these connections which would be very useful in order to reduce the number of cases of renal patients and to prevent or control the complications of this condition. Considering the data published by Eriksen O. et al. [5], old age and the male gender are predictors of worsening kidney disease and the rapid progression to severe kidney failure. In the study of Giandalia A et al. [8] the authors concluded that men are more affected, because in their studies, the number of men enrolled is higher than the number of women, results similar to our study where the percentage of men was 64.86%.

Weight is an important parameter in assessing the nutritional status of the patient with CKD. In obese people with CKD, the progression of the disease to renal failure is faster compared to cases of normal weight. Also kidney transplantation or hemodialysis, which may become necessary in advanced stages of the disease, may be more difficult and may have more frequent complications in obese people [9]. After initiating treatment with hemodialysis or peritoneal dialysis, in patients with a glomerular filtration rate below 35 ml/min/1.73 m^2, in stage 3 CKD, weight loss is a factor that increases the risk of mortality by 54% compared to with patients whose weight remains constant. Even in patients with moderate renal impairment, weight loss should be closely monitored and the patient's nutritional status monitored before dialysis is required [10].

In the final stages of the kidney disease, hypoalbuminemia is common, caused by factors that act simultaneously: reduced liver synthesis, increased degradation and renal loss and the glomerular filter membrane is damaged and becomes more permeable allowing the passage of negative albumin molecules from plasma. In our study we observed that separately, in women and men, the plasma concentrations of albumin do not correlate significantly with age or weight. However, applying the Correl test to the whole group, resulted in a significant correlation between albuminemia and weight. We made this determination, knowing that the albumin dosage is a test to assess the general state of nutrition, especially in elderly patients with various conditions, in this category falling most of the patients with CKD in the lot we studied. According to Haller C. et al. [11] the inflammatory phenomenon in kidney disease is also involved in the pathogenesis of hypoalbuminemia. Inflammation correlates with the risk of mortality, hypoalbuminemia is not in itself a pathogenic factor of renal impairment, but is a major predictor of unfavorable prognosis.

Diabetes mellitus is one of the main negative prognostic factors for chronic diseases [12]. According to Anders HJ. et al. [13] the prevalence of type 2 diabetes and CKD increases the incidence of diabetic kidney disease, also known as diabetic nephropathy. The combination of the two diseases can be in the form of diabetic kidney disease if the kidney damage is the consequence of diabetes, it can be a kidney disease caused by individual mechanism in the diabetic patient or it can be a coincidence of simultaneous occurrence of the two diseases. The involvement of the membrane-glucose cotransporter membrane and hyperglycemia are associated with the activation of the renin-angiotensin-aldosterone system, glomerular hyperfiltration and tubular hyperabsorption occur, which creates a particular situation that requires appropriate treatment. Whatever the mechanism, the patient's evolution is more unfavorable compared to those who have only one of the conditions [13,14]. Our study results for 35,13% of the patients, that were diagnosed with diabetus mellitus type II, found that 69.23% of them have further developed diabetic nefropathy.

In the study of Tsimihodimos V et al. [15] it was shown that lipid metabolism is disrupted in patients with CKD even in the early stages and can be very severe in some cases, further impairing kidney function. The pathogenic mechanisms are not fully understood, but studies show that dyslipidemia is a pathogenic factor for both kidney disease and the associated cardiovascular disease [16]. In our study dyslipidemia was identified in only 8,1% of the patients.

Atherosclerosis sets in early and progresses rapidly in patients with CKD. It usually increases the risk of cardiovascular disease and, as the severity of kidney disease increases, so does the mortality rate, according to Olechnowicz-Tietz S. et al. [17]. In our study population 27.02% were found to suffer from atherosclerosis.

Considering the cardiovascular risk, compared to the general population, patients with CKD, according to the study of Matsushita K et al. [16] have a constant increased cardiovascular events risk. Studies identified that 70% of CKD patients, and up to 80-90% of those on dialysis, are diagnosed with high blood pressure [18,19]. Considering all these data, our studies results that 91.9% of CKD subjects were suffering of/diagnosed with hypertensive disease are consistent with the data in the literature.

As shown in the study of Maric-Bilkan C. et al. [20], in the absence of diabetes, women have a considerably lower risk than men of vascular, microvascular or macrovascular diseases. The opposite trend is observed in diabetic patients: women are at higher risk for these complications. Microvascular lesions that occur in nephropathy, neuropathy, and retinopathy are more common in men. Macrovascular lesions consisting of chronic coronary heart disease, myocardial infarction, peripheral artery damage, and stroke are more common in women. To explain this, the focus has been on sex steroids, which play an important role in regulating heart function. Estrogens are generally considered hormones with cardioprotective effects, and androgens with adverse cardiac effects. But the effects of these hormones on the target organs are complex and different, and the presence of diabetes interferes with the pathophysiological mechanisms that cause vascular damage [14,18,19].

Renal comorbidities are, in part, primary kidney disease that has progressed unfavorably, with the patient reaching the stage of CKD that requires dialysis in order to survive [20]. Another part of these comorbidities is secondary, the pre-existing disease being another. This is the case with nephropathy, which develops during the course of hypertensive disease or diabetes [14]. The percentage of incidence of nephropathy of any etiology in the study population was 45.94%. It is a high percentage, which is explained by the fact that, as we have shown above, diabetes and hypertension are precursors or caused by CKD.

Our results are consistent with literature data showing that 11.2% of patients in a large group of with CKD present with kidney stones. The same study by Chuang TF. et al [21] shows that patients with kidney stones are 1.82 times more likely to develop CKD.

Secondary hyperparathyroidism is found in chronic dialysis patients, the incidence of this comorbidity being reduced by treatments with phosphorus chelators, calcium supplements and calcitriol. As shown in the study of Llach F. et al [22] there are situations when parathyroidectomy is necessary, namely when clinical signs such as persistent hypercalcemia, pruritus, metastatic calcifications appear [23]. In our study hyperparathyroidism was found in only 5,4% of the patients but out of the whole lot, 10.81% of the patients had already been treated with parathyroidectomy. The latter can cause insufficiency of many organs.

Blood characteristics such as blood group or different blood components (eg. lymphocytes) have been linked to chronic diseases or their treatments toxicity [24-27]. According to the study of Ueda N. et al. [28] hemoglobin levels below 12 g/dl for women and below 13 g/dl for men are considered anemia. Iron and erythropoietin deficiency causes anemia in patients with CKD. Hepcidin is a hormone produced by the liver in inflammation or when the body's iron deposits in the form of ferritin or hemosiderin are saturated. Hepcidin binds to and inhibits ferroportin, thereby decreasing the release of iron from the enterocyte and reticuloendothelial cells and decreases plasma iron. Inflammatory status in CKD increases hepcidin regardless of the availability of iron to the body, so it becomes necessary to administer iron, but also to reduce inflammatory phenomena [29]. It was a frequent occurence in our study as 62.5% of our patients were diagnosed with anemia.

Hepatitis B or C virus infections can cause chronic kidney damage but do not have a high incidence according to Chacko EC et al. [30]. If the infection occurs in patients with CKD, anti-infective treatment has many side effects and may worsens the prognosis. Similar results were shown in our study as only 10.81% of the patients presented with concurrent viral hepatitis and CKD.

CONCLUSIONS

Following the analysis of the information about the patients with CKD in the dialysis program from our study weight has been found to be significantly higher in men, compared to women. Albuminemia was within normal limits in all patients, significantly correlating with the body weight. Patients' comorbidities identified in our study were similar to the ones found in the current literature to be related to CKD. The most common comorbidity is high blood pressure, identified in over 90% of the subjects. Anemia is the second most common comorbidity, occurring in almost 60% of patients. With high frequency, in over 20% of patients, we observed the presence of diabetes, atherosclerosis, ischemic coronary heart disease, nephropathy of various etiologies, autosomal dominant polycystic disease and chronic glomerulonephritis.

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Review

The Role of Coronary CT Angiography in the Management of Patients with Coronary Atherosclerotic Disease

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Abstract: *Coronary CT angiography is a non-invasive method of analyzing the coronary lumen through which the atheromatous bur-den can be evaluated, with the analysis of the type of plaque (soft, calcified, mixed) and the impact on the arterial lumen (stenosis, occlusion). The anatomical evaluation by coronary CT is indicated in symptomatic patients with low and medium risk factors for coronary atherosclerotic disease, in those with inconclusive laboratory and EKG results, patients with un-certain stress test results, in the evaluation of coronary grafts and intrastent stenoses. Depending on the result of the angiography, the severity of the stenosis and the management of the patient are established. In the case of mild and medium stenoses, risk factors management and drug treatment are recommended. For severe stenoses, patients are referred for interventional coronary angiography or functional evaluation. Coronary CT angiography increases the certainty of coronary atherosclerotic disease diagnosis. It has a superior discriminative capacity of plaques with low attenuation, the main predictor of myocardial infarction, with almost five-fold higher risk. Coronary CT angiography increases the adaptation of medication, and the inclusion of statin therapy decreases the risk of fatal and nonfatal myocardial infarction at 5 years and increases the quality of life.*

Keywords: *coronary CT angiography, coronary atherosclerotic disease, calcium score, CAD-RADS*

INTRODUCTION

Atherosclerotic disease is the main cause of death in Europe [1]. Lifestyle factors (smoking, diet, level of physical activity) but also biological risk factors (hypertension, hypercholesterolemia, hyperglycemia) contribute to this [2].

The management of patients with coronary atherosclerotic disease is complex, including numerous investigative methods. Of these, coronary CT angiography has been analyzed more and more, over the last decade, due to technological progress. Coronary CT angiography is a non-invasive method of evaluating the heart and coronary arteries. Coronary CT angiography is performed using a computer tomograph that involves high-speed scans for moving organs and contrast material containing iodine, administered in injectable intravenous form to obtain the best images of the coronary arteries and the heart. Thus, the atheroma plaques in the coronary arteries can be identified, the type of plaques analyzed (soft, calcified, mixed), and the consequences on the arterial lumen (stenoses, occlusions) evaluated. Coronary anomalies can be detected, valve morphology can be analyzed, as well as the degree of myocardial perfusion.

MATERIALS AND METHODS

The main reference studies used in evaluating and managing patients with coronary atherosclerotic disease are extensive studies that have contributed to the current state of knowledge in this field, demonstrating the usefulness of the investigation in diagnosis, monitoring, and recommendations for the treatment of the disease.

The PROMISE study (Prospective Multicentre Imaging Study for Evaluation of Chest Pain) – from the year 2017 – showed that coronary CT angiography is a superior means of evaluation for identifying patients at risk with nonobstructive coronary atherosclerotic disease, compared to classical functional exploration means, in patients with stable angina pectoris and with an average probability of coronary atherosclerotic disease. The patients were randomly divided into two groups, 4500 patients were investigated by coronary CT angiography and 4602 patients were evaluated by functional means (exercise electrocardiogram, exercise ultrasound, and exercise myocardial scintigraphy). The patients were subsequently monitored for 26 months. The significantly lower incidence of coronary events in patients investigated by coronary CT angiography compared to those evaluated by functional means (33.4% versus 78.0%) demonstrates that coronary CT angiography had a significantly higher discriminative capacity for predicting coronary events than functional explorations (c -index, 0.72; 95% CI, 0.68-0.76 versus 0.64; 95% CI, 0.59-0.69; P=0.04) [3].

The SCOT HEART study (Scottish Computer Tomography of the Heart) is large, started in 2010, and is still ongoing, which will continue until 2027, with some of the results already published. It is a randomized parallel study, which included, between 2010-2014, 9848 participants with angina pectoris suggestive of coronary atherosclerotic disease and with mild and medium risk factors. The patients were divided into two groups that were evaluated differently: 4146 patients by coronary CT angiography and the rest were managed by standard anatomical and functional means. The main diagnostic results showed that coronary CT angiography increased the certainty of the diagnosis of coronary atherosclerotic disease (relative risk [RR] = 1.79, 95% confidence interval [CI] 1.62-1.96, p < 0.001) and reduced the frequency of the diagnosis of angina due coronary atherosclerotic disease (RR = 0.93, 95% CI 0.85-1.02, p = 0.13) compared to standard care [4]. The main clinical result was a decrease in the risk of fatal and nonfatal myocardial infarction at 5 years, which was 2.3% in the coronary CT angiography group, compared to 3.9% in the group managed by standard means. The secondary results consisted of an increase in the quality of life, the adaptation of medication, and the inclusion of statin therapy. In those cases where the lesions were severe, patients were referred for angiography and stenting [5].

Other secondary, very important results emerged in 1769 patients, followed for 4-5.7 years, which proved that the presence of plaques with low attenuation is the main predictor of myocardial infarction, independent of the presence of cardiovascular disease risk factors, of the score of calcium or area of stenosis. Patients with low-attenuation plaques had an almost 5-fold higher risk of myocardial infarction (95% CI, 2.06-10.5; P<0.001). In 41 patients who suffered an acute myocardial infarction, both low-attenuation plaque load and calcium score were increased (336 [62-1064] versus 19 [0-217] Agatston units; P<0.001), and the presence of obstructive coronary artery disease (54% versus 25%; P<0.001) [6].

The UK's National Institute for Health and Care Excellence's 2016 NICE chest pain guideline recommended coronary CT angiography as the first line of evaluation in patients with new stable chest pain. Emphasis is placed on anatomic explorations over functional ones, a choice to which cost-effectiveness also contributes [7,8].

However, this approach raised concerns that, after the first evaluation with coronary CT angiography, there would be an increased referral to further investigations, such as invasive coronary angiography. To test this, a retrospective study was conducted on 652 patients who underwent coronary CT angiography from 2017 to 2018. The results showed that 92 patients had moderate (50-70%) or severe stenosis (>70 %). Of these, 69 were directly referred for invasive coronary angiography, of which 63 underwent the investigation, and severe atherosclerotic coronary disease was confirmed in 40 patients (63%). Among the patients with moderate stenosis, 18 were referred to cardiac stress echography, which was positive in one case. The remaining patients had mild stenosis in 62 cases, 36 studies were inconclusive, and the rest of them (462 patients) were normal or had minimal stenosis [9]. The study's conclusions showed that coronary CT angiography is an effective first-line method in the evaluation of patients with newly appeared stable angina pectoris, most of them having normal results or minimal coronary atherosclerotic disease. In patients with severe stenosis, it had an increased detective capacity, the stenosis being confirmed by invasive coronary angiography in 63% of cases.

The positive predictive value for coronary stenosis of coronary CT angiography, compared with stress tests, was evaluated and published in a study from 2019. A total of 800 patients with stable angina pectoris from two centers were included, one in the UK where 400 patients were investigated with coronary CT angiography and a center in Portugal where 400 patients were investigated with exercise tests (treadmill exercise stress test EST or stress imaging test). The patients in the two groups were equally distributed in terms of gender and BMI. Of the 400 coronary CT angiographic examinations, 387 (96%) were diagnostic. Of these, 92 patients (23%) had CAD-RADS 3-5 coronary obstruction, of which 67 (72%) underwent coronary angiography and 61 patients (91%) had atherosclerotic coronary obstructive disease confirmed. In the Portuguese group, 400 patients with stable angina evaluated by stress test with the positive result, recommended for interventional angiography, were included. On interventional angiography, the obstructive coronary disease was diagnosed in 51% of patients. In conclusion, the positive predictive value of coronary CT angiography is 91%, statistically significantly higher (p<0.0001) than stress tests (51%) [10]. The study concludes that coronary CT angiography, due to its significantly higher predictive value than exercise tests, has a good indication as a first test for evaluating coronary stenosis, and could mean the avoiding of unnecessary interventional angiographic tests.

The AGATSTON coronary artery calcium score is a semi-automatic measurement of the degree of calcification of the coronary arteries. It is an investigation performed with a low dose of radiation at the beginning of the coronary CT angiographic examination, before the administration of contrast substance. The main role is an early stratification of the degree of coronary atherosclerotic infiltration.

An automatic calculation of coronary calcifications is made according to the density in Hounsfield units and the area occupied by the plaque.

Depending on the value of the total calcium score, coronary artery disease is divided into five degrees: no evidence of disease (calcium score 0), minimal damage (1-10), mild (11-100), moderate (101-400), severe (>400), the risk of major cardiac events increases in patients with an Agatston score greater than 160 (Table1) [11].

Table 1: Calcium score according to the Agatston scale

Agatston score	Coronary artery disease
0	no evidence of calcium
1-10	minimal
11-99	mild
100-399	moderate
400-999	severe
1000+	extensive calcium deposits increased risk of coronary events in the next year

The calcium score showed a good correlation with coronary CT angiography in the diagnosis of coronary plaques, with the limitation of not being able to evaluate the soft component of the plaque.

Special emphasis is given to the Agatston score of zero, there is the concept of "the power of zero", which means that patients are considered low risk and if there are no other risk factors, that leads to the avoidance of certain preventive drug therapies, such as statins. Another important role of the calcium score is personalizing the prescription of statins and aspirin among the general population but also the population with special conditions such as severe hypercholesterolemia or diabetes [12].

Coronary calcium score (CAC) has become a popular investigation due to its accessibility, speed, tolerability, low cost, reproducibility, and financial benefits due to the exclusion of complex coronary pathologies that involve more expensive investigations [13].

There are practice guidelines that include structured recommendations for the treatment and prevention of coronary atherosclerotic disease but there are some variations between countries in using coronary calcium score as a reference for inpatient treatment. However, there is a common agreement that coronary calcium scoring assessment is to begin in patients over 40 years. In asymptomatic patients with an intermediate risk of atherosclerotic coronary disease and a calcium score of over 100, the initiation of statin treatment is recommended. In patients with a calcium score of 0, the risk is decreased and the administration of statins is retained, with a repeat of the calcium score after 10 years. These conclusions were reached following the MESA study (Multi-Ethnic Study of Atherosclerosis) in which the 10-year risk of cardiac events was between 1.3-5.6% for a calcium score of 0 and 13-26.6% for a calcium score over 300. The MESA study concluded that in conditions of other cardiovascular disease risk factors maintained constant, the relative risk of cardiac events increases proportionally with the increment of the calcium score [14].

For the good management of patients with coronary atherosclerotic disease, the Guide of the European Society of Cardiology (ESC) was updated in 2019. It established coronary CT angiography as the first line of anatomical evaluation of patients with coronary atherosclerotic disease. The previous recommendations (ESC guide from 2013) proposed a combined approach between anatomical and functional means.

The current management of patients with angina pectoris and suspected obstructive coronary disease consists of a 6-step approach.

1. In the first stage, patients are evaluated through a clinical examination to identify patients with unstable angina or acute coronary syndrome, with the determination of symptoms.

2. The second step involves the evaluation of the general condition and the quality of life - patients with unstable angina or acute coronary syndrome are not included.

3. The third step includes performing some basic tests (blood tests, resting electrocardiogram, resting cardiac ultrasound) and evaluating the function of the left ventricle.

4. Step four consists of assessing the risk of clinical probability of obstructive coronary disease.

5. The fifth step involves diagnostic tests, which are chosen depending on the clinical probability of obstructive coronary disease but also multiple clinical factors, comorbidities, expertise, and access to examinations.

Thus, the anatomical evaluation by coronary CT is recommended as an initial diagnostic test in symptomatic patients in whom the coronary atherosclerotic disease cannot be excluded by clinical evaluation, in those with a low and medium probability of coronary atherosclerotic disease.

Functional tests are recommended in situations where coronary CT angiography is non-diagnostic or has shown stenoses whose functional significance is uncertain.

Invasive angiography is recommended for those with very high clinical probability, symptoms that do not respond to drug treatment, and typical angina at a low level of effort, in patients with an uncertain diagnosis on functional tests.

Coronary CT angiography is not recommended in extensive calcifications, irregular heart rhythm, significant obesity, the impossibility of performing respiratory commands, and any other situations in which qualitative images cannot be obtained.

6. The sixth step, depending on the result of the above tests, the management of the patient and the drug treatment are decided.

Severe calcifications and poor clinical images can lead to an overestimation of the severity of stenoses. In patients that present revascularization through stents and bypass grafts, the accuracy of the coronary CT angiographic examination is impaired in some cases due to the presence of blooming artifacts and the incomplete evaluation of the native vessels. Coronary CT angiography is not recommended in any situations in which qualitative images cannot be obtained: extensive calcifications, significant obesity, and the impossibility of performing respiratory commands [15].

CAD-RADS Coronary Artery Disease Reporting and Data System is a method of evaluating coronary atheromatous plaques developed by the American Cardiology Society with the main purpose of standardizing the reporting of coronary atherosclerotic disease.

Also, the method includes recommendations for patient management depending on the result of coronary CT angiography (Table 2).

Table 2: CAD Rads score for the quantification of stenoses and patient management

CAD-Rads	Stenosis	Additional test
0	0%	-
1	1-24% minimum	-
2	25-49% mild	-
3	50-70% moderate	functional evaluation
4A	70-90% in 1 or 2 vessels	functional evaluation or interventional coronary angiography
4B	>50% in the left main trunk >70% in 3 vessels	interventional coronary angiography
5	100% total occlusion	interventional coronary angiography and viability assessment
N	Non-diagnostic study	additional evaluations

Thus, stenoses are divided into 5 categories, as follows:

- CAD-RADS 0 means the absence of plaques or stenoses and implies the search for other causes of angina pectoris;
- CAD-RADS 1 are plaques without stenosis or minimal stenosis (1%-24%) and imply preventive therapy and risk factor management;
- CAD-RADS 2 includes mild stenoses (25-49%) and includes preventive therapy and risk factor management, especially in patients with non-obstructive plaques in several segments;
- CAD-RADS 3 are moderate stenoses (50-69%). Patient management consists of anti-ischemic pharmacological treatment, preventive with the treatment of risk factors;
- CAD-RADS 4 are severe stenoses, which include two sub-categories: A = severe stenoses (70-99%) and B = tri coronary stenoses (≥70%) or left common coronary trunk (>50%). For category A, interventional coronary angiography or functional evaluation is recommended and for category B, interventional coronary angiography is recommended, to which anti-ischemic drug treatment and treatment of risk factors are added;
- CAD-RADS 5 is total occlusion (100%), and interventional coronary angiography and/or viability assessment is recommended, to which anti-ischemic drug treatment and treatment of risk factors are added;
- CAD-RADS N includes non-diagnostic studies that cannot exclude obstructive coronary disease, and require additional/alternative investigation.

All vessels larger than 1.5 mm in diameter must be included in the classification.

CAD-RADS 1 category also includes plates with positive remodeling and no evidence of stenosis.

There are four modifiers of the CAD-RADS score that are mentioned in the result with the "/" symbol, these are 1 N (non-diagnostic), 2 S (stent), 3 G (graft), and 4 V (vulnerability)[16].

RESULTS

The selected studies show that coronary CT angiography increases the certainty of the diagnosis of coronary atherosclerotic disease. It can predict coronary events, decreases the risk of fatal and nonfatal myocardial infarction at 5 years, and increases the quality of life. Coronary CT angiography has a good indication for being the first test for evaluating coronary stenosis due to its higher predictive value than exercise tests, thus avoiding several unnecessary interventional angiographic tests. The management of patients with coronary atherosclerotic disease can be conducted depending on the result of coronary CT angiography. The detection of severe lesions by coronary CT angiography makes intervention possible in the next stage by angiography and angioplasty.

DISCUSSION

The indications for coronary CT angiography are numerous - from retrosternal pain, clarification of inconclusive non-invasive studies, and assessment of coronary atherosclerosis and by-passes or stents.

The management of the patient with coronary atherosclerotic disease involves a clinical examination and basic tests (blood tests, resting electrocardiogram, resting cardiac ultrasound). Anatomic evaluation by coronary CT is recommended as an initial diagnostic test in symptomatic patients with low and intermediate probability of atherosclerotic coronary artery disease. Functional testing is recommended in situations where coronary CT angiography is nondiagnostic or has revealed stenoses of uncertain functional significance. Depending on the severity of the stenosis, the management of the patient is established. For minimal and small stenoses, preventive therapy and the management of risk factors are chosen. For moderate stenoses, anti-ischemic pharmacological treatment is added, and for severe stenoses, interventional coronary angiography or functional evaluation is recommended.

Also, with the help of coronary CT angiography, other structures such as bones, soft tissues, lungs, or blood vessels can be visualized simultaneously during the scan, being useful for a possible differential diagnosis.

There are limitations of coronary CT angiography such as pregnancy or chronic renal failure, and other contraindications: cardiac arrhythmias, hyperthyroidism, and iodine allergy. In patients with revascularization through stents and bypass grafts, the accuracy of the coronary CT angiographic examination can be impaired due to blooming artifacts and incomplete evaluation of the native vessels. Coronary CT angiography is not recommended in any situations in which qualitative images cannot be obtained: extensive calcifications, significant obesity, and the impossibility of performing respiratory commands.

CONCLUSION

Numerous studies have proven coronary CT angiography as the main investigation for coronary anatomical evaluation. Coronary CT angiography provides important information about the presence and extent of coronary atheroma plaques. A major advantage is the early diagnosis of coronary atherosclerosis, based on which preventive treatment can be administered. Coronary CT angiography decreases the risk of myocardial infarction, allows drug treatment adaptation, and increases the quality of life.

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All authors have read and agreed to the published version of the manuscript.
The respective roles of each author are: ASC first/lead author performed the research, wrote the paper, and is the corresponding author, secondary authors DC and SMS supervised the paper, implemented feedback, and conceptualized some ideas.

Ethics approval and consent to participate
The manuscripts contain a study that hasn't been performed on humans, ethical review and approval were not applicable.

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Not applicable.

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Original Article

Does Intravenous Immunoglobulin Therapy Have a Role in the Treatment of Severe Coronavirus-19 Patients?

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Abstract: *We wanted to evaluate the efficacy of intravenous immunoglobulin (IVIG) treatment in Covid-19 patients in the intensive care unit (ICU). Sixteen (66.7%) male and 8 (33.3%) female adult severe Covid-19 patients who were treated with IVIG in the tertiary ICU between April 2020 and October 2021 at Ankara City Hospital were retrospectively reviewed. The median age of the patients was 63 (interquartile range (IQR):19). The patients were evaluated in terms of laboratory values and clinical results before and after IVIG treatment. There was no statistically significant difference between survived and deceased Covid-19 patients treated with IVIG in terms of age, gender, the onset of symptoms, presence of concomitant diseases, APACHE-II scores, duration of mechanical ventilator support, and length of stay in the ICU. When the laboratory values before and after IVIG treatment were compared, a significant decrease was identified only in alanine transaminase levels (p=0.01). However, there was a statistically significant difference in mortality in 5 patients with Guillain-Barré syndrome who all survived (p=0.001). IVIG treatment was effective in Covid-19 patients with Guillain-Barré syndrome. However, IVIG treatment did not have a statistically significant effect on pre and post-treatment laboratory values, morbidity, and mortality of severe Covid-19 patients in the ICU.*

Keywords: *Covid-19, critical care, Guillain-Barré syndrome, Intravenous immunoglobulin*

INTRODUCTION

Coronavirus disease 2019 (Covid-19) is caused by a RNA virus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1,2]. Covid-19 can lead to asymptomatic infection, mild symptoms, or severe pulmonary symptoms with hyperinflammation [3]. In severe Covid-19 patients, management of hyperinflammation and cytokine storm with appropriate antiinflammatory agents is recommended [4]. Different anti-inflammatory agents have been tried on critically ill patients during the Covid-19 pandemic.

IVIG is a biological agent which contains pooled polyclonal immunoglobulin G from the plasma of approximately one thousand healthy blood donors [1]. IVIG is used for the treatment of immunodeficiency, autoimmune and inflammatory diseases [5]. IVIG has not only anti-inflammatory effects but also the ability to neutralize bacterial toxins [6]. IVIG treatment has also been tried in epidemic diseases related to viruses such as influenza A H1N1, Middle East Respiratory Syndrome (MERS-CoV-2), and Severe Acute Respiratory Syndrome (SARS), and it has been reported that IVIG treatment decreased viral load, caused clinical improvement and decreased mortality in patients with a severe course [7,8]. In light of experience, IVIG treatment has been recommended to be used for the management of patients with Covid-19 [1,9-13]. However, the role of IVIG treatment in patients with severe Covid-19 remains unclear. Thus, this study aimed to evaluate the efficacy of IVIG treatment administered to severe Covid-19 patients in the ICU.

MATERIALS AND METHODS

Local ethics committee approval for this study was obtained from Ankara City Hospital (E2-21-835). The electronic records of 640 Covid-19 patients who were followed up between April 2020 and October 2021 in tertiary ICU were retrospectively reviewed. Thereafter, sixteen (66.7%) male and 8 (33.3%) female adult Covid-19 patients who were treated with IVIG in the ICU between April 2020 and October 2021 at Ankara City Hospital were included in this study for further analysis. The definitive Covid-19 diagnosis was based on both real-time reverse transcription polymerase chain reaction (RT-PCR) test results and ground glass appearance with pneumonia on thorax computed tomography. These severe Covid-19 patients had also respiratory distress with peripheral capillary oxygen saturation (SPO2) ≤ 93% in room air before admission to the ICU.

IVIG treatment was administered due to sepsis in 6 (25%) patients, Guillain-Barré syndrome in 5 (20.8%) patients, hematological disorders such as hemophagocytic syndrome, chronic lymphocytic leukemia and autoimmune hemolytic anemia in 9 (37.5%) patients, rheumatologic and immunological disorders such as immunodeficiency, systemic lupus erythematosus, myositis and vasculitis in 4 (16.7%) patients. In all patients, IVIG was administered at 0.4 g/kg/day for 3 consecutive days. These 24 patients were evaluated in terms of laboratory values before the first IVIG treatment session and after the third IVIG treatment session. Thus, neutrophil, lymphocyte and platelet count, neutrophil/lymphocyte ratio, lactate dehydrogenase, d-dimer, fibrinogen, ferritin, procalcitonin, c-reactive protein (CRP), interleukin-6, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were noted. Demographic data, the onset of symptoms, concomitant diseases, APACHE II scores, duration of mechanical ventilator support, length of ICU stay, and mortality rates were also recorded.

For all statistical analysis, Statistical Packages for the Social Sciences (v17.0 SPSS 24 Inc. Chicago, IL) software was used. Categorical data were presented as numbers and percentages, while quantitative data were presented as mean standard deviation or median and interquartile range (IQR), as appropriate. The differences between the two groups were evaluated with the Mann-Whitney U test and the chi-square test. The statistical significance level was accepted as p<0.05.

RESULTS

The median age of the patients included in this study was 63 (IQR:19), and the median time to symptom onset was 6.5 days (IQR:6). The median APACHE II score of these patients was 16 (IQR:9). The median length of stay in the ICU was 22.5 (IQR:34) days, whereas the median mechanical ventilator time was 19 days (IQR:29) (Table 1). Eighteen (75%) patients had concomitant diseases such as diabetes mellitus type 2, hypertension, and coronary artery disease. The most common disease accompanying Covid-19 was hypertension in 13 (54.2%) patients. The most common symptom was dyspnea in 17 (70.8%) patients. Fever was present in 7 (29.1%) and cough in 5 (20.8%) patients. Other less frequent symptoms were headache, sore throat, nausea, vomiting, impaired taste and smell, myalgia, and weakness. Complications, which were not related to IVIG treatment, such as gastrointestinal bleeding in 2 patients, cerebrovascular accident in 1 patient, myocardial infarction in 1 patient, pneumothorax in 6 patients, and seizures in 2 patients were observed in total in 12 (50%) patients. Antibiotics were empirically administered in 15 (62.5%) of the patients. Unfortunately, 16 (66.7%) patients passed away due to complications related to Covid-19 (Table 1).

Table 1: Characteristics of the Intravenous Immunoglobulin Treated Covid-19 Patients

Characteristics of Covid-19 patients	Survived	Deceased	Total	*P* value
Gender (n, %) Male Female	 6 (75) 2 (25)	 10 (62.5) 6 (37.5)	 16 (66.7) 8 (33.3)	0.67*
Age (years) (median, IQR)	61.5 (29)	63.5 (19)	63 (19)	0.85**
The onset of symptoms (days) (median, IQR)	6.5 (3)	6.5 (6)	6.5 (6)	0.60**
Length of ICU stay (days) (median, IQR)	29.5 (42)	22.0 (23)	22.5 (34)	0.42**
Mechanical ventilator duration (days) (median, IQR)	26 (59)	16 (27)	19 (29)	0.32**
Presence of concomitant disease (n, %)	7 (87.5)	11 (68.8)	18 (75)	0.62*
Diabetes mellitus type 2	4 (50)	4 (25)	8 (33.3)	0.36*
Hypertension	6 (75)	7 (43.8)	13 (54.2)	0.21*
Coronary artery disease	-	5 (100)	5 (20.8)	0.13*
Complications (n, %)	5 (62.5)	7 (43.8)	12 (50)	0.67**
Antibiotic use (n, %)	4 (50)	11 (68.8)	15 (62.5)	0.41**
APACHE II score (median, IQR)	15 (9)	18.5 (12)	16 (9)	0.13**

*†IQR: interquartile range. ‡ICU: intensive care unit; *Chi-square test was used. **Mann-Whitney U test was used*

There was no statistically significant difference between survived and deceased patients in terms of gender, age, the onset of symptoms, length of stay in the ICU, duration of mechanical ventilator support, accompanying symptoms, concomitant diseases such as diabetes mellitus type 2, hypertension, coronary artery disease, complication development, antibiotic use, and APACHE II scores. However, a significant difference was identified in mortality when the effect of IVIG treatment administered for Guillain-Barré syndrome in 5 patients and other disorders were compared (p=0.001). All patients with Guillain-Barré syndrome survived after IVIG treatment. All 6 patients, who were treated with IVIG for sepsis, died. Three patients died and 1 patient survived with rheumatological and immunological diseases. Seven of 9 patients with hematological diseases passed away, whereas 2 patients survived (Table 1). When the laboratory values before and after IVIG treatment were compared, a significant decrease was found only in ALT levels (p=0.01) (Table 2).

Table 2: Laboratory Parameters of Covid-19 Patients Before and After IVIG Treatment

Laboratory parameters	Before IVIG treatment	After IVIG treatment	*P* value*
Neutrophil count x10^9/L	8.4 (6.5)	8.0 (7.8)	0.84
Lymphocyte count x10^9/L	0.59 (0.49)	0.59 (0.45)	0.26
Platelet count x10^9/L	206.5 (152)	210 (142)	0.48
Neutrophil/lymphocyte ratio	13.0 (10.4)	11.8 (7.0)	0.39
Lactate dehydrogenase (U/L)	456.5 (349)	459.5 (193)	0.18
D-dimer (mg/L)	2.79 (4.91)	2.69 (6.05)	0.74
Fibrinogen (g/L)	5.4 (2.9)	4.4 (2.8)	0.22
Ferritin (µg/L)	1011 (1417)	715 (1198)	0.38
Procalcitonin (µg/L)	0.30 (0.35)	0.51 (1.17)	0.10
C-reactive protein (g/L)	66 (136)	95 (151)	0.76
Interleukin-6 (pg/mL)	31.1 (34.3)	38.2 (61.4)	0.40
Aspartate aminotransferase (U/L)	44.5 (50)	40.5 (26)	0.21
Alanine aminotransferase (U/L)	52.5 (48)	34.5 (46)	0.01

*In all patients, IVIG was administered 0.4 g/kg/day for 3 consecutive days. Numerical data are given as a median and interquartile range. IVIG: intravenous immunoglobulin. *Wilcoxon test was used.*

DISCUSSION

The severity of the cases, the IVIG dose, and the time of administration should be considered when evaluating the effect of IVIG on Covid-19. In our study, the patients who underwent IVIG therapy were severe Covid-19 patients and all of the patients received IVIG treatment at the same dose and duration (0.4 g/kg/day for 3 consecutive days).

Huang et al. reported that 45 non-severe Covid-19 patients who were treated with IVIG had no benefit beyond standard therapy in terms of duration of fever, virus clearance time, length of hospital stay, and use of antibiotics [14]. However, after the initiation of IVIG therapy in Covid-19 patients, there have been several studies published reporting that IVIG treatment reduced mortality in Covid-19 patients. Gharebaghi et al. reported that 30 Covid-19 patients were treated with IVIG four vials daily for three days and the in-hospital mortality rate was significantly lower in the IVIG group compared to the control group (6 (20.0%) vs.14 (48.3%), p=0.022) [15]. Sakoulas et al. administered IVIG 0.5 g/kg/d for 3 days to 16 Covid-19 patients, reporting that patients treated with IVIG had shorter median ICU stay (2.5 vs 12.5 days, p=0.006) in addition to improvement of hypoxia compared to the control group [16]. Raman et al. reported that 50 Covid-19 patients were treated with IVIG, who had a significantly shorter hospital stay, and faster recovery with an improvement in oxygen saturation. Moreover, Raman et. al claimed that positive RT-PCR tests of Covid-19 patients treated with IVIG became negative in a shorter period compared to the control group (7 vs 18 days) [17]. Ali et al. administered each group with 10 patients with different doses of IVIG 0.15, 0.20, 0.25, and 0.30 g/kg. Even 10 of 40 participants (25%) passed away, Ali et al. reported that IVIG increased survival chances and reduced the risk of Covid-19 progression [18]. Esen et al. reported that 51 Covid-19 patients who received IVIG had prolonged median survival time and reduced CRP levels [19]. Shao et al. stated that early administration (admission ≤ 7 days) of high-dose IVIG (> 15 g per day) not only improved the prognosis but also reduced 60-day mortality in severe Covid-19 patients [20].

The latest Surviving Sepsis Campaign Guidelines did not recommend IVIG therapy for the treatment of sepsis due to the low quality of evidence from previous studies [21]. All of our 6 patients treated with IVIG for sepsis died, however, all patients with Guillain-Barré syndrome survived after IVIG treatment in this study. In addition, there are several case reports of Covid-19 patients who had neurological disorders such as acute disseminated encephalomyelitis and Guillain-Barré syndrome which were reported to be successfully treated with IVIG [22-27]. Moreover, IVIG treatment has been reported to be effective in Covid-19 related immunological diseases such as multisystem inflammatory disease, myocarditis, and Kawasaki disease [28].

However, there are several studies claiming that IVIG treatment did not affect the need for mechanical ventilation, improvement in chest tomography, and reduction in mortality in severe Covid-19 patients [29]. Tabarsi et al. administered 400 mg/kg IVIG daily for three days to 52 patients, in addition to hydroxychloroquine and lopinavir/ritonavir treatment. IVIG treatment was initiated after 3.84 ± 3.35 days of admission and Tabarsi et al. did not identify any significant difference between the IVIG and control group in terms of the need for mechanical ventilation and mortality [29]. Hou et al. reported that 113 Covid-19 patients were treated with IVIG. Patients who received IVIG had a longer hospital stay of 23.0 days (19.0–31.0) with a higher mortality rate than the control group (9 (19.1%) vs 4 (6.1%), p= 0.032) [30]. Hou et al. stated that IVIG improved neither in-hospital mortality nor the need for mechanical ventilation in severe Covid-19 patients. Liu et al. stated that they treated 421 severe Covid-19 patients with IVIG and they observed no significant differences in 28-day mortality, acute respiratory distress syndrome, acute hepatic injury, acute kidney injury, diffuse intravascular coagulation, myocardial injury, shock and invasive mechanical ventilation between the IVIG and control group [31]. Focosi et al. evaluated 2401 Covid-19 patients from 10 studies and concluded that IVIG was not associated with reduced death risk, thus the current evidence from the medical literature did not support IVIG therapy in Covid-19 patients [32]. In a recently published retrospective, multi-center cohort study of critically ill Covid-19 patients, 109 patients treated with standard therapy and 74 patients who received IVIG therapy were compared [33]. Salehi et al. reported that there was no difference in length of stay in the ICU, duration of mechanical ventilation, and mortality between standard therapy and IVIG therapy groups. Thus, Salehi et al. concluded that IVIG therapy was not beneficial in critically ill Covid-19 patients [33].

Within our study, there was no statistically significant difference in mechanical ventilation duration and length of stay in the ICU between critically ill Covid-19 patients who survived or died (p=0.32 and p=0.42, respectively). However, we did not have the opportunity to compare critically ill Covid-19 patients who received IVIG therapy plus standard treatment and only standard treatment.

Moreover, the effect of IVIG on mild Covid-19 cases requires further research. Huang et al. reported that 45 non-severe Covid-19 patients who were treated with IVIG had no benefit beyond standard therapy in terms of duration of fever, virus clearance time, length of hospital stay, and use of antibiotics [14].

In this study, laboratory parameters before and after IVIG treatment were evaluated. There was neither a statistically significant increase nor decrease in acute phase reactants such as CRP, procalcitonin, ferritin, and fibrinogen before and after IVIG therapy (p=0.76, p=0.10, p=0.38, and p=0.22, respectively.) The only laboratory parameter which had a statistically significant decrease after IVIG therapy was ALT in critically ill Covid-19 patients (p=0.01) (Table 2).

IVIG may slightly increase liver function tests. However, Chen et al. reported that IVIG was effective in alleviating hepatic ischemia-reperfusion injury in a rat model by reducing ALT, AST, IL-6, and TNF-alpha [34]. Moreover, Omma et al. reported improvement in liver function tests including ALT after IVIG treatment [3].

IVIG treatment is an expensive therapy option in developing countries. Therefore, we had to administer IVIG as a salvage therapy only to severe Covid-19 patients. Unfortunately, 16 (66.7%) of 24 severe Covid-19 patients passed away despite IVIG treatment.

IVIG therapy has the risk of several side effects such as thromboembolism, renal failure, meningoencephalitis, and anaphylaxis [35]. We have encountered no side effects during IVIG therapy in our ICU.

The main limitation of this retrospective study was the small sample size. A larger sample size will help to learn more about the positive and negative effects of IVIG therapy in Covid-19 patients. As our intensive care unit was reserved for only severe Covid-19 patients during the pandemic, this study lacked a control group. The effect of IVIG on severe Covid-19 cases requires further well-designed prospective randomized controlled studies.

CONCLUSION

IVIG treatment was effective in Covid-19 patients with Guillain-Barré syndrome. However, IVIG treatment did not have a statistically significant effect on pre and post-treatment laboratory values, morbidity, and mortality of severe Covid-19 patients in the ICU.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.
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Authors' contribution

AConceptualization, S.G.I., C.M.A., M.E.Y., S.I.; methodology, M.E.Y., S.I.; software, M.E.Y.; validation, S.G.I., C.M.A., M.E.Y., S.I.; formal analysis, M.E.Y.; investigation, M.E.Y.; resources, S.G.I., C.M.A., M.E.Y., S.I.; data curation, S.G.I, C.M.A, M.E.Y., S.I.; writing-original draft preparation, M.E.Y.; writing-review and editing, S.G.I., C.M.A., M.E.Y., S.I.; visualization, M.E.Y.; supervision, S.I.; project administration, S.I. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Ankara City Hospital (E2-21-835).

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Original Article

Deregulation of the Hippo Signaling Pathway in Virus-Associated Cancer

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Abstract: *Hippo signaling has been recognized as a newly identified tumor suppressor signaling pathway that can regulate cellular processes including regeneration, cell death, differentiation, and development. Its dysregulation through overexpression of YAP (Yes-associated protein) and TAZ (PDZ-binding motif) as two main oncogenic factors of the Hippo pathway has a crucial role in several cancers. Because of the limited prognosis and therapeutic targets, further understanding of molecular pathways involved in tumorigenesis is one of the interesting issues in studies. Here, we demonstrated that some viruses, through dysregulation of the Hippo signaling pathway can be implicated in transformation, metastasis, and chemotherapeutic drug resistance in virus-related cancer and also help processes involved in the pathogenesis of viral infection such as persistence.*

Keywords: *Hippo signaling pathway, virus, cancer*

INTRODUCTION

The Hippo signaling pathway was discovered to be involved in controlling organ size and cell differentiation in Drosophila melanogaster for the first time, and it has recently been recognized as a critical tumor-suppressor network [1]. Consequently, the correlation of deregulated Hippo pathway signaling in several cancer types such as breast, liver, lung, prostate, gastric and colorectal tumors has been reported. In addition to this role, the Hippo pathway is involved in the conservation of tissue-specific stem cells, tissue renewal, and damage recovery [2,3].

The Hippo pathway is a kinase cascade that includes kinases MST1 and MST2 (also known as STK4 and STK3), large tumor suppressors 1 and 2 (LATS1 and LATS2) and adaptor proteins SAV1 (Salvador homolog 1), and MOB kinase activator 1A and 1B (MOB1A and MOB1B). MST1 and MST2 induce LATS1 and LATS2 phosphorylation, which in turn facilitates LATS-dependent phosphorylation of the homologous oncoproteins YAP (Yes-associated protein; encoded by YAP1) and TAZ (PDZ-binding motif, also known as WWTR1) that inhibit the activation of transcriptional co-activators Yorkie (Yki), YAP, and TAZ [4-6].

Hippo pathway main effectors YAP and TAZ, phosphorylation by the Hippo core complex can retain them in the cytoplasm where they expose to proteasomal degradation [7]. Since TAZ/YAP mutations rarely happen other mechanisms are more likely responsible for Hippo's relevance in cancer. Growing evidence suggests that a complex network of interactions with critical pathways can modulate YAP/TAZ activity such as TGFB, GPCR, Notch, EGFR, and angiogenesis [8].

In the case of modulation of Hippo signaling, stimulation of G protein-coupled receptors (GPCRs) associated with Gas might lead to phosphorylation of LATS and consequently down-regulation of YAP. GPCRs as integral membrane receptors activated by external ligands their binding leads to the activation of the alpha-subunits of subclasses of G proteins. In contrast, stimulation of Ga12/13-coupled GPCRs is associated with suppression of LATS and up-regulation of YAP [9,10].

Other instances of membrane receptors that reported to modulate YAP expression are the receptor tyrosine kinases EGFR and ERBB4 [11]. The EGFR ligand amphiregulin (AREG) is an important YAP target that provides a positive cycle that mediates YAP and EGFR signaling and can stimulate intensifying proliferative signals from both pathways. Also, ERBB4 as a member of the EGFR family, via interaction of YAP and possibly TEAD1 with its intracellular domain in the nucleus, demonstrated either induction of YAP activation or increase expression of YAP target genes [12].

Oncoviruses are associated with about 12% of all human cancers. People carry at least one of these oncoviruses in their life, but these infections only a small proportion of these individuals go on to develop cancer [13]. The interaction between viral factors and the host is a complex process to provide a desirable microenvironment for oncogenesis [14]. The aim of this review is the improvement of our understanding of mechanisms of oncogenesis modulation by different viruses and a better understanding of tumorigenesis that may result in more effective targeted therapy in the future. By the recent rises in viral-related carcinomas and the link between Hippo and viruses, we assessed to determine if Hippo signaling is the main target in such cancers.

HEPATITIS B Virus

In recent decades human hepatocellular carcinoma (HCC) with approximately 746,000 deaths per year, identified as the sixth most common cancer and the third leading reason for cancer-related deaths in human populations [15].

Hepatocarcinogenesis, due to several genetic and epigenetic changes which occur through different stages of the disease is known as a complex process [16]. As regards the fact that hepatitis B virus (HBV) infection has a key role in the establishment of HCC, it is important to know its proteins' roles in Hepatocarcinogenesis. HBV encoded four proteins and among these proteins, the HBV X protein (HBx) is a multifunctional protein and has a critical role in hepatocellular carcinogenesis. Multiple signaling pathways are participating in the HCC progression [17,18].

Many studies are designed to assess the molecular and cellular mechanism of HCC because finding a suitable treatment for HCC is an immediate task for healing patients suffering from HCC. Recently, numerous researches have shown the role of the Hippo pathway in organ size control, cell proliferation, cell death, and tumor progression [19,20]. Although the role of YAP, which is a downstream factor of the Hippo signaling pathway in HCC, remains unclear, this review provides a vision for the regulation of YAP upstream and downstream effectors involved in hepatic cancer [21].

Interestingly, the hyperexpression of YAP can result in epithelial-to-mesenchymal transition (EMT) and may inhibit apoptosis, growth factor-independent proliferation, and anchorage-independent growth [22]. The liver has an amazing characterization that can lead to having unique features such as Growth control which in the liver has some unusual features. The liver has been known for its noticeable capacity to renew consequently to a two-third partial hepatectomy (PHx) or injury. The natural liver in an adult is composed of two parenchymal cell types — hepatocytes and cholangiocytes [23].

Dedifferentiated hepatocytes as multipotent stem cells are capable to be the source of tissue renovation in the damaged liver [24]. Factors of the Hippo signaling pathway including MST1 and MST2 have been demonstrated to have key roles in adjusting or restricting liver progenitor cells. Thus, it is predicted that the Hippo can be involved in the suppression of stem/progenitor cell proliferation in the mature liver [25]. Furthermore, some studies also showed that knocking out YAP resulted in hepatocyte and cholangiocyte injury and loss [26].

In synergy with the functional roles of Hippo signaling, any change of upstream effectors of the hippo pathway like Mst1/2 can result in over-proliferation and sometimes Hepatocarcinogenesis. Since HBV is commonly the major reason for HCC; YAP possibly has an important role in its carcinogenesis. Interestingly, YAP expression is frequently shown to be increased in HBV infections resulting in HCC, infected cell lines, and HBx transgenic mice. Through the CRE-dependent HBx attaching to the YAP promoter, HBx can increase YAP and follow stimulate YAP target genes which leads to the proliferation of hepatoma cells. Findings revealed the importance of the Hippo signaling pathway in cancer and suggested new treatments for HBV-infected patients to inhibit progression to HCC [27].

Studies have revealed that the loss of Mst1/2 may result in the promotion of several tumors in the liver of mice. The loss of both Mst1/2 is required to affect the liver since the loss of Mst1 or Mst2 is not effective on liver size or the number of liver tumors [28]. These findings firmly revealed the effect of Mst1/2 on YAP activity. Interestingly, lack of YAP phosphorylation and impairment of Mst1/2 activity that occurs in carcinogenesis have been reported in 30% of HCCs [29].

The liver-specific NF2/merlin inactivation may lead to an increase in hepatic stem cell number with a lack of any changes in differentiated hepatocytes [30]. The studies on Sav or Mst1/2 positive livers for assessment of the effectiveness of

YAP/TAZ and Lats1/2 phosphorylation in the hippo pathway displayed complex results regarding the need for Mst1/2 or Sav in Lats1/2 phosphorylation [29,31] and since in mouse fibroblasts, for YAP phosphorylation, presence of Mst1/2 was not crucial, it is considered that the pathway may be specific in hepatitis [29].

In a study on transgenic mice, it was shown that YAP induction can lead to abnormal hepatocyte expansion after 2 months and consequently several nodes have been reported in hepatitis [32]. The nodes were reported to be a collection of hepatocytes in a generative condition that condensed the near parenchyma and revealed severe HCC. Also, this liver-specific loss of either Mst1/2 in hepatocytes leads to noticeable liver growth because of its deregulated cell proliferation [28].

On the other hand, when transgenic mice models with YAP hyperactivity crossed with mice lost its DNA-binding domain TEAD2 do not promote HCC. Lacking YAP overexpression may lead to having mice with livers deficiency in Nf2/merlin after crossing with mice that have the defective TEAD2 gene, confirming the therapeutic concept of using the TEAD with YAP in the complex. Verteporfin injection has been demonstrated to reduce liver mass in YAP transgenic animal models and also has no side effect on the wild mice tissue homeostasis, defining the potency of compounds for therapeutic administration in patients with HCC. Verteporfin displayed numerous important features that make its clinical execution for HCC promising [32].

In HCC cell lines induction of YAP results in further resistance to doxorubicin-induced apoptosis; while, inhibition of YAP expression by RNA interference has been reported to reverse YAP-increased chemoresistance. These processes are mediated by the MAP kinase signaling pathway induction rather than an AKT-dependent mechanism of chemoresistance [33]. This process of chemoresistance occurs after treatment by the MEK1/2 suppressor U0126 before doxorubicin injection, which noticeably reduces cell vitality in YAP overexpressing HCC lines [34].

Survivin is accepted as an antiapoptotic protein and in HCC patients identified to be associated with a weak prognosis. Recent studies have shown YAP overexpression is in associate with survivin induction in patients with HCC. It seems that YAP has this oncogenic capacity on the cell with a survivin increase leading to carcinogenesis [35].

HUMAN PAPILLOMAVIRUS

Cervical cancer considers the most important cancer in women because it is the fourth leading reason of cancer-related death in women. Due to the detection of human papillomavirus (HPV) DNA in the majority of cervical cancer patients, high-risk HPV types recognize as the cause of cervical cancer [2].

In a study by Chunbo He et al results implicated that YAP1, one of the main oncogenic effectors of the Hippo pathway, was (11%) amplified several times, whereas LATS1/2, MST1, and FATs, that identified as upstream tumor inhibitors of the Hippo pathway, were not detectable or mutated in cervical cancer patients. YAP1 induction in cervical cancer cells noticeably triggers the expression of EGFR and its related ligands AREG and TGFa. On the other hand, TGFa and AREG, through EGFR signaling, can block the Hippo pathway and induce YAP protein (dephosphorylated LATS1, MOB1, and YAP1) to promote cervical cancer cell growth. Recognition of this circumstance between Hippo/YAP and EGFR in human cervical cancer provides an opportunity to combine targeting of the Hippo/YAP and EGFR pathways to have an efficient way to treat cervical cancer [36].

In addition, hyperactivation of YAP1 can be an important mediator to the persistence of HPV infection. First, studies report that hyperactivated YAP1 increases the expression of HPV receptor molecules such as ITGRA6, SDC1, and EGFR, which through the early stage of HPV infection can help the HPV entry process. Second, is that hyperactivation of YAP1 in cervical epithelial cells may reduce viral pattern recognition by the innate immune system. Some studies report that TLR2 and TLR4 and adaptor proteins MYD88 and TRIF were suppressed, which could facilitate HPV infection. Third, there is evidence that ectopic expression of YAP may suppress the activation of transcriptional factors that are essential for the production of type I IFNs, including IRF1, IRF3, and IRF7, leading to the reduction of IFNA1, IFNB1, and IFNE in hers. IFNs can activate antiviral factors of the immune system and play their role in the progress of innate and adaptive immune responses. Also, some studies showed that the expression and activation of main components of the type I IFN pathway, like IFNAR2, STAT1, JAK1, and IRF9, are suppressed by the expression of YAPS127A in the epithelium of the cervix. Suppression of the IFNAR2/JAK1/STAT1 signaling pathway leads to decreasing of antiviral ISGs, such as MX1, CH25H, and IFITMs (blockers of virus entry), and APOBEC3G, OAS1, ISG15, and IFI44L (suppressors of virus translation and replication) [37].

The HPV oncoproteins E6 and E7 expression can result in the suppression of some essential tumor inhibitors such as TP53 and RB1 in many types of cells [38,39]. Suppression of TP53 and RB1 extends cell life (by inhibition of death) and may lead to immortality in cervical epithelial cells, facilitating malignancy of HPV-infected cells by an oncogenic signal (including hyperactivation of YAP1) [40,41].

In epithelial cell cultures, cell-cell contact development is a key step in differentiation before the formation of distinguished apicobasal surfaces. The transcriptional co-activator YAP plays a key role in this event [42,43]. Numerous reports have shown that cell-cell contacts activate the LATS1/2 serine/threonine kinases which can phosphorylate YAP [44,45]. During phosphorylation, YAP restricts from the nucleus and it inhibits the transcription of key genes in the cell cycle [42,46]. A cervical cancer study recently demonstrated that in high-grade cervical lesions levels of nuclear YAP are increased [47].

There are some studies demonstrating that HPV16-E6 or HPV16-E6/E7 double-transgenic mice treated with estrogen may promote cervical cancer [48,49]. Related to that result, other studies report that YAP was increased in the E6 and E6/E7-induced cervical tumor cells, and YAP was localized in the nucleus of these cells [36]. The human foreskin keratinocyte(HFK) “raft” culture infected with HPV16 makes a unique model for the assessment of the HPV16 proliferation cycle [48,50].

YAP can be inactivated by two mechanisms: first, the Ser127 phosphorylation-mediated spatial adjustment (system of nuclear-cytoplasmic shuttling); and second, the Ser397 (Ser381 in YAP protein isoform 2) phosphorylation-mediated adjustment (the phosphodegron-induced degradation). These two mechanisms are applicable directly to prevent YAP oncogenic activity [51,52]. It has been observed that CK1d/ε and SOCS6 proteins are crucial for YAP degradation [52,53]. Although expression of HPV E6 in HT3 cells causes a noticeable increase in CK1d/ε and a reduction in p-YAP (S397), it suggests that the CK1d/ε pathway may not participate in the HPV E6-mediated YAP protein turning [40]. HPV E6 increases YAP protein levels, but also it upregulates the expression and activation of AREG protein in the cell culture. These observations showed a possibility that HPV E6 protein may be associated with the Hippo-ERBB signaling circle and also implicating the transformation and development of cervical cancer. Interestingly, HPV16 E6 and E7 could transcriptionally activate EGFR expression [40,54]. The HPV16 E6 makes receptor protein tyrosine kinase to signaling and facilitates the internalization of phosphorylated receptors [55] (Figure 1).

Figure 1: Schematic presentation of reciprocal interactions between HPV and Hippo signaling pathway

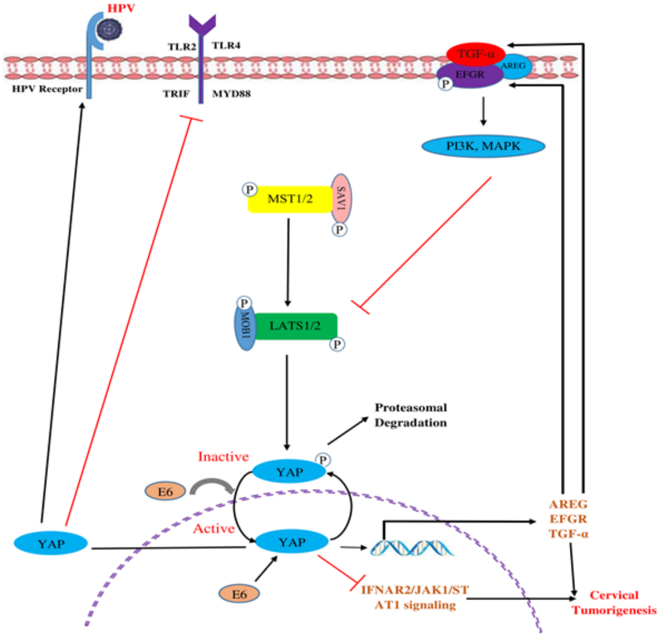

POLYOMAVIRUSES

Through identification of SV40 tumorigenesis in specific laboratory animals and transforms several types of cultured cells offered an opportunity to discover the molecular basis of the tumor. Viral oncogenes were introduced as key tools in the recognition of tumor inhibitor pathways in human primary cells. The p53 and p16/pRb tumor inhibitor pathways have been introduced as targets of SV40 large T. Hahn’s study indicates a transformation model [56], expressed HRas V12, and a construct encoding SV40 large T to target the p14ARF/p53 and p16INK4A/pRb pathways. Recently it is discovered that SV40 small T (ST) (encoded in the large T construct) is needed without requiring large T to enhance anchorage-independent growth [57].

Murine polyomavirus also has frequently presented visions into tumorigenesis by demonstrating important modulation mechanisms including tyrosine phosphorylation and phosphoinositide 3-kinase (PI3K) signaling. Previous studies showed that polyomavirus small T antigen (ST) associates with YAP, an effector of the Hippo pathway, to modulate differentiation. Other studies introduced YAP as a target of middle T antigen (MT) required for transformation [58,59]. Wild-type MT via a surface containing residues R103 and D182 binds to the YAP WW domains. Mutation in each one of R103 or D182 of MT disrupts YAP binding. This happens without disturbing bindings to other signaling factors or the strength of PI3K or Ras signaling. One of the other genetic disruptions of MT binding to YAP or stopping of YAP via short hairpin RNA (shRNA) decreased MT transformation, offering that YAP has an important participation in the transformed phenotype. MT targets YAP via triggering signaling pathways that affect it and consequently bind to it. The signaling of either wild-type MT or the YAP associate MT mutant develops YAP phosphorylation at S127 and S381/397 (YAP2/YAP1) [58]. In addition to previous roles of these phosphorylated serines, MT signaling results in the reduction of YAP from the nucleus and degradation. When MT Binds to YAP, it associates YAP with protein phosphatase 2A (PP2A), resulting in the YAP dephosphorylation together with the MT complex. Furthermore, this process results in the increase of YAP in membranes. Overall, these data demonstrate that YAP develops MT transformation by mechanisms that may be due to YAP's established major transcriptional activation functions [60].

The capability of ST to discuss anchorage-independent growth brings with it its ability to inactivate the PP2A function. Suppression of PP2A may bypass the need for ST in cellular transformation models, and mutations inactivating PP2A subunits have been recognized in tumors [61,62]. PP2A has several substrates including, AKT, b-catenin, and c-Myc which are defined to be activated by ST. Although the total overexpression of these proteins only incompletely replaced ST in cellular transformation, it suggests that undefined PP2A effector pathways may be involved [62].

Hippo/MST kinase acts by a membrane-associated complex including the tumor inhibitor NF2/Merlin [63]. MST kinases enhance activating LATS kinases, which subsequently phosphorylation of the transcriptional coactivators YAP and TAZ, resulting in their inactivation of them via cytoplasmic isolation and increased degradation [64-66]. Suppression of YAP and TAZ restricts proliferation and triggers apoptosis [67].

Interestingly, it is observed that overexpression of YAP is enough to result in tumorigenesis in mice. Both SV40 ST and the Merkel cell polyomavirus ST induce endogenous YAP. Some studies provide data that ST acts through PAK1 to inactivate the NF2 tumor suppressor to alleviate the inactivation of YAP. Activation of PAK1 at the cell membrane is sufficient to bypass the requirement for ST. Restricting PAK1 activity disrupts the transforming capability of the ST protein. Therefore, ST detaches YAP from modulation via the Hippo tumor inhibitor pathway. Induction of Active YAP is required and enough to replace ST in cellular transformation. Therefore, the YAP proto-oncogene is an important mediator of the transforming properties of the ST oncoproteins [68] (Figure 2).

Figure 2: Schematic presentation of reciprocal interactions between Polyomaviruses and Hippo signaling pathway

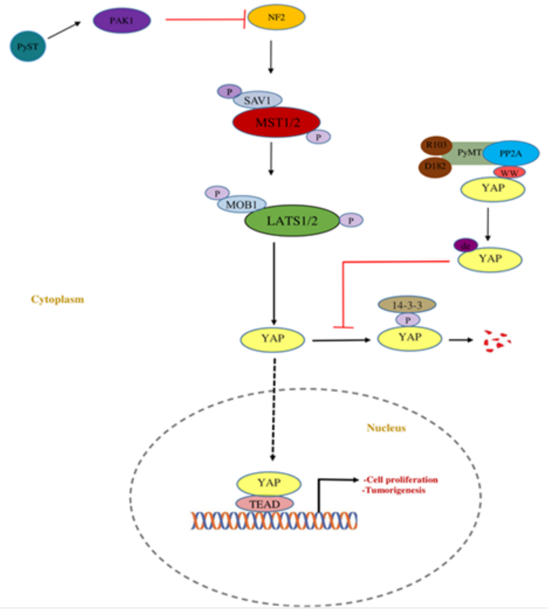

EPSTEIN-BARR VIRUS

Epstein-Barr virus (EBV) is recognized as a linear dsDNA virus and a member of the human gamma-herpesvirus family that observed to correlate with various B-cell lymphomas, such as Burkitt's lymphoma, Hodgkin's lymphoma, and other tumors like nasopharyngeal carcinoma, T-cell non-Hodgkin lymphomas, and gastric carcinomas. These cancers are firmly correlated with latent Epstein-Barr virus (EBV) infection, genetic factors, and environmental factors thus EBV can regulate mechanisms affecting carcinogenesis, proliferation, death, migration of cells, epigenetic changes in lymphocyte-specific development, and induce cell immortalization. The main mechanisms related to the carcinogenic effectors of EBV consist of LMP1, LMP2, and microRNA that we will introduce bellow [69-71].

The capacity of LMP1 to immortalize and transform cells possibly depends on simultaneously regulating cellular signaling pathways which suppress apoptosis or mediate proliferative, growth factor-like effects. Furthermore, LMP1 can induce a cancer progenitor cells (CPC)-like appearance in epithelial cells, offering that LMP1-induced phenotypic alterations can be related to the promotion of cancer [72]. Therefore, the mechanism and role of LMP1 in immortalizing and transforming cells is complex and several important questions remain to be answered.

Previous studies demonstrated that LMP1 could induce actin remodeling [73]. The actin cytoskeleton is a key factor for the modulation of the Hippo pathway. These data make us investigate How LMP1 can upregulate TAZ expression by inhibition of the Hippo pathway. In a study, findings determined that the LMP1-targeted CNE1-LMP1 cells decreased TAZ expression. The target genes of TAZ are CTGF and Cyr61. Other researches demonstrate that LMP1 can trigger the mRNA expression of CTGF and Cyr61 [74]. There is the possibility that TAZ may be involved in the positive modulation of cell proliferation and survival associated with the obtaining of cancer stem cell (CSC) properties and epithelial-to-mesenchymal transition (EMT) [75, 76]. Previously, some studies have been done to determine if LMP1 can develop EMT by TAZ and define if the morphology alters. The result of these studies has shown some morphological variations from epithelial to mesenchymal phenotypes. LMP1 can develop cell proliferation, EMT, and CSC-like characteristics by TAZ and give us some visions and rationales for the LMP1-mediated resistance to cancer treatments [74].

On the other hand, it has been shown that phosphorylation of the WW domain of the transactivator TAZ by activated LATS1/2, leads to TAZ degradation [77]. Studies have shown that LMP1 can elevate the level of TAZ and increase TAZ nucleus translocation. Thus, it is possible that LMP1 could suppress LATS1/2 activation, resulting in the enhancement of TAZ stability [74] (Figure 3).

Figure 3: Schematic presentation of reciprocal interactions between EBV and Hippo signaling pathway

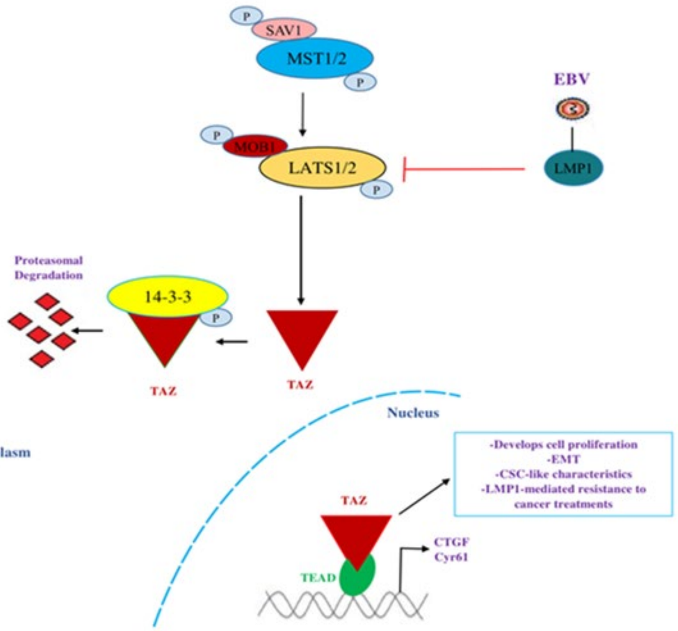

KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

Kaposi sarcoma-associated herpesvirus (KSHV) is a double-stranded linear DNA virus of the human gamma-herpesvirus family from rhadinovirals and also an oncogenic virus that is responsible for human Kaposi sarcoma (KS) and is associated with an AIDS-defining malignancy. One of the main viral proteins of KSHV is a viral G-protein-coupled receptor (vGPCR). KSHV encodes vGPCR which is essential for the initiation and progression of Kaposi sarcoma [78-80]. Previous studies identified that YAP/TAZ, two homologous oncoproteins that can be suppressed by the Hippo tumor inhibitor pathway, activates in KSHV-infected cells in in vitro conditions, KS-like tumors in mice, and clinical human KS samples [81].

The molecular process to which vGPCR contributes in KSHV-induced oncogenesis remained uncertain but it is suggested to be involving both direct and paracrine oncogenic effects, that are activated by growth factors including VEGF and PDGF induced by vGPCR [82,83]. The KSHV protein (vGPCR) activity is via Gq/11 and G12/13 to suppress the Hippo pathway kinases Lats1/2, enhancing the activation of YAP/TAZ. Also, clearance of YAP/TAZ inhibits vGPCR-induced cell proliferation and tumorigenesis in a xenograft mice model. The transformation of cells mediated by vGPCR is sensitive to pharmacological blockers of YAP [84,85].

It is demonstrated that the Knockdown of YAP or TAZ can inhibit the vGPCR-induced CTGF expression, offering that YAP/TAZ enhances the biological functions of vGPCR. In addition to the Hippo pathway, studies have shown other signaling pathways that have also been related to the oncogenic effect of vGPCR [85]. For instance, PI3Kγ is activated by vGPCR and has a key role in vGPCR-induced sarcomas, whereas NFkB has an indirect role in vGPCR-induced paracrine neoplasia. It seems the activation of YAP/TAZ is associated with other vGPCR downstream signaling events, including PI3K and NFkB, which generally participates in KS tumorigenesis in KSHV-infected patients. It is noticeable that YAP/TAZ also upregulates the expression of several growth factors, including CTGF and insulin-like growth factor (IGF) [86,87], that can be associated with neoplasia caused by vGPCR in a paracrine condition [81, 88].

Some studies revealed that Hippo pathway activation has a key role in the promotion of the lytic cycle and transition from latency. Inactivation of the Hippo pathway modulator YAP1 may promote reactivation from latency, lytic protein increase, and production of infectious virions. Interestingly, expression of the YAP1-inactivating kinase LATS1 consequently induces reactivation from latency. Conversely, LATS1 silencing decreases viral protein expression along the late phase of lytic replication. However the detailed mechanism of switching of KSHV latent/lytic by the Hippo signaling pathway is still unclear, increased amounts of nuclear centers containing the viral latency-associated nuclear antigen (LANA) have been reported in YAP1-silenced cells [89] (Figure 4).

Figure 4: Schematic presentation of reciprocal interactions between KSHV and Hippo signaling pathway

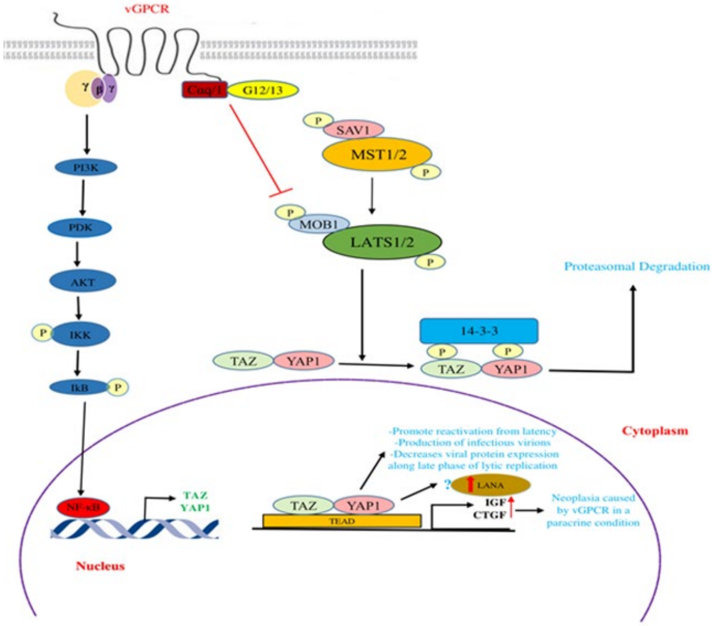
vGPCR
γ
β
γ
Gαq/11
G12/13
P
SAV1
MST1/2
P
PI3K
PDK
AKT
P
IKK
IkB
P
P
MOB1
LATS1/2
P
Proteasomal Degradation
14-3-3
P
P
TAZ
YAP1
TAZ
YAP1
Cytoplasm
-Promote reactivation from latency
-Production of infectious virions
-Decreases viral protein expression along late phase of lytic replication
?
LANA
NF-κB
TAZ
YAP1
TAZ
YAP1
TEAD
IGF
CTGF
Neoplasia caused by vGPCR in a paracrine condition
Nucleus

HEPATITIS C VIRUS

Hepatitis C virus (HCV), a member of the Flaviviridae family, is an enveloped RNA virus with a positive sense single-strand RNA genome [90]. Chronic HCV infection is one of the main risk factors for disease progression to metabolic disorders, fibrosis, cirrhosis, and HCC [91]. Epithelial-to-mesenchymal transition (EMT) is a physiological process by which epithelial cells lose their cell polarity and cell-to-cell junctions, and achieve migratory and invasive properties to become mesenchymal cells [92]. Previous literature reviews have shown that EMT plays a crucial role in promoting metastasis in various human cancers including HCC [93]. During the process of EMT, loss of epithelial markers, especially E-cadherin is mediated by transcriptional repressor SNAIL that binds to the E-box motif on the promoter region of E-cadherin [94]. HCV non-structural 4B (NS4B) protein is a 27kDa hydrophobic protein that role in HCC pathogenesis mediate interaction networks with cellular host proteins [95]. A study recently showed that interaction NS4B with scribble protein, a scaffold protein involved in regulating cell polarity and cell proliferation in epithelial cells, suppresses the Hippo signal pathway that subsequently results in the induction of the PI3K/AKT pathway. Induction of the PI3K-Akt pathway upregulates Snail expression via translocation to the nucleus. Finally, the up-regulation of Snail induces EMT progression by reducing E-cadherin expression which contributes to HCV-caused HCC progression, invasion, and metastasis [96].

HCVs entry to hepatocytes is recognized to be mediated by CD81 [97]. Ezrin is a member of a membrane-cytoskeleton linking protein that increases in HCC and is identified in correlation with malignant HCC [98]. In normal hepatocytes, CD81 overexpression results in the phosphorylation of ezrin and increasing nuclear YAP. As the CD81-SYK-ezrin complex causes signals to modify cell construction through protein interactions, gene expression is mediated by YAP via downstream regulation of the Hippo signaling pathway [99]. Also deregulation of the Hippo signaling pathway and increased YAP activity have been demonstrated in 50% of HCC cases [100]. In contrast since Glypican (GPC)-3 is one of the main ligands for CD81, it upregulates the expression of CD81 in HCC cells enhances activation of Hippo, a reduction in ezrin phosphorylation, and a reduction in YAP. Interestingly HCV E2 protein mimics the function of GPC3, enhances the activation of the Hippo signaling pathway, and subsequently reduces YAP and cell proliferation. Consequently, although any hepatocytes which have missing CD81 expression are considered to be resistant to HCV infection but can expand faster than the normal hepatocytes, loss of CD81 is a usual event in the promotion of hepatic neoplasia [101] (Figure 5).

Figure 5: Schematic presentation of reciprocal interactions between HCV and Hippo signaling pathway

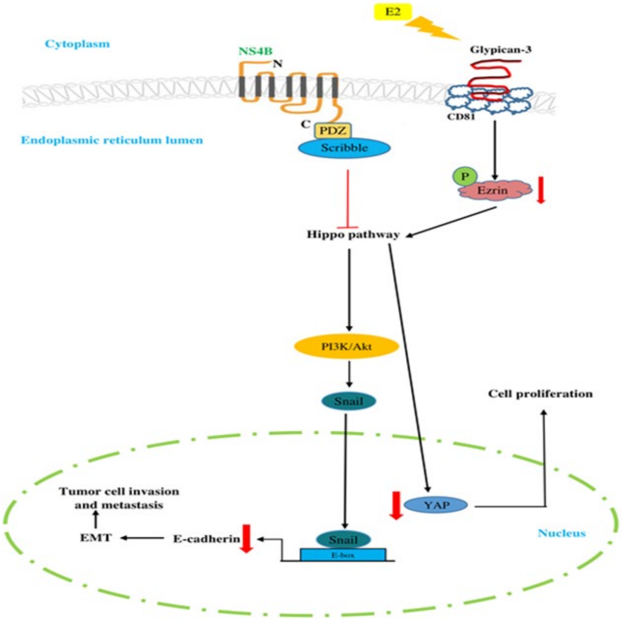

CONCLUSION

The Hippo signaling is an emerged evolutionarily conserved tumor suppressor pathway that regulates proliferation, apoptosis, differentiation, and development, whose dysregulation overexpresses YAP and TAZ as two major oncogenic downstream effectors of Hippo pathways that play a crucial role in multiple human pathological diseases, such as cancer (Table 1).

Table 1: The summary of the deregulation of the hippo signaling pathway by oncoviruses

Virus	Viral protein	Component of hippo signaling	Mechanism/Effect	References
HBV	HBx	YAP	HBx can increases YAP via the CRE-dependent HBx binding to the YAP promoter and following stimulation of YAP target genes	[27]
HPV	-	YAP1	Hyperactivation of YAP1 can be an important mediator in the persistence of HPV infection	[37]
	-	YAP1	Hyperactivation YAP1 increases the expression of HPV receptor molecules such as ITGRA6, SDC1, and EGFR	[37]
	-	YAP1	Hyperactivation of YAP1 in cervical epithelial cells may reduce viral patterns recognition TLR2 and TLR4 and adaptor proteins MYD88 and TRIF of the innate immune system	[37]
	-	YAP1	Expression of YAP may suppress the activation of transcriptional factors that are essential for the production of type I IFNs, including IRF1, IRF3, and IRF	[37]
	E6/E7	YAP	Expression and activation of main components of the type I IFN pathway, like IFNAR2, STAT1, JAK1, and IRF9, suppressed by expression of $YAPS^{127A}$	[37]
		YAP	YAP was increased in the E6 and E6/E7-induced cervical tumor cells and YAP was localized in the nucleus of these cells	[36]
PyVs		ST YAP	Polyomavirus small T antigen (ST) associates with YAP, an effector of the Hippo pathway, to modulate differentiation	[58]
		MT YAP	MT Binds to YAP, it associates YAP with protein phosphatase 2A (PP2A), resulting in the YAP dephosphorylation together with the MT complex	[60]
		ST YAP	ST acts through PAK1 to inactivate the NF2 tumor suppressor to alleviate the inactivation of YAP	[68]
EBV		LMP1 TAZ	LMP1 can upregulate TAZ expression by inhibition of Hippo pathway proteins	[74]
		LMP1 TAZ	LMP1 can develop cell proliferation, EMT, and CSC-like characteristics by TAZ	[74]
		LMP1 LATS1/2	LMP1 could suppress LATS1/2 activation, resulting in the enhancement of TAZ stability	[74]
KSHV		vGPCR Lats1/2	The KSHV protein (vGPCR) activity is via Gq/11 and G12/13 to suppress the Hippo pathway kinases Lats1/2, enhancing the activation of YAP/TAZ	[85]
		vGPCR YAP/TAZ	Activation of YAP/TAZ is associated with other vGPCR downstream signaling events, including PI3K and NF-kB	[86,87]
		- YAP1	Inactivation of the Hippo pathway modulator YAP1 may promote reactivation from latency, lytic protein increasing, and production of infectious virions	[89]
		LATS1	Expression of the YAP1-inactivating kinase LATS1 consequently induces reactivation from latency	[89]
HCV	*NS5B*	-	*Interaction NS4B with scribble protein suppresses the Hippo signal pathway that subsequently resulting in the induction of PI3K/AKT pathway*	[96]
	E2	YAP	HCV E2 protein mimics the function of GPC3, enhances the activation of the Hippo signaling pathway, and subsequently reduces YAP	[101]

The activation of YAP and TAZ contributes to cancer initiation, metastasis, and anticancer drug resistance via interactions with several signaling pathways in many human tumors. Oncoviruses either directly or indirectly by modulating a variety of cellular signaling pathways lead to the dysregulation of the Hippo signaling pathway to increase the expression of the downstream effectors TAZ and YAP which can be implicated in transformation, metastasis, and chemotherapeutic drug resistance in virus-related cancer and also to facilitate some processes involved in the pathogenesis of viral infection such as entry, persistence, escape from the immune system, and latency. However, in some cases inactivation of YAP can aid the pathogenesis of KSHV and HCV. (Figure 6).

Figure 6: Schematic presentation of reciprocal interactions between oncoviruses and Hippo signaling pathway

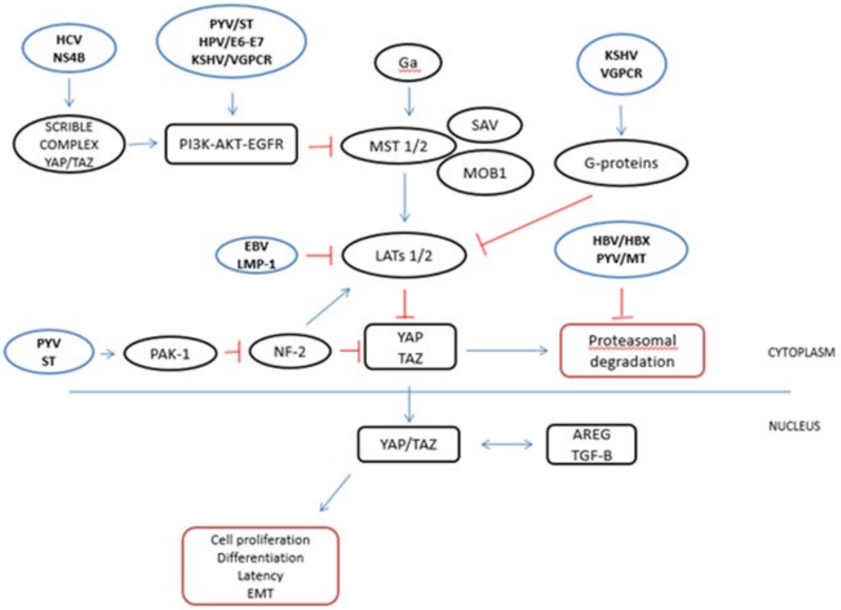

With these interpretations, we recommend that the overexpression of the major downstream effectors of the Hippo pathway, TAZ, and YAP can be used as prognosis markers in cancers caused by oncoviruses. Overall, Hippo signaling is a new pathway in viral infection, and in this way, better understanding interaction between oncoviruses with Hippo signaling can be used as a new therapeutic target in virus-caused cancers.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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Original Article

Relationship between Mental Health, Life Satisfaction, and Job Satisfaction of Nurses

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Abstract: *Improving the mental health of nurses and investigating the factors affecting it leads to the improvement of job performance and the quality of nursing care. Aim: The present study aimed to determine the relationship between mental health, life satisfaction, and job satisfaction of nurses in Ilam city in 2022. The present correlational study was carried out on 160 nurses working in educational hospitals of Ilam city and was selected by stratified random sampling in 2022. A four-part questionnaire was used: the first part included a question on demographic information. Minnesota Satisfaction Questionnaire (MSQ) (1977), Diener et al.'s Satisfaction with Life Scale (SWLS) (1985), and Derogatis et al.'s Symptom Checklist-90 (SCL-9) (1973) were included in the second, third and the fourth parts, respectively. Results showed no significant relationship between the total score of mental health and the total score of job satisfaction. There was a significant relationship between the total job satisfaction score and life satisfaction score (r=0.267, p=0.001). There was also an inverse and significant relationship between the total mental health score and the life satisfaction score (r=0.294, p=0.001). The collected data was analyzed using descriptive tests (frequency, mean percentage, and standard deviation) and non-parametric Spearman and Kruskal-Walli's correlation tests in SPSS ver. 22. P-value<0.05 was considered as the significance level in all tests. Job satisfaction is effective in creating motivation, efficiency, responsibility, and social accountability in the personnel of any organization. Job dissatisfaction can lead to complications such as job burnout, illness, absenteeism, the job left, leaving many nursing jobs, and the reluctance of the young workforce to attend the wards. Therefore, it is necessary to make further efforts in this field by healthcare policymakers.*

Keywords: *mental health, life satisfaction, job satisfaction*

INTRODUCTION

Mental health has an expanded definition. But every person who can deal with his/her deep issues, get along with him/herself and others, and can face his/her inevitable internal conflicts, and does not isolate him/herself from society, can be stated to have mental health [1-4]. Nurses are directly related to all social strata and touch people's problems closely. This close relationship doubles their serious responsibility toward people's health [5]. Nursing is one of the most stressful professions so the National Institute for Occupational Safety & Health has introduced it among the top 40 stressful professions [6].

There are many sources of occupational stress in the nursing work environment. Overwork, urgency, patient death, independent practice, lack of social support, poor job fit, lack of necessary specialized knowledge, unsafe workplace and rapidly changing healthcare environment are known as nursing stressors [7]. Nurses are prone to health threats in different dimensions due to long work shifts and the resulting fatigue. Nurses with no good general health are not able to provide good care such as physical and psychological support to patients. This increases the risk of mistakes and occupational accidents, and subsequent consequences affect the patient and the nurse [8].

Nurses' stress and fatigue can affect the vital and critical aspects of their performance, including problem-solving ability, decision-making ability, and creativity, and ultimately lead to disruption in medical care. Because patient safetyو health and recovery depend on the safe and optimal performance of nurses [9]. Many researchers have confirmed that job-related stress can have harmful effects on the physical and mental health of individuals [10,11]. Job stress is one of the factors affecting the health of employees. Employees' health is at risk when they face various stressors. This issue is more evident in the nursing profession [12]. The impact of job dissatisfaction on the mental health of employees has been a public concern in various societies, especially in recent years. Perceived impacts of job dissatisfaction on people's mental health from an economic point of view is also an important issue and play an important role in the quality of professional and personal life of nurses [13].

Another variable that plays a key role in improving nurses' mental health is life satisfaction. Life satisfaction is one of the mental health-related factors and refers to a person's general attitude and evaluation toward life as a whole [14]. It also reflects the balance between a person's aspirations and his/ her current situation. The greater this balance, the greater the life satisfaction will be [7]. According to previous studies, this concept is in full interaction with health and there is a close relationship between physical and mental health with life satisfaction [15]. Lyubomirsky believes that people who have higher life satisfaction, use more effective and appropriate coping methods, experience deeper positive emotions and feelings and have higher general health [16]. Nurses are among the vulnerable employees of society, whose health and balance in their personal and family life is related to occupational variables. Work-family conflict causes psychological symptoms such as higher stress, depression, chronic physical problems, increased physical complaints, lower life satisfaction, low quality of family life, and low energy levels [17]. Suzuki et al. stated that nurses were in 27th place in the evaluation of 130 stressful jobs sorted according to their reference to doctors for health problems [18].

Improving the mental health of nurses and investigating the factors affecting it leads to the improvement of job performance and the quality of nursing care. Since nursing is one of the sensitive professions and nurses spend an important part of their lives with people and patients, their mental health leads to optimal job performance and guarantees the health and improvement of many patients and people [19].

Having job satisfaction, life satisfaction and mental health leads to an improvement in the quality of patient care and increasing productivity and improving nursing services, therefore, conducting relevant studies and using their results in various aspects of the nursing profession can have useful results in care, education, management, therapy and also the personal life of a nurse [20]. Therefore, the present study aimed to determine the relationship between mental health, life satisfaction, and job satisfaction of nurses in Ilam city in 2022.

MATERIALS AND METHODS

The present correlational study was carried out on 160 nurses working in educational hospitals of Ilam city and was selected by stratified random sampling in 2022. Inclusion criteria included working in one of the above-mentioned educational hospitals and having informed consent to participate in the study. Failure to complete one of the questionnaires, an incomplete questionnaire, and unwillingness to cooperate were among the exclusion criteria. A four-part questionnaire was used: the first part included a question on demographic information. Minnesota Satisfaction Questionnaire (MSQ) (1977), Diener et al.'s Satisfaction with Life Scale (SWLS) (1985), and Derogatis et al.'s Symptom Checklist-90 (SCL-9) (1973) were included in the second, third and the fourth parts, respectively. Demographic information of nurses included age, gender, years of work experience, marital status, education level, hospital, ward, position, income level, employment status, number of children, and home type.

Minnesota Satisfaction Questionnaire (MSQ) consists of 19 items and 6 subscales of the payment system, type of job, achievement opportunities, organizational atmosphere, leadership style, and physical conditions. MSQ is scored based on a 5-point Likert scale, ranging from "Completely Disagree" (Score 1) to "Completely Agree" (Score 5). To calculate the overall score of the questionnaire, the scores of all the items are added together. Scores 19-38, 38-57, and 38-57 indicate poor, moderate, and very good job satisfaction, respectively. This questionnaire has been used in many types of research in the world [21-26]. Its internal consistency reliability was measured α = 0.87.

Diener et al.'s Satisfaction with Life Scale (SWLS) is a 5-item instrument designed to measure a person's cognitive and overall judgment of life. This instrument is scored based on a 7-point Likert scale ranging from 1 (Completely disagree) to 7 (Completely agree). The overall score of this scale is calculated by summing the answers and the possible score range is 5 and 35. A higher score indicates higher levels of life satisfaction. This questionnaire has also been used in many types of research in the world [27-32]. The Cronbach's α reliability was 0.86.

Derogatis et al.'s Symptom Checklist-90 (SCL-9) can be completed within approximately 25 minutes. This questionnaire is the short form of SCL-90 and was extracted by Najarian & Davoudi based on the original form and through exploratory factor analysis. It consists of 25 items and 9 subscales of somatization (6 questions), obsession-compulsion (3 questions), interpersonal sensitivity (3 questions), depression (2 questions), anxiety (3 questions), phobia (3 questions), paranoid thoughts (1 question), psychosis (3 questions), hostility (1 question). SCL was answered according to its original form and based on a 5-point Likert swale ranging from None or Rarely (0), A little (1), To some extent (2), A lot (3), Very much or Strongly (4). The overall score of general psychological injuries was extracted. Higher scores indicate more damage [30]. The Cronbach's alpha reliability of the tool was calculated α = 0.94.

The collected data was analyzed using descriptive tests (frequency, mean percentage, and standard deviation) and non-parametric Spearman and Kruskal-Walli’s correlation tests in SPSS ver. 22. P-value<0.05 was considered as the significance level in all tests.

Ethical considerations: The present study was approved by the Ethics Committee of Ilam University of Medical Sciences with the code IR.MEDILAM.REC.1400.229. The contents of the Nuremberg and Helsinki declarations and the 26 research ethics codes were observed.

RESULTS

The results showed that most of the participants were female (61.2%), aged 26-29 (40%), single (52.5%), childless (75%), owning their own house (72.5%), and had 6-10 million-toman income (70%). Also, the majority of participants had a bachelor's degree (88.8%), less than 6 years of work experience (70%), were ward nurses (91.2%), were permanent nurses (45%), and worked in ICUs (45%).

Table 1 shows the correlation matrix of job satisfaction, life satisfaction, and mental health. This table determines the relationship between job satisfaction, life satisfaction, and mental health of research subjects. The table shows no significant relationship between the total score of mental health and the total score of job satisfaction. There was a significant relationship between the total job satisfaction score and life satisfaction score (r=0.267, p=0.001). There was also an inverse and significant relationship between the total mental health score and the life satisfaction score (r=0.294, p=0.001).

Kruskal-Walli’s test shows the relationship between job satisfaction, life satisfaction, and mental health according to demographic characteristics (Table 2). This table shows that the highest frequency of the variables of work experience, education level, medical training center, hospital ward, income level, and employment status are related to less than 3 years (p=0.017, df=4, k=11.98), BA (p=0.014, df=1, k=6.07), Ayat-Allah-Taleghani hospital (P=0.004, df=2, k=11.03), ICUs (P=0.001, df=2, k=55.91), 6-10 million tomans(P=0.022, df=2, k=7.62), and contractual employment (P=0.001, df=2, k=14.37), which are all had a statistically significant relationship with job satisfaction. The highest frequency of gender, marital status, and employment status were related to females (P=0.037, df=1, k=4.34), married individuals (P=0.034, df=1, k=4.47), and contractual individuals (p=0.032, df=2, k=6.88), which all had a statistically significant relationship with life satisfaction. Also, the female gender (k=11.75, p=0.001, df=1) had a statistically significant relationship with mental health.

DISCUSSION

Mental health affects health-related variables related to [4,33-35]. The results of the present research showed no significant relationship between each of the mental health components and the total score of job satisfaction. There was also no significant relationship between the total scores of job satisfaction and mental health. However, there was an inverse and significant relationship between the two components of job satisfaction and some mental health components, that is between the payment system with physical complaints, interpersonal sensitivity, phobia, and the overall mental health score, and also between the type of job with obsessive-compulsive and paranoid thought.

Kahe et al. revealed a statistically significant relationship between overall job satisfaction with employees' mental health, and people with high job satisfaction obtained a better mental health score (below average) (p=0.02). In other words, the lower the level of mental health, the lower the job satisfaction and vice versa [36].

Table 1: Correlation matrix of job satisfaction, life satisfaction, and mental health (Spearman correlation)

Variable			*Correlation*	
Variable	**Life satisfaction**	**Mental health**	**Meaningful**	
Job Satisfaction	0/267	-0/063	r	*1*
	*0/001	0/428	P	-
Life satisfaction	1	-0/294	r	
	-	*0/001	P	
Mental health		1	r	
		-	P	

Table 2: Relationship between job satisfaction, life satisfaction, and mental health according to demographic characteristics (Kruskal Walli's)

Variable		Mental health				Life satisfaction				Job Satisfaction			
		P	df	K	Mean	P	df	K	Mean	P	df	K	Mean
Gender	Man	0/001	1	11/75	64/73	0/037	1	4/34	70/92	0/262	1	1/25	75/34
	Female				90/48				86/56				83/77
Age	Less than 25 years	0/420	3	2/82	79/84	0/209	3	4/53	76/97	0/181	3	4/87	85/39
	26-29 years				86/58				87/72				86/63
	30-34 years				79/29				66/21				75/57
	More than 34 years				69/50				82/90				65/83
Marital status	Single	0/64	1	0/213	82/11	0/034	1	4/47	73/14	0/071	1	3/26	86/79
	Married				78/72				88/63				73/55
Number of children	0	0/357	3	3/23	82/43	0/647	3	1/65	78/47	0/202	3	4/61	85
	1				62/61				93/5				66/72
	2				82/13				81				65/5
	3				91/17				80/83				71/83
Type of house	Rental	0/227	1	1/46	87/68	0/927	1	0/008	79/95	0/836	1	0/043	81/73
	Personal				77/78				80/71				80/03
Work experience	Less than 3 years	0/105	4	7/64	89/33	0/985	4	0/37	81/09	0/017	4	11/98	94/17
	6-3 years				83/14				79/43				82/29
	9-6 (9-12) years				60/93				86/36				65/5
	< 3 years, > 12 years				50/83				74/5				53/5
	6-3 years				74/14				79/93				63/71
Level of Education	Masters	0/198	1	1/65	82/18	0/423	1	0/64	81/54	0/014	1	6/07	83/71
	MSc				67/28				72/28				55/17
Section	Public section	0/265	3	13/45	83/08	0/254	3	13/63	68/24	0/001	2	55/91	64/24
	Special section				76/95				89/79				91/39
	Emergency room				85/4				79/3				66/75

Veysey et al. showed an inverse relationship between the general health questionnaire and job satisfaction and a direct relationship with the increase in cases of stress [37]. In her study in teaching hospitals of Hormozgan, Mastaneh et al. (2013) also showed a significant negative correlation between mental health pay satisfaction (p<0.005) and type of work (P<0.01) [38].

Sarafis et al. also showed a significant relationship between the stress management domain of health-promoting lifestyle and the payment system domain of job satisfaction (p< 0.005) [39].

Ghasemipirbalooti et al. found a statistically significant relationship between mental health and job satisfaction of the employees of Shahrekord University of Medical Sciences (p=0.001) [40].

Mei et al. (2015) investigated the effect of psychological characteristics of employees on their performance and job satisfaction and the indirect effect of feelings on their relationships. They concluded a positive and direct relationship between job satisfaction with psychological characteristics and positive feelings of employees [41].

Many studies have shown a direct relationship between mental health and job satisfaction [42-47].

The results of this study show an inverse and significant relationship between life satisfaction and each of the components of mental health except for the "paranoid thought" component. The results also show that the level of mental health of nurses increases and mental injuries decrease with increasing life satisfaction scores. Salimi also showed in research titled "Investigating mental health and its relationship with job burnout and life satisfaction of employees" that there is a significant relationship between mental health and life satisfaction.

The consistency between the results of the present research with Salimi's study is attributed to the correlation between mental health and life satisfaction. That is, life satisfaction prerequisite for mental health, and if there is no satisfaction, negative mood and despair dominate the person and make him/her susceptible to all kinds of mental illnesses [48].

In a study on nurses in Ahvaz government hospitals, it was found that life satisfaction is correlated with high mental health (Birgani et al.). The higher the level of life satisfaction, the more likely a person is to experience positive emotions and feelings [49]. Geravand also showed in a study that mental health has a direct effect on life satisfaction.

Changes in mental health and emotions can lead to changes in life satisfaction and a person's attitude [50]. The results of various research show a significant relationship between mental health and life satisfaction. The results of Ferguson's study indicated the interaction between mental health and life satisfaction [51].

In a study of the level of job satisfaction of nurses in hospitals affiliated with Kashan University of Medical Sciences and related factors, Tagharobi et al. showed a statistically significant relationship between the level of education, major stress in the last six months, the workplace, the level of life satisfaction, mental health satisfaction health, pay satisfaction, satisfaction with the number of employees in each shift, satisfaction with the feedback of patients and their families, satisfaction with the treatment and performance of doctors, satisfaction with the treatment and performance of head nurses and colleagues, satisfaction with the treatment and performance of nursing office managers, satisfaction with workplace amenities and happiness with job satisfaction [52].

As was pointed out by Grant, nurses are among the most vulnerable working group in society, and their health and personal-family life balance are strongly related to occupational variables. Work and family life conflict and subsequent stress cause psychological symptoms such as higher stress, depression, chronic physical problems, increased physical complaints, lower life satisfaction, low quality of family life, and low energy levels in these people [17].

CONCLUSION

According to the results of the present study, nurses' life satisfaction score increases with the increase in their job satisfaction score. But there is no significant relationship between the total mental health score and the total job satisfaction score. However, there is an inverse and significant relationship between mental health and life satisfaction. On the other hand, the level of mental health of nurses increases, and mental injuries decrease with the increase in life satisfaction scores. Considering the vital role of nurses in achieving the goals of the health system and public health, neglecting the job dissatisfaction of nurses may have consequences such as reducing productivity, reducing the level of responsibility, reducing the level of quality of their performance, reducing life satisfaction and increasing the number of psychological injuries. Job satisfaction is effective in creating motivation, efficiency, responsibility, and social accountability in the personnel of any organization. Job dissatisfaction can lead to complications such as job burnout, illness, absenteeism, the job left, leaving many nursing jobs, and the reluctance of the young workforce to attend the wards. Therefore, it is necessary to make further efforts in this field by healthcare policymakers.

Acknowledgments
Ilam University of Medical Sciences

Ethics approval and consent to participate

IR.MEDILAM.REC.1400.229

Informed written consent was obtained from all participants included in the study. All participants were informed about the purpose of the study and assured of confidentiality and anonymity. The respondents were informed of their freedom to withdraw from the study at any time.

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Original Article

Survey the Effect of Educational Intervention Based on Mobile Short Message Service (SMS) on Self-Care in Patients with Type 2 Diabetes Referring to the Diabetes Clinic in Khorram Abad City

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Abstract: *Introduction: Self-care behaviors are very important to control type 2 diabetes. The current study was conducted aiming at determining the effect of the educational intervention based on mobile short message service on the self-care behaviors in patients with type 2 diabetes in Khorram Abad. Methods: This study was a semi-experimental study on 191 patients with type 2 diabetes (45case and 45 controls). Data collection tools included a demographic information form, International Physical Activity Questionnaire, Self-Care Scale, and Self-Efficacy Scale. Three to four educational messages were sent daily through mobile phones as an educational intervention. Data were analyzed with SPSS V24 at a significance level of 0.05. Findings: The data analysis indicated that the average score of self-care, self-efficacy, and blood sugar was significantly higher in the case group than in the control group before and after the intervention (P<0.001). However, in terms of the physical activity variable, this relationship was not statistically significant in the case and control groups. Conclusion: Educational interventions to empower diabetic patients by strengthening their self-care can be an effective way to improve the health of diabetic patients. Therefore, it is suggested to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.*

Keywords: *self-care, self-efficacy, physical activity, blood sugar, type 2 diabetes*

INTRODUCTION

Diabetes is the most common disease caused by metabolic disorders, which is mainly characterized by a chronic increase in blood sugar and disturbed carbohydrate, fat, and protein metabolism. In general, diabetes is a multifactorial disorder diagnosed by a chronic increase in blood sugar or hyperglycemia (according to diagnostic criteria) and is caused by a disorder in insulin secretion or function or both [1]. Retinal damage and blindness, peripheral neuropathy, leg pain, stroke, peripheral vascular problems, end-stage renal disease, and limb amputation are among the most serious complications of diabetes [2]. The most common type of diabetes is type 2 diabetes, which accounts for approximately 90-95% of diabetes cases and is usually associated with metabolic disorders, weight gain, and physical inactivity [3]. About 422 million people worldwide suffer from diabetes, the majority of whom live in low- and middle-income countries, and in 2019, diabetes was introduced as the direct cause of 1.5 million deaths [4]. According to the International Diabetes Federation (IDF), the number of diabetic patients will reach 439 million people in 2030, and the prevalence of diabetes in developing countries will be 69% [5]. More than 3 million people with diabetes live in Iran [6]. By 2030, Iran will be one of the most prevalent regions in the world in terms of diabetes [7]. Paying attention to the chronic and costly nature of this disease for the general health of society and the creation of a large financial burden, it is essential to pay serious attention to this problem and its consequences [8]. According to the results obtained from the studies, the long-term complications of this disease, including neuropathy, nephropathy, and retinopathy can be prevented, or at least the occurrence of these complications can be postponed if blood sugar is controlled [9, 10]. Many studies have found physical activity and exercise to be effective in reducing blood sugar levels and controlling diabetes [11,12]. The World Health Organization considers education to be the basis of diabetes treatment. Therefore, self-care is one of the important topics of diabetes treatment. Self-care is a learned regulation function in humans, which is based on the ability of people to perform care actions on themselves [13]. It includes the behaviors of following a proper diet, taking medicine on time, self-monitoring of blood sugar, performing regular physical activity, and physical care of the feet [14]. Studies show that 67% of patients with type 2 diabetes do not monitor their blood sugar according to the recommendations. Also, only 19-30% of diabetic patients use the recommended physical activities and compliance with nutritional behaviors in these patients is 65%, and only 7% of diabetic patients fully adhere to all aspects of self-care behaviors [15, 16]. Self-efficacy is also known as a strategy to improve self-care in diabetic patients, which results in self-confidence and adherence to care recommendations to control blood sugar in these patients [17].

Nowadays, to follow up and educate diabetic people, new communication systems are required to direct the focus of care and treatment from the clinic to the patient's daily life. Researchers have pointed out the use of mobile phone service as a method of education and follow-up in chronic diseases, that short message is one of the main functions of mobile phone in the education of diabetic patients [18,19].

Considering the importance of self-care in controlling the blood sugar of diabetic patients and the value of self-efficacy as one of the self-care approaches, it seems necessary to design educational interventions on the platform of mobile phones and in the form of short messages as a relatively new educational method in controlling the disease of diabetics. Thus, the present study was conducted to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad.

MATERIALS AND METHODS

The present study was a semi-experimental intervention research with a pre-test and post-test design. The sample size was specified as 90 people who were selected from the research population of patients with type 2 diabetes referring to the diabetes clinic in Khorramabad, Lorestan province. The participants were selected according to the inclusion criteria using random sampling. Then, they were divided into case and control groups using a simple randomization method (45 ones in each group). The inclusion criteria include informed consent to participate in the research, having a minimum level of literacy and understanding of the content sent through text messages, having a fixed personal mobile number to receive educational SMS, lack of any serious diabetic complication, and not having special diets because of specific diseases or physical problems limiting physical activity. Exclusion criteria include suffering from serious complications of diabetes during the study, suffering from diseases that limit physical activity or nutrition, and the patient's unwillingness to receive educational messages.

Data collection tools in this study included a demographic information form, International Physical Activity Questionnaire (IPAQ), a Self-Care Scale developed by Tubert, and Self-Efficacy Scale. Demographic information included: age, height, weight, gender, educational level, employment status, marital status, and type of treatment. The IPAQ with seven items measured the duration of physical activity in the last week in four categories: moderate physical activity, intense physical activity, walking, and sitting activities. According to the scoring protocol in this questionnaire, the intensity of physical activity based on the ratio of energy consumption in terms of metabolic equivalent of task (MET) in the last seven days is classified into three levels: low, medium, and high. It was proposed by the World Health Organization and the Centers for Disease Control and Prevention in 1998 to measure the physical activity of people aged 15 to 69 years and its validity and reliability have been confirmed in 12 countries worldwide, including Iran [20,21]. A self-care questionnaire was used in this study that included 15 items in the areas of diet compliance, physical activity, blood sugar monitoring, insulin injection or diabetes pill consumption, foot care, and smoking, which was translated and standardized by Hamdzadeh [22,23]. The self-efficacy questionnaire, which is one of the constructs of the standard Health Belief Model Questionnaire, validity and reliability of which have been confirmed [24].

After presenting a letter of introduction from the Iran University of Medical Sciences and obtaining the necessary permits, the researcher referred to a diabetes clinic in Khorram Abad and selected 90 people from among 200 eligible people using random sampling. People who were willing to participate in the study and met the inclusion criteria were assigned to the case and control groups (45 in each group) using a simple randomization method. After random allocation, blood sugar samples were taken in all groups four days before the start of the intervention. In the first session, the researcher explained the purpose of the meeting to the participants, and written informed consent was obtained. Then, the personal information form and questionnaires were completed by the case and control groups. The case group received an educational program in the form of short educational messages via mobile phone, for four weeks and on average, at least three to four messages per day related to the educational content, including exercise, self-monitoring of blood sugar, diet, insulin therapy, medication use, and foot care. For example, short messages about physical activity and blood sugar monitoring were "for people with diabetes, walking is the best exercise" or "it is better for people with diabetes to measure their fasting blood sugar after waking up". To improve self-efficacy, encouraging messages about adherence to self-care were used.

Finally, after two months, the same questionnaires (demographic, physical activity, self-care, and self-efficacy scales) were distributed and completed by the participants, and the blood sugar levels of the two groups were measured again. During this period, the control group did not receive any educational intervention.

However, to comply with the ethical principles, an educational CD containing self-care training was given to the control group after completing the research. The data of the two groups before and after the intervention were entered into SPSS version 24 software and analyzed using Wilcoxon, Mann-Whitney, Chi-square test, independent t-test, and paired t-test at a significant level of 0.05.

RESULTS

The participants' average age was 51.01 ± 0.967, and the average height and weight were 164.57 ± 1.031 and 75.69 ± 1.101, respectively.

Before the implementation of the educational program, the case and control groups were matched in terms of demographic variables, including age, height, weight, gender, education, employment status, marital status, and type of treatment, and no statistically significant difference was observed between the two groups (Table 1).

Table 1: Comparison of the absolute and relative frequency distribution of demographic variables in case and control groups

Variable		Case		Control		P value
		No.	%	No.	%	
Gender	Female	31	69	26	57/7	0.796
	Male	14	31	19	42/2	
Employment	Unemployed	5	11	2	4	0.686
	Housewife	26	58	24	53	
	Self-employed	9	20	9	20	
	Public	5	11	10	22	
Education	Illiterate	5	11	4	9	0.479
	Primary school	17	38	10	22	
	Secondary school	9	20	5	11	
	High school	4	9	11	24	
	High school diploma	4	9	10	22	
	Higher education	6	9	5	11	
Marital status	Single	2	4	1	2	0.800
	Divorced	1	2	0	0	
	Widow	9	20	5	11	
	Married	33	73	39	87	
Type of treatment	Insulin	21	47	21	47	0.920
	Oral medication	20	45	22	49	
	Diet	4	9	2	4	
	Exercise	0	0	0	0	

As observed in Table 2, there is no statistically significant difference in terms of the average score of physical activity, self-care, self-efficacy, and blood sugar between the case and control groups before the intervention, while after the intervention, the average scores of these variables, except for physical activity (P=0.8), was significantly higher in the case group compared to the control group (P<0.001). In the control group, before and after the intervention, no significant difference was observed in the average scores of physical activity, self-care, self-efficacy, and blood sugar (P>0.05).

Table 2: Comparison of the average scores of physical activity, self-care, self-efficacy, and blood sugar, before and after the intervention in case and control groups

Scale	Group	Case	Control	p-value
		Mean ± SD	Mean ± SD	
Physical activity	Before intervention	677.5±954.938	307.5±461.75	0.6
	After intervention	903.75±973.125	655±925.625	0.8
	p-value	0.8	0.4	_
Self-care	Before intervention	2.31±0.813	2.38±0.844	0.12
	After intervention	3.38±0.688	2±0.906	0<0001
	p-value	0<001	0.22	_
Self-efficacy	Before intervention	2.7±0.4	2.7±0.3	0.15
	After intervention	3.7±0.7	2.7±0.7	0<0001
	p-value	0<001	0.286	_
Blood sugar	Before intervention	180±80.5	185±51	0.8
	After intervention	170±87.5	180±70.5	0<001
	p-value	0.009	0.491	_

Table 3: Comparison of blood sugar scores before and after the educational intervention in the case group

Method of treatment	Blood sugar level before the intervention	Blood sugar level after the intervention	Level of change
Insulin	227.09	217.92	-9.17
Oral medicine	173.48	170.65	-2.83
Diet	181	180.44	-0.56
Exercise	194	194.5	0.5
Medium	193.89	190.88	-3.02

Table 3 indicates that the blood sugar level in all patients with different treatment methods (insulin, oral medication, diet) in the case group reduced after the educational intervention compared to before intervention, except for the patients who only used exercise to control their blood sugar.

DISCUSSION

The present study was conducted aiming to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad. The research findings suggest that the educational intervention through mobile phone short messages improved self-care in the case group, which is consistent with findings by Abaza et al. [25] and Agbede et al. [26] on the effect of the intervention based on mobile phone short messages in the self-care of diabetic patients and the findings by Yari et al. [27] and Rahnavard et al. [28] concerning SMS-based and self-care education of diabetic patients. Krishna et al. [29] reported that technologies such as mobile phones and text messages, which are currently part of people's daily lives, have the potential to influence self-care and improve people's health. The approach of health education through SMS to promote self-care in diabetic patients is more welcomed than traditional approaches that require reading pamphlets or attending lectures [25,30].

In this study, self-efficacy also there was a significant difference in the case group in terms of self-efficacy after the educational intervention. Katz and Nordwall [31] and Peymani et al. [32] also found that interventions based on mobile phone messages improve self-efficacy and self-care. Similar studies [33,34] regarding the self-care of diabetic patients also indicate a significant difference in the average score of self-efficacy and self-care in the case group compared to the control group after the educational intervention. These studies are consistent with our work in that increasing self-efficacy causes the adoption of self-care behaviors in diabetic patients. Self-efficacy is an important prerequisite for behavior change [35]. An individual with low self-efficacy is less likely to make an effort to perform a new health behavior or to change the behavior he is used to [36]. Researchers believe that self-efficacy has a suitable framework for understanding and predicting and patient's commitment to self-care behavior in the treatment of diabetes [37].

The results of the present study showed that education through mobile phone short messages does not affect increasing physical activity in the case group, which is in line with the findings by Bakhshian et al[38] regarding the effect of education through mobile phone short messages on self-care of patients with type 2 diabetes. It is also in agreement with the findings by Kim et al. [39] on adherence to diabetes control recommendations, while our findings are not consistent with the study of Paul et al. [40] regarding the use of software that can be installed on mobile phones to improve the amount of daily walking in patients with a history of stroke and the study Martha et al.[41] concerning mobile phone SMS intervention on diabetes self-care activities, cardiovascular disease risk awareness, and food choices among type 2 diabetic patients. This discrepancy can be due to the lack of sports facilities and parks in the current study or the addition of chronic diseases to the patients participating in this research, as well as social and cultural factors that prevent people from doing physical activities in public environments. In the present study, since the number of women is more than men, the problem of social and cultural factors could be more marked.

The use of mobile phone messages in the current study showed a significant positive effect on the reduction of blood sugar in the case group after the educational intervention, which is consistent with the findings of similar studies in this field [42,43,44]. In patients suffering from diabetes, education is as valuable as medicine, exercise, and diet, because the treatment will never be effective unless the patient is well aware of the nature of his disease and takes positive steps to deal with it.

CONCLUSION

The results of this study demonstrated that educational interventions based on mobile phone short messages can be effective in enhancing self-care in patients with diabetes. Educational interventions aimed at empowering diabetic patients by strengthening self-care in them can be an effective way to improve the health of patients with diabetes. Therefore, it is recommended to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research.

The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

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Authors' contribution

All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Vice President of Research and Technology of Iran University of Medical Sciences with code IR.IUMS.REC.1397.501. Informed consent was obtained from all individual participants included in the study before conducting.

Consent to publish

All participants consented verbally to the publication of the interview data.

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Original Article

A Comparative Study of Patients' Trust in Physicians and Nurses in COVID-19 and Non-COVID-19 Wards in a Reference Hospital in Tehran, Iran

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Abstract: *Trust in health care professionals is considered as a basis for patients' care. This study aimed to compare the level of patients' trust in physicians and nurses in both general and COVID-19 wards in a reference hospital in Tehran during the pandemic. This was a cross-sectional descriptive study. The study population comprised patients from Baqiyatallah Hospital in 2022. The data collection tool was the scale of patients' trust in nurses and the Wake-Forest physician trust scale. Data analysis was performed using SPSS-26 software. The level of patients' trust in physicians in COVID-19 wards was significantly lower than in Non-COVID-19 wards (P<0.001), but there was no difference between the two groups in the trust of nurses. The lowest level of trust was in the receiving of required information from nurses, which had a lower score compared to other questions in the questionnaire, and the average amount of receiving required information for patients in general wards was significantly lower than that of patients in COVID-19 (P=0.006). Trust in nurses and physicians in both COVID-19 and general wards was optimum. During the COVID-19 pandemic, the medical staff worked tirelessly and despite the difficulties and complications of the pandemic, patients have high trust in physicians and nurses.*

Keywords: *trust; nurses, physicians; COVID-19; coronavirus*

INTRODUCTION

Physicians and nurses, as the main members of the medical staff, take on different roles, considering the physical, psychological, and social needs of patients. They may act as educators, counselors, mentors, informers, advocates, and act as experts. To play such roles, physicians and nurses need to establish a good relationship with their patients [1,2]. Trust forms the basis of the relationship between the physician or nurse [3] and patient and provides the conditions for the physician or nurse and patient to work together for a better care plan [4]. The relationship between trust and health outcomes is different in individual studies [5]. For example, in a sample of patients with diabetes, trust in a healthcare professional was positively associated with objective and mental health outcomes (glycemic control, health-related quality of life, and patient satisfaction] [6].

The hypothetical relationship between trust and health outcomes is also reinforced by the ethical claims derived from trustworthy and patient-centered relationships in clinical settings [7]. Studies show that patients who have more trust in their therapist are more satisfied with the care and are more likely to follow treatment recommendations [8,9]. Patients' experiences of trust in nursing depend on nurses' knowledge, and the commitment to conversation to build and develop relationships [10]. Therapeutic staff must receive appropriate training to build a bond of trust between themselves and the patient to establish a trustable relationship between themselves and the patient [11]. Trust between patient and therapist is essential to reduce patients' anxiety and build the capacity to control them [12]. The COVID-19 epidemic has created special conditions in the relationship between the medical staff and patients [13]; furthermore, the need for social distancing and the use of protective equipment on the one hand, as well as an increasing number of patients, especially those with severe illnesses, epidemics, illnesses and dementia and lack of time to provide medical care, on the other hand, has created a different kind of doctor-nurse relationship with the patient that may affect patients' trust [14]. Considering the importance of patients 'trust in physicians and nurses in adhering to treatment and the effectiveness of medical care, this study was designed to compare the level of patients' trust in physicians and nurses in general and COVID-19 wards in a reference hospital in Tehran during the pandemic.

MATERIALS AND METHODS

This study was descriptive and cross-sectional. The study population consisted of patients admitted to both the general and COVID-19 wards of Baqiyatallah Hospital, Tehran, Iran in 2022. Inclusion criteria included the age range of age: 20-70, hospitalization for more than three days in hospital wards, having no serious psychological disorders, and submitting consent to participate in the study. The data collection tools included the patient's trust scale in the nurse and the Wake-Forest physician trust scale. The Nurse Trust Scale has been validated by Nouripour et al [15]. The Physician Trust Questionnaire has been validated by Forati et al [16].

The scale of patient trust in the nurse contains five questions about Responding to the need, Usefulness of measures, Trust in measures and Practice of treatment" and Giving sufficient information to the patient by the nurse with the scales Never, Rarely, Sometimes, Most of the time, Usually, Always". The Physician Trust Questionnaire includes nine questions about Trust in Skills, Treatment Selection, and Physician Decisions on a scale of "Strongly Agree, Agree, Neither Agree Nor Disagree, Strongly Disagree and Disagree".

Patients with more than three days of hospitalization were randomly selected from the ward list. First, patient satisfaction with the study was obtained and then a questionnaire about patients' trust in physicians and nurses was filled in by an interviewer. Data analysis was performed using SPSS-26 software. Descriptive analysis was performed with mean, standard deviation, frequency, percentage, and analytical analysis depending on the type of data with t-test and Chi-square test.

RESULTS

Four hundred patients participated in this study. Descriptive values related to the age of these patients by group and in general are displayed in Table 1. The mean age of patients in the COVID-19 group was 53.3 years and in the Non-COVID-19 group was 53.8 years. This study included 257 male patients (64.3%). In addition, the sex distribution was the same in the two groups of patients with COVID-19 and Non-COVID-19 and no significant difference was observed in the sex distribution of the two groups (P=0.132).

Table 1: Descriptive characteristics of the patients admitted to Covid-19 and non-Covid-19 wards

Variable		COVID-19	Non-COVID-19	P Value
Age (Mean ±SD]		5.3 ± 14.53	3.8 ± 17.53	0.11
Sex	Male	120	137	13.0
	Female	79	65	
Education	Less than a Bachelor's degree	99 (54.1 %)	147 (8.72 %)	<0.001
	Bachelor degree	57 (1.31 %)	49 (3.24 %)	
	Senior	22 (0.12 %)	5 (5.2 %)	
	PhD	5 (7.2 %)	1 (5.0 %)	

In total, 198 patients (49.5%) with COVID-19 and 202 (50.5%) Non-COVID-19 patients were considered in this study. The overall mean of trust in nurses in the COVID-19 group was not significantly different from that of the Non-COVID-19 group.

Most people in both COVID-19 (85.3%) and Non-COVID-19 (86.6%) groups reported that nurses were available, most of the time or always when they needed them.

Most patients in both COVID-19 (99.4%) and Non-COVID-19 (97.4%) groups believed that the nurse's activity was useful to them, most of the time or always. The mean usefulness of therapeutic activities in patients in general wards was 0.1 higher than in patients in COVID-19 wards but this difference was not significant (P>0.05).

Most patients in both COVID-19 (99%) and Non-COVID-19 (95%) groups believed that they trusted the care of nurses always. The average level of trust in medical care for patients in general wards was 0.1% lower than that of patients in COVID-19 wards. But the difference was not significant (P>0.05).

In the COVID-19 group, 93% of patients often or always followed the advice of the nurses, while in the Non-COVID-19 group, 96.5% of patients most of the time or always followed the statements and recommendations of the nurses. The mean rate of treatment of patients with therapeutic recommendations in general wards was significantly higher (0.4) than patients admitted to the COVID-19 ward (P<0.001).

In the COVID-19 group, most patients (71.8%) believed that most of the time, or always, nurses provided enough accurate information about their disease. While in the Non-COVID-19 group, about half of the patients (55%) believed that most of the time or always the nurses provided them with sufficient accurate information about their disease. The average amount of information required for patients in general wards was 0.3 less than for patients admitted to the COVID-19 ward. There was a significant difference in the amount of information required for patients in general wards and COVID-19.

Table 2: Descriptive values related to the trust variables of the patients admitted in the Covid-19 and non-covid-19 wards

Variable	COVID-19			Non-COVID-19		
	SD ± Min	Min	Max	SD ± Min	Min	Max
Responsibility for patient needs	4.6 ± 1.1	2	6	4.6 ± 1.1	2	6
The usefulness of therapeutic activities	8.2 ± 0.5	3	6	8.3 ± 0.5	1	6
Reliability of medical care	3.5 ± 8.0	3	6	2.5 ± 1.1	1	6
Following the recommendations	5±0.9	3	6	4.5±0.9	2	6
Patients' access to the necessary information	4.2± 1.3	1	6	3.9± 1.6	1	6
Satisfaction with the nurse	7.0 ± 9.4	2.6	6	7.0 ± 9.4	2.6	6
Satisfaction with the physicians	3.5±0.3	2.8	4.3	3.7±0.3	2.2	4.5

DISCUSSION

Patients’ trust in nurses and physicians is important and at the same time complex phenomenon that to achieve it, some professional characteristics must be created and strengthened and some ethical principles must be observed [17]. In the study of patients' trust in nurses of Baqiyatallah Hospital, Tehran, Iran, we used the questionnaire of patients’ trust (P=0.006). The results of the study assessing the patients’ trust in the physicians showed that most patients participating in the study in both groups of COVID-19 (92.4%) and Non-COVID-19 (97.5%) had a high level of trust in physicians. Considering the mean difference, it can be said that the average trust in physicians in COVID-19 patients was significantly lower by 0.14 than in Non-COVID-19 patients (P<0.001).

Ravdin and Cabral nurses, which was validated by Nouripour et al [15]. This questionnaire had five questions, and in what follows, we will review the results obtained from the questions.

The level of responding to patients' needs and being available to them when needed, the level of use of treatment activities, and the level of trust in medical care in both general and COVID-19 wards were desirable. The rate of follow-up to treatment recommendations was favorable in both groups, but the mean rate of follow-up to treatment recommendations of patients in general wards was significantly higher than patients admitted to the COVID-19 ward. Studies have shown that there is a positive relationship between the level of trust in the nurses and the level of treatment adherence [18]. Trust in the health care specialist has been proposed as a basis for effective and basic treatments for patient-centered care [19-21]. However, the novelty of COVID-19 and the fact that there is still no definitive cure for the disease is a problem noted in the study by Baker [22]. The lack of definitive treatment and protocol for this disease and frequent changes in health protocols during this period may reflect scientific uncertainty; meanwhile, the uncertainty of scientific evidence reduces patients' trust to follow the recommendations of medical staff compared to other diseases and overshadows their treatment adherence.

In this study, the lowest level of trust was in the average amount of information required for patients, which had a lower score compared to other questions in the questionnaire, and the average amount of information required for patients in general wards was 0.3 less than patients in the COVID-19 ward. Nurses in both the COVID and general wards have the lowest level of patients’ trust in providing the necessary information. In Ozaras and Abaan study, the use of medical terms or terms that the patient does not fully understand, the lack of anticipation or understanding of patients' information needs, and neglecting responsibilities and factors related to nursing work such as busyness, etc. have been mentioned as important causes in reducing the provision of the necessary information to patients [23]. It seems that to improve the provision of information to patients, some programs should be implemented in the hospital and the nursing department.

The level of patients' trust in physicians in COVID-19 wards was significantly lower than in Non-COVID-19 patients. Gupta et al. showed that the pandemic coronavirus disease 2019 (COVID-19) has posed new threats to the trust in medical staff [24]. Factors such as the high prevalence and involvement of doctors during the epidemic peaks, as well as the specific way of communicating with the patient (personal protective equipment and social distancing, etc.] and that the patient does not even know and see the face of the treating physician well, are involved in this issue [25]. Identifying the causes of this difference requires further study and investigation of the issue from different respective.

CONCLUSION

Trust in nurses and physicians in both COVID-19 and general wards was optimum. This finding indicates that during the COVID-19 pandemic, the medical staff worked tirelessly and despite the difficulties and complications of the pandemic, patients have high trust in physicians and nurses.

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Original Article

Covid-19 Risk Perception in a Sample of Iranian Population

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Abstract: *How people behave in an emergency depends on many social, cultural, and contextual factors. This study aimed to determine the COVID-19 risk perception in Iranian people. This study was descriptive. The study population was the assistant of patients who had come to Baqiyatallah Hospital in 2021. The number of participants was 500. The study tool was the COVID-19 risk assessment questionnaire. Data were analyzed using SPSS-22 software. The mean risk perception score was 2.78(±0.6) on a 7-point scale. The lowest and highest mean of the predictors of risk perception in COVID-19 was trust in government and personal efficacy, respectively. There was a significant difference between men and women in the mean of collective efficacy (P= 0.032). Social knowledge (p=0.007), trust in science (p=0.001), and trust in medical professionals (p=0.050) in positive direct experience people were significantly more than in people with negative direct experience of COVID-19. The most important predictors of risk perception in the COVID-19 epidemic were gender, education, social knowledge, and trust in science (P<0.05). People's perception of the risk of COVID-19 depends on several various factors, the most important factors were gender, level of education, social knowledge, and people's trust in science.*

Keywords: *COVID-19; coronavirus; risk perception*

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and has spread around the world quickly, emerging as a significant challenge of the current century for public health, the economy, markets, and policies in many countries [1].

The coronavirus outbreak was declared a public health emergency of international concern by the World Health Organization on 30 January 2020. Today, most people around the world have understood the outcomes of COVID-19 infection and they have accepted the need for hand hygiene and social distancing to prevent its spread. But, some of them ignore or delay hygienic actions, government laws, and social distances in public places, or homes. The fact that individuals in the same situation of common challenge act so differently indicates that the persons' risk perception relating to this new and chronic challenge is strongly different [2].

How people behave in an emergency depends on their understanding of the severity and depth of the risk and the extent of their vulnerability, but many social, cultural, and contextual factors may influence their risk perception [3]. Little is known about how people perceive the risk associated with emerging infectious diseases compared to other areas of risk. Risk perception leads to the adoption of preventative health behaviors so misperception of COVID-19 risk in this condition can lead to negative consequences of pandemic control [4].

Personal experience with the virus, individualistic and prosocial values, hearing about the virus from friends and family, trust in government, science, and medical professionals, personal knowledge of government strategy, and personal and collective efficacy were all significant predictors of risk perception, in the first global assessment of public risk perception of COVID-19 [5].

Previous studies have examined differences in risk perception of COVID-19 in diverse populations and races. Health workers reported higher risk perception, level of worry, and knowledge as related to COVID-19 infection compared to the general population [6]. In some studies, females were found to perceive risks higher, complied more with the government's guidelines, and coped better than males in response to COVID-19 [7]. But Self-reported anxiety over the pandemic is a crucial predictor in explaining risk perceptions in all age and gender groups [8]. And overall public initial concerns and trust can play an essential role in improving the perceived risk of a pandemic and increasing public participation in adopting preventive measures [9]. This study aimed to determine the risk perception score and its related factor in Iranian people.

MATERIALS AND METHODS

This study was descriptive-analytical. The study population was the assistant of patients who had come to the clinic of Baqiyatallah Hospital in 2021. The inclusion criteria were 15 to 80 years old, no serious and acute illness, and consent to participate in the study. The number of participants in the study was 500, with a 5% alpha error and 20% beta error. The study tool was the COVID-19 risk assessment questionnaire, which was designed by Dryhurst [10] using previous studies to identify all dimensions of risk perception covering affective, cognitive, and temporal-spatial dimensions to provide a holistic measure of risk perception. The questionnaire included items on COVID-19 experience, demographic variables (age, gender, and education) knowledge, both personal and social knowledge, social amplification, prosociality, individualism, trust in l three domain of science, medical professionals, and government, personal efficacy, and collective efficacy. The validity of the COVID-19 risk assessment questionnaire was assessed using face and content validity approaches. The impact score of each item was assessed to evaluate the face validity. The questionnaire was distributed among 10 technical experts who were asked to evaluate items based on the mentioned criterion. Then the importance of each item was calculated using the impact score formula and the results were compared with the standard score (1.5), and items whose scores were higher than the standard score were included in the questionnaire. Next, the content validity of the instrument was analyzed using the Content Validity Ratio (CVR) and Content Validity Index (CVI). 10 respondent experts also participated in this phase. Experts were asked to group items as "essential", "essential but not useful", or "not essential" to calculate CVR. Their responses were calculated based on the CVR formula and the results were compared with Lawshe's Table. Numbers higher than 0.6 were approved. CVI survey was done based on the Waltz and Basel content validity index [11]. A questionnaire was distributed among the experts who were asked to rank 24 items based on a four-part Likert scale as compatible with 3 criteria of relevancy, simplicity, and clarity. (For example, unrelated items, partially related items, related items, and fully related items were scored 1, 2, 3, and 4, respectively). Items higher than 0.6 were approved according to the CVI score. Cronbach's alpha method was used to assess the questionnaire's reliability. Data collection was started after the standardization of the questionnaire.

Patients completed the study questionnaire, after written consent. The questionnaire was filled out using the interview method and by a trained interviewer. All information obtained from patients was confidential and the study received an ethics code from the ethics committee of Baqiyatallah Hospital. Primary data were analyzed using SPSS-22 software. Descriptive data analysis was performed using mean and standard deviation in quantitative data and frequency and percentage in qualitative data. Analytical analysis was performed according to the normality of the data with parametric and non-parametric tests. Linear and logistic regression were used to measure the relationship between variables according to the type of data.

RESULTS

The validity and reliability of the instrument of this study

In the first step, the COVID-19 risk assessment questionnaire was validated. Tables 1 and 2 show that the scores of all items of the questionnaire were higher than the criterion score (i.e. 1.5) and experts approved the quantitative face validity of all items. Moreover, the results of CVI indicate that all items of the questionnaire obtained a score higher than 0.6, and content validity was considered appropriate by experts. The reliability of the questionnaire based on Cronbach's alpha was 0.79, 0.69, and 0.77 for risk perception, individualism, and trust in science, respectively.

Study findings

In the study samples, 500 people participated, of whom 253 (50.6%) were women and 247 (49.4%) were men.

The mean age of participants was 38.9 (±11.7) and 147 (29.4%) of the people have experienced the disease. The descriptive characteristics are listed in Tables 3 and 4.

The mean risk perception score was 2.78(±0.6) on a 7-point scale. The lowest mean of the predictors of risk perception in COVID-19 was in trust in the government and the highest mean was in personal efficacy (Table 4).

Table 1: Results of calculating quantitative face validity (impact score) of items of the designed instrument

Item	Frequency	Importance	Impact score
Q1: How worried are you personally about the following issues at present? Coronavirus/COVID-19	0.7	4.4	3.08
Q2: How likely do you think it is that you will be directly and personally affected by the following in the next 6 months? - Catching the coronavirus/COVID-19	0.6	4.1	2.46
Q3: How likely do you think it is that your family and friends in the country you are currently living in will be directly affected by the following in the next 6 months? Catching the coronavirus/COVID-19	0.6	3.6	2.16
Q4: How much do you agree or disagree with the following statements? – The coronavirus/COVID-19 will NOT affect very many people in the country I'm currently living in	0.5	3.6	1.8
Q5: How likely do you agree or disagree with the following statements? - I will probably get sick with the coronavirus/ COVID-19	0.7	3.8	2.66
Q6: How much do you agree or disagree with the following statements? - Getting sick with the coronavirus/COVID-19 can be serious	0.6	3.3	1.98
Q7: How much do you feel you understand the government's strategy to deal with the coronavirus/COVID-19 pandemic?	0.6	3.5	2.1
Q8: To what extent do you think scientists have a good understanding of the coronavirus/ COVID-19?	0.5	3.6	1.8
Q9: Have you ever had, or thought you might have, the coronavirus/COVID-19?	0.6	3.1	1.92
Q10: Have you come across information about coronavirus/COVID-19 from: - Friends and family	0.6	3.4	2.04
Q11: To what extent do you think it's important to do things for the benefit of others and society even if they have some costs to you personally?	0.7	2.2	1.54
Q12: The government interferes far too much in our everyday lives.	0.8	2.6	2.2
Q13: How much do you trust the country's politicians to deal effectively with the pandemic?	0.6	2.5	1.5
Q14: How much do you trust each of the following? – Scientists	0.7	3.3	2.31
Q15: How much do you trust each of the following? - Medical doctors and nurses	0.6	3.2	1.92
Q16: To what extent do you feel that the personal actions you are taking to try to limit the spread of coronavirus make a difference?	0.6	3	1.8
Q17: To what extent do you feel the actions that your country is taking to limit the spread of coronavirus make a difference?	0.7	3	2.1

Table 2: Content validity index of questionnaires through CVR and CVI

Item	CVR	CVI		
		simplicity	perspicuity	relevance
Q1: How worried are you personally about the following issues at present? Coronavirus/COVID-19	1	0.9	0.9	0.9
Q2: How likely do you think it is that you will be directly and personally affected by the following in the next 6 months? - Catching the coronavirus/COVID-19	0.6	0.9	0.8	0.8
Q3: How likely do you think it is that your family and friends in the country you are currently living in will be directly affected by the following in the next 6 months? Catching the coronavirus/COVID-19	0.8	0.7	0.8	0.8
Q4: How much do you agree or disagree with the following statements? – The coronavirus/COVID-19 will NOT affect very many people in the country I'm currently living in	0.8	0.8	0.8	0.9
Q5: How likely do you agree or disagree with the following statements? - I will probably get sick with the coronavirus/ COVID-19	0.6	0.9	0.9	0.7
Q6: How much do you agree or disagree with the following statements? - Getting sick with the coronavirus/COVID-19 can be serious	0.8	0.8	0.8	0.7

Q7: How much do you feel you understand the government's strategy to deal with the coronavirus/COVID-19 pandemic?	0.9	0.8	0.7	0.9
Q8: To what extent do you think scientists have a good understanding of the coronavirus/COVID-19?	0.7	0.7	0.8	0.8
Q9: Have you ever had, or thought you might have, the coronavirus/COVID-19?	0.9	0.8	0.7	0.7
Q10: Have you come across information about coronavirus/COVID-19 from: - Friends and family	0.8	0.9	0.9	0.9
Q11: To what extent do you think it's important to do things for the benefit of others and society even if they have some costs to you personally?	0.6	0.7	0.8	0.7
Q12: The government interferes far too much in our everyday lives.	0.6	0.6	0.6	0.7
Q13: How much do you trust the country's politicians to deal effectively with the pandemic?	0.9	0.7	0.7	0.8
Q14: How much do you trust each of the following? – Scientists	1	1	1	0.9
Q15: How much do you trust each of the following? - Medical doctors and nurses	1	1	1	1
Q16: To what extent do you feel that the personal actions you are taking to try to limit the spread of coronavirus make a difference?	0.7	0.8	0.7	0.9
Q17: To what extent do you feel the actions that your country is taking to limit the spread of coronavirus make a difference?	0.9	0.7	0.7	0.9

Table 3: Descriptive characteristics of the participants in the study of risk perception of Covid-19

		Frequency	Percent
Gender	Female	253	50.6
	Male	247	49.4
	Total	500	100.0
Education	Under Diploma	37	7.4
	Diploma	149	29.8
	BSc	178	35.6
	MSc	91	18.2
	PhD	42	8.4
	Total	497	99.4
Direct experience with the disease	Yes	147	29.4
	No	353	70.6
	Total	500	100.0

Table 4: The scores of the risk perception questionnaire in participants in the Covid-19 risk perception study

		Number	Minimum	Maximum	Mean	Std. Deviation
Age		497	15	78	38.97	11.731
Risk perception		500	1	4	2.78	0.60669
Personal knowledge		500	1	7	4.78	1.50084
Social knowledge.		500	1	7	4.34	1.43105
Social amplification	Yes	426			85.2*	
	No	74			14.8*	
Prosociality		500	1	7	5.33	1.39061
Individualism		500	1	5	2.73	0.80308
Trust in government		500	1	5	2.20	1.071
Trust in science		500	1	5	3.73	0.73161
Trust in medical professionals		500	1	5	3.94	0.814

Personal efficacy	500	1	7	5.35	1.23006
Collective efficacy	500	1	7	4.02	1.20094

**: Percent*

Next, we ran a Spearman's rho to assess the correlation of the predictors that play a role in COVID-19 risk perception (Table 5). Social knowledge, trust in science, and trust in medical professionals were all significantly associated with risk perception refer to Table 3.

Table 5: The statistics of the predictors of COVID-19 risk perception

		Age	Risk perception	Personal knowledge	Social knowledge	Prosociality	Individualism	Trust in government	Trust in science	Trust in medical professionals	Personal efficacy	Collective efficacy
Age	Correlation Coefficient		.006	.000	.010	.092*	.030	.096*	-.005	.054	.006	.086
	Sig. (2-tailed)		.889	.999	.825	.040	.498	.033	.917	.229	.886	.055
Risk perception	Correlation Coefficient	.006		.044	-.266**	.009	-.078	.018	-.227**	-.156**	-.005	-.006
	Sig. (2-tailed)	.889		.329	.000	.833	.082	.694	.000	.000	.915	.891
Personal knowledge	Correlation Coefficient	.000	.044		-.061	.158**	-.218**	-.069	-.022	.100*	.296**	.069
	Sig. (2-tailed)	.999	.329		.171	.000	.000	.123	.618	.025	.000	.122
Social knowledge	Correlation Coefficient	.010	-.266**	-.061		-.028	.065	.026	.250**	.079	-.039	.097*
	Sig. (2-tailed)	.825	.000	.171		.532	.149	.567	.000	.076	.385	.029
Prosociality	Correlation Coefficient	.092*	.009	.158**	-.028		-.338**	-.031	.131**	.173**	.316**	.069
	Sig. (2-tailed)	.040	.833	.000	.532		.000	.486	.003	.000	.000	.125
Individualism	Correlation Coefficient	.030	-.078	-.218**	.065	-.338**		.052	-.061	-.134**	-.359**	-.122**
	Sig. (2-tailed)	.498	.082	.000	.149	.000		.243	.170	.003	.000	.006
Trust in government	Correlation Coefficient	.096*	.018	-.069	.026	-.031	.052		.088*	.074	-.022	.339**
	Sig. (2-tailed)	.033	.694	.123	.567	.486	.243		.048	.098	.623	.000
Trust in science	Correlation Coefficient	-.005	-.227**	-.022	.250**	.131**	-.061	.088*		.407**	.121**	.128**
	Sig. (2-tailed)	.917	.000	.618	.000	.003	.170	.048		.000	.007	.004
Trust in medical professionals	Correlation Coefficient	.054	-.156**	.100*	.079	.173**	-.134**	.074	.407**		.249**	.179**
	Sig. (2-tailed)	.229	.000	.025	.076	.000	.003	.098	.000		.000	.000
Personal efficacy	Correlation Coefficient	.006	-.005	.296**	-.039	.316**	-.359**	-.022	.121**	.249**		.277**
	Sig. (2-tailed)	.886	.915	.000	.385	.000	.000	.623	.007	.000		.000
Collective efficacy	Correlation Coefficient	.086	-.006	.069	.097*	.069	-.122**	.339**	.128**	.179**	.277**	
	Sig. (2-tailed)	.055	.891	.122	.029	.125	.006	.000	.004	.000	.000	

** Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).*

All predictors were analyzed based on gender variables (the Mann-Whitney U test was used due to abnormal distribution). There was a statistically significant difference between men and women in the mean of collective efficacy (P= 0.032) and the rest were not.

Specifically, people who have had direct personal experience of COVID-19 were different in some predictors of risk perception from those who had no direct experience of disease and social knowledge (p=0.007), trust in science (p=0.001) and trust in medical professionals (p=0.050) in positive direct experience people were significantly more than in people with negative direct experience of COVID-19.

We investigated the determinants of risk perception in regression models. The correlations of demographic variables with risk perception were assessed and gender, as well as education, were significantly associated with risk perception of COVID-19, such that males perceive less risk compared to females (Table 6) and the most important predictors of risk perception in COVID-19 epidemic were gender, education, social knowledge and trust in science (Table 7).

Table 6: Regression output of demographic variables of risk perception in COVID-19

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.991	0.142		21.078	0.000
	Age	-0.001	0.002	-0.017	-0.371	0.711
	Gender	0.115	0.055	0.095	2.081	0.038
	Education	-0.118	0.026	-0.205	-4.452	0.000

a. Dependent Variable: Risk perception

Table 7: Regression output of predictors of risk perception in COVID-19

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	4.141	.339		12.226	.000
Age	-.001	.002	-.018	-.419	.675
Gender	.116	.053	.096	2.187	.029
Education	-.111	.026	-.193	-4.219	.000
Personal knowledge	.021	.018	.053	1.181	.238
Social knowledge	-.099	.019	-.234	-5.303	.000
Direct experience	-.031	.058	-.024	-.544	.586
Social amplificatio	.033	.074	.020	.448	.654
Prosociality	-.001	.021	-.001	-.029	.977
Individualism	-.061	.037	-.081	-1.636	.103
Trust in government	.024	.026	.043	.935	.350
Trust in science	-.113	.042	-.136	-2.697	.007
Trust in medical professionals	-.068	.037	-.091	-1.843	.066
Personal efficacy	-.016	.025	-.033	-.661	.509
Collective efficacy	.017	.024	.033	.690	.490

a. Dependent Variable: Risk perception

DISCUSSION

Risk perception is important in determining health-protective behavior. Perception of risk in critical experiences like epidemics is influenced by social, cultural, and contextual factors. Previous experiences such as SARS, influenza, and swine flu epidemic showed that the strategies performed and the results of activities often depend on recognizing the predictors of risk perception and coordinating programs with the important predictors [12]. It should be considered the management of risk perception predictors directs by governments in all programs and activities. The risk perception model recommends in COVID-19 epidemics consist of clusters of variables that correspond to the cognitive tradition (e.g. people's knowledge and understanding of risks), the emotional and experiential tradition (e.g. personal experience), the social-cultural paradigm (e.g. the social amplification of risk, cultural theory, trust, and values), and relevant individual differences (e.g. gender, education, ideology). The mean risk perception score was 2.78(±0.6) in this study which is slightly different from the average risk perception score in other countries. The study of risk perception in ten countries found that the risk perception score of COVID-19 epidemics varied between 4.78 and 5.45 on a 7-point scale, and it is relatively high in countries of the U.K., Spain, and North America. Therefore, in this study, the score of risk perception was significantly lower than in all ten countries. High-risk perceptions may enhance the public's active response to the epidemic. Risk perception is a dynamic process and it changes over time. In this study, more than a year had passed from the beginning of the epidemic, which can be an influential factor in the perception of risk.

In demographic factors, gender was the significant predictor of risk perception. The risk perception score of women was higher than men on all the components of risk perception. Other studies reported the same result and it seems that gender was included as the moderator of risk perception and protective behavior [13]. In similar crises of the past decades, women are constantly seen to be more precautionary behaviors than their male counterparts. Another basic demographic predictor is the level of education of the individual, especially in positive experience persons. In other studies, a high level of educated is more likely to improve the preparedness level and adherence to COVID-19 preventive and control guidelines [14], and poor education was significantly associated with lower risk perception [15], similar to our findings.

The level of education could affect knowledge as well as social knowledge; therefore social knowledge might be an important predictor of risk perception, which was observed in this study. Although studies on COVID-19 have reported associations between social knowledge and risk perception [16], some of them did not find this result [17]. Social knowledge is acquired through socialization and lived experience; however educational guidelines in the media at the national level could affect social knowledge, especially in critical situations such as the COVID-19 epidemic. The basic role of social knowledge in predicting the level of risk perception can be considered in the planning and implementation of national protocols.

This study found that scientific sources were the most trusted sources for COVID-19 information. Similar results were reported in Kwok's study on the COVID-19 pandemic [18] and the prior outbreak of SARS in 2003 [19] and the influenza A (H1N1) pandemic in 2009 [20].

Trust in science and experts in this study was greater than trust in government. The lowest mean of the predictors of risk perception was trust in the government. Trust in the government has been found to influence people's willingness to be accepting preventive activities [21], social limitations [22], and vaccination programs [23]. When people lack the necessary knowledge to evaluate the risk of a hazard, trust becomes an important cue regarding whom to believe and behavior [24]. People need to rely on scientists, professionals, and government agencies to listen and realize the information and the people's trust in these sources might be an important factor contributing to people's acceptance of the difficult precautionary measures needed to eradicate a pandemic [25].

This study might be consisting of some limitations. It should be noted that the study population was limited to those who were referred to the hospital. These people may not be a perfect example of the general population, and it is better to do such research at the national level and with diverse populations. The duration of the research was limited, and it is better to do this research several times.

CONCLUSION

People's perception of the risk of COVID-190 depends on several various factors, the most important of which were gender, level of education, social knowledge, and people's trust in science. It is recommended that studies be performed with larger and heterogeneous populations and longer time.

Acknowledgments

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REVIEW

Carcinoid Heart Disease – A 2022 Retrospective

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Abstract: *We aim to introduce a 2022 update on CHD (carcinoid heart disease) considering a multidisciplinary perspective. This is a narrative mini-review. We searched English, full-length papers (PubMed). Inclusion criteria: original articles regardless of the level of statistical significance. We identified 44 papers and manually selected those with CHD, as follows: 8 original studies, 1 case series (N=9 patients), 16 case reports (N=1 patient/paper), and a total of 1,030 patients on published studies. The most remarkable results are the longest period of enrolment of 3-4 decades; CHD ratio among carcinoid syndrome of 37%; 30-day mortality post-cardiac surgery of 12%; median survival in CHD from 1.3 to 3.9 years (more than 2 years if valve intervention is provided); most useful prognostic markers: 5HIIA, NT-pro-BNP, but, potentially, chromogranin A. The specific protective role of somatostatin analogs against developing CHD is yet to be determined. CHD in the absence of carcinoid syndrome/liver metastasis may be related to ovarian NEN (neuroendocrine neoplasia) with a better outcome if prompt intervention. Remarkably, 3 guidelines are released regarding CHD on behalf of ENETS (European Neuroendocrine Tumor Society), and ESC (European Society of Cardiology). CHD still represents a most challenging entity situated at the crossroads of surgery, cardiology, oncology, endocrinology, and gastroenterology.*

Keywords: *carcinoid heart disease, neuroendocrine, serotonin, neuroendocrine neoplasia, carcinoid syndrome, 5HIIA, valve, tricuspid, echocardiography*

INTRODUCTION

The complex field of neuroendocrine neoplasia (NEN), a rare condition with an annual incidence of 2 to 5.1 cases per 100,000 individuals, represents one of the most spectacular domains with massive recent progress from a multidisciplinary perspective [1,2]. The two main direct cardiac complications of NENs' evolution are either heart metastasis or carcinoid heart disease (CHD), also, recently named carcinoid valvular heart disease [3,4]. Primary cardiac NENs are mostly uncommon [5]. While myocardial and pericardial NENs' spreading is unusual (representing less than 1-3% of NENs-related metastases), CHD represents the hallmark of a very severe disease, typically embracing a very poor outcome [6,7]. The advances in cardiac surgery and interventional once-cardiology which have been registered within the latest years improved the overall CHD prognostic, as recently seen in other cardiac surgical procedures [8,9].

CHD is related to hormonally active NENs that increase overall morbidity and mortality; nevertheless, valve intervention might associate with numerous benefits, but carriers of such interventions display additional risks, including the need for post-operative pacing or connected infectious risks [10,11]. The statistical confirmation that the surgical approach to CHD improves survival is currently based on observational data rather than controlled studies [12].

The pathogenesis of CHD is still poorly understood. However, the presence of CHD is closely correlated with abnormal levels of biomarkers (blood and urinary neuroendocrine markers, also, named vasoactive substances which are more than 40 types, despite not testing all of them in daily practice), especially serotonin, and its urinary metabolite 5-HIIA (5-hydroxy-indole acetic acid), but, also, chromogranin A, neuron-specific enolase, etc. which serve as diagnostic tools and prognostic markers as well as part of the surveillance protocols [13-15]. Novel biomarkers like those based on mRNA transcript are currently under development for daily practice implementation [13]. Cardiac imaging is essential for recognizing CHD, from traditional ultrasound to advanced techniques such as speckle-tracking echocardiography, 68 DOTA peptide PET-CT (Positronic Emission Tomography – Computed Tomography) scans, and cardiac magnetic resonance or CT [12,16-18].

CHD almost exclusively targets the right cardiac side with consecutive heart failure, but in the early stages, the complication might be underdiagnosed [19]. The left side of the heart is usually not affected (unless abnormal intra-cardiac shunts) because the fact that the main contributor of CHD, namely serotonin (5-hydroxytryptamine), but, also, histamine, bradykinins, and tachykinins, are enzymatically inactivated into pulmonary bloodstream [19]. Remarkably, in 2018 three cases of quadruple valve replacement were reported in addition to the prior four concerning CHD, which represents exceptional situations in NEN-CHD pointing out that the left heart side may be affected, as well [20].

Usually, at the moment when CHD is identified, the patients already have clinically manifested NEN in terms of traditional carcinoid syndrome (flush, diarrhea, wheezing, loss of appetite and malnutrition, non-specific abdominal pain or cramps, hypotension, telangiectasia, edema, etc.) that is presented in correlation with liver metastases, grossly affecting 30-40% of all NENs [21]. Most patients with CHD are aged between 50 and 70 years (males being slightly more affected than females), and present chronic fatigue and dyspnea, independent of carcinoid syndrome (but may be initially misdiagnosed as carcinoid syndrome or even carcinoid crisis) [19]. The most frequent type of NEN causing CHD is gut-derivate (noting that the small intestine is the most frequent NEN up to 65-67% followed by lung NEN of 25%) and approximately one out of five or two cases (depending on the studied population) is prove to develop CHD which finally influence the prognostic even more than the primary tumor growth itself (in some cases) [12,22]. One meta-analysis from 2021 (including 9 studies, N=416 adults with NEN, mean age of 63 years, 53% males) showed that 75% of CHD – related NENs originate from the small intestine and colon, and 98% of them have synchronous hepatic metastases. Tricuspid regurgitation was confirmed in 97% of individuals of different severities, respective 72% concerning pulmonary valve, while right heart failure was identified in 48% of all cases. After having a valve replacement (99% of tricuspid, and 59% of pulmonary valve), a 30-day mortality of 9% was identified (with a median post-surgery survival of 3 years) which points out an acceptable safety profile of valves intervention in CHD [23].

Overall, the multidisciplinary management of CHD includes cardiologic therapy for heart failure, the control of serotonin excess by using high doses of somatostatin analogs such as octreotide or lanreotide (and/or telotristat ethyl) that act on tumor growth as well, peptide receptor radionuclide therapy in addition to surgery for primary tumor and metastases, especially at the liver site (if feasible) to reduce the tumor burden, and, finally, surgical – based valves replacement or catheter-directed valves intervention in selected patients, depending on various criteria that take into consideration the underlying NEN, the heart status, and the access to cardiac surgery or interventional cardiology [24-28]. Globally, patients with carcinoid syndrome (thus metastatic NENs) and CHD have a more severe prognostic when compare to those without CHD, knowing that NEN individuals with clinically manifested carcinoid syndrome have a more severe prognostic versus those carcinoid free [28,29]. Valve intervention in CHD improves long-term outcomes despite the current peri-operatory mortality rate being considered at 10-20% [29]. Of note, there is no specific medication for CHD other than the medical approach for the originating tumor and associated hormonal panel (NEN's management), on one hand, and cardiologic therapy for heart failure in addition to interventional procedures targeting the affected valves [28-30] (Figure 1).

Figure 1: Sneak peek – carcinoid valvular heart disease

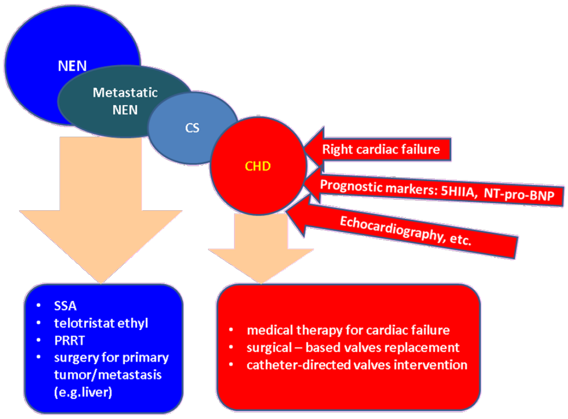
NEN
Metastatic NEN
CS
CHD
Right cardiac failure
Prognostic markers: 5HIIA, NT-pro-BNP
Echocardiography, etc.
SSA
telotristat ethyl
PRRT
surgery for primary tumor/metastasis (e.g.liver)
medical therapy for cardiac failure
surgical – based valves replacement
catheter-directed valves intervention

NEN = neuroendocrine neoplasia; CS = carcinoid syndrome; CHD = cardiac heart disease; 5HIIA = 5-hydroxyindoleacetic acid; NT-pro-BNP = NT-pro-brain natriuretic peptide; SSA = somatostatin analogues; PRRT = peptide receptor radionuclide therapy

MATERIALS AND METHODS

Aim

Our purpose is to introduce a 2022 update on CHD considering a multidisciplinary perspective.

Method

This is a narrative mini-review of literature. We searched English, full-length papers published in 2022 concerning journals indexed in PubMed (by using the terms "carcinoid heart disease"). Inclusion criteria are represented by original articles particularly/specifically addressing CHD (in vivo) regardless of the level of statistical significance (original studies or case reports/series). Exclusion criteria: papers considering heart metastasis in NENs or primary cardiac NENs.

According to mentioned methodology, we identified 44 papers in 2022, and manually selected those with CHD, as follows: 8 original studies, 1 case series (N=9 patients), 16 case reports (N=1 patient/paper), a total of 1,030 patients on published studies, in addition to 8 reviews, 3 guidelines (one with duplicate). We excluded 3 articles concerning non-CHD, one in vitro study, and 3 papers regarding withdrawal (Figure 2).

CLINICAL STUDIES PUBLISHED IN 2022 CONCERNING CHD (accessed via PubMed)

We identified 8 original studies in 2022 (Table 1). A multicenter, retrospective study on 275 patients analyzed the clinical presentation and the outcome in NENs, confirming that more than one-third of the individuals with carcinoid syndrome are affected by CHD. All the patients had carcinoid syndrome (mean age of 57 years, aged between 21 and 84 years; metastatic disease was found in 94.6% of cases). CHD was identified in 37.3% of them (N=104) by using echocardiography, with the tricuspid valve being the most affected cardiac site, single or in combination with other valves. CHD correlated with a poor prognostic in individuals with carcinoid syndrome (similarly, male sex was found to be a risk factor); median survival in the studied population was of 9 years) [31].

Figure 2: The articles included according to the methodology of research

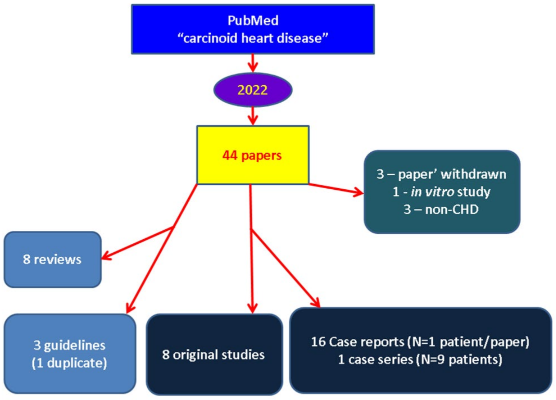

Table 1: Original studies published in 2022 considering carcinoid heart disease

First author/ Reference number	Type of Study	Number of patients	Results
Bergsten J [32]	retrospective (1986 - 2019)	60	30-day mortality (after cardiac surgery)=12% median post-operatory survival = 2.2 years
Levy S [34]	retrospective (1984 - 2021)	84	median survival since CHD diagnostic varying from 1.3 to 3.9 years
Levy S (bis) [35]	validation (case-control) study (1984 - 2021)	114	NT-pro-BNP has the best predictive value for CHD
Kostiainen I [36]	cross-sectional cohort study	65	low rate of CHD (5%) highly sensitive value of echocardiography (not BNP)
Shah HA [33]	retrospective study (2010 - 2019)	17	a 35% decline rate of 5-HIIA in association with liver function improvement
Macfie R [39]	retrospective study (2001 - 2021)	282	overall survival is lower in CHD *versus* non-CHD (N1=40 *versus* N2=242, p<0.001) valve replacement improves survival in CHD + inoperable liver metastasis
Konsek-Komorowska SJ [38]	retrospective study	108	chromogranin A inversely correlates with overall survival (p<0.05) in patients with carcinoid syndrome overall survival is lower in CHD *versus* non-CHD + carcinoid syndrome (67.22 versus 73 months)
Fijalkowski R [31]	retrospective study	275	prevalence of CHD 37.3% among patients with carcinoid syndrome overall survival is lower in CHD *versus* non-CHD

Abbreviations: CHD=cardiac heart disease; BNP=brain natriuretic peptide; N=number of patients

Bergsten J. et al. studied 60 patients (between 1986 and 2019, a total of 3 decades) with a median age of 64 years with CHD and cardiac surgery (N1=42 persons underwent both tricuspid and pulmonary valve replacement; N2=18 individuals had only tricuspid valve replacement with/without pulmonary valvotomy). The procedures (which exclusively used bioprosthetic valves) associated a 30-day mortality of 12%; and a median post-operatory survival of 2.2 years (a maximum of 21 years; statistically significantly higher in N1 versus N2, respective of 3 versus 0.9 years, p=0.02). A most useful tool of prognostic was echocardiography, either as a pre-operatory assessment (but with the limitation of underestimating pulmonary involvement) or post-operatory surveillance. Additional poor prognostic markers are high pre-surgery levels of 5-HIIA and NT-pro-BNP (NT-pro-brain natriuretic peptide). Overall, the double valve is superior to single tricuspid replacement [32]. Moreover, a retrospective UK study (between 2010 and 2019) on 17 patients with CHD who became candidates for valve replacement surgery had a single valve procedure (3/17) versus double valve (13/17), respective triple valve (1/17). Post-operatory, a 35% decline of 5-HIIA witnesses the improvement of general status in association with hepatic function recovery which suggests an indirect benefit of cardiac surgery [33].

Levy S. et al. introduced a cohort study on 84 patients with CHD (58% males) followed for 4 decades (between 1984 and 2021) at Netherlands Cancer Institute; the median age of NEN confirmation was of 62.3 years with a consecutive CHD diagnostic after a median of 1.1 years and an associated median survival varying from 1.3 to 3.9 years (considering the patients who were most recently admitted when valve replacement surgery was more often applied). Prognostic markers are NT-proBNP (positively correlated with tricuspid regurgitation), respective to the implementation of valve replacement surgical procedures (which increases the survival rate) [34]. The same researchers conducted a validation (case-control) study on CHD – associated biomarkers. 114 individuals (N1 with CHD versus N2 without CHD) showed similar age (median age of N1 – 57.9 years, respective of N2 – 59.7 years, p=0.29). Among studied assessments like circulating serotonin, activin A, sST2 (soluble suppression of tumorigenicity 2), CTGF (connective tissue growth factor), and NT-pro-BNP had the best predictive value for CHD in individuals with high serotonin [35].

As opposed to these results, we mention a cross-sectional cohort study on 65 patients with small intestinal NETs (neuroendocrine tumors) (45% with carcinoid syndrome and 5% with CHD). The results showed that both categories had a statistically significant increase in hepatic tumor load, serum levels of chromogranin A, and urinary 5HIIA when compare to the patients without carcinoid syndrome (p<0.005). However, the authors identified a lower rate of CHD (5%) than expected in persons with carcinoid syndrome (30-50% – according to data from the literature). Moreover, they did not confirm the predictive value of proBNP but identified the highly sensitive value of echocardiography in subjects with elevated chromogranin A and 5HIIA [36].

Another classical biomarker in NENs is chromogranin A, very sensitive, but less specific [37]. A retrospective study focusing on blood chromogranin A values (N=108 patients with carcinoid syndrome; N'=84 individuals were followed-up and 27 of them complicated with CHD) showed that its increased levels inversely correlate with overall survival (p<0.05) in patients with carcinoid syndrome. Also, the presence of CHD is statistically significantly associated with a reduced overall survival (67.22 versus 73 months in patients with carcinoid syndrome but CHD-free) [38].

Some authors consider the protective effect of somatostatin analogs like octreotide for CHD, but a large study on 282 patients with carcinoid syndrome (between 2001 and 2021) did not sustain this observation. Overall survival was lower in patients with CHD versus non-CHD (N1=40 versus N2=242, p<0.001). Considering the patients with inoperable hepatic metastasis, the survival rate was lower in patients without undergoing valve replacement when compared to the individuals with CHD and cardiac surgery or the individuals CHD-free [39]. Similarly, we mention a case series of 9 subjects with NEN who followed for 39 months; initially, the patients were CHD-free and started therapy with somatostatin analogs (median time from NEN diagnostic to therapy initiation of one month; median time from starting the medical treatment to CHD confirmation of 23 months) [40]. As far as we currently know, we cannot predict that using somatostatin analogs is a protection factor against CHD, but, of course, this medication has a proven effect in controlling the hormonal and tumor burden [41].

INTERESTING CASE REPORTS

We identified according to our methodology 16 case reports (a total of 16 patients with CHD). We mention the case of a 70-year-old female with torrential tricuspid regurgitation due to CHD regarding an ovarian teratoma with neuroendocrine differentiation. The subject had a heterotopic tricuspid valve replacement with a stable disease within the following 6 months. Mostly particular, the patient did not experience carcinoid syndrome, and neither imaging assays proved any metastatic NEN (including hepatic) after gynecological surgery [42]. Similarly, another case of a large insular primary ovarian NEN was reported on a 40-year-old female admitted for virilization syndrome in association with unexpected CHD – related heart failure; the panel of increased neuroendocrine markers improved after gynecological intervention, but CHD aggravated even in the absence of liver metastasis requiring valve replacement within 6 years [43]. This scenario is extremely unlikely among the typical evolution of NENs, neither ovarian NEN is recognized as a common source of serotonin over-production. However, ovarian carcinoid tumors are among the rarest forms of CHD in the absence of carcinoid syndrome and/or liver metastases, thus their early removal massively improves the survival rate and does not follow within the general frame concerning the very poor outcome in CHD-NENs [44-46].

adequate diagnostic/recognition when she was identified with CHD and had cardiac surgery. The identification of primary NEN was after the intervention for CHD [47]. This case brings out at least two atypical aspects: CHD in young adults, as prior mentioned, which is rather atypical unless a clear genetic background is present, and CHD diagnostic before confirmation of primary NEN [48]. Generally, one-third of NENs remain with unknown primary sources [49]. Theoretically, younger patients with CHD and NEN might have a better post-cardiac surgery prognostic, but further statistical evidence is needed.

A 44-year-old male with neurofibromatosis type 1 had a 6-year history of gastric NET with hepatic metastasis. He was admitted for sensory neuronopathy and a meningocele. After glucocorticoid treatment was empirically initiated, additional decompressive laminectomy was done, but the patient soon developed CHD with a fatal outcome within 3 months (due to pneumonia) [50]. Of note, currently, we do not know the potential role of glucocorticoids (probably recommended for incidental co-morbidities) in aggravating CHD. Moreover, there are no consistent data to point out that individuals with genetic syndromes underlying NEN have a higher risk of developing CHD [51].

As consideration amid the recent COVID-19 pandemic, we mention one case of a 55-year-old male who delayed the presentation and was admitted for severe CHD which was confirmed synchronously with a high serotonin-producing primary NEN [52]. Another case brings out the particular aspects of cardio-oncology practice following valve replacement in subjects with CHD [53]. This is the case of a 58-year-old female who experienced a carcinoid crisis peri-operatory [54]. Another aspect concerns the post-surgery risks, for instance, a 77-year-old woman was offered a successful ablation therapy for recurrent atrial flutter developed after the surgical procedure of replacing the tricuspid valve [55]. Moreover, another case of an 81-year-old male introduces the idea of consecutive interventions in CHD considering the right side of the heart (severe pulmonary regurgitation was successfully approached by trans-catheter implantation 4 years after initial surgery for tricuspid) [56].

DISCUSSIONS

Pathogenic traits of CHD

Hedinger syndrome, tricuspid insufficiency in addition to pulmonary stenosis due to CHD still represents a challenging condition nowadays [57]. CHD is a progressive condition in subjects with carcinoid syndrome where the right side of the heart is transformed in terms of fibrosis, degeneration, inflammation, desmoplastic reactions causing valvar dam-age (stenosis or regurgitation), and cardiac insufficiency [58,59]. The formation of plaque-like deposits (myo-proliferative plaques) on endocardial areas of the valves and subvalvular system impairs the blood flow dynamics [60-62]. Similarly to CHD, mesenteric fibrosis might develop in individuals with carcinoid syndrome [60]. Common pathogenic factors are described like TGF-β (Transforming growth factor beta) or PDGF (platelet-derived growth factor), etc [60].

The clinical approach of CDD

The main predictors of CHD are an advanced stage of NEN with high levels of circulating serotonin but mostly increased 24-hour urinary 5HIIA in addition to the presence of carcinoid syndrome and other clinical features [63-65]. Other collateral elements may involve particular cardiac anatomy, for instance, patent foramen ovale is reported in half of the individuals with CHS (twice more frequently than in the general population) which causes a right-to-left shunting [66,67]. Intra-cardiac shunt explains the left heart involvement in CHD as one case was published in 2022 with four valves involvement [68,67].

Since initially, CHD is subclinical or completely asymptomatic, a strategy of periodic check-ups is necessary, including performing differential diagnostics with other complications of NENs [60,63,69]. Cardiac imaging represents a strong point in CHD management. Ultrasound is useful for serial evaluations and early assessment; CT exams might show various elements like early contrast opacification of inferior cave vein as an indicator of tricuspid regurgitation; modern imaging procedures like 68Ga-DOTATATE PET are helpful for adequate positive diagnostic, especially for cases with a low index of suspicion, when a differentiation with myocardial metastasis or non-NEN incidental conditions is needed [70-72]. One case from 2022 introduced 203Pb-VMT-α-NET Scintigraphy as a new alternative to PET/CT [73].

Full blown picture of heart valve disease – associated clinical manifestations and related cardiovascular and pulmonary complications of NENs including carcinoid crisis represents a sign of advanced disease and prompt multi-disciplinary intervention is required [74,75]. The overall experience with cardiac surgery for CHD is limited when compared to many of the non-CHD causes, thus there are still some areas of open discussion like predictors of successful intervention, the timing of the procedure, criteria of candidates selection, the type replacement (single or double valve) [76-79].

Key messages on behalf of highly specialized medical and surgical societies

In 2022 three papers of major importance were released concerning CHD. European Neuroendocrine Tumor Society (ENETS) published excellent “guidance” regarding carcinoid syndrome and CHD. We mention a few major aspects: NT-proBNP remains the most important marker of CHD at the first-ever diagnostic (in patients who experience high serotonin status with or without clinical manifestation like carcinoid syndrome) and during follow-up. A cutoff value of 260 pg/mL indicates further investigations with echocardiography. In terms of ultrasound, the transthoracic approach is indicated, stress echocardiography might not bring additional information; while inadequate ultrasound data (including the evaluation of pulmonary vein damage) or planning for cardiac surgery may require supplementary imaging procedures like cardiac CT or magnetic resonance. Once identified, CHD requires serial NT-proBNP assays and cardiac ultrasound every 6 to 12 months, noting that sudden progress might occur anytime. Surgery (bioprosthetic valves are advised) should be done in severe CHD with more than 1 year of expected post-surgery survival; some specific criteria are recommended considering the adequate timing of intervention which should not be delayed. Double valve (tri-cuspid and pulmonary) replacement is better than a single one. Minimally invasive procedures are not yet studied enough [80]. Additionally, ENETS introduced a "synoptic" concerning the major role of echocardiography in CHD [81]. A collaboration of ESC (European Society of Cardiology) with other societies provided a guideline on cardio-oncology, also, addressing CHD. We mention some aspects, such as, for instance, echocardiography is recommended in patients with clinical evidence of CHD, carcinoid syndrome, and high BNP followed by a check-up every 3 to 6 months (level IB). BNP is assessed at baseline and every 6 months (level IB). A specific preparation is required considering the surgical/interventional risk of triggering a carcinoid crisis. Valve replacement, as in the ENETS guide-line, is recommended in patients with an expected survival of more than 1 year after intervention if they have symptomatic CHD or asymptomatic CHD (tricuspid or pulmonary) but with progressive dysfunction (level IC, respective IIaC) or severe mitral and/or aortic involvement in symptomatic cases (level IC) [82,83].

CONCLUSIONS

CHD represents a most challenging entity situated at the crossroads of surgery, cardiology, oncology, endocrinology, and gastroenterology. Until a few years ago, CHD was synonymous with a very severe form of NEN/carcinoid syndrome, with an expected poor outcome and limited resources. Recent cardio-oncology progress in addition to multiple shaped interventions for NEN allows a new level of approach in CHD to the benefit of the patients. However, many aspects are still open issues even in 2022.

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Authors' contribution
Conceptualization, CEN, and MC; methodology, CEN, and MC; software, MC; validation, CEN, MC, AER, and AC; formal analysis, AC; investigation, AER, and AC; resources, AER, and AC; data curation, AC; writing—original draft preparation, CEN, MC, and AC; writing—review and editing, CEN, MC, AER, and AC; visualization, CEN, and MC; supervision, CEN; project administration, CEN, and AC.
All authors have read and agreed to the published version of the manuscript.

List of Abbreviations
5-HIIA = 5-hydroxyindoleacetic acid
NEN = neuroendocrine neoplasia
CHD = carcinoid heart disease
NT-pro-BNP = NT-pro-brain natriuretic peptide
PET-CT = Positronic Emission Tomography – Computed Tomography
TGF = Transforming Growth Factor
PDGF = platelet-derived growth factor
NET = neuroendocrine tumor
NEN = neuroendocrine neoplasia
sST2 = soluble suppression of tumorigenicity 2
CTGF = Connective Tissue Growth Factor
ENETS=European Neuroendocrine Tumor Society
ESC=European Society of Cardiology

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REVIEW

Collision Tumors of the Thyroid

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Abstract: *Collision tumors, rare neoplasms underlying two distinct histological types of carcinomas (with different origins) separated by normal tissue, are described in many organs including the thyroid gland, but to a lesser extent. We aim to overview the current published data concerning collision tumors of the thyroid (CTT). This is a narrative review considering papers published on PubMed through a search starting from the terms "collision" and "thyroid". Inclusion criteria are full-length articles, the English language, the timeline from inception to December 2022, and original studies with different levels of statistical evidence. We followed the main types of thyroid carcinomas: differentiated thyroid carcinoma of papillary and follicular type, anaplastic/ undifferentiated/poorly differentiated carcinoma, respectively medullary thyroid carcinoma. We identified 92 papers based on the mentioned key words criteria. We excluded 54 papers (reviews or non-thyroid CTT or other topics outside our purpose). We included in the final analysis 38 papers: 34 case reports (1 patient per paper), 2 case series (2 individuals per each article), one case series of 3 subjects, and a single study of retrospective type (including 21 subjects) – a total of 62 patients on published cases which represents the largest study on published cases to our knowledge (according to our methodology).*

Keywords: *collision, thyroid, thyroid cancer, papillary carcinoma, follicular carcinoma, medullary thyroid cancer, thyroidectomy, pathological report*

INTRODUCTION

Collision tumors, rare neoplasms underlying (at least) two distinct histological types of carcinomas (with different origins) separated by normal tissue (and no mixture areas), are described in many organs (for instance, lung, stomach, breast, and ovary, etc.) including the thyroid gland, but to a lesser extent than seen in others sites (affecting less than 1% of all thyroid malignancies) [1-3]. The most common combination is papillary and medullar carcinomas, but any type of primary thyroid malignancy is reported in collision with a secondary spreading to the thyroid from a non-thyroid primary cancer or another primary type [4-6].

Collision tumors of the thyroid (CTT) are regarded as challenging in terms of recognition, poor outcome elements, and particular behavior in addition to the fact that there are still areas of the unknown regarding this chapter on thyroid neoplasms [7-9]. A distinction with composite tumors should be done (where the same mutation causes histologically different entities) [10-12]. Also, metachronous tumors (syndromic or not) represent a different category [13-14].

We aim to overview the current published data concerning CTT**.**

MATERIALS AND METHODS

This is a narrative review considering papers published on PubMed through a search starting from the terms "collision" and "thyroid". Inclusion criteria are full-length articles, the English language, the timeline from inception to December 2022, and original studies with different levels of statistical evidence. Exclusion criteria are non-thyroid collision tumors, non-English papers, and reviews. We followed the main types of thyroid carcinomas: differentiated thyroid carcinoma of papillary and follicular type, anaplastic/undifferentiated/ poorly differentiated carcinoma, respectively medullary thyroid carcinoma**.**

RESULTS

We identified 92 papers based on the mentioned key words criteria. We excluded 54 papers (reviews or non-thyroid CTT or other topics outside our purpose). We included in the final analysis 38 papers including 34 case reports (1 patient per paper), 2 case series (2 individuals per each article), one case series of 3 subjects, and a single study of retrospective type (including 21 subjects) – a total of 62 patients on published cases which represents the largest study on published cases to our knowledge (according to previously mentioned methodology) (Figure 1).

Figure 1: The method of research considering collision tumors of the thyroid

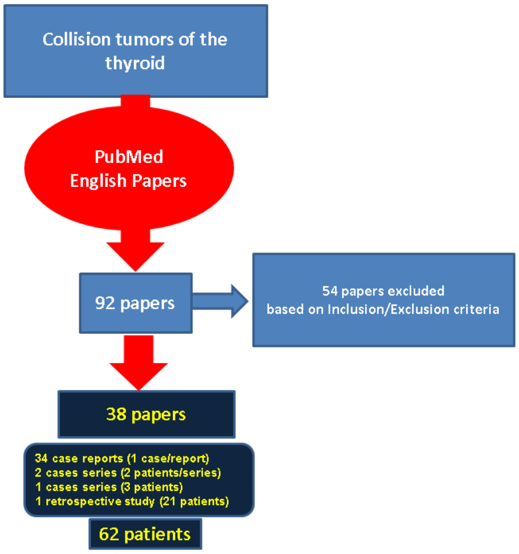
Collision tumors of the thyroid
PubMed
English Papers
92 papers
54 papers excluded
based on Inclusion/Exclusion criteria
38 papers
34 case reports (1 case/report)
2 cases series (2 patients/series)
1 cases series (3 patients)
1 retrospective study (21 patients)
62 patients

Papillary thyroid carcinoma

Papillary thyroid carcinoma is the most frequent histological type in CTT, but, generally as a single entity, it represents the most common thyroid neoplasia [15]. Apart from the most often association of medullary thyroid neoplasia, follicular malignancy is reported as well [16-19]. The combination with thyroid metastasis (typically from lung, breast, or renal cancers, etc.) is reported at the level of case reports (the spreading may be by local extension or via blood mainstream) [20-21]. For example, a case from 2007 introduced a CTT consisting of metastasis from lung adenocarcinoma and papillary thyroid cancer in a 63-year-old female [20]. A case report from 2021 included a 60-year-old female who was identified with the same carcinoma and metastasis from osteosarcoma within a CTT. This is the first case with bone origin as the primary source reported in a CTT [21]. The combination with a second primary cancer which is not originating from the thyroid is also reported. We identified a single case of a 67-year-old male who was diagnosed with a CTT associated a papillary thyroid cancer (with lymph node metastases) and parathyroid carcinoma clinically manifested with primary hyperparathyroidism [22]. The co-existence of a squamous cell carcinoma was reported, as well, originating from different sites or of unknown origin [23-27]. In 2004, a most unusual association with metastatic liposarcoma was published (this represents a most unique combination) [28].

Follicular thyroid carcinoma

This is the case of a 62-year-old female with follicular carcinoma (with capsular and vascular invasion) in association with a mucoepidermoid carcinoma and distant metastasis. Immunohistochemistry report might identify the mucoepidermoid differentiation of follicular malignancy over a different histological type (like mucoepidermoid malignancy) [29]. Hürthle cell carcinoma (regarded as a type of follicular group of differentiated thyroid cancers) is reported in less than 3% of thyroid malignancies, with the same ratio (of 3%) being reported for medullary thyroid carcinoma, as well. Their combination was identified on a 70-year-old female as a CTT (of note, the medullary cancer was intra-thyroidal, while the lymph nodes extension was derivate from Hürthle cell carcinoma) [30].

Interestingly, one retrospective study on 8 patients diagnosed with a primary thyroid malignancy identified the co-presence of the second histological type, meaning a follicular thyroid adenoma in association with papillary thyroid carcinoma (7/8), respectively medullary thyroid carcinoma (1/8) [31]. However, the current definition of CTT includes two malignant tumors, either primary or secondary, not a benign one like a follicular adenoma (which cannot be differentiated from a follicular carcinoma through the cytological report and it requires histological assessment) or a non-tumor – related cyst [31,32].

Medullary thyroid carcinoma

CTT may concern the thyroid gland and/or associated lymph nodes [33,34]. For instance, there is a case published in 2021 involving a 27-year-old male presenting CTT at lymph nodes (central compartment) from papillary thyroid cancer and non-syndromic medullary thyroid cancer. The growth speed was evaluated and the authors suggested that it may be similar to what we prior know concerning each of the two malignancies [35]. Generally, collision metastasis (mostly at cervical lymph nodes) is rarely reported in the absence of a thyroid tumor itself. We identified two papers concerning collision metastasis from papillary thyroid cancer and breast malignancy in female subjects [36,37]. In another case, a 73-year-old male was admitted for collision metastasis (cervical lymph nodes) from squamous cell carcinoma of nasal origin and B-cell lymphoma [38]. A previous report from 2009 included a lymph node spreading from a primary follicular malignancy and a squamous cell carcinoma of unknown origin [39]. Moreover, we mention the case of a 47-year-old male with collision metastasis from oral tongue–related squamous cell carcinoma and accidentally detected thyroid papillary carcinoma [40].

However, we currently do not have enough statistical evidence to compare the evolution in CTT versus non – CTT cases. The largest study particularly addressing CTT is a retrospective analysis that included the experience from 2009 to 2019 (a 10-year experience) on a tertiary care cancer. CTT (medullary + papillary) represented 4.7% of the medullary cancer series (N=21 individuals, the sex ratio of 1; mean age of 45.33 years, range between 26 and 77 years). 66.66% of the papillary cancers were situated at the right thyroid lobe; 85.7% of them were of micro-papillary type. The mean size of medullary cancer was three times larger than differentiated thyroid tumors. The contribution to lymph node spreading (which was confirmed in 76.2% of cases) was done by medullary type (87.5%), papillary cancer (12.5%), respectively both malignancies (12.5%) [41].

Anaplastic, undifferentiated, and poorly differentiated thyroid carcinoma

In 2022 Gupta RK. et al. reported the case of a 55-year-old female with a most unusual CTT: anaplastic thyroid malignancy (osteoclastic variant) and primary squamous cell carcinoma, each affecting a different thyroid lobe in addition to distant metastases at the level of bone, muscle and lymph nodes. However, according to WHO (World Health Organization) 2022, these two types are parts of the same malignancy spectrum thus their combination is nowadays regarded as a CTT-like lesion [42].

The "late anaplastic shift" theory concerns the identification of anaplastic thyroid carcinoma on a subject with a long history of differentiated thyroid carcinoma (either papillary or follicular) [43]. However, the synchronous identification of a differentiated tumor in association with a poorly differentiated cancer respecting a border area between them is consistent with the current concept of CTT. Here we mention the case published in 2021 by Vlad M. et al. (a 56-year-old female admitted amid the COVID-19 pandemic with a post-thyroidectomy confirmation of anaplastic cancer on the left lobe and lymph nodes spreading and a papillary micro-carcinoma on the right lobe) [44].

The presence of two highly aggressive malignancies in CTT makes the prognostic extremely severe. There is the case of a 63-year-old female confirmed with a triple CTT: poorly differentiated carcinoma, papillary carcinoma (classical and hobnail variant – this is the first case report underlying this subtype in CTT), and oncocytic (Hürthle cell) carcinoma. The hobnail pattern has been reported in anaplastic and poorly differentiated thyroid malignancies, representing a sign of a very aggressive profile with rapid evolution [45].

DISCUSSION

Pathogenic loops in collision

The field of thyroid surgery is extremely complex, while the domains of pathological reports underlying thyroid neoplasia are also extremely vast and dynamic [46-48]. The collision phenomenon has been described at many levels within multiple organs of the human body [49]. "Chance" theory is one pathogenic hypothesis [41]. CTT might be underdiagnosed, especially micro PTC [41]. Fine needle aspiration (cytological report) might not identify both tumors. However, immunohistochemistry and molecular studies might help the diagnostic [50].

Whether different immune or genetic co-morbidities might act as a trigger or epigenetic elements for CTT development there is still an open question. We mention a case of CTT (papillary and medullary neoplasia) in a 46-year-old female with multiple sclerosis and prior therapy with interferon. This is the first case of CTT on a patient with this neurologic condition (published in 2020) and there is still missing information about a potential pathogenic loop [51].

Common genetic background of both tumors underlying CTT is reported in some cases. For instance, there is the case of a 69-year-old woman with Hürthle cell carcinoma (on the right lobe) and multi-centric papillary cancer (on both thyroid lobes), associated with BRAF mutations (V600E gene) [52].

Also, in 2010, a case of non-multiple endocrine neoplasia medullary cancer in addition to papillary malignancy underlying CTT was reported in a female carrying a particular RET mutation (familial medullary thyroid cancer) [53]. A RET somatic point mutation in association with mixt medullary–papillary (including a follicular variant of papillary cancer) neoplasia was described in a 59-year-old male (a case published in 2007) [54]. Earlier data also suggest this theory [55]. Moreover, the co-presence of granulomatous inflammation in a case of papillary–medullary malignancy might represent the hallmark of common pathways [56].

Early reports of CTT

Of historical note, we identified the first report regarding CTT on PubMed in 1977 (non-English paper) [57]. The first English paper concerning a single case with medullary and papillary neoplasia is dated 1989 [58]. The first case series (2 out of 3 cases) on PubMed with the same mix of neoplasia is published in 1994 [59]. The first CTT with collision metastasis published in a PubMed – indexed journal dates from 1996 [60]. The first triple CTT (on PubMed) was reported on a 27-year-old female with papillary, follicular, and medullary carcinoma [61].

The literature-based analysis

Overall, we identified 62 cases on published data concerning CTT, thus we may say that this particular entity remains at the level of case reports. However, paying attention to this most unusual neoplasia might improve the prognostic and help early detection of at least one of the tumors. With one exception, all cases included at least one differentiated thyroid malignancy, either papillary or follicular (including 3 cases of Hürthle cell/oncocytic carcinoma). Five reports were consistent with the concept of collision metastasis concerning local lymph nodes (near the thyroid). 16/38 articles included medullary thyroid cancers. Based on these data, following differentiated (papillary being the most frequent) and medullary types, elements of squamous cell carcinoma originating from different organs were more frequent than anaplastic/poorly differentiated carcinoma. As mentioned, the most uncommon elements in CTT were: metastatic liposarcoma and osteosarcoma, respectively, and parathyroid carcinoma. All reported cases are adults (we did not identify any pediatric cases). The age varied between 26 and 88 years. More than half of the papers included female patients, but the single study we have so far reported an equal prevalence between males and women (Table 1).

Table 1: Published cases on collision tumors of the thyroid according to our methodology; the cases are displayed from the most recent starting with 2022 (please see references in the text according to first column indices)

First author, Number, Reference number	Studied population	DTC	ATC	MTC	Other types of carcinoma
Vijayvergiya G., 1, [29]	1 case (62-y, F)	FTC			mucoepidermoid carcinoma
Yeap ZH., 2, [30]	1 case (70-y, F)	Hürthle cell carcinoma		MTC	
Vlad M., 3, [44]	1 case (56-y, F)	micro-PTC	ATC		
Li H., 4, [35]	1 case (27-y, M)	PTC		sporadic MTC	
Toyoshima MTK., 5, [45]	1 case (63-y, F)	oncocytic carcinoma classical and hobnail PTC	poorly differentiated carcinoma		
Koufopoulos N., 6, [21]	1 case (63-y, F)	PTC		MTC	metastasis from osteosarcoma
Foppiani L., 7, [22]	1 case (67-y, M)	PTC			parathyroid carcinoma
Thomas A., 8, [41]	retrospective (N=21, F/M=1)	PTC		MTC	
Feng JW, 9, [16]	1 case (40-y, F)	PTC + FTC			
Sirbu AM., 10, [51]	1 case (46-y, F)	PTC		MTC	
Pishdad R., 11, [17]	1 case (79-y, M)	PTC + FTC			
Dikbas O., 12, [18]	1 case (35-y, F)	PTC		MTC	
Alhanafy AM., 13, [33]	1 case (73-y, F)	PTC			moderately differentiated squamous cell carcinoma
Sinno S., 14, [52]	1 case (69-y, F)	PTC + Hürthle cell carcinoma			
Fulciniti F., 15, [50]	1 case (#)	PTC			mucoepidermoid carcinoma
Gao Q., 16, [36]	1 case (59-y, F)	PTC			breast cancer ##
Ryan N., 17, [23]	1 case (88-y, F)	PTC			squamous cell carcinoma
Gurkan E., 18, [6]	1 case (44-y, M) 1 case (77-y, M)	PTC		MTC	
Plauche V., 19, [19]	1 case (62-y, F)	PTC + FTC			
Wang X., 20, [24]	1 case (55-y, M)	PTC			laryngeal squamous cell carcinoma
Kataria K., 21, [56]	1 case (35-y, M)	PTC		MTC	
Zeng H, 22, [37]	1 case (49-y, F)	PTC			breast cancer ##
Sadat Alavi M., 23, [34]	1 case (25-y, M)	PTC		MTC	

Kakarala K., 24, [38]	1 case (73-y, M)				squamous cell carcinoma low-grade B cell lymphoma ##
Warman M., 25, [25]	1 case (84-y, F)	PTC			squamous cell carcinoma
Gul K., 26, [53]	1 case (F, #)	PTC		MTC	
Jacobson AS., 27, [26]	1 case (#)	PTC			squamous cell carcinoma
Mattioli F., 28, [39]	1 case (#)	FTC			squamous cell carcinoma ##
Lim YC., 29, [40]	1 case (47-y, M)	PTC			squamous cell carcinoma##
Rekhi B., 30, [54]	1 case (59-y, M)	PTC		MTC	
Nabili V., 31, [20]	1 case (63-y, F)	PTC			metastasis from lung adenocarcinoma
Walvekar RR., 32, [27]	1 case (65-y, F)	PTC			squamous cell carcinoma
Rossi S., 33, [55]	1 case (61-y, F) 1 case (27-y, M) 1 case (34-y, M)	PTC		MTC	
Brandwein-Gensler M., 34, [28]	1 case (86-y, F)	PTC			metastatic liposarcoma
Wu CJ., 35, [61]	1 case (27-y, F)	PTC + FTC		MTC	
Pastolero GC., 36, [60]	1 case (#)	PTC		MTC	
Lax SF., 37, [59]	2 cases (#)	PTC		MTC	
Gero MJ., 38, [58]	1 case (#)	PTC		MTC	

We currently do not know if any data concerning the age or the sex of the subjects should be regarded as risk factors. In addition to the lack of poor survival indices (which are relevant in metastatic cases, those with late diagnostic, and anaplastic/poorly differentiated tumors), the underlying mechanisms are controversial and we need further histological, molecular, and genetic studies.

CONCLUSIONS

The current limits of the CTT field are correlated with the small size of published data until the present time. We still need markers of poor prognostic. Awareness is essential for early detection with a consecutive potential improvement of the outcome.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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None

Authors' contribution
Conceptualization, A.D., and M.C.; methodology, M.C.; software, A.P.C.; validation, A.D., E.P., and M.C.; formal analysis, D.T.; investigation, A.P.C., D.T.; resources, A.D., M.C.; data curation, E.P.; writing—original draft preparation, M.C.; writing—review and editing, A.D., A.P.C, D.T., M.C.; visualization, M.C.; supervision, A.D.; project administration, A.D., M.C.
All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Diagnosis of Chronic Kidney Disease in the Elderly – A Challenge for the Practitioner

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Abstract: *The recent increase in life expectancy is the main argument for a better understanding of the pathophysiological mechanisms underlying aging. These, once known, can provide possible links to therapies to prevent aging or slow down the process. Normal aging is associated with a progressive decrease in the glomerular filtration rate. Accurate estimation of GFR in the elderly is under the suspicion of multiple errors mainly due to sarcopenia and decreased protein intake. Differentiation between chronic kidney disease and the physiological decline of GFR might be a challenge in clinical practice and this has consequences on the evolution and treatment of the numerous comorbidities of the elderly. The current trend to use non-invasive diagnostic techniques explains the need to identify a serological marker to help differentiate between decreased GFR secondary to kidney aging or the development of chronic kidney disease.*

Keywords: *elderly, normal kidney aging, chronic kidney disease*

INTRODUCTION

Globally, there is a progressive aging of the population.

According to the United Nations Report, 1 in 11 people was over 65 years old in 2019 and it is estimated that in 2050, 1 in 6 people will be this age [1].

The aging process affects the whole body and is characterized by structural changes and functional decline in all organs, leading to an increased incidence of many diseases considered "specific to the elderly". Among these changes, the aging-associated alterations in both structure and function of the kidney are particularly important, having consequences on the evolution and treatment of the numerous comorbidities of the elderly.

Accurate quantification of kidney function in the elderly is difficult, both overestimating and underestimating the glomerular filtration rate (GFR) being possible with currently available serum creatinine-based calculation formulas.

AGING KIDNEY PROCESS

An accurate description of histological changes in the senile kidney, i.e. changes attributed to advanced age per se, is difficult because the elderly usually accumulate important comorbid burdens with impact on the kidney structure and function: diabetes, hypertension, heart failure, etc. Many of the terms that describe physiological changes in the aged kidney superimpose with terms that describe renal pathological processes: nephrosclerosis, tubulointerstitial sclerosis, glomerulosclerosis, arteriosclerosis, etc.

Macroscopically, starting from the 4th decade of age, the kidneys begin to decrease in size and weight progressively, so that around the 7th decade there is a reduction by about 20-30% of kidney weight compared to the age of 40 years [2]; in the elderly without kidney disease, the decrease in kidney size is symmetrical. Also, with age, the kidney's cortex becomes thinner and rougher, the number and size of benign cysts increases, and the pelvis is enlarged with more fatty infiltration. The scarring is more advanced when associated with aortic and renal atherosclerosis (Figures 1 – 5) [3].

Microscopically, starting with the 5th decade of life, there is a progressive decrease in the total number of nephrons due to sclerosis of all nephron compartments [3]: glomerulosclerosis, tubulointerstitial sclerosis, and arteriosclerosis.

The prevalence of glomerulosclerosis increased from 25% in the 3rd decade of life to 82% in the 8th decade [4], the main cause being arteriosclerosis of the small intrarenal arteries.

Figure 1: 87 years old male: granular surface with increased scarring

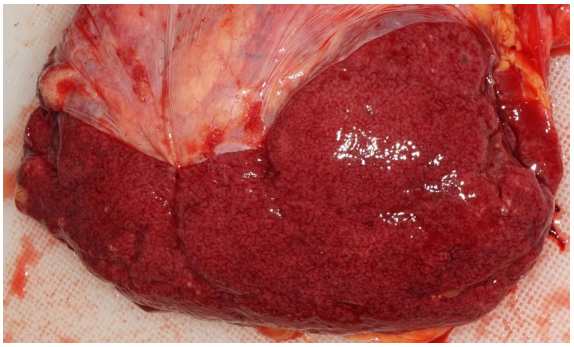

Figure 2: Same case, yellow arrows: small stones, thinning of the cortex

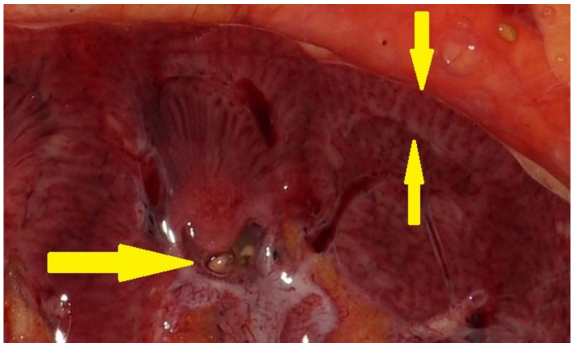

Figure 3: Same case, yellow arrows: glomerulosclerosis (10X HE)

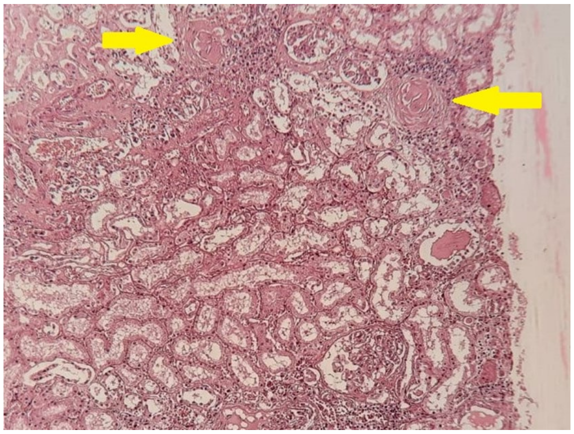

Figure 4: Same case, yellow arrows: arterial wall thickening (10X HE)

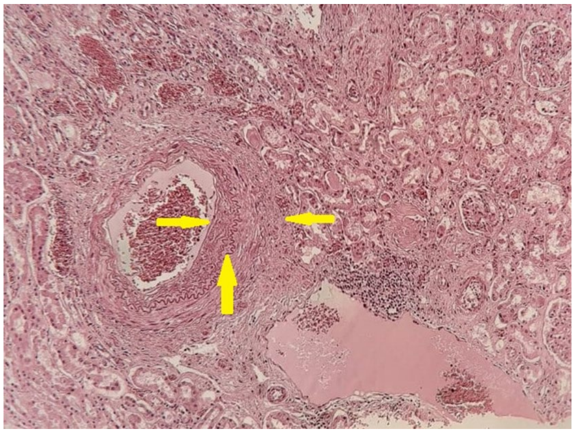

Figure 5: Same case: interstitial sclerosis (10X HE)

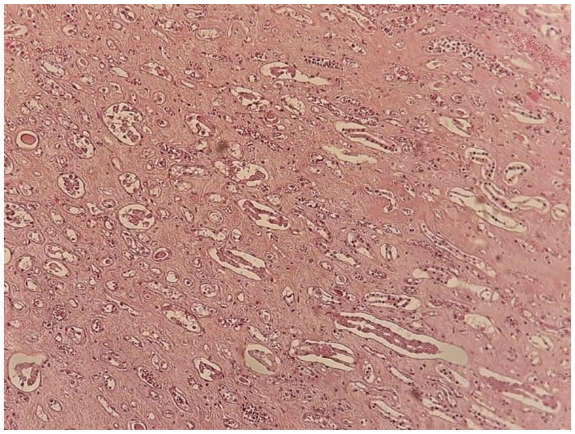

The hallmark of the functional decline of the senile kidney is a progressive decrease in the glomerular filtration rate (GFR) [5]. The decline in GFR begins around the 4th decade of life; between the 8th and 9th decades, the GFR is over 45% lower than at the age of 40 [3,6].

ESTIMATED GLOMERULAR FILTRATION RATE (eGFR) IN ELDERLY

There is no consensus regarding the optimal equation for the estimation of GFR in the elderly. Formulas used in clinical practice in adults – MDRD (Modification of Diet in Renal Disease, CKD-EPI (Chronic Kidney Disease – Epidemiology Collaboration), Cockroft-Gault are all based on serum creatinine levels and they have not been all validated for individuals older than 70 years.

Creatinine-based equations for GFR estimation have several drawbacks in older individuals [7]. Due to age-associated sarcopenia, there is a reduced release of creatinine from the muscles, therefore also a reduced urinary excretion of creatinine [8]. In elderly diabetics, especially in the late-elderly group (75 years or older), it has been noted a more decreased serum creatinine when compared with non-diabetics and this observation was explained by the increased loss of muscle mass promoted by diabetes [9]. Also, the elderly tend to have a reduced intake of proteins and a decreased basal metabolism, therefore, they have supplementary causes for the reduced production of creatinine [10]. Increased metabolism of creatinine by intestinal microorganisms may be an additional cause for reduced levels of serum creatinine, as there are proven age-related alterations of gut microbiota [11]. Formulas using the patient’s weight besides serum creatinine give more errors due to increased fat percentage in the organism of the elderly. Several researchers had concluded that both MDRD [12] and CKD-EPI [13] formulas tend to overestimate GFR in the elderly, while Cockroft Gault's [14] formula underestimates it [15,16].

Serum creatinine alone is often used in clinical practice not especially for estimation of GFR, but for monitoring the patient during treatment with a potential nephrotoxic agent (aminoglycosides for example) or a potential ischemic renal insult (cardiac surgery for example). Besides the fact that this conduct is not correct due to the late and modest increase of serum creatinine level compared to the moment and degree of kidney insult, in the elderly, it is a source of supplementary severe misjudgments. The elderly have often lower basal serum creatinine and during an injury renal process, an increase of serum creatinine remains in the range of the normal values of the laboratory and this result may be misinterpreted as a stable state, i.e. no iatrogenic. Moreover, the creatinine increase may be blunted by intensive hydration of the patient applied to minimize the kidney insult.

Formulas based on both serum creatinine and serum cystatin C have been proposed to overcome the disadvantages of formulas based only on creatinine values [17]. Nevertheless, serum cystatin C is influenced not only by kidney function, but also by several diseases often present in the elderly, such as atherosclerosis, inflammatory estates, or obesity [18,19].

Several alternative equations have been developed for older individuals. The HUGE formula (Hematocrit Urea GEnder) was developed in 2011 by a group of researchers from Spain to differentiate patients without CKD from those with CKD [20]; this formula was validated in some studies [21,22].

L = 2.505458 - (0.264418 x hematocrit) + (0.111800 x blood urea); 1.383960 is added for the male patient. For L <0, CKD is excluded; for L> 0, it is classified as CKD.

In 2012, the Berlin Initiative Study (BIS) introduced two new formulas based on serum creatinine and serum creatinine plus serum cystatin C, respectively, for estimating GFR in people over 70 years of age [23].

BIS 1: eGFR = 3736 x serum creatinine-0.87 x age-0.95; in women, the result is multiplied by 0.82.

BIS 2: eGFR = 767 x cystatin C-0.61 x serum creatinine-0.40 x age-0.57; in women, the result is multiplied by 0.87.

Although these formulas have been validated in some studies, there are no large trials to conclude their superior performance, therefore meanwhile guidelines recommend using the same equation as in the general adult population.

NORMAL KIDNEY AGING VERSUS CKD IN ELDERLY

Reported data on CKD prevalence in the elderly is impressive. A highly cited source in the literature is a meta-analysis performed by Hills and collab. which comprises more than 100 reports from all continents; according to this meta-analysis, the prevalence of CKD in all stages in the general population is 13.4% (11,7-15.1%), but increases to 27.6% (range 26.7-28.5%) in the 6th decade and 34.3% (31.9-36.7%) in the 7th decade of life. The same source also revealed increased CKD stage 3-5 in the elderly: from 10.6% in the general population (9.2-12.2%) to 11.3% (8.1-14.5%) in the 6th decade and respectively to 27.9% (16.4-39.3%) in the 7th decade [24]. Nevertheless, a large proportion of the geriatric population meets the KDIGO criteria for CKD even without markers of renal impairment [25,26]. Regardless of the formulas utilized to estimate GFR, an important question needs to get answered in the face of an older patient with abnormal GFR and no markers of kidney disease. Is it CKD without markers of kidney disease or is it normal kidney aging? Several reasons require a response to this question. Establishing a diagnosis of CKD imposes close monitoring of the patient and adequate preparation for conservative or dialysis treatment. Most of the patients aged >65 years have estimated GFR between 45-60 mL/min/1.73m^2; older patients with estimated GFR between 45-60 mL/min/1.73m^2 have, in comparison with younger adults, decreased risk to progress to end-stage renal disease (ESRD), but increased risk to die from other diseases until they reach ESRD [27,28]. Close nephrological monitoring of all these patients would be a significant burden for physicians, and also for the health systems. On the other hand, CKD, even oligosymptomatic, is associated from early stages with increased risk for cardiovascular events and cardiovascular mortality; specific complications of CKD, such as acidosis or increased parathormone (PTH) have early onset and need to be controlled.

There are several subtle clues allowing the differentiation between the physiologic decrease of GFR and CKD (Table 1).

Serum urea and creatinine increase late in the course of CKD in all patients, but especially in elderly patients. Low serum creatinine levels are observed due to decreased basal metabolism, reduced protein intake, and sarcopenia [3], therefore CKD may be present with normal values of serum creatinine in the elderly. However, the aging kidney excretes increased amounts of urea, due to the reduced density of urea channels in the collecting tubes [29]; a normal serum urea level is associated with normal aging [15].

Table 1: CKD versus normal kidney aging

	CKD	***Aging kidney***
Nitrogen waste products in the blood	Increased	Normal
Albuminuria	Present	Absent
Anemia	Present (erythropoietin deficiency)	May be present (nutritional anemia)
Acid-base balance	Metabolic acidosis	Normal
Ca-P metabolism	Hypocalcemia decreased active vitamin D, increased PTH	Possible hypocalcemia, normal PTH, normal active vitamin D

No anemia should be present as in healthy older populations erythropoietin (EPO) production is normal or even increased secondary to increased erythrocyte turnover, increased EPO resistance, or as a response to occult digestive blood losses [30,31]. Nevertheless, in clinical practice, the elderly have often nutritional mild/moderate anemia that may be misinterpreted as CKD-associated anemia. Reduced secretion of EPO in CKD is associated with normocytic and normochromic anemia, while nutritional anemia is often hypochromic and microcytic [32], but they may coexist.

No abnormalities of mineral metabolism are present in healthy older individuals. Renal tubular excretion of calcium, magnesium, and phosphorus is not altered in healthy elderly [5,33]. The elderly are prone to hypomagnesemia, but this seems to be mainly due to reduced dietary intake, decreased intestinal absorption of magnesium, and/or laxative abuse [5]. Although the elderly present more often with mild hypocalcemia, it occurs mainly secondary to native vitamin D deficiency through reduced sun exposure or decreased skin synthesis of vitamin D [34]. In contrast with CKD, there is no deficiency of active vitamin D, nor an increased synthesis of parathormone in healthy elderly.

Under basal conditions, the kidneys of the elderly have a normal capacity for the excretion of acids and the acid-base balance does not undergo significant changes even with a normal- or hyper-protein diet [35]. However, the renal reserve of the elderly is reduced and a rapidly installed high acid load may be difficult to be compensated by the senile kidney; the elderly are highly vulnerable to the side effects of acute metabolic acidosis (uncontrolled diabetes, sepsis, acute kidney injury).

On the other hand, the presence of albuminuria is a strong argument for CKD even if the patient has other comorbidities that may explain this finding (systemic atherosclerosis, diabetes, cardiac failure). Albuminuria is associated with an increased risk of progression of CKD [36], therefore applying antiproteinuric measures might have the same benefits in the elderly as in younger adults, i.e., attenuating the progression of CKD and reducing cardiovascular events [37]. Nevertheless, overzealous administration of RAAS inhibitors in the elderly is associated with an increased risk of side effects (hypotension, hyperkaliemia, acute kidney injury) in the presence of reduced GFR and systemic atherosclerosis or other comorbidities; another reason why these drugs are no longer needed in elderly without proteinuria is the fact that they become useless, not having time to install their renoprotective effect over time [38,39]. The elderly are prone to hyperkaliemia even in the absence of reduced GFR due to the low sensitivity of distal tubules to aldosterone [31,40].

Taking into account that the elderly have a higher risk of death due to various complications than to progress to ESRD, there is a need to assess individual risk for these outcomes to analyze treatment options and make early plans for conservative treatment (diet, prevention, and control of uremic syndrome with medication) or renal replacement therapy. CKD has an extremely heterogeneous presentation in the elderly; between an independent full-functioning person without cognitive impairment and without major comorbidities at one pole to a frail, immobilized in-bed individual with severe cognitive dysfunction, multiple disabling comorbidities and total dependence for medical care at the other pole, there are numerous clinical pictures. One such equation was proposed by Tangri et al in 2011 who developed the Kidney Failure Risk Equation (KFRE) using variables like age, sex, estimated GFR, albuminuria, serum calcium, serum phosphate, serum bicarbonate, and serum albumin to predict progression toward ESRD after 1, 3 and 5 years respectively for patients with CKD stages 3 to 5 [41]; a variant using only 4 variables (age, sex, estimated GFR and albuminuria) may be used, but the results offer less accuracy [41]. This model has not been designed especially for older individuals, but it has been validated both for patients < 65 years and those above this age and for various races [42,43]. Calculation of this score is available on the internet at http://www.qxmd.com/Kidney-Failure-Risk-Equation. KFRE is recommended by European Renal Best Practice Group to be used in elderly with an estimated GFR < 45 mL/min/1.73 m^2 to assess progress toward ESRD [44]. Nevertheless, a lot of old individuals are placed in the range of GFR between 45-60mL/min/1.73 m^2 and have no markers of kidney disease (albuminuria) [45]; there are no available validated prediction models for this situation and members of European Renal Best Practice Group opine for the urgent need of such prediction models.

Regarding the prediction of death, in the literature there are numerous scores specially designated for the old population and most of them also include, besides demographic variables (age, gender) and comorbidities (heart failure, diabetes, active neoplasia, anemia, history of stroke, chronic obstructive pulmonary disease, etc.), a marker of kidney dysfunction such as estimated GFR or urinary albumin/creatinine ratio [46-49]. These models estimate the risk of death at periods ranging from 3 months to 5 years. The European Good Practice Guideline recommends the use of the Bansal score in current practice for assessing the risk of mortality of non-fragile, un-institutionalized elderly people with CKD >stage 3b [44,47]; this score uses 9 variables - age, sex, GERD, urinary albumin/ creatinine ratio, smoking, diabetes, heart failure, history of stroke; according to this predictive model, the 5-year mortality varies between 3.8% for individuals with lower scores to 83.6% for individuals with all 9 items reached. Nevertheless, again, none of these models took into analysis the particular situation of individuals with normal kidney aging, i.e. those patients without markers of kidney damage and GFR between 45-60 mL/min/1.73m^2. We found in the literature just one publication, that of Van Pottelbergh and collab. who analyzed mortality of very old (>85 years) individuals with GFR> 60 mL/min versus those with GFR between 45-60 mL/min [50]. According to the authors of this paper, the combination of the baseline estimated GFR and the estimated GFR slope over 3 years can be used to assess the risk of death; in the subgroup of patients having estimated baseline GFR between 45-60 mL/min only those with a GFR decrease ≥ 5 mL/min/year had an increased risk of death, while among patients with baseline GFR > 60 mL/min, a decrease of ≥ 3mL/min/year was associated with increased risk of death. The same authors explained that these changes are a reflection of cardiovascular damage. Also, the authors share the opinion of a lot of others those older adults with GFR between 45-60 mL/min should not be classified as moderate stage 3 CKD.

CONCLUSIONS

Aging associates the functional decline of all tissues and organs secondary to degenerative structural changes. Normal kidney aging is a complex process that is difficult to be differentiated in clinical practice from chronic kidney disease with contemporary available criteria; both share the reduced glomerular filtration rate as the main feature. Nevertheless, it is important to make this differentiation.

Establishing a diagnosis of CKD imposes periodic nephrology consultation for monitoring the progression of the disease, treatment of complications, early planning of conservative or dialysis treatment, and also adjustment of treatment of multiple comorbidities of the elderly. CKD has multiple complications and requires often interdisciplinary care: cardiologists, neurologists, psychiatrists, nutritionists, etc. On the other hand, proving that reduced GFR is only a sign of normal aging and that there are no markers of kidney damage allows gerontologists to provide holistic care for slowing the aging process and minimizing the incidence of high-risk diseases in the elderly.

Thus, misclassifying an elderly person as a patient with CKD involves an unnecessary psychological burden for the patient and his family, as well as unnecessary medical care and increased financial expenses.

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The authors declare no conflict of interest.

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Authors' contribution
Conceptualization, D.R., C.D., I.A.V., G.E.L.; methodology, A.L.C., R.F.T.; software, M.O.D.L.; investigation, I.A.V., D.R., C.D.; writing—original draft preparation, A.L.C., F.P.; writing—review and editing, S.I., I.A.V.; supervision, A.L.C., D.I.
All authors have read and agreed to the published version of the manuscript.

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REVIEW

Concise Review on Modulation Mechanism of Matrix Metalloproteinase via Oncoviruses in Aggressiveness of Malignancies

Ali Ramezani[1], Emad Behboodi[2], Seyed A. Mirhosseini[1], Mohammad S. Hashemzadeh[3]

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Abstract: *Matrix metalloproteinases (MMPs) are a member of the protein family intimately associated with zinc-containing endopeptidases, which are the main proteases implicated in extracellular matrix (ECM) degradation. The high ability of MMPs in the degradation of extracellular proteins is an important event in many normal biological as well as pathologic processes, including tumor progression, tissue repair, wound healing, developmental morphogenesis, and inflammatory diseases. This review is focused on the description of the modulation mechanism of MMPs via oncoviruses. Up to now, a large number of oncogenic viruses have been detected and recognized as etiologic factors of multiple human cancers. Actually, by expression of several viral oncoproteins of tumorigenic viruses with the simple genetic system, we are able to deregulate or modulate the pathways of cell transformation procedure in malignancy. In brief, upregulated expression of MMPs genes by direct (through DNA binding) , as well as indirect interactions (by activating PI-3K/AKT, ERK/MAPK, IFN/JAK/STAT, JNK, NF-κB signaling pathways and/or immune response) with tumorigenic viruses, have a noticeable role in the proliferation of cells, as well as angiogenesis, mobility, EMT, invasiveness, and metastatic features. In fact, the growth of tumor-related researchers offers a future perspective for the efficiency of targeted, molecular-based therapies and its anticancer effects via diverse mechanisms such as the suppression of viral oncoproteins.*

Keywords: *Matrix Metalloproteinases (MMPs), Tumor Viruses, Tumor Progression*

INTRODUCTION

Matrix metalloproteinases (MMPs), form a family of zinc-dependent extracellular matrix (ECM) remodeling endopeptidase enzymes that play a role in a number of physiological processes, like the healing of wounds, organismal development, and effectors of immune responses, as well as in pathological conditions, like pulmonary dysfunctions, autoimmune diseases, and cancer [1,2]. To date, almost 25 various vertebrate MMPs have been recognized, including 23 members in humans [3]. Based on sequence homology and specificity for substrate, the MMPs family divides into six categories of stromelysins, collagenases, matrilysins, gelatinases, membrane-type as well as other non-classified MMPs [4]. Each of these groups has its subunits that perform unique functions [5]. Chemokines, cytokines, as well as growth factors, cell-to-to-cell adhesion molecules, and binding proteins, can be mentioned as other potential targets for MMP substrates [6]. The classification of MMPs is indicated in Table 1 shown [7-11]. Matrix metalloproteinase (MMPs) activity might be regulated at three transcription levels, production of enzymes like zymogens, which require activation, as well as co-expression of tissue inhibitors of metalloproteinases (TIMPs) [12,13]. MMPs transcription is perfectly controlled, and their expression level of them is low. TIMPs are endogenous suppressors of metalloproteinases that can inhibit the MMM's catalytic activity, therefore maintaining ECM homeostasis [14]. MMPs are produced in their inactive zymogenic forms (proenzymes or pro-MMPs), which are subsequently proteolytically activated by removing propeptides by other proteases [15]. The majority of MMPs are activated in the extracellular space via plasma, tissues, and microbial proteases. The rest of the activated MMPs like MT-MMPs, MMP-11, 23, and 28 possess a furin-dependent cleavage motif RXK/RR in the propeptide sequence, which are intracellularly activated via furin-like proteases of Golgi [16]. Any disruption in the regulation of MMPs modulatory mechanisms may associate with the progression of the disease in pathological conditions [12].

Table 1: Classification of the main members of the MMP family by chromosomal location and the group of substrates

MMP number	MMP subclass/ Family/Group	MMP name	Substrates	Chromosome
MMP-1	Collagenases	Interstitial collagenase	Collagens (I,II,III,VIII a X); gelatin; aggrecan; L-selectin; IL-1β proteoglycans, entactin; ovostatin; MMP-2; MMP-9	11q22-q23
MMP-2	Gelatinases	Gelatinase A	Collagens (I,IV,V,VII,X,XI a XIV); gelatin; elastin; fibronectin; aggrecan; MBP; osteonectin; laminin-1; MMP-1; MMP-9; MMP-13	16q13
MMP-3	Stromelysins	Stromelysin 1	Collagens (III,IV.V, a IX); gelatin; aggrecan; perlecan; decorin; laminin; elastin; casein; osteonectin; ovostatin; antactin; plasminogen; MBP; IL-1β; MMP-2/TIMP-2; MMP-7; MMP8; MMP-9; MMP-13	11q23
MMP-7	Matrilysins	Matrilysins	Collagens (IV a X); gelatin; aggrecan; decorin; fibronectin; laminin; entactin; elastin; casein; transferrin; plasminogen; MBP; β4-integrin; MMP-1; MMP-2; MMP-9; MMP-9/TIMP-1	11q21-q22
MMP-8	Collagenases	Neutrophil collagenase	Collagens (I,II,III,V,VII,VIII a X); gelatin; aggrecan; fibronectin	11q21-q22
MMP-9	Gelatinases	Gelatinase B	Collagens (IV,V,VII,X a XIV); gelatin; entactin; aggrecan; elastin; fibronectin; osteonectin; plasminogen; MBP; IL-1β	20q11.2-q13.1
MMP-10	Stromelysins	Stromelysin 2	Collagens (III-V); gelatin; casein; aggrecan; elastin; MMP-1; MMP-8	11q22.3-q23
MMP-11	Stromelysins	Stromelysin 3	Unknown (casein)	22q11.2
MMP-12	Elastase	Metalloelastase	Collagen IV; gelatin; elastin; casein; fibronectin; vitronectin; laminin; entactin; MBP; fibrinogen; fibrin; plasminogen	11q22.2-q22.3
MMP-13	Collagenases	Collagenase-3	Collagens (I,II,III,IV,IX,X,a XIV); gelatin; plasminogen; aggrecan; perlecan; fibronectin; osteonectin; MMP-9	11q22.3
MMP-14	Membrane-MMP	MT1-MMP	Collagens (I-III); gelatin; casein; fibronectin; laminin; vitronectin; entactin; proteoglycans; MMP-2; MMP-13	14q11-q12
MMP-15	Membrane-MMP	MT2-MMP	Fibronectin; entactin; laminin; perlecan; MMP-2	16q12.2-q21
MMP-16	Membrane-MMP	MT3-MMP	Collagen III; gelatin; casein; fibronectin; MMP-2	8q21
MMP-17	Membrane-MMP	MT4-MMP	Gelatin	12q24
MMP-18	Collagenases	Collagenase-4	Unknown	Unknown
MMP-19	Other	RASI-1	Gelatin; tenascin; fibronectin; collagen IV; laminin; entactin; fibrin/fibrinogen; aggrecan	12q14
MMP-20	Other	Enamelysin	Amelogrenein; aggrecan; COMP	11q22
MMP-23	Other	Unnamed	Amelogenin	11q22
MMP-24	Membrane-MMP	MT5-MMP	Pro-MMP 2	20q11.2
MMP-25	Membrane-MMP	MT6-MMP	Pro-gelatinase A; fibrin; fibronectin; collagen IV; gelatin	16p/3.3
MMP-26	Matrilysins	Endometase, matrilysin-2	Gelatin Iα; P1; fibrinogen; fibronectin; vitronectin; β-casein	Unknown

MMPs AND TUMOR PROGRESSION

Cancer is the second factor of death globally, which is estimated to account for 9.6 million deaths in 2018 [17]. Recent two decades of biomedical research have led to large amounts of information about the molecular events which occur during carcinogenesis as well as signaling pathways involved in the progression of cancer. Complex molecular mechanisms play different roles in the tumor cells and the tumor environment associated with this process [18]. Several interactions of tumor cells with the involved environment of the tumor are crucial for the pathological changes observed during the transformation of cells and carcinogenesis in the extracellular matrix (ECM), as well as cytokines, growth factors associated with ECM, along with neighboring cells (neutrophils, endothelial cells, mast cells, macrophages, fat cells, and pericytes) [19-21]. Different steps of cancer consisting of migration, attack, metastasis, and angiogenesis are in interaction with the neighboring microenvironment [22]. MMPs are the main molecules in these processes, and since they break down numerous cell adhesion molecules, indicating their roles in the regulation of cell-to-cell, and cell-ECM interactions [23,24]. Previous researchers have identified that MMPs play an essential role in all phases of tumor maintenance and growth, including invasiveness, migration of tumor cells, evasion from apoptosis as well as immune surveillance, angiogenesis, and metastasis, so that all these major stages may be modulated by MMPs [25].

Interferon, interleukins, growth, factors, and extracellular MMP inducers secreted from tumor cells in a paracrine, manner stimulating adjacent host cells to generate the required MMPs. MMPs produced by normal cells can be bound and used on the surface of tumor cells [26]. MMPs gene expression and its activity increase in many types of cancers in humans. And this increased expression causes an increase in tumor size, invasion, and decreased survival [27]. Thus, MMPs are used as a novel and capable therapeutic target in different types and cancer phases [28]. MMPs use their proteolytic function to remove physical barriers, developing angiogenesis and tumor metastasis [29]. Tumor development and angiogenesis depend on the facilitated accessibility of signaling molecules. For example, cytokines and growth factors by MMPs enhance by making them available for tumor cells and their microenvironment [18]. This process is performed by releasing these factors from the ECM (VEGF, IGF, and bFGF) or via secreting them from the surface of the cell (HB-EGF, EGF, TGF-a) [4]. The release of endogenous angiogenesis suppressers can regulate angiogenesis perfectly, for instance, angiogenesis inhibitors like tumstatin, endostatin, angiostatin, and endorepellin [30]. Disruptions in the regulation of cell-cell and cell-ECM interactions by MMPs during the processing of E-cadherin and integrins, respectively, affect both cell phenotype (EMT) as well as increasing cell migration [31,32]. Almost all members of the MMP family have been reported to be dysregulated in human tumors, although MMPs such as MMP-1, -2, -7, -9, -13, and 14 are more important [33]. We have demonstrated the most noticeable MMPs, which are related to the phases of tumor growth, biological effects, and their consequences in Table 2.

Table 2: Key MMPs in relation to the stages of cancer progression, biological effect, and consequences

Function	MMP	Biological effect	Consequence	Reference
Cancer cell invasion	MT1-MMP, MMP-2 and MMP-9	Proteolytic	Breakdown physical barriers	[144]
Cancer cell proliferation	MMP-1, -2, -3, -7, -9, -11, -19	Cleavage of IGF binding proteins, Shedding of membrane, anchored ligands of EGFR, including HB-EGF, TGF-a and amphiregulin, Activation of TGF-β, Shedding of HB-EGF	Proliferation	[145-147] [148] [149] [150]
Cancer cell apoptosis	MMP-7 Several MMPs	Cleaving the Fas ligand Indirect activation of the serine/serine/threonine Akt ⁄ protein kinase B through the signaling cascades of EGFR and IGFR	Anti-apoptotic	[151] [147, 152]
Tumor angiogenesis	MMP-2, -9 MMP-3, -10, -11 MMP-1, -8, -13	Degradation of COL-IV, perlecan; release of VEGF and bFGF, respectively Degradation of COL-IV, COL-XVIII, perlecan; generation of tumstatin, endostatin, angiostatin and endorepellin, respectively	Upregulation of angiogenesis Downregulation of angiogenesis	[153, 154] [4, 155]
Cell adhesion, migration, and EMT	MMP-2 MT1-MMP MMPs MMP-2, -3, -9, -13, -14 MMP-1 and -7 MMP-28	Degradation of COL-IV; generation of cryptic peptides Degradation of laminin-5; generation of cryptic peptides Integrins as substrates Overexpression this MMPs associated with EMT, a highly conserved and fundamental process of morphological transition By cleavage E-cadherincontribute to morphological transition Proteolytic activation of TGF-β	Promote migration Induction of EMT; cell migration Powerful inducer of EMT, leading to cell migration	[156, 157] [158] [159] [160] [161]
Immune surveillance	MMP-9 MMP-9, -2, -14 MMP-7, -11, -1, -8, -3 MMP-7, -8	Cleavage of IL-2R alpha Release active TGF- β Release of a1-proteinase inhibitor Cleave several members of the CC (β-chemokine) and CXC (α-chemokine) chemokine subfamilies or to regulate their mobilization	Repress T-lymphocyte proliferation Abolish T-lymphocyte reaction against cancer cells Decrease cancer cell sensitivity to NK cells Affecting leukocyte infiltration and migration	[162] [163] [164] [165, 166]

ONCOVIRUSES

Approximately 20% of all human malignancies worldwide are associated with viruses [34]. Seven viruses have been implicated as oncogenic, including HPV, EBV, HCV, HBV, Kaposi's sarcoma-associated herpesvirus (KSHV), HTLV-1, and Merkel cell polyomavirus (MCV), which have been reported to be etiologically implicated in the maintenance and progression of human cancers [35]. However, viral oncogenesis processes are a complex process, with induction of chronic inflammation conditions, disruption of cellular DNA repair mechanisms, interrupting host genetic and epigenetic integrity, and homeostasis, viral infection may result in genome variation and cell cycle deregulation. Viral ‘oncoproteins’ are capable of modulating or deregulating cellular signaling pathways, making changes in the expression of genes and microRNAs in either transcription or post-transcription, and disrupting tumor suppressor factors and proteins, which regulate cell polarity, immune response, signal transduction, and cell apoptosis. Genetic and epigenetic modifications caused via oncoviruses may be resulting in the morphology and variation of cancer stem cells, which are essential for the primary initiation, development, migration, invasiveness, and chemotherapy resistance of tumors. These molecular events underlying the interactions between oncoviruses that use specific cellular genes and cellular signaling pathways are important issues to research efforts [36-38]. MMPs have several specific biological actions in every cancer phase, from the beginning to the extension of pertinent metastases. Accordingly, this review’s focus is on characterizing the mechanism of modulation or deregulation of MMPs by oncoviruses.

INTERACTION BETWEEN ONCOVIRUSES AND MMPs

Epstein-Barr virus

Epstein-Barr virus (EBV) is a linear dsDNA virus that belongs to the human gamma-herpesvirus family, playing a role in different B-cells lymphomas, including Hodgkin’s lymphoma, Burkitt’s lymphoma, post-transplant lymphoproliferative disorders, as well as other tumors like T-cell non-Hodgkin lymphomas and nasopharyngeal carcinoma [39]. In tumors associated with EBV, the virus leads to a latent infection expressing only a subset of 12 latent viral transcripts, two early RNAs (EBERs), three latent membrane proteins (LMPs), six nuclear antigens (EBNAs), and BARF transcripts. Out of these genes, EBNA1, EBNA-2, EBNA-3A, EBNA-3C, EBNA-LP, and LMP1 enable the virus to control a number of cellular signaling pathways associated with oncogenesis [40,41]. EBV LMP1 increases MMP-9 expression by recruitment of Sp-1, AP-1, and NF-kappa B transcription factors on MMP-9 promoter that is related to invasiveness and metastasis in EBV-associated NPC [42]. Previous research demonstrated that C-terminal activation regions 1 and 2 (CTAR-1 and CTAR-2), two crucial signaling domains within the carboxy terminus of LMP1, might activate the MMP-9 promoter and stimulate MMP-9 activity [43]. TANG Jian showed EBV-induced MMP-9 upregulation correlates with lymph node metastasis and clinical stage in NPC tumors [44]. Lee demonstrated that upregulation of MMP1 and MMP3 expression in LMP1-positive NPC tumors led to the enhancement of invasiveness in NPC cells [45]. BZLF1 (also known as Zta) is one of the critical inducers of EBV lytic cycle alteration and has been reported to trigger metastasis in epithelial cells [46, 47]. In vitro studies demonstrated that Zta stimulates MMP3 and MMP9 expression in epithelial cells derived from NPC tumors. Zta can activate the MMP3 promoter by AP-1 elements, and its DNA-binding domain is necessary for the process of promoter binding and MMP3 stimulation. Zta-enhanced cell migration requires MMP3 but not MMP9. Zta-elevated cell migration needs MMP3, but not MMP9. Therefore, the expression of both MMP3 and MMP9 is important for the co-expression of the two MMPs (MMP3 and MMP9) induced by Zta, which simultaneously promotes cell invasiveness of epithelial cells differentiated from NPC tumor [48]. Moreover, Zta protein via binding AP-1 site of its promoter increases TIMP-1 expression in EBV-infected B-cells as well as EBV-immortalized lymphoblastoid cell lines (LCLs). Upregulation of TIMP-1 expression through Zta enhances the maintenance of EBV-infected B-cells and promotes the progression of EBV-associated cancer [49]. EBNA3C regulates the transcription of numerous viral as well as cellular genes important for the process of immortalization by EBV in primary B-lymphocytes [50]. Nm23-H1 is a well-known metastasis inhibitor, and its low expression is correlated with metastatic tumor capability in different types of human carcinoma [51]. In vitro research showed the interaction of EBNA3C with Nm23-H1 leads to the translocation of Nm23-H1 to the nucleus and inhibits the antimotility effect. However, studies have shown that this interaction also can increase transcriptional activity on a related promoter of the target gene [52]. Interaction between EBNA3C and Nm23-H1 upregulates the expression of MMP-9 by binding the transcription factors AP-1 and NF-kB on its promoter in B-cells that were isolated from Burkitt’s lymphoma [53]. LMP2A promotes cell transformation, migration, and invasion by affecting host cellular signaling pathways, mainly by its N-terminus, intracellular domain, which consists of an immunoreceptor tyrosine-based activation motif (ITAM), a YEEA motif, and two PPPPY (PY) motif [54,55]. LMP2A promotes invasion of NPC cells by the PY motif via stimulation of extracellular signal-regulated kinase (ERK)-1/2 as well as the downstream transcription factor Fra-1, therefore leading to upregulation of MMP9 expression [56]. WW domain-containing oxidoreductase WOX1, also called WWOX or FOR, is a candidate tumor inhibitor encoded by the human fragile WWOX gene, related to several signaling pathways modulating embryonic development, metabolism, cell proliferation, and apoptosis [53]. The function of WOX1 in cancer suppression is commonly used by stimulating apoptosis and sequestering some oncoproteins [57,58]. According to a previous study, the interaction of the tyrosine residue 33 of WOX1 and the PY motif of LMP2A is needed for the LMP2A-induced ERK1/2-Fra-1-MMP9 pathway in NPC cells (Figure 1) [59]. Two SNPs (rs8113877T>G or rs17576A>G) of MMP-9 in ab_EBV positive individuals were associated with more possibility of progression to chronic lymphocytic leukemia in contrast to those individuals who were negative [60]. In summary, the upregulation of MMPs by EBV plays a significant role in EBV-triggered metastasis.

Figure 1: Schematic model of deregulation of MMPs by EBV virus

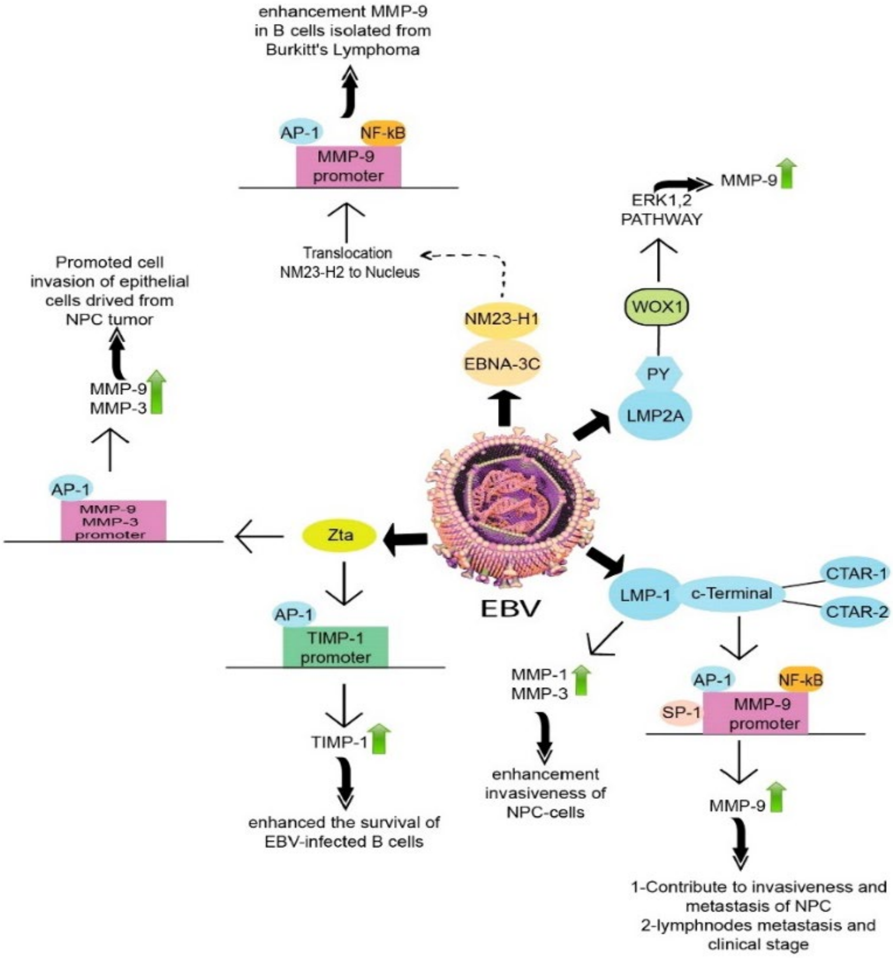
enhancement MMP-9 in B cells isolated from Burkitt's Lymphoma
AP-1
NF-kB
MMP-9 promoter
MMP-9
ERK1,2 PATHWAY
Translocation NM23-H2 to Nucleus
Promoted cell invasion of epithelial cells drived from NPC tumor
WOX1
NM23-H1
EBNA-3C
PY
LMP2A
MMP-9
MMP-3
AP-1
MMP-9 MMP-3 promoter
Zta
EBV
CTAR-1
LMP-1
c-Terminal
CTAR-2
AP-1
TIMP-1 promoter
MMP-1
MMP-3
AP-1
NF-kB
SP-1
MMP-9 promoter
TIMP-1
enhancement invasiveness of NPC-cells
MMP-9
enhanced the survival of EBV-infected B cells
1-Contribute to invasiveness and metastasis of NPC
2-lymphnodes metastasis and clinical stage

Human papillomavirus

Human papillomaviruses (HPVs), namely double-stranded DNA viruses, are recognized as one of the main agents that relate to the progression of numerous cancers such as head and neck, cervix, vagina, vulva, anal and penile carcinomas [61]. Continuing infection with high-risk human papillomavirus (HPV) types, especially HPV type 16 or 18, is essential for the development of a cancer precursor lesion and invasive cervical cancer, which is identified through deregulated and high-level expression of HPV early genes (E6 and E7) [62]. The high-risk HPV E6 and E7 oncoproteins result in transformation by a disruption in the activity of cellular tumor suppressor proteins p53 and RB, respectively, and several cellular proteins [63,64]. Induction of MT1-MMP expression in HaCaT cells, an immortalized human keratinocyte line, by the E7 protein of high-risk HPV types 16 and 8 contributes to cellular proliferation, angiogenesis, and, malignant progression, for the invasion of HPV-positive tumor cells [65]. In human keratinocytes and organotypic skin cultures, HPV8 E2 protein overexpresses the expression of MMP-9 by direct activation of its promoter, resulting in increased squamous fragility involved in facilitating viral escape [66]. A recent study demonstrated that HPV-16 E6/E7 oncoproteins cause upregulation of MT1-MMP, MMP-2, and MMP-9 to promote cervical cancer cells [67]. Previous studies reported that HPV 16 infection might lead to lung tumor cells through increasing in interleukin-17 (IL-17) expression. IL-17 along with its downstream signaling mediator, interleukin-8 (IL-8), play an efficient role in tumor angiogenesis and metastasis by modulation of various pro-angiogenic factors [68]. Induction of IL-8 by HPV upregulates the expression of MMP-2 and MMP-9 in lung adenocarcinoma that correlated with progressive mechanisms of growth, and metastatic and angiogenic characteristics [69]. HPV-16 E6/E7 overexpressed the expression of MT1-MMP and MMP-2, which are correlated with increased cell invasion in cervical cancer [70]. According to recent research (Haukioja), low salivary levels of MMP-8 are due to continued oral HPV infection, which appears like a failure of the anti-inflammatory defense of the oral mucosa to allow an incident oral HPV infection to become a chronic infection [71]. Common skin extract immunoblots and zymographic analysis revealed increased expression of MMP-9, MMP-13, and MT1-MMP in HPV8-positive mice rather than HPV8-negative mice, which are involved in the development of HPV8-induced cutaneous tumors (Figure 2) [72]. Singh et al. have shown that SNPs -1306CC (rs243865: C>T) in the promoter of the MMP2 gene, enhanced transcription and enzymatic activity of the MMP-2 gene is associated with the incidence possibility of cervical cancer. A significant relationship between the −1306CC genotype and HPV infection in increasing the risk of cervical cancer was observed [73].

Figure 2: Schematic model of deregulation of MMPs by HPV virus

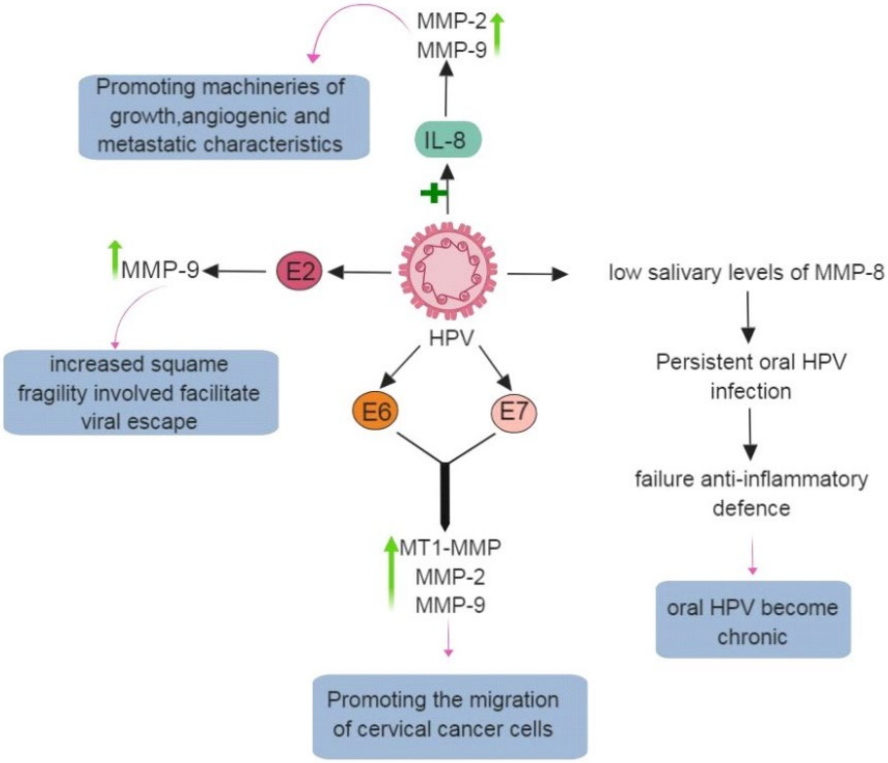
MMP-2
MMP-9
Promoting machineries of growth,angiogenic and metastatic characteristics
IL-8
MMP-9
E2
HPV
low salivary levels of MMP-8
increased squame fragility involved facilitate viral escape
Persistent oral HPV infection
E6
E7
failure anti-inflammatory defence
MT1-MMP
MMP-2
MMP-9
oral HPV become chronic
Promoting the migration of cervical cancer cells

HBV

Chronic hepatitis B (CHB), an important agent responsible for liver cirrhosis and hepatocellular carcinoma, is among the primary health problems of the world [74,75]. HBV transactivator protein (HBx) enables the invasive capacity of the virus, which is mediated by increased the expression of membrane-type one matrix metalloproteinase (MT1-MMP) that subsequently activates matrix metalloproteinase-2. HBx is able to induce both MT1-MMP expression and cell invasion through dependence on cyclooxygenase-2 (COX-2) activity. Furthermore, HBx increases COX-2 expression through the activation of COX-2 gene promoter transcription in a nuclear factor of an activated T-cell-dependent (NF-AT–dependent) manner. These data indicated the capacity of HBx to augment tumor cell invasion by a mechanism consisting of MT1-MMP and COX-2 upregulation and led to new insights into HBx protein mechanism of action, its involvement in tumor metastasis and hepatocellular carcinoma resulting from intrahepatic metastasis to HBx protein expression following establishment of tumor [76]. HBx protein plays a crucial role in the progression of hepatocellular carcinoma (HCC). HBx is often the only HBV protein that would be detected in hepatic tumor cells, which enhances tumor cell invasion and metastasis. HBx, by inducing the PI-3K/AKT and ERK pathways except for the P38 MAPK pathway, leads to the activation of NF-kB and AP-1 transcription factors. HBx triggers the translocation of NF-kB and AP-1 into the nucleus, leading to increasing MMP-9 expression. Thus, HBx modulates the transcriptional function of MMP-9 that is essential to the incursion and metastasis of tumors through PI-3 K/AKT and

ERK pathways. These findings suggest that HBx could induce MMP-9 and, in turn, in the invasion and metastasis of cancer, by activating ERK and PI-3K signaling pathways (Figure 3) [77].

Figure 3: Molecular mechanism of HBX-activated MMP-9 expression. HBx induces PI-3K/AKT and ERK pathways but not the p38 MAPK pathway. This process leads to the stimulation of NF-kB and AP-1 transcription factors. Translocation of NF-kB and AP-1 activated by HBx into the nucleus leads to an increase of MMP-9 expression. Therefore, HBx via regulation of transcriptional activation of MMP-9, plays an important role in the invasion and metastasis of tumors via PI-3K/AKT and ERK pathways

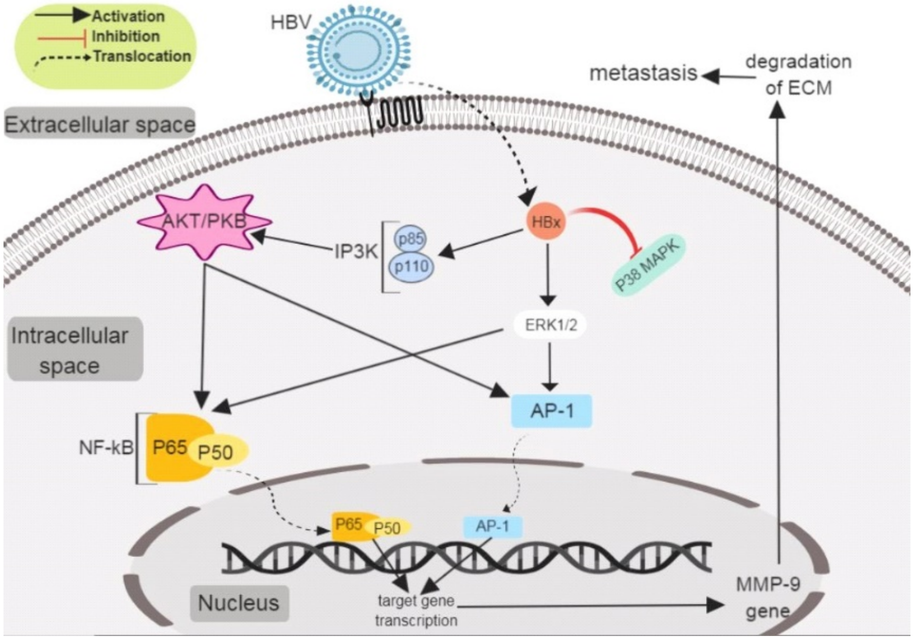

Genes that were defined to be modulated in the presence of HBx include those genes involved in signal transduction, cell cycle, cytokine production, metabolism, apoptosis, cell adhesion, and oncogenesis. The functions of other less-defined genes also were affected. Transactivation by HBx upregulates the translation, transcription, and release of matrix metalloproteinase-3 (MMP-3), which manifests as a cell migratory phenotype. This effect of HBx was suppressed in the presence of an MMP-3-specific peptide inhibitor. HBx applies selective control of transcription in hepatoma cells as well as inducing cell migration via activation of MMP-3. Therefore, by upregulation of MMPs, HBx increases the invasion of tumor cells and the destruction of the extracellular matrix that is reported to increased cell migration [78]. The progression of tumors tightly depends on a permissive microenvironment that is highly correlated with the destiny of tumor cells. The normal structure of ECM is of high importance in the modulation of cell growth. For invasiveness, cancer cells must overwhelm the mechanical barriers caused by the ECM. It has been demonstrated which conformational changes in the ECM by MMPs have a noticeable role in the mobility and spread of tumor cells, resulting in invasion and metastasis. The activation of MMP induced by HBx signifies a role of HBx protein in the development of HCC metastasis. However, HBx is demonstrated to relieve transcriptional stimulation activity, it is not defined in what way HBx induces the expression of MT1-MMP [79,80]. HBV replication is assisted by MMP-9 through repression of IFN/JAK/STAT signaling as well as IFNAR1 function. Thus, these outcomes have discovered a novel role for MMP-9 in viral replication, revealing a new mechanism by which HBV escapes host immunity to maintain persistent infection. Indeed, there is a positive feedback loop between the injection of HBV and the production of MMP-9. Firstly, HBV activates MMP-9 production in PBMCs and macrophages. Subsequently, HBV replication would be facilitated by MMP-9 through repressing IFN/JAK/STAT signaling, interacting with IFNAR1 to prevent its binding to IFN-α and promoting the ubiquitination phosphorylation, degradation of IFNAR1 and ISRE, subcellular distribution, and interferon-stimulated response element. Thus, MMP-9 would benefit HBV by blocking innate immunity to launch or maintain chronic infection (Figure 4) [81].

In hepatitis B infection, apoptosis of liver cells that is induced by varying degrees of inflammation plays an essential role in the outcome of CHB [82]. The MMP-9 expression level is directly related to TNF-α and IL-6. Because IL-6 and TNF-α can regulate MMP-9 activity in a concentration- and time-dependent mode, overexpression of MMP-9 can degrade ECM to destroy the basement membrane and lead to disorganized and loose tissue structures, leading to further penetration of TNF-α and IL-6. These factors can interact with each other and create a vicious cycle, resulting in pathological changes in the liver. In conclusion, the MMP-9 level is increased remarkably in patients with varying degrees of CHB, it would be important in the pathological progress of the liver, and it has a close correlation with inflammation, which can provide a theoretical basis for clinical treatment [83].

Figure 4: MMP-9 assists HBV replication by suppressing IFN/JAK/STAT signaling and IFNAR1. Indeed, HBV induces MMP-9 in infected patients and in HBV-stimulated leukocytes. Then, MMP-9 comforts HBV replication through repressing IFN/JAK/STAT signaling, interacting with IFNAR1 to block its binding to IFN-α, and promoting the phosphorylation, ubiquitination, subcellular distribution, and degradation of IFNAR1

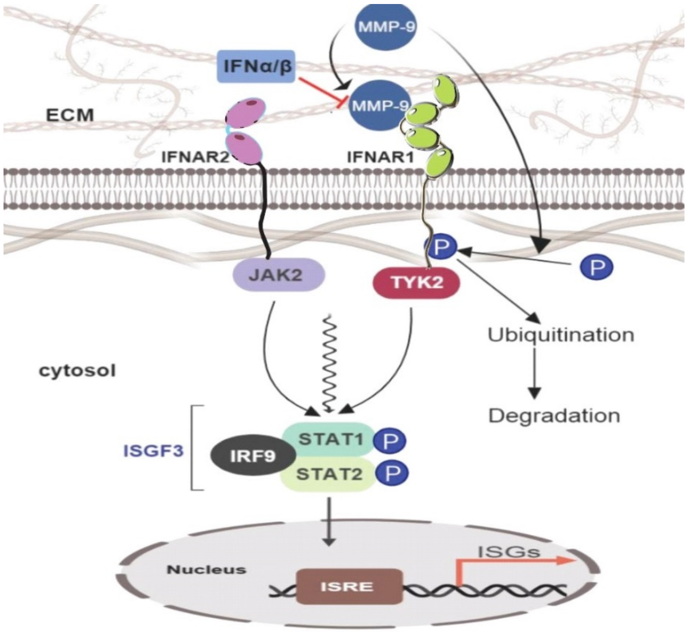

HCV

Hepatic inflammation, cirrhosis, developing liver fibrosis, and hepatocellular carcinoma (HCC) are common problems in HCV patients [84,85]. In the liver, the main reason for fibrogenesis is pathological repletion of the extracellular matrix (ECM), which leads to an imbalance in the rate of enhanced matrix synthesis to reduce the degradation of connective tissue proteins that can be controlled by matrix metalloproteinases (MMPs). Recent studies showed that increasing levels of hepatic gelatinases, including MMP-9, and MMP-2, are related to the progression of HCC and the fibrotic index of chronic HCV patients [86,87]. The disparity between matrix metalloproteinases (MMPs) and tissue suppressors of matrix metalloproteinases (TIMPs) is remarked to be a significant index of degradation of the extracellular matrix. MMPs are members of gene families that can degrade collagen and play an essential role in the natural mechanism of tissue repair in pathological states, including liver fibrosis. Thus, an increase of the MMP-1/TIMP-1 ratio by IFN treatment can relieve liver fibrosis of chronic hepatitis C that demonstrates the anti-fibro genetic effect of IFN on the factors modulating hepatic fibrosis [88]. During chronic liver harm, activated hepatic stellate cells (HSC) turned backward into myofibroblast-like cells and proliferated. This stage is an important factor in the creation of fibrosis and subsequent cirrhosis [89]. HSCs are considered important sources of fibrillar as well as nonfibrillar collagens of the liver, matrix-degrading proteases (MMPs), and their specific blockers (TIMPs). Briefly, recent studies on patients with chronic hepatitis C reported important variations in hepatic TIMP and MMP expression. It is likely that TIMP expression was affected via the inflammatory conditions of the disease, which can reflect the effects of the disease. Promoted fibrosis enhances the expression of MMP-7 and MMP-2 and provides a condition for the destruction assessment of connective tissue [86]. The primary activity in the biosynthesis of different prostaglandins (PG) and thromboxanes catalyzes via cyclooxygenases [90,91]. There are two cyclooxygenases (COX) isoforms: COX-1 is basically expressed in several cell types and is the main factor in the homeostatic activity of PG, while COX-2 is triggered by different proinflammatory stimuli, including lipopolysaccharide (LPS) and cytokines [92-94]. COX-2 is involved in fibrogenesis, inflammation, and carcinogenesis [95-97]. Therefore, different researchers reported that increased COX-2 expression and PG production might be associated with liver dysfunction and tumorigenesis in various animal models [98-100], in addition to fibrosis and hepatocellular carcinoma (HCC) among humans [101,102]. HCV core and NS5A proteins, both alone and in association with the impact of endotoxin and proinflammatory cytokines, are capable of regulating the expression of COX-2 and MMP-9 genes through chronic HCV infection. Therefore, this mechanism may be considered evidence for this process that chronic HCV infection may result in liver fibrosis. Thus, it seems reasonable that much PGs generated in hepatocytes via the correlated effect of HCV proteins in addition to inflammatory factors may affect neighboring liver cells by autocrine and/or paracrine pathways, therefore participating in hepatocarcinogenesis [103]. The E2 envelope glycoprotein of the hepatitis C virus (HCV) is an important modulator of HCV interaction with cell surface receptors. E2 interacts with an extracellular loop of human CD81; it's a receptor expressed on various cell types such as hepatocytes and B-cells. CD81 on the cell surface is expressed in human hepatic stellate cells (HSC) at a high level, Matrix metalloproteinase-2 (MMP-2; gelatinase A), an important enzyme responsible for the degradation of the normal hepatic extracellular matrix, upregulates subsequently after binding E2 toCD81. Furthermore, E2/CD81 interaction in human HSC causes an increasing amount of MMP-2 via increasing the binding activity of activator protein-2/DNA via ERK/MAPK phosphorylation. Increased degradation of the hepatic extracellular matrix in HCV-concentrated regions may be associated with inflammatory as well as parenchymal damage [104]. HCV replication may be regulated by signal cascades, cellular factors, and viral proteins [105-108]. The HCV nonstructural protein 3 (NS3) has a serine protease required for the synthesis of viral protein and helicase activity needed for viral replication [109,110]. NS3 also is a major protein in modulating cell proliferation, indicating its role in carcinogenesis [111,112]. HCV NS3 protein facilitates tumor cell invasion in two ways. Firstly, NS3 induces several signaling pathways like protein kinase D2 (PKD2), JNK, and ERK1/2 to modulate the transcription factor NF-κB, and subsequently, it regulates the expression of COX-2 [113], and COX-2, in turn, activates MMP-9 expression. Secondly, NS3 induces the ERK1/2–p38 signal pathway to modulate the activity of NF-κB, which triggers the translocation of NF-κB to the nucleus and binding of NF-κB to MMP-9 promoter, which consequently upregulates MMP-9 expression. MMP-9 (an important enzyme in degrading the extracellular matrix), and COX-2 (the main enzyme in prostaglandin E2 production) exert key roles in tumor invasion and tumorigenesis. Therefore, NS3 enhanced tumor cell metastasis by MMP-9 and COX-2, leading to tumorigenesis (Figure 5) [114]. The MMP2, MMP9, and TNF-α levels have an effective increase in chronic HCV patients. However, no considerable correlation has been reported in the levels of MMPs and TNF-α between fibrotic and cirrhotic cases. In consequence, MMP2, MMP9, and TNF-α revealed high reproduction capacity in distinct patients afflicted with liver fibrosis (F1–F4) compared to the control group. In contrast, MMP9, MMP2, and TNF-α were not distinguishable factors for liver fibrosis in patients with chronic HCV. Therefore it is likely that they would be used as prognostic markers for liver fibrosis [115].

Figure 5: The mechanism of hepatoma cell invasion by HCV NS3 protein. First, NS3 activates multiple signaling components such as protein kinase D2 (PKD2), JNK, and ERK1/2 to regulate the activity of transcription factor NF-κB, which in turn regulates the expression of COX-2, and then COX-2 activates MMP-9 expression. Second, NS3 activates ERK1/2–p38 signaling pathway to regulate NF-κB activity, stimulates the translocation of NF-κB from the cytosol to the nucleus and the binding of NF-κB to the MMP-9 promoter, and thus activate MMP-9 expression.Thus, NS3 enhances tumor cell migration through COX-2 and MMP-9, resulting in oncogenesis or tumorigenesis

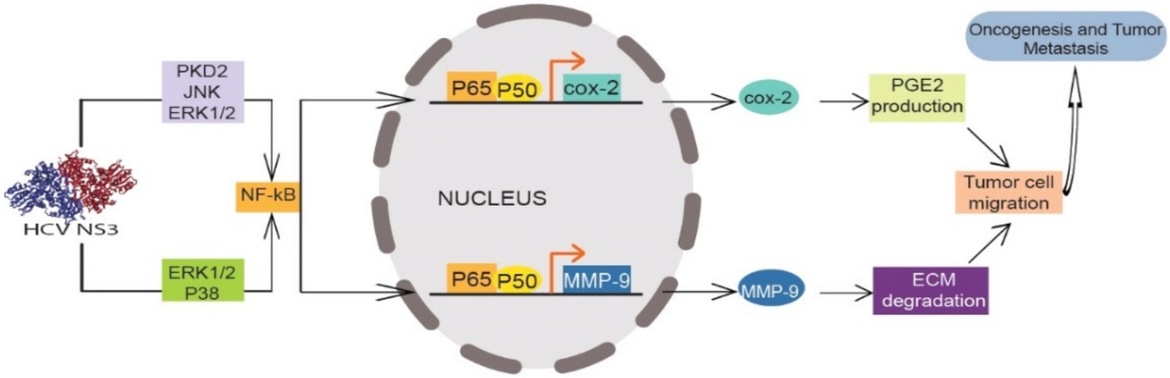

KSHV

Human herpesvirus-8 (HHV-8) or Kaposi sarcoma-associated herpesvirus (HHV-8 /KSHV) is a ds DNA virus with 165 kb in length. The HHV-8 life cycle consists of two states, lytic replication, and a latent phase. After primary infection, HHV-8 uses the cellular machinery of the host for replication and is subsequently observed in a latent phase as an episome. Like all herpesviruses, the B lymphocyte is the primary source of HHV-8 latency. The second sites for viral latency are endothelial cells, but these cells are the first infected cells in Kaposi sarcoma (KS). During latency, HHV-8 can regulate host cellular signaling pathways by different cellular mechanisms. Latent proteins, which include viral Cyclin a (v-Cyclin), LANA, kaposin, viral FLICE inhibitory protein (v-FLIP), and several miRNAs contribute to the pathogenesis of malignancies associated with HHV-8 [116]. HHV-8 has been identified in a Kaposi sarcoma lesion in an AIDS patient in 1994. This virus was reported to be associated with many B cell lymphoproliferative disorders (LPDs) such as posttransplant and germinotropic LPDs, multicentric Castleman disease (MCD), as well as primary effusion lymphoma (PEL) [117]. MMPs possibly associate with the mechanism of invasive KS growth and can be a proper target for effective therapeutic measures [118]. Developing KS lesional cells express gelatinases (MMP-2, MMP-9), stromelysin-1 (MMP-3), matrilysin (MMP-7), and collagenase (MMP-1, MMP-13) [119]. Cheng F. et al. demonstrated KSHV induces endothelial-to-mesenchymal transition (EMT). EMT was triggered by the viral proteins vGPCR and vFLIP via activation of the Notch pathway, which leads to a gain of membrane-type-1 matrix metalloproteinase (MT1-MMP) (Figure 6) [120]. The transcription factor PROX1 expression was recently investigated in KS. PROX1 binds the MMP14 promoter to repress its transcription. PROX1 negatively regulates MMP14 in normal tissues. Silvia Gramolelli et al. revealed that infection of lymphatic endothelial cells (LECs) with KSHV decreases PROX1 expression [121].

Figure 6: Schematic model of deregulation of MMPs by KSHV virus

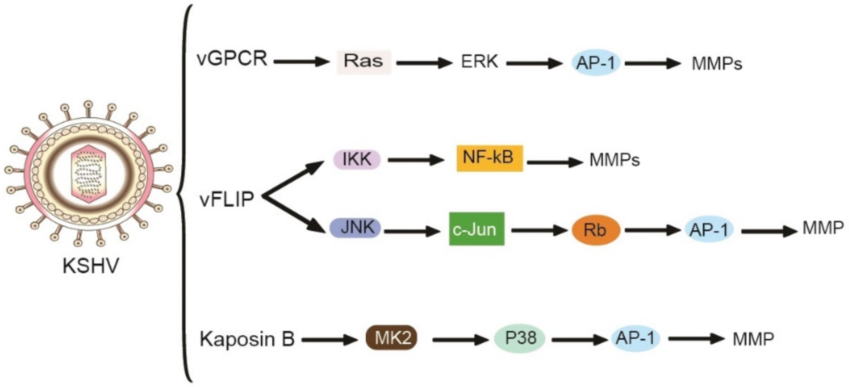

Human T-cell lymphotropic virus type I (HTLV-I)

HTLV1 is a member of the retroviridae family, which is an etiologic factor of Adult T-cell Leukemia (ATL).ATL is a malignancy responsible for the extremely aggressive proliferation of CD4+ cells with no known cure [122]. ATL commonly originates from an infection that is acquired in early life [123]. Also, besides ATL, other manifestations of infiltrated T-cells, matrix metalloproteinases (MMPs), and cytokines of viral infection are Sjogren syndrome with an inflammatory appearance and HAM/TSP associated with neurological symptoms and incomplete paralysis of limbs [124-126]. The 3’ end of the genome (pX region) encodes some specific genes, including Rex, Tax, p12, p13, p30, and HTLV-1 bZIP factor (HBZ) [127,128]. HTLV-1 encodes two important genes, basic leucine zipper factor (HBZ), and tax, that are recognized to exert key roles in the infectivity of HTLV-1, survival, and growth of leukemic cells. Unlike HTLV-1 tax, HBZ escapes the antiviral response and is not detectable via the immune system. HTLV-1 provirus pX region encodes HBZ protein and in contrast to Tax, expressed in 100% of ATLL cells [123,129,130]. Although the proliferation of T cells is increased by HBZ, its function is interestingly antagonist to many tumorigenic activities of tax, such as activation of classical signaling pathways such as NFAT, AP-1, CREB, and NF-κB. Tax Repression through HBZ provides a condition for HTLV-1 to develop ATL by evading the immune system [123,131]. Tax may interact with cellular factors including AP-1, CBP/p300, CREB/ATF, and NF-κB. Both HTLV-1-replication and T-cell proliferation are generally enhanced by activating of IκB kinase (IKK)-NF-κB that is stimulated by viral Tax oncoproteins. Viral transactivator Tax is a presumed E3 ubiquitin ligase that is unitedly related with UbcH7, UhcH5c, and UbcH2 and is responsible for direct activation of IKK via binding to the NEMO subunit [132,133]. Bazarbachi et al. proved the interaction of HTLV-I-transformed cells in relation to endothelial cells triggers the gelatinase activity of MMP-9 and MMP-2 in endothelial cells and decreases the tissue blocker of MMP. In a study by Szymocha et al. on infected astrocytes (T cells) with HTLV-1, contact with T cells is the factor that the astrocyte obtains a typical phenotype of gliosis and causes the release of proinflammatory cytokines (i.e. IL-1 α, IL-6, TNF-α) and matrix metalloproteinases (MMP-3, MMP-9). The high expression of MMPs and their endogenous suppressors (TIMP-3 and TIMP-1) cause MMP/TIMP imbalance. It changes the extracellular matrix [134]. Giraudon et al. showed cytokines secreted by infected glial cells with HTLV-1 are responsible for increasing the expression of TIMP-3, and MMP-3, MMP-9, while MMP-2, TIMP-1, and TIMP-2 remained stable (Figure 7) [135].

Figure 7: The demographic characteristics of the nurses (average score and standard deviation of nurses' clinical competence and moral reasoning)

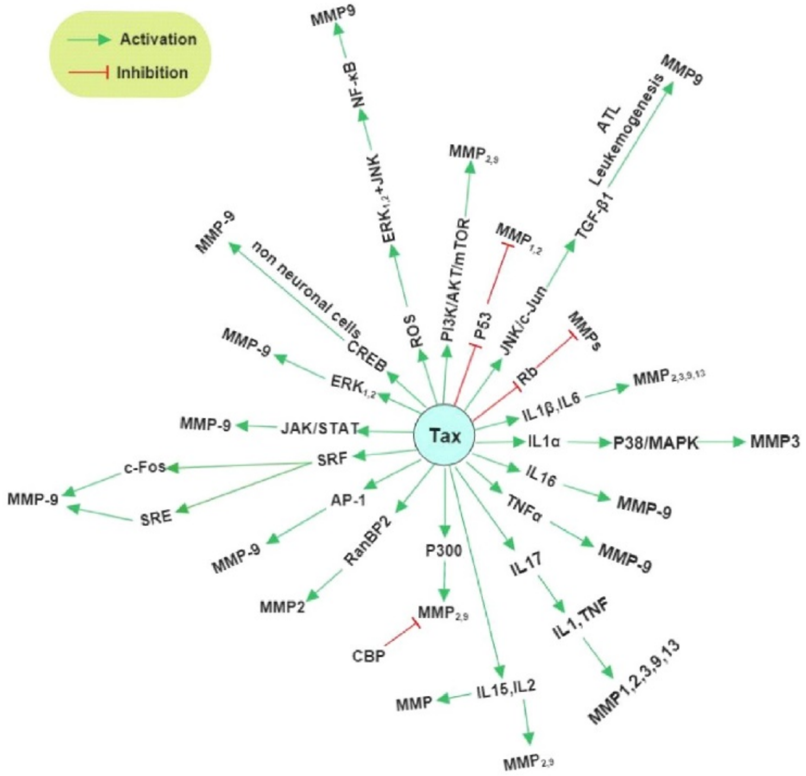
Activation
Inhibition
MMP9
NF-κB
ERK1,2+JNK
MMP2,9
PI3K/AKT/mTOR
MMP1,2
P53
ROS
MMP9
ATL
Leukemogenesis
TGF-β1
JNK/c-Jun
MMP-9
non neuronal cells
CREB
MMPs
Rb
MMP-9
ERK1,2
IL1β,IL6
MMP2,3,9,13
MMP-9
JAK/STAT
Tax
IL1α
P38/MAPK
MMP3
c-Fos
SRF
MMP-9
SRE
AP-1
IL16
MMP-9
TNFα
MMP-9
MMP-9
RanBP2
P300
IL17
MMP2
MMP2,9
IL1,TNF
CBP
IL15,IL2
MMP
MMP1,2,3,9,13
MMP2,9

MATRIX METALLOPROTEINASE INHIBITORS

Nowadays, MMPS targeting suppression of the activity of MMPs in the extracellular matrix as a novel anticancer strategy could be reconsidered. TIMPs, which are natural inhibitors of MMPs, at first, were the compounds to be developed for medical research. Hypothetically, TIMPs with potent inhibitory activity specifically can suppress the function of some MMPs which could lead to a useful therapeutic effect. However, their clinical application is still hampered by the absence of an efficient gene delivery style that has restricted the clinical use of this method [136]. Nevertheless, the progress of several types of natural synthetic suppressors of MMPs has been completely adopted and broadly investigated in clinical trials and researched in various cancers during the past decade (Table 3). Generally, synthetic suppressors of MMPs divide into three distinct pharmacologic groups including collagen non-peptidomimetics and peptidomimetics, tetracycline derivatives, and bisphosphonates [137].

THE RELATION BETWEEN INHIBITING OF MAJOR SIGNALING PATHWAYS BY MMP INHIBITORS AND ONCOVIRUSES

As previously mentioned, in HBV infection, HBx controls the transcription induction of MMP-9 which plays a key role in migration as well as metastasis of the tumor via PI-3 K/AKT and ERK pathways. Therefore, these data show that HBx plays a major role in inducing MMP-9 and, in turn, in the invasion and metastasis of cancer via activating ERK and PI-3K signaling pathways [77]. On the other hand, all the findings of one study already implied that 3-azidoWA suppresses 3-azidoWA-mediated MMP-2 expression through the attenuation of internal phospho-ERK as well as phospho-Akt expression in a dose-dependent condition that may have a significant role in the prevention of mouse angiogenesis as a natural inhibitor [138].

Table 3: An overview of the mechanisms deregulation MMPs by oncoviruses

Virus	Viral Factor	Number MMP	Mechanism/Effect	References
EBV	LMP1	MMP-9	Enhanced MMP-9 expression through induction of the MMP9 promoter by recruitment transcription factors AP-1, Sp-1, and NF-kappa B on MMP-9 promoter	[42]
	LMP1	MMP-1, MMP3	Upregulated MMP-1 and MMP-3 expression in NPC tumors	[45]
		MMP-9	Upregulated MMP-9 expression correlation with lymph nodes metastasis and clinical stage	[44]
	Zta	MMP-9, MMP-3	Overexpressed MMP-9 and MMP-3 expression in epithelial cells derived from NPC tumor	[48]
	EBNA3C	MMP-9	The interaction between EBNA3C and Nm23-H1 upregulated expression of MMP-9	[53]
	LMP2A	MMP-9	Upregulated MMP-9 expression by activation of extracellular signal-regulated kinase (ERK)-1/2 and a downstream transcription factor Fra-1	[56]
	Zta		Zta protein also up-regulated TIMP-1 gene expression in EBV-infected B cells and EBV-immortalized LCLs	[49]
HPV	E7	MT-1 MMP	E7 protein of the high-risk HPV types 16 and 8 lead to the induction of MT-1 MMP in in HaCaT cells	[65]
	E2	MMP-9	Increases the expression of MMP-9 in human keratinocytes and organotypic skin cultures	[66]
	E6/E7	MT1-MMP, MMP-2 and MMP-9	Upregulated expression of MT1-MMP, MMP-2 and MMP-9 in cervical cancer	[67]
		MMP-2, MMP-9	Up-Regulates MMP-2 and MMP-9 Expression and Activity by Inducing Interleukin-8 in Lung Adenocarcinomas	[69]
		MMP-8	Persistent oral HPV infection was associated with a low salivary MMP-8 concentration	[71]
		MMP-2, MMP-9, MMP-13 and MT1-MMP	Increased expression of MMP-9, MMP-13 and MT1-MMP in HPV8-positive mice than HPV8-negative animals	[72]
		MMP-2	A significant relationship was observed between the −1306C>T functional polymorphism in MMP-2 gene and HPV infection in increasing the cervical cancer risk	[73]
HBV	HBX	MT1-MMP, MMP-2	Up-regulated MT1-MMP expression by cyclooxygenase-2 (COX-2) activity and activates MMP-2	[76]
	HBX	MMP-9	Increased expression and regulate the transcriptional activaton of MMP-9 by activation of ERK and PI-3K signal pathways	[77]
	HBX	MMP-3	Up-regulated thetranscription,translationandsecretionofMMP-3	[78]
HCV	CORE AND NS5A	MMP-9	Up-regulated expression of MMP-9 with the synergistic effect of endotoxin and proinflammatory cytokines during chronic HCV infection	[103]
	E2	MMP-2	Up-regulation of MMP-2 by interaction between E2 and CD81and E2/CD81 interaction induced the up-regulation of MMP-2 by increasing activator protein-2/DNA binding activity via ERK/MAPK phosphorylation	[104]
	NS3	MMP-9	Activates MMP-9 expression by activates PKD2, JNK, and ERK1/2 to regulate NF-κB which regulates COX-2 expression , finally cox-2 activates mmp-9 expression	[114]
			Activates ERK1/2–p38 signal pathway to regulate NF-κB activity, stimulates the translocation of NF-κB from cytosol to the nucleus and the binding of NF-κB to the MMP-9 promoter	
KSHV	vGPCR and vFLIP	MT1-MMP	Increases of MT-MMP by induced EMT through activation of Notch pathway, leading to gain of MT1-MMP	[120]
		MMP-14	KSHV reduced prox-1 expression(Prox-1 binds to MMP14 promoter and represses its transcription),therefore prevents the repression of MMP-14 by prox-1 and induces mmp-14 expression	[121]
HTLV-1	TAX	MMP-2 MMP-9	Induces the gelatinase activity of MMP-9 and MMP-2 in endothelial cells and downregulates the tissue inhibitor of MMP	[134]
		MMP-9 MMP-3	Increases expression of MMP-9 and MMP-3 bysecreted proinflammatory cytokines (IL-1alpha, IL-6, TNF-alpha) by infected glial cells with HTLV-1	[134, 135]

Moreover, the results of a study by LU et al. provide molecules for the molecular signaling basis of the effects of newly synthesized quinazolinone MJ-29 on the inhibition of metastasis and invasion that could be beneficial as a therapeutic strategy to treat human oral cancer [139].

Accordingly, it can be concluded that 3-azidoWA and MJ-29 as natural MMP-9 inhibitors may have a more effective inhibitory ability on HBV, and HCV-related cancers by attenuating the internal phospho-ERK and phospho-Akt protein expression, which led to the repression of ERK and PI-3K signal pathways.

Other natural compounds such as curcumol via activation of JNK1/2, Akt, and nuclear translocation of NF-κB p65 [140], as well as magnolol through the inhibition of TNF-α by suppressing NF-κB binding activity [141], both caused the reduction of MMP-9 expression in MDA-MB-231 cells, breast cancer cell metastasis, and human urinary bladder cancer 5637 cells, respectively. Meanwhile, there is a possibility that these natural inhibitors can also modulate MMP-9 protein expression through multiple signaling pathways such as JNK, Akt, and TNF-α in HCV-related cancers. Antcin-H, a pure compound that is isolated from A.cinnamomea, can downregulate MMPs through inhibition of the FAK-ERK-C/EBP-/β/c-Fos-MMP-7 pathway, especially MMP-7 expression in renal cancer cell [142] (Table 4).

Table 4: Natural and synthetic inhibitors of MMPs in inhibiting targets of major signaling pathways in various types of cancer

Inhibitor	Class	Selectivity	Type of cancer studied	Inhibiting targets of major signaling pathways	Reference
Batimastat	Peptidomimetic	Broad spectrum	malignant ascites and malignant pleural effusion		[33]
Marimastat	Peptidomimetic	Broad spectrum	Breast cancer, small cell lung cancer, on-small cell lung cancer		[33]
BB-2516	Peptidomimetic	Broad spectrum	Head and neck squamous cell carcinoma (HNSCC)	through interference with autocrine loops in EGFR/c-erbB signaling pathways	[143]
BAY 12-9566	Nonpeptidomimetic inhibitor	MMP-2, MMP -3	advanced ovarian cancer, Breast cancer		[167]
AG3340	Nonpeptidomimetic inhibitor	MMP-2, MMP -3	non-small cell lung cancer		[168]
BMS-275291	Nonpeptidomimetic inhibitor	MMP-2, MMP -9	Non-small-cell lung cancer, prostate cancer, colorectal cancer, Breast cancer		[169, 170]
CGS 27023A	Small molecule	Broad spectrum	Advanced solid cancer		[33]
Col-3 (metastat)	Chemically modified tetracycline	MMP-2, MMP -9	Soft-tissue sarcoma, AIDS-related Kaposi's sarcoma, high-grade gliomas		[171, 172]
Prinomastat	Small molecule	Broad spectrum	Non-small cell lung cancer, , prostate cancer		[136]
Tanomastat	Small molecule	MMP-2, MMP-3, MMP-9	Ovarian cancer, adenocarcinoma of the pancreas, non-small cell lung cancer		[136]
Doxycycline	Tetracycline derivative	Broad spectrum	polycystic ovarian cancer		[136]
SB-3CT	Small molecule	MMP-2, MMP-9	T-cell lymphoma and prostate cancer models		[173]
ND-322	Small molecule	MMP-2/MT1-MMP	melanoma		[174]
curcumol	Major active components in the essential oil of RhizomaCurcumae	MMP-9	Breast cancer cell metastasis	via JNK1/2 and Akt(Ser473)-dependent NFκB signaling pathways	[140]
3 -azidoWA	derivative of Withaferin A	MMP-2	human cervical HeLa and prostate PC-3 cells	concomitant marked reduction in pAkt and pERK signaling by modulating extracellular par-4	[138]
MJ-29	2-phenyl6-pyrrolidinyl-4-quinazolinone derivatives	MMP-2/9	Oral squamous cell carcinoma	Down-regulation of MAPK and AKT Signaling	[139]
antcin-H	pure compound isolated from A. cinnamomea	MMP-7	Renal Cancer Cell	Partly through Inactivation of FAK-ERK-C/EBP-/β/c-Fos-MMP-7 Pathways	[142]
magnolol	the main constituent of Magnoliaceae	MMP-9	human urinary bladder cancer 5637 cells	Inhibition TNF-α-induced MMP-9 expression by suppressing NF-κB binding activities	[141]

The presence of MMP-inhibitory effects in virus-related cancers is still unknown. It has been determined that BB-2516, among all synthetic inhibitors, decreases the protein expression of a broad spectrum of MMPs by interfering with autocrine loops of EGFR/c-erbB signaling pathway in head and neck squamous cell carcinoma (HNSCC) [143]. However, more studies are necessary to understand the connections between MMP inhibitors and these signaling pathways in oncovirus-related cancers, making it especially important to examine how the relationships are.

CONCLUSIONS

In summary, matrix metalloproteinases as cellular proteases play the main role in biological processes as well as pathological conditions, including tissue repair, wound healing, developmental morphogenesis, inflammatory diseases, and tumor progression. Oncoviruses, in many cases, promote tumor progression through multiple mechanisms that affect the expression of these cell proteases. As oncoviruses upregulate the expression of MMPs genes through direct (via DNA binding) as well as indirect interactions (via activating PI-3K/AKT, ERK/MAPK, IFN/JAK/STAT, JNK, NF-κB signaling pathways or immune response), causing escape from the immune system and play a key role in cell proliferation, migration, angiogenesis, EMT, invasion, metastatic features, and subsequent tumor progression. Today, several compounds have been studied as matrix metalloproteinase inhibitors. Various studies have shown that these inhibitors activate the immune system and suppress tumor progression by inhibiting signaling pathways activated by viruses. With these interpretations, these cell proteases may be used as biomarkers to detect different stages of the development of oncovirus-related tumors, soon followed by inhibitors of these proteases in the treatment of viral tumors.

Conflict of interest

The authors report no conflicts of interest.

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Ethics approval and consent to participate

The study protocol was reviewed and approved by the ethics committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1399.520).

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REVIEW

Vascular Changes in Hypertension: Is There Cell Death Involved? – A Narrative Review

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Abstract: *Background: Cardiovascular disease can be triggered and accelerated by hypertension and the underlying changes in the structure and function of arteries. The purpose of this review is to explore some vascular changes that occur in hypertension as a consequence of the imbalance between cell proliferation and cell death, processes that play an important role in stabilizing the thickness of the arterial wall during vascular remodeling. Methods: The authors conducted research through PubMed and Google Scholar databases using the following search formula: (hypertensive*) AND ((vascular modifications)) OR (vascular changes)) AND (cell death). Results: From 40 articles, only 17 publications were included in this study, taking into account four processes that can be preceded or followed by inflammation and depend on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli: Cell proliferation and growth; Cell migration; Cell death; Degradation or reorganization of extracellular matrix. Conclusions: To summarize, maladaptive vascular changes in hypertension can represent a major argument for prompt and maximal therapeutic intervention in patients with high cardiovascular risk. Moreover, they can represent an important step in discovering new markers of cardiovascular risk and in the development of new targeted therapies for different pathways of cellular signaling through which the reversibility of abnormal vascular changes could be obtained.*

Keywords: *cell death; inflammation; cellular metabolism alteration; vascular remodeling; hypertension; pathophysiology*

INTRODUCTION

Cardiovascular disease, one of the leading causes of death worldwide, can be triggered and accelerated by hypertension and the underlying changes in the structure and function of arteries. Knowing the pathophysiological mechanisms that are responsible for the maladaptive changes occurring at the vascular level in the context of hypertension, can represent a first step in the development of new targeted therapies for different pathways through which the reversibility of the abnormal vascular changes can be obtained.

Some studies have shown that hypertension linked to the imbalance between cell death and cell proliferation could explain vascular remodeling: accelerates endothelial apoptosis, promotes cell migration, and accelerates the proliferation of vascular smooth muscle cells [1] resulting in the thickening of the middle tunic of the arteries [1,2]. The role of cell death in blood pressure charge is still unknown [3]. The prevalence of hypertension increases with age and the proposed pathophysiological mechanisms are represented by hemodynamic changes, arterial stiffness (due to elastic lamina disruption and intimal hyperplasia), and neurohormonal and renal dysfunction [4]. In the elderly, vascular injuries can heal through the formation of vascular scars due to the increase in the proliferation of smooth muscle cells and the reduction of their apoptosis and decrease in sensitivity to apoptotic stimuli in fibroblasts and myofibroblasts, with secondary fibrosis [5,6].

In terms of the cell death process, some major pathways have been described (apoptosis, necrosis, and autophagy), depending on the injury and the type of cell [7]. Programmed cell death and necrotic cell death sometimes have mechanisms that can be overlapped [5]. The initiation of apoptosis is the result of death signaling pathways, intrinsic (mechanisms triggered by cellular stress through hypoxia, oxidative stress, and ADN damage) or extrinsic (mediated by transmembrane receptors of the TNF receptors family). In apoptosis, an essential process in maintaining tissue homeostasis, as a result of caspase cascade activation and imbalance between pro and antiapoptotic factors, the cell is broken for-ming membrane-bound vesicles and apoptotic bodies (after the shrinkage of the cell, fragmentation of nucleus and chromatin and plasma membrane blebbing [7]) responsible for clearance of dead cells and also for intercellular communication [8]. Necrosis is an accidental form of cell death but in certain instances is initiated or modulated under programmed control mechanisms. Unlike apoptosis, which requires energy consumption, necrosis is a process in which cells die without energy consumption; in the necrotic cell death process, as a result of the breaking of the cell membrane and the elimination of the cell content in the extra-cellular space, inflammation occurs. It has been described as a form of programmed necrosis (necroptosis), caspase-independent, with mitochondrial changes involvement [5]. Another type of cell death, autophagy, is the process through a staving or deprivation of growth factors cell generate energy and metabolites by digesting its organelles or macromolecules. There were also described multiple sub-types of cell death, depending on caspase involvement or the presence of some of the necrosis criteria: aponecrosis, paraptosis, anoikis, cornification, etc [5].

Recent studies examine how certain factors affect endothelial apoptosis in patients with hypertension and other comorbidities [1,4,9]. Endothelial cell apoptosis was found in patients with hypertension and peripheral arterial disease by studying endothelial cell cultures [9]. Vascular cell remodeling, an active process, involves changes in at least 4 cellular processes: cell growth and cell migration, inflammation, cell death, and degradation or reorganization of the extracellular matrix. It is dependent on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli [10].

Although the in vitro studies [11] demonstrated a relationship between the level of some plasma membrane receptors involved in apoptosis induction (named death receptors) [12] and the phenomenon of apoptosis, the causal link between cell death and vascular changes was not proved yet [11].

Considering that the phenomenon of cell death and cardiovascular outcomes are linked through metabolic risk factors intervention and exogenous causes of cellular stress that lead to cell destruction via different pathways and demonstrating to what extent the alteration of cellular signaling, the alteration of the extracellular environment and the triggering of cell death mechanisms are responsible for the abnormal remodeling of the arterial wall in the hypertensive patients, the research could be directed to discover new markers of cardiovascular risk and new therapies that can be targeted in patients with cardiovascular risk factors. The benefit could be major, the measurement of death receptors levels related to cell death phenomena that can predict cardiovascular outcomes and death [11], in patients with high cardiovascular risk, became a major argument for prompt and maximal therapeutic intervention in this specific populational group.

The purpose of this review is to explore some vascular changes that occur in hypertension as a consequence of an imbalance between cell proliferation and cell death, processes that play an important role in stabilizing the thickness of the arterial wall during vascular remodeling.

MATERIALS AND METHODS

A comprehensive search through PubMed and Google Scholar using the following search formula: (hypertension*) AND ((vascular modifications)) OR (vascular changes)) AND (cell death) was performed. Only full-text freely available articles published in English between 2000 and 2021 were used. Articles that were most relevant to the subject of this review were included after thorough consideration. Inclusion criteria were published in English, publication within the mentioned period, and inclusion of hypertensive models or animal models, or cell cultures studies. From 40 articles, only 17 publications were included in this study. All the selected papers were reviewed by an independent reviewer to check that the interpretations were correct.

Hypertension has a great impact on the cardiovascular system; the thickness of the arterial wall during the remodeling process could be the result of cell death mechanisms triggered by factors potentially involved in the etiology of arterial hypertension. Considering that the connection between cell proliferation and cell death is responsible by a vascular remodeling process in hypertension, we take into account for our study four processes that can be preceded or followed by inflammation and depend on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli:
- Cell proliferation and growth
- Cell migration
- Cell death
- Degradation or reorganization of extracellular matrix

RESULTS

The search strategy identified 40 articles of which 17 were deemed to be eligible for inclusion in this systematic review. Of these, 12 studies addressed markers of cell proliferation/growth, 7 studies addressed cell migration, 12 studies addressed cell death processes/markers, 7 studies focused on the degradation of extracellular matrix, and 13 studies addressed inflammation markers (shown in Table 1).

Table 1: Type of processes involved in vascular remodeling

Reference	First author, year of publishing	Study type	Pathophysiological mechanisms, Type of process					
			Arterial remodelling	**Inflammation**	**Cell proliferation/ cell growth**	**Cell migration**	**Cell death**	**Reorganization/ degradation of extracellular matrix**
1	Brown, I.A.M *et al,* 2018	Review	X	X	VSMC hypertrophy and hyperplasia	X	X	
2	Xu, C. *et al,* 2001	Experimental study	X		X		X	
16	Martinez-Quinones, P. *et al,* 2018	Review	X	X	VSMC hypertrophy and hyperplasia	X		X
10	Renna, N.F. *et al,* 2013	Review	X	X	X	X	X	X
21	Schiffrin, E.L. 2012	Review	X	X	VSMC hypertrophy and hyperplasia		X (apoptosis; anoikis – in endotelial cells)	X
19	Virdis, A. *et al*, 2014	Review	X	X	X	X		
15	Sun, H.J *et al,* 2020	Review	X			X		X
18	Munoz-Durango, N. *et al,* 2016	Review	X	X	X	X	X	X
17	Bruno, R.M. *et al*, 2017	Research article	X	X	X		X (apoptosis)	
3	Abdelbary, M. *et al*, 2019	Comparative study		X	X		X (necrosis, apoptosis)	
22	Intengan, H.D.; Schiffrin, E.L. , 2001	Review	X	X	X		X (apoptosis)	X
9	Gardner, A.W. *et al*, 2019	Randomized Controlled Trial		X			X (apoptosis)	
26	Krishna, S. M. *et al*, 2021	Randomized Controlled Trial	X	X			X (apoptosis)	X
27	Fekete, A. A. *et al*, 2016	Randomized Controlled Trial	X	X				
28	Li, X. *et al*, 2022	Review	X	X	X	X	X (apoptosis)	X
32	Xu, W.;Erzurum, S.C., 2011	Review	X		X		X (apoptosis)	
33	Levy, M. *et al*, 2007	Clinical research	X	X	X		X (apoptosis)	

In hypertension, the connection between cell proliferation and cell death could be critical in determining the thickness of the arterial wall during the remodeling process. There are 2 types of criteria for classifying cell remodeling:

- according to the size of the middle tunic: hypertrophic, eutrophic, and hypotrophic
- according to the size of the lumen: internal and external remodeling.

The consequences of cell remodeling are:

- high pulse pressure,
- stiffening of the arterial wall
- changes in the extracellular matrix.

All consequences of cell remodeling are determined by the release of growth factors and cytokines. The immune system, namely T lymphocytes, plays a key role in remodeling, more precisely based on a T-reg/T-effector imbalance.

Because of its ability to modify the vascular tone, vascular endothelium plays a significant role in hypertension. Endothelial dysfunction is caused by a reduction in nitric oxide (NO) availability, which causes endothelial dysfunction. The main cause of the decline in NO is the excessive production of reactive oxygen species (ROS)[13].

Blood pressure is affected by the phenomenon of cell death that can occur at different levels of the arterial wall, however, in hypertension the role of different types of vascular cell death is unclear. Researchers discovered that vascular smooth muscle cell growth is increased by cell death [1]. The cell death process is thought to play a key part in remodeling, but it is unclear whether it is the main process or a growth-related compensatory mechanism, being influenced by reactive oxygen species, NO, angiotensin type 2 (AT-II), and endothelins.

Due to vascular damage, elevated blood pressure values may alter the integrity of the endothelium and may also represent a risk factor for the onset and progression of vascular atherosclerosis changes leading to the destabilization of atheroma plaques [14]. Atherosclerosis favors the processes of inflammation and necrosis of the blood vessel and plaque can become unstable without proper treatment and cause even more complications.

DISCUSSION

In our study, we collected a lot of information about vascular modifications in hypertension. From the 17 selected articles, in 15 we found information about vascular remodeling (88.23%), in 14 articles we found arguments for the presence of the inflammatory phenomenon (82.35%), 13 articles (77.47%) argued for the presence of cell death and also 13 described cell proliferation, 7 articles (41.17%) described the phenomenon of cell migration, and 8 articles (47.05%) described reorganization of the extracellular matrix.

Starting from the information provided by the articles included in our study, our hypothesis related to the involvement of cell death at the vascular level in arterial hypertension seems to be valid. Maintaining a balance between the processes of cell proliferation, cell migration, cell death, and degradation of the extracellular matrix could ensure adequate vascular elasticity and delay the installation of specific hypertensive vascular changes. It seems that cells from all layers of the vascular wall are involved and can be affected by different types of death, the damage to each cell type leading to cellular metabolic changes and changes in the extracellular environment.

Vascular tone and vascular remodeling

The endothelial cells which form the tunica interna of the arterial wall, are essential for regulating hemostasis, vascular tone, and host defense [15]. Secretion, protection as a barrier, and local metabolism are the endothelium's three primary activities, each of which is altered by hypertension. The endothelial cells produce vasoactive substances such as nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H2S)[15], prostacyclin (PGI2), endothelin 1 (ET-1), AT-II, prostaglandins. Normally, there is an equilibrium between these substances to maintain proper blood flow. In hypertension, at the vascular level is an increased production of vasoconstrictor factors (ET-1, AT-II, prostaglandins) which determine endothelium dysfunction (increased permeability, induce inflammation, and edema). It was discovered that the substances and structures that are released from a cell when it dies can affect the endothelial function in an autocrine, paracrine or endocrine model [16].

The endothelium is important for both short-term vascular tone modulation and long-term vascular remodeling. Nitric oxide is the most significant endothelium-derived vasodilator molecule which, under healthy conditions, is capable of inhibiting the primary fundamental pathways driving the formation of atherosclerosis and thereby improving vascular health. Reduced NO availability and endothelial dysfunction are caused by NO breakdown by reactive oxygen species (ROS) in physiological aging and many pathological diseases, including arterial hypertension.

Essential hypertension patients (HT) had a lower endothelial response to acetylcholine (Ach) than normotensive people (NT) in each age group, but it was proved that the vascular response to Ach in both HT and NT patients decreases as they become older [17]. In the pathogenesis of endothelial dysfunction and the cardiovascular consequences related to endothelial inflammation [18], apart from the effects of nitric oxide and carbon monoxide, the role of hydrogen sulfide has been also described. H2S formation was detected in smooth muscle cells, vascular endothelial cells, and cardiomyocytes and it was shown that H2S could protect endothelial cells against apoptosis by attenuating the overproduction of ROS and inhibiting mitochondrial apoptosis [15].

The vascular smooth muscle cells (VSMCs) that form tunica media are responsible for vasoconstriction, vasodilatation, and the process of remodeling [19]. The hyperplasia and hypertrophy of VSMCs are the processes that underlie vascular remodeling. The most frequent type of remodeling in hypertension is the inward type causing the narrowing of the arterial lumen. The outward type of remodeling occurs during anti-hypertensive treatment and is also caused by increased blood flow[16]. Some studies have revealed that a marked cell death influences and accelerates the proliferation of vascular smooth muscle cells, resulting in the thickening of the middle tunic of the arteries [2]. As a result of cell death through necrosis and the breakdown of plasma membrane integrity, the contents of necrotic cells are released, triggering a proinflammatory response [3].

Fibroblasts that form the tunica externa secrete chemokines, cytokines, and growth factors that contribute to normal vascular function and participate in the degradation of the extracellular matrix components. They play an important role in remodeling as a response to injury. However, fibroblasts suffer structural changes that are accompanied by proliferation and migration into the tunica media during hypertension [16].

Cell remodeling

Cell remodeling involves cell growth and cell migration, cell death, and degradation of the extracellular matrix and is dependent on multiple factors [10]. The media-to-lumen ratio (M/L), modifying the vessel wall width for increasing muscle mass, the reorganization of cellular and noncellular elements and the lumen dimensions are the most prominent manifestations of these adaptations [20]. As a result, remodeling can be classified based on the degree of change of the medium arterial tunic in hypertrophic, which is the most common, hypotrophic, or eutrophic remodeling, as well as the size of the lumen in internal or exterior remodeling [21]. With age, large arteries undergo outward hypertrophic remodeling and stiffening [4], and in hypertension, this process may be accelerated, resulting in higher pulse pressure [22-24]. The elastic laminae, on the other hand, develop multiplication and fragmentation in severe hypertension, with increased deposition of collagen and fetal fibronectin, leading to greater stiffness [23]. Small artery remodeling has typically been associated with increased media thickness in hypertension, but new studies have shown that there are two forms of remodeling, inward eutrophic and inward hypertrophic, depending on whether the media cross-sectional area is enlarged [23]. The discovery that inward eutrophic remodeling may be prominent in some kinds of hypertension has raised the prospect that countervailing mechanisms in hypertension, including apoptosis, may compensate for or modify growth [23,25]. The outer and lumen diameters are decreased, the media cross-sectional area is unchanged and the M/L is increased without stiffening in inward eutrophic remodeling [25]. Inward eutrophic remodeling reduces the outer diameter of the vessel through a mix of growth and apoptosis, with apoptosis concentrated to the periphery, whilst inward growth decreases lumen diameter, which may explain the preservation of media volume. It is uncertain whether apoptosis is a growth-related compensatory mechanism or a primary process[25]. Mechanical forces exerted on the arterial wall also play a significant role in remodeling. Changes in the extracellular matrix, the cellular release of growth factors and cytokines, and vascular sensitivity to circulating humoral factors are all caused by them. Force duration, load, and force properties all have an impact on all of these factors [1].

Low-grade inflammation

In hypertension and cardiovascular disease, low-grade inflammation plays a critical pathogenic function [26-28]. Innate and adaptive immune systems, specifically T cells, are implicated, according to a significant body of research. T-lymphocytes play a key role in the development of hypertension-related vascular remodeling. The balance between T-effector and Treg lymphocytes is a critical regulatory system that, when disrupted, favors blood pressure increase and the development of organ damage [21]. Treg lymphocytes have potent anti-inflammatory characteristics, ensuring vascular homeostasis and protecting the arterial wall against the onset of atherosclerosis [21,29]. In humans, the majority of data establishing essential hypertension as a chronic low-grade inflammatory state found a clear and independent link between c-reactive protein (CRP), tumor necrosis factor (TNF), Interleukin 1 beta (IL-1 beta)[30], Interleukin-6 (IL-6), and adhesion molecules and vascular alterations in essential hypertensive patients [21].

An important role in the process of vascular remodeling is played by the renin-angiotensin-aldosterone system (RAAS)[29,31] because of its role in VSMC migration and inflammation [24,32]. This system acts through 2 types of angiotensin II receptors AT1-R and AT2-R, but the AT1-R is thought to be the primary receptor that causes Ang II's proliferative and profibrotic actions [20,33]. The AT2-Rs are thought to cause opposite effects, preventing vascular deterioration. But there are times when AT2-R acts similarly to AT1-R (for example, in renovascular hypertension it causes tonic renal medullary vasoconstriction) [32,34]. Recent studies showed that there exists an AT1-R autoantibody (AT1-AA) which is an agonist of AT1-R. AT1-AA raises AT2-R expression and encourages cell migration through cytoskeletal remodeling [34]. The RAAS is implicated in physiological and pathological actions when it comes to vascular changes. It reduces ROS generation and modifies vascular tone via vasoconstriction and vasodilatation. During hypertension, this RAA system induces fibroblast proliferation, collagen deposition, and vascular hypertrophy [20].

Cell death

Apoptosis is a type of gene-controlled cell death that was first discovered in development as a mechanism for fine-tuning growth. The vascular system has a large number of apoptosis modulators. Reactive oxygen species, NO, angiotensin type 2 receptors, and the endothelin system are all possible candidates. Reactive oxygen species play a key role in the pathogenesis of hypertension and vascular cell apoptosis [35], with O_2^- inducing proliferation and H_2O_2 inducing apoptosis through a protein kinase C–dependent pathway, respectively. Angiotensin II is also a potential apoptotic trigger [25,31,36].

Recent studies examine how certain factors affect endothelial apoptosis in patients with hypertension and other comorbidities. Endothelial cell apoptosis was found in patients with hypertension and peripheral arterial disease (PAD) by studying endothelial cell cultures. They demonstrated that physical activity can lower this type of cell death[9]. PAD patients present increased cardiovascular stress, inflammation, and oxidative stress. Inflammation and oxidative stress speed up the progression of myopathy and increase the risk of apoptosis and sarcopenia. In older persons, exercise decreases oxidative stress and improves reactivity to oxidative processes [9].

Inflammation, VSMC apoptosis, and oxidative stress in patients with hypertension [1,18,19] can also play an important role in the pathology of abdominal aortic aneurysm AAA [37,38]. According to some studies [39] a member of the seric proteinase inhibitor superfamily, Human Kallistatin, acts as a protective factor by blocking the pathway that stimulates AAA generation [39], and even whey-protein supplementation has a beneficial effect, improving vascular function by inhibiting the serum angiotensin-converting enzyme [40].

Lesions that appear on the arterial wall and the dysfunction of endothelial cells determined by high blood pressure values are factors that contribute to atherosclerosis. In the injured artery intima, extra low-density lipoprotein (LDL) accumulated suffer processes of oxidation and can attract monocytes and macrophages, forming the foam cells. The local inflammatory reaction is severe [41] and it triggers a cascade of events [38,42]. Foam cell secretion, deposition, and apoptosis [43] are crucial steps in the occurrence of atheroma plaque [44]. In advanced stages, the macrophage function of phagocytosis is destabilized, the atheromatous plaque continues to grow and, in the end, it will become unstable, promoting local inflammation and necrosis without the proper treatment [41]. Studies have revealed that at the cellular level microRNA plays a role in the control of atherosclerosis [41,45], acting on the TGF-β signaling pathway, inducing proliferation, migration, and apoptosis of endothelial cells, VSMCs, and macrophages [37,43,46].

Sometimes, associated with long-term uncontrolled hypertension and left ventricular dysfunction (or other conditions such as left heart disease, thromboembolic pulmonary condition, lung disease, liver disease, hematologic disease, metabolic disease, connective tissue disease, human immunodeficiency virus infection, thyroid diseases, etc.), pulmonary hypertension may occur [47,48]. In this situation, through the lungs arteries that have a reduced lumen and thickened walls, blood will flow slowly. Pulmonary arterial hypertension (PAH) is defined by mean pulmonary arterial pressure (mPAP)>20 mmHg at rest assessed by right heart catheterization [47-49]. Histopathological changes at the pulmonary level are neointima formation in pulmonary arteries, wall thickness, and plexiform lesions formed by the proliferation of endothelial cells. There is an increased level of ROS that contributes to oxidative stress and vascular remodeling [50], similar to the changes found in the arteries of hypertensive patients. It has been discovered that there is a deficient activity of NO-synthase, the level of NO is low and so are the vasodilatory effects. The role of proapoptotic and antiapoptotic proteins has been incriminated in the initiation of the pathogenesis of PAH and the proliferation of apoptosis-resistant vascular cells [51,52]. Both reversible and irreversible PAH express the apoptotic markers (caspase-3 and p-53) [51,53], while irreversible PAH expresses BCL-2 only in severely wounded endothelial cells [51]. Apoptosis plays an important role in pulmonary hypertension among the proliferation of VCMCs and plexiform lesions [51,52].

In our study we highlighted some evidence in favor of the hypothesis that cell death is involved and has consequences on vascular changes in people with arterial hypertension; the vascular changes occurring in the hypertensive patient may be due to the alteration of the cellular metabolism, which may represent a triggering factor of cell death processes by activating abnormal cellular signaling pathways. However, there are insufficient data to show the cell death types involved and the types of cells in the vascular walls involved in the process of vascular remodeling, therefore we consider that further studies are necessary to elucidate these aspects

CONCLUSIONS

Cell death and cardiovascular outcomes seem to be linked through metabolic risk factors intervention and exogenous causes of cellular stress. Demonstrating that alteration of cellular signaling, the alteration of the extracellular environment, and abnormal cell death mechanisms are responsible for the abnormal remodeling of the arterial wall in hypertensive patients could direct future research toward the identification of new molecules as plasma membrane receptors involved in apoptosis and new therapies that can be targeted in patients with cardiovascular risk factors. Once the vascular impact of imbalances between cell proliferation and cell death will be demonstrated, its early detection could become an argument for maximum therapeutic intervention in this population group.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution
EB conceived of the presented idea. AMD, EB contributed to documentation on the topic of interest, work conception and revised the manuscript. EB, ŞSB, OA performed the final revision of the manuscript. All authors discussed the results and contributed to the final manuscript. ŞSB and OA gave final approval. All authors have read and agreed to the published version of the manuscript. EB, ŞSB, OA had equal contributions.

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ORIGINAL ARTICLE

Is Still Neurology Desirable as a Specialty for Aspiring Physicians?

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Abstract: *This article aims to highlight the burnout burden among neurologists and provide information on some of its causes and consequences. We report an original article that reviews our current understanding of burnout matter in the neurology field. The main search engine through which we performed extensive literature research was PubMed, Scopus, and Google Scholar. The following information was collected: triggers of burnout and factors that perpetuate it, major complaints, values, and goals that dictate how coaching with it. Physician burnout is an umbrella term characterized by symptoms determined by exposure to chronic workplace stressors. Simply put, it encompasses many dimensions: emotional exhaustion, feelings of cynism, detachment (depersonalization), and a sense of ineffectiveness at work (low personal accomplishment). Burnout is common among all medical specialties, but neurology ranks at the top of the list. Several identified factors continue to fuel this problem. Properly addressing them, rethinking practice, and implementing wise, modern strategies can make significant contributions to reducing it. Aside from the chronic harm to the physician, burnout puts at risk the healthcare community at many levels including patients, other professionals, finances, etc. This paper wants to emphasize that burnout in neurology should be regarded as a global crisis. Thus, some strategies are proposed.*

Keywords: *neurology, burnout, work-life, emotional exhaustion, stress, fatigue, depersonalization, second-victim phenomenon, complaints, functional neurology*

INTRODUCTION

Physician burnout is an umbrella term characterized by symptoms in response to prolonged exposure to chronic stressors related to the workplace. This topic attracted interest from researchers since it exhibits a concerning prevalence. In the field of medicine, we can consider burnout a pandemic that has a negative impact both on doctors and their patients, but most of the time this reaches far beyond that, to families and the overall healthcare system. All medical specialties are affected, but some of them carry a higher percentage of burnout. Over 40% of general physicians experienced at least one symptom of burnout, neurology being ranked the second specialty affected, with more than 60% of neurologists signaling symptoms [1,2]. Medscape 2022 report on Physician Burnout and Depression showed that 47% of neurology specialists are experiencing mental health challenges, which represents a substantial increase in its prevalence compared with the previous year's reports when it was 42% [3]. The purpose of this article is to highlight the burnout burden among neurologists and provide information on some of its causes and consequences.

BURNOUT AND NEUROLOGY

To date, many research studies have contributed to a comprehensive overview and understanding of the root causes that fuel physician burnout.

The prevalence of neurological disorders increased in the last two centuries and the increased burden that comes along can be attributed to explosive population growth, people reaching older ages, unhealthy lifestyles, and unawareness of the importance of prophylactic measures [4].

In opposition to this growth, the global shortage of neurologists led to inequalities in the availability of patient care with those already existing clinicians being overwhelmed. The Association of American Medical Colleges biannually investigates the number of patients per active physician. Reports published two years ago indicated that the number was higher in neurology (23.429) than in ophthalmology (17.162), cardiovascular diseases (14.717), radiology (11.896), psychiatry (8.544), emergency medicine (7.332), internal medicine (2.758), and is approaching specialties with the highest burnout rates such us urology (32.490) [5]. Thus, the low number of practicing neurologists per patient increases the amount of work, compromises patient care, and exacerbates burnout.

Switching to professional working hours per week, an interesting study concluded that neurologists worked 57.1 hours per week, with 42.3 hours being spent performing inpatient activities. In our neurology department, the average number of professional working hours per week is 59, with a peak of 100 on rare occasions (absenteeism of other colleagues because of medical conditions/vacations or lack of staff) [6].

From a holistic point of view, burnout can be regarded as an indicator of quality and duration of life, given that it has a direct damaging effect on health: overnight shifts, exposure to all kinds of infective agents, irregular and exaggerated effort, lack or inadequate physical exercise, emotional detachment, interrupted meals or maladaptive coping strategies such as excessive caffeine intake or other power drinks, alcohol, and drug abuse. Johann Ludwig Casper, a prominent name in forensic medicine stated that there is no profession with a comparable physical and mental force demand as the medical one, an observation comparable with recent studies [7]. It is also worth mentioning that being on call requires working very fast or accomplishing more tasks being short on time. Very similar ideas are presented in a meta-analysis of 17 studies that in people with high demands, the risk of mortality is 20% higher than in the reference group [8]. Similar opinions are found in a study involving 1.671 American neurologists, where 60.1% of the responders had at least one symptom of burnout [2].

According to the World Health Organisation and International Classification of Diseases, burnout has become 2019 a legitimate medical disorder and has been given its ICD-10 code (Z73.0 - Burn-out state of vital exhaustion). Burnout is a state of chronic stress, that occurs after the body system is repeatedly triggered. It can exacerbate mental conditions like anxiety disorders, depression, and sleep problems. Some authors focused on the cortisol secretion parameters and concluded burnout is a mental syndrome with unique pathophysiology [9]. Moreover, as it often happens, the presence of a disease leads to a higher chance for another one to develop. Burnout influences not only subjective well-being but other biological processes as well. In a large study of nearly 9000 employed adults, was found that burnout is a significant risk factor for developing coronary heart disease and finally, heart attacks [10]. These findings support the idea that physicians with high demands as neurologists are exposed to challenges regarding their health and are more vulnerable to stress and burnout.

There is an excessive workload and clerical duties, including finishing notes, phone calls, ordering tests and reviewing results, responding to phone calls and patient requests, and prescribing medication. Assessing indicators related to burnout in a national survey on 9561 neurology residents and fellows after studying 354 responses to the survey the following was revealed: regarding the amount of time spent on clerical tasks directly related to patient care" only 26.2% of residents and 29.7% of fellows agreed that it is reasonable [2]. Another particular challenge is finding meaning in work and this often translates to helping others. Some neurological disorders do not come with treatments or cures or are associated with a significant burden of disability. In the same study, in the matter of, satisfaction with the job" only 68.9% of residents and 63.1% of fellows were more likely to be satisfied with their career choice and in line with, work being meaningful", 78.8 % of residents and 85.6% of fellows had positive feelings towards their job [11]. In comparison to attending physicians, an important number of neurology residents experience burnout due to extra workload and the challenges that come with a new environment, 73% of residents mentioned at least one symptom from the constellation of burnout. Furthermore, both residents and practicing physicians are affected by the second-victim phenomenon, which refers to feelings of guilt caused by medical errors which result in poor patient outcomes [12,13].

Some authors focused instead on sources and outcomes of burnout, on potential interventions to treat it or to prevent the onset. It must be mentioned that burnout is a consequence of systemic deficiencies rather than a result of poor uncoping skills. Increasing trainee resilience and cutting clerical tasks through hiring administrative workers or through using user-friendly technology [14], positive reinforcement and support during residency, and implementing regular burnout evaluations such as Maslach Burnout Inventory. The last one mentioned is the most widely accepted evaluation of burnout and outlines three key aspects of this response: the state of feeling emotionally worn out, feelings of cynicism toward work, and a personal sense of inefficiency in the workplace [15].

The supply-and-demand issue that perpetuates burnout and challenges patient care in neurology could be overcome by recruiting other medical staff. For example, nurse practitioners could get in charge of some medical tasks, without actually exceeding their abilities, as the healthcare oath foremost promotes abstaining from all intentional wrong-doing and harm. This topic with potential usefulness has already been addressed in an article published in the JAMA journal, but it aroused a lot of controversies [16]. Most complaints came from those in need of help, like physicians, stating that to figure out the pathology, prove competency, and be able to give medical assistance one has to go for over a decade in training. This amount of time is spent in school only by doctors, to fully become licensed, and no other alternative shorter programs can replace this training. But maybe they can approach it. An answer to this problem could be by enhancing nonphysician practitioners in medical student courses or by attending residents, giving them templates and free access to educational programs. In conjunction with this, in the UK for example, specialist nurses are known for their crucial role. They can choose and qualify for various neurological conditions (multiple sclerosis, Parkinson's disease, and epilepsy). Sometimes they can order tests, administer basic treatments, and in some states even prescribe medications.

Another indicator of burnout and shortage of neurologists is also projected by the difficulty in making an appointment for new patients and by lengthy waiting lists. Data from 626.000 first appointments scheduled in 2016 with 1.044 providers concluded that wait times for new patients are longer for high-demand specialties, with neurology patients waiting approximately 32.3 days for first appointments [17]. This is particularly relevant since waiting for more than a month often determines patients to cancel their appointments, and never return to the clinic with consequences resulting from delayed diagnosis and treatment. Moreover, significant information was revealed by 2021 research on NHS data conducted by Neurological Alliance – a coalition with over 80 organizations including the MS Society, Parkinson’s UK, and MND Association. Thus, they showed that by the end of March 2021, over 150.000 people were waiting for an assessment by a specialist and over 10.000 of these have been waiting for over a year [18]. These numbers indicate how overwhelmed are the neurology services, putting a lot of pressure on existing physicians, and prolonging a state of excessive stress and fatigue that is burnout.

As a consequence of different workplace stressors and poor coping mechanisms, it was estimated that 10-15% of doctors will develop a problem with substance addiction at some point in their careers [19]. Regarding the neurology field, in a published survey among all specialties, answers from 470 chronic pain/neurology subjects as well, it was shown that in this category of physicians there is a prevalence of 3.7% marijuana use. The issue is not limited to illegal drugs, pain medicine, and alcohol count as well. The anonymous questionnaire indicated a prevalence of 88.6% of alcohol use in physicians from the category of chronic pain/neurology, also being the group with the highest prevalence for benzodiazepine of 15.5%. In addition to this, they found 16.3% prevalence for minor opiates and 1.9% for major opiates, hence being ranked the third specialty. Neurologists frequently come across chronic pain patients in need of benzodiazepines and opiates and somehow this exposes them to these drugs. They ranked second only to psychiatrists in the matter of the risk of using benzodiazepines [20].

Many neurologic diseases require interdisciplinary care and this sometimes creates the setting for conflict between providers offering healthcare services [21]. To reduce the burnout burden, neurologists should try to understand and develop the concept of teamwork. Literature describing the issue of individual vs. teamwork in neurology is scarce, but limited evidence suggests there are more benefits from the adoption of a teamwork approach, where feasible. By avoiding professional isolation, rethinking professional roles, and abandoning territorial behavior.

Receiving complaints is another issue that lies at the heart of burnout in neurology as well. Many of these complaints arise from the limits that go along with disabling or cureless illnesses, giving a false feeling that the healthcare workers did not do anything possible to resolve the case. This situation can be overcome by improving the informative part of the medical act with better communication skills with the patient and through offering honest expectations for patients and their families. Moreover, it is not uncommon that patients to have a low level of medical education and not understand the limits of medicine, priorities, or medical procedures, so this is another important factor that promotes complaints [22,23].

We propose some strategies for the prevention of burnout on neurology specialists (Table 1). There are three categories of factors: related to patient education, related to the workplace, and the healthcare system, and factors related to patient education. An important contributor that fuels burnout is the offering of patients by medical professionals who come across neurological diseases in their practice and whose residency programs do not provide minimum knowledge. Many of these patients have functional neurological disorders which do not always require neurologist interventions. The same problem is with incidental findings on brain imaging such as minimal white matter changes with the improbability of multiple sclerosis or other significant CNS diseases. Poor medical knowledge, not understanding the limits of medicine, medical priorities, or investigations are factors of complaints between relatives. Time-consuming work, the complexity of neurological diseases, communicating a difficult diagnosis, or spending more time doing bureaucratic tasks than caring for patients, all of these are frustrating. Adequate working conditions, generous salaries, creation of functional interdisciplinary teams, filling vacancies, and introduction of telemedicine and artificial intelligence in the field of neurology, would be some solutions for attracting young doctors to this specialty.

Table 1: Significant factors contributing to burnout among neurologists and strategies for prevention

Main factors related to burnout	Proposed solutions
Factors related to the patient education	
Many referrals to neurology that do not need neurology intervention Eg: Functional neurology (often, patients with functional conditions tend to show more dissatisfaction) [22].	Educational initiatives/ online courses targeting other medical professionals that send patients and have minimal to no knowledge of common neurological diseases. Early neuroscience curricula in medical school.
The pandemic of cardiovascular risk factors: high blood pressure and cholesterol, obesity, smoking, and poor diet - put a person at high risk for neurological diseases as well.	Preventive medicine. Screening programs and campaigns to raise awareness.
Factors related to the workplace and healthcare system	
Complaints from patients/families arise from: - the poor medical knowledge - not understand the limits of medicine, medical priorities, or investigations (23)	Legal assistance for the doctors involved.
Shortage of neurologists because of not choosing the specialty [24].	Programs in medical school promote and value neurology. Adequate salary.
Neurologists choose urban over rural settings [25].	Improving rural health settings. Providing medical tools/gadgets/making technology available so the rural residents have equal opportunities to receive medical treatment.
The complexity of neurological diseases creates a job mismatch.	Development of team-based neurologic care Increased need for subspecialty medical care Eg: In Romania, there are no specialists in movement disorders or neuro-ophthalmology
Responsibility fatigue at the workplace.	The schedule of work must be dependent on the complexity of the tasks.
The neutral attitude of the hospital management board [26].	Providing support, understanding human needs, expand vacation policies.
Many rescheduling of appointments decreases physician and healthcare system efficiency overall.	Telemedicine and e-consults increase the capacity to respond to the demand for care and help patients with difficulties in coming to their clinic visits [27].
Mounds of paperwork - spending more time doing bureaucratic tasks than caring for patients. Lack of access to medical records - especially in older patients with many investigations.	Digitalization and artificial intelligence are the future of healthcare. Enabling tracking system for ordered tests/ follow-ups.
Time-consuming tasks. Eg: documenting a history of falls in Parkinson	Artificial intelligence Eg: smartwatches or other wearables that quantify falls or periods of inactivity and already provide a pattern or a conclusion.
Factors related to the physician	
Communicating a difficult diagnosis (often the first interaction occurs already at the time of critical illness).	Programs/Training courses that will help doctors improve their medical communication with patients.
The emotional burden of the specialty itself.	Providing necessary psychological assistance to guide neurologists facing mental health problems like depression or anxiety.

CONCLUSIONS

Overall, burnout can be regarded as a matter of specialty, and it has built up slowly over time, creating a vicious cycle, perpetuated by workforce dropout or choosing other medical specialties with a better lifestyle. Identification of burnout effects in neurology is policy relevant for some reasons: it affects the overall healthcare system with all its components, work-life integration (family), emotional and physical well-being, and social engagement. To conclude, it must be regarded as a worldwide issue.

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REVIEW

Prognostic Value of Serum Biomarkers in Secondary Peritonitis - A Literature Review

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Abstract: *Background: Infection and sepsis represent major complications after abdominal surgery for secondary peritonitis. Finding new tools that can help identify patients at risk for developing postoperative complications is of the utmost importance for clinicians. We tried to evaluate the use of different biomarkers in evaluating the postoperative prognosis of patients with secondary peritonitis. Methods: We searched the available literature on the usefulness of serum biomarkers in evaluating prognosis in patients with secondary peritonitis. Results: Elevated postoperative lactate levels and high procalcitonin (PCT) levels were associated with negative outcomes. C-reactive protein values increase rapidly in response to the surgical insult but have no value in assessing the overall prognosis. Conclusion: Procalcitonin (PCT) is superior to other biomarkers in predicting severe septic complications and overall mortality in patients with secondary peritonitis.*

Keywords: *biomarkers, peritonitis, C-reactive protein, lactate, procalcitonin, presepsin*

INTRODUCTION

Secondary peritonitis is a condition caused by the spread of bacteria and contents of intraabdominal organs into the peritoneal cavity [1] and is the second leading cause of sepsis in intensive care units worldwide [2]. Overall mortality is 6%, but mortality rises to 35% in patients who develop severe sepsis [2]. Secondary peritonitis requires immediate surgical intervention but after the initial procedure, persisting or new-onset abdominal sepsis continues to be a major problem in the postoperative course [3].

Using clinical symptoms such as fever, tachycardia, tachypnea, non-specific laboratory findings, and scoring systems are valuable tools to assess overall severity and prognosis but will not allow a differentiation between an infectious and non-infectious etiology of a systemic inflammatory response. The clinical assessment of sepsis biomarkers has gained considerable interest in identifying patients at risk for severe complications following surgical intervention for secondary peritonitis [1].

This review aimed to identify the most important biomarkers evaluated in patients with secondary peritonitis and their association with prognosis.

MATERIALS AND METHODS

We searched in three databases using the keywords „peritonitis“ and „prognosis“ in the title or abstract AND specific biomarkers by full name and abbreviated form in full text: lactate, C-reactive protein (CRP), procalcitonin (PCT), presepsin.

Due to the scarcity of the results, a broader search was conducted using the keywords „peritonitis“ AND „biomarker“ to ensure that all relevant literature is covered. The search was last updated on 24/06/2022. A total of 361 publications were primarily retrieved in PubMed, Embase, and Web of Science. No articles were added after manually searching major scientific journals and other references. After excluding 80 duplicates, 281 titles were screened for eligibility and 43 articles were assessed for full-text evaluation. Only English-written human studies were accepted and most articles were excluded due to irrelevancy. We focused only on circulating biomarkers for prognosis in secondary peritonitis. No cut-off point was set for the date of publishing due to the paucity of articles. At the end of the process, seven articles were included in the review. The search strategy is detailed in Figure 1.

Figure 1: Search strategy – identification of studies via databases and registers

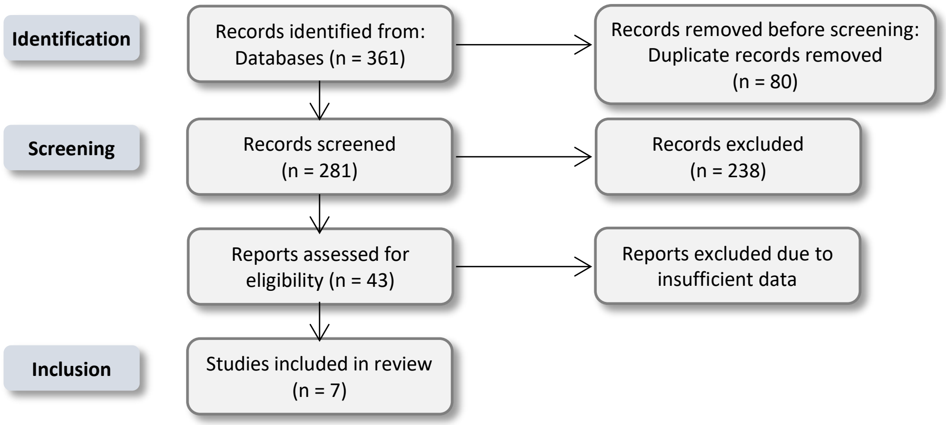
Identification
Records identified from: Databases (n = 361)
Records removed before screening: Duplicate records removed (n = 80)
Screening
Records screened (n = 281)
Records excluded (n = 238)
Reports assessed for eligibility (n = 43)
Reports excluded due to insufficient data
Inclusion
Studies included in review (n = 7)

OVERVIEW OF THE SELECTED ARTICLES

Seven articles were included in the current review. Four studies were prospective and three were retrospective, and one study included a control group. Biomarkers of interest were lactate, procalcitonin, and C-reactive protein. No study evaluating presepsin was identified. All studies focused on mortality and severity of septic shock induced by secondary peritonitis.

Further details on the selected studies are summarized in Table 1.

SERUM BIOMARKERS CORRELATED WITH PROGNOSIS IN SECONDARY PERITONITIS

Lactate

Lactate is the metabolic end product of anaerobic glycolysis. Approximately 1500 mmol of lactate is produced daily in all tissues, but mostly in skeletal muscle, brain, intestine, and red blood cells [6,9]. The concentration of arterial lactate is a reflection of the delicate balance between net production and clearance. Disturbances in lactate production, clearance, or both lead to hyperlactatemia and it is seen in a variety of conditions such as sepsis, shock, cardiac arrest, tissue hypoxia, burns, or after some pharmacological agents [6].

The prognostic value of lactate in patients with secondary peritonitis was evaluated in four studies. Negi et al. conducted a prospective study to evaluate the utility of arterial lactate measured on admission (AL1) and 24h after surgery (AL24) as well as lactate clearance as prognostic indicators for mortality in patients with secondary peritonitis. The study concluded that mean values of initial lactate, postoperative lactate, and percentage lactate clearance correlated significantly with higher morbidity and mortality [6]. Of these three lactate indices, postoperative lactate (AL24) was the most accurate in predicting morbidity and mortality [6]. Elevated lactate levels persisting postoperatively probably indicate irreversible pathophysiological changes and global tissue hypoperfusion, indicative of poor lactate clearance and higher morbidity and mortality. Andreevich et al., after conducting a retrospective study, concluded that increased lactate levels both before and 72h post-operatively were associated with a negative prognostic[8]. In the study conducted by Suarez-de-la-Rica et al., 24h postoperative lactate concentrations predicted mortality in the Surgical Critical Care Units, but overall mortality was predicted better by concurrent high procalcitonin and lactate peak concentrations [7].

Jung et al. conducted a retrospective study that aimed to compare the performance of the qSOFA score alone with a combined score derived from qSOFA and serum lactate levels for predicting in-hospital mortality for patients with secondary peritonitis. The study results showed that the qSOFA score alone has poor sensitivity and a modest predictive performance in patients with secondary peritonitis [5]. The addition of one point for hyperlactatemia (plasma lactate level > 2.0 mmol/L) resulted in improved sensitivity (5). The use of the qSOFA + lactate score can be useful to assist clinicians in immediately assessing the risk of mortality in patients with secondary peritonitis [5].

Most studies agreed that elevated post-operative lactate levels correlate better with negative outcomes than preoperative lactate levels.

Procalcitonin

Procalcitonin (PCT) is the 116-amino acid pro-peptide of the biologically active hormone calcitonin [10]. The clinical value of PCT assessment in infection and sepsis has been investigated in almost every medical discipline so far. Most studies have shown that, in general, only in the presence of systemic host response to bacterial or fungal stimuli, does PCT concentration rise above the reference range of 0.5 ng/mL. However, new data demonstrated an early production of PCT related to tissue damage after trauma and the magnitude of the hypovolemic challenge, yet unrelated to infection (4, 10). We analyzed four studies on the usefulness of PCT measurements in predicting negative outcomes in patients with secondary peritonitis. In 246 patients with intraoperatively proven secondary peritonitis of various origins, Reith et al. showed that PCT is a sensitive parameter for the prediction of severe septic complications. Moreover, the study demonstrated that PCT reflects the effectiveness of the surgical procedure in eliminating the septic intra-abdominal focus in the postoperative course (4, 10). PCT was of excellent prognostic value and achieved a sensitivity of 84% and a specificity of 91% in discriminating survivors from non-survivors within the first four postoperative days [4]. Similar observations have been reported in a study conducted by Rau et al. on 82 patients with intraoperatively proven secondary peritonitis. Pro-calcitonin values were elevated in patients who developed septic multiple organ dysfunction syndromes (MODS) with peak levels on the third and fourth days after the onset of symptoms. Comparing the preoperative and postoperative courses, PCT concentrations were significantly elevated in patients with septic MODS during the immediate postoperative course. A persistent procalcitonin elevation beyond the first week after the onset of symptoms was evident in non-survivors, whereas normal concentrations were measured in survivors [3].

Table 1: Studies focused on mortality and severity of septic shock induced by secondary peritonitis

Author	Study design	Biomarker	No. of patients	Correlation with prognosis
Akcay et al (2014)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	84	Serum PCT levels follow a falling trend after surgery as long as the postoperative course remains uneventful. PCT was a better marker than CRP in following postoperative complications and overall prognosis.
Reith et al (2000)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	246	A reduction in the PCT concentration correlated with improvement in the infection and sepsis and was found in all surviving patients. Compared with the values for CRP, PCT showed significant differences between survivors and patients who died, while CRP did not [4].
Rau et al (2007)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	82	The cumulative analysis of all PCT and CRP values measured in the first, second, and third to fourth weeks identified persisting PCT levels greater than 1 ng/mL as an excellent means to assess the overall outcome. Irrespective of the interval, PCT was significantly better than CRP in assessing overall prognosis [3].
Jung et al (2018)	retrospective	lactate	457	The qSOFA score alone has poor sensitivity and a modest predictive performance in patients with secondary peritonitis. The addition of one point for hyperlactatemia (plasma lactate level > 2.0 mmol/L) resulted in improved sensitivity. The use of the qSOFA + lactate score will assist clinicians in immediately assessing the risk of mortality in patients with secondary peritonitis [5].
Negi et al (2020)	prospective	lactate	224	Mean values of initial lactate, postoperative lactate, and percentage lactate clearance were found to correlate significantly with higher morbidity and mortality in patients with secondary peritonitis. Of these three lactate indices, postoperative lactate was the most accurate in predicting morbidity and mortality [6].
Suarez-de-la-Rica et al (2014)	retrospective	lactate, C-reactive protein (CRP), procalcitonin (PCT)	221	Both PCT and lactate provided early prognostic information, more accurate than clinical scores, for overall mortality [7].
Andreevich et al (2021)	retrospective	lactate, C-reactive protein (CRP)	136	Increased lactate and CRP values both before and 72 h after surgery were associated with negative prognostic. PCT value before surgery was insignificant and only an increase in PCT 72h after surgery was associated with a negative outcome [8].

In a prospective study conducted on 84 patients, Akcay et al. were able to demonstrate that serum PCT levels follow a falling trend after surgery as long as the postoperative course remains uneventful. In addition, they showed that after the surgical intervention PCT was a better marker than CRP in following postoperative complications and overall prognosis in patients with secondary peritonitis [1].

Suarez-de-la-Rica et al. compared the prognostic value of lactate, procalcitonin, and c-reactive protein. Peak lactate (>1,8 mmol/L) and PCT (>100 ng/mL) concentrations were associated with mortality, but peak PCT level was the best biomarker for overall mortality. In this study, concomitant high peak lactate and peak PCT concentrations considered as a single dummy variable were highly predictive of overall mortality [7].

C-Reactive Protein

C-reactive protein (CRP), named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae, was the first acute-phase protein to be described [11]. CRP is an evolutionarily conserved protein. From arthropods to humans, CRP has been found in every organism where its presence has been sought [12].

C-reactive protein is a useful marker of systemic inflammation without discriminating infection from other inflammatory processes [7]. When it comes to the value of CRP in evaluating prognosis in patients with secondary peritonitis, after searching the available literature, we found five studies that included this biomarker. All studies agreed that measured CRP values, which increase rapidly in response to the surgical insult, had no value in assessing overall prognosis. C-reactive protein did not reveal any differences between patients with or without septic MODS or in non-survivors and survivors in the perioperative course [3].

CONCLUSIONS

Although a multitude of biomarkers is elevated in patients with secondary peritonitis in the perioperative course, only a few are reliable parameters for assessing the course and prognosis in this category of patients. Many studies have clearly shown that procalcitonin (PCT) is superior to other biomarkers in predicting severe septic complications and overall mortality. In combination with careful clinical patient assessment, PCT offers distinct advantages for the evaluation of response to treatment and prediction of outcome in patients with secondary peritonitis. In regards to the fact that procalcitonin is a well-known „sepsis" parameter, this may not embrace the real properties of PCT, which remain to be defined.

Further studies must be conducted to obtain more knowledge about the importance of serum biomarkers in patients with secondary peritonitis and their correlation with prognosis.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Etiology of Chronic Kidney Disease and Comorbidities in Chronic Hemodialysis Patients

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Abstract: *Introduction: As general population tends to have increasing life expectancy, the risk associated with developing chronic kidney disease (CKD) with multiple incapacitating concequences, also increases. Method: For the present study, we registered the data from the observation files of 37 patients diagnosed with CKD undergoing treatment by chronic hemodialysis and noted the CKD associated diagnoses included in the notion of comorbidities. We monitored their statistical incidence both in the whole group and separately, in women and men using TTEST and CORREL. Results: The median age of the subjects was 55.86 (± 12.00) years. The study population mean weight was 74.90 (± 14.44) kg, with a mean weight of 69.33 kg for female subjects, and 77.92 kg for males, respectively. Diabetes was identified in 35.13% of patients, whilst heart failure was present in 16.21% of patients. Conclusions: Following the analysis of the information about the patients with CKD in the dialysis program, which we included in the study group, we observed the existence of variations that occur with age, significant correlations between age and weight and between albuminemia and weight. The most common comorbidity is high blood pressure followed by anemia.*

Keywords: *chronic kidney disease; chronic hemodialysis patients; comorbidities; plasma albumin*

INTRODUCTION

Chronic kidney disease (CKD) can develop due to a multitude of pathologies that all have in common the evolution to kidney failure, with gradual or rapid loss of the main function of the kidneys, that is to remove waste products and excess fluid from the body [1]. The last stage of the development of CKD, especially in patients who are treated by peritoneal dialysis or hemodialysis, is characterized by multiple organs and systems damage [2].

Not only CKD, but also the treatment of its various manifestations, mainly affect the body tissues and systems, at all levels: nervous, cardiovascular, respiratory, endocrine, hematopoietic, digestive, renourinary [2].

The tendency to increase life expectancy of the population is also associated with an increase of the incidence and prevalence of CKD, considering the already known relationship between aging and decreased kidney function, which is seen as a consequence of the physiological aging of the kidneys [2,3]. An even steeper increase in the number of patients with CKD is expected in the coming years. In this context, the medical goal becomes to slow down the progression to terminal stages, achievable primarily by diagnosing the condition as early as possible [1].

Compared to conservative treatment, extrarenal treatment methods provided clear advantages for patients with chronic end-stage renal disease, the duration and quality of life being considerably superior. There are several methods of extrarenal purrification: peritoneal dialysis, hemodialysis, hemodiafiltration. The decision for each type of treatment is based primarily on medical considerations, depending on the underlying disease and the comorbidities present [4]. Diabetic patients are usually at higher risk of developing more severe forms of chronic diseases (e.g. cancer) [5,6,7]. Peritoneal dialysis is preferred for diabetic patients with CKD as this method of treatment ensures the maintenance of a residual diuresis for a longer time, the prevention of complications due to the present vascular damage, the possibility of intraperitoneal administration of insulin and a better hemodynamic stability than chronic hemodialysis. In cases where the patient has a poor state of hygiene or if peritoneal dialysis does not allow a satisfactory volume control to be maintained, chronic hemodialysis is preferred [4].

MATERIALS AND METHODS

For the study we performed, we registered the data from the observation files of 37 patients diagnosed with CKD undergoing treatment by chronic hemodialysis, in the Nephrology Department of the Bucharest University Emergency Hospital.

We noted their general data and the presence of associated comorbidities, as well as the determined values of plasma albumin concentration.

The statistical data processing regarding the incidence of comorbidities revealed important information for understanding the clinical and biochemical context of this particular type of patient in the hemodialysis department.

Among the tests performed on the patients that had presented for chronic dialysis, we noted the value of plasma albumin concentration, because it is a parameter that provides information about the loss of protein in the urine and the nutritional status of renal patients. We determined the statistical differences between the values from women and men, as well as the correlations between this parameter and age, respectively weight.

We performed the statistical processing using the Student test (t-test) to determine the statistical significance of the differences in the values of the parameters determined in women and men and the Correl test to determine whether there are correlations with statistical significance between the parameters we tracked.

We noted the diagnoses associated with CKD, included in the notion of comorbidities and we monitored their statistical incidence both in the whole group and separately, in women and men.

RESULTS

In terms of gender distribution, of the 37 patients, 13 (35.13%) were women and 24 (64.86%) were men.

The median age of the subjects was 55.86 (± 12.00) years. For the women, the median age was 56.46 (± 10.86) years, whereas for the men it 55.54 (± 12.78) years. Although there is a slight gender difference regarding age, this failed to be statistically significant (p> 0.05).

Regarding weight, as presented in Table 1, the study population mean weight was 74.90 (± 14.44) kg, with a mean weight of 69.33 kg for female subjects, and 77.92 kg for males, respectively. As presented in Table 1, this difference was found to be statistically significant, p = 0.01.

Table 1: Average weight and the statistical difference between men and women

	Average weight in women (kg)	Average weight in men (kg)
Average value	56.46	52.27
Standard Deviation	10.87	17.16
p (t-test) 0.43		

Applying the Correl test, we observed that age and weight are two parameters that correlate statistically significantly (p = 0.04) (Figure 1).

Figure 1: Significant correlation between the values of weight and age

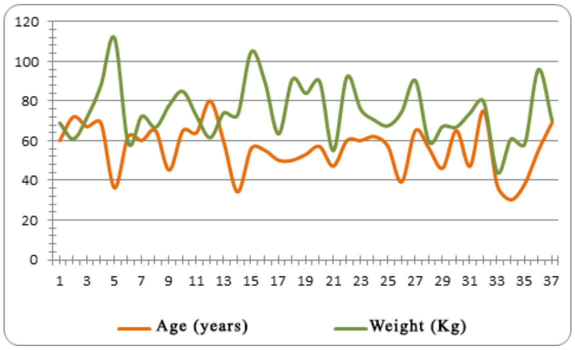

For the 37 patients, we noted the value of the plasma albumin concentration and determined the difference in values according to the gender of the patients and the statistical significance of this difference, using the Student test (t-test) (Table 2).

It is observed that the albuminemia values that were determined in these patients are not outside the normal laboratory limits of 3.5-5.2 g/dl (Table 2). By applying the

Correl test, we calculated the statistical significance of the correlations of albuminemia with age and weight (Table 3).

Table 2: Statistical significance of differences in plasma albumin concentrations in patients in the group by sex

Albuminemia(g/dl)		
	Women	Men
Average value	4.10	4.25
Standard deviation	0.24	0.27
t-test	p = 0.41	

Table 3: Statistical significance of correlations between plasma albumin concentrations with age and weight in patients in the group and by sex

Significance of the correlation between plasma albumin concentration and		
	Age	Weight
Women	p = 0.1	p = -0.2
Men	p = -0.37	p = 0.1
Total	p = -0.24	p = 0.02

Figure 2: Correlation weight-albuminemia

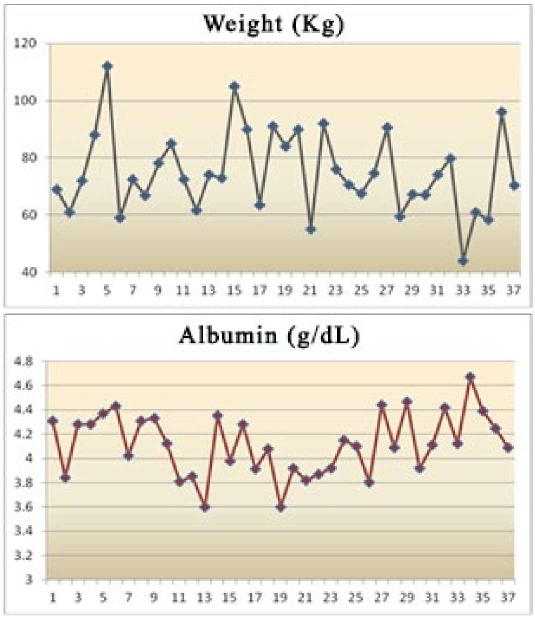

We observed that separately, in women and men, the plasma concentrations of albumin do not correlate significantly with age or weight, but, after using the Correl test for the whole study group, a significant correlation between albuminemia and weight has been identified.

We made this determination, knowing that the albumin dosage is a test to assess the general state of nutrition, especially in elderly patients with various conditions, in this category falling most of the patients with CKD in the lot we studied.

COMORBIDITIES DIAGNOSED IN THE STUDIED GROUP

Diabetes mellitus, dyslipidemia, atherosclerosis, hypertension, arteriopathy obliterans of the lower limbs

Diabetes is present in 35.13% of patients, 3 of women and 10 of men, which means 23.07% and 41.66%, respectively. Dyslipidemia occurs in a number of 3 male patients, which means a percentage of 8.1% of the total group. Atherosclerosis is noted in a female patient with kidney disease and in 9 male patients, the incidence rate being 27.02% in the whole group. High blood pressure is a more common comorbidity in patients in the study group: 11 out of 13 women are hypertensive and 23 out of 24 men with CKD are hypertensive. Lower limb arteriopathy is present in only 2 of the male patients, which means 5.4% of the group. Both patients also have diabetes and high blood pressure.

Heart failure, cardiomyopathy, arrithmia, valvulopathy, chronic coronary heart disease

Heart failure is present in 5 of the women and only 1 of the men, this comorbidity is therefore found in 16.21% of patients. Cardiomyopathy is rarer, 1 female patient and 2 of the men in the group, ie 8.1% also have this diagnosis. Arrhythmias are more common in women, 5 out of 14, ie 35.7%, presenting this manifestation in the form of atrial fibrillation with medium or large ventricular allure or in the form of supraventricular tachycardia. Among men, only 2.33% have atrial fibrillation with a medium ventricular allure. Thus, 18.91% of the total group have a form of arrhythmia. Valvular damage is identified in 4 patients out of a total of 37, ie 10.81%, more frequently in women 3 out of 14 and only in 1 male patient. Coronary ischemic disease is diagnosed in 3 women and 6 men, the incidence being 24.32%.

Diabetic/hypertensive/mixed nephropathy, chronic tubule-interstitial nephropathy, autosomal dominant polycystic kidney disease, single kidney, chronic pyelonephritis, chronic glomerulonephritis

Chronic tubulointerstitial nephropathy is diagnosed in a single male patient. The incidence at group level is 2.7%. Out of 13 women in the group, hypertensive nephropathy is present in 2 and diabetic nephropathy in 3 of them, a total of 38.46% with nephropathy. Of the 24 men in the group, 6 have diabetic nephropathy, 4 have hypertensive nephropathy and 2 have mixed nephropathy, a total of 50% of patients with nephropathy. A number of 4 of the 13 women, 30.76%, are also diagnosed with autosomal dominant polycystic kidney disease. Of the 24 men, 4 also have this diagnosis, representing

16.66%. At the level of the whole group, the percentage is 21.62%. Only one male patient has a single kidney, the cause being congenital. The incidence at group level is 2.7%. And pyelonephritis rarely occurs, only one patient, a woman, has this diagnosis. The incidence at group level is 2.7%. More common is chronic glomerulonephritis, which is present in 6 of the men, respectively 25%, and in 2 of the women, respectively 15.38%, with an incidence in the whole group of 21.62%.

Kidney stones, parathyroidectomy, secondary hyperparathyroidism, anemia, chronic viral hepatitis

Kidney stones are present in 2 women and 3 men in the group, which means an incidence of 13.51%. Secondary hyperparathyroidism is diagnosed in only 2 patients, a man and a woman, the incidence is 5.4% in the whole group. Surgical removal of the parathyroid glands was performed in 3 women and 1 man in the group, the frequency being 10.81%. Anemia is more common, 7 of the 13 women, ie 53.8% and 15 of the 24 men, ie 62.5%, have pathologically low hemoglobin. Out of the whole lot 59.45% of patients have anemia. Chronic hepatitis B infection is uncommon as viral hepatitis B is present in 2 women, 1 man in the group is infected with hepatitis B virus and 1 is coinfected with hepatitis B + C viruses. The frequency of chronic viral hepatitis in the group is 10.81%.

DISCUSSIONS

Gender and age are factors that influence some characteristics related to CKD in patients with type 1 or type 2 diabetes, and certain particular aspects of pathogenesis, diagnosis and treatment management have been identified, but the mechanisms are incompletely known and more studies are needed to clarify these connections which would be very useful in order to reduce the number of cases of renal patients and to prevent or control the complications of this condition. Considering the data published by Eriksen O. et al. [5], old age and the male gender are predictors of worsening kidney disease and the rapid progression to severe kidney failure. In the study of Giandalia A et al. [8] the authors concluded that men are more affected, because in their studies, the number of men enrolled is higher than the number of women, results similar to our study where the percentage of men was 64.86%.

Weight is an important parameter in assessing the nutritional status of the patient with CKD. In obese people with CKD, the progression of the disease to renal failure is faster compared to cases of normal weight. Also kidney transplantation or hemodialysis, which may become necessary in advanced stages of the disease, may be more difficult and may have more frequent complications in obese people [9]. After initiating treatment with hemodialysis or peritoneal dialysis, in patients with a glomerular filtration rate below 35 ml/min/1.73 m^2, in stage 3 CKD, weight loss is a factor that increases the risk of mortality by 54% compared to with patients whose weight remains constant. Even in patients with moderate renal impairment, weight loss should be closely monitored and the patient's nutritional status monitored before dialysis is required [10].

In the final stages of the kidney disease, hypoalbuminemia is common, caused by factors that act simultaneously: reduced liver synthesis, increased degradation and renal loss and the glomerular filter membrane is damaged and becomes more permeable allowing the passage of negative albumin molecules from plasma. In our study we observed that separately, in women and men, the plasma concentrations of albumin do not correlate significantly with age or weight. However, applying the Correl test to the whole group, resulted in a significant correlation between albuminemia and weight. We made this determination, knowing that the albumin dosage is a test to assess the general state of nutrition, especially in elderly patients with various conditions, in this category falling most of the patients with CKD in the lot we studied. According to Haller C. et al. [11] the inflammatory phenomenon in kidney disease is also involved in the pathogenesis of hypoalbuminemia. Inflammation correlates with the risk of mortality, hypoalbuminemia is not in itself a pathogenic factor of renal impairment, but is a major predictor of unfavorable prognosis.

Diabetes mellitus is one of the main negative prognostic factors for chronic diseases [12]. According to Anders HJ. et al. [13] the prevalence of type 2 diabetes and CKD increases the incidence of diabetic kidney disease, also known as diabetic nephropathy. The combination of the two diseases can be in the form of diabetic kidney disease if the kidney damage is the consequence of diabetes, it can be a kidney disease caused by individual mechanism in the diabetic patient or it can be a coincidence of simultaneous occurrence of the two diseases. The involvement of the membrane-glucose cotransporter membrane and hyperglycemia are associated with the activation of the renin-angiotensin-aldosterone system, glomerular hyperfiltration and tubular hyperabsorption occur, which creates a particular situation that requires appropriate treatment. Whatever the mechanism, the patient's evolution is more unfavorable compared to those who have only one of the conditions [13,14]. Our study results for 35,13% of the patients, that were diagnosed with diabetus mellitus type II, found that 69.23% of them have further developed diabetic nefropathy.

In the study of Tsimihodimos V et al. [15] it was shown that lipid metabolism is disrupted in patients with CKD even in the early stages and can be very severe in some cases, further impairing kidney function. The pathogenic mechanisms are not fully understood, but studies show that dyslipidemia is a pathogenic factor for both kidney disease and the associated cardiovascular disease [16]. In our study dyslipidemia was identified in only 8,1% of the patients.

Atherosclerosis sets in early and progresses rapidly in patients with CKD. It usually increases the risk of cardiovascular disease and, as the severity of kidney disease increases, so does the mortality rate, according to Olechnowicz-Tietz S. et al. [17]. In our study population 27.02% were found to suffer from atherosclerosis.

Considering the cardiovascular risk, compared to the general population, patients with CKD, according to the study of Matsushita K et al. [16] have a constant increased cardiovascular events risk. Studies identified that 70% of CKD patients, and up to 80-90% of those on dialysis, are diagnosed with high blood pressure [18,19]. Considering all these data, our studies results that 91.9% of CKD subjects were suffering of/diagnosed with hypertensive disease are consistent with the data in the literature.

As shown in the study of Maric-Bilkan C. et al. [20], in the absence of diabetes, women have a considerably lower risk than men of vascular, microvascular or macrovascular diseases. The opposite trend is observed in diabetic patients: women are at higher risk for these complications. Microvascular lesions that occur in nephropathy, neuropathy, and retinopathy are more common in men. Macrovascular lesions consisting of chronic coronary heart disease, myocardial infarction, peripheral artery damage, and stroke are more common in women. To explain this, the focus has been on sex steroids, which play an important role in regulating heart function. Estrogens are generally considered hormones with cardioprotective effects, and androgens with adverse cardiac effects. But the effects of these hormones on the target organs are complex and different, and the presence of diabetes interferes with the pathophysiological mechanisms that cause vascular damage [14,18,19].

Renal comorbidities are, in part, primary kidney disease that has progressed unfavorably, with the patient reaching the stage of CKD that requires dialysis in order to survive [20]. Another part of these comorbidities is secondary, the pre-existing disease being another. This is the case with nephropathy, which develops during the course of hypertensive disease or diabetes [14]. The percentage of incidence of nephropathy of any etiology in the study population was 45.94%. It is a high percentage, which is explained by the fact that, as we have shown above, diabetes and hypertension are precursors or caused by CKD.

Our results are consistent with literature data showing that 11.2% of patients in a large group of with CKD present with kidney stones. The same study by Chuang TF. et al [21] shows that patients with kidney stones are 1.82 times more likely to develop CKD.

Secondary hyperparathyroidism is found in chronic dialysis patients, the incidence of this comorbidity being reduced by treatments with phosphorus chelators, calcium supplements and calcitriol. As shown in the study of Llach F. et al [22] there are situations when parathyroidectomy is necessary, namely when clinical signs such as persistent hypercalcemia, pruritus, metastatic calcifications appear [23]. In our study hyperparathyroidism was found in only 5,4% of the patients but out of the whole lot, 10.81% of the patients had already been treated with parathyroidectomy. The latter can cause insufficiency of many organs.

Blood characteristics such as blood group or different blood components (eg. lymphocytes) have been linked to chronic diseases or their treatments toxicity [24-27]. According to the study of Ueda N. et al. [28] hemoglobin levels below 12 g/dl for women and below 13 g/dl for men are considered anemia. Iron and erythropoietin deficiency causes anemia in patients with CKD. Hepcidin is a hormone produced by the liver in inflammation or when the body's iron deposits in the form of ferritin or hemosiderin are saturated. Hepcidin binds to and inhibits ferroportin, thereby decreasing the release of iron from the enterocyte and reticuloendothelial cells and decreases plasma iron. Inflammatory status in CKD increases hepcidin regardless of the availability of iron to the body, so it becomes necessary to administer iron, but also to reduce inflammatory phenomena [29]. It was a frequent occurence in our study as 62.5% of our patients were diagnosed with anemia.

Hepatitis B or C virus infections can cause chronic kidney damage but do not have a high incidence according to Chacko EC et al. [30]. If the infection occurs in patients with CKD, anti-infective treatment has many side effects and may worsens the prognosis. Similar results were shown in our study as only 10.81% of the patients presented with concurrent viral hepatitis and CKD.

CONCLUSIONS

Following the analysis of the information about the patients with CKD in the dialysis program from our study weight has been found to be significantly higher in men, compared to women. Albuminemia was within normal limits in all patients, significantly correlating with the body weight. Patients' comorbidities identified in our study were similar to the ones found in the current literature to be related to CKD. The most common comorbidity is high blood pressure, identified in over 90% of the subjects. Anemia is the second most common comorbidity, occurring in almost 60% of patients. With high frequency, in over 20% of patients, we observed the presence of diabetes, atherosclerosis, ischemic coronary heart disease, nephropathy of various etiologies, autosomal dominant polycystic disease and chronic glomerulonephritis.

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Review

The Role of Coronary CT Angiography in the Management of Patients with Coronary Atherosclerotic Disease

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Abstract: *Coronary CT angiography is a non-invasive method of analyzing the coronary lumen through which the atheromatous bur-den can be evaluated, with the analysis of the type of plaque (soft, calcified, mixed) and the impact on the arterial lumen (stenosis, occlusion). The anatomical evaluation by coronary CT is indicated in symptomatic patients with low and medium risk factors for coronary atherosclerotic disease, in those with inconclusive laboratory and EKG results, patients with un-certain stress test results, in the evaluation of coronary grafts and intrastent stenoses. Depending on the result of the angiography, the severity of the stenosis and the management of the patient are established. In the case of mild and medium stenoses, risk factors management and drug treatment are recommended. For severe stenoses, patients are referred for interventional coronary angiography or functional evaluation. Coronary CT angiography increases the certainty of coronary atherosclerotic disease diagnosis. It has a superior discriminative capacity of plaques with low attenuation, the main predictor of myocardial infarction, with almost five-fold higher risk. Coronary CT angiography increases the adaptation of medication, and the inclusion of statin therapy decreases the risk of fatal and nonfatal myocardial infarction at 5 years and increases the quality of life.*

Keywords: *coronary CT angiography, coronary atherosclerotic disease, calcium score, CAD-RADS*

INTRODUCTION

Atherosclerotic disease is the main cause of death in Europe [1]. Lifestyle factors (smoking, diet, level of physical activity) but also biological risk factors (hypertension, hypercholesterolemia, hyperglycemia) contribute to this [2].

The management of patients with coronary atherosclerotic disease is complex, including numerous investigative methods. Of these, coronary CT angiography has been analyzed more and more, over the last decade, due to technological progress. Coronary CT angiography is a non-invasive method of evaluating the heart and coronary arteries. Coronary CT angiography is performed using a computer tomograph that involves high-speed scans for moving organs and contrast material containing iodine, administered in injectable intravenous form to obtain the best images of the coronary arteries and the heart. Thus, the atheroma plaques in the coronary arteries can be identified, the type of plaques analyzed (soft, calcified, mixed), and the consequences on the arterial lumen (stenoses, occlusions) evaluated. Coronary anomalies can be detected, valve morphology can be analyzed, as well as the degree of myocardial perfusion.

MATERIALS AND METHODS

The main reference studies used in evaluating and managing patients with coronary atherosclerotic disease are extensive studies that have contributed to the current state of knowledge in this field, demonstrating the usefulness of the investigation in diagnosis, monitoring, and recommendations for the treatment of the disease.

The PROMISE study (Prospective Multicentre Imaging Study for Evaluation of Chest Pain) – from the year 2017 – showed that coronary CT angiography is a superior means of evaluation for identifying patients at risk with nonobstructive coronary atherosclerotic disease, compared to classical functional exploration means, in patients with stable angina pectoris and with an average probability of coronary atherosclerotic disease. The patients were randomly divided into two groups, 4500 patients were investigated by coronary CT angiography and 4602 patients were evaluated by functional means (exercise electrocardiogram, exercise ultrasound, and exercise myocardial scintigraphy). The patients were subsequently monitored for 26 months. The significantly lower incidence of coronary events in patients investigated by coronary CT angiography compared to those evaluated by functional means (33.4% versus 78.0%) demonstrates that coronary CT angiography had a significantly higher discriminative capacity for predicting coronary events than functional explorations (c -index, 0.72; 95% CI, 0.68-0.76 versus 0.64; 95% CI, 0.59-0.69; P=0.04) [3].

The SCOT HEART study (Scottish Computer Tomography of the Heart) is large, started in 2010, and is still ongoing, which will continue until 2027, with some of the results already published. It is a randomized parallel study, which included, between 2010-2014, 9848 participants with angina pectoris suggestive of coronary atherosclerotic disease and with mild and medium risk factors. The patients were divided into two groups that were evaluated differently: 4146 patients by coronary CT angiography and the rest were managed by standard anatomical and functional means. The main diagnostic results showed that coronary CT angiography increased the certainty of the diagnosis of coronary atherosclerotic disease (relative risk [RR] = 1.79, 95% confidence interval [CI] 1.62-1.96, p < 0.001) and reduced the frequency of the diagnosis of angina due coronary atherosclerotic disease (RR = 0.93, 95% CI 0.85-1.02, p = 0.13) compared to standard care [4]. The main clinical result was a decrease in the risk of fatal and nonfatal myocardial infarction at 5 years, which was 2.3% in the coronary CT angiography group, compared to 3.9% in the group managed by standard means. The secondary results consisted of an increase in the quality of life, the adaptation of medication, and the inclusion of statin therapy. In those cases where the lesions were severe, patients were referred for angiography and stenting [5].

Other secondary, very important results emerged in 1769 patients, followed for 4-5.7 years, which proved that the presence of plaques with low attenuation is the main predictor of myocardial infarction, independent of the presence of cardiovascular disease risk factors, of the score of calcium or area of stenosis. Patients with low-attenuation plaques had an almost 5-fold higher risk of myocardial infarction (95% CI, 2.06-10.5; P<0.001). In 41 patients who suffered an acute myocardial infarction, both low-attenuation plaque load and calcium score were increased (336 [62-1064] versus 19 [0-217] Agatston units; P<0.001), and the presence of obstructive coronary artery disease (54% versus 25%; P<0.001) [6].

The UK's National Institute for Health and Care Excellence's 2016 NICE chest pain guideline recommended coronary CT angiography as the first line of evaluation in patients with new stable chest pain. Emphasis is placed on anatomic explorations over functional ones, a choice to which cost-effectiveness also contributes [7,8].

However, this approach raised concerns that, after the first evaluation with coronary CT angiography, there would be an increased referral to further investigations, such as invasive coronary angiography. To test this, a retrospective study was conducted on 652 patients who underwent coronary CT angiography from 2017 to 2018. The results showed that 92 patients had moderate (50-70%) or severe stenosis (>70 %). Of these, 69 were directly referred for invasive coronary angiography, of which 63 underwent the investigation, and severe atherosclerotic coronary disease was confirmed in 40 patients (63%). Among the patients with moderate stenosis, 18 were referred to cardiac stress echography, which was positive in one case. The remaining patients had mild stenosis in 62 cases, 36 studies were inconclusive, and the rest of them (462 patients) were normal or had minimal stenosis [9]. The study's conclusions showed that coronary CT angiography is an effective first-line method in the evaluation of patients with newly appeared stable angina pectoris, most of them having normal results or minimal coronary atherosclerotic disease. In patients with severe stenosis, it had an increased detective capacity, the stenosis being confirmed by invasive coronary angiography in 63% of cases.

The positive predictive value for coronary stenosis of coronary CT angiography, compared with stress tests, was evaluated and published in a study from 2019. A total of 800 patients with stable angina pectoris from two centers were included, one in the UK where 400 patients were investigated with coronary CT angiography and a center in Portugal where 400 patients were investigated with exercise tests (treadmill exercise stress test EST or stress imaging test). The patients in the two groups were equally distributed in terms of gender and BMI. Of the 400 coronary CT angiographic examinations, 387 (96%) were diagnostic. Of these, 92 patients (23%) had CAD-RADS 3-5 coronary obstruction, of which 67 (72%) underwent coronary angiography and 61 patients (91%) had atherosclerotic coronary obstructive disease confirmed. In the Portuguese group, 400 patients with stable angina evaluated by stress test with the positive result, recommended for interventional angiography, were included. On interventional angiography, the obstructive coronary disease was diagnosed in 51% of patients. In conclusion, the positive predictive value of coronary CT angiography is 91%, statistically significantly higher (p<0.0001) than stress tests (51%) [10]. The study concludes that coronary CT angiography, due to its significantly higher predictive value than exercise tests, has a good indication as a first test for evaluating coronary stenosis, and could mean the avoiding of unnecessary interventional angiographic tests.

The AGATSTON coronary artery calcium score is a semi-automatic measurement of the degree of calcification of the coronary arteries. It is an investigation performed with a low dose of radiation at the beginning of the coronary CT angiographic examination, before the administration of contrast substance. The main role is an early stratification of the degree of coronary atherosclerotic infiltration.

An automatic calculation of coronary calcifications is made according to the density in Hounsfield units and the area occupied by the plaque.

Depending on the value of the total calcium score, coronary artery disease is divided into five degrees: no evidence of disease (calcium score 0), minimal damage (1-10), mild (11-100), moderate (101-400), severe (>400), the risk of major cardiac events increases in patients with an Agatston score greater than 160 (Table1) [11].

Table 1: Calcium score according to the Agatston scale

Agatston score	Coronary artery disease
0	no evidence of calcium
1-10	minimal
11-99	mild
100-399	moderate
400-999	severe
1000+	extensive calcium deposits increased risk of coronary events in the next year

The calcium score showed a good correlation with coronary CT angiography in the diagnosis of coronary plaques, with the limitation of not being able to evaluate the soft component of the plaque.

Special emphasis is given to the Agatston score of zero, there is the concept of "the power of zero", which means that patients are considered low risk and if there are no other risk factors, that leads to the avoidance of certain preventive drug therapies, such as statins. Another important role of the calcium score is personalizing the prescription of statins and aspirin among the general population but also the population with special conditions such as severe hypercholesterolemia or diabetes [12].

Coronary calcium score (CAC) has become a popular investigation due to its accessibility, speed, tolerability, low cost, reproducibility, and financial benefits due to the exclusion of complex coronary pathologies that involve more expensive investigations [13].

There are practice guidelines that include structured recommendations for the treatment and prevention of coronary atherosclerotic disease but there are some variations between countries in using coronary calcium score as a reference for inpatient treatment. However, there is a common agreement that coronary calcium scoring assessment is to begin in patients over 40 years. In asymptomatic patients with an intermediate risk of atherosclerotic coronary disease and a calcium score of over 100, the initiation of statin treatment is recommended. In patients with a calcium score of 0, the risk is decreased and the administration of statins is retained, with a repeat of the calcium score after 10 years. These conclusions were reached following the MESA study (Multi-Ethnic Study of Atherosclerosis) in which the 10-year risk of cardiac events was between 1.3-5.6% for a calcium score of 0 and 13-26.6% for a calcium score over 300. The MESA study concluded that in conditions of other cardiovascular disease risk factors maintained constant, the relative risk of cardiac events increases proportionally with the increment of the calcium score [14].

For the good management of patients with coronary atherosclerotic disease, the Guide of the European Society of Cardiology (ESC) was updated in 2019. It established coronary CT angiography as the first line of anatomical evaluation of patients with coronary atherosclerotic disease. The previous recommendations (ESC guide from 2013) proposed a combined approach between anatomical and functional means.

The current management of patients with angina pectoris and suspected obstructive coronary disease consists of a 6-step approach.

1. In the first stage, patients are evaluated through a clinical examination to identify patients with unstable angina or acute coronary syndrome, with the determination of symptoms.

2. The second step involves the evaluation of the general condition and the quality of life - patients with unstable angina or acute coronary syndrome are not included.

3. The third step includes performing some basic tests (blood tests, resting electrocardiogram, resting cardiac ultrasound) and evaluating the function of the left ventricle.

4. Step four consists of assessing the risk of clinical probability of obstructive coronary disease.

5. The fifth step involves diagnostic tests, which are chosen depending on the clinical probability of obstructive coronary disease but also multiple clinical factors, comorbidities, expertise, and access to examinations.

Thus, the anatomical evaluation by coronary CT is recommended as an initial diagnostic test in symptomatic patients in whom the coronary atherosclerotic disease cannot be excluded by clinical evaluation, in those with a low and medium probability of coronary atherosclerotic disease.

Functional tests are recommended in situations where coronary CT angiography is non-diagnostic or has shown stenoses whose functional significance is uncertain.

Invasive angiography is recommended for those with very high clinical probability, symptoms that do not respond to drug treatment, and typical angina at a low level of effort, in patients with an uncertain diagnosis on functional tests.

Coronary CT angiography is not recommended in extensive calcifications, irregular heart rhythm, significant obesity, the impossibility of performing respiratory commands, and any other situations in which qualitative images cannot be obtained.

6. The sixth step, depending on the result of the above tests, the management of the patient and the drug treatment are decided.

Severe calcifications and poor clinical images can lead to an overestimation of the severity of stenoses. In patients that present revascularization through stents and bypass grafts, the accuracy of the coronary CT angiographic examination is impaired in some cases due to the presence of blooming artifacts and the incomplete evaluation of the native vessels. Coronary CT angiography is not recommended in any situations in which qualitative images cannot be obtained: extensive calcifications, significant obesity, and the impossibility of performing respiratory commands [15].

CAD-RADS Coronary Artery Disease Reporting and Data System is a method of evaluating coronary atheromatous plaques developed by the American Cardiology Society with the main purpose of standardizing the reporting of coronary atherosclerotic disease.

Also, the method includes recommendations for patient management depending on the result of coronary CT angiography (Table 2).

Table 2: CAD Rads score for the quantification of stenoses and patient management

CAD-Rads	Stenosis	Additional test
0	0%	-
1	1-24% minimum	-
2	25-49% mild	-
3	50-70% moderate	functional evaluation
4A	70-90% in 1 or 2 vessels	functional evaluation or interventional coronary angiography
4B	>50% in the left main trunk >70% in 3 vessels	interventional coronary angiography
5	100% total occlusion	interventional coronary angiography and viability assessment
N	Non-diagnostic study	additional evaluations

Thus, stenoses are divided into 5 categories, as follows:

- CAD-RADS 0 means the absence of plaques or stenoses and implies the search for other causes of angina pectoris;
- CAD-RADS 1 are plaques without stenosis or minimal stenosis (1%-24%) and imply preventive therapy and risk factor management;
- CAD-RADS 2 includes mild stenoses (25-49%) and includes preventive therapy and risk factor management, especially in patients with non-obstructive plaques in several segments;
- CAD-RADS 3 are moderate stenoses (50-69%). Patient management consists of anti-ischemic pharmacological treatment, preventive with the treatment of risk factors;
- CAD-RADS 4 are severe stenoses, which include two sub-categories: A = severe stenoses (70-99%) and B = tri coronary stenoses (≥70%) or left common coronary trunk (>50%). For category A, interventional coronary angiography or functional evaluation is recommended and for category B, interventional coronary angiography is recommended, to which anti-ischemic drug treatment and treatment of risk factors are added;
- CAD-RADS 5 is total occlusion (100%), and interventional coronary angiography and/or viability assessment is recommended, to which anti-ischemic drug treatment and treatment of risk factors are added;
- CAD-RADS N includes non-diagnostic studies that cannot exclude obstructive coronary disease, and require additional/alternative investigation.

All vessels larger than 1.5 mm in diameter must be included in the classification.

CAD-RADS 1 category also includes plates with positive remodeling and no evidence of stenosis.

There are four modifiers of the CAD-RADS score that are mentioned in the result with the "/" symbol, these are 1 N (non-diagnostic), 2 S (stent), 3 G (graft), and 4 V (vulnerability)[16].

RESULTS

The selected studies show that coronary CT angiography increases the certainty of the diagnosis of coronary atherosclerotic disease. It can predict coronary events, decreases the risk of fatal and nonfatal myocardial infarction at 5 years, and increases the quality of life. Coronary CT angiography has a good indication for being the first test for evaluating coronary stenosis due to its higher predictive value than exercise tests, thus avoiding several unnecessary interventional angiographic tests. The management of patients with coronary atherosclerotic disease can be conducted depending on the result of coronary CT angiography. The detection of severe lesions by coronary CT angiography makes intervention possible in the next stage by angiography and angioplasty.

DISCUSSION

The indications for coronary CT angiography are numerous - from retrosternal pain, clarification of inconclusive non-invasive studies, and assessment of coronary atherosclerosis and by-passes or stents.

The management of the patient with coronary atherosclerotic disease involves a clinical examination and basic tests (blood tests, resting electrocardiogram, resting cardiac ultrasound). Anatomic evaluation by coronary CT is recommended as an initial diagnostic test in symptomatic patients with low and intermediate probability of atherosclerotic coronary artery disease. Functional testing is recommended in situations where coronary CT angiography is nondiagnostic or has revealed stenoses of uncertain functional significance. Depending on the severity of the stenosis, the management of the patient is established. For minimal and small stenoses, preventive therapy and the management of risk factors are chosen. For moderate stenoses, anti-ischemic pharmacological treatment is added, and for severe stenoses, interventional coronary angiography or functional evaluation is recommended.

Also, with the help of coronary CT angiography, other structures such as bones, soft tissues, lungs, or blood vessels can be visualized simultaneously during the scan, being useful for a possible differential diagnosis.

There are limitations of coronary CT angiography such as pregnancy or chronic renal failure, and other contraindications: cardiac arrhythmias, hyperthyroidism, and iodine allergy. In patients with revascularization through stents and bypass grafts, the accuracy of the coronary CT angiographic examination can be impaired due to blooming artifacts and incomplete evaluation of the native vessels. Coronary CT angiography is not recommended in any situations in which qualitative images cannot be obtained: extensive calcifications, significant obesity, and the impossibility of performing respiratory commands.

CONCLUSION

Numerous studies have proven coronary CT angiography as the main investigation for coronary anatomical evaluation. Coronary CT angiography provides important information about the presence and extent of coronary atheroma plaques. A major advantage is the early diagnosis of coronary atherosclerosis, based on which preventive treatment can be administered. Coronary CT angiography decreases the risk of myocardial infarction, allows drug treatment adaptation, and increases the quality of life.

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All authors have read and agreed to the published version of the manuscript.
The respective roles of each author are: ASC first/lead author performed the research, wrote the paper, and is the corresponding author, secondary authors DC and SMS supervised the paper, implemented feedback, and conceptualized some ideas.

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Original Article

Does Intravenous Immunoglobulin Therapy Have a Role in the Treatment of Severe Coronavirus-19 Patients?

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Abstract: *We wanted to evaluate the efficacy of intravenous immunoglobulin (IVIG) treatment in Covid-19 patients in the intensive care unit (ICU). Sixteen (66.7%) male and 8 (33.3%) female adult severe Covid-19 patients who were treated with IVIG in the tertiary ICU between April 2020 and October 2021 at Ankara City Hospital were retrospectively reviewed. The median age of the patients was 63 (interquartile range (IQR):19). The patients were evaluated in terms of laboratory values and clinical results before and after IVIG treatment. There was no statistically significant difference between survived and deceased Covid-19 patients treated with IVIG in terms of age, gender, the onset of symptoms, presence of concomitant diseases, APACHE-II scores, duration of mechanical ventilator support, and length of stay in the ICU. When the laboratory values before and after IVIG treatment were compared, a significant decrease was identified only in alanine transaminase levels (p=0.01). However, there was a statistically significant difference in mortality in 5 patients with Guillain-Barré syndrome who all survived (p=0.001). IVIG treatment was effective in Covid-19 patients with Guillain-Barré syndrome. However, IVIG treatment did not have a statistically significant effect on pre and post-treatment laboratory values, morbidity, and mortality of severe Covid-19 patients in the ICU.*

Keywords: *Covid-19, critical care, Guillain-Barré syndrome, Intravenous immunoglobulin*

INTRODUCTION

Coronavirus disease 2019 (Covid-19) is caused by a RNA virus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1,2]. Covid-19 can lead to asymptomatic infection, mild symptoms, or severe pulmonary symptoms with hyperinflammation [3]. In severe Covid-19 patients, management of hyperinflammation and cytokine storm with appropriate antiinflammatory agents is recommended [4]. Different anti-inflammatory agents have been tried on critically ill patients during the Covid-19 pandemic.

IVIG is a biological agent which contains pooled polyclonal immunoglobulin G from the plasma of approximately one thousand healthy blood donors [1]. IVIG is used for the treatment of immunodeficiency, autoimmune and inflammatory diseases [5]. IVIG has not only anti-inflammatory effects but also the ability to neutralize bacterial toxins [6]. IVIG treatment has also been tried in epidemic diseases related to viruses such as influenza A H1N1, Middle East Respiratory Syndrome (MERS-CoV-2), and Severe Acute Respiratory Syndrome (SARS), and it has been reported that IVIG treatment decreased viral load, caused clinical improvement and decreased mortality in patients with a severe course [7,8]. In light of experience, IVIG treatment has been recommended to be used for the management of patients with Covid-19 [1,9-13]. However, the role of IVIG treatment in patients with severe Covid-19 remains unclear. Thus, this study aimed to evaluate the efficacy of IVIG treatment administered to severe Covid-19 patients in the ICU.

MATERIALS AND METHODS

Local ethics committee approval for this study was obtained from Ankara City Hospital (E2-21-835). The electronic records of 640 Covid-19 patients who were followed up between April 2020 and October 2021 in tertiary ICU were retrospectively reviewed. Thereafter, sixteen (66.7%) male and 8 (33.3%) female adult Covid-19 patients who were treated with IVIG in the ICU between April 2020 and October 2021 at Ankara City Hospital were included in this study for further analysis. The definitive Covid-19 diagnosis was based on both real-time reverse transcription polymerase chain reaction (RT-PCR) test results and ground glass appearance with pneumonia on thorax computed tomography. These severe Covid-19 patients had also respiratory distress with peripheral capillary oxygen saturation (SPO2) ≤ 93% in room air before admission to the ICU.

IVIG treatment was administered due to sepsis in 6 (25%) patients, Guillain-Barré syndrome in 5 (20.8%) patients, hematological disorders such as hemophagocytic syndrome, chronic lymphocytic leukemia and autoimmune hemolytic anemia in 9 (37.5%) patients, rheumatologic and immunological disorders such as immunodeficiency, systemic lupus erythematosus, myositis and vasculitis in 4 (16.7%) patients. In all patients, IVIG was administered at 0.4 g/kg/day for 3 consecutive days. These 24 patients were evaluated in terms of laboratory values before the first IVIG treatment session and after the third IVIG treatment session. Thus, neutrophil, lymphocyte and platelet count, neutrophil/lymphocyte ratio, lactate dehydrogenase, d-dimer, fibrinogen, ferritin, procalcitonin, c-reactive protein (CRP), interleukin-6, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were noted. Demographic data, the onset of symptoms, concomitant diseases, APACHE II scores, duration of mechanical ventilator support, length of ICU stay, and mortality rates were also recorded.

For all statistical analysis, Statistical Packages for the Social Sciences (v17.0 SPSS 24 Inc. Chicago, IL) software was used. Categorical data were presented as numbers and percentages, while quantitative data were presented as mean standard deviation or median and interquartile range (IQR), as appropriate. The differences between the two groups were evaluated with the Mann-Whitney U test and the chi-square test. The statistical significance level was accepted as p<0.05.

RESULTS

The median age of the patients included in this study was 63 (IQR:19), and the median time to symptom onset was 6.5 days (IQR:6). The median APACHE II score of these patients was 16 (IQR:9). The median length of stay in the ICU was 22.5 (IQR:34) days, whereas the median mechanical ventilator time was 19 days (IQR:29) (Table 1). Eighteen (75%) patients had concomitant diseases such as diabetes mellitus type 2, hypertension, and coronary artery disease. The most common disease accompanying Covid-19 was hypertension in 13 (54.2%) patients. The most common symptom was dyspnea in 17 (70.8%) patients. Fever was present in 7 (29.1%) and cough in 5 (20.8%) patients. Other less frequent symptoms were headache, sore throat, nausea, vomiting, impaired taste and smell, myalgia, and weakness. Complications, which were not related to IVIG treatment, such as gastrointestinal bleeding in 2 patients, cerebrovascular accident in 1 patient, myocardial infarction in 1 patient, pneumothorax in 6 patients, and seizures in 2 patients were observed in total in 12 (50%) patients. Antibiotics were empirically administered in 15 (62.5%) of the patients. Unfortunately, 16 (66.7%) patients passed away due to complications related to Covid-19 (Table 1).

Table 1: Characteristics of the Intravenous Immunoglobulin Treated Covid-19 Patients

Characteristics of Covid-19 patients	Survived	Deceased	Total	*P* value
Gender (n, %) Male Female	 6 (75) 2 (25)	 10 (62.5) 6 (37.5)	 16 (66.7) 8 (33.3)	0.67*
Age (years) (median, IQR)	61.5 (29)	63.5 (19)	63 (19)	0.85**
The onset of symptoms (days) (median, IQR)	6.5 (3)	6.5 (6)	6.5 (6)	0.60**
Length of ICU stay (days) (median, IQR)	29.5 (42)	22.0 (23)	22.5 (34)	0.42**
Mechanical ventilator duration (days) (median, IQR)	26 (59)	16 (27)	19 (29)	0.32**
Presence of concomitant disease (n, %)	7 (87.5)	11 (68.8)	18 (75)	0.62*
Diabetes mellitus type 2	4 (50)	4 (25)	8 (33.3)	0.36*
Hypertension	6 (75)	7 (43.8)	13 (54.2)	0.21*
Coronary artery disease	-	5 (100)	5 (20.8)	0.13*
Complications (n, %)	5 (62.5)	7 (43.8)	12 (50)	0.67**
Antibiotic use (n, %)	4 (50)	11 (68.8)	15 (62.5)	0.41**
APACHE II score (median, IQR)	15 (9)	18.5 (12)	16 (9)	0.13**

*†IQR: interquartile range. ‡ICU: intensive care unit; *Chi-square test was used. **Mann-Whitney U test was used*

There was no statistically significant difference between survived and deceased patients in terms of gender, age, the onset of symptoms, length of stay in the ICU, duration of mechanical ventilator support, accompanying symptoms, concomitant diseases such as diabetes mellitus type 2, hypertension, coronary artery disease, complication development, antibiotic use, and APACHE II scores. However, a significant difference was identified in mortality when the effect of IVIG treatment administered for Guillain-Barré syndrome in 5 patients and other disorders were compared (p=0.001). All patients with Guillain-Barré syndrome survived after IVIG treatment. All 6 patients, who were treated with IVIG for sepsis, died. Three patients died and 1 patient survived with rheumatological and immunological diseases. Seven of 9 patients with hematological diseases passed away, whereas 2 patients survived (Table 1). When the laboratory values before and after IVIG treatment were compared, a significant decrease was found only in ALT levels (p=0.01) (Table 2).

Table 2: Laboratory Parameters of Covid-19 Patients Before and After IVIG Treatment

Laboratory parameters	**Before IVIG treatment**	**After IVIG treatment**	***P* value***
Neutrophil count x10^9/L	8.4 (6.5)	8.0 (7.8)	0.84
Lymphocyte count x10^9/L	0.59 (0.49)	0.59 (0.45)	0.26
Platelet count x10^9/L	206.5 (152)	210 (142)	0.48
Neutrophil/lymphocyte ratio	13.0 (10.4)	11.8 (7.0)	0.39
Lactate dehydrogenase (U/L)	456.5 (349)	459.5 (193)	0.18
D-dimer (mg/L)	2.79 (4.91)	2.69 (6.05)	0.74
Fibrinogen (g/L)	5.4 (2.9)	4.4 (2.8)	0.22
Ferritin (µg/L)	1011 (1417)	715 (1198)	0.38
Procalcitonin (µg/L)	0.30 (0.35)	0.51 (1.17)	0.10
C-reactive protein (g/L)	66 (136)	95 (151)	0.76
Interleukin-6 (pg/mL)	31.1 (34.3)	38.2 (61.4)	0.40
Aspartate aminotransferase (U/L)	44.5 (50)	40.5 (26)	0.21
Alanine aminotransferase (U/L)	52.5 (48)	34.5 (46)	0.01

*In all patients, IVIG was administered 0.4 g/kg/day for 3 consecutive days. Numerical data are given as a median and interquartile range. IVIG: intravenous immunoglobulin. *Wilcoxon test was used.*

DISCUSSION

The severity of the cases, the IVIG dose, and the time of administration should be considered when evaluating the effect of IVIG on Covid-19. In our study, the patients who underwent IVIG therapy were severe Covid-19 patients and all of the patients received IVIG treatment at the same dose and duration (0.4 g/kg/day for 3 consecutive days).

Huang et al. reported that 45 non-severe Covid-19 patients who were treated with IVIG had no benefit beyond standard therapy in terms of duration of fever, virus clearance time, length of hospital stay, and use of antibiotics [14]. However, after the initiation of IVIG therapy in Covid-19 patients, there have been several studies published reporting that IVIG treatment reduced mortality in Covid-19 patients. Gharebaghi et al. reported that 30 Covid-19 patients were treated with IVIG four vials daily for three days and the in-hospital mortality rate was significantly lower in the IVIG group compared to the control group (6 (20.0%) vs.14 (48.3%), p=0.022) [15]. Sakoulas et al. administered IVIG 0.5 g/kg/d for 3 days to 16 Covid-19 patients, reporting that patients treated with IVIG had shorter median ICU stay (2.5 vs 12.5 days, p=0.006) in addition to improvement of hypoxia compared to the control group [16]. Raman et al. reported that 50 Covid-19 patients were treated with IVIG, who had a significantly shorter hospital stay, and faster recovery with an improvement in oxygen saturation. Moreover, Raman et. al claimed that positive RT-PCR tests of Covid-19 patients treated with IVIG became negative in a shorter period compared to the control group (7 vs 18 days) [17]. Ali et al. administered each group with 10 patients with different doses of IVIG 0.15, 0.20, 0.25, and 0.30 g/kg. Even 10 of 40 participants (25%) passed away, Ali et al. reported that IVIG increased survival chances and reduced the risk of Covid-19 progression [18]. Esen et al. reported that 51 Covid-19 patients who received IVIG had prolonged median survival time and reduced CRP levels [19]. Shao et al. stated that early administration (admission ≤ 7 days) of high-dose IVIG (> 15 g per day) not only improved the prognosis but also reduced 60-day mortality in severe Covid-19 patients [20].

The latest Surviving Sepsis Campaign Guidelines did not recommend IVIG therapy for the treatment of sepsis due to the low quality of evidence from previous studies [21]. All of our 6 patients treated with IVIG for sepsis died, however, all patients with Guillain-Barré syndrome survived after IVIG treatment in this study. In addition, there are several case reports of Covid-19 patients who had neurological disorders such as acute disseminated encephalomyelitis and Guillain-Barré syndrome which were reported to be successfully treated with IVIG [22-27]. Moreover, IVIG treatment has been reported to be effective in Covid-19 related immunological diseases such as multisystem inflammatory disease, myocarditis, and Kawasaki disease [28].

However, there are several studies claiming that IVIG treatment did not affect the need for mechanical ventilation, improvement in chest tomography, and reduction in mortality in severe Covid-19 patients [29]. Tabarsi et al. administered 400 mg/kg IVIG daily for three days to 52 patients, in addition to hydroxychloroquine and lopinavir/ritonavir treatment. IVIG treatment was initiated after 3.84 ± 3.35 days of admission and Tabarsi et al. did not identify any significant difference between the IVIG and control group in terms of the need for mechanical ventilation and mortality [29]. Hou et al. reported that 113 Covid-19 patients were treated with IVIG. Patients who received IVIG had a longer hospital stay of 23.0 days (19.0–31.0) with a higher mortality rate than the control group (9 (19.1%) vs 4 (6.1%), p= 0.032) [30]. Hou et al. stated that IVIG improved neither in-hospital mortality nor the need for mechanical ventilation in severe Covid-19 patients. Liu et al. stated that they treated 421 severe Covid-19 patients with IVIG and they observed no significant differences in 28-day mortality, acute respiratory distress syndrome, acute hepatic injury, acute kidney injury, diffuse intravascular coagulation, myocardial injury, shock and invasive mechanical ventilation between the IVIG and control group [31]. Focosi et al. evaluated 2401 Covid-19 patients from 10 studies and concluded that IVIG was not associated with reduced death risk, thus the current evidence from the medical literature did not support IVIG therapy in Covid-19 patients [32]. In a recently published retrospective, multi-center cohort study of critically ill Covid-19 patients, 109 patients treated with standard therapy and 74 patients who received IVIG therapy were compared [33]. Salehi et al. reported that there was no difference in length of stay in the ICU, duration of mechanical ventilation, and mortality between standard therapy and IVIG therapy groups. Thus, Salehi et al. concluded that IVIG therapy was not beneficial in critically ill Covid-19 patients [33].

Within our study, there was no statistically significant difference in mechanical ventilation duration and length of stay in the ICU between critically ill Covid-19 patients who survived or died (p=0.32 and p=0.42, respectively). However, we did not have the opportunity to compare critically ill Covid-19 patients who received IVIG therapy plus standard treatment and only standard treatment.

Moreover, the effect of IVIG on mild Covid-19 cases requires further research. Huang et al. reported that 45 non-severe Covid-19 patients who were treated with IVIG had no benefit beyond standard therapy in terms of duration of fever, virus clearance time, length of hospital stay, and use of antibiotics [14].

In this study, laboratory parameters before and after IVIG treatment were evaluated. There was neither a statistically significant increase nor decrease in acute phase reactants such as CRP, procalcitonin, ferritin, and fibrinogen before and after IVIG therapy (p=0.76, p=0.10, p=0.38, and p=0.22, respectively.) The only laboratory parameter which had a statistically significant decrease after IVIG therapy was ALT in critically ill Covid-19 patients (p=0.01) (Table 2).

IVIG may slightly increase liver function tests. However, Chen et al. reported that IVIG was effective in alleviating hepatic ischemia-reperfusion injury in a rat model by reducing ALT, AST, IL-6, and TNF-alpha [34]. Moreover, Omma et al. reported improvement in liver function tests including ALT after IVIG treatment [3].

IVIG treatment is an expensive therapy option in developing countries. Therefore, we had to administer IVIG as a salvage therapy only to severe Covid-19 patients. Unfortunately, 16 (66.7%) of 24 severe Covid-19 patients passed away despite IVIG treatment.

IVIG therapy has the risk of several side effects such as thromboembolism, renal failure, meningoencephalitis, and anaphylaxis [35]. We have encountered no side effects during IVIG therapy in our ICU.

The main limitation of this retrospective study was the small sample size. A larger sample size will help to learn more about the positive and negative effects of IVIG therapy in Covid-19 patients. As our intensive care unit was reserved for only severe Covid-19 patients during the pandemic, this study lacked a control group. The effect of IVIG on severe Covid-19 cases requires further well-designed prospective randomized controlled studies.

CONCLUSION

IVIG treatment was effective in Covid-19 patients with Guillain-Barré syndrome. However, IVIG treatment did not have a statistically significant effect on pre and post-treatment laboratory values, morbidity, and mortality of severe Covid-19 patients in the ICU.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.
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Authors' contribution

AConceptualization, S.G.I., C.M.A., M.E.Y., S.I.; methodology, M.E.Y., S.I.; software, M.E.Y.; validation, S.G.I., C.M.A., M.E.Y., S.I.; formal analysis, M.E.Y.; investigation, M.E.Y.; resources, S.G.I., C.M.A., M.E.Y., S.I.; data curation, S.G.I, C.M.A, M.E.Y., S.I.; writing-original draft preparation, M.E.Y.; writing-review and editing, S.G.I., C.M.A., M.E.Y., S.I.; visualization, M.E.Y.; supervision, S.I.; project administration, S.I. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Ankara City Hospital (E2-21-835).

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Original Article

Deregulation of the Hippo Signaling Pathway in Virus-Associated Cancer

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Abstract: *Hippo signaling has been recognized as a newly identified tumor suppressor signaling pathway that can regulate cellular processes including regeneration, cell death, differentiation, and development. Its dysregulation through overexpression of YAP (Yes-associated protein) and TAZ (PDZ-binding motif) as two main oncogenic factors of the Hippo pathway has a crucial role in several cancers. Because of the limited prognosis and therapeutic targets, further understanding of molecular pathways involved in tumorigenesis is one of the interesting issues in studies. Here, we demonstrated that some viruses, through dysregulation of the Hippo signaling pathway can be implicated in transformation, metastasis, and chemotherapeutic drug resistance in virus-related cancer and also help processes involved in the pathogenesis of viral infection such as persistence.*

Keywords: *Hippo signaling pathway, virus, cancer*

INTRODUCTION

The Hippo signaling pathway was discovered to be involved in controlling organ size and cell differentiation in Drosophila melanogaster for the first time, and it has recently been recognized as a critical tumor-suppressor network [1]. Consequently, the correlation of deregulated Hippo pathway signaling in several cancer types such as breast, liver, lung, prostate, gastric and colorectal tumors has been reported. In addition to this role, the Hippo pathway is involved in the conservation of tissue-specific stem cells, tissue renewal, and damage recovery [2,3].

The Hippo pathway is a kinase cascade that includes kinases MST1 and MST2 (also known as STK4 and STK3), large tumor suppressors 1 and 2 (LATS1 and LATS2) and adaptor proteins SAV1 (Salvador homolog 1), and MOB kinase activator 1A and 1B (MOB1A and MOB1B). MST1 and MST2 induce LATS1 and LATS2 phosphorylation, which in turn facilitates LATS-dependent phosphorylation of the homologous oncoproteins YAP (Yes-associated protein; encoded by YAP1) and TAZ (PDZ-binding motif, also known as WWTR1) that inhibit the activation of transcriptional co-activators Yorkie (Yki), YAP, and TAZ [4-6].

Hippo pathway main effectors YAP and TAZ, phosphorylation by the Hippo core complex can retain them in the cytoplasm where they expose to proteasomal degradation [7]. Since TAZ/YAP mutations rarely happen other mechanisms are more likely responsible for Hippo's relevance in cancer. Growing evidence suggests that a complex network of interactions with critical pathways can modulate YAP/TAZ activity such as TGFB, GPCR, Notch, EGFR, and angiogenesis [8].

In the case of modulation of Hippo signaling, stimulation of G protein-coupled receptors (GPCRs) associated with Gas might lead to phosphorylation of LATS and consequently down-regulation of YAP. GPCRs as integral membrane receptors activated by external ligands their binding leads to the activation of the alpha-subunits of subclasses of G proteins. In contrast, stimulation of Ga12/13-coupled GPCRs is associated with suppression of LATS and up-regulation of YAP [9,10].

Other instances of membrane receptors that reported to modulate YAP expression are the receptor tyrosine kinases EGFR and ERBB4 [11]. The EGFR ligand amphiregulin (AREG) is an important YAP target that provides a positive cycle that mediates YAP and EGFR signaling and can stimulate intensifying proliferative signals from both pathways. Also, ERBB4 as a member of the EGFR family, via interaction of YAP and possibly TEAD1 with its intracellular domain in the nucleus, demonstrated either induction of YAP activation or increase expression of YAP target genes [12].

Oncoviruses are associated with about 12% of all human cancers. People carry at least one of these oncoviruses in their life, but these infections only a small proportion of these individuals go on to develop cancer [13]. The interaction between viral factors and the host is a complex process to provide a desirable microenvironment for oncogenesis [14]. The aim of this review is the improvement of our understanding of mechanisms of oncogenesis modulation by different viruses and a better understanding of tumorigenesis that may result in more effective targeted therapy in the future. By the recent rises in viral-related carcinomas and the link between Hippo and viruses, we assessed to determine if Hippo signaling is the main target in such cancers.

HEPATITIS B Virus

In recent decades human hepatocellular carcinoma (HCC) with approximately 746,000 deaths per year, identified as the sixth most common cancer and the third leading reason for cancer-related deaths in human populations [15].

Hepatocarcinogenesis, due to several genetic and epigenetic changes which occur through different stages of the disease is known as a complex process [16]. As regards the fact that hepatitis B virus (HBV) infection has a key role in the establishment of HCC, it is important to know its proteins' roles in Hepatocarcinogenesis. HBV encoded four proteins and among these proteins, the HBV X protein (HBx) is a multifunctional protein and has a critical role in hepatocellular carcinogenesis. Multiple signaling pathways are participating in the HCC progression [17,18].

Many studies are designed to assess the molecular and cellular mechanism of HCC because finding a suitable treatment for HCC is an immediate task for healing patients suffering from HCC. Recently, numerous researches have shown the role of the Hippo pathway in organ size control, cell proliferation, cell death, and tumor progression [19,20]. Although the role of YAP, which is a downstream factor of the Hippo signaling pathway in HCC, remains unclear, this review provides a vision for the regulation of YAP upstream and downstream effectors involved in hepatic cancer [21].

Interestingly, the hyperexpression of YAP can result in epithelial-to-mesenchymal transition (EMT) and may inhibit apoptosis, growth factor-independent proliferation, and anchorage-independent growth [22]. The liver has an amazing characterization that can lead to having unique features such as Growth control which in the liver has some unusual features. The liver has been known for its noticeable capacity to renew consequently to a two-third partial hepatectomy (PHx) or injury. The natural liver in an adult is composed of two parenchymal cell types — hepatocytes and cholangiocytes [23].

Dedifferentiated hepatocytes as multipotent stem cells are capable to be the source of tissue renovation in the damaged liver [24]. Factors of the Hippo signaling pathway including MST1 and MST2 have been demonstrated to have key roles in adjusting or restricting liver progenitor cells. Thus, it is predicted that the Hippo can be involved in the suppression of stem/progenitor cell proliferation in the mature liver [25]. Furthermore, some studies also showed that knocking out YAP resulted in hepatocyte and cholangiocyte injury and loss [26].

In synergy with the functional roles of Hippo signaling, any change of upstream effectors of the hippo pathway like Mst1/2 can result in over-proliferation and sometimes Hepatocarcinogenesis. Since HBV is commonly the major reason for HCC; YAP possibly has an important role in its carcinogenesis. Interestingly, YAP expression is frequently shown to be increased in HBV infections resulting in HCC, infected cell lines, and HBx transgenic mice. Through the CRE-dependent HBx attaching to the YAP promoter, HBx can increase YAP and follow stimulate YAP target genes which leads to the proliferation of hepatoma cells. Findings revealed the importance of the Hippo signaling pathway in cancer and suggested new treatments for HBV-infected patients to inhibit progression to HCC [27].

Studies have revealed that the loss of Mst1/2 may result in the promotion of several tumors in the liver of mice. The loss of both Mst1/2 is required to affect the liver since the loss of Mst1 or Mst2 is not effective on liver size or the number of liver tumors [28]. These findings firmly revealed the effect of Mst1/2 on YAP activity. Interestingly, lack of YAP phosphorylation and impairment of Mst1/2 activity that occurs in carcinogenesis have been reported in 30% of HCCs [29].

The liver-specific NF2/merlin inactivation may lead to an increase in hepatic stem cell number with a lack of any changes in differentiated hepatocytes [30]. The studies on Sav or Mst1/2 positive livers for assessment of the effectiveness of

YAP/TAZ and Lats1/2 phosphorylation in the hippo pathway displayed complex results regarding the need for Mst1/2 or Sav in Lats1/2 phosphorylation [29,31] and since in mouse fibroblasts, for YAP phosphorylation, presence of Mst1/2 was not crucial, it is considered that the pathway may be specific in hepatitis [29].

In a study on transgenic mice, it was shown that YAP induction can lead to abnormal hepatocyte expansion after 2 months and consequently several nodes have been reported in hepatitis [32]. The nodes were reported to be a collection of hepatocytes in a generative condition that condensed the near parenchyma and revealed severe HCC. Also, this liver-specific loss of either Mst1/2 in hepatocytes leads to noticeable liver growth because of its deregulated cell proliferation [28].

On the other hand, when transgenic mice models with YAP hyperactivity crossed with mice lost its DNA-binding domain TEAD2 do not promote HCC. Lacking YAP overexpression may lead to having mice with livers deficiency in Nf2/merlin after crossing with mice that have the defective TEAD2 gene, confirming the therapeutic concept of using the TEAD with YAP in the complex. Verteporfin injection has been demonstrated to reduce liver mass in YAP transgenic animal models and also has no side effect on the wild mice tissue homeostasis, defining the potency of compounds for therapeutic administration in patients with HCC. Verteporfin displayed numerous important features that make its clinical execution for HCC promising [32].

In HCC cell lines induction of YAP results in further resistance to doxorubicin-induced apoptosis; while, inhibition of YAP expression by RNA interference has been reported to reverse YAP-increased chemoresistance. These processes are mediated by the MAP kinase signaling pathway induction rather than an AKT-dependent mechanism of chemoresistance [33]. This process of chemoresistance occurs after treatment by the MEK1/2 suppressor U0126 before doxorubicin injection, which noticeably reduces cell vitality in YAP overexpressing HCC lines [34].

Survivin is accepted as an antiapoptotic protein and in HCC patients identified to be associated with a weak prognosis. Recent studies have shown YAP overexpression is in associate with survivin induction in patients with HCC. It seems that YAP has this oncogenic capacity on the cell with a survivin increase leading to carcinogenesis [35].

HUMAN PAPILLOMAVIRUS

Cervical cancer considers the most important cancer in women because it is the fourth leading reason of cancer-related death in women. Due to the detection of human papillomavirus (HPV) DNA in the majority of cervical cancer patients, high-risk HPV types recognize as the cause of cervical cancer [2].

In a study by Chunbo He et al results implicated that YAP1, one of the main oncogenic effectors of the Hippo pathway, was (11%) amplified several times, whereas LATS1/2, MST1, and FATs, that identified as upstream tumor inhibitors of the Hippo pathway, were not detectable or mutated in cervical cancer patients. YAP1 induction in cervical cancer cells noticeably triggers the expression of EGFR and its related ligands AREG and TGFa. On the other hand, TGFa and AREG, through EGFR signaling, can block the Hippo pathway and induce YAP protein (dephosphorylated LATS1, MOB1, and YAP1) to promote cervical cancer cell growth. Recognition of this circumstance between Hippo/YAP and EGFR in human cervical cancer provides an opportunity to combine targeting of the Hippo/YAP and EGFR pathways to have an efficient way to treat cervical cancer [36].

In addition, hyperactivation of YAP1 can be an important mediator to the persistence of HPV infection. First, studies report that hyperactivated YAP1 increases the expression of HPV receptor molecules such as ITGRA6, SDC1, and EGFR, which through the early stage of HPV infection can help the HPV entry process. Second, is that hyperactivation of YAP1 in cervical epithelial cells may reduce viral pattern recognition by the innate immune system. Some studies report that TLR2 and TLR4 and adaptor proteins MYD88 and TRIF were suppressed, which could facilitate HPV infection. Third, there is evidence that ectopic expression of YAP may suppress the activation of transcriptional factors that are essential for the production of type I IFNs, including IRF1, IRF3, and IRF7, leading to the reduction of IFNA1, IFNB1, and IFNE in hers. IFNs can activate antiviral factors of the immune system and play their role in the progress of innate and adaptive immune responses. Also, some studies showed that the expression and activation of main components of the type I IFN pathway, like IFNAR2, STAT1, JAK1, and IRF9, are suppressed by the expression of YAPS127A in the epithelium of the cervix. Suppression of the IFNAR2/JAK1/STAT1 signaling pathway leads to decreasing of antiviral ISGs, such as MX1, CH25H, and IFITMs (blockers of virus entry), and APOBEC3G, OAS1, ISG15, and IFI44L (suppressors of virus translation and replication) [37].

The HPV oncoproteins E6 and E7 expression can result in the suppression of some essential tumor inhibitors such as TP53 and RB1 in many types of cells [38,39]. Suppression of TP53 and RB1 extends cell life (by inhibition of death) and may lead to immortality in cervical epithelial cells, facilitating malignancy of HPV-infected cells by an oncogenic signal (including hyperactivation of YAP1) [40,41].

In epithelial cell cultures, cell-cell contact development is a key step in differentiation before the formation of distinguished apicobasal surfaces. The transcriptional co-activator YAP plays a key role in this event [42,43]. Numerous reports have shown that cell-cell contacts activate the LATS1/2 serine/threonine kinases which can phosphorylate YAP [44,45]. During phosphorylation, YAP restricts from the nucleus and it inhibits the transcription of key genes in the cell cycle [42,46]. A cervical cancer study recently demonstrated that in high-grade cervical lesions levels of nuclear YAP are increased [47].

There are some studies demonstrating that HPV16-E6 or HPV16-E6/E7 double-transgenic mice treated with estrogen may promote cervical cancer [48,49]. Related to that result, other studies report that YAP was increased in the E6 and E6/E7-induced cervical tumor cells, and YAP was localized in the nucleus of these cells [36]. The human foreskin keratinocyte(HFK) "raft" culture infected with HPV16 makes a unique model for the assessment of the HPV16 proliferation cycle [48,50].

YAP can be inactivated by two mechanisms: first, the Ser127 phosphorylation-mediated spatial adjustment (system of nuclear-cytoplasmic shuttling); and second, the Ser397 (Ser381 in YAP protein isoform 2) phosphorylation-mediated adjustment (the phosphodegron-induced degradation). These two mechanisms are applicable directly to prevent YAP oncogenic activity [51,52]. It has been observed that CK1d/ε and SOCS6 proteins are crucial for YAP degradation [52,53]. Although expression of HPV E6 in HT3 cells causes a noticeable increase in CK1d/ε and a reduction in p-YAP (S397), it suggests that the CK1d/ε pathway may not participate in the HPV E6-mediated YAP protein turning [40]. HPV E6 increases YAP protein levels, but also it upregulates the expression and activation of AREG protein in the cell culture. These observations showed a possibility that HPV E6 protein may be associated with the Hippo-ERBB signaling circle and also implicating the transformation and development of cervical cancer. Interestingly, HPV16 E6 and E7 could transcriptionally activate EGFR expression [40,54]. The HPV16 E6 makes receptor protein tyrosine kinase to signaling and facilitates the internalization of phosphorylated receptors [55] (Figure 1).

Figure 1: Schematic presentation of reciprocal interactions between HPV and Hippo signaling pathway

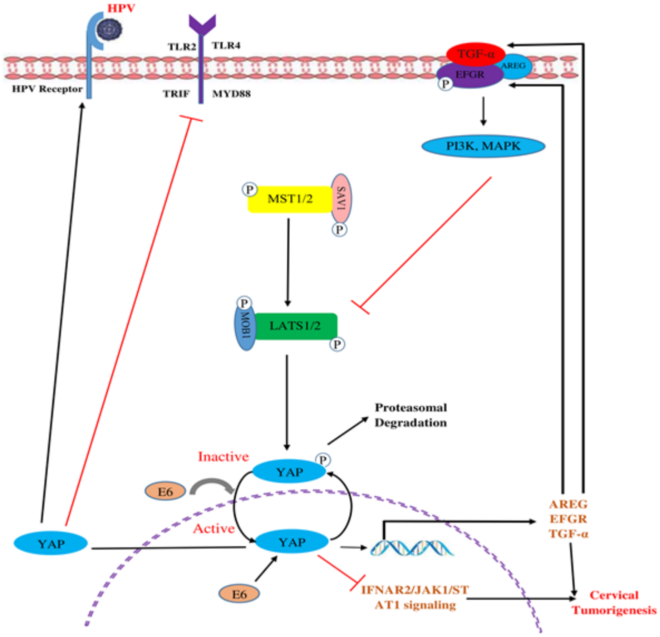

POLYOMAVIRUSES

Through identification of SV40 tumorigenesis in specific laboratory animals and transforms several types of cultured cells offered an opportunity to discover the molecular basis of the tumor. Viral oncogenes were introduced as key tools in the recognition of tumor inhibitor pathways in human primary cells. The p53 and p16/pRb tumor inhibitor pathways have been introduced as targets of SV40 large T. Hahn's study indicates a transformation model [56], expressed HRas V12, and a construct encoding SV40 large T to target the p14ARF/p53 and p16INK4A/pRb pathways. Recently it is discovered that SV40 small T (ST) (encoded in the large T construct) is needed without requiring large T to enhance anchorage-independent growth [57].

Murine polyomavirus also has frequently presented visions into tumorigenesis by demonstrating important modulation mechanisms including tyrosine phosphorylation and phosphoinositide 3-kinase (PI3K) signaling. Previous studies showed that polyomavirus small T antigen (ST) associates with YAP, an effector of the Hippo pathway, to modulate differentiation. Other studies introduced YAP as a target of middle T antigen (MT) required for transformation [58,59]. Wild-type MT via a surface containing residues R103 and D182 binds to the YAP WW domains. Mutation in each one of R103 or D182 of MT disrupts YAP binding. This happens without disturbing bindings to other signaling factors or the strength of PI3K or Ras signaling. One of the other genetic disruptions of MT binding to YAP or stopping of YAP via short hairpin RNA (shRNA) decreased MT transformation, offering that YAP has an important participation in the transformed phenotype. MT targets YAP via triggering signaling pathways that affect it and consequently bind to it. The signaling of either wild-type MT or the YAP associate MT mutant develops YAP phosphorylation at S127 and S381/397 (YAP2/YAP1) [58]. In addition to previous roles of these phosphorylated serines, MT signaling results in the reduction of YAP from the nucleus and degradation. When MT Binds to YAP, it associates YAP with protein phosphatase 2A (PP2A), resulting in the YAP dephosphorylation together with the MT complex. Furthermore, this process results in the increase of YAP in membranes. Overall, these data demonstrate that YAP develops MT transformation by mechanisms that may be due to YAP's established major transcriptional activation functions [60].

The capability of ST to discuss anchorage-independent growth brings with it its ability to inactivate the PP2A function. Suppression of PP2A may bypass the need for ST in cellular transformation models, and mutations inactivating PP2A subunits have been recognized in tumors [61,62]. PP2A has several substrates including, AKT, b-catenin, and c-Myc which are defined to be activated by ST. Although the total overexpression of these proteins only incompletely replaced ST in cellular transformation, it suggests that undefined PP2A effector pathways may be involved [62].

Hippo/MST kinase acts by a membrane-associated complex including the tumor inhibitor NF2/Merlin [63]. MST kinases enhance activating LATS kinases, which subsequently phosphorylation of the transcriptional coactivators YAP and TAZ, resulting in their inactivation of them via cytoplasmic isolation and increased degradation [64-66]. Suppression of YAP and TAZ restricts proliferation and triggers apoptosis [67].

Interestingly, it is observed that overexpression of YAP is enough to result in tumorigenesis in mice. Both SV40 ST and the Merkel cell polyomavirus ST induce endogenous YAP. Some studies provide data that ST acts through PAK1 to inactivate the NF2 tumor suppressor to alleviate the inactivation of YAP. Activation of PAK1 at the cell membrane is sufficient to bypass the requirement for ST. Restricting PAK1 activity disrupts the transforming capability of the ST protein. Therefore, ST detaches YAP from modulation via the Hippo tumor inhibitor pathway. Induction of Active YAP is required and enough to replace ST in cellular transformation. Therefore, the YAP proto-oncogene is an important mediator of the transforming properties of the ST oncoproteins [68] (Figure 2).

Figure 2: Schematic presentation of reciprocal interactions between Polyomaviruses and Hippo signaling pathway

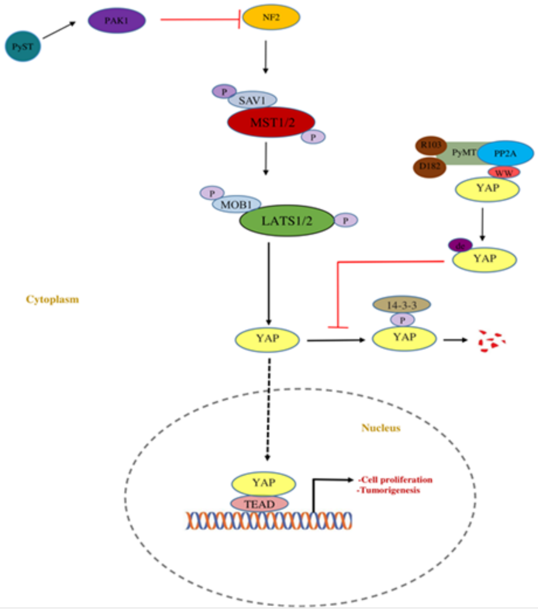

EPSTEIN-BARR VIRUS

Epstein-Barr virus (EBV) is recognized as a linear dsDNA virus and a member of the human gamma-herpesvirus family that observed to correlate with various B-cell lymphomas, such as Burkitt's lymphoma, Hodgkin's lymphoma, and other tumors like nasopharyngeal carcinoma, T-cell non-Hodgkin lymphomas, and gastric carcinomas. These cancers are firmly correlated with latent Epstein-Barr virus (EBV) infection, genetic factors, and environmental factors thus EBV can regulate mechanisms affecting carcinogenesis, proliferation, death, migration of cells, epigenetic changes in lymphocyte-specific development, and induce cell immortalization. The main mechanisms related to the carcinogenic effectors of EBV consist of LMP1, LMP2, and microRNA that we will introduce bellow [69-71].

The capacity of LMP1 to immortalize and transform cells possibly depends on simultaneously regulating cellular signaling pathways which suppress apoptosis or mediate proliferative, growth factor-like effects. Furthermore, LMP1 can induce a cancer progenitor cells (CPC)-like appearance in epithelial cells, offering that LMP1-induced phenotypic alterations can be related to the promotion of cancer [72]. Therefore, the mechanism and role of LMP1 in immortalizing and transforming cells is complex and several important questions remain to be answered.

Previous studies demonstrated that LMP1 could induce actin remodeling [73]. The actin cytoskeleton is a key factor for the modulation of the Hippo pathway. These data make us investigate How LMP1 can upregulate TAZ expression by inhibition of the Hippo pathway. In a study, findings determined that the LMP1-targeted CNE1-LMP1 cells decreased TAZ expression. The target genes of TAZ are CTGF and Cyr61. Other researches demonstrate that LMP1 can trigger the mRNA expression of CTGF and Cyr61 [74]. There is the possibility that TAZ may be involved in the positive modulation of cell proliferation and survival associated with the obtaining of cancer stem cell (CSC) properties and epithelial-to-mesenchymal transition (EMT) [75, 76]. Previously, some studies have been done to determine if LMP1 can develop EMT by TAZ and define if the morphology alters. The result of these studies has shown some morphological variations from epithelial to mesenchymal phenotypes. LMP1 can develop cell proliferation, EMT, and CSC-like characteristics by TAZ and give us some visions and rationales for the LMP1-mediated resistance to cancer treatments [74].

On the other hand, it has been shown that phosphorylation of the WW domain of the transactivator TAZ by activated LATS1/2, leads to TAZ degradation [77]. Studies have shown that LMP1 can elevate the level of TAZ and increase TAZ nucleus translocation. Thus, it is possible that LMP1 could suppress LATS1/2 activation, resulting in the enhancement of TAZ stability [74] (Figure 3).

Figure 3: Schematic presentation of reciprocal interactions between EBV and Hippo signaling pathway

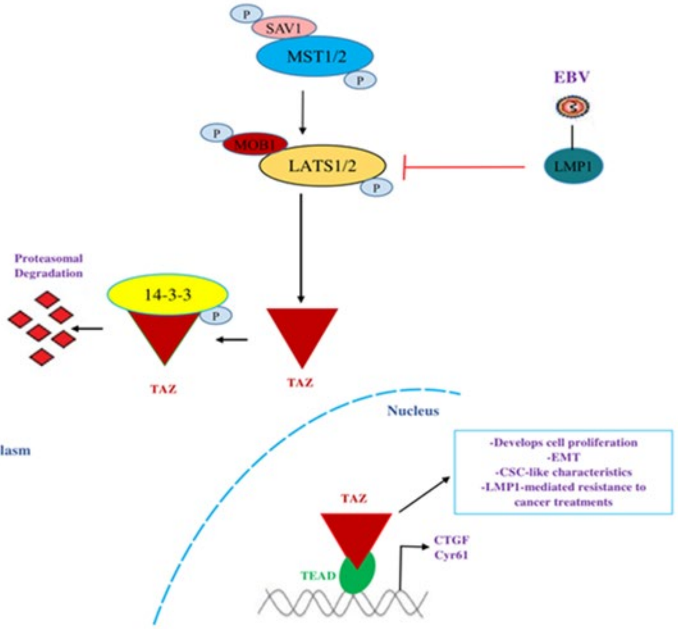

KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

Kaposi sarcoma-associated herpesvirus (KSHV) is a double-stranded linear DNA virus of the human gamma-herpesvirus family from rhadinovirals and also an oncogenic virus that is responsible for human Kaposi sarcoma (KS) and is associated with an AIDS-defining malignancy. One of the main viral proteins of KSHV is a viral G-protein-coupled receptor (vGPCR). KSHV encodes vGPCR which is essential for the initiation and progression of Kaposi sarcoma [78-80]. Previous studies identified that YAP/TAZ, two homologous oncoproteins that can be suppressed by the Hippo tumor inhibitor pathway, activates in KSHV-infected cells in in vitro conditions, KS-like tumors in mice, and clinical human KS samples [81].

The molecular process to which vGPCR contributes in KSHV-induced oncogenesis remained uncertain but it is suggested to be involving both direct and paracrine oncogenic effects, that are activated by growth factors including VEGF and PDGF induced by vGPCR [82,83]. The KSHV protein (vGPCR) activity is via Gq/11 and G12/13 to suppress the Hippo pathway kinases Lats1/2, enhancing the activation of YAP/TAZ. Also, clearance of YAP/TAZ inhibits vGPCR-induced cell proliferation and tumorigenesis in a xenograft mice model. The transformation of cells mediated by vGPCR is sensitive to pharmacological blockers of YAP [84,85].

It is demonstrated that the Knockdown of YAP or TAZ can inhibit the vGPCR-induced CTGF expression, offering that YAP/TAZ enhances the biological functions of vGPCR. In addition to the Hippo pathway, studies have shown other signaling pathways that have also been related to the oncogenic effect of vGPCR [85]. For instance, PI3Kγ is activated by vGPCR and has a key role in vGPCR-induced sarcomas, whereas NFkB has an indirect role in vGPCR-induced paracrine neoplasia. It seems the activation of YAP/TAZ is associated with other vGPCR downstream signaling events, including PI3K and NFkB, which generally participates in KS tumorigenesis in KSHV-infected patients. It is noticeable that YAP/TAZ also upregulates the expression of several growth factors, including CTGF and insulin-like growth factor (IGF) [86,87], that can be associated with neoplasia caused by vGPCR in a paracrine condition [81, 88].

Some studies revealed that Hippo pathway activation has a key role in the promotion of the lytic cycle and transition from latency. Inactivation of the Hippo pathway modulator YAP1 may promote reactivation from latency, lytic protein increase, and production of infectious virions. Interestingly, expression of the YAP1-inactivating kinase LATS1 consequently induces reactivation from latency. Conversely, LATS1 silencing decreases viral protein expression along the late phase of lytic replication. However the detailed mechanism of switching of KSHV latent/lytic by the Hippo signaling pathway is still unclear, increased amounts of nuclear centers containing the viral latency-associated nuclear antigen (LANA) have been reported in YAP1-silenced cells [89] (Figure 4).

Figure 4: Schematic presentation of reciprocal interactions between KSHV and Hippo signaling pathway

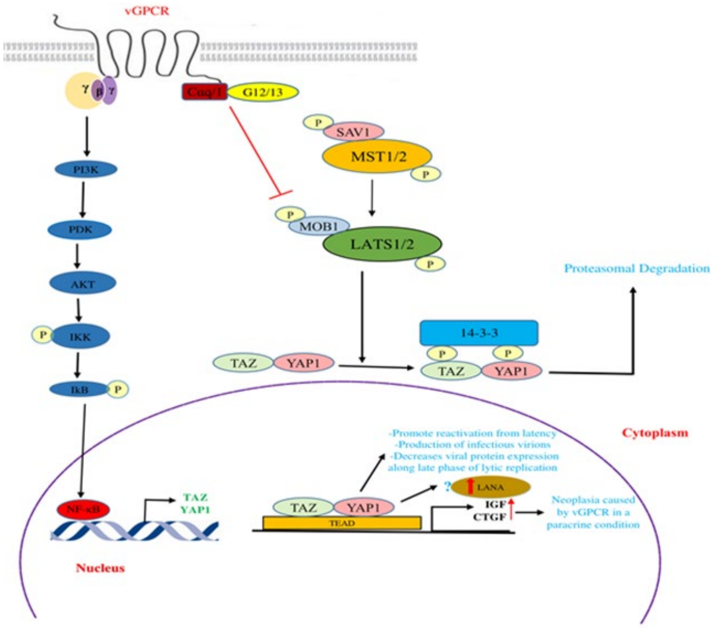
vGPCR
γ
β
γ
Gαq/11
G12/13
PI3K
PDK
AKT
P
IKK
IkB
P
P
SAV1
MST1/2
P
P
MOB1
LATS1/2
P
Proteasomal Degradation
14-3-3
P
P
TAZ
YAP1
TAZ
YAP1
Cytoplasm
-Promote reactivation from latency
-Production of infectious virions
-Decreases viral protein expression along late phase of lytic replication
?
LANA
NF-κB
TAZ
YAP1
TAZ
YAP1
TEAD
IGF
CTGF
Neoplasia caused by vGPCR in a paracrine condition
Nucleus

HEPATITIS C VIRUS

Hepatitis C virus (HCV), a member of the Flaviviridae family, is an enveloped RNA virus with a positive sense single-strand RNA genome [90]. Chronic HCV infection is one of the main risk factors for disease progression to metabolic disorders, fibrosis, cirrhosis, and HCC [91]. Epithelial-to-mesenchymal transition (EMT) is a physiological process by which epithelial cells lose their cell polarity and cell-to-cell junctions, and achieve migratory and invasive properties to become mesenchymal cells [92]. Previous literature reviews have shown that EMT plays a crucial role in promoting metastasis in various human cancers including HCC [93]. During the process of EMT, loss of epithelial markers, especially E-cadherin is mediated by transcriptional repressor SNAIL that binds to the E-box motif on the promoter region of E-cadherin [94]. HCV non-structural 4B (NS4B) protein is a 27kDa hydrophobic protein that role in HCC pathogenesis mediate interaction networks with cellular host proteins [95]. A study recently showed that interaction NS4B with scribble protein, a scaffold protein involved in regulating cell polarity and cell proliferation in epithelial cells, suppresses the Hippo signal pathway that subsequently results in the induction of the PI3K/AKT pathway. Induction of the PI3K-Akt pathway upregulates Snail expression via translocation to the nucleus. Finally, the up-regulation of Snail induces EMT progression by reducing E-cadherin expression which contributes to HCV-caused HCC progression, invasion, and metastasis [96].

HCVs entry to hepatocytes is recognized to be mediated by CD81 [97]. Ezrin is a member of a membrane-cytoskeleton linking protein that increases in HCC and is identified in correlation with malignant HCC [98]. In normal hepatocytes, CD81 overexpression results in the phosphorylation of ezrin and increasing nuclear YAP. As the CD81-SYK-ezrin complex causes signals to modify cell construction through protein interactions, gene expression is mediated by YAP via downstream regulation of the Hippo signaling pathway [99]. Also deregulation of the Hippo signaling pathway and increased YAP activity have been demonstrated in 50% of HCC cases [100]. In contrast since Glypican (GPC)-3 is one of the main ligands for CD81, it upregulates the expression of CD81 in HCC cells enhances activation of Hippo, a reduction in ezrin phosphorylation, and a reduction in YAP. Interestingly HCV E2 protein mimics the function of GPC3, enhances the activation of the Hippo signaling pathway, and subsequently reduces YAP and cell proliferation. Consequently, although any hepatocytes which have missing CD81 expression are considered to be resistant to HCV infection but can expand faster than the normal hepatocytes, loss of CD81 is a usual event in the promotion of hepatic neoplasia [101] (Figure 5).

Figure 5: Schematic presentation of reciprocal interactions between HCV and Hippo signaling pathway

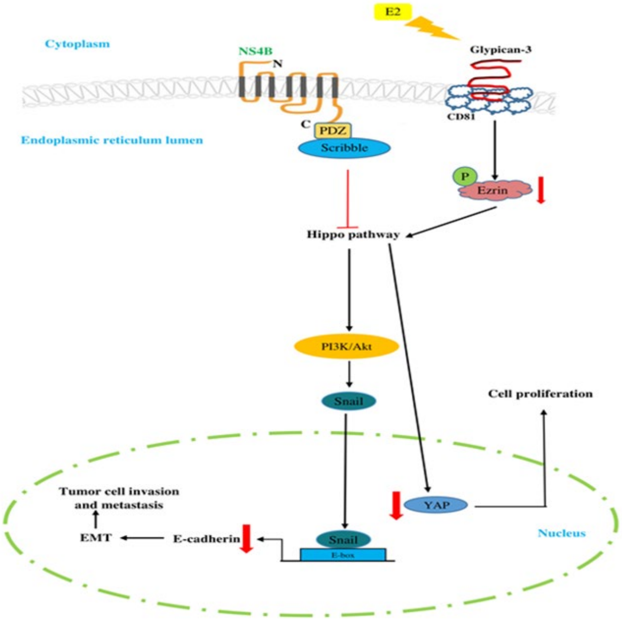

CONCLUSION

The Hippo signaling is an emerged evolutionarily conserved tumor suppressor pathway that regulates proliferation, apoptosis, differentiation, and development, whose dysregulation overexpresses YAP and TAZ as two major oncogenic downstream effectors of Hippo pathways that play a crucial role in multiple human pathological diseases, such as cancer (Table 1).

Table 1: The summary of the deregulation of the hippo signaling pathway by oncoviruses

Virus	Viral protein	Component of hippo signaling	Mechanism/Effect	References
HBV	HBx	YAP	HBx can increases YAP via the CRE-dependent HBx binding to the YAP promoter and following stimulation of YAP target genes	[27]
HPV	-	YAP1	Hyperactivation of YAP1 can be an important mediator in the persistence of HPV infection	[37]
	-	YAP1	Hyperactivation YAP1 increases the expression of HPV receptor molecules such as ITGRA6, SDC1, and EGFR	[37]
	-	YAP1	Hyperactivation of YAP1 in cervical epithelial cells may reduce viral patterns recognition TLR2 and TLR4 and adaptor proteins MYD88 and TRIF of the innate immune system	[37]
	-	YAP1	Expression of YAP may suppress the activation of transcriptional factors that are essential for the production of type I IFNs, including IRF1, IRF3, and IRF	[37]
	E6/E7	YAP	Expression and activation of main components of the type I IFN pathway, like IFNAR2, STAT1, JAK1, and IRF9, suppressed by expression of YAPS^{127A}	[37]
		YAP	YAP was increased in the E6 and E6/E7-induced cervical tumor cells and YAP was localized in the nucleus of these cells	[36]
PyVs		ST YAP	Polyomavirus small T antigen (ST) associates with YAP, an effector of the Hippo pathway, to modulate differentiation	[58]
		MT YAP	MT Binds to YAP, it associates YAP with protein phosphatase 2A (PP2A), resulting in the YAP dephosphorylation together with the MT complex	[60]
		ST YAP	ST acts through PAK1 to inactivate the NF2 tumor suppressor to alleviate the inactivation of YAP	[68]
EBV		LMP1 TAZ	LMP1 can upregulate TAZ expression by inhibition of Hippo pathway proteins	[74]
		LMP1 TAZ	LMP1 can develop cell proliferation, EMT, and CSC-like characteristics by TAZ	[74]
		LMP1 LATS1/2	LMP1 could suppress LATS1/2 activation, resulting in the enhancement of TAZ stability	[74]
KSHV		vGPCR Lats1/2	The KSHV protein (vGPCR) activity is via Gq/11 and G12/13 to suppress the Hippo pathway kinases Lats1/2, enhancing the activation of YAP/TAZ	[85]
		vGPCR YAP/TAZ	Activation of YAP/TAZ is associated with other vGPCR downstream signaling events, including PI3K and NF-kB	[86,87]
		- YAP1	Inactivation of the Hippo pathway modulator YAP1 may promote reactivation from latency, lytic protein increasing, and production of infectious virions	[89]
		LATS1	Expression of the YAP1-inactivating kinase LATS1 consequently induces reactivation from latency	[89]
HCV	*NS5B*	-	*Interaction NS4B with scribble protein suppresses the Hippo signal pathway that subsequently resulting in the induction of PI3K/AKT pathway*	[96]
	E2	YAP	HCV E2 protein mimics the function of GPC3, enhances the activation of the Hippo signaling pathway, and subsequently reduces YAP	[101]

The activation of YAP and TAZ contributes to cancer initiation, metastasis, and anticancer drug resistance via interactions with several signaling pathways in many human tumors. Oncoviruses either directly or indirectly by modulating a variety of cellular signaling pathways lead to the dysregulation of the Hippo signaling pathway to increase the expression of the downstream effectors TAZ and YAP which can be implicated in transformation, metastasis, and chemotherapeutic drug resistance in virus-related cancer and also to facilitate some processes involved in the pathogenesis of viral infection such as entry, persistence, escape from the immune system, and latency. However, in some cases inactivation of YAP can aid the pathogenesis of KSHV and HCV. (Figure 6).

Figure 6: Schematic presentation of reciprocal interactions between oncoviruses and Hippo signaling pathway

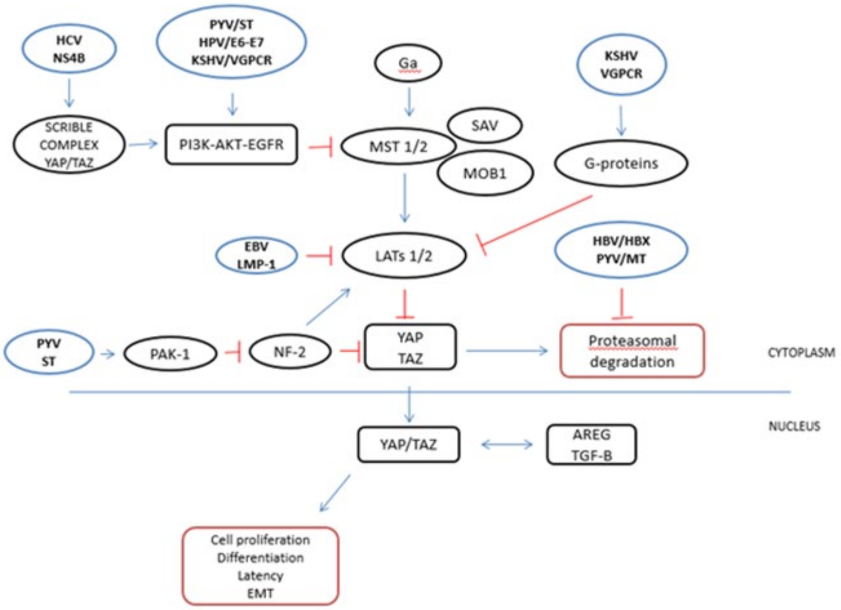

With these interpretations, we recommend that the overexpression of the major downstream effectors of the Hippo pathway, TAZ, and YAP can be used as prognosis markers in cancers caused by oncoviruses. Overall, Hippo signaling is a new pathway in viral infection, and in this way, better understanding interaction between oncoviruses with Hippo signaling can be used as a new therapeutic target in virus-caused cancers.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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Original Article

Relationship between Mental Health, Life Satisfaction, and Job Satisfaction of Nurses

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Abstract: *Improving the mental health of nurses and investigating the factors affecting it leads to the improvement of job performance and the quality of nursing care. Aim: The present study aimed to determine the relationship between mental health, life satisfaction, and job satisfaction of nurses in Ilam city in 2022. The present correlational study was carried out on 160 nurses working in educational hospitals of Ilam city and was selected by stratified random sampling in 2022. A four-part questionnaire was used: the first part included a question on demographic information. Minnesota Satisfaction Questionnaire (MSQ) (1977), Diener et al.'s Satisfaction with Life Scale (SWLS) (1985), and Derogatis et al.'s Symptom Checklist-90 (SCL-9) (1973) were included in the second, third and the fourth parts, respectively. Results showed no significant relationship between the total score of mental health and the total score of job satisfaction. There was a significant relationship between the total job satisfaction score and life satisfaction score (r=0.267, p=0.001). There was also an inverse and significant relationship between the total mental health score and the life satisfaction score (r=0.294, p=0.001). The collected data was analyzed using descriptive tests (frequency, mean percentage, and standard deviation) and non-parametric Spearman and Kruskal-Walli's correlation tests in SPSS ver. 22. P-value<0.05 was considered as the significance level in all tests. Job satisfaction is effective in creating motivation, efficiency, responsibility, and social accountability in the personnel of any organization. Job dissatisfaction can lead to complications such as job burnout, illness, absenteeism, the job left, leaving many nursing jobs, and the reluctance of the young workforce to attend the wards. Therefore, it is necessary to make further efforts in this field by healthcare policymakers.*

Keywords: *mental health, life satisfaction, job satisfaction*

INTRODUCTION

Mental health has an expanded definition. But every person who can deal with his/her deep issues, get along with him/herself and others, and can face his/her inevitable internal conflicts, and does not isolate him/herself from society, can be stated to have mental health [1-4]. Nurses are directly related to all social strata and touch people's problems closely. This close relationship doubles their serious responsibility toward people's health [5]. Nursing is one of the most stressful professions so the National Institute for Occupational Safety & Health has introduced it among the top 40 stressful professions [6].

There are many sources of occupational stress in the nursing work environment. Overwork, urgency, patient death, independent practice, lack of social support, poor job fit, lack of necessary specialized knowledge, unsafe workplace and rapidly changing healthcare environment are known as nursing stressors [7]. Nurses are prone to health threats in different dimensions due to long work shifts and the resulting fatigue. Nurses with no good general health are not able to provide good care such as physical and psychological support to patients. This increases the risk of mistakes and occupational accidents, and subsequent consequences affect the patient and the nurse [8].

Nurses' stress and fatigue can affect the vital and critical aspects of their performance, including problem-solving ability, decision-making ability, and creativity, and ultimately lead to disruption in medical care. Because patient safetyو health and recovery depend on the safe and optimal performance of nurses [9]. Many researchers have confirmed that job-related stress can have harmful effects on the physical and mental health of individuals [10,11]. Job stress is one of the factors affecting the health of employees. Employees' health is at risk when they face various stressors. This issue is more evident in the nursing profession [12]. The impact of job dissatisfaction on the mental health of employees has been a public concern in various societies, especially in recent years. Perceived impacts of job dissatisfaction on people's mental health from an economic point of view is also an important issue and play an important role in the quality of professional and personal life of nurses [13].

Another variable that plays a key role in improving nurses' mental health is life satisfaction. Life satisfaction is one of the mental health-related factors and refers to a person's general attitude and evaluation toward life as a whole [14]. It also reflects the balance between a person's aspirations and his/ her current situation. The greater this balance, the greater the life satisfaction will be [7]. According to previous studies, this concept is in full interaction with health and there is a close relationship between physical and mental health with life satisfaction [15]. Lyubomirsky believes that people who have higher life satisfaction, use more effective and appropriate coping methods, experience deeper positive emotions and feelings and have higher general health [16]. Nurses are among the vulnerable employees of society, whose health and balance in their personal and family life is related to occupational variables. Work-family conflict causes psychological symptoms such as higher stress, depression, chronic physical problems, increased physical complaints, lower life satisfaction, low quality of family life, and low energy levels [17]. Suzuki et al. stated that nurses were in 27th place in the evaluation of 130 stressful jobs sorted according to their reference to doctors for health problems [18].

Improving the mental health of nurses and investigating the factors affecting it leads to the improvement of job performance and the quality of nursing care. Since nursing is one of the sensitive professions and nurses spend an important part of their lives with people and patients, their mental health leads to optimal job performance and guarantees the health and improvement of many patients and people [19].

Having job satisfaction, life satisfaction and mental health leads to an improvement in the quality of patient care and increasing productivity and improving nursing services, therefore, conducting relevant studies and using their results in various aspects of the nursing profession can have useful results in care, education, management, therapy and also the personal life of a nurse [20]. Therefore, the present study aimed to determine the relationship between mental health, life satisfaction, and job satisfaction of nurses in Ilam city in 2022.

MATERIALS AND METHODS

The present correlational study was carried out on 160 nurses working in educational hospitals of Ilam city and was selected by stratified random sampling in 2022. Inclusion criteria included working in one of the above-mentioned educational hospitals and having informed consent to participate in the study. Failure to complete one of the questionnaires, an incomplete questionnaire, and unwillingness to cooperate were among the exclusion criteria. A four-part questionnaire was used: the first part included a question on demographic information. Minnesota Satisfaction Questionnaire (MSQ) (1977), Diener et al.'s Satisfaction with Life Scale (SWLS) (1985), and Derogatis et al.'s Symptom Checklist-90 (SCL-9) (1973) were included in the second, third and the fourth parts, respectively. Demographic information of nurses included age, gender, years of work experience, marital status, education level, hospital, ward, position, income level, employment status, number of children, and home type.

Minnesota Satisfaction Questionnaire (MSQ) consists of 19 items and 6 subscales of the payment system, type of job, achievement opportunities, organizational atmosphere, leadership style, and physical conditions. MSQ is scored based on a 5-point Likert scale, ranging from "Completely Disagree" (Score 1) to "Completely Agree" (Score 5). To calculate the overall score of the questionnaire, the scores of all the items are added together. Scores 19-38, 38-57, and 38-57 indicate poor, moderate, and very good job satisfaction, respectively. This questionnaire has been used in many types of research in the world [21-26]. Its internal consistency reliability was measured $\alpha = 0.87$.

Diener et al.'s Satisfaction with Life Scale (SWLS) is a 5-item instrument designed to measure a person's cognitive and overall judgment of life. This instrument is scored based on a 7-point Likert scale ranging from 1 (Completely disagree) to 7 (Completely agree). The overall score of this scale is calculated by summing the answers and the possible score range is 5 and 35. A higher score indicates higher levels of life satisfaction. This questionnaire has also been used in many types of research in the world [27-32]. The Cronbach's α reliability was 0.86.

Derogatis et al.'s Symptom Checklist-90 (SCL-9) can be completed within approximately 25 minutes. This questionnaire is the short form of SCL-90 and was extracted by Najarian & Davoudi based on the original form and through exploratory factor analysis. It consists of 25 items and 9 subscales of somatization (6 questions), obsession-compulsion (3 questions), interpersonal sensitivity (3 questions), depression (2 questions), anxiety (3 questions), phobia (3 questions), paranoid thoughts (1 question), psychosis (3 questions), hostility (1 question). SCL was answered according to its original form and based on a 5-point Likert swale ranging from None or Rarely (0), A little (1), To some extent (2), A lot (3), Very much or Strongly (4). The overall score of general psychological injuries was extracted. Higher scores indicate more damage [30]. The Cronbach's alpha reliability of the tool was calculated α = 0.94.

The collected data was analyzed using descriptive tests (frequency, mean percentage, and standard deviation) and non-parametric Spearman and Kruskal-Walli's correlation tests in SPSS ver. 22. P-value<0.05 was considered as the significance level in all tests.

Ethical considerations: The present study was approved by the Ethics Committee of Ilam University of Medical Sciences with the code IR.MEDILAM.REC.1400.229. The contents of the Nuremberg and Helsinki declarations and the 26 research ethics codes were observed.

RESULTS

The results showed that most of the participants were female (61.2%), aged 26-29 (40%), single (52.5%), childless (75%), owning their own house (72.5%), and had 6-10 million-toman income (70%). Also, the majority of participants had a bachelor's degree (88.8%), less than 6 years of work experience (70%), were ward nurses (91.2%), were permanent nurses (45%), and worked in ICUs (45%).

Table 1 shows the correlation matrix of job satisfaction, life satisfaction, and mental health. This table determines the relationship between job satisfaction, life satisfaction, and mental health of research subjects. The table shows no significant relationship between the total score of mental health and the total score of job satisfaction. There was a significant relationship between the total job satisfaction score and life satisfaction score (r=0.267, p=0.001). There was also an inverse and significant relationship between the total mental health score and the life satisfaction score (r=0.294, p=0.001).

Kruskal-Walli's test shows the relationship between job satisfaction, life satisfaction, and mental health according to demographic characteristics (Table 2). This table shows that the highest frequency of the variables of work experience, education level, medical training center, hospital ward, income level, and employment status are related to less than 3 years (p=0.017, df=4, k=11.98), BA (p=0.014, df=1, k=6.07), Ayat-Allah-Taleghani hospital (P=0.004, df=2, k=11.03), ICUs (P=0.001, df=2, k=55.91), 6-10 million tomans(P=0.022, df=2, k=7.62), and contractual employment (P=0.001, df=2, k=14.37), which are all had a statistically significant relationship with job satisfaction. The highest frequency of gender, marital status, and employment status were related to females (P=0.037, df=1, k=4.34), married individuals (P=0.034, df=1, k=4.47), and contractual individuals (p=0.032, df=2, k=6.88), which all had a statistically significant relationship with life satisfaction. Also, the female gender (k=11.75, p=0.001, df=1) had a statistically significant relationship with mental health.

DISCUSSION

Mental health affects health-related variables related to [4,33-35]. The results of the present research showed no significant relationship between each of the mental health components and the total score of job satisfaction. There was also no significant relationship between the total scores of job satisfaction and mental health. However, there was an inverse and significant relationship between the two components of job satisfaction and some mental health components, that is between the payment system with physical complaints, interpersonal sensitivity, phobia, and the overall mental health score, and also between the type of job with obsessive-compulsive and paranoid thought.

Kahe et al. revealed a statistically significant relationship between overall job satisfaction with employees' mental health, and people with high job satisfaction obtained a better mental health score (below average) (p=0.02). In other words, the lower the level of mental health, the lower the job satisfaction and vice versa [36].

Table 1: Correlation matrix of job satisfaction, life satisfaction, and mental health (Spearman correlation)

Variable			*Correlation*	
Variable	**Life satisfaction**	**Mental health**	**Meaningful**	
Job Satisfaction	0/267	-0/063	r	*1*
	*0/001	0/428	P	-
Life satisfaction	1	-0/294	r	
	-	*0/001	P	
Mental health		1	r	
		-	P	

Table 2: Relationship between job satisfaction, life satisfaction, and mental health according to demographic characteristics (Kruskal Walli's)

Variable		Mental health				Life satisfaction				Job Satisfaction			
		P	df	K	Mean	P	df	K	Mean	P	df	K	Mean
Gender	Man	0/001	1	11/75	64/73	0/037	1	4/34	70/92	0/262	1	1/25	75/34
	Female				90/48				86/56				83/77
Age	Less than 25 years	0/420	3	2/82	79/84	0/209	3	4/53	76/97	0/181	3	4/87	85/39
	26-29 years				86/58				87/72				86/63
	30-34 years				79/29				66/21				75/57
	More than 34 years				69/50				82/90				65/83
Marital status	Single	0/64	1	0/213	82/11	0/034	1	4/47	73/14	0/071	1	3/26	86/79
	Married				78/72				88/63				73/55
Number of children	0	0/357	3	3/23	82/43	0/647	3	1/65	78/47	0/202	3	4/61	85
	1				62/61				93/5				66/72
	2				82/13				81				65/5
	3				91/17				80/83				71/83
Type of house	Rental	0/227	1	1/46	87/68	0/927	1	0/008	79/95	0/836	1	0/043	81/73
	Personal				77/78				80/71				80/03
Work experience	Less than 3 years	0/105	4	7/64	89/33	0/985	4	0/37	81/09	0/017	4	11/98	94/17
	6-3 years				83/14				79/43				82/29
	9-6 (9-12) years				60/93				86/36				65/5
	< 3 years, > 12 years				50/83				74/5				53/5
	6-3 years				74/14				79/93				71/63
Level of Education	Masters	0/198	1	1/65	82/18	0/423	1	0/64	81/54	0/014	1	6/07	83/71
	MSc				67/28				72/28				55/17
Section	Public section	0/265	3	13/45	83/08	0/254	3	13/63	68/24	0/001	2	55/91	64/24
	Special section				76/95				89/79				91/39
	Emergency room				85/4				79/3				66/75

Veysey et al. showed an inverse relationship between the general health questionnaire and job satisfaction and a direct relationship with the increase in cases of stress [37]. In her study in teaching hospitals of Hormozgan, Mastaneh et al. (2013) also showed a significant negative correlation between mental health pay satisfaction ($p<0.005$) and type of work ($P<0.01$) [38].

Sarafis et al. also showed a significant relationship between the stress management domain of health-promoting lifestyle and the payment system domain of job satisfaction ($p< 0.005$) [39].

Ghasemipirbalooti et al. found a statistically significant relationship between mental health and job satisfaction of the employees of Shahrekord University of Medical Sciences ($p=0.001$) [40].

Mei et al. (2015) investigated the effect of psychological characteristics of employees on their performance and job satisfaction and the indirect effect of feelings on their relationships. They concluded a positive and direct relationship between job satisfaction with psychological characteristics and positive feelings of employees [41].

Many studies have shown a direct relationship between mental health and job satisfaction [42-47].

The results of this study show an inverse and significant relationship between life satisfaction and each of the components of mental health except for the "paranoid thought" component. The results also show that the level of mental health of nurses increases and mental injuries decrease with increasing life satisfaction scores. Salimi also showed in research titled "Investigating mental health and its relationship with job burnout and life satisfaction of employees" that there is a significant relationship between mental health and life satisfaction.

The consistency between the results of the present research with Salimi's study is attributed to the correlation between mental health and life satisfaction. That is, life satisfaction prerequisite for mental health, and if there is no satisfaction, negative mood and despair dominate the person and make him/her susceptible to all kinds of mental illnesses [48].

In a study on nurses in Ahvaz government hospitals, it was found that life satisfaction is correlated with high mental health (Birgani et al.). The higher the level of life satisfaction, the more likely a person is to experience positive emotions and feelings [49]. Geravand also showed in a study that mental health has a direct effect on life satisfaction.

Changes in mental health and emotions can lead to changes in life satisfaction and a person's attitude [50]. The results of various research show a significant relationship between mental health and life satisfaction. The results of Ferguson's study indicated the interaction between mental health and life satisfaction [51].

In a study of the level of job satisfaction of nurses in hospitals affiliated with Kashan University of Medical Sciences and related factors, Tagharobi et al. showed a statistically significant relationship between the level of education, major stress in the last six months, the workplace, the level of life satisfaction, mental health satisfaction health, pay satisfaction, satisfaction with the number of employees in each shift, satisfaction with the feedback of patients and their families, satisfaction with the treatment and performance of doctors, satisfaction with the treatment and performance of head nurses and colleagues, satisfaction with the treatment and performance of nursing office managers, satisfaction with workplace amenities and happiness with job satisfaction [52].

As was pointed out by Grant, nurses are among the most vulnerable working group in society, and their health and personal-family life balance are strongly related to occupational variables. Work and family life conflict and subsequent stress cause psychological symptoms such as higher stress, depression, chronic physical problems, increased physical complaints, lower life satisfaction, low quality of family life, and low energy levels in these people [17].

CONCLUSION

According to the results of the present study, nurses' life satisfaction score increases with the increase in their job satisfaction score. But there is no significant relationship between the total mental health score and the total job satisfaction score. However, there is an inverse and significant relationship between mental health and life satisfaction. On the other hand, the level of mental health of nurses increases, and mental injuries decrease with the increase in life satisfaction scores. Considering the vital role of nurses in achieving the goals of the health system and public health, neglecting the job dissatisfaction of nurses may have consequences such as reducing productivity, reducing the level of responsibility, reducing the level of quality of their performance, reducing life satisfaction and increasing the number of psychological injuries. Job satisfaction is effective in creating motivation, efficiency, responsibility, and social accountability in the personnel of any organization. Job dissatisfaction can lead to complications such as job burnout, illness, absenteeism, the job left, leaving many nursing jobs, and the reluctance of the young workforce to attend the wards. Therefore, it is necessary to make further efforts in this field by healthcare policymakers.

Acknowledgments
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Ethics approval and consent to participate
IR.MEDILAM.REC.1400.229
Informed written consent was obtained from all participants included in the study. All participants were informed about the purpose of the study and assured of confidentiality and anonymity. The respondents were informed of their freedom to withdraw from the study at any time.

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Original Article

Survey the Effect of Educational Intervention Based on Mobile Short Message Service (SMS) on Self-Care in Patients with Type 2 Diabetes Referring to the Diabetes Clinic in Khorram Abad City

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Abstract: *Introduction: Self-care behaviors are very important to control type 2 diabetes. The current study was conducted aiming at determining the effect of the educational intervention based on mobile short message service on the self-care behaviors in patients with type 2 diabetes in Khorram Abad. Methods: This study was a semi-experimental study on 191 patients with type 2 diabetes (45case and 45 controls). Data collection tools included a demographic information form, International Physical Activity Questionnaire, Self-Care Scale, and Self-Efficacy Scale. Three to four educational messages were sent daily through mobile phones as an educational intervention. Data were analyzed with SPSS V24 at a significance level of 0.05. Findings: The data analysis indicated that the average score of self-care, self-efficacy, and blood sugar was significantly higher in the case group than in the control group before and after the intervention (P<0.001). However, in terms of the physical activity variable, this relationship was not statistically significant in the case and control groups. Conclusion: Educational interventions to empower diabetic patients by strengthening their self-care can be an effective way to improve the health of diabetic patients. Therefore, it is suggested to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.*

Keywords: *self-care, self-efficacy, physical activity, blood sugar, type 2 diabetes*

INTRODUCTION

Diabetes is the most common disease caused by metabolic disorders, which is mainly characterized by a chronic increase in blood sugar and disturbed carbohydrate, fat, and protein metabolism. In general, diabetes is a multifactorial disorder diagnosed by a chronic increase in blood sugar or hyperglycemia (according to diagnostic criteria) and is caused by a disorder in insulin secretion or function or both [1]. Retinal damage and blindness, peripheral neuropathy, leg pain, stroke, peripheral vascular problems, end-stage renal disease, and limb amputation are among the most serious complications of diabetes [2]. The most common type of diabetes is type 2 diabetes, which accounts for approximately 90-95% of diabetes cases and is usually associated with metabolic disorders, weight gain, and physical inactivity [3]. About 422 million people worldwide suffer from diabetes, the majority of whom live in low- and middle-income countries, and in 2019, diabetes was introduced as the direct cause of 1.5 million deaths [4]. According to the International Diabetes Federation (IDF), the number of diabetic patients will reach 439 million people in 2030, and the prevalence of diabetes in developing countries will be 69% [5]. More than 3 million people with diabetes live in Iran [6]. By 2030, Iran will be one of the most prevalent regions in the world in terms of diabetes [7]. Paying attention to the chronic and costly nature of this disease for the general health of society and the creation of a large financial burden, it is essential to pay serious attention to this problem and its consequences [8]. According to the results obtained from the studies, the long-term complications of this disease, including neuropathy, nephropathy, and retinopathy can be prevented, or at least the occurrence of these complications can be postponed if blood sugar is controlled [9, 10]. Many studies have found physical activity and exercise to be effective in reducing blood sugar levels and controlling diabetes [11,12]. The World Health Organization considers education to be the basis of diabetes treatment. Therefore, self-care is one of the important topics of diabetes treatment. Self-care is a learned regulation function in humans, which is based on the ability of people to perform care actions on themselves [13]. It includes the behaviors of following a proper diet, taking medicine on time, self-monitoring of blood sugar, performing regular physical activity, and physical care of the feet [14]. Studies show that 67% of patients with type 2 diabetes do not monitor their blood sugar according to the recommendations. Also, only 19-30% of diabetic patients use the recommended physical activities and compliance with nutritional behaviors in these patients is 65%, and only 7% of diabetic patients fully adhere to all aspects of self-care behaviors [15, 16]. Self-efficacy is also known as a strategy to improve self-care in diabetic patients, which results in self-confidence and adherence to care recommendations to control blood sugar in these patients [17].

Nowadays, to follow up and educate diabetic people, new communication systems are required to direct the focus of care and treatment from the clinic to the patient's daily life. Researchers have pointed out the use of mobile phone service as a method of education and follow-up in chronic diseases, that short message is one of the main functions of mobile phone in the education of diabetic patients [18,19].

Considering the importance of self-care in controlling the blood sugar of diabetic patients and the value of self-efficacy as one of the self-care approaches, it seems necessary to design educational interventions on the platform of mobile phones and in the form of short messages as a relatively new educational method in controlling the disease of diabetics. Thus, the present study was conducted to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad.

MATERIALS AND METHODS

The present study was a semi-experimental intervention research with a pre-test and post-test design. The sample size was specified as 90 people who were selected from the research population of patients with type 2 diabetes referring to the diabetes clinic in Khorramabad, Lorestan province. The participants were selected according to the inclusion criteria using random sampling. Then, they were divided into case and control groups using a simple randomization method (45 ones in each group). The inclusion criteria include informed consent to participate in the research, having a minimum level of literacy and understanding of the content sent through text messages, having a fixed personal mobile number to receive educational SMS, lack of any serious diabetic complication, and not having special diets because of specific diseases or physical problems limiting physical activity. Exclusion criteria include suffering from serious complications of diabetes during the study, suffering from diseases that limit physical activity or nutrition, and the patient's unwillingness to receive educational messages.

Data collection tools in this study included a demographic information form, International Physical Activity Questionnaire (IPAQ), a Self-Care Scale developed by Tubert, and Self-Efficacy Scale. Demographic information included: age, height, weight, gender, educational level, employment status, marital status, and type of treatment. The IPAQ with seven items measured the duration of physical activity in the last week in four categories: moderate physical activity, intense physical activity, walking, and sitting activities. According to the scoring protocol in this questionnaire, the intensity of physical activity based on the ratio of energy consumption in terms of metabolic equivalent of task (MET) in the last seven days is classified into three levels: low, medium, and high. It was proposed by the World Health Organization and the Centers for Disease Control and Prevention in 1998 to measure the physical activity of people aged 15 to 69 years and its validity and reliability have been confirmed in 12 countries worldwide, including Iran [20,21]. A self-care questionnaire was used in this study that included 15 items in the areas of diet compliance, physical activity, blood sugar monitoring, insulin injection or diabetes pill consumption, foot care, and smoking, which was translated and standardized by Hamdzadeh [22,23]. The self-efficacy questionnaire, which is one of the constructs of the standard Health Belief Model Questionnaire, validity and reliability of which have been confirmed [24].

After presenting a letter of introduction from the Iran University of Medical Sciences and obtaining the necessary permits, the researcher referred to a diabetes clinic in Khorram Abad and selected 90 people from among 200 eligible people using random sampling. People who were willing to participate in the study and met the inclusion criteria were assigned to the case and control groups (45 in each group) using a simple randomization method. After random allocation, blood sugar samples were taken in all groups four days before the start of the intervention. In the first session, the researcher explained the purpose of the meeting to the participants, and written informed consent was obtained. Then, the personal information form and questionnaires were completed by the case and control groups. The case group received an educational program in the form of short educational messages via mobile phone, for four weeks and on average, at least three to four messages per day related to the educational content, including exercise, self-monitoring of blood sugar, diet, insulin therapy, medication use, and foot care. For example, short messages about physical activity and blood sugar monitoring were “for people with diabetes, walking is the best exercise” or “it is better for people with diabetes to measure their fasting blood sugar after waking up”. To improve self-efficacy, encouraging messages about adherence to self-care were used.

Finally, after two months, the same questionnaires (demographic, physical activity, self-care, and self-efficacy scales) were distributed and completed by the participants, and the blood sugar levels of the two groups were measured again. During this period, the control group did not receive any educational intervention.

However, to comply with the ethical principles, an educational CD containing self-care training was given to the control group after completing the research. The data of the two groups before and after the intervention were entered into SPSS version 24 software and analyzed using Wilcoxon, Mann-Whitney, Chi-square test, independent t-test, and paired t-test at a significant level of 0.05.

RESULTS

The participants’ average age was 51.01 ± 0.967, and the average height and weight were 164.57 ± 1.031 and 75.69 ± 1.101, respectively.

Before the implementation of the educational program, the case and control groups were matched in terms of demographic variables, including age, height, weight, gender, education, employment status, marital status, and type of treatment, and no statistically significant difference was observed between the two groups (Table 1).

Table 1: Comparison of the absolute and relative frequency distribution of demographic variables in case and control groups

Variable		Case		Control		P value
		No.	%	No.	%	
Gender	Female	31	69	26	57/7	0.796
	Male	14	31	19	42/2	
Employment	Unemployed	5	11	2	4	0.686
	Housewife	26	58	24	53	
	Self-employed	9	20	9	20	
	Public	5	11	10	22	
Education	Illiterate	5	11	4	9	0.479
	Primary school	17	38	10	22	
	Secondary school	9	20	5	11	
	High school	4	9	11	24	
	High school diploma	4	9	10	22	
	Higher education	6	9	5	11	
Marital status	Single	2	4	1	2	0.800
	Divorced	1	2	0	0	
	Widow	9	20	5	11	
	Married	33	73	39	87	
Type of treatment	Insulin	21	47	21	47	0.920
	Oral medication	20	45	22	49	
	Diet	4	9	2	4	
	Exercise	0	0	0	0	

As observed in Table 2, there is no statistically significant difference in terms of the average score of physical activity, self-care, self-efficacy, and blood sugar between the case and control groups before the intervention, while after the intervention, the average scores of these variables, except for physical activity (P=0.8), was significantly higher in the case group compared to the control group (P<0.001). In the control group, before and after the intervention, no significant difference was observed in the average scores of physical activity, self-care, self-efficacy, and blood sugar (P>0.05).

Table 2: Comparison of the average scores of physical activity, self-care, self-efficacy, and blood sugar, before and after the intervention in case and control groups

Scale	Group	Case	Control	p-value
		Mean ± SD	Mean ± SD	
Physical activity	Before intervention	677.5±954.938	307.5±461.75	0.6
	After intervention	903.75±973.125	655±925.625	0.8
	p-value	0.8	0.4	_
Self-care	Before intervention	2.31±0.813	2.38±0.844	0.12
	After intervention	3.38±0.688	2±0.906	0<0001
	p-value	0<001	0.22	_
Self-efficacy	Before intervention	2.7±0.4	2.7±0.3	0.15
	After intervention	3.7±0.7	2.7±0.7	0<0001
	p-value	0<001	0.286	_
Blood sugar	Before intervention	180±80.5	185±51	0.8
	After intervention	170±87.5	180±70.5	0<001
	p-value	0.009	0.491	_

Table 3: Comparison of blood sugar scores before and after the educational intervention in the case group

Method of treatment	Blood sugar level before the intervention	Blood sugar level after the intervention	Level of change
Insulin	227.09	217.92	-9.17
Oral medicine	173.48	170.65	-2.83
Diet	181	180.44	-0.56
Exercise	194	194.5	0.5
Medium	193.89	190.88	-3.02

Table 3 indicates that the blood sugar level in all patients with different treatment methods (insulin, oral medication, diet) in the case group reduced after the educational intervention compared to before intervention, except for the patients who only used exercise to control their blood sugar.

DISCUSSION

The present study was conducted aiming to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad. The research findings suggest that the educational intervention through mobile phone short messages improved self-care in the case group, which is consistent with findings by Abaza et al. [25] and Agbede et al. [26] on the effect of the intervention based on mobile phone short messages in the self-care of diabetic patients and the findings by Yari et al. [27] and Rahnavard et al. [28] concerning SMS-based and self-care education of diabetic patients. Krishna et al. [29] reported that technologies such as mobile phones and text messages, which are currently part of people's daily lives, have the potential to influence self-care and improve people's health. The approach of health education through SMS to promote self-care in diabetic patients is more welcomed than traditional approaches that require reading pamphlets or attending lectures [25,30].

In this study, self-efficacy also there was a significant difference in the case group in terms of self-efficacy after the educational intervention. Katz and Nordwall [31] and Peymani et al. [32] also found that interventions based on mobile phone messages improve self-efficacy and self-care. Similar studies [33,34] regarding the self-care of diabetic patients also indicate a significant difference in the average score of self-efficacy and self-care in the case group compared to the control group after the educational intervention. These studies are consistent with our work in that increasing self-efficacy causes the adoption of self-care behaviors in diabetic patients. Self-efficacy is an important prerequisite for behavior change [35]. An individual with low self-efficacy is less likely to make an effort to perform a new health behavior or to change the behavior he is used to [36]. Researchers believe that self-efficacy has a suitable framework for understanding and predicting and patient's commitment to self-care behavior in the treatment of diabetes [37].

The results of the present study showed that education through mobile phone short messages does not affect increasing physical activity in the case group, which is in line with the findings by Bakhshian et al[38] regarding the effect of education through mobile phone short messages on self-care of patients with type 2 diabetes. It is also in agreement with the findings by Kim et al. [39] on adherence to diabetes control recommendations, while our findings are not consistent with the study of Paul et al. [40] regarding the use of software that can be installed on mobile phones to improve the amount of daily walking in patients with a history of stroke and the study Martha et al.[41] concerning mobile phone SMS intervention on diabetes self-care activities, cardiovascular disease risk awareness, and food choices among type 2 diabetic patients. This discrepancy can be due to the lack of sports facilities and parks in the current study or the addition of chronic diseases to the patients participating in this research, as well as social and cultural factors that prevent people from doing physical activities in public environments. In the present study, since the number of women is more than men, the problem of social and cultural factors could be more marked.

The use of mobile phone messages in the current study showed a significant positive effect on the reduction of blood sugar in the case group after the educational intervention, which is consistent with the findings of similar studies in this field [42,43,44]. In patients suffering from diabetes, education is as valuable as medicine, exercise, and diet, because the treatment will never be effective unless the patient is well aware of the nature of his disease and takes positive steps to deal with it.

CONCLUSION

The results of this study demonstrated that educational interventions based on mobile phone short messages can be effective in enhancing self-care in patients with diabetes. Educational interventions aimed at empowering diabetic patients by strengthening self-care in them can be an effective way to improve the health of patients with diabetes. Therefore, it is recommended to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research.

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Authors' contribution

All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Vice President of Research and Technology of Iran University of Medical Sciences with code IR.IUMS.REC.1397.501. Informed consent was obtained from all individual participants included in the study before conducting.

Consent to publish

All participants consented verbally to the publication of the interview data.

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Original Article

A Comparative Study of Patients' Trust in Physicians and Nurses in COVID-19 and Non-COVID-19 Wards in a Reference Hospital in Tehran, Iran

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Abstract: *Trust in health care professionals is considered as a basis for patients' care. This study aimed to compare the level of patients' trust in physicians and nurses in both general and COVID-19 wards in a reference hospital in Tehran during the pandemic. This was a cross-sectional descriptive study. The study population comprised patients from Baqiyatallah Hospital in 2022. The data collection tool was the scale of patients' trust in nurses and the Wake-Forest physician trust scale. Data analysis was performed using SPSS-26 software. The level of patients' trust in physicians in COVID-19 wards was significantly lower than in Non-COVID-19 wards (P<0.001), but there was no difference between the two groups in the trust of nurses. The lowest level of trust was in the receiving of required information from nurses, which had a lower score compared to other questions in the questionnaire, and the average amount of receiving required information for patients in general wards was significantly lower than that of patients in COVID-19 (P=0.006). Trust in nurses and physicians in both COVID-19 and general wards was optimum. During the COVID-19 pandemic, the medical staff worked tirelessly and despite the difficulties and complications of the pandemic, patients have high trust in physicians and nurses.*

Keywords: *trust; nurses, physicians; COVID-19; coronavirus*

INTRODUCTION

Physicians and nurses, as the main members of the medical staff, take on different roles, considering the physical, psychological, and social needs of patients. They may act as educators, counselors, mentors, informers, advocates, and act as experts. To play such roles, physicians and nurses need to establish a good relationship with their patients [1,2]. Trust forms the basis of the relationship between the physician or nurse [3] and patient and provides the conditions for the physician or nurse and patient to work together for a better care plan [4]. The relationship between trust and health outcomes is different in individual studies [5]. For example, in a sample of patients with diabetes, trust in a healthcare professional was positively associated with objective and mental health outcomes (glycemic control, health-related quality of life, and patient satisfaction] [6].

The hypothetical relationship between trust and health outcomes is also reinforced by the ethical claims derived from trustworthy and patient-centered relationships in clinical settings [7]. Studies show that patients who have more trust in their therapist are more satisfied with the care and are more likely to follow treatment recommendations [8,9]. Patients' experiences of trust in nursing depend on nurses' knowledge, and the commitment to conversation to build and develop relationships [10]. Therapeutic staff must receive appropriate training to build a bond of trust between themselves and the patient to establish a trustable relationship between themselves and the patient [11]. Trust between patient and therapist is essential to reduce patients' anxiety and build the capacity to control them [12]. The COVID-19 epidemic has created special conditions in the relationship between the medical staff and patients [13]; furthermore, the need for social distancing and the use of protective equipment on the one hand, as well as an increasing number of patients, especially those with severe illnesses, epidemics, illnesses and dementia and lack of time to provide medical care, on the other hand, has created a different kind of doctor-nurse relationship with the patient that may affect patients' trust [14]. Considering the importance of patients 'trust in physicians and nurses in adhering to treatment and the effectiveness of medical care, this study was designed to compare the level of patients' trust in physicians and nurses in general and COVID-19 wards in a reference hospital in Tehran during the pandemic.

MATERIALS AND METHODS

This study was descriptive and cross-sectional. The study population consisted of patients admitted to both the general and COVID-19 wards of Baqiyatallah Hospital, Tehran, Iran in 2022. Inclusion criteria included the age range of age: 20-70, hospitalization for more than three days in hospital wards, having no serious psychological disorders, and submitting consent to participate in the study. The data collection tools included the patient's trust scale in the nurse and the Wake-Forest physician trust scale. The Nurse Trust Scale has been validated by Nouripour et al [15]. The Physician Trust Questionnaire has been validated by Forati et al [16].

The scale of patient trust in the nurse contains five questions about Responding to the need, Usefulness of measures, Trust in measures and Practice of treatment" and Giving sufficient information to the patient by the nurse with the scales Never, Rarely, Sometimes, Most of the time, Usually, Always". The Physician Trust Questionnaire includes nine questions about Trust in Skills, Treatment Selection, and Physician Decisions on a scale of "Strongly Agree, Agree, Neither Agree Nor Disagree, Strongly Disagree and Disagree".

Patients with more than three days of hospitalization were randomly selected from the ward list. First, patient satisfaction with the study was obtained and then a questionnaire about patients' trust in physicians and nurses was filled in by an interviewer. Data analysis was performed using SPSS-26 software. Descriptive analysis was performed with mean, standard deviation, frequency, percentage, and analytical analysis depending on the type of data with t-test and Chi-square test.

RESULTS

Four hundred patients participated in this study. Descriptive values related to the age of these patients by group and in general are displayed in Table 1. The mean age of patients in the COVID-19 group was 53.3 years and in the Non-COVID-19 group was 53.8 years. This study included 257 male patients (64.3%). In addition, the sex distribution was the same in the two groups of patients with COVID-19 and Non-COVID-19 and no significant difference was observed in the sex distribution of the two groups (P=0.132).

Table 1: Descriptive characteristics of the patients admitted to Covid-19 and non-Covid-19 wards

Variable		COVID-19	Non-COVID-19	P Value
Age (Mean ±SD]		5.3 ± 14.53	3.8 ± 17.53	0.11
Sex	Male	120	137	13.0
	Female	79	65	
Education	Less than a Bachelor's degree	99 (54.1 %)	147 (8.72 %)	<0.001
	Bachelor degree	57 (1.31 %)	49 (3.24 %)	
	Senior	22 (0.12 %)	5 (5.2 %)	
	PhD	5 (7.2 %)	1 (5.0 %)	

In total, 198 patients (49.5%) with COVID-19 and 202 (50.5%) Non-COVID-19 patients were considered in this study. The overall mean of trust in nurses in the COVID-19 group was not significantly different from that of the Non-COVID-19 group.

Most people in both COVID-19 (85.3%) and Non-COVID-19 (86.6%) groups reported that nurses were available, most of the time or always when they needed them.

Most patients in both COVID-19 (99.4%) and Non-COVID-19 (97.4%) groups believed that the nurse's activity was useful to them, most of the time or always. The mean usefulness of therapeutic activities in patients in general wards was 0.1 higher than in patients in COVID-19 wards but this difference was not significant (P>0.05).

Most patients in both COVID-19 (99%) and Non-COVID-19 (95%) groups believed that they trusted the care of nurses always. The average level of trust in medical care for patients in general wards was 0.1% lower than that of patients in COVID-19 wards. But the difference was not significant (P>0.05).

In the COVID-19 group, 93% of patients often or always followed the advice of the nurses, while in the Non-COVID-19 group, 96.5% of patients most of the time or always followed the statements and recommendations of the nurses. The mean rate of treatment of patients with therapeutic recommendations in general wards was significantly higher (0.4) than patients admitted to the COVID-19 ward (P<0.001).

In the COVID-19 group, most patients (71.8%) believed that most of the time, or always, nurses provided enough accurate information about their disease. While in the Non-COVID-19 group, about half of the patients (55%) believed that most of the time or always the nurses provided them with sufficient accurate information about their disease. The average amount of information required for patients in general wards was 0.3 less than for patients admitted to the COVID-19 ward. There was a significant difference in the amount of information required for patients in general wards and COVID-19.

Table 2: Descriptive values related to the trust variables of the patients admitted in the Covid-19 and non-covid-19 wards

Variable	COVID-19			Non-COVID-19		
	SD ± Min	Min	Max	SD ± Min	Min	Max
Responsibility for patient needs	4.6 ± 1.1	2	6	4.6 ± 1.1	2	6
The usefulness of therapeutic activities	8.2 ± 0.5	3	6	8.3 ± 0.5	1	6
Reliability of medical care	3.5 ± 8.0	3	6	2.5 ± 1.1	1	6
Following the recommendations	5±0.9	3	6	4.5±0.9	2	6
Patients' access to the necessary information	4.2± 1.3	1	6	3.9± 1.6	1	6
Satisfaction with the nurse	7.0 ± 9.4	2.6	6	7.0 ± 9.4	2.6	6
Satisfaction with the physicians	3.5±0.3	2.8	4.3	3.7±0.3	2.2	4.5

DISCUSSION

Patients’ trust in nurses and physicians is important and at the same time complex phenomenon that to achieve it, some professional characteristics must be created and strengthened and some ethical principles must be observed [17]. In the study of patients' trust in nurses of Baqiyatallah Hospital, Tehran, Iran, we used the questionnaire of patients’ trust (P=0.006). The results of the study assessing the patients’ trust in the physicians showed that most patients participating in the study in both groups of COVID-19 (92.4%) and Non-COVID-19 (97.5%) had a high level of trust in physicians. Considering the mean difference, it can be said that the average trust in physicians in COVID-19 patients was significantly lower by 0.14 than in Non-COVID-19 patients (P<0.001).

Ravdin and Cabral nurses, which was validated by Nouripour et al [15]. This questionnaire had five questions, and in what follows, we will review the results obtained from the questions.

The level of responding to patients' needs and being available to them when needed, the level of use of treatment activities, and the level of trust in medical care in both general and COVID-19 wards were desirable. The rate of follow-up to treatment recommendations was favorable in both groups, but the mean rate of follow-up to treatment recommendations of patients in general wards was significantly higher than patients admitted to the COVID-19 ward. Studies have shown that there is a positive relationship between the level of trust in the nurses and the level of treatment adherence [18]. Trust in the health care specialist has been proposed as a basis for effective and basic treatments for patient-centered care [19-21]. However, the novelty of COVID-19 and the fact that there is still no definitive cure for the disease is a problem noted in the study by Baker [22]. The lack of definitive treatment and protocol for this disease and frequent changes in health protocols during this period may reflect scientific uncertainty; meanwhile, the uncertainty of scientific evidence reduces patients' trust to follow the recommendations of medical staff compared to other diseases and overshadows their treatment adherence.

In this study, the lowest level of trust was in the average amount of information required for patients, which had a lower score compared to other questions in the questionnaire, and the average amount of information required for patients in general wards was 0.3 less than patients in the COVID-19 ward. Nurses in both the COVID and general wards have the lowest level of patients’ trust in providing the necessary information. In Ozaras and Abaan study, the use of medical terms or terms that the patient does not fully understand, the lack of anticipation or understanding of patients' information needs, and neglecting responsibilities and factors related to nursing work such as busyness, etc. have been mentioned as important causes in reducing the provision of the necessary information to patients [23]. It seems that to improve the provision of information to patients, some programs should be implemented in the hospital and the nursing department.

The level of patients' trust in physicians in COVID-19 wards was significantly lower than in Non-COVID-19 patients. Gupta et al. showed that the pandemic coronavirus disease 2019 (COVID-19) has posed new threats to the trust in medical staff [24]. Factors such as the high prevalence and involvement of doctors during the epidemic peaks, as well as the specific way of communicating with the patient (personal protective equipment and social distancing, etc.] and that the patient does not even know and see the face of the treating physician well, are involved in this issue [25]. Identifying the causes of this difference requires further study and investigation of the issue from different respective.

CONCLUSION

Trust in nurses and physicians in both COVID-19 and general wards was optimum. This finding indicates that during the COVID-19 pandemic, the medical staff worked tirelessly and despite the difficulties and complications of the pandemic, patients have high trust in physicians and nurses.

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Original Article

Covid-19 Risk Perception in a Sample of Iranian Population

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Abstract: *How people behave in an emergency depends on many social, cultural, and contextual factors. This study aimed to determine the COVID-19 risk perception in Iranian people. This study was descriptive. The study population was the assistant of patients who had come to Baqiyatallah Hospital in 2021. The number of participants was 500. The study tool was the COVID-19 risk assessment questionnaire. Data were analyzed using SPSS-22 software. The mean risk perception score was 2.78(±0.6) on a 7-point scale. The lowest and highest mean of the predictors of risk perception in COVID-19 was trust in government and personal efficacy, respectively. There was a significant difference between men and women in the mean of collective efficacy (P= 0.032). Social knowledge (p=0.007), trust in science (p=0.001), and trust in medical professionals (p=0.050) in positive direct experience people were significantly more than in people with negative direct experience of COVID-19. The most important predictors of risk perception in the COVID-19 epidemic were gender, education, social knowledge, and trust in science (P<0.05). People's perception of the risk of COVID-19 depends on several various factors, the most important factors were gender, level of education, social knowledge, and people's trust in science.*

Keywords: *COVID-19; coronavirus; risk perception*

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and has spread around the world quickly, emerging as a significant challenge of the current century for public health, the economy, markets, and policies in many countries [1].

The coronavirus outbreak was declared a public health emergency of international concern by the World Health Organization on 30 January 2020. Today, most people around the world have understood the outcomes of COVID-19 infection and they have accepted the need for hand hygiene and social distancing to prevent its spread. But, some of them ignore or delay hygienic actions, government laws, and social distances in public places, or homes. The fact that individuals in the same situation of common challenge act so differently indicates that the persons' risk perception relating to this new and chronic challenge is strongly different [2].

How people behave in an emergency depends on their understanding of the severity and depth of the risk and the extent of their vulnerability, but many social, cultural, and contextual factors may influence their risk perception [3]. Little is known about how people perceive the risk associated with emerging infectious diseases compared to other areas of risk. Risk perception leads to the adoption of preventative health behaviors so misperception of COVID-19 risk in this condition can lead to negative consequences of pandemic control [4].

Personal experience with the virus, individualistic and prosocial values, hearing about the virus from friends and family, trust in government, science, and medical professionals, personal knowledge of government strategy, and personal and collective efficacy were all significant predictors of risk perception, in the first global assessment of public risk perception of COVID-19 [5].

Previous studies have examined differences in risk perception of COVID-19 in diverse populations and races. Health workers reported higher risk perception, level of worry, and knowledge as related to COVID-19 infection compared to the general population [6]. In some studies, females were found to perceive risks higher, complied more with the government's guidelines, and coped better than males in response to COVID-19 [7]. But Self-reported anxiety over the pandemic is a crucial predictor in explaining risk perceptions in all age and gender groups [8]. And overall public initial concerns and trust can play an essential role in improving the perceived risk of a pandemic and increasing public participation in adopting preventive measures [9]. This study aimed to determine the risk perception score and its related factor in Iranian people.

MATERIALS AND METHODS

This study was descriptive-analytical. The study population was the assistant of patients who had come to the clinic of Baqiyatallah Hospital in 2021. The inclusion criteria were 15 to 80 years old, no serious and acute illness, and consent to participate in the study. The number of participants in the study was 500, with a 5% alpha error and 20% beta error. The study tool was the COVID-19 risk assessment questionnaire, which was designed by Dryhurst [10] using previous studies to identify all dimensions of risk perception covering affective, cognitive, and temporal-spatial dimensions to provide a holistic measure of risk perception. The questionnaire included items on COVID-19 experience, demographic variables (age, gender, and education) knowledge, both personal and social knowledge, social amplification, prosociality, individualism, trust in l three domain of science, medical professionals, and government, personal efficacy, and collective efficacy. The validity of the COVID-19 risk assessment questionnaire was assessed using face and content validity approaches. The impact score of each item was assessed to evaluate the face validity. The questionnaire was distributed among 10 technical experts who were asked to evaluate items based on the mentioned criterion. Then the importance of each item was calculated using the impact score formula and the results were compared with the standard score (1.5), and items whose scores were higher than the standard score were included in the questionnaire. Next, the content validity of the instrument was analyzed using the Content Validity Ratio (CVR) and Content Validity Index (CVI). 10 respondent experts also participated in this phase. Experts were asked to group items as "essential", "essential but not useful", or "not essential" to calculate CVR. Their responses were calculated based on the CVR formula and the results were compared with Lawshe's Table. Numbers higher than 0.6 were approved. CVI survey was done based on the Waltz and Basel content validity index [11]. A questionnaire was distributed among the experts who were asked to rank 24 items based on a four-part Likert scale as compatible with 3 criteria of relevancy, simplicity, and clarity. (For example, unrelated items, partially related items, related items, and fully related items were scored 1, 2, 3, and 4, respectively). Items higher than 0.6 were approved according to the CVI score. Cronbach's alpha method was used to assess the questionnaire's reliability. Data collection was started after the standardization of the questionnaire.

Patients completed the study questionnaire, after written consent. The questionnaire was filled out using the interview method and by a trained interviewer. All information obtained from patients was confidential and the study received an ethics code from the ethics committee of Baqiyatallah Hospital. Primary data were analyzed using SPSS-22 software. Descriptive data analysis was performed using mean and standard deviation in quantitative data and frequency and percentage in qualitative data. Analytical analysis was performed according to the normality of the data with parametric and non-parametric tests. Linear and logistic regression were used to measure the relationship between variables according to the type of data.

RESULTS

The validity and reliability of the instrument of this study

In the first step, the COVID-19 risk assessment questionnaire was validated. Tables 1 and 2 show that the scores of all items of the questionnaire were higher than the criterion score (i.e. 1.5) and experts approved the quantitative face validity of all items. Moreover, the results of CVI indicate that all items of the questionnaire obtained a score higher than 0.6, and content validity was considered appropriate by experts. The reliability of the questionnaire based on Cronbach's alpha was 0.79, 0.69, and 0.77 for risk perception, individualism, and trust in science, respectively.

Study findings

In the study samples, 500 people participated, of whom 253 (50.6%) were women and 247 (49.4%) were men.

The mean age of participants was 38.9 (±11.7) and 147 (29.4%) of the people have experienced the disease. The descriptive characteristics are listed in Tables 3 and 4.

The mean risk perception score was 2.78(±0.6) on a 7-point scale. The lowest mean of the predictors of risk perception in COVID-19 was in trust in the government and the highest mean was in personal efficacy (Table 4).

Table 1: Results of calculating quantitative face validity (impact score) of items of the designed instrument

Item	Frequency	Importance	Impact score
Q1: How worried are you personally about the following issues at present? Coronavirus/COVID-19	0.7	4.4	3.08
Q2: How likely do you think it is that you will be directly and personally affected by the following in the next 6 months? - Catching the coronavirus/COVID-19	0.6	4.1	2.46
Q3: How likely do you think it is that your family and friends in the country you are currently living in will be directly affected by the following in the next 6 months? Catching the coronavirus/COVID-19	0.6	3.6	2.16
Q4: How much do you agree or disagree with the following statements? – The coronavirus/COVID-19 will NOT affect very many people in the country I'm currently living in	0.5	3.6	1.8
Q5: How likely do you agree or disagree with the following statements? - I will probably get sick with the coronavirus/ COVID-19	0.7	3.8	2.66
Q6: How much do you agree or disagree with the following statements? - Getting sick with the coronavirus/COVID-19 can be serious	0.6	3.3	1.98
Q7: How much do you feel you understand the government's strategy to deal with the coronavirus/COVID-19 pandemic?	0.6	3.5	2.1
Q8: To what extent do you think scientists have a good understanding of the coronavirus/ COVID-19?	0.5	3.6	1.8
Q9: Have you ever had, or thought you might have, the coronavirus/COVID-19?	0.6	3.1	1.92
Q10: Have you come across information about coronavirus/COVID-19 from: - Friends and family	0.6	3.4	2.04
Q11: To what extent do you think it's important to do things for the benefit of others and society even if they have some costs to you personally?	0.7	2.2	1.54
Q12: The government interferes far too much in our everyday lives.	0.8	2.6	2.2
Q13: How much do you trust the country's politicians to deal effectively with the pandemic?	0.6	2.5	1.5
Q14: How much do you trust each of the following? – Scientists	0.7	3.3	2.31
Q15: How much do you trust each of the following? - Medical doctors and nurses	0.6	3.2	1.92
Q16: To what extent do you feel that the personal actions you are taking to try to limit the spread of coronavirus make a difference?	0.6	3	1.8
Q17: To what extent do you feel the actions that your country is taking to limit the spread of coronavirus make a difference?	0.7	3	2.1

Table 2: Content validity index of questionnaires through CVR and CVI

Item	CVR	CVI		
		simplicity	perspicuity	relevance
Q1: How worried are you personally about the following issues at present? Coronavirus/COVID-19	1	0.9	0.9	0.9
Q2: How likely do you think it is that you will be directly and personally affected by the following in the next 6 months? - Catching the coronavirus/COVID-19	0.6	0.9	0.8	0.8
Q3: How likely do you think it is that your family and friends in the country you are currently living in will be directly affected by the following in the next 6 months? Catching the coronavirus/COVID-19	0.8	0.7	0.8	0.8
Q4: How much do you agree or disagree with the following statements? – The coronavirus/COVID-19 will NOT affect very many people in the country I'm currently living in	0.8	0.8	0.8	0.9
Q5: How likely do you agree or disagree with the following statements? - I will probably get sick with the coronavirus/ COVID-19	0.6	0.9	0.9	0.7
Q6: How much do you agree or disagree with the following statements? - Getting sick with the coronavirus/COVID-19 can be serious	0.8	0.8	0.8	0.7

Q7: How much do you feel you understand the government’s strategy to deal with the coronavirus/COVID-19 pandemic?	0.9	0.8	0.7	0.9
Q8: To what extent do you think scientists have a good understanding of the coronavirus/COVID-19?	0.7	0.7	0.8	0.8
Q9: Have you ever had, or thought you might have, the coronavirus/COVID-19?	0.9	0.8	0.7	0.7
Q10: Have you come across information about coronavirus/COVID-19 from: - Friends and family	0.8	0.9	0.9	0.9
Q11: To what extent do you think it’s important to do things for the benefit of others and society even if they have some costs to you personally?	0.6	0.7	0.8	0.7
Q12: The government interferes far too much in our everyday lives.	0.6	0.6	0.6	0.7
Q13: How much do you trust the country’s politicians to deal effectively with the pandemic?	0.9	0.7	0.7	0.8
Q14: How much do you trust each of the following? – Scientists	1	1	1	0.9
Q15: How much do you trust each of the following? - Medical doctors and nurses	1	1	1	1
Q16: To what extent do you feel that the personal actions you are taking to try to limit the spread of coronavirus make a difference?	0.7	0.8	0.7	0.9
Q17: To what extent do you feel the actions that your country is taking to limit the spread of coronavirus make a difference?	0.9	0.7	0.7	0.9

Table 3: Descriptive characteristics of the participants in the study of risk perception of Covid-19

		Frequency	Percent
Gender	Female	253	50.6
	Male	247	49.4
	Total	500	100.0
Education	Under Diploma	37	7.4
	Diploma	149	29.8
	BSc	178	35.6
	MSc	91	18.2
	PhD	42	8.4
	Total	497	99.4
Direct experience with the disease	Yes	147	29.4
	No	353	70.6
	Total	500	100.0

Table 4: The scores of the risk perception questionnaire in participants in the Covid-19 risk perception study

		Number	Minimum	Maximum	Mean	Std. Deviation
Age		497	15	78	38.97	11.731
Risk perception		500	1	4	2.78	0.60669
Personal knowledge		500	1	7	4.78	1.50084
Social knowledge.		500	1	7	4.34	1.43105
Social amplification	Yes	426			85.2*	
	No	74			14.8*	
Prosociality		500	1	7	5.33	1.39061
Individualism		500	1	5	2.73	0.80308
Trust in government		500	1	5	2.20	1.071
Trust in science		500	1	5	3.73	0.73161
Trust in medical professionals		500	1	5	3.94	0.814

Personal efficacy	500	1	7	5.35	1.23006
Collective efficacy	500	1	7	4.02	1.20094

**: Percent*

Next, we ran a Spearman's rho to assess the correlation of the predictors that play a role in COVID-19 risk perception (Table 5). Social knowledge, trust in science, and trust in medical professionals were all significantly associated with risk perception refer to Table 3.

Table 5: The statistics of the predictors of COVID-19 risk perception

		Age	Risk perception	Personal knowledge	Social knowledge	Prosociality	Individualism	Trust in government	Trust in science	Trust in medical professionals	Personal efficacy	Collective efficacy
Age	Correlation Coefficient		.006	.000	.010	.092*	.030	.096*	-.005	.054	.006	.086
	Sig. (2-tailed)		.889	.999	.825	.040	.498	.033	.917	.229	.886	.055
Risk perception	Correlation Coefficient	.006		.044	-.266**	.009	-.078	.018	-.227**	-.156**	-.005	-.006
	Sig. (2-tailed)	.889		.329	.000	.833	.082	.694	.000	.000	.915	.891
Personal knowledge	Correlation Coefficient	.000	.044		-.061	.158**	-.218**	-.069	-.022	.100*	.296**	.069
	Sig. (2-tailed)	.999	.329		.171	.000	.000	.123	.618	.025	.000	.122
Social knowledge	Correlation Coefficient	.010	-.266**	-.061		-.028	.065	.026	.250**	.079	-.039	.097*
	Sig. (2-tailed)	.825	.000	.171		.532	.149	.567	.000	.076	.385	.029
Prosociality	Correlation Coefficient	.092*	.009	.158**	-.028		-.338**	-.031	.131**	.173**	.316**	.069
	Sig. (2-tailed)	.040	.833	.000	.532		.000	.486	.003	.000	.000	.125
Individualism	Correlation Coefficient	.030	-.078	-.218**	.065	-.338**		.052	-.061	-.134**	-.359**	-.122**
	Sig. (2-tailed)	.498	.082	.000	.149	.000		.243	.170	.003	.000	.006
Trust in government	Correlation Coefficient	.096*	.018	-.069	.026	-.031	.052		.088*	.074	-.022	.339**
	Sig. (2-tailed)	.033	.694	.123	.567	.486	.243		.048	.098	.623	.000
Trust in science	Correlation Coefficient	-.005	-.227**	-.022	.250**	.131**	-.061	.088*		.407**	.121**	.128**
	Sig. (2-tailed)	.917	.000	.618	.000	.003	.170	.048		.000	.007	.004
Trust in medical professionals	Correlation Coefficient	.054	-.156**	.100*	.079	.173**	-.134**	.074	.407**		.249**	.179**
	Sig. (2-tailed)	.229	.000	.025	.076	.000	.003	.098	.000		.000	.000
Personal efficacy	Correlation Coefficient	.006	-.005	.296**	-.039	.316**	-.359**	-.022	.121**	.249**		.277**
	Sig. (2-tailed)	.886	.915	.000	.385	.000	.000	.623	.007	.000		.000
Collective efficacy	Correlation Coefficient	.086	-.006	.069	.097*	.069	-.122**	.339**	.128**	.179**	.277**	
	Sig. (2-tailed)	.055	.891	.122	.029	.125	.006	.000	.004	.000	.000	

** Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).*

All predictors were analyzed based on gender variables (the Mann-Whitney U test was used due to abnormal distribution). There was a statistically significant difference between men and women in the mean of collective efficacy (P= 0.032) and the rest were not.

Specifically, people who have had direct personal experience of COVID-19 were different in some predictors of risk perception from those who had no direct experience of disease and social knowledge (p=0.007), trust in science (p=0.001) and trust in medical professionals (p=0.050) in positive direct experience people were significantly more than in people with negative direct experience of COVID-19.

We investigated the determinants of risk perception in regression models. The correlations of demographic variables with risk perception were assessed and gender, as well as education, were significantly associated with risk perception of COVID-19, such that males perceive less risk compared to females (Table 6) and the most important predictors of risk perception in COVID-19 epidemic were gender, education, social knowledge and trust in science (Table 7).

Table 6: Regression output of demographic variables of risk perception in COVID-19

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.991	0.142		21.078	0.000
	Age	-0.001	0.002	-0.017	-0.371	0.711
	Gender	0.115	0.055	0.095	2.081	0.038
	Education	-0.118	0.026	-0.205	-4.452	0.000

a. Dependent Variable: Risk perception

Table 7: Regression output of predictors of risk perception in COVID-19

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	4.141	.339		12.226	.000
Age	-.001	.002	-.018	-.419	.675
Gender	.116	.053	.096	2.187	.029
Education	-.111	.026	-.193	-4.219	.000
Personal knowledge	.021	.018	.053	1.181	.238
Social knowledge	-.099	.019	-.234	-5.303	.000
Direct experience	-.031	.058	-.024	-.544	.586
Social amplificatio	.033	.074	.020	.448	.654
Prosociality	-.001	.021	-.001	-.029	.977
Individualism	-.061	.037	-.081	-1.636	.103
Trust in government	.024	.026	.043	.935	.350
Trust in science	-.113	.042	-.136	-2.697	.007
Trust in medical professionals	-.068	.037	-.091	-1.843	.066
Personal efficacy	-.016	.025	-.033	-.661	.509
Collective efficacy	.017	.024	.033	.690	.490

a. Dependent Variable: Risk perception

DISCUSSION

Risk perception is important in determining health-protective behavior. Perception of risk in critical experiences like epidemics is influenced by social, cultural, and contextual factors. Previous experiences such as SARS, influenza, and swine flu epidemic showed that the strategies performed and the results of activities often depend on recognizing the predictors of risk perception and coordinating programs with the important predictors [12]. It should be considered the management of risk perception predictors directs by governments in all programs and activities. The risk perception model recommends in COVID-19 epidemics consist of clusters of variables that correspond to the cognitive tradition (e.g. people's knowledge and understanding of risks), the emotional and experiential tradition (e.g. personal experience), the social-cultural paradigm (e.g. the social amplification of risk, cultural theory, trust, and values), and relevant individual differences (e.g. gender, education, ideology). The mean risk perception score was 2.78(±0.6) in this study which is slightly different from the average risk perception score in other countries. The study of risk perception in ten countries found that the risk perception score of COVID-19 epidemics varied between 4.78 and 5.45 on a 7-point scale, and it is relatively high in countries of the U.K., Spain, and North America. Therefore, in this study, the score of risk perception was significantly lower than in all ten countries. High-risk perceptions may enhance the public's active response to the epidemic. Risk perception is a dynamic process and it changes over time. In this study, more than a year had passed from the beginning of the epidemic, which can be an influential factor in the perception of risk.

In demographic factors, gender was the significant predictor of risk perception. The risk perception score of women was higher than men on all the components of risk perception. Other studies reported the same result and it seems that gender was included as the moderator of risk perception and protective behavior [13]. In similar crises of the past decades, women are constantly seen to be more precautionary behaviors than their male counterparts. Another basic demographic predictor is the level of education of the individual, especially in positive experience persons. In other studies, a high level of educated is more likely to improve the preparedness level and adherence to COVID-19 preventive and control guidelines [14], and poor education was significantly associated with lower risk perception [15], similar to our findings.

The level of education could affect knowledge as well as social knowledge; therefore social knowledge might be an important predictor of risk perception, which was observed in this study. Although studies on COVID-19 have reported associations between social knowledge and risk perception [16], some of them did not find this result [17]. Social knowledge is acquired through socialization and lived experience; however educational guidelines in the media at the national level could affect social knowledge, especially in critical situations such as the COVID-19 epidemic. The basic role of social knowledge in predicting the level of risk perception can be considered in the planning and implementation of national protocols.

This study found that scientific sources were the most trusted sources for COVID-19 information. Similar results were reported in Kwok's study on the COVID-19 pandemic [18] and the prior outbreak of SARS in 2003 [19] and the influenza A (H1N1) pandemic in 2009 [20].

Trust in science and experts in this study was greater than trust in government. The lowest mean of the predictors of risk perception was trust in the government. Trust in the government has been found to influence people's willingness to be accepting preventive activities [21], social limitations [22], and vaccination programs [23]. When people lack the necessary knowledge to evaluate the risk of a hazard, trust becomes an important cue regarding whom to believe and behavior [24]. People need to rely on scientists, professionals, and government agencies to listen and realize the information and the people's trust in these sources might be an important factor contributing to people's acceptance of the difficult precautionary measures needed to eradicate a pandemic [25].

This study might be consisting of some limitations. It should be noted that the study population was limited to those who were referred to the hospital. These people may not be a perfect example of the general population, and it is better to do such research at the national level and with diverse populations. The duration of the research was limited, and it is better to do this research several times.

CONCLUSION

People's perception of the risk of COVID-190 depends on several various factors, the most important of which were gender, level of education, social knowledge, and people's trust in science. It is recommended that studies be performed with larger and heterogeneous populations and longer time.

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REVIEW

Carcinoid Heart Disease – A 2022 Retrospective

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Abstract: *We aim to introduce a 2022 update on CHD (carcinoid heart disease) considering a multidisciplinary perspective. This is a narrative mini-review. We searched English, full-length papers (PubMed). Inclusion criteria: original articles regardless of the level of statistical significance. We identified 44 papers and manually selected those with CHD, as follows: 8 original studies, 1 case series (N=9 patients), 16 case reports (N=1 patient/paper), and a total of 1,030 patients on published studies. The most remarkable results are the longest period of enrolment of 3-4 decades; CHD ratio among carcinoid syndrome of 37%; 30-day mortality post-cardiac surgery of 12%; median survival in CHD from 1.3 to 3.9 years (more than 2 years if valve intervention is provided); most useful prognostic markers: 5HIIA, NT-pro-BNP, but, potentially, chromogranin A. The specific protective role of somatostatin analogs against developing CHD is yet to be determined. CHD in the absence of carcinoid syndrome/liver metastasis may be related to ovarian NEN (neuroendocrine neoplasia) with a better outcome if prompt intervention. Remarkably, 3 guidelines are released regarding CHD on behalf of ENETS (European Neuroendocrine Tumor Society), and ESC (European Society of Cardiology). CHD still represents a most challenging entity situated at the crossroads of surgery, cardiology, oncology, endocrinology, and gastroenterology.*

Keywords: *carcinoid heart disease, neuroendocrine, serotonin, neuroendocrine neoplasia, carcinoid syndrome, 5HIIA, valve, tricuspid, echocardiography*

INTRODUCTION

The complex field of neuroendocrine neoplasia (NEN), a rare condition with an annual incidence of 2 to 5.1 cases per 100,000 individuals, represents one of the most spectacular domains with massive recent progress from a multidisciplinary perspective [1,2]. The two main direct cardiac complications of NENs' evolution are either heart metastasis or carcinoid heart disease (CHD), also, recently named carcinoid valvular heart disease [3,4]. Primary cardiac NENs are mostly uncommon [5]. While myocardial and pericardial NENs' spreading is unusual (representing less than 1-3% of NENs-related metastases), CHD represents the hallmark of a very severe disease, typically embracing a very poor outcome [6,7]. The advances in cardiac surgery and interventional once-cardiology which have been registered within the latest years improved the overall CHD prognostic, as recently seen in other cardiac surgical procedures [8,9].

CHD is related to hormonally active NENs that increase overall morbidity and mortality; nevertheless, valve intervention might associate with numerous benefits, but carriers of such interventions display additional risks, including the need for post-operative pacing or connected infectious risks [10,11]. The statistical confirmation that the surgical approach to CHD improves survival is currently based on observational data rather than controlled studies [12].

The pathogenesis of CHD is still poorly understood. However, the presence of CHD is closely correlated with abnormal levels of biomarkers (blood and urinary neuroendocrine markers, also, named vasoactive substances which are more than 40 types, despite not testing all of them in daily practice), especially serotonin, and its urinary metabolite 5-HIIA (5-hydroxy-indole acetic acid), but, also, chromogranin A, neuron-specific enolase, etc. which serve as diagnostic tools and prognostic markers as well as part of the surveillance protocols [13-15]. Novel biomarkers like those based on mRNA transcript are currently under development for daily practice implementation [13]. Cardiac imaging is essential for recognizing CHD, from traditional ultrasound to advanced techniques such as speckle-tracking echocardiography, 68 DOTA peptide PET-CT (Positronic Emission Tomography – Computed Tomography) scans, and cardiac magnetic resonance or CT [12,16-18].

CHD almost exclusively targets the right cardiac side with consecutive heart failure, but in the early stages, the complication might be underdiagnosed [19]. The left side of the heart is usually not affected (unless abnormal intra-cardiac shunts) because the fact that the main contributor of CHD, namely serotonin (5-hydroxytryptamine), but, also, histamine, bradykinins, and tachykinins, are enzymatically inactivated into pulmonary bloodstream [19]. Remarkably, in 2018 three cases of quadruple valve replacement were reported in addition to the prior four concerning CHD, which represents exceptional situations in NEN-CHD pointing out that the left heart side may be affected, as well [20].

Usually, at the moment when CHD is identified, the patients already have clinically manifested NEN in terms of traditional carcinoid syndrome (flush, diarrhea, wheezing, loss of appetite and malnutrition, non-specific abdominal pain or cramps, hypotension, telangiectasia, edema, etc.) that is presented in correlation with liver metastases, grossly affecting 30-40% of all NENs [21]. Most patients with CHD are aged between 50 and 70 years (males being slightly more affected than females), and present chronic fatigue and dyspnea, independent of carcinoid syndrome (but may be initially misdiagnosed as carcinoid syndrome or even carcinoid crisis) [19]. The most frequent type of NEN causing CHD is gut-derivate (noting that the small intestine is the most frequent NEN up to 65-67% followed by lung NEN of 25%) and approximately one out of five or two cases (depending on the studied population) is prove to develop CHD which finally influence the prognostic even more than the primary tumor growth itself (in some cases) [12,22]. One meta-analysis from 2021 (including 9 studies, N=416 adults with NEN, mean age of 63 years, 53% males) showed that 75% of CHD – related NENs originate from the small intestine and colon, and 98% of them have synchronous hepatic metastases. Tricuspid regurgitation was confirmed in 97% of individuals of different severities, respective 72% concerning pulmonary valve, while right heart failure was identified in 48% of all cases. After having a valve replacement (99% of tricuspid, and 59% of pulmonary valve), a 30-day mortality of 9% was identified (with a median post-surgery survival of 3 years) which points out an acceptable safety profile of valves intervention in CHD [23].

Overall, the multidisciplinary management of CHD includes cardiologic therapy for heart failure, the control of serotonin excess by using high doses of somatostatin analogs such as octreotide or lanreotide (and/or telotristat ethyl) that act on tumor growth as well, peptide receptor radionuclide therapy in addition to surgery for primary tumor and metastases, especially at the liver site (if feasible) to reduce the tumor burden, and, finally, surgical – based valves replacement or catheter-directed valves intervention in selected patients, depending on various criteria that take into consideration the underlying NEN, the heart status, and the access to cardiac surgery or interventional cardiology [24-28]. Globally, patients with carcinoid syndrome (thus metastatic NENs) and CHD have a more severe prognostic when compare to those without CHD, knowing that NEN individuals with clinically manifested carcinoid syndrome have a more severe prognostic versus those carcinoid free [28,29]. Valve intervention in CHD improves long-term outcomes despite the current peri-operatory mortality rate being considered at 10-20% [29]. Of note, there is no specific medication for CHD other than the medical approach for the originating tumor and associated hormonal panel (NEN's management), on one hand, and cardiologic therapy for heart failure in addition to interventional procedures targeting the affected valves [28-30] (Figure 1).

Figure 1: Sneak peek – carcinoid valvular heart disease

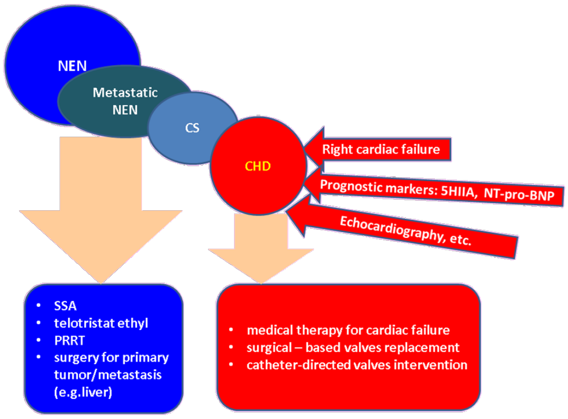
NEN
Metastatic NEN
CS
CHD
Right cardiac failure
Prognostic markers: 5HIIA, NT-pro-BNP
Echocardiography, etc.
SSA
telotristat ethyl
PRRT
surgery for primary tumor/metastasis (e.g.liver)
medical therapy for cardiac failure
surgical – based valves replacement
catheter-directed valves intervention

NEN = neuroendocrine neoplasia; CS = carcinoid syndrome; CHD = cardiac heart disease; 5HIIA = 5-hydroxyindoleacetic acid; NT-pro-BNP = NT-pro-brain natriuretic peptide; SSA = somatostatin analogues; PRRT = peptide receptor radionuclide therapy

MATERIALS AND METHODS

Aim

Our purpose is to introduce a 2022 update on CHD considering a multidisciplinary perspective.

Method

This is a narrative mini-review of literature. We searched English, full-length papers published in 2022 concerning journals indexed in PubMed (by using the terms "carcinoid heart disease"). Inclusion criteria are represented by original articles particularly/specifically addressing CHD (in vivo) regardless of the level of statistical significance (original studies or case reports/series). Exclusion criteria: papers considering heart metastasis in NENs or primary cardiac NENs.

According to mentioned methodology, we identified 44 papers in 2022, and manually selected those with CHD, as follows: 8 original studies, 1 case series (N=9 patients), 16 case reports (N=1 patient/paper), a total of 1,030 patients on published studies, in addition to 8 reviews, 3 guidelines (one with duplicate). We excluded 3 articles concerning non-CHD, one in vitro study, and 3 papers regarding withdrawal (Figure 2).

CLINICAL STUDIES PUBLISHED IN 2022 CONCERNING CHD (accessed via PubMed)

We identified 8 original studies in 2022 (Table 1). A multicenter, retrospective study on 275 patients analyzed the clinical presentation and the outcome in NENs, confirming that more than one-third of the individuals with carcinoid syndrome are affected by CHD. All the patients had carcinoid syndrome (mean age of 57 years, aged between 21 and 84 years; metastatic disease was found in 94.6% of cases). CHD was identified in 37.3% of them (N=104) by using echocardiography, with the tricuspid valve being the most affected cardiac site, single or in combination with other valves. CHD correlated with a poor prognostic in individuals with carcinoid syndrome (similarly, male sex was found to be a risk factor); median survival in the studied population was of 9 years) [31].

Figure 2: The articles included according to the methodology of research

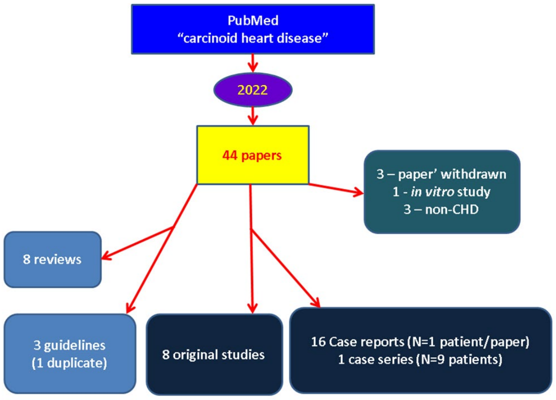

Table 1: Original studies published in 2022 considering carcinoid heart disease

First author/ Reference number	Type of Study	Number of patients	Results
Bergsten J [32]	retrospective (1986 - 2019)	60	30-day mortality (after cardiac surgery)=12% median post-operatory survival = 2.2 years
Levy S [34]	retrospective (1984 - 2021)	84	median survival since CHD diagnostic varying from 1.3 to 3.9 years
Levy S (bis) [35]	validation (case-control) study (1984 - 2021)	114	NT-pro-BNP has the best predictive value for CHD
Kostiainen I [36]	cross-sectional cohort study	65	low rate of CHD (5%) highly sensitive value of echocardiography (not BNP)
Shah HA [33]	retrospective study (2010 - 2019)	17	a 35% decline rate of 5-HIIA in association with liver function improvement
Macfie R [39]	retrospective study (2001 - 2021)	282	overall survival is lower in CHD *versus* non-CHD (N1=40 *versus* N2=242, p<0.001) valve replacement improves survival in CHD + inoperable liver metastasis
Konsek-Komorowska SJ [38]	retrospective study	108	chromogranin A inversely correlates with overall survival (p<0.05) in patients with carcinoid syndrome overall survival is lower in CHD *versus* non-CHD + carcinoid syndrome (67.22 versus 73 months)
Fijalkowski R [31]	retrospective study	275	prevalence of CHD 37.3% among patients with carcinoid syndrome overall survival is lower in CHD *versus* non-CHD

Abbreviations: CHD=cardiac heart disease; BNP=brain natriuretic peptide; N=number of patients

Bergsten J. et al. studied 60 patients (between 1986 and 2019, a total of 3 decades) with a median age of 64 years with CHD and cardiac surgery (N1=42 persons underwent both tricuspid and pulmonary valve replacement; N2=18 individuals had only tricuspid valve replacement with/without pulmonary valvotomy). The procedures (which exclusively used bioprosthetic valves) associated a 30-day mortality of 12%; and a median post-operatory survival of 2.2 years (a maximum of 21 years; statistically significantly higher in N1 versus N2, respective of 3 versus 0.9 years, p=0.02). A most useful tool of prognostic was echocardiography, either as a pre-operatory assessment (but with the limitation of underestimating pulmonary involvement) or post-operatory surveillance. Additional poor prognostic markers are high pre-surgery levels of 5-HIIA and NT-pro-BNP (NT-pro-brain natriuretic peptide). Overall, the double valve is superior to single tricuspid replacement [32]. Moreover, a retrospective UK study (between 2010 and 2019) on 17 patients with CHD who became candidates for valve replacement surgery had a single valve procedure (3/17) versus double valve (13/17), respective triple valve (1/17). Post-operatory, a 35% decline of 5-HIIA witnesses the improvement of general status in association with hepatic function recovery which suggests an indirect benefit of cardiac surgery [33].

Levy S. et al. introduced a cohort study on 84 patients with CHD (58% males) followed for 4 decades (between 1984 and 2021) at Netherlands Cancer Institute; the median age of NEN confirmation was of 62.3 years with a consecutive CHD diagnostic after a median of 1.1 years and an associated median survival varying from 1.3 to 3.9 years (considering the patients who were most recently admitted when valve replacement surgery was more often applied). Prognostic markers are NT-proBNP (positively correlated with tricuspid regurgitation), respective to the implementation of valve replacement surgical procedures (which increases the survival rate) [34]. The same researchers conducted a validation (case-control) study on CHD – associated biomarkers. 114 individuals (N1 with CHD versus N2 without CHD) showed similar age (median age of N1 – 57.9 years, respective of N2 – 59.7 years, p=0.29). Among studied assessments like circulating serotonin, activin A, sST2 (soluble suppression of tumorigenicity 2), CTGF (connective tissue growth factor), and NT-pro-BNP had the best predictive value for CHD in individuals with high serotonin [35].

As opposed to these results, we mention a cross-sectional cohort study on 65 patients with small intestinal NETs (neuroendocrine tumors) (45% with carcinoid syndrome and 5% with CHD). The results showed that both categories had a statistically significant increase in hepatic tumor load, serum levels of chromogranin A, and urinary 5HIIA when compare to the patients without carcinoid syndrome (p<0.005). However, the authors identified a lower rate of CHD (5%) than expected in persons with carcinoid syndrome (30-50% – according to data from the literature). Moreover, they did not confirm the predictive value of proBNP but identified the highly sensitive value of echocardiography in subjects with elevated chromogranin A and 5HIIA [36].

Another classical biomarker in NENs is chromogranin A, very sensitive, but less specific [37]. A retrospective study focusing on blood chromogranin A values (N=108 patients with carcinoid syndrome; N'=84 individuals were followed-up and 27 of them complicated with CHD) showed that its increased levels inversely correlate with overall survival (p<0.05) in patients with carcinoid syndrome. Also, the presence of CHD is statistically significantly associated with a reduced overall survival (67.22 versus 73 months in patients with carcinoid syndrome but CHD-free) [38].

Some authors consider the protective effect of somatostatin analogs like octreotide for CHD, but a large study on 282 patients with carcinoid syndrome (between 2001 and 2021) did not sustain this observation. Overall survival was lower in patients with CHD versus non-CHD (N1=40 versus N2=242, p<0.001). Considering the patients with inoperable hepatic metastasis, the survival rate was lower in patients without undergoing valve replacement when compared to the individuals with CHD and cardiac surgery or the individuals CHD-free [39]. Similarly, we mention a case series of 9 subjects with NEN who followed for 39 months; initially, the patients were CHD-free and started therapy with somatostatin analogs (median time from NEN diagnostic to therapy initiation of one month; median time from starting the medical treatment to CHD confirmation of 23 months) [40]. As far as we currently know, we cannot predict that using somatostatin analogs is a protection factor against CHD, but, of course, this medication has a proven effect in controlling the hormonal and tumor burden [41].

INTERESTING CASE REPORTS

We identified according to our methodology 16 case reports (a total of 16 patients with CHD). We mention the case of a 70-year-old female with torrential tricuspid regurgitation due to CHD regarding an ovarian teratoma with neuroendocrine differentiation. The subject had a heterotopic tricuspid valve replacement with a stable disease within the following 6 months. Mostly particular, the patient did not experience carcinoid syndrome, and neither imaging assays proved any metastatic NEN (including hepatic) after gynecological surgery [42]. Similarly, another case of a large insular primary ovarian NEN was reported on a 40-year-old female admitted for virilization syndrome in association with unexpected CHD – related heart failure; the panel of increased neuroendocrine markers improved after gynecological intervention, but CHD aggravated even in the absence of liver metastasis requiring valve replacement within 6 years [43]. This scenario is extremely unlikely among the typical evolution of NENs, neither ovarian NEN is recognized as a common source of serotonin over-production. However, ovarian carcinoid tumors are among the rarest forms of CHD in the absence of carcinoid syndrome and/or liver metastases, thus their early removal massively improves the survival rate and does not follow within the general frame concerning the very poor outcome in CHD-NENs [44-46].

adequate diagnostic/recognition when she was identified with CHD and had cardiac surgery. The identification of primary NEN was after the intervention for CHD [47]. This case brings out at least two atypical aspects: CHD in young adults, as prior mentioned, which is rather atypical unless a clear genetic background is present, and CHD diagnostic before confirmation of primary NEN [48]. Generally, one-third of NENs remain with unknown primary sources [49]. Theoretically, younger patients with CHD and NEN might have a better post-cardiac surgery prognostic, but further statistical evidence is needed.

A 44-year-old male with neurofibromatosis type 1 had a 6-year history of gastric NET with hepatic metastasis. He was admitted for sensory neuronopathy and a meningocele. After glucocorticoid treatment was empirically initiated, additional decompressive laminectomy was done, but the patient soon developed CHD with a fatal outcome within 3 months (due to pneumonia) [50]. Of note, currently, we do not know the potential role of glucocorticoids (probably recommended for incidental co-morbidities) in aggravating CHD. Moreover, there are no consistent data to point out that individuals with genetic syndromes underlying NEN have a higher risk of developing CHD [51].

As consideration amid the recent COVID-19 pandemic, we mention one case of a 55-year-old male who delayed the presentation and was admitted for severe CHD which was confirmed synchronously with a high serotonin-producing primary NEN [52]. Another case brings out the particular aspects of cardio-oncology practice following valve replacement in subjects with CHD [53]. This is the case of a 58-year-old female who experienced a carcinoid crisis peri-operatory [54]. Another aspect concerns the post-surgery risks, for instance, a 77-year-old woman was offered a successful ablation therapy for recurrent atrial flutter developed after the surgical procedure of replacing the tricuspid valve [55]. Moreover, another case of an 81-year-old male introduces the idea of consecutive interventions in CHD considering the right side of the heart (severe pulmonary regurgitation was successfully approached by trans-catheter implantation 4 years after initial surgery for tricuspid) [56].

DISCUSSIONS

Pathogenic traits of CHD

Hedinger syndrome, tricuspid insufficiency in addition to pulmonary stenosis due to CHD still represents a challenging condition nowadays [57]. CHD is a progressive condition in subjects with carcinoid syndrome where the right side of the heart is transformed in terms of fibrosis, degeneration, inflammation, desmoplastic reactions causing valvar dam-age (stenosis or regurgitation), and cardiac insufficiency [58,59]. The formation of plaque-like deposits (myo-proliferative plaques) on endocardial areas of the valves and subvalvular system impairs the blood flow dynamics [60-62]. Similarly to CHD, mesenteric fibrosis might develop in individuals with carcinoid syndrome [60]. Common pathogenic factors are described like TGF-β (Transforming growth factor beta) or PDGF (platelet-derived growth factor), etc [60].

The clinical approach of CDD

The main predictors of CHD are an advanced stage of NEN with high levels of circulating serotonin but mostly increased 24-hour urinary 5HIIA in addition to the presence of carcinoid syndrome and other clinical features [63-65]. Other collateral elements may involve particular cardiac anatomy, for instance, patent foramen ovale is reported in half of the individuals with CHS (twice more frequently than in the general population) which causes a right-to-left shunting [66,67]. Intra-cardiac shunt explains the left heart involvement in CHD as one case was published in 2022 with four valves involvement [68,67].

Since initially, CHD is subclinical or completely asymptomatic, a strategy of periodic check-ups is necessary, including performing differential diagnostics with other complications of NENs [60,63,69]. Cardiac imaging represents a strong point in CHD management. Ultrasound is useful for serial evaluations and early assessment; CT exams might show various elements like early contrast opacification of inferior cave vein as an indicator of tricuspid regurgitation; modern imaging procedures like 68Ga-DOTATATE PET are helpful for adequate positive diagnostic, especially for cases with a low index of suspicion, when a differentiation with myocardial metastasis or non-NEN incidental conditions is needed [70-72]. One case from 2022 introduced 203Pb-VMT-α-NET Scintigraphy as a new alternative to PET/CT [73].

Full blown picture of heart valve disease – associated clinical manifestations and related cardiovascular and pulmonary complications of NENs including carcinoid crisis represents a sign of advanced disease and prompt multi-disciplinary intervention is required [74,75]. The overall experience with cardiac surgery for CHD is limited when compared to many of the non-CHD causes, thus there are still some areas of open discussion like predictors of successful intervention, the timing of the procedure, criteria of candidates selection, the type replacement (single or double valve) [76-79].

Key messages on behalf of highly specialized medical and surgical societies

In 2022 three papers of major importance were released concerning CHD. European Neuroendocrine Tumor Society (ENETS) published excellent “guidance” regarding carcinoid syndrome and CHD. We mention a few major aspects: NT-proBNP remains the most important marker of CHD at the first-ever diagnostic (in patients who experience high serotonin status with or without clinical manifestation like carcinoid syndrome) and during follow-up. A cutoff value of 260 pg/mL indicates further investigations with echocardiography. In terms of ultrasound, the transthoracic approach is indicated, stress echocardiography might not bring additional information; while inadequate ultrasound data (including the evaluation of pulmonary vein damage) or planning for cardiac surgery may require supplementary imaging procedures like cardiac CT or magnetic resonance. Once identified, CHD requires serial NT-proBNP assays and cardiac ultrasound every 6 to 12 months, noting that sudden progress might occur anytime. Surgery (bioprosthetic valves are advised) should be done in severe CHD with more than 1 year of expected post-surgery survival; some specific criteria are recommended considering the adequate timing of intervention which should not be delayed. Double valve (tri-cuspid and pulmonary) replacement is better than a single one. Minimally invasive procedures are not yet studied enough [80]. Additionally, ENETS introduced a "synoptic" concerning the major role of echocardiography in CHD [81]. A collaboration of ESC (European Society of Cardiology) with other societies provided a guideline on cardio-oncology, also, addressing CHD. We mention some aspects, such as, for instance, echocardiography is recommended in patients with clinical evidence of CHD, carcinoid syndrome, and high BNP followed by a check-up every 3 to 6 months (level IB). BNP is assessed at baseline and every 6 months (level IB). A specific preparation is required considering the surgical/interventional risk of triggering a carcinoid crisis. Valve replacement, as in the ENETS guide-line, is recommended in patients with an expected survival of more than 1 year after intervention if they have symptomatic CHD or asymptomatic CHD (tricuspid or pulmonary) but with progressive dysfunction (level IC, respective IIaC) or severe mitral and/or aortic involvement in symptomatic cases (level IC) [82,83].

CONCLUSIONS

CHD represents a most challenging entity situated at the crossroads of surgery, cardiology, oncology, endocrinology, and gastroenterology. Until a few years ago, CHD was synonymous with a very severe form of NEN/carcinoid syndrome, with an expected poor outcome and limited resources. Recent cardio-oncology progress in addition to multiple shaped interventions for NEN allows a new level of approach in CHD to the benefit of the patients. However, many aspects are still open issues even in 2022.

Conflicts of interest and sources of funding
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None

Authors' contribution

Conceptualization, CEN, and MC; methodology, CEN, and MC; software, MC; validation, CEN, MC, AER, and AC; formal analysis, AC; investigation, AER, and AC; resources, AER, and AC; data curation, AC; writing—original draft preparation, CEN, MC, and AC; writing—review and editing, CEN, MC, AER, and AC; visualization, CEN, and MC; supervision, CEN; project administration, CEN, and AC.
All authors have read and agreed to the published version of the manuscript.

List of Abbreviations

5-HIIA = 5-hydroxyindoleacetic acid
NEN = neuroendocrine neoplasia
CHD = carcinoid heart disease
NT-pro-BNP = NT-pro-brain natriuretic peptide
PET-CT = Positronic Emission Tomography – Computed Tomography
TGF = Transforming Growth Factor
PDGF = platelet-derived growth factor
NET = neuroendocrine tumor
NEN = neuroendocrine neoplasia
sST2 = soluble suppression of tumorigenicity 2
CTGF = Connective Tissue Growth Factor
ENETS=European Neuroendocrine Tumor Society
ESC=European Society of Cardiology

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REVIEW

Collision Tumors of the Thyroid

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Abstract: *Collision tumors, rare neoplasms underlying two distinct histological types of carcinomas (with different origins) separated by normal tissue, are described in many organs including the thyroid gland, but to a lesser extent. We aim to overview the current published data concerning collision tumors of the thyroid (CTT). This is a narrative review considering papers published on PubMed through a search starting from the terms "collision" and "thyroid". Inclusion criteria are full-length articles, the English language, the timeline from inception to December 2022, and original studies with different levels of statistical evidence. We followed the main types of thyroid carcinomas: differentiated thyroid carcinoma of papillary and follicular type, anaplastic/ undifferentiated/poorly differentiated carcinoma, respectively medullary thyroid carcinoma. We identified 92 papers based on the mentioned key words criteria. We excluded 54 papers (reviews or non-thyroid CTT or other topics outside our purpose). We included in the final analysis 38 papers: 34 case reports (1 patient per paper), 2 case series (2 individuals per each article), one case series of 3 subjects, and a single study of retrospective type (including 21 subjects) – a total of 62 patients on published cases which represents the largest study on published cases to our knowledge (according to our methodology).*

Keywords: *collision, thyroid, thyroid cancer, papillary carcinoma, follicular carcinoma, medullary thyroid cancer, thyroidectomy, pathological report*

INTRODUCTION

Collision tumors, rare neoplasms underlying (at least) two distinct histological types of carcinomas (with different origins) separated by normal tissue (and no mixture areas), are described in many organs (for instance, lung, stomach, breast, and ovary, etc.) including the thyroid gland, but to a lesser extent than seen in others sites (affecting less than 1% of all thyroid malignancies) [1-3]. The most common combination is papillary and medullar carcinomas, but any type of primary thyroid malignancy is reported in collision with a secondary spreading to the thyroid from a non-thyroid primary cancer or another primary type [4-6].

Collision tumors of the thyroid (CTT) are regarded as challenging in terms of recognition, poor outcome elements, and particular behavior in addition to the fact that there are still areas of the unknown regarding this chapter on thyroid neoplasms [7-9]. A distinction with composite tumors should be done (where the same mutation causes histologically different entities) [10-12]. Also, metachronous tumors (syndromic or not) represent a different category [13-14].

We aim to overview the current published data concerning CTT**.**

MATERIALS AND METHODS

This is a narrative review considering papers published on PubMed through a search starting from the terms "collision" and "thyroid". Inclusion criteria are full-length articles, the English language, the timeline from inception to December 2022, and original studies with different levels of statistical evidence. Exclusion criteria are non-thyroid collision tumors, non-English papers, and reviews. We followed the main types of thyroid carcinomas: differentiated thyroid carcinoma of papillary and follicular type, anaplastic/undifferentiated/ poorly differentiated carcinoma, respectively medullary thyroid carcinoma**.**

RESULTS

We identified 92 papers based on the mentioned key words criteria. We excluded 54 papers (reviews or non-thyroid CTT or other topics outside our purpose). We included in the final analysis 38 papers including 34 case reports (1 patient per paper), 2 case series (2 individuals per each article), one case series of 3 subjects, and a single study of retrospective type (including 21 subjects) – a total of 62 patients on published cases which represents the largest study on published cases to our knowledge (according to previously mentioned methodology) (Figure 1).

Figure 1: The method of research considering collision tumors of the thyroid

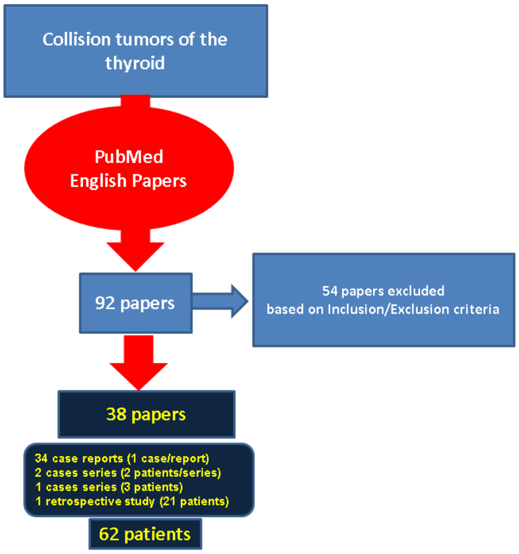
Collision tumors of the thyroid
PubMed English Papers
92 papers
54 papers excluded based on Inclusion/Exclusion criteria
38 papers
34 case reports (1 case/report)
2 cases series (2 patients/series)
1 cases series (3 patients)
1 retrospective study (21 patients)
62 patients

Papillary thyroid carcinoma

Papillary thyroid carcinoma is the most frequent histological type in CTT, but, generally as a single entity, it represents the most common thyroid neoplasia [15]. Apart from the most often association of medullary thyroid neoplasia, follicular malignancy is reported as well [16-19]. The combination with thyroid metastasis (typically from lung, breast, or renal cancers, etc.) is reported at the level of case reports (the spreading may be by local extension or via blood mainstream) [20-21]. For example, a case from 2007 introduced a CTT consisting of metastasis from lung adenocarcinoma and papillary thyroid cancer in a 63-year-old female [20]. A case report from 2021 included a 60-year-old female who was identified with the same carcinoma and metastasis from osteosarcoma within a CTT. This is the first case with bone origin as the primary source reported in a CTT [21]. The combination with a second primary cancer which is not originating from the thyroid is also reported. We identified a single case of a 67-year-old male who was diagnosed with a CTT associated a papillary thyroid cancer (with lymph node metastases) and parathyroid carcinoma clinically manifested with primary hyperparathyroidism [22]. The co-existence of a squamous cell carcinoma was reported, as well, originating from different sites or of unknown origin [23-27]. In 2004, a most unusual association with metastatic liposarcoma was published (this represents a most unique combination) [28].

Follicular thyroid carcinoma

This is the case of a 62-year-old female with follicular carcinoma (with capsular and vascular invasion) in association with a mucoepidermoid carcinoma and distant metastasis. Immunohistochemistry report might identify the mucoepidermoid differentiation of follicular malignancy over a different histological type (like mucoepidermoid malignancy) [29]. Hürthle cell carcinoma (regarded as a type of follicular group of differentiated thyroid cancers) is reported in less than 3% of thyroid malignancies, with the same ratio (of 3%) being reported for medullary thyroid carcinoma, as well. Their combination was identified on a 70-year-old female as a CTT (of note, the medullary cancer was intra-thyroidal, while the lymph nodes extension was derivate from Hürthle cell carcinoma) [30].

Interestingly, one retrospective study on 8 patients diagnosed with a primary thyroid malignancy identified the co-presence of the second histological type, meaning a follicular thyroid adenoma in association with papillary thyroid carcinoma (7/8), respectively medullary thyroid carcinoma (1/8) [31]. However, the current definition of CTT includes two malignant tumors, either primary or secondary, not a benign one like a follicular adenoma (which cannot be differentiated from a follicular carcinoma through the cytological report and it requires histological assessment) or a non-tumor – related cyst [31,32].

Medullary thyroid carcinoma

CTT may concern the thyroid gland and/or associated lymph nodes [33,34]. For instance, there is a case published in 2021 involving a 27-year-old male presenting CTT at lymph nodes (central compartment) from papillary thyroid cancer and non-syndromic medullary thyroid cancer. The growth speed was evaluated and the authors suggested that it may be similar to what we prior know concerning each of the two malignancies [35]. Generally, collision metastasis (mostly at cervical lymph nodes) is rarely reported in the absence of a thyroid tumor itself. We identified two papers concerning collision metastasis from papillary thyroid cancer and breast malignancy in female subjects [36,37]. In another case, a 73-year-old male was admitted for collision metastasis (cervical lymph nodes) from squamous cell carcinoma of nasal origin and B-cell lymphoma [38]. A previous report from 2009 included a lymph node spreading from a primary follicular malignancy and a squamous cell carcinoma of unknown origin [39]. Moreover, we mention the case of a 47-year-old male with collision metastasis from oral tongue–related squamous cell carcinoma and accidentally detected thyroid papillary carcinoma [40].

However, we currently do not have enough statistical evidence to compare the evolution in CTT versus non – CTT cases. The largest study particularly addressing CTT is a retrospective analysis that included the experience from 2009 to 2019 (a 10-year experience) on a tertiary care cancer. CTT (medullary + papillary) represented 4.7% of the medullary cancer series (N=21 individuals, the sex ratio of 1; mean age of 45.33 years, range between 26 and 77 years). 66.66% of the papillary cancers were situated at the right thyroid lobe; 85.7% of them were of micro-papillary type. The mean size of medullary cancer was three times larger than differentiated thyroid tumors. The contribution to lymph node spreading (which was confirmed in 76.2% of cases) was done by medullary type (87.5%), papillary cancer (12.5%), respectively both malignancies (12.5%) [41].

Anaplastic, undifferentiated, and poorly differentiated thyroid carcinoma

In 2022 Gupta RK. et al. reported the case of a 55-year-old female with a most unusual CTT: anaplastic thyroid malignancy (osteoclastic variant) and primary squamous cell carcinoma, each affecting a different thyroid lobe in addition to distant metastases at the level of bone, muscle and lymph nodes. However, according to WHO (World Health Organization) 2022, these two types are parts of the same malignancy spectrum thus their combination is nowadays regarded as a CTT-like lesion [42].

The "late anaplastic shift" theory concerns the identification of anaplastic thyroid carcinoma on a subject with a long history of differentiated thyroid carcinoma (either papillary or follicular) [43]. However, the synchronous identification of a differentiated tumor in association with a poorly differentiated cancer respecting a border area between them is consistent with the current concept of CTT. Here we mention the case published in 2021 by Vlad M. et al. (a 56-year-old female admitted amid the COVID-19 pandemic with a post-thyroidectomy confirmation of anaplastic cancer on the left lobe and lymph nodes spreading and a papillary micro-carcinoma on the right lobe) [44].

The presence of two highly aggressive malignancies in CTT makes the prognostic extremely severe. There is the case of a 63-year-old female confirmed with a triple CTT: poorly differentiated carcinoma, papillary carcinoma (classical and hobnail variant – this is the first case report underlying this subtype in CTT), and oncocytic (Hürthle cell) carcinoma. The hobnail pattern has been reported in anaplastic and poorly differentiated thyroid malignancies, representing a sign of a very aggressive profile with rapid evolution [45].

DISCUSSION

Pathogenic loops in collision

The field of thyroid surgery is extremely complex, while the domains of pathological reports underlying thyroid neoplasia are also extremely vast and dynamic [46-48]. The collision phenomenon has been described at many levels within multiple organs of the human body [49]. "Chance" theory is one pathogenic hypothesis [41]. CTT might be underdiagnosed, especially micro PTC [41]. Fine needle aspiration (cytological report) might not identify both tumors. However, immunohistochemistry and molecular studies might help the diagnostic [50].

Whether different immune or genetic co-morbidities might act as a trigger or epigenetic elements for CTT development there is still an open question. We mention a case of CTT (papillary and medullary neoplasia) in a 46-year-old female with multiple sclerosis and prior therapy with interferon. This is the first case of CTT on a patient with this neurologic condition (published in 2020) and there is still missing information about a potential pathogenic loop [51].

Common genetic background of both tumors underlying CTT is reported in some cases. For instance, there is the case of a 69-year-old woman with Hürthle cell carcinoma (on the right lobe) and multi-centric papillary cancer (on both thyroid lobes), associated with BRAF mutations (V600E gene) [52].

Also, in 2010, a case of non-multiple endocrine neoplasia medullary cancer in addition to papillary malignancy underlying CTT was reported in a female carrying a particular RET mutation (familial medullary thyroid cancer) [53]. A RET somatic point mutation in association with mixt medullary–papillary (including a follicular variant of papillary cancer) neoplasia was described in a 59-year-old male (a case published in 2007) [54]. Earlier data also suggest this theory [55]. Moreover, the co-presence of granulomatous inflammation in a case of papillary–medullary malignancy might represent the hallmark of common pathways [56].

Early reports of CTT

Of historical note, we identified the first report regarding CTT on PubMed in 1977 (non-English paper) [57]. The first English paper concerning a single case with medullary and papillary neoplasia is dated 1989 [58]. The first case series (2 out of 3 cases) on PubMed with the same mix of neoplasia is published in 1994 [59]. The first CTT with collision metastasis published in a PubMed – indexed journal dates from 1996 [60]. The first triple CTT (on PubMed) was reported on a 27-year-old female with papillary, follicular, and medullary carcinoma [61].

The literature-based analysis

Overall, we identified 62 cases on published data concerning CTT, thus we may say that this particular entity remains at the level of case reports. However, paying attention to this most unusual neoplasia might improve the prognostic and help early detection of at least one of the tumors. With one exception, all cases included at least one differentiated thyroid malignancy, either papillary or follicular (including 3 cases of Hürthle cell/oncocytic carcinoma). Five reports were consistent with the concept of collision metastasis concerning local lymph nodes (near the thyroid). 16/38 articles included medullary thyroid cancers. Based on these data, following differentiated (papillary being the most frequent) and medullary types, elements of squamous cell carcinoma originating from different organs were more frequent than anaplastic/poorly differentiated carcinoma. As mentioned, the most uncommon elements in CTT were: metastatic liposarcoma and osteosarcoma, respectively, and parathyroid carcinoma. All reported cases are adults (we did not identify any pediatric cases). The age varied between 26 and 88 years. More than half of the papers included female patients, but the single study we have so far reported an equal prevalence between males and women (Table 1).

Table 1: Published cases on collision tumors of the thyroid according to our methodology; the cases are displayed from the most recent starting with 2022 (please see references in the text according to first column indices)

First author, Number, Reference number	Studied population	DTC	ATC	MTC	Other types of carcinoma
Vijayvergiya G., 1, [29]	1 case (62-y, F)	FTC			mucoepidermoid carcinoma
Yeap ZH., 2, [30]	1 case (70-y, F)	Hürthle cell carcinoma		MTC	
Vlad M., 3, [44]	1 case (56-y, F)	micro-PTC	ATC		
Li H., 4, [35]	1 case (27-y, M)	PTC		sporadic MTC	
Toyoshima MTK., 5, [45]	1 case (63-y, F)	oncocytic carcinoma classical and hobnail PTC	poorly differentiated carcinoma		
Koufopoulos N., 6, [21]	1 case (63-y, F)	PTC		MTC	metastasis from osteosarcoma
Foppiani L., 7, [22]	1 case (67-y, M)	PTC			parathyroid carcinoma
Thomas A., 8, [41]	retrospective (N=21, F/M=1)	PTC		MTC	
Feng JW, 9, [16]	1 case (40-y, F)	PTC + FTC			
Sirbu AM., 10, [51]	1 case (46-y, F)	PTC		MTC	
Pishdad R., 11, [17]	1 case (79-y, M)	PTC + FTC			
Dikbas O., 12, [18]	1 case (35-y, F)	PTC		MTC	
Alhanafy AM., 13, [33]	1 case (73-y, F)	PTC			moderately differentiated squamous cell carcinoma
Sinno S., 14, [52]	1 case (69-y, F)	PTC + Hürthle cell carcinoma			
Fulciniti F., 15, [50]	1 case (#)	PTC			mucoepidermoid carcinoma
Gao Q., 16, [36]	1 case (59-y, F)	PTC			breast cancer ##
Ryan N., 17, [23]	1 case (88-y, F)	PTC			squamous cell carcinoma
Gurkan E., 18, [6]	1 case (44-y, M) 1 case (77-y, M)	PTC		MTC	
Plauche V., 19, [19]	1 case (62-y, F)	PTC + FTC			
Wang X., 20, [24]	1 case (55-y, M)	PTC			laryngeal squamous cell carcinoma
Kataria K., 21, [56]	1 case (35-y, M)	PTC		MTC	
Zeng H, 22, [37]	1 case (49-y, F)	PTC			breast cancer ##
Sadat Alavi M., 23, [34]	1 case (25-y, M)	PTC		MTC	

Kakarala K., 24, [38]	1 case (73-y, M)				squamous cell carcinoma low-grade B cell lymphoma ##
Warman M., 25, [25]	1 case (84-y, F)	PTC			squamous cell carcinoma
Gul K., 26, [53]	1 case (F, #)	PTC		MTC	
Jacobson AS., 27, [26]	1 case (#)	PTC			squamous cell carcinoma
Mattioli F., 28, [39]	1 case (#)	FTC			squamous cell carcinoma ##
Lim YC., 29, [40]	1 case (47-y, M)	PTC			squamous cell carcinoma##
Rekhi B., 30, [54]	1 case (59-y, M)	PTC		MTC	
Nabili V., 31, [20]	1 case (63-y, F)	PTC			metastasis from lung adenocarcinoma
Walvekar RR., 32, [27]	1 case (65-y, F)	PTC			squamous cell carcinoma
Rossi S., 33, [55]	1 case (61-y, F) 1 case (27-y, M) 1 case (34-y, M)	PTC		MTC	
Brandwein-Gensler M., 34, [28]	1 case (86-y, F)	PTC			metastatic liposarcoma
Wu CJ., 35, [61]	1 case (27-y, F)	PTC + FTC		MTC	
Pastolero GC., 36, [60]	1 case (#)	PTC		MTC	
Lax SF., 37, [59]	2 cases (#)	PTC		MTC	
Gero MJ., 38, [58]	1 case (#)	PTC		MTC	

We currently do not know if any data concerning the age or the sex of the subjects should be regarded as risk factors. In addition to the lack of poor survival indices (which are relevant in metastatic cases, those with late diagnostic, and anaplastic/ poorly differentiated tumors), the underlying mechanisms are controversial and we need further histological, molecular, and genetic studies.

CONCLUSIONS

The current limits of the CTT field are correlated with the small size of published data until the present time. We still need markers of poor prognostic. Awareness is essential for early detection with a consecutive potential improvement of the outcome.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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None

Authors' contribution

Conceptualization, A.D., and M.C.; methodology, M.C.; software, A.P.C.; validation, A.D., E.P., and M.C.; formal analysis, D.T.; investigation, A.P.C., D.T.; resources, A.D., M.C.; data curation, E.P.; writing—original draft preparation, M.C.; writing—review and editing, A.D., A.P.C, D.T., M.C.; visualization, M.C.; supervision, A.D.; project administration, A.D., M.C.
All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Diagnosis of Chronic Kidney Disease in the Elderly – A Challenge for the Practitioner

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Abstract: *The recent increase in life expectancy is the main argument for a better understanding of the pathophysiological mechanisms underlying aging. These, once known, can provide possible links to therapies to prevent aging or slow down the process. Normal aging is associated with a progressive decrease in the glomerular filtration rate. Accurate estimation of GFR in the elderly is under the suspicion of multiple errors mainly due to sarcopenia and decreased protein intake. Differentiation between chronic kidney disease and the physiological decline of GFR might be a challenge in clinical practice and this has consequences on the evolution and treatment of the numerous comorbidities of the elderly. The current trend to use non-invasive diagnostic techniques explains the need to identify a serological marker to help differentiate between decreased GFR secondary to kidney aging or the development of chronic kidney disease.*

Keywords: *elderly, normal kidney aging, chronic kidney disease*

INTRODUCTION

Globally, there is a progressive aging of the population.

According to the United Nations Report, 1 in 11 people was over 65 years old in 2019 and it is estimated that in 2050, 1 in 6 people will be this age [1].

The aging process affects the whole body and is characterized by structural changes and functional decline in all organs, leading to an increased incidence of many diseases considered "specific to the elderly". Among these changes, the aging-associated alterations in both structure and function of the kidney are particularly important, having consequences on the evolution and treatment of the numerous comorbidities of the elderly.

Accurate quantification of kidney function in the elderly is difficult, both overestimating and underestimating the glomerular filtration rate (GFR) being possible with currently available serum creatinine-based calculation formulas.

AGING KIDNEY PROCESS

An accurate description of histological changes in the senile kidney, i.e. changes attributed to advanced age per se, is difficult because the elderly usually accumulate important comorbid burdens with impact on the kidney structure and function: diabetes, hypertension, heart failure, etc. Many of the terms that describe physiological changes in the aged kidney superimpose with terms that describe renal pathological processes: nephrosclerosis, tubulointerstitial sclerosis, glomerulosclerosis, arteriosclerosis, etc.

Macroscopically, starting from the 4th decade of age, the kidneys begin to decrease in size and weight progressively, so that around the 7th decade there is a reduction by about 20-30% of kidney weight compared to the age of 40 years [2]; in the elderly without kidney disease, the decrease in kidney size is symmetrical. Also, with age, the kidney's cortex becomes thinner and rougher, the number and size of benign cysts increases, and the pelvis is enlarged with more fatty infiltration. The scarring is more advanced when associated with aortic and renal atherosclerosis (Figures 1 – 5) [3].

Microscopically, starting with the 5th decade of life, there is a progressive decrease in the total number of nephrons due to sclerosis of all nephron compartments [3]: glomerulosclerosis, tubulointerstitial sclerosis, and arteriosclerosis.

The prevalence of glomerulosclerosis increased from 25% in the 3rd decade of life to 82% in the 8th decade [4], the main cause being arteriosclerosis of the small intrarenal arteries.

Figure 1: 87 years old male: granular surface with increased scarring

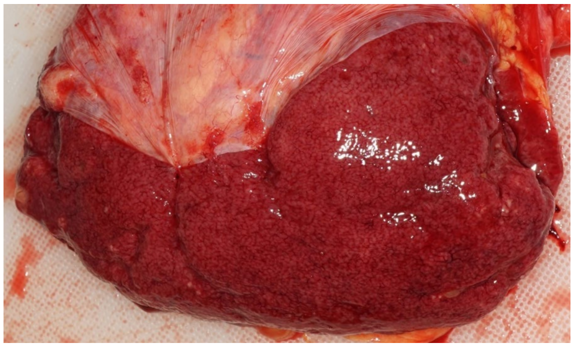

Figure 2: Same case, yellow arrows: small stones, thinning of the cortex

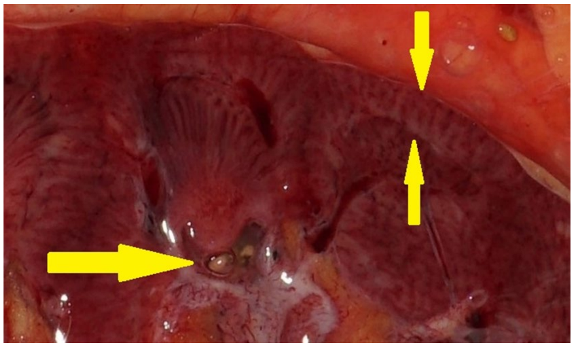

Figure 3: Same case, yellow arrows: glomerulosclerosis (10X HE)

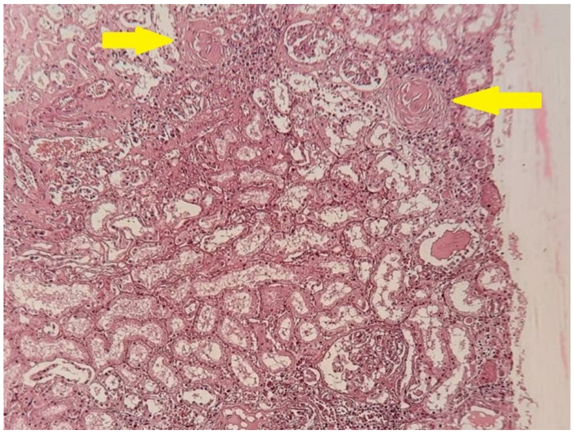

Figure 4: Same case, yellow arrows: arterial wall thickening (10X HE)

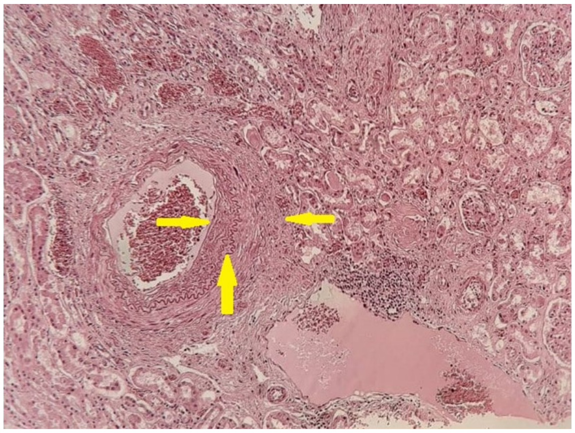

Figure 5: Same case: interstitial sclerosis (10X HE)

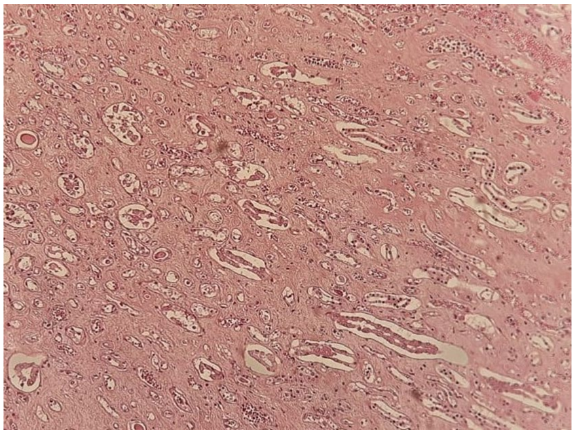

The hallmark of the functional decline of the senile kidney is a progressive decrease in the glomerular filtration rate (GFR) [5]. The decline in GFR begins around the 4th decade of life; between the 8th and 9th decades, the GFR is over 45% lower than at the age of 40 [3,6].

ESTIMATED GLOMERULAR FILTRATION RATE (eGFR) IN ELDERLY

There is no consensus regarding the optimal equation for the estimation of GFR in the elderly. Formulas used in clinical practice in adults – MDRD (Modification of Diet in Renal Disease, CKD-EPI (Chronic Kidney Disease – Epidemiology Collaboration), Cockroft-Gault are all based on serum creatinine levels and they have not been all validated for individuals older than 70 years.

Creatinine-based equations for GFR estimation have several drawbacks in older individuals [7]. Due to age-associated sarcopenia, there is a reduced release of creatinine from the muscles, therefore also a reduced urinary excretion of creatinine [8]. In elderly diabetics, especially in the late-elderly group (75 years or older), it has been noted a more decreased serum creatinine when compared with non-diabetics and this observation was explained by the increased loss of muscle mass promoted by diabetes [9]. Also, the elderly tend to have a reduced intake of proteins and a decreased basal metabolism, therefore, they have supplementary causes for the reduced production of creatinine [10]. Increased metabolism of creatinine by intestinal microorganisms may be an additional cause for reduced levels of serum creatinine, as there are proven age-related alterations of gut microbiota [11]. Formulas using the patient's weight besides serum creatinine give more errors due to increased fat percentage in the organism of the elderly. Several researchers had concluded that both MDRD [12] and CKD-EPI [13] formulas tend to overestimate GFR in the elderly, while Cockroft Gault's [14] formula underestimates it [15,16].

Serum creatinine alone is often used in clinical practice not especially for estimation of GFR, but for monitoring the patient during treatment with a potential nephrotoxic agent (aminoglycosides for example) or a potential ischemic renal insult (cardiac surgery for example). Besides the fact that this conduct is not correct due to the late and modest increase of serum creatinine level compared to the moment and degree of kidney insult, in the elderly, it is a source of supplementary severe misjudgments. The elderly have often lower basal serum creatinine and during an injury renal process, an increase of serum creatinine remains in the range of the normal values of the laboratory and this result may be misinterpreted as a stable state, i.e. no iatrogenic. Moreover, the creatinine increase may be blunted by intensive hydration of the patient applied to minimize the kidney insult.

Formulas based on both serum creatinine and serum cystatin C have been proposed to overcome the disadvantages of formulas based only on creatinine values [17]. Nevertheless, serum cystatin C is influenced not only by kidney function, but also by several diseases often present in the elderly, such as atherosclerosis, inflammatory estates, or obesity [18,19].

Several alternative equations have been developed for older individuals. The HUGE formula (Hematocrit Urea GEnder) was developed in 2011 by a group of researchers from Spain to differentiate patients without CKD from those with CKD [20]; this formula was validated in some studies [21,22].

L = 2.505458 - (0.264418 x hematocrit) + (0.111800 x blood urea); 1.383960 is added for the male patient. For L <0, CKD is excluded; for L> 0, it is classified as CKD.

In 2012, the Berlin Initiative Study (BIS) introduced two new formulas based on serum creatinine and serum creatinine plus serum cystatin C, respectively, for estimating GFR in people over 70 years of age [23].

BIS 1: eGFR = 3736 x serum creatinine-0.87 x age-0.95; in women, the result is multiplied by 0.82.

BIS 2: eGFR = 767 x cystatin C-0.61 x serum creatinine-0.40 x age-0.57; in women, the result is multiplied by 0.87.

Although these formulas have been validated in some studies, there are no large trials to conclude their superior performance, therefore meanwhile guidelines recommend using the same equation as in the general adult population.

NORMAL KIDNEY AGING VERSUS CKD IN ELDERLY

Reported data on CKD prevalence in the elderly is impressive. A highly cited source in the literature is a meta-analysis performed by Hills and collab. which comprises more than 100 reports from all continents; according to this meta-analysis, the prevalence of CKD in all stages in the general population is 13.4% (11,7-15.1%), but increases to 27.6% (range 26.7-28.5%) in the 6th decade and 34.3% (31.9-36.7%) in the 7th decade of life. The same source also revealed increased CKD stage 3-5 in the elderly: from 10.6% in the general population (9.2-12.2%) to 11.3% (8.1-14.5%) in the 6th decade and respectively to 27.9% (16.4-39.3%) in the 7th decade [24]. Nevertheless, a large proportion of the geriatric population meets the KDIGO criteria for CKD even without markers of renal impairment [25,26]. Regardless of the formulas utilized to estimate GFR, an important question needs to get answered in the face of an older patient with abnormal GFR and no markers of kidney disease. Is it CKD without markers of kidney disease or is it normal kidney aging? Several reasons require a response to this question. Establishing a diagnosis of CKD imposes close monitoring of the patient and adequate preparation for conservative or dialysis treatment. Most of the patients aged >65 years have estimated GFR between 45-60 mL/min/1.73m^2; older patients with estimated GFR between 45-60 mL/min/1.73m^2 have, in comparison with younger adults, decreased risk to progress to end-stage renal disease (ESRD), but increased risk to die from other diseases until they reach ESRD [27,28]. Close nephrological monitoring of all these patients would be a significant burden for physicians, and also for the health systems. On the other hand, CKD, even oligosymptomatic, is associated from early stages with increased risk for cardiovascular events and cardiovascular mortality; specific complications of CKD, such as acidosis or increased parathormone (PTH) have early onset and need to be controlled.

There are several subtle clues allowing the differentiation between the physiologic decrease of GFR and CKD (Table 1).

Serum urea and creatinine increase late in the course of CKD in all patients, but especially in elderly patients. Low serum creatinine levels are observed due to decreased basal metabolism, reduced protein intake, and sarcopenia [3], therefore CKD may be present with normal values of serum creatinine in the elderly. However, the aging kidney excretes increased amounts of urea, due to the reduced density of urea channels in the collecting tubes [29]; a normal serum urea level is associated with normal aging [15].

Table 1: CKD versus normal kidney aging

	CKD	***Aging kidney***
Nitrogen waste products in the blood	Increased	Normal
Albuminuria	Present	Absent
Anemia	Present (erythropoietin deficiency)	May be present (nutritional anemia)
Acid-base balance	Metabolic acidosis	Normal
Ca-P metabolism	Hypocalcemia decreased active vitamin D, increased PTH	Possible hypocalcemia, normal PTH, normal active vitamin D

No anemia should be present as in healthy older populations erythropoietin (EPO) production is normal or even increased secondary to increased erythrocyte turnover, increased EPO resistance, or as a response to occult digestive blood losses [30,31]. Nevertheless, in clinical practice, the elderly have often nutritional mild/moderate anemia that may be misinterpreted as CKD-associated anemia. Reduced secretion of EPO in CKD is associated with normocytic and normochromic anemia, while nutritional anemia is often hypochromic and microcytic [32], but they may coexist.

No abnormalities of mineral metabolism are present in healthy older individuals. Renal tubular excretion of calcium, magnesium, and phosphorus is not altered in healthy elderly [5,33]. The elderly are prone to hypomagnesemia, but this seems to be mainly due to reduced dietary intake, decreased intestinal absorption of magnesium, and/or laxative abuse [5]. Although the elderly present more often with mild hypocalcemia, it occurs mainly secondary to native vitamin D deficiency through reduced sun exposure or decreased skin synthesis of vitamin D [34]. In contrast with CKD, there is no deficiency of active vitamin D, nor an increased synthesis of parathormone in healthy elderly.

Under basal conditions, the kidneys of the elderly have a normal capacity for the excretion of acids and the acid-base balance does not undergo significant changes even with a normal- or hyper-protein diet [35]. However, the renal reserve of the elderly is reduced and a rapidly installed high acid load may be difficult to be compensated by the senile kidney; the elderly are highly vulnerable to the side effects of acute metabolic acidosis (uncontrolled diabetes, sepsis, acute kidney injury).

On the other hand, the presence of albuminuria is a strong argument for CKD even if the patient has other comorbidities that may explain this finding (systemic atherosclerosis, diabetes, cardiac failure). Albuminuria is associated with an increased risk of progression of CKD [36], therefore applying antiproteinuric measures might have the same benefits in the elderly as in younger adults, i.e., attenuating the progression of CKD and reducing cardiovascular events [37]. Nevertheless, overzealous administration of RAAS inhibitors in the elderly is associated with an increased risk of side effects (hypotension, hyperkaliemia, acute kidney injury) in the presence of reduced GFR and systemic atherosclerosis or other comorbidities; another reason why these drugs are no longer needed in elderly without proteinuria is the fact that they become useless, not having time to install their renoprotective effect over time [38,39]. The elderly are prone to hyperkaliemia even in the absence of reduced GFR due to the low sensitivity of distal tubules to aldosterone [31,40].

Taking into account that the elderly have a higher risk of death due to various complications than to progress to ESRD, there is a need to assess individual risk for these outcomes to analyze treatment options and make early plans for conservative treatment (diet, prevention, and control of uremic syndrome with medication) or renal replacement therapy. CKD has an extremely heterogeneous presentation in the elderly; between an independent full-functioning person without cognitive impairment and without major comorbidities at one pole to a frail, immobilized in-bed individual with severe cognitive dysfunction, multiple disabling comorbidities and total dependence for medical care at the other pole, there are numerous clinical pictures. One such equation was proposed by Tangri et al in 2011 who developed the Kidney Failure Risk Equation (KFRE) using variables like age, sex, estimated GFR, albuminuria, serum calcium, serum phosphate, serum bicarbonate, and serum albumin to predict progression toward ESRD after 1, 3 and 5 years respectively for patients with CKD stages 3 to 5 [41]; a variant using only 4 variables (age, sex, estimated GFR and albuminuria) may be used, but the results offer less accuracy [41]. This model has not been designed especially for older individuals, but it has been validated both for patients < 65 years and those above this age and for various races [42,43]. Calculation of this score is available on the internet at http://www.qxmd.com/Kidney-Failure-Risk-Equation. KFRE is recommended by European Renal Best Practice Group to be used in elderly with an estimated GFR < 45 mL/min/1.73 m^2 to assess progress toward ESRD [44]. Nevertheless, a lot of old individuals are placed in the range of GFR between 45-60mL/min/1.73 m^2 and have no markers of kidney disease (albuminuria) [45]; there are no available validated prediction models for this situation and members of European Renal Best Practice Group opine for the urgent need of such prediction models.

Regarding the prediction of death, in the literature there are numerous scores specially designated for the old population and most of them also include, besides demographic variables (age, gender) and comorbidities (heart failure, diabetes, active neoplasia, anemia, history of stroke, chronic obstructive pulmonary disease, etc.), a marker of kidney dysfunction such as estimated GFR or urinary albumin/creatinine ratio [46-49]. These models estimate the risk of death at periods ranging from 3 months to 5 years. The European Good Practice Guideline recommends the use of the Bansal score in current practice for assessing the risk of mortality of non-fragile, un-institutionalized elderly people with CKD >stage 3b [44,47]; this score uses 9 variables - age, sex, GERD, urinary albumin/ creatinine ratio, smoking, diabetes, heart failure, history of stroke; according to this predictive model, the 5-year mortality varies between 3.8% for individuals with lower scores to 83.6% for individuals with all 9 items reached. Nevertheless, again, none of these models took into analysis the particular situation of individuals with normal kidney aging, i.e. those patients without markers of kidney damage and GFR between 45-60 mL/min/1.73m^2. We found in the literature just one publication, that of Van Pottelbergh and collab. who analyzed mortality of very old (>85 years) individuals with GFR> 60 mL/min versus those with GFR between 45-60 mL/min [50]. According to the authors of this paper, the combination of the baseline estimated GFR and the estimated GFR slope over 3 years can be used to assess the risk of death; in the subgroup of patients having estimated baseline GFR between 45-60 mL/min only those with a GFR decrease ≥ 5 mL/min/year had an increased risk of death, while among patients with baseline GFR > 60 mL/min, a decrease of ≥ 3mL/min/year was associated with increased risk of death. The same authors explained that these changes are a reflection of cardiovascular damage. Also, the authors share the opinion of a lot of others those older adults with GFR between 45-60 mL/min should not be classified as moderate stage 3 CKD.

CONCLUSIONS

Aging associates the functional decline of all tissues and organs secondary to degenerative structural changes. Normal kidney aging is a complex process that is difficult to be differentiated in clinical practice from chronic kidney disease with contemporary available criteria; both share the reduced glomerular filtration rate as the main feature. Nevertheless, it is important to make this differentiation.

Establishing a diagnosis of CKD imposes periodic nephrology consultation for monitoring the progression of the disease, treatment of complications, early planning of conservative or dialysis treatment, and also adjustment of treatment of multiple comorbidities of the elderly. CKD has multiple complications and requires often interdisciplinary care: cardiologists, neurologists, psychiatrists, nutritionists, etc. On the other hand, proving that reduced GFR is only a sign of normal aging and that there are no markers of kidney damage allows gerontologists to provide holistic care for slowing the aging process and minimizing the incidence of high-risk diseases in the elderly.

Thus, misclassifying an elderly person as a patient with CKD involves an unnecessary psychological burden for the patient and his family, as well as unnecessary medical care and increased financial expenses.

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The authors declare no conflict of interest.

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Authors' contribution
Conceptualization, D.R., C.D., I.A.V., G.E.L.; methodology, A.L.C., R.F.T.; software, M.O.D.L.; investigation, I.A.V., D.R., C.D.; writing—original draft preparation, A.L.C., F.P.; writing—review and editing, S.I., I.A.V.; supervision, A.L.C., D.I.
All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Laparoscopic Wedge Resection for Gastric Mesenchymal Tumor – Small Case Series

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Abstract: *Introduction: Gastric mesenchymal tumors are a pathology that is increasing in incidence in recent years but the treatment is not yet standardized. The laparoscopic approach to these lesions is a modern option, with the patient benefiting from the advantages of minimally invasive surgery. Methods: We included in the study 4 patients operated laparoscopically for suspicion of gastric GISTs between Mars 2019 and May 2022. From a prospectively kept database, we extracted the following characteristics: age, sex, length of postoperative hospital admission, operation time, tumor location, tumor size, histopathological assessment of resection margins, intraoperative blood loss, and incidence of postoperative complications. Results: Average tumor size was 4.3 cm, ranging from 4 to 6 cm. Laparoscopic wedge resection was possible in all cases. There was no conversion, no episode of tumor rupture or spillage, and no major intraoperative complications. Histology confirmed a GIST in 3 of 4 cases. In one case histological workup showed gastric schwannoma. Conclusion: Laparoscopic wedge resection for gastric stromal tumors, even larger than 5 cm, appears to be safe and feasible in selected patients, with acceptable operative time and oncological outcome after an intermediate follow-up. More important than the size of tumors that are approached laparoscopically is their location and the possibility of a stenosis-free resection.*

Keywords: *laparoscopic wedge resection, Gastrointestinal Stromal Tumors (GIST), gastric mesenchymal tumors, Schwannoma*

INTRODUCTION

Gastric tumors can be divided into epithelial and subepithelial tumors based on their origin. 90% of gastric tumors including adenoma and adenocarcinoma originate from epithelium [1]. Various tumors occur as subepithelial lesions, including mainly gastrointestinal stromal tumors (GISTs) and leiomyomas, less often neuroendocrine tumors, schwannoma, lymphangioma [1], lipomas, granular cell tumors, and ectopic pancreatic tissue [2].

Epidemiology

GIST is the most common mesenchymal tumor of the gastrointestinal tract [3], with an incidence of about 1-1.5/100,000 persons/year [4,5]. Few studies have indicated that the incidence of GIST is greater in patients undergoing bariatric surgery (0.6%- 0.8%) than in the general population [6]. Increased body mass index has been linked with a higher risk for several types of cancer. Another possible hypothesis for the high incidence found in these studies is that many small, asymptomatic GISTs are underdiagnosed in the general population [7,8].

Gastric stromal tumors account for about 60-70% of all GISTs [4,9,10]. The term is relatively recent, it was first used in 1983, to reclassify a subset of tumors previously identified as leiomyomas or leiomyosarcomas [11,12]. Typically, GISTs are solitary tumors identified slightly more often in males than females of middle to advanced age (between 40 and 80 years) [6,7]; however, the estimated mean age at diagnosis is in the sixth decade of life [13].

Histological Findings

GIST originates from the interstitial cells of Cajal, the pacemakers of the gastrointestinal tract [14], or their stem cell precursors [15]. Their defining characteristic is a gain-of-function mutation in genes coding for the KIT tyrosine kinase receptor, which is considered the driving force of cell proliferation in this tumor [16].

Diagnosis of GIST was based on microscopic analysis, including immunohistochemistry with a panel of antibodies: Cluster of

Differentiation-117 (CD117), Cluster of Differentiation-34 (CD34) and Discovered on GIST-1 (DOG1), S-100 protein, desmin, and smooth muscle actin [17,18].

Natural Evolution

GISTs are not classified as either benign or malignant but are rather stratified by their clinical risk of malignancy: very low, low, intermediate, or high [13]. The malignant potential depends on tumor cell mitosis, tumor size, tumor location [17, 19], and tumor rupture [5]. Approximately 10% to 30% of GISTs have a malignant clinical course [13] because they can metastasize hematogenously to the solid viscera, including the liver, small bowel, and lungs [14]. In these conditions, the only absolute signs of malignancy are metastasis or invasion into adjacent organs [16].

Biologically, tumors grow locally within the stomach wall (intra- or extraluminal expansion) and can also spread along the smooth muscle planes within the stomach or can rupture into the peritoneal cavity causing sarcomatosis [10].

Clinical presentation and incidental GIST

The most common symptoms of GISTs are gastrointestinal bleeding, including acute melena and hematemesis [13] with subsequent anemia, weakness, and abdominal pain [18], distension, and discomfort due to a tumor-induced mass effect or metastasizing tumors [16]. Tumor hemorrhage commonly occurs when large tumors develop an ischemic, punctate ulcer. Usually, the bleeding can be controlled using endoscopic sclerotherapy or electrosurgical coagulation techniques [10].

15% to 30% of patients with GISTs are asymptomatic and are diagnosed incidentally, during examinations for other reasons, during surgery for other diseases [6,15], or during postmortem autopsy [13]. In these asymptomatic patients, tumors grow insidiously and can sometimes reach large sizes.

Diagnostic Process

With the popularity of screening endoscopy, increasing gastric submucosal tumors are incidentally detected [20]. The main endoscopic finding of GISTs is common to all subepithelial lesions: a nonspecific smooth bulge covered with normal mucosa [13].

Diagnostic workup can be challenging and consists of endoscopy, ultrasonography, computed tomography, magnetic resonance imaging, and endoscopic ultrasonography with fine needle aspiration [13]. However, a biopsy specimen is often negative [15].

Treatment

The treatment strategy for immunohistologically confirmed GISTs is as follows: surgical resection is the first choice for resectable GISTs without metastasis and administration of imatinib is the primary approach for unresectable, metastatic, or recurrent GISTs [13].

Medical Treatment

In 2000, imatinib (first-line tyrosine kinase inhibitor) was first used to treat patients with advanced GIST, achieving unprecedented treatment responses in a tumor type that – when metastatic – was previously rapidly lethal [12]. The drug was approved for use as adjuvant treatment after the surgical removal of high-risk GISTs in 2012 when it is administered in an attempt to reduce the recurrence rate [8]. Sunitinib (second-line tyrosine kinase inhibitor) and Regorafenib (third-line multikinase inhibitor) can be used in advanced cases after treatment failure with Imatinib. However, it is difficult to obtain a permanent cure with tyrosine kinase inhibitors [13].

Surgical Treatment

In contrast to gastric adenocarcinoma, where gastrectomy with wide margins and lymphadenectomy is recommended [10], limited gastric resection is the recommended surgical approach for GIST, because it grows by expansion rather than diffuse infiltration and rarely invades nearby lymph nodes [5, 14,19]. Wide margins and lymph node dissection do not improve survival or decrease recurrence [11].

The surgery aims to achieve a complete resection with no tumor residue [17,21]. Moreover, strict avoidance of intraoperative tumor rupture is crucial for preventing tumor relapse [16].

Traditionally gastric GISTs resection was done with open surgery, but, more recently, less invasive methods have been reported [18]. The first description of laparoscopic resection of a GIST was in 1992 when Lukaszczyk discovered and resected an incidental gastric mass during a laparoscopic cholecystectomy [11]. The choice of the laparoscopic or open method was made by the surgeon based on his evaluation and the patient's consent [19].

As experience accumulated, initially tumors up to 2 cm [18, 22], then between 2 and 5 cm [4], and more recently between 5 and 10 cm were approached laparoscopically [21]. Prerequisites for the laparoscopic approach were the absence of metastases and the presence of a well-defined tumor on CT scanning without the involvement of adjacent organs, the esophagogastric junction, or the pylorus of the stomach [18, 19]. However, wedge resection sometimes involves inadvertent resection of additional gastric tissue, which can produce gastric deformation. Anatomic gastrectomy (subtotal or total gastrectomy) is reserved for large tumors that involve a significant portion of the stomach [10].

METHODS

After obtaining our institutional review board approval, we retrospectively reviewed the medical records of our patients operated laparoscopically for gastric GISTs between March 2019 and May 2022.

Study Population and Data Analysis

We extracted the following characteristics: age, sex, length of postoperative hospital admission duration, operation time, tumor location, tumor size, histopathological assessment of resection margins, intraoperative blood loss, and incidence of postoperative complications (surgical and non-surgical). Patients were eligible for laparoscopic surgery if preoperative staging (endoscopy and CT scan) showed a localized, non-metastatic extra mucosal gastric lesion (Figures 1, 2). Tumors were required to be of a diameter and in a position that suggested resectability through segmental or wedge resection. Preoperative histological confirmation of the diagnosis was not a prerequisite for inclusion.

Figure 1: Subepithelial gastric tumor with a clot on the surface (Case 4)

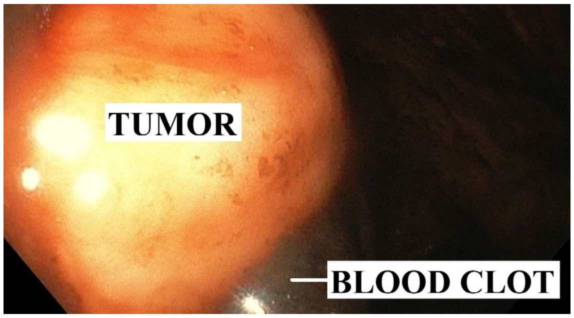

Figure 2: CT scan image (Case 1)

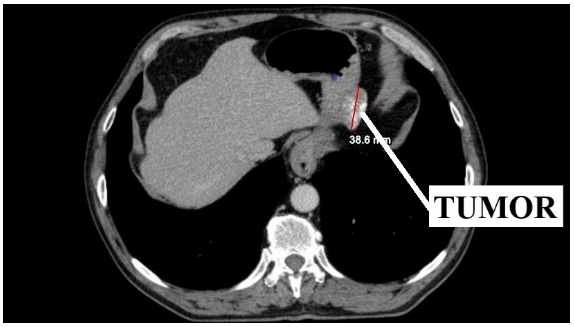

Surgical Resection Procedures

All patients were treated by laparoscopy while under general anesthesia. The patient was placed supine in the anti-Trendelenburg position and the surgeon stood between the legs. Using a Veress needle, the pneumoperitoneum was achieved with CO_2 at 12 mmHg pressure. The first trocar was placed on the median line, about 15 cm from the xiphoid process. Under visual control, another 3 or 4 work trocars were introduced. After checking all the abdominal cavities for metastases, the lesion was identified through visualization and palpation. In the case of tumor location at the posterior wall of the stomach, the gastrocolic ligament was dissected and the stomach was inverted to display the lesion. The tumor-bearing gastric segment was resected with several 60 mm endoscopic linear staplers (EndoGIA) (Figure 3). To avoid stenosis of the stomach during wedge resection, a calibration tube (36 Fr) was inserted by the anesthesiologist. Special care has to be taken to prevent peritoneal seeding and the possibility of capsular rupture during manipulation.

Figure 3: Intraoperative image, after the first EndoGIA cartridge is placed (Case 4)

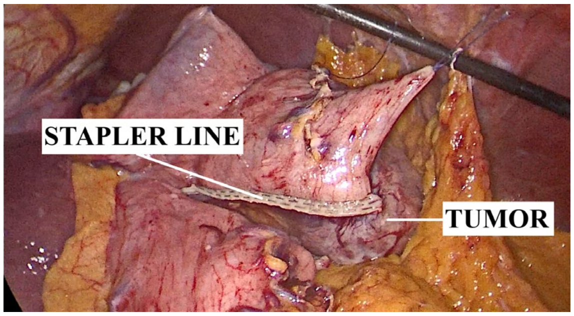

The resection specimen was placed into a plastic retrieval bag and removed through one of the extended port incisions. Stapler lines were visually controlled and, if deemed necessary by the surgeon, their integrity was assessed through the application of methylene blue solution via a nasogastric tube (blue test). At the end of the operation, a drain tube was placed in the vicinity of the staple line.

Pathological evaluation

The specimens were fixed in 10% formalin solution and sent for pathological evaluation. The characteristics of gross specimens were described, including tumor size, gross appearance, ulceration (Figures 4, 5). After sectioning, hematoxylin and eosin staining was regularly performed followed by immunohistochemical staining.

Figure 4: The specimen, after extraction (Case 3)

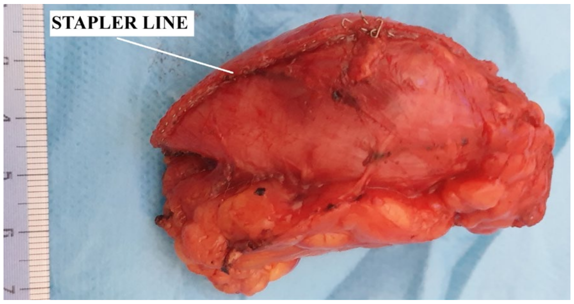

Figure 5: The specimen, with the suture line open (for inspection of the resection margins) (Case 2)

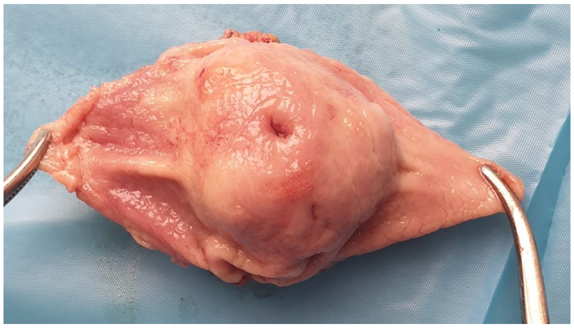

Follow up

Patients with immunohistochemically confirmed GIST were referred to the oncology department for therapeutic management

RESULTS

Four patients were planned for a laparoscopic wedge resection of a suspected gastric GIST. The presented cases were 3 females and 1 male with a median age of 70.25 years (between 64 and 74 years).

The reasons for admission to Gastroenterology Department were abdominal pain (3 patients), weakness (2 patients), anemia (2 patients), and gastrointestinal bleeding (1 patient).

All patients were investigated by endoscopy and CT scan. The average tumor size was 4.3 cm, ranging from 4 to 6 cm. The main patient data are summarized in Table 1.

The average operation time was 77.5 min, ranging from 60 to 100 min. Resection was performed using between 2 and 3 cartridges. Because the operated lesions were large, there was no need for intraoperative endoscopy to identify the tumor's location. Laparoscopic wedge resection was possible in all cases. There was no conversion, no episode of tumor rupture or spillage, and no major intraoperative complications. Intraoperative blood loss was below 50 mL in all patients and no patient required blood transfusions. The surgical margin of all tumors was confirmed using the extracted specimen.

All tumors were resected with macroscopic and pathologically negative margins. Histology confirmed a GIST in 3 of 4 cases. In one case histological workup showed gastric schwannoma.

The median postoperative hospital stay was 5.5 days, ranging from 5 to 7 days. Hospitalization was 5 days for 3 of the patients and 7 days for one patient who had a postoperative complication. One patient (with a tumor size of 3.5 cm) experienced a postoperative hemorrhage (grade II complication after Clavien-Dindo classification). This was treated conservatively with proton pump inhibitors. We had not identified any other postoperative complication.

Table 1: Patients data

Case number	1	2	3	4
Age	74	71	64	72
Sex	Male	Female	Female	Female
Histology	GIST	GIST	Schwannoma	GIST
Location	Fundus/ Greater curvature/ Anterior wall	Body of the stomach/ Lesser curvature/ Posterior wall	Body of the stomach/ Lesser curvature/ Anterior wall	Body of the stomach/ Greater curvature/ Posterior wall
Morphology	Pediculated	Extraluminal	Extraluminal	Extraluminal
Operation time	70 minutes	100 minutes	80 minutes	60 minutes
Number of cartridges	2	2	2	3
Dimension of resection	6x5 cm	7x6 cm	6.2x5 cm	9x8 cm
Tumor size	4 cm	3.5 cm	4 cm	6 cm
Tumor staging	pT_2	pT_2	-	pT_3
Risk of malignancy	Very low	Low	-	High

In the same period, outside this study, in our department, a patient with a massive tumor (18.8/18/13 cm) was treated by open subtotal gastrectomy.

All patients were referred for oncological consultation, but only 2 patients did it. Due to the favorable histopathological exam, absence of distant metastases at the time of diagnosis and on the subsequent follow-up (annually CT), and absence of local recurrence on follow-up endoscopy (6 months postoperatively, then annually), the patients did not indicate adjuvant chemotherapy.

All patients were alive at the end of this study and in good health.

DISCUSSION

Open surgery was the traditional and most widely used treatment for gastric stromal tumors. When possible, the endoscopic or laparoscopic approach is safe and leads to non-inferior outcomes compared to the open approach [21].

Although our experience is limited, we can state that the cases we have treated by laparoscopic wedge resection and presented have progressed favorably, benefiting from the well-known advantages of laparoscopic surgery. Among the other variants of the laparoscopic approach found in the literature, we list the following: non-anatomic full-thickness removal of a disk of tissue with the closure of the resulting gastrotomy, anatomic partial gastrectomy with reconstruction, and transgastric resection [11].

The choice of laparoscopic approach must take into account both the size of the tumor and its location. Visible lesions on the anterior gastric wall are most suitable for wedge resection, consisting of lifting the lesion and performing a wedge resection by application of one or more endoscopic linear stapler devices. But selected cases can be approached laparoscopically even if they are not located on the anterior wall. On the other hand, patients with lesions near cardia or pyloric antrum are not proper candidates for wedge resection due to potential stenosis. Therefore, limited gastrectomy is usually used, but alterations of anatomic structures may lead to functional disturbance and impaired quality of life [20]. In our patients, we did not find any early postoperative stenosis. Concerning long-term functional results, we were not confronted with symptoms or endoscopic signs of reflux or stenosis during postoperative follow-up.

As experience with minimally invasive surgery has increased over the past decade, laparoscopic surgery for gastrointestinal malignancies has become increasingly common [5]. As in other laparoscopic procedures, the existence of a learning curve must be assumed. However, due to the experience gained by the two senior surgeon authors in oncological surgery and bariatric surgery (sleeve gastrectomy), the surgical interventions for the presented cases were carried out in good conditions. Being tumors with malignant potential, all measures must be taken to prevent tumor injury.

In our small case series, 1 out of 4 tumors preoperatively suspected to be a GIST were histologically diagnosed as gastric schwannoma. Schwannomas are benign, slow-growing, encapsulated nerve-sheath tumors arising from Schwann cells, meant for the myelin sheath formation in the peripheral nervous system. Gastric schwannoma is a rare gastric mesenchymal tumor, which accounts for 6.3% of the gastric mesenchymal tumor, 4% of all benign gastric tumors, and 0.2% of all gastric tumors. Being different from GISTs, they are considered as being benign, slowly growing, and with excellent prognosis [20]. Retrospectively, in this case, surgical resection would have not been required for oncological reasons. We also found this situation in the literature studied [14,16,23], and it's explainable because we didn't have a preoperative histological diagnosis. On the other hand, it is considered that all localized resectable gastric stromal tumors with a diameter of 2 cm or more need resection [2,4]. Preoperative histological diagnosis of submucosal gastric lesions is however not always feasible through endoscopic biopsy [16]. With the benign and mostly asymptomatic nature of gastric schwannomas, endoscopic follow-up is also an alternative method. But it is not widely adopted due to the lack of a standardized surveillance strategy, the risk and cost-effectiveness of repeated endoscopic procedures, and patients’ stress and compliance [20].

CONCLUSIONS

In conclusion, laparoscopic wedge resection for gastric GIST, even larger than 5 cm, appears to be safe and feasible in selected patients, with acceptable operative time and oncological outcome after an intermediate follow-up.

More important than the size of tumors that are approached laparoscopically is their location and the possibility of a stenosis-free resection.

Conflicts of interest and sources of funding

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REVIEW

The Role of Inflammation in the Pathogenesis of Psoriasis

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Abstract: *Psoriasis is a chronic inflammatory skin condition with genetic determinism characterized by the presence of well-defined, erythematous plaques, covered by white, pearly, stratified scales, located on the extension areas, the skin of the scalp, intertriginous regions. The origin of psoriasis is multifactorial, involving hereditary and environmental pathogenic mechanisms. It is triggered by various risk factors involving a variety of processes, such as inflammation, antigen presentation, cell signaling, and transcriptional regulation.*

Keywords: *psoriasis vulgaris, inflammation, immune system*

OVERVIEW

Definition

To have a starting point and be able to go deeper into a subject, as in any field, and in the case of diseases, we must first know the definition. A definition must be as complete as possible, to create an overall picture of the disease.

Psoriasis is a chronic inflammatory skin condition [1], proliferative [2], with genetic determinism [3], characterized from a clinical point of view by the presence of well-defined, erythematous plaques [4], covered by white, pearly, stratified scales [5], located on the extension areas and in the skin of the scalp.

If it was initially considered a benign dermatological condition, psoriasis is now accompanied by multiple comorbidities, being considered a multisystemic inflammatory disease [6]. In addition to the impact it has from an aesthetic point of view, psoriasis also significantly influences patients' quality of life [7].

A recent study estimated that the psychological impact in patients with psoriasis is severe, between 10-62% [8]. Among the most common symptoms are: low self-esteem, sexual dysfunction, suicidal ideation, which occurs in up to 10% of patients with psoriasis, decreased professional opportunities, stress – which can trigger psoriasis flare-ups, depression [8].

Incidence

Worldwide, the prevalence of psoriasis is between 0.5%-11.4% in adults [9], and this increases with increasing distance from the Equator [10]. Recent studies have shown that in Australia the prevalence of psoriasis in adults is between 2.3% - 6.6% and 1.3% - 2.2% in the United Kingdom [11].

While most cases of psoriasis often appear before age 35, it can develop at any age [12], and men and women are equally affected [13].

ETIOPATHOGENESIS

The cause of psoriasis is not known, but it was found to be a condition with multifactorial determinism [14], which to manifest itself clinically requires the presence of genetic and environmental factors [12].

About 40% of people with psoriasis or psoriatic arthritis have a family member with psoriasis [15].

The major susceptibility locus in psoriasis: PSORS 1 (6p21.3) within the MHC gene complex is present in approximately 30% of patients with psoriasis [15].

In patients with genetic predisposition, environmental factors can have a triggering or aggravating effect on the disease, and among the most frequently involved environmental factors are: trauma, infections, endocrine factors, certain drugs, alcohol, smoking, and obesity [16].

Pathogenic hypotheses

As mentioned, the origin of psoriasis is multifactorial, involving hereditary and environmental pathogenic mechanisms [17]. At the epidermal level, there is hyperproliferation and abnormal differentiation of keratinocytes, as well as activation of the skin's immune system [18].

Human histocompatibility system (HLA)

The major histocompatibility complex (MHC) also known as human leukocyte antigen (HLA) is one of the most genetically diverse regions in the genomes of different species [18].

More than 500 associations have been identified in the HLA region for approximately 200 phenotypes, including immune deficiencies, autoimmune diseases, susceptibility to infections, malignancies, and psychiatric conditions (Welter et al. 2014) [18].

The function of gene families at the level of MHC in the occurrence of autoimmune diseases has been the subject of research for decades [19].

Psoriasis is characterized by epidermal hyperproliferation and dermal inflammation [20], mediated by T cells [21]. Efforts to study genetics in psoriasis continue, with nine susceptibility loci (PSORS1-9) being identified [22]. The PSORS1 locus is considered the genetic determinant of psoriasis [23].

From the human genome, MHC is one of the most studied regions [24], with multiple genetic variants at this level that have contributed to the emergence of diseases such as psoriasis, Crohn's disease, systemic lupus erythematosus, rheumatoid arthritis [25].

MHC is located on chromosome 6p21.3 and is divided into 3 regions: the centromeric region (MHC III) and the telomeric regions (MHC I and II) [26].

The genome-wide association study (GWAS) demonstrated that at the MHC level there are SNPs associated with the occurrence of psoriasis [27].

The major locus involved in the development of psoriasis – PSORS 1 – located at the MHC level is probably associated with non-MHC loci with common alleles [28], which determines the genetic heterogeneity of psoriasis [29].

Certain gene combinations increase the risk of psoriasis, while others decrease it. Patients with psoriasis have been observed to have a higher prevalence of HLA-Cw6, HLA-B13, HLA-Bw17, and HLA-B37 antigens [27].

Major susceptibility locus (PSORS1)

While the major susceptibility locus is PSORS1, other susceptibility loci seem to also play important roles: PSORS2 (17q24-q25) [26].

Molecular studies revealed the following "candidate" genes for psoriasis:
- Gene S located on HLA-Cw6 (expresses the duration of keratinocyte differentiation) [26].
- Genes from the differentiation complex PSORS4 (1q21-q22) - encoding molecules involved in the terminal differentiation of keratinocytes [28].
- PSORS 5 (predisposition in Swedish families) [26].
- PSORS 8 (susceptibility to Crohn's disease and psoriatic arthritis [27].
- PSORS 9 (predisposition in Chinese families) [28].

The immune system

Psoriasis is an incurable autoimmune disease with genetic determinism, mediated by T lymphocytes…

Immunity includes all the physiological mechanisms that allow the body to recognize and eliminate a "non-self" antigen [30]. To be effective, the immune system must differentiate between "self" – cells, proteins that belong to the body and "non-self" – pathogens or substances foreign to the body [31].

There are two types of immune system response:
- Innate immunity = non-specific (which generates an immediate response)
- Acquired immunity = specific

Innate immunity includes two main lines of defense:
A) Physical barriers: skin, mucous membranes, cilia.
B) Chemical barriers: saliva, mucus, acid environment in the stomach, sweat, lysozyme.

Acquired immunity involves:
- The inflammatory response
- The complement system
- Cellular components

The innate immune response is naturally present in humans and is not pathogen-specific. The acquired immune response triggers a specific cell-mediated response through T lymphocytes, which secrete cytokines, and humoral through B lymphocytes, which secrete antibodies [31].

Psoriasis, the chronic inflammatory skin disease [1], is characterized by abnormal innate and acquired immunity [30], which is associated with aberrant differentiation and hyperproliferation of keratinocytes [31].

Pathogenic hypotheses

Psoriasis is a complex genetic disorder that is triggered by various risk factors involving a variety of processes, such as inflammation, antigen presentation, cell signaling, and transcriptional regulation [33]. Persistent inflammation in psoriasis causes uncontrolled differentiation and proliferation of keratinocytes [34]. It is considered that both keratinocytes and other cell types are involved in the appearance of the psoriatic plaque, from whose interaction in the superficial epidermal layers the persistent inflammation results [35].

From a histological point of view, the changes that appear in psoriasiform skin are acanthosis, parakeratosis, and hyperkeratosis [36].

In the most recent meta-analysis performed on genome-wide association studies, 15 new PSORS-type loci were identified, bringing their number to 36 [37]. Among the loci identified are several that encode the NFkB signal transduction cascade, such as REL, NFKBIA, TRAF6, CARD14, and ILF3 [37].

Studies at the level of the entire genome emphasize the complexity of gene changes involved in the occurrence of psoriasis [38].

In several reports, the role of the nervous system in the pathogenesis of psoriasis was emphasized, and its involvement was based on the symmetrical distribution of lesions on the body and the interaction of sensitive peripheral nerves with immunomodulatory networks [39].

Cases have also been reported in which surgical denervation of psoriasis lesions or local anesthesia would decrease local inflammation [39].

Emotional stress, as well, has been reported as being involved in the onset of psoriasis or its exacerbation [40].

Over time, research has shown that the immune system has a key role in the development of psoriasis [41].

Classically, psoriasis is a systemic inflammatory disease [1], controlled by T cells [3], and modulated by genetic and environmental factors [2].

The presence of macrophages, lymphocytes, and neutrophils in the skin is a characteristic sign of psoriasis [7]. Therefore, the disruption of the innate and adaptive skin immune response, to which is added the presence of non-immune cells, determines the support of inflammation [9].

THE ACTIVE MOLECULAR ENVIRONMENT IN PSORIASIS

The activity of the immune system does not consist only in its specificity, but also in the elaborated communication network through the cytokines secreted by the activated immune cells [42].

Cytokines are small, soluble proteins that allow the recruitment of other immune cells, such as T lymphocytes or dendritic cells, and are involved in identifying the site of infection [43].

The key groups of cytokines are represented by: IL, interferon (IFN), and tumor necrosis factors (TNF) [42].

IL-1 is produced by macrophages, monocytes and other types of cells, being composed of IL-alpha and IL-beta, with a key role in the inflammatory response [44].

It binds to the specific receptor – IL-1R and its accessory protein (IL-1RAcP) [43], further activating a complex intracellular signaling network [45].

This ultimately leads to the induction of transcription factors, such as NFκB, resulting in inflammatory immune responses [46].

The IL-beta subunit has an important role in psoriasis, inducing the proliferation of T cells, which produce IL-17 and thus maintain the inflammatory processes in psoriasis [47].

Therapies targeting IL-1 have not demonstrated satisfactory results in plaque psoriasis, but satisfactory results have been obtained in generalized pustular psoriasis [45].

IL-23 is a cytokine formed by two subunits, p19 and p40 [48], involved in antifungal and antibacterial defense [49]. It is produced by macrophages and dendritic cells, and the receptor to which it binds consists of IL-23 R and IL-12Rbeta1 [50]. Furthermore, it determines the activation of transcription factors, including STAT-3 [51].

It cannot activate naive T cells alone, because they do not have specific receptors, but it is involved in the pathogenesis of psoriasis through Th 17 cells [52].

The development of anti-IL-23 therapies has shown excellent efficacy in the treatment of psoriasis [51].

Th17 cells, neutrophils, mast cells, Nk cells, and B cells are the source of IL-17 [52], The IL-17 family has 6 members (IL-17A – IL-17F) that bind to specific receptors [53].

In macrophages and fibroblasts, IL-17 induces the expression of pro-inflammatory molecules (IL-1p, IL-6, GM-CSF, TNF-alpha) [52].

Having a pro-inflammatory role, it can cause autoimmunity in the immune system, which has been demonstrated for rheumatoid arthritis, psoriasis, and inflammatory bowel disease [53].

IL-17 is involved in the inflammatory process from metabolic disorders, such as hepatic steatosis and arteriosclerosis [54] so IL-17 antagonists can also intervene in the improvement of comorbidities, not only in the treatment of psoriasis [55].

IL-22 is part of the same family of cytokines as IL-10, and the active form is a monomer that binds to its specific receptor, located at the level of epithelial cells and fibroblasts of various cells [56]. It consists of heterodimeric subunits (IL-22R1 and IL-10R2) [57].

IL-22 is produced by CD4 (Th1, Th17, Th22) and CD8 lymphocytes, innate lymphoid cells, and NK cells [58].

The transcription factor RORγt is essential for IL-22 production because CD4 + cells secrete IL-22 only in the presence of this receptor [59].

The serum level of IL-22 is correlated with the activity of the disease [57] and in the skin with psoriasis lesions, the level of IL-22 is increased [58]. This causes keratinocytes to migrate, preventing their differentiation, and leading to the thickening of the epidermis [59]. Therefore, IL-22 could represent a potential target for the treatment of psoriasis [57].

IL-12 is produced by dendritic cells, macrophages, and neutrophils, being a heterodimer formed by the p35 unit and the p40 unit [62]. Its receptor is mostly found on T cells and N cells [65] and consists of IL-12 Rbeta 1 and IL-12Rbeta2 subunit [63]. The binding of IL-12 to the receptor activates the JAK/STAT pathway [67]. Although IL-12 increases IFN-gamma and TNF-alpha [68] molecules involved in the pathogenesis of psoriasis, an increased level of the p35 subunit was not detected in the skin of patients with psoriasis [69], and targeting the p40 subunit exerted its effects by inhibiting IL-23 [70].

The main sources of interferon-gamma - type 2 interferon (IFN-γ) are CD4+ and CD8+ cells, NK cells, but also antigen-presenting cells and B cells [69]. It is involved in antiviral and antibacterial defense [67]. The IFN-gamma receptor consists of two IFNGR1 chains and two IFNGR2 chains responsible for signal transduction [71], which leads to the activation of the JAK/STAT signaling pathway [72]. The serum level correlates with the activity of the disease, being higher in patients with psoriasis [70]. Greater decreases in serum IFN-gamma are associated with longer periods of disease remission [67], but anti-IFN-gamma therapy has not demonstrated efficacy on psoriasis lesions [70].

The role of TNF alpha in the inflammatory mechanisms of psoriasis

TNF-α is produced by macrophages, monocytes, keratinocytes, and lymphocytes, being a pro-inflammatory cytokine [72], involved in both acute and chronic inflammation [71], with an antibacterial and antitumor role [70].

Receptors for TNF are found in all nucleated cells [69] and are formed by two main isoforms: TNFR1 and TNFR [73].

TNF alpha is involved in the proliferation of CD4 cells [72], in the production of chemokines and cytokines [74] (IL-1), and is also involved in the process of apoptosis in certain types of cells [73].

The level of TNF-alpha correlates with disease activity [71], and TNF-alpha inhibitors have proven effective in the treatment of plaque psoriasis [72].

INFLAMMATORY CELLS IN PSORIASIS

Keratinocytes and cells of innate immunity in the skin

At the level of the skin, which is the largest organ of the body, consisting of the epidermis, dermis, and hypoderm [3], there is a large number of cells with various roles.

In psoriasis, the interaction between cells of the innate immune system (macrophages, neutrophils), cells belonging to adaptive immunity (T and B cells), and resident cells (keratinocytes, melanocytes) support chronic inflammation [73].

In the early stage of inflammation, an important role is played by dendritic cells, which are antigen-presenting [75]. The mechanism by which these cells are activated is incompletely elucidated, but it seems that there are certain microbial peptides, such as LL37, and S100, released by keratinocytes [76]. In psoriatic skin, these peptides are overexpressed and bind to the DNA of damaged cells, which causes dendritic cells to be activated to produce IFNα [77]. It (IFNα) causes the activation of myeloid dendritic cells [75].

Activated dendritic cells transform into antigen-presenting cells to be able to interact with naïve T cells and begin to produce large amounts of TNF-alpha, IL-23, IL-6, and IL-12 [77].

These cytokines activate the inflammatory cascade by recruiting neutrophils to the site of inflammation [78] and the proliferation of keratinocytes [79].

Keratinocytes, in turn, produce antimicrobial peptides [74], cytokines (IL-6, TNF-alpha), and chemokines (CCL20, CXCL5, CXCL9, CXCL10) [76].

IL-36, secreted mainly by keratinocytes and dendritic cells [73], accumulates neutrophil granules, present in large numbers in psoriatic skin [77]. IL-36 binds to its receptor IL-36Ra [78] and is involved in the transcription of other inflammatory mediators, by activating NF kappa B [79]. Also, IL-36 increases inflammation through its interaction with other inflammatory cytokines, such as IL-17 [79]. Some data suggest that decreasing the number of neutrophils significantly improves inflammation [76].

Macrophages are also antigen-presenting cells [75]. They derive from monocytes [77] and are involved in the occurrence of psoriasis inflammation [78], being an important source of TNF-alpha [77].

Within the innate immune system, cells without antigen-specific receptors (ILCs) are involved in antimicrobial defense and create a balance between pro- and anti-inflammatory factors [80].

ILC 3 can produce IL-17 and IL-2 [81], and their number decreases in the affected skin during the remission period of the disease [80], emphasizing the involvement in the pathogenesis of psoriasis, which requires additional data [81].

T cells

T cells have an important role in the defense against pathogens, and in the pathogenesis of psoriasis, there is an interaction with a central role between T cells and dendritic cells.

Dendritic cells release cytokines such as IL-12 and IL-23 that promote Th1, Th17, and Th22 responses.

At the epidermal level, T helper cells support keratinocyte hyperproliferation, leading to decreased apoptosis.

By carrying out human skin transplantation in SCID immunodeficient mice, followed by the injection of CD 4 T cells from patients with psoriasis, the role of these cells in the pathogenesis of psoriasis was demonstrated.

Th 1 (CD4+) cells produce large amounts of TNF-alpha and IFN-gamma involved in triggering psoriasis.

In mice with IL-17 receptor deficiency, skin inflammation is reduced, emphasizing the importance of IL-17 in the occurrence of psoriasis lesions.

Thus, elevated serum levels of IL-17 and increased expression in lesions are typical features of psoriasis.

After it was demonstrated that IL-23, produced by dendritic cells, is involved in the development of Th17, it became obvious that the IL-23/Th17 axis has an important role in the pathogenesis of psoriasis.

B cells

B cells are involved in the adaptive immune response through antibody production and antigen presentation [23].

Using a model of intestinal inflammation, Mizoguchi et al. [58] in a model of intestinal inflammation, showed that B cells produce IL-10 and thus have a protective role against inflammation.

Also through the secretion of IL-10, B regs cells are involved in alleviating various allergic and inflammatory diseases [82]. They do not represent a distinct lineage of B cells but are rather a subset of them [81]. It is not elucidated which factors are involved in the development of B cells in a type B regs [80].

The B regs cell population is characterized by the presence of CD5+CD1d hi on the surface [81].

Other subsets of B cells involved in the production of IL-10 are those in the marginal zone – CD19+CD21 hiCD23-CD24hi and CD138+ plasma cells [80].

Compared to T cells and studies regarding their role in the pathogenesis of psoriasis, in the case of B cells, data are limited regarding the involvement of these cells in psoriasis, as they rarely appear in lesional skin [83].

The decrease in the number of B lymphocytes in patients treated with anti-CD20 (Rituximab) for other autoimmune diseases led to the appearance of psoriasis skin lesions in individual cases [84].

B cells secrete inflammatory cytokines such as IL-6, IL-4, and IFN-gamma, being key effector cells [83].

Also, in a recent study, it was demonstrated that a large number of IL-6-producing B cells are found in inflamed mouse skin [80]. Blocking IL-6 resulted in reduced inflammatory symptoms [84].

DISCUSSION

In the case of mild psoriasis, the inflammatory process is confined to the skin, on the other hand, a more extensive active disease should be regarded as a systemic inflammatory disease, impacting internal diseases, including arthritis and metabolic syndrome [88].

Patients suffering from moderate to severe forms of psoriasis have increased serum levels of multiple proinflammatory cytokines, including TNF-a, IFN-c, IL-6, IL-8, IL-12, IL-17A, and IL-18 [89].

All the subtypes of T cells involved in the pathogenesis of psoriasis are also involved in atherosclerosis [90]. Sherlock et al. demonstrated that IL-23 drives inflammation in the aortic root through the activation of CD3+CD4−CD8− T-lymphocytes [91].

TNF alpha plays an important role in the inflammatory pathways of metabolic syndrome, diabetes, and cardiovascular disease. TNF alpha inhibitor therapy is associated with a lower risk of major adverse cardiovascular events (MACE) independent of traditional cardiovascular risk factors [92]. Recent research shows the importance of Th17 and other IL-17-producing cell types in the pathogenesis of cardiovascular events [90].

We lack large well-designed studies which prove the improvement of comorbidities by treating psoriasis with biologics.

In short term, there is evidence that early treatment with biologics reverses damage caused by inflammation. New biomarkers and early diagnosis help healthcare workers in better evaluation of patients' responses to systemic therapies [93].

CONCLUSIONS

Psoriasis is a recurrent, chronic, T-cell-mediated, polygenic disease characterized by erythematous squamous lesions in specific body sites. The pathogenesis of psoriasis is complex and still undergoing study. Biologic therapy is the key to reversing inflammations and preventing associated comorbidities.

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ORIGINAL ARTICLE

Postirradiation Angiosarcoma – A Late, Severe Complication of Radiotherapy for Mammary Neoplasm

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Abstract: *Angiosarcoma is a malignant tumor derived from the endothelium of the blood and lymphatic vessels. Its aggressive behavior results in a poor prognosis for the patients. Radiation-associated angiosarcoma is a rare recently described particular subtype of angiosarcoma best shown in patients after treatment of breast cancer. The latency period of development is approximately 8 years, thus the 5-year standard follow-up after mammary neoplasm therapy needs to be extended up to 10 years. Radiotherapy is an independent risk factor for angiosarcoma development. Rapid diagnosis is essential for prompt therapeutical intervention to improve the patient's prognosis. The importance of early diagnosis is emphasized by a case of a 54 years old woman, with a diagnosis of breast cancer treated by sectorectomy and radiotherapy 7 years prior, presenting to our Dermatology Department for the recent occurence of erythemato-violaceous cutaneous patches on the area subjected to radiotherapy.*

Keywords: *angiosarcoma, radiation, radiotherapy, breast cancer, conservative surgery, follow-up*

INTRODUCTION

Angiosarcoma is a malignant tumor that originates in the endothelium of the blood and lymphatic vessels [1]. It usually develops in the skin, soft tissue, breast, or liver [2]. It comprises 1-2% of the soft tissue sarcomas that appear in humans [3], it is uncommon and affects mostly Caucasian men, with the highest incidence in adults over 70 years.

When it comes to younger ages such as children or adolescents, angiosarcomas are rare and are prone to develop in viscera or nearby, usually the heart, or they are associated with other conditions such as immuno-suppression, chronic radiodermatitis or lymphedema [4].

Risk factors associated with angiosarcoma include chronic lymphedema, radiation history, carcinogens (eg vinyl chloride, arsenic), and specific familial syndromes [3].

Angiosarcomas cells have endothelial differentiation and they clone while undergoing a malignant transformation. Unlike other sarcomas, angiosarcomas have a different upregulation of the genes responsible for the vascular receptors [4]. In angiosarcomas that have been induced by radiation or lymphedema, the amplification of MYC is high which in the end leads to the downregulation of thrombospondin-1, a protein that inhibits angiogenesis [4]. MYC is overexpressed in patients with radiation-induced angiosarcoma and this can help distinguish it from primary angiosarcoma [5]. Angiosarcomas can be well- or poorly differentiated; in the first case, one can observe vascular channels that are not regular in shape, they are lined by endothelial cells and they dissect the fibers of collagen; the latter has ill-defined vascular spaces, the cells are spindled, polygonal, epithelioid and round and they show multiple mitosis and big nuclei [1].

Immunohistochemistry is used when diagnosing less differentiated angiosarcomas. The cells express vascular as well as endothelial antigens such as Factor-VIII, CD31, CD34, and VEGF. CD31 is seen in more than 50% of the cases and has great sensitivity and specificity [3]. Recently, there has been used a marker called ERG, which is a member of the ETS family of DNA-binding transcription factors and which offers greater sensitivity and specificity for endothelial cell differentiation

[1].

PATHOGENESIS AND TYPES OF ANGIOSARCOMAS

The mechanism through which angiosarcoma may appear in chronic lymphedema is not well known, but theories postulate that some carcinogens build up in the lymphatic fluid and because the lymphatic network is lost, the affected area is spared by the immune system and this fact can furtherly favor the neoplastic modified cells.

An independent risk factor for the development of angiosarcoma is radiotherapy which is best shown in patients with breast cancer [4].

There are essentially 3 instances for the occurrence of cutaneous angiosarcoma: idiopathic angiosarcoma of the scalp, face, and neck, angiosarcoma associated with chronic lymphedema (Stewart-Treves syndrome), and post-irradiation angiosarcoma. Some authors describe a fourth type which occurs in any other settings than the previous ones [1].

Angiosarcoma of the scalp, face, and neck is most frequent in elderly individuals. Men are two times more affected than women. The lesion begins as a bluish macule that can be confused with a bruise.

Angiosarcoma associated with chronic lymphedema is also called Stewart-Treves syndrome and this type is seen in areas with long-lasting lymphedema as is the case of the upper arm after the patient underwent a mastectomy. The tumor develops 11-22 years after surgery, having though a low incidence of 0.45% of the cases, but a poor prognosis [6]. Other instances of chronic lymphedema may be parasitic infections or congenital lymphedema [3].

Post-irradiation angiosarcoma is a vascular tumor that appears in sites that have been irradiated in the past. If the affliction for which the patient has received radiotherapy was benign, the average time for angiosarcoma to develop is 23 years, but if the condition treated with radiation was malignant, then the time reduces to only 12 years [6].

The fourth type is uncommon and may appear for example in benign vascular tumors, in cases of immunosuppression after organ transplantation.

RADIATION ASSOCIATED ANGIOSARCOMA

The angiosarcomas that arise in areas of previous radiation sites are difficult to be differentiated from atypical vascular lesions. Sometimes even histologically, the diagnosis can be challenging since well-differentiated angiosarcomas which exhibit only minor cytologic atypia despite their aggressive behavior, may be similar to microcystic lymphatic malformation. Also histologically, the angiosarcoma must be differentiated from other vascular neoplasms such as PILA (papillary intra-lymphatic angioendothelioma), RHE, epithetlioid hemangioendothelioma, KHE (Kaposiform hemangio-endothelioma) [4].

Imaging tools such as CT, and MRI are helpful, but to have a precise diagnosis for angiosarcoma, histologic examination and immunohistochemistry of the tissue confirm the disease.

Post-irradiation angiosarcoma is not seen very often [1]. It is considered a late severe complication of radiotherapy [7]. The majority of cases emerge in the skin after it has been irradiated for breast cancer, or not so frequently, for a neoplasm of the internal organs [1]. Post-irradiation angiosarcoma has a cumulative incidence of 0.9-3.2 per 1000 cases of mammary neoplasm. In terms of prognosis, a 5-year survival rate lies between 27 to 48% [8]. If the patient had radiation therapy for breast cancer, the period after which a sarcoma in this area may appear is 5-10 years and this means that the usual 5-year follow-up needs to be extended [3]. It appears that other radiation-induced sarcomas take more than 10 years to develop [9]. Some studies link the usage of radiation in high doses with the incidence of angiosarcoma [3]. In the case of breast angiosarcoma, the latest population-based studies have shown the risk to be about 0.1%. It has been hypothesized that the replacement of radical mastectomies with conservatory surgery has led to secondary angiosarcomas in the mammary gland [10]. Breast angiosarcoma can be categorized into primary (de novo) if it develops without any known trigger, or secondary if it appears at the site of previously irradiated integument [8].

It appears that in the last few years, of all the subtypes of sarcomas of the breast induced by radiation, angiosarcoma had the highest occurrence [10].

In a retrospective Dutch population-based study from 2019 of 296,577 patients with angiosarcoma after breast cancer treatment (1989-2015), the median follow-up after diagnosis of breast cancer was 7.7 years, and the median age when diagnosed with angiosarcoma was 58 years. Of the 184,823 patients that received radiotherapy, only 209 had angiosarcoma of the breast and/or chest wall. As a note, angiosarcoma did not occur in any of the patients that did not receive radiotherapy (111,754). The median period between breast neoplasm treatment and the appearance of post-radiotherapy angiosarcoma was 8 years. In this study, 5- and 10-year survival rate was 40.5% and 25.2%, respectively, regardless of the type of treatment (surgery alone, surgery and radiotherapy combined sometimes with hyperthermia, chemotherapy, and radiotherapy). The inclusion of radiotherapy compared to only surgery had not shown a significant survival [7]. Another study from 2013, that evaluated 48 patients with angiosarcoma treated between 1990 and 2004 (7 with breast cancer) showed a 7 years latency period for angiosarcoma of the breast. [11].

Clinical presentation of cutaneous angiosarcoma includes single or multiple red, bluish or violaceous macules, patches, and nodules, that can further ulcerate or present intra-tumoral hemorrhage [6].

The 2022 diagnostic criteria for radiation-associated angiosarcoma are a) sarcoma that develops in a previously irradiated field or the tissue volume comprised in the 5% isodose line; b) minimum 4 years between radiotherapy and the arising of the angiosarcoma; c) histological difference between primary neoplasm and secondary sarcoma; d) the exclusion of patients with cancer syndromes [5].

The prognosis is poor because angiosarcoma is an aggressive tumor; patients' survival rate without treatment is between 6 months and 2 years after diagnosis [6]. If it is a low-grade tumor, it behaves similarly to atypical vascular lesions, but a high-grade angiosarcoma is aggressive, has violent growth, and local recurrences arise after a few months [9]. However, with an incidence so low, one can assume that it is worth risking the arising of an angiosarcoma in a patient with breast cancer for the benefit of radiotherapy on the primary neoplasm [8].

Treatment consists of surgery, radiotherapy, and chemotherapy as options, but the choices and results vary on tumor size, location, and type, and last, but not least, resectability [3]. Currently, the main choice is surgical removal [8]. For localized angiosarcoma, the mainstay of the treatment is surgical resection along with adjuvant radiotherapy. Recurrences are difficult to avoid; chemotherapy is the first option in case of metastatic angiosarcoma [3]. The recurrence rate of post-radiation angiosarcoma of the breast is high (96%) and metastases are most often found in the lungs and liver and they can coexist with the local recurrence or appear soon after [8].

In a case of a 54-year-old woman who was referred to our clinic for ecchymotic and purpuric macules localized on the right breast, postradiotherapy angiosarcoma was diagnosed based on clinical aspect, history, histology, and immunohistochemistry. She had a previous history of sectorectomy for breast cancer 7 years prior and she also underwent radiotherapy. Clinical examination identified well-defined ecchymotic and purpuric macules, oval or with irregular polycyclic borders, 1-3 cm diameter; a similar macule located in the lower-inner quadrant, 4 cm diameter, with purple nodules on the surface, resembling Kaposi sarcoma (Figure 1).

Figure 1: Clinical aspect of the breast lesion

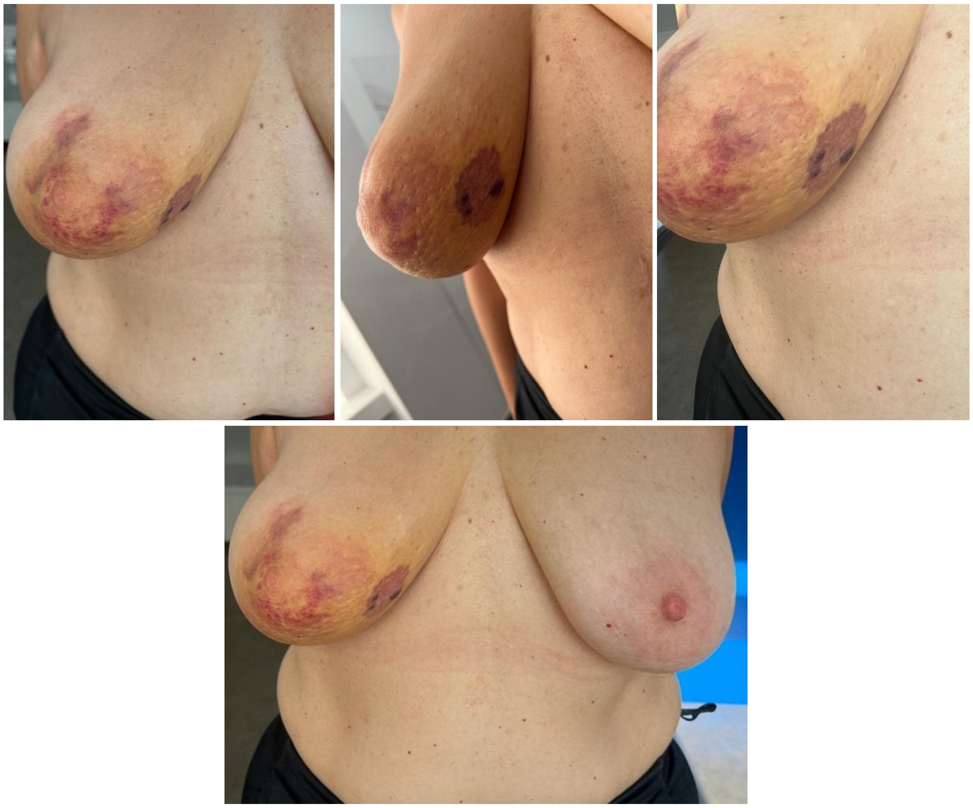

The lesions appeared 2 months before presentation, with progression in terms of dimensions and the number of lesions. The dermoscopic examination showed a combination of red, blue, and purple: a violaceous hue of the lesion that had purple clods with white structures, red and blue areas, and reddish-brown crusts that covered the central part of the nodules (Figure 2).

Figure 2: The dermoscopic examination

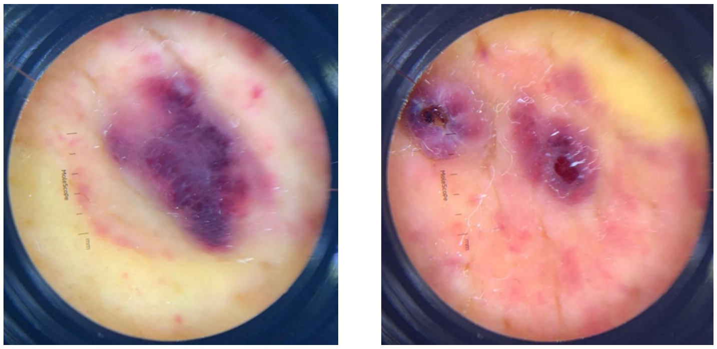

Skin biopsy of two sites was performed: a cutaneous nodule from the lower-inner quadrant of the right breast and a cutaneous fragment from the upper-outer quadrant of the right breast. The histology examination revealed: the first site showed ill-defined tumoral proliferation made up of randomly located oval/fusiform cells with elongated nuclei or grouped in short anastomotic bundles that defined small vascular spaces or intracytoplasmic slits without walls, some of them containing blood; moderated lymphoplasmacytic infiltrate and hemosiderin associated deposits. The second site of the biopsy had the same histopathologic description (Figures 3 and 4).

Figure 3: Dermal vascular proliferation, mimicking Kaposi sarcoma (HE X 10)

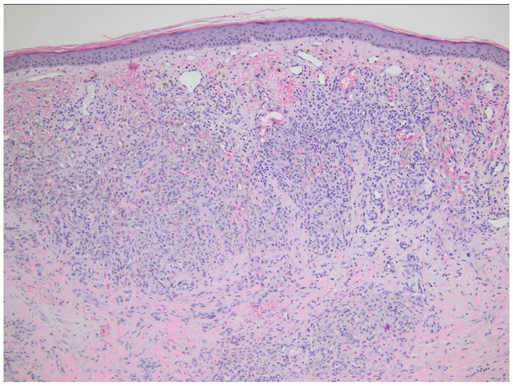

Figure 4: Dermal vascular proliferation, mimicking Kaposi sarcoma (HE X 20)

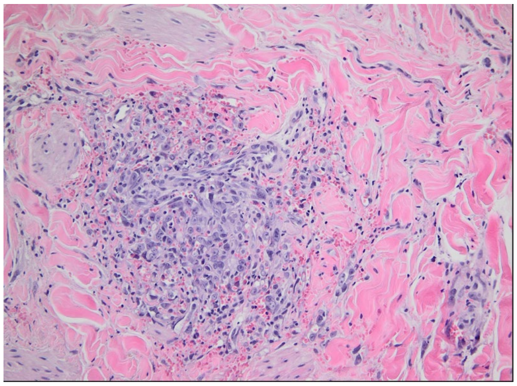

Was then performed immunohistochemistry tests for a positive diagnosis. These tests showed on both blocks HHV-8 negative and ERG and SMA positive, but c-MYC positive on the second piece of tissue (Figures 5-7).

The conclusion was that of a postradiotherapy angiosarcoma. The patient was then referred to Oncology Department for further investigations and therapeutic intervention.

Figure 5: C-MYC positive within numerous tumor nuclei

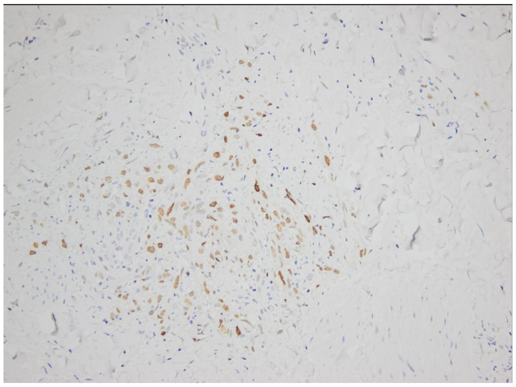

Figure 6: SMA positive within scattered tumor cells

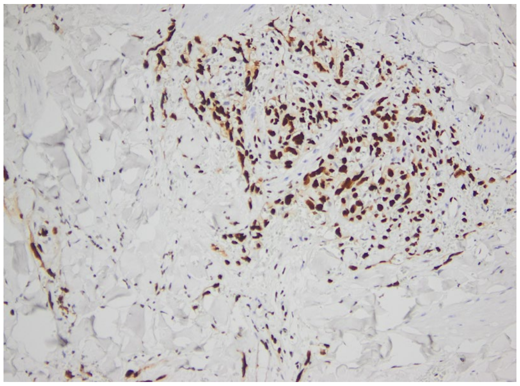

Figure 7: ERG positive within numerous tumor nuclei

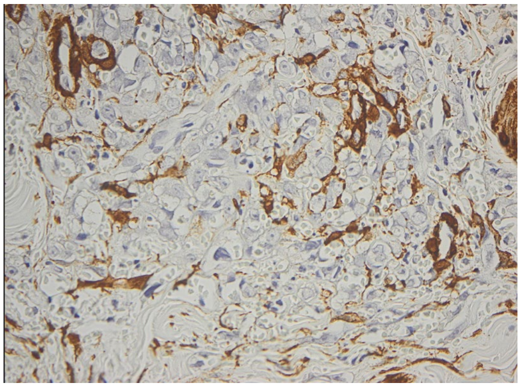

CONCLUSIONS

- Radiotherapy is an independent risk factor for the development of angiosarcoma, best shown in patients with breast cancer.
- Post-irradiation angiosarcoma has a cumulative incidence of 0.9-3.2 per 1000 cases of mammary neoplasm so it is a rare complication of radiotherapy, proving to be a challenging clinical diagnosis.
- The diagnosis of angiosarcoma can also be histologically challenging – it can exhibit only minor cytologic atypia in well-differentiated cases; immunohistochemistry testing is essential for differentiation from other types of sarcoma (including Kaposi sarcoma).
- The average period between breast neoplasm treatment and the appearance of post-radiotherapy angiosarcoma is 8 years.
- Prognosis of RAASB is poor, 5-year survival rate lying between 27 to 48% and thus it needs rapid diagnosis and therapeutic measures.
- 5-year standard follow-up after breast cancer needs to be extended up to 10 years to capture the development of an angiosarcoma.

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ORIGINAL ARTICLE

Tips and Tricks in Laparoscopic Adrenalectomy for Pheochromocytoma. How We Do It

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Abstract: *Pheochromocytomas are rare neuroendocrine tumors that frequently have genetic involvement and can present a particular risk in terms of the anesthetic-surgical procedure. Current worldwide medical recommendations state that laparoscopic adrenalectomy is the conventional treatment for pheochromocytoma, however, the ideal surgical strategy is still being developed as specialized surgeons gain experience with these rare tumors. The study's purpose was to thoroughly evaluate our film library and identify the best surgical steps that represent our standardized procedure. We developed a comprehensive set of surgical critical strategies for either a left or right adrenalectomy, providing helpful advice on patient positioning, port placement, the adrenal gland approach, as well as other surgical methods specifically designed for tumor mobilization and dissection. The current paper aims to synthesize our clinical experience in terms of preoperative preparation, patient positioning, intraoperative complications, causes of conversion, and specimen extraction. The main conclusions concern both the best surgical approach and the importance of surgical experience in the clinical management of these rare tumor types.*

Keywords: *pheochromocytomas, laparoscopic adrenalectomy, surgical strategy*

INTRODUCTION

Pheochromocytomas are relatively uncommon tumors often with genetic aggregation that is accompanied by a high risk of overall morbidity and mortality, especially during surgical procedures precipitated by important blood pressure and heart rate variations. Although laparoscopic adrenalectomy has become a standard procedure for pheochromocytoma, the optimal approach continues to evolve as surgeons develop expertise in this pathology.

MATERIALS AND METHODS

Our experience starts almost 20 years ago, counting 436 laparoscopic adrenalectomies, from which 96 were performed for pheochromocytoma. We systematically reviewed our video database and highlighted the best surgical steps that exemplify our standardized approach.

RESULTS

From our experience, we managed to synthesize detailed surgical key steps for the overall procedure when performing left or right adrenalectomy, offering valuable tips starting from the patient positioning, and port placement and continuing to the adrenal approach, along with different strategies specifically tailored for pheochromocytoma mobilization and dissection. Taking into consideration our experience in dealing with the challenges of this procedure the current article aims to lead to improvement of overall outcomes for this procedure.

Preoperative preparation

From the anesthesiologist's point of view, surgical resection of pheochromocytoma can be divided into two phases based on the ligation of the tumoral blood supply [1]. Communication between the surgeon and the anesthesiologist regarding the surgical steps, in particular the ligation of the central adrenal vein, allows a proactive attitude, foreseeing the eventual intraoperative hemodynamic variations.

The first part of the procedure refers to all the surgical and anesthetic steps before ligation of the central adrenal vein in which severe episodes of cardiac arrhythmias and hypertension can occur [1].

The second part refers to all the surgical events that follow the central adrenal vein seal and it might be characterized by a sudden decrease of arterial blood pressure and in intravascular volume that might require vasopressor support [1].

Keeping in mind the possible cardio-circulatory outcomes during this surgical procedure, large-bore venous access should always be obtained [2], the guidelines also recommend continuous intraarterial blood pressure monitoring for all patients with pheochromocytoma or at least for those with persistent preoperative hypertension [3–5].

Adding a central venous catheter allows secure and effective administration of vasoactive medication to promptly counterbalance the hemodynamic variations, especially since there are no accurate predictors of which patients are most likely to need central administration of vasopressors [2,4].

In our experience, the availability of 2 large-bore venous access associated with good preoperative management with stress minimization and anxiolytic administration, decreases the sympathetic nervous stimulation, that might trigger a hypertensive crisis [6,7], was enough to ensure rapid and effective administration of intraoperative drugs. Frequent non-invasive blood pressure measurement (every 3 minutes) and close communication with the surgeon regarding the current, the following, and the particularities of the ongoing surgical steps supply all the data needed to fine-tune vasoactive drug administration even without intra-arterial continuous blood pressure monitoring.

Although not routinely recommended, at the beginning of our experience we chose to perform a mild bowel preparation the day before the surgery (polyethylene glycol), a practice that we later abandoned as we saw no intraoperative benefit in terms of bowel injury or postoperative bowel movements. More than that, some of the cases presented a distended lumen at the time of surgery, especially for the left colonic flexure, and this was disturbing the surgical dissection.

Patient positioning

Our experience refers only to the transperitoneal approach mainly due to the familiarization with the peritoneal cavity as general surgeons. In all our cases we used the lateral trans peritoneal approach because it usually offers a larger surgical field that is particularly useful in the case of large tumors such as pheochromocytomas and morbidly obese patients.

The standard patient position for laparoscopic adrenalectomies is in contralateral decubitus [8,9] with the possibility of a backward tilt up to 30° [5]. After anesthesia induction, a nasogastric tube (especially useful in left-sided adrenalectomies), and urinary catheter insertion, the patient is placed in lateral decubitus. Prevention of contralateral brachial plexus injury was done using a soft roll under the contralateral axilla. The other arm is can be elevated and secured on an elevated arm board [8] but, in our experience, we placed the ipsilateral arm slightly bent, in a relaxed position over the other one without needing rigid support or any other fixation method.

Keeping in mind the unpredictable hemodynamic events that characterize pheochromocytomas during adrenal surgery, the patient's position has to offer both good access to the tumor and also the possibility to finish the surgery openly due to conversion in an emergency setting. The umbilicus is placed above the surgical table breakpoint that is next flexed and tilted in an anti-Trendelenburg position until the ipsilateral flank contour is stretched to a straight line which we position parallel to the horizontal line. Over-flexing the operating table can cause supplementary tension of the abdominal wall thus causing less compliance, less working space, and consequently need for increased pneumoperitoneum pressure [8]. These patient position landmarks provide a maximum distance from the anterior superior iliac spine and costal margin offering a large operating field and increased ergonomic for the surgical team even for large tumors – the patient “needs to open” as much as possible the space between rib’s rim and the iliac crest. Depending on the high conversion probability (large tumoral size, retrocaval positioning, and hemodynamic variability) we tilted the patient back up to 30° using shoulder and hip supports. The patient’s legs are a little bit flexed to avoid stretching the crural nerve. Additional fixation was achieved using cloth straps at the hip and leg level.

According to the literature, trocar placement is variable. All ports are inserted at least 2 cm from the rib’s margin. Pneumoperitoneum is achieved in open maneuver, and then the first 10 mm trocar is inserted between the anterior axillary line and midclavicular line, depending on the exposure of the operating field (first, we palpate the inferior edge of the liver and – when is possible – the fundus of the gallbladder, then we decide to insert the trocar laterally to the gallbladder with a parallel direction to the trajectory of the inferior vena cava). Other three 10 mm trocars are inserted under direct visualization equidistant from each other (at least one hand distance), in the lateral border of the rectus sheath, another in the midaxillary line, and for retraction, one below the xiphoid process [10]. In all the laparoscopic adrenalectomies performed in our hospital, we used the Veress technique for the pneumoperitoneum, and the first skin incision was made in the midclavicular line at 3-4 cm from the rib’s margin. Only in obese patients, pneumoperitoneum was realized at 2 cm from the rib margin because CO_2 abdominal distension would move the incision further caudally causing a suboptimal visualization of the surgical field. All surgeries were performed with 4 trocars (we didn't experience a surgical adrenalectomy with 5 or more additional trocars): below xiphoid process for retraction, on the axillary line below the 10th rib (12 mm – used also to extract the specimen), and one at the half distance between the sub-xiphoid and optic trocar. The peritoneal space is insufflated to 12 mmHg using slow or medium flow insufflation as it is known that, intra-abdominal insufflation during laparoscopic pheochromocytoma excision may cause an increase in serum catecholamines [5,11] – Consequently, we strongly advise for taking enough time to realize the proper intra-abdominal pressure using small quantities of CO_2 inflated in at least 5 minutes (break times can be used!). During this time, the blood pressure of the patient should be carefully observed by the anesthesiologist and a trend to increase should be instantly corrected but not using too many anti-hypertensive drugs (risk for severe hypotension and rebound). Usually, the failure of this operative time is solved by conversion to open surgery, with rapid recovery of the blood tension being noticed with no major anesthesia intervention.

It is known that during surgery for pheochromocytoma, there is a risk of a hypertensive event due to catecholamine secretion – it could be beneficial for the patient to use a decreased level of pneumoperitoneum – like 11 mmHg, even 10 mmHg – if the working camera is allowing you proper movements of the instruments. Many surgeons highlighted that early ligation of the adrenal vein is an important goal in adrenalectomy, but studies have shown that delayed vein ligation does not appear to cause significant hemodynamic variation during laparoscopic adrenalectomy for pheochromocytoma [12,13] and taking into consideration that the dissection of the adrenal vein as the first step of the adrenalectomy, can be more demanding in case of large size adrenal lesions such as pheochromocytomas, we did not routinely approach the adrenal vein first (unless it was identified from the very first beginning), and we did not encounter intraoperative cardiovascular complications. Laparoscopy offers a real-time surgical overview to the anesthetic team that can anticipate when the adrenal vein is ligated and take steps to avoid cardiopulmonary complications.

Even from the insertion of the camera, a full video inspection of the peritoneal cavity should be done to evaluate the availability of other trocar entry sites. The presence of intraperitoneal postoperative adhesions can change the trocar order so that the operating field and other trocar entry sites can be conveniently exposed. In particular, placement of the lateral port in left adrenalectomies requires, in most cases mobilization of the splenic flexure and descending colon incising the peritoneum along the white line of Toldt. The viscera surrounding the adrenal gland are inspected for invasion: the spleen, the diaphragm and the greater gastric curvature (on the left side) and ascending colon, the liver, the right kidney, the diaphragm, and the duodenum (on the right side) are inspected and if there are suggestive signs of malignancy, conversion is mandatory [8]. Before beginning the laparoscopic adrenalectomy we should keep in mind that pheochromocytomas are larger than other adrenal tumors and, in our experience, they also have a frail consistency with a thin capsule and an increased number of arteries and veins that easily bleed on manipulation. This is the reason we advise having proper laparoscopic equipment and also alternatives to ensure good hemostasis and vessel sealing as well as to strictly follow the rule of "dissection of the body from the gland instead of dissection of the gland from the body" (Prof. J Marescaux, Strasbourg, France – off-the-record).

On the right side, while the liver is retracted cranially and medially so that the peritoneal reflection is seen and the left-right triangular ligament is tensioned and divided; the peritoneum is incised along the liver as far on the diaphragm so that the right lobe of the liver gently falls away medially. The posterior peritoneum is incised along its reflection on the inferior liver margin. The inferior vena cava (IVC) is identified and the peritoneum along its lateral margin is incised until it meets the hepatic parenchyma. The literature defines the caudal limit for the peritoneal division along the IVC as the superior edge of the renal vein [14] in our experience identifying this landmark might require unnecessary touching of the pheochromocytoma, especially in patients with well-represented retroperitoneal fatty tissue. When dealing with pheochromocytoma we use the macroscopic inferior border of the adrenal tumor as a landmark for inferior peritoneal division, as these tumors tend to be larger, carefully not to damage the inferior arm of the adrenal gland.

On the left side once the hepatic flexure and descending colon have been retracted medially by following the space between the Gerota's fascia and the mesocolon [8]. The next step ensures a good mobilization of the spleen and is carried out by division of the phrenicosplenic ligament starting at the posterior and inferior margin of the spleen and continuing cranially until the fundus of the stomach is visualized. Although the literature mentions visualization of the left diaphragmatic crus as the end landmark of this dissection [8], in our experience this was rarely needed. Medial mobilization of the spleen during this step can be performed with gentle retraction or, as some authors recommend, leaving a 2 cm peritoneal margin attached to the inferior margin of the spleen to grasp [8]. Usually, this is the moment when accidentally puncture of the splenic parenchyma is performed due to aggressive manipulation of the laparoscopic instruments – minor bleeding can occur and immediate hemostasis should be performed using hemostatic agents like oxidized regenerated cellulose or other topical fibrin sealant patch (sometimes, when a simple injury of the spleen capsule is noticed, it is enough to apply a pad pressure onto the injured area for a few minutes). Once the spleen is fully mobilized it will fall medially along with the tail of the pancreas exposing the lateral edge and anterior portion of the left adrenal gland [9]. Correctly fulfilling this surgical step ensures access to the right dissection plane thus avoiding blind dissection into retroperitoneal fatty tissue which is prone to excessive bleeding and capsular breaking, forbidden when resecting pheochromocytomas.

Partial adrenalectomy avoids the need for life-long hormone replacement and it is usually useful for patients with bilateral gland involvement. This procedure is performed in small tumors located on the periphery of the affected adrenal limb. Performed in pheochromocytoma has a high risk for intraoperative hypertensive crisis due to tumor manipulation and thus early identification of the adrenal vein is mandatory before partial resection [15]. For partial adrenalectomy to be feasible the remnant gland to have a good vascularization, so laparoscopic ultrasonography to evaluate the adrenal gland viability must be recommended [16]. Because of the risk of bleeding in partial adrenalectomy, it is preferred safety surgery and total adrenalectomy be performed.

Once the periglandular dissection is started it is important to keep in mind a principle postulated even from the first adrenalectomies, and demonstrated in later studies: a mean increase in plasmatic catecholamines up to 34-fold during tumor manipulation [17-19]. To minimize hemodynamic instability, these techniques require minimal to no direct manipulation or compression of the tumoral gland [5], achieved in many cases by gentle retraction of the surrounding viscera or tissue such as the kidney, pancreas, and liver or by using periglandular fatty tissue, the Gerota's fascia and/or the disconnected vein stump, to manipulate the gland accordingly. Comparing pheochromocytoma ablation with other types of adrenal tumors, it is mandatory to respect the principle of "dissecting the patient away from the tumor" to achieve this important goal [20]. In large tumors with which we encounter technical difficulties prompt conversion has to be taken into consideration to reduce the patient's risk for capsular disruption and following iatrogenic disease recurrence [5] or even pheochromocytomatosis [14]. As mentioned before, pheochromocytomas have numerous tributary vessels that combined with the frail vascular wall and increased circulatory catecholamines represent the optimal recipe for possible uncontrollable bleeding, so achieving a proper vessel seal before tissue dividing is mandatory even with the usage of clips as a complementary method to the electrocoagulation and sealing devices [5].

For the right-sided adrenalectomy, the lateral border of the IVC continues to be the main landmark during the procedure allowing lateral retraction of the adrenal gland and thus exposing the central adrenal vein. Even though it is more demanding, many authors concluded that once the peritoneum is incised, as mentioned before, the tumoral gland should be approached first on the medial border – detaching the tumor from the IVC, exposing, clipping, and dividing the central adrenal vein [8]. The dissection continues along the inferior border of the adrenal gland and might include the peri-adrenal fat. The adrenal is gently lifted and the posterior aspect is released and then the lateral one. The final step is detaching the superior border of the tumor [8]. We share the opinion that first freeing the inferior and posterior aspect of the gland assures enough mobility to safely push the gland laterally, without manipulation or grasping, increasing the space to the IVC, exposing the central adrenal vein, and more important, pulling the adrenal tissue situated posterior to the IVC. While this approach carries a higher risk of capsular breaking when beginning the dissection, it offers more control and better retraction in dealing with the main vascular pedicle and retro-caval adrenal tissue. The dissection continues cephalad until the medial side of the gland is released and further on the superior edge.

Once the central adrenal vein is secured, we can better manipulate the tumoral gland. In large tumors, some authors recommend starting the dissection from the lateral and superior border of the tumor and then moving inferior to the IVC [8].

For the left adrenalectomy, it is generally accepted that the dissection begins along with the medial border proceeding from the upper to the lower adrenal pole, keeping close to the posterior muscular plane. This maneuver allows lateral rotation of the adrenal and exposes the left adrenal vein that will be ligated early in the dissection. During the dissection of the medial border of the adrenal gland, the inferior diaphragmatic vein might be encountered and it represents a landmark for the central adrenal vein. Particularly in pheochromocytomas, starting the dissection from the lateral border should be avoided since it will cause the gland to rotate inferiorly and medially thus requiring increased manipulation and pulling the adrenal gland, in order to access the central adrenal vein. After securing the central adrenal vein, the gland is lifted and the dissection continues at the posterior and lateral aspect of the gland, leaving the upper adrenal pole to be approached as last surgical gesture [8,14].

Several factors as the lack of major anatomic landmarks, the relative profound in the retroperitoneal fat of the left adrenal gland, with the main vein within the retroperitoneal fat, the proximity and tissue similarity with the pancreas tail, may render the left adrenalectomy more difficult [8], especially in the settings of mandatory minimal glandular manipulation and compression, and forbidden cellular spillage. So, it is recommended for beginners to start laparoscopic removal of a pheochromocytoma on the right side, then the left side.

Taking into consideration that the retroperitoneal space contains the kidney, adrenal gland, and fatty tissue, it is very important to localize the adrenal gland within the surrounding fat. To avoid capsular breaking, especially in smaller-sized tumors perinephric fat containing the adrenal gland should be dissected away from the transversus abdominis muscle (laterally), the diaphragm (above), the *quadratus lumborum* muscle (posteriorly), and the pancreas or liver (medially on the left and right sides, respectively) just inside Gerota's fascia. During the separation of the perinephric fat from the diaphragm at the upper pole of the adrenal gland, the superior adrenal vessels are usually encountered and must be, carefully sealed and transected (especially in patients with large pheochromocytomas). During en bloc dissection, the perinephric fat is divided between the adrenal gland and the upper pole of the kidney using a vessel-sealing device. Next, the adrenal gland is lifted by grasping the periadrenal fat. The inferior and posterior adrenal vessels are in this way intercepted in the periglandular fat, sealed, and transected leaving the adrenal vein exposed between the gland and the inferior vena cava (right) or renal vein (left) [22]. At this moment (depending on the surgeon's experience, vessel diameter, and available surgical devices), the adrenal vein can be clipped or sealed leaving only the division of the remaining peri-adrenal fat to completion of the adrenalectomy. As a result, the adrenal gland will be removed along with its surrounding fatty tissue.

The technical feasibility of laparoscopic adrenalectomy for large pheochromocytomas in today's surgical activity is no longer a rarity [23,24]. Large right-sided tumors may be technically more challenging because of the operating space, which is limited by the liver with the inferior vena cava compressed and displaced anteriorly by the tumor, sometimes taking a lenticular shape on the medial margin of the tumor. However, large left-sided pheochromocytomas are also a source of complications. Our experience counts 23 pheochromocytomas larger than 6 cm and our intraoperative strategy is in concordance with other authors' opinions performing systemic peritumoral dissection, without manipulation of the tumoral gland, without the previous approach of the adrenal vein [25]. The purpose is to progressively free the tumor from the surrounding tissue and to take maximum advantage of gravity in achieving exposure. Another useful tool, in our experience, was the blunt 10 mm vessel seal and dividing device that helped not only achieve good hemostasis in the peritumoral fat but also protect the capsule from rupture, not mentioning also a speedy surgery timing.

Intraoperative complications

Laparoscopic adrenalectomy is considered to be the gold standard for surgery to remove the adrenal gland. Still one of the factors for intraoperative complication occurrence is pheochromocytoma along with the tumor size, surgical inexperience, patient age, and body mass index [26]. The most common intraoperative complication is hemorrhage which is in most cases possible to control in the hands of a surgeon with good laparoscopic skills and experience. Conversion to open surgery dropped from 6% in some early studies [27] to 0.6% [28]. Bleeding in a hypertensive crisis can occur from small vessels that are why special attention has to be paid to hemostasis. If proper electric hemostasis cannot be achieved and if it affects the safe dissection, applying a large gauze and pressing it into the bleeding area, was in our experience the best way to manage this situation until a clip was applied to control the bleeding. Apart from general complications during laparoscopic adrenalectomies specific aspects are related in particular to the pheochromocytoma, large tumors with important retro-caval development that compress the IVC anteriorly, present a high-risk caval injury that mandate conversion to laparotomy in most cases. Other vascular or visceral lesions can be caused by the large tumoral volume, specific to pheochromocytomas, and the need to mobilize the surrounding tissues instead of the gland, to avoid both catecholamines release or capsular breaking are the renal and the splenic veins. Major vascular injuries are recognized instantly during dissection, and it is important to evaluate the chance of controlling them laparoscopically, and not waste time converting to an open procedure [29]. Liver, pancreatic and splenic injuries are mostly due to adhesions, the presence of a large tumor or unexpected excessive tissue retraction to protect or better access the pheochromocytoma. In our experience, no solid organ injury during pheochromocytoma minimal invasive surgery forced us to convert to open surgery.

Causes of conversion

A large multicenter study showed that unplanned conversion to laparotomy during laparoscopic unilateral adrenalectomy for pheochromocytoma was independently associated with increased intraoperative hemodynamic instability episodes and postoperative complications rate [30,31]. Studies revealed that the ratio of intraoperative systolic blood pressure >200 mmHg and the total anesthesia time was an independent factor associated with conversion [32]. Although controversial intraoperative hemodynamic instability remains an important marker of the quality of pheochromocytoma manipulation during the surgery [32,33].

Another particular case of conversion in this type of tumor is represented by tumoral spillage not only due to the high risk of relapse [5,23] but also because of the high risk of undiagnosed malignancy [34], especially taking into consideration that in pheochromocytoma radiographic and histological information, including intraoperative frozen section examination, are unreliable predictors of malignancy [35,36].

However, conversion to an open procedure is warranted when laparoscopic dissection cannot be performed safely or a complete resection cannot be performed without undue trauma to the gland.

In our experience, conversion during laparoscopic adrenalectomy for pheochromocytoma was needed in 9 cases: 1 case of severe cardiac arrhythmia with cardiac arrest that required resuscitation on the operating table, 3 cases of invasive malignant pheochromocytoma, 2 cases large tumors that could not be dissected safely, and 3 cases of major vessel lesions: two inferior vena cava lacerations and one left renal vein injury.

Specimen extraction

Special care should be paid to the adrenal specimen since it might spill and contaminate the peritoneal cavity causing disease recurrence [5] or even pheochromocytomatosis [14]. This is why immediately following specimen detachment it is introduced in a retrieval bag for the following surgical steps like controlling hemostasis. Manipulation during this step should follow the dissection principles since the pheochromocytoma capsule can rupture under its weight if it is uncarefully manipulated. We recommend grasping the specimen using periglandular tissue, fascia, or vascular stumps to place it in the bag. We retrieved the bag always through the lateral incision that is enlarged accordingly to the specimen size, avoiding morcellation and contamination of the wall.

We also routinely drained the adrenal lodge, the drainage tube being exteriorized through the same incision from the specimen was retrieved. The tube stays in place for 24 hours if no bleeding is noticed.

Special attention to malignant pheochromocytoma was given to achieve an en-bloc excision of the tumor with wide surgical margins and as much peri-adrenal tissue as possible. The need to operate around the renal artery and vein or the renal hilum for achieving an en-bloc resection along with the peri-adrenal does not mandate conversion to laparotomy. However, conversion to an open procedure is warranted when laparoscopic dissection cannot be safely performed or a complete resection cannot be performed without undue trauma to the gland [5]. Hand-assisted laparoscopy is a good alternative to open conversion if a difficult dissection is encountered intraoperatively [37].

CONCLUSIONS

The best way to improve is to repeatedly follow a correct surgical pathway even from the beginning, especially when approaching a pathology with great morbidity related strictly to the delicacy and correctness of the surgical act. Experience of the high volume centers translates into excellent outcomes, achieved by minimizing complications, decreasing blood loss, and shortening hospital stays.

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REVIEW

Melanoma: A Historical Walk-through from Palliative Treatment to Modern-day Practice

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Abstract: *Melanoma is one of the most common types of malignancy in the world, and one known to carry a very poor prognosis until recent years. This review aims to outline the events in the history of the disease and the impact made by the discoveries along the way, as well as the modern-day consensus by referencing the updated literature regarding the present approach and future directions. In the last two decades, several studies and research have brought significant improvement in the diagnosis and clinical management of melanomas. The development of sentinel lymph node biopsy has brought major changes to the surgical approach to the disease, and modern therapies based on recently developed knowledge changed the death sentence this diagnosis once meant to a manageable condition despite its aggressiveness, keeping in mind that early diagnosis and safe margin excision remain the best and most optimistic course of treatment.*

Keywords: *melanoma, surgery, Breslow, Clark, lymphadenectomy, BRAF, immunotherapy, sentinel lymph node biopsy*

INTRODUCTION

Malignant skin tumors are among the top 10 causes of disease burden worldwide. 18.8 million new cases of cancer have been diagnosed in 2018 alone, with breast, lung, and colorectal cancer being the most frequent. It is also the second most frequent cause of death, preceded only by ischemic heart disease [1]. While skin cancer is not among the highest incidence malignancies overall, they do represent the most common malignancy in the white population. Furthermore, melanoma has had the highest increase in incidence rates – 5-fold in the past 30 years [2]. Therefore, reducing disease burden and mortality has become public health priority. Numerous studies and research have brought significant improvement in the diagnosis and clinical management of skin cancers, especially in the case of melanomas, which carried a very poor prognosis until recent years. Part of these improvements is due to the development of genetic testing for specific mutations and the subsequent development of targeted therapies. These have led to the increased survival of numerous patients and to important changes in the way this pathology is managed. Surgical excision has always held a central role in melanoma therapy, and it naturally changed and adapted along with newer treatments.

HISTORY OF MELANOMA DIAGNOSIS AND TREATMENT

The word "melanoma" originates from Greek ("melas" meaning "dark" and "oma" meaning "tumor") and it was described for the first time in recorded history by Hippocrates in the 5th century BC. The earliest physical evidence of melanotic tumors dates from around the same time and was found in the bones of pre-Colombian mummies [3]. Throughout the following centuries, more reports of such pigmented tumors were made, though not much was understood about their origin and development. In 1806, Rene Laennec introduced the term melanosis to describe the disease as a separate entity and demonstrated the difference in its structure from other dark-colored lesions found in autopsies, such as carbon deposits in the lungs [4]. The first thorough study of the disease was made by William Norris in 1830, who followed up a patient for 3 years, described the condition clinically and the autopsy findings in detail, including the vast number of metastases, their macroscopic structure, and the hereditary character of that particular case. He was also the first one who, in later studies, highlighted a possible link between "melanosis", and noticed a correlation between the condition and light-colored skin and hair [5]. The term "melanoma" was introduced in 1838 by Robert Carswell, who studied and described melanoma metastases, including brain localization, in detail [6]. A comprehensive analysis of primary tumors was also provided by James Paget in 1853, including the first description of the transition between a radial growth phase and a vertical growth phase [3,7].

In the 20th century, research techniques improved, and more effort was made to understand the disease and structure its treatment. In 1953, Spitz and Allen observed that nodular (or “deep”) melanomas have a worse prognosis than superficial melanomas [8].

In 1954 Ackerman and Del Regato were the first to propose a staging system, comprising four groups: a – patients with the generalized disease, b – patients with clinical lymph node disease, c – patients with infra-clinical (histological) lymph node involvement, and d – patients with primary tumors. The system was based on the observation that the latter group demonstrated improved survival compared to the first group, but not much difference was observed between the two middle groups [9,10].

With time, other stage systems were developed and proposed for clinical practice, such as the one designed by McNeer and Das Gupta in 1964, which comprised three stages based on disease extension and is regarded as a precursor to the modern TNM staging system [11]. This system was expanded and improved by the MD Anderson Hospital in 1976 [12], but it still had some shortcomings, such as failing to consider the pathology of the primary lesion.

In 1969, Clark, improving earlier systems proposed by Petersen (1962)[13] and Mehnert and Heard (1965)[14], based on the microscopic aspect of the primary tumor, presented the five levels of invasion still in use today [15]. One year later, in 1970, Breslow demonstrated the importance of primary tumor thickness in overall prognosis and survival [16].

With later studies corroborating their findings, such as McGovern’s for the Clark index [17] and Balch’s for Breslow – which also proved that tumor thickness is more relevant for prognosis than the level of invasion [18] – these characteristics of the primary lesion were added to later staging systems. Over time, the American Joint Committee for Cancer (AJCC) and the Union Internationale Contre le Cancer (UICC) have made several attempts to establish a simple, relevant, and coherent staging system that could be applied internationally.

Following a series of proposed systems that were only partially satisfactory, and after adapting to encompass new findings, in 2002 the AJCC proposed the modern staging system, whose updated version (8th edition) is in use today [19,20]. It includes ulceration of the primary lesion as a prognosis factor, uses the Breslow index as a primary determinant of T staging, with the Clark level used only for thin tumors (<1 mm), and makes a clear distinction between clinical and pathological staging. It also takes into consideration additional prognostic factors such as age, gender, tumor location, and histological subtype.

Melanoma treatment evolved as the understanding of the disease grew. The first surgical resection of such a lesion in Western medicine was performed by John Hunter in London in 1787 [3]. By the end of the 19th century, it became clearer that surgical excision is an appropriate course of treatment, with some authors such as Herbert Snow advising adjacent lymph node resection, while William Handley suggested a 5 cm of healthy tissue around the tumor along with extensive lymph node excision [3]. This remained the standard for melanoma surgery well into the 20th century.

After Clark and Breslow’s studies on the importance of primary lesion thickness in the prognosis of melanoma, surgical therapy was gradually adapted to decrease morbidity after extended excisions. A series of studies in the 1990s up to the early 2000s aimed to determine the optimal resection margin for each primary tumor stage.

Eventually, a relative consensus was established for safety margins of 0.5 cm for melanoma in situ, 1 cm for invasive melanoma <1 mm thick, and 2 cm for a Breslow index of >2 mm with some debate still ongoing about the optimal resection margin for tumors between 1 and 2 mm thick [21].

As mentioned previously, complete radical lymphadenectomy was performed as part of the surgical treatment for melanoma for most of the 20th century. The argument behind the indication was based on extensive studies on lymphatic drainage made by Rudolph Virchow and by Marie Sappey in the later part of the 19th century, and was supported and practiced by prominent surgeons like William Halsted for breast cancer in the US and, as mentioned before, by Herbert Snow for melanoma, in Britain [22].

Lymphadenectomy became an essential step in the oncological resection of various tumors for almost a century. By 1990, lymph node dissection was still performed as standard of care in melanoma treatment, whether electively (for clinically normal nodes) or therapeutically (for clinically evident lymph node involvement), but the need to find a better approach was frequently discussed, especially for small primary tumors and no clinical lymph node involvement [23].

In 1992, Morton and Cochran published a study that demonstrated the dissemination of tumor cells through lymphatic vessels first to a specific, identifiable node, called the sentinel node, then to other regional nodes [24]. They were not the first authors to use the term “sentinel node”, the term had been used previously by several authors to describe the first nodes draining specific areas on the body, but based primarily on their anatomy rather than physiology [22]. Morton used the technique described by Gerota who used a blue dye to map lymphatic vessels and introduced lymphoscintigraphy for patients with axial melanoma to determine the primary draining basin [25]. Their studies were not accepted easily, but as data continued to support their findings, sentinel lymph node biopsy became an integrated part of melanoma treatment.

GENETIC MUTATIONS IN MELANOMA

It is now understood that cancers are the consequence of a series of mutations in genes that control the proliferation, differentiation, and programmed death of the affected cells. In 2002, Davies and colleagues demonstrated a significant presence of BRAF mutations in human cancers [26]. BRAF (v-RAF murine sarcoma viral oncogene homolog B1), a proto-oncogene, is a RAS-regulated serine-threonine kinase and an activator of the MAPK signaling cascade. It plays an important role in cell differentiation, proliferation, and death through extracellular signals. The mutation was found in several malignancies, including melanoma, colorectal cancer, adenocarcinomas of the lung, sarcomas, ovarian cancers, and liver cancers, and it prompted the research for other targets and pathways. Its identification in a large proportion of benign demonstrated that this mutation may be a step in melanoma development and prompted further studies [27].

Among the most frequent mutations responsible for oncogenesis in melanoma are observed in the MAPK signaling pathway (mitogen-activated protein kinase). This pathway promotes cell cycle progression, transformation, and cell survival and, in non-cancerous cells, it is activated through cytokine receptors or tyrosine kinase receptors. Ligand binding to the receptors leads to the activation of GTPase switch proteins known as RAS, which in turn activate a series of serine/threonine kinases, which ultimately activate MAPK. Permanently activated MAPK signaling has been observed in almost all melanomas [28].

BRAF is a kinase whose only known activity is in the MAPK pathway and is highly expressed in melanocytes but also hematopoietic tissue and neural cells. The most common BRAF mutation in melanoma is the V600E – a valine to glutamic acid substitution in exon 5, codon 600, a mutation that permanently activates the kinase domain [26]. These mutations are usually found in cutaneous melanoma, specifically in those tumors arising on intermittently sun-exposed skin, despite it not being a typical UV-induced DNA mutation [29].

Other mutations involved in cell proliferation are relatively rare in melanomas, such as mutations that increase RAS activity. They occur mostly in amelanotic nodular subtypes and makeup only about 10-20% of melanomas, with NRAS being the most commonly affected [30]. Interestingly, these two mutations, BRAF and NRAS seldom occur simultaneously in melanomas [31], and in the rare situations when they do, the BRAF mutation is not the typical V600E type [32], suggesting that they have overlapping oncogenic activity and either of them could activate the MAPK pathway.

The above-described mutations can also appear in benign nevi, as well as in melanomas. BRAF mutations can be found in 70-80% of dysplastic nevi [33] and NRAS in congenital nevi [34]. Given these findings, it was at first believed that the BRAF mutation was the initial oncogenic event that triggered all other changes in BRAF-positive melanoma. However, data showed that only 6% of in situ melanomas have a BRAF mutation [26], and that seems to be polyclonal (containing melanocytes with and without the BRAF mutation, or with BRAF wild type), metastatic melanoma tends to be monoclonal [35], suggesting that the mutation might occur later in the oncogenic process. Still, at any moment it occurs, BRAF activation leads to cell growth and dysregulation of the MAPK signaling pathway [36]. Other mutations or alterations can lead to the same result, like overexpression of growth factor receptors like KIT or EGFR (epidermal growth factor receptor)[37,38]. NF1 (neurofibromatosis 1, a tumor suppressor) mutations lead to melanoma proliferation through the MAPK and PI3K pathways [39].

The MTIF gene, responsible for a melanocyte-inducing transcription factor is another essential regulator of melanocyte development, survival, and function, and it can be linked to melanoma development. Its amplification can be found in 15-20% of melanomas, especially metastatic tumors, and it has been shown to contribute, alongside BRAF, to melanoma development [40].

An interesting occurrence in melanoma is the genetic heterogeneity of these tumors, both intralesional and occurring during disease progression. These findings partially explain treatment resistance, on one hand, and can influence therapeutic decisions, on the other [41]. One study investigated both benign and malignant melanocytic lesions and interestingly found that the benign lesions only showed BRAF-V600E mutations [42]. As the disease progressed, more mutations developed. In more advanced lesions, NRAS and subsequently TERT promoter mutations were found. In invasive and advanced melanomas, inactivation of CDKN2A and loss of PTEN and TP53 were observed [41,42]. All these findings suggest that the BRAF-V600E mutation may require other mutations that inhibit tumor suppressors to ultimately lead to melanoma development.

TARGETED THERAPY IN MELANOMAS

The first targeted agent for BRAF V600 was Vemurafenib, which showed significantly higher response rates than the previously used chemotherapeutic agent dacarbazine [43]. Dabrafenib was the second targeted agent to be developed, and despite some side effects such as rash, fatigue, or fever, the therapy was generally well tolerated [44]. Along with a better understanding of the BRAF gene and its interconnected activity with MEK, MEK inhibitors were developed, with trametinib being the first. Simultaneously, studies into BRAF-inhibitor resistance lead to a better understanding of the importance of MEK in the process, and the connection between the two pathways [45]. This eventually led to the association of the two agents, a BRAF inhibitor and a MEK inhibitor, which was studied in a series of clinical trials, for both dabrafenib plus trametinib, as well as for vemurafenib plus cobimetinib, with encouraging results showing a lower rate of cutaneous adverse reactions and increased progress-free survivals [46,47]. This has led to attempts to treat non-BRAF mutated melanoma with a MEK inhibitor in monotherapy, but the results were not promising, suggesting that further genetic studies into the MEK activity and its possible association with other mutations such as NRAS are necessary and could lead to new therapies [43].

Vemurafenib is a selective kinase inhibitor of the MAPK pathway in BRAF-mutated cancer cells, leading to decreased proliferation and apoptosis, while dabrafenib is a reversible, ATP-competitive BRAF inhibitor [48]. Cobimetinib is a non-ATP-competitive inhibitor of MEK1, with a high selectivity for MEK 1 over MEK2. Similarly, trametinib is an allosteric small molecule that binds preferentially to unphosphorylated MEK, preventing Raf-dependent MEK phosphorylation and activation.

In clinical practice, the RT-PCR method is widely used for BRAF mutation detection. The cobas 4800 BRAF V600 kit and the ThxID-BRAF kit have been developed together with the targeted therapeutic agents' vemurafenib and cobimetinib and dabrafenib and trametinib respectively, and are frequently used due to their accessibility, reproducibility and relatively rapid results. These tests use two different primer sets to detect the V600E mutation and the wild-type sequence and then compare the results. While this is sufficient for usual testing, other methods are available for more in-depth analysis and also for rare BRAF types (about 2% of cases), such as Sanger sequencing, pyrosequencing, and high-resolution melt analysis (HRMA)[41].

Systemic therapy for non-mutated melanoma

For decades, melanoma has been considered an incurable disease, especially in later stages, partially because both classic chemotherapy and radiation therapy are not significantly effective, with the drug Dacarbazine showing the best overall response rates of about 10-20% [49]. Research into the development of this malignancy and specific studies on metastatic melanoma has led to the discovery of genetic testing and targeted therapies on one hand, and also to the development of immunotherapies for non-mutated tumors.

There are four main types of immunotherapy: biological agents such as cytokines, interferon, and granulocyte-monocyte colony-stimulating factors; vaccination therapy; adoptive cell therapy and immune checkpoint inhibitors [50]. Biological immunotherapy such as interferons and IL2 were the first alternative to classic chemotherapeutic agents and were frequently used together, to amplify their action. Vaccination therapy is a type of specific immunotherapy that is currently studied. Oncolytic viruses are modified strains of various viruses that maintain their replication capacity but lose their virulence, and such strains have led to cell death in melanoma cell lines in vitro and animal models, by activating antitumoral immunity through the production of cytokines [51]. However, not all types of vaccination are very effective in vivo, such as DNA-based vaccines, despite proving safe and effective in laboratory settings [52]. Adoptive cell therapy is an experimental treatment alternative based on the patient's lymphocytes, which are then activated in vitro and reinjected into the patient to induce an immune response. The technique is time-consuming and elaborate, requiring specialized personnel and equipment [50]. The most effective immunotherapy studied has been the immune checkpoint inhibitors. They are designed to suppress the mechanism of self-regulation in natural immune response and target specific overexpressed molecules in the tumor they are used against. In melanoma, they target PD-1, a cell-surface molecule that downregulates B and T lymphocyte activity (Nivolumab and Pembrolizumab), or CTLA-4, a protein receptor that acts as a negative regulator of T-cell activation (Ipilimumab).

As previously mentioned, most trials for immunotherapy agents have been conducted on regionally advanced and metastatic diseases. Interferon-α has been used for over 20 years before the development of modern therapies to treat advanced disease, with moderate results, as shown in a meta-analysis by Mocellin in 2010 [54], while pegylated IFN-α demonstrated to prolong disease-free survival without improving overall survival in regionally advanced disease [55]. The treatment was associated with significant side effects varying from myalgia, fatigue, fever, headaches, and chills to depression, which could be severe and require treatment [56].

Ipilimumab has been in use since 2015. Its efficacy was demonstrated initially in unresectable metastatic melanoma and subsequently began being used as an adjuvant treatment.

Initial studies proved it increases overall survival in patients who had undergone complete lymph node resection for node metastases (overall survival at 5 years 65.4% compared to 54.4% in the placebo group) but was associated with significant adverse events (in 41.6% of patients)[57], even though these adverse reactions didn't significantly lower quality of life [58]. Ipilimumab is not currently in use as the first line of treatment in regionally advanced or metastatic melanoma.

Nivolumab was approved in 2014 and most studies compared its efficacy with Ipilimumab in the same category of patients, showing a lower rate of recurrence (recurrence-free survival at 18 months of 66.4% vs 52.7% for Ipilimumab and 70.5% vs 63.7% at 24 months) and significantly fewer adverse reactions (17.5% vs 40.4%)[57].

Pembrolizumab was approved in 2019 and showed similar results, disease-free survival of 71.4% vs 53.2% for placebo, and 14.5% moderate to severe adverse reactions vs 3.4% in the placebo group [58].

Combination therapy has proven to be more efficient than monotherapy, the association of Ipilimumab and Nivolumab led to better results in several studies(50), although toxicity may increase, with cutaneous reactions (over 50% of patients), gastrointestinal and thyroid disorders being the most frequent [56].

CURRENT RECOMMENDATIONS IN MELANOMA DIAGNOSIS AND TREATMENT

Any suspicious lesion of the skin should be carefully examined. The surrounding skin should also be checked for satellite lesions and regional lymph nodes should be palpated. Physical examination of the lesion can be completed with dermoscopy by a specialized medical professional for improved accuracy [59]. A patient's personal history of sun exposure, other skin lesions, or cancers is also important, as well as a careful history of the lesion in question. Excisional biopsy with subsequent microscopic examination of suspicious lesions is the only way to accurately establish a melanoma diagnosis. The excisional biopsy should include margins of 1-2 mm around the lesions and into the subcutaneous tissue. Other surgical methods like incisional biopsy are not recommended with very few exceptions and superficial shave biopsies should be avoided because accurate lesion thickness cannot be correctly established [60]. If suspicious palpable masses are identified around the lesion or node basin, further evaluation may be necessary, the most accessible of which is soft-tissue ultrasonography, which can be paired with a fine-needle aspiration.

Once a melanoma diagnosis has been established through biopsy, wide local excision of the scar and surrounding tissue is recommended, the margins of which are established based on Breslow thickness [61]. For in situ melanomas – 0.5 to 1.0 cm; for Breslow < 1.0 mm – 1.0 cm; for Breslow between 1.0 and 2.0 mm – 1.0 to 2.0 cm; and for Breslow > 2.0 mm – 2.0 cm margins are recommended. The histological examination should be performed on permanent sections, given that frozen sections may lead to false negative results [62]. Mohs surgery is also not recommended, with certain exceptions in very superficial tumors located in delicate anatomical sites [61]. Complex reconstruction involving skin flaps should be postponed until microscopic margins are established. Some guidelines accept covering the defect with non-adherent dressing until the final histological result is obtained [61,63].

The spread of malignant cells from the primary tumor occurs primarily through lymphatic vessels to distant sites, through regional lymph nodes. The most valuable predictors for nodal dissemination are Breslow thickness and the presence or absence of ulceration and they are the basis for the indication of a sentinel lymph node biopsy [64]. Thus, all tumors thicker than 1.0mm should be considered for lymph node biopsy. Smaller tumors should be evaluated for poor prognosis factors such as ulceration or microvascular invasion and a decision can be made on that basis [65]. Recent studies have also evaluated the role of complete lymph node dissection in the case of a positive SLN, which was the initial standard of care. They did not demonstrate improved survival and metastasis-free survival in the case of early completion of lymph node dissection, as opposed to monitoring and conducting the procedure in a delayed fashion, when the nodes become identifiable clinically or ultrasonographically [66].

Staging aims to be a simple and practical method to determine a patient's prognosis and guide treatment. It is also helpful in reporting data for future studies and clinical trials. The latest American Joint Committee for Cancer (AJCC) TNM staging system defines separate clinical and pathological staging [20,65]. Clinical staging (cTNM) is based on imaging and clinical examination and pTNM takes into account histological results obtained after surgery. Stage 0 represents melanoma in situ. Stage I is a localized disease, with IA representing tumors of less than 1 mm thickness with or without ulceration (T1a/T1b) or 1-2 mm without ulceration (T2a). Stage II melanoma is also localized, but with larger primary lesions, or ulceration. IIA denotes a 1-2 mm ulcerated lesion, IIB a >2 mm lesion with or without ulceration. Stage III is regional melanoma, with node involvement or satellite or in-transit lesions, with subtypes "a" to "d" for the extent of invasion. It is the subgroup with the most variable 5-year survival rates, ranging from 93% for IIIa to 32% for IIId [68]. Stage IV signifies generalized disease, with distant site dissemination. No subgroups were defined for this stage, given that the overall survival is similar among these patients [50].

Other prognostic factors can have an impact on prognosis. The female gender appears to be associated with a better prognosis, while melanomas of the head and neck are associated with a worse prognosis [69]. Older patients have overall higher mortality [70], nodular and amelanotic melanomas have a worse prognosis [71] while melanoma developed on pre-existing nevi are less aggressive than de novo melanoma [72].

Adjuvant therapy is essential for the outcome of stages III and IV melanoma, the stages with the highest rates of recurrence and dissemination. For earlier stages – stages I and IIA – surgical excision and follow-up seem to be the optimal treatment. For stages, IIB and IIC the risk of adverse reactions is greater than the risk of recurrence, so adjuvant treatments are generally not indicated. For stage III and IV, immunotherapy with Nivolumab or Pembrolizumab, alternatively, if BRAF-V600E genetic test is positive, Dabrafenib and Trametinib is the first-line option [73].

CONCLUSIONS AND FUTURE DIRECTIONS

Diagnosis and treatment of melanoma have evolved greatly to the benefit of patients, especially in later stages, and is even more important as the incidence of melanomas continues to rise. The development of sentinel lymph node biopsy brought major changes to the surgical approach to the disease, and the discovery of the genetic and immune basis of melanoma development changed the treatment protocols that have been used for decades. Classic chemotherapeutic agents are relatively ineffective, recently studied chemotherapy presents more promising results, nonetheless, newer agents also present side effects, sometimes leading to interrupting the treatment, such as colitis and diarrhea for immunotherapy, or keratoacanthoma and squamous-cell carcinoma for targeted therapies [56]. Experimental studies are being conducted with T-cell-based adoptive cell transfer immunotherapy, which proved effective in a laboratory setting [74]. Simultaneously, there is also a need to optimize screening. At the moment, screening and follow-up of low-risk melanomas are based on clinician-led surveillance, but there are proposals to increase patient education on self-examination [75]. Results are yet inconclusive, some studies already show that patients tend to detect melanomas when they are thicker [76], yet the value of self-examination has been proven before [77]. Modern technology is also being researched to aid early diagnosis. Gene profiling can identify low or high risk of developing melanoma [78]. Dermoscopy can be used with computer software to analyze suspicious lesions more efficiently [79]. Finally, the increasing number of smartphone apps focusing on healthcare, including dermatology, should be assessed for accuracy, as many potentially dangerous lesions can go overlooked [80-82].

In conclusion, understanding melanoma has improved spectacularly, which benefits the increasing number of new patients and the therapies developed based on this knowledge changed the death sentence this diagnosis meant until 20 years ago to a manageable condition despite its aggressiveness, keeping in mind altogether that early diagnosis and safe margin excision remain the best and most optimistic course of treatment.

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ORIGINAL ARTICLE

The Impact of the Hypercaloric Diet versus the Mediterranean Diet on Insulin Sensitivity

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Abstract: *Obesity affects the population worldwide. A hypercaloric diet associated with a sedentary life, stress, and genetic background, triggers various metabolic disorders, such as metabolic syndrome, diabetes mellitus, cancer, cardiovascular diseases, non-alcoholic fatty liver disease, and cognitive impairment. A healthy diet correlated with physical activity, not smoking, and moderate alcohol consumption reduces the risk of developing metabolic diseases. The Mediterranean diet contains antioxidants, fiber, polyunsaturated fats, and compounds with anti-inflammatory, anti-oxidant, anti-cancer, and anti-obesity properties. In a wide variety of species including humans, the reduction of calories between 20-40% significantly improves health, increases longevity, and delays the development of various pathologies. The main aim of this review is to present the comparative effects of the Mediterranean diet versus the hypercaloric diet on insulin sensitivity.*

Keywords: *obesity, diet, MetS, insulin, metaflammation*

INTRODUCTION

Currently, obesity affects the young and adult population worldwide. Hypercaloric diet, sedentary life, and stress lead to obesity [1-3]. Sugar has a well-established metabolic effect. Therefore, the direct pathway includes fructose, which induces lipid and carbohydrate metabolism dysregulation. Indirectly, an excessive sugar intake leads to body weight and fat gain, which further induces the perturbation of lipid and carbohydrate metabolism [4].

A hypercaloric diet triggers dyslipidemia, a key player in the pathogenesis of metabolic diseases [5]. Hyperinsulinemia is installed when insulin secretion is increased, insulin pulses are decreased, and/or hepatic insulin clearance is reduced [6]. This metabolic state is induced by several factors such as the modern Western diet, over-nutrition, and genetic background. Once installed, hyperinsulinemia perturbs the balance of the insulin-growth hormone (GH)-insulin-like growth factor (IGF)-axis and switches to an insulin: GH ratio, leading to lipogenesis and inhibition of lipolysis and further to obesity [6]. In mammals, GH stimulates the production of IGF [7]. The hypothalamus, the pituitary gland, and the liver regulate the GH/IGF-1 system. GH is primarily synthesized by the somatotrophs from the anterior pituitary gland, but reproductive tissues, lymphoid tissues, and the gastrointestinal tract may also syntheses it. Moreover, other endocrine axes may have permissive actions on GH and stimulate IGF-1 expression [8].

Ceramides are lipids from the sphingolipid group, which can act at the plasma membrane, endoplasmic reticulum, and mitochondria levels, being involved in insulin signaling and glucose homeostasis [9]. Under certain metabolic dysregulation (low androgen levels in males and high androgen levels in women) IGF signaling is perturbated leading to metabolic dysfunction and type 2 diabetes [10].

The most common metabolic disorders correlated with hypercaloric diets include metabolic syndrome (MetS) [11,12] diabetes mellitus type 1 and 2 [13], obesity [2,3,14], cardiovascular diseases (CVD)[15], various cancer types [16-

21] infertility, non-alcoholic fatty liver disease (NAFLD) and cognitive impairment [16,22].

HYPERCALORIC DIET AND INSULIN SENSITIVITY

Clinically, insulin resistance (IR) was defined as the incapacity of insulin to increase cellular glucose uptake [23]. Several factors such as age, body weight, genetic predisposition, sedentary life, stress, overstimulation of the sympathetic nervous system, and inflammation may induce the development of IR [24]. Currently, a wide range of populations worldwide have a sedentary life, which correlated with unhealthy diets will conduce to an increased incidence of obesity development related to Diabetes mellitus type 2 [25]. Epigenetic modifications were detected: altered DNA methylation of type 2 diabetes-specific genes such as IRS1, KCNQ1, PPARG, and TCF7L2 [25].

In muscle and liver, IR is correlated with increased circulating levels of free fatty acids, and mitochondrial and endoplasmic dysfunctions [26]. It is well known that type 2 diabetes is installed as a consequence of IR, a process that is frequently associated with obesity. In obesity, hyperglycemia induces excessive insulin production that further conduces to increased beta-cell mass and at the end beta-cell apoptosis. In the last decade, obesity has been named a low-grade inflammatory state, known as metiflammation [27].

In overweight adults, a diet poor in vegetables harms gut microbiota, leading to IR [28].

The muscle, the liver, and the adipose tissue may have increased amounts of ceramide, which is further correlated with IR. The high carbohydrate diet leads to an increased serum level of triglycerides (TG) and decreased levels of HDL-cholesterol, events that are correlated with an increased risk of CVD [25]. Chronic hyperglycemia induces subclinical inflammation, which is the major factor of endothelial damage and represents the link between diabetes and CVD [29].

It is well known that overnutrition conduces to weight gain, which further creates a vicious cycle characterized by IR/hyperinsulinemia. This event will conduce to atherogenic dyslipidemia development, illustrated by hypertriglyceridemia, decreased HDL, and increased LDL [30]. IR affects 25-35 % of the Westernized population [16,22].

Metabolic syndrome (MetS) is defined as a multi-factorial disorder, which received several names such as IR syndrome, metabolic syndrome, Reaven's syndrome, Syndrome X, and the deadly quartet [12,31]. MetS are characterized by abnormal obesity, dyslipidemia, decreased levels of HDL-cholesterol, IR, and hypertension. This metabolic disorder is mainly characterized by obesity and IR. The risk of coronary heart disease and type 2 diabetes is significantly reduced by treating MetS [32].

One of the most common disorders of pregnancy is gestational diabetes mellitus (GDM), which is developed in the second and third trimesters of pregnancy, leading to hyperglycemia of various severity [22,33]. GDM development harms both mother and child, resulting in cesarean delivery, macrosomia, and neonatal hypoglycemia. Moreover, women with GDM have a huge after-pregnancy risk of CVD and type 2 diabetes development [22]. A decreased amount of vegetables and an increase in animal products may cause IR. Pre-pregnancy intake of processed and unprocessed meat is associated with an increased risk of GDM development [34].

Short-chain fatty acids (SCFAs) produced by our gut microbiota can affect the cardiometabolic phenotype because they can enter the systemic circulation and may directly affect the metabolism or the peripheral tissue function [35]. Therefore, SCFAs stimulate the sodium/glucose transporter-1 expression leading to increased absorption of monosaccharides. SCFAs directly influence the host's satiety, because they can promote the glucagon-like peptide-1 (GLP-1) and peptide YY release. This is possible after the interaction of SCFAs with G-proteins expressed by enteroendocrine cells. More than that, SCFAs promote lipogenesis and inhibit fatty acid oxidation, triggering obesity [36].

MEDITERRANEAN DIET AND INSULIN SENSITIVITY

A healthy lifestyle includes a healthy diet, physical activity, not smoking, and moderate consumption of alcohol, especially for women [37]. A vegan diet based only on the consumption of grains, vegetables, and fruits, reduces the risk of MetS, diabetes, and [28, 38], and prevents CVD [39].

A diet rich in fiber, more than 25 g/day in women and more than 38 g/day in men decreases the risk of type 2 diabetes. In addition, a diet that contains nonviscous compounds such as whole grains and insoluble cereal fibers may influence the postprandial glucose response or could be very well fermented by the colon gut microbiota [40]. The results of 30 randomized clinical trials performed on men and women, reported that the consumption of milk and dairy milk products is associated with a decreased activity of insulin and fasting insulin levels [41].

In the case of healthy subjects, an acute endurance exercise in the fasted state causes important changes: stable blood glucose amount, low blood insulin levels, increased lipolysis with an increased blood-free fatty acid concentration, maintenance of the muscular glycogen and high intramyocellular TG oxidation [42].

Recent studies illustrate that replacing the carbohydrates from the diet with fat will decrease fasting TG and will increase the HDL level. Therefore, the results of the meta-analysis that included obese patients who received a high-fat versus low-fat diet, reported after the consumption of a low-fat diet a significantly elevated level of TG and a decreased level of HDL-cholesterol [43].

The Mediterranean diet (MD) consists of antioxidants, fiber, polyunsaturated fats, and compounds with anti-antioxidant, anti-inflammatory, anti-cancer, and anti-obesity properties [44]. Fats intake, especially monounsaturated fat (MUFA) helps the body to reduce the level of insulin. Moreover, 3–4 g of MUFA will decrease the TG levels and blood pressure (BP) in subjects with MetS. Cohort studies reported that a dairy fat consumption of 50% saturated fat leads to a decreased incidence of MetS [43]. A Mediterranean-style diet has positive effects in reducing the MetS prevalence and cardiovascular complications [45]. Even World Health Organisation (WHO) recommends a diet poor in carbohydrates, for adults and children. Their consumption reduces circulating insulin levels and decreases body weight and TG levels [46]. On the other hand, the decreased level of circulating insulin conduces to an increased level of circulating fatty acids, utilized for oxidation and further ketone bodies synthesis. However, this diet has negative effects on the human body in long term [46].

Willems AEM and his research team, published 2021 the results of a meta-analysis regarding the impact of low-carbohydrate and low-fat consumption on markers of MetS in obese patients without cardiometabolic disease [47]. The studies reported that weight loss is the most efficient way to reduce fasting glucose levels independent of macronutrient intake. Moreover, the specific studies reported that carbohydrate and fat intake, but without changing the percentage in the intake of these macronutrients, improved diastolic blood pressure (DPB) and circulating TG levels, without any effect on weight loss [47]. The IR was also improved with both diets, while HDL was only improved in the low-carbohydrate diet. This study concluded that the reduction of carbohydrate and fat intake are not the only factors that improve the MetS markers. This suggests that the guidelines for the prevention of MetS must be re-evaluated [47].

Cellular studies reported that the consumption of polyphenols, such as green tea catechins and epigallocatechin gallates, resveratrol, and curcumin reduce TG accumulation, and stimulate lipolysis and fatty acid-beta oxidation, leading to the reduction of obesity-related inflammation [48].

Ros E and his research team, observe the long-term effects of the MD without any energy restriction on CVD patients [49]. The study reported that MD had good effects on oxidative stress, atherosclerosis, blood pressure, lipids, and inflammation. The consumption of high-unsaturated fat and antioxidant-rich diets such as the MD one may prevent CVD development [49]. Therefore, olive oil, red wine, and nuts have beneficial effects on human health [50]. Dietary polyphenols including fruits, vegetables, whole grains, coffee, tea, and nuts, influence glycemia and type 2 diabetes, by promoting the tissue's glucose uptake, and therefore improving insulin sensitivity [51].

Ryan MC and co-workers conducted a randomized study that included twelve non-diabetic NAFLD patients (6 females/6 males) across 6 weeks. The study participants received MD, rich in MUFA. The study conclusions indicated that MD reduces liver steatosis and improves insulin sensitivity [52]. Abenavoli L and colleagues obtained similar results in NAFLD patients who consumed MD [53].

The clinical study Coronary Diet Intervention conducted by Delgado-Lista J, which enrolled 1,002 coronary patients from Spain, compare the effects of MD rich in virgin oil versus the low-fat diet on the impact of cardiovascular events. At the beginning of the study, 70.6% of the patients had LDL-cholesterol <100 mg/dL, 56.3% of them were obese, 64.4% were former smokers and only 9.6% were active smokers. However, between patients who received MD and a low-fat diet, no difference regarding weight, hypertension, fasting plasma glucose, or lipid profile was observed [54].

The preliminary results of the ongoing trial conducted by Sayón-Orea C regarding the effects of MD and physical activity on 6874 men and women (aged 55 to 75 years) with MetS and no CVD history enrolled between September 2013 and December 2016, illustrated a significant change. The change was visible in BMI, body weight, waist circumference, and lipid profile after 12 months compared with the control group [55].

The scientific group of Papadaki reported changes in 18 of 28 MetS components and risk factors [body weight, BMI, waist circumference, SBP, DBP, glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR) index, total cholesterol, LDL-cholesterol, HDL-cholesterol, TG, alanine transaminase, hepatic fat mass, C-reactive protein, interleukin-6, tumor necrosis factor-a, and flow-mediated dilatation] and lower risk of CVD incidence, after the consumption of MD [56]. Moreover, low-caloric MD and exercise training are important targets in the treatment of NAFLD [57].

CALORIC RESTRICTION (CR)

CR represents the reduction of kilocalorie (kcal) intakes between 20 - 40%. Therefore, food restriction has been demonstrated to improve health and increase longevity in various groups of species, including humans, dogs, nematodes, rodents, rotifers, and non-human primates. Moreover, CR may delay the development of various pathologies such as autoimmune diseases, CVD, cancer, diabetes, renal diseases, neurodegenerative diseases, and respiratory diseases [58].

The term intermittent fasting (IF) refers to extended periods (16-48 hours) in which people eat little or nothing, alternating with periods of normal food intake. Moreover, periodic fasting (PF) means the periods of fasting or fasting-mimicking performed between 2 to 21 days or more. Studies performed on experimental models (mice and rats) summited on IF and PF, reported a decreased incidence of age-related disorders such as diabetes, CVD, cancer, and neurological disorders [59]. Therefore, a 60% energy restriction 2 days per week, and a five-day diet of 750-1100 kcal for normal and overweight patients conduces to weight loss and improves IR, and reduces the risk of CVD. Thus, IF improves health and counteracts diseases by the activation of several pathways involved in adaptive cellular response, mitochondrial death, autophagy, and DNA repair [59]. Taking into account all the aspects presented, the inflammation generated by obesity can be a key element for the development of oral pathologies such as periodontal disease [60-63]. Depression-related obesity is very dangerous, which may conduce to antidepressants consumptions [64,65].

Figure 1: The relationship between obesity-inducible factors, the Mediterranean diet, caloric restriction, and various metabolic pathologies

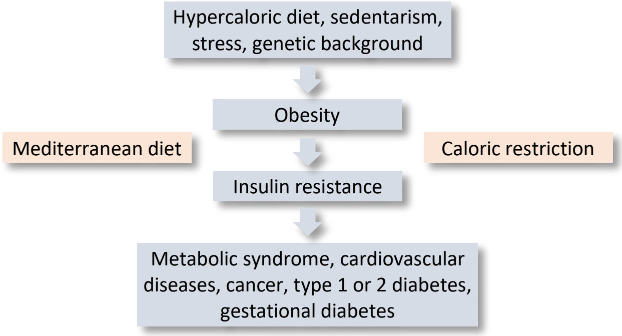
Hypercaloric diet, sedentarism, stress, genetic background
Obesity
Mediterranean diet
Caloric restriction
Insulin resistance
Metabolic syndrome, cardiovascular diseases, cancer, type 1 or 2 diabetes, gestational diabetes

CONCLUSIONS

IR is a consequence of many factors such as body weight, stress, genetic predisposition, inflammation, and over-stimulation of the sympathetic nervous system. Hypercaloric diets lead to obesity and IR, pathologies associated with MetS installation, and further CVD development. Healthy diets such as the MD, in combination with weight loss, especially for individuals who are overweight or obese, can drastically improve the cardiometabolic risk factors. MD reduces the level of TG and stimulates lipolysis and fatty acid-beta oxidation. CR, IF and PF improve general health and may delay the development of age-related disorders.

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ORIGINAL ARTICLE

Progress and Challenges of Diabetic Foot Diagnostic and Treatment

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Abstract: *Diabetic foot is one of the main long-term complications of diabetes, having a significant impact on a patient's quality of life. However, prevention by daily checking of the limbs and observance of the rules of care remains particularly important to avoid total or partial amputation. This paper aims to highlight the main challenges and progress in the treatment of the diabetic foot. To achieve this, we analyzed a database consisting of more than 100 articles published in recent years, approaching the treatment of the diabetic foot. The main problems that occur with the diabetic foot are peripheral vascular disease and diabetic neuropathy. These can lead to blisters and skin lesions that can eventually lead to ulcers in the lower limbs and even amputation. In conclusion, prevention is the main method of treatment for diabetic foot, followed by the treatment of each complication depending on when it is identified and its severity.*

Keywords: *diabetic foot; treatment of diabetic ulcers, diabetic foot*

INTRODUCTION

Diabetic foot is one of the most common complications of diabetes, being the leading cause in the world of lower limb amputation [1], as well as frequent hospitalizations in patients with diabetes [2,3]. According to the World Health Organization, diabetic foot syndrome is defined as "ulceration of the foot (distally from the ankle and including the ankle) associated with neuropathy and different grades of ischemia and infection" [4]. The main factors involved in the pathogenesis of diabetic foot ulcers are neuropathy and peripheral vascular disease [5]. However, other factors intervene in the occurrence of diabetes complications such as poor control of blood sugar and blood pressure, dyslipidemia, obesity, smoking, old age, inadequate prevention strategies, and long-term diabetes [5,6]. Considering that there are some limitations of specific treatment, attention remains focused on prevention and foot care education [4]. Careful management of the diabetic foot, which requires a good doctor-patient relationship, is a sure way to prevent amputation and maintain body integrity. Focusing on educating the patient can lead to a better understanding of his pathology, being a very important step in preventing the complications of the disease [5,7]. On the other hand, initiating the most appropriate treatment of complications depending on the stage at which they are discovered is essential in the healing process and even to prevent a possible amputation [3,6,8].

MATERIALS AND METHODS

This review aims to highlight the progress of treatment methods and best strategies providing a quick and correct diagnosis, as well as the complications of the diabetic foot.

We have selected 100 studies in English, German, and Romanian that presented information on methods of treating diabetic foot, difficulties encountered in the differential diagnosis and treatment of diabetic foot, as well as the complications of diabetic foot. Articles presenting the pathophysiology of the diabetic foot were also excluded.

Databases such as PubMed, Up-to-date, Medline, and NCBI were used to perform the search using keywords such as diabetic foot, diabetic foot complications, diabetic foot treatment, differential diagnosis of diabetic foot, and advances in diabetic foot treatment. To select the papers, exclusion criteria were imposed, such as the article publication date should not exceed 20 years old, and the publications to be in English, German, and Romanian treatment protocols were also analyzed.

RESULTS

Diagnostic and differential diagnosis of diabetic neuropathy

Diabetic neuropathy is one of the most common complications of diabetes, with about 50% of patients with type 1 or type 2 diabetes showing symptoms specific to diabetic neuropathy including pain, numbness, tingling, weakness, and difficulties with balance [9]. The most prevalent of diabetic neuropathies is generalized, symmetrical polyneuropathy, in which case may occur sensitive, motor, and autonomic manifestations [10,11].

There is also a high percentage of asymptomatic diabetic peripheral neuropathies, so recognition and prompt intervention are extremely important and reduce the risk of an insensitive foot injury, as well as a possible amputation [8]. Diabetic neuropathy is a diagnosis of exclusion, which is why other possible etiologies must be excluded, given that neuropathy in diabetic patients may have other causes (Table 1) [12]. In addition, some diabetics may have several causes of neuropathy at the same time [10]. The Rochester Diabetic Neuropathy Study reported that nondiabetic peripheral neuropathies were present in about 10% of patients [13].

Table 1: Different diagnoses of diabetic neuropathies [12]

Metabolic disease	Thyroid disease (common) Renal disease
Systemic disease	Systemic vasculities Nonsystemic vasculities Paraproteinemia Amyloidosis
Infectious	HIV, VHB, Lyme
Inflammatory	Chronic inflammatory demyelinating polyradiculoneuropathy
Nutritional	Deficit of B12, post-gastroplasty Pyridoxine, Thiamine, Tocopherol
Industrial agents, drugs, and metal	Industrial agents: Acrylamide, Organophosphorous agents Drugs: Alcohol, Amiodarone, Colchicine, Dapsone, Vinka alkaloids, Platinum, Taxol Metals: Arsenic, Mercury
Hereditary	Hereditary motor, sensory, and autonomic neuropathies

Thus, to optimize outcomes and lead to specific treatment in the diabetic patient, rapid recognition and proper management are essential.

Treatment

When we talk about a diabetic foot, we inevitably think of infection, hospital admission, amputation, and emotional and financial impact [14]. To achieve the best results for a diabetic patient it is necessary to implement a good prevention strategy [15,16]. As we all know, prevention is not always easy, especially for older patients with other comorbidities, but it is essential for a better prognostic and good quality of life [15,17]. The prevention of diabetic neuropathy is based on two goals: good glycemic control and a balanced lifestyle [7,12].

The patient's education is extremely important, in this process it is necessary to present his pathology, the complications that may occur along the way, and simple techniques to follow, such as checking and daily care of the feet, wearing special shoes, signaling any new foot mark and avoiding foot trauma [14,18]. The management of diabetic foot requires a rapid intervention in front of any type of wound, if possible by a multidisciplinary team [16,19]. Daily diabetic foot screening is the best prevention [7,20].

Numerous advances have been made in the management of diabetic foot in recent years, many of which have been applied especially in clinics dedicated to diabetic foot care [14,18]. Most important in the management of diabetic foot is a multidisciplinary team consisting of specialists to act quickly in the face of any type of wound [16,19].

To determine the best treatment method, it is important to determine the severity of the diabetic foot [18]. The treatment can be conservative or surgical [21]. As long as conservative treatment is chosen, it can be performed in an outpatient setting as long as the patient is compliant, the ulceration is superficial and there are no signs of systemic damage [20]. Also, during the treatment period, it is very important to maintain the glycemic values within normal limits and to respect the nutritional regime and the daily exercise program [16,22].

The first step in treating foot ulcer is inspection, to stabilize the degree of damage and the existence or not of the infection, then the wound is cleaned, and if necessary local anesthesia can be performed (many patients have polyneuropathy and no anesthesia is needed) to debridement [23]. In the case of superficial ulcers, a multidisciplinary approach is important to prevent evolution (Table 2) [22].

There is currently a modern local treatment method that involves the use of hydro active dressings, which promote healing through cell proliferation processes in a humid environment. This is done to prevent infection and/or reduce the microbial load, prevent necrosis or eliminate existing ones and adapt to each stage of the wound healing process (cleaning, granulation, and epithelialization) [24].

Table 2: Treatment for superficial uncomplicated foot ulcers [22]

Active ulceration Peripheral neuropathy +/- PAD	**Clinic evaluation**	**Patients evaluation**	**Core**	**Adjuncts**
Non-limb threatening Ulcer	- Superficial - Minimal to no cellulitis - No bone or joint involvement - No significant ischemia - No toxicity	- Reliable - Will conform to treatment - Good support system - Evaluation of comorbidities	- Offloading - Debridement, Dressing - Management of infections - Vascular evaluation if needed - Foot-sparing surgery	- Control blood sugar - Treat comorbidities - Nutrition - Control edema - Physical and emotional therapy
Limb threatening ulcer	Check both feet and legs, and compare Before any intervention, the ulcer probes deeply Note location May involve bone or joint Cellulitis >2cm Lymphangitis, gas, crepitation Ischemia +/-foot pulses, Doppler Systemic toxicity, temperature, white blood cell, lymph nodes Escher, *callor*, granulation tissues	- Unreliable - Poor support systems - Poor control of comorbidities	Therapy - Admit to hospital – complete bed rest - Employ core and adjunct - control blood- stabilize comorbidities - Especially consult (cardiology, renal, infectious disease, etc) - Empirical broad-spectrum antibiotics – adjust by culture and sensitivity - Labs and X-ray Early vascular evaluation and Rx after control of infarction - Foot-sparing surgical vs amputation/revisions - Close follow up	
Septic ulcer	- Same as for limb-threatening ulcer - Be sure to check for undermining deep tissue exposure, abscess, bone, and gas	- Same as limb-threatening ulceration	- Immediate admission – complete bed rest – protect wound and foot, esp heel - Employ core and adjuncts - Intravenous broad-spectrum antibiotics/fluid replacement and resuscitation - Labs and X-rays - Culture and sensitivity – blood culture - Control edema - Emergency aggressive surgical debridement - control all activity infarction, including possible amputations - Stabilizing medically – blood glucose, cardiac(MI, CHF) renal, esp consult (cardiology, renal, ID, etc.) - Evaluate later for antihypertensive, beta-blockers, calcium channel blockers, ACE inhibitors - Early vascular evaluation and Rx after control of infarction - Close follow up	

If the foot ulcer is infected it is especially important to determine if there is a systemic or only local infection [25]. The signs of local infection are erythema, edema, suppuration, and unpleasant odor, but often if the patient has kidney failure they do not show [26].

Depending on the degree of damage and the level of infection, the method of treatment is decided, but the basis of any method of treatment is the initiation of antibiotic therapy and wound care [25].

To choose the best antibiotic therapy, the following criteria are taken into account:

- Local bacterial resistance pattern,
- Proper antibiotic history,
- Gram stains of deep exudate,
- Appearance of wound and pus,
- Associated co-morbid conditions like renal, hepatic, and cardiac impairment [22]

DISCUSSIONS

Clinical aspects and the pathophysiology of diabetic foot

Because there are multiple etiologies underlying this concept, the diabetic foot is considered to be a "syndrome” [27]. It is one of the most common complications of diabetes which in the long-term, if not treated properly, can lead to amputations, disability, and reduced quality of life [4,28]. It is well known that around 25% of diabetic patients will develop a foot ulcer in their lives [28,29]. The pathophysiology of a diabetic foot ulcer is based on triad neuropathy, trauma with secondary infection, and arterial occlusive disease, which appears in various forms [17].

Due to the nerve damage, there is a loss of pain and thermal sensitivity that makes the feet vulnerable to minor injuries caused by excessive pressure, and mechanical or thermal damage [30,31]. Furthermore, motor neuropathy leads to intrinsic muscle atrophy, and foot deformities by altering the biomechanics of the foot, as well as limited joint mobility and postural and coordination deviation [32]. In the end, the formation of calluses comes as an expression of the plantar excessive pressure [28].

One of the goals of treating any ulcer with neuropathy is to reduce weight, regardless of the presence of ischemia [6,33]. Last, the effects of the onset of autonomic neuropathy are diminished sweating, altered blood flow regulation, and microvascular dysfunction, which will lead to dry skin and cracks [21,34]. Repetitive trauma and impaired proprioceptive and exteroceptive sensitivity predispose to skin injury leading to atrophy and dislocation of plantar fat, followed by ulceration and infection [28,35].

There are situations in which, inattention to certain signs (redness, blister formation) or lack of routine foot care, leads to the formation of ulcers and the appearance of infections, undesirable things in diabetic patients with neuropathy, or a history of foot ulcers [3]. Studies have shown that the risk of ulceration increases in diabetic patients with neuropathy, a history of amputations, or foot deformity [16,17].

There are advantages and disadvantages to each kind of wound treatment and multiple solutions for wound dressing are present (Miculickz pomade, 1% silver sulfadiazine cream, zinc hyaluronate solution, bovine collagen, hyperbaric oxygen therapy, hydrocolloid dressing or synthetic skin substitutes)[15,16]. Most of them need real confirmation in randomized studies.

Adjuvant care of diabetic foot ulcers implies systemic factors control of hyperglycemia, control of infection, treatment of vasculopathy (sulodexide, cilostazol) or local factors-peripheral neuropathies, control of pressure at foot level (custom-made ortheses and shoes)[36].

Charcot neuro-osteoarthropathy

Charcot neuro-osteoarthropathy or the Charcot Foot is one of the most severe complications of diabetes [37]. The main factors that lead to this disease are a combination of neuropathy, repeated microtrauma caused by increased weight, and an abnormal bone metabolism leading to inflammatory reactions [35,38]. The result is the destruction of the normal architecture of the foot, subluxation, dislocation, and deformity, most commonly in the ankle and foot [39]. Charcot's Foot is an urgent condition, often associated with ulcers and osteomyelitis, and requires proper management by a foot specialist [40].

Osteomyelitis

Osteomyelitis represents a severe infection involving bone, which occurs due to non-healing ulcers, and if not treated properly may lead to major amputation [27,28]. Two-thirds of the patients with diabetic ulcers may be affected by osteomyelitis, so this complication requires increased attention [38].

In addition, patients with a diabetic ulcers may develop osteomyelitis without any signs of pain, but there may also be a decrease in local signs of infection caused by neuropathy and peripheral arterial disease [41].

CONCLUSIONS

Diabetes mellitus is a chronic disease, characterized by high blood glucose levels [42].

The long-term complications of diabetes are vascular and neurological, and of these, diabetic foot syndrome is one of the most serious consequences in terms of the health complications it generates and the impact on quality of life. It includes ulceration, infection, and osteoarthropathy, known as Charcot's foot. Proper prevention and care of the feet are essential for the early identification of problems and mitigation of consequences. This includes patient education, prophylactic skin, nail care, and proper footwear.

Depending on the severity of the condition, the best method of treatment for the diabetic foot is decided. There are several options, both invasive and non-invasive, for treating this disease.

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ORIGINAL ARTICLE

Image of a Junior Officer in Military Personnel Participating in Combat Operations with Symptoms of Post-traumatic Stress Disorder

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Abstract: *According to the results of the conducted study there were distinguished the peculiarities of perception of the image of a junior officer by servicemen who participated in hostilities and had various PTSD symptoms. The study included 233 male servicemen with combat experience. The age of the participants varied from 20 to 55 years. It was determined that the image perception of the direct commander by the servicemen who did not have any PTSD symptoms had been influenced by both the key features of the modern way of fighting and the way a protective mechanism of consciousness acted, which gave the possibility to maintain mental health under combat conditions. These servicemen considered the awareness, kindness, and physical strength of their commander to be not important. The first stage was occupied by his/her ability to adequately fulfill his/her leadership function and the power of his/her spirit – the courage and love of the Motherland, which formed his/her reliability. Servicemen who had PTSD symptoms were less satisfied with the role of a junior officer and considered him/her to be less capable of meeting their expectations. They were less interested in commanders who could involve them in situations that required quick, brave action and complete responsibility for the entrusted part of common affairs. The most important factors in the structure of the direct commander image in both studied groups were the power of personality (the ability of self-regulation); however, the servicemen with PTSD symptoms had a specific orientation of these factors – to help their subordinates. They were quite selfishly aimed at using their direct commanders as a way of getting help.*

Keywords: *leader, serviceman, service and combat activity, combat experience, post-traumatic stress disorder, psychological recover*

INTRODUCTION

It was quite difficult to overestimate the role of a junior officer who was a direct commander during the fight. He/she shared with his/her subordinates all the complexities of the service, informed them about orders of senior officers, implemented the coordination of actions of small military groups, made relevant decisions related to the life of the unit, and resolved conflict situations in the team [1]. The behavior of warlords had a deep effect on the mental health of those whom they had to lead [2]. According to Tedeschi and Mcnally, the perception of their leaders influenced not only the way they responded to stressful events, such as hostilities, but also an individual's desire to apply for psychological help in times of personal crisis [3].

The implementation of combat tasks and the preservation of the lives of entrusted him/her servicemen fully depended on the competence of the direct commander's actions under combat conditions. According to Baran and Scott to make a unit's actions effective the subordinates had to trust in the actions of their commander, trust that he would not pose them to the actions with unjustified risk [4]. The research conducted by Sweeney determined that leaders who showed competence before combat actions had a higher level of trust among their subordinates, while those who demonstrated a lack of competence and a lack of previous combat experience could feel distrust from the side of their subordinates [5]. Fisher, et al. [6] received similar conclusions to Sweeney [5] and proposed to add that successful leadership under combat conditions was possible due to previous successes on the battlefield. According to them the experience of participation in hostilities strengthened the leader's confidence and formed appropriate beliefs about self-efficacy.

The experience of joint actions on the battlefield imposed a significant imprint on the perception of the direct commander by his/her subordinates. In particular, the studies conducted by Xue, et al. [7] and Ramchand, et al. [8] proved that servicemen’s perception of their commander (leader) directly related to post-traumatic stress disorder. Other conducted studies (Castro and McGurk; Du Preez, Sundin, Wessely, and Fear; Iversen, Fear, Ehlers, et al.; Jones, Seddon, Fear, et al.; McGurk, Sinclair, Thomas, et al.) also figured out that the perception of the leader by servicemen on the battlefield related to the fair and equal attitude, praise, interest, and concern [9-13]. However, in general, the studies lacked theoretical explanations of the relations which could help to make the image of leadership the only factor that could be changed. The only exception was the study by McGurk, et al. [13] who used the theory of resources conservation that was proposed by Hobfll as an explanation of how positively and negatively leaders could contribute to obtaining or losing the resources, which could explain the connection with PTSD [14].

However, the experienced stressful events could interfere with the acquired combat experience as well as the features and qualities which the serviceman realized in him/herself, his/her comrades, and the commander overcoming life challenges. The negative experience caused by the mental traumatization was painful (Kolesnichenko), having the desire to weaken its power a serviceman could want both to find help and to find the perpetrators [15]. He/she could become isolated, believing that people around him/her could not understand and help. The inability of the serviceman to accept his/her combat experience (Kalesnichenko) [16], and loss of his/her mental health (Kolesnichenko) [17] could have a negative influence on the interaction within the unit, on the perception of the image and the role of the commander (Jansen and Delahaij) [18]. Although the military hierarchy regulated the relations between the leaders and subordinates, unofficially servicemen could abandon their leaders. Such refusal, according to Pierro, Cicero, and Bonaiuto, et al., could cause floats and counterproductive behavior within the military teams [19].

METHOD

Participants and Procedure

The study was conducted in 2022, it was a part of another bigger study related to the determination of important qualities of military leaders. The study included 252 servicemen (42.16% of them were mobilized; 57.84% of them were servicemen under the contract) who participated in hostilities. All the participants were divided following the scale “Mississippi Scale for Combat-Related PTSD” into two groups – group A included the servicemen (155 people) whose PTSD symptoms did not exceed the norm (up to 78 points); group B included the servicemen (97 people) whose indicators exceeded the norm (more than 78 points). This study was based on the presence of definite PTSD symptoms of servicemen who, on request, received qualified individual consulting help from military psychologists and participated in group works aimed at restoring their resilience ability as well as at increasing their ability to accept their own combat experience. It was not about the formed PTSD.

All procedures carried out in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants gave consent for their data to be used in this research.

Instruments and Measures

To identify the peculiarities of perception of the ideal representation (image) of a junior officer by the servicemen who participated in hostilities and had PTSD symptoms based on the analysis of modern and foreign studies there were distinguished more than 50 descriptions of the characteristics of military leaders. After the first stage of the Delphi estimation method, where the experts were selected from the military leaders with different ranks, subordinates including those with considerable combat experience, military instructors, and lecturers from the higher military educational establishments. The total number of experts was 65 people. There were made up separate lists of 30 characteristics to evaluate the sergeants, junior and senior officers. It had to be highlighted that the descriptions of the leader’s characteristics in the lists were significantly reduced and edited due to the experts’ recommendations. Due to that definite list of 30 characteristics, which was formed to evaluate the junior officer by the 11-point scale (from 0 to 10 points), the participants who took part in hostilities were asked to evaluate the importance of those characteristics in the "ideal" junior officer, according to their point of view.

To evaluate the degree of PTSD expressiveness of the servicemen who participated in the study there was used "Mississippi Scale for Combat-Related PTSD" [20]. Nowadays, it was one of the most common methods used to measure the signs of PTSD. The scale included 35 statements, each of which can be estimated on a five-point scale. The total indicator which was made up of this method gave the possibility to estimate the degree of influence of the traumatic experience transferred by an individual.

Data Analysis

To represent the data, there was used the main descriptive statistics (arithmetic mean M and standard deviation SD). To compare the estimates provided in the selected groups there was used the T-criteria of the Student. Moreover, there was used the procedure of factor analysis by Principal Components to distinguish the structure of the image of the junior officer due to the given points. Mathematical data was processed with the help of SPSS 17.0.

RESULTS

The points given to the selected groups of servicemen who participated in hostilities were proposed by 30 qualities used to make the perfect image of a junior officer. The points were compared with the help of the T-criterion of the Student. This information was presented in Table 1.

According to Table 1, due to all 30 characteristics which were used to describe the ideal junior officer, the service-men without PTSD symptoms had much higher indicators than the servicemen of the second group. The data indicated not only the presence of different ideas of the representatives of these groups concerning the ideal of a junior commander but also the more negative attitude of the group representatives with PTSD symptoms towards a junior commander in general and towards the role he performed.

It should be noted that both groups had differences between the most important quality and the least important which were minimal. Thus, in the first group, the most important was considered the ability to “adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation" (9.16 ± 1.51), and the least important was “own achievements, professionalism and character traits made him/her an attractive model to follow” (8.50 ± 1.97).

In the group with PTSD symptoms, the most important characteristic was "a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it" (8.58 ± 1.90), and the least important, as in the first group, was "own achievements, professionalism and character traits made him/her an attractive model to follow” (7.60 ± 2.54). Thus, despite the different evaluation tactics in these groups, the least important were both It should be noted that both groups had differences between the most important quality and the least important which were minimal. Thus, in the first group, the most important was considered the ability to “adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation" (9.16 ± 1.51), and the least important was “own achievements, professionalism and character traits made him/her an attractive model to follow” (8.50 ± 1.97).

In the group with PTSD symptoms, the most important characteristic was "a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it" (8.58 ± 1.90), and the least important, as in the first group, was "own achievements, professionalism and character traits made him/her an attractive model to follow” (7.60 ± 2.54). Thus, despite the different evaluation tactics in these groups, the least important were both the status and exemplarity of the commander (his/her achievements).

However, if the first group put in the first place a specific function of the leader – the ability to adequately perform the role of intermediate (between the senior commander and subordinates) leader, which ensured the coordination of small units’ actions, the second group valued his/her civil position and his/her own choice – love of the Motherland and the ability to concentrate all strength to protect it; it seemed that only that could justify all the sufferings which they had survived with their commander, under his/her direct guidance.

A lower quantity of points could indicate less satisfaction with the role of junior officers, and their inability to meet expectations. It was necessary to point out that the commander's patriotism was highly appreciated by the representatives of the first group in which he/she, conditionally, occupied the second stage. However, it could be assumed that the servicemen of the first group reflected the protective mechanism on the estimation of the direct commander.

The protective mechanism ensured the preservation of their mental health – the focus on the process of performing their functions under combat conditions – hence occurred a specific view of the role of the commander.

To clarify the assumptions which were made, there occurred a need to look more precisely at the most and the least important qualities of junior officers in both groups. It was inappropriate to consider in detail the whole hierarchy of the characteristics of a junior officer in the distinguished groups due to the minimal differences in the estimations of the most and the least important qualities.

Table 1: The Characteristics of the ideal image of a junior officer in the groups of servicemen with different PTSD signs (in standard points)

№	Variable	Studied		$t_{кр}$	p
		group A	group B		
1	He/she worried about the welfare of his/her subordinates and found opportunities to organize the most convenient conditions for their service in the current circumstances	9.02±1.77	8.14±2.43	3.09	.01
2	He/she was brave and strong-willed, and with his/her example he/she inspired the subordinates to overcome difficulties	9.08±1.54	8.14±2.49	3.33	.01
3	He/she was able to take any situation under his/her control, to make up-to-date decisions	9.03±1.68	8.24±2.44	2.79	.01
4	He/she was reliable, did not give up, and acted effectively even having minimal resources	8.96±1.67	8.10±2.31	3.16	.01
5	He/she saw the prospects that his/her unit had and could unite the subordinates to achieve these prospects	9.01±1.63	8.06±2.46	3.36	.01
6	His/her achievements, professionalism, and character traits made him/her an attractive model to follow	8.50±1.97	7.60±2.54	2.96	.01
7	He/she adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation	9.16±1.51	8.31±2.34	3.17	.01
8	He/she had good communication skills and could adequately resolve conflict situations	9.04±1.62	8.46±2.23	2.21	.05
9	He/she could explain, teach, and help to form the necessary skills as well as the traits of character	8.92±1.67	8.00±2.34	3.34	.001
10	He/she was aware, well-informed, and could easily acquire the necessary knowledge as well as professional experience	8.84±1.76	7.91±2.39	3.31	.001
11	He/she could form the team – everyone in the unit was "in his/her place" both due to professional skills as well as due to personal traits, which ensured his/her effective work	9.05±1.62	8.03±2.37	3.72	.001
12	He/she formed confidence in the better future that which individual wanted to follow with his/her brethren	8.83±1.62	8.00±2.22	3.15	.01
13	He/she was confident in him/herself, in his/her decisions, and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity	9.01±1.61	8.13±2.26	3.33	.001
14	He/she adequately evaluated the importance of solving certain problems. To solve them he/she showed the appropriateness of persistence, honesty, and stubbornness	9.04±1.52	8.25±2.06	3.25	.01
15	He/she was quite independent – made up-to-date decisions and took responsibility for the given orders	8.95±1.78	7.90±2.33	3.82	.001
16	He/she was friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones	8.82±1.78	8.22±2.13	2.30	.05
17	He/she was emotionally balanced and stress-resistant and could control his/her negative experience	8.97±1.62	8.29±1.96	2.84	.01
18	His/her morality, belief perseverance, and ability to defend those beliefs gave him/her the possibility to be a role model for his/her subordinates	8.89±1.60	8.17±2.08	2.92	.01
19	He/she was demanding to others as well as to him/herself and inspired the others to imitate him/her by own self-discipline	8.98±1.47	8.10±2.23	3.42	.001
20	He/she was tactful being demanding to his/her subordinates and taking care of them. He/she represented respect and maintained a sense of dignity in his/her subordinates	8.91±1.66	8.16±2.17	2.91	.01
21	He/she was able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation	9.08±1.51	8.17±2.29	3.48	.001
22	He/she formed the feeling of "we" in servicemen: "We are defenders", "If not we, then who?"	9.03±1.64	8.22±2.30	2.99	.01
23	He/she carefully considered his/her decisions, calculated all the possible complications within the process of their implementation	8.98±1.69	8.23±2.16	2.90	.01
24	He/she formulated his/her opinion, gave consistent orders, and agreed with other executives	9.05±1.49	8.42±2.06	2.63	.01
25	He/she maintained good physical form, could bear the hardness of work on par with everybody in the unit if it was necessary, he/she could take on the professional functions of any of the subordinates	8.76±1.77	8.27±2.15	1.87	.1
26	He/she could take on full responsibility for the actions of his/her subordinates	8.96±1.68	8.29±2.26	2.51	.05
27	He/she ensured the information security of his/her unit, did not allow the leakage of information related to the servicemen and their families	9.05±1.64	8.50±1.97	2.29	.05
28	He/she was a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it	9.12±1.77	8.58±1.90	2.22	.05

29	He/she could adequately evaluate the physical and psychological state of his/her subordinates, could organize the necessary help for them	9.11±1.50	8.40±2.09	2.92	.01
30	He/she was professional – had good professional knowledge, skills, and experience in their implementation under combat conditions	9.05±1.70	8.44±2.20	2.33	.05

There was a need to highlight that such minimality of differences of estimations of the most and the least important characteristics of the direct commander in both studied groups of the participants in hostilities testified the syncretic (fusion) of the perception of his/her image, as a significant individual in the life of a serviceman, while surviving under combat conditions directly depended on him/her.

In the first group, the most important (those who occupied leading positions and ranks in the hierarchy) were the following qualities:

1) adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation (9.16 ± 1.51);

2) a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it (9.12 ± 1.77);

3) could adequately evaluate the physical and psychological state of his/her subordinates, could organize the necessary help for them (9.11 ± 1.50);

4) able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation (9.08 ± 1.51);

5) brave and strong-willed, with his/her example he/she inspired the subordinates to overcome difficulties (9.08 ± 1.54).

Conditionally the least important in the first group (those who occupied the least leading positions and ranks in the hierarchy) were the following qualities:

1) own achievements, professionalism, and character traits made him/her an attractive model to follow (8.50 ± 1.97);

2) maintained good physical form, could bear the hardness of work on par with everybody in the unit, if it was necessary, he/she could take on the professional functions of any of the subordinates (8.76 ± 1.77);

3) friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones (8.82 ± 1.78);

4) formed confidence in the better future that which individual wanted to follow with his/her brethren (8.83 ± 1.62);

5) aware, well-informed, and could easily acquire necessary knowledge as well as professional experience (8.84 ± 1.76).

As could be seen, it was not so important for servicemen, who retained their mental health throughout the fight, which achievements their direct commander had, and whether he was well-informed, friendly, and strong. Among all the personal traits, only his/her patriotism and courage were important. For these servicemen, the first place was occupied by his/her ability to adequately fulfill his/her leadership functions – to set the combat tasks with adequate consideration of the combat situation and the subordinates he/she had.

In the group that had PTSD symptoms, the following characteristics received the highest points:

1) a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it (8.58 ± 1.90);

2) ensured the information security of his/her unit, did not allow the leakage of information related to the servicemen and their families (8.50 ± 1.97);

3) had good communication skills and could adequately resolve conflict situations (8.46 ± 2.23);

4) professional – had good professional knowledge, skills, and experience in their implementation under combat conditions (8.44 ± 2.20);

5) formulated his/her opinion, gave consistent orders, and agreed with other executives (8.42 ± 2.06).

The participants of hostilities who had PTSD symptoms considered the following characteristics to be the least important for junior officers:

1) own achievements, professionalism, and character traits made him/her an attractive model to follow (7.60 ± 2.54);

2) quite independent – made up-to-date decisions and took responsibility for the given orders (7.90 ± 2.33);

3) aware, well-informed and could easily acquire necessary knowledge as well as professional experience (7.91 ± 2.39);

4) could explain, teach, and help to form the necessary skills as well as the traits of character (8.00 ± 2.34);

5) formed confidence in the better future that which individual wanted to follow with his/her brethren (8.00 ± 2.22).

For the servicemen of the second group as well as for the first one the achievements of their commander and his/her awareness were not important. Moreover, they did not believe that he/she could bring them to a better future, or help them to overcome their problems related to their personal life (to form the necessary traits). It seemed that those psychologically injured servicemen transferred to the image of the direct commander their confidence that their lives had no prospects, and no one could help them (one of the PTSD signs) neither due to their initiative (due to the love to their relatives) nor due to their functional responsibilities.

However, among the most important characteristics of the direct commander, three out of five in their formulation in one way or another indicated their ability to protect their subordinates. The other two were related to the cognitive functions which, among other things, were able to ensure the definite success of servicemen who, due to the loss of mental health, had problems with attention and order understanding. Thus, the attitude towards the direct commander by the servicemen who had PTSD symptoms showed opposite tendencies – on the one hand, they sought protection and help to keep the success in performing the given tasks and not lose the remains of self-esteem, on the other hand – they did not believe the fact that their direct commanders themselves or by the order of senior officers could help them to cope with their negative experience, and could direct them to the path leading to the "better future." The negativity of their estimations related to junior officers was caused by internal contradictions concerning their attitude towards them, the attitude which combined the search for protection and helping with the despair in the fact that they could help them (such PTSD characteristics as the belief in the uniqueness of their traumatic experience and the unpromising life).

Moreover, there were several characteristics in the process of their estimation in two groups, there had been distinguished the highest differences (more than 10 positions/ranks).

Thus, the servicemen whose PTSD symptoms did not exceed the norm, higher positions were occupied with the following characteristics:

- able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation (4th position in contrast to 16.5th position in the second group);

- brave and strong-willed, with his/her example he/she inspired the subordinates to overcome difficulties (5th position in contrast to 19th position in the second group);

- could form the team – everyone in the unit was "in his/her place" both due to professional skills as well as due to personal traits, which ensured his/her effective work (7.5th position in contrast to the 25th position in the second group).

Instead, in the second group, higher positions were occupied with the following characteristics:

- could take on full responsibility for the actions of his/her subordinates (8.5th position in contrast to 20.5th in the first group);

- emotionally balanced and stress-resistant, and could control his/her negative experience (8.5th position in contrast to 19th in the first group);

- maintained good physical form, could bear the hardness of work on par with everybody in the unit, if it was necessary, he/she could take on the professional functions of any of the subordinates (10th position in contrast to 29th in the first group);

- friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones (14th position in contrast to 28th in the first group).

This data could indicate that servicemen with PTSD symptoms were less interested in commanders who could involve them in situations that required quick, brave actions, and complete responsibility for the entrusted part of the common affairs; instead, they required a commander who could take on the responsibility for their failures as well as a part of their direct responsibilities, and at the same time he/she would not condemn (express any negative attitude).

There was a need to consider the structure of characteristics of junior officers formed with the help of a factor analysis due to the estimations made by the servicemen from the selected groups.

In the group of servicemen, where the indicators of PTSD symptoms did not exceed the norm, after the simplification procedure, there was formed a three-factors structure (see Table 2), described 78.47% of the symptoms' dispersion. This structure was presented in Table 2.

The content of the first and the most important factor (40.56%) was determined with the following variables "emotionally balanced and stress-resistant, and could control his/her negative experience" (0.815), "able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation"(0.771), "maintained good physical form, could bear the hardness of work on par with everybody in the unit, if it was necessary, he/she could take on the professional functions of any of the subordinates"(0.771), "confident in him/herself, in his/her decisions and in the unit itself – he/she did not allow any doubts, uncertainty, and the apathy which could undermine the efficiency of the unit activity"(0.743), and "demanding to others as well as to him/herself, and inspired the others to imitate him/her by own self-discipline" (0.663). This factor was interpreted as the power of "I" and as the power of personality based on a developed ability to self-regulation.

The second factor (23.05%) was determined by the variables such as "had good communication skills and could adequately resolve conflict situations" (0.796) and "adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation" (0.781). This factor could be marked as professional communicative competence, which showed up, both in the ability to formulate a combat mission and convey information as well as in the ability to resolve the conflict.

Table 2: The structure of the characteristics of junior officers formed following the estimations made by servicemen who did not have clearly expressed signs of PTSD (in standard points).

Variable	Factors		
	1	2	3
He/she adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation	0.275	0.781	0.235
He/she had good communication skills and could adequately resolve conflict situations	0.343	0.796	0.208
He/she was confident in him/herself, in his/her decisions, and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity	0.743	0.273	0.435
He/she was emotionally balanced and stress-resistant and could control his/her negative experience	0.815	0.359	0.139
He/she was demanding to others as well as to him/herself and inspired the others to imitate him/her by own self-discipline	0.663	0.234	0.291
He/she was able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation	0.794	0.342	0.186
He/she maintained good physical form, could bear the hardness of work on par with everybody in the unit if it was necessary, he/she could take on the professional functions of any of the subordinates	0.771	0.259	0.302
He/she was professional – had good professional knowledge, skills, and experience in their implementation under combat condition	0.413	0.397	0.819

The third factor was completely correlated with the variable "was professional – had good professional knowledge, skills, and experience in their implementation under combat conditions" (0,819). Some clarifications of the content of this factor were made by a variable "was confident in him/herself, in his/her decisions and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity" (0.435), which emphasized the active energetic position of the servicemen who had professional knowledge and experience. Accordingly, this factor was marked as professional efficiency – the ability to act effectively based on professional knowledge and experience.

For the servicemen who had expressed PTSD symptoms, the structure of the characteristics of junior officers was simpler and had only 2 factors, which described 79.88% of the signs' dispersion. This structure was presented in Table 3.

The content of the first factor (59.55%) was defined with the following variables "clearly saw the prospects that his/her unit had and could unite the subordinates to achieve these prospects" (0.887), "was brave and strong-willed, with his/her personal example he/she inspired the subordinates to overcome difficulties"(0.877), "was reliable, did not give up, acted effectively even having minimal resources" (0.859), "was professional – had good professional knowledge, skills, and experience in their implementation under combat conditions" (0.844), "adequately evaluated the importance of solving certain problems. To solve them he/she showed the appropriateness of persistence, honesty, and stubbornness" (0.837), "own achievements, professionalism and character traits made him/her an attractive model to follow" (0.832), "was able to take any situation under his/her control, to make up-to-date decisions"(0.827), "was confident in him/herself, in his/her decisions and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity" (0.815), "adequately brings to subordinates leadership, their importance, capable of ensuring their implementation adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation" (0.805), and "worried about the welfare of his/her subordinates and found opportunities to organize the most convenient conditions for their service in the current circumstances" (0.761). This factor had a weight that exceeded the weight of the second factor and combined the variables, the vast majority of which described the ability of a junior officer to self-regulation, but unlike the situation in the first group, this variable received a specific direction – to ensure a prospective development and well-being of their subordinates.

The second factor (20.33%) was determined by two variables – "was friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones" (0.885) and "was emotionally balanced and stress-resistant, and could control his/her negative experience" (0.847). Those were the variables that occupied much higher positions (ranks) in servicemen with the signs of PTSD than in the servicemen of the first group which were already marked as a tendency not to condemn their subordinates but to help them.

Table 3: The structure of the characteristics of junior officers was formed following the estimations made by servicemen who had expressed PTSD symptoms (in standard points).

Variables	Factors	
	1	2
He/she worried about the welfare of his/her subordinates and found opportunities to organize the most convenient conditions for their service in the current circumstances	0.761	0.363
He/she was brave and strong-willed, and with his/her example he/she inspired the subordinates to overcome difficulties	0.877	0.238
He/she was able to take any situation under his/her control, to make up-to-date decisions	0.827	0.296
He/she was reliable, did not give up, and acted effectively even having minimal resources	0.859	0.209
He/she saw the prospects that his/her unit had and could unite the subordinates to achieve these prospects	0.887	0.254
His/her achievements, professionalism, and character traits made him/her an attractive model to follow	0.832	0.332
He/she adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation	0.805	0.305
He/she was confident in him/herself, in his/her decisions, and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity	0.815	0.369
He/she adequately evaluated the importance of solving certain problems. To solve them he/she showed the appropriateness of persistence, honesty, and stubbornness	0.837	0.360
He/she was friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones	0.272	0.885
He/she was emotionally balanced and stress-resistant and could control his/her negative experience	0.310	0.847
He/she was professional – had good professional knowledge, skills, and experience in their implementation under combat conditions	0.844	0.294

As it could be seen, the structure of the characteristics of a junior officer within the servicemen who had the expressed PTSD symptoms had some similarity with the structure given by the first group, it also referred to the power of personality (ability to self-regulation) and definite communicative competence; however, the mentioned factors had a specific orientation in this group – to help his/her subordinates. They were quite selfishly aimed at the use of their direct commanders to help them, their structure "omitted" professional efficiency, achievement of professionally important goals, and so on.

DISCUSSIONS

Although the study conducted by Sheerin C.M., et al. [21] which used a more abstract research plan did not reveal any connections between PTSD and negative attitude towards the world in general, the study was conducted gave the possibility to suggest that having expressed PTSD symptoms the people around were perceived more negatively (at least those who were responsible for the lives of a serviceman – their direct commanders).

In the study by Bartholomew, et al. it was determined that servicemen who had signs of PTSD were less focused on their future [22]. In particular, the servicemen who had the signs of PTSD had disbelief in the achievement of a better future which extended to others, to those who had to direct the achievements of this better future to the whole team – to the direct commander.

It was interesting that real and perceived social support, as a factor that affected stress overcoming, had been already studied earlier, for example, it was discussed in works by Smith, Benight, and Cieslak [23]. An interesting phenomenon was identified in the study which was related to internal conflict attitude towards an individual the one who had to help, who was responsible for that morally and formally – that individual was a direct commander. He was perceived both as the one whom they expected to get help from and as the one whose real ability to help they had poorly trusted.

Caska-Wallace et al. worked with the negative changes in servicemen who were under PTSD influence [24]. They determined that the increase in problems in relationships between veterans and their partners referred to effective communication as well as communication related to the address of problems' solutions. The results of the conducted research suggested that not only a partner, but also a commander, and probably another significant person who surrounded a serviceman with PTSD symptoms, was taken as one who had to help, to solve the problems of psychologically traumatized servicemen, however, he/she could not solve those problems. That became a reason for the conflicting attitude of the serviceman towards definite surroundings.

Moreover, it had to be noted that if this study of an individual, who did not have signs of PTSD in the structure of the commander image, distinguished his/her strong personality and the ability to self-regulation as leading factors, the research by Rahman, et al. figured out that the perception of self-efficacy was a kind of traumatic stress prevention [25]. Conducting a certain analogy, it could be assumed (even if that required further studies) that the perception of the figure of the commander, as self-effective and capable of self-regulation was a kind of prevention of PTSD formation in his/her subordinates in the future. The procedure of study did not give any possibility to suggest whether the perception of the commander as an effective one was the prevention of PTSD symptoms in subordinates, or whether the absence of signs of PTSD reduced the negativity in the perception of the commander's figure, of his/her self-efficacy, of his/her "I", or of the experience became a reason for the posttraumatic growth and perception of oneself and of the one who ran the process of overcoming the life troubles, as those which had a significant individual power (Tedeschi and Calhoun) [26]. Also, the format of the study did not give the possibility to reveal whether the presence of PTSD symptoms caused the situation that servicemen were less interested in the commander who could involve them in dangerous swift-fleeting conditions, or whether servicemen who avoided danger lost their opportunities for productivity and growth following the study by Jamieson, et al. [27].

Unfortunately, the study did not give any possibility to identify cause-effect relationships, it did not give the possibility to determine whether PTSD symptoms had an influence on the image of the military leader, or whether the attitude towards the commander became one of the reasons for acquiring PTSD symptoms during the fight. Thus, the determination of those relationships would be the purpose of the feather studies.

CONCLUSIONS

Thus, the conducted study showed that the existing extreme experience of participating in hostilities led to the syncretic perception of the image of the junior commander – all qualities were perceived as extremely important.

In servicemen who did not have signs of PTSD (able to accept their combat experience as well as experience of interaction under combat conditions), the perception of the direct commander image had been influenced both by the peculiarities of the modern way of battle conduction (the actions made by small groups which were coordinated by the leader – that was where significant importance of the ability to adequately inform about the leaders' orders and to implement the role of a coordinator came from) and by the action of a protective mechanism of consciousness, which gave the possibility to maintain mental health under combat conditions, so-called focus on the process of performing their combat tasks.

For these servicemen, the awareness, kindness, and physical strength of their commander were less important. His/her ability to adequately fulfill his/her leadership function (pose combat tasks with adequate consideration of the combat situation and his/her subordinate forces) and the power of his/her spirit including the courage and love of the Motherland, which formed his/her reliability (gave the possibility to perform the combat tasks under extreme conditions and not to give those tasks up).

The servicemen with expressed PTSD symptoms were less satisfied with the role of a junior officer. They considered him/her less capable of meeting their expectations. They were less interested in commanders who could involve them in situations that required quick, brave action and complete responsibility for the entrusted part of the common affair, they required more the commander who would take care of them. Moreover, that commander would not blame the subordinates for their failures, but also, he/she would take on a part of their professional duties and responsibilities for their activity and inactivity.

In the servicemen with expressed PTSD symptoms the negativism of the attitude towards the role of the direct commander was caused by internal contradictions – on the one hand, they looked for the commander who could protect and help, on the other hand – they did not believe that he/she could help them, considering their experience to be unique, which was not appropriate for knowledge and experience of overcoming others.

In the structure of the direct commander image in both studied groups the most important factor was the power of personality (ability to self-regulation), however, in the servicemen with the expressed PTSD symptoms, these factors had specific orientation – to help their subordinates. They were quite selfishly aimed at using their direct commanders to help them.

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Authors' contribution

Conceptualization, YaM. and O.K; managed the project, I.P.; methodology, A.B.; research, O.Z.; writing—original draft preparation, K.M., M.K., Yu.R., and I.T. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

All procedures followed were following the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

Patient consent for publication

Informed consent was obtained from all subjects for inclusion in the study while maintaining their confidentiality. Written informed consent has been obtained from all subjects to publish this paper.

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REVIEW

The Role of Vitamin D in the Development and Progression of Osteoarthritis

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Abstract: *Low levels of vitamin D in patients with osteoarthritis (OA) rather adversely affect the structure and function of articular cartilage. Low levels of vitamin D are also associated with joint pain, limited physical activity, quality of life, as well as decreased muscle strength, primarily in the lower extremities with adverse OA progression. A high percentage of low vitamin D levels was found in patients with OA and joint arthroplasty as well as a less satisfactory postoperative follow-up in patients with low vitamin D levels. The administration of vitamin D supplementation in patients with knee osteoarthritis has been associated with improved articular cartilage architecture, and reduced joint pain, combined with improved functionality and quality of life in patients with OA.*

Keywords: *arthroplasty; vitamin D; osteoarthritis; vitamin D supplementation; vitamin D receptors*

INTRODUCTION

Osteoarthritis (OA) is defined as a heterogeneous group of conditions, leading to symptoms and signs associated with a disorder of joint cartilage, as well as with lesions of the subchondral bone and joint boundaries [1-3]. It is distinguished into primary OA when it cannot be attributed to a distinct pathological disease and secondary OA when it is related to other conditions such as injury, congenital bone disease, metabolic diseases, crystal deposition disease, or neuropathic arthropathy (Charcot's joint) [4-6]. In this review, we will only deal with the primary OA.

Regarding the role of vitamin D in OA development and progression, we will deal with the following important fields based on information from the PubMed database we searched for using the keywords osteoarthritis, vitamin D, vitamin D receptor, vitamin D supplement, joint replacement, until October 30, 2019:

1. Correlation of OA development with genetic polymorphisms of vitamin D receptor.
2. Effect of vitamin D on the structure and function of articular cartilage.
3. Correlation of low levels of vitamin D with the risk of OA development and progression.
4. Effect of vitamin D on OA clinical manifestations.
5. Correlation of vitamin D levels with muscle strength in patients with OA.
6. Correlation of vitamin D levels with the risk of arthroplasty and postoperative progress of OA patients' joints.
7. The role of vitamin D supplementation in patients with OA.

Before referring to the above, it should be noted that desired levels of vitamin D [as estimated by measuring the levels of 25-hydroxyvitamin D [25(OH)D] are >30 ng/mL. Levels <10 ng/mL mean insufficiency, while 10-30 ng/mL consist of a level lower than the considered optimal amount of vitamin D. In 2011 the Institute of Medicine (IOM) defined as insufficiency when the of 25(OH)D level was <20 ng/mL [7], while the Endocrine Society (ES) when the levels were at 20-29 ng/mL [8].

It should also be noted that vitamin D has a significant effect on the immune system function, as it inhibits the synthesis and action of prostaglandins, activates monocytes and macrophages, and has an inhibitory effect on the maturation and function of antigen-presenting progenitor cells and production of inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8), etc [9-13]. Vitamin D deficiency in individuals with a genetic predisposition may affect the immune system's response and tolerance process by affecting T lymphocytes and B lymphocyte function [12,13].

CORRELATION OF OA DEVELOPMENT WITH GENETIC POLYMORPHISMS OF VITAMIN D RECEPTOR

The possible correlation between gene polymorphisms that encode the synthesis of the vitamin D receptor (VDR) and the risk of developing OA has been studied. However, the conclusions of those studies are contradictory. In a meta-analysis, it was reported that the active metabolite of vitamin D, 1,25(OH)2D3 binds to its receptors in the articular cartilage and may lead to hypertrophy and impairment of chondrocyte function [14]. A study found an association between the risk of OA of the hand extremities with polymorphisms of the vitamin D receptor [15], while another study in postmenopausal women with OA of the knee, it was found a correlation between this risk and a TaqI polymorphism of vitamin D receptor [16]. On the other hand, in another meta-analysis of 10 studies, no association was found between TaqI, BsmI, and ApaI polymorphisms and the risk of developing OA in the knees, hips, hands, and lumbar spine [17]. In the meta-analysis of Z.-H. Zhu et al., a weak association of ApaI polymorphism with the risk of developing OA in Asian (but not in European) patients with OA were found [18]. No correlation between vitamin D receptor polymorphisms and the risk of developing OA in the knees was found in the meta-analysis of Rodriguez-Fontenla et al. in 9 Genome-wide studies [19] as well as in other studies [20,21]. Another interesting study carried out by Hansen AK et al. found that 25(OH)D3 and 1,25(OH)2D3 led to an increase of chondrocytes proliferation, in combination with a reduction of proteoglycans synthesis, and thus with a reduction of the intercellular substance [22].

EFFECT OF VITAMIN D ON ARTICULAR CARTILAGE STRUCTURE AND FUNCTION

In male Sprague-Dawley rats, Pascual-Garrido et al. used Micro-CT analysis to detect adverse effects of vitamin D deficiency on articular cartilage. Of particular importance is Jiang X et al.’s study in rats, in which it was found that vitamin D promotes function, the differentiation of the bone marrow-derived mesenchymal stem cells (BMSCs) in mature chondrocytes, enhanced by the action of transforming growth factor-β1 (TGF-β1) and their migration to the articular cartilage [24].

Low levels of vitamin D in OA patients have been shown to adversely affect the structure and function of articular cartilage. In a study in which patients with knee OA participated, low levels of vitamin D were found to be associated with loss of knee articular cartilage as assessed by the decreased joint space [odds ratio-OR=2.3 (Cl, 0.9-5.5)] and the increased size of the osteophytes [OR=3.1 (Cl, 1,3-7,5)] [25]. Ding et al. randomly selected patients (aged 51-79 years, 50% women) in whom the volume of articular cartilage, the presence of articular cartilage deficits in knees using X-rays and magnetic resonance imaging (MRI), as well as the 25(OH)D levels, were assessed at the beginning. 2.9 years later in 353 of these individuals, a correlation between low levels of 25(OH)D and articular cartilage loss was found [26].

In a study in 2014, 80 patients were classified according to 25(OH)D levels into three groups (<10, 10-20, and ≥20 ng/mL). It was found that vitamin D deficiency (<10 ng/mL) may significantly affect the articular cartilage thickness of the peripheral part of the femur (lateral femoral condyle of the right knee and the medial femoral condyle of the left knee) as found by ultrasound [27].

Moreover, in Zheng S et al. study of 340 patients with knee OA, the results showed a correlation between low levels of 25(OH)D with the decreased knee articular cartilage volume, as well as the presence of articular cartilage deficits, bone marrow damage and an increase of synovial membrane thickness (a finding compatible with inflammation) as assessed by MRI [28].

Lastly, in another study of 340 patients with symptomatic OA of the knee and vitamin D deficiency, 25(OH)D levels were measured at the start of the study as well as 3 and 24 months later. It was found that patients with stable normal levels of vitamin D compared to patients with low levels (25(OH)D >50 nmol/L vs ≤50 nmol/L) had a lower loss of joint cartilage volume, thinner synovial membrane and less articular fluid collection [28].

Low levels of vitamin D have also been linked to increased levels of cytokines involved in inflammation in patients with OA. A study by Askari A et al. in 131 patients with OA who compared 262 healthy controls found that lower vitamin D levels were associated with higher levels of interleukin 17A (IL-17A) and interleukin 23 (IL-23), cytokines that promote inflammation in patients with OA [29].

CORRELATION OF VITAMIN D LOW LEVELS WITH THE RISK OF OA DEVELOPMENT AND PROGRESSION

Several studies have focused on the possibility and to what extent low levels of vitamin D in OA patients are associated compared to individuals of the same sex and similar age in the general population and OA progression, with the results being controversial. In Bergink AP et al. meta-analysis, no significant correlation was found between vitamin D levels [25(OH)D] and the frequency as well as the evolution of knee, hip or hand OA. In 3 cross-sectional studies, however, there was found a correlation between the low levels of 25(OH)D and the stenosis of knee joint space (OR=1.52, 95% CI:1.15-2.01). The meta-analysis of the above-mentioned three studies did not show a significant correlation between the low levels of

25(OH)D and the evolution of knee OA (OR=1.37, 95% CI:0.97-1.92). However, when only patients with progressive knee OA development were assessed, an increased risk was found in patients with low levels of 25(OH)D (OR=2.40, 95% CI:1.22-4.72) [30]. In a study of 149 patients (133 women, mean age 63.6 years) with knee OA, the vitamin D deficiency rate [25(OH)D<10 ng/mL] was estimated at 90% [31]. Chaganti RK et al. conducted a study in 1104 elderly men who participated in the "Osteoporotic Fractures in Men Study", measuring 25(OH)D levels at the beginning of the study and performing a radiographic hip examination 4.6 years later. It was found that higher levels of 25(OH)D at the beginning of the study were associated with a lower risk for 25[OH]D decrease, while men with vitamin D deficiency compared to men with normal vitamin D levels had a significantly higher risk (OR=2.19, 95% CI: 1.21-3.97) of developing hip OA [32]. Zhang FF et al. in 418 patients with clinical and radiographic knee OA found a correlation between low 25(OH)D levels and the risk of developing OA radiographically as assessed by evaluating the joint space stenosis (<15 μg/L compared to ≥15μg/L) [33,34]. On the other hand, Lane NE et al. radiographically attended the hips of 237 individuals (with an interval of 8 years) and found no correlation between levels of 25(OH)D and serum 1.25(OH)2D and OA risk progression (development of osteophytes and/or reduction of the joint space) [35]. In addition, Bergink AP et al., in 1248 elderly people (728 women and 520 men) who participated in the prospective study (Rotterdam Study) assessed the development and progression of OA knee radiographically (average follow-up time 6.5 years), found no correlation between 25(OH)D levels (at the beginning of the study) and the risk of subsequent knee OA [36]. In a cross-sectional study of 99 patients (90 women and 9 men, aged 36-80 years) with clinical and radiographic knee OA, no correlation of low 25(OH)D levels with clinical and radiographic OA severity was found [37].

EFFECT OF VITAMIN D ON CLINICAL MANIFESTATIONS OF OA

Some studies have found a correlation between low serum vitamin D levels in patients with OA and joint pain as well as limited physical activity, and quality of life, while some other studies did not. In Zheng S et al.'s study, in 340 patients with OA knees, a correlation of low levels of 25(OH)D was found with severe limitation of the patient's functionality [28]. Levinger P et al. in a study of 24 patients (68.6 ± 6.2 years) with clinical symptoms of knee OA, found a correlation between low levels of 25(OH)D (deficiency ≤50 nmol/L) and knee pain and restriction of patients' functionality [38]. In another study of 100 patients with knee OA, it was found that the vitamin D deficiency group had more severe knee pain, more severe physical limitations, a more intense feeling of fatigue, and a worse quality of life [39]. On the other hand, Lee A et al. evaluated 3824 people aged >65 years (Korean National Health and Nutrition Examination Survey) and found that the levels of 25(OH) D were not related to knee pain [40]. Also in another study of 149 patients (133 women, mean age 63.6 years) with knee OA, no correlation was found between low levels of 25(OH)D and knee pain [31].

CORRELATION OF VITAMIN D LEVELS WITH MUSCLE STRENGTH IN PATIENTS WITH OA

Of particular clinical importance is the association between vitamin D levels and muscle strength (primarily of the lower extremities muscles such as quadriceps that support the weight-bearing joints) of patients with OA (primarily knee OA). A study found that normal levels of vitamin K and vitamin D were associated with better lower extremity function [41]. Javadian Y et al. found a positive correlation between 25(OH)D levels and quadriceps muscle strength in 92 patients with knee OA knee (≥40 years old) (r=0.304, r=9.24%, p=0.005). For every increase of 25(OH)D levels by 1 ng/mL, the strength of the quadriceps increased by 14.2%±3.5% (p=0.014) [42]. In a 24 patients study (68.6 ± 6.2 years) with OA knee clinical signs, a correlation between low 25(OH)D levels (deficiency in ≤50 nmol/L) and the reduction of quadriceps muscle strength was found [39].

However, the effect of other factors, such as body mass index, should be also assessed. In a cross-sectional study of 319 patients with knee OA, a significant correlation between serum levels 25(OH)D and strength of the upper tibial muscles were found, which, however, ceased to be observed when the effect of the body mass index was estimated [43].

CORRELATION BETWEEN VITAMIN D LEVELS AND THE POSSIBILITY OF AN UNDERGOING ARTHROPLASTY IN PATIENTS WITH OA

Numerous researchers have investigated whether vitamin D levels in patients with OA are associated with the possibility of undergoing arthroplasty (mainly hip and/or knee arthroplasty) as well as their postoperative follow-up. A high percentage of low vitamin D levels was found as well as less satisfactory follow-up in patients with low levels. In a recent literature review (12 studies, 2593 patients) the percentage of patients with OA who underwent hip or knee arthroplasty with hypovitaminosis D was estimated at 47% (34.02% -59.9%) [44]. In another study, in 164 patients with hip or knee OA who were to undergo arthroplasty, vitamin D deficiency was found in 81.7%, especially in men [45]. Elevated vitamin D levels are likely to reduce the risk of OA patients undergoing total hip arthroplasty. Hussain SM et al. studied 9135 patients ≥40 years old, who participated in the Australian Diabetes, Obesity and Lifestyle Study, in which the levels of 25(OH)D during 1999-

2000, as well as the percentage of hip arthroplasty in 2002-2011, was estimated (Australian Orthopedic Association National Joint Replacement Registry). 201 patients with OA (90 men and 111 women) underwent hip arthroplasty. In men for any one-standard-deviation increase in levels of 25(OH)D, the probability of undergoing the operation was reduced by 25% (HR=1.25 and 95% CI 1.02-1.56) but not found in women (HR=1.10 and 95% CI 0.87-1.39) [46].

Regarding the effect of low vitamin D levels in patients with OA who have undergone arthroplasty, it appears that they are associated with less satisfactory postoperative results. In Zhang H et al.'s review, it was found that patients with OA who underwent hip or knee arthroplasty, and hypovitaminosis D adversely affected the postoperative results of these patients (Harris Hip Score score was higher in these patients compared to patients with normal vitamin D levels) [44]. In a study conducted in 2010, 62 patients who underwent total hip arthroplasty were compared with 62 patients of the same sex and similar age from the control group, it was found that the patients with vitamin D deficiency had less satisfactory postoperative follow-up results (p=0,038) as well as they had the lowest preoperative Harris Hip Scores (Mann-Whitney test, p=0.018) [47]. Maniar RN et al. also studied 120 patients with OA who underwent total knee arthroplasty [53.3% had preoperative levels 25(OH)D<30 ng/mL]and they assessed their functional ability before and 3 months after surgery. Before surgery, in patients with vitamin D deficiency, their functional ability was limited [Western Ontario and McMaster Osteoarthritis Index (WOMAC), p=0.040]. After surgery, in patients who received vitamin D supplementation, their functional ability was restored, which was similar to the functional ability of patients with normal vitamin D levels [48]. On the other hand, in the study of Visser E et al. in 87 patients with OA who underwent total hip arthroplasty, no correlation was found between levels of vitamin D and restoration of hip function. However, an increase in levels of 25(OH)D was observed six months after surgery, which was associated with an improved hip function (Pearson's r =-0.471, p=0.018) [49].

Regarding the dosage of postoperative vitamin D administration in patients with OA who underwent arthroplasty, there are interesting results from a randomized, double-blind 24-month study of Bischoff-Ferrari et al. in which participated 273 patients underwent knee arthroplasty. 137 patients received 2000 IU of vitamin D while 136 patients received 800 IU per os daily. Even though the administration of 2000 IU was accompanied by a significant increase in 25(OH)D levels (45.6 ng/mL vs 37.1 ng/mL of the 800 IU dose after 24 months, p <0.0001). No difference regarding the restoration of operated knee function, nor on the radiographic progression of the other knee OA, between the group of patients who were administered 2000 IU and 800 IU respectively (30,5% vs 31,3%, p=0.88) [50].

THE ROLE OF VITAMIN D SUPPLEMENTATION IN PATIENTS WITH OA

Animal studies have shown that vitamin D has a protective effect on the articular cartilage architecture [51,52]. In an animal study, micro swings that were on a diet rich in cholesterol, it was found that vitamin D had a protective effect on the architecture of articular cartilage (reduction of inflammation and fat infiltration) [51]. In Li S et al.'s animal (ovariectomized rats) study, was also found that vitamin D deficiency was associated with deterioration of the knee articular cartilage, while the administration of 1,25(OH)2D3 with a protective effect on articular cartilage [52].

Vitamin D supplementation in patients with knee OA has been associated with a reduction in synovial membrane thickness and joint effusion associated with the OA development, as well as an improvement in knee articular cartilage architecture [53,54]. In a study of 413 women with symptomatic OA knee and low 25(OH)D, 209 women were supplemented with vitamin D (50,000 IU per os monthly) while 204 women were administered placebo for 24 months. This study investigates the effect of this administration on the articular membrane thickness and joint effusion using MRI. The rate of increase of the above-mentioned variables was lower in the group supplemented with vitamin D compared to the placebo group (risk ratio (RR)=0.87, 95% CI: 0.77-0.97) [53]. Veronese N et al. in 783 patients with knee OA (59.8% women, mean age 62.3 years), vitamin D supplementation was evaluated for its effect on the volume and thickness of the medial and lateral femoral condyle cartilage with MRI. Using linear regression analysis in which the effect of 10 potential confusing factors was assessed, it was found that vitamin D supplementation led to an increase in the volume and thickness of the medial and lateral femoral condyle cartilage, suggesting an improvement in its architecture [54].

Several studies have investigated the effect of vitamin D supplementation on joint pain, quality of life, physical function, joint stiffness, muscle strength (such as quadriceps femoris), joint space range, articular cartilage volume, and deficits as well as bone marrow lesions, etc, with conflicting results [55]. Moreover, in a systematic review and meta-analysis conducted in 2017, it was found to be small as a moderate result of vitamin D supplementation in knee pain [standardized mean difference (SMD)=-0.32, 95% CI: -0.63 to -0.02]. However, no effect was found on tibial articular cartilage volume/thickness (SMD=0.12, 95% CI: -0.05 to 0.29) or joint space range (SMD=0.07, 95% CI: -0,08 to 0.23). In other words, vitamin D supplementation had a rather favorable effect on knee pain but not on OA severity [56].

In all randomized controlled trials, 25(OH)D levels were found to increase in patients supplemented with vitamin D compared to patients that took the placebo. However, no reduction in pain (except for one study) or knee stiffness (evaluation with WOMAC scale) was found. On the other hand, in 2 of 3 studies that assessed knee pain using a visual analog scale (VAS), vitamin D supplementation led to an improvement in knee pain [57]. In Heidari B et al.'s study, 67 patients (aged ≥30 years with knee OA) received a supplement of 50,000 IU of cholecalciferol per week orally for at least two months. It was found that the cholecalciferol supplementation led to an increase in the normal level of 25(OH)D ($p=0.007$) as well as to a significant increase in quadriceps strength ($p=0.002$) and a significant decrease in knee pain ($p=0.001$) to all patients (except for one patient) [58].

In a multicenter randomized double-blind study, 340 patients with symptomatic knee OA were distinguished into one group of patients who received vitamin D (50,000 IU per month orally) and another group that received a placebo two years ago. The purpose of the above-mentioned study was the evaluation of this administration's effect on tibial articular cartilage volume, the presence of defects in tibial articular cartilage, bone marrow lesions, and knee pain. There was a significant increase in 25(OH)D levels in the supplemented group compared to the placebo group (40.6 nmol/L vs 6.7 nmol/L, $p<0.001$). However, no effect was found on tibial joint cartilage volume, the presence of tibial joint cartilage deficits, or bone marrow lesions as assessed by MRI [59].

Additionally, in a study of 175 patients with primary knee OA and low levels of 25(OH)D (<30 ng/mL), they were supplemented with 40,000 IU of vitamin D (ergocalciferol) per os once a week for six months. It was found significant increase in 25(OH)D levels ($p<0.001$), improved quality of life ($p=0.005$) and patients' functionality ($p<0.05$), as well as reduced knee pain ($p=0.002$) [60]. On the other hand, in a three-year double-blind, randomized study of 474 patients aged>50 years with radiological signs of knee osteoarthritis, it was found that the group of patients supplemented with vitamin D led to an increase at 25(OH)D levels compared to the placebo group, without affecting the narrowing of the joint space, pain, function, or stiffness of the knee joint [61].

The administration of high-dose vitamin D supplements has a rather beneficial effect on reducing knee pain. A meta-analysis of 4 randomized group studies with 1,136 patients, evaluated whether and to what extent the administration of a vitamin D supplement affects pain, function, stiffness, and tibial joint cartilage volume of patients with knee OA. It was found that this administration was associated with pain reduction and improved function but did not improve knee stiffness or tibial joint cartilage volume. Particularly favorable was the effect on pain and knee function in patients receiving a dose of natural vitamin D higher than 2000 IU [62].

It was also studied whether vitamin D supplementation and maintaining adequate levels affected inflammatory markers and metabolic biomarkers associated with OA pathogenesis. Zheng S et al. studied 413 patients with symptomatic knee OA and vitamin D deficiency, who were randomly assigned to one group receiving 1.25 mg of vitamin D3 and to another group receiving a placebo, once a month for 24 months. The researchers concluded that vitamin D supplementation as well as maintaining adequate levels did not affect inflammatory markers or metabolic biomarkers [63].

CONCLUSIONS

In conclusion, low levels of vitamin D in patients with OA rather adversely affect the structure and function of articular cartilage. They are associated with joint pain, limited physical activity, quality of life, etc, as well as decreased muscle strength, primarily of the lower extremities with adverse OA progression. A high percentage of low vitamin D levels was found in patients with OA and joint arthroplasty as well as less satisfactory postoperative progress in patients with these low levels. The administration of vitamin D supplementation in patients with knee OA has been associated with improved articular cartilage architecture and reduced joint pain in combination with improved function and quality of life.

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The author declares no conflict of interest.

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REVIEW

Biotribology of the Total Hip Replacement – Review of the Current Bearing Surfaces

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Abstract: *Total hip replacement surgery (THR) is one of the most common surgeries performed in modern orthopedics, and in most cases offers a significant improvement in the quality of the patient's life. However, after some time, wear and osteolysis do appear, which leads to the need for revision surgery. With the indication being widened to a more young and active population, the need for extending the lifespan of the prosthesis is mandatory. Although several bearing surfaces have been studied over the years, and a lot of couplings are in use, the choice of the right combination of materials is still controversial. Biotribology tries to answer some of the current questions regarding the best bearing surface to use, being the branch of science and technology that studies friction, wear, and lubrication of different materials in the human body. In the present day, a large number of materials are in use for total hip replacement, but choosing the right combination of materials is still controversial, and is being done on one hand by the surgeon's experience and on the other hand by the age and activity of the patient. The objective of this review is to try to present the main advantages and disadvantages of different materials and try to answer the most difficult question of them all: What is the right material to use for THR-bearing surfaces?*

Keywords: *total hip replacement, tribology, biotribology, arthroplasty*

INTRODUCTION

More the 350,000 total hip replacements are done each year in the United States [1], with a constantly increasing rate. The survival rate of these implants is, according to the British National Joint Registry, 93.2% at 13 years, and 80% up to 20 years [2]. Although these rates seem to be pretty good, about 13% of these hip replacements are revision arthroplasties [3]. This high rate can be attributed to the younger and more active patients who were treated with this procedure.

The most important aspect is still worn, which limits the lifespan of this prosthesis to 10 – 15 years. With a lot of materials and couplings to choose from, such as hard-on-soft bearings like metal-on-polyethylene (MoP) and ceramic-on-polyethylene (CoP), and hard-on-hard bearings such as ceramic-on-ceramic (CoC), metal-on-metal (MoM) and ceramic-on-metal (CoM), it can be confusing for both the patient and the physician, all of these materials showing specific wear mechanisms and characteristics that will be discussed in the present paper. Wear patterns like burnishing, abrasion, pitting, and scratches were observed for ultra-high molecular weight polyethylene (UHMWPE) and cross-link polyethylene (XLPE) [4], stripe wear was observed for ceramic components [5], changes in surface characteristics like roughening and roundness for metal-on-polyethylene couplings [6], gouging, scratching and polishing for metal-on-metal couplings [7].

Metal-on-metal couplings

With the first implantation in 1938 by Wiles, the MoM coupling was the first bearing used for THR. In conventional metal-on-metal hip replacements, the prosthesis is made up of a large diameter metallic femoral head on a standard femoral stem that articulates with a metal acetabular cup or liner, CoCrMo alloy was used widely for these bearings (Figure 1).

The advantages of this type of coupling are that they could be engineered to be extremely smooth and hard, properties that can reduce the wear rate, and the wear rate of MoM is estimated to be 60 times less than conventional MoP implants [8]. Other advantages include increased resistance to dislocation associated with large-diameter heads and reduced osteolysis due to the absence of polyethylene particles [8].

Figure 1: Metal on-metal coupling [10]

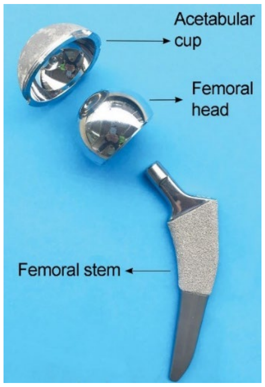

On the other hand, MoM materials come with a series of disadvantages. The metal alloys used in orthopedic implants degrade due to wear and, unlike polyethylene, also corrosion. This can lead to soft tissue inflammatory reactions to metal debris, known as adverse reactions to metal debris. The rate of wear depends largely on the contact force between the two surfaces and the sliding distance, and thus increases with physical activity, weight gain, larger implants, and the roughness of the implant surface [9].

The **inflammatory pseudotumors** (Figure 2) represent the existence of an aseptic mass in the periprosthetic tissue that can be solid or cystic and associated with local inflammation [11]. It is believed that it develops as a result of an adverse reaction to the metal ions released from the wear of metal articular bearing surfaces [12]. Implanting the acetabular cup at abnormal inclination angles increases the edge loading which leads to increased prosthetic wear [13]. The incidence of pseudotumors is up to 32% in some studies [14]. In a study conducted in 2009 on 1224 patients, it was shown that female patients and those under 40 years of age had an increased risk of revision due to pseudotumors [15].

Figure 2: The inflammatory pseudotumors [16]

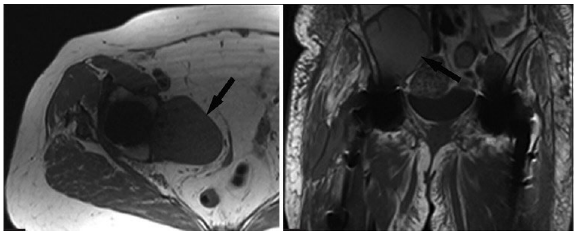

Aseptic lymphocytic vasculitis-associated lesion (ALVAL), consists of cellular changes that occur as a response to cobalt and chromium ions. The mechanism is believed to be a T lymphocyte-mediated type 4 hypersensitivity reaction, with tissue damage occurring as a result of cytotoxic T cells and activated monocytes/macrophages].

Metallosis, represents aseptic fibrosis, local necrosis, or aseptic loosening of a device because of metallic corrosion and release of wear debris [18].

Trunnionosis (Figure 3), the metal wear at the head-neck junction of a total hip implant is believed to be an important contributor to the release of metal particles [19]. It is present in the modular design prosthesis, both in MoM and MoP, because in both cases the head-neck junction is made up of metal-on-metal surfaces. This is the main reason why trunnions cannot be blamed to be the only cause of the increased rate of adverse reactions in MoM hip replacements.

Figure 3: Trunnionosis [20]

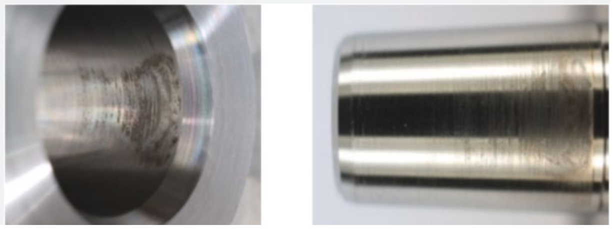

Revision rates for MoM at 10 years for cemented and uncemented hip replacements are 19.68% and 21.92% by comparison with MoP, which was 3.13% and 3.98% [21]. There seems to be a direct connection between the size of the femoral head that was used and wear, in THR that used a head size above 40mm the revision rate at 10 years was 25.5%, by comparison to those that used a femoral head diameter below 28mm with a revision rate at 10 years of just 5.7%.

Serum and urine ion levels produced by MoM bearings are similar to the ones produced by the modular junctions of femoral components. The toxicologic importance of these trace metal elevations has not been established. Because the wear of a THR with an MoM bearing cannot be evaluated radiographically, metal ion concentrations in serum and urine can be indicators of wear [22].

Considering the renal excretion of Co-Cr ions, a potential for increased risk of bladder and other cancers in patients with metal-on-metal couplings was formulated. Two large studies were conducted in England and Wales and Finland that examined nearly 330.000 patients [23,24], but no connection between MoM bearings and the high risk of developing a type of cancer was discovered. The follow-up protocol within those studies was seven and four years. So, we think that studies with a longer follow-up period are needed to completely declare that MoM implants do not increase the risk of cancer development.

Due to all of these considerations, in the present day, the MoM THR is almost entirely abandoned by surgeons and withdrawn from the market by manufacturers [25].

Polyethylene

Conventional and cross-linked polyethylene liners are the most common choice in THR. First used by Charnley in 1962 [26], this polymer is a high molecular weight polyethylene with extremely long chains. Until the mid-2000s, the MoP bearings (Figure 4), with head diameters between 22 mm and 32 mm we're considered the standard. The linear wear for PE is between 100 to 300 µm/year [27], which corresponds to volumetric wear of 20 – 150 mm^3/year for 28 mm heads [28]. It was shown that a correlation exists between volumetric wear and the risk for osteolysis, every increase in the amount of volumetric wear of 40 mm^3/year triples the long-term risk of osteolysis [29].

Figure 4: Metal on polyethylene coupling [10]

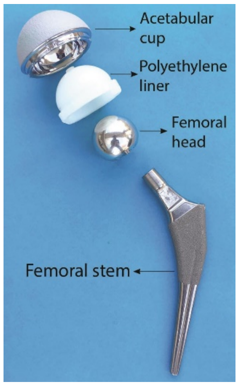

When using polyethylene liners, not only the material is important, but also the sterilization technique, because this too can influence the mechanical properties and biological effects. The most common sterilization method used was gamma irradiation. Some studies [30] show that this sterilization method increases cross-linking between polyethylene molecules offering a more wear-resistant material. But, when this is done in the presence of oxygen, free radicals are released that can oxidate and make the liner more brittle with reduced resistance and increased wear. So, thermal sterilization with gas plasma was shown to improve wear and oxidation resistance [30, 31], and oxygen-free gamma irradiation showed less head penetration and less osteolysis [32].

Because of the wear debris of the conventional polyethylene, cross-linked polyethylene was developed (Figure 5). It is obtained by irradiation of conventional polyethylene with at least 50 kGy of gamma beam radiation, which induces the cross-linking between PE molecules, which increases wear resistance [33]. The rate of revision at 16 years for XLPE was 6.2% vs. conventional PE which was 11.7% [34]. The process of cross-linking can however decrease the mechanical properties so that the liner can become at risk for fatigue fracture because the irradiation process releases free oxygen radicals [35]. In addition, steep positioning of the acetabular cup leads to stress concentration or impingement and it is considered also a risk factor [36]. This problem was solved by introducing vitamin E into the liners, which is an antioxidant, and the mechanical properties are saved. Also, the liners can be now thinner and the femoral head bigger, which can improve joint stability.

Figure 5: Cross-link polyethylene–electron microscopy [37]

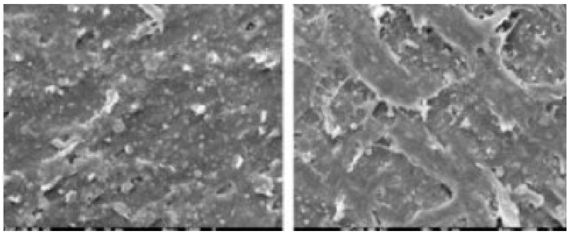

When evaluating the long-term resistance of PE, conventional or cross-linked, we must take into consideration also the femoral head material and diameter. We know that the larger the femoral head diameter the bigger the wear, especially when coupled with conventional PE [38]. It seems that this does not apply to XLPE, where the wear rate is not higher for bigger head diameters [38].

In the matter of ceramic vs. metallic femoral heads, in early studies, the average wear rates with the metal femoral head (stainless steel and cobalt chrome) invariably were higher than with ceramic femoral heads [39-41]. In a more recent study based on a systematic analysis of registry data, an overall equal long-term performance was found between metal and ceramic heads, although a lower revision rate was discovered with CoXLPE than MoXLPE [38]. The advantages of ceramic heads are higher hardness with less risk of damage during implantation, the surface finishing with only negative peaks due to grains detachment rather than deformation, and higher wettability which means better lubrication of ceramic, less friction and scratching and less linear wear [27]. Last, but not least, alumina is biologically inert and has the best biocompatibility.

Ceramic on ceramic

Alumina and zirconia ceramic is used in THR surgery (Figure 6). Alumina has a very low friction coefficient, is biocompatible, and its material properties are not affected by aging it was also shown that it reduces volumetric wear [33]. With the risk of fracture being the most severe concern, alumina (82%) was mixed with zirconia (17%), chromium oxide (0.5%), and strontium crystals, and a higher resistance to crack and enhanced hardness were obtained. With this breakthrough, the fracture rates of the femoral head were reduced from 0.021% to only 0.003% [42]. However, the risk of ceramic head fracture is still lower than the risk of ceramic liner fracture (0.003% vs. 0.03%) [43]. The survival rate at 10 years of these implants in excellent, varying from 98% to 99.3% [44], 92.8% at 15 years [34], and 84.4% at 21 years [45].

Figure 6: Ceramic on ceramic coupling [46]

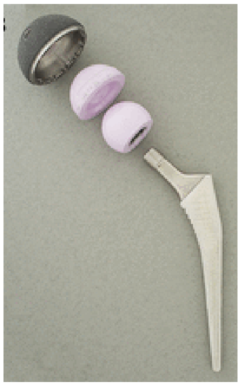

The advantages of CoC bearings are a very low friction coefficient and very low wear rates, due to high wettability and hardness. If however, the ceramic implant does suffer wear, the particles released are biologically inert and produce a less biological reaction. Another great advantage is that the wear is not dependent on the diameter of the femoral head, a large femoral head will provide a more stable artificial joint with a low risk of dislocation. A recent study showed that 36mm ceramic femoral heads had a lower revision rate than smaller 28mm ceramic femoral heads [47]

Like any other bearing surface, the CoC coupling also has a series of drawbacks. Although the risk of fracture was lowered as presented, the risk remains and is a catastrophic complication that requires revision surgery. Factors that can increase the liner fracture risk are misalignment during insertion of the liner, metal back damage or acetabular component malposition that can lead to edge loading, and the most important factor, excessive anteversion.

Another disadvantage is the noise that this coupling can produce. Clicking, grinding, clunking, and squeaking has been described. The incidence varies from 0.7% to 20.9% [48], this adverse event can even be a cause of revision, with a rate of 0.2% [49]. Risk factors for squeaking are age, obesity, activity level, soft tissue balancing, and positioning of the acetabular cup.

It is recommended that in cases with severe posttraumatic acetabular deformities or severe developmental dysplasia of the hip, the CoC should be avoided, because of the difficulty of correct positioning of the acetabular component.

Another drawback of the CoC bearing is the cost. It is the most expensive choice of coupling, but also the most advanced and resistant to wear when used correctly.

DISCUSSION

After the literature data has been analyzed, we concluded that the preferred choice when it comes to total hip arthroplasty bearings is a hard-on-soft coupling like the metal (cobalt-chrome) on polyethylene or ceramic on polyethylene. MoPs are still the most widely used coupling covering about 60% of all interventions, but the use of CoP is increasing. But which of the two is better?

If we consider the studies of wear screening using water-based lubrication and linear tracking, there is a 20-to-80-fold advantage for ceramic-on-polyethylene when compared with metal-on-polyethylene [50]; however newer wear studies with high protein concentrations have shown similar wear with alumina or with met-al-on-polyethylene [51]. Because of the biological advantages of alumina which is biologically inert and with the amount of literature that supports the superiority of the ceramic femoral head, we too recommend the use of ceramic-on-polyethylene bearings over the metal-on-polyethylene ones.

Considering the type of polyethylene that should be used, we feel that is enough literature that proves that conventional polyethylene has a much higher wear rate than cross-link polyethylene. More and more manufacturers are offering just the cross-link version now, the only advantage of the standard polyethylene is the lower cost of production. However, we feel that because of the high wear rate that requires early revision, there is practically no cost advantage, and therefore we cannot recommend using conventional polyethylene anymore.

How about hard-on-hard surface bearings? We know that the manufacturers do not provide the MoM bearing anymore, due to the pseudotumors caused by toxic metallic ions, so the only valid option for this kind of bearing is the CoC.

Although the wear is insignificant with this coupling and is not associated with femoral head diameter, there is still concern about motion sounds like squeaking, which has a revision rate that varies between 1 and 21%. Another drawback is the risk of liner fracture; although is a quite low risk, it has a catastrophic impact and requires urgent revision surgery. So, there is still room for improvement.

CONCLUSIONS

Deciding what coupling to choose is mostly dependent on the surgeon's preference and experience. Considering the literature mentioned in the present paper we recommend the use of CoC bearing if the cost is not an issue, in young and high-activity demanding patients, with a large diameter femoral head, up to 36 mm, as wear is not related to large head diameter femoral heads and can improve joint stability. Care must be taken in cases where the correct positioning of the acetabular component may be an issue, as the malposition of the acetabular cup can lead to squeaking and increase the risk of fracture of the ceramic liner.

CoXLPE is a good option for CoC, with a lower cost and offers comparable results. It can be successfully used in active patients over 60 years of age, care must be taken in choosing the right femoral head, larger diameter head being associated with a higher wear risk. We cannot find any reason for using conventional PE, as it proved to be less reliable and more susceptible to wear, with a higher risk of releasing debris.

The choice of MoXLPE remains a viable option in a situation in which cost is an issue. Other than cost-related issues, the ceramic bearings are a more reliable option over the metallic on PE.

Even if the best bearing surface is available, we should not forget that coupling is not everything, and careful patient selection is still mandatory. More than that other factors influence the overall behavior of the THR, like age, level of activity, anatomic deformities of the acetabulum, positioning of the components, bone quality, soft tissue balancing, and patient compliance. So, to have a successful THR all of these factors must be carefully quantified, and the surgeon must use the correct surgical technique.

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ORIGINAL ARTICLE

Aesthetic Principles in Nasal Reconstruction after Squamous Cell Carcinoma Resection

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Abstract: *A successful nose reconstruction is represented by a normal look and nasal breathing preservation. The choice of method is based on the site, size, depth of the defect, donor availability, and surgeon experience. There are multiple methods of restoring nasal cover, from primary repair to healing by secondary intention, from primary suture, skin graft to locoregional flaps, but all of them should be integrated into a personalized approach, keeping the nasal functions, a good aesthetic outcome and without any tumor infiltration. This paper consisting of a retrospective study of 9 patients diagnosticated with squamous cell carcinoma revealed the importance of skin properties and skin changes during aging. Despite all aesthetic principles, skin is the most important resource in facial reconstruction.*

Keywords: *nose reconstruction, aesthetic, squamous cell carcinoma*

INTRODUCTION

Cutaneous squamous cell carcinoma is one of the most common skin cancer with an increasing incidence [1]. Most frequently on sun-exposed sites, like the face, it could be a challenge for a surgeon [2]. One, because of its aggressiveness and the possible requirement of large excisions, and second, because of the psychological impact on the patient. The nose is one of the most visible anatomical sites so, an aesthetic result is even more required. The nasal restoration should be based on keeping the contouring, color, and texture, minimalizing the scars, and preserving the breathing function [3].

MATERIALS AND METHODS

A retrospective study performed in the Plastic Surgery Department of Emergency University Hospital, Bucharest, Romania evaluated 9 patients diagnosticated with cutaneous squamous cell carcinoma at the nose level through the histological exam, two of them with local relapse. The age average was 63 years old, with a minim of 44 years old and a maxim of 90 years old. It was identified as a male prevalence.

In all cases, the tumor resection was performed with free macroscopically oncological edges. Depending on the tumor size, site, and patient age, multiple reconstruction techniques were required. In 4 cases primary suture was practiced, in 3 cases local flaps were required, and in 2 cases skin grafts.

All the patients signed the Informed Consent before surgery and the study was approved by Ethics Committee.

DISCUSSION

There are nine aesthetic subunits of the nose, dorsum, paired sidewalls, nasal tip, paired alae, columella, and paired soft tissue facets (Figure 1). The concept of aesthetic units in nasal reconstruction was first described by Millard to improve surgical results. Then, Burget and Menick introduced the principles of nose aesthetic subunits which improved the aesthetic results in nose reconstruction [4].

The skin quality is different in every aesthetic subunit, being thicker at the nasal tip, nasal alae, and cranial dorsum, and upper nasal sidewall and mid-lower dorsum being the thinnest. In all facial reconstructions, the skin patient is an important factor. During aging, the skin properties are changing and the skin becomes looser.

Nose reconstruction is a complex process in which the first step should be defect identification. A classification of nasal defects is based on size. There are small defects, with a diameter of less than 1.5 cm, and large defects, with a diameter over 1.5 cm, which require a skin graft or a local flap [5].

Figure 1: Nose aesthetic subunits

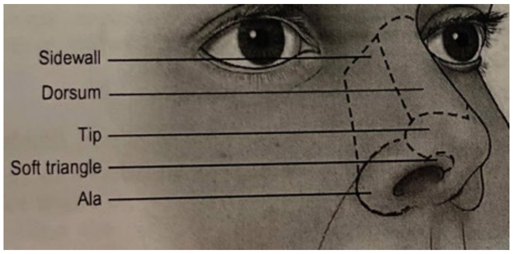

Depending on the depth of injury, there are superficial defects where the skin is missing and also, a small underlying subcutaneous fat and nasalis muscle could be missing. Deep defects are represented by the absence of an underlying supportive framework or lining. The cartilage could be affected, so a composite skin graft could be required, cartilage grafts, or vascularized regional flap for cover [6].

A more complex reconstruction is required by composite defects which are represented by nose defects extended to the cheek or upper lip (Figure 2). The challenge consists of the fact that they are different aesthetic units. The reconstruction of composite defects should be based on keeping the quality, outline, contour, projection, and function of each one, not only to “fill the hole”.

Figure 2: Nasogenian tumor that left a composite defect including sidewall, ala, dorsum, and cheek, which was covered by a local cheek flap

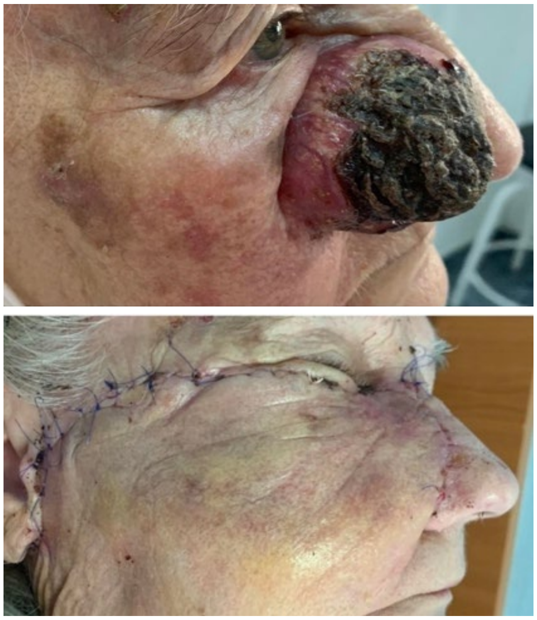

Choosing the reconstruction method should be based on the shape, size, location, and depth of the defect and the aesthetic unit involved [7]. The bilobed flap is one of the most common local flaps used in nose reconstruction, especially for tip and ala defects, when the diameter of the defect doesn`t count more than 1.5cm [8]. The Bipedicle mucosal flap could be useful in small alar reconstruction to keep the lining of the nose [9].

Another common flap in nose reconstruction is the septal hinge flap which is used for tip, ala, and sidewall defects [10]. The technique is based on the anterior septal artery. When the nasal tip and columella are missing, and also a part of the septum, bilateral septal flaps could restore the lining of the ala and nasal vestibule [11,12,13].

In cases of full-thickness defects, especially for lateral defects, the inferior turbinate flap could be a good option, alone or together with other techniques [14].

Forehad flap is useful for any significant nasal defect over 2.5 cm. It can be also used when the septal hinge flap and inferior turbinate flap failed. The technique is based on the supratrochlear vessels that pierce the orbital bone at about 2 cm lateral to the midline [15].

Melolabial flap in an interpolated flap is used for defects less than 4 cm, especially for cutaneous nasal ala defects with or without modest extension into the tip and sidewall [16].

The aesthetic outcomes between local flaps and skin grafts are depending on patients' selection. Sapathavee, Munaretto, and Toriumi proved that a skin graft provides an aesthetic result in patients with Fitzpatrick types 1 and 2 skin, also with thin and less sebaceous skin. The preauricular skin offers a good match in nose reconstructions. Some authors are using “skin fat grafts” to fill small deep defects of the nose [17].

Keeping the structure of the nose is an important part of the reconstruction. Cartilage grafts are useful tools in maintaining nose support. They prevent also overlaying soft tissue contraction, minimize pincushion deformity and reduce nasal obstruction. The donor sites could be costal cartilage or calvarial bone for restoring the contour and support of total or subtotal nasal defects, and conchal cartilage for alar reconstruction due to its curvature shape.

To keep the internal lining, a composite graft from the conchal site could be used. Also, a composite graft from the helix root could be used in nasal ala restoration [18].

In our case, we used skin grafts in 2 cases of female patients, about 63 and 69 years old. The tumors had the same localization, at the dorsum level, and has a diameter over 3 cm, without bone invasion.

In 2 cases of the cutaneous tumor with approximately 1.5 diameters, at the sidewall and ala levels, local flaps were performed. The nasolabial transposition flap was the reconstruction method for ala defect, while for sidewall defect a dorsal nasal flap was performed. Rieger described the dorsal nasal flap as a laterally-based rotation flap with a wide pedicle. The technique consists in designing a "V" in an upside-down fashion over the glabella and then a curvilinear line on the nasal–cheek junction till it reaches the defect site. Marchac and Toth updated the Rieger version by narrowing the pedicle in an axial flap based on the vessel arising from the inner canthus. The advantages consist in increasing mobility, decreasing wound closure tension, and a more precise adjustment [19].

One case of a cutaneous tumor involving multiple nasal subunits, with a diameter over 4 cm, without bone invasion, required multiple techniques of reconstruction, frontal flap for dorsum defect, and primary suture for secondary defects.

Figure 3: Intraoperatively picture with dorsum defect size after squamous carcinoma resection for which the skin allowed a primary suture.

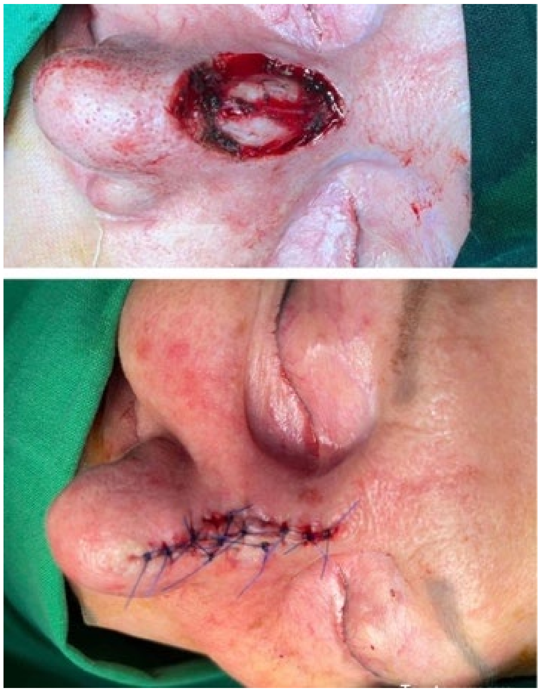

In 4 cases, the primary suture was enough for closing the defect. Three of them had a diameter less than 1.5 cm, one at ala level and 2 at dorsum level, with ages between 59 and 80 years old. There was another case of 77 years old female, with a diameter tumor of approximately 2.5 cm at the dorsum level, for which the primary suture was enough (Figure 3). Usually, a defect of 2.5 cm isn`t suitable for primary suture and a local flap is mandatory. For defects of 1.5 cm – 2.5 cm, a dorsal nasal flap, a bilobed flap, or a glabellar flap could be used [20].

So, depending on the aesthetic unit involved and defect size, nasal reconstruction methods differ. Choosing the reconstruction method should be based on restoring the aesthetic unit, not on filling the defects. Keeping a natural look, the borderline, the skin quality, and preserving the breathing function are variables that we should take into account in nose reconstruction, but free tumor edge is the most important medical approach. Dorsum and sidewalls have common skin characteristics, so the reconstruction methods are similar. Defects less than 1.5 cm could be closed primarily, with a glabellar flap or a transposition one. Defects with a diameter of 1.5-2.5 cm require a local flap, a glabellar flap, a bilobed flap, or a dorsal nasal flap. The last one can be also used for defects more than 2.5 cm [21].

In case of nasal tip defects, a bilobed flap can be used for defects less than 1.5 cm, a dorsal nasal flap under 2.5 cm, and forehead flaps for defects of or bigger than 2.5 cm (Figure 4).

Figure 4: Skin tumor involving ala unit which required a forehead flap

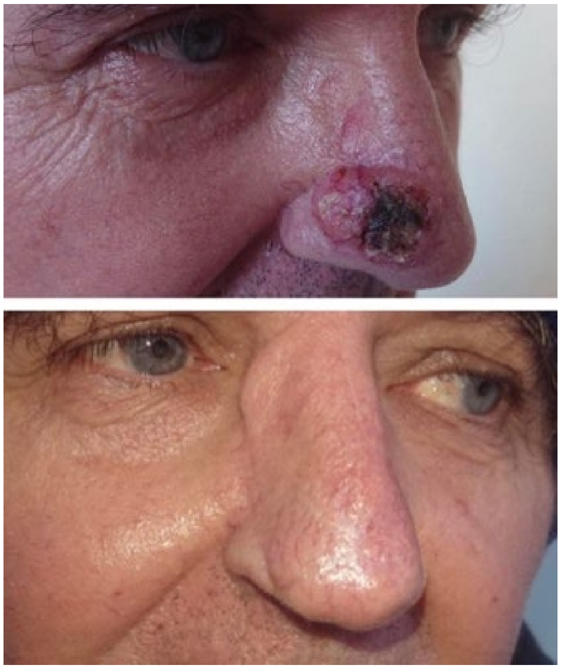

In case of nasal ala defects, a full-thickness skin graft and a cartilage graft to keep the shape and to avoid breathing obturation is a good choice for superficial defects, while in case of deeper defects, a more complex reconstruction like nasolabial flap or interpolated melolabial flap is required [22].

Despite all the reconstruction science of skin defects, skin properties are changing during aging which requires a personalized surgical approach.

Even if the resection was performed with free macroscopical tumor edges, the histological exam revealed a deep tumor infiltration in 5 cases, which showed the limitation of anatomical site between safe tumor resection with free tumor edges and aesthetic result.

CONCLUSIONS

Since the nose is the most visible part of the body, its reconstruction has an important role in the life quality of patients. Behind the science, plastic surgery is also about art and understanding that skin suffers changes during aging which affects its properties, its structure and requires a personalized approach. Every nose reconstruction should be based on keeping a three-dimensional contour, keeping the quality of the skin, the border outline, and breathing function. All the techniques should be focused on restoring the aesthetic units, not on filling the defects. Sometimes the preservation of the aesthetic aspect interferes with the preservation of nose function and complete resection of the tumor, which is a challenge for the doctor to make the patient understand the prioritization of health over the aesthetic aspect and to help him to manage this situation without decreasing quality of life.

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Authors' contribution
A.G. conceptualization and writing (original draft preparation), A.M.O. methodology and writing (review and editing), I.P.F. data curation and supervision. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
Approval of the local Ethics Review Board of Emergency University Hospital of Bucharest was obtained for the use of the patient data.

Patient consent for publication
Informed consent was obtained from all subjects involved in the study

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ORIGINAL ARTICLE

The Prevalence of Cerebral Aneurysms in Patients with Polycystic Liver Disease

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Abstract: *Background: It is well known that patients with polycystic kidney disease (PKD) are at increased risk of developing cerebral aneurysms, however, this association has not been well studied for patients with polycystic liver disease (PLD). Material and methods: Cross-sectional descriptive study, which included 15 adult patients diagnosed with polycystic liver disease at the Gastroenterology and Hepatology Department of Fundeni Clinical Institute. Standard neurological exam and brain MRI were performed in all patients on a 1.5 Tesla MRI. Brain imaging protocol included T1/T2, T1SE, T2-FLAIR, DWI, SWI, 2D-TOF, 3D-TOF. Results: The majority of patients (93%) were females. The mean age was 53 ± 5 years old. Patients with AD-PKD and polycystic hepatic disease predominated (60%). Aneurysms were found in only one patient diagnosed with AD-PKD (in whom three aneurysms were described). The overall prevalence of cerebral aneurysms in our patient group was 7%. However, none of the patients with isolated PLD was found to have intracranial aneurysms. Other brain imaging abnormalities were frequent but nonspecific (mostly attributable to vascular-degenerative changes). Conclusions: Given these results, and due to the small number of patients in our study, it is hard to appreciate if polycystic liver disease is indeed associated with an increased risk of cerebral aneurysms.*

Keywords: *polycystic liver disease, cerebral aneurysm, brain MRI*

INTRODUCTION

Polycystic liver disease (PLD) is a genetic disorder characterized by the replacement of normal hepatic tissue with fluid-filled cysts. This is due to genetic mutations of the proteins that are involved in epithelial growth and transport of fluid in the liver [1]. Some authors have defined PLD by the presence of more than 10 cysts in the liver, while others have defined it as a liver containing over 20 cysts [2,3]. PLD can occur in isolation or can be associated with autosomal dominant polycystic kidney disease (AD-PKD), in which case patients present with cysts involving both the liver and the kidneys.

The latter combined form (AD-PKD and PLD) is more common, accounting for 80-90% of all patients with multiple liver cysts [4]. It is well-known that patients with PKD are at an increased risk of developing cerebral aneurysms [5,6], however, this association has not been well-studied for patients with PLD.

MATERIALS AND METHODS

We performed a cross-sectional descriptive study, in which we recruited 15 adult patients diagnosed with PLD at the Gastroenterology and Hepatology Department of Fundeni Clinical Institute. All patients were included on the liver transplantation waiting list (or have already benefited from liver transplantation). The evaluation comprised a standard neurological examination and electroencephalogram. Brain MRI was performed in all patients on a 1.5 Tesla MRI. Brain imaging protocol included T1/T2, T1SE, T2-FLAIR, DWI, SWI, 2D-TOF, 3D-TOF. To better characterize PLD, abdominal CT scans with contrast enhancement and volumetric assessment of the liver were performed in all patients. Laboratory workup included liver function parameters (albumin, total bilirubin, conjugated bilirubin, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, ammonia, blood coagulation tests), kidney function parameters (urea, creatinine, uric acid), complete blood count, inflammatory markers and tumoral markers (alpha-fetoprotein, CA 125, CA 15.3, CA 19.9, CEA). Liver disease severity was measured using the MELD score, while kidney function was measured using serum creatinine levels and creatinine clearance (Cockcroft-Gault Equation).

Patients were included only if an informed consent form was signed and if the inclusion and exclusion criteria were respected (see Table 1). The study protocol was approved by the Ethics Committee of Fundeni Clinical Institute.

Table 1: Inclusion and exclusion criteria used for patients in our study.

Exclusion criteria	Inclusion criteria
Patients diagnosed with other cystic lesions of the liver (e.g., Caroli disease)	Age > 18 years-old
Patients diagnosed with isolated polycystic kidney disease	Diagnosis of polycystic liver disease ± polycystic kidney disease

Relevant medical information (imaging results, laboratory test results) was accessed using the hospital's informatic system Hipocrate. The patient database was created with Microsoft Office 365.

RESULTS

93% of the patients were females (n=14). The mean age of patients was 53 ± 5 years old. The mean age at the time of PLD diagnosis was 38 ± 12 years old. The majority of patients (60%) had PLD associated with AD-PKD (see Figure 1). The mean hepatic volume as assessed by abdominal CT volumetry was 7406.38 ± 2939.83 cm3. The mean MELD score was 8 ± 2 points (patient range: 6-12 points). The mean creatinine level was 1.0 ± 0.3 mg/dL (patient range: 0.68 – 1.72 mg/dL). Only two patients had serum creatinine above the upper normal limit and were diagnosed with chronic kidney disease in the setting of polycystic kidney disease. The mean level of alkaline phosphatase was 102 ± 47 units/liter, while the mean level of gamma-glutamyl transferase was 70 ± 70 units/liter. Two patients benefited from a kidney transplant in the past, and one of them received a liver transplant as well.

Figure 1: Proportion of patients with isolated versus mixed hepatic-renal polycystic disease

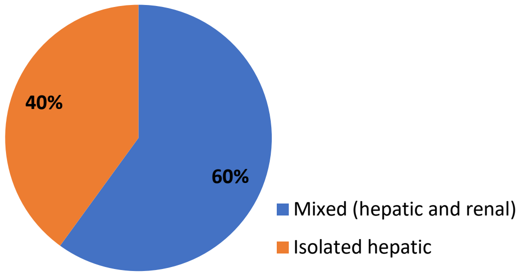

Clinical neurological exam was normal in almost all patients. One patient was deaf-mute, another patient had a positive left-sided Lasègue's sign and diminished Achilles reflexes, and another patient had diminished Achilles reflexes. Apart from these findings, all neurological examinations were unremarkable. Regarding brain MRI abnormalities, unruptured cerebral aneurysms were found in only one patient (in whom three aneurysms were described). However, it should be mentioned that this patient was diagnosed with autosomal-dominant polycystic kidney disease, and not with isolated polycystic liver disease. Of the three aneurysms, the first two were saccular: one measured 3 mm and involved the M1 segment of the left middle cerebral artery, and the other one measured 9.7 mm and involved the M1 segment of the right middle cerebral artery. The third aneurysm was fusiform, had a diameter of 5.5 mm, and involved the right vertebral artery. The overall prevalence of cerebral aneurysms in our patient group was 7%.

Nonspecific supratentorial demyelinating lesions were described in the majority of patients (73%) (see Figure 2). More than a quarter of patients presented with filiform (hypoplastic) posterior communicating arteries. Dilated perivascular spaces were described in 47% of patients (n=7).

DISCUSSION

The prevalence of isolated polycystic liver disease (PLD) varies in the literature. While autosomal dominant polycystic kidney disease (AD-PKD) affects up to 0.2% of the population, the frequency of isolated PLD is much lower (under 0.01%) [7]. Some authors have cited a prevalence of 1 up to 9 cases per 100 000 persons for isolated PLD [8].

On the other hand, the hepatic cystic disease is one of the main extrarenal manifestations of AD-PKD, and its prevalence is age-related, increasing from 20% in the third decade to 70% in the seventh decade [9]. The prevalence of hepatic cysts in patients with AD-PKD may be as high as 83% (if properly sought by MRI), as was highlighted in one study [10]. The number and size of hepatic cysts have been shown to correlate not only with the age of patients but also with female sex, history of pregnancy, the severity of renal cystic disease, and decreased renal function (as measured by the creatinine clearance) [11].

Figure 2: Imaging findings in patients with PLD. Note that cerebral aneurysms were detected in only one patient

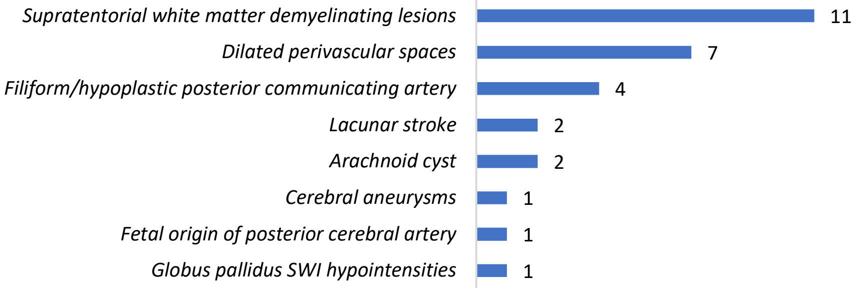

When looking at unruptured cerebral aneurysms, we find an overall prevalence in the general population of approximately 3%, and only a fraction of them progresses toward rupture (with subsequent subarachnoid hemorrhage) [12]. In a systematic review that analyzed 23 studies (comprising more than 56000 patients), the prevalence of intracranial aneurysms varied considerably according to study design and study population, from 0.4% in retrospective autopsy series, to 3.6% in prospective autopsy series, to 3.7% in retrospective angiography series and up to 6% in prospective angiography series [13]. However, when all available evidence was taken into account (including factors that overestimate or underestimate the risk), a frequency of about 2% for cerebral aneurysms was calculated in adults without risk factors for subarachnoid hemorrhage.

The prevalence of intracranial aneurysms in patients with AD-PKD is higher than in the general population [14,15]. Perrone et al mentioned a frequency of about 10% in asymptomatic patients and up to 25% in patients with a family history of intracranial aneurysms or subarachnoid hemorrhage. Likewise, Sanchis et al noted a prevalence of 9% for cerebral aneurysms after evaluating 812 patients with AD-PKD using screening with MR angiography. In another paper, in which 355 patients with AD-PKD underwent screening by 3D-TOF MR-angiography, the prevalence of intracranial aneurysms was found to be 12.4% [16].

Data regarding the frequency of cerebral aneurysms in patients with isolated PLD are lacking and only a few published papers have addressed this issue. In one study, an asymptomatic cerebral aneurysm was found in one out of the 10 patients with PLD who received screening studies of the cerebral vessels. [17]. Schievink et al described an instance of a familial association between PLD and cerebral aneurysms, in which a father with isolated PLD died of a ruptured cerebral aneurysm, while two of his six children also presented brain aneurysms (one of whom was diagnosed with isolated PLD) [18]. There is also a published case of a ruptured cerebral aneurysm associated with bilateral carotid dissection in a normal-weight woman who presented upon clinical examination with an enlarged abdomen. Abdominal CT highlighted a massively enlarged polycystic liver (with only a few cysts in the kidneys), while subsequent genetic analysis was positive for homozygote mutations in the PKD1 gene (for AD-PKD) and ADPLD gene (for PLD) [19]. Aneurysms situated in other parts of the circulatory system have also been described concerning PLD. Such is the case published about a 51 years-old male with PLD, who was discovered to have bilateral popliteal aneurysms [20]. The clinical features of patients with isolated PLD have been compared to those of patients with polycystic liver in the setting of AD-PKD. Patients with isolated PLD have greater numbers and larger sizes of cysts but have a more benign disease course and less associated morbidities than patients with AD-PKD [21]. It remains to be seen if this last statement can be extrapolated to the risk of intracranial aneurysms. Given that their frequency increase with age (both in the general population and in patients with AD-PKD), large prospective studies are needed to assess the prevalence of intracranial aneurysms in patients with isolated PLD.

The rupture rate of cerebral aneurysms in patients with AD-PKD appears to be similar to that of the general population [22]. In the review performed by Cagnazzo et al, the rupture rate, growth rate, and incidence of new aneurysm formation were 0.4% per patient-year, 0.4% per patient-year, and 1.4% per patient-year, respectively.

The main risk factor for the presence of unruptured intracranial aneurysms is a positive family history of intracranial aneurysms. However, most cases of ruptured cerebral aneurysms occur in the absence of a positive family history. In a study discussing the necessity of screening for cerebral aneurysms in patients with AD-PKD, about one-third of nephrologists were in favor of systematic screening for all patients with AD-PKD [23]. However, after reviewing 15 studies (comprising almost 1500 patients with AD-PKD), Zou et al concluded that screening should be recommended only for patients with a positive family history of hemorrhagic stroke or intracranial aneurysm and if they originate from China, Japan, and Europe (since the studies from these countries reported a higher prevalence of aneurysms). The calculated incidences of aneurysm rupture, aneurysm growth, and new aneurysm formation were 0.13%, 1.84%, and 0.57%, respectively [24]. Poor outcomes among treated aneurysms were seen in one study only with the ruptured presentation and did not differ significantly between treatment modalities (open surgery versus endovascular approach) [25].

CONCLUSIONS

In our study, cerebral aneurysms were found in only one patient, who was diagnosed with autosomal-dominant polycystic kidney disease. None of the patients with isolated polycystic liver disease was found to have intracranial aneurysms.

Given these results, and due to the small number of patients in our study, it is hard to appreciate if polycystic liver disease is indeed associated with an increased risk of cerebral aneurysms. Other brain imaging abnormalities were frequent but nonspecific (mainly attributable to vascular-degenerative changes).

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Authors' contribution:
All authors contributed individually and equally to the writing of this article. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication:
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Drug Discovery – A New Bioactive Compounds Isolated from Natural Sources

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Abstract: *A rise in mortality due to fungal infections in an immunocompromised population has been observed lately. Nowadays, due to increased fungal infections, the limitations encountered in their treatment like resistance, side-effects, and high toxicity, the rising prescription and overuse of conventional antifungals all stimulate a search for alternative natural drugs therefore we are in dire need of natural newer strategies that involve reliable agents for the treatment of fungal diseases such as essential oils are known for their anti-microbial properties and are multi-component. Materials & Methods: Soil samples (66 samples) were collected from different agricultural fields and animals habitat of Saharanpur (U.P.) Isolation of keratinophilic fungi was carried out by the hair-baiting technique. Extraction of Mentha piperita & Cinnamonum vernum essential oil was carried out by hydrodistillation method and the chemical composition of both extracted essential oils was determined by Gas Chromatography-Mass Spectrophotometry. Antimycotic studies of essential oils were done by the standard disc diffusion method. Results & Discussion: In the present study, the antifungal potential of Mentha piperita & Cinnamonum vernum essential oils were evaluated against three human pathogenic fungi isolated from the soil of agricultural field and animals habitat of Saharanpur (U.P.) i.e Trichophyton mentagrophytes, T.tonsurans, and T.equinum. The chemical composition of Mentha piperita & Cinnamonum vernum EO was analyzed by GC-MS. Menthol (53.28%) was the major compound of the Mentha piperita EO followed by Menthyl acetate (15.1%) and Menthofuran (11.18%). Major constituents of Cinnamonum vernum EO were linalool (8%), (E)-cinnamaldehyde (7.2%), β-caryophyllene (7.4%), eucalyptol (6.4%), and eugenol (5.6%). Conclusion: Essential oils of Mentha piperita & Cinnamonum vernum have been found to have remarkable & excellent antifungal activity against these pathogenic fungi. Present findings conclude that natural products like plant-derived EOs instead of chemotherapy and the emergence of resistance to antifungal drugs can be regarded as an environmental safety mode of disease control against pathogens.*

Keywords: *essentials oils, antimicrobial, drug, resistance, natural, products*

INTRODUCTION

Keratinophilic fungi are a group of microorganisms that can decompose keratin remains in the environment and are pathogenic to humans and animals. The soil that is rich in keratin materials is most conducive for the growth and occurrence of keratinophilic fungi distribution is variable with the environment and depends on different environmental factors such as humans' and animals' presence. Keratinophilic fungi include a variety of filamentous fungi mainly comprising hyphomycetes and several other taxonomic groups [1-2]. Dermatophytes cause humans and animal mycoses and thus have drawn the attention of medical and veterinary epidemiologists. The majority of dermatophytes can live saprophytically and keratinophilic fungi can be considered as a potential pathogen [3]. They occur in many natural and man-made habitats. Keratinophilic fungi are unique in the sense that they require and utilize keratin for growth [4-5]. Studies on keratinophilic fungi are now of considerable significance for their important role in the breakdown of keratinous debris of animals and man in nature and they have a worldwide distribution [6,7]. Studies have shown that soils are important sources of dermatophytes and keratinophilic fungi [8-10]. In the past two decades, the emergence of resistance to various antifungal drugs has accelerated dramatically. Azole-resistant Candida and Aspergillus species are the top pathogens responsible for nosocomial or food-borne infections [11,12]. In addition, the formation of biofilms by Candida species has raised concerns due to their increased resistance to antifungal therapy, and protects the microbial cells within biofilms from the host immune defenses [13,14]. Moreover, EOs especially those with known antibacterial effects have the potential to be used in the food industry as preservatives and to increase the shelf life of products. Therefore, determining the antimicrobial properties of EOs might help to overcome microorganism resistance to antibiotics. The chemical composition of aromatic plants depends largely on individual genetic variability and different plant parts. The presence and concentration of certain chemical constituents of EOs also fluctuate according to the season, climatic condition, and site of plant growth [15]. A review of the literature reveals that many essential oils acquire antifungal activity [16-19]. The development of antifungal resistance is complex and depends on multiple hosts and microbial factors [20]. According to Moreira et al, lipophilic compounds of the oils bond the phospholipid bilayer of the cell membrane increasing its permeability and spreading out the intracellular contents or damaging the enzymatic system of the cell [21-22]. M. piperita oil is one of the most popular and widely used essential oils, mostly because of its main components, Menthol, and menthone [23]. Previous studies have shown antiviral, antibacterial, antifungal, antibiofilm formation, radio-protective, angioedema, analgesic, and antioxidant activities of the EO [24-26]. Cinnamon is a good source of the powerful antioxidant manganese. Therefore, much attention has been paid to the discovery and development of new antimicrobial agents that might act against these resistant microorganisms, and cinnamon could be an interesting candidate [27-28]. Concerning volatile components, the chemical composition of cinnamon essential oils depends on the part of the plant from which they are extracted [29-31]. The presence and concentration of certain chemical constituents of EOs also fluctuate according to the season, climatic condition, and site of plant growth [15,32]. The objective of this work was to evaluate the antifungal efficacy of Mentha piperita & Cinnamonum vernum EOs against drug-resistant three widely pathogenic fungal strains that cause superficial skin infections in humans and trying to find more safely hygienic natural plant products. The goal of this study was to investigate the chemical composition and in vitro antifungal activities of essential oils in the leaves of M. piperita & C. vernum.

MATERIALS AND METHODS

Collection of Soil Samples

Sixty-six soil samples were collected from different agricultural fields and animals habitat of Saharanpur (U.P.) India. For this present study, different soil samples were collected in sterile polyethylene bags and brought to the laboratory for further microbiological analysis. Keratin substrates (Ks) were collected from different sources such as chicken feathers, buffalo hairs, goat hairs, dog hairs, pig hairs, ship hairs, and horse hairs in clean sterilized plastic bags and transferred to the laboratory. Samples were transmitted to the laboratory as early as possible for further analysis like pH, temperature, humidity, salinity, and total dissolved solids.

Isolation of keratinophilic fungi by Hair Bait Technique

Isolation of keratinophilic fungi was done using the hair bait technique (Vanbreuseghem, R. 1952) [33]. Moist chambers were prepared using sterile soil samples. 2-3 cm short strands of sterilized defatted baits were spread over soil samples. 10-15 ml of sterile water was added to the soil to facilitate the germination of fungal spores. Petri dishes were then incubated at room temperature at 28◦C for 15-20 days. Plates were examined periodically for the development of mycelium.

Identification of Keratinophilic fungi

Every single fungal colony was taken from the mixed culture. The pure culture was developed by repeated subculturing which was transferred to an agar slant and subcultured and then stored at 4°Ċ temperature. The fungal colonies were identified by microscopic and macroscopic characteristics. The macroscopic examination of fungi was characterized by duration of growth, surface morphology, and pigment production on the reverse and microscopically, the isolated fungi were stained with Lactophenol cotton blue staining and observe under the microscope at high power of the objective lens.

Gas chromatography Mass Spectrometry (GC-MS) analysis of M. piperita and C. vernum essential oils.

Quantitative and qualitative analyses of the essential oils were performed using a GC-MS apparatus. The analysis was performed with an Rtx 5 MS column. For the GC-MS detection, an electron ionization system with an ionization energy of 70eV was used. Nitrogen gas was used as carrier gas with a flow rate of 1.21 ml/min. The column was raised from 50ºC to 320ºC at a rate of 3ºC/min. The relative percentage of the oil constituents was expressed as a percentage by peak areas and the identification of oil components was based on their retention time with available literature values.

Antimycotic Studies of Essential Oils against Selected Fungi by Disc Diffusion

Oil was screened for its antifungal activity against T. mentagrophytes, T. equinum, and T. tonsurans by disc diffusion method [34]. Standard size Whatman no. 1 filter paper discs 6.0 mm in diameter, sterilized by dry heat at 140°C in an oven for one hour were used to determine antifungal activity. SDA medium for the disc diffusion test was prepared. After sterilization, it was poured into sterilized Petri plates and allowed to solidify. Then one-day old fresh culture of isolated fungi was used for inoculums preparation. A suspension was just turbid (~0.5 McFarland standard) by visual inspection and was prepared by suspending fungus in 0.9% NaCl solution and the homogeneous suspension was used for inoculation. Using a sterile cotton swab, fungal cultures were swabbed on the surface of sterile Sabourauds Dextrose Agar plates. Sterilized filter paper discs were soaked in neat, undiluted (100%, 75%, 50%, 25%) concentrations of oils. Using ethanol-dipped and flamed forceps, oil-saturated discs of 100%, 75%, 50%, and 25% concentration were aseptically placed over Sabourauds dextrose agar plates seeded with the respective test microorganism. The antibiotic discs of Clotrimazole and Ketoconazole (10 mcg per disc) were also aseptically placed over the seeded Sabouraud's dextrose agar plates as a standard drug for comparison of antifungal activity with essential oils. The plates were incubated at 28ºC for 48-72 hours. The diameter of the inhibition zones was measured in mm and the activity index was calculated based on the size of the inhibition zone. Three replicates were kept in each case and average values were calculated.

RESULTS

The present study reported that out of sixty-six soil samples collected, three species of keratinophilic fungi were predominantly isolated from different animal habitats and agricultural fields with different keratin substrates. These habitats contain lots of keratin debris. In the present work, T. mentagrophytes, T. tonsurans, and T. equinum were isolated predominantly from the agricultural and animal habitat soils of Saharanpur (U.P) and also found the main etiological agent of zoonotic dermatophytosis in the investigated area. Macroscopic & Microscopic characteristics of isolated fungi are shown in Table.1. Various earlier workers reported the diverse population of keratinophilic fungi and dermatophytes from Indian soil [35,36]. The high prevalence of keratinophilic fungi from the soil of Saharanpur is due to buffalo hairs, human hairs, and dog hairs which come to the soil either as dead or dropped off serve as substrates, and are subjected to microbial decomposition. The present study indicates that the diverse existence of keratinophilic fungi in the soil of Saharanpur plays a major role in bioremediation in the natural environment. The present study coincides with other workers who also reported diverse soil habitats have been screened from different countries Brazil, Kuwait, Iran, and India indicating that these groups of fungi are distributed worldwide [37-39]. In urban areas, where there are high concentrations of people and animals, soil rich in organic matter may constitute a permanent or occasional reservoir for fungi and during this fungi can be a potential source of skin infection for animals and humans. These animals serve as reservoirs of zoophilic dermatophytes and their zoonotic infections have considerable importance [40-42]. Sandpits are thought to play a role in the epidemiology of human and animals mycoses and reported special care needs to be paid to identify potential pathogenic fungal species such as species from Fusarium, isolated from soil samples of recreational parks and in all elementary schools [43]. Some workers reported that when infections due to other opportunistic fungal agents occur can lead to very unfavorable and even serious outcomes of infection by fungi [44].

Table 1: Macroscopic & microscopic characteristics of isolated fungi

S. No	Isolated Fungi	Macroscopic Characteristics	Microscopic Characteristics
1	*Trichophyton tonsurans*	Colonies on Sabouraud's Dextrose Agar at 28°C grow moderately and the color of the colony is initially white than creamy yellow rose at age with conidial production. Suede-like with radial folds sometimes powdery or velvety. The reverse is a pale lemon yellow to mahogany brown may dark diffusing in the agar.	Many pyriform microconidia on stalks ballon forms. Rare distorted macroconidia. Hyphae are relatively broad irregular, and much branched with numerous septa. Microconidia are varying in size and shape from long clavate to broad pyriform is borne right.
2	*Trichophyton mentagrophytes*	Colonies on Sabouraud's Dextrose Agar at 28°C grow moderately and velvety, flat, and thin with powder and pale powder dry & white powder in color.	Round microconidia in grape-like clusters, Spiral hyphae, and macroconidia narrowly attached to hyphae.
3	*Trichophyton equinum*	Growth rate moderately rapid texture suede-like to downy flat. Thallus color cream to pale yellow deep to pinkish or brown is in the center. Reverse deep yellow becoming red-brown or dark pink may produce brown soluble pigment.	Pyriform microconidia lateral may be produced on stalks. Rare club-shaped macroconidia smooth thin-walled.

The leaves of Mentha piperita and Cinnamonum vernum were collected from Saharanpur (U.P) and essential oil was extracted by Clevenger apparatus based on the hydro-distillation method as shown in Figures 1 and 2. Hence, antimycotic studies and chemical composition of essential oils of Cinnamon & Peppermint were carried out against selected fungi to eco-friendly control fungal infections. In the present study, thirteen (13) compounds representing 99.37% area of the oil, were identified. The qualitative and quantitative compositions of the EO of M. piperita by GC-MS analysis and antifungal activity carried out by disc diffusion method are presented in Tables 2, 3, and Figure 3.

Figure 1: Mentha piperita (Peppermint oil)

M. Peppermint

Figure 2: Cinnamon oil (Cinnamomum Verum)

Cinnamon oil

Figure 3: Antidermatophytic activity of M.piperita essential oil at 100% concentration against selected fungal strains

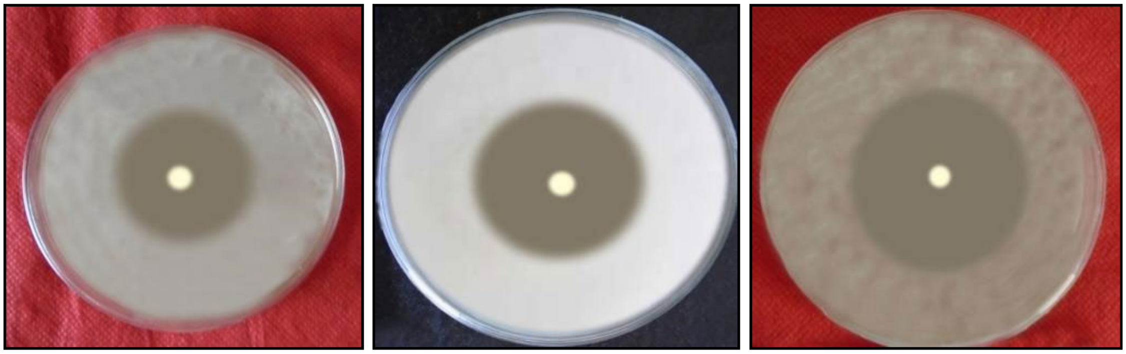

(A) T. equinum (B) T. mentagrophytes (C) T. tonsurans

GC/MS analyses showed that the main constituent of the M. piperita EO was Menthol (53.28%) followed by Menthyl acetate (15.1%) and Menthofuran (11.18%). The compositions of the EOs might be affected by the developmental stages of the plant.

Some authors reported alpha terpinene as the dominant component of M. piperita EO (19.7%) while other previous studies identified Menthol as one of the main constituents of the Eos [23,45]. The higher concentration of Menthol in this study may reflect variations due to the geographical location from which the plants were collected.

In this study, the EOs of Mentha piperita exhibited strong antimycotic activities against T. mentagrophytes (60 mm), T. equinum (67 mm), and T. tonsurans (50 mm) at 100% concentration of pure essential oil than standard drugs.

Table 2: Chemical components (%) of the essential oils distilled from Mentha piperita

Compounds	RI	% oil
α-Pinene	930	0.32
Sabinene	970	0.26
β-pinene-1	978	0.58
β-pinene-2	1035	6.69
Ci$-sabinene hydrate	1072	0.50
Menthone	1155	2.45
Menthofuran	1168	11.18
Neomenthal	1166	2.79
Menthol	1178	53.28
Neomenthy acetate	1274	0.65
Menthyl acetate	1298	15.10
Isomenthyl acetate	1308	0.61
β -Bourbonene	1389	0.37

Table 3: Antifungal activity of Mentha piperita oil against selected isolated fungal strains

S. No	Oil	Test strain	Concentration of oil	IZ of sample
1.	*Mentha piperita Oil (Peppermint oil)*	*T. mentagro-phytes*	100 %	60 mm
			75 %	50 mm
			50 %	29 mm
			25 %	12 mm
2.		*T. tonsurans*	100 %	67 mm
			75 %	40 mm
			50 %	30 mm
			25 %	15 mm
3.		*T. equinum*	100 %	50 mm
			75 %	39 mm
			50 %	29 mm
			25 %	15 mm

IZ = Inhibition zone (in mm) including the diameter of the disc (6 mm).

IZ of Ketoconazole & Clotrimazole (10 mcg/disc) is 32 mm & 30 mm against T. mentagrophytes

IZ of Ketoconazole & Clotrimazole (10 mcg/disc) is 28 mm & 25 mm against T. tonsurans

IZ of Ketoconazole & Clotrimazole (10 mcg/disc) is 24 mm & 22 mm against T. equinum

Similarly second oil i.e. Cinnamonum verum, chemical constituents were identified by GC-MS analysis resulting in the identification of ten (10) chemical compounds for C. vernum essential oil, as indicated in Table 4 and Figure 4.

(E)-cinnamaldehyde (7.2), linalool (8.00%), β-caryophyllene (7.40%), eucalyptol (6.40%), and eugenol (5.60%) were the main components of the C. vernum essential oil. The other important constituents were p-cymene (1.90%), α-humulene (1.70%), δ-cadinene (1.40%), α-pinene (1.30%), and limonene (1.20%). Following our results, several studies have reported that cinnamaldehyde is the major chemical compound of C. zeylanicum bark essential oil [46,47].

Table 4: Chemical components (%) of the essential oils distilled from Cinnamonum vernum

Compounds	RI	% oil
E-cinnamaldehyde	15.22	7.2
α-Pinene Linalool	5.66	1.3
Linalool	9.86	8
β-Caryophyllene	18.58	7.4
Eucalyptol	8.08	6.4
Eugenol	16.90	5.6
α-Humulene	19.47	1.7
δ-Cadinene	20.97	1.4
p-Cymene	7.82	1.9
Limonene	7.93	1.2

Figure 4: Antidermatophytic activity of C.vernum essential oil at 100% concentration against selected fungal strains

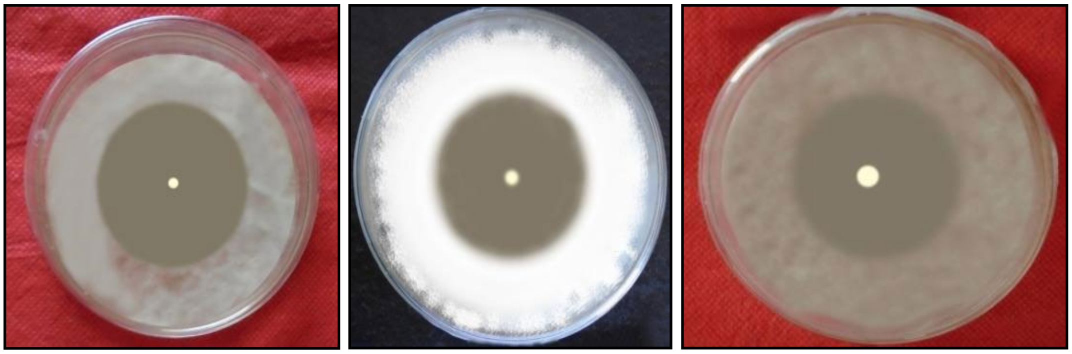

(A) T. equinum

(B) T. mentagrophytes

(C) T. tonsurans

Antimycotic activity of C. vernum essential oil was also evaluated by disc diffusion method and found excellent inhibition of zone at 100% concentration of pure essential oil against T. tonsurans (60 mm), T. mentagrophytes (59 mm) & T. equinum (58 mm) as compared to standard antifungal drugs i.e. Ketoconazole & Clotrimazole (Table 5).

Table 5: Antifungal activity of Cinnamonum vernum oil against selected fungi

S. No	Oil	Test strain	Concentration of oil	IZ of sample
1.	*Mentha piperita Oil (Peppermint oil)*	*T. mentagro-phytes*	100 %	59 mm
			75 %	50 mm
			50 %	30 mm
			25 %	20 mm
2.		*T. tonsurans*	100 %	60 mm
			75 %	46 mm
			50 %	31 mm
			25 %	19 mm
3.		*T. equinum*	100 %	58 mm
			75 %	45 mm
			50 %	31 mm
			25 %	19 mm

IZ = Inhibition zone (in mm) including the diameter of the disc (6 mm).

The traditional use of plants as medicines provide the basis for indicating which essential oils may be useful for specific medical conditions. It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds. The present findings are similar to Wuthi-udomlert et al. (2000) who reported the antifungal activity of turmeric oil against 29 clinical strains of dermatophytes and in the screening of turmeric oil, the diameter of the inhibition zone was found to vary from 26.1 mm to 46 mm against 29 clinical strains of dermatophytes [48].

Numerous scientific studies proved the inhibitory effect of essential oils against different fungi [49]. It is important to analyze the plants which have been used in the medicines as a potential source of normal antimicrobial compounds. The present work coincides with the work of Sharma et al. (2011) who also reported the additive and inhibitory effect of Curcuma longa (Turmeric) and Zingiber officinale (Ginger) essential oils against dermatophytes causing superficial skin infections [50]. The use of essential oils in the treatment and prevention of infection has been in demand in the field of research from the past. Natural products have served as a research resource for most drugs, providing a basis for chemical research and the discovery of new drugs [51].

Several reasons have been offered to explain the success of natural products, among them are their great chemical diversity, the effects of evolutionary pressure in creating biologically active molecules, and the structural similarity of protein targets in different species, among others [52]. The treatment of fungal disease is limited and part of the reason is due to the limited spectrum of the currently antifungal drugs and the expensive treatment particularly due to the need for prolonged therapy. Jayaprakasha et al., (2003) also reported some alternative therapies including natural products are necessary to control fungal infections [53].

Various workers have reported that due to the high volatility and lipophilicity of the essential oils, they are readily attached to penetrate into the cell membrane to exert their biological effect. Ali & Ali (2019) in Saudi Arabia investigate the antifungal activity of plants from the Jeddah region (A. indica, Z. spina-christi, and O. europaea), P. dactylifera seed, neem oil, and other oils [54]. Another interesting investigation reports the antibacterial activity of cinnamon bark essential oil and its main constituents, trans-cinnamaldehyde and eugenol against Cronobacter sakazakii and C. malonaticus, which are opportunistic pathogens that cause infection in children and immunocompromised adults. Kizil et al (2010) reported the EOs exhibited fungistatic and fungicidal activities against both the standard and clinical strains of Candida species at concentrations ranging from 0.5 µL/mL to 8 µL/Ml [55].

According to the study of Saharkhiz et al, (2010), essential oils killed the standard strain of C. neoformance is a well-known primarily opportunistic pathogen that produces chronic and life-threatening meningitis at a concentration of 4 µL/mL [56]. The antifungal activities of the essential oils (EOs) of Acantholippia seriphioides, Artemisia mendozana, Gymnophyton polycephalum, Satureja parvifolia, Tagetes mendocina, Lippia integrifolia, collected in the Central Andes area, Province of San Juan, Argentina, were investigated against M. gypseum, T. mentagrophytes and T. rubrum and were inhibited by the EOs of G. polycephalum, L. integrifolia, and S. parvifolia, with minimum inhibitory concentrations (MICs) between 31.2 and 1000 µg/ml.

This study shows that these Central Andean area species might be used to treat superficial fungal infections [57]. The study of Agarwal et al. (2008) and Saharkhiz et al. (2010) found that the formation of biofilm by C. albicans was inhibited completely at a concentration of up to 2 µL/mL in a dose-dependent manner [56,58]. Shiming et al (2006) reported antifungal mechanism of essential oils, inhibits the synthesis of ergosterol in fungal and cholesterol in mammalian cells [59]. The present results showed the excellent antidermatophytic efficacy of M.piperita and C.vernum against T. mentagrophytes. T. tonsurans and T. equinum as compared to standard antifungal drugs and can be used to control the superficial fungal infections of the skin as an eco-friendly agent and to combat the mechanism of antimicrobial drug resistance.

The tested Essential oil in the present study was rich in Menthol. It has been shown that this phenolic monoterpene has a hydroxyl group around the phenolic ring and exhibits its antimicrobial activity through the disruption of the cytoplasmic membrane due to the main characteristics of EOs is their hydrophobicity, which enables their incorporation into the cell membrane. [60,61]. The great importance of natural products in developing new therapeutic tools is evident. In this aspect, medicinal plants and their derivatives are important for pharmacological research and drug development. Special attention has been directed to natural derivatives, based on the knowledge of antifungal compound production in nature.

CONCLUSIONS

The polluted area of agricultural fields of soil is a dynamic medium in which a large number of keratinophilic and non-keratinophilic fungi live in close association. Some keratinophilic fungi are pathogenic to man and animals. Dermatophytic infections are highly common and endemic diseases in developing countries due to poverty, improper health facilities, illiteracy, and other poorly developed diagnostic techniques. Also, some microorganisms are resistant to newer or modern antifungal synthetic drugs and in some severely immunosuppressed patients. M.piperita and C.vernum essential oils can be suggested as an alternative to synthetic antibiotics, especially for the treatment of antibiotic-resistant infections. These EOs can also be considered for developing products to control fungal infections. Thus, there is a need for an alternative therapy that is safe, cheaper, economical & easily available so a wide range of essential oils from plants are still unexplored for their antifungal activity. Hence, the present study concludes to explore new antifungal agents alternative to synthetic drugs for the treatment of dermatophytic & other fungal diseases. This research study will be beneficial for human health, the human economy, environment safety, and antimicrobial drug resistance for proper and timely treatment of fungal infections in immunocompromised patients.

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ORIGINAL ARTICLE

Comparison of Serum Vitamin D Levels with Glucose, Lipid, and the Other Biochemical Parameters: A Cross-Sectional Study

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Abstract: *Since vitamin D is synthesized from cholesterol, it is claimed to be associated with insulin resistance, suggesting that there may be an association between vitamin D glucose and lipids. For this reason, we aimed to investigate a relationship between vitamin D and biochemical parameters, including glucose and lipids. This cross-sectional study included 198 patients .admitted to the family medicine outpatient clinic between June and December 2016. There was no significant correlation between vitamin D and serum glucose, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, calcium, magnesium, sodium, TSH, and body mass index values in both men and women. There was a correlation between vitamin D and total cholesterol and LDL cholesterol levels in the younger subjects below 41 years (p=0.002). Regression analysis with other variables showed no statistical significance between vitamin D levels on LDL-C levels (P=0.309). Despite previous studies, our study did not confirm the effects of vitamin D on serum glucose, lipid, calcium, and other biochemical parameters. However, these results suggest that the standard up-to-date literature showing the relationship between vitamin D and various metabolic and hormonal disorders may need to be confirmed by new large-scale studies.*

Keywords: *vitamin D; glucose; lipid profile; calcium; biochemical parameters*

INTRODUCTION

The prevalence of vitamin D deficiency is reported to be between 30% to 50% globally in the general population. Similarly, in Turkey, this ratio was found to be 51% in Ankara and 46.9% in İzmir [1-3]. Also, in a study conducted among female university students in Istanbul, vitamin D. levels were found below normal values in 92.4% of the participants [4]. A study conducted in Konya detected vitamin D deficiency in 88,4% of the patients admitted to the family medicine outpatient clinic for any reason [5].

Vitamin D is usually found in foods from animal sources in the form of cholecalciferol and small amounts in plant sources in the form of ergosterol. It is primarily synthesized in the skin under the influence of sunlight [6,7]. Cholecalciferol is synthesized in the epidermis from 7-dehydrocholesterol by the effect of ultraviolet B rays or can be taken with food. First, it transforms into 25-hydroxycholecalcidiol in the liver and then into 1.25-hydroxycholecalcitriol, the active form, in the kidneys [8]. Vitamin D deficiency paves the way for diseases such as rickets in children and osteomalacia in adults [9,10]. It has been suggested to prevent colorectal and prostate cancers and the formation of disorders such as prediabetes and metabolic syndrome by decreasing insulin resistance when taken at high doses [11,12]. In addition, it has been stated that vitamin D deficiency may affect cardiac functions such as diastolic function and aortic elasticity [13].

Vitamin D affects calcium absorption and metabolism by regulating receptor-mediated gene expression [14]. In addition to intestinal and bone tissue, vitamin D receptors are found in many tissues, including the brain, heart, stomach, pancreas, skin, gonads, and T and B lymphocytes. It is accepted that Vitamin D inhibits the proliferation of both normal and hyperproliferative cells and promotes the maturation of these cells through its receptors [7]. Controversial results have been reported on the effects of vitamin D other than calcium metabolism; while some studies suggest a positive effect on the lipid profile, some found no association between vitamin D and lipid parameters [15,16].

We hypothesized that because vitamin D is synthesized from cholesterol and has numerous receptors in tissues, it might interact with biochemical parameters, including glucose and lipids. Therefore, we aimed to investigate the relationships between vitamin D levels and other biochemical parameters, including glucose and lipids.

MATERIALS AND METHODS

Study Design

This study is a cross-sectional study. The sample size was calculated to be at least 162 according to the formula n = t2pq/ d2. In determining the sample size, the p-value was accepted as 0.12, the q-value was 0.88, the d-value was 0.05, α error level was 0.05, and the t-value according to the error level was 1.96.

Population selection

The study included one hundred ninety-eight patients selected from the patient records who applied to the family medicine outpatient clinic for general control between May and October 2016. Individuals who did not use any medication and were found to be healthy were included in the study. Patients with previously defined diseases, such as depression, anxiety, hypertension, diabetes, hyperlipidemia, thyroid dysfunction, vitamin B12 deficiency, liver disease, and kidney disease, were excluded from the study. Demographic data, including age, sex, body mass index (BMI), and biochemistry laboratory parameters, were obtained from patient records. The subjects were then divided into two groups for age using the simple arithmetic mean method.

Statistical analysis

The data were analyzed using SPSS software (PASW Statistics for Windows, Version 16.0. Chicago: SPSS Inc.). Categorical data are expressed in numbers and percentages. Numerical data were expressed as means and analyzed by the Mann-Whitney U test in independent groups. Spearman Correlation analysis was used for correlation analysis. The linear regression test was used to show any relationship between the parameters. The Kolmogorov-Smirnov test was used to determine homogeneity for the variants.

Ethical Considerations

Ethical approval was obtained from the İstanbul Training Research Hospital Clinical Research Ethics Committee (Decision no. 09.08.2019, 1944).

RESULTS

A total of 198 patients aged 18-75 years (41.81±13.43), 71.2% female (141) and 28.8% male (57), were included in the study. The mean age of women was significantly lower than that of men (45.70 versus 40.20). Serum vitamin D, Body Mass Index (BMI), glucose, triglyceride, calcium, and magnesium levels were lower, and serum HDL cholesterol (HDL-C) levels were significantly higher in females compared to males (Table 1). Vitamin D levels had no significant correlation between serum glucose, total cholesterol, triglyceride, HDL-C, LDL cholesterol (LDL-C), calcium, magnesium, sodium, and TSH levels, and BMI values in both groups (Table 2).

Table 1: Comparison of serum vitamin D, glucose, lipid, calcium, magnesium, sodium, TSH, and BMI parameters between women and men

Parameters	Min-Max	Total	Women	Men	Z	p
		Mean ± SD (n)				
Vitamin D (ng/ml)	4.4-89.9	18.49±11.01(198)	16.92±9.67(141)	22.38±13.28(57)	-3.87	0.001*
Age (years)	18-75	41.81±13.43(198)	40.20±13.30(141)	45.79±13.04(57)	-2.88	0.004*
Glucose (mg/dl)	72-188	96.10±15.20(198)	93.28±11.32(141)	103.07±20.58(57)	-4.24	0.001*
Cholesterol (mg/dl)	99-331	207.72±44.07(198)	209.12±44.21(141)	204.25±43.91(57)	-0.54	0.585
Triglyceride (mg/dl)	29-417	118.78±65.32(198)	103.91±46.17(141)	155.56±88.02(57)	-3.93	0.001*
HDL-C (mg/dl)	28-104	53.54±12.63(198)	56.75±12.72(141)	45.60±8.18(57)	-6.18	0.001*
LDL-C (mg/dl)	44-215	131.44±35.97(198)	132.67±36.82(141)	128.41±33.92(57)	-0.35	0.720
Calcium (mg/dl)	7,6-10,4	9.46±0.46 (129)	9.40±0.42 (93)	9.60±0.51(36)	-2.63	0.008*
Magnesium (mg/dl)	1,0-2,8	1.99±0.19 (138)	1.97±0.20 (96)	2.04±0.17(42)	-2.11	0.034*
Sodium (mmol/L)	133-143	139.14±1.98(116)	139.00±1.97(83)	139.48±1.98(33)	-1.46	0.142
TSH (mIU/L)	0.3-5.1	1.79±0.96(140)	1.81±0.98(98)	1.73±0.94(42)	-0.42	0.672
BMI	16-40.4	25.84±4.40(158)	25.45±4.49(109)	26.71±4.10(49)	-2.05	0.040*

*SD: Standard Deviation; BMI: Body Mass Index; *Mann-Whitney U test is significant at 0.05 level (2-tailed); Min: minimum value; Max: maximum value*

When age groups were compared, serum glucose, total cholesterol, triglyceride, LDL-C, sodium levels, and BMI scores were statistically lower in young individuals below 41 years of age versus the elderly group over 42 years old. There was no difference between age groups regarding vitamin D, HDL-C, calcium, magnesium, and TSH levels (Table 3).

Table 2: Correlation of vitamin D3 with biochemical and other parameters

Vitamin D	Total	Women	Men
	r_s value; p-value (n)		
Age (years)	0.103; 0.148 (198)	0.058; 0.493 (141)	0.036; 0.790 (57)
Glucose (mg/dl)	-0.045; 0.525 (198)	-0.144; 0.089 (141)	-0.197; 0.143 (57)
Cholesterol (mg/dl)	-0.125; 0.079 (198)	-0.221; 0.153 (141)	-0.129; 0.340 (57)
Triglycerides (mg/dl)	-0.90; 0.208 (198)	-0.161; 0.056 (141)	-0.257; 0.053 (57)
HDL-C (mg/dl)	0.000; 0.993 (198)	0.148; 0.079 (141)	0.119; 0.378 (57)
LDL-C (mg/dl)	-0.090; 0.208 (198)	-0.111; 0.189 (141)	-0.029; 0.831 (57)
Calcium (mg/dl)	0.108; 0.223 (129)	0.058; 0.582 (93)	0.051; 0.769 (36)
Magnesium (mg/dl)	-0.007; 0.930 (138)	-0.058; 0.576 (96)	-0.054; 0.732 (42)
Sodium (mmol/L)	0.019; 0.838 (116)	-0.029; 0.796 (83)	0.46; 0.798 (33)
TSH (mIU/L)	0.003; 0.975 (140)	0.014; 0.888 (98)	0.013; 0.935 (42)
BMI	0.021; 0.792 (158)	-0.014; 0.882 (109)	-0.171; 0.240 (49)

*r_s value: Spearman correlation; BMI: Body Mass Index; *Correlation is significant at 0.05 (2-tailed).*

Table 3: Comparison of serum vitamin D, glucose, lipid, calcium, magnesium, sodium, TSH, BMI, and parameters according to age groups

Parameters	*< 41 years*	> 42 years	Z	p
	Mean ± SD (n)			
Vitamin D (ng/ml)	18.35±10.78(92)	18.61±11.39(106)	-0.19	0.847
Glucose (mg/dl)	90.64±7.61(92)	100.83±18.30(106)	-5.42	0.001*
Cholesterol (mg/dl)	188.13±38.97 (92)	224.72±41.23 (106)	-6.24	0.001*
Triglyceride (mg/dl)	94.98±53.16 (92)	140.20±67.08 (106)	-5.85	0.001*
HDL-C (mg/dl)	53.65±12.32 (92)	53.44±12.96 (106)	-0.36	0.718
LDL-C (mg/dl)	115.17±31.67 (92)	145.57±33.54 (106)	-6.34	0.001*
Calcium (mg/dl)	9.46±0.49 (68)	9.46±0.42 (61)	-0.09	0.923
Magnesium (mg/dl)	1.97±0.19 (73)	2.01±0.19 (65)	-1.85	0.064
Sodium (mmol/L)	138.41±1.95 (58)	139.86±1.74 (58)	-3.81	0.001*
TSH (mIU/L)	1.81±0.98(70)	1.73±0.94(70)	-0.22	0.819
BMI	24.23±3.89 (81)	27.55±4.28 (77)	-4.80	0.001*

*SD: Standard Deviation; BMI: Body Mass Index; *Mann-Whitney U test is significant at the 0.05 level (2-tailed).*

Table 4: Correlation of vitamin D with biochemical and other parameters in age groups

Vitamin D	*< 41 years*	> 42 years
	r_s value; p-value (n)	
Glucose (mg/dl)	-0.061; 0.561 (92)	-0.040; 0.685 (106)
Cholesterol (mg/dl)	-0.251; 0.016 (92)*	-0.086; 0.379 (106)
Triglycerides (mg/dl)	-0.179; 0.088 (92)	-0.077; 0.430 (106)
HDL-C (mg/dl)	0.133; 0.207 (92)	-0.106; 0.278 (106)
LDL-C (mg/dl)	-0.313; 0.002 (92)**	0.025; 0.796 (106)
Calcium (mg/dl)	0.018; 0.881 (68)	0.213; 0.099 (61)
Magnesium (mg/dl)	0.072; 0.547 (73)	-0.077; 0.541 (65)
Sodium (mmol/L)	-0.037; 0.782 (58)	0.033; 0.803 (58)
TSH (mIU/L)	-0.100; 0.408 (70)	0.101; 0.404 (70)
BMI	-0.071; 0.526 (81)	0.024; 0.883 (77)

*r_s value: Spearman correlation; BMI: Body Mass Index; *Correlation is significant at the 0.05 level (2-tailed) **Correlation is significant at the 0.01 level (2-tailed)*

A statistically significant correlation was found between vitamin D and total cholesterol and LDL-C in the young age group. However, serum vitamin D had no significant correlation with serum glucose, triglyceride, HDL-C, calcium, magnesium, sodium, TSH levels, and BMI scores (Table 4).

A linear regression analysis was performed to determine the vitamin D level affecting LDL-C levels in the younger age group below 41 years old. Variables correlated with LDL-C, including vitamin D, age, glucose, triglycerides, and sex, were added to the model for analysis (Table 5).

According to the regression analysis results, in model one, (R = 0.218, R2 = 0.048, F (1, 90) = 4.91, P = 0.037) and in model two, (R = 0.566, R2 = 0.321, F (4, 86) = 8.652, P < 0.001) were found to be significant. Also, in the regression analysis, the decrease in vitamin D level is associated with 0.048% of the increase in LDL-C. However, when other parameters were included in the analyses, the association of vitamin D level with LDL-C level did not reach statistical significance (P=0.309) (Table 6).

Table 5: Correlation of LDL-C with vitamin D, age, sex, glucose, and triglycerides in age groups

LDL-C	**41 years and below**	**42 years and above**
	r_s value; p-value (n)	
Vitamin D (ng/ml)	-0.313; 0.002 (92)**	0.025; 0.796 (106)
Age (years)	0.294; 0.004 (92)**	0.119; 0.224 (106)
Sex	-0.160; 0.128 (92)	-0.135; 0.168 (106)
Glucose (mg/dl)	0.236; 0.024 (92)*	-0.001; 0.993 (106)
Triglycerides (mg/dl)	0.455; 0.001 (92)**	0.100; 0.390 (106)

*r_s value: Spearman correlation; *Correlation is significant at 0.05 level (2-tailed) **Correlation is significant at 0.01 level (2-tailed)*

Table 6: Regression analysis for the association between LDL-C and vitamin D, age, sex, glucose, and triglycerides in the young age group 41 years and below

Coefficients ^{a}							**95.0% CI for B**	
	Variable	**B**	**SE**	**Beta**	**t**	**p**	**Lower**	**Upper**
Model 1								
R = 0.218; R^2 = 0.048 F (1, 90) = 4.91; P = 0.037	(Constant)	126.92	6.43		19.76	<0.001	114.16	139.69
	Vitamin D	-0.64	0.30	-0.22	-2.12	0.037*	-1.24	-0.04
Model 2								
R = 0.566; R^2 = 0.321 F (4, 86) = 8.652, P < 0.001	(Constant)	19.01	35.73		0.53	0.596	-52.04	90.06
	Vitamin D	-0.29	0.28	-0.09	-1.02	0.309	-0.85	0.27
	Age	1.20	0.39	0.28	3.09	0.003*	0.43	1.98
	Sex	-25.70	8.07	-0.33	-3.19	0.002*	-41.75	-9.66
	Glucose	0.83	0.40	0.20	2.11	0.038*	0.05	1.62
	Triglycerides	0.23	0.06	0.38	3.92	<0.001*	0.11	0.34

^{a}Dependent Variable: LDL-C; B: Unstandardized Coefficients; Beta: Standardized Coefficients; CI:Confidence Interval; R^2 = R square; SE: Standard Error; Linear regression analysis is significant for P< 0.05 level (2-tailed)

DISCUSSION

In our study, serum vitamin D, calcium, and magnesium levels were lower in women than in men, whereas HDL-C was higher in women. In addition, serum glucose, total cholesterol, triglyceride, LDL-C and sodium levels, and BMI scores were lower in young individuals than in the elderly. These findings show that the population evaluated within the scope of our study conforms to normal distribution. In addition, lower serum glucose, triglyceride levels, and BMI scores found in women could be explained by the lower mean age of women.

Findings of no correlation between serum vitamin D with total cholesterol, triglyceride, HDL-C, LDL-C, glucose, calcium, magnesium, sodium, and TSH levels and BMI scores in either men or women in our study contradicts the common view in the literature. In a meta-analysis, it has been reported that vitamin D supplementation decreases total serum cholesterol, triglyceride, and LDL-C levels in patients with type 2 diabetes [15]. Supporting this, in one study conducted on patients with type 2 diabetes, vitamin D supplementation was suggested to reduce serum glucose and triglyceride levels [17]; in another study, higher serum triglyceride levels were associated with lower serum vitamin D levels [18].

Contrary to the findings about vitamin D levels and lower LDL associations, a meta-analysis study found that vitamin D supplementation increased serum LDL-C levels but did not affect HDL-C and triglyceride levels [16]. In a study conducted in the general population in Spain, a statistically significant association was found between total cholesterol and LDL-C levels with vitamin D levels [19]. In another study in Turkey partially similar to our findings, no statistically significant difference was found between total cholesterol, LDL-C, and triglyceride levels compared to vitamin D levels. [20]. Although a negative correlation was found between serum vitamin D and total cholesterol and LDL-C levels in young people in our study, this correlation did not reach significance when other parameters were included.

However, a human epidermal keratocyte cell culture study suggests an indirect effect of vitamin D on cholesterol synthesis, in which total cholesterol synthesis and LDL-C organization were increased in cells exposed to low calcium concentrations [21]. Our study did not support this, and there was no correlation between vitamin D and calcium levels.

The correlation between vitamin D and LDL-C in young people did not persist in the multiple linear regression analysis. In a large-scale study on 11734 individuals from the adult population, LDL-C and calcium levels were significantly higher in the group with high vitamin D levels, and patients with co-morbid situations, type 2 diabetes, hypertension, dyslipidemia, and atherosclerotic heart disease were unlikely to have less Vitamin D deficiency (<20 ng/mL) [22]. However, these studies contradict the widespread belief that vitamin D affects LDL-C and calcium levels.

CONCLUSIONS

As a result, our findings indicate no significant effect of vitamin D on calcium metabolism, and contradictory findings regarding its effects on glucose and lipid parameters suggest that the common view asserting a relationship between vitamin D and several metabolic and hormonal disorders may be inaccurate. Studies suggesting an association between vitamin D and some diseases were usually conducted on limited relevant patient groups. Thus, the margin of error is expected to be high in generalizing the results from limited studies.

For this reason, with the findings of this study and the current literature, it would be necessary to further elaborate on the cause-effect relationships and to conduct new studies.

Conflicts of interest and sources of funding
The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. This research received no external funding.

Authors' contribution
All authors made substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of data for the work.

Ethics approval and consent to participate
Ethical approval was obtained from the İstanbul Training Research Hospital Clinical Research Ethics Committee (Decision no. 09.08.2019, 1944).

Patient consent for publication
Because is a retrospective study, patient consent was not obtained.

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ORIGINAL ARTICLE

The Current State of Knowledge Regarding the Use of Double J Catheters in Treating Obstructive Urolithiasis

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Abstract: *Urinary lithiasis is a common pathology in the modern era. Its significance lies in the possible complications that may arise as well as in its potential for recurrence. The treatment and prevention of recurrences of urinary lithiasis often require the intervention of several specialists: urologists, nephrologists, endocrinologists, nutritionists, biochemists, etc. In the last 20 years, the treatment strategy for urinary lithiasis has changed, with minimally invasive methods replacing laparoscopic or open surgery. These are effective and have rare complications. Whichever treatment method is chosen, it may be necessary to temporarily divert the upper urinary tract by inserting double J catheters for preventive, curative, or palliative purposes. Ureteral catheters have had to be improved over time to avoid two major incidents: their migration and colonization. Various materials were used, varying the shape, size, length, guide as well as approaches. The urinary infection-urolithiasis association is frequent, without always being able to specify the cause-effect relationship. The rate of urinary colonization appears to be influenced by the presence of stent colonization as well as the time since the implant. The association of chronic diseases or emergency insertion is associated with an increased risk of urinary colonization. Complications induced by the time of the double J catheter being implanted are rare, and minor and disappear with its removal. In the case of failure to insert a double J ureteral catheter, the alternative would be to perform an ultrasound-guided percutaneous nephrostomy. Double J ureteral catheter insertion is an effective minimally invasive option in the treatment of obstructive urolithiasis.*

Keywords: *urolithiasis, double J catheter, obstructive urolithiasis, urinary colonization*

INTRODUCTION

Urinary lithiasis is a frequent and often recurring pathology, with a changing evolution (asymptomatic or with a noisy clinical picture). Its incidence is increasing in developed countries where the prevalence in the general population varies between 8 and 10%, tripling in the last 40 years.

By the end of the 19th century, phosphate-uric urinary lithiasis with bladder localization prevailed, and starting from the 20th century the oxalic-calcium urinary lithiasis predominates (70-80% of cases) with renal localization. It mainly affects the population between 20 and 60 years old, with a double rate for men compared to those women.

The recurrence rate in this population is approximately 50% between 5 and 10 years, with approximately 10% of patients presenting multiple episodes of lithiasis recurrence [1,2].

Urinary lithiasis is often revealed by nephrotic colic. Acute nephrotic colic represents about 1-2% of examinations in Emergency Departments, and about 6% of them require hospitalization due to their complications [2,3].

The treatment of a simple, uncomplicated nephrotic colic consists of analgesic treatment (NSAIDs in the absence of contraindications), while a complicated nephrotic colic requires hospitalization and often emergency urinary drainage. The technique is usually chosen according to the size, location, and chemical composition of the stones as well as the morphology of the excretory urinary tract.

Prophylaxis of urolithiasis recurrences requires a general metabolic examination as well as a morpho-constitutional and IR spectrophotometric examination of removed stones.

Urological treatments available today are extracorporeal lithotripsy, rigid or flexible ureteroscopy, and percutaneous nephrolithotomy. They use different energies to destroy the calculus: shock waves, pneumatic ballistic energy, Holmium laser, and ultrasounds. The results of these treatments can sometimes be improved by expulsive medical therapy and post-urotherapy.

The new technological discoveries, their effectiveness, as well as the few complications of urological instrumental treatments in urinary lithiasis have led to a decrease in the indications for surgery (laparoscopic or open). They remain used in case of failure of instrumental treatments or cases of giant stones. Thus, in less than 20 years, the urolithiasis treatment strategy has changed through the use of mini-invasive and highly effective methods [4].

The indications for instrumental urological treatment of urinary stones are:
- symptomatic stones: pain, hematuria, infection;
- complicated stones: urinary obstruction, infection;
- evolutionary stones: the increase of calculus size measured by imaging means;
- stones of significant size: over 1.5 cm for kidney stones and over 6 mm for ureteral stones;
- evolutionary lithiasis disease: the presence of more than two episodes per year;
- infected or infectious stones;
- at the request of the patient;
- in fragile or complex patients (single kidney, kidney failure, heart failure, anticoagulated, etc).

DOUBLE J URETERAL CATHETERS

Regardless of the treatment method approach for urinary lithiasis, it may be necessary to insert a double J endo-prosthesis or catheter for a defined period in case of complete obstruction or secondary infection [5].

Today, the use of double J ureteral catheters is common practice in urology departments.

According to medical dictionaries, a stent is defined either as a device used to maintain an opening, cavity, or contour or as a catheter with a tubular structure intended to maintain an open lumen. The word stent became common in the specialized literature when Montie explicitly defined it as "an intraluminal device to maintain patency of the ureter until healing occurs" [6].

The concept of ureteral stent appears as an adjunct to open surgery to achieve drainage of the upper urinary tract ensuring the continuity of the ureters. In the 19th century, Gustav Simon places a tube in the ureters during a cystostomy and in 1839 Joaquin Albarrano creates the first ureteral catheter made of varnished textile material. In 1952, Tulluch described the use of polyethylene tubes to "repair" ureters and fistulas, and in 1967 Zimskind endoscopically inserted the first silicone ureteral catheter. Their main defect seems to be the migration of the catheters in both directions, and to eliminate it, in 1978 Finney describes for the first time the double J ureteral catheter, provided with loops at both ends [6].

The JJ ureteral catheter used today is an auto-static, flexible tube made of polyurethane or silicone, with curved ends to be able to maintain it between the kidney at the upper extremity and urinary bladder at the lower extremity [7,8,9].

Double J ureteral catheters must comply with the following conditions:
- to be radio-opaque for good visibility on radiography;
- to be provided with visible marks during endoscopy for good positioning;
- have a low surface tension to limit friction during insertion;
- to be inserted anterograde or retrograde;
- to be simple to insert, change, extract, biocompatible and resistant;
- to have a good caliber for urine drainage and at the same time a low risk of obstruction;
- have a low price.

Regardless of the purpose of its insertion (preventive, curative, palliative) or its indications, the double J catheter must ensure the derivation of the upper urinary system by physiological pathways and the flow of urine from the kidney to the bladder [10,11]. It suppresses the pressure due to the lithic obstruction eliminating the pain and by increasing the caliber of the ureter facilitates the migration of stones or their extraction by ureteroscopy.

The indications of the double J catheter are:
- ureteral obstruction by calculus responsible for nephrotic colic;
- preventively, before extra-corporeal lithotripsy to avoid possible obstruction with residual fragment;
- in the case of complicated pelvic surgeries (urological, digestive, gynecological);
- in the case of extrinsic compression of the ureter (tumors, fibrosis, etc.);
- in congenital anomalies, trauma, or iatrogenic insults.

Over time, methods have been sought to decrease the colonization rate of ureteral catheters by using different materials (silicone, polyethylene, polyurethane, biodegradable materials) or by covering the catheters with thin protective films (heparin, silver, polytetrafluoroethylene, antibiotics) [12]. Some urease inhibitors have been used to reduce and prevent stent encrustation or obstruction [13].

In the case of urolithiasis with complete obstruction, there is an indication for the derivation of the upper urinary system by inserting double J catheters, and in case of failure, a percutaneous nephrostomy guided by ultrasound will be performed [14]. This more aggressive technique aims to obtain a kidney without residual fragments at the end of the intervention.

The catheters vary in size (from 3 to 9), length (between 12 and 30 cm), guide (simple metal, Teflon, lubricated polyurethane monofilament), material (polyvinyl chloride, silicone) [15,16, 17].

The catheter is inserted under general anesthesia or loco-regional anesthesia [18].

Two approaches can be used [19]:
- naturally through cystoscopy under radiological control;
- percutaneously after renal puncture (nephrostomy) under radiological control.

The J catheter can be kept from a few days to a few months (generally 3 months) depending on the indications. Removing too early can be ineffective and complications (calcifications, obstructions) can occur if kept too long [20].

During catheter indwelling, various unwanted effects can be mentioned [21,22,23]:
- frequent urination (indication of anticholinergics);
- lumbar pain during urination (indication of minor analgesics);
- bladder discomfort (indication of antispasmodics);
- hematuria (indication of correct hydration – 1.5 l/24 h and avoidance of physical efforts);
- urinary infections (indication for antibiotic treatment).

The ablation of the JJ catheter is generally done by cystoscopy, under local anesthesia, or in ambulatory care (takes less than 5 min).

COMPLICATIONS

There is a frequent association between urinary infection and urolithiasis. However, it is difficult to determine if the calculus became infected secondarily or if the infection preceded the calculus and was responsible for its formation.

According to European and American scientific societies, urinary infections are defined by the association of significant bacteriuria with symptoms evocative of a urinary infection in the absence of another infectious focus. Symptoms and clinical signs compatible with a urinary infection can be varied, from functional urinary signs such as pollakiuria, and burning during urination – to non-specific symptoms such as fever, chills, psychomotor retardation, confusion, spasticity, etc. Urinary colonization corresponds to the presence of one or more microorganisms in the urinary tree without generating clinical manifestations. A urinary infection is considered if the clinical signs and symptoms are associated with a bacteriuria higher than 105 CFU/ml (103 CFU/ml for women and 104 CFU/ml for children). It is known that immunosuppressed, pregnant women, alcoholics, and malnourished, patients with chronic renal or neurological diseases have a higher rate of urinary colonization.

The risk of urinary colonization is significantly higher in patients with colonized JJ catheters, especially if the indwelling time is over 45 days.

Complications related to the maintenance of the double-J catheter are rare (hematuria, infection and/or colonization, catheter fragmentation, encrustation) and occur especially with long-term stent indwelling (more than 30 days) and disappear with its ablation.

Failing to remove all residual fragments after instrumental urological treatment of urolithiasis is a major factor of recurrence (50%), even for residual fragments smaller than 2 mm. Under these conditions, any instrumental treatment must be systematically imaging evaluated every 3 months with a reno-vesical CT or ASP + reno-vesical ultrasound.

In the case of residual fragments after a urological instrumental treatment, there are two possibilities:

- expulsive medical treatment: the administration of an alpha-blocker (tamsulosin) would favor the elimination of the fragments and reduce the painful crises associated with their elimination;

- post-urotherapy performed in ventral decubitus and incline at 30o - 45o to favor the elimination of sedimented fragments in the lower calyces.

Any urinary calculus requires a complex examination consisting of a morpho-constitutional analysis and IR spectrophotometry, metabolic balance, dietary survey, hygienic-dietary measures, and possibly medical treatment.

CONCLUSIONS

The treatment strategy for obstructive urolithiasis has changed in the last 20 years with the new high-performance, mini-invasive techniques.

Double J ureteral catheter insertion in obstructive urolithiasis is a minimally invasive option for high urinary tract diversion, regardless of the treatment method chosen for urolithiasis.

The double J catheter can be inserted for different purposes: preventive, curative, and palliative.

The risk of urinary colonization is significantly higher in patients with colonized JJ ureteral stents.

The prevalence of urinary colonization increases with the indwelling time, especially in the case of emergency insertion.

The association of chronic diseases such as diabetes, hypertension, CKD, and disabling neurological diseases is associated with an increased risk of urinary colonization.

In pelvic ureteral lithiasis, spontaneous expulsion of the stone is possible, probably due to ureteral dilatation secondary to the presence of the ureteral prosthesis.

Complications related to the indwelling of the double J catheter are rare and occur especially in the case of long-term indwelling (more than 30 days) and disappear with its ablation.

In case of failure to insert a double J ureteral catheter, the alternative for a high urinary system derivation is an ultrasound-guided percutaneous nephrostomy.

Abbreviations

CKD - Chronic Kidney Disease

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EDITORIAL AND CONTENT CONSIDERATIONS

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

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– Open to all prospective registrants free of charge or at minimal cost;

– Validates registered information;

– Identifies trials with a unique number; and

– Includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of the last follow-up, a target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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Acknowledgments

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ORIGINAL ARTICLE

Laparoscopic Wedge Resection for Gastric Mesenchymal Tumor – Small Case Series

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Abstract: *Introduction: Gastric mesenchymal tumors are a pathology that is increasing in incidence in recent years but the treatment is not yet standardized. The laparoscopic approach to these lesions is a modern option, with the patient benefiting from the advantages of minimally invasive surgery. Methods: We included in the study 4 patients operated laparoscopically for suspicion of gastric GISTs between Mars 2019 and May 2022. From a prospectively kept database, we extracted the following characteristics: age, sex, length of postoperative hospital admission, operation time, tumor location, tumor size, histopathological assessment of resection margins, intraoperative blood loss, and incidence of postoperative complications. Results: Average tumor size was 4.3 cm, ranging from 4 to 6 cm. Laparoscopic wedge resection was possible in all cases. There was no conversion, no episode of tumor rupture or spillage, and no major intraoperative complications. Histology confirmed a GIST in 3 of 4 cases. In one case histological workup showed gastric schwannoma. Conclusion: Laparoscopic wedge resection for gastric stromal tumors, even larger than 5 cm, appears to be safe and feasible in selected patients, with acceptable operative time and oncological outcome after an intermediate follow-up. More important than the size of tumors that are approached laparoscopically is their location and the possibility of a stenosis-free resection.*

Keywords: *laparoscopic wedge resection, Gastrointestinal Stromal Tumors (GIST), gastric mesenchymal tumors, Schwannoma*

INTRODUCTION

Gastric tumors can be divided into epithelial and subepithelial tumors based on their origin. 90% of gastric tumors including adenoma and adenocarcinoma originate from epithelium [1]. Various tumors occur as subepithelial lesions, including mainly gastrointestinal stromal tumors (GISTs) and leiomyomas, less often neuroendocrine tumors, schwannoma, lymphangioma [1], lipomas, granular cell tumors, and ectopic pancreatic tissue [2].

Epidemiology

GIST is the most common mesenchymal tumor of the gastrointestinal tract [3], with an incidence of about 1-1.5/100,000 persons/year [4,5]. Few studies have indicated that the incidence of GIST is greater in patients undergoing bariatric surgery (0.6%- 0.8%) than in the general population [6]. Increased body mass index has been linked with a higher risk for several types of cancer. Another possible hypothesis for the high incidence found in these studies is that many small, asymptomatic GISTs are underdiagnosed in the general population [7,8].

Gastric stromal tumors account for about 60-70% of all GISTs [4,9,10]. The term is relatively recent, it was first used in 1983, to reclassify a subset of tumors previously identified as leiomyomas or leiomyosarcomas [11,12]. Typically, GISTs are solitary tumors identified slightly more often in males than females of middle to advanced age (between 40 and 80 years) [6,7]; however, the estimated mean age at diagnosis is in the sixth decade of life [13].

Histological Findings

GIST originates from the interstitial cells of Cajal, the pacemakers of the gastrointestinal tract [14], or their stem cell precursors [15]. Their defining characteristic is a gain-of-function mutation in genes coding for the KIT tyrosine kinase receptor, which is considered the driving force of cell proliferation in this tumor [16].

Diagnosis of GIST was based on microscopic analysis, including immunohistochemistry with a panel of antibodies: Cluster of

Differentiation-117 (CD117), Cluster of Differentiation-34 (CD34) and Discovered on GIST-1 (DOG1), S-100 protein, desmin, and smooth muscle actin [17,18].

Natural Evolution

GISTs are not classified as either benign or malignant but are rather stratified by their clinical risk of malignancy: very low, low, intermediate, or high [13]. The malignant potential depends on tumor cell mitosis, tumor size, tumor location [17, 19], and tumor rupture [5]. Approximately 10% to 30% of GISTs have a malignant clinical course [13] because they can metastasize hematogenously to the solid viscera, including the liver, small bowel, and lungs [14]. In these conditions, the only absolute signs of malignancy are metastasis or invasion into adjacent organs [16].

Biologically, tumors grow locally within the stomach wall (intra- or extraluminal expansion) and can also spread along the smooth muscle planes within the stomach or can rupture into the peritoneal cavity causing sarcomatosis [10].

Clinical presentation and incidental GIST

The most common symptoms of GISTs are gastrointestinal bleeding, including acute melena and hematemesis [13] with subsequent anemia, weakness, and abdominal pain [18], distension, and discomfort due to a tumor-induced mass effect or metastasizing tumors [16]. Tumor hemorrhage commonly occurs when large tumors develop an ischemic, punctate ulcer. Usually, the bleeding can be controlled using endoscopic sclerotherapy or electrosurgical coagulation techniques [10].

15% to 30% of patients with GISTs are asymptomatic and are diagnosed incidentally, during examinations for other reasons, during surgery for other diseases [6,15], or during postmortem autopsy [13]. In these asymptomatic patients, tumors grow insidiously and can sometimes reach large sizes.

Diagnostic Process

With the popularity of screening endoscopy, increasing gastric submucosal tumors are incidentally detected [20]. The main endoscopic finding of GISTs is common to all subepithelial lesions: a nonspecific smooth bulge covered with normal mucosa [13].

Diagnostic workup can be challenging and consists of endoscopy, ultrasonography, computed tomography, magnetic resonance imaging, and endoscopic ultrasonography with fine needle aspiration [13]. However, a biopsy specimen is often negative [15].

Treatment

The treatment strategy for immunohistologically confirmed GISTs is as follows: surgical resection is the first choice for resectable GISTs without metastasis and administration of imatinib is the primary approach for unresectable, metastatic, or recurrent GISTs [13].

Medical Treatment

In 2000, imatinib (first-line tyrosine kinase inhibitor) was first used to treat patients with advanced GIST, achieving unprecedented treatment responses in a tumor type that – when metastatic – was previously rapidly lethal [12]. The drug was approved for use as adjuvant treatment after the surgical removal of high-risk GISTs in 2012 when it is administered in an attempt to reduce the recurrence rate [8]. Sunitinib (second-line tyrosine kinase inhibitor) and Regorafenib (third-line multikinase inhibitor) can be used in advanced cases after treatment failure with Imatinib. However, it is difficult to obtain a permanent cure with tyrosine kinase inhibitors [13].

Surgical Treatment

In contrast to gastric adenocarcinoma, where gastrectomy with wide margins and lymphadenectomy is recommended [10], limited gastric resection is the recommended surgical approach for GIST, because it grows by expansion rather than diffuse infiltration and rarely invades nearby lymph nodes [5, 14,19]. Wide margins and lymph node dissection do not improve survival or decrease recurrence [11].

The surgery aims to achieve a complete resection with no tumor residue [17,21]. Moreover, strict avoidance of intraoperative tumor rupture is crucial for preventing tumor relapse [16].

Traditionally gastric GISTs resection was done with open surgery, but, more recently, less invasive methods have been reported [18]. The first description of laparoscopic resection of a GIST was in 1992 when Lukaszczyk discovered and resected an incidental gastric mass during a laparoscopic cholecystectomy [11]. The choice of the laparoscopic or open method was made by the surgeon based on his evaluation and the patient's consent [19].

As experience accumulated, initially tumors up to 2 cm [18, 22], then between 2 and 5 cm [4], and more recently between 5 and 10 cm were approached laparoscopically [21]. Prerequisites for the laparoscopic approach were the absence of metastases and the presence of a well-defined tumor on CT scanning without the involvement of adjacent organs, the esophagogastric junction, or the pylorus of the stomach [18, 19]. However, wedge resection sometimes involves inadvertent resection of additional gastric tissue, which can produce gastric deformation. Anatomic gastrectomy (subtotal or total gastrectomy) is reserved for large tumors that involve a significant portion of the stomach [10].

METHODS

After obtaining our institutional review board approval, we retrospectively reviewed the medical records of our patients operated laparoscopically for gastric GISTs between March 2019 and May 2022.

Study Population and Data Analysis

We extracted the following characteristics: age, sex, length of postoperative hospital admission duration, operation time, tumor location, tumor size, histopathological assessment of resection margins, intraoperative blood loss, and incidence of postoperative complications (surgical and non-surgical). Patients were eligible for laparoscopic surgery if preoperative staging (endoscopy and CT scan) showed a localized, non-metastatic extra mucosal gastric lesion (Figures 1, 2). Tumors were required to be of a diameter and in a position that suggested resectability through segmental or wedge resection. Preoperative histological confirmation of the diagnosis was not a prerequisite for inclusion.

Figure 1: Subepithelial gastric tumor with a clot on the surface (Case 4)

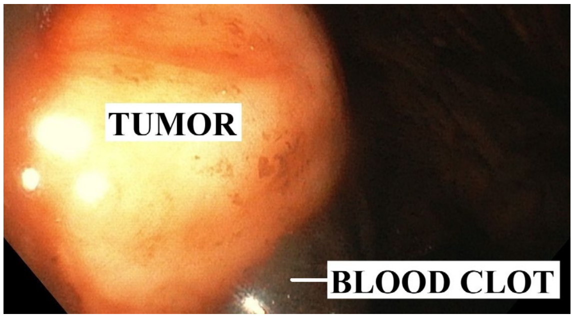

Figure 2: CT scan image (Case 1)

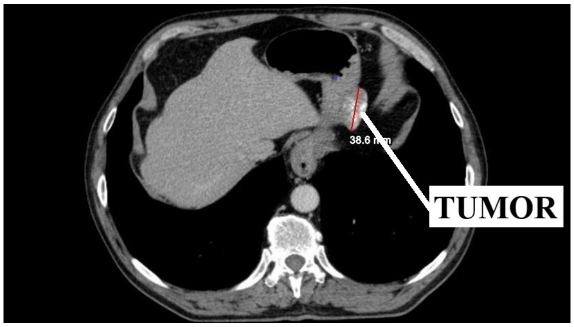

Surgical Resection Procedures

All patients were treated by laparoscopy while under general anesthesia. The patient was placed supine in the anti-Trendelenburg position and the surgeon stood between the legs. Using a Veress needle, the pneumoperitoneum was achieved with CO_2 at 12 mmHg pressure. The first trocar was placed on the median line, about 15 cm from the xiphoid process. Under visual control, another 3 or 4 work trocars were introduced. After checking all the abdominal cavities for metastases, the lesion was identified through visualization and palpation. In the case of tumor location at the posterior wall of the stomach, the gastrocolic ligament was dissected and the stomach was inverted to display the lesion. The tumor-bearing gastric segment was resected with several 60 mm endoscopic linear staplers (EndoGIA) (Figure 3). To avoid stenosis of the stomach during wedge resection, a calibration tube (36 Fr) was inserted by the anesthesiologist. Special care has to be taken to prevent peritoneal seeding and the possibility of capsular rupture during manipulation.

Figure 3: Intraoperative image, after the first EndoGIA cartridge is placed (Case 4)

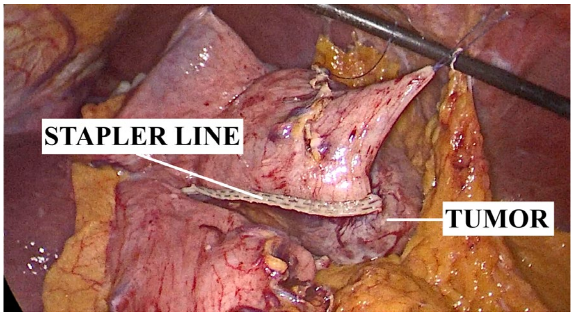

The resection specimen was placed into a plastic retrieval bag and removed through one of the extended port incisions. Stapler lines were visually controlled and, if deemed necessary by the surgeon, their integrity was assessed through the application of methylene blue solution via a nasogastric tube (blue test). At the end of the operation, a drain tube was placed in the vicinity of the staple line.

Pathological evaluation

The specimens were fixed in 10% formalin solution and sent for pathological evaluation. The characteristics of gross specimens were described, including tumor size, gross appearance, ulceration (Figures 4, 5). After sectioning, hematoxylin and eosin staining was regularly performed followed by immunohistochemical staining.

Figure 4: The specimen, after extraction (Case 3)

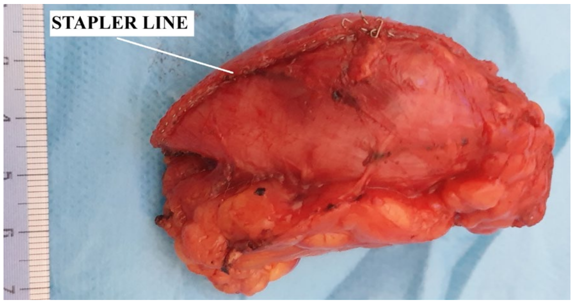

Figure 5: The specimen, with the suture line open (for inspection of the resection margins) (Case 2)

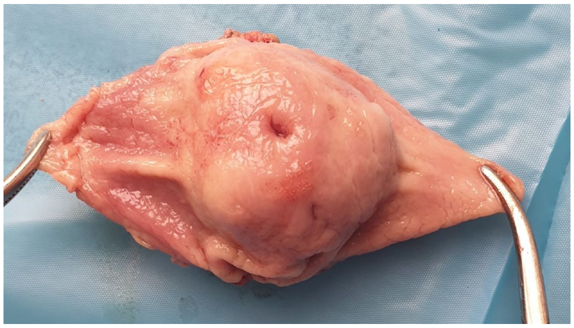

Follow up

Patients with immunohistochemically confirmed GIST were referred to the oncology department for therapeutic management

RESULTS

Four patients were planned for a laparoscopic wedge resection of a suspected gastric GIST. The presented cases were 3 females and 1 male with a median age of 70.25 years (between 64 and 74 years).

The reasons for admission to Gastroenterology Department were abdominal pain (3 patients), weakness (2 patients), anemia (2 patients), and gastrointestinal bleeding (1 patient).

All patients were investigated by endoscopy and CT scan. The average tumor size was 4.3 cm, ranging from 4 to 6 cm. The main patient data are summarized in Table 1.

The average operation time was 77.5 min, ranging from 60 to 100 min. Resection was performed using between 2 and 3 cartridges. Because the operated lesions were large, there was no need for intraoperative endoscopy to identify the tumor's location. Laparoscopic wedge resection was possible in all cases. There was no conversion, no episode of tumor rupture or spillage, and no major intraoperative complications. Intraoperative blood loss was below 50 mL in all patients and no patient required blood transfusions. The surgical margin of all tumors was confirmed using the extracted specimen.

All tumors were resected with macroscopic and pathologically negative margins. Histology confirmed a GIST in 3 of 4 cases. In one case histological workup showed gastric schwannoma.

The median postoperative hospital stay was 5.5 days, ranging from 5 to 7 days. Hospitalization was 5 days for 3 of the patients and 7 days for one patient who had a postoperative complication. One patient (with a tumor size of 3.5 cm) experienced a postoperative hemorrhage (grade II complication after Clavien-Dindo classification). This was treated conservatively with proton pump inhibitors. We had not identified any other postoperative complication.

Table 1: Patients data

Case number	1	2	3	4
Age	74	71	64	72
Sex	Male	Female	Female	Female
Histology	GIST	GIST	Schwannoma	GIST
Location	Fundus/ Greater curvature/ Anterior wall	Body of the stomach/ Lesser curvature/ Posterior wall	Body of the stomach/ Lesser curvature/ Anterior wall	Body of the stomach/ Greater curvature/ Posterior wall
Morphology	Pediculated	Extraluminal	Extraluminal	Extraluminal
Operation time	70 minutes	100 minutes	80 minutes	60 minutes
Number of cartridges	2	2	2	3
Dimension of resection	6x5 cm	7x6 cm	6.2x5 cm	9x8 cm
Tumor size	4 cm	3.5 cm	4 cm	6 cm
Tumor staging	pT_2	pT_2	-	pT_3
Risk of malignancy	Very low	Low	-	High

In the same period, outside this study, in our department, a patient with a massive tumor (18.8/18/13 cm) was treated by open subtotal gastrectomy.

All patients were referred for oncological consultation, but only 2 patients did it. Due to the favorable histopathological exam, absence of distant metastases at the time of diagnosis and on the subsequent follow-up (annually CT), and absence of local recurrence on follow-up endoscopy (6 months postoperatively, then annually), the patients did not indicate adjuvant chemotherapy.

All patients were alive at the end of this study and in good health.

DISCUSSION

Open surgery was the traditional and most widely used treatment for gastric stromal tumors. When possible, the endoscopic or laparoscopic approach is safe and leads to non-inferior outcomes compared to the open approach [21].

Although our experience is limited, we can state that the cases we have treated by laparoscopic wedge resection and presented have progressed favorably, benefiting from the well-known advantages of laparoscopic surgery. Among the other variants of the laparoscopic approach found in the literature, we list the following: non-anatomic full-thickness removal of a disk of tissue with the closure of the resulting gastrotomy, anatomic partial gastrectomy with reconstruction, and transgastric resection [11].

The choice of laparoscopic approach must take into account both the size of the tumor and its location. Visible lesions on the anterior gastric wall are most suitable for wedge resection, consisting of lifting the lesion and performing a wedge resection by application of one or more endoscopic linear stapler devices. But selected cases can be approached laparoscopically even if they are not located on the anterior wall. On the other hand, patients with lesions near cardia or pyloric antrum are not proper candidates for wedge resection due to potential stenosis. Therefore, limited gastrectomy is usually used, but alterations of anatomic structures may lead to functional disturbance and impaired quality of life [20]. In our patients, we did not find any early postoperative stenosis. Concerning long-term functional results, we were not confronted with symptoms or endoscopic signs of reflux or stenosis during postoperative follow-up.

As experience with minimally invasive surgery has increased over the past decade, laparoscopic surgery for gastrointestinal malignancies has become increasingly common [5]. As in other laparoscopic procedures, the existence of a learning curve must be assumed. However, due to the experience gained by the two senior surgeon authors in oncological surgery and bariatric surgery (sleeve gastrectomy), the surgical interventions for the presented cases were carried out in good conditions. Being tumors with malignant potential, all measures must be taken to prevent tumor injury.

In our small case series, 1 out of 4 tumors preoperatively suspected to be a GIST were histologically diagnosed as gastric schwannoma. Schwannomas are benign, slow-growing, encapsulated nerve-sheath tumors arising from Schwann cells, meant for the myelin sheath formation in the peripheral nervous system. Gastric schwannoma is a rare gastric mesenchymal tumor, which accounts for 6.3% of the gastric mesenchymal tumor, 4% of all benign gastric tumors, and 0.2% of all gastric tumors. Being different from GISTs, they are considered as being benign, slowly growing, and with excellent prognosis [20]. Retrospectively, in this case, surgical resection would have not been required for oncological reasons. We also found this situation in the literature studied [14,16,23], and it's explainable because we didn't have a preoperative histological diagnosis. On the other hand, it is considered that all localized resectable gastric stromal tumors with a diameter of 2 cm or more need resection [2,4]. Preoperative histological diagnosis of submucosal gastric lesions is however not always feasible through endoscopic biopsy [16]. With the benign and mostly asymptomatic nature of gastric schwannomas, endoscopic follow-up is also an alternative method. But it is not widely adopted due to the lack of a standardized surveillance strategy, the risk and cost-effectiveness of repeated endoscopic procedures, and patients’ stress and compliance [20].

CONCLUSIONS

In conclusion, laparoscopic wedge resection for gastric GIST, even larger than 5 cm, appears to be safe and feasible in selected patients, with acceptable operative time and oncological outcome after an intermediate follow-up.

More important than the size of tumors that are approached laparoscopically is their location and the possibility of a stenosis-free resection.

Conflicts of interest and sources of funding

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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REVIEW

The Role of Inflammation in the Pathogenesis of Psoriasis

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Abstract: *Psoriasis is a chronic inflammatory skin condition with genetic determinism characterized by the presence of well-defined, erythematous plaques, covered by white, pearly, stratified scales, located on the extension areas, the skin of the scalp, intertriginous regions. The origin of psoriasis is multifactorial, involving hereditary and environmental pathogenic mechanisms. It is triggered by various risk factors involving a variety of processes, such as inflammation, antigen presentation, cell signaling, and transcriptional regulation.*

Keywords: *psoriasis vulgaris, inflammation, immune system*

OVERVIEW

Definition

To have a starting point and be able to go deeper into a subject, as in any field, and in the case of diseases, we must first know the definition. A definition must be as complete as possible, to create an overall picture of the disease.

Psoriasis is a chronic inflammatory skin condition [1], proliferative [2], with genetic determinism [3], characterized from a clinical point of view by the presence of well-defined, erythematous plaques [4], covered by white, pearly, stratified scales [5], located on the extension areas and in the skin of the scalp.

If it was initially considered a benign dermatological condition, psoriasis is now accompanied by multiple comorbidities, being considered a multisystemic inflammatory disease [6]. In addition to the impact it has from an aesthetic point of view, psoriasis also significantly influences patients' quality of life [7].

A recent study estimated that the psychological impact in patients with psoriasis is severe, between 10-62% [8]. Among the most common symptoms are: low self-esteem, sexual dysfunction, suicidal ideation, which occurs in up to 10% of patients with psoriasis, decreased professional opportunities, stress – which can trigger psoriasis flare-ups, depression [8].

Incidence

Worldwide, the prevalence of psoriasis is between 0.5%-11.4% in adults [9], and this increases with increasing distance from the Equator [10]. Recent studies have shown that in Australia the prevalence of psoriasis in adults is between 2.3% - 6.6% and 1.3% - 2.2% in the United Kingdom [11].

While most cases of psoriasis often appear before age 35, it can develop at any age [12], and men and women are equally affected [13].

ETIOPATHOGENESIS

The cause of psoriasis is not known, but it was found to be a condition with multifactorial determinism [14], which to manifest itself clinically requires the presence of genetic and environmental factors [12].

About 40% of people with psoriasis or psoriatic arthritis have a family member with psoriasis [15].

The major susceptibility locus in psoriasis: PSORS 1 (6p21.3) within the MHC gene complex is present in approximately 30% of patients with psoriasis [15].

In patients with genetic predisposition, environmental factors can have a triggering or aggravating effect on the disease, and among the most frequently involved environmental factors are: trauma, infections, endocrine factors, certain drugs, alcohol, smoking, and obesity [16].

Pathogenic hypotheses

As mentioned, the origin of psoriasis is multifactorial, involving hereditary and environmental pathogenic mechanisms [17]. At the epidermal level, there is hyperproliferation and abnormal differentiation of keratinocytes, as well as activation of the skin's immune system [18].

Human histocompatibility system (HLA)

The major histocompatibility complex (MHC) also known as human leukocyte antigen (HLA) is one of the most genetically diverse regions in the genomes of different species [18].

More than 500 associations have been identified in the HLA region for approximately 200 phenotypes, including immune deficiencies, autoimmune diseases, susceptibility to infections, malignancies, and psychiatric conditions (Welter et al. 2014) [18].

The function of gene families at the level of MHC in the occurrence of autoimmune diseases has been the subject of research for decades [19].

Psoriasis is characterized by epidermal hyperproliferation and dermal inflammation [20], mediated by T cells [21]. Efforts to study genetics in psoriasis continue, with nine susceptibility loci (PSORS1-9) being identified [22]. The PSORS1 locus is considered the genetic determinant of psoriasis [23].

From the human genome, MHC is one of the most studied regions [24], with multiple genetic variants at this level that have contributed to the emergence of diseases such as psoriasis, Crohn's disease, systemic lupus erythematosus, rheumatoid arthritis [25].

MHC is located on chromosome 6p21.3 and is divided into 3 regions: the centromeric region (MHC III) and the telomeric regions (MHC I and II) [26].

The genome-wide association study (GWAS) demonstrated that at the MHC level there are SNPs associated with the occurrence of psoriasis [27].

The major locus involved in the development of psoriasis – PSORS 1 – located at the MHC level is probably associated with non-MHC loci with common alleles [28], which determines the genetic heterogeneity of psoriasis [29].

Certain gene combinations increase the risk of psoriasis, while others decrease it. Patients with psoriasis have been observed to have a higher prevalence of HLA-Cw6, HLA-B13, HLA-Bw17, and HLA-B37 antigens [27].

Major susceptibility locus (PSORS1)

While the major susceptibility locus is PSORS1, other susceptibility loci seem to also play important roles: PSORS2 (17q24-q25) [26].

Molecular studies revealed the following "candidate" genes for psoriasis:
- Gene S located on HLA-Cw6 (expresses the duration of keratinocyte differentiation) [26].
- Genes from the differentiation complex PSORS4 (1q21-q22) - encoding molecules involved in the terminal differentiation of keratinocytes [28].
- PSORS 5 (predisposition in Swedish families) [26].
- PSORS 8 (susceptibility to Crohn's disease and psoriatic arthritis [27].
- PSORS 9 (predisposition in Chinese families) [28].

The immune system

Psoriasis is an incurable autoimmune disease with genetic determinism, mediated by T lymphocytes…

Immunity includes all the physiological mechanisms that allow the body to recognize and eliminate a "non-self" antigen [30]. To be effective, the immune system must differentiate between "self" – cells, proteins that belong to the body and "non-self" – pathogens or substances foreign to the body [31].

There are two types of immune system response:
- Innate immunity = non-specific (which generates an immediate response)
- Acquired immunity = specific

Innate immunity includes two main lines of defense:
A) Physical barriers: skin, mucous membranes, cilia.
B) Chemical barriers: saliva, mucus, acid environment in the stomach, sweat, lysozyme.

Acquired immunity involves:
- The inflammatory response
- The complement system
- Cellular components

The innate immune response is naturally present in humans and is not pathogen-specific. The acquired immune response triggers a specific cell-mediated response through T lymphocytes, which secrete cytokines, and humoral through B lymphocytes, which secrete antibodies [31].

Psoriasis, the chronic inflammatory skin disease [1], is characterized by abnormal innate and acquired immunity [30], which is associated with aberrant differentiation and hyperproliferation of keratinocytes [31].

Pathogenic hypotheses

Psoriasis is a complex genetic disorder that is triggered by various risk factors involving a variety of processes, such as inflammation, antigen presentation, cell signaling, and transcriptional regulation [33]. Persistent inflammation in psoriasis causes uncontrolled differentiation and proliferation of keratinocytes [34]. It is considered that both keratinocytes and other cell types are involved in the appearance of the psoriatic plaque, from whose interaction in the superficial epidermal layers the persistent inflammation results [35].

From a histological point of view, the changes that appear in psoriasiform skin are acanthosis, parakeratosis, and hyperkeratosis [36].

In the most recent meta-analysis performed on genome-wide association studies, 15 new PSORS-type loci were identified, bringing their number to 36 [37]. Among the loci identified are several that encode the NFkB signal transduction cascade, such as REL, NFKBIA, TRAF6, CARD14, and ILF3 [37].

Studies at the level of the entire genome emphasize the complexity of gene changes involved in the occurrence of psoriasis [38].

In several reports, the role of the nervous system in the pathogenesis of psoriasis was emphasized, and its involvement was based on the symmetrical distribution of lesions on the body and the interaction of sensitive peripheral nerves with immunomodulatory networks [39].

Cases have also been reported in which surgical denervation of psoriasis lesions or local anesthesia would decrease local inflammation [39].

Emotional stress, as well, has been reported as being involved in the onset of psoriasis or its exacerbation [40].

Over time, research has shown that the immune system has a key role in the development of psoriasis [41].

Classically, psoriasis is a systemic inflammatory disease [1], controlled by T cells [3], and modulated by genetic and environmental factors [2].

The presence of macrophages, lymphocytes, and neutrophils in the skin is a characteristic sign of psoriasis [7]. Therefore, the disruption of the innate and adaptive skin immune response, to which is added the presence of non-immune cells, determines the support of inflammation [9].

THE ACTIVE MOLECULAR ENVIRONMENT IN PSORIASIS

The activity of the immune system does not consist only in its specificity, but also in the elaborated communication network through the cytokines secreted by the activated immune cells [42].

Cytokines are small, soluble proteins that allow the recruitment of other immune cells, such as T lymphocytes or dendritic cells, and are involved in identifying the site of infection [43].

The key groups of cytokines are represented by: IL, interferon (IFN), and tumor necrosis factors (TNF) [42].

IL-1 is produced by macrophages, monocytes and other types of cells, being composed of IL-alpha and IL-beta, with a key role in the inflammatory response [44].

It binds to the specific receptor – IL-1R and its accessory protein (IL-1RAcP) [43], further activating a complex intracellular signaling network [45].

This ultimately leads to the induction of transcription factors, such as NFκB, resulting in inflammatory immune responses [46].

The IL-beta subunit has an important role in psoriasis, inducing the proliferation of T cells, which produce IL-17 and thus maintain the inflammatory processes in psoriasis [47].

Therapies targeting IL-1 have not demonstrated satisfactory results in plaque psoriasis, but satisfactory results have been obtained in generalized pustular psoriasis [45].

IL-23 is a cytokine formed by two subunits, p19 and p40 [48], involved in antifungal and antibacterial defense [49]. It is produced by macrophages and dendritic cells, and the receptor to which it binds consists of IL-23 R and IL-12Rbeta1 [50]. Furthermore, it determines the activation of transcription factors, including STAT-3 [51].

It cannot activate naive T cells alone, because they do not have specific receptors, but it is involved in the pathogenesis of psoriasis through Th 17 cells [52].

The development of anti-IL-23 therapies has shown excellent efficacy in the treatment of psoriasis [51].

Th17 cells, neutrophils, mast cells, Nk cells, and B cells are the source of IL-17 [52], The IL-17 family has 6 members (IL-17A – IL-17F) that bind to specific receptors [53].

In macrophages and fibroblasts, IL-17 induces the expression of pro-inflammatory molecules (IL-1p, IL-6, GM-CSF, TNF-alpha) [52].

Having a pro-inflammatory role, it can cause autoimmunity in the immune system, which has been demonstrated for rheumatoid arthritis, psoriasis, and inflammatory bowel disease [53].

IL-17 is involved in the inflammatory process from metabolic disorders, such as hepatic steatosis and arteriosclerosis [54] so IL-17 antagonists can also intervene in the improvement of comorbidities, not only in the treatment of psoriasis [55].

IL-22 is part of the same family of cytokines as IL-10, and the active form is a monomer that binds to its specific receptor, located at the level of epithelial cells and fibroblasts of various cells [56]. It consists of heterodimeric subunits (IL-22R1 and IL-10R2) [57].

IL-22 is produced by CD4 (Th1, Th17, Th22) and CD8 lymphocytes, innate lymphoid cells, and NK cells [58].

The transcription factor RORγt is essential for IL-22 production because CD4 + cells secrete IL-22 only in the presence of this receptor [59].

The serum level of IL-22 is correlated with the activity of the disease [57] and in the skin with psoriasis lesions, the level of IL-22 is increased [58]. This causes keratinocytes to migrate, preventing their differentiation, and leading to the thickening of the epidermis [59]. Therefore, IL-22 could represent a potential target for the treatment of psoriasis [57].

IL-12 is produced by dendritic cells, macrophages, and neutrophils, being a heterodimer formed by the p35 unit and the p40 unit [62]. Its receptor is mostly found on T cells and N cells [65] and consists of IL-12 Rbeta 1 and IL-12Rbeta2 subunit [63]. The binding of IL-12 to the receptor activates the JAK/STAT pathway [67]. Although IL-12 increases IFN-gamma and TNF-alpha [68] molecules involved in the pathogenesis of psoriasis, an increased level of the p35 subunit was not detected in the skin of patients with psoriasis [69], and targeting the p40 subunit exerted its effects by inhibiting IL-23 [70].

The main sources of interferon-gamma - type 2 interferon (IFN-γ) are CD4+ and CD8+ cells, NK cells, but also antigen-presenting cells and B cells [69]. It is involved in antiviral and antibacterial defense [67]. The IFN-gamma receptor consists of two IFNGR1 chains and two IFNGR2 chains responsible for signal transduction [71], which leads to the activation of the JAK/STAT signaling pathway [72]. The serum level correlates with the activity of the disease, being higher in patients with psoriasis [70]. Greater decreases in serum IFN-gamma are associated with longer periods of disease remission [67], but anti-IFN-gamma therapy has not demonstrated efficacy on psoriasis lesions [70].

The role of TNF alpha in the inflammatory mechanisms of psoriasis

TNF-α is produced by macrophages, monocytes, keratinocytes, and lymphocytes, being a pro-inflammatory cytokine [72], involved in both acute and chronic inflammation [71], with an antibacterial and antitumor role [70].

Receptors for TNF are found in all nucleated cells [69] and are formed by two main isoforms: TNFR1 and TNFR [73].

TNF alpha is involved in the proliferation of CD4 cells [72], in the production of chemokines and cytokines [74] (IL-1), and is also involved in the process of apoptosis in certain types of cells [73].

The level of TNF-alpha correlates with disease activity [71], and TNF-alpha inhibitors have proven effective in the treatment of plaque psoriasis [72].

INFLAMMATORY CELLS IN PSORIASIS

Keratinocytes and cells of innate immunity in the skin

At the level of the skin, which is the largest organ of the body, consisting of the epidermis, dermis, and hypoderm [3], there is a large number of cells with various roles.

In psoriasis, the interaction between cells of the innate immune system (macrophages, neutrophils), cells belonging to adaptive immunity (T and B cells), and resident cells (keratinocytes, melanocytes) support chronic inflammation [73].

In the early stage of inflammation, an important role is played by dendritic cells, which are antigen-presenting [75]. The mechanism by which these cells are activated is incompletely elucidated, but it seems that there are certain microbial peptides, such as LL37, and S100, released by keratinocytes [76]. In psoriatic skin, these peptides are overexpressed and bind to the DNA of damaged cells, which causes dendritic cells to be activated to produce IFNα [77]. It (IFNα) causes the activation of myeloid dendritic cells [75].

Activated dendritic cells transform into antigen-presenting cells to be able to interact with naïve T cells and begin to produce large amounts of TNF-alpha, IL-23, IL-6, and IL-12 [77].

These cytokines activate the inflammatory cascade by recruiting neutrophils to the site of inflammation [78] and the proliferation of keratinocytes [79].

Keratinocytes, in turn, produce antimicrobial peptides [74], cytokines (IL-6, TNF-alpha), and chemokines (CCL20, CXCL5, CXCL9, CXCL10) [76].

IL-36, secreted mainly by keratinocytes and dendritic cells [73], accumulates neutrophil granules, present in large numbers in psoriatic skin [77]. IL-36 binds to its receptor IL-36Ra [78] and is involved in the transcription of other inflammatory mediators, by activating NF kappa B [79]. Also, IL-36 increases inflammation through its interaction with other inflammatory cytokines, such as IL-17 [79]. Some data suggest that decreasing the number of neutrophils significantly improves inflammation [76].

Macrophages are also antigen-presenting cells [75]. They derive from monocytes [77] and are involved in the occurrence of psoriasis inflammation [78], being an important source of TNF-alpha [77].

Within the innate immune system, cells without antigen-specific receptors (ILCs) are involved in antimicrobial defense and create a balance between pro- and anti-inflammatory factors [80].

ILC 3 can produce IL-17 and IL-2 [81], and their number decreases in the affected skin during the remission period of the disease [80], emphasizing the involvement in the pathogenesis of psoriasis, which requires additional data [81].

T cells

T cells have an important role in the defense against pathogens, and in the pathogenesis of psoriasis, there is an interaction with a central role between T cells and dendritic cells.

Dendritic cells release cytokines such as IL-12 and IL-23 that promote Th1, Th17, and Th22 responses.

At the epidermal level, T helper cells support keratinocyte hyperproliferation, leading to decreased apoptosis.

By carrying out human skin transplantation in SCID immunodeficient mice, followed by the injection of CD 4 T cells from patients with psoriasis, the role of these cells in the pathogenesis of psoriasis was demonstrated.

Th 1 (CD4+) cells produce large amounts of TNF-alpha and IFN-gamma involved in triggering psoriasis.

In mice with IL-17 receptor deficiency, skin inflammation is reduced, emphasizing the importance of IL-17 in the occurrence of psoriasis lesions.

Thus, elevated serum levels of IL-17 and increased expression in lesions are typical features of psoriasis.

After it was demonstrated that IL-23, produced by dendritic cells, is involved in the development of Th17, it became obvious that the IL-23/Th17 axis has an important role in the pathogenesis of psoriasis.

B cells

B cells are involved in the adaptive immune response through antibody production and antigen presentation [23].

Using a model of intestinal inflammation, Mizoguchi et al. [58] in a model of intestinal inflammation, showed that B cells produce IL-10 and thus have a protective role against inflammation.

Also through the secretion of IL-10, B regs cells are involved in alleviating various allergic and inflammatory diseases [82]. They do not represent a distinct lineage of B cells but are rather a subset of them [81]. It is not elucidated which factors are involved in the development of B cells in a type B regs [80].

The B regs cell population is characterized by the presence of CD5+CD1d hi on the surface [81].

Other subsets of B cells involved in the production of IL-10 are those in the marginal zone – CD19+CD21 hiCD23-CD24hi and CD138+ plasma cells [80].

Compared to T cells and studies regarding their role in the pathogenesis of psoriasis, in the case of B cells, data are limited regarding the involvement of these cells in psoriasis, as they rarely appear in lesional skin [83].

The decrease in the number of B lymphocytes in patients treated with anti-CD20 (Rituximab) for other autoimmune diseases led to the appearance of psoriasis skin lesions in individual cases [84].

B cells secrete inflammatory cytokines such as IL-6, IL-4, and IFN-gamma, being key effector cells [83].

Also, in a recent study, it was demonstrated that a large number of IL-6-producing B cells are found in inflamed mouse skin [80]. Blocking IL-6 resulted in reduced inflammatory symptoms [84].

DISCUSSION

In the case of mild psoriasis, the inflammatory process is confined to the skin, on the other hand, a more extensive active disease should be regarded as a systemic inflammatory disease, impacting internal diseases, including arthritis and metabolic syndrome [88].

Patients suffering from moderate to severe forms of psoriasis have increased serum levels of multiple proinflammatory cytokines, including TNF-a, IFN-c, IL-6, IL-8, IL-12, IL-17A, and IL-18 [89].

All the subtypes of T cells involved in the pathogenesis of psoriasis are also involved in atherosclerosis [90]. Sherlock et al. demonstrated that IL-23 drives inflammation in the aortic root through the activation of CD3+CD4−CD8− T-lymphocytes [91].

TNF alpha plays an important role in the inflammatory pathways of metabolic syndrome, diabetes, and cardiovascular disease. TNF alpha inhibitor therapy is associated with a lower risk of major adverse cardiovascular events (MACE) independent of traditional cardiovascular risk factors [92]. Recent research shows the importance of Th17 and other IL-17-producing cell types in the pathogenesis of cardiovascular events [90].

We lack large well-designed studies which prove the improvement of comorbidities by treating psoriasis with biologics.

In short term, there is evidence that early treatment with biologics reverses damage caused by inflammation. New biomarkers and early diagnosis help healthcare workers in better evaluation of patients' responses to systemic therapies [93].

CONCLUSIONS

Psoriasis is a recurrent, chronic, T-cell-mediated, polygenic disease characterized by erythematous squamous lesions in specific body sites. The pathogenesis of psoriasis is complex and still undergoing study. Biologic therapy is the key to reversing inflammations and preventing associated comorbidities.

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ORIGINAL ARTICLE

Postirradiation Angiosarcoma – A Late, Severe Complication of Radiotherapy for Mammary Neoplasm

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Abstract: *Angiosarcoma is a malignant tumor derived from the endothelium of the blood and lymphatic vessels. Its aggressive behavior results in a poor prognosis for the patients. Radiation-associated angiosarcoma is a rare recently described particular subtype of angiosarcoma best shown in patients after treatment of breast cancer. The latency period of development is approximately 8 years, thus the 5-year standard follow-up after mammary neoplasm therapy needs to be extended up to 10 years. Radiotherapy is an independent risk factor for angiosarcoma development. Rapid diagnosis is essential for prompt therapeutical intervention to improve the patient's prognosis. The importance of early diagnosis is emphasized by a case of a 54 years old woman, with a diagnosis of breast cancer treated by sectorectomy and radiotherapy 7 years prior, presenting to our Dermatology Department for the recent occurence of erythemato-violaceous cutaneous patches on the area subjected to radiotherapy.*

Keywords: *angiosarcoma, radiation, radiotherapy, breast cancer, conservative surgery, follow-up*

INTRODUCTION

Angiosarcoma is a malignant tumor that originates in the endothelium of the blood and lymphatic vessels [1]. It usually develops in the skin, soft tissue, breast, or liver [2]. It comprises 1-2% of the soft tissue sarcomas that appear in humans [3], it is uncommon and affects mostly Caucasian men, with the highest incidence in adults over 70 years.

When it comes to younger ages such as children or adolescents, angiosarcomas are rare and are prone to develop in viscera or nearby, usually the heart, or they are associated with other conditions such as immuno-suppression, chronic radiodermatitis or lymphedema [4].

Risk factors associated with angiosarcoma include chronic lymphedema, radiation history, carcinogens (eg vinyl chloride, arsenic), and specific familial syndromes [3].

Angiosarcomas cells have endothelial differentiation and they clone while undergoing a malignant transformation. Unlike other sarcomas, angiosarcomas have a different upregulation of the genes responsible for the vascular receptors [4]. In angiosarcomas that have been induced by radiation or lymphedema, the amplification of MYC is high which in the end leads to the downregulation of thrombospondin-1, a protein that inhibits angiogenesis [4]. MYC is overexpressed in patients with radiation-induced angiosarcoma and this can help distinguish it from primary angiosarcoma [5]. Angiosarcomas can be well- or poorly differentiated; in the first case, one can observe vascular channels that are not regular in shape, they are lined by endothelial cells and they dissect the fibers of collagen; the latter has ill-defined vascular spaces, the cells are spindled, polygonal, epithelioid and round and they show multiple mitosis and big nuclei [1].

Immunohistochemistry is used when diagnosing less differentiated angiosarcomas. The cells express vascular as well as endothelial antigens such as Factor-VIII, CD31, CD34, and VEGF. CD31 is seen in more than 50% of the cases and has great sensitivity and specificity [3]. Recently, there has been used a marker called ERG, which is a member of the ETS family of DNA-binding transcription factors and which offers greater sensitivity and specificity for endothelial cell differentiation

[1].

PATHOGENESIS AND TYPES OF ANGIOSARCOMAS

The mechanism through which angiosarcoma may appear in chronic lymphedema is not well known, but theories postulate that some carcinogens build up in the lymphatic fluid and because the lymphatic network is lost, the affected area is spared by the immune system and this fact can furtherly favor the neoplastic modified cells.

An independent risk factor for the development of angiosarcoma is radiotherapy which is best shown in patients with breast cancer [4].

There are essentially 3 instances for the occurrence of cutaneous angiosarcoma: idiopathic angiosarcoma of the scalp, face, and neck, angiosarcoma associated with chronic lymphedema (Stewart-Treves syndrome), and post-irradiation angiosarcoma. Some authors describe a fourth type which occurs in any other settings than the previous ones [1].

Angiosarcoma of the scalp, face, and neck is most frequent in elderly individuals. Men are two times more affected than women. The lesion begins as a bluish macule that can be confused with a bruise.

Angiosarcoma associated with chronic lymphedema is also called Stewart-Treves syndrome and this type is seen in areas with long-lasting lymphedema as is the case of the upper arm after the patient underwent a mastectomy. The tumor develops 11-22 years after surgery, having though a low incidence of 0.45% of the cases, but a poor prognosis [6]. Other instances of chronic lymphedema may be parasitic infections or congenital lymphedema [3].

Post-irradiation angiosarcoma is a vascular tumor that appears in sites that have been irradiated in the past. If the affliction for which the patient has received radiotherapy was benign, the average time for angiosarcoma to develop is 23 years, but if the condition treated with radiation was malignant, then the time reduces to only 12 years [6].

The fourth type is uncommon and may appear for example in benign vascular tumors, in cases of immunosuppression after organ transplantation.

RADIATION ASSOCIATED ANGIOSARCOMA

The angiosarcomas that arise in areas of previous radiation sites are difficult to be differentiated from atypical vascular lesions. Sometimes even histologically, the diagnosis can be challenging since well-differentiated angiosarcomas which exhibit only minor cytologic atypia despite their aggressive behavior, may be similar to microcystic lymphatic malformation. Also histologically, the angiosarcoma must be differentiated from other vascular neoplasms such as PILA (papillary intra-lymphatic angioendothelioma), RHE, epithetlioid hemangioendothelioma, KHE (Kaposiform hemangioendothelioma) [4].

Imaging tools such as CT, and MRI are helpful, but to have a precise diagnosis for angiosarcoma, histologic examination and immunohistochemistry of the tissue confirm the disease.

Post-irradiation angiosarcoma is not seen very often [1]. It is considered a late severe complication of radiotherapy [7]. The majority of cases emerge in the skin after it has been irradiated for breast cancer, or not so frequently, for a neoplasm of the internal organs [1]. Post-irradiation angiosarcoma has a cumulative incidence of 0.9-3.2 per 1000 cases of mammary neoplasm. In terms of prognosis, a 5-year survival rate lies between 27 to 48% [8]. If the patient had radiation therapy for breast cancer, the period after which a sarcoma in this area may appear is 5-10 years and this means that the usual 5-year follow-up needs to be extended [3]. It appears that other radiation-induced sarcomas take more than 10 years to develop [9]. Some studies link the usage of radiation in high doses with the incidence of angiosarcoma [3]. In the case of breast angiosarcoma, the latest population-based studies have shown the risk to be about 0.1%. It has been hypothesized that the replacement of radical mastectomies with conservatory surgery has led to secondary angiosarcomas in the mammary gland [10]. Breast angiosarcoma can be categorized into primary (de novo) if it develops without any known trigger, or secondary if it appears at the site of previously irradiated integument [8].

It appears that in the last few years, of all the subtypes of sarcomas of the breast induced by radiation, angiosarcoma had the highest occurrence [10].

In a retrospective Dutch population-based study from 2019 of 296,577 patients with angiosarcoma after breast cancer treatment (1989-2015), the median follow-up after diagnosis of breast cancer was 7.7 years, and the median age when diagnosed with angiosarcoma was 58 years. Of the 184,823 patients that received radiotherapy, only 209 had angiosarcoma of the breast and/or chest wall. As a note, angiosarcoma did not occur in any of the patients that did not receive radiotherapy (111,754). The median period between breast neoplasm treatment and the appearance of post-radiotherapy angiosarcoma was 8 years. In this study, 5- and 10-year survival rate was 40.5% and 25.2%, respectively, regardless of the type of treatment (surgery alone, surgery and radiotherapy combined sometimes with hyperthermia, chemotherapy, and radiotherapy). The inclusion of radiotherapy compared to only surgery had not shown a significant survival [7]. Another study from 2013, that evaluated 48 patients with angiosarcoma treated between 1990 and 2004 (7 with breast cancer) showed a 7 years latency period for angiosarcoma of the breast. [11].

Clinical presentation of cutaneous angiosarcoma includes single or multiple red, bluish or violaceous macules, patches, and nodules, that can further ulcerate or present intra-tumoral hemorrhage [6].

The 2022 diagnostic criteria for radiation-associated angiosarcoma are a) sarcoma that develops in a previously irradiated field or the tissue volume comprised in the 5% isodose line; b) minimum 4 years between radiotherapy and the arising of the angiosarcoma; c) histological difference between primary neoplasm and secondary sarcoma; d) the exclusion of patients with cancer syndromes [5].

The prognosis is poor because angiosarcoma is an aggressive tumor; patients' survival rate without treatment is between 6 months and 2 years after diagnosis [6]. If it is a low-grade tumor, it behaves similarly to atypical vascular lesions, but a high-grade angiosarcoma is aggressive, has violent growth, and local recurrences arise after a few months [9]. However, with an incidence so low, one can assume that it is worth risking the arising of an angiosarcoma in a patient with breast cancer for the benefit of radiotherapy on the primary neoplasm [8].

Treatment consists of surgery, radiotherapy, and chemotherapy as options, but the choices and results vary on tumor size, location, and type, and last, but not least, resectability [3]. Currently, the main choice is surgical removal [8]. For localized angiosarcoma, the mainstay of the treatment is surgical resection along with adjuvant radiotherapy. Recurrences are difficult to avoid; chemotherapy is the first option in case of metastatic angiosarcoma [3]. The recurrence rate of post-radiation angiosarcoma of the breast is high (96%) and metastases are most often found in the lungs and liver and they can coexist with the local recurrence or appear soon after [8].

In a case of a 54-year-old woman who was referred to our clinic for ecchymotic and purpuric macules localized on the right breast, postradiotherapy angiosarcoma was diagnosed based on clinical aspect, history, histology, and immunohistochemistry. She had a previous history of sectorectomy for breast cancer 7 years prior and she also underwent radiotherapy. Clinical examination identified well-defined ecchymotic and purpuric macules, oval or with irregular polycyclic borders, 1-3 cm diameter; a similar macule located in the lower-inner quadrant, 4 cm diameter, with purple nodules on the surface, resembling Kaposi sarcoma (Figure 1).

Figure 1: Clinical aspect of the breast lesion

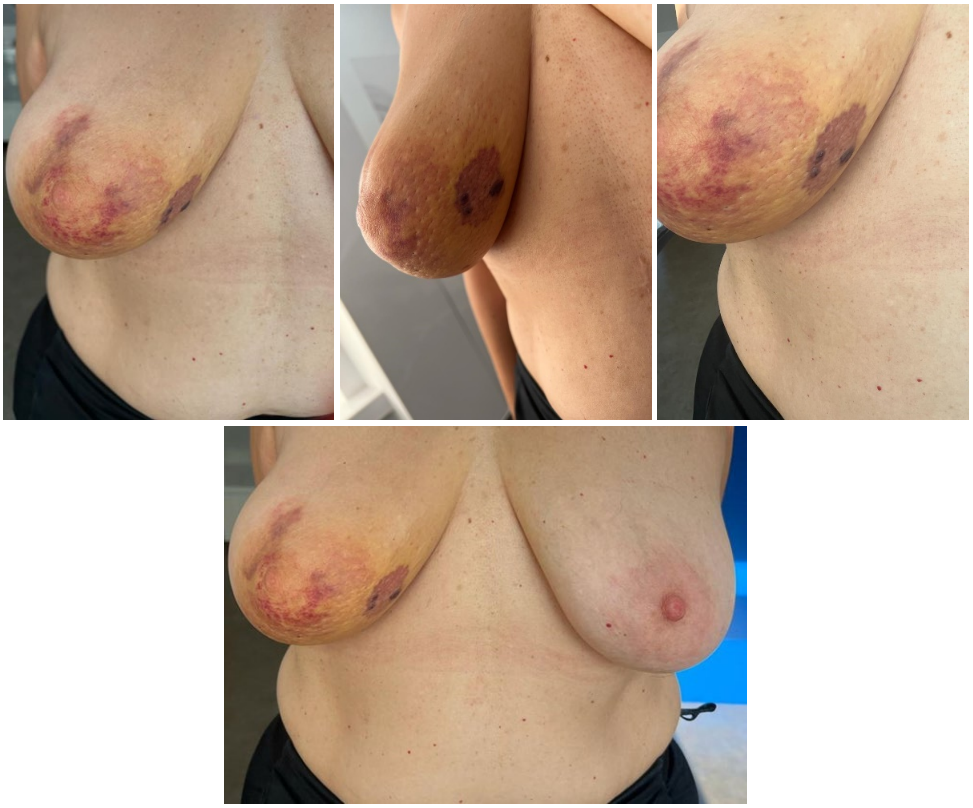

The lesions appeared 2 months before presentation, with progression in terms of dimensions and the number of lesions. The dermoscopic examination showed a combination of red, blue, and purple: a violaceous hue of the lesion that had purple clods with white structures, red and blue areas, and reddish-brown crusts that covered the central part of the nodules (Figure 2).

Figure 2: The dermoscopic examination

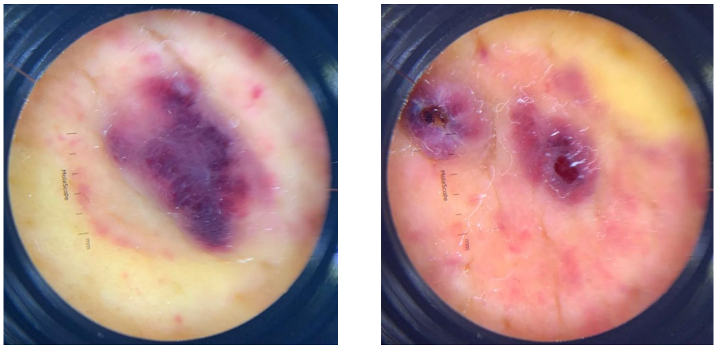

Skin biopsy of two sites was performed: a cutaneous nodule from the lower-inner quadrant of the right breast and a cutaneous fragment from the upper-outer quadrant of the right breast. The histology examination revealed: the first site showed ill-defined tumoral proliferation made up of randomly located oval/fusiform cells with elongated nuclei or grouped in short anastomotic bundles that defined small vascular spaces or intracytoplasmic slits without walls, some of them containing blood; moderated lymphoplasmacytic infiltrate and hemosiderin associated deposits. The second site of the biopsy had the same histopathologic description (Figures 3 and 4).

Figure 3: Dermal vascular proliferation, mimicking Kaposi sarcoma (HE X 10)

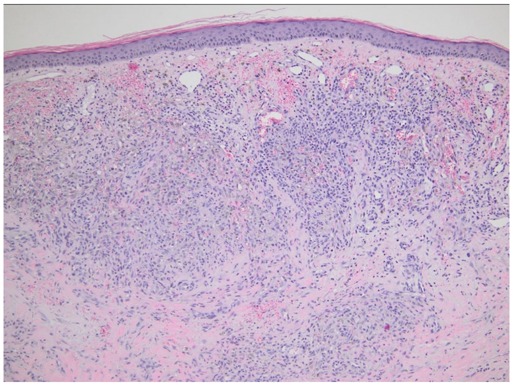

Figure 4: Dermal vascular proliferation, mimicking Kaposi sarcoma (HE X 20)

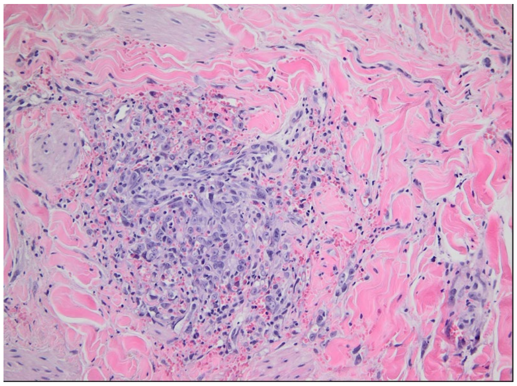

Was then performed immunohistochemistry tests for a positive diagnosis. These tests showed on both blocks HHV-8 negative and ERG and SMA positive, but c-MYC positive on the second piece of tissue (Figures 5-7).

The conclusion was that of a postradiotherapy angiosarcoma. The patient was then referred to Oncology Department for further investigations and therapeutic intervention.

Figure 5: C-MYC positive within numerous tumor nuclei

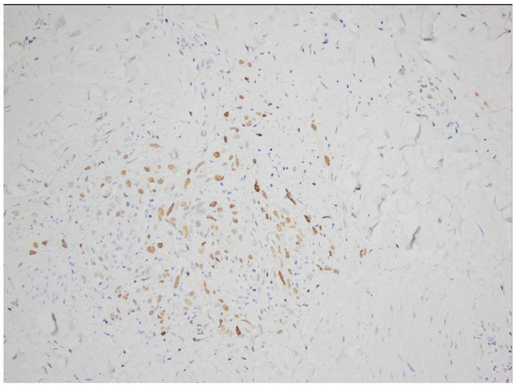

Figure 6: SMA positive within scattered tumor cells

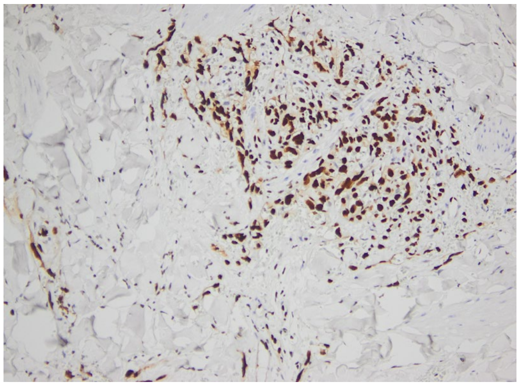

Figure 7: ERG positive within numerous tumor nuclei

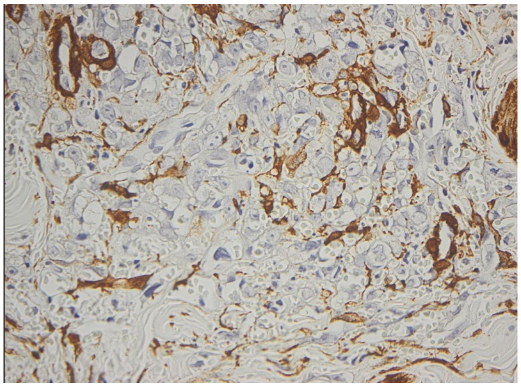

CONCLUSIONS

- Radiotherapy is an independent risk factor for the development of angiosarcoma, best shown in patients with breast cancer.
- Post-irradiation angiosarcoma has a cumulative incidence of 0.9-3.2 per 1000 cases of mammary neoplasm so it is a rare complication of radiotherapy, proving to be a challenging clinical diagnosis.
- The diagnosis of angiosarcoma can also be histologically challenging – it can exhibit only minor cytologic atypia in well-differentiated cases; immunohistochemistry testing is essential for differentiation from other types of sarcoma (including Kaposi sarcoma).
- The average period between breast neoplasm treatment and the appearance of post-radiotherapy angiosarcoma is 8 years.
- Prognosis of RAASB is poor, 5-year survival rate lying between 27 to 48% and thus it needs rapid diagnosis and therapeutic measures.
- 5-year standard follow-up after breast cancer needs to be extended up to 10 years to capture the development of an angiosarcoma.

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ORIGINAL ARTICLE

Tips and Tricks in Laparoscopic Adrenalectomy for Pheochromocytoma. How We Do It

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Abstract: *Pheochromocytomas are rare neuroendocrine tumors that frequently have genetic involvement and can present a particular risk in terms of the anesthetic-surgical procedure. Current worldwide medical recommendations state that laparoscopic adrenalectomy is the conventional treatment for pheochromocytoma, however, the ideal surgical strategy is still being developed as specialized surgeons gain experience with these rare tumors. The study's purpose was to thoroughly evaluate our film library and identify the best surgical steps that represent our standardized procedure. We developed a comprehensive set of surgical critical strategies for either a left or right adrenalectomy, providing helpful advice on patient positioning, port placement, the adrenal gland approach, as well as other surgical methods specifically designed for tumor mobilization and dissection. The current paper aims to synthesize our clinical experience in terms of preoperative preparation, patient positioning, intraoperative complications, causes of conversion, and specimen extraction. The main conclusions concern both the best surgical approach and the importance of surgical experience in the clinical management of these rare tumor types.*

Keywords: *pheochromocytomas, laparoscopic adrenalectomy, surgical strategy*

INTRODUCTION

Pheochromocytomas are relatively uncommon tumors often with genetic aggregation that is accompanied by a high risk of overall morbidity and mortality, especially during surgical procedures precipitated by important blood pressure and heart rate variations. Although laparoscopic adrenalectomy has become a standard procedure for pheochromocytoma, the optimal approach continues to evolve as surgeons develop expertise in this pathology.

MATERIALS AND METHODS

Our experience starts almost 20 years ago, counting 436 laparoscopic adrenalectomies, from which 96 were performed for pheochromocytoma. We systematically reviewed our video database and highlighted the best surgical steps that exemplify our standardized approach.

RESULTS

From our experience, we managed to synthesize detailed surgical key steps for the overall procedure when performing left or right adrenalectomy, offering valuable tips starting from the patient positioning, and port placement and continuing to the adrenal approach, along with different strategies specifically tailored for pheochromocytoma mobilization and dissection. Taking into consideration our experience in dealing with the challenges of this procedure the current article aims to lead to improvement of overall outcomes for this procedure.

Preoperative preparation

From the anesthesiologist's point of view, surgical resection of pheochromocytoma can be divided into two phases based on the ligation of the tumoral blood supply [1]. Communication between the surgeon and the anesthesiologist regarding the surgical steps, in particular the ligation of the central adrenal vein, allows a proactive attitude, foreseeing the eventual intraoperative hemodynamic variations.

The first part of the procedure refers to all the surgical and anesthetic steps before ligation of the central adrenal vein in which severe episodes of cardiac arrhythmias and hypertension can occur [1].

The second part refers to all the surgical events that follow the central adrenal vein seal and it might be characterized by a sudden decrease of arterial blood pressure and in intravascular volume that might require vasopressor support [1].

Keeping in mind the possible cardio-circulatory outcomes during this surgical procedure, large-bore venous access should always be obtained [2], the guidelines also recommend continuous intraarterial blood pressure monitoring for all patients with pheochromocytoma or at least for those with persistent preoperative hypertension [3–5].

Adding a central venous catheter allows secure and effective administration of vasoactive medication to promptly counterbalance the hemodynamic variations, especially since there are no accurate predictors of which patients are most likely to need central administration of vasopressors [2,4].

In our experience, the availability of 2 large-bore venous access associated with good preoperative management with stress minimization and anxiolytic administration, decreases the sympathetic nervous stimulation, that might trigger a hypertensive crisis [6,7], was enough to ensure rapid and effective administration of intraoperative drugs. Frequent non-invasive blood pressure measurement (every 3 minutes) and close communication with the surgeon regarding the current, the following, and the particularities of the ongoing surgical steps supply all the data needed to fine-tune vasoactive drug administration even without intra-arterial continuous blood pressure monitoring.

Although not routinely recommended, at the beginning of our experience we chose to perform a mild bowel preparation the day before the surgery (polyethylene glycol), a practice that we later abandoned as we saw no intraoperative benefit in terms of bowel injury or postoperative bowel movements. More than that, some of the cases presented a distended lumen at the time of surgery, especially for the left colonic flexure, and this was disturbing the surgical dissection.

Patient positioning

Our experience refers only to the transperitoneal approach mainly due to the familiarization with the peritoneal cavity as general surgeons. In all our cases we used the lateral trans peritoneal approach because it usually offers a larger surgical field that is particularly useful in the case of large tumors such as pheochromocytomas and morbidly obese patients.

The standard patient position for laparoscopic adrenalectomies is in contralateral decubitus [8,9] with the possibility of a backward tilt up to 30° [5]. After anesthesia induction, a nasogastric tube (especially useful in left-sided adrenalectomies), and urinary catheter insertion, the patient is placed in lateral decubitus. Prevention of contralateral brachial plexus injury was done using a soft roll under the contralateral axilla. The other arm is can be elevated and secured on an elevated arm board [8] but, in our experience, we placed the ipsilateral arm slightly bent, in a relaxed position over the other one without needing rigid support or any other fixation method.

Keeping in mind the unpredictable hemodynamic events that characterize pheochromocytomas during adrenal surgery, the patient's position has to offer both good access to the tumor and also the possibility to finish the surgery openly due to conversion in an emergency setting. The umbilicus is placed above the surgical table breakpoint that is next flexed and tilted in an anti-Trendelenburg position until the ipsilateral flank contour is stretched to a straight line which we position parallel to the horizontal line. Over-flexing the operating table can cause supplementary tension of the abdominal wall thus causing less compliance, less working space, and consequently need for increased pneumoperitoneum pressure [8]. These patient position landmarks provide a maximum distance from the anterior superior iliac spine and costal margin offering a large operating field and increased ergonomic for the surgical team even for large tumors – the patient “needs to open” as much as possible the space between rib’s rim and the iliac crest. Depending on the high conversion probability (large tumoral size, retrocaval positioning, and hemodynamic variability) we tilted the patient back up to 30° using shoulder and hip supports. The patient’s legs are a little bit flexed to avoid stretching the crural nerve. Additional fixation was achieved using cloth straps at the hip and leg level.

According to the literature, trocar placement is variable. All ports are inserted at least 2 cm from the rib’s margin. Pneumoperitoneum is achieved in open maneuver, and then the first 10 mm trocar is inserted between the anterior axillary line and midclavicular line, depending on the exposure of the operating field (first, we palpate the inferior edge of the liver and – when is possible – the fundus of the gallbladder, then we decide to insert the trocar laterally to the gallbladder with a parallel direction to the trajectory of the inferior vena cava). Other three 10 mm trocars are inserted under direct visualization equidistant from each other (at least one hand distance), in the lateral border of the rectus sheath, another in the midaxillary line, and for retraction, one below the xiphoid process [10]. In all the laparoscopic adrenalectomies performed in our hospital, we used the Veress technique for the pneumoperitoneum, and the first skin incision was made in the midclavicular line at 3-4 cm from the rib’s margin. Only in obese patients, pneumoperitoneum was realized at 2 cm from the rib margin because CO_2 abdominal distension would move the incision further caudally causing a suboptimal visualization of the surgical field. All surgeries were performed with 4 trocars (we didn't experience a surgical adrenalectomy with 5 or more additional trocars): below xiphoid process for retraction, on the axillary line below the 10th rib (12 mm – used also to extract the specimen), and one at the half distance between the sub-xiphoid and optic trocar. The peritoneal space is insufflated to 12 mmHg using slow or medium flow insufflation as it is known that, intra-abdominal insufflation during laparoscopic pheochromocytoma excision may cause an increase in serum catecholamines [5,11] – Consequently, we strongly advise for taking enough time to realize the proper intra-abdominal pressure using small quantities of CO_2 inflated in at least 5 minutes (break times can be used!). During this time, the blood pressure of the patient should be carefully observed by the anesthesiologist and a trend to increase should be instantly corrected but not using too many anti-hypertensive drugs (risk for severe hypotension and rebound). Usually, the failure of this operative time is solved by conversion to open surgery, with rapid recovery of the blood tension being noticed with no major anesthesia intervention.

It is known that during surgery for pheochromocytoma, there is a risk of a hypertensive event due to catecholamine secretion – it could be beneficial for the patient to use a decreased level of pneumoperitoneum – like 11 mmHg, even 10 mmHg – if the working camera is allowing you proper movements of the instruments. Many surgeons highlighted that early ligation of the adrenal vein is an important goal in adrenalectomy, but studies have shown that delayed vein ligation does not appear to cause significant hemodynamic variation during laparoscopic adrenalectomy for pheochromocytoma [12,13] and taking into consideration that the dissection of the adrenal vein as the first step of the adrenalectomy, can be more demanding in case of large size adrenal lesions such as pheochromocytomas, we did not routinely approach the adrenal vein first (unless it was identified from the very first beginning), and we did not encounter intraoperative cardiovascular complications. Laparoscopy offers a real-time surgical overview to the anesthetic team that can anticipate when the adrenal vein is ligated and take steps to avoid cardiopulmonary complications.

Even from the insertion of the camera, a full video inspection of the peritoneal cavity should be done to evaluate the availability of other trocar entry sites. The presence of intraperitoneal postoperative adhesions can change the trocar order so that the operating field and other trocar entry sites can be conveniently exposed. In particular, placement of the lateral port in left adrenalectomies requires, in most cases mobilization of the splenic flexure and descending colon incising the peritoneum along the white line of Toldt. The viscera surrounding the adrenal gland are inspected for invasion: the spleen, the diaphragm and the greater gastric curvature (on the left side) and ascending colon, the liver, the right kidney, the diaphragm, and the duodenum (on the right side) are inspected and if there are suggestive signs of malignancy, conversion is mandatory [8]. Before beginning the laparoscopic adrenalectomy we should keep in mind that pheochromocytomas are larger than other adrenal tumors and, in our experience, they also have a frail consistency with a thin capsule and an increased number of arteries and veins that easily bleed on manipulation. This is the reason we advise having proper laparoscopic equipment and also alternatives to ensure good hemostasis and vessel sealing as well as to strictly follow the rule of "dissection of the body from the gland instead of dissection of the gland from the body" (Prof. J Marescaux, Strasbourg, France – off-the-record).

On the right side, while the liver is retracted cranially and medially so that the peritoneal reflection is seen and the left-right triangular ligament is tensioned and divided; the peritoneum is incised along the liver as far on the diaphragm so that the right lobe of the liver gently falls away medially. The posterior peritoneum is incised along its reflection on the inferior liver margin. The inferior vena cava (IVC) is identified and the peritoneum along its lateral margin is incised until it meets the hepatic parenchyma. The literature defines the caudal limit for the peritoneal division along the IVC as the superior edge of the renal vein [14] in our experience identifying this landmark might require unnecessary touching of the pheochromocytoma, especially in patients with well-represented retroperitoneal fatty tissue. When dealing with pheochromocytoma we use the macroscopic inferior border of the adrenal tumor as a landmark for inferior peritoneal division, as these tumors tend to be larger, carefully not to damage the inferior arm of the adrenal gland.

On the left side once the hepatic flexure and descending colon have been retracted medially by following the space between the Gerota's fascia and the mesocolon [8]. The next step ensures a good mobilization of the spleen and is carried out by division of the phrenicosplenic ligament starting at the posterior and inferior margin of the spleen and continuing cranially until the fundus of the stomach is visualized. Although the literature mentions visualization of the left diaphragmatic crus as the end landmark of this dissection [8], in our experience this was rarely needed. Medial mobilization of the spleen during this step can be performed with gentle retraction or, as some authors recommend, leaving a 2 cm peritoneal margin attached to the inferior margin of the spleen to grasp [8]. Usually, this is the moment when accidentally puncture of the splenic parenchyma is performed due to aggressive manipulation of the laparoscopic instruments – minor bleeding can occur and immediate hemostasis should be performed using hemostatic agents like oxidized regenerated cellulose or other topical fibrin sealant patch (sometimes, when a simple injury of the spleen capsule is noticed, it is enough to apply a pad pressure onto the injured area for a few minutes). Once the spleen is fully mobilized it will fall medially along with the tail of the pancreas exposing the lateral edge and anterior portion of the left adrenal gland [9]. Correctly fulfilling this surgical step ensures access to the right dissection plane thus avoiding blind dissection into retroperitoneal fatty tissue which is prone to excessive bleeding and capsular breaking, forbidden when resecting pheochromocytomas.

Partial adrenalectomy avoids the need for life-long hormone replacement and it is usually useful for patients with bilateral gland involvement. This procedure is performed in small tumors located on the periphery of the affected adrenal limb. Performed in pheochromocytoma has a high risk for intraoperative hypertensive crisis due to tumor manipulation and thus early identification of the adrenal vein is mandatory before partial resection [15]. For partial adrenalectomy to be feasible the remnant gland to have a good vascularization, so laparoscopic ultrasonography to evaluate the adrenal gland viability must be recommended [16]. Because of the risk of bleeding in partial adrenalectomy, it is preferred safety surgery and total adrenalectomy be performed.

Once the periglandular dissection is started it is important to keep in mind a principle postulated even from the first adrenalectomies, and demonstrated in later studies: a mean increase in plasmatic catecholamines up to 34-fold during tumor manipulation [17-19]. To minimize hemodynamic instability, these techniques require minimal to no direct manipulation or compression of the tumoral gland [5], achieved in many cases by gentle retraction of the surrounding viscera or tissue such as the kidney, pancreas, and liver or by using periglandular fatty tissue, the Gerota’s fascia and/or the disconnected vein stump, to manipulate the gland accordingly. Comparing pheochromocytoma ablation with other types of adrenal tumors, it is mandatory to respect the principle of “dissecting the patient away from the tumor” to achieve this important goal [20]. In large tumors with which we encounter technical difficulties prompt conversion has to be taken into consideration to reduce the patient's risk for capsular disruption and following iatrogenic disease recurrence [5] or even pheochromocytomatosis [14]. As mentioned before, pheochromocytomas have numerous tributary vessels that combined with the frail vascular wall and increased circulatory catecholamines represent the optimal recipe for possible uncontrollable bleeding, so achieving a proper vessel seal before tissue dividing is mandatory even with the usage of clips as a complementary method to the electrocoagulation and sealing devices [5].

For the right-sided adrenalectomy, the lateral border of the IVC continues to be the main landmark during the procedure allowing lateral retraction of the adrenal gland and thus exposing the central adrenal vein. Even though it is more demanding, many authors concluded that once the peritoneum is incised, as mentioned before, the tumoral gland should be approached first on the medial border – detaching the tumor from the IVC, exposing, clipping, and dividing the central adrenal vein [8]. The dissection continues along the inferior border of the adrenal gland and might include the peri-adrenal fat. The adrenal is gently lifted and the posterior aspect is released and then the lateral one. The final step is detaching the superior border of the tumor [8]. We share the opinion that first freeing the inferior and posterior aspect of the gland assures enough mobility to safely push the gland laterally, without manipulation or grasping, increasing the space to the IVC, exposing the central adrenal vein, and more important, pulling the adrenal tissue situated posterior to the IVC. While this approach carries a higher risk of capsular breaking when beginning the dissection, it offers more control and better retraction in dealing with the main vascular pedicle and retro-caval adrenal tissue. The dissection continues cephalad until the medial side of the gland is released and further on the superior edge.

Once the central adrenal vein is secured, we can better manipulate the tumoral gland. In large tumors, some authors recommend starting the dissection from the lateral and superior border of the tumor and then moving inferior to the IVC [8].

For the left adrenalectomy, it is generally accepted that the dissection begins along with the medial border proceeding from the upper to the lower adrenal pole, keeping close to the posterior muscular plane. This maneuver allows lateral rotation of the adrenal and exposes the left adrenal vein that will be ligated early in the dissection. During the dissection of the medial border of the adrenal gland, the inferior diaphragmatic vein might be encountered and it represents a landmark for the central adrenal vein. Particularly in pheochromocytomas, starting the dissection from the lateral border should be avoided since it will cause the gland to rotate inferiorly and medially thus requiring increased manipulation and pulling the adrenal gland, in order to access the central adrenal vein. After securing the central adrenal vein, the gland is lifted and the dissection continues at the posterior and lateral aspect of the gland, leaving the upper adrenal pole to be approached as last surgical gesture [8,14].

Several factors as the lack of major anatomic landmarks, the relative profound in the retroperitoneal fat of the left adrenal gland, with the main vein within the retroperitoneal fat, the proximity and tissue similarity with the pancreas tail, may render the left adrenalectomy more difficult [8], especially in the settings of mandatory minimal glandular manipulation and compression, and forbidden cellular spillage. So, it is recommended for beginners to start laparoscopic removal of a pheochromocytoma on the right side, then the left side.

Taking into consideration that the retroperitoneal space contains the kidney, adrenal gland, and fatty tissue, it is very important to localize the adrenal gland within the surrounding fat. To avoid capsular breaking, especially in smaller-sized tumors perinephric fat containing the adrenal gland should be dissected away from the transversus abdominis muscle (laterally), the diaphragm (above), the *quadratus lumborum* muscle (posteriorly), and the pancreas or liver (medially on the left and right sides, respectively) just inside Gerota's fascia. During the separation of the perinephric fat from the diaphragm at the upper pole of the adrenal gland, the superior adrenal vessels are usually encountered and must be, carefully sealed and transected (especially in patients with large pheochromocytomas). During en bloc dissection, the perinephric fat is divided between the adrenal gland and the upper pole of the kidney using a vessel-sealing device. Next, the adrenal gland is lifted by grasping the periadrenal fat. The inferior and posterior adrenal vessels are in this way intercepted in the periglandular fat, sealed, and transected leaving the adrenal vein exposed between the gland and the inferior vena cava (right) or renal vein (left) [22]. At this moment (depending on the surgeon's experience, vessel diameter, and available surgical devices), the adrenal vein can be clipped or sealed leaving only the division of the remaining peri-adrenal fat to completion of the adrenalectomy. As a result, the adrenal gland will be removed along with its surrounding fatty tissue.

The technical feasibility of laparoscopic adrenalectomy for large pheochromocytomas in today's surgical activity is no longer a rarity [23,24]. Large right-sided tumors may be technically more challenging because of the operating space, which is limited by the liver with the inferior vena cava compressed and displaced anteriorly by the tumor, sometimes taking a lenticular shape on the medial margin of the tumor. However, large left-sided pheochromocytomas are also a source of complications. Our experience counts 23 pheochromocytomas larger than 6 cm and our intraoperative strategy is in concordance with other authors' opinions performing systemic peritumoral dissection, without manipulation of the tumoral gland, without the previous approach of the adrenal vein [25]. The purpose is to progressively free the tumor from the surrounding tissue and to take maximum advantage of gravity in achieving exposure. Another useful tool, in our experience, was the blunt 10 mm vessel seal and dividing device that helped not only achieve good hemostasis in the peritumoral fat but also protect the capsule from rupture, not mentioning also a speedy surgery timing.

Intraoperative complications

Laparoscopic adrenalectomy is considered to be the gold standard for surgery to remove the adrenal gland. Still one of the factors for intraoperative complication occurrence is pheochromocytoma along with the tumor size, surgical inexperience, patient age, and body mass index [26]. The most common intraoperative complication is hemorrhage which is in most cases possible to control in the hands of a surgeon with good laparoscopic skills and experience. Conversion to open surgery dropped from 6% in some early studies [27] to 0.6% [28]. Bleeding in a hypertensive crisis can occur from small vessels that are why special attention has to be paid to hemostasis. If proper electric hemostasis cannot be achieved and if it affects the safe dissection, applying a large gauze and pressing it into the bleeding area, was in our experience the best way to manage this situation until a clip was applied to control the bleeding. Apart from general complications during laparoscopic adrenalectomies specific aspects are related in particular to the pheochromocytoma, large tumors with important retro-caval development that compress the IVC anteriorly, present a high-risk caval injury that mandate conversion to laparotomy in most cases. Other vascular or visceral lesions can be caused by the large tumoral volume, specific to pheochromocytomas, and the need to mobilize the surrounding tissues instead of the gland, to avoid both catecholamines release or capsular breaking are the renal and the splenic veins. Major vascular injuries are recognized instantly during dissection, and it is important to evaluate the chance of controlling them laparoscopically, and not waste time converting to an open procedure [29]. Liver, pancreatic and splenic injuries are mostly due to adhesions, the presence of a large tumor or unexpected excessive tissue retraction to protect or better access the pheochromocytoma. In our experience, no solid organ injury during pheochromocytoma minimal invasive surgery forced us to convert to open surgery.

Causes of conversion

A large multicenter study showed that unplanned conversion to laparotomy during laparoscopic unilateral adrenalectomy for pheochromocytoma was independently associated with increased intraoperative hemodynamic instability episodes and postoperative complications rate [30,31]. Studies revealed that the ratio of intraoperative systolic blood pressure >200 mmHg and the total anesthesia time was an independent factor associated with conversion [32]. Although controversial intraoperative hemodynamic instability remains an important marker of the quality of pheochromocytoma manipulation during the surgery [32,33].

Another particular case of conversion in this type of tumor is represented by tumoral spillage not only due to the high risk of relapse [5,23] but also because of the high risk of undiagnosed malignancy [34], especially taking into consideration that in pheochromocytoma radiographic and histological information, including intraoperative frozen section examination, are unreliable predictors of malignancy [35,36].

However, conversion to an open procedure is warranted when laparoscopic dissection cannot be performed safely or a complete resection cannot be performed without undue trauma to the gland.

In our experience, conversion during laparoscopic adrenalectomy for pheochromocytoma was needed in 9 cases: 1 case of severe cardiac arrhythmia with cardiac arrest that required resuscitation on the operating table, 3 cases of invasive malignant pheochromocytoma, 2 cases large tumors that could not be dissected safely, and 3 cases of major vessel lesions: two inferior vena cava lacerations and one left renal vein injury.

Specimen extraction

Special care should be paid to the adrenal specimen since it might spill and contaminate the peritoneal cavity causing disease recurrence [5] or even pheochromocytomatosis [14]. This is why immediately following specimen detachment it is introduced in a retrieval bag for the following surgical steps like controlling hemostasis. Manipulation during this step should follow the dissection principles since the pheochromocytoma capsule can rupture under its weight if it is uncarefully manipulated. We recommend grasping the specimen using periglandular tissue, fascia, or vascular stumps to place it in the bag. We retrieved the bag always through the lateral incision that is enlarged accordingly to the specimen size, avoiding morcellation and contamination of the wall.

We also routinely drained the adrenal lodge, the drainage tube being exteriorized through the same incision from the specimen was retrieved. The tube stays in place for 24 hours if no bleeding is noticed.

Special attention to malignant pheochromocytoma was given to achieve an en-bloc excision of the tumor with wide surgical margins and as much peri-adrenal tissue as possible. The need to operate around the renal artery and vein or the renal hilum for achieving an en-bloc resection along with the peri-adrenal does not mandate conversion to laparotomy. However, conversion to an open procedure is warranted when laparoscopic dissection cannot be safely performed or a complete resection cannot be performed without undue trauma to the gland [5]. Hand-assisted laparoscopy is a good alternative to open conversion if a difficult dissection is encountered intraoperatively [37].

CONCLUSIONS

The best way to improve is to repeatedly follow a correct surgical pathway even from the beginning, especially when approaching a pathology with great morbidity related strictly to the delicacy and correctness of the surgical act. Experience of the high volume centers translates into excellent outcomes, achieved by minimizing complications, decreasing blood loss, and shortening hospital stays.

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REVIEW

Melanoma: A Historical Walk-through from Palliative Treatment to Modern-day Practice

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Abstract: *Melanoma is one of the most common types of malignancy in the world, and one known to carry a very poor prognosis until recent years. This review aims to outline the events in the history of the disease and the impact made by the discoveries along the way, as well as the modern-day consensus by referencing the updated literature regarding the present approach and future directions. In the last two decades, several studies and research have brought significant improvement in the diagnosis and clinical management of melanomas. The development of sentinel lymph node biopsy has brought major changes to the surgical approach to the disease, and modern therapies based on recently developed knowledge changed the death sentence this diagnosis once meant to a manageable condition despite its aggressiveness, keeping in mind that early diagnosis and safe margin excision remain the best and most optimistic course of treatment.*

Keywords: *melanoma, surgery, Breslow, Clark, lymphadenectomy, BRAF, immunotherapy, sentinel lymph node biopsy*

INTRODUCTION

Malignant skin tumors are among the top 10 causes of disease burden worldwide. 18.8 million new cases of cancer have been diagnosed in 2018 alone, with breast, lung, and colorectal cancer being the most frequent. It is also the second most frequent cause of death, preceded only by ischemic heart disease [1]. While skin cancer is not among the highest incidence malignancies overall, they do represent the most common malignancy in the white population. Furthermore, melanoma has had the highest increase in incidence rates – 5-fold in the past 30 years [2]. Therefore, reducing disease burden and mortality has become public health priority. Numerous studies and research have brought significant improvement in the diagnosis and clinical management of skin cancers, especially in the case of melanomas, which carried a very poor prognosis until recent years. Part of these improvements is due to the development of genetic testing for specific mutations and the subsequent development of targeted therapies. These have led to the increased survival of numerous patients and to important changes in the way this pathology is managed. Surgical excision has always held a central role in melanoma therapy, and it naturally changed and adapted along with newer treatments.

HISTORY OF MELANOMA DIAGNOSIS AND TREATMENT

The word “melanoma” originates from Greek (“melas” meaning “dark” and “oma” meaning “tumor”) and it was described for the first time in recorded history by Hippocrates in the 5th century BC. The earliest physical evidence of melanotic tumors dates from around the same time and was found in the bones of pre-Colombian mummies [3]. Throughout the following centuries, more reports of such pigmented tumors were made, though not much was understood about their origin and development. In 1806, Rene Laennec introduced the term melanosis to describe the disease as a separate entity and demonstrated the difference in its structure from other dark-colored lesions found in autopsies, such as carbon deposits in the lungs [4]. The first thorough study of the disease was made by William Norris in 1830, who followed up a patient for 3 years, described the condition clinically and the autopsy findings in detail, including the vast number of metastases, their macroscopic structure, and the hereditary character of that particular case. He was also the first one who, in later studies, highlighted a possible link between “melanosis”, and noticed a correlation between the condition and light-colored skin and hair [5]. The term “melanoma” was introduced in 1838 by Robert Carswell, who studied and described melanoma metastases, including brain localization, in detail [6]. A comprehensive analysis of primary tumors was also provided by James Paget in 1853, including the first description of the transition between a radial growth phase and a vertical growth phase [3,7].

In the 20th century, research techniques improved, and more effort was made to understand the disease and structure its treatment. In 1953, Spitz and Allen observed that nodular (or "deep") melanomas have a worse prognosis than superficial melanomas [8].

In 1954 Ackerman and Del Regato were the first to propose a staging system, comprising four groups: a – patients with the generalized disease, b – patients with clinical lymph node disease, c – patients with infra-clinical (histological) lymph node involvement, and d – patients with primary tumors. The system was based on the observation that the latter group demonstrated improved survival compared to the first group, but not much difference was observed between the two middle groups [9,10].

With time, other stage systems were developed and proposed for clinical practice, such as the one designed by McNeer and Das Gupta in 1964, which comprised three stages based on disease extension and is regarded as a precursor to the modern TNM staging system [11]. This system was expanded and improved by the MD Anderson Hospital in 1976 [12], but it still had some shortcomings, such as failing to consider the pathology of the primary lesion.

In 1969, Clark, improving earlier systems proposed by Petersen (1962)[13] and Mehnert and Heard (1965)[14], based on the microscopic aspect of the primary tumor, presented the five levels of invasion still in use today [15]. One year later, in 1970, Breslow demonstrated the importance of primary tumor thickness in overall prognosis and survival [16].

With later studies corroborating their findings, such as McGovern's for the Clark index [17] and Balch's for Breslow – which also proved that tumor thickness is more relevant for prognosis than the level of invasion [18] – these characteristics of the primary lesion were added to later staging systems. Over time, the American Joint Committee for Cancer (AJCC) and the Union Internationale Contre le Cancer (UICC) have made several attempts to establish a simple, relevant, and coherent staging system that could be applied internationally.

Following a series of proposed systems that were only partially satisfactory, and after adapting to encompass new findings, in 2002 the AJCC proposed the modern staging system, whose updated version (8th edition) is in use today [19,20]. It includes ulceration of the primary lesion as a prognosis factor, uses the Breslow index as a primary determinant of T staging, with the Clark level used only for thin tumors (<1 mm), and makes a clear distinction between clinical and pathological staging. It also takes into consideration additional prognostic factors such as age, gender, tumor location, and histological subtype.

Melanoma treatment evolved as the understanding of the disease grew. The first surgical resection of such a lesion in Western medicine was performed by John Hunter in London in 1787 [3]. By the end of the 19th century, it became clearer that surgical excision is an appropriate course of treatment, with some authors such as Herbert Snow advising adjacent lymph node resection, while William Handley suggested a 5 cm of healthy tissue around the tumor along with extensive lymph node excision [3]. This remained the standard for melanoma surgery well into the 20th century.

After Clark and Breslow's studies on the importance of primary lesion thickness in the prognosis of melanoma, surgical therapy was gradually adapted to decrease morbidity after extended excisions. A series of studies in the 1990s up to the early 2000s aimed to determine the optimal resection margin for each primary tumor stage.

Eventually, a relative consensus was established for safety margins of 0.5 cm for melanoma in situ, 1 cm for invasive melanoma <1 mm thick, and 2 cm for a Breslow index of >2 mm with some debate still ongoing about the optimal resection margin for tumors between 1 and 2 mm thick [21].

As mentioned previously, complete radical lymphadenectomy was performed as part of the surgical treatment for melanoma for most of the 20th century. The argument behind the indication was based on extensive studies on lymphatic drainage made by Rudolph Virchow and by Marie Sappey in the later part of the 19th century, and was supported and practiced by prominent surgeons like William Halsted for breast cancer in the US and, as mentioned before, by Herbert Snow for melanoma, in Britain [22].

Lymphadenectomy became an essential step in the oncological resection of various tumors for almost a century. By 1990, lymph node dissection was still performed as standard of care in melanoma treatment, whether electively (for clinically normal nodes) or therapeutically (for clinically evident lymph node involvement), but the need to find a better approach was frequently discussed, especially for small primary tumors and no clinical lymph node involvement [23].

In 1992, Morton and Cochran published a study that demonstrated the dissemination of tumor cells through lymphatic vessels first to a specific, identifiable node, called the sentinel node, then to other regional nodes [24]. They were not the first authors to use the term "sentinel node", the term had been used previously by several authors to describe the first nodes draining specific areas on the body, but based primarily on their anatomy rather than physiology [22]. Morton used the technique described by Gerota who used a blue dye to map lymphatic vessels and introduced lymphoscintigraphy for patients with axial melanoma to determine the primary draining basin [25]. Their studies were not accepted easily, but as data continued to support their findings, sentinel lymph node biopsy became an integrated part of melanoma treatment.

GENETIC MUTATIONS IN MELANOMA

It is now understood that cancers are the consequence of a series of mutations in genes that control the proliferation, differentiation, and programmed death of the affected cells. In 2002, Davies and colleagues demonstrated a significant presence of BRAF mutations in human cancers [26]. BRAF (v-RAF murine sarcoma viral oncogene homolog B1), a proto-oncogene, is a RAS-regulated serine-threonine kinase and an activator of the MAPK signaling cascade. It plays an important role in cell differentiation, proliferation, and death through extracellular signals. The mutation was found in several malignancies, including melanoma, colorectal cancer, adenocarcinomas of the lung, sarcomas, ovarian cancers, and liver cancers, and it prompted the research for other targets and pathways. Its identification in a large proportion of benign demonstrated that this mutation may be a step in melanoma development and prompted further studies [27].

Among the most frequent mutations responsible for oncogenesis in melanoma are observed in the MAPK signaling pathway (mitogen-activated protein kinase). This pathway promotes cell cycle progression, transformation, and cell survival and, in non-cancerous cells, it is activated through cytokine receptors or tyrosine kinase receptors. Ligand binding to the receptors leads to the activation of GTPase switch proteins known as RAS, which in turn activate a series of serine/threonine kinases, which ultimately activate MAPK. Permanently activated MAPK signaling has been observed in almost all melanomas [28].

BRAF is a kinase whose only known activity is in the MAPK pathway and is highly expressed in melanocytes but also hematopoietic tissue and neural cells. The most common BRAF mutation in melanoma is the V600E – a valine to glutamic acid substitution in exon 5, codon 600, a mutation that permanently activates the kinase domain [26]. These mutations are usually found in cutaneous melanoma, specifically in those tumors arising on intermittently sun-exposed skin, despite it not being a typical UV-induced DNA mutation [29].

Other mutations involved in cell proliferation are relatively rare in melanomas, such as mutations that increase RAS activity. They occur mostly in amelanotic nodular subtypes and makeup only about 10-20% of melanomas, with NRAS being the most commonly affected [30]. Interestingly, these two mutations, BRAF and NRAS seldom occur simultaneously in melanomas [31], and in the rare situations when they do, the BRAF mutation is not the typical V600E type [32], suggesting that they have overlapping oncogenic activity and either of them could activate the MAPK pathway.

The above-described mutations can also appear in benign nevi, as well as in melanomas. BRAF mutations can be found in 70-80% of dysplastic nevi [33] and NRAS in congenital nevi [34]. Given these findings, it was at first believed that the BRAF mutation was the initial oncogenic event that triggered all other changes in BRAF-positive melanoma. However, data showed that only 6% of in situ melanomas have a BRAF mutation [26], and that seems to be polyclonal (containing melanocytes with and without the BRAF mutation, or with BRAF wild type), metastatic melanoma tends to be monoclonal [35], suggesting that the mutation might occur later in the oncogenic process. Still, at any moment it occurs, BRAF activation leads to cell growth and dysregulation of the MAPK signaling pathway [36]. Other mutations or alterations can lead to the same result, like overexpression of growth factor receptors like KIT or EGFR (epidermal growth factor receptor)[37,38]. NF1 (neurofibromatosis 1, a tumor suppressor) mutations lead to melanoma proliferation through the MAPK and PI3K pathways [39].

The MTIF gene, responsible for a melanocyte-inducing transcription factor is another essential regulator of melanocyte development, survival, and function, and it can be linked to melanoma development. Its amplification can be found in 15-20% of melanomas, especially metastatic tumors, and it has been shown to contribute, alongside BRAF, to melanoma development [40].

An interesting occurrence in melanoma is the genetic heterogeneity of these tumors, both intralesional and occurring during disease progression. These findings partially explain treatment resistance, on one hand, and can influence therapeutic decisions, on the other [41]. One study investigated both benign and malignant melanocytic lesions and interestingly found that the benign lesions only showed BRAF-V600E mutations [42]. As the disease progressed, more mutations developed. In more advanced lesions, NRAS and subsequently TERT promoter mutations were found. In invasive and advanced melanomas, inactivation of CDKN2A and loss of PTEN and TP53 were observed [41,42]. All these findings suggest that the BRAF-V600E mutation may require other mutations that inhibit tumor suppressors to ultimately lead to melanoma development.

TARGETED THERAPY IN MELANOMAS

The first targeted agent for BRAF V600 was Vemurafenib, which showed significantly higher response rates than the previously used chemotherapeutic agent dacarbazine [43]. Dabrafenib was the second targeted agent to be developed, and despite some side effects such as rash, fatigue, or fever, the therapy was generally well tolerated [44]. Along with a better understanding of the BRAF gene and its interconnected activity with MEK, MEK inhibitors were developed, with trametinib being the first. Simultaneously, studies into BRAF-inhibitor resistance lead to a better understanding of the importance of MEK in the process, and the connection between the two pathways [45]. This eventually led to the association of the two agents, a BRAF inhibitor and a MEK inhibitor, which was studied in a series of clinical trials, for both dabrafenib plus trametinib, as well as for vemurafenib plus cobimetinib, with encouraging results showing a lower rate of cutaneous adverse reactions and increased progress-free survivals [46,47]. This has led to attempts to treat non-BRAF mutated melanoma with a MEK inhibitor in monotherapy, but the results were not promising, suggesting that further genetic studies into the MEK activity and its possible association with other mutations such as NRAS are necessary and could lead to new therapies [43].

Vemurafenib is a selective kinase inhibitor of the MAPK pathway in BRAF-mutated cancer cells, leading to decreased proliferation and apoptosis, while dabrafenib is a reversible, ATP-competitive BRAF inhibitor [48]. Cobimetinib is a non-ATP-competitive inhibitor of MEK1, with a high selectivity for MEK 1 over MEK2. Similarly, trametinib is an allosteric small molecule that binds preferentially to unphosphorylated MEK, preventing Raf-dependent MEK phosphorylation and activation.

In clinical practice, the RT-PCR method is widely used for BRAF mutation detection. The cobas 4800 BRAF V600 kit and the ThxID-BRAF kit have been developed together with the targeted therapeutic agents' vemurafenib and cobimetinib and dabrafenib and trametinib respectively, and are frequently used due to their accessibility, reproducibility and relatively rapid results. These tests use two different primer sets to detect the V600E mutation and the wild-type sequence and then compare the results. While this is sufficient for usual testing, other methods are available for more in-depth analysis and also for rare BRAF types (about 2% of cases), such as Sanger sequencing, pyrosequencing, and high-resolution melt analysis (HRMA)[41].

Systemic therapy for non-mutated melanoma

For decades, melanoma has been considered an incurable disease, especially in later stages, partially because both classic chemotherapy and radiation therapy are not significantly effective, with the drug Dacarbazine showing the best overall response rates of about 10-20% [49]. Research into the development of this malignancy and specific studies on metastatic melanoma has led to the discovery of genetic testing and targeted therapies on one hand, and also to the development of immunotherapies for non-mutated tumors.

There are four main types of immunotherapy: biological agents such as cytokines, interferon, and granulocyte-monocyte colony-stimulating factors; vaccination therapy; adoptive cell therapy and immune checkpoint inhibitors [50]. Biological immunotherapy such as interferons and IL2 were the first alternative to classic chemotherapeutic agents and were frequently used together, to amplify their action. Vaccination therapy is a type of specific immunotherapy that is currently studied. Oncolytic viruses are modified strains of various viruses that maintain their replication capacity but lose their virulence, and such strains have led to cell death in melanoma cell lines in vitro and animal models, by activating antitumoral immunity through the production of cytokines [51]. However, not all types of vaccination are very effective in vivo, such as DNA-based vaccines, despite proving safe and effective in laboratory settings [52]. Adoptive cell therapy is an experimental treatment alternative based on the patient's lymphocytes, which are then activated in vitro and reinjected into the patient to induce an immune response. The technique is time-consuming and elaborate, requiring specialized personnel and equipment [50]. The most effective immunotherapy studied has been the immune checkpoint inhibitors. They are designed to suppress the mechanism of self-regulation in natural immune response and target specific overexpressed molecules in the tumor they are used against. In melanoma, they target PD-1, a cell-surface molecule that downregulates B and T lymphocyte activity (Nivolumab and Pembrolizumab), or CTLA-4, a protein receptor that acts as a negative regulator of T-cell activation (Ipilimumab).

As previously mentioned, most trials for immunotherapy agents have been conducted on regionally advanced and metastatic diseases. Interferon-α has been used for over 20 years before the development of modern therapies to treat advanced disease, with moderate results, as shown in a meta-analysis by Mocellin in 2010 [54], while pegylated IFN-α demonstrated to prolong disease-free survival without improving overall survival in regionally advanced disease [55]. The treatment was associated with significant side effects varying from myalgia, fatigue, fever, headaches, and chills to depression, which could be severe and require treatment [56].

Ipilimumab has been in use since 2015. Its efficacy was demonstrated initially in unresectable metastatic melanoma and subsequently began being used as an adjuvant treatment.

Initial studies proved it increases overall survival in patients who had undergone complete lymph node resection for node metastases (overall survival at 5 years 65.4% compared to 54.4% in the placebo group) but was associated with significant adverse events (in 41.6% of patients)[57], even though these adverse reactions didn't significantly lower quality of life [58]. Ipilimumab is not currently in use as the first line of treatment in regionally advanced or metastatic melanoma.

Nivolumab was approved in 2014 and most studies compared its efficacy with Ipilimumab in the same category of patients, showing a lower rate of recurrence (recurrence-free survival at 18 months of 66.4% vs 52.7% for Ipilimumab and 70.5% vs 63.7% at 24 months) and significantly fewer adverse reactions (17.5% vs 40.4%)[57].

Pembrolizumab was approved in 2019 and showed similar results, disease-free survival of 71.4% vs 53.2% for placebo, and 14.5% moderate to severe adverse reactions vs 3.4% in the placebo group [58].

Combination therapy has proven to be more efficient than monotherapy, the association of Ipilimumab and Nivolumab led to better results in several studies(50), although toxicity may increase, with cutaneous reactions (over 50% of patients), gastrointestinal and thyroid disorders being the most frequent [56].

CURRENT RECOMMENDATIONS IN MELANOMA DIAGNOSIS AND TREATMENT

Any suspicious lesion of the skin should be carefully examined. The surrounding skin should also be checked for satellite lesions and regional lymph nodes should be palpated. Physical examination of the lesion can be completed with dermoscopy by a specialized medical professional for improved accuracy [59]. A patient's personal history of sun exposure, other skin lesions, or cancers is also important, as well as a careful history of the lesion in question. Excisional biopsy with subsequent microscopic examination of suspicious lesions is the only way to accurately establish a melanoma diagnosis. The excisional biopsy should include margins of 1-2 mm around the lesions and into the subcutaneous tissue. Other surgical methods like incisional biopsy are not recommended with very few exceptions and superficial shave biopsies should be avoided because accurate lesion thickness cannot be correctly established [60]. If suspicious palpable masses are identified around the lesion or node basin, further evaluation may be necessary, the most accessible of which is soft-tissue ultrasonography, which can be paired with a fine-needle aspiration.

Once a melanoma diagnosis has been established through biopsy, wide local excision of the scar and surrounding tissue is recommended, the margins of which are established based on Breslow thickness [61]. For in situ melanomas – 0.5 to 1.0 cm; for Breslow < 1.0 mm – 1.0 cm; for Breslow between 1.0 and 2.0 mm – 1.0 to 2.0 cm; and for Breslow > 2.0 mm – 2.0 cm margins are recommended. The histological examination should be performed on permanent sections, given that frozen sections may lead to false negative results [62]. Mohs surgery is also not recommended, with certain exceptions in very superficial tumors located in delicate anatomical sites [61]. Complex reconstruction involving skin flaps should be postponed until microscopic margins are established. Some guidelines accept covering the defect with non-adherent dressing until the final histological result is obtained [61,63].

The spread of malignant cells from the primary tumor occurs primarily through lymphatic vessels to distant sites, through regional lymph nodes. The most valuable predictors for nodal dissemination are Breslow thickness and the presence or absence of ulceration and they are the basis for the indication of a sentinel lymph node biopsy [64]. Thus, all tumors thicker than 1.0mm should be considered for lymph node biopsy. Smaller tumors should be evaluated for poor prognosis factors such as ulceration or microvascular invasion and a decision can be made on that basis [65]. Recent studies have also evaluated the role of complete lymph node dissection in the case of a positive SLN, which was the initial standard of care. They did not demonstrate improved survival and metastasis-free survival in the case of early completion of lymph node dissection, as opposed to monitoring and conducting the procedure in a delayed fashion, when the nodes become identifiable clinically or ultrasonographically [66].

Staging aims to be a simple and practical method to determine a patient's prognosis and guide treatment. It is also helpful in reporting data for future studies and clinical trials. The latest American Joint Committee for Cancer (AJCC) TNM staging system defines separate clinical and pathological staging [20,65]. Clinical staging (cTNM) is based on imaging and clinical examination and pTNM takes into account histological results obtained after surgery. Stage 0 represents melanoma in situ. Stage I is a localized disease, with IA representing tumors of less than 1 mm thickness with or without ulceration (T1a/T1b) or 1-2 mm without ulceration (T2a). Stage II melanoma is also localized, but with larger primary lesions, or ulceration. IIA denotes a 1-2 mm ulcerated lesion, IIB a >2 mm lesion with or without ulceration. Stage III is regional melanoma, with node involvement or satellite or in-transit lesions, with subtypes “a” to “d” for the extent of invasion. It is the subgroup with the most variable 5-year survival rates, ranging from 93% for IIIa to 32% for IIId [68]. Stage IV signifies generalized disease, with distant site dissemination. No subgroups were defined for this stage, given that the overall survival is similar among these patients [50].

Other prognostic factors can have an impact on prognosis. The female gender appears to be associated with a better prognosis, while melanomas of the head and neck are associated with a worse prognosis [69]. Older patients have overall higher mortality [70], nodular and amelanotic melanomas have a worse prognosis [71] while melanoma developed on pre-existing nevi are less aggressive than de novo melanoma [72].

Adjuvant therapy is essential for the outcome of stages III and IV melanoma, the stages with the highest rates of recurrence and dissemination. For earlier stages – stages I and IIA – surgical excision and follow-up seem to be the optimal treatment. For stages, IIB and IIC the risk of adverse reactions is greater than the risk of recurrence, so adjuvant treatments are generally not indicated. For stage III and IV, immunotherapy with Nivolumab or Pembrolizumab, alternatively, if BRAF-V600E genetic test is positive, Dabrafenib and Trametinib is the first-line option [73].

CONCLUSIONS AND FUTURE DIRECTIONS

Diagnosis and treatment of melanoma have evolved greatly to the benefit of patients, especially in later stages, and is even more important as the incidence of melanomas continues to rise. The development of sentinel lymph node biopsy brought major changes to the surgical approach to the disease, and the discovery of the genetic and immune basis of melanoma development changed the treatment protocols that have been used for decades. Classic chemotherapeutic agents are relatively ineffective, recently studied chemotherapy presents more promising results, nonetheless, newer agents also present side effects, sometimes leading to interrupting the treatment, such as colitis and diarrhea for immunotherapy, or keratoacanthoma and squamous-cell carcinoma for targeted therapies [56]. Experimental studies are being conducted with T-cell-based adoptive cell transfer immunotherapy, which proved effective in a laboratory setting [74]. Simultaneously, there is also a need to optimize screening. At the moment, screening and follow-up of low-risk melanomas are based on clinician-led surveillance, but there are proposals to increase patient education on self-examination [75]. Results are yet inconclusive, some studies already show that patients tend to detect melanomas when they are thicker [76], yet the value of self-examination has been proven before [77]. Modern technology is also being researched to aid early diagnosis. Gene profiling can identify low or high risk of developing melanoma [78]. Dermoscopy can be used with computer software to analyze suspicious lesions more efficiently [79]. Finally, the increasing number of smartphone apps focusing on healthcare, including dermatology, should be assessed for accuracy, as many potentially dangerous lesions can go overlooked [80-82].

In conclusion, understanding melanoma has improved spectacularly, which benefits the increasing number of new patients and the therapies developed based on this knowledge changed the death sentence this diagnosis meant until 20 years ago to a manageable condition despite its aggressiveness, keeping in mind altogether that early diagnosis and safe margin excision remain the best and most optimistic course of treatment.

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ORIGINAL ARTICLE

The Impact of the Hypercaloric Diet versus the Mediterranean Diet on Insulin Sensitivity

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Abstract: *Obesity affects the population worldwide. A hypercaloric diet associated with a sedentary life, stress, and genetic background, triggers various metabolic disorders, such as metabolic syndrome, diabetes mellitus, cancer, cardiovascular diseases, non-alcoholic fatty liver disease, and cognitive impairment. A healthy diet correlated with physical activity, not smoking, and moderate alcohol consumption reduces the risk of developing metabolic diseases. The Mediterranean diet contains antioxidants, fiber, polyunsaturated fats, and compounds with anti-inflammatory, anti-oxidant, anti-cancer, and anti-obesity properties. In a wide variety of species including humans, the reduction of calories between 20-40% significantly improves health, increases longevity, and delays the development of various pathologies. The main aim of this review is to present the comparative effects of the Mediterranean diet versus the hypercaloric diet on insulin sensitivity.*

Keywords: *obesity, diet, MetS, insulin, metaflammation*

INTRODUCTION

Currently, obesity affects the young and adult population worldwide. Hypercaloric diet, sedentary life, and stress lead to obesity [1-3]. Sugar has a well-established metabolic effect. Therefore, the direct pathway includes fructose, which induces lipid and carbohydrate metabolism dysregulation. Indirectly, an excessive sugar intake leads to body weight and fat gain, which further induces the perturbation of lipid and carbohydrate metabolism [4].

A hypercaloric diet triggers dyslipidemia, a key player in the pathogenesis of metabolic diseases [5]. Hyperinsulinemia is installed when insulin secretion is increased, insulin pulses are decreased, and/or hepatic insulin clearance is reduced [6]. This metabolic state is induced by several factors such as the modern Western diet, over-nutrition, and genetic background. Once installed, hyperinsulinemia perturbs the balance of the insulin-growth hormone (GH)-insulin-like growth factor (IGF)-axis and switches to an insulin: GH ratio, leading to lipogenesis and inhibition of lipolysis and further to obesity [6]. In mammals, GH stimulates the production of IGF [7]. The hypothalamus, the pituitary gland, and the liver regulate the GH/IGF-1 system. GH is primarily synthesized by the somatotrophs from the anterior pituitary gland, but reproductive tissues, lymphoid tissues, and the gastrointestinal tract may also syntheses it. Moreover, other endocrine axes may have permissive actions on GH and stimulate IGF-1 expression [8].

Ceramides are lipids from the sphingolipid group, which can act at the plasma membrane, endoplasmic reticulum, and mitochondria levels, being involved in insulin signaling and glucose homeostasis [9]. Under certain metabolic dysregulation (low androgen levels in males and high androgen levels in women) IGF signaling is perturbated leading to metabolic dysfunction and type 2 diabetes [10].

The most common metabolic disorders correlated with hypercaloric diets include metabolic syndrome (MetS) [11,12] diabetes mellitus type 1 and 2 [13], obesity [2,3,14], cardiovascular diseases (CVD)[15], various cancer types [16-21] infertility, non-alcoholic fatty liver disease (NAFLD) and cognitive impairment [16,22].

HYPERCALORIC DIET AND INSULIN SENSITIVITY

Clinically, insulin resistance (IR) was defined as the incapacity of insulin to increase cellular glucose uptake [23]. Several factors such as age, body weight, genetic predisposition, sedentary life, stress, overstimulation of the sympathetic nervous system, and inflammation may induce the development of IR [24]. Currently, a wide range of populations worldwide have a sedentary life, which correlated with unhealthy diets will conduce to an increased incidence of obesity development related to Diabetes mellitus type 2 [25]. Epigenetic modifications were detected: altered DNA methylation of type 2 diabetes-specific genes such as IRS1, KCNQ1, PPARG, and TCF7L2 [25].

In muscle and liver, IR is correlated with increased circulating levels of free fatty acids, and mitochondrial and endoplasmic dysfunctions [26]. It is well known that type 2 diabetes is installed as a consequence of IR, a process that is frequently associated with obesity. In obesity, hyperglycemia induces excessive insulin production that further conduces to increased beta-cell mass and at the end beta-cell apoptosis. In the last decade, obesity has been named a low-grade inflammatory state, known as metiflammation [27].

In overweight adults, a diet poor in vegetables harms gut microbiota, leading to IR [28].

The muscle, the liver, and the adipose tissue may have increased amounts of ceramide, which is further correlated with IR. The high carbohydrate diet leads to an increased serum level of triglycerides (TG) and decreased levels of HDL-cholesterol, events that are correlated with an increased risk of CVD [25]. Chronic hyperglycemia induces subclinical inflammation, which is the major factor of endothelial damage and represents the link between diabetes and CVD [29].

It is well known that overnutrition conduces to weight gain, which further creates a vicious cycle characterized by IR/hyperinsulinemia. This event will conduce to atherogenic dyslipidemia development, illustrated by hypertriglyceridemia, decreased HDL, and increased LDL [30]. IR affects 25-35 % of the Westernized population [16,22].

Metabolic syndrome (MetS) is defined as a multi-factorial disorder, which received several names such as IR syndrome, metabolic syndrome, Reaven's syndrome, Syndrome X, and the deadly quartet [12,31]. MetS are characterized by abnormal obesity, dyslipidemia, decreased levels of HDL-cholesterol, IR, and hypertension. This metabolic disorder is mainly characterized by obesity and IR. The risk of coronary heart disease and type 2 diabetes is significantly reduced by treating MetS [32].

One of the most common disorders of pregnancy is gestational diabetes mellitus (GDM), which is developed in the second and third trimesters of pregnancy, leading to hyperglycemia of various severity [22,33]. GDM development harms both mother and child, resulting in cesarean delivery, macrosomia, and neonatal hypoglycemia. Moreover, women with GDM have a huge after-pregnancy risk of CVD and type 2 diabetes development [22]. A decreased amount of vegetables and an increase in animal products may cause IR. Pre-pregnancy intake of processed and unprocessed meat is associated with an increased risk of GDM development [34].

Short-chain fatty acids (SCFAs) produced by our gut microbiota can affect the cardiometabolic phenotype because they can enter the systemic circulation and may directly affect the metabolism or the peripheral tissue function [35]. Therefore, SCFAs stimulate the sodium/glucose transporter-1 expression leading to increased absorption of monosaccharides. SCFAs directly influence the host's satiety, because they can promote the glucagon-like peptide-1 (GLP-1) and peptide YY release. This is possible after the interaction of SCFAs with G-proteins expressed by enteroendocrine cells. More than that, SCFAs promote lipogenesis and inhibit fatty acid oxidation, triggering obesity [36].

MEDITERRANEAN DIET AND INSULIN SENSITIVITY

A healthy lifestyle includes a healthy diet, physical activity, not smoking, and moderate consumption of alcohol, especially for women [37]. A vegan diet based only on the consumption of grains, vegetables, and fruits, reduces the risk of MetS, diabetes, and [28, 38], and prevents CVD [39].

A diet rich in fiber, more than 25 g/day in women and more than 38 g/day in men decreases the risk of type 2 diabetes. In addition, a diet that contains nonviscous compounds such as whole grains and insoluble cereal fibers may influence the postprandial glucose response or could be very well fermented by the colon gut microbiota [40]. The results of 30 randomized clinical trials performed on men and women, reported that the consumption of milk and dairy milk products is associated with a decreased activity of insulin and fasting insulin levels [41].

In the case of healthy subjects, an acute endurance exercise in the fasted state causes important changes: stable blood glucose amount, low blood insulin levels, increased lipolysis with an increased blood-free fatty acid concentration, maintenance of the muscular glycogen and high intramyocellular TG oxidation [42].

Recent studies illustrate that replacing the carbohydrates from the diet with fat will decrease fasting TG and will increase the HDL level. Therefore, the results of the meta-analysis that included obese patients who received a high-fat versus low-fat diet, reported after the consumption of a low-fat diet a significantly elevated level of TG and a decreased level of HDL-cholesterol [43].

The Mediterranean diet (MD) consists of antioxidants, fiber, polyunsaturated fats, and compounds with anti-antioxidant, anti-inflammatory, anti-cancer, and anti-obesity properties [44]. Fats intake, especially monounsaturated fat (MUFA) helps the body to reduce the level of insulin. Moreover, 3–4 g of MUFA will decrease the TG levels and blood pressure (BP) in subjects with MetS. Cohort studies reported that a dairy fat consumption of 50% saturated fat leads to a decreased incidence of MetS [43]. A Mediterranean-style diet has positive effects in reducing the MetS prevalence and cardiovascular complications [45]. Even World Health Organisation (WHO) recommends a diet poor in carbohydrates, for adults and children. Their consumption reduces circulating insulin levels and decreases body weight and TG levels [46]. On the other hand, the decreased level of circulating insulin conduces to an increased level of circulating fatty acids, utilized for oxidation and further ketone bodies synthesis. However, this diet has negative effects on the human body in long term [46].

Willems AEM and his research team, published 2021 the results of a meta-analysis regarding the impact of low-carbohydrate and low-fat consumption on markers of MetS in obese patients without cardiometabolic disease [47]. The studies reported that weight loss is the most efficient way to reduce fasting glucose levels independent of macronutrient intake. Moreover, the specific studies reported that carbohydrate and fat intake, but without changing the percentage in the intake of these macronutrients, improved diastolic blood pressure (DPB) and circulating TG levels, without any effect on weight loss [47]. The IR was also improved with both diets, while HDL was only improved in the low-carbohydrate diet. This study concluded that the reduction of carbohydrate and fat intake are not the only factors that improve the MetS markers. This suggests that the guidelines for the prevention of MetS must be re-evaluated [47].

Cellular studies reported that the consumption of polyphenols, such as green tea catechins and epigallocatechin gallates, resveratrol, and curcumin reduce TG accumulation, and stimulate lipolysis and fatty acid-beta oxidation, leading to the reduction of obesity-related inflammation [48].

Ros E and his research team, observe the long-term effects of the MD without any energy restriction on CVD patients [49]. The study reported that MD had good effects on oxidative stress, atherosclerosis, blood pressure, lipids, and inflammation. The consumption of high-unsaturated fat and antioxidant-rich diets such as the MD one may prevent CVD development [49]. Therefore, olive oil, red wine, and nuts have beneficial effects on human health [50]. Dietary polyphenols including fruits, vegetables, whole grains, coffee, tea, and nuts, influence glycemia and type 2 diabetes, by promoting the tissue's glucose uptake, and therefore improving insulin sensitivity [51].

Ryan MC and co-workers conducted a randomized study that included twelve non-diabetic NAFLD patients (6 females/6 males) across 6 weeks. The study participants received MD, rich in MUFA. The study conclusions indicated that MD reduces liver steatosis and improves insulin sensitivity [52]. Abenavoli L and colleagues obtained similar results in NAFLD patients who consumed MD [53].

The clinical study Coronary Diet Intervention conducted by Delgado-Lista J, which enrolled 1,002 coronary patients from Spain, compare the effects of MD rich in virgin oil versus the low-fat diet on the impact of cardiovascular events. At the beginning of the study, 70.6% of the patients had LDL-cholesterol <100 mg/dL, 56.3% of them were obese, 64.4% were former smokers and only 9.6% were active smokers. However, between patients who received MD and a low-fat diet, no difference regarding weight, hypertension, fasting plasma glucose, or lipid profile was observed [54].

The preliminary results of the ongoing trial conducted by Sayón-Orea C regarding the effects of MD and physical activity on 6874 men and women (aged 55 to 75 years) with MetS and no CVD history enrolled between September 2013 and December 2016, illustrated a significant change. The change was visible in BMI, body weight, waist circumference, and lipid profile after 12 months compared with the control group [55].

The scientific group of Papadaki reported changes in 18 of 28 MetS components and risk factors [body weight, BMI, waist circumference, SBP, DBP, glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR) index, total cholesterol, LDL-cholesterol, HDL-cholesterol, TG, alanine transaminase, hepatic fat mass, C-reactive protein, interleukin-6, tumor necrosis factor-a, and flow-mediated dilatation] and lower risk of CVD incidence, after the consumption of MD [56]. Moreover, low-caloric MD and exercise training are important targets in the treatment of

NAFLD [57].

CALORIC RESTRICTION (CR)

CR represents the reduction of kilocalorie (kcal) intakes between 20 - 40%. Therefore, food restriction has been demonstrated to improve health and increase longevity in various groups of species, including humans, dogs, nematodes, rodents, rotifers, and non-human primates. Moreover, CR may delay the development of various pathologies such as autoimmune diseases, CVD, cancer, diabetes, renal diseases, neurodegenerative diseases, and respiratory diseases [58].

The term intermittent fasting (IF) refers to extended periods (16-48 hours) in which people eat little or nothing, alternating with periods of normal food intake. Moreover, periodic fasting (PF) means the periods of fasting or fasting-mimicking performed between 2 to 21 days or more. Studies performed on experimental models (mice and rats) summited on IF and PF, reported a decreased incidence of age-related disorders such as diabetes, CVD, cancer, and neurological disorders [59]. Therefore, a 60% energy restriction 2 days per week, and a five-day diet of 750-1100 kcal for normal and overweight patients conduces to weight loss and improves IR, and reduces the risk of CVD. Thus, IF improves health and counteracts diseases by the activation of several pathways involved in adaptive cellular response, mitochondrial death, autophagy, and DNA repair [59]. Taking into account all the aspects presented, the inflammation generated by obesity can be a key element for the development of oral pathologies such as periodontal disease [60-63]. Depression-related obesity is very dangerous, which may conduce to antidepressants consumptions [64,65].

Figure 1: The relationship between obesity-inducible factors, the Mediterranean diet, caloric restriction, and various metabolic pathologies

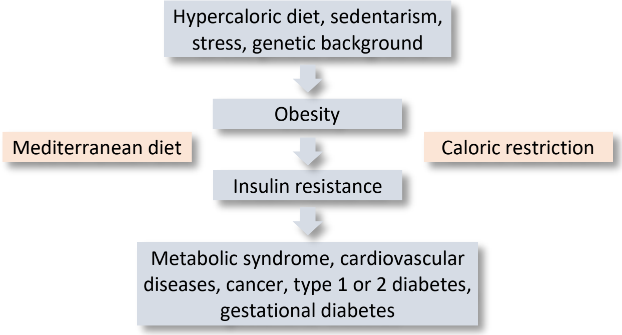
Hypercaloric diet, sedentarism, stress, genetic background
Obesity
Mediterranean diet
Caloric restriction
Insulin resistance
Metabolic syndrome, cardiovascular diseases, cancer, type 1 or 2 diabetes, gestational diabetes

CONCLUSIONS

IR is a consequence of many factors such as body weight, stress, genetic predisposition, inflammation, and over-stimulation of the sympathetic nervous system. Hypercaloric diets lead to obesity and IR, pathologies associated with MetS installation, and further CVD development. Healthy diets such as the MD, in combination with weight loss, especially for individuals who are overweight or obese, can drastically improve the cardiometabolic risk factors. MD reduces the level of TG and stimulates lipolysis and fatty acid-beta oxidation. CR, IF and PF improve general health and may delay the development of age-related disorders.

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ORIGINAL ARTICLE

Progress and Challenges of Diabetic Foot Diagnostic and Treatment

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Abstract: *Diabetic foot is one of the main long-term complications of diabetes, having a significant impact on a patient's quality of life. However, prevention by daily checking of the limbs and observance of the rules of care remains particularly important to avoid total or partial amputation. This paper aims to highlight the main challenges and progress in the treatment of the diabetic foot. To achieve this, we analyzed a database consisting of more than 100 articles published in recent years, approaching the treatment of the diabetic foot. The main problems that occur with the diabetic foot are peripheral vascular disease and diabetic neuropathy. These can lead to blisters and skin lesions that can eventually lead to ulcers in the lower limbs and even amputation. In conclusion, prevention is the main method of treatment for diabetic foot, followed by the treatment of each complication depending on when it is identified and its severity.*

Keywords: *diabetic foot; treatment of diabetic ulcers, diabetic foot*

INTRODUCTION

Diabetic foot is one of the most common complications of diabetes, being the leading cause in the world of lower limb amputation [1], as well as frequent hospitalizations in patients with diabetes [2,3]. According to the World Health Organization, diabetic foot syndrome is defined as "ulceration of the foot (distally from the ankle and including the ankle) associated with neuropathy and different grades of ischemia and infection" [4]. The main factors involved in the pathogenesis of diabetic foot ulcers are neuropathy and peripheral vascular disease [5]. However, other factors intervene in the occurrence of diabetes complications such as poor control of blood sugar and blood pressure, dyslipidemia, obesity, smoking, old age, inadequate prevention strategies, and long-term diabetes [5,6]. Considering that there are some limitations of specific treatment, attention remains focused on prevention and foot care education [4]. Careful management of the diabetic foot, which requires a good doctor-patient relationship, is a sure way to prevent amputation and maintain body integrity. Focusing on educating the patient can lead to a better understanding of his pathology, being a very important step in preventing the complications of the disease [5,7]. On the other hand, initiating the most appropriate treatment of complications depending on the stage at which they are discovered is essential in the healing process and even to prevent a possible amputation [3,6,8].

MATERIALS AND METHODS

This review aims to highlight the progress of treatment methods and best strategies providing a quick and correct diagnosis, as well as the complications of the diabetic foot.

We have selected 100 studies in English, German, and Romanian that presented information on methods of treating diabetic foot, difficulties encountered in the differential diagnosis and treatment of diabetic foot, as well as the complications of diabetic foot. Articles presenting the pathophysiology of the diabetic foot were also excluded.

Databases such as PubMed, Up-to-date, Medline, and NCBI were used to perform the search using keywords such as diabetic foot, diabetic foot complications, diabetic foot treatment, differential diagnosis of diabetic foot, and advances in diabetic foot treatment. To select the papers, exclusion criteria were imposed, such as the article publication date should not exceed 20 years old, and the publications to be in English, German, and Romanian treatment protocols were also analyzed.

RESULTS

Diagnostic and differential diagnosis of diabetic neuropathy

Diabetic neuropathy is one of the most common complications of diabetes, with about 50% of patients with type 1 or type 2 diabetes showing symptoms specific to diabetic neuropathy including pain, numbness, tingling, weakness, and difficulties with balance [9]. The most prevalent of diabetic neuropathies is generalized, symmetrical polyneuropathy, in which case may occur sensitive, motor, and autonomic manifestations [10,11].

There is also a high percentage of asymptomatic diabetic peripheral neuropathies, so recognition and prompt intervention are extremely important and reduce the risk of an insensitive foot injury, as well as a possible amputation [8]. Diabetic neuropathy is a diagnosis of exclusion, which is why other possible etiologies must be excluded, given that neuropathy in diabetic patients may have other causes (Table 1) [12]. In addition, some diabetics may have several causes of neuropathy at the same time [10]. The Rochester Diabetic Neuropathy Study reported that nondiabetic peripheral neuropathies were present in about 10% of patients [13].

Table 1: Different diagnoses of diabetic neuropathies [12]

Metabolic disease	Thyroid disease (common) Renal disease
Systemic disease	Systemic vasculities Nonsystemic vasculities Paraproteinemia Amyloidosis
Infectious	HIV, VHB, Lyme
Inflammatory	Chronic inflammatory demyelinating polyradiculoneuropathy
Nutritional	Deficit of B12, post-gastroplasty Pyridoxine, Thiamine, Tocopherol
Industrial agents, drugs, and metal	Industrial agents: Acrylamide, Organophosphorous agents Drugs: Alcohol, Amiodarone, Colchicine, Dapsone, Vinka alkaloids, Platinum, Taxol Metals: Arsenic, Mercury
Hereditary	Hereditary motor, sensory, and autonomic neuropathies

Thus, to optimize outcomes and lead to specific treatment in the diabetic patient, rapid recognition and proper management are essential.

Treatment

When we talk about a diabetic foot, we inevitably think of infection, hospital admission, amputation, and emotional and financial impact [14]. To achieve the best results for a diabetic patient it is necessary to implement a good prevention strategy [15,16]. As we all know, prevention is not always easy, especially for older patients with other comorbidities, but it is essential for a better prognostic and good quality of life [15,17]. The prevention of diabetic neuropathy is based on two goals: good glycemic control and a balanced lifestyle [7,12].

The patient's education is extremely important, in this process it is necessary to present his pathology, the complications that may occur along the way, and simple techniques to follow, such as checking and daily care of the feet, wearing special shoes, signaling any new foot mark and avoiding foot trauma [14,18]. The management of diabetic foot requires a rapid intervention in front of any type of wound, if possible by a multidisciplinary team [16,19]. Daily diabetic foot screening is the best prevention [7,20].

Numerous advances have been made in the management of diabetic foot in recent years, many of which have been applied especially in clinics dedicated to diabetic foot care [14,18]. Most important in the management of diabetic foot is a multidisciplinary team consisting of specialists to act quickly in the face of any type of wound [16,19].

To determine the best treatment method, it is important to determine the severity of the diabetic foot [18]. The treatment can be conservative or surgical [21]. As long as conservative treatment is chosen, it can be performed in an outpatient setting as long as the patient is compliant, the ulceration is superficial and there are no signs of systemic damage [20]. Also, during the treatment period, it is very important to maintain the glycemic values within normal limits and to respect the nutritional regime and the daily exercise program [16,22].

The first step in treating foot ulcer is inspection, to stabilize the degree of damage and the existence or not of the infection, then the wound is cleaned, and if necessary local anesthesia can be performed (many patients have polyneuropathy and no anesthesia is needed) to debridement [23]. In the case of superficial ulcers, a multidisciplinary approach is important to prevent evolution (Table 2) [22].

There is currently a modern local treatment method that involves the use of hydro active dressings, which promote healing through cell proliferation processes in a humid environment. This is done to prevent infection and/or reduce the microbial load, prevent necrosis or eliminate existing ones and adapt to each stage of the wound healing process (cleaning, granulation, and epithelialization) [24].

Table 2: Treatment for superficial uncomplicated foot ulcers [22]

Active ulceration Peripheral neuropathy +/- PAD	Clinic evaluation	Patients evaluation	Core	Adjuncts
Non-limb threatening Ulcer	- Superficial - Minimal to no cellulitis - No bone or joint involvement - No significant ischemia - No toxicity	- Reliable - Will conform to treatment - Good support system - Evaluation of comorbidities	- Offloading - Debridement, Dressing - Management of infections - Vascular evaluation if needed - Foot-sparing surgery	- Control blood sugar - Treat comorbidities - Nutrition - Control edema - Physical and emotional therapy
Limb threatening ulcer	Check both feet and legs, and compare Before any intervention, the ulcer probes deeply Note location May involve bone or joint Cellulitis >2cm Lymphangitis, gas, crepitation Ischemia +/-foot pulses, Doppler Systemic toxicity, temperature, white blood cell, lymph nodes Escher, *callor*, granulation tissues	- Unreliable - Poor support systems - Poor control of comorbidities	Therapy - Admit to hospital – complete bed rest - Employ core and adjunct - control blood- stabilize comorbidities - Especially consult (cardiology, renal, infectious disease, etc) - Empirical broad-spectrum antibiotics – adjust by culture and sensitivity - Labs and X-ray Early vascular evaluation and Rx after control of infarction - Foot-sparing surgical vs amputation/revisions - Close follow up	
Septic ulcer	- Same as for limb-threatening ulcer - Be sure to check for undermining deep tissue exposure, abscess, bone, and gas	- Same as limb-threatening ulceration	- Immediate admission – complete bed rest – protect wound and foot, esp heel - Employ core and adjuncts - Intravenous broad-spectrum antibiotics/fluid replacement and resuscitation - Labs and X-rays - Culture and sensitivity – blood culture - Control edema - Emergency aggressive surgical debridement - control all activity infarction, including possible amputations - Stabilizing medically – blood glucose, cardiac(MI, CHF) renal, esp consult (cardiology, renal, ID, etc.) - Evaluate later for antihypertensive, beta-blockers, calcium channel blockers, ACE inhibitors - Early vascular evaluation and Rx after control of infarction - Close follow up	

If the foot ulcer is infected it is especially important to determine if there is a systemic or only local infection [25]. The signs of local infection are erythema, edema, suppuration, and unpleasant odor, but often if the patient has kidney failure they do not show [26].

Depending on the degree of damage and the level of infection, the method of treatment is decided, but the basis of any method of treatment is the initiation of antibiotic therapy and wound care [25].

To choose the best antibiotic therapy, the following criteria are taken into account:

- Local bacterial resistance pattern,
- Proper antibiotic history,
- Gram stains of deep exudate,
- Appearance of wound and pus,
- Associated co-morbid conditions like renal, hepatic, and cardiac impairment [22]

DISCUSSIONS

Clinical aspects and the pathophysiology of diabetic foot

Because there are multiple etiologies underlying this concept, the diabetic foot is considered to be a "syndrome" [27]. It is one of the most common complications of diabetes which in the long-term, if not treated properly, can lead to amputations, disability, and reduced quality of life [4,28]. It is well known that around 25% of diabetic patients will develop a foot ulcer in their lives [28,29]. The pathophysiology of a diabetic foot ulcer is based on triad neuropathy, trauma with secondary infection, and arterial occlusive disease, which appears in various forms [17].

Due to the nerve damage, there is a loss of pain and thermal sensitivity that makes the feet vulnerable to minor injuries caused by excessive pressure, and mechanical or thermal damage [30,31]. Furthermore, motor neuropathy leads to intrinsic muscle atrophy, and foot deformities by altering the biomechanics of the foot, as well as limited joint mobility and postural and coordination deviation [32]. In the end, the formation of calluses comes as an expression of the plantar excessive pressure [28].

One of the goals of treating any ulcer with neuropathy is to reduce weight, regardless of the presence of ischemia [6,33]. Last, the effects of the onset of autonomic neuropathy are diminished sweating, altered blood flow regulation, and microvascular dysfunction, which will lead to dry skin and cracks [21,34]. Repetitive trauma and impaired proprioceptive and exteroceptive sensitivity predispose to skin injury leading to atrophy and dislocation of plantar fat, followed by ulceration and infection [28,35].

There are situations in which, inattention to certain signs (redness, blister formation) or lack of routine foot care, leads to the formation of ulcers and the appearance of infections, undesirable things in diabetic patients with neuropathy, or a history of foot ulcers [3]. Studies have shown that the risk of ulceration increases in diabetic patients with neuropathy, a history of amputations, or foot deformity [16,17].

There are advantages and disadvantages to each kind of wound treatment and multiple solutions for wound dressing are present (Miculickz pomade, 1% silver sulfadiazine cream, zinc hyaluronate solution, bovine collagen, hyperbaric oxygen therapy, hydrocolloid dressing or synthetic skin substitutes)[15,16]. Most of them need real confirmation in randomized studies.

Adjuvant care of diabetic foot ulcers implies systemic factors control of hyperglycemia, control of infection, treatment of vasculopathy (sulodexide, cilostazol) or local factors-peripheral neuropathies, control of pressure at foot level (custom-made ortheses and shoes)[36].

Charcot neuro-osteoarthropathy

Charcot neuro-osteoarthropathy or the Charcot Foot is one of the most severe complications of diabetes [37]. The main factors that lead to this disease are a combination of neuropathy, repeated microtrauma caused by increased weight, and an abnormal bone metabolism leading to inflammatory reactions [35,38]. The result is the destruction of the normal architecture of the foot, subluxation, dislocation, and deformity, most commonly in the ankle and foot [39]. Charcot's Foot is an urgent condition, often associated with ulcers and osteomyelitis, and requires proper management by a foot specialist [40].

Osteomyelitis

Osteomyelitis represents a severe infection involving bone, which occurs due to non-healing ulcers, and if not treated properly may lead to major amputation [27,28]. Two-thirds of the patients with diabetic ulcers may be affected by osteomyelitis, so this complication requires increased attention [38].

In addition, patients with a diabetic ulcers may develop osteomyelitis without any signs of pain, but there may also be a decrease in local signs of infection caused by neuropathy and peripheral arterial disease [41].

CONCLUSIONS

Diabetes mellitus is a chronic disease, characterized by high blood glucose levels [42].

The long-term complications of diabetes are vascular and neurological, and of these, diabetic foot syndrome is one of the most serious consequences in terms of the health complications it generates and the impact on quality of life. It includes ulceration, infection, and osteoarthropathy, known as Charcot's foot. Proper prevention and care of the feet are essential for the early identification of problems and mitigation of consequences. This includes patient education, prophylactic skin, nail care, and proper footwear.

Depending on the severity of the condition, the best method of treatment for the diabetic foot is decided. There are several options, both invasive and non-invasive, for treating this disease.

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ORIGINAL ARTICLE

Image of a Junior Officer in Military Personnel Participating in Combat Operations with Symptoms of Post-traumatic Stress Disorder

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Abstract: *According to the results of the conducted study there were distinguished the peculiarities of perception of the image of a junior officer by servicemen who participated in hostilities and had various PTSD symptoms. The study included 233 male servicemen with combat experience. The age of the participants varied from 20 to 55 years. It was determined that the image perception of the direct commander by the servicemen who did not have any PTSD symptoms had been influenced by both the key features of the modern way of fighting and the way a protective mechanism of consciousness acted, which gave the possibility to maintain mental health under combat conditions. These servicemen considered the awareness, kindness, and physical strength of their commander to be not important. The first stage was occupied by his/her ability to adequately fulfill his/her leadership function and the power of his/her spirit – the courage and love of the Motherland, which formed his/her reliability. Servicemen who had PTSD symptoms were less satisfied with the role of a junior officer and considered him/her to be less capable of meeting their expectations. They were less interested in commanders who could involve them in situations that required quick, brave action and complete responsibility for the entrusted part of common affairs. The most important factors in the structure of the direct commander image in both studied groups were the power of personality (the ability of self-regulation); however, the servicemen with PTSD symptoms had a specific orientation of these factors – to help their subordinates. They were quite selfishly aimed at using their direct commanders as a way of getting help.*

Keywords: *leader, serviceman, service and combat activity, combat experience, post-traumatic stress disorder, psychological recover*

INTRODUCTION

It was quite difficult to overestimate the role of a junior officer who was a direct commander during the fight. He/she shared with his/her subordinates all the complexities of the service, informed them about orders of senior officers, implemented the coordination of actions of small military groups, made relevant decisions related to the life of the unit, and resolved conflict situations in the team [1]. The behavior of warlords had a deep effect on the mental health of those whom they had to lead [2]. According to Tedeschi and Mcnally, the perception of their leaders influenced not only the way they responded to stressful events, such as hostilities, but also an individual's desire to apply for psychological help in times of personal crisis [3].

The implementation of combat tasks and the preservation of the lives of entrusted him/her servicemen fully depended on the competence of the direct commander’s actions under combat conditions. According to Baran and Scott to make a unit’s actions effective the subordinates had to trust in the actions of their commander, trust that he would not pose them to the actions with unjustified risk [4]. The research conducted by Sweeney determined that leaders who showed competence before combat actions had a higher level of trust among their subordinates, while those who demonstrated a lack of competence and a lack of previous combat experience could feel distrust from the side of their subordinates [5]. Fisher, et al. [6] received similar conclusions to Sweeney [5] and proposed to add that successful leadership under combat conditions was possible due to previous successes on the battlefield. According to them the experience of participation in hostilities strengthened the leader's confidence and formed appropriate beliefs about self-efficacy.

The experience of joint actions on the battlefield imposed a significant imprint on the perception of the direct commander by his/her subordinates. In particular, the studies conducted by Xue, et al. [7] and Ramchand, et al. [8] proved that servicemen’s perception of their commander (leader) directly related to post-traumatic stress disorder. Other conducted studies (Castro and McGurk; Du Preez, Sundin, Wessely, and Fear; Iversen, Fear, Ehlers, et al.; Jones, Seddon, Fear, et al.; McGurk, Sinclair, Thomas, et al.) also figured out that the perception of the leader by servicemen on the battlefield related to the fair and equal attitude, praise, interest, and concern [9-13]. However, in general, the studies lacked theoretical explanations of the relations which could help to make the image of leadership the only factor that could be changed. The only exception was the study by McGurk, et al. [13] who used the theory of resources conservation that was proposed by Hobfll as an explanation of how positively and negatively leaders could contribute to obtaining or losing the resources, which could explain the connection with PTSD [14].

However, the experienced stressful events could interfere with the acquired combat experience as well as the features and qualities which the serviceman realized in him/herself, his/her comrades, and the commander overcoming life challenges. The negative experience caused by the mental traumatization was painful (Kolesnichenko), having the desire to weaken its power a serviceman could want both to find help and to find the perpetrators [15]. He/she could become isolated, believing that people around him/her could not understand and help. The inability of the serviceman to accept his/her combat experience (Kalesnichenko) [16], and loss of his/her mental health (Kolesnichenko) [17] could have a negative influence on the interaction within the unit, on the perception of the image and the role of the commander (Jansen and Delahaij) [18]. Although the military hierarchy regulated the relations between the leaders and subordinates, unofficially servicemen could abandon their leaders. Such refusal, according to Pierro, Cicero, and Bonaiuto, et al., could cause floats and counterproductive behavior within the military teams [19].

METHOD

Participants and Procedure

The study was conducted in 2022, it was a part of another bigger study related to the determination of important qualities of military leaders. The study included 252 servicemen (42.16% of them were mobilized; 57.84% of them were servicemen under the contract) who participated in hostilities. All the participants were divided following the scale “Mississippi Scale for Combat-Related PTSD” into two groups – group A included the servicemen (155 people) whose PTSD symptoms did not exceed the norm (up to 78 points); group B included the servicemen (97 people) whose indicators exceeded the norm (more than 78 points). This study was based on the presence of definite PTSD symptoms of servicemen who, on request, received qualified individual consulting help from military psychologists and participated in group works aimed at restoring their resilience ability as well as at increasing their ability to accept their own combat experience. It was not about the formed PTSD.

All procedures carried out in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants gave consent for their data to be used in this research.

Instruments and Measures

To identify the peculiarities of perception of the ideal representation (image) of a junior officer by the servicemen who participated in hostilities and had PTSD symptoms based on the analysis of modern and foreign studies there were distinguished more than 50 descriptions of the characteristics of military leaders. After the first stage of the Delphi estimation method, where the experts were selected from the military leaders with different ranks, subordinates including those with considerable combat experience, military instructors, and lecturers from the higher military educational establishments. The total number of experts was 65 people. There were made up separate lists of 30 characteristics to evaluate the sergeants, junior and senior officers. It had to be highlighted that the descriptions of the leader’s characteristics in the lists were significantly reduced and edited due to the experts’ recommendations. Due to that definite list of 30 characteristics, which was formed to evaluate the junior officer by the 11-point scale (from 0 to 10 points), the participants who took part in hostilities were asked to evaluate the importance of those characteristics in the "ideal" junior officer, according to their point of view.

To evaluate the degree of PTSD expressiveness of the servicemen who participated in the study there was used "Mississippi Scale for Combat-Related PTSD" [20]. Nowadays, it was one of the most common methods used to measure the signs of PTSD. The scale included 35 statements, each of which can be estimated on a five-point scale. The total indicator which was made up of this method gave the possibility to estimate the degree of influence of the traumatic experience transferred by an individual.

Data Analysis

To represent the data, there was used the main descriptive statistics (arithmetic mean M and standard deviation SD). To compare the estimates provided in the selected groups there was used the T-criteria of the Student. Moreover, there was used the procedure of factor analysis by Principal Components to distinguish the structure of the image of the junior officer due to the given points. Mathematical data was processed with the help of SPSS 17.0.

RESULTS

The points given to the selected groups of servicemen who participated in hostilities were proposed by 30 qualities used to make the perfect image of a junior officer. The points were compared with the help of the T-criterion of the Student. This information was presented in Table 1.

According to Table 1, due to all 30 characteristics which were used to describe the ideal junior officer, the service-men without PTSD symptoms had much higher indicators than the servicemen of the second group. The data indicated not only the presence of different ideas of the representatives of these groups concerning the ideal of a junior commander but also the more negative attitude of the group representatives with PTSD symptoms towards a junior commander in general and towards the role he performed.

It should be noted that both groups had differences between the most important quality and the least important which were minimal. Thus, in the first group, the most important was considered the ability to “adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation" (9.16 ± 1.51), and the least important was “own achievements, professionalism and character traits made him/her an attractive model to follow” (8.50 ± 1.97).

In the group with PTSD symptoms, the most important characteristic was "a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it" (8.58 ± 1.90), and the least important, as in the first group, was "own achievements, professionalism and character traits made him/her an attractive model to follow” (7.60 ± 2.54). Thus, despite the different evaluation tactics in these groups, the least important were both It should be noted that both groups had differences between the most important quality and the least important which were minimal. Thus, in the first group, the most important was considered the ability to “adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation" (9.16 ± 1.51), and the least important was “own achievements, professionalism and character traits made him/her an attractive model to follow” (8.50 ± 1.97).

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However, if the first group put in the first place a specific function of the leader – the ability to adequately perform the role of intermediate (between the senior commander and subordinates) leader, which ensured the coordination of small units’ actions, the second group valued his/her civil position and his/her own choice – love of the Motherland and the ability to concentrate all strength to protect it; it seemed that only that could justify all the sufferings which they had survived with their commander, under his/her direct guidance.

A lower quantity of points could indicate less satisfaction with the role of junior officers, and their inability to meet expectations. It was necessary to point out that the commander's patriotism was highly appreciated by the representatives of the first group in which he/she, conditionally, occupied the second stage. However, it could be assumed that the servicemen of the first group reflected the protective mechanism on the estimation of the direct commander.

The protective mechanism ensured the preservation of their mental health – the focus on the process of performing their functions under combat conditions – hence occurred a specific view of the role of the commander.

To clarify the assumptions which were made, there occurred a need to look more precisely at the most and the least important qualities of junior officers in both groups. It was inappropriate to consider in detail the whole hierarchy of the characteristics of a junior officer in the distinguished groups due to the minimal differences in the estimations of the most and the least important qualities.

Table 1: The Characteristics of the ideal image of a junior officer in the groups of servicemen with different PTSD signs (in standard points)

№	Variable	Studied		$t_{кр}$	p
		group A	group B		
1	He/she worried about the welfare of his/her subordinates and found opportunities to organize the most convenient conditions for their service in the current circumstances	9.02±1.77	8.14±2.43	3.09	.01
2	He/she was brave and strong-willed, and with his/her example he/she inspired the subordinates to overcome difficulties	9.08±1.54	8.14±2.49	3.33	.01
3	He/she was able to take any situation under his/her control, to make up-to-date decisions	9.03±1.68	8.24±2.44	2.79	.01
4	He/she was reliable, did not give up, and acted effectively even having minimal resources	8.96±1.67	8.10±2.31	3.16	.01
5	He/she saw the prospects that his/her unit had and could unite the subordinates to achieve these prospects	9.01±1.63	8.06±2.46	3.36	.01
6	His/her achievements, professionalism, and character traits made him/her an attractive model to follow	8.50±1.97	7.60±2.54	2.96	.01
7	He/she adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation	9.16±1.51	8.31±2.34	3.17	.01
8	He/she had good communication skills and could adequately resolve conflict situations	9.04±1.62	8.46±2.23	2.21	.05
9	He/she could explain, teach, and help to form the necessary skills as well as the traits of character	8.92±1.67	8.00±2.34	3.34	.001
10	He/she was aware, well-informed, and could easily acquire the necessary knowledge as well as professional experience	8.84±1.76	7.91±2.39	3.31	.001
11	He/she could form the team – everyone in the unit was "in his/her place" both due to professional skills as well as due to personal traits, which ensured his/her effective work	9.05±1.62	8.03±2.37	3.72	.001
12	He/she formed confidence in the better future that which individual wanted to follow with his/her brethren	8.83±1.62	8.00±2.22	3.15	.01
13	He/she was confident in him/herself, in his/her decisions, and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity	9.01±1.61	8.13±2.26	3.33	.001
14	He/she adequately evaluated the importance of solving certain problems. To solve them he/she showed the appropriateness of persistence, honesty, and stubbornness	9.04±1.52	8.25±2.06	3.25	.01
15	He/she was quite independent – made up-to-date decisions and took responsibility for the given orders	8.95±1.78	7.90±2.33	3.82	.001
16	He/she was friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones	8.82±1.78	8.22±2.13	2.30	.05
17	He/she was emotionally balanced and stress-resistant and could control his/her negative experience	8.97±1.62	8.29±1.96	2.84	.01
18	His/her morality, belief perseverance, and ability to defend those beliefs gave him/her the possibility to be a role model for his/her subordinates	8.89±1.60	8.17±2.08	2.92	.01
19	He/she was demanding to others as well as to him/herself and inspired the others to imitate him/her by own self-discipline	8.98±1.47	8.10±2.23	3.42	.001
20	He/she was tactful being demanding to his/her subordinates and taking care of them. He/she represented respect and maintained a sense of dignity in his/her subordinates	8.91±1.66	8.16±2.17	2.91	.01
21	He/she was able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation	9.08±1.51	8.17±2.29	3.48	.001
22	He/she formed the feeling of "we" in servicemen: "We are defenders", "If not we, then who?"	9.03±1.64	8.22±2.30	2.99	.01
23	He/she carefully considered his/her decisions, calculated all the possible complications within the process of their implementation	8.98±1.69	8.23±2.16	2.90	.01
24	He/she formulated his/her opinion, gave consistent orders, and agreed with other executives	9.05±1.49	8.42±2.06	2.63	.01
25	He/she maintained good physical form, could bear the hardness of work on par with everybody in the unit if it was necessary, he/she could take on the professional functions of any of the subordinates	8.76±1.77	8.27±2.15	1.87	.1
26	He/she could take on full responsibility for the actions of his/her subordinates	8.96±1.68	8.29±2.26	2.51	.05
27	He/she ensured the information security of his/her unit, did not allow the leakage of information related to the servicemen and their families	9.05±1.64	8.50±1.97	2.29	.05
28	He/she was a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it	9.12±1.77	8.58±1.90	2.22	.05

29	He/she could adequately evaluate the physical and psychological state of his/her subordinates, could organize the necessary help for them	9.11±1.50	8.40±2.09	2.92	.01
30	He/she was professional – had good professional knowledge, skills, and experience in their implementation under combat conditions	9.05±1.70	8.44±2.20	2.33	.05

There was a need to highlight that such minimality of differences of estimations of the most and the least important characteristics of the direct commander in both studied groups of the participants in hostilities testified the syncretic (fusion) of the perception of his/her image, as a significant individual in the life of a serviceman, while surviving under combat conditions directly depended on him/her.

In the first group, the most important (those who occupied leading positions and ranks in the hierarchy) were the following qualities:

1) adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation (9.16 ± 1.51);

2) a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it (9.12 ± 1.77);

3) could adequately evaluate the physical and psychological state of his/her subordinates, could organize the necessary help for them (9.11 ± 1.50);

4) able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation (9.08 ± 1.51);

5) brave and strong-willed, with his/her example he/she inspired the subordinates to overcome difficulties (9.08 ± 1.54).

Conditionally the least important in the first group (those who occupied the least leading positions and ranks in the hierarchy) were the following qualities:

1) own achievements, professionalism, and character traits made him/her an attractive model to follow (8.50 ± 1.97);

2) maintained good physical form, could bear the hardness of work on par with everybody in the unit, if it was necessary, he/she could take on the professional functions of any of the subordinates (8.76 ± 1.77);

3) friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones (8.82 ± 1.78);

4) formed confidence in the better future that which individual wanted to follow with his/her brethren (8.83 ± 1.62);

5) aware, well-informed, and could easily acquire necessary knowledge as well as professional experience (8.84 ± 1.76).

As could be seen, it was not so important for servicemen, who retained their mental health throughout the fight, which achievements their direct commander had, and whether he was well-informed, friendly, and strong. Among all the personal traits, only his/her patriotism and courage were important. For these servicemen, the first place was occupied by his/her ability to adequately fulfill his/her leadership functions – to set the combat tasks with adequate consideration of the combat situation and the subordinates he/she had.

In the group that had PTSD symptoms, the following characteristics received the highest points:

1) a patriot, who proved by his/her actions his/her love of the Motherland and the ability to protect it (8.58 ± 1.90);

2) ensured the information security of his/her unit, did not allow the leakage of information related to the servicemen and their families (8.50 ± 1.97);

3) had good communication skills and could adequately resolve conflict situations (8.46 ± 2.23);

4) professional – had good professional knowledge, skills, and experience in their implementation under combat conditions (8.44 ± 2.20);

5) formulated his/her opinion, gave consistent orders, and agreed with other executives (8.42 ± 2.06).

The participants of hostilities who had PTSD symptoms considered the following characteristics to be the least important for junior officers:

1) own achievements, professionalism, and character traits made him/her an attractive model to follow (7.60 ± 2.54);

2) quite independent – made up-to-date decisions and took responsibility for the given orders (7.90 ± 2.33);

3) aware, well-informed and could easily acquire necessary knowledge as well as professional experience (7.91 ± 2.39);

4) could explain, teach, and help to form the necessary skills as well as the traits of character (8.00 ± 2.34);

5) formed confidence in the better future that which individual wanted to follow with his/her brethren (8.00 ± 2.22).

For the servicemen of the second group as well as for the first one the achievements of their commander and his/her awareness were not important. Moreover, they did not believe that he/she could bring them to a better future, or help them to overcome their problems related to their personal life (to form the necessary traits). It seemed that those psychologically injured servicemen transferred to the image of the direct commander their confidence that their lives had no prospects, and no one could help them (one of the PTSD signs) neither due to their initiative (due to the love to their relatives) nor due to their functional responsibilities.

However, among the most important characteristics of the direct commander, three out of five in their formulation in one way or another indicated their ability to protect their subordinates. The other two were related to the cognitive functions which, among other things, were able to ensure the definite success of servicemen who, due to the loss of mental health, had problems with attention and order understanding. Thus, the attitude towards the direct commander by the servicemen who had PTSD symptoms showed opposite tendencies – on the one hand, they sought protection and help to keep the success in performing the given tasks and not lose the remains of self-esteem, on the other hand – they did not believe the fact that their direct commanders themselves or by the order of senior officers could help them to cope with their negative experience, and could direct them to the path leading to the "better future." The negativity of their estimations related to junior officers was caused by internal contradictions concerning their attitude towards them, the attitude which combined the search for protection and helping with the despair in the fact that they could help them (such PTSD characteristics as the belief in the uniqueness of their traumatic experience and the unpromising life).

Moreover, there were several characteristics in the process of their estimation in two groups, there had been distinguished the highest differences (more than 10 positions/ranks).

Thus, the servicemen whose PTSD symptoms did not exceed the norm, higher positions were occupied with the following characteristics:

- able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation (4th position in contrast to 16.5th position in the second group);

- brave and strong-willed, with his/her example he/she inspired the subordinates to overcome difficulties (5th position in contrast to 19th position in the second group);

- could form the team – everyone in the unit was "in his/her place" both due to professional skills as well as due to personal traits, which ensured his/her effective work (7.5th position in contrast to the 25th position in the second group).

Instead, in the second group, higher positions were occupied with the following characteristics:

- could take on full responsibility for the actions of his/her subordinates (8.5th position in contrast to 20.5th in the first group);

- emotionally balanced and stress-resistant, and could control his/her negative experience (8.5th position in contrast to 19th in the first group);

- maintained good physical form, could bear the hardness of work on par with everybody in the unit, if it was necessary, he/she could take on the professional functions of any of the subordinates (10th position in contrast to 29th in the first group);

- friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones (14th position in contrast to 28th in the first group).

This data could indicate that servicemen with PTSD symptoms were less interested in commanders who could involve them in situations that required quick, brave actions, and complete responsibility for the entrusted part of the common affairs; instead, they required a commander who could take on the responsibility for their failures as well as a part of their direct responsibilities, and at the same time he/she would not condemn (express any negative attitude).

There was a need to consider the structure of characteristics of junior officers formed with the help of a factor analysis due to the estimations made by the servicemen from the selected groups.

In the group of servicemen, where the indicators of PTSD symptoms did not exceed the norm, after the simplification procedure, there was formed a three-factors structure (see Table 2), described 78.47% of the symptoms' dispersion. This structure was presented in Table 2.

The content of the first and the most important factor (40.56%) was determined with the following variables "emotionally balanced and stress-resistant, and could control his/her negative experience" (0.815), "able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation"(0.771), "maintained good physical form, could bear the hardness of work on par with everybody in the unit, if it was necessary, he/she could take on the professional functions of any of the subordinates"(0.771), "confident in him/herself, in his/her decisions and in the unit itself – he/she did not allow any doubts, uncertainty, and the apathy which could undermine the efficiency of the unit activity"(0.743), and "demanding to others as well as to him/herself, and inspired the others to imitate him/her by own self-discipline" (0.663). This factor was interpreted as the power of "I" and as the power of personality based on a developed ability to self-regulation.

The second factor (23.05%) was determined by the variables such as "had good communication skills and could adequately resolve conflict situations" (0.796) and "adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation" (0.781). This factor could be marked as professional communicative competence, which showed up, both in the ability to formulate a combat mission and convey information as well as in the ability to resolve the conflict.

Table 2: The structure of the characteristics of junior officers formed following the estimations made by servicemen who did not have clearly expressed signs of PTSD (in standard points).

Variable	Factors		
	1	2	3
He/she adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation	0.275	0.781	0.235
He/she had good communication skills and could adequately resolve conflict situations	0.343	0.796	0.208
He/she was confident in him/herself, in his/her decisions, and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity	0.743	0.273	0.435
He/she was emotionally balanced and stress-resistant and could control his/her negative experience	0.815	0.359	0.139
He/she was demanding to others as well as to him/herself and inspired the others to imitate him/her by own self-discipline	0.663	0.234	0.291
He/she was able to quickly and adequately evaluate the professional situation and its changes, to make necessary decisions for the normalization of the situation	0.794	0.342	0.186
He/she maintained good physical form, could bear the hardness of work on par with everybody in the unit if it was necessary, he/she could take on the professional functions of any of the subordinates	0.771	0.259	0.302
He/she was professional – had good professional knowledge, skills, and experience in their implementation under combat condition	0.413	0.397	0.819

The third factor was completely correlated with the variable "was professional – had good professional knowledge, skills, and experience in their implementation under combat conditions" (0,819). Some clarifications of the content of this factor were made by a variable "was confident in him/herself, in his/her decisions and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity" (0.435), which emphasized the active energetic position of the servicemen who had professional knowledge and experience. Accordingly, this factor was marked as professional efficiency – the ability to act effectively based on professional knowledge and experience.

For the servicemen who had expressed PTSD symptoms, the structure of the characteristics of junior officers was simpler and had only 2 factors, which described 79.88% of the signs' dispersion. This structure was presented in Table 3.

The content of the first factor (59.55%) was defined with the following variables "clearly saw the prospects that his/her unit had and could unite the subordinates to achieve these prospects" (0.887), "was brave and strong-willed, with his/her personal example he/she inspired the subordinates to overcome difficulties"(0.877), "was reliable, did not give up, acted effectively even having minimal resources" (0.859), "was professional – had good professional knowledge, skills, and experience in their implementation under combat conditions" (0.844), "adequately evaluated the importance of solving certain problems. To solve them he/she showed the appropriateness of persistence, honesty, and stubbornness" (0.837), "own achievements, professionalism and character traits made him/her an attractive model to follow" (0.832), "was able to take any situation under his/her control, to make up-to-date decisions"(0.827), "was confident in him/herself, in his/her decisions and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity" (0.815), "adequately brings to subordinates leadership, their importance, capable of ensuring their implementation adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation" (0.805), and "worried about the welfare of his/her subordinates and found opportunities to organize the most convenient conditions for their service in the current circumstances" (0.761). This factor had a weight that exceeded the weight of the second factor and combined the variables, the vast majority of which described the ability of a junior officer to self-regulation, but unlike the situation in the first group, this variable received a specific direction – to ensure a prospective development and well-being of their subordinates.

The second factor (20.33%) was determined by two variables – "was friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones" (0.885) and "was emotionally balanced and stress-resistant, and could control his/her negative experience" (0.847). Those were the variables that occupied much higher positions (ranks) in servicemen with the signs of PTSD than in the servicemen of the first group which were already marked as a tendency not to condemn their subordinates but to help them.

Table 3: The structure of the characteristics of junior officers was formed following the estimations made by servicemen who had expressed PTSD symptoms (in standard points).

Variables	Factors	
	1	2
He/she worried about the welfare of his/her subordinates and found opportunities to organize the most convenient conditions for their service in the current circumstances	0.761	0.363
He/she was brave and strong-willed, and with his/her example he/she inspired the subordinates to overcome difficulties	0.877	0.238
He/she was able to take any situation under his/her control, to make up-to-date decisions	0.827	0.296
He/she was reliable, did not give up, and acted effectively even having minimal resources	0.859	0.209
He/she saw the prospects that his/her unit had and could unite the subordinates to achieve these prospects	0.887	0.254
His/her achievements, professionalism, and character traits made him/her an attractive model to follow	0.832	0.332
He/she adequately informed the subordinates concerning the commanders' orders, pointing out their importance, and could ensure their implementation	0.805	0.305
He/she was confident in him/herself, in his/her decisions, and in the unit itself – he/she did not allow any doubts, uncertainty, and apathy which could undermine the efficiency of the unit activity	0.815	0.369
He/she adequately evaluated the importance of solving certain problems. To solve them he/she showed the appropriateness of persistence, honesty, and stubbornness	0.837	0.360
He/she was friendly and available for the subordinates to communicate, they could share their problems as well as their doubts. He/she was also available for their families who were concerned about the lives of their loved ones	0.272	0.885
He/she was emotionally balanced and stress-resistant and could control his/her negative experience	0.310	0.847
He/she was professional – had good professional knowledge, skills, and experience in their implementation under combat conditions	0.844	0.294

As it could be seen, the structure of the characteristics of a junior officer within the servicemen who had the expressed PTSD symptoms had some similarity with the structure given by the first group, it also referred to the power of personality (ability to self-regulation) and definite communicative competence; however, the mentioned factors had a specific orientation in this group – to help his/her subordinates. They were quite selfishly aimed at the use of their direct commanders to help them, their structure "omitted" professional efficiency, achievement of professionally important goals, and so on.

DISCUSSIONS

Although the study conducted by Sheerin C.M., et al. [21] which used a more abstract research plan did not reveal any connections between PTSD and negative attitude towards the world in general, the study was conducted gave the possibility to suggest that having expressed PTSD symptoms the people around were perceived more negatively (at least those who were responsible for the lives of a serviceman – their direct commanders).

In the study by Bartholomew, et al. it was determined that servicemen who had signs of PTSD were less focused on their future [22]. In particular, the servicemen who had the signs of PTSD had disbelief in the achievement of a better future which extended to others, to those who had to direct the achievements of this better future to the whole team – to the direct commander.

It was interesting that real and perceived social support, as a factor that affected stress overcoming, had been already studied earlier, for example, it was discussed in works by Smith, Benight, and Cieslak [23]. An interesting phenomenon was identified in the study which was related to internal conflict attitude towards an individual the one who had to help, who was responsible for that morally and formally – that individual was a direct commander. He was perceived both as the one whom they expected to get help from and as the one whose real ability to help they had poorly trusted.

Caska-Wallace et al. worked with the negative changes in servicemen who were under PTSD influence [24]. They determined that the increase in problems in relationships between veterans and their partners referred to effective communication as well as communication related to the address of problems' solutions. The results of the conducted research suggested that not only a partner, but also a commander, and probably another significant person who surrounded a serviceman with PTSD symptoms, was taken as one who had to help, to solve the problems of psychologically traumatized servicemen, however, he/she could not solve those problems. That became a reason for the conflicting attitude of the serviceman towards definite surroundings.

Moreover, it had to be noted that if this study of an individual, who did not have signs of PTSD in the structure of the commander image, distinguished his/her strong personality and the ability to self-regulation as leading factors, the research by Rahman, et al. figured out that the perception of self-efficacy was a kind of traumatic stress prevention [25]. Conducting a certain analogy, it could be assumed (even if that required further studies) that the perception of the figure of the commander, as self-effective and capable of self-regulation was a kind of prevention of PTSD formation in his/her subordinates in the future. The procedure of study did not give any possibility to suggest whether the perception of the commander as an effective one was the prevention of PTSD symptoms in subordinates, or whether the absence of signs of PTSD reduced the negativity in the perception of the commander's figure, of his/her self-efficacy, of his/her "I", or of the experience became a reason for the posttraumatic growth and perception of oneself and of the one who ran the process of overcoming the life troubles, as those which had a significant individual power (Tedeschi and Calhoun) [26]. Also, the format of the study did not give the possibility to reveal whether the presence of PTSD symptoms caused the situation that servicemen were less interested in the commander who could involve them in dangerous swift-fleeting conditions, or whether servicemen who avoided danger lost their opportunities for productivity and growth following the study by Jamieson, et al. [27].

Unfortunately, the study did not give any possibility to identify cause-effect relationships, it did not give the possibility to determine whether PTSD symptoms had an influence on the image of the military leader, or whether the attitude towards the commander became one of the reasons for acquiring PTSD symptoms during the fight. Thus, the determination of those relationships would be the purpose of the feather studies.

CONCLUSIONS

Thus, the conducted study showed that the existing extreme experience of participating in hostilities led to the syncretic perception of the image of the junior commander – all qualities were perceived as extremely important.

In servicemen who did not have signs of PTSD (able to accept their combat experience as well as experience of interaction under combat conditions), the perception of the direct commander image had been influenced both by the peculiarities of the modern way of battle conduction (the actions made by small groups which were coordinated by the leader – that was where significant importance of the ability to adequately inform about the leaders' orders and to implement the role of a coordinator came from) and by the action of a protective mechanism of consciousness, which gave the possibility to maintain mental health under combat conditions, so-called focus on the process of performing their combat tasks.

For these servicemen, the awareness, kindness, and physical strength of their commander were less important. His/her ability to adequately fulfill his/her leadership function (pose combat tasks with adequate consideration of the combat situation and his/her subordinate forces) and the power of his/her spirit including the courage and love of the Motherland, which formed his/her reliability (gave the possibility to perform the combat tasks under extreme conditions and not to give those tasks up).

The servicemen with expressed PTSD symptoms were less satisfied with the role of a junior officer. They considered him/her less capable of meeting their expectations. They were less interested in commanders who could involve them in situations that required quick, brave action and complete responsibility for the entrusted part of the common affair, they required more the commander who would take care of them. Moreover, that commander would not blame the subordinates for their failures, but also, he/she would take on a part of their professional duties and responsibilities for their activity and inactivity.

In the servicemen with expressed PTSD symptoms the negativism of the attitude towards the role of the direct commander was caused by internal contradictions – on the one hand, they looked for the commander who could protect and help, on the other hand – they did not believe that he/she could help them, considering their experience to be unique, which was not appropriate for knowledge and experience of overcoming others.

In the structure of the direct commander image in both studied groups the most important factor was the power of personality (ability to self-regulation), however, in the servicemen with the expressed PTSD symptoms, these factors had specific orientation – to help their subordinates. They were quite selfishly aimed at using their direct commanders to help them.

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Authors' contribution

Conceptualization, YaM. and O.K; managed the project, I.P.; methodology, A.B.; research, O.Z.; writing—original draft preparation, K.M., M.K., Yu.R., and I.T. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

All procedures followed were following the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

Patient consent for publication

Informed consent was obtained from all subjects for inclusion in the study while maintaining their confidentiality. Written informed consent has been obtained from all subjects to publish this paper.

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REVIEW

The Role of Vitamin D in the Development and Progression of Osteoarthritis

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Abstract: *Low levels of vitamin D in patients with osteoarthritis (OA) rather adversely affect the structure and function of articular cartilage. Low levels of vitamin D are also associated with joint pain, limited physical activity, quality of life, as well as decreased muscle strength, primarily in the lower extremities with adverse OA progression. A high percentage of low vitamin D levels was found in patients with OA and joint arthroplasty as well as a less satisfactory postoperative follow-up in patients with low vitamin D levels. The administration of vitamin D supplementation in patients with knee osteoarthritis has been associated with improved articular cartilage architecture, and reduced joint pain, combined with improved functionality and quality of life in patients with OA.*

Keywords: *arthroplasty; vitamin D; osteoarthritis; vitamin D supplementation; vitamin D receptors*

INTRODUCTION

Osteoarthritis (OA) is defined as a heterogeneous group of conditions, leading to symptoms and signs associated with a disorder of joint cartilage, as well as with lesions of the subchondral bone and joint boundaries [1-3]. It is distinguished into primary OA when it cannot be attributed to a distinct pathological disease and secondary OA when it is related to other conditions such as injury, congenital bone disease, metabolic diseases, crystal deposition disease, or neuropathic arthropathy (Charcot's joint) [4-6]. In this review, we will only deal with the primary OA.

Regarding the role of vitamin D in OA development and progression, we will deal with the following important fields based on information from the PubMed database we searched for using the keywords osteoarthritis, vitamin D, vitamin D receptor, vitamin D supplement, joint replacement, until October 30, 2019:

1. Correlation of OA development with genetic polymorphisms of vitamin D receptor.
2. Effect of vitamin D on the structure and function of articular cartilage.
3. Correlation of low levels of vitamin D with the risk of OA development and progression.
4. Effect of vitamin D on OA clinical manifestations.
5. Correlation of vitamin D levels with muscle strength in patients with OA.
6. Correlation of vitamin D levels with the risk of arthroplasty and postoperative progress of OA patients' joints.
7. The role of vitamin D supplementation in patients with OA.

Before referring to the above, it should be noted that desired levels of vitamin D [as estimated by measuring the levels of 25-hydroxyvitamin D [25(OH)D] are >30 ng/mL. Levels <10 ng/mL mean insufficiency, while 10-30 ng/mL consist of a level lower than the considered optimal amount of vitamin D. In 2011 the Institute of Medicine (IOM) defined as insufficiency when the of 25(OH)D level was <20 ng/mL [7], while the Endocrine Society (ES) when the levels were at 20-29 ng/mL [8].

It should also be noted that vitamin D has a significant effect on the immune system function, as it inhibits the synthesis and action of prostaglandins, activates monocytes and macrophages, and has an inhibitory effect on the maturation and function of antigen-presenting progenitor cells and production of inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8), etc [9-13]. Vitamin D deficiency in individuals with a genetic predisposition may affect the immune system's response and tolerance process by affecting T lymphocytes and B lymphocyte function [12,13].

CORRELATION OF OA DEVELOPMENT WITH GENETIC POLYMORPHISMS OF VITAMIN D RECEPTOR

The possible correlation between gene polymorphisms that encode the synthesis of the vitamin D receptor (VDR) and the risk of developing OA has been studied. However, the conclusions of those studies are contradictory. In a meta-analysis, it was reported that the active metabolite of vitamin D, 1,25(OH)2D3 binds to its receptors in the articular cartilage and may lead to hypertrophy and impairment of chondrocyte function [14]. A study found an association between the risk of OA of the hand extremities with polymorphisms of the vitamin D receptor [15], while another study in postmenopausal women with OA of the knee, it was found a correlation between this risk and a TaqI polymorphism of vitamin D receptor [16]. On the other hand, in another meta-analysis of 10 studies, no association was found between TaqI, BsmI, and ApaI polymorphisms and the risk of developing OA in the knees, hips, hands, and lumbar spine [17]. In the meta-analysis of Z.-H. Zhu et al., a weak association of ApaI polymorphism with the risk of developing OA in Asian (but not in European) patients with OA were found [18]. No correlation between vitamin D receptor polymorphisms and the risk of developing OA in the knees was found in the meta-analysis of Rodriguez-Fontenla et al. in 9 Genome-wide studies [19] as well as in other studies [20,21]. Another interesting study carried out by Hansen AK et al. found that 25(OH)D3 and 1,25(OH)2D3 led to an increase of chondrocytes proliferation, in combination with a reduction of proteoglycans synthesis, and thus with a reduction of the intercellular substance [22].

EFFECT OF VITAMIN D ON ARTICULAR CARTILAGE STRUCTURE AND FUNCTION

In male Sprague-Dawley rats, Pascual-Garrido et al. used Micro-CT analysis to detect adverse effects of vitamin D deficiency on articular cartilage. Of particular importance is Jiang X et al.’s study in rats, in which it was found that vitamin D promotes function, the differentiation of the bone marrow-derived mesenchymal stem cells (BMSCs) in mature chondrocytes, enhanced by the action of transforming growth factor-β1 (TGF-β1) and their migration to the articular cartilage [24].

Low levels of vitamin D in OA patients have been shown to adversely affect the structure and function of articular cartilage. In a study in which patients with knee OA participated, low levels of vitamin D were found to be associated with loss of knee articular cartilage as assessed by the decreased joint space [odds ratio-OR=2.3 (Cl, 0.9-5.5)] and the increased size of the osteophytes [OR=3.1 (Cl, 1,3-7,5)] [25]. Ding et al. randomly selected patients (aged 51-79 years, 50% women) in whom the volume of articular cartilage, the presence of articular cartilage deficits in knees using X-rays and magnetic resonance imaging (MRI), as well as the 25(OH)D levels, were assessed at the beginning. 2.9 years later in 353 of these individuals, a correlation between low levels of 25(OH)D and articular cartilage loss was found [26].

In a study in 2014, 80 patients were classified according to 25(OH)D levels into three groups (<10, 10-20, and ≥20 ng/mL). It was found that vitamin D deficiency (<10 ng/mL) may significantly affect the articular cartilage thickness of the peripheral part of the femur (lateral femoral condyle of the right knee and the medial femoral condyle of the left knee) as found by ultrasound [27].

Moreover, in Zheng S et al. study of 340 patients with knee OA, the results showed a correlation between low levels of 25(OH)D with the decreased knee articular cartilage volume, as well as the presence of articular cartilage deficits, bone marrow damage and an increase of synovial membrane thickness (a finding compatible with inflammation) as assessed by MRI [28].

Lastly, in another study of 340 patients with symptomatic OA of the knee and vitamin D deficiency, 25(OH)D levels were measured at the start of the study as well as 3 and 24 months later. It was found that patients with stable normal levels of vitamin D compared to patients with low levels (25(OH)D >50 nmol/L vs ≤50 nmol/L) had a lower loss of joint cartilage volume, thinner synovial membrane and less articular fluid collection [28].

Low levels of vitamin D have also been linked to increased levels of cytokines involved in inflammation in patients with OA. A study by Askari A et al. in 131 patients with OA who compared 262 healthy controls found that lower vitamin D levels were associated with higher levels of interleukin 17A (IL-17A) and interleukin 23 (IL-23), cytokines that promote inflammation in patients with OA [29].

CORRELATION OF VITAMIN D LOW LEVELS WITH THE RISK OF OA DEVELOPMENT AND PROGRESSION

Several studies have focused on the possibility and to what extent low levels of vitamin D in OA patients are associated compared to individuals of the same sex and similar age in the general population and OA progression, with the results being controversial. In Bergink AP et al. meta-analysis, no significant correlation was found between vitamin D levels [25(OH)D] and the frequency as well as the evolution of knee, hip or hand OA. In 3 cross-sectional studies, however, there was found a correlation between the low levels of 25(OH)D and the stenosis of knee joint space (OR=1.52, 95% CI:1.15-2.01). The meta-analysis of the above-mentioned three studies did not show a significant correlation between the low levels of

25(OH)D and the evolution of knee OA (OR=1.37, 95% CI:0.97-1.92). However, when only patients with progressive knee OA development were assessed, an increased risk was found in patients with low levels of 25(OH)D (OR=2.40, 95% CI:1.22-4.72) [30]. In a study of 149 patients (133 women, mean age 63.6 years) with knee OA, the vitamin D deficiency rate [25(OH)D<10 ng/mL] was estimated at 90% [31]. Chaganti RK et al. conducted a study in 1104 elderly men who participated in the "Osteoporotic Fractures in Men Study", measuring 25(OH)D levels at the beginning of the study and performing a radiographic hip examination 4.6 years later. It was found that higher levels of 25(OH)D at the beginning of the study were associated with a lower risk for 25[OH]D decrease, while men with vitamin D deficiency compared to men with normal vitamin D levels had a significantly higher risk (OR=2.19, 95% CI: 1.21-3.97) of developing hip OA [32]. Zhang FF et al. in 418 patients with clinical and radiographic knee OA found a correlation between low 25(OH)D levels and the risk of developing OA radiographically as assessed by evaluating the joint space stenosis (<15 μg/L compared to ≥15μg/L) [33,34]. On the other hand, Lane NE et al. radiographically attended the hips of 237 individuals (with an interval of 8 years) and found no correlation between levels of 25(OH)D and serum 1.25(OH)2D and OA risk progression (development of osteophytes and/or reduction of the joint space) [35]. In addition, Bergink AP et al., in 1248 elderly people (728 women and 520 men) who participated in the prospective study (Rotterdam Study) assessed the development and progression of OA knee radiographically (average follow-up time 6.5 years), found no correlation between 25(OH)D levels (at the beginning of the study) and the risk of subsequent knee OA [36]. In a cross-sectional study of 99 patients (90 women and 9 men, aged 36-80 years) with clinical and radiographic knee OA, no correlation of low 25(OH)D levels with clinical and radiographic OA severity was found [37].

EFFECT OF VITAMIN D ON CLINICAL MANIFESTATIONS OF OA

Some studies have found a correlation between low serum vitamin D levels in patients with OA and joint pain as well as limited physical activity, and quality of life, while some other studies did not. In Zheng S et al.'s study, in 340 patients with OA knees, a correlation of low levels of 25(OH)D was found with severe limitation of the patient's functionality [28]. Levinger P et al. in a study of 24 patients (68.6 ± 6.2 years) with clinical symptoms of knee OA, found a correlation between low levels of 25(OH)D (deficiency ≤50 nmol/L) and knee pain and restriction of patients' functionality [38]. In another study of 100 patients with knee OA, it was found that the vitamin D deficiency group had more severe knee pain, more severe physical limitations, a more intense feeling of fatigue, and a worse quality of life [39].

On the other hand, Lee A et al. evaluated 3824 people aged >65 years (Korean National Health and Nutrition Examination Survey) and found that the levels of 25(OH) D were not related to knee pain [40]. Also in another study of 149 patients (133 women, mean age 63.6 years) with knee OA, no correlation was found between low levels of 25(OH)D and knee pain [31].

CORRELATION OF VITAMIN D LEVELS WITH MUSCLE STRENGTH IN PATIENTS WITH OA

Of particular clinical importance is the association between vitamin D levels and muscle strength (primarily of the lower extremities muscles such as quadriceps that support the weight-bearing joints) of patients with OA (primarily knee OA). A study found that normal levels of vitamin K and vitamin D were associated with better lower extremity function [41]. Javadian Y et al. found a positive correlation between 25(OH)D levels and quadriceps muscle strength in 92 patients with knee OA knee (≥40 years old) (r=0.304, r=9.24%, p=0.005). For every increase of 25(OH)D levels by 1 ng/mL, the strength of the quadriceps increased by 14.2%±3.5% (p=0.014) [42]. In a 24 patients study (68.6 ± 6.2 years) with OA knee clinical signs, a correlation between low 25(OH)D levels (deficiency in ≤50 nmol/L) and the reduction of quadriceps muscle strength was found [39].

However, the effect of other factors, such as body mass index, should be also assessed. In a cross-sectional study of 319 patients with knee OA, a significant correlation between serum levels 25(OH)D and strength of the upper tibial muscles were found, which, however, ceased to be observed when the effect of the body mass index was estimated [43].

CORRELATION BETWEEN VITAMIN D LEVELS AND THE POSSIBILITY OF AN UNDERGOING ARTHROPLASTY IN PATIENTS WITH OA

Numerous researchers have investigated whether vitamin D levels in patients with OA are associated with the possibility of undergoing arthroplasty (mainly hip and/or knee arthroplasty) as well as their postoperative follow-up. A high percentage of low vitamin D levels was found as well as less satisfactory follow-up in patients with low levels. In a recent literature review (12 studies, 2593 patients) the percentage of patients with OA who underwent hip or knee arthroplasty with hypovitaminosis D was estimated at 47% (34.02% -59.9%) [44]. In another study, in 164 patients with hip or knee OA who were to undergo arthroplasty, vitamin D deficiency was found in 81.7%, especially in men [45]. Elevated vitamin D levels are likely to reduce the risk of OA patients undergoing total hip arthroplasty. Hussain SM et al. studied 9135 patients ≥40 years old, who participated in the Australian Diabetes, Obesity and Lifestyle Study, in which the levels of 25(OH)D during 1999-

2000, as well as the percentage of hip arthroplasty in 2002-2011, was estimated (Australian Orthopedic Association National Joint Replacement Registry). 201 patients with OA (90 men and 111 women) underwent hip arthroplasty. In men for any one-standard-deviation increase in levels of 25(OH)D, the probability of undergoing the operation was reduced by 25% (HR=1.25 and 95% CI 1.02-1.56) but not found in women (HR=1.10 and 95% CI 0.87-1.39) [46].

Regarding the effect of low vitamin D levels in patients with OA who have undergone arthroplasty, it appears that they are associated with less satisfactory postoperative results. In Zhang H et al.'s review, it was found that patients with OA who underwent hip or knee arthroplasty, and hypovitaminosis D adversely affected the postoperative results of these patients (Harris Hip Score score was higher in these patients compared to patients with normal vitamin D levels) [44]. In a study conducted in 2010, 62 patients who underwent total hip arthroplasty were compared with 62 patients of the same sex and similar age from the control group, it was found that the patients with vitamin D deficiency had less satisfactory postoperative follow-up results (p=0,038) as well as they had the lowest preoperative Harris Hip Scores (Mann-Whitney test, p=0.018) [47]. Maniar RN et al. also studied 120 patients with OA who underwent total knee arthroplasty [53.3% had preoperative levels 25(OH)D<30 ng/mL]and they assessed their functional ability before and 3 months after surgery. Before surgery, in patients with vitamin D deficiency, their functional ability was limited [Western Ontario and McMaster Osteoarthritis Index (WOMAC), p=0.040]. After surgery, in patients who received vitamin D supplementation, their functional ability was restored, which was similar to the functional ability of patients with normal vitamin D levels [48]. On the other hand, in the study of Visser E et al. in 87 patients with OA who underwent total hip arthroplasty, no correlation was found between levels of vitamin D and restoration of hip function. However, an increase in levels of 25(OH)D was observed six months after surgery, which was associated with an improved hip function (Pearson's r =-0.471, p=0.018) [49].

Regarding the dosage of postoperative vitamin D administration in patients with OA who underwent arthroplasty, there are interesting results from a randomized, double-blind 24-month study of Bischoff-Ferrari et al. in which participated 273 patients underwent knee arthroplasty. 137 patients received 2000 IU of vitamin D while 136 patients received 800 IU per os daily. Even though the administration of 2000 IU was accompanied by a significant increase in 25(OH)D levels (45.6 ng/mL vs 37.1 ng/mL of the 800 IU dose after 24 months, p <0.0001). No difference regarding the restoration of operated knee function, nor on the radiographic progression of the other knee OA, between the group of patients who were administered 2000 IU and 800 IU respectively (30,5% vs 31,3%, p=0.88) [50].

THE ROLE OF VITAMIN D SUPPLEMENTATION IN PATIENTS WITH OA

Animal studies have shown that vitamin D has a protective effect on the articular cartilage architecture [51,52]. In an animal study, micro swings that were on a diet rich in cholesterol, it was found that vitamin D had a protective effect on the architecture of articular cartilage (reduction of inflammation and fat infiltration) [51]. In Li S et al.'s animal (ovariectomized rats) study, was also found that vitamin D deficiency was associated with deterioration of the knee articular cartilage, while the administration of 1,25(OH)2D3 with a protective effect on articular cartilage [52].

Vitamin D supplementation in patients with knee OA has been associated with a reduction in synovial membrane thickness and joint effusion associated with the OA development, as well as an improvement in knee articular cartilage architecture [53,54]. In a study of 413 women with symptomatic OA knee and low 25(OH)D, 209 women were supplemented with vitamin D (50,000 IU per os monthly) while 204 women were administered placebo for 24 months. This study investigates the effect of this administration on the articular membrane thickness and joint effusion using MRI. The rate of increase of the above-mentioned variables was lower in the group supplemented with vitamin D compared to the placebo group (risk ratio (RR)=0.87, 95% CI: 0.77-0.97) [53]. Veronese N et al. in 783 patients with knee OA (59.8% women, mean age 62.3 years), vitamin D supplementation was evaluated for its effect on the volume and thickness of the medial and lateral femoral condyle cartilage with MRI. Using linear regression analysis in which the effect of 10 potential confusing factors was assessed, it was found that vitamin D supplementation led to an increase in the volume and thickness of the medial and lateral femoral condyle cartilage, suggesting an improvement in its architecture [54].

Several studies have investigated the effect of vitamin D supplementation on joint pain, quality of life, physical function, joint stiffness, muscle strength (such as quadriceps femoris), joint space range, articular cartilage volume, and deficits as well as bone marrow lesions, etc, with conflicting results [55]. Moreover, in a systematic review and meta-analysis conducted in 2017, it was found to be small as a moderate result of vitamin D supplementation in knee pain [standardized mean difference (SMD)=-0.32, 95% CI: -0.63 to -0.02]. However, no effect was found on tibial articular cartilage volume/thickness (SMD=0.12, 95% CI: -0.05 to 0.29) or joint space range (SMD=0.07, 95% CI: -0,08 to 0.23). In other words, vitamin D supplementation had a rather favorable effect on knee pain but not on OA severity [56].

In all randomized controlled trials, 25(OH)D levels were found to increase in patients supplemented with vitamin D compared to patients that took the placebo. However, no reduction in pain (except for one study) or knee stiffness (evaluation with WOMAC scale) was found. On the other hand, in 2 of 3 studies that assessed knee pain using a visual analog scale (VAS), vitamin D supplementation led to an improvement in knee pain [57]. In Heidari B et al.’s study, 67 patients (aged ≥30 years with knee OA) received a supplement of 50,000 IU of cholecalciferol per week orally for at least two months. It was found that the cholecalciferol supplementation led to an increase in the normal level of 25(OH)D ($p=0.007$) as well as to a significant increase in quadriceps strength ($p=0.002$) and a significant decrease in knee pain ($p=0.001$) to all patients (except for one patient) [58].

In a multicenter randomized double-blind study, 340 patients with symptomatic knee OA were distinguished into one group of patients who received vitamin D (50,000 IU per month orally) and another group that received a placebo two years ago. The purpose of the above-mentioned study was the evaluation of this administration's effect on tibial articular cartilage volume, the presence of defects in tibial articular cartilage, bone marrow lesions, and knee pain. There was a significant increase in 25(OH)D levels in the supplemented group compared to the placebo group (40.6 nmol/L vs 6.7 nmol/L, $p<0.001$). However, no effect was found on tibial joint cartilage volume, the presence of tibial joint cartilage deficits, or bone marrow lesions as assessed by MRI [59].

Additionally, in a study of 175 patients with primary knee OA and low levels of 25(OH)D (<30 ng/mL), they were supplemented with 40,000 IU of vitamin D (ergocalciferol) per os once a week for six months. It was found significant increase in 25(OH)D levels ($p<0.001$), improved quality of life ($p=0.005$) and patients' functionality ($p<0.05$), as well as reduced knee pain ($p=0.002$) [60]. On the other hand, in a three-year double-blind, randomized study of 474 patients aged>50 years with radiological signs of knee osteoarthritis, it was found that the group of patients supplemented with vitamin D led to an increase at 25(OH)D levels compared to the placebo group, without affecting the narrowing of the joint space, pain, function, or stiffness of the knee joint [61].

The administration of high-dose vitamin D supplements has a rather beneficial effect on reducing knee pain. A meta-analysis of 4 randomized group studies with 1,136 patients, evaluated whether and to what extent the administration of a vitamin D supplement affects pain, function, stiffness, and tibial joint cartilage volume of patients with knee OA. It was found that this administration was associated with pain reduction and improved function but did not improve knee stiffness or tibial joint cartilage volume. Particularly favorable was the effect on pain and knee function in patients receiving a dose of natural vitamin D higher than 2000 IU [62].

It was also studied whether vitamin D supplementation and maintaining adequate levels affected inflammatory markers and metabolic biomarkers associated with OA pathogenesis. Zheng S et al. studied 413 patients with symptomatic knee OA and vitamin D deficiency, who were randomly assigned to one group receiving 1.25 mg of vitamin D3 and to another group receiving a placebo, once a month for 24 months. The researchers concluded that vitamin D supplementation as well as maintaining adequate levels did not affect inflammatory markers or metabolic biomarkers [63].

CONCLUSIONS

In conclusion, low levels of vitamin D in patients with OA rather adversely affect the structure and function of articular cartilage. They are associated with joint pain, limited physical activity, quality of life, etc, as well as decreased muscle strength, primarily of the lower extremities with adverse OA progression. A high percentage of low vitamin D levels was found in patients with OA and joint arthroplasty as well as less satisfactory postoperative progress in patients with these low levels. The administration of vitamin D supplementation in patients with knee OA has been associated with improved articular cartilage architecture and reduced joint pain in combination with improved function and quality of life.

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REVIEW

Biotribology of the Total Hip Replacement – Review of the Current Bearing Surfaces

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Abstract: *Total hip replacement surgery (THR) is one of the most common surgeries performed in modern orthopedics, and in most cases offers a significant improvement in the quality of the patient's life. However, after some time, wear and osteolysis do appear, which leads to the need for revision surgery. With the indication being widened to a more young and active population, the need for extending the lifespan of the prosthesis is mandatory. Although several bearing surfaces have been studied over the years, and a lot of couplings are in use, the choice of the right combination of materials is still controversial. Biotribology tries to answer some of the current questions regarding the best bearing surface to use, being the branch of science and technology that studies friction, wear, and lubrication of different materials in the human body. In the present day, a large number of materials are in use for total hip replacement, but choosing the right combination of materials is still controversial, and is being done on one hand by the surgeon's experience and on the other hand by the age and activity of the patient. The objective of this review is to try to present the main advantages and disadvantages of different materials and try to answer the most difficult question of them all: What is the right material to use for THR-bearing surfaces?*

Keywords: *total hip replacement, tribology, biotribology, arthroplasty*

INTRODUCTION

More the 350,000 total hip replacements are done each year in the United States [1], with a constantly increasing rate. The survival rate of these implants is, according to the British National Joint Registry, 93.2% at 13 years, and 80% up to 20 years [2]. Although these rates seem to be pretty good, about 13% of these hip replacements are revision arthroplasties [3]. This high rate can be attributed to the younger and more active patients who were treated with this procedure.

The most important aspect is still worn, which limits the lifespan of this prosthesis to 10 – 15 years. With a lot of materials and couplings to choose from, such as hard-on-soft bearings like metal-on-polyethylene (MoP) and ceramic-on-polyethylene (CoP), and hard-on-hard bearings such as ceramic-on-ceramic (CoC), metal-on-metal (MoM) and ceramic-on-metal (CoM), it can be confusing for both the patient and the physician, all of these materials showing specific wear mechanisms and characteristics that will be discussed in the present paper. Wear patterns like burnishing, abrasion, pitting, and scratches were observed for ultra-high molecular weight polyethylene (UHMWPE) and cross-link polyethylene (XLPE) [4], stripe wear was observed for ceramic components [5], changes in surface characteristics like roughening and roundness for metal-on-polyethylene couplings [6], gouging, scratching and polishing for metal-on-metal couplings [7].

Metal-on-metal couplings

With the first implantation in 1938 by Wiles, the MoM coupling was the first bearing used for THR. In conventional metal-on-metal hip replacements, the prosthesis is made up of a large diameter metallic femoral head on a standard femoral stem that articulates with a metal acetabular cup or liner, CoCrMo alloy was used widely for these bearings (Figure 1).

The advantages of this type of coupling are that they could be engineered to be extremely smooth and hard, properties that can reduce the wear rate, and the wear rate of MoM is estimated to be 60 times less than conventional MoP implants [8]. Other advantages include increased resistance to dislocation associated with large-diameter heads and reduced osteolysis due to the absence of polyethylene particles [8].

Figure 1: Metal on-metal coupling [10]

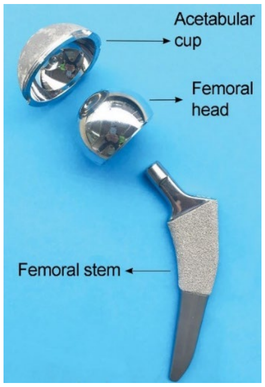

On the other hand, MoM materials come with a series of disadvantages. The metal alloys used in orthopedic implants degrade due to wear and, unlike polyethylene, also corrosion. This can lead to soft tissue inflammatory reactions to metal debris, known as adverse reactions to metal debris. The rate of wear depends largely on the contact force between the two surfaces and the sliding distance, and thus increases with physical activity, weight gain, larger implants, and the roughness of the implant surface [9].

The **inflammatory pseudotumors** (Figure 2) represent the existence of an aseptic mass in the periprosthetic tissue that can be solid or cystic and associated with local inflammation [11]. It is believed that it develops as a result of an adverse reaction to the metal ions released from the wear of metal articular bearing surfaces [12]. Implanting the acetabular cup at abnormal inclination angles increases the edge loading which leads to increased prosthetic wear [13]. The incidence of pseudotumors is up to 32% in some studies [14]. In a study conducted in 2009 on 1224 patients, it was shown that female patients and those under 40 years of age had an increased risk of revision due to pseudotumors [15].

Figure 2: The inflammatory pseudotumors [16]

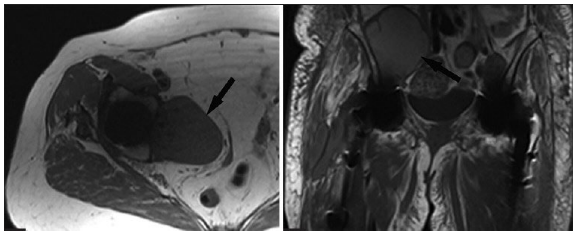

Aseptic lymphocytic vasculitis-associated lesion (ALVAL), consists of cellular changes that occur as a response to cobalt and chromium ions. The mechanism is believed to be a T lymphocyte-mediated type 4 hypersensitivity reaction, with tissue damage occurring as a result of cytotoxic T cells and activated monocytes/macrophages].

Metallosis, represents aseptic fibrosis, local necrosis, or aseptic loosening of a device because of metallic corrosion and release of wear debris [18].

Trunnionosis (Figure 3), the metal wear at the head-neck junction of a total hip implant is believed to be an important contributor to the release of metal particles [19]. It is present in the modular design prosthesis, both in MoM and MoP, because in both cases the head-neck junction is made up of metal-on-metal surfaces. This is the main reason why trunnions cannot be blamed to be the only cause of the increased rate of adverse reactions in MoM hip replacements.

Figure 3: Trunnionosis [20]

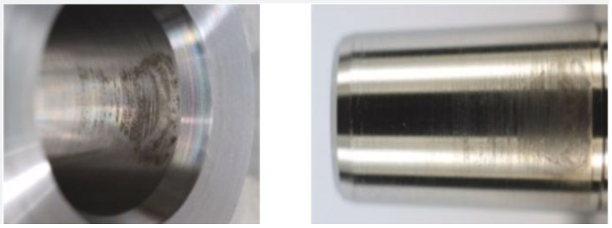

Revision rates for MoM at 10 years for cemented and uncemented hip replacements are 19.68% and 21.92% by comparison with MoP, which was 3.13% and 3.98% [21]. There seems to be a direct connection between the size of the femoral head that was used and wear, in THR that used a head size above 40mm the revision rate at 10 years was 25.5%, by comparison to those that used a femoral head diameter below 28mm with a revision rate at 10 years of just 5.7%.

Serum and urine ion levels produced by MoM bearings are similar to the ones produced by the modular junctions of femoral components. The toxicologic importance of these trace metal elevations has not been established. Because the wear of a THR with an MoM bearing cannot be evaluated radiographically, metal ion concentrations in serum and urine can be indicators of wear [22].

Considering the renal excretion of Co-Cr ions, a potential for increased risk of bladder and other cancers in patients with metal-on-metal couplings was formulated. Two large studies were conducted in England and Wales and Finland that examined nearly 330.000 patients [23,24], but no connection between MoM bearings and the high risk of developing a type of cancer was discovered. The follow-up protocol within those studies was seven and four years. So, we think that studies with a longer follow-up period are needed to completely declare that MoM implants do not increase the risk of cancer development.

Due to all of these considerations, in the present day, the MoM THR is almost entirely abandoned by surgeons and withdrawn from the market by manufacturers [25].

Polyethylene

Conventional and cross-linked polyethylene liners are the most common choice in THR. First used by Charnley in 1962 [26], this polymer is a high molecular weight polyethylene with extremely long chains. Until the mid-2000s, the MoP bearings (Figure 4), with head diameters between 22 mm and 32 mm we're considered the standard. The linear wear for PE is between 100 to 300 µm/year [27], which corresponds to volumetric wear of 20 – 150 mm^3/year for 28 mm heads [28]. It was shown that a correlation exists between volumetric wear and the risk for osteolysis, every increase in the amount of volumetric wear of 40 mm^3/year triples the long-term risk of osteolysis [29].

Figure 4: Metal on polyethylene coupling [10]

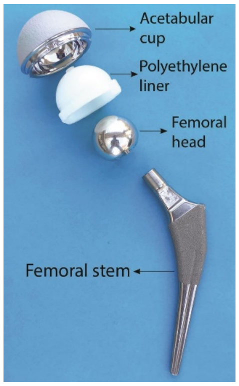

When using polyethylene liners, not only the material is important, but also the sterilization technique, because this too can influence the mechanical properties and biological effects. The most common sterilization method used was gamma irradiation. Some studies [30] show that this sterilization method increases cross-linking between polyethylene molecules offering a more wear-resistant material. But, when this is done in the presence of oxygen, free radicals are released that can oxidate and make the liner more brittle with reduced resistance and increased wear. So, thermal sterilization with gas plasma was shown to improve wear and oxidation resistance [30, 31], and oxygen-free gamma irradiation showed less head penetration and less osteolysis [32].

Because of the wear debris of the conventional polyethylene, cross-linked polyethylene was developed (Figure 5). It is obtained by irradiation of conventional polyethylene with at least 50 kGy of gamma beam radiation, which induces the cross-linking between PE molecules, which increases wear resistance [33]. The rate of revision at 16 years for XLPE was 6.2% vs. conventional PE which was 11.7% [34]. The process of cross-linking can however decrease the mechanical properties so that the liner can become at risk for fatigue fracture because the irradiation process releases free oxygen radicals [35]. In addition, steep positioning of the acetabular cup leads to stress concentration or impingement and it is considered also a risk factor [36]. This problem was solved by introducing vitamin E into the liners, which is an antioxidant, and the mechanical properties are saved. Also, the liners can be now thinner and the femoral head bigger, which can improve joint stability.

Figure 5: Cross-link polyethylene–electron microscopy [37]

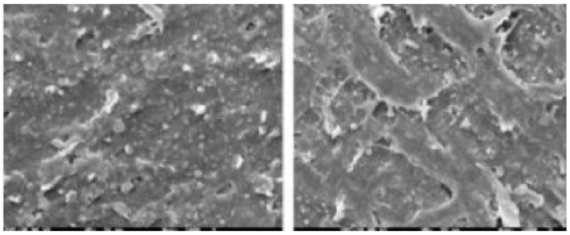

When evaluating the long-term resistance of PE, conventional or cross-linked, we must take into consideration also the femoral head material and diameter. We know that the larger the femoral head diameter the bigger the wear, especially when coupled with conventional PE [38]. It seems that this does not apply to XLPE, where the wear rate is not higher for bigger head diameters [38].

In the matter of ceramic vs. metallic femoral heads, in early studies, the average wear rates with the metal femoral head (stainless steel and cobalt chrome) invariably were higher than with ceramic femoral heads [39-41]. In a more recent study based on a systematic analysis of registry data, an overall equal long-term performance was found between metal and ceramic heads, although a lower revision rate was discovered with CoXLPE than MoXLPE [38]. The advantages of ceramic heads are higher hardness with less risk of damage during implantation, the surface finishing with only negative peaks due to grains detachment rather than deformation, and higher wettability which means better lubrication of ceramic, less friction and scratching and less linear wear [27]. Last, but not least, alumina is biologically inert and has the best biocompatibility.

Ceramic on ceramic

Alumina and zirconia ceramic is used in THR surgery (Figure 6). Alumina has a very low friction coefficient, is biocompatible, and its material properties are not affected by aging it was also shown that it reduces volumetric wear [33]. With the risk of fracture being the most severe concern, alumina (82%) was mixed with zirconia (17%), chromium oxide (0.5%), and strontium crystals, and a higher resistance to crack and enhanced hardness were obtained. With this breakthrough, the fracture rates of the femoral head were reduced from 0.021% to only 0.003% [42]. However, the risk of ceramic head fracture is still lower than the risk of ceramic liner fracture (0.003% vs. 0.03%) [43]. The survival rate at 10 years of these implants in excellent, varying from 98% to 99.3% [44], 92.8% at 15 years [34], and 84.4% at 21 years [45].

Figure 6: Ceramic on ceramic coupling [46]

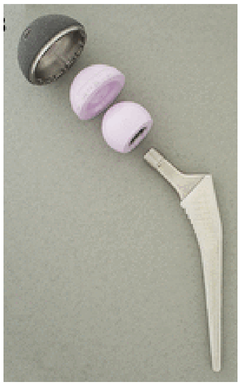

The advantages of CoC bearings are a very low friction coefficient and very low wear rates, due to high wettability and hardness. If however, the ceramic implant does suffer wear, the particles released are biologically inert and produce a less biological reaction. Another great advantage is that the wear is not dependent on the diameter of the femoral head, a large femoral head will provide a more stable artificial joint with a low risk of dislocation. A recent study showed that 36mm ceramic femoral heads had a lower revision rate than smaller 28mm ceramic femoral heads [47]

Like any other bearing surface, the CoC coupling also has a series of drawbacks. Although the risk of fracture was lowered as presented, the risk remains and is a catastrophic complication that requires revision surgery. Factors that can increase the liner fracture risk are misalignment during insertion of the liner, metal back damage or acetabular component malposition that can lead to edge loading, and the most important factor, excessive anteversion.

Another disadvantage is the noise that this coupling can produce. Clicking, grinding, clunking, and squeaking has been described. The incidence varies from 0.7% to 20.9% [48], this adverse event can even be a cause of revision, with a rate of 0.2% [49]. Risk factors for squeaking are age, obesity, activity level, soft tissue balancing, and positioning of the acetabular cup.

It is recommended that in cases with severe posttraumatic acetabular deformities or severe developmental dysplasia of the hip, the CoC should be avoided, because of the difficulty of correct positioning of the acetabular component.

Another drawback of the CoC bearing is the cost. It is the most expensive choice of coupling, but also the most advanced and resistant to wear when used correctly.

DISCUSSION

After the literature data has been analyzed, we concluded that the preferred choice when it comes to total hip arthroplasty bearings is a hard-on-soft coupling like the metal (cobalt-chrome) on polyethylene or ceramic on polyethylene. MoPs are still the most widely used coupling covering about 60% of all interventions, but the use of CoP is increasing. But which of the two is better?

If we consider the studies of wear screening using water-based lubrication and linear tracking, there is a 20-to-80-fold advantage for ceramic-on-polyethylene when compared with metal-on-polyethylene [50]; however newer wear studies with high protein concentrations have shown similar wear with alumina or with met-al-on-polyethylene [51]. Because of the biological advantages of alumina which is biologically inert and with the amount of literature that supports the superiority of the ceramic femoral head, we too recommend the use of ceramic-on-polyethylene bearings over the metal-on-polyethylene ones.

Considering the type of polyethylene that should be used, we feel that is enough literature that proves that conventional polyethylene has a much higher wear rate than cross-link polyethylene. More and more manufacturers are offering just the cross-link version now, the only advantage of the standard polyethylene is the lower cost of production. However, we feel that because of the high wear rate that requires early revision, there is practically no cost advantage, and therefore we cannot recommend using conventional polyethylene anymore.

How about hard-on-hard surface bearings? We know that the manufacturers do not provide the MoM bearing anymore, due to the pseudotumors caused by toxic metallic ions, so the only valid option for this kind of bearing is the CoC.

Although the wear is insignificant with this coupling and is not associated with femoral head diameter, there is still concern about motion sounds like squeaking, which has a revision rate that varies between 1 and 21%. Another drawback is the risk of liner fracture; although is a quite low risk, it has a catastrophic impact and requires urgent revision surgery. So, there is still room for improvement.

CONCLUSIONS

Deciding what coupling to choose is mostly dependent on the surgeon's preference and experience. Considering the literature mentioned in the present paper we recommend the use of CoC bearing if the cost is not an issue, in young and high-activity demanding patients, with a large diameter femoral head, up to 36 mm, as wear is not related to large head diameter femoral heads and can improve joint stability. Care must be taken in cases where the correct positioning of the acetabular component may be an issue, as the malposition of the acetabular cup can lead to squeaking and increase the risk of fracture of the ceramic liner.

CoXLPE is a good option for CoC, with a lower cost and offers comparable results. It can be successfully used in active patients over 60 years of age, care must be taken in choosing the right femoral head, larger diameter head being associated with a higher wear risk. We cannot find any reason for using conventional PE, as it proved to be less reliable and more susceptible to wear, with a higher risk of releasing debris.

The choice of MoXLPE remains a viable option in a situation in which cost is an issue. Other than cost-related issues, the ceramic bearings are a more reliable option over the metallic on PE.

Even if the best bearing surface is available, we should not forget that coupling is not everything, and careful patient selection is still mandatory. More than that other factors influence the overall behavior of the THR, like age, level of activity, anatomic deformities of the acetabulum, positioning of the components, bone quality, soft tissue balancing, and patient compliance. So, to have a successful THR all of these factors must be carefully quantified, and the surgeon must use the correct surgical technique.

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ORIGINAL ARTICLE

Aesthetic Principles in Nasal Reconstruction after Squamous Cell Carcinoma Resection

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Abstract: *A successful nose reconstruction is represented by a normal look and nasal breathing preservation. The choice of method is based on the site, size, depth of the defect, donor availability, and surgeon experience. There are multiple methods of restoring nasal cover, from primary repair to healing by secondary intention, from primary suture, skin graft to locoregional flaps, but all of them should be integrated into a personalized approach, keeping the nasal functions, a good aesthetic outcome and without any tumor infiltration. This paper consisting of a retrospective study of 9 patients diagnosticated with squamous cell carcinoma revealed the importance of skin properties and skin changes during aging. Despite all aesthetic principles, skin is the most important resource in facial reconstruction.*

Keywords: *nose reconstruction, aesthetic, squamous cell carcinoma*

INTRODUCTION

Cutaneous squamous cell carcinoma is one of the most common skin cancer with an increasing incidence [1]. Most frequently on sun-exposed sites, like the face, it could be a challenge for a surgeon [2]. One, because of its aggressiveness and the possible requirement of large excisions, and second, because of the psychological impact on the patient. The nose is one of the most visible anatomical sites so, an aesthetic result is even more required. The nasal restoration should be based on keeping the contouring, color, and texture, minimalizing the scars, and preserving the breathing function [3].

MATERIALS AND METHODS

A retrospective study performed in the Plastic Surgery Department of Emergency University Hospital, Bucharest, Romania evaluated 9 patients diagnosticated with cutaneous squamous cell carcinoma at the nose level through the histological exam, two of them with local relapse. The age average was 63 years old, with a minim of 44 years old and a maxim of 90 years old. It was identified as a male prevalence.

In all cases, the tumor resection was performed with free macroscopically oncological edges. Depending on the tumor size, site, and patient age, multiple reconstruction techniques were required. In 4 cases primary suture was practiced, in 3 cases local flaps were required, and in 2 cases skin grafts.

All the patients signed the Informed Consent before surgery and the study was approved by Ethics Committee.

DISCUSSION

There are nine aesthetic subunits of the nose, dorsum, paired sidewalls, nasal tip, paired alae, columella, and paired soft tissue facets (Figure 1). The concept of aesthetic units in nasal reconstruction was first described by Millard to improve surgical results. Then, Burget and Menick introduced the principles of nose aesthetic subunits which improved the aesthetic results in nose reconstruction [4].

The skin quality is different in every aesthetic subunit, being thicker at the nasal tip, nasal alae, and cranial dorsum, and upper nasal sidewall and mid-lower dorsum being the thinnest. In all facial reconstructions, the skin patient is an important factor. During aging, the skin properties are changing and the skin becomes looser.

Nose reconstruction is a complex process in which the first step should be defect identification. A classification of nasal defects is based on size. There are small defects, with a diameter of less than 1.5 cm, and large defects, with a diameter over 1.5 cm, which require a skin graft or a local flap [5].

Figure 1: Nose aesthetic subunits

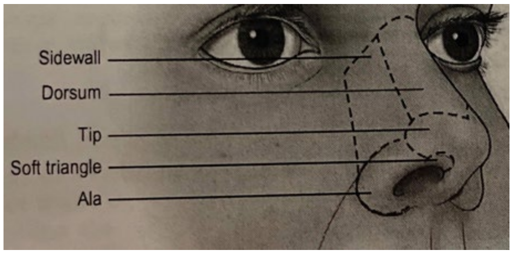

Depending on the depth of injury, there are superficial defects where the skin is missing and also, a small underlying subcutaneous fat and nasalis muscle could be missing. Deep defects are represented by the absence of an underlying supportive framework or lining. The cartilage could be affected, so a composite skin graft could be required, cartilage grafts, or vascularized regional flap for cover [6].

A more complex reconstruction is required by composite defects which are represented by nose defects extended to the cheek or upper lip (Figure 2). The challenge consists of the fact that they are different aesthetic units. The reconstruction of composite defects should be based on keeping the quality, outline, contour, projection, and function of each one, not only to “fill the hole”.

Figure 2: Nasogenian tumor that left a composite defect including sidewall, ala, dorsum, and cheek, which was covered by a local cheek flap

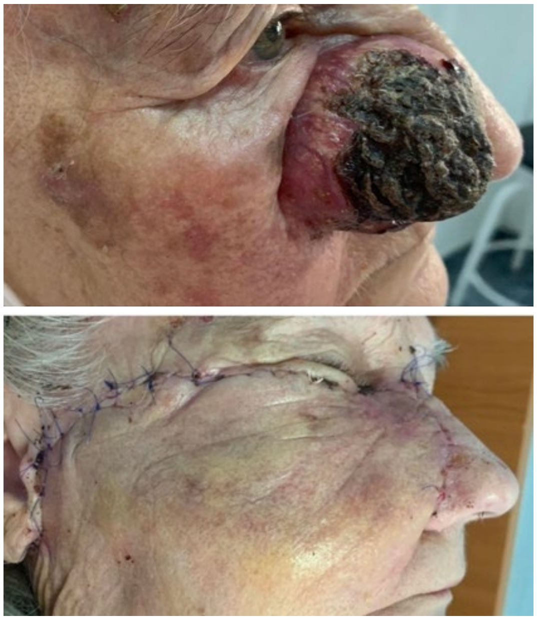

Choosing the reconstruction method should be based on the shape, size, location, and depth of the defect and the aesthetic unit involved [7]. The bilobed flap is one of the most common local flaps used in nose reconstruction, especially for tip and ala defects, when the diameter of the defect doesn`t count more than 1.5cm [8]. The Bipedicle mucosal flap could be useful in small alar reconstruction to keep the lining of the nose [9].

Another common flap in nose reconstruction is the septal hinge flap which is used for tip, ala, and sidewall defects [10]. The technique is based on the anterior septal artery. When the nasal tip and columella are missing, and also a part of the septum, bilateral septal flaps could restore the lining of the ala and nasal vestibule [11,12,13].

In cases of full-thickness defects, especially for lateral defects, the inferior turbinate flap could be a good option, alone or together with other techniques [14].

Forehad flap is useful for any significant nasal defect over 2.5 cm. It can be also used when the septal hinge flap and inferior turbinate flap failed. The technique is based on the supratrochlear vessels that pierce the orbital bone at about 2 cm lateral to the midline [15].

Melolabial flap in an interpolated flap is used for defects less than 4 cm, especially for cutaneous nasal ala defects with or without modest extension into the tip and sidewall [16].

The aesthetic outcomes between local flaps and skin grafts are depending on patients' selection. Sapathavee, Munaretto, and Toriumi proved that a skin graft provides an aesthetic result in patients with Fitzpatrick types 1 and 2 skin, also with thin and less sebaceous skin. The preauricular skin offers a good match in nose reconstructions. Some authors are using “skin fat grafts” to fill small deep defects of the nose [17].

Keeping the structure of the nose is an important part of the reconstruction. Cartilage grafts are useful tools in maintaining nose support. They prevent also overlaying soft tissue contraction, minimize pincushion deformity and reduce nasal obstruction. The donor sites could be costal cartilage or calvarial bone for restoring the contour and support of total or subtotal nasal defects, and conchal cartilage for alar reconstruction due to its curvature shape.

To keep the internal lining, a composite graft from the conchal site could be used. Also, a composite graft from the helix root could be used in nasal ala restoration [18].

In our case, we used skin grafts in 2 cases of female patients, about 63 and 69 years old. The tumors had the same localization, at the dorsum level, and has a diameter over 3 cm, without bone invasion.

In 2 cases of the cutaneous tumor with approximately 1.5 diameters, at the sidewall and ala levels, local flaps were performed. The nasolabial transposition flap was the reconstruction method for ala defect, while for sidewall defect a dorsal nasal flap was performed. Rieger described the dorsal nasal flap as a laterally-based rotation flap with a wide pedicle. The technique consists in designing a "V" in an upside-down fashion over the glabella and then a curvilinear line on the nasal–cheek junction till it reaches the defect site. Marchac and Toth updated the Rieger version by narrowing the pedicle in an axial flap based on the vessel arising from the inner canthus. The advantages consist in increasing mobility, decreasing wound closure tension, and a more precise adjustment [19].

One case of a cutaneous tumor involving multiple nasal subunits, with a diameter over 4 cm, without bone invasion, required multiple techniques of reconstruction, frontal flap for dorsum defect, and primary suture for secondary defects.

Figure 3: Intraoperatively picture with dorsum defect size after squamous carcinoma resection for which the skin allowed a primary suture.

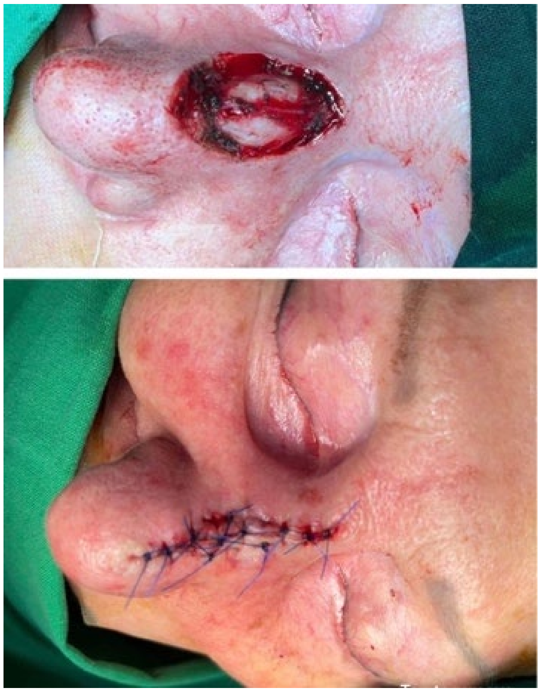

In 4 cases, the primary suture was enough for closing the defect. Three of them had a diameter less than 1.5 cm, one at ala level and 2 at dorsum level, with ages between 59 and 80 years old. There was another case of 77 years old female, with a diameter tumor of approximately 2.5 cm at the dorsum level, for which the primary suture was enough (Figure 3). Usually, a defect of 2.5 cm isn`t suitable for primary suture and a local flap is mandatory. For defects of 1.5 cm – 2.5 cm, a dorsal nasal flap, a bilobed flap, or a glabellar flap could be used [20].

So, depending on the aesthetic unit involved and defect size, nasal reconstruction methods differ. Choosing the reconstruction method should be based on restoring the aesthetic unit, not on filling the defects. Keeping a natural look, the borderline, the skin quality, and preserving the breathing function are variables that we should take into account in nose reconstruction, but free tumor edge is the most important medical approach. Dorsum and sidewalls have common skin characteristics, so the reconstruction methods are similar. Defects less than 1.5 cm could be closed primarily, with a glabellar flap or a transposition one. Defects with a diameter of 1.5-2.5 cm require a local flap, a glabellar flap, a bilobed flap, or a dorsal nasal flap. The last one can be also used for defects more than 2.5 cm [21].

In case of nasal tip defects, a bilobed flap can be used for defects less than 1.5 cm, a dorsal nasal flap under 2.5 cm, and forehead flaps for defects of or bigger than 2.5 cm (Figure 4).

Figure 4: Skin tumor involving ala unit which required a forehead flap

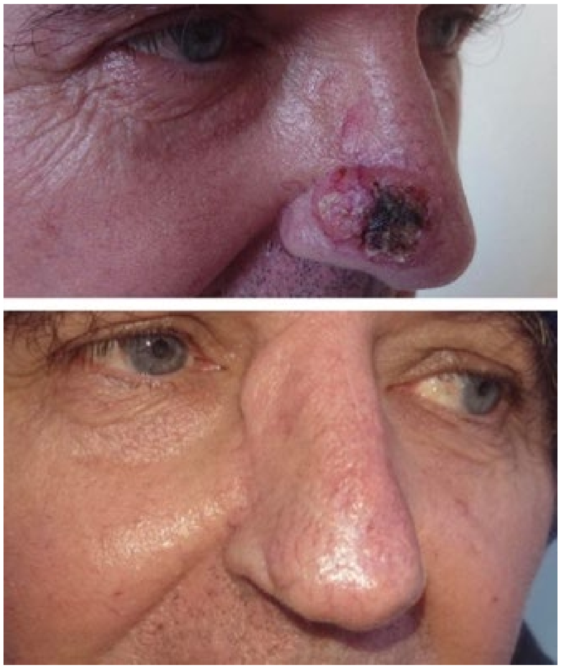

In case of nasal ala defects, a full-thickness skin graft and a cartilage graft to keep the shape and to avoid breathing obturation is a good choice for superficial defects, while in case of deeper defects, a more complex reconstruction like nasolabial flap or interpolated melolabial flap is required [22].

Despite all the reconstruction science of skin defects, skin properties are changing during aging which requires a personalized surgical approach.

Even if the resection was performed with free macroscopical tumor edges, the histological exam revealed a deep tumor infiltration in 5 cases, which showed the limitation of anatomical site between safe tumor resection with free tumor edges and aesthetic result.

CONCLUSIONS

Since the nose is the most visible part of the body, its reconstruction has an important role in the life quality of patients. Behind the science, plastic surgery is also about art and understanding that skin suffers changes during aging which affects its properties, its structure and requires a personalized approach. Every nose reconstruction should be based on keeping a three-dimensional contour, keeping the quality of the skin, the border outline, and breathing function. All the techniques should be focused on restoring the aesthetic units, not on filling the defects. Sometimes the preservation of the aesthetic aspect interferes with the preservation of nose function and complete resection of the tumor, which is a challenge for the doctor to make the patient understand the prioritization of health over the aesthetic aspect and to help him to manage this situation without decreasing quality of life.

Conflicts of interest and sources of funding

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Authors' contribution

A.G. conceptualization and writing (original draft preparation), A.M.O. methodology and writing (review and editing), I.P.F. data curation and supervision. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Approval of the local Ethics Review Board of Emergency University Hospital of Bucharest was obtained for the use of the patient data.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study

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ORIGINAL ARTICLE

The Prevalence of Cerebral Aneurysms in Patients with Polycystic Liver Disease

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Abstract: *Background: It is well known that patients with polycystic kidney disease (PKD) are at increased risk of developing cerebral aneurysms, however, this association has not been well studied for patients with polycystic liver disease (PLD). Material and methods: Cross-sectional descriptive study, which included 15 adult patients diagnosed with polycystic liver disease at the Gastroenterology and Hepatology Department of Fundeni Clinical Institute. Standard neurological exam and brain MRI were performed in all patients on a 1.5 Tesla MRI. Brain imaging protocol included T1/T2, T1SE, T2-FLAIR, DWI, SWI, 2D-TOF, 3D-TOF. Results: The majority of patients (93%) were females. The mean age was 53 ± 5 years old. Patients with AD-PKD and polycystic hepatic disease predominated (60%). Aneurysms were found in only one patient diagnosed with AD-PKD (in whom three aneurysms were described). The overall prevalence of cerebral aneurysms in our patient group was 7%. However, none of the patients with isolated PLD was found to have intracranial aneurysms. Other brain imaging abnormalities were frequent but nonspecific (mostly attributable to vascular-degenerative changes). Conclusions: Given these results, and due to the small number of patients in our study, it is hard to appreciate if polycystic liver disease is indeed associated with an increased risk of cerebral aneurysms.*

Keywords: *polycystic liver disease, cerebral aneurysm, brain MRI*

INTRODUCTION

Polycystic liver disease (PLD) is a genetic disorder characterized by the replacement of normal hepatic tissue with fluid-filled cysts. This is due to genetic mutations of the proteins that are involved in epithelial growth and transport of fluid in the liver [1]. Some authors have defined PLD by the presence of more than 10 cysts in the liver, while others have defined it as a liver containing over 20 cysts [2,3]. PLD can occur in isolation or can be associated with autosomal dominant polycystic kidney disease (AD-PKD), in which case patients present with cysts involving both the liver and the kidneys.

The latter combined form (AD-PKD and PLD) is more common, accounting for 80-90% of all patients with multiple liver cysts [4]. It is well-known that patients with PKD are at an increased risk of developing cerebral aneurysms [5,6], however, this association has not been well-studied for patients with PLD.

MATERIALS AND METHODS

We performed a cross-sectional descriptive study, in which we recruited 15 adult patients diagnosed with PLD at the Gastroenterology and Hepatology Department of Fundeni Clinical Institute. All patients were included on the liver transplantation waiting list (or have already benefited from liver transplantation). The evaluation comprised a standard neurological examination and electroencephalogram. Brain MRI was performed in all patients on a 1.5 Tesla MRI. Brain imaging protocol included T1/T2, T1SE, T2-FLAIR, DWI, SWI, 2D-TOF, 3D-TOF. To better characterize PLD, abdominal CT scans with contrast enhancement and volumetric assessment of the liver were performed in all patients. Laboratory workup included liver function parameters (albumin, total bilirubin, conjugated bilirubin, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, ammonia, blood coagulation tests), kidney function parameters (urea, creatinine, uric acid), complete blood count, inflammatory markers and tumoral markers (alpha-fetoprotein, CA 125, CA 15.3, CA 19.9, CEA). Liver disease severity was measured using the MELD score, while kidney function was measured using serum creatinine levels and creatinine clearance (Cockcroft-Gault Equation).

Patients were included only if an informed consent form was signed and if the inclusion and exclusion criteria were respected (see Table 1). The study protocol was approved by the Ethics Committee of Fundeni Clinical Institute.

Table 1: Inclusion and exclusion criteria used for patients in our study.

Exclusion criteria	Inclusion criteria
Patients diagnosed with other cystic lesions of the liver (e.g., Caroli disease)	Age > 18 years-old
Patients diagnosed with isolated polycystic kidney disease	Diagnosis of polycystic liver disease ± polycystic kidney disease

Relevant medical information (imaging results, laboratory test results) was accessed using the hospital's informatic system Hipocrate. The patient database was created with Microsoft Office 365.

RESULTS

93% of the patients were females (n=14). The mean age of patients was 53 ± 5 years old. The mean age at the time of PLD diagnosis was 38 ± 12 years old. The majority of patients (60%, n=9) had PLD associated with AD-PKD (see Figure 1). The mean hepatic volume as assessed by abdominal CT volumetry was 7406.38 ± 2939.83 cm3. The mean MELD score was 8 ± 2 points (patient range: 6-12 points). The mean creatinine level was 1.0 ± 0.3 mg/dL (patient range: 0.68 – 1.72 mg/dL). Only two patients had serum creatinine above the upper normal limit and were diagnosed with chronic kidney disease in the setting of polycystic kidney disease. The mean level of alkaline phosphatase was 102 ± 47 units/liter, while the mean level of gamma-glutamyl transferase was 70 ± 70 units/liter. Two patients benefited from a kidney transplant in the past, and one of them received a liver transplant as well.

Figure 1: Proportion of patients with isolated versus mixed hepatic-al polycystic disease

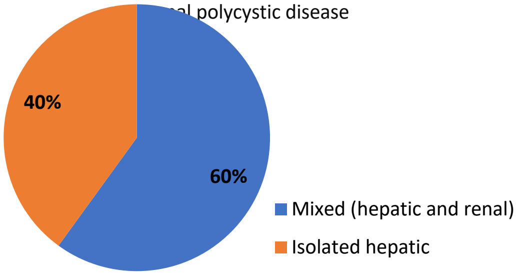

Clinical neurological exam was normal in almost all patients. One patient was deaf-mute, another patient had a positive left-sided Lasègue's sign and diminished Achilles reflexes, and another patient had diminished Achilles reflexes. Apart from these findings, all neurological examinations were unremarkable. Regarding brain MRI abnormalities, unruptured cerebral aneurysms were found in only one patient (in whom three aneurysms were described). However, it should be mentioned that this patient was diagnosed with autosomal-dominant polycystic kidney disease, and not with isolated polycystic liver disease. Of the three aneurysms, the first two were saccular: one measured 3 mm and involved the M1 segment of the left middle cerebral artery, and the other one measured 9.7 mm and involved the M1 segment of the right middle cerebral artery. The third aneurysm was fusiform, had a diameter of 5.5 mm, and involved the right vertebral artery. The overall prevalence of cerebral aneurysms in our patient group was 7%.

Nonspecific supratentorial demyelinating lesions were described in the majority of patients (73%) (see Figure 2). More than a quarter of patients presented with filiform (hypoplastic) posterior communicating arteries. Dilated perivascular spaces were described in 47% of patients (n=7).

DISCUSSION

The prevalence of isolated polycystic liver disease (PLD) varies in the literature. While autosomal dominant polycystic kidney disease (AD-PKD) affects up to 0.2% of the population, the frequency of isolated PLD is much lower (under 0.01%) [7]. Some authors have cited a prevalence of 1 up to 9 cases per 100 000 persons for isolated PLD [8].

On the other hand, the hepatic cystic disease is one of the main extrarenal manifestations of AD-PKD, and its prevalence is age-related, increasing from 20% in the third decade to 70% in the seventh decade [9]. The prevalence of hepatic cysts in patients with AD-PKD may be as high as 83% (if properly sought by MRI), as was highlighted in one study [10]. The number and size of hepatic cysts have been shown to correlate not only with the age of patients but also with female sex, history of pregnancy, the severity of renal cystic disease, and decreased renal function (as measured by the creatinine clearance) [11].

Figure 2: Imaging findings in patients with PLD. Note that cerebral aneurysms were detected in only one patient

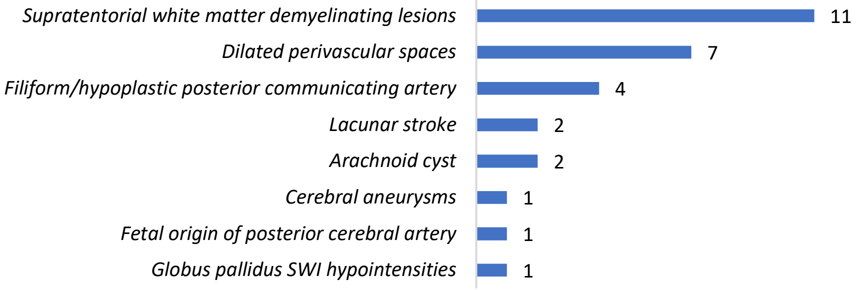

When looking at unruptured cerebral aneurysms, we find an overall prevalence in the general population of approximately 3%, and only a fraction of them progresses toward rupture (with subsequent subarachnoid hemorrhage) [12]. In a systematic review that analyzed 23 studies (comprising more than 56000 patients), the prevalence of intracranial aneurysms varied considerably according to study design and study population, from 0.4% in retrospective autopsy series, to 3.6% in prospective autopsy series, to 3.7% in retrospective angiography series and up to 6% in prospective angiography series [13]. However, when all available evidence was taken into account (including factors that overestimate or underestimate the risk), a frequency of about 2% for cerebral aneurysms was calculated in adults without risk factors for subarachnoid hemorrhage.

The prevalence of intracranial aneurysms in patients with AD-PKD is higher than in the general population [14,15]. Perrone et al mentioned a frequency of about 10% in asymptomatic patients and up to 25% in patients with a family history of intracranial aneurysms or subarachnoid hemorrhage. Likewise, Sanchis et al noted a prevalence of 9% for cerebral aneurysms after evaluating 812 patients with AD-PKD using screening with MR angiography. In another paper, in which 355 patients with AD-PKD underwent screening by 3D-TOF MR-angiography, the prevalence of intracranial aneurysms was found to be 12.4% [16].

Data regarding the frequency of cerebral aneurysms in patients with isolated PLD are lacking and only a few published papers have addressed this issue. In one study, an asymptomatic cerebral aneurysm was found in one out of the 10 patients with PLD who received screening studies of the cerebral vessels. [17]. Schievink et al described an instance of a familial association between PLD and cerebral aneurysms, in which a father with isolated PLD died of a ruptured cerebral aneurysm, while two of his six children also presented brain aneurysms (one of whom was diagnosed with isolated PLD) [18]. There is also a published case of a ruptured cerebral aneurysm associated with bilateral carotid dissection in a normal-weight woman who presented upon clinical examination with an enlarged abdomen. Abdominal CT highlighted a massively enlarged polycystic liver (with only a few cysts in the kidneys), while subsequent genetic analysis was positive for homozygote mutations in the PKD1 gene (for AD-PKD) and ADPLD gene (for PLD) [19]. Aneurysms situated in other parts of the circulatory system have also been described concerning PLD. Such is the case published about a 51 years-old male with PLD, who was discovered to have bilateral popliteal aneurysms [20]. The clinical features of patients with isolated PLD have been compared to those of patients with polycystic liver in the setting of AD-PKD. Patients with isolated PLD have greater numbers and larger sizes of cysts but have a more benign disease course and less associated morbidities than patients with AD-PKD [21]. It remains to be seen if this last statement can be extrapolated to the risk of intracranial aneurysms. Given that their frequency increase with age (both in the general population and in patients with AD-PKD), large prospective studies are needed to assess the prevalence of intracranial aneurysms in patients with isolated PLD.

The rupture rate of cerebral aneurysms in patients with AD-PKD appears to be similar to that of the general population [22]. In the review performed by Cagnazzo et al, the rupture rate, growth rate, and incidence of new aneurysm formation were 0.4% per patient-year, 0.4% per patient-year, and 1.4% per patient-year, respectively.

The main risk factor for the presence of unruptured intracranial aneurysms is a positive family history of intracranial aneurysms. However, most cases of ruptured cerebral aneurysms occur in the absence of a positive family history. In a study discussing the necessity of screening for cerebral aneurysms in patients with AD-PKD, about one-third of nephrologists were in favor of systematic screening for all patients with AD-PKD [23]. However, after reviewing 15 studies (comprising almost 1500 patients with AD-PKD), Zou et al concluded that screening should be recommended only for patients with a positive family history of hemorrhagic stroke or intracranial aneurysm and if they originate from China, Japan, and Europe (since the studies from these countries reported a higher prevalence of aneurysms). The calculated incidences of aneurysm rupture, aneurysm growth, and new aneurysm formation were 0.13%, 1.84%, and 0.57%, respectively [24]. Poor outcomes among treated aneurysms were seen in one study only with the ruptured presentation and did not differ significantly between treatment modalities (open surgery versus endovascular approach) [25].

CONCLUSIONS

In our study, cerebral aneurysms were found in only one patient, who was diagnosed with autosomal-dominant polycystic kidney disease. None of the patients with isolated polycystic liver disease was found to have intracranial aneurysms.

Given these results, and due to the small number of patients in our study, it is hard to appreciate if polycystic liver disease is indeed associated with an increased risk of cerebral aneurysms. Other brain imaging abnormalities were frequent but nonspecific (mainly attributable to vascular-degenerative changes).

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Patient consent for publication:
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Drug Discovery – A New Bioactive Compounds Isolated from Natural Sources

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Abstract: *A rise in mortality due to fungal infections in an immunocompromised population has been observed lately. Nowadays, due to increased fungal infections, the limitations encountered in their treatment like resistance, side-effects, and high toxicity, the rising prescription and overuse of conventional antifungals all stimulate a search for alternative natural drugs therefore we are in dire need of natural newer strategies that involve reliable agents for the treatment of fungal diseases such as essential oils are known for their anti-microbial properties and are multi-component. Materials & Methods: Soil samples (66 samples) were collected from different agricultural fields and animals habitat of Saharanpur (U.P.) Isolation of keratinophilic fungi was carried out by the hair-baiting technique. Extraction of Mentha piperita & Cinnamonum vernum essential oil was carried out by hydrodistillation method and the chemical composition of both extracted essential oils was determined by Gas Chromatography-Mass Spectrophotometry. Antimycotic studies of essential oils were done by the standard disc diffusion method. Results & Discussion: In the present study, the antifungal potential of Mentha piperita & Cinnamonum vernum essential oils were evaluated against three human pathogenic fungi isolated from the soil of agricultural field and animals habitat of Saharanpur (U.P.) i.e Trichophyton mentagrophytes, T.tonsurans, and T.equinum. The chemical composition of Mentha piperita & Cinnamonum vernum EO was analyzed by GC-MS. Menthol (53.28%) was the major compound of the Mentha piperita EO followed by Menthyl acetate (15.1%) and Menthofuran (11.18%). Major constituents of Cinnamonum vernum EO were linalool (8%), (E)-cinnamaldehyde (7.2%), β-caryophyllene (7.4%), eucalyptol (6.4%), and eugenol (5.6%). Conclusion: Essential oils of Mentha piperita & Cinnamonum vernum have been found to have remarkable & excellent antifungal activity against these pathogenic fungi. Present findings conclude that natural products like plant-derived EOs instead of chemotherapy and the emergence of resistance to antifungal drugs can be regarded as an environmental safety mode of disease control against pathogens.*

Keywords: *essentials oils, antimicrobial, drug, resistance, natural, products*

INTRODUCTION

Keratinophilic fungi are a group of microorganisms that can decompose keratin remains in the environment and are pathogenic to humans and animals. The soil that is rich in keratin materials is most conducive for the growth and occurrence of keratinophilic fungi distribution is variable with the environment and depends on different environmental factors such as humans' and animals' presence. Keratinophilic fungi include a variety of filamentous fungi mainly comprising hyphomycetes and several other taxonomic groups [1-2]. Dermatophytes cause humans and animal mycoses and thus have drawn the attention of medical and veterinary epidemiologists. The majority of dermatophytes can live saprophytically and keratinophilic fungi can be considered as a potential pathogen [3]. They occur in many natural and man-made habitats. Keratinophilic fungi are unique in the sense that they require and utilize keratin for growth [4-5]. Studies on keratinophilic fungi are now of considerable significance for their important role in the breakdown of keratinous debris of animals and man in nature and they have a worldwide distribution [6,7]. Studies have shown that soils are important sources of dermatophytes and keratinophilic fungi [8-10]. In the past two decades, the emergence of resistance to various antifungal drugs has accelerated dramatically. Azole-resistant Candida and Aspergillus species are the top pathogens responsible for nosocomial or food-borne infections [11,12]. In addition, the formation of biofilms by Candida species has raised concerns due to their increased resistance to antifungal therapy, and protects the microbial cells within biofilms from the host immune defenses [13,14]. Moreover, EOs especially those with known antibacterial effects have the potential to be used in the food industry as preservatives and to increase the shelf life of products. Therefore, determining the antimicrobial properties of EOs might help to overcome microorganism resistance to antibiotics. The chemical composition of aromatic plants depends largely on individual genetic variability and different plant parts. The presence and concentration of certain chemical constituents of EOs also fluctuate according to the season, climatic condition, and site of plant growth [15]. A review of the literature reveals that many essential oils acquire antifungal activity [16-19]. The development of antifungal resistance is complex and depends on multiple hosts and microbial factors [20]. According to Moreira et al, lipophilic compounds of the oils bond the phospholipid bilayer of the cell membrane increasing its permeability and spreading out the intracellular contents or damaging the enzymatic system of the cell [21-22]. M. piperita oil is one of the most popular and widely used essential oils, mostly because of its main components, Menthol, and menthone [23]. Previous studies have shown antiviral, antibacterial, antifungal, antibiofilm formation, radio-protective, angioedema, analgesic, and antioxidant activities of the EO [24-26]. Cinnamon is a good source of the powerful antioxidant manganese. Therefore, much attention has been paid to the discovery and development of new antimicrobial agents that might act against these resistant microorganisms, and cinnamon could be an interesting candidate [27-28]. Concerning volatile components, the chemical composition of cinnamon essential oils depends on the part of the plant from which they are extracted [29-31]. The presence and concentration of certain chemical constituents of EOs also fluctuate according to the season, climatic condition, and site of plant growth [15,32]. The objective of this work was to evaluate the antifungal efficacy of Mentha piperita & Cinnamonum vernum EOs against drug-resistant three widely pathogenic fungal strains that cause superficial skin infections in humans and trying to find more safely hygienic natural plant products. The goal of this study was to investigate the chemical composition and in vitro antifungal activities of essential oils in the leaves of M. piperita & C. vernum.

MATERIALS AND METHODS

Collection of Soil Samples

Sixty-six soil samples were collected from different agricultural fields and animals habitat of Saharanpur (U.P.) India. For this present study, different soil samples were collected in sterile polyethylene bags and brought to the laboratory for further microbiological analysis. Keratin substrates (Ks) were collected from different sources such as chicken feathers, buffalo hairs, goat hairs, dog hairs, pig hairs, ship hairs, and horse hairs in clean sterilized plastic bags and transferred to the laboratory. Samples were transmitted to the laboratory as early as possible for further analysis like pH, temperature, humidity, salinity, and total dissolved solids.

Isolation of keratinophilic fungi by Hair Bait Technique

Isolation of keratinophilic fungi was done using the hair bait technique (Vanbreuseghem, R. 1952) [33]. Moist chambers were prepared using sterile soil samples. 2-3 cm short strands of sterilized defatted baits were spread over soil samples. 10-15 ml of sterile water was added to the soil to facilitate the germination of fungal spores. Petri dishes were then incubated at room temperature at 28◦C for 15-20 days. Plates were examined periodically for the development of mycelium.

Identification of Keratinophilic fungi

Every single fungal colony was taken from the mixed culture. The pure culture was developed by repeated subculturing which was transferred to an agar slant and subcultured and then stored at 4°Ċ temperature. The fungal colonies were identified by microscopic and macroscopic characteristics. The macroscopic examination of fungi was characterized by duration of growth, surface morphology, and pigment production on the reverse and microscopically, the isolated fungi were stained with Lactophenol cotton blue staining and observe under the microscope at high power of the objective lens.

Gas chromatography Mass Spectrometry (GC-MS) analysis of M. piperita and C. vernum essential oils.

Quantitative and qualitative analyses of the essential oils were performed using a GC-MS apparatus. The analysis was performed with an Rtx 5 MS column. For the GC-MS detection, an electron ionization system with an ionization energy of 70eV was used. Nitrogen gas was used as carrier gas with a flow rate of 1.21 ml/min. The column was raised from 50ºC to 320ºC at a rate of 3ºC/min. The relative percentage of the oil constituents was expressed as a percentage by peak areas and the identification of oil components was based on their retention time with available literature values.

Antimycotic Studies of Essential Oils against Selected Fungi by Disc Diffusion

Oil was screened for its antifungal activity against T. mentagrophytes, T. equinum, and T. tonsurans by disc diffusion method [34]. Standard size Whatman no. 1 filter paper discs 6.0 mm in diameter, sterilized by dry heat at 140°C in an oven for one hour were used to determine antifungal activity. SDA medium for the disc diffusion test was prepared. After sterilization, it was poured into sterilized Petri plates and allowed to solidify. Then one-day old fresh culture of isolated fungi was used for inoculums preparation. A suspension was just turbid (~0.5 McFarland standard) by visual inspection and was prepared by suspending fungus in 0.9% NaCl solution and the homogeneous suspension was used for inoculation. Using a sterile cotton swab, fungal cultures were swabbed on the surface of sterile Sabourauds Dextrose Agar plates. Sterilized filter paper discs were soaked in neat, undiluted (100%, 75%, 50%, 25%) concentrations of oils. Using ethanol-dipped and flamed forceps, oil-saturated discs of 100%, 75%, 50%, and 25% concentration were aseptically placed over Sabourauds dextrose agar plates seeded with the respective test microorganism. The antibiotic discs of Clotrimazole and Ketoconazole (10 mcg per disc) were also aseptically placed over the seeded Sabouraud's dextrose agar plates as a standard drug for comparison of antifungal activity with essential oils. The plates were incubated at 28ºC for 48-72 hours. The diameter of the inhibition zones was measured in mm and the activity index was calculated based on the size of the inhibition zone. Three replicates were kept in each case and average values were calculated.

RESULTS

The present study reported that out of sixty-six soil samples collected, three species of keratinophilic fungi were predominantly isolated from different animal habitats and agricultural fields with different keratin substrates. These habitats contain lots of keratin debris. In the present work, T. mentagrophytes, T. tonsurans, and T. equinum were isolated predominantly from the agricultural and animal habitat soils of Saharanpur (U.P) and also found the main etiological agent of zoonotic dermatophytosis in the investigated area. Macroscopic & Microscopic characteristics of isolated fungi are shown in Table.1. Various earlier workers reported the diverse population of keratinophilic fungi and dermatophytes from Indian soil [35,36]. The high prevalence of keratinophilic fungi from the soil of Saharanpur is due to buffalo hairs, human hairs, and dog hairs which come to the soil either as dead or dropped off serve as substrates, and are subjected to microbial decomposition. The present study indicates that the diverse existence of keratinophilic fungi in the soil of Saharanpur plays a major role in bioremediation in the natural environment. The present study coincides with other workers who also reported diverse soil habitats have been screened from different countries Brazil, Kuwait, Iran, and India indicating that these groups of fungi are distributed worldwide [37-39]. In urban areas, where there are high concentrations of people and animals, soil rich in organic matter may constitute a permanent or occasional reservoir for fungi and during this fungi can be a potential source of skin infection for animals and humans. These animals serve as reservoirs of zoophilic dermatophytes and their zoonotic infections have considerable importance [40-42]. Sandpits are thought to play a role in the epidemiology of human and animals mycoses and reported special care needs to be paid to identify potential pathogenic fungal species such as species from Fusarium, isolated from soil samples of recreational parks and in all elementary schools [43]. Some workers reported that when infections due to other opportunistic fungal agents occur can lead to very unfavorable and even serious outcomes of infection by fungi [44].

Table 1: Macroscopic & microscopic characteristics of isolated fungi

S. No	Isolated Fungi	Macroscopic Characteristics	Microscopic Characteristics
1	*Trichophyton tonsurans*	Colonies on Sabouraud's Dextrose Agar at 28°C grow moderately and the color of the colony is initially white than creamy yellow rose at age with conidial production. Suede-like with radial folds sometimes powdery or velvety. The reverse is a pale lemon yellow to mahogany brown may dark diffusing in the agar.	Many pyriform microconidia on stalks ballon forms. Rare distorted macroconidia. Hyphae are relatively broad irregular, and much branched with numerous septa. Microconidia are varying in size and shape from long clavate to broad pyriform is borne right.
2	*Trichophyton mentagrophytes*	Colonies on Sabouraud's Dextrose Agar at 28°C grow moderately and velvety, flat, and thin with powder and pale powder dry & white powder in color.	Round microconidia in grape-like clusters, Spiral hyphae, and macroconidia narrowly attached to hyphae.
3	*Trichophyton equinum*	Growth rate moderately rapid texture suede-like to downy flat. Thallus color cream to pale yellow deep to pinkish or brown is in the center. Reverse deep yellow becoming red-brown or dark pink may produce brown soluble pigment.	Pyriform microconidia lateral may be produced on stalks. Rare club-shaped macroconidia smooth thin-walled.

The leaves of Mentha piperita and Cinnamonum vernum were collected from Saharanpur (U.P) and essential oil was extracted by Clevenger apparatus based on the hydro-distillation method as shown in Figures 1 and 2. Hence, antimycotic studies and chemical composition of essential oils of Cinnamon & Peppermint were carried out against selected fungi to eco-friendly control fungal infections. In the present study, thirteen (13) compounds representing 99.37% area of the oil, were identified. The qualitative and quantitative compositions of the EO of M. piperita by GC-MS analysis and antifungal activity carried out by disc diffusion method are presented in Tables 2, 3, and Figure 3.

Figure 1: Mentha piperita (Peppermint oil)

M Peppermint

Figure 2: Cinnamon oil (Cinnamomum Verum)

Cinnamon oil

Figure 3: Antidermatophytic activity of M.piperita essential oil at 100% concentration against selected fungal strains

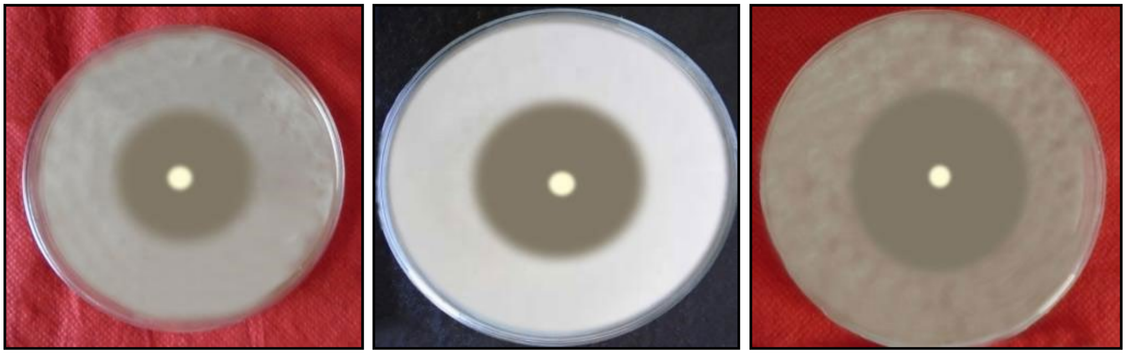

(A) T. equinum (B) T. mentagrophytes (C) T. tonsurans

GC/MS analyses showed that the main constituent of the M. piperita EO was Menthol (53.28%) followed by Menthyl acetate (15.1%) and Menthofuran (11.18%). The compositions of the EOs might be affected by the developmental stages of the plant.

Some authors reported alpha terpinene as the dominant component of M. piperita EO (19.7%) while other previous studies identified Menthol as one of the main constituents of the Eos [23,45]. The higher concentration of Menthol in this study may reflect variations due to the geographical location from which the plants were collected.

In this study, the EOs of Mentha piperita exhibited strong antimycotic activities against T. mentagrophytes (60 mm), T. equinum (67 mm), and T. tonsurans (50 mm) at 100% concentration of pure essential oil than standard drugs.

Table 2: Chemical components (%) of the essential oils distilled from Mentha piperita

Compounds	RI	% oil
α-Pinene	930	0.32
Sabinene	970	0.26
β-pinene-1	978	0.58
β-pinene-2	1035	6.69
Ci$-sabinene hydrate	1072	0.50
Menthone	1155	2.45
Menthofuran	1168	11.18
Neomenthal	1166	2.79
Menthol	1178	53.28
Neomenthy acetate	1274	0.65
Menthyl acetate	1298	15.10
Isomenthyl acetate	1308	0.61
β -Bourbonene	1389	0.37

Table 3: Antifungal activity of Mentha piperita oil against selected isolated fungal strains

S. No	Oil	Test strain	Concentration of oil	IZ of sample
1.	*Mentha piperita Oil (Peppermint oil)*	*T. mentagro-phytes*	100 %	60 mm
			75 %	50 mm
			50 %	29 mm
			25 %	12 mm
2.		*T. tonsurans*	100 %	67 mm
			75 %	40 mm
			50 %	30 mm
			25 %	15 mm
3.		*T. equinum*	100 %	50 mm
			75 %	39 mm
			50 %	29 mm
			25 %	15 mm

IZ = Inhibition zone (in mm) including the diameter of the disc (6 mm).

IZ of Ketoconazole & Clotrimazole (10 mcg/disc) is 32 mm & 30 mm against T. mentagrophytes

IZ of Ketoconazole & Clotrimazole (10 mcg/disc) is 28 mm & 25 mm against T. tonsurans

IZ of Ketoconazole & Clotrimazole (10 mcg/disc) is 24 mm & 22 mm against T. equinum

Similarly second oil i.e. Cinnamonum verum, chemical constituents were identified by GC-MS analysis resulting in the identification of ten (10) chemical compounds for C. vernum essential oil, as indicated in Table 4 and Figure 4.

(E)-cinnamaldehyde (7.2), linalool (8.00%), β-caryophyllene (7.40%), eucalyptol (6.40%), and eugenol (5.60%) were the main components of the C. vernum essential oil. The other important constituents were p-cymene (1.90%), α-humulene (1.70%), δ-cadinene (1.40%), α-pinene (1.30%), and limonene (1.20%). Following our results, several studies have reported that cinnamaldehyde is the major chemical compound of C. zeylanicum bark essential oil [46,47].

Table 4: Chemical components (%) of the essential oils distilled from Cinnamonum vernum

Compounds	RI	% oil
E-cinnamaldehyde	15.22	7.2
α-Pinene Linalool	5.66	1.3
Linalool	9.86	8
β-Caryophyllene	18.58	7.4
Eucalyptol	8.08	6.4
Eugenol	16.90	5.6
α-Humulene	19.47	1.7
δ-Cadinene	20.97	1.4
p-Cymene	7.82	1.9
Limonene	7.93	1.2

Figure 4: Antidermatophytic activity of C.vernum essential oil at 100% concentration against selected fungal strains

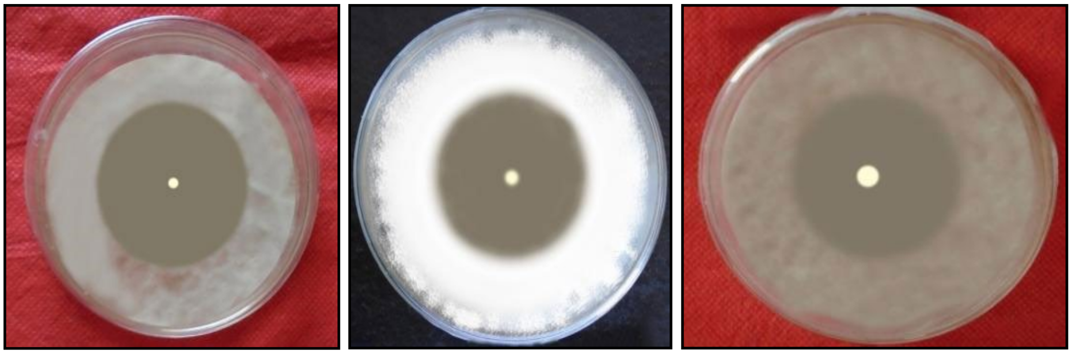

(A) T. equinum

(B) T. mentagrophytes

(C) T. tonsurans

Antimycotic activity of C. vernum essential oil was also evaluated by disc diffusion method and found excellent inhibition of zone at 100% concentration of pure essential oil against T. tonsurans (60 mm), T. mentagrophytes (59 mm) & T. equinum (58 mm) as compared to standard antifungal drugs i.e. Ketoconazole & Clotrimazole (Table 5).

Table 5: Antifungal activity of Cinnamonum vernum oil against selected fungi

S. No	Oil	Test strain	Concentration of oil	IZ of sample
1.	*Mentha piperita Oil (Peppermint oil)*	*T. mentagro-phytes*	100 %	59 mm
			75 %	50 mm
			50 %	30 mm
			25 %	20 mm
2.		*T. tonsurans*	100 %	60 mm
			75 %	46 mm
			50 %	31 mm
			25 %	19 mm
3.		*T. equinum*	100 %	58 mm
			75 %	45 mm
			50 %	31 mm
			25 %	19 mm

IZ = Inhibition zone (in mm) including the diameter of the disc (6 mm).

The traditional use of plants as medicines provide the basis for indicating which essential oils may be useful for specific medical conditions. It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds. The present findings are similar to Wuthi-udomlert et al. (2000) who reported the antifungal activity of turmeric oil against 29 clinical strains of dermatophytes and in the screening of turmeric oil, the diameter of the inhibition zone was found to vary from 26.1 mm to 46 mm against 29 clinical strains of dermatophytes [48].

Numerous scientific studies proved the inhibitory effect of essential oils against different fungi [49]. It is important to analyze the plants which have been used in the medicines as a potential source of normal antimicrobial compounds. The present work coincides with the work of Sharma et al. (2011) who also reported the additive and inhibitory effect of Curcuma longa (Turmeric) and Zingiber officinale (Ginger) essential oils against dermatophytes causing superficial skin infections [50]. The use of essential oils in the treatment and prevention of infection has been in demand in the field of research from the past. Natural products have served as a research resource for most drugs, providing a basis for chemical research and the discovery of new drugs [51].

Several reasons have been offered to explain the success of natural products, among them are their great chemical diversity, the effects of evolutionary pressure in creating biologically active molecules, and the structural similarity of protein targets in different species, among others [52]. The treatment of fungal disease is limited and part of the reason is due to the limited spectrum of the currently antifungal drugs and the expensive treatment particularly due to the need for prolonged therapy. Jayaprakasha et al., (2003) also reported some alternative therapies including natural products are necessary to control fungal infections [53].

Various workers have reported that due to the high volatility and lipophilicity of the essential oils, they are readily attached to penetrate into the cell membrane to exert their biological effect. Ali & Ali (2019) in Saudi Arabia investigate the antifungal activity of plants from the Jeddah region (A. indica, Z. spina-christi, and O. europaea), P. dactylifera seed, neem oil, and other oils [54]. Another interesting investigation reports the antibacterial activity of cinnamon bark essential oil and its main constituents, trans-cinnamaldehyde and eugenol against Cronobacter sakazakii and C. malonaticus, which are opportunistic pathogens that cause infection in children and immunocompromised adults. Kizil et al (2010) reported the EOs exhibited fungistatic and fungicidal activities against both the standard and clinical strains of Candida species at concentrations ranging from 0.5 µL/mL to 8 µL/Ml [55].

According to the study of Saharkhiz et al, (2010), essential oils killed the standard strain of C. neoformance is a well-known primarily opportunistic pathogen that produces chronic and life-threatening meningitis at a concentration of 4 µL/mL [56]. The antifungal activities of the essential oils (EOs) of Acantholippia seriphioides, Artemisia mendozana, Gymnophyton polycephalum, Satureja parvifolia, Tagetes mendocina, Lippia integrifolia, collected in the Central Andes area, Province of San Juan, Argentina, were investigated against M. gypseum, T. mentagrophytes and T. rubrum and were inhibited by the EOs of G. polycephalum, L. integrifolia, and S. parvifolia, with minimum inhibitory concentrations (MICs) between 31.2 and 1000 µg/ml.

This study shows that these Central Andean area species might be used to treat superficial fungal infections [57]. The study of Agarwal et al. (2008) and Saharkhiz et al. (2010) found that the formation of biofilm by C. albicans was inhibited completely at a concentration of up to 2 µL/mL in a dose-dependent manner [56,58]. Shiming et al (2006) reported antifungal mechanism of essential oils, inhibits the synthesis of ergosterol in fungal and cholesterol in mammalian cells [59]. The present results showed the excellent antidermatophytic efficacy of M.piperita and C.vernum against T. mentagrophytes. T. tonsurans and T. equinum as compared to standard antifungal drugs and can be used to control the superficial fungal infections of the skin as an eco-friendly agent and to combat the mechanism of antimicrobial drug resistance.

The tested Essential oil in the present study was rich in Menthol. It has been shown that this phenolic monoterpene has a hydroxyl group around the phenolic ring and exhibits its antimicrobial activity through the disruption of the cytoplasmic membrane due to the main characteristics of EOs is their hydrophobicity, which enables their incorporation into the cell membrane. [60,61]. The great importance of natural products in developing new therapeutic tools is evident. In this aspect, medicinal plants and their derivatives are important for pharmacological research and drug development. Special attention has been directed to natural derivatives, based on the knowledge of antifungal compound production in nature.

CONCLUSIONS

The polluted area of agricultural fields of soil is a dynamic medium in which a large number of keratinophilic and non-keratinophilic fungi live in close association. Some keratinophilic fungi are pathogenic to man and animals. Dermatophytic infections are highly common and endemic diseases in developing countries due to poverty, improper health facilities, illiteracy, and other poorly developed diagnostic techniques. Also, some microorganisms are resistant to newer or modern antifungal synthetic drugs and in some severely immunosuppressed patients. M.piperita and C.vernum essential oils can be suggested as an alternative to synthetic antibiotics, especially for the treatment of antibiotic-resistant infections. These EOs can also be considered for developing products to control fungal infections. Thus, there is a need for an alternative therapy that is safe, cheaper, economical & easily available so a wide range of essential oils from plants are still unexplored for their antifungal activity. Hence, the present study concludes to explore new antifungal agents alternative to synthetic drugs for the treatment of dermatophytic & other fungal diseases. This research study will be beneficial for human health, the human economy, environment safety, and antimicrobial drug resistance for proper and timely treatment of fungal infections in immunocompromised patients.

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ORIGINAL ARTICLE

Comparison of Serum Vitamin D Levels with Glucose, Lipid, and the Other Biochemical Parameters: A Cross-Sectional Study

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Abstract: *Since vitamin D is synthesized from cholesterol, it is claimed to be associated with insulin resistance, suggesting that there may be an association between vitamin D glucose and lipids. For this reason, we aimed to investigate a relationship between vitamin D and biochemical parameters, including glucose and lipids. This cross-sectional study included 198 patients .admitted to the family medicine outpatient clinic between June and December 2016. There was no significant correlation between vitamin D and serum glucose, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, calcium, magnesium, sodium, TSH, and body mass index values in both men and women. There was a correlation between vitamin D and total cholesterol and LDL cholesterol levels in the younger subjects below 41 years (p=0.002). Regression analysis with other variables showed no statistical significance between vitamin D levels on LDL-C levels (P=0.309). Despite previous studies, our study did not confirm the effects of vitamin D on serum glucose, lipid, calcium, and other biochemical parameters. However, these results suggest that the standard up-to-date literature showing the relationship between vitamin D and various metabolic and hormonal disorders may need to be confirmed by new large-scale studies.*

Keywords: *vitamin D; glucose; lipid profile; calcium; biochemical parameters*

INTRODUCTION

The prevalence of vitamin D deficiency is reported to be between 30% to 50% globally in the general population. Similarly, in Turkey, this ratio was found to be 51% in Ankara and 46.9% in İzmir [1-3]. Also, in a study conducted among female university students in Istanbul, vitamin D. levels were found below normal values in 92.4% of the participants [4]. A study conducted in Konya detected vitamin D deficiency in 88,4% of the patients admitted to the family medicine outpatient clinic for any reason [5].

Vitamin D is usually found in foods from animal sources in the form of cholecalciferol and small amounts in plant sources in the form of ergosterol. It is primarily synthesized in the skin under the influence of sunlight [6,7]. Cholecalciferol is synthesized in the epidermis from 7-dehydrocholesterol by the effect of ultraviolet B rays or can be taken with food. First, it transforms into 25-hydroxycholecalcidiol in the liver and then into 1.25-hydroxycholecalcitriol, the active form, in the kidneys [8]. Vitamin D deficiency paves the way for diseases such as rickets in children and osteomalacia in adults [9,10]. It has been suggested to prevent colorectal and prostate cancers and the formation of disorders such as prediabetes and metabolic syndrome by decreasing insulin resistance when taken at high doses [11,12]. In addition, it has been stated that vitamin D deficiency may affect cardiac functions such as diastolic function and aortic elasticity [13].

Vitamin D affects calcium absorption and metabolism by regulating receptor-mediated gene expression [14]. In addition to intestinal and bone tissue, vitamin D receptors are found in many tissues, including the brain, heart, stomach, pancreas, skin, gonads, and T and B lymphocytes. It is accepted that Vitamin D inhibits the proliferation of both normal and hyperproliferative cells and promotes the maturation of these cells through its receptors [7]. Controversial results have been reported on the effects of vitamin D other than calcium metabolism; while some studies suggest a positive effect on the lipid profile, some found no association between vitamin D and lipid parameters [15,16].

We hypothesized that because vitamin D is synthesized from cholesterol and has numerous receptors in tissues, it might interact with biochemical parameters, including glucose and lipids. Therefore, we aimed to investigate the relationships between vitamin D levels and other biochemical parameters, including glucose and lipids.

MATERIALS AND METHODS

Study Design

This study is a cross-sectional study. The sample size was calculated to be at least 162 according to the formula n = t2pq/ d2. In determining the sample size, the p-value was accepted as 0.12, the q-value was 0.88, the d-value was 0.05, α error level was 0.05, and the t-value according to the error level was 1.96.

Population selection

The study included one hundred ninety-eight patients selected from the patient records who applied to the family medicine outpatient clinic for general control between May and October 2016. Individuals who did not use any medication and were found to be healthy were included in the study. Patients with previously defined diseases, such as depression, anxiety, hypertension, diabetes, hyperlipidemia, thyroid dysfunction, vitamin B12 deficiency, liver disease, and kidney disease, were excluded from the study. Demographic data, including age, sex, body mass index (BMI), and biochemistry laboratory parameters, were obtained from patient records. The subjects were then divided into two groups for age using the simple arithmetic mean method.

Statistical analysis

The data were analyzed using SPSS software (PASW Statistics for Windows, Version 16.0. Chicago: SPSS Inc.). Categorical data are expressed in numbers and percentages. Numerical data were expressed as means and analyzed by the Mann-Whitney U test in independent groups. Spearman Correlation analysis was used for correlation analysis. The linear regression test was used to show any relationship between the parameters. The Kolmogorov-Smirnov test was used to determine homogeneity for the variants.

Ethical Considerations

Ethical approval was obtained from the İstanbul Training Research Hospital Clinical Research Ethics Committee (Decision no. 09.08.2019, 1944).

RESULTS

A total of 198 patients aged 18-75 years (41.81±13.43), 71.2% female (141) and 28.8% male (57), were included in the study. The mean age of women was significantly lower than that of men (45.70 versus 40.20). Serum vitamin D, Body Mass Index (BMI), glucose, triglyceride, calcium, and magnesium levels were lower, and serum HDL cholesterol (HDL-C) levels were significantly higher in females compared to males (Table 1). Vitamin D levels had no significant correlation between serum glucose, total cholesterol, triglyceride, HDL-C, LDL cholesterol (LDL-C), calcium, magnesium, sodium, and TSH levels, and BMI values in both groups (Table 2).

Table 1: Comparison of serum vitamin D, glucose, lipid, calcium, magnesium, sodium, TSH, and BMI parameters between women and men

Parameters	Min-Max	Total	Women	Men	Z	p
		Mean ± SD (n)				
Vitamin D (ng/ml)	4.4-89.9	18.49±11.01(198)	16.92±9.67(141)	22.38±13.28(57)	-3.87	0.001*
Age (years)	18-75	41.81±13.43(198)	40.20±13.30(141)	45.79±13.04(57)	-2.88	0.004*
Glucose (mg/dl)	72-188	96.10±15.20(198)	93.28±11.32(141)	103.07±20.58(57)	-4.24	0.001*
Cholesterol (mg/dl)	99-331	207.72±44.07(198)	209.12±44.21(141)	204.25±43.91(57)	-0.54	0.585
Triglyceride (mg/dl)	29-417	118.78±65.32(198)	103.91±46.17(141)	155.56±88.02(57)	-3.93	0.001*
HDL-C (mg/dl)	28-104	53.54±12.63(198)	56.75±12.72(141)	45.60±8.18(57)	-6.18	0.001*
LDL-C (mg/dl)	44-215	131.44±35.97(198)	132.67±36.82(141)	128.41±33.92(57)	-0.35	0.720
Calcium (mg/dl)	7,6-10,4	9.46±0.46 (129)	9.40±0.42 (93)	9.60±0.51(36)	-2.63	0.008*
Magnesium (mg/dl)	1,0-2,8	1.99±0.19 (138)	1.97±0.20 (96)	2.04±0.17(42)	-2.11	0.034*
Sodium (mmol/L)	133-143	139.14±1.98(116)	139.00±1.97(83)	139.48±1.98(33)	-1.46	0.142
TSH (mIU/L)	0.3-5.1	1.79±0.96(140)	1.81±0.98(98)	1.73±0.94(42)	-0.42	0.672
BMI	16-40.4	25.84±4.40(158)	25.45±4.49(109)	26.71±4.10(49)	-2.05	0.040*

*SD: Standard Deviation; BMI: Body Mass Index; *Mann-Whitney U test is significant at 0.05 level (2-tailed); Min: minimum value; Max: maximum value*

When age groups were compared, serum glucose, total cholesterol, triglyceride, LDL-C, sodium levels, and BMI scores were statistically lower in young individuals below 41 years of age versus the elderly group over 42 years old. There was no difference between age groups regarding vitamin D, HDL-C, calcium, magnesium, and TSH levels (Table 3).

Table 2: Correlation of vitamin D3 with biochemical and other parameters

Vitamin D	**Total**	**Women**	**Men**
	r_s value; p-value (n)		
Age (years)	0.103; 0.148 (198)	0.058; 0.493 (141)	0.036; 0.790 (57)
Glucose (mg/dl)	-0.045; 0.525 (198)	-0.144; 0.089 (141)	-0.197; 0.143 (57)
Cholesterol (mg/dl)	-0.125; 0.079 (198)	-0.221; 0.153 (141)	-0.129; 0.340 (57)
Triglycerides (mg/dl)	-0.90; 0.208 (198)	-0.161; 0.056 (141)	-0.257; 0.053 (57)
HDL-C (mg/dl)	0.000; 0.993 (198)	0.148; 0.079 (141)	0.119; 0.378 (57)
LDL-C (mg/dl)	-0.090; 0.208 (198)	-0.111; 0.189 (141)	-0.029; 0.831 (57)
Calcium (mg/dl)	0.108; 0.223 (129)	0.058; 0.582 (93)	0.051; 0.769 (36)
Magnesium (mg/dl)	-0.007; 0.930 (138)	-0.058; 0.576 (96)	-0.054; 0.732 (42)
Sodium (mmol/L)	0.019; 0.838 (116)	-0.029; 0.796 (83)	0.46; 0.798 (33)
TSH (mIU/L)	0.003; 0.975 (140)	0.014; 0.888 (98)	0.013; 0.935 (42)
BMI	0.021; 0.792 (158)	-0.014; 0.882 (109)	-0.171; 0.240 (49)

*r_s value: Spearman correlation; BMI: Body Mass Index; *Correlation is significant at 0.05 (2-tailed).*

Table 3: Comparison of serum vitamin D, glucose, lipid, calcium, magnesium, sodium, TSH, BMI, and parameters according to age groups

Parameters	***< 41 years***	**> 42 years**	**Z**	**p**
	Mean ± SD (n)			
Vitamin D (ng/ml)	18.35±10.78(92)	18.61±11.39(106)	-0.19	0.847
Glucose (mg/dl)	90.64±7.61(92)	100.83±18.30(106)	-5.42	0.001*
Cholesterol (mg/dl)	188.13±38.97 (92)	224.72±41.23 (106)	-6.24	0.001*
Triglyceride (mg/dl)	94.98±53.16 (92)	140.20±67.08 (106)	-5.85	0.001*
HDL-C (mg/dl)	53.65±12.32 (92)	53.44±12.96 (106)	-0.36	0.718
LDL-C (mg/dl)	115.17±31.67 (92)	145.57±33.54 (106)	-6.34	0.001*
Calcium (mg/dl)	9.46±0.49 (68)	9.46±0.42 (61)	-0.09	0.923
Magnesium (mg/dl)	1.97±0.19 (73)	2.01±0.19 (65)	-1.85	0.064
Sodium (mmol/L)	138.41±1.95 (58)	139.86±1.74 (58)	-3.81	0.001*
TSH (mIU/L)	1.81±0.98(70)	1.73±0.94(70)	-0.22	0.819
BMI	24.23±3.89 (81)	27.55±4.28 (77)	-4.80	0.001*

*SD: Standard Deviation; BMI: Body Mass Index; *Mann-Whitney U test is significant at the 0.05 level (2-tailed).*

Table 4: Correlation of vitamin D with biochemical and other parameters in age groups

Vitamin D	***< 41 years***	**> 42 years**
	r_s value; p-value (n)	
Glucose (mg/dl)	-0.061; 0.561 (92)	-0.040; 0.685 (106)
Cholesterol (mg/dl)	-0.251; 0.016 (92)*	-0.086; 0.379 (106)
Triglycerides (mg/dl)	-0.179; 0.088 (92)	-0.077; 0.430 (106)
HDL-C (mg/dl)	0.133; 0.207 (92)	-0.106; 0.278 (106)
LDL-C (mg/dl)	-0.313; 0.002 (92)**	0.025; 0.796 (106)
Calcium (mg/dl)	0.018; 0.881 (68)	0.213; 0.099 (61)
Magnesium (mg/dl)	0.072; 0.547 (73)	-0.077; 0.541 (65)
Sodium (mmol/L)	-0.037; 0.782 (58)	0.033; 0.803 (58)
TSH (mIU/L)	-0.100; 0.408 (70)	0.101; 0.404 (70)
BMI	-0.071; 0.526 (81)	0.024; 0.883 (77)

*r_s value: Spearman correlation; BMI: Body Mass Index; *Correlation is significant at the 0.05 level (2-tailed) **Correlation is significant at the 0.01 level (2-tailed)*

A statistically significant correlation was found between vitamin D and total cholesterol and LDL-C in the young age group. However, serum vitamin D had no significant correlation with serum glucose, triglyceride, HDL-C, calcium, magnesium, sodium, TSH levels, and BMI scores (Table 4).

A linear regression analysis was performed to determine the vitamin D level affecting LDL-C levels in the younger age group below 41 years old. Variables correlated with LDL-C, including vitamin D, age, glucose, triglycerides, and sex, were added to the model for analysis (Table 5).

According to the regression analysis results, in model one, (R = 0.218, R2 = 0.048, F (1, 90) = 4.91, P = 0.037) and in model two, (R = 0.566, R2 = 0.321, F (4, 86) = 8.652, P < 0.001) were found to be significant. Also, in the regression analysis, the decrease in vitamin D level is associated with 0.048% of the increase in LDL-C. However, when other parameters were included in the analyses, the association of vitamin D level with LDL-C level did not reach statistical significance (P=0.309) (Table 6).

Table 5: Correlation of LDL-C with vitamin D, age, sex, glucose, and triglycerides in age groups

LDL-C	**41 years and below**	**42 years and above**
	r_s value; p-value (n)	
Vitamin D (ng/ml)	-0.313; 0.002 (92)**	0.025; 0.796 (106)
Age (years)	0.294; 0.004 (92)**	0.119; 0.224 (106)
Sex	-0.160; 0.128 (92)	-0.135; 0.168 (106)
Glucose (mg/dl)	0.236; 0.024 (92)*	-0.001; 0.993 (106)
Triglycerides (mg/dl)	0.455; 0.001 (92)**	0.100; 0.390 (106)

*r_s value: Spearman correlation; *Correlation is significant at 0.05 level (2-tailed) **Correlation is significant at 0.01 level (2-tailed)*

Table 6: Regression analysis for the association between LDL-C and vitamin D, age, sex, glucose, and triglycerides in the young age group 41 years and below

Coefficients [a]							**95.0% CI for B**	
	Variable	**B**	**SE**	**Beta**	**t**	**p**	**Lower**	**Upper**
Model 1								
R = 0.218; R^2 = 0.048 F (1, 90) = 4.91; *P* = 0.037	(Constant)	126.92	6.43		19.76	<0.001	114.16	139.69
	Vitamin D	-0.64	0.30	-0.22	-2.12	0.037*	-1.24	-0.04
Model 2								
R = 0.566; R^2 = 0.321 F (4, 86) = 8.652, *P* < 0.001	(Constant)	19.01	35.73		0.53	0.596	-52.04	90.06
	Vitamin D	-0.29	0.28	-0.09	-1.02	0.309	-0.85	0.27
	Age	1.20	0.39	0.28	3.09	0.003*	0.43	1.98
	Sex	-25.70	8.07	-0.33	-3.19	0.002*	-41.75	-9.66
	Glucose	0.83	0.40	0.20	2.11	0.038*	0.05	1.62
	Triglycerides	0.23	0.06	0.38	3.92	<0.001*	0.11	0.34

[a]Dependent Variable: LDL-C; B: Unstandardized Coefficients; Beta: Standardized Coefficients; CI:Confidence Interval; R^2 = R square; SE: Standard Error; Linear regression analysis is significant for P< 0.05 level (2-tailed)

DISCUSSION

In our study, serum vitamin D, calcium, and magnesium levels were lower in women than in men, whereas HDL-C was higher in women. In addition, serum glucose, total cholesterol, triglyceride, LDL-C and sodium levels, and BMI scores were lower in young individuals than in the elderly. These findings show that the population evaluated within the scope of our study conforms to normal distribution. In addition, lower serum glucose, triglyceride levels, and BMI scores found in women could be explained by the lower mean age of women.

Findings of no correlation between serum vitamin D with total cholesterol, triglyceride, HDL-C, LDL-C, glucose, calcium, magnesium, sodium, and TSH levels and BMI scores in either men or women in our study contradicts the common view in the literature. In a meta-analysis, it has been reported that vitamin D supplementation decreases total serum cholesterol, triglyceride, and LDL-C levels in patients with type 2 diabetes [15]. Supporting this, in one study conducted on patients with type 2 diabetes, vitamin D supplementation was suggested to reduce serum glucose and triglyceride levels [17]; in another study, higher serum triglyceride levels were associated with lower serum vitamin D levels [18].

Contrary to the findings about vitamin D levels and lower LDL associations, a meta-analysis study found that vitamin D supplementation increased serum LDL-C levels but did not affect HDL-C and triglyceride levels [16]. In a study conducted in the general population in Spain, a statistically significant association was found between total cholesterol and LDL-C levels with vitamin D levels [19]. In another study in Turkey partially similar to our findings, no statistically significant difference was found between total cholesterol, LDL-C, and triglyceride levels compared to vitamin D levels. [20]. Although a negative correlation was found between serum vitamin D and total cholesterol and LDL-C levels in young people in our study, this correlation did not reach significance when other parameters were included.

However, a human epidermal keratocyte cell culture study suggests an indirect effect of vitamin D on cholesterol synthesis, in which total cholesterol synthesis and LDL-C organization were increased in cells exposed to low calcium concentrations [21]. Our study did not support this, and there was no correlation between vitamin D and calcium levels.

The correlation between vitamin D and LDL-C in young people did not persist in the multiple linear regression analysis. In a large-scale study on 11734 individuals from the adult population, LDL-C and calcium levels were significantly higher in the group with high vitamin D levels, and patients with co-morbid situations, type 2 diabetes, hypertension, dyslipidemia, and atherosclerotic heart disease were unlikely to have less Vitamin D deficiency (<20 ng/mL) [22]. However, these studies contradict the widespread belief that vitamin D affects LDL-C and calcium levels.

CONCLUSIONS

As a result, our findings indicate no significant effect of vitamin D on calcium metabolism, and contradictory findings regarding its effects on glucose and lipid parameters suggest that the common view asserting a relationship between vitamin D and several metabolic and hormonal disorders may be inaccurate. Studies suggesting an association between vitamin D and some diseases were usually conducted on limited relevant patient groups. Thus, the margin of error is expected to be high in generalizing the results from limited studies.

For this reason, with the findings of this study and the current literature, it would be necessary to further elaborate on the cause-effect relationships and to conduct new studies.

Conflicts of interest and sources of funding
The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. This research received no external funding.

Authors' contribution
All authors made substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of data for the work.

Ethics approval and consent to participate
Ethical approval was obtained from the İstanbul Training Research Hospital Clinical Research Ethics Committee (Decision no. 09.08.2019, 1944).

Patient consent for publication
Because is a retrospective study, patient consent was not obtained.

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ORIGINAL ARTICLE

The Current State of Knowledge Regarding the Use of Double J Catheters in Treating Obstructive Urolithiasis

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Abstract: *Urinary lithiasis is a common pathology in the modern era. Its significance lies in the possible complications that may arise as well as in its potential for recurrence. The treatment and prevention of recurrences of urinary lithiasis often require the intervention of several specialists: urologists, nephrologists, endocrinologists, nutritionists, biochemists, etc. In the last 20 years, the treatment strategy for urinary lithiasis has changed, with minimally invasive methods replacing laparoscopic or open surgery. These are effective and have rare complications. Whichever treatment method is chosen, it may be necessary to temporarily divert the upper urinary tract by inserting double J catheters for preventive, curative, or palliative purposes. Ureteral catheters have had to be improved over time to avoid two major incidents: their migration and colonization. Various materials were used, varying the shape, size, length, guide as well as approaches. The urinary infection-urolithiasis association is frequent, without always being able to specify the cause-effect relationship. The rate of urinary colonization appears to be influenced by the presence of stent colonization as well as the time since the implant. The association of chronic diseases or emergency insertion is associated with an increased risk of urinary colonization. Complications induced by the time of the double J catheter being implanted are rare, and minor and disappear with its removal. In the case of failure to insert a double J ureteral catheter, the alternative would be to perform an ultrasound-guided percutaneous nephrostomy. Double J ureteral catheter insertion is an effective minimally invasive option in the treatment of obstructive urolithiasis.*

Keywords: *urolithiasis, double J catheter, obstructive urolithiasis, urinary colonization*

INTRODUCTION

Urinary lithiasis is a frequent and often recurring pathology, with a changing evolution (asymptomatic or with a noisy clinical picture). Its incidence is increasing in developed countries where the prevalence in the general population varies between 8 and 10%, tripling in the last 40 years.

By the end of the 19th century, phosphate-uric urinary lithiasis with bladder localization prevailed, and starting from the 20th century the oxalic-calcium urinary lithiasis predominates (70-80% of cases) with renal localization. It mainly affects the population between 20 and 60 years old, with a double rate for men compared to those women.

The recurrence rate in this population is approximately 50% between 5 and 10 years, with approximately 10% of patients presenting multiple episodes of lithiasis recurrence [1,2].

Urinary lithiasis is often revealed by nephrotic colic. Acute nephrotic colic represents about 1-2% of examinations in Emergency Departments, and about 6% of them require hospitalization due to their complications [2,3].

The treatment of a simple, uncomplicated nephrotic colic consists of analgesic treatment (NSAIDs in the absence of contraindications), while a complicated nephrotic colic requires hospitalization and often emergency urinary drainage. The technique is usually chosen according to the size, location, and chemical composition of the stones as well as the morphology of the excretory urinary tract.

Prophylaxis of urolithiasis recurrences requires a general metabolic examination as well as a morpho-constitutional and IR spectrophotometric examination of removed stones.

Urological treatments available today are extracorporeal lithotripsy, rigid or flexible ureteroscopy, and percutaneous nephrolithotomy. They use different energies to destroy the calculus: shock waves, pneumatic ballistic energy, Holmium laser, and ultrasounds. The results of these treatments can sometimes be improved by expulsive medical therapy and post-urotherapy.

The new technological discoveries, their effectiveness, as well as the few complications of urological instrumental treatments in urinary lithiasis have led to a decrease in the indications for surgery (laparoscopic or open). They remain used in case of failure of instrumental treatments or cases of giant stones. Thus, in less than 20 years, the urolithiasis treatment strategy has changed through the use of mini-invasive and highly effective methods [4].

The indications for instrumental urological treatment of urinary stones are:
- symptomatic stones: pain, hematuria, infection;
- complicated stones: urinary obstruction, infection;
- evolutionary stones: the increase of calculus size measured by imaging means;
- stones of significant size: over 1.5 cm for kidney stones and over 6 mm for ureteral stones;
- evolutionary lithiasis disease: the presence of more than two episodes per year;
- infected or infectious stones;
- at the request of the patient;
- in fragile or complex patients (single kidney, kidney failure, heart failure, anticoagulated, etc).

DOUBLE J URETERAL CATHETERS

Regardless of the treatment method approach for urinary lithiasis, it may be necessary to insert a double J endo-prosthesis or catheter for a defined period in case of complete obstruction or secondary infection [5].

Today, the use of double J ureteral catheters is common practice in urology departments.

According to medical dictionaries, a stent is defined either as a device used to maintain an opening, cavity, or contour or as a catheter with a tubular structure intended to maintain an open lumen. The word stent became common in the specialized literature when Montie explicitly defined it as "an intraluminal device to maintain patency of the ureter until healing occurs" [6].

The concept of ureteral stent appears as an adjunct to open surgery to achieve drainage of the upper urinary tract ensuring the continuity of the ureters. In the 19th century, Gustav Simon places a tube in the ureters during a cystostomy and in 1839 Joaquin Albarrano creates the first ureteral catheter made of varnished textile material. In 1952, Tulluch described the use of polyethylene tubes to "repair" ureters and fistulas, and in 1967 Zimskind endoscopically inserted the first silicone ureteral catheter. Their main defect seems to be the migration of the catheters in both directions, and to eliminate it, in 1978 Finney describes for the first time the double J ureteral catheter, provided with loops at both ends [6].

The JJ ureteral catheter used today is an auto-static, flexible tube made of polyurethane or silicone, with curved ends to be able to maintain it between the kidney at the upper extremity and urinary bladder at the lower extremity [7,8,9].

Double J ureteral catheters must comply with the following conditions:
- to be radio-opaque for good visibility on radiography;
- to be provided with visible marks during endoscopy for good positioning;
- have a low surface tension to limit friction during insertion;
- to be inserted anterograde or retrograde;
- to be simple to insert, change, extract, biocompatible and resistant;
- to have a good caliber for urine drainage and at the same time a low risk of obstruction;
- have a low price.

Regardless of the purpose of its insertion (preventive, curative, palliative) or its indications, the double J catheter must ensure the derivation of the upper urinary system by physiological pathways and the flow of urine from the kidney to the bladder [10,11]. It suppresses the pressure due to the lithic obstruction eliminating the pain and by increasing the caliber of the ureter facilitates the migration of stones or their extraction by ureteroscopy.

The indications of the double J catheter are:
- ureteral obstruction by calculus responsible for nephrotic colic;
- preventively, before extra-corporeal lithotripsy to avoid possible obstruction with residual fragment;
- in the case of complicated pelvic surgeries (urological, digestive, gynecological);
- in the case of extrinsic compression of the ureter (tumors, fibrosis, etc.);
- in congenital anomalies, trauma, or iatrogenic insults.

Over time, methods have been sought to decrease the colonization rate of ureteral catheters by using different materials (silicone, polyethylene, polyurethane, biodegradable materials) or by covering the catheters with thin protective films (heparin, silver, polytetrafluoroethylene, antibiotics) [12]. Some urease inhibitors have been used to reduce and prevent stent encrustation or obstruction [13].

In the case of urolithiasis with complete obstruction, there is an indication for the derivation of the upper urinary system by inserting double J catheters, and in case of failure, a percutaneous nephrostomy guided by ultrasound will be performed [14]. This more aggressive technique aims to obtain a kidney without residual fragments at the end of the intervention.

The catheters vary in size (from 3 to 9), length (between 12 and 30 cm), guide (simple metal, Teflon, lubricated polyurethane monofilament), material (polyvinyl chloride, silicone) [15,16, 17].

The catheter is inserted under general anesthesia or loco-regional anesthesia [18].

Two approaches can be used [19]:
- naturally through cystoscopy under radiological control;
- percutaneously after renal puncture (nephrostomy) under radiological control.

The J catheter can be kept from a few days to a few months (generally 3 months) depending on the indications. Removing too early can be ineffective and complications (calcifications, obstructions) can occur if kept too long [20].

During catheter indwelling, various unwanted effects can be mentioned [21,22,23]:
- frequent urination (indication of anticholinergics);
- lumbar pain during urination (indication of minor analgesics);
- bladder discomfort (indication of antispasmodics);
- hematuria (indication of correct hydration – 1.5 l/24 h and avoidance of physical efforts);
- urinary infections (indication for antibiotic treatment).

The ablation of the JJ catheter is generally done by cystoscopy, under local anesthesia, or in ambulatory care (takes less than 5 min).

COMPLICATIONS

There is a frequent association between urinary infection and urolithiasis. However, it is difficult to determine if the calculus became infected secondarily or if the infection preceded the calculus and was responsible for its formation.

According to European and American scientific societies, urinary infections are defined by the association of significant bacteriuria with symptoms evocative of a urinary infection in the absence of another infectious focus. Symptoms and clinical signs compatible with a urinary infection can be varied, from functional urinary signs such as pollakiuria, and burning during urination – to non-specific symptoms such as fever, chills, psychomotor retardation, confusion, spasticity, etc. Urinary colonization corresponds to the presence of one or more microorganisms in the urinary tree without generating clinical manifestations. A urinary infection is considered if the clinical signs and symptoms are associated with a bacteriuria higher than 105 CFU/ml (103 CFU/ml for women and 104 CFU/ml for children). It is known that immunosuppressed, pregnant women, alcoholics, and malnourished, patients with chronic renal or neurological diseases have a higher rate of urinary colonization.

The risk of urinary colonization is significantly higher in patients with colonized JJ catheters, especially if the indwelling time is over 45 days.

Complications related to the maintenance of the double-J catheter are rare (hematuria, infection and/or colonization, catheter fragmentation, encrustation) and occur especially with long-term stent indwelling (more than 30 days) and disappear with its ablation.

Failing to remove all residual fragments after instrumental urological treatment of urolithiasis is a major factor of recurrence (50%), even for residual fragments smaller than 2 mm. Under these conditions, any instrumental treatment must be systematically imaging evaluated every 3 months with a reno-vesical CT or ASP + reno-vesical ultrasound.

In the case of residual fragments after a urological instrumental treatment, there are two possibilities:

- expulsive medical treatment: the administration of an alpha-blocker (tamsulosin) would favor the elimination of the fragments and reduce the painful crises associated with their elimination;

- post-urotherapy performed in ventral decubitus and incline at 30o - 45o to favor the elimination of sedimented fragments in the lower calyces.

Any urinary calculus requires a complex examination consisting of a morpho-constitutional analysis and IR spectrophotometry, metabolic balance, dietary survey, hygienic-dietary measures, and possibly medical treatment.

CONCLUSIONS

The treatment strategy for obstructive urolithiasis has changed in the last 20 years with the new high-performance, mini-invasive techniques.

Double J ureteral catheter insertion in obstructive urolithiasis is a minimally invasive option for high urinary tract diversion, regardless of the treatment method chosen for urolithiasis.

The double J catheter can be inserted for different purposes: preventive, curative, and palliative.

The risk of urinary colonization is significantly higher in patients with colonized JJ ureteral stents.

The prevalence of urinary colonization increases with the indwelling time, especially in the case of emergency insertion.

The association of chronic diseases such as diabetes, hypertension, CKD, and disabling neurological diseases is associated with an increased risk of urinary colonization.

In pelvic ureteral lithiasis, spontaneous expulsion of the stone is possible, probably due to ureteral dilatation secondary to the presence of the ureteral prosthesis.

Complications related to the indwelling of the double J catheter are rare and occur especially in the case of long-term indwelling (more than 30 days) and disappear with its ablation.

In case of failure to insert a double J ureteral catheter, the alternative for a high urinary system derivation is an ultrasound-guided percutaneous nephrostomy.

Abbreviations

CKD – chronic kidney disease

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ORIGINAL ARTICLE

Assessment of Low-Doses Doxorubicin Effects in Mice Using Chromosomal Aberration Assay

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Abstract: *The cytogenetic aberrations induced by doxorubicin (DOX) may be influenced by diet. Investigation regarding effects of low doses of DOX on chromosomal aberrations frequency in mice bone marrow. The study explored a possible relationship between diet, follow-up period, and cytostatic doses. Four mice groups received normal or high-fat (pork) diet. At the beginning of the 8th day mice from each group received a single intraperitoneal injection with DOX (dose range: 1.7-7.7 mg/kg b.w.) or isotonic saline solution (0.02 mL/g control mice). In the 10th (groups I, II) and 14th (groups III, IV) days from the beginning of the experiment, the mice were euthanized and chromosome aberrations were tested in bone marrow cells. The body weight of all mice who received DOX decreases, especially at higher doses. In the first three days post-injection, weight loss percentage was significantly influenced by DOX doses and/or type of diet ($p<0.003$). The interaction between the type of diet and DOX doses was not statistically significant in the follow-up period (p interactions >0.05). DOX doses were able to increase the frequency of chromosomal aberrations. DOX acts as a potent inductor of cytogenetic aberrations in bone marrow cells, regardless of the type of diet.*

Keywords: *mice, animals, injections, intraperitoneal, cytostatic agents, Doxorubicin, chromosome aberrations*

INTRODUCTION

Doxorubicin (Adriamycin, DOX) belongs to the anthracycline family of antitumor antibiotics which is prescribed for several decades in a variety of tumors [1-3]. Doses used for chemotherapy can determine serious, multiorgan or systemic, side effects in many patients [1,4] and in animal models of chemotherapy [5,6]. Most specialists consider that its antineoplastic and cytotoxic effects are the consequence of the drug's insertion between DNA strands base pairs (thus interfering with replication and gene transcription), inhibition of topoisomerase II (which predisposes to single and double-strand breaks) [7,8] and generation of reactive oxygen species (ROS) [9-11].

The DNA damage induced by this drug seems to affect preferentially some tissues (e.g. cardiac myocytes) [10,12] and it can represent an early event in doxorubicin-induced death, at least for some types of cells [13,14]. All these effects can be influenced by different factors, doses, rhythms, and routes of DOX administration [15].

Currently, studies performed on animals explore the effects of diet on DOX-side effects. Thus, long-term milk or kefir ingestion performed on old male Sprague Dawley rats may be helpful before and during DOX treatment [16]. Moreover, creatine is involved in managing Dox-induced skeletal muscle side effects [17]. Regarding the fat diet, experimental results revealed that switching to a low-fat diet 1 week before and during DOX treatment, may attenuate the DOX-increased cardiotoxicity [18]. Instead, the high-fat diet may decrease the efficacy of treatment with DOX [19].

Moreover, a hypercaloric diet will not affect only the doxorubicin efficacy, but also has a negative impact on the human body, leading to various human and animal pathologies [20-25].

Taking all these aspects into consideration, our study aimed to test the effects of low-dose DOX on the frequency of chromosomal aberrations in mice bone marrow, and whether these are influenced by diet and follow-up period.

DOX as a chemotherapeutic agent has various side effects in human and animal models. Therefore, the main aim of our study was to explore the effects of low doses of DOX on male mice fed with normal food of pork cracklings (PC).

MATERIALS AND METHODS

Animal care and doxorubicin treatment

Eight to nine weeks old BALBc male mice weighing 21.33±2.19 g were included in this study. They were accustomed to their environment for at least 10 days before the initiation of the experiment. During the experiment, the mice were maintained in the National Institute of Research and Development for Food Bioresources facility, in rooms with 20–24°C, 55–65% humidity, on a 12:12-h dark/light cycle. Mice were housed 2 in plastic cages. They received ad libitum a commercial pelleted food and filtered drinking water. The food and water were changed every other day throughout the experiment. During the experimental period, the hygienic conditions were kept constant. All mice were weighed on the 1st, 8th, and 10th day of the experiment; the mice from groups III and IV were also weighed on the 14th day (Table 1).

Table 1: Study design. NF – normal food, PC – pork cracklings

Days	1-7	8	10	14
Intervention	**Diet**	**Injection**	**Euthanasia**	**Euthanasia**
Groups	NF	i.p.	Group I	Group III
	PC	i.p.	Group II	Group IV

On the first day of the experiment, mice were distributed into 2 groups that received a normal diet (groups NFI, NFIII) and 2 groups that received only pork cracklings – PC (groups PCII, PCIV). Subsequently, the type of diet did not change until the end of the experiment. At the beginning of the 8th day, mice received a single intraperitoneal (i.p.) injection with DOX (2 mg/ml, Actavis). Eight mice from each group received one of the following doses of DOX: 1.7; 1.9; 2.2; 2.6; 3.1; 3.8; 5.1; 7.7 mg/kg b.w. In addition, four mice from each group were considered controls (C) and received a single i.p. injection with isotonic saline solution (0.02 mL/g). On the 10th (groups I, II) and 14th (groups III, IV) days from the beginning of the experiment the mice were euthanized (Table 1).

Cytogenetic analysis

Mice were injected i.p. with an aqueous solution of colchicine (0.05 %) two hours before euthanasia. For each mouse, both femurs were removed and the medullary canal was washed with 1.5 ml of NaCl hypotonic solution (75 mM). Bone marrow cells were re-suspended with a Pasteur pipette and then were centrifuged at 1000 rpm/10 minutes. The supernatant was discarded and the resulting sediment was re-suspended in 1.5 ml NaCl (75 mM). Cells were fixed in a fresh fixative solution (methanol: acetic acid 3:1) and after 20 min storage at 4°C the fixation step was repeated. After fixation, cells were re-suspended in a small volume of the fixative solution and were dropped onto clean slides that were dried at room temperature for 24 h. Slides were stained by Giemsa stain (4%) for 5-10 min and were subsequently washed with tap water and air-dried. Stained slides were coded and scored by light microscopy (at X100 magnification).

The structural and numerical chromosomal aberrations were counted for 100 well-spread metaphase/animal. The numerical (polyploidy) and structural chromosomal aberrations (e.g., deletions, translocations, acentric fragments, gaps, and chromosome stickiness) were counted. Some of these cytogenetic abnormalities (e.g., gaps, stickiness) were scored but they were not included in the total number of aberrations or the subsequent statistical analyses because we considered that they are not good indicators of chromosome damage.

Statistical analysis

Results are reported as means ± SE. The percentage weight gain was calculated as (weight gain/previous weight) x 100. Statistical analysis was performed using R statistical software (version 4.2.0). The cutoff point for significance was Bonferroni/Yates corrected.

RESULTS

Body weight, diet, and treatment

The weight of mice from the four groups was similar at the beginning of the experiment (one-way ANOVA: p=0.23). The change in body weight during the experiment can be influenced by diet and the dose of cytostatic administrated

(Figure 1).

Figure 1: Average body weight of mice (g) for the groups who received DOX (DOX injection – continuous lines) or saline solution (controls – dotted lines). The type of diet is indicated by line color: red lines – NF; blue lines – PC. NF – normal food, PC – pork chops, C – control.

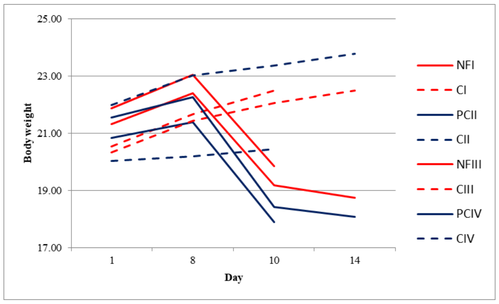

Thus, during the first 7 days of the experiment, the percentage of weight gain was positive but significantly lower, for mice who received PC compared with those who received NF (I, III vs. II, IV: 5.26±1.57% vs. 2.98±1.66%, $p<0.00001$). In the next three days, the percentage of weight gain for control mice remains positive and much lower in PC than in NF (3.36±0.56 vs. 1.41±0.51, p=0.0009).

In the interval between the 10th-14th day, this tendency was maintained, yet the differences were no longer significant (2.03±0.71% vs. 1.76±1.02%, $p>0.05$). The percentage of weight loss in the first three days after administration of DOX was influenced by either dose of cytostatic (one-way ANOVA: p=1.7E-28) (Figure 2 a) and type of diet (groups I, III vs. II, IV: -14.47±4.56 vs. -16.83±4.15, p=0.003) (Figure 1).

Post hoc test reveals significant differences in percentage weight loss (Tukey HSD $p<0.05$) when comparing different DOX doses, except for the two lowest doses (1.7 and 2.9 mg/kg b.w.). For the interval between days 10th-14th, this parameter was neither associated with the dose of cytostatic (groups III vs. IV: -2.25±2.88 vs. -1.83±0.98, p=0.52) nor with the type of diet (Figure 2 b).

The interaction between the type of diet and DOX doses was not significant neither in the first three days after DOX administration (groups I-IV two-ways ANOVA: p interactions = 0.68) nor the following four days (groups III and IV Two ways Anova: p interactions = 0.81).

Chromosomal aberrations

The number of cytogenetic changes and deletions identified on days three (-0.76 and -0.7 respectively) and seven (-0.66) post-injection of DOX has a negative linear correlation with weight change. The frequency of cytogenetic aberrations may be influenced by DOX doses or their interaction with the type of diet, or the post-administration follow-up interval.

Doxorubicin doses between 1.7–7.7 mg/kg b.w. were found to increase the frequency of polyploidy, chromatid deletion, acentric fragments, and Robertsonian translocations (Figures 3 and 4) ($p<0.05$).

Figure 2: Percentage of weight change post-injection of DOX at three (groups I-IV) (a) or seven (groups III-IV) (b) days post-injection. Data are presented as means ± SE for both charts. p< 0.05 for overall difference (one-way ANOVA). Significant differences ($p<0.05$) identified by post hoc Tukey HSD test are indicated by letters [compared with DOX dose 1.7 (a), 1.9 (b), 2.2 (c), 2.6 (d), 3.1 (e) or 3.8 (f) mg/kg b.w.].

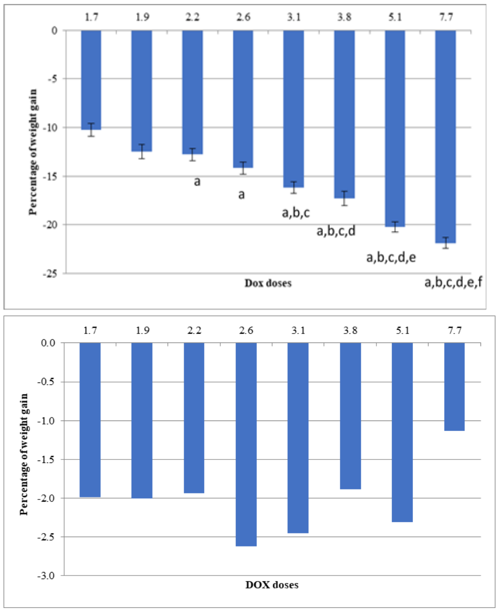

The total number of cytogenetic aberrations (Figure 3a), polyploidies (Figure 3b), and deletion (Figure 3c) showed significant differences between mice treated with different DOX doses (one-way ANOVA $p<0.0003$); the differences were more important when comparing values corresponding to lowest and highest DOX doses (5.1 or 7.7 mg/kg b.w., post hoc Tukey HSD $p<0.05$).

Interactions

A significant interaction between DOX dose and diet was found in the case of polyploidy (two ways Anova p= 4.5E-11); its frequency significantly increased in mice who received the highest DOX doses (5.1 or 7.7 mg/kg b.w.) and PC compared with those who received NF ($p<0.009$) (Figure 4). The interaction between DOX doses and the post-administration follow-up interval was found to not influence the frequency of cytogenetic aberration scored in this study ($p>0.05$).

DISCUSSION

Chromatin remodeling plays an essential role in cell proliferation, differentiation, and DNA repair as a consequence of gene expression regulation. Many studies on different biological models have revealed that chromatin remodeling complexes and enzymes that target histone modifications could sensitize cells to selective cytotoxic stress [26,27]. Investigation of epigenetic, cytotoxic, genotoxic, and/or mutagenic potential of cytostatics is useful for a better insight into the mechanisms of both drugs' actions and adverse effects. The reversal or accumulation of DNA lesions induced by cytostatic play an important role in cell survival and the efficiency of chemotherapy [28,29].

Figure 3: The distribution of an average number of cytogenetic aberrations according to DOX dose. Data are presented as means ± SE for all charts. $p< 0.05$ for overall difference (one-way ANOVA) were detected for the number of cytogenetic ab-errations (a), polyploidies (b), and deletion (c). Significant differences ($p<0.05$) identified by post hoc Tukey HSD test are indicated by letters (compared with 1.7 (a), 1.9 (b), 2.2 (c), 2.6 (d) 3.1 (e), 3.8 (f) or 5.1 (g) mg/kg b.w.).

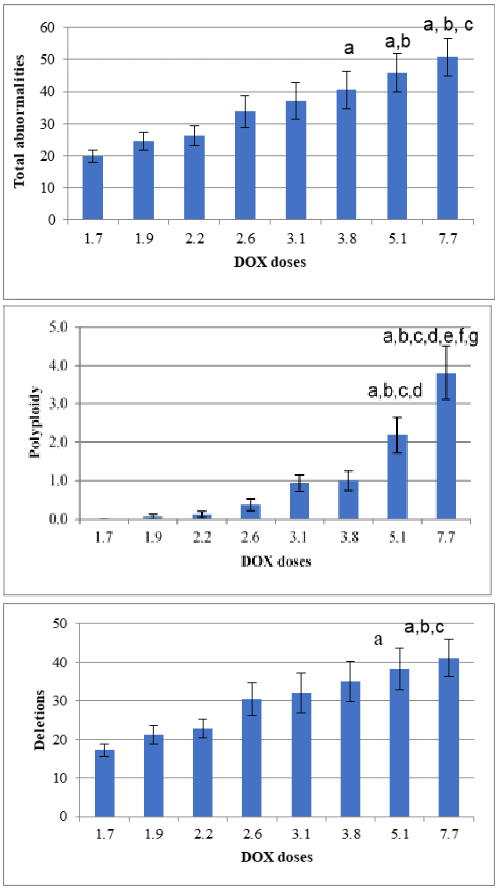

We set out to test the clastogenic effects of eight low doses of DOX in mice bone marrow cells detected after several rounds of cell cycles.

The LD50 is related to the particularities of both expo-sure and testing systems [15]. The LD50 values for adult mice treated i.p. with DOX were estimated to be in the range of 11.98 [30] and 56.87 mg/kg b.w. [31].

Figure 4: The distribution of an average number of polyploidies according to DOX dose and type of diet. Data are presented as means ± SE for all charts. $p< 0.05$ for overall difference (two-way ANOVA) were detected. NF – normal food, PC – pork chops

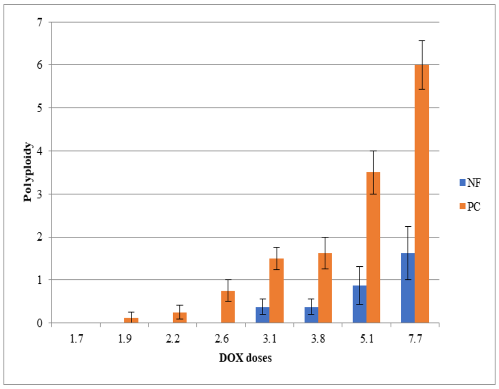

DOX can promote localized tissue damage at the site of administration that can interfere with the absorption of the subsequent doses of the drug [32]. After exposure to high doses of cytostatic, some animals died [31] or exhibited multiple tissue toxicity [33-35]. In an attempt to mitigate some of these effects, we decided to test the impact of a single dose of cytostatic administrated i.p. to each mouse. The tested doses (the range: 1.7-7.7 mg/kg b.w.) were comparable lower to those to which human patients [36] or mice [37] are frequently exposed (20 mg/kg b.w.).

The body weight change registered during the experiment in the four groups reflects the compliance of the mice with the type of diet and the effects of the cytostatic.

In the first week of the experiment, the percentage of weight gain was positive yet much lower in mice that received PC than in those who remained on a normal diet ($p=0.00001$). The control mice kept this trend in the interval between the 8th-10th ($p=0.0009$) and the 10th-14th day ($p>0.05$). These results suggested that PC was not well appreciated by mice, at least in the first 10 days of the experiment (Figure 1).

The body weight of all mice decreases after DOX administration, especially at higher doses of cytostatic. In the first three days post-injection, but not in the next four days, the percentage of weight loss was significantly influenced by doses of cytostatic (Figure 2a) and/or type of diet ($p<0.003$) (Figure 1). The interaction between the type of diet and DOX doses was not statistically significant in the follow-up period (p interactions >0.05).

Our results are in accordance with previous studies in which i.p. administration of DOX was associated with a decrease in food and water consumption [38] and a decline of mice body weight to over three days [39]. The impact of DOX on patients' body weight was also reported [40]. The effects of DOX at the nuclear level may reflect the ability of cytostatic to alkylate DNA, to inhibit DNA denaturation, replication, and topoisomerase II activity [41].

The aneugenic and clastogenic [42,43] potential of DOX was shown in different test systems (e.g., human lymphocytes, albino mice) [44,45].

Previous studies performed on mice found that the frequencies of chromatid and chromosome type aberrations detected during the first 24h post-administration were directly correlated with DOX doses (3, 12, or 24 mg/kg b.w.) [46,47]. Also, 24 h after administration of two injections with 0.8 mg/kg DOX on consecutive days, chromosome breaks (12.6%), acentric fragments (11.6%), ring chromosomes (8.3%), Robertsonian translocations (4%) and polyploidy (2%) were identified [48].

In our study, especially in the case of higher doses of cytostatic, the frequency of chromatids breaks, polyploidy, and Robertsonian translocations had a higher frequency. Doses tested in this study were associated with a low frequency of acentric fragments. Fragmented chromosomes may be attributed to the clastogenic effects of xenobiotics on DNA strands. Differences between our results and those previously reported can be explained by the comportment of acentric fragments during mitosis and by the number of cell cycles performed by the bone marrow cells during the post-administration follow-up period (1 day vs. 3- or 7- days post-administration).

In a previous experiment with 3 or 12 mg/kg DOX, similar results for breakage (20% or 26%) and cells with translocations (0% and 1%) were estimated [49].

Some cells can become giant, polyploid, and polymorphic after exposure to a low dose of DOX (e.g., HCT116, MCF7). [47,50,51]. Twenty-four hours after 0.8 mg/kg of DOX was injected i.p. in consecutive days, the frequency of polyploidy in the bone marrow cells of mice was 0.2% (47). We observed that polyploidy was more frequent in mice that received PC vs. NF and the differences are more evident at high DOX doses (Figure 4).

Division of polyploid cells may be relevant for the generation of cancer stem cells [52] and repopulation with tumoral cells after therapy [53].

Chromosome stickiness may reflect the highly toxic effects of xenobiotics and it can lead to cell death [54-56]. In our experiment, this abnormality increases with cytostatic doses. Even though the frequency of some aberrations like gaps, complex interchange, Robertsonian translocations, and chromosomal stickiness seems to increase when reaching the end range of dosage, there are no significant results that would imply a correlation between dose and frequency for aforementioned aberrations.

Limitations of the study

Some of the DNA lesions induced by mutagens can lead to apoptosis or can be fixed. It was estimated that in 12 h after DOX treatment, some bone-marrow cells went through two cell cycles [46]. Consequently, in our experiment during the post-administration period, some cells have come through a significant number of cell cycles (up to 28). During these cycles, the chromatid deletion could be converted into chromosomal deletion and some acentric fragments can be lost. Thus, the results we have obtained using conventional cytogenetic analysis highlight only a limited spectrum of cytogenetic modifications induced by DOX.

CONCLUSIONS

A brutal shift of diet from normal feed to high fat (pork cracklings) is associated with significant weight loss. The main conclusion which emerges from our study is that treatment of mice with low drug doses significantly increases the number of chromosomal aberrations compared with controls. These aberrations are an indication of DOX toxicity for the bone marrow cells and their clastogenic impact. This clastogenic potential of DOX was not influenced by diet.

The frequencies of polyploidy and deletions were significantly higher in mice who received 5.7-7.1 mg/kg and those treated with lower doses. In addition, our results suggested that some cytogenetic damage induced by low doses of DOX can be retained even three – or seven – days post-administration.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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Authors' contribution

Conceptualization: RSC, RN, SN, DC, and LB; methodology: RN, RMS, SSD, and OC; software: RMS and MIN; validation: SSD, IR, and MO; formal analysis: RMS, SSD, MIN, and DC; investigation: SSD, MIN, and IR; resources: RN, SN, and LB; data curation: RSC, RN, SN, and DC; writing: RN, RMS, MIN; writing- review and editing, RN, and SN; project administration, LB. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The animal study protocol was approved by the National Institute of Research and Development for Food Bioresources Ethics Committee with registration number 342/16.5.2014.

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ORIGINAL ARTICLE

Relationship between Glycemic Control and Triglyceride-Glucose Index in Patients with Type 2 Diabetes Mellitus

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Abstract: *The triglyceride-glucose index is a newly discovered parameter that indicates insulin resistance. This study aims to investigate the relationship between the triglyceride-glucose index and triglyceride/high-density lipoprotein ratio with glycemic control in patients with type 2 diabetes mellitus. This study was performed retrospectively in 296 patients with type 2 diabetes mellitus. Patients were divided into two equal groups according to their HbA1c levels as having good glycemic control (HbA1c 7 and below) and poor glycemic control (HbA1c above 7). Age, gender, body mass index, fasting blood glucose, HbA1c, total cholesterol, serum triglyceride level, serum HDL level, serum LDL level, triglyceride-glucose index, and triglyceride/HDL ratio were compared between the groups. Fasting blood glucose, HbA1c, triglyceride, HDL, triglyceride-glucose index, and triglyceride/HDL ratio was significantly different between patient groups with good and poor blood glucose regulation (p<0.05). Patients with poor glycemic control had lower fasting blood glucose, HbA1c, triglycerides, triglyceride-glucose index, and HDL values compared to patients with high triglyceride/HDL ratio and good glycemic control. There was no difference between the groups in terms of other variables (p>0.05). Triglyceride-glucose index and triglyceride/HDL ratio may be indicators of poor glycemic control in diabetic individuals. These indices are cheaper, universal, and easy-to-measure parameters compared to HbA1c and can be used to predict glycemic regulation.*

Keywords: *type 2 diabetes mellitus, triglyceride-glucose index, HbA1c*

INTRODUCTION

Diabetes mellitus is a global health problem whose incidence and morbidity continue to increase significantly in all countries of the world.

The most important mechanism in pathogenesis is a decrease in the sensitivity of target tissues to insulin [1]. Persistent hyperglycemia and insulin resistance are associated with long-term damage to organs, particularly the eye, kidney, nervous system, and cardiovascular system [2].

Type 2 diabetic patients have atherogenic dyslipidemia and obesity, which markedly increase the risk of coronary artery disease. Lipid profile changes in these patients increase insulin resistance and insulin resistance-related complications [3].

Regulating glycemic control and reducing insulin resistance reduces diabetic complications. Glycated hemoglobin (HbA1c) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), which are predictors of microvascular and macrovascular complications, are the best-known parameters for retrospective long-term glycemic control and insulin resistance [4].

In several studies, indicators such as Triglyceride/Glucose index, Triglyceride/HDL ratio, body mass index (BMI), waist circumference (WC), and visceral adiposity index have shown promising results in the assessment of insulin resistance [5,6]. In addition, a recent study showed that the triglyceride-glucose index was correlated with HOMA-IR and hyperinsulinemic-euglycemic clamp test [7]. There are also studies showing the association of triglyceride-glucose index with cardiovascular diseases [8,9].

The present study aimed to examine the relationship between HbA1c, which is currently the most commonly used parameter that retrospectively shows glycemic blood control, and the triglyceride-glucose index. In addition to HbA1c, the relationship between the height-weight index and the triglyceride-glucose index was also examined.

MATERIALS AND METHODS

Study Design

The study was conducted retrospectively with a total of 296 patients with diabetes mellitus 2 who applied to the Internal Medicine outpatient clinic of Mersin Şehir Training and Research Hospital between 20.06.2021 and 20.06.2022. Patients with type 2 diabetes mellitus were selected from those who met the American Diabetes Association criteria. Patients with coronary artery disease, cancer, rheumatologic diseases, type 1 diabetes mellitus, pregnant women, patients with chronic kidney disease, patients with chronic liver disease, patients with autoimmune diseases, patients with hypothyroidism or hyperthyroidism, patients receiving anti-hyperlipidemic therapy, and patients with diabetic complications were excluded.

Demographic data of patients such as height, weight, duration of diabetes, age, and smoking status were obtained from the electronic information system. Body-mass index (BMI) was calculated by weight in kilograms divided by the square of height in meters. Regarding previous studies, the TyG index was calculated separately for each individual with the formula = ln [fasting TG (mg/dL) x fasting glucose (mg/dL)/2] [6,10]. Serum triglyceride level (mg/dL) was divided by serum HDL level (mg/dL) to obtain the Triglyceride/HDL ratio (THR). Fasting plasma glucose, HbA1c, and lipid profile were studied from venous blood samples obtained after fasting for at least 8 hours.

The 296 patients included in the study were divided into two equal groups: patients with good glycemic control (N=148, HbA1c:7 and below) and patients with poor glycemic control (N=148, HbA1c above 7). Age, gender, duration of diabetes, smoking status, body mass index, fasting blood glucose, HbA1c, total cholesterol, serum triglyceride level, serum HDL level, serum LDL level, triglyceride - glucose index, and triglyceride/HDL ratio were compared between the groups.

Ethics committee approval was obtained from Mersin University Ethics Committee before starting the study (dated 20.07.2022 and numbered 2022/491).

Statistical Analysis

Shapiro-Wilk test was used to test whether the variables conformed with normal distribution. Normally distributed variables were presented as mean±standard deviation and an independent sample t-test was used for comparisons between two independent groups. Non-normally distributed variables were presented as median (minimum-maximum). Mann-Whitney U test was used to compare non-normally distributed variables between two independent groups and the Kruskal Wallis H test was used for comparisons between three independent groups. Categorical variables were presented as frequency and percentage values (n(%)) and the Pearson chi-square test was used for intergroup comparisons.

Statistical analyses were performed in IBM SPSS Statistics 22.0 program. P<0.05 was accepted as statistically significant in all analyses.

RESULTS

A total of 298 patients were included in the study. The median age was 57 (33-75) years in the good glycemic control group and 56 (27-75) years in the poor glycemic control group. Basic demographic data and biochemical blood parameters of the patients included in the study are shown in Table 1.

Although the duration of diabetes was less than 20 years in both groups, there was a statistically significant difference between the groups (P <0.001). There was no statistically significant difference between the good and poor glycemic control groups in terms of gender and age (P= 0.201, 0.390). There was no statistically significant difference between the two groups in terms of smoking (P=0.118). No statistically significant difference was found between the two groups in terms of body mass index (P=0.118).

DM duration, fasting blood glucose, HbA1c, triglyceride, HDL, triglyceride-glucose index, and triglyceride/HDL ratio was significantly different between the patient groups with good and poor blood glucose regulation (p<0.05). DM duration, fasting blood glucose, HbA1c, triglycerides, triglyceride-glucose index, and triglyceride/HDL ratio was higher in patients with poor glycemic control while HDL values were lower compared to patients with good glycemic control. No difference was found in other variables between the groups (p>0.05). Statistical results showing the differences in biochemical parameters and triglyceride-glucose index and triglyceride-HDL ratio between the good and poor glycemic control groups are presented in Table 2.

When the relationship between triglyceride-glucose index, triglyceride/HDL ratio, and body mass index was analyzed, it was found that the TyG index and THR values did not differ between the groups concerning body mass index. (p>0,05). The results of the analysis are shown in Table 3**.**

DISCUSSION

Type 2 diabetes mellitus is a global health problem whose prevalence continues to rise. Dyslipidemia and insulin resistance are the main mechanisms involved in the development of microvascular and macrovascular complications. HbA1c is still the most widely used blood parameter that retrospectively shows blood glucose regulation over the longest period. The present study aimed to evaluate various metabolic indices that may be associated with HbA1c, which may indicate glycemic control, are economical and easily applicable.

Table 1: Anthropometric Data and Biochemical Blood Tests of the Groups with Good and Poor Diabetic Control

	Well glycemic controlled group (N=148)	**Poor glycemic controlled group (N=148)**
	$\bar{X}$+SS/Median (Min-Max)	**$\bar{X}$+SS/Median (Min-Max)**
Age (year)	57 (33-75)	56 (27-75)
Gender (n, %)	Female: 83 (56%) Male: 66 (44%)	Female: 77 (52%) Male: 71 (48%)
Smoking (n, %)	No (122, 82.4%) Yes (26, 17.6%)	No (111, 75%) Yes (37, 25%)
Diabetes duration(year)	6 (1-17)	8 (1-20)
Body Mass Index (kg/m^2)	Normal 33 (22.3%) Overweight 57 (38.5%) Obese 38 (29.2%)	Normal 49 (33.1%) Overweight 50 (33.8%) Obese 49 (33.1%)
Fasting blood sugar (mg/dL)	128 (71-303)	215 (98-445)
HbA1c (%)	6.8 (5.6-7)	9.4 (7.1-14.7)
Total cholesterol (mg/dL)	194±36.55	203.52±42.79
HDL (mg/dL)	45 (27-98)	43 (25-80)
LDL (mg/dL)	109.89±27.63	111.73±36.29
Triglyceride (mg/dL)	175 (61-780)	201.5 (72-982)
Triglyceride-glucose index (TGI)	9.28 (6.62-10.69)	9.95 (8.64-12)
Triglyceride/HDL ratio (THR)	3.76 (0.3-20.53)	4.57 (1.29-28.88)

Table 2: Comparison of Biochemical Parameters Between Groups

		N	**$\bar{x}$+SS/Median (Min-Max)**	**Z/2t**	**p**
Diabetes duration	Well Regulated group	148	6(1-17)	-4,022	<0,001
	Poorly Regulated group	148	8(1-20)		
Fasting blood sugar	Well Regulated group	148	128(71-303)	-12,811	<0,001
	Poorly Regulated group	148	215(98-445)		
HbA1c	Well Regulated group	148	6,8(5,6-7)	-14,928	<0,001
	Poorly Regulated group	148	9,4(7,1-14,7)		
Total cholesterol	Well Regulated group	148	194±36,55	-1,944	0,053
	Poorly Regulated group	148	203,52±42,79		
Triglyceride	Well Regulated group	148	175(61-780)	-3,101	0,002
	Poorly Regulated group	148	201,5(72-982)		
HDL	Well Regulated group	148	45(27-98)	-2,355	0,019
	Poorly Regulated group	148	43(25-80)		
LDL	Well Regulated group	148	109,89±27,63	-0,491	0,624
	Poorly Regulated group	148	111,73±36,29		
TGİ	Well Regulated group	148	9,28(6,62-10,69)	-9,079	<0,001
	Poorly Regulated group	148	9,95(8,64-12)		
THR	Well Regulated group	148	3,76(0,3-20,53)	-3,645	<0,001
	Poorly Regulated group	148	4,57(1,29-28,88)		

Table 3: Analysis Results Showing the Relationship Between Body Mass Index and TGI and THR

		N	Median (Min-Max)	Ki-Kare	P
TGI	Normal	82	9.76 (8.25-12)	4.88	0.087
	Overweight	107	9.6 (8.38-11.44)		
	Obese	107	9.46 (6.62-11.49)		
THR	Normal	82	4.79 (1.03-28.88)	3.729	0.155
	Overweight	107	4.22 (1.47-14)		
	Obese	107	3.77 (0.3-28.88)		

Good glycemic control, i.e. an HbA1c level below 7, plays a very important role in reducing the complications of diabetes mellitus [10]. In type 2 diabetes mellitus, hyperglycemia and decreased insulin sensitivity be associated with hypertriglyceridemia and low HDL levels when blood glucose regulation is impaired. Increased plasma cholesterol ester transfer protein (CETP) increases VLDL-C levels by facilitating the transfer of triacylglycerol and cholesterol esters between HDL and LDL particles [11,12]. Increased small dense LDL is associated with increased diabetic dyslipidemia and cardiovascular complications. Estimation of small dense LDL particles is not useful in clinical practice; the triglyceride/HDL ratio may be an indirect indicator of these LDL particles [6,13].

Previous studies have shown that the triglyceride/HDL ratio is correlated with HbA1c [13,14].

One study found higher triglyceride-glucose index and lower HDL levels in patients with poor glycemic control [14]. The result obtained in the present study is similar. The group with high HbA1c levels, the poor glycemic control group, had a higher Triglyceride-glucose index and higher triglyceride-HDL ratio compared to patients with good glycemic control (P<0.001). Similar to the other study, HDL level was significantly lower in patients with poor glycemic control in the present study [14]. Low HDL levels may be the result of increased oxidative stress associated with impaired glycemic control.

Poor glycemic control is known to increase microvascular and macrovascular complications of diabetes. Triglyceride-glucose index and triglyceride/HDL ratio are correlated with HbA1c and thus may be predictive markers of complications in diabetic patients. In a study on this subject, triglyceride/glucose index was shown to be associated with cardiac autonomic neuropathy [15]. Another study in hospitalized diabetic patients showed that nephrotic and vascular complications were associated with elevated triglyceride-glucose index [16]. Other studies have shown that the triglyceride-glucose index is higher in patients with diabetic nephropathy, retinopathy, and coronary artery disease [17,18]. Since patients with known diabetic complications (diabetic retinopathy, nephropathy, coronary artery disease, diabetic neuropathy) were excluded from the present study, it can be concluded that triglyceride-glucose index and triglyceride/HDL ratio in correlation with HbA1c is already high even before diabetic complications occur. This group of patients should be monitored more closely for the occurrence of diabetes complications. However, there may be possible micro-complications and atherosclerotic processes that have not been evaluated. Further studies may clarify this subject.

In the present study, the relationship between triglyceride-glucose index, triglyceride/HDL ratio, and body mass index was also evaluated. In both groups, patients were divided into three sub-groups according to body mass index: normal weight (body mass index of 18.5 and below), overweight (body mass index of 25-29.9), and obese (body mass index of 30 and above). When the relationship between triglyceride-glucose index, triglyceride/HDL ratio, and body mass index was analyzed, it was found that the TyG index and THR values did not differ between the groups concerning body mass index. (p>0,05). This result suggests that the triglyceride-glucose index and triglyceride/HDL ratio are not associated with obesity. That is, they are correlated with insulin resistance but not with body mass index. A previous cohort study has also shown that the triglyceride glucose index is associated with the development of diabetes in young, non-obese individuals [19]. Considering the relationship between the triglyceride-glucose index and insulin resistance, the TyG index is expected to be higher in obese individuals compared to non-obese individuals. Another study suggested that the TyG index in non-diabetic individuals can be used as a diagnostic criterion for identifying metabolically obese but normal-weight individuals [20]. In contrast to our results, another study in the literature showed that the triglyceride-glucose index was correlated with the body mass index in diabetic individuals [14]. Further investigations are needed to explain why the results are not similar in diabetic individuals.

There were two important limitations in this study. The study was conducted retrospectively and the number of patients was relatively small.

In conclusion, triglyceride-glucose index and triglyceride/HDL ratio may be indicators of poor glycemic control in diabetic individuals. These indices are cheap, universal, easily accessible parameters similar to HbA1c and can be used to predict blood glucose regulation. In addition, although these two indexes were associated with insulin resistance, they were not found to be associated with the body-mass index in diabetic individuals. Further studies are needed to clarify and validate the results. Since patients with known diabetic complications were excluded from the present study, it can be concluded that the triglyceride-glucose index and triglyceride/HDL ratio in correlation with HbA1c is already high before diabetic complications occur. This group of patients should be monitored more closely for the occurrence of diabetes complications.

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ORIGINAL ARTICLE

Value of Electrocardiogram Algorithms Predicting Right Ventricular Outflow Tract Origin in the Era of Catheter Mapping

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Abstract: *Background: The 12-lead electrocardiogram (ECG) is an extremely useful tool for analyzing cardiac arrhythmias and numerous ECG algorithms have been developed to predict the origin of right ventricular outflow tract (RVOT) arrhythmias. Aim: To determine if using complex, time-consuming analysis, and difficult-to-memorize ECG algorithms is feasible and preferable before the ablation procedure when compared with the use of multipolar mapping catheter use during the ablation procedure. Methods: 47 consecutive patients admitted for radiofrequency catheter ablation of idiopathic PVC were included prospectively in this study. Of the algorithms selected for comparison, three have a memorable design and easy applicability (Dixit, Joshi, Pytkowski) and the fourth has a complex, detailed design and is more difficult to memorize (Zhang). Results: The greatest accuracy was observed for the algorithms proposed by Dixit and Pytkowski – 92. 3%. However, when the algorithms were compared for their accuracy, specificity, and sensitivity, no significant differences were found (p=0. 96). Conclusions: 3-D guided mapping using a conventional ablation catheter in a narrower, precise area of interest in the RVOT, based on ECG algorithms is not inferior compared to the multielectrode catheter mapping in the whole RVOT, in terms of mapping time, total procedural time, and radiofrequency application time.*

Keywords: *electrocardiogram (ECG) algorithm; multielectrode mapping catheter; premature ventricular contraction (PVC) ablation; right ventricular outflow tract*

INTRODUCTION

Idiopathic ventricular arrhythmias originating in the right ventricular outflow tract (RVOT) represent 10% of all ventricular tachycardia (VT) admissions [1,2]. The most common presentation scenario is represented by frequent premature ventricular complexes (PVC) [3], but non-sustained or sustained ventricular tachycardias are other scenarios. Ventricular arrhythmias may cause heart failure and ventricular dysfunction [4]. PVCs originating from the RVOT pre-sent on 12 lead electrocardiogram (ECG) had a left bundle branch block (LBBB) morphology and an inferior axis. The exact cut-off of ventricular burden that causes PVC-induced cardiomyopathy is not yet known. Several studies showed a correlation between PVC burden and the development of cardiomyopathy [5,6]. Baman's study [6] revealed that in patients with normal LV dimensions and systolic left ventricular (LV), a PVC burden cut-off of 24% may identify the patients at risk to develop left ventricular (LV) dysfunction.

The most common site of origin of RVOT arrhythmias is below the pulmonary valve, in the anterior septal region [7]. Less common sites of PVC are found in the right ventricular free wall, posterior septal area, and other rare areas [8,9,10]. The current guidelines for symptomatic patients with RVOT arrhythmias, recommend catheter ablation as first-line therapy [11,12]. In experienced centers, for patients without structural heart disease, the acute success rate of catheter ablation is usually high, >95% [13,14,15]. It is paramount to correctly predict the precise origin of RVOT arrhythmias to establish the appropriate ablation approach before the procedure. As a 12-lead electrocardiogram (ECG) is an extremely useful tool for analyzing cardiac arrhythmias, numerous ECG algorithms have been developed to predict the origin of RVOT arrhythmias [16-27].

The major endpoint of this study was to evaluate the differences between mapping the RVOT arrhythmias using a 3-D electroanatomical system as Ensite (Abbott Laboratories, Abbott Park, IL, USA) and a quadripolar catheter (Abbott Laboratories, Abbott Park, IL, USA) only in the region of interest based on ECG algorithms shortens significantly the mapping time, the total procedural time, the radiofrequency application time,. when compared to the use of a multipolar mapping catheter (16 electrodes) (Abbott Laboratories, Abbott Park, IL, USA) in the entire RVOT.

Another endpoint was to determine if using complex, time-consuming analysis, and difficult-to-memorize is feasible and preferable instead of determining a wider location of RVOT ectopies (septal or free wall location) before the ablation procedure. Other objectives were to evaluate the concordance between the arrhythmia location based on each algorithm and the location obtained on the 3-D mapping system and to define the level of accuracy of each algorithm used in our study.

MATERIALS AND METHODS

Study population

Between January 2019 and October 2022, 47 consecutive patients admitted to two different centers for radiofrequency catheter ablation of idiopathic PVC were included prospectively in this study. PVCs with LBBB morphology and an inferior axis were documented for all patients. Usually, symptomatic patients have a high PVC burden (>10000 PVCs/24h). Because symptoms are not found exclusively in this category, patients with a reduced PVC burden (5000-10 000/24h) are frequently highly symptomatic and warrant ablation[28]. In this study, we included symptomatic with PVCs refractory or intolerant to an antiarrhythmic drug (beta-blocker and/or at least one class IC anti-arrhythmic agent), with a ventricular burden above 5% on a 24-hour Holter monitoring. All patients presented normal left ventricular ejection fraction.

Of the algorithms selected for comparison, three algorithms have a memorable design and easy applicability (Dixit [26], Joshi [3], Pytkowski [27]). The fourth algorithm has a complex, detailed design and is more difficult to memorize (Zhang [24]).

Evaluated algorithms

1. The Dixit algorithm (2003) evaluates the presence or the absence of “notching” in leads II, III, and aVF, and pre-cordial transition (R ≥ S at or beyond lead V4) to predict the arrhythmia origin. QRS polarity in lead aVL is further assessed to determine arrhythmia locations in the anterior to the posterior plane.

2. The Joshi algorithm (2005) is based on three criteria for differentiating the free wall from septal wall origin (presence or absence of R-wave “notching” in inferior leads, a QRS duration ≥140 ms, an R/S ratio ≤1 in lead V3). According to the QRS polarity in lead I, the anterior or posterior location of arrhythmias is determined. Next, the QRS polarity in aVL lead determines a cranial versus a caudal origin.

3. The Zhang algorithm (2009) allows the location of the ventricular arrhythmias in four different sites (RV septal, or free wall origin, near the His, LV). It begins with the determination of the QRS morphology and axis. LBBB morphology with the inferior axis suggests an RVOT origin. The transitional zone in V4, the R wave duration index, the R/S amplitude index, and RR’/RSR’ pattern in lead aVL are evaluated. The PVC-QRS duration/the QRS duration of the preceding sinus beat and QRS pattern in lead I am also evaluated. Arrhythmia origin is further described as anterior, posterior, and mid-region.

4. The Pytkowski algorithm (2013) uses RVOT arrhythmia location in one of the 9 sub-regions created by 3 vertical and 3 horizontal zones. It starts by evaluating the presence or the absence of “notching” in leads II, III, and aVF. A low R wave amplitude in the inferior leads suggests a free wall origin. RVOT is next divided into 3 vertical zones (posterior to anterior) based on the lead I polarity. Then, 3 horizontal zones are defined, depending on the transitional zone in the precordial leads, with the horizontal intermediate zone (R[r] = S[s] in V4) occupying a zone in the median part of the RVOT which delineates the superior and the inferior zones.

All the ECG tracings were analyzed at a paper speed of 25 mm/s and were reviewed by two experienced electrophysiologists to locate the origin of the arrhythmia based on the 4 presented algorithms. Each electrophysiologist used ECG assessment one consecutive algorithm for every consecutive patient. Furthermore, based on the precise or wider location obtained from ECG evaluation, the ablation procedure was performed using the same 3-D mapping system, mapping, and ablation catheter. The algorithm used for evaluation, the time from the beginning till the end of the mapping with the multielectrode catheter and next with the mapping and ablation catheter, the success rate, procedural time, and radiofrequency application time during ablation procedures were registered for each patient.

Based on Dixit and Zhang algorithm, a less precise sub-localization in the RVOT was performed – septal vs free wall and on the horizontal plane (posterior, median, and anterior zones). When Zhang or Pytkowski’s algorithms were used, the location was identified in one of the nine regions of the RVOT divided by 3 vertical and 3 horizontal lines.

Electrophysiological procedure

The anti-arrhythmic therapy was interrupted for all patients at least five half-lives before the procedure. Spontaneous PVC at baseline and during intravenous isoprenaline (1-4 µg/min) infusion were recorded. A multipolar (16 poles) electrode catheter was inserted into a femoral vein and positioned in the RVOT. The 3D geometry of RVOT was created by navigating the mapping catheter within the RVOT using the non-contact electro-anatomical mapping system. To identify the earliest activation (EA) site, a broad color setting with a high pass filter set at 2 Hz was used. The EA site was identified by stepping further back in time where the red color zone shrinks down to the blue. For each patient, the EA site obtained from the mapping with the multi-polar catheter was noted, as well as the time from the insertion of the mapping catheter in the RVOT until the final definition of the earliest activation zone. Next, an eight-French quadripolar catheter with a 3. 5 mm distal electrode, sensor-enabled interelectrode spacing of 1-4-1mm, and a flexible tip (Abbott Laboratories, Abbott Park, IL, USA) was placed in the RVOT for mapping the earliest activation zone in the region of interested obtained from the ECG algorithm. The time from the insertion of the ablation catheter in the RVOT until the final definition of the earliest activation zone was also noted. Unipolar electro-grams at this site were reconstructed. The presence of a QS morphology was used as an additional criterion for the EA site.

For the Ensite system, several modules can be used to facilitate the localization of ventricular arrhythmia origin - Template Score Matching (Figure 1, Figure 2) and Real Time PVC Map Score. Template Score Matching analyses the QRS morphology of the clinical PVC in all 12 leads ECG during mapping and creates a compatibility score for the PVC. This way, all PVCs that are not in the interest zone are eliminated, either non-clinical PVC or PVC induced by the catheter movement.

The filter settings for the intracardiac electrograms were set at 30 –500 Hz. The pacing was performed at twice the diastolic threshold with a programmable stimulator using stimuli with a duration of 2ms. Pace mapping for each of the targeted sites was also performed to ensure at least 11 of 12 matching on the 12-lead ECG.

Concordance between the earliest activation zone obtained from the multielectrode catheter and the mapping and ablation electrode for each patient was evaluated. In the case of concordance, the earliest activation site was targeted by radiofrequency catheter ablation. In case of mismatch, radiofrequency energy was delivered through the ablation catheter in temperature-control mode (Stockert, Biosense Webster, Diamond Bar, CA, USA) in the earliest activation zone obtained from the multielectrode catheter. The power output was titrated to as high as 40 W to achieve a target temperature of 48°C for 60 – 120 s.

Figure 1: 3-D reconstructed image of the right ventricular outflow tract with activation map revealing the origin of ventricular in the white zone (with the red dots added) in the septal area of RVOT. The compatibility score of 100% and the earliest activation time (LAT) of 24 ms (the time from the earliest ventricular EGM to the onset of QRS) are displayed in the inferior part of the image. In the right panel, the 12 lead ECG shows a PVC characterized by an LBBB morphology with late transition in V4 and R/S ratio > 1 in lead II, III, and AVF. The presence of QRS notching and the lower R wave amplitude in the inferior leads is highly suggestive of a free wall origin.

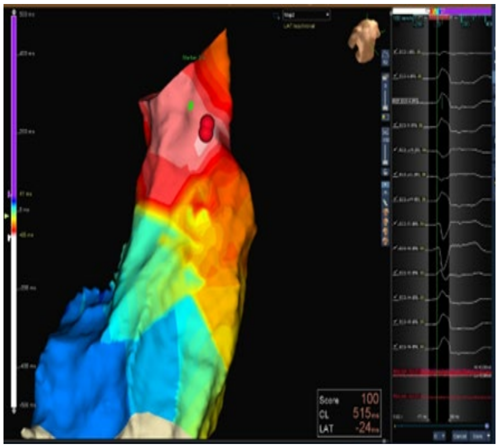

Figure 2: 3-D reconstructed image of the right ventricular outflow tract with activation map revealing the origin of ventricular ectopy in the white zone in the septal area of RVOT. The compatibility score of 99% and the earliest activation time (LAT) of 21 ms (the time from the earliest ventricular EGM to the onset of QRS) are displayed in the inferior part of the image. In the right panel, the 12 lead ECG shows a PVC characterized by an LBBB morphology with late transition in V4 and R/S ratio > 1 in lead II, III, and AVF. The lack of QRS notching and a higher R wave amplitude in the inferior leads suggest a septal origin.

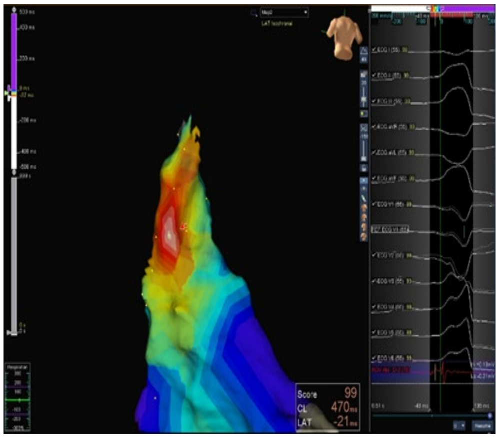

An observational period of 30 min was applied for all patients. In case of incomplete elimination of VAs, surrounding sites were further investigated by activation and pace map-ping to find alternative ablation sites. Next, subsequent ablations were applied to those sites. The ablation procedure was considered successful if clinical PVC disappeared during RF and didn't reoccur within 30 minutes after the last RF application, and if PVCs were non-inducible after Isoproterenol infusion (1-10 μg/min) [29,30].

Based on the 3D electroanatomical non-contact mapping, RVOT was divided through vertical and horizontal imaginary lines into nine distinct regions to facilitate the description of the origin of PVC based on the successful ablation site.

Statistical analysis

For data analysis, IBM SPSS Statistics for Windows (version 20. 0; IBM Corp. , Armonk, NY, USA) was used. Continuous variables are presented as median and mean ± standard deviation (SD). Categorical variables were presented as counts and percentages.

RESULTS

47 patients with RVOT arrhythmias were enrolled in this study. Patient clinical characteristics are presented in Table 1. Ablation was delivered at 14 free wall sites and 33 septum sites.

The 3D electroanatomic maps were created for all patients. The precise origin of RVOT was identified as it is presented in Table 2. The RVOT chamber was correctly identified by the evaluators in 47/47 (100%) patients. Furthermore, the concordance between the origin obtained from the multielectrode catheter also targeted for ablation, and the origin obtained by the mapping and ablation catheter guided by the ECG algorithm was evaluated. The results are presented in Table 3.

Table 1: Clinical characteristics (PVC, premature ventricular contraction; LVEF, left ventricular ejection fraction)

Age (years; median)	42. 76
Female/ Male (n)	27/20
PVC burden (%; median)	17. 95
LVEF (%; median)	62. 1
Use of antiarrhythmic drugs(%) - Beta-blockers - Class I C	76. 59% 44. 68% 31. 91%

Table 2: Localization of arrhythmia in RVOT based on mapping with the multielectrode catheter when performed by both electrophysiologists (EP1 and EP2).

Location in RVOT	EP1	EP2	Total
Free wall	6	8	14
Anterior	1	0	1
Mid	1	2	2
Posterior	4	6	11
Septal wall	17	16	33
Anterior	8	7	15
Mid	2	2	4
Posterior	7	7	14

Table 3: The concordance between the origin obtained from the multielectrode catheter, also targeted for ablation and the origin obtained by the mapping and ablation catheter guided by ECG algorithm.

Algorithm	Electrophysiologist 1 Concordance - number of patients/percentages	Electrophysiologist 2 Concordance - number of patients/percentages	Concordance - number and percentage in our study
Dixit	5/6 patients 83. 33%, P=0. 68	6/6 patients 100%, P=0. 54	11/12 patients 91. 6%, P=0. 77
Joshi	4/6 patients 66. 66%, P=0. 51	5/6 patients 83. 33%, P=0. 68	9/12 patients 75%, P=0. 75
Zhang	4/5 patients 80%, P=0. 42	3/6 patients 50%, P=0. 55	7/11 patients 63. 63%, P=0. 94
Pytkowski	5/6 patients 83. 33%, P=0. 51	6/6 patients 100%, P=0. 55	11/12 patients 91. 66%, P=0. 96
Total	18/23 patients	20/24 patients	38/47 patients 80. 85%, P=0. 88

Acute procedural success was achieved for 45 out of 47 patients (95. 74%). Failure of RF ablation was observed in 2 out of 47 patients, both patients in the concordance origin group. The diagnostic accuracy evaluations showed that the greatest accuracy was observed for the algorithms proposed by Dixit and Pytkowski – 92. 3%. However, no significant differences were found when the algorithms were compared for their accuracy, specificity, and sensitivity (p=0. 96). The diagnostic accuracy, sensitivity, and specificity of each algorithm are presented in Table 4.

Table 4: Accuracy, sensitivity, and specificity of each algorithm in our study

Algorithm	Sensitivity	Specificity	Accuracy
Dixit	100%	80%	92. 3%
Joshi	87. 5%	71. 43%	80%
Zhang	80%	60%	73. 33%
Pytkowski	100%	66. 67%	92. 3%

The mean time for mapping with the multielectrode catheter was 14. 89±8. 15 min, whereas the time used for mapping with the ablation catheter in the region of interest obtained from the ECG algorithms was. 12. 27±7. 70 min. The mean total procedural time was. 95. 5±24. 42 min. The mean total RF application time was 264. 46±134. 12 seconds. Ectopy recurrence during the waiting time occurred in 5 out of 47 patients (10. 63%).

DISCUSSION

The predicted accuracy of the ECG algorithms in our study varied from 73. 3% to 92. 3%. The accuracy of algorithms was similar to the results previously reported [9-27] despite the differences in the population, and the heterogeneity between the assessors in our study and the developers of algorithms, a narrower sub-localization of the arrhythmias compared with algorithms differentiating only septal from the free wall and a more complex design of the Zhang algorithm.

Though the diagnostic accuracy was similar for the algorithms proposed by Dixit and Pytkowski – 92. 3%, the Pytkowski scheme allows a more precise location, not only septal vs free wall location, but also a more exact location in the horizontal and vertical plane.

Several major issues were observed when comparing the Dixit algorithm from our study and the Dixit publication. Dixit algorithm predicts the arrhythmia origin based on pace mapping and not based on activation maps or a combination of activation and pace mapping as it is known that pace mapping in RVOT exhibits a lower spatial resolution (2 cm). Secondly, our study allowed arrhythmia localization in one of the 9 regions of RVOT, while the Dixit algorithm is refined to 6 zones.

One major issue was also detected when comparing our evaluation based on the Pytkowski algorithm with the one from the Pytkowski study In the Pytkowski study, the precise location of arrhythmia was fluoroscopy only based, without the use of 3-D mapping.

The analysis of mapping time, total procedural, time, and RF application time performed in the concordance and non-concordance origin groups of the multielectrode catheter and ablation catheter mapping showed no significant differences (p=0. 15) – Table 5. Next, the impact of ventricular burden during the procedure and especially during the mapping interval on the mapping time, total procedural time, and RF application time performed in the concordance and non-concordance origin groups of the multielectrode catheter and ablation catheter mapping, was evaluated and results are presented in Table 6.

Table 5: The impact of concordance or non-concordance origin of RVOT arrhythmia on mapping time with a multielectrode catheter, ablation catheter, total procedural time, and RF application time.

Mean time ± SD	Concordance group	Non-concordance group	P value
Multielectrode catheter mapping time	16. 29 ± 7. 97 min	11 ± 7. 46 min	0. 71
Ablation catheter mapping time	12. 94 ± 7. 08min	10. 53 ± 9. 2 min	0. 43
Total procedural time	94. 17 ± 25 min	99. 15 ± 23. 4 min	0. 67
RF application time	243. 08 ± 127. 14 sec	320. 38 ± 140. 53 sec	0. 23

The evaluation revealed that a low ventricular burden du-ring mapping is associated with a longer total procedural time (P=0. 04) and a longer RF application time (p=0. 01). These findings can be explained by a cumulation of factors. A low ventricular burden during mapping can prolong the mapping time (though statistical differences were not observed in our study). The suppression of clinical PVC during catheter manipulation during low ventricular burden can be more frequently unnoticed. The waiting period after RF application in these cases can also be prolonged, either due to early or late recurrence of PVC, either by transient suppression caused by an incomplete lesion or ablation in an adjacent zone near the real focus.

Based on these findings we can conclude that there are no significant differences in terms of mapping time, total procedural time, and RF application time when mapping is performed with a multielectrode catheter or a conventional ablation catheter in a narrower, precise area of interest in the RVOT, based on ECG algorithms.

The ECG-based algorithms for a narrower localization in the RVOT may exhibit acceptable to excellent accuracy, simplify the mapping process, reduce the procedural and RF application time, and improve clinical outcomes, resulting in greater clinical utility. Algorithms with a more elementary and easy-to-memorize design as Pytkowski can be routinely used to precisely localize the RVOT arrhythmia origin instead of other algorithms with a less precise sub-localization of arrhythmia origin.

Table 6: The impact of ventricular burden during the procedure on the total mapping time, total procedural time, and RF application time performed in the concordance and non-concordance origin groups of the multielectrode catheter and ablation catheter mapping.

Mean time	Concordance group	Non concordance group	P value
Multielectrode catheter mapping time PVC : 1- 5/ min PVC : 6-10/min PVC : >11/min	22. 14 min 15. 38 min 5. 5 min	19. 75 min 9. 75 min 5 min	0. 79
Ablation catheter mapping time PVC : 1- 5/ min PVC : 6-10/min PVC : >11/min	18. 5 min 11. 07 min 4. 2 min	19. 75 min 7. 75 min 5. 4 min	0. 7
Total procedural time PVC : 1- 5/ min PVC : 6-10/min PVC : >11/min	115. 15 min 87. 92 min 64. 66 min	105. 5 min 93. 25 min 98. 8 min	**0. 04**
RF application time PVC : 1- 5/ min PVC : 6-10/min PVC : >11/min	318. 92 sec 198. 14 sec 171 sec	362 sec 323. 5 sec 284. 6 sec	**0. 01**

Meanwhile, it is paramount to understand that the algorithms with a discriminating and stepwise analysis may lessen the inaccurate results. Detailed algorithms are not easy to remember, and the orientation of the subsequent steps can be frequently challenging. Time-consuming and complex analyses of such algorithms may be more helpful while predicting the arrhythmia location and may lead to better preparation before the procedure. Also, in our study, in case of erroneous predictions of the RVOT arrhythmia origin based on ECG algorithms, the effectiveness of ablation was not impacted, and no inappropriate approaches were detected.

Limitations

The number of patients in our study is relatively small, which may affect the results. Several limitations associated with the ECG algorithms were found both in our and other studies such as inconsistencies and heterogeneities between the assessors, the fact that some algorithms require an accurate measurement of many ECG parameters, and the lack of electronic calipers or wandering baselines [20-22]. Variable spatial relationships of the heart in the thorax and variable lead placements are prone to create inaccuracies in ECG algorithms [13,31,32]. In our study, we did not perform magnetic resonance imaging (MRI) for all patients so right ventricular dysplasia cannot be ruled out. The patients' age range was 24 to 69 years for this study, and we did not include more pediatric or older patients, thus the age-related changes of PVC morphology are not discussed.

CONCLUSION

3-D guided mapping using a conventional ablation catheter in a narrower, precise area of interest in the RVOT, based on ECG algorithms is not inferior compared to the multi-electrode catheter mapping in the whole RVOT, in terms of mapping time, total procedural time, and RF application time. In case of misleading predictions of the RVOT arrhythmia origin based on ECG algorithms, the effectiveness of ablation is not impacted.

In our study, although both Dixit and Pytkowski algorithms have the best accuracy among the evaluated algorithms, the Pytkowski scheme is more precise in localizing the RVOT arrhythmias origin, not only in septal vs free wall but also in the horizontal and the vertical plane, has a more basic and easy-to-memorize design.

Conflicts of interest and sources of funding
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To the Cardiology teams of both hospitals

Authors' contribution

Conceptualization, R.S.P., and L.R.; methodology, R.S.P., and L.R.; software, M.A, O.P.; validation, R.S.P., L.R., and I.M.; formal analysis, R.S.P., and L.R.; investigation, R.S.P., and L.R.; resources, R.S.P., and L.R; data curation, R.S.P., and L.R.; writing - original draft preparation, R.S.P., and L.R.; writing - review and editing, R.S.P., and L.R.; visualization, I.M.; supervision, I.M.; project administration, R.S.P., and L.R. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the hospital's ethics committee, number 11/21/11/2018, and is performed under the ethical standards laid down by the Declaration of Helsinki.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Risk Factors for Urinary Colonization in Patients with Double J Catheters Implanted for Treating Obstructive Urolithiasis

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Abstract: *Background: The highlighting of possible risk factors for urinary colonization in patients with obstructive urolithiasis that needed double J catheters implanted to preserve renal function. Methods: We performed a descriptive, retrospective study, carried out in the Urology Department of the Bucharest Central Military Hospital, between January 2020 and January 2022 and included 168 patients with urolithiasis who required the insertion of double J catheters. We studied the bacteriological profile, using both urine and JJ catheter samples. Results: We obtained a double J catheter colonization rate of 32% (54 patients) and 29% of urinary colonization (49 patients). The rate of urinary colonization is higher in patients with colonized ureteral catheters regardless of sex, age, and associated comorbidities. At the same time, we noticed an increased rate of urinary colonization in patients associated with diabetes, hypertension, and chronic kidney disease. Conclusions: The prevalence of urinary colonization in patients with double J catheters was 29%. The colonization of the JJ catheters, as well as the association with chronic diseases, such as diabetes, hypertension, and CKD (Chronic Kidney Disease), show an increased risk of urinary colonization.*

Keywords: *double J catheters, obstructive urolithiasis, urinary colonization, risk factors*

INTRODUCTION AND OBJECTIVES

Urolithiasis is a frequent pathology; in developed countries, its prevalence in the general population has tripled in the last 40 years. Acute Renal Colic is common in the Emergency Department, and approximately 6% of these require hospitalization due to subsequent complications [4,8].

Today we have multiple urological treatments available: extracorporeal lithotripsy, rigid or flexible ureteroscopy, and percutaneous nephrolithotomy, and their results can be improved by expulsive medical therapy and posturotherapy. Minimally invasive procedures have become common practice in urology departments, being very effective and having the advantage of rare post-procedure complications.

The method of treatment of urolithiasis depends on multiple factors: the patient's morphology and comorbidities; the size, chemical composition, and location of the calculus, urinary tract anatomy, and/or other possible complications.

In complicated urolithiasis with complete obstruction and/or secondary infection, an endo-prosthesis may be required temporarily, whatever the surgical method subsequently approached.

An ultrasound-guided percutaneous nephrostomy would be performed in case of physiological urinary drainage failure [3,6].

The urinary tract is normally sterile, except for the distal portion of the urethra. Urinary tract infections can occur through different mechanisms, but the contamination is ofte006E endo or extra-luminal. The use of catheters favors the colonization process by the means of an extracellular polysaccharide matrix to which germs adhere, resulting in a biofilm that will protect them from both defense mechanisms and microbial agents [2,5].

New methods have been constantly seeking to decrease the colonization rate of ureteral catheters by using different materials (silicone, polyethylene, polyurethane, biodegradable materials) or by covering the catheters with thin protective films (heparin, silver, polytetrafluoroethylene, antibiotics) [7].

Urinary colonization, unlike bacterial urinary infections, does not indicate systematic antibiotic treatment, even if the patient has an inserted catheter or not, is diabetic, elderly, with a neurological bladder. In special situations, antibiotic treatment is justified:

- if urinary colonization is a risk factor for morbidity and mortality: neutropenia, immunosuppressed, pregnant women;

- pre-operative: surgery and urological examinations;

- patients with joint, vascular, or cardiac prostheses that require invasive investigations;

- epidemic with multiresistant bacteria in a hospital unit.

Over time, many studies have been conducted on urinary infection and colonization [1], without looking for their prevalence amongst JJ ureteral catheter carriers, regardless of indication, comorbidities, or complications.

The objectives of the study were to identify possible risk factors of urinary colonization in patients with double J catheters inserted for complicated urolithiasis.

MATERIALS AND METHODS

This descriptive, retrospective study, was carried out in the Urology Department of the "Dr. Carol Davila" University Central Emergency Military Hospital, between January 2020 and January 2022.

The batch included 168 patients with obstructive urolithiasis who were inserted with double J ureteral catheters, regardless of sex, age, comorbidities, and complications.

Patients who did not agree to be included in the study, pregnant women (there is no integrated Obstetrics - Neonatology department within the Central Military Emergency University Hospital "Dr. Carol Davila"), and patients for whom transfer to other departments was necessary were excluded.

Preoperative biochemical evaluation and bacteriological examinations (urinary and JJ catheter sampling) were performed on nonselective media (BCP = bromocresol purple lactose and CLED = cystine – lactose – electrolyte – deficient).

It has been used as the standard method of insertion of JJ ureteral catheters - retrograde endoscopically under spinal anesthesia.

The following parameters were studied:

- sex and age;

- laterality of the catheter: right/left, both;

- associated comorbidities: general (high blood pressure, diabetes), reno-urinary (renal malformations, adenoma/ prostate cancer, bladder cancer), or immunosuppressed (grafts, Covid 19, other cancers);

- type and location of urinary infection: asymptomatic/ symptomatic, low/high;

- type and conditions of insertion of the ureteral catheter: emergency/appointment;

- indwelling time of the catheter: under/over 30 days;

- occurrence of post-stenting complications: calcifications, hematuria.

RESULTS

The study group included 168 patients, of which 101 men (60%) and 67 women (40%) with a sex-ratio M/W of 1.50. Urinary colonization is more frequent in women, 46% (31 patients) compared to 31% in men (31 patients), possibly explained by the shorter urethra in women and the proximity of the digestive flora.

The average age of the patients was 59 years, with extremes of 19 and 85 years.

The indication for the double J catheter insertion was ureteral-hydronephrosis, bilateral in 39% of cases (66 patients) and unilateral in 61% of cases (102 patients).

The distribution, from the point of view of stone laterality, was: 28% on the right (47 patients) and 72% on the left (121 patients); 52% pelvic ureteral stones (87 patients) and 48% pyelitic lithiasis (81 patients).

Many patients associated other comorbidities too (Table 1).

Table 1: Associated comorbidities with obstructive urinary lithiasis

Comorbidities	Number of patients	Rate [%]
High Blood Pressure	55	33
Diabetes	39	23
Obesity	27	16
Covid 19	18	11
Adenoma/Prostate cancer	28	17
Bladder cancer	10	6
Other cancers	7	4
Recurrent Urinary Tract Infections	49	29
CKD	32	19

Monitoring the bacteriological profile of the urine for the group of 114 patients with non-colonized catheters revealed that only 19 patients (17%) presented subsequent urinary colonization, and 83% (95 patients) remained non-colonized.

Amongst patients with colonized catheters (54 patients), only 12 (22%) remained bacteriologically negative throughout the study, regardless of comorbidities or associated complications (Table 2 and Figure 1).

The ureteral catheters colonized have a primary contributor to the pathogenesis of urinary infections, increasing their occurrence, regardless of sex, age, and comorbidities of patients in our batch.

Table 2: Urinary bacteriological profile depending on the JJ catheter bacteriological profile

Group	Number of patients	
	Urinary colonized	Urinary non-colonized
Non-colonized JJ catheters	19 (17%)	95 (83%)
Colonized JJ catheters	42 (78%)	12 (22%)

Figure 1: (a) Distribution of urinary colonization in patients with colonized catheters; (b) Distribution of urinary colonization in patients with non-colonized catheters

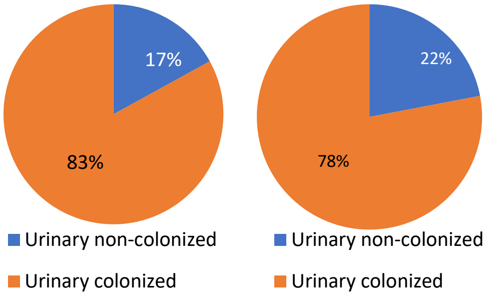

From a bacteriological point of view, different germs were identified, with the predominance of Escherichia Coli, and Klebsiella pneumoniae, both, at the urinary level and in the ureteral stents (Table 3).

We studied the urinary bacteriological profile in patients with urolithiasis and chronic diseases such as Diabetes, High Blood Pressure and CKD regardless of the ureteral catheters' bacteriological profile (Table 4).

Table 3: Germs identified at urinary and ureteral catheters level.

Bacterial germ	Number of patients with urinary colonized	Number of patients with JJ catheters colonized
Escherichia Coli	13	18
Klebsiella Pneumoniae	10	8
Enterococcus Faecalis	8	9
Enterobacter Cloaecae	1	2
Serratia Marcescens	1	0
Staphylococcus Hominis	3	0
Staphylococcus Aureus	1	6
Others	5	5

Table 4: Urinary profile of patients with associated chronic diseases

Associated chronic diseases	Number of patients	Urinary Colonization	
		Positive	Negative
Diabetes	39	22 (57%)	17
High Blood Pressure	55	26 (47%)	29
CKD	32	16 (50%)	16

In patients with chronic diseases such as diabetes, high blood pressure, and CKD we noticed an increased risk of urinary colonization regardless of the bacteriological profile of JJ catheters.

We noticed a higher rate of acute kidney failure in patients with obstructive urolithiasis associated with diabetes, high blood pressure, or chronic kidney failure, probably determined by the presence of secondary angionephrosclerosis.

It is already known that the immunosuppressed, pregnant women, alcoholics, and the elderly have a higher risk of bacterial colonization (urinary and catheters) regardless of associated comorbidities**.**

CONCLUSIONS

Double J (ureteral) catheter implants represent a minimally invasive alternative for deriving the upper urinary system to preserve the renal function, in case of acute or chronic, intrinsic or extrinsic obstruction, regardless of its type.

The study shows that 29% of the patients with urolithiasis carrying double J catheters have urinary colonization.

The colonization of double J catheters, regardless of the amount of time since they were inserted, plays an important role in the pathogenesis of urinary colonization.

The prevalence of urinary colonization increases with the indwelling time and especially in the case of emergency insertion of ureteral catheters.

Chronic diseases, such as Diabetes, High Blood Pressure, and CKD, are associated with increased risks of urinary colonization, requiring strict bacteriological monitoring.

Complications related to the indwelling of the double J catheter are rare and occur especially in the case of long-term indwelling (more than 30 days) and disappear with its ablation.

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ORIGINAL ARTICLE

Survey the Effect of Educational Intervention Based on Mobile Short Message Service (SMS) on Self-Care in Patients with Type 2 Diabetes Referring to the Diabetes Clinic in Khorram Abad City

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Abstract: *Background: Self-care behaviors are very important to control type 2 diabetes. The current study was conducted aiming at determining the effect of the educational intervention based on mobile short message service on the self-care behaviors in patients with type 2 diabetes in Khorram Abad. Methods: This study was a semi-experimental study on 191 patients with type 2 diabetes (45 cases and 45 control). Data collection tools included a demographic information form, International Physical Activity Questionnaire, Self-Care Scale, and Self-Efficacy Scale. Three to four educational messages were sent daily through mobile phones as an educational intervention. Data were analyzed with SPSS V24 at a significance level of 0.05. Results: The data analysis indicated that the average score of self-care, self-efficacy, and blood sugar was significantly higher in the case group than in the control group before and after the intervention (P<0.001). However, in terms of the physical activity variable, this relationship was not statistically significant in the case and control groups. Conclusion: Educational interventions to empower diabetic patients by strengthening their self-care can be an effective way to improve the health of diabetic patients. Therefore, it is suggested to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.*

Keywords: *self-care, self-efficacy, physical activity, blood sugar, type 2 diabetes*

INTRODUCTION

Diabetes is the most common disease caused by metabolic disorders, which is mainly characterized by a chronic increase in blood sugar and disturbed carbohydrate, fat, and protein metabolism. In general, diabetes is a multifactorial disorder diagnosed by a chronic increase in blood sugar or hyperglycemia (according to diagnostic criteria) and is caused by a disorder in insulin secretion or function or both [1]. Retinal damage and blindness, peripheral neuropathy, leg pain, stroke, peripheral vascular problems, end-stage renal disease, and limb amputation are among the most serious complications of diabetes [2]. The most common type of diabetes is type 2 diabetes, which accounts for approximately 90-95% of diabetes cases and is usually associated with metabolic disorders, weight gain, and physical inactivity [3]. About 422 million people worldwide suffer from diabetes, the majority of whom live in low- and middle-income countries, and 2019, diabetes was introduced as the direct cause of 1.5 million deaths [4]. According to the International Diabetes Federation (IDF), the number of diabetic patients will reach 439 million people in 2030, and the prevalence of diabetes in developing countries will be 69% [5]. More than 3 million people with diabetes live in Iran [6]. By 2030, Iran will be one of the most prevalent regions in the world in terms of diabetes [7]. Paying attention to the chronic and costly nature of this disease for the general health of society and the creation of a large financial burden, it is essential to pay serious attention to this problem and its consequences [8]. According to the results obtained from the studies, the long-term complications of this disease, including neuropathy, nephropathy, and retinopathy can be prevented or at least the occurrence of these complications can be postponed if blood sugar is controlled [9, 10]. Many studies have found physical activity and exercise to be effective in reducing blood sugar levels and controlling diabetes [11,12]. The World Health Organization considers education to be the basis of diabetes treatment. Therefore, self-care is one of the important topics of diabetes treatment. Self-care is a learned regulation function in humans, which is based on the ability of people to perform care actions on themselves [13]. It includes the behaviors of following a proper diet, taking medicine on time, self-monitoring blood sugar, performing regular physical activity, and physical care of the feet [14]. Studies show that 67% of patients with type 2 diabetes do not monitor their blood sugar according to the recommendations. Also, only 19-30% of diabetic patients use the recommended physical activities and compliance with nutritional behaviors in these patients is 65%, and only 7% of diabetic patients fully adhere to all aspects of self-care behaviors [15,16]. Self-efficacy is also known as a strategy to improve self-care in diabetic patients, which results in self-confidence and adherence to care recommendations to control blood sugar in these patients [17].

Nowadays, to follow up and educate diabetic people, new communication systems are required to direct the focus of care and treatment from the clinic to the patient's daily life. Researchers have pointed out the use of mobile phone service as a method of education and follow-up in chronic diseases, that short message is one of the main functions of mobile phone in the education of diabetic patients [18,19].

Considering the importance of self-care in controlling the blood sugar of diabetic patients and the value of self-efficacy as one of the self-care approaches, it seems necessary to design educational interventions on the platform of mobile phones and in the form of short messages as a relatively new educational method in controlling the disease of diabetics. Thus, the present study was conducted to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad.

MATERIALS AND METHODS

Population of study

The present study was a semi-experimental intervention research with a pre-test and post-test design. The sample size was specified as 90 people who were selected from the research population of patients with type 2 diabetes referring to the diabetes clinic in Khorramabad, Lorestan province. The participants were selected according to the inclusion criteria using random sampling. Then, they were divided into case and control groups using a simple randomization method (45 ones in each group).

Inclusion and Exclusion Criteria

The inclusion criteria include informed consent to participate in the research, having a minimum level of literacy and understanding of the content sent through text messages, having a fixed personal mobile number to receive educational SMS, lack of any serious diabetic complication, and not having special diets because of specific diseases or physical problems limiting physical activity. Exclusion criteria include suffering from serious complications of diabetes during the study, suffering from diseases that limit physical activity or nutrition, and the patient's unwillingness to receive educational messages.

Questionnaires

Data collection tools in this study included a demographic information form, International Physical Activity Questionnaire (IPAQ), Self-Care Scale developed by Tubert, and Self-Efficacy Scale. Demographic information included: age, height, weight, gender, educational level, employment status, marital status, and type of treatment. The IPAQ with seven items measured the duration of physical activity in the last week in four categories: moderate physical activity, intense physical activity, walking, and sitting activities. According to the scoring protocol in this questionnaire, the intensity of physical activity based on the ratio of energy consumption in terms of metabolic equivalent of task (MET) in the last seven days is classified into three levels: low, medium, and high. It was proposed by the World Health Organization and the Centers for Disease Control and Prevention in 1998 to measure the physical activity of people aged 15 to 69 years and its validity and reliability have been confirmed in 12 countries worldwide, including Iran [20,21]. A self-care questionnaire was used in this study that included 15 items in the areas of diet compliance, physical activity, blood sugar monitoring, insulin injection or diabetes pill consumption, foot care, and smoking, which was translated and standardized by Hamdzadeh [22,23]. The self-efficacy questionnaire, which is one of the constructs of the standard Health Belief Model Questionnaire, validity and reliability of which have been confirmed [24].

Data collection

After presenting a letter of introduction from the Iran University of Medical Sciences and obtaining the necessary permits, the researcher referred to a diabetes clinic in Khorram Abad and selected 90 people from among 200 eligible people using random sampling. People who were willing to participate in the study and met the inclusion criteria were assigned to the case and control groups (45 in each group) using a simple randomization method. After random allocation, blood sugar samples were taken in all groups four days before the start of the intervention. In the first session, the researcher explained the purpose of the meeting to the participants and written informed consent was obtained. Then, the personal information form and questionnaires were completed by the case and control groups. The case group received an educational program in the form of short educational messages via mobile phone, for four weeks and on average, at least three to four messages per day related to the educational content, including exercise, self-monitoring of blood sugar, diet, insulin therapy, medication use, and foot care. For example, short messages about physical activity and blood sugar monitoring were “for people with diabetes, walking is the best exercise” or “it is better for people with diabetes to measure their fasting blood sugar after waking up”. To improve self-efficacy, encouraging messages about adherence to self-care were used. Finally, after two months, the same questionnaires (demographic, physical activity, self-care, and self-efficacy scales) were distributed and completed by the participants, and the blood sugar levels of the two groups were measured again. During this period, the control group did not receive any educational intervention. However, to comply with the ethical principles, an educational CD containing self-care training was given to the control group after completing the research.

Statistical Analysis

The data of the two groups before and after the intervention were entered into SPSS version 24 software and analyzed using Wilcoxon, Mann-Whitney, Chi-square test, independent t-test, and paired t-test at a significant level of 0.05.

RESULTS

The participants’ average age was 51.01 ± 0.967, and the average height and weight were 164.57 ± 1.031 and 75.69 ± 1.101, respectively.

Before the implementation of the educational program, the case and control groups were matched in terms of demographic variables, including age, height, weight, gender, education, employment status, marital status, and type of treatment, and no statistically significant difference was observed between the two groups (Table 1).

Table 1: Comparison of the absolute and relative frequency distribution of demographic variables in case and control groups

Variable		Case		Control		P value
		No.	%	No.	%	
Gender	Female	31	69	26	57/7	0.796
	Male	14	31	19	42/2	
Employment	Unemployed	5	11	2	4	0.686
	Housewife	26	58	24	53	
	Self-employed	9	20	9	20	
	Public	5	11	10	22	
Education	Illiterate	5	11	4	9	0.479
	Primary school	17	38	10	22	
	Secondary school	9	20	5	11	
	High school	4	9	11	24	
	High school diploma	4	9	10	22	
	Higher education	6	9	5	11	
Marital status	Single	2	4	1	2	0.800
	Divorced	1	2	0	0	
	Widow	9	20	5	11	
	Married	33	73	39	87	
Type of treatment	Insulin	21	47	21	47	0.920
	Oral medication	20	45	22	49	
	Diet	4	9	2	4	
	Exercise	0	0	0	0	

There is no statistically significant difference in terms of the average score of physical activity, self-care, self-efficacy, and blood sugar between the case and control groups before the intervention, while after the intervention, the average scores of these variables, except for physical activity (P=0.8), was significantly higher in the case group compared to the control group (P<0.001). In the control group, before and after the intervention, no significant difference was observed in the average scores of physical activity, self-care, self-efficacy, and blood sugar (P>0.05) (Table 2).

The blood sugar level in all patients with different treatment methods (insulin, oral medication, diet) in the case group reduced after the educational intervention compared to before intervention, except for the patients who only used exercise to control their blood sugar (Table 3).

Table 2: Comparison of the average scores of physical activity, self-care, self-efficacy, and blood sugar, before and after the intervention in case and control groups

Scale	Group	Case	Control	p-value
		Mean ± SD	Mean ± SD	
Physical activity	Before intervention	677.5±954.938	307.5±461.75	0.6
	After intervention	903.75±973.125	655±925.625	0.8
	p-value	0.8	0.4	_
Self-care	Before intervention	2.31±0.813	2.38±0.844	0.12
	After intervention	3.38±0.688	2±0.906	0<0001
	p-value	0<001	0.22	_
Self-efficacy	Before intervention	2.7±0.4	2.7±0.3	0.15
	After intervention	3.7±0.7	2.7±0.7	0<0001
	p-value	0<001	0.286	_
Blood sugar	Before intervention	180±80.5	185±51	0.8
	After intervention	170±87.5	180±70.5	0<001
	p-value	0.009	0.491	_

Table 3: Comparison of blood sugar scores before and after the educational intervention in the case group

Method of treatment	Blood sugar level before the intervention	Blood sugar level after the intervention	Level of change
Insulin	227.09	217.92	-9.17
Oral medicine	173.48	170.65	-2.83
Diet	181	180.44	-0.56
Exercise	194	194.5	0.5
Medium	193.89	190.88	-3.02

DISCUSSION

The present study was conducted aiming to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad. The research findings suggest that the educational intervention through mobile phone short messages improved self-care in the case group, which is consistent with findings by Abaza et al. and Agbede et al. on the effect of the intervention based on mobile phone short messages in the self-care of diabetic patients and the findings by Yari et al. and Rahnavard et al. concerning SMS-based and self-care education of diabetic patients [25-28]. Krishna et al. reported that technologies such as mobile phones and text messages, which are currently part of people's daily lives, have the potential to influence self-care and improve people's health [29]. The approach of health education through SMS to promote self-care in diabetic patients is more welcomed than traditional approaches that require reading pamphlets or attending lectures [25,30].

In this study, self-efficacy also there was a significant difference in the case group in terms of self-efficacy after the educational intervention. Katz and Nordwall and Peymani et al. also found that interventions based on mobile phone messages improve self-efficacy and self-care [31,32]. Similar studies regarding the self-care of diabetic patients also indicate a significant difference in the average score of self-efficacy and self-care in the case group compared to the control group after the educational intervention. These studies are consistent with our work in that increasing self-efficacy causes the adoption of self-care behaviors in diabetic patients

[33,34]. Self-efficacy is an important prerequisite for behavior change [35]. An individual with low self-efficacy is less likely to make an effort to perform a new health behavior or to change the behavior he is used to [36]. Researchers believe that self-efficacy has a suitable framework for understanding and predicting and patient's commitment to self-care behavior in the treatment of diabetes [37].

The results of the present study showed that education through mobile phone short messages does not affect increasing physical activity in the case group, which is in line with the findings by Bakhshian et al regarding the effect of education through mobile phone short messages on self-care of patients with type 2 diabetes [38]. It is also in agreement with the findings by Kim et al. on adherence to diabetes control recommendations [39], while our findings are not consistent with the study of Paul et al. regarding the use of software that can be installed on mobile phones to improve the amount of daily walking in patients with a history of stroke [40] and the study Martha et al. concerning mobile phone SMS intervention on diabetes self-care activities, cardiovascular disease risk awareness, and food choices among type 2 diabetic patients [41]. This discrepancy can be due to the lack of sports facilities and parks in the current study or the addition of chronic diseases to the patients participating in this research, as well as social and cultural factors that prevent people from doing physical activities in public environments. In the present study, since the number of women is more than men, the problem of social and cultural factors could be more marked.

The use of mobile phone messages in the current study showed a significant positive effect on the reduction of blood sugar in the case group after the educational intervention, which is consistent with the findings of similar studies in this field [42,43,44]. In patients suffering from diabetes, education is as valuable as medicine, exercise, and diet, because the treatment will never be effective unless the patient is well aware of the nature of his disease and takes positive steps to deal with it.

CONCLUSIONS

The results of this study demonstrated that educational interventions based on mobile phone short messages can be effective in enhancing self-care in patients with diabetes. Educational interventions aimed at empowering diabetic patients by strengthening self-care in them can be an effective way to improve the health of patients with diabetes. Therefore, it is recommended to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research.

The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

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Authors' contribution

All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Vice President of Research and Technology of Iran University of Medical Sciences with code IR.IUMS.REC.1397.501. Informed consent was obtained from all individual participants included in the study before conducting.

Consent to publish

All participants consented verbally to the publication of the interview data.

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ORIGINAL ARTICLE

Peculiarities of Military Values of Combatants with Signs of Post-traumatic Stress Reactions and Moral Traumas

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Abstract: *According to the results of the study, there were determined the peculiarities of military values of servicemen who participated in hostilities and had signs of PTSD and moral trauma. The study included 397 servicemen (aged 20 to 55 years) with combat experience. The study showed that PTSD and violation of moral normativity were closely related to unfavorable social conditions of performing combat tasks. Unlike PTSD, the moral normativity indicator was less tightly related to the immediate threat to life. The structure of values of the servicemen had no signs of PTSD as well as no violation of moral normativity consisted of factors that indicated the value of teamwork, responsibilities for decisions and actions made based on ideas related to loyalty to the oath and the Motherland, resistance, and professionalism. For the servicemen who participated in hostilities who had violations of moral normativity, it was more important to have the ability to act following their conscience, not to lose dignity, and to remain honest in the faces of their brothers than to have professionalism, loyalty to the oath and the Motherland.*

Keywords: *values, violation of moral normativity, serviceman, combat experience, post-traumatic stress disorder*

INTRODUCTION

The topic of the moral suffering of a serviceman who participated in hostilities had always been acquiring relevance when there occurred armed conflicts. However, after the Second World War [1], Wars in Vietnam [2], Korea [3], Iraq, and Afghanistan [4-5], etc. the topic of moral suffering was largely replaced by the study of psychiatric disorders of servicemen and, in particular, the disorders of combatants. However, the practice of providing psychiatric and psychological assistance showed that the approaches and activities proposed to overcome the symptoms of PTSD were ineffective to overcome the moral injury of the combatant. Thus, Gray, Schorr, Nash, and others [6], Griffin, Purcell, Burkman, and others [7], Maguen, Lucenko, Reger, and others [8] indicated that there was not any confirmed treatment for mental health disorders for today.

Thus, the construct of moral trauma was offered to describe the suffering that some veterans experienced while carrying out the actions during the fight that violated their belief in their kindness and benevolence of the world [9], and could undermine confidence in themselves, others and the world in general [10]. It gave the possibility to identify them as potentially traumatic experiences which differed from the traumas which were based on fear and were related to post-traumatic stress disorder. Thus, Bryan, Roberge, Leifker, and Rozek [11] found out that the servicemen, who faced a life-threatening injury and developed post-traumatic stress disorder, had the profile of symptoms that primarily included loss of memory, nightmares, memories, and exaggerated reaction concerning fright. Griffin et al. and Williamson et al. suggested that the profile of symptoms of those who underwent moral damages more often included a higher level of guilt, anger, shame, depression, and social isolation [12-13].

Frankfurt and Frazier believed that the study of moral damage had to occur in three directions: a) the definition, prevalence, and potential correlation of actions (e.g., military training, leadership, combat actions, and personality); b) the relationship between actions and syndrome of moral trauma (e.g., demoralization and self-injuring), and c) mechanisms of the genesis of moral trauma (e.g., shame, guilt, social isolation, and self-disposal) [9].

Joseph Wiinikka-Lydon pointed out that the nature of moral damage would be misunderstood if the determination of moral injury did not take into account the relationship which provoked the appearance of damage, so due to his words it was advisable to determine moral injury as the injury that occurred among people within moral ecology of wartime considering it to be a form of intersubjectivity [10].

Shay highlighted that the army could be considered a moral structure. The general expectations and values of the army formed the moral universe, which servicemen considered "legitimate", "natural" and "obligatory for themselves" [14]. Thus, the morality of the servicemen was tightly related to the military organization and depended on it, close to the fact that the army could make a serviceman ready to risk his/her own life. Due to the specific and strong moral connection between the serviceman and the army, according to Shay, its violation could have catastrophic consequences.

Williamson, Greenberg, and Murphy in their study determined that moral injury had negative consequences for psychological well-being. Among the examples of potentially morally injurious events (PMIE), which caused moral trauma and were described by British military veterans in the course of their study – bad treatment of civilians or hostile combatants, obtaining an order to violate the rules of combat, and disrespect for corpses [15].

It was quite clear that having such reasons, veterans did not readily resort to the discussion of the experience, which was often used in PTSD. Thus, Shay pointed out that civilians would rather prefer outspoken stories about injuries, as that could lead to doubts related to their moral beliefs and "could bring them back and disorient them" [14]. Although according to Shay, this attitude was understandable, as it came from the moral construction of a "normal human life" [14], he emphasized that it could interfere with treatment.

British researchers [15] found out that even discussing PMIE with friends, colleagues, or family members was considered catharsis; veterans did not report that it helped to solve their moral dissonance. Veterans did not consider social support to be a way to solve problems. The study by Williamson with colleagues showed that the solution of moral dissonance that arose in the sphere of value was described by veterans as a key turning point and led to a decrease in their emotional stress and improvement of daily functioning [15]. These researchers considered it quite promising to overcome the consequences of moral injury by finding ways to resolve the conflict between systems of values for example, military values against civilians), as well as conflicts within the system of values (for example, the conflict between military moral obligations such as respect for civilian life and combatants of opponents and respect for the value of colleagues' protection and successful mission performance.

Overcoming a moral conflict that arose was also important because the moral conflict, which was associated with a particular event, could generate fundamental moral conflicts that went beyond this event [16].

Describing existing studies of the category "moral trauma", it was necessary to note that from the very beginning of its research, the talk was about moral conflict, conflict in the sphere of values, but they were preceded by the studies conducted at the beginning of 2000s related to the peculiarities of value orientations of servicemen who had signs of PTSD after deployment.

Thus, almost decades ago, German researchers Zimmermann, Firnkes, Kowalski, Backus, Siegel, Willmund, and Maercer, worried that empirical studies of the peculiarities of valuable orientations of servicemen who had post-traumatic disorder after their deployment were very limited in time; the factors which were related to the deployment provided ideal scientific conditions" [17]. The studies which were available at that time concerning post-traumatic symptoms and personal values were devoted to the victims of crimes and people with adaptation disorders after critical life circumstances [18].

However, having formulated the topic of the study devoted to the values of the servicemen who had post-traumatic stress disorders due to deployment-related servicemen P. Zimmermann, Firnkes, Kowalski, Backus, Siegel, Willmund, and Maercer focused on personal values and did not pay attention to specific military values.

Almost at the same time as there was formulated the problem of value orientations by servicemen with signs of PTSD, there occurred studies were based on the study of values of servicemen who survived traumatic events. The studies were related to the differentiation between post-traumatic stress disorder and moral injury [16].

It could be confidently said that the study of the sphere of values and mental traumatization of servicemen became one of the reasons for reviving interest in the topic of moral injury of servicemen.

METHOD

Participants and Procedure

The presented article research had a fairly simple plan with a minimum of used psychodiagnostic techniques and groups, which was caused by the peculiarities of its conduct. The study was conducted within the frames of the program of restoration of servicemen who participated in hostilities. The examination was carried out before the rehabilitation to determine the depth of traumatization and peculiarities of the state of servicemen. The study had a searching basis and was intended to formulate problems important for the development of effective programmers related to the prevention of psychological traumatization of combatants.

The study was conducted in 2022. The study included 397 servicemen of combatants. The studied group consisted of officers – 29.84% (from a junior lieutenant to a colonel) and contract servicemen and mobilized – 70.16% (from a private to a senior warrant officer). The age of the studied participants ranged from 20 to 55 years.

Using the results obtained according to the methods "Maladjustment" and "Mississippi Scale for Combat-Related PTSD", a sample of studied servicemen who participated in hostilities was divided into three groups: group 1 – servicemen who did not have signs of maladjustment and PTSR – 277 individuals; group 2 – servicemen who had signs of violation of moral normativity (5 or more points according to the scale of VMN and more than 15 points according to the total indicator of maladjustment) – 59 individuals; group 3 – servicemen who had signs of post-traumatic stress reactions (78 or more points according to the Mississippi Scale) – 61 individuals. It had to be noted that all the servicemen, without any exceptions, who had signs of VMN servicemen also had signs of PTSD (78 or more points according to the Mississippi Scale). The third group included the servicemen who had signs of PTSD but did not have the peculiarities of VMN and maladjustment in general which exceeded the norms. Unfortunately, available tools did not give the possibility to divide the participants into groups without being specified in the second group "crossroad".

All procedures carried out in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants gave consent for their data to be used in this research.

Instruments and Measures

One of the reasons that there was a small number devoted to the studies of the "moral trauma" of servicemen was the lack of development of psychodiagnostic tools. In this study, as a criterion of moral trauma there was used a "violation of moral normativity" (VMN) – the scale of "maladjustment" techniques [19], which gave the possibility to differentiate individuals who had doubts about the expediency of using morals standards to regulate the relationships between individuals, as a result of which there was a loss of feeling of psychological well-being and signs of maladjustment arose.

The "maladjustment" technique was a rapid version of the "adaptability" technique [20], which had a long tradition of use in the Armed Forces of Ukraine. The "adaptability" technique was concluded by the Russian military psychologist Maklakov [20] to determine the adaptation potential of servicemen based on individual MMPI scales [21]. The developer reported the satisfactory psychometric characteristics of the concluded methodology. Since the beginning of hostilities in eastern Ukraine, the Maklakov methodology modification "Adaptability 200", formed by Chermyanin and Kondratiev, was widespread among military psychologists of Ukraine [22]. Unlike the previous methodology, it was supplemented by several issues that allowed us to distinguish "military-professional orientation", "tendency to deviant behaviors" and "suicidal risk" and to calculate "the level of resistance to combat stress". This technique consisted of 200 questions, so there was a need for a simpler and more reliable toolkit, which was the basis for the development of an express version of the methodology. The developed method of "maladjustment" [19] was aimed at the identification of specific and the most influential ability to adequately perform the tasks, and violations of adaptation potential. Thus, the technique had 4 scales, each with 10 issues – "violation of behavioral regulation", "probability of committing suicidal attempts", "violation of moral normativity", and "loss of communicative potential". There was the proposed calculation of the total indicator of "maladjustment". This calculation was simple and convenient and could be carried out in the field without any additional tools. Although the work with the technique involved the presence of trust in a psychologist, the technique had a scale of sincerity. The developers provided data on satisfactory psychometric indicators of the technique.

There was used the method "Mississippi Scale for Combat-Related PTSD" to determine the presence of post-traumatic stress reaction" [21]. At this time, it was one of the most common techniques used to measure PTSD symptoms of servicemen. The scale included 35 statements, each of which could be estimated under a five-point scale. The technique involved the process of distinguishing 3 levels – up to 77 points were normative indicators; 78-97 points were separate symptoms of PTSD; 98-145 points were the clinical variant of PTSD.

To determine the connection of violation of moral normativity with the action of combat stress factors, there was used the procedure of correlation analysis, and the method of "assessment of psychological trauma of combat experience of servicemen" by Doctor of Psychological Sciences Kolesnichenko [23] based on American methodology "Scales of assessment of combat experience" [24]. While developing the method there was taking into account the traumatic events and their intensity which was typical for the hostilities in Ukraine with the beginning of the Russian invasion in 2014. Unlike the "Scale of Evaluation of Combat Experience", this technique was a list of phenomena that reduced the trauma of the impact of combat stress factors. The technique had 5 scales that corresponded to 3 groups of combat stress factors and 2 factors that described internal and external resources to counteract stress factors. In the process of standardizing the technique, there was used regression analysis procedure, which gave the possibility to determine the contribution of each scale to acute stress disorder due to the action of combat stress factors. It was reported that the technique had satisfactory psychometric characteristics.

To determine the military values there was used a purposefully developed questionnaire.

At the end of 2021, there was developed a "questionnaire of determining the military values of servicemen of the National Guard of Ukraine" based on ideas related to the military values that existed in the armed formations of Ukraine, ethical codes of servicemen of European countries [25-26], historical information about the Cossack Ethical Code of the Warrior, availability of law enforcement and combat functions in the professional activity of NGU servicemen, a new experience of participating in the hybrid war and counteracting the numerically predominant opponent [27]. The questionnaire included a list of 11 generalized values including their content. The serviceman needed to evaluate the importance of values from this list using a scale in which 0 was the least important and necessary value for military service, and 10 was the most important and necessary value for military service. The scores had to be repeated.

In the proposed list for evaluation, there were generalized military values that united some values that described the experience of performing service and combat activity by servicemen:

1. To be physically brave – capable of controlling their fear, showing courage in situations that involved risks, injuries, and death; to be ready to give life for their Motherland as well as for their sworn brother.

2. To be morally brave – to find the strength to act correctly, and fairly, not to be indifferent to the injustice, grief, and sufferings of those whom they, being law enforcement, must protect; be human, humane, to be an example for imitation.

3. To be disciplined – to be guided by the legal requirements of the commander and the statutes; the execution of the commander's orders was the basis for the confidence of each member of the unit that would not be supplied, they would perform a part of their work accurately and in time, it was the basis for the coherence of the unit's actions, the basis for effective interaction with other units, and the basis for the unification of forces to achieve a common result.

4. To be self-disciplined – to perform their work without reminders, to meet high standards of professionalism and morality, so that their sworn brothers could trust them and rely on them, know that they would not fail, would not betray, and would not disappoint; to make the right choice and to be able to control their fear.

5. To be honest – one that could be trusted by fellow people; did not hide their shortcomings from sworn brothers, but corrected them, constantly professionally improving and developing. If necessary, they could be able to ask and receive help from their sworn brothers to get rid of defects, shortcomings in personal development, and vocational training. To report the conflicts of interest, about changes in their physical or mental state, so that the commander and sworn brothers could take this into account while performing the task, they had to know to what extent they could rely on the servicemen. Not to perform or hide the illegal and immoral actions of the sworn brothers, as it could potentially be the basis for blackmail, distrust, and a sense of guilt and, as a consequence, it could destroy the team, jeopardize the lives of other brothers and fail the accomplishment of the task.

6. To be worthy – to meet moral and professional requirements as well as to meet the standards of the profession. To follow their conscience and to follow the principles of Cossack chivalry. To improve constantly and professionally, and to acquire the necessary knowledge and skills required for the profession. To remain open to new professional experiences, not to dwell in their self-development, and to be self-critical.

7. To be a team member – to be able to rely on a sworn brother, to trust in a friend on weapons; be the one whom sworn brothers could rely on (competent, honest, faithful, disciplined, and predictable for a friend); respect sworn brothers and the commander, clearly obey the orders, as they were the basis for the unit control, the basis for concentrating the forces of the whole unit on the task and adjusting the forces of different units; to use humor to support and stimulate the development of sworn brothers, not to humiliate them; to prevent discrimination of comrades on weapons by nationality, race, and gender, etc.; to be an example and assistant for recruits. Being loyal to their unit did not mean

that they had to cover the illegal or immoral actions of the sworn brothers, as such actions made them and their unit open for manipulations and blackmail from the side of enemies. Their actions did not have to throw a shadow at their unit as well as at their sworn brothers. To be guided by the principle – "sworn brothers took the first place, then went the task (mission), and only after – their interests." Remember that military affairs were teamwork; it was effective when everyone did their part of the work completed.

8. To be faithful – to be faithful to the military oath – not to betray members of their unit; not to participate in any actions that could harm their weapons, not to provide any information related to the countries that were disloyal to Ukraine, not to express judgments that could devour the honor and dignity of the Ukrainian Army (National Guard), to harm its reputation; to be faithful to the Ukrainian people – to be faithful to the principles of independence, democracy, and protection of the rights and freedoms of the people of Ukraine; to be faithful to the traditions of the Ukrainian people, to imitate the traditions of Ukrainian Cossacks; to protect Ukraine and the way of life of the Ukrainians, to serve the interests of the Ukrainian people; to be faithful to themselves – not to betray themselves, their principles, to be reliable, integral, and predictable for sworn brothers (as a basis for effective interaction)

9. To be resistant – not to give up to the enemy, as long as there was strength and opportunities to resist themselves or as long as there was strength and opportunities to resist at least one of their companions on weapons; not to accept mercy from the enemy. Bravely face all the difficulties of service (not to complain, not to spread the information concerning household difficulties, and workload, as it could be used as a way of manipulation by opposing forces and of undermining the image of military service).

10. To be free – to take responsibility for their actions, to be capable of self-thinking and decision-making, to assert and maintain their point of view, to realize their values and principles, not to be manipulated, blackmailed, etc.

11. To be professional – to perform tasks at a high professional level, to constantly acquire and improve professional knowledge, skills, and possession of personal weapons; to maintain a physical form at a level that did not interfere with the tasks; to maintain their awareness of modern weapons, tactics, including those used by the enemy; to acquire and share professional experience with brothers, to be susceptible to the professional experience of others.

In 2022 there was published a study, which presented the peculiarities of the values of servicemen with different military-professional statuses [27].

Data Analysis

There was conducted the comparison of values in the studied groups using the Student's T-criterion. To determine the structure of the sphere of values there was used the procedure of factor analysis by Principal Components. The statistical analysis was performed using the SPSS 22 statistical package.

RESULTS

At the first stage of the study, it was necessary to consider the way how correlated psychological traumatization, the indicators of VMN (maladjustment), and PTSD with the factors of psychological traumatization of servicemen. The results of this stage of the study were presented in Table 1.

Table 1: Relationship between maladjustment and PTSD with the indicators according to the methodology “Evaluation of Psychological Traumatism of Combat Experience of Servicemen who participated in hostilities".

Scales	Unfavorable social conditions for performing combat tasks	Specific combat conditions which become traumatic	The unfavorable social situation of participation in hostilities	Internal resources of resistance – professional and psychological training	External resources – friendship, respect	General indicator, traumatization of experience
«Maladjustment»						
Violation of behavioral regulation	0.55***	0.29***	0.26***	-0.15**	-0.17**	0.40***
Likelihood of committing suicidal attempts	0.44***	0.13*	0.20***	-0.20***	-0.21***	0.41***
Violation of moral normativity	0.39***	0.16**	0.23***	-0.09	0.00	0.37***
Loss of communication potential	0.32***	0.14*	0.19***	-0.09	-0.27***	0.36***
General indicator of maladjustment	0.50***	0.22***	0.27***	-0.16**	-0.20***	0.42***
«Mississippi Scale»						
PTSD	0.57***	0.34***	0.26***	-0.18***	-0.26***	0.48***

*Note: *p≤ .05; **p≤ .01; ***p≤ .001*

As it could be seen from the proposed data, PTSD as well as violation of moral norms was the most closely related to "Unfavorable social conditions of performing combat tasks", such as the attitude of the population on the territories where there were the hostilities, media coverage of events, etc. However, unlike PTSD, VMN was weakly related to the direct threat to life – the number of shelling, bombings, encirclements, etc., which was described by the second scale of the methodology "Evaluation of Psychological Traumatism of Combat Experience of Servicemen who Participated in Hostilities" (r= 0.16, p≤ .01). Moreover, it was possible to highlight the data related to the relationship between the indicator of violation of moral norms (VMN) and the external resource that protected against traumatization – friendship, respect, attitude to the relatives – r=0.00. To some extent, they agreed with the presented data from a semi-structured interview with British researchers, who determined that social support was not crucial while overcoming moral trauma [15]. However, the relationship between the indicators of PTSD and "External resource", which was defined in this methodology as the support of family and friends, being statistically significant (r=-0.26; p≤ .001), was weak in terms of its density.

As could be seen, the VMN indicator was related to combat stress factors and factors that mitigated their impact, as well as moral trauma according to the existing descriptions of this psychological category. Therefore, it was considered to be possible to use the VMN indicator as one of the indicators of the presence of moral trauma in a serviceman, taking into account that currently there was practically no psychodiagnostic toolkit for its determination.

The next stage of the study was a comparison of military values and average ranks in selected research groups. These results were presented in Table 2.

As could be seen from the provided above data, the hierarchies of military values were quite similar in all three groups. The leading place in them was occupied by courage, and the least important was "Will" and "Resistance". However, each group had its characteristics.

Such peculiarities of the first group included the absence of expressed peaks and depreciations in the hierarchy – the gap between the largest ("Moral courage" (6.09±3.09)) and the smallest ("Will" (3.50±3.29)) indicators in the hierarchy of military values was minimal in comparison with others in the groups Moreover, in this group the indicators of courage ("Moral courage" (6.09±3.09), "Physical courage" (5.77±2.99)) and "Dignity" (4.68±2.89) had the lowest indicators among the compared groups (the latest feature also had the lowest rank in the hierarchy in comparison with other groups), and the indicators of "Professionalism" (5.03±3.47) and "Loyalty" (5.73±3.13) were the highest (the latest feature also had the highest ranks in the hierarchy of values in comparison with other groups). It was described in detail in comparison with other research groups.

Table 2: Indicators of military values among servicemen who participated in hostilities and had signs of VMN, PTSD, and those who did not have any disorders which occurred under the influence of combat stress factors

Military value	Studied individuals						The significance of difficulties		
	group 1		group 2		group 3		t_{1-2}	t_{1-3}	t_{2-3}
	without any disorders		with signs of VMN		with signs of PTSD				
	average	rank	average	rank	average	rank			
Physical courage	5.77±2.99	2	6.06±3.19	2	6.41±2.63	2	0.62	1.660	0.66
Moral courage	6.09±3.09	1	7.39±2.59	1	6.52±2.91	1	3.37**	1.03	1.730
Discipline	5.03±3.11	6.5	4.56±3.48	8	5.30±3.07	6	0.96	0.62	1.23
Self-discipline	4.90±2.89	8	4.72±3.49	6	4.44±3.00	9	0.37	1.08	0.47
Honesty	5.12±2.89	5	5.67±3.07	4	5.52±3.08	4	1.26	0.93	0.26
Dignity	4.68±2.89	9	4.78±2.56	5	6.19±3.22	3	0.27	3.37**	2.65*
Teamwork	5.59±2.91	4	5.83±2.71	3	5.41±2.93	5	0.63	0.43	0.83
Loyalty	5.73±3.13	3	4.67±2.06	7	4.85±3.00	8	3.25**	2.05*	0.40
Resistance	4.52±3.04	10	4.33±3.03	9.5	4.15±2.32	10	0.44	1.07	0.38
Will	3.50±3.29	11	4.33±3.51	9.5	3.15±3.07	11	1.660	0.81	1.96*
Professionalism	5.03±3.47	6.5	4.22±3.17	11	4.93±3.34	7	1.740	0.21	1.18

*Note: 0p≤ .1; *p≤ .05; **p≤ .01*

In the second group, such military value as "Moral Courage" (7.39±2.59) was emphasized and significantly elevated above the others. According to this indicator, servicemen of this group differed from the first group at a statistically significant level (t1-2=3.37, p≤ .01) and at the trend level from the group with signs of PTSD (t2-3=1.73, p≤ .1). Other most important values of servicemen with signs of VMN included "Physical courage" (6.06±3.19), "Teamwork" (5.83±2.71), and "Honesty" (5.67±3.07). In this group, there was no expressed depreciation of any values (average scores did not fall below 4). However, in contrast to other groups, the lowest scores, which occupied the last 11th position in the hierarchy of values, in this group belonged to such military value as "Professionalism" (4.22±3.17). The peculiarities of this group included significantly lower "Loyalty" indicators than in the first group (4.67±2.06; t1-2=3.25, p≤ .01) and slightly higher indicators of "Will" than in the other compared groups (4.33±3.51; t1-2=1.66, p≤ .1 and t2-3=1.96, p≤ .05), which only in this group did not occupy the last position in the hierarchy of values.

Comparing the ranks of servicemen who had signs of VMN (group 2) and servicemen who did not have signs of maladjustment and PTSD (group 1), the following information had to be noted: servicemen of group 2 had significantly higher ranks of "Dignity" (5 vs. 9). However, these ranks were lower than those of servicemen with signs of PTSD (group 3) and significantly lower ranks of "Loyalty" (7 vs. 3) and "Professionalism" (11 vs. 6.5).

Forming a general ethical portrait of this group, it was necessary to point out that for servicemen who had VMN, the ability to act following conscience, not to lose dignity, and to remain honest in the faces of their fellow men was more important than abstract ideas about professionalism, and loyalty to the oath and the Motherland. The typical peculiarity of this group was a higher level of personal responsibility for their actions, and reluctance to shift them on the actions posed by the orders or by the irresistible circumstances.

Although among servicemen with signs of PTSD (group 3), courage, as in other compared groups, occupied the highest position in the hierarchy of military values; however, in this group the indicators of "Physical courage" (6.41±2.63) were slightly higher than in the others. Thus, the differences with the group without any signs of VMN and PTSD occupied the level of the tendency to statistical significance – t1-3=1.66, p ≤ 0.1. Moreover, in this group, the differences between "Moral Courage" (6.52±2.91) and "Physical courage" (6.41±2.63) were minimal, which suggested that there was no expressed differentiation of these concepts in this group. Among the three most important military values of representatives of this group, in addition to "Moral" and "Physical courage", was "Dignity" (6.19±3.22). Such a high position of this value (rank 3) and significantly higher average values (t1-3=3.37, p ≤ .01 and t2-3=2.65, p ≤ .05) were a defining (significant) peculiarity of this group. Moreover, in this group, in comparison with all other groups, the indicators of "Will" were the lowest (3.15±3.07), according to which they differed at a statistically significant level from the group with signs of VMN – t2-3=1.96, p ≤ .05.

Comparing the ranks, it had to be pointed out that this group, in addition to the already indicated position of "Dignity", which differed from other groups, had a lower rank of "Loyalty" than in group 1 (8 against 3) and higher rank of "Professionalism" than in group 2 (7 against 11).

Thus, for the ethical portrait of group 3, it was typical not to allow oneself to be suspected of cowardice, they demonstrated courage that was not significantly guided by ideals – the protection of the Motherland or loyalty to the oath – but which was guided by the fear of not meeting the expectations of others, the fear of being accused of cowardice; for them, it was extremely important not to lose a sense of their dignity (respect of others and self-respect).

Perhaps the fact that servicemen without signs of maladjustment and PTSD were characterized by a lower level of "Courage" and "Dignity" and a greater orientation to "Professionalism" and "Loyalty" made their behavior more flexible and less dangerous – they were not so dependent on patterns of behavior, did not aspire to "heroism" and conflict, they focused on professional actions and loyalty to the oath and to the Motherland, which did not only reduce their victimhood but also made them more adequate concerning combat situations.

To clarify the obtained results, Table 3 there was presented the data of the factor analysis (with the procedure of simplifying the factor structure) for a better understanding of the structure of the sphere of values of servicemen who did not have signs of maladjustment and PTSD.

This factorial structure described 90.58% of dispersion peculiarities.

The first factor had a share of 21.91%. In other factors, the share was approximately the same and varied from 17.44 to 16.82%. The content of the first factor was determined by such variables as "teamwork" (0.819) and "loyalty" (0.794). This factor was marked as the conscious value of teamwork, belonging to the team, and brotherhood. The share of this factor reaffirmed that servicemen were aware of the importance and value of teamwork, and were aware of their strength as a group formation.

Table 3: The structure of military values of servicemen who did not have signs of maladjustment and PTSD

Variables	Factors				
	1	2	3	4	5
Dignity	-0.027	-0.010	-0.079	-0.080	0.984
Teamwork	0.819	-0.140	0.272	0.179	0.087
Loyalty	0.794	0.307	-0.130	-0.174	-0.141
Resistance	0.085	0.113	0.961	0.045	-0.083
Will	0.072	0.957	0.114	0.070	-0.006
Professionalism	0.010	0.065	0.046	0.977	-0.081

The second factor (17.44%) was closely linked to the variable "will" (0.957). It was interesting, that the content of this factor specified the variable "loyalty" (0.307). This factor was interpreted as their responsibility for the decisions made and actions taken based on their own beliefs about loyalty (oath, home, brothers, etc.).

The content of the third factor, the share of which was 17.26%, was correlated with the variable "resistance" (0.961). Some clarification of the content was made by the variable "teamwork" (0.272). This factor was marked as an awareness of the importance to keep up (being resistant) so as not to let their sworn brothers down.

The fourth factor (17.16%) was almost entirely determined by the variable "professionalism" (0.977), the reverse pole of this factor indicated the variable "loyalty" (-0.174). This factor was marked as the ability to act in the interests of the case, despite personal affection.

The fifth factor (16.82%) correlated with the variable "dignity" (0.984), such variables as "loyalty", "resistance", "will", and "professionalism" had weak feedback with this factor. This factor was marked as the ability to consciously comply with the given standards and standards of the profession and morality, which implied the ability to develop them, openness to the new, and the ability to self-criticism – "the ability to meet the changing situation". The presence of this factor in the structure of military values gave the possibility to be appropriate to changes that occurred during crucial events such as war.

There was developed a four-factorial structure of military values of servicemen with signs of VMN and PTSR (see Table 4).

Table 4: The structure of military values of servicemen who had signs of violation of moral normativity

Variables	Factors			
	1	2	3	4
Physical courage	-0.021	-0.100	-0.095	0.916
Discipline	0.915	-0.074	-0.007	0.030
Self-discipline	0.876	0.008	0.095	-0.271
Honesty	-0.210	0.870	0.138	0.101
Dignity	0.084	0.860	-0.162	-0.324
Loyalty	-0.713	0.078	0.043	-0.446
Resistance	-0.369	-0.386	-0.727	0.110
Will	-0.143	-0.178	0.927	-0.049

The factorial structure presented in Table 4 described 83.96% of dispersion peculiarities. The first factor had the highest share – 29.02%. Its content was determined by the direct connection between the values of "discipline" (0.915) and "self-discipline" (0.876). The reverse pole of the factor determined the value of "loyalty" (-0.713). The inverse relationship with “resistance” (-0.369) clarified the content of the factor. This factor was marked as the ability to comply with the requirement or internal conditions. This factor was not connected with beliefs or with endurance. The talk was mostly about the "habit" to be reliable for others to perform the assigned tasks, but not about the actions committed following abstract ideals – love for the Motherland, and loyalty to the oath.

The second factor (21.24%) was determined by the variables "honesty" (0.870) and "dignity" (0.860). Its content was clarified with the peculiarity "resistance" (-0,386). Unlike the fifth factor in the structure of servicemen who did not have signs of maladjustment and PTSD, this factor indicated not the correspondence of the current situation, but the correspondence of the existing relationships to the desire to meet the concept of decency in relations. It was the idea to preserve someone’s decency in the relationship that regulated their activity.

The positive pole of the third factor (18.17%) was almost entirely determined by the variable "will" (0.927), which described the importance of their responsibility for the decisions which were made as well as for the committed actions. The negative pole of the factor, like the first two, was associated with "resistance", but the third factor was much denser (-0.727). It was likely that servicemen, with the violated moral normativity, were aware that the maintenance of discipline and decent behavior as well as taking on full responsibility for their decisions and actions reduced their ability to be resistant which led to moral suffering.

The content of the fourth factor (15.53%) was interesting, which indicated that "physical courage" (0.916) was achieved by representatives of this group at the expense (resource of inverse relationship) of such values as "loyalty" (-0.446), "dignity” (-0.324), and “self-discipline” (0.271). Showing physical courage on the battlefield, these servicemen tried to maintain a previously created image of themselves, as decent, reliable people they used to consider themselves. It was possible that namely these resourceful connections could lead to exhaustion and moral crisis – a war that required brave actions, "squeezed" a drop of their usual ideas about themselves as reliable and decent people.

The structure of military values of servicemen who had signs of PTSD were given in Table 5.

Table 5: The structure of military values of servicemen who had signs of post-traumatic stressful reactions

Variables	Factors			
	1	2	3	4
Physical courage	0.782	-0.205	0.242	-0.090
Moral courage	0.887	-0.210	-0.046	0.028
Discipline	0.194	-0.196	0.194	-0.697
Self-discipline	-0.246	-0.268	0.772	0.055
Dignity	-0.763	-0.304	0.075	-0.091
Teamwork	0.255	-0.225	0.109	0.806
Honesty	-0.357	-0.032	-0.804	0.155
Resistance	-0.192	0.815	-0.066	0.173
Professionalism	0.067	0.810	-0.141	-0.179

DISCUSSION

The analysis of the results gave the possibility to describe servicemen who did not have signs of maladjustment and PTSR, as they were able to meet the difficult situation of participation in hostilities. These servicemen were not guided by the distorted ideas of courage and did not try to prove to anyone that they were not cowards, but instead, they acted being guided by their ideas related to professionalism and loyalty to the oath and the Motherland, due to that they were less victimized and more appropriate to the combat situations. Probably, such characteristics of the sphere of values of these servicemen could be reflected at the personal level of view of the army as a unique "moral structure" described by Shay [14], which gave it a possibility to be integrated into servicemen not only to maintain mental health but also to sacrifice their lives. These data confirmed previously made conclusions concerning the importance of relevant secondary socialization (professionalization) of servicemen, as prevention of their psychological traumatization under combat conditions [28] (p. 125).

Unlike the servicemen who were integrated into the moral construction of the army, combatants with signs of VMN had less abstract ideas about loyalty and become hostages to their image of a decent person, which could be counted by their sworn brothers. They were aware that keeping this image under the conditions of the war was the path of suffering and consciously they followed it. Every manifestation of physical courage for these servicemen became a step that took them away from themselves – from the ideas of their honesty and humanity. And despite this, they did not try to transfer responsibility for the actions taken into insurmountable circumstances and did not try to justify themselves with the need to act by order. These data expanded the idea of moral conflict, as the basis for moral trauma, described by Williamson with colleagues [15].

The obtained results also demonstrated that for servicemen with signs of VMN, as one of the manifestations of moral injury, social support was not a resource capable of solving their problems. These data agreed with the British researchers who determined that social support was not crucial in overcoming moral injury [15]. Moreover, the existing data showed that the construct of "moral injury" was more related to the social context of the situation, than the immediate threat to life.

The obtained results in the group of servicemen with signs of PTSD were quite interesting. Their traumatization, as expected, was related not only to the social context of participation in hostilities but also to the immediate threat to life. However, it was an unexpected assumption of the greater number of victims of these servicemen in comparison with those who had no signs of maladjustment and PTSD. It could be assumed that these servicemen could be hostage to their ideas about the need to manifest courage during hostilities, it was extremely important for them that others did not suspect them of cowardice.

For these servicemen, participation in hostilities was a life on the verge of their ideas about their capabilities, it was a test. It could be assumed that they had "excessive" expectations from their participation in hostilities, the requirements of the profession of a serviceman, which contributed to their exaggerated response to the situation of participation in hostilities (greater courage, aggressiveness, suspicion, etc.) and, accordingly, to the higher level of exhaustion. And although the assumption of "excessive expectations" as a peculiarity of their greater victimization was only an assumption related to the possible mechanism of formation of psychological traumatization of a military serviceman who participated in hostilities, its formulation, and further testing were important for the development of effective PTSD prevention programs.

Nowadays it was quite traditional to study the changes in the relationship under the influence of PTSD, but mainly they are related to the civilian life of veterans. Thus, in particular, Caska-Wallace, Smith, Renshaw, and Allen investigated the impact of PTSD on the marital relations of servicemen [29], Ellon, Colvonen, Haller, Norman, and Angkaw determined that the severity of PTSD was the only significant sign. The topic of the relationship between servicemen and the peculiarities of PTSD in the military team and with the commander was less studied. Moreover, these and other studies did not determine the role of values as regulators of relationships that were changed as a result of the psychological traumatization of servicemen.

Harper, Stanley, Exline, Pargament, Fletcher, and Teng found out that betrayal perception was positively linked to post-traumatic stress disorder. They suggested that the mechanism of communication of a potentially morally injurious event (PMIE) and symptoms of post-traumatic stress disorder was social separation (“promiscuity” from Russian). The mentioned researchers emphasized that the study of this mechanism was extremely limited. In the secondary analysis, they were able to distinguish that the interaction between betrayal and perceived social support (another significant issue and family) gave the possibility to predict the symptoms of post-traumatic stress disorder [31]. In the conducted research it was found that such values as "loyalty" of servicemen with signs of post-traumatic stressful reactions had the opposite position in the hierarchy of military values in comparison with the servicemen who had no signs of PTSD. And although the format of the study did not give the possibility to conclude whether the "loyalty" factor contributed to the development of PTSD or, conversely, the acquisition of signs of post-traumatic stress reactions led to the depreciation of "loyalty", it was also possible to assume that "social disconnection" was an important mechanism for the development of PTSD symptoms and there was a need in further study of the role of value regulation in the implementation of this mechanism.

Nowadays there were widespread studies that demonstrated that the cohesion of the military team was connected with a lower level of severity of post-traumatic stress disorder [32]. The conducted research gave the possibility to formulate another problem of the research – psychologically injured servicemen could reduce the coordination of the military team and the coordination of its actions.

The given data demonstrated that the PTSD indicator had a statistically significant, but the not very dense connection with social support as an external overcoming resource. These and other results of the study gave the possibility to suggest that more promising for determination of the ways of prevention of psychological traumatization of servicemen was not the study of social cohesion of military teams, but the study of the peculiarities of professional socialization of servicemen in which the cohesion of the military team was a derivative characteristic. In light of this, it was quite possible to assume that the study of the content and process of internalization of military values, as a means of professional socialization of servicemen, and their acquisition of professional identity, could expand the idea of ways of preventing psychological traumatization of servicemen who participated in hostilities.

CONCLUSIONS

The conducted study demonstrated that PTSD and VMN indicators were most closely related to adverse social conditions available for combat task performance. Unlike PTSD, VMN was less densely related to a direct threat to life. Social support had no preventive effect on VMN. The connection of a factor that described social support with PTSD was weak but statistically significant.

The servicemen who participated in hostilities and had no signs of PTSD and VMN had a slightly lower level of indicators of the value “bravery” in comparison with other studied groups, while they had a higher level of “professionalism” than in other groups of the study. Their structure of values consisted of the factors that testified to the value of teamwork; responsibilities for decisions and actions made based on ideas related to loyalty to the oath and the Motherland; resistance; professionalism as the ability to act in the interests of the case, despite personal affection; and the ability to be appropriate to the changing conditions. Such peculiarities made their pain integrated into the "moral construction of the army" and more appropriate (adaptive) to the combat situations, where dominated teamwork and professionalism as opposed to unjustified risks and excessive reflection.

For the servicemen who participated in hostilities and had VMN, it was more important to have the ability to act consciously, not to lose dignity, and to remain honest in the faces of their sworn brothers than to have professionalism and loyalty to the oath and the Motherland. The typical peculiarity of this group was a higher level of personal responsibility for their actions, an aversive to transfer it to the actions by the order or too insurmountable circumstances. In the structure of their values, the leading place was occupied by the factors that described the ability to comply with the standards; to remain honest in front of their sworn brothers; to be quite responsible for their actions. These factors in the group of servicemen who had VMNs were related to the realization that they made them vulnerable in combat. The process of following them led to suffering. The need to manifest physical courage (as the ability to be aggressive against another person) "squeezed" a drop of their usual ideas about themselves as reliable and decent people.

The "excessive" courage of servicemen who had signs of PTSD was a consequence not only of the desire to be guided by the ideals – the protection of the Motherland or faithfulness to oath, etc. – but the consequence of the fear not to meet the expectations of others, the desire not to lose their sense of dignity (respect of others and self-esteem). In the structure of their values, a leading place was occupied with courage and professionalism. Both of these factors in the group of servicemen who had the symptoms of PTSD involved the attitude toward them as if they required actions on the verge of their capabilities. Other factors in the structure of their military values indicated that they had a sense of unity with their team – leadership of their ideas concerning the relevance of actions, which made them less controlled and unreliable for brothers-in-arms as well as commanders under the combat conditions.

The presented study was mainly directed to theoretical research, and its main results were the formulation of research problems that were important for the development of an effective program for the prevention of psychological traumatization of servicemen who participated in hostilities. Unfortunately, the used format of the study did not give the possibility to state whether the peculiarities of the sphere of values made servicemen sensitive to the action of combat stress factors and to the specific reaction concerning them in the form of PTSD or VMN (moral injury), or vice versa, changes in the sphere of values were the results of the formation of signs of PTSD and VMN by servicemen who participated in hostilities. Moreover, there was a need for further research to clarify the role of military values in the mechanism of PTSD and VMN formation. Similarly, it was required to study and verify the suggestion related to the greater number of victims of servicemen who, participating in hostilities, acquired signs of psychological traumatization (maladjustment and PTSD). It was also advisable to study the impact of the peculiarity of secondary (professional) socialization of servicemen and their acquisition of military-professional identity as a factor in the prevention of psychological traumatization of a serviceman under combat conditions.

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Authors’ contribution
Conceptualization, YaM. and O.K; managed the project, I.P.; research, K.M.; writing – original draft preparation, Yu.R., I.T., and R.G.; methodology, D.P. All authors read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
All procedures followed were by the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

Consent to publish
Informed consent was obtained from all subjects for inclusion in the study while maintaining their confidentiality. Written informed consent was obtained from all subjects to publish this paper.

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ORIGINAL ARTICLE

Symptom Severity from Posttraumatic Stress is Not Correlated to C-reactive Protein Level or Altered by Ketone Salt Supplementation

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Abstract: *This study aimed to determine if: 1) C-reactive protein (CRP) level correlated with PTSD symptom severity as measured by PTSD Checklist-5 (PCL-5), and 2) ketone supplement reduced PTSD symptoms. Twenty participants with PTSD were randomly assigned to a ketone supplementation or placebo group and evaluated for 6-weeks for PTSD symptom severity and CRP levels. The Spearman Rank correlation between PCL-5 and CRP was positive, but not statistically significant. Mixed models on the ranks of the data were used to examine the difference in PCL-5 and CRP between ketone and control groups over time. Median PCL-5 at the pre-test was higher than at the post-test in the ketone group but not in the control. The reduction of PCL-5 medians between ketone and control groups at the post-test was not different. The median pre-test CRP was not different from than post-test in the ketone group or control group. This pilot study did not find a correlation between CRP levels and PTSD symptom severity but was limited by a lack of CRP data collection due to the loss of follow-up. PTSD symptom severity was not altered by 6 weeks of chronic ketone salt supplementation compared to the control group but should be examined in a larger sample size for further verification.*

Keywords: *PTSD; inflammation; PTSD Checklist-5; ketone salts; anxiety*

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a severe mental illness that can develop after an individual is exposed to a traumatic event. Traumatic events which can trigger PTSD include, but are not limited to, exposure to combat, sexual violence, interpersonal violence, and accidental violent death. The lifetime prevalence of PTSD ranges from 7-8% in the United States [1].

PTSD is associated with recurrent, involuntary, and intrusive memories and nightmares of the traumatic event. Individuals often feel that the traumatic event is recurring, which can be triggered by exposure to stimuli that evoke memories of the event. These symptoms are incredibly distressing and can promote avoidance of situations that involve triggers, potentially leading to impairment in daily activities [2]. PTSD is also associated with significant cognitive and mood dysfunction [3]. Furthermore, patients suffering from PTSD have higher rates of both psychiatric and physical comorbid disorders than the general population. Psychiatric comorbidities include substance use disorder, anxiety disorders, and depressive disorders [4]. Physical comorbidities found in veterans include greater incidences of cardiovascular, musculoskeletal, metabolic, and neurological diseases [5].

While the symptoms and morbidity associated with PTSD are well-documented, treatment continues to present a challenge, due to the lack of clear understanding of the pathophysiology of the disorder, and the barriers to access to treatments that have been proven to be effective. Current treatment guidelines vary, but generally recommend psychotherapy as a first-line therapy [6,7], with the addition of SSRIs or SNRIs if refractory, although some suggest that SSRIs should be part of initial therapy [8-12]. Prazosin has also been shown to be effective in reducing symptoms of PTSD, especially in individuals with elevated blood pressure [13]. However, all of these treatments have considerable drawbacks and barriers. Psychotherapy of any kind is time-consuming and often expensive, and many patients with PTSD do not have access to the consistent care necessary for improvement; even with improved access to care, many people do not utilize resources available due to internal factors, such as lack of motivation or fear of embarrassment due to stigma associated with the disorder and mental health in general [14]. SSRIs and SNRIs have a wide range of side effects, including weight gain and sexual dysfunction, and are sometimes discontinued for these reasons [15]. Additionally, because individuals with PTSD are at higher risk of comorbidities such as diabetes and obesity, these side effects are especially unfavorable. For these reasons, a nutritional supplement that could reduce the symptoms of PTSD in affected individuals would be of considerable value in reducing the burden of the disorder in many individuals.

Past research has shown that there are higher levels of pro-inflammatory cytokines in combat-exposed males with PTSD than in combat-exposed males without PTSD, suggesting that inflammation may play a role in PTSD symptomatology [16, 17]. Another study found that higher levels of C-reactive protein (CRP) were associated with increased PTSD symptom severity in African American women with Type 2 diabetes mellitus [18]. Several other studies have found that PTSD is correlated with higher levels of CRP, but the populations only included African Americans and veterans, respectively [19,20]. For the results to be generalizable, studies that identify the relationship of CRP (and inflammation) with a mixed demographic diagnosed with PTSD are needed.

Ketones are molecules naturally produced by the liver during limited carbohydrate availability that act as both a fuel source and signaling molecule. They are widely available as commercial supplements and are used for a variety of purposes. Ketones possess physiologic properties such as anti-inflammatory actions that may provide benefits for PTSD symptoms. Beta-hydroxybutyrate, a ketone, has been shown to inhibit NLRP3 inflammasome activity in human monocytes, displaying the potential anti-inflammatory effects of ketogenic diets/supplements [21]. In a case report, a ketogenic diet was shown to treat Crohn's disease and significantly reduce levels of CRP [22]. In addition, current evidence suggests that ketogenic diet therapies can be therapeutic in treating epilepsy, nonmotor Parkinson's disease, cognition and cerebral blood flow in Alzheimer's patients, and Type 2 diabetes [23]. However, there are no studies that investigate the effect of ketone supplementation on CRP levels in PTSD patients. Therefore, the questions posed by this preliminary report are:

(1) Do high levels of CRP—as an inflammatory blood marker—correlate with PTSD symptom severity (measured by PTSD Check List – 5 questionnaires)? We hypothesize that CRP levels positively correlate with PTSD symptom severity.

(2) Is there a difference between the level of CRP in blood in the group of participants receiving adjunct ketone supplements versus placebo? We hypothesize that patients on a ketone supplement have lower CRP levels compared to those on a placebo.

MATERIALS AND METHODS

Experimental Design

In this randomized, double-blinded, placebo-controlled clinical trial, participants were assigned to one of two groups—an experimental group receiving an adjunct ketone supplement to their usual treatment (or no treatment) and a control group receiving an adjunct placebo. The duration of the trial was 6 weeks, with either the ketone or placebo supplementation consumed twice daily. Phone calls, adherence logs, and text messages were used to monitor compliance with the regimen. Participants visited the laboratory for a familiarization visit which included an explanation of the study, proper completion of the informed consent, and collection of demographic and medical history information. Participants were also advised to fast for 10 hours (only water was allowed) and withhold from exercise, caffeine, and nicotine for 12 hours before the following data collection visits. Following familiarization, participants visited the laboratory twice for data collection: once at baseline and then again at the end of the 6-week intervention. The study was approved by the University's Institutional Review Board, and all procedures performed complied with the institutional guidelines. This study was registered as a clinical trial (ClinicalTrials.gov Identifier: NCT04083352) and a CONSORT 2010 Flow Diagram is provided in Figure 1.

Participants

A total of 20 participants were recruited. Participants were recruited via the Veteran Success department, paper flyers on the University's campus and in the local community, a local news interview, the Mood and Trauma Outpatient Clinical Program, Veteran organizational meetings, and the local Warrior Project. The study inclusion criteria were: (1) a diagnosis of PTSD and (2) men and non-pregnant women from 18-65 years old. No exclusion of PTSD patients who were taking medication for psychiatric comorbidities. Exclusion criteria were pregnancy, chronic smoking, and/or taking cardiovascular-related medications 12 hours before and during evaluations. Patients who were taking prescribed medication continued doing so at the same dosage throughout the trial.

Figure 1: CONSORT 2010 Flow Diagram.

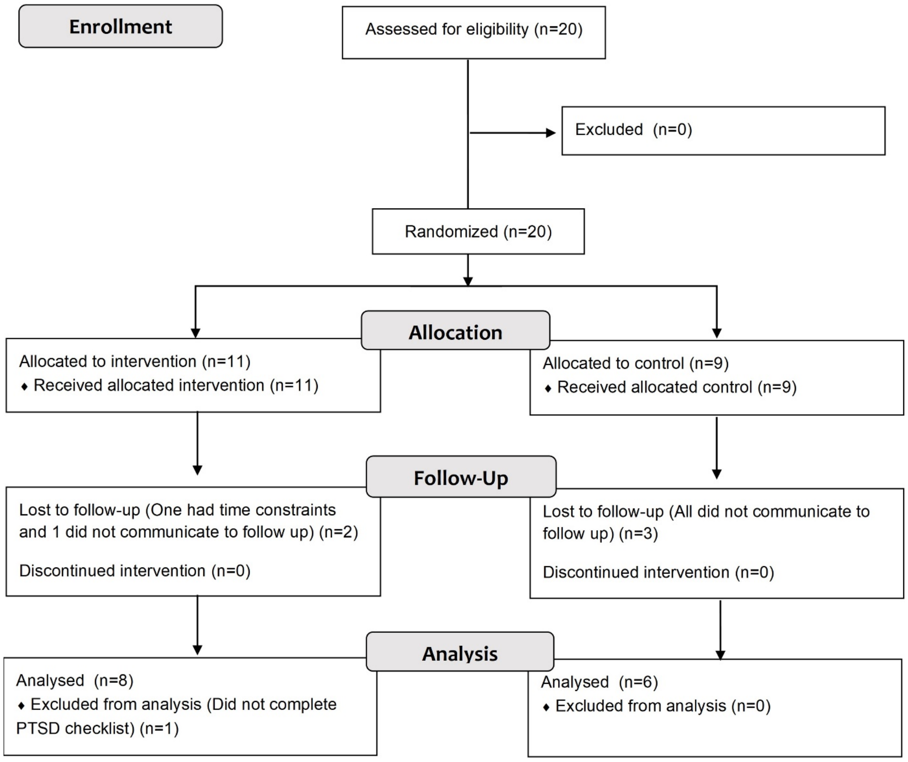
Enrollment
Assessed for eligibility (n=20)
Excluded (n=0)
Randomized (n=20)
Allocation
Allocated to intervention (n=11)
♦ Received allocated intervention (n=11)
Allocated to control (n=9)
♦ Received allocated control (n=9)
Follow-Up
Lost to follow-up (One had time constraints and 1 did not communicate to follow up) (n=2)
Discontinued intervention (n=0)
Lost to follow-up (All did not communicate to follow up) (n=3)
Discontinued intervention (n=0)
Analysis
Analysed (n=8)
♦ Excluded from analysis (Did not complete PTSD checklist) (n=1)
Analysed (n=6)
♦ Excluded from analysis (n=0)

Measures

To determine PTSD symptom severity, the PTSD checklist (PCL-5) was completed by each participant at the beginning and the end of the trial with an interviewer present for questions or clarification. C-Reactive Protein (CRP) was also measured for each participant at both the beginning and end of the trial. To measure CRP, venous blood samples were collected by an investigator and immediately transported to the Clinical Pathology Laboratory at the University's Hospital for subsequent analyses. The CRP analysis was performed on an automated chemistry analyzer (ADVIA 1800 Chemistry System; Siemens Healthineers, Erlangen, Germany).

Supplement

Participants were randomized to consume a ketone salt supplement or a placebo in conjunction with their normal diet and medication regimen. The ketone salt and placebo supplements were in powder form. The participants were instructed to take two packets a day mixed with water, once in the morning and once in the afternoon, for 6 weeks. The ketone salt supplement included 7 g of beta-hydroxybutyrate combined with erythritol, L-Taurine, fermented L-Leucine, citric acid, natural flavor, vegetable juice color, stevia, xanthan gum, beta carotene and approximately 920 mg of sodium (KETO//OS® MAX, Prüvit Ventures, Inc., Melissa, TX). The placebo supplement included maltodextrin and similar color, sodium, and flavoring, as the ketone salt supplement. Both supplements contained 41 kcal per serving.

Statistical Methods

All statistical analysis was performed using SAS 9.4 and statistical significance was assessed using an alpha level of 0.05. Due to the small sample size, rank-based and exact methods were used for all analyses. Descriptive statistics were determined by the ketone group and included frequencies and percentages for categorical variables and medians and interquartile ranges for continuous variables.

Fisher's Exact tests for categorical variables and Wilcoxon rank sum tests for continuous variables were used to examine differences by ketone groups for baseline values. Spearman Rank correlations between PCL-5 and CRP at baseline were determined.

Mixed models using the ranks of the data using the minimum variance quadratic unbiased estimation of covariance parameters method of estimation and assuming an unstructured covariance structure were used to examine changes over time between ketone groups for PCL-5 and CRP.

The denominator degrees of freedom were calculated using the Kenward-Roger method for factors in the model that did not involve time, and for factors that did involve time the denominator degrees of freedom were infinity. Each model included fixed effects of the ketone group and time as well as the two-factor interaction.

The F-test for the two-factor interaction was the statistical test of interest and the statistical significance of this F-test was determined using the Brunner, Domhof, and Langer (2002) method [24].

A Bonferroni adjustment to the overall alpha level was used to examine post hoc pairwise differences.

RESULTS

Table 1 gives the descriptive statistics by ketone status and the results for examining differences in baseline values. Briefly, 27% of those in the ketone group and 11% in the control group were male. The average age in the ketone group was 44 yrs and 50 yrs in the control group. There were no statistically significant differences between the ketone and control groups for gender, age, PCL-5, or CRP. The Spearman Rank correlation between PCL-5 and CRP was positive, but weak with a value of 0.21 (n=12, p=0.5149), and was not statistically different from zero.

Table 1: The descriptive statistics by ketone status and the results for examining differences in baseline values are shown in this table. *Abbreviations: CRP, c-reactive protein; n, number; PCL-5, PTSD Checklist 5; Q, quartile; SD, standard deviation*

Variable	Ketone N=11 (55%)	Control N=9 (45%)	P-value
Male Gender – n (%)	3 (27.3)	1 (11.1)	0.5913
Age – mean (SD)	44.8 (9.1)	40.0 (12.7)	0.3017
Baseline			
PCL-5 – median (Q1-Q3), n	58.5 (38.0-67.0) N=10	34.0 (26.0-60.0) N=9	0.1207
CRP – median (Q1-Q3), n	0.39 (0.30-0.73) N=6	0.47 (0.09-1.62) N=6	0.8099
Follow-Up			
PCL-5 – median (Q1-Q3)	54.0 (30.5-60.0) N=8	34.0 (19.0-36.0) N=6	
CRP – median (Q1-Q3)	0.62 (0.26-0.87) N=5	0.27 (0.09-0.46) N=2	

Table 2: The repeated measures non-parametric mixed model results examining differences in PCL-5 or CRP over time between groups on the ranks of the data are shown in this table. *Abbreviations: CRP, c-reactive protein; PCL-5, PTSD Checklist 5; Post, after the 6-week supplementation period; Pre, baseline; Q, quartile*

Outcome	Ketone Group	Time	Unadjusted Median	Unadjusted Q1-Q3	F	p-value
PCL-5	Ketone		55	38-67	2.77	0.1231
	Control		34	25-49		
		Pre	49	29-66	10.99	0.0009
		Post	38.5	20-57		
	Ketone	Pre	58.5	38-67	0.07	0.7988
		Post	54	30-60		
	Control	Pre	34	26-60		
		Post	34	19-36		
CRP	Ketone		0.47	0.26-0.87	0.15	0.7120
	Control		0.40	0.09-1.11		
		Pre	0.41	0.19-0.80	0.00	0.9580
		Post	0.46	0.09-0.87		
	Ketone	Pre	0.39	0.30-0.73	0.51	0.4520
		Post	0.62	0.26=0.87		
	Control	Pre	0.47	0.09-1.62		
		Post	0.27	0.09-0.46		

The results examining differences in PCL-5 or CRP over time between groups on the ranks of the data are given in Table 2.

The interaction between ketone and time was not statistically significant for PCL-5 indicating that the changes over time in the median PCL-5 scores in the ketone and control groups were not different.

The median PCL-5 at the pre-test was statistically, but not clinically, significantly higher than at the post-test (p=0.0003) in the ketone group but not in the control group (p=0.4418). There were no statistically significant differences in the PCL-5 medians between the ketone and control groups at the pre-test (p=1.0000) or post-test (p=0.6020). The interaction between ketone and time was not statistically significant for CRP indicating that the changes over time in the median PCL-5 scores in the ketone and control groups were not different. The median CRP at the pre-test was not significantly different from than post-test (p=1.0000) in the ketone group nor in the control group (p=1.0000). There were no statistically significant differences in CRP between the ketone and control groups at the pre-test (p=1.0000) or post-test (p=1.0000).

DISCUSSION

In this study, we did not find a statistically significant or clinically meaningful correlation between CRP levels and PTSD symptom severity. In addition, there was no significant difference in baseline CRP levels in patients in the ketone supplement group compared to the placebo group.

Several studies have examined the relationship between inflammation and PTSD. Lindqvist et al. examined the relationship between pro-inflammatory cytokines and PTSD in combat-exposed males. The study included 51 participants with PTSD and 51 participants without PTSD, comparing their "pro-inflammatory score", which consisted of summed standardized scores of Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), Tumor Necrosis Factor-alpha (TNFα), Interferon-gamma (IFNγ), and CRP. PTSD symptom severity was measured using the Clinician-Administered PTSD Scale (CAPS). The study found that subjects with PTSD had significantly higher pro-inflammatory scores compared to those without PTSD, with a p-value of 0.002 after controlling for early life trauma, depression diagnosis and severity, ethnicity, asthma/allergies, body mass index, education, possible confounding medications, and time since combat exposure. However, within the PTSD group, the pro-inflammatory scores were not significantly correlated with CAPS total score [16].

Lindqvist et al. conducted a replication study with a different cohort (31 combat-exposed participants with PTSD and 30 combat-exposed participants without PTSD). Again, they found that those with PTSD had higher total pro-inflammatory scores compared to those without PTSD. In addition, there was no significant correlation between pro-inflammatory score and PTSD symptom severity [17].

More recently, a study by Powers et al. examined the relationship between inflammation and PTSD. The study consisted of 55 traumatized African American women with type 2 diabetes mellitus. Participants were selected from a publicly funded hospital in Atlanta, Georgia, drawing from a low socioeconomic, urban minority population. The Clinician-Administered PTSD Scale (CAPS) was used to measure PTSD symptom severity, and CRP levels were taken as a measure of inflammation. The study found that CRP levels were positively correlated with PTSD symptom severity (p<0.01) [18].

Our study results indicated that CRP levels were not correlated with PTSD symptom severity. However, there are several differences to note. First, we measured PTSD symptom severity with PCL-5 rather than CAPS, which was the assessment used in previous studies. In addition, our study had a very small sample size—only 7 participants had data on both baseline and follow-up CRP levels. Because of this, it is difficult to draw any meaningful conclusions regarding CRP from the results obtained from this study.

One finding of note was that there was a statistically significant difference between pre-test and post-test PCL-5 scores in the ketone group, but not in the control group. However, because the difference was small and the difference in pre-test mean scores was large, this finding is not clinically significant. However, it does indicate that ketone supplementation should be further studied as a method of symptom reduction for PTSD patients. Future studies should aim to have a larger sample size to hold more statistical power, as the small sample size was a limitation of this pilot study.

CONCLUSIONS

This pilot study did not demonstrate a correlation between CRP level and PTSD severity; a statistically significant nor clinically meaningful correlation was not found between CRP levels and PTSD symptom severity measured by the PCL-5. In addition, there were no differences in CRP levels between the ketone salt supplementation and placebo groups, which suggests that ketone salt supplementation may not alter the levels of this inflammatory marker in individuals diagnosed with PTSD. However, as mentioned in the discussion, the study was limited by small sample size and ketones as a method for symptom reduction in patients with PTSD merit follow-up with a larger scale study due to their potential to provide more accessible relief if they are proven to be effective.

Conflicts of interest and sources of funding

The authors declare no conflicts of interest. Angelia Holland-Winkler received funding for this project from Augusta University's College of Education and Human Development and Prüvit Ventures, Inc. Ketone and placebo supplements were provided by Prüvit Ventures, Inc.

Authors' contribution

Authors include Langston Gilmore, Nagy Youssef, Jennifer Waller, Rebecca Nalloor, Max Wieder, and Angelia Maleah Holland-Winkler (PI). AMH-W designed the research, AMH-W and RIN collected the data, JLW analyzed the data, LBG, NAY, JLW, RIN, MAW, and AMH-W drafted and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by Augusta University's Institutional Review Board with approval number 907405. All subjects signed an approved and stamped informed consent document before participating.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Investigating the Relationship Between Social Responsibility and COVID-19 Morbidity in the Population Covered by Health Centers in Iran

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Abstract: *Introduction: Social responsibility and, is an indicator that is important in the epidemic of diseases with high prevalence and death rates. The current study aims at determining the relationship between social responsibility and COVID-19 in the population covered by health centers in Iran. Methods: 500 people participated in this descriptive-analytical study. The data was collected using a researcher-made social responsibility questionnaire in the context of the Covid-19 pandemic in three areas general, individual, and social responsibility. The quantitative content validity method was used to measure the validity of the questionnaire and Cronbach's alpha calculation method was used to measure its reliability. The data were analyzed using SPSS-21 software. Findings: Among the participants, 4.32% of them had a history of covid-19, and the history of complications was more in men (51.9%) and the mean social responsibility score of women was higher (P<0.001). The average responsibility score was higher in people without a history of Covid-19 than in people with a history of Covid-19 (P=0.006). Conclusion: Individuals with higher social responsibility observed more health protocols and had lower rates of covid-19 infection among them. Therefore, in the design of educational interventions, planning should be done to increase people's sense of social responsibility.*

Keywords: *social responsibility, health, COVID-19*

INTRODUCTION

The prevalence and incidence of various infectious diseases throughout history have been one of the most serious threats to human health; the most recent of which is the emerging COVID-19 virus from the coronavirus family [1]. COVID-19, which appeared in Johann, China in December 2019 [2] is considered a threat to the health and lives of millions of people worldwide [3]. This disease has changed the lifestyle of millions of people around the world [3], and in Iran, it is very prevalent, endangering the physical and mental health of people [4]. Individuals' responsibility for health is one of the most important aspects of a healthy lifestyle, with which a person chooses a lifestyle to maintain and promote his health and prevent diseases [5].

Since a human is a social being and lives in society from the moment of birth to the end of life and is in constant interaction with other human beings, he/she must always learn how to satisfy his/her life and needs in society. This requires learning social skills. One learns these skills within the socialization process, and if these skills are learned properly, it can lead to the formation of social responsibility [6].

According to Glaser, responsibility is formulated in the form of three components: first, learning to choose behaviors that meet our basic needs, second, accepting the consequences of our behavior, and third, it should not be an obstacle to meeting the needs of others [7]. Considering that social responsibility improves social abilities, academic motivation, attention to moral and social concepts, personal adaptation, sense of accountability to others, commitment to society, etc [8]. and given that responsibility is the outcome of three characteristics of recognition, decision, and creativity, so responsible individuals are conscientious, confident, serious, and possibly disciplined [6]. Besides, the goal of the health system is to promote the health of the community, and the factors that shape the behavior cause prevention and reduction of diseases and improvement of the community health, and the best platform and solution to achieve this goal are measures related to social responsibility [9]. Thus, considering previous research findings, and given the importance of the subject of social responsibility in the health of individuals, and since no study has been conducted on the relationship between social responsibility and COVID-19 in Iran, the present research was carried out for investigating the relationship between social responsibility and COVID-19 morbidity in the population covered by health centers of Iran in 2021.

MATERIALS AND METHODS

The present study is a cross-sectional research (descriptive-analytical) that was conducted on 500 individuals covered by Andimeshk city health centers in 2021. Andimeshk city is located in the north of Khuzestan province and southwest of Iran.

Inclusion criteria were having informed consent to participate in the study and literacy of completing the questionnaire, and exclusion criteria included the reluctance of the participant in each stage of the study and incomplete completion of the questionnaire.

The questionnaire used in this study is an author-made questionnaire on social responsibility during the COVID-19 pandemic. The designed questionnaire includes 13 demographic items and 32 items related to responsibility. With a review of related literature, two areas were considered including self-health responsibility for own health (individual area) and health responsibility for the health of others (social area).

Items of individual health responsibility area were designed according to the health responsibility dimension of the Health-Promoting Lifestyle Profile II (HPLP II) questionnaire established by Walker, which consisted of 12 items (items 1-12). The options are scored on a Likert scale ranging as strongly agree, agree, no opinion, disagree, and strongly disagree from 5 to 1, respectively. The range of scores in this area is between12-60. The items of the questionnaire related to the social responsibility area were designed according to the Social Responsibility Questionnaire developed by Harrison Goff, which included 20 items (items 13-32). To measure social responsibility in the social area, personality traits such as conscientiousness, sense of commitment, hardworking, seriousness, trustworthiness, discipline-based behavior, and behavior following the sense of responsibility towards family, society, and the world were considered. The items 19-20-25-27-28-29-30-31 were scored as strongly agree, agree, no opinion, disagree, and strongly disagree from 5 to 1, respectively. Items 16-17-18-21-22-23-24-26-32 were scored inversely, i.e., strongly agree, agree, no opinion, disagree, and strongly disagree from 1 to 5, respectively. Therefore, the range of scores in this area is between20-100. To assess the validity of the questionnaire, the quantitative content validity method was used by the panel of experts, and Cronbach's alpha calculation method was used to evaluate the reliability of the questionnaire, which was obtained as 0.78.

The sample size was calculated based on Cochran's sample size formula. To determine the sample size, by considering alpha as 0.05 accuracy as d = 0.4, and standard deviation as 6, the sample size was determined to be 484, which was increased to 500 for increasing the validity of the study, and 500 individuals completed the questionnaire as the research sample. Due to the problems related to the COVID-19 pandemic and the impossibility of face-to-face visits to collect information, the samples were selected using the convenience sampling method. So, to conduct the study, the questionnaire was initially developed The data were analyzed by SPSS software version 21. Mean and standard deviation as well as frequency distribution tables were used to describe the characteristics of the subjects. An Independent t-test was used for the comparison of responsibility scores in terms of the history of COVID-19 morbidity. To investigate the relationship between overall responsibility, as well as health responsibility for one's health (individual area) and health responsibility for the health of others (social area) with the chance of COVID-19 morbidity, Simple Logistic Regression was used at a significant level of 0.05. Then, the relationship between baseline characteristics variables and the chance of COVID-19 morbidity was measured at a significance level of 0.2. Variables that had a statistically significant relationship with the chance of COVID-19 morbidity were individually entered into the Multiple Logistic Regression model at a significance level of 0.2 to eliminate their confounding effect on the relationship between responsibility and history of COVID-19 morbidity. In case of the presence of variables with a confounding effect on the relationship between different areas of responsibility and the chance of COVID-19 morbidity (more than 10% change in the COVID-19 chance associated with responsibility), they are entered in the final model (simultaneously) at the significance level of 0.05. The variables of age and home space have entered the models as centering for interpretability of intercept of the regression model collected virtually through the questionnaire design website (https://porsline.ir/), and then the questionnaire link was sent to the participants through the largest virtual page of Andimeshk city news agency on Instagram. This page is mostly followed by residents of Andimeshk city, and it has about 70k members with a high rate of daily likes and visits. Following the completion of the questionnaire, it was available to us through the Porsline website.

RESULTS

Finally, 500 individuals were analyzed in this study, of which 162 (32.4%) had a history of COVID-19, and 338 (67.6%) did not report a history of COVID-19 morbidity. Of those with a history of COVID-19, 42 reported transmitting the disease to others.

Out of the total number of people surveyed, 321 (64.2%) reported that at least one of those around them had been infected by COVID-19 in the past. 210 (42%) were registered in the self-declaration system of the Ministry of Health (Table 1).

Table 1: Baseline characteristics of the participants in the study

Variable		No.	Percent
Incidence of COVID-19	No	338	67.6
	Yes	162	32.4
Gender	Male	240	48
	Female	260	52
Education	Illiterate	13	2.6
	Below high school degree	46	9.2
	High school diploma	95	19
	Higher education	346	69.2
Number of Household members	1	5	1
	2	50	10
	3	67	13.4
	Above 3	378	75.6
Proper ventilation of the residential house	No	124	24.8
	Yes	376	75.2
Registration in the COVID-19 self-declaration system	No	290	58
	Yes	210	42
Frequency of morbidity of COVID-19	No morbidity	340	68
	once	139	27.4
	Twice	19	3.8
	More than 2 times	4	0.8
Morbidity of COVID-19 in relatives	No	179	35.8
	Yes	321	64.2
Behavior leading to COVID-19	travel	4	2.5
	Going to a party	45	28
	Not wearing mask	38	23.6
	No hand sanitization	5	3.1
	Non-observance of social distance	18	11.2
	Contaminated work environment	35	21.7
	Caring for a patient with COVID-19	16	9.9
Transmitting your disease to others	No	379	75.8
	Yes	42	8.4
	I don’t know	79	15.8
Having underlying disease	No	56	11.2
	Yes	443	88.8

In the total subjects, the means of overall responsibility, responsibility in the individual area, and responsibility in the social area were equal to 126.17 ± 22.85, 44 ± 10.68, and 82.17 ± 14.77, respectively. In all areas, the mean score of responsibility of individuals with a history of COVID-19 was lower than those without a history of COVID-19, so that, the scores of overall responsibility, individual responsibility, and social responsibility in individuals with a history of COVID-19 were 5.93, 2.43, and 3.50 less than the scores of those without a history of COVID-19 (P <0.05) (Table 2).

Table 2: Comparison of the mean scores of different areas of responsibility in terms of the history of COVID-19 morbidity

Different areas	History of morbidity of COVID-19	No.	Mean ± SD	Difference of means	95% confidence interval		P-value*
					Lower limit	Upper limit	
Overall responsibility	Non-infected	338	128.09±22.23	5.93	1.67	10.20	0.008
	infected	162	122.15±23.67				
Individual responsibility	Non-infected	338	44.79±10.60	2.43	0.43	4.42	0.017
	infected	162	42.36±10.70				
Social responsibility	Non-infected	338	83.30±13.87	3.50	0.74	6.26	0.013
	infected	162	79.80±16.28				

** Independent Sample T Test*

As shown by the results of univariate analysis, for one unit increase in score of overall responsibility, individual responsibility, and social responsibility, the chances of morbidity of COVID-19 decreased by 2%, 3%, and 2%, respectively, which was statistically significant (P <0.05) (Table 3). There were statistically significant relationships between variables of literacy level, home space, history of COVID-19 in others, the transmission of COVID-19 to others, the presence of underlying disease, and age of individuals and the chance of morbidity of COVID-19 (P<0.20) (Table 3).

Table 3: Relationship between different areas of responsibility and baseline characteristics with the morbidity of COVID-19

Variable		OR (95% CI)	P-Value
Overall responsibility		0.98 (0.98, 0.99)	0.007
Individual responsibility		0.97 (0.96, 0.99)	0.018
Social responsibility		0.98 (0.97, 0.99)	0.013
Variable		**OR (80% CI)**	**P-Value**
Gender	Female (reference)	1	
	Male	1.25 (0.98, 1.60)	0.233
Level of literacy	Elementary school (ref.)	1	
	Below school	0.33 (0.14, 0.77)	0.093
	High school	0.41 (0.19, 0.89)	0.141
	Higher education	0.40 (0.19, 0.83)	0.111
Household dimension		1.04 (0.97, 1.11)	0.385
Ventilation of the residential place	No (ref.)	1	
	Yes	0.87 (0.65, 1.15)	0.532
House space		0.99 (0.99, 1.00)	0.134
Registration in the system of the Ministry of Health	No (ref.)	1	
	Yes	0.79 (0.62, 1.02)	0.243
History of morbidity of COVID-19 in others	No (ref.)	1	
	Yes	2.69 (2.03, 3.57)	< 0.001
Transmitting your disease to others	No (ref.)	1	
	I don't know	2.23 (1.60, 3.11)	0.002
	Yes	23.08 (12.29, 43.32)	< 0.001
Underlying disease	No (ref.)	1	
	Yes	1.74 (1.20, 2.51)	0.052
Age		1.02 (1.00, 1.03)	0.037

Table 4: Adjusting the confounding effect baseline characteristics variables on the relationship between different areas of responsibility and the morbidity of COVID-19

Adjusted variable	Overall responsibility			Individual responsibility			Social responsibility		
	aOR* (80% CI)	P-Value	% of change **	aOR* (80% CI)	P-Value	% of change **	aOR* (80% CI)	P-Value	% of change **
Level of literacy	0.98 (0.99, 0.98)	0.010	0%	0.97 (0.99, 0.96)	0.023	0%	0.98 (0.99, 0.97)	0.019	0%
Space of the house	0.98 (0.99, 0.98)	0.008	0%	0.98 (0.99, 0.96)	0.023	0.01%	0.98 (0.99, 0.97)	0.015	0%
History of coronary heart disease in those around	0.98 (0.99, 0.98)	<0.001	0%	0.97 (0.99, 0.96)	<0.001	0%	0.97 (0.99, 0.96)	<0.001	0.01%
Transmission of coronary heart disease to others	0.99 (0.98, 1)	0.170	0.01%	0.98 (0.97, 1)	0.186	0.01%	0.99 (0.98, 1)	0.245	0.01%
Underlying disease	0.99 (0.99, 0.98)	0.014	0.01%	0.98 (0.99, 0.96)	0.024	0.01%	0.98 (0.99, 0.97)	0.029	0%
Age	0.98	0.002	0%	0.97	0.007	0%	0.98	0.005	0%

** adjusted Odds Ratio for the relationship between responsibility and COVID-19 morbidity*

*** Percentage of OR change for relationship between responsibility and COVID-19 morbidity compared to its unadjusted state*

In the multiple logistic regression model, it was found that none of the variables of literacy level, home space, history of COVID-19 in others, the transmission of COVID-19 to others, presence of underlying disease, and age of individuals had a confounding effect on the relationship between overall responsibility, individuals responsibility, and social responsibility and morbidity of COVID-19 (Table 4).

DISCUSSION

The present study was conducted aiming at investigating the relationship between social responsibility and COVID-19 disease. A total of 500 residents of Andimeshk city in Khuzestan province in the southwest of Iran were surveyed.

The results of our study showed that individuals with a history of COVID-19 have lower scores of overall responsibility, individual responsibility, and social responsibility than those without a history of COVID-19 disease. Besides, the mean score of individuals' responsibilities for their health in the social area was higher than the individual area, and this finding is consistent with the study of Khajeh Nouri [8].

The mean score of responsibility in all areas surveyed was higher in individuals without a history of COVID-19 disease and those who showed better adherence to health protocols. In general, individual and social responsibility is a very important variable since it shows a person's commitment to his/her behavior, and the higher the commitment of individuals to an issue, its achievement would be easier realized through one's individual and social responsibility. Moreover, Thome maintains that social responsibility is based on a sense of commitment in individuals [10].

The results of the univariate analysis of our study showed that for one unit increase in score of overall responsibility, individual responsibility, or social responsibility, the chance of developing COVID-19 is decreased. The results of the present study also showed that men were more likely than women to get infected by COVID-19. This is in line with the findings of Brodin on the safety factors determining the manifestation and severity of COVID-19 disease. According to his results, he believes that men are more prone to COVID-19 disease than women [11]. Of course, the presence of men in crowded areas is a factor in increasing infection. Besides, men have poorer performance in terms of following health protocols than women [12], which increases the chances of getting the disease and this indicates less responsibility and commitment of men.

In this study, those registered in the COVID-19 self-declaration system of the Ministry of Health had a lower chance of getting infected by COVID-19, and this implies higher social commitment and responsibility of people to comply with health protocols and recognition of the infected cases and cutting the transmission chain. Because people are more committed to health, related behaviors are manifested in them earlier for anxiety management, and this suggests a responsibility to their health and those around them. It was also mentioned in the study of Fathi et al., and the author believes that individuals with higher responsibility experience less COVID-19 anxiety [13].

Our results demonstrated that for every year of age increase, the chances of getting infected by COVID-19 increase. The age variable itself is a determining factor in COVID-19 morbidity. This finding is in agreement with the findings of other studies [14-16].

In the present study, people who transmitted the disease to others had a higher chance of getting infected again because these people usually have a lower risk perception of the disease and also have lower health responsibility. This finding is consistent with the findings of Robinson's study [17].

In the present study, those with the underlying disease were more likely to develop COVID-19 disease, which is consistent with the study of Dadgari [14]. In our study, individuals with literacy levels below high school, high school, and higher education levels showed a lower chance of COVID-19 morbidity compared to those with an elementary school degree. They performed better following the health instructions and social responsibility, and this finding was in line with the study of Kharamin et al [12].

Usually, when people around you are not responsible, they create obstacles and problems for others that can increase their chances of risk. Our findings also indicated those with a history of COVID-19 morbidity among those around them are more prone to be infected, which this finding is consistent with the findings of Sahebi [7].

The results of this study demonstrated that in the case of COVID-19 morbidity, the most common causes of COVID-19 disease were related to behaviors, going to parties, not wearing a mask, and the contaminant work environment, respectively. Moreover, the chance of COVID-19 morbidity was less in less populated households and families with larger homes and more well-ventilated houses.

Consequently, given that the average score of social responsibility is higher in individuals without a history of COVID-19 disease and with better adherence to health protocols, research findings indicate that the more individuals take responsibility, the more attention they pay to the consequences of their actions, take their action and issues more seriously and try harder to solve them [18].

CONCLUSIONS

According to the results obtained in different studies, further research can be done to deepen the existing knowledge in the field of the correlation between social responsibility and health and different diseases and COVID-19 morbidity. In general, the component of responsibility should be institutionalized at different levels of society from the younger ages with different strategies, so that it can play an effective role in human health and the prevention of events and diseases.

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The personal interests of the authors were not related to the results of this research. The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

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Authors' contribution
All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate
This study was approved by the Research Ethics Committee, Iran University of Medical Sciences with the code IR.IUMS.REC.1399.1199. Informed consent was obtained from all individual participants included in the study before conducting.

Patient consent for publication
All participants consented verbally to the publication of the interview data.

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ORIGINAL ARTICLE

A Family-based Association Test of the VDR Gene in Proximal Spinal Muscular Atrophy

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Abstract: *Different factors may be involved in the clinical heterogeneity of spinal muscular atrophy disease. The vitamin D receptor (VDR) might be a candidate gene for this disease. Our study aimed to assess the preferential transmission of VDR polymorphisms from parents to SMA children. We genotyped 261 subjects (87 SMA nuclear families) for VDR FokI, BsmI, ApaI, and TaqI polymorphisms. The transmission of the genetic marker was estimated with Plink and FBAT software. It detected a preferential transmission of the rs731236 and rs7975232 variants to SMA1 patients and of rs1544410 variants to SMA2 patients. The variants of rs2228570 were preferentially transmitted to parents of all SMA patients. Haplotype analysis identified that haplotypes C-C-G-A and T-A-A-G seem to be involved in the booth type of SMA whereas the impact of T-A-A-A seems to be limited only to SMA2. Strong linkage disequilibrium (LD) between rs7975232 and rs1544410 was detected in samples from parents. Even though we investigated a small number of nuclear families the results suggest a potential link between VDR polymorphisms and SMA disease.*

Keywords: *SMA, TDT analysis, VDR gene*

INTRODUCTION

Spinal muscular atrophy (SMA) refers to a group of hereditary recessive neuromuscular diseases leading to muscle weakness and infant mortality. The clinical onset of spinal muscular atrophy 1 (SMA1 or Werdnig Hoffmann disease) is evident in the first 6 months of life whereas for spinal muscular atrophy type II (SMA2 or Dubowitz disease) it is observed at ages 6 - 12 months.

The main symptoms of SMA1 and SMA2 are degeneration of anterior spinal cord motoneurons, muscular hypotonia, or atrophy. They are determined by mutations (e.g. homozygous deletion, punctiform mutations, or complex rearrangements) in the SMN1 locus (5q13.2) [1,2] but are influenced by other factors (e.g., mutations in SMN2, NAIP, PLS3) [3,4,5].

Vitamin D is involved in mineral metabolism and interferes with immune response, apoptotic and anti-proliferative processes, or neuromuscular function [6,7,8,9]. Vitamin D deficiency may predispose to reversible muscle weakness, wasting, and instability of gait [10,11], whereas supplementation with this vitamin may increase muscle strength in athletes [12] and reduce the risk of cancer death [13].

The vitamin D receptor (VDR), encoded by the VDR gene (12q.13.1), is a nuclear receptor belonging to class 2 of the family of steroid receptors. Several VDR polymorphisms (e.g. VDR FokI, BsmI, Apa, TaqI) have been associated or in linkage with different human diseases (e.g. diabetes mellitus type 1, osteoporosis, allergic diseases), including carcinomas and neurological disease (e.g. Parkinson's disease, Alzheimer's disease, multiple sclerosis, SMA1) [14,15,16,17,18,19]. However, the association between VDR polymorphisms and neurological diseases remains a subject of debate.

This study aimed to investigate the transmission of VDR polymorphisms in nuclear families with SMA1 or SMA2 patients.

MATERIALS AND METHODS

Subjects

For families with SMA type, 1 (n=43) or type 2 (n=44) patients who were referred to several hospitals from Bucharest (Romania), 24 of them were also included in a previous publication [19]. Diagnosis of SMA was based on age at clinical onset and of homozygous deletion of SMN1 exon 7. SMA1 patients never achieve sitting whereas those with SMA2 could sit but were not able to walk, according to classification criteria [20,21].

Genotyping

DNA was isolated using a commercial kit (The Wizard® Genomic DNA Purification Kit) from peripheral blood (0.5 ml) collected on EDTA anticoagulant tubes. The homozygous deletions of SMN1 exon 7 or 8 were tested by PCR -RFLP and the homozygous deletion of NAIP exon 5 was tested by PCR and electrophoresis, according to the previously published protocols [22,23,24]. VDR polymorphisms Taq (rs731236), Apa (rs7975232), Bsm (rs1544410), and Fok (rs2228570) were genotyped using PCR-RFLP method, as was previously described [19,25,26]. Restriction products were resolved in electrophoresis. The genotype of each sample was established independently by two investigators.

Statistical analysis

The frequencies of alleles and haplotypes, linkage disequilibrium (LD) between markers, and the pattern of genetic markers transmission from parents to affected offspring (Transmission disequilibrium test – TDT) was performed with Plink [27] and the FBAT (Family Based Association Test) software [28]. The linkage between VDR markers in samples from parents and the LD plot was estimated with Haploview 4.1 [29]. A p-value of 0.05 was considered statistically significant.

RESULTS

The gender distribution was similar in SMA1 and SMA2 patients (51.16% and 52.27%, respectively were boys).

The parents of children with SMA1 (25.76±3.42; 19-36 years old) or SMA2 (25.86±3.55; 19-35 years old) had similar ages. The age of patients with SMA1 was significantly lower than that of SMA2 (8.39±4.21 months vs. 13.33±3.54 months, p< 0.0001). The genetic test showed that all investigated parents have at least one copy of the SMN1 gene. The homozygous deletion of SMN1 exon 8 or NAIP exon 5 was detected more frequently in patients with SMA1 than in those with SMA2 (53.48% vs. 40.91%; and 25.58% vs. 20.45% of cases).

The distribution of all VDR genotypes in the sublot of parents and rs1544410 in the sublot of SMA patients are not in agreement with Hardy Weinberg's principle (Table 1). Only the Minor Allele Frequency (MAF) of rs1544410 is in the interval of values reported in 1000 Genomes for VDR polymorphisms (Table 2) [30].

The results obtained with FBAT and Plink software reveal a preferential transmission of the VDR alleles from parents to SMA children. The calculated statistical significance was much stronger for rs731236 and rs7975232 and more reduced for rs1544410 and rs2228570. These results depend on the type of disease; it was detected a preferential transmission of the rs731236 and rs7975232 variants to SMA1 patients and of rs1544410 variants to SMA2 patients (Table 3, Table 4).

Table 1: The distribution of VDR polymorphisms in sublots of SMA patients and parents

Polymorphisms	Lot of subjects	Genotypes		
rs731236		TT	TC	CC
	patients	18	43	26
	parents*	37	120	17
rs7975232		AA	AC	CC
	patients	18	39	30
	parents*	34	113	27
rs1544410		GG	GA	AA
	patients*	29	50	8
	parents*	32	127	15
rs2228570		GG	GA	AA
	patients	17	38	32
	parents*	29	112	33

** Distributions of genotypes that are not following HWE in sublot of parent*

Table 2: The MAF of VDR polymorphisms in sublots of patients and parents

VDR polymorphisms	Variants	1000 genomes MAF		SMA Patients	Parents
		Europeans	All samples		
rs731236	C	0.400	0.277	0.546	0.443
rs7975232	C	0.445	0.485	0.569	0.480
rs1544410	A	0.404	0.296	0.379	0.451
rs2228570	A	0.378	0.328	0.586	0.511

Table 3: TDT test calculated for VDR polymorphism (FBAT software)

SNPs	Allele	Families	afreq	Fam#	Z; p
rs731236	C	SMA all	0.547	78	3.31; 0.0009
		SMA1	0.570	39	2.78; 0.005
		SMA2	0.523	39	1.92; >0.05
rs7975232	C	SMA all	0.477	78	3.02; 0.003
		SMA1	0.483	36	2.66; 0.008
		SMA2	0.472	42	1.66; >0.05
rs1544410	A	SMA all	0.451-	78	2.34; 0.02
		SMA1	0.470	37	1.19; >0.05
		SMA2	0.433	41	2.077; 0.04
rs2228570	G	SMA all	0.491	77	2.46; 0.01
		SMA1	0.494	37	1.57; 0.11
		SMA2	0.489	40	1.89; >0.05

Note: # Fam: number of informative families, Z: standard normal variable. p: probability

Table 4: TDT test calculated for VDR polymorphism (Plink software).

SNPs	A1 allele	A2 allele	Families	Transmitted A1 allele count	Untransmitted A1 allele count	OR; p
rs731236	C	T	All	76	41	1.85; 0.001
			SMA1	38	16	2.38; 0.003
			SMA2	38	23	1.65; >0.05
rs7975232	C	A	All	71	40	1.78; 0.003
			SMA1	34	14	2.43; 0.004
			SMA2	37	24	1.54; >0.05
rs1544410	A	G	All	48	75	0.64; 0.02
			SMA1	23	31	0.74; >0.05
			SMA2	25	42	0.6; 0.04
rs2228570	G	A	All	42	69	0.61; 0.01
			SMA1	17	30	0.56; >0.05
			SMA2	24	39	0.62; >0.05

Note: The results of the TDT test performed for families with SMA cases (plink)

The frequency of haplotypes in SMA families was estimated with FBAT software (Table 5). The most frequent three (rs731236-rs7975232-rs1544410-rs2228570) haplotypes estimated for SMA1 and SMA2 families were C-C-G-A (0.120 vs. 0.147), T-A-G-A (0.116 vs. 0.145), T-A-A-G (0.115 vs. 0.124) and, the rarest was C-A-G-A (0.009 vs. 0.003). Statistically significant results were identified between C-C-G-A and both types of SMA, T-A-A-G haplotypes, and SMA1 disease and between T-A-A-A and SMA2 (Table 5).

We found a strong linkage disequilibrium (LD) measure between rs7975232 and rs1544410 and a less significant linkage between rs7975232 and rs731236 (Figure 1).

Table 5: VDR haplotype analysis in SMA families (FBAT software)

Haplotypes	Families	Frequency of haplotype	fam#	S-E(S)	Var(S)	Z;P
C-C-G-A	SMA All	0.133	36	12.589	10.339	3.91; 0.00009
	SMA 1	0.120	12	6.082	4.166	2.98; 0.003
	SMA2	0.147	21	7.464	6.647	2.89; 0.004
T-A-A-G	SMA All	0.101	34	10.205	7.728	3.67; 0.0002
	SMA 1	0.115	14	5.256	3.823	2.69; 0.007
	SMA2	0.124	19	1.808	5.08	0.80; 0.42
T-A-A-A	SMA All	0.068	28	4.713	4.886	2.13; 0.03
	SMA2	0.082	17	3.877	3.640	2.03; 0.04

Note: Haplotype: rs731236- rs7975232- rs1544410- rs2228570; # Fam: number of informative families, Z: standard normal variable. p: probability

Figure 1: VDR LD plot estimated for the four polymorphisms identified in samples from parents

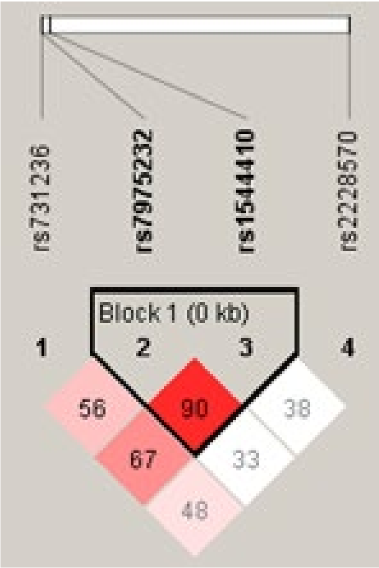

DISCUSSION

We performed a family-based association test, to bring new data regarding the relationship between VDR gene polymorphisms and SMA disease [19]. The transmission disequilibrium test, unlike the case-control association study, is not susceptible to population stratification. Eighty-seven families with children diagnosed with SMA1 or SMA2 were included in this study. The observed distribution of mutations may reflect some characteristics of families included in this study. Thus, the homozygous deletion of SMN1 exon 8 (53.48% and 40.91% respectively) in blood samples from our SMA1 and SMA2 patients was less frequent than in other populations (100% and 91.48%). These differences may be explained by the results of Vijzelaar R. and colleagues who considered that the distribution of SMN mutations is influenced by population background [31].

The homozygous deletion of NAIP exon 5 in SMA1 and SMA2 patients (25.58% and 20,45% respectively) was in the range of values reported in other studies (9.5%-82.85% respectively) [32,33,34].

The distribution of VDR polymorphisms in the sublot of parents is not in agreement with Hardy Weinberg equilibrium. In addition, the distribution of rs731236, rs1544410, and rs2228570 in a subgroup of parents differs from those reported in public databases [35]. However, the MAF values for rs2228570 estimated in this study and previous research performed in the population from South Romania are similar (0.378 vs 0.365) [36]. In this sublot of subjects, we also detected much stronger linkage disequilibrium (LD) between rs7975232 and rs1544410 than between rs7975232 and rs731236. This result is not following data from public databases [30]. These results could be explained, at least partially, by the size of this sublot of subjects and by the criteria used for selection.

In families investigated in this study, the C-C-G-A and T-A-A-G haplotypes were linked with booth types of SMA whereas T-A-A-A could be in relation only with SMA2 (Table 5). In a previous case-control study, we found an association between SMA1 and VDR haplotypes C-C-G-A (OR= 2.8, p=0.02) or T-A-A-A (OR= 0.27, p=0.03) [19].

The rs1544410, rs7975232, and rs731236, located near the 3‘end of the VDR gene, were supposed to be non-functional. We detected a preferential transmission of the rs731236 and rs7975232 variants to SMA1 patients, and rs1544410 variants to SMA2 patients. These markers may be in linkage disequilibrium with a potential functional VDR polymorphism.

The rs2228570 was mapped in exon 2 of the VDR gene, in a potential translation initiation start site. Its variants were associated with two receptor variants: a long version of the protein (the T variant) and a shortened variant by three amino acids (the C variant). The VDR C variants seem to interact more efficiently with transcription factor TFIIB [37]. The variants of rs2228570 were preferentially transmitted to parents of all

SMA patients (Table 5).

This is the first report of a TDT test performed for VDR gene polymorphisms in SMA diseases. The results indicated that some VDR polymorphisms may be preferentially transmitted from parents to SMA patients. This result is completing the literature reports about the preferential transmission of VDR gene polymorphisms alleles in various diseases like diabetes [38, 39], rheumatoid arthritis [40], tuberculosis [41], or bone mineral density [42].

In conclusion, our data indicated a potential transmission of VDR variants from patients to SMA1 or SMA2 patients.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research. The authors declare that they have no competing interests. This study did not receive any grant from funding agencies.

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ORIGINAL ARTICLE

First Romanian Tele-Echography Network – Preliminary Results

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Abstract: *The evolution of society, the development of the Internet, and the increased need for medical services generated the emergence of telemedicine. The visual and auditory connection between the doctor and the patient allowed good communication, but the limitation of the clinical examination remained a problem. Tele-echography complements tele-consults and brings valuable information in many specialties. Our study is conducted on data obtained by organizing the first network of telemedicine with tele-echography in the country. The ultrasound examinations targeted only the abdomen, but the structure also allows tele-echocardiography as well as the extension to other services at distance. The purpose of the study is to objectively assess the ability of tele-echography to complete the teleconsultation with information on which we can rely. The national tele-echography network was created by distributing 40 tele-echographs in the country, one in each county, in the family doctors' offices. The obtained data were analyzed statistically. The study shows that tele-echography can be performed with very good accuracy in synchronous mode but low in asynchronous mode, which required re-examination or resumption of examination in the reference medical center. The widespread use of tele-echography associated with teleconsultations can have favorable consequences on the medical act, the quality of life of the patients and the doctors, and society by reducing pollution and urban agglomerations.*

Keywords: *tele-echography; teleconsultation; abdominal echography*

INTRODUCTION

Telemedicine is defined as a technique that comprises the usage of information and communication technologies (ICT) to enhance the access of patients to distant healthcare providers and technologies [1].

Tele-echography or tele-ultrasonography (Tele US) uses remote transmission to provide enhanced US diagnosis, based on the opinion of remote experts. TELUS is frequently performed at the point-of-care (PoC) by primary care doctors, with a profound impact on patients located at remote destinations [2].

The aim of the organization of the national network telemedicine with tele-echography is to provide patient-centered care, with enhancement in the clinical decision-making for individual cases. The procedures' results are amplified by the involvement of specialists as remote experts [3].

The results of previous studies have motivated the organization of the first tele-echography network in Romania [4,5,6].

In the studies carried out in the field of telemedicine with tele-echography, the following disadvantages are identified: the deficit of specialized medical staff, the difficulties of time management, and the costs of equipment and the internet [7,8,9].

The national telemedicine network with tele-echography is made up of 40 tele-echography centers organized as follows: In each county, the Lotus Image medical center from Targu Mures provided a tele-echography through which tele-consultations, including tele-echography, were performed with the specialists.

In this preliminary retrospective study, we present the details of this structure and the statistical analysis of the accumulated data from abdominal examinations. The presented data demonstrate the increase in the quality of the medical service when tele-echography is associated with teleconsultation and motivates the extension to the other specialties: cardiology, obstetrics and gynecology, pediatrics, endocrinology, and senology.

MATERIALS AND METHODS

The components of the tele-echography network are a doctor, portable POCUS device -Telemed LS64 FLT-1T with EchoWave II (EWII) software, a virtual private network (VPN)-secure, private, and encrypted online connection, remote expert. The two main parts of the process are the transmission and reception of ultrasound images. The transmission is done by the ultrasound device and the internet connection. The reception is made up of personal computers or smartphones with dedicated soft. In asynchronous mode, the third component intervenes, the server where echography films are stored and downloaded later by experts. In synchronous mode, the examinations are made at the same time and the result of the examination is issued immediately after its completion.

Figure 1: Tele-echography network

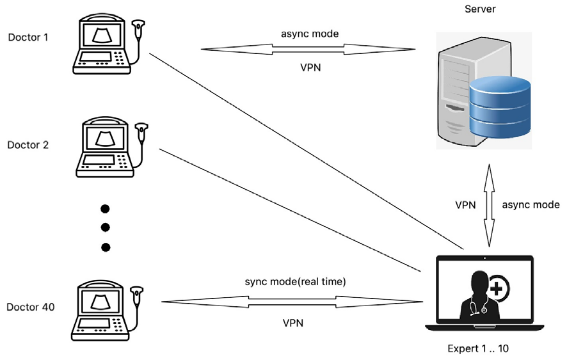

Synchronous examination or real-time is the most appreciated method of examination by patients, examining physicians, and experts because it alleviates the possibility of timely interaction between the three of them, the adjustment by verbal guidance of the examination to obtain the correct images and their interpretation in the clinical context and release the results immediately after the end of the examination.

Asynchronous examination or store-and-forward has the advantage that the videos and results are saved digitally and can constitute both an image database and forensic documentation in case of malpractice process. The second advantage is that it allows greater flexibility in the working time for the patient-examining physician and expert but is subject to the risk of incomplete or incorrect results with the possible consequence of requesting the resumption of the examination from the expert.

The national tele-echography network that was created within this project had both options. As a result, through the activity in the network, a database was established consisting of both the storage of echography videos and of the standardized results.

By entering the standardized elements in an excel database, a complex statistical analysis was possible, which included the following elements: the reasons for the patients' presentation for tele-echography, the number of organs completely or incorrectly reviewed, the ability to visualize the spleen and kidney, the collaboration with the patient to obtain and maintaining apnea necessary for a correct examination of the upper abdomen.

RESULTS

The study group resulted from the selection of approximately 3000 tele-echography abdominal examinations performed in synchronous and asynchronous modes within the created network. The results of the examinations were drafted and standardized, and the parameters were entered into the excel database. Incomplete results and re-examinations were excluded. The created database contains both medical documents and video recordings. The distributions of the ”reasons for presentation” for the tele-echography examination are shown in Table 1, Figure 2. A large proportion of persons presented themselves to the specialist for screening (30.3%). This parameter illustrates the awareness of the population for prevention and confirms the role of this method to meet the needs of the patients. Chronic abdominal pain syndrome and nonspecific syndromes were the reasons for teleconsultation with tele-echography in another third of the patients.

Figure 2: Reasons for the presentation at the tele-echography

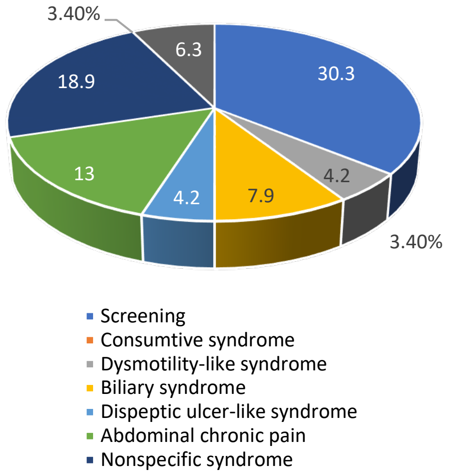

Table 1: The reasons for the presentation at the ultrasound

Reasons for the presentation	Number	Percentage
Screening	144	30.3
Consumptive Syndrome[1]	16	3.4
Dysmolity-Like Syndrome[2]	20	4.2
Biliary Syndrome[3]	38	7.9
Dyspeptic Ulcer-Like Syndrome[4]	60	12.6
Abdominal Pain Syndrome[5]	62	13
Nonspecific Syndrome[6]	90	18.9
High Urinary Syndrome[7]	16	3.4
Lower Urinary Syndrome[8]	30	6.3
Total	477	100

[1] Consumptive syndrome – clinical syndrome characterized by consuming the body's reserves, secondary to the evolution of serious chronic diseases.
[2] Dysmotility-like dyspepsia – bloating, abdominal distention, flatulence, and prominent nausea.
[3] Biliary Syndrome – abdominal pain, especially in the upper right side of the abdomen under the rib cage, nausea or vomiting
[4] Dyspeptic Ulcer-Like Syndrome – discomfort or pain that occurs in the upper abdomen, often after eating or drinking.
[5] Abdominal Pain Syndrome – continuous or almost continuous abdominal pain, slightly related to intestinal function, associated with decreased daily tone, and which has been present for at least the last six months.
[6] Nonspecific Syndrome – Indigestion, abdominal pain with bowel movements, feeling full after eating a small amount of food, nausea, vomiting.
[7] High Urinary Syndrome – Painful urination (dysuria), frequent urination, especially during the day, pain in upper abdomen and back
[8] Lower Urinary Syndrome – blood in your urine (hematuria), difficulty urinating (urinary retention), feeling an immediate urge to urinate, frequent urination, especially during the day, painful intercourse (dyspareunia), painful urination (dysuria), pain in your genitals, lower abdomen and lower back.

Correlation between the parameters in pathology where the ultrasound examination has a major role in the diagnosis such as lithiasis and proliferative disease

Correlations between biliary syndrome and cholelithiasis

Out of 38 patients who complained of biliary syndrome, only 4 were found biliary lithiasis ultrasound aspects, while out of 439 without the biliary syndrome, 25 (5.7%) had cholelithiasis Table 2. The differences are statistically insignificant (p -0.27). The absence of a syndrome in the anamnesis does not exclude a certain type of pathology. The usefulness of the ultrasound examination is obvious in this case.

Table 2: The relationship between biliary syndrome and cholelithiasis

P -0.27			Cholelithiasis		Total
			not	yes	
Biliary Syndrome[1]	not	number	414	25	439
		%	94.3%	5.7%	100.0%
	yes	number	34	4	38
		%	89.5%	10.5%	100.0%
Total		number	448	29	477
		%	93.9%	6.1%	100.0%

[1] Biliary syndrome: Abdominal pain, especially in the upper right side of the abdomen under the rib cage. Nausea or vomiting with or without icter

Correlations between high urinary syndrome and renal lithiasis

Of the 16 people who complained of a high urinary syndrome, only 2 were labeled with renal lithiasis. Of the 461 people who did not report a high urinary syndrome, only 18 were labeled with renal lithiasis. The differences are statistically insignificant (p -0.14), Table 3.

Table 3: The relationship between high urinary syndrome and renal lithiasis

P -0.14			Renal lithiasis		Total
			not	yes	
High urinary syndrome[1]	not	number	443	18	461
		%	96.1%	3.9%	100.0%
	yes	number	14	2	16
		%	87.5%	12.5%	100.0%
Total		number	448	457	20
		%	93.9%	95.8%	4.2%

[1] High urinary syndrome: upper back pain, dysuria, polakiuria, with or without macroscopic hematuria, fever, chills

Correlations between lower urinary syndrome – hypertrophy of the prostate

Of the 31 people who reported down urinary syndrome, 7 (22.6%) were labeled benign hypertrophy of the prostate (BPH). Of the 446 people who did not report a urinary syndrome down, only 43 (9.6%) were labeled with BPH. The differences are statistically significant (p -0.03), Table 4.

Table 4: The relationship between lower urinary syndrome and benign hypertrophy of the prostate (BPH)

P -0.03			**BPH**		**Total**
			not	**yes**	
Lower urinary syndrome[1]	not	number	403	43	446
		%	90.4%	9.6%	100.0%
	yes	number	24	7	31
		%	77.4%	22.6%	100.0%
Total		number	448	427	50
		%	93.9%	89.5%	10.5%

[1] *Lower urinary syndrome: difficulty urinating (urinary retention), feeling an immediate urge to urinate, frequent urination, especially during the day, painful intercourse (dyspareunia), painful urination (dysuria), pain in your genitals, lower abdomen and lower back pain with or without hematuria, fever, chills*

Tele-ultrasound examination skills

We analyzed the examiner's ability to highlight the abdominal organs that present difficulties of ultrasound examination at the classical, direct examination. One of the factors that makes ultrasound examination difficult is obesity. That's why we analyzed the number of unchecked organs and the body mass index (BMI).

Number of unvisualized organs and body mass index (BMI)

In 41 cases an organ was not revealed, in 5 situations two organs were not visualized, in 4 cases – 3 organs, in one case – 5, respectively 9 organs. In cases where an organ has not been visualized, 4 (13.3%) people with obesity grade II are noted, or 17 (10.4%) overweight people. Situations with two unvisualized organs were identified in normal or overweight people Table 5.

Table 5: Relationship between BMI and number of organs not viewed

P -0.98			**No invisible org**						**Total**
			0	**1**	**2**	**3**	**5**	**9**	
BMI[1]	under ponderal	number	18	0	0	0	0	0	18
		%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%
	normo ponderal	number	123	11	2	1	0	1	138
		%	89.1%	8.0%	1.4%	0.7%	0.0%	0.7%	100.0%
	Supra ponderal	number	144	17	2	1	0	0	164
		%	87.8%	10.4%	1.2%	0.6%	0.0%	0.0%	100.0%
	obesity grade I	number	103	8	1	2	1	0	115
		%	89.6%	7.0%	0.9%	1.7%	0.9%	0.0%	100.0%
	obesity grade II	number	26	4	0	0	0	0	30
		%	86.7%	13.3%	0.0%	0.0%	0.0%	0.0%	100.0%
	obesity degrees III	number	11	1	0	0	0	0	12
		%	91.7%	8.3%	0.0%	0.0%	0.0%	0.0%	100.0%
Total		number	425	41	5	4	1	1	477
		%	89.1%	8.6%	1.0%	0.8%	0.2%	0.2%	100.0%

[1] *BMI = weight (kg)/height (cm^2)*

Relationship between tele-ecographist and spleen visualization

The spleen has the same characteristics in ultrasound examinations as kidneys: intestinal gaseous overlaps and the chest cage make it difficult to examine these organs. Therefore, we evaluated the feasibility of the method by establishing the vision rate of the spleen at each of the examiners included in the study. The results, contained in the table below, demonstrate the uniformity of this parameter and support the promotion of the patient's examination method (Table 6).

Number of unidentified organs during an examination

The first condition for correct examination is the honesty of the examiner; declaring the parameters unidentified during the examination makes sure that they will be evaluated in other conditions (e.g. after the treatment of reducing bloating, examination of the bladder in the replete or gallbladder of fasting) or with other imaging means: retroperitoneum by CT or MRI, prostate by an endorectal probe, genital organs by an endovaginal probe (Table 7).

Table 6: Relationship between tele-ecographist and spleen visualization

P -0.96			View the spleen		Total
			not	yes	
Teleecographer	Other	number	0	19	19
		%	0.0%	100.0%	100.0%
	CJ	number	2	59	61
		%	3.3%	96.7%	100.0%
	PV	number	11	358	369
		%	3.0%	97.0%	100.0%
	RS	number	1	27	28
		%	3.6%	96.4%	100.0%
Total		number	14	463	477
		%	2.9%	97.1%	100.0%

Table 7: Relationship between the tele-ecographer and the number of unvisualized organs

P -0.38			No invisible organs						Total
			0	1	2	3	5	9	
Tele ecographer	Other	number	18	1	0	0	0	0	19
		%	94.7%	5.3%	0.0%	0.0%	0.0%	0.0%	100.0%
	CJ	number	57	4	0	0	0	0	61
		%	93.4%	6.6%	0.0%	0.0%	0.0%	0.0%	100.0%
	PV	number	325	35	3	4	1	1	369
		%	88.1%	9.5%	0.8%	1.1%	0.3%	0.3%	100.0%
	RS	number	25	1	2	0	0	0	28
		%	89.3%	3.6%	7.1%	0.0%	0.0%	0.0%	100.0%
Total		number	425	41	5	4	1	1	477
		%	89.1%	8.6%	1.0%	0.8%	0.2%	0.2%	100.0%

The two cases in which no organ could be visualized were correlated with very low acoustic impedance, increased BMI, and difficulties in maintaining clinostatism and apnea, due to the accusation of comorbidities, in conditions of postprandial examination and with the bladder in vacuity. In these cases, the limits of the method are declared and the patient was guided to imaging methods compatible with their particularities.

DISCUSSION

The terms used in this study are based on the recommendations of EFSUMB 2018 in which it is specified that the ultrasound examination suggests diagnostic hypotheses, but does not make a diagnosis [10]. To extend the accumulated experience to other specialties, we analyzed the studies published in the following areas: emergency medicine, obstetrics, gynecology, cardiology, pediatrics, musculo-skeletal, and pneumology.

Portable ultrasound units were used in the initial phase of trauma resuscitation for Focused Assessment with Sonography for Trauma, based on triage protocols for trauma patients by paramedics emergency physicians: infield FAST or e-FAST examinations. The study demonstrated similar quality with original movies; after 20 min lecture on FAST and US system presentation TeleUS (FAST) for US naive paramedics by emergency physicians was performed FAST on a patient with real-time EP connection. The result of the study was 100% complete views, with a mean time of 262 seconds (< 5 minutes) [11].

Obstetrics and gynecology: lack of fetal radiation exposure make teleUS the perfect tool for geographically isolated pregnant patients. Confirmation of intrauterine pregnancy, monitoring of fetal growth, and evaluation of pregnancy-related complications, with markedly reduced costs, were demonstrated in the studies [12,13,14].

Echocardiography is indispensable in clinical decision-making remote fetal echocardiography congenital prenatal heart disease left ventricular systolic function, LV ejection fraction [15].

Remote spatial applications International Space Station applications, 2h pre-flight training modules, 60 min in-flight session, e-learning system for US operators, no consistent significant effects of long-duration space flight on echocardiographic variables [16,17,18].

The use of ultrasound in the examination of musculoskeletal structures exceeded the limit of the penetrability of ultrasound through the bone and allowed the opening of new horizons both in direct examination and by tele-echography including self-examination [19].

In pediatrics, the use of point-of-care ultrasonography (POC US) is very useful [20].

During the COVID-19 pandemic, it was necessary to use all diagnostic means including ultrasound, which proved to be a low-cost tool very useful in remotely guided pulmonary resuscitation [21].

Tele-echography in addition to teleconsultations is a unanimously accepted aid.

The main problem is the number of specialists needed to solve the increasing demand for tele-echography services. The solution can be to accredit the overspecialization of the nurses in synchronous tele-echography, and the use of artificial intelligence in the field of tele-ultrasound.

On the existing structure, other types of ultrasound examinations are carried out: echo cardio, thyroid echo, and peripheral eco doppler, which is the subject of ongoing studies.

In the future, networks, such as those described, can be organized in human communities such as militia units, refugee camps, children's camps, and theaters of battle.

CONCLUSION

Portable hand-held ultrasound devices will move this valuable medical investigation near the patient and the medical society must prepare for this stage.

Future research directions may be a combination of tele-consultation with other investigations that can participate in the diagnosis of the remote patient: tele-EKG, tele EEG, tele-dermatoscopy, tele-otoscopy, telemedicine stethoscope.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Authors' contribution

Conceptualization, Nicolau Carmen Daniela and Luiza Enache methodology, Cormos Bogdan; software, Adrian Tarta validation, and formal analysis, Adrian Saftoiu, investigation, Remus Sipos resources, Nicolau Carmen Daniela; writing—Nicolau Carmen Daniela; writing—review and editing, Adrian Saftoiu.; project administration, Bogdan Cormos. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted following the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Lotus Image Medical Center (protocol code 10 / 22.10.2022)." for studies involving humans.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Assessment of Cerebral Tumors and Metastases by Two-Photon Excitation Microscopy

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Abstract: *Objective: The study subject approaches a topic of cerebral pathology, namely the development of a pathological diagnosis that is as accurate as possible for the cerebral tumors and the metastases, using a new method that complements the optical microscopic examination and immunohistochemistry to correctly diagnose cerebral malignancies. Methods: Two-photon excitation (TPE) microscopy techniques are non-linear optical imaging methods that are gaining momentum in the investigation of fixed tissue sections, fresh tissue, or even for in vivo experiments. This method can be used for offering complementary information on the tissue architecture. We argue that using non-linear optical microscopy (which includes TPE microscopy) images collected on this category of brain tissue samples, can facilitate the interpretation of brain tumors and metastases, thus it is a solution worthy to explore. Scanning large areas of the lesions is important to avoid false-negative diagnoses because lesions are often non-uniform. Finding potential invasion sites is also restricted when using a narrow field-of-view imaging method. Results: Our study demonstrated that TPE microscopy can detect specific cellular features of cerebral tumors and metastases in good correlation with histopathological results. This approach can improve the accuracy of the cerebral tumors diagnosis and possibly other neoplasms. Conclusion: TPE microscopy is very important for future research because it could prevent the false or inadequate diagnosis of specific lesions and differentiate a primary tumor from a metastasis. The significance of the findings is attributable to cross-disciplinary cooperation and the constant use of a working standard across all of the experiments in this study.*

Keywords: *Two-photon excitation (TPE) microscopy, cerebral tumors, metastases, non-linear optical microscopy*

INTRODUCTION

Cerebral tumors are common in the general population and can present with focal neurological symptoms, seizures, or headaches, while some are asymptomatic and found incidentally. Cerebral metastases, meningiomas, and gliomas, particularly glioblastoma, are the most frequent types of cerebral tumors. Complex coordination of treatment between medical oncologists, radiation oncologists, neurosurgeons, and primary care physicians is necessary for the management of brain tumors and their consequences.

The term "primary cerebral tumors" refers to primary intracranial tumors that originate from the meninges, neuroepithelial tissues, pituitary and associated structures, cranial nerves, germ cells, blood-forming organs, or a distant subclinical primary tumor. In the United States, with an estimated 23,380 new cases identified in 2014 and 14,320 fatalities, malignant tumors are uncommon in adults. They accounted for 1.4% of all new cases of cancer and 2.4% of all cancer deaths. New cerebral tumors occur at a rate of 6.4 per 100,000 people annually, and their overall five-year survival rate is 33.4%. The peak occurrence follows between the ages of 55 and 64, and men are slightly more likely than women to experience it [1].

Primary cerebral tumors have a complex etiology, and genetics undoubtedly play a part in how they arise. The only environmental risk factor for primary brain cancers that has been scientifically validated is high-dose ionizing radiation exposure. There is little evidence that additional toxins or exposures raise the risk [2,3].

The World Health Organization assigns malignancy-grade tumors based on a combination of morphological characteristics, growth patterns, and molecular profiles. Primary cerebral tumors are categorized based on histopathologic criteria and immunohistochemistry data [4].

Primary cerebral tumor clinical symptoms might be general or specific. The cause of general symptoms like headaches and seizures is elevated intracranial pressure [5]. The compression or tissue breakdown of specific regions causes focal symptoms, such as unilateral weakness or personality abnormalities [6]. Early signs of low-grade tumors or early stages of illness are frequently focal before becoming more generalized as the tumor grows and spreads [7].

The typical perception of a tumor headache is that it is intense, worse in the morning, and accompanied by nausea and vomiting. But bifrontal, tension-type headaches are more frequently reported in people with cerebral tumors [8].

There is no distinct pathognomonic marker on imaging that distinguishes between main cerebral tumors and metastatic or nonneoplastic disease, despite the fact that imaging considerably reduces the differential diagnosis of primary cerebral tumors. Chest and abdomen CT may be helpful in situations of suspected or pathologically confirmed metastatic cancer, but pinpointing the location of the source tumor is frequently challenging, particularly when there are no clinical cues from the history and physical examination [9].

A skilled interdisciplinary team made up of experts in neurosurgery, radiation oncology, and medical oncology makes customized treatment decisions for each patient. The nature and location of the tumor, its likelihood of being malignant, the patient's age, and general health all factor into treatment considerations. Although surgery, radiotherapy, chemotherapy, or a combination of these may be used as the sole form of treatment, registration in clinical trials should be made available as an option for some high-grade malignancies [10].

The most safe and effective surgical removal of the tumor, followed by radiotherapy and chemotherapy, is the preferred course of treatment for primary brain tumors. Although the size of the resection is a predictive factor, the size of a safe tumor resection depends on the location of the tumor, the patient's performance, and most crucially, the patient's age [11].

After surgical resection, radiotherapy may be administered as the main treatment or as an adjuvant. Although brachytherapy, fractional stereotactic radiation, and stereotactic radiosurgery are other alternatives, standard fractionated external beam radiotherapy is the most used method. In some circumstances, combining radiation and chemotherapy has been demonstrated to increase survival [12].

In addition, it was studied with two-photon excitation (TPE) microscopy on mouse brains, even in-vivo, which may have an important role in our study [19].

This microscopy technique can be applied in many fields including physiology, neurobiology, embryology, and tissue engineering. Thin, almost transparent tissues (such as tegument cells) can be visualized in sharp detail high-speed imaging capabilities can also be used in the non-invasive optical biopsy. In cell biology, TPE microscopy has been used to produce localized chemical reactions. To detect cells and collagen in the cornea using intrinsic fluorophores (autofluorescence and second harmonic generation) as well as using extrinsic fluorophores [18].

Fluorescence microscopy based on (TPE) is a fluorescence-based imaging technique that allows imaging of tissue up to about one millimeter in depth due to reduced absorption and much lower scattering of emitted light. It has recently been applied in pre-clinical studies [18].

Fluorescence microscopy based on TPE does not require a filter in the emission path because it minimizes the loss of emitted radiation. The attenuation of excitation radiation due to scattering is also reduced because the scattering cross-section decreases with increasing wavelength. Near-infrared radiation used in two-photon excitation has several orders of magnitude less absorption in biological tissue than UV or blue-green radiation. A clear separation is achieved between the excitation and emission spectrum, ensuring the removal of excitation radiation and Raman scattering [20].

The discovery of potential sites of invasion is restricted by a limited wide field of view. One option for increasing the field of view, to be used in future research, is the acquisition of adjacent images and stitching these by using mosaicking algorithms. High penetration depth is important for imaging abnormal cells in cerebral tissue samples [20].

Over the past two decades, significant efforts have been made to advance the state-of-the-art in the clinical diagnosis of malignant dermatological disorders (e.g., non-melanoma skin cancers) [18]. However, the gold standard for the identification of such disorders continues to be conventional histopathology assays that call for the removal of small tissue fragments, fixation, staining, and subsequent bright-field microscopy examination. However, it must be remembered that these methods have significant drawbacks, including sample error, sluggishness, high costs, and interpretation unpredictability [13]. Due to these restrictions, there is a significant demand for faster, less invasive alternative diagnosis approaches that maintain the benefits of traditional histopathology, particularly those that relate to simple picture interpretation. These would steadily enhance the diagnostic workflows, lower related expenses, and finally result in increased survival rates of the patients [14].

Magnetic resonance imaging, optical coherence tomography, and more recently non-linear optical microscopy are some of the most promising approaches that have been put out to date for brain diseases. TPE microscopy is a prominent non-linear optical technique that offers supplemental data that has been shown to date to be extremely beneficial for determining tissue conditions. TPE microscopy entails the simultaneous absorption of two photons whose combined energy is sufficient to cause a transition to an excited state and the ensuing emission of a single photon with an energy that is only marginally less than the combined energy of the two excitation photons as a result of non-radiative processes (e.g., vibrational relaxation) [15].

Optical microscopy is a technology that can collect size and shape details from human tissues, but when the cell structure becomes poorly differentiated, the features of this technique are limited. The main limitations of optical microscopy are related to resolution (implicit in light diffraction) and the wavelength of the radiation used. So, the modifications that can be made to improve the spatial resolution and image contrast are by using radiation with shorter wavelengths, increasing the numerical aperture, or using a medium with a high refractive index. Using even a microscope with very good quality lenses, a well-aligned system, and the largest aperture, the resolution remains limited to half the wavelength used. Confocal scanning microscopy and multi-photon microscopy bring an improvement in both spatial and axial resolution limits [20].

The TPE microscopy technique is a non-linear optical imaging method. It is based on the excitation of a fluorophore by the simultaneous absorption of two photons in a single event. This method can be used to provide complementary information on tissue architecture [16].

In the case of two photons excitation, it is necessary that the wavelength of the photons is twice the wavelength of the photons used in the case of excitation using a single photon to obtain the same excitation energy, which corresponds to the difference between the energy of the ground state of the molecule and the of the excited state. At the same time, the two photons must reach the sample in a very short time (less than 10-16 s).

Since the emission of fluorescence by two-photon excitation depends on the simultaneous absorption of two photons with infrared wavelengths, the probability of two-photon absorption by a fluorescent molecule is a quadratic function of the excitation radiance [17].

MATERIALS AND METHODS

The use of nonlinear optical microscopy images (which includes TPE microscopy) collected on this category of cerebral tissue samples can facilitate the interpretation of cerebral tumors and metastases and is thus a solution worth exploring. Scanning large areas of lesions is important to avoid false-negative diagnoses, as lesions are often non-uniform.

In order to achieve an excitation based on the coupling of two photons (which has a low probability), it is necessary to have a lot of photons (thus strong laser radiation) at the same time and in the same place. This thing is achieved using a Ti: Sapphire laser with femtosecond pulses, where basically the photons will be in these pulses, and placing them in the same place is done with high numerical aperture objective lenses that focus the radiation in very narrow areas. As an excitation source, we used a Ti: Sapphire laser (Chameleon Ultra II, Coherent) tuned at 870 nm, with ~140 fs pulses and a repetition rate of 80 MHz. A 10× magnification and 0.3 numerical aperture (NA) objective was used for focusing the laser beam on the sample and for collecting the two-photon excitation signals.

The material was collected from patients who had a histopathological diagnosis after surgery ("Dr. Carol Davila" Central Military Emergency Hospital Bucharest). Tissue fragments were fixed in 10% formalin, embedded in paraffin, with paraffin block sections – 4-5 microns thick. Images were acquired on labeled samples (Hematoxylin & Eosin stained) on the basis of which histopathological diagnoses were made under a conventional light microscope and fluorescence was from Eosin.

The macroscopy technique has been adapted to the particularities of each individual surgical specimen provided that the following requirements are:
- macroscopic description of the specimen and all macroscopic lesions;
- taking fragments of all macroscopically identified lesions, as far as possible including both macroscopically pathological and normal tissue in the same fragment;
- harvesting of fragments from the resection margin; if the macroscopically identified lesion was at a great distance from the resection margin (not being possible to include both lesion and resection margin in the same tissue fragment at the same time), the resection margin could be harvested parallel to the

plane of the incision; if the lesion was located close to the resection margin, harvesting perpendicular to the plane of the incision so that, after microscopic examination, the distance between the lesion and the resection margin could be measured. In the case of tumor pathology, this type of approach makes it possible to establish the completeness of the resection within oncological safety limits.

The final product is the permanent histopathological preparation which allows correct interpretation to establish the pathological diagnosis.

It worked according to the protocol of the Pathology Department of the Central University Emergency Military Hospital. For example, different reagents may be used, and the time of action and concentrations mentioned in the recipes are indicative and are adapted according to local conditions (ambient temperature, hardness, and pH of tap water). The technique used is considered correct if the results expected by the staining are obtained. Variations in the recommended staining recipes are allowed, depending on the structure to be revealed.

RESULTS

The standard of the histopathological technique requires the transformation of an organ or tissue specimen from an opaque tissue mass into a fine, translucent preparation that allows microscopic visualization of tissue structural details.

We made an analysis of the images acquired with fluorescence microscopy based on TPE and we'll try in future research to develop the earliest possible diagnosis of cerebral tumors based on distribution and tissue architecture. We acquired images for both a primary cerebral tumor and a secondary (metastatic) tumor.

Figure 1: Cerebral adenocarcinoma metastasis - (a), (b), (c) - Bright-field microscopy image of Hematoxilin&Eosin-stained section - low magnification: (a) cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (top left corner) (b) cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (in the center of the image), (c) cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (bottom left corner); (d) TPE microscopy image for stained section (a), which clearly shows the desmoplastic stroma with collagen fibers by the bright orange-yellow; (e) TPE microscopy image for stained section (b); (f) TPE microscopy image for stained section (c).

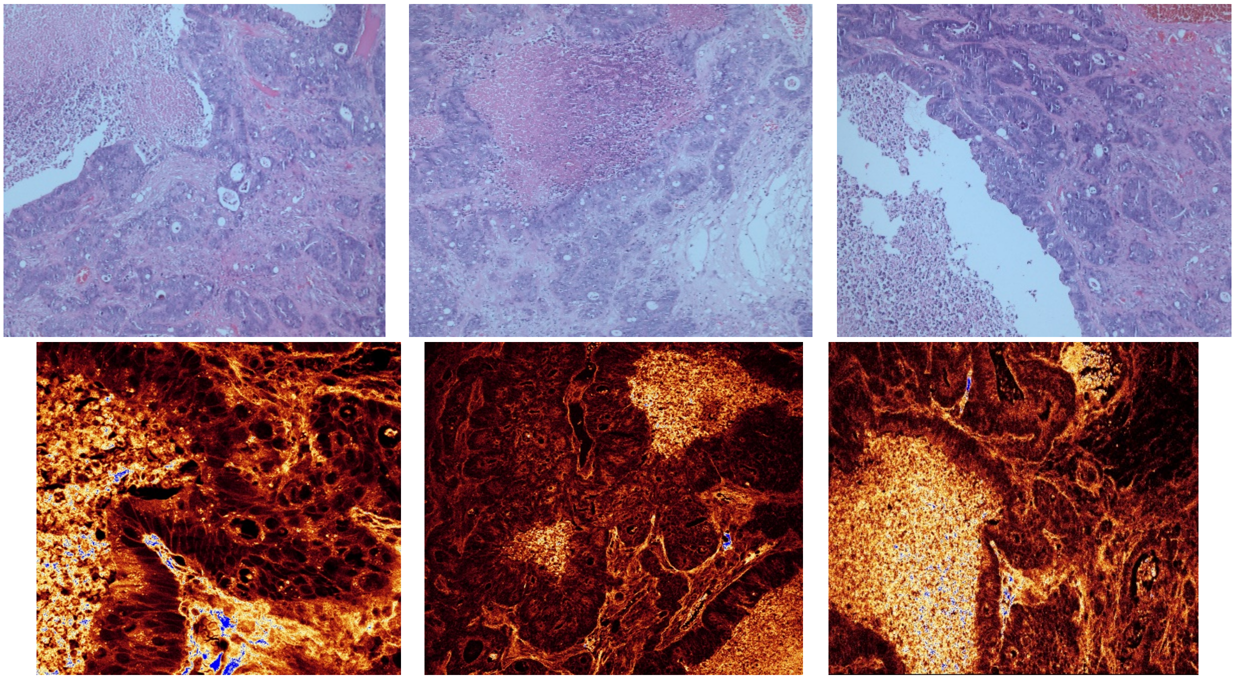

In Figure 1 (a) – cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (top left corner), 1(b) – cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (in the center of the image) and 1 (c) – cerebral adenocarcinoma metastasis with necrotic debris inside the luminal of tumoral glands (bottom left corner) acquired images, we can visualize tumor proliferation composed of medium-sized cells grouped in glandular structures with a cribriform, tubular and solid pattern, associated with extensive areas of necrotic debris and desmoplastic stroma – like a colonic adenocarcinoma metastasis in the right occipital lobe, with a history of a colon neoplasm.

In Figure 1 (d) (e) (f) acquired TPE microscopy images we can observe the glandular tumor structures represented in dark red, and the lakes of necrotic debris and desmoplastic stroma with collagen fibers in bright orange-yellow, with the mention that the areas of necrotic debris are punctate and brighter, and the collagen fibers in the desmoplastic stroma are represented by linear-wavy curves.

Figure 2: Atypical meningioma - (a)(b)(c) - Bright-field microscopy image of Hematoxilin&Eosin - stained section - low magnification; (a) Atypical meningioma with spindle cells with increased cell density areas; (b) Atypical meningioma with true psammoma body (black arrow); (c) Atypical meningioma with early ages psammoma bodies according to their level of calcification) and vascular spaces; (d) TPE microscopy image for stained section (a); (e) TPE microscopy image for stained section (b) – psammoma body (white arrow); (f) TPE microscopy image for stained section (c), but with high magnification to highlight the vascular spaces detectable on the Hematoxilin&Eosin-stained section (light blue).

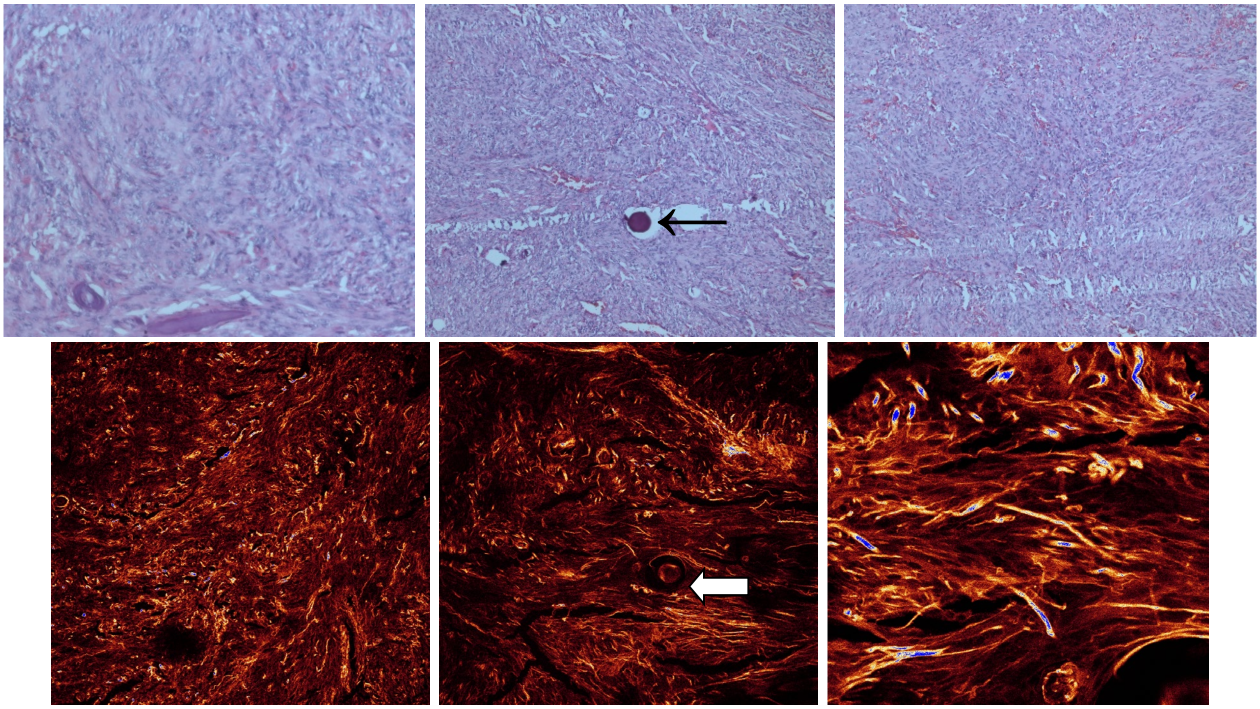

In Figure 2 (a), (b), (c) images, we detect a primary cerebral tumor in the left parietal lobe. We can observe on the Hematoxilin&Eosin-stained section, a tumor proliferation consisting of spindle cells with increased cell density, cellular atypia, and the presence of calcospherites well represented also in two-photon excitation microscopy from Figure 2 (d), (e), (f). Calcospherites or psamomma bodies are concentric calcifications of meningothelial cell nests (concentric laminated eosinophilic bodies) with different ages depending on their level of calcification. A true calcospheritis is well represented (black arrow) in a Bright-field microscopy image of Hematoxilin&Eosin – Figure 2(b). Histopathological features are consistent with atypical meningioma.

In Figure 2 (f) Corresponding two-photon microscopy image for stained section (c) – low magnification, we acquired two-photon excitation microscopy images at high magnification to highlight the vascular spaces (light blue) detectable in the standard Hematoxylin&Eosin-stained section.

DISCUSSION

Our study demonstrated that TPE microscopy can detect specific cellular features of atypical meningioma (primary cerebral tumor) and colonic adenocarcinoma (cerebral metastases) in good correlation with histopathological findings and even differentiate them.

Although such efforts are very valuable and play an important role in facilitating the interpretation of nonlinear optical data, it should be noted that a universal image processing or artificial intelligence method capable of generalizing and accurately addressing all tissue types is still lacking.

The use of this technique will also avoid the overuse of other relatively expensive complementary diagnostic techniques currently available (molecular biology techniques, genetic tests, IHC tests, electron microscopy, etc.).

The importance of the results obtained is due to interdisciplinary collaboration and the consistent application of a working standard throughout the experiments in this work. TPE microscopy has allowed us to obtain a global image of tissue because fluorescence has this advantage. Used as an additional technique with applicability in pathology could help to avoid underdiagnosis or overdiagnosis of some lesions, whose correct diagnosis is essential for proper and effective treatment.

CONCLUSION

Our research demonstrates that TPE microscopy can differentiate a cerebral metastasis from an atypical meningioma (primary cerebral tumor). Also with the help of two-photon excitation microscopy, it is possible to detect cellular, glandular, and vascular spaces, calcospherites in different stages, necrotic debris, and desmoplastic stroma structures. All of these are important tissue elements in diagnosing and differentiating certain tumor entities.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution
All authors had equal contributions to this study elaboration.

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ORIGINAL ARTICLE

An Overview of Panniculitis – A Diagnostic Challenge for Dermatologists and Pathologists

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Abstract: *The term "panniculitis" refers to a heterogeneous group of conditions that involve inflammation of the subcutaneous cellular tissue. Although there is no universally accepted classification of panniculitis, to facilitate the diagnosis, they were divided into two large classes, based on the histopathological criteria: predominantly lobular and predominantly septal panniculitis. Depending on the presence or absence of vascular damage, each of the two classes mentioned above is divided in turn into panniculitis without vasculitis and panniculitis with vasculitis. The diagnosis of various types of panniculitis can sometimes be very difficult, due to the histopathological changes that occur in the various evolutionary stages of the lesions. Thus, the positive diagnosis relies on the corroboration of clinical data, histopathological examination, and ancillary laboratory tests. We provide an overview of the clinical and histopathological features of panniculitis, classified according to the histopathological criteria.*

Keywords: *panniculitis; inflammation; vasculitis; necrosis; subcutaneous tissue*

INTRODUCTION

The term "panniculitis" refers to a heterogeneous group of conditions that involve inflammation of the subcutaneous cellular tissue. The clinical and histopathological diagnosis of panniculitis is difficult to establish, this group of conditions being considered "the ugly duckling of dermatopathology", firstly because their deep localization does not allow the clinician to identify characteristic aspects for each type of panniculitis and secondly because of the inadequate sampling of the biopsy piece, which is often not deep enough.

Clinically, most panniculitis is characterized by the occurrence of erythematous nodules, that are not pathognomonic for a particular clinical subtype. A careful anamnesis, location, and evolution of the lesions, symmetry, age of onset, and association with systemic signs and symptoms must be taken into consideration for the clinical diagnosis.

Histopathological examination is a useful tool for a positive diagnosis. It is necessary to perform a deep incisional skin biopsy, which should also include subcutaneous cellular tissue, from an early-stage, evolving nodular lesion. Lesions in advanced stages usually show microscopic changes of lipoatrophy and sclerosis, leading to an inconclusive result of the histopathological examination [1,2].

Several histopathological features need to be considered in order to correctly classify panniculitis, mainly the location of the inflammatory infiltrate (septal, lobular) and the presence or absence of associated vasculitis. Based on these criteria, panniculitis is divided into septal and lobular panniculitis with or without vasculitis and if it exists, the size of the affected vessels can guide the diagnosis. Further details such as the type of adipocyte necrosis, the type of inflammatory cells, or the presence of granulomas may contribute to a more precise diagnosis. Although it is considered that all panniculitis is mixed panniculitis since the inflammatory infiltrate can be highlighted both in the lobules and the septa, based on where it predominates the condition may be classified into a predominantly lobular or a predominantly septal form [1,3].

Necrosis of adipocytes is another aspect that can be observed during the histopathological examination of panniculitis, these appearing either in the form of elements that have completely lost their cellular structure or in the form of anucleated cells, depending on the type of necrosis.

The most frequently encountered type of adipocyte necrosis is lipophagic necrosis, the so-called foamy macrophages, loaded with lipids, obtained by digesting dead adipocytes, being objectified during microscopic evaluation. Their cytoplasm can be granular or pale, with multiple microvacuoles. Lipodermatosclerosis and traumatic panniculitis are typical examples of this type of necrosis [1,3,4].

The hyalinizing necrosis of the adipose tissue consists of adipocytes with a homogeneous glassy protein material in the cytoplasm, altering the cellular structure. This type of necrosis is most often found in lupus panniculitis [1,3,4].

Another type of adipocyte necrosis is represented by liquefactive necrosis, which consists of granules formed by amphophilic detritus. This type of necrosis is frequently encountered in pancreatic panniculitis and in alpha-1-antitrypsin deficiency panniculitis. In pancreatic panniculitis, a subtype of liquefactive necrosis is objectified, the so-called enzymatic necrosis, which consists in the saponification of the cytoplasmic lipids in the adipocytes by the circulating pancreatic lipase [1,3,4].

Ischemic necrosis of the adipose tissue usually occurs in the center of the lobules, initially as anucleated adipocytes, but if the ischemia persists, lipophagic necrosis changes can also be observed, as seen in erythema induratum of Bazin [1,3,4].

Membranous necrosis of adipocytes represents an advanced stage of necrosis that consists of the appearance of an amphophilic or eosinophilic border of degraded cellular organelles. This type of necrosis is a histopathological change constantly encountered in sclerodermiform dermohypodermitis, but it is not specific to it, as it can be observed in almost all advanced stages of panniculitis [1,3,4].

The final aspect to take into account during the microscopic examination of the skin fragment is the type of cells in the inflammatory infiltrate [1,3,4].

In predominantly lobular panniculitis, in which the histiocytic inflammatory infiltrate predominates, septal sclerosis associated with a history of radiotherapy is suggestive of post-radiation panniculitis, while the microscopic objectification of crystals may be suggestive of panniculitis associated with gout, fat necrosis of the newborn, crystal storing panniculitis or panniculitis after administration of corticosteroids. Evidence of an inflammatory infiltrate rich in histiocytes, with no evidence of crystal, should direct the diagnosis towards posttraumatic panniculitis, lipoatrophy, or subcutaneous sarcoidosis, while cytophagocytosis suggests a possible cytophagic panniculitis [5].

In predominantly lobular panniculitis, in which an inflammatory infiltrate rich in neutrophils associated with foreign bodies is evident, we lean towards factitial panniculitis, while the presence of bacteria, fungi, or protozoa suggests infectious panniculitis. In the case of a predominantly lymphocytic infiltrate, the presence of lymphoid follicles and plasma cells is suggestive of the diagnosis of lupus panniculitis, while the location of infiltrate perivascularly in the deep dermis and subcutaneous adipose tissue tilts the scale towards the diagnosis of cold panniculitis. Last, but not least, the infiltrate without inflammatory cells leads to the diagnosis of sclerema neonatorum, if it is associated with needle-shaped crystals located in the center of fat cells, to calciphylaxis or oxalosis if calcifications are observed in the blood vessels or to sclerosing panniculitis if the microscopic examination shows necrosis in the center of the fat lobules [5].

In predominantly lobular panniculitis associated with vasculitis, the size of the affected blood vessels must be determined, venules damage suggesting either neutrophilic nodular panniculitis, Lucio phenomenon, or erythema nodosum leprosum, while damage of the arteries and veins is highly suggestive for erythema induratum, also known as nodular vasculitis [4,5].

In predominantly septal panniculitis that does not associate vascular damage, in which the histiocytic inflammatory infiltrate predominates, the radial granulomas in the septa are suggestive of erythema nodosum, while the microscopic objectification of cholesterol clefts may be suggestive of xanthogranulomatous necrobiosis. Evidence of an inflammatory infiltrate rich in histiocytes, associated with fibrin directs the diagnosis toward a rheumatoid nodule, while the association with mucin suggests a possible subcutaneous annular granuloma [5].

The histopathological examination of an inflammatory infiltrate made up of lymphocytes and plasma cells, associated or not with granulomatous infiltrate, in a patient with predominantly septal panniculitis tilts the scale towards the diagnosis of necrobiosis lipoidica, respectively deep morphea.

Predominantly lobular panniculitis is divided into forms with vasculitis of large vessels, such as nodular vasculitis, and forms without vasculitis, such as alpha-1-antitrypsin deficiency, cold panniculitis, sclerosing panniculitis, calciphylaxis, lupus panniculitis, pancreatic panniculitis, infectious panniculitis, traumatic panniculitis, polymeric microemboli from the coating of intravascular devices and histiocytic panniculitis with cytophagy and subcutaneous T-cell lymphoma.

Predominantly septal panniculitis without vasculitis may be found in erythema nodosum, rheumatoid nodule, lipoid necrobiosis, and eosinophilic fasciitis [6,7].

Predominantly septal panniculitis associated with vasculitis is divided according to the caliber of the affected vessels into panniculitis with vasculitis of large vessels and panniculitis with vasculitis of small vessels. If the affected vessels are represented by arteries, the suspected diagnosis is cutaneous polyarteritis nodosa, while the damage of veins suggests potential superficial thrombophlebitis. Venule involvement in a patient with panniculitis indicates leukocytoclastic vasculitis [8].

MOSTLY LOBULAR PANNICULITIS WITH VASCULITIS

Nodular vasculitis (Erythema induratum)

Nodular vasculitis is the most common form of predominantly lobular panniculitis with vasculitis, firstly described by Bazin in 1861, as firm, erythematous, indurated plaques and nodules, located predominantly on the dorsal side of the calves, more frequently in middle-aged obese women, with a history of thrombophlebitis, chronic venous stasis or cooling [9].

Although tuberculosis is the most frequent infectious triggering factor incriminated in the etiopathogenesis of nodular vasculitis, this being considered a "reactive disorder", there are also cases of the disease in which no trigger factor has been identified [10,11]. Many factors have been cited in the etiopathogenesis of erythema induratum, both non-infectious (hypothyroidism, chronic lymphocytic leukemia, superficial thrombophlebitis, rheumatoid arthritis, various drugs) and infectious (tuberculosis, group B and C hepatitis, Fusarium, Nocardia, Pseudomonas).

Clinically, erythema induratum appears as recurrent episodes of firm, indurated, erythematous nodules and plaques, located symmetrically on the dorsal side of the limbs, where the adipose tissue is well represented and the covering skin is cyanotic and cold. Nodules appear slowly, usually during winter and some of them ulcerate, discharge an oily material, then slowly heal, leaving atrophic scars.

Nodular vasculitis is characterized by a granulomatous type of inflammation that occurs as a specific response mediated by T lymphocytes to an antigen or as a type IV hypersensitivity reaction [12].

Acute cases of erythema induratum and infectious panniculitis present similar microscopic features, such as basophilic zonal necrosis and neutrophilic inflammatory infiltrate, which is why cultures and special stains for bacteria and other microorganisms are performed to exclude an infectious cause of panniculitis. In nodular vasculitis, microorganisms are never detected on special stains [13].

Histopathologically, nodular vasculitis is predominantly lobular panniculitis. In the early stages, a discrete inflammatory infiltrate with neutrophils can be detected in the lobules, but during the course of the disease, extensive areas of ischemic necrosis of the lobular adipocytes can be observed. The necrotic adipocytes are ingested by the histiocytes that later transform into foam cells. The histopathological examination of the nodular vasculitis lesions shows a characteristic aspect of granulomatous inflammation, the inflammatory infiltrate is composed of multinucleated giant cells, epithelioid histiocytes, and lymphocytes. In case of severe vascular damage, extensive areas of caseous necrosis can be noted, microscopically similar to tuberculosis lesions [14]. The type of inflammatory infiltrate also varies depending on the age of the lesions, so that in the early stages an inflammatory infiltrate predominantly composed of neutrophils is observed, while in the advanced stages of the disease, the inflammation is lymphocytic or granulomatous.

Once the histopathological diagnosis is established, it is necessary to evaluate a possible association with tuberculosis. In this sense, a chest x-ray, a Mantoux tuberculin test, and/or an interferon-gamma release assay test are performed. If one of the last two is positive, the patient will follow 9 months of triple tuberculosis therapy [15,16].

MOSTLY LOBULAR PANNICULITIS WITHOUT VASCULITIS

Panniculitis due to alpha-1-antitrypsin deficiency

Alpha-1-antitrypsin (AAT) is a circulating inhibitor of serine proteases. It inhibits the enzymes involved in the proteolytic degradation of tissues, has effects on the immune system, and inhibits complement activation. Severe deficiency can be associated with systemic diseases, such as pulmonary emphysema, pancreatitis, liver cirrhosis, glomerulonephritis, rheumatoid arthritis, vasculitis, and/or angioedema. Rarely, AAT deficiency is associated with recurrent lobular panniculitis and necrosis of the subcutaneous fat tissue. Patients with a partial deficiency may present panniculitis without systemic symptoms.

Clinically, panniculitis due to alpha-1-antitrypsin deficiency appears as recurrent, painful, erythematous plaques and nodules, with a proximal distribution on the hips, thighs, and buttocks, being most often induced by local trauma.

Characteristic of this type of panniculitis is the fact that the nodules ulcerate and suppurate, discharging an oily fluid, and heal with scarring.

The diagnosis is established on clinical, paraclinical, and histopathological criteria. The dosage of the serum AAT level and the evaluation of the AAT phenotype is indicated.

Histopathology shows in the early stages neutrophilic panniculitis in the reticular dermis and between the collagen bundles, followed by necrosis and destruction of adipocytes. Even though primary leukocytoclastic vasculitis is absent, secondary lymphocytic vasculitis may be found in areas with heavy neutrophilic infiltration and thrombosis [17].

Cold panniculitis

The cold panniculitis is a form of panniculitis that occurs most frequently in children exposed to extremely low temperatures or during winter, in people wearing tight pants that do not provide adequate thermal insulation and restrict blood supply to the subcutaneous fatty tissue [18,19].

Clinically, one can notice marbled, reddish-blue plaques similar to frostbite, located on the thighs and buttocks. If the affected skin areas are kept warm, the skin lesions disappear after a few days, without scars.

During the histopathological examination, an inflammatory infiltrate consisting of histiocytes and lymphocytes located in the adipose lobules can be noted, sometimes associated with edema in the papillary dermis and an extension of the inflammatory infiltrate in the dermis, the microscopic appearance being similar to that of perniosis [20].

Sclerosing panniculitis

Sclerosing panniculitis, also known as sclerodermiform dermohypodermitis or lipodermatosclerosis, is a complication of venous stasis, more common in women characterized by chronic inflammation, fibrosis, and progressive induration [21, 22].

Clinically, in the early stages, lipodermatosclerosis appears as erythematous-purple, indurated, imprecisely demarcated, painful plaques, similar to cellulitis, while in late stages, it appears as a woody plaque, with well-defined edges, hard on palpation. Fibrosis is accentuated in the subcutaneous tissue, and eventually, the leg takes on the appearance of an inverted champagne bottle.

The main etiological factor of lipodermatosclerosis is chronic venous insufficiency of the lower limbs, causing sludging in the lobular capillaries, with ischemia and necrosis of the central portion of the adipose lobules. [23]

Other factors involved in the etiopathogenesis of lipodermatosclerosis are represented by reduced plasma fibrinolytic activity, protein S and C deficiency, and excessive collagen production [24,25].

Histopathologically, the characteristic changes of stasis dermatitis are noticeable in the superficial dermis, while in the papillary dermis, there is a proliferation of capillaries and venules, fibrosis, and abundant deposition of hemosiderin. In the early stages, small amounts of inflammatory infiltrate, predominantly composed of lymphocytes, distributed among the collagen bundles of the septa are found, while in the center of the lobules, "ghost" outlines of the anucleated necrotic adipocytes can be observed. In the advanced stages of the disease, septal sclerosis is the main histopathological change. Other histopathological changes that can be objectified are represented by foci of necrotic keratinocytes, simultaneously with areas of extravasated erythrocytes and vascular congestion [24,25].

The diagnosis of lipodermatosclerosis is based on clinical criteria, the biopsy not be indicated due to the difficult healing given by the vascular damage and the risk of developing ulcers at this level. In the advanced stages of the disease, sclerosing panniculitis requires a differential diagnosis with conditions associated with diffuse induration in the lower limbs, such as scleromyxedema, morphea, erythema nodosum, myxedema, necrobiosis lipoidica, eosinophilic fasciitis, as well as another panniculitis.

Calciphylaxis

Calciphylaxis is a condition with a high mortality rate that occurs frequently in patients with chronic kidney disease undergoing dialysis. Calcium deposits in the middle tunic of the small arteries from the skin lead to thrombosis and ischemic necrosis with the appearance of refractory ulcers involving the lower extremities, the abdomen, and the gluteal region [26]. Major risk factors for calciphylaxis are end-stage renal disease, phospho-calcium metabolism imbalance, and primary and secondary hyperparathyroidism, while non-uraemic risk factors include female sex, obesity, diabetes, hypo-albuminemia, autoimmune diseases, warfarin use, protein C and S deficiency, active neoplasia or liver damage in a toxic-metabolic context [27].

Clinically, the lesions are similar to those in livedo reticularis, appearing as marbled, red-violet teguments, with reticular discoloration, reflecting the impaired vascular flow from the superficial dermis. Another variant of the appearance of calciphylaxis lesions can be painful erythema, with a marbled appearance and a sudden onset on the calves or on the feet, evolving into hardened plaques that later become necrotic and ulcerated. Other skin signs may include pathergia (ulceration triggered by minor trauma), recurrent digital ischemia, penile necrosis, and subcutaneous nodules on the breasts and abdomen. Extremely rare, patients can associate concomitant systemic damage such as proximal myopathy, aortic valve damage, or pulmonary calciphylaxis [26,27].

The main histopathological aspects objectified in patients with calciphylaxis are calcifications in small arteries, vascular thrombosis, and intimal hyperplasia (endarteritis obliterans) [28].

Lupus panniculitis

Lupus panniculitis is a chronic form of recurrent panniculitis, which affects women more frequently, the skin lesions being predominantly located on the buttocks, arms, shoulders and face [29].

Clinically, it presents itself as deeply located subcutaneous plaques or nodules, while the adjacent skin may present lesions of discoid lupus erythematosus. The regression of lupus panniculitis lesions can result in the loss of subcutaneous cellular tissue, with atrophy, this being a deforming complication. Subcutaneous nodules can calcify or ulcerate [30].

During the histopathological examination, approximately half of the cases of lupus panniculitis show changes in the epidermis and dermis similar to those in discoid lupus erythematosus, such as vacuolization at the dermo-epidermal junction, epidermal atrophy, mucin interposition between the collagen fibers in the dermis, thickening of the basement membrane or the presence of a lymphocytic inflammatory infiltrate in the dermis, while in the other half of the cases, the pathological changes of lobular panniculitis are located strictly in the subcutaneous adipose tissue [31,32]. A quite characteristic aspect of this type of panniculitis is represented by the presence of lymphoid follicles, with a germinal center and numerous plasma cells on the periphery and between the collagen fibers of the septa of the subcutaneous adipose tissue [33]. Although they are quite specific for lupus panniculitis, they can be objectified on microscopic examination in other conditions as well, such as erythema nodosum, erythema induratum of Bazin, morphea, subcutaneous panniculitis-like T-cell lymphoma or in panniculitis associated with dermatomyositis. In the late stages of this disease, hyaline fibrosis and calcification can be noticed. Membranous necrosis is also common in this stage of the disease. In the microscopic examination of the lesions collected from these patients, an inflammatory infiltrate rich in eosinophils can be objectified. The low titer of antinuclear antibodies does not exclude the diagnosis of lupus panniculitis [34,35].

Dermatomyositis is another systemic collagenosis that can be associated with panniculitis and requires a differential diagnosis with lupus panniculitis. Vasculitis with lymphocytic or neutrophilic infiltrate, the thickening of the walls of the subcutaneous vessels, and the presence of calcifications tip the scaling towards the diagnosis of panniculitis associated with dermatomyositis [36].

Eosinophilic fasciitis and deep morphea present histopathological diagnostic criteria (plasma cell-rich lymphoid infiltrates, fibrous thickening of the interlobular septa, eosinophils occasionally) and clinical (nodules and indurated plaques) are similar to lupus panniculitis. However, the absence of hyaline necrosis and the absence of dermo-epidermal changes (epidermal atrophy, thickening of the basal membrane, orthokeratosis, vacuolar degeneration of the basal layer, prominent dermal mucin) exclude the diagnosis of lupus panniculitis.

Subcutaneous panniculitis-like T-cell lymphoma is characterized by the "rimming" of adipocytes by atypical cytotoxic T cells, which get intensely colored when in contact with proliferation markers. The absence of lupus skin signs and nodular aggregates of lymphoid B cells excludes the diagnosis of lupus panniculitis. From a clinical point of view, there are no differences between lupus panniculitis and subcutaneous panniculitis-like T-cell lymphoma, but the persistence of the lesions, their rapid evolution, and absence of regression point the diagnosis towards lymphoma.

Immunofluorescence studies can be performed, objectifying the presence of linear deposits of C3 and IgM along the dermo-epidermal junction [31].

Pancreatic panniculitis

The pancreatic panniculitis is a form of panniculitis rarely associated with certain pancreatic diseases, such as pancreatic islet cell tumors, acinar cell carcinoma, pancreatic pseudocysts, acute or chronic pancreatitis, ductal adenocarcinoma or post-traumatic pancreatitis [38].

Clinically, pancreatic panniculitis appears as erythematous or red-brown subcutaneous nodules, painful or not, sometimes with a soft center, usually located on the lower limbs, but they can also appear on the arms, buttocks, or trunk. In mild cases of the disease, they heal with atrophic scars, while in severe cases, they can ulcerate and discharge a sterile, brownish, oily material, representing necrotic fat [39]. It can be associated with pleurisy or necrosis of the periarticular tissue [40]. Skin signs can appear a few months before the manifestation of the pancreatic disease. Circulating lipase and amylase are responsible for adipose tissue necrosis in pancreatic panniculitis. Disseminated necrosis of fatty tissue is associated with a high rate of mortality [41].

In pancreatic panniculitis, unlike other predominantly lobular panniculitis, it is preferable to take a skin biopsy from an established lesion. The histology of pancreatic panniculitis is characteristic, an important necrosis of adipose tissue being observed, as well as the appearance of the so-called "ghost cells", which are the former adipocytes that now present a thick, ghostly wall, the cell membrane not being destroyed by lipase. The latter present in the cytoplasm a granular, basophilic material, called calcium soap, which resulted from the combination of fatty acids with calcium ions [42,43].

In the early stages of pancreatic panniculitis, the characteristics of predominantly septal panniculitis determined by the pancreatic enzymes that reach this level through the permeable vascular walls are noted. Identifying the specific microscopic aspects of pancreatic panniculitis can be difficult also in the advanced stages of the disease, in which the structure of the fat lobule is completely replaced by a granulomatous inflammatory infiltrate, made up of multinucleated giant cells and foamy macrophages.

Severe cases of pancreatic panniculitis, associated with a history of pancreatic damage, are relatively easy to diagnose, but the same does not happen in mild cases. These lend themselves to differential diagnosis with erythema induratum of Bazin, infectious panniculitis, erythema nodosum, panniculitis associated with alpha-1 antitrypsin deficiency, aspergillosis, subcutaneous mucormycosis, and subcutaneous metastatic deposits [44,45].

MOSTLY SEPTAL PANNICULITIS WITH VASCULITIS OF THE LARGE VESSELS

Cutaneous polyarteritis nodosa

Cutaneous polyarteritis nodosa is a necrotizing vasculitis that affects the muscular layer of the small and medium arteries in the deep dermis and subcutaneous tissue. The vital organs are not involved, but the disease is recurrent and chronic [46].

Clinically, subcutaneous polyarteritis nodosa lesions manifest as painful skin nodules, associated with palpable purpura, ulcers, and livedo reticularis in the lower limbs. Usually, the presence of a pseudo-influenza syndrome characterized by low fever, unwell general condition, myalgia, and arthralgia is also observed [47].

During the histopathological examination, vasculitis lesions are observed in the small and medium-sized arteries and arterioles from the septa of the upper half of the subcutaneous adipose tissue. The affected vessels have thickened walls, presenting an inflammatory infiltrate composed predominantly of neutrophils. The intima of the affected vessels has a target appearance on the cross-section, presenting an eosinophilic ring of fibrinoid necrosis. Cutaneous polyarteritis nodosa is characterized by the segmental damage of the arterial vessels and by the simultaneous existence of lesions in different stages of evolution [46].

Direct immunofluorescence studies objectify the presence of complement and IgM deposits in the walls of the affected blood vessels [48].

MOSTLY SEPTAL PANNICULITIS WITHOUT VASCULITIS

Erythema nodosum

Erythema nodosum is the most common type of panniculitis in general and the main type of predominantly septal panniculitis without vasculitis. Most cases appear between the second and fourth decade of life, more frequently in women [49]. Erythema nodosum may be a delayed hypersensitivity response to infection or another inflammatory disease. In approximately one-third of the cases of erythema nodosum, a cause could not be identified, but in the rest of the cases, bacterial, viral, fungal, and protozoal infections, drugs, inflammatory bowel diseases, Behcet's disease, sarcoidosis, pregnancy, various neoplasms were blamed as triggering factors [50-52]. Streptococcal infection is the most common cause in children, while in adults different drugs, pregnancy, and sarcoidosis have been reported as the main triggering factors [53].

From a clinical point of view, erythema nodosum lesions appear abruptly as firm, tender, erythematous, non-purpuric, warm nodules, approximately 1-5 cm in diameter, symmetrically distributed on the calves, pretibial, ankles, and knees, after a prodromal period characterized by altered general condition, fever, headache, arthralgia, and myalgia. Rarely, nodules can also appear on the arms or trunk. The nodules from this pathology never ulcerate and they heal without scars. Sometimes, gastrointestinal symptoms can be associated, such as nausea, vomiting, abdominal pain, or diarrhea. In evolution, the nodular lesions in erythema nodosum flatten, leading to the appearance of hematoma-like lesions with a blue-green hue. The lesions fade within 2-6 weeks, even faster in children, leaving behind lesions with a purple appearance, similar to bruises, which disappear slowly [54].

A particular chronic form of erythema nodosum is represented by subacute migratory nodular panniculitis or erythema nodosum migrans, much less frequent, characterized by the appearance of a single firm, indurated, erythematous plaque, which slowly expands to the periphery while it heals centrally [55].

Histopathologically, in the early stages, the microscopic evaluation can objectify an abundant inflammatory infiltrate, rich in neutrophils, which could create diagnostic difficulties. The thickening of the septa is not very important, being caused by the accumulation of inflammatory infiltrate, edema and hemorrhage at this level, and cells around the cleft-like spaces in Miescher's radial granulomas are predominantly neutrophils. In the advanced stages, the inflammation is much reduced, being replaced by lipoatrophy and fibrosis [56].

Chronic erythema nodosum lesions are characterized by thickening of the septa and a slightly abundant inflammatory infiltrate, associating phlebitis and extravasation of erythrocytes, while in erythema nodosum migrans there is marked thickening of the septa from adipose tissue, the presence of multiple granulomas made up of granulation tissue, multinucleated giant cells, and an important capillary proliferation, but without signs of phlebitis [57]. Currently, most authors consider that chronic erythema nodosum and erythema nodosum migrans represent different stages of evolution of the same condition [3]. A quite specific histopathological aspect for chronic erythema nodosum is represented by Miescher's radial granulomas, which consist of small nodular aggregates, well delimited, of histiocytes, agglutinated around a banana- or stellate-shaped fissure, but it can also be identified in other conditions such as Behcet's disease, erythema induratum, Sweet syndrome, necrobiosis lipoidica or nephrogenic fibrosing dermopathy [58].

Necrobiosis lipoidica

Lipoid necrobiosis is a condition characterized by a granulomatous inflammatory process that extends from the deep dermis to the subcutaneous cellular tissue, leading to the appearance of predominantly septal panniculitis. There were no cases of lipoid necrobiosis affecting only the subcutaneous adipose tissue cited in the literature, its inflammation being always secondary to the inflammation of the deep dermis. The pathogenesis of granulomatous inflammation is not fully understood, but many cases of necrobiosis lipoidica have been associated with diabetes mellitus, although it does not occur exclusively in diabetic people [59].

The clinical picture consists in the appearance of oval, reddish-brown, well-demarcated papules and plaques, that may present telangiectasias on the surface, with clearly defined, erythematous, raised, shiny edges, usually located pretibial. The lesions progressively expand and they have a tendency to merge. In older plaques, the center is more yellow and can be atrophic, the subcutaneous vessels being visible through its transparency. The plaques can ulcerate, but the surrounding skin remains intact, showing, in general, some characteristics of necrobiosis lipoidica [59].

The histopathological features of necrobiosis lipoidica, which overlap with those of granuloma annulare, include palisading granulomas composed mainly of histiocytes, which surround the modified collagen fibers from the dermis, the inflammation being able to extend to the subcutaneous cellular tissue, affecting mainly the septa [59].

The immunofluorescence studies performed on lesions in the early stages of necrobiosis lipoidica objectify the presence of complement and IgM deposits in the walls of the vessels from the affected territories and sometimes even along the dermo-epidermal junction [60].

Eosinophilic fasciitis (Shulman’s syndrome)

Eosinophilic fasciitis is a form of localized scleroderma, which affects the deep fascia and sets in suddenly, most of the time being triggered by intense physical exercises. In the initial phase, it can mimic cellulite [61].

From a clinical point of view, eosinophilic fasciitis appears as symmetrical, edematous, reddish-brown plaques, which do not show sensitivity to palpation, usually located on the limbs, which evolve into brown, indurated plaques with a pale center. The extension of sclerosis in the subcutaneous adipose tissue restricts the movements of the tendons of the affected limb [62].

From a histopathological point of view, the lesions in eosinophilic fasciitis are similar to those in subcutaneous scleroderma. The microscopic examination shows thickening and edema of the interlobular fibrous septa and the deep fascia. Both in the septa and the fascia, an abundant inflammatory infiltrate is observed, consisting of eosinophils, lymphocytes, plasma cells, and histiocytes. Occasionally, lymphoid follicles are also present in the septa. The lesions extend deeply, to the underlying fascia, but unlike scleroderma, which usually does not present an inflammatory infiltrate, Shulman's syndrome is characterized by an abundant eosinophil infiltrate in the fascia [63,64].

CONCLUSION

One of the first steps that should be performed in the histopathological examination of lesions suspected of being panniculitis, is to establish whether it is primarily a panniculitis or if it is a dermatitis with extension to the subcutaneous fatty tissue, especially in the septa, as it happens in conditions such as sarcoidosis, lipoid necrobiosis or scleroderma.

Although there is no universally accepted classification of panniculitis, to facilitate the diagnosis, they were divided into two large classes, based on the histopathological criteria: predominantly lobular and predominantly septal panniculitis. Depending on the presence or absence of vascular damage, each of the two classes mentioned above is divided in turn into panniculitis without vasculitis and panniculitis with vasculitis.

Once these two criteria have been established, the type of inflammatory infiltrate in which histiocytes, neutrophils, lymphocytes, or no inflammatory cells may predominate, is also an important element in the diagnosis of panniculitis that does not associate vascular damage, but this may not bring additional information, or it can even delay the diagnosis, since, in this group of conditions, the histopathological picture varies according to the clinical age of the lesions. Most panniculitis lesions begin with an inflammatory infiltrate predominantly composed of neutrophils, after which they pass either through a granulomatous phase or through a phase in which the histiocytic inflammatory infiltrate predominates, while finally, in the late stages, the inflammation is reduced or even absent. Since it is a difficult mission for the clinician to identify with the naked eye the lesions of panniculitis in early stages, those that end up being biopsied are, most of the time, in advanced stages, which is why they do not provide specific information regarding the type of panniculitis.

The diagnosis of panniculitis is difficult, but there are clinical diagnostic keys (location of lesions, symmetry, age of onset) and histopathological ones (type and location of inflammatory infiltrate, presence or not of vasculitis, presence or not of liponecrosis, membranocystic changes, atypia or cellular necrosis). The corroboration of clinical data with histopathological ones and with the results of additional paraclinical explorations depending on the histological classification leads to a positive diagnosis.

The nosological classification of panniculitis is essential for the optimal therapeutic approach, with some cases only needing follow-up, and others having a severe, potentially fatal course in the context of systemic diseases, serious infectious diseases, or neoplasias.

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Authors' contribution

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REVIEW

Pulmonary Hypertension in Autoimmune Diseases – A Review of New Concepts in Screening, Diagnosis and Therapeutic Management

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Abstract: *Pulmonary hypertension (PH) has gained more and more attention in the last years given the increased mortality and morbidity rate and in particular pulmonary arterial hypertension associated with autoimmune diseases (a specific subgroup of PH), entities with an increasing incidence and prevalence globally. Among these, systemic sclerosis is of particular interest, being the most common among connective tissue diseases (CTDs) with PH as a major complication. Other CTDs such as mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE), primary Sjogren's syndrome, and dermatomyositis may also develop PH as a complication of these severe and complex conditions, which can have a major impact on prognosis and quality of life. PH has also been reported in rheumatoid arthritis and ankylosing spondylitis but further studies are necessary. Several studies have shown that the 3-year survival rate for patients with systemic sclerosis-associated with pulmonary hypertension (SSc-PH) is between 31% and 52%, while the 5-year survival rate is below <50%. Therefore, an early and accurate diagnosis as well as subsequent management of PH in these conditions is mandatory, since survival remains suboptimal despite treatment advances. The aim of this article is to provide an overview of different autoimmune diseases associated with PH and to provide an easy reference source on current best practices.*

Keywords: *pulmonary hypertension; autoimmune diseases; connective tissue diseases; vasodilators; Raynaud phenomenon'*

INTRODUCTION

Pulmonary hypertension (PH) is a severe, progressive, and life-threatening disease that can often lead to premature death. Pulmonary hypertension (PH) describes a heterogeneous group of conditions that are defined by an increase in the mean pulmonary arterial pressure (mPAP) and pulmonary vascular resistance leading to an increase in the right ventricular (RV) afterload which results in RV overload and finally to right heart failure and even death. The most common causes of PH are left heart diseases and chronic lung diseases [1,2].

Patients with connective tissue diseases (CTDs) such as systemic sclerosis (SSc), mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE), primary Sjogren's syndrome, and dermatomyositis may develop PH as a complication of these complex conditions, which can have a major impact on prognosis and quality of life. In this review, PH is discussed among connective tissue diseases, with an emphasis on systemic sclerosis, which is the most common of all collagenoses that have PH as a complication. Therefore, new findings regarding epidemiology, early screening/ diagnosis, new therapeutic perspectives, and prognosis are brought to attention [3].

CLASSIFICATION

The current classifications of PH are based on clinical, physiopathological, and etiological similarities along with hemodynamic and treatment characteristics. PH is classified into five groups based on etiology: 1 – pulmonary arterial hypertension (PAH); 2 – pulmonary hypertension associated with left heart diseases; 3 – pulmonary hypertension associated with lung diseases; 4 – chronic thromboembolic pulmonary hypertension; 5 – pulmonary hypertension due to systemic or multifactorial conditions. The first group is also known as primary PH and the other four are under the common name of secondary PH [4].

Patients with CTDs such as SSc, MCTD, and SLE are at high risk of developing PH, especially with type 1-PAH (pulmonary arterial hypertension) as it is the most common and important due to its aggressiveness, short survival, and limited treatment options. In the last decades, the survival rate in this type of PH has improved, reaching 57% from the moment of diagnosis based on cardiac catheterization, but it is still suboptimal and continues to be a huge challenge [5]. At the same time, other forms of PH may appear in these patients, possibly an overlap of different forms of PH probably due to the autoimmune status of these conditions (Figure 1).

Figure 1: Different phenotypes of pulmonary hypertension in SSc-overlap. Abbreviations: CTEPH, chronic thromboembolic pulmonary hypertension; ILD, interstitial lung disease; LHD, left heart disease; PAH, pulmonary arterial hypertension, PVOD, pulmonary veno-occlusive disease; SSc, systemic sclerosis [Almaaitah, 2020].

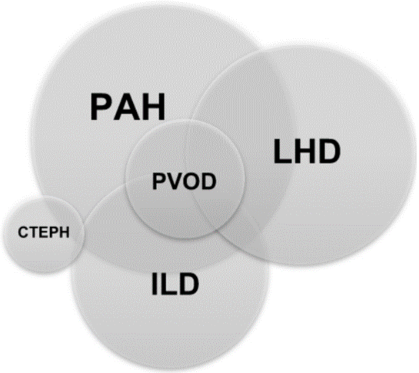

Connective tissue diseases represent the second major cause of PAH, after idiopathic ones, occupying about a quarter of the total population with PAH. Group 2 of PH, which is associated with left heart diseases can also occur in these collagenoses. Interstitial lung disease (pulmonary fibrosis) is another common complication of these autoimmune diseases, especially SSc and MCTD, so type 3 PH may be present to the same extent in these patients. Of note is pulmonary veno-occlusive disease which is associated with a worse prognosis due to the lack of response to PAH therapy and the risk of pulmonary edema due to these treatments. This form of PAH occurs especially in patients with SSc [3].

Another definition refers to the hemodynamic component, thus PH was defined in the past as mean pulmonary artery pressure (mPAP) of ≥25 mmHg, but recently it has been updated according to the 6th World Symposium on Pulmonary Hypertension (WSPH) to an mPAP value >20 mmHg. Studies have shown that normal mPAP in healthy individuals is approximately 14±3.3 mmHg and the upper limit of normal is 20 mmHg. This new definition appears to be less practical but may be useful in diagnosing more individuals with PH, as symptoms usually begin at a much higher value above the normal upper limit (i.e. >50mmHg). Nonetheless, there is a major concern regarding inclusion in this group of healthy individuals.

Another major hemodynamic change recommended by the 6th WSPH is the inclusion of pulmonary vascular resistance (PVR) in the classification and definition of PH. Thus, PH is further subclassified as pre-capillary PH (pre-PH), isolated post-capillary PH (IpcPH), or combined pre- and post-capillary PH (CpcPH) based on pulmonary arterial pressure and PVR [5].

EPIDEMIOLOGY

The lungs are often affected in connective tissue diseases (CTDs), because of the systemic nature of these autoimmune pathologies and due to the complex anatomic-functional patterns of the respiratory apparatus. Among the CTDs, systemic sclerosis (SSc) has the highest prevalence of pulmonary involvement (between 70% and 90% of patients with CTDs) and PAH is developed in 8-12% of patients with SSc. The incidence of PAH in SSc is about 0,61 cases per 100 patients per year. The prevalence of PAH among other CTDs remains to be determined due to the lack of large studies, but it is estimated to be lower than in patients with SSc. The prevalence of PAH in SLE is <4% and for the other diseases in this group is <1% [3,7].

PATHOGENESIS AND DIFFERENT FEATURES OF PULMONARY HYPERTENSION IN DIFFERENT AUTOIMMUNE DISEASES

Systemic sclerosis (SSc) or scleroderma is a rare and complex systemic autoimmune disease that affects the skin and the internal organs through multiple processes including fibrosis and thickening of these structures and vascular abnormalities [8]. The two major complications that might occur are pulmonary fibrosis and pulmonary arterial hypertension (PAH). The presence of both complications is not random since they have overlapping mechanisms. It seems to appear an abnormal fibroproliferation that results from a vascular injury such as endothelial cell injury and consecutive dysfunction [8]. Pulmonary fibrosis and PAH might appear as a consequence of an increased number of vasoconstrictors and proliferative mediators (endothelin-1) and a decrease in vasodilators (nitric oxide and prostacyclin), therefore leading to progressive pulmonary arterial remodeling and increase in pulmonary vascular resistance [6].

Systemic lupus erythematosus (SLE) is a chronic, autoimmune disorder that presents with a multitude of signs and symptoms and it affects multiple internal organs including the lungs. The most common lung manifestation is pleuritis but the parenchyma and the vasculature may also be involved causing thickening of the arterial media and subintima and immune complexes deposition in the arterial wall. PAH can rarely be present in SLE but it is a life-threatening pulmonary manifestation caused by left ventricular dysfunction or congestive cardiac failure and also by interstitial lung disease and chronic thromboembolic disease. Nevertheless, there is an increased risk of PAH and pulmonary venous thromboembolism (VTE) in SLE patients especially when it is associated with antiphospholipid antibody syndrome. Among SLE patients, most cases with PAH developed in the presence of Raynaud's phenomenon (60% of cases) and positive rheumatoid factor [9]. Another association was found between PAH and anti-RNP antibodies [7,9].

Primary Sjogren syndrome (pSS) is an autoimmune disease characterized by impairment of exocrine glands (lacrimal and salivary) due to inflammatory infiltrate but it can also affect extra glandular organs, including the respiratory tract and lung involvement which are one of the most common complications leading to increased mortality. PAH can rarely appear in pSS but can lead to right heart failure and death. The exact prevalence of PAH in pSS is difficult to estimate but one study has shown a value of 22% of patients based on echocardiographic evidence, although right heart catheterization is the gold standard for the diagnosis; There is an association between pSS-PAH and hypergamma-globulinemia, positive ANA and anti-RNP antibodies [7].

Rheumatoid arthritis (RA) associated pulmonary hypertension is very rare and can be attributed to parenchymal lesions, vascular disease such as rheumatoid vasculitis or it can develop due to chronic thromboembolic disease. But rheumatoid arthritis can also be associated with other autoimmune diseases such as scleroderma, SLE, and other CTDs in overlap syndromes, therefore the diagnosis of PH can be more relatable to these conditions.

Moreover, even though there is no disease-modifying treatment of RA (DMARDs or bDMARDs) suspected of causing PH, it is common knowledge that Methotrexate can cause pulmonary fibrosis that can lead to PH. The role of these drugs on PH can not be evaluated due to a lack of data. PH seems to be more common in patients with seropositive forms and advanced radiological signs. Systematic screening of PH is not necessary for RA patients but if is suspected further diagnostic testing should be pursued. In the case of RA, the prevalence of PH is identical to that seen in the general population [11,12].

Ankylosing spondylitis (AS) is a systemic inflammatory disease affecting the axial skeleton and/or peripheral joints and can be associated with extra-articular manifestations including uveitis, aortitis, and valvular heart disease. The association between PH and ankylosing spondylitis (AS) is not so common and a few case reports have described a high incidence based on echocardiography but without right heart catheterization and no other connective tissue disease was excluded [13]. Only one case from 2022 has confirmed the diagnosis of PAH based on transthoracic echocardiography and right heart catheterization [14]. In conclusion, further studies are needed to establish a causal relationship between PH and ankylosing spondylitis.

Mixed connective tissue disease and PH. It is very difficult to estimate the exact prevalence of PH in mixed CTD because of the very frequent overlap syndromes where SSc may contribute to the high rates of PH [15]. The estimated prevalence is somewhere around 8%.

CLINICAL PRESENTATION

Patients usually present at first with non-specific signs and symptoms such as fatigue, weakness, dyspnoea on exertion, and dizziness and are usually described to appear due to connective tissue diseases per se. Other symptoms such as syncope, angina, ankle edema, and abdominal distention appear only after the vasculopathy has advanced and are signs of right heart failure [3].

PH associated with left heart disease (type 2 of PH) may be present among patients with SSc since cardiac dysfunction can develop in more than 40% of patients with SSc leading to myocardial and conduction system fibrosis which determines arrhythmias and diastolic heart failure. Atherosclerotic coronary vessel disease and hypertensive crisis may also appear.

More often, interstitial lung disease (ILD) can be present as a complication of SSc (SSc-ILD) leading to PH in 31% of cases. It is associated with the diffuse cutaneous form of SSc and the presence of positive Scl-70 (anti-topoisomerase) antibodies. Although these antibodies seem to have a protective role against PAH, type 3 PH may still appear due to interstitial lung disease [6].

DIAGNOSIS

Screening procedures. Early diagnosis is essential to improve survival in PAH patients. Therefore, early screening for diagnosis leads to adequate treatment at the right moment. The proper screening for PAH in SSc with a disease duration of more than 3 years and a modified DLCO (diffusion capacity of the lung for carbon monoxide) value is by using the DETECT algorithm based on non-invasive clinical and laboratory tests in order to reduce the missed diagnosis of PAH. Among patients with concomitant pulmonary emphysema, the utility of lung function tests is reduced as it might increase FVC (forced vital capacity) and diminish DLCO. The DETECT algorithm is based on 2 steps diagnosis: in step 1 clinical status, laboratory findings, and lung function tests (FVC, telangiectasia, anticentromere antibody positivity, electrocardiogram right axis deviation, and serum levels of Nt-proBNP and urate) should be determined and based on the results, echocardiographic parameters might be recommended in the second step to generate a final score. Based on the results of these steps, a right heart catheterization might be performed. The sensitivity of this method is high (96%) but it has a low specificity for detecting PH in SSc patients (48%). Screening for PH should be performed at least once a year. For other CTDs, there is no current systematic screening for PAH because of its low prevalence, although careful monitoring of these patients is recommended [8,10].

Biomarkers such as NT-proBNP and uric acid, which reflect cardiac dysfunction, are used in the DETECT algorithm as part of a screening method for PAH in SSc patients or other CTDs although these are not specific. One study identified other circulating biomarkers that might have a future impact on the diagnosis of PH and reduce the necessity of invasive procedures. Besides NT-proBNP, other proteins such as collagen IV, endostatin, insulin-like growth factor binding protein (IGFBP)-2, IGFBP-7, matrix metallopeptidase-2, neuropilin-1 and RAGE (receptor for advanced glycation end products) could improve the DETECT algorithm for screening of PH and understanding of different mechanisms in the pathogenesis of PAH. These markers are known to be involved in the process of pulmonary vascular remodeling, angiogenesis, and cellular growth but further data is needed in order to establish the exact role of these markers in PH [16].

Spirometry with DLCO estimation is important to evaluate the state of pulmonary function since DLCO is reduced in PAH (<50%) but FVC has a normal value and they have a high specificity (90%) and a high positive predictive value for SSc-PAH. In pulmonary fibrosis, both parameters are low [10]. This occurs due to a low blood volume in the pulmonary capillary in PAH patients and a diminution in the transfer capacity of the alveolar-capillary membrane. The best screening method for PAH among lung function tests is the FVC (% predicted)/DLCO (% predicted) [17].

Autoantibodies: Several SSc-specific antibodies are thought to be associated with PAH such as high anti-centromere ab (ACA) titers and some of the ANA panel. One study reported a significant correlation between SSc-PAH and the positivity of anti-U1-RNP and Th/To antibodies. Although AECAs (anti-endothelial cell antibodies) are involved in the vascular pathology of SSc further data is needed to demonstrate the exact implications of these antibodies in SSc-PAH. Another study reported the presence of anti-endothelin receptor type A antibodies (ETAR) and anti-angiotensin receptor type I antibodies (AT1R) as being the main factor of pulmonary vascular inflammation. The level of these antibodies was higher in the SSc-PAH patients compared to other forms of PH.

Transthoracic echocardiography (TTE) is recommended in all SSc patients once a year and it is the most common tool for screening SSc-PAH although it has a low predictive value [10]. There are many parameters that can be modified on TTE in case of PH: 1. right ventricle/left ventricle basal diameter ratio >1.0 or flattening of the interventricular septum; 2. early diastolic pulmonary regurgitation velocity >2.2 m/sec or increase in the tricuspid regurgitant jet velocity (TRV) and/or systolic pulmonary artery pressures (sPAP) and/or pulmonary artery diameter > 25mm; 3. inferior cava diameter >21 mm with decreased inspiratory collapse (18 cm) [17, 18].

Right Heart Catheterization (RCH): with the help of this procedure the diagnosis of PAH is established with certainty and the measurements that can be performed are the mPAP and the pulmonary capillary wedge pressure (PCWP), PVR, right atrium (RA) volume, and transpulmonary pressure gradient (TPG), by using a catheter inserted into the right femoral vein. RHC is the most precise method for the diagnosis of PH and it can offer information about the severity of the disease and prognosis. RHC is necessary when there is a moderate or high risk of developing PH based on the results of TTE [10,19].

TREATMENT

Because pulmonary hypertension due to autoimmune disease is a relatively rare syndrome and because it is challenging to treat, patients are probably best served if they are referred to a center that has experience with treating pulmonary hypertension. Close communication should be maintained between the referring physician and the treating physician [20].

No standard guidelines exist for the treatment of pulmonary hypertension associated with connective tissue disease.

Treatment recommendations, however, are modeled from the available literature on patients with PPH for several reasons. First, the clinical presentations and histopathologic features of PPH and autoimmune disease–associated pulmonary hypertension may be quite similar. Second, some patients who are initially diagnosed with PPH later manifest symptoms and signs of systemic autoimmune disease [21]. A further link between PPH and collagen-vascular disease–associated pulmonary hypertension is suggested by Isern et al, who found that 9 of 31 patients (29%) with PPH had specific autoantibodies associated with connective tissue diseases and 39% had Raynaud's phenomenon or positive antinuclear antibody results. Anti-Ku antibodies were found in 23% of patients with PPH [22]. Although the outcome still remains poor, many advances have been made in the last 30 years in vasodilator therapy, supportive care, and, more recently, the use of lung transplantation. These advances have improved survival and quality of life for PPH patients to a considerable extent. Further study of these therapies' usefulness among patients with pulmonary hypertension associated with systemic autoimmune disease is forthcoming.

General Supportive Therapy

The treatment of pulmonary hypertension should begin with the reversal of any contributing factors, including hypoxemia, hypercarbia, and acidosis. Such treatment is intended to reduce right heart strain with hopes of increasing survival. Oxygen supplementation to maintain arterial oxygen tension above 60 mm Hg or oxyhemoglobin saturation over 90% reduces mortality from cor pulmonale and improves cognitive function and quality of life in patients with the severe chronic obstructive pulmonary disease [23,24,25]. Others have shown that in primary pulmonary hypertension, low arterial oxygen saturation is the single best predictor of poor survival, as it is associated with a higher incidence of sudden death.

Digitalis, diuretics, and sodium restriction should be considered when heart failure persists. Although the use of digitalis in right-heart failure has been controversial and the drug is known to increase pulmonary vascular resistance and has a high incidence of toxicity in patients with cor pulmonale, it is shown to improve right heart function when left heart failure is also present, and it may offset the negative inotropic effects of calcium-channel blockers when they are administered concomitantly. Diuretics are useful in improving peripheral edema and hepatic congestion. Cautious administration is warranted, because it may lead to metabolic alkalosis, which, in patients with derangements in ventilatory control, may depress ventilatory response to carbon dioxide and lead to hypercarbia. Aggressive diuresis may also reduce right ventricular blood volume and reduce cardiac output. Finally, blood viscosity may increase due to a rise in hematocrit associated with diuresis. Serial phlebotomy may be required to decrease blood volume and viscosity in patients who have polycythemia associated with cor pulmonale.

Anticoagulation has been shown to provide survival benefits in patients with PPH. In situ, thrombosis due to endothelial injury and venous stasis and, sometimes, due to the presence of anticardiolipin antibodies may contribute to pulmonary hypertension in some patients by reducing the cross-sectional area of the pulmonary vascular bed [26]. In situ, thrombosis in pulmonary vessels has been shown to occur in all forms of pulmonary hypertension. Moreover, right heart failure, by promoting venous stasis, may further increase the risk of pulmonary thromboembolism. As such, anticoagulation with warfarin or with adjusted-dose or low-molecular-weight subcutaneous heparin in patients at risk for bleeding may benefit patients with pulmonary hypertension resulting from systemic autoimmune disease. Alternatively, the use of antiplatelet drugs such as aspirin or dipyridamole may be helpful; this recommendation is based on the fact that levels of urinary thromboxane A2, indicating platelet activation, are increased in some patients with pulmonary hypertension. Speculation exists that the beneficial effects of chronic prostacyclin infusion in some patients with in situ thrombosis may occur because of its potent anti-platelet aggregating activity rather than through vasodilatation because improved outcomes are seen even in patients who do not experience substantial acute hemodynamic changes. The same theoretic supposition applies to the suspicion that heparin may be preferred to warfarin because it inhibits smooth muscle and endothelial cell proliferation. The risk of anticoagulation must be considered, particularly because more than half of all patients with severe pulmonary hypertension experience syncope, and 15% experience hemoptysis [27].

Vasodilators

Vasodilator therapy has been used for more than 30 years in patients with PPH under the premise that pulmonary vasoconstriction, at least in part, contributes to the pathogenesis of this disease. Although it is not clear whether the vasoconstriction is the primary cause of increased pulmonary vascular resistance or results from distension of pulmonary blood vessels by increased intraluminal pressures, the premise also exists that even a small reduction in right ventricular afterload leads to clinically significant increases in right ventricular output. Not all patients will respond to vasodilators, and trials of vasodilator therapy are indicated to distinguish those patients who have a reversible vasoconstrictive component of pulmonary hypertension from those who have fixed pulmonary hypertension. While we await the results of a forthcoming prospective trial investigating the use of long-term therapy with prostacyclin among patients with pulmonary hypertension from systemic autoimmune disease, we include a discussion of vasodilator therapy for two reasons: first, some patients diagnosed with PPH later will manifest specific evidence for systemic autoimmune disease; and second, some patients with autoimmune disease–associated pulmonary hypertension have histopathologic changes on lung biopsy that are identical to those of PPH. As such, the two forms of pulmonary hypertension may, in fact, represent different points along the same continuum of disease progression, or may represent a common final pathway of injury following some inciting stimulus. The optimal response of vasodilator therapy is a reduction in PA pressure without a change in pulmonary capillary wedge pressure, along with an increase in cardiac output and thus a decrease in pulmonary vascular resistance. These changes should occur without a decrease in systemic arterial pressure or oxygenation. Only 15% to 30% of patients will experience such a response, however [28].

A second pattern is exhibited by approximately half of all patients with PPH, who experience a reduction in pulmonary vascular resistance in association with an increase in cardiac output, without a change in PA pressure. Although these patients also have increased exercise tolerance and improved right ventricular function, the duration of these improvements is unknown, as is the presence of any long-term survival advantage. In some patients, these hemodynamic improvements are most pronounced during exercise rather than rest, and so clinical benefits may exceed those predicted by responses measured during the resting state. The remaining quarter of patients are those in whom vasodilator therapy is contraindicated: they suffer a reduction in systemic blood pressure with acute vasodilator challenge, whereas PA pressure and cardiac output remain unchanged or increase. In these patients, vasodilators affect the systemic vasculature more than they do the pulmonary vessels. In response to the decrease in systemic vascular resistance, cardiac output may increase or remain unchanged. If cardiac output increases in response to systemic vasodilation, the increased flow through the pulmonary vessels will achieve the undesirable effect of a further increase in PA pressure. If the cardiac output does not respond to systemic vasodilation, then systemic hypotension will result, and this outcome may be resistant to most therapies, including discontinuation of the vasodilator. Volume resuscitation is often not helpful, and vasopressors may worsen pulmonary hypertension and increase right ventricular afterload. Such systemic hypotension may also precipitate acute angina or heart failure. The patients who experience a reduction in systemic blood pressure without reduction in PA pressure on acute vasodilator challenge are classified as having fixed vascular disease. Many drugs have been used to evaluate pulmonary vascular responsiveness [28]. The ideal drug, however, is one that: (1) elicits pulmonary vasodilation at a dose that has little or no effect on the systemic circulation; (2) is short-acting and is rapidly cleared from the circulation, so that baseline hemodynamics are reestablished soon after the drug is discontinued; (3) is easy to administer accurately in an incremental dosing regimen; (4) has hemodynamic and other side effects that accurately predict the response to longer-acting agents; and (5) is able to identify those patients who will not respond to therapy. In summary, a screening agent must be able to accurately reproduce the risk-benefit profile a patient will experience with longer-acting agents. This means identifying patients who will benefit from further trials with long-acting agents, as well as delineating those who should be spared from further therapeutic trials because of potential adverse outcomes of therapy.

Several agents have been used for screening: prostacyclin and its analog iloprost, acetylcholine, adenosine, and nitric oxide. Nitric oxide is administered by the inhaled route, whereas the others are given intravenously. The most intensively studied of these agents is prostacyclin, a metabolite of arachidonic acid produced by endothelial cells. Although it has some effect on systemic vascular resistance, it has almost all of the properties of an ideal screening agent. Its half-life is only 2 to 3 minutes, it has a rapid onset of action, its effects resolve rapidly after it is discontinued, and it is amenable to incremental intravenous dosing. Acute administration of prostacyclin has been shown to predict pulmonary vasodilator response to traditional long-term agents and identify those patients with PPH in whom long-term therapy may not be useful [29].

The recent approval by the Food and Drug Administration (FDA) for continuous long-term intravenous infusion in primary pulmonary hypertension has resulted in increased use of this drug as an alternative to traditional oral medications in selected patients [29]. Iloprost is a short-acting, stable carbocyclic derivative of prostacyclin with a longer half-life of more than 10 minutes. It has similar pharmacologic effects on the pulmonary circulation of patients with PPH. Recently, it has been used in long-term therapy for patients with pulmonary hypertension in the setting of connective tissue diseases. Acetylcholine is also short-acting and well-tolerated but may be a less potent vasodilator than prostacyclin, although this observation may in part be explained by the use of lower doses of acetylcholine [30]. Adenosine is a very short-acting pulmonary vasodilator, with a half-life of less than 10 seconds. It has been shown to have acute hemodynamic effects similar to those of prostacyclin in PPH [31].

Inhaled nitric oxide has selective pulmonary vasodilatory properties, with little effect on systemic circulation. It has an interesting additional effect of reducing intrapulmonary shunt and increasing systemic arterial oxygen saturation.

Transplantation

Transplantation has generally been reserved for those patients whose symptoms progress despite aggressive medical management, and who have New York Heart Association functional class III or IV heart failure [32]. The presence of severe right-sided heart failure that is accompanied by hepatomegaly, hyperbilirubinemia, and ascites confers high mortality, however, and these patients are not suitable candidates for transplantation. Transplantation has received attention in the recent past as a potential panacea for patients with severe forms of pulmonary hypertension, as it produced a dramatic reduction in PA pressure and reversal of right heart failure [33]. As experience has accrued, however, many centers have found that the incidence of acute complications following lung transplantation is greater among patients with systemic autoimmune disease than those with PPH, and some have abandoned it as a therapeutic option. Moreover, the incidence of postoperative Raynaud's phenomenon may be higher in these patients, and the vasoconstrictive effects of cyclosporine, which is used in posttransplant immunosuppression, are evoked as a putative mechanism. Other potential complications include bronchiolitis obliterans, which occurs with an incidence of 25% to 40%, and opportunistic infection [34,35]. Other limitations of transplantation include its confinement to certain specialized centers, its expense, and, regrettably, the paucity of donor organs [36]. Treatment of underlying systemic autoimmune disease

When pulmonary hypertension occurs in the setting of significant parenchymal lung involvement with systemic autoimmune disease, or due to vasculitis, the response to therapies targeted toward the underlying autoimmune disorder has been variable [37,38,39]. Small-scale studies suggest that traditional vasodilators alone may be ineffective. Most patients do not respond to corticosteroids, but some may [39]. In one series of three patients who had pulmonary hypertension in the setting of vasculitis, pneumonitis, and thrombosis, an aggressive combination of corticosteroids, anticoagulation, and either cyclophosphamide or azathioprine appeared to be beneficial [40]. In patients with rheumatoid arthritis who exhibit pulmonary hypertension because of pulmonary arteritis, which is thought to be due to deposition of immune complexes in vessel walls, the use of high-dose corticosteroids or cytotoxic agents has been investigated. Evidence has been unclear on whether such therapy improves overall outcomes.

Calcium-Channel Blockers

Although there is a paucity of data regarding their use in autoimmune disease–associated pulmonary hypertension, nifedipine, verapamil, diltiazem, and recently, nicardipine have been used against PPH. They are thought to act by reducing calcium influx into pulmonary vascular smooth muscle cells, thereby blocking muscle contraction and promoting vasodilation. Nifedipine is the most frequently used of the four, although nicardipine and diltiazem are useful when adverse effects of nifedipine occur. Among 10 patients with diffuse systemic sclerosis, CREST syndrome, or mixed connective tissue disease, oral nifedipine has been shown to produce both acute and chronic reduction (at 3 to 6 months) in pulmonary vascular resistance [41].

Prostaglandins

Prostacyclin is now FDA-approved for continuous intravenous infusion for the treatment of PPH, and may soon be routinely used for pulmonary hypertension associated with collagen vascular disease if results of a proposed clinical trial show therapeutic efficacy in this setting. Although it was initially proposed for patients who had severe forms of pulmonary hypertension as a bridge to transplantation, it has since been continued in patients who exhibit pronounced response to prostacyclin (PGI2) infusion, for a duration exceeding 5 years, with consideration for transplantation only if symptoms recur or if complications occur. The drug must be delivered by an infusion pump through an indwelling central venous catheter. Frequent dosing increments are required to maintain responses; moreover, complications including death have occurred because of failures in the drug delivery system, including thrombosis or infection of the catheter, pump malfunction, or interruption in the infusion system due to accidental disconnection. Nevertheless, studies have shown that long-term prostacyclin infusion offers symptomatic and hemodynamic improvement [42,43] and confers a survival advantage in advanced PPH [44,45].

Iloprost is a stable prostacyclin analog that has been studied in a number of settings. In the acute situation, high doses have been shown to reduce pulmonary vascular resistance in healthy persons, patients with PPH, and those with toxic oil syndrome, which results from ingestion of contaminated rapeseed oil and resembles scleroderma. Recently, de la Mata et al have studied the effects of long-term iloprost treatment in patients with severe pulmonary hypertension due to systemic sclerosis and primary antiphospholipid syndrome. They followed prospectively three patients with systemic sclerosis and two with antiphospholipid syndrome who did not respond to oral vasodilator therapy for pulmonary hypertension. They found that acute infusion of maximum tolerated doses reduced pulmonary vascular resistance and raised cardiac index and pulmonary arterial oxygen saturation in a statistically significant manner. After 32 weeks, all patients had improved exercise tolerance and New York Heart Association functional class. This improvement in exercise tolerance, however, was not accompanied by improvements in resting hemodynamics nor in pulmonary oxygen saturation, and one patient died. Further studies are needed to determine whether iloprost is more beneficial for patients who have less severe degrees of pulmonary hypertension than those in this study. In addition, long-term intravenous therapy continues to pose problems associated with the drug delivery system—one patient in this study died of bacteremia [30].

Oral iloprost has also been studied as a potential long-term therapeutic alternative to intravenous administration. In a European multicenter, double-blind, placebo-controlled trial of 63 patients with Raynaud's phenomenon, iloprost given orally at the maximum tolerated dose for 10 days resulted in reduced duration and severity of attacks of vasospasm. The results did not reach statistical significance, however, and this study did not include any assessment of the effects of this therapy on pulmonary vascular disease [46].

CONCLUSION

PH can be caused by several conditions in patients with systemic sclerosis or other CTDs and it is an important cause of mortality and morbidity, therefore early diagnosis and annual screening in asymptomatic patients is mandatory in order to provide adequate treatment. Even though significant efforts are being made regarding treatment options, long-term survival remains an important issue. The mortality rate of PAH in SSc patients is worse than the one in idiopathic PH and other CTDs due to a lack of response to treatment and the severity of the disease. More studies are needed in order to see progress in elucidating the pathogenesis and treatment regimens, especially the immunosuppressive therapy, and new non-invasive tools are required to simplify the diagnosis of PH and therefore improve patients' survival rate and quality of life [18,47].

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

All the authors contributed to the following aspects of this study: Conceptualization: D.A., I.N.N, and L.I.M; resources: D.A., R.S.C., and D.O.B.; writing- original draft preparation: D.A., I.N.N., and L.I.M.; writing – review and editing: I.N.N., O.G.P., V.C.B., D.O.B., and F.I.R.; writing- finalization: D.A., D.O.B., N.I.N, and L.I.M. The authors have given their approval for this article to be published. All of the authors have participated sufficiently in the work and take public responsibility for appropriate portions of the content. All of the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ORIGINAL ARTICLE

The Role of Chromogranin A Assay in the Diagnosis of Pheochromocytomas

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Abstract: *Background: Pheochromocytomas (Pheo) are rare neuroendocrine tumors with a suggestive clinical picture, characterized by hypersecretion of catecholamines and other neuroendocrine biomarkers. Methods: The purpose of the study was to analyze the diagnostic features of Pheo and investigate the role played by different neuroendocrine and hormonal markers in diagnosing Pheo.The retrospective study involved a group of 69 patients diagnosed and treated with Pheo, who had both urinary and plasma catecholamines and neuroendocrine markers measured pre- and postoperatively. Results: After comparing pre- and postoperative hormonal parameters and neuroendocrine biomarker changes, numerous statistically significant findings were found. The analysis of the relationships between chromogranin A (CgA) levels, plasma and urine metanephrines, and normetanephrine and Pheo tumor size was included in the study. Additionally, we evaluated Cg A's diagnostic efficacy in comparison to plasma metanephrines, normetanephrine, and neuron-specific enolase (NSE) for Pheo. Conclusions: We obtained statistically significant data on pre- and postoperative differences for plasma and urinary catecholamines, CgA and NSE. Pheo tumor size is interdependent with serum levels of Cg A, plasma and urinary metanephrines, and normetanephrine. The best diagnostic power for Pheo was plasma normetanephrine, followed by plasma metanephrines and CgA.*

Keywords: *pheochromocytomas, catecholamines, chromogranin A, neuron-specific enolase*

INTRODUCTION

The term "neuroendocrine tumors" (NETs) refers to a wide range of extremely rare neoplasms that fall under the category of borderline endocrinology. They have the capacity to secrete a wide variety of physiologically active peptides and amines, which may result in a variety of biological behaviors and, in turn, metabolic and systemic consequences.

Advances in genetic studies have had a major impact in characterizing the hereditary aetiopathogenesis of pheochromocytomas (Pheo). In the 2000s years, it was thought that only 10% of these tumors, were recognized with a genetic cause, but now more than 18 genes have been described, whose mutations lead to the development of these tumor types in more than 30-40% of cases. In addition, large-scale studies target the genetic fingerprint of these tumor types as a possible pathophysiological key to further developing personalized medical therapy [1].

Currently, Pheo is defined as a catecholamine-secreting tumor originating from neuroendocrine chromaffin cells of the adrenal gland. In terms of pathophysiological mechanisms, Pheo can synthesize, store, secrete and metabolize catecholamines (noradrenaline and adrenaline, respectively their metabolites normetanephrine and metanephrine), thanks to their enzymatic background, namely tyrosine hydroxylase and dopamine β-hydroxylase enzymes. These two enzymes are found in both the adrenal medulla and sympathetic nerve ganglia and are responsible for the production of catecholamines and their storage in dense secretory vesicles together with CgA, adenosine triphosphate, calcium, magnesium, adrenomedullin, and other neuropeptides. CgA was first described as originating from adrenal chromaffin cells. It is a member of the granin family together with chromogranin B, chromogranin C (secretogranin II), secretogranins III-VI and vascular endothelial growth factor (VEGF) and it is considered a pro-hormone that plays multiple biological roles, including calcium homeostasis and mediating the secretion of catecholamines and other hormones [2-4].

In addition, the adrenal medulla is uniquely capable of producing and secreting adrenaline (epinephrine) and its metabolite metanephrine, thanks to a specific glucocorticoid-dependent enzyme of its own called phenylethanolamineN-methyltransferase (PNMT), which converts noradrenaline (norepinephrine) to adrenaline by methylation [5,6].

Pheo is characterized by clinical signs and symptoms specific to catecholamine hypersecretion (paroxysmal hypertensive crisis, diaphoresis, palpitations, headache, weight loss, hyperglycemia, etc.). Moreover, for Pheo, the clinical picture may include the specific classic triad: headache, sweating, and palpitations. However, non-specific symptoms may also occur, such as anxiety, weight loss, insomnia, or life-threatening cardiovascular events: rupture of an aortic aneurysm or stroke during a hypertensive crisis, hypertensive encephalopathy, catecholamine cardiomyopathy, etc [7].

The general biochemical diagnosis of catecholamines secreting NETs is characterized by the determination of specific markers: catecholamines and their plasmatic and urinary metabolites, chromogranin A (CgA), neuron-specific enolase (NSE) as well as by identifying the immunohistochemical expression of synaptophysin, CgA, etc. [8].

Serum CgA dosing and monitoring in Pheo is a topic of great interest in the literature and is currently included in current diagnostic and treatment guidelines. According to the body’s literature, CgA levels are correlating with tumor volume, disease recurrence, and the presence of secondary determinations, especially hepatic [9,10].

MATERIALS AND METHODS

The aim of the study was to analyze the diagnostic features of Pheo and to identify possible correlations between various clinical and hormonal factors with an impact on clinical outcome and prognosis. Subjects were diagnosed with Pheo based on the following data:

- Suggestive symptomatology (hypertensive crisis, palpitations, sweating, headache, weight loss)
- Values that exceeded the upper limit of the reference range of plasma or urinary metanephrines and normetanephrine at least four times were considered diagnostic values
- Identification and localization of adrenal tumors by imaging techniques
- Pathological examination confirmed the diagnosis of Pheo

The study involved data collection and inclusion of 69 patients evaluated, diagnosed, and treated for Pheo of sporadic or genetic etiology. We investigated hormonal levels pre and post-operative, neuroendocrine biomarkers, and their correlations. According to the inclusion and diagnostic criteria, after obtaining informed consent, we enrolled in the study 69 patients with clinical suspicion of Pheo supported on the basis of biological and hormonal evidence, as well as on the basis of imaging investigations in whom histopathological examination obtained postoperatively confirmed the diagnosis. Clinical, biochemical, and hormonal parameters were monitored dynamically, i.e. preoperatively (at the time of diagnosis) and immediately postoperatively (at 2-4 weeks, the date of the first endocrinological evaluation after surgery). Our study included subjects evaluated and diagnosed in C.I. Parhon National Institute of Endocrinology Bucharest, during the period 2000-2017. We performed retrospective data collection of patients diagnosed and treated by Pheo from clinical observation sheets and electronic patient files. The collected data were entered into a database. Statistical interpretation of the data obtained was performed using IBM SPSS Statistics 20.0 and MedCalc 12. Results were expressed as mean ± standard deviation for numerical variables and as the frequency for categorical variables. The statistical tests used to interpret the data were the usual ones (minimum, maximum, mean, median values) as well as complex ones such as t-test (with the assumption of equal and unequal variances, respectively) for comparing the variance of means and F-test for comparing standard deviations. For statistical hypothesis testing, a p-value < 0.05 was used to assert a statistical correlation. The diagnostic power of the parameters was tested using the ROC curve.

RESULTS

Comparative analysis of pre- and postoperative hormonal parameters and neuroendocrine biomarkers changes for the Pheo group revealed numerous statistically significant data. The results of the statistical analysis of the dependent sample T-test, taking into account pre and postoperative values are reported in Table 1. Thus, in the Pheo study group, the mean plasma CgA level preoperatively was 390.43±173.83 ng/ml, and postoperatively it decreased statistically significantly ($p<0.001$) to 56.43±63 ng/ml. The NSE concentration also showed a statistically significant change ($p=0.004$) decreasing from a preoperative mean value of 12.98±1.69 ng/ml to 10.3±3.01. Plasma metanephrines recorded a mean value at diagnosis of 937.82±1201.51 pg/ml and decreased statistically significantly ($p<0.001$) postoperatively to 41.2±22.7 pg/ml. Plasma normetanephrine ranged from a preoperative mean value of 1712.11±1893.39 pg/ml to a postoperative mean value of 94.94±205.85 pg/ml, the difference being statistically significant ($p<0.001$). Regarding urinary metanephrines, they ranged from a mean value at diagnosis of 1798.81±2259.44 μg/24 hours to mean postoperative values of 96.1±80.9 μg/24 hours, reaching the threshold of statistical significance. Similarly, urinary normetanephrine had preoperative mean values of 3212.8±3284.07 µg/24 hours and postoperative mean values of 178±188.01 µg/24 hours, the difference being statistically significant (p<0.001, n=50).

Table 1: T-test for paired samples – Evolution of pre- and postoperative parameters in the Pheo group.

Variable	Pheo		N	Sig.	Correlation	Mean Diff.	Sig (2 tailed)
	Preop. mean	Postop. mean					
CgA (ng/ml)	390.43±173.83	56.43±63	28	0.046	0.38	334±110.83	<0.001
NSE (ng/ml)	12.98±1.69	10.3±3.01	12	0.08	0.698	2.68±1.32	0.004
Plasma metanephrines (pg/ml)	937.82±1201.51	41.2±22.7	69	0.92	-0.12	896.62±1178.8	<0.001
Plasma normetanephrine (pg/ml)	1712.11±1893.39	94.94±205.85	69	0.017	0.286	1617.17±1687.5	<0.001
Urinary metanephrines (µg/24 ore)	1798.81±2259.44	96.1±80.9	52	0.218	0.151	1702.7±2178.54	<0.001
Urinary normetanephrine (µg/24 ore)	3212.8±3284.07	178±188.01	50	0.06	0.177	3034.8±3096.06	<0.001

We also statistically analyzed the correlations of CgA levels and plasma and urinary metanephrines and their metabolites with Pheo tumor size.

Regarding the correlation of preoperative serum Cg A levels with Pheo tumor size, the results of the statistical analysis showed a positive association, with high statistical significance (P<0.0001, r=0.86), being plotted in Figure 1.

Figure 1: Correlation of CgA and tumor size in the Pheo group

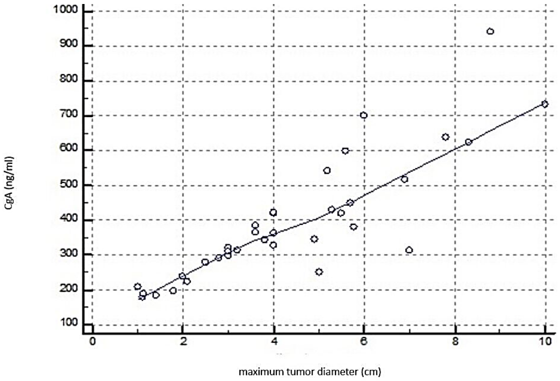

In addition, by studying the correlation of mean plasma metanephrines level with tumor size, we obtained a statistically significant positive correlation (P<0.0001, r=0.57), plotted in Figure 2.

In addition, serum plasma normetanephrine levels also showed statistically significant positive correlations with Pheo size (P=0.0002, r=0.43), plotted in Figure 3.

Similarly, regarding urinary metabolites of catecholamines, in Figure 4 it can be seen the presence of a positive association of urinary metanephrine level with Pheo maximum tumor diameter (P<0.0001, r=0.55).

In addition, urinary normetanephrine levels at the time of diagnosis showed a positive correlation as shown in Figure 5, with statistical significance (p<0.001, r=0.53).

Moreover, in terms of genetic or sporadic etiology in Pheo, the statistical data did not show a statistically significant difference (P=0.411, F-ratio=0.691) in plasma levels of Cg A. Thus, the mean serum CgA in genetic cases was 341.18±140.09 ng/ml (n=7), and in sporadic Pheo cases mean serum CgA was 402.32±181.15 ng/ml (n=29), without statistical significance.

Figure 2: Correlation of plasma metanephrines with tumor size in Pheo

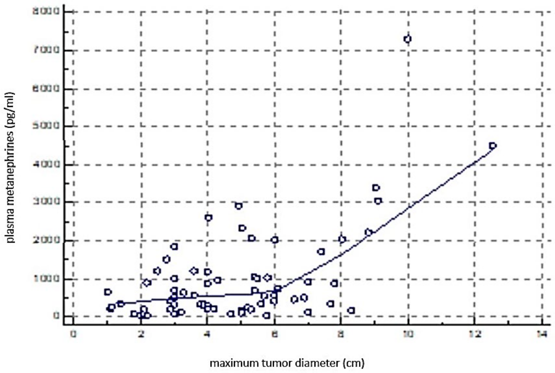

Figure 3: Correlation of plasma normetanephrine with tumor size in Pheo

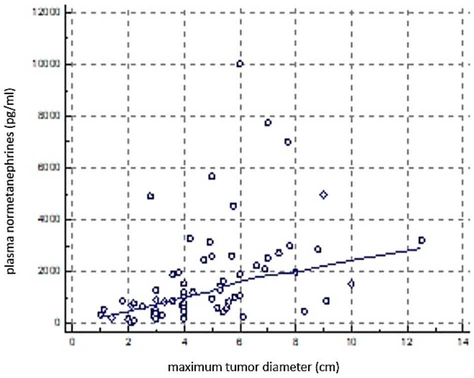

Figure 4: Correlation of urinary metanephrines with tumor size in Pheo

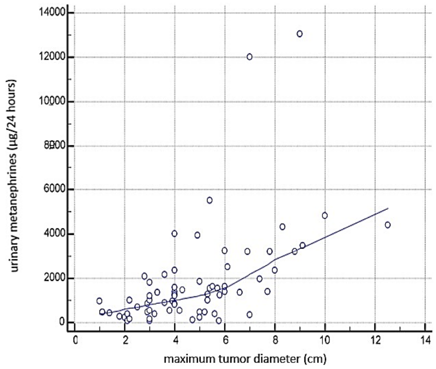

Figure 5: Correlation of urinary normetanephrine with tumor size in Pheo

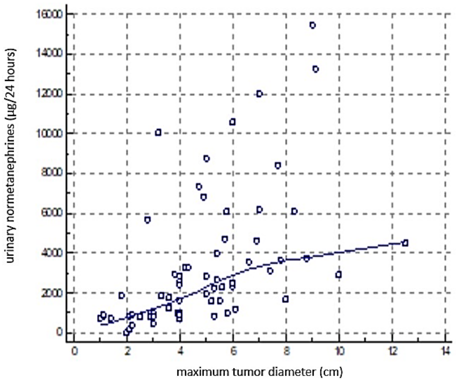

We also investigated correlations between CgA levels and hormone levels.

We identified a statistically significant positive correlation (p=0.02, r=0.37) between serum CgA and plasma metanephrine levels at the time of Pheo diagnosis, illustrated in Figure 6.

Figure 6: Correlation between preoperative plasma CgA and metanephrine levels in Pheo

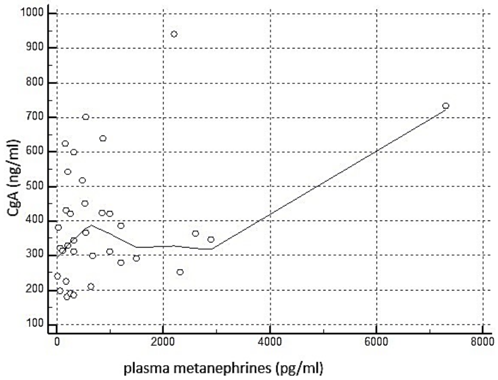

Similarly, regarding the correlation of urinary metanephrine levels with serum Cg A concentration, there was a statistically significant positive correlation (P=0.0001, r=0.62), as shown in Figure 7.

Figure 7: Correlation between serum CgA and preoperative urinary metanephrines in Pheo

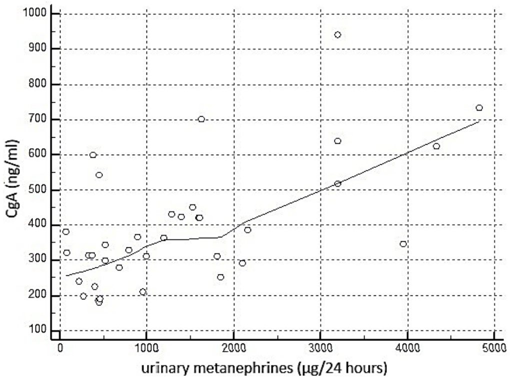

In contrast, we did not obtain statistically significant data implying correlations between preoperative serum CgA and plasma normetanephrine levels (p=0.18, r=0.22) nor urinary normetanephrine (p=0.12, r=0.26).

Regarding the correlations between serum NSE level, we identified a preoperative mean value of 12.98±1.7 ng/ml (n=18) which correlated positively, statistically significantly (p<0.0001, r=0.76) with Pheo tumor size, being illustrated in Figure 8.

Figure 8: Correlation between tumor diameter and serum NSE level in Pheo

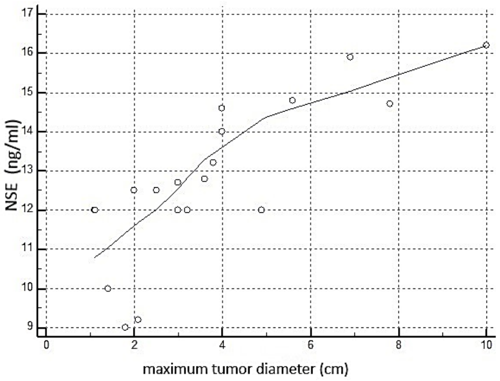

Similarly, we found no statistical significance for the correlation of serum NSE levels with plasma metanephrines at the time of Pheo diagnosis (p=0.10, r=0.35), urinary metanephrines (p=0.2, r=0.48) and urinary normetanephrine (p=0.23, r=0.26).

However, the correlation of preoperative serum NSE levels with plasma normetanephrine showed a positive line and was statistically significant (p=0.03, r=0.44), as shown in Figure 9.

Figure 9: Correlation of preoperative serum levels between serum NSE and plasma normetanephrine in Pheo

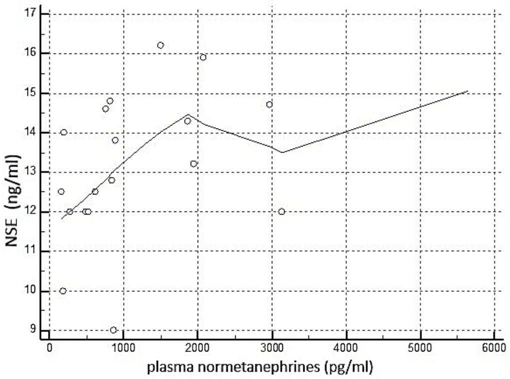

Regarding the analysis of NSE concentration according to Pheo etiopathogenesis, we found no statistically significant differences (p=0.74, r=1.332) between genetic Pheo whose mean serum NSE concentration was 12.75±1.19 ng/ml (n=4) and sporadic Peo whose mean NSE concentration was 13.06±2.22 ng/ml (n=14).

We performed also the analysis of the diagnostic power of Cg A versus plasma metanephrines and normetanephrine for Pheo.

Regarding the sensitivity and specificity of plasma metanephrines for Pheo diagnosis, being plotted in Figure 10, the results of statistical analysis indicated that a plasma metanephrines cut-off value >58 pg/ml has a sensitivity of 94.2% and a specificity of 100% for Pheo diagnosis, being highly statistically significant (p<0.0001) and AUC=0.970.

Figure 10: Sensitivity and specificity of metanephrines in the diagnosis of Pheo

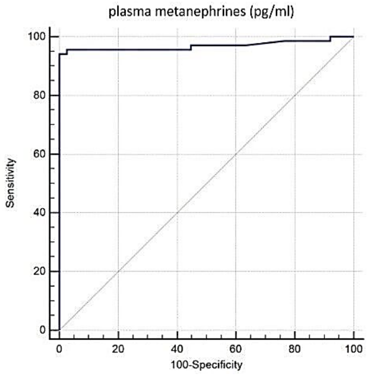

Similarly, analyzing the sensitivity and specificity of plasma normetanephrine for the diagnosis of Pheo, illustrated in Figure 11, the results indicated that a plasma normetanephrine cut-off value > 72 pg/ml has a sensitivity of 98.55% and a specificity of 100% for the diagnosis of Pheo, with high statistical significance (p<0.0001) and AUC=0.990.

Figure 11: Sensitivity and specificity of normetanephrine in the diagnosis of Pheo

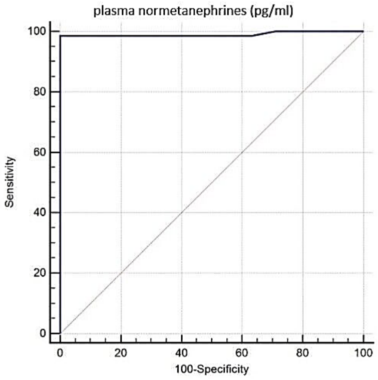

Additionally, combining plasma metanephrines and normetanephrine as a diagnostic element, there are no statistically significant differences between the two ROC curves (p=0.31), suggesting statistically similar diagnostic power, as shown in Figure 12.

Figure 12: Sensitivity and specificity of metanephrines and normetanephrine in the diagnosis of Pheo

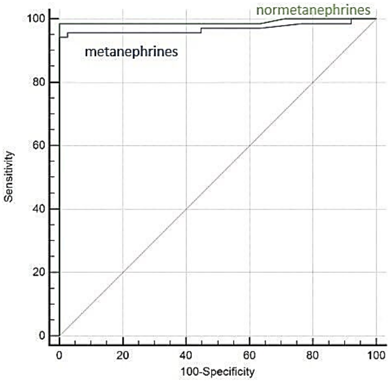

Figure 13: Sensitivity and specificity of CgA for the diagnosis of Pheo

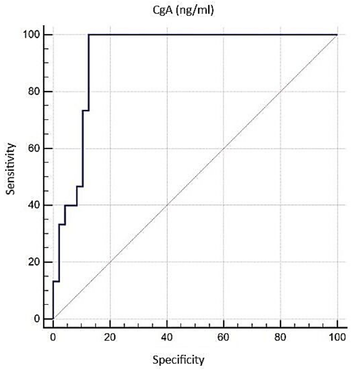

Analyzing the sensitivity and specificity of CgA for Pheo diagnosis, according to Figure 13, we obtained a sensitivity of 100% for cut-off values of CgA>197 ng/ml and a specificity of 87.5%, with high statistical significance (p<0.0001) and

AUC=0.926.

In Figure 14, it can be seen the sensitivity and specificity of CgA compared to that of plasma metanephrines and normetanephrine in the Pheo group at the time of diagnosis, but without a statistically significant difference (p=0.16, p=1) compared to the other two.

Figure 14: Sensitivity and specificity of plasma CgA, metanephrines, and normetanephrine in the diagnosis of Pheo

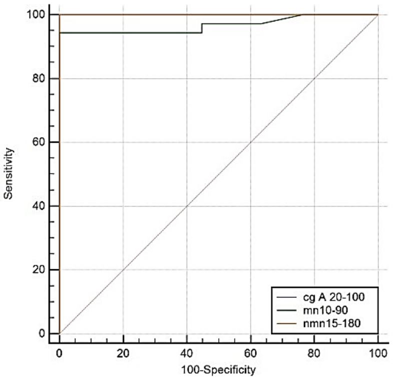

DISCUSSION

The biochemical diagnosis of Pheo is based on the determination of plasma and urinary metanephrines and normetanephrine and secondarily on the determination of neuroendocrine markers [11]. An important theme of our study was the dynamic (pre and postoperative) assessment of hormonal parameters. CgA is a glycoprotein that belongs to the granin family and Its dosing is recommended for the diagnosis and monitoring of NET [12]. Dynamic pre- and postoperative CgA assessment in Pheo was studied by others who reported a mean preoperative CgA ranging from 600- 632 ng/ml with a significant decrease postoperatively (p<0.001) [13-15]. Our data are consistent with the findings of the studies already mentioned, but the mean preoperative CgA value was 390.43±173.83 ng/ml, the difference may derive from different CgA assay methods (radioimmunoassay or ELISA technique), but the significant decrease postoperatively is conclusive. At the same time, the NSE dynamics recorded in our study (12.98±1.69 ng/ml preoperatively and 10.3±3.01 postoperatively) are within the literature studies [16]. Additionally, the course of plasma and urinary metanephrine and normetanephrine values showed significant decreases in the study, consistent with the data from the literature [17-19].

Analyzing the correlations between Pheo size and the amount of plasma and urinary metanephrines and normetanephrine, respectively neuroendocrine markers secreted at the time of diagnosis, we found statistically significant positive correlations for serum CgA levels (P<0. 0001, r=0.86), NSE (P<0.0001, r=0.76), as well as with plasma (P<0.0001, r=0.57); P=0.0002, r=0.4) and urinary (P<0.0001, r=0.55; p<0.001, r=0.5) metanephrines and normetanephrine levels. Our results correspond with data reported by the study of Unger N. et al. [18] which identified a positive correlation between CgA levels and tumor volume (p<0.001), as well as research of d'Herbomez et al. [20] that highlighted positive correlations between both preoperative CgA concentration and plasma and urinary fractional metanephrine levels with tumor volume. In contrast, the research of Guerrero M et al. [21] showed only a positive correlation between urinary normetanephrine levels with tumor size. In addition, similar to data confirmed in the literature, we obtained statistically significant data regarding positive correlations between preoperative CgA levels with plasma (p=0.02, r=0.37) and urinary (P=0.0001, r=0.62) metanephrines levels but we found no correlations with urinary and plasma normetanephrine. Similar to the study of Okamura T et al. [22] identified interdependence between CgA and urinary metanephrine levels (r= 0.80; P<0.0001), but as in our case, they did not reach the threshold of statistical significance for correlations of urinary and plasma normetanephrine with CgA.

Regarding the analysis of differences in biochemical parameters and genetic or sporadic etiopathogenesis of Pheo, we did not find statistically significant correlations for CgA (p=0.411, r=0.6) or NSE (p=0.74, r=0.1). In parallel, studies of Eisenhofer G et al. [23] and Zuber S et al. [24] reported a statistically significant difference in CgA values, whereas the research of d'Herbomez M et al. [14] reported a statistically insignificant difference (p=0.35) between CgA values between genetic and sporadic Pheo cases. We can assume that our data did not reach the threshold of statistical significance due to the small number of patients with Pheo of genetic cause in whom we had CgA and NSE assays available.

Regarding the sensitivity and specificity of plasma metanephrines for the diagnosis of Pheo, the results of our analysis indicated that a plasma metanephrine cut-off value >58 pg/ml has a sensitivity of 94.2% and a specificity of 100%, with AUC=0.970 for the diagnosis of Pheo. Similarly, the study of d'Herbomez M et al. [14] indicated a sensitivity of 71% and a specificity of 89.9%, and the study of Unger N et al. [18] indicated values of 70% respectively 90%, with AUC=0.8. Similarly, analyzing the sensitivity and specificity of plasma normetanephrine for Pheo diagnosis, our results indicated a sensitivity of 98.55% and a specificity of 100% for Pheo diagnosis, with AUC=0.990. Literature reports converge towards the same values, thus the study of d'Herbomez M et al. [14] reported a sensitivity of 95.2% and a specificity of 98.6% and the study of Unger N et al. [18] indicated a sensitivity of 89.5%, and a specificity of 98.3%, with AUC=0.91. Additionally, combining plasma metanephrines and normetanephrine as a diagnostic element, in our research we did not obtain statistically significant differences between the two ROC curves (p=0.31), suggesting statistically similar diagnostic power. Similarly, the research of Unger N et al. [18] reported a sensitivity of 89.5% and a specificity of 95% when combining the two diagnostic tests. Analyzing the sensitivity and specificity of CgA for Pheo diagnosis, we obtained a sensitivity of 100% and a specificity of 87.5%, with AUC=0.926. The study of Grossrubatscher E et al. [20] revealed a sensitivity of 91% and a specificity of 95% for CgA, while the study of d'Herbomez M et al. [14] reported a sensitivity of 98.3% and a specificity of 97% and the study of Unger N et al. [18] reported a sensitivity of 73.9% and a specificity of 74.2% with AUC=0.74.

CONCLUSIONS

We obtained statistically significant data on pre- and postoperative differences for the following hormonal and neuroendocrine parameters: CgA, plasma metanephrines and normetanephrine, urinary metanephrines, and normetanephrine. Pheo tumor sizes are interdependent with serum levels of Cg A, plasma and urinary metanephrines, and normetanephrine. In terms of inter-hormonal correlations, serum NSE levels correlate significantly with plasma normetanephrine levels and serum CgA levels correlate with plasma and urinary metanephrine levels. The best diagnostic power for Pheo was plasma normetanephrine (AUC=0.99), followed by plasma metanephrines (AUC=0.97) and CgA (AUC=0.92).

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ORIGINAL ARTICLE

Pitfalls of Ovarian Dysgerminoma

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Abstract: *This is a narrative review based on published articles, using the following criteria: key words "ovarian dysgerminoma" and "MRI (Magnetic Resonance Imaging)"; PubMed access; full-length English articles since inception to May 2023; original studies and case reports/series. Exclusion criteria were ovarian tumors other than dysgerminomas, non-English papers, non-human studies, and reviews. Dysgerminomas are the most common ovarian malignant germ cell tumors, accounting for 1-2% of all primary ovarian neoplasms.*

Keywords: *dysgerminomas, germ cell tumor, malignant tumor, MRI*

INTRODUCTION

The frame of ovarian dysgerminoma

Up to one-third of all ovarian malignancies are represented by germ cell tumors, but only 5% of these are malignant [1,2].

Ovarian germ cell tumors include, as proposed in the 2020 World Health Organization Classification of Tumors, teratomas, mature or immature, dysgerminomas, yolk sac tumors, embryonal carcinoma, non-gestational chorio-carcinoma, mixed germ cell tumors, mono dermal teratomas, and germ cell-sex cord-stromal tumors [3].

Dysgerminomas are the most common ovarian malignant germ cell tumors, accounting for 1-2% of all primary ovarian neoplasms [4,5]. In 75% of the cases, they occur after the onset of puberty [4]. Key features are the lack of precursor lesions and of specific differentiation patterns [5], a less invasive behavior with a good prognosis after surgical treatment, with a nearly 100% 5-year survival [6,7,8] and almost 90% at 10 years [9,10,11].

Although the age range reported for ovarian dysgerminoma is 7 months to 70 years old, their peak incidence occurs after puberty, more specifically in the second and third decades of life [12].

They are believed to develop from primordial germ cells that present spontaneous transmembrane tyrosine kinase receptor c-kit (CD 117) mutations. These mutations are usually observed in unilateral dysgerminomas, probably occurring after the cellular migration to the gonadal ridges [13,14].

Histologically, ovarian dysgerminomas, considered the microscopical female correspondent of the male testicular seminomas and extragonadal germinoma, are composed of undifferentiated germ cells with well-defined cytoplasmic borders, large nuclei, numerous mitoses, and clear cytoplasm, who are typically arranged in sheets or elongated nests with a palisaded peripheral cell arrangement, frequently infiltrating the stroma. The gross appearance of a well-encapsulated solid mass with an average diameter of 15 cm, but there are 50 cm cases reported, with a grey-white or light tan fleshy lobe-like pattern on section [2,3,6,7,14,15].

Immunohistochemistry shows positive testing for CD117, thus correlating with KIT mutations, while positive expression of organic cation/carnitine transporter 4 (OCT 4), spalt-like transcription factor 4, and D2–40 can also be determined. 30% of the tumours are present with vimentin (+), lactate dehydrogenase -LDH (+) and neuronal specific enolase- NSE (+). Although these tumors are not hormone-producing, still, human chorionic gonadotrophin (hCG) and ihibin are found positive in syncytiotrophoblastic cells [2,5,6,16,17].

These tumors have a propensity for bilateral involvement, with up to 5-10 % of the cases presenting as bilateral ovarian masses [18].

Their management involves surgery, and, in stages beyond IA chemo- and or radiotherapy are added as a mandatory therapeutic step [19,20].

Since these patients are usually under 30 years old and there is a time frame of at least 5 years between the diagnosis and the conception moment, optimal preoperative diagnosis is mandatory, and fertility preservation surgery becomes a key issue in their management [13,19,21,22]. 15%–20% are diagnosed during pregnancy or post-childbirth and are the most frequent tumors in pregnancy [23].

Diagnostic workup includes imaging studies, such as transabdominal ultrasound (in children), a transvaginal ultrasound, computed tomography scan (CT scan), and magnetic resonance imaging (MRI) studies, along with serum markers assay for differential diagnosis of the abdominal mass. Flourine-18 fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) is not a usual diagnostic tool, but rather used for relapse, or follow-up after chemotherapy or surgery [24, 25, 26]. Dysgerminomas are associated with various elevated serum markers. Elevated LDH, alkaline phosphatase is frequently found in these patients' assays [27].

Despite the progress of ultrasound imaging and the introduction of IOTA criteria for distinguishing between benign and malignant lesions, up to 25% of the lesions remain indeterminate, requiring further imaging investigation [28].

Clinical presentation of these rapid-growth abdominal masses ranges from incidental findings on imaging studies to the more common abdominal enlargement, associated pain, or menstrual abnormalities. Precocious puberty, metrorrhagia, and acute abdomen due to tumor rupture, torsion, or hemorrhage are also noted.

Differential diagnosis includes but is not limited to other histological types of ovarian tumors (benign or malignant), ovarian metastases, or ovarian myomas.

Ovarian dysgerminoma complicated with torsion, rupture can mimic other acute abdominal conditions, such as acute appendicitis, diverticulitis, tubo-ovarian abscess, ectopic pregnancy, or ruptured follicular cyst [29].

According to NCCN (National Comprehensive Cancer Network) Guidelines 2020, in patients who desire to preserve fertility, fertility-sparing surgery following comprehensive surgical staging is mandatory and consists of unilateral salpingo-oophorectomy along with all visible tumors' resection [30,31].

MATERIALS AND METHODS

This is a narrative review based on published articles, using the following criteria: keywords "ovarian dysgerminoma" and "MRI"; PubMed access; full-length English articles since inception to May 2023; original studies and case reports/ series. Exclusion criteria were ovarian tumors other than dysgerminomas, non-English papers, non-human studies, and reviews.

According to our four main criteria, we identified 40 papers that we manually checked and excluded 3 papers, 1 veterinary study, and 2 articles in another language than English. We considered the various imaging aspects with consideration to the clinical practice (Figure 1).

Figure 1: Flowchart according to our methodology (n = number of articles).

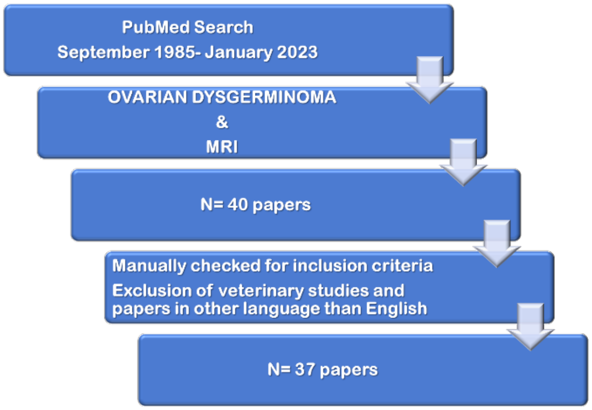
PubMed Search
September 1985- January 2023
OVARIAN DYSGERMINOMA
&
MRI
N= 40 papers
Manually checked for inclusion criteria
Exclusion of veterinary studies and papers in other language than English
N= 37 papers

RESULTS

Ultrasound features

Ultrasound features involve a well-defined, highly vascularized, heterogenous multilobulated solid mass with significantly vascularized septa [32].

MRI features

MRI aspects include intermediate signal intensity on T2-weighted imaging (T2WI) and high the lobulated aspect seen on ultrasound is also found on CT scans, with enhanced septa. Cystic areas represent either hemorrhage or necrosis. Speckled calcification can be found, especially in hypercalcemia patients. MRI scan shows a similar solid lobulated mass with fibrovascular septa, with low intensity on T1-weighted images (T1WI) and intermediate signal intensity on T2-weighted imaging (T2WI). They usually have high signal intensity on DW imaging and low ADC value. Fibrovascular septa are hyperintense on T2W1 in the presence of edema [32-34].

Zhao et al. divided septa into four categories, according to the degree of edema: Type I consists of thin non-oedematous hypointense T2W1 septa, which are unrecognizable on pre-contrast CT scans. Type II are thin T2W1 hyperintense oedematous septa. Type III are included thick oedematous T2W1 hyperintense septae with hypoattenuation in pre-contrast CT scan. Type IV consists of map-like T2W1 oedematous morphous septae that are usually found in large tumors [35].

Multiplicity can be found as small nodules with high signals on DWI. Large tumor vessels can be found at the center of the lesions with radiant peripheral distribution through the vascular septae. In a case series, the multiplicity detected in the MRI studies is considered to be most likely a sign of the transformation of gonadoblastoma to germinoma [32-36].

According to the same authors, the central vasculature found in MRI studies is most likely a typical finding of dysgerminoma, but further studies are required [36].

18F-FDG PET/CT

The role of 18F-FDG PET/CT is not currently set for ovarian malignant germ cell tumors; it is usually used for the restaging of residual disease after chemotherapy, after surgery, or follow-up in apparently disease-free patients. A negative 18FDG PET/CT might be useful to reduce the number of excessive invasive procedures [37].

In a case of a 21-year-old female with ultrasound evidence of a large solid mass in the abdomen and elevated CA-125, right large pleural effusion on CT-scan, the 18F-fluoro-2-deoxy-D-glucose positron emission tomography maximum standardized uptake was 15,8. Areas of tumor mass reduced metabolism were secondary to tumor necrosis [38].

Associated conditions

Ovarian dysgerminoma can be associated with various other conditions. Patients with disorders of sex development or dysgenic gonads can associate malignant transformation. There are reported cases of Turner syndrome, triple X syndrome, or testicular feminization [39,40].

Patients with disorders of sex development, such as 46, XY with SRY mutations in the high mobility group domain have an increased risk of ovarian malignant transformation, requiring specific investigations [39,40].

Demon Meigs syndrome, consisting of co-occurrence of an ovarian mass, pleural effusion, and elevated serum cancer antigen CA-125 levels, can associate with ovarian dysgerminoma. In the case of a 19-year-old patient presenting with dyspnea and fever, a large right pleural effusion, a small number of ascites, and a huge (29.2 cm × 11.8 cm × 8.4 cm) abdominopelvic tumor were diagnosed. Elevated serum markers- CA-125, LDH, ROMA score suggested the presence of a malignant ovarian mass. The surgical specimen- solid, fleshy, cream-colored, creatine kinase (+), leukocyte common antigen (lymphocyte, +), placental alkaline phosphatase (+), CD30 (−), CD117 (+), α-fetoprotein (−), podoplanin (+), human chorionic gonadotropin (HCG) (+), and inhibin-α (−) was confirmed as dysgerminoma [41, 42].

Unlike seminoma in males, the association of Down syndrome with ovarian dysgerminoma is rather unusual, with only five cases being reported so far in the literature. There is no proven link so far between the two conditions, but the possible association might be secondary to an effect of genetic overexpression, hormonal imbalance, and a higher affinity of the trisomic cells to carcinogens [41,42].

Pregnancy-associated dysgerminoma

Pregnancy-associated dysgerminoma is an extremely rare presentation, and according to a literature review, only 21 cases were published in the last 7 decades. The symptoms are similar to non-pregnant patients, ranging from no symptoms to abdominal distension, and growing abdominal mass. In 2 cases, obstructed labor was reported leading to tumor incarceration. The diagnosis was made based on ultrasound or MRI reports along with elevated serum tumor markers- LDH, the most sensitive, AFP or CA-125. In pregnant patients, MRI studies are second-line imaging tools, used only for confirmation/further investigation of the cases where ultrasound was not sufficient for diagnosis. Several complications can occur during vaginal delivery- incarceration or a caesarean section- tumor rupture. Fetal death can occur in up to one-quarter of the cases [43,44].

Lymph node metastasis

The metastatic pattern in advanced dysgerminoma was only reported isolated in several studies, although this tumor is more metastatic than other histological types of ovarian cancer. It is more likely to develop para-aortic lymph node metastasis than in other stations.

FDG PET CT might help in the identification of these metastatic lymph nodes, reducing the burden associated with extensive tumor relapse treatment. A case series of two patients who presented with an initial FIGO IA stage showed how an improper initial surgery might lead to inadequate assessment and staging of the tumor. The first patient received surgery and lymph node sampling (who was negative) and had a subsequent nodal relapse. The second patient was offered surgery without lymph node sampling/lymphadenectomy and thirteen months later she presented with a para-aortic tumor that required extensive surgery and treatment [45,46].

A case of cervical lymph node metastasis was confirmed by fine needle aspiration after 18FDG PET-CT in a 20-year-old woman who presented with a large abdominal mass and cervical swelling [47].

Rare associations

A case of ovarian dysgerminoma was reported in association with Mullerian agenesis, in a 20-year-old woman who presented with symptoms of pregnancy, and a positive urinary pregnancy test. Clinical and ultrasound evaluation showed the lack of a right ovary and uterus with a normal-appearing left ovary. She confirmed primary amenorrhea. Seriated pelvic MRI initially showed a 2 cm low T1 and low T2 signal mass adjacent to the normal-appearing right ovary, corresponding to a 3/1.5 cm solid pink smooth tumor invading the ovarian hilum. The pathology report confirmed a dysgerminoma containing syncytiotrophoblast cells and microscopic tumoral foci away from the main tumor. Immunohistochemistry was positive for placental alkaline phosphatase and weekly positive for beta HCG [48].

A unicornuate uterus, non-communicating rudimentary horn, and right homogenous mass- dysgerminoma, was reported in an 18 -year- old woman [49].

Ovarian dysgerminoma complications

Dysgerminoma usually presents rapid growth with subsequent torsion and rupture of hemorrhage. Patients present abdominal pain with a sudden increase in intensity, abdominal distension, and a palpable mass revealed on clinical examination. Loss of appetite, nausea, or vomiting can be associated. The size of the tumor determines the intensity and the rapidity of the onset of the symptoms. In such emergency cases diagnosis is usually based on ultrasound and CT-scan, MRI requiring a longer acquisition time. A CT scan might show an area of necrosis or edema with low attenuation, the presence of pelvic free fluid, and the twisted vascular pedicle originating from the adnexa [50,51].

An unusual case of aggressive systemic mastocytosis was reported in a 16-years-old female who was diagnosed with ovarian dysgerminoma 2 years before and received 4 cycles of neoadjuvant chemotherapy (bleomycin, etoposide, and cisplatin) followed by surgery and adjuvant carboplatin and etoposide, with normalization of alpha-fetoprotein levels. 2 years later she complained of bone pain, skin rash, fever, and anaphylaxis and imaging studies revealed spine osteosclerosis and osteolysis, yet no tumor mass was present. AFP levels were normal and immunohistochemistry staining was positive for mast cell tryptase, CD25, CD33, and c-KIT. The serum tryptase level was elevated. Her symptoms were alleviated with prednisolone, but significant improvement was achieved with tyrosine kinase inhibitor – nilotinib [52].

Hypercalcemia can be found in 5% of gynecologic neoplasms, including dysgerminoma, with clinical symptoms depending on the baseline general status, level of calcemia, or rapidity of progression [53]. Two-thirds of ovarian cancer associated with hypercalcemia can be found in small cell carcinoma of the ovary, dysgerminoma being the second associated most common pediatric ovarian malignancy. Fifteen cases of dysgerminoma-associated hypercalcemia have been reported so far. Symptoms include the presence of tumoral mass, abdominal pain/distension, unintentional weight loss, dyspnoea on exertion, nausea constipation, lethargy, polydipsia, and polyuria [54].

Unlike hypercalcemia associated with breast cancer, where a local osteolytic mechanism is involved, in ovarian cancer associated with hypercalcemia, a systemic secretion of parathormone-related peptide (PTHrP) is the cause of the humoral abnormality. Abnormal tumoral expression of 1-alpha-hydroxylase can also be incriminated. 1,25 dihydroxy vitamin D might be involved in malignant-associated hypercalcemia by increasing both osteoclastic bone resorption and intestinal calcium absorption. Surgery is usually followed by the resolution of the symptoms [55,56].

DISCUSSION

Ovarian dysgerminoma is a rare malignant ovarian tumor, diagnosed in the first three decades of life, with a favorable prognosis if the appropriate treatment is given. According to FIGO staging, the initial treatment can either be surgery or neoadjuvant chemotherapy. Since patients are very young, fertility preservation is a major concern and current surgical options include fertility-sparing techniques [13,19,30,31,57].

Due to the scarcity of the literature data, imaging criteria are not very clear. The ultrasound IOTA criteria were introduced to distinguish between benign and malignant ovarian masses [28,31]. Additional imaging studies are further required in possible malignant tumors. CT scan is more likely to be used in emergency situations including acute abdomen due to rupture, hemorrhage, or ovarian torsion. In such cases, an MRI examination would be a time-consuming investigation and doesn’t really add supplementary information. Regarding the evaluation of lymph node status, both CT scan and MRI can give information on para-aortic nodes, as these are the most frequent metastatic site. 18FDG PET-CT might be helpful in identifying the metabolically active areas (lymph node stations) that should undergo surgery [37,38]. MRI studies are valuable for the evaluation of ovarian tissue and vascularization and can add important data on other abnormalities, such as disorders of sex development, and other associated soft tissue anomalies [39,40].

There are two specific aspects that are described in MRI scans. The first one, the multiplicity defined as the presence of small nodules that can be found in section studies, is indicative of early stages or slow-growing tumors. The central vascular vessels, the second characteristic, also seems to be kind of specific for these tumors, as it is usually not described in other histological types of ovarian malignancies, but further studies are required.

Considering the possibility of ovarian dysgerminoma-associated hypercalcemia, the measurement of serum levels of PTH, PTHrP, and 1,25-hydroxyvitamin D seem to be useful, as neurocognitive dysfunction, volume depletion, and renal failure might prove to be serious complications.

Given the reviewed data, the management algorithm in ovarian dysgerminoma patients should include several mandatory aspects. A careful history of all the symptoms is the first step in the evaluation of the patient, followed by ultrasound, abdominal or transvaginal. Serum markers assessment along with supplementary investigations, such as upper and lower GI endoscopy, and cystoscopy can be used in selected cases. A thorough description of the lesion, of the lymph nodes, and the evaluation of possible complications can be achieved through imaging studies – chest X-Ray for pleural effusions, CT/MR scan, and 18FDG PET-CT if available for lymph node evaluation. Neoadjuvant chemotherapy can be used in advanced metastatic disease, followed by surgery. According to current guidelines for non-epithelial ovarian tumors, in the early stages, initial minimally invasive fertility-sparing surgery is the treatment of choice [58,59].

Although ovarian dysgerminoma has a good prognosis with appropriate treatment, optimal diagnostic work-up is mandatory to achieve the best results in young women.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution
Conceptualization, N.I.A. and C.E.N.; methodology, C.A.P.; software, N.F. and S.N.M.E.D.; validation, N.I.A., C.A.P. and N.C.E.; formal analysis, N.F.; investigation, C.A.P.; resources S.N.M.E.D.; data curation N.F.; writing—original draft preparation, N.I.A., C.A.P. and C.E.N.; writing—review and editing, N.I.A, C.A.P. and N.C.E.; visualization, N.I.A.; supervision, N.C.E.; project administration, N.I.A and N.C.E.
All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Should Cervical Cancer Screening with Papanicolaou Smear be Recommended for Female Patients with Psoriasis before Biological Agent Treatment?

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Abstract: *Chronic inflammation and immunosuppressive treatment may play a role in the etiopathogenesis of cancer in psoriasis patients. We wanted to evaluate the Papanicolaou smear (Pap smear) results of female patients with psoriasis which were performed for cervical cancer screening before the initiation of biological agent treatment. Between April 2019 and November 2021, Pap smear results of female patients with psoriasis were reviewed retrospectively. This study included 70 female psoriasis patients with a mean age of 48.41±11.62 years. 49 (70%) patients were biologically naive, whereas 21 (30%) patients previously received biological agents. Obscuring inflammation was reported in 33 (47.1%) patients. Atrophy, intermediate cell predominance, reactive cellular changes due to inflammation, and both atrophy and reactive cellular changes were detected in 15 (21.4%), 7 (10%), 3 (4.3%) patients, and 1 (1.4%) patient, respectively. No malignancy or intraepithelial lesion was observed in 69 (98.6%) patients, whereas atypical squamous cells of undetermined significance were reported in 1 (1.4%) patient. Infections such as bacterial vaginosis, candidiasis, and actinomycosis were detected in 13 (18.6%) patients. Pap smear tests should be performed in female psoriasis patients to detect premalignant lesions of the cervix before the initiation of biological agent treatment. Moreover, psoriasis treatment guidelines should be updated accordingly.*

Keywords: *biological agents; cancer; cervical; psoriasis*

INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder that has been associated with systemic comorbidities such as psoriatic arthritis, cardiovascular disorders, and metabolic syndrome [1,2]. Furthermore, increased risk of developing cancer and cancer mortality have been reported in patients with psoriasis [3-6]. Lymphoma, lung, bladder, colorectal, liver, and kidney cancers have been related to psoriasis [4,7]. It has been suggested that predisposition to obesity, lifestyle factors such as smoking, chronic inflammation, and immunosuppressive drugs used for the treatment of psoriasis might play a role in the etiopathogenesis of cancer in patients with psoriasis [4,8]. Cancer risk increases with disease severity [9]. Patients with severe psoriasis are usually treated with systemic immunosuppressive drugs for a long period of time to control disease [8].

Biological agents such as tumor necrosis factor-α (TNF-α) inhibitors and interleukin (IL) 12/23, IL-23, and IL-17 inhibitors are choices of treatment in psoriasis [10]. TNF-α is an inflammatory cytokine that plays a role in the regulation of cell proliferation and apoptosis [11]. IL-12 and IL-23 show anti-tumor effects, whereas IL-17 has both anti-tumor and tumorigenic effects [10]. Therefore, concerns have been raised that treatment with biological agents might lead to increased cancer risk in psoriasis patients [10,12]. Joint American Academy of Dermatology-National Psoriasis Foundation guidelines recommends performing age-appropriate cancer screening tests in psoriasis patients treated with biological agents [13].

Cervical cancer is the third most common cancer in women globally. Moreover, cervical cancer is the second leading cause of cancer-related mortality in women. Papanicolaou (Pap) smear is the main test used to screen for cervical cancer. Colposcopy and biopsy may be performed in patients with atypical epithelial cells on Pap smear results [14].

Within this study, we evaluated Pap smear results of female patients with psoriasis which were performed to detect premalignant lesions of the cervix and cervical cancer before the initiation of biological agent treatment.

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained prior to this study (approval number: 2021-1067). Between April 2019 and November 2021, Pap smear results of female patients with psoriasis which were performed for cervical cancer screening before the initiation of biological agent treatment were reviewed retrospectively.

Specimen types such as liquid-based and conventional Pap smear, presence of endocervical transformation zone component, quality indicators such as obscuring inflammation and blood, non-neoplastic findings such as atrophy and reactive cellular changes, general categorization, and detected microorganisms were evaluated. Patients with inadequate Pap smear specimens for evaluation were excluded from the study.

Statistical analysis was performed using SPSS version 20.0. Continuous variables were defined as the mean (±) standard deviation and categorical variables were expressed as percentages. Differences between the two groups were detected with a chi-square test. P<0.05 was considered statistically significant.

RESULTS

This study included 70 female patients with psoriasis with a mean age of 48.41±11.62 years (range: 25-74 years). 54 (77.1%) patients had psoriasis vulgaris, 8 (11.4%) patients had generalized pustular psoriasis, 5 (7.1%) patients had palmoplantar psoriasis, 2 (2.9%) patients had palmoplantar pustular psoriasis and 1 (1.4%) patient had inverse psoriasis. The mean disease duration was 15.71±11.51 years (range: 1-50 years). 26 (37.1%) patients had psoriatic arthritis. Past medical history was unremarkable in 37 (52.9%) patients. Moreover, 24 (34.3%) patients had hypertension, 16 (22.9%) patients had diabetes, 5 (7.1%) patients had chronic hepatitis B virus infection, 4 (5.7%) patients had hypothyroidism, 4 (5.7%) patients had coronary artery disease, 3 (4.3%) patients had hyperlipidemia, 2 (2.9) patients had chronic obstructive pulmonary disease, 2 (2.9) patients had asthma and 1 (1.4%) patient had steatohepatitis. 69 (98.6%) patients were treated with conventional systemic treatments such as methotrexate, cyclosporine, and acitretin, and 18 (25.7%) patients were previously treated with phototherapy. 49 (70%) patients were biologic naive, however, 21 (30%) patients were previously treated with biological agents. Among them, 6 (8.6%) patients received infliximab, 5 (7.1%) patients received adalimumab, 3 (4.3%) patients received etanercept, 2 (2.9%) patients received ustekinumab, 1 (1.4%) patient received ixekizumab, 1 (1.4%) patient received secukinumab and 1 (1.4%) patient received certolizumab pegol. In addition, 1 (1.4%) patient was treated with both adalimumab and etanercept, and 1 (1.4%) patient was previously treated with both infliximab and etanercept.

Specimen type was liquid-based in 46 (65.7%) patients and conventional Pap smear in 24 (34.3%) patients (Table 1). The presence of endocervical transformation zone components was reported in 47 (67.1%) patients.

Table 1: Pap smear results of the patients with psoriasis before biological agent treatment

	n (%)			
Specimen type	Liquid-based 46 (65.7%)	Conventional 24 (34.3%)		
Endocervical transformation zone	Present 47 (67.1%)	Absent 23 (32.9%)		
The other quality indicators	Obscuring inflammation 33 (47.1%)	Obscuring blood 1 (1.4%)		
Non-neoplastic findings	Atrophy 15 (21.4%)	Intermediate cell predominance 7 (10%)	Reactive cellular changes due to inflammation 3 (4.3%)	Atrophy+reactive cellular changes 1 (1.4%)
General categorization	Negative for malignancy 69 (98.6%)	Atypical squamous cells 1 (1.4%)		
Microorganisms	None 57 (81.4%)	Bacterial vaginosis 6 (8.6%)	Candida 6 (8.6%)	Bacterial vaginosis+ Actinomyces 1 (1.4%)
3-dimensional cell groups	1 (1.4%)			

Atypical squamous cells of undetermined significance were reported in 1 (1.4%) patient. Fungal organisms consistent with Candida were encountered in 6 (8.6%) patients, 3 (4.3%) of them had fungal organisms morphologically consistent with Candida glabrata. In addition, three dimensional groups of endocervical cell shedding which might be associated with benign cervical lesions such as polyp or papillary cervicitis was reported in 1 (1.4%) patient.

However, the endocervical transformation zone component was absent and thus squamous epithelial cells were evaluated in 23 (32.9%) patients. Among other quality indicators, obscuring with inflammation was reported in 33 (47.1%) patients, and obscuring with blood was reported in 1 (1.4%) patient. As a non-neoplastic finding, atrophy was detected in 15 (21.4%) patients, intermediate cell predominance was detected in 7 (10%) patients, and reactive cellular changes due to inflammation were detected in 3 (4.3%) patients. In addition, 1 (1.4%) patient showed both atrophy and reactive cellular changes. No malignancy or intraepithelial lesion was observed in 69 (98.6%) patients, however, epithelial cell abnormality was detected in 1 (1.4%) patient. Atypical squamous cells of undetermined significance (ASC-US) [15] were reported in the latter. The patient with ASC-US was a 44-year-old female with a 36-year history of psoriasis vulgaris and psoriatic arthritis who had been previously treated with methotrexate and infliximab. No microorganism was detected in the Pap smear results of 57 (81.4%) patients. However, 6 (8.6%) patients showed flora change suggesting bacterial vaginosis. Fungal organisms consistent with Candida were encountered in 3 (4.3%) patients and fungal organisms morphologically consistent with Candida glabra-ta were encountered in 3 (4.3%) patients. Pap smear result of 1 (1.4%) patient revealed both flora changes suggesting bacterial vaginosis and microorganisms consistent with Actinomyces. Three-dimensional groups of endocervical cell shedding which might be associated with benign cervical lesions such as polyp or papillary cervicitis were reported in 1 (1.4%) patient.

Patients with atrophy on Pap smear were statistically significantly older than those who did not have atrophy on Pap smear (the mean age: 60.44±6.25 and 44.85±10.41 years, respectively) ($p<0.001$). On the other hand, the mean age of the patients with inflammation on Pap smear (44.88±8.98 years) was statistically significantly lower than the mean age of the patients without inflammation on Pap smear (51.57±12.87 years) ($p=0.015$). Inflammation on Pap smear was observed in 26 (37.1%) patients with psoriasis vulgaris, 3 (4.3%) patients with palmoplantar psoriasis, 2 (2.9%) patients with generalized pustular psoriasis, 1 (1.4%) patient with palmoplantar pustular psoriasis and 1 (1.4%) patient with inverse psoriasis. No statistically significant difference was detected between psoriasis type and inflammation on Pap smear ($p=0.549$). The mean disease duration with and without inflammation on Pap smear was 13.67±10.33 and 17.54±12.31 years, respectively ($p=0.161$). 13 (18.6%) patients with inflammation on Pap smear had psoriatic arthritis, and 20 (28.6%) patients with inflammation on Pap smear did not have psoriatic arthritis. No statistically significant difference was detected between psoriatic arthritis and inflammation on Pap smear ($p=0.713$). 11 (15.7%) patients with inflammation on Pap smear had accompanying comorbidities whereas 22 (31.4%) of the patients with inflammation had an unremarkable past medical history ($p=0.029$). 25 (35.7%) patients with inflammation on Pap smear were biologic naive, whereas 8 (11.4%) patients with inflammation on Pap smear received former biological agent treatment ($p=0.321$). Among 33 patients with inflammation, only 8 of them had Pap smear results suggesting infection such as bacterial vaginosis or candidiasis ($p=0.249$).

DISCUSSION

Cervical cancer screening aims for the early detection of premalignant and malignant lesions in order to reduce cervical cancer-related mortality [16]. US Preventive Services Task Force (USPSTF) recommends performing cervical cytology every 3 years in females between the ages of 21 and 29. Between the ages of 30 and 65, it is recommended to perform cervical cytology every 3 years, high-risk human papillomavirus (HPV) testing every 5 years, or both tests every 5 years. Cervical cancer screening is not recommended before 21 years of age and in females older than 65 without high-risk factors for cervical cancer.

According to USPSTF, immunocompromised individuals, patients with human immunodeficiency virus (HIV) infection, and individuals who were exposed in utero to diethylstilbestrol and previously treated for a high-grade precancerous lesion or cervical cancer are at high risk for cervical cancer. Individualized programs for cervical cancer screening have been recommended in the follow-up of these patients [17]. The American Cancer Society (ACS) recommends initiating cervical cancer screening at the age of 25. Between ages 25 and 65, primary HPV tests such as Onclarity HPV are preferred every 5 years. When primary HPV testing is unavailable, both Pap smear and HPV tests such as Digene hybrid capture 2 are recommended every 5 years, or Pap smear alone may be performed every 3 years. Cervical cancer screening should be ceased in women older than 65 with normal screening results for the last 10 years. However, these individuals should not have been diagnosed with cervical intraepithelial neoplasia 2 or more advanced dis-ease for the last 25 years [18]. World Health Organization (WHO) recommends starting cervical cancer screening at the age of 30 in the general population and in women aged 25 with HIV infection. Primarily, HPV DNA detection is recommended every 5 to 10 years in the general population and every 3 to 5 years in women with HIV infection. If HPV-DNA testing is unavailable, either visual inspection with acetic acid (VIA) or cytology may be performed in both groups.

Screening is discontinued both in the general population and in women with HIV infection after 50 years of age [19]. According to the American College of Obstetricians and

Gynecologists (ACOG), cervical cancer screening should be started at 21 years of age. Cytology is preferred for cervical cancer screening in women aged 21-29 years. However, women aged 25-29 with an average risk for cervical cancer can also be screened by high-risk HPV testing every 5 years [20].

The age to start cervical cancer screening, primarily preferred screening tests, screening intervals, and age to stop screening differ in WHO and cervical cancer screening guidelines [17-19]. Moreover, recommendations about immunocompromised individuals such as patients with HIV infection and solid organ transplantation have not been detailed by USPSTF, whereas, in these patients, ACS recommends cytology annually between ages 21-29 and cytology as well as high-risk HPV testing every 3 years between ages 30-65 [21]. Therefore, we suggest that guidelines for the treatment of psoriasis with biological agents should include detailed information on cervical cancer screening in order to guide both physicians and female patients with psoriasis [19].

Studies investigating the risk of cervical cancer in women with psoriasis are inadequate and conflicting results have been reported on the risk of cervical cancer in patients with systemic inflammatory diseases [22,23]. For instance, Mos-cicki et al. reviewed the risk of cervical cancer in patients with immunosuppression for reasons other than HIV infection and in patients with autoimmune diseases. Moscicki et al. suggested that the risk for the development of cervical cancer increased in patients with inflammatory bowel disease and rheumatoid arthritis who were under immuno-suppressive treatment and in patients with systemic lupus erythematosus regardless of treatment [22]. On the other hand, Kim et al. reported that the risk of cervical dysplasia and cervical cancer were higher in patients with systemic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus but not different in patients with psoriasis and inflammatory bowel disease compared to women without systemic inflammatory diseases. Kim et al. suggested that the results might be affected by the variety in systemic inflammation levels between patient groups and low rates of administration of immunosuppressive medication in patients with psoriasis and inflammatory bowel disease [23].

Patients with psoriasis who are treated with biological agents may not undergo necessary cancer screening tests [24]. Kim et al. revealed that only 55% of the patients with psoriasis were admitted to a gynecologist at least once within an approximately two-year follow-up [23]. Silveira et al. suggested that 25.2% of the patients with psoriasis who were under biological agent treatment did not have necessary laboratory tests during the previous year [25]. Barbieri et al. reported that the rate of attendance of cervical cancer screening in psoriasis patients treated with biological agents was similar in patients with hypertension, however, 9% higher than in the general population [24].

CONCLUSION

Within this study, Pap smear results of 70 women with psoriasis were evaluated before the initiation of biological agent treatment. Atypical squamous cells were detected in 1 (1.4%) patient, whereas Pap smear results revealed infections such as bacterial vaginosis, candidiasis, and actinomycosis in 13 (18.6%) patients. Furthermore, obscuring with inflammation was reported in 33 (47.1%) patients. Inflammation on Pap smear was more commonly detected in younger patients, however, no statistically significant association was observed between inflammation on Pap smear and psoriasis type, disease duration, the existence of psoriatic arthritis, and previous biological agent treatment (p=0.549, p=0.161, p=0.713, p=0.321, respectively). Hence, we suggest that a Pap smear test should be performed in female psoriasis patients to detect premalignant lesions of the cervix before the initiation of biological agent treatment. Moreover, psoriasis treatment guidelines should be updated accordingly.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.
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Authors' contribution
Conceptualization, F.T., I.O., A.G.; methodology, F.T.; software, F.T.; validation, F.T., I.O., A.G.; formal analysis, F.T.; investigation, F.T., I.O.; resources, F.T., I.O., A.G.; data curation, F.T., I.O., A.G.; writing—original draft preparation, F.T.; writing—review and editing, F.T., I.O., A.G.; visualization, F.T.; supervision, A.G.; project administration, F.T., I.O., A.G.
All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2021-1067).

Patient consent for publication
Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Haemodialysis Patients with Type 2 Diabetes Mellitus and COVID-19: An Observational Study

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Abstract: *Background and Objectives: Chronic Kidney Disease (CKD) affects 6,7% of the adult population in Romania and is associated with high morbidity. About one out of three adults with diabetes has kidney disease. According to current literature data, the prevalence of diabetes is very high, up to 11,6%, of whom 2,4% had undiagnosed diabetes, and is the leading cause of kidney damage and the need for renal replacement therapy (RRT). COVID-19 has brought with it a lot of unanswered questions, regarding the risk factors, the disease evolution, and the treatment possibilities. It became clear that diabetic kidney disease (DKD) is among the independent risk factors that predict unfavorable outcomes upon SARS-CoV-2 infection, so we aimed to evaluate the characteristics of diabetic and non-diabetic dialyzed patients, COVID-19 positive. Materials and Methods: It is an observational, single-center study that analyzed type 2 diabetes mellitus and non–diabetic patients in maintenance hemodialysis hospitalized for SARS CoV-2 infection. Results: A total of 101 adult dialyzed patients were admitted with a SARS-CoV-2 RT-PCR positive test, out of which 42 had a long history of diabetes mellitus type 2 and 59 of them have been known with other etiologies of CKD. Hypertension and heart disease were the most commonly associated comorbidities. Inflammatory markers and anemia were significantly increased in diabetic patients compared to non-diabetic. Conclusions: We found that anemia was more severe in patients COVID-19-positive MHD T2DM patients.*

Keywords: *COVID-19; diabetic nephropathy; diabetic kidney disease; mortality*

INTRODUCTION

The introduction of the term chronic kidney disease (CKD) was followed by the discovery of an unexpectedly high prevalence of CKD. According to PREDATORR study, the prevalence of CKD in Romania was estimated at 6.7% [1]. The real problem with this finding is the high morbidity and mortality burden associated with advanced stages of CKD. During COVID-19 pandemics, the mortality rates associated with SARS-CoV-2 infection were higher in maintenance hemodialysis patients (MHD) than in the non-renal population, varying between 27-41% [2], reaching 50% in other studies. [3]. The maintenance hemodialysis patients are even at a greater risk for death, as was observed in recent studies [4].

Another risk factor for unfavorable outcomes in COVID-19 infection is type 2 diabetes mellitus (T2DM) [5]. According to the International Diabetes Federation, there are 1.2 million patients with diabetes mellitus in Romania [6].

The association between diabetes and chronic kidney disease is extremely common, being the main cause of end-stage kidney disease (ESKD), in more than 40% of patients worldwide [7], even more than the traditional nephrological pathology, such as glomerular diseases. Poor glycemic control has been proven to be a risk factor for progression to ESKD, renal replacement therapy (RRT), and fatal cardiovascular events. During the COVID-19 pandemic, studies have reported diabetes mellitus (DM) to be associated with a two-fold increase in mortality as well as increased severity of COVID-19 disease, as compared to non-DM patients [8]. Maintaining optimal glycemic control is crucial for avoiding severe complications, and continuous glucose monitoring devices could improve glycemic control for patients with type 2 diabetes, thus decreasing the rates of high morbidity as seen in recent studies [9]. Given the fact that diabetes is a major concern worldwide, new biomarkers for glycemic control need to be found for better diagnosis and treatment [10]

The aim of the present study was to evaluate the characteristics of COVID-19 patients with DM as compared to non-DM COVID-19 population.

MATERIALS AND METHODS

It is an observational, single-center study conducted in the Nephrology Clinic of Saint John Emergency Clinical Hospital Bucharest and includes data on MHD patients admitted between November 2021-March 2022. COVID-19 disease was confirmed by positive real-time polymerase chain reaction (RT-PCR). The data was collected from the hospital's electronic medical database. The study was conducted according to the Declaration of Helsinki on human rights.

The protocol of the study was approved by the Institutional Ethics Committee. Considering the observational character of the study, the informed consent was waived by the Ethics Committee.

We collected data regarding age, gender, disease severity, oxygen supplementation need, etiology of chronic kidney disease (CKD), laboratory tests (hemoglobin levels, leukocyte, lymphocyte and platelet counts, serum albumin, C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6) and data about infectious and non-infectious complications. We divided the study population into two groups based on the T2DM status (T2DM vs. non-DM) and compared continuous variables from the two groups using the nonparametric Mann-Whitney U test and parametric t-test. We considered statistically significant a p-value of less than 0.05. The software used was Graph Pad Prism 6.0.

The limits of this study are represented by its retrospective character, including the small number of patients.

RESULTS

A complete physical examination was required for assessing the clinical status of patients and the performance status was evaluated according to the ECOG scale. Most patients (61.7%) presented with good performance status (ECOG 0), 35.6% with ECOG 1, and 0.7% with ECOG 2.

The study group consisted of 101 consecutive adults (>18 years of age) MHD patients who were admitted with a SARS-CoV-2 RT-PCR positive test, out of which 42 (48.5%) had a diagnosis of diabetic kidney disease (DKD), and 59 (51.5%) have been known with other etiologies of CKD, predominantly represented by hypertensive nephropathy (33.89%), chronic obstructive tubulointerstitial nephropathy (11.86%), and autosomal dominant polycystic kidney disease (8.47%).

For the study population, demographic and clinical characteristics are presented in Table 1.

In the T2DM group, the median age was 65.69±31.87 years (CI 95%) and the majority of the patients were men (66.6%). More than 50% of patients were obese and the most common comorbidities were represented by hypertension (100%), chronic coronary heart disease (61.9%), atherosclerosis (61.9%), followed by pulmonary disease (11.9%), and active malignancies (7.1%). In the T2DM study population, 42.8% of patients required noninvasive oxygen therapy at admission, out of which 11.1% needed positive pressure ventilation in evolution, while 11.9% needed positive pressure ventilation from admission and 23.8% required mechanical ventilation. In the T2DM group, hemorrhagic complications were observed in 16.6% of patients. We identified thrombosis in 4.76% of patients (arteriovenous fistula thrombosis, lower limb deep vein thrombosis, and splenic infarction). Sepsis was identified in 30.9% of the cases and one patient developed Clostridioides difficile colitis. The recorded mortality rate for the T2-DM patients was 42.5%, out of which 72.2% of the deaths were due to sepsis (Figure 1).

In the non-diabetes study group, the median age was 64.33 ±3.08 years (CI 95%), the majority being men (64.4%). The obesity rates were lower (30.5%) than those in the T2DM group and 89.8% were hypertensive. Chronic coronary heart disease (47.4%) and atherosclerosis (40.6%) were observed at lower rates, with higher rates of chronic pulmonary diseases (18.6%) and active malignancies (20.3%).

Regarding respiratory insufficiency management, we observed that when compared with T2DM patients, 49.1% of non-DM required non-invasive oxygen therapy at admission, with a statistically significant difference, but the rates for assisted ventilation (positive pressure ventilation (PPV) and mechanical ventilation (MV) were lower, 10.1% and 18.6%, respectively.

Table 1: Demographic and clinical characteristics and the significant differences

	Overall	T2DMgroup	Non-DM group	P value
Patient number (N)	101	42	59	
Demographic data				
Age (mean, years)	64.901 ±2.241	65.69 ±31.87	64.339 ±3.08	0.4111
Gender (%)				
Female (N, %)	35	14 (33%)	21 (35.6%)	0.6573
Male (N, %)	66	28 (67%)	38 (64.4%)	0.2390
Risk factors				
Obesity (N, %)	45	27 (64.2%)	18 (30.5%)	0.5796
Atherosclerosis (N, %)	50	26 (61.9%)	24 (40.6%)	0.3712
Comorbidities				
Hypertension (N, %)	95	42 (100%)	53 (89.8%)	0.8127
Ischemic heart disease (N, %)	54	26 (61.9%)	28 (47.4%)	0.2450
History of neoplasm (N, %)	15	3 (7.14%)	12 (20.3%)	0.7672
Pulmonary disease (N, %)	16	5 (11.9%)	11 (18.6%)	0.7732
Supplemental oxygen				
Nasal cannula (N, %)	47	18 (42.8%)	29 (49.1%)	0.0440
PPV (N, %)	13	7 (16.6%)	6 (10.1%)	0.7854
MV (N, %)	21	10 (23.8%)	11 (18.6%)	0.7310
Complications				
Hemorrhagic (N, %)	24	7 (16.6%)	17 (28.8%)	0.6509
Thrombosis (N, %)	4	2 (4.7%)	2 (3.3%)	0.8230
Infection (N, %)	23	13 (30.9%)	10 (25.4%)	0.7258
Laboratory testing				
Hemoglobin <12 g/dL (N,%)	85	34 (80.9%)	51 (86.4%)	0.0173
WBC > 11 x 10^3/mm^3 (N, %)	21	11 (26.9%)	10 (16.9%)	0.0001
CRP > 5 ng/mL (N, %)	98	40 (95.2%)	58 (98.3%)	0.0001
Procalcitonin >0,05 ng/mL (N, %)	101	42 (100%)	59 (100%)	0.0001
Interleukin-6 > 7 pg/mL (N, %)	98	41 (97.6%)	57 (93.2%)	0.0001
Mortality (N, %)	39	19 (45.2%)	20 (33.8%)	0.5685

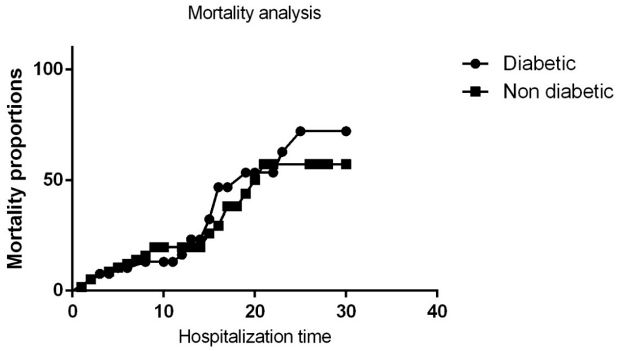

Figure 1: Mortality analysis between diabetic and non-diabetic patients

Hemorrhagic complications were observed in 28.8% of patients in the non-DM group, more frequently with soft tissue hematoma and only two cases of thrombosis. Infectious complications were observed in 25.4% of the non-DM patients (66.6% represented by sepsis and 33.3% by Clostridioides difficile colitis). The mortality rates for the non-DM group were lower (32.2%) when compared to the T2DM group.

We analyzed biological parameters in both groups. In the

T2DM group, 80.9% of patients had anemia (Hgb<12 g/dL), with a higher proportion in the non-DM group, accounting for 86.4%. Inflammatory status was assessed by leukocyte count, lymphocyte count, C-Reactive Protein level, procalcitonin, and Interleukin-6 (IL-6) levels. Leukocytosis (>11.000/mm^3) was present at admission in 26.9% of cases, while lymphopenia in 61.9% of cases.

C- Reactive Protein, procalcitonin, and IL-6 levels were increased at admission in almost all T2DM patients namely 95.2%, 100%, and 97.6% respectively. The non-DM-group also accounted for high inflammatory parameters, leukocytosis in 16.9%, C-Reactive Protein >5 mg/L in 98.3%, procalcitonin >0,05 ng/mL in 100%, and IL-6 >7 pg/mL in 93.2%. We found statistically significant differences when comparing the two groups ($p<0.001$). Hypoalbuminemia (serum albumin level <3.4 mg/dL) was noted in higher rates in the non-DM group (27.1%) as compared with T2DM patients (23.8%), with statistically significant differences (Table 1).

DISCUSSION

Diabetes is a major cause of chronic kidney disease, accounting for more than 50% of MHD patients all around the world, exceeding traditional kidney diseases [11,12]. Early COVID-19 reports mentioned DM among the most important risk factors for severe disease and increased mortality due to SARS-CoV-2 infection. The majority of COVID-19 reports stated that CKD is independently a risk factor for poor outcomes, with adjusted mortality in CKD and HD patients being described to be 2 to 3 times higher [4].

All of the 42 T2DM patients were known with hypertension. It is well known the equipotent relationship between CKD and hypertension: hypertension can cause CKD and CKD is a risk factor for hypertension. Also, DM and hypertension frequently occur together, with a substantial overlap between DM and hypertension in disease mechanisms. In the non-DM group, 89.8% were hypertensive, with lower rates when compared with the T2DM group, approaching the literature data that DM and CKD cause high blood pressure with poor control [13], and with a high mortality risk in SARS-CoV-2 infection [14,15].

Obesity rates were higher in the T2DM group, more than 50% vs 30.5%. In the first meta-analysis of obese patients and COVID-19, was stated that higher body mass index was associated with intensive care unit admission and critical disease [16], by decreasing expiratory reserve volume, diaphragm excursion, and restricting ventilation.

In DM patients, the risk of fatal cardiovascular events is much higher compared to the general population. A close link exists between diabetes and cardiovascular disease which is the most prevalent cause of morbidity and mortality in diabetic patients [17]. In the presented study, coronary heart disease was more frequently observed in T2DM patients as compared to non-DM patients. Also, atherosclerosis was seen with higher prevalence in the DM group, approaching the literature and the current evidence that diabetes dramatically accelerates atherosclerosis [18], all of this constellation increasing the COVID-19 mortality [19].

Some malignancies are shown to increase the risk of kidney failure [20,21,22] In our study, patients with active malignancies were in a higher proportion in the non-DM group.

Anemia is more commonly associated with diabetic kidney disease [23]. When comparing the two study groups, we found that anemia is a risk factor for poor outcomes in MHD T2DM patients.

In general, infectious diseases are more frequent and severe in patients with DM, due to the chronic hyperglycemic status that promotes immune dysfunction [24]. Studied T2DM patients presented with significantly altered inflammation markers compared to the non-DM group. Hypoalbuminemia has been observed in the T2DM group, to a lesser extent compared to non-DM, but the differences were statistically significant. T2DM status and impaired immune system are risk factors for poor outcomes in SARS-CoV-2 infected patients. The mortality rates were higher in the T2DM group, although the differences were not statistically significant.

CONCLUSION

In conclusion, we found that anemia is more severe in COVID-19 MHD T2DM patients. The severity of anemia in the T2DM group was consistent with significantly altered inflammatory markers. New biomarkers and studies are needed for better anemia management and more specific biomarkers that correlate the inflammatory status with the infectious disease.

We aim for a future in which we have as many studies as possible that include hemodialysis patients, a special category of patients due to the high morbidity and mortality associated with it, and also due to the important social impact.

The patient`s data used to support the findings of this study is available from the corresponding author upon request.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.
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Authors' contribution
Conceptualization: IAV, LFF; Methodology: IAV, LFF, EC and AEBS; Software: IAV and LFF; Validation: IAV, ALC. and DR; Formal analysis: IAV, EC, LR and CD; Investigation: EC, LFF and AEBS; Resources: IAV, DR and CD; Data curation: AEBS, ALC, MTG, SI and LR; Writing—original draft preparation, IAV and LFF; Writing—

review and editing, IAV, LFF, EC, and ALC; Visualization, CD, LR, MTG, SI, DR; Supervision EC, ALC; Project administration IAV, LFF

All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Descriptive Analysis of Internal Registry Data on Cemented and Uncemented Total Hip Arthroplasty

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Abstract: *Background: As total hip replacement cases increase year after year, registry-wide data becomes increasingly important in order to assess pathology distribution, reimbursement efficiency as well as physician performance. In order to assess all these factors patient evolution needs to be documented after surgery as well, but the main impediment to doing this is losing patients to follow-up. For this reason, the present study aims to perform a descriptive analysis of the hospital's internal registry data as well as to determine what percentage of patients can be expected to return to follow-up after total hip replacement. Methods: A query of the hospital's internal registry data in the last years was performed. Patient demographics, county distribution as well as ICD-10 code usage were documented. Data standardization was performed to determine the probability and cumulative distribution of the patient's age. The chi2 test of independence was used to assess whether there is a correlation between the patient's gender and ICD-10 codes. Pot hoc power analysis was performed to assess the accuracy of correlation analysis. Results: Only 29.6% of the patients undergoing total hip replacement are from the county within which the hospital is located. 25% of the patients were under 60 years old. Conclusions: Current registry data reveals that most patients undergoing total hip replacement are around 67.5 ± 11 years old, suffer from unilateral or bilateral primary or secondary hip osteoarthritis and only 29.6% of them are likely to return to follow-up.*

Keywords: *total hip replacement; ICD-10; registry; statistical analysis*

INTRODUCTION

Over 1 million total hip arthroplasty (THA) surgeries have been performed globally in the last year and this number is rising for both primary and revision procedures [1]. In the United States, the rate of primary total hip arthroplasties increased by 50%, from 47 per 100 000 people to 69 per 100 000 people, with a market size of nearly 2.30 billion USD [2]. With over 305 operations per 100,000 residents, Switzerland continues to lead the list of countries in Europe with the highest hip replacement rate, according to a 2016 report from the World Organization for Cooperation and Regional Development (OECD) [3].

In Romania, the annual rate of primary hip replacement has climbed dramatically during the previous 15 years, 51.8 out of every 100,000 people underwent a main hip replacement procedure in 2015, whereas 2.7 out of every 100,000 people required a hip revision surgery [4,5].

The demand for hip implants is expected to increase as a result of adequate health insurance coverage and reimbursement laws for joint replacement surgeries [6].

In our country, THAs were performed mostly on patients between 60 and 69 years old. Male patients are more prevalent in the 50-59 age range and younger age groups, while female patients are more likely to have THAs after the age of 59. With a total of 29,497 female patients and 19,675 male patients, women over the age of 59 make up the clear majority [5].

Although THA has demonstrated outstanding short and long-term results, the revision rate has remained constant over the past few decades despite improvements in surgical technique and implant design [7,8].

In the US the most common causes for re-intervention were: instability (22%), mechanical loosening (20%), infection (15%), implant failure (10%), osteolysis (7%), and periprosthetic fracture (6%) [9,10]. In Romania, the most common reasons for revision surgery are acetabular defects (mostly acetabular loosening and osteolysis), femoral defects, and other (un-standardized reasons for revision in 20%-25% of the cases), followed by were, luxation, infection, periprosthetic fracture and broken implants [11].

It has been established that primary surgeries use fewer financial resources than revision surgeries [12]. In order to maintain the sustainability of their finances, institutions must comprehend the costs and durability of these interventions [13]. Implants cost to make up a significant portion of the entire hospitalization bill (for revision surgery especially) and are a key area for cost-cutting overall which demands proper financial planning [14].

There is a wide range in reimbursements for both primary and revision surgeries, possibly in part because of the diagnosis-related group (DRG), intrinsic unpredictability, and complexity of these procedures with a rising need to reconcile the clinical challenges with the available technical stock [6,13]. That is why we consider that analyzing and supporting the cost-effectiveness of surveillance measures is a necessity. Orthopedic specialists generally concur that some follow-up after THA is important [11]. Nowadays there are no clear standardized instructions regarding the timing of the subsequent visits following hip replacement interventions [15]. The identification of asymptomatic patients who need revision surgery remains one of the primary justifications for early and late routine follow-ups [16].

There are only five recommended protocols for THA follow-up visits that are well described, according to a systematic review of the literature and the content of orthopedic associations' websites, with a lot of variation in the suggested follow-up frequency and duration [17]. The latter is simply mentioning the necessity of routine follow-up visits without defining their timing, duration, or their cost-effectiveness [18,19].

According to earlier research, patients who don't follow up with their surgeon regularly are more likely to experience worse outcomes and require additional surgical intervention at a different location [18,20]. Regardless of its importance, postoperative evaluation places a heavy load and financial burden on patients, families, and tertiary healthcare systems which have to manage a rising number of new patients [21,22].

This study's goal was to assess patient compliance with clinical follow-up in a line of patients who underwent hip surgery in different surgical centers in our country. In addition, we aim to analyze the positive and negative predictors for clinical follow-up compliance.

MATERIALS AND METHODS

Prior to the study's inception, ethical approval was sought and received. The aim of the study was to investigate the hospital's internal hip arthroplasty registry between 2018 and 2022 in order to determine patient gender and age distribution as well as how many patients would be likely to return to follow-up after surgery. An arbitrary distance boundary was set to only include the county in which the hospital is situated (B, Bucharest County). A descriptive analysis of ICD-10 codes frequency and gender distribution was also generated. Patients undergoing cemented and uncemented total hip arthroplasty were eligible for inclusion.

In order to plot an age frequency distribution, z-scores were generated and from them, the frequency distribution was plotted in Microsoft Excel.

A chi2 test of independence was performed to assess whether there is a gender propensity for any ICD-10 scores. Posthoc power analysis was also conducted to judge the appropriateness of the chi2 test results.

Further stratification based on relevant age groups has been performed in order to identify diagnostic patterns. Relevant age groups were constructed as follows: "Under 30", "30-39", "40-49", "50-59", and "60 and above". A histogram was used to present diagnostic patterns in each age group.

Data gathering, analysis, and storage were performed using SPSS v.26, Microsoft Excel, and Python programming language v.2.7.

RESULTS

After ethical approval, 929 patients were detected within the specified period. Patient age and gender distribution are presented in Table 1. Figure 1 presents the patient's distribution as a function of the county with 29.6% of the patients undergoing cemented or uncemented hip arthroplasty coming from the county in which the hospital is located, Bucharest. Figure 2 and Table 1 show that most often, patients 67.5 years or older undergo total hip arthroplasty. At the same time, a significant proportion of the included patients are under the age of 60, 25%. ICD-10 codes M16.1 "Unilateral primary osteoarthritis of the hip" and M16.7 "Other unilateral secondary osteoarthritis of the hip" were the most used, accounting for almost 45% of cases. ICD-10 codes M16.6 "Other bilateral secondary osteoarthritis of the hip" and M16.0 "Bilateral primary osteoarthritis of the hip" were the second most used codes accounting for 22.4% of cases.

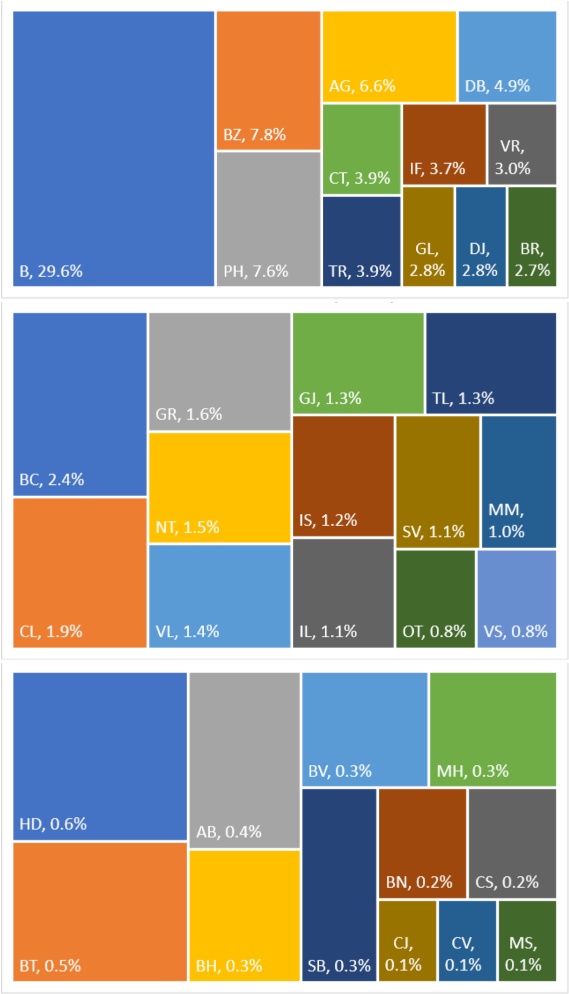

Figure 1: Visual representation of patient distribution by county. The figure is divided into three components for ease of interpretation. Each county name was abbreviated according to its national code

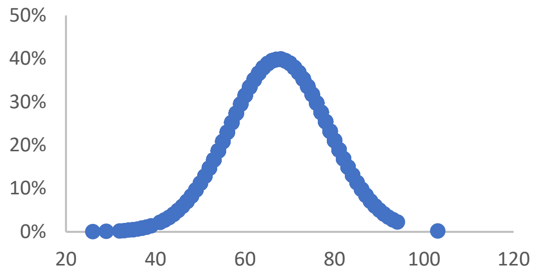

Figure 2: Patient age-standardized probability distribution

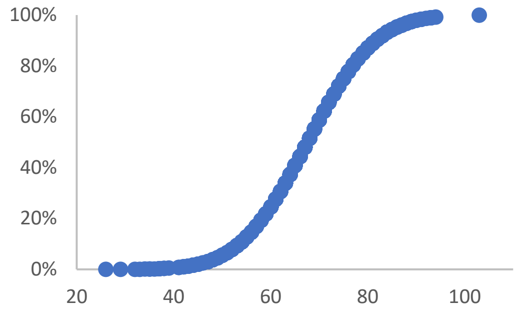

Figure 3: Patient age-standardized cumulative distribution

Figure 5 presents a descriptive analysis of each used ICD-10 code as a function of gender distribution. The chi2 test of independence has not identified any significant correlations between gender and any ICD-10 code. However, post hoc power analysis has shown that the sample size in each subgroup was insufficient to detect a meaningful effect. For this reason, we cannot draw any meaningful conclusions from the subgroup analysis. Age group diagnostic patterns reveal that the most often encountered patients are the ones at 60 years or above followed by the ones within the 50 to 59 age group. Diagnostic patterns do not reveal any significant differences between groups, M16.1 being the most often encountered diagnostic code as depicted in Figure 6.

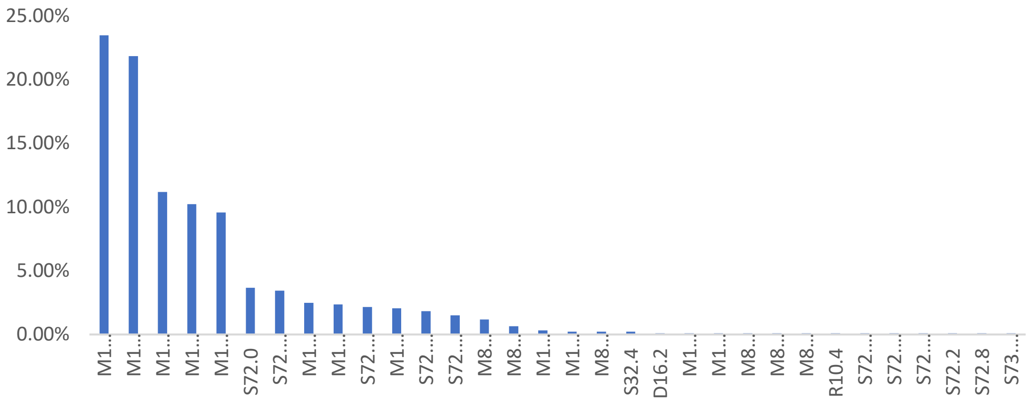

Figure 4: Frequency distribution of ICD-10 diagnostic codes used for patients undergoing cemented or uncemented total hip arthroplasty

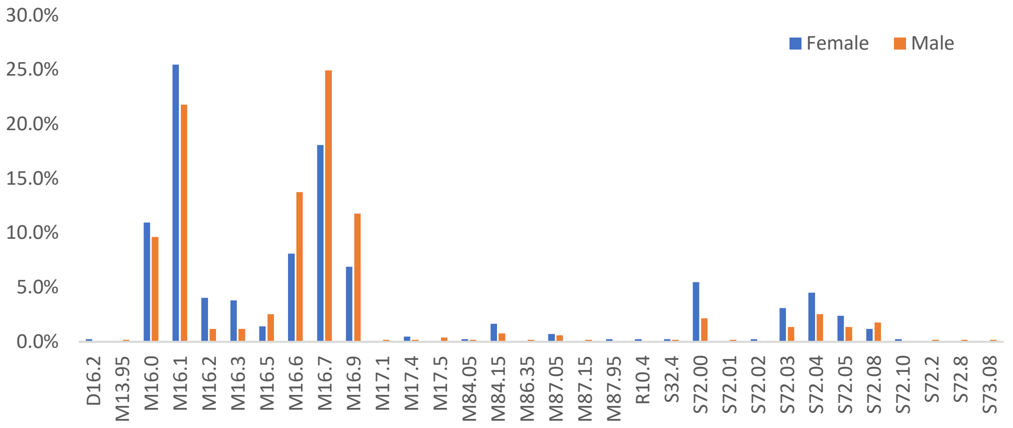

Figure 5: Frequency distribution of ICD-10 diagnostic codes as functions of patient gender

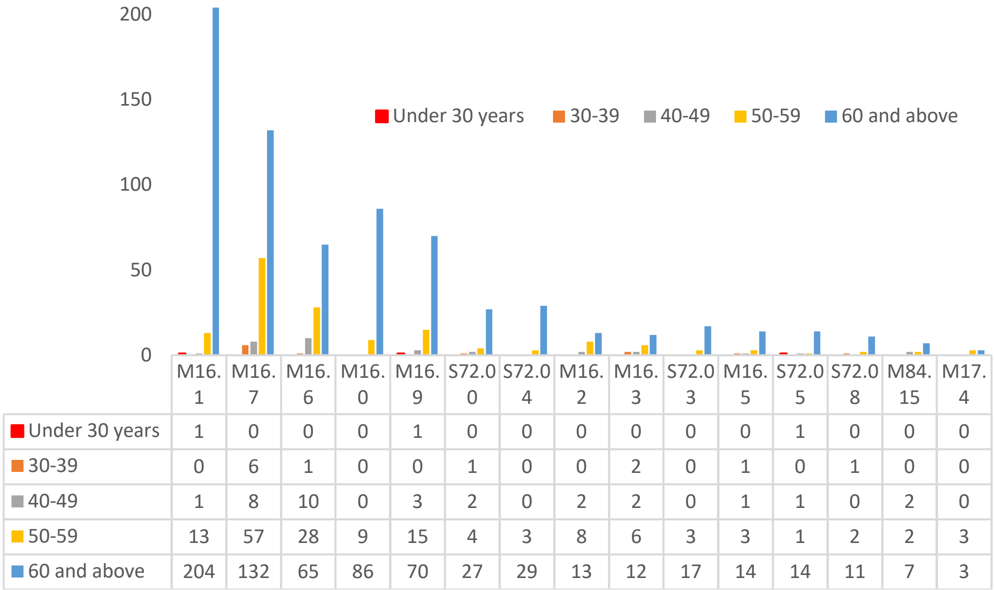

	M16.1	M16.7	M16.6	M16.0	M16.9	S72.00	S72.04	M16.2	M16.3	S72.03	M16.5	S72.05	S72.08	M84.15	M17.4
🟥 Under 30 years	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0
🟧 30-39	0	6	1	0	0	1	0	0	2	0	1	0	1	0	0
■ 40-49	1	8	10	0	3	2	0	2	2	0	1	1	0	2	0
🟨 50-59	13	57	28	9	15	4	3	8	6	3	3	1	2	2	3
🟦 60 and above	204	132	65	86	70	27	29	13	12	17	14	14	11	7	3

Figure 6: A brief descriptive analysis of ICD-10 code distribution in the 5 main patient age groups. Furthermore, a data table is provided with the main 15 ICD-10 codes for data transparency

Table 1: Patient demographics presenting gender frequency and age mean and standard deviation (SD).

	F, %	**M, %**
Gender	420, 45%	509, 55%
	Mean	**SD**
Age	67.5	11

DISCUSSION

The results presented indicate that patient follow-up poses significant challenges, as only 29.6% of the patients included were deemed suitable for returning to the surgical center for follow-up. In addition, it is anticipated that these patients will experience the typical follow-up loss rate of 10-20%, regardless of their proximity to the surgical center. The significance of this report lies in its importance for upcoming prospective studies at our hospital, which will enable the precise evaluation of implant durability and patient-reported outcome measures. It is crucial to monitor adverse events during follow-up, as changes in patient-reported outcomes may indicate the likelihood of such events occurring [23].

Tertiary hospitals often face a common issue where patients from various regions across the country are referred to them, making it challenging to monitor their symptoms and track their progress over an extended period. In light of this, remote reporting could serve as a viable solution for these hospitals to maintain contact with their patients and minimize follow-up loss rates, resulting in higher patient reporting rates during prospective studies [24,25].

The age probability and cumulative distributions provided in the report are extremely beneficial for the orthopedics department and healthcare providers, as they enable them to determine when patients are likely to require hip replacement surgery. Simultaneously, these distributions enable all concerned parties to anticipate future budget allocations based on patient age, as older patients are more susceptible to degenerative hip conditions, whereas younger patients are more prone to developmental hip diseases such as dysplasia. As a result, budget allocation can be optimized through informed decision-making, allowing for the advance procurement of specific resources to serve patients promptly after their initial consultation [26,27].

By implementing ICD-10 code reporting and rigorous oversight over its reporting, additional budget optimization can be achieved by providing decision-makers with information on which implants are likely to be required at each hospital [28,29]. Present results highlight the fact that in 45% of cases patients either suffer from “Unilateral primary osteoarthritis of the hip” or “Other unilateral secondary osteoarthritis of the hip”. Patients suffering from primary unilateral osteoarthritis who are 67 years or older most likely require standard uncemented total hip replacements for this reason at least 23.4% (Figure 4) of cases will require standard total hip implants. At the same time, patients suffering from unilateral secondary osteoarthritis most likely developed osteoarthritis secondary to aseptic osteonecrosis of the femoral head [30] while a smaller proportion of younger patients are expected to develop osteoarthritis secondary to acetabular or proximal femoral deformities like acetabular retroversion, excessive femoral anteversion with ace-tabular under coverage, CAM, pincer or mixed femoral-acetabular impingement (FAI) [31–33]. This kind of patient often requires specialized implants in order to accurately reconstruct hip offset and anatomical center of rotation or when associated with the need for femoral derotation or shortening osteotomy [34]. Furthermore, the following 22.4% of cases comprised of “Other bilateral secondary osteoarthritis of the hip”, and “Bilateral primary osteoarthritis of the hip”, signal an important need for sequential or single-step bilateral total hip replacement. Each option has to be considered against its risks. Sequential bilateral hip replacements can be done through any approach and are more suited for patients who do not have a reliable support network and for whom the disease process is not as debilitating [35,36]. Innovative anterior-based hip approaches have made single-step total hip replacement possible, enabling patients to be placed in a supine position while preserving their abductors and external hip rotators. As a result, rehabilitation can be easily carried out, even in patients with bilateral implants [35,36].

Unfortunately, the current employed ICD-10 codes do not allow for a more in-depth analysis of causative relations leading to hip degenerative disease. The prevalent problem is that "secondary" causes of hip osteoarthritis involve intricate clinical conditions that involve spinopelvic interactions, pelvic and femoral orientation, and their interrelationship. Thus, labeling a case as "secondary" hip osteoarthritis alone does not furnish healthcare providers and practitioners with sufficient information to formulate predictable models to attain economic and treatment efficiency [37].

The report emphasizes the significance of registry data while underscoring the current inadequacies in reporting standards that impede advanced clinical and economic planning. These inadequacies underscore the necessity for clinicians and healthcare providers to actively engage with technological advancements, particularly with respect to patient reporting standards and stringent follow-up [24,38–41].

Future research efforts should concentrate on enhancing ICD-10 reporting standards, devising innovative methods for maintaining contact with patients after an intervention, particularly in tertiary hospitals, and furnishing healthcare providers with reliable data that can facilitate efficient planning and procurement, ensuring the utmost standard of healthcare for patients.

CONCLUSION

Registry data provides valuable information to healthcare providers and is a key source of data for healthcare improvements. Healthcare practitioners will most of the time encounter patients 67 ± 11 years old most likely suffering from either unilateral primary or secondary osteoarthritis. Based on the available data surgeons could expect that standard total hip implants will most often than not meet patient needs and be sufficient to reconstruct the native hip anatomy.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

This research received no external funding.

Authors’ contribution

Conceptualization, A.G.M.; methodology, A.G.M.; software, M.D.; validation, M.D., A.G.M., and L.T.M.; formal analysis, A.G.M.; investigation, A.G.M; data curation, A.S.; writing—original draft preparation, A.G.M.; writing—review and editing, A.G.M., L.T.M., A.S., M.D.; visualization, A.G.M., M.D.; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Prior to the study’s inception, a research protocol was submitted to and approved by the hospital’s ethics committee, registration number 521/05.05.2022.

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ORIGINAL ARTICLE

Multipurpose Hall for CBRNe Incidents

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Abstract: *A multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes plays a vital role in enhancing preparedness, response, and recovery capabilities in the face of CBRN incidents. A multipurpose hall for CBRN purposes provides a dedicated and specialized environment to support CBRN preparedness, response, and recovery efforts. It enhances coordination, facilitates training and exercises, ensures proper equipment storage, and fosters collaboration, ultimately strengthening the ability to protect personnel, mitigate risks, and minimize the impact of CBRN incidents. This study is qualitative research based on the content analysis method. To design and build multipurpose halls for CBRN events, this study can be used as a checklist. The purpose of this study is to collect important factors and sub-factors in the design, construction, and equipping of buildings and multi-purpose CBRN structures.*

Keywords: *Multipurpose Hall, CBRNe, Disaster, Emergency*

INTRODUCTION

A multi-purpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes plays an important role in handling CBRN incidents and supporting preparedness, response, and recovery efforts. Among the important reasons for the design and construction of such structures can be mentioned the centralization of operations, which specifies a multi-purpose hall provides a centralized place for CBRN operations, and the various resources, equipment, and personnel involved in the preparation, bring together CBRN response and recovery. It acts as a command center where decision-makers can effectively coordinate and manage activities and ensure a coordinated and effective response. In addition to training, the multi-purpose hall can serve as a dedicated place for CBRN training and exercises [1-4]. It provides a controlled environment where personnel can simulate and practice response procedures, test equipment and enhance their skills in handling CBRN incidents. Training and exercises conducted in the multi-purpose hall help ensure readiness and improve responsiveness. It can also be for proper storage, organization, and maintenance of CBRN equipment and supplies, which includes specialized protective equipment, detection and monitoring devices, disinfection equipment, and other essential tools used in CBRN incidents [5-7]. Having a designated place for equipment storage ensures quick access and proper care, minimizes response time, and optimizes equipment performance. Multipurpose halls for CBRN are equipped with decontamination facilities, including showers, washing stations, and appropriate disinfectant solutions. These facilities are critical for the rapid decontamination of people, equipment, and materials exposed to hazardous substances. Effective decontamination helps protect personnel, prevent cross-contamination, and maintain operational readiness. The multipurpose hall serves as a hub for communication and information sharing during CBRN incidents. It incorporates communication systems, command centers, and information management tools to facilitate real-time communication, coordination, and data exchange between response teams, external agencies, and other stakeholders. The important ones mentioned can support research and development efforts related to defense and CBRN technologies. They provide a controlled environment for testing and evaluating new equipment, protocols, and techniques for CBRN preparedness and response [8-10]. Research conducted at this center helps advance knowledge and improve CBRN capabilities. Also, its importance is in facilitating cooperation between different organizations involved in CBRN operations, where it brings together experts from different disciplines including emergency management, public health, law enforcement, and fire services, strengthening partnerships and increasing inter-agency coordination. This joint approach improves the effectiveness of response efforts and ensures a comprehensive and integrated response to CBRN incidents. Having a dedicated multi-purpose hall for CBRN operations demonstrates a commitment to public safety and preparedness. It instills confidence in the community by demonstrating the ability to effectively respond to CBRN incidents. The facility serves as a visible symbol of preparedness and preparedness, assuring the public that measures are in place to mitigate the risks associated with CBRN events [11-13].

Multipurpose halls are designed to accommodate a wide range of CBRN-related activities and can adapt to evolving needs and emerging threats. The versatility of the facility enables the integration of new technologies, the implementation of innovative approaches, and the ability to address a wide range of CBRN scenarios, ensuring the facility remains relevant and effective over time. The multipurpose hall provides a controlled environment for CBRN activities. It may have specialized ventilation and air filtration systems to prevent the release of hazardous agents, ensure personnel safety, and limit the impact of CBRN incidents. Facility design and infrastructure help contain CBRN materials and maintain a safe environment for operations [14-17].

MATERIALS AND METHODS

This research is qualitative research based on the content analysis method with a deductive and guided approach. A multipurpose hall for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes would need to be designed to accommodate various activities related to CBRN defense, response, and training. By studying and reviewing some sources including books, articles, and documents, some considerations for the design of such facilities were collected and reviewed as follows:

1. Location

The hall should be situated in a secure area away from densely populated areas, with easy access for emergency vehicles and personnel. The location of a multipurpose hall for CBRN (Chemical, Biological, Radiological, and Nuclear) activities is crucial and carries significant importance [18-20].

1.1. Safety and Security: The primary concern when dealing with CBRN hazards is the safety and security of personnel and the surrounding environment. Choosing an appropriate location away from densely populated areas helps minimize the risk of exposing a large number of people to potential CBRN incidents. It allows for better control, containment, and response to emergencies, reducing the potential for casualties and damage.

1.2. Proximity to Response Resources: The location of the hall should take into account its proximity to essential response resources, such as hospitals, emergency services, and specialized CBRN response teams. Being located within a reasonable distance of these resources enables faster and more efficient emergency response and medical assistance in case of an incident.

1.3. Access for Emergency Vehicles and Personnel: The hall should have suitable access points that allow easy entry and exit for emergency vehicles, such as ambulances and fire trucks. It should also provide convenient access for response personnel, enabling rapid deployment during emergencies. The location should consider factors like road infrastructure, transportation routes, and proximity to major highways or airports for logistical support.

1.4. Environmental Considerations: The location should take into account environmental factors that may affect the effectiveness of CBRN response operations. For instance, prevailing wind patterns, geographical features, and nearby bodies of water should be considered to assess the potential dispersion patterns of CBRN agents. This information helps in determining the best location to minimize the impact and spread of hazardous substances.

1.5. Zoning and Legal Requirements: Different jurisdictions may have specific zoning regulations and legal requirements for facilities handling CBRN materials. The location should comply with these regulations to ensure the facility's legality and appropriate adherence to safety protocols. Zoning restrictions may dictate areas suitable for CBRN-related activities, ensuring compatibility with neighboring properties and land use.

1.6. Training and Collaboration Opportunities: The location can also provide opportunities for collaboration and training with nearby CBRN response organizations, research institutions, or military installations. Proximity to such entities facilitates knowledge sharing, joint exercises, and the exchange of expertise, enhancing overall preparedness and response capabilities.

2. Structural Integrity

The building should be constructed to withstand potential CBRN incidents, including blasts and chemical or radiological hazards. Reinforced concrete walls and ceilings, as well as blast-resistant windows and doors, may be necessary. When designing a multipurpose hall for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes, ensuring the structural integrity of the facility is of utmost importance [21-24].

2.1. Robust Construction: The hall should be constructed using robust materials and techniques to withstand potential CBRN incidents. Reinforced concrete or other sturdy materials are commonly used for walls, floors, and ceilings. These materials provide strength and resistance to impacts, blasts, and structural damage.

2.2. Blast Resistance: The structure should be designed to resist the effects of explosions that may occur during CBRN incidents. This involves incorporating blast-resistant features, such as reinforced walls, windows, and doors. The design should consider blast loadings, shockwaves, and fragments, and ensure that the structure can withstand these forces without compromising its integrity.

2.3. Containment and Isolation: The hall should have the ability to contain and isolate CBRN hazards within designated areas. This can be achieved by using airtight seals, robust partitions, and barriers to prevent the spread of contaminants. The facility should also have separate zones to minimize cross-contamination and allow for efficient isolation of affected areas.

2.4. Protection from External Hazards: The design should consider potential external hazards that can impact the structural integrity, such as extreme weather events (e.g., hurricanes, earthquakes) or intentional attacks. Adequate measures, such as reinforced roofs, impact-resistant windows, and structural reinforcements, should be implemented to protect the facility from these external threats.

2.5. Structural Sealing: To prevent the ingress of CBRN agents, the hall should have a well-sealed structure. This includes ensuring tight seals around windows, doors, ventilation systems, and other openings. High-quality seals and gaskets should be used to maintain airtight integrity and prevent the infiltration of hazardous substances.

2.6. Access Points: The design should include controlled access points with appropriate security measures. These access points should be designed to withstand potential attacks or unauthorized entry attempts. Secure doors, locks, and surveillance systems should be employed to maintain the integrity of the facility.

2.7. Reinforced Utilities: Critical utilities, such as electrical systems, water supply, and HVAC (Heating, Ventilation, and Air Conditioning) systems, should be designed and positioned to withstand CBRN incidents. They may require additional reinforcement or protective measures to ensure continued operation during emergencies.

2.8. Structural Monitoring: Implementing structural monitoring systems can help detect and assess any potential structural vulnerabilities or damage. Structural sensors, such as strain gauges or vibration sensors, can be installed to monitor the health of the building and provide early warning of any compromised areas.

2.9. Compliance with Standards: The design should adhere to relevant building codes, safety regulations, and industry standards related to CBRN facilities. These standards ensure that the structural integrity meets specified requirements and guidelines for CBRN defense and response.

3. Ventilation and Filtration

The hall should have a robust ventilation system capable of providing a controlled airflow to prevent the spread of contaminants. High-efficiency air filtration systems, including HEPA filters, should be installed to remove CBRN agents from the air. Ventilation and filtration systems are critical components of a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. They play a vital role in maintaining a safe and controlled environment by minimizing the spread of CBRN agents. [25-29].

3.1. Controlled Airflow: The hall should have a well-designed ventilation system capable of providing controlled airflow. This ensures that fresh air is supplied to occupied areas while maintaining appropriate pressure differentials to prevent the migration of CBRN agents to unaffected areas. The ventilation system should be designed with adjustable airflow rates to accommodate different scenarios and response requirements.

3.2. Air Filtration Systems: High-efficiency air filtration systems, including High-Efficiency Particulate Air (HEPA) filters, should be an integral part of the ventilation system. HEPA filters are highly effective in removing particulates, including biological agents, radioactive particles, and many chemical contaminants, from the air. These filters should be properly sized, installed, and maintained to ensure optimal filtration efficiency.

3.3. Filtration System Redundancy: Redundancy is crucial in CBRN facilities to ensure the continuous operation of filtration systems. The inclusion of backup or redundant filtration units and provisions for rapid filter replacement or change-out are important considerations. This helps maintain filtration performance in the event of filter saturation or system malfunctions.

3.4. Air Pressurization: In CBRN scenarios, it may be necessary to create areas with positive or negative air pressure to prevent the migration of CBRN agents. Positive pressure areas ensure that air flows out of the controlled area, reducing the risk of contaminants entering. Negative pressure areas draw air into the controlled area, preventing the release of hazardous agents to the outside environment. The ventilation system should be capable of maintaining the desired pressure differentials between different zones within the facility.

3.5. Isolation and Containment: The ventilation system should support isolation and containment measures during CBRN incidents. This can include the ability to seal off affected areas, establish differential pressure zones, and control airflow between different sections of the hall. These features help contain and prevent the spread of CBRN agents to unaffected areas.

3.6. Air Monitoring and Detection: Integrating air monitoring and detection systems into the ventilation system allows for continuous monitoring of air quality and the presence of CBRN agents. These systems can provide early warnings and trigger appropriate response actions if hazardous substances are detected. Air monitoring devices, such as gas detectors or biological sensors, should be strategically placed throughout the facility.

3.7. Maintenance and Monitoring: Regular maintenance, testing, and inspection of the ventilation and filtration systems are crucial to ensure their continued effectiveness. Filters should be replaced or cleaned at recommended intervals, and the functionality of the ventilation system, including airflow rates, pressure differentials, and alarms, should be regularly tested and verified.

3.8. Emergency Shutdown Procedures: The ventilation system should have emergency shutdown procedures in place to quickly stop airflow in case of emergencies or incidents. These shutdown procedures should be easily accessible and understood by authorized personnel to minimize the spread of CBRN agents during critical situations.

4. Decontamination Facilities

Dedicated decontamination areas should be included within the hall. These areas would typically have showers, washbasins, and appropriate decontamination solutions for individuals or equipment exposed to CBRN agents. Decontamination facilities are a crucial component of a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. These facilities are necessary for the safe and effective decontamination of individuals and equipment that may have been exposed to CBRN agents [30, 31].

4.1. Showers and Washbasins: Dedicated areas with showers and washbasins should be included in the decontamination facility. These areas allow individuals to thoroughly wash their bodies, hair, and exposed skin to remove any contaminants. The showers should have an adequate water supply and temperature control to ensure effective decontamination.

4.2. Decontamination Solutions: The decontamination facility should provide appropriate decontamination solutions or agents to neutralize or remove CBRN agents from the skin, clothing, and equipment. These solutions should be selected based on the specific CBRN agents anticipated and their effectiveness in decontamination. Guidelines for the proper use and disposal of these solutions should be clearly provided.

4.3. Separate Areas for Contaminated and Clean Zones: The decontamination facility should have separate areas or designated zones for contaminated and clean areas to prevent cross-contamination. Individuals and equipment should enter the facility through the contaminated zone and proceed to the clean zone after decontamination.

4.4. Personal Protective Equipment (PPE): Adequate storage space should be provided within the decontamination facility for storing personal protective equipment (PPE) used during the decontamination process. This includes items such as gloves, masks, goggles, and disposable suits. The storage area should be easily accessible and well-organized for quick retrieval of PPE.

4.5. Waste Management: Proper waste management procedures should be in place to handle and dispose of contaminated waste generated during the decontamination process. This may include designated waste collection containers, hazardous waste disposal protocols, and compliance with local regulations and guidelines for waste management.

4.6. Water Drainage and Containment: The decontamination facility should have appropriate water drainage and containment systems to prevent the spread of contaminants. This includes the installation of floor drains, impervious flooring, and adequate water collection and containment mechanisms to prevent the runoff of contaminated water into other areas.

4.7. Accessibility and Safety: The decontamination facility should be designed to be accessible to individuals with disabilities and consider their specific needs during the decontamination process. Additionally, safety measures such as slip-resistant flooring, proper lighting, and emergency communication systems should be in place to ensure the safety of individuals using the facility.

4.8. Training and Procedures: Clear operating procedures and training materials should be developed and provided to personnel using the decontamination facility. This includes instructions on proper decontamination techniques, the sequence of actions, handling of equipment, and safety protocols to ensure effective decontamination and minimize the risk of recontamination.

4.9. Maintenance and Inspection: Regular maintenance, inspection, and testing of decontamination facilities are essential to ensure their continued functionality and compliance with safety standards. This includes routine checks of water supply, showers, drainage systems, PPE storage, and decontamination solutions to verify their effectiveness and readiness for use.

5. Equipment Storage

Adequate storage space should be provided to house CBRN-related equipment, such as personal protective equipment (PPE), detection devices, decontamination supplies, and specialized response tools. Equipment storage is a critical aspect of a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. Proper storage facilities are essential for organizing and safeguarding CBRN-related equipment, ensuring readiness and quick access when needed [32-35].

5.1. Dedicated Storage Areas: Designate specific areas within the multipurpose hall for equipment storage. These areas should be easily accessible and organized, allowing for efficient retrieval and inventory management. Depending on the size and quantity of equipment, different storage options such as cabinets, shelves, lockers, or specialized storage racks may be utilized.

5.2. Equipment Categorization: Categorize CBRN equipment based on its type, purpose, and level of protection. This helps in organizing and locating items quickly during emergencies or training exercises. For example, respiratory protection equipment, protective suits, detection devices, decontamination supplies, and specialized response tools may each have their designated storage area or section.

5.3. Secure Storage: Implement appropriate security measures to ensure the integrity and protection of stored equipment. This may include restricted access to storage areas, lockable cabinets or containers, surveillance systems, and inventory control protocols. Access should be limited to authorized personnel only, minimizing the risk of theft, tampering, or unauthorized use.

5.4. Equipment Maintenance: Provide space and resources for equipment maintenance, cleaning, and inspection within the storage area. This includes workbenches, tools, cleaning agents, and dedicated areas for equipment servicing. Regular maintenance ensures that the equipment remains in good working condition and is ready for use when needed.

5.5. Proper Ventilation: Ensure adequate ventilation within the storage area to prevent the buildup of moisture, humidity, or other environmental factors that may damage the equipment. Proper airflow helps maintain the longevity and functionality of sensitive equipment, especially when stored for extended periods.

5.6. Labeling and Inventory Management: Clearly label storage areas and equipment to facilitate easy identification. Implement an inventory management system that tracks the location, condition, and usage of equipment. This enables efficient inventory control, timely replacement of expired or damaged items, and ensures readiness during CBRN incidents or training exercises.

5.7. Compliance with Safety Regulations: Ensure compliance with safety regulations and guidelines for the storage of CBRN-related equipment. These regulations may include specific requirements for hazardous materials, controlled substances, or sensitive equipment. Adhering to these regulations promotes safety, minimizes risks, and maintains legal compliance.

5.8. Training and Familiarization: Provide appropriate training and familiarization to personnel on the location, access procedures, and handling protocols for stored CBRN equipment. This ensures that authorized personnel are knowledgeable about the equipment, understand its usage, and can retrieve or store it safely.

5.9. Emergency Preparedness: Develop emergency response plans that outline procedures for accessing and utilizing stored CBRN equipment during emergencies. This includes provisions for quick retrieval, distribution, and deployment of equipment to relevant personnel or response teams.

5.10. Regular Audits and Inspections: Conduct regular audits and inspections of the equipment storage areas to ensure compliance, proper organization, and readiness. This involves checking expiration dates, conducting inventory reconciliation, and assessing the overall condition of the stored equipment.

6. Training and Simulation

The hall should offer versatile training capabilities, including simulation scenarios for CBRN incidents. This may involve the use of specialized training props, mock environments, and immersive simulation technologies. Training and simulation capabilities are vital components of a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. These features allow personnel to acquire the necessary knowledge, skills, and experience in a controlled environment to effectively respond to CBRN incidents [32, 36-41].

6.1. Training Areas: Designate specific areas within the multipurpose hall for training exercises. These areas should be versatile and adaptable to simulate different CBRN scenarios, such as mock incident sites, contaminated zones, or confined spaces. The layout and features of these areas can be modified to replicate real-life scenarios and provide a realistic training environment.

6.2. Simulation Tools: Incorporate various simulation tools and equipment to enhance training effectiveness. This may include smoke generators, simulated hazardous substances, training mannequins, or specialized props that mimic CBRN hazards. The use of these tools helps create realistic scenarios, enabling trainees to practice response procedures and decision-making skills.

6.3. Training Props and Equipment: Provide training props and equipment that replicate actual CBRN response gear, including personal protective equipment (PPE), detection devices, decontamination equipment, and specialized response tools. These props should closely resemble the functionality and characteristics of real equipment, allowing trainees to become familiar with their use and limitations.

6.4. Emergency Communication Systems: Install reliable emergency communication systems throughout the training areas to facilitate coordination and communication between trainees, instructors, and control personnel. These systems may include intercoms, radios, or other communication devices that mimic real-world communication protocols used during CBRN incidents.

6.5. Monitoring and Feedback: Incorporate monitoring systems to capture trainees' actions, responses, and performance during training exercises. This may involve video recording, sensors, or observation booths for instructors to assess trainee actions and provide feedback. Monitoring and feedback play a crucial role in identifying areas for improvement and enhancing trainee proficiency.

6.6. Training Scenarios and Drills: Develop a variety of realistic training scenarios and drills that cover different aspects of CBRN incidents, such as detection, containment, decontamination, and response protocols. These scenarios should be designed to challenge trainees' knowledge and skills progressively, allowing them to practice different response strategies and experience various levels of complexity.

6.7. Safety Protocols: Implement strict safety protocols during training exercises to minimize risks to trainees and instructors. This includes setting clear safety guidelines, conducting risk assessments, and providing appropriate personal protective equipment. Safety measures should be strictly enforced to ensure the well-being of all participants during training activities.

6.8. Training Curriculum and Instructors: Develop a comprehensive training curriculum that covers theoretical knowledge, practical skills, and decision-making exercises related to CBRN incidents. The curriculum should be designed to cater to different levels of expertise, ranging from basic awareness to advanced response techniques. Qualified instructors with relevant expertise in CBRN defense and response should lead the training sessions.

6.9. After-Action Reviews: Conduct after-action reviews following each training exercise or drill to evaluate performance, identify strengths and areas for improvement, and facilitate continuous learning. These reviews should involve all participants, including trainees, instructors, and control personnel, to gather valuable feedback and insights for future training enhancements.

6.10. Integration with Emergency Response Plans: Ensure that the training and simulation activities align with established emergency response plans and procedures. This integration helps familiarize trainees with the actual protocols and coordination mechanisms that would be used during real CBRN incidents, enhancing overall preparedness and effectiveness.

7. Communication Systems

Robust communication infrastructure, including radio systems, internet connectivity, and intercoms, should be integrated into the facility to support coordination during emergencies. Effective communication systems are essential in a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes. These systems enable efficient coordination, information sharing, and emergency response during CBRN incidents [42-44].

7.1. Intercom Systems: Install intercom systems throughout the multipurpose hall to facilitate direct communication between different areas, such as control rooms, training areas, storage facilities, and decontamination zones. Intercoms allow for quick and clear communication, enabling effective command and control during CBRN incidents.

7.2. Two-Way Radios: Equip personnel with two-way radios to enable real-time communication between individuals or teams within the multipurpose hall. Two-way radios provide mobile communication capabilities, allowing personnel to stay connected while moving within the facility. Ensure that the radio system has sufficient coverage within the hall and is compatible with existing communication infrastructure.

7.3. Emergency Broadcast Systems: Implement emergency broadcast systems that can disseminate critical announcements, instructions, or alerts to all personnel within the multipurpose hall. These systems can include loudspeakers strategically positioned throughout the facility, enabling mass notification and ensuring that important information reaches all occupants, even in noisy or crowded environments.

7.4. Digital Communication Platforms: Utilize digital communication platforms, such as email, instant messaging, or mobile applications, to enhance communication and information sharing among personnel. These platforms can be particularly useful for non-emergency situations, such as routine updates, scheduling, or sharing documents related to CBRN operations.

7.5. Video Conferencing: Integrate video conferencing capabilities into designated areas, such as command centers or control rooms, to facilitate remote communication and coordination with external stakeholders or off-site experts. Video conferencing allows for real-time visual communication, enabling efficient collaboration and decision-making during CBRN incidents.

7.6. Data and Network Connectivity: Ensure reliable data and network connectivity throughout the multipurpose hall to support effective communication. This includes providing sufficient Wi-Fi coverage, Ethernet ports, and network infrastructure to facilitate the use of communication devices, access to online resources, and data exchange among personnel.

7.7. Emergency Communication Protocols: Establish clear emergency communication protocols and procedures for CBRN incidents. These protocols should outline communication chains of command, designated emergency communication channels, and reporting mechanisms. Ensure that personnel are trained on these protocols and familiar with the appropriate use of communication systems during emergencies.

7.8. Integration with External Communication Networks: Coordinate with external emergency response agencies, such as fire departments, law enforcement, or medical services, to integrate the multipurpose hall's communication systems with their networks. This allows for seamless communication and coordination during joint response efforts or when seeking external assistance.

7.9. Backup Power and Redundancy: Implement backup power systems, such as uninterruptible power supplies (UPS) or emergency generators, to ensure communication systems remain operational during power outages or emergencies. Additionally, consider redundancy in communication systems to minimize the risk of single points of failure and maintain continuous communication capabilities.

7.10. Testing and Maintenance: Regularly test and maintain communication systems to ensure their reliability and functionality. Conduct periodic drills or simulations to validate the effectiveness of communication systems during various scenarios. Routinely check equipment, perform system checks, and update software or firmware to address any vulnerabilities or issues.

8. Safety Systems

The hall should be equipped with safety features, such as emergency alarms, evacuation routes, emergency lighting, and fire suppression systems, to ensure the safety of personnel during CBRN incidents. Safety systems play a crucial role in ensuring the protection of personnel and minimizing risks in a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes [42-50].

8.1. Fire Suppression Systems: Install fire suppression systems, such as sprinklers or specialized suppression agents, throughout the multipurpose hall. These systems should be designed to quickly detect and suppress fires, minimizing the risk of fire-related incidents. Ensure that the fire suppression systems are compatible with the CBRN activities and do not pose additional risks during CBRN incidents.

8.2. Emergency Lighting: Implement reliable emergency lighting systems that automatically activate during power outages or emergencies. Emergency lighting helps maintain visibility, especially in areas critical for evacuation or emergency response. Ensure that emergency lighting is strategically placed to illuminate evacuation routes, exits, and important safety equipment.

8.3. Emergency Power Backup: Provide emergency power backup systems, such as uninterruptible power supplies (UPS) or emergency generators, to ensure essential safety systems remain operational during power failures or emergencies. Critical systems, including emergency lighting, communication systems, fire suppression systems, and ventilation, should be connected to the emergency power backup.

8.4. Alarm Systems: Install comprehensive alarm systems that include audible and visual alarms throughout the multipurpose hall. These alarms should be triggered automatically in the event of CBRN incidents, fires, or other emergencies, alerting personnel to take appropriate actions. Consider integrating the alarm systems with the overall facility's alarm system to ensure a coordinated response.

8.5. Ventilation and Air Filtration Systems: Implement robust ventilation and air filtration systems designed to handle CBRN contaminants. These systems should be capable of providing a continuous supply of fresh air, removing airborne contaminants, and controlling the circulation of hazardous substances. Regular maintenance and filter replacement should be carried out to ensure their effectiveness.

8.6. Emergency Evacuation Plans: Develop and communicate clear emergency evacuation plans for the multipurpose hall. These plans should include designated evacuation routes, assembly points, and procedures for personnel to follow during CBRN incidents or other emergencies. Display evacuation maps prominently throughout the facility and conducts regular drills to familiarize personnel with evacuation procedures.

8.7. Personal Protective Equipment (PPE): Ensure that an adequate supply of appropriate personal protective equipment (PPE) is readily available throughout the multipurpose hall. This includes respiratory protection, protective clothing, gloves, goggles, and other necessary equipment for personnel to protect themselves during CBRN incidents or when handling hazardous substances.

8.8. Emergency Response Equipment: Equip the multipurpose hall with emergency response equipment such as first aid kits, stretchers, eye wash stations, and emergency showers. These provisions ensure that immediate medical attention can be provided in case of injuries or exposure to hazardous substances. Regularly check and replenish the emergency response equipment as needed.

8.9. Safety Signage: Install clear and visible safety signage throughout the multipurpose hall. This includes signs indicating the location of emergency exits, first aid stations, fire extinguishers, hazardous material storage areas, and other critical safety equipment. The signage should use internationally recognized symbols and be easily understandable in high-stress situations.

8.10. Safety Training and Education: Provide comprehensive safety training and education programs for all personnel working in the multipurpose hall. This includes CBRN safety protocols, proper use of safety equipment, evacuation procedures, and the recognition of potential hazards. Regularly refresh and update training programs to ensure personnel are well-prepared and informed.

9. Accessibility

The facility should be designed to accommodate individuals with disabilities and provide ease of access for emergency responders and equipment. Ensuring accessibility in a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes is important to accommodate individuals with disabilities or mobility challenges [51-54].

9.1. Accessibility Standards: Adhere to local accessibility standards and regulations when designing and constructing the multipurpose hall. These standards typically outline requirements for ramps, door widths, corridor widths, accessible parking spaces, and other accessibility-related features. Compliance with these standards promotes inclusivity and provides equal access for all individuals.

9.2. Ramps and Elevators: Install ramps or elevators at entrances and other areas with level changes to ensure wheelchair users and individuals with mobility aids can access different parts of the facility. Ramps should be designed with appropriate slopes, handrails, and tactile indicators to enhance safety and usability.

9.3. Accessible Parking Spaces: Designate accessible parking spaces near the entrance of the multipurpose hall. These spaces should be wide enough to accommodate accessible vehicles and provide sufficient clearance for wheelchair users to enter and exit the vehicles comfortably. Accessible parking spaces should also be conveniently located and clearly marked.

9.4. Wide Doorways and Corridors: Ensure that doorways and corridors within the multipurpose hall are wide enough to accommodate individuals using wheelchairs or mobility aids. The minimum width should comply with accessibility standards to allow for easy passage and maneuverability. Install automatic doors or door openers where feasible to facilitate independent access.

9.5. Accessible Restrooms: Provide accessible restrooms that meet the requirements of accessibility standards. These restrooms should have appropriate door widths, grab bars, accessible sinks, and other necessary features to ensure usability for individuals with disabilities. Signage indicating the location of accessible restrooms should be clearly visible.

9.6. Tactile and Visual Cues: Incorporate tactile and visual cues throughout the multipurpose hall to assist individuals with visual impairments or hearing impairments. This may include braille signage, tactile floor indicators, contrasting colors for wayfinding, and visual alarms or flashing lights for emergency notifications.

9.7. Seating Arrangements: Plan seating arrangements within the multipurpose hall to accommodate individuals with disabilities. Ensure that there are designated spaces for wheelchair users, as well as companion seating options for individuals with disabilities attending events or training sessions.

9.8. Assistive Listening Devices: Provide assistive listening devices, such as induction loop systems or FM systems, to assist individuals with hearing impairments during presentations, meetings, or training sessions. These devices transmit audio directly to compatible hearing aids or personal receivers, improving sound clarity and accessibility.

9.9. Training and Awareness: Train staff and personnel on disability awareness, including best practices for assisting individuals with disabilities and addressing their specific needs. Sensitize employees on the importance of inclusive practices and provide them with the knowledge and skills to ensure a welcoming and accessible environment.

9.10. Continuous Evaluation and Improvement: Regularly assess the accessibility features and gather feedback from individuals with disabilities or accessibility experts. Identify areas for improvement and implement necessary modifications to enhance accessibility in the multipurpose hall. This may include adjusting door widths, adding additional signage, or addressing any barriers that may impede accessibility.

10. Security Measures

Strict security measures should be implemented to prevent unauthorized access to CBRN materials and ensure the facility's integrity. Implementing robust security measures in a multipurpose hall designed for CBRN (Chemical, Biological, Radiological, and Nuclear) purposes is crucial to protect personnel, prevent unauthorized access, and safeguard sensitive materials and equipment [51, 55-59].

10.1. Perimeter Security: Establish a secure perimeter around the multipurpose hall to control access and deter unauthorized entry. This may include fencing, gates, access control systems, surveillance cameras, and signage indicating restricted access. Implement measures to monitor and control vehicle and pedestrian access points.

10.2. Access Control Systems: Install access control systems at entry points to regulate and monitor personnel entering and exiting the multipurpose hall. This can include electronic key card systems, biometric readers, or other secure authentication methods. Access control systems enable authorized personnel to enter while restricting access to unauthorized individuals.

10.3. Video Surveillance: Deploy a comprehensive video surveillance system throughout the multipurpose hall. Install high-quality cameras in strategic locations, including entrances, exits, critical areas, storage facilities, and external perimeters. Monitor and record video footage to deter potential security breaches and assist in investigations if necessary.

10.4. Intrusion Detection Systems: Implement intrusion detection systems, including motion sensors, door/window sensors, and glass break detectors, to detect and alert security personnel about any unauthorized access attempts or suspicious activities within the multipurpose hall. Integrate these systems with the overall security infrastructure for a prompt response.

10.5. Security Personnel: Assign trained security personnel to monitor and patrol the multipurpose hall. They should be responsible for enforcing security policies, performing regular security checks, responding to alarms, and maintaining a visible security presence. Security personnel should be adequately trained in CBRN security protocols and emergency response procedures.

10.6. Restricted Areas: Designate specific areas within the multipurpose hall as restricted zones, accessible only to authorized personnel with appropriate security clearance. Use physical barriers, access control systems, or biometric authentication to ensure limited access to sensitive areas, such as storage facilities, control rooms, or laboratories.

10.7. Alarm and Alert Systems: Install audible and visual alarm systems throughout the multipurpose hall to notify personnel of emergencies, security breaches, or CBRN incidents. These systems should be interconnected and capable of providing specific alerts and instructions based on the nature of the incident.

10.8. Cybersecurity Measures: Implement robust cybersecurity measures to protect digital systems, networks, and sensitive information within the multipurpose hall. This includes firewalls, secure authentication protocols, regular software updates, data encryption, and employee awareness training on cybersecurity best practices. Protecting digital assets is crucial to prevent unauthorized access or manipulation of critical information.

10.9. Incident Response Plan: Develop a comprehensive incident response plan that outlines procedures and protocols to address security incidents, including CBRN-related events. The plan should cover roles and responsibilities, communication protocols, evacuation procedures, and coordination with external emergency response agencies. Regularly review and update the plan to reflect changing security needs and emerging threats.

10.10. Training and Awareness: Conduct regular security training sessions for all personnel working in the multipurpose hall. This should include CBRN security awareness, identification of suspicious activities, proper use of access control systems, and response to security incidents. Regularly reinforce security practices and encourage personnel to report any security concerns promptly.

RESULTS

The location of a multipurpose hall for CBRN activities significantly impacts the safety, response efficiency, accessibility, compliance with regulations, and collaboration opportunities. Careful consideration of these factors during the selection process ensures that the facility is strategically positioned to mitigate risks and effectively respond to CBRN incidents.

By incorporating these considerations, a multipurpose hall for CBRN purposes can be designed to provide a structurally sound and resilient environment that can withstand CBRN incidents and protect occupants from potential hazards. It is important to consult with structural engineers and professionals experienced in CBRN facility design to ensure compliance with safety standards and best practices.

Other important points are consulted with ventilation and filtration experts, as well as professionals with expertise in CBRN defense and response, to design and install appropriate systems that meet safety standards and requirements specific to your location and the intended use of the multi-purpose hall.

Experts have an important role in decontamination procedures, CBRN defense, and response when designing and implementing decontamination facilities. They can guide best practices, safety standards, and compliance with local regulations to ensure the decontamination facility meets the specific requirements for CBRN-related activities in the multipurpose hall.

The equipment storage in a multipurpose hall for CBRN purposes can be efficiently organized, secure, and readily accessible. Consulting with CBRN defense and response experts can provide valuable insights and guidance on specific storage requirements, safety protocols, and best practices.

A multipurpose hall for CBRN purposes can provide a realistic and effective training environment. It is crucial to collaborate with CBRN defense and response experts, experienced instructors, and relevant stakeholders to design and implement a comprehensive training and simulation program that meets specific training objectives and regulatory requirements.

A multipurpose hall for CBRN purposes can establish robust communication systems that enable effective coordination, information sharing, and emergency response. It is important to consult with communication experts and incorporate best practices specific to CBRN operations to ensure the implementation of reliable and efficient communication capabilities.

It is crucial to consult with safety experts, regulatory guidelines, and CBRN defense professionals to ensure the implementation of appropriate safety systems in the multipurpose hall. Regular inspections, maintenance, and adherence to safety regulations are essential to maintain a safe environment for personnel involved in CBRN activities.

A multipurpose hall for CBRN purposes can be designed to provide equal access and inclusion for individuals with disabilities or mobility challenges. Collaboration with accessibility experts and engaging individuals with disabilities during the design process can offer valuable insights and ensure that the facility meets its specific needs.

DISCUSSION

Creating a multi-purpose hall that is specifically designed for CBRNE incidents is necessary for several reasons, such as: having a dedicated facility, the possibility of a centralized response to CBRNE incidents, the need for CBRNE incidents to have specialized infrastructure and facilities to manage the unique challenges caused by Chemical, biological, radiological, nuclear and explosive risks.

Provide a dedicated controlled environment theater for training and preparedness activities where responders can simulate CBRNE incidents, practice response methods, test equipment, and coordinate between different agencies and organizations. improve Regular training exercises in a realistic environment to enhance preparedness and enable responders to develop the skills and knowledge necessary to effectively manage CBRNE incidents. Many other reasons are given in this study, in addition to the description of the necessity of building such structures, which deals with the detailed study of the required factors in order to be used as a minimum checklist for the establishment and creation of such multi-purpose halls.

Consequently, a multi-purpose theater designed specifically for CBRNE incidents, due to its ability to centralize response efforts, provide specialized infrastructure, facilitate training and preparedness, support decontamination and treatment, encourage research and development, enhance safety public, and promote cooperation between stakeholders is essential. Such facilities will significantly improve the overall response to CBRNE incidents and increase the resilience of communities to these complex and dangerous events.

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ORIGINAL ARTICLE

Validity of Photoplethysmography Mobile Analysis to Test the Autonomic Stress Status of Tactical Athletes

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Abstract: *Background: The effect of stress on sympathetic modulation measured by HRV has been studied in various population groups exposed to different stressors. HRV measurement tools are based on the electrocardiogram (ECG), portable tools such as POLAR V800, and recently photoplethysmography (PPG). The aim of this study was to analyze the validity of a PPG mobile app to measure HRV with a validated V800 system in a tactical athlete population. Methods HRV of 53 professional militaries were analyzed by POLAR V800 and an HRV Camera app with a smartphone during 5 min and 30 s protocol respectively. Results: The HRV values obtained by the PPG presented a significant negative significant correlation in the HRVI index, LF, HF, LF (n.u.), HF (n.u.), and a positive significant correlation in TINN index by the Polar V800 system with the application of a regression equation over the data obtained. Conclusions: HRV Camera PPG App 30s record analysis protocol could be applied using a regression formula depending on the HRV variables after the comparison with a standard 5-minute test conducted with a validated Polar V800. In future research, it is suggested to validate PPG protocols with longer durations.*

Keywords: *Heart rate variability, military, autonomic modulation, photoplethysmography*

INTRODUCTION

Heart rate variability (HRV) represents the physiological response of the variation in the interval of time between each heartbeat. It is measured by the variation in the interval from beat to beat [1]. This modulation is controlled by the balance between the activation of the Parasympathetic Nervous System (PNS), and Sympathetic Nervous System (SNS) which is named autonomic modulation [2]. This physiology response is related to the effect of the stress or to exposure to stressors agents due to experiences or the type of work performed, [3] showing a variation in HRV variables that is related to a low parasympathetic activity, which is characterized by an increased sympathetic activity [1].

The effect of stress on sympathetic modulation measured by HRV has been studied in various population groups exposed to different stressors as ultra-endurance athletes [4], skydivers [5], firefighters [6], policemen [7], soldiers [8], pilots [9], journalists [10], students [11], professors [12], and sanitary [13]. Independently of the population, an increased sympathetic modulation was observed when they were exposed to the stressor, a fact related to the activation of the fight-flight response [14]. The fight or flight response is a psychophysiological reaction that is produced by the presence of a threat, in which the body responds by activating the sympathetic nervous system (SNS) and the adren-al-cortical system [15]. Military personnel, due to their duty and the exigences of their operation are considered tactical athletes and are a clear example of a high-stress exposure population group. Previous research found a high activation of the SNS in melee combat simulations [16], as well as in asymmetrical [17] and urban combat [14]; the organic response was also evaluated in light infantry and heavy infantry in combat simulations [18].

Along the same line, HRV measurement can be an efficient device to understand the state of the autonomic nervous system in sports athletes, which showed changes in their HRV and stress level due to the stress that competition can produce [19]. In this way, HRV should be a tool to avoid the state of overload of the athlete, and at the same time provide us with how the state of an athlete and ad equating a control of the workout loads [20,21].

HRV measurement tools are based on the electrocardiogram (ECG) analysis which has proven effective for stress detection [22]. There are also portable tools such as the POLAR V800 heart rate monitor that showed its effectiveness to evaluate HRV and stress response [23,24]. Recently there are new tools such as photoplethysmography (PPG), which could be an alternative evaluation tool to the ECG and heart rate monitors. Previous studies showed how PPG could be an alternative to evaluated HRV signal in ECGs derivation and analysis in ambulatory cardiac monitoring of healthy individuals [25–27]. This kind of tool is proven in new technologies such as smartphones as fingertip recording mobile-app where there are some researchers that tested this kind of technology as a useful tool [28–31].

New technologies as smartphones or smartwatches, could be an interesting tool for the military who work in austere environments and are already familiar with the use of these devices. There are actually applications of weather impact in military corps [32], prototypes of military medical applications for combat medics and other health care providers [33], a behavioral health screening app [34] or tools for posttraumatic stress diseases [35], but not to analyze the acute autonomic status of this personnel. Then, the aim of this study was to analyze the validity of a PPG mobile app to measure HRV with a validated V800 system in a tactical athlete population. The initial hypothesis was that PPG mobile app would be a valid system to analyze HRV compared with a validated V800 system.

MATERIALS AND METHODS

53 professional military of the Spanish Army (age: M = 35.4 years, SD = 5.88 years; height: M = 174.89 cm, SD = 6.87 cm; body mass: M = 77.33 kg, SD = 11.95 kg; Military Duty= 14.44years, SD=6.43; Military Operation= 4 months, SD= 4.25 months) were analyzed. Procedures and protocols were explained to all the subjects who gave the voluntary written informed in accordance with the Declaration of Helsinki and approved by the European University Ethics Committee (CIPI/18/093).

HRV was measured by a validated Polar V800 heart rate monitor (Polar, Kempele)[36] and the PPGV mobile app for Android HRV by Camera. For this aim the Polar electrode belt was placed following Polar's guidelines in the middle of the chest. Then, participants remained seated for 5 minutes in a quiet room while the Polar V800 record the signal minutes before a maneuver meeting. After 2 minutes of starting recording, they place the index finger in the camera of a mobile device following the guideline of the HRV by Camera ECG Android App supported for BLE devices in order to analyze the HRV PPG based. The PPGV analysis lasted 30 seconds in which the participant remained sited, with the right arm flexed at 90º supported in an armrest.

The following HRV variables were obtained by the two devices:

Mean R-R; the standard deviation of successive differences (SDNN); mean HR; root mean square of successive differences (RMSSD); the number of adjacent intervals varying by more than 50ms (PNN50); HRV triangular index (HRVI); interval histogram (TINN); low-frequency power (LF); high-frequency power (HF); the low-frequency band in normalized units (LFun); the high-frequency band in normalized units (HFun); the sensitivity of the short-term variability (SD1) and the sensitivity long-term variability (SD2).

Statistical analysis

The SPSS statistical package (version 21.0; SPSS, Inc. Chica-go, Illinois) was used to analyze the data. Normality assumptions were checked with a Kolmogorov-Smirnov test. It evaluated an Intra-class Correlation (ICC) and 95% Confidence Interval (CI), Pearson correlation, and a regression equation. The level of significance for all the comparisons was set at $P < 0.05$.

RESULTS

The results are reported with their mean and standard deviation. Table 1 shows the results of HRV for each test. A negative significant correlation was found in HRVI, LF, HF, LF(n.u.), HF (n.u.) values. Also was found a significant positive correlation in TINN values. No correlations were found between other parameters.

DISCUSSION

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible.

Future research directions may also be highlighted.

CONCLUSION

The aim of this study was to analyze the validity of a PPG mobile app to measure HRV with a validated polar V800 system in the athlete population. The initial hypothesis was partially fulfilled since some PPG mobile app values presented positive and negative correlations than a validated V800 system.

HRV measurement is an efficient and important approach to assess autonomic modulation, which shows the stress state of the organism [37].

Table 1: This is a table.

	Polar		HRV Camera		ICC (CI 95%)	Pearson	Regression	p
	M	SD	M	SD				
MeanRR (ms)	916.72	136.10	941.37	165.15	.009 (-1.029, .519)	.005	0.003x+957.970 (p>0.05)	0.252
SDNN (ms)	101.54	45.96	416.79	1770.56	.002 (-1.071, .519)	-.173	0.000x+101.650 (p>0.05)	0.329
MeanHR (bpm)	68.06	9.88	64.58	12.50	-.401 (-1.967, .331)	.022	-0.126x+76.460 (p>0.05)	0.417
RMSSD (ms)	48.28	15.54	44.64	20.10	.252 (-.563, .640)	.148	0.130x + 43.057 (p>0.05)	0.204
pNN50 (%)	20.62	10.61	27.90	22.67	.126 (-.779, .574)	.091	0.041x+19.071 (p>0.05)	0.221
HRVI	17.43	4.07	3.90	1.94	-.024 (-.087, .095)	-.177	-0.382x+19.724 (p>0.05)	0.000
TINN	337.5	151.36	162.74	107.63	.063 (-.241, .378)	.082	0.142x+318.684 (p>0.05)	0.000
LF(ms2)	74.22	14.73	740.03	1207.76	-.006 (-.812, .487)	-.163	-0.002x+76.709 (p>0.05)	0.014
HF(ms2)	25.72	14.71	426.49	417.62	-.002 (-.444, .397)	-.023	-0.001x+24.775 (p>0.05)	0.000
LF (n.u.)	77.79	13.34	60.94	87.55	-.189 (-.664, .284)	-.224	-0.139x+83.575 (p>0.05)	0.000
HF (n.u.)	22.16	13.33	61.88	58.65	-.192 (-.674, .284)	-.226	-0.140x+30.308 (p>0.05)	0.000
SD1 (-)	34.20	11.01	43.20	52.76	.227 (-.630, .630)	.158	0.078x+31.195 (p>0.05)	0.562
SD2 (-)	138.65	65.35	225.45	476.18	-.093 (-.919, .567)	.150	0.025x+134.856 (p>0.05)	0.545

M: Mean. SD: Standard Deviation. F: Fisher-Snedecor test. ICC: Intraclass correlation. SDNN: Standard deviation of normal-to-normal R-R intervals. RMSSD: Square root of the mean of the sum of the squared differences between adjacent normal R-R intervals. pNN50: The percentage of differences between R-R intervals higher than 50 ms.HRVI: HRV triangular index. TINN: interval histogram. LF: Low frequency. HF: High frequency. n.u.: Normalised unit. SD1: Poincaré Plot index of instantaneous recording of the variability. SD2: Poincaré plot index of overall variability.

The benefits of evaluating heart rate variability have been observed in psychological disorder as depression and posttraumatic stress disorder [38] and other medical diseases that are accompanied by a dysregulation of the autonomic nervous system such as asthma, heart failure, or hypertension [39]. The utility in sports has been also demonstrated due to the organic alterations suffered by athletes performing physical training. In this line, HRV has been shown as an efficient tool to control the athlete's internal load as a stress marker [40], being useful in designing conditioning programs that ensure a sufficient load that corresponds to the specific capacity of the athlete [21] and prevent states of overload and fatigue of the athlete [21].

HRV measurements have been also used to evaluate the anxiety level of the military population[41,42]. In this line, we found similitudes between soldiers' RMSSD PPG smartphone app values and values of non-elite soldiers found in previous research (44.64 ± 20.10 ms and 41.82 ± 22.95 ms respectively) [43]. In this line, we found RMSSD similar values in military pilots in repose (44.28 ± 25.96 ms) where could be similar HRV response due to stressors before a military operative flight training [44]. Although, it is observed lower RMSSD results than helicopter flight-fighter before a night maneuver (93.4±71.1 ms) [9] and some higher than military corps before underwater evacuation training (33.11 ± 18.73 ms)[45]. Regarding the behavior of this parameter in other athletes. It is observed higher values in athletes in the competition period (61.8 ± 51.73 ms)[46] which indicate that the similarity of RMSSD values in different scenarios is not an exact predictive.

Regarding other PPG system values, we found similar results in the LF values (n.u.) with Special Operation soldiers population in basal conditions before a military operation maneuver (60.94 ± 87.55 vs 60.61 ± 6.66 respectively) [47]. Similar results were shown by a rescue helicopter crew before a flight maneuver (63.6 ± 21.5) [48]. In this line, it has found similarities as athletes in the competition period (64.2 ± 18.70) [46]. Although all cases showed a standard deviation minor than showed in this investigation it presented a similar response in basal conditions before exposure to stressors.

The great contrasts in the frequency values also are observed if we compare to previous literature in basal LF values (740.03 ± 1207.76 vs 64.44 ± 18.00) and basal HF values (426.49 ± 417.62 vs 35.49 ± 18.01) in pilots before a real and simulated flight maneuver [44]. This large discrepancy in the LF and HF values could be produced by an error in the measurement procedure of the mobile app.

In this line, other values in basal conditions show differences compared to previous research, such as the mean RR values, which we observed lower values than thirty-one male subjects from the Spanish Army prior to simulated combat (941.37±165.15 vs 872.8±150.4) [49]. This discrepancy of responses showed in the HRV values in basal conditions may not be produced by the stressor that the subject will face, if not by the difference of subject experience or research protocols.

A disagreement is also observed in the HRV values obtained by the PPG mobile application compared to those obtained with the validated Polar V800 tool. We found a discrepancy to the values obtained by the Polar V800 system where, there was a large discrepancy in the LF values (740.03 ± 1207.76 vs 74.22 ± 14.73 respectively), HF values (426.49 ± 417.62 vs 25.7± 214.71 respectively). The disparity in the HRV data analyzed in our investigation could be due to the APP's short-time analysis protocol, of 30 seconds. This duration was selected in order to find the protocol that would require the least time from the participants due to the low availability of time in the measured population, trying to find the most efficient protocol and enough time to provide HRV measurements as the application sentence. The protocol of the Polar V800 system was 5 minutes, which is more reliable due to the duration of the measurement, which is similar to those seen in the rest of the protocols in investigations with other PPG technology devices.

On the contrary, to solve the discrepancy in the data, the application of a regression equation produces the correlation of the values. Positive and negative correlations in frequency values, HRVI index, and TINN index have been observed between both devices. In this case, the PPG system could provide us with reliable results in a short time record.

This fact is related to previous research that validated different PPG tools with standard systems such as HRV4Training [31] which has been proven a systematic tool to measure to optimize individualized training in professional endurance runners [50] and cyclists [51]. This kind of app delivers efficient results comparable to the ECG for measuring HRV in basal conditions where measurements protocols consisted at least of a 1 min recording [52]. Therefore, there could be greater efficiency in terms of HRV recording as long as the measurement time is greater than that proposed in our research without the need to apply any type of equation.

The evaluation and measurement of HRV have also been evaluated in PPG technology in other devices such as wristband devices. It has been demonstrated satisfactory HRV-related mental stress prediction taking LF/HF feature and with a similar record protocol as our research, 30 seconds of registration, but in this case with more records, three times per day in wristband PPG device [53]. In addition, the Apple Watch showed a significant decrease of values related to increased stress, HF, and RMSSD, with a protocol of 2 sets of 5 minutes [54] and Wrist-Worn Polar Vantage V2 PPG which is regarded as an HRV PPG system record efficient in athletic and healthy population with long time period protocols,7 minutes in supine position in laboratory and 4 hours recording period during nocturnal sleep time [55]. Showing in all the cases better results when the measurement time is longer in time or with several records in a period of time.

Practical application

Present results joining other research could help refine an exact protocol for using the PPG smartphone apps, and become an effective and easy-to-use tool in harsh conditions. And provide us with the correlation equation for the validity of the data obtained through this application with a protocol with a short time record.

Limitations of the study and future research lines

The main limitation of this study is the low sample of participants. Also, it could be recommended the use of ECG to compare values as in previous research. In future research, it is suggested to validate PPG protocols with longer durations.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution

Conceptualization, A.C.-R., V.J.C.-S and A.B.-S.; methodology and formal analysis A.C.-R., A.B.-S and V.J.C.-S investigation, A.C.-R., A.B.-S and V.J.C.-S.; data curation, V.J.C.-S.; Writing—Original draft preparation, , A.C.-R., A.B.-S and V.J.C.-S.; Writing—Review and Editing, A.C.-R., A.B.-S and V.J.C.-S.; visualization, LPA, A.C.-R., A.B.-S and V.J.C.-S.; supervision and funding acquisition, V.J.C.-S.

Ethics approval and consent to participate

Procedures and protocols were explained to all the subjects who gave the voluntary written informed in accordance with the Declaration of Helsinki and approved by the Euro-pean University Ethics Committee (CIPI/18/093).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Predictive Factors for Death among Patients with Clostridium difficile Infection – A Single Center Experience Study

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Abstract: *Clostridium difficile infection (CDI) is one of the most common healthcare-associated infections and an important cause of morbidity and mortality worldwide. The objective of the study was to identify the predictive factors for unfavorable evolution and deaths among patients with CDI. Materials and methods: We conducted a retrospective, observational, non-randomized study on 202 patients diagnosed with CDI in the Clinical Emergency Hospital of Bucharest, Romania, for a period of two years. For the statistical analysis, the R program was used, with p-values <0.05 being considered statistically significant. Results: The average age of the patients included in the study was 70.8 years, the average duration of hospitalization was 18.4 days, and the mortality rate was 25.7%. During the follow-up period, only 5% of the patients presented a recurrence of CDI. Multiple binomial logistic regression defined as independent risk factors for death among CDI patients: advanced age, comorbidities such as chronic obstructive pulmonary disease (p=0.008), chronic kidney disease (p=0.007), or COVID-19 (p=0.036), leukocytes ≥ 16,755/mm^3 (p=0.009) and serum albumin < 2.83 g/dL (p<0.001). Conclusions: The identification of certain negative prognostic factors in patients with CDI requires a careful follow-up of the patients, for an early identification of complications and adaptation of therapeutic management.*

Keywords: *Clostridium difficile; chronic obstructive pulmonary disease; COVID-19; chronic kidney disease; prognosis*

INTRODUCTION

Clostridium difficile infection (CDI) is one of the most common healthcare-associated infections and an important cause of morbidity and mortality worldwide [1]. The latest epidemiological data provided by the European Center for Disease Prevention and Control (ECDC), in 2008, reported an incidence of approximately 4.2 cases of CDI per 10.000 patient-days per hospital, with a range of variation of 0.0-36.3 [2]. According to this report, the incidence of CDI increased dramatically compared to 2005 when an incidence of approximately 2.45 cases of CDI per 10,000 patient-days per hospital was reported, with a range of variation of 0.13-7.1 [3,4]. In the United States of America (USA), in 2012, approximately half a million cases of CDI and 29.000 deaths secondary to this disease were registered [5]. However, because of the implementation of a surveillance and infection prevention program in 10 USA states, the health-care-associated CDI decreased from 92.76 cases per 100,000 inhabitants in 2011, to 66.96 cases per 100,000 inhabitants in 2017 [6]. Regarding the incidence of community-associated CDI in the USA, it increased slightly between 2011 and 2017 (55.3 cases per 100,000 inhabitants in 2011 vs. 70.4 cases per 100,000 inhabitants in 2017) [6]. Another problem is represented by the important recurrence rate of CDI, respectively 20-30% [7]. Beyond the negative impact on the quality of life and life expectancy, the healthcare of these patients involves very high costs. For example, in the USA, 5.4-6.3 billion dollars per year are consumed by these patients [8].

It is also reported a Clostridium difficile carriage rate of 8-10% among hospitalized adults, compared to 3% among healthy adults [9]. Carriers, but asymptomatic patients, represent reservoirs of germs, which can eliminate spores and transmit the infection to other patients [10,11]. A study that followed the prevalence of colonization with Clostridium difficile, at admission, among 250 patients who did not present diarrhea, reported a percentage of 79% of patients colonized with this bacteria [12]. Among them, in 15% of cases, a toxigenic strain was identified, and in 6% of cases a non-toxigenic strain [12]. No differences were identified regarding recent antibiotic use or contact with medical care units between patients colonized with a toxigenic strain vs. uncolonized patients [12]. Also, the concentrations of toxins in stools have been shown to be similar in asymptomatic carriers vs. symptomatic patients [13].

CDI is a public health problem with a major impact on the healthcare system. This can be explained by the significant increase in the incidence after the year 2000, the existence of CDI outbreaks in some hospitals, but also by the appearance of hypervirulent strain NAP1/B1/027 [14,15].

The most common risk factors for CDI are represented by hospitalization, antibiotic use, and advanced age [16-18]. However, there are other risk factors that can modify the evolution and prognosis of these patients, respectively: obesity, severe comorbid illness, inflammatory bowel disease, chemotherapy, enteral feeding, hematopoietic stem cell transplantation, cirrhosis, and gastric acid suppression [19-23]. Regarding the risk of complications, a study that followed 1,380 patients with CDI identified a series of predictive factors for unfavorable evolution: older age, abnormal vital signs (respiratory rate over 20/minute and heart rate over 90 beats per minute), and abnormal blood tests (C-reactive protein ≥ 150 mg/L, albumin < 25 g/L, white blood cell count < 4,000/L or ≥ 20,000/L) [24]. According to data from the specialized literature, all cases' death rate at 30 days in patients with CDI varies between 9% and 38% [25].

Given the burden and the important death rates associated with CDI, we aimed to identify the predictive factors for an unfavorable evolution among patients with CDI. The primary endpoint of the study was the in-hospital mortality rate.

MATERIALS AND METHODS

We conducted a retrospective, observational, non-randomized study in which we evaluated the mortality rate associated with CDI and the risk factors for an unfavorable evolution. We followed 202 patients diagnosed with CDI between January 2020-January 2022 during hospitalization in the Clinical Emergency Hospital of Bucharest, Romania. The study was approved by the Ethics Committee of the Clinical Emergency Hospital of Bucharest (approval no. 10002/ 04.10.2022). The study respected the ethical standards of the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

The inclusion criteria consisted of the existence of clinical and paraclinical evidence attesting CDI. For the diagnosis, we applied the 2019 European Center for Disease Prevention and Control (ECDC) definition of CDI, respectively: diarrhea or toxic megacolon and a positive test for Clostridium difficile toxins A/B in stool samples or positive polymerase chain reaction (PCR) test for Clostridium difficile; colonoscopic examination that highlighted pseudomembranous colitis; histopathological examination that identified characteristic elements of CDI in a tissue sample obtained by endoscopy from a patient with or without diarrhea [4]. The exclusion criteria were represented by the absence of some clinical-paraclinical data that were followed in the study and the absence of the patient's informed consent. None of the patients initially included in the study were subsequently excluded.

The variables followed in the study are epidemiological data (age, sex), presence of comorbidities (chronic obstructive pulmonary disease, COVID-19, chronic kidney disease, heart failure, atrial fibrillation, diabetes mellitus, arterial hypertension, neoplasms), CDI recurrence, treatment with proton pump inhibitors, a series of biological variables (hemoglobin, number of leukocytes, C-reactive protein, erythrocyte sedimentation rate, fibrinogen, albumin), hospitalization length and death rate.

Statistical analysis

Patient data were obtained from the electronic medical records of the hospital and were centralized in an Excel database. For the statistical analysis, we used the R program, version 4.0.2 [26]. We used a simple univariate binomial logistic regression, the dependent variable being death during hospitalization, and the independent variables' demographic-clinical parameters monitored in the study. Later, the predictors that had statistical significance in the simple model were used in a multiple univariate binomial logistic regression, the model being built with the help of a retrograde selection algorithm of the predictors. We also performed a descriptive statistical analysis of the variables followed in the study. P-values lower than 0.05 were considered statistically significant.

RESULTS

The average age of the patients included in the study was 70.8 years, with a minimum value of 18 years and a maximum value of 95 years. Regarding gender, the proportion was approximately equal, respectively 47% women and 53% men. The average duration of hospitalization was 18.4 days, reaching up to 145 days in more severe cases. During the 2-year follow-up period, only 10 patients (5%) presented a recurrence of Clostridium difficile colitis. Regarding comorbidities, 58.4% of the patients had arterial hypertension (AH), 33.2% heart failure (HF), 29.7% chronic kidney disease (CKD), 27.7% diabetes mellitus, 26.2% atrial fibrillation (AF), 22.8% neoplasms, 22.3% COVID-19 and 3.96% chronic obstructive pulmonary disease (COPD). Among the patients included in the study, 45% were treated with proton pump inhibitors (PPI) (Table 1).

Table 1: Demographic, clinical, and paraclinical analysis of the study group

Variable	Number of patients (N=202)
Age	
Average (SD)	70.8 (15.7)
Median [Min, Max]	73.5 [18.0, 95.0]
Sex	
F	95 (47.0%)
M	107 (53.0%)
Length of hospital stay	
Average (SD)	18.4 (18.4)
Median [Min, Max]	14.0 [1.00, 145]
COPD	
Yes	8 (3.96%)
No	194 (96.04%)
Recurrence of CDI	
Yes	10 (5.0%)
No	192 (95.0%)
COVID-19	
Yes	45 (22.3%)
No	157 (77.7%)
PPI	
Yes	91 (45.0%)
No	111 (55.0%)
CKD	
Yes	60 (29.7%)
No	142 (70.3%)
HF	
Yes	67 (33.2%)
No	135 (66.8%)
AF	
Yes	53 (26.2%)
No	149 (73.8%)
DM	
Yes	56 (27.7%)
No	146 (72.3%)
AH	
Yes	118 (58.4%)
No	84 (41.6%)
Neoplasms	
Yes	46 (22.8%)
No	156 (77.2%)
Number of leukocytes	
Average (SD)	18600 (14000)
Median [Min, Max]	14500 [2800, 94000]
Fibrinogen (mg/dL)	
Average (SD)	498 (173)
Median [Min, Max]	469 [107, 1020]
CRP (mg/dL)	
Average (SD)	118 (73.8)
Median [Min, Max]	102 [0.710, 371]
ESR (mm/hr)	
Average (SD)	43.6 (22.0)
Median [Min, Max]	45.0 [2.00, 98.0]
Hemoglobin (g/dL)	
Average (SD)	10.6 (2.28)
Median [Min, Max]	10.4 [5.50, 18.3]
Albumin (g/dL)	
Average (SD)	2.91 (0.797)
Median [Min, Max]	2.90 [1.00, 5.10]
Death	
No	150 (74.3%)
Yes	52 (25.7%)

Legend: COPD = chronic obstructive pulmonary disease; CDI = Clostridium difficile infection; PPI = proton pump inhibitors; CKD = chronic kidney disease; HF = heart failure; AF = atrial fibrillation; DM = diabetes mellitus; AH = arterial hypertension; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

Also, a series of biological variables were analyzed, such as the number of leukocytes and the serum values of fibrinogen, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin, and albumin.

The average value of leukocytes was 18,600/mm^3, varying between 2,800/mm^3 and 94,000 mm^3. Also, increased values of inflammatory markers were identified, namely an average value of fibrinogen of 498 mg/dL (107-1,020 mg/dL), an average value of CRP of 118 mg/dL (0.710-371 mg/dL), and an average value of ESR of 43.6 mm/hr (2.00-98.00 mm/hr).

The mean serum hemoglobin was 10.6 g/dL and the mean serum albumin was 2.91 g/dL (Table 1). 52 patients died during follow-up, the death rate being 25.7% (Table 1).

Later, we performed a simple univariate binomial logistic to identify the risk factors for unfavorable prognosis and death (Table 2).

This statistical analysis identified a direct proportional relationship between the mortality rate and the following risk factors: advanced age, association with COVID-19, CKD or COPD, serum hemoglobin < 9.85 g/dL, leukocyte count ≥ 16,755/mm^3, CRP ≥ 104.02 mg/dL, serum albumin < 2.83 g/dL. Increasing age by 1 year was associated with a 2% increase in the death rate (p=0.048, 95% CI 1.02 (1.00-1.05)).

Table 2: Simple univariate binomial logistic regression to determine the risk factors for poor prognosis and death among patients with Clostridium difficile colitis.

Predictor	N	OR (95% CI)[1]	P value
Age	202	1.02 (1.00 la 1.05)	0.048
Sex	202		
F		—	
M		1.05 (0.56 la 1.98)	0.88
Recurrence of CDI	202		
Yes		—	
No		1.41 (0.34 la 9.54)	0.67
COVID-19	202		
Yes		—	
No		0.47 (0.23 la 0.97)	0.039
PPI	202		
Yes		—	
No		0.69 (0.36 la 1.30)	0.25
CKD	202		
Yes		—	
No		0.29 (0.15 la 0.57)	<0.001
HF	202		
Yes		—	
No		0.52 (0.27 la 1.01)	0.051
COPD	202		
Yes		—	
No		0.04 (0.00 la 0.25)	0.004
AF	202		
Yes		—	
No		0.57 (0.29 la 1.15)	0.11
DM	202		
Yes		—	
No		0.57 (0.29 la 1.13)	0.10
Neoplasms	202		
Yes		—	
No		0.49 (0.24 la 1.01)	0.050
Hemoglobin (g/dL)[2]	202		
< 9.85		—	
≥ 9.85		0.31 (0.16 la 0.60)	<0.001
AH	202		
Yes		—	
No		0.67 (0.34 la 1.29)	0.24

Predictor	N	OR (95% CI)[1]	P value
Number of leukocytes[2] (/mm³)	202		
< 16755		—	
≥ 16755		3.85 (2.01 la 7.56)	<0.001
Fibrinogen (mg/dL)	202	1.00 (1.00 la 1.00)	0.13
CRP[2] (mg/dL)	202		
< 104.02		—	
≥ 104.02		2.27 (1.20 la 4.39)	0.013
ESR (mm/hr)	202	1.01 (1.00 la 1.03)	0.17
Albumin[2] (g/dL)	191		
< 2.83		—	
≥ 2.83		0.21 (0.10 la 0.42)	<0.001

[1] OR = Odds Ratio, CI = Confidence Interval, [2] Cut-off determined by a ROC analysis

Legend: COPD = chronic obstructive pulmonary disease; CDI = Clostridium difficile infection; PPI = proton pump inhibitors; CKD = chronic kidney disease; HF = heart failure; AF = atrial fibrillation; DM = diabetes mellitus; AH = arterial hypertension; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

The predictors that had statistical significance in the simple model were later used in a multiple univariate binomial logistic regression, the model being built with the help of a retrograde selection algorithm of the predictors (Table 3). This statistical analysis identified COVID-19 as an independent risk factor, with an approximately 3-fold increase in the risk of death (95% CI 0.39 (0.16 – 0.94); p=0.036). Other factors associated with an independent increase in the risk of death, among the patients included in the study, were: the presence of CKD, with a 3-fold increase in the risk of death (95% CI 0.33 (0.15 – 0.73); P=0.007), the presence of COPD, with a 20-fold increase in the risk of death (95% CI 0.05 (0.00 – 0.33); p=0.008), the number of leukocytes ≥ 16,755/mm³, which led to an approximately 3-fold increase in the risk of death (95% CI 2.82 (1.31 – 6.22); p=0.009) and the value of serum albumin < 2.83 g/dL, which led to a 4-fold increase in the risk of death (95% CI 0.23 (0.09 – 0.50); p<0.001) (Table 3).

Table 3: Binomial univariate multiple logistic regression with predictors for death among patients with Clostridium difficile colitis

Predictors	OR (95% CI)[1]	P-value
COVID-19		
Yes	—	
No	0.39 (0.16 la 0.94)	0.036
CKD		
Yes	—	
No	0.33 (0.15 la 0.73)	0.007
COPD		
Yes	—	
No	0.05 (0.00 la 0.33)	0.008
Number of leukocytes[2] (/mm³)		
< 16755	—	
>= 16755	2.82 (1.31 la 6.22)	0.009
Albumin[2] (g/dL)		
< 2.83	—	
>= 2.83	0.23 (0.09 la 0.50)	<0.001

[1] OR = Odds Ratio, CI = Confidence Interval, [2] Cut-off determined by a ROC analysis

CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease

DISCUSSION AND CONCLUSIONS

Clostridium difficile colitis is one of the most common nosocomial infections in developed countries, for which higher morbidity and mortality rates were reported after 2000 [27]. In the USA, approximately half a million patients are diagnosed with CDI annually [28]. This phenomenon is partially explained by the spread of a more virulent strain, ribotype (RT) 027, after the year 2000, in the context of the increase in life expectancy and population aging [27,29]. However, the global burden associated with CDI decreased by 24% between 2011 and 2017 in the USA, due to the reduction of healthcare-associated infections [6]. The rates of reinfection with Clostridium difficile and mortality associated with this condition did not change significantly during this period [6, 30]. An explanation for the reduction of the global burden of CDI was represented by the natural decrease of RT 027 or as a result of the large-scale reduction in the use of fluoroquinolones [30-32]. Also, other strains that can be associated with a possible unfavorable evolution, such as RT 244 or 078, were rarely identified in the USA [33,34]. In 2016, ECDC reported 7711 cases of CDI in 20 member states of the European Union, 74.6% being associated with nursing [35]. The most frequent RT were 027 (22.9%), 001 (7.5%), 014 (6.7%), 078 (5.1%), 002 (4.2%), and 020 (4.2%) [35]. The all-cause mortality rate among these patients was 20.7% [35]. In Romania, the average prevalence of CDI is 5.2 cases per 10,000 patient days, increasing to approximately 25 cases per 10,000 patient days in the departments of gastroenterology or infectious diseases [36]. The dominant RT in Romania is 027. A study that followed 393 patients with CDI identified RT 027 in 82.6% of cases (95% CI: 73.6%-89%) [36]. Other identified RT were: 002, 018, 087, 014, 001, 011, 012, 017, 020, and 106 [36].

In our study, the average age of patients diagnosed with CDI was 70.8 years, and the death rate from all causes was 25.7. These results correspond to those reported in the literature. Redelings et al. reported an increase in the mortality rate associated with CDI in the USA from 5.7 deaths per 1 million individuals in 1999 to 23.7 deaths per 1 million individuals in 2004 [37]. The same authors reported an average age of patients whose death was associated with CDI of 82 years [37]. Another study that followed 66 patients with CDI, aged over 65 years, reported the following mortality rates: 11.5% at 7 days, 26.2% at 30 days, 38.1% at 90 days, and 41.4% at 180 days. Mitchell et al. highlighted a variability of all-cause mortality at 30 days in patients who had associated CDI between 9% and 38% and of in-hospital mortality between 8% and 37.2% [38]. The directly proportional relationship between advanced age and poor prognosis among patients diagnosed with CDI was also highlighted by a multicenter study that followed patients from hospitals in Quebec [39]. These authors highlighted the increase in the 1-year mortality rate among patients with CDI from 4.7% in the 18-64 years age group to 21.1% in the 65-74 years age group [39]. In our study, increasing age by one year was associated with increasing the risk of death by 2%.

Regarding the average length of hospitalization of the patients included in our study, it varied between one day and 145 days, with an average value of 18.4 days. This result corresponds to data from the literature [40-42]. Thus, Hebbinckuys et al. reported an average length of hospitalization of 18.5 days for patients with CDI, which can vary between 6 and 132 days [40]. Another study compared two groups of patients hospitalized for various ailments: the first group consisted of 38 patients who had associated CDI and the second group consisted of 76 patients who did not have associated CDI [41]. In the first batch, the average duration of hospitalization was significantly higher compared to the second batch (31.5 days vs. 5.5 days) [41]. The conclusion of this study was the increase of the average duration of hospitalization by approximately 4 days by the association of CDI ($p<0.001$) [41]. During the study, only 5% of the patients presented a recurrence of infection with Clostridium difficile. According to ECDC, the recurrence rate of CDI in Europe is approximately 7.9% [35]. Other studies reported higher rates of recurrence of CDI, respectively 15-30% [43,44]. The factors associated with the risk of CDI recurrence are advanced age, infection with hypervirulent strains, history of CDI and their severity, longer duration of hospitalization, use of antibiotics for non-CDI after establishing the diagnosis of CDI, use of gastric acid suppression drugs or renal failure [44,46,47]. An explanation for the lower recurrence rate of CDI in the population included in our study can be represented by the absence of an infectious disease department in the hospital where the study was carried out. Under these conditions, there is the probability that in case of relapse, some of the patients would have presented to other hospitals.

Among the comorbidities of patients included in our study, we list in order of their frequency: arterial hypertension, heart failure, CKD, diabetes mellitus, atrial fibrillation, neoplasms, COVID-19, and COPD. This wide range of comorbidities can be explained by the advanced age of the patients, the average age being approximately 71 years. Feuerstadt et al. identified among the most frequent comorbidities in CDI patients: HF, COPD, diabetes without complications, peripheral vascular disease, and renal disease ($p < 0.01$) [48]. Our study validates these results, adding to the comorbidities also COVID-19, being performed during the pandemic. According to data from the literature, the association of other diseases in patients with CDI leads to an increase in the disease burden, by increasing the risk of polypharmacy, compromising the immune status, and increasing the duration of hospitalization [49]. Almost half of the patients had treatment with proton pump inhibitors (PPI). A recent meta-analysis that included eight studies from the USA, Canada, Europe, and Asia highlighted a statistically significant association between PPI use and the risk of developing CDI [50]. Inghammar et al. also highlighted the fact that the risk of developing CDI remains high even one year after stopping PPI treatment [51]. Another meta-analysis that included 356,683 patients concluded that the use of PPIs leads to an increase in the risk of developing CDI in both groups, inpatients, and outpatients [52].

From a paraclinical point of view, the statistical analysis identified leukocytosis, inflammatory syndrome, mild anemia, and hypoalbuminemia among the patients included in our study. A prospective study that followed 60 patients with unexplained leukocytosis identified the presence of Clostridium difficile toxins in fecal specimens in 58% of them [53]. The data currently available suggest the need to perform paraclinical tests in order to identify CDI in hospitalized patients who develop leukocytosis [53,54]. A series of studies demonstrated the correlation between elevated CRP values, anemia, hypoalbuminemia, and the unfavorable prognosis of patients with CDI [55-60]. Ahmed et al. have highlighted the fact that the decrease in serum albumin values and the increase in the ATLAS score (which includes age, albumin and creatinine values, the number of leukocytes, and treatment with antibiotics during CDI therapy) among patients with Clostridium difficile colitis reflect the worsening of the disease [58].

The main objective of our study was to identify the predictive factors for unfavorable evolution and death among patients with CDI. The simple univariate binomial logistic regression identified the following risk factors for death: advanced age, comorbidities such as COVID-19, CKD, and COPD, but also the values of some paraclinical markers, such as serum hemoglobin < 9.85 g/dL, leukocyte count $\geq 16{,}755/mm^3$, CRP ≥ 104.02 mg/dL and serum albumin < 2.83 g/dL. Later, the multiple binomial logistic regression analysis defined advanced age, the association of COPD, CKD, or COVID-19, the number of leukocytes $\geq 16{,}755/mm^3$, and the serum albumin value < 2.83 g/dL as independent risk factors for death. A study carried out in Taiwan, which followed 401 patients with CDI, highlighted a 30-day mortality rate of 23.4%; among the predictive factors for death were age > 65 years, serum albumin value < 2.5 g/dL, and increased creatinine by 1.5 times [61]. De Miguel-Diez et al. highlighted a doubling of the incidence of CDI and an increase in mortality among patients with COPD compared to the general population [62].

Regarding the evolution of patients who associate CDI and COVID-19, our study validates other data from the literature [63,64]. Thus, a study that followed 2065 patients with COVID-19, of whom 40 had CDI, highlighted a more severe evolution among patients who combined both diseases, with 45% of them developing complications, and 22.5% dying in hospital [63]. The extensive use of antibiotics during the COVID-19 pandemic increased the risk of CDI development [63]. According to other studies, CKD increases both the prevalence and severity of Clostridium difficile colitis [65-67]. This phenomenon can be explained by the frequent hospitalizations of patients with CKD, but also by other factors such as the frequent use of antibiotics or PPIs, alteration of the immune system, or intestinal dysmotility [65]. Keddis et al. highlighted a higher prevalence of CDI among CKD patients compared to the general population (1.49% vs 0.70%) [66]. The same authors identified in the group of patients who had both CKD and CDI, compared to patients with CKD without CDI, longer average hospital stays (7 days vs. 4 days), higher rates of colectomy (0.67% vs. 0.28%) and higher death rates (6.62% vs. 3.99%) [66].

The peculiarity of our study consists of the follow-up of patients with CDI during the COVID-19 pandemic. The mortality rate did not show significant differences compared to the one recorded before the pandemic, but the CDI recurrence rate was slightly lower compared to that reported by the literature. This result can be explained by the patients' fear of infectious diseases and their presentation in case of relapse in infectious disease clinics. The main limitation of our study is the lack of differentiation between community cases of CDI and those associated with medical assistance.

In conclusion, CDI is a public health problem due to both high incidence rates and high mortality rates, especially among elderly patients and those with important comorbidities. To improve the therapeutic management and prognosis of these patients, more effective systems for risk identification are needed. In this regard, our study sought to identify a series of predictive factors of death among patients with CDI. Patients who present the clinical and paraclinical profiles described above as being at high risk of unfavorable evolution and death require more careful monitoring for the early identification of possible complications and optimal treatment. Also, these risk factors can contribute to the correct selection between ambulatory treatment, hospitalization, or the need for intensive therapy measures.

Author Contributions

Conceptualization, V.A.I. and G.G.; methodology, R.S.C. and V.A.I.; software, F.G.; validation, C.C.D.; formal analysis, A.G.A. and F.G; investigation, F.G.; resources, R.S.C; data curation, A.G.A.; writing—original draft preparation, G.G.; writing—review and editing, C.C.D.; visualization, C.C.D.; supervision, C.C.D. and R.S.C.; project administration, V.A.I..; funding acquisition, R.S.C. All authors have read and agreed to the published version of the manuscript.

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The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Clinical Emergency Hospital of Bucharest, Romania (approval no 10002/ 04.10.2022).

Informed Consent Statement

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Not applicable.

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Conflicts of Interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Self-esteem, Self-efficacy, and Smoking Prevalence: A Cross-sectional Study Among Military and Civilian Medical Students

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Abstract: *Background: The prevalence of smoking among students is a growing concern, particularly among medical students. Being associated with stress, competitiveness, and various social influences, smoking can have detrimental effects on self-esteem and self-efficacy and may create a vicious cycle that may contribute to student's well-being and academic performance outcomes. We aimed to investigate the relationship between self-esteem, self-efficacy, and smoking habits among medical students and their sociodemographic correlates. Methods: A cross-sectional study on 456 medical students was conducted via an online survey using structured questionnaires on socio-demographic characteristics, smoking habits, nicotine dependence, self-esteem, and self-efficacy. Results: The prevalence of smoking was 31.1% The scores of self-esteem and self-efficacy were positively correlated with smoking habits (W1=0.957, W2=0.975, P<0.05). Students presented rather low smoking addiction with male students having slightly higher scores than female students on self-esteem and self-efficacy, military students had higher self-esteem scores than civilians with physical activity having a positive correlation with non-smoking behavior. Conclusion: According to our results, self-esteem and self-efficacy were slightly positively correlated with smoking habits. Furthermore, there was no statistically significant association between other social factors and smoking. Our results may develop a theoretical basis for medical students' psychological variables studying for further implementation of university educational programs and preventive interventions empowering students towards an overall better quality of life, addressing, among others, smoking behaviors.*

Keywords: *prevalence, smoking, medical students, self-esteem, self-efficacy*

INTRODUCTION

Stress is a common experience for most medical students due to the rigorous demands of their training with medical education being challenging and students often facing heavy workloads and competition pressure situations. Having to absorb and retain a large volume of complex information, time pressure, and expecting to academically perform at a high level can create a significant amount of mental distress. Major years students can have even more challenging and stressful experiences as they are often exposed to the presentation of critically ill patients and life-threatening emergencies with supplementary emotional input. With less time for socializing, hobbies, or self-care and other socio-economic difficulties, medical students can develop various stages of burnout and use unhealthy coping mechanisms, especially after the COVID-19 lockdown when coping mechanisms deteriorated with smoking and drinking, among others, important factors of the stress perceived [1,2,3].

Smoking is one way, among others, that people usually cope with stress with nicotine providing a temporary sense of relaxation. However, smoking has been linked to a variety of negative health outcomes, including lung cancer, heart disease, and stroke, comprising 16% of non-communicable diseases, in Romania [4,5]. In addition, smoking can increase stress levels over the long term by disrupting sleep, reducing exercise tolerance, and impairing lung function [6,7].

There have been several studies that have looked at the link between smoking and stress in medical school. One study found that medical students who reported high levels of stress were more likely to smoke, with around 30% of students reporting smoking, and that smoking was more common among male students [8]. In Europe, the prevalence of smoking among medical students varies from 28.02% in Germany to 31.3% in Italy, which is higher than in the general population [9] and compared to students from other faculties [10]. Stress and symptoms of anxiety and depression increase in the first two years of medical studies but decrease towards their end [11]. In doctors, stress and burnout are rather typical. Both issues could start during medical school and continue during residency and then across a lifetime. Medical schools need to be informed about the persistent and important effects of stress and burnout among their students.

The effects of smoking on various psychological variables have been a topic of interest in recent years with studies investigating the relationship between smoking and mental health, including self-esteem and self-efficacy. Smoking was negatively associated with self-esteem and self-efficacy in medical students. This negative relationship is believed to be due to the harmful effects of smoking on both physical and mental health. Smoking may affect one's social and emotional well-being, leading to feelings of isolation and reduced self-worth. Smokers may also experience academic problems, which can further reduce their self-esteem and self-efficacy [12].

Unfortunately, smoking is not the only factor that comes along with stress. A vicious cycle is formed between smoking, low self-esteem, and low self-efficacy. A study published found that these factors were significant predictors of academic performance among medical students. Higher levels of stress were associated with lower academic performance, while higher levels of self-esteem were associated with better academic performance. The study also found that there was a significant negative correlation between stress and self-esteem [13]. Medical students who smoked had lower self-esteem than non-smoking students. Similarly, a study found that smoking was associated with lower self-esteem in medical students. The concerning part is that self-esteem and self-efficacy were negatively associated with smoking and that higher levels of self-efficacy were associated with a lower likelihood of smoking. [14].

The importance of self-esteem and self-efficacy was highlighted by their influence as mediating the relationship between motor proficiency and internalizing problems along with social support. This represents an important aspect of the concept of the environmental stress hypothesis which may also relate to unhealthy coping mechanisms such as smoking [15]. In recent years, there are no studies including all three variables as included in our research. This may represent a more complete understanding of how these psychological variables impact smoking behavior. Even if there are some studies regarding military medical students [16] we found no approaches concerning this type of students in aspects of self-esteem or self-efficacy. The social component is not discussed enough as loneliness and social circle may influence smoking behavior [17].

Thus, this study aimed to examine the relationship between smoking prevalence, self-esteem, and self-efficacy in medical students. Additionally, this study`s secondary aims were to compare these factors based on socio-demographic characteristics and investigate their potential correlation. Our hypothesis was based on correlations between variables but no presumptions as to whether they were negative or positive were made in advance. Also, there is likely that coping mechanisms may represent an additional factor for smoking behavior prevalence. Moreover, military students are expected to have lower self-esteem and self-efficacy scores as their military service goes along with their medical training with stress levels being considered higher.

MATERIALS AND METHODS

Study design and participants

The design of the study was cross-sectional, comprising a survey of 22 questions and three standardized psychometric instruments. The cross-sectional design was successfully used in previous literature on addictive behaviors [18,19], including in Romania [20,21].

456 medical students from the first three years of study (33.1% men, 65.7% women, 1,09% other; mean age = 20.8, SD = 2.24) agreed to participate in the study and answered the psychometric tests.

The inclusion criteria were as follows: (1) students currently studying at the university (2) obtained informed consent and willingness to participate in the study.

Context and Procedure

The data were gathered in April–May 2023 through the administration of an online survey containing the study instruments. Firstly, we informed the participants of the study’s aims. Participation was anonymous, and students’ responses did not affect the teacher’s evaluation of the student’s academic performance.

The study procedure was run via a SurveyMonkey® interface (One Curiosity Way, San Mateo, CA, USA). Online testing in university settings is reported to ensure the speed and representativeness of the collected data [22,23].

Methods in the study were designed according to the World Medical Association`s Declaration of Helsinki. The study protocol was approved by the UMFCD Ethics Review Board (no. 14873/2023). All responses were processed anonymously and a numerical code was assigned to each respondent. The data were accessible exclusively to study researchers (LM, CGI, MS). The interpretation of the questionnaires was performed independently by two researchers (MS, CGI). Results were included in a JASP version 0.10.2 statistical software (Department of Psychological Methods the University of Amsterdam, Amsterdam, The Netherlands, https://jasp-stats.org/) database.

Questionnaires

Three standardized psychometric instruments were used to measure their smoking addiction, self-esteem, and self-efficacy scales.

(1) The Fagerstrom Test for Nicotine Dependence (FTND) is a questionnaire used to measure the degree of nicotine dependence in cigarette smokers. The answers to the 6 questions are used to calculate a total score, with higher scores indicating greater nicotine dependence. Across literature studies, the scale has been shown to have good internal consistency, good validity, and a fairly stable factorial structure [24,25]. Even if sometimes it has a low consistency (0,4-0,6), it is rather acceptable in literature [26].

(2)The Rosenberg Self-Esteem Scale (RSES) is a commonly used scale for measuring self-esteem. It consists of ten statements, to which the respondent rates their level of agreement on a 4-point scale ranging from strongly agree to strongly disagree. The scale measures both positive and negative feelings about oneself, with higher scores indicating higher levels of self-esteem. Responses are scored from 0 to 3, with the total score ranging from 0 to 30. A higher score indicates higher self-esteem [27,28].

(3)The Schwarzer and Mathias self-efficacy scale, also known as the General Self-Efficacy Scale (GSE), is a psychometric tool designed to measure an individual's self-belief in their ability to handle difficult situations and achieve their goals. It consists of ten statements that participants rate on a four-point scale, with higher scores indicating greater self-efficacy. Scores are then calculated and interpreted to provide an overall measure of an individual’s self-efficacy. [29,30].

In addition to these three psychometric tests, the participants provided information about their age, place of origin, current academic year, type of training-medical or civilian, current residence, specialty, and coping mechanisms.

Data analysis

We first described the demographics of our participants. The internal consistency of the smoking addiction, self-esteem, and self-efficacy scales was assessed using Cronbach’s alpha coefficient and all instruments had coefficients above 0.7, which is considered to be the minimum acceptable value for proper reliability [31].

We made descriptive statistics using all the data depending on the smoker/non-smoker status. Afterward, we performed T-tests to compare various groups on the three main variables studied: smoking addiction, self-esteem, and self-efficacy. Furthermore, we used Chi-Squared tests to quantify differences in smoking/non-smoking depending on the place of living and gender. Other T-tests were used to compare the three scales depending on situations when smoking, coping mechanisms, and type of cigarettes smoked.

Data management and descriptive analyses were performed using JASP statistical software, version 0.17.2 statistical software [32].

Ethical Considerations

- Participants were appropriately informed about the nature of the research.
- Informed consent was taken from the participants.
- Anonymity of participants and confidentiality of the information was maintained.
- Participants were given the right to withdraw from research at any time.
- The responses were accurately represented

RESULTS

Sociodemographic data and smoking correlates

The response rate was 10.08% (456 out of 4521 medical students invited). Data was collected from 456 students who agreed to participate in the study (151 men, 300 women, 5 not specified gender; mean age = 20.88, SD = 2.24). A total of 86.8% of the participants were Romanian module students with 14.2% reported being Military Medicine students and only 27.4 % reporting living in a student campus (complete detailed results in Table S1. Supplementary Materials). Table 1 summarizes the descriptive indicators of the socio-demographic data.

The Fagerstrom nicotine dependence test showed a low mean level of nicotine addiction among medical students (m = 1.45, SD =1.406) [33]. The data reveal interesting patterns in smoking behavior across different sociodemographic categories.

As expected, the majority of both smokers and non-smokers are from Civil Medicine (being the majority), but the proportion of smokers in Military Medicine is noticeably higher than that met in Civil Medicine students. This could suggest a potential association between the stressors or lifestyle factors specific to Military Medicine and smoking behavior. In terms of residence, while the majority of both groups reside in personal properties, there is a slightly higher proportion of smokers residing on campus, probably due to frequent casual socialization. Details can be seen in Table 2.

Table 1: Sociodemographic characteristics of the sample

Variable	Condition	Participants (n)	Percent (%)
Gender	Female	300	65.79
	Male	151	33.11
	Other	5	1.10
Study module	Romanian module	396	86.84
	English module	56	12.28
	Missing	4	0.88
Specialty	Civil Medicine	391	85.75
	Military Medicine	65	14.25
Origin	Urban	371	81.36
	Rural	85	18.64
Current residence	Campus	125	27.41
	Personal propriety	331	72.59
Smoker	No	309	67.76
	Yes	142	31.14
	Missing	5	1.10
Smoking before college	No	30	6.58
	Yes	112	24.56
	Missing	314	68.86
Psychotropic treatment	No	431	94.52
	Yes	25	5.48
Psychological treatment	No	406	89.04
	Yes	50	10.97
Total	-	**456**	**100.00**

Table 2: Smoking and some of its sociodemographic correlates

Smoker	Categories	Participants (n)	Percent (%)
No	Civil Medicine	271	87.70
	Military Medicine	38	12.30
	Campus	85	27.51
	Personal propriety	224	72.49
	Total	**309**	100.00
Yes	Civil Medicine	115	80.99
	Military Medicine	27	19.01
	Campus	40	28.17
	Personal propriety	102	71.83
	Total	**142**	100.00

Patterns in smoking: risk situations and alternative coping

Next, we will deal with two opposing factors relevant to smoking: contexts that increase the risk of smoking behaviors and coping strategies that may lower its frequency or diminish its impact. Concerning the types of contexts that trigger smoking the results are presented in Table 3. The data reveal that the majority of smokers reported experiencing academic stress (78.17%) and social smoking (90.14%) as extremely common contexts for smoking. In contrast, emotional conflicts were reported by a smaller, yet still substantial, proportion of participants (66.90%).

Table 3: Types of situations for smoking

Situations	Present	Participants (n)	Percent (%)
Academic stress	Yes	111	78.17
	No	31	21.83
	Total	142	
Social smoking	Yes	128	90.14
	No	14	9.86
	Total	142	
Emotional conflicts	Yes	95	66.90
	No	47	33.10
	Total	142	

The alternative coping to smoking can be seen in Table 4. Not surprisingly, the mean score for smoking within the group engaged in physical activities is lower (1.260) compared to the group not engaged in physical activities, with a mean score of 1.774.

Table 4: Coping Strategies for Smoking

Strategy	Present	Participants (n)	Percent (%)
Mindfulness	Yes	43	30.28
	No	99	69.72
	Total	142	
Relaxation	Yes	89	62.68
	No	53	37.32
	Total	142	
Physical activity	Yes	66	46.48
	No	76	53.52
	Total	142	

Other methods for coping with stress reported verbatim by participants are music therapy, reading, and some negative methods such as alcohol, marijuana, and binge eating. There is also another series of apparently linked factors that are not actually related to smoking behavior. We found no significant correlation of Fagerstrom nicotine dependence results with gender, language module, military vs civilian module, area, living place, previous psychotropic treatment, previous psychotherapy, and starting before entering university (All the statistical analyses results are attached in Supplementary Materials).

There is no significant difference in smoking addiction scores between students who practice mindfulness and those who do not (p>0.05). Based on these p-values, there is no convincing evidence to suggest a significant difference in the measured variables between the relaxation and no-relaxation groups.

Self-Esteem, Self-Efficacy, and their socio-demographic correlates

The Self-Esteem assessment showed practically no difference between smokers/non-smokers (30.09 vs 30.98). The prototypical participant with high self-esteem is male, a Romanian module student, a Military Medicine student, from urban areas, living on campus, with no previous psychotropic treatment and no psychotherapy.

Overall, the differences in mean scores between individuals engaged in physical activities and those who are not are relatively small across the variables.

Differences in Self-Esteem and Self-Efficacy between Smokers and Non-Smokers

Significant differences were found between non-smokers and smokers in terms of self-efficacy and self-esteem. Smokers reported higher levels of self-efficacy (p = .001, rpb = -0.198) and self-esteem (p = .044, rpb = -0.123) compared to non-smokers. The effect sizes for the significant results were small to medium, suggesting the practical significance of the findings (Table 5).

The evaluation of the effort capacity from 0 initial value has reached 10 minutes without effort disorders so that later on the final evaluation on the best day of the patient will be 15 minutes without effort disorders. We mention that every day the patient's dynamics undergo changes from one hour to the next and the time of day matters a lot.

DISCUSSION

Smoking prevalence

Only one-third of the students responding to our survey were smokers, but rather low addiction scores were reported as confirmed by the Global Adult Tobacco Survey (GATS) 2018 study in a sample of the Romanian general population, remaining unchanged since 2011 [34]. There were no gender differences regarding smoking addiction with most of the smokers living in their own houses. Also, academic stressful situations and intense emotional conflicts were reported as the most frequent situations when students smoke as another study previously reported [35]. This may represent a norm for young adults as addictive behaviors were previously reported as adopted in various stressful situations, especially work and family-related ones [36].

Table 5: Comparison of Self-Efficacy, Rosenberg Self-Esteem, and Fagerstrom Test for Nicotine Dependence Scores Between Non-Smokers and Smokers

Dependent Variable	Non-Smokers			Smokers			U	p	r_{pb}
	N	M	SD	N	M	SD			
Self-Efficacy	271	30.705	4.155	132	32.265	4.608	14350.5	.001	-0.198
Self-Esteem	265	30.098	5.1	134	30.985	5.61	15571.5	.044	-0.123
Nicotine Dependence		-	-	81	1.457	1.406	-	-	-

*Note. M and SD represent the mean and standard deviation, respectively. U represents the Mann-Whitney U test statistic. p represents the p-value for the Mann-Whitney U test. rpb represents the rank biserial correlation, which is the effect size for the Mann-Whitney U test. All p-values are two-tailed. Effect sizes were calculated using the formula rpb = 1 - (2U / (n1 * n2)), where n1 and n2 are the sample sizes for the two groups*

Otherwise, our results may present an optimistic outcome concerning the relatively low degree of addiction even in those starting smoking before entering university and taking into consideration the growth in consuming heat-ed-tobacco products (HTP) as Romania is the second Euro-pean country reporting the use of HTPs [37,38]. This perspective is confirmed by studies including Romania as being the 9th EU member state on smoking prevalence and 10th on pack-year prevalence but persisting on a descendent trend in contrast to other Eastern European countries like Bulgaria or Greece [39,40].

Concerning coping mechanisms, only physical activity had a significant correlation to low smoking addiction scores. Physical activity, among others, was highlighted as an important predictor of health outcomes across life stages, including smoking [41,42].

Self-esteem, self-efficacy, and smoking prevalence

The present study aimed to investigate the relationship between smoking addiction with self-reported self-esteem and self-efficacy among medical students. The results showed that correlations between smoking addiction and self-esteem/ self-efficacy were positive, indicating that higher levels of smoking addiction were associated with higher levels of self-esteem and self-efficacy. However, it is important to note that the correlations were relatively weak and not statistically significant. It is important to retain that only one-third of the students declared smoking with 78.8% starting before entering university. The mean scores on the smoking scale were showing rather low addiction. This may represent a point of interest in discussing why the majority of the students did not develop a higher addiction since they began smoking a few years now. Based on the results of this study and the literature as well, it seems it is necessary to conduct more studies in this area, especially longitudinal and interventional studies to practically determine the effect of self-esteem and self-efficacy improvement programs on smoking prevention or cessation.

Most of the studies rank medical students among the students with low self-esteem and self-efficacy [43,44] this leads to various mental health issues like depression, anxiety, and burnout [45]. Also, most of the studies rank them lower than non-medical students regarding smoking [46,47,48]. Few studies tried to correlate the variables of self-esteem and self-efficacy with smoking addiction levels.

Our study found results that may explain the protective role high self-esteem and self-efficacy hay have in stopping students not to develop higher levels of addiction as the years go by. Also, the finding might be a result of cumulated factors including social interactions, breaks between learning sessions, but also other coping mechanisms that interfere with these results (mindfulness, relaxation, physical therapy, etc.)

With most of the studies focused on smoking cessation and self-esteem and self-efficacy [49-52] having predictor roles for successful abstinence and not advancing in higher stages of addiction, our study stays in line with those findings. Also, happiness was associated with lower scores of smoking in young adults [53] confirming our relatively low addiction scores in our research.

While few studies correlate high self-esteem and self-efficacy with habits like smoking [54] others show negative correlations, especially among young adults progressing in smoking [55-58] with negative social capital being also linked with smoking behavior [59]. In contrast, a study shows that self-esteem may not represent a predictor for smoking [60]. This may be considered important in the light of social, economic, and cultural characteristics of the Romanian population.

Gender differences

Gender differences were observed in the study, with males tending to have slightly higher self-efficacy and self-esteem scores compared to females which is supported by previous research [61]. Also, gender can influence social expectations and perceptions, which may further affect self-esteem and self-efficacy [62]. There was no significant difference in smoking addiction scores between genders. This suggests that gender alone may not be a significant factor influencing smoking addiction.

Other social correlates

The study found that individuals in the military group had higher self-esteem scores compared to the civilian group. This suggests that the context of military service may positively influence self-esteem.

Studies have shown that military medical students may vary in matters of well-being, depression, burnout, or the tendency to leave medical school while trying to adopt more coping strategies [63,64]. Military medical students are a unique population to study in relation to smoking, self-esteem, and self-efficacy, knowing that military service members and veterans have been found to have higher rates of smoking than the general population. Military medical students may face additional stressors related to their military obligations, such as deployments, training exercises, and other demands of military service. These stressors may impact their self-esteem and self-efficacy and may also influence their smoking behavior.

Students living on campus had higher self-esteem scores compared to those who lived alone. The social environment and support provided in the campus setting may contribute to higher self-esteem as highlighted before [65].

Individuals engaged in physical activities had slightly higher self-efficacy scores and lower smoking addiction scores. Physical activity and exercise can positively impact self-esteem and self-efficacy through improved physical well-being and feelings of accomplishment. Students engaged in physical activities had lower smoking addiction scores compared to those who were not engaged in physical activities. This suggests that being physically active may have a positive influence on reducing smoking addiction and improving overall quality of life as previous research showed [66,45,67].

CONCLUSIONS

In our study, self-esteem and self-efficacy were rather high among smokers and non-smokers as well. Our study suggests that there may be other psychological variables worth taking into consideration concerning smoking habits, like personality, cultural background, social attitudes, and mental individual variables. There is a need to investigate the cultural and social aspects of smoking in Eastern European countries like Romania where smoking policy and education still have a lot of space for improvement.

Self-esteem and self-efficacy were reported as high in most of our participants. Medical students are required to have higher intellectual effort and engagement than most of the superior education systems thus there is a need for confidence and self-enhancement to perform academically at the expected standards.

Smoking may not represent at the moment, in their first three years of study, a matter of concern regarding physical or mental aspects but the lifetime perspective needs to be addressed.

So, medical students need to be aware of the negative effects of smoking on their mental and physical health, as well as their academic performance. Quitting smoking and adopting healthy lifestyle habits can lead to improvements in self-esteem and self-efficacy, which can ultimately lead to better academic performance and overall well-being. Further research is needed to explore the specific mechanisms underlying the relationship between smoking and self-esteem/self-efficacy in medical students. It becomes crucial to address this issue through preventive interventions. By promoting smoking cessation and the adoption of healthy lifestyle habits, it is possible to break the cycle and potentially improve self-esteem and self-efficacy among students. These improvements, in turn, can lead to positive outcomes in academic performance and overall well-being.

Smoking is a major public health concern and understanding the factors that contribute to smoking behavior can help inform interventions and policies aimed at reducing smoking prevalence considering Romania already signed the WHO Framework Convention on Tobacco Control (FCTC), when a national campaign was launched as “the First Generation without Tobacco” endorses the WHO ‘tobacco end game’ by 2035 [68,69,70].

Understanding the relationship between smoking, self-esteem, and self-efficacy can help identify potential targets for intervention and provide insights into the psychological processes underlying smoking behavior. Medical students are at a critical point in their education and career development; addressing smoking behavior among medical students can have important implications for their future patients, as well as for the broader medical profession.

Limitations

This study has several limitations. The sample size was rather small, and the participation in the survey was based on self-selection. The sample was asymmetric in terms of gender and origin while the design of the study was cross-sectional, not allowing the assessment of the study variables in their long-term perspective. Also, the sample size only included the first three years of medical students mainly from Bucharest and Muntenia southern region of Romania. This may reduce the generalizability of the findings, taking into consideration cultural, social, and attitude aspects toward smoking.

Future Research

Next, studies on this subject could be run longitudinally and include other potentially relevant variables (e.g., pertaining to personality or other psychopathological variables). Moreover, a distinct line of development could be represented by the creative use of the qualitative methodology among medical students.

The in-depth evaluation of personality traits, mental and physical well-being, and social circle employing, for example, self-enhancement or counseling groups, could not only supplement the data, but also offer a valuable opportunity for students to reflect on the complex etiology of smoking and other addictive behaviors, and assess them in relation to their life and academic goals.

Author Contributions

M.L. and M.S. contributed to the design of the work. M.S. conducted the data collection. M.S. and C.G.I. conducted the data analyses. M.S. contributed to the interpretation of the results. C.G.I., S.P., M.S., and M.L. contributed to drafting the manuscript. M.L. and S.P. supervised the development of the work and helped with manuscript evaluation. M.S., S.P., and C.G.I. helped to evaluate and edit the manuscript.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the „Carol Davila" University of Medicine and Pharmacy — Bucharest, Romania (no. 7872/2021).

Informed Consent Statement

Written informed consent has been obtained from the participants to publish this paper" if applicable.

Data Availability Statement

The data presented in this study are available on reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare that there is no conflict of interest

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REVIEW

Cardiotoxicity of Chemotherapy in Lynch Syndrome – A Literature Review

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Abstract: *Chemotherapy is an important treatment in oncological disease, with a vast number of side effects. The cardiotoxicity of several chemotherapeutic agents and appropriate risk stratification and patient follow-up must be ensured by a multidisciplinary team which must include an oncologist and a cardiologist. Lynch syndrome is associated with younger-onset malignant tumors of various localizations, requiring aggressive chemotherapy. FOLFOX chemotherapy which is frequently used in Lynch syndrome-associated colorectal cancer has several cardiotoxic effects with mechanisms ranging from increased reactive oxidative species to Krebs cycle blockade or coronary vasospasm. These complex effects on the cardiovascular system have varied clinical effects, such as heart failure, arrhythmias, or acute ischemic events*.

Keywords: *heart failure, bradycardia, cardiotoxicity, chemotherapy, Lynch syndrome, FOLFOX, colon cancer, cardio-oncology*

INTRODUCTION

Cancer therapy is life-saving for patients requiring such medication. However, a wide array of side effects have been described, depending on the treatment used and the history and risk factors of the patient. Cardiotoxicity has been described as a side effect of many chemotherapic drugs.

In young patients with oncological disease, the diagnosis is usually determined at a later stage, requiring more aggressive chemotherapy. Moreover, younger-onset cancers might be associated with hereditary diseases, such as Lynch syndrome. This syndrome is associated with early-onset cancers of varied localizations, from colorectal cancer to endometrial cancer.

We present Lynch syndrome chemotherapy cardiovascular side-effects for the most commonly associated cancers.

DISCUSSION

Cancer is no longer a pathology reserved for the elderly. Several studies reveal an alarming increase in the incidence of colorectal cancers for young patients. Colorectal cancers at a younger age are more often localized on the left colon, are less differentiated, more advanced at diagnosis, and have a higher prevalence of signet cells. 30% of early-onset patients have hereditary cancer-predisposing syndrome mutations and 20% have familial colorectal cancer [1,2]. In clinical practice, still, in a young patient, cancer is often a diagnostic of exclusion, rarely taken into consideration due to its low incidence and nonspecific signs and symptoms. There are several distinct characteristics of cancer in the young population. These people often have no other diseases requiring frequent medical visits which might lead to a delayed diagnosis, with no potential surgical treatment. Moreover, the chemotherapy regimen used in advanced cancers is more aggressive, both in dosage and in the agents required, leading to various potential side effects and drug interactions that have to be managed by the medical team. The cardiotoxicity of these compounds is variable and has to be taken into consideration and monitored accordingly [3].

To assess the cardiac toxicity of various chemotherapy agents, several tests can be used – ECG, biomarkers (troponin, natriuretic peptides), and imaging. Cardiac echography is the most used imagistic test. Advanced echocardiographic methods using myocardial deformation analysis are important, as they can detect early alterations of cardiac function. Using Color Doppler tissue imaging, strain, and strain rate data can be collected and plotted to assess global and regional cardiac systolic function. Diastolic strain and strain rate data can be obtained, but it is not widely used. Global longitudinal strain reduction has been used as a parameter of global cardiotoxicity because it is modified earlier than LVEF in patients undergoing chemotherapy. However, there is significant inter-vendor variability in GLS cut-offs. Moreover, some agents such as anthracyclines may cause peak strain rate reduction even before inducing diastolic dysfunction, which suggests that some chemotherapic agents may cause initial systolic dysfunction without diastolic dysfunction [4].

An important cause of cancer in young patients with no other comorbidities is Lynch syndrome, which is the most common cause of inherited colorectal cancer (hereditary nonpolyposis colorectal cancer). It is an autosomal dominant disorder caused by mutations in various DNA mismatch repair genes, such as MLH1 (3p22.2), MSH2 (2p21-16), MSH6 (2p16.3), PMS2 (7p22.1), leading to early carcinogenesis. Mutations in the MSH2 gene are the most frequent, followed by MLH1 mutations [5], [6]. Loss of MLH1 expression is exemplified in Figure 1.

Figure 1: Loss of MLH1 expression in the tumoral cell mass

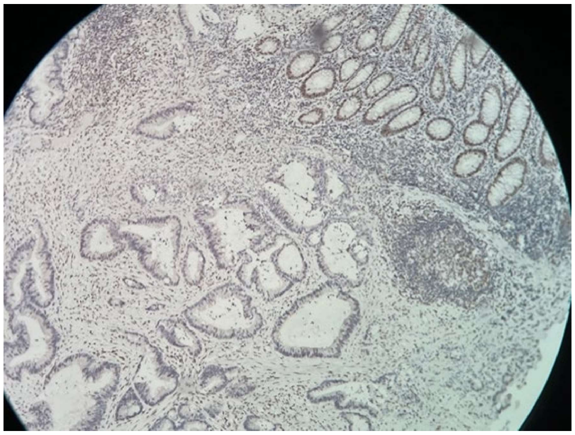

There are several cancers associated with Lynch syndrome, such as colorectal, endometrial, gastric, ovarian, renal/ ureteral, prostatic or skin cancers. The incidence of each type of cancer varies with the mutation involved in the etiology of Lynch syndrome. The classical aspect of Lynch syndrome associated with colon cancer can be seen in Figure 2. The prognostic of the affected individuals and the personalized screening program varies according to several variables. These variables include the pathogenic variant involved, the personal and familial history of cancer, and age at the first oncologic diagnostic. The most frequent malignancy is colon cancer, which is most frequently right-sided and can be associated with synchronous or metachronous tumors of different localization. Furthermore, the adenoma-carcinoma sequence in Lynch syndrome is accelerated and the cancer can develop directly from mucosal crypts in the colon [7].

Figure 2: HE, 10X, Mucinous colon adenocarcinoma with lymphocytic infiltration

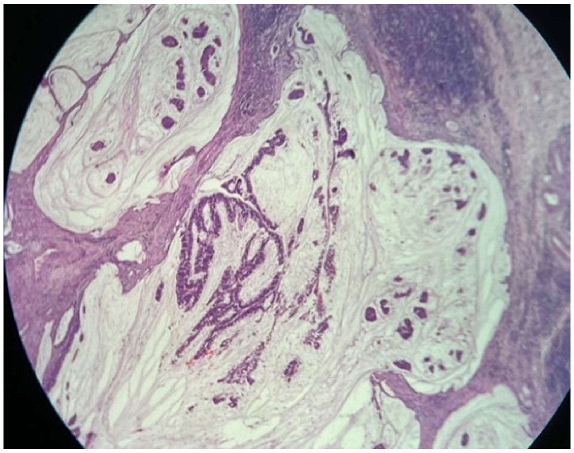

For about 50% of females that carry one of the LS mutations, the first malignant lesion is an endometrial carcinoma. Women with Lynch syndrome have a lifetime cumulative risk is 40-60%. This risk is similar to or exceeds their risk of colorectal cancer [8].

In young individuals with malignant tumors, genetic testing of the patient and their family members is paramount. All patients with colorectal cancer must be evaluated for DNA mismatch repair gene deficits by immunohistochemistry or microsatellite instability (MSI) by PCR [9]. Revised Bethesda Guidelines can be used to assess the need for oncogenetic screening.

According to the aforementioned guidelines, oncogenetic screening should be performed to [10]:

1. Patients with a diagnosed colorectal cancer before 50 years of age;

2. Patients with synchronous or metachronous colorectal tumors or with any other HNPCC-associated tumors, regardless of the patient's age;

3. Patients with colorectal cancer with MSI-H histology before 60 years of age;

4. Patients with colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one cancer being diagnosed before 50 years of age;

5. Patients with colorectal cancer are diagnosed in at least two first or second-degree relatives with HNPCC-related tumors,

regardless of age.

Amsterdam criteria have been modified since their original proposal, however the 3-2-1 rule remains useful in clinical practice, due to its' simple applicability: at least three relatives should have histologically confirmed CRC, spanning at least two successive generations and with at least one colorectal cancer being diagnosed before 50 years of age [11]. However, in current practice, the guidelines recommend the use of the Revised Amsterdam criteria. These criteria diagnose Lynch syndrome in patients with at least three relatives with HNPCC-associated cancer (colorectal cancer, endometrial cancer, enteral cancer, ureteral cancer, or renal pelvis cancer) associated with [12]:

1. One relative should be a first-degree relative to the other two;
2. At least two successive generations should be affected;
3. At least one patient should be diagnosed before 50 years of age;
4. Familial adenomatous polyposis should be excluded;
5. Tumors should be confirmed by pathological examination.

Cardiotoxicity of chemotherapy agents has been classified into type 1 and type 2 agents. Class 1 agents have dose-related cardiotoxic effects, that are mostly irreversible. They are cytotoxic and cause necrosis and apoptosis of cardiac cells, increasing troponin levels. Class 1 agents have a variable incidence of LV dysfunction, its' incidence increasing in the following order: antimicrotubule agents (docetaxel/paclitaxel) <alkylating agents (cyclophosphamide, 5-FU) <anthracyclines (doxorubicin, idarubicin, etc.). Cyclo-fosfamide may cause hypertension, myocardial infarction, Raynaud phenomenon, or hepatic vein occlusion [13]. Class 2 agents have variable cardiotoxicity even in the same category. Class 2 agents have a more complex pathophysiology, but their effects are generally reversible. In this class, anti-HER2 agents, VEGF inhibitors, and TK inhibitors are to be mentioned. They cause cellular dysfunction, leading to transient LV dysfunction [14–18].

One of the most important chemotherapy regimens in colon cancer is the FOLFOX regimen, which includes 5-fluorouracil, Folinic acid, and Oxaliplatin. The addition of oxaliplatin reduces relapse in stage III colorectal cancer compared to a regimen of 5-Fluorouracil and folinic acid [19]. To prevent cardiotoxicity development in cancer patients, the new guidelines for Cardio-oncology recommend risk stratification tools, such as the HFA-ICOS risk stratification [20]. In our presented case, VEGFi therapy cardiotoxic risk stratification could be guided by this score. Risk factors and their stratification in VEGFi therapy are exemplified in Table 1.

Table 1: Risk stratification for VEGFi

M1 risk factors	M2 risk factors	H risk factors	VH risk factors
Elevated baseline cTn or NP	Arrhythmia	DVT/PE	HF/Cardiomyopathy/CTRCD
Radiotherapy to left chest/mediastinum	450 ≤ QTc < 480 ms (men) 460 ≤ QTc < 480 ms (women)	QTc ≥ 480 ms	MI/PCI/CABG/Stable angina
Age 65-74	LVEF 50-54%	LVEF < 50%	Arterial vascular disease
CKD, Proteinuria, DM or hyperlipidemia		Age > 75 years	
Smoker/smoking history Obesity (BMI>30 kg/m^2)		HTN	

cTn, cardiac troponin; NP, natriuretic peptides; CKD, chronic kidney disease; DM, diabetes mellitus, BMI, body mass index, QTc, corrected QT; LVEF, left ventricle ejection fraction; DVT, deep venous thrombosis; PE, pulmonary embolism; HTN, arterial hypertension; HF, heart failure; CTRCD, cancer therapy-related cardiac dysfunction; MI, myocardial infarction; PCI, percutaneous intervention; CABG, coronary artery bypass graft;
Risk level: low risk (no risk factors or one M1 risk factor), moderate risk (moderate risk factors with a total of 2-4 points; M1 = 1 point, M2 = 2 points), high risk (moderate risk factors with a total of at least 5 points or any high-risk factor), very-high risk (any very high-risk factor)

Fluoropyrimidines are a class of chemotherapy drugs frequently used in digestive neoplasia and breast cancer. This class includes drugs such as 5-Fluorouracil (administered IV), its' metabolic precursor capecitabine (administered po), and others, such as Tegafur, or TAS-102. They act by inhibiting thymidylate synthase, blocking the synthesis of nucleic acids, and causing the insertion of 5-FU metabolites in cells' nucleic acid. Not only do these effects occur in cancer cells, but also in normal cells of various localizations, causing cellular dysfunction in many tissues [21]. The most frequent cause of fluoropyrimidine toxicity is caused by a deficiency of dihydropyrimidine dehydrogenase, an important enzyme in the metabolism of fluorouracil, which is the first-rate limiting step in its catabolic pathway. The deficiency of this enzyme's activity leads to the accumulation of fluoropyrimidine in the body, increasing the risk of dose-related chemotoxicity. This enzyme is encoded by the DPYD gene, which has important interindividual variability [22]. In clinical practice, dose adjustment must be taken into consideration by testing for DPYD polymorphisms or by testing the patient's pharmacokinetics of the drug.

Moreover, the duration of 5-FU infusion is also important to prevent toxic accumulation of 5-FU. Elastomeric chemotherapy pumps are very important to ensure a slow release of the chemotherapic agent, with an infusion duration of 46 hours. The medical engineering of newer, more accurate elastomeric pumps may lead to a lower incidence of over- or under-dosing. Furthermore, different methods can be implemented to detect and reduce pump dysfunction, such as pump weighing by the patient [23]. Differences between 5-FU and capecitabine-related cardiotoxicity have been reported, with studies reporting a higher incidence of cardiotoxicity in the capecitabine group [24]. However, whereas exposure to capecitabine was more often associated with chronic heart failure compared to 5-FU, exposure to the latter was associated with cardiovascular diseases [25]. However, in the literature, there have been cases of severe reversible LVEF reduction described, from 65% to 15%, after the first dose of 5-FU containing chemotherapy [26].

Mechanisms of 5-FU-related cardiotoxicity have not been completely determined. Various mechanisms have been suggested, such as coronary vasospasm, direct myocardial injury, vascular endothelium dysfunction with associated thrombosis, or a-fluoro-b-alanine accumulation which causes a Krebs cycle blockade due to fluorocitrate accumulation [27–30]. Focal coronary vasospasm was documented in the early 1990s by Luwaert and co after 2 boluses of 5-FU. Moreover, in both animal and human models, endo-thelium-independent vasoconstriction has been associated with the administration of progressively higher doses of 5-FU [31]. Moreover, fluoropyrimidine-induced diffuse coronary spasm is caused by endothelial cytotoxicity. This has been demonstrated by cell edema, cytolysis, and vessel wall and cell contraction, associated with platelet adhesion, aggregation, and fibrin formation. A proposed mechanism is via endothelial nitric oxide synthase (eNOS), which is an important factor of vascular tonus. Furthermore, patients with 5-FU cardiotoxicity have increased levels of free radicals and endothelin-1, which is an important vasoconstrictor [32,33]. Dihydropyrimidine dehydrogenase, an enzyme involved in the 5-FU catabolism, also plays an important role in the myocardial impairment associated with fluoropyrimidine therapy [34].

3 platinum-based drugs are frequently used in the treatment of cancer: cisplatin, carboplatin, and oxaliplatin. Platinum-based drugs cause crosslinking of DNA, inhibiting nucleic acid replication and transcription. Moreover, it inhibits DNA repair and synthesis, leading to cell apoptosis. There are various side effects reported for this class of drugs, such as nephrotoxicity (AKI, renal tubular acidosis, hyperuricemia, hypomagnesemia), ototoxicity, neurotoxicity (peripheral sensory neuropathy), and cardiotoxicity. Various cardiological side effects of platinum-based drugs have been reported in the last decade, such as arrhythmias (ranging from sinus bradycardia to tachyarrhythmias or complete heart block), vasospasm (which might result in angina, acute coronary syndrome, or stroke), heart failure or thromboembolic events, hypertension, thrombosis (including cerebrovascular events). More data suggests that vascular toxicity is very important in platinum-based chemotherapy. Persisting circulating platinum causes endothelial dysfunction, increasing endothelial injury markers such as von Willebrand factor, tissue-type plasminogen activator, and plasminogen activator inhibitor-1. Moreover, an increased carotid-intima index has been reported [35–40].

Folinic acid (leucovorin) is added to 5-FU because it potentiates the effects of the latter [41]. Being used as an enhancer for 5-FU, the cardiotoxic effects of folinic acid alone have not yet been described.

Vascular endothelial growth factor signaling pathways are involved in both normal and pathological angiogenesis and ensure appropriate vascular permeability. VEGF binds to tyrosine kinase receptors, having different effects in different cells depending on the type of VEGF receptor [42]. Vascular endothelial growth factor inhibitors such as bevacizumab are used in the treatment of numerous cancer types with various localizations, ranging from colon to thyroid. However, VEGF inhibitors are associated with cardiovascular morbidity. The most frequently reported cardiovascular side effect is arterial hypertension, which is dose-dependent and reversible upon drug discontinuation. The mechanism is related to the effect of anti-VEGF drugs on cancer cells, by inhibiting VEGFR2, leading to reduced NO production in vessels, increasing ROS, and determining vasoconstriction [43]. Several mechanisms involved in VEGF inhibitors-induced hypertension were also described: vascular remodeling along with a decrease in microcapillary density (vascular rarefaction) that subsequently leads to an increased systemic vascular resistance, increased endothelin-1 levels, or autonomic nervous system toxicity [44].

Other side effects are heart failure, acute vascular events (arterial events such as aortic dissection, stroke, vasospasm, arterial and venous thrombosis, and thromboembolism), and significant QTc prolongation [42,45–49].

Even if VEGFi associated FOLFOX regimen has been efficient in HNPCC-associated colorectal cancer, this is not a specific treatment. Lynch syndrome is associated with microsatellite instability and deficient mismatch repair, and specific immunotherapy with immune checkpoint blockers is highly efficient. Molecules such as Pembrolizumab (anti-PD-1 antibody, FDA approved in 2017) and Nivolumab [50] are available in the US and might come into current practice and national guidelines in Romania in the following years. In animal studies, PD-1 deletion causes autoimmune myocarditis in murine models, which was confirmed in some human trials. Anti-PD-1 treatments promote myocardial infiltration with both CD4+ and CD8+ T cells, with an increased activation level of the latter (which are also called cytotoxic lymphocytes). This increases the risk of autoimmune reactions in the myocardium and a dysregulated cellular metabolism, with proteomal alteration [51]. More PD-1 inhibitor-associated cardiovascular side effects are arrhythmias, cardiomyopathy (such as dilatative cardiomyopathy or Tako-Tsubo syndrome), pericarditis, or myocardial/cerebral ischemia. The proarrhythmic mechanism is considered to be autoimmune when myocarditis causes intracardiac conduction system alterations. This can lead to atrioventricular blocks. Another proarrhythmic mechanism is indirect. Immune checkpoint inhibitors can cause autoimmune thyroiditis which is associated with atrial fibrillation. The monitoring of cardiac side effects is very important, as CV events have a significant mortality. Tropoponin is a sensitive test for ICI-associated myocarditis, however non-specific, particularly in late-presentation patients [52,53]. Higher cardiovascular toxicity has been reported in dual ICI-therapy-treated patients (ipilimumab and nivolumab) [54–57]. Another important side effect that has been documented in immune checkpoint inhibitor anticancer therapy has been vasculitis, the most frequent one being temporal arteritis [58].

Endometrial cancer is also a frequent Lynch syndrome-associated neoplasia, with an incidence similar to CRC in women with Lynch syndrome. The chemotherapy regimens required to treat these malignancies are extremely potent, however, the patients develop significant side effects. The classes used are more varied than in CRC. Alongside already discussed alkylating agents, VEGFi, and platinum salts, we mention:

- Anthracyclines (such as Doxorubicin);

- Immune checkpoint inhibitors (Pembrolizumab, Dostarlimab);

- Taxanes (Paclitaxel, Docetaxel);

- HER2-inhibitors (Trastuzumab);

- TKI (Pazopanib, Lenvatinib).

These classes are not used at the same time, however, the arsenal of agents that can be used is more vast and the cardiovascular side effects must be taken into consideration. NCCN guidelines recommend cisplatin + radiotherapy + carboplatin/paclitaxel in chemoradiation therapy and carboplatin + paclitaxel + another agent (pembrolizumab or dostarlimab or trastuzumab depending on the stage of the tumor or its' HER-2 status).

Anthracyclines are frequently associated with left ventricular dysfunction and act by altering topoisomerase enzymes. These enzymes are responsible for DNA unwinding to allow nucleic acid replication. When topoisomerases are inhibited, tissue growth is impaired, causing oxidative stress pathways to become more active (such as MAPKs or SAPKs), which are important in cell survival, leading to programmed cell death in many tissues, such as the myocardium. [59] There are several other mechanisms proposed for anthracycline cardiotoxicity, such as impaired mitochondrial function, calcium homeostasis dysfunction, and autophagy. Moreover, it downregulates DNA-methyltransferase 1 (DNMT1), leading to hypomethylation and mitochondrial gene dysfunction (peroxisome proliferator-activated receptor γ coactivator-1-α/PGC-1a, nuclear respiratory factor 1/NRF-1, mitochondrial transcription factor A/TFAM). In cardiac cells, oxidative stress associated with doxorubicin damages the CKMB isoenzyme, generating peroxynitrite free radicals. Furthermore, cardiomyocyte apoptosis is stimulated by increasing heat shock factor 1 (HSF-1) which induces heat shock protein 25 (HSP-25). HSP-25 induction stabilizes the p53 protein, generating proapoptotic factors such as FasL, Fas, and c-Myc [60,61].

Taxanes can cause edema, bradyarrhythmias, or hypotension. They frequently potentiate anthracycline toxicity by increasing plasma levels of the latter, but may directly cause cellular lesions in cardiac cells by microtubule alterations, leading to an ineffective cytoskeleton required for cell regulation and proliferation [62]. In animal models, Paclitaxel increased TNF-α and Jun N-terminal kinase JNK expression, leading to cardiomyocyte apoptosis [63].

HER-2 inhibitor Trastuzumab (which is the prototype of class 2 chemotherapy drugs regarding cardiovascular toxicity) is associated with left ventricular dysfunction or heart failure. This is very important in anthracycline-treated patients, as they cause increased expression of HER2-HER4 survival pathways which are targeted by Trastuzumab, leading to cardiac dysfunction by ineffective HER2-HER4 pathways [64]. One suggested mechanism that has been observed in animal models is ferroptosis, which is a form of iron-induced cell death. Herceptin causes increased cellular in mitochondrial ROS and altered protein expression of glutathione peroxidase and recombinant solute carrier family 7 member 11 (SLC7A11) which were reversed by ferroptosis inhibitor ferrostatin-1 [65]. Another observed mechanism of HER cardiotoxicity has been by trastuzumab phosphorylation of HER1 and HER2 which triggers the autophagy-associated inhibitory Erk pathway in human cardiomyocytes, leading to cellular toxins accumulation in these cells [66]. However, the most likely mechanism of Herceptin cardiotoxicity is binding to an extracellular domain of HER2, inhibiting HER2-ErbB4 dimerization, which downregulates HER2 expression and triggers signaling of the PI3K-AKT-mTOR pathway. This pathway is involved in cell growth and proliferation. Furthermore, in anthracycline-exposed patients, myocardial cells are already undergoing apoptotic or necrotic cellular modifications, being more susceptible to trastuzumab cardiotoxicity and myocardiocyte death during HER-2 inhibitor treatment [67,68].

TKI has a wide range of side effects, ranging from hypertension or ischemia to QT prolongation, arrhythmias, or thromboembolism (venous or arterial) [69]. These effects can be explained by the complex intracellular mechanisms that can be altered in tyrosine kinase inhibitor treatment. One of these mechanisms is the increased endoplasmic reticulum stress pathways activation, inducing cellular change and increased reactive oxygen species and lipid peroxidation [70].

Endometrial cancer survivors have an overall higher cardiovascular risk, even after finishing chemotherapy. These patients have a higher incidence of arterial hypertension and vascular disease. Moreover, endometrial cancer survivors had a 47% higher risk of heart disease compared with patients with no endometrial cancer history [71]. This higher risk can be caused by early menopause induced by hormonal treatment (which is associated with increased cardiovascular risk) and surgical treatment by hysterectomy with bilateral adnexectomy (except for stage IA G1 endometrial cancer, according to the ESMO clinical practice guidelines) [72]. The cessation of estrogen cardiovascular protection leads to vascular dysfunction, arterial hypertension, and redistribution of body fat [73]. Thus, patients with Lynch syndrome-associated endometrial cancer are at significant risk of cardiovascular disease because of various factors – early menopause – surgical hormonal, and chemotherapy.

To prevent cardiotoxicity development in cancer patients, the new guidelines for Cardio-oncology recommend risk stratification tools, such as the HFA-ICOS risk stratification [20]. Risk factors and their stratification in VEGFi therapy are exemplified in Table 1.

CONCLUSION

Lynch syndrome-associated cancers are aggressive and require significant chemotherapy regimens. Some effects are transient; however, some may be permanent. This is an important reason for the necessity of introducing a consistent interdisciplinary team that includes a cardiologist and an oncologist in the management of oncological patients, especially young ones, such as patients with Lynch syndrome.

Conflicts of interest and sources of funding

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Patient consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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REVIEW

Emotional Regulation Strategies in Lynch Syndrome Diagnosis Psychological Adjustment - A Review

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Abstract: *The purpose of this article is to investigate the role of emotional regulation (ER) as a process underlying a variety of symptoms associated with Lynch syndrome (depression, anxiety, fear of cancer recurrence (FCR), insomnia, fatigue, pain, and subjective cognitive difficulties in patients with Lynch syndrome. Firstly, we provided an overview of the main psychopathological symptoms found in cancer patients and their impact, and then an overview of emotional regulation strategies and discussed the main impact ER strategies have on cancer patients, especially emotional suppression and experiential avoidance, as found in evidence-based studies. Recent research has shown that two ER strategies generally considered maladaptive, emotional suppression and experiential avoidance were significantly associated with higher levels of anxiety, depression, FCR, fatigue, and cognitive difficulties. We propose that experiential avoidance and emotional suppression may be common transdiagnostic mechanisms for a common set of psychological symptoms in Lynch syndrome patients, a hypothesis to be tested in a future study*.

Keywords: *emotional regulation; experiential avoidance; emotional suppression; Lynch syndrome*

INTRODUCTION

With genetic diagnosis being available for a high number of hereditary syndromes the number of families with knowledge about hereditary cancer is also increasing. Lynch Syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is a multi-cancer syndrome caused by inherited DNA mismatch-repair gene mutations that pose particularly high risks for colorectal, gynecological, stomach, small bowel, pancreas, hepatobiliary tree, brain and urinary tract cancers.

Genetic counseling provides information about traits, possibilities, and recommendations for cancer-preventive programs.

Particularly, in Lynch Syndrome, the diagnosis is requested by most members of a family and it is beneficial since, for example, colonoscopy reduces morbidity and mortality in colorectal cancer [1-5].

Family members with hereditary cancer often experience death from cancer from close relatives and it can be assumed this may represent a burden for them with studies addressing psychological well-being in those individuals with increased risk of developing cancer. Studies are primarily focusing on genetic counseling time framework and found increased levels of distress and anxiety with the return to baseline 6-12 months after the diagnosis.

10% maintained distress related to the diagnosis after the same period [6-8]. Meanwhile, there are few studies concerning the psychological impact of Lynch Syndrome diagnosis.

Thus, our research questions focused on highlighting the main psychological symptoms that are associated with cancer diagnosis and specifically, Lynch Syndrome patients. At the same time, we wanted to assess the literature concerning the main emotional regulation strategies used by patients with cancer and/or Lynch Syndrome patients.

After assessing the main variables described above our main research question concerned the possible relationship between specific emotional regulation strategies and psychopathological symptoms in cancer patients and, specifically, in Lynch Syndrome patients.

MATERIALS AND METHODS

Studies included in the review consisted of published English-language peer-reviewed articles focused on assessing correlations between various emotional regulation strategies and mental health outcomes in patients diagnosed with Lynch Syndrome. We utilized the following databases: Google Scholar, Elicit, PsycINFO, and conducted electronic searches from June 2023 to August 2023, examining articles published at any time prior to June 15, 2023. Searches were conducted with terms related to Emotional Regulation strategies (i.e., “emotional regulation,” “suppression”, „experiential avoidance” and Lynch Syndrome patients (i.e., “Lynch Syndrome,” “cancer genetic testing” “Lynch Syndrome mental health”).

The concepts of „emotional regulation strategies” and „Lynch Syndrome diagnosis” were scarcely mentioned throughout the literature thus we included broader concepts like „cancer patients”, „emotional regulation in cancer patients” and „mental health in cancer patients” to attain a broader perspective on our primary objectives. All published articles found using the above search terms and deemed to be related to the topic of focus were included based on their relevance and familiarity with the hypothesis asked.

EMOTIONAL REGULATION (ER) AND LYNCH SYNDROME

ER may play a significant role in the mental adjustment of individuals diagnosed with Lynch syndrome. It may represent a key factor in mental adaptation to this diagnosis in various contexts as follows: a) coping strategies, where ER helps individuals with Lynch syndrome cope with the stressors within the disease.

Communal coping, which involves perceiving stressors as a shared problem and mobilizing adaptive resources together, can be an effective strategy for families affected by Lynch syndrome [9]; b) psychopathology and well-being where ER strategies can be adaptive or maladaptive depending on their function, context, and timing. Effective ER can contribute to better mental well-being and reduce psychopathological symptoms in individuals with mental disorders [10,11].

Also, ER can help individuals manage the psychological impact of the condition and maintain a better quality of life; c) social support and emotional expression, where ER is closely linked to situations, for example, when individuals perceive their social network as less responsive, they may suppress their emotions and not express them openly. This emotional suppression can contribute to distress in individuals facing an increased genetic risk of cancer [12]; d) trauma and emotion regulation. Previous research suggests that trauma, including early life stress, can affect the acquisition of appropriate ER skills. Individuals who have experienced trauma may have difficulties understanding and regulating their emotions. This can have implications for individuals with Lynch syndrome who may have experienced traumatic events related to their condition [13,14].

Overall, emotional regulation may play a crucial role in the mental adaptation of individuals with Lynch syndrome. It may help them cope with stress, maintain mental well-being, navigate social support, and manage the psychological impact of their condition. Developing effective emotional regulation skills can contribute to better mental health outcomes for individuals with this diagnosis.

MENTAL HEALTH AND LYNCH SYNDROME

Lynch syndrome can have a significant impact on the mental health of affected individuals and their families. Individuals, whether they have had cancer, may experience an increase in depression and anxiety scores post-disclosure [15]. A study found that 10-12% of Lynch syndrome carriers displayed depressive symptoms [16].

Long-term psychosocial and behavioral adjustment in individuals receiving genetic test results in Lynch syndrome showed that participants who underwent genetic testing for Lynch Syndrome (LS) had higher levels of anxiety and depression compared to those who did not undergo testing [17]. Also, individuals with Lynch syndrome may experience long-term psychosocial and behavioral adjustment issues [17]. This can include difficulties with coping, emotional regulation, and social support.

An important part of the mental health impact factors is represented by the structure of emotional support networks in families, which can be affected. Communal coping, which involves perceiving stressors as a shared problem and mobilizing adaptive resources together, can be an effective strategy for families affected by LS [17,18]. However, if individuals perceive their social network as less responsive, they may suppress their emotions and not express them openly. This emotional suppression can contribute to distress in individuals facing an increased genetic risk of cancer, such as LS [19]. Regarding a study found that individuals with LS may have a lower sense of coherence (SOC) and self-concept. Low SOC has been found to be associated with anxiety and depression after genetic counseling [20].

In conclusion, LS can have a significant impact on the mental health of affected individuals and their families. It can lead to depression, anxiety, difficulties with psychosocial and behavioral adjustment, social support, and self-concept.

PSYCHOPATHOLOGICAL SYMPTOMS ASSOCIATED WITH CANCER AND CANCER TREATMENTS

Anxiety and depression

In the oncological context, psychological suffering is defined as a multifactorial and unpleasant experience of a psychological nature (either cognitive, affective, or behavioral), social or spiritual, which may affect the ability to cope effectively with cancer, physical symptoms, and its treatments [21].

Psychological suffering occurs along a continuum that varies from feelings of vulnerability, sadness, and concern about the disease, which are normal responses to cancer, to problematic and disabling symptoms such as depression, anxiety, and adaptive disorders [21].

Symptoms of a depressive disorder include a depressed mood and/or decreased interest in or pleasure in activities, accompanied by fluctuations in weight or appetite, sleep disturbances, psychomotor agitation or slowness, fatigue or loss of energy, feelings of worthlessness or excessive or inappropriate guilt, concentration difficulties, and recurring thoughts of death or suicidal [22].

Anxiety disorders, on the other hand, manifest themselves in the form of cognitive disorders (e.g., attentional bias toward threatening stimuli), affective (e.g., anxiety, nervousness), behavioral (e.g., avoidance), and physical (e.g., muscle tension). Symptoms of depression and anxiety are often experienced on a transient basis, which is part of the normal process of adapting to cancer. However, the simultaneous occurrence of several symptoms (5 of 9, including one of the first two in the case of major depression), accompanied by clinically significant distress or marked impairment of the person's social, occupational, or other functioning, indicates the presence of a psychological disorder [22].

When a person experiences excessive psychological suffering in relation to what is considered normal or which significantly affects normal functioning, within three months of exposure to an identifiable stressor, such as LS, an adaptation disorder may be diagnosed. Adaptation disorder may be accompanied by depressive and/or anxiety symptoms (but may not meet the criteria for a depressive disorder and/or anxiety or other emotional and behavioral disorders [22].

Prevalence

According to a meta-analysis of 70 studies of 10,071 patients with different types of cancer, 38% of patients suffered from depression, an anxiety disorder, or an adjustment disorder [23]. In comparison, the annual prevalence of major depressive disorder in the general population is 4% [24].

Fear of recurrence/Fear of cancer recurrence (FCR)

It is a more specific form of anxiety or concern about the idea that the disease/cancer returns or progresses [25]. In the original formulation [26] FCR is not an exclusively emotional phenomenon. It is rather a multidimensional construct in which the emotion of fear can result from negative interpretations or cognitions about cancer, which in turn would be triggered by certain internal causes (for example, physical symptoms) or external (for example, a cancer television program [26,27,28]. FCR can lead to maladaptive reactions (for example, anxiety concerns, avoidance, self-examination, and the search for excessive reassurance), which in turn are likely to increase the extent of this FRC [26]. Most patients consider this symptom to be a major concern and declare that they have not received enough help to manage it [29].

Prevalence

Due to the lack of consensus on the definition and measurement of FRC, the prevalence rates of this symptom vary considerably in the literature, ranging from 24% to 70% in breast cancer patients [30,31]. FRC is reported to be higher in younger patients, who have more physical symptoms related to cancer or its treatment, or who have a less favorable prognosis (head or neck cancer, cancer recurrence, more advanced stage of cancer) [32,33].

Fatigue

Several authors identify this symptom as being the most reported one in relation to cancer and its treatments, regardless of the location or treatment received [34]. Fatigue is present during the entire course of the disease [35]. The most commonly used definition of cancer-related fatigue is that provided by the National Cancer Comprehensive Network (NCCN), which describes fatigue as a subjective and persistent feeling of exhaustion associated with cancer or oncological treatments that is disproportionate to activity level (the level of daily activity) and that does not subside with rest and interferes with usual daily activities [35,36]. Fatigue affects between 38% and 84% of breast cancer (female) patients during cancer treatment and up to 40% after treatment ends [37,38].

Insomnia (I)

This is the most reported sleep disturbance in cancer patients [39]. It is characterized by dissatisfaction related to sleep or sleep quality, which may include difficulty falling asleep, maintaining sleep through the night, or waking in the morning with an inability to fall asleep again [22]. When symptoms of insomnia are severe (minimum 30 minutes to fall asleep or nighttime awakenings) occurring at least 3 nights per week and more than 3 months and recurring despite adequate opportunities for sleep and eliciting marked distress or significant impairment in functioning, then a sleep disorder may be assumed [22]. Insomnia can occur independently (primary insomnia) or in comorbidity with another sleep disorder, with another psychopathological disorder (e.g. depression), or can be related to the effects of a substance (e.g. caffeine) or to a disease such as cancer (comorbid insomnia).

Prevalence in the overall population, 30% of people have insomnia symptoms, while 10-15% have insomnia syndrome [40,41]. In breast cancer patients, these rates range from 16% to 58% for insomnia symptoms and 26% to 46% for insomnia syndrome [42,43].

Pain (P)

Pain is a multifaceted phenomenon resulting from a complex interaction between different physiological (e.g. tissue damage), psychological (e.g. psychological stress related to pain), cognitive (e.g. the meaning attributed to pain), and social factors. Pain can be caused by the tumor itself, by its metastases (e.g., bone), but also due to oncological treatments [44,45].

Prevalence

Between 44% and 64% of breast cancer patients report pain during or after their oncological treatment [46,47]. Pain is thought to increase with disease progression, affecting up to 64% of patients with advanced or terminal cancer. More than a third of patients who experience cancer-associated pain describe its intensity as moderate or severe [47].

Cognitive difficulties (CD)

Cognitive functioning includes a range of functions such as verbal and non-verbal memory, language, attention and concentration, psychomotor functioning, reading, and writing. These include reasoning, problem-solving, mental flexibility, and working memory [48]. These functions are assessed either objectively using standardized neuropsychological tests or subjectively using self-assessment questionnaires. Cognitive difficulties may be related to cancer either directly (e.g. tumor or metastases located in the central nervous system) or indirectly (e.g. metabolic or endocrine abnormalities, infections, and oncological treatments such as chemotherapy; [49]. The main cognitive difficulties reported by patients following cancer treatments include difficulties in learning added information, memory problems, and decreased attention and concentration [50,51,52]. It should be noted that, in general, both objective and subjective measures of cognitive functioning in cancer patients are poorly associated with each other [53]. Moreover, subjective complaints of cognitive impairment, rather than the results of objective neuropsychological tests, are more closely correlated with depression, anxiety, and fatigue. Therefore, it seems that cognitive performance and perception of one's cognitive abilities are two different phenomena. In this study, only subjective cognitive difficulties will be evaluated.

Prevalence

Between 21% and 90% of breast cancer patients report subjective cognitive difficulties [52]. Considering all types of cancer combined, the highest proportions of cognitive difficulties are observed during oncological treatment [54]. While some results indicate that cognitive functioning returns to normal 6 to 12 months after the end of treatment [55], others suggest that cognitive difficulties may persist for up to 10 or even 20 years after the end of treatment [56].

THE IMPACT OF PSYCHOPATHOLOGICAL SYMPTOMS ASSOCIATED WITH CANCER AND CANCER TREATMENTS

Psychopathological symptoms associated with cancer and oncology therapy have consequences for patients. Depression, anxiety, fear of cancer recurrence, fatigue, insomnia, pain, and cognitive difficulties are all associated with poorer quality of life and impaired daily functioning [57,58]. The presence of certain symptoms, such as depression and fatigue, is also related to the increased use of healthcare [57]. In addition, depressive symptoms, hopelessness, fatigue, and poorly controlled pain are risk factors for early death wishes and /or suicidal thoughts and actions in cancer patients [59,60].

Emotional Regulation (ER)- concept definition and strategies

Emotional regulation (ER), which has been the subject of increasing interest in the field of clinical psychology in recent years, maybe a psychological mechanism that could contribute to the etiology of psychological symptoms associated with cancer. In fact, problems related to emotions or emotional regulation characterize about 75% of the mental disorders listed in the Diagnostic and Statistical Manual of Mental Disorders [61]. An increasing number of studies suggest that difficulties with ER play a central role in developing and maintaining psychopathology [62,63,64].

Some psychopathologies such as anxiety and mood disorders are diagnosed primarily based on emotional disorders, including a predominance of negative affect [65]. ER also influences perception and adaptation to acute and chronic pain, fibromyalgia, and chronic fatigue [66-69].

Emotional problems are also considered to be associated with insomnia. On the one hand, emotions that have not been adequately regulated during the day tend to reappear when it is time to go to bed, fueling concern and anxiety and also affecting sleep and creating hyperactivation that is harmful to sleep [70]. On the other hand, sleep problems could be harmful to the emotional regulation of the next day by creating emotional hyperactivation [71]. This suggests that emotional regulation problems could underpin several symptoms associated with cancer, a hypothesis that has not yet been studied [61].

Emotional problems are also thought to be associated with insomnia. On the one hand, emotions that have not been adequately regulated during the day tend to resurface when it is time to go to sleep, fueling concern and anxiety affecting sleep, and creating hyperarousal that is detrimental to sleep [70,71]. On the other hand, sleep problems could be detrimental to daytime emotional regulation by creating affective hyperactivity [70]. This suggests that problems with emotional regulation may underlie more symptoms associated with cancer, a hypothesis that has not yet been studied [61].

To fully understand ER, it is important to first define the concept of emotion. The definition varies greatly in the literature, but different authors seem to agree on three general points. First, emotions involve changes in the fields of subjective experience, behavior, and physiology. Second, emotions are dynamic phenomena that occur over a period of time that varies from a few seconds to a few minutes [72].

Third, the same emotion can be beneficial or harmful, depending on the context [72]. Fear, which leads to the avoidance of a potentially serious threat, and joy, which strengthens social relationships, are examples of beneficial emotions that adapt to their context [72].

Emotions are harmful when their intensity, duration, frequency or type are inadequate to the situation and when they give rise to maladaptive behaviors or cognitions.

The modal pattern of emotions [72] schematically illustrates how the situation, attention, evaluation appraisal and response follow one another. First, an emotion is directed towards a specific object: it has a trigger, or external situational antecedents (e.g. watching a sad movie) or internal antecedents (e.g. anticipation of an exam). Second, the trigger must receive the person's attention for there to be an emotional response. Third, the trigger is assessed according to the person's priorities (e.g. passing an exam, pleasing the other person). Fourth, several physiological, behavioral, and experiential systems coordinate to produce an emotional response. Fifth, emotion is flexible, meaning that it can be overridden (nullified) by other imperatives or responses to the situation. This flexibility is what allows emotion to be regulated [61].

ER refers to the strategies by which individuals influence the nature of the emotions they experience, as well as when and how they experience these emotions [61,72]. ER strategies can be automatic or controlled, conscious or unconscious, and can occur at any point in the sequence of the production of the emotional response illustrated in the modal pattern of emotion [61]. ER can be used to reduce, maintain, or even increase the intensity of an emotion [61].

ER has some similarities to coping strategies. Adaptation is defined as the cognitive and behavioral efforts that are undertaken to cope with external or internal demands deemed to exceed the individual's resources. Both ER and coping are processes for regulating affective processes [72]. However, coping is generally defined as a global reaction that occurs in response to a stress factor and includes a set of reactions aimed at both changing a stressful situation, as well as stress regulation related to the stressor. ER, on the other hand, incorporates reactions to specific emotions, both positive and negative, that occur in a variety of contexts, including stressful situations [72]. Both ER and coping are dynamic processes that change over time [73]. Nevertheless, compared to ER, which occurs more limited in time, coping is described as a process that can take place over a long period of time (for example, coping with mourning over several months). As mentioned above, ER may include both automatic and conscious processes, while coping is considered to primarily reflect conscious processes [73]. In addition, while ER strategies may occur just before an emotion is generated, (for example, by avoiding exposure to a situation), coping occurs after exposure to stress [72,73]. ER and coping would therefore be separate constructions that are partially juxtaposed, their main common ground is that they involve efforts to regulate emotions in response to stressful events or circumstances [73]. ER strategies can target different components of the emotional response, namely the experiential (subjective experience of the emotion), behavioral (e.g. facial expression specific to an emotion such as disgust, fear, or joy), and physiological (e.g. activation of the sympathetic nervous system [72]. ER strategies were classified into five groups, based on the point in the emotional response sequence at which they occur. Situation selection strategies refer to the decision whether or not to expose oneself to a situation that might elicit an emotion, (i.e.) which is the choice to avoid or go to certain places, people, or activities. Subsequently, coping strategies make it possible to act on the situation to change its emotional impact.

Attentional deployment strategies involve directing attention to a specific aspect of the situation. Cognitive reappraisal strategies involve changing the meaning of the situation that the person attributes to it in order to change the associated emotions. These four types of strategies are initiated before the emotional response is fully deployed (e.g., behavioral change or physiological activation), as opposed to the fifth type of strategy, response modulation strategies, which aim to modify the emotional response already produced.

Each type of strategy can be used adaptively or maladaptively, depending on the context in which it is used [72]. The later ER occurs in the sequence of producing the emotional response, the more effort it requires [61]. Intervention as early as possible in the sequence of producing the emotional response would make it possible to adjust the emotion more efficiently [74]. ER is maladaptive when it does not alter the emotional response in the desired way (e.g., does not reduce negative affect), when the long-term costs of a strategy (e.g., disruption of functioning, interpersonal relationships) exceed the benefits associated with short-term emotional changes, or when the strategy used is not contextually appropriate [61].

ER can be evaluated either subjectively, using self-assessment questionnaires, or objectively, using physiological indicators, such as heart rate variability. The ER strategies that have been most studied in both the general population and cancer patients are cognitive reappraisal, suppression of emotional expression, and experiential avoidance [61].

Cognitive reappraisal

Cognitive reappraisal involves changing the appreciation of a situation to reduce or increase its emotional intensity [61]. In Gross and Thompson's model, this strategy occurs before the emotional response is fully unfolded [75].

In the general population, the use of reappraisal is associated with higher levels of effectivity, lower levels of negative affect, fewer depressive symptoms, higher self-esteem, greater satisfaction with life, better interpersonal functioning, and increased well-being [61]. In patients suffering from depression, less frequent use of cognitive reappraisal has been associated with higher levels of depressive symptoms [76]. Other results indicate that this strategy moderates the relationship between stress and depressive symptoms, in that, in high-stress situations, the use of cognitive reappraisal makes it possible to regulate negative emotional states likely to lead to depressive symptoms [77]. In addition, reappraisal has a low impact on physiological activation and cognitive resources [61]. It is also associated with lower levels of inflammation and reduced risk of cardiovascular disease [78]. Therefore, cognitive reappraisal is considered a generally appropriate CR strategy [79]. Learning this strategy is also a key element of several psychotherapies, including cognitive-behavioral therapy [80]. Although several clinical trials have demonstrated the effectiveness of CBT in reducing anxiety, depression, pain, insomnia, and fatigue in cancer patients [81], the specific impact of cognitive reappraisal on a range of psychological symptoms associated with cancer has been poorly studied. Only one study investigated the association between this strategy (Emotion regulation questionnaire; ERQ) and anxiety and depression in 144 patients with different cancers [82]. Lower levels of cognitive reappraisal were cross linked to more anxiety and depression three months after cancer diagnosis. Other studies have evaluated the effect of a subtype of cognitive reappraisal, positive reassessment, which involves changing one's evaluation of a situation so as to give it a positive meaning [83].

In a prospective study of a sample of 509 women with breast cancer, the use of cognitive reappraisal was evaluated using the Cognitive Emotion Regulation Questionnaire, a questionnaire that assesses various cognitive ER strategies under stressful conditions (including positive reappraisal). Positive reappraisal and depressive symptoms were assessed five to seven days after oncological surgery (T1), and then depressive symptoms were re-evaluated one month later (T2). Higher levels of positive reappraisal at T1 were associated with lower levels of depressive symptoms at T2. In a cross-sectional study it was analyzed the effect of certain social strategies (avoidance of talking to loved ones about cancer concerns and unsupportive behaviors from loved ones) and cognitive strategies (positive reappraisal, self-esteem, and confidence in one's ability to cope with cancer; COPE inventory) on CRP and depressive symptoms in 150 women diagnosed with gynecological cancer. Self-esteem has significantly mediated the relationship between CRP and depressive symptoms. A higher level of positive reappraisal has been associated with lower depressive symptoms, but this strategy was not significantly associated with CRP [84].

Results from another cross-sectional study that included 353 patients diagnosed with breast cancer showed a weak correlation between positive reappraisal (Mini-Mental Adjustment to Cancer Scale) and cancer-related fatigue [85]. Nevertheless, this association was significant only in a multivariate analysis model that also included age, depression, and grief: the unique relationship between positive reappraisal and fatigue was not significant. Finally, in another study, positive reappraisal (COPE Inventory), positive mood (Vigor subscale of the Profile of Mood States) and psychological distress (Anger, Depression, Tension, Fatigue and Confusion subscales of the Profile of Mood States Scale) were measured in 92 breast cancer patients at three-time points, namely 20 weeks after the end of oncological treatment (T1), at 3 months (T2) and 12 months (T3). The higher levels of positive reappraisal in T1 significantly predicted an improvement in the positive mood at subsequent measurement time points in participants who had low or medium levels of positive mood at T1. Positive reappraisal did not predict psychological distress.

In conclusion, existing studies suggest that cognitive reappraisal is an adaptive and cognitively somewhat expensive ER strategy. It is associated with lower levels of psychopathology (depression and anxiety) in the general population. In cancer patients, only five studies have evaluated the possible impact of this strategy. The results are contradictory and the literature has its limitations. First, only one study specifically investigated cognitive reappraisal in this population, while the others focused on a subtype of cognitive reappraisal, namely positive reappraisal. Thus, little is known about the impact of cognitive reassessment in general on adaptation to cancer. Results in the general population suggest that this strategy may be associated with better adaptation to cancer, but more studies are needed on this question. In addition, the existing literature is limited to depression, anxiety, CRF, and fatigue. The relationship between cognitive reappraisal and other isolated symptoms (pain, insomnia, cognitive difficulties) remains unknown, as does its association with symptom clusters.

Emotional suppression (ES)

Emotional suppression is defined as an individual's effort to reduce an unwanted emotional experience. Specifically, suppression consists of inhibiting behaviors related to emotional expressions, such as facial expression (e.g., maintaining a neutral face, suppressing crying or laughing) or verbal expression (e.g., avoiding mentioning the experienced emotion) in order not to communicate to others information about the emotional states of the person [86]. This strategy also includes suppressing thoughts associated with emotional reactions [87]. Suppression is not a highly effective RE strategy and involves various short- and long-term costs. Although this strategy may reduce the subjective experience of positive emotions, it has no effect on the subjective experience of negative emotions [72]. In many cases, suppression has the paradoxical effect of increasing the negative emotions that the individual is trying to suppress [88]. Therefore, suppression is associated with important levels of negative affect, reduced levels of positive affect, poorer social functioning, and a diminished sense of personal well-being [72]. It can also lead to cognitive overload, reduced cognitive performance, and impaired memory and attention [87,88]. Suppression would be less effective and require more effort than cognitive reassessment [72]. In addition, it appears that suppression is used more frequently in people with a mood or anxiety disorder than in those without a psychological disorder [89]. Several studies also associate suppression with poorer adjustment to pain and chronic fatigue syndrome, increased risk of insomnia, higher levels of inflammation, and an increased risk of cardiovascular disease [90]. Suppression may also be associated with an increased risk of death (cancer, cardiovascular mortality, and all-cause mortality), although more studies are needed to confirm and explain this association [91]. Conversely, a good ability to flexibly regulate (i.e., suppress and enhance) the expression of joy and sadness has been associated with less interference with physical symptoms (e.g., headaches, heart problems). Although the individual using suppression seeks to reduce the intensity of the emotions felt, this strategy is often associated instead with activation of the sympathetic nervous system, manifested by changes in skin conductance and temperature as well as the pulse amplitude at the finger [72]. For example, 180 participants were asked to watch either emotionally charged or uncharged movies (sad, neutral, and funny) in the usual way or using suppression. Suppression was not associated with any physiological effect for the neutral movie, but for the amusing one, it was associated with an increase in sympathetic activity as measured by digital pulse amplitude, finger pulse transmission time, and digital temperature, and a reduction in heart rate. For the sad film, the suppression was associated with an increase in skin conductance and sympathetic and respiratory activation [92].

In cancer patients, the results of the few studies available indicate that suppression is generally associated with higher psychological distress. In a cross-sectional study previously described, higher suppression levels were associated with higher levels of anxiety and depression [82]. A study evaluated the relationship between suppression (measured with the Courtauld Emotional Control Scale), anxiety (Manifest anxiety scale), and emotional distress (Mood profile scale) in 93 women who completed questionnaires before and after being diagnosed with cancer or benign tumor to the breast. High levels of anxiety and suppression measured before cancer diagnosis have been associated with significantly higher scores of emotional distress before and after diagnosis. Of the 21 participants diagnosed with cancer, those with higher levels of suppression before diagnosis reported more psychological suffering after diagnosis. The same researchers then evaluated the effects of suppression on the negative emotions of 14 breast cancer patients on their first visit to the outpatient clinic, immediately after being diagnosed with cancer, after surgery, and at a follow-up visit three months later [93]. Participants with higher levels of suppression measured before diagnosis reported more general psychological distress, anxiety, depression, and anger at all measurement times except immediately after surgery. In addition, higher levels of suppression before diagnosis were associated with more fatigue after diagnosis. In another prospective study involving 31 breast cancer patients, suppression (Courtauld Emotional Control Scale) and psychological distress (Mood Profile Scale) were measured before surgery and between one and six months after surgery. A higher level of suppression measured before surgery was associated with more psychological distress after surgery (higher scores on stress-anxiety, depression, anger-hostility, fatigue-inertia, confusion, and total scores subscales) [94]. A similar cross-sectional association between suppression (Courtauld Emotional Control Scale) and psychological distress (all subscales of the Profile of Mood States Scale except the anger subscale) was obtained in 27 breast cancer patients who had completed their oncological treatments. High levels of suppression have also been associated with stress symptoms (for example, muscle tension, and sympathetic activation), assessed by the Calgary Stress Symptoms Inventory (C-SOSI) [95]. The results of another cross-sectional study in 139 patients in remission from various cancers for at least five years showed an association between suppression (Control Courtauld scale emotional) and higher levels of stress (Perceived stress scale), anxiety, and depression (Hospital anxiety and depression scale; HADS) and poorer adaptation to cancer (Mini-Mental scale for cancer adaptation) [96]. Suppression predicted the level of perceived stress, even after controlling depressive symptoms. However, contradictory data were also obtained. In a study that included 241 patients with different types of cancer, suppression (Courtauld Emotional Control Scale) has been associated with less self-reported psychological suffering on Distress Thermometer and HADS (total scale and depression subscale, but not on the anxiety sub-scale) [97]. To explain this unexpected result, the authors suggest that the reluctance to report psychological suffering is consistent with the use of suppression because completing a questionnaire about psychological symptoms can be perceived as an emotional manifestation. For the time being, these results have not been replicated and this hypothesis has not received empirical support.

In summary, the literature about the general population shows that suppression is associated with increased symptoms of psychological distress, pain, fatigue and insomnia, cognitive difficulties, and activation of the sympathetic nervous system. In the oncological context, several studies have investigated the relationship between suppression and psychological suffering, stress, anxiety, depression, and fatigue in a transversal and prospective manner. Therefore, these results suggest that suppression could be involved in the development of psychological and psychophysiological symptoms associated with cancer. However, the number of studies that have investigated the role of suppression in the development of cancer-associated symptoms is limited, and these studies are restricted to symptoms of depression, anxiety, and fatigue. The relationship between suppression and other symptoms that are highly prevalent in people with cancer, such as pain, insomnia, CRP, and cognitive difficulties, remains unknown. Finally, existing studies have assessed the relationship between suppression and isolated symptoms: the relationship between suppression and symptom clusters does not appear to have been studied up to the present time.

Experiential avoidance

Experiential avoidance is defined as a tendency to avoid or flee from certain psychological experiences (e.g., emotions, but also thoughts, physiological sensations, memories, impulses, and cognitions) by attempting to alter their form, frequency, or intensity, even when these attempts are futile or interfere with the individual's goals or values [98]. Experiential avoidance incorporates strategies like those used in emotional suppression. However, while emotional suppression is clearly an emotional response modulation strategy, experiential avoidance can be used at any point in the emotion production sequence [98]. In this respect, experiential avoidance is a broader concept that involves the use of strategies relevant to the five groups identified [72], namely situation selection (e.g., avoiding starting an apprehensive project), situational modification (e.g., using reassurance from a friend or family member), and situation modulation (e.g., use of a moderator to modulate the emotional response), use of reassurance by an anxious person to tolerate an uncomfortable situation), attention deployment (e.g., avoiding paying attention to thoughts, sensations or memories likely to provoke unpleasant emotions), cognitive reappraisal (e.g., attempting to rephrase a thought associated with an emotion): (e.g., "This university interview is a test of my value as a student and as a person "and replace it with "This interview is an opportunity for me to learn more about this university and see if it is really right for me") and response modulation (e.g., attempts to control verbal, behavioral, and physiological expression of emotions, including emotion suppression) [98]. In the general population, experiential avoidance is associated with poorer quality of life, poorer perceived physical health, and higher levels of general psychopathology, depression, anxiety, and substance abuse [99]. This strategy is even considered by several authors to be a fundamental mechanism in the development and maintenance of psychopathology [88]. A study evaluated the relationship between experiential avoidance (acceptance and action questionnaire), cognitive suppression and reevaluation (Emotion regulation questionnaire), and several indicators of psychological well-being and psychopathology [88]. Results suggest that experiential avoidance is a better predictor of psychological distress and anxiety symptoms than suppression and cognitive reappraisal. In addition, experiential avoidance mediated the relationship between suppression and reappraisal, on the one hand, and positive and negative daily experiences and functioning, life satisfaction and social anxiety, on the other. It is also interesting to note that the results from clinical trials suggest that reducing experiential avoidance is a significant mediator of the effectiveness of mindfulness-based therapies and acceptance and commitment therapy for several mental (e.g., stress) and physical (smoking cessation) health problems [98].

Some studies already assessed the possible influence of experiential avoidance on cancer-related symptoms [100,101] In a clinical study aimed to measure the effect of a group psychological intervention targeting anxiety, depression, and stress, 54 breast cancer patients completed the Acceptance and Action Questionnaire (AAQ-II), which measures experiential avoidance before and after the surgical intervention. A smaller decrease in experiential avoidance between the period before and after treatment predicted a weaker improvement in depressive symptoms and anxiety. Other papers have focused on acceptance, an ER strategy that is thought to be the opposite of experiential avoidance [102].

CONCLUSIONS

Although there is limited information on LS emotional regulation interventions. some studies have explored the structure of emotional support networks in families affected by Lynch syndrome and others have mapped psychosocial interventions in familial colorectal cancer. One study suggested that practitioners may assess cancer-risk-related distress before genetic testing and offer distressed individuals interventions focused on changing emotion regulation [105-107]. Another systematic review of studies involving families with BRCA1, BRCA2, and LS found that proband-mediated interventions, such as genetic counseling with additional telephone or in-person follow-up, letters, videos, and decision aids, have the potential to increase disclosure of genetic risk in families with a BRCA or LS condition [108]. However, none of these studies specifically focused on emotional regulation interventions for LS. It is worth noting that some studies have explored emotion regulation in the context of cancer survivorship more broadly. For example, researchers have called for the study of emotion regulation in breast cancer survivorship [109]. The Difficulties in Emotion Regulation Scale (DERS) and the Emotion Regulation Questionnaire (ERQ) are two tools that have been used to assess emotion regulation strategies [109]. However, these studies are not specific to LS.

Future perspectives

Cancer patients suffer from a range of psychological symptoms (depression, anxiety, CRF, insomnia, fatigue, pain, and subjective cognitive difficulties). Several authors suggest that a common psychological mechanism may contribute to the development and maintenance of symptoms, but no study has investigated this hypothesis so far. One possible underlying mechanism is ER, which has been the subject of increasing interest in clinical psychology in recent years. One possible underlying mechanism is RE, which has been the subject of increasing interest in clinical psychology in recent years. Previous work has shown that two RE strategies, emotional suppression (inhibition of emotion-related thoughts and behaviors, such as facial expression of emotions) and experiential avoidance (avoidance of feelings, physical sensations, and emotion-related thoughts), have been associated with anxiety, depression, and fatigue in patients treated for cancer. In contrast, cognitive reappraisal, i.e. changing the meaning associated with a situation to alter its emotional load, appears to be associated with better adaptation to cancer, in particular with fewer depressive and anxiety symptoms. Therefore, studies are needed to determine the role of ER as a common psychological mechanism for a broader set of cancer-associated symptoms, both cross-sectionally and prospectively. The study of psychological mechanisms that may underlie clusters of symptoms associated with cancer is relevant both theoretically and clinically. From a theoretical point of view, it is necessary to better understand why and how these symptoms develop. Clinically, several authors have emphasized that it is vital to understand the processes underlying the emergence of symptom clusters in order to provide more effective treatments [110]. For example, transdiagnostic psychological treatments for psychopathologies (for example, anxiety and depressive disorders) are innovative interventions that are of growing interest to psychologists and clinicians. These treatments make it possible to treat multiple comorbid psychological disorders simultaneously because they target the affective, cognitive, and behavioral processes underlying the disorders (e.g., avoidance, rumination, and suppression, which are found in a variety of anxiety and mood disorders), rather than targeting each disorder individually [111]. The effectiveness of several transdiagnostic treatment protocols developed for mood and anxiety disorders has received a great deal of empirical support [112]. However, these protocols cannot be applied directly to cancer patients, as they do not include certain symptoms common to this population (e.g., CRF, fatigue, pain, insomnia, and cognitive difficulties).

Identifying a mechanism underlying psychological disorders in LS would be the first step in adding real help to classical treatments.

Author Contributions

ML and CGI contributed to the conception, and structure of the paper, and contributed to the analysis, and interpretation of available literature. FG and CGI contributed to the development initial draft. ML and FG reviewed and critiqued

the output for important intellectual content. All the authors contributed to the article and approved the submitted version.

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ORIGINAL ARTICLE

Overview of the Genetic Aspects of Lynch Syndrome

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Abstract: *Lynch syndrome (LS) is one of the most common inherited cancer predisposition syndromes and the most important cause of hereditary colorectal and endometrial cancers. It is inherited in an autosomal dominant pattern, caused by a pathogenic germline variant in one of the mismatch repair (MMR) genes (MLH1, MSH2, MSH6, or PMS2), which encode proteins responsible for maintaining genome stability. A defective MMR system confers an elevated risk of developing certain types of cancer, at a younger age than in the general population and at a high rate of multiple primary neoplasia. Lynch syndrome is not just one disease, but a collection of different subtypes of underlying molecular aspects with specific clinical implications. A better understanding of these subgroup profiles could offer better care for each patient, leading to more personalized risk-reduction and surveillance strategies. Lynch syndrome is a frequent but yet underdiagnosed condition that needs multidisciplinary increased awareness for correct diagnosis and management. The aim of this review is to provide a summary of available literature data on the genetic aspects of Lynch syndrome together with geno-type-phenotype correlations and its clinical implications in the detection, genetic counseling, genetic testing, risk stratification, and management of this condition***.**

Keywords: *Lynch syndrome; cancer predisposition; hereditary cancer; mismatch repair deficiency*

INTRODUCTION

Cancer is a disease of the genome that originates from complex interactions between various genetic, epigenetic, and environmental factors. Even though most of the genetic alterations in cancer are acquired somatic events leading to sporadic cancers, common and rare germline variants have important effects on tumor development [1,2].

About 5-10% of all cancers are hereditary. In those cases, a major inherited genetic risk factor could be identified through molecular genetic testing [3,4].

Colorectal cancer is the second most common type of cancer (after breast cancer) and the second cause of cancer death (after lung cancer) [5]. Approximately 20% to 30% of colorectal cancer cases could be associated with genetic factors and hereditary predisposition syndromes constitute 3% to 5% of all cases [3].

Lynch syndrome is the most frequent cause of hereditary colorectal cancer that accounts for 1%–3% of all colorectal cancer diagnoses [6].

According to Globocan estimated data, the burden of all types of cancer will increase in our country as well as in the entire Europe by 2040, including colorectal cancer [7]. Therefore, even if there seems to be a small proportion of hereditary causes, given the high incidence of cancer, a significant number of selected patients should benefit from hereditary cancer risk assessment, genetic counseling, and genetic testing for a correct diagnosis and management.

LS affects 1 in 279 individuals predisposing to colorectal, endometrial, ovarian, stomach cancers, and other malignancies at an earlier onset age than in the general population and at a high rate of multiple primary cancers [8,9]. It still remains an underdiagnosed condition despite all the recommendations for the identification of LS cases [10,11].

MOLECULAR GENETICS OF LS

Microsatellites, or short tandem repeats (STRs) are structures consisted of one to six nucleotides structures dispersed all over the genome, with a highly variable number of repetitions [12]. At this level, the DNA polymerase enzyme is prone to errors during DNA replication. MMR proteins are important in the recognition and correction of mispaired nucleotides, small insertions, or deletions generated by slippage of DNA polymerase [12].

A compromised or defective MMR system leads to microsatellite instability (MSI) and prone DNA to the accumulation of somatic mutations that often promote cancer development [9]. It has been demonstrated that the presence of MSI increases the mutation rate to 100-fold [13,14].

Immunohistochemical (IHC) staining of the MMR proteins and molecular testing of microsatellite instability by polymerase chain reaction (PCR) constitute the basis for the identification of MMR deficiency [15]. MSI and/or immunohistochemical loss of expression of MMR proteins could be identified frequently in colorectal and endometrial cancers and represents hallmarks for Lynch syndrome [16]. Alterations of MMR genes result in LS-associated tumors lacking the IHC expression of the corresponding MMR protein [6].

Since 2009, The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group recommended testing all colorectal cancer cases for MSI or MMR deficiency through immunohistochemistry as a screening method for LS [17]. Furthermore, the National Comprehensive Cancer Network (NCCN) now recommends universal testing of endometrial carcinomas for MMR proteins/MSI [18,19]. Although being important red flags for Lynch syndrome, IHC loss of expression of MMR proteins and/or microsatellite instability could not differentiate between germline and somatic origin of the defects [9].

Germline and somatic defects of the MMR genes

Mismatch repair deficient tumors may develop from acquired, somatic events in sporadic cancers or in the context of a cancer predisposition syndrome that necessitates a genetic defect of a germline origin, present from birth in all the cells of an organism [20].

LS is caused by a pathogenic heterozygous germline variant in one of the mismatch repair (MMR) genes (MLH1, MSH2, MSH6, or PMS2), which encode proteins responsible for maintaining genome stability [8].

A deficient MMR system determined by a pathogenic constitutional variant of one allele requires a second somatic hit in the wild-type (normal) allele in order to confer the cell a mutator phenotype and MSI [13,15]. This second somatic defect could be acquired through a two-hit inactivation process (based on Knudson's two-hit hypothesis for tumor suppressor genes) or by other mechanisms such as epigenetic inactivation that may confer haploinsufficiency [21].

Despite presenting as a recessive condition at the cellular level, Lynch syndrome displays an autosomal dominant pattern of inheritance [9].

Most of the MMR variants causing LS are inherited from one parent [22,23]. De novo occurrence of MMR mutations was described but appears to be a rare presentation [24]. One study focused on determining the frequency of these cases reported that 2.3 percent of LS diagnoses occurred due to de novo germline variants [24].

Nevertheless, MSI/MMR deficiency may be attributable to somatic events/biallelic somatic inactivation [20]. For example, 15% of MSI-high/MMRd colorectal tumors are determined by hypermethylation of MLH1 promoter on both alleles (homozygous), associated with somatic BRAF V600E mutated status [16,25]. Therefore, MLH1 promoter hypermethylation analysis and/or BRAF V600E variant testing should be performed in tumors lacking MLH1 protein expression prior to germline testing to assess for sporadic origin of the MSI [23,26].

A unique somatic positive methylation result without BRAF testing in colorectal cancer could not exclude a predisposition syndrome caused by germline hypermethylation of MLH1 [23]. Although rare, this mechanism has been described and some authors recommend MLH1 constitutional epimutation testing using normal tissue in cases with methylated MLH1 that fulfil the revised Bethesda guidelines [23,27]. Similarly, just a BRAF V600E negative result could not differentiate between constitutional epimutations and germline mutations of MLH1 [23,27].

In endometrial tumors, the usefulness of BRAF testing is unclear [28]. MLH1 promoter methylation analysis is important in discriminating between somatic and inherited cases. Nevertheless, cases of coexistence of both MLH1 promoter hypermethylation and MLH1 germline mutations have been described to such an extent that Lynch syndrome could not be completely excluded in the case of positive somatic methylation of MLH1 [28].

On the other hand, the presence of two germline (homozygous or compound heterozygous) MMR defects give rise to a distinct phenotype, constitutional mismatch repair deficiency syndrome (CMMRD) a very rare presentation, with an autosomal recessive inheritance and severe clinical implications. In these cases, MMR deficiency could be encountered in any of the normal tissues of the patients, not just in the tumor cells [21].

Germline genetic testing and genetic spectrum of LS defects

Genetic germline testing in patients or healthy individuals at a high risk of carrying a pathogenic variant in one of the MMR genes is a mandatory step in the diagnosis of Lynch syndrome.

Approximately 50% of Lynch syndrome cases are determined by germline pathogenic variants in MLH1 and 40% are caused by MSH2 defects. MSH6 gene is responsible for 7-10% of LS cases and PMS2 accounts for fewer than 5% of cases [23]. In a small proportion of cases (1-3%), LS is caused by a deletion in the EPCAM (epithelial cell adhesion molecule) gene that confers loss of expression of the MSH2 gene due to hypermethylation of its promoter [23,29]. Other rare mechanisms that could be involved in the pathogenesis of LS are germline or constitutional epigenetic silencing of one of the MMR genes present in normal tissues [9,30,31].

Most of the germline defects could be identified through sequencing of the DNA or deletion/duplication assays. In contrast, epimutations are a particular type of genetic alterations that do not alter the DNA sequence but determine the transcriptional silencing of active genes [32].

Germline epimutation occurs in the germ cells, is maintained in fertilization and embryonic development, and is present in all the cells of an individual. In contrast, constitutional epimutations arise in the early stages of embryonic development, inducing a mosaic of affected and unaffected somatic cells [31]. This separation interferes with the inheritance of epimutations. Germline epimutations could be reversible between generations depending on the gender of the affected parent [33]. Studies have shown that epimutations could be inherited from the mother because there is unlikely to be lost in oogenesis whilst they disappear during spermatogenesis [31]. On the contrary, constitutional epimutations are not inherited [31]. Epigenetic silencing could also be inherited when determined by a secondary germline defect in another gene within their vicinity [30,33,34].

Germline or constitutional MLH1 and MSH2 epimutations have been described in some cases although negative for germline defects in the MMR genes [30,33–36]. The methylation of MHS6 and PMS2 genes has never been described in the literature [37].

The pathogenic spectrum of MMR variants in Lynch syndrome includes missense, nonsense, frameshift, or splice site mutations, although copy-number variants and structural rearrangements have also been described [6,23]. Therefore, to cover the most frequent alterations responsible for LS, genetic germline testing should include DNA sequencing and copy-number analysis of MMR genes as well as copy-number variations (CNVs) detection of the EPCAM gene. This approach can detect up to 99% of LS causes [23]. Testing for germline or constitutional epimutations requires different types of genetic analyses that are not routinely included in the diagnostic approach of Lynch syndrome [30]. Molecular testing of PMS2 is technically challenging considering the presence of multiple highly homologous PMS2 pseudogenes as well as balanced large rearrangements [23,38,39]. Additional analyses may be required to cover all of the genetic alterations in this gene [38,40].

Over the last few years, next-generation sequencing (NGS) testing costs and turnaround time significantly decreased. [41]. Available multigene panels comprising the MMR and EPCAM genes represent a comprehensive and more efficient testing approach than single-gene testing [19,23]. Different laboratories now offer a lot of commercial NGS panels for patients suspected of Lynch syndrome or even larger panels for hereditary colorectal cancer predisposition. These assays may present small differences in terms of performance characteristics and limitations [41].

Interpretation of genetic results

Next-generation sequencing does not provide a simple positive or negative type of results. High-throughput sequencing, also known as NGS opens up a large amount of data. That comes up with challenges in interpretation and understanding the clinical significance of the identified sequence variants [42,43]. More than 15,000 different variants were reported in the MMR genes [23].

The American College of Medical Genetics and Genomics (ACMG) guidelines provide a classification of genetic variants into five categories of interpretation: Pathogenic (P), Likely pathogenic (LP), Uncertain significance (VUS), Likely benign (LB), and Benign (B) depending on a series of criteria [43,44]. In addition, laboratories should use a uniform reporting nomenclature as a clear, common language regardless of language or country of origin [43].

Benign and likely benign variants are considered harmless and do not require additional intervention. Pathogenic and likely pathogenic variants conversely, entail specific recommendations for the prevention and surveillance of Lynch syndrome-associated malignancies and enable cascade-testing of at-risk family members and family planning strategies [22].

Besides its recognized clinical utility, very often NGS brings to light variants of uncertain significance (VUS), a challenging, grey area of results with a high potential of misunderstanding [45,46]. A VUS result means that at the time of reporting, there is insufficient populational-based or functional evidence to determine the pathogenicity of the variant or there is conflicting data in the databases or the literature about the variant's role in the disease [45,46].

As the databases and the literature are enriched with relevant data, a VUS could be reclassified into pathogenic or benign. In addition, family segregation analysis (genotyping the family members to evaluate if they have the same variant) and assessing for de novo occurrence could aid in the reclassification process [43]. Furthermore, in the process of assessing the pathogenicity of a variant, a detailed patient's phenotype is very important to be provided by the ordering clinician [43,44].

ACMG stated that "a variant of uncertain significance (VUS) should not be used in clinical decision-making" and efforts should be made to reclassify the variant into pathogenic or benign [43]. A large retrospective study that included 1.45 million individuals tested for hereditary cancer risk reported that 7.7% of unique variants of uncertain significance were reclassified [47]. Among these, 91.2% were downgraded to benign or likely benign variants 8.7% were upgraded to pathogenic or likely pathogenic variants and the median time for reclassification was less than 2 years [47].

CLINICAL SPECTRUM OF LS-ASSOCIATED MALIGNANCIES AND GENOTYPE-PHENOTYPE CORRELATIONS

Lynch syndrome is associated with an increased lifetime risk for colorectal and endometrial cancer, as well as for other extracolonic malignancies (gastric, small bowel, pancreatic, hepato-biliary, prostate, ovarian, skin, central nervous system or upper urinary tract neoplasia) [10,21].

The risk of developing breast cancer is highly debated. There are conflicting results in the literature, still, some studies point out that pathogenic variants in MSH2/PMS2 may confer a 2 to 3-fold increased breast cancer occurrence [48–50].

Lynch syndrome is a highly heterogeneous disease with important variability within the molecular germline defects, somatic profile of the tumors, as well as the onset age of cancer diagnosis and penetrance for different types of cancer [6,15].

Genotype-phenotype correlations provide individualized surveillance protocols according to subsequent genetic alteration.

Mutation carriers of MSH2 and MLH1 have a significantly higher lifetime risk of cancer compared to MSH6 or PMS2 carriers. The lifetime cancer risk by age 70 for MSH2 is 57-80%, followed by MLH1 with 59-65%, 25% for MSH6, and 25-30% for PMS2 [21]. Moreover, MSH6 and PMS2 mutation carriers display higher ages of onset for different types of LS-associated cancers [21]. Therefore, MSH6 and PMS2 confer a milder phenotype and reduced penetrance of cancer compared with MLH1 and MSH2 carriers. This fact has important implications in surveillance strategies according to each gene's corresponding risk. Better tolerability of MSH6 and PMS2 variants compared to MLH1 or MSH2 could also explain their frequent presentation in a biallelic state in CMMRD syndrome [22]. In addition, variability regarding age at first cancer diagnosis is observed between families with the same affected gene as well as within the same family affected by the same variant [51]. A variety of environmental and genetic factors contributing to the different onset ages of cancer in Lynch syndrome families have been proposed [52].

Genetic anticipation, a phenomenon characterized by progressively earlier age at the onset of a hereditary disorder and/or increased severity in subsequent generations, has also been described in Lynch syndrome [51–53]. It was also observed that patients with de novo mutations have a younger age of onset than those with an inherited variant [24].

LS patients also manifest a variable expressivity depending on MMR underlying the affected gene. Particularly, mutation carriers of MLH1 display the highest risk of developing colorectal cancer among all patients diagnosed with LS, and MSH2 carriers have the most elevated risk for extracolonic malignancies [10,21]. A large study based on data from the prospective Lynch syndrome database (PLSD) reported that the cumulative risks for colorectal cancer at 70 years of age are 46% for MLH1 carriers, 35% for MSH2, 20% for MSH6 and 10% for PMS2. The corresponding risks for developing endometrial cancer were 34%, 51%, 49% and 24% [54].

Muir-Torre syndrome is a phenotypic variant of LS most frequently determined by MSH2 germline defects. It is characterized by a high incidence of cutaneous sebaceous tumors (sebaceous adenomas/carcinomas and keratoacanthomas) [9].

Turcot syndrome, another phenotypic variant of LS, describes patients presenting with colorectal cancers and central nervous system tumors (glioblastomas) [6,9]. However, Turcot syndrome could also be part of the familial adenomatous polyposis (FAP) syndrome caused by an APC germline defect when colorectal cancer is associated with medulloblastoma [6,9,21].

Besides these larger associated risks for any of the MMR genes, it was observed that even for the same type of cancer, different mutational patterns were described in the same organ partly depending on the germline MMR-affected gene [15].

According to the "three-pathway model of carcinogenesis", LS colorectal cancers could develop from MMR-proficient adenomas, MMR-deficient adenomas, or MMR-deficient crypts [15]. NGS analyses of colorectal tumors in LS have shown highly heterogenous molecular somatic defects that could be associated with different affected MMR genes [2,15] For example, colorectal cancers due to MLH1 defects have been associated frequently with CTNNB1 mutations that could explain nonpolyposis immediate invasive growth through MMR-deficient carcinoma pathway resulting in interval carcinoma presentation [15]. On the contrary, PMS2-associated colorectal tumors do not harbor CTNNB1 defects and do not show interval colorectal cancers [15].

This variability shows that LS is not just one disease with a unique profile. It could be divided into distinct subgroups, with different associated risks and surveillance strategies depending on the germline MMR-affected gene [15] A better understanding of these subgroup profiles could offer better care for each patient, leading to more personalized surveillance strategies.

STRATEGIES FOR DIAGNOSIS OF LYNCH SYNDROME

The evaluation of individuals at high risk of carrying one LS pathogenic variant could encompass the following scenario:

- traditional testing strategy based on clinicopathological criteria (Amsterdam I/II criteria and Revised Bethesda guidelines)
- personal history of MMR-deficient tumor;
- increased risk for LS based on clinical prediction models;
- presence of confirmed LS pathogenic variant in the family [9,10,42].

In the 20th century, before the discovery of the genetic background of LS, family history of cancer represented the main tool in identifying familial aggregates of malignancies [9].

Amsterdam criteria (I and II) and Bethesda Guidelines, based on family and personal history of cancer and age of cancer onset were used to identify individuals eligible for molecular analysis [3,10]. Requiring three or more family members diagnosed with cancers from the LS spectrum at an early age, Amsterdam criteria were very stringent [3,22]. This method of screening targeted families with highly penetrant and dominant cancers, thus being less sensitive in detecting LS cases determined by pathogenic MSH6 or PMS2 variants [55,56]. Subsequently, less stringent Bethesda Guidelines (based on patient and/or family history of LS cancers and MSI-high pathologic features of colorectal tumors) were formulated to provide wider clinical criteria for screening of LS [3,9,22,57].

Despite these family-history-based prediction tools, just some selected patients were offered testing for LS [55,58]. Studies have shown that families meeting one of the clinical criteria have 82% sensitivity and 77% specificity in detecting LS and that Bethesda guidelines may miss up to 25% of LS cases [9,59].

Later on, because of the low sensitivity of family-history-based clinical criteria, “universal tumor screening” of all newly diagnosed colorectal cancer cases for MMR-deficiency by immunohistochemistry testing or for MSI was proposed as an alternative strategy to guide Lynch syndrome evaluation [3,10]. This approach demonstrated improved sensitivity compared to revised Bethesda guidelines [3]. Afterward, universal tumor testing was expanded to include recommendations for screening all endometrial cancers for LS if not limited by resources to maximize the sensitivity of LS detection [3,58,60].

Computational prediction models such as PREMM5, MMRPro, and MMRPredict represent effective tools in guiding germline testing, especially in the absence of available tumor tissue [8,57–59]. These models incorporate information like family history of cancer in order to estimate the likelihood of carrying MMR pathogenic variants [57,58]. For example, PREMM5 provides comprehensive risk estimation for all MMR genes [61]. Referral for genetic evaluation and germline genetic testing is recommended for individuals with overall predicted probability scores ≥2.5% [61].

If a pathogenic family variant is known, genetic testing for both affected and at-risk family members should be performed targeting that particular mutation [9,42]

Individuals meeting any of these scenarios should be referred for genetic counseling and genetic testing [42].

GENETIC COUNSELING IN LS FAMILIES

Patients following any germline genetic testing should receive appropriate pre-test and post-test genetic counseling [42].

Collecting detailed personal and family medical history (with at least three-generation pedigree on each side of the family) is an important step in understanding hereditary risk in a family [42,57]. Comprehensive cancer risk assessment should be focused on the type of diagnosed cancers in the family members (colorectal cancer, endometrial cancer, and other extracolonic LS-associated malignancies) as well as subtypes, pathology details, age of diagnosis, age of death, and causes of death, details about the patient or family’s country of origin or ancestry, and any known possible consanguinity [42]. Still, a negative family history of LS cancers should not exclude a possible LS diagnosis. De novo occurrence of a germline variant, constitutional epimutations, reduced penetrance, cancers not yet manifested in carriers, or early death of family members by other causes could represent sources of negative family history in LS cases [24,31,62]. Therefore, overreliance only on family history of LS-associated cancers to guide the selection of families for LS testing could contribute to general underdiagnosis of this condition [22,24].

Universal testing of colorectal and endometrial tumors could efficiently identify symptomatic patients at risk of Lynch syndrome. Genetic counseling is not required prior to testing of the tumor tissue for MMR proteins/MSI, but if a positive screening test result is obtained, patients should receive specialist counseling [42,58].

Pre-test genetic counseling is focused on providing essential information about the possible outcomes of germline testing, including positive or negative results as well as variants of uncertain significance (VUS), unexpected incidental findings, and limitations of the test [42,57].

Education of the patient or asymptomatic at-risk individuals on possible management options depending on the genetic test result, inheritance patterns, heterogeneity of the disease, penetrance, and variable expressivity helps in the informed, voluntary decision-making process [9,42,57].

Genetic testing should be performed after obtaining informed consent in individuals at high risk where a positive result will impact the medical management of the case and/or their family members [4,42,58].

After disclosure of results, post-test genetic counseling is highly recommended in order to explain genetic results and their implications and to discuss possible management options.

Patients diagnosed with Lynch syndrome should be offered intensive screening and follow-up according to genetic test results, family history, and personal history of cancer [4,6,60]. Because of the increased lifetime risk of colorectal, endometrial, and other extra-colonic cancers, LS patients and their families require lifelong coordinated multidisciplinary care [55].

Preventive measures (i.e. risk-reducing chemo-preventive agents and risk-reducing surgery) and surveillance protocols such as colonoscopy for early detection of cancers are proven to have a positive impact on patient outcomes and facilitate early diagnosis in individuals with confirmed LS diagnosis [6,42,55].

Genetic counseling and cascade testing of family members at risk of carrying LS-causing variants is highly recommended. Patients should be advised to inform their relatives about the risk of inheriting the identified P/LP variant [42]. In case of a VUS result, family testing could be recommended just for segregation studies to help in variant reclassification, but not for risk estimation or any clinical purposes [43].

Lynch syndrome is typically inherited in an autosomal dominant manner. Each child of an individual diagnosed with LS has a 50% (1/2) risk of inheriting the same P/LP variant [62]. Usually, germline testing for hereditary cancer predisposition of pre-symptomatic individuals is not recommended under 18 years old unless the risk of developing cancer is very high at younger ages and results would impact medical care in childhood [42,62]. Constitutional MMR deficiency (CMMRD) syndrome is one of the exceptions in which testing prior to age 18 is recommended to guide medical care [42].

Prenatal diagnosis or preimplantation genetic testing with selective implantation of unaffected zygotes is technically feasible if a pathogenic variant is known in the family [57,62-64].

CONCLUSIONS

Lynch syndrome is the most common hereditary colorectal cancer syndrome, characterized by high genetic and clinical heterogeneity. Being associated with the early onset of colorectal cancer and other extracolonic malignancies, individuals diagnosed with LS and their families require multidisciplinary care during their entire lifetime. Comprehensive genetic counseling educates patients and at-risk family members about their risks, genetic testing advantages and limitations, and implications of diagnosis. Specific genotype-phenotype correlations were observed and germline molecular testing helps individualize risk-reduction and surveillance strategies according to underlying genetic defect.

Conflicts of interest and sources of funding

The author declares no conflict of interest.

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REVIEW

Lynch Syndrome: Approach from the Gastroenterologist's Point of View

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Abstract: *Colorectal cancer is one of the main causes of death by cancer in the European Union and in Romania. Lynch Syndrome is an autosomal dominant inherited disease that affects around 3% of all colorectal cancer patients, defined by mutations in the mismatch repair genes (MLH1, MSH2, MSH6, PMS2). Aside from the high risk for colorectal cancer (CRC), they exhibit a high risk for endometrial cancer, ovarian cancer, gastric cancer, pancreatic cancer, brain cancer, small bowel cancer, and sebaceous gland cancer. Diagnosis is often made by the gastroenterologist, usually when he meets a young patient with symptoms suggesting the presence of a colonic tumor and a family history of CRC. The role of the gastroenterologist is to investigate the best practices in order to have an early diagnosis and to prevent interval cancers according to the newest guidelines or other gastrointestinal tract tumors associated with Lynch Syndrome. Registries play an important role in the optimal surveillance of these particular categories of patients and should be put into place in every country. They will allow clinicians to have a better understanding of the disease and a more standardized quality of care for them*.

Keywords: *Lynch Syndrome, colonoscopic surveillance, patient registry*

INTRODUCTION

Colorectal cancer (CRC) represents the second leading cause of death by cancer after lung cancer in the European Union and the second one by frequency of occurrence, making it an important problem of public health in many countries [1]. In Romania, the National Institute of Public Health estimates an incidence rate of 34.6‰$_{00}$ people in 2020, representing 12.7 % of the newly diagnosed malignancies in the oncologist's office. Analyzing past data, we can observe a marked increase in the incidence of new cases from 31.0‰$_{00}$ in 2012 to 34.6‰$_{00}$ in 2020 which medical professionals should take into account [2].

It is estimated that 3-5% of CRC diagnoses are associated with a genetic syndrome, but what is really concerning, is that 35% of CRC have a hereditary predisposition. Lynch Syndrome (LS) is the most common hereditary CRC syndrome and it's the cause of approximately 3 % of all CRC, with an estimate of 1/279 (95% CI, 192–403) of the population carrying a mutation in the mismatch repair genes [3].

LS is an autosomal dominant inherited syndrome genetically diagnosed by a mutation in one of the mismatch repair (MMR) genes (MLH1, MSH2/EPCAM, MSH6, PMS2) [4].

It is characterized by an overall increased risk of CRC, manifested by a predominance of diagnosed right-sided tumors, and a high incidence of metachronous or synchronous tumors [5]. Patients who carry a pathogenic variant of the MMR genes are also at high risk of developing endometrial cancer and some other cancers based on the respective gene defect [6].

Recent guidelines recommend that all newly discovered CRC regardless of age of diagnosis, should undergo testing for mismatch deficiency by immunohistochemistry (IHC) staining for MMR proteins, or microsatellite instability (MSI) by polymerase chain reaction (PCR). If the tumor is either MMR deficient or presents high instability, the patient should automatically be tested for LS. Ideally, genetic diagnosis should happen before chemotherapy is started, considering that, immunotherapy is associated with an improved prognosis in this category of patients compared with the ones having an MMR-proficient tumor. In cases of early-onset CRC, all patients should undergo genetic counseling and be tested for germline genetic mutations regardless of the results of IHC/PCR testing [6].

When faced with a young individual, the patient's history is very important. The clinician should try to find out all the family history details, making a comprehensive review and ideally also a family pedigree. All causes of death in the family should be noted in order to understand how many of them were caused by the underlying condition and to observe the penetrance of the gene in the respective case [7].

MATERIALS AND METHODS

The studies that we included in this review, are peer-reviewed articles, published in English focused on gastroenterological surveillance in Lynch Syndrome. We used the PubMed database, Springer Link, and Google Scholar and we selected articles published before the 1st of August 2023. We use terms like "Lynch Syndrome" combined with "Colonoscopic surveillance", "Endoscopic Surveillance", "colon cancer", "Pancreatic cancer", and "non-invasive surveillance" and selected relevant articles to the subject. We didn't include articles that presented small lots of patients and had insufficient data presented. The articles that complied with the search and contained relevant information for our study were included and presented in our study. We also researched the newest guidelines on hereditary colorectal cancer such as the NCNN latest guideline version, the ESMO guidelines but also the Mallorca consensus, and the new Delphi initiative on early-onset colorectal cancer.

RESULTS

As gastroenterologists, we are concerned about the early detection of Lynch-associated tumors and how to best prevent premalignant lesions. New studies discussed the pathways that lead to CRC cancer and what gastroenterologists can do to prevent new malignant lesions. Also, concerning the rarer GI tract cancers associated with Lynch Syndrome, there are new surveillance recommendations published very often and gastroenterologists need to stay up to date in order to offer the best care.

We are going to focus on the best practices in gastrointestinal surveillance, therefore we are going to discuss the early detection of patients, surveillance, and better ways to improve patients' adherence to a lifetime of screenings.

Detection of patients

When talking about the gastroenterologist's perspective, it is usually concerning a CRC case at diagnosis. The most frequent motives for a doctor's appointment are hematochezia (46%), iron deficiency anemia (13%), and weight loss (10%) [8-11]. Physicians have a tool by applying the Amsterdam II Criteria or the revised Bethesda Guidelines when assessing a patient's risk for Lynch Syndrome [12-13] (Table 1).

Table 1: Amsterdam II Criteria and Revised Bethesda Guidelines

Amsterdam II criteria
At least three relatives must have cancer associated with LS (colorectal, endometrial, small bowel, ureter, or renal pelvis); all of the following criteria should be present:
One must be a first-degree relative of the other two
At least two successive generations must be affected
At least one relative with cancer associated with LS should be diagnosed before age 50
Familial adenomatous polyposis should be excluded in the CRC cases (if any)
Tumors should be verified whenever possible
Revised Bethesda Guidelines
Tumors from individuals should be tested for MSI in the following situations
CRC diagnosed in a patient who is younger than age 50 years
Presence of synchronous or metachronous colorectal or other LS-related tumors regardless of age
CRC with MSI-high histology diagnosed in a patient who is younger than age 60 years
CRC diagnosed in a patient with one or more first-degree relatives with LS-related cancer, with one of the cancers being diagnosed below age 50
CRC diagnosed in a patient with two or more first or second-degree relationships with LS-related cancer regardless of age

Although throughout the years they proved themselves to be useful tools in the office, the more recently recommended approach is to define the MMR status via IHC staining or MSI using the polymerase chain reaction. Furthermore, the molecular alternative plays an important part in the aftermath of the diagnosis by being a useful indicator of a positive response to the use of immune checkpoint inhibitors [14].

In particular situations, where no tumor tissue is available, like patients with a high suspicion of Lynch Syndrome that don't have a cancer diagnosis, clinical prediction models have been developed, in order to assess the indication for genetical testing.

The PREMM model ((http://premm.dfci.harvard.edu/), is a useful tool for clinicians, and easy to incorporate in day-to-day work. Recently Harvard developed the new PREMM5 model with an increased chance of predicting whether a patient might carry a germline variant of the genes involved in LS. If the prediction is above the 2.5% limit in the case of the PREMM5 model or more than 5% in older versions, the patient should be referred for genetic testing and further investigations in a geneticist's office [15].

From a gastroenterologist's point of view, the interview is based on onset symptoms, previous lab results, family history, and personal risk factors such as smoking status, gender, and age at diagnosis. Afterward, depending on the situation, whether there is a patient from a Lynch family or a newly identified patient, there are different ways to approach the screening. Patients may be referred to a geneticist in order to receive genetic counseling and to be explained familial risk. A study was conducted in Denmark on the manner in which individuals were informed about their cancer risk. There is a Lynch Syndrome registry implemented since 1991 with continuous improvements, where there is approval from the authorities to send unsolicited letters to patients at risk of familial cancer in order to include them in a surveillance program. The reactions to these letters were studied, and while the results were good, there still was a category of patients upset or angry to be informed of their genetic heritage. Even though the standard recommendation is that family members inform one another, depending on familial situations, and personal preferences, it was decided that the unsolicited letters were helpful in reaching as many members as possible. Potential individuals at risk were invited to genetic counseling and genetic testing in order to assess their further need for surveillance [16].

Colonoscopic surveillance

When talking about colonoscopic surveillance in LS there are several issues to be discussed. First, there are multiple recommendations regarding the screening both concerning the onset age to begin colonoscopic surveillance and also the interval between the procedures. The consensus is that the interval between colonoscopies should not be longer than 3 years as it was demonstrated in some studies that a 3-year window was associated with metachronous cancer [17]. A study conducted by Ahadova et al opened the window to new challenges regarding colonoscopy surveillance. The study presents a new theory that states the fact that carcinogenesis in Lynch Syndrome may occur via more pathways opposite to the previous theory stipulating that it's an accelerated road from adenoma to adenocarcinoma (Table 2). While the classic pathway with the adenoma to adenocarcinoma is still present, they show that adenocarcinomas can develop on macroscopically normal tissue, concluding that a schedule with more frequent colonoscopies won't necessarily prevent the development of new tumors [18]. Even though endoscopists will perform polyp resections when deemed necessary, they can't analyze the normal tissue. In summary, colonoscopy screening with polypectomies performed when necessary did not guarantee that the patient would be risk-free and tumor could appear on apparently normal mucosa. This new information makes colonoscopy surveillance even more important, and respecting the recommended intervals with no more than 2 years interval between exams is crucial in detecting tumors early.

Table 2: Pathways to Carcinoma in Lynch syndrome as stated by Ahadova et al. [18]

Pathway 1	Normal mucosa to Adenoma MMR proficient to MMR deficient Adenoma to Carcinoma
Pathway 2	Normal mucosa to MMR deficient crypt foci to MMR deficient Adenoma to Carcinoma
Pathway 3	Normal mucosa to MMR deficient crypt foci to Carcinoma

Seeing as new information appears every year, new guidelines were published with different recommendations regarding the timing of colonoscopy surveillance, and also the interval between the examinations. Recent data concerning LS patients shows that the on-set age should be different depending on the mutation, with the latest NCCN recommendations stating that in the case of MLH 1 or MSH 2, colonoscopy should start at age 20-25 years or 2-5 years before the earliest CRC diagnosis if it was diagnosed before 25 years of age, with an interval of 1-2 years between examinations. When talking about MSH 6 and PMS2, the guidelines are more relaxed, recommending starting at 30-35 years of age or 2-5 years before the earliest CRC diagnosed before the age of 30 years with an accepted interval of 1-3 years between examinations. If adenocarcinoma is detected in one of the examinations, colonoscopy on the remaining part of the colon should be made every 1-2 years, the same indication going in cases of adenomas that were successfully resected endoscopically. If there are situations when adenomas aren't suitable for endoscopic resection, surgery may be indicated depending on the particular situation [19].

The British Society of Gastroenterology published a new guideline in 2020 on the management of hereditary colorectal cancer making similar recommendations, the difference being in intervals between examinations. It is recommended a 2-year interval between colonoscopies and they also state that screening should end at age 75 years [20]. The ESMO 2019 guidelines are in consensus with the previous ones, making recommendations based on the known gene mutation with a

1-2 years interval between examinations [21]. The ACG guidelines state a slightly different approach to screening with a recommended age at onset between 20-25 years at least every 2 years with a suggestion to perform it annually in confirmed mutation carriers [22] (Table 3).

Table 3: Summary of guideline recommendations for colonoscopic surveillance

Guideline	Age	Interval
NCCN	MLH1/MSH2: 20-25 years or 2-5 years before earliest CRC diagnosis if prior to 25 years of age MSH6/PMS 2: 30-35 years or 2-5 years before earliest CRC diagnosis if prior to 30 years of age	1-2 years 1-3 years
ESMO	MLH1/MSH2: 25 years MSH6/PMS2: 35 years	1-2 years
BSG	MLH1/MSH2: 25 years MSH6/PMS2: 35 years	2 yearly until age 75 years
ACG	Age 20-25 years	1-2 years

One study conducted by the Dutch in 2022 [23] on colonoscopic surveillance of diagnosed LS patients revealed some difficulties encountered on the way regarding adherence to follow-up by the patients. One important difference that was acknowledged was the call for an appointment. Patients who were called by the hospital in order to make the appointment were more likely to undergo colonoscopic surveillance than the ones who were supposed to initiate the call themselves. Another issue at hand was the burden of bowel preparation. Patients were more open to smaller volume preparations than larger ones. Another issue that should be taken into account is the psychological burden. Out of the 197 patients included in the study, 41 answered that they considered quitting the colonoscopic surveillance. Out of them, 6 complained that it reminded them of having LS, 8 said that it did not fit into their schedule, and for 17 of them, the colonoscopy was burdensome. This was mostly found in populations under 40 years of age. The findings suggested that individuals would be more likely to undergo screening colonoscopy when the hospital is the one initiating the discussion in the context of a centralized cancer registry like the one already in place in the Netherlands because it was revealed that many of the patients didn't know which was the right schedule to appoint a new examination. They concluded by recommending new approaches to surveillance in Lynch Syndrome and exploring less invasive methods like noninvasive biomarkers, that would make patients more likely to adhere to a surveillance program.

Another study from the Prospective Lynch Syndrome Database shows interesting results that somehow contradict the before-known recommendations in the matter of interval between examinations. They compared results from different countries that have different examination schedules from yearly in Melbourne to every 3 years in Finland. They concluded that irrespective of the time since the last colonoscopy was performed, the tumor stage distribution was similar. They proposed another theory, one that exists in breast cancer from before, the overdiagnosis [24]. There are cases described in the literature that formulate the hypothesis that the host’s immune system could detect neoantigens present on the surface of MSI cells and destroy them. So, performing colonoscopies too often could result in additional unnecessary surgical procedures that could be handled by the immune system [25].

Noninvasive surveillance

Over the last few years, there have been several studies conducted in order to try to find a suitable noninvasive screening method to be implemented in order to avoid performing colonoscopy yearly or every 2 years depending on the country of origin. There have been small studies concerning imaging methods, blood biomarkers, or fecal biomarkers that researchers hoped would help patients avoid the burden of undergoing colonoscopy.

In Australia, Lim et al conducted a small prospective study to compare Magnetic resonance colonography with colonoscopy results, in patients undergoing both procedures on the same day. The MR colonography images were reviewed by two radiologists. They managed to detect three lesions in three patients that were large, 70, 36, and 17 mm respectively, but failed to discover one small-sized adenocarcinoma sized 3 mm, two tubulo-villous adenomas, and one sessile serrated adenoma. They concluded that the examination still had a poor sensitivity in detecting small-sized polyps even though they detected the larger ones. Comparing the methods by patient comfort, even though other studies confirmed that patients admit a lower burden in performing MR colonography than colonoscopy, the burden of bowel preparation remains [26]. There have been other studies made on small sets of patients regarding other noninvasive methods such as stool tests (fecal immunochemical test or microbiota) or blood tests but at the moment none of them have proven to be able to replace screening colonoscopy [27-29].

Upper gastrointestinal (GI) tract surveillance

While most of the gastroenterologist’s efforts are driven towards a high quality lower gastrointestinal tract surveillance, considering the high risk of colorectal cancer, gastric cancer, and small bowel cancer are two entities that should be taken into account when talking about Lynch Syndrome surveillance. There is no consensus on onset, interval, or even recommendation to perform upper GI endoscopy up until present. Recent guidelines recommend an individualized approach to gastric cancer screening depending on personal risk; that includes, family members with a diagnosis of gastric cancer, male sex, or increasing age. However, there were cases where risk factors weren't present, which made this a clinician's decision [30]. There is one common point that most guidelines agree upon, universal Helicobacter Pylori testing should be performed, the infection eradicated when discovered, and ulterior testing to confirm the eradication to be done [31]. Recent studies advocate for an early first Upper GI Endoscopy, at 30 years of age or 2-5 years earlier than the first upper GI tract cancer diagnosed in the family, with multiple biopsies taken in order to assess the presence of atrophic gastritis or a presumable Upper GI infection. If symptoms appear, an earlier examination should be performed. While there is no consensus on the best interval between exams, a 2-3 years interval could be a good option if there are no other risk factors to be taken into consideration. Also, the best way to ensure patient adherence to screening is to perform both colonoscopy and upper endoscopy in the same session [32].

Pancreas surveillance

While rarer than the other pathologies mentioned above, pancreatic cancer presents a higher risk in LS patients than in the general population (3.7% vs. 1.5%) [33]. With MLH 1 and MSH 2 mutations being most frequently involved. Another difference between LS patients and the general population is the age at diagnosis, with a mean age of 70 years, in this particular situation it may be diagnosed at a younger age. [34] Unfortunately, there is no standardized screening method for this pathology with the most useful tools at the moment being endoscopic ultrasound and magnetic resonance imaging, both of them being more accurate than a CT scan [35].

DISCUSSION

Lynch Syndrome is a diagnosis with a high impact burden on the patient, that became more common in the past few years with the implementation of standard IHC in all colorectal tumors that are surgically resected. Unfortunately, over the years many patients were missed because of the high cost of the investigations in underdeveloped countries, and physicians made everything possible to try not to miss any patients that may have been at risk for any hereditary cancer syndrome. While there are programs for some genetic mutations to be tested for free, germline mutation for Lynch Syndrome is at the moment available only if patients support the full cost, this situation making many patients refuse it for the moment being. Romania is a country undergoing medical developments with new projects to create national registries set into motion. Seeing as in other countries registries helped medical professionals follow certain categories of patients and improve their outcome, all countries are trying to achieve the same results.

The gastroenterologist is an important part of the diagnostic equation in this syndrome, alongside the gynecologist is usually the first medical professional that identify potential LS patients. That being considered, it is very important that all gastroenterologists should know the clinical features of LS in order to correctly identify patients at risk and send them to a geneticist. Colonoscopic surveillance, but also upper GI surveillance should not be performed at any center but only in the highly specialized ones by trained endoscopists with experience in screening colonoscopies for particular populations. A high-quality colonoscopy should be performed, ideally with the help of chromoendoscopy and of high definition white light endoscopy. Trained specialists know how to assess lesions properly when discovered and to decide which one should be excised endoscopically and which should go to surgery. It is a field in continuous development where new information appears every year. The existence of patient registries is very important and should be made a priority in every country, to allow patients to have high-quality surveillance in specialized centers and receive the best care possible to improve their lifetime expectancy and quality of life.

CONCLUSIONS

Surveillance of Lynch Syndrome patients is very challenging, with new information emerging every day, making the physician's job even more interesting. That is why it is very important to have more gastroenterologists specialized in the pathology associated with LS to ensure an appropriate standard of care for this category of patients.

Conflicts of interest and sources of funding

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Authors' contribution

L.S.D. – writing, original draft preparation, case research, C.G. – review and editing, supervision.

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ORIGINAL ARTICLE

An Endocrine Perspective on Lynch Syndrome

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Abstract: *Background: A germline mutation of the MisMatch Repairgene that causes a malfunctioning DNA system is the defining feature of the hereditary illness known as Lynch syndrome. This review will discuss the endocrine aspects of LS and highlight current advancements in the area. Methods: We searched the available literature of the last 10 years for terms such as endocrine tumors and LS. Our goal is to provide a summary of the most recent information available on the endocrine perspective in Lynch syndrome. Results: The hormonal chemoprevention methods cited are the use of combined oral contraceptives, the use of progestogen-releasing intrauterine devices, and the use of progesterone-only drugs. In addition, after surgical exclusion of the uterus and ovaries, a method frequently adopted for LS patients, it is necessary to start hormonal menopausal therapy, taking into account certain age-specific features. In addition, numerous LS-associated endocrine tumor types have been described. Conclusions: Many hormonal variants are available that are useful in the chemoprevention involved in the treatment of LS. Menopausal hormone therapy is imperative for LS patients who need it. Clinicians need to be aware of the possible association of certain types of aggressive endocrine cancers associated with LS.*

Keywords: *Lynch syndrome, endocrine tumors, menopausal hormone therapy*

INTRODUCTION

The most prevalent hereditary colorectal cancers are those caused by Lynch syndrome (LS), also known as hereditary non-polyposis colon cancer. This autosomal-dominant disorder causes colorectal cancers to develop on average 10 years sooner than sporadic colorectal neoplasia [1].

Lynch syndrome is caused by inherited mutations or pathogenic variations of the genes of the MisMatch Repair (MMR) system involved in the DNA repair system: in the mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6) and PMS1 homolog 2 (PMS2) genes. In addition, epithelial cell adhesion molecule (EpCAM) deletions of the 3' end may result in the epigenetic silencing or inactivation of MSH2. More rarely, germline hypermethylation of the MLH1 promoter area might result in hereditary inactivation of the MMR system. Failure of the MMR system causes a loss of expression of one or two MMR proteins with a consecutive increase in the number of MMR proteins and leads to an increase in errors during DNA replication. In addition, tumors that develop in the spectrum of LS also exhibit microsatellite instability and loss of expression of one or two MMR proteins [1,2]. The future of LS screening may involve using next-generation sequencing to check for microsatellite instability and find mutations in the MMR genes [3].

This syndrome is associated with a significantly increased risk of colorectal cancers along with carcinomas of the endometrium, upper urinary tract (mainly renal pelvis and ureter), ovary and small intestine, stomach, sebaceous tumors (adenomas and sebaceous neoplasms, keratoacanthomas), brain tumors (gliomas), pancreatic and bile duct tumors [4]. Furthermore, there have been observed genotype-phenotype connections, such as an increased incidence of extracolonic tumors or multiple malignancies in individuals with MSH2 mutations [3].

From the endocrinologist's perspective, patients with Lynch syndrome may require clinical and therapeutic management of surgically induced primary menopause with all its associated risks, as well as screening and possible treatment of other associated endocrine cancers.

Additionally, it's critical to accurately and promptly diagnose LS using medical genetic techniques because it affects both the patient and their family in terms of the moment and type of surgery, long-term monitoring, and screening tests for various tumor types.

MATERIAL AND METHODS

We searched PubMed for English and French full-length publications, aiming to compile a narrative mini-review, through a search starting from the terms ‘’lynch syndrome’’, and associated “endocrine tumors” searching for “thyroid tumors”, “adrenal tumors”, “pituitary tumors”, “testicular tumors”. We used as inclusion criteria original publications, regardless of the level of statistical significance, full-length articles, and the French and English language, for the last 10 years. Exclusion criteria were non-English papers, non-French articles, and non-endocrine disorders associated with LS, usually not referred to the endocrinologist. We followed the main endocrine tumors associated with LS, as well as the importance of hormonal treatment in women with LS. Searching for the term "thyroid tumors" associated with Lynch syndrome, we found 17 publications, including 4 case reports and 3 cohort studies. In addition, searching for the term "adrenal tumors" associated with Lynch syndrome in the last years, we found 21 articles, 5 of which were case reports. Similarly, when searching for the term pituitary tumors, the search resulted in 6 publications of which 3 case reports. Searching for the term "testicular tumors" associated with Lynch syndrome in the last 5 years, we found 7 articles, 2 of which were case reports. In addition, searching for other endocrine tumors described with Lynch syndrome we found a case report with a parathyroid carcinoma and one clinical case of thymic carcinoma.

RESULTS

From the endocrinologist's perspective, of interest are how to optimally treat and monitor premature menopause associated with LS treatment and the clinical approach to endocrine tumors developed within this syndrome, especially thyroid, adrenal, but also testicular, pituitary, parathyroid, and thymic tumors.

Premature menopause may develop in patients with LS either induced by prophylactic or curative surgery or secondary to chemo and radiotherapy procedures that may be part of the therapy. The impact on patients, often young women under the age of 45 years, has numerous implications both endocrinologically, on fertility, as well as metabolically, cardiovascular, cognitive, bone, and not least psychologically.

The clinical management of premature menopause is carried out as a team with the gynecologist who is responsible for the diagnosis of ovarian and endometrial pathology associated with the LS. Our article will not focus on the diagnosis and treatment of gynecological pathology, but only on the hormonal and non-hormonal treatment of endocrine consequences, i.e. the treatment of premature menopause and the possible hormonal approach to LS patients. After scanning the specialized literature we will systematize the information found according to endocrine disorders.

Thus, we will first discuss the endocrine consequences of the clinical management of ovarian or endometrial cancer and the possible hormonal and non-hormonal treatments of premature menopause associated with LS. Then, we will detail other endocrine tumor types associated with this syndrome.

1. Ovarian and endometrial cancer

The foremost conditions associated with gynecologic cancers include LS and hereditary breast and ovarian cancer. About 2-3% of all endometrial cancers are considered to be associated with LS [5].

LS genes vary in clinical penetrance, with a lifetime risk of developing endometrial cancer of up to 60% and a risk of up to 24% for developing ovarian cancer, especially in the carriers of MSH2 [6]. On the other hand, PMS2 carriers, in particular, have a lower lifetime risk of cancer than other LS genes[7].

In terms of clinical approaches, we will discuss in the following both general methods of lifestyle optimization and methods of chemoprevention, as well as surgical options to reduce the risk of gynecological cancer.

Regarding lifestyle suggestions, patient education includes methods to lower cancer risk factors generally, such as quitting smoking, getting regular physical exercise (150-300 minutes of moderate-intensity weekly exercise), maintaining a healthy weight, eating a healthy diet (rich in vegetables and fruits and less red meat), limiting or eliminating alcohol, guarding against sexually transmitted diseases, avoiding sun exposure, and getting adequate cancer screenings [8]. However, no particular research has been done on how this broad advice may affect LS patients.

Chemoprevention

The first drug therapy used as chemoprevention in LS is the use of aspirin, as it can reduce the incidence of colorectal cancer. Studies indicate starting with a dose of 325 mg of aspirin per day, with consecutive increases to a dose of 650 mg per day after several months of treatment if aspirin is tolerated without side effects. In patients who have side effects following aspirin treatment or those with a personal and family history suggesting a lower risk of cancer, a lower dose of aspirin may be used [9]. In addition, taking aspirin diminishes the risk of developing all neoplasia in LS gene carriers. Aspirin appears to reduce the risk of endometrial neoplasia in obese women with LS compared to non-obese women [10].

In addition, in terms of hormonal chemoprevention, combined oral contraceptive pills (COC), progestin-only pills, or progesterone-containing intrauterine devices (IUD), are an alternative form of risk-reduction for those with LS, before to completion of the childbearing period and prior to risk-reducing surgery.

Moreover, data on the prevention of endometrial cancer with hormonal therapies in women with LS are limited and most of the evidence is extrapolated from studies in the general population.

Regarding COC, their use is known to decrease the lifetime risk of endometrial and ovarian neoplasia by 50%. Importantly, the protective effects of using COC become stronger the longer the use [11]. A retrospective cohort study showed that patients with LS who received hormonal contraceptives for more than 1 year had a reduced risk of endometrial cancer [12]. However, COC use raises the risk of breast cancer by up to 38% in the general population, if used for more than 10 years [13].

In a retrospective analysis, women with LS who used hormonal contraception for at least a year — in the form of a COC progestin-only pill, or an implant, had a 61% lower risk of developing endometrial cancer [14].

Table 1: Non-surgical prevention methods in LS *(adapted after Lynch syndrome for the gynecologist, Neil AJ Ryan et al)*

	Combined oral contraceptives	**Menopausal hormone therapy**	**Levonogestrel Intrauterine Device**	**Depo Provera Medroxyprogesterone Acetate**	**Lifestyle measures***	**Aspirin**
Timing	Before surgical prophylactic surgery, 2 years after menarche	After surgical excision until the normal age of menopause	Method of chemoprevention, 2 years after menarche	Method of chemoprevention, over 18 years old	Any time	Method of chemoprevention, From 18 years old
Mechanism of action	Prevention of ovulation decreased endometrial proliferation	Anti-inflammatory impact decreased endometrial proliferation	Decreased endometrial proliferation	Decreased endometrial proliferation	Decrease of cancer risk factors	Not fully described
Contraindications	Hypertension, breast cancer, ischemic heart disease, migraines with auras, endometrial cancer, cirrhosis, hepatocellular adenoma, malignant hepatoma, history of venous thromboembolism	History of estrogen-dependent or breast cancer, active arterial thromboembolic disease, undiagnosed vaginal bleeding, thrombophilia disorder, history of venous thromboembolism	Sexually transmitted infection, congenital uterine abnormality, pelvic inflammatory disease, Confirmed or suspicion of uterine or cervical malignancy, abnormal uterine bleeding	Undiagnosed vaginal bleeding, active thrombophlebitis or past history of thromboembolic disorders or cerebral vascular disease, gallbladder disease, history of diabetes, coronary artery disease, or a current tobacco smoker age ≥ 35	Those who have health issues that would prevent them from doing too much physical activity	Severe heart failure, hemophilia, peptic ulcer disease, active abuse of alcohol
Adverse effects	Breakthrough bleeding, nausea, headaches, abdominal cramping, breast tenderness, decreased libido	Dysuria, skin reactions, mood alterations	Headache, acne, breast tenderness, weight gain, cramps or pain during menstruation, depression	Headache, acne, hirsutism, weight gain, depression	-	Dyspepsia, digestive haemorrhage

** reduce weight, increase physical exercise, healthy diet, smoking cessation*

Moreover, the use of depo-medroxyprogesterone acetate (depo MPA) or COC was specifically evaluated in LS patients, showing evidence of a marked decrease in endometrial proliferation [15]. However further investigation of these hormonal agents in this high-risk population is needed.

The levonorgestrel intrauterine device (IUD) releases progestin directly into the endometrium, attenuating the stimulatory effects of estrogen and it is frequently used to treat endometrial intraepithelial neoplasia and low-grade endometrial cancers. Studies have shown that all levonorgestrel IUD users have a reduced risk of endometrial and ovarian cancer in the general population, without an increase in the risk of breast cancer and this decreased risk persists for 5 years following discontinuation [14,16]. Unfortunately, POET, a randomized trial that aimed to ascertain whether the levonorgestrel IUD was effective at preventing endometrial neoplasia in women with LS, was prematurely stopped due to a lack of enrollment [8].

Thus, data on general lifestyle measures and chemoprevention methods used in LS have been systematized in Table 1.

Risk-reducing surgery

Regarding the option of preventive surgery, the Manchester Consensus Group recommends that total hysterectomy with bilateral salpingo-oophorectomy should be offered to patients no earlier than 35-40 years after cessation of the childbearing period in carriers of proven pathogenic MLH1, MSH2, and MSH6 genes. Furthermore, for pathogenic PMS2 heterozygotes, the usefulness of preventive surgery is not proven. It is also recommended to consider a woman's personal risk in order to provide individualized advice on the optimal timing of the surgical procedure [2].

Moreover, women with LS, especially when they are premenopausal and/or women over 40 who have chosen not to undergo total hysterectomy with bilateral salpingo-oophorectomy and who opt for periodic surveillance should have an annual follow-up with a gynecological specialist with knowledge of the syndrome [8]. In addition, a study reported that the risk is higher in the case of MSH6 gene mutation compared to MLH1 and MSH2 gene mutations [17].

The largest study comparing patients with LS concluded that prophylactic hysterectomy with bilateral salpingo-oophorectomy is an effective strategy for preventing endometrial and ovarian cancer in women with LS [18].

Currently, there is no recommended screening strategy for gynecologic cancers with demonstrated efficacy in the setting of LS. The annual association of the clinical examination and the endovaginal ultrasound is proposed [8]. In addition, some studies indicate the performance of the annual endometrial biopsy, is correlated with the monitoring of the CA-125 marker levels [2]. In any case, women should be warned that the appearance of metrorrhagia should lead to a specialist consultation as soon as possible and a diagnostic examination. Most endometrial cancers are diagnosed at an early stage, with a favorable prognosis similar to sporadic ones. In particular, the distribution of uterine tumor localization seems to be different in patients with LS [19]. It is worth noting that while in the case of endometrial cancer patients without LS, the tumor develops in the uterine body, for patients with this syndrome there is a greater risk of tumors of the lower segment of the uterus, which can be misdiagnosed as cervical adenocarcinomas [5].

These risk-reducing operations might lead to early menopause, infertility, and surgical complications, which would have a major negative impact on general health and quality of life. Patients need to be informed about the advantages and disadvantages of this gynecological surgery in terms of the morbidity of the operation and especially the medium and long-term consequences of surgically induced menopause.

The surgical complications rate varies by 1.6-9%, including events such as bowel and urinary tract injuries, bleeding, and infections [18]. Patients should be informed about the symptoms of surgically induced premature menopause, including vasomotor symptoms, sleep disturbances, decreased libido, symptoms of vaginal atrophy but also long-term systemic implications such as osteoporosis, the risk of cerebrovascular disease, cardiovascular disease, and accelerated neurodegenerative aging [20] .

However, studies continue to advise risk-reducing surgery for LS patients who have finished the childbearing period rather than cancer surveillance and chemoprevention [8].

Impact on Fertility

In addition, patients with LS should be referred shortly after diagnosis to clinicians with expertise in female reproductive endocrinology and gynecologic oncology for counseling on gynecologic cancer surveillance. In addition, patients need counseling on fertility, fertility preservation options, use of assisted human reproduction techniques (ART) as well as other options regarding the desire to have children, such as adoption or surrogacy. Additionally, in the case of ART, preimplantation genetic testing to prevent implantation of an affected embryo or prenatal diagnosis to determine if the fetus is affected is recommended in LS [21].

Consideration of fertility preservation options is an important component of the management of patients with LS. Early referral of patients known to carry pathogenic gene variants to reproductive endocrinology specialists at age 20-30 is recommended so that they can be informed about their own follicular reserve, fertility preservation, and potential for preimplantation genetic testing. This allows early assessment of baseline ovarian reserve and enables interested women to achieve spontaneous pregnancies at younger ages or to access ART when the methods are most likely to be successful. Through ART, patients have the option of cryopreserving and storing oocytes, embryos, or both [22].

Generally, during a cycle of controlled ovarian hyperstimulation, the ovaries are stimulated with exogenous gonadotropins, followed by ultrasound-guided transvaginal needle aspiration of the oocytes. However, there is some concern in patients carrying gene mutations about the effect of high levels of oestradiol generated during ovarian hyperstimulation on the risk of developing gynecological cancer. Fortunately, data from the literature indicate that the use of fertility drugs does not appear to increase the risk of breast cancer in patients with prior breast cancer or BRCA gene mutation carriers, having no impact on survival rates, at least in the short term. However, in these patients, it is recommended to minimize the increase in oestradiol levels by using an aromatase inhibitor (letrozole) in addition to using the gonadotropin stimulation protocol, which leads to lower, physiologically similar oestradiol levels. In the case of a patient who has cryopreserved oocytes or embryos prior to bilateral adnexectomy, pregnancy can still be achieved by hormonal preparation of the preimplantation uterus and embryo transfer. However, if a woman does not have her own oocytes or embryos available, she may consider using donor oocytes, or donor embryos, or turning to adoption [21].

For a woman of reproductive age who has opted for total hysterectomy either for treatment or as a preventive method to reduce the risk of cancer, who wants a child from her own gametes, ART can be used to obtain her own oocytes, which are then used in a gestational carrier. Medical, legal, and psychological counseling should be considered for both the prospective parents and the gestational carrier. Uterine transplantation is also a procedure that has recently resulted in live births, but its role in preserving fertility in patients at high risk of hereditary gynecological cancer has not yet been determined [5].

For patients in whom testing reveals a low ovarian reserve, we would recommend that pregnancy is unlikely and that options for becoming parents include adoption or the use of donor eggs or embryos.

Concerns regarding the long-term safety of fertility drugs and procedures have been raised due to the usage of reproductive treatments that may lead to repeated ovulation and changes in endogenous hormone levels, especially in hereditary gynecologic cancers, such as LS [23]. Although certain clinical research has revealed a connection between fertility medications and the risk of cancer, it can be challenging to evaluate the findings of these investigations. Based on the available data, the risk of invasive ovarian cancer is not significantly increased after the use of fertility drugs in infertile women [21].

Offering choices for prenatal testing, including pre-implantation genetic diagnosis, and carrier testing to persons of reproductive age is crucial. About 50% of a parent's kids can be affected by the autosomal dominant condition LS, which can be passed on from either parent. Couples should also be informed of the risks and potential outcomes of conceiving a child with constitutional MMR deficiency syndrome if both spouses have pathogenic mutations in the same MMR gene. To find out if the spouse carries pathogenic mutations in the same MMR gene, carrier testing might be made available. This is especially important since MSH6 and PMS2 mutations are widespread in the community and may not be strongly correlated with a substantial family history of cancer, even though they may be linked to a more moderate penetrance phenotype [24].

Menopausal hormone treatment

In terms of treatment decisions, endocrinologists generally form a clinical management team with gynecologists.

Menopausal hormone treatment (MHT), is a general term that refers to both unopposed estrogen usage for women who have had hysterectomies and also the combination estrogen-progestin therapy for women who still have their uteruses intact but require a progestin treatment to avoid estrogen-associated endometrial hyperplasia. MHT is sometimes referred to as "hormone therapy", although in the past it was known as "hormone replacement therapy". MHT can prevent and relieve most of the symptoms associated with surgically induced premature menopause. Non-hormonal treatment strategies are also available for women who have contraindications to MHT for the treatment of vasomotor symptoms and for the alleviation of genitourinary syndrome symptoms, including selective serotonin reuptake inhibitors (SSRIs), alpha 2 adrenergic agonists, and alternative medicine approaches such as the use of black cohosh, soy, flaxseed, vitamin E , yoga exercise and meditation [25].

Decisions about whether or not to use MHT should be individualized, taking into account the potential risk-benefit balance, severity of estrogen deprivation symptoms, personal and family history of cancer, and therapeutic goals [25].

In addition, recommendations in the Endocrine Society's 2015 Clinical Practice Guidelines suggest calculating cardiovascular and breast cancer risks before initiating MHT for any patient [26].

Although it has not been specifically researched in a Lynch population, MHT is generally regarded as safe for the treatment of surgical menopause after prophylactic surgery in premenopausal women. Women also benefit from requiring only estrogen replacement therapy, which is also advised after hysterectomy for endometrial cancer risk reduction in LS. In particular, for women with LS who undergo premenopausal surgery, experts advise considering MHT [8,14]. It's interesting to note that there is evidence that MHT protects against the development of colorectal cancer in the general population, which may be beneficial for women with LS, regardless of the type of oral or cutaneous MHT product, although further studies in this specific population would be needed [27]. Furthermore, researchers stated that MHT is advantageous in survivors of colorectal cancer [23].

Thus, in the case of young LS patients who opt for preventive surgery with full knowledge of the consequences, MHT should be started as soon as possible. In the absence of contraindications, MHT is recommended for clinical management of relief symptoms as well as for bone, cardiac, and cerebral health.

In addition, the guidelines recommend initiating MHT immediately after the diagnosis of premature menopause and prescribing higher doses of estrogens to mimic endogenous estrogen levels in younger, menstruating women compared to women who are menopausal at a normal age in their 50s. In addition, it states that MHT should be continued until the age of natural menopause, at which point the appropriateness of extending MHT should be reassessed [20].

In the absence of contraindications, MHT is recommended for relief of clinical symptoms as well as for cardiac, cerebral, and bone health and benefits [23].

It is recommended to start treatment with transdermal oestradiol which is biochemically similar to endogenous oestradiol. In addition, the use of the transdermal route avoids hepatic metabolism, with less metabolic impact. Alternatively, oral estrogens or a vaginal ring through which systemic doses of estrogen are administered can be used. Depending on the patient's preferences and whether she has a uterus, endometrial protection can be provided in several ways. If the patient wishes to become pregnant, it is recommended to use micronized progesterone rather than a synthetic progestogen for 12 to 14 days per month[28]. The absence of bleeding at the end of therapy or near the end of cyclic progestin therapy is an indication of a pregnancy test. If conception has occurred, MHT should be discontinued. For women without a uterus, established evidence does not justify the concomitant use of progesterone because endometrial protection is not necessary [5,20,26].

Women who underwent bilateral salpingo-oophorectomy before the age of 45 and were treated with estrogens until at least 45 years of age had a decreased risk of osteoporosis [29].

However, following basic guidelines for preserving bone health, bone mineral density should be assessed as soon as the start of premature menopause [20].

In addition, in a study of the Women's Health Initiative, women assigned to receive estrogen therapy who underwent bilateral salpingo-oophorectomy before the age of 45 years had a reduction in long-term all-cause mortality [30].

Guidelines for the prevention of bone and cardiovascular disease also recommend the adoption of general healthy lifestyle measures: weight regulation, exercise, restful sleep, smoking cessation, moderation of alcohol consumption, adequate calcium and vitamin D intake, and management of blood pressure, lipid and glucose levels [20].

Thus, it should be noted that the use of estrogen comes with a number of warnings and precautions, including a higher risk of gallbladder disease; if severe hypercalcemia, eyesight loss, or cholestatic jaundice develops, estrogen usage should be stopped. In addition, women getting thyroid replacement treatment should have their thyroid function checked regularly [5].

2. Endocrine tumors associated with LS

Adrenocortical carcinoma

Adrenocortical carcinoma (ACC) is a rare, aggressive cancer that starts in the adrenal gland's outer layer. ACC was associated with LS, even though it is known that it belongs to the Li Fraumeni syndrome tumor spectrum due to a TP53 mutation [31]. In a recent study, 3 patients out of a total of 634 patients carrying a germline mutation in the MSH2 gene were diagnosed with non-secretory ACC, corresponding to an incidence of 0.47% of all patients and 1.7% of all patients with MSH2 mutation, similar to other less common neoplasms considered to be part of the LS spectrum.

The association of ACC and LS was initially described in 2013 in a study that reported that 3.2% of LS patients had ACC [32]. Moreover, in 2016, the first case of familial ACC in association with an MSH2 pathogenic mutation was reported [33].

However, at this time, there is no need to advocate routine ACC screening for individuals with LS; nevertheless, physicians should have ACC on their minds for LS patients who have adrenal masses or other clinical signs of overactive adrenal glands [34].

Thyroid cancer

The first case described in the literature was an LS patient with a germline MSH2 mutation who developed undifferentiated thyroid cancer as an index case. Another case described was a thyroid cancer case in a patient with LS with anaplastic thyroid cancer as index cancer.

Another case reported was a synchronous association of papillary thyroid carcinoma and metachronous breast cancer with progression to metastasis [35]. In addition, a cohort study of 383 participants showed co-occurrence of familial non-medullary thyroid cancer (FNMTC) with LS, showing a heterozygous MSH2 gene modification exclusively in the FNMTC-LS family. FNMTC was more prevalent in females in 68.3% and was characterized by a mean tumor size of 1 cm, having multifocal growth in 44% of cases and extrathyroidal extension in 11.3% of cases [36].

Pituitary tumors

Additionally, a patient with LS who had an MSH2 mutation and had significant clinical Cushing disease was documented. He had corticotroph pituitary carcinoma. Furthermore, an invasive nonsecreting macroadenoma and a microprolactinoma, two clinically significant pituitary tumors, were described in a cohort study of 910 patients with LS from Sweden [37].

Testicular tumors

Moreover, a histopathological case report also described a clinical case that led to the broadening of the clinical tumor spectrum of LS by a clinical case of testicular seminoma associated with loss of MSH2 function [38]. Although there have been reports of MSI and MMR deficiency in testicular cancers that are resistant to chemotherapy and have a shorter time to recurrence, testicular cancer is not considered a member of the LS tumor spectrum. Despite the conflicting data reports, it is probably still desirable to investigate individuals with chemoresistant or recurrent testicular cancer for a potential link with LS [34].

Parathyroid carcinoma

Even while parathyroid carcinoma is extremely uncommon — it only makes up 0.005% of all malignancies — and isn't typically seen of being a member of the group of cancers that are connected with LS, one case report was recorded. Thus, a patient with severe hypercalcemia resistant to zoledronic acid accompanied by hyperparathyroidism and MSH2 and MSH6 deficiency was noted, demasking metastatic parathyroid carcinoma [39].

Thymoma and thymic cancer

Thymoma in LS patients has also been documented, as well as a unique occurrence of thymic cancer in a patient who carries the MLH1 heterozygous mutation [40].

CONCLUSIONS

Endometrial and ovarian cancer are more common in people with LS. Hormonal therapies, including combined oral contraceptive pills, only progestin treatment, or progesterone-containing intrauterine devices, are an alternative form of chemoprevention for those with LS. The risk of passing on hereditary pathogenic gene variants to their offspring, future fertility options, and the potential use of hormone replacement therapy after risk-reducing surgery should all be discussed with women who have LS.

Bone mineral density should be measured as early as the onset of premature menopause, following general recommendations for maintaining bone health. In addition, cardiovascular risks should be assessed and managed, and the patient should be encouraged to follow healthy lifestyle measures.

Further studies are needed on the role played by drugs involved in the chemoprevention of endometrial and ovarian cancers, to decide the appropriate time for prophylactic surgery and the ideal time for fertility, in order to enable physicians to prevent and identify Lynch-associated cancers earlier, thus reducing morbidity and mortality caused by this syndrome.

Other endocrine tumors have been described such as adrenal carcinoma, thyroid cancer, aggressive pituitary tumors, parathyroid, testicular, and thymic tumors.

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The author declares no conflict of interest.

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SYSTEMATIC REVIEW

Lynch Syndrome – An Overview of the Worldwide Management of This Silent Killer Within Our Genes

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Abstract: *Lynch syndrome, also known as hereditary non-polyposis cancer syndrome, represents the most common autosomal dominant genetic predisposition for the early onset development of several malignancies. Nearly three decades ago, the discovery of microsatellite instability, a distinctive feature of pathogenic variants within genes encoding mismatch repair proteins, marked a significant leap forward in understanding cancer biology and the underlying spectrum of cancers triggered by these mutations – typical of Lynch syndrome. In recent years, a new treatment paradigm, using immune checkpoint inhibitors, as well as preventive measures has drastically improved the survival rates. Identifying individuals with an inherent predisposition to cancer, through diagnostic protocols followed by personalized screening and treatment algorithms, holds the potential to mitigate premature cancer-related fatalities as well as preventable mortality. It is estimated that only a limited number of patients have been diagnosed, underscoring the importance of implementing specific screening programs for early detection of malignancies to which these patients are susceptible. This article aims to underline the importance of a national protocol tailored to guide a Western-inspired practice for Lynch syndrome patient management, the main aim of the Romanian Society for Lynch Syndrome, by providing an overview of similar initiatives throughout the world.*

Keywords: *Lynch syndrome, national registry; international consortium*

INTRODUCTION

In this article, we provide an overview of the key historical milestones in the management of Lynch's syndrome. Our review spans global geography, with the aim of highlighting the imperative for a national Romanian registry of Lynch syndrome, alongside tailored management initiatives. Additionally, we aim to identify the most straightforward and expeditious approaches to implementing global guidelines within the Romanian healthcare system.

Hereditary non-polyposis cancer syndrome was first studied by American pathologist A. S. Warthin, in 1895, in the family of a seamstress, with a generous background of cancers. [1] Warthin didn't manage to convince the scientific community that cancer prevalence within this family was not a coincidence, despite having published the oncological background of the seamstress's German family, known as "Family G", that had a history of very specific cancers, mostly digestive and gynecological. As such, the topic was left dormant for years. [2] In 1971, Henry T. Lynch, MD revisited "Family G", gathering data on 929 descendants. The clear presence of an autosomal dominant cancer predisposition pattern prompted the initiation of molecular investigations. Subsequent analysis revealed microsatellite instability as a distinguishing feature of these tumors. The underlying reason was attributed to a deficiency in the Mismatch Repair (MMR) system, with the responsible genes duly identified, reporting on the specific mutation in the MSH2 gene for the "G Family". [3,4] Henry T. Lynch formulated the fundamental principles of oncogenetic syndromes (heritable cancers): an early age of cancer onset, a distinct pattern of primary cancers, and a Mendelian inheritance model observed in families worldwide. He emphasized that identifying the genetic predisposition to develop cancers holds both prognostic and therapeutic implications. [4]

The acknowledgment of a genetic predisposition that substantially increases the risk of cancer, predictably affecting specific organs, has prompted global efforts to identify susceptible patients; this is driven by an understanding that closely monitoring organs prone to such risks – the digestive tract, reproductive and urinary systems, as well as the skin – represents the optimal approach for bolstering survival rates. To aid in the identification of potential patients, the Amsterdam Criteria were introduced in 1990. [4]. Understanding the biology of deficient MMR tumors, their accelerated growth rate [5], and the tumor immunogenicity carrying therapeutic implications, through the widespread employment of immunotherapy [6] or prophylactic measures like vaccines which are already in advanced stages of clinical studies [7,8] form the foundation for early detection and standardized management of these patients.

MATERIAL AND METHODS

We conducted a comprehensive search on PubMed for English full-length publications with the intention of creating a concise narrative review. Our query used the keyword “Lynch syndrome management”, aiming to encompass information from several continents. Our inclusion criteria featured original publications from the past 10 years, provided they were in full-length format and written in the English language. We excluded non-English papers, articles focusing solely on isolated tumors associated with Lynch syndrome, and those lacking a systemic impact. Throughout our research, we adhered to prominent international guidelines and consensus related to the diagnosis, screening, and treatment of Lynch syndrome. We found 872 relevant publications for the search term “Lynch syndrome management”. Furthermore, when exploring the intersection of "consortium" and Lynch syndrome over the recent years, we identified 84 articles. Similarly, our query for “Lynch syndrome management guidelines” yielded a total of 182 relevant publications, narrowed down to 95 when focusing on the last 5 years. When we searched for “Henry T Lynch” as an author, we found 294 publications, from which 82 regarded Lynch syndrome pathogenesis, manifestations spectrum, or management options.

RESULTS

The prodigious scientific activity of Henry Lynch is explicitly synthetized in almost 100 articles in which he aimed to find an optimal methodology for diagnosis, being aware that the new paradigm in oncology will impact in a significant way the survival of these patients. Describing the biological particularities of tumors attributed to Lynch Syndrome, he was a visionary regarding tailored screening programs, novel therapeutic options, and chemoprevention. Several international consortiums were coagulated aiming to find families and to study their particularities.

The Birth of Diagnostic Criteria

The Netherlands took the first step by identifying 22 families with a genetic predisposition to develop cancers. In 1983, a registry was set up in The Netherlands, overseen by a committee comprising multiple hospitals. The foundation’s objectives include enhancing monitoring within families with predispositions to hereditary tumors, ensuring consistent periodic assessment, and providing guidance to both general practitioners and medical specialists on screening approaches. [9] Dutch researcher Hans Vasen, MD was one of the leading minds behind the inception of the “International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer” (ICG-HNCPCC). This initiative was conceived during a CRC conference in Jerusalem in 1989 and held its inaugural gathering in Amsterdam in 1990. It was during this meeting that Vasen introduced the initial Amsterdam Criteria, also known as the 3 2 1 rule. Following this, the ICG-HNPCC would meet every year until 2001. [4]

The inception of a prominent new organization, InSiGHT (International Society for Gastrointestinal Hereditary Tumors), stemmed from the merger of ICG-HNPCC with the Leeds Castle Polyposis Group (LCPG), carried out over several meetings, in 1999 in Lorne, Australia, in 2000 in Tiberias, Israel, and in 2001 in Venice, Italy, respectively. [10,11]

These guidelines played a crucial role in pinpointing familial groups that ultimately unveiled the connection between the HNPCC syndrome and inherited mismatch repair gene mutations (MMR) [12-14]. However, the guidelines do not account for extracolonic cancers or apply to smaller family groups.

Both sets of criteria were later revised, as their original variants presented insufficient diagnostic sensibilities and specificity, leading to the omission of numerous patients with Lynch syndrome.

The Revised Amsterdam Criteria (Amsterdam II)

The identification of at least three relatives with HNPCC-associated cancer (cancer of the colon or rectum, endometrium, small bowel, ureter, or renal pelvis) and:
I. One should be a first-degree relative to the other two;
II. At least two successive generations should be affected;

III. At least one should be diagnosed before age 50;
IV. Familial adenomatous polyposis should be excluded;
V. Tumors should be verified by pathological examination. [14]

The Revised Bethesda Guidelines
I. Individual with CRC diagnosed by age 50;
II. Individual with synchronous or metachronous CRC or other HNPCC-associated tumors, regardless of age;
III. Individual with CRC and MSI-H histology diagnosed by age 60;
IV. Individual with CRC and more than one FDR with an HNPCC-associated tumor, with one cancer diagnosed by age 50;
V. Individuals with CRC and more than two FDRs or SDRs with an HNPCC-associated tumor, regardless of age. [15,16]

In November 1996, the Early Detection Branch of the U.S. National Cancer Institute held a global workshop titled "The Intersection of Pathology and Genetics in the Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Syndrome", in Bethesda, Maryland. The workshop sought to elucidate the genetic influence on HNPCC pathology, discussing topics like genomic instability, multistep carcinogenesis, mismatch repair gene involvement, HNPCC histopathology, potential molecular correlations, cell proliferation markers, their relevance to early diagnosis and prognosis, and potential clinicopathologic criteria for identifying more HNPCC patients. [17]

The diagnostic landscape of Lynch syndrome has seen significant advancements through the formulation of the Amsterdam and Bethesda criteria (and their revised versions). As we delve into this realm, it becomes relevant to also consider the contributions of the Ontario Familial Colon Cancer Registry (OFCCR). The organization is an innovative database gathering family histories, epidemiological records, blood samples, and tumor specimens from a relevant sample of colorectal cancer patients and their families. Achieving substantial response rates in genetic family investigations is particularly difficult, due to time demands and confidentiality concerns. Notably, the inaugural year of the registry saw a 61% response rate, with 1,395 probands taking part. [18]

The main goal of the Colon Cancer Family Registry (CCFR), an international consortium comprising 7 institutions from the USA, Canada, and Australasia. [18]

This database allowed for the differentiation between sporadic and familial forms of colorectal cancer in the subsequent year. These distinctions formed the basis for preventive, early diagnostic, and therapeutic strategies for colorectal cancers. [19]

Scientific research into the distinction between sporadic and hereditary cancers within these databases has yielded numerous conclusions that now play a crucial role in primary and secondary prophylaxis, optimal screening methods, and treatment approaches:

- Assessing the therapeutic effectiveness of extensive colectomies [20];

- Creating a model for distinguishing hereditary colorectal cancers from sporadic ones that exhibit microsatellite instability based on hyper-methylation, as well as morphological and clinical criteria [21];

- Comparing the diagnostic values obtained through IHC (Immunochemistry) tests against PCR (Polymerase Chain Reaction) [22];

- Factors that influence the adherence to screening programs [23]

- Smoking as a risk factor and NSAIDs emerging as a protective factor [24];

- The potential reduction of colorectal cancer risk through phytoestrogen intake [25];

- Estrogen-progesterone therapy [26];

- BMI, as underweight and obese postmenopausal women with colon cancer face an elevated risk of death, with comorbidities potentially contributing to this increased risk [27];

- Multiparity and pregnancy decrease the risk of colorectal cancer; studies showed an approximately 30% – 50% lower risk of gravidi-ty-associated colon cancer when considering parity. A rise in gravidity and parity appeared to show a declining trend in rectal cancer risk (p for trend = 0.07). In comparison to women with an equal number of pregnancies and live births, those who were nulligravid and nulliparous faced a 40% - 60% increased risk of colon cancer. Additionally, having one more pregnancy than live birth hinted at a potential decrease in both colon cancer and rectal cancer risk. Furthermore, there was a suggestion of an increased risk of MSI-high tumors in connection with nulligravidity and nulliparity. [28]

- Smoking increases the risk of rectal cancers in patients with Lynch syndrome [29];

- Physical activity decreases the risk of colon cancer [30].

In 2007, the registry of familial colorectal cancers became an international resource for the epidemiological study of colorectal cancers, employing two distinct recruitment strategies: population-based (tracking patients with colorectal cancer and identifying the proband through MSI testing) and clinic-based (identifying families with a genealogical history of cancers). This resource supports an ongoing, comprehensive research initiative that spans various fields and disciplines. The extensive and well-defined resource is open to the wider scientific community for studies on colorectal cancer. [31]

Western Countries Which Have Adopted Best Practice Guidelines

United States of America

In the USA, the implementation of screening recommendations for Lynch syndrome involved the collaboration of the CDC in 2009, resulting in the "Evaluation of Genomic Applications in Practice and Prevention (EGAPP) recommendations for Lynch syndrome. [32]

Figure 1: xxx

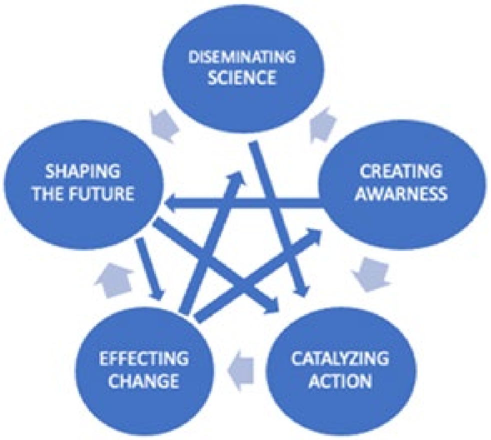

Health outcomes are the ultimate goal, driven by the 5 domains of influence. Disseminating science can include publication of findings in peer-reviewed journals, presentation at conferences, or through other media channels. Creating awareness involves receiving recognition and may include awards, general awareness, or acceptance of a concept or findings by the scientific community or policymakers, generating new discussion. Catalyzing action may include partnerships and collaborations, technology creation, congressional hearings or bills, or introduction in practice. Effecting change may include building public health capacity, legal or policy change, cultural, social, or behavioral change, or economic change. Shaping the future may include new hypotheses or strategies, implementation of new programs/ initiatives, or quality improvement." [33,34]

The genetic testing standard for hereditary colorectal cancers and polyps, set by the American College of Medical Genetics and Genomics, underwent its latest revision in 2021. It mandates universal testing for colorectal and endometrial cancers, irrespective of age at diagnosis, using techniques such as immunohistochemistry (dMMR testing) or molecular biology (microsatellite instability testing). Both methods are considered interchangeable alternatives.

Simultaneously, The International Mismatch Repair Consortium (IMRC) operates as a global collaboration comprising over 115 researchers from 59 centers/clinics across Africa, Asia, Australasia, Europe, North, and South America. These researchers are engaged in clinical care and/or research related to Lynch syndrome families. Professors Jenkins, Macrae, and Haile have established this consortium, harnessing the expertise of leading groups worldwide, facilitated by the International Society for Gastrointestinal Hereditary Tumors (InSiGHT) and the Collaborative Group of the Americas on Inherited Colorectal Cancer (CGA). Cumulatively, the IMRC possesses pedigree data for more than 8,800 Lynch syndrome families, encompassing over 20,000 confirmed carriers of germline mutations in mismatch repair genes. [34]

Canada

Canada initiated the registry for hereditary colorectal cancers in 2011 following a multidisciplinary consensus involving experts in genetics, gastroenterology, surgery, oncology, pathology, and healthcare systems. This collaboration resulted in the establishment of five consensus statements:

- Consensus Statement #1: Hereditary colorectal cancer registries will improve the identification of individuals with an elevated risk of hereditary colorectal cancer.
- Consensus Statement #2: Hereditary colorectal cancer registries will increase access to suitable clinical and genetic screening for individuals at increased risk of colorectal cancer.
- Consensus Statement #3: Increased access to clinical and genetic screening will contribute to reducing the occurrence of colorectal cancer and enhancing survival rates among relatives who are carriers at risk.
- Consensus Statement #4: Every province's population should have access to a provincial hereditary colorectal cancer registry.
- Consensus Statement #5: A network of hereditary colorectal cancer registries in Canada would facilitate both clinical care and collaborative research. [35]

The latest recommendation derived from the Canadian hereditary colorectal cancer registry is that incorporating universal screening within a publicly funded system will enhance the effectiveness of identifying Lynch syndrome patients.

Given potential limitations in genetic testing and counseling resources within public systems, the integration of secondary screening involving BRAF and MLH1 promoter hypermethylation testing is crucial for further enhancing efficiency. [36,37,38]

United Kingdom

In February 2017, the National Institute for Health and Care Excellence (NICE) issued updated diagnostic guidance: Pathology departments at 14 hospital trusts in the Yorkshire and Humber region of the United Kingdom were requested to submit material from patients aged 50 years or older with newly diagnosed colorectal cancer between April 1, 2017, and March 31, 2019, for Lynch syndrome screening. The testing protocol included immunohistochemistry for MLH1, PMS2, MSH2, and MSH6, followed by BRAF mutation analysis with or without MLH1 promoter methylation testing if MLH1 loss was identified. A total of 3,141 individual specimens were submitted from 12 departments, comprising 3,061 distinct tumors and 2,791 prospectively collected CRC patients. dMMR was observed in 15% of cases. Among cases with MLH1 loss, 76% exhibited a detectable BRAF mutation, while 77% of the remaining cases showed MLH1 promoter hypermethylation. Among patients included in the final analysis, 81 (2.9%) had indications for germline testing.

The implementation of LS screening through the NICE diagnostics guidance pathway can be effectively executed on a large scale, identifying a substantial cohort of patients with dMMR. This data is used to refer patients to regional clinical genetics services and also plays a crucial role in shaping treatment routes, including decisions regarding adjuvant/ neoadjuvant chemotherapy and immunotherapy. [39]

Australia

The first case of Lynch syndrome was identified by the Genetic Services Western Australia in 1994, shortly after pathogenic mutations in MMR genes were established as the underlying cause of the condition previously referred to as HNPCC. Over the following 11 years until 2004, there were 1 to 4 new cases identified annually. This observation was deemed lower than anticipated, given that the annual incidence of CRC in WA was approximately 1,000 cases during this period and considering the assumption that LS contributes to 1–3% of all CRC cases.

Australia has a single registry for familial cancers, which, starting in 1994, compiled data accompanied by Lynch syndrome screening results using routine IHC testing of MMR or MSI through PCR for cancers in patients under 60 years old. This approach quadrupled the annual number of identified LS cases. [40]

The Netherlands

The Dutch Hereditary Cancer Registry was established in 1985 with the support of the Ministry of Health (VWS). The main goals of the registry are:

I. Facilitating the identification of families with hereditary cancer;

II. Encouraging high-risk individuals' participation in surveillance programs;

III. Ensuring the continuity of lifelong surveillance examinations;

IV. Promoting research, especially for enhancing surveillance protocols.

Currently in the Netherlands, the diagnosis of Lynch Syndrome relies on a nationwide guideline for MSI analysis, introduced in January 2008 by the Netherlands Comprehensive Cancer Organization. Leveraging the Netherlands Cancer Registry [41], this guideline facilitates dynamic enhancements in Lynch Syndrome Management. This guideline closely resembles the MIPA (Multitesting Indicated by a Pathologist) criteria. MSI analysis (along with IHC of MMR proteins) is requested by pathologists for patients recently diagnosed with CRC or endometrial carcinoma under the age of 50, or those with two LS-associated tumors (including synchronous and metachronous CRCs) before 70. Cases with multiple LS-associated cancers are documented in PALGA, the nationwide network and registry of histopathology and cytopathology in the Netherlands [42]. For MSI analysis prompted by a positive family history, referral to a clinical geneticist is advised. In these instances, MSI analysis is generally conducted when the (revised) Bethesda or Amsterdam Criteria are met, contingent upon the availability of archival paraffin-embedded tumor tissue. [43,44]

Sweden

The National Swedish Registry (now known as the SCRCR) was founded in 1995, with a focus on rectal cancers, and later expanded to include colon cancers in 2007. The use of a unique personal ID number by the SCRCR enables the tracking of patient data over time. The study indicated that a minority of young CRC patients received genetic counseling, which goes against clinical guidelines. Consequently, hereditary CRC is likely to be underdiagnosed, even within the younger population. [45,46]

In Sweden, families suspected of having Lynch syndrome are directed to the regional department of clinical genetics in Umeå, Uppsala, Stockholm, Linköping, Gothenburg, or Lund for counseling and genetic testing.

Switzerland

The present approach to Lynch syndrome screening in Switzerland incorporates two tumor-based assessments (IHC and BRAF V600), followed by DNA sequencing. Germline DNA screening exhibits almost 100% accuracy, thus identifying all instances of Lynch syndrome and eradicating false negative and false positive diagnoses. This strategy also averts the possibility of CRC patients being lost to follow-up and prevents deaths stemming from undetected LS cases. Additionally, it identifies a greater number of cancer-free relatives and new CRC cases at early stages through colonoscopy screening. Despite the notable expenses linked to providing DNA testing for all CRC patients, universal LS screening enhances the overall effectiveness of genetic testing at a reasonable cost. [46]

Iceland

Iceland introduced universal screening for Lynch syndrome by conducting immunohistochemistry for MMR proteins on resected colorectal carcinomas and endometrial carcinomas in 2017. The primary challenge involves establishing a robust implementation of the screening algorithm by pathologists and ensuring accurate referrals for genetic counseling. One suggested solution is the automatic application of IHC staining on all CRC and EC cases, with any abnormal staining outcomes promptly reported to designated pathologists overseeing the ordering and interpretation of additional molecular tests. When genetic counseling is recommended, effective communication with the surgeon in charge, and ideally, the genetic counselor, is of paramount importance, preferably through verbal means [47].

Austria

The Austrian AGO (the workgroup for oncological gynecology) recommends IHC tissue screening for both type-I and type-II endometrial cancers for patients below the age of 70, and for endometrioid and clear-cell ovarian cancers, irrespective of patient age. If necessary, IHC can be supplemented with MLH1 promoter hypermethylation testing and/or microsatellite instability analysis on tissue samples. To confirm a Lynch syndrome diagnosis, identifying a germline mutation through molecular genetic testing of the mismatch repair genes using the patient's blood is required. This genetic examination should be conducted directly without prior tissue screening if the family history of LS-associated cancer patients aligns with either the Amsterdam II or the revised Bethesda criteria.

There are two distinct tumor tissue-based screening processes that significantly raise suspicion of Lynch syndrome:

- IHC identification of the absence of any of the four relevant MMR proteins: MLH1, MSH2, MSH6, or PMS2 in tumor tissue.

- Detection of MSI by examining five specific microsatellites in tumor DNA using RT-PCR; the presence of two or more positive markers indicates a strong suspicion of Lynch syndrome.

However, for individuals with a confirmed LS-associated carcinoma and a positive family history in accordance with either Amsterdam II or the revised Bethesda criteria, the aforementioned screening protocol should be bypassed, and a germline analysis should be promptly provided.

In alignment with the 2014 National Comprehensive Cancer Network guidelines, AGO Austria advises the testing of tumor tissue for Lynch syndrome in all women with endometrial carcinoma (both types I and II) who are under the age of 70. [48]

Furthermore, AGO Austria also advocates for the performance of these tests regardless of the age at diagnosis for endometrioid and clear-cell ovarian cancers. Even in cases where all screening outcomes yield negative results, if the family history still strongly indicates the possibility of Lynch syndrome (i.e., Amsterdam II or revised Bethesda criteria are fulfilled), comprehensive molecular genetic testing of all identified LS-associated genes should be contemplated.

Japan

Until the early 2000s, Lynch syndrome screening predominantly targeted cancer patients with high-risk attributes as per the Amsterdam II criteria or the revised Bethesda guidelines. However, in subsequent years, the practice of universal screening, involving MMR IHC and/or MSI analysis (with or without age restrictions), gradually took precedence over these population-based selections. Consequently, the approach to identifying Lynch syndrome has transitioned towards widespread universal screening [49].

Today, the advent of cancer genomic-based precision medicine has ushered in the use of Next-Generation Sequencing (NGS) for comprehensive somatic and germline MMR sequencing, spanning selected-gene or genome-wide assessments.

However, the main challenge of universally applying sequencing for germline MMR analysis pertains to the assessment of pathogenicity, which relies on the public genome database. This arises due to the substantial presence of Variants of Uncertain Significance (VUSs), and the eventual transition to a pathogenic level over time.

High rates of gastric cancer were observed in Japanese individuals affected by Lynch syndrome, and this occurrence was linked to Helicobacter pylori infection. The deficiency of MMR proteins was found to contribute to the formation of malignant tumors in LS patients. [50]

Advancing Lynch Syndrome Management

Results of Universal Screening

Presently, there are differing approaches to tumor testing methods across hospitals, resulting in a range of 0 - 19% of patients having incomplete mismatch repair deficiency test results. Due to the existing gaps in Lynch syndrome testing and referrals, additional efforts are required to establish effective approaches for seamlessly integrating LS testing into clinical practice; this could serve as a blueprint not only for hereditary cancers but also for broader genomic medicine. The implementation of standardized reporting may assist clinicians in comprehending tumor test outcomes and initiating appropriate subsequent steps.

Extending Testing to Other Organs

In 2020, Stanford University proposed the implementation of MMR testing for all digestive tract cancers, not solely limited to colon cancers. This recommendation stemmed from a study involving 1,425 consecutive malignant neoplasms that underwent MMR testing post-surgery. Surprisingly, 7.2% of these cases exhibited dMMR. The highest prevalence rates were observed for gastric cancers at 10%, followed by colorectal cancers at 9.1%, pancreatic cancers at 5.3%, and gastroesophageal cancers at 4.5%. [51]

Improving the Implementation of Diagnostic Guidelines

Based on these findings, and starting in 2008, routine MSI and/or IHC testing was recommended for all CRC patients under 60 years of age in Western Australia, regardless of their family cancer history. However, the systematic implementation of these tests at a population level for LS identification has proven to be challenging due to the need for collaboration and effective communication among various disciplines, including gastroenterology, pathology, surgery, oncology, and medical genetics. By the end of 2012, GSWA had identified 115 unrelated LS families with MMR gene mutations, comprising 40 MLH1, 51 MSH2, 11 MSH6, and 9 PMS2 germline mutations.

A significant number of Lynch syndrome patients detected through screening did not have a family history of cancer meeting the Amsterdam or Bethesda criteria, highlighting the importance of laboratory screening tests in their identification. Findings strongly suggest that the routine use of MSI and IHC screening for CRC has substantially improved LS detection in Western Australia.

Drawing from past experience with population-based screening in Western Australia, three crucial components take shape that are essential for the successful implementation of LS screening in other states or regions:

I. Reflex IHC testing should be conducted through accredited pathology services with consistent quality control systems in place.

II. The establishment of a centralized reference laboratory at the state or regional level is essential for MSI testing, serving to validate any abnormal or uncertain IHC results initially detected by pathology service providers. This is a crucial mechanism in preventing unnecessary referrals of individuals with false-positive IHC results to familial cancer clinics. In previous observations, the MSI test displayed reduced susceptibility to interpretational errors and greater potential for standardization, compared to IHC.

III. Thirdly, a pivotal aspect is the presence of state or regional-wide LS coordinators, ensuring that all red flag cases discovered by laboratory testing are promptly referred to familial cancer clinics before patients' health deteriorates significantly or life-threatening situations arise. [51]

Defining the Phenomenon of Genetic Anticipation

Genetic anticipation is a phenomenon where symptoms of a hereditary disease appear at an earlier age and/or are more severe in successive generations. [52]

A 2009 study examined the evolution of 125 families, encompassing 824 individuals (with 427 being female). This study yielded a quantified inference about anticipation. It revealed that the cancer appeared 3 years earlier from generation to generation. [53]

Since Alfred Whartin's initial description of Lynch syndrome in 1925, there has been documentation of a gradual reduction in the age of CRC diagnoses across successive generations. Over the years, a multitude of studies have examined the possibility of anticipation in this syndrome, showing variances from 3 to 13 years. [54]

Cost-Effectiveness Analysis for Universal Screening

A cost-effectiveness analysis conducted in Switzerland examined universal screening for Lynch syndrome in individuals diagnosed with colorectal cancer, followed by cascade genetic testing of their relatives. The Evaluation of Genomic Applications in Practice and Prevention working group has endorsed the practice of screening for LS among all newly diagnosed cases of CRC, regardless of age and family history, followed by the sequential testing of blood relatives. [55] The study assesses the economic viability of the alternative approach to identifying LS cases, considering the perspective of the Swiss healthcare system.

An analytical framework was formulated, merging decision trees with Markov modeling to calculate the cost-effectiveness of universal LS screening for individuals newly diagnosed with CRC, followed by cascade testing. Markov models encompassed four states: well-being, CRC, metachronous CRC (mCRC), and death. When diminishing the CRC risk in LS-diagnosed relatives to 25%, the program's cost rose to CHF

103,385 per QALY, surpassing the cost-effectiveness threshold. Universal LS screening combined with the sequential testing of relatives is cost-effective in around 80% of cases. The number of averted deaths is linked to yearly shifts from a state of health to CRC, and from CRC to death, which are significantly lower in populations subjected to testing and screening. [56]

Contemporary Challenges

Among path_MMR carriers undergoing colonoscopy surveillance, deaths resulting from non-CRC LS surpassed those from CRC. Addressing mortality associated with non-CRC presents a significant contemporary medical challenge in managing Lynch syndrome. [57]

International consortia

Genturis

The European Reference Network (ERN) is a network that connects healthcare providers and specialized healthcare centers, aiming to enhance access to diagnosis, treatment, and the delivery of exceptional healthcare services to patients diagnosed with rare diseases, regardless of their location in Europe. The governance of ERN also involves the participation of patient representatives. ERN employs international consensus guidelines for patient recruitment:

- Endorsed guidelines for Lynch, and polyposis, and hereditary CRC. The Manchester International Consensus Group recommendations for the management of gynecological cancers in Lynch syndrome

- Endorsed guidelines for Lynch, and polyposis, and hereditary CRC. Endoscopic management of Lynch syndrome and of familial risk of colorectal cancer – the European Society of Gastrointestinal Endoscopy (ESGE) Guideline.

- Endorsed guidelines for Lynch, and polyposis, and hereditary CRC. European guidelines from the EHTG and ESCP for Lynch syndrome – an updated third edition of the Mallorca guidelines based on gene and gender.

Middle East Network on Hereditary Colorectal Cancer

An association of medical professionals from Middle Eastern and North African (ME/NA) countries was established to enhance the identification and care of LS families. The organization conducted a survey in order to assess the existing healthcare services for families with LS in this region.

The acknowledgment and management of Lynch syndrome within ME/NA countries are suboptimal, leading to the majority of LS families in the area remaining unidentified. Future initiatives should focus on raising awareness about Lynch syndrome among both the public and medical practitioners and on improving the infrastructure in these countries. [58]

Future initiatives should concentrate on raising LS awareness among the public and medical professionals and enhancing the infrastructure within these nations.

Moreover, it's crucial to establish registries for families with hereditary cancer. This step aims to motivate participation in prevention programs and ensure the seamless continuation of lifelong surveillance initiatives. [59]

Countries With Limited Adoption of Western Best Practice Guidelines

Numerous countries continue to exclusively employ clinical diagnostic criteria, namely Amsterdam and Bethesda. The majority are in Asia and Africa, although most of these countries lead the diagnosis until genetic confirmation.

In Taiwan only a small number of medical centers offer Lynch syndrome diagnostics [60]; For a long time, China relied solely on the clinical diagnostic criteria. [61] In 2021, knowledge about LS-related EEC among the Chinese population remained scarce. The most extensive Chinese multi-center population study was undertaken in 2021, involving an analysis of 95 cases of dMMR. [62]; Based on the Singapore Polyposis Registry, an analysis was conducted on 59 unrelated families meeting the clinical diagnostic criteria, specifically Amsterdam II, and revised Bethesda. All identified genetic abnormalities involved MLH1 and MSH2, leading to the detection of LS in 17 families. [63] In a study conducted at a tertiary referral center focused on the rising cases of early-onset colorectal cancer, histopathologically processed tumor specimens revealed a potential increase in Lynch Syndrome incidence among CRC patients in Indonesia. They identified 32 likely cases of LS. [64]; The 2011-founded Russian Society for Clinical Oncology lacks specific recommendations for hereditary colon cancer. Lynch syndrome treatment seems to lean towards immunotherapy, with no clear genetic counseling recommendations. [65]

In South Korea, the largest study, which analyzed colo-rectal cancers recorded over a span of 10 years involving 5,520 patients, applied the Amsterdam II criteria to identify patients with LS. Only 1.3% of participants met these criteria, and their tumors were tested for microsatellite instability. Out of the latter group, only 25 patients, representing 6.9%, tested positive for MSI. Genetic tests were conducted on half of this latter group, yielding positive results for only 11 patients. [66]

Qatar, Saudi Arabia, and Pakistan conducted studies that represent foreplay for genetic testing, but no standardization is available for the moment [67, 68, 69].

African countries

South Africa consists of 9 provinces, with an approximate mid-year population of 58.78 million.

Genetic services are primarily extended to recently diagnosed LS patients and their family members within these areas through academic institutions situated in the Western Cape. Additional initiatives reach out to smaller neighboring towns and rural regions. [70-73]

Colonoscopic surveillance for at-risk family members is arduous and costly in resource-limited settings. Nevertheless, previous LS studies in the Western Cape region of South Africa have demonstrated that identifying the causative mutation significantly streamlines and reduces surveillance expenses. [74]

Morocco lacks a national protocol, the largest study involved 385 patients; for 214, MMR IHC testing was conducted; 159 underwent BRAF mutation testing as well. Among those diagnosed with LS, 35.6% were under 50 years old. These findings underscore the significant prevalence of CRC in young patients and the frequent occurrence of rectal localizations within the Moroccan population. [75]. In Tunisia, diagnosis is primarily based on clinical criteria. [76]

Identification of Lynch Syndrome Risk Variants in the Romanian Population

So far, two important research initiatives have been conducted in Romania.

After sequencing 61 CRC patients, 11 uncommon coding variants were identified in CRC genes: six in MLH1, one in MSH6, one in PMS2, and three in APC. ClinVar19's literature and clinical trial submission data for pathogenicity assessment were cross-referenced. Among the 11 variants, six were previously documented in ClinVar, each with a frequency below 1% in the ExAC database. [77]

Paget's Disease of the Bone and Lynch Syndrome: An Exceptional Finding

The patient's medical record included a diagnosis of well-differentiated rectal adenocarcinoma at the age of 34, which was treated with chemotherapy and radiotherapy, resulting in secondary amenorrhea, as well as the removal of an oesophageal papilloma through endoscopy. Furthermore, she had undergone surgery for three lumbar herniated discs and dealt with autoimmune chronic thyroiditis while maintaining normal thyroid function.

Concerning her family's medical history, the patient's mother had been diagnosed with polyostotic PDB, impacting her skull, femur, and spine at 44. Additionally, her father passed away from colorectal cancer at the age of 47. As of now, no targeted genetic testing has been conducted.

Although the simultaneous occurrence of PDB and LS has not been documented previously, instances of two distinct autosomal dominant conditions co-occurring, albeit rare, have been previously reported. [78].

DISCUSSION

The Romanian Society for Lynch Syndrome is working, through the national Lynch syndrome network, to align Romania with the countries practicing optimized management of LS patients.

CONCLUSIONS

Globally, colorectal cancer ranks as the first or second most common cancer in both sexes. Lynch syndrome, the most prevalent autosomal dominant genetic syndrome, predisposes to the development of numerous cancers, primarily driven by a genetic defect in MMR proteins. The colon is most frequently affected, with up to an 80% elevated risk of neoplasia compared to the general population. Multiple organs can be involved, including the endometrium, ovaries, and other parts of the digestive tract, identified since the syndrome's inception. Early diagnosis, both for the proband and their family, is pivotal for primary prevention and tailored screening programs. As of 2018, distinct therapeutic approaches have been introduced, including immunotherapy [79, 80].

Currently, there are numerous guideline recommendations and countries that have implemented them. However, widespread adoption of these recommendations has the potential to enhance cancer-related epidemiological indices (incidence, prevalence, morbidity, mortality), as well as economic profiles. Additionally, the significance of enriched databases enriches our understanding of the disease's biology, which still harbors many unknowns – prognostic factors for cancer onset, immuno-logical or genetic means (vaccines) of prevention, and more.

Conflicts of interest and sources of funding
The author declares no conflict of interest.

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REVIEW

A systematic review on a comparison between intravenous paracetamol and intravenous morphine sulfate for management of patients with trauma injuries

Ali Ebrahimi[1], Mohammad R. Ghane[1], Seyed N. Naghibi[1]

Abstract: *Introduction – Pain is known the most common complaint in emergency departments. Several studies are administered many different protocols to control pain in trauma patients. The aim of the study was to review the major relevant articles in case of application of intravenous paracetamol and intravenous morphine sulfate for management of patients with trauma injuries.*

Methods – We searched the online databases such as PubMed, MEDLINE, EMBASE, ISI Web of Knowledge. We applied keywords included pain, acute, chronic, morphine sulfate, paracetamol, trauma, limb, wounds, and injuries, damage and etc.

Results – We included four eligible randomized clinical trials in this study. Three studies reported superior effects in intravenous paracetamol compared to intravenous morphine sulfate in controlling pain in trauma patients (limb trauma and trauma headache). However, a study reported no significant differences between the two groups in knee arthroscopy in trauma patients. All studies used from VAS methods for measurement of pain score.

Conclusion – The results indicated that IV paracetamol presents a superior analgesia than IV morphine sulfate particularly after acute limb trauma and trauma headache. However, further studies should be done that clinician could be have better decision for control pain in trauma patients.

Keywords: *intravenous, paracetamol, morphine sulfate, pain, trauma injuries*

INTRODUCTION

Pain is known the most common complaint in emergency departments [1, 2]. Studies have reported about 70% of all patients attending emergency departments to suffer different levels of pain. Several studies are administered many different protocols to control pain in trauma patients [3-7]. However, stand for an ideal suggestion for an analgesic to be administered as the main part of the treatment is a debatable issue yet [4, 7, 8].

Regarding control different levels of in both acute and chronic pain, opioids have many beneficial [9, 10]. Morphine sulfate has known the prototype utilized in patients suffering from different levels of pain because of trauma injuries [8, 11, and 12].

Morphine sulfate has an acceptable analgesia by several controlling modalities in the trauma patient for controlling acute pain [11]. However, several complications such as constipation sedation, confusion, miscellaneous neuro-psychological symptoms, nausea/vomiting and respiratory depression & hallucinations have been reported in patients taking morphine sulfate intravenously [13]. Considering some limits and side effects may result in applications in order to quest for more efficient and less disadvantageous alternative analgesics [4, 8, 14].

N-Acetyl-p-Aminophenol which is acknowledged as paracetamol is an inhibitor of cyclooxygenases which has been recognized globally as a safe and effective substance for pain drug and decreasing fever [12-16]. Paracetamol is one of the leading generally used analgesic and antipyretic factors. Since 2001, intravenous paracetamol has been available in Europe under the trade name Perfalgan and is accessible in many countries [15]. It is generally applied for reducing different rates of the pain of trauma patients from

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mild to moderate [15]. Also, it is used to control fever in neonates, children, and adults [8, 15, 17].

Studies reported paracetamol has fewer complications than opioids or nonsteroidal anti-inflammatory drugs (NSAIDs) [8, 12]. Studies have reported several complications such as opioid administration such as nausea, emesis, histamine release/pruritus, hypotension, sedation, respiratory depression, and constipation following administering this analgesic [16]. Also, hepatotoxicity may happen as a consequence of overdose or exceed therapeutic dosage medicine [15, 16].

Several investigations were carried to analyze the effectiveness of applying paracetamol and morphine either in separated groups or in combination with each other or alternative adjuvant therapy, including postoperative pain relief, cancer pain control or intravenous regional anesthesia [14-17]. A few studies are carried to study the comparative efficacy of prescription of morphine sulfate versus paracetamol in trauma injuries [8, 12]. So it should be realized that further studies may be required to confirm the findings. In this study, we aimed to review the major relevant articles in case of application of intravenous paracetamol and intravenous morphine sulfate for management of patients with trauma injuries to support clinicians to have a better understanding of these technologies and choosing the better healing choice.

METHODS

We searched the online databases such as PubMed, MEDLINE, Wiley, EMBASE, ISI Web of Knowledge and Scopus. We applied keywords included pain, acute, chronic, morphine sulfate, paracetamol, trauma, limb, wounds, and injuries, damage and etc. All relevant randomized clinical trial studies were included in this study. Two reviewers independently searched and evaluated the titles and abstracts of all articles.

RESULTS

In a randomized, clinical trial by Vahdati et al. (2014) evaluated the analgesic and safety of paracetamol compared to morphine in the management of a headache in patients with a trauma headache. Patients were randomly classified into two groups to get either intravenous paracetamol [Apotel 1 g (paracetamol 1 g made by Unipharm factory, Greece) normal saline (1 g/100 mL/10 minutes)] or morphine (0.1 mg/kg/100 mL/10 minutes NaCl). They concluded intravenous paracetamol is an effective and safe treatment than morphine [16].

Craig et al. (2012) compared the effect of intravenous paracetamol versus morphine in patients with moderate to severe traumatic limb pain. They included patients (16 to 65 years) with isolated limb trauma and in moderate to severe pain (pain score of 7 or more) received either 1 g intravenous paracetamol or 10 mg intravenous morphine sulfate over 15 min. They measured pain score via visual analog scale at 0, 5, 15, 30 and 60 min following beginning drug treatment. They reported there was no difference in analgesic effect and rescue analgesia between the two groups at any time interval. But there were significantly more adverse reactions in the morphine group than the paracetamol group. They concluded intravenous paracetamol seems to give a level of analgesia comparable to intravenous morphine in isolated limb trauma patients [17].

Jalili et al (2016) did a randomized double-blinded clinical trial for comparison of intravenous 0.1 mg/kg morphine sulfate with 1 g intravenous paracetamol in order to pain reduction in sixty patients with pain score of greater than 3/10 in the limb trauma. They reported the mean pain intensity scores for paracetamol (3.86) and for morphine (2.16) ($P < 0.001$). 15 patients (morphine group) plus four patients (paracetamol group) required rescue analgesia ($P = 0.05$). They concluded that IV paracetamol present superior analgesia than IV morphine in these traumatic patients. However, further studies should be done that confirms this issue [18].

Hashemi et al (2015) evaluated comparison of IV paracetamol with morphine for the pain administration after diagnostic knee arthroscopy in sixty traumatic patients in a randomized double-blinded clinical trial. They measured the nausea and vomiting, pain level, and sedation. They reported no considerable difference between the two groups regarding to the pain level. The complications were not seen in the paracetamol group; although, the postoperative nausea was seen in 22.3% of PCA morphine group. Hashemi et al presented clinical advantages for IV paracetamol including stable hemodynamic parameters, reduction of analgesics management, improved postoperative pain, no nausea and vomiting, lower complications, reduction length of hospitalization, and increased patient comfort and satisfaction [19].

Dijkstra et al (2013) did a systematic review in order to recognize efficient and safe primary pain interventions for traumatic patients with acute pain in the chain of emergency care, accessible in the Netherlands. They reported that Paracetamol (both IV and orally) and IV opioids (fentanyl and morphine) comfirmed to be efficient. They not recommended non-steroidal anti-inflammatory drugs (NSAIDs) for use in pre-hospital ambulance or emergency medical services because NSAIDs showed mixed results [20].

Table 1: Characteristics of the reviewed studies

Author(s) (year)	Medication	Design	Trauma	N	Setting	Pain score outcome measure	Pain relief results	Conclusion
Jalili et al (2016) [18]	intravenous 0.1 mg/ kg morphine sulfate with 1 g intravenous paracetamol.	randomized double-blinded clinical trial	Limb Trauma	60	trauma center of a university hospital (emergency department)	VAS	The mean reduction in numerical rating scale pain intensity scores at 30 minutes was 3.86 (± 1.61) for paracetamol, and 2.16 (± 1.39) for morphine	They concluded that IV paracetamol present superior analgesia than IV morphine in these traumatic patients. However, further studies should be done that confirms this issue
Hashemi et al (2015) [19]	intravenous infusion of 1 g paracetamol within 15 minutes after surgery and every 6 hours to 24 hours in the paracetamol group. The patient-controlled analgesia group received morphine through PCA infusion pump at 2 mL/h base rate and 1mL bolus every 15 minutes. Pain level, nausea and vomiting, and sedation were measured and recorded during entering the recovery, 15 and 30 minutes after entering the recovery, 2, 6, and 24 hours after starting morphine pump infusion in the morphine and paracetamol in the paracetamol groups.	randomized double-blinded clinical trial	knee arthroscopy in trauma patients	60	trauma center of a university hospital (tertiary hospital)	VAS	They reported no considerable difference between the two groups regarding to the pain level. The complications were not seen in the paracetamol group; although, the postoperative nausea was seen in 22.3% of PCA morphine group	Hashemi et al presented clinical advantages for IV paracetamol including stable hemodynamic parameters, reduction of analgesics management, improved postoperative pain, no nausea and vomiting, lower complications, reduction length of hospitalization, and increased patient comfort and satisfaction
Vahdati et al. (2014) [16]	intravenous paracetamol [Apotel 1 gr. (paracetamol 1 gr. made by Unipharm factory, Greece) normal saline (1 g/100 mL/10 minutes)] or morphine (0.1 mg/kg/100 mL/10 minutes NaCl).	randomized, clinical trial	Trauma Headache	60	trauma center of a university hospital (tertiary hospital)	VAS	After 15 minutes, pain was 31.7 ± 18.0 and 48.3 ± 14.1 mm in Paracetamol and Morphine Respectively (P<0.005).	They concluded intravenous paracetamol is an effective and safe treatment than Morphine
Craig et al. (2012) [17]	1 g intravenous paracetamol or 10 mg intravenous morphine sulfate over 15 min.	randomised, double-blind pilot study	traumatic limb	55	Emergency department	VAS	After 60 minutes, pain was 52.9± 27.4 and 44.0± 22.6 in Paracetamol (and Morphine Respectively (P=0.28).	They concluded intravenous paracetamol seems to give a level of analgesia comparable to intravenous morphine in isolated limb trauma patients

DISCUSSION

Efficient management of pain is an important concern for anesthesiologists. The patients still endure insufficient postoperative pain administration in spite of the progression of pain control methods. The management of pain by opioids has some complications such as vomiting, nausea, urinary retention, pruritus, and respiratory depression [21]. Consequently, the non-opioids have become more accepted to avoid of these complications [22]. Hashemi et al reported that paracetamol and morphine equally reduced the intensity of postoperative pain. Decreasing of opioids usage is crucial for the reason that it dues to a reduction in complications and side-effects. In addition, any method that reduces admittedly total opioid consumption dose is considered an advantage. It demonstrated that intravenous paracetamol be as successful as ketorolac, diclofenac, and morphine in the some clinical studies. Therefore, it is favorable for the management of moderate to severe pain after surgery [23, 24].

The correct mechanism of function of paracetamol has not been found. The paracetamol might cause activation of serotonin receptor II and subsequently prevention of cyclooxygenase pathway for the production of prostaglandins with double-barreled impacts [25-27]. The paracetamol can be related to bleeding complications through preventing the synthesis of prostaglandins and associated compounds [25]. Paracetamol may possibly reduce peripheral vascular resistance and cardiac output [28]. Hashemi et al presented clinical advantages for IV paracetamol including stable hemodynamic parameters, reduction of analgesics management, improved postoperative pain, no nausea and vomiting, lower complications, reduction length of hospitalization, and increased patient comfort and satisfaction.

Different studies discuss the advantages of IV paracetamol such as; Sinatra et al [29] reported that IV paracetamol is a valuable analgesic, although Haddow et al [30] commented that it is more readily accessible than morphine, which is an effective and restricted drug. Todd et al [31] demonstrated that no difference between IV morphine and IV paracetamol in the analgesic effects on patients. Also, Van Aken et al [32] demonstrated that IV paracetamol is superior tolerated after dental surgery. Craig et al [33] demonstrated that approximately no difference in pain scores for comparison of the effects of 10 mg of IV morphine with 1 g IV paracetamol in the UK ED patients. Paracetamol can reduce opioid supplies by between 20% and 30% because it is an efficient adjunct to opioids [34]. The researches indicated that IV morphine would have intensified the iatrogenic influences of fentanyl, especially its potential to cause respiratory depression and hypotension, while IV paracetamol be able to decrease the amount of fentanyl required [35].

Institute for Clinical Systems Improvement reported that paracetamol is extensively applied in clinical practice and is an effective safe, simple, and analgesic with few side effects. Hoogewijs et al and Viallon et al [36-37] reported that intravenous management of paracetamol in the ED seemed to have a quicker beginning, while paracetamol controlled p.o. does not need an i.v. catheter supplement and is consequently easier and less time-consuming for emergency care professionals. Feldman et al indicated rectal usage of paracetamol has a inconsistent bioavailability, and it cannot be simply appropriate in pre-hospital emergency conditions; consequently, this method of management is not suggested in emergency care. Dijkstra et al reported that Paracetamol and opioids including morphine and fentanyl proved and verified to be effective and safe analgesics for trauma patients [20].

Jalili et al demonstrate and suggested that acetaminophen is a safe and effective nonopioid analgesic for ED patients with acute traumatic limb pain. They showed IV morphine sulfate is efficient for pain administration in these patients, although 1-gram dose of IV paracetamol presented a superior level of analgesia comparison to morphine. In the other hand, the morphine consumption is considerably lower in the paracetamol group [18]. Several studies confirmed prospective efficiency of intravenous paracetamol in acute pain administration. Nevertheless, the majority of them demonstrated that the analgesic outcome of IV paracetamol is analogous to effect of IV morphine sulfate. Craig et al comprised IV paracetamol and IV morphine sulfate for pain administration of acute limb trauma in the ED, they showed no considerable difference in analgesic outcome between the morphine and paracetamol groups at any time point [33]. However, amount of morphine sulfate was greater than the used morphine dose by Jalili et al. Bektas et al. evaluated the analgesic outcome of IV morphine and IV paracetamol for the cure of renal colic, while were observed no considerable reductions in the pain intensity between paracetamol and morphine group [38]. Sinatra et al. assessed the safety and efficacy of paracetamol for pain administration following major orthopedic surgery [38]. They reported the pain intensity scores for consumption of paracetamol and morphine is considerably reduced while no complications were seen during 24 hours after surgery in comparison to the placebo group [38].

CONCLUSIONS

The results indicated that IV paracetamol presents a superior analgesia than IV morphine sulfate particularly after acute limb trauma and trauma headache. However, further studies should be done that clinician could be have better decision for control pain in trauma patients.

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REVIEW

A comprehensive review of the factors affecting the work-life balance of nurses

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Abstract: *The field of nursing has a lot of responsibilities and workload and is considered as a hard job. If a person does not have the spirit of being responsible for work or interest in service, he cannot survive in the difficult conditions that exist in the field of nursing, such as fatigue, work shifts, stress. Occupational concerns such as insufficient staff, high workload, long working hours and work pressure make nurses unable to play their role in front of their families. On the other hand, nurses are usually unable to perform their duties in the workplace due to family problems. In this study, related articles from the years 2000-2020 were reviewed. The results of studies showed that due to the importance of work-life balance in nurses, it is recommended that managers create the conditions for creating effective methods and techniques for job flexibility for nurses and help nurses to better perform these approaches with the necessary training. Taking care of nurses living conditions, question and answer sessions and interviews with nurses, establishing kindergartens with long working hours can also be effective in creating balance*.

Keywords: *work-life balance, nurse, management, satisfaction*

INTRODUCTION

Nurses are important members of the health care team that human society needs and they exert their influence in the health system by playing different roles [1]. For nurses, like other sections of the working community, the profession is considered as the most important factor in shaping social identity, the source of providing the family and shaping social relationships [2]. But despite all the positive effects that work and the resulting income have on the personal life of every human being, the concept of work has changed imperceptibly over time, exposing human social and family relationships to wide-ranging changes and causing problems for employed people [3].

One of the biggest problems that almost every working person complains about is the lack of balance between work and family activities, so much so that it can be said that the lack of balance between work and life has become a new companion for today's families [4]. Today, the nursing community around the world faces many problems such as insufficient staff, high workload, long working hours, irregular work schedule and work pressure. In studies on job stress over the past 32 years, the nursing profession has been considered as the first source of stress due to insufficient number of nurses and as a result of high work pressure on nurses [5]. Stress is a well-known component of modern nursing, which in the long run leads to chronic diseases such as hypertension, cardiovascular disease and asthma. In today's competitive world, the issue of work-life balance has attracted the attention of many researchers and academics around the world [6]. Today, the challenge of balancing work and family roles is one of the main concerns for individuals. They have the most and strongest connection with the individual and society, so that creating a balance between them is of great value and importance, and if the relationship between work and life is not managed, the conflict between them will cause irreparable damage to the individual, organization and society [7].

A study by Boston Consulting Group on HR managers across Europe found that balancing work and life is one of the three major challenges facing HR managers. Work-life balance is a self-identifying term that is achieved by a person who is able to manage responsibilities in the field of work and family, and this balance is maintained by physical and mental health by preventing grief and other negative effects on life [8].

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Researcher shows that failure to achieve the right balance between effort and rest leads to a feeling of lack of control over workload and lack of energy to achieve individual goals and commitment, resulting in imbalance leading to fatigue, poor performance and reduced quality of life [9]. There are several definitions of work-life balance, with some studies defining work-life balance as follows: The extent to which an individual's effectiveness and satisfaction in work and family roles are consistent with his or her life priorities. Balancing work and life is one of the most important concerns of employees in organizations today. In other words, the most successful employees are those who can balance work and life [10]. One of the tools to balance work-family life is to create flexible programs in organizations. Hospitals are one of the organizations that have more justification for implementing flexible programs among their employees (nurses) [11]. The purpose of this study is a comprehensive review of the factors affecting the balance between work and life of nurses.

LITERATURE REVIEW

The Table 1 lists the most important studies conducted in the field of work-life balance.

Table 1: Literature review

Study specifications	Conclusion
Clark AJ. Nasica and fides. The Classical Quarterly. 2007 May;57(1):125-31.	Defines work-life balance as good and satisfying performance at work and at home, regardless of role conflicts (12)
Raisinghani M, Goswami R. Model of Work Life Balance Explaining Relationship Constructs. Int J Appl Innov Eng Manage 2014;3(2):46-59.	Work-life balance is defined as the satisfactory and appropriate performance of work at work and at home with minimal role conflict (13).
Littig B. Work Life Balance-catchword or catalyst for sustainable work? Vienna: Institute for Advanced Studies 2008.	On the other hand, work-life balance is mainly related to issues related to employee productivity, performance and job satisfaction (14).
Fapohunda TM. An exploration of the effects of work life balance on productivity. J Hum Resour Manage Labor Stud 2014;2(2):71-89	Various studies show that when employees are happy at home, workplaces are free of conflict and become enjoyable places that ultimately lead to increased employee productivity (15).
Engel CC, Bray RM, Jaycox LH, Freed MC, Zatzick D, Lane ME, et al. Implementing collaborative primary care for depression and posttraumatic stress disorder: design and sample for a randomized trial in the U.S. military health system. Contemp Clin Trials. 2014;39(2):310-9	Excessive workload and poor work environments severely affect the psychological aspects of employees and make it difficult for them to strike the right balance between work and private life (16).
Grzywacz JG, Frone MR, Brewer CS, Kovner CT. Quantifying work-family conflict among registered nurses. Res Nurs Health. 2006;29(5):414-26.	Hospital staff are more exposed to work-life conflict due to occupational conditions such as low organizational support, number of shifts, hard work, low work flexibility and long working hours (17).
Buerhaus PI, DesRoches CM, Dittus R, Donelan K. Practice characteristics of primary care nurse practitioners and physicians. Nurs Outlook. 2015;63(2):144-53	Long working hours in medical settings for nurses can cause severe depression and hormonal imbalances. As a result, nurses often tend to eat more, use drugs to relieve fatigue, stress, and drowsiness in their lives (18).
Kunaviktikul W, Wichaikhum O, Nantsupawat A, Nantsupawat R, Chontawan R, Klunklin A, et al. Nurses' extended work hours: Patient, nurse and organizational outcomes. Int Nurs Rev. 2015;62(3):386-93	Various studies show that nurses' mistakes at work, such as mistakes in injections and medication errors, cause more problems that may cause patients dissatisfaction (19).
Poulose S, Sudarsan N. Assessing the influence of work-life balance dimensions among nurses in the healthcare sector. J Manage Dev 2017;36(3):427-37.	Nurses also have responsibilities in relation to their family and social life, so that an imbalance between nurses' personal and professional activities can lead to reduced organizational commitment, burnout, increased work stress, severe job dissatisfaction and disruption in personal, family and life (20).
Konrad AM, Mangel R. The impact of work-life programs on firm productivity. Strategic Manage J. 2000;21(12):1225-37.	Balance programs in three types of organizations have significant profit margins, which are: organizations that have a high percentage of skilled labor, have made large investments in connection with the specific skills of their organization, and a high percentage of their workforce are women (21).

Study specifications	Conclusion
Wong SC-k, Ko A. Exploratory study of understanding hotel employees' perception on work– life balance issues. Int J Hosp Manage 2009;28(2):195-203	In their research, the factors affecting work-life balance are important, including having free time, work support in case of family problems, work commitment, job flexibility, attention to life issues and voluntary reduction of working hours in order to solve family problems (22).
Hasanqulipour T, Seyed Javadin S, Safiri K, Roshannezhad M. [Presentation and explanation of a model for causes and factors affecting the balance of work life]. J Public Adm. 2012;5(2):37-54.	In their research, they identified job flexibility, work and family support, and individual skills as factors affecting work-life balance (23).
Hasanqulipour T, Seyed Javadin S, Safiri K, Roshannezhad M. [Presentation and explanation of a model for causes and factors affecting the balance of work life]. J Public Adm. 2012;5(2):37-54.	Free time, work support, work commitment, job flexibility, attention to life issues and reduction of working hours voluntarily (24).
Rahimaghaee F, Dehghan Naiery N, Mohammadi E. [Iranian nurses perception of their professional growth and development]. Online J Issues Nurs. 2010;9(10).	In their research, they concluded that many nurses believe that in no other profession are conditions such as the nursing profession possible for them to help others, this attitude is largely rooted in the nature of Iranian society. Iranian society is associated with spirituality and religion, so that some nurses participating in the study of their presence in the nursing profession considered it a divine destiny that caused them to become closer to spirituality, so the commitment to the nursing profession regardless of material needs (25).
Hinami K, Whelan CT, Wolosin RJ, Miller JA, Wetterneck TB. Worklife and satisfaction of hospitalists: toward flourishing careers. J Gen Intern Med. 2012;27(1):28-36.	In their study, they showed that 1.63% of the nurses studied are weak in balancing work and life, which will lead to high job transfer rates, poor job satisfaction and ultimately low quality of life for nurses (26).
Hebrany P, Behdani F, Mobtaker M. [Exploring of stressors in nurses]. J Ment Health 2010;10(3):231-7.	The study identified job stressful factors for nurses including job insecurity, financial problems, communication conflicts, time constraints, irregular working hours, high work pressure, problems with psychological contracts, unequal job opportunities, inadequate vocational training and hospital policies. And introduced one of the ways to reduce these problems to support nurses (27).
Almalki MJ, Fitzgerald G, Clark M. Quality of work life among primary health care nurses in the Jazan region, Saudi Arabia: a cross-sectional study. Hum Resour Health. 2012;10(1):30.	In their study, they concluded that 91% of the nurses studied in the hospital environment need times or places away from patients to be free from any stress for hours (28).
Bahrami M. [Quality of life of nurses working in internal-surgical wards of Oncology center of medical university Isfahan]. J Isfahan Univ Med Sci. 2016;3(7):36-46.	In her research, she showed that the quality of life of nurses is far from the desired situation (29).
Baljani E, Rahimi J, Safari S. [Health Promotion Needs, Stress Resources, and Nurses' Workplace Problems]. J Hamedan Nurs Midwifery Fac. 2013;21(1):49-56	Showed that more than half of the nurses participating in the study needed help and promotion in the areas of exercise and physical activity, stress management, improving nutrition and avoiding continuous work (30).
Alhani F, Seyyed Nematollah Roshan F. [Explaining the Problems of Improving Nursing Healthy Lifestyle and Providing Solutions]. Razi Med J. 2014;21(125):1-8.	The biggest problem of nurses in promoting a healthy lifestyle related to the managerial-executive field was the high working hours of nurses with a score of 1.72 (31).

CONCLUSION

Considering the importance of nurses 'health as the main member of the country's health system, which is responsible for maintaining the health of themselves, family and society, and since nurses' health is directly related to the quality of their performance in caring for patients and playing family roles The importance of conducting this research in identifying the factors affecting the work-life balance of nurses is becoming increasingly clear. According to various studies in the present study, the factors affecting work-life balance, respectively, the importance of the effect, including job flexibility, commitment, work support, free time, attention to life issues and reduction of working hours were voluntarily extracted. Therefore, if hospital managers provide conditions for nurses to have some control over their working hours. In order to reduce the problems of nurses in this dimension, it is recommended that managers provide less working conditions for nurses in exchange for lower salaries and maintaining job security. This approach can be useful in solving many of the problems mentioned by nurses. The findings of this study emphasize the importance of creating a balance in the family and professional life of nurses, so that by informing the officials of the results of this study, an important step can be taken to reduce the problems of nurses and increase the quality of services provided to them.

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ORIGINAL ARTICLE

The COVID-19 pandemic – A global statistical interpretation in first year dynamics

Viorel Ordeanu[1,2], Lucia E. Ionescu[1]

Abstract: *The novel coronavirus, identified in December 2019, has caused a pandemic that will remain in the medical literature as SARS-CoV-2, the etiological agent of the Covid-19 disease. It was initially stated that this disease would not be more serious than the flu, in terms of prevalence, frequency of complications and mortality. Although in the country of origin the epidemic was only local, the virus spread successively to other countries, until it quickly spread to all inhabited continents and was declared a pandemic.*

Being a new disease and a new virus, the world panicked. The World Health Organization released guidelines to member countries, which they have implemented. Because the disease sped out of control, it became not only a health crisis but also a socio-economic one. A year later, we are still facing this disease, about which we have learned a lot but not yet all that is necessary and so the time has come for a certain summing up.

The Covid-19 pandemic is a reality of current times, the year 2020 being portrayed as the "COVID year". The international scientific community has shown that, when needed, it can collaborate beyond classical barriers. Statistics show that the virus is not very aggressive, the disease is not very serious but there are also serious, sometimes fatal complications to which appropriate measures must be applied. The risk of serious illness and life-threatening complications is statistically reduced over time, the disease being less severe now than at the beginning, so it is on a downward slope.

Keywords: *Covid-19, SARS-CoV-2, pandemic, statistics, public health, fake news*

THE OVERALL SITUATION

The coronavirus identified by the Chinese in December 2019 caused an epidemic and then a pandemic that would remain in the medical literature as the severe acute respiratory syndrome virus coronavirus 2 (SARS-CoV-2) as the etiological agent of Covid-19 disease (coronarovirus from December 2019) by analogy with the SARS coronarovirus from 2002. It was stated from the beginning that this disease would not be more serious than influenza, both in prevalence and in the frequency of complications and mortality.

Although in the supposed country of origin the epidemic was only local (but in the size of a medium-sized country) and was "obstructed" in a short period of time by drastic, medical and non-medical measures, the virus spread successively to other countries, until it quickly spread to all inhabited continents and was declared a pandemic.

Being a new disease (although viral pneumonia is known and treatable) and a new virus (although coronaviruses are known with hundreds of species with different pathogens and virulence) the world panicked. The World Health Organization (WHO) has accounted for the situation and released guidelines to member countries which they have more or less implemented, sooner or later, according to "national specifics". Because the evil was not pulled out by the roots suddenly but rather gradually, the disease got out of control and became not only a health crisis but also a socio-economic one. It seems that some countries would have had an interest in maximizing the disease in order for it to be considered the main cause of the current economic crisis, which had started anyway. The impact on the population was variable, from absurd denial and protests to unjustified fear that culminated in suicides for fear of the novel coronavirus.

A year later, we are still facing this disease, about which we

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have learned a lot but not yet all that is necessary and so the time has come for a certain summing up, from the onset to the present. The assessment is useful for evaluating through critical points in the dynamics, because in fact we do not even know when and how this complex crisis that mainly dominated Western civilization will end.

The statistically processed data were recorded before the appearance of new variants of SARS-CoV-2 discovered in the UK (known as 20I/501Y.V1, VOC 202012/01, or B.1.1.7) and South Africa (SARS-CoV-2 known as 20H/501Y.V2 or B.1.351).

MATERIAL AND METHOD

The medical literature on Covid-19 and the information released in the official and unofficial public space about the current complex crisis caused by the pandemic were studied. Data published in the media and on the Internet by credible organizations were statistically analyzed: WHO, CDC (Center for Disease Control, USA), ECDC (European Center for Disease Control), Worldometer (USA), communiqués from national governments and specialised journals. Of these, the most complete and systematized data, representing the US point of view, are updated and published daily on the website and can be accessed free of charge by anyone interested in accurate information [1, 2].

The primary statistical interpretation was possible by dividing quantitative intervals, percentage processing by absolute and relative values per million people [3], by applying the 2 alpha significance test, etc. corroborated with medical logic, which has lately come into contradiction with "pseudo-evidence-based medicine". In general, infectious diseases on Earth and consecutive epidemics have a certain pattern of evolution, which is learned in medicine and is found in the history of medicine. The Romanian historian Nicolae Iorga said "Learn history to know what will come!". Obviously, when it comes to microbes brought, with or without intention, from other celestial bodies, we will not know what we have to do, being in front of absolute news, and the risk will be maximum.

RESULTS AND COMMENTS

The listing of the data provided by Worldometer on world statistics for all the landmarks considered important, grouped by fields, presents a comprehensive, dynamic and realistic figure of the current society. The listing of data provided by Worldometer on 13.12.2020 on the Covid-19 pandemic shows that data on morbidity, prevalence, diagnosis, severity and mortality from 120 territorial entities on five continents were reported and centralized: the 195 independent member countries UN/WHO, 23 permanently inhabited dependent territories and two extraterritorial ones (cruise ships quarantined for epidemic on board) but not including the territories that did not declare the presence of this disease.

The centralised data published on a daily basis are in permanent dynamics, apparently amplified, so for any statistical interpretation the corresponding date must be mentioned. In general, the trend is mathematically increasing, but through the biostatistical interpretation of the primary and secondary data, different parameters of the pandemic can be objectively highlighted.

2020 statistical processing on case studies

The exhaustive table is ordered by the absolute number of cases of Covid-19 disease recorded from the beginning of the year until now, with linear and logarithmic graphical processing. The total number of 72,197,885 cases registered on 13.12.2020 represents a prevalence of the disease of 0.92% of the world's population (7,832,803,872 inhabitants). Compared to the overall mortality of the world population (55,995,505), 2.88% of mortality is due to/with Covid-19 (1,613,401). We statistically analyzed the percentage distribution of mortality (as the maximum severity factor of the disease) compared to the total number of Covid-19 cases registered by country, the world average being 2.24%, so a total of 1 death of covid or with covid at 4 855 inhabitants, which means a cause of mortality with a relatively low frequency. It is far outweighed by other diseases, such as HIV/AIDS, under-five infant mortality or toxic use (tobacco, alcohol, drugs).

The graphical distribution by percentage statistical intervals of the countries did not create a normal bell-shaped Gaussian curve, but a steep downward curve. Thus, for the interval 0 (inclusive) – 1 (exclusive) % there are 75 countries, about a third of the total, and of these 24 countries/territories have no covid mortality at all. In general, these countries have a relatively small population (below the world average) and the total number of covid patients is relatively small, below 1,000, except for Turkey. At the 1-2% range are 62 countries, including "world champions" such as the USA, India, Brazil and Russia. In the median range of 2-3% are 50 countries, including Romania which is slightly above the world average (2.18%) with 2.40% mortality from the number of covid patients. Then the frequency decreases dramatically, so at 3-4% there are 14 countries, at 4-5% there are 6 countries, at 5-6% there are 4 countries, at 6-7% there are 3, at 7-8% it is only one country, at 9-10% one, at 10-11% one and at 29-30% one. A total of 64 countries out of the 220 statistically monitored entities (states and territories) have a mortality rate due to Covid-19 above the world average.

It should be noted that Yemen holds the "absolute record" of 29.9%, but it is in a prolonged war with Saudi Arabia (which could be considered a "proxy" war between the US and Iran) which has induced a major humanitarian crisis, as never before, for the solution of which the "international community" has not been effectively involved. The other excessive values are for Mexico (9.16%) which apparently would have no special reason but also for Western Sahara, which has a mortality of 10%, with 2 deaths out of a total of 10 cases, so it is not statistically significant (Figure 1).

Figure 1: The percentage distribution of the mortality rate due to Covid-19 compared to the total number of Covid-19 cases registered by country (website www.worldometer on 13.12.2020)

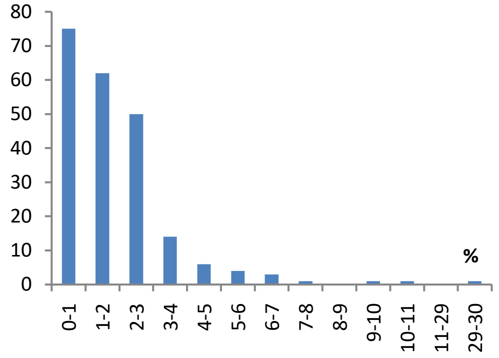

Statistical processing on data from the present situation - active cases

The percentage distribution of Covid-19 active cases (so, which were hospitalized on 13.12.2020) at approx. one year after the initial declaration of the epidemic, reports cases of serious illness (serious and critical) compared to the current active cases (mild and asymptomatic), according to Worldometer. The total incidence of serious cases in the total number of cases under treatment is 0.42%, which compared to the historical situation for a year of 2.24% mortality shows a major improvement in the situation. This favorable situation may be due to several causes: either due to a decrease in the virulence of the virus by its adapting to the human host (no pathogen has the "interest" to kill its host), or by optimizing therapy (although we do not yet have effective specific drugs), either due to the extension of collective immunity (so contemptuously called "herd" in English literature) or a combination of these. In fact, the WHO estimates that 99.5% of current cases are mild and only 0.5% are severe, which means that mortality in Covid-19 cases should not be higher than 0.2%, i.e. below influenza mortality.

It is interesting to note that in the "year of covid" we do not have a flu epidemic or acute upper respiratory infections (URI), which is remarkable because there is a hypothesis of the possibility of hybridization of the novel coronavirus with the influenza virus, which would have led to an increase in the risk to public health. The explanation could be that the measures of individual and collective prophylaxis were effective: wearing a protective mask both healthy (so as not to be contaminated) and sick (so as not to spread the virus), physical/social distancing, and compliance with hygiene rules. Individual and collective, preventive medical control (including mass thermometry), specific tests, isolation, quarantine, compulsory hospitalization, etc. Another possible explanation could be that in previous influenza and/or IACRS outbreaks some of the cases were actually coronaviruses, including the current undiagnosed Covid, or that we currently have undiagnosed influenza that is erroneously recorded with the diagnosis of Covid- 19.

The global prevalence has dropped from 0.92% per year to 0.26% up to the present moment (13.12.2020), and 78 countries (more than a third of the total) currently have no serious cases in evolution. Calculated as a percentage of serious cases from the total number of active Covid-19 cases, between 0 (inclusive) ÷ 1% (exclusive) are 147 countries, for example the USA, the absolute “champion”, has only 0.42% so with little above the world average. Of these, the 78 countries and territories mentioned do not have serious cases at all, so they do not require intensive care in the ICU. This includes the median of the group. In the range of 1÷ 2% there are 28 countries, including Germany with 1.32% and Romania with 1.48% (so well above the world average). At 2÷3% there are 13 countries, at 3÷4% there are 8 countries, at 4÷5% there are 3 countries, at 5÷6% there are 4 countries, at 6÷7% there are 4 countries, at 7÷8 % is a country, 8÷9% are 2 countries, 9÷10% is a country and over 10% are 5 countries. It should be mentioned that 4 countries no longer report for various reasons, so they cannot be fully included in the statistical study: Great Britain, Spain, the Netherlands and Sweden. We may only wonder how some of these countries would have reacted if another country would have acted as such under the circumstances. A total of 101 countries out of the 220 monitored have reported serious active cases (current, serious, critical) above the world average.

It should be mentioned that Yemen has a severity incidence of 24.47% of the reasons shown above, and Saudi Arabia of 15.36%, a country with which it is at war. These are followed by Liechtenstein with 12.65%, Suriname with 100% (in 2 cases out of 2) and Antigua with 33.33% (in 2 cases out of 6) which with a relatively small population and low casuistry show no individual statistical significance, but only within the world average (Figure 2).

Figure 2: Percentage distribution of current (active) cases of Covid-19 with serious forms (serious, critical) compared to the total number of active cases currently registered by country (website www.worldometer on 13.12.2020)

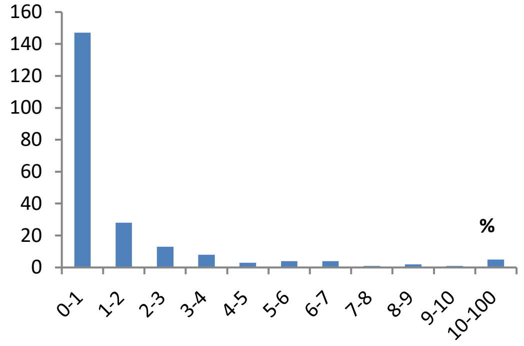

Comment on closed communities

In the early days of the pandemic, there were two cases of cruise ships (Diamond Princess and MS Zandam) of interest as a "situational experiment" in which international personnel benefiting from extraterritoriality suffered the effects of the epidemic, without other external factors. Peculiarities of the situation: a crew of thousands of healthy adults and passengers thousands of other adults and especially healthy elderly or with various comorbidities, but with permanent medical assistance (for surveillance, quarantine, treatment and evacuation), with maximum congestion and reduced isolation possibilities. A total of 721 Covid-19 cases were registered, with 15 deaths and 706 cures, so a contagiousness of max. 20% with mortality 2.08% of the total number of cases, so max. 0.4% mortality from the existing population. It can be estimated that under conditions of strictly supervised medical and non-medical countermeasures, Covid-19 is neither very contagious nor has a high mortality, even if there is a population in the risk group: the elderly, chronic diseases, etc (Figure 3).

Figure 3: Proportion of sick and deceased aboard the cruise ships, registered by country (website www.worldometer on 13.12.2020)

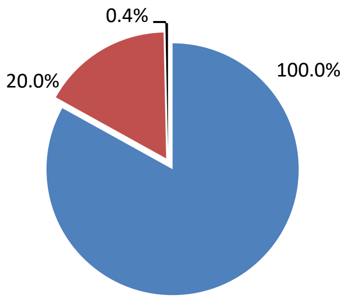

Data significance validity and probability

The entry of the statistically processed data from the Worldometer tables primarily processed on the grid's graph with the probability of significance "2 alpha" of 95%, shows that the general prevalence of fatal cases of/with Covid-19 compared to the total cases of Covid-19 throughout 2020 (A) is significantly higher than the prevalence of severe cases compared to the total current active cases of Covid-19 (B). Because the size of the sublots (cohort) can influence the statistical interpretation, we completed the graphical matrix once in descending order of number of cases and separately in reverse order, respectively in ascending order of number of cases and compared the two curves. These showed that the severity of group A is higher than that of group B with a statistical probability of 95%. In order to obtain the statistically significant result, in the first situation 20 cases were necessary and in the second 34 cases out of a total of 220 available. It was clear that the situation before December was worse, so we are currently facing a milder disease, which may be the "beginning of the pandemic's end." (Figure 4).

Figure 4: Comparison of the Covid-19 percentage mortality curve by country in 2020 compared to serious active cases in December 2020 registered by country (website www.worldometer on 13.12.2020)

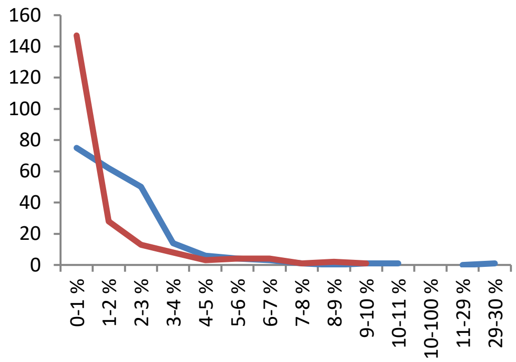

There is a decrease in the severity of the disease in the pandemic's dynamic context, which could mean that: the virus has become less virulent (adapts to the host), the treatment is more effective (more effective therapeutic strategies are being identified), (specific and/or cross) collective immunity develops or all of these in different proportions.

The United Nations (UN) centralizes worldwide data on the Covid-19 pandemic, namely the 230 countries (195 independent and 35 inhabited dependent territories) and the data are updated on a daily basis and published on the Internet via www.worldometercoronavirus. The world's population, which currently consists of nearly 7.8 billion people, is extremely unevenly distributed in these countries and is in ongoing dynamics. Some have over 1 billion inhabitants (China, India), about 150 countries have a population between 1 million and 1 billion, and about 70 entities have a population between 1 thousand and 1 million inhabitants and the rest under 1 thousand (e.g. the Vatican). The distribution of the population is uneven even between continents, but the discrepancies are much attenuated. Asia has the majority of the world's population, about 60% and the other six continents have the remaining 40% but Antarctica has no permanent inhabitants.

Of the countries in the world, 218 reported at least one case of Covid-19 (the US and India each have over 10 million cases) and 24 of them have no fatal cases of Covid-19. Countries free of Covid-19 or without mortality are generally small and sparsely populated and most are located in areas with warm climates. It should be recalled that four major countries, which are severely affected by the pandemic, no longer report up-to-date data on the evolution of the disease (UK, the Netherlands, Spain and Sweden) which may lead to some erroneous statistical conclusions. Among the countries monitored for Covid-19 are 23 (i.e. 10.45%) that have reported cases but have no mortality, and currently there are 78 countries (i.e. 35.45%) that do not have serious cases. Statistically, it can be concluded that the pandemic is on a downward trend.

The results of the statistical data processing, contained in Subchapter 3.1. could support the alarmists and Subchapter 3.2. the moderates, but in fact show a dynamic of the epidemic that is common in all epidemics. We could quote what the Germans jokingly say: "Statistics is a science that can prove anything, absolutely anything."

It seems that the current pandemic also holds a record for unfounded, contradictory and fake news depending on different interests, this being doubled by an "infodemic".

Comparative public health implications by geographical area

The representation of the real situation compared on different geographical areas, respectively compared to the world total with the percentage comparison (to mitigate extreme quantitative differences) on continents, which broadly represent one or more types of civilizations in the sense described by Huntingdon is systematised in tables 1-10. The comparison of statistical data is illustrative for the severity and evolution in the pandemic's dynamics, on large geographical areas with the respective population (Table no. 1).

Table 1: World population with numerical and percentage distribution by continents (www.worldometer on 01.07.2020)

No.	Geographical area	Population	%	Observations regarding the population
1	Worldwide	7,794,798,739	100.0	
2	Europe	747,636,026	9.6	
3	North America	368,829,647	4.7	
4	Asia	4,641,054,775	59.5	majority
5	South America	653,962,331	8.4	
6	Africa	1,340,518,147	17.2	
7	Oceania	42,677,813	0.5	minority
8	Antarctica	0	0	uninhabited

It is observed that the area of the extended western civilization (1 + 2 + 4 + 6) represents about 23%, and the area of the other civilizations about 77%, i.e. the absolute majority.

The percentage prevalence of the total cases of Covid-19 from the whole year 2020 compared to the total population, morbidity, is presented in Table 2, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 2: Covid-19 prevalence in the population, by geographical areas

No.	Geographical area	Population	%	Covid-19 Cases	% prevalence in population	Observations on morbidity
1	Worldwide	7,794,798,739	100.0	81027859	1.04	average
2	Europe	747,636,026	9.6	22684299	3.03	extremely high
3	North America	368,829,647	4.7	22400648	6.07	maximum
4	Asia	4,641,054,775	59.5	20362013	0.44	low
5	South America	653,962,331	8.4	12856856	1.97	high
6	Africa	1,340,518,147	17.2	2675424	0.20	low
7	Oceania	42,677,813	0.5	48222	0.11	minimum
8	Antarctica	0	0	0	0.0	

The area of restricted western/Euro-Atlantic civilization has about 14% of the population and about 56% of the cases

The percentage prevalence of Covid-19 fatal cases throughout 2020 compared to the total population, mortality, is presented in Table 3, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 3: Prevalence of Covid-19 mortality in the population, by geographical areas

No.	Geographical area	Population	%	Fatal Cases of Covid-19	% prevalence in population	Observations on morbidity
1	Worldwide	7,794,798,739	100.0	1770254	0.023	average
2	Europe	747,636,026	9.6	521434	0.070	extremely high
3	North America	368,829,647	4.7	496982	0.135	maximum
4	Asia	4,641,054,775	59.5	332002	0.007	low
5	South America	653,962,331	8.4	355935	0.054	high
6	Africa	1,340,518,147	17.2	62834	0.005	low
7	Oceania	42,677,813	0.5	1058	0.002	minimum
8	Antarctica	0	0	0	0.0	

North America has a Covid-19 mortality rate among its population of almost 6 times higher than the global rate.

The percentage incidence of mortality due to Covid-19 in the total cases of Covid-19 in 2020 is presented in Table 4, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 4: Incidence of mortality in patients with Covid-19, by geographical areas

No.	Geographical area	Population	%	Fatal Cases of Covid-19	Total Covid-19 Cases	% prevalence in population	Observations on morbidity
1	Worldwide	7,794,798,739	100.0	1770254	81027859	2.18	average
2	Europe	747,636,026	9.6	521434	22684299	2.30	
3	North America	368,829,647	4.7	496982	22400648	2.22	
4	Asia	4,641,054,775	59.5	332002	20362013	1.63	minimum
5	South America	653,962,331	8.4	355935	12856856	2.77	maximum
6	Africa	1,340,518,147	17.2	62834	2675424	2.35	high
7	Oceania	42,677,813	0.5	1058	48222	2.19	
8	Antarctica	0	0	0	0	0.0	

South America and Africa have high mortality and Asia has low mortality.

The percentage incidence of mortality due to Covid-19 in the new cases of Covid-19 in December 2020 is presented in Table 5, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

South America, Africa and Europe have high mortality and Oceania no longer has mortality.

The percentage prevalence in the population of the current active cases of Covid-19 in December 2020 is presented in Table 6, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

North America and Europe have a high prevalence (totaling 87% in a population of about 14%) and Asia, Africa and Oceania currently have a low prevalence.

The prevalence of serious cases (serious and critical) of current assets in December 2020 expressed as a percentage of the population is presented in Table 7, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

With an average prevalence of active cases of 1/100 000 it can be considered that at this moment Covid-19 is a "rare disease" and drugs intended for the prophylaxis and treatment of this disease can be considered as "orphan drugs". North America and Europe have a relatively higher prevalence and Africa and Oceania currently have a very low prevalence.

Table 5: Incidence of mortality in new patients with Covid-19 (December), by geographical areas

No.	Geographical area	Population	%	New deaths due to Covid-19	New Covid-19 cases	% Incidence in patients	Observations on mortality
1	Worldwide	7,794,798,739	100.0	5830	316402	1.84	average
2	Europe	747,636,026	9.6	2699	122940	2.20	high
3	North America	368,829,647	4.7	1321	102110	1.29	
4	Asia	4,641,054,775	59.5	1348	72428	1.86	minimum
5	South America	653,962,331	8.4	99	2938	3.37	maximum
6	Africa	1,340,518,147	17.2	363	15955	2.28	high
7	Oceania	42,677,813	0.5	0	34	0.0	minimum
8	Antarctica	0	0	0		0.0	

Table 6: Percentage prevalence in the population of Covid-19 active cases (December), by geographical areas

No.	Geographical area	Population	%	Covid-19 Active Cases	% Incidence in patients	Observations on prevalence
1	Worldwide	7794798739	100.0	22074490	0.283	average
2	Europe	747636026	9.6	11083322	1,482	high
3	North America	368829647	4.7	8220611	2,229	maximum
4	Asia	4641054775	59.5	1244003	0.027	minimum
5	South America	653962331	8.4	1136032	0.174	
6	Africa	1340518147	17.2	377067	0.028	low
7	Oceania	42677813	0.5	13727	0.032	low
8	Antarctica	0	0	0	0.0	

Table 7: Percentage prevalence in the population of severe active cases of Covid-19 (December), by geographical areas

No.	Geographical area	Population	%	Covid-19 Serious Active Cases	% Incidence in population	Observations on severity
1	Worldwide	7,794,798,739	100.0	105246	0.001	average
2	Europe	747,636,026	9.6	26548	0.004	high
3	North America	368,829,647	4.7	34193	0.009	maximum
4	Asia	4,641,054,775	59.5	25284	0.001	minimum
5	South America	653,962,331	8.4	16758	0.003	high
6	Africa	1,340,518,147	17.2	2442	0.0002	low
7	Oceania	42,677,813	0.5	22	0.00005	minimum
8	Antarctica	0	0	0	0.0	

The incidence of serious cases (serious and critical) that require ICU from the active cases of Covid-19 in December 2020 (treatable at home or in hospital) is presented in Table 8, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Asia, South America and Africa have a high incidence of serious cases and Europe and Oceania have a low incidence.

The evolution of morbidity by comparing the percentage of current active cases (December 2020) compared to the total cases of Covid-19 in 2020 is presented in Table 9, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 8: Incidence of serious cases in Covid-19 active cases by geographical areas

No.	Geographical area	Population	%	Covid-19 Serious Active Cases	Covid-19 Active Cases	% Incidence in patients	Incidence observations
1	Worldwide	7794798739	100.0	105246	22074490	0.48	average
2	Europe	747636026	9.6	26548	11083322	0.24	low
3	North America	368829647	4.7	34193	8220611	0.42	
4	Asia	4641054775	59.5	25284	1244003	2.03	maximum
5	South America	653962331	8.4	16758	1136032	1.48	high
6	Africa	1340518147	17.2	2442	377067	0.65	
7	Oceania	42677813	0.5	22	13727	0.16	
8	Antarctica	0	0	0	0	0.0	

Table 9: Evolution of morbidity by percentage comparison of current active cases (December 2020) compared to the total number of Covid-19 cases in 2020, by geographical area

No.	Geographical area	Population	%	Covid-19 Active Cases	Covid-19 Total Cases	% Incidence in patients	Evolution observations
1	Worldwide	7,794,798,739	100.0	105246	81027859	27.24	average
2	Europe	747,636,026	9.6	26548	22684299	48.86	maximum
3	North America	368,829,647	4.7	34193	22400648	36.70	high
4	Asia	4,641,054,775	59.5	25284	20362013	6.11	minimum
5	South America	653,962,331	8.4	16758	12856856	8.84	low
6	Africa	1,340,518,147	17.2	2442	2675424	14.09	
7	Oceania	42,677,813	0.5	22	48222	28.47	
8	Antarctica	0	0	0	0	0.0	

By a reductio ad absurdum, in a more balanced distribution, December should have represented less than 10% of the total for 2020, but it is almost 3 times higher worldwide, so the epidemic is "accelerating" especially in Europe and North America.

By comparing the percentage of serious (serious and critical) current cases (December 2020) to the total mortality of Covid-19 cases in 2020, the evolution of the severity of the cases is presented in Table 10, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 10: The evolution of the severity of the cases by comparing the percentage of the serious (serious and critical) current active cases (December 2020) to the total mortality of Covid-19 cases in 2020, by geographical areas

No.	Geographical area	Population	%	Covid-19 death rate % on 2020	% Severity Covid-19 active	% Difference	% Decrease in severity	Evolution observations
1	Worldwide	7,794,798,739	100.0	2.18	0.48	1.78	22.02	average
2	Europe	747,636,026	9.6	2.30	0.24	2.06	10.43	good
3	North America	368,829,647	4.7	2.22	0.42	1.80	18.92	
4	Asia	4,641,054,775	59.5	1.63	2.03	-0.40	124.54	Is getting worse!
5	South America	653,962,331	8.4	2.77	1.48	1.29	53.43	worse

No.	Geographical area	Population	%	Covid-19 death rate % on 2020	% Severity Covid-19 active	% Difference	% Decrease in severity	Evolution observations
6	Africa	1,340,518,147	17.2	2.35	0.65	1.70	27.66	
7	Oceania	42,677,813	0.5	2.19	0.16	2.03	7.31	good
8	Antarctica	0	0	0	0	0.0	0	

It is observed that the severity of the pandemic increases in Asia and South America and decreases in Europe and Oceania.

The published data are updated on a daily basis showing, as expected, a permanent dynamic of the pandemic. However, the general evolution is maintained and falls within the usual predictions in epidemiology, but with inherent features dependent on the biological agent, the disease, the sick, the population, the environment, the medical system, etc.

DISCUSSIONS

Statistically, it is found that worldwide the prevalence of the disease and the incidence of mortality are unequally represented in different countries and territories, presenting higher than average values in Europe and North America, followed by South America and Australia, so in the predominantly western civilization area and especially in the Euro-Atlantic one. Although an initial flu-like severity was estimated in the initial epidemic, the consecutive pandemic had an upward and then downward trend, now reaching below the severity of the pandemic flu epidemic. The factors favoring the pandemic can be incriminated as well as the relatively effective control measures, and currently the pandemic is decreasing both quantitatively and qualitatively. On the daily world case series (Worldometer graphs) there are three periods of increasing the frequency of the disease (16.03-13.05.2020, 08.07. - 02.09.2020 and 28.10-present) consistent with three consecutive periods of increasing mortality (12.04. - 09.05.2020, 29.07. - 21.09.2020 and 14.11-present). So it was a first wave in the spring of 2020, a second in the summer and a third in the fall-winter, so we expect a fourth wave next spring overlapping with the season of acute respiratory viruses. It means that the media warning that a second or third wave will come is not based on reality, they have already come and nothing special has happened. But the genetic variability of many viruses creates the risk of mutations in contagiousness, virulence and/or immunogenicity.

The global Covid-19 pandemic was not particularly serious, as some experts have questioned the declaration of the pandemic for medical reasons, and its continuation will be, as world statistics indicate, easier, even below the level of seasonal flu. We currently benefit from specific diagnostic tools (for viruses, antibodies and clinical interpretation), etiological, adjuvant and complementary treatment (even if we do not yet have specific antivirals) and vaccine prophylaxis (under authorization) which will be applied en masse in the near future. The optimistic variant is that the pandemic will end in the summer, but there are also various pessimists that sustain it will last until 2022 or that we will have to live with the virus. Which is actually the case with influenza viruses, but no one is scared of them now.

At the mentioned date, out of the total of 72,197,885 cases diagnosed with Covid-19 for the year 2020, representing a world prevalence of 0.92%, there are 1,613,401 deaths, so the incidence of mortality was of 2.29%. Compared to these, there are currently 27.71% of current cases (with a global prevalence of 0.26%) under treatment in December. Thus, the frequency of Covid-19 diseases increased, but severe cases (requiring ICU treatment) accounted for only 0.53% of the cases. It turns out that the severity of Covid-19 has now been reduced by more than four times.

After the date on which the presented data were counted, some major events in the evolution of the pandemic occurred: the urgent authorization of the new anti-Covid-19 vaccines and the identification of an N501Y mutation that could change the evolution of this disease, but in what sense it is not yet known.

The exaggeration of the danger presented by this virus can be explained by: the novelty of the disease and its causative agent, which could have evolved unpredictably; by the desire of the sanitary decision makers not to trivialize a possible potentially serious epidemic; the desire of policy makers to show their concern for the population (especially if it is an election year); the desire of global finance to reap benefits primarily through Big Pharma and related fields; the desire of the press to hunt "hot" subjects; the desire of politicians to mask the incipient world economic crisis and even the attempt to economically suffocate the "factory of the world" in order to block the change of the New World Order.

It is paradoxical that the most civilized countries and with the best medicine have suffered the most, both through the health crisis (sick, deaths, involvement of forces and health) and the subsequent socio-economic crisis. There is an explanation that these countries have the longest-lived population, so aged and with comorbidities, which are ranked in "risk groups". Nevertheless, it can be estimated that the best performing medicine, and therefore the most expensive, is not as effective in the field of public health. Compared to the health insurance system, the territorial rounding of health care allows better hygienic and epidemiological surveillance and an epidemiological investigation on the epidemiological chain: source-transmission-receiver, which has proven to be the most effective means of stopping the epidemic. However, the implementation of some measures would be in contradiction with the law, with the rights and freedoms of the individual and with the interests of the health insurance system.

Under these conditions, it would have been possible for any other viral, bacterial or other epidemic / pandemic to occur, with a single or multiple etiological agent, known or not yet known, with even more serious consequences. All this requires the timely preparation of appropriate, comprehensive but resilient Action Plans to cover any foreseeable situation in the future, including that of biological warfare, bioterrorism, biocrime or biological accident.

From the multitude of objective data published daily by the worldometer, interesting conclusions can be drawn for the evolution of the pandemic and public health, and each person interested in the real situation can ultimately reach his own conclusions.

The current pandemic has generated a complex health, social, economic crisis but also a "psychodemia" through direct and indirect effects. It is interesting that many doctors have let themselves get drawn into this collective fear, some have resigned shamefully, looking only at the particular cases in their field and at the multitude of information and misinformation in the environment subjectively and uncritically, rather criticizing interestedly or uninterestedly the realistic ones. American writer and journalist Mark Twain said, "If you don't read the newspaper, you're uninformed. If you read the newspaper, you're mis-informed." As recommended by some specialists (some of whom have lost their jobs, the freedom or their lives) we need to turn off the TV and analyze the statistical data provided by reliable scientific sources; we mainly chose WHO, Nature, Science and Worldometer. At the same time, the false news spread by conspiratorial negativists who do not see the virus, do not see the disease, do not see the epidemic, and do not see the reality and try to convince others of their aberrations must be rejected.

Regarding the diagnosis, treatment and prophylaxis, it can be considered that something that works now is more useful than something perfect in the future. It is important to respect the Hippocratic principles: „Primum non nocere" and „We treat the sick and not diseases". But as the military say, „You go to war with what you have, not with what you want."

At the same time, the year 2020 is one of the most prolific for medical science: the number of bio-medical scientific researches has reached a record hard to match, books have been written, articles about the various medical and non-medical aspects of Covid-19, an international collaboration took form with fast publications that are freely available online, just before the peer-review (which resulted in a record number of false researches that misled the medical world), a counter-clockwise competition for diagnostic reagents, drugs and specific vaccines, protective equipment, disinfectants, etc.

An English journalist and writer wrote, "I often thought again and again how unprepared the whole community had been for the calamity that had paled it, and to what extent the ensuing disaster was due to the fact that no public and personal measures and provisions had been taken in time. If everything had been done properly, the number of victims would have been much lower. I say all this as a warning and as a lesson for posterity." This warning was written by Daniel Defoe (1660-1731) in the book "A Journal of the Plague Year" published in 1722 [4] and is still valid today, and posibly also for the future...

The Covid-19 pandemic is a reality of current times, the year 2020 being portrayed as the "COVID year". The international scientific community has shown that, when needed, it can collaborate beyond classical barriers. The novel coronavirus was studied as a coronavirus by biologists, as a new disease by doctors, as the new reagents by biochemists, as the new drugs from the perspective of pharmacologists, and so forth. Thus, in record time, faster than in any other similar situation, we already have a lot of knowledge about them and we have an adequate diagnostic, therapeutic and prophylactic means, but not everything.

Statistics show that the virus is not very aggressive, the disease is not very serious but there are also serious, sometimes fatal complications to which appropriate measures must be applied, including ICU. The risk of serious illness and life-threatening complications is statistically reduced over time, the disease being less severe now than at the beginning, so it is on a downward slope.

It can be estimated that there are premises for the end of this pandemic in the near future and that if no other disruptive factors intervene, it will return to normal, or at least the ordinary, in terms of social and economic life.

Comments

After one year, the Covid-19 is present disease, and the pandemic is a reality of current times, the year 2020 being portrayed as the "COVID year". The medical literature on Covid-19 and the information released in the public space allow statistical analysis, the most complete and systematized data, representing the US point of view, are updated and published on a daily basis, including on the website www.worldometercoronarovirus.

The primary and secondary statistical interpretation of quantitative data is possible by dividing on quantitative intervals, percentage processing by absolute and relative values, by applying the significance test, etc. and obtaining quantitative and qualitative data that corroborate with medical logic according to the principles of "evidence-based medicine".

The graphical distribution by percentage statistical intervals of the countries did not create a normal bell-shaped Gaussian curve, but a steep downward curve, so most countries had few or no cases and the high prevalence was in a small number of countries. The global prevalence has dropped from 0.92% per year to 0.26% up to the present moment (13.12.2020), and 78 countries (more than a third of the total) currently have no serious cases in evolution.

From a statistical point of view, confirmed by the "situational experiment" presented, it can be estimated that in the conditions of strictly supervised medical and non-medical countermeasures Covid-19 is neither very contagious nor has a high mortality, even if there is a population in the risk group: the elderly, chronic diseases, etc. The entry of the statistically processed data from the Worldometer tables primarily processed on the grid’s graph with the probability of significance “2 alpha” of 95%, shows that the general prevalence of fatal cases of/with Covid-19 compared to the total cases of Covid-19 throughout 2020 (A) is significantly higher than the prevalence of severe cases compared to the total current active cases of Covid-19 (B). There is a decrease in the severity of the disease in the pandemic's dynamic context, which could mean that: the virus has become less virulent (adapts to the host), the treatment is more effective (more effective therapeutic strategies are being identified), (specific and / or cross) collective immunity develops or all of these in different proportions.

The area of the extended western civilization (2 + 3 + 5 + 7) represents about 23%, and the area of the other civilizations about 77%, i.e. the absolute majority, and the area of restricted western, mainly Euro-Atlantic, has about 14% of the population and about 56% of cases. North America has a Covid-19 mortality rate among its population of almost 6 times higher than the global rate. The incidence of mortality in new Covid-19 patients in South America and Africa is high and low in Asia. The incidence of mortality in new Covid-19 patients is high in South America, Africa and Europe and Oceania no longer has mortality. The percentage prevalence in the population of Covid-19 active cases is high in North America and Europe (totalling 87% of active cases in a population of about 14%) and Asia, Africa and Oceania currently have a low prevalence.

With an average prevalence of active cases of 1/100,000 it can be considered that at this moment Covid-19 is a "rare disease" and drugs intended for the prophylaxis and treatment of this disease can be considered as "orphan drugs". North America and Europe have a relatively higher prevalence and Africa and Oceania currently have a very low prevalence. Asia and South America and Africa have a high incidence of serious cases and Europe and Oceania have a low incidence. By a reductio ad absurdum, in a more balanced distribution, December should have represented less than 10% of the total for 2020, but it is almost 3 times higher worldwide, so the epidemic is "accelerating" especially in Europe and North America. It is observed that the severity of the pandemic increases in Asia and South America and decreases in Europe and Oceania.

CONCLUSION

Statistics show that the virus is not very aggressive, the disease is not very serious but there are also serious, sometimes fatal complications to which appropriate measures must be applied, including ICU. The risk of serious illness and life-threatening complications is statistically reduced over time, the disease being less severe now than at the beginning, so it is on a downward slope. With prophylaxis, vaccination and treatment we hope the finish of present epidemia.

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ORIGINAL ARTICLE

Inadequate perioperative control of modifiable risk factors in total knee arthroplasty and implications on functional results

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Abstract: *Diabetes and increased body mass index are two of the most associated comorbidities in patients undergoing total knee arthroplasty. With the number of patients undergoing knee arthroplasty increasing and with the incidence of diabetes and increased BMI, we can expect that in the coming decades the association of the two comorbidities will be frequent. An ethical issue arises when the patients who are refused for total knee arthroplasty are instructed to change the risk factors, and they fail either because of a mental problem or because of a physical problem.*

This study analyzes the effects of controlled, uncontrolled, uncomplicated and complicated diabetes on the rates of post-knee arthroplasty complications. The study proposes to evaluate obese patients in terms of functional outcomes and postoperative complications after total knee arthroplasty.

Keywords: *total knee arthroplasty, diabetes, body mass index, total knee arthroplasty complications, periprosthetic joint infection*

INTRODUCTION

Diabetes affects 8.5% of the Europe's population, and its prevalence increases with age [1]. It is one of the common comorbidities associated with patients over 65 years old undergoing total knee arthroplasty. The literature data suggest that one of 10 patients that are admitted for a total knee arthroplasty has associated diabetes, but the prevalence of diabetes is constantly increasing [2, 3]. This has important implications in the perioperative management of the total knee arthroplasty, with an increase in the number of patients with associated diabetes [4].

The risks of the association between total knee arthroplasty and diabetes are periprosthetic infections, deep vein thrombosis and stroke [5-9]. The risks are even higher for stroke and death when the diabetes is uncontrolled [10]. The risk of complications increases directly proportional to the value of glycated hemoglobin (HbA1c) [11]. This information allows the surgeon to change the risk even from the preoperative period.

Approximately 48% to 56% of patients with diabetes are developing osteoarthritis, which leads to decreased functionality, decreased physical activity and high mortality rates [12]. The current standard of treatment for knee osteoarthritis is total knee arthroplasty [13, 14, 15].

Given the increasing prevalence of both diabetes and osteoarthritis of the knee as they age, these patients are forced to coexist with these associated pathologies, but also with developed complications, which may result in the postoperative period [16, 17].

Obesity is one of the most important modifiable risk factors and at the same time is one of the most discussed in terms of increasing postoperative complications in total knee arthroplasty. Regarding the postoperative complications, there are data available that indicate the fact that an increased body mass index (BMI) will increase the risk of total knee arthroplasty, but there is no cut-off value to help

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us make the decision to perform surgery on a patient or not [18-21]. Literature data indicate that patients with high BMI benefit from a functional outcome and an improved quality of life more than normal-weight patients [22].

An ethical issue arises when the patients who are refused for total knee arthroplasty are instructed to change the risk factors, and they fail either because of a mental problem or because of a physical problem.

This study analyzes the effects of controlled, uncontrolled, uncomplicated and complicated diabetes on the rates of post-knee arthroplasty complications. The study proposes to evaluate obese patients in terms of functional outcomes and postoperative complications after total knee arthroplasty.

MATERIALS AND METHODS

The study ran over a period of three years from January 2017 until Decembre 2019 and included a number of 446 patients. The following patient data were collected: age, sex, body mass index, preoperative blood glucose, preoperative HbA1c, diabetes status (controlled/uncontrolled, complicated/uncomplicated), WOMAC score (preoperative, 3 months postoperatively and 12 months postoperatively), history of periprosthetic infection or complications of the wound.

In the statistical processing, the data were classified into quantitative, such as WOMAC score, and qualitative, respectively, sex, age range, controlled or uncontrolled diabetes. The qualitative or nominal data were represented as graphs. The graphs were of the column type (Column) and point cloud (Scatter Plot). In addition, Box Plots were used to represent the WOMAC score at different periods of time, preoperatively, at 3 months and 12 months depending on the patient's BMI.

The inclusion criteria in the study were patients with total knee arthroplasty, patients with and without diabetes and those with known BMI. In addition, the exclusion criterion from the study was the loss of the patient's records.

RESULTS

The study included a number of 446 patients, 20 of whom had periprosthetic infections.

The distribution of patients BMI shows that 173 were normal weight, 234 overweight and 39 obese. The analysis of the data shows that the mean WOMAC score was:

- at onset according to the patients' BMI was not variable. Specifically, the normal weight patients had a mean WOMAC score of 51, overweight patients had an average WOMAC score of 51, and obese patients had an average WOMAC score of 47.
- at 3 months according to the patients' BMI was for normal weight 74, overweight patients had an average WOMAC score of 69, and obese patients had an average WOMAC score of 60.
- at 12 months according to the patients' BMI was for the normal weight 88, the overweight patients had an average WOMAC score of 80, and the obese patients had an average WOMAC score of 69.

The distribution of patients with periprosthetic infection according to BMI shows that 1 normoponderal patient, 7 overweight patients and 12 obese patients presented with periprosthetic infection.

The distribution of patients with controlled and uncomplicated diabetes according to periprosthetic infections shows:

- The category of overweight patients with controlled and uncomplicated diabetes also had periprosthetic infections. The evolution of the WOMAC score for patients with controlled and uncomplicated diabetes and who presented periprosthetic infections is 38.5 preoperatively, 43 at 3 months and 53 at 12 months.
- The category of obese patients with controlled and uncomplicated diabetes also had periprosthetic infections. The evolution of the WOMAC score for patients with controlled and uncomplicated diabetes and who presented periprosthetic infections is 45 preoperatively, 50 at 3 months and 55 at 12 months.

The distribution of patients with controlled and complicated diabetes according to periprosthetic infections presents:

- The category of overweight patients with controlled and complicated diabetes did not show periprosthetic infections. The evolution of the WOMAC score for the patients with controlled and complicated diabetes and who did not have periprosthetic infections is 45 preoperatively, 66 at 3 months and 75 at 12 months.
- The category of obese patients with controlled and complicated diabetes also had periprosthetic infections.
- The evolution of the WOMAC score for the patients with controlled and complicated diabetes and who presented periprosthetic infections is 41 preoperatively, 45 at 3 months and 50 at 12 months.

The distribution of patients with uncontrolled diabetes according to periprosthetic infections shows:

- The category of overweight patients with uncontrolled diabetes also had periprosthetic infections. The evolution of the WOMAC score for the patients with uncontrolled diabetes and who presented periprosthetic infections is 38 preoperatively, 46.5 at 3 months and 50 at 12 months.

- The category of obese patients with uncontrolled diabetes also had periprosthetic infections. The evolution of the WOMAC score for the patients with uncontrolled diabetes and who presented periprosthetic infections is 42 preoperatively, 48.5 at 3 months and 57.5 at 12 months.

The ANOVA test with repeated measurements was used to investigate the evolution of the WOMAC score according to BMI. The Mauchly test showed that there are statistically significant differences both in BMI, WOMAC and in the evolution of BMIx WOMAC. Thus, for BMI, the Mauchly test had the value 0.97, p = 0.03, for WOMAC, it had the value 0.21, p = 0.001 and for WOMAC x BMI it had the value 0.04, p = 0.001.

The differences in BMI were as follows:
- Normal weight Vs. overweight (p = 0.001, 77.55 vs. 66.57);
- Overweight vs. obese (p = 0.001, 66.57 vs. 92.17);
- Normal weight Vs. obese (p = 0.001, 77.55 vs. 92.17)

The differences at the WOMAC level were as follows:
- WOMAC at baseline vs. WOMAC 3 months (p = 0.001, 68.11 vs. 80.34);
- WOMAC 3 months vs. WOMAC 12 months (p = 0.001, 80.34 vs. 87.84);
- WOMAC at baseline vs. WOMAC at 12 months (p = 0.001, 68.11 vs. 87.84).

The average ANOVA score varies significantly depending on the BMI, respectively the WOMAC score at 12 months reaches the highest point for all types of BMI (Figure 1).

Figure 1: The evolution of the WOMAC score according to patient's BMI

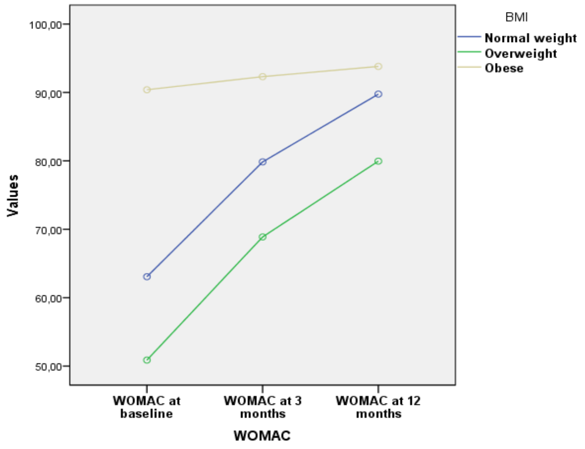

The distribution of patients' blood glucose according to BMI and WOMAC score reveals that the increased blood glucose levels at the WOMAC score time points were of obese patients (Figure 2).

Figure 2: The evolution of the WOMAC score according to patient's BMI

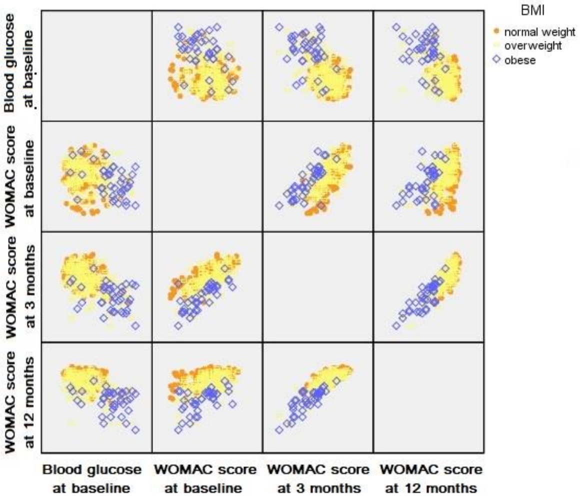

The distribution of glycated hemoglobin of patients according to BMI and WOMAC score reveals that the increased level of glycated hemoglobin at the time points of the WOMAC score were of overweight and obese patients (Figure 3).

Figure 3: The distribution of glycated hemoglobin of patients according to BMI and WOMAC score

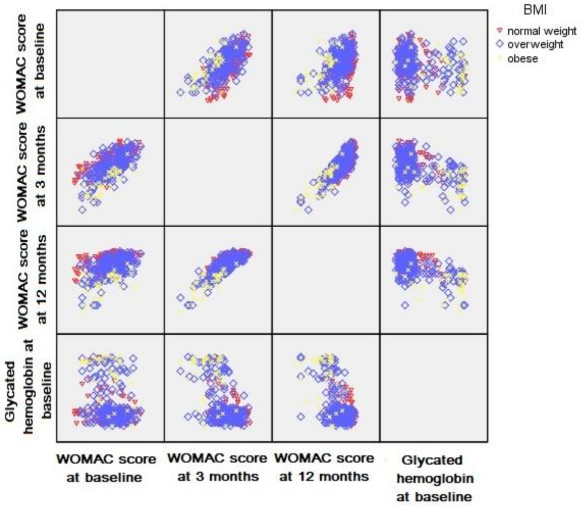

DISCUSSIONS

Perioperative risks assessment is a standard in arthroplasty procedures. Careful exposure of the risks to the patient is essential for physician-patient communication. Total knee arthroplasty is not a risk-free intervention, as risks can range from the smallest (e.g. a small wound dehiscence) to complications with major functional impact such as periprosthetic infections.

Given that the incidence of gonarthrosis increases from year to year and the incidence of diabetes reaches alarming levels, the association of the two is inevitable. Diabetes is associated with a number of important complications for the patient. When a diabetic patient is proposed for arthroplasty, the risks are much higher than in a non-diabetic, which must be fully understood by the patient. In this discussion, an increased BMI, another associated risk factor, often occurs.

Most often, these patients are advised by current orthopedic doctors to follow a hygiene and diet regimen for weight loss along with trying to control diabetes and then move on to a knee arthroplasty. When providing such indications, it should be borne in mind that increased BMI along with uncontrolled diabetes are the result of many years of negligence and any attempts to change the weight or level of glycated hemoglobin will fail in most cases. The patient will return for arthroplasty with a level of glycated hemoglobin above normal, with BMI unchanged from the last visit, but with gonarthrosis that advanced due to the passage of time, which will lead to a possible arthroplasty with a risk of complications higher than initially.

It is ideal for these modifiable risk factors to be kept under control through multiple and careful consultations by patients with their specialists in diabetes. However, it should not be overlooked that sometimes this is impossible to achieve at an optimal level. An elderly patient, with a high BMI and uncontrolled diabetes, in the best-case scenario, after an average period, they will manage a moderate control of diabetes and the absence of its complications.

Adapting surgical techniques and perioperative care along with the assumption of potential risks, both by the attending physician and by the patient in such cases, is the optimal conduct.

CONCLUSIONS

Early diagnosis and correct treatment of diabetes are essential for optimal postoperative results after total knee arthroplasty. Immediate postoperative functional outcomes are also influenced by glycemic control. Optimal methods of preoperative glycemic control are needed to maintain a high level of functional outcomes as well as to limit the complication rates.

The functional results analyzed using the WOMAC score showed low values at 3 months postoperatively for overweight and obese patients compared to normal weight, but at 12 months they showed a significant improvement in functionality. The increases scores remain important compared to the preoperative level for these patients.

Periprosthetic infections occurred mainly in overweight and obese patients, obviously associated with elevated glycemic levels and elevated glycated hemoglobin.

The WOMAC score was low in all categories of patients with periprosthetic infection regardless of diabetes status. Overweight and obese patients with periprosthetic infection had a significantly higher level of glycated hemoglobin.

It can be said that the incidence of periprosthetic infections was predominantly higher in overweight and obese patients who associated diabetes regardless of status, but the functional improvements of these patients are remarkable compared to the preoperative level.

These perioperative and modifiable risk factors are becoming more common. The obese patient, with diabetes in various forms and with gonarthrosis is a current reality of the times and cannot be ignored. The decision to operate on such a patient should not be made by highlighting the potential complications that may occur postoperatively, the potential for a possible arthroplasty to improve the patient's quality of life and functionality are in the forefront and should dictate the operability decision.

Improving surgical techniques and the use of specific perioperative care techniques specific to an obese and diabetic patient should be considered to minimize the risk of a possible periprosthetic infection.

The diabetic, overweight, or obese patient with gonarthrosis will represent in the future an important share of all gonarthrosis patients. Thus, adapting the surgical approach of these patients to reduce the rates of periprosthetic infection represent one of the main solutions that can be applied.

The attempts to modify the lifestyle and eating behavior of an overweight or obese and diabetic person often fail for their diabetologists, which will lead to an advance of gonarthrosis, resulting in an arthroplasty grafted with a much greater potential for complications.

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ORIGINAL ARTICLE

Mg-1Ca alloy intramedullary nailing influence on bone callus formation and on vital organs functions, as an alternative to bioplastics

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Abstract: *Magnesium alloys represent a new research direction in the field of orthopedic resorbable metal materials, because it has several important properties that recommend it as an excellent resorbable biomaterial for implants. From in vitro studies, Magnesium has osteoconductive properties, promoting the formation of new bone by stimulating the adhesion of osteoblastic cells, this leading to the idea that it will induce formation of hard callous at fracture site. The present study followed the in vivo evaluation of the biodegradation of pins made of Mg-1Ca alloy introduced centromedulary in rabbit tibiae, with previously induced experimental fractures. The influence of the biodegradation process on the formation of the bone callus was evaluated through radiological images and histopathological examinations. At the same time, we evaluated the function of vital organs through histopathological examinations and blood tests. We chose intramedulary nailing (IMN) with pins, because in orthopaedic surgery this is the golden standard for fractures of tibia or femur. In the radiological images obtained, we notice callus formation at the level of the experimental fracture at 6 weeks after surgery. The histological sections made from the samples collected from the fracture zone adjacent to the Mg1Ca alloy implant, confirm the formation of the bony callus. The trichrome Masson sections show a normal bone development with newly formed bone tissue at the tissue - alloy interface. In the sections harvested from the vital organs, no pathological changes are observed. These observations suggest that the biodegradation process of the Mg-1Ca alloy do not interfere negatively with the biological process of bone formation, allowing the formation of the bone callus and do not confirm the suspicion of pathological changes induced by systemically released corrosion products and gas embolism in vital organs.*

Keywords: *magnesium, alloy, implant, pins, biodegradation*

INTRODUCTION

Magnesium alloys represent a new research direction in the field of orthopedic resorbable metal materials. Use ofbioabsorbable materials will eliminate the need for a second ablation intervention leading to reduction of patient stress, reduction of postoperative infection risk and medical cost reduction. Also, it will have the advantage of reduction of bone/implant contact stress effect (stress shielding effect) and load transfer, gradually during resorption, toward the newly formed bone in the same time with resorption, decreasing the risk of fracture after surgery, as in the case of metal one

Main requirements of bioabsorbable metal materials are biocompatibility and biological functionality, physiological material without rejection reaction, that will assist the bone repair. In the same time it will need to have physicochemical properties consistent with the physiology of the region, meaning, no toxicities when resorption occurs, bone adhesion and allowing bone cell proliferation (osteoconductive), resorption by biodegradation and bone remodeling. Other recent studies in biomaterials have yet to take into account the possibility of bioabsorbable metal as an option [1], while others rely on biocompatibility without bioresorbtion [2, 3].

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Mg1Ca alloy

Mg-1Ca alloy consists in two natural elements of the human body, being able to dissolve in the body without inducing toxicities and foreign body reactions. Magnesium has osteoconductive properties, promoting the formation of new bone by stimulating the adhesion of osteoblastic cells, this leading to the idea that will induce formation of hard callous at fracture site.

Calcium is an important component of bone tissue and is an essential element in biochemical processes. It have a low density, 1.55 g/cm^3, which enables Mg-Ca alloys to achieve a density similar to bone, wich is 1.8 – 2.1 g/cm^3. Also, Magnesium has the potential to improve the incorporation of calcium into bone. Mg-Ca alloys are the most used and researched alloys, the solubility of Ca in Mg is 1.34 wt% [4].

The main drawbacks of Mg alloys are rapid biodegradation, increased rate of corrosion in the body, at a physiological pH of 7.4 to 7.6. Rapid corrosion will lead to loss of mechanical capabilities, the bending strength being able to decreasein a first stage of erosion from 652 MPa to 390 MPa [5].

Also, rapid corrosion will increase ph in the corrosion environment, thus producing a alkalizing effect of peri-implant tissues, with cytotoxic effect. According to ISO 10993-5:2009, a reduction of cell viability more than 30% it is considered a cytotoxic effect [6].

During corrosion process of Mg-Ca, hydrogen gas will be released. High hydrogen evolution rate corresponding to a high corrosion rate, will lead to gas bubbles formation into the surrounding tissues, leading to tissue necrosis, surgical incision dehiscence [7] and risk of gas embolisation in vital organs [8].

Mg-Ca alloys have a binary microstructure, consisting of a primary phase, α Mg, and an eutectic secondary phase, with lamellar structure, composed of Mg_2Ca [9]. Mg-Ca alloys corrosion process is the result of a galvanic circuitformation, due to the electrochemical potential difference between the primary phase α Mg and the secondary phase Mg_2Ca. Mg-Ca alloys undergo in SBF the corrosion process shown in figure 1:

a) galvanic corrosion of Mg and Mg_2Ca phase;

b) partially protective layer covering the surface of the Mg-Ca alloy;

c) absorption of chlorine ions for the conversion of $Mg(OH)_2$ to $MgCl_2$;

d) hydroxyapatite formation by consuming Ca^{2+} and $PO4^{3-}$ ions;

e) particulate residues decay emerging from the substrate volume.

Figure 1: Mg-Ca alloy corrosion in SBF (adapted after 8)

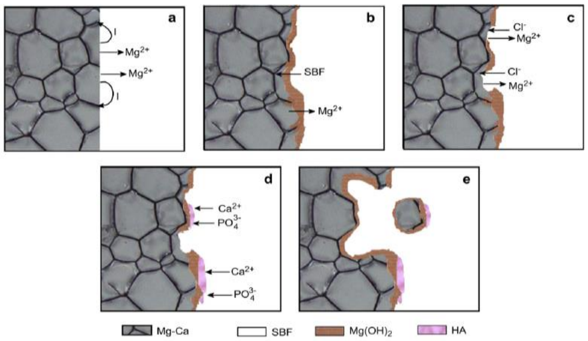

Based on this type of corrosion, literature studies concluded that controlling the amount of calcium in the alloy will influence corrosion rate and mechanical properties. Thus results obtained on alloys with low calcium concentrations, 1, 2 and 3% were better than those with high concentrations (5% - 20% Ca) both in terms of mechanical properties and corrosion rate [4, 10, 11], Mg-Ca 1% alloy being considered to be optimal for orthopedic implants [12].

Another method of improving the mechanical properties of Mg-1Ca alloys in the molded state is secondary mechanical processing. The tensile strength of the alloy has recorded, initially, a value of 71.38 ± 3.01 MPa. Through the process of hot rolling it reached a value of 166.7 ± 3.01 MPa, and by hot extrusion it was improved up to a value of 239.63 ± 7.21 MPa [12]. In the same time, this method improved hydrogen emissions meaning that corrosion rate is improved (high corrosion rate equals high hydrogen release). Diminished corrosion rate of the Mg-1Ca alloy, processed by hot extrusion has been expressed by a low hydrogen emission rate, from 35 ml/cm^2 to 10 ml/cm^2 [12].

During corrosion process, a hydroxyapatite layer is formed on the surface of the alloy, having a positive role on bone cell adhesion. Also, magnesium ions resulting from the degradation of the Mg-1Ca alloy, Zreiqat [11], could increase cell adhesion of HBDC (Human Body Derived Cells) in cell cultures. At the level of HBDC cells grown on the surface of the alloy, increased levels of α5b1 and b1 integrin receptors were discovered, which play a role in mediating cell adhesion to the surface of biomaterials. Thus, it is assumed that the increased viability expressed by Mg-1Ca alloys in cell cultures can be explained by facilitating the early response of osteoblasts, and it can be inferred that the release of Mg^{2+} ions from the alloy can stimulate bone cell adhesion and proliferation, being raised the hypothesis of the positive role of Mg-1Ca alloys in bone healing.

This research comes in suite with other areas of development in material science for orthopedic use in the local geographic area focusing on the interaction between the organic and the inorganic and also focusing on the impact of this research on the patients' lives [13, 14].

MATERIAL AND METHOD

Four specimens of domestic rabbit "Oryctologus Cuniculus", adult, minimum weight 3.5 kg, were used, divided in two groups. Group 1, included 3 specimens, with experimental fractures. Two rabbits with experimental fracture al anterior tibial tuberosity level and one with tibial diaphysis accidental fracture, during Mg-1Ca implant insertion. Fracture site was stabilized by the cylindrical implant followed by spica cast for three weeks. Group 2, control one, included one rabbit with a subtrochanteric fracture, occurred during a Mg-1Ca implant insertion (another experiment). In order not to lose the specimen, we extracted the magnesium alloy and continued with the hip osteosynthesis, plate and screws, austenitic steel.

We had used cylindrical implants of Mg-1Ca alloy, cast, with a density of 1.78 g/cm^3 and dimensions: L 40 mm, diameter 3.2 mm (Figure 2).

Figure 2: Mg-1Ca nail

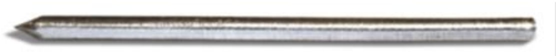

We chose intramedulary nailing (IMN), pins, because in orthopaedic surgery this is the golden standard for fractures of tibia or femur. This hypothesis is supported by biomechanical and biological factors.

Because of its design and mode of insertion, in the center of the bone, respecting the anatomical and mechanical axis of the bone, IMN is a load sharing implant compared to plates that are load bearing implants. This will increase the load bearing capacity of the implant, in the same time decreasing the stress shielding effect, fractures around the implant being less. Considering these characteristics, load bearing will be allowed earlier compared to plates osteosynthesis.

Regarding the biological factors , IMN will lead to less soft tissue damages compared to plates osteosynthesis. Fracture healing is a periosteum depended process and IMN will preserve it and also it will preserve the hematoma formed around the fracture site, that is a source of mediators for the bone callus formation process. Also, IMN is less invasive and the infection rate is low.

Another reason why we chose the intramedullary nailing system method is that the main objective of the study is to investigate the interaction of Mg-1Ca biomaterial with bone tissue. As we have shown in preliminary studies, the corrosion rate of Mg 1Ca alloy in contact with bone tissue is different from that in contact with muscle tissue. Unlike IMN which provides contact of the alloy only with bone tissue, plate and screw osteosynthesis will lead to two areas of contact of the alloy, with bone tissue and muscle tissue. This can generate false negative or false positive data, which can alter the results of the study.

The study was carried out with approval of the Ethics Committee for Scientific Research, no 92/20.04.2016, code PO-35-F-03. Animal care and clinical evaluation were performed according to FELASA (Federation of European Laboratory Animal Science Associations) guidelines and recommendations [15].

Preop procedures: Lower limbs radiographs, in two incidents, anterior-posterior and laterally, Figures 3 and 4. The animals were dewormed using Ivermectin, at a dose of 0.1 ml/body kg, subcutaneously (SC). Preoperative antibiotic therapy , one dose of Enrofloxacin (Boytril 5%) at a dose of 10 mg/body kg, subcutaneous.

Figure 3: Preoperative Xray

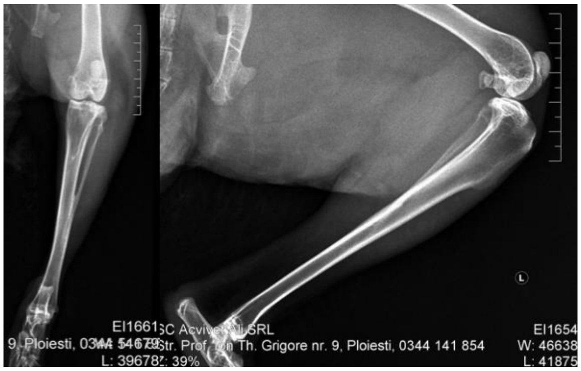

Figure 4: Preoperative Xray control group

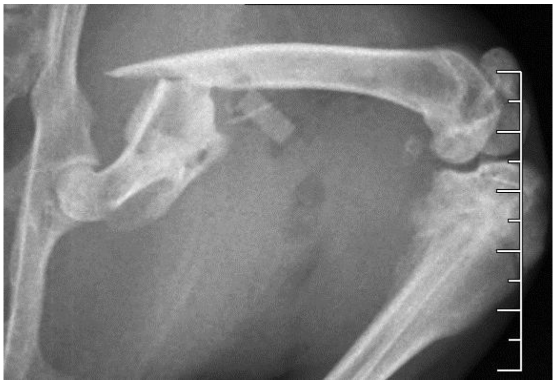

Blood tests: are needed to evaluate the possible impairment of the function of the vital organs, kidney, liver or ionogram by the corrosion of the alloy.For the blood samples we used as a source the auricular artery. All determinations of blood parameters were performed using the services of accredited laboratories. The determined parameters and their physiological values are represented in the following Tables (1 and 2).

Table 1: Ionogram

Element	Normal value
Magnesium	2.19 – 4.14 (mg/dL)
Calcium	12.4 – 15.6 (mg/dL)
Sideremia	111.7 – 329.6 (µg/dL)
Sodium (Na^+)	138 – 155 (mmol/L)
Potassium (K^+)	3.7 – 6.3 (mmol/L)

Table 2: Vital organ tests

Element	Normal value
Serum urea	12.6 - 50.3 (mg/dL)
Creatinine	0.38 - 1.88 (mg/dL)
Alanaminotransferase (ALT)	<61 (IU/L)
Aspartataminotransferase (AST)	<28 (IU/L)

Surgical procedures: The implants were chemically sterilized by ethylene oxide sterilization method, thus avoiding damage to the alloy.

The type of anesthesia chosen was, mask inhalation anesthesia, veterinary use (Figure 5). For induction Ketamine 20mg/body Kg, subcutaneous (s.c.) was used. The anesthetic agent used was: Sevoflurane.The anesthesia was maintained by sevoflurane delivered in oxygen (1.5–3.5% oxygenmixture; 1.5 L/min) Throughout the surgery we monitored cardiac activity and respiratory movements. Infusion of Ringer's lactate solution(10 mL/kg/h) during the entire surgical procedure ensured cardiovascular function.

Figure 5: Anesthesia

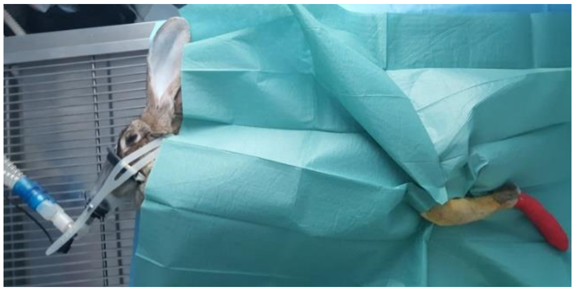

The surgery consisted of centromedular implantation, proximal tibia, cylindrical implant Mg-1Ca alloy, nail type by approach over the anterior tibial tuberosity (Figures 6, 7), followed by an experimental fracture, with oscillating saw, in tibial diaphysis at a distance of 1 cm distal of anterior tibial tuberosity (Figure 8).

Figure 6: Mg alloy implant insertion

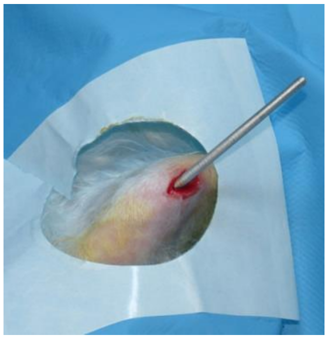

Figure 7: Mg alloy implant insertion

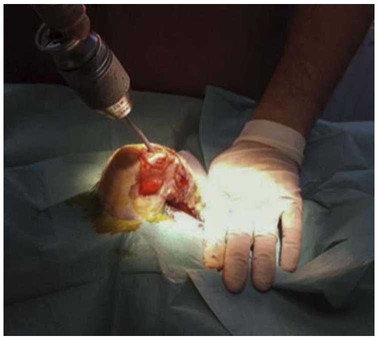

Figure 8: Experimental fracture

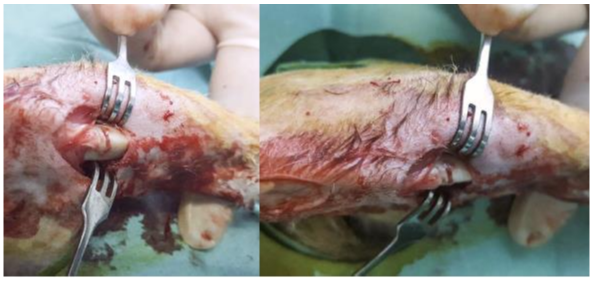

Postoperative procedures: Prophylactic antibiotic therapy, two days post-operatively. Enrofloxacin (Boytril 5%) at a dose of 5 mg/body Kg/12 hours, (s.c.) was used. Postoperative pain was controlled using Meloxicam at 0.2 mg/body Kg/24 hours (s.c.).

Two incidents radiographs, anterior-posterior and lateral, were used, with the same radiological device and the same technical data as in the case of pre-operative radiographs. Radiological follow up was for 6 weeks, and it is necessary to highlight the resorption of implants, and bone callus evolution.

At 6 weeks postoperatively, blood samples were collected.

Blood parameters and harvest conditions were similar to those performed preoperatively.

Euthanasia of rabbits was performed 6 weeks after implantation. In order to eliminate the suffering of the animals, we chose as an initial procedure their anesthesia, performed by s.c. Ketamine injection at a dose of 20 mg/body Kg. After this, euthanasia was performed by injecting 1 ml T61 (Etambutramide + Mebezonium Iodide + Tetracaine hydrochloric) intravenous (i.v.).

Post-euthanasia procedures: proximal tibialhalf, wasblock harvested, including bone callus formation area, and Mg-1Ca implant included insidethe bone piece, directly after sacrifice and fixated in 4% formaldehyde for postmortem histological analysis. We aimed to highlight the formation of bone callus in the fracture site, to highlight the cell viability, the morphology of the bone cells in the immediate vicinity of the Mg-1Ca alloy implant and those from the bone callus. The slides were analyzed using a Niko Eclipse E200 optical microscope, connected to a computer for the extraction and analysis of the obtained images. Bone tissue samples were decalcified in 10% EDTA solution. After decalcification, 4 µm thick sections were fixed in paraffin blocks, from which microtomes were cut. The sections were fixed on examination blades and colored. The staining method was Hematoxylin-Eosin, Van Gieson and Trichrome Masson. We also harvested tissue samples for histological examination from vital organs such as lungs, kidney and liver due to the suspicion of gas embolization in the vital organs and the influence of the resorption process of the Mg-1Ca alloy on the function of these organs, the same that I assessed through the laboratory tests. The slides were analyzed using the same type of microscope as before, and the staining method was Hematoxylin-Eosin.

RESULTS

The radiological follow-up after surgery in shown in Figure 9.

Figure 9: ATT fracture group, Mg-1Ca implant marked with no 1, fracture site marked with no 2.

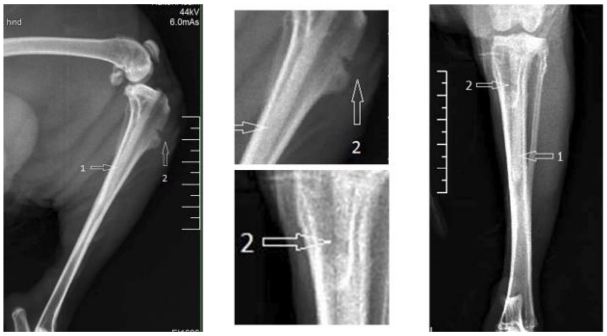

Figure 10 shows the accidental fracture of the tibial diaphysis that occurred during the introduction of Mg alloy implant. Figure 11 shows the control specimen, austenitic steel plate and screws osteosynthesis, marked with no 1.

Figure 10: Accidental fracture

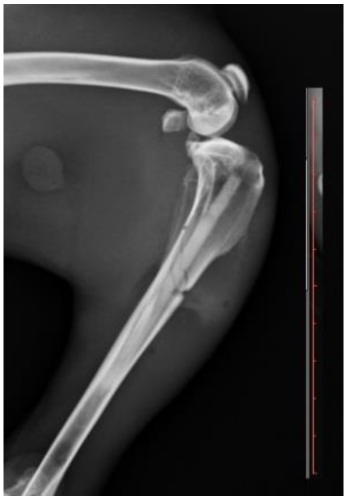

Figure 11: Control specimen

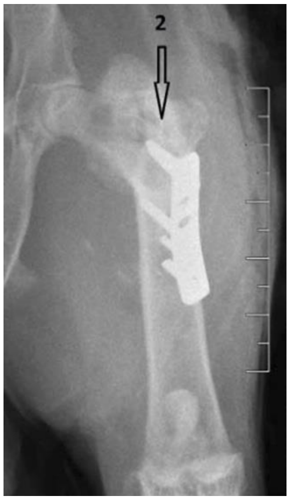

Six weeks after surgery

The situation of the experimental fracture at 6 weeks is presented in Figure 12. The fracture is healed, bone callus is noted with no 2. The implant from the Mg-1Ca alloy, Figure 1, is partially resorbed, especially in the metaphyseal area, but the shape is still preserved. The gas bubbles, no 3, are present in a small quantity, especially at the joint level. In the area adjacent to the implant, in contact with its surface, opaque areas, no 4, are observed, signs of mineralization. Figure 13 shows the accidental fracture, healed with a large callus formation.

Figure 12: ATT fracture

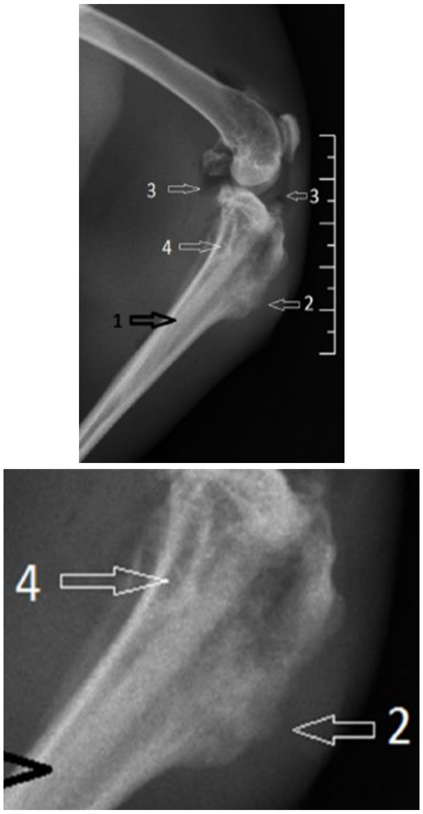

Figure 13: Accidental fracture

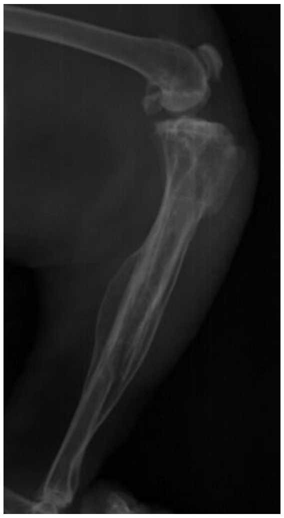

Histological results

Callus formation

Figure 14 shows a section from ATT callus, Hematoxylin-Eozine dye, magnitude 10X10. Bone tissue with nonuniform disposition, viable osteoblasts, with present intra-cytoplasmic nucleus and cartilaginous areas with chondroblasts with intracytoplasmic visible nuclei are observed, specific to bone callus formation. Figure 15 shows an overview of bone callus, Hematoxylin-Eosin dye, magnitude 4X10, from the accidental fracture callus site. Bone areas are irregularly arranged, alternating with cartilaginous areas, image of bone remodeling, specific to bone callus such as in Figure 14. Figure 16, is a detailed image of bone callus remodeling process, Hematoxylin-Eosin dye, magnitude 40X10, digital zoom. Multinucleate osteoclasts are visible, no 1, surrounded by bone tissue, with viable osteoblasts, intracellular nucleus visible, no 2. Figure 17 shows bone callus from the control specimen, Hematoxylin Eosin dye, 4X magnification. Bone remodeling areas are identified, being visible osteoclasts surrounded by newly formed bone tissue, with viable osteoblasts, similar to the previously presented samples.

Figure 14: ATT fracture

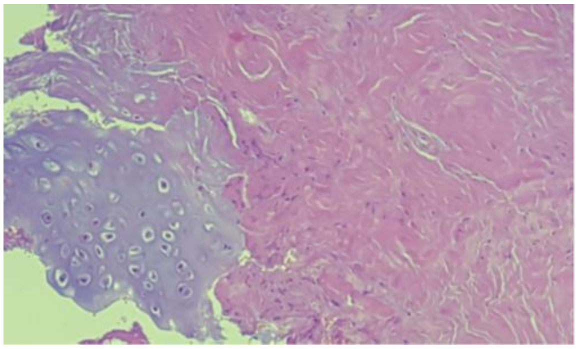

Figure 15: Accidental fracture callus

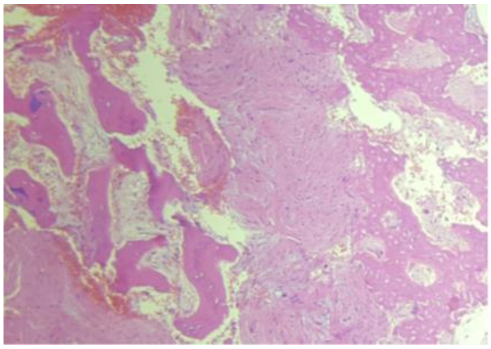

Figure 16: Bone remodeling

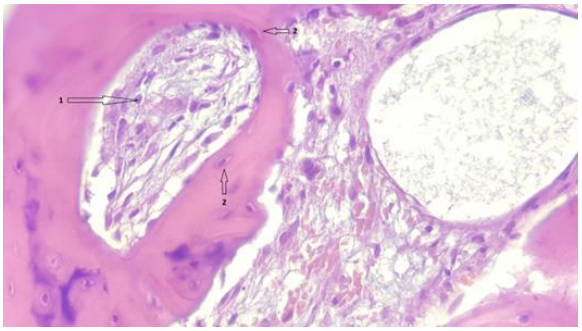

Figure 17: Control specimen callus

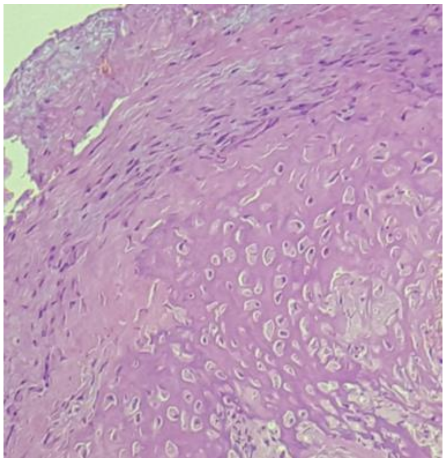

Bone alloy interface, it is showed in Figure 18, Tricrome Masson dye, magnitute 10x40. In this section we highlight viable bone cells, with intracytoplasmic nucleus, number 1 and collagen fibers, colored in blue, noted with no 2, arranged both pericellularly and in bone mass, sign of osteoblastic activity.The Mg-1Ca alloy, noted with no 4, which is in the process of resorption, is directly adherent to the bone surface, without identifying interposing tissues at the bone-alloy interface, no 5. In the immediate vicinity of the implant, the bone tissue is disposed in parallel lines, no 3, remineralization sign, bone formation.

Figure 18: Bone tissue and Mg-1Ca alloy, 6 weeks, Tricrome Masson dye

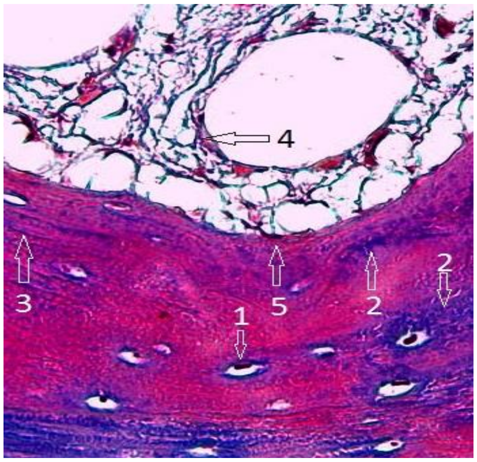

Vital organs

Figure 19 shows a section through the lung tissue. The alveoli appear in the form of empty spaces, marked with number 1. No intra-alveolar secretions or alveolar collabs are observed. Interstitial spaces, marked with no 2, have a normal appearance, without interstitial infiltration. A bronchioleis marked with no 3, with normal appearance and structure, and blood vessels are identified with no 4. No signs of gaseous embolism are observed.

Figure 20 shows a section through the renal tissue. It looks normal, with no pathological signs. The glomeruli of normal appearance and structure are marked with number 1, and with number 2 the collecting channels.

Figure 21 shows a section through the liver tissue. Liver cells, marked with number 1 have a viable appearance, with a visible nucleus inside. These are placed in columns delimiting the biliary drainage channels, marked with no 2. The tissue, does not have pathological aspects.

Figure 19: Lung, 6 weeks

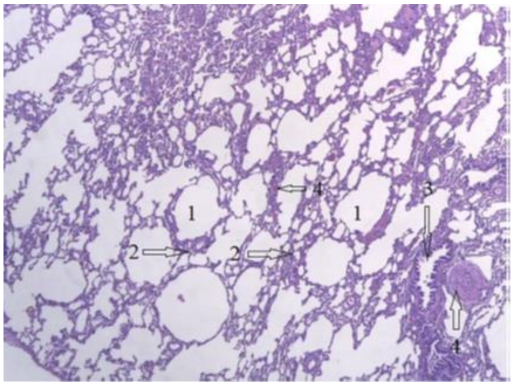

Figure 20: Kidney, 6 weeks

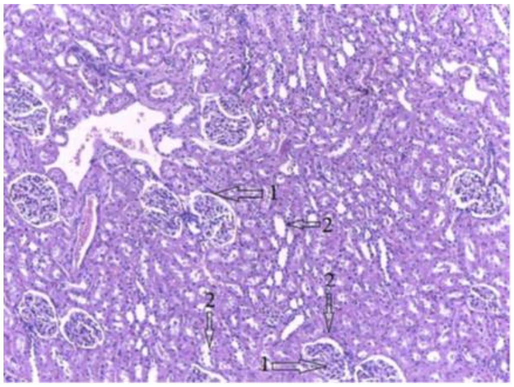

Figure 21: Liver, 6 weeks

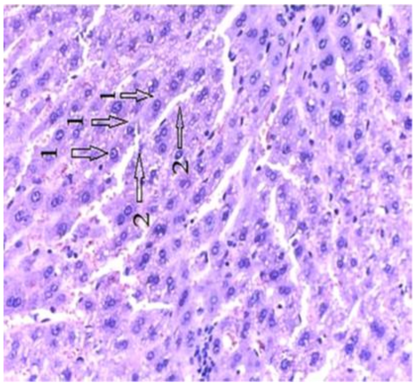

Blood tests results

The values obtained by monitoring the physiological blood parameters, preoperatively and at 6 weeks, are summarized in the following Table and Figures 22-25.

Table 3: Ionogram evolution

No	Magnesium (mg/dL)		Calcium (mg/dL)		Sideremia (µg/dL)		Sodium (mmol/L)		Potassium (mmol/L)	
	Preop	6 week	Preop	6 week	Preop	6 week	Preop	6 week	Preop	6 week
1	2.41	2.72	14.75	13.75	185.51	180.2	146.31	151.8	7	3.43
2	2.99	2.2	13.78	16.8	109.86	175.2	150	151.2	4.24	4.47
3	2.55	2.67	18.41	17.49	111.41	120.8	147.95	150.7	6.13	5.89

Figure 22: Mg evolution

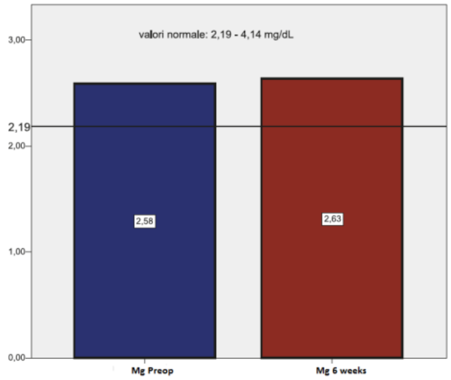

Figure 23: Ca evolution

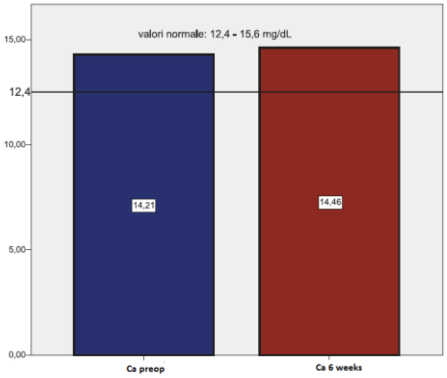

Figure 24: Na evolution

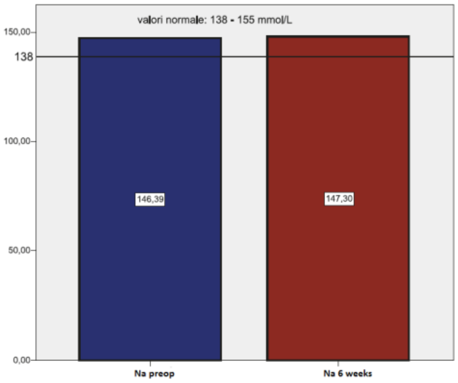

Figure 25: K evolution

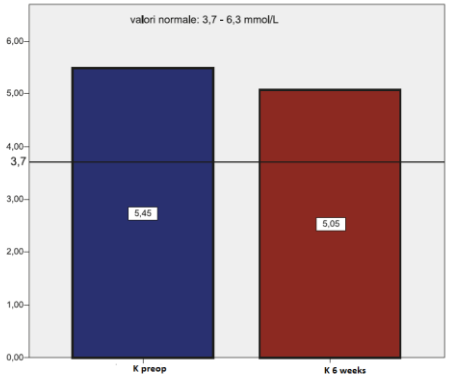

The studied parameters, magnesium, calcium, sodium and potassium, had registered average values preoperatively and at 6 weeks, within the range of normal, physiological values, characteristic to their species.

Urea has a slightly higher preoperative mean value than normal, 24.09 mg/dL compared to 23.50 mg/dL, but at 6 weeks we recorded an average value of 23.35 mg/dL, which is within the the normal, physiological limits of the species.

Creatinine has a preoperative mean value of 0.73 mg/dL and 0.74 mg/dL at 6 weeks, both values being within normal range.

Regarding the evolution of hepatic function indicators, both ALT and AST have preoperative average values and at 6 weeks, within the normal range, being from 25.8 IU to 23.2 IU for ALT and from 23, 3 IU to 24 IU.

Table 4: Vital organ function evolution

No	Serum urea (mg/dL)		Creatinine (mg/dL)		ALT (µg/dL)		AST (mmol/L)	
	Preop	6 week	Preop	6 week	Preop	6 week	Preop	6 week
1	16.7	17.2	0.56	0.59	21	23	22	23
2	28.6	27.5	0.69	0.72	18	19	27	25
3	33	16.1	0.53	0.7	17	23	25	26

Figure 26: Serum urea evolution

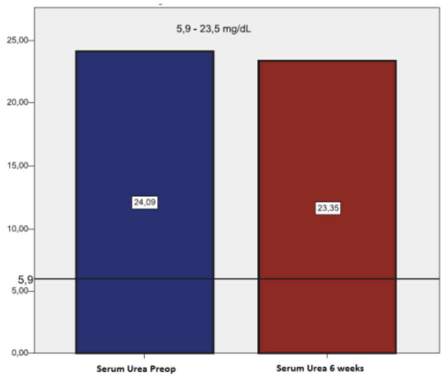

Figure 27: Creatinine evolution

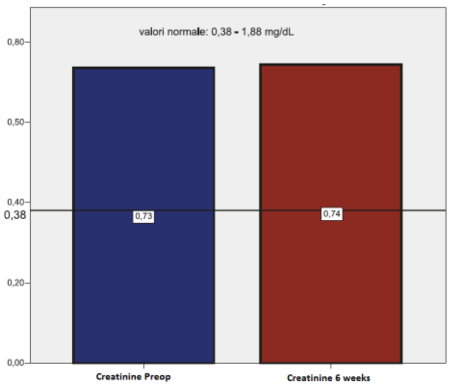

Figure 28: ALT evolution

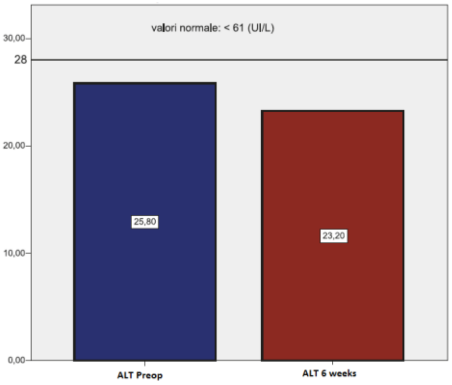

Figure 29: AST evolution

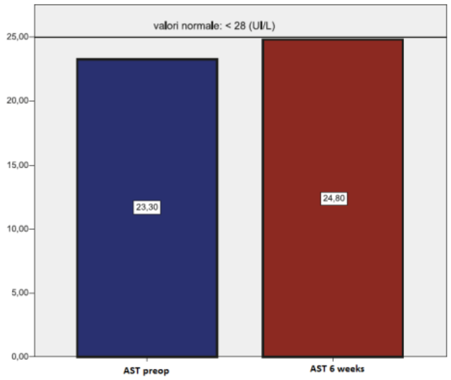

DISCUSSIONS

In vivo studies represent a superior stage of research required to certify the results obtained through in vitro studies. The present study, in vivo, performed on laboratory animals, rabbits, aimed at evaluating the biocompatibility of a Mg-1Ca binary alloy by evaluating the influence on the formation of bone callus, cell viability and systemic effects, impairing the function of vital organs.For this, we radiologically followed the evolution of the implant and the adjacent bone, we performed blood tests and histological sections

In the radiological images obtained, we notice callus formation both, at the level of the experimental fracture and in accidental fracture, at6 weeks after surgery. No radiologically transparent lines were identified at the bone implant interface, suggesting that there is no local osteolytic process or development of fibro-cartilaginous tissue at the bone implant interface. This observation suggests that the biodegradation process of the Mg-1Ca alloy does not adversely interfere with the biological process of bone tissue formation.

Histological examination is an important element in determining the biocompatibility of the Mg-1Ca alloy. Sample collection was performed at 6 weeks postoperatively.

The histological sections made from the samples collected from the fracture zone adjacent to the Mg-1Ca alloy implant, confirm the formation of the bony callus, showing areas of endochondral ossification, newly formed bone with disordered disposition, areas of bone remodeling, in which many osteoclasts and osteoblasts are identified. We can notice a cellularity specific to the formation of a normal bone callus, with no tissue necrosis. The trichrome Masson sections, shows a normal bone development with newly formed bone tissue at the alloy interface.

In the sections harvested from the vital organs, no pathological changes are observed.

This observations suggests that the biodegradation process of the Mg-1Ca alloy does not interfere negatively with the biological process of bone formation, allowing the formation of the bone callus in the fracture site, in a normal time period.

The study of blood parameters plays an important role in evaluating the systemic effects of the corrosion process of the Mg-1Ca alloy. Magnesium and calcium release from the alloy during the corrosion process did not lead to pathological changes in the serum values of the two ions. At the same time, we do not encounter pathological changes in serum sodium and potassium, ions with a very important physiological role.

Renal function is not influenced during the corrosion process, which is supported by the evolution of urea and creatinine values. Similarly, the liver function was normal, during the study, the ALT and AST transaminases recording average values within normal limits, at both evaluation times.

These data do not confirm the suspicion of pathological changes induced by systemically released corrosion products and gas embolism in vital organs.

The shape of the implant (pin) is basic, but reproducible; 3D printing of a patient-specific bioresorbable implant may be an option, as in modern knee surgery [16].

CONCLUSIONS

Thus, as a final conclusion, I can say that the Mg-1Ca alloy has been proven to be:

- Safe, with a high degree of biocompatibility
- It shows good capacity for osteointegration and osteo-conductivity
- With no local toxic effects, no impairment with bone callus formation
- No systemic toxic effects, no impairment with vital organs function
- A promising future solution for orthopedic surgery.

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ORIGINAL ARTICLE

The importance of Greek primary healthcare reconstruction during the COVID-19 outbreak

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Abstract: *Family doctors and general practitioners have long been those who were to be the first to deal with family's health problems. After primary care systems administration, general practitioners acquired a special role in the operation and efficiency of these systems. Their existence as institution contributes to the most complete and better prevention and treatment of health issues, as well as to the resources saving for the insurance systems. In Greece, since the formation of the Greek National Health System in 1983, numerous interventions have been legislated, most of them referring to the institution of family doctor. However, factors related to political will, financial interests, administrative and educational inadequacies, financial problems and geographical particularities did not allow the establishment of a strong primary healthcare. COVID-19 outbreak has exposed the weaknesses of all types of health systems. However, health systems based on primary care seem to better cope with the pandemic, as well as the continuity of care and quality of health services have been secured. In order, that Greek primary healthcare be organized from now on, many actions are required such as the establishment of electronic health records, the creation of properly staffed and equipped health centers, as well as more governmental financing programs about the modernization of primary care. Last but not least, pressure should also be exerted on governments to pass more groundbreaking bills and implement some of the older provisions.*

Keywords: *family doctor, general practitioner, Greek primary healthcare, COVID-19, legislative interventions*

INTRODUCTION

Primary Care (PC) is the first point of contact between people and the health system [1]. According to the Alma-Ata declaration, PC bases its operation on a group of health workers that has direct contact with patients, so that they respond appropriately to their needs [2]. Therefore, family doctor (FD)/general practitioner (GP) plays an integral role to PC promotion. The role of GPs is determined by the need for patients to contact health services that are near where they live and work, for prevention or health issues and, if necessary, to be addressed to more specialized health services [3].

In Greece, in one of the first legislative initiatives of the law 1397/83 for the Greek National Health System formation, FDs' existence was first mentioned. However, especially in urban areas, the full development of PC has not been fully implemented, while at the same time there are organizational issues regarding the cooperation of PC with hospital care [4, 5].

COVID-19 pandemic highlighted health system weaknesses and vulnerability, both in the field of public health and primary health. Overall, it is reported that countries with universal health coverage, updated strategy and readiness regarding the pandemic including primary care, as well as the level of governmental aid for public health measures, are found to best cope with the COVID-10 outbreak [6]. Moreover, past evidence regarding pandemics management, indicates that when community stops receiving health services due to its fear about the probability of getting infected, the health of population is endangered [7].

The aim of this article is to describe the multidimensional advantages of a solid PC system and the existence of FDs, especially during the COVID-19 pandemic. Moreover the legislative initiatives in Greece towards the formation of Greek PC while the reasons that have not been implemented will be analyzed. Lastly, the main reasons of the failure of the

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Greek PC will be descripted, with the aim of that solutions be suggested, so that the next waves of the pandemic may be faced in a better way.

GENERAL PRACTITIONER AS A KEY FACTOR OF PRIMARY CARE

The strengthening of PC systems, with FD playing a central role is of paramount importance [8]. The implementation of PC based on FDs has been associated with improved patient health, and reduction of mortality rates [9, 10], a result independent of confounding factors such as income [11, 12]. The quality of interfacing and the continuity of care between primary and secondary healthcare is improved, as well as the development of doctor-patient interpersonal relationships [13, 14]. Patients are also more satisfied with the quality of services provided [15, 16]. The advantages of a GP/FD-based care may be explained due to its low cost, accessibility, holistic perception and treatment of health problems and comorbidities and knowledge of family and social peculiarities [17, 18]. Last but not least, it is well documented that FDs' delivery of primary care is correlated with better outcomes [17].

Such a health system relies more on prevention and the holistic treatment of patients' health issues, reducing the cost of health services [19]. The contribution of FDs is also important in reducing access to hospitals and hospitalization (gatekeeping) [20, 21]. However, the results are not proportionately positive in regards of reducing the likelihood of re-admission to the hospital [22]. Moreover, relative equality in the quality of health services regardless of income and social class is ensured, while the vaccination coverage of the population is improved [3, 23, 24].

COVID-19 PANDEMIC AND THE IMPORTANCE OF PRIMARY HEALTHCARE

The wide spread of the SARS-CoV-2 outbreak (COVID-19) has been a challenging situation for all types of health systems globally. The uncertain epidemiology of this new infectious disease, makes the action plans to be based mainly on the containment of the disease and the delay of dissemination through social distancing [25]. The role of PC in the management of the pandemic is of paramount importance [26] as only the critical or severe patients admitted to the hospital, while GPs are those who mainly make the initiate diagnosis. At the same time, it is crucial that patients with chronic as well as acute health problems be monitored (follow-up), especially those who are disabled or frail at home or health institutions [27].

Not only has increased non-COVID-19 mortality been mentioned, but it is reported that numerous primary care offices have been closed as well as reduced capacity in primary care in general. Access to primary care has been reduced due to lockdowns, continuity of health services have been dramatically affected, as COVID-19 cases were in priority over non-COVID-19 patients with other health conditions [28].

Hospitals and major health institutions have to focus on the critical cases, while PC has to target on less severe patients as well as on the health of the whole population, with triage and gatekeeping playing a key role. Consequently, there are three main goals that PC has to accomplish during the pandemic: the first goal of PC providers is the healthcare workers' protection from being infected as well as to prevent COVID-19 transmission [6]. The second goal is informing community, identifying and protecting individuals-mainly the vulnerable-from becoming infected as well as protecting the tertiary care level (hospitals), are some other crucial responsibilities of PC providers [6]. Last but not least, PC providers have to continue providing health services for non COVID-19 patients so that there is no discontinuity of their medical condition. Moreover, there are reports that demonstrate the vital role of PC regarding influenza surveillance as well as important information and data about public health [29-31]. As a result, PC may be an important tool as far as COVID-19 management and surveillance are concerned. Nevertheless, this could only be accomplished whether PC has adequate resources and capacity to retain its services [6].

France is one European country that dealt with the problem of rapid escalation of COVID-19 cases. Despite the fact that in the initial national guidelines, published at February 2020, PC providers were not involved, on March 2020, when the first version of a national guideline engaging the role of PC was implemented [27, 32].

Furthermore, as far as Iceland is concerned, the PC providers have the role as the first point of management for people with COVID-19 like symptoms. Instructions and/or indications for tests were published by the Directorate of Health. The majority of the COVID-19 tests were taken in PC centers. COVID-19 confirmed patients were admitted to a special Hospital of Iceland, being monitored until their discharge. People who were under quarantine, were monitored by the PC providers (GPs). The whole structure of the Icelandic PC system was adjusted, in order to be adapted to the new criteria and needs for the COVID-19 pandemic.

In United Kingdom and Australia, the national health system relies on the integrated and well-structured primary health care. When a serious health emergency occurs, e.g. the

COVID-19 outbreak, it is of outmost importance that general practice as well as other national PC services be supported, in aim that the continuity of health care for the community be secured and the entire health system be better prepared to cope with the emergency [33]. During the COVID-19 pandemic, telehealth services and PC in general were funded with $2.4 billion by the Australian government, in order to secure the uninterrupted access to health services [33, 34].

LONGITUDINAL SURVEY OF LEGISLATIVE INITIATIVES FOR PC ESTABLISHMENT IN GREECE

In Greece, there is not a well-organized PC system, especially in urban areas [5]. Outpatient healthcare is provided, without an solid coordination, by many specialized physicians of both various PC services and physicians from the private sector, leading to a piecemeal approach/treatment of health issues [35]. In Greece, the institution of family doctor has been institutionalized with various laws in the past, but it has not gone into operation, despite all the efforts that have been attempted from time to time. Legislations that foresee the provision of PC services in Greece begin from very early times, but the first remarkable effort for the development of an organized national health system is held with a bill at 1983. Since then, despite the fact that numerous laws have been voted regarding the provision and organization of PC and the institution of FD, they have not been fully implemented [36]. In 1983, the foundations of PC network were created, especially in rural areas. It enabled residents of remote areas to obtain their own doctor, a form of "FD". However, the inability to organize and record patients and their problems did not allow that the system be improved and established at levels similar to those abroad [36]. From the above, it may be distinguished that despite the existence of successive bills concerning the development of PD and FD, neither is completed. Actually, the Greek PC system remains one of the weakest in Europe [37]. Numerous laws and bills were passed, many of which were often contrary to each other.

Another main reason concerning the insufficiency of Greek PC system is the small number of GPs. In 2018, Greece had the highest number of doctors with 6.1 per 1,000 population, but this number is a over-estimation as it includes all doctors licensed to practice (including retired physicians and those who might have emigrated to other countries) (Table 1) [38]. In many countries, the main concern has been about growing shortages of general practitioners, particularly in rural and remote regions. Whereas the overall number of doctors per capita has increased in nearly all countries, the share of general practitioners (GPs) has come down in most countries. On average across EU countries, only about one in five doctors were GPs in 2018.

Table 1: Practicing doctors per 1 000 population, 2008 and 2018

	2008	2018
Greece[1]	5.71	6.10
Austria	4.60	5.24
Portugal[1]	3.61	5.15
Lithuania	3.88	4.60
Germany	3.54	4.31
Sweden	3.68	4.27
Bulgaria	3.66	4.22
Denmark	3.58	4.19
Cyprus	2.78	4.07
Czech Republic	3.56	4.04
Spain	3.54	4.02
Italy	3.79	3.98
Malta	3.05	3.97
EU27	3.32	3.83
Netherlands		3.67
Slovak Republic[2]	3.37	3.52
Estonia	3.34	3.48
Croatia	2.74	3.44
Hungary	3.09	3.38
Latvia	3.23	3.30
Ireland		3.28
Finland	2.72	3.21
Slovenia	2.40	3.18
France	3.07	3.17
Belgium	2.92	3.13
Romania	2.32	3.05
Luxembourg	2.72	2.98
Poland	2.16	2.38
Norway	4.00	4.81
Switzerland	3.82	4.34
Iceland	3.64	3.89
North Macedonia[2]	2.62	3.00
Serbia		2.98
United Kingdom	2.57	2.84
Montenegro	2.10	2.76
Turkey[2]	1.59	1.88

[1] Data refer to all doctors licensed to practice, resulting in a large over-estimation of the number of practicing doctors (e.g. of around 30% in Portugal).

Figure 1: Share of different categories of doctors, 2018

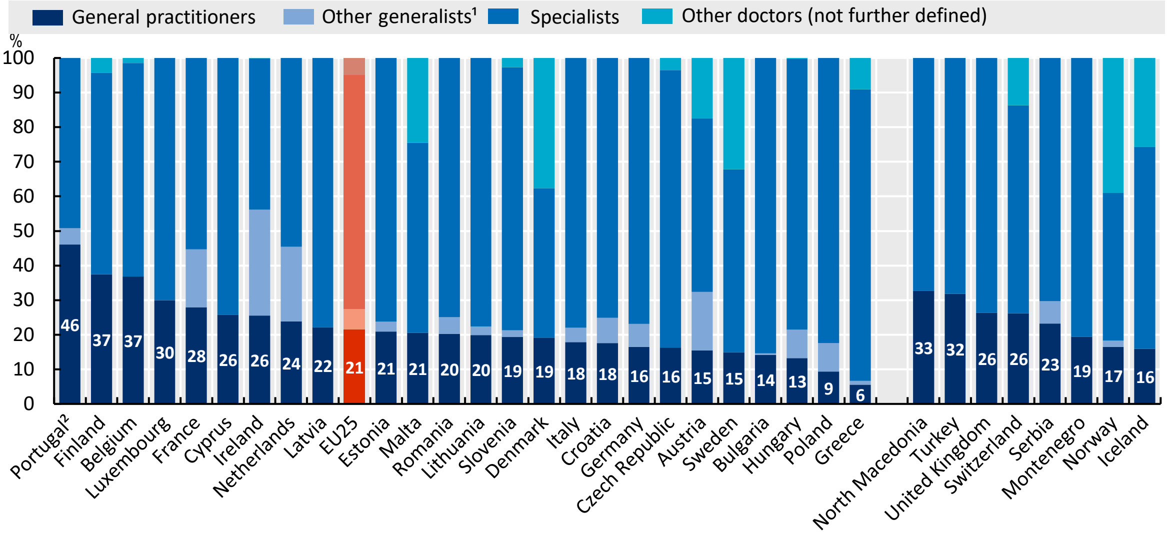

Greece and Poland have the lowest share of GPs, while Portugal, Finland and Belgium have been able to maintain a better balance between GPs and specialists. Several countries have taken steps over the past decade to increase the number of postgraduate training places in general medicine. For example, in France, about 40% of all new postgraduate training places have been allocated to general medicine since 2017, a greater proportion than in most other EU countries [38]. According to most recent data from Hellenic Statistical Authority, there are 3.8 GPs per 10.000 inhabitants (Figure 2) [39]. The number of GPs is smaller than other specialties such as cardiologists, microbiologists, obstetricians-gynecologists (Table 2).

Figure 2: Percentage of general practitioners in physicians' total number in the various regions of Greece

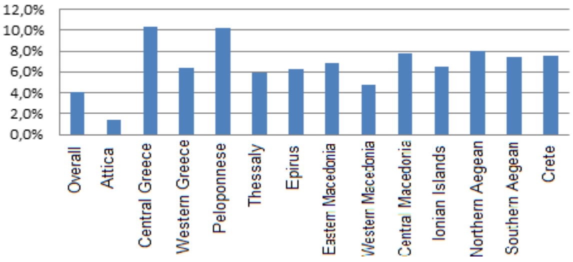

Table 2: Number of doctors per 10.000 inhabitants per specialty in Greece, 2019

Doctors total number	General Medicine	Cardiologists	Microbiologists	Internal Medicine	Paediatrists
66,058	3,232	3,508	3,493	4,604	3,719

An additional challenge in many European countries is the uneven geographic distribution of doctors as well as the various difficulties in recruiting and retaining doctors in remote and sparsely populated areas [38, 40]. In all countries, the density of physicians is generally greater in urban regions, reflecting the concentration of specialized services such as surgery in urban centers as well as physicians' preferences to live and practice in cities. Differences in the density of doctors between urban and rural regions are highest in the Slovak Republic, the Czech Republic and Greece (Figure 3) [38, 40].

Apart from Ireland and Portugal (physicians licensed to practice); there were more specialist physicians than generalist physicians in 2018 in each of the EU Member States [41]. Over time, there has been a widespread movement towards more specialist physicians relative to generalist physicians. In a majority of the EU Member States, there was an increase in the ratio of the number of specialists relative to the number of generalists between the years shown in Figure 5. In relative terms, this was particularly clear in Ireland, Romania and Croatia. By contrast, there were seven Member States, Greece included, where there was a decline in the ratio of specialists to

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[2] Data include not only doctors providing direct care to patients, but also those working in the health sector as managers, educators, researchers, etc. (adding another 5-10% of doctors)

generalists [41].

As far as the training of GPs is concerned, it is not appropriate, given the range of medical knowledge and practice in which they must immerse themselves. The specialty of General Practice/Medicine lasts five years in Greece. However, there is insufficient education regarding the most common diseases of the population, their diagnosis and treatment, as well as the methods of preventive medicine. Lastly, there are no methods of ensuring continuing education for general practitioners after the end of their specialty [42]. All these reasons make the specialty of General Practice in Greece unattractive.

Another reason of the failure of Greek PC system is the absence of an electronic/digital health record, leading to the inability of a continuous monitoring of the community health (continuity of care) [43]. Furthermore, the absence of urban-type PC centers or a network of GPs/FDs also contributes to the latter. These networks could record each patient's medical history, in order to facilitate the work of health professionals [44].

The different types of PC administration between urban centers and the province as well as between public and private sector, create difficulties in the health services provided. Although, PC services in urban areas are provided by doctors of different specialties, in the province (rural areas) there are primary health centers and peripheral clinics staffed mainly by GPs. In both cases there is no reliable system for linking primary to secondary healthcare. Last but not least, the geographical peculiarities of Greece as a country with numerous islands create additional problems.

Figure 3: Physicians by specialty, 2018

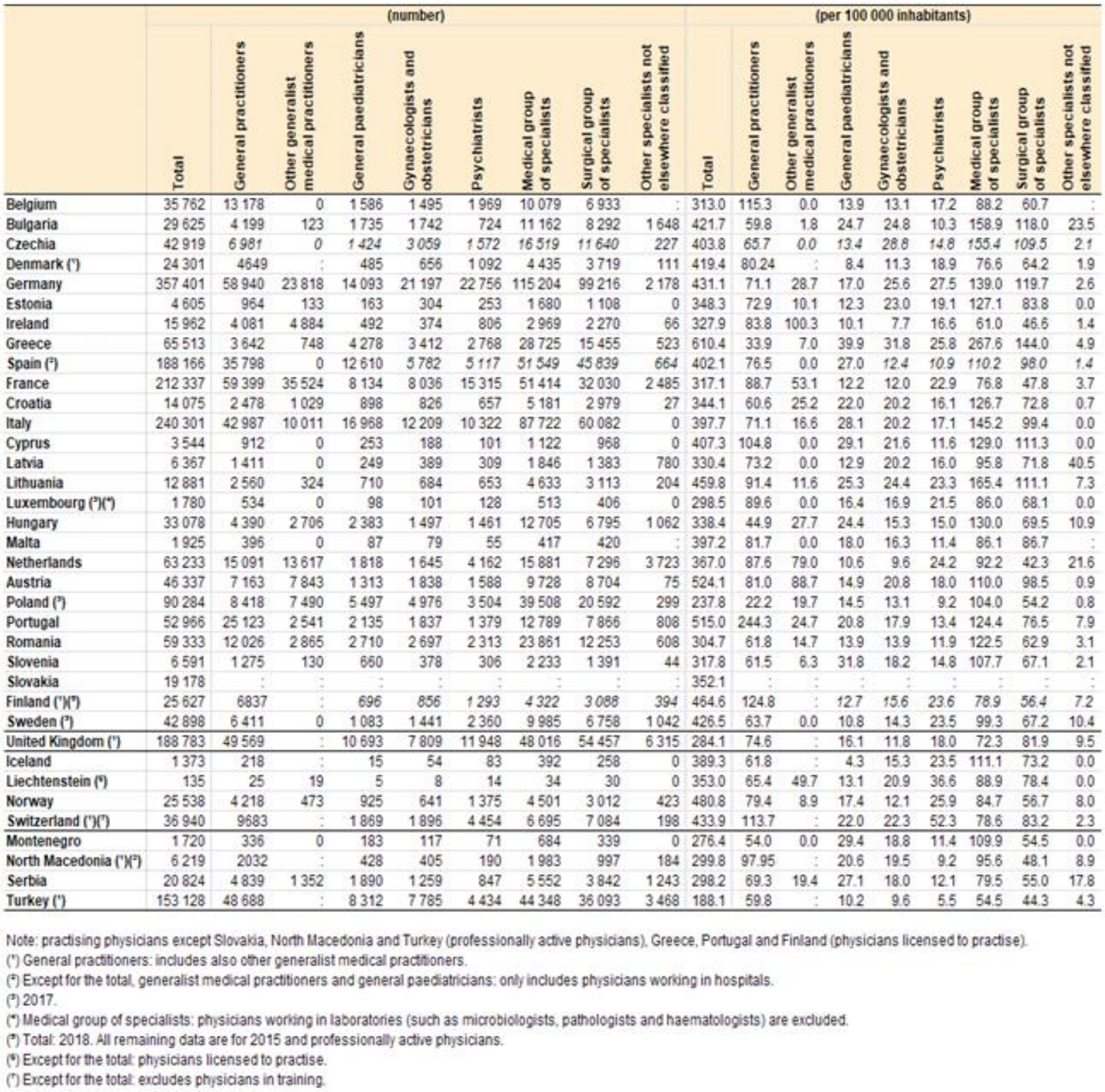

	(number)										(per 100 000 inhabitants)									
	Total	General practitioners	Other generalist medical practitioners	General paediatricians	Gynaecologists and obstetricians	Psychiatrists	Medical group of specialists	Surgical group of specialists	Other specialists not elsewhere classified	Total	General practitioners	Other generalist medical practitioners	General paediatricians	Gynaecologists and obstetricians	Psychiatrists	Medical group of specialists	Surgical group of specialists	Other specialists not elsewhere classified
Belgium	35 762	13 178	0	1 586	1 495	1 969	10 079	6 933	:	313.0	115.3	0.0	13.9	13.1	17.2	88.2	60.7	:
Bulgaria	29 625	4 199	123	1 735	1 742	724	11 162	8 292	1 648	421.7	59.8	1.8	24.7	24.8	10.3	158.9	118.0	23.5
Czechia	42 919	*6 981*	*0*	*1 424*	*3 059*	*1 572*	*16 519*	*11 640*	227	403.8	*65.7*	*0.0*	*13.4*	*28.8*	*14.8*	*155.4*	*109.5*	2.1
Denmark (1)	24 301	4649	:	485	656	1 092	4 435	3 719	111	419.4	80.24	:	8.4	11.3	18.9	76.6	64.2	1.9
Germany	357 401	58 940	23 818	14 093	21 197	22 756	115 204	99 216	2 178	431.1	71.1	28.7	17.0	25.6	27.5	139.0	119.7	2.6
Estonia	4 605	964	133	163	304	253	1 680	1 108	0	348.3	72.9	10.1	12.3	23.0	19.1	127.1	83.8	0.0
Ireland	15 962	4 081	4 884	492	374	806	2 969	2 270	66	327.9	83.8	100.3	10.1	7.7	16.6	61.0	46.6	1.4
Greece	65 513	3 642	748	4 278	3 412	2 768	28 725	15 455	523	610.4	33.9	7.0	39.9	31.8	25.8	267.6	144.0	4.9
Spain (2)	188 166	35 798	0	12 610	*5 782*	*5 117*	*51 549*	*45 839*	*664*	402.1	76.5	0.0	27.0	*12.4*	*10.9*	*110.2*	*96.0*	*1.4*
France	212 337	59 399	35 524	8 134	8 036	15 315	51 414	32 030	2 485	317.1	88.7	53.1	12.2	12.0	22.9	76.8	47.8	3.7
Croatia	14 075	2 478	1 029	898	826	657	5 181	2 979	27	344.1	60.6	25.2	22.0	20.2	16.1	126.7	72.8	0.7
Italy	240 301	42 987	10 011	16 968	12 209	10 322	87 722	60 082	0	397.7	71.1	16.6	28.1	20.2	17.1	145.2	99.4	0.0
Cyprus	3 544	912	0	253	188	101	1 122	968	0	407.3	104.8	0.0	29.1	21.6	11.6	129.0	111.3	0.0
Latvia	6 367	1 411	0	249	389	309	1 846	1 383	780	330.4	73.2	0.0	12.9	20.2	16.0	95.8	71.8	40.5
Lithuania	12 881	2 560	324	710	684	653	4 633	3 113	204	459.8	91.4	11.6	25.3	24.4	23.3	165.4	111.1	7.3
Luxembourg (3)(4)	1 780	534	0	98	101	128	513	406	0	298.5	89.6	0.0	16.4	16.9	21.5	86.0	68.1	0.0
Hungary	33 078	4 390	2 706	2 383	1 497	1 461	12 705	6 795	1 062	338.4	44.9	27.7	24.4	15.3	15.0	130.0	69.5	10.9
Malta	1 925	396	0	87	79	55	417	420	:	397.2	81.7	0.0	18.0	16.3	11.4	86.1	86.7	:
Netherlands	63 233	15 091	13 617	1 818	1 645	4 162	15 881	7 296	3 723	367.0	87.6	79.0	10.6	9.6	24.2	92.2	42.3	21.6
Austria	46 337	7 163	7 843	1 313	1 838	1 588	9 728	8 704	75	524.1	81.0	88.7	14.9	20.8	18.0	110.0	98.5	0.9
Poland (3)	90 284	8 418	7 490	5 497	4 976	3 504	39 508	20 592	299	237.8	22.2	19.7	14.5	13.1	9.2	104.0	54.2	0.8
Portugal	52 966	25 123	2 541	2 135	1 837	1 379	12 789	7 866	808	515.0	244.3	24.7	20.8	17.9	13.4	124.4	76.5	7.9
Romania	59 333	12 026	2 865	2 710	2 697	2 313	23 861	12 253	608	304.7	61.8	14.7	13.9	13.9	11.9	122.5	62.9	3.1
Slovenia	6 591	1 275	130	660	378	306	2 233	1 391	44	317.8	61.5	6.3	31.8	18.2	14.8	107.7	67.1	2.1
Slovakia	19 178	:	:	:	:	:	:	:	:	352.1	:	:	:	:	:	:	:	:
Finland (1)(5)	25 627	6837	:	*696*	*856*	*1 293*	*4 322*	*3 088*	*394*	464.6	124.8	:	*12.7*	*15.6*	*23.6*	*78.9*	*56.4*	*7.2*
Sweden (3)	42 898	6 411	0	1 083	1 441	2 360	9 985	6 758	1 042	426.5	63.7	0.0	10.8	14.3	23.5	99.3	67.2	10.4
United Kingdom (1)	188 783	49 569	:	10 693	7 809	11 948	48 016	54 457	6 315	284.1	74.6	:	16.1	11.8	18.0	72.3	81.9	9.5
Iceland	1 373	218	:	15	54	83	392	258	0	389.3	61.8	:	4.3	15.3	23.5	111.1	73.2	0.0
Liechtenstein (6)	135	25	19	5	8	14	34	30	0	353.0	65.4	49.7	13.1	20.9	36.6	88.9	78.4	0.0
Norway	25 538	4 218	473	925	641	1 375	4 501	3 012	423	480.8	79.4	8.9	17.4	12.1	25.9	84.7	56.7	8.0
Switzerland (1)(7)	36 940	9683	:	1 869	1 896	4 454	6 695	7 084	198	433.9	113.7	:	22.0	22.3	52.3	78.6	83.2	2.3
Montenegro	1 720	336	0	183	117	71	684	339	0	276.4	54.0	0.0	29.4	18.8	11.4	109.9	54.5	0.0
North Macedonia (1)(3)	6 219	2032	:	428	405	190	1 983	997	184	299.8	97.95	:	20.6	19.5	9.2	95.6	48.1	8.9
Serbia	20 824	4 839	1 352	1 890	1 259	847	5 552	3 842	1 243	298.2	69.3	19.4	27.1	18.0	12.1	79.5	55.0	17.8
Turkey (1)	153 128	48 688	:	8 312	7 785	4 434	44 348	36 093	3 468	188.1	59.8	:	10.2	9.6	5.5	54.5	44.3	4.3

Note: practising physicians except Slovakia, North Macedonia and Turkey (professionally active physicians), Greece, Portugal and Finland (physicians licensed to practise).

(1) General practitioners: includes also other generalist medical practitioners.

(2) Except for the total, generalist medical practitioners and general paediatricians: only includes physicians working in hospitals.

(3) 2017.

(4) Medical group of specialists: physicians working in laboratories (such as microbiologists, pathologists and haematologists) are excluded.

(5) Total: 2018. All remaining data are for 2015 and professionally active physicians.

(6) Except for the total: physicians licensed to practise.

(7) Except for the total: excludes physicians in training.

Figure 4: Medical doctors employed in hospitals (2008, 2013, 2018)

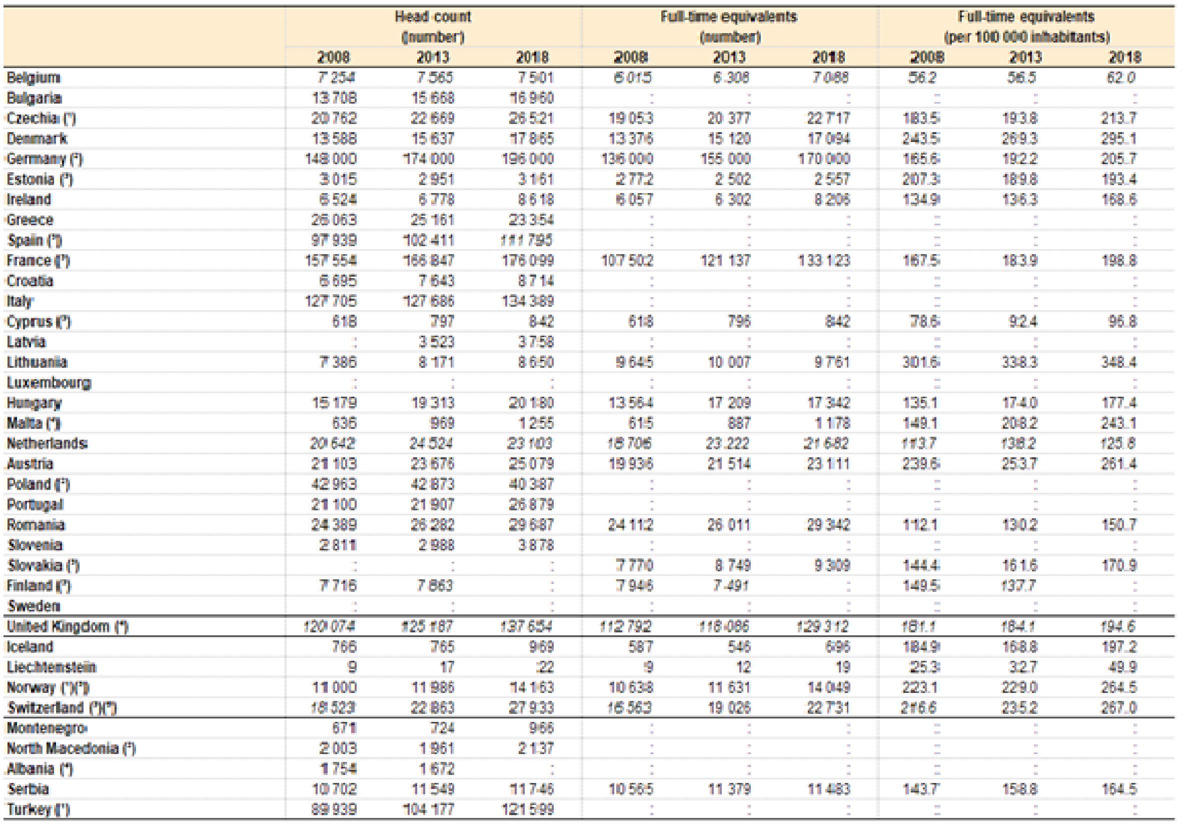

	Head count (number)			Full-time equivalents (number)			Full-time equivalents (per 100 000 inhabitants)		
	2008	2013	2018	2008	2013	2018	2008	2013	2018
Belgium	*7 254*	*7 565*	*7 501*	*6 015*	*6 308*	*7 088*	*56.2*	*56.5*	*62.0*
Bulgaria	13 708	15 668	16 960	:	:	:	:	:	:
Czechia (*)	20 762	22 669	26 521	19 053	20 377	22 717	183.5	193.8	213.7
Denmark	13 588	15 637	17 865	13 376	15 120	17 094	243.5	269.3	295.1
Germany (*)	148 000	174 000	196 000	136 000	155 000	170 000	165.6	192.2	205.7
Estonia (*)	3 015	2 951	3 161	2 772	2 502	2 557	207.3	189.8	193.4
Ireland	6 524	6 778	8 618	6 057	6 302	8 206	134.9	136.3	168.6
Greece	26 063	25 161	23 354	:	:	:	:	:	:
Spain (*)	97 939	102 411	111 795	:	:	:	:	:	:
France (*)	157 554	166 847	176 099	107 502	121 137	133 123	167.5	183.9	198.8
Croatia	6 695	7 643	8 714	:	:	:	:	:	:
Italy	127 705	127 686	134 389	:	:	:	:	:	:
Cyprus (*)	618	797	842	618	796	842	78.6	92.4	96.8
Latvia	:	3 523	3 758	:	:	:	:	:	:
Lithuania	7 386	8 171	8 650	9 645	10 007	9 761	301.6	338.3	348.4
Luxembourg	:	:	:	:	:	:	:	:	:
Hungary	15 179	19 313	20 180	13 564	17 209	17 342	135.1	174.0	177.4
Malta (*)	636	969	1 255	615	887	1 178	149.1	208.2	243.1
Netherlands	*20 642*	*24 524*	*23 103*	*18 706*	*23 222*	*21 682*	*113.7*	*138.2*	*125.8*
Austria	21 103	23 676	25 079	19 936	21 514	23 111	239.6	253.7	261.4
Poland (*)	42 963	42 873	40 387	:	:	:	:	:	:
Portugal	21 100	21 907	26 879	:	:	:	:	:	:
Romania	24 389	26 282	29 687	24 112	26 011	29 342	112.1	130.2	150.7
Slovenia	2 811	2 988	3 878	:	:	:	:	:	:
Slovakia (*)	:	:	:	7 770	8 749	9 309	144.4	161.6	170.9
Finland (*)	7 716	*7 863*	:	7 946	*7 491*	:	149.5	*137.7*	:
Sweden	:	:	:	:	:	:	:	:	:
United Kingdom (*)	*120 074*	*125 187*	*137 654*	*112 792*	*118 086*	*129 312*	*181.1*	*184.1*	*194.6*
Iceland	766	765	969	587	546	696	184.9	168.8	197.2
Liechtenstein	9	17	22	9	12	19	25.3	32.7	49.9
Norway (*)(*)	11 000	11 986	14 163	10 638	11 631	14 049	223.1	229.0	264.5
Switzerland (*)(*)	*18 523*	22 863	27 933	*16 563*	19 026	22 731	*216.6*	235.2	267.0
Montenegro	671	724	966	:	:	:	:	:	:
North Macedonia (*)	2 003	1 961	2 137	:	:	:	:	:	:
Albania (*)	1 754	1 672	:	:	:	:	:	:	:
Serbia	10 702	11 549	11 746	10 565	11 379	11 483	143.7	158.8	164.5
Turkey (*)	89 939	104 177	121 599	:	:	:	:	:	:

(*) 2018: break in series.
(*) 2017 instead of 2018.
(*) 2013: break in series.
(*) 2009 instead of 2008.
(*) Excluding self-employed doctors.

Figure 5: Physicians – ratio of specialists to generalists (2008, 2013, 2018)

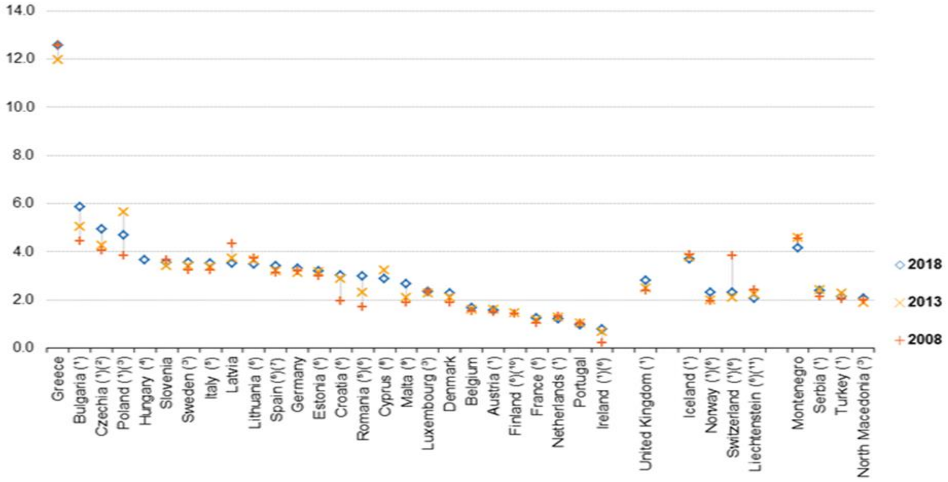

Note: practising physicians except Slovakia, Finland, North Macedonia and Turkey (professionally active physicians), Czechia, Greece and Portugal (physicians licensed to practise). Slovakia: not available.
(¹) 2018: break in series.
(²) 2018: estimate.
(³) 2017 instead of 2018.
(⁴) 2008 and 2013: not available.
(⁵) 2008: estimate.
(⁶) 2013: break in series.
(⁷) 2018: provisional. Data on specialists refer only to physicians working in hospitals.
(⁸) 2008: not available. 2014 instead of 2013.
(⁹) 2009 instead of 2008.
(¹⁰) 2013: estimate. 2018: not available.
(¹¹) 2008: data on generalists exclude interns.

CONCLUSION

The COVID-19 pandemic has revealed the fragility of all types of health systems around the globe. It is obvious that there is a great need to protect global health with a solid and sufficient PC within a properly organized health system, under a strong and continuous governmental support. PC physicians are the "gatekeepers" of the health system, they are responsible for the provision of a quality care as far as the common infections and diseases (COVID-19 cases included) are concerned, as well as undertaking important public health functions.

A strong PC system may lead to a better health level of the community. Furthermore, FD as an institution is accepted by the society and where it is applied, the citizens are more satisfied with the health services provided, as well as a primary care system based on FD is cost-effective, qualitative and efficient especially in times of pandemics. The reasons why a organized PC network has not been applied in Greece are mostly economic, political, social and geographic.

In conclusion, in order that PC be organized from now on, many actions are required. First, pressure should be exerted in aim that reforms be completed such as the electronic health record. Workshops and web events should be held in order public be informed about the benefits of PC. It is of paramount importance that urban-type health centers be made and be staffed properly, as well as documents to the EU be sent in order to seek financing programs. Last but not least, pressure should also be exerted on governments to pass more groundbreaking bills and implement some of the older provisions.

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ORIGINAL ARTICLE

Vulvovaginal atrophy – the impact on the quality of life and self-regard. Review of literature

Natalia Turcan[1], Delia Gradinaru-Fometescu[1], Alexandru Baros[2], Violeta Coravu[3], Gheorghe Turcan[4], Monica M. Cirstoiu[2]

Abstract: *Vulvovaginal atrophy is a condition frequently encoutered among menopausal women that often is ignored, remains undiscussed with the physician and the patient adapts the life style is such matter that offers a run from the specific symptomathology. According to the specialized published data, only 25% of the patients with vulvovaginal atrophy receive adequate therapy. The therapeutic perspective is quite extensive but the patient should receive recommendations and try different products until they find one that meets their needs and the one they like best. Proper estrogen therapy leads to a restoration of the acidic, normal vaginal pH, of the vaginal microflora, thickens the epithelium, increases vaginal lubrication and decreases vaginal dryness, also, adverse effects of vaginal estrogen therapy are uncommon. Early active detection of this pathological condition and the provision of an appropriately selected therapy can prevent the progression of the symptomatology and can significantly improve the sexuality of women.*

Keywords: *vulvovaginal atrophy, therapy, quality of life*

INTRODUCTION

Menopause is a physiological condition that occurs in women with an average age of 51 years; 95% of women become climacteric at an age between 45 and 55 years. Characteristic for menopausal period is the marked decrease of the ovarian estrogen production [1]. The vulvovaginal correspondent of this hormonal change is vulvovaginal atrophy. This is the condition that is frequently ignored, undiscussed with the physician and the patient remains in the position where he does not recognize the symptoms and adapts the life style is such matter that offers a run from this pathology. Therefore, most women forget that at 50 years old are young and they prefer to have a precarious sexual life, adopting in the same context the idea of aging.

The Genitourinary Syndrome of Menopause (GSM) was a term introduced for the first time in 2014 by the International Society for the Study of Women's Sexual Health and the North American Menopause Society [1, 2]. Being defined as the collection of symptoms and signs associated with decreased estrogen and other sex steroids, the GSM includes the totality of atrophic symptoms that can occur in the bladder-urethral area and vulvovaginal area (labia majora/minora, clitoris, vestibule/introitus, vagina) due to the loss of estrogen in the menopausal period. These symptoms include vaginal dryness, burning, pruritus, dyspareunia, vaginal discharge, bleeding/spotting and urinary tract symptoms like dysuria, increased urinary frequency, urethral discomfort and, infrequently, hematuria [3]. In Table 1 the main signs associated with GSM are summarized.

The aspects of the life quality must always be assessed; these include the degree of discomfort, the behavior adapted to the symptoms, the impact of these symptoms on daily activities, sexual life and partner relationship. No matter what management protocol is targeted, this must be

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adapted to the woman's expectations regarding its results [4]. According to the specialized published data, only 25% of the patients with vulvovaginal atrophy receive adequate therapy [5]. One explanation of this is that many patients feel ashamed to discuss intimate complains with a specialist and furthermore, some patients consider symptoms of GMS to be manifestations of the natural aging process and do not seek help for this.

Table 1: Clinical signs associated with GMS

Suggestive signs for GSM	Labia minora resorption or fusion
	Tissue fragility/fissures/petechiae
	Introital retraction
	Loss of hymenal remnants
	Prominence of urethral meatus
	Urethral eversion or prolapse
	Vulvovaginal pallor/erythema
	Loss of vaginal rugae
	Decreased vulvovaginal secretions/lubrication
	Decreased elasticity

Regarding the cascade of events involved in the pathophysiological mechanism, in hypoestrogenic conditions, the vaginal epithelium becomes thinner, the epithelial barrier function is lost, tissue elasticity decreases and the secretory activity of the Bartholin glands decreases, all this leading to trauma to the vaginal mucosa and pain [6].

In addition, under conditions of estrogen deficiency, the balance of the vaginal microbiota is disturbed, pathogenic gram-negative fecal flora and other bacteria prevail in its composition increasing the risk for vaginal and urinary tract infections.

Specific to the sexual intercourse in post-menopause are the following aspects [7]: narrowing of the vulvo-vaginal copulatory space with rigidity of vaginal walls, frigidity in the context of dyspareunia and local injury, maintaining sexual activity is associated with attenuation of the involutional process and decreased sensitivity to erogenous factors with changes in sexual behavior.

In Romania, this problem is even more serious as, for example, in the biggest Hospital of Bucharest with a median of 10,000 hospitalizations per year in the Obstetrics and Gynecology Department, in the last 10 years only about 400 cases of vulvovaginal atrophy, as a secondary diagnosis, were recorded.

Having this data, our aim was to summarize aspects related to the clinical symptomatology, approach and management and highlight the impact of vulvovaginal atrophy of the quality of life.

The extent of the pathology and recommendations

In 2018, the results of a significant study were published [8]. According to this study, which included more than 2000 patients, valvovaginal atrophy affects more than 90% of menopause women, the great majority of them combining a mean of five symptoms. Also, the authors of the same study state that in approximatively 10% of symptomatic cases, the signs of atrophic vulvo-vaginitis can lack. This cases have one advantage as the structural changes can be prevented trough specific topic therapy. Summarizing, the authors highlight the progressive trend of this condition and the great importance of early instituted therapy.

As regards the impact of GMS on the women's daily living, in 2019 Nappi et al [9] published an extensive study including 2160 climacteric, evaluable women in whom, clinical confirmation of vulvovaginal atrophy was possible in more than 90% and over 66% of this patients presented severe vaginal symptoms. Along with the symptomatology adapted questionnaire (EQ-5D-3L) a Day-to-Day Impact of Vaginal Aging (DIVA) questionnaire was used. The results showed a direct relation between the clinical presentation of vulvovaginal atrophy and the impact on the quality of life [6]. In the context of this results, it is to mention that the effect on the life quality of GMS should be regarded as equal to the impact of other conditions on daily activity of patients and treated with the same interest.

The topic regarding sexual activity in menopause is surrounded by reluctance on both sides, clinician and patient. It is a known fact that active sexual function at midlife has a significant positive impact on personal and relational quality and improves longevity. Simon and al [10], recommend as a solution a conversation guide for helping the practitioner to open and direct the discussion and to offer an appropriate treatment to enhance the sexual desire, treat orgasmic dysfunction and attenuate dyspareunia. According to another specific study [11] more than half of all menopausal women suffer from at least one sexual symptom along to other conditions related with hormonal changes. Psychosocial factors should be regarded a solution for maintaining sexual satisfaction and practitioners have to offer adequate counselling and proactively address sexual symptoms from the beginning of the menopausal period.

TREATMENT OPTIONS

Women should try different products until they find one that meets their needs and the one they like best. The first intention is non-hormonal lubricants. Glycerin lubricants can lead to an increased risk of vulvovaginal candidiasis. These products should be used routinely, usually two or three days a week and not just during sexual activity. Vaginal moisturizer and lubricating therapy appear to be just as effective as vaginal estrogen. These agents improve vaginal comfort, but do not reverse most atrophic vaginal changes. Thus, they are especially useful for women with mild symptoms, and many women with severe symptoms will need hormonal or alternative medications.

Mitchel et al [12] conducted a randomized clinical trial and found that the effect of local hormonal therapy is comparable equal to that of non-hormonal, the differences between case-placebo being minimal. The only statistically significant difference was the slightly better improvement of pain at penetration with low dose-vaginal estradiol. Therefore, local non-hormonal therapy is an extremely effective treatment for symptoms of dryness or vulvovaginal discomfort, tissue fragility, vaginal discharge if the only etiology is hypoestrogenism.

Vaginal estrogen therapy is the first line therapy for GSM that does not respond to vaginal moisturizers and lubricants. Proper estrogen therapy leads to a restoration of the acidic, normal vaginal pH, of the vaginal microflora, thickens the epithelium, increases vaginal lubrication and decreases vaginal dryness. Adverse effects of vaginal estrogen therapy are uncommon. Vaginal estrogen therapy at recommended doses results in the systemic absorption, although to a lesser extent than oral or transdermal estrogen therapy. A higher level of absorption with the initial therapy may be due to the atrophic thin vaginal epithelium. Low-dose vaginal estrogen is the most effective treatment for moderate to severe symptoms of vaginal atrophy that do not respond to non-hormonal intervention. Among patients, discussing hormone therapy causes fear, a phobia, justified on the one hand, but acceptable, and recommended on the other. Thus, successive studies have been conducted on the impact of local, hormonal therapy on chronic heart disease, the risk of thrombotic accidents, and breast cancer. The common conclusion was that the risk in the presence of these diseases outweighs the benefit, regardless of age [13]. However, the amount of circulating estrogen obtained through properly administered vaginal hormone therapy provides a low and constant systemic concentration and can be safely applied for a period of 3 months [14].

A systematic review of 19 randomized trials, including over 4,000 women, investigated topic estrogen treatment and found that creams, vaginal tablets, and rings were equally effective in relieving the symptoms of vaginal atrophy. In the first 2 weeks of use, the daily use of the above-mentioned groups of drugs is recommended, followed by a switch to maintenance therapy with a dose of two to three times a week. Continuous-release vaginal rings are preferred for women for whom daily medication is unacceptable. Vaginal rings are inserted for up to 90 days and can be placed and removed by the patient. The use of vaginal rings is not recommended for women with genital prolapse. It is also necessary to warn the patient about the possible expulsion of the devise. Such a route of administration allows the shunt of hepatic metabolism, which minimizes the risk of adverse reactions specific to systemic estrogen therapy and excludes the effects on the endometrium and breast tissue. So it is not necessary to prescribe progesterone preparations to prevent endometrial hyperplasia and adenocarcinoma. Local estrogen therapy allows the rapid elimination of vulvovaginal atrophy symptoms, but does not relieve the vasomotor symptoms and does not reduce the risk of osteoporosis. The safety of vaginally administered estrogen on the endometrium and breast was successively studied and demonstrated [15, 16, 17, 18]. Estrogen therapy is prescribed after a thorough clinical examination and identification of risk factors for possible complications. The risk of overdose is increased by using vaginal creams. Excessive estrogen levels in postmenopausal women are associated with an increased risk of heart disease, breast cancer, thromboembolic complications and cerebrovascular disease. For women with additional risk factors for any of these complications, it is necessary to refer to another therapeutic method. Low doses of estrogen are preferred for use, as high doses of estrogen at menopausal age is associated with an increased risk of endometrial adenocarcinoma.

Dehydroepiandrosterone (DHEA) is a steroid prohormone obtained through the biosynthesis of testosterone and estradiol. Vaginal metabolism of DHEA leads to the activation of estrogen and androgen receptors in the three layers of the vaginal wall. Due to local inactivation of this prohormone, the increase in serum estrogen and androgen levels is minimal. It was proven by reference clinical trials the local positive effect of intravaginal DHEA on mild and severe dyspareunia [19].

Laser therapy is beneficial trough the enhancement of vascularization of the vaginal mucosa, stimulation of collagen synthesis, the formation of new vaginal papillae, reformation of glycogen stores in the vaginal epithelium, restauration of the balance of the vaginal mucosa and therefore improves the symptoms of atrophy caused by lack of estrogen [20]. Finally, laser vaginal therapy was associated with a significant improvement in quality of life and sexual activity.

From the same new therapy spectrum, platelet-rich plasma injection it is based on the principle of activating and improving the body's natural ability to regenerate. It consists of the injection of autologous platelet-rich plasma (PRP) into the upper wall of the vagina and clitoris, triggering the multiplication of stem cells and the growth of "younger" tissue. Growth factors released from activated PRP, promote the production of collagen and elastin inside the vagina and around the clitoris, causing an increase in its elasticity, easier stimulation, positive effect on dyspareunia and vaginal lubrication [21].

Vaginal lipofilling is an effective and minimally invasive way to restore tissue volume in both cosmetic and reconstructive surgery. The disadvantages of lipofilling are unpredictability and variable reabsorption rate [22].

Our experience complies with the data presented above; the clinical diagnosis can be established during a simple gynecological examination were the signs of vulvovaginal atrophy can be identified especially in low to moderate cases accompanied by symptomatology (Figure 1), in severe cases were the diagnosis is instantly clear (Figure 2) or in cases in complex case where the signs and symptoms of vulvovaginal atrophy are overshadowed by the underlying pathology (Figures 3 and 4).

Figure 1: Moderate vaginal and cervical atrophy with loss of vaginal rugae, fissures and petechiae (authors' personal collection)

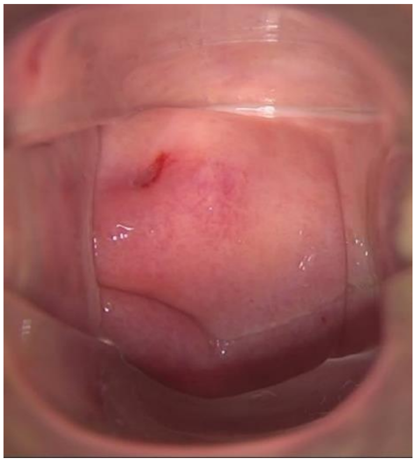

Figure 2: Severe vaginal and cervical atrophy with endocervical squamocolumnar junction, petechiae and tissue fragility

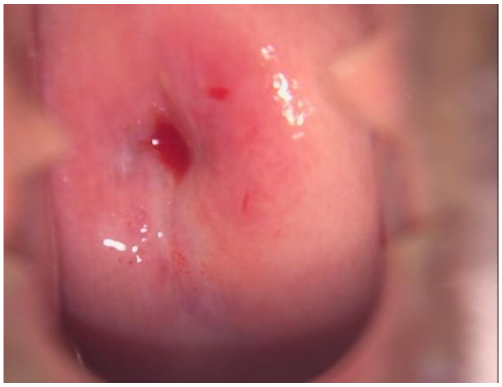

Figure 3: Ignored vulvovaginal atrophy superimposed on a grade 4 cystocele

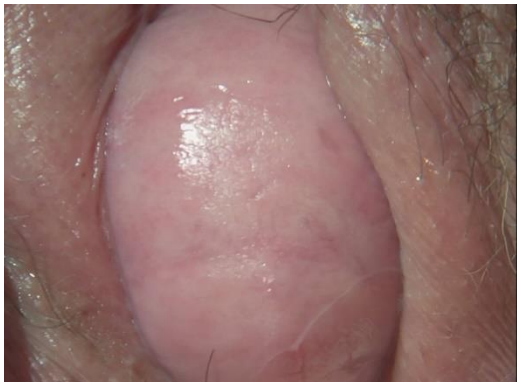

Figure 4: Severe vulvovaginal atrophy with labia minora resorption and prominence of urethral meatus, six years after a surgically induced menopause for cervix uteri neoplasia, six months after a PRP and topic lubricants.

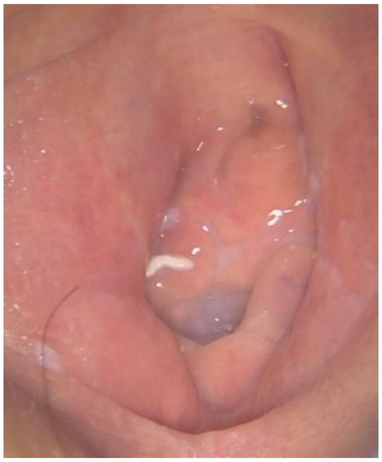

CONCLUSION

There are several ways to prevent severe vulvovaginal atrophy and to improve postmenopausal sex life. Some recommendations are:

o avoiding overeating, alcohol and spicy foods;
o avoiding smoking;
o psychological support;
o cotton underwear and loose clothing;
o sexually active status improves blood circulation in the

vagina and stimulates natural lubrication;

o sexual activity has no effect on serum estrogen levels. However, by improving blood circulation, it keeps the sexual organs healthier for longer time;

o giving the necessary time for prelude is an important point in maintaining the sexual function;

o the systemic impact of active sex life consists of an increased level of androgens and gonadotropins.

Many women with GSM do not seek medical advice, and clinicians should include questions about these symptoms in the history of menopausal women. The management of symptoms is guided by the patient's symptoms and goals. In the context of atrophic vulvovaginitis, low doses of vaginal estrogen are preferred compared to systemic hormonal therapy. This condition complicates the postmenopausal period in more than half of women worldwide and the manifestations bring discomfort in a woman's daily life, negatively influencing the quality of life and the couple's relationship.

Early active detection of this pathological condition and the provision of an appropriately selected therapy can prevent the progression of the symptomatology and can significantly improve the sexuality of women. A superior positive effect in the treatment of women with vulvovaginal atrophy can be obtained by combining hormonal and non-hormonal methods.

The role of the gynecologist is crucial by facilitating the discussion, recognizing the condition, being sensitive to the impact on sexual activity and quality of life, educating about risk factors and consequences, understanding the needs and expectations, removing barriers, fears, misconceptions, considering all the potential options according to the benefits and risks balance and according to the Psychosocial profile, having the objective to effectively treat VVA, starting early to obtain a better response to treatments and improving long-term adherence.

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ORIGINAL ARTICLE

Obesity is not related to individuals but to environmental conditions; from the perspective of obese patients

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Abstract: *Background: The Beliefs About Obese Persons Scale (BAOP) is used among non-obese people in English speaking countries to evaluate their opinions on obese people. Nevertheless, the data are scarce from the view of obese people. Therefore, we wanted to test BAOP on obese patients in order to evaluate their opinions from the perspective of obese patients.*

Methods: Between August 2019-June 2020, 115 (76.7%) female and 35 (23.3%) male, a total of 150 obese patients who were admitted to the general surgery department to be evaluated for bariatric surgey, were included in this study. Local ethics committee approval was obtained (2019/08-10). These obese patients were asked to fill out BAOP, which included 8 statements in which each statement was valued between -3 to +3 points.

Results: Mean score of BAOP within all obese patients was 34.87 ± 8.12 (range: 5-48). The average score of BAOP was 34.33 ± 8.60 (range: 5-48) in females and 36.62 ± 6.06 (range: 21-48) in males ($p = 0.14$). The frequency of the answers given by the male and female patients to the 5th statement, which was "Most obese people eat more than non-obese people", showed a statistically significant difference ($p = 0.03$).

Conclusions: Obese patients had higher BAOP scores compared to previous BAOP studies performed with non-obese people. Within this study, obese patients considered obesity as a condition which was not under their control.

Keywords: *bariatric, belief, obesity, surgery*

INTRODUCTION

Obesity is a worldwide public health problem causing predisposition to diabetes, heart disease and malignancy such as breast and colon cancer [1-3]. In the United States, more than 20% of the population has obesity [4]. Moreover, the number of people with obesity is rapidly increasing in Turkey. Patients who suffer from obesity not only have health related problems but also experience weight-related discrimination [5]. Patients with obesity often claim that they are accused of lacking self-discipline, uncontrolled binge eating, sedentary lifestyle, inadequate exercise and laziness [6].

Until now, different kinds of measuring scales have been introduced to evaluate the thoughts and beliefs of non-obese people over obese people [7-14]. Allison et al. published the article entitled "The measurement of attitudes toward and beliefs about obese persons" in 1991 [15]. One of the aims of Allison et al. was to develop reliable measures of beliefs about obese people, thus The Belifs About Obese Persons Scale (BAOP) was generated. Furthermore, Dedeli et al. tested and confirmed the validity and reliability of BAOP to measure beliefs about obese people in Turkish speaking countries [16].

Most of these previous studies with BAOP were performed on non-obese people. Nevertheless, the data are scarce from the point of view of obese people. Commenting on somebody else without having the same or similar conditions might be easy, however comments of obese people are of utmost importance to identify and solve obesity related problems within the society. Moreover, medical doctors should not only present a patient-oriented

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approach to investigate the causes of obesity and treatment options but also include patients in the decisions about their health [3, 17]. Therefore, we wanted to test BAOP on obese Turkish patients in order the understand their beliefs about obesity and obese people. For this purpose, we asked 150 obese patients, who were admitted to the general surgery department to be evaluated for bariatric surgery such as sleeve gastrectomy, to fill out BAOP.

METHODS

Between August 2019-June 2020, 115 (76.7%) female and 35 (23.3%) male, a total of 150 obese patients who were admitted to the general surgery department to be evaluated for bariatric surgey, were included in this study. Aksaray University Ethics Committee approval was obtained (2019/08-10). The patients with body mass index (BMI) between 25 and 29.9 kg/m^2 were regarded as overweight and patients with BMI 30 kg/m^2 and above were regarded as obese [5, 18]. In addition, BMI between 35–39.9 kg/m^2, 40-49.9 kg/m^2 and over 50 pointed out severe obesity, morbid obesity and severe morbid obesity, respectively [19].

These 150 obese patients were asked to fill out BAOP. The Beliefs About Obese Persons Scale is an 8-statement questionnaire [15, 16]. The participants were asked to mark each statement according to how much they agreed or disagreed with it, using a scale between, -3 to +3 points (-3: I strongly disagree, -2: I moderately disagree, -1: I slightly disagree, +1: I slightly agree, +2: I moderately agree, +3: I strongly agree). These eight statements are: "1. Obesity often occurs when eating is used as a form of compensation for lack of love or attention., 2. In many cases, obesity is the result of a biological disorder., 3. Obesity is usually caused by overeating., 4. Most obese people cause their problem by not getting enough exercise., 5. Most obese people eat more than non-obese people., 6. The majority of obese people have poor eating habits that lead to their obesity., 7. Obesity is rarely caused by a lack of willpower., 8. People can be addicted to food, just as others are addicted to drugs, and these people usually become obese."

All of the statements should be responded by the participants by the help of BAOP scale. In BAOP, points given to each statement by the participant are summed up. This final number is added to 24 in order to reach a total score, which varies between 0-48 [9, 20].

Statistical analysis was performed using the SPSS 20.0 statistical package programme. Numerical variables were stated as the mean (±) standard deviation and medians (minimum–maximum). Categorical variables were stated as percentages. The chi-square test and independent samples t test were used to compare differences between two groups. The p values <0.05 were considered significant.

RESULTS

This study included 150 obese patients, 115 (76.7%) were female and 35 (23.3%) were male. The mean age of the patients was 38.68 ± 12.09 years (range: 18-66). The mean age of women was 37.81 ± 11.53 years (range: 18-66), while the mean age of men was 41.51 ± 13.59 years (range: 21-66) (p = 0.11). The mean BMI of the patients was 42.03±7.24 kg/m^2 (range: 30.10-65.30 kg/m^2). Average BMI was 42.29±7.20 (range: 30.10-64.30 kg/m^2) in women and 41.16±7.41 (range: 30.16-65.30 kg/m^2) in men (p = 0.42).

The mean score of BAOP within all obese patients was 34.87 ± 8.12 (range: 5-48). The mean score of BAOP was 34.33 ± 8.60 (range: 5-48) in females and 36.62 ± 6.06 (range: 21-48) in males (p = 0.14). The answers given by all patients, female patients, and male patients separately to the questions on the scale of beliefs about obese people are shown in Table 1, Table 2 and Table 3, respectively.

Table 1: The frequency of the answers given by the patients to the questions of the Beliefs About Obese Persons Scale

	Patients (n/%)							
Score	**Q-1**	**Q-2**	**Q-3**	**Q-4**	**Q-5**	**Q-6**	**Q-7**	**Q-8**
-3	23 (15.3)	10 (6.7)	12 (8)	8 (5.3)	19 (12.7)	4 (2.7)	14 (9.3)	14 (9.3)
-2	13 (8.7)	11 (7.3)	7 (4.7)	7 (4.7)	13 (8.7)	4 (2.7)	8 (5.3)	4 (2.7)
-1	8 (5.3)	16 (10.7)	10 (6.7)	13 (8.7)	9 (6)	7 (4.7)	11 (7.3)	12 (8)
1	34 (22.7)	31 (20.7)	23 (15.3)	28 (18.7)	21 (14)	21 (14)	37 (24.7)	25 (16.7)
2	24 (16)	37 (24.7)	29 (19.3)	32 (21.3)	20 (13.3)	28 (18.7)	29 (19.3)	29 (19.3)
3	48 (32)	45 (30)	69 (46)	62 (41.3)	68 (45.3)	86 (57.3)	51 (34)	66 (44)

†Q: Question

48 (32%) patients gave 3 points to the first question, while 8 (5.3%) patients gave -1 points. (The first statement with "Obesity often occurs when eating is used as a form of compensation for lack of love or attention" has been answered as "I strongly agree" by 48 (32%) obese patients). 45 (30%) patients gave 3 points to the second question, while 10 (6.7%) patients gave -3 points. However, the patients gave most frequently 3 points ''I strongly agree'' to all questions.

Table 2: Replies of female patients to questions about Beliefs About Obese Persons Scale

Female Patients (n/%)								
Score	**Q-1**	**Q-2**	**Q-3**	**Q-4**	**Q-5**	**Q-6**	**Q-7**	**Q-8**
-3	17 (14.8)	8 (7)	10 (8.7)	8 (7)	15 (13)	4 (3.5)	13 (11.3)	11 (9.6)
-2	9 (7.8)	9 (7.8)	6 (5.2)	5 (4.3)	13 (11.3)	4 (3.5)	7 (6.1)	4 (3.5)
-1	5 (4.3)	13 (11.3)	7 (6.1)	12 (10.4)	5 (4.3)	7 (6.1)	10 (8.7)	10 (8.7)
1	26 (22.6)	23 (20)	18 (15.7)	21 (18.3)	16 (13.9)	17 (14.8)	25 (21.7)	18 (15.7)
2	20 (17.4)	26 (22.6)	17 (14.8)	25 (21.7)	19 (16.5)	19 (16.5)	21 (18.3)	22 (19.1)
3	38 (33)	36 (31.3)	57 (49.6)	44 (38.3)	47 (40.9)	64 (55.7)	39 (33.9)	50 (43.5)

†Q: Question

38 (33%) of the female patients gave 3 points, while 5 (4.3%) patients gave -1 points to the first question. Most of the female patients gave 3 points ''I strongly agree'' to all eight questions.

Table 3: Replies of male patients to questions within Beliefs About Obese Persons Scale

Male Patients (n/%)								
Score	**Q-1**	**Q-2**	**Q-3**	**Q-4**	**Q-5**	**Q-6**	**Q-7**	**Q-8**
-3	6 (17.1)	2 (5.7)	2 (5.7)	0	4 (11.4)	0	1 (2.9)	3 (8.6)
-2	4 (11.4)	5 (5.7)	1 (2.9)	2 (5.7)	0	0	1 (2.9)	0
-1	3 (8.6)	3 (8.6)	3 (8.6)	1 (2.9)	4 (11.4)	0	1 (2.9)	2 (5.7)
1	8 (22.9)	8 (22.9)	5 (14.3)	7 (20)	5 (14.3)	4 (11.4)	12 (34.3)	7 (20)
2	4 (11.4)	11 (31.4)	12 (34.3)	7 (20)	1 (2.9)	9 (25.7)	8 (22.9)	7 (20)
3	10 (28.6)	9 (25.7)	12 (34.3)	18 (51.4)	21 (60)	22 (62.9)	12 (34.3)	16 (45.7)

†Q: Question

While 10 (28.6%) of the male patients gave 3 points to the first question, 3 (8.6%) of them gave -1 points. Most patients (31.4%) gave 2 points to the second question. Twelve patients gave 2 points to the third question and the other 12 patients gave 3 points. None of the male patients gave -3 points to the fourth question and -2 points to the fifth question. None of the patients gave -3, -2 and -1 points to the sixth question. The seventh question was answered as 1 point and 3 points by 12 patients equally. Most of the male patients (45.7%) gave 3 points to the eighth question.

The frequency of the answers given by the male and female patients to the 5th question showed a statistically significant difference (p = 0.03). However, the frequency of their answers to the 1st, 2nd, 3rd, 4th, 6th, 7th and 8th questions did not show a statistically significant difference (p = 0.82, p = 0.89, p = 0.18, p = 0.35, p = 0.28 p = 0.32).

DISCUSSION

BAOP scale is used to evaluate the beliefs about obese people. A low score on BAOP suggests that obesity is controllable as obese individuals cause their obesity by themselves. Nevertheless, a high score on BAOP scale indicates a stronger belief that obesity is not under the control of the obese person [20, 21]. Moreover, lower BAOP score points out tendency towards anti-obese attitudes such as accusing obese individuals as lazy and deprived of self-control [22]. Mean score of 17.9 with BAOP scale was reported to be associated with anti-obesity attitude [21].

Sonneville et al. explored weight bias among 322 public health trainees by an cross-sectional online survey assessing explicit beliefs about the controllability of obesity using BAOP [8] The mean BAOP scores within public health trainees was 22.9. Relative to male students, female students had a stronger belief that obesity was not within the control of the individual (p = 0.03) [8]. Soto et al. performed BAOP scale on 528 students (250 medical students, 278 psychology students), where 56.3% of the participants were women. The average age was 20.7 ± 3.0. The mean BAOP scores were 18.6±5.7 and 16.6±5.5 in psychology and medical students, respectively. No differences were observed between BAOP scores of the participants related to age, gender or BMI [23]. Darling et al. investigated BAOP scale scores of 403 nursing students, 35 graduate education students, and 88 graduate social work students. BAOP scores were inbetween 14.35-25.39 which indicated that there was room for improvement for the belief that obesity was not under the individual's control. BAOP scores were not signifficantly different between male and female students. Moreover, Darling et al. reported that perception of one's own body as obese and having a friend or family member who was obese did not lead to more positive beliefs that obesity was not under the individual's control [3]. Usta et al. aimed to determine beliefs of 658 nursing students about obese people [9]. The 76.6% of the students was female and the mean age of the students was 20.63 ± 1.52. The percentage of the students who were overweight was 15.2%. In addition, 18.2% of the students claimed to have had discrimimation related to their weight. More interestingly, 17.5% of the nursing students did not want to get involved in daily care of obese patients. The mean BAOP score was 23.36 ± 0.83 (minimum 21.38, maximum 26). Usta et al. found out that nursing students who had a previous history of diet and regular physical exercise believed that obesity was controlled by individuals [9]. Kadar et al. tried to assess the obesity bias among 143 preclinical and clinical chiropractic students and 30 faculty members [24]. Average scores of BAOP scale were 31.68±5.19, 31.26±4.83, 27.93± 4.85 and 30.07±7.85 in the student and faculty member groups, respectively. BAOP scores of preclinical faculty group was significantly higher than each student groups. As a result, obesity bias was common among students and faculty members [24]. Yilmaz et al. aimed to assess whether there was prejudice towards obese people among 190 student nurses and 189 registered nurses using BAOP [14]. The most of both student nurses and registered nurses had negative beliefs about obese people. The mean scores of BAOP scale were 17.4± 4.8 and 15.7±5.8 in student nurses and registered nurses, respectively which revealed that both groups had negative beliefs about obese people [14].

Within our study, the mean score of BAOP within all obese patients was 34.87 ± 8.12 which revealed that obese patients considered obesity as a condition that was not under their control. In addition, we identified that majority of obese patients gave 3 points to each question of the 8-item survey as they strongly agreed. Therefore, obese patients within this study had higher BAOP scores compared to BAOP studies performed with non-obese people. The results obtained from this study revealed that non-obese and obese people had different opinions about the reasons of obesity. Morever, mean BAOP scores within our study did not differ statistically in relation to gender as male and female. Nevertheless, the fifth statement within BAOP which questioned if most obese ate more than non-obese people was replied by female patients statistically different from male patients (p = 0.03). Thus, this might be a clue pointing out the different eating habits of male and female obese patients.

Previous BAOP scale-related studies performed on non-obese people revealed low BAOP scores which indicated that obesity was related solely to individuals. Therefore, obese people were accused of lacking self-control, for example not controlling enough their eating habits. However, in our study, obese individuals reached high BAOP scores which indicated that obesity was not related to individuals but to environmental conditions. This discrepancy within the BAOP scale points of obese and non-obese individuals require detailed investigation of the beliefs towards obese people and reasons of obesity. We hope these results encourage our colleagues not only to present a patient-oriented approach to investigate causes of obesity but also include obese patients in the decision process of their further medical or surgical treatment options.

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All the authors agreed on the content of the manuscript.

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ORIGINAL ARTICLE

Comparison of complications of surgical repair in inguinal hernia with Bassini, Lichtenstein, and preperitoneal methods

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Abstract: *Introduction: Complication after inguinal hernia repair is one of the challenging problems after inguinal hernia repair. This study aimed to compare the complications of three common surgical methods in inguinal hernia repair, Bassini, Lichtenstein, and Preperitoneal methods (with anterior approach).*

Methods: In this prospective study, patients who underwent inguinal hernia repair surgery in Baqiyatallah Hospital from June 2019 to May 2020 were included. Patients were allocated in three groups: Bassini, Lichtenstein, and Preperitoneal (with anterior approach), based on surgeons' opinions. Postoperative pain, hematoma and seroma, wound infection, recurrence, and also we assed postoperative satisfaction after surgery.

Results: The mean age of patients was 49.90 ± 16.44 years, and 92.5% of patients were male. Of the total patients, 6.8% had an infection and there was no significant difference in the distribution of infection in the groups (P=0.230). The mean of pain in all patients was 2.37 ± 1.10. There was no significant difference between the three groups in the mean of pain (P = 0.253). There was no significant difference in the distribution of recurrence in the groups (P=0.356). There was no significant difference in the distribution of testicular swelling in the groups (P=0.421). No postoperative hematoma and seroma were reported in the groups. The mean of satisfaction in all patients was 93.61±19.63. There was no significant difference between the three groups in the mean of satisfaction (P = 0.156).

Conclusion: The results showed that infection, pain, and recurrence rate in the Preperitoneal group were lower than the other groups, although this difference was not significant. Also, testicular swelling, hematoma, and seroma as well as patients' satisfaction were similar between groups.

Keywords: *inguinal hernia repair, Bassini, Liechtenstein, preperitoneal*

INTRODUCTION

Operation of inguinal hernia is one of the most common procedures in the general surgery field. The prevalence of inguinal hernia is between 15-45% in different ages [1-3]. There are two categories for inguinal hernia, direct and indirect, that included 24 to 50% of all types of hernia, respectively [4-7]. Some studies reported chronic obstructive pulmonary disease, chronic cough, benign prostatic hyperplasia, chronic constipation, family history of hernia, previous right lower quadrant incision, smoking, collagen diseases, physical activity, and bearing the burden can trigger inguinal hernias [5-10].

The inguinal hernias can develop discomfort, pain, and dysfunction in the patient, and also it can cause a cosmetic problem for most patients [2-4]. Severe complications of this disorder, which often occur following hernia entrapment and suppression, require emergency intervention. These include obstruction, ischemia, and gangrene of the bowel or any organ trapped inside the hernia sac [4-7].

Surgical procedure is the standard treatment of this disease. Today, there are several techniques of surgery for it, and surgeons associated with circumstances chose a surgery

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strategy. The main purpose of surgery is to treat patients and decrease the recurrence chance of the disorder [11-15].

Inguinal hernia repair surgery has several potential complications such as hernia recurrence, pain, osteitis of the pubis, Damage to the cord and testicles, hematoma, and testicular swelling [1-3, 5]. There are two main approaches to operation including open surgery and laparoscopy. Also, there are several techniques to repair the herniation site such as classical and preperitoneal techniques. The classical method is a simpler procedure than other methods of repair conducted by most surgeons. Various conventional methods like Bassini's applying suture material are in usage despite the laparoscopic hernia repair [16-18]. Suture repair is related to a significant tension on the suture line. This is possible to induce ischemia of the tissues and final failure of repair causing recurrence [18-20].

Open surgical techniques include two categories of tissue repair and mesh repair methods. More traditional methods are based on initial tissue repair, but today with the production and presentation of industrial meshes, non-traction hernia repair with mesh is the standard method of inguinal hernias surgery [9-6]. Lichtenstein et al. reported the concept of tension-free hernia repair through a synthetic proline mesh that ensuring promising outcomes of hernia repair in the long term [1-3].

Preperitoneal repair is one of the pre-peritoneal methods, the mesh is placed under the transversal fascia and right on the peritoneum, and especially in cases of recurrent hernias after tissue repair methods. In this method, the peritoneal space is released through the anterior access, a 15 by 15 cm mesh is implanted on the peritoneum and fixed to the umbilicus and symphysis of pubis with non-absorbable thread, and then tissue repair with nylon loop thread is done [13-14].

This study aimed to compare the complications of Surgical Repair in inguinal hernia with Bassini, Lichtenstein, and Preperitoneal methods.

METHOD

In this prospective study, patients who underwent inguinal hernia repair surgery in Baqiyatallah Hospital from June 2019 to May 2020 were included.

Patients were allocated to Bassini, Lichtenstein, and Preperitoneal methods (with anterior approach) based on surgeons' opinions. Postoperative pain, hematoma, seroma, infection, recurrence, and postoperative satisfaction were assessed after surgery.

Sufficient information was given to patients about the type of different surgical techniques.

All cases were assessed and followed up according to the standard protocol. All the patients were followed up for 18 months postoperatively to assess complications. Excisional sac hernias were sent to a pathologist to examine their tissue for any malignancies. All cases were normal.

All operations were performed by surgeons in this research with a similar technique. The pain was measured according to the visual analog scale (VAS) post-operative. VAS was described for patients as a ruler that is calculated from 0 to 10. A score of zero shows painlessness and a score of 10 shows unbearable pain possible by the patients.

The protocol of the study was confirmed by the ethics committee of Baqiyatallah University of Medical Sciences with the IR.BMSU.BAQ.REC.1399.026 code. Consent forms were received from all participants.

Mean, standard deviation, frequency, and percent were reported to describe the data. Chi-square and Fisher's exact tests were used to determine the differences between qualitative variables. Data were analyzed by SPSS-20 software. The statistical significance level of all tests was considered less than 0.05.

RESULTS

Overall, 56 patients in the Bassini group, 50 patients in the Liechtenstein group, and 40 patients in the Reed-Reeves group were included.

The mean age of patients was 49.90 ± 16.44 years, and 92.5% of patients were male. The mean BMI of all patients was 25.52±3.56. The mean duration of surgery in all patients was 28.49 ± 75.97 minutes.

There was no significant difference between the groups in the sex distribution of patients (P=0.117). The mean age in the Bassini group was 18.59 ± 45.28 years and was significantly lower than the other groups (P = 0.003). The mean BMI in the Liechtenstein group was 26.95±4.13 and was significantly higher than the other groups (P = 0.002) (Table 1).

The mean duration of surgery in all patients was 28.49±75.97 minutes and there was no significant difference between the three groups in the mean duration of surgery (P = 0.226).

The mean length of hospital stay in all patients was 0.89. 1.02 days and in the Reed-Reeves group was 0.80 ± 0.50 days which was significantly less than the Bassini group (P=0.003).

There was no significant difference in the distribution of history of diabetes, COPD, and smoking between the three groups (P> 0.05) (Table 2).

Table 1: Patients' characteristics according to three groups of the study

Items	Bassini	Liechtenstein	Reed-Reeves	P-value
Age	45.28 ± 18.59	55.66±11.76	50.43±15.45	0.003
BMI	24.90±3.17	26.95±4.13	24.56±2.65	0.002
Sex	7 (12.1%)	1 (2.0%)	3 (7.5%)	0.002
	49 (87.9%)	48 (98.0%)	37 (92.5%)	

Table 2: Duration of surgery, length of hospital stay, diabetes, smoking, COPD and constipation in patients

Items		Bassini	Liechtenstein	Reed-Reeves	P-value
Duration of surgery		75.44±33.75	85.27±17.69	70.00±22.79	0.226
Duration of hospitalization		1.27±0.93	0.93±0.71	0.57±0.80	0.003
Diabetes	Yes	5 (9.1%)	4 (8.0%)	3 (7.5%)	0.002
	No	51 (90.9%)	46 (92.0%)	37 (92.5%)	
Smoking	Yes	11 (19.7%)	10 (20.0%)	7 (15.6%)	0.360
	No	45 (80.3%)	38 (76.0%)	31 (78.1%)	
	History	0 (0.0%)	2 (4.0%)	2 (6.2%)	
COPD	Yes	2 (3.1%)	6 (12.0%)	0 (0.0%)	0.050
	No	54 (96.9%)	44 (88.0%)	40 (100.0%)	
Constipation	Yes	1 (1.6%)	6 (12.0%)	8 (18.8%)	0.008
	No	55 (98.4%)	44 (88.0%)	32 (81.2%)	

The frequency of constipation in Reed-Reeves and Liechtenstein groups was significantly higher than Bassini group (P = 0.008).

Of the total patients, there was 6.8% postoperative wound infection and there was no significant difference in the distribution of postoperative infection in the three groups (P=0.230) (Table 3). No postoperative hematoma and seroma was reported in the groups (P=0.999). The mean of pain in all patients was 2.37 ± 1.10. There was no significant difference between the three groups in the mean of pain (P = 0.253).

Table 3: Infection, hematoma, postoperative pain, recurrence, and patients satisfaction

Items		Bassini	Liechtenstein	Reed-Reeves	P-value
Pain (VAS)		1.42±2.81	1.00±2.21	0.59±1.41	0.252
Satisfaction		96.96±11.22	91.42±26.45	89.65±20.61	0.156
Wound infection	Yes	5 (9.1%)	4 (8.0%)	0 (0.0%)	0.230
	No	51 (90.9%)	46 (92.0%)	40 (100.0%)	
Hematoma and seroma	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.999
	No	56 (90.9%)	50 (100%)	40 (100.0%)	
Testicular swelling	Yes	3 (6.1%)	2 (4.0%)	0 (0.0%)	0.421
	No	53 (93.9%)	48 (96.0%)	40 (100.0%)	
Recurrence	Yes	7 (12.1%)	5 (10.0%)	2 (5.0%)	0.356
	No	49 (87.9%)	45 (90.0%)	38 (95.0%)	

There was no significant difference in the distribution of recurrence in the groups (P=0.356). There was no significant difference in the distribution of testicular swelling in the groups (P=0.421). The mean of satisfaction in all patients was 93.61. 19.63. There was no significant difference between the three groups in the mean of satisfaction (P = 0.156) (Tables 3).

DISCUSSION

This research assessed the complications of Surgical Repair in inguinal hernia with Bassini, Lichtenstein, and Preperitoneal methods. Among different surgical methods for the repair of inguinal hernia, the rate of recurrence and complications in the three methods was low. Inguinal hernia is a common disorder in the field of general surgery. The results showed that infection, pain, testicular swelling, and recurrence rate were lower in the Reed-Reeves group than the other groups, although this difference was not significant. Also, there was no significant difference between the three groups in the mean of satisfaction.

Muldoon et al. (2004) conducted a clinical trial on patients with an inguinal hernia by Liechtenstein and Reed-Reeves methods. The average recurrence in the Liechtenstein method was 4.3% and in the Reed-Reeves method less than 1%, but this rate was not statistically significant [16]. The results of this study are in agreement with the results of the present study.

Akhavan Moghadam et al. (2011) in Iran compared the results between Liechtenstein and Reed-Reeves surgery in unilateral inguinal hernia repair. Postoperative pain and return to work time were significantly shorter in the Reed-Reeves group. The duration of surgery and postoperative recurrence were similar in both groups, but the rate of postoperative infection was higher in the Liechtenstein group [17]. The results of this study in terms of recurrence rate and wound infection rate confirmed the results of the present study.

In the study by Van Nieuwenhove et al. (2007), Which was performed on patients with inguinal hernia using the preperitoneal and Liechtenstein methods, the mean operation time in the preperitoneal method was 20±9 minutes and in the Liechtenstein method was 35±4 [18]. In another study by Nienhuijs et al. (2007), Compared Liechtenstein and pre-peritoneal methods, 41 and 54 minutes were reported for the preperitoneal and Liechtenstein methods, respectively. The recurrence rate was 1.1% in the Liechtenstein group and 2.3% in the peritoneal group [19]. In the present study, the mean duration of surgery in all patients was 28.49 ± 75.97 minutes and there was no significant difference between the three groups in the mean duration of surgery.

Asgari et al. (2013) conducted a study on patients with an inguinal hernia by Liechtenstein and preperitoneal methods. They found that the preperitoneal method was a shorter and more significant incision length and operation time than the Liechtenstein technique. They concluded that The peritoneal technique can be a suitable and safe alternative to the conventional Liechtenstein method [20].

A study by Ghoseiri et al. (2014) in a study On 240 inguinal hernia patients and the reconstructive method of Liechtenstein and preperitoneal showed that preperitoneal surgery is a suitable alternative to conventional Liechtenstein [21].

Jayesh Gohe et al. (2016) on 80 patients with an inguinal hernia in India examined Bassine, Liechtenstein, peritoneal, and the Shouldice method in terms of time and recurrence after surgery. The recurrence rate was reported at around 2.5% with no difference between the methods [22].

A study conducted by Gatabi et al. (2018) compared long-term complications of the two methods of Basini and Liechtenstein on patients with an inguinal hernia. They found that the results of the two methods Basini and Liechtenstein are very similar It is similar, so both methods can be used as a safe method for patients [23].

Mohebbi et al. (2009) compared the quality of life and complications after preperitoneal anterior approach (Rives), herniorrhaphy, and Lichtenstein methods of unilateral inguinal hernia surgery. They reported that herniorrhaphy had a lower quality of life and more complications than Liechtenstein and Reeves methods [24]. In the present study, it was shown that postoperative complications and pain in Liechtenstein and Reed-Reeves methods are less than Bassini surgery.

CONCLUSION

The results showed that infection, hematoma, pain, testicular swelling, and recurrence rate in the Preperitoneal group were lower than the other groups, although this difference was not significant. Satisfaction was similar between the three groups.

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ORIGINAL ARTICLE

Prevalence of rheumatic disorders in Iranian chemical injured patients: a WHO–ILAR COPCORD study

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Abstract: *Background: Rheumatic disorders are one of the most prevalent human diseases which commonly become chronic and cause different types of disabilities. Chemical veterans may suffer from chronic difficulties caused by rheumatic disorders that can lead to decrease of their quality of life. The main purpose of this study was to evaluate the prevalence of rheumatic disorders among Iranian chemical warfare patient's long-years after sulfur mustard (SM) exposure.*

Methods: This cross-sectional study was conducted from 2013 to 2014 on 245 Iranian chemical veterans. In order to evaluate rheumatic disorders, a special questionnaire for COPCORD studies were filled by veterans and then general and special rheumatology examinations were applied under the supervision of internists and rheumatologists. Data from all questionnaires were analyzed by SPSS software and the results were reported as percentage or mean ±SD.

Results: Osteoarthritis (69.9%) was the most common complications diagnosed in these patients. After that back pain (30.5%) and pre-arthritis (27.2) were the other popular complications among veterans. The percentage of frozen shoulder and moderate carpal tunnel syndrome was 13.2% and 11.5%, respectively. Tennis elbow and fibromyalgia (3.7%), rheumatoid arthritis (0.8%) and gouty arthritis with 0.4% were the lowest complications among these patients.

Conclusion: Rheumatic associated complications in Iranian chemical injured veterans are higher than the mean of whole community. This data provided the possibility of calculating the disease's burden and making efficient decisions in the sanitary and treatment systems.

Keywords: *chemical veterans, rheumatic disease, prevalence, COPCORD*

INTRODUCTION

Rheumatic disorders are among the most prevalent human diseases. One of seven referring patients to physicians suffer from rheumatic diseases [1]. Several factors such as bacterial infections, life style, and environmental factors can be correlated to rheumatic disorders [2, 3]. This disease can cause different types of important disabilities which in turn adversely influence the quality of life, physical and social activities in affected individuals [4]. It was estimated that ~40% of all chronic diseases and ~54% of long term disabilities are caused by rheumatic diseases [5]. For instance, Disability Adjusted Life Year (DALY) in cases of osteoarthritis was increased from 13275 patients in 1990 to 14230 patients in 2000 [6]. These patients are significantly susceptible to other disorders [7] and diseases like hepatitis B [8].

Back into the 7th decade, the manuscript of the community oriented program for the control of rheumatic diseases (COPCORD) was provided in the non-communicable disease section of WHO [9]. This manuscript describing a model of consumable agenda in rural and urban areas of developed and developing countries, was evaluated in the first meeting of the ILAR and WHO in January 1981 in Genève and formed as COPCORD [10].

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COPCORD is one of the most comprehensive epidemiologic studies worldwide. The first stage of the agenda was approached in Iran which was applied by the Rheumatology Research Center of Tehran University of Medical Sciences on the adult population of Tehran's urban areas via cluster sampling method. It was also followed by other studies from other areas of the country in the recent years [11].

A joint study by the Iranian and Bangladeshi researchers with the objective of providing and improving a questionnaire consisting of identification of knee osteoarthritis risk factors in developing countries, reported a pilot survey in the phase III of the COPCORD questionnaire. They suggested that these questionnaires must be validated for identifying knee osteoarthritis among Asian and Oceania populations [12].

Mustard gas (Sulfur mustard) is one of the warfare agents which has been utilized during chemical wars and used by Iraq against Iran during 1980-1988. During Iraq-Iran war, SM injured more 100,000 veterans that many of whom are still suffering from its late effects. Chronic infection, purulent bronchitis, spread tightened trends, bronchial hyper activity, severe asthma, bronchitis obliteran, psychophysiological asthma and vascular-pulmonary problems are the most prevalent implications caused by sulfur mustard exposure in veterans [15, 16]. Chemical veterans may suffer from some chronic difficulties such as rheumatic disorders which can lead to decrease of their quality of life, their working abilities, social relationships and physical activities. However, there is little information about the prevalence of rheumatic disorders among chemical veterans.

Since nutrition, medicine, economic, activity status and lifestyle of the chemical veterans are different from other communities, the frequency of rheumatic diseases among this population may be different.

Therefore, the aim of this study is to evaluate the prevalence of rheumatic diseases among chemical veterans exposed to SM during the imposed war of Iraq against Iran using a community oriented program for the control of rheumatic diseases.

METHODS

This cross-sectional survey was conducted on the 245 Iranian chemical veterans from Tehran and Karaj cities in 2013 to 2015. The veterans who fulfilled the following criteria were included: (1) having a documented history of sulfur mustard exposure (confirmed by related authorities), (2) male gender, (3) being over 40 years old, (3) moderate and negligible involvement of lungs and (4) giving a consent letter for participating in the study.

Sampling in Tehran city was accomplished by introducing the initial list of certain chemical veterans from the hospitalization sections of Baqiyatallah Hospital, lung clinic and the medical commission of the Baqiyatallah University of Medical Sciences. Due to the restrictions of veterans visiting, only the information of 97 chemical veterans from Tehran was obtained successfully.

For sampling in Karaj city, a list of 1050 chemical veterans was prepared by the coordination of the Alborz province Veterans and the Martyrs Affair Foundation (VMAF) primarily. From the initial list, 148 subjects who fulfilled the inclusion criteria were invited to participate in the study. In order to evaluate rheumatic diseases, special questionnaire for COPCORD studies were filled by veterans and general and special rheumatology examinations were applied under the supervision of internists and rheumatologists.

All data from 245 filled questionnaires were analyzed by SPSS software and the results were reported as percentage or mean ±SD. The COPCORD questionnaire used in this study was provided by the Rheumatology Research Center at Baqiyatallah University of Medical Sciences.

RESULTS

Among the 245 studied veterans, 97 subjects were resident in Tehran and 148 patients lived in Alborz Provinces. The mean of age and BMI of all patients was 51.1 years and 26.7 kg/m^2, respectively. Other demographic data such as smoking habitat, race and marriage status are presented in Table 1.

After examinations by specialists and assessing medical histories, diseases which were responsible for the mentioned implications were diagnosed. Figure 1 shows the percentage of complaints associated with rheumatoid arthritis in SM-exposed veterans. Osteoarthritis (69.9%) was the most common complications diagnosed in these patients.

After that back pain (30.5%) and pre-arthritis (27.2) were the other popular complications among veterans. The percentage of frozen shoulder and moderate carpal tunnel syndrome was 13.2% and 11.5%, respectively. Tennis elbow and fibromyalgia (3.7%), rheumatoid arthritis (0.8%) and gouty arthritis with 0.4% were the lowest complications among these patients.

The frequency of rheumatic disease associated complications for each age group and the whole studied population can be seen in Tables 2, 3 and 4.

Table 1: The frequency distribution of marriage status, race, occupation status and smocking background in veterans

Variables				Frequency	Percentage
Marriage status	Single			0.4	1
	Married			98.8	237
	Spouse dead			0.4	1
	Separated or divorced			0.4	1
Race	Fars			62.1	149
	Azeri			27.9	67
	Kurd			4.6	11
	Lor			2.9	7
	Other			2.5	6
Occupational status	Employee			34.1	76
	Worker			2.7	6
	Retired			34.1	76
	Self-employed			22	49
	Unemployed			6.7	15
	Disabled			0.4	1
Smoking		**Average of time (years)**	**Daily average use**		
	Never	--	--	75.4	178
	I smocked	19.9	10.9	9.7	23
	I used to	6.8	12.7	14.8	35

Table 2: The relative frequency of pre-arthritis (at least one type) based on different age groups

Age Group (Years)	COPCORD Males Tehran	Veterans	
	%	%	Frequency
40-49	4.19	23.7	28
50-59	7.21	31.7	33
Over 60	10.78	23.8	5
Total	---	27.2	66

Table 3: The relative frequency of moderate carpal tunnel syndrome based on different age groups

Age Group (Years)	COPCORD Males Tehran	Veterans	
	%	%	Frequency
40-49	0.69	11.7	14
50-59	0.69	12.5	13
Over 60	0.28	4.8	1
Total	---	11.5	28

Table 4: The relative frequency of rheumatoid arthritis based on different age groups

Age Group (Years)	COPCORD Males Tehran	Veterans	
	%	%	Frequency
40-49	0.25	0.8	1
50-59	0	0	0
Over 60	0	4.8	1
Total	---	0.8	2

The percentage of pre-arthritis in veterans with 51-59 years-old was higher than that in other age groups (Table 2). Veterans over 60 year-olds showed higher percentage of rheumatoid arthritis (4.8%) and moderate carpal tunnel syndrome (4.8%) compared to the other age groups (Table 3 and Table 4).

DISCUSSION

With the objective of identification, prevention and controlling rheumatoid diseases in the developing countries, WHO and ILAR coordinated in 1981 in order to organize COPCORD [9]. This program has been performed in many countries such as Australia, Bangladesh, Brazil, Chili, China, Cuba, Indonesia, India, Kuwait, Malaysia, Mexico, Pakistan, Egypt, Guatemala, Lebanon, Tunisia, Philippines, Tailed, Vietnam, as well as Iran [9, 12, 17-30].

Figure 1: The frequency of diagnosed joint diseases in chemical veterans

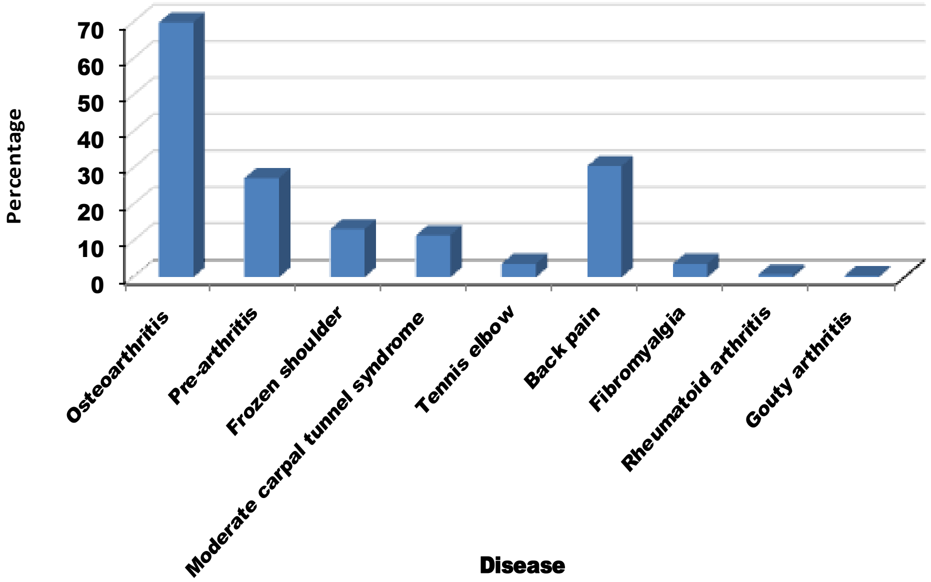

Degenerative joint diseases are among the most prevalent rheumatoid disorders in human populations worldwide [31]. Studies indicating the relationship between age and knee osteoarthritis have demonstrated that along with aging, some levels of degenerative changing occur in joints such as knee joints [32]. In spite of several studies which emphasized rheumatoid disorders in Iran, our study is the first survey in which the percentage of rheumatoid disorders was evaluated in Iranian chemical veterans. Our data have revealed that osteoarthritis and pre-arthritis with the prevalence of 69.9% and 27.2% were the most prevalent among the studied population, respectively. The results of a study conducted in Tehran confirmed these results [11]. The results of a study on rural areas of Iran revealed that about 20% of patients suffered from osteoarthritis in at least one joint. Also, the COPCORD results of 17 countries indicated that with the prevalence of 16.9%, Iran stands in the second level of osteoarthritis prevalence [33].

Our results showed that the prevalence of pre-arthritis or soft tissue rheumatoid among veterans was remarkably higher than the general population of Iran. Similarly, previous studies in Iran showed that the prevalence of pre-arthritis in Sanandaj, Tehran and for the whole country was 5.5%, 4.62% and 4.6%, respectively [11, 24, 34].

The prevalence of rheumatoid arthritis in our study was 0.8%, which was close to the results reported previously in the southeast areas of Iran (0.98%) [35]. Meanwhile, the prevalence of rheumatoid arthritis among veterans was higher than the population of Tehran (0.33%) and Sanandaj (0.51%) [11, 24]. The comparison of the following results with the general population of other countries revealed a higher prevalence of the mentioned diseases among veterans.

The prevalence of gouty arthritis in this study was 0.4%, while it was previously reported as 0.13% in Tehran and 0.12% for Sanandaj [24, 36]. Dawatchi et al. indicated that Iran is ranked 11th among 17 countries in regards to the prevalence of this disease [34]. The other statistics revealed that Indonesia (1.6%) and Australia (1.5%) had the highest frequency for this disease [34].

CONCLUSIONS

This study evaluated the prevalence of rheumatic complications and disorders among Iranian chemical veterans community. Our results have demonstrated that total rheumatic complaints and disorders among this population are higher than the mean of the whole community. These data provided the possibility of calculating the disease's burden and making efficient decisions in the sanitary and treatment systems. Due to the restrictions and special treatment needs of this community, approaching periodic screening tests, designing beneficial nutrition, athletic and treatment interventions can improve and meliorate rheumatic diseases among chemical veterans. Therefore, this study has performed the initiating steps in the case of identifying mentioned complications among chemical veterans and following steps must be taken to investigate the preventing applications of rheumatoid disorders among this population. Also, medicine prescription as well as the intervention of the prescribed drugs must be evaluated through a widespread study.

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ORIGINAL ARTICLE

The relationship between intestinal dysbiosis and multiple sclerosis

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Abstract: *Multiple sclerosis is a chronic autoimmune disease, characterized by the appearance of demyelinating lesions with inflammatory character in the CNS. Given the unpredictable evolution of the disease, numerous attempts have been initiated to find some pathogenic factors and by default targeted treatment methods. The intestinal microbiota of these patients is often significantly altered compared to the healthy population and given the complex connections between the intestine and the central nervous system, the idea of causality between dysbiosis and this condition was launched. The intestinal mucosa represents the interface between the external environment and the organism, so that it is considered the starting point of some local immunological processes but with systemic resonance, especially neurological.*

The classic medication scheme widely used to treat MS nowadays can also affect the integrity of the intestinal mucosa by breaking the fine balance at this level. Data from recent studies around the subject open new treatment opportunities around preserving the intestinal microbiota and strategies like vitamin A intake and fat-soluble vitamin D supplementation, probiotic complexes or faecalis microbiota transplantation appear more promising and easier to apply in daily clinical practice.

Keywords: *intestinal microbiome; gut-brain axis; multiple sclerosis; disease-modifying therapies (DMTs), neuroinflammation*

INTRODUCTION

Multiple sclerosis (MS) is a chronic disease with an autoimmune substrate, characterized by the appearance of acute demyelinating lesions with inflammatory character in the CNS, is the main neurological illness with onset among young. MS affects about 2 million people, and the number is constantly growing, with a 3-fold higher prevalence among women. The most common form of the disease, often the onset one, is relapsing remitting (RRMS), characterized by full recovery of the deficit after an acute episode of the disease and may sometimes pass, after years of evolution, in secondary progressive form, with a progressive, irreversible accumulation of deficit (30-40%) [1-5].

Despite efforts in recent decades to find a certain pathogenic factors, the research has been in vain, concluding that the evolution of the disease involves both genetic and environmental factors, such as geographical conditions, obesity, diet, smoking, infections with viruses such as Epstein-Barr, vitamin D deficiency and finally, the intestinal microbiota. The last one that recently attracted attention, is considered the biggest risk factor in triggering multiple neurological diseases such as Parkinson's, Alzheimer's disease, amyotrophic lateral sclerosis and optic neuromyelitis [2, 6].

The gastrointestinal tract is populated by an invaluable collection of microorganisms, 3.8 × 1013 bacteria, 1014

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different species, building up the microbiota, in perfect harmony with the host organism. Considered an organ itself, the microbiota fulfils numerous roles, not only locally, but also systemically: it helps to ensure the entirety of the intestinal microbiome and the generation of antimicrobial substances, supports motility, prevents colonization with pathogens, ensures essential bowel functions, absorption and secretion, provides metabolic support by producing vitamins such as vitamin K, folate, vitamin B complex, bone reabsorption, peptides, DNA synthesis/repair and, more recently, the maturation of the immune system [2, 6, 7].

THE GUT/CNS AXIS

About two centuries ago, connections were discovered between CNS and intestinal microorganisms, bidirectional links with other systems, such as immune, endocrine, or nervous; they are recognized today in the form of Gut-CNS axis. As mentioned earlier, the microbiota has a secretory role, being able to synthesize, among many others, cytokines and neurotransmitters that act at a central level, such as GABA (gamma-aminobutyric acid), histamine, serotonin, or dopamine. Through neuroactive molecules, the microbiota stimulates the vagus nerve, modulates hypothalamic-pituitary-adrenal connection and catecholamine production. Also, SCFA (short-chain fatty acids), internally produced and released systemically, can adjust the activity of NK cells, macrophages, or dendritic cells. Given the strong links at this level, it is easy to understand why the alteration of the composition of the microbiota, called dysbiosis, leads to the onset of many diseases of the nervous system [1, 5, 7, 8].

ETIOPATHOGENIC MECHANISMS AND IMMUNOLOGICAL INTERACTIONS

In both MS and other autoimmune diseases, numerous studies have attempted to identify the connection between intestinal dysbiosis and exacerbated autoimmunity, concluding the existence of a mechanism of molecular mimicry, a detrimental interaction of the immune system with the intestinal microbiome that leads to the expansion of Th1/Th17 cells [9]. In addition, any minor infection of the gastrointestinal tract can lead to intestinal dysbiosis, which is common today, given that digestive infections are among the most common infections [10].

Butyric acid, the main SCFA produced by the intestinal microbiota, along with acetic and propionic acid, have immunomodulatory and anti-inflammatory properties, favoring the differentiation of Treg cells (Regulatory CD4 + cells), TH1 and TH17. These, released in the proximal colon following the fermentation of non-digestible carbohydrates and subsequently transferred to the peripheral blood, have been found in small quantities in the population suffering from multiple sclerosis [5, 11].

MAIT cells (Mucosal-associated invariant T) represent a subset of cells belonging to the innate immunity, found on the surface of the mucous membranes, but also in the blood and liver, whose dysfunction is found both in MS and in other diseases with an immunological substrate such as type 1 diabetes, Sjogren syndrome, rheumatoid arthritis or systemic lupus erythematosus; MAIT cells, through their ability to synthesize IL17, play an essential proinflammatory role in defending the body against bacteria, fungi or certain viruses. A recent study, led by Willing et al., Found the existence of a circulating inflammatory subset of CD8 + CD161high MAIT cells in the blood of patients with early onset of MS. In addition, in a study that aimed to track circulating MAIT cells as a biomarker in the evolution of MS, the same cells were found in excess in the CNS of patients known to have MS. It should be noted that a reduction in CD8 + CD161 + MAIT cells was observed after treatment with dimethyl fumarate [12].

IgA is an antibody found mainly on the mucosal surface, ensuring intestinal homeostasis. Recent studies suggest the involvement of IgA as a possible pathogen in several extraintestinal autoimmune diseases. Regarding MS, elevated IgA levels were found in both patients' blood and cerebrospinal fluid during periods of disease activity, compared to healthy controls [13].

Demyelinating plaques within the MS are the result of the neurodegeneration process the following infiltration with microglia, astrocytes, and leukocytes. Microglia, known as "brain macrophages", are directly involved in the myelination and maturation of synapses, phagocytose debris and secrete neurotrophic factors. Astrocytes, extremely numerous in the nervous tissue, control the function of synapses, regulate blood flow through the blood-brain barrier (BBB) and maintain the pro and anti-inflammatory process. The activity of these cells and the interaction between them are controlled by the microbiota, so that any change leads to compromising this fine balance [1].

The small intestine represents an interface between the internal and external environment, a surface for the development of interactions between various cells so that its integrity is essential for the proper functioning of the whole organism. Recent studies have noted the existence of a connection between the exaggerated permeability of the small intestine and MS, by affecting the BBB through receptors for Zonulin. Used as a marker of intestinal permeability, Zonulin is a protein that ensures the integrity of epithelial junctions, strengthening the immune tolerance of the intestine. According to the "leaky gut" theory, intestinal dysbiosis causes alteration of the composition of the mucus in the small intestine, but also to enterocyte apoptosis and altered cell junctions at this level, followed by bacterial infiltration of the lamina propria, being considered the immune protection barrier of the intestinal wall. Consequently, lymphocytes will be attracted to these areas, with the induction of an inflammatory status that will maintain lymphocyte production and the secretion of cytokines (IL17, tumor necrosis factor) thus forming a vicious circle that will predispose the host body to aberrant autoimmune responses at the local level as well as at the systemic level [2, 5, 14]. Regarding MS, elevated levels of Zonulin were detected in the serum of patients with RR form, being correlated with BBB disruption, excessive activation of microglia and astrocytes with inflammatory neuro-status, a fact confirmed by the lesions observed on MRI images [2, 5].

IMPAIRMENT OF THE INTESTINAL MICROBIOTA IN MS

Detailed studies in recent years managed to describe the immunological status of patients with MS and reveal the microbiome alteration in these patients compared to the healthy control group [2]. Thus, a study conducted by Reynders et al., confirms the abundance of Faecalicoccus, Ruminococcus, Gemmiger, Alistipes and Anaerotroncus in the samples obtained from laboratory mice [15]. Other recent research suggests a low level of Faecalibacterium while Streptococcus thermophilus/Salivarius and Eggerthella lenta were significantly increased in faecal samples obtained directly from patients with this pathology [6, 16].

It seems that the basic medication in MS, disease-modifying therapies (DMTs), prescribed both for prophylactic purposes and for ameliorating the symptoms, would also act on the composition of the microbiota, raising the question of whether their therapeutic effect is exerted by also acting on the intestinal barrier [2, 14, 17].

Interferon-β (IFN- β). Interferon has been used in clinical practice for many years, proving effective in many other pathologies. Interferon-β is considered a major cytokine of the organism that participates in the innate immune response of the host because of bacterial or viral aggression, ensuring the integrity of the intestinal epithelial junctions. Between the microbiota and the intestinal barrier, there is bidirectional communication mediated by IFN-β, together forming a trio that ensures junctional integrity, stimulates the recruitment of Treg cells to the mucosa and increases local defence in the presence of an antigenic stimulus; Studies in this direction show an increase in the Provotella species, with a known probiotic role, among MS patients treated with Interferon-β similar to the healthy population, while untreated patients with the same pathology have a deficiency of probiotic species in the intestine [2,14,17,18].

Glatiramer acetate (GA). GA, a first line DMT, has anti-inflammatory properties, participating in modulating the immune response by reducing TNF alpha activity, increasing anti-inflammatory mediators (IL-10, TGF-b) and also recruiting Treg cells to the intestinal mucosa. The effectiveness of GA has also been demonstrated in laboratory models, improving colonic lesions in ulcerative colitis. Regarding patients with MS, GA induces changes of Faecalibacterium, Ruminococcus, Lactobacillaceae, Bacteroidaceae and Clostridium. Recent studies on laboratory animals show an improvement in the concentration of Prevotella in the gastrointestinal tract after the administration of GA, and the concomitant administration of this preparation with live Prevotella has led to an improvement in the symptoms of the disease [2, 14].

Dimethyl Fumarate (DMF). DMF, long used in the treatment of psoriasis and, more recently, as a first-line treatment in MS, exerts its immunomodulatory effects by inducing T cell apoptosis and inducing a Th2 cell response. In addition, DMF supports the integrity of the intestinal wall, decreases intestinal permeability by supporting epithelial junctions. In patients with MS, DMF appears to decrease Clostridia and Firmicutes and increase Bacteroidetes and other SCFA-producing species concomitantly. It should be noted that recent experimental studies show that the abundance of SCFA-producing microbes potentiates the anti-inflammatory effect of DMF at the systemic level [2, 14]. This observation naturally leads us to conclude the benefit of the simultaneous administration of this DMT with probiotics.

Teriflunomide. Teriflunomide is another DMT used as a first-line in MS, having an anti-inflammatory effect by reversibly inhibiting dihydroorotate dehydrogenase, significantly influencing the number of lymphocytes entering the central nervous system, acting in the intestine by inside stimulation of Tregs cells (CD39+); Notwithstanding the beneficial effects against MS, in laboratory studies on porcine intestinal cells, this drug has been shown to decrease the ability of epithelial cells to defend against infections [2, 14].

Natalizumab (NTZ). The Natalizumab, a second line DMT, is a monoclonal antibody directed against integrins, thus limiting the number of T cells entering the CNS, suppressing immune activity in areas affected by MS. Given the non-selective activity, NTZ acts not only on the T lymphocytes in the CNS but also on those in the intestine, proving effective in inflammatory bowel disease (IBD). Thus, NTZ has been proposed for patients with highly active RRMS, being even preferred in those patients who also suffer from IBD. In the context of existing intestinal dysbiosis in MS, in the case of NTZ treatment, the aberrant exposure of the local immune system to antigenic stimuli will be ameliorated and this may be considered an additional mode of action of this drug [2, 14].

Fingolimod. Like NTZ, Fingolimod, a second line DMT used in RRMS, acts as a sphingosine 1-phosphate receptor (S1P) antagonist, a receptor needed for the maturation and release of T and B lymphocytes from the lymph nodes into the peripheral blood, improving the systemic immune response [14]. By the same mechanism, it is experimentally effective in relieving ulcerative colitis, and many studies are underway in this direction. Regarding the intestinal microbiome, it appears that Fingolimod regulates the maturation of IgA blasts in the intestinal Peyer's patches and acts as the first line of defence against local microbial colonization. The same antimicrobial effect has been demonstrated in vitro, inhibiting the growth of Clostridium and other associated endotoxins [2].

Alemtuzumab. Initially used for treating various forms of leukaemia and newly approved in the therapeutic scheme of RRMS, Alemtuzumab is a monoclonal antibody focused against CD-52 that induces the depletion of B and T lymphocytes in peripheral blood. Despite its proven effectiveness, used for a long time, it alters the repopulation of peripheral blood with immune cells and predisposes to the development of various autoimmune diseases such as membranous glomerulonephritis, autoimmune thrombocytopenic purpura, thyroid disease, autoimmune hepatitis, etc. It should be noted that following the administration of Alemtuzumab, one patient developed, due to Clostridium, fatal colitis, and another patient presented pancolitis after the first dose of treatment. Subsequent studies in laboratory animals show the harmful effects of Alemtuzumab on the gastrointestinal tract by altering the microbiota, affecting the integrity of the epithelial barrier and the consequent increase in intestinal permeability. These findings make us wonder if the profound effects on intestinal integrity balance the benefit of fighting MS [2, 14].

DIET AND ITS CONNECTION TO THE MICROBIOTA AND MS

Diet is considered an important trigger in the development of multiple diseases and may even influence the intestinal microbiota and implicitly, multiple sclerosis. Given the developed network of connections between diet and this disease, it is important to understand the mechanisms of action of many food triggers to treat the causes or find new therapeutic options for the future.

The Swank Diet. The Swank Diet is a low-fat diet that arose from Dr Swank's observations that the occurrence of MS can be correlated with increased fat intake. The data collected from the study concluded that patients who consume a higher percentage of fat also have a higher degree of disability. However, this study presents its limitations, the data is reported directly by the patients, questioning their accuracy [19].

Ketogenic diet. This diet, which is based on high-fat-low-carbohydrate principles, has been shown to improve Th1 and Th17 cells by promoting Treg cell proliferation. Studies based on the ketogenic diet have improved the quality of life of these patients, depression and fatigue for a period of 3 to 6 months, being a pragmatic starting point for future research [20-24].

Vitamin D. Recent epidemiological studies have found a higher percentage of MS patients in areas far from the equator, suggesting that vitamin D may have a protective role against this disease. Data from in vivo studies show the ability of this vitamin to limit experimental autoimmune encephalomyelitis (EAE), the most common preclinical model of MS in mice. In vitro, the data suggest that vitamin D reduces lymphocyte proliferation and antibody production induced by antigenic stimulation, induces the suppression of pathological Th1 cells, and increases the protective activity of Tregs. However, laboratory mice that do not have a vitamin D receptor or have been on a vitamin D-poor diet appear to experience milder forms of EAE [25-28]. These conflicting results underestimate the importance of vitamin D in the immune response, although it is the most studied dietary risk factor in MS. However, several studies show that vitamin D supplementation during pregnancy reduces the risk of developing MS among newborns [8,20,29,30].

Vitamin A. Vitamin A can modulate the immune response according to its local concentration and specially to reduce neuroinflammation. Thus, it has been observed that 13-cis-retinoic acid, a precursor of vitamin A, decreases the production of IL-2 and limits leukocyte proliferation, improving the evolution of EAE [31-33]. Retinoids are already used in clinical practice to treat acne or psoriasis, and although the usefulness of vitamin A in the treatment of MS is not yet clear, the data obtained so far suggest that patients may benefit from the immunomodulatory effects of supplementation with this vitamin [19].

Excess salt. The excess salt characteristic of the Western diet but also the significantly increased percentage of MS patients in the Western world have led to the incrimination of this compound as a possible etiological factor. Studies show that a hypertonic environment due to excess salt and consequent osmotic stress causes inflammatory status due to increased cytokine production [34, 35]. This fact is also supported by the evidence obtained in the laboratory; following the administration of an excess of salt, it was noticed the increase of the production of Th17 cells and the aggravation of EAE. However, other studies following the evolution of patients with the clinically isolated syndrome (CIS) have not found causal connections between salt and the subsequent development of MS so extensive research is needed to clarify things [2, 20].

FUTURE DIRECTIONS

Probiotics. Several studies have analyzed the therapeutic potential of probiotic administration, even supporting their effectiveness by decreasing the peripheral inflammatory response in MS, the most widely used belonging to the genera Lactobacillus and Bifidobacterium [1, 36-38]. Moreover, a single study has shown an improvement in the EDSS score among patients treated with probiotics, but the results of more detailed studies, both clinically and imaging, are expected [20].

Faecal microbiota transplantation (FMT). Initially used to treat Clostridium difficile infections, FMT appears to be a possible innovative treatment for patients with epilepsy, Parkinson's disease and MS, according to recent research. A study by K. Li et al. proved that, in the case of mice with EAE, FMT succeeded the improvement of the symptoms, the improvement of the clinical scores and slowing down of the disease progression [39]. The mechanisms underlying these results are the maintenance of BBB integrity, protection of axons and myelin sheaths, and reduction of microglia and astrocyte activation, proven in FMT-treated EAE to be lower than those treated with a simple saline solution [4]. Currently, studies are underway to obtain the safety of FMT use, which is considered the most direct way to restore the intestinal microbiota [1, 8, 21].

Vitamin D. Elevated vitamin D levels are inversely associated with the number of new lesions on MRI investigations, with high doses (between 10,000-40,000 IU/day) being effective in reducing proinflammatory cytokines and suppressing T lymphocytes in the serum of MS patients. In addition, vitamin D ensures the integrity of intestinal epithelial junctions, ensures optimal intestinal permeability, and reduces local cellular apoptosis [8, 14, 19].

Oral antibiotics. Oral antibiotics are useful in ameliorating the symptoms of mice with EAE by modulating the composition of the intestinal microbiome [40-42]. However, to avoid the exacerbation of dysbiosis in the evolution of MS, detailed studies are needed to discover the optimal preparations and doses to be applied in these cases [8].

CONCLUSIONS

The intestinal barrier plays an extremely important role in the development of various diseases, being the interface between the external environment and the body and a starting point for complex immune interactions that maintain communication with the CNS. Correction of the intestinal microbiota seems a promising option as a future treatment direction, requiring multidirectional actions to restore balance at this level in patients with MS. Thus, the administration of probiotics, vitamins, dietary measures or FMT are viable options to reduce neuroinflammation and alleviate the disease, but studies are still needed to build the answers.

Authors' contribution

All the authors substantially contributed to each of the following aspects of this review paper: conception and design of the study (mainly CAS, RMD, TMV and CS), literature research and analysis and interpretation of the relevant data (mainly CAS, AER, AMS and ADA). In addition, all authors were involved in the drafting of the manuscript (mainly CAS, RMD, TVM, CS, AMS and ADA) and revised it critically for important intellectual content (mainly RMD, TMV, CS and AMS). All authors have read and approved the final version of the manuscript. The authors have participated sufficiently in the study, take public responsibility for appropriate portions of the content, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest:

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Content, hierarchy, intensity of motives and their possibility to be implemented in servicemen with various levels of effectiveness of service activity

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Abstract: *In accordance with the results of conducted research there were figured out the content, hierarchy, intensity of motives and their possibility to be implemented in servicemen with various levels of effectiveness of service activity. 2435 male servicemen participated in the research, they equally presented all the operational and territorial military unifications and units of the National Guard of Ukraine. Each unit proportionately presented officers as well as military personnel under contract who included the participants of hostilities and those who did not have such experience. Examination of various aspects of servicemen motivation was conducted with the help of the following the methods which were standardized being based on the Ukrainian selection "Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs", "Questionnaire of Estimation of the Effectiveness of Military Tasks' Execution by Servicemen of the National Guard of Ukraine", questionnaire on "Sense-Bearing and Life Orientations", the methods of "Studying the Motivational Profile of Personality", and "Locus of Control". There was conducted the cluster analysis which gave the possibility to figure out six motivational types connected with the effectiveness of servicemen operational activity. Motivational types were located between the poles which were introduced by two dimensions: self-efficacy (autonomous and controlled motivation) and motivation of helping others (motivations of public service - selfishness). Those motivational types of servicemen were the following: motivated by public service, stagnant, prosocial, romantic, deficient, and dependent. Distinguished types included the content, hierarchy, intensity of motives and their possibility to be implemented.*

Keywords: *motive, motivational sphere, effectiveness of service, servicemen, public service*

INTRODUCTION

Motivation as a factor of professional as well as personal development was significant but was not completely discussed aspect of a complicated issue of professiographical examination of servicemen operational activity; though, thenecessity of examining the proposed topic was mostly caused by a need of practice (Prykhodko I., Matsehora J., Lipatov I., Tovma I., Kostikova I., 2019). Experience of using military units and formations of the Armed Forces of Ukraine (AFU) and of the National Guard of Ukraine (NGU) during combat operations in Ukraine since 2014 strongly convinced that with a considerable degree successful conduction of combat and operational tasks depended on the level of personnel's motivation. Its independent level during operations devaluated any advantages of military formations in logistics and professional development; moreover, it provoked combat stress (Prykhodko I., 2018). Before the combat conflict on the East of Ukraine daily service of servicemen faced difficult times including low level of logistics and salary, absence of prospects to receive their private accommodation, and plenty of other social and

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domestic problems which brought the situation to the line where servicemen made inappropriate choice (Prykhodko et alia, 2019). Insufficient level of motivation occurred in negative facts of refusal of some parts of the Ukrainian military formations' personnel to continue the service on the territory of the Autonomous Republic of Crimea, of Donetsk and Luhansk regions at the beginning of 2014 as well as in adequate efficiency of units' operation which were manned by mobilized servicemen, in significant quantity of suicides, crimes, and violations of military discipline among servicemen at the Anti-Terrorist Operation zone (further Joint Forces Operations) (Prykhodko et alia, 2019).

Besides the fact that there were many scientific researches of peculiarities of personal motivation concerning professional activity under extreme conditions, namely: combat operations constantly made adjustments to components of its diagnostics and formation. Especially that issue was related to the sphere of professional activity of the Ukrainian servicemen. Military service included participation in combat operations, execution of operational tasks connected with vital risks, combat trainings, and daily activities (Kolesnichenko, 2018). Motivation concerning the military activity included a range of various social and psychological conditions, and determined the complex of positive as well as negative aspects of military service (Kapliuchenko, Prykhodko, 2019). Motivation included the reveal and actualization of inner resources of servicemen and creation of positive motivation in combination with external conditions (Prykhodko et alia, 2019). Its specifics depended on personal peculiarities as well as on professional activity.

The vast majority of modern foreign researches on individual motivation concerning professional activity, namely, under extreme conditions, was focused on the paradigm of the theory of self-determination. In 1985 this paradigm was proposed by Deci and Ryan (1985) after that it was accepted as a reliable empiric theory. According to its core postulates human motivation was divided into external and internal, internal included three congenital universal psychological needs: competency (need of people to show their abilities), autonomy (right of choice to choose the profession), and interrelationship (need of feeling their own involvement into social groups and/or spheres of activity). This theory led to the conclusion that different regulating processes which, being the core of achieving goals, were correlated with the effective functioning and welfare. Different contents of goals were correlated with the quality of behaviour, mental health, and with the level of needs' satisfactionin different ways.

To prove the key notions of the theory of self-determination Gillet, Becker, Lafrenière, Huart, and Fouquereau (2017) analyzed interrelationship between motivating profiles of servicemen and civil workers. The results of their research showed that profiles were differently connected with accepted organizational support as well as with involvement into the activity of both selections includingcommunication and support from the side of management and with positive and negative effects in the second selection. Moreover, servicemen with the highest indicators of autonomous motivation presented the highest levels of organizational support acceptanceand involvement into activity. Nevertheless, the highestlevel of autonomous motivation was connected with the highest level of communication, support of commander, and positive influence.

Using the theory of self-determination as a theoretical basis, Chambel, Castanheira, Oliveira-Cruz, and Lopes (2015) created a model that included the servicemen's perception of organizational support on exchange between commanders and members of military staff, professional motivation and welfare. The results showed that motivation concerning autonomous activity was significantly connected with both contextual factors. Besides that, motivation concerning autonomous work was positively connected with its involvement into activity and was negatively connected with professional burnout while controlled motivation demonstrated the opposite reaction.

Foreign researchers (Griffith, 2008; Helmus et alia, 2018) to prove main aspects of the theory of self-determination distinguished institutional and pragmatic motivation as well as their combination. Internal motivation was included into the process of launching the activity because that was interesting and could self-satisfy despite the activities turned to the achievement of external goal. Though, Graffith (2008) using the analysis of motivation of the US National Guard servicemen included into the list of institutional type such internal motives as: patriotism, desire to test themselves, desire to be useful for country and society. Pragmatic motivation to serve was demonstrated through general attitude to army as if it was an employer who made delightful proposition.

In another research Helmus et alia (2018) from RAND center distinguished key motives of entering the US Army and revealed the specificity of army life reality. The results showed that the choice of future servicemen selection in the USA was influenced by two groups of motives which were interrelated with each other: institutional and pragmatic. The key motives to choose the profession of the US Army serviceman were "adventures and travelling", "advantages", "stability of the work and salary", "avoidance of negative surrounding", and "professional education".

Moreover, Helmus et alia (2018) figured out that on the contrary those servicemen who were motivated by pragmatic incentives tended to long-term military service in contrast with those who chose the service as a “call of their heart” being based on institutional motives. Thus, the most of candidates chose the military service in the US Army due to pragmatic (material) reasons, but for the majority of recruits the military service became the work and only after that – call of their hearts.

The research of Bury (2017) which was based onthe determination of motives of recruiting and retention in logistics’ divisions of the British Army reserve demonstrated that servicemen who served because of institutional motives were focused on longer service. Servicemen with pragmatic motivation were less satisfied with the conditions of their service and entered the military sphere to follow the contract only.

Woodruff (2017) had the same conclusion which pointed out that internal motives created stronger connections with army and military valuesthan external motives such as: salary, experience for future professional activity, finances for future education, etc.

The Norwegian experts Johansen, Laberg, and Martinussen (2014) proved that institutional and pragmatic motives could cohabit and a serviceman who was interested in salary, help and economic welfare was not obligatory a bad soldier. The authors pointed out that during the international mission beyond the borders of their own country it was hard to maintain the ideal images of military service; however, mainly the loyalty to traditional military ideals were the best motives of serving and factors of effective military organization.

Grigorov and Spirdonov (2018) confirmed the theses that there was institutional and pragmatic motivation. They conducted the research to distinguish core motives of choosing the military profession in the Republic of Bulgaria and suggested that the dominant motivational structures in the process of choosing the profession was the coexistence of leading factors which were connected with pragmatic motivation in comparison with internal factors which related to one definite work, independence, achievement, and recognition.

According to Taiyang (2010) the system of motivation to serve in the Chinese Army was determined with the factors of tradition and culture: national flag as a symbol of honour, courage, role of model caused the involvement into nobility and caring about other people; non-material motivated stimulation.

Analyzing the motivation of the Brazilian servicemen, Rodrigues-Goulart (2006) highlighted that it always had crucial importance for servicemen who executed tasks under combat (extremal) conditions which posed risks for their life and health. According to the researcherfor many situations motivation was a core to succeed in battle. Rodrigues-Goulart distinguished the following motives of military profession: sense of obligation, belonging to military sphere, responsibility, sacrificing, need of fate, desire of adventures, leadership, working abilities, tendency to recognition and awards, ideas of legitimacy of war, hope and victory, hatred of enemies, and need of self-preservation.

In accordance with Catignani (2004) “strong spirit” of military division was equal to high combat motivation. It was impossible to overestimate the importance of combat motivation to succeed in execution of military operations such as patrolling as well as full-scale war. Combat motivation was key factor that gave possibility to ordinary army to succeed in conflicts; talking about Israel it was called “secret weapon” of the army. Catignani (2004) pointed out that there was “a tough connection between cohesion, motivational level of servicemen and combat effectiveness”.

Analysis of modern scientific works indicated unbelievable urgency of the problem of servicemen motivation to act under extreme conditions in different countries all around the world. Systematization of analyzing the peculiarities of professional motivation of servicemen gave the possibility to conclude that motivation was one of the most significant factors for military division to achieve the posed goals in many situations which occurred in the process of executing combat operations under extreme conditions.

The aim of the research was to determine the content, hierarchy, intensity of motives and their possibility to implement them in servicemen with various levels of effectiveness of service activity.

MATERIALS AND METHODS

Respondents

The research was conducted from 2019 to 2021. There participated 2435 servicemen of the NGU who equally presented all the operational and territorial divisions and military units of the NGU. Each unit equally presented their officers – 29.84% (from Second Lieutenant to Colonel) and servicemen under contract – 70.16% (from Crewman to Warrant Officer), participants of hostilities and those who did not have such experience. The age of the respondents was from 20 to 55. All procedures carried out in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants gave consent for their data to be used in this research.

Instruments

To determine various aspects of servicemen motivation there were used the methods which were standardized being based on the Ukrainian selection.

1. “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” by Moskalienko (2002). This questionnaire was developed by the Ukrainian researcher for professional psychological selection for future policemen education, it gave possibility to evaluate: “Motives Related to Objective Socially Posed Tasks and to Content of Profession”, “Motives Related to Personal and Professional Development”, “Independent Profession Selection”, “Dependent Profession Selection”, “Motives Related to External Prestigious Professions and Financial Welfare”, “Motives Related to Romanticization of Profession”, “Motives Related to Eagerness of Compensation of Temper Flaws”, “Antisocial Motives (attitude to profession was as if it was a way to satisfy own antisocial needs)”. The instruction of this method proposed to answer the question concerning the reason of choosing this profession where the respondents using a scale made of 5 points could estimate 40 statements which described the profession of law enforcements.

2. “Methods of Studying the Motivational Profile of Personality”by Ritchie and Martin (1999). The use of this method gave the possibility to determine the urgent motivation of professional activity. It had the following scales: “Need of High Salary and Financial Benefits”, “Need of High Working Conditions and Comfortable Surrounding”, “Need of Accurate Structurization of Work and Feedback”, “Need of Social Relations”, “Need of Forming and Supporting Long-Term Stable Relations”, “Need of Obtaining the Recognition by Other People”, “Need of Posing Brave and Tough Goals and of Achieving Them”, “Need of Interesting and Socially Beneficial Work”, “Need of Self-Improvement and Development as Personality”, “Need of Being Creative, Thoughtful, and Open for further Ideas Employee”, “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”, “Need of Manipulation and Power, Eagerness to Control the Other People”. This questionnaire included 33 questions with 4 variants of answers where the respondents had to replace 11 points in accordance with the importance of the answers.

3. Method of “Will-Regulated Personality” by Hutkin and Mikhalchenko (2000) gave the possibility to determine general indicator which characterized willed organization and willed peculiarities of personality: organization of will and sense, organization of activity, determination, perseverance, self-control, and self-reliance. This method included 56 questions with 4 variants of answers.

4. Method of “Locus of Control” in modification proposed by Ksienofontova (1999) which gave the possibility to analyze internal orientation of serviceman personality, his/her ability to recognize and take responsibility for his/her decisions and actions. This method included 40 statements which respondent could agree or disagree with. This questionnaire determined 17 options of internality: general internality, internality in thoughts about life in general, internality in description of personal experience, internality in the sphere of achievements, internality in the sphere of fails, tendency to self-incrimination, internality in the sphere of professional activity, professional and social aspect of internality, professional and processual aspects of internality, internality in interpersonal communication, competence in the sphere of interpersonal relations, responsibility in the sphere of interpersonal relations, internality in the sphere of family relations, internality in the sphere of health, negation of activity, readiness for activities which was connected with difficulties overcoming, readiness for self-planning, acting and responsibility for their activities.

5. Test of “Sense-Bearing and Life Orientations”in adaptation made by Muzdybaieva (1998). This test was the adapted version of test “Goal of Life” by Crumbaugh, Maholick (1988). Within the further work with the method there was developed a scale of searching for sense-bearing and life goals (Crumbaugh and Maholick, 1969). It was planned as an additional scale for the test devoted to life understanding. According to the ideas of the authors the scale had to measure the strength of motivational tendency to search for sense of life; thus, it was opposite to the scale “Goal of Life” – the more goals of life there were the less need for their search.

Adaptation of this text into the Russian language was completed by Muzdybaiev (1998). The factor structure of the Russian version of the text was determined during special research. This research demonstrated that despite the small quantity (20 points) during the factor analysis there were distinguished six factors on thebasis of which there were marked out some subscales in the final variant of sense-bearing and life orientations.

6. “Questionnaire of Estimation of the Effectiveness of Military Tasks’ Execution by Servicemen of the National Guard of Ukraine” by Prykhodko (2013) gave the possibility to estimate the level of development of important professional qualities and peculiarities of executing the servicemen’s combat operations. Experts were the commanders of servicemen who participated in the

research.

The questionnaire included 37 criteria of estimation. In the process of estimation experts used the scale with 5 points: 5 points was the maximal possible level of development of estimated professional important quality or maximal level of effectiveness of the estimated aspect of service. 0 points was used to determine the absence, undevelopment of estimated important professional quality or full inefficiency of subordinate individual in estimated aspect of combat operations. Informative paragraphs of this questionnaire revealed all the processual aspects of work, peculiarities of professional communication and motives, professional skills, and possibilities of productive work.

Mentioned methods were standardized for the Ukrainian selections, they were often used to determine the peculiarities of professional motivation of servicemen and law enforcements of Ukraine.

Data Analysis

A cluster analysis was used to compare the data of psychodiagnostics scales in the study groups. To present the data, we used the main descriptive statistics (arithmetic mean M and standard deviation SD). To reliably detect significant differences between comparative groups, the t-Student criterion was used (significance level p<.1, p<.05, p<.01). Mathematical data were processed through SPSS 17.0

RESULTS

Conducted cluster analysis gave possibility to distinguish 6 groups which differeddue to their ways of estimation of combat operations effectiveness as well as typical sphere of motivation and will. The indicators for psychodiagnostic methods that determine the characteristics of the identified types of motivation in servicemen are showed in Table 1.

Table 1: Indicators for psychodiagnostic methods that determine the characteristics of the identified types of motivation in servicemen (in standard points)

Psychodiagnostic methods	MWE	MHE	HE	WE	MLE2	MLE1
"Questionnaire of Estimation of the Effectiveness of Military Tasks' Execution by Servicemen of the National Guard of Ukraine"						
General indicator of the effectiveness of service and combat activities	19.43±3.22	20.43±2.16	21.05±2.59	18.52±1.93	20.23±1.88	20.40±1.71
"Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs"						
"Motives Related to Objective Socially Posed Tasks and to Content of Profession"	20.71±3.40	17.13±4.13	21.47±2.98	23.69±1.75	19.61±2.92	23.22±2.34
"Motives Related to Personal and Professional Development"	18.50±4.30	13.53±4.38	17.60±4.52	22.38±3.99	20.04±2.29	20.04±3.70
"Independent Profession Selection"	17.15±4.66	12.34±4.91	18.73±3.96	23.00±1.53	18.74±2.82	20.74±2.95
"Dependent Profession Selection"	13.20±4.97	9.87±4.53	10.41±4.34	21.31±4.94	18.17±3.07	11.98±4.89
"Motives Related to External Prestigious Professions and Financial Welfare"	18.20±3.58	13.51±3.76	17.00±3.28	23.62±1.89	19.26±3.66	18.93±3.27
"Motives Related to Romanticization of Profession"	15.71±4.51	11.00±3.99	13.68±4.41	22.69±2.72	18.96±3.35	14.83±5.01
"Motives Related to Eagerness of Compensation of Temper Flaws"	19.12±3.61	13.66±4.67	17.44±4.22	23.08±2.06	19.91±3.07	20.57±3.66
"Antisocial Motives (attitude to profession was as if it was a way to satisfy own antisocial needs)"	14.43±4.99	9.89±3.99	10.30±3.62	21.46±3.48	18.70±3.10	13.30±5.67
"Methods of Studying the Motivational Profile of Personality"						
"Need of High Salary and Financial Benefits"	32.49±6.74	36.11±9.65	32.67±8.12	30.69±4.13	39.17±7.62	34.56±7.53
"Need of High Working Conditions and Comfortable Surrounding"	30.72±5.40	31.70±5.99	28.96±7.36	31.08±2.56	30.26±4.27	28.50±5.80
"Need of Accurate Structurization of Work and Feedback"	33.58±7.92	31.30±6.81	33.17±5.53	32.00±4.00	33.52±4.33	32.70±6.53
"Need of Social Relations"	28.07±5.24	27.85±5.13	29.42±4.68	28.23±3.96	27.61±4.98	30.83±4.98

Psychodiagnostic methods	MWE	MHE	HE	WE	MLE2	MLE1
“Need of Forming and Supporting Long-Term Stable Relations”	28.41±5.45	28.02±4.55	28.64±6.66	31.23±2.74	28.83±3.65	29.89±4.47
“Need of Obtaining the Recognition by Other People”	31.29±5.93	30.87±5.03	30.95±5.76	29.38±4.13	34.09±6.16	32.39±6.96
“Need of Posing Brave and Tough Goals and of Achieving Them”	30.47±4.82	29.53±4.98	31.14±4.18	30.31±3.22	30.83±4.49	28.98±4.11
“Need of Manipulation and Power, Eagerness to Control the Other People”	26.66±7.54	26.85±5.42	26.35±5.28	28.85±2.51	26.61±5.24	26.70±4.45
“Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”	29.59±4.66	29.42±6.01	28.81±4.67	31.08±3.01	27.52±3.82	27.56±5.22
“Need of Being Creative, Thoughtful, and Open for further Ideas Employee”	29.34±3.93	29.02±5.09	29.23±4.12	30.62±6.21	31.30±4.35	28.59±5.00
“Need of Self-Improvement and Development as Personality”	31.27±5.03	30.60±4.09	30.87±4.59	30.62±1.85	28.43±3.81	31.22±4.11
“Need of Interesting and Socially Beneficial Work”	31.12±5.57	31.74±6.02	32.79±6.31	28.92±4.35	24.83±4.98	31.07±5.16
Test of “Sense-Bearing and Life Orientations”						
General indicator of “Sense-Bearing and Life Orientations”	90.41±11.21	95.23±14.75	116.60±12.03	105.38±20.48	78.91±12.02	120.09±9.84
“Goal”	27.76±5.08	29.62±5.23	36.35±4.39	29.08±8.90	24.13±5.89	38.17±2.95
“Process”	25.85±4.55	28.72±4.75	32.90±4.86	33.92±7.25	23.91±5.52	32.69±4.64
“Result”	22.92±4.20	24.26±4.19	30.17±3.27	26.54±5.90	19.17±4.99	31.72±2.68
“Locus of Control-I”	18.84±3.60	19.83±3.51	24.29±2.76	20.85±4.88	18.04±4.67	25.98±1.68
“Locus of Life Control”	27.42±4.69	28.30±5.57	35.73±4.23	28.85±6.73	24.57±5.81	37.81±3.24
Method of “Locus of Control”						
General internality	25.71±3.74	20.64±2.94	29.60±3.55	24.15±3.29	20.43±3.70	20.44±3.50
Internality in thoughts about life in general	12.07±2.33	9.81±2.39	14.05±2.24	10.31±1.49	9.87±2.51	9.37±1.83
Internality in description of personal experience	13.64±2.38	10.83±2.68	15.56±2.12	13.85±2.97	10.57±2.84	11.07±2.68
Internality in the sphere of achievements	5.76±1.68	4.55±1.29	6.82±1.21	6.46±1.45	4.17±1.44	4.76±1.53
Internality in the sphere of fails	4.59±1.24	3.40±1.46	4.68±1.47	5.15±1.41	4.04±1.19	3.04±1.44
Internality to self-incrimination	-1.16±1.91	-1.15±1.97	-2.15±1.72	-1.31±1.55	-0.13±1.74	-1.72±2.18
Internality in the sphere of professional activity	10.63±1.75	7.66±1.80	11.83±1.85	9.85±1.52	8.13±1.96	7.78±2.00
Professional and social aspect of internality	5.43±1.13	3.81±1.23	6.04±1.25	4.69±0.75	4.13±1.36	4.06±1.41
Professional and processual aspects of internality	5.20±1.23	3.85±1.34	5.79±1.33	5.15±1.28	4.00±1.65	3.72±1.46
Internality in interpersonal communication	9.34±1.88	7.75±2.08	11.15±1.90	8.00±1.83	8.09±2.09	7.74±1.67
Competence in the sphere of interpersonal relations	5.02±1.67	3.98±1.50	6.40±1.38	3.77±1.74	3.70±1.46	4.24±1.48
Responsibility in the sphere of interpersonal relations	4.31±1.46	3.77±1.22	4.76±1.16	4.23±0.83	4.39±1.47	3.50±1.46
Internality in the sphere of family relations	4.15±1.17	3.74±1.18	4.90±1.22	3.69±0.85	3.87±1.10	3.69±1.30
Internality in the sphere of health	5.60±1.28	4.28±1.26	6.29±1.17	5.54±0.66	4.30±1.40	4.37±1.57
Negation of activity	5.14±2.12	7.55±1.67	3.30±1.86	7.46±0.78	8.13±1.74	7.83±2.39

Psychodiagnostic methods	MWE	MHE	HE	WE	MLE2	MLE1
Readiness for activities which was connected with difficulties overcoming	5.19±1.63	3.96±1.37	6.63±1.25	2.54±1.45	3.22±1.62	4.13±1.89
Readiness for self-planning, acting and responsibility for their activities	5.67±1.58	4.49±1.09	6.07±1.45	6.00±1.78	4.65±1.87	4.04±1.67
Method of “Will-Regulated Personality”						
Value-semantic organization of personality	14.02±3.25	13.19±3.73	17.60±2.85	13.54±3.67	11.04±2.40	19.39±2.69
Organization of activity	14.56±2.37	13.32±2.41	15.53±2.17	13.69±1.89	11.39±2.10	16.00±2.64
Determination	15.16±3.21	14.72±2.75	18.56±2.99	13.85±3.89	11.22±2.26	20.11±2.56
Perseverance	15.85±2.97	14.72±3.77	19.25±2.81	14.15±4.04	10.87±2.26	20.46±2.45
Self-control	15.30±2.84	14.42±3.32	18.44±2.64	13.23±4.68	10.91±2.43	19.31±2.39
“Self-Reliance”	12.88±3.15	13.85±3.00	14.66±2.38	9.15±4.20	8.35±2.90	14.54±3.54
Lies index	10.72±2.38	8.79±3.32	11.70±3.04	11.08±3.12	10.13±2.62	13.43±2.98
General indicator characterizing the volitional organization of the personality	87.78±12.20	84.21±12.48	104.04±10.10	77.62±18.06	63.78±5.33	109.81±7.75

Notes: MWE – middle level of effectiveness with tendency to weak indicator of effectiveness; MHE – middle level of effectiveness with tendency to high indicator of effectiveness; HE – highly effective; WE – weakly effective; MLE2 – middle level of effectiveness; MLE1 – middle level of effectiveness

Table 2 show the significance of differences between the proposed types according to the scales of methods for describing the features of the motivation of military personnel.

The group of highly effective (HE) servicemen included 35% of the general selection of the research. This group consisted of 1/3 from every category which participated in the research: officers, military personnel under contract, and participants of hostilities (PH).

The group of weakly effective (WE) servicemen included 4% of the general selection of the research. This group consisted of 85% of military personnel under contract and 15% of officers. 85% of this group were the participants of hostilities.

There were two groups with middle level of effectiveness of operational activity. Group number 1 with middle level of effectiveness (MLE1) was more numerical – 15% of the general selection of the research. This group consisted of 2/3 of military personnel under contract and 1/3 of officers. The status of the participants of hostilities belonged to 3/4 of respondents.

Group number 2 with middle level of effectiveness (MLE2) was less numerical – 7% of the general selection of the research. The indicator of combat operations’ effectiveness was distinguished as 20.23±1.88. This group consisted of 87% of military personnel under contract and 13% of officers. The status of the participants of hostilities belonged to 48% of respondents.

There was distinguished one more group which took intermediate place between the groups with high effectiveness and with middle level of effectiveness. It was marked as a group with middle level of effectiveness with tendency to high indicator of effectiveness (MHE). The group included 15% of the general selection of the research. This group consisted of 70% of military personnel under contract and 30% of officers. The status of the participants of hostilities belonged to 50% of respondents.

Moreover, there was detected the group which indicator of combat operations’ effectiveness took intermediate place between the group with middle level of effectiveness and the group with weaklevel of effectiveness. It was marked as a group with middle level of effectiveness with tendency to weak indicator of effectiveness (MWE). The group include done-in-four participant of the research. This group consisted of 73% of military personnel under contract and 27% of officers. The status of the participants of hostilities belonged to 45% of respondents.

According to “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” (Moskalienko, 2002) there were highlightedthe motives of choosing military profession by group HE, but their indicators were significantly weaker than those of groups WE and MLE1.

The peculiarity of motives of choosing military profession in this group was determined by hierarchy indicators.

Thus, the most expressed were “Motives Related to Objective Socially Posed Tasks and to Content of Profession”.

Table 2: The significance of the differences between the proposed types according to the scales of methods for describing the features of the motivation of military personnel (in standard points)

Psychodiagnostic methods	$t_{MWE-MHE}$	t_{MHE-HE}	t_{MWE-WE}	$t_{MWE-MLE2}$	$t_{MWE-MLE1}$	t_{MHE-HE}	t_{MHE-WE}	$t_{MHE-MLE2}$	$t_{MHE-MLE1}$	t_{HE-WE}	$t_{HE-MLE2}$	$t_{HE-MLE1}$	$t_{WE-MLE2}$	$t_{WE-MLE1}$	$t_{MLE2-MLE1}$
Questionnaire of Estimation of the Effectiveness of Military Tasks’Execution by Servicemen of the National Guard of Ukraine															
General indicator of the effectiveness of service and combat activities	2.19*	3.89**	1.42	1.52	2.32*	1.65^0	3.12**	0.41	0.09	4.34**	1.81^0	1.99*	2.57*	3.21**	0.37
Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs															
Motives Related to Objective Socially Posed Tasks and to Content of Profession	5.30**	1.67^0	4.91**	1.55	5.17**	6.91**	8.78**	2.98**	9.35**	4.01**	2.80**	4.22**	5.24**	0.81	5.26**
Motives Related to Personal and Professional Development	6.54**	1.47	3.24**	2.32*	2.25*	5.60**	7.03**	8.48**	8.29**	4.06**	3.91**	3.77**	1.94^0	1.93^0	0.01
Independent Profession Selection	5.72**	2.56*	8.90**	2.05*	5.58**	8.37**	13.38**	7.16**	10.70**	7.72**	0.02	3.75**	5.88**	3.87**	2.81**
Dependent Profession Selection	4.06**	4.21**	5.51**	5.96**	1.42	0.74	7.60**	9.31**	2.32*	7.65**	10.36**	2.04*	2.07*	6.12**	6.71**
Motives Related to External Prestigious Professions and Financial Welfare	7.27**	2.47*	8.31**	1.24	1.24	5.87**	13.72**	6.24**	7.94**	10.98**	2.77**	3.61**	4.70**	6.81**	0.38
Motives Related to Romanticization of Profession	6.43**	3.24**	7.78**	3.81**	1.05	3.96**	12.54**	8.96**	4.38**	10.58**	6.57**	1.47	3.63**	7.73**	4.22**
Motives Related to Eagerness of Compensation of Temper Flaws	7.27**	3.08**	5.73**	1.06	2.31*	5.08**	10.96**	6.89**	8.51**	8.22**	3.32**	5.01**	3.69**	3.30**	0.81
Antisocial Motives (attitude to profession was as if it was a way to satisfy own antisocial needs)	5.91**	6.57**	6.36**	5.07**	1.21	0.65	10.43**	10.40**	3.60**	10.96**	11.62**	3.58**	2.38*	6.61**	5.37**
Methods of Studying the Motivational Profile of Personality															
Need of High Salary and Financial Benefits	2.40*	0.18	1.32	3.83**	1.65	2.28*	3.09**	1.48	0.93	1.46	3.72**	1.50	4.33**	2.51*	2.44*
Need of High Working Conditions and Comfortable Surrounding	0.97	2.00*	0.39	0.43	2.26*	2.59*	0.57	1.19	2.81**	2.18*	1.17	0.45	0.72	2.43*	1.48
Need of Accurate Structurization of Work and Feedback	1.80^0	0.42	1.13	0.05	0.71	1.76^0	0.48	1.71^0	1.09	0.96	0.34	0.46	1.06	0.49	0.65
Need of Social Relations	0.24	1.92^0	0.13	0.39	3.13**	1.91^0	0.29	0.19	3.05**	1.01	1.62	1.77^0	0.41	2.02*	2.60*
Need of Forming and Supporting Long-Term Stable Relations	0.45	0.27	2.94**	0.44	1.75^0	0.71	3.26**	0.82	2.14*	2.68**	0.20	1.47	2.23*	1.38	1.09
Need of Obtaining the Recognition by Other People	0.45	0.41	1.45	1.95^0	0.96	0.10	1.11	2.21*	1.30	1.25	2.27*	1.33	2.73**	2.02*	1.06
Need of Posing Brave and Tough Goals and of Achieving Them	1.09	1.05	0.15	0.34	1.94^0	2.06	0.69	1.12	0.62	0.86	0.31	3.20**	0.40	1.26	1.69^0

Psychodiagnostic methods	$t_{MWE-MHE}$	t_{MHE-HE}	t_{MWE-WE}	$t_{MWE-MLE2}$	$t_{MWE-MLE1}$	t_{MHE-HE}	t_{MHE-WE}	$t_{MHE-MLE2}$	$t_{MHE-MLE1}$	t_{HE-WE}	$t_{HE-MLE2}$	$t_{HE-MLE1}$	$t_{WE-MLE2}$	$t_{WE-MLE1}$	$t_{MLE2-MLE1}$
Methods of Studying the Motivational Profile of Personality – cont.															
Need of Manipulation and Power, Eagerness to Control the Other People	0.17	0.33	2.04*	0.04	0.04	0.56	1.96^0	0.18	0.15	2.96**	0.21	0.45	1.73^0	2.32*	0.08
Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine	0.18	1.20	1.52	2.20**	2.34*	0.66	1.42	1.65	1.71^0	2.43*	1.43	1.52	3.08**	3.21**	0.03
Need of Being Creative, Thoughtful, and Open for further Ideas Employee	0.39	0.18	0.72	1.96^0	0.93	0.27	0.86	2.00^0	0.44	0.78	2.11*	0.83	0.35	1.09	2.39*
Need of Self-Improvement and Development as Personality	0.85	0.58	0.87	2.94**	0.06	0.38	0.02	2.23*	0.78	0.39	2.72**	0.51	2.30*	0.80	2.87**
Need of Interesting and Socially Beneficial Work	0.61	2.03*	1.63	5.25**	0.05	1.05	1.92^0	5.21**	0.61	2.90**	6.73**	1.90^0	2.57*	1.54	4.98**
Test of Sense-Bearing and Life Orientations															
General indicator of Sense-Bearing and Life Orientations	2.04*	16.16**	2.58**	4.13**	16.46**	9.31**	1.68^0	5.06**	10.24**	1.94^0	13.81**	2.03*	4.26**	2.52*	14.49**
Goal	2.07*	12.75**	0.52	2.70**	15.34**	8.21**	0.21	3.86**	10.38**	2.91**	9.48**	3.22**	1.79^0	3.63**	10.87**
Process	3.51**	10.74**	3.90**	1.55	8.55**	5.33**	2.46*	3.63**	4.37**	0.50	7.31**	0.28	4.32**	0.59	6.68**
Result	1.84^0	13.44**	2.13*	3.30**	15.14**	9.14**	1.31	4.28**	10.95**	2.19*	10.18**	3.32**	3.80**	3.09**	11.39**
Locus of Control-I	1.60	11.85**	1.43	0.76	15.88**	8.22**	0.71	1.65	11.53**	2.50*	6.22**	5.02**	1.68^0	3.74**	7.94**
Locus of Life Control	0.96	13.12**	0.74	2.17*	15.49**	8.69**	0.27	2.61*	10.78**	3.61**	8.78**	3.59**	1.92^0	4.68**	10.27**
Method of Locus of Control															
General internality	8.89**	7.58**	1.56	6.06**	8.44**	17.44**	3.52**	0.24	0.32	5.64**	10.98**	15.98**	3.11**	3.61**	0.01
Internality in thoughts about life in general	5.47**	6.14**	3.64**	3.79**	7.64**	11.01**	0.94	0.09	1.07	8.12**	7.45**	14.62**	0.66	1.94^0	0.86
Internality in description of personal experience	6.26**	6.00**	0.24	4.76**	5.75**	11.40**	3.34**	0.38	0.47	2.02*	8.02**	10.88**	3.23**	3.08**	0.73
Internality in the sphere of achievements	4.76**	5.05**	1.60	4.52**	3.61**	10.92**	4.35**	1.07	0.77	0.87	8.32**	8.79**	4.56**	3.76**	1.61
Internality in the sphere of fails	4.97**	0.45	1.36	1.95^0	6.56**	5.34**	4.01**	2.03^0	1.28	1.16	2.26*	6.94**	2.41*	4.85**	3.19**
Internality to self-incrimination	0.03	3.81**	0.30	2.47*	1.55	3.20**	0.31	2.26*	1.43	1.83^0	5.11**	1.27	2.09*	0.79	3.40**
Internality in the sphere of professional activity	9.55**	4.79**	1.69^0	5.55**	8.62**	14.02**	4.48**	0.98	0.32	4.38**	8.39**	12.73**	2.92**	4.13**	0.72
Professional and social aspect of internality	7.78**	3.68**	3.06**	4.21**	6.05**	11.03**	3.29**	0.97	0.96	5.70**	6.27**	8.95**	1.60	2.25*	0.22

Psychodiagnostic methods	$t_{MWE-MHE}$	t_{MHE-HE}	t_{MWE-WE}	$t_{MWE-MLE2}$	$t_{MWE-MLE1}$	t_{MHE-HE}	t_{MHE-WE}	$t_{MHE-MLE2}$	$t_{MHE-MLE1}$	t_{HE-WE}	$t_{HE-MLE2}$	$t_{HE-MLE1}$	$t_{WE-MLE2}$	$t_{WE-MLE1}$	$t_{MLE2-MLE1}$
Method of Locus of Control – cont.															
Professional and processual aspects of internality	5.95**	3.32**	0.12	3.24**	6.17**	8.87**	3.26**	0.39	0.47	1.70^0	4.91**	8.93**	2.33*	3.52**	0.70
Internality in interpersonal communication	4.51**	6.85**	2.45*	2.60*	5.23**	10.20**	0.42	0.64	0.04	5.90**	6.56**	12.00**	0.13	0.47	0.70
Competence in the sphere of interpersonal relations	3.81**	6.27**	2.43*	3.75**	2.90**	10.04**	0.40	0.78	0.90	5.27**	8.22**	9.12**	0.13	0.90	1.49
Responsibility in the sphere of interpersonal relations	2.35*	2.35*	0.30	0.22	3.21**	4.99**	1.60	1.77^0	1.05	2.08*	1.13	5.59**	0.42	2.40*	2.44**
Internality in the sphere of family relations	2.02*	4.49**	1.71^0	1.08	2.14*	5.97**	0.15	0.48	0.21	4.64**	4.07**	5.85**	0.54	0.02	0.64
Internality in the sphere of health	5.97**	3.95**	0.29	4.04**	4.86**	9.90**	4.98**	0.06	0.32	3.56**	6.42**	8.06**	3.59**	4.15**	0.18
Negation of activity	7.44**	6.51**	7.40**	6.98**	6.78**	14.97**	0.27	1.36	0.72	15.28**	12.10**	12.39**	1.59	0.95	0.61
Readiness for activities which was connected with difficulties overcoming	4.75**	6.92**	6.03**	5.17**	3.39**	12.15**	3.21**	1.92^0	0.52	9.80**	9.57**	8.89**	1.29	3.33**	2.14**
Readiness for self-planning, acting and responsibility for their activities	5.22**	1.85^0	0.62	2.40*	5.76**	7.98**	2.93**	0.39	1.67^0	0.14	3.45**	7.76**	2.14*	3.61**	1.36
Method of Will-Regulated Personality															
Value-semantic organization of personality	1.35	8.23**	0.45	4.87**	10.58**	7.70**	0.31	3.00**	9.85**	3.87**	11.65**	4.01**	2.20*	5.41**	13.44**
Organization of activity	2.96**	3.03**	1.49	6.23**	3.27**	5.76**	0.60	3.51**	5.49**	3.29**	8.63**	1.15	3.37**	3.64**	8.13**
Determination	0.87	7.77**	1.16	6.76**	10.08**	8.31**	0.76	5.80**	10.50**	4.24**	13.57**	3.52**	2.23*	5.52**	15.20**
Perseverance	1.86^0	8.33**	1.45	8.73**	9.98**	7.86**	0.46	5.49**	9.33**	4.44**	15.66**	2.90**	2.70*	5.40**	16.61**
Self-control	1.62	8.09**	1.55	7.41**	8.99**	7.83**	0.86	5.14**	8.75**	3.95**	13.45**	2.19*	1.66	4.55**	13.96**
Self-Reliance	1.81^0	4.43**	3.07**	6.54**	2.81**	1.75^0	3.80**	7.52**	1.09	4.65**	9.84**	0.24	0.61	4.27**	8.00**
Lies index	3.68**	2.62**	0.39	0.98	5.63**	5.48**	2.33*	1.88^0	7.59**	0.69	2.58*	3.53**	0.92	2.46*	4.85**
General indicator characterizing the volitional organization of the personality	1.65	10.17**	1.96^0	13.94**	13.07**	10.23**	1.25	10.00**	12.73**	5.19**	28.08**	4.15**	2.70*	6.29**	30.06**

Notes: MWE – middle level of effectiveness with tendency to weak indicator of effectiveness; MHE – middle level of effectiveness with tendency to high indicator of effectiveness; HE – highly effective; WE – weakly effective; MLE2 – middle level of effectiveness; MLE1 – middle level of effectiveness; $^0p\leq .1$; $^*p\leq .05$; $^{**}p\leq .01$

The next stage of hierarchy was occupied by “Independent Profession Selection”, “Motives Related to Personal and Professional Development”, “Motives Related to Eagerness of Compensation of Temper Flaws”, “Motives Related to External Prestigious Professions and Financial Welfare”. Law there were “Motives Related to Romanticization of Profession”. The lowest stage of hierarchy was occupied by “Dependent Profession Selection” and “Antisocial Motives”. The indicators of “Dependent Profession Selection” and “Antisocial Motives” were the lowest among the compared groups (they were the lowest only in participants of group MHE).

According to “Methods of Studying the Motivational Profile of Personality” (Ritchie and Martin, 1999) there were determined the highest points of the scale“Need of Accurate Structurization of Work and Feedback” that demonstrated their responsible attitude to accurate realization of the goal as well as eagerness to satisfy the expectations. The second stage of the hierarchy was occupied by “Need of Interesting and Socially Beneficial Work” and “Need of High Salary and Financial Benefits”. The proximity of indicators concerning those indicators showed that for servicemen of the NGU socially beneficial work considered to be the source of income. The next stage was occupied by “Need of Posing Brave and Tough Goals and of Achieving them”, “Need of Obtaining the Recognition by Other People”, “Need of Self-Improvement and Development as Personality” which characterized those servicemen as brave, ambitious, and desiring to develop people.

In accordance with “Sense-Bearing and Life Orientations” (Muzdybaiev, 1998) servicemen of group HE had one of the highest indicators of “Life Understanding”. They had significant prospects of life and one of the highest indicators concerning the scale “Locus of Control-I” and “Locus of Life Control”.

During the use of the method “Locus of Control” (Ksienofontova, 1999) servicemen of group HE had the highest indicators of internality among all the compared groups: “General Internality”, “Internality in the Sphere of Achievements”, “Internality in the Sphere of Professional Activity”, “Internality in Interpersonal Communication”. Furthermore, they had high indicators of the scale “Internality in the Sphere of Health”, “Readiness for Activities which was Connected with Difficulties Overcoming”, “Readiness for Self-Planning”. This group had the lowest indicator of a return scale “Negation of Activity”. Servicemen of this group did not eager to shift the blame of fails on others “Internality in the Sphere of Fails”.

Due to the method of “Will-Regulated Personality” (Hutkin and Mikhalchenko, 2000) members of group HE also had one of the highest indicators they conceded only before group MLE1. The highest indicators of group HE were “Perseverance”, “Determination” and “Self-Control”. The lowest indicators were “Self-Reliance” and “Organization of Activity”. The latest indicators were the highest among the compared groups and had sufficient level of development.

The less effective group was distinguished on the basis of other significantly high indicators ofthe method which described the motivation. Thus, due to the data of“Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” (Moskalienko, 2002) the participants of group WE differed from all other groups on statistically significant level. The exceptions were the indicators of group MLE1 in the scale “Motives Related to Objective Socially Posed Tasks and to Content of Profession”, “Motives Related to External Prestigious Professions and Financial Welfare” and the lowest and minimal indicators were presented by the scales “Dependent Profession Selection”, “Antisocial Motives”. This data demonstrated thepassion of those servicemen and their high emotionality.

To the core of “Methods of Studying the Motivational Profile of Personality” (Ritchie and Martin, 1999) there was incorporated the mechanism of overcoming social eagerness to get answers. It gave more information concerning the hierarchy of motives of this group; however, the distance from the most significant motive to the least significant stayed the shortest among all the compared groups. The most expressed motives of this group as well as in group HE of servicemen were “Need of Accurate Structurization of Work and Feedback”, but the next stage was occupied by motives which described comfortable conditions of work including normal circle of communication: “Need of Forming and Supporting Long-Term Stable Relations”, “Need of High Working Conditions and Comfortable Surrounding”, “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”. The least important in that group were the motives which described the interest in society: “Need of Obtaining the Recognition by Other People”, “Need of Interesting and Socially Beneficial Work”, “Need of Manipulation and Power, Eagerness to Control the Other People”, “Need of Social Relations”.

According to the data proposed by “Sense-Bearing and Life Orientations” (Muzdybaiev, 1998) the participants of group WE had middle indicators of life overthinking and control localization (that level was higher than in groups MWE, MHE, and MLE2 and lower than in groups MLE1 and HE). Peculiarities of this group were in concentration on “Life Process”, in accordance with this scale there were distinguished the highest indicators among all the compared groups.

Moreover, there was a need to highlight the indicators of group WE concerningthe method of “Locus of Control” (Ksienofontova, 1999) and their combination with indicators on the method “Will-Regulated Personality” (Hutkin and Mikhalchenko, 2000). Thus, on the “General Scale” those servicemen with low efficiency had one of the highest indicators giving priority only to group HE. That showed absolute connection between their “I” and motives including desires and needs. However, the scale “Readiness for Difficulties Overcoming” demonstrated the lowest indicators among all the distinguished groups.

Participants of this group had one of the lowest indicators of the scale “Will-Regulated Personality”, they gave priority only to group MLE2. Among all the compared groups the least effective servicemen had the lowest indicators of the scale “Self-Reliance” that way they developed the dichotomywith highly effective servicemen whose indicators of this scale were the highest among all the groups.

Participants of group WE as well as of MLE1 had the highest points of the scale “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” (Moskalienko, 2002). However, the hierarchy of motives of choosing the profession in group MLE1 differed from group WE. The most expressed and significant in comparison with other groupswere the “Motives Related to Objective Socially Posed Tasks and to Content of Profession”. The next hierarchy stage was developed by three types of motives which were marked with the eagerness of self-regulation: “Independent Profession Selection”, “Motives Related to Eagerness of Compensation of Temper Flaws” and “Motives Related to Personal and Professional Development”.

“Motivational Profile of Personality” of this group was built around the vector: “Need of High Salary and Financial Benefits”, “Need of Manipulation and Power, Eagerness to Control the Other People”. That characterized those servicemen as performers, so called “employee”.

The second hierarchy stage was formed by: “Need of Accurate Structurization of Work and Feedback”, “Need of Obtaining the Recognition by Other People”. Such a high position of desire to be recognized highlighted this group among all the other groups.

The third stage was developed with the help of the following motives: “Need of Self-Improvement and Development as Personality”, “Need of Interesting and Socially Beneficial Work”. Their combination demonstrated the importance of professional honour for those servicemen.

Specific stage was occupied by the motives of social cooperation “Need of Social Relations”, “Need of Forming and Supporting Long-Term Stable Relations”. Indicators of the scale “Need of Social Relations” of this group were the highest among all the compared groups (differences reached the level of statistic importance and tendency to these motives with other groups except group HE).

The next stage included four types of motives due to which servicemen of group MLE1 had the lowest indicators among all the compared groups: “Need of Posing Brave and Tough Goals and of Achieving Them” – 28.98±4.11, “Need of Being Creative, Thoughtful, and Open for Further Ideas Employee”, “Need of High Working Conditions and Comfortable Surrounding”, and “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”.

The peculiarities of group MLE1 included incredibly high indicators of the general scale “Sense-Bearing and Life Orientations” which statistically differed from other groups. Sense of life in this group was focused on the future – those servicemen had the highest indicators of the scale “Goal”. However, group MLE1 had the highest indicators among all the compared groups of the scale “Result” that highlighted the importance of recognizing their achievements by the participants.

Group MLE1 had an interesting combination of scales “Locus of Control” and “Will Regulation”. Thus, group MLE1 had the highest indicators among all the compared groups of both scales of locus of control method of “Sense-Bearing and Life Orientations” (Muzdybaiev, 1998): “Locus of Control – I”, “Locus of Control – Life”. Although, according to the method“Locus of Control” in group MLE1 there were one of the lowest indicators of the general scale and according to the scales which differentiated that indicator: “Internality in Thoughts about Life in General”, “Professional and Processual Aspects of Internality”, “Responsibility in the Sphere of Interpersonal Relations”, “Readiness for Self-Planning”, and “Internality in the Sphere of Fails”.

According to all the scales “Will-Regulated Personality” the servicemen of group MLE 1 had the highest points. The exception was made by the scale “Self-Reliance” which was the highest in group HE. Among the components of will regulation of servicemen from group MLE1 the most expressed were: “Perseverance” and “Determination”. The least expressed indicators were: “Self-Reliance” and “Organization of Activity”.

Such combination demonstrated the executive motivation in the sphere of professional activity and cooperation concerning the conscious perception of the role of “a hard-working performer”. Servicemen of group MLE1 realized their actions and were able to overcome difficulties to achieve the posed goals, they trusted the decisions of their commanders, and they were assured in their actions that gave them the possibility to express determination.

Group MLE1 also had the highest points of the indicator “Insincerity” that did not demonstratethe insincerity, but their being orientated on socially approved “correct” answer on the commander’s claims.

Participants of group MLE2 were the only individuals whose motives connected with socially posed goals and with the content of profession were not leading during the military profession selection. Other peculiarities included slight differentiated motives of profession selection. Thus, the most highlighted were: “Motives Related to Personal and Professional Development”, “Motives Related to Eagerness of Compensation of Temper Flaws”. The least expressed were motives “Dependent Profession Selection”. The second stage was occupied by “Motives Related to Objective Socially Posed Tasks and to Content of Profession”. Penultimate stage was obtained by “Motives Related to Romanticization of Profession”, “Independent Profession Selection”, and “Antisocial Motives”. Nevertheless, romantic and antisocial motives were significantly higher than in other compared groups, but they gave priority only to the indicators of group WE.

Received indicators of the method “Motivational Profile of Personality” (Ritchie and Martin, 1999) were protected from the effects’ activity which negatively influenced the differentiation of motives. According to the data of this method group MLE2 had the following significant indicators: “Need of High Salary and Financial Benefits”. The least expressed and lagged indicators were “Need of Interesting and Socially Beneficial Work”. Motivational vector, which they developed, depicted their expressive, useful, and selfish direction.

The second stage in hierarchy of motives was occupied by “Need of Obtaining the Recognition by Other People”, “Need of Accurate Structurization of Work and Feedback”. The data represented by the scale “Need of Obtaining the Recognition by Other People” showed that on statistical level servicemen of group MLE2 differed from other groups except group MLE1 with which they had almost identical indicators.

The next stage in hierarchy of motives “Need of Being Creative, Thoughtful, and Open for further Ideas Employee”, “Need of Posing Brave and Tough Goals and of Achieving them”, and “Need of High Working Conditions and Comfortable Surrounding”. Group MLE2 had one of the highest indicators of “Need of Being Creative, Thoughtful, and Open for Further Ideas Employee” due to which on statistic level they differed from all other groups except group WE.

On the next stage there was a combination of “Need of Forming and Supporting Long-Term Stable Relations” and “Need of Self-Improvement and Development as Personality” which demonstrated definite benefits of relations. Servicemen of group MLE2 wanted to choose the surrounding which could help their development.

The next stage was occupied by “Need of Social Relations”, “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”, it determined that social relations were used by the participants of group MLE2 for fun, rest, and spare time spending.

Servicemen of group MLE2 had the lowest indicators among all the compared groups on the scales of the method “Sense-Bearing and Life Orientations”. Despite that, the indicators of this group on the scale “Goal” were and “Process”, those results were almost identical that demonstrated their orientation on the nearest future.

Group MLE2 among the other groups washighlighted with the highest indicator of the scale “Negation of Activity” that demonstrated the confidence in futility of eagerness to achieve the goals of life.

Moreover, group MLE2 had the lowest indicators of all the scales of the method “Will-Regulated Personality” among all the compared groups. Extremely low indicators were inthe scale “Self-Reliance”.

Indicators of the motivation of choosing the military profession in group MHE were weakly expressed in the scale of the method “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs”. The motives which were diagnosed with the help of this method developed the vector from “Dependent Profession Selection”, “Antisocial Motives” to “Motives Related to Objective Socially Posed Tasks and to Content of Profession”. The second stage in hierarchy of motives of profession selection showed the struggle for professional development in the past: “Motives Related to Eagerness of Compensation of Temper Flaws”, “Motives Related to Personal and Professional Development” and “Motives Related to External Prestigious Professions and Financial Welfare”.

Due to the “Methods of Studying the Motivational Profile of Personality” (Ritchie and Martin, 1999) the group MHE had the most expressed indicators which made them significantly different from others in “Need of High Salary and Financial Benefits” and the least expressed were indicators of “Need of Manipulation and Power, Eagerness to Control the Other People”.

The second stage in hierarchy of motives was occupied by “Need of Interesting and Socially Beneficial Work”, “Need of High Working Conditions and Comfortable Surrounding”, “Need of Accurate Structurization of Work and Feedback”. Then there were motives which demonstrated their ambitions “Need of Obtaining the Recognition by Other People”, “Need of Self-Improvement and Development as Personality”.

Less important for group MHE were the motives which described the eagerness to get new and non-standard issues in their work: “Need of Posing Brave and Tough Goals and of Achieving Them”, “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”, and “Need of Being Creative, Thoughtful, and Open for further Ideas Employee”. Even less important for them was the desire to be engaged into social cooperation: “Need of Forming and Supporting Long-Term Stable Relations”, “Need of Social Relations”.

Due to the scales of the method “Sense-Bearing and Life Orientations” (Muzdubaiev, 1998) servicemen of group MHE had statistically lower indicators than groups HE and MLE1, but higher than group MLE2. Besides that, they as well as the mentioned groups had almost identical indicators of the scales “Goal” and “Process”. There was not any increase of the scale “Result”.

Due to the indicators of the method “Locus of Control” (Ksenofontova, 1999) servicemen of group MHE had identically low quantity of general points as well as groups MLE1 and MLE2. The peculiarities of group MHE included the lowest indicators among all the compared groups “Internality in the Sphere of Professional Activity” which were formed because of weak indicators “Professional and Social Aspect of Internality”. Moreover, other indicators related to the control in cooperation were decreased “Internality in Interpersonal Communication”, “Competence in the Sphere of Interpersonal Relations” and “Responsibility in the Sphere of Interpersonal Relations”.

In accordance with the data of the method “Will-Regulated Personality” (Hutkin and Mikhalchenko, 2000) servicemen of group MHE had indicators which were maximally close to group WE. However, their indicators of scales were weakly differentiated that did not demonstrate any urgency of will regulation, they substituted it with postarbitrary activity to make professional activities automatic.

Servicemen of group MHE had the lowest indicator of “Insincerity” that showed not only the desire to give completely honest answers, but also demonstrated slightly weaker social tolerance and communicative correctness.

In the structure of motivation of choosing the military profession group MWE had the most expressed motives such as: “Motives Related to Objective Socially Posed Tasks and to Content of Profession”. Close to them there were “Motives Related to Eagerness of Compensation of Temper Flaws”. Further there occurred “Motives Related to Personal and Professional Development” and “Motives Related to External Prestigious Professions and Financial Welfare”. On the other side of hierarchy there were Dependent Profession Selection”, “Antisocial Motives”, “Motives Related to Romanticization of Profession”. In group MWE intermediate position between values and motives which depreciated was occupied by the motives “Independent Profession Selection”.

The participants of group MWE came close to the indicators of group HE: “Motives Related to Objective Socially Posed Tasks and to Content of Profession”, “Motives Related to Personal and Professional Development”. According to the scales “Dependent Profession Selection”, “Motives Related to External Prestigious Professions and Financial Welfare”, “Motives Related to Romanticization of Profession” and “Antisocial Motives” participants of group MWE moved closer to group MLE1.

Due to the data presented by the “Methods of Studying the Motivational Profile of Personality” (Ritchie and Martin, 1999) group MWE had the most expressed indicators of “Need of Accurate Structurization of Work and Feedback”, “Need of High Salary and Financial Benefits”. The next dense group of motives revealed in group MWE the urgency of needs of improving social status with their own powers “Need of Obtaining the Recognition by Other People”, “Need of Self-Improvement and Development as Personality” and “Need of Interesting and Socially Beneficial Work”.

The eagerness to change life for better and to make it more comfortable distinguished the place in hierarchy of motives of group MWE due to results received from the scales: “Need of High Working Conditions and Comfortable Surrounding”, “Need of Posing Brave and Tough Goals and of Achieving Them”.

Group MWE was quite low in hierarchy of motives “Need of Diversity, Breaks and Stimulations, Eagerness to Avoid Everyday Routine”, “Need of Being Creative, Thoughtful, and Open for further Ideas Employee”.

Less important for group MWE were indicators of “Need of Forming and Supporting Long-Term Stable Relations”, “Need of Social Relations" and "Need of Manipulation and Power, Eagerness to Control the Other People" that demonstrated the eagerness to avoid social relations.

According to the scales of the method "Sense-Bearing and Life Orientations" (Muzdybaiev, 1998) servicemen of group MWE had one of the lowest indicators (lower than in the 5th group). Their indicators of the methods "Locus of Control" (Ksenofontova, 1999) and "Will-Regulated Personality" (Hutkin and Mikhalchenko, 2000) moved indicators closer to group WE (it had the lowest quantity of statistically important differences). Unlike group WE they had significantly better indicators of the scale "Negation of Activity", they had more readiness to act to overcome the differences, as well they had higher level of confidence in possibility to run their own lives, they did not tend to shift their responsibility on others due to results of cooperation, and they had significantly higher indicators of "Self-Reliance". That indicated their high level of self-reliance and low level of dependency in professional and interpersonal relations in comparison with participants from group WE

DISCUSSION

The research demonstrated that servicemen with the lowest effectiveness of combat operations had the highest indicators through all the motives unlike those with higheffectiveness. However, this case wasnot the realization of the law of Yerkes and Dodson (1908). It was likely that the received data demonstrated the phenomenon of forming the tangentiality of awakening and of free motivation which were described by the Eastern European psychology by Lieontievym (1975) and Byhotskym (1983), by phenomenon of self-efficiency proposed by the American psychology Bandura (1994) who considered that the success of activity was determined not only by the attractiveness of result, but also by the trust in their personal powers.

Thus, to estimate the motivation appropriately there was a need to include servicemen of distinguished groups into motivational types which consisted of content, hierarchy, intensity of motives and their possibility to be implemented.

In that way highly effective servicemen choosing profession considered its content, expressed eagerness to realize themselves in their profession, comply with it, develop in profession, and acquire the professional skills. They rejected dependency and unlawful benefit as motives of profession selection. For relevant motivation of HEit was typical to have eagerness to execute posed tasks and to meet expectations. It was important for them to do socially beneficial work which was understood by them as the only source of income. Important peculiarity of their motivation was the preservation of high professional ambitions – they struggled for professional development, tough tasks, and high estimation of their achievements. Motives which described the conditions andregime of work, social cooperation were non-essential. Servicemen with high effectiveness were characterized as those who had high level of life understanding, significant life prospects, and considered themselves to be strong personality with sufficient will of selection. They were assured that people could control their lives, could make decisions by themselves, and could implement them. Existing content of motivation was supported by high level of internality and developed qualities of will. Thus, servicemen of group HE took ultimate responsibility for their actions having analyzed them, they did not avoid any work, could plan, and put efforts by themselves to achieve the results they struggled for and whichthey were not ashamed of. Their responsibility spreadon the results of professional activity and interpersonal communication as well as on the health. Their obtained level of internality was, first of all, their own experience of successes and fails. Strong-willed quality of servicemen from the group with high efficiency were well-developed and, first of all, met the requirements of their professional activity including tough and unsafe character of their profession as well as cruel normative settlement.

Servicemen with weak effectiveness, as it was already mentioned, had significantly high indicators of all the scales which described the motives of activity and its selection and slight differentiation of those motives. This data gave possibility to talk about insufficiently formed randomness of activity which was presented by inability to cope with desires, low level of self-reliance and reluctance to overcome difficulties. Servicemen from group WE depended on their desires as well as on their surrounding. They wanted to have simple, comfortable work which was usually occupied by them thanks to the patronage of people from their close surrounding.

Presented features of servicemen from groups HE and WE on the indicators of combat activity correlated with the situations concerning the connection between Public Service Motivation (PSM) with work effectiveness and its mediated self-efficiency (Breaugh, Ritz, and Alfes, 2018).

According to it servicemen from group HE was depicted as the type which was guided by motivation of public service, and servicemen from group WE as dependent motivational type.

Servicemen from group MLE1 had highly expressed motivation of a performer which was based on conscious trust in their commanders and marked qualities of will.

Those servicemen were orientated namely on military profession acquiring (high points for the motives of professional selection) and on making efforts to master it. They were responsible and determined concerning the execution of their tasks. They agreed to execute tough tasks, routine actions, and were undemanding concerning the working conditions. It was quite important for those servicemen to have high salary, recognition of their achievements, conformity of social expectations, and support of friendly relations.

Having compared motives of groups HE and MLE1 it was possible to conclude that they had plenty of common aspects concerning their motives of public service and prosocial motivation (Schott, Neumann, Baertschi, and Ritz, 2019). According to that data servicemen from group MLE1 were included into prosocial motivational type. Besides that, the description of this type was supported by the data received by Gillet, Becker, Lafrenière, Huart, and Foiquereau (2017). They determined that servicemen with the highest indicators of autonomous motivationfelt high organizational support, commander's support, positive experience, and high level of communicationbecause they were engaged into professional activity due to their pleasure, personal choice, and personal will, demonstrated the highest level of well-being.

Servicemen of group MLE2 were the only participants among all the distinguished types who chose their profession being led by their own mercenary interests, possibility to work with things they liked and were interested in, those activities were full of romantic, but not of objective content of the profession. They used social relations to be "on good standing" with their commanders or to gain satisfaction from communication. Those servicemen did not want to realize their strong-willed qualities. Such servicemen required constant control from the side of commanders because they could be addicted to the "romantic" features of their profession neglecting its core sense. The description of this group of servicemen was close to the definition of motivational type proposed by Stéphanie Vincent Lyk-Jensen and Ane Glad (2018) – "Personal Benefit". Thus, servicemen from group MLE2 were included into romantic (struggle for adventures) motivational type. Servicemen of this type searched for adventures, challenges; however, they were unstable concerning the hardships of service, the actions of combat factors of stress and, usually, these servicemen resigned after the first contract expired.

Servicemen of group MHE choosing the military profession considered its content and were ready to make everything possible to acquire it; however, this motivation lost its urgency. Acquiring the profession they concentrated on financial benefits, personal ambitions, and comfortable conditions of work; they were weakly motivated to put efforts to acquire new profession. It was useful for them to be orientated on the nearest future. Most of professional actions were executed automatically without any strong-willed efforts. They did not get along with their surrounding and showed low level of tolerance. This type was determined as a stagnated one. Nevertheless, even if this type had quite high indicators of effectiveness the general prospects for this type were pessimistic. In accordance with Voet, Steijn, and Kuipers (2017), who distinguished positive relations between the eagerness of changes and prosociality, group MHE was likely to decrease its altruistic behaviour.

Servicemen of group MWE chose the military profession because of its content as well as of his/her own desire to change the life and social status for better. In urgent situation it was important for them to have understandable and well-paid activity which would give them the possibility to improve their social status and conditions of lifeinternally. These servicemen did not feel themselves ready for intellectual and creative work. They did not cooperate with their surrounding and that fact detained the process of professional socialization. Despite the desire to help other people, work, endeavour, and change, they did not achieve high effectiveness in professional activity as a serviceman due to their concentration on defeating the difficulties of life. This type having analogy with Maslow (1943) was marked as a deficient type of motivation. The determination of deficient type of motivation of servicemen who joined the army to change their lives for better, to get new prospects, all those factors were not something new in military psychology. That was the way the American researchers pointed out that military service was often joined by Afro-Americans and people from Latin America, not so often it was joined by people who positively studied at school, planned to enter the college and by parents who graduated from the Higher Educational Establishments (Bachman, Segal, Freedman-Doan, and O'Malley, 2000).

CONCLUSION

Conducted research gave the possibility to determine 6 motivational types related to the effectiveness of servicemen's operational activity. These motivational types were located between the poles which were introduced by two dimensions: self-efficacy (autonomous and controlled motivation) and motivation of helping others (motivations of public service – selfishness). Those motivational types of servicemen were motivated by public service, stagnant, prosocial, romantic, deficient and dependent. Distinguished types included the content, hierarchy, intensity of motives and their possibility to implement them.

Developmentof the motivation diagnostics of the applicants to the military service on the basis of their determinationto one of the distinguished motivational types would help to decrease the quantity of mistakes during the prediction of effectiveness of operational activity.

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ORIGINAL ARTICLE

Post-traumatic stress disorder and cardiovascular disease

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Abstract: *Posttraumatic stress disorder (PTSD) is a psychological-psychiatric state caused by exposure to stressful, traumatic events that manifests as a vivid reminiscence of them by flashbacks, nightmares, reccurent memories, emotional and physical manifestations. Cardiovascular diseases (CVD) are of interest in patients suffering from PTSD because there is an increasing body of evidence that these pathologies are linked. Hypertension, dyslipidemia, diabetes, and other cardiovascular risk factors are common in patients with PTSD and all of them increase the incidence of coronary syndromes, both chronic and acute. Posttraumatic stress disorder has a major impact on the lifestyle and health of subjects exposed to trauma or stress. Cardiovascular diseases tend to appear sooner in PTSD diagnosed patients due to a variety of reasons: poor habits, low addressability to health care systems, chronic inflammation status, changes at molecular levels, etc. Otherwise, PTSD tends to be induced by CVD, thus inclining the balance towards whis association. According with data published until now, there is a strong pathophysiologic relatonship between PTSD and some CVD; there is also outlining a vice versa relationship, from some CVD to PTSD.*

Keywords: *posttraumatic, stress, disorder, cardiovascular, disease*

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a psychological-psychiatric state caused by exposure to stressful, traumatic events that manifests as a vivid reminiscence of them by flashbacks, nightmares, recurrent memories, emotional and physical manifestations.

Cardiovascular disease (CVD) is of interest in patients suffering from PTSD because there is an increasing body of evidence that these pathologies are linked.

Posttraumatic stress disorder seems to be associated to an increased risk of CVD due to its psychosocial consequences that become cardiovascular risk factors and due to its ability to cause cardiovascular structural changes and alter the neuroendocrine mechanisms involved in maintaining body functions. This disorder is mainly associated with hypertension and coronary artery disease but it can also lead to arrhythmia or stroke, with recent studies bringiging into attention the pathological link at molecular and genetic levels. The shortcomings of existing studies are that they do not report the incidence rate of each CVD after exposure to stressful events but rather tell us about the incidence rate of cardiovascular risk factors in populations suffering from PTSD (mostly military field workers, either veterans or active soldiers).This systematic review of the literature aims to bring forward the extent to which PTSD can cause damage to the cardiovascular system and to present the pathological link between PTSD and CVD as well as to present the vice-versa relationship: PTSD induced by CVD.

MATERIALS AND METHODS

We conducted the present systematic review in concordance with updated Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [1]. A literature search using a prespecified MeSH combination of words (“posttraumatic stress disorder " AND "cardiovascular disease”) was performed in PubMed and Google Academic. Two essential inclusion criteria were defined and applied for retrieved citations which guided the eligibility assessment: (1) randomized controlled and non-randomized clinical trials

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with available abstract (for PubMed) and containing ”the exact phrase” (for Google Academic); (2) studies which enrolled at least 50 patients or more (due to the high probability of not having a significant statistical power). Additionally, studies were excluded from the analysis if they met at least one of the following criteria: articles that could’t be retrieved because of access to the editorial, doubles, articles written in languages other than english, other types of literature (books, e-books, disertation thesis, article in press), studies conducted on animals and articles that have not treated the issue of our search. All studies without PTSD diagnosis defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-III), DSM-IV, DSM-5 or International Classification of Diseases (ICD-10) were excluded. We taking into consideration the reviews, systematic reviews and meta-analysis only in discussions, to avoid duplications and bias selection.

Two independent investigators decided if studies fulfilled the inclusion criteria and any disagreements were solved by discussion and consensus.

RESULTS

The flow-chart of the search process as well as complete databases search strategies were provided in Figure 1. We retrieved a total number of 244,976 articles from both databases out of which were removed 244,589 due to automation tools presented above, 11 doubles and other 118 articles that were other types of literature or whose abstract was written in other language than english, resulting in 258 articles for screening.

Figure 1: Systematic search of the literature: PRISMA flowchart

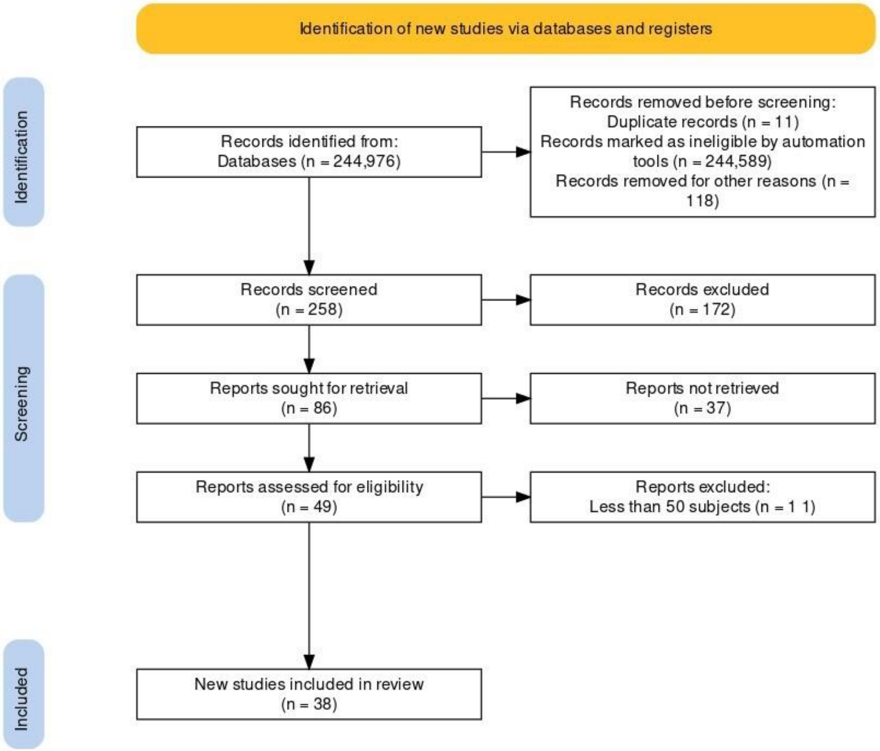
Identification of new studies via databases and registers
Identification
Records identified from: Databases (n = 244,976)
Records removed before screening: Duplicate records (n = 11) Records marked as ineligible by automation tools (n = 244,589) Records removed for other reasons (n = 118)
Screening
Records screened (n = 258)
Records excluded (n = 172)
Reports sought for retrieval (n = 86)
Reports not retrieved (n = 37)
Reports assessed for eligibility (n = 49)
Reports excluded: Less than 50 subjects (n = 1 1)
Included
New studies included in review (n = 38)

After exclusion of 172 articles (19 presenting studies conducted on animals and the rest not being of interest because they did not debate the relationship between PTSD and CVD), another 37 could not be retrieved and 11 not being taking in consideration because they used less than 50 subects in trials.

The final number of articles used in our systematic review was 38 (n=2,507,724 patients): 25 prospective (n=1,451,924 patients) and 13 retrospective (n=1,055,800 patients) studies.

Study types and their aims, along with demographic, methodology of each and results are presented in Table 1 [2-39].

Twenty three prospective (n=1,449,992 patients; 99.9%) and nine retrospective (n=1,043,266 patients; 98.8%) studies showed a relationship between PTSD and CVD.

Table 1: Studies taken into consideration.

Author	Study type and aim	No. of patients	Methodology	Results
Scherrer JF et al., 2020 [2]	Prospective study Primary objective:estimate the risk of incident CVD (CVD) in patients with vs. without a clinically meaningful PTSD (PTSD) improvement. Secondary objective: if results differed by depression status and age group.	1079	Eligible patients, were 30–70 years old, diagnosed with PTSD and used Veterans Health Affairs PTSD specialty clinics. Patients had a PTSD Checklist score (PCL) ≥ 50 between 2008 and 2012 and a second PCL score within 12 months and at least 8 weeks after the first PCL ≥ 50. Clinically meaningful PTSD improvement was defined by ≥20 point PCL decrease between the first and second PCL score. Patients were free of CVD diagnoses for 1 year prior to index. Index date was 12 months following the first PCL.	Patients with vs. without PTSD improvement did not differ in CVD risk (HR = 1.08; 95%CI: 0.72–1.63). Results did not change after stratifying by age group or depression status.
Kubzansky LD et al., 2014 [3]	Prospective observational To determine if women who develop PTSD symptoms are more likely to gain weight and become obese relative to trauma-exposed women who do not develop PTSD symptoms or women with no trauma exposure	54224	Trauma and PTSD Screening Questionnaire was used to determine whether women present traumatic exposure according to the DSM-IV PTSD diagnosis. These women were divided in 2 separate groups for analysis depending on when trauma exposure/PTSD occurred. Data on weight and height was reported biennially. The BMI was calculated.	If trauma leads to the development of PTSD symptoms, the patient has an increased risk of weight gain. Women with PTSD should be monitored or undergo screening for development of adverse cardiometabolic outcomes. It is important to expand PTSD treatments to attend to behavioral alterations.
Bourassa KJ et al., 2020 [4]	Retrospective study A secondary analysis of a larger intervention study of active duty soldiers with PTSD The aim of this study is to see if first-line exposure therapies are associated with improvements in participants cardiovascular functioning and reactivity compared to not receiving treatment patients.	104	Active duty soldiers with PTSD were randomized to an exposure therapy—either prolonged exposure or virtual reality exposure (VRE) or a waitlist control condition. The interest variables were: change in participants' resting heart rate (HR) and HR reactivity from baseline (prior to randomization) to mid- and post-treatment.	Decreased resting HR (B = -5.06, p = .024) and HR reactivity (B = -2.46, p = .005) from baseline to post-treatment for PE and VRE relative to waitlist. Exploratory analyses found that change in resting HR and HR reactivity were not significantly correlated with either self-reported or clinician-rated PTSD symptom change.
Burg MM et al., 2017 [5]	Prospective study The aim is to investigate the prospective relationship of PTSD to incident hypertension risk and the effect of PTSD treatment on hypertension risk.	194319	Data from a nationally representative sample of 194,319 veterans were drawn from the Veterans Administration roster of United States service men and women. The variables were: incident hypertension, PTSD treatment	The incident hypertension risk independently associated with PTSD ranged from HR=1.12 (95% CI 1.08 – 1.17, p<0.0001) to HR=1.30 (95% CI 1.26 – 1.34, p<0.0001). The interaction of PTSD and treatment revealed that treatment reduced the PTSD associated hypertension risk (e.g., from HR=1.44 [95% CI 1.38 – 1.50, p<0.0001] for those untreated, to HR=1.20 [95% CI 1.15 – 1.25, p<0.0001] for those treated)
Dennis PA et al., 2014 [6]	Prospective study The aim o this study is to demonstrate that: 1) PTSD symptoms would be associated with lower HRV; 2) PTSD symptoms would be associated with greater smoking, higher rates of lifetime alcohol dependence, higher rates of abdominal obesity, and more sleep disturbance; and 3) each of these behavioral risk	227	Short-term laboratory-based and 24-hour ambulatory measures of HRV were collected from 227 young adults (18-39 years old), 107 of whom were diagnosed with PTSD. They assessed the relationship of PTSD symptoms with HRV along with potential behavioral health mediators.	PTSD symptoms were associated with reduced HRV, β = -.21, p = .002. This association was reduced in models that adjusted for cigarette consumption and history of alcohol dependence, and was rendered non-significant in a model adjusting for sleep disturbance. Abdominal obesity was not a

Author	Study type and aim	No. of patients	Methodology	Results
	factors would partially mediate the association between PTSD symptoms and HRV.			significant mediator.
Chang JC et al., 2017 [7]	Prospective study The present study estimated incident PTSD and comorbid diseases for over a decade in a cohort from a community-based integrated screening program.	76417	The cohort was derived from a population of about 390 thousands adults (aged 30 years and above) in the northeastern part of Taiwan. The enrolled participants were interviewed to collect social demographics, health behaviors (e.g., smoking or alcohol drinking), and family history of major illnesses (e.g., psychiatric disorders).	Compared with non-PTSD participants, those with PTSD tended to be female, younger, more highly educated, non-smokers, and non-drinkers. Furthermore, participants with PTSD had a higher proportion of CVD.
Meli L et al., 2018 [8]	Prospective study They tested whether younger age was associated with greater threat perceptions during ACS evaluation in the ED and greater posttraumatic stress symptoms (PSS) in the frst week after ED enrollment.	871	Participants completed measures of demographics, including age, sex, and race/ethnicity, and perceived threat during evaluation for suspected acute coronary syndrome in the ED. During inpatient stay or by phone after discharge (median 3 days after enrollment), participants reported PSS (i.e., symptoms of re-experiencing and/or avoiding reminders of the trauma, and hyperarousal) in response to the event that brought them to the emergency department.	Younger, versus older individuals are at risk for greater PSS after acute coronary syndrome evaluation, and elevated perceived threat partially mediated this association.
Rosman L et al., 2019 [9]	Retrospective study They assessed the association between PTSD and incident transiemt ischemic attack (TIA) and ischemic stroke	987855	They assessed the incidence of TIA and ischemic stroke in a cohort of 987855 young and middleaged Veterans (mean age of 30.29±9.19 years; 87.8% men, 64.4% white) who first accessed care through the Veterans Health Administration from October 2001 to November 2014 and were free of TIA and ischemic stroke at baseline.	PTSD was significantly associated with new-onset TIA (hazard ratio [HR], 2.02; 95% CI, 1.62–2.52) and ischemic stroke (HR, 1.62; 95% CI, 1.47–1.79). The effect of PTSD on ischemic stroke risk was stronger in men than in women (HR, 0.63; 95% CI, 0.47–0.86; P=0.003), but no effect of sex was found for TIA.
Giesinger I et al., 2020 [10]	Prospective study To assess whether 9/11-related probable PTSD is associated with increased mortality risk	63666	PTSD assessments at baseline (wave 1: 2003-2004) and 3 follow-up time points (wave 2: 2006-2007, wave 3: 2011-2012, wave 4: 2015-2016). PTSD was defined using the 17-item PTSD Checklist–Specific (PCL-S) self-report measure (score≥50) at each wave (waves 1-4	Comparable findings between responders and civilians suggest that 9/11-related PTSD is associated with an increased mortality risk.
Lima BB et al., 2020 [11]	Prospective study. To evaluate the association of PTSD with mental stress–induced myocardial ischemia among individuals who survived a myocardial infarction (MI).	303	Participants had a verified history of MI within 8 months. Patients received technetium 99m–labeled sestamibi myocardial perfusion imaging at rest, with mental stress (ie, a speech task) and conventional stress (ie, exercise or pharmacologic)	PTSD was associated with the development of myocardial ischemia with mental stress.
Lima BB et al., 2019 [12]	Prospective study The aim is to demonstrate that survivors of a myocardial infarction (MI) who have PTSD would show an enhanced inflammatory response to acute psychological stress compared to those without PTSD.	271	Individuals with a verified history of MI within 8 months and a clinical diagnosis of current PTSD underwent a mental stress speech task. Inflammatory biomarkers including interleukin-6 (IL-6), high-sensitivity C reactive protein (HsCRP), matrix metallopeptidase 9 (MMP-9), intercellular adhesion molecule	MI patients with current PTSD exhibit enhanced IL-6 response to psychosocial stress, suggesting a mechanistic link between PTSD and adverse cardiovascular outcomes

Author	Study type and aim	No. of patients	Methodology	Results
			(ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and monocyte chemoattractant protein (MCP)-1 were measured at rest and 90 min after mental stress.	
Britvic D et al., 2015 [13]	Retrospective study The aim of this study was to investigate the differences in the prevalence of somatic diseases among combat veterans and their contemporaries who were not exposed to the traumatic experience at the battlefield, and to determine whether socio-demographic factors, exposure to war-time trauma and/or injury might predict individual somatic diseases	1326	501 male combat veterans with posttraumatic stress disorder (PTSD) and the corresponding control group of 825 men who were not exposed to combat experience. All participants were assessed systematically.	Veterans with PTSD suffered more often from cardiovascular, dermatological, musculoskeletal, pulmonary and metabolic diseases than corresponding control subjects who were not exposed to combat experience. PTSD as a result of exposure to war trauma increases the likelihood of developing somatic diseases.
von Känel R et al., 2021 [14]	Retrospective study This study explored whether a preventive behavioral intervention leads to a decrease in myocardial infarction-induced PTSS between two follow-up assessments.	104	104 patients with MI were randomized to a 45-min one-session intervention of either trauma-focused counseling or stress counseling (active control). Sociodemographic, clinical, and psychological data were collected at baseline, and PTSS were assessed with the Clinician-Administered Post-traumatic Stress Disorder Scale 3 and 12 months post-MI.	PTSS severity showed no change over time from 3 to 12 months post-MI, either in all patients or through the intervention.
Agarwal S et al., 2019 [15]	Prospective study Compared 1-year all-cause mortality and major adverse cardiovascular events in cardiac arrest survivors with and without PTSD symptomatology at hospital discharge.	114	Compare ACM or MACE— hospitalization for nonfatal myocardial infarction, unstable angina, congestive heart failure, emergency coronary revascularization, or urgent implantable defibrillators/permanent pacemaker placements within 12 months of discharge, in case of cardiac arrest suvivors with/without PTSD.	PTSD symptomatology is common after cardiac arrest (CA), and CA-induced PTSS was associated with significantly higher risk of death and cardiovascular events.
Goetz M et al., 2014 [16]	Retrospective study They examined whether PTSD and combat exposure were associated with CIMT (Carotid Intima-Media Thickness) in VietnamWar–era twins after controlling for shared genetic and childhood factors.	465	465 middle-aged twins from the Vietnam Era Twin Registry who were free from CVD were included. PTSD was diagnosed using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition and CIMT was measured by ultrasound.	Among Vietnam War–era veterans, combat exposure and PTSD are associated with CIMT, though the associations are largely mediated by shared childhood factors
Jiang C, 2018 [17]	Prospective study Revealed the prevalence, associated risk factors, and long-termoutcomes of PTSD in patients after intracerebral hemorrhage (ICH)	64	64 patients with a first ICH were included. PTSD was determined with Diagnostic and Statistical Manual of Mental Disorders (fifth edition; DSM-5), and questionnaires on mental and physical status were used. Patients were followed at 3 and 12 months after ICH	Patients with PTSD were more likely to be female (67% vs. 29%, p ¼ 0.01), and had higher rates of receiving minimally invasive surgery (MIS; 47% vs. 18%, p ¼ 0.04) and and had poorer quality of life and worse stroke-related disability.
Rosman L et al., 2019 [18]	Prospective study The study assessed the incidence of atrial fibrillation over a 13-year period among 988 090 young and middleaged veterans	988090	988090 young and middleaged veterans (mean age, 30.299.19 years; 87.8% men, 64.5% white) free of AF, atrial flutter, or atrial tachycardia at baseline were included and followed for 13 years	Patients with PTSD had a higher overall incidence of AF (P<0.0001) and were more likely to develop AF at a younger age than those without PTSD (P=0.004).

Author	Study type and aim	No. of patients	Methodology	Results
von Känel R et al., 2018 [19]	Prospective study Assessed if trauma-focused counseling prevents the incidence of ACS (Acute Coronary Syndrome)-induced PTSS.	190	190 patients with ACS were randomized to a single session intervention of either trauma-focused counseling or an active control intervention (in first 48 hours) and additional health outcomes were assessed at 3 months of follow-up.	Self-rated PTSS indicated less beneficial effects with trauma-focused (6.54; 4.95–8.14) versus stress counseling (3.74; 2.39–5.08; p = 0.017).
Seligowski AV et al., 2020 [20]	Prospective study Assessed the cardiovascular response to extinction in a sample of trauma-exposed individuals.	51	51 trauma-exposed women underwent a fear conditioning paradigm. Heart rate variability (HRV) was calculated at baseline and during the extinction session.	Higher baseline HRV was significantly associated with greater fear bradycardia during early extinction. Fear bradycardia may be a relevant biomarker in predicting how individuals will respond to extinction-based psychotherapy.
Chen YY et al., 2021 [21]	Prospective study Investigated the associations of PTSD (PTSD) manifestation and early risk factors for cardiovascular physiological measures among adolescence.	606	606 adolescents aged 14-19 years old were included. They were asked to rate their possible trauma experiences and PTSD symptoms via questionnaires. The measured variables were: blood pressure, heart rate and body mass index.	PTSD symptoms had a significant effect on blood pressure and heart rate
Cave B et al., 2019 [22]	Retrospective study Determined, based on prior arterial blood pressure monitoring (ABPM) studies, if PTSD diagnosed veterans have abnormal nocturnal dipping patterns compared with the general veteran population	470	Retrospective chart review was performed on all archived ABPM studies and classified by nocturnal dipping status and BP control rates.	Veterans with PTSD had similar distributions of dipping patterns and rates of overall, awake, and nocturnal hypertension compared with the general veteran population.
Campara TM et al., 2014 [23]	Prospective study The aim of this study was to determine how lipid component act after a certain time distance after effects of multifactorial war stress, in the postwar period among the members of the police department.	300	300 police officers were divided in 2 groups :150 as experimental and 150 respondents as a control group. They were investigated the same way: detailed history, physical examination,complete laboratory evaluation, lipid electrophoresis, ultrasound of the abdomen and Color Doppler of the neck vascular structures.	A significant increase in levels of cholesterol and triglyceride levels was noticed in experimental compared to the control group. The ultrasound of the abdomen showed fatty infiltrated liver in 16% of respondents from the experimental and 2% of the respondents in the control group.
Yu S et al., 2018 [24]	Retrospective study The aim of this study was to investigate the association between 9/11-related PTSD (PTSD), dust cloud exposure, and subsequent development of stroke.	42527	Using data from the World Trade Center Health Registry surveys, they employed Cox proportional hazards regression models to assess the associations.	Incidence of stroke was higher among those with PTSD or intense dust cloud exposure than those without, and it was even higher for those who had experienced both
Baranyi A et al., 2021 [25]	Prospective study 1) Determine whether blood levels of trimethylamine N-oxide (TMAO) vary immediately after acute myocardial infarction (AMI) in patients with/without AMI-induced PTSD symptomatology (2) Investigated whether TMAO is a potential biomarker that might be useful in the prediction of PTSD.	114	114 AMI patients were included. The Clinician Administered PTSD Scale for DSM-5 was used to explore PTSD-symptoms at the time of AMI and 6 months after AMI. To assess patients' TMAO status, serum samples were collected at hospitalization and 6 months after AMI.	Participants with PTSD-symptomatology had significantly higher TMAO levels immediately after AMI than patients without PTSD-symptoms. An elevated TMAO level might be a biological marker for severe stress that is associated with vulnerability to PTSD.
Eslami B et al., 2017 [26]	Prospective study Compared the level of PTSD between adults with and without congenital heart disease and examined the correlates of PTSD	853	Patients were divided in two groups: 347 patients with congenital heart disease and 353 without. PTSD was assesed using PTSD symptom scale	52% of patients with congenital heart disease had PTSD while 48% of those without congenital heart disease had PTSD; p=0.183

Author	Study type and aim	No. of patients	Methodology	Results
Ratcliff CG et al.. 2017 [27]	Retrospective study Examined the association between COPD and CHF in patients with mental health disorder, among them PTSD (based on patient electronic medical records and self-report data)	180	Patients with COPD and CHF were assessed for PTSD (and other mental health issues) using Beck Anxiety Inventory and Patient Health Questionnaire -9	13% of patients with COPD or CHF were diagnosed with PTSD
Jane Irvine J et al., 2011 [28]	Prospective study Assessed the relationship between ICD (implantable cardioverter defibrillator) and PTSD (implantable defibrillator carriers may develop PTSD)	193	96 patients with ICD had received cognitive behaviour therapy while 97 were adressed to standard clinical care; both groups were assessed regarding PTSD and other mental health issues at baseline, 6 and 12 months	Use of cognitive behavioural therapy showed an improvement in PTSD symptoms in patients with ICD (p<0.01)
Shemesh E et al., 2006 [29]	Retrospective study Evaluated the symptoms of PTSD and adherence to aspirine in patients with PTSD which received cognitive behavioral treatment	65	14 patients with a previous MI and diagnosed PTSD: 6 patients received cognitive beahavioral treatment while 8 had received standard care	PTSD symptoms improved in those following cognitive behavioral treatment; adherence to aspirine improved in patients submited to standard care
Gilsanz P et al., 2017 [30]	Prospective study Assessed the association of PTSD symptom severity and trauma exposure to the risk of CVD	49859	The subjects (female nurses enrolled in 1989) were followed up by biennial questionnaires regarding risk factors and health conditions and two supplementary questionnaires assessing exposure to violence, trauma, PTSD symptoms onset, severity and remision	Women exposed to trauma and those with PTSD presented a higher risk of CVD (OR 1.69, 95% CI1.08-2.63) Minimizing the symptoms of PTSD attenuates the cardiovascular risk
Gradus JL et al., 2015 [31]	Prospective study Examined the relationship between PTSD and adjustment disorder and four CVDs: myocardial infarction, stroke, ischaemic stroke and venous thromboembolism	4724	Data from Danish national registries were analysed	There is an association between PTSD and adjustment disorder and all four types of CVD
Remch M et al., 2018 [32]	Prospective study Determined PTSD as a risk factor for myocardial infarction and stroke	6481	Subjects were involved in cleaning the World Trade Centers debris PTSD and depression were self reported, and a 4 year follow up sought for myocardial infarction and stroke among subjects using interview or hospitalizations	The cummulative incidence of myocardial infarction or stroke was higher in PTSD patients
Vaccarino V et al., 2013 [33]	Prospective study Determined the association between PTSD and coronary heart disease	562	Subjects were monizygotic and dyzigotic male twins who were military active in Vietnam era subjected for a 13 years follow up At least one twin of each pair should have had PTSD or depresion or none of them for both but in either case, no previous CVD	In twins with PTSD the incidence of coronary heart disease was higher (p<0.001)
Vidal C et al., 2018 [34]	Retrospective study Determined the risk for CVD in different specific groups	10165	Two datasets that form part of the Collaborative Psychiatric Epidemiology Surveys - the National Latino and Asian American Study and the National Comorbidity Survey Replication were analyzed	Cardiovascular events rate was higher in patients with a diagnosis of PTSD: OR=2.10, 95% CI, 1.32-3.33; all ehnical groups, except for asian responders, had a higher likelihood of cardiovascular events associated with PTSD
Chen MH et al., 2015 [35]	Prospective study Assessed PTSD induced strok	5217	-	PTSD subjects have higher risk for any stroke (HR 3.37, 95% CI 2.44-4.67) and ischaemic stroke (HR=3.47, 95% CI 2.44-4.67)

Author	Study type and aim	No. of patients	Methodology	Results
Goldfinger JZ et al., 2014 [36]	Retrospective study Measured the prevalence of stroke induced by PTSD	535	PTSD Checklist Specific for stroke was applied to adult patients who suffered a stroke or a transient ischaemic attack within 4 years; a PTSD was diagnosed based on score of 50 or more	18% patients had a score of 50 or more Stroke induced by PTSD was more frequently in young patients
Scherrer JF et al., 2019 [37]	Retrospective study Sought for the relationship between PTSD and CVD based on other comorbid associations	11856	Data for 5940 patients with PTSD and 5916 without PTSD, aged 30-70 years, were obtained from electronic health records	PTSD depends on other factors to determine CVD: it was of signifficant statistic value just the association adjusted based on age (HR=1.41; 95% CI: 1.21-1.63) and metabolic conditions (HR=1.23; 95 CI:1.06-1.44)
Cyr S et al., 2021 [38]	Retrospective study Assessed the type of trauma inducing PTSD in patients with CVD	148	37 patients with PTSD and CVD were compared with 111 patients with adjustment disorder and CVD	In 51% cases PTSD was induced by a medical trauma
Spitzer C et al., 2020 [39]	Prospective study Assessed the relationship between PTSD and traumatic stress to carotid plaque and intima-media thickness	3119	General population based study with the patients subdivided in five age groups; each subject underwent a carotid arteries ultrasound PTSD and trauma exposure was assessed by interview	Carotid intima media thickness was higher in subjects with trauma experiences compared to the group without trauma exposure (p<0.001) In the group age 40-49 years carotid intima media thickness was higher in the PTSD group (p=0.005)

ABPM: arterial blood pressure measuement; ACS: acute coronary syndrome; AMI: acute myocardial infarction; CA: cardiac arrest; CHF: congestive heart failure; CIMT: Carotid Intima-Media Thickness; COPD: chronic obstructive pulmonary disease; CVD: CVD; ED: emergency department; HR: heart rate; HRV: heart variability rate; ICD: implantable cardiac deffibrilator; ICH: intracerebral hemorrhage; MACE: Major Adverse Cardiovascular Events; MI: myocardial infarction; PTSD: PTSD; PTSS: posttraumatic stress symptoms; VRE: virtual reality exposure.

DISCUSSIONS

1. PTSD and cardiovascular disease – mechanisms and pathways

1.1. General mechanisms

Emerging literature (Table 2) postulates that there are several mechanisms that contribute to the occurrence of cardiovascular disorders in people suffering from PTSD, none of them acting alone but rather together (Figure 2). It is already known that anxiety and depression as well as other psychosocial factors are modifiers of the calculated total cardiovascular risk as we find in the CVD prevention guidelines [41]. These eventually lead to poor health behavior: sedentary life, obesity, smoking and alcohol consumption that contribute to hypertension, diabetes mellitus and dyslipidemia.

Figure 2: Mechanisms of the PTSD-cardiovascular disease relationship. IL6: interleukin 6; PTSD: Posttraumatic stress disorder

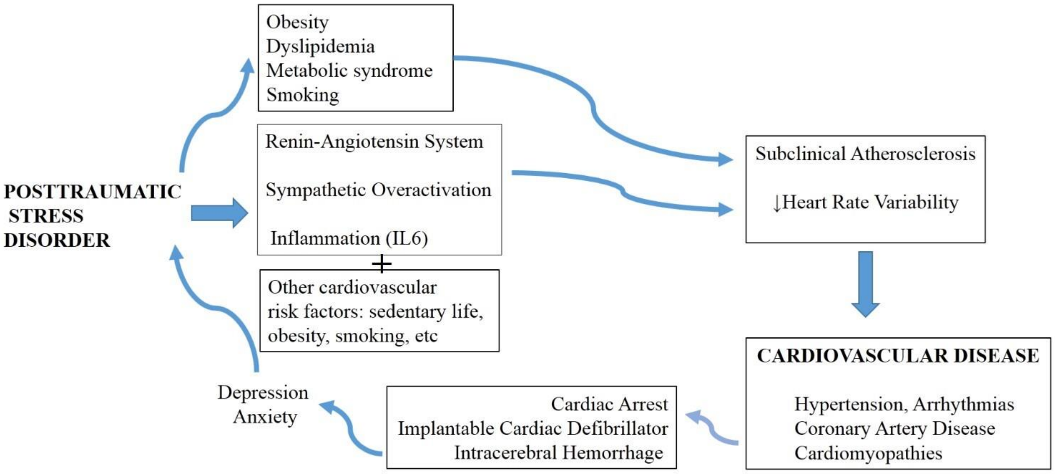
Obesity
Dyslipidemia
Metabolic syndrome
Smoking
Renin-Angiotensin System
Sympathetic Overactivation
Inflammation (IL6)
+
Other cardiovascular risk factors: sedentary life, obesity, smoking, etc
POSTTRAUMATIC STRESS DISORDER
Subclinical Atherosclerosis
↓Heart Rate Variability
CARDIOVASCULAR DISEASE
Hypertension, Arrhythmias
Coronary Artery Disease
Cardiomyopathies
Cardiac Arrest
Implantable Cardiac Defibrillator
Intracerebral Hemorrhage
Depression
Anxiety

Table 2: Studies for general mechanisms and pathways in PTSD generating cardiovascular disease

Author, year	Conclusion
Lima BB et al., 2019, (12)	MI patients with PTSD exhibit an increased inflammatory response to stress (enhanced IL-6 response), suggesting a mechanistic link between PTSD and adverse cardiovascular outcomes.
Brudey C et al., 2015 (40)	This study demonstrates a link between PTSD and CVD. The renin-angiotensin system, sympathetic overactivation and inflammation contribute to compromised cardiovascular health in PTSD.

CVD: cardiovascular disease; MI: myocardial infarction; PTSD: Posttraumatic stress disorder; IL-6: interleukin 6

Chronic systemic inflammation associated with PTSD is the cause for accelerated atherosclerosis that causes vascular disease (peripheral, cerebrovascular) as well as coronary artery disease. Studies have shown that in patients with PTSD there is an imbalance between proinflammatory and anti-inflammatory cytokines in favor of the first ones, with high levels of IL-1β, TNFα, IL-6 and CRP and low levels of IL-4 and IL-10 [42]. In accordance to this pathway, Lima and collaborators measured the levels of inflammaftory markers in survivors of a myocardial infarction with and without PTSD and found out that, after using a mental stress test (public speaking), levels of IL-6 were 68% higher in the PTSD group (p=0.01) [12].

Exposure to a chronic stress and inflammatory state as that in PTSD induces cardiac structural changes at the level of gap junctions. Studies have shown a decrease in the gap junction protein connexin 43 (Cx43) that is physiologically involved in intercellular communication and thus in action potential conduction through the heart [43]. Decreased levels of Cx43 may be involved in triggering cardiac arrhythmia since this protein is present in both atrial and ventricular cardiomyocytes. Neuroendocrine disturbances are also involved in increasing the risk for CVD. In people suffering from PTSD there is an increased activity of renin-angiotensin system as well as overactivity of sympathetic nervous system, these two together contributing to structural cardiovascular changes, hypertension and its consequences [40].

1.2. PTSD as a two edge sword – PTSD induced by cardiovascular disease

More recently, PTSD is seen as a two edge sword because emerging literature is determining that CVD are acting as its triggers (Table 3). Vice versa, in cardiac patients suffering from an acute coronary event, PTSD can occur after the event. Meli L and her collaborators evaluated the association between PTSD symptoms and age during acute coronary syndrome evaluation in the emergency department.

Table 3: Studies debating cardiovascular disease induced stroke

Author, year	Conclusion
Schultebraucks et al., 2020 [44]	Screening for stroke-induced PTSD in the emergency department has an important role in the potential implementation of primary and secondary interventions to improve patient outcomes.
Chang J-C et al., 2017 [7]	Cardiovascular heart disease, bipolar disorder and majore depressive disorder are associated with an increased risk of developing PTSD.
Meli L et al., 2018 [8]	Younger patients are at risk for greater posttraumatic stress symptoms than older ones, after acute coronary syndrome evaluation in the emergency department.
Lima BB et al., 2020 [11]	In myocardial infarction survivors, PTSD is associated with developing ischemia with mental stress. Traumatic reminders can provoke frequent episodes of mental stress-induced myocardial ischemia, aspect that can lead to worse cardiovascular outcomes in these patients.
von Känel R et al., 2021 [14]	The preventive one-session intervention of trauma-focused counseling had no effect in myocardial infarction survivors (posttraumatic stress symptoms were persistent in the first year after the event).
Agarwal S et al., 2019 [15]	PTSD symptoms are frequent after cardiac arrest. Cardiac arest -induced PTSS is associated with elevated mortality and cardiovascular events.
Jiang C, 2018 [17]	Intracerebral haemorrhage is associated with an increased risk for PTSD in the chinese population, especialy in those who are female, more severely disabled, or received minimally invasive surgery.
von Känel R et al., 2018 [19]	In myocardial infarction survivors, trauma-focused counseling had no effect in preventing acute coronary syndome-induced PTSD.
Greenman PS et al., 2018 [45]	Mental health history, the tendency to stifle negative emotional experiences (known as ‘‘type D personality’’), and social support in patients with heart disease are related to the onset of or protection from depression, anxiety, and posttraumatic stress.
Baranyi A et al., 2021 [25]	An elevated TMAO level might be a biological marker of acute myocardial infarction-related severe stress that is associated with vulnerability to PTSD.
Eslami B et al., 2017 [26]	Half of the adults with congenital heart disease present PTSD diagnosis, which is associated with elevated depressive symptoms and history of cardiac surgery.
Ratcliff CG et al., 2017 [27]	Veterans with cardiopulmonary disease experiencing mental health problems are

	underrecognized and improvment of menthal health screening is necessary.
Jane Irvine J et al., 2011 [28]	The cognitive behavior therapy intervention in combination with usual cardiac care is associated with a greater improvment in psychological functioning of implantable cardiac defibrillator patients.
Shemesh E et al., 2006 [29]	PTSD symptoms and cardovascular risk improved in individuals who received trauma-focused cognitive-behavior treatment.
Edmondson D et al., 2012 [46]	Symptoms of acute coronary syndrome induced PTSD are moderately prevalent and are associated with increased risk for recurrent cardiac events and mortality.

PTSD: Posttraumatic stress disorder; PTSS: posttraumatic syndrome symptoms; TMAO: Trimethylamine N-Oxide

They found that younger versus older subjects are at risk for greater posttraumatic stress symptoms after acute coronary syndrome evaluation and elevated perceived threat partially mediated this association (p < .0001) [8].

An observational prospective cohort study (114 subjects) compared prognosis (1-year all-cause mortality and major adverse cardiovascular events) in cardiac arrest survivors with and without PTSD symptomatology at hospital discharge. After adjustment for demographic and medical covariates, they concluded that cardiac arrest-induced PTSD is associated with greater risk of death and significant cardiovascular risk (HR=3.2; CI, 1.7–6.1). Cardiac arrest induced-PTSD may be considered a distinct type of medical-event induced PTSD, because cardiac arrest survivors rarely remember the event or early post-event period [15].

Recent studies are focusing on acute coronary syndrome-induced PTSD prevention. von Känel and his collaborators concluded that self-rated posttraumatic stress symptoms indicated less beneficial effects with single session trauma-focused counseling versus active control intervention [19]. Implantable cardiac defibrillator (ICD) carriers may develop PTSD and a study conducted on 193 ICD patients showed that cognitive behavior therapy intervention in combination with usual cardiac care is associated with a greater improvment in psychological functioning of these patients [28].

Cerebrovascular pathology can induce PTSD. A study on 64 chinese subjects showed that intracerebral hemorrhage is associated with increased risk of PTSD and the more likely profile of these patients is: female, more severely disabled and subjects that received minimally invasive surgery [17]. Goldfinger measured the prevalence of stroke induced PTSD in patients with a history of stroke or transient ischemic attack within 5 years (mean age was 63 years) and defined the portrait of stroke-induced PTSD patient: young, disabled, with recurrent strokes and comorbidities [36].

A study conducted by Eslami compared the prevalence of PTSD among adults with (347 adults) and without (353 adults) congenital heart disease and found that half of the congenital heart disease patients presented a likely PTSD diagnosis, which was associated with greater depressive symptoms (OR=1.27) and history of cardiac surgery (OR=2.02) [26].

2. PSTD and cardiovascular disease

2.1. PTSD and hypertension

As we know hypertension is a cardiovascular risk factor and also a disorder itself that results from the addition of other risk factors (obesity, sedentarism, smoking, dyslipidemia, alcohol abuse) that, in subjects suffering from PTSD prevail due to bad habits that this people have as a mechanism of coping. Hypertension is the second most studied cardiovascular associated disease in PTSD, after coronary artery disease.

Table 4: PTSD and hypertension

Author, year	Conclusion
Burg MM et al., 2017 [5]	Patients with untreated PTSD have a greater risk to develop incident hypertension.
Chen YY et al., 2021 [21]	PTSD may be a risk factor for elevated blood pressure and heart rate.
Cave B et al., 2019 [22]	There were no differences in distribution of dipping patterns and rates of overall, awake,and nocturnal hypertension in veterans with PTSD compared with the general veteran population.
Sumner JA et al., 2021 [47]	Significant effects of PTSD symptoms were detected for SBP and DBP.

DBP: diastolic blood pressure; PTSD: Posttraumatic stress disorder; SBP: systolic blood pressure

The main studies attributed to hypertension as a consequence of PTSD or a stressful event are presented in Table 4. Burg MM et al. investigated the relationship between PTSD and incident hypertension in a large, nationally representative sample of almost 200,000 Veterans of Operations Iraqi Freedom, Enduring Freedom, and New Dawn and proved that patients with untreated PTSD have a 24-46% greater risk for incident hypertension [5]. Interestingly, another study showed that systolic blood pressure and heart rate modifications present a linear relation with the number of trauma episodes (p =0.003 and p=0.046 respectively), aspect that strengthens the supposition that PTSD is linked to CVD [21]. Despite these evidences, the study of Cave found that there were no differences in distribution of dipping patterns and rates of overall, awake and nocturnal hypertension in veterans with PTSD compared with the general veteran population, but this study had important limitations (retrospective design; variable dipping statuses were not evaluated) [22].

2.2. PTSD and coronary artery disease

Coronary artery disease is the most studied associated pathology to PTSD (Table 5). Proinflammatory status and hyperlipidemia associated with chronic stress contribute to accelerating the process of atherosclerosis thus leading to coronary events. Remch and collaborators found that World Trade Center attack–related PTSD is a risk factor for myocardial infarction and stroke with the same impact in men and women, independent of depression and traditional cardiovascular risk factors [32]. Behavioral mechanisms (smoking, obesity, alcohol overuse) may represent the link between PTSD and elevated risk of coronary artery disease, but the majority of the included studies made adjustments for these variables. All selected studies concluded that PTSD is independently associated with increased risk for incident coronary heart disease and it may be a modifiable risk factor for this pathology [32, 33, 48].

Table 5: PTSD and coronary artery disease

Author, year	Conclusion
Akosile W et al., 2018 [49]	This study demonstrates a possible association between PTSD and incident coronary artery disease.
Gradus JL et al., 2015 [31]	Stress-related psychopathology (including PTSD) is associated with CVD events (myocardial infarction, stroke, ischaemic stroke, venous thromboembolism).
Edmondson D et al., 2013 [48]	PTSD is independently associated with increased risk for incident coronary heart disease and it may be a modifiable risk factor for coronary heart disease.
Remch M et al., 2018 [32]	WTC attack–related PTSD is a risk factor for myocardial infarction and stroke of similar magnitude in men and women, independent of depression and traditional cardiovascular risk factors.
Vaccarino V et al., 2013 [33]	Among Vietnam-era veterans, PTSD is associated with an increased risk of coronary heart disease. This risk is not explained by adverse health behaviors, established risk factors for coronary heart disease or by familial risk factors.

PTSD: posttraumatic syndrome disorder; WTC: World Trade Center

2.3. PTSD and other cardiovascular disease

Stroke represents the leading cause of long term disability and in the same time the leading preventable cause of disability. The mechanisms of the relationship between PTSD and incident stroke are not completely understood until now in the literature (Table 6). One theory is that PTSD implies chronic inflammation, endothelial dysfunction, platelet activation and aggregation, autonomic dysregulation and all of these may have a significant role to atherothrombotic manifestations [9]. The association between PTSD and incident transient ischemic attack (TIA) and ischemic stroke was assessed in a cohort of 987855 veterans (mean age of 30.29±9.19 years) which found that PTSD is associated with a significant increased risk of early incident TIA (HR, 1.61; 95% CI,1.27–2.04) and ischemic stroke (HR, 1.36; 95% CI,1.22–1.52), risk that persisted after adjustment for established stroke risk factors, coexisting psychiatric disorders and healthcare utilization. Also, the same study concluded that PTSD may be a risk factor for stroke at younger ages and also a target for stroke prevention strategies in this category of patients [9].

Table 6: PTSD and other cardiovascular disease

Author, year	Conclusion
Rosman L et al., 2019 [9]	PTSD is independently associated with increased risk for early incident TIA and ischemic stroke in young and middle-aged Veterans. Sex moderated the relationship for adults with ischemic stroke (the effect of PTSD on ischemic stroke risk was stronger in men than in women) but no effect of sex in case of TIA.
Yu S et al., 2018 [24]	9/11-related PTSD and intense dust exposure were each independently associated with an increased risk of stroke.
Kastaun S. et al., 2016 [50]	Studies supporting a link between psychiatric disorders and Takotsubo syndrome. Anxiety and depressive disorders seemed to be more common in patients with Takotsubo syndrome than patients with ST-segment elevation myocardial infarction. Major depression and generalized anxiety disorder were also diagnosed more often in the Takotsubo syndrome cohort than in patients with acute coronary syndrome. Optimal management and treatment of Takotsubo syndrome should involve integration of the psychoneuroendocrinological aspects of the syndrome with the well-established functional abnormalities of the disease.
Edmondson D et al., 2013 [51]	Stroke-induced PTSD is common (1 in 4 stroke/ transient ischemic attack survivors in the first year develop PTSD symptoms, and after that 1 in 9 may experience chronic PTSD) and may represent a new risk factor for recurrent stroke.

Gradus JL et al., 2015 [31]	Stress-related psychopathology (including PTSD) is associated with CVD events (myocardial infarction, stroke, ischaemic stroke, venous thromboembolism).
Remch M et al., 2018 [32]	WTC attack–related PTSD is a risk factor for myocardial infarction and stroke of similar magnitude in men and women, independent of depression and traditional cardiovascular risk factors.
Chen MH et al., 2015 [35]	Patients with PTSD have an increased risk of developing any stroke and ischaemic stroke.
Goldfinger JZ et al. [36]	Stroke-induced PTSD is related with younger age, recurrent strokes, greater disability, and comorbidities.
Perkins JD et al., 2021 [52]	PTSD independently predicts stroke and is seems to be linked to many major stroke risk factors.

PTSD: Posttraumatic stress disorder; PTSS: posttraumatic syndrome symptoms; TMAO: Trimethylamine N-Oxide

Another cohort study that included 6481 subjects who were involved in cleaning the World Trade Centers debris, concluded that PTSD is a potent determinant for myocardial infarction and stroke of similar importance in men and women, independent of depression and traditional cardiovascular risk factors [32]. A longitudinal follow-up study (5217 patients with PTSD and 20868 age – and gender-matched controls; mean age 36.65 years with 79.1% women) aimed to determine the temporal association between PTSD and stroke and found that the PTSD goup presented an increased incidence of any stroke (3.82 versus 1.00 per 1000 person-years, P<0.001) or ischemic stroke (2.07 versus 0.53 per 1000 person-years, P<0.001) at a younger age compared to control group [35].

2.4. PTSD and arrhythmia

The relationship between PTSD and potential arrrhythmia is yet an unknown field because most studies are focusing on other aspects of CVD related to PTSD (Table 7). Trials looking forward to bring in attention this link are rather aiming for changes in heart rate variability (HRV) as a consequence to PTSD than to look for specific arrhythmia. As presented by Dennis and his collaborators in their study conducted on 227 subjects, 107 of whom were diagnosed with PTSD (49 of which were veterans), those in the late category presented a decreased HRV (p=0.002). Interestingly, despite the fact that sleep disorders, abdominal obesity and poor health behavior (alcohol consumption and smoking) were more frequently encountered in the PTSD group compared to nonPTSD group (p<0.001 for each), when adjusting for each of it, analysis was either non-significant (as in the case of sleep disorders and abdominal obesity) or the relationship between PTSD symptoms and decreased HRV was attenuated (for smoking and alcohol consumption) yet, when taken together (smoking, sleep disorder and alcohol consumption), they were responsible for 94% of the effects of PTSD symptoms on decreasing HRV [6].

Table 7: PTSD and arrhythmias

Author, year	Conclusion
Bourassa KJ et al., 2019 [4]	The exposure therapy (prolonged exposure or virtual reality exposure) for PTSD may reduce cardiovascular risk in these patients, improving cardiovascular functioning.
Dennis PA et al., 2014 [6]	The behavioral health risks (smoking, alcohol overuse, sleep disturbance) mediate the association between PTSD and HRV. A healthy lifestyle may enhance the benefits from psychiatric and psychological interventions in PTSD.
Ge F et al.. 2019 [54]	The reduced HRV may be regarded as an endophenotype in PTSD research.
Rosman L et al., 2019 [18]	In this cohort of young and middle-aged veterans, PTSD is associated with an increased risk for early incident atrial fibrillation.
Seligowski AV et al., 2020 [20]	Fear bradycardia may be indicative of greater vagal control (i.e., HRV), both of which are psychophysiological biomarkers that may influence cardiovascular and autonomic disease risk in trauma-exposed individuals.

PTSD: Posttraumatic stress disorder; HRV: heart rate variability

More recently, a prospective study of middle age veterans sought for atrial fibrillation and found out that those with a diagnosis of PTSD had a higher incidence of it (p<0.0001) in unadjusted and adjusted models. Oddly, development of atrial fibrillation was associated with younger ages (p=0.0004) [18].

Arrhythmia may be more frequently in people suffering from PTSD due to a few aspects which are substracted from the former studies: first, there is an increase in sympathetic activity as the PTSD was proved to be associated with increased plasma levels of norepinephrine [53]; secondly, PTSD may generate an unfavorable lifestyle with increasing obesity which favors the appearance of obstructive sleep apnea that, in turns, is also responsible for increasing the sympathetic activity and thirdly, this poor health behavior along with sympathetic overactivity increases the risk of coronary artery disease which can generate life threatening arrhythmia.

3. PTSD – modifiable cardiovascular risk factors and prophilaxy

Posttraumatic stress disorder is associated with a greater risk of cardiometabolic disease (Table 8) and the involved mechanisms are complex: increased inflammation, dysfunction of autonomic nervous system and of hypothalamic-pituitary-adrenal axis, poor behavioral factors (alcohol overuse, smoking, sedentary lifestyle, increased caloric intake) [55]. Rosenbaum's study reinforces this supposition by founding that PTSD patients have a greater risk to develop metabolic syndrome compared to age and sex matched controls (almost twice) and a routine screening for behavioral factors plus an adequate management is needed [56]. Obesity in relationship with PTSD was evaluated in a group of 54224 subjects (ages 24-44 years in 1989) which concluded that women with at least 4 PTSD symptoms had a greater risk of becoming overweight ($p < .001$) [3]. The authors concluded that PTSD treatment should include correction of behavioral alteration and women with a PTSD diagnosis may undergo screening for development of adverse cardiometabolic outcomes [3].

Table 8: PTSD – modifyable cardiovascular risk factors and prophilaxy

Author, year	Conclusion
Bartoli F et al., 2020 [57]	PTSD may be considered a systemic condition and assesment, monitoring and treatment of metabolic variations may determine a significant improvement in its management and prognosis.
Kubzansky LD et al., 2014 [3]	PTSD is associated with an elevated risk of becoming overwight/obese.
Bartoli F et al., 2013 [58]	PTSD is associated with a greater risk of metabolic syndrome.
Goetz M et al., 2014 [16]	Among Vietnam War–era veterans, PTSD is associated with carotid artery intima-media thickness (measure of subclinical atherosclerosis), though the association is largely mediated by shared childhood factors.
Campara TM et al., 2014 [23]	Elevated levels of cholesterol and triglycerides were found in the blood serum of the policemen who have spent the entire war in Sarajevo (experimental), compared to the police officers who spent the war in other areas of Bosnia and Herzegovina (control group). The war determine changes in lipid profile of persons employed at risk professions, such as police officers.
van den Berk-Clark C et al., 2018 [55]	PTSD is associated with reduced healthy eating and physical activity, and increased obesity and smoking. The relation between PTSD, metabolic and CVD may be due to poor diet, sedentary lifestyle, high prevalence of obesity and co-occurring smoking in these patients.
Rosenbaum S et al., 2015 [56]	PTSD is associated with metabolic syndrome (PTSD patients are almost twice as likely to have metabolic syndrome compared to age and sex matched controls).
Spitzer C et al., 2020 [39]	Association between PTSD and atherosclerosis is inconclusive.
Chen YY et al., 2021 [21]	PTSD may be a risk factor for elevated blood pressure and heart rate.
Solter V et al., 2002 [59]	Patients with PTSD have a higher risk of atherosclerosis.

CVD: cardiovascular disease; PTSD: Posttraumatic stress disorder

CONCLUSIONS

Posttraumatic stress disorder has a major impact on the lifestyle and health of subjects exposed to trauma or stress. Cardiovascular disease tend to appear sooner in PTSD diagnosed patients due to a variety of reasons: poor habits, low addressability to health care systems, chronic inflammation status, changes at molecular levels, etc. Otherwise, PTSD tends to be induced by CVD, thus inclining the balance towards whis association. Prevention of PTSD by proper psychological and/or psychiatric intervention in subjects exposed to trauma or stress, including that generated by CVD, might be of help in preventing the appearance or worsening of cardiovascular disorders in this population. Regarding arrhythmic events, we suspect that, PTSD patients may be at risk of developing arrhythmic events, atrial fibrillation probably being the most common. In the light of the above mentioned facts, looking for arrhythmic patients in people suffering from PTSD may be of particular interest in future studies.

Study limitations

Most of the studies analyzed CVD events (defined as myocardial infarction, stroke, ischaemic stroke, venous thromboembolism) not separately on each CVD. Due to the inhomogeneity of the data about CVD type associated with PTSD and the limited data on each type of CVD type (hypertension, coronary artery disease, arrhythmias, or cardiomyopathies like Takotsubo syndrome) and the parameters that was analyzed for this CVD, there was not possible to make an extensive statistical analysis.

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Major radiological threats and emergencies requiring public health preparedness (the example of Greece)

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Abstract: *Radiological emergencies carry the potential for major disruptions to public health care provision, due to their infrequency, unpredicted nature, delay in appearance of adverse effects and the grave psychological impact on sufferers and society. This study aims to describe and analyze the main categories of radiological hazards and emergencies that require hospital response and preparedness at a nation-wide level, taking into specific account the example of Greece. These radiological threats are either accidental, like reactor emergencies and accidents in hospital or industrial units implementing radiological sources of high activities, or deliberate, like detonation of nuclear devices and malicious use of various radionuclides.*

Keywords: *public health, radiological incidents, radiation emergencies, preparedness, nuclear terrorism*

INTRODUCTION

Even though natural disasters and biological hazards are currently the most frequent hazards for massive casualties, recent data suggest that radiological emergencies from various nuclear incidents may not be a reassuringly remote possibility. Till the year 2015, more than 420 radiation incidents of various degrees of severity have occurred, thus surging the global preparedness to a steady ascent [1], whilst specific deficits in training and experience may lead to devastating increase in the victims and mortality rate in the case of a major radiological event, either accidental or malicious. Furthermore, there are roughly ten million sealed sources of radionuclides used for peaceful applications worldwide; only between 1995 and 2008, more than 600 of them were either lost or stolen and less than the half were eventually retrieved [2].

COMMONEST TYPES OF RADIATION EMERGENCIES

Greece does not have a nuclear power industry; thus, Greek radiation preparedness is directed mainly to radiological scenarios of low probability, with rather minor health and social impact compared to major accidents from power reactors. However, a wide variety of radioactive sources is used in medicine, industry and research. Several types of severe radiological emergencies that would necessitate a special medical response are bellow analyzed in detail.

Nuclear detonation

The detonation of a nuclear weapon constitutes the most catastrophic radiological scenario; the enormous number of casualties from the shock-wave, the thermal burns and the ionizing radiation would probably lead the health network of any urban target in total collapse. Although the probability of a terrorist attack involving the detonation of a nuclear device is considered rather unlikely, the scenario of a single, low-yield nuclear weapon, stolen from a military facility, for a major attack in a densely populated modern metropolis, appears as a quite feasible and thinkable perspective of the 21st century’s terrorism, according to US strategic analysts [3].

Reactor Emergencies

Following a reactor emergency, it is expected that a radiological contamination will occur to the surrounding environment and also external exposure and contamination through inhalation or ingestion may affect the general

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public. Although many radionuclides are produced in a nuclear accident, the most important to human health are cesium-137 (Cs-137), strontium-90 (Sr-90) and iodine-131 (I-131) [4]. Apart from radioactivity, reactor emergencies may disperse long-lasting psychological consequences to enormously large populations [5].

The two most catastrophic reactor emergencies were the case of Chernobyl, Ukraine (April 1986), when enormous quantities of radioactive contaminants were dispersed across all continents [6], causing an extensive increase in neoplastic diseases and genetic malformations to the affected population [7], and that of Fukushima Daiichi, Japan (March 2011) which affected heavily the pacific ocean [8]. The size of the consequences of this kind of radiological incidents is vastly influenced by the meteorological conditions (mainly winds) prevailing in the area that would facilitate the movement of the contaminated cloud and deposits; in 1986 disaster, it took only ten days for the radio-contaminated cloud from Chernobyl nuclear power plant to cross southern Europe and arrive in Greece. Therefore, all nuclear power plants and radiological waste storage sites in Europe pose an existing threat. Yet, seismic activity is another factor that needs to be considered, especially concerning the seismogenic arc of the Asia Minor coast. Regarding the Balkan neighbors of Greece:

a. There are currently no active nuclear power plants in Greek territory and the low-power experimental reactor supervised by the Greek Institute of Nuclear & Radiological Sciences and Technology is under permanent non-operation state since 2001.

b. Bulgaria operates one of the oldest commercial nuclear reactors in Europe in Kozloduy since 1974 and according to the Belgium report [9] the vulnerability to failure and accidents of such units has steadily grown in the last years. In Germany, an accident in a poorly maintained nuclear facility out-borders is regarded the most probable major radiation incident [10]. Yet, as the effects of nuclear disasters have no borders, that old Bulgarian unit located only 260 km far from the northern borders of Greece, poses a significant radiological threat for all Balkan countries. The construction of a second nuclear unit in Belene was officially terminated in 2012 [11].

c. Romania operates a nuclear power plant in Cernavodae, located 325 km far from the northern borders of Greece (Figure 1), and in the indefinite future it plans to construct a second, larger unit probably in Transylvania.

d. Turkey's first nuclear power plant, at Akkuyu, commenced construction in April 2018. Although rather remote from the Hellenic inland, a severe radiological accident in Akkuyu will heavily affect Cyprus (Figure 2). Plans for two more power stations in Sinop (Northern Turkey, by the Black Sea) and İğneadain (in the European part of Turkey) have currently stuck, mainly due to economic reasons [12].

Nonetheless, since there are so many nuclear power plants, nuclear weapons and nuclear waste storage facilities within EU member states, advanced preparedness and countermeasures are necessary, under the threat of a European version of the 9/11 scenario, presumably in a more sophisticated implementation, e.g. a cyber-sabotage that would affect or interfere in the function of a nuclear plant (e.g. in the cooling or control sections).

Figure 1: Nuclear power plants neighboring to Greece; the green islets illustrate seismogenic locations during the period 1972-1997

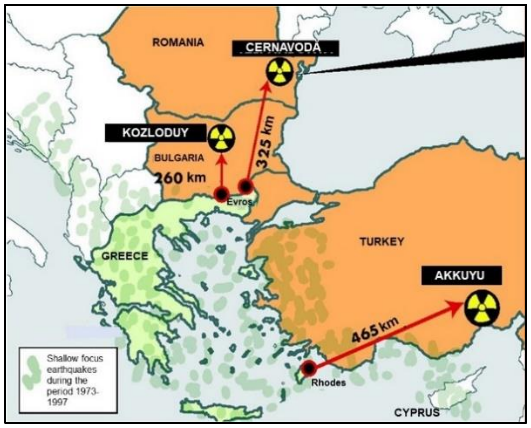

Figure 2: In the scenario of a severe radiological accident in the planned-to-operate nuclear power plant in Akkuyu, Cyprus would be inside the red zone of the disaster.

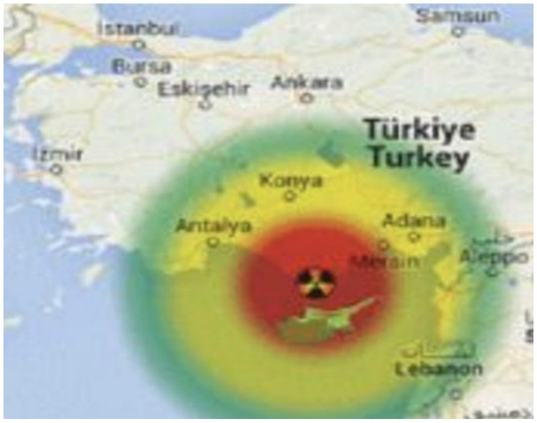

Accidental Medical overexposure

Medical teletherapy devices, though currently outdated by linear accelerators, are still used in many hospitals worldwide for cancer radiotherapy, by applying high energy gamma radiation focused on tumor targets. These devices typically use a single Co-60 source in a solid metal form, though a limited number of teletherapy devices use Cs-137 sources, which are in the form of a water soluble chloride salt, a much more dangerously prone to dispersion chemical form, thus making easier the contamination of people and environment [13]. The teletherapy device is securely encapsulated in heavy shielding and only highly trained personnel may operate it, because it poses extreme hazard if not properly handled. According to the decay energy and the physical half-life of the radionuclide, the equivalent dose for that radionuclide is 13 mSv per Ci per hour at 1 meter from the source; that may hypothetically lead a person to receive a lethal dose from a full 10,000 Ci unshielded Co-60 source when standing at one meter distance, within only a few minutes [14]. More realistic are of course scenarios of accidental medical overexposure from Co-60 teletherapy devices due to miscalculation of the radiotherapy treatment planning or improper function of such devices, or even when higher activities of radionuclides are accidentally administered in Nuclear Medicine departments. Although all above situations are considered as serious medical problems, they may not be classified as radiological incidents requiring hospital preparedness, because medical and radiation protection guidelines are regularly provided by health and physics specialists in each hospital.

Abandoned, lost or stolen radiation sources

Radiation sources may be improperly discarded, accidentally lost or even stolen for financial or malicious reasons. In many historical cases, decommissioned teletherapy units were unshielded, as a rule by people irrelevant to radiological education; the radioactive source was then stored or discarded and the metal shielding was scrapped. As a consequence of that, the perpetrators and sometimes the public, were either externally exposed to various levels of radiation or/and were heavily contaminated, resulting in serious injuries, illness or even death.

The worst of that kind of accidents occurred in 1987 in Goiânia, Brazil when an abandoned teletherapy source of a long-lived beta and gamma emitter, Cs-137 with activity of 1375 Ci, was unshielded by a group of metal scrapers. Four people received lethal doses of radiation, 28 suffered radiation injuries and 249 had been contaminated [15]. The delay in the verification of the radiological incident led to an uncontrolled and widespread radiological contamination in the urban district of Goiânia, including buildings, vehicles and soil [16].

Another potential radiological hazard comes from blood irradiators; these heavy-shielded devices contain Cs-137 with activity of 3,000-7,000 Ci [13] and are used in hospitals for the irradiation of blood prior to infusion. The most significant radiological incident concerning this type of device occurred in 2011 in a hospital in Finland where a blood irradiator containing 60.1 Tbq Cs-137 was exposed in high temperatures after a fire outbreak. Although no dangerous radioactivity was unleashed, quite a lot shortages in the flow of crisis information among the first responders, the radiation safety officer and the radiation safety authority were afterwards ascertained; furthermore defective preventive, storage and shielding protocols of such dangerous radiation sources were among the many lessons learned in the aftermath of a nearly disaster [17].

Incidents from dangerous industrial sources

Apart from medical sources, there are currently several hundreds of large industrial facilities worldwide that utilize high activities of ionizing radiation. The most dangerous of such industrial facilities is the gamma sterilization plant; such irradiation plants are usually located in industrial areas and are used to sterilize various products, most often food and medical devices, by using extremely high doses of ionizing radiation. A typical irradiation plant includes a large number of high activity Co-60 rod sources, housed in an array immersed in a water pool inside a shielded chamber.

The product to be irradiated is exposed by remote control to the sources for the time required to kill bacteria. Indicative of the high activities that are used in industrial irradiation is that a typical such facility contains from hundreds of thousands, up to some millions curies of Co-60 [13], that is roughly a thousand times more than the typical activity of a Co-60 medical teletherapy source.

Therefore, health emergencies occurring in industrial irradiation plants often lead to lethal levels of whole body absorbed doses after exposure of just some seconds [18]. Although such plants are highly secured and physically inviolable, operating according to globally standardized specifications and their safety to be established through decades of operational experience, training, supervision and safety systems technology, some sporadic serious accidents have taken place, like in Italy, 1975; Norway, 1982; [19]; El Salvador, 1989 [20]; Soreq, Israel, 1990 [19] and Nesvizh, Belarus, 1991 [18].

The thorough evaluation of all foreseeable hazards resulting from industrial facilities that use ionizing radiation needs to consider natural and anthropogenic disasters, failures in installation, operation procedures, supplies, machinery, technological infrastructures and safety education. Importantly also, users of radioactive material with longer experience possessed a surprisingly lower degree of nuclear security awareness compared to less experienced ones [21].

Malicious use of radioactive materials

Nowadays, it is widely accepted that international terrorism may have acquired the resources and technical capabilities to obtain or manufacture improvised nuclear devices or other types of radiological dispersal devices coupling conventional explosives and radioactive material [22]. The latter are known as “dirty bombs” and are much easier to be prepared than a nuclear detonation device and yet the raw materials can be found in industry, hospitals or research institutes. The appropriateness of a radiological source for malevolent applications depends on various factors like the size, accessibility and ease of portability of the source, the total radioactivity and physical half-life of the radionuclide it contains, the type of the radiation emitted (for example gamma emitters are suitable for external exposure, whilst alpha emitters only for radiological contamination) and also the physical and chemical form of the radioactive material [5]. For practical reasons the radioactive sources suitable for nuclear terrorism are classified into two general categories:

a. *Radiation exposure devices (RED)*; these usually involve small-sized sources with radioactive material emitting gamma radiation. In RED, the primary radiation source(s) is/are in a relatively intact physical state and shape, thus the radiological threat comes rather from external radiation exposure and usually raises minimal or no aspects of disseminated contamination. When in large quantity, like for example in the case of an unshielded medical teletherapy Co-60 source, the RED raises hazards of acute or chronic radiation syndromes to the victims. When of small quantity, the exposure dose to individuals is expected to be too low to allow for acute effects; however, the psychosocial fear of future cancers raises concerns of diffuse or uncontrolled public anxiety.

b. *Radiation dispersal devices (RDD)*; these involve larger quantity of radioactive material which is dispersed in micro-granular, airborne or water soluble form (as is usually the case for the radio-cesium chloride salt) over large areas, often by the use of conventional explosives, coupled with the radioactive substance. In this way, the contamination risk, either external (at the environment, skin, or cloths), or internal (by ingestion or inhalation) usually overwhelms the exposure threat. Furthermore, RDD carry the potential to cause complicated injuries, like blast and burn traumas from a chemical explosion combined with radiological contamination. And again, the psychological impact of such an attack, especially when affecting a large urban area, might provoke extensive economic and social disruption to a whole country.

Various radionuclides require enhanced nuclear security measures, since even gram or milligram quantities of some, assuming perfectly dispersed, might be enough to contaminate large areas above acceptable levels for habitation. The number of affected people in such a scenario also varies, according to the type of the bomb, the quantity of the nuclear material, the time and location of the attack. According to a scenario of U.S. Homeland Security Council [23], the concurrent blast of Cs-137 “dirty bombs” at three different locations in a densely populated urban area might cause 180 fatalities, 270 injured and 20,000 contaminated people at each site; hundreds of radiation induced cancers would also occur in the future unless tens of thousands people were relocated from the city [10]. The most common radionuclides that might be used in a RDD with corresponding thresholds to contaminate 1 square km are presented in Table 1.

Table 1: Common radionuclides that might be used in a RDD

Radionuclide	Threshold (grams) to contaminate 1 square km*
Co-60	0.01
Ir-192	0.01
Sr-90	0.37
Cs-137	0.48
Ra-226	13
Am-241	22.7
Pu-239	220

** refers to the theoretical amount of radionuclides in pure and perfectly dispersed form required to contaminate that area to the level that a person living there for one year would receive a cumulative dose of 20 mSv*

Concerning Po-210, there is always the threat of targeted assassinations like the case of Alexander Litvinenko, the first recorded victim of Po-210 radiation poisoning [24]; much scarier though is the theoretical scenario of a massive food poisoning, e.g. in grand hospitals, congress venues, cruise ships, etc. with lethal or pathogenic doses of radionuclides. Radio-contamination of the water is a far remoter perspective, due to both the sophisticated control mechanisms in central water supply reservoirs of the modern Metropolises and the vast quantities of radiological factors required to cause significant contamination of the water reservoirs. However, that is not always the case in smaller tanks irrigating small communities or sensitive infrastructures (e.g. hospitals) In any case crisis communication will play a significant role in dealing with such incidents, which may well overwhelm the health capacity and capability of the whole national medical network.

Furthermore, the enormous financial disaster that would result from a long-lasting radiological contamination of critical infrastructures, such as ports, train stations, government buildings, central squares, etc, and the economical loss of the consequent shutdown after such an incident for a large city has been estimated to tens of billions U.S. dollars [25]. Table 2 illustrates the theoretical probabilities for deterministic effects (acute or chronic radiation syndromes), concerning the threats described above whilst Table 3 presents the medical and public health characteristics of the three major categories of radiological events.

Table 2: Theoretical probabilities for deterministic effects (acute or chronic radiation syndromes), for radiation exposure and for radiological contamination

		Theoretical probability	Source of radiation	Acute radiation syndrome	Chronic radiation syndrome	Radiation exposure	Contamination	
							External	Internal
Accidental Incidents	Reactor Emergencies	√	Cs-137, I-131	no	no	no	no	√
	Industrial Steriliz. Accidents	very low	Co-60	√	√	√	no	no
	Co-60 Brachytherapy Accidents	√	Co-60	√	√	√	no	no
	Blood Irradiator Accidents	√	Cs-137	√	√	√	no	no
	X-ray Medical Accidents	√	X-rays	√	√	√	no	no
	Medical Radionuclide accidents	√	Med. Radionuclides	no	no	√	√	√
Malicious Incidents	Nuclear Detonation	very low	Fission products	√	√	√	√	√
	Co-60 RED	√	Co-60 RED	√	√	√	no	no
	Co-60 RDD	√	Co-60 RDD	no	no	√	√	√
	Cs-137 RED	√	Cs-137 RED	√	√	√	?	?
	Cs-137 RDD	√	Cs-137 RDD	√	√	√	√	√
	Po-210 RDD	√	Po-210 RDD	√	√	no	√	√
	Am-241 RDD, etc.	very low	Am-241 RDD	no	no	no	no	?

RED: Radiation Exposure Device, RDD: Radiation Dispersal Device

Table 3: Medical and public health characteristics of the main categories of radiological events (modified from Canestra, 2012)

Type of incident	Relative casualty-type distribution			
	Lethal injuries		Non-lethal injuries	
	Prompt	Delayed	Exposed individuals	Psychologically affected non-exposed individuals
Nuclear weapon	+++	+++	+++	+++
RED	+	++	+++	+++
RDD	+	+	++	+++

+++/++/+: *high/intermediate/low probability; RED: Radiation Exposure Device, RDD: Radiation Dispersal Device*

Contamination of food and water

The radiological contamination of food and water or any other goods of public consumption is another health emergency radiation scenario. That could result by two main reasons:

a. As a consequence of an accidental radiological accident, e.g. from a reactor emergency, that might release radiological contaminants to the environment and then to the food chain, or from a damaged radiological source, which might then be dispersed and contaminate the surrounding area. In the reactor emergency scenario it is expected a widespread contamination, leading to low level absorbed doses to a large population. Yet there is usually expected to be time for precautionary measures for the public protection, whilst as regards accidents involving radiological sources, these occasionally may be latent for a longer period of time [16].

b. As a malevolent act; that is the intentional contamination of public supplies with radioactive materials, or the attempt to assassinate specific individuals. In the case of a massive deliberate contamination of consumable public supplies, it is expected that the public would receive a rather low dose of radiation per person due to the dilution of the radioactivity load into a large pool of mass or consumed volume and consequently, due to the extremely large amounts of radioactive material required to achieve high levels of large-scale contamination. However, targeted assassinations [24] are another gloomy perspective of radiological terrorism; high doses of extremely dangerous radionuclides (Po-210, in that case), incorporated in the human body can cause genuine lethal deterministic radiation effects.

In any such case, fast actions from the public authorities involving radiological experts and the media are mandatory to quickly assess the health impact and reduce the consequences. Concerning the level of radionuclides in water supplies, the World Health Organization (WHO) has developed detailed guidelines [26]. On the social level however, an uncontrollable public concern or even panic is to be expected in such cases, as past experience has shown [27]. Furthermore, massive national or international financial consequences are expected concerning the distribution chain of food merchandise.

CONCLUSIONS

The key point concerning all above described cases of radiological emergencies, is that appropriate assessment of potential public threats and required preparedness or response actions should be applied before the appearance of the consequences of such incidents. Thus, continuous awareness and preparedness for such hazards is crucial for all public health stakeholders.

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VARIA

Typology of individual values of combatants of the War in Eastern Ukraine

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Abstract: *The individual values of combatants determine life goals, how to achieve them, and perform an important adaptive and protective function under conditions of combat stress. Understanding them enables mental health professionals to create an effective system of Combat and Operational Stress Control for military personnel.*

The purpose of this research was to determine the personal value sphere typology of individual values of combatants of the War in Eastern Ukraine, and, on its basis, to predict the vector of PTSD development.

The value sphere (Rokeach Value Scale) and peculiarities of mental status (“Traumatic Stress Questionnaire”) of nine hundred forty three Ukrainian combatants were investigated.

Cluster analysis made it possible to create a typology of combatants' values, consisting of five types: “Passionary”, “The one who does not leave the comfort zone”, “Romantic”, “Formal military man”, “Routine”. The most resistant to combat stress are combatants who are aimed at interiorizing values, expanding the boundaries of existing norms, and public recognition (“Passionary” (2.19%), “Romantic” (3.82%)), and the most vulnerable are those who are “Routine” (15.30%) and “Formal military man” (4.37%). The majority of Ukrainian combatants have a defined type of “The one who does not leave the comfort zone” type (74.32%). For them, safety and well-to-do life are the main priorities.

Keywords: *values, posttraumatic stress, hostilities, combatants, Ukraine*

INTRODUCTION

Values are the core motivators behind actions that underlie individual decision-making and affect human interaction. They are a strong guiding force, consistently determining behavior and shaping attitudes [1-2]. The values of the military personnel of the armies of different countries are based not only on individual universal human values but also on specific military values, such as loyalty, duty, unselfish service, respect, honor, courage, self-sacrifice, patriotism, etc [3-5]. Despite the national differences, there is one international military culture in the world that reflects common values [6].

In combat conditions, the value sphere of a soldier performs an adaptation function [7]. It contributes to the preservation of basic individual and military values [8], their change [9-10], as well as the formation of new value orientations [11-12]. The value sphere of the personality allows us to experience the influence of trauma softer and determines the vector of development (posttraumatic growth) [13-14] or personality regression [15].

Modern research on the value sphere of a personality after exposure to various extreme situations is based on four fundamental theories of values of C. Kluckhohn, G. Allport, M. Rokeach, and S. Schwartz. These studies focus on the relationship between posttraumatic stress symptoms and

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personal values of crime victims, and as well as between adjustment disorder symptoms in individuals exposed to critical life events [16-17]; changes in the value sphere of the personality of military personnel due to the stressful or traumatic events [10, 18]; the influence of the soldiers' personal values on the occurrence of depression, anxiety and somatoform symptoms [19]; the influence of traditional and modern values on human mental health [16, 20-21]; the influence of values on pathological addictions [22-23] and etc.

Researchers claim that values do shed light on the behavior, actions, and attitudes of individuals and have a wide variety of predictive qualities [24]. Maercker et al. [16] have found out that modern values (achievements, hedonism, stimulation) protect against stress by mediating the processes of social interaction. At the same time, traditional values (conformity, benevolence, customary orientation) increase stress through the same mechanism. It disproved the popular assumption that traditional values contribute to the preservation of health, and modern values worsen it [20-21].

The above is also supported by studies by Zimmermann et al. [19-25], who found that a strong and conscious personality value system can help to protect military personnel from developing psychogenic depression, and posttraumatic stress disorder (PTSD) in a combat situation. At the same time, values that are formed from a deep sense of altruism and partnership increase subjective distress if PTSD occurs [19]. The military personnel with such values is more psychologically vulnerable. If they witness torment (worry) during the service, then they experience them as their own [19].

Thus, it is clear that the values of combatants perform an important adaptive and protective function under conditions of combat stress. Understanding of them enables military mental health professionals to create an effective system of Combat and Operational Stress Control (COSC) for military personnel.

Since 2014, full-scale hostilities have been waged in Eastern Ukraine between the official defense structures of Ukraine and illegal armed groups of the self-proclaimed Donetsk and Luhansk republics. Military personnel of the Armed Forces of Ukraine and the National Guard of Ukraine (NGU) is taking part in this war against the separatists. As of 2019, more than 370 thousand Ukrainian military men took part in the hostilities [4]. All of them were exposed to combat stress, which caused many changes in their mental state, impaired adaptation and behavior, the appearance of various mental disorders, as well as the transformation of the value sphere of the individual [7, 11, 26]. The researchers also found out that the value orientations of combatants, in terms of their significance and meaningfulness, are more differentiated than those of military personnel without combat experience [27-28]. After participating in hostilities, most combatants began to value life and control it better, in contrast to military personnel without combat experience [28]. At the same time, they developed a pronounced polarity in their assessments of life values: they began to comprehend life-meaning orientations more deeply or on the contrary, lose their meaning in them [29].

The researchers suggested that military personnel with different value spheres will react differently to combat stress that will make it possible to perform a more differentiated choice of tactics for providing first psychological aid in a combat zone and determine measures for the rehabilitation and prevention of PTSD [30-31].

Consequently, the purpose of this research was to determine the typology of the personal value sphere of military personnel who took part in hostilities in the East of Ukraine, and on its basis to predict the vector of PTSD development.

Hypothesis 1. Combatants with different personal value spheres can be grouped into separate clusters (types) depending on the content of individual value constructs, indicators of acute stress disorder (ASD), depression, and PTSD.

Hypothesis 2. Combatants with different types of personal value spheres have different susceptibility to combat stress, which will make it possible to predict the severity of an acute stress reaction, a tendency to develop depression, and the further development of mental disorders, in particular PTSD.

METHOD

The research was conducted in 2016-2017. Nine hundred forty three male NGU military personnel of different categories (officers, contract military servicemen) were involved in this research, they all took part in hostilities in the East of Ukraine. The duration of a trip to the combat zone ranged from one to three months.

All combatants were informed about the nature of the research of values and manifestations of traumatic stress before completing the method forms.

All participants have given consent for their data to be used in this research.

The features of the value sphere of the combatants were studied using the Rokeach Value Scale (RVS), adapted into

Russian [32]. The respondents received two lists of values: terminal (goals that a person wants to achieve throughout life) and instrumental (ways to achieve terminal values). Each list consisted of 18 values, which were arranged in alphabetical order. Each value was accompanied by short phrases that explained its content. The research was conducted on paper. The respondents ranked the values on each list from most important (1) to least important (18). The respondents built a hierarchy out of their individual value priorities. The processing of the data obtained was carried out by comparing the selected ranks of different types of individual value spheres. The average value for each ranked value was calculated by counting (summing) the individual assessments of the respondents. For the data presentation, we used basic descriptive statistics (frequency n (%), arithmetical mean M, standard deviation SD).

The types of combatants' individual value spheres were determined using the cluster analysis procedure (K-means).

The features of the mental status of combatants who underwent the impact of extreme (combat) factors were studied using the "Traumatic Stress Questionnaire" by I.A. Kotenev [33]. The presence of such manifestations of traumatic stress in the posttraumatic period as hypervigilance, hypertrophied reaction, aggressiveness, impaired memory and concentration, the level of depression, anxiety, drug abuse, hallucinatory experiences, and sleep disturbances were determined in combatants. The research was conducted on paper. The questionnaire contains 110 statements which were rated by the respondents according to a 5-point Likert scale. The maximum (5 points) to the greatest extent corresponded to the mental state of the respondent at the present time, the minimum (1 point) absolutely did not correspond to the mental state of the respondent. The average value for each indicator of the combatant's mental state was calculated by counting (summing) the individual assessments of the respondents. As a result, three generalizable indicators were calculated: ASD, depression, and PTSD. For the data presentation, we used basic descriptive statistics (frequency n (%), arithmetical mean M, standard deviation SD).

The statistical analysis of the results of the study was carried out using the program SPSS 17.0.

We presented the ratio of the identified types of the value sphere of combatants and the vector of development of PTSD in the form of a circular configuration proposed in the theory of values of Schwartz [34]. Values that appear across from each other on the circle are values directly in conflict, whereas values next to or near each other on the circle are more closely related.

RESULTS

The conducted cluster analysis made it possible to distinguish five types of the value sphere of personality among combatants: "Passionary" (Type 1), "The one who doesn't leave the comfort zone" (Type 2), "Romantic" (Type 3), "Formal military man" (Type 4), "Routine" (Type 5). The types of the value sphere of the combatants' personality are presented in Table 1.

As can be seen from the Table, terminal values of the most common second type (74.32%) of the value sphere of the personality of combatants conditionally form several groups. The most significant value for them is "Health (physical and mental)", followed by a group that combines "Love" and "Happy family life". The third block is "Financially secure life", "Faithful friends", "Life wisdom" and "Active life". The fourth group is formed by "Interesting job", "Public mission", "Development" and "Self-confidence". The lowest indicators of terminal values have "Productive life", "Freedom", "Cognition", "Beauty of nature and art, "Happiness of others", "Entertainment" and "Creativity". In achieving life goals (terminal values), combatants of the second type give preference to such methods of achieving them (instrumental values) as "Good breeding", "Sensitiveness", "Responsibility", "Self-control" and "Cheerfulness". Low values are "Effectiveness in business", "Sincerity", "Open-minded", "High demands" and "Intolerance to own and others drawbacks".

The fifth type of combatants is the next most widespread (15.30%). The most significant terminal values for this type of combatants are "Active life" and "Life wisdom". Important values also include "Interesting job", "Health (physical and mental)" and "Love". The least significant terminal values are "Cognition", "Creativity", "Faithful friends", "Freedom", "Public mission", "Happy family life" and "Entertainment". Military men of the fifth type prefer such instrumental values as "Carefulness", "Tolerance" and "Cheerfulness". Representatives of this type have the lowest ranks according to such instrumental values as "Courage in defending their own views", "Independence", "Open-minded", "Honesty (sincerity)", "Sensitivity" and "Efficiency in business".

Combatants of the fourth type (4.37%) are in third place in terms of prevalence. They prefer such terminal values as "Interesting job", "Financially secure life" and "Love". High ranks also have "Public mission", "The beauty of nature and art" and "Faithful friends". The least significant terminal values for this type of combatants are "Freedom", "Creativity", "Cognition", "Productive life" and "Development". Among the instrumental values, the preference is given to "Self-control" and "Discipline".

“Cheerfulness”, “Independence” and “Education” are also highly rated. The lowest ranks were received by the instrumental values: “Sensitivity”, “Honesty (sincerity)”, “Firm will” and “Effectiveness in business”.

Table 1: Typology of individual values of combatants (in points).

------ Indicators	Type 1 (n = 2.19%)	Type 2 (n = 74.32%)	Type 3 (n = 3.82%)	Type 4 (n = 4.37%)	Type 5 (n = 15.30%)
	M±SD	M±SD	M±SD	M±SD	M±SD
Terminal values					
Active life	3.75±4.19	7.60±4.60	11.71±3.15	8.88±4.82	4.93±4.29
Life wisdom	10.00±5.29	7.38±3.98	14.29±3.73	9.00±5.81	5.71±3.89
Health (physical and mental)	14.75±2.87	3.73±3.79	11.43±4.12	10.257.81	6.68±5.09
Interesting job	5.25±3.77	8.96±4.91	14.57±3.10	4.00±5.76	6.68±4.50
The beauty of nature and art	12.00±6.93	12.68±5.18	13.29±1.98	6.75±4.33	9.61±4.47
Love	13.75±5.85	5.94±4.66	13.43±2.37	5.75±3.41	7.14±5.01
Financially secure life	13.25±4.27	6.51±4.62	12.71±5.62	4.25±1.91	8.32±5.93
Faithful friends	10.00±5.72	7.07±3.81	4.14±3.63	7.38±4.27	11.54±4.96
Public mission	11.50±2.08	9.49±3.79	4.71±4.31	6.50±4.14	11.64±3.83
Cognition	10.00±4.55	11.73±4.04	6.14±3.29	12.25±4.95	11.07±4.20
Productive life	9.25±5.56	11.04±3.67	4.14±4.41	13.88±3.04	10.96±4.22
Development	7.25±4.92	9.71±4.03	7.29±4.72	14.13±4.02	9.96±4.56
Entertainment	9.75±5.62	13.46±3.56	10.00±4.62	9.38±5.21	11.79±4.78
Freedom	5.25±1.71	11.37±4.03	4.14±3.67	11.88±3.83	11.61±4.65
Happy family life	9.75±4.92	5.96±4.96	8.29±4.15	9.50±4.60	11.64±5.54
Happiness of others	4.75±2.22	12.81±4.44	9.71±4.79	10.63±4.24	13.04±4.13
Creativity	10.00±5.94	15.28±3.05	9.71±5.65	11.88±4.49	11.29±4.39
Self-confidence	10.50±6.24	10.03±4.67	11.14±4.41	11.25±3.69	8.46±4.53
Instrumental values					
Carefulness	14.75±2.87	6.86±5.32	3.14±3.13	10.38±5.55	4.39±2.94
Good breeding	12.75±2.99	6.67±5.02	5.00±3.37	8.75±6.48	8.50±5.51
High demands	9.75±6.95	13.10±5.07	5.57±3.69	7.38±5.24	8.29±4.65
Cheerfulness	12.25±5.12	7.67±4.66	8.14±4.22	6.13±2.80	7.14±4.15
Discipline	13.25±7.54	8.35±4.72	10.29±4.86	5.25±3.92	9.39±4.80
Independence	8.25±7.85	9.08±4.82	6.14±3.34	6.13±3.94	10.54±4.49
Intolerance to own and others drawbacks	8.00±5.77	13.63±4.20	11.00±5.57	7.63±4.10	9.46±6.29
Education	9.00±5.03	8.87±4.81	8.00±4.55	6.50±3.66	8.68±4.56
Responsibility	15.75±1.50	7.20±4.41	11.43±4.16	9.38±4.44	9.14±4.86
Rationalism	14.00±2.16	9.24±4.44	5.00±3.00	7.13±4.61	8.39±4.33
Self-control	10.25±5.12	7.35±4.45	12.29±3.77	4.88±2.90	8.71±4.77
Courage in defending own views	6.75±3.10	9.74±4.23	11.29±4.96	11.38±5.18	10.32±3.87
Firm will	7.25±3.20	8.89±4.49	12.29±5.94	14.25±2.71	8.54±4.94
Tolerance	5.50±0.58	9.71±4.43	12.00±4.00	11.38±4.03	6.00±4.21
Open-minded	6.00±2.45	12.16±4.57	10.71±5.35	11.50±3.93	11.64±4.44

Indicators	Type 1 (n = 2.19%)	Type 2 (n = 74.32%)	Type 3 (n = 3.82%)	Type 4 (n = 4.37%)	Type 5 (n = 15.30%)
	M±SD	M±SD	M±SD	M±SD	M±SD
Honesty (sincerity)	3.25±3.30	9.50±5.69	12.57±3.60	14.25±2.25	12.39±5.34
Efficiency in business	6.00±4.24	11.29±4.94	10.86±5.98	15.38±2.50	14.79±3.68
Responsiveness	5.75±4.92	11.51±5.14	13.29±4.27	14.00±3.42	13.50±4.27
Features of the mental status of combatants					
PTSD	106.00±20.05	107.43±17.76	109.43±18.20	124.25±18.38	143.64±15.77
ASD	100.50±21.06	98.55±17.99	95.57±19.24	116.75±18.59	138.36±16.94
Depression	35.25±6.85	39.65±5.28	40.43±3.41	42.63±6.09	49.39±6.06

In the structure of terminal values of the third type (3.82%) preference is given to such values as “Faithful friends”, “Freedom”, “Productive life”, “Public mission”. However, they rate low the importance of such values as “Self-confidence”, “Health (physical and mental)”, “Active life”, “Financially secure life”, “Beauty of nature and art”, “Love”, “Life wisdom” and “Interesting job”. Among the instrumental values, representatives of this type distinguish “Carefulness”, “Rationalism”, “Good breeding”, “High demands” and “Independence”. Low positions in the hierarchy of instrumental values have “Courage in defending own views”, “Responsibility”, “Tolerance”, “Self-control”, “Firm will”, “Honesty (sincerity)” and “Sensitivity”.

The least widespread type was identified (2.19%) of combatants, for whom the most significant terminal values are “Active life”, “Happiness of others”, “Freedom”, “Interesting job” and “Development”. For them, “Public mission”, “Beauty of nature and art”, “Financially secure life”, “Love” and “Health (physical and mental)” are of little significance. To achieve them, combatants of this type consider it important for themselves to use such instrumental values as “Honesty”, "Tolerance", “Sensitivity”, “Open-minded”, “Efficiency in business”, “Courage in defending own views” and “Firm will”. Combatants of this type have the lowest indicators for such instrumental values as “Cheerfulness”, “Good breeding”, “Discipline”, “Rationalism”, “Carefulness” and “Responsibility”.

As can be seen from the table, in the NSU servicemen of the first, second and third types of the value sphere, the indicators of depression, ASD, and PTSD practically coincide and indicate the absence or insignificant manifestation of symptoms described using these scales. Combatants of the fourth type of value sphere differ from the first three in higher ASD indicators. This gives grounds to assume the formation of their individual symptoms of post-traumatic stress. In terms of depression and PTSD, the fourth type of combatants occupies an intermediate position between the first three and the fifth type of value sphere of combatants. The fifth type of combatant has higher rates of depression, ASD, and PTSD than the rest. With such indicators, it is possible to predict a high probability of the onset (development) of PTSD of the fifth type of combatants.

The five types of the value sphere of the combatants' personality that we have identified can be placed between the two axes of the Schwartz [34] model:

− “common goal of life”, which has poles “for the happiness of others” – “for oneself” (it also includes “for the sake of one's social/professional group”, “reference group”, “family”, etc.), or “transcendence – individualism”;

− “a way to achieve a goal”, which has poles “the use of social norms to expand boundaries” – “the use of social norms to narrow the boundaries”.

The ratio of the types of the value sphere of the personality and the vector of the development of PTSD in combatants is shown in the Figure 1.

Figure 1: Correlation between the types of values and the vector of development of PTSD of the NGU combatants.

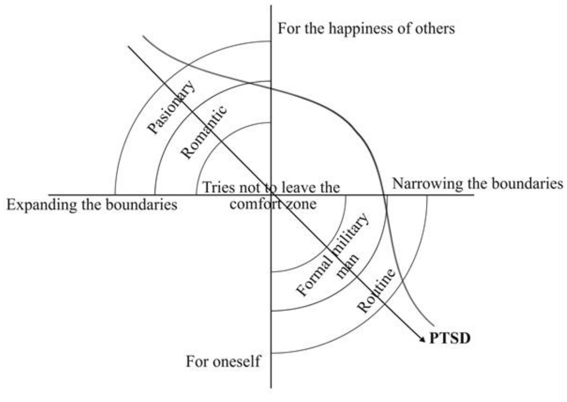

In the Figure, the axes are set by the directions of the development of the value sphere of the individual. The PTSD vector indicates the direction in which the PTSD indicators increase (from the absence of symptoms to the formation of a posttraumatic symptom complex with clinical manifestations). The curve above it schematically describes the frequency of the corresponding PTSD indicators in the sample of combatants.

DISCUSSION

The most widespread type of value sphere, identified in 74.32% of NGU combatants, we designated it as “The one who doesn’t leave the comfort zone”. Military personnel of this type strive for a balanced, comfortable, prosperous life, observing for this the norms and methods of interaction with the outside world, accepted in society. They do not place high demands on themselves and their lives. Thus, in a study by Prykhodko et al. [7] it was proved that compliance with the norms of society, conflict-free interpersonal interaction is an important component of the psychological safety of a person. Confirmation of the obtained results is the conclusions of researchers that resourcefulness (strategies of conservation, avoidance, procrastination) [35] and satisfaction of spiritual needs (Spiritual Needs), in particular, religion [36] are a preventive factor in the development of PTSD.

The “routine” personality type, identified in 15.30% of combatants, is characterized as a person who constantly works to improve his/her own life but does not feel happiness and pleasure from it. This structure of the value sphere describes military men who believe that life is a hard routine, daily work. They are not convinced that life should necessarily be happy and that work should be interesting, aimed at something important. According to McAllister, Callaghan, & Fellin [37], such military personnel adheres to generally accepted norms of interaction to avoid conflict and other problems. They limit themselves in emotional manifestations. Fulfillment of tasks in combat conditions does not leave the room to react to stress, provokes in them the development of depressive manifestations, and in the future, PTSD.

The structure of the value sphere of the personality of combatants of the fourth type (found in 4.37%) can be described as “Formal military men”. They are narrowly focused on their profession as a means of satisfying material needs. Moreover, their professional identification does not reach the level of mastery, stopping at the level of the performer. The profession of a military man is not perceived by them as contributing to self-development, knowledge, creativity, and richness of life. According to Johansen, Laberg, & Martinussen [38], such features of the professionalization of military men determine the leading role of self-control and discipline in the structure of instrumental values and explain in it the low importance of result orientation. With such identification, the external form of the soldier's actions is copied, but not the meaning of the profession, its mission. The limitations and lack of freedom that characterize the identification of the “performer” level make the military men vulnerable to the occurrence of ASD and increase the development of depression and PTSD.

Military men of the third type (3.82%) can be characterized as “Romantics”. They are focused on the close social environment, strive to be significant for society, share their values, and are able to refuse from comfort, love, health, and their favorite profession for the sake of public recognition. According to Jones & Wessely [39] and Noy [40], this personal value structure contains the well-known potential for coping with combat stress: using the help of peers, mutual understanding, and trust of combat brothers.

The least common (2.19%) identified is the first type of personality of combatants, which is called “Passionary”. Its feature is an active struggle for the happiness of others, the expansion of the boundaries of existing norms. For this, they are ready to refuse from a comfortable, safe life and their own interests. Military personnel of this type is free in their thoughts and actions. They are sincere, sensitive, tolerant, aimed at achieving a result, but they neglect restrictions and conventions. According to Banducci, Connolly, Vujanovic, Alvarez, & Bonn-Miller [41], a person’s high tolerance to stress, perceived or actual ability to tolerate negative emotional or physical states are back proportional to the symptoms of PTSD. Research by Bandura [42] and Schwarzer [43] has shown that motivated people are better able to cope with problems and solve new, more complex problems. Military men of this type can often find themselves in stressful situations, sometimes they create them by themselves. However, this does not lead to the development of symptoms of depression or PTSD in the future.

Thus, the research confirmed our hypothesis 1 about the possibility of typing the individual values of combatants, taking into account the indicators of acute stress disorder (ASD), depression, and PTSD.

An analysis of the results of the correlation between the types of values and the vector of development of PTSD of combatants (see the Figure) showed that belonging to such types of personal value spheres as “Routine” and “Formal military men”, which imply a narrowing of the goal and the method of achieving it, significantly reduces the ability of military men to resist combat stress, and increases the possibility of the formation of a symptom complex of PTSD. At the same time, the interiorizing of values, which involve expanding the range of ways to achieve the goal, going beyond the personal interests inherent of combatants of such types as “Passionary” and “Romantic”, is the prevention of the development of PTSD. These military personnel will be potentially more resistant to combat stress.

At the same time, attention is drawn to the fact that the absolute majority of NGU combatants (74.32%) belong to the type “The one who does not leave the comfort zone”. These military men are in an equidistant position from opposite poles of the circumplex of values and the vector of PTSD development. Therefore, it can be assumed that some of them will be more resistant to the effects of combat stress, taking into account their individual psychological characteristics (good psychological resilience, adaptation to extreme situations, etc.). The other part will be more susceptible to stress and the occurrence of psychogenic disorders. In general, the identified problem of studying the value sphere of combatants, in our opinion, is relevant and requires in-depth research.

The results of our research support hypothesis 2 partially. Based only on belonging to a certain type of personal value sphere, it is impossible to predict with a high degree of probability the risk of development and the severity of behavioral and clinical manifestations of an acute reaction to stress, susceptibility to depression, and other mental disorders, including PTSD.

CONCLUSIONS

Values determine the life goals of a person and how to achieve them. The individual values of combatants perform an important adaptive and protective function under conditions of combat stress. Understanding of them enables military mental health professionals to create an effective system of COSC for military men.

As a result of the research, a typology of the value sphere of combatants has been developed, consisting of five types: “Passionary”, “The one who does not leave the comfort zone”, “Romantic”, “Formal serviceman”, “Routine”. The most resistant to combat stress are military men who are aimed at internalizing value orientations, expanding the boundaries of existing norms, striving to be significant for society and are able to refuse from comfort, love, health, and their favorite profession for the sake of public recognition (“Passionary” (2.19%), “Romantic” (3.82%)). The most vulnerable to combat stress and the occurrence of PTSD are combatants belonging to the “Routine” (15.30%) and “Formal soldier” (4.37%) types. For the Ukrainian combatants of the “The one who does not leave the comfort zone” type (74.32%), safety and stable well-to-do life are the main priorities.

Ethical standards and informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all subjects for inclusion in the study while maintaining their confidentiality.

Disclaimer

No conflict of interest to declare. No financial support was received from any governmental institution, economic or non-governmental organization. No personal information was collected through the survey, the anonymity was preserved for all the respondents.

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VARIA

The effect of leadership style on the productivity of hospital's staff with moderating effect of job promotion opportunity and job description

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Abstract: *Background: Human resource is the most important criteria of today organizations. In considering a human resource, performance and productivity are very fundamental. One of the most important criteria that affect human resources is leadership style.*

Aims: This study aimed to assess the effect of leadership style (Autocratic, Democratic, and Laissez-faire) on productivity that in this relationship two phenomena (job promotion opportunity and job description) plays the roles of moderating variable.

Methods: The data were collected through the distribution of standard questionnaires among 170 employees of a hospital in Tehran and analyzed with structural equation methods with smart PLS software. The reliability and validity of questionnaires are confirmed through software examination and elites' opinions. For examining the hypothesis, factor loading and path coefficient are considered.

Results: Democratic and laissez-faire styles have a significant effect on productivity (with 5.51 and 2.65 loading factor and positive path coefficient), but the autocratic style has a negative effect (due to 5.54 loading factor and -0.35 path coefficient) on productivity. Neither job promotion opportunity nor job description plays the role of moderating variables according to the results of the analyzes.

Conclusion: Leadership style has a significant impact on productivity. The autocratic style has a negative effect on productivity, but democratic and laissez-faire have a positive effect. Job promotion opportunities and job descriptions don't play a moderating role in leadership style and productivity relationships.

Keywords: *leadership style. productivity. job promotion. job description*

INTRODUCTION

"Human resource" is one of the most valuable assets of organizations, especially in organizations that knowledge is their first priority [1]. In fact, managers should pay more attention to human resources rather than ordinary managing, hiring, or financial incentives. Nowadays, the generation gap between employees and also hiring educated people, make managing more challenging. Therefore, organizations and managers should create a dashboard so they can observe human resources criteria like efficiency and productivity. Without this kind of Mechanism, employees can't show their creativity and also they lose their enthusiasm for work [2].

Matsushita in his book "In Iron Hand" said that Human is like a gem, his value is depending on how it processed. He believed that everyone who is responsible for people in the organization should know how to extract the best of them [3].

In this research, nevertheless, there are lots of ways to measuring productivity, but methods of Taylor and Steel [4]. Has chosen, they took efficiency and effectiveness for measuring productivity.

Personnel productivity has a positive impact on the

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productivity of the organization. Thus, if managers want to have a better approach to organizational culture, strategies, policies, and prospect, they should understand that productivity is vital for them [5].

On the other hand, leadership style has a great impact on human resource performance and function [6]. With this view, the management means the process of planning, organizing, "leading" and controlling to reach the goals of the organization [7]. In fact, leadership has a bigger concept and meaning than management. Management is a subset of leadership and leadership mostly means how effect and influence people to make them be in direction of reaching goals for themselves or the organization [8]. With this meaning, it seems that there is some relationship between leadership and productivity.

For considering leadership style and also the existence of three famous leadership styles (Autocratic, Democratic and Laissez-faire) that are most common in the atmosphere of Iran organization, researchers decided to choose Kurt Lewin theory of leadership that presented in Iowa University of USA [9]. Levin and his colleagues describe these three leadership styles:

1- Autocratic: A leader who decides just by himself and mostly dictates responsibilities to employees.

2- Democratic: Democratic: This style mostly refers to managers who share the power of choice with his/her human resource and just illustrate the whole vision of the job and give employees the right to choose what should be the method to reach goals.

3- Laissez-faire: Laissez-faire: Managers who have this style of management, don't participate in groups and just intervene when groups have problems and challenges.

The result of Kurt Lewin's research shows that among these three leadership styles, Laissez-faire style has the least Efficiency [9].

Moderating variables, job description opportunities, and job description, considered from the perspective of incentives. Job promotion opportunity describes as when an employee in a specific period of time does duties and responsibilities well and also has knowledge and competency for the higher level in the organization; does he/she promote or not? [10]. In this context, the absence of gender discrimination is very important for job promotion [11]. In a holistic view, if employees know there is a job promotion opportunity in the organization, their performance becomes well [12] so researchers decided to survey how it works in the field of productivity.

Definition of the job description is "a document that has main responsibilities, functions, and duties of a person that works in the organization. This document maybe has the little portion of job criteria to very specific things like even to whom an employee must give her/him reports" [13].

With all these illustrations, eventually, we decided to discuss the relationship between these three leadership styles and productivity with moderating role of job promotion opportunity and job description. Conceptual model of this study is presented in Figure 1.

Figure 1: Conceptual model

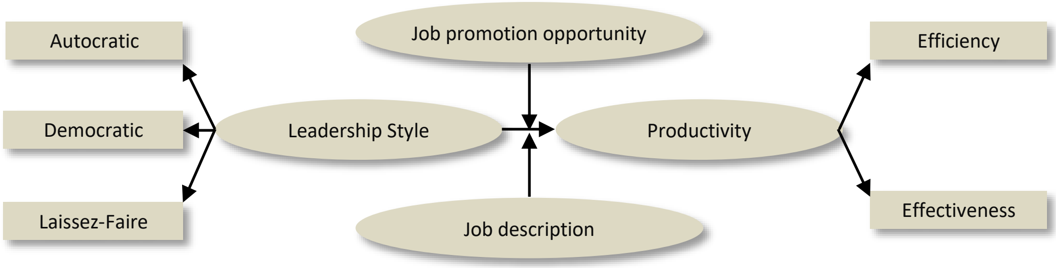

METHODS

The type of study is cross-sectional that conducted on employees of a hospital in Tehran from April of 2018 to May of 2018. Data for analyzing collected through the distribution of productivity and leadership style. Questionnaire of productivity as it said before, is from Taylor and Steel [4]. and questionnaire of leadership style is from Kurt Lewin theory [9]. Number of the questionnaire that has to be gain is extracted from Cochrane formula [14] and Morgan table [15]. According to these two criteria for sampling in the statistical universe, 170 questionnaires randomly distributed among 370 employees of the morning shift. Gathered data analyzed by Smart PLS and through path coefficient, factor loadings, and other important outputs. Inclusive criteria in this study are two main things. First, an employee must be officially hired, and second is she/he working the morning shift.

The questionnaire of leadership has three leadership styles altogether, but at the time of analyzing, they separated and each style analyzed by its questions separately. After analyzing the relationship between our two main variables with all consideration of modifying the inner and outer model in software, moderating variables analyzed. Due to binary moderating variables, the method of analyzing moderating variable [16] formula which is operational for this research. Consequently, in this research, nine different analyzes took place; three for the main path between leadership styles and productivity and six analyzes for moderating variables (because each leadership has two moderating analyzes).

Reliability and validity in statistics sample were confirmed by elites and also methods of confirming in Smart PLS [17]. Results of this tests are illustrated in Table 1.

Table 1: Results of model examination in PLS

Purpose of Test	Name of Test	Confirmation Status	Power Status
Consistency	Consistency of outer model variables	Confirmed (with modification)	Normal
Validity	Cross Loading Test	Confirmed (with modification)	Normal
	Fornell - Larcker	Confirmed	Strong
	Factor Loading Significantly	Confirmed	Strong
	Factor Loading	Confirmed	Strong
	AVE>0.5	Confirmed	Strong
	CR>AVE	Confirmed	Strong
Reliability	*Delvin-Goldstein (Composite reliability)*	Confirmed	Strong
	Communality	Confirmed	Strong
Model Quality	Outer Model Quality	Confirmed	Strong
	Inner Model Quality	Confirmed	Strong
	Generalized Model	Confirmed	Strong

The precision [16] that says if in a research, the sample volume is above of 168, research has 97 percent power and accuracy of covariance structure analysis. Accordingly, because of 170 samples of this study, the analyzes and outputs are accurate.

With all this explanation, three main hypothesizes of this research are:

H1: Leadership style has a significant effect on productivity.

H2: Leadership style with moderating effect of job promotion opportunity has a significant effect on productivity.

H3: Leadership style with moderating effect of job description has a significant effect on productivity.

RESULTS

First analyze is without any modification in the inner and outer model in smart PLS. Raw analyze of autocratic leadership is presented in Figure 2.

After model modification and results that are shown in Table 1, three basic leadership analyzes of path coefficient are presented in Figures 3, 4 and 5.

Results of analyzes without moderating variables in path coefficient and factor loading mode are presented in Table 2.

Table 2: Results of analyze without moderating variables

Leadership	Productivity	
	Factor Loading	Path Coefficient
Autocratic	5.541563	-0.358867
Democratic	5.511342	0.371835
Laissez-Faire	2.654519	0.167206

According to this table, all three leadership has a significant effect on productivity, but the path coefficient shows that autocratic leadership style affects negatively on productivity, but two other leadership has a positive path coefficient.

Figure 2: Autocratic leadership raw analyze

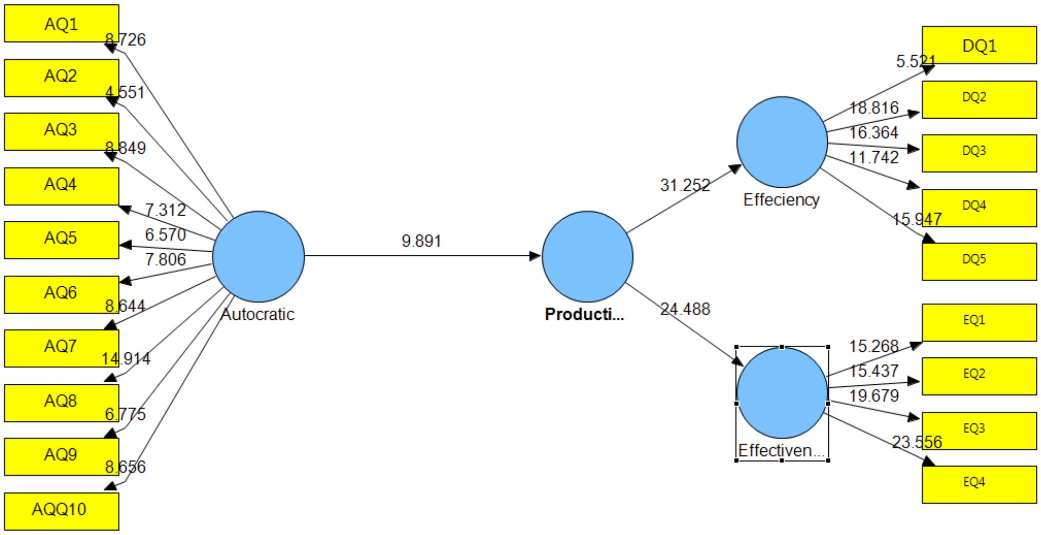
AQ1
AQ2
AQ3
AQ4
AQ5
AQ6
AQ7
AQ8
AQ9
AQQ10
8.726
4.551
8.849
7.312
6.570
7.806
8.644
14.914
6.775
8.656
Autocratic
9.891
Producti...
31.252
24.488
Effeciency
Effectiven...
5.521
18.816
16.364
11.742
15.947
15.268
15.437
19.679
23.556
DQ1
DQ2
DQ3
DQ4
DQ5
EQ1
EQ2
EQ3
EQ4

Figure 3: Autocratic leadership analyze in path coefficient output

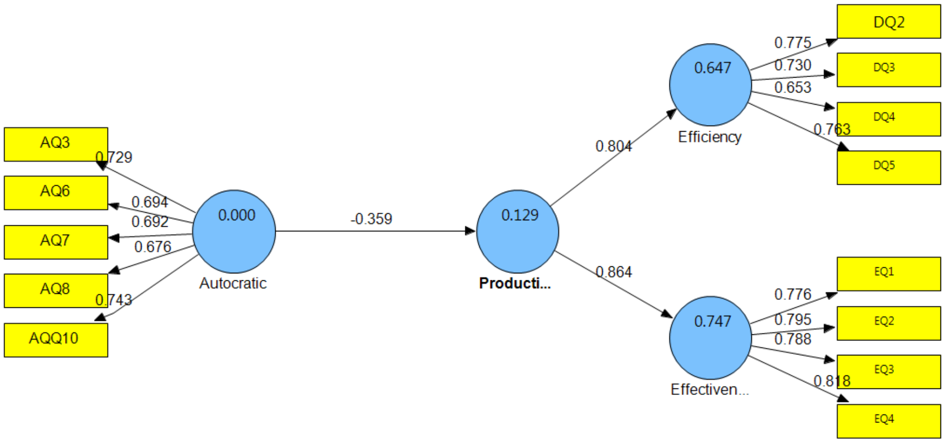
AQ3
AQ6
AQ7
AQ8
AQQ10
0.729
0.694
0.692
0.676
0.743
0.000
Autocratic
-0.359
0.129
Producti...
0.804
0.864
0.647
Efficiency
0.747
Effectiven...
0.775
0.730
0.653
0.763
0.776
0.795
0.788
0.818
DQ2
DQ3
DQ4
DQ5
EQ1
EQ2
EQ3
EQ4

Figure 4: Democratic leadership analyze in path coefficient output

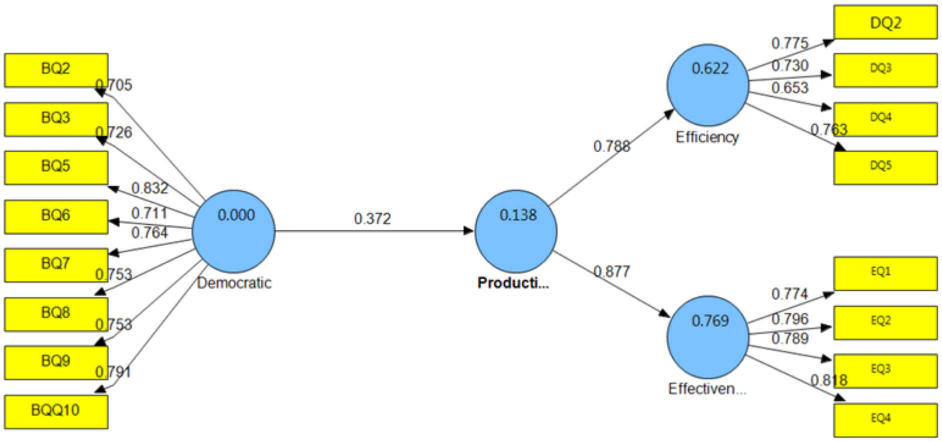
BQ2
BQ3
BQ5
BQ6
BQ7
BQ8
BQ9
BQQ10
0.705
0.726
0.832
0.711
0.764
0.753
0.753
0.791
0.000
Democratic
0.372
0.138
Producti...
0.788
0.877
0.622
Efficiency
0.769
Effectiven...
0.775
0.730
0.653
0.763
0.774
0.796
0.789
0.818
DQ2
DQ3
DQ4
DQ5
EQ1
EQ2
EQ3
EQ4

Figure 5: Laissez-Faire leadership analyze in path coefficient output

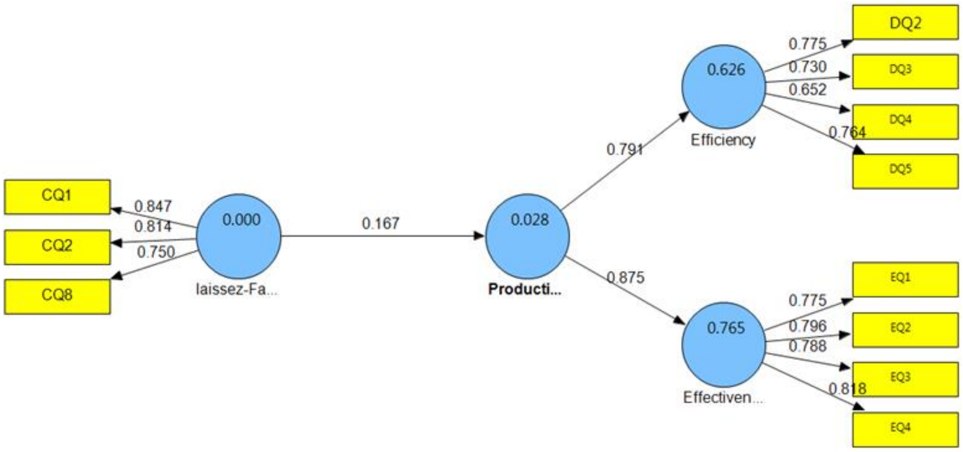

For analyzing moderating variables, as presented before, the Hair formula used in this research. The result of all six moderating variables analyzes are in Table 3.

Table 3: Results of moderating effects

Moderating Variable	Leadership Style	Result	Confirmation Status
Job promotion opportunity	Autocratic	0.18	NOT confirmed
	Democratic	0.159	NOT confirmed
	Laissez-Faire	0.058	NOT confirmed
Job Description	Autocratic	-0.144	NOT confirmed
	Democratic	0.259	NOT confirmed
	Laissez-Faire	0.19	NOT confirmed

Eventually, R^2 and model quality were analyzed. The model [18] and R^2 are according to Chin [19]. Model quality comes with main formula:

$$\text{Model Quality Formula} = \sqrt{\overline{\text{Communality}} \times \overline{R^2}}$$

and for autocratic leadership is 0.53, for democratic leadership is 0.542 and for laissez-faire leadership is 0.533. These results show all three models have good quality according to numbers of Tenenhaus and Vinzi (Table 4).

Table 4: Results of R^2

Variable	R^2		
	Autocratic	Democratic	Laissez-Faire
Efficiency	0.646507	0.621514	0.625817
Effectiveness	0.746749	0.768643	0.765111
Productivity	0.128785	0.138261	0.027958

According to numbers of chin, in all models, efficiency and effectiveness have high value, but productivity in laissez-faire model has bad value and two other models have something between bad and medium value.

DISCUSSION

First, we considering research hypothesis. According to results, H1 of this research, that's mean "Leadership style has the significant effect on productivity", in democratic and laissez-faire leadership confirmed [20]. In autocratic leadership because of negative path coefficient, not even confirmed, but also shows that this kind of leadership has the negative effect on productivity and managers have to know that if they want to rise up their employee's productivity, it's better to survey all consequences that come with autocratic leadership style. As results shows, and also other research confirmed, different leadership style has a different effect on productivity [6, 21].

H2 and H3 of this research, which are "Leadership style with moderating effect of job promotion opportunity has significant effect on productivity" and "Leadership style with moderating effect of job description has significant effect on productivity", not confirmed due to lack of the significance effect of both moderating variables in all three leadership styles [9]. Laissez-faire leadership has less efficiency for managing in comparison with autocratic and democratic. This claim is not in conformity with results of this research. In return [22]. Is confirms our results. Furthermore, democratic leadership is the best style among these three styles that considered in this survey and this outcome is in conformity with Walumbwa [23]. Who researched ethical leadership and shows one of the ethical leadership is democratic leadership that affects productivity. Due to the average variance extracted of laissez-faire leadership, we cannot certainly talk about this style and expand it to all environments. But even with this result, we can say managers should be cautious in choosing this style, cause some researches to show that this style is harmful to organizations like research of Skogstad [24] especially traditional and complex organizations. Nevertheless, the result of this research about laissez-faire leadership is in conformity with Yang [25] research's outcome. That found out job description has the significant effect on productivity. Job promotion opportunity mostly collaborate with concepts like sexuality in Groot [26] research or educational perspective [27] research, that in both of these research, job promotion opportunity has incentive role. We discussed and figured out that H2 and H3 are not confirmed. With this result, among all six moderating variables, one of them is really unexpected. Job description in laissez-faire model analysis is the controversial point here. As we know, laissez-faire leadership is the act of delegation. This view proves that employees who work under the supervision of this leadership, need some job description to do their works better (in comparison with two other leaderships that some interaction of leader exists). However, the job description doesn't play the role of moderating variable in this relationship, and because of that, maybe more research needed for conformity or opposition to this result.

Future research

According to the result that we achieved, we suggest to researchers to consider two things in their future study if this article is their source. First, examine other moderating variables with these two main variables (leadership style and productivity) and discuss is any other concepts exist for playing the role of moderating effect or not. Second, because of the low average extracted variances for some aspects of this research, it is helpful to test the topic of this survey in another atmosphere. This suggestion is for clearance of results of this research.

CONCLUSIONS

Among three leadership style that discussed, autocratic, democratic and laissez-faire have the significant effect on productivity (democratic leadership has a better impact rather than laissez-faire leadership). Autocratic leadership has a negative impact on productivity due to the negative path coefficient. Neither job promotion opportunity, nor job description plays the role of moderating variable in this survey.

Acknowledgment

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VARIA

Electrodiagnostic studies in carpian tunnel syndrome

Hazim Elayan[1], Ana M. Oproiu[2], Radu C. Jecan[2], Ioan P. Florescu[2]

Abstract: *The diagnosis in Carpian tunnel syndrom is based on clinical symptoms and electrodiagnostic studies. Electrodiagnostic studies help to stage both motor and sensory nerve damage.*

Keywords: *electrodiagnosis, nerve conduction, motor nerve, sensory nerve*

INTRODUCTION

Electrodiagnostics represents a test or a paraclinical study of analysis of the muscular and nervous system, which evaluates the transmission of the motor and sensory nerve impulse, respectively, which helps the specialist doctor to obtain a diagnosis or prognosis, after the anamnesis and clinical examination have been performed [1].

When we talk about electrodiagnostics we refer to both Electromyography (EMG) and Electroneurography (ENG) [1].

Figure 1: Electrodiagnostics (https://share.upmc.com/2017/09/facts-electrodiagnostic-medicine/)

There are several advantages of electrodiagnostic [2]:

1. May specify the location of the neurological or muscular injury.
2. Can provide data on the type and severity of the lesion (demyelinating, axonal or mixed lesion).
3. It can specify the degree of axonal injury (neurapraxia, axonotmezis, neurotmezis).
4. It can stage affection (acute, subacute, chronic stage).
5. Can diagnose and monitor the progress of the disease over time.
6. It can formulate a prognosis of the disease and can be used in monitoring the effects of treatment (radiculopathy).
7. Represents an objective method of investigating the neuro-muscular system.

Limitations of electrodiagnostic studies

In chronic nerve compression, the first changes that occur in nerve fibers are changes in demyelinated nerve fibers, which cannot be evaluated with electrodiagnostic studies and tests [3]. Example: early symptoms of pain and paresthesia cannot be objectified with electrodiagnostic studies [4].

Another limitation of the nerve conduction study is the location of the nerve lesion in the extremity. Nerve problems occur very distally or very proximal and are difficult to assess [4].

Dynamic changes in blood flow that may intermittently cause changes in peripheral nerve function may not be detected with electrodiagnostic studies [4].

The timing of nerve conduction velocity studies also influences their usefulness because even complete sectioning of the nerves will not become evident for 26

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weeks after injury [4].

In situations with more than one level of injury or when systemic polyneuropathy is present, nerve conduction studies may be less reliable [4].

Electrodiagnostic studies in carpal tunnel syndrome

Electroneurography (ENG)

1. Nervous motor conduction:

The median nerve is electrically stimulated at the wrist and the motor response from the short abductor muscle of the thumb is recorded, with the help of surface electrodes; usually, the motor response occurs 4 msec after the application of the stimulus, an interval called motor distal latency (DL) (latency is the time required for the impulse to travel the distance from the stimulation site to the collection site); if a distal latency over 4.5 msec is observed, it means that we are dealing with a carpal tunnel syndrome [5, 6, 7].

Nerv conduction velocity (NCV = s/t = S/PL - DL)[1] in the motor nerve fibers is 45 - 65 m/s, in the nerve fibers, depending on the thickness of the nerve (Figure 2).

Figure 2: Compound muscle action potential (CMAP). Distal latency is measured from the stimulus to the onset of the negative response. The amplitude is measured from the base value to the negative peak (https://musculoskeletalkey.com/the-electrodiagnostic-examination/; Jinny Tavee, Kerry H. Levin)

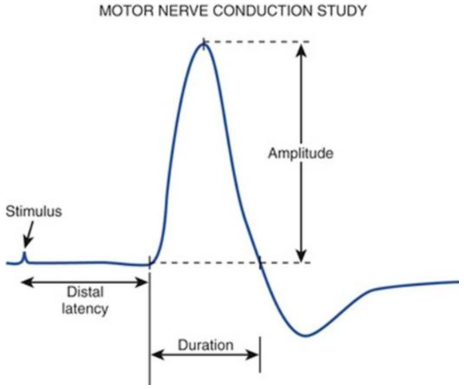

Observation: It can also stimulate the median motor nerve both palmar and at the wrist, evaluating the amplitude of the motor response thus providing information on the lesion: conduction block or axonal lesion [5, 6, 7].

The two-point stimulation method is preferred because it has the advantage that it eliminates the inaccuracies generated by the fact that the measured time represents not only the conduction in the nerve fiber, but also the conduction through the motor plate.

2. Nervous sensitive conduction:

Most frequently, it is measured antidromically, electrically stimulating the median nerve in the wrist and recording with surface electrodes in the fingers (most often, in the index and middle); a decrease in the sensitive conduction velocity (obtained by dividing the distance between the stimulator cathode and the active recording electrode at the sensitive distal latency) below 40 m/s is diagnostic for carpal tunnel syndrome [5, 6, 7].

Figure 3: Sensory nerve action potential (SNAP). The peak latency is measured at the beginning of the negative phase. The amplitude is measured from the base value to the negative peak (https://musculoskeletalkey.com/the-electrodiagnostic-examination/; Jinny Tavee, Kerry H. Levin)

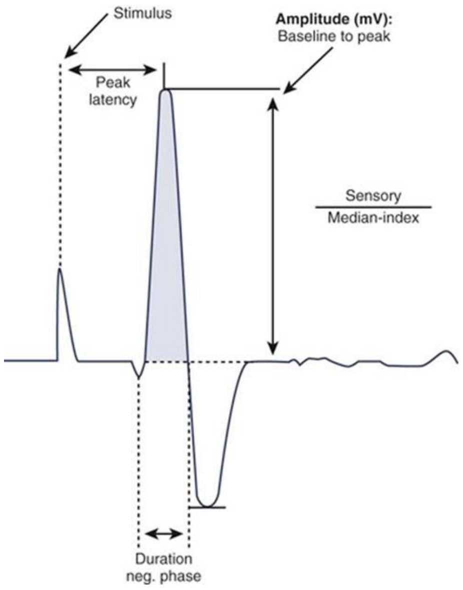

Another method applied when the changes are smaller is to stimulate from the same distance the ulnar and median nerves at the wrist, with the recording of the response from the IV finger – ring, which has mixed sensory innervation; a distal latency when stimulating the median nerve by more than 0.5 msec higher than when stimulating the ulnar nerve has diagnostic significance [5, 6, 7].

Electromyography (EMG)

When the existence of an axonal suffering of the median nerve is suspected, by prolonged compression, electromyography with needle is performed of the short abduction muscle of the thumb to detect early (before the installation of clinically obvious amyotrophy) the denervation changes; in this case, surgery is mandatory [5, 6, 7].

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[1] S = distance, t = time required for the impulse to move between the two stimulation points

Muscles are also tested electromyographically, when necessary for differential diagnosis (radial flexor of the carpus – median nerve injury above the carpal tunnel; adductor police – ulnar nerve injury, upper thoracic opening syndrome, etc.) [5, 6, 7].

CONCLUSIONS

Electrodiagnostic studies include electromyography (EMG) and nerve conduction studies.

Tests are not a substitute for a detailed clinical examination by a specialist.

Unfortunately, some doctors rely on these studies without recognizing the major limitations of electrodiagnostics examination [8].

Electrodiagnostic studies are useful to eliminate others associated problems such as cervical disc disease, motor neuron problems, myopathy or overlapping polyneuropathy.

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Cecostomy vs ileostomy for protection of anastomoses in colorectal surgery

Dan Bratu[1], Alin Mihețiu[1], Radu Chicea[2], Alexandru Sabău[1]

Abstract: *Background: The most frequently encountered complication of anastomotic surgery is fistula. In addition to the medical history of the patient, local conditions, the operative moment and surgical technique that were chosen, a series of some other factors can interfere, leading to suture disunity.*

Aim: The aim of the study was to analyze the existence of a relationship between the use of cecostomy (as a mechanism of temporary diversion) and the frequency of anastomotic fistulas.

Materials and methods: We conducted a retrospective study, over a period of 7 years, between 2011-2018 taking into consideration 194 patients who benefited from resections completed with anastomoses in the transverse colon, descending colon, sigmoid and rectum. They were divided into two categories: patients who benefited from cecostomy and patients for whom we did not use this technique. The frequency of fistulas was analyzed for the two main groups and in association with each type of surgery.

Results: It was observed the existence of a relationship between the appearance of fistulas and the use of a decompression mechanism such as cecostomy in the sense of a lower number of fistulas for the first situation (2.66%) compared to 11.76% for cases without cecostomy (p=0.034). The duration of hospitalization in the group with cecostomy was shorter than in those without this derivative stoma and the postoperative recovery was good.

Conclusions: Cecostomy is a useful protection for the anastomosis line, with the advantage that, when the tube is suppressed, the stoma will spontaneously close.

Keywords: *colon and colorectal anastomosis, intraluminal pressure, fistula, cecostomy*

INTRODUCTION

Colon and rectal surgery have encountered multiple complications over time, the most severe and frequent being the fistula. First fistula records come from antiquity (Old Testament and Hindu texts) and Middle Age with enterocutaneous fistulas being described after abdominal injuries. With the occurrence of anastomotic surgery, the first post-anastomotic fistulas were also described, with the initial frequency and mortality being at high levels. Risk of colonic anastomotic fistula continues to range between 1.5% and 23%, with low colorectal and coloanal anastomoses posing the highest risk [1].

Due to the need for achieving mechanical protection of the suture line, various ways of decompressing the perianastomotic colon were imagined. Colostomy, ileostomy and cecal stoma are thus used. These methods, however, have been associated with complications specific to the stomas in general, and often they remain in use for a long time. Anesthetic and surgical associated risk should also be considered, especially in elderly, due to the aged related frailty [2]. It is therefore necessary to find an anastomotic protection method that is effective, with as few as possible of the stoma-specific complications and which can be easily abolished, preferably without surgical reintervention.

MATERIALS AND METHODS

A retrospective study, over a 7-year period extending from January 2011 to December 2018, was conducted at Second Surgical Department at the County Clinical Emergency

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Hospital of Sibiu. The data of patients with this type of cancer were obtained from the electronic database, clinical files and operating registers. The aim of the study was to analyze the existence of a relationship between the use of cecostomy (as a mechanism of temporary diversion) and the frequency of anastomotic fistulas.

194 patients were included in the study, patients suffering from cancer located on transverse colon, splenic flexure, descending colon, sigmoid and rectum, on which various types of resections and anastomoses were used.

Data analysis was classified by age, gender, body mass index (BMI), ASA score, tumor location, type of resection and anastomosis, the presence or absence of cecostomy, frequency of fistulas, duration of surgery, and average time of hospitalization.

All the patients were positively diagnosed using colonoscopy, colonoscopic biopsy and computed tomography. There was no neoadjuvant treatment prior to surgery. Comorbidity factors included diabetes mellitus, cardiac pathology, obstructive pulmonary disease, chronic renal failure, liver cirrhosis, hypoproteinemia, anemia. Postoperative complications were followed according to the Dindo Clavien classification. The statistical data and analysis was organized and conducted using the Microsoft Excel Office 365 software. The differences were considered significant if $p < 0.05$.

Surgical technique

After the anastomosis has been completed, the cecum is mobilized, released from adhesion (if any), a Pezzer tube with a caliber between 16 and 24 Fr is prepared and a purse string suture is applied on the anterior surface of cecum (Figure 1). In principle, the place of cecostomy should correspond to the appendicular stump, a maneuver that would keep the patient away from appendicular inflammatory complications. However, even if this was not done, we did not encounter local complications in our study. The cecum is pierced with the scalpel and the tube is inserted into the colon. The suture is tightened and knotted around the Pezzer tube (Figure 2).

A breach is made in the right iliac fossa through which the Pezzer tube is exteriorized. The cecum is fixed to the peritoneum and the probe to the skin (Figure 3). All cases were completed with peritoneal and perianastomotic drainage. In all cases we preferred a post-anastomotic decompression like manual anal dilatation or sphincterotomy. Intraluminal tube was not used (Figures 4-6).

Figure 1: Surgical intervention in relation with leakage site

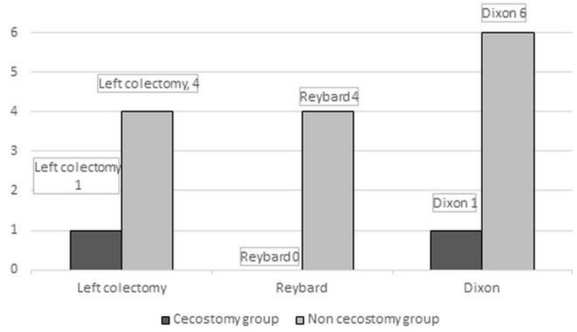

Figure 2: Anastomotic leak complication in Clavien-Dindo classification

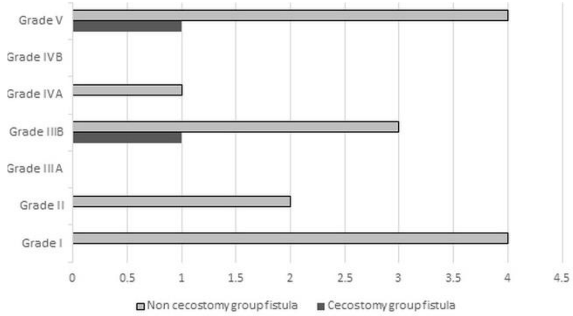

Figure 3: Fistula incidence in our study and in overall literature

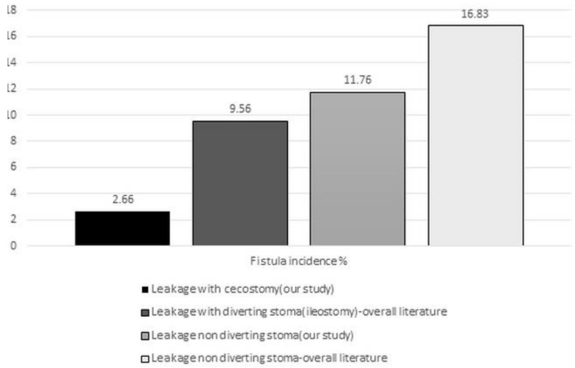

RESULTS

The distribution of cancer on studied colon segments was as follow: transverse colon (9.27%), splenic flexure (11.34%) descending colon (22.16%), sigmoid (39.69%) and rectum (17.52%). Gender distribution in general was 47.93% male and 54.06% women. For the cecostomy group, the distribution was 61.33% in women and 38.66% in male (Table 1).

Of the total number of patients, 75 patients (38.65%) had cecostomy, 119 patients (61.34%) were with no diverting stoma. In cecostomy group cancer repartition was 8% transverse colon, 9.33% splenic flexure, 16% descendent colon, 49.33% sigmoid and 17.33% rectal. Also 28% cases in the first group and 32.77% cases in the second one were anastomosis performed after a previous resection completed with stoma.

Table 1: Characteristics of the patients in the study group

	Cecostomy group	Non cecostomy group
Age (years) median range	62 (38-86)	64.5 (44-85)
Gender female/male	35/40	61/58
BMI kg/m²	27.75 (18.3-37.2)	28 (18.6-37.4)
ASA score (%)		
I	16%	22.68%
II	54.66%	59.66%
III	29.33%	17.64%
Tumor placement		
Transverse colon	6	10
Splenic flexure	7	12
Descendent colon	12	24
Sigmoid	37	48
Rectum	13	25
Blood transfusion	21	39

For the first group surgical techniques applied were segmental transverse colon resection (4%), left colectomy (25.33%), Reybard operation (46.66%) and Dixon operation (24.01%). The statistical analysis obtained was p = 0.042. In the second group the percentage for segmental transverse colon resection was 4.20%, 22.68% for left colectomy, 47.05% in Reybard operation and 26.07% for Dixon technique (p = 0.03).

All cases were resolved by laparotomy, the anastomoses being hand sewn. The average duration of surgery for the group in which cecostomy was used was 146 minutes and 133 minutes for the second group. Three hemorrhagic intraoperative complications were registered being identified and solved. In all cases, hand sewn anastomosis was performed with separate threads in one layer. The most common types of anastomoses for cecostomy group were end to end 77.33%, side to side 13.33% and end to side (9.33%), with a p value of 0.026. Repartition for the other group was end to end 50.42%, side to side 29.41%, and end to side 20.17% (p = 0.011).

The frequency of the fistula in the cecostomy group was 2.66%, with two cases, one with left colectomy status for splenic flexure cancer and the other one with Dixon low rectal resection.

Figure 4: Purse string suture applied on the anterior cecal wall

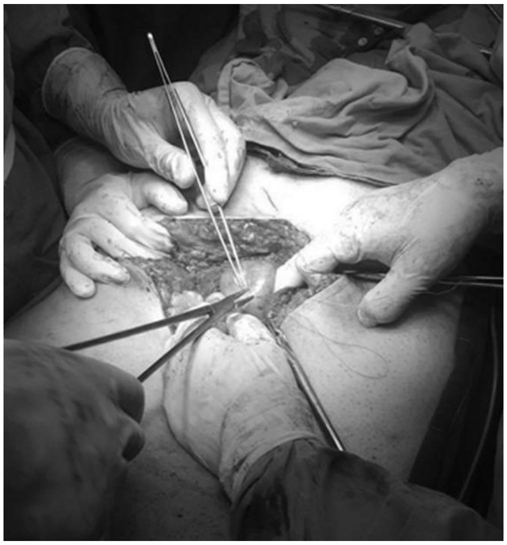

Figure 5: Cecum pierced and prepared for tube insertion

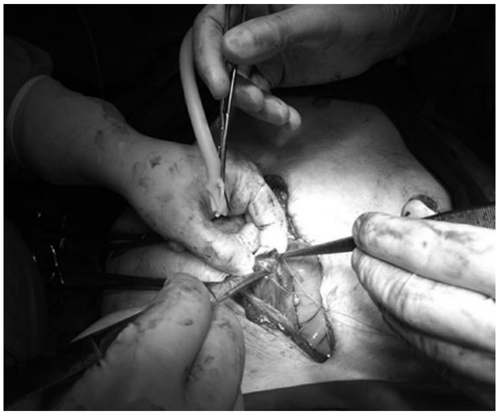

Figure 6: Cecum is fixed to the peritoneum

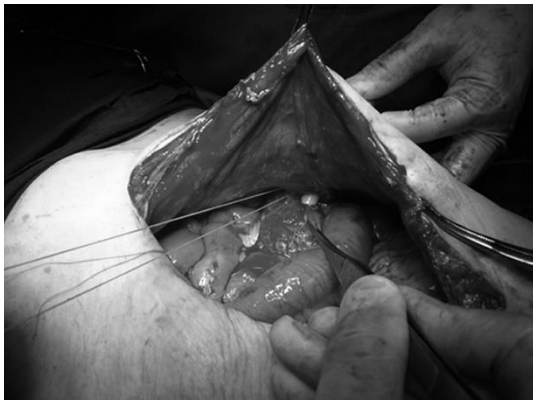

The first case was conservatively treated, the second one was surgically managed by practicing a stoma upstream of the dehiscence. Of the 14 cases with the fistula in the second group, 11 cases were resolved by conservative treatment, in 3 cases surgical approach was preferred, recording 4 deaths.

A statistically significant value p=0.034 was obtained, taking into account the standard value p <0.05. The duration of surgical technique for cecostomy alone was not significantly or statistically increased, the average duration being between 11 min and 5 min with an average of 7.2 minutes. Average length of hospital stay for the cecostomy group was 9.8 days by comparison with 10.3 days in the other group. In the group of patients who presented anastomotic fistula, with cecostomy previously performed the average period of hospitalization was 27.5 days, for the non-cecostomy group being 25.66 days.

The Pezzer tube was removed on average after 8.6 days. No local complications were encountered. The maximum duration of cecostomy drainage was of 14 days after extraction of the Pezzer tube, closing surgery procedures being unnecessary.

DISCUSSION

Colorectal anastomotic leak rates vary from 1.5 to 23% depending on numerous of risk factors such as malnutrition (due to the negative effect in fibroblast proliferation or collagen synthesis) and serum albumin level less than 3.5 mg/dl [1]. Immunosuppression, especially in patients with inflammatory bowel disease on the background of corticosteroid therapy for the underlying pathology and nonsteroidal anti-inflammatory drugs are also known to interfere with anastomotic consolidation [3-5]. Body mass index higher than 25 is associated with a higher risk of fistula.

Fat tissue, diabetes due to chronic complications, low blood levels of hemoglobin (ineffective tissue perfusion) and an ASA score higher than 3 is commonly met along with an increased risk for fistula [6-8]. Mechanical bowel preparation, a controversial topic, with studies that support the usefulness of this maneuver and studies that contradict it. We consider such a maneuver to be useful, it makes a reduction in septicity of the colon. Our experience recommends it to an unprepared colon mentioning that the intervention does not take place immediately after the mechanical preparation of the colon due to edema observed in the colorectal walls after a recent washout [9].

Suture line placement may be the most important factor in the occurrence of a fistula observing that the lower the anastomosis is located, the higher is the risk for leakage [10], many studies link a larger tumor size to a higher risk of fistula [7, 11]. The surgical technique differs according to the surgeon's experience and intraoperative conditions, but the following principles must be respected: achieving a tension free anastomosis and maintaining an effective perfusion on an unobstructed colon [10].

Surgical options include a handsewn or stapled anastomosis in various types, side-to-side, end-to-end, side-to-end, or end-to-side. Additional options include suture material, staple heights, number of layers. It has been demonstrated that regardless of whether the anastomosis has one or two layers, this does not affect the incidence of anastomotic leak, as long as the serosa is apposed and submucosa is incorporated [8]. Beside this particularities, intraluminal postoperative pressure has also its key role in the occurrence of anastomotic breaches.

Thus, postoperatively, the intra-abdominal pressure and the intraluminal pressure increase (by 10-15 mmHg for intraluminal pressure) especially because of paresis of the digestive tract during the period immediately following surgery. Frequently, the moment the fistula occurs corresponds to 5-8 days after surgery, at which time the swelling of the anastomosis is at maximum level, and the intraluminal pressure is at a high level, corresponding to the period in which the intestinal transit is not yet resumed.

Edema of the suture line may predispose to dehiscence, intraluminal pressure intervenes as a decisive factor in causing a further strain on an area of weak resistance with consequent production of leak [12-16].

To prevent pressure effects in the anastomotic area it has been argued that a diverting stoma is an effective and safe procedure to prevent anastomosis leakage complications after colorectal surgery, being imagined different ways of achieving a type of decompression through a diverting stoma such as loop or end ileostomy, colon stoma, cecal stoma, appendicostomy (using vermiform appendix as diverting stoma) or cecostomy [17]. Postanastomotic decompression using manual anal dilatation, sphincterotomy and intraluminal tube used alone are sometimes insufficient. Fistula rates when these procedures are used varies between 5-6% [12, 16, 18]. Intraluminal tube has the disadvantage that it can be mounted only in low colorectal resections. Manual anal dilatation or sfincterotomy are considered useful and had been used in all cases of the study.

The most widely used diverting stoma is ileostomy, more often the loop ileostomy type. Some studies have demonstrated a reduction in the fistula rate in those with ileostomy at values ranging from 3% to 10.9% [10, 11]. On a number of 41 prospective, retrospective and randomized control trials, between 2000-2017, researches that studied anastomotic leakage in patients with colon and colorectal anastomosis for stoma diverting group, the percentage of fistula was 9.56 % and for the non-diverting stoma group was 15.83% [16-22].

The benefits of this method have to be balanced with the specific complications of each type of stoma. The most common complications described were ileus, adhesion syndromes, wound infection and incisional hernias. Mortality associated with anastomotic leakage is raging between 2,1% to 22%. In addition to these, it must be recognized that this type of diverting stoma requires the patient to undergo two surgeries [13, 23-29].

Thus, cecostomy becomes superior to other ways of decompression. Intermittently used in surgery, it comes back in actuality based on an increasingly frequent intention to choose an anastomotic surgery instead of one ended through stoma. Technically it is easy to perform, does not require excessive extension of surgery time, it becomes functional quickly, reducing relating suture-pressure issues (especially the gas pressure) and during its use does not cause difficulty in handling, behaving like a drainage tube. It is recommended to maintain the Pezzer tube for 8-10 days, during which the adhesions process is complete, with the formation of adhesions between the cecum and the parietal peritoneum, the insulation of cecostomy from the abdominal cavity being meanwhile achieved. Suppression of the Pezzer probe does not require any special technique, a simple traction being enough. The area where the cecostomy is placed is covered with a colostomy bag, becoming progressively unproductive - at this time, the cecostomy is closed with a simple bandage on the area. Maintaining a good nutritional support and the use of probiotics improves the patients' quality of life [30, 31].

The advantages of cecostomy are that it is easy to perform, realizes a partial diversion, in transit it is easy to handle and cared by the patient, removal is done without the need for another surgical procedure, and local drainage stops spontaneously after a short time. Temporary diversion ileostomy, compared to cecostomy, implies in our opinion additional statements borne by both the patient and the medical system. In addition to the difficulties of management and care of an ileal stoma compared to a cecostomy, the risk of dysfunctionalities and stenosis of the anastomosis must be taken into account. Also, there is still no consensus regarding the time of instituting oncological treatment in relation to the abolition of ileostomy, the patient may be put in a position to delay chemotherapy. Possible absorption deficits in an ileostomy may increase during chemotherapy, leading to decreased compliance to therapy and increased duration of recovery, or may even affect the outcome of therapy.

The surgical gesture, as well as the anesthesia, especially the general anesthesia in the case of abdominal or pelvic surgery, is an aggression for the body [32]. It is the double exposure of the patient to the risks associated with surgical procedures and immunosuppression associated with additional surgery and anesthesia that we find inappropriate when it comes to two serial interventions. Those risks may be lowered if a minimally invasive, temporary option which does not interfere with cancer treatment and does not require reintervention, is considered [33].

CONCLUSION

We consider mini-invasiveness to be a must in the surgical approach whenever possible. Ileostoma has an additional disabling character, especially for a certain type of patients: the elderly, patients with neuro degenerative or neuro-mental disorders. In addition to all these elements, the cost of stoma care, stoma bags, a new hospitalization, the cost of anesthesia-surgery or postoperative care in the event of complications must be taken into account.

Although out of frequent surgical use, we believe that cecostomy has its advantages over other methods of diversion, and we believe that the tactical option and surgical technique should not be in line with a trend but should be strictly dictated by what brings the most benefits to the patient. The previously mentioned as well as personal experience make us recommend cecostomy as a protection mechanism in colorectal anastomotic surgery because of its safety, spontaneous reversibility and minimal invasivity.

Cecostomy is a safe and effective alternative for diverting stoma in colon and colorectal anastomosis, allowing a proper decompression that protects the anastomosis area, without requiring any surgical reintervention. Anastomotic colorectal surgery is thus safer with certain benefits for patients.

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VARIA

Evaluation of morbid obese patients with attitudes toward obese persons scale prior to bariatric surgery: Obesity from the perspective of real patients

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Abstract: *Background: The number of studies with the application of scales, such as Attitudes Toward Obese Persons Scale (ATOP), on morbid obese patients are limited. Therefore, we wanted to examine the attitudes toward obese people through the eyes of morbid obese patients.*

Methods: Between October 2019 - April 2020, 70 female and 20 male, morbid obese patients, who were admitted to the general surgery department to be evaluated for bariatric surgery were included in this study. These 90 morbid obese patients were asked to fill out ATOP, which included 20 statements.

Results: The mean ATOP score of all morbid obese patients was 63.44±18.58 (range:19-116). The mean ATOP score of female patients was 63.47±20.34 (range:19-116), whereas the mean ATOP score of male patients was 63.35±10.69 (range:43-80) (p=0.98). The frequency of the responses given by the male and female patients to the third statement, which was "Most obese people are more self-conscious than other people" pointed out a statistically significant difference (p=0.02).

Conclusions: Based on ATOP scores gained from morbid obese patients, 60% of morbid obese patients did not feel as happy as non-obese people, whereas 74.4% of the morbid obese patients thought that obese people were not as healthy as non-obese people. Moreover, ATOP scores which were slightly higher than 60 points in both female and male morbid obese patients indicated that morbid obese patients had neither negative nor highly positive attitudes toward obese people.

Keywords: *attitude, bariatric, morbid, obesity, surgery*

INTRODUCTION

Obesity is a chronic disorder that leads to an increasing public health problem worldwide [1, 2]. Obesity has a substantial psychosocial effect on a person's life [3]. It has been suggested that 20-60% of obese patients have a psychiatric disorder.

Depression, eating disorders such as binge eating, anxiety, low self-esteem, decreased quality of life, emotional neglect, stigma and discrimination have been associated with obesity. As weight loss improves the psychosocial functioning, psychosocial effects of obesity should be considered in the management of patients especially prior to bariatric surgery [4].

Obese patients may encounter stigmatization and weight bias not only at school and work but also within their social environment. Stigmatization refers to presence of negative perceptions toward obese patients, whereas weight bias refers to negative attitudes and beliefs toward obese patients without a reason. Attitudes of stigmatization and weight bias have been investigated in various study populations such as healthcare professionals, medical students and university students [5]. However, the number of studies with the application of scales, such as Attitudes Toward Obese Persons Scale (ATOP), on morbid obese patients are limited. Attitudes Toward Obese Persons Scale is used to evaluate whether people have favorable attitudes against obese patients or not [5]. Within this study, we wanted to examine the attitudes towards obese people through the eyes of morbid obese patients using ATOP.

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METHODS

Between October 2019 - April 2020, 70 (77.8%) female and 20 (22.2%) male, a total of 90 morbid obese patients, who were admitted to the general surgery department to be evaluated for bariatric surgery were included in this study. Local ethics committee approval was obtained prior to this study (2019/08-10). Patients with body mass index (BMI) greater than 40 kg/m^2 were regarded as morbid obese, and patients with BMI greater than 50 kg/m^2 were regarded as severe morbid obese [6]. These 90 morbid obese patients were asked to fill out ATOP, which included 20 statements. Each statement was valued between -3 to +3 points [7].

The participants were asked to mark each statement according to how much they agreed or disagreed with it, using a scale between, -3 to +3 points (-3: I strongly disagree, -2: I moderately disagree, -1: I slightly disagree, +1: I slightly agree, +2: I moderately agree, +3: I strongly agree).

These twenty statements are:
1. Obese people are as happy as nonobese people.
2. Most obese people feel that they are not as good as other people.
3. Most obese people are more self-conscious than other people.
4. Obese workers cannot be as successful as other workers.
5. Most nonobese people would not want to marry anyone who is obese.
6. Severely obese people are usually untidy.
7. Obese people are usually sociable.
8. Most obese people are not dissatisfied with themselves.
9. Obese people are just as self-confident as other people.
10. Most people feel uncomfortable when they associate with obese people.
11. Obese people are often less aggressive than nonobese people.
12. Most obese people have different personalities than nonobese people.
13. Very few obese people are ashamed of their weight.
14. Most obese people resent normal weight people.
15. Obese people are more emotional than nonobese people.
16. Obese people should not expect to lead normal lives.
17. Obese people are just as healthy as nonobese people.
18. Obese people are just as sexually attractive as nonobese people.
19. Obese people tend to have family problems.
20. One of the worst things that could happen to a person would be for him to become obese.

All of these statements were responded by the participants by the help of ATOP scale. In ATOP, points given to each statement by the participant are summed up. This final number is added to 60 in order to reach a total score, which varies between 0-120 [8].

Statistical analysis was performed using the SPSS 20.0 statistical package programme. Numerical variables were stated as the mean (±) standard deviation and medians (minimum–maximum). Categorical variables were stated as percentages. The Mann Whitney U test and independent samples t test were used to compare differences between two groups. The p-value <0.05 was considered significant.

RESULTS

This study included 90 – 70 female (77.8%) and 20 male (22.2%) – morbid obese patients. The mean age of the patients was 36.26±10.28 years (range: 18-65). The mean age of female patients was 36.77±10.22 years (range: 18-65), while the mean age of male patients was 34.50±10.54 years (range: 21-51). The mean BMI of the patients was 45.83±5.70 kg/m^2 (range: 40-65.3). The mean BMI of the female patients was 45.54±5.34 kg/m^2 (range: 40-64.3), while the mean BMI of the male patients was 46.84±6.86 kg/m^2 (range: 40-65.3) (p=0.57). The mean ATOP score of all morbid obese patients was 63.44±18.58 (range: 19-116). The mean ATOP score of female patients was 63.47±20.34 (range: 19-116), whereas the mean ATOP score of male patients was 63.35±10.69 (range: 43-80) (p=0.98). The responses of the patients to the statements within ATOP are depicted are in Tables 1, 2, 3.

The frequency of the responses given by the male and female patients to the third statement pointed out a statistically significant difference (p=0.02). Nevertheless, the frequency of the responses of the patients to the rest of the statements did not show any statistically significant difference (s1:p=0.95, s2:p=0.58, s4:p=0.40, s5:p=0.08, s6:p=0.38, s7:p=0.11, s8:p=0.40, s9:p=0.59, s10:p=0.98, s11:p=0.50, s12:p=0.68, s13:p=0.33, s14:p=0.97, s15:p=0.69, s16:p=0.44, s17:p=0.48, s18:p=0.66, s19:p=0.28, s20:p=0.73. s: statement)

Statement 1 was given -3 points by 42 (46.7%), -2 points by 4 (4.4%), -1 point by 8 (8.9%), 1 point by 11 (12.2%), 2 points by 9 (10%) and 3 points by 16 (17.8%) patients, respectively. The majority of morbid obese patients (60%) did not agree with the statement that obese people were as happy as non-obese people.

Statement 2 was given -3 points by 7 (7.8%) , -2 points by 6 (6.7%), -1 point by 8 (8.9%), 1 point by 10 (11.1%), 2 points by 12 (13.3%), 3 points by 47 (52.2%) patients, respectively. 76.6% of morbid obese patients agreed with the statement that most obese peopl felt that they were not as good as other people.

Table 1: Points given by the patients to the statements within attitudes toward obese persons scale. 60 (66.7%) patients strongly disagreed and 4 (4.4%) patients strongly agreed with the 8th statement, which was “most obese people are not dissatisfied with themselves”. In addition, most patients (58.9%) strongly disagreed with the 17th statement, which was “obese people are just as healthy as nonobese people” while only 7.8% of the morbid obese patients stated that they strongly agreed with this statement.

Score	-3	-2	-1	1	2	3
Statement	Patients n (%)					
S-1	42 (46.7%)	4 (4.4%)	8 (8.9%)	11 (12.2%)	9 (10%)	16 (17.8%)
S-2	7 (7.8%)	6 (6.7%)	8 (8.9%)	10 (11.1%)	12 (13.3%)	47 (52.2%)
S-3	17 (18.9%)	7 (7.8%)	3 (3.3%)	8 (8.9%)	10 (11.1%)	45 (50%)
S-4	28 (31.1%)	5 (5.6%)	6 (6.7%)	9 (10%)	13 (14.4%)	29 (32.2%)
S-5	13 (14.4%)	5 (5.6%)	10 (11.1%)	6 (6.7%)	21 (23.3%)	35 (38.9%)
S-6	17 (18.9%)	8 (8.9%)	7 (7.8%)	14 (15.6%)	20 (22.2%)	24 (26.7%)
S-7	19 (21.1%)	14 (15.6%)	11 (12.2%)	21 (23.3%)	15 (16.7%)	10 (11.1%)
S-8	60 (66.7%)	12 (13.3%)	5 (5.6%)	3 (3.3%)	6 (6.7%)	4 (4.4%)
S-9	25 (27.8%)	9 (10%)	11 (12.2%)	14 (15.6%)	12 (13.3%)	19 (21.1%)
S-10	18 (20%)	13 (14.4%)	15 (16.7%)	12 (13.3%)	15 (16.7%)	17 (18.9%)
S-11	20 (22.2%)	9 (10%)	11 (12.2%)	15 (16.7%)	14 (15.6%)	21 (23.3%)
S-12	12 (13.3%)	14 (15.6%)	15 (16.7%)	19 (21.1%)	8 (8.9%)	22 (24.4%)
S-13	24 (26.7%)	9 (10%)	7 (7.8%)	9 (10%)	6 (6.7%)	35 (38.9%)
S-14	11 (12.2%)	9 (10%)	6 (6.7%)	11 (12.2%)	12 (13.3%)	41 (45.6)
S-15	7 (7.8%)	3 (3.3%)	3 (3.3%)	17 (18.9%)	15 (16.7)	45 (50%)
S-16	27 (30%)	8 (8.9%)	9 (40%)	8 (8.9%)	14 (15.6%)	24 (26.7%)
S-17	53 (58.9%)	3 (3.3%)	11 (12.2%)	10 (11.1%)	6 (6.7%)	7 (7.8%)
S-18	47 (52.2%)	12 (13.3%)	10 (11.1%)	9 (10%)	3 (3.3%)	9 (10%)
S-19	16 (17.8%)	15 (16.7%)	12 (13.3%)	7 (7.8%)	11 (12.2%)	29 (32.2%)
S-20	12 (13.3%)	5 (5.6%)	7 (7.8%)	14 (15.6%)	13 (14.4%)	39 (43.3%)

Statement 3 was given -3 points by 17 (18.9%) , -2 points by 7 (7.8%), -1 point by 3 (3.3%), 1 point by 8 (8.9%), 2 points by 10 (11.1%), 3 points by 45 (50%) patients, respectively. 70% of morbid obese patients agreed that most obese people were more self-conscious than other people.

Statement 4 was given -3 points by 28 (31.1%), -2 points by 5 (5.6%), -2 points by 6 (6.7%), 1 point by 9 (10%), 2 points by 13 (14.4%) and 3 points by 29 (32.2%) patients, respectively. 56.6% of morbid obese patients belived that obese workers could not be as successful as other workers.

Statement 5 was given -3 points by 13 (14.4%), -2 points by 5 (5.6%), -1 point by 10 (11.1%), 1 point by 6 (6.7%), 2 points by 21 (23.3%), 3 points by 35 (38.9%) patients, respectively. The majority of the morbid obese patients (68.9%) thought that most non-obese people would not want to marry anyone who was obese.

Statement 6 was given -3 points by 17 (18.9%), -2 points by 8 (8.9%), -1 point by 7 (7.8%), 1 point by 14 (15.6%), 2 points by 20 (22.2%) and 3 points by 24 (26.7%), respectively. The majority of the morbid obese patients (64.5%) believed that severely obese people were usually untidy.

Statement 7 was given -3 points by 19 (21.1%), -2 points by 14 (15.6%), -1 point by 11 (12.2%), 1 point by 21 (23.3%), 2 points by 15 (16.7%) and 3 points by 10 (11.1%) patients, respectively. These results revealed that tendency of morbid obese patients was towards thinking that obese people were usually sociable (51.1%).

Statement 8 was given -3 points by 60 (66.7%), -2 points by 12(13.3%), -1 point by 5(5.6%), 1 point by 3(3.3%), 2 points by 6(6.7%) and 3 points by 4(4.4%) patients, respetively. 85.6% of morbid obese patients strongly disagreed with the statement that “most obese people are not dissatisfied with themselves”.

Table 2: Points given by the female patients to the statements within attitudes toward obese persons scale. 57.1% of the female patients strongly agreed with the 3rd statement, which was “most obese people are more self-conscious than other people”, while only 15.7% of them stated that they strongly disagreed with this statement.

Score	-3	-2	-1	1	2	3
Statement	**Female patients n (%)**					
S-1	33 (47.1%)	2 (2.9%)	6 (8.6%)	9 (12.9%)	9 (12.9%)	11 (15.7%)
S-2	6 (8.6%)	5 (7.1%)	7 (10%)	3 (4.3%)	11 (15.7%)	38 (54.3%)
S-3	11 (15.7%)	5 (7.1%)	2 (2.9%)	6 (8.6%)	6 (8.6%)	40 (57.1%)
S-4	21 (30%)	4 (5.7%)	4 (5.7%)	6 (8.6%)	11 (15.7%)	24 (34.3%)
S-5	8 (11.4%)	4 (5.7%)	5 (7.1%)	6 (8.6%)	18 (25.7%)	29 (41.4%)
S-6	15 (21.4%)	7 (10%)	6 (8.6%)	9 (12.9%)	14 (20%)	19 (27.1%)
S-7	16 (22.9%)	13 (18.6%)	9 (12.9%)	14 (20%)	11 (15.7%)	7 (10%)
S-8	48 (68.6%)	11 (15.7%)	2 (2.9%)	1 (1.4%)	4 (5.7%)	4 (5.7%)
S-9	18 (25.7%)	8 (11.4%)	10 (14.3%)	12 (17.1%)	10 (14.3%)	12 (17.1%)
S-10	14 (20%)	11 (15.7%)	11 (15.7%)	9 (12.9%)	11 (15.7%)	14 (20%)
S-11	16 (22.9%)	7 (10%)	9 (12.9%)	9 (12.9%)	9 (12.9%)	20 (28.6%)
S-12	9 (12.9%)	10 (14.3%)	14 (20%)	13 (18.6%)	5 (7.1%)	19 (27.1%)
S-13	21 (30%)	8 (11.4%)	6 (8.6%)	5 (7.1%)	2 (2.9%)	28 (40%)
S-14	8 (11.4%)	7 (10%)	6 (8.6%)	8 (11.4%)	9 (12.9%)	32 (45.7%)
S-15	7 (10%)	3 (4.3%)	3 (4.3%)	9 (12.9%)	11 (15.7)	37 (52.9%)
S-16	23 (32.9%)	3 (4.3%)	6 (8.6%)	5 (7.1%)	12 (17.1%)	21 (30%)
S-17	42 (60%)	2 (2.9%)	10 (14.3%)	8 (11.4%)	3 (4.3%)	5 (7.1%)
S-18	37 (52.9%)	11 (15.7%)	6 (8.6%)	7 (10%)	2 (2.9%)	7 (10%)
S-19	11 (15.7%)	12 (17.1%)	9 (12.9%)	6 (8.6%)	7 (10%)	25 (35.7%)
S-20	9 (12.9%)	5 (7.1%)	5 (7.1%)	9 (12.9%)	11 (15.7%)	31 (44.3%)

Statement 9 was given -3 points by 25(27.8%), -2 points by 9(10%), -1 point by 11(12.2%), 1 point by 14(15.6%), 2 points by 12(13.3%) and 3 points by 19(21.1%) patients, respectively. According to these results, half of the morbid obese agreed with the view that obese people were just as self-confident as other people, while the other half of morbid obese patients did not.

Statement 10 was given -3 points by 18(20%), -2 points by 13(14.4%), -1 point by 15(16.7%), 1 point by 12(13.3%), 2 points by 15(16.7%), 3 points by 17(18.9%) patients, respectively. As a result, 50% of the morbid obese patients were in favor of the statement that most people felt uncomfortable when they associated with obese people, while the other 50% disagreed with this statement.

Statement 11 was given -3 points by 20 (22.2%), -2 points by 9 (10%), -1 point by 11 (12.2%), 1 point by 15 (16.7%), 2 points by 14 (15.6%) and 3 points by 21 (23.3%) patients, respectively. 40 patients (44.4%) disagreed while 50 patients (55.6%) agreed with the statement that obese peopole were often less aggressive than non-obese people.

Statement 12 was given -3 points by 12(13.3%), -2 points by 14(15.6%), -1 points by 15(16.7%), 1 point by 19(21.1%), 2 points by 8(8.9%) and 3 points by 22(24.4%) patients, respectively. The majority of the morbid obese patients (54.4%) indicated that most obese people had different personalities than non-obese people.

Statement 13 was given -3 points by 24(26.7%), -2 points by 9(10%), -1 point by 7(7.8%), 1 point by 9(10%), 2 points by 6(6.7%) and 3 points by 35(38.9%), respectively. According to these scores, 50 (55.6%) morbid obese patients had a positive tendency toward the statement that very few obese people were ashamed of their weight.

Table 3: Points given by the male patients to the statements within attitudes toward obese persons scale. Most of the male patients (30%) strongly disagreed with the 3rd statement, which was “most obese people are more self-conscious than other people”. Statistically significant difference was observed between the frequency of the responses given by the male and female patients to the third statement (p=0.02).

Score	-3	-2	-1	1	2	3
Statement	**Male patients n (%)**					
S-1	9 (45%)	2 (10%)	2 (10%)	2 (10%)	0 (0%)	5 (25%)
S-2	1 (5%)	1 (5%)	1 (5%)	7 (35%)	1 (5%)	9 (45%)
S-3	6 (30%)	2 (10%)	1 (5%)	2 (10%)	4 (20%)	5 (25%)
S-4	7 (35%)	1 (5%)	2 (10%)	3 (15%)	2 (10%)	5 (25%)
S-5	5 (25%)	1 (5%)	5 (25%)	0 (0%)	3 (15%)	6 (30%)
S-6	2 (10%)	1 (5%)	1 (5%)	5 (25%)	6 (30%)	5 (25%)
S-7	3 (15%)	1 (5%)	2 (10%)	7 (35%)	4 (20%)	3 (15%)
S-8	12 (60%)	1 (5%)	3 (15%)	2 (10%)	2 (10%)	0 (0%)
S-9	7 (35%)	1 (5%)	1 (5%)	2 (10%)	2 (10%)	7 (35%)
S-10	4 (20%)	2 (10%)	4 (20%)	3 (15%)	4 (20%)	3 (15%)
S-11	4 (20%)	2 (10%)	2 (10%)	6 (30%)	5 (25%)	1 (5%)
S-12	3 (15%)	4 (20%)	1 (5%)	6 (30%)	3 (15%)	3 (15%)
S-13	3 (15%)	1 (5%)	1 (5%)	4 (20%)	4 (20%)	7 (35%)
S-14	3 (15%)	2 (10%)	0 (0%)	3 (15%)	3 (15%)	9 (45%)
S-15	0 (0%)	0 (0%)	0 (0%)	8 (40%)	4 (20%)	8 (40%)
S-16	4 (20%)	5 (25%)	3 (15%)	3 (15%)	2 (10%)	3 (15%)
S-17	11 (55%)	1 (5%)	1 (5%)	2 (10%)	3 (15%)	2 (10%)
S-18	10 (50%)	1 (5%)	4 (20%)	2 (10%)	1 (5%)	2 (10%)
S-19	5 (25%)	3 (15%)	3 (15%)	1 (5%)	4 (20%)	4 (20%)
S-20	3 (15%)	0 (0%)	2 (10%)	5 (25%)	2 (10%)	8 (40%)

Statement 14 was given -3 points by 11(12.2%), -2 points by 9(10%), -1 point by 6(6.7%), 1 point by 11(12.2%), 2 points by 12(13.3%), 3 points by 41(45.6) patients, respectively. Majority of the morbid obese patients (71.1%) accepted that most obese people resented normal weight people.

Statement 15 was given -3 points by 7(7.8%), -2 points by 3(3.3%), -1 point by 3(3.3%), 1 point by 17(18.9%), 2 points by 15(16.7%) and 3 points by 45(50%) patients, respectively. The vast majority of the morbid obese patients (80.6%) shared the idea that obese people were more emotional than non-obese people.

Statement 16 was given -3 points by 27(30%), -2 points by 8(8.9%), -1 point by 9(40%), 1 point by 8(8.9%), 2 points by 14(15.6%) and 3 points by 24(26.7%) patients. The vast majority of the morbid obese patients (78.9%) did not agree with the statement that obese people should not expect to lead normal lives.

Statement 17 was given -3 points by 53 patients by 53(58.9%), -2 points by 3(3.3%), -1 point by 11(12.2%), 1 point by 10(11.1%), 2 points by 6(6.7%) and 3 points by 7(7.8%) patients. 74.4% of the morbid obese patients did not agree with the statement that obese people were just as healthy as non-obese people.

Statement 18 was given -3 points by 47(52.2%), -2 points by 12(13.3%), -1 point by 10(11.1%), 1 point by 9(10%), 2 points by 3(3.3%) and 3 points by 9(10%) patients, respectively. 76.6% of morbid obese patients did not agree with the statement that obese people were just as sexually attractive as non-obese people.

Statement 19 was given -3 points by 16(17.8%), -2 points by 15(16.7%), -1 point by 12(13.3%), 1 point by 7(7.8%), 2 points by 11(12.2%), and 3 points by 29(32.2%) patients, respectively. 52.2% of the morbid obese patients agreed with the statement that obese people tended to have family problems.

Statement 20 was given -3 points by 12(13.3%), -2 points by

5(5.6%), -1 point by 7(7.8%), 1 point by 14(15.6%), 2 points by 13(14.4%) and 3 points by 39(43.3%). 58.9% of the patients agreed with the statement that becoming obese would be one of the worst things that could happen to a person.

DISCUSSIONS

Hereditary, social, economic and environmental factors have been included in the etiology of obesity [9, 10]. In accordance with increased obesity rates, bias about obese people has become more prevalent [11, 12]. Negative attitudes toward obese people which lead to prejudice and discrimination have a detrimental impact on the mental health of obese individuals (9). Obese people may experience discrimination in various aspects of life such as work, education and medical care [13-22].

In order to evaluate attitudes toward obese people, ATOP scale has been used in several studies [5]. However, ATOP scale which was used on different populations revealed conflicting results. Puhl et al. compared ATOP scores between female obese patients with and without binge eating disorder. The mean ATOP scores were similar in both groups. Puhl et a. suggested that obesity increased vulnerability to negative weight-based attitudes regardless of eating disorder [8]. Kadar et al. reported that obesity bias was common in chiropractic students and faculty members. Students and faculty members had anti-obesity attitudes according to ATOP. In addition, ATOP scores did not show significant difference between preclinical and clinical students [23]. Soto et al. reported more positive attitudes toward obese individuals in psychology students compared to medical students. Moreover, positive attitudes of the students according to ATOP were correlated with frequent contact with obese people [24]. Darling et al. investigated attitudes toward obese people in graduate and undergraduate nursing students, graduate education and graduate social work students. Darling et al. reported worse attitudes in nursing students, younger participants and participants without an obese family member or a friend [5]. Ambwani et al. reported that 92.5% of young adults aged 18 to 29 approved at least one negative attitude within ATOP. However, approval of negative attitudes did not show significant difference according to BMI [11].

It has been suggested that negative attitudes toward obese people might be observed in women more commonly than in men. However, Glenn et al. reported that women were significantly more positive in their attitude toward obese people compared to men [25]. On the other hand, Darling et al. reported similar ATOP scores in females and males, thus no gender difference in the attitudes toward obese individuals was identified [5]. Moreover, several studies revealed that individuals who had a relative or a friend with obesity or had contact to obese people commonly tended to have more positive attitudes toward obese people [5, 24, 26]. However, attitudes of obese people toward other obese people have not been investigated in depth, yet.

Since the ATOP score ranges from 0 to 120 and higher scores reveal more positive attitudes, the mean ATOP score of our patients (63.44±18.58) which was slightly higher than midpoint indicated that morbid obese had positive attitudes toward obese people. Previous studies reported the mean ATOP score was 60 in obese women, whereas 59.6 in obese women with binge eating disorder [8], 57.6 in preclinical chiropractic students, 59.7 in clinical chiropractic students, 49 in preclinical faculty members, 52.2 in clinical faculty members [8] and 68.5 in nursing students, 77.3 in social work students, 71.4 in graduate education students [5].

Within this study, the majority of the patients (77.8%) admitted to the general surgery clinic for bariatric surgery evalution were female. This result may point out two different findings. First of all, obesity is more common in female than male individuals. Secondly, the number of obese women seeking medical help to fight against obesity is more than the number of male patients. Within this study, no significant difference was observed in mean ATOP scores of the female and male morbid obese patients (p=0.98). Therefore, our results revealed that both women and men with morbid obesity had similar attitudes toward obese people. However, points given by morbid obese patients to the 3rd statement ‘‘Most obese people are more self-conscious than other people’’ showed gender difference. More than half of female morbid obese patients (57.1%) stated that they strongly agreed to the 3rd statement while most of the male morbid obese patients (30%) stated that they strongly disagreed to the same statement. This result might reveal that morbid obesity might affect female and male patients differently in building relationship or in communicating with other people. Thefore, novel and gender specific scales about obesity may be helpful in evaluating obese patients before making an appropriate treatment plan prior to bariatric surgery.

Moreover, with the help of ATOP applied on morbid obese patients, we have gained insight into the attitudes of morbid obese patients on obese individuals. In addition, we had the opportunity to evaluate obesity from the perspective of morbid obese patients. Unfortunately, 60% of morbid obese patients did not feel as happy as non-obese people. 74.4% of the morbid obese patients thought that obese people were not as healthy as non-obese people. Moreover, 76.6% of morbid obese patients replied that obese people were not as sexually attractive as non-obese people. Nevertheless, 80.6% of morbid obese patients shared the idea that obese people were more emotional than non-obese people.

In conclusion, ATOP has been investigated in various studies in order to determine whether non-obese individuals had negative attitudes toward obese individuals or not. However, the data are scarce in the English medical literature about the attitudes of morbid obese patients toward obese individuals. Thefore, we evaluated ATOP in morbid obese individuals to gain a sight whether morbid obese patients had negative perceptions about other obese people. Any ATOP score above the midpoint, which is 60 points, has been reported to indicate that the individual had positive attitudes toward obese people [27, 28]. However, higher scores than 60 indicate more positive attitudes toward obese individuals. Therefore, ATOP scores identified within our study which were slightly higher than 60 in both our female and male patients indicated that morbid obese patients had neither negative nor highly positive attitudes toward obese people.

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All the authors agreed on the content of the manuscript.

Local ethics committee approved this study.

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VARIA

Medical intervention in the biological attack with Bacillus anthracis

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Abstract: *The biological agent most likely to be used as a biological weapon is Bacillus anthracis, which causes the anthrax infectious disease. During the Cold War, it was present in large quantities in the military arsenals of some states, and at the beginning of the 21st century was used in a series of small bioterrorist attacks. In case of occurrence of events using Bacillus anthracis, urgent and adequate qualitative and quantitative response measures are needed to manage the consequences of the attack and to reduce the effects on public health. These include complex and well-coordinated activities to ensure medical intervention for the prophylaxis, treatment and recovery of patients. General prophylaxis is achieved by vaccination, individual and collective CBRN protection, decontamination, partial and total sanitary treatment, microbiological analysis for the detection, identification and confirmation of the biological agent. Therapy for disease induced by weaponized B. anthracis differs from classic antrax disease regarding antibiotics, doses and time, resulting a large antibiotic consumption per case. Complete recovery of the patients is achieved not only in terms of restoring normal biological parameters, but also in terms of the organism's "sterilisation" regarding the causal biological agent.*

Keywords: *biological agent, antibiotherapy, anthrax, Bacillus anthracis, pharmacoeconomics, medical intervention*

INTRODUCTION

The biological attack, as an act of biological warfare or bioterrorism, occurs by attack with biological weapons, the biological agent being living (bacteria, viruses, etc.) or nevi (toxin, intermediates, etc.).

The most used biological agent, also confirmed by the existence of military arsenals or bioterrorist attacks in the 20th and early 21st centuries, is Bacillus anthracis, (sporogenous gram-positive bacillus) that causes anthrax infectious disease (Figure 1).

Anthrax spores can be disseminated by various means to produce a military attack with biological warfare agents (BWA), a limited bioterrorist attack, or a bio-crime against established targets (as they were in the US and EU in 2001 and 2002).

In order to reduce the effects on public health, urgent and adequate qualitative and quantitative response measures are needed to manage the consequences of the attack. These include medical intervention for the prophylaxis, treatment and recovery of patients [1, 2].

Figure 1: Bacillus anthracis 1119 R Stamatin – culture examined under a microscope x1500, (original)

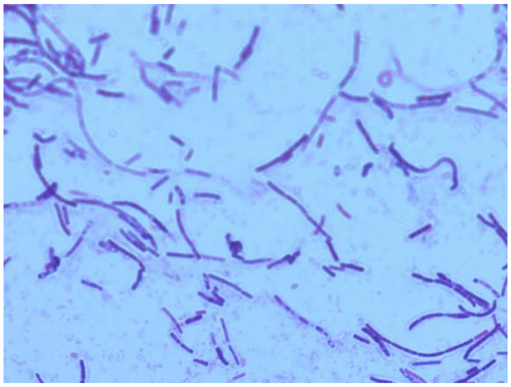

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The general prophylaxis is achieved through health education, hygiene, vaccination, individual and collective CBRN protection, decontamination, partial and total health treatment, surveillance of suspects and contacts and, as the case may be, evacuation or isolation. It means that the medical service must collaborate with the veterinary service, the specialized military units, law enforcement agencies, local authorities and media representatives to disseminate accurate information, in order to reduce the epidemic and panic in the targeted community.

In practice, after a biological attack, the decontamination of the environment on large areas does not become an issue in terms of the excessive costs in using important forces and means, but only in terms of the personnel, the objects of strict necessity and of the access routes. The experience of the British army, on the island of Gruinard, is illustrative in this regard [3].

Although some authors consider (including in military textbooks) anthrax to be a non-contagious infectious disease, the risk of secondary contamination by manure, coughing and objects contaminated with bacteria in vegetative or sporadic form should not be overlooked. As a result, general precautions for anti-infective prophylaxis should be applied.

The diagnosis involves, as the case may be, an environmental stage with microbiological analysis for the detection, identification and confirmation of the biological agent in the air (if aerosolized), water, food, soil or different objects. This stage involves not only sanitary units (eg public health) but also military defense units against chemical, biological, radiological and nuclear weapons of mass destruction (WMD-CBRN), emergencies, secret services, etc. If contamination and disease are suspected, the task is of the human and/or veterinary medical service, which collects biological samples, cultivates, isolates and identifies with classical and/or modern means as well as confirmation by Polymerase chain reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA) and Mass Spectrometry (Figure 2). Immediately after shall be established the pattern of susceptibility/resistance of the vegetative bacteria to antibiotics, for the orientation of the antibiotherapy. Some anthrax strains may be less pathogenic, anthrax-like or vaccine strains, which would lead to false alarms and the loss of credibility of the CBRN health and surveillance/defense service [4].

Figure 2: Bacillus anthracis - mass spectrometry diagram (original)

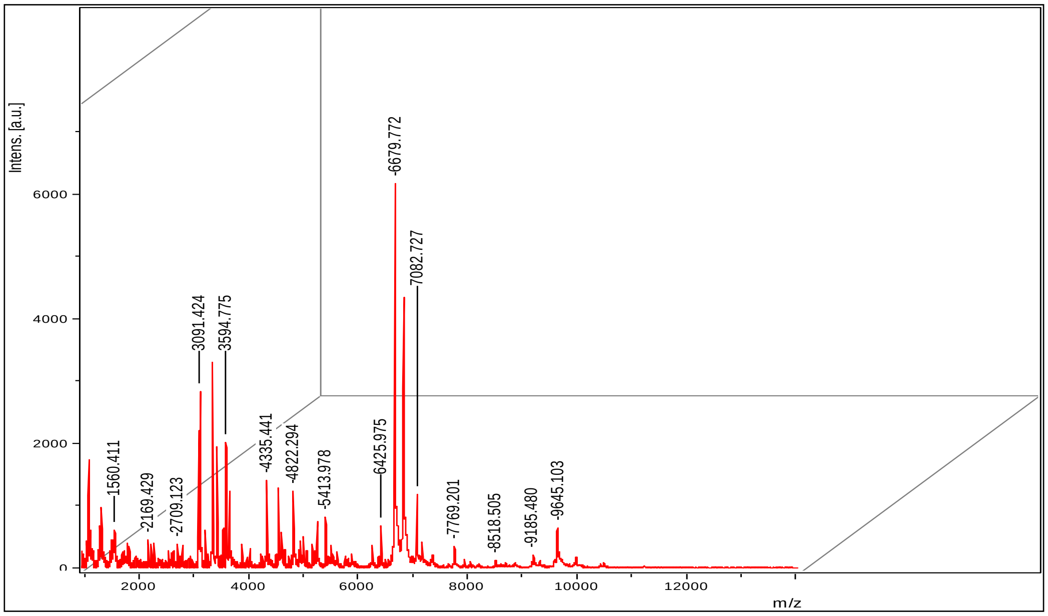

CLINICAL

Anthrax, also called charcoal, pustule, black sore, etc. is an acute infectious disease that naturally affects herbivores (horses, cattle, sheep, etc.) as zoonoses and epizootics, but can also be transmitted to humans, such as zooantroponosis and epidemic. Because the etiological agent B. anthracis is a telluric bacterium, a gram-positive bacillus that spores, therefore highly resistant to the environment, B. anthracis spores are considered the most likely biological agent for biological weapons and for bioterrorism, being confirmed by recent attacks. The spore itself is not pathogenic, because it does not have a metabolism and does not multiply, but it finds favorable living conditions (humidity, nutrients, heat, etc.) in the body that it contaminates and passes into vegetative form, which is pathogenic and causes the infection. For the biological attack on humans, domestic and wild animals or for the environment's contamination, it is estimated the use of strains of B. anthracis that are highly pathogenic, virulent, toxigenic, resistant to the environment and to the treatment with antibiotics (multiple antibioresistance), obtained either by selection either by genetic manipulation (Figure 3). As a result, biosafety measures must be respected, and treatment is intensive and prolonged [5, 9, 11-17].

Figure 3: Simulation of the dispersion of the biological agent Bacillus anthracis, 40 minutes report after aerosolization (original)

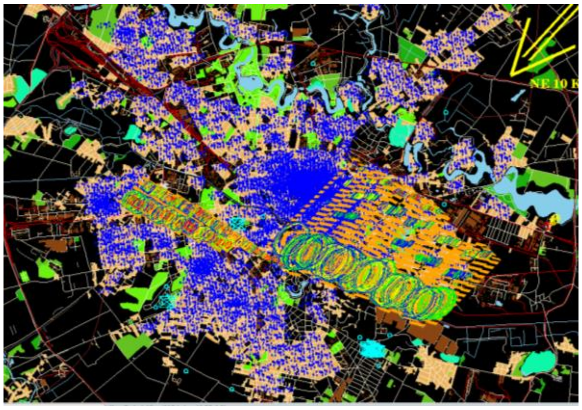

Depending on the pathway into the body and the location of the infection, the cutaneous anthrax (which has average severity) and the visceral anthrax are differentiated, with four clinical forms: gastrointestinal, pulmonary, meningeal and septicemic, which have a very serious prognosis.

Cutaneous anthrax the most common form of natural infection, most often occurs in animal breeders, is manifested by one or more characteristic "black sore", with a "charcoal" appearance (red-hot coal) with a red area and edematized around, painful, with medium-serious infectious and toxemic manifestations, but with reduced interhuman contagiousness. Classical antibiotherapy is applied (according to the infectious diseases manuals), symptomatic medication (anti-inflammatory, pain relievers, antipyretics), local antiseptics, dressing, possibly minor surgery.

Gastrointestinal anthrax (intestinal) caused by the ingestion of bacteria or spores with contaminated food or water that infects the gastrointestinal tract, with infectious and toxemic manifestations of high severity, with reduced human contagiousness, but with risk of environmental contamination. Classical antibiotherapy (according to infectious diseases manuals), symptomatic medication (anti-inflammatory, pain relievers, antipyretics), intestinal antiseptics, antidiarrheal and antiemetics are applied with caution. The waste will be collected and decontaminated energetically with conventional sporicidal disinfectants (chloride of lime, chloramine or formaldehyde).

Pulmonary anthrax (inhalation), caused by the inhalation of spores, in professional context or in aerosolization attacks for the deliberate release of spores. It is the most effective form of the biological attack, with infectious and toxemic manifestations of extreme severity: it is considered that morbidity is 99%, mortality over 90% in the untreated patients and about 50% in the treated patients. It is considered that it is not transmitted interhumanly, but we must also consider secondary aerosolization (from the patient) that can spread bacteria and spores through coughs, sneezes, saliva, etc. therefore precautions for air transmission must also be applied.

Other clinical forms of anthrax, more serious but rare, are actually complications of untreated disease, insufficiently treated or in immunocompromised organisms: meningeal anthrax and septicemic anthrax, in which mortality can reach 99%.

The incubation period for pulomonary anthrax varies between 1 and 60 days, depending on the host and the biological agent, and the patients often present: fever, discomfort, salivation, fatigue, low productive cough, nausea, vomiting and asfixia.

TREATMENT

The therapy for the disease resulting in a biological attack differs greatly from the classic recommendations, such as antibiotics (AB), doses and duration, achieving a very high consumption for each treated case. Our intention is to explain and complete the therapeutic procedures in the cases of anthrax resistant to treatment, as it can be the case in regards to BWA, bioterorist and biocrime attacks, with multi-resistant bacteria, in the patients with complications or immunosuppressed (by irradiation, intoxication or infections) etc. The old treatments were replaced or supplemented with antibiotherapy (ABT), which was a real breakthrough for the infectious disease clinic. But in the case of BWA, although this therapy is not wrong, it may not be effective: the bacterium is multi-resistant (AMR antimicrobial resistance), the clinical form is more serious, there should be a mass application treatment, so the therapeutic protocol differs. The causative agent of anthrax can persist in the body as a harmless spore and may become vegetative, thus pathogenic, later or even after the completion of the classic treatment. For this reason, after the attack with anthrax spores, post-exposure prophylaxis to contaminants and the anti-infective treatment in patients shall be continued without interruption for 60 days, according to the recommendations of the European Medicines Agency [6, 7, 8]. There is a risk of relapse and fallback, so treatment must be complex and individualized, but sometimes under mass therapy conditions. These conflicting requirements require continuous medical supervision and adequate health logistics [9].

There are no studies on humans for post-exposure prophylaxis with antibiotics, but experiments on guinea pigs and monkeys show that antibiotics from the cyclin and fluorinated quinolone groups, respectively Doxycycline and Ciprofloxacin, are the most effective in the prophylactic and curative treatment of anthrax [10]. However, any anti-infective treatment is important, because in the event of an epidemic, reserves of a certain antibiotic will be quickly depleted.

In the case of natural pulmonary anthrax (with "wild" bacillus) can be applied a complex treatment with classical antibiotherapy (according to the infectious diseases manuals), an adjunctive therapy (general or specific immunotherapy), complementary, symptomatic medication (anti-inflammatory, pain relievers, antipyretics), antiseptics, supporting vital functions (oxygen therapy, prosthetic respiration, etc.) and hospitalization in the AIC ward for infectious diseases.

In the case of the attack with aerosolized BWA, will be applied the principles of modern antibiotherapy, with antibiotic of choice or alternatives, for 60 days according to the recommendations of the EMA 2014 and the complex treatment depending on the specific situation. It turns out that in the case of an epidemic caused by a biological attack, the large number of patients who require the same special medical care and the same medicines creates a great problem of medical logistics.

Medical countermeasures may not be effective due to insufficient forces and means, which requires interinstitutional cooperation at local, national, European or international level. It should be noted that where the therapeutic recommendations state that "there is no recommended treatment" this does not mean that there is no treatment, but only that there is no specific treatment yet. So the doctor will apply any available treatment, which according to the medical logic and his experience, will heal his patient or will improve his condition, but in compliance with the classic principle "Primum non nocere" (Hippocrates). Regardless of the situation, no patient should be left without treatment.

Ciprofloxacin is recommended in the first line of treatment in two daily doses for 60 days [8], with all the pharmacological disadvantages it presents but the WHO has recommended caution with high and/or prolonged doses of ciprofloxacin treatment due to side effects such as tendon damage [18].

Other quinolones, such as Ofloxacin and Levofloxacin, offer options for alternative treatment, but dosage recommendations exist only for adults. Doxycycline and penicillins may be alternative therapies only if confirmed by susceptibility tests; Penicillin is not a safe bactericide on B. anthracis, but oral Amoxicillin is an alternative or continuation option if tolerated by the patient and if in vitro susceptibility is confirmed. B. anthracis can produce betalactamases, enzymes that hydrolyze penicillins [19].

Broad-spectrum antibiotics in prolonged treatment cause intestinal dysmicrobism and the microbiota should be protected with probiotics and the administration of useful bacteria if necessary.

The same antibacterial chemotherapics are recommended for the post-exposure prophylaxis, but Amoxicillin will be the drug of choice for pregnant women and young children, but only if the susceptibility tests are positive for penicillins.

Due to the high mortality in pulmonary anthrax, it is recommended to combine two or more antibiotics considered to be effective, the "therapeutic umbrella", but we do not have clinical trials for the multimedication therapy. Other active in vitro antibiotics that could be used in combination with Ciprofloxacin and Doxycycline are: protein synthesis inhibitors (Rifampicin, Chloramphenicol, Clindamycin, Clarithromycin, Erythromycin, Gentamicin, Streptomycin, etc.) and Vancomycin, but there is insufficient data to confirm their therapeutic efficacy in pulmonary anthrax [19]. Penicillins will not be used in combination with Ciprofloxacin. B. anthracis has a natural resistance to Sulfamethoxazole, Trimethoprim, Cefuroxime, Cefotaxime, Aztreonam, Ceftazidime etc., so these antibiotics cannot be used in the prophylactic or curative treatment of anthrax [10].

The injectable anti-infective treatment, although more effective, cannot be applied practically in the case of mass disease caused by a biological attack. Oral treatment is recommended if the patient can swallow and bioavailability is appropriate. As an example, Ciprofloxacin and Doxycycline are recommended for oral use [8].

Contraindications are those provided in the package leaflet, and as recommended by Prof. Ludovic Păun: "When prescribing an antibiotic, at least read the package leaflet!"

In pregnant and lactating women, shall be avoided the adminstration of risk products, breastfeeding will be discontinued and the treatment schedule shall be adapted accordingly. When applying any treatment, the axiom "There are no diseases, but patients" (Hippocrates) will be taken into account, and the complexity of the possible situations reminds us that medicine is equally science and art.

The present synthesis proposes therapeutic schemes for patients confirmed or suspected of pulmonary, gastrointestinal or cutaneous anthrax, and for the post-exposure prophylaxis in the case of confirmed or suspected exposure to B. anthracis, according to the studied bibliography and to the personal experience of scientific research of in vitro and in vivo anti-infective medical protection [19] and are presented by: International Common Denominator (ICD), role in therapy and prophylaxis, contraindications, dosage in treatment of suspected or clinically confirmed cases, post-exposure prophylaxis in confirmed or suspected cases of exposure to B. anthracis and duration of curative or prophylactic treatment. Depending on the clinical manifestations and situation, other anti-infective treatments, therapeutic serum (anti-carbonation serum, Cantacuzino I.), for supporting vital functions, immunomodulators, adjuvants, hygienic-dietary, psychotherapy, etc. will be applied.

In cutaneous anthrax with mild or medium forms, in which there are cases of spontaneous healing, the treatment will be done with the same dosage, but only for 7-10 days, and if there is a risk of inhalation of spores, the prophylaxis will last 60 days. In severe cases of cutaneous anthrax, with signs of systemic impairment, locally pronounced edema, with injuries to the head or the neck, a combined treatment will be applied [8].

The contraindications are those provided in the package leaflet, and in pregnant women and in lactation, the risk products will be avoided, breastfeeding will be discontinued and the treatment schedule adapted.

The dosage for children is calculated in mg/kg body weight, corresponding to a pharmacological reality, but without exceeding the adult dosage. From this it can be deduced that in adults with body weights significantly different from the average weight of 70 kg, therapeutic doses should be recalculated and we propose, for example, that individuals over 140 kg (without being obese) should be given double doses, if possible.

Antibiotherapy schemes according to EMA 2014

In the case of the BWA anthrax attack, the classical ABT is unlikely to be effective, due to changes in the "militarization" of the biological agent. As a result, AB will be used according to the EMA 2014 recommendations for biological agents, but also the antibiogram (ABG) will be used to confirm the spectrum of the isolated strain susceptibily/resistance from the patient.

Quinolones are the most important antibacterial chemotherapics in the treatment of anthrax.

Ciprofloxacin is the treatment of choice and is an alternative to continuing oral treatment, and is the first choice in prophylaxis until confirmation of the susceptibility of other antibiotics.

The dosage for the curative treatment of pulmonalry and gastrointestinal anthrax in adults (over 15 years) is initially 400 mg i.v. twice daily, continued with 500 mg p.o. twice a day for 60 days, totaling 60 g of the active substance. In children (under 15 years) is initially 15 mg/kg body weight i.v. twice a day, continued with 15 mg/kg body weight p.o. twice a day, (the daily dose should not exceed the adult dose) for 60 days.

Posology for the prophylactic post-exposure treatment in adults is 500 mg p.o. two times a day, for 60 days, totaling 60 g active substance, and in children 15 mg/kg body weight daily twice a day, (the daily dosage shall not exceed the adult dose), for 60 days.

It is known that antibiotics are among the most widely used drugs, and their costs are not very high. But in countries where compulsory health insurance operates, costs per dose can be increased by an order of magnitude. As a result, the costs per treatment must be compared, because for a large number of patients the total costs can be very high, so the principles of pharmacoeconomics will apply.

Ofloxacin is an alternative to Ciprofloxacin. The dosage for the curative treatment of pulmonary and gastrointestinal anthrax in adults (over 15 years) is initially 400 mg i.v. twice a day, continued with 400 mg p.o. twice a day for 60 days, totaling 48 g of active substance. The dosage for prophylactic post-exposure treatment in adults is 400 mg p.o. twice a day for 60 days, totaling 48 g of active substance.

Levofloxacin is an alternative to Ciprofloxacin. The dosage for the curative treatment of pulmonary and gastrointestinal anthrax in adults (over 15 years) is initially 500 mg i.v. once a day, continued with 500 mg p.o. once a day for 60 days, totaling 30 g of active substance. The dosage for prophylactic post-exposure treatment in adults is 500 mg p.o. once a day for 60 days, totaling 30 g of active substance.

Modern cyclins represent the optimal alternative antibacterial treatment.

Doxycycline is the alternative choice treatment and continuation of oral treatment, and is the first choice in prophylaxis if the sensitivity of the B. anthracis strain is confirmed.

The dosage for the curative treatment of pulmonalry and gastrointestinal anthrax in adults (over 15 years) and in older children 8-15 years, over 45 kg) is initially 100 mg i.v. twice a day, continued with 100 mg p.o. twice a day for 60 days, totaling 12 g of the active substance. In older children (8-15 years, under 45 kg) and in younger children (under 8 years) is initially 2.2 mg/kg body weight i.v. twice a day, (the daily dose should not exceed the adult dose) continued with the same oral dosage for 60 days, totaling 12 g of active substance.

The dosage for prophylactic post-exposure treatment in adults (over 15 years and in older children 8-15 years, over 45 kg) is initially 100 mg i.v. twice a day, for 60 days, totaling 12 g of the active substance and in children 15 mg/kg body weight twice a day (the daily dose should not exceed the adult dose). In older children (8-15 years, under 45 kg) and in younger children (under 8 years) is initially 2.2 mg/kg body weight i.v. twice a day, continued with the same oral dosage for 60 days, totaling 12 g of active substance.

Some beta-lactams may be used as an alternative antibacterial treatment, depending on the susceptibility of the B. anthracis strain.

Penicillin G is an alternative to first-line treatment if bacterial susceptibility is confirmed and penicillin allergy is excluded.

The posology for the curative treatment of pulmonary and gastrointestinal anthrax in adults (over 15 years and in older children 12-15 years) is 3 g (approx. 3 MUI) i.v. (preferably brannule infusion) 6 times a day (every 4 hours), for 60 days, totaling 1,080 g in 360 sessions (!?). In children (under 12 years) 30 mg/kg body weight, 5 times a day, for 60 days. It is not administered for post-exposure prophylaxis.

Amoxicillin is an alternative to first-line treatment and oral prophylaxis if bacterial susceptibility is confirmed.

Posology for the curative treatment of pulmonary and gastrointestinal anthrax in adults (over 15 years) 1 g i.v. 3 times a day, followed by 500 mg p.o. 3 times a day for 60 days, totaling 91.5 g of the active substance. In children (under 15 years), 80 mg/kg body weight per day, i.v. divided into 3 doses, followed by 80 mg/kg body weigh per day, p.o. divided into 3 doses (without exceeding the adult dose) for 60 days.

Posology for the prophylactic post-exposure treatment in adults (over 15 years) is 500 mg p.o. three times a day, for 60 days, totaling 90 g active substance, and in children is given 80 mg/kg body weight daily, p.o. divided into 3 doses, without exceeding the adult dose, for 60 days.

The recovery of the patients shall be considered not only in terms of restoring health but also in terms of the "sterilization" of the causal biological agent, so it is long lasting even in the case of an apparent cure.

Pharmacoeconomics

In case of a mass contamination or illness, very large quantities of specific antibacterial chemotherapics are needed, and the supply and replenishment of the sanitary units involved will impose significant financial costs, sometimes prohibitive. It turns out that, in addition to the pharmacological requirements, it is compulsory to take into account the criteria of pharmacoeconomics, for which we present below some examples, which can be found and/or updated on dedicated sites, on the Internet [20, 32, 33, 34].

“The wholesale cost of Ciprophloxacine in the developing world is between US$0.03 and US$0.13 a dose” [14]. In the United States it is sold for about US$0.40 per dose.

“Ofloxacin was patented in 1980 and approved for medical use in 1985 [21]. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system”. Ofloxacin is available as a generic medication [22]. The wholesale cost in the developing world is about US$3.27 per month [23]. In the United States a course of treatment costs about $50–100. The wholesale cost in the developing world is about US$3.27 per month [24]. In the United States a course of treatment costs about $50–100.

“Levofloxacin was approved for medical use in the United States in 1996. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system [25]. It is available as a generic medication” [24]. “The wholesale cost in the developing world is about US$0.44–0.95 per week of treatment. In the United States a week of treatment costs about $50–100. The wholesale cost in the developing world is about US$0.44–0.95 per week of treatment” [26]. In the United States a week of treatment costs about $50–100.

Doxycycline is available as a generic medicine and is generally inexpensive [27, 28]. “The wholesale cost in the developing world is between US$0.01 and $0.04 per pill. In the United States 10 days of treatment is about US$14; however, some in 2014 were selling it for more than $3.00–10.00 per pill. The wholesale cost in the developing world is between US$0.01 and $0.04 per pill” [29]. In the United

States 10 days of treatment is about US$14; however, some in 2014 were selling it for more than $3.00–10.00 per pill.

Benzylpenicillin was discovered in 1929 by Alexander Fleming and came into commercial use in 1942 [30]. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system [31]. The wholesale cost in the developing world is about US$0.24–2.72 per day [32]. In the United States a course of treatment costs $100–200.

Amoxicillin is available as a generic medication [33]. It has a wholesale cost in the developing world of between US$0.02 and 0.05 per pill [34]. In the United States, ten days of treatment costs about US$16 ($0.40 per pill).

OBSERVATION

The medical intervention for the prophylaxis, treatment and recovery of the patients includes an antibiotherapy that differs much from the classic recommendations, like antibiotics, doses and duration, realizing very high consumptions for each treated case and indeed huge for the winding up of the consequences of the biological attack. The biological agent must be detected, identified and confirmed as soon as possible, in the environment, in humans and animals and in patients for etiological diagnosis and antibiogram. Depending on the epidemiological triage, it is necessary to determine exactly who needs curative treatment, who is taking prophylactic treatment and who should be kept under medical supervision.

The anti-infective treatment should be personalized and supplemented as appropriate with other medicines: specific biological products (anti-carbon serum for the seriously ill, vaccine for those at risk of exposure), non-steroidal anti-inflammatory and pain relievers (aspirin, paracetamol etc.), antipyretics, antitussive, oxygen with or no assisted breathing, antiseptics, disinfectants, etc. Currently, there is a big problem at national level: the main producer in Romania, of biological products for treatment and diagnosis, INCDMM Cantacuzino, no longer has the marketing authorization for prophylactic and therapeutic vaccines, therapeutic and immunomodulatory sera, being undergoing re-technologization.

The seriously ill should be hospitalized, preferably in hospitals with existing infectious-disease wards or improvised in civilian sanitary units through civil-military cooperation (CIMIC) or by transforming some field hospitals (specialized in the management of polytrauma). The other patients, suspects and contacts, if large in number, will be treated and supervised by existing CIMIC forces and means, and if these shall not suffice, with international support from EU, NATO, WHO, etc.

The therapeutic schemes presented in this paper have been extracted from the specialized literature, being suggested as treatment alternatives in the case of pulmonary anthrax with resistant germs, or for the situations in which the resources have been exhausted and other therapeutic strategies should be used to avoid high risk situations for the public health, in the case of biological attack with B. anthracis. Pharmacoeconomic comparisons are presented, both for the guidance of clinicians and health decision-makers [35-49].

It should be noted that in the case of other bacterial biological agents, including BWA, the therapeutic schemes (post-exposure prophylaxis and curative treatment) range between 10-20 days, with usual antibiotics, ensuring medical countermeasures being much easier to achieve from a logistical standpoint.

CONCLUSIONS

The biological attack with B. anthracis is possible, as much as biological warfare, bioterrorist attack or biological accident. The management of the consequences also includes medical intervention, which is essential in combating and eliminating the effects of the attack with B. anthracis. The biological agent must be detected, identified and confirmed as soon as possible, in the environment, in humans and animals and in patients for etiological diagnosis and antibiogram.

The medical intervention for the prophylaxis, treatment and recovery of the patients includes an antibiotherapy that differs much from the classic recommendations, like antibiotics, doses and duration, achieving very high consumption per treated case and indeed huge for the liquidation of the consequences of the biological attack. Specific therapeutic schemes should be known by physicians and pharmacists for practical application, but also by the decision makers for setting up health stocks and could be integrated in the management of the consequences of the biological attack.

We believe that by applying the specific complex measures presented, the morbidity and mortality following the biological attack with B. anthracis can be reduced to an acceptable level, common for infectious diseases.

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3D reconstruction protocol in complex acetabular defects

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Abstract: *The current paper describes the protocol used in our clinic for 3D imaging, reconstruction and printing of complex acetabular defects that are used for surgical planning of complex acetabular defects. Surgical guides have also been developed to aid optimal acetabular reamer placement in order to recreate the optimal hip rotation centre for each patient undergoing revision hip arthroplasty that has impaired acetabular bone stock.*

Keywords: *3D printing, 3D reconstruction, acetabular defects, surgical guides, revision, convergent modelling*

INTRODUCTION

Acetabular bone defects are the main sources of prosthesis instability after total hip arthroplasty (THA) revision surgery if not addressed correspondingly. There are several areas important from a biomechanics point of view like the posterior and posterior-superior rims that are high stress areas that cannot be overlooked during surgery in comparison with the anterior and superior rim defects that can be ignored given that they do not generate instability [1].

Medialisation of the prosthesis is of key interest since this process minimizes prosthesis loading while excessive lateralization due to insufficient medialization or large medial wall defect increases joint reactive forces (JRF) that will eventually destabilize the cup starting from its inferior pole and then if the problem is not addressed the destabilized cup causes bone lysis at its upper pole creating a superior rim defect [1–3]. Other types of intraoperative prosthesis implantation difficulty require different surgical solutions [4]. As the acetabular defect complexity increases the required surgery becomes increasingly complicated requiring extensive soft tissue dissection with a higher risk of complications [3, 5, 6]. With these in mind the focus is oriented towards methods of increasing surgical success rate one such method represents the shift from conventional roentgenographic studies towards computed tomography (CT) that enables a better bone stock evaluation while at the same time offering the opportunity of 3D defect reconstruction, augmenting surgical planning and decision making [7–9]. In the following section we will describe our protocol for 3D reconstruction and printing of patient specific concept-guides for complex acetabular defects.

MATERIALS AND METHODS

CT image acquisition and later processing of acetabular bone stock prior to revision surgery has been performed according to ISO standard 12052 [10]. In the following section we will provide a detailed walk-through of each step employed from image processing to 3D printing the personalized surgical block [11, 12].

Image acquisition protocol

While CT offers arguably more information on acetabular bone stock in comparison to radiographic studiesit does possess the inconvenience of metal-related artifacts generated by competing mechanisms like photon starvation, beam hardening, scattering, partial volume effects, under-sampling and patient motion [13]. Our protocol focuses on the major mechanisms namely photon starvation and beam hardening. We have used Computed Tomography X-Ray machine (SIEMENS, SOMATON, serial no. 111382). The acquisition parameters that we use are exposure time of 1 second, nominal single collimation width of 0.7 mm and mean CT dose index (CTDI) 4.72mGy. CT X-ray source parameters are kVp 111 kV and exposure 273 mAs. Metal-artifact reduction (MAR) protocol used was a image based metal segmentation method named iterative MAR (iMAR).

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Image processing

After successful acquisition the images were processed using the picture archiving and communication system (PACS).

After this step, image storage and transfer was performed by converting the data to Digital Imaging and Communications in Medicine format (DICOM).

3D Reconstruction using DICOM data

The first step in the 3D reconstruction is the processing of DICOM data into stereolithography (STL) format. This process is done automatically during the 3D reconstruction and for this purpose we have used 3D Slicer. DICOM data was imported in the 3D Slicer database and opened in the segment editor module. Two different approaches were used:

The first technique called the two layers technique two segments were created, one named bone and the another one named other. With the bone layer selected the paint tool can be activated. Using the sphere brush the zones of interest for the bone were worked through each slice.

This technique can be extended to other layers for different bones in the same scan. With the other layer active and the sphere brush selected, zones that do not contain bone that are intended for 3D reconstruction are marked through some of the DICOM slices.

After proper marking, the grow from seeds tool is used. After processing, the DICOM slices should be marked accordingly (Figure 1). If the marking is not correct in some slices, it can be corrected by marking the correct layer in the DICOM slices and checking the live update box in the grow from seeds tool. The marked results can be observed in the 3D view (Figure 2). After proper marking the 3D we recommend to use the smoothing tool and generate the final 3D model.

Figure 1: Coronal view with marked bone segment (green) and other marked segment (yellow), processed in 3D Slicer with the "grow from seed" function (*this image is from the author's personal database*)

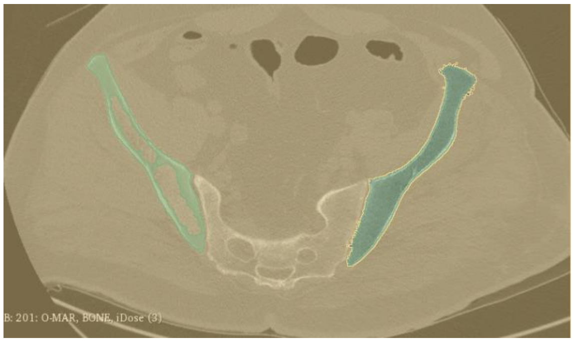

Figure 2: 3D view of bone segments processed in 3D Slicer (*this image is from the author's personal database*)

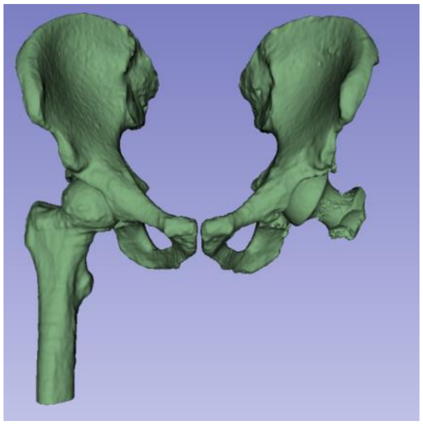

The second technique is simpler and faster but the 3D model quality can be inferior to the previous presented method due to noise in the CT scan or different bone interactions. The main tool for this method is the threshold tool. By opening this tool, an interval can be chosen for the intensity of the pixels. The program will select all the pixels with the intensity in the selected interval and will mark them. In order to edit specific zones or to add and delete pixels based on a threshold value the brush tool can be combined with the threshold function in order to do zone marking or subtraction based on the selected threshold interval. After proper marking were commend the use of the smoothing tool and to generate the final 3D model.

3D Reconstruction post-processing

After the desired 3D model is obtained post-processing of the triangle meshes is required. For the post-processing of the 3D model we recommend Autodesk Meshmixer (Autodesk Meshmixer, RRID:SCR_015736) software. The 3D model is imported in Meshmixer and using the Select tool all the surfaces are selected by double clicking on the model. Using the Separate command, the model is separated by connected surfaces. Using the object browser, bodies that are not required are deleted. After this preliminary clean-up the model is checked for errors using the inspect tool from the analyse tab. If errors are detected the geometry is repaired with the help of the Inspect tool.

For easy and fast handling of the 3D model we recommend to reduce the complexity of the model using the reduce tool from the Edit tab (the model needs to be selected). The reduce variables need to be adjusted until a result like the one in Figures 3 and 4.

The goal is to reduce the geometry of the model as much as possible but also maintain the shape. This model is saved and exported in STL.

Figure 3: Wireframe view of acetabulum before reduced geometry, processed in Autodesk Meshmixer (*this image is from the author's personal database*)

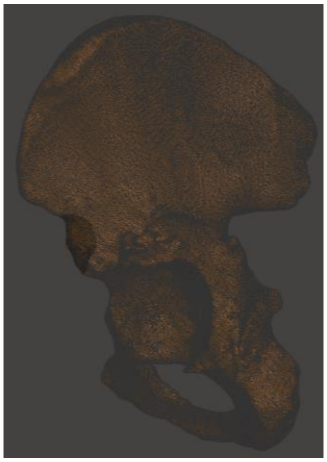

Figure 4: Wireframe view of acetabulum after reduced geometry, processed in Autodesk Meshmixer (*this image is from the author's personal database*)

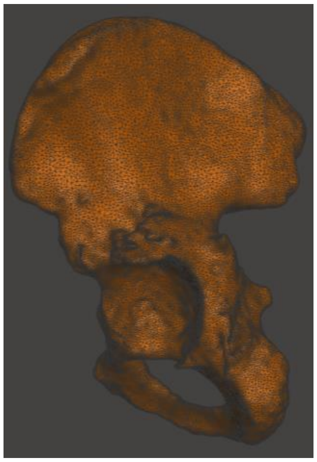

Convergent modellingTM

After post-processing we further edited the STL file into Solid Edge (Siemens AG).In this step we recommend doing a remeshing by using the re-mesh tool from the Reverse Engineering tab and align the model. After remeshing the identify regions tool is used. By selecting the automatic tool and choosing the body of the femur from the model, the program will automatically identify the surfaces from the model. Alternatively, in some cases is faster to use the manual tool from the Identify Regions section and paint the zones of interest on the model. By using the Fit tool, choosing the sphere entity and selecting the surfaces that describe the femural head, a sphere will be created on the femural head.

Using the 3D line command, 3 axes are placed between the 2 acetabula in the sagittal (Figure 5), coronal and axial planes. These axes will be later used to properly define the acetabular cup placement during surgery (Figure 6).

Figure 5: Lines placement for sagital plane construction, image processed in Solid Edge (*this image is from the author's personal database*)

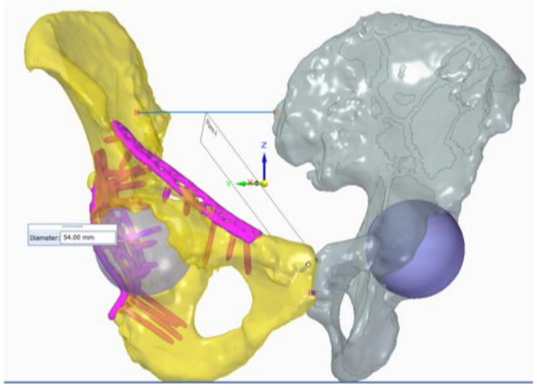

Figure 6: Abduction angle, image processed in Solid Edge (*this image is from the author's personal database*)

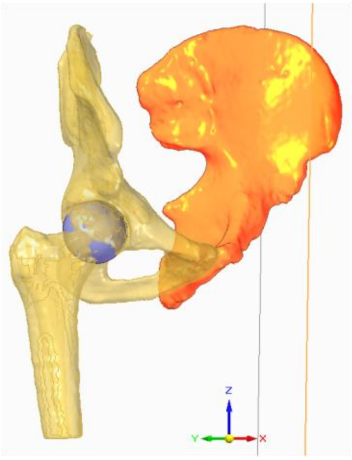

By creating a plane parallel to the median plane and rotate it 90 degree we built a plane parallel to the coronal axis. This plane can be moved in the centre of the constructed spheres using the steering wheel. Two more planes were created and placed parallel to the sagittal and axial axes. After all three planes were created, specific angles were defined to determine physiologic anteversion and abduction angles. In this procedure by taking the healthy acetabulum as reference we have defined 45 degrees cup abduction and 29 degrees cup anteversion (Figure 7).

After completion the model can be added into an assembly together with the pre-designed guide. The guide is constrained by using the Axial Align tool. The guide can be placed and rotated on the acetabulum by using the acetabular rim as support (Figure 8).

Figure 7: Anteversion planes, image processed in Solid Edge (*this image is from the author's personal database*)

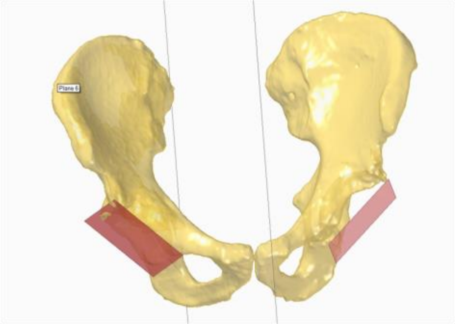

Figure 8: The pre-designed guide, image processed in Solid Edge (*this image is from the author's personal database*)

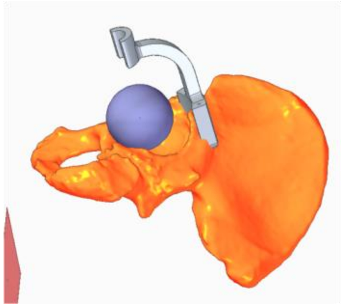

After placement the acetabulum can be subtracted from the guide by using the boolean tool, or the model can be exported in STL and the boolean operation can be made in Meshmixer. In this case we have employed (which one?)…

After the boolean operation the guide is prepared for printing. The STL model of the guide is imported into the printer slicer. In this case we have used PrusaSlicer.

In order to print the segmented acetabulum, we have used a Prusa i3 MK3 printer using a default profile of 0.15 mm with a 60% rectangular infill. The filament used for printing was made of poly-lactic acid (PLA).

RESULTS

After all the processing steps we have constructed a personalized guide for acetabular reaming and cup placement. The obtained guide construct is based on the local acetabular anatomy in order to allow the best obtainable stability. In order to obtain intra-operative stability, we have designed a locking mechanism that makes use of the superior acetabular rim anatomy. After proper acetabular exposure we can place the reaming guide on the superior acetabular rim and rotate it clock-wise in order to lock it into the bony landmarks that have been identified during the CT processing steps. This locking mechanism will make the acetabular reaming independent of patient positioning because of its improved stability which is an important problem to deal with especially in patients with higher body mass indexes (BMIs).

DISCUSSION

Several authors have published mixed results on patient specific guide preparation and outcomes [7–9]. Most relevant variables in direct connection with surgical utility are represented by blood loss, surgical time, dislocation rate, infection rate and overall patient reported outcome [14–16]. On the other hand, there are several factors that need to be considered when trying to implement new technologies into the operating room. Based on our current research experience, we are inclined to evaluate such a technology based on 1) tangible and 2) intangible results. We refer to tangible results when talking about implementing 3D printing in avoiding unnecessary surgical steps and evaluating relevant surgical variables like the ones mentioned above. On the other hand, intangible results are best evaluated based on patient reported outcomes.

Other researchers in the orthopaedic field have reached similar results in different areas where preoperative planning augmented through the use of modern technology has lead to a superior outcome for the patient, be it in surgical time, recovery time or overall patient satisfaction [17–19].

CONCLUSION

3D printing of patient-specific guides offers a new opportunity to innovate surgical practice by offering an augmented surgical planning with an enhanced control over surgical instrument placement. At this time the literature on the matter reports mixed results regarding the actual improvements, but we are confident that as the technology comes closer to the surgical needs we will see an increased use of patient-specific 3D printed guides especially in orthopaedic surgery practice. The current paper offers a comprehensive approach to personalized surgical guide creation using CT scans of complex acetabular defects.

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VARIA

Vitamin D status and cholecalciferol supplementation on patients with active Basedow disease: a clinical study

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Abstract: *This study aims to analyze the 25-hydroxyvitamin D (25OHD) corelations with TRab (anti-TSH receptor Antibodies) at baseline and after corections of 25OHD deficit.*

Method: Prospective, interventional, controled, single-centre, clinical study on 62 patients with active Basedow disease (BD), admitted at C.I. Parhon National Institute of Endocrinology, Bucharest, Romania, between 2013 and 2020. Patients were divided into two groups: one group (N=37) received 2000 IU of daily cholecalciferol and a second one (N=25) without vitamin D supplementation, all subjects being under anti-thyroid drugs (standard guideline therapy). 25OHD and TRab levels were assessed at start and after 6, respective 12 months.

Results: Initial evaluation confirmed vitamin D deficiency (mean 25OHD of 18.33±6.45 ng/mL). At start, TRab negatively correlates with 25OHD (N=62, r= -0.22, p=0.08). The decrease in TRab was statistically significant higher in group 1 versus group 2 after 6 months (35.84% versus 3.53%, p=0.03). After 12 months, TRab decreased with 56.28% in group 1, respective 27.16% in group 2 (p=0.03). In group 1, inverse correlation between 25OHD and TRab was consistent at 6 and 12 months.

Conclusion: In patients with active BD, TRab values were negatively correlated with 25OHD at baseline and during follow-up. Correction of vitamin D deficiency is correlated with the decrease of TRab levels.

Keywords: *25-hydroxyvitamin D, Basedow disease, anti-TSH receptor antibody, vitamin D deficiency, hyperthyroidism, vitamin D, autoimmune thyroid disease, TSH*

INTRODUCTION

Autoimmune thyroid diseases are among the most common organ-specific autoimmune disorders, Hashimoto's chronic thyroiditis and Basedow disease (BD) being the most common clinical expressions [1]. BD is considered to be the most common form of thyrotoxicosis, following anti-TSH receptor autoantibodies (TRab) secretion and actions [1, 2]. Besides the well known treatment of this disorder (antithyroid drugs followed by radioiodine therapy or thyroidectomy), current studies demonstrate the effectiveness of complementary therapies for obtaining and maintaining a prolonged remission of the disease [1, 2]. In this context, the extra-skeletal roles of vitamin D are subject of intensive debate [1, 2]. Vitamin D is incriminated in regulating cell functions, its deficiency being considered a

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risk factor for the development of different autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, diabetes mellitus type 1 and thyroid autoimmune disorders [2, 3]. In addition to its established role in calcium-phosphate metabolism, vitamin D is involved in multiple disorders, the vitamin D receptor (VDR) being widespread and its polymorphisms extensively studied [2, 3]. Vitamin D helps regulate cell growth, differentiation and apoptosis, being incriminated in regulation of cell functions in many systems, like cardiovascular, renal, central and peripheral nervous system [2, 3]. Correction of vitamin D deficiency prevents the onset or unfavourable evolution of some of these disorders; however, controversies around the role of vitamin D supplements are still described [4, 5]. In thyroid autoimmune conditions, correlations between vitamin D levels as expressed by serum levels of 25-hydroxyvitamin D (25OHD) and ATPO (Anti-thyroperoxidase antibodies) or TRab status have been found [3, 6, 7]. Moreover, levels of 25OHD <20 ng/mL appear to be an independent risk factor for treatment failure in patients receiving radioiodine for BD [8]. Recent studies have shown a high incidence of vitamin D deficiency but in BD subjects, 25OHD-TRab corrections data are still controversial and the collateral role of vitamin D in achieving and maintaining drug remission in BD remains an unanswered question [3].

Aim of the study

The primary endpoint is to establish the correlation between 25OHD and TRab in patients with active BD, at baseline and after vitamin D supplementation in addition to the classical anti-thyroid drugs. The second endpoint is to identify the prevalence of vitamin D deficiency in adult patients with BD.

MATERIALS AND METHODS

Study design: prospective, interventional, single centre, controlled study in 62 hospitalized patients from C.I. Parhon National Institute of Endocrinology, Bucharest, Romania, enrolled between 2013 and 2020.

Inlusion criteria: adults aged over 18 years, with newly onset BD and a TRab level >1.5 U/mL, with or without active ophtalmopathy, patient' consent for the study.

Exclusion criteria: current medication targeting mineral metabolism (calcium and/or vitamin D suplements, glucocorticoids, antiepileptics), steroids treatment, malabsorbtion or acute/chronic renal disease and pregnancy. Patients were divided into two groups: one group received 2000 IU of cholecalciferol daily and a second one without vitamin D supplementation. We investigated the effects of vitamin D deficiency correction on the level of TRAb after 6 and 12 months (group 1). Blood samples were collected under fasting conditions (between 8:00 and 10:00 AM). TRab was measured by electrochemiluminiscence (ECLIA Cobas e601), with a lower detection limit of 0.3 UI/L. 25OHD was measured by electro-chemiluminiscence (ECLIA, Cobas e601, Roche) with a lower detection limit of 4.2 ng/mL. The 25OHD categories are as follows: severe deficit < 10 ng/mL; insufficient: 10-30 ng/mL; optimum: 30-100 ng/mL; toxicity: >100 ng/mL. The intra- and inter-assay variation coefficients in our laboratory were 7.6% and 11.4% for TRAb, respectively 3.27% and 9.1% for 25OHD. Serum concentrations of TSH (Thyroid Stimulating Hormone), fT4 (Free Levothyroxine) and ATPO were determined by direct immuno-chemiluminiscence assay (Siemens Immulite). The statistical interpretation was performed using Microsoft Office Excel 2010 and its applications. We used the following statistical tests: Student's t test and Spearman coefficients. Statistical tests were considered significant if $p < 0.05$.

RESULTS

The study was performed on 62 patients, aged between 21 and 80 years (group 1: N=37, group 2: N=25). The study enrolled 48 women (77.42%) and 14 men (22.58%). There were no significant differences in terms of age distribution between the two groups and the standard anti-thyroid medication regimes and target hormonal values. The maximum number of patients, of either group, belongs to the 40-49 age categories. The evaluation of the initial status of 25OHD in all 62 patients confirmed vitamin D deficiency; the mean 25OHD was 18.33± 6.45 ng/mL; all the patients, regardless of age group, had 25OHD values below 30 ng/mL. The age group with the lowest value of 25OHD was the 50-59 years, with a mean 25OHD of 15.76± 8.1 ng/mL (Figure 1).

Figure 1: Age distribution depending on the initial status of vitamin D in studied patients with active Basedow disease

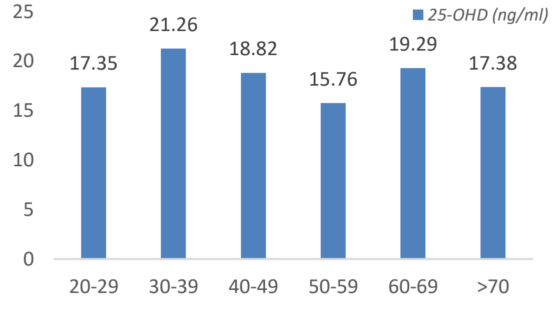

In the first group, after vitamin D supplementation, the percentage of patients with vitamin D deficiency (25OHD <20 ng/mL) diminished and the percentage of patients with optimal values of vitamin D (25OHD above 40 ng/ml) increased after first 6 months, but statistically significant only after first 12 months. After daily administration of 2000 UI of cholecalciferol, mean 25OHD increased with 50.5% (8.4±6.51 ng/mL) after the first 6 months, and with 88.53% (14.73±10.43 ng/mL) after 1 year (Figure 2).

Figure 2: Vitamin D status in patients receiving cholecalciferol 2000 U/day (group 1 – number of patients receiving vitamin D supplementation) and active Basedow disease

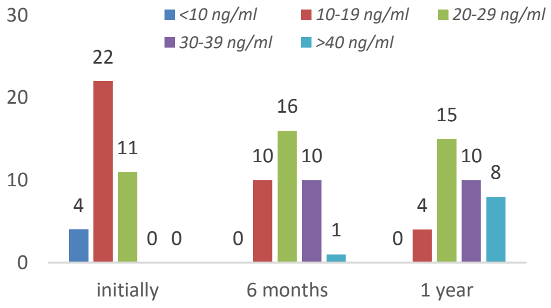

During follow-up of group 2, 25OHD levels had a decrease at 6, respective 12 months (without statistical significance) (Figure 3).

Figure 3: Vitamin D status in patients without vitamin D suplementation (group 2 – patients without cholecalciferol supplimentation) and active Basedow disease

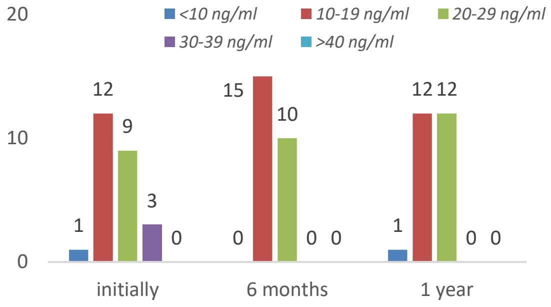

Comparative evolution of vitamin D levels between group 1 and group 2 is shown in Figure 4.

At start, TRab negatively correlates with 25OHD (N=62, r=-0.22; p=0.08). The decrease of TRab was higher in group 1 versus group 2 after 6 months: 35.84% versus 3.53% (the levels after 6 months of 6.18 ±7.27 UI/L versus 0.58±10.95 UI/L, p=0.03).

After 12 months, TRab decreased with 56.28% (9.7±7.7 UI/L) in group 1 and with 27.16% (4.43±10.21 UI/L) in group 2 (p=0.03).

During follow-up of group 1, TRab decreased, while 25OHD increased at 6 and 12 months (Figure 5).

These changes (decrease of TRab, increase of 25OHD) were not observed in the second group (Figures 6 and 7).

Figure 4: Comparative evolution of 25-OHD between group 1 and group 2 after 6, respective 12 months (with daily 2000 U of cholecalciferol in group 1, without vitamin D supplementation in group 2 in association with standard therapy for active Basedow disease)

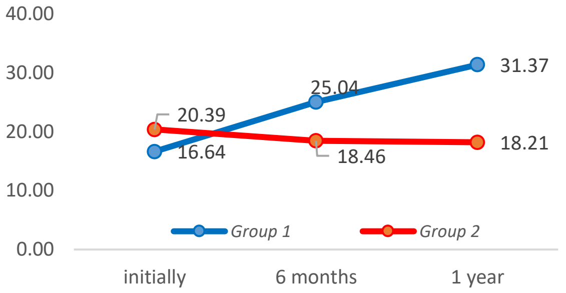

Figure 5: 25-OHD and TRab mean values in adult patients receiving daily 2000U of cholecalciferol (group 1) at baseline, after 6, respective 12 months (in addition to standard anti-thyroid medication) – values in ng/mL

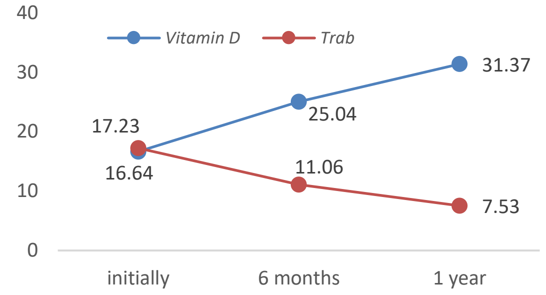

Figure 6: 25-OHD and TRab mean values in adult patients without vitamin D supplementation (group 2) at baseline, after 6, respective 12 months (under standard anti-thyroid medication)

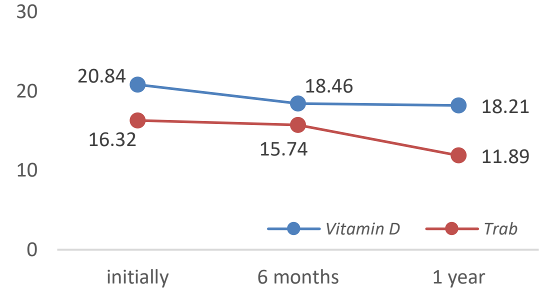

Figure 7: 25-OHD and TRab values evolution in enrolled patients

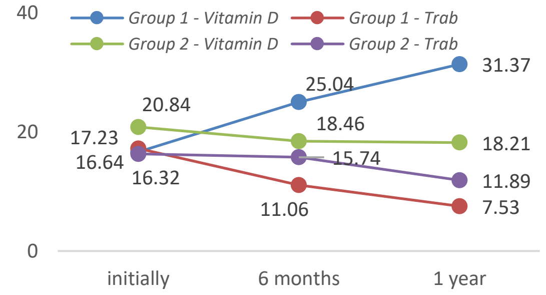

DISCUSSION

BD, caused by autoantibodies actually has a multifactorial pathogeny, including the potential role of vitamin D [9-11]. There are studies showing that the treatment with methimazole in combination with vitamin D supplementation in patients with BD attained the euthyroid status faster compared to those treated only with anti-thyroid drugs [12]. In addition, supplementation with vitamin D has been shown to inhibit inflammatory responses in the thyroid and T cells [12]. It has also been found that vitamin D deficiency is associated with increased thyroid volume in patients with newly onset GD [12]. Several allelic variants (polymorphisms) have been described in the VDR gene on chromosome 12 and have been reported to be associated with a variety of physiological and pathological phenotypes, including in thyroid autoimmune diseases and thyroid cancer [13-16]. However, the actual mechanisms of vitamin D receptor polymorphisms are still a matter of debate [15, 17]. Our study confirmed the high incidence of vitamin D deficiency in general adult, Caucasian population. Daily supplementation with 2000 IU of vitamin D does not provide full correction of vitamin D deficiency (meaning achieving a level of 25OHD > 30 ng/mL) at 6 and 12 months, but a statistically significant improvement is observed. For this correction, current guidelines recommend 25000-50000 IU/week for first 8-12 weeks with a subsequent lower maintenance dose, but the high levels are also necessary to continue under special conditions (like COVID-19 pandemic, chronic diseases like autoimmune conditions involving different medical specialities, obesity, uncorrected hypocalcemia, high doses of glucocorticoids or elevated osteoporotic fracture risk status, etc.) [18-22]. However, we highlighted the role of vitamin D in reducing the TRab titre, objectified by the negative correlation between antibodies and vitamin D levels after correcting its deficiency. In group 2, the minimal decrease in TRab concentration was considered to be a consequence of the immunosuppressive effect of thiamazole and of the natural evolution of the disease.

CONCLUSION

This study highlights the low levels of 25OHD in adults with active Basedow disease. TRab is inversely correlated with 25OHD in newly diagnosed patients. Daily supplementations with 2000 U of cholecalciferol statistically significant increase 25OHD while TRab decreases during follow-up. Vitamin D should be considered as a possible complementary therapy under these circumstances. Therefore, public health policies should aim for prevention, diagnosis and correction of its deficit in adult population.

Authors' contributions

FNP drafted the manuscript and critically revised the final form, MG researched the literature, BP researched the literature, AG drafted the manuscript, FS researched the literature, MC drafted the manuscript and is the corresponding author, RSB revised the final form and wrote the conclusions, AF researched the literature, and MCD approved the final form.

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A marketing perspective on the efficient strategies in Romanian family medicine

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Abstract: *Family Medicine has always been regarded as an interdisciplinary specialty, but lately, in most Eastern-European countries, such as Romania, it has been supported by many laws and regulations. The outcome of this prolific background has brought both advantages and disadvantages. Some advantages relate to a sustainable structure, and, at the same time, Family Medicine has been recognized as being the gate-keeper in almost any health care system. Further, due to the privatization of most family practice offices, in Romania, appeared a modern organizational approach, in the shape of a consortium of family physician practices. Some family physicians consider providing their services in a private office as a benefit, but for others the freedom and autonomy to become responsible of their own practice is considered to be a drawback because, apart from the clinical activity, many family physicians have to deal with managerial and marketing issues. In Romania, a consortium of family practice offices has to overcome a very competitive environment and some Marketing specific trends. The aim of this paper is to investigate the usefulness of marketing strategies in the Romanian family physician practice.*

Keywords: *family medicine, family physician, primary care, marketing, word-of-mouth, WOM*

INTRODUCTION

From its beginnings, the Family Medicine specialty has raised considerable discussion and debates regarding its scope and borders from an interdisciplinary perspective [1-3]. Since the Alma Ata conference in 1978 [4] and the World Health Organization's program, called "Health for All" [5], the importance of efficient primary care has been recognized as being the foundation of health systems in many parts of the world [6]. However, today Family medicine continues to face an unclear identity, as the scope of the practice is not well-defined and its consequences may trigger issues that relate to medical malpractice, healthcare coverage and insurance reimbursement [7].

In Central and Eastern European countries, health care systems are very much specialist and hospital oriented but the importance of family care still remains in the background, and it is not fully supported by focused strategies [8]. In the pandemic context, most of these countries have undergone rapid political changes and challenges that led to the necessity of developing and supporting the activities of the Family Medicine.

Since the collapse of the communist regime, the Eastern-European countries have made a radical transformation in the family care practice [9]. Once, under the umbrella of the impersonal polyclinic services and the purview of the law, the family practice offices have been privatized and are often managed by the family physicians themselves. Moreover, this privatization triggered an emergent decentralized primary care sector, which lacks the team-based care approach.

Although in most of this region many countries still struggle for the on-going acceptance and recognition, in Romania, there has been an upsurge of laws supporting the family practice activity and discipline-specific associations that recognize its fundamental position in the health care system. For instance, solo family practices have mostly disappeared in the Romanian urban areas, being replaced by a modern perspective, namely, building consortium of family physician practices [10]. In 2019, in Romania, there were 11,682 family physicians, who provided around 30,720,485 receipts [11].

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Further, the organizations that promote and support the modern Family Medicine are: The Romanian Society of Family Medicine/General Medicine, The Family Medicine Commission of the Medical College of Physicians and the National Centre for Studies in Family Medicine. The most important organization is the Romanian Society of Family Medicine/General Medicine because it is the biggest association of family physicians and has a long tradition in supporting the development of Family Medicine, as a distinct specialty in Romania [12].

The self-employed status of a family physician has brought with it a wide range of other responsibilities besides the clinical activity. The family physician has to take responsibility for the equipment and supporting staff as well as for the management of the practice. In fact, family physicians had to learn about management and marketing strategies in order to develop and implement a modern concept of their practice and offer a competitive advantage.

The aim of this paper is to investigate the usefulness of marketing strategies in the Romanian family physician practice

THE PARTICULARITIES OF FAMILY MEDICINE IN ROMANIA

Family physicians are the core stakeholders in delivering any health policy by providing primary care services, and the first contact of patients with the health system [13]. Although, the World Health Organization has defined their role in the primary health practice and their importance is widely accepted, there may be registered some differences between countries [14]. Specifically for the Family Medicine, the scientific literature revealed that the most often encountered differences relate to the incentives, practice information capacity, access, the use of quality indicators, staff and patient safety and the number of allowed patients in a portfolio of a family physician [15].

In Romania, the family physician is a person responsible with the provision of comprehensive and continuing care to every individual in need, regardless of age, gender and illness, but who is under his supervision [16]. Broadly, family physicians integrate in their professional practice activities that focus on the health and prevention measures as well as activities that concentrate on the provision of cure, care or palliation. A family physician provides these services either directly or through the services of other specialists, according to the health needs and the resources available within a community.

The particularities of Family Medicine in Romania are [16]:

- The family practice office is normally the point of first medical contact within the health care system, which provides open and unlimited access to its users, dealing with all health problems regardless of the age, sex, or any other characteristic of an individual;
- The family physician makes efficient use of health care resources through coordinated care, working with other professionals in the primary care setting, having an advocacy role for the patient in need;
- The family physician has a person-centred approach, orientated towards both the individual, his/her family, and their community;
- The family physician has a unique consultation process, which should establish a long-term relationship with the patients, through effective communication strategies;
- The family physician is responsible for the provision of longitudinal continuity of care as determined by the needs of the patient;
- The family physician has a specific decision making process determined by the prevalence and incidence of illness in the community;
- The family physician manages simultaneously both acute and chronic health problems of patients;
- The family physician manages illnesses at early stages of evolution;
- The family physician promotes health and well-being ideas for individuals through appropriate and effective interventions.
- The family physician deals with health problems in their physical, psychological, social, cultural and existential dimensions.

According to the Primary Health Care Activity Monitor for Europe [17], Romania has one of the strongest primary care structures, along with Denmark, Finland, Italy, Portugal and United Kingdom, but the critical issues in a family physician activity resumes to buying modern equipment, competence involvement and using appropriate appointment systems [18].

Despite having an interdisciplinary approach, family physicians have identified the following challenges in providing their specialty [18]:

- Less attractive – Due to the economical and managerial freedom, most family physicians no longer find this medical specialty attractive. The former enjoyment of working on a private basis has been replaced by disappointment because the workload has not been reduced, especially in the pandemic context.

- Constant need to buy modern equipment – In order to deliver quality health care services, family physicians need to keep track of the latest equipment and, as the patient becomes more empowered with information, they need to understand that the patients' needs have been raised as well. In Romania, some family physician offices have been equipped with computers and printers, electrocardiographs, spirometers, CT scanners and even office-based laboratory tests.

- Raise the continuing professional development credits – The family physician's activity is influenced by the quality improvement initiatives of their practice in the shape of a constant participation to conferences and other specific events or publish certain articles, books and other materials that would help them build their knowledge and learn about the latest medical information. Yearly, getting the credits imposed by the laws, leads to a re-certification of the family physician.

- Impairment in accessing the academic field – Although the academic positions opened for family practitioners have improved significantly, there are a few issues that need to be addressed, as for instance the salience of Family Medicine as a discipline and its connections with other medical specialties [19].

Moreover, the indicators specific to Family Medicine relate to health care quality, and the elements that need to be addressed are linked to continuity, coordination, comprehensiveness of care and community orientation. [20] Continuity is described by informational, relational and management activities, while the coordination is reflected in the organization and the monitoring of the services delivery. In Romania, the family physician's continuity and coordination are connected to the medical records and electronic medical records and with the collaboration with other specialists. The comprehensiveness is reflected in the scope of the practice and in the range of services provided, whereas the community orientation is important to be ensured for the sustainability of the health care system. In Romania, both the comprehensiveness and the community orientation are extremely low because the family physician provides the basic services or ancillary services, with a specific interest, for ultrasound or gynaecology related services, and the community orientation is non-existent because family physicians are too busy with their clinical activities in order to have other voluntary activities.

All four elements reduce to the doctor-patient relationship, this being the core characteristic of the Family Medicine [21]. If the practice gets influenced in a negative direction by the doctor-patient relationship, than there would be significant implications at the image level and the patients' desirability to continue going to the family physician. In addition, since 1999, when the family physician's form of payment switched from a salary paid by the system to a yearly contract based model, the family physicians became the gate keepers of the health system but was also introduced the freedom of patient choice of a family physician. Therefore, family physicians have to catch the attention of patients through different strategies and build their portfolios as well as offer a competitive advantage. In fact, family physicians had to implement efficient marketing strategies in order to survive on a market.

CURRENT CHALLENGES OF FAMILY MEDICINE IN ROMANIA

Health care is a field in a permanent and constant evolution, being filled with a wide range of opportunities that should stimulate creativity and enthusiasm of the specialists in the field. Still, health care services are not like other services because they are unwanted, and this may be considered highly troublesome by patients and, at the same time, critically important for their well-being [22]. Actually, health care services have always been archetypal in nature because consumers lack the technical knowledge to understand it, and have to rely on credence qualities [23]. Consequently, entropy of information is formed and consumers will assess the quality of the provided health care services based on their perceptions and not on their real experiences. If we take into consideration the pressure which consumers face in making health care decisions, we may conclude that patients confront with a challenging lack of accessible and medically verified information that would, eventually, help them in rationally think about the benefits and drawbacks of the medical procedures [24]. Moreover, frequently, the desires and the needs of health care consumers come in conflict, resulting in their reluctance to play the role of co-producers [25], but physicians motivate them to assume their part in the health care process by using ethics, compassion, empathy, and solidarity [26].

Doyle [27] mentioned that patient experience consists of two aspects: rational and functional. The rational aspect focuses on the 'interpersonal aspects of care', such as the physicians' ability to treat patients with respect and compassion, to empower patients with information in order to prevent other illnesses, as well as, to highly engage patients and their family members in the decision-making process, whereas the functional aspect relates to the patient's basic expectations about the health care service, which includes elements such as the effectiveness and efficiency of health care delivery and the cleanliness and safety of the health care background.

Due to the emergence of technology, patient emancipation or patient empowerment became the most substantial and visible trend [28], prior to sickness in the shape of prevention methods or upon its primary form, in the shape of diagnosis, treatment, and during the treatment period, shaped by the degree of compliance. This trend has fundamentally changed not only the doctor–patient relationships, but also the relationships established between doctor–society– patient, as a whole. As such, the passive patient has changed and became involved and proactive in designing the process of care and taking decisions. Also, this trend triggered several changes in the providers' perspectives that emphasized the necessity for greater authority and involvement in care from the regulatory and funding bodies as well as from the stakeholders, who had to change their behaviors, through financial, structural, and organizational mechanisms. These strategies had to significantly rely on patient expectations, and basically, to improve patient support by using personalized communication about preventive advice and reorientation based on consumption analyses, and, implement Marketing instruments and principles [29]. Marketing plays an important role in helping health care professionals to create, communicate, and provide value to their target market- the health care consumers- by building a sustainable relationship [30].

Although many experts have considered the emergence of Health Marketing as a distinct field, few specialists have provided clear and integrative definitions [31].

Over the past decade, some market specific trends contributed to integrating Marketing in the health care field. These trends are the following [32]:

- From a mass-marketing approach to a more specific approach;
- From "one measure for all" to personalization;
- From the emphasis on a health episode to a long-lasting relationship;
- From low-technology market to high-technology market.

From a Marketing perspective, the process of providing health care services must be conducted according to the patient requirements, because these activities are designed to satisfy their needs and wants. The main argument in adopting a Marketing perspective in health care services steams from the objectives of the health care systems around the world, namely, assessing a balance of financial-territorial resource allocation and the control of costs, reducing the inadequate technology employment and introducing modern managerial methods [33]. Further, a high performing health system is characterized by the following features: accessibility, pertinence toward the needs, equity, chance of selection, efficacy and efficiency, and large scale social accessibility [30]. So, everyone has the right to non- discriminant medical attendance and health care organizations should satisfy the needs of the health care consumers in a timely manner. In addition, along with these challenges, competition in the health care market has increased in certain medical specialties, as in the case of Family Medicine.

In Family Medicine, Marketing strategies should be tailored differently for patients and families, communities, other physicians with whom they work with and other categories of medical personnel, in the shape of promotion strategies. As such, promotion strategies include a carefully designed set of activities intended to induce change with the help of a message.

Frequently, promotion strategies concentrate on various elements such as specific communication objectives for each target audience, guidelines for designing attention-catching and effective messages, and designing appropriate communication channels [34]. However, promotion is considered discreditable among the medical profession because there is a strong social norm against advertising in the health care field [35]. Although, consumers typically receive most of the information about health care services from Mass-Media, in fact, the most important information comes from recommendations from family or peers, which take the form of word-of-mouth.

WOM is the most powerful form of Marketing that can be easily used by physicians [36], and that may influence consumer expectations [37], pre-usage attitudes [38] and post-usage perceptions of a service [39]. In Family Medicine, WOM may be useful for consumer decision-making because it is quick, convenient, cost-free and it saves time and effort. Actually, the two categories of sources have complementary functions in Family Medicine, namely, Mass-Media informs and builds awareness, whereas the personal sources raise the potential evaluation process in looking for information. In the pandemic context, many family physicians made use of the WOM in the online environment by keeping up-to-date informative blogs and articles published on Social Media. Hence, the main benefits of implementing promotion strategies in a Family Medicine are the following:

- to improve the competitive advantage;
- to increase visibility;
- to create a positive reputation among patients;

- to monitor the expectations of consumers;
- to build a brand.

eWOM generated by patients is based on their referrals, and these recommendations, in their turn, are the outcomes of physician-patient interactions or experiences [40]. For instance, negative eWOM in health care services is expressed in the shape of online messages, in which are embedded emotions, such as anger [40]. In order to avoid the spread of negative eWOM, family physicians may elaborate or contribute to the design of a patient journey.

Patient journey maps [41] are used to measure patient experience after an interaction with a physician from the patient's perspective. The aim of a patient journey map is to capture the key elements of a journey, consisting of patient persona, medical timeline, medical pathway, relationships between elements and rules of getting from the starting point to the ending point. Going further with an in-depth analysis, the patient persona offers details about the user group and their emotional, physical and technological journey, and each interaction with the physician should be divided into tasks.

CONCLUSION

Health Care Marketing strategies have proved to be useful in Family Medicine. Marketing strategies may focus on the referral or recommendation solutions generated by patients, known as word-of-mouth. The outcome of WOM is to build a positive reputation and to attract more patients for a portfolio of a family physician. In order to avoid negative WOM, family physicians should design a patient journey map.

Conflict of interest

The authors declare that there is no conflict of interest.

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Challenges of the next normality

Victor L. Purcarea, Daniel O. Costache

At the beginning of last year, Professor Eliot Sorel continued to plead for a successful exit from the devastating pandemic, highlighting, among other aspects, the role played by the creative integration of the artificial and human intelligence in accelerating post-pandemic innovations (Sorel E. COVID-19 Vaccines, Accelerated Innovations, and 21st Century Social Contracts Challenges. World Soc Psychiatry. 2021; 3:7). At the same time, a study conducted by the McKinsey Global Institute (part of McKinsey & Company, involved in business and economic research) highlighted the *three groups of consumption and business trends accelerated by COVID-19*: distance working and virtual interactions, e-commerce, and digital transactions, as well as implementation of automation and AI (McKinsey Global Institute, The future of work after COVID-19, The post pandemic economy, Executive summary, February 2021).

In an article published by McKinsey & Company in November 2021 (Bujor, M., Cierkowska, A., Dorling, G., Dragan, R., Filip, A., Remes, J. and Vukojevic, M. How prioritizing health can be a prescription for Romania's prosperity, McKinsey & Company, November 2021), the aspects highlighted were: *the hard lessons learned in the context of the COVID-19 pandemic*, the opportunity of promoting health and welfare, taking into account the weaknesses of the health system and the indissoluble relationship between health and economy; a number of encouraging lessons on the ability to respond effectively in the context of the crisis (rapid development of COVID-19 vaccines, adoption of digital health tools and companies implementation of rapid operational changes in order to adapt to the pandemic).

In December 2021, in an episode from the McKinsey podcasting about health care, the executive director of Datavant company (which is about to become one of the world's largest healthcare data ecosystems, enabling patients, providers, payers, healthcare data analysis companies, patient applications, government applications and institutions dealing with living organisms and life processes *to exchange patient data safely*), Pete McCabe, talked about *current challenges: data fragmentation and access, the benefits of tokenization* (process of substituting a sensitive data element with a non-sensitive equivalent, called token; tokens can be individual words, phrases and even whole sentences; the process allows the division of a sequence into pieces, some characters, such as punctuation marks, being removed), *value-based care and the need for neutrality* (Reddy, P., 2021. How can healthcare unlock the power of data connectivity? McKinsey & Company, Healthcare Systems & Services Practice, December 2021). According to McCabe, for example, innovative technologies, such as tokenization, can close data connectivity gaps (this connectivity places together disparate data sets and applications, including data from different identity spaces, allowing collaboration between different parts with data control, ensuring secure and efficient activation across the wider ecosystem); the foundation of value-based care requires data and this can be as good as the basic data that supports it; value-based care is just the beginning of potential leadership, so, if the internet can recommend a piece of clothing based on consumer's buying history, why would not a doctor be able to recommend a treatment based on a holistic view of individual life data of a patient?

Also in December 2021, CB Insights highlighted the agile adaptation of technology giants such as Amazon, Microsoft, Google, and Apple to the challenges in the pharmaceutical industry caused by the COVID-19 pandemic, respectively, *the answer of these large technology companies materialized in building technology for drug discovery, clinical trials, production, and supply chains, as well as patients' remote monitoring and their health records* (CB Insights a, 2021. The Big Tech In Pharma Report: How Amazon, Microsoft, Apple, And Google Are Capitalizing On The Digital Transformation, December 14, 2021). Those who are familiar with the field are also accustomed to the developments that allow patients to manage their own health based on both data records from the Electronic Health Record/EHR, containing the medical history based on the physician's records (including lab results and care conclusions), and patient-generated data (such as symptoms). According to CB Insights, *the above-mentioned technology giants make the most of their expertise in software, data, and artificial intelligence to capitalize opportunities along the value chain* (its management considers the bottom-up flow, that is from the consumer/patient to the source; the value chain management 4.0 means generating and keeping value throughout the chain, using the technology usually associated with industry 4.0 everywhere, not just in the factory). CB Insights insists that *reducing the cost of drug development is a top priority, a way of making this possible by using digital solutions* (software), and, in this context, big technology companies pay close attention to the digital transformation of the pharmaceutical industry: Amazon builds the drugstore of the future using its supply chain in order to create a customer experience similar to its e-commerce business; Microsoft enters the pharmaceutical market with SaaS healthcare solutions and partnerships with the already existing players; Google uses patient data and artificial intelligence expertise to build drug research and development capabilities; Apple uses its hardware and user database to its advantage, in order to build a secure, centralized location for patient health data (CB Insights b, 2021. Apple's greatest contribution, Healthcare Insights, Thu 23/12/2021 14:04).

Obviously, the COVID-19 pandemic forced the reimagining of all aspects related to healthcare services, with *considerable changes in telehealth, digital therapy, and medical devices* (CB Insights c, 2021. Our Top 2021 Healthcare Reports, December 21, 2021). Thus, healthcare providers have considered innovative solutions to improve clinical workflows and decided for a wide range of remote care options (from consultations to therapies and mental health support). A confirmation of this concern in our increasingly digital world is the evolution of global funding for healthcare amid the continuing waves of COVID-19 pandemic (which reached $97,8 billion in the third quarter of this year, a figure that keeps on growing, breaking the total record of 2020 by 21%).

There is no doubt that the use of information and communication technologies in medicine (as well as in other medical professions, in order to manage disease and health risks and to promote well-being) will continue to play an increasingly critical role in improving health care. But, digital transformation (generally understood only as a shift to supporting more business functions with the help of technology) does not mean just switching to digital methods of communicating with potential clients (patients). "Digital" does not only refer to technology in business models, it refers just as much to people and mentalities, to humanization, as the effects of the COVID-19 pandemic have changed the habits of clients/users, and the providers of health care services have been forced to adapt their strategies on the continuous improvement of their relationship with the patient.

This improvement also depends on optimizing operational efficiency throughout the supply chain, to provide the client/ patient a frictionless, convenient, and personalized decision in all aspects, for a better understanding of all the moments (and even the micro moments) of the interaction in the "digital" space. The essence of an agile transformation is the reimagining of an organization as a network of high-quality teams, supported by an efficient and stable backbone of strategy, structure, processes, people, and technology. Digital adoption means reaching a state in which users gain the ability to use digital tools to the fullest and in the way they are intended, software adoption being just a part of the digital adoption process.

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EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

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REVIEW

Intention to vaccinate against COVID-19 in patients with autoimmune diseases

Elisabeta Candrea[1], Cristina Pamfil[1], Jeno L. Vargha[2], Maria Crisan[1], Adrian Baican[1], Adrian Neacsu[3,4], Roxana M. Toader[5], S. Rednic[1]

Abstract: *Understanding COVID-19 vaccination attitudes is of paramount importance in order to estimate and influence the willingness to vaccinate.*

A cross-sectional study was performed in the spring of 2021 in Romania by sending a web-based questionnaire to patients with rheumatic autoimmune diseases in order to assess their willingness to vaccinate against SARS CoV-2 and to identify factors that might influence their decision. Variables derived from the questionnaire were analyzed in univariable and multivariable analysis.

71 patients, of which 60 (84.5%) women, were included in the study, with a 53.5% (38 subjects) willingness to vaccinate. A positive association was observed between the willingness to vaccinate and the patients' trust in authorities, trust in the healthcare system and the choice of scientific sources of information in their decision making. A positive correlation was observed between the intention to vaccinate and the global VAX score, as well as the subscale VAX scores (p<0.05). Global VAX score emerged as a predictive factor for the intention to vaccinate on logistic regression (p<0.05).

The present study underlines the importance of reliable scientific sources of information for pa-tients with autoimmune diseases and how the general attitude about vaccination becomes a pre-dictive factor for accepting COVID-19 vaccine.

Keywords: *COVID-19, vaccination, autoimmune diseases*

INTRODUCTION

The SARS-CoV-2 infection accounts for a high morbidity and mortality, having caused the death of more than 3 million people all over the world since the onset of the pandemic [1]. The COVID-19 disease particularly endangers people with comorbidities, leaving patients with autoimmune diseases in a place of uncertainty and vulnerability, enhanced by the fear of an increased risk for infection in the context of immunosuppression.

The current increasing prevalence of coronavirus worldwide will unfortunately provide the necessary data on the follow-up of autoimmune conditions. From an endocrinological point of view, patients with autoimmune thyroiditis have a higher risk of developing other antibody-related conditions such as vitiligo, alopecia areata, dermatitis herpatiformis, hypophysitis, autoimmune hepatitis, Sjogren's syndrome, Raynaud's syndrome, premature ovarian failure, primary adrenal insufficiency, type 1 diabetes mellitus, rheumatoid arthritis, atrophic gastritis, lupus, sclerodermia, vasculitis, and celiac disease [2].

The rapid development of vaccines against the SARS-CoV-2 infection provides the opportunity of acquiring immunity without the need of going through the disease. However, the novelty of the vaccine and of the technological platforms were paralleled by a gap in knowledge regarding the potential side effects of vaccination. Patient with autoimmune diseases thus struggled in a battle where on

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one hand was the desire for protection against a virus that might aggravate a serious, chronic disease and, on the other hand, was the concern regarding safety associated with vaccination. In this light, our aim was to assess the intention to vaccinate among patients with rheumatic diseases.

MATERIALS AND METHODS

A cross-sectional study was performed in the spring of 2021 in Cluj-Napoca, Romania. A web-based questionnaire was sent via a Google docs platform to patients with autoimmune diseases before a patient-dedicated virtual event, in order to assess their willingness to vaccinate against SARS CoV-2 and to identify factors that might influence their decision.

The questionnaire comprised a small section dedicated to general data, including a personal and family history of COVID-19 infection; a section evaluating the anxiety regarding COVID-19 as elaborated by Lee [3], a section evaluating the general attitudes toward vaccination, addressed by the means of the Vaccination Attitudes Examination (VAX) scale, developed in English by Martin and Petrie [4] and validated in Romanian by Huza [5]; VAX scale is comprised by the following four subscales: mistrust of vaccine benefit, worries about unforeseen future effects, concerns about commercial profiteering and preference for natural immunity.

Supplementary questions regarding the attitudes of patients towards the government, healthcare system and guideline compliance were imported from an article that published the data of the UCL COVID-19 Social Study [6]. One question evaluated whether the decision to accept the COVID-19 vaccine was due to information drawn from non-scientific sources (the opinion of a public person, opinion of friends, possible travel restrictions, others) or from scientific sources (scientific books and journals, opinion of an expert in the field).

Participants received no compensation for completing the questionnaire, however, they had enhanced access to communicate with the physician to address further inquiries regarding their autoimmune pathology or COVID-19 disease, including vaccination.

For the statistical analysis, variables were derived from the questionnaire and included in a bivariate analysis. Chi square test was used to assess the relationship between the intention to vaccinate and most of the variables considered for analysis. Fisher test was used to assess a relationship between the intention to vaccinate and COVID-19 guideline compliance and Freeman-Halton test for the variables type of immunosuppressive therapy and COVID-19 disease experienced by the subject, the family, close friends and acquaintances. We used Fisher and Freeman-Hallton instead of Chi-Square when the minimum of 5 expected cases was not met, with Fisher being used for 2x2 matrices and Freeman Gallton for 2x3 matrices. Significance threshold was considered under 0.05. Multivariable analysis was subsequently performed by the aid of logistical regression. Statistical analysis was performed using SPSS v21.

RESULTS

Of 75 answers that were submitted, 71 were included in the study after a quality control check. The study sample consisted mostly of women, 60 (84.5%), participants having a mean age of 46 years, mostly from urban areas 58 (81.68%), with various autoimmune diseases, such as dermatomyositis 4 (5.6%), systemic lupus erythematosus 13 (18.3%), rheumatoid arthritis 28 (39.43%), systemic sclerosis 12 (16.9%), Sjogren syndrome 3 (4.2%) and other autoimmune diseases 13 (18.3%). The diverse forms of autoimmune diseases necessitate diverse treatments, ranging from hydroxychloroquine 10 subjects (14%) and biologic therapies such as belimumab, rituximab, tocilizumab 7 (9.8%) to other immunosuppressive drugs such as prednisone, methotrexate, mycophenolate mofetil 54 (76%).

The proportion of patients with autoimmune pathology willing to get vaccinated was 53.5% (38 subjects). Upon statistical analysis, a positive association was observed between the willingness to vaccinate and the patients' trust in authorities, their trust in the healthcare system and the choice of scientific sources of information in their decision making ($p<0.05$). A positive correlation was observed between the intention to vaccinate and the global VAX score, as well as the subscale VAX scores ($p<0.05$).

No association was found between the intention to vaccinate and a history of in-fluenza vaccination. Furthermore, the intention to vaccinate was not influenced by a prior vaccination refusal, by the preference for a certain type of vaccine technology (ARN-based, viral vector, inactivated virus or no preference) nor COVID-19-related anxiety.

No statistically significant relationship was noted between the intention to vaccinate and whether the subject or the family/friends of the subject experienced the burden of the COVID-19 disease or whether they are compliant to COVID-19 prevention guidelines recommended by authorities.

Upon multivariable analysis, global VAX score emerged as a predictive factor for the intention to vaccinate ($p<0.05$).

DISCUSSION

The current pandemic day's prescription is marked by access through digital medicine. The access to investigations might be reduced but drug administration including vaccines should not be postponed, if considered necessary [7]. The willingness to vaccinate in our study (53.5%) was similar to the one reported in a cross-country study (54.2%) with more than 1500 participants with systemic autoimmune or inflammatory rheumatic diseases [8], but slightly lower compared to a willingness of 61% in a cohort of patients with rheumatic diseases in The Netherlands [9].

Our results are significantly lower compared to a 92.9% preparedness for vaccination against COVID-19 in Danish patients with systemic lupus erythematosus and rheumatoid arthritis [10]. The association between pancreatitis, panniculitis and polyarthritis, entitled PPP syndrome, is a special category that need to be included in future studies to see if the percentage is the same like the others categories [11].

The scarcity of clinical trials studying the adverse effects of vaccination on rheumatic diseases, at the time of the study, and the time gap from publication to patient information may be important factors that account for the lack of willingness to vaccinate.

Studies like the one published by Achiron et al [12] revealed the fact that disease relapse in another autoimmune disease, multiple sclerosis, was similar in patients compared to controls; the rates of adverse events was higher in younger patients and in subjects with lower disability, orienting the physician toward a possible subgroup of subjects that might possibly be at risk of developing unwanted side-effects [13].

Hypercortisolemia found in glucocorticoid osteoporosis is a well known cause of obesity-related type of secondary osteoporosis more frequent in obese subjects without Cushing’s syndrome that display chronic high values of basal cortisol which seems suppressible at dexamethasone inhibition test and the chronic autonomous secretion of cortisol is identified in patients with adrenal incidentaloma with an age-dependent incidence and this has been reported to increase the risk of osteoporosis independent of age, so, this category can be affected by the fear of getting the infection because they are immunosuppressed by their pathology, so the vaccination will be more wished [14].

Data reported in a short-term observational study assessing the safety of SARS-CoV-2 mRNA vaccines in patients with rheumatic and musculoskeletal diseases indicates mainly mild local and systemic adverse events [15,16]. Patients with regression melanomas, a phenomenon that occurs as a result of host immune response that attacks the primary melanocytic tumor cells through lymphocytes determining a fibrotic process, can be also a difficult category of persons that can not wish the vaccination, because they can fear of a faster evolution of the regression and feel the a faster dissemination of the neoplastic cells [17].

Patients with an autoimmune disease are known to have high rates of anxiety and depression [18]. In our cohort, the proportion of subjects with COVID-19-related anxiety was slightly higher than the one obtained from a questionnaire distributed to students enrolled in the healthcare system: 33.8% (24 subjects) vs 28.5% (article submitted). In our sample, choosing scientific sources of information correlated with the positive intention to vaccinate, in agreement with previously published data stating that not relying upon social media for virus information is a significant predictor for the intention to vaccinate [19].

We note that the majority of subjects in our cohort chose scientific resources, 47 subjects (66.19%); this may be attributed to a selection error, participants having been recruited with the occasion of a patient-dedicated virtual event.

In our opinion, the association between the intention to vaccinate and the global VAX score, as well as subscale VAX scores is an important finding since it reveals the way the general attitude toward vaccination influences a particular vaccination choice, in our case the SARS-CoV-2vaccine. The relationship between general vaccination attitudes and the intention to vaccinate against COVID-19 was highlighted in a study having students as a target group (Candrea et al, submitted), but also in a large study involving more than 32.000 participants of various social, ethnical and educational backgrounds [6,20].

The above-mentioned association has a broader impact on the healthcare of patients with autoimmune diseases since they have recommendation for the administration of other types of vaccines, including pneumococcal immunization [21].

Limitations of our study can be considered the psychiatric conditions in these patients, for example the diagnosis of depression as well as the treatment for depression were significantly more frequently associated with psoriasis, an immune mediated disease. Patients with mild psoriasis can experience psychiatric comorbidities; however, depression is more common in patients with severe psoriasis or psoriatic arthritis.

So, father research is needed to se if any psychiatric comorbidities can influence the patient decision to be vaccinated against COVID-19 [22].

In conclusion, the present study highlights the importance of reliable scientific sources of information for patients with autoimmune diseases and shows how the general attitude about vaccination becomes a predictive factor for the de decision to vaccinate against COVID-19. Therefore, it is recommendable to make efforts to shift patients' general attitude toward vaccination.

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Authors' contributions
Conceptualization, EC, CP, VJL; methodology, EC, CP, VJL, SR; validation, all authors; formal analysis, EC, CP; investigation, CP, CM, AB, SR.; data curation, EC, CP, AB; writing—original draft preparation, EC, CP, CM, TRM; writing—all authors; supervision, SR. All authors have read and agreed to the published version of the manuscript.

Ethics approval
The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the 'I. Hatieganu' University of Medicine and Pharmacy, approval number 99.

Informed consent
Statement: By completing the questionnaire, subjects consented to participate in the study.

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REVIEW

A review of biovitroceramic PAW1 – Romanian bioactive implant material for ossiculoplasty

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Abstract: *A successful ossiculoplasty (reconstruction of the ossicular chain of the middle ear), even now, almost a hundred years after the first attempts and countless tested materials, remains a challenge through the complexity of the surgical act and through the repeated attempts to find the most adequate prosthesis to re-establish, in the long term, the sound conductive mechanism.*

In this article, we present a review of the main implanted materials, including the biotechnological aspects of ossicular chain reconstruction with an emphasis on the Romanian contribution to this field of study.

Keywords: *prostheses, implants, ossiculoplasty, ceramics, ossicular chain, biotechnology, biocompatibility, biovitroceramic PAW1*

INTRODUCTION

Ossiculoplasty is a surgical procedure used to restore hearing loss by reconstructing the ossicular chain mechanism between the tympanic membrane and the oval window, in the presence of a discontinuity or fixation, with or without a concomitant disease. The medium-term and especially the long-term success of this intervention depends on several important factors: the skill of the surgeon, the material used in the reconstruction of the ossicular chain, and, most importantly, a preserved, quality middle ear.

Chronic suppurative otitis media (COM), with or without cholesteatoma [1], is the most common pathology that influences the outcome of surgery and the long-term functional and anatomic results. The functional results become even more important if you consider that hearing loss of variable etiology represents one of the most challenging public health issues confronting the world's population [2]. "The integrity of the auditory system is one of the prerequisites for the acquisition and the proper development of oral language and a person suffering from hypoacusis is more likely to have poorer professional results than his colleagues, and will be less competitive in the labor market, and will have smaller chances to complete higher education" [3,4].

The next most important factor influencing results is the type and the quality of the material. Over time many types of materials have been tested (biological and synthetic), some entering into medical practice a shorter period, others remaining in current use, but the surgeon's choice for the ossicular chain reconstructions depends on their own experience in the field, the ossicular deficiency or the patient's immunological status [5]. The purpose of all these attempts is to implant a type of material with excellent biocompatibility, and stability, to provide natural sound transmission with the minimum risk of potential bacterial colonization or graft rejection [6].

THEORY AND CONCEPT

Ossicular substitution or reconstruction has been attempted with an array of materials but the only conclusion that has been drawn is that "the ideal prosthesis for ossicular reconstruction should be made of a material that maintains

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its shape, rigidity, and acoustic properties, as well as being cost-effective. This material should also be biocompatible, safe, and easily inserted and modified” [7].

From the acoustical point of view, a prosthesis is well designed, if its shape and material reach a high stiffness combined with a low mass but is also small, relatively cheap, and easy to modify [8,9,10,11].

From this point of view, titanium has advantages compared with gold, ceramics, or plastics because of its low density and high mechanical strength. These properties of titanium significantly reduce possible loss in sound transfer due to internal resonances. The higher mass of gold and ceramic prostheses decreases the motion of the entire system above the first resonance.

This inertia influence can also be seen by a decrease in the first resonance frequency of the (reconstructed) middle ear system. “By contrast, the low mass of the light titanium prostheses contributes to a better sound transfer performance, especially in the high-frequency range.” [12]

A further significant parameter is the quality of prosthesis coupling to both tympanic membrane/malleus and stapes. The dynamics of the reconstructed middle ear are very sensitive to where and how the prosthesis is coupled to the tympanic membrane and ossicles, to the spatial direction in which the prosthesis is oriented, and to the prosthesis attachment (near rigid or rather loose attachment). In a patient, the prosthesis attachment may be influenced by the resulting tension that is achieved both by the surgeon and by postoperative wound healing.

Prosthesis placement is paramount for obtaining good functional results, but this represents no guarantee that postoperative migration relative to the malleus handle, TM, or stapes will not occur [13,14]. This is highly dependent on the skill of the surgeon; the type of prosthesis and we should never forget that medical research can sometimes require a high degree of abstraction and theorization [15].

“Ossicular reconstruction materials are categorized as autografts, homografts, and alloplastic prosthetics. Each of these materials has advantages and disadvantages for use in the middle ear” [7].

BIOLOGICAL MATERIALS

Hall and Rytzner (1957) performed the first chain reconstruction using autogenous incus [16]. Several otologists, including Farrion, Sheehy, and Guilford, began reporting on the success of using autografts for ossicular reconstruction, especially for the body of the incus [17]. However, the patient’s incus is not always available for repositioning and Farrion first advocated the use of cortical bone as an ossicular substitute [17,18].

As a biological alternative to ossicles, cartilage has been extensively used in tympanoplasty and ossiculoplasty [18]. Autologous tragal and/or conchal cartilage is, however, not always suitable for ossicular chain reconstruction, especially in cases where the stapes arch is missing since they do not have the required mass, stiffness, and thickness [19]. Zini et al. (1984) introduced ossicular prostheses made from costal cartilage and reported no extrusion at 10 years of follow-up with stable functional results over the years [19].

Autologous materials presented a high degree of tolerance within the middle ear and provided reliable hearing results. Their disadvantages were the laborious and time-consuming sculpting into the required shape and the scarceness of material in chronically diseased ears [4] loss of rigidity (especially with cartilage), and possible fixation to the soft tissues of the middle ear [17].

Homografts (as an option for unreliable autografts) were first used for middle ear reconstruction in 1966 by House and colleagues and later led to the use of “irradiated ossicles, cartilage, and even homograft tympanic membranes with en bloc ossicles” [20,21]. The hearing results and biocompatibility of homografts and autografts proved similar but the risk of disease transmission of HIV and prions (i.e., Creutzfeldt-Jakob disease) brought their decline in use – see Table 1.

Donor (D) – Receiver (R) Relationship:
1. Autologous: D ≡ R
2. Isologous: D·R Homozygotic Twins
3. Homologous (Alloplastic): D·R Same species
4. Heterologous (Xenoplastic): D·R Different species

Donor Situs (D.S.) to Receiving (R.S.) situs Relationship:
1. Orthotopic (same situs): D.S. ≡ R.S.
2. Heterotopic (different situs): D.S. ≠ R.S.

Although many surgeons preferred biological prostheses due to increased compatibility with the middle ear and low rate of rejection, the lack of materials and prolonged intraoperative time led the researchers to look for the ideal material in the reconstruction of sound conducting mechanisms.

SYNTHETIC MATERIALS

Other than the natural or biological materials (autologous and homologous), polymers, ceramics, and metals are currently used for ossicular chain reconstruction, each with its advantages and disadvantages. These synthetic materials are defined as biomaterials.

In 1958, Shea used for the first-time polyethylene prostheses (Polytef – Teflon and silicone elastomer – Silastic) for middle ear reconstruction [17] with excellent short-term hearing results. The high extrusion rate, significant middle ear reactivity towards synthetic materials, and risk of penetration into the inner ear [17] caused this material to gradually drop out of use.

Table 1: Types of natural biomaterials used in ossiculoplasty

Natural Biomaterials (Grafts, Transplants)				
Type	**Status**	**Donor–Receiver Relationship**	**Species**	**Author, Year**
Autologous	Fresh	Orthotopic	Remodeled malleus	Wullstein, 1953
		Heterotopic	Cartilage Tragal cartilage Septal cartilage Rib cartilage Mastoid cortical bone	Williams, 1958 Utech, 1960 Jansen, 1962
Homologous (Allo)	Preserved	Orthotopic	Isolated ossicles Ossicular chain	Portmann, 1959 Marquet, 1966
		Heterotopic	Mastoid cortical bone Septal cartilage Rib cartilage Meniscus cartilage Dentine	Wullstein, 1952 Jansen, 1960 Zini, 1967
Heterologous (Xeno)	Preserved	Orthotopic	Veal incus	Sultan, 1983
		Heterotopic	Veal rib cartilage	Zini, 1959
	Normal	Normal	Coral	Robier, 1987 Schmorll, 1990

After almost 20 years of research, in 1976, Shea reported on the use of a high-density polyethylene sponge (Plastipore), a porous, nonreactive material that could allow the in-growth of tissue [22]. “A similar thermal-fused porous polyethylene (Polycell) was developed later and allowed the prosthesis to be coupled to other materials, such as stainless steel thus enabling various prosthesis designs” [17]. The most-reported problem of such porous materials was the high extrusion rate when in direct contact with the TM. However, a disc of cartilage placed between the head of the prosthesis and the tympanic membrane gives excellent long-term results even today [22,23].

In 1979 the era of ceramics in ossicular reconstruction begins by introducing a few categories of alloplastic materials, either bioinert or bioactive. Implantable materials that do not release detectable trace substances are termed bioinert. The prototype bioinert material is dense aluminum oxide ceramic (Al_2O_3), popular in Germany and Japan in the 1970s. The implant can be fitted to the under-surface of the tympanic membrane without cartilage coverage [24].

By studying the way, the body reacts to the implanted materials, there can be three major recipients defined (Tables 2, 3, 4):

● “bioinert” – the body does not react to the implant material.

● “bio-tolerated” – the local tissue regards the implant as a foreign body but does not react, the material is not excluded.

● “bioactive” – the local tissue reacts and tends to create a stable bound to integrate it [6].

“Bioactive implants, such as glass-ceramic (Ceravital), were biocompatible and reacted with surrounding soft tissue and adjacent bone allowing a coupling between the implant and the ossicle” [19,25] and were used, with various results, around the world, even in the former communist bloc, including Romania [26].

Hydroxyapatite, found in the mineral matrix of living bone, was introduced for tympanoplasty surgery in 1984 by Grote (calcium phosphate ceramic) [27]. It is a bioactive material that can be easily integrated into surrounding tissues (bone). The first results by Wehrs et al. reported functional success and a low extrusion rate 4 years after implanting hydroxyapatite incus or incus/stapes prosthesis. Since that time, this material has been adapted to various uses and prosthesis designs [17,20].

Titanium prostheses were developed and first used in Germany in 1993 and have been growing in popularity ever since. The material is rigid and biocompatible just like hydroxyapatite, but much lighter. It is also nonmagnetic and allows it to be manufactured in many precise shapes. “Most of the titanium prostheses possess an open head, allowing better visualization during placement” [17].

Table 2: Types of synthetic bioinert materials used in ossiculoplasty.

Name	Composition, Chemical structure, Family, Order	Author, Year
ALUMINA FRIALIT CORINDON	Al_2O_3 Oxide Ceramics	Plester & Jahnke, 1979
BIOCERAM	Al_2O_3 Y_2O_3	Yammamoto & Ywanaga, 1983
MACOR CERAMIC	Fluor phlogopite mica-Borosilicate glass $SiO_2 \cdot MgO \cdot Al_2O_3 \cdot K_2O \cdot B_2O_3 \cdot F$	Pauler & Jahnke, 1984
VITROCARBON	Glass - Carbone	Blemke et al. 1987

Table 3: Types of synthetic bioactive materials used in ossiculoplasty.

Name	Composition, Chemical structure, Family, Order	Author, Year
BIOGLASS 45-S-5	Silicon-Phosphorus Bio-glass $SiO_2 \cdot CaO \cdot Na_2O \cdot P_2O_5$	Hench et al., 1970
CERAVITAL	Silicon-Phosphorus Bio-vitro-ceramic (Hydroxyapatite – Glass) $Li_2O \cdot ZnO \cdot SiO_2$	Brömer et al., 1973
CERABONE A-W	Hydroxyapatite – Glass- β-Wollastonite ($CaO \cdot SiO_2$)	Kokubo et al., 1982
IMPLANT L-I	Hydroxyapatite – Glass- β-Wollastonite ($CaO \cdot SiO_2$)	
BIOVERIT I	Mica – Apatite – Glass $SiO_2 \cdot (Al_2O_3) \cdot MgO \cdot Na_2O \cdot K_2O \cdot F \cdot KO \cdot P_2O_5$	Holland &Vogel, 1987
BIOVERIT II	Mica – Glass – Corderite	Holland & Vogel, 1993
BIOVITROCERAMICS PAW-1	Fluro-hydroxyapatite – Wollastonite – Glass $B_2O_3Fl_2$	Rădulescu & Negreanu, 1993

Table 4: Types of synthetic bio-tolerated materials used in ossiculoplasty.

Name	Composition, Chemical structure, Family, Order	Author, Year
PALAVIT	Vinyl-acrylic resin	Wullstein, 1952
SUPRAMID	Polyamide (Nylon)	Kley, 1955
POLYETHYLENE	Non-porous, low-density polyethylene	House, 1960
TEFLON	Polytetrafluoroethylene (C2F4)n (PTFE)	Shea, 1962
NON-OXIDATIVE STEEL TANTALUM SILVER ZIRCONIUM TITANIUM	Non-oxidative metals	Schucknecht, 1971
PROPLAST	Polytetrafluoroethylene (TEFLON) – Carbon fibre composite	Shea, 1974
PLASTIPORE	High-density polyethylene sponge (HDSP)	Shea, 1974
POLYCEL	Thermal fused HDSP	Fisch, 1981

Classification of Synthetic Biomaterials:

1. Destination:
   1.1. Tissue
   1.2. Organ
   1.3. Function

2. Composition:
   2.1. Monocomponent
   2.2. Policomponent

3. Structure – Mass - Form:
   3.1. Monostructure
   3.2. Polistructure

4. Relationship to Host (Recipient):
   4.1. Biotolerated
   4.2. Bioinert
   4.3. Bioreactive (Active, Bio-reactive, Bioactive)
   4.4. Composite (e.g., Tolerate + Active)

BIOVITROCERAMIC PAW-1 AND THE ROMANIAN CONTRIBUTION

Synthesized in 1990 by Dr. Ing. Popescu-Negreanu, PAW-1 was tested physically, chemically, and biologically (in vitro and in vivo) between 1991 and 1992 and homologated on March 31st, 1993.

Bio-vitro-ceramic PAW 1 is a solid, bio-reactive material, made up of fluoro-hydroxyapatite and wollastonite microcrystals encompassed into a vitreous mass (glass). Hydroxyapatite, which is the mineral matrix of living bone, is a well-tolerated osteoinductive material that does not produce necrotic phenomena in adjacent tissues and does not give foreign body reactions [26] – see Table 5.

Table 5: Taxonomy of PAW1.

General Taxonomy of PAW1
Class: Solids with biphasic structure (crystalline and non-crystalline)
Order: Ceramics
Family: Bio-vitro-ceramics
Genus: Silicon – Phosphorus
Species: Controlled porosity
Variety: α - closed porosity (dense) β– opened porosity (porous)
Special Taxonomy of PAW1
Synthetic
Destination: Organ
Composition: Poli-component
Structure – Mass – Form: Monostructure
Relationship to Host (Recipient): Bioactive

Medical use:

1. Dentistry: Central Military Hospital, Bucharest since June 1993
- ● Parodontopathy
- ● Granules

2. Oral and maxillofacial surgery/Esthetics: Prof. Burlibașa, Prof. Gănuță since 1993
- ● Granules
- ● Shaped implants

3. Orthopedics: Central Military Hospital, Bucharest and Foișor Orthopedics Hospital, Bucharest, Prof. Popescu Mihai since 1993
- ● Granules
- ● Shaped implants

4. Neurosurgery: “Bagdasar-Arseni” Neurosurgical Hospital, Bucharest, Prof. Dănăilă since 1994
- ● Shaped implants

5. Otorhinolaryngology (Otology): IFACF-ORL Prof. Dorin Hociota, Dr. Marian Rădulescu since June 1993
- • Ossicular chain implants (PORP, TORP)

Chemical composition:
1. Fluorohydroxyapatite: $[Ca_{10}(PO_4)_6(OH)xFl_2\text{-}x]$
2. β-Wollastonite ($CaO \cdot SiO_2$)
3. Boron trioxide: B_2O_3
4. Fluoride: Fl_2

The material is obtained by thermally controlled crystallization (ceramization) of glass from the Silicium-Calcium-Magnesium-Phosphorus system ($SiO_2 \cdot CaO \cdot MgO \cdot P_2O_5$) with minute additions of Borum trioxide (B_2O_3) and molecular Fluoride.

Biological properties:

1. Biocompatibility:
● Predictive: Good, due to the identity of the crystalline phase of the material to the mineral phase of the mature bone.
● Experimental: tested through the low rejection rate of middle-ear implants.
2. Biodegradation:
● Predictive: Null without inflammation/infection. Osteogenesis on the implant-tissue interface.
Identical to the bone with inflammation/infection.
● Experimental: Clinical – the study of rejected implants degradation
Paraclinical – Rx for integrated implants/HP for rejected.
3. Bioactivity:
● Predictive: Good, based on the physical and chemical structure.
Porosity – cell colonization and mechanical anchorage.
Chemical structure: Chemical anchorage (direct) = covalent ties at the implant-tissue interface. Osteogenesis on the interface.
● Experimental: tested through the rate of histological integration and rate of tympanic membrane perforation.
4. Bio-functionality:
● Predictive: Good, based on mechanical and vibro-mechanical properties.
● Experimental: tested through therapeutic efficiency (audiological gain – dB): Air-bone Gap (ABG) pre-operatively vs. post-operatively.

CONCLUSION

Although numerous techniques and materials have been used for ossicular reconstruction, the quest for the ideal prosthesis and the best-suited materials is ongoing. This research took place all over the world during the past century and even developing countries such as Romania played a role.

The autochthonous bio-vitro-ceramic PAW1 proved to be a reliable, resilient material with a high degree of biocompatibility and good functional results.

The best combination of biocompatibility, toughness, ease of shaping, and inserting are still to be found.

Today, autogenous, and alloplastic prostheses are equally used with good outcomes, but the final decision remains to the surgeon, and it would be wise that he chooses the one that is most comfortable for him and provides consistent and favorable results.

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None declared.

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Authors' contribution
Conceptualization: RM, AIM. Data curation: AIM, RM. Formal analysis: AIM, RM. Methodology: RM. Project administration: AIM. Visualization: AIM. Writing - original draft: AIM, RM. Writing - review & editing: RM, AIM. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present work represents a review of the literature and therefore requires no agreement from the Research Ethics Committee.

Patient consent for publication
Not applicable.

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REVIEW

Should Hoffa's fat pad be resected during total knee arthroplasty? A review of literature

Symeon Naoum[1]

Abstract: *Knee osteoarthritis is caused by the degeneration of joint tissues through mechanical loading as well as inflammatory effects. The number of total knee arthroplasties is increased and is expected to further be increased by 2050 compared to 2021. The infrapatellar fat pad, also known as Hoffa's fat pad, is often partially or fully resected during total knee arthroplasty to improve the surgeon's view in the upper tibia as well may reduce the risk of soft tissue involvement, when placing the prosthesis. The purpose of this study is to present the importance and functions of Hoffa's fat pad, as well as the latest data on the two conflicting views, on the removal or not of Hoffa's fat pad in total knee arthroplasty. However, there is not a consensus regarding Hoffa's fat pad resection, as its preservation has been associated with increased postoperative anterior knee pain. Due to the fact, that there are no current official guidelines for this issue, it would be preferable that surgeons try to preserve it, but only if there are no visibility and/or access problems during surgery. The choice of complete or partial resection or preservation is based on the surgeon's preferences, experience as well as patient's needs. More high-quality research and randomized clinical trials are needed for better guidance regarding Hoffa's fat pad management at total knee replacement.*

Keywords: *Hoffa's fat pad, infrapatellar fat pad, total knee arthroplasty, osteoarthritis of the knee, anterior knee pain, patellar tendon*

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease and the leading cause of chronic pain and disability in many developed countries [1]. Knee OA is caused by the gradual degeneration of joint tissues through mechanical loading as well as inflammatory effects [2]. However, two recent theories are considered to be the predominant causal factors: firstly, as the prevalence of knee OA increases with age, the increased life expectancy is considered to have led to high knee OA levels, with the joint tissues accumulating more damage than continuous loading [3]. Secondly, obesity and high body mass index (BMI) have become an “epidemic” in the western world, being a significant risk factor for the development of knee OA, possibly due to the combined effects of joint overload and inflammation [4].

The result of the above is the increased rates of total knee arthroplasty (TKA), as the definitive treatment for osteoarthritis of the knee. Typically, in the elderly patient alone in the United States, the per capita number of primary TKAs doubled from 1991 to 2010 [5,6]. Finally, the number of TKAs conducted annually in the United States is expected to increase by 143% by 2050 compared to 2012 [7,8].

The infrapatellar fat pad, also known as Hoffa's fat pad (HFP), is often removed, either partially or completely, during TKA to improve the surgeon's field in the upper tibia and may reduce the risk of soft tissue involvement, when placing the prosthesis. However, there are proponents of the opposite view, arguing that the "sacrifice" of HFP may jeopardize the perfusion of the patellar tendon, which may be reduced in the future.

The purpose of this review is to first present the importance and functions of HFP, as well as the latest data on the two conflicting views, the removal or not of HFP in TKA.

ANATOMY AND FUNCTION OF THE HOFFA'S FAT PAD

HFP is composed of fibrous adipose tissue and is located in the anterior compartment of the knee joint, constituting an intracapsular but extraarticular structure. It is bordered by

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the lower pole of the patella, the patellar tendon, the tibial plateau, and the femoral condyles. Adipose tissue (and the fat pad) is generally recognized as an endocrine organ, with sensory and immune functions capable of producing inflammatory mediators and growth factors. There is considerable evidence that HFP plays an important role in inflammatory processes associated with the pathology of the anterior surface of the knee, including OA [9,10]. In addition, the blood vessels that supply the patella and the patellar tendon travel through HFP, with the resection of the latter endangering the vascularity of these structures, with the ultimate result of the narrowing of the patellar tendon [11].

HFP has an abundant peripheral anastomotic perfusion network, with the superior medial and superior external iliac arteries providing 2 vertical arteries and connecting horizontally with 2-3 arteries located more distally [12]. In addition, there are rich local anastomotic branches to the menisci and patellar tendon onward, as well as to the periosteum of the tibia backward. Finally, anatomical studies revealed a large, mainly transverse contribution of vessels to the posterior surface and the central third of the patellar tendon, while the central area is relatively vascular [13].

Regarding HFP neurosis, the supply comes mainly from the posterior tibial nerve [14]. In addition, free nerve endings (type Via) have been identified in the fat pad. These free nerve endings may be activated by mechanical deformity or specific immunoreactive chemical agents and can send pain, pressure, and thermal stimuli to the central nervous system and are active through both "fast" and "slow" pain pathways [15]. The contribution of substance P to both pain generation and pro-inflammatory response induction is well known. Several authors report the presence of abundant substance P nerve fibers in HFP [12,16]. Moreover, in patients who experience anterior knee pain, the number of substance P nerve fibers in HFP increases significantly [12]. The presence and number of afferent immunoreactive nerve fibers as well as the ability to release pro-inflammatory cytokines have led to the hypothesis that HFP is a possible causative agent in patellar tendonitis [17]. Finally, there are indications that it plays a regulatory role in the inflammatory pathways involved in OA [18].

THE CONTRIBUTION OF HOFFA'S FAT PAD TO THE BIOMECHANICS OF THE KNEE

Studies have shown a positive correlation between HFP size and patient height, possibly leading to a biomechanical role in the knee joint [19]. In a study, it is reported that this structure facilitates the distribution of synovial fluid and may act to absorb various loads-actions created through the joint [20].

Patients suffering from anterior knee pain have been reported to show reduced coordination of motor units between the medialis and lateralis vastus muscles [21]. In one study, the authors concluded that after hypertonic saline injection into the fat pad, there was a significant subsequent activation as well as a reduction in the contraction width of the quadriceps muscle as the patient climbed the stairs [22]. This quadriceps inactivation is thought to increase patellar loading, which leads to an increased incidence of cartilage degeneration of the patellar articular joint [23]. Additionally, in a human cadaver model study, HFP resection was found to reduce medialization of the patella and reduce tibial rotation during bending [24]. Using the same model in a later study, the authors attempted to simulate swelling and the effect of the HFP [25]. The increase in simulated edema again reduced the area as well as the pressure of the patellar joint surfaces, while the measured pressure within HFP was also increased [26].

Finally, in a study that studied the biomechanical behavior of HFP in patients with OA, the results indicated the true biomechanical relevance of HFP in the knee as well as the loss of the proper "strain" behavior of HFP in OA knees, under mechanical loads [20].

THE ROLE OF HOFFA'S FAT PAD IN THE DEVELOPMENT OF OSTEOARTHRITIS

There is little scientific evidence of Hoffa's body's role in the development of osteoarthritis in the international literature. However, several reports are linking anterior knee pain syndrome to changes in the fatty body, such as hypertrophy, inflammation, and fibrosis [26].

Cartilage damage as well as a mechanical overload of the joints associated with abnormal movements, lead to the activation of mechanical receptors on chondrocytes and osteoblasts. The association between obesity and joint degeneration consists not only of deformed biomechanics but also of the ability of adipose tissue to activate the inflammatory process. In response to mechanical stimuli, cells activate inflammatory processes, resulting in the release of mediators causing further articular cartilage degeneration [26]. White adipose tissue is made up of highly specialized and well-differentiated cells and contains fat cells, fibroblasts, leukocytes, macrophages, and other cells that play a role in inflammation. In patients with OA, both the number of C fibers and the number of medium and large fibers containing substance P is greatly increased. The procedure leads to swelling, tissue ischemia, and partial necrosis. All of these mechanisms also cause changes in the structure and metabolism of HFP [26].

In patients with knee OA, HFP and synovial fluid contain significant amounts of inflammatory agents such as FGF-2, VEGF, TNF-α, and IL-6. Finally, HFP plays an important role in the onset and progression of OA through the activation and release of pro-inflammatory mediators.

EXCLUSION OF HOFFA'S FAT PAD DURING TOTAL KNEE ARTHROPLASTY: RECENT DATA

The exclusion, or not, of HFP during TKA, has been a common subject of research. A study conducted in the United Kingdom investigating the awareness of orthopedic surgeons on this issue had particularly interesting results [27]. Of the 173 respondents, 86.7% were consultants. 62.4% of the respondents underwent a partial resection of HFP, 23.1% underwent a complete resection while 9.8% of the surgeons maintained it. In addition, only 23% said they were aware of the proposed guidelines.

Studies supporting the maintenance of HFP during TKA report an increased risk of scarring and consequently a reduction in the length of the patellar tendon caused by disturbances of the anastomotic connections presented in HFP [28]. The resulting length reduction of the patellar tendon is considered to contribute to increased stiffness of the knee, patella, and impaired kinematics [24,28,29].

Respectively, resection of HFP is also thought to disrupt patellar perfusion and thus increase the fracture risk [30]. Increased pain is also reported to be associated with HFP resection [31]. The majority (62.4%) of surgeons in the above study performed a partial resection, while the majority also stated that adequate exposure to the outer part of the tibia was the main reason for total or partial resection. It is worth mentioning that in the same study several surgeons who preferred to maintain HFP stated that in cases of difficult exposure, they would proceed to a partial resection. A small number of surgeons considered that maintenance was associated with increased anterior pain postoperatively. This idea has been widely discussed, as HFP is believed to be involved in inflammatory processes, which makes it a possible source of anterior knee pain [32].

In a cohort study, the possible difference in the Insall-Salvati ratio of knees undergoing TKA was studied between the group of patients in which HFP had been retained and the one in which it had been completely removed [9]. The difference in the Insall-Salvati index (ratio) from the preoperative to the immediate postoperative period of the group in which HFP was retained compared to the group in which it was removed, showed a significant difference (p=0.010). However, the change in the Insall-Salvati ratio after 1-year of follow-up did not differ significantly between the groups (p=0.059). Therefore, this study does not clearly support any preference between the two techniques.

In another study involving 90 patients undergoing TKA, patients were divided into 2 groups (n1=45, n2=45) [31]. In the first group, patients underwent minimally invasive total knee arthroplasty (MIS TKA) with complete resection of HFP, while in the other group, 45 patients underwent MIS TKA without resection. The authors measured the reduction in patellar tendon length, knee flexion, anterior knee pain, Knee Society Score-KSS scale, functionality, and complications of the patella in preoperative and postoperative (6 weeks, 3 months, 6 months, and 1 year). The results of this study showed no significant differences in the reduction of patellar tendon length, KSS scale, functionality, or knee flexion in either group. However, patients with HFP resection had more anterior knee pain (8.3% vs. 0%, p=0.03, CI 95%, -0.007 to 0.174) at the end of the study, and no complications were found to be related to the patella in any group, concluding that surgeons should maintain HFP if excellent exposure and visibility can be achieved, but it could be removed if necessary to improve exposure during TKA.

An anatomical study of vascularization showed that the majority of HFP mass could be removed without causing injury to the lower part of the blood vessel network [33]. Discontinuation of HFP’s vascularity may not affect the vascularity of the patella. The study by Pinsornsak et al. does not report any evidence of vascular necrosis or patella fracture in any patient with HFP resection [31,33].

In a prospective randomized study of 68 patients undergoing TKA for OA, no significant differences in the length of the patellar tendons were found from radiographs immediately after surgery, nor after 1 month or after 6 months [34]. This study demonstrated a statistically significant association between Hoffa adipose body fibrosis and anterior knee pain during the first six postoperative months in TKA. The authors also report that resection of HFP with severe fibrosis has a beneficial effect on pain at six months follow-up and, therefore, the authors recommend systematic resection of HFP during TKA.

In a retrospective observational study of 78 TKAs for OA, the researchers found a significant reduction of the patellar tendon in the group in which HFP was removed compared with the group in which it was maintained at 1-year follow-up [35]. These changes continued in all 3 years of follow-up. In another retrospective observational study of 720 patients (1055 TKA), the researchers found no differences in patellar tendon length before and after surgery but found increased postoperative pain in patients who had HFP resected [36]. In addition, a study of 1,401 patients (1,417 TKA) used the five-dimensional Oxford Knee Score and EuroQol questionnaire (Eq. 5D) to compare results for HFP resection and retention groups [37]. At 1 year follow-up postoperatively, those with the retained HFP had significantly better subjective pain scores and functionality scores compared to the group in which it was removed.

A systematic review of 2013, included studies that reported anterior knee pain, patellar tendon length, range of motion, patellar vascularity, or functional outcomes [38]. This review reports that HFP resection during TKA gives favorable outcomes in patients with OA. Particularly, after HFP resection, for patients with OA, no differences in functionality, range of motion, and anterior knee pain were found. Regarding patients with rheumatoid arthritis (RA), there was a tendency for greater discomfort and reduced functionality, while in patients with OA and RA there was a reduction in patellar tendon length.

Another subsequent systematic review, which included ten studies with a total of 10,163 patients, studied 6 parameters: knee pain, patellar vascularity, range of motion (ROM), patellar tendon length, trauma complications, and patient satisfaction [39]. There was no clear evidence of increased knee pain in patients undergoing fat resection during TKA. In addition, conflicting evidence was found regarding patellar vascularity and patellar tendon length after surgery, and insufficient evidence was found for differences in ROM. However, there is moderate evidence that HFP resection increases postoperative knee pain. It should be noted that this systematic review is limited by the lack of level 1 randomized clinical trials.

Another systematic review and meta-analysis conducted in 2016 included 7 studies (databases: Embase, PubMed, and Cochrane Library) and a total of 2,734 patients (3,258 knees) [40]. Excision tends to increase the incidence of postoperative anterior knee pain within 2 months postoperatively, compared with patients in whom HFP was retained ([OR] 2.12 [0.95, 4.73], p=0.07). In addition, there was an increased incidence of anterior knee pain in the resection group >12 months postoperatively, but the difference was not statistically significant ([OR] 3.69 [0.81, 16.82], p=0.07).

Reported causes of anterior knee pain include low HFP mass (due to previous surgery or injury), postoperative scarring, impingement syndrome, low patella position (patella baja), and misalignment of the patella, while excessive secretion of inflammatory mediators is also recognized as a causative agent [41-43]. There was also a tendency for a greater reduction in the length of the patellar tendon in the resection group, while no significant results were found regarding the postoperative function of the knee. Reduction of the patellar tendon is one of the most common complications after TKA. There is a tendency reported in the literature for a greater reduction in patellar tendon length in the group of patients with HFP resection compared with the conservation group. Many factors are responsible for the reduction, including scarring/thinning of surrounding soft tissues, disruption of the surrounding arterial network, and quadriceps muscle weakness [40,44]. The cavity formed after HFP excision is filled with fibrous tissue, while its preservation reduces scar formation/shedding. Based on the results of previous studies, maintaining HFP not only reduces the cessation of patellar tendon perfusion but also reduces the incidence of scarring and patellar tendon rupture. In addition, reduction of the patellar tendon is also responsible for postoperative anterior knee pain and misalignment of the patella [25]. In conclusion, the above systematic review and meta-analysis report that HFP retention has better results than resection in patients undergoing TKA due to the relatively lower rate of pain after short-term follow-up.

In a recent systematic review on whether or not to maintain HFP, published in 2019, including seven studies (two of which were randomized clinical trials) involving 2,815 patients (3,312 knees), the authors focused on the length of the patellar tendon, postoperative pain, KSS scale, and knee function [45]. The meta-analysis identified a tendency for a larger reduction of the patellar tendon after the HFP resection. The systematic review also showed a tendency that the HFP resection group was related to a greater reduction in patellar tendon length. When HFP is injured, there are fibrotic lesions. The fibrous tissue bands pass through this area and form a rigid structure [45]. This fact, in turn, leads to a narrowing of the patellar tendon. Excision was associated with a lower incidence of postoperative pain at one to two months of follow-up; however, at three to six months the pain scores were higher in this group. HFP is believed to play a role in the inflammatory process and contains pain nerve fibers and is, therefore, a possible source of anterior knee pain. In conclusion, its maintenance may explain the tendency of pain to increase at the beginning of one to two months postoperative period in the preservation team. At three to six months, this tendency is reversed with a higher incidence of knee pain in the resection group. One possible explanation for this may be due to the remaining nerve fibers injured during TKA as a source of ongoing pain in the resection group. Finally, no statistical difference was found in either the KSS scale or the functionality score.

Last but not least, in the most recent meta-analysis conducted in 2020 [46], 9 randomized controlled trials, involving 783 TKAs (722 patients), were included. The meta-analysis identified a trend regarding the shortening of the patellar tendon after HFP resection at both 6 months and 12 months. As far as the Insall-Salvati ratio, anterior knee pain within 6-12 months, Knee Society Scores at 12 months, and knee flexion within 6-12 months are concerned, no statistical difference between HFP resection and HFP preservation was found. Consequently, the authors of the above-mentioned meta-analysis reported that surgeons are advised to keep the HFP as far as sufficient exposure is achieved [46].

CONCLUSION

There are currently no formal guidelines for maintaining or resecting HFP during TKA. Removal versus retention during TKA is a controversial issue with the available literature not convincing. This review argues that retention is associated with an increased incidence of anterior knee pain up to two months postoperatively, while resection tends to reduce the length of the patellar tendon as well as a higher incidence of moderate to anterior knee pain. It would be preferable to make an effort to maintain HFP, but its preservation mustn't cause visibility and access issues during TKA.

However, the management of HFP during TKA is the subject of ongoing discussion without clear conclusions. The choice of complete resection, retention, or partial resection varies depending on the surgeon's preferences, experience, and patient needs. Last but not least, further studies of high quality and randomized clinical trials are needed to provide better guidance on the management of HFP during TKA.

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ORIGINAL ARTICLE

Anatomic-surgical study on the interparietoperitoneal space from a laparo-endoscopic perspective

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Abstract: *The interparietoperitoneal (IPP) space, by a broad definition, is the space between the musculo-fascial walls of the abdomen and the parietal peritoneum. A review of the literature on this subject has been performed through a search in the databases according to the following keywords: Bogros space, Retzius space, preperitoneal approach, and urogenital fascia. We have also analyzed the video recordings of the dissections of the inguinopreperitoneal region during TAPP, conducted by a single team, evaluating the dissection planes in the two compartments (medial and lateral) of the IPPS. Based on the latest data from the literature, as well as on our own experience in laparo-endoscopic herniation surgery, we aim to provide answers to several questions. IPPS from the inguinal region is an area as complex as it is narrow, but of great current surgical interest (approaches of hernias, vessels, prostate). The fascial distribution has its origin in the embryological development of the urogenital apparatus. The basic fascial structure in understanding IPPS compartmentalization is the UGF, along with its extensions.*

Keywords: *interparietoperitoneal space, Bogros space, Retzius space, preperitoneal approach, urogenital fascia*

INTRODUCTION

The interparietoperitoneal (IPP) space, by a broad definition, is the space between the musculo-fascial walls of the abdomen and the parietal peritoneum.

In classic anatomy descriptions, it is depicted as a virtual space, filled with adipose/fibroareolar tissue [1]. It is Bogros' merit to have broadened the perspective on a portion of the IPPS in 1823, one of only 13.5/15.5 mm, through which continuity between the anterior and posterior IPP space is obtained [2].

Bogros' initial description was in relation to the inferior epigastric artery and went along the lines of: “a loose layer of cellular tissue separating the inferior epigastric artery from the transversalis fascia anteriorly. Posterior, a thicker layer of the same tissue separates it from the peritoneum” [3].

We could therefore call this space located immediately below the FT (Fascia transversalis), where Bogros recommends the approach of the epigastric artery, as "original sp. of Bogros". The term Bogros space was given to it by Poirier as late as 1912 [4]. In 1858 the Swiss Anders Adolph Retzius described the so-called prevesical space as a compartment of the extraperitoneal space (a division of the at-large extraperitoneal space), as it turns out, without the knowledge of Bogros' work [5]. Since then, the two compartments of the IPPS have long been debated in anatomical literature.

A “historical review” of the authors who endeavored with the anatomical clarification of the IPPS, in terms of

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delimitations, content, and surgical applications, was made by R.C. Read [6]. The laparo-endoscopic approach of interparietoperitoneal space has brought many clarifications concerning regional anatomy, through in vivo exploration, obtained by magnification using specific optical instrumentation.

Based on the latest data from the literature, as well as on our own experience in laparo-endoscopic herniation surgery, we aim to provide answers to several questions:

- Which is the structure described by Bogros as a "loose layer of cellular tissue" in the first part of the quote?
- Which is structure described as a “thicker layer”?
- Is there an anteroposterior division of the Bogros space?
- Is the Retzius space a continuation within the same plane of the Bogros space?
- Which are the landmarks in terms of the fasciae for optimal dissection during the transabdominal preperitoneal laparoscopic (TAPP)/totally extraperitoneal laparoscopic (TEP) approach?

MATERIALS AND METHODS

A review of the literature on this subject has been performed through a search in the databases according to the following keywords: Bogros space, Retzius space, preperitoneal approach, urogenital fascia.

We have also analyzed the video recordings of the dissections of the inguinopreperitoneal region during TAPP, conducted by a single team, evaluating the dissection planes in the two compartments (medial and lateral) of the IPPS.

RESULTS

Ever since 1965, R Stoppa has approached the inguinal pro-peritoneal space by median sub umbilical incision, to carry out the prosthetic procedure called giant prosthetic reinforcement of the visceral sac (GPRVS). Dissecting the sperm cord elements in the IPPS, he described the internal spermatic fascia as an extension of the urogenital fascia [7].

This latter structure is the key to understanding the layout of the anatomical elements within Bogros and Retzius spaces. Embryology data show that the two layers of the UGF are a direct continuation of the renal fasciae, the anterior one (Gerota) and the posterior one (Zukerkandl), in-between themselves containing the urogenital elements developed from the mesonephros (kidneys, adrenal glands, gonads, ureters, ductus deferens), as well as the vessels and nerves that serve them, being compared to a border of these organs [8]. In the 5-week-old embryo, the mesonephros is attached to the posterior abdominal wall through a "broad urogenital mesentery" [9]. One must mention here that the urinary bladder and the urachus have another embryonic origin, which is the cloaca and allantois respectively, so they are located behind the UGF (Figure 1).

Figure 1: IPPS from the “new perspective” of TAPP

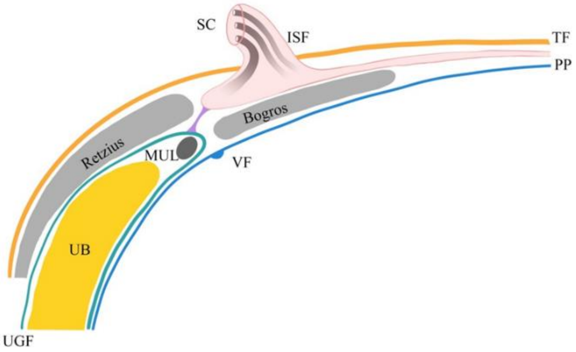

UGF – Urogenital fascia; MUL – Medial umbilical ligament; UB –Urinary bladder; SC –Spermatic cord; ISF – Internal spermatic fascia; TF – Fascia transversalis; PP – Parietal peritoneum; VF – Vesicular fold

Therefore, the presence of the UGF in the IPPS sub-divides the two compartments – medial and lateral into another two – superficial and deep, as shown in the following diagram (Table 1):

1 – the superficial medial compartment is the actual Retzius space, previously delimited by the FT (superior)/pubic (inferior) and posterior to it by the UGF.

2 – the deep medial compartment is limited by the UGF anterior to it and peritoneum posterior to it, containing the urinary bladder/urachus and the medial umbilical ligament (MUL), and also known as the perivesical space.

3 – the superficial lateral compartment between the FT (bilaminated) in the anterior plane and the UGF contains the initial portion of VEI, femoral vessels, and genito-femoral nerves. It is the compartment in which Bogros suggested the approach of the inferior epigastric artery be performed.

4 – the deep lateral compartment, delimited by the UGF in the anterior plane and the parietal peritoneum in the posterior plane, i.e., "the new Bogros space".

Table 1: IPPS – Interparietoperitoneal Space Compartments

	Medial	Lateral
Superficial	Retzius prevesical space	Old Bogros space
Deep	Prevesical space	New Bogros space

From this perspective, the structure described by Bogros as “a loose layer separates the inferior epigastric artery from the transversalis fascia" is now understood to be the FT's posterior layer, as described by Cooper. The structure described as "a thicker layer" is identified today as the UGF, having as well a bilaminated structure [10].

Anatomical descriptions of the IPP are sometimes confusing because the posterior layer of FT is often described as fused with or identical to the anterior layer of the UGF [11].

The two extensions of the UGF – the spermatic fascia and the umbilical-prevesical fascia have a connection/continuity area at the level of the umbilical artery origin point in the internal iliac artery located in front of the sacroiliac joint. This could correspond to the peritoneal fold visible during TAPP exploration and is described as a "transverse vesicular fold" [12].

From the laparo-endoscopic perspective of inguinal hernia, the Retzius space was located anterior to the UGF, and the new Bogros space was posterior to it (Figure 2).

Figure 2: IPPS Diagram

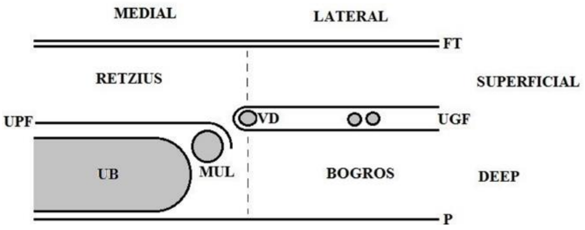

In the TAPP approach of indirect inguinal hernia, medial dissection is therefore conducted anterior to the UGF, the mark being the MUL located posterior to it. In TEP, one arrives from the retromuscular space in the avascular Retzius space penetrating the posterior rectus sheath [13].

Wandering medial to the posterior space of the UGF leads to bleeding from perivesical venous plexus or even to opening the bladder!

Regarding the creation of the new Bogros space during the laparo-endoscopic approach, our own experience has shown that the most suitable place to initiate the lateral compartment dissection is lateral to the deep inguinal opening, advancing towards the iliac spine in strict contact with the parietal peritoneum. If the dissection is started from the iliac spine, it most often determines the sectioning of the entire peritoneo-fascial cover, revealing the muscular fibers of the transversus abdominis and a variable degree of bleeding from the muscular branches. As one descends towards the ilio-pubic tract, the risk of bleeding from "the Bendavid venous circle" is added [14]. Therefore, lateral compartment dissection should aim strictly for the space between the peritoneum and UGF.

There are authors [15] with descriptions of the dissection plane in the two compartments (medial and lateral) similar to ours, but who use the term "preperitoneal tissue" or "areolar tissue" to denote what we consider to be the UGF or its prolongations.

In a sagittal section, the two spaces, Retzius and Bogros, are located anterior and posterior to the UGF, hence the confusion of some authors who speak, in general, of IPPS and properitoneal space without specifying the differences between the two compartments, the medial and the lateral [16].

Laparoscopic anatomy data of the IPP are confirmed imagistically in various pathological entities involving this region [17-22]. Osteoporosis of different etiology, especially in obese people has multiple anatomical implications for this region through fractures and defective post-healing remodeling, so the surgical approach to this region must be correlated with the condition of the patient [23]. An interesting area of future research is the possibility that the miscarriages occurring in the context of a uterine septum may be explained by the poor vascularization of fibromuscular tissue composing the septum and the approach of the IPPS from the inguinal region can treat the problem with less invasive surgical procedures [24]. Therefore, the prosthesis placed via TAPP or TEP in the Retzius and Bogros spaces is located anterior and posterior to the UGF [25].

DISCUSSION

- The IPPS from the inguinal region is an area as complex as it is narrow, but of great current surgical interest (approaches of hernias, vessels, prostate).
- The fascial distribution has its origin in the embryological development of the urogenital apparatus.
- The basic fascial structure in understanding IPPS compartmentalization is the UGF, along with its extensions.
- Starting from the requirement of reuniting the anatomical terms in this matter we suggest the following equivalents:

- preperitoneal/properitoneal space = interparietoperitoneal space (IPPS)
- old Retzius space= is the space between the UB and FT/pubic branch
- new Retzius space = the space between the UGF and FT/ pubic branch
- old Bogros space = the space between FT and the UGF
- new Bogros space = the space between the UGF and the parietal peritoneum
- UGF = preperitoneal fascia = retroperitoneal fascia.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

DA drafted the manuscript and critically revised the final form, AB researched the literature, RDSB researched the literature and revised the initial form, OA is the corresponding author, AMO drafted the manuscript, AP critically revised the data from the literature, MCD researched the literature, DNP approved the final form. All authors have contributed equally to writing, reading, and approving the final manuscript.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests

Abbreviations

FT: Transversalis Fascia; UGF: Urogenital fascia; P: Peritoneum; UPF: umbilical prevesical fascia; MUL: Medial umbilical ligament; VD: Vas Deferens; UB: Urinary bladder

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ORIGINAL ARTICLE

Climate change and public health – past, present, future

Viorel Ordeanu[1,2], Lucia E. Ionescu[1]

Abstract: *The evolution of the planet's climate is multifactorial influenced and has a dynamic over time. Many scientists have approached this controversial field, and each relies on objective but divergent statistics.*

Geographers have established that planet Earth is in a period of slight slow cooling, not at the level of the four prehistoric ice ages, but only at the level of the Little Ice Age from the Middle Ages which probably caused the great migration of peoples from Asia to Europe. This slow cooling is only slowed down and may even be partially reversed by anthropogenic activities. The industry produces large amounts of carbon dioxide from burning fossil fuels and other greenhouse gases. Animals produce carbon dioxide through respiration and digestion, some also methane. The waste degradation, fires, volcanic eruptions, swamps, and thawing of permafrost release carbon dioxide, methane, and other greenhouse gases into the atmosphere.

Climate change exit and has always existed, but there is no scientific evidence for global warming or for climate risks other than those we already know. Public health is already facing danger, directly and indirectly, for multiple reasons, to which „climate change" is added.

Keywords: *global warming, climate change, public health, greenhouse effect, ecology, climate warfare*

INTRODUCTION

Towards the end of the twentieth century, the written and audiovisual media bombarded us with alarmist information about „global warming", but this did not come true. At the beginning of this century, the same media informs us about „climate change", the shortcomings it creates and the risks we will be exposed to. But as American writer and journalist Mark Twain humorously wrote, „If you don't read the newspaper, you're uninformed. If you read the newspaper, you're misinformed"

Is the climate changing?

It is known that the evolution of the climate on the planet is multifactorial influenced and has a dynamic over time, from one geological era to another, from one age to another, from one geographical region to another, from one year to another, according to the season, the circadian cycle and even from one moment to the next. Many scholars have approached this controversial field, some for, some against or simply trying to be impartial. Each relied on objective statistics, those mathematical tools about which the Germans say only half-jokingly: „Statistics is a science that can prove anything, absolutely anything...".

In our country, the geographer Prof. Elena Teodoreanu, who was also a scientific researcher in medical geography, tried to answer this question, systematizing the knowledge in the field in her book entitled „Is the climate changing? A question at the beginning of the millennium" [1]. She stated: „It is strange how a simple look at the climates of the Middle Ages shows us the existence of calamities in past centuries as well – droughts, floods, destructive frosts – and yet, nowadays, a certain state of unrest about climate anomalies, is made felt in almost all countries. It is obvious that the possibilities of information today are far superior to those of the historical past, which allows knowledge in the shortest time, of events of all kinds around the globe and especially of those that attract attention, disturbance, fear."

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Other scientists, some of our own, have asked the same question. Thus, the physicist Ştefan Hepites, who founded in 1884 the Romanian Meteorological Institute, published in 1898 the article „Has the climate changed?” in which it is well documented that it has not. Another head of this institution, the meteorologist Nicolae Topor, published in 1967 the article „Is the climate changing?” in which he demonstrates that the climate does not change and concludes that: „what we are commenting on today are aspects of the same climate, which can change only partially in millennia, and only after millions of years.”

CLIMATE WARFARE

The great catastrophes in Earth's history have not caused human casualties, because humans had not yet appeared on the planet. But the small local catastrophes that ensued left millions or even billions of human casualties in total: dead, wounded, sick, and refugees. In addition, man has created a series of anthropic or even artificial catastrophes: wars, revolutions, attacks, collective or mass accidents, changes in the environment, and much more...

At the time, Walter Orr Roberts, president of the American Association for the Advancement of Science, said: „We aim to put the entire globe under constant observation of the weather until 1970 with reasonable expenses. We assume that we will be able to obtain incomparably better forecasts on storms, frosts, droughts, and smog and we will be able to avoid disasters. But behind these advantages that science brings us lies the danger of a potentially terrible weapon of war - the deliberate manipulation of the weather in favor of a small number of people in power, to the detriment of the enemy and perhaps those who stand aside.” [1]. This conclusion does nothing but reminds us of the threats made by the heads of the Great Powers, Stalin and Bush jr., summarized in the expression: „He who is not with us is against us.”

In 2004, the French writer Jouzel Debroise published the book „Climate – a dangerous game”. The following year, the „Final Report, Air University of the US Air Force, AF 2025”, is published, stating: „Climate change will become part of domestic and international security and can be done unilaterally. The ability to generate rain, fog, hurricanes and to change or produce certain weather conditions is part of the same set of technologies that can weaken the opponent and allow access to domination, wealth and power.” [2]

It is estimated that the enemy's combat actions, logistics, and transport may be hampered, food crises may be created to starvation, the outbreak and spread of natural or biological-induced epidemics may be favored, and medical countermeasures may no longer act effectively. It seems that the United States and Russia already have the capacity for climate warfare, and politicians are interfering in climate research. In 2009, General Emil Străinu published the book „Geophysical Warfare. Techniques for modifying the environment for military purposes” [3] which draws attention to the risk following attempts to change the weather and presents some situations of alleged climate attack on some countries, including Romania.

Geophysical warfare, which also includes climate change or climate catastrophes, would also be a major challenge for military medicine and public health in general. New problems of pathology and epidemiology, diagnosis, treatment and prophylaxis, health logistics would appear, specialized forces and means would be needed, and in their absence, the situation could quickly degenerate into a biological crisis.

THE PHENOMENON OF „GLOBAL WARMING”

Although the natural change of the climate is imperceptible and the anthropic change is very discreet, the observation of seemingly frequent catastrophic consequences has launched the hypothesis of global warming, which should be stopped. It seems that initially, the fight against „global warming” was an attempt initiated by the US to slow down the boom of the Chinese R.P. economy, whose energy was based on coal. The Chinese have large deposits of coal, but no oil and gas, which they are currently looking for in the South China Sea. However, in the meantime, China has become the world's second-largest economic power, and the expansion of the fight against carbon dioxide (greenhouse gas) produced by burning fossil fuels (including oil and gas) is also hitting the US economy, largest producer and consumer of energy. As a result, Donald Trump, as US president, no longer supported scientific research on „global warming” and the United States withdrew from international organizations that oppose this controversial phenomenon. But at the same time, NASA publishes alarming studies showing that global warming tends to become irreversible, with serious consequences, including Arctic ice melting, rising sea levels, and flooding areas, with the cities of London, New York, Sydney, etc. [4]. The areas populated by „natives” did not get the attention of these world science leaders.

If we think about the planet's distant past, the atmosphere was much different: more oxygen, much more carbon dioxide than now, excess water vapor and heat, all in one place favoring the giant Carboniferous plants and the giant Jurassic animals. But even today the richest fauna and microfauna, as well as the richest flora and microflora are found in the equatorial jungles. So we can think that the danger is not so much for the thriving biosphere as for the business world that will have to adapt to the new conditions.

Due to the lack of scientific evidence on global warming (it can neither be clearly shown that it exists nor that it does not) another paradigm has been used: climate change. For now, specialists in the field are reluctant and do not express firm opinions, whatever is possible, in the near or distant future.

WHAT IS REALLY HAPPENING?

Geographers have established that planet Earth is in a period of slight slow cooling, not at the level of the four prehistoric ice ages, but only at the level of the Little Ice Age from the Middle Ages (when, in one winter, the Nile froze) which probably caused the great migration of peoples from Asia to Europe. This slow cooling is only slowed down and may even be partially reversed by anthropogenic activities. The industry produces large amounts of carbon dioxide from burning fossil fuels, as well as other greenhouse gases. Animals produce carbon dioxide through respiration and digestion, some also methane. The waste degradation, fires, volcanic eruptions, swamps, and thawing of permafrost release carbon dioxide, methane, and other greenhouse gases into the atmosphere. All this contributes to slight heating, especially in the most industrialized or zoo-intensive areas.

Meteorologists' measurements are very accurate, their thermometers measure air and water temperatures by tenths of a degree Celsius, and the records and reports are very rigorous, at least in recent centuries. But the place of measurement, the location of the weather station, remained in place although the anthropic area (housing and industry) is constantly expanding and warming the environment. As an example, the first Romanian weather station was in Bucharest, on Filaret Hill, where the Astronomical Observatory was located. As the area became more and more populated, and the station was now in the city, a second weather station was set up, in Băneasa, outside the city, in the North-West direction, where the predominant wind comes from. So the weather in the city could be compared with the weather outside the city. In the beginning, the recorded temperature differences were about 2°C, but now they are on average about approx. 1°C, due to the expansion of urbanization in this area as well. Almost all weather stations are currently included in urban areas, so they report very accurately measured temperatures, but with systematic error in addition to the respective geographical area, an involuntary falsehood.

For some, the mild winters of recent years, through early February, would be evidence of „global warming”. But I could give some examples from personal memories: after the winter of 1971-1972, when the Black Sea froze on the shore and seaports including Mangalia were blocked (river ports are the ones blocked every year), the old sailors said they had never seen anything like it since they were young. But in the winter of 1973-1974, it was spring-like weather; on February 11th (my birthday) I went for a walk along the seafront wearing just a suit. And ten years ago, when my niece was born, on February 9th, 2009, I went to the maternity ward in a suit, in spring-like weather. But that hasn't stopped the Black Sea from freezing again in 2011 as well as in other, more recent years. All this means that the climate is neither heating nor cooling, but that it is only a random variation between certain limits, a statistical dynamic with a sawtooth pattern. And the slightly ascending „regression line” could just be the result of the systematic error I pointed out before, or it could be the „sawtooth” pattern of statistics applied to another time scale.

ASSUMPTIONS AND EVIDENCE

Many see as objective evidence the partial melting of mountain glaciers and polar caps, especially in the Arctic, and the rising of the worldwide ocean levels. Anyways, every summer the polar caps fragment on the edges and partially melt, and during the polar winter they grow back. The phenomenon is so repeatable that nautical charts mark the minimum and maximum limits of the polar ice caps. If the alarm is real, major potential hazards arise from this. If the mountain glaciers would melt completely (the most important being in the Himalayas), the rivers and streams in Asia will no longer be supplied with water and there will be a shortage of freshwater, which will affect billions of people. This phenomenon could lead to water wars involving billions of people.

If the polar caps melt in time, the level of the Planetary Ocean will increase by approx. 10 m due to the Arctic and approx. 80 m because of Antarctica. [5]. It is believed that if all the solidified water on the planet melted, the sea level could reach almost +100 m isobaths, and the land area would be reduced by about a third. The land surface will be reduced from almost 30% to less than 20% of the globe's surface. But these major changes, which would take thousands of years, are hypothetical, just some apocalyptic scenarios imagined by some journalists (who want the public's attention) and some politicians (who want to show that their country could save the planet) or by others as such, which are not seriously taken by scientists.

For now, we see that the partial melting of the Arctic polar cap allows ships from the far North to move, during the boreal summer, on the Arctic Ocean (Northern Frozen Ocean), both through the East Passage, between the Atlantic to the West and the Pacific to the East north of Europe and Asia, as well as through the Western Passage, between the Pacific to the West and the Atlantic to the East (North of Canada). These new sea routes shorten freight routes and favor the great maritime powers (USA, Russia, China, etc.). Unfortunately, Romania, which was the 11th maritime power in the world Knowledge Horizons – Economics, no longer has many ships, so it will not be able to benefit from this opportunity [6].

The thawing in Northern Canada, Greenland, and Siberia also facilitates mining, oil, gas, etc., thus favoring economic development. But this is where the serious problem arises. The thawing of the Siberian permafrost (swamps frozen for thousands of years or still frozen since the last glaciation) allows the slow emanation of methane in the soil (natural biogas) and it has a greenhouse effect [7]. The complex industrial pollution in the area of the North Polar Circle, in continuous development, reduces the snow's albedo, which instead of reflecting most of the solar radiation, absorbs it and melts rapidly.

At present, this phenomenon may be intentionally potentiated by humans. The US military's High-Frequency Active Auroral Research Program (HAARP) aims to bombard the ionosphere with strong radio waves, heat it, and reflect it to the ground, causing heat and devastating phenomena. If this biophysical weapon becomes operational, it may also be used for peaceful purposes, for the benefit of the Arctic industry but aggravating the phenomenon of melting ice. It may not be a coincidence that specific US facilities are located in Alaska, Greenland, and Norway, and the Russian ones in Siberia [8, 9].

At the same time, it is observed that on the contrary, the Antarctic cap is not reduced there is an increase in area and thickness. And the Great Powers are already disputing the division of Antarctica to begin mining for minerals under the ice cap. Political, economic, financial, and military forces, state and supranational, as well as large multinationals, are repositioning themselves for the new negotiations on the Law of the Sea, the polar caps, and the outer space. The multipolar world is complex, unpredictable, and dangerous, especially for the little ones. As the poet Gr. Alexandrescu said: „We want equality, but not for puppies”.

PUBLIC HEALTH CONSEQUENCES

Overall, the prosperity of a nation and its bio-psycho-social health are climate-dependent. The World Health Organization has defined the concept of human health as „physical, mental, intellectual and social well-being”, or all these are interdependent and multifactorial conditioned. Holistically speaking, everything around us and everything that happens to us is reflected, directly or indirectly, in public health, in the broadest sense [10].

It is considered that it would be possible that if the average temperature increased by 4°C, the level of the Planetary Ocean would increase by 9 m. The areas with the largest population and the most developed economy would be flooded; all beaches, all low plains, all ocean and sea ports, most islands, and even entire countries such as the Netherlands, Bangladesh, and others would disappear. This hypothetical global catastrophe would lead to a population exodus of billions of people, with poverty, epidemics, and wars [11]. In our country, too, major changes would occur: the Romanian coast, as we know it, would disappear and return to its former form, the Danube Delta will once again be a bay of the Black Sea, and the Danube would flow into the sea through a long estuary from Calarasi to Galati, and northern Dobrogea could be an island or a peninsula. In this unlikely situation, the land area of Romania would be reduced to only approx. 200,000 km2 but the water area would increase [12].

If this global catastrophe were to occur in the coming centuries, the consequences for public health would be particularly serious for all states and all nations. It is already believed that climate change, with warming in some regions, such as Europe, favors the emergence of exotic and tropical diseases, which although at the beginning, only „import diseases”, will spread and could become endemic diseases or epidemics in the temperate zone, so in populations that do not have natural protection against new pathogens. This creates a pandemic potential for exotic and tropical infectious diseases. Examples can be found permanently in the epidemiological communiqués of the World Health Organization (WHO), Center for Diseases Control, USA (CDC), European Center for Disease Control (ECDC), Ministries of Health (MoH), etc. and we will not insist on them. We know and sometimes we even face these diseases. But we will have to reconsider the entire public health monitoring system to deal, at least conceptually, with any present or future situation [13].

It should also be mentioned that if our Roman ancestors were afraid of the heat (heat wave – dog weather), we Romanians are afraid of the cold (dog weather), and jokers say that our Dacian ancestors, “the bravest of the Thracians”, were not afraid of anything, only of draught and that's why they walked around wearing little hats covering their ears both in winter and during summer. Anyway, the

Romanian expressions “to stay warm”, “tucked up nice and warm”, and “someplace warm” express the preferences of Romanians, unlike those of Americans for whom being “cool” is a good thing. Let's not forget that the Romanian greeting formula “salut” comes from the Latin greeting of health (salus), which, as the Romanian people say, "there is nothing more important than good health". And the Romanian rulers used to welcome their equals and subjects with the greeting: "May all those present be healthy!" a wish that we also address to our readers. The international importance of climatic change for public health shows over 35 000 scientific articles indexed on the Web of Science [14].

A very special issue in this context, and which can become a global threat to humanity, is posed by biological agents of war and those of bioterrorism. Biological warfare was formally banned, meaning „with proper documents”. But this is only a formality. The Convention on the Prohibition of Biological Weapons, launched by the United Nations (UN) in Geneva (BTWC 1972) follows other conventions that prohibited, implicitly or explicitly, the use of biological weapons. But there are still no real control mechanisms and biological agents have been used repeatedly for biological attacks. The convention was accepted by approx. 90% of the countries of the world (including all the states of the European Union), which seems to be a very good situation. But the remaining 10%, who have not signed or ratified the Convention, represent about 20 states with about a quarter of the world's population [15].

Moreover, the Great Powers do not allow international control over their territory or military bases. On the contrary, Iraq, which had biological weapons but also destroyed them, was accused of possessing biological weapons, was attacked and invaded, almost destroyed, but no biological weapons were confirmed. The foreign invasion troops (peacemaking) were replaced by occupation troops (peacekeeping) and did not leave completely even after 17 years. Currently, Iraq is considered by the international community as one of the "failed states" but no guilt has been established so far and the "international community" and "public opinion" have not been effectively involved in resolving this critical situation.

Biological warfare agents (BWA) are living microorganisms (bacteria and viruses) and toxins obtained from living organisms, which are disseminated with the help of biological weapons: aircraft or spray aerosolization, explosive munitions such as missiles, air bombs, and artillery shells, or diversionary agents. In a broad sense, the disease itself, usually infectious-contagious and/or zooanthroponotic caused by biological agents is considered a biological weapon. Officially, 7 bacterial species, 3 groups of viruses, and 5 toxins are listed. This list is exhaustive due to the characteristics required for effective military use. In contrast, bioterrorists can also use any other pathogens for humans, animals, and/or plants (bacteria, viruses, prions, fungi, parasites, etc.) to make people sick, reduce agricultural or animal production, to create food crises. It should be noted that biological warfare viruses are not listed as species, but only as groups, which means that in hemorrhagic fevers, for example, Ebola, Marburg, and many more are implicitly included. This will technically (not legally) allow the use of exotic and tropical viruses in areas affected by global warming and explain the medical-military interest of some states for medical intervention in these epidemics [16].

THE NEW EUROPEAN UNION POLICY FOR ENVIRONMENTAL PROTECTION

The current President of the European Commission (EC), a kind of Prime Minister of the Government of the European Union (EU), and the former German Minister of Defense (Bundeswehr), Ursula von der Leyen, is a doctor by profession. In 2019, at the moment of her election, she published the document “A Union that strives for more. My agenda for Europe” in which she stated that “for the generation of my children, Europe is the only aspiration” [17]. Of the six priority goals she has proposed for the next 5 years, the first is A European Green Deal (an ecological Europe) and she said “I want Europe to strive for more by being the first climate-neutral continent”, partly by 2030 and partly by 2050. This requires investment in innovation and research, redesigning the economy, updating industrial policy, and a new industrial strategy. Some complied and immediately found a "minimum method" of application, without investment: they take some extra money from drivers and other small polluters, and that's about all for now [18].

But the new ecological conception was already rooted in the world for many years. We pay more for electricity because we also use more expensive, unprofitable, but environmentally friendly technologies. Electric cars are in vogue, since 1905 exceeded the speed of 100 km/h, but they were not imposed for technical and cost reasons, but there are fears that they will become mandatory. For the emotional awareness of the public opinion, at the end of 2019, an ecological skit was broadcast, in which the filmmakers did not shy away from using an autistic teenager. Was that politically correct? And maybe some were wondering, „What's next?” This was followed on February 1st, 2020 by the World Economic Forum (WEF) in Davos (Switzerland), with the main theme of the year:

„Stakeholders for a Cohesive and Sustainable World” (united shareholders for a sustainable and cohesive world), under the slogan „How to Save the Planet”. 3,000 delegates from the largest private companies participated. Work has been conducted to prepare for the massive reorientation of global capital flows, through which financial giants, including the World Bank and the European Central Bank, are repositioning themselves to maximize their profits. As an example, only the promoter of this initiative, the BlackRock investment fund, has to invest over 7 trillion dollars (i.e. thousands of billions), more than the annual budget of most countries. The Davos Forum has been strongly involved in the Climate Change Agenda. Although it is acknowledged that it is impossible to answer the question: „when will climate risk have an impact on the planet's economy?”. It is considered that climate risk is also a risk for investments and that there will be a massive reallocation of capital through the Green Finance Initiative. Thousands of billions of dollars of investment funds will be channeled to „green” projects, so funding is not for everything and especially not for everyone. But in the meantime, the outbreak of the Covid-19 pandemic partially canceled the old plans, replacing them with the Great Reset of the world economy for the benefit of the Great Powers, the world's great finances, and multinationals. The US has an ambivalent position [19].

It seems that the „climate change” paradigm will be imposed to become the „new religion” of the 21st century, with a God whom, although we do not perceive, His priests are threatening us in His name. There is a risk that money, not work, will become the engine of progress and well-being, including the source of public health. But the investment will be directed at „rule of law” states that stand upright in the face of great global finance. However, we have the advantage that we are members of the European Union, we shall rise with it and, God forbid, we will fall with it [20].

In 2019 the „international community” says that the present situation is a „climate emergency” and in 2020 Roumanian Academy publish a „point of view” about climate change [21].

CONCLUSIONS

Climate change exists and has always existed, but there is no scientific evidence for global warming or for climate risks other than those we already know. But we find them better and faster, and the damage is greater because the population is larger and the human environment is more extensive, including in risk areas. Public health is already facing danger, directly and indirectly, for multiple reasons, to which "climate change" is added. It seems that the problem is mainly political, with economic and financial implications, and will be used as financial leverage at the global level, for global interests. For now, let's remain optimistic, but also prepared and vigilant.

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ORIGINAL ARTICLE

Comparison of rinsing with chlorhexidine and matrica mouthwashes on the ventilator-associated pneumonia

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Abstract: *Introduction: The present study aimed to compare the effect of two methods of rinsing with chlorhexidine Oral hygiene in patients undergoing endotracheal intubation is one of the most important tasks of nurses, and Matrica mouthwashes on the incidence of ventilator-induced pneumonia (VAP) in intensive care unit (ICU) patients.*

Materials and Methods: This is a clinical trial carried out on 80 patients undergoing oral endotracheal intubation. Patients were included in the study using simple sampling and randomly divided into chlorhexidine mouthwash and the matrica mouthwash groups. Data collection was carried out using a demographic information questionnaire and clinical pulmonary infection score (CPIS).

Results: The results also showed that most participants in the chamomile group (75%) did not have pneumonia, but the chlorhexidine group had pneumonia in 77.5% of cases. The Chi-square test showed a statistically significant difference between the groups in terms of the incidence and non-incidence of pneumonia (p = 0). Also, the odd ratio of developing pneumonia in the chamomile mouthwash to chlorhexidine mouthwash was 0.30%.

Conclusion: Considering the advantages of herbal compounds such as their low price and low risk, matrica can be considered a suitable alternative to chlorhexidine.

Keywords: *ventilator-associated pneumonia, mouthwash, chlorhexidine, matrica, intensive care*

INTRODUCTION

Pneumonia is the second most common nosocomial infection in ICU patients, affecting 27% of all patients. Nosocomial pneumonia develops in hospitalized patients for more than 48 hours and its incubation period is usually not more than 2 days. Among nosocomial pneumonia, VAP develops in ICU patients undergoing mechanical ventilation for more than 48 hours. Various studies reported VAP-related mortality between 0 and 50% [1].

Risk factors for VAP are divided into three categories; the first category included host-related factors such as old age, physical conditions, immobility, underlying disease, nutrition, and level of consciousness. The second category includes factors related to devices such as endotracheal tube and tracheostomy and intubation time, ventilator route, and gastric-nasal tubes. Finally, the third category includes factors related to employees such as poor hand hygiene and non-use of gloves [2].

Aspiration, intubation due to low level of consciousness or cardiorespiratory arrest, cardiopulmonary resuscitation, emergency intubation, and Glasgow Coma Scale (GCS)< 9 are important risk factors for VAP in the first 48 hours of intubation [3]. In intubated patients, lack of eating, reduced salivary flow, and spontaneous cleaning properties of the mouth and teeth lead to an increase in plaque accumulation [4] as well as the probability of inflammation [5,6].

Adherence to oral hygiene and care in ICU patients is one of the main tasks of health care providers and one of the basic

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aspects of ICU nursing care [7,8]. Dental plaque is a good place for the accumulation of microorganisms. These plaques can be a good reservoir for pathogenic microorganisms in patients with poor oral hygiene (such as patients undergoing endotracheal intubation).

Fourier et al. demonstrated a significant relationship between dental plaque microorganisms and tracheal secretions. The plaque provides the opportunity for germs and other microorganisms to adhere to tooth surfaces. Bacteria in dental plaque act as pathogens when aspirated into the lower airways [9], and can cause VAP [10].

Pittet et al. believe that plaques increase during the first 7 days of intubation [11]. Therefore, oral care is considered one of the most important ways to prevent dental plaque formation and microbial contamination of the mouth [12].

There are two methods to eliminate dental plaque and its associated germs: the mechanical method (brushing) and the medical method (including antibiotics). One of the most effective methods to control plaque is to use mouthwash. Chlorhexidine is an antiseptic that is effective against a wide range of bacteria, some fungi, and some viruses [13].

Two studies have shown the effect of chlorhexidine mouthwash on reducing respiratory infections in patients after heart surgery, but it didn't decrease the rate of VAP in other special departments of a hospital [14,15]. Although chlorhexidine has been recommended as the most effective plaque-killing agent in some studies [16], it is still not strongly recommended due to insufficient evidence for its effectiveness [17].

Various studies have investigated the effect of different chlorhexidine concentrations on the reduction of VAP, but the results have not been consistent [18]. Guideline from the Infectious Diseases Society of America also does not recommend routine use of chlorhexidine for VAP [4]. Besides, chlorhexidine has side effects that prevent its long-term use that incudes discoloration of teeth and mucous membranes, desquamation of oral mucosa, mouth irritation, stimulation of salivary gland stones (calculi), changes in taste, oral burning and dryness, peeling gums, and negative systemic effects on swallowing. Germ formation is also exacerbated by chlorhexidine due to the creation of an alkaline environment [12,19,20].

Therefore, to overcome the side effects of chemical drugs, World Health Organization (WHO) recommends research to find suitable natural substances such as herbal extracts [20]. For this reason, it is recommended to use non-chemical mouthwashes, including herbal mouthwashes [21]. Research has shown that the use of herbal mouthwashes such as chamomile has fewer side effects [22]. Chamomile extract has antibacterial, antiviral, and antifungal properties. The extract of this herb contains compounds that have strong antimicrobial properties against gram-positive and gram-negative bacteria [23].

Firoozian and Darvish Khezri (2015) showed that chamomile mouthwash not only reduces the number of oral bacteria colonies in healthy people but also affects the number of bacteria with different flora and higher resistance in patients undergoing mechanical ventilation than in healthy people [24]. VAP has been identified as a challenge for ICU personnel [25], therefore, prevention is a priority in ICUs [26].

Grap and Monro stated that oral hygiene and care as one of the most important components of nursing care should be taken into account [27]. Therefore, nurses who are in direct contact with the patient are expected to play an important role in preventing nosocomial infections, especially VAP [28]. Despite the high prevalence of VAP in ICU and the importance of mouthwash in its prevention, the prevalence of this dangerous complication has not been lower than expected despite the use of various solutions [26,29].

Also, considering the importance of using methods with minimal complications and risk that promote oral hygiene in the care of ICU patients, the widespread use of chlorhexidine mouthwash as a common solution in ICUs and due to its side effects and also the absence of these side effects in chamomile and instead its beneficial effects such as antibacterial, soothing and anti-inflammatory properties of this herb, it is recommended to use this herb if it is as effective as chlorhexidine in terms of antimicrobial performance.

It should be noted that antimicrobial effects of these mouthwashes on pathogens were investigated in vitro more frequently, and since there is a difference between ICU patients with outpatients in terms of the type of microorganisms, body resistance, and oral immunity, on the one hand, and despite the importance of preventing VAP in reducing mortality, reducing care costs, and reducing the length of stay [30], on the other hand, the present study aimed to compare the effect of two methods of rinsing with chlorhexidine and matrica (chamomile) mouthwashes on the incidence of VAP among patients admitted to ICU Department of Imam Reza Hospital in Birjand, Iran.

MATERIALS AND METHODS

This clinical trial was performed on ICU patients in Birjand in 2020 to compare the effect of rinsing with chlorhexidine and matrica mouthwashes on the incidence of VAP. The sample size was estimated at 36 people per group based on the formula $n = (Z_\alpha + Z_\beta) \times \left(\frac{(p_1+q_1)+(p_z+q_z)}{(p_1+q_1)^2}\right)$ and taking into account the result of Rakhshani et al.'s (31) study on the incidence of pneumonia before and after the intervention in chlorhexidine and matrica groups ($p_1 = 0.93$, $p_2 = 0.80$, $\alpha = 0.05$, and $\beta = 0.1$). The minimum sample size per group was increased to 40 people to increase the accuracy and power of the test, considering the special conditions of patients, and the duration of the study.

Inclusion criteria included obtaining the consent of the patient's guardian to participate in the intervention, patients aged over 18 years of age and under 65 years of age, no oral and maxillofacial injury, no cancer or diabetes and chronic obstructive pulmonary disease, no immunosuppression by drugs or specific diseases, intubation in the first 24 hours and patients who are expected to remain intubated for at least 72 hours, CPIS< 6 upon admission, GCS < 9, no antibiotics before and during hospitalization, natural teeth and having no more than 6 artificial teeth, no use of anticoagulants, no history of allergies to herbal compounds. Exclusion criteria also included death or transfer of the patient from the studied ward, removal of the endotracheal tube before the end of the study, the occurrence of overt aspiration during the study period, reintubation due to the removal of the endotracheal tube for any reason during the first 5 days, sensitivity to mouthwash, the patient's guardian stated unwillingness to continue the study.

In this study, 80 anesthetized eligible ICU patients were selected by convenience sampling method and assigned to one of the two groups of chlorhexidine mouthwash (n=40), chamomile mouthwash (n = 40) in the order of time of admission using simple random sampling and a random number table. Oral care and rinsing were performed using chlorhexidine mouthwash in one group of patients and chamomile mouthwash in the other group. The frequency, duration, and manner of using mouthwash were the same in both groups. At the baseline, informed consent was obtained from the patient's guardian or legal guardian, after explaining the study process and informing him/her. Then the demographic information, medical history, cause of hospitalization, and drugs used were recorded in the information list. All patients were evaluated using CPIS and the results were recorded in the relevant form.

This instrument consists of 6 subscales including:

1. Temperature (score 0 if temperature $36.5 \leq T \leq 38.4$, score 1 if temperature $38.5 \leq T \leq 38.9$, score 2 if temperature $T < 36.5$ or $39 \leq T$ 2 points)

2. White blood cell (WBC) count (score 0 if $4000 \leq WBC \leq 11000$, score 1 if WBC> 11000 or WBC <4000, score 2 if WBC> 1100 or WBC <4000)

3. Oxygenation (score 0 if 240 <or ARDS, score 2 if ≤ 240 or no ARDS)

4. Chest radiography (No infiltration (score 0), diffuse infiltration (score 1), local infiltration (score 2)

5. Tracheal secretion culture (pathogenic bacteria did not grow or there is a small amount of them (score 0), moderate to high growth of pathogenic bacteria (score 1), growth of pathogenic bacteria of the same type seen in gram staining (score 2)

6. Volume and appearance of tracheal secretions (absence of pulmonary secretions (score 0), presence of non-purulent pulmonary secretions (score 1), presence of purulent pulmonary secretions (score 2).

Each subscale is scored between 0 and 2, and the total score is 12. In this model, scores ≥ 6 at the baseline or after the entry of microbiological results were considered pneumonia.

Chlorhexidine gluconate mouthwash 0.2% (Behsa Pharmaceutical Co.) was used in the chlorhexidine group. In the chamomile group, chamomile solution (10% Matricaria Chamomila) (Adonis Gol Darou Co.) prepared from the flower of the plant was used according to the opinion of a counseling expert.

In both groups, the patients were placed in the supine position during each intervention, and if there is no prohibition, the head of the bed was raised by 30° and the face was placed to one side. Before performing oral care to ensure the suitability of occlusive endotracheal tube cuff pressure, it was set at 20-25 cmH_2O using a pressure manometer. The intervention was started using a soft pediatric toothbrush soaked with the desired mouthwash and all surfaces of the mouth including teeth and gums per quarter, tongue, inside of the cheeks, hard palate, and soft palate, and the endotracheal tube was brushed. Moreover, 2.5 cc chlorhexidine mouthwash 0.2 and 2.5 ccs chamomile mouthwash were used each time for 30 seconds on each of the aforementioned surfaces and a total mouthwash solution used was 20 cc (10 ccs in the first time and 10 ccs in the second time). The tongue was also brushed gently and away from the end of the tongue. For all patients in both groups, secretions were suctioned and performed at each stage of the intervention, if necessary, and at the end, the lips were cleaned and the mouth became dry. The endotracheal tube was removed and cleaned and placed in the patient's mouth again, the endotracheal tube strip was replaced and some Vaseline was swabbed to lubricate the mucosal surfaces and lips, and then the patient was returned to a comfortable previous state. It took a total of 6 minutes to perform the whole process of oral cleaning and rinsing in both stages. In addition, aseptic tips such as thorough and frequent hand washing and disinfection were observed by the researcher before, during, and after the intervention, and the use of examination gloves when rinsing the mouth and suctioning secretions.

Mouthwashes were used in both groups twice a day for 12 days. At the time interval between mouth-washing interventions, patients' mouth was rinsed with normal saline every 2-3 hours and was suctioned, if necessary. All patients were assessed on the fifth day after the intervention, based on the clinical lung infection assessment checklist that includes the following sections: 1. Temperature control with mercury thermometer (axillary temperature was measured in all patients due to intubation) 2. WBC was performed by CBC test. 3. Oxygenation which was measured by measuring arterial blood gases and pulse oximetry. 4. Chest radiograph was performed by a portable device and seen by an anesthesiologist. 5. Pulmonary secretions were cultured using sterile gloves and a special container by a researcher and another nurse to maintain the sterility of the procedure. 6. Examination of pulmonary secretions for presence or absence, purulence or non-purulence of endotracheal secretions.

The intervention duration was the same for patients in both groups and was performed by the researcher at an interval of 12 hours.

The collected data was entered into SPSS ver. 22 for data analysis. P-value <0.05 was considered the significance level. Independent t-test was used to compare the mean of quantitative variables between the two groups, such as age, and Chi-square and Fisher's exact test were also used to compare the frequency of qualitative variables between the two groups, such as comparing the mean frequency of positive endotracheal and oropharyngeal cultures.

RESULTS

The present study was performed on 80 ICU patients undergoing mechanical ventilation in chlorhexidine and chamomile groups (n= 40 people). There was no statistically significant difference between the two groups in terms of their mean age. The mean age was 39.85 ± 9.55 and 45.10 ± 9.29 years in the chlorhexidine and chamomile groups, respectively. There was also no statistically significant difference between the two groups in terms of the frequency distribution of sex, cause of hospitalization, marital status, place of residence, and insurance status (p> 0.05).

Regarding the prevalence of VAP, the results showed that most participants in the chamomile group (75%) did not have pneumonia and the chlorhexidine group suffered from this complication in 77.5% of cases. The Chi-square test showed statistically significant differences between the two groups in terms of the absence and presence of pneumonia (p = 0). Also, the odd ratio of developing pneumonia in the chamomile mouthwash to chlorhexidine mouthwash was 0.30%. In other words, the risk of pneumonia in the chamomile group was reduced by 30% compared to the chlorhexidine group, and chamomile reduced the risk of pneumonia by up to 70% (Table 1).

Table 1: Distribution of absolute frequency and frequency percentage of subjects in terms of presence and absence of pneumonia in chamomile and chlorhexidine groups

Pneumonia	Number(%)		Z score	p-value
	Cm	Ch		
Presence	10 (25%)	31 (77.5%)	<0.001	22.06
Absence	30 (75%)	9 (22.5%)		

Cm – Chamomile, Ch - Chlorhexidine

The most common microorganism in tracheal culture was Pseudomonas (12.5%) in the chamomile group and Enterobacter (27.5%) in the chlorhexidine group. There was also no microorganism in the tracheal culture of the chamomile group in 75% of cases. The Chi-square test showed statistically significant differences between the two groups in terms of the frequency of microorganisms (Table 2).

Table 2: Absolute frequency distribution and frequency percentage of research subjects in terms of microorganisms in chamomile and chlorhexidine groups

Microorganism	Number (n, %)		Z score	p-value
	Cm	Ch		
Enterobacter	2 (5)	11 (27.5)	26.29	0
Pseudomonas	5 (12.5)	6 (15)		
Klebsiella	0 (0)	7 (17.5)		
Aureus	1 (2.5)	2 (5)		
E-coli	1 (2.5)	3 (7.5)		
Acinetobacter	1 (2.5)	2 (5)		
Absence of microorganisms	30 (75)	9 (22.5)		

Cm – Chamomile, Ch - Chlorhexidine

DISCUSSION

The results of the present research show that chamomile mouthwash was more effective than chlorhexidine mouthwash in preventing VAP so that the CPIS was reduced significantly in patients of the two groups on the fifth day (p <0.001) and these differences were statistically significant.

In a study comparing the effects of chlorhexidine and tooth brushing on the prevention of VAP, Samani et al. found no significant difference between chlorhexidine and brushing or both of them in terms of incidence of VAP and they had no advantage over each other in reducing the incidence of pneumonia [32].

In a clinical trial on the effect of providing oral health services (including 0.12% chlorhexidine digluconate) with or without brushing on the incidence of VAP, Lorente et al. found no significant differences between the two groups in terms of incidence of VAP [33]. Khalifazadeh et al. also found that 0.2% chlorhexidine and swab in comparison with toothbrush and chlorhexidine solution had no significant effect on reducing the incidence of VAP in ICU patients [34]. The results of the study performed by Nideelman et al. showed that brushing 4 times a day with 0.2% chlorhexidine was not able to significantly reduce the incidence of VAP [35].

In a study, Salehi et al. reported that chlorhexidine was more effective on the number of oral bacterial colonies than persica and matrica, but matrica was more effective in reducing the number of bacterial colonies than persica [36].

In a study comparing the antifungal and antibacterial effects of persica, matrica, and Iral wax mouthwashes with chlorhexidine in vitro, Atai et al. showed that among herbal mouthwashes, matrica mouthwash had the strongest antimicrobial effects on Streptococcus sanguis, Streptococcus salivarius, Streptococcus sobrinus, Actinomycosis viscosus and Candida albicans [37].

In a study titled “Comparing the effect of chlorhexidine with chamomile and normal saline on oral bacterial colonization, Azimi et al. reported that chlorhexidine was more effective than both chamomile mouthwash and normal saline in reducing oral bacterial colonization, and also none of the mouthwashes was able to remove Staphylococcus aureus, Pseudomonas, Klebsiella, and Acinetobacter [38].

Another study aimed at determining the effect of brushing, chlorhexidine mouthwash, and brushing + and chlorhexidine on the incidence of VAP among patients undergoing mechanical ventilation showed that chlorhexidine reduced early-onset VAP. Brushing + chlorhexidine was not effective. However, chlorhexidine significantly reduced the incidence of pneumonia among patients with baseline CPIS< 6 on the third day, and brushing could not affect CPIS and increase the effect of chlorhexidine, which is not consistent with the results of the present study [26].

The researchers stated that the type of mouthwash solution is probably less important than using a toothbrush to clean the mouth, which has not been addressed in many studies. In the present study, in addition to mouthwash, toothbrushes were used for both groups of patients. Teeth were brushed using carefully using a soft children's toothbrush soaked in mouthwash. All surfaces of the mouth, gums, tongue, throat, and teeth were brushed. Moreover, all of the areas of the mouth, including both interior and exterior surfaces of the teeth (rotational movements), gums, and tongue (back and forth movements) were brushed. Brushing has a great effect on preventing plaque formation. In this regard, Estaji et al. conducted a study aimed at comparing the effect of chlorhexidine solution and toothbrush in the prevention of oral lesions of 30 ICU patients undergoing endotracheal intubation.

The results showed that toothbrushes and chlorhexidine are effective in the prevention of oral lesions, but a better improvement was observed in oral health in cases where toothbrushes have been used [39].

CONCLUSION

Considering the results of the present study and other relevant studies, we conclude that the use of toothbrushes with mouthwash may reduce the incidence of VAP to some extent directly or indirectly by acting on oral pathogens, however, it still cannot prevent its incidence. This may be because several factors are involved in the development of pneumonia in these patients. For example, there is a significant relationship between GCS< 9 and the incidence of pneumonia [14,40]. There are several other factors many risk factors for VAP in ICUs such as observing other aspects of care such as hand washing, open suction, patients with different immune systems, and the presence of resistant pathogens. Therefore, the limitation of the present study was the lack of control over all these aspects of care due to the limited time and small sample size.

Therefore, considering the advantages of herbal compounds over chemical compounds, the positive effect of chamomile as a mouthwash in critically ill or anesthetized patients can be considered a suitable alternative to chlorhexidine. However, there is a need to further studies on the effect of chamomile mouthwash, especially in the prevention of pneumonia among ventilator-dependent critically-ill patients.

According to the results of the present study, chamomile mouthwash was more effective than chlorhexidine mouthwash on the incidence of VAP in ICU patients and according to the benefits of herbal compounds, chamomile mouthwash can be considered a suitable alternative to chlorhexidine.

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Conflict of Interests:

There is no conflict of interest in the present study.

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ORIGINAL ARTICLE

Diagnosis of malignant neoplasms of the skin in servicemen of the Armed Forces of Ukraine

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Abstract: *Objective: Malignant neoplasms of the skin are diagnosed in high-risk groups, among which the military is special. The article is devoted to the analysis of diagnostics, and risk factors for malignant skin tumors in servicemen of the Armed Forces of Ukraine (AFU)*

Materials and methods: An analysis of 23 cases of diagnosed malignant neoplasms of the skin in servicemen of AFU for the period 1999-2021. The study included cases in which the diagnosis was confirmed histologically in two laboratories. Clinical, laboratory, and histological data of each case were analyzed, and further analysis took into account the length of military service and the risks of professional activity.

Results: Among the 23 cases, the majority - 22 were servicemen, and only 1 case of basal cell carcinoma in a 52-year-old employee. Among other servicemen, the age range was 25-55 years, with a mean age of diagnosis of 42 years. The period of military service at the time of diagnosis was 23.7 years, most cases were melanoma (15 of 23 cases), and in other cases - basal cell carcinoma. Patients were dominated by Air Force personnel (10 out of 23 cases)

Conclusion: The prevalence of malignant skin neoplasms in the Armed Forces corresponds to global trends. A group of increased risk of developing malignant skin neoplasms is servicemen of the Armed Forces of Ukraine with a long service life (more than 20 years), older than 40 years. However, there is a high probability of developing melanoma of the skin in servicemen of shorter service life, which requires enhanced dermatological supervision in servicemen of any age and service life.

Keywords: *malignant neoplasms of the skin, servicemen, armed forces of Ukraine*

INTRODUCTION

Malignant neoplasms of the skin are diagnosed in many age, national and occupational groups, due to numerous risk factors studied in great detail by dermatologists. Among them are insolation, contact with carcinogens, genetic factors, and many others [1]. However, among professional groups, the high-risk group – the military – attracts attention, which clearly demonstrates the acquired nature of the development of skin cancer [2]. After all, as a standard, at enlistment in military service, people with potential risk factors and precancerous diseases are eliminated and development in the future of such new growths is caused by features of professional activity.

It is proved [3] increased risk of malignant neoplasms of the skin and servicemen who had increased contact with ultraviolet radiation, and this trend has been observed over the past 100 years in participants of World War I and II, the Vietnam War, and other armed conflicts of the 20th century [4]. Among the military, two groups are known to have high levels of skin cancer: Caucasians and men over 50 [5]. Among the military, the Air Force has the highest rate of both melanoma and non-melanoma skin cancer [6]. Accordingly, officers, especially senior officers, have an increased incidence of melanoma and non-melanoma skin cancer compared to non-military personnel [7]. The significant role of ultraviolet radiation is indicated by the fact that pilots have the highest risk of developing malignant skin tumors among other groups of servicemen [8]. It is noted that the levels of ultraviolet radiation increase to 24 percent with each increase in altitude by 1000 meters [9]. The publications on the diagnosis and manifestations of malignant skin neoplasms are mainly based on data from the

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US or other NATO countries [2-9], we have not found publications on the analysis of diagnosis, risk factors for malignant skin tumors in the Armed Forces of Ukraine (AFU). Based on this, we set a goal – to analyze the features of diagnosis and manifestations of malignant neoplasms of the skin in the Armed Forces.

MATERIALS AND METHODS

We analyzed 23 cases of diagnosed malignant skin neoplasms in the Armed Forces of Ukraine for the period 1999-to 2021.

The data were obtained from the reports of surgeons and dermatologists in the hospitals of the Armed Forces of Ukraine. The study included cases in which the diagnosis was confirmed histologically in two laboratories. Clinical, laboratory, and histological data of each case were analyzed, and further analysis took into account the length of military service and the risks of professional activity. The study did not include suspected cases of malignant neoplasms not confirmed by repeated histological examination.

RESULTS

Among the 23 cases, the majority - 22 were servicemen and only 1 case of basal cell carcinoma in a 52-year-old employee.

Among other servicemen, the age range was 25-55 years, with a mean age of diagnosis of 42 years. There were 13 and 9 officers and sergeants, respectively, and servicemen, which does not allow to define of this indicator as a risk factor. The term of military service turned out to be a more predictable risk factor, as it amounted to 23.7 years, which practically corresponds to the average term of service of a serviceman of the Armed Forces. However, we note the presence of three cases of diagnosis of malignant neoplasms that occurred after 1-3 years of military service. These were cases of solid basal cell carcinoma in a 37-year-old soldier, a case of anonymous melanoma in a 25-year-old soldier, which was detected in the stage of widespread metastasis after 2 years of service, and primary multiple melanomas in a 44-year-old soldier, which was detected in 3 years of service. All these cases required radical treatment and led to dismissal with subsequent disability of patients.

Officers were dominated by skin melanoma in 9 of 13 cases, which was detected after 20 years of service and did not require urgent radical treatment, and in 5 cases did not lead to dismissal.

Further analysis of nosology and its prevalence revealed that most cases were melanoma (15 of 23 cases), in other cases – basal cell carcinoma. Other risk factors were predominantly in the Air Force (10 out of 23 cases), consistent with other studies on the increased risk of developing skin neoplasms in military personnel involved in flight missions and a significant increase in ultraviolet radiation.

DISCUSSION

Due to the prevalence of malignant skin tumors among APU servicemen relative to other population groups, it is difficult to make an adequate comparison. According to our data, the studied population of sick servicemen was in the age range of 25-55 years and mostly male, according to data for 2020 the incidence of melanoma in men was 7.4 per 100 thousand population, and non-melanoma malignancies 38.2 per 100 thousand population [10]. However, no data have been found on age, and it should be borne in mind that servicemen are a group of enhanced surveillance, in which early diagnosis is present and potential risk groups are excluded. In our opinion, it is not correct to compare the civilian population and the military.

In our opinion, it is the consideration of the peculiarities of the factors of supervision and closes medical examination of servicemen that should be taken into account when comparing the data on the incidence of malignant skin neoplasms in this category of the population and other populations. In particular, there are data [2] on the absence of a difference in the incidence of melanoma between the general population of the United States and the US Navy or Air Force, and even on reduced age-adjusted rates compared to civilians [5].

However, there is evidence of an increased risk of melanoma in US servicemen over the age of 45 [4], which is consistent with our data on a significant percentage of patients in this age group. The predominance of Air Force patients among patients is in line with the global trend and indicates the need for more careful supervision in this group of servicemen. Also noteworthy is the presence of a group of patients with relatively short service life in the Armed Forces and young people, who were diagnosed with malignant skin tumors with signs of disease progression, which is not a standard situation in this age group, but described the development of melanoma in 27-year-old US serviceman [11], which indicates the need for close medical examination of young servicemen and short service life.

CONCLUSIONS

The prevalence of malignant skin neoplasms in the Armed Forces corresponds to global trends. A group of increased risk of developing malignant skin neoplasms is servicemen of the Armed Forces of Ukraine with a long service life (more than 20 years), older than 40 years. However, there is a high probability of developing melanoma of the skin in servicemen of shorter service life, which requires enhanced dermatological supervision in servicemen of any age and service life.

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ORIGINAL ARTICLE

Effect of different physical stressors on soldier's psychophysiological response and marksmanship

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Abstract: *Shooting impairment may occur whereas there is either central or peripheral fatigue. However, the effects of different physical stressor stimuli on fine motor skills as shooting are yet not fully understood. The present research aimed to analyze the effect of endurance and resisted physical stressors on the psychophysiological response and pistol marksmanship of novel soldiers, and the possible differences in gender and BMI. Variables of heart rate, isometric hand strength, rate of perceived exertion, and pistol marksmanship were analyzed in 56 soldiers in endurance and resisted stress protocols. Isometric hand strength, heart rate, and RPE were significantly higher in both resisted and endurance physical stress protocols than in the basal sample. Gender differences were seen in strength, cardiovascular and RPE values. Also, subjects with larger BMI presented significantly higher isometric hand strength and higher marksmanship in all moments evaluated. We conclude that an endurance stress protocol produced a higher cardiovascular and perceived exertion than a resisted one, not affecting hand strength, shooting heart rate, and marksmanship. Females presented lower hand strength and marksmanship while higher rated of perceived exertion and heart rate during the endurance and resisted stress protocols than males. Overweight participants presented higher heart rates during both physical stress protocols, but higher hand strength and marksmanship while similar shooting heart rates than normal-weight participants.*

Keywords: *marksmanship; stress; heart rate; fine motor skills; fatigue*

INTRODUCTION

The effect of different stressor stimuli on fine motor skills is an area with wide applications, yet not highly developed. The stress effects may differ regarding the characteristics of the stressor stimuli. In the case of physical exercise, the intensity, duration, and type of exercise have a direct impact either on fine or gross motor skills [1].

Authors found how after a bout of high-intensity, lower-body resistance training passing accuracy was decreased [2]; after cycling in an ergometer at 100% of maximal capacity, significant attenuation on reaction times was found, like at lower intensities (70%) [3], suggesting an inverted-U function between exercise intensity and motor control.

Specifically, in combat, previous researchers found no effect on marksmanship despite the increased combat stress, neither with rifles and pistols independently of the caffeine ingestion [4,5]. The physiological mechanisms underlying the decrease of performance on fine motor skills may be related to i. the accumulation of hydrogen ions, which affect glycolytic enzymes due to low Ph, impairing ATP production and compromising the contractile capacity of the muscle [6]; ii. the high inorganic phosphate concentration, which inhibits Ca ++ reuptake increasing its concentration in the sarcoplasm and hindering muscle contraction; and iii. the ammonia concentration, which is related to the affection of neurobiological processes, affecting the quality of neural connections and electrical activity of the muscle [7].

In addition, all these acute metabolic responses are directly modulated by the intensity and type of exercise [8]. Related to shooting accuracy and marksmanship, previous authors showed that high-intensity exercise affected central aspects

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of shooting performance, shooting accuracy, shooting precision, and stability of hold, being accentuated when shooting in a standing position, compared with shooting in a prone position [9]. Acute protocols designed to fatigue the upper body flexor muscle groups (muscles involved in stabilizing and supporting a firearm), such as lifting, climbing, and pulling, presented significant decreases in rapid-fire rifle shooting performance in the standing position in soldiers immediately after these exercises [10]. In this line, independently of the type of exercise (concentric, eccentric, or a combination of both) or the location (knee, ankle, or entire lower extremity), acute fatigue may induce a loss of stability[11]. This fact, directly affects postural, standing, and gun stability, key factors connected to rifle shooting accuracy 12 since body sway and imbalance has been directly correlated with poor shooting score[13,14]. Superior shooting performance requires good postural balance, as well as, an ability to steady the gun while aiming[12]. However, the effect of different acute stress exercise-induced peripheral fatigue on fine motor skills such as shooting is yet not well documented. Most of the studies have focused either on soldiers or experienced subjects, showing how lower fitness levels, experience, and training increased fatigue and decrease marksmanship [15,16]. In addition, previous studies were the focus on the rifle, showing lower research on pistols, leading to greater muscular fatigue due to the farther center of mass to the shooter's body and the increase in joint angle [17]. Thus, we proposed the present research to analyze the effect of endurance and resisted physical stressors on the psychophysiological response and pistol marksmanship of non-experienced shooters. As a second aim, we aimed to analyze differences in gender and weight. We hypothesized that i. resisted physical stressor would produce a higher psychophysiological stress response, decreasing the marksmanship more than the endurance exercise stressor; ii. Subjects with higher body mass index (BMI) would present higher psychophysiological response and lower marksmanship; iii. There would be gender differences in the psychophysiological stress response and marksmanship.

MATERIALS AND METHODS

Participants

A total of 56 soldiers belonging to light military forces (23.6±3.7 years; 175.8±9.1 cm; 71.3±8.7kg; 23.1±2.2 BMI) were analyzed. Inclusion Criteria were: Professional Experience in the army. Exclusion Criteria for were: Presence of any medical condition or injury that would be a limiting factor for intense physical activity and the use of firearms (e.g. cardiovascular diseases) at the moment or during the protocol, intake of any dietary supplement, stimulants, or other ergogenic aids. Participants were informed of the characteristics of the study and the potential risks derived from those who were exposed. All the procedures were conducted following the Helsinki Declaration (as revised in Brazil, 2013), all the participants filled out an informed consent form previous to starting the research, and all the procedures were approved by the University Ethic Committee.

Methods

Procedures

Before the research started all the subjects received instructions/familiarization regarding the protocol and tests. Then 3 days of evaluation were distributed as follows: 1º Day: basal sample of all the variables measured in the study; 2º Day: 48h after, subjects performed randomly an endurance or resisted physical stress protocol; 3º Day: 48h after, subjects performed the remaining protocol.

For both physical stress protocols, participants conducted a warm-up composed by:

i) 2 min of joint mobility

ii)5 min of light aerobic running (50-60% Maximum Heart Rate)

iii) 2 series of 20 m of progressive running intensity

After the warm-up, participants performed the physical stress protocol, which was supervised and encouraged by sports science professionals:

Resisted Physical Stress Protocol

- 20 sets of burpees at maximum intensity

Endurance Physical Stress Protocol

- 5 min maximal running test in a track field.

Before the research, body weight was assessed with a SECA scale model 714 with a precision of 100 grams (range 0.1-130 Kg), located on a flat and smooth surface and calibrated at zero. Subjects were barefoot and with minimal clothes. Once located in the center of the platform, they remained without their body being in contact with surrounding objects, with the weight evenly distributed on both feet facing forward. Height, with a SECA scale model 714 with a precision of 0.1 mm (range 60-200 cm). The subject stood up, barefoot with the head oriented in the Frankfurt plane. Arms on both sides of the trunk and fully extended with palms touching the external face of the thighs, heels together touching the lower end of the vertical surface with the inner edge of the feet, occipital area, scapular, buttocks, posterior face of the knees and calves touching the vertical surface of the anthropometer.

Before and after the exercise stress protocols, we measured:

- Rating of perceived exertion (RPE), 6–20 scale.

- Isometric hand-grip strength using a TKK 5402 dynamometer (Takei Scientific Instruments CO. LTD). Each participant's grip strength was measured on the shooting hand. Participants were standing with 0 degrees of shoulder flexion, 90 degrees of elbow flexion, and the forearm in neutral. Idem, was done pre-post and among series.

- Heart Rate (HR) was measured before and during the entire protocol by a Polar V800 HR monitor (Kempele, Finland).

- Marksmanship was measured by the sum of three airsoft pistol shots to a target at a distance of 7 m, following procedures of previous research [4,5]. A gas blowback Beretta 92X Centurion airsoft gun (450 fps) was used.

Statistical analyses

To analyze the data, we used the SPSS statistical package (version 24.0; SPSS, Inc., Chicago, Ill.). Means and standard deviation (SD) were calculated using traditional statistical techniques. Normality and homoscedasticity assumptions were checked with a Kolmogorov-Smirnov test. To analyze differences between pre and post a T-test was administered, and to analyze differences between experimental groups (gender and BMI) and differences between moments of evaluation, an ANOVA test was conducted since variables presented a parametric distribution.

RESULTS

The results are reported as mean±SD. Isometric hand strength, HR, and RPE were significantly higher in both Resisted and Endurance physical stress protocols than in basal samples (Table 1).

According to gender differences, males present significantly higher isometric hand strength at all moments evaluated. HR at shooting at Basal and Endurance physical stress protocol of females was statistically higher than males. Female RPE was higher than males at Resisted and Endurance physical stress protocols (Table 2) (between parenthesis significant differences (p≤0.05) between males vs females).

Subjects with larger BMI (BMI ≥25 overweight) presented significantly higher isometric hand strength at Basal and Resisted physical stress protocol than subjects with lower BMI (BMI<25). Subjects with larger BMI also presented higher marksmanship in all moments evaluated (Table 3) (between parenthesis significant differences (p≤0.05) between BMI<25 vs BMI≥25).

Table 1: Psychophysiological parameters analyzed before and after the physical stress protocols

	Basal	Resistance Training	Endurance Training	F	P	η2	Moment comparison
Isometric Hand Strength	42.8 ± 8.4	44.9 ± 6.4	42.7 ± 2.1	8.481	.001	.239	2>1
Marksmanship	12.8 ± 9.5	14.5 ± 10.6	15.2 ± 10.1	1.844	.168	.068	-
Heart rate during protocol	-	166.1 ± 13.3	178.9 ± 11.7	68.156	.013	.711	3>2
Shooting Heart Rate	90.3 ± 17.5	125.2 ± 19.0	124.3 ± 17.6	50.122	.000	.650	2>1; 3>1
Rated of Perceived Exertion	6.6 ± 1.1	15.2 ± 2.0	16.8 ± 1.8	739.846	.000	.965	3>2>1; 3>1

Table 2: Psychophysiological gender differences of the parameters analyzed before and after the physical stress protocols

		Basal	Resistance based exercise	Endurance based exercise	F	P	η2	Moment comparison
Male	Isometric Hand Strength	44.8±7.8	46.3±6.2	43.9±9.5	3.973	.026	.153	2>1
	Marksmanship	13.6±9.5	16.0±10.8	15.7±10.7	1.940	.156	.081	-
	Heart rate during protocol	-	165.8±9.2	178.9±6.1	71.012	.021	.781	3>2
	Shooting Heart Rate	87.7±17.0	128.4±16.0	121.6±16.5	65.007	.000	.747	2>1;2>3;3>1
	Rated of Perceived Exertion	6.6±1.1	14.9±2.0	16.5±1.8	604.148	.000	.965	3>2>1; 3>1
Female	Isometric Hand Strength	33.4±2.4 (.000)	38.2±1.0 (.000)	36.9±2.1 (.025)	53.399	.000	.930	3>1; 2>1
	Marksmanship	9.2±9.1 (.011)	7.4±5.5 (.018)	13.2±7.3 (.024)	2.533	.141	.388	-
	Heart rate during Protocol	-	174.4±8.2 (.011)	191.2±7.1 (.006)	71.231	.000	.961	3>2
	Shooting Heart Rate	102.6±15.0 (.014)	113.6±27.1	136.6±17.9 (.014)	69.514	.000	.946	3>1; 3>2
	Rated of Perceived Exertion	6.6±1.2	16.2±1.8 (.009)	18.2±1.0 (.007)	392.302	.000	.990	3>1; 2>1

Table 3: Psychophysiological body mass index differences of the parameters analyzed before and after the physical stress protocols

		Basal	Resistance based exercise	Endurance based exercise	F	P	η2	Moment comparison
BMI <25	Isometric Hand Strength	41.7±3.5	43.7±7.0	42.9±7.7	1.197	.318	.238	-
	Marksmanship	11.4±9.7	12.4±10.6	12.5±8.8	.380	.686	.019	-
	Heart rate during protocol	-	163±8.5	178±7.2	67.145	.032	.631	3>2
	Shooting Heart Rate	91.8±15.2	125.9±14.7	126.8±17.2	28.322	.000	.685	1<2; 1<3
	Rated of Perceived Exertion	6.5±1.0	14.7±1.9	16.8±1.5	910.743	.000	.979	1<2; 1<3; 2<3
BMI ≥25	Isometric Hand Strength	44.5±2.8 (.019)	48.5±5.5 (.011)	39.7±12.9	22.960	.000	.793	1<2
	Marksmanship	16.8±7.8 (.001)	20.8±7.7 (.009)	23.4±9.8 (.000)	2.169	.157	.266	-
	Heart rate during Protocol	-	171±9.7 (.031)	188±9.1 (.021)	79.256	.038	.720	3>2
	Shooting Heart Rate	92.7±23.5	122.2±24.3	118.4±16.9	3.996	.047	.400	1<2; 1<3
	Rated of Perceived Exertion	7.0±1.3	16.4±1.7 (0.007)	18.8±2.4 (0.021)	112.325	.000	.949	1<2; 1<3

DISCUSSION

The present research aimed to analyze the effect of endurance and resisted physical stressors on the psychophysiological response and pistol marksmanship of non-experienced shooters. Our hypotheses were partially completed since i. There were no significant differences in stress protocols in the psychophysiological stress response or marksmanship. ii. A similar psychophysiological response was found independently of the BMI, but subjects with larger BMI presented higher marksmanship values. iii. There were gender differences in the psychophysiological stress response and marksmanship.

The cardiovascular response monitored at the basal time was higher than the considered standard for the population evaluated (60-70 bpm) [18]. This was consistent with an anticipatory anxiety response, a defense organic response modulated by the activation of the sympathetic nervous system that prepares individuals for any uncontrolled or new stimuli [19]. Previous authors reported a significant increase in the heart rate from a standing resting position to a standing position firing a gun, in line with the results obtained in the present research [20].

The cardiovascular response has a direct impact on fine motor skills as shooting, the higher HR shortens the diastolic phase and increases the systolic one, increasing body tremor [21], which in addition to the one produced by physical activity, marksmanship could be compromised [22,23]. After resisting and endurance physical stress protocols we found a significant increase in the cardiovascular response to shooting (38.6% and 46% respectively), however, marksmanship was not affected, suggesting no negative effect on shooting HR and fine motor skills.

In this line, previous authors obtained similar cardiovascular results, without a marksmanship compromise in archers [22] and pistol shooters [24]. The lack of significant differences in marksmanship between the two stress protocols highlighted the no effects of peripheral fatigue on shooting performance.

There are three possible hypotheses which would explain this: i) Subjects performed both resisted and endurance physical stress protocols with an optimal arousal level [25,26], in accordance with the Inverted-U hypothesis which predicts that performance is best at a moderate optimal level and becomes progressively worse with either increases or decreases in arousal, and comes to confirm that incorporating psychological factors in training (e.g. pressure), may enhance performance [27,28]; ii) Since subjects have none previous experience and despite performing a familiarization, as consequence of low total acquired experience, fatigue did not affect their marksmanship values; iii) Among the familiarization, breathing and holding techniques were taught to the subjects, which are essential to attenuate the postural tremors created by possible high HR at the moment of shooting, technique which has proven essential for rifle, pistol [15] and arch [22], the use of these techniques could be another possible explanation.

The ability to stabilize a gun is crucial for performance in pistol shooting, being related to the shooter's muscular strength [29]. The maintenance of isometric hand strength can play an important role in this fact, highlighting the importance of physical fitness for athletes and professionals that have to use fire weapons (such as biathlon athletes, or police and military respectively).

Previous authors proposed integrated operative high-intensity interval training as one of the best training tools for this aim since it allows participants to improve both, muscular and cardiovascular performance, two factors directly related to shooting performance [30,31]. In this line, both protocols elicited a significant increase in HR during its execution, being higher in the endurance stress protocol.

This cardiovascular response could be related to the continuous impact, load, and physiological demand of this type of work, a fact consequent with previous studies which compared similar exercise-based protocols [31,32]. The HR reached during the two stress protocols is associated with a decrease in fine motor abilities and specifically in shooting, a fact basic for any shoot actions independently performed by arch [33] pistol or rifle [24].

Nevertheless, at the shooting moment the HR decreased below 140 bpm, HR is not related to this fine motor abilities impairment.

These results highlighted the importance of cardiovascular stress response control in these activities, emphasizing the importance of a good aerobic physical condition for these activities. Females presented lower isometric hand strength values at all moments evaluated. This could be explained due to the female phenotype since they present lower body and muscle mass than males, lower baseline testosterone levels, and lower strength in the upper body muscles [34]. Previous authors also found similar results when comparing the hand grip of active handball and judo female athletes with healthy male subjects, presenting males with higher hand grip strength than females [35].

RPE seems to be also influenced by gender. Females presented higher RPE in the endurance and resisted physical stress protocols. These gender differences were also found in weight-bearing as treadmill [36,37,38], partial weight-bearing as ski machine [38], and non-weight-bearing as cycle [39]. Mechanisms underlying this apparent gender difference cannot be specified, however, there are several hypotheses as information processing [40] and physiological [41] differences as plausible explanations. In this line, previous authors suggested that gender differences in RPE may exist in the fact that females rate exercise as more effortful than males at absolute levels of power output [42].

Finally, a higher cardiovascular response during the protocols and while shooting at basal and endurance stress protocol was presented by females. These differences are related firstly to their smaller myocardium, beating slightly faster to make up for its size. While the average basal male heart beat is around 70-72 bpm, women's beats are 78-82 bpm [43]. Secondly, the increased basal heart rate would explain the aforementioned anticipatory anxiety response, which increases the heart rate due to perceived stress and novelty of the stimuli [19]. The response has been also evaluated in sports competition tasks [44] or novel paratroopers before jumping [45] with a similar physiological response. Thirdly, the lower recovery capacity after short burst high-intensity endurance activities of females [46], may explain the significantly higher shooting heart rate than males after the endurance stress protocol. BMI cutline was established at normal-weight (<25 BMI) and overweight (25 BMI >).

Overweighed subjects presented greater significant values of isometric hand strength, probably explained due to the larger body mass, consequent with previous studies comparing the strength of obese and non-obese subjects of either upper [47] and lower body [48] strength. In addition, overweighed subjects present greater marksmanship values evaluated at all moments, reinforcing the relationship between hand strength and shooting marksmanships [49].

However, the cardiovascular response of overweighed subjects was significantly higher during both physical stress protocols, explained due to inferior cardiovascular performance, since for the same stimuli and intensity greater cardiovascular output is needed compared to normal-weight subjects.

Nevertheless, during shooting actions their HR was similar to the normal-weight group, decreasing to HR values related with no negative effect on fine motor skills. In addition, RPE was also higher in overweighed subjects after both protocols, suggesting greater effort and exposing inferior fitness, explained due to worst physical conditioning, or the fact that being overweight is an added organic stressor 50. In this line, previous authors correlated larger BMI with reduced cardiovascular output and lower performance, showing greater sympathetic, stress, and RPE baseline values [51], supporting our present data.

CONCLUSIONS

An endurance stress protocol produced a higher cardiovascular and perceived exertion than a resisted one, not affecting hand strength, shooting heart rate, and marksmanship. Females presented lower hand strength and marksmanship while higher rated of perceived exertion and heart rate during the endurance and resisted stress protocols than males.

Overweight participants presented higher heart rates during both physical stress protocols, but higher hand strength and marksmanship while similar shooting heart rates than normal-weight participants.

Limitations of the study practical applications

The small number of subjects is one of the main limitations of the present study, however, there were no more available subjects at the moment. A second limitation was the lack of evaluation of hormones such as testosterone, alpha-amylase, and cortisol to control the hormonal stress response. These limitations were subjected to financial and technological lack.

Practical applications

The information of the present research could be used for specific training, allowing further individualization of the training stimulus according to gender, anthropometric and experience characteristics. In addition, soldiers, police, and other security crops, as well as those sport modalities in which shooting is present, for example, biathlon, could potentially benefit from this information.

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ORIGINAL ARTICLE

Evaluation of the protective effect of coenzyme Q10 on the growth process of embryos from in vitro fertilization in laboratory mice treated with cyclophosphamide

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Abstract: *Cyclophosphamide is an anticancer drug that acts as an alkylating agent after metabolism in the liver. Despite its many clinical applications in cancer treatment, this drug has toxic effects on the body's organs, especially the genitals. One of the most critical side effects is a change in the function of the female reproductive system, which can lead to infertility. This study aimed to investigate the antioxidant effects of coenzyme Q10 on cyclophosphamide-induced toxicity in vitro fertilized embryos in mice. In this experimental study, 24 female mice weighing 25 g 4 groups of 6 were divided and treated for 21 days. The first group (control group), solvent (cyclophosphamide) including DMSO and PBS (0.1 ml intraperitoneally), the second group (sham group), cyclophosphamide at a dose of 10 mg/kg was injected as a single dose, and the third group (experimental group), along with single-dose cyclophosphamide, coenzyme Q10 at a dose of 200 mg/kg/day was injected intraperitoneally and the fourth group (positive control group), only coenzyme Q10 at a dose of 200 mg/kg/day was injected intraperitoneally. At the end of the treatment period, ovulation stimulation was performed using PMSG and HCG hormones. Six adult male mice were used to prepare normal sperm. The animals were facilitated after anesthesia. After extraction of regular eggs and sperm and fertilization in HTF + 4 mg BSA medium, the fertilized eggs were incubated for 120 hours, and the embryonic developmental stages were examined during this period. Were analyzed by ANOVA and comparison of ratios. Cyclophosphamide significantly reduced oocyte quality, fertilization rate, pre-implantation embryonic development, and embryo quality. Coenzyme Q10 (Co Q10) significantly reduced the adverse effects of cyclophosphamide.*

The present study showed that crocin could protect the fertility of the female sex against damage caused by cyclophosphamide.

Keywords: *cyclophosphamide, ovary, coenzyme Q10, mice, fertility*

INTRODUCTION

Operation of inguinal hernia is one of the most common procedures in the general surgery field. The prevalence of inguinal hernia is between 15-45% at different ages [1-3]. There are two categories of inguinal hernia, direct and indirect, that included 24 to 50% of all types of hernia, respectively [4-7].

Some studies reported chronic obstructive pulmonary disease, chronic cough, benign prostatic hyperplasia, chronic constipation, family history of hernia, previous right lower quadrant incision, smoking, collagen diseases, physical activity, and bearing the burden can trigger inguinal hernias [5-10].

The inguinal hernias can develop discomfort, pain, and dysfunction in the patient, and also it can cause a cosmetic problems for most patients [2-4]. Severe complications of this disorder, which often occur following hernia entrapment and suppression, require emergency intervention. These include obstruction, ischemia, and gangrene of the bowel or any organ trapped inside the hernia sac [4-7].

A surgical procedure is the standard treatment for this disease. Today, there are several techniques of surgery for it, and surgeons associated with circumstances chose a surgery strategy. The main purpose of surgery is to treat patients and decrease the recurrence chance of the disorder [11-15].

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Inguinal hernia repair surgery has several potential complications such as hernia recurrence, pain, osteitis of the pubis, Damage to the cord and testicles, hematoma, and testicular swelling [1-3, 5]. There are two main approaches to operation including open surgery and laparoscopy. Also, there are several techniques to repair the herniation site such as classical and preperitoneal techniques. The classical method is a simpler procedure than other methods of repair conducted by most surgeons. Various conventional methods like Bassini's applying suture material are in usage despite the laparoscopic hernia repair [16-18]. Suture repair is related to a significant tension on the suture line. This is possible to induce ischemia of the tissues and final failure of repair causing recurrence [18-20].

Open surgical techniques include two categories of tissue repair and mesh repair methods. More traditional methods are based on initial tissue repair, but today with the production and presentation of industrial meshes, non-traction hernia repair with mesh is the standard method of inguinal hernias surgery [9-6]. Lichtenstein et al. reported the concept of tension-free hernia repair through a synthetic proline mesh that ensures promising outcomes of hernia repair in the long term [1-3].

Preperitoneal repair is one of the pre-peritoneal methods, the mesh is placed under the transversal fascia and right on the peritoneum, especially in cases of recurrent hernias after tissue repair methods. In this method, the peritoneal space is released through the anterior access, a 15 by 15 cm mesh is implanted on the peritoneum and fixed to the umbilicus and symphysis of pubis with non-absorbable thread, and then tissue repair with nylon loop thread is done [13-14].

This study aimed to compare the complications of Surgical Repair in inguinal hernia with Bassini, Lichtenstein, and Preperitoneal met Exposure to exogenous materials, whether natural or man-made, has a profound effect on vital processes and reduces or destroys reproductive capacity [1]. In the female animal, if it comes in contact with chemicals, it affects the oocyte cell, which is in various stages of primary, primary, secondary, or ovulation. Most chemotherapeutic agents are mutagenic and cause defects in sex cell chromosomes. Alkylating agents such as cyclophosphamide are among the significant chemotherapeutic agents used to treat cancers and autoimmune diseases [2]. This compound is a cytostatic drug from the oxazaphosphorine group and alkylated nitrogen mustard derivatives [3].

Cyclophosphamide is converted by its oxidase enzymes in the liver to its active metabolite, 4-hydroxy cyclophosphamide, which interacts with its totomer called aldophosphamide. Most aldophosphamide is oxidized by the enzyme aldehyde dehydrogenase (ALDH) to carboxy-phosphamide, and only a small amount of aldophosphamide is converted to mustard phosphor amid acrolein [4]. Despite its wide range of clinical applications, this drug causes reproductive toxicity. Studies have shown that cyclophosphamide disrupts the process of spermatogenesis by increasing sperm DNA damage [5]. Ovarian dysfunction decreased sex hormones, and reduced follicle count have also been reported with cyclophosphamide [6]. Cyclophosphamide also destroys primary follicles and, due to reduced follicular reserve, harms the reproductive system, and causes infertility and premature menopause in females [7]. Cyclophosphamide products chromatin and some of its effects have been observed in the next generation of F2 [8]. Injection of cyclophosphamide into pregnant mice on the thirteenth day of the fetus can cause a defect in the synaptic process as partial synapses or no bilateral synapses on the eighteenth day of the fetus [9].

Oxidative stress results from an imbalance between the production of oxygen free radicals versus the antioxidant defense capacity of the cell, leading to the production of free radicals, including superoxide anion (O_2), hydroxyl radicals (OH), and non-radical derivatives of oxygen such as hydrogen. Peroxide (H_2O_2). These free radicals are highly unstable and react rapidly and non-specifically with biomolecules, leading to the development and spread of cellular damage including plasma membrane peroxidation, amino acid, nucleic acid oxidation, apoptosis, and necrosis. Finally, it leads to a decrease in the viability and development of embryos in the culture medium [10,11]. Reproductive disorders in both males and females caused by oxidative stress have been reported. The effect of reactive oxygen species (ROS) levels on zygotes or early embryonic development has been identified. Oxidative stress may be responsible for fetal toxicity in the hydrosalpinx [12]. The addition of antioxidants such as vitamins C and E to the culture medium improves the blastocyst development ratio in mouse embryos [13]. It enhances the fertility ratio and increases implantation [11]. Cyclophosphamide, as an important and effective drug in the treatment of cancer, despite its improvement in cancer patients, acts as an oxidative stress-producing agent in the body and increases the production of ROS. Therefore, to modulate and prevent the effects of oxidative stress caused by cyclophosphamide, coenzyme Q10, which has an antioxidant role, can be helpful. Coenzyme Q10 (CoQ10), a fat-soluble molecule in almost all cell membranes, is located inside the mitochondrial membrane. CoQ10 is an essential component for transporting electrons in the mitochondrial respiratory chain to produce cellular energy. In addition, the reduced form of CoQ10, known as ubiquinol, acts as an antioxidant in cellular metabolism by inhibiting lipid peroxidation, protein, and DNA oxidation [14,15]. The amount of CoQ10 in tissues is often reduced in many system-related pathological conditions such as diabetes and Alzheimer's and prion diseases, as well as the use of chemicals. Although CoQ10 is synthesized in almost all normal tissues, CoQ10 tissue levels gradually decrease with age [16]. Defects in CoQ10 synthesis with specific clinical disorders associated with high-energy consuming tissues, including skeletal muscle, endocrine glands, and nervous system [17]. Studies have shown that CoQ10 supplementation causes a significant increase in CoQ10 levels in some tissues such as muscle, sperm, and plasma, and a substantial increase in the adrenal glands and ovaries [18,19]. Therefore, CoQ10 supplementation protects cells against ROS-induced damage due to its antioxidant properties, strengthening endogenous cell antioxidant systems.

Since no study has been performed on the effect of coenzyme Q10 as an antioxidant in reducing the effects of oxidative stress induced by cyclophosphamide on the growth of in vitro fertilization embryos, in this study, the protective effect of coenzyme Q10 on Improving the growth process of in vitro fertilization embryos was studied in cyclophosphamide-treated mice.

METHOD

In this experimental study, 24 female rats weighing approximately 20-25 g were randomly divided into four equal groups of 6 and treated for 21 days. Animals under standard conditions with a temperature of 22.2° C, humidity 30-60%, and the light cycle, 14 hours of light and 10 hours of darkness were maintained. Water and food were freely available.

The control group (cyclophosphamide) including DMSO and PBS (0.1 ml intraperitoneally), the sham group with a dose of 10 mg/kg was injected as a single dose of only cyclophosphamide, experimental group 1, with a single dose of cyclophosphamide, coenzyme Q10 at a dose of 200 mg/kg/day was The control group (cyclophosphamide) including DMSO and PBS (0.1 ml intraperitoneally), the sham group with a dose of 10 mg/kg was injected as a single dose of only cyclophosphamide, experimental group 1, with a single dose of cyclophosphamide, coenzyme Q10 at a dose of 200 mg/kg/day was injected intraperitoneally into the positive control group, only coenzyme Q10 at a dose of 200 mg/kg/day was injected intraperitoneally. Ovulation induction was required for this study. For this purpose, after the end of the treatment period and 72 hours before in vitro fertilization (IVF), 5.7 units of HCG (made by Folligon, Netherlands) in a volume of 0.1 ml was injected a volume of 0.1 ml and 48 ml. An hour later, 5.7 units of HCG (made by Folligon, Netherlands) in a volume of 0.1 ml was injected intraperitoneally. Ovulation was performed 13 hours after HCG injection.

The first step in IVF was to prepare normal sperm from 6 adult male laboratory mice by injecting ketamine (25 mg/kg body weight, intraperitoneally) under anesthesia with a high dose of easy ketamine. The skin of the abdomen was then sterilized with 70% ethanol. After making an incision in the abdomen and separating the connective tissues, we saw around the tail of the epididymis with some vas deferens from the testicle and into a 3 cm dish containing a medium. HTF culture, containing 4 mg/kg BSA (Sigma, USA), was previously transferred to the incubator for exchange. After making several incisions in the tail of the epididymis and pressure in the vas deferens to expel sperm, Petri the dishes were placed inside a carbon dioxide incubator. After 30 minutes, the sperm were released and spread in the environment. The sperms were then washed and separated using a flotation method. In this study, standard sperm samples with motility above 80% and one million per ml of culture medium were used. The second stage of work was ovulation in female mice. For this purpose, 13 hours after HCG injection (following day), animals after anesthesia with ketamine injection (25 mg/kg body weight, intraperitoneal) and injection. The high dose of ketamine was facilitated, then the fallopian tubes were removed and placed in HTF + 4mg/ml BSA culture medium at 37° C, which was prepared in advance, and the eggs were removed using an autopsy method. After washing, they were transferred to the fertilization medium under mineral oil containing HTF culture medium (containing 4 mg/ml BSA). Then the sperms were transferred to the culture medium in the amount of one million per ml of culture medium. It took about 3 to 5 hours after the sperm were added and thus, the zygotes were obtained. Zygotes from all groups were cultured in HTF medium for 120 h and embryonic developmental stages were evaluated under the inverted microscope to evaluate the effect of oxidative stress due to cyclophosphamide administration and the protective effect of coenzyme Q10. Fission rate was assessed 24 hours after culture and in zygotes in each group, embryos in terms of fragmentation, embryonic development, and the number of embryos stopped due to various factors such as embryonic slippage, necrotic, fragmentation, and the presence of cytoplasmic vesicles occurred. Embryo quality, number of embryos grown, and incision percentage were evaluated by examining the percentage of two-cell embryos, the number of stopped embryos, and the rate of blastocysts formed during 120 hours of incubation in each group.

RESULTS

The control group differentiated the percentage of embryos from blastocysts with appropriate quality after 120 hours of incubation, and the percentage of embryos that stopped at different stages of development. B, cyclophosphamide group, a small percentage of embryos have reached the blastocyst stage and the resulting blastocysts are not of good morphological quality. C, a cyclophosphamide-treated group with coenzyme Q10 (experimental) compared with the cyclophosphamide control group, a high percentage of embryos reached the blastocyst stage and the resulting blastocysts are of good morphological quality, and a small percentage of embryos are stopped. D, coenzyme Q10% higher percentage of embryos reach blastocyst and percentage of embryos are stopped at different stages (invert microscope, magnification × 400) (Figure 1).

Figure 1: Fetal growth photomicrograph: A: Control group, B: Cyclophosphamide group, C: Cyclophosphamide with coenzyme Q10, D: Coenzyme Q10

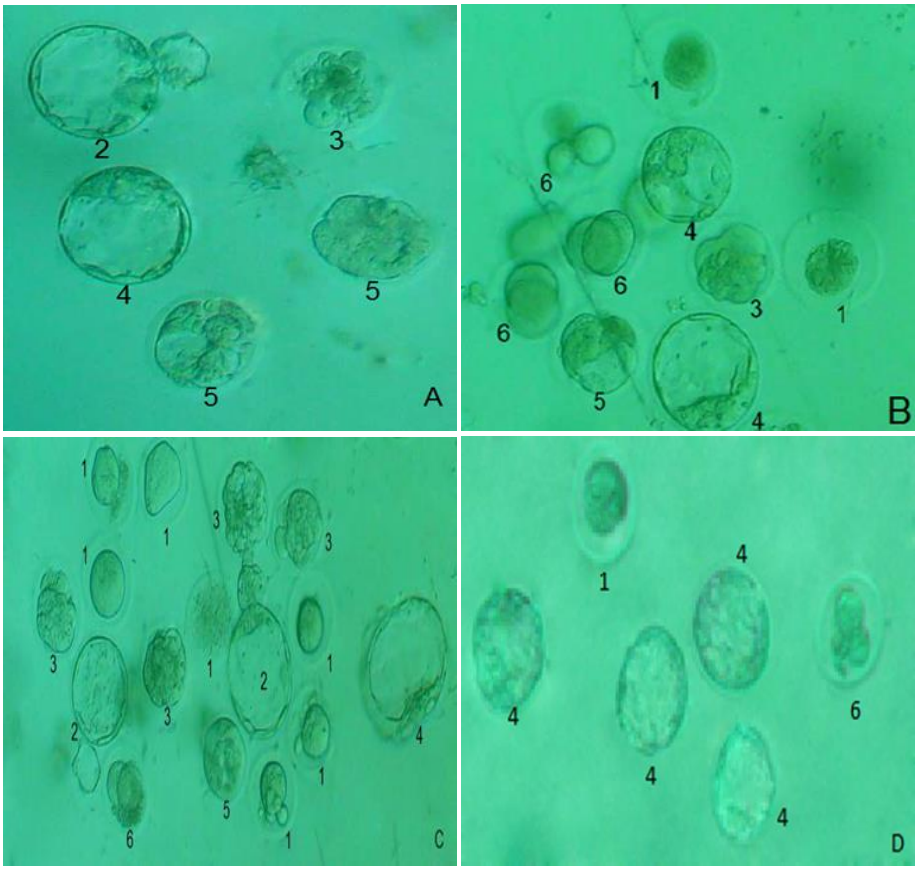

1. Oocyst; 2. The fetus is being hatched; 3. Merola; 4. Blastocyst; 5. Primary blastocyst; 6. Fetal fetus

The interaction of different drug groups and antioxidants on the measured parameters showed that the effect of drug treatment on the number of oocytes was significant (P <0.05), so the highest number of oocytes was obtained from the control group and the lowest amount belongs to the cyclophosphamide group only. It was also shown that the mean number of oocytes in the cyclophosphamide group with coenzyme Q10 was almost similar to the control group, and there was no significant difference with this group (Figure 2).

The effect of antioxidant treatment in the cyclophosphamide group with coenzyme Q10 on the mean number of zygotes was significant compared to the cyclophosphamide group (P <0.05), but this increase was not significantly different from the control group among the treated groups. The highest number of zygotes belonged to the group that received coenzyme Q10 alone and was not substantially different from the control group; the lowest number of zygotes was obtained from the group that received only cyclophosphamide (Figure 3).

The mean of two-cell zygotes in cyclophosphamide-receiving groups with coenzyme Q10 was not significantly different from that of cyclophosphamide despite increasing the number of two-celled zygotes (Figure 4).

Figure 2

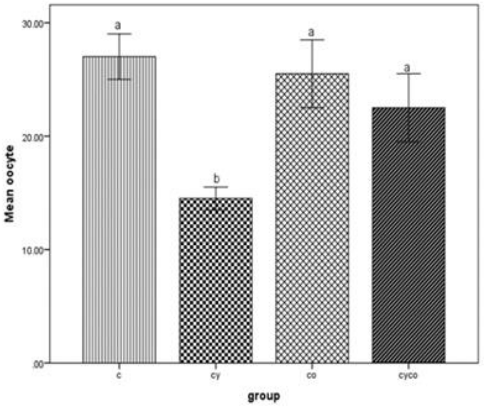

Figure 3

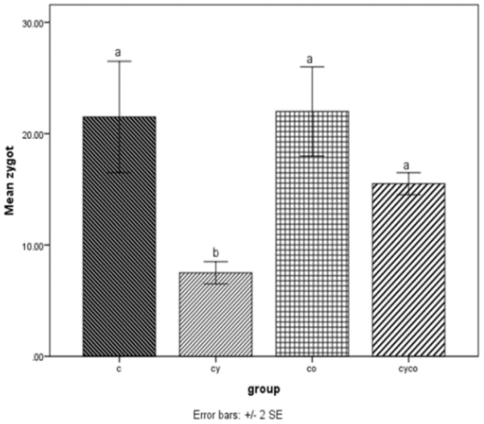

Figure 4

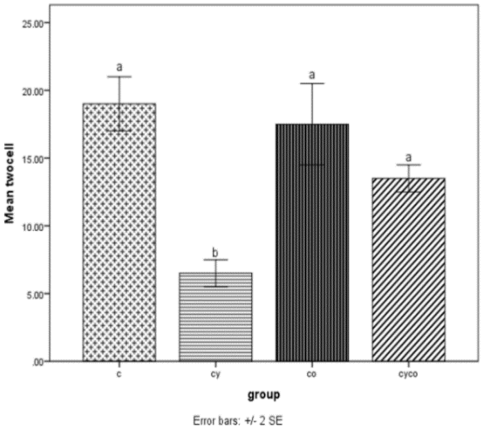

The effect of antioxidant treatments on the number of blastocysts was. The highest amount belonged to the control group and the lowest amount belonged to the group that received only cyclophosphamide (Figure 5).

The mean number of blastocytes in all groups receiving cyclophosphamide along with coenzyme Q10 was significantly different from the cyclophosphamide group (P <0.05). Regarding this parameter, the group receiving coenzyme Q10 alone did not have a significant difference from the control group (Figure 6).

Figure 5

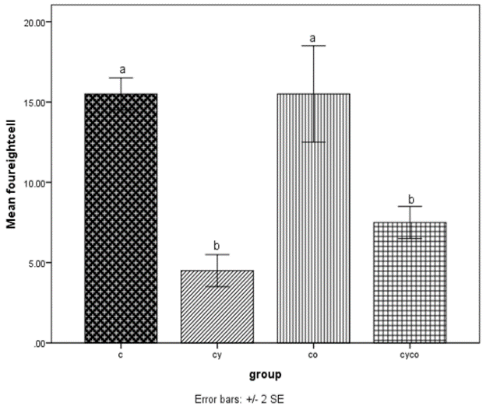

Figure 6

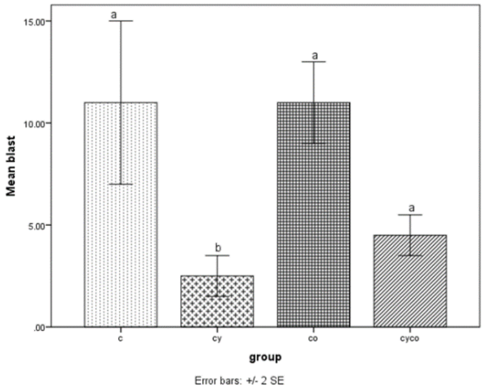

The highest number of discontinued embryos was observed in the cyclophosphamide group and the use of coenzyme Q10 with cyclophosphamide significantly reduced the mean number of discontinued embryos (P <0.05) (Figure 7).

Figure 7

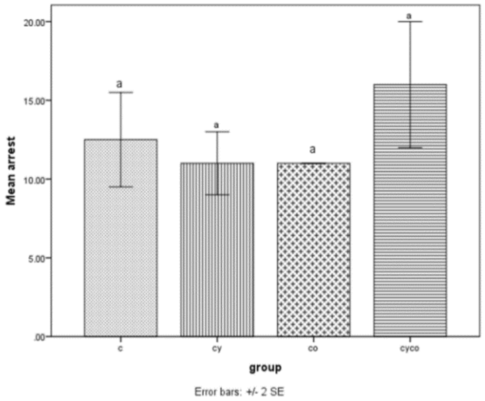

DISCUSSION

Cyclophosphamide causes reproductive toxicity by inducing oxidative stress, which is reduced by the concomitant administration of antioxidants. The ability of cyclophosphamide to produce free radicals, lipid peroxidation, and oxidative stress has been reported in laboratory mice [20].

Previous studies have shown that low doses and long-term use of cyclophosphamide in male and female mice impair fertility [21,22]. It changes the development of the next generation [23]. Intracellular thiol levels appear to play an essential role in determining cell susceptibility to cyclophosphamide-induced damage [24].

4-Hydroxycyclophosphamide can react with cellular thiols to produce a thio-alkyl derivative. Derivatives produced in equilibrium with 4-hydroxycyclophosphamide are considered alkylating agents [25] and can induce oxidative stress. Cyclophosphamide has also been shown to have immunosuppressive properties, which are related to its induction of oxidative stress [26].

Due to the effect of cyclophosphamide on chromatin of sex cells and DNA failure in the nucleus of oocyte cells, some of its effects appear in the next generation [8]. One study found that in cyclophosphamide-affected larvae, after extraction in a culture medium, the meiotic division was inhibited 48 hours later, whereas, in animals, fertility may continue in humans. As a result, under the influence of chemotherapy agents, the development of embryos is disrupted and anomalies develop in them [27]. This study was performed to modulate the side effects of oxidative stress exerted by cyclophosphamide on the growth process of in vitro fertilization embryos using coenzyme Q10 as an antioxidant. In this study, the growth rate and quality of embryos obtained from in vitro fertilization in the control group showed a significant decrease compared to the control group.

These observations indicate the toxic effect of cyclophosphamide on reproductive activity through oxidative stress, which is co-administered. Coenzyme Q10 (Co Q10), as an antioxidant, attenuated this toxic effect of the drug and increased the quality of fetal growth in the laboratory. Injection of high-dose cyclophosphamide into female mice mainly affects the primary follicles and disrupts other ovarian characteristics such as ovulation, egg transfer, and end endocrine regulation [28].

In a study conducted on the effect of Khuzestani Marzeh essential oil on in vitro fertilization of adult female mice treated with cyclophosphamide, administration of safflower extract with cyclophosphamide improved the growth of embryos obtained from in vitro fertilization.

Other studies on the modification or prevention of side effects of chemotherapy drugs by antioxidant agents have shown the toxic effects of cyclophosphamide on sperm morphology and ovarian tissue, are protected by lycopene and alginic acid as antioxidants [29].

The protective role of vitamin E against oxidative stress induced by cyclophosphamide has been demonstrated in mice. This action of vitamin E may be the result of the destruction of free radicals as well as the stimulation of the release of anterior pituitary gonadotropins [30]. Also, the presence of antioxidants in culture media improves the blastocyst development ratio in mouse embryos and improves the fertility ratio, and increases implantation [31].

CONCLUSION

Considering all the results obtained along with their interpretation, the present study reinforces the idea that the reproductive toxicity caused by cyclophosphamide administration occurs due to oxidative stress, and that a potent antioxidant such as coenzyme Q10 can regenerate the reproductive system. Protects against oxidative stress damage caused by cyclophosphamide, which often causes infertility. In the present study, coenzyme Q10 was able to increase the quality of germ cells, and fertilization rate, improve fetal growth in the laboratory, and reducing cessation at different stages of embryonic development. effective.

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ORIGINAL ARTICLE

Frequency and genotyping of human papillomavirus in patients with DNA tracking technique during 1996-99

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Abstract: *The prevalence of human papillomavirus (HPV) infections and their role in cervical cancer have been well documented in numerous articles, but it seems that a study in this field in developing countries and the Middle East, including Iran. It's not done. Therefore, the present study was designed and performed to investigate the frequency and genotyping of human papillomavirus in patients by DNA tracking technique. This study was a population-based study in which the frequency and genotyping of human papillomavirus in patients by tracking technique was designed and performed on 534 men and women living in Tehran by DNA tracking technique. Sequences obtained using the NCBI site were analyzed at (http://www.ncbi.nlm.nih.gov/BLAST/LaserGene) and Bio edit software. So that in three low-risk groups including 6 and 11 genotypes, Intermediate risk includes 26-31-33-35-39-40-42-45-49-51-52-53 genotypes -55-56-58-59-66-63-83 and High risk includes 16 & 18 genotypes were divided. According to the results, the prevalence of the papilloma virus in the study population was 21.16%, of which 19.46% were in the high-risk group, 69.89% were in the intermediate-risk group and 50.43% were in the low-risk group. According to the results of the present study, although the prevalence of HPV in the study population is less than in many countries in the world, it is suggested that conducting epidemiological studies in the country to extract the true prevalence and plan to prevent and control this disease.*

Keywords: *human papillomavirus, frequency, genotype, DNA tracking technique*

INTRODUCTION

Cervical cancer is the fourth most common malignancy and a major cause of death in women worldwide. Numerous reports show an association between a group of DNA oncogenic viruses and the development of malignancies in the human population, including human papillomavirus [1].

More than 200 different types of human papillomavirus have been found, of which about 40 are capable of infecting the genital tract. Human papillomavirus is classified into two low-risk and high-risk groups based on the degree of malignancy and cancer [2].

In the latest classification published by the World Health Organization, the International Agency for Research on Cancer, genotypes 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, and 59 as high-risk types, other cultivars including genotype 26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85 and 97 are also known to be carcinogenic types and genotypes 6 and 11, which are known to cause benign lesions of the genital tract. Introduced as low risk types [3].

The progression of cancer requires the expression of viral genes, the entry of viral DNA into host chromosomes, and changes in the genes and gene products of the host cell. This group of viruses requires the DNA polymerase of host cells to replicate the genome, and from the entry points created through fine scratches in the stratum corneum cell layers to reach the basal layer of the epithelium, the location of basal cells and pre-cells. They use instruments that can be reproduced [4]. The E6 and E7 oncoproteins in HPV are key players in carcinogenesis and target tumor-suppressing proteins in host cells. In high-risk HPVs, the E6 protein forms a complex with a ubiquitin ligase [5]. This complex binds to p53, which is followed by the breakdown of p53, which inactivates it. P53 acts as a transcription factor and induces

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the expression of genes involved in cell cycle arrest and apoptosis. When E6 is not inactive, the p53 protein increases, causing p53-dependent apoptosis and killing the infected cell before the virus multiplies [6]. E7 protein also exerts its oncogenic effect by inactivating retinoblastoma protein and two proteins related to retinoblastoma protein, p106 and p130 [7].

Retinoblastoma protein suppresses tumors and plays a key role in regulating the eukaryotic cell cycle. In the case of high-risk HPV infection, the E7 protein breaks down the E2F-Rb complex, releasing E2F, resulting in the cell entering the S phase outside the original time, which disrupts the cell cycle [8].

The human papillomavirus completes its evolution as it crosses the basal layer with the superficial cell layers, which increases the expression of the virus's L1 and L2 proteins, which play an important role in their identification. High-risk types of HPV cause lesions in the cervical epithelium, the severity of which varies depending on the extent to which squamous epithelial cells have been replaced by basaloid cells in infected species [9].

Grading CIN III, CIN II, CIN I High-risk HPV is a necessary and insufficient factor in the development of cervical cancer, so other factors associated with cervical cancer include smoking, age, nutrition, race, geographical location, having Multiple sexual partners noted long-term use of birth control pills, weakened immune systems, and host-related factors [10].

The global prevalence of HPV infections in 2012 is estimated at 440 million. In 2012 alone, these infections led to 550,000 cases of cervical cancer and approximately 288,000 deaths [11]. Also, according to published statistics on the diagnosis of human papillomavirus in various diseases, infection of this virus in 99% of cervical cancers, 97% of anal cancers, 70% of vaginal cancers, 47% of penile cancers, 40% of gastrointestinal cancers, 47 The percentage of urological cancer and 11% of cases of oral cancer have been observed [12].

In most of the results obtained from the studies of researchers inside and outside the country, the abundance of 6, 11, 16, and 18 is more than other genotypes. Therefore, the frequency and determination of HPV genotype due to the identification of low-risk and high-risk types have diagnostic and therapeutic value and can be prevented by screening and timely diagnosis of lesions that lead to malignancy. Therefore, it is recommended that women between the ages of 30 and 65 have a screening test every 5 years [13].

Human papillomavirus is known to be one of the most important causes of cervical cancer [14]. HPV infection is a sexually transmitted disease that the prevalence and type of virus that causes it varies depending on the area where people live. Studies in different human populations show that more than 50% of women become infected with at least one type of virus during their lifetime.

Human papillomavirus is a small, uncoated virus with double-stranded DNA of at least 200 different types. About 35 to 40 types of this virus infect the skin epithelium or mucous membranes in the female reproductive system [15]. Most viral infections do not cause specific symptoms or diseases and heal on their own. If the infection persists, the variety of lesions of the virus is high, ranging from benign skin warts that are self-limiting and often appear on the hands and feet to malignant and invasive lesions manifesting as fatal cervical cancer [16].

Condyloma acuminata is one of the most common sexually transmitted diseases. Mucosal types can be divided into high-risk and low-risk according to the risk of disease. High-risk types of human papillomavirus play a major role in causing cervical cancer. Among these viruses, at least 15 types of high-risk viruses cause more than 95% of cervical cancers and 80% of vaginal cancers [17]. Therefore, its accurate diagnosis will have a significant impact on the control and management of this cancer and the promotion of women's health in society.

Currently, 170 HPV genotypes are classified according to the type of biological wall and oncogenicity and their type of racial evolution, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are known to be high-risk, and types 26, 53, and 66 may also be oncogenic [18]. Abnormal cervical epithelial cells can be detected by Pap smear staining and microscopic observation. However, this diagnostic method is only used for women at risk for advanced disease, and about 50% of the lesions will not be detected if only a Pap smear is performed on them, as the HPV virus does not grow in culture and serological tests are of limited accuracy.

HPV infection triggers a humoral immune response against the capsid protein, which is an antibody that stays in the body for many years (serological tests are not suitable for diagnosing present and past infections [19]. As a result, accurate diagnosis of HPV infection relies heavily on molecular methods.

Molecular techniques can be used to accurately diagnose and identify HPV, as well as to inform the patient and follow up after treatment. Accurate HPV genotyping is essential to classify patients into high-risk or low-risk groups.

Evidence also suggests that the presence of multiple HPV types may reflect repeated exposure to the virus and may be associated with an increased risk of disease progression [20].

Prevalence and genotyping of human papillomavirus in precancerous and cancerous samples with DNA tracking technique from 1396 to 1399 in Tehran to evaluate the health of the female community.

This study aimed to investigate the frequency and genotyping of human papillomavirus in precancerous and cancerous samples by DNA tracking technique.

METHODS

This case-control study was performed by collecting 534 biopsy specimens from men and women. All biopsy specimens were examined for the presence of the human papillomavirus genome.

DNA extraction

DNA extraction was performed according to the protocol of a High pure viral nucleic acid kit from Roche (Germany). The steps are summarized in Figure 1.

Figure 1: General process of DNA extraction protocol by High pure viral nucleic acid kit

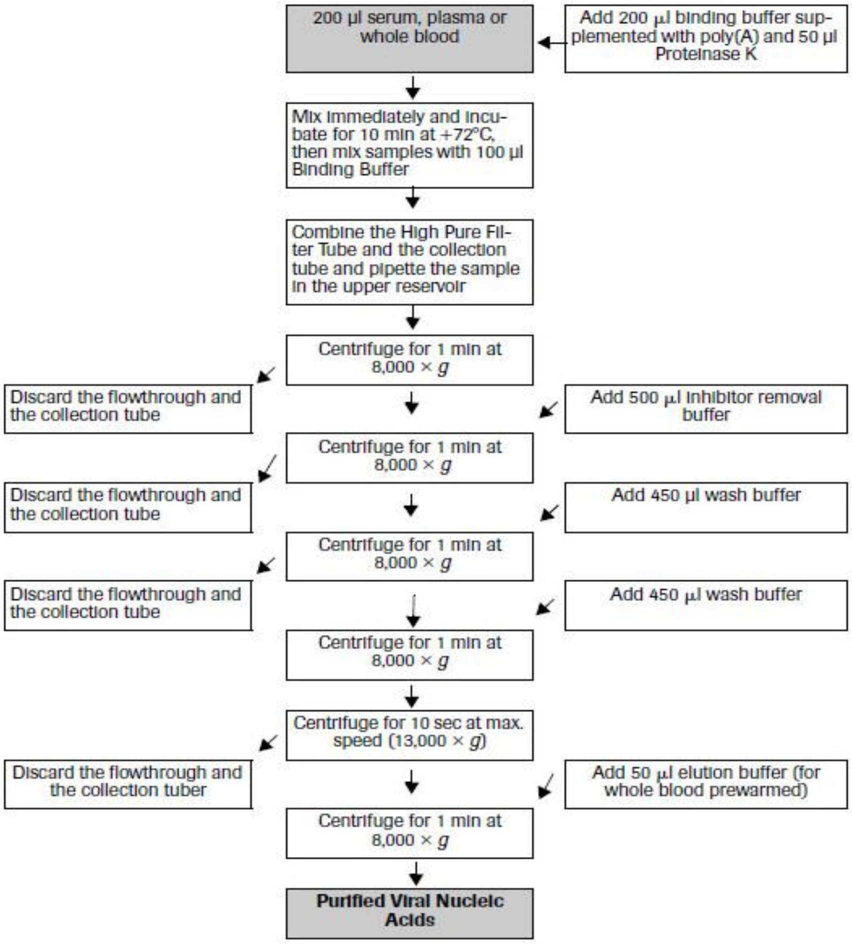
200 µl serum, plasma or whole blood
Add 200 µl binding buffer supplemented with poly(A) and 50 µl Proteinase K
Mix immediately and incubate for 10 min at +72°C, then mix samples with 100 µl Binding Buffer
Combine the High Pure Filter Tube and the collection tube and pipette the sample in the upper reservoir
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 500 µl inhibitor removal buffer
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 450 µl wash buffer
Centrifuge for 1 min at 8,000 × g
Discard the flowthrough and the collection tube
Add 450 µl wash buffer
Centrifuge for 1 min at 8,000 × g
Centrifuge for 10 sec at max. speed (13,000 × g)
Discard the flowthrough and the collection tuber
Add 50 µl elution buffer (for whole blood prewarmed)
Centrifuge for 1 min at 8,000 × g
Purified Viral Nucleic Acids

Quality control of extracted DNA

At this stage, using the PCR technique and targeting the β-Globin gene as a housekeeping gene, quality control was performed, so that samples that were positive for the β-globin gene were considered suitable samples for testing in later stages. Were taken. Samples that were negative for this gene were retested, and if we did not respond again, we would have to retrieve the patient sample.

Table 1: β-Globin gene primers

Pco3	Forward	5'-ACACAACTGTGTTCACTAGC-3'
Pco4	Reverse	5'-CAACTTCATCCACGTTCACC-3'

Nested PCR for human papillomavirus genome

Nested PCR is a modified model of PCR that reduces nonspecific connections in the reaction product (due to propagation of components that are not expected). In this method, two pairs of primers are used to increase the sensitivity of PCR. First, with a pair of first primers over 15-30 cycles, specific pieces of target DNA are amplified. The resulting PCR product is then transferred to another tube and used as a template, and the second stage of PCR is performed by a pair of second primers. In the Nested PCR test to detect HPV, we first used the pairs of primers mentioned in Tables 2 and 3.

Table 2: Sequence of MY09/11 primers (Outer Primers)

MY09	Forward	5′-CGTCC(A/C)A(A/G)(A/G)GGA(A/T)ACTGATC-3′
MY11	Reverse	5′-GC(A/C)CAGGG(A/T)CTATAA(C/T)AATGG-3′

Table 3: Sequence of GP5+/6+ primers (Inner Primers)

GP5+	Forward	5′-TTTGTTACTGTGGTAGATACTAC-3′

The PCR product with MY09/11 and GP5+/6+ primers are 450 and 150 bp, respectively.

Sequencing

To determine the genotype of HPV positive samples, Biosystems ABI 911 automatic device was used using the cyclic sequencing method.

For this purpose, 4 separate PCR reactions are performed, each containing a similar primer and a different ddNTP terminator labeled with fluorescence dye.

Genetic analysis of HPV sequences

Sequences obtained using the NCBI site were analyzed at (http://www.ncbi.nlm.nih.gov/BLAST/LaserGene) and Bioedit software. So that in three low-risk groups including 6 and 11 genotypes, Intermediate risk includes 26-31-33-35-39-40-42-45-49-51-52-53 genotypes -55-56-58-59-66-63-83 and High risk includes 16 & 18 genotypes were divided.

RESULTS

The data obtained from 534 participants in the study were analyzed using descriptive statistical methods (mean, standard deviation, frequency, and percentage) and the results were obtained according to the objectives and hypotheses of this study, respectively.

Table 4 shows the age distribution of the research community in terms of quantitative variables.

The minimum age for men is 24 and the maximum age is 65, the minimum age for women is 19 and the maximum age for women is 75.

The mean and standard deviation of the age of men and women are equal to (35.12 ±77.44), (43.11 ±23.15).

Table 4: Descriptive statistics of quantitative variables

Variables	At least	Maximum	Mean	Standard deviation
Age of men	24	65	35.77	12.44
Age of women	19	75	43.23	11.15

Table 5 shows the PCR findings based on positive and negative criteria. Of the 18 men referred, 3 were negative for papillomavirus, and 15 were positive. Out of 516 female patients, 418 were negative for papillomavirus, and 98 were positive.

According to the results, the prevalence of the papilloma virus in the study population was 21.16%.

Table 5: Report of PCR findings based on positive and negative criteria

Variables	Positive number	Negative number	Percent
Age of men	15	3	13.27
Age of women	98	418	86.72

According to Table 6, 3 men were simultaneously High risk, Intermediate risk, and Low risk, 8 were only Intermediate risk, 3 were Intermediate risk and Low risk and 3 were Low risks.

According to Table 7, 34 women were Low risk, 36 were Intermediate risk, 7 were Intermediate risk and Low risk, 15 were High risk, 3 were High risk and Low risk, and 3 were High risk and Intermediate risk.

Table 6: Report PCR findings based on risk criteria – men

Low risk	Intermediate risk	High risk	Number of people
*	*	*	**1**
	*		**2**
	*		**3**
	*		**4**
	*		**5**
	*		**6**
	*		**7**
	*		**8**
	*		**9**
*	*		**10**
*	*		**11**
*	*		**12**
*			**13**
*			**14**
*			**15**

Table 7: Report PCR findings based on risk criteria – women

Low risk	Intermediate risk	High risk	Positive number
	*	*	1
	*	*	2
	*	*	3
*		*	4
*		*	5
		*	6
		*	7
		*	8
		*	9
		*	10
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*			95
*			96
*			97
*			98

Table 8 shows the frequency of different genotypes of papillomavirus in the study population.

Table 8: Report of PCR findings based on the frequency of papillomavirus genotype

Percent	Frequency	Genotype	Risk
34.51	39	HPV6	Low risk
15.92	18	HPV11	
0.88	1	HPV26	Intermediate risk
0.88	1	HPV30	
6.19	7	HPV31	
7.07	8	HPV32	
7.96	9	HPV33	
0.88	1	HPV34	
12/38	14	HPV35	
0.88	1	HPV39	
1.76	2	HPV40	
6.53	4	HPV42	
0.88	1	HPV43	
1.76	2	HPV44	
0.88	1	HPV51	
2.65	3	HPV52	
1.76	2	HPV54	
2.65	3	HPV56	
4.42	5	HPV58	
2.65	3	HPV66	
0.88	1	HPV68	
2.65	3	HPV70	
1.76	2	HPV81	
1.76	2	HPV87	
19.46	22	HPV16	High risk

DISCUSSION

Today, cancer is a dangerous and deadly disease that is one of the most important causes of death worldwide. In 2012, 14.1 million new cases of the disease were reported worldwide, and the disease has killed 8.2 million people [21]. While the mortality rate and cancer incidence in 2008 was 7.6 million and 12.7 million people, respectively [22]. Approximately 47% of new cancers and 55% of deaths from them occur in developing and less developed countries.

In Iran, cancer is the third leading cause of death and more than 30,000 people die of cancer every year [23]. With the increase in life expectancy and the percentage of aging in the country's population, the advancement of medical science and technology, and the departure of lifestyles from a clean environment free of industrial carcinogens, cancer incidence is expected to double in the next two decades. According to the World Health Organization, the incidence of cancer in Iran in 1399 will reach about 86,000 cases in the total population and the resulting mortality rate will reach about 63,000 cases [24].

Various factors involved in cancer include lifestyle, heredity, environment, host factors, and infectious agents. Infectious agents are one of the most important controllable factors in cancer. Studies show that 17.8% of cancers are associated with infectious agents, among which viral agents are of great importance because about 12.1% of cancers are associated with viral agents [25]. Annually, 1.3 million new cases of cancer are attributed to viruses, and cancers caused by viral agents lead to the death of 1 million people each year [26].

One of the viral families associated with cancer is viruses belonging to the papilloma viridae family. Cervical cancer is the fourth most common cancer in women worldwide [27] and has received much attention today due to the large social and economic costs imposed on society and the health system. Given that cervical cancer has a long and slow progression, early detection, especially in precancerous stages, can help reduce mortality from this important cancer. Current methods for screening, diagnosis, and follow-up of this disease are based on cytological and histological methods; for example, Pap smear, which has been used for many years and is unchanged [28].

Due to the limitations of these methods, including insufficient sensitivity in a study and the need for repeated studies, and high dependence on individual skills and sampling conditions, as well as relying on the principle of increasing our knowledge of this disease and understanding the causes of creating and Its development (mentioned in the introduction), and the invention of new diagnostic methods as a complement or even alternative to the methods currently used have been of interest to many researchers in this field. The study of the pathogenesis and the attempt to find biomarkers of cervical cancer have led to the emergence of new ideas for early diagnosis and treatment [29].

Recent studies in the field of cervical cancer

Scientific information is focused on the initial diagnosis and targeted treatment of cancer. HPV induces metastasis and related events by affecting cellular signaling pathways. Human papillomavirus increases the expression of many cellular genes, including genes involved in reproduction and survival, the transition from epithelial to mesenchymal, substrate-independent growth, and oxidative stress [30]. Research on cervical cancer shows that changes in the function of several genes lead to abnormal proliferation and invasion of tumor cells.

Cervical cancer has a global prevalence and is the most common type of cancer in developing countries. It is the fourth most common cancer among women and annually more than 400,000 cases of this malignancy are reported in the world, which accounts for approximately 12% of the most common cancers among women [31]. Researchers point to several risk factors for cervical cancer. In a case-control study conducted in Mexico, factors such as the number of deliveries, the age of onset of sexual activity, and the use of oral contraceptives were examined. The prevalence of this cancer was 5 times higher in women who had given birth 12 times than in women who had given birth up to 3 times. It was also twice as common in women who started having sex after the age of 19 than in women who were under 15 when they started having sex. In addition, the use of hormonal contraceptives has been shown to increase the chances of developing cervical cancer [32].

According to many case studies, HPV infection has been identified as a major factor in the development of neoplasia and malignancy in the genital epithelium, and this is especially evident when there is a persistent infection with high-risk types of the virus [33]. Research over the past two decades has shown a strong association between cervical cancer and some types of human papillomavirus. In more than 90% of cases of invasive cervical cancer, the HPV genome is detected by molecular methods [34].

Pap smear test is used as a common test for screening patients around the world due to its relative ease and cheapness, but unfortunately, the number of false negatives in this test is very high [35]. HPV infections are usually asymptomatic, so methods to improve the screening process are needed to diagnose the infection. Epidemiological studies and efforts to develop vaccines require reliable methods to diagnose HPV genital infections and to determine the common genotypes in the infection [36].

Methods such as PCR, which detect the DNA of the virus, actually identify the cause of the infection, while cytological tests, such as Pap smear, identify the lesions and complications of the infection [37, 38]. PCR technique is considered one of the most sensitive methods for detecting HPV in genital tissues due to its ability to amplify viral DNA using specific primers, and its combination with sequencing as a confirmatory test increases its accuracy by 100%. Therefore, its use along with cytological methods can be significantly effective in increasing the resolution and accuracy of clinical examinations of patients [39].

Determining the common genotypes of the virus in a geographical area can also be very important in epidemiological studies and HPV vaccination development programs [40]. Experimental findings on the diagnosis of HPV genome in the samples indicate that the prevalence of carcinogenic types of HPV in patients studied in this study was 19.46%, which is consistent with the results of studies in some other regions of Iran.

In a study conducted in northern Iran, the presence of HPV in cervical cancer samples was shown to be 81.4%. This study shows that the frequency of HPV infection in cases of cervical cancer in Iran is also significant and comparable to other parts of the world [41]. Type 6 was one of the most common genotypes found in this study. Since cervical cancer usually occurs after 10-12 years after HPV infection, it is known as a complication that occurs in middle age. Findings show that in Yazd province, the highest prevalence of HPV in age groups (31-50 years) is 60%, while in many other countries, the highest prevalence of this virus is usually observed in lower age groups [21]. This difference is related to the onset of sexual intercourse.

In Iran, sexual intercourse usually begins with marriage and usually occurs between 20-30 years, but in Western societies, sexual activity usually begins after puberty. This could explain the incidence of cervical cancer in older Iranian women with HPV compared to many countries where the age of HPV infection is usually lower and in the age group of 21-39 years [33].

In some studies, some statistical populations are diagnosed in terms of patient pathology but show negative results in the HPV PCR test. There are several possible justifications for this: One is that the formation of malignant and cancerous lesions in these people was due to a carcinogen other than the HPV virus. Another is that according to a series of studies, the cause of false-negative results in PCR tests, especially in cases where pathological tests indicate the presence of malignant and invasive cancers, may be related to how the virus genome is replaced in the host cell chromosome [36].

According to one hypothesis, the cyclic genome of the virus is replaced in the chromosome of the host cell in such a way that it transforms the region of the gene that was the target of the PCR process and therefore changes the PCR results. Although the E2 gene region of the virus genome is usually degraded during the replacement process, in some cases the PCR primer binding sequences in the L1 gene region may be contiguous with the degraded regions of the virus genome, resulting in HPV DNA detection by PCR.

Another factor that can negatively affect the falsification of PCR results is the problems that arise in the process of preparation and extraction of DNA from formalin and paraffin-stabilized samples [22]. It has sometimes been observed that in studies using fresh tissue samples for PCR testing, the amount and percentage of HPV reported are usually higher than that of formalin-stabilized samples, which is probably due to abnormalities.

In the present study, the nested PCR technique was used to evaluate the frequency of the HPV genome. The results showed a significant relationship in the presence of the human papillomavirus genome. The present study, confirming the reports of some researchers, showed that the selection of an appropriate extraction method is important in improving the quality and quantity of DNA obtained and is very effective in the reliability of diagnostic results. In the DNA extraction stage, the cross-sectional preparation of tissue is very important. Cervical epithelial tissue has a high cell density. Therefore, the thickness of the cut should be appropriate and appropriate. If the thickness of tissue sections is not appropriate, PCR may interfere. If the cut thickness is low, the DNA concentration is low and if the thickness is increased, the protein degradation is incomplete and the impurity increases [16].

DNA extracted from samples is often high in concentration and contains factors that attenuate PCR, so it is best to use multiple dilutions of DNA in each sample for PCR to reduce the likelihood of false-negative responses. The presence of false-negative reactions can be due to the presence of many inhibitors in biological samples that have the potential to interfere with the PCR reaction. Inhibitors inhibit cofactor activity in PCR. On the other hand, these inhibitors can bind to the target site of Taq DNA Polymerase enzyme or directly inhibit the activity of this enzyme [11].

Therefore, these inhibitors can cause slightly erroneous results or false-negative results. In this case, the first step is to dilute the sample to reduce contaminants. In preparing the dilutions, it is necessary to observe the sterile conditions. In this study, we faced this challenge and this problem was solved by diluting the sample. The use of Nested PCR, which increases the sensitivity and accuracy of diagnosis, especially in low-titer samples of the virus, and also reduces the concentration of inhibitors that interfere with PCR performance due to its two-step concentration, can be very efficient [8].

High concentrations of primers with non-specific bonding cause dimers and non-specific products. Low primer also reduces bandwidth. Differences in the results of human papillomavirus genome identification in different studies may be due to the different populations studied, the sexual habits of the people in that community and the age of onset of sexual intercourse, the rate of human papillomavirus exposure, laboratory techniques and methods. Be used [42]. According to Table 3-4 (a), 3 men were simultaneously High risk, Intermediate risk, and Low risk, 8 were only Intermediate risk, 3 were Intermediate risk and Low risk and 3 were Low risk. According to Table 7, there were 34 low-risk women, 36 Intermediate risk, 7 Intermediate risk, and Low risk, 15 High risk, 3 High risk, and Low risk, and 3 High risk and Intermediate risk.

The largest and most comprehensive study to investigate the presence of the HPV genome in cervical cancer specimens was performed according to a standard protocol by the International Agency for Research on Cancer (IARC). About 1,000 women with confirmed cervical cancer from 22 countries around the world were included in the study. Frozen biopsy specimens of tumors in the central laboratory were examined for the presence of human papillomavirus DNA by PCR. The results of this study proved the presence of HPV-DNA in about 99.7% of the samples, which is a reason for the causal role of HPV in the development and progression of cervical cancer [43, 44]. This study was performed on 66 paraffin blocks of cervical cancer tissue by the Nested Multiplex-PCR method. Type 16 human papillomavirus was detected in 60% of the samples [45]. In several other separate studies conducted in Iran, the presence of the HPV genome in cervical cancer samples were reported to be 71.8%, 83.7%, and 77.7% [46].

In a combined analysis and a meta-analysis performed by the IARC, 3,000 and 10,000 cervical cancer samples were examined for the distribution of HPV types, respectively. Eight types of the most common types of HPV were identified in both series of studies, which are in descending order: HPV types 16, 18, 45, 31, 33, 52, 58, and 35. Among these eight common types, HPV-16 is the most common. Outbreaks and HPV-35 have the lowest prevalence. These eight types are responsible for about 90% of all cervical cancers worldwide [47].

Persistent infection with the human papillomavirus is the most important cause of the development and progression of cervical cancer, but only a small percentage of women infected with HPV develop cervical cancer. Therefore, there may be other risk factors for having an independent or boosting role of HPV in the occurrence of cervical cancer, such as having multiple sexual partners, initiating sexual intercourse at an early age, contracting the human immunodeficiency virus (HIV), smoking, oral contraceptives, and other pathogenic factors, although the exact mechanism of each is still unknown [48].

Therefore, it is necessary to study the molecular mechanisms of cervical cancer and the role of HPV in creating and facilitating the process of this disease [49].

CONCLUSIONS

Several types of vaccines have been proposed to control cervical cancer, some of which have been licensed by the World Health Organization. HPV vaccination is common in countries such as the United States and Canada. The structure of these vaccines consists of L1 and L2 viruses of the virus and they are made using the common epitopes 6,18,16,11 HPV. Given that some types of papilloma viruses have a specific regional prevalence and are also identified in cervical cancer; therefore, some researchers believe that epidemiological studies can be an effective aid in determining the appropriate structure of the vaccine. Therefore, in Iran, such studies will be very useful in selecting or designing a suitable vaccine.

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ORIGINAL ARTICLE

From the discovery of combat gases to insecticides and bioterrorism – a brief history

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Abstract: *The identification of organophosphate (OP) compounds dates back to the 1800s. OPare largely used pesticides in the agricultural field and households but prior use as a chemical weapon was considered during wars. Their toxicity on humans has been previously studied and addresses both acute intoxication and low-level chronic exposure. Symptoms of acute exposure describe a cholinergic syndrome with bradycardia, miosis, confusion, fasciculations, tearing, sialorrhea, diarrhea, bronchorrhea, and bronchospasm. The intermediate syndrome causes paralysis of the gait muscles, neck flexors, and respiratory failure. Moreover, unrestricted access to OP has increased the rate of suicide, accounting for around 30% of cases, increasing concern about OP availability. Western countries have drastically restricted access to OP by imposing regulations regarding handling these hazardous substances, but efforts have to be made to globally reduce this struggle.*

Keywords: *organophosphates, pesticides, combat gases, bioterrorism, poisoning*

INTRODUCTION

Organophosphates (OP) are substances that contain carbon and phosphorus atoms in their structure (Figure 1), but not all compounds with this component inhibit acetylcholinesterase [1]. Depending on their chemical substrate, they can be divided into four classes with individual physical features. OP has been widely used as a pesticide, compounds being spread both in the agricultural field and in households [2].

Figure 1: Structure of organophosphate compounds (ro.wikipedia.org)

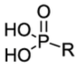

Their specific physical and chemical characteristics determine the duration of the pesticide effect, as well as the duration of persistence on applied surfaces or soil [3]. As a result, the degradation mechanisms differ from one OP compound to another [4].

Out of the OP, chlorpyrifos (Figure 2), is the world's most widely used organophosphorus insecticide, being effective against mosquitoes, and lice, while dichlorvos has shown beneficial in controlling plant lice (aphids), flies or spiders [1,5].

Figure 2: Chemical structure of Chlorpyryphos (cen.acs.org)

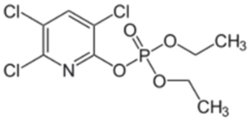

Diazinon (Figure 3), a thiophosphate compound, is effective against silverfish, fleas or ants, malathion is indicated against mosquitoes, wasps or insects that affect fruit trees [1]. Parathion (Figure 4) is a broad-spectrum insecticide, but its use has been restricted due to its toxic effects [6].

In addition to their use as pesticides, ester OP compounds are used as flame retardants or plasticizers in various industries. They are utilized for coating electronic instruments, such as antifoams or adhesives [7]. The OP

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esters are mixed with the other elements in the composition of the desired substances, without making any chemical bonds with them [8].

Figure 3: Chemical structure of Diazinon (en.wikipedia.org)

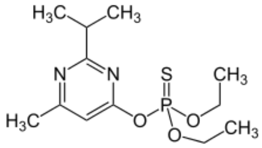

Figure 4: Chemical structure of Parathion (en.wikipedia.org)

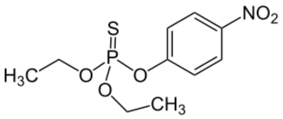

OP poisoning is a major cause of morbidity and mortality worldwide. Due to its widespread use in agriculture and domestic use, contact with OP substances has become very common. In countries where there are no imposed regulations on OP use in agriculture, the number of cases of intoxication is significantly higher [9].

A special aspect regarding the incidence of OP poisoning is their use as a method of suicide. Globally, this type of intoxication is considered one of the main methods of suicide, accounting for approximately a third of all cases, raising it as a top priority health problem [10].

HISTORY OF ORGANOPHOSPHATE COMPOUNDS – FROM DISCOVERY TO HAZARDOUS USE

With the perfection of ether synthesis methods by the chemist Alexander Williamson, the synthesis of organophosphates (OP) has been possible since the middle of the 19th century [11,12].

The initial discoverers of the OP class are traditionally considered to be the French chemist Philippe de Clermont and the Russian Wladimir P Moschnin, but the contribution of the Swiss Franz Anton Vögeli must not be forgotten [11]. In his laboratory, Gustav Magnus managed to synthesize two products resulting from the combination of ether with phosphoric acid [11]. Other sources claim that the Swedish Bo Holmstedt was the first to describe the structure of the first OP, namely tetraethyl pyrophosphate (Figure 5), in a letter to the French chemist Clermont [13].

However, there is evidence that indicates that several OP were synthesized by the French Jean Louis Lassaigne well before those mentioned above [13,14]. In the early 1800s, he succeeded to demonstrate the existence of phosphovinic acid, which was later synthesized by Jules Pelouze [12]. Later on, Anton Vögeli used these studies to elaborate on diethyl- and triethyl phosphate [11,12] (Figures 6 and 7).

Figure 5: Chemical structure of tetraethyl pyrophosphate (en.wikipedia.org)

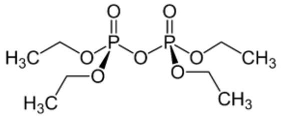

Figure 6: Chemical structure of Diethyl Phosphate (selleckchem.com)

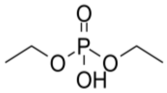

Figure 7: Chemical structure of Triethylphosphate (en.wikipedia.org)

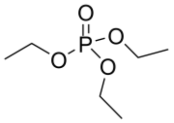

Although in recent research the French chemist Clermont has been invested with a major role in laying the foundations of OP, studies of the history of medicine indicate that W.P. Moschnin contributed to at least the same importance [15]. Moreover, there is evidence of joint activity of the two chemists in the same laboratory in Paris [13,15]. Despite being considered the parents of OP, Clermont and Moschnin were unable to understand and describe the effects of these substances [13]. After obtaining tetraethyl pyrophosphate, the first product that could inhibit acetylcholinesterase, Clermont's only description, obtained after tasting, distinguished that it is "a thick and sticky liquid, with an intense taste and a special smell" [11].

The chemists Ernst Ratzlaff and Adolf Schall identified, under the guidance of prestigious professor August Michaelis, a compound named diethylphosphoramidocyanate, similar to what would be called decades later "tabun"-diethylphosphoramidocyanate (Figure 8) [16].

Figure 8: Chemical structure of Tabun (researchegate.net)

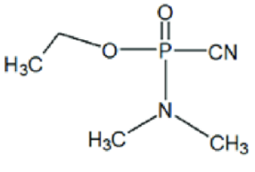

Research on the effects of OP was subsequently continued, at the beginning of the 20th century, by the Germans Willy

Lange and Gerda von Krüger, who first described the toxicity of these substances [17].

During the preparation of his doctoral thesis, Gerda von Krüger, together with his coordinating professor, Willy Lange, elaborated their most important scientific work: the description of toxic effects of OP, namely shortness of breath, photosensitivity, and disturbance of consciousness, which disappear within hours after ending exposure [17]. With the introduction of the Nazi regime, Willy Lange is forced to stop their study and reaches the United States, where he will devote himself to other research work [17].

The study of the toxic effects of OP was continued by the chemist Gerhard Schräder, who, in the attempt to obtain an OP-based insecticide, is exposed to it and needed medical care for several days [16]. When he returns to the laboratory, Schräder identifies the substance that caused the manifestations as ethyl-N-N-dimethylphosphoramido-cyanate, which he called “tabun” (from the German word "taboo") [16].

Throughout his activity Schräder successfully synthesized many OPs and created the first OP insecticide, octamethylpyrophosphoramide (Figure 9), marketed under the name E605 and used in agriculture [13]. With the end of World War II, this substance was taken over by the allied countries, where it was widely marketed under the name of parathion [13].

Figure 9: Chemical structure of Octamethylpyrophosphoramide (fischersci.com)

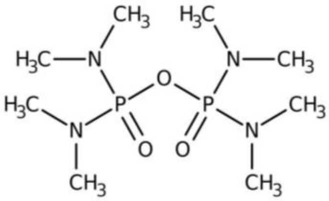

Although Nazi Germany secretly tried to create a chemical weapon based on OP, it has never been used on battlefields, despite repeated accusations from Stalin [13,18]. At the same time, other countries searched for chemical weapons based on tabun, sarin, or phosgene, but they were not used during World War II [13,18,19]. Although it has been proven that Japan used this type of weapon during World War II, it did not contain OP [19].

After the war, in the United Kingdom, the chemist Ranajit Ghosh, synthesized a new product called VX (Figure 10), thus creating a new class of OP (class V – from the English word "venomous") which was used as a chemical weapon [20].

Figure 10: Chemical structure of Vx (Wikiwand.com)

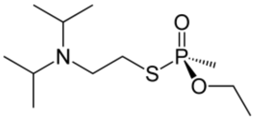

Meanwhile, the Soviet Union developed a similar compound VX, called VR or Russian VX (84). Despite international regulations regarding the use of combat gases (tabun, sarin, or phosgene), they have been used countless times during the First Gulf War between Iran and Iraq – tabun, Yemen – phosgene, Cambodia – phosgene, Angola – VX or Syria – sarin [21,22].

In addition to the armed attacks that used the combat gases, chemical terrorism has also developed, characterized by a series of attacks on the civilian population, the best known being the sarin attack on the Tokyo subway (Figure 11) which resulted in about 640 victims [23].

Figure 11: Sarin Attack in Tokyo (brittanica.com)

With the dissolution of the Soviet Union in the early 1990s, many documents were declassified, showing that during the Cold War, the Russian authorities secretly led a chemical weapon development program, called FOLIANT [20]. Thus it appears that VR served as a prototype for another class of OP called Novichok or class A [20]. This class of agents, with chemical weapon potential, came back to attention in 2018 when Russian spy Sergei Skripal and his daughter were attacked with a Novichok (Figure 12) compound [20].

Figure 12: Chemical structure of Novichok (chm.bris.ac.uk)

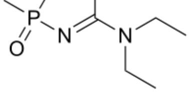

Beyond the development of chemical weapons, research continued to obtain insecticides that can be widely used. At the same time, the mechanism of action of OP has been discovered, as well as the possible antidotes that can be used in case of acute intoxication [13].

OP production continues to grow, with more than 200,000 tones of insecticides being used each year in the European Union [10]. According to Eurostat statistics between 2011 and 2016, pesticide consumption was steadily increasing, and only six countries reported a decrease in pesticide sales [24]. The largest decrease was in Denmark, at about 50%, followed by Ireland, Italy, Malta, Portugal, and Romania [24]. There are also countries in which the sale of pesticides has increased, the spike was in Austria (over 25%), followed by France, Slovakia, and Hungary [24].

The country with the highest pesticide production and consumption worldwide is China, where both imports and exports grow annually [10,25]. In the United States, the tendency to increase pesticide consumption is maintained, the most used compound being glyphosate [26]. In Brazil, pesticide consumption increased by more than 200% from 2000 to 2013 [27].

ORGANOPHOSPHATE TOXICITY AND CURRENT REGULATIONS

Toxicokinetics refers to the behavior of toxic substances in the body from the moment of penetration to elimination. Absorption, distribution, metabolism, and elimination of OP pesticides depend on the physical properties of the compounds, but also the dose involved [28]. Most OP compounds are fat-soluble and not ionized, which promotes rapid absorption [29]. OP is rapidly absorbed orally and by inhalation. The fat solubility of OP compounds favors the crossing of lipid membranes in the body, so they have a wide systemic distribution in all organs [29,30].

In the case of acute intoxication with pesticides, the onset of clinical manifestations depend on the characteristics of the agent involved, and the mode and duration of exposure [31].

Manifestations of OP poisoning are mainly characterized by a parasympathetic vegetative dysfunction, induced by the accumulation of acetylcholine [31]. According to the distribution of cholinergic receptors, specific signs and symptoms of acute intoxication correspond to the stimulation of nicotinic and muscarinic receptors [32].

Classically, manifestations of acute exposure are described as a cholinergic syndrome characterized mainly by bradycardia, miosis, confusion, fasciculations, tearing, sialorrhea, nausea, diarrhea, bronchorrhea, and bronchospasm [33].

The main features of the intermediate syndrome are paralysis of the gait muscles, neck flexors, cranial nerves, and respiratory failure caused by diaphragmatic paralysis and intercostal muscles [34].

Late poisoning manifestations manifest as polyneuropathy and occur more rarely than the intermediate syndrome [35]. Nevertheless, endocrine and metabolic dysfunction may occur even after low-dose OP exposure [36,37].

Considering that at present OP is widely used, we try to limit consumption by strict regulations regarding the quantities and concentrations applied to agricultural crops or in households [38,39]. For example, if in the early 1990s the number of pesticides exceeded 10 kg/ha in many Western European countries, European Union standards currently limit these quantities below 4.6 kg/ha [39]. Moreover, the use and marketing of OP are subject to the rules of the Rotterdam Convention which assessed, based on national reports, the risks associated with them [40]. In addition, the Convention had done a classification of these substances, which led to the development of worldwide use and labeling rules. Last but not least, substances considered to be at high risk for the general population were banned by the committee of the Convention [40].

Furthermore, in the European Union, there are regulations developed by the Parliament and the European Council, whose application has been mandatory in all member countries (Regulation (EC) No 1185/2009 of the European Parliament and of the Council of 25 November 2009 on pesticide statistics) [40].

The United States reinitiated regulatory processes of OP exposure since raising the possibility of OP's risk to children. Thus, programs targeting to assess exposure risk to pesticides were made as well as considering the menace of co-exposure to pesticides with similar toxicity mechanisms [41].

The United Kingdom has revealed neuro-psychiatric disorders in participants of the Gulf War that were designated as OP poisoning. Moreover, identifying increasing rates of depression in farmers exposed to OP made new regulations eliminate OP and carbamates off the market [41].

Despite European efforts to decrease the accessibility to OP, Japan has only imposed restrictions on the most toxic compound, namely methamidophos. Regarding other OP compounds, indications are of correct use without imposing limitations in agriculture or domestic fields [42].

CONCLUSIONS

The discovery of OP compounds involved various scientists that would first describe their action, namely inhibiting acetylcholinesterase, and then describe toxic effects [43]. OP toxicity proved useful in acting as pesticides in agriculture but the risk of developing a chemical weapon based on OP compounds has dominated wars [44].

Exposure to OP is a significant public health problem associated with long-term repercussions like affecting human brain development [9]. Despite an incompletely known toxicity mechanism, several countries have issued strict rules regarding OP use. Although Romania is one of the countries that has reported a global decline in pesticide trade, the increased accessibility of this type of product for people untrained in handling and using it suggests the need for stricter regulations.

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ORIGINAL ARTICLE

Futurology and monitoring in the field of virology to deal with emerging diseases

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Abstract: *Every year we see many changes in the world around us. One of these changes is the climate and weather, and it could be one of the reasons for changing behaviors between humans and animals, which could cause zoonotic diseases. We usually face new diseases that infect a growing population leading to a pandemic. Different reasons are leading to an increase in the emergence of new infections. Some of these diseases can be associated with zoonotic diseases and the diverse world of viral genetics and its failures. Furthermore, properties like mutations and also unknown effects of these mutations on the virulence of the agents make it challenging to trace new diseases. As we know, preventing emerging diseases is inevitable and viral diseases are dangerous, and some viruses can spread quickly and cause an endemic or pandemic. As was noticed during the recent pandemic, the need to study emerging diseases in the field of virology has become more and more apparent to us. In this review, we want to look at how we could monitor and control these diseases with new approaches and what should be done when a disease emerges. Moreover, we will investigate risk factors, ways to deal with emerging viral infections, new treatments that make the condition better when a person gets infected, and the future of these diseases. We will also study the ways to deal with pandemics with the new generations of vaccines.*

Keywords: *emerging-disease, viral disease, futurology, vaccination*

BACKGROUND

The world has witnessed various emerging infectious diseases (EIDs) that reemerge and cause multiple health and financial issues. For instance, in 2010, an unusual clinical image showed severe inflammatory syndrome which was along with vascular conditions similar to transient ischemic attacks or venous thromboembolism. There were several reports of this issue, and almost ten cases were recognized. The patients having underlying autoimmune diseases or hematologic malignancies were the primary targets for this condition [1,2].

Emerging infectious diseases are recent phenomena that affect humans with a high rate of spreading in different geographical areas. We have experienced more than 50 EIDs in the last 40 years. Most of these infections are re-emerging after a period, which has led to the adoption of the term “emerging and reemerging infectious diseases.” The term is used for infectious diseases that occur once in a region or among a population. It appears again with more virulence or with resistance to the drugs once used to eradicate them. We should also note that the re-emergence of infectious diseases happens at a higher occurrence rate than the initial emergence [3].

Various new and reemerging infectious diseases are threatening people all over the world, inflicting higher financial costs to societies along with increasing the risk of mortality. Some of the most recent infectious diseases include pneumonia, SARS, swine flu (H1N1), influenza, malaria, tuberculosis (TB), cholera, hepatitis, meningitis, food-borne gastroenteritis, chikungunya, Ebola virus diseases, campylobacteriosis, and salmonellosis. The most recent infectious disease threatening people all over the world is SARS-COV2 (Covid-19) [3–6].

There exist more than 50 EIDs in the world at the moment with varying levels of threat to public health. These infectious diseases can target human beings, animals, and plants. Studies indicate that climate change can also lead to the emergence of many of these diseases. Thus, controlling

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climate change and environmental factors can help control and eradicate infectious diseases[7].

Population growth has a fundamental role in the spread of diseases. It is also concurrent with the rise in speed and rate of travel across the earth which leads to the rapid dissemination of pathogens across the globe. This is partly due to globalization which has increased international trade, migration, and reduction in travel costs. Furthermore, an increase in leisure travels to previously unknown destinations is also one of the main factors in exposing people to various infectious agents [2,6–8].

During the last 20 years, researchers have encountered various species which were resistant to drugs, and some of them are classified as species that are resistant to several drugs, including methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci, multidrug-resistant or extensively resistant tuberculosis, carbapenemase-encoding Gram-negative bacteria, and extended-spectrum β-lactamase E. Coli. These are considered emerging pathogens and can rapidly disseminate among hospitalized patients and people of varying ages; hence, they require considerable attention [2].

These infectious diseases are a source of frustration among the general public. Notably, the emergence and re-emergence of all these diseases are dependent on the economic and geographical position of the region [3,9]. The good news is that most of these diseases are controlled through new chemotherapeutic methods, but that is not the case with all of them, since some of these agents are not controlled due to unknown etiology and lack of proper management methods [3,10,11]. Scientists have cited various problems as the reason for the onset of these infections, among which one can note population, improper food-processing methods, lack of awareness about hygiene measures, and problems in water distribution systems and sewage pipelines. Furthermore, the production of ineffective antimicrobial agents by pharmaceutical companies is another cause that makes it difficult to manage these infectious diseases. Infections acquired in the hospital and pathogens which are resistant to various drugs are also issues that can make the result of treatments uncertain [3,12,13].

Countries need to change their object of focus from healthcare and prevention to research on the determinants of health and primary healthcare provision. Political empowerment and creating a global initiative to increase the capacity of the health sector in developed and developing nations is another alternative that government officials need to consider to stop the spread and emergence of various infectious diseases [14,15].

Various drastic changes have been experienced during the last three decades in clinical virology laboratories. Direct diagnosis of the virus in clinical specimens is one of the main issues noticed in the last decades, which means the demonstration of virus particles (including viral antigens and nucleic acids) [16,17]. The application of new methods and reformulation of the old ones, demand for an easier test with higher accuracy and multiple applications, the trends existing in developing countries along with the detection of new concepts regarding microbial interactions are among the factors shaping molecular assays, development of diagnostic approaches, and changes in the interpretation of data obtained in the laboratory [18].

A considerable number of emerging infectious diseases are the result of zoonotic agents, and in some cases, vector-borne viruses are responsible for their emergence. Although resistance to antimicrobial drugs is another troubling issue, it is less likely to have bacterial or fungal pathogens as the cause of a pandemic [19].

In this study, we want to clarify how emerging diseases can be monitored and controlled and how we can deal with them in times of occurrence to stop or prevent the occurrence of a pandemic or endemic disease.

MONITORING AND OBSERVATION

Our local environment and global landscape is changing all the time. Due to the higher error rate of virus enzymes replicating their genomes, viruses containing RNA can adapt themselves to different conditions. That is the reason for the higher rate of emergence and re-emergence of diseases that are caused by RNA viruses [14]. For instance, the pandemics caused by influenza virus strains are caused by viruses that reside in host animals before infecting humans. Humans, horses, pigs, birds (domestic and wild), and sea mammals are among the species that can be infected with the influenza virus.

The present century has witnessed the death of over 20 million people in different countries due to Spanish influenza, which had a higher prevalence compared to HIV [18]. The ways to recognize the pathogens and distinguish test positive individuals from the negative ones are the first steps to controlling and monitoring emerging diseases.

New technologies and approaches to microbial interaction

CRISPR and next-generation sequencing (NGS) are among the recent technologies emerging in the market. A single reaction in NGS can parallel sequence a large number of DNA templates. It is quite fast and cheap at sequencing the complete genome of many microbial samples. Virology has various applications such as management of viral infections, figuring out the infection sources, investigating the immune response of the host, and selecting the proper intervention and treatment strategies [18,20,21].

NGS makes use of three methods to sequence the genetic material of the virus. The first one is identical to the Sanger method and is done through amplification and sequencing of the target. The second method is done through nonspecific amplification of the genetic content of the sample before NGS. The third approach enriches the genetic material of the virus and makes use of NGS to sequence them subsequently. NGS has higher throughput besides being fully automated and can find the genome mutations causing drug resistance. It is also able to target several viruses at the same time. The added benefit of NGS is that it can detect both known and new microbes despite being pathogen or commensal. NGS is mainly used for the detection of different drug-resistant variants of HIV and HBV in clinical virology.

The advantage of detecting the undetectable variants is not yet clear, but the evidence obtained from resistance tests of patients with low levels of virus and rare mutations (including patients with HIV) shows that they will have a great influence on routine clinical practice and platform for nucleic acid detection based on nucleic acid amplification and Cas13a-mediated cleavage of a reporter RNA which enables the detection of the target in real-time [22–24] SHERLOCK is among the best techniques which equal PCR essays in terms of sensitivity. It is used to detect one or several RNAs residing in the microbial strains. One of the best features of SHERLOCK is its ability to be combined with non-PCR methods used for amplification. SHERLOCK is widely used for the detection of nucleic acids from other viral threats, including the dengue virus and Zika. SHERLOCK is also important in terms of commercial usage since its reagents can be lyophilized and reconstituted on paper.

Researchers can also run it on body fluids without the need to be extracted before and provides the results in less than two hours [18,25,26]. DETECTOR (DNA endonuclease targeted CRISPR trans-reporter) is another technology that is similar to SHERLOCK in using Cas12a. The mere difference is that it works on DNA, and shows unspecific collateral single-stranded DNA cleavage activity once it recognizes the target sequence. DETECTOR has attomolar sensitivity in detecting DNA. Furthermore, it is widely used for detecting human papillomavirus 16 and 18, which are two human papillomaviruses that have a high tendency to neoplastic transformation. DETECTOR is easy to use and much faster than the existing methods of human papillomavirus detection. Moreover, you do not need to provide any special equipment for conducting DETECTOR. Hence, it can be considered a useful platform for clinics that lack resources, and it is a good option for conducting molecular diagnostics rapidly [18,27].

Previous multiplex assays combined the detected viruses according to various parameters, including primer and reagents, amplification reaction, sample processing compatibility, etc. Hence, the tests were mainly based on analytical methods.

Novel methods are capable of detecting a wide range of germs and viruses with the same clinical manifestations, entry method, and tissue tropism. This is the main reason for multianalyte tests known as syndromic tests.

There are some proprietary panels produced by pharmaceutical companies which are dedicated to infections of gastro intestine, respiratory system, central nervous system, and the infections that are transmitted sexually [18,28–30] Biocontainment laboratories that are located far from EVD treatment centers usually use RT-PCR as their main diagnostic test. Recent approaches make use of Point-of-Care ELISA-based assays for VP40 matrix protein. It provides considerable operational advantages and leads to the timely isolation of positive cases. Hence, it is a great tool to clear suspect cases with higher accuracy and sensitivity compared to the methods used earlier. However, the role it plays in field conditions is uncertain [31,32]

The existence of a centralized infrastructure for sequencing in areas that have a higher biological diversity which acts as the beginning sources of new human infections can help fast and timely detection of emerging pathogens. Furthermore, these centralized infrastructures can act as hubs to send resources to unknown locations in cases of new outbreaks. This infrastructure has the potential to search the environment regularly to figure out the spectrum of viral strains and species existing within phylogenetic families that can infect humans. Furthermore, it could be used for high-throughput sequencing to define all existing viruses [19].

An initiative of WHO named Research and Development (R&D) Blueprint works on decreasing the period from the initial reports of a public health emergency to the provision of test kits and diagnostic methods, treatments, and vaccines to rescue people’s lives and prevent the initiation of the health crisis. It also takes severe emerging diseases into account that lack any type of countermeasures to prevent their expansion. It was at the request of 194 Member States in May 2015 that WHO asked a group of specialists to develop a Research and Development (R&D) Blueprint for Action to Prevent Epidemics.

It was on 31 December 2019 that reports were sent to WHO about several pneumonia cases without a definite cause in Wuhan City, Hubei Province of China. Chinese authorities identified the coronavirus disease (COVID-19) as the causative agent on 7 January 2020 [4]. The R&D blueprint started its activity on the day it was informed of the pandemic to work on diagnosing, vaccinating, and therapeutic measures for eradication of the virus [4]. The infectious agents occurring for the first time pose various challenges due to a lack of knowledge about their identity, epidemiology, and pathogenesis, which are the case invariants of influenza or COVID-19. It is also difficult to identify the zoonotic reservoir which makes it more challenging to foretell the time, place, and possibility of an outbreak [33]. Most of the reports regarding emerging diseases are from developed countries. The United States accounts for more than a third of reports about emerging diseases [34] which shows the important role of geographical location in the emergence and spread of an infectious agent.

CONTROL, TREAT, AND FUTURE STUDY

Probable approaches to control and treat

Gene-based antigen-delivery technologies are the best methods to fight EIDs; however, some of these methods have not received the required licenses to be used in human medicine. Furthermore, in times of crisis when time plays an important role, these technologies can provide vaccines in the shortest time possible based on the information obtained from target-gene sequences compared to other methods which require the virus to be isolated and grown along with considerable containment [19]. Interference in the transmission of the virus from animals to human beings can help control zoonosis which is possible via vaccines that are self-disseminating. These vaccines can act on insects, animals, and also environmental reservoirs of the virus.

These methods work by the replication of the existing vectors of the virus that are engineered for the expression of the disease’s antigens. They are also used in cases when clinicians want to target specific groups of animals. The objective of animal vaccination is to keep pathogens of the disease in the animal reservoirs to prevent them from infecting humans. There are various issues including costs, possibilities, and the ensuing safety concerns that should be kept in mind during this method which are necessary for controlling the sources of these pathogens. Moreover, it is not possible to predict which animal pathogen can cause disease in humans. It should be noted that the usage of a CMV-based vector to vaccinate apes was suggested as a way to curb the transmission of the Ebola virus to people. The same method was also effective in protecting the animals against death [44,45].

Hospitals are the first place to have a measure to control the transmission of viruses. The staffs are at a higher risk in the initial outbreak; however, providing most centers with personal protective equipment and kits has prevented further transmission. It was also difficult to reduce the risk of transmission during the burial rituals. The main difficulty stemmed from making burial consistent with social and familial norms [31]. The world has witnessed different emerging and reemerging infectious diseases which have threatened it in terms of security, economy, and biodefense [46,47].

There were several diseases in the past few years including H1N1 (2009-Mexico) [48], SARS (2002-China) [49], MERS (2012-Saudi Arabia) [50], Ebola (EBOV) (2013-Africa) [51], Lassa virus (2018-Nigeria) [52], Zika virus (2015-Brazil) [53], and the COVID-19 pandemic (2019) [54] has made different scientists eager to devise strategies for quicker prevention, treatment, and/or controlling these epidemic viruses [44]. The number of fatal cases (case fertility rate) due to emerging and reemerging infectious diseases was high during the specified time, which indicated the need for immediate action to prevent their spread [44,55].

Various factors, including globalization, urbanization, travel, commerce, climate change, and the increasing elderly population are also influencing factors in the emergence of these diseases. Zoonotic diseases are also on the rise due to increasing contact with zoonotic sources. Traveling has also been on the rise due to rapid and comfortable means of traveling, and it makes it increases the distribution of pathogens all over the world. Furthermore, the elderly are far more at risk of these infections, which leads to a higher mortality rate. All these issues affect the way pathogens are distributed all over a previously endemic zone [56,57]. Due to the nonexistent nature of licensed antiviral drugs to prevent or treat emerging viruses, the treatment method is restricted to care and fluid- and electrolyte therapies. However, these prevention methods are also absent in most developing countries [58].

Cytokine-storm

Cytokine storm is regarded as one of the most lethal immune syndromes with a quick-spreading ability and highly activated T cells, natural killer cells, and macrophages. It also causes overproduction and release of many inflammatory cytokines and chemical mediators through immune or non-immune defense cells. Induction of hypercytokinemia, also known as cytokine storm (CS), is the main cause of the high fatality rate in immunocompetent individuals. It can also engender different pathological side effects such as shock, damage to the tissues, sepsis, the failure of several organs of the body, and edema. In some cases, lack of attention to these symptoms can lead to death.

Moreover, cytokine storm is concurrent with hemorrhagic fevers caused by a virus, and these immunopathological conditions can be due to several viruses including Lassa, Crimean, Congo hemorrhagic fever, Ebola, and Dengue fever (DF), and Marburg viruses [58,59].

Currently, there is no standard treatment to cure cases of cytokine storm and we lack drugs that are approved specifically to treat cytokine storm. Corticosteroids were not successful in treating cytokine storm in clinical testing. Furthermore, non-steroidal anti-inflammatory medicine was not able to treat cases of cytokine storm while used besides antiviral drugs. Hence, further randomized control is required in this regard. Some experiments are being conducted on new treatment options and they are in the preclinical testing stage. We hope that these methods prove effective in treating cytokine storm [58]. Although oseltamivir was being used in some countries of South Asia during the infection of the area with the HPAI virus, there is no report about how effective this method was. Furthermore, these clinical studies did not show a considerable reduction in the number of death cases due to using oseltamivir. The reports indicated that the rate of survival in patients receiving oseltamivir equaled 30% (13 of 34); while the survival rate in untreated cases was 25% (4 of 16). Hence, it can be said that the efficacy of oseltamivir treatment was dependent on timely administration. In cases when the treatment was within 5 days after the initiation of the symptoms, the rate of survival was 53%; however, postponing the oseltamivir administration to 6 days reduced the rate of survival up to 26% [60,61].

Several anti-inflammatory drugs (being administered alone or in combination with other therapies) have been assessed for the treatment of cytokine storm in influenza infections. However, it was seen in one of the studies that the utilization of anti-inflammatory drugs as a single treatment to cure cytokine storm comes with various challenges. The same study showed that curbing cytokine production by glucocorticoids did not lead to any form of protection for the mice infected with HPIA (A/Vietnam/ 1203/04 H5N1) [62]. The results obtained from another study indicate that combining COX-2 inhibitors with zanamivir has significant effects on survival since it acts by stopping the emergence of apoptosis and decreasing cytokine dysfunction [63,64]. Sphingosine-1-phosphate-1 receptor (S1P1R) agonists have been used for both suppressions of cytokine storm and treatment of pathogenic influenza virus infection in mice. The protection level was higher in this method in comparison to oseltamivir, and the rate of survival equaled 50%. However, the protection was the highest in cases receiving oseltamivir and AAL-R, and the reports delineate the survival rate equaling 96%. Being licensed, these drugs can help us in combating various pandemics, and they can be used to suppress various viruses including MERS, Ebola, and SARS which are the prominent causes inducing cytokine storm [65].

One of the novel approaches to treating cytokine storm involves the down-regulation of main signaling pathways or genes that are responsible for the regulation of various host responses including apoptosis or inflammation. The signaling pathway named C-Jun N-terminal Kinase plays a role in different mechanisms such as apoptosis, inflammation, and proliferation. The reports indicate that they are activated during the infection and replication of the influenza A virus [66]. Treatment of mice with short-term heat (39°C for 4 hours) led to a significant increase in HSP70, and it also increased the amounts of cytokines TNF-α, IFN-β, IFN-γ, and IL-6. The study showed that 57% of treated mice were alive after 14 days of being infected with HPVI. It is suggested that the positive effects of heat shock treatment on HPAI are correlated with the increased expression and protective effects of HSP70, which leads to transient down-regulation of the over-expressions of pro-inflammatory cytokines in the lungs [67]. It also makes interferon and TNF-alpha be expressed in response to infection and activates pathways to secrete specific proteins and antiviral cytokines. The results show that macrophages can be a good biomarker to assess the severity of influenza infection [68,69].

Following pathology identification, it is necessary to develop preclinical models to study the interactions between the virus and the host and select vaccination volunteers to define its features. Furthermore, the identification of the epitopes is required for the guidance of the vaccine and it has a role in the production of antibodies and lowering Nabs. All in all, these lead to antibody-dependent enhancement of the infection caused by the virus. Furthermore, the agencies, governments, and companies working in the field should share data and collaborate to have a strategic approach to the prevention of the next pandemic threats [70,71]. Due to the lack of effective drugs or vaccines to work against the COVID-19 pathogens, social distancing is the only method to hinder the spread of the virus. Hygiene measures are also necessary to prevent contact with patients. Using soap and water to wash hands along with using alcohol-based sanitizers are among the other preventive measures in the absence of effective drugs. It is noteworthy that COVID-19 pathogens can survive on surfaces for up to a few days;

hence, using disinfectants is usually effective in eliminating the pathogens residing on the surfaces [72].

The first step during a pandemic or endemic disease is to limit the contact between populations to stop transmission between human beings. In some cases, the diseases could be zoonotic and the animal vectors should be avoided. As said earlier about viral emerging diseases, we know that viral diseases mostly do not respond to drug-based treatments; hence, vaccination is the most effective choice to control emerging diseases [54].

Challenges in dealing with emerging diseases via vaccination

As noted earlier, vaccination remains the only effective method to stop the spread of viral emerging diseases. Vaccines can cause protective responses in the body against pathogens and reduce the causes of morbidity and mortality. Rapid vaccine production is essential to manage the outbreak and prevent the spread of the viral agent [44]. The history of using the vaccine to deal with viral emerging diseases dates back to 1938 when the U.S. army soldiers received influenza vaccines during World War II [73].

Vaccines have higher efficacy than other treatment options; hence, it is necessary to develop vaccines for all emerging infectious diseases. However, vaccine production is not readily possible for some viral infections due to the unknown identity of emerging pathogens, inadequate animal models, cost and time required for production, licensing, and global distribution, to name a few [74].

Vaccines are tested on young, healthy volunteers. This method was used for Dengue and Influenza virus; however, there should be an accepted treatment option if any problem occurs. Licensure and final approval of the vaccines are other issues that need to be considered. Rapid development and administration of a safe and effective vaccine is another challenge [75,76].

Long-lasting protection by making the body produce antibodies against the pathogens in question is the criterion to measure the efficacy of vaccines. The study conducted by Nakaya et al. analyzed the antibody responses following influenza vaccination on day 28 vs. 180. They emphasized finding pathways that were related to the waning or persistent reaction of the antibody [77].

The phrase ‘synthetic vaccinology’ is used in cases where only sequencing data are enough to start designing vaccine antigens and reagent production. Recent developments and advances in the field of protein engineering, understanding of the assembly of virus-like particles (VLPs), and the identification of other self-assembling molecules that improve immunogenicity by optimizing antigen display make it easier to adjust the vaccine-induced immune responses to specific antigens [19,78].

Some viruses can escape the trap of the immune system. The same strategies cannot be used for all cases of virus emergence, and there need to be new strategies for antigen design and formulation, pathway, sequence, and the timing of administration in some cases, such as extreme genetic plasticity and antigenic variability (HIV and Influenza virus), persistent infection and latency (HIV or Herpesviruses), or multiple distinct serotypes (rhinoviruses). Removing these biological obstacles of 'difficult' viral pathogens can provide methods to use in pre-pandemic vaccine development or rapid vaccine development in cases of emerging viral pathogens [19].

There are two types of mRNA vaccines developed so far. The first is a conventional non-replicating vaccine, and the other is a self-amplifying vaccine. A mature mRNA molecule is produced by the IVT method conducted a plasmid of the of a DNA template, which has the sequence for RNA polymerase that is dependent on DNA. It includes an open reading frame that can encode the required antigen, the 50 and 30 flanking UTRs, the terminal poly(A) tail, and the 50 caps. The host's cellular machinery translates the vaccine in vivo after receiving it and provides the corresponding post transnationally modified antigen. This is the process through which the vaccine mimics being infected naturally in vivo. These vaccines can activate the immune system by attaching to receptors, thus creating immune responses. The produced mRNA vaccines can make the body immune for a long time against various infections caused by viruses such as Ebola, Zika, influenza, Dengue, and other viral diseases [44,74,79].

Adenoviruses are mostly used viral vectors for priming the immune system. They can lead to both cellular and humoral responses. In clinical trials with adenoviruses, the existence of an anti-vector can challenge the vaccine response. To prevent immunity, the vaccine platforms for human beings are usually taken from adenoviral vectors obtained from animals. In some cases, rare species are also used for these purposes. Two vaccines are made of viral vectors: Imojev and Dengvaxia. The former is a vaccine used for JEV and the latter is a Dengue vaccine. Both these vaccines are produced by Sanofi Pasteur. Chimeric YFV is used as a vector in both of them. They work by propagating chimeric viruses inside the cell culture [44,80].

There are also protein-based recombinant vaccines that contain the immunogenic protein of the pathogen it aims to target. The production of these vaccines requires the identification of the pathogen. Bioreactors are used to produce these vaccines on a large scale depending on the posttranscriptional pattern of modification required by the use of heterologous expression systems, like yeasts, insects, bacteria, mammalian cell lines, or plants.

These vaccines are safe due to the lack of genetic information derived from the pathogen, and there is no need to live pathogen manipulation during the manufacturing process. They are suitable in times when a quick response is needed to an epidemic condition. It is because the production process can begin after the virus genome has been sequenced, and there is no need for virus isolation. Vaccines based on protein can be obtained during the production of recombinant virus subunits (SUVs). It is also possible to administer them along with adjuvants for enhancing the immune reaction of the host against the recombinant viral antigens [44,81].

CONCLUSION

It is estimated that zoonosis accounts for about 60% of diseases stemming from infections. The source of most infectious agents is wildlife, and increasing contact with wildlife can increase the occurrence of infectious diseases. Climate change is another issue leading to vector-borne diseases; however, it needs to be further evaluated to see the exact influence of climate alterations on the occurrence of the agents causing infections. Recently, various platforms are used to design vaccines which include viral-vector vaccines, nucleic acid vaccines, and recombinant protein-based vaccines.

Studies have revealed the overlap between animal and human ecosystems as one of the reasons for COVID-19 to pass the species barrier. Hence, there is a need for coordination with veterinary experts and the submission of laws to control the animal trade.

Our suggestion is the establishment of working groups and scientific consortia to govern the design process of vaccine antigens. The produced antigens should lead to the production of cross-reactive antibodies. These working groups should also look for novel vaccine administration methods to directly target the agents affecting immunity. Each pandemic enhances our scientific knowledge regarding the induction of cytokine storm by viruses. Identification of novel therapies for the treatment and control of infections requires enhancing our knowledge about the complicated interaction between antiviral immune responses of the host and the ability of the virus to change these responses and lead to immune dysfunction.

It is predicted that the world will witness the emergence and re-emergence of various zoonotic and mosquito-borne viruses in the future. The natural mutation will be the process through which these viruses evolve, and they will pose a real threat to public health. It will be difficult to protect against them due to the lack of proper therapeutic procedures including vaccines or drugs. Hence, it is necessary to be prepared for them and work on novel approaches for the treatment and control of these pathogens. In particular, the development of methods targeting the route taken advantage of by viruses provides the opportunity for effective treatment methods in different viral strains.

Almost all outbreaks have been with the nosocomial transmission, and there are several cases of transmission in the hospital. Hence, healthcare sector workers should be among the first groups receiving vaccination when a pandemic or endemic occurs. Our study showed that zoonotic diseases play the main role in emerging diseases, especially emerging viral diseases. Hence, the role of the veterinarian as the frontline confrontation with emerging diseases is quite clear in preventing the occurrence of a pandemic. Because there is no effective cure for viral emerging diseases other than prevention and control, all nations should have a group of veterinarian scientists to control and deal with the animal reservoirs and vectors to prevent a pandemic [19,78,82–88].

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Authors’ Contributions

Dr. H. Esmaeili and Mr. M. Baqerkhani both developed the concept, design, and definition of the intellectual content of this article, Dr. A. Soleimanzadeh and Mr. M. Baqerkhani both developed the literature search, data acquisition, data analysis and statistical analysis of this article. Dr. M. Farzanehpour and Mr. M. Baqerkhani both developed manuscript preparation for this article. Dr. M. Farzanehpour developed manuscript editing and manuscript review. Dr. M. Farzanehpour is in charge to take responsibility for the integrity of the work as a whole from inception to published article and is designated as 'guarantor'.

Ethics approval and consent to participate

This study was executed under the supervision of the ethical committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (ethical code IR.BMSU.REC.1399.555 (.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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ORIGINAL ARTICLE

Investigating the effect of methylprednisolone pulse on the treatment of back pain

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Abstract: *Background: Pain is a common problem afflicting humanity. Back pain is a musculoskeletal disorder with a high prevalence; this pain may occur to anyone at least once during their lifetime.*

Aim: the present study was performed to investigate the effect of the use of methylprednisolone pulse on the pain status of patients suffering from back pain in Ilam city.

Methods: This study is a clinical trial conducted as double-blind in 2021 in Ilam city with pretest and post-test groups plus a control group. The patients referred with vertebral disc herniation diagnosis suffering from diffuse pain in the right leg were assigned into test and control groups. A methylprednisolone 500 mg IV would be administered to the patient as pulse inside DW 500cc serum. The extent of pain was compared before and after the intervention at one, two, three, and seven days later. Placebo was applied for the control group patients. The data were analyzed by SPSS 16 as well as based on descriptive and analytical tests.

Results: According to the findings, no statistically significant difference was observed between the demographic characteristics of patients receiving methylprednisolone and patients in the control group ($p<0.05$). Also, the result showed the comparison of pain scores of patients in the two groups. According to the findings, the M (SD) pain score in the experimental group was 6.59 (0.88) before the intervention and 1.76 (0.69) in the week after the intervention, which was statistically significant ($P=0.000$). Also, the changes in the control group's score before intervention were 6.82 (0.71) and after the intervention was 6.77 (1.12) ($P>0.05$).

Conclusions: Considering the effect of methylprednisolone on mitigating the pain of patients suffering from back pain, it is suggested to use injective methylprednisolone for reducing the pain of patients suffering from back pain.

Keywords: *methylprednisolone, back pain, pain*

BACKGROUND

Pain is a common problem afflicting humanity [1]. Pain causes the development of numerous problems and constraints for the patient including reduction of quality of life, psychological health, life expectancy, daily activities, spiritual health plus related factors [2-5], as well as the development of depression, disability, and stress [6,7]. Back pain is a musculoskeletal disorder with a high prevalence; this pain may occur to anyone at least once during their lifetime [8]. Today, with changes that have occurred in the lifestyle of people, different groups may experience this pain, and this disease may happen in response to ergonomic-mechanical, as well as individual and social factors [9]. Back pain is a common cause of visits and medical interventions. Chronic back pain has considerable effects on functional status, constraining occupational activity, and certain socioeconomic consequences [10,11]. Among skeletal disorders, the damage related to back pain claims the largest share and in addition to healthcare aspects, it has also attracted the public attention economically [12]. The causes of this disease are numerous and its precise identification is sometimes difficult. Back pain is considered an important health problem that is common in all countries, especially industrial ones. Musculoskeletal disorders are typically

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associated with symptoms such as pain as well as permanent disorders in muscles, joints, nerves, and tendons. The effects of back pain may go beyond pain, which can be very serious and profound, causing incurrence of indirect costs, diminished capacity of individual ability, and increased risk of suffering from other medical conditions [13-15].

In pain etiology, various factors are involved, and one of the common features in all of them is neck movements; as these movements increase, so does the pain in that region [16]. Radiculopathy refers to nerve root disorders that may develop in response to structural injuries such as disk, tumor, trauma, vascular disorders, or infection such as viruses [17]. The radicular back pain is a kind of neuralgia that develops following sensory root stimulation or the dorsal root ganglion of the spinal nerve. Radicular back pains occur because of the generation of abnormal impulses in the dorsal root ganglion and its transfer through the peripheral nerve axon [18,19].

Different studies have been performed for the treatment of back pain and various therapeutic protocols have been criticized and examined. These methods include nonpharmacological methods such as educational intervention [20], exercise interventions [21], spiritual care [21], distraction [22], as well as pharmacotherapy [23]. There are different drugs for mitigating the pain in patients suffering from radicular pain including methylprednisolone. Prescription of this drug can help treat spinal nerve injury given its anti-inflammatory properties, immunosuppression, and improving neurological recovery [24].

OBJECTIVES

Identification of pain mitigation methods and especially attempts in choosing the best pain alleviation method in neurosurgery patients is one of the most important duties of neurosurgeons. This can be effectuated only through clinical research and performing interventional studies in this regard. Accordingly, the present study was performed to investigate the effect of the use of methylprednisolone pulse on the pain status of patients suffering from back pain in Ilam city.

METHODS

Study Design

This study is a clinical trial conducted as double-blind in 2021 in Ilam city with pretest and post-test groups plus a control group. The patients referred with vertebral disc herniation diagnosis suffering from diffuse pain in the right leg were assigned into test and control groups. A methylprednisolone 500 mg IV would be administered to the patient as pulse inside DW 500cc serum. The extent of pain was compared before and after the intervention at one, two, three, and seven days later. Placebo was applied for the control group patients.

Study Population

The statistical population in this study consisted of patients referring to hospitals, clinics, and practice offices of physicians in Ilam where the radicular pain. The sample size consisted of 94 patients; after the intervention, 12 of them were excluded, and eventually, the data were analyzed by 42 patients in the experimental group and 40 patients in the control group.

Inclusion and Exclusion Criteria

Inclusion Criteria

Informed consent for participation in the study, aged older than 20 years, at least three months past the disease, definite diagnosis of the disease by a neurosurgeon, and patients suffering from diffuse pain in the right leg were the inclusion criteria.

Exclusion Criteria

Desire to leave the study at any time, history of consuming medications other than the drugs of the intervention, history of any other disease affecting pain, and requiring surgery for radicular pain treatment constituted the exclusion criteria. Furthermore, patients with underlying problems, spondylolisthesis, spinal tumors, and congenital lesions were also excluded.

Ethical Approval

Receiving ethics code in the research with the code IR.MEDILAM.REC.1400.118, voluntary participation or lack of participation in the study, freeness of all visits, medications, and costs related to the interventions, confidentiality of patient information, explaining the objectives of pharmacological intervention with an understandable language for patients.

Study Tools

Demographic characteristics form and pain scale were used. VAS pain scale was applied to determine the severity of patients' pain. This scale is a 10 cm graduated ruler, which was used here for scoring the most severe pain and zero score according to the patient's report. In this instrument, acquiring a score 1-3 represents mild pain, a score 4-7 moderate pain, and a score 8-10 severe pain (25).

Statistical Analysis

The data were analyzed by SPSS 16 as well as based on descriptive and analytical tests.

RESULTS

According to the findings of Table 1, no statistically significant difference was observed between the demographic characteristics of patients receiving methylprednisolone and patients in the control group (p<0.05).

Table 2 showed the comparison of pain scores of patients in the two groups. According to the findings, the M (SD) pain score in the experimental group was 6.59 (0.88) before the intervention and 1.76 (0.69) in the week after the intervention, which was statistically significant (P=0.000). Also, the changes in the control group's score before intervention were 6.82 (0.71) and after the intervention was 6.77 (1.12) (P>0.05).

Table 1: Comparison of demographic characteristics of patients under study

Pain		Experimental Group	Control Group	P
		N (%)	N (%)	
Gender	Male	23 (54.8)	25 (62.5)	0.48
	Female	19 (45.2)	15 (37.5)	
Marital status	Married	29 (69)	28 (70)	0.92
	Single	13 (31)	12 (30)	
Economic situation	Weak	19 (45.2)	10 (25)	0.11
	Medium	18 (42.9)	24 (60)	
	Excellent	5 (11.9)	6 (15)	
Age, M (SD)		51.87 (18.46)	51.52 (9.40)	0.91

Table 2: Comparison of demographic characteristics of patients under study

Pain	Experimental Group	Control Group	P, F
	M (SD)	M (SD)	
Pain pre intervention	6.59(0.88)	6.82(0.71)	P=0.2, F=2.38
pain after 1 days	4.88(1.08)	6.72(0.59)	P=0.000, F=9.34
pain after 2 days	3.83(0.62)	6.45(0.95)	P=0.000, F=11.43
pain after 3 days	2.50(0.63)	6.35(1.23)	P=0.000, F=14.04
pain after 7 days	1.76(0.69)	6.77(1.12)	P=0.000, F=4.66

DISCUSSIONS

This study was conducted to explore the effect of methylprednisolone on pain mitigation among patients suffering from radicular back pain. The group of patients suffering from back pain claims a considerable percentage; in the meta-analysis by Azizpour et al., it was found that the prevalence of back pain in 20 papers with a total sample size of 5670 subjects was 51%, mostly afflicting 15-50-year-old age group [26] as the largest prevalence. As such, this group of patients requires special attention.

In this study, the usage of methylprednisolone drug could lead to a reduction of patient back pain. In a retrospective study by Dobohue et al. to compare methylprednisolone and dexamethasone drugs on pain mitigation, it was found that although both drugs could reduce the patient's pain, there was no significant difference between the two drugs against the patient pain mitigation. Further, methylprednisolone at two months following the intervention had caused 65% pain reduction for patients, which was 75% for dexamethasone [27]. In the study by Li et al., to explore the effect of methylprednisolone on neuropathic pain, they found that methylprednisolone could lead to improvement of quality of life and mitigation of pain. In that study, the extent of pain reduction was reported as about 50% or more [28].

The findings of other studies have also concurred with the present study results. In the meta-analysis by Liu et al. analyzing six clinical trial studies, 350 patients met the inclusion criteria. The results of this meta-analysis showed that usage of methylprednisolone at 6 and 24 h post-injection could result in pain alleviation for patients. Further, injection of this drug contributed to reduced nausea and vomiting for the researched patients [29]. Also, in the study by Lunn et al. on patients with total knee arthroplasty, again methylprednisolone had led to a reduction of pain and fatigue as well as an improvement in sleep quality [30]. The results of the mentioned studies [29,30] have been in line with the findings of the present study regarding the effect of methylprednisolone on pain alleviation in patients.

CONCLUSIONS

Considering the effect of methylprednisolone on mitigating the pain of patients suffering from back pain, it is suggested to use injective methylprednisolone for reducing the pain of patients suffering from back pain.

Authors' Contribution:

MH, KK did study conception, data analysis, and manuscript writing. MH, KK did data collection and manuscript writing. MH, KK did data collection and manuscript writing. Both authors (MH, KK) contributed to all stages of the article.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Approval:

The Ethics Committee of the Ilam University of Medical Sciences approved (IR.MEDILAM.REC.1400. 118).

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ORIGINAL ARTICLE

Musculoskeletal sports injuries of the upper limb in children

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Abstract: *The participation of children and adolescents in sports is advantageous in many ways regarding their physical and mental health, promotes cooperation and socialization between them, prevents diseases, such as obesity, and also sets the base of a healthy lifestyle in adulthood. In addition, childhood per se is characterized by an increased level of physical activity. Nevertheless, despite the multiple benefits of physical activity, it does not lack the possibility of unfortunate events or harmful conditions. The inner need of the athlete for excellence and recognition, the effort to reach or keep on an elite level of sports, early sports specialization, and pressure derived from family and coaches often lead to sports injuries-diseases either acute or chronic. Many of these conditions sometimes are underestimated or undiagnosed in the Emergency Department, especially if the main complaining symptom does not correlate with the disease. This non-systematic literature review aimed to identify the most common upper limb sport related-injuries and conditions among pediatric athletes. These injuries are described into groups depending on the anatomical area e.g shoulder girdle, elbow joint, and wrist.*

Keywords: *sport-related injuries in children, sport-related injuries, pediatric athletes, musculoskeletal injury in sports, sports medicine, little league shoulder*

INTRODUCTION

Nowadays, children are encouraged to participate in sports at higher levels at a much younger age than before [1]. As a consequence, there are increasing numbers of young athletes visiting their pediatricians because of sports-related injuries or asking for a piece of advice regarding injury prevention [2]. However, children are not "small adults". Growth plates act as stress risers in the distal upper extremity, resulting in injuries that may have a serious impact on a child's overall growth, joint alignment, and/or extremity length [1]. Injuries of the ligaments in children usually present as avulsion injuries. Moreover, attention should be paid to comprehension of the soft tissue attachments of any bony spot seen in imaging studies.

From an economic standpoint, the economic burden of the above-mentioned injuries is quite considerable, especially among children and adolescents. Additionally, these injuries may have a long-term impact on the musculoskeletal system, resulting in decreased levels of physical activity [3].

This non-systematic literature review aimed to identify the most common upper limb sport related-injuries and conditions among pediatric athletes. The inclusion criteria for the selection of the articles were concerning pediatric athletes, musculoskeletal sport-related injuries, English language, published until October 2021 and access to the full text. The PubMed, Scopus and Google Scholar database were searched with the terms "musculoskeletal sport-related injuries in children", "youth" or "pediatric athletes", "musculoskeletal injury in sports". Both review articles and original research articles were used to synthesize this review with a focus on contemporary and seminal papers.

SHOULDER GIRDLE

Little League Shoulder

Most often seen in the dominant shoulder, this injury refers to an undisplaced type 1 Salter-Harris fracture (epiphysiolysis) of the proximal humerus and affects mostly young athletes involved in throwing sports (e.g. baseball pitchers) or in excessive overhead activity (e.g.

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tennis)[1,2,4,5]. Young males are more commonly affected than females, while the peak prevalence of the disease presents at the time of the most rapid skeletal growth gain (11-16 years old for males) [4-6]. This injury is the result of the repetitive rotational and traction forces applied on the epiphysis, especially during the late cocking phase, with the humerus at maximum external rotation and abduction, and acceleration phase, where strong internal rotation and adduction forces are applied [7]. The increased overall number of throwing events is considered the most important risk factor for the prevalence of the disease. The patient's history may be remarkable for shoulder pain during sports that are alleviated at rest or correlated with an increase in practice intensity. Another complaint may be the deterioration of their athletic performance due to a decrease in throwing accuracy or velocity. On physical examination, glenohumeral internal rotation deficit (GIRD) may be noted. Radiographs are remarkable for physeal widening [5]. Sometimes, a radiographic evaluation of the contralateral shoulder is essential for the diagnosis [4]. At the late stages of the disease, premature fusion of the physis is noted, accompanied by angular deformity of the proximal humerus. Magnetic Resonance Imaging (MRI) of the shoulder is necessary for the diagnosis of the disease in patients with normal radiographs. In addition, MRI is useful for the evaluation of other soft tissue lesions, such as a rotator cuff (RC) tear, subacromial impingement, Superior Labrum Anterior to Posterior (SLAP) lesions, labral tears, etc [4,8]. The first line of treatment should be the cessation of throwing for at least 3 months. If symptom resolution is achieved, physical therapy is recommended with an emphasis on the strengthening of the shoulder girdle and posterior capsule stretching. Return to the sport should be gradual, and only after improvement of throwing technique through the appropriate practice sessions. Complications are rare thought serious, because of the developmental deformities of the physis that are included (premature physeal closure, growth arrest, upper limb length discrepancy, angular deformity, humeral head osteonecrosis) [4,5,8].

Glenohumeral Internal Rotation Deficit – GIRD

Young throwing athletes, such as baseball pitchers, even as young as 10 years old, are susceptible to this disease [9]. By definition, GIRD results in loss of internal rotation of the affected shoulder (more than 20°), compared to the contralateral shoulder [10]. Responsible for this range of motion (ROM) loss is considered the posterior capsular-rotator cuff tightness, which in turn causes abnormal shoulder deceleration during throwing [9,10]. At first, tightness results in a posterosuperior migration of the humeral head, as well as anterior capsule stretching, and, if left unaddressed, it leads to increased humeral head retroversion and abnormal shoulder functionality [5]. This condition predisposes young athletes to serious conditions, such as rotator cuff tears, internal impingement, labral tears, SLAP lesions, scapular dyskinesia, and Ulnar Collateral Ligament (UCL) injury of the ipsilateral elbow [5,9,10]. The patient's complaints include vague shoulder pain, stiffness, and the necessity for extended warmup or deterioration of their athletic performance. Physical examination should include meticulous shoulder ROM assessment and comparison with the contralateral limb in every thrower, who reports shoulder pain [6]. Furthermore, the physician should verify that the internal rotation ROM loss compensates with an equivalent external rotation ROM increase [4,10]. Plain radiographs rarely reveal abnormal findings, while MRI assessment of the soft tissue may reveal concomitant lesions. Management of GIRD requires rest from throwing activities for 6 months and physical therapy focused on posteroinferior capsular stretching, as well as, rotator cuff strengthening, and, fortunately, the prognosis is excellent for the majority of young patients [5][6]. Conservative treatment failure may require surgical management. Surgical management is, also, indicated, if concomitant lesions (e.g. rotator cuff tear) are present.

Rotator Cuff Disease

Rotator cuff tear is considered mainly an adult disease due to its rareness in underaged patients. Only 1.4% of children addressing a physician for shoulder pain are affected by rotator cuff tear [11]. Nevertheless, increased sports participation and early specialization in overhead activities result in a rising tendency of rotator cuff pathology at these ages [4,5,11,12]. Swimmers, baseball pitchers, throwers, tennis and volleyball players are particularly vulnerable to rotator cuff internal impingement, tendinitis, and, ultimately, tear because of the repetitive overhead and abduction-external rotation motion [5,11]. As far as swimmers are concerned, the term "swimmer's shoulder" is used to describe an overuse condition associated with impingement of the humeral head and the rotator cuff on the coracoacromial ligament [5]. It is difficult to make management recommendations on the rotator cuff tear in young patients because of the rarity of the disease. Even in the best available literature, information derives from case reports or small case series [11,12]. Each patient should be managed individually, and it is essential to take into consideration the level of the athlete, the necessity of a return to the pre-injury level, the injury as an entity (e.g. number of tendons affected, partial or full-thickness tears, concomitant soft tissue lesions, etc) [11]. Excellent functional outcomes in rotator cuff surgical repair are reported in limited case series studies although overhead athletes may not be able to play in the same position after surgery [11,12].

Shoulder Instability

Anterior shoulder dislocation is much more common (95%) than posterior dislocation or luxatio erecta [13,14]. About 1.7% of the general population is affected and 20% of the patients are younger than 20 years old, with a higher incidence in male patients [15]. Recurrence chance increases as the age of first dislocation decreases. The recurrence rate is as high as 90-95% for the age of the first dislocation under 25 years old [16], and in individuals with open physes escalates to almost 100% [13]. Nevertheless, some studies report significantly lower recurrence rates (33%) in ages of the first dislocation under 13, while for ages 14-17 recurrence exceeds 90% [17]. Risk factors for re-dislocation are age as abovementioned, male sex, contact sports, ligamentous laxity, and glenoid bone loss [4, 6, 13, 16, 17].

Patients with shoulder dislocation on examination present with asymmetry due to loss of the normal round shape of the shoulder, moderate to severe pain, and numbness, while the dislocated arm is usually supported by the healthy arm. The physical examination should always include the neurovascular assessment (especially the axillary nerve) of the affected arm. Plain radiographs are essential not only to confirm the diagnosis but also to exclude the fracture, before the reduction [6]. Many reduction manipulations have been described, such as the Hippocrates method, Kocher, or Stimson [16]. Some of them include the use of hard objects (e.g. the doctor's heel in the Hippocrates method) to apply the necessary reduction force on the humeral head in the patient's axilla [14]. Those techniques present an increased risk for neurovascular iatrogenic injuries due to the compression-traction of the axillary vessels, as well as the brachial plexus, and should be approached with caution [14]. The patient requires adequate analgesia and muscle relaxation medication, accompanied by psychological support, to achieve an effortless and painless reduction as soon as possible, with the fewest required manipulations. In case of failure to achieve a reduction in a conscious patient, general anesthesia might be necessary, especially in patients with heavy musculature. Reduction is usually felt by both the patient and the physician, by palpation, hearing, and, also, visually as the shoulder regains its normal round shape. Post-reduction neurovascular assessment should be performed, and a plain radiograph is necessary to confirm reduction and exclude any manipulation-related fracture [14]. After the reduction, a shoulder sling is used [6,16]. Position of immobilization in external rotation and adduction, despite support by many authors in the past, seems to have similar results in soft tissue healing and recurrence rate compared to immobilization in internal rotation and adduction [13,16]. The duration of immobilization is, also, a matter of debate [13,16].

Existing literature supports surgical stabilization after the first episode of shoulder dislocation, as far as active young adults are concerned. On the other hand, such a literature unanimity does not exist, regarding underaged active patients, who experience their first dislocation episode. According to Franklin et al. [15], conservative management of underaged patients leads to a higher recurrence rate (approximately 70-73). In addition, return to play rates are significantly higher for the athletes who underwent surgery (95.3% operative vs 41,3% nonoperative, Zaremski et al.) [18]. In conclusion, young active underaged patients are more prone to re-dislocation, so the benefits and drawbacks of both operative and nonoperative management should be thoroughly presented and discussed with the patient and the parents, to choose the best available method of treatment [15].

ELBOW

Ulnar Collateral Ligament Injury

Growth plate injuries are much more common in skeletally immature patients than injuries of the attached ligaments [4,5,19]. However, due to single-sport specialization and the intensity of throwing programs at an early age, ulnar collateral ligament (UCL) injury rates seem to rise among pediatric and adolescent overhead athletes population [20,21]. In pediatric patients, injuries to the anterior bundle of UCL are more common than in the posterior bundle [21] and might refer to partial or full tears, attenuation, or insufficiency of the ligament [5,22]. In cases of acute injuries, the patient usually experiences a popping sensation associated with swelling, ecchymosis, and pain on the medial or posterior elbow aggravated during late cocking and acceleration phases of throwing [5,13,22]. The valgus stress test of the elbow may indicate instability [22]. Patients with chronic injuries complain of vague medial elbow pain and deterioration of their throwing performance [22]. Plain radiographs are necessary to exclude pathologic findings, such as fractures-epiphysiolyses or avulsion of the medial epicondyle. MRI is indicated in the evaluation of the UCL in the case of normal radiographs. Initial treatment consists of cessation of throwing, ice therapy bracing and analgesics, followed by physical therapy and gradual return to sports [5,21]. In elite adult athletes, in case of conservative treatment failure, UCL reconstruction is preferred over UCL repair. Nevertheless, optimal surgical management is less clear regarding adolescent patients even if athletes between 15-19 years represent the largest patient population, in which UCL reconstruction is performed [22]. The newest available data present an 80% and 84% rate of return to play at the same or higher level of competition for javelin throwers and baseball players respectively, following UCL reconstruction [20].

Little League Elbow

Little League Elbow is an umbrella term used to describe medial elbow painful pathologic conditions that affect skeletally immature overhead athletes, such as medial epicondyle apophysitis, avulsions or stress fractures, UCL injuries, and flexor-pronator mass strains [4-6,8, 19, 23]. Tension overload of the medial elbow, as a result of the repetitive valgus stress experienced by these athletes during throwing, is the responsible mechanism [19]. Pitching despite the symptoms, high-speed fastballs, throwing breaking pitches, increased pitches per game, and year-round play are considered risk factors for the prevalence of the disease [19]. Apart from medial elbow pain, the young throwers complain of deterioration of their throwing accuracy or velocity. On plain radiographs, the medial epicondyle may be displaced. In case of doubt, an examination of the contralateral elbow may be required [6]. Management of flexor-pronator muscle strains and apophysitis of the medial epicondyle is conservative and includes cessation of throwing and physical therapy [19]. Avulsion fractures with a displacement of less than 5mm should be initially immobilized and follow an early range of motion physical therapy protocol. Displacement of more than 5mm may require open reduction and internal fixation of the affected medial epicondyle [5,6,19].

Osteochondritis dissecans (OCD)

Repetitive microtrauma, associated with axial compression forces or valgus stress, is considered to be the main etiologic factor for the development of the disease. This notion is amplified by the fact that OCD is more common in skeletally immature throwers and gymnasts. The elbows of skeletally mature athletes are rarely involved, probably due to the greater blood supply of the mature distal humeral epiphysis. The capitellum of the dominant upper limb is affected more often and males are more prone to elbow OCD than females [5,23-25]. Patients usually complain of lateral elbow pain and, as the disease progresses, mechanical symptoms may present, such as locking, clicking, decreased ROM, and swelling [25,26]. Early diagnosis is essential to avoid chondral fragmentation and loose body formation, which may lead to degenerative lesions at a young age [26]. Late-stage OCD may appear as increased radiolucency of the capitellum or loose intraarticular bodies on plain radiographs [19,25]. In the case of radiographs with normal findings and strong suspicion of OCD, an elbow MRI should be obtained, which provides the optimal evaluation of the lesion stability [27, 28]. In general, the stability of the lesion indicates roughly the conservative or surgical management of the disease [28]. Most of the early-stage lesions in patients with open physes are best managed nonoperatively [5,25,28]. Unstable symptomatic lesions (pain, decreased ROM, locking, fragmentation, closed physes) require surgical intervention. Although a consensus does not exist due to insufficient reliable data and many treatment modalities have been reported (arthroscopic debridement, abrasion chondroplasty, microfractures, fragment fixation, osteochondral autograft transplantation-OAT, costal osteochondral transplantation-CAT), it seems that osteochondral autografting techniques may be superior in restoring the local anatomy and elbow function.

OCD of the elbow should not be confused with Panner's disease, which is a self-limiting osteochondrosis of the capitellum, with an excellent prognosis. It affects more commonly children of younger age (4-9 years old). On physical examination, symptoms may be mimicking those of the OCD. Conservative management (cessation of activities, analgesics, and immobilization) is sufficient [19].

Olecranon apophysitis

Similar to the Osgood-Schlatter (tibial tubercle apophysitis) disease, olecranon apophysitis is the result of traction microtrauma due to the repetitive contraction of the extension mechanism, in skeletally immature athletes. Apophysitis may progress to avulsion fractures in younger patients or stress fractures in older individuals. Posterior elbow tenderness, swelling, and decreased ROM are the most common complaints. Conservative management with rest, activity modification, and analgesics is often the treatment of choice [5,19,29]. In the case of olecranon apophyseal nonunion surgical management may provide satisfactory results [30].

WRIST

Distal Radius Fractures

These fractures are the most common children's fractures (20% of all fractures), with a predominance in male patients, and happen usually in contact sports, such as soccer [1, 5,31]. A developing skeleton is characterized by unique fracture patterns, such as buckle fractures, greenstick fractures, plastic deformation, and epiphysiolysis [1,31]. Children's fractures, also, present a great healing and remodeling potential. Remodeling is more eminent near the most active physes, in the plane of joint motion, and younger skeletal age [31]. The pediatric trauma surgeon should consider the abovementioned, as well as the patient's and parents' capability of compliance, before applying the proper treatment. Surgical intervention must be performed with minimal physeal trauma. Growth arrest is a devastating complication of physeal injuries, affecting up to 10% of physeal fractures and may cause deformities, such as length discrepancy, angulation, and joint incongruency, ultimately leading to lifelong disability [1,31].

Gymnast's Wrist

The distal radial physis of the young gymnast not only does suffer from fractures or epiphysiolyses, but also chronic overuse injuries, such as the "gymnast's wrist" [4,5]. Due to the needs of this particular sport, the wrist of the pediatric athlete is forced to bear heavy axial loads with the wrist in full extension multiple times during practice or competition [1,23]. The repetition of this abnormal loading pattern is responsible for microtrauma to the physis, which may even lead to physeal arrest [1,5]. Plain wrist radiographs in young gymnasts with wrist pain and contralateral comparison, regardless of the physical examination signs, are suggested by some authors [1]. Abstaining from gymnastics may be a necessity if any abnormality, such as irregularity or physeal widening, is noticed on radiographs. A cast may also be applied [1,5]. In the case of normal x-rays, an MRI may reveal bone marrow edema on both the proximal and distal side of the physis [4]. Management of patients with physeal arrest might be quite challenging and depends on the years remaining until expected physeal closure, the portion of physis with physeal arrest, and the existence of deformity (ulnar positive variance, bowing), and the articular congruency. Surgical treatment options include recurrent radial lengthening, both radial, and ulnar lengthening, radial osteotomies, and ulnar epiphysiodesis. Despite the good results reported, most of the patients will not be able to return to their previous level of gymnastics [1].

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ORIGINAL ARTICLES

Addressability to the Emergency Department of patients with acute post-COVID 19 clinical symptoms

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Abstract: *The long-distance post-SARS-CoV2 infection effects were partially known. The study aims to follow the evolution of the patients with acute post-COVID 19 clinical symptoms presented in the Emergency Department. Methods: We analyzed 262 presentations more than 14 days after the initial COVID-19 diagnosis and with at least one acute COVID-19-related symptom. This study included presented between 01.01 and 31.05.2021 in the Emergency Department of Bucharsti-Emergency University Hospital. Data were obtained in real-time, at the presentation, from the patients' medical charts, and afterward from SUUB's database. Results. Out of the total of 262 presentations, 40.84% required patient hospitalization. The highest number of presentations with (18.70%) or without (22.90%) hospitalization was recorded in the first month after the COVID-19 diagnosis. Patients with less than 30 days between presentations had significantly fewer hospitalization days, compared to those with more than 30 days between presentations (8.3 vs 14.14 days, p=0.009). We found a high amount of hospitalization days (1093 days), with 29.88% deaths. Conclusions: COVID-19 complications require prolonged hospitalization, with implications on the patients' quality of life, the overcrowding of intensive care facilities, high-cost treatment, and unfavorable evolution for a great number of patients.*

Keywords: *long COVID, ongoing COVID, COVID-19 complications, hospitalization*

INTRODUCTION

Worldwide, the Emergency Departments were at the front line of the fight against the SARS-CoV2 pandemic, which began on March 11, 2020 [1]. Presently, there are published many COVID-19 studies, which bring information regarding the physiopathological mechanisms of action, the long-term effects, and complications of the disease, treatment, and the potential leverage of prevention, as well as the psychologic and socio-economic implications of COVID-19 [2]. Males seem to be more frequently affected by COVID-19 whereas the risk for poor prognosis is increased by age and chronic illnesses [3]. These factors seem to contribute to the extreme variability of COVID-19, from asymptomatic to severe illness and death, as well as to its unpredictable response to treatment.

In the Czech Republic, a study over 2 ½ months, that enrolled 7,455 patients with positive RT-PCR SARS-CoV2 tests shows that 15.9% required hospitalization and 6.2% developed severe complications [4].

The term "ongoing symptomatic COVID-19" is used when the symptoms persist between 4 and 12 weeks whereas the frequently used terms "post-COVID syndrome" or "long COVID" implies the persistence of symptoms over 12 weeks, in the absence of other causes [5,6]. The Office for National Statistics (UK) estimates that 1 of 10 persons with positive COVID-19 tests has symptoms for a period of more than 12 weeks, and 1 of 5 persons for a period of more than 5 weeks. In England, an ample review of 508,707 COVID-19 patients identified several risk factors for the persistence of symptoms for more than 12 weeks (e.g. age, female gender, obesity, and hospitalization for COVID-19) [7].

National Institute for Health Research mentions a study

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conducted on patients discharged from a hospital in Rome with “ongoing COVID-19 symptoms”. Of them, 87% had at least one symptom present 60 days after the debut of SARS-CoV2 infection, 55% had 3 or more symptoms, and 40% had a reduced quality of life [8].

NHS England estimated that out of 95,000 patients admitted to hospitals until June 5, 2020, 45% had “ongoing support” and up to 50% required rehabilitation services [9].

Long COVID affects multiple and different body systems, influences daily routine activity [10], impacts family life, and decreases workplace productivity, with psychological and socio-economic repercussions. A study that followed 3,762 patients from 56 countries, which had symptoms for more than 28 days, showed that 90% of them had symptoms for up to 60 days and 91% needed more than 35 weeks to recover; the most frequently reported symptoms after 6 months were fatigue, decreased resistance to exertion, and cognitive disfunction [11].

Persistent clinical charges for up to 6 months after COVID-19 were reported mostly for inpatients, but also for those who did not require hospitalization [12]. A cohort of patients, discharged in the period 07.01-29.05.2020, mentioned fatigue, weakness, sleeping disorders, anxiety, and depression, 6 months after acute infection [13]. The persistence of symptoms (e.g. fatigue, shortness of breath, memory loss, hair loss) was correlated with the number of days of hospitalization [14]. A New York City study that evaluated 929 survivors out of 1,190 hospitalized COVID-19 patients at 3 months and 6 months after discharge, found the persistence of cardiopulmonary symptoms (35.7%, respectively 28%), especially shortness of breath (22.1%, respectively 15%). After 6 months from discharge, general (26.4%) and neurological (24.2%) symptoms persist. There were also 33 deaths during the 6 months [15]. A study conducted in Rome, on 143 patients, shows that a high number of patients still have more than 2 symptoms at discharge. The patients entered a recovery program after discharge and, after 60 days from the debut 12.6% had no symptoms, 32% had 1-2 symptoms, and 55% had more than 3 symptoms [16].

A review of 24 articles published until 01.01.2021 concludes that the presence of more than 5 symptoms in the first week of acute infection is associated with the development of long COVID, regardless of age or gender. Increased risk of symptoms’ persistence is associated with advanced age, hospitalization at the debut, shortness of breath at debut, chest pain, and comorbidities [17].

Hypertension, considered independent or in association with diabetes mellitus, predisposes to longer persistence of symptoms [18] and the increased mortality after COVID-19 [19]. COVID-19 also is a risk factor for other diseases, like stroke, myocardial infarction, and thrombosis [20].

Taking all this info into account, we followed the patients with previous COVID-19 diagnoses presented, in the Emergency Department of the University Emergency Hospital in Bucharest (SUUB).

METHOD

We analyzed 262 emergency presentations, unique or multiple, in the period January-May 2021, with at least one acute COVID-19 related symptom, that necessitated or did not hospitalization. Clinical data were obtained in real-time, at the moment of the presentation and afterward, from SUUB’s database.

Inclusion criteria were: at least 14 days after prior COVID-19 diagnosis, confirmed by RT-PCR test, age over 18 years, and the presence of at least one persistent, acute, or newly appeared symptom associated with SARS-CoV2 infection. Presentations in Emergency Department that are not related to the previous COVID-19 diagnosis were not included in this study.

Statistical analysis was performed using the StatsDirect software.

RESULTS

The total number of presentations with acute post-SARS-CoV2 infection symptoms in the Emergency Department of SUUB was 262 (Figure 1); out of these, 155 (59.16%) were discharged and 107 (40.84%) were hospitalized.

Figure 1: Total number of presentations (262) in the period 01.01.-31.05.2021

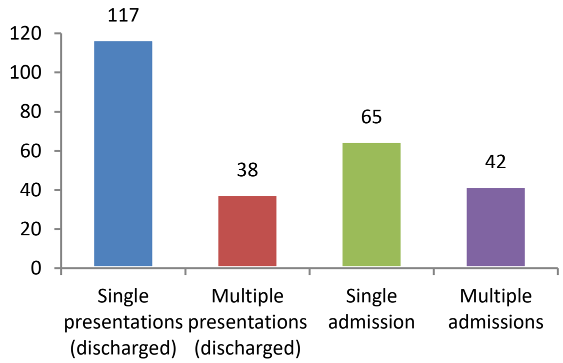

117 patients didn’t need hospitalization, and 2 of them had 2 presentations. 87 patients were hospitalized; out of these, 65 patients had a single presentation with admission and the other 22 patients had multiple presentations (in total 78

presentations) out of which 42 presentations were followed by admission, and 36 by home discharge (Figure 1).

There is a high variability regarding the 22 patients with multiple presentations, as follows: 12 patients with 2 presentations, 1 patient with 3 presentations, 7 patients with 4 presentations, 1 patient with 8 presentations, and another with 15 presentations, all of them with at least 1 admission.

Most presentations are in the first month after the initial diagnosis, regardless of severity: 60 presentations with discharge (22.90%), and 49 presentations with hospitalization (18.70%).

The decreasing trend is maintained at 4 months distance for both types of patients and continues after 4 months for the patients that needed hospitalization.

There is an increase in presentations that do not require hospitalization, with less severe symptoms, like fatigue, decreased resistance to exertion, sleeping disorders, physical and mental asthenia, influencing daily activity, after 4 months from the COVID-19 diagnosis (Figure 2).

Figure 2: Interval between COVID-19 diagnosis and ED presentation

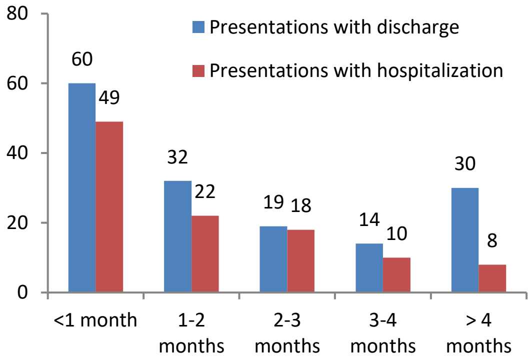

Concerning the time of hospitalization, there is high variability, from 1 to 36 days. The 65 patients with single presentation and admission cumulate a total of 643 days of hospitalization.

In the case of the 22 patients with multiple presentations, they have a variable number of hospitalizations and hospitalization days, as follows: 7 patients with 1 hospitalization (5-36 days), 11 patients with 2 hospitalization periods (6-46 days), 3 patients with 3 hospitalizations (15-31 days), and 1 patient with 4 hospitalizations (30 days). The total number of hospitalization days for all the patients in the study was 1093 days (Figure 3).

Figure 3: Number of presentations with admission and hospitalization days

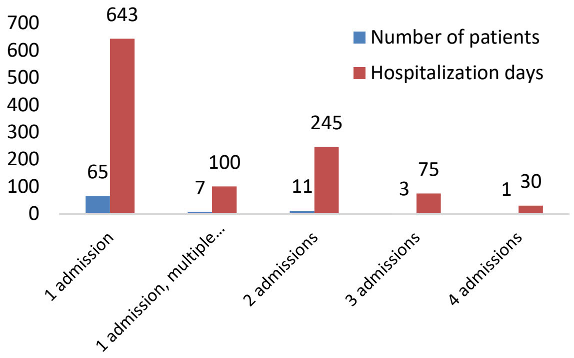

The analysis of the sublot of patients with multiple presentations and admissions shows that the average value of hospitalization time for presentations in the first 30 days from COVID-19 diagnosis is 8.3 (±4.5) days, respectively 14.14 (±4.98) days for presentations after the first 30 days (0.01). Out of the 87 hospitalized patients, 61 (70.12%) were discharged, and the rest 26 (29.88%) died.

The 117 patients that didn't require admission were 63 (53.85%) males and 54 (46.15%) females. The highest age group was 40-59 years, respectively 49 patients. There were no patients aged less than 20 years. 5 patients had between 80-89 years (Figure 4). The most frequent symptoms for the discharged patients were: respiratory (36.77%), cardiac (19.35%), digestive (18.06%), neurological (18.06%), ENT (4.52%), anxiety (3.23%).

Figure 4: Age distribution for discharged/admitted patients

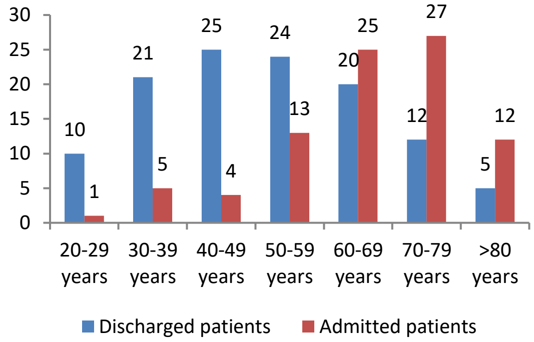

Out of the 87 admitted patients, 16 (18.39%) males and 10 (11.49%) females died. Most deaths occurred in men (43.75%) aged 60-69 years and in women in the age group 70-79 years (50% of female deaths) (Figure 5).

Out of 107 admissions in the period covered by the study, 46 patients (43%) had a positive RT-PCR test at the time of admission. During hospitalization, 15 patients (32.6%) from this lot; 11 patients (18.03%) of the 61 patients with negative

RT-PCR at the admission died ($p>0.05$).

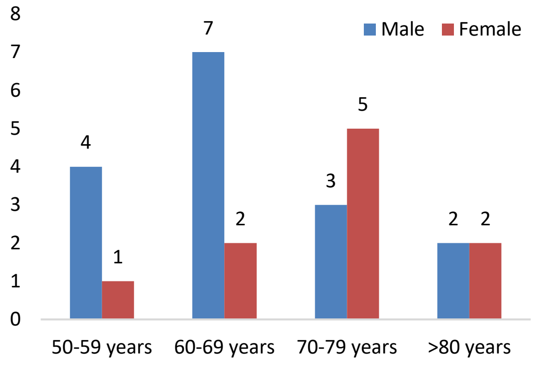
Figure 5: Age distribution for deaths

Out of the 22 patients with multiple admissions, only 8 had positive RT-PCR at first admission (5 of them had 2 admissions and 3 patients had 3 admissions). The other 14 patients had negative RT-PCR tests. Eighty-seven patients (81.30%) had comorbidities versus 20 patients (18.69%) without comorbidities (Figure 6). The most frequent conditions diagnosed at the admission of patients needing hospitalizations were an acute respiratory failure (25.68%), stroke (22.47%), acute decompensated heart failure (6.42%), Clostridioides difficile infection (6.42%), acute coronary syndrome (5.35%), pulmonary thromboembolism (4.28%), and deep venous thrombosis (4.28%). Out of the deceased patients, 5 (25%) had no medical history before the SARS-CoV2 infection, 2 males (53 years and 68 years old), and 3 females (68, 70, and 81 years old).

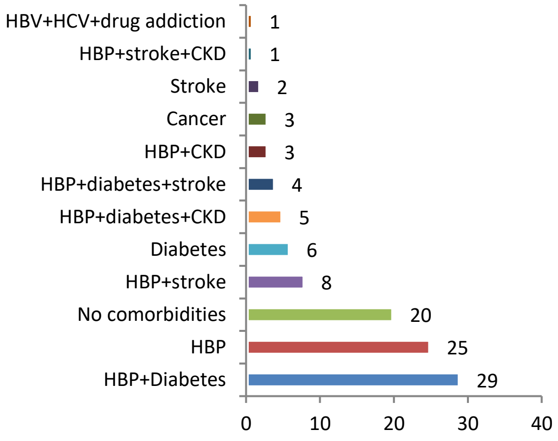
Figure 6: Comorbidities in hospitalized patients

Neutrophilia was found in 66.5% of patients with a single presentation. History of stroke, neutrophilia, heart rhythm disorders, and neurological diagnoses at admission presented significant differences between patients stratified according to the number of presentations (Table 1). The most deaths were in patients with a single which suggests the severity of the post-COVID 19 pathologies.

Table 1: Differences identified between subjects who required hospitalization (n=87)

Characteristics	Number of hospitalizations		Statistical significance (p)
	Single	Multiple	
Neutrophilia (normal/yes)	22/43	14/8	<0.05
History of stroke (no/yes)	46/19	20/2	<0.05
Neurological diagnoses at admission (no/yes)	44/21	20/2	<0.05
Heart rhythm disorders	18/47	0/22	0.002
Discharge/Death	43/22	21/1	0.004

DISCUSSION

In the third wave of the pandemic, at the same time with newly infected COVID-19 patients, there were 262 post-COVID presentations in the Emergency Department, in the first 5 months of the year 2021.

Out of these, 59.16% of presentations did not require hospitalizations, and 40.84% were hospitalized. For both categories of patients, most came to the hospital with acute or persistent symptoms in the first month after the initial COVID-19 episode. 22 patients came repeatedly to the ED, out of which 7 patients had 4 presentations, 1 patient had 8 presentations and 1 patient had 15 presentations.

The patients presented in the first 30 days after SARS-CoV2 infection had significantly fewer hospitalization days, versus those presented after 30 days or more (8.3 vs 14.14 days, $p=0.01$).

The number of hospitalization days is variable, from 1 day to 36 days, for a single admission. 15 patients (17.24%) required multiple hospitalizations (2-4). Four of these patients cumulated over 30 days of hospitalization (maximum 46 days for 1 patient). The total number of hospitalization days for an small number of patients (87) was 1093.

Out of the total number of admissions, those with negative RT-PCR tests at the time of admission were more (57%) than those with positive RT-PCR tests. 18.03% of patients with negative RT-PCR died, vs 32.60% of those with positive RT-PCR. The results show that a negative RT-PCR test after the initial COVID-19 episode isn't equivalent to favorable evolution or total recovery.

Our study, like other studies, argues that associated comorbidities increase the risk of COVID-19 symptoms' persistence, the risk of complications, and is a factor in poor prognosis.

Hypertension is the most common comorbidity (28.73%), with the highest risk of unfavorable evolution, followed by diabetes (6.89%). In hospitalized patients, the most frequent comorbidities are hypertension and diabetes (33.33%), hypertension and stroke (9.19%), or hypertension, diabetes mellitus, and chronic kidney disease (5.75%) [21].

Acute stroke was diagnosed in 22.47% of admitted patients [22].

Treatment included interventional procedures, like mechanical thrombectomy for 2 patients with acute stroke, or coronary angioplasty, for 1 patient with STEMI.

The high percentage of deaths among patients who presented with post-COVID 19 symptoms and required hospitalization (29.88%) shows the severity of the complications of COVID-19.

The hospitalized patients without prior medical history (18.69% - 20 patients) are apparently few, but 25% of them (5 patients) died. It is therefore important to monitor the evolution of post-COVID-19 patients without prior comorbidities.

The limitations of the study are the small number of presentations, the study being conducted in a single emergency department, and a lack of data related to the vaccination status of patients (vaccination began in Romania at the end of December 2020).

CONCLUSIONS

The overcrowding of emergency departments and hospitals caused by post-COVID-19 complications becomes a real logistic problem in the context of the currently ongoing SARS-CoV2 pandemic. It appears from the study that late presented patients have unfavorable evolution, with significant mortality and prolonged hospitalization. Some patients died after just 1-3 days of hospitalization.

The importance of the study is great, data being collected from the biggest emergency department in Romania, with the highest number of presentations. The high number of hospitalization days has multiple implications, both for the patients and the medical personnel. The results of the study point out the frequency and severity of post-COVID-19 pathology.

Prompt intervention, targeting cases investigated clinically and radiologically in an outpatient setting, could go the relief of emergency departments overcrowding and a decrease in the rate of hospitalization, and implicitly a decrease in morbidity and mortality, with positive effects on each patient's health, and also in their familial and socio-economic circumstances.

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ORIGINAL ARTICLES

Patients' knowledge and attitudes regarding human papillomavirus disease and the effectiveness of education in patients referred to the Dermatology Clinic of Baqiyatallah Hospital in 2020

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Abstract: *Introduction: Human papillomavirus infection considers one of the leading causes of infection-related cancer worldwide. The general public's range of knowledge and understanding of human papillomavirus is low; the present study was done intending to evaluate the knowledge and attitude of patients with human papillomavirus and the effectiveness of educational intervention in those patients.*

Methods: This was a quasi-experimental study. The study population included patients with genital human papillomavirus referred to Dermatology Clinic of Baqiyatallah Hospital in 2020. The patients filled out a pre-test questionnaire. In the next step, they were trained by the treating physician about the disease, transmission ways, complications, treatment methods, and laboratory tests, and available vaccines. After that, an educational pamphlet was given to the patient to complete and review the information. Moreover, a post-test was performed in the patient's next visit. Data were analyzed using SPSS software version 22.

Results: 92 patients were included in the study. The levels of knowledge and attitude of patients were between 50 to 65% before training and training had a significant impact on improving the level of knowledge and attitude of patients (P <0.001) so that the level of knowledge and attitude of patients reached above 95%. Demographic factors such as age, gender, marital status, and level of education were not related to the initial knowledge and attitude of individuals based on the statistical analysis (P <0.05). However, the knowledge of married people about the complications of the disease was more than single ones (P = 0.032).

Conclusion: the level of knowledge about human papillomavirus and its complications, transmission ways, prevention and treatment is low, which has increased with training significantly.

Keywords: *human papillomavirus, education, knowledge, attitude*

INTRODUCTION

Human papillomavirus infection as one of the main causes of infection-related cancers worldwide also causes other diseases like genital warts and respiratory papillomatosis [1]. According to the International Agency for Research on Cancer, the high-risk genotypes of Human Papillomavirus include types 16, 18, 31, 33, 45, 52, and 58 are responsible for about 90% of anogenital positive Human Papillomavirus cancers worldwide, however, Human Papillomavirus types 6 and 11, low-risk genotypes, are responsible for 90% of genital warts [2]. Human papillomavirus, besides anogenital cancers, is responsible for some cancers of the head and neck [3]. About 80% of sexually active people become infected with human papillomavirus during their lifetime. The immune system of the body controls most of these infections within one to two years. If the infection persists, however, it can cause cellular changes that may lead to

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certain types of cancer [4]. The overall prevalence of human papillomavirus in a global meta-analysis of women with normal cervical cytology is estimated at 10.4%, which varies by geographical area [5]. Most human papillomavirus infections do not cause a problem and the body can clear them within two years. This is recommended for specific clinical manifestations of human papillomavirus-related disease (e.g., anogenital warts, precancerous lesions, or cancers [6].

The human papillomavirus vaccine is for the prevention of this disease, which reduces the progression of cervical cancer by up to 70%, and for better effects should be administered before or shortly after sexual intercourse [4]. According to the European Center for Disease Control and Prevention, 19 European countries have started vaccinating against human papillomavirus in women, as of 2012, and ten countries have initiated initiatives in this regard [7]. The coverage of human papillomavirus vaccination programs, despite these advances, varies greatly between countries [7]. Further, the least aware groups of the infection include racial and ethnic minorities, low-income women, with low education levels and no insurance, which are at significant risk for morbidity and mortality from the disease consequences [8]. The general public has little knowledge and understanding of human papillomavirus. It seems that the stress of diagnosing human papillomavirus infection is related to the lack of understanding of human papillomavirus [9].

Researches in Western countries suggest that most women, adolescents, and young people, even those with higher education, are not much aware of Human Papillomavirus infection [10]. In Iran, there is no public education program for human papillomavirus and vaccination for the general public.

The patients who have experienced the disease play a vital role in maintaining the disease transmission chain, and raising patients' awareness of disease transmission ways. Moreover, the treatment approaches can be very effective in decreasing disease transmission. Moreover, the relationship between human papillomavirus and cervical cancer considers important information which people must be aware of which may not even be taken seriously by the medical staff [11]. A study was conducted for surveying the attitude of patients with genital warts towards their disease in patients referred to the dermatology clinic of Razi Hospital in Iran, which indicated that patients' attitudes about various dimensions of the aforementioned disease are impaired [12]. The evolution of current knowledge and attitudes about human papillomavirus in patients is vital in trying to improve it.

This study was conducted with the aim of evaluating the knowledge and attitude of patients with Human Papillomavirus, transmission ways, treatment and its complications, training of patients moreover re-evaluating the knowledge and attitude of patients.

METHODS AND MATERIAL

The present study was a quasi-experimental one. The study population was patients with genital papillomavirus referred to the skin and hair clinic of Baqiyatallah Hospital in the year 2020. The sample size was obtained according to the prevalence of 38.6% [13].

Moreover the alpha of 5%, the beta of 20% and the sample size of 92 was obtained. The inclusion criteria of the study of human genital papillomavirus affection was confirmed and the satisfaction of participating in the study was related to the age range of 14 to 65 years. The exclusion criteria of the study was dissatisfaction of continuing the study. A researcher-made questionnaire was according to the desired trainings for patients that their validity of was questioned by referring questions to five experts. The reliability of this questionnaire was calculated using SPSS and Cronbach's alpha statistical method, which was higher than 70%, so the reliability of the aforementioned study was confirmed. The patients were asked about having satisfaction participating in the study before starting the study. At first, the patients filled out a pre-test questionnaire. Then the patients were trained about human papillomavirus in two parts. First, The doctor explained the cause of the disease, transmission ways of the disease, ways of prevention and treatment of the disease moreover its complications for the patients through face-to-face training. Second, A pre-designed pamphlet was provided for the patients, which contained complete explanations of the mentioned cases. Moreover, a phone number was given to the patient to ask any probable questions. the post-test questionnaire was filled out again after one month of revisit. Patients were included in the study in the form of encoding. The patient information was confidential. The individuals in the project adhered to all Helsinki’s moral principles.

Data were analyzed using SPSS software version 22. The results were expressed as the mean ± SD for quantitative variables and as a percentage for qualitative variables. The Chi-square and McNemar tests were used.

RESULTS

In this study, 92 infected patients with human papillomavirus were assessed about the knowledge and attitude of the disease before and after training in patients referred to the skin and hair clinic of Baqiyatallah Hospital in the year 2020. Table 1 indicates the distribution of demographic variables in patients.

The factors such as age, gender, marital status, and level of education were not related to the initial knowledge and attitude of individuals based on the statistical analysis (P <0.05), although, the knowledge of married people about the complications of the disease (P = 0.032), was more than single ones (70.8% vs. 45.5%).

The level of knowledge and attitude of patients was between 50% and 65% before training. The results indicated that training had a significant impact on improving the level of knowledge and attitude of patients as it reached above 95 at all levels after training (P <0.001) (Table 2).

Table 1: Distribution of demographic variables of age, gender, marital status and literacy level in the patient

Leadership Productivity	Factor Loading	Percentage	Frequency
Age	<30	33.3%	29
	30-40	52.9%	46
	>40	13.8%	12
Gender	Female	18.4%	16
	Male	71.6%	71
Marital status	Single	25.3%	22
	Married	76.4%	65
Education level	Less than diploma	22.9%	19
	Diploma	42.2%	35
	More than diploma	34.9%	29
History of STDs	Yes	3.4%	3
	No	96.6%	85

DISCUSSION

Human papillomavirus by silencing apoptosis (program cell death) and producing the inhibitor proteins of p53 and Retinoblastoma genes (inhibitor genes of cell growth) provides the conditions for the production of cellular cancer masses [14]. The human papillomavirus is involved in 30% of oropharynx cancers, 45% to 95% of anal cancers, 60% to 65% of vaginal cancers moreover 40% to 60% of vulvar cancers [15, 16]. The incidence of uterine and cervical cancers is about seven in one hundred thousand, based on the reports of the Cancer Institute. This cancer normally occurs between the ages of 30 and 55, even though there have been numerous reports among young women recently. [17] For this purpose, the existence of sufficient knowledge about the aforementioned virus and methods of its prevention and treatment can have a significant impact on promoting public health.

The present study surveyed the basic knowledge about human papillomavirus, its prevention, and treatment among patients referred to the dermatology clinic of a reference hospital and tried to indicate that the best solution for improving patients' knowledge and attitude is training them by conducting face-to-face training using educational pamphlet moreover providing some pieces of training. The findings indicated that the level of knowledge and attitude of patients was between 50% to 65% before training which was accompanied by improvement of knowledge and attitude of clients in all of these cases using training (P<0.001).

The present study indicated that the age factor does not have any impact on the knowledge and attitude of individuals about the causative agent, transmission ways, complications of the disease, the role of vaccines in disease prevention moreover the treatment way. Another study surveyed the information of people about this disease in various age groups. The amount of people's information has increased with the increment in the range of age (up to 40 years) [18]. The aforementioned study was not in line with the present study, which could be because of the wide age range mentioned in the study.

It was indicated in the current study, that education was not associated with knowledge and attitude of people about the cause of warts, transmission ways of disease, complications, disease, vaccine role in disease prevention moreover ways of treatment. Such an issue indicates that there is not any difference in knowledge and attitude of patients with human papillomavirus, even among highly educated individuals. On the other hand, it indicates a lack of adequate information or education in the community and at the general level too.

The present study surveyed the impact of gender on attitudes toward the disease, but any difference was not seen in this case. Another study conducted in this field which indicated that the gender of individuals doesn’t have any impact on their awareness of Human Papillomavirus moreover its related factors. [19]

63% of patients were aware of the cause of genital wart before being trained. This rate reached about 98% after revision (P <0.001). A similar study was conducted by Zu et al during which it was determined that the awareness of people of human papillomavirus was 15.5% while the awareness of people was 18.9% for human papillomavirus vaccines (20). Another study, conducted on Italian students to learn about human papillomavirus and its vaccination, indicated that 60% and 70.3% of pre-school students have relative and little knowledge about these cases, respectively. [21]

Table 2: Frequency of answers to questions before and after training

Questions	Answer	Pre-test	Post-test	P value
Identifying of the cause of the disease	Right	58 (63.04٪)	90 (97.9٪)	<0.001
	Wrong	34 (36.96%)	2 (2.1٪)	
Understanding the ways of disease transmission				
Direct and indirect skin contact	Right	57 (60.8%)	91 (99.0٪)	<0.001
		35 (38.2)	1 (1.0٪)	
Virus-infected instruments	Right	57 (60.8%)	89 (96.8٪)	<0.001
	Wrong	35 (38.2)	3 (3.2٪)	
Blood and blood products transfusions	Right	57 (60.8%)	91 (99.0٪)	<0.001
	Wrong	35 (38.2)	1 (1.0٪)	
The gyms	Right	57 (60.8%)	89 (96.8٪)	<0.001
	Wrong	35 (38.2)	3 (3.2٪)	
Hairdressers	Right	50 (54.3%)	90 (97.9٪)	<0.001
	Wrong	40 (44.7)	2 (2.1٪)	
Sexual contact	Right	55 (59.8%)	91 (99.0٪)	<0.001
	Wrong	37 (40.2)	1 (1.0٪)	
Shared facilities	Right	53 (57.6%)	90 (97.9٪)	<0.001
	Wrong	39 (43.4)	2 (2.1٪)	
Disease complications and treatment				
The complications of the disease	Right	54 (58.7%)	89 (96.8%)	<0.001
	Wrong	38 (41.3)	3 (3.2%)	
The vaccine for this disease?	Right	52 (56.5%)	90 (97.9%)	<0.001
	Wrong	40 (43.5)	2 (2.1%)	
Treatment methods	Right	46 (50.0%)	91 (99.0%)	<0.001
	Wrong	46 (50.0%)	1 (1.0%)	
Identifying of prevention				
vaccination	Right	48 (52.1%)	88 (95.6%)	<0.001
	Wrong	44 (47.9%)	4 (4.3%)	
Education	Right	46 (50.0%)	89 (96.8%)	<0.001
	Wrong	46 (50.0%)	3 (3.2%)	
Boosting of immunity system	Right	49 (53.3%)	91 (99.0%)	<0.001
	Wrong	43 (46.7%)	1 (1.0%)	
Prevention by medication	Right	47 (51.1%)	90 (97.9%)	<0.001
	Wrong	45 (48.9%)	2 (2.1%)	

The knowledge of the subjects in the present study about vaccination was 52% before training which reached 95% after training. The amount of training and advertising about Vaccines in our country has been poor. The present study is designed to indicate the importance of training about vaccines while the amount of training about this vaccine in other countries is much more extensive and is being conducted with a variety of tools.

Clients were informed about the transmission ways of the disease. Various factors including direct and indirect skin contact, virus-infected equipment, blood transfusion, and blood component, gyms, hairdressers through waxing, sexual contact, and common equipment in the dormitory were examined during conducting this study. The knowledge of people was between 57-50% before training which reached 91-89% after training.

Other studies have surveyed the amount of awareness of people of transmission ways of the disease, which indicated a variety of various results [22]. A study by Joshi et al. found that 44.3% of subjects were aware of the transmission ways of the disease [18]. Moreover, it was concluded in this study that both the level and type of education of individuals have significant impacts on their awareness of the aforementioned virus as well as its vaccination and treatment.

CONCLUSIONS

The knowledge and attitude of patients with human papillomavirus disease, despite its importance, seem not to be enough. However, effective and well-planned education can have a significant impact on the knowledge and attitude of patients with this disease. Thus, it is suggested that in public education programs, the important educational points about Human Papillomavirus must be given priority.

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ORIGINAL ARTICLES

Prediction of anxiety, depression and social function among mothers of NICU-hospitalized infants compared to healthy infants' mothers in Baqiyatallah and Imam Hossein Hospitals

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Abstract: *The main objective of this research is to predict anxiety, depression and social function of NICU-hospitalized infants' mothers compared to healthy infants; mothers. The methodology of research is descriptive in type of correlative and causative-comparative. Population included all mothers of NICU-hospitalized infants and healthy infants' mothers in two Tehran Hospitals from June 2018 to September 2019. The main sample size was randomly selected. Sample size was calculated as 120 at this level based on Cochran's table. The instruments were Beck's (1969) anxiety and depression inventories, and Lekomet et al. (2014) social functioning questionnaire (SFQ). Data was analyzed using analysis of variance, multivariate and step-wise regression. The results implied that those NICU-hospitalized infants' mothers experienced higher levels of depression and anxiety than healthy infants' mothers. Moreover, formers had lower social functioning than latters. On the other hand, depression and anxiety were suitable predictors of mother' social functioning and there was a significant relationship between depression and social functioning mediated by mother's anxiety.*

Keywords: *anxiety, depression, social functioning, infants' mothers, NICU ward*

INTRODUCTION

Low-weight and immature infants are at-risk groups socially and they would experience more physical, psychological and mental diseases than natural infants. Because of physiological specifications they have, immature infants need regulated care to survive and regain their natural growth [1]. On average, 9% of the infants need NICU caregiving processes [2] most of whom are immature infants [3].

Pregnancy, giving birth and postpartum periods are sensitive instances during the woman's life. Thisperiod of time incudes natural and mental transformations in woman's both social roles and familial role [4]. Pre-term parturition is usually traumatic and a source of the parents' distress. This may be recognized as an emotional crisis which often is diagnosed by sense of lacking and sorrow. Sometimes, these statuses remain for a long time after the child birth. For many mothersadaptation to having preterm infants resembles the adaptation to having disabled child [5].

The potential stressor that affect the parents of preterm infants include problems associated with infants' health in hospital as well as after being discharged, reduced responsiveness and concerns about the infants' mental development [6].

Approximately, 28 to 70 percent of preterm infants' mothers experience high levels of the psychological pressures. The damaging influence of preterm infant on mother disrupts her subjective abilities increasing other tensions of having high-risked child. High levels of stress transform and disrupt mother-child interactions. On the other hand, parents with preterm child -may experience wrong and sad ideas as their infant is disabled and is vulnerable against any type of diseases and damages. This parents' idea causes undesirable parents-child interactions and, as a result, leads to their depression and anxiety disorders causing disruptions in

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parents cannot play their parenting role well. Consequently, this adversely affects the child's growth and interactive abilities.

One of the variables studied associated with mothers is anxiety. Anxiety is a resisting and chronic phobia of a particular situation or objects which spontaneously is not fearful and this phobia leads the individual to avoid from that situation or object; while individual understands his/her phobia is illogical [7]. Anxiety is referred to as a part of every human's life and everyone experiences it to some balanced extent. To this extent, anxiety is deemed a compromised response [8]. Lack of anxiety may expose individuals to significant risks and issues but anxiety may reach chronic and constant aspects. In this case, this can no longer be a compromised response but it has to be taken as a failure, uncompromised reaction or extensive despair which established a large spectrum of cognitive and physical disorders rather than unjustified phobias [9]. There have been various definitions provided for anxiety. In definition, "excessive phobia in real or imaginative situations" is anxiety. However, anxiety and phobia are similar in many aspects, they indicate apparent differences. Phobia signifies adverse event occurring or experience which will occur soon while anxiety signified an unjustified phobia. Main feature of anxiety is concern including worrying excessively in situations with uncertain outcomes. Excessive concern is harmful since it disrupts the problem-solving capability [10]. Generally, anxiety means resisting and chronic phobia of something or of situations which in fact are not damaging. Such phobialeads to acute tendency to avoid anxious situations; however, individual understands his/her phobias and avoidance are illogical [11].

Studies on epidemiology of this disorder implied that about 8-12% of children and 5-10% of adolescents are exposed to one of the criteria of anxiety disorder diagnosis to the extent that their natural and daily life and functionality are disrupted. This is while girls experience higher levels of anxiety than boys [12]. These issues are often manifested more from pre-school years and highlightedduring the school years behaviorally [13]. Typically, anxiety accompanies with other problems such as eating disorders and substance abuse. Anxious children are less academically achieved and successful, experience interpersonal relationship with same-aged children and are weak in social abilities. Excessive absences during school years and defective relationship with same-aged children will result in future in weak job adaptation and issues associated with self-image as well as psychological disorders [14].

General forms of anxiety disorders are separation anxiety which is diagnosed by excessive level of anxiety due to being distanced from main caregivers, generalized or extended anxiety, phobia of open spaces, social phobia which includes excessive anxiety due to being judged by others or behaviors in a group, phobia disorders which are diagnosed by unpredictable fearful reactions, particular phobias like phobia of objects, animals or particular situations, OCD disorder which is diagnosed by constant and iterative thoughts and habitual behaviors, post-trauma stress disorder which is the result of a shocking event like disasters, sexual rape or death of someone [15].

Howeverall disorders share the anxiety as their main feature, contextual or particular situational factors make them isolated. Particular phobias as King, Hin and Elending (2005) put refer to a wide range of phobias including the five groups mentioned below:
- Zoophobia
- Nature phobia
- Ampule phobia
- Situational phobias
- Other types of phobia [16]

The other variable studied in this research is depression. Depression is a prevalent illness to the extent which is called psychiatry cold. At any certain time section, 15-20% of adults suffer from depressive signs significantly and at least, 12% have got depression to the extent they need therapy. It is guessed that about 75% of hospitalized cases in Psychiatric hospitals consist of depressed cases. Due to reasons not clarified yet, depression among women is approximately two times more than men in western societies [17].

It seems impossible that only one factor can explain depression, but depression is result of several factors interactions. It is shown that depression starting point and development depends on various variables like biology, background of depression, socio-psychological variables and environment among which followings are mentioned:
- Neurotransmitters dysfunction
- Depression background or alcoholism in family
- Losing parents during childhood
- Being neglected by parents
- Current negative life events
- Marrying a criticizer or aggressive spouse
- Lack of closed and reliable relationship
- Lack of social support
- Lack of long-term sense of being valuable

Scientists believe that acute depression is highly prevalent among children. About 2% of children in 6-12 years and 4% of adolescents are depressed. Based on DSM TR-IV criteria for children and adolescents substantial depression, although increasing among children, they are not enlisted among having depressive disorders due to lack of particularity among these age groups. Australian international psychological health and safety provided a checklist of children's behavioral assessment which classified behavioral issues in two general groups and 7 categories. In this classification, anxiety and depression among children are listed as specialized disorders [18].

Practically, four sets of signs are introduced for depression. In addition to behavioral or emotional signs, the intellectual or cognitive signs, motivational signs and physical signs are also mentioned:

• Emotional signs: sense of disappoint, dissatisfaction, lack of values, humility are emotional depression sign but sorrow is the most important and frequent emotional sign of depression.

• Cognitive signs: depressed individual thinks negatively about him/herself. These negative ideas toward him/herself underlie his/her present and future self-image. Depressed individual often experiences low self-esteem. He/she believes in his failure and knows him/herself responsible for the failures. He/she believes in his/her humiliation, incompetence and unworthy.

• Motivational signs: depressed individual face many difficulties to start their activities. In chronic type, lack of response is willingness paralysis. Such patient cannot even take necessary actions for his life. In acute depression, there may be psycho-motor slowness.

• Physical signs: This includes weight loss, reduction or increase of appetite and sexual tendency reduction. Depressed individual is isolated and his body does not attract anything and increasing concerns occur about pains and suffrages [19].

However, there are not sufficient studies on stress and anxiety among NICU-hospitalized infants' parents in Iran and present resources are mostly focusing on effect of psychological diseases on mother due to preterm parturition. Therefore, based on the significance of early diagnosis of anxiety and depression and low social functioning, interventions to increase the preterm infants' parents' coping power, effect of these disorders on mother and infants' health and lack of sufficient studies in Iran, present study aims to compare the anxiety and depression and social functioning of preterm infants' mothers hospitalized in NICU with healthy infants' mothers.

Fliz Arlan &Koel Koyko (2011) in a research in Anadolu University, Turkey, studied the issues of public hospital NICU-hospitalized infants' families and found out that unreliability of adaptation with child's status, executive processes on child and instruments used, lack of visiting or limited time of visiting, lack of sufficient information from medical team, lack of parenting role implementation when child is hospitalized are variables which lead to anxiety among the families of these children and infants [20].

Beheshti Pour et al. (2014) studied compared the stress level of parents with preterm infants hospitalized in NICU and implied that mothers' stress level was higher than that of fathers. Therefore, providing more information and paying more attention to and supporting for parents when their infants are hospitalized should be more focused by medical team particularly the NICU nurses [21].

Jebrayili et al. (2009) in their study investigated the stressors from hospitalized infants' mothers' perspective in Tabriz and indicated that most stressors for these mothers were appearance and behavior of their infants and their parenting role changes [22].

Bastani & Haghighi (2012) in a research found that parents with hospitalized infants in NICU experience depression, disappoint, sense of isolation, stress, anxiety, emotional duality and reduced control on situations [23].

Greene et al. (2015) analyzed depression, anxiety due to prenatal (PPTS) situation among mothers with low-weighted infants hospitalized in NICU and concluded that psychological screening is important in NICU and PPTS symptoms indicated that there may be need for Primiparas therapy for intervention [24].

Bozkurt et al. (2017) studied whether mothers' psychological disorder are effective on preterm infants' birth in pregnancy over 32 weeks and found that mother's depression is negatively related to preterm infants' nervous development during the pregnancy over 32 weeks and during the long-term infants' follow-up of preterm infants, mothers psychological health has to be taken into consideration [25].

METHODOLOGY

Present research is a descriptive study in term of methodology and since the research predicts the anxiety, depression and social functioning of NICU-hospitalized infants' mothers compared to healthy infants mothers, itis a non-pilot study because the inter-variables associations are not changed. This research compares the anxiety, depression and social functioning of NICU-hospitalized infants' mothers with healthy infants' mothers; therefore, this study is a causative-comparative one. Population included all mothers of NICU-hospitalized infants and healthy infants' mothers in Baqiyatallah and Imam Hossein Hospitals from June 2018 to September 2019.

Because the population studied was extensive and this was not possible to provide a list of all members of this population, 120 mothers and their infants were selected as sample size using available random sampling in 2 hospitals, Tehran. The sample size was obtained using Cochran's formula used for large populations with reliability coefficient of 0.95 and accuracy coefficient of 0.05 and the final sample size was 120 participants. The information required for this research was collected from primary resources like theoretical studies for theoretic discussions including specialized books, articles, journals and specialized researches and secondary recourses including information and comments obtained from healthy and NICU-hospitalized infants' mothers

To find these comments, a questionnaire -for this purpose was distributed among the participants. The questionnaires included Beck's anxiety inventory (BAI) involving 21 items. The participants answer multiple choice questions as their level of anxiety. This questionnaire includes scoring and scores interpretation table.

This scale measures particularly the chronic signs of clinical anxiety among individuals with high reliability and validity. The internal homogeneity coefficient (Alpha-coefficient) is 0.92 whose validity was confirmed to be 0.75 in a reexamination after one week and items correlation was from 0.30 to 0.76.

Beck's depression inventory (BDI): this scale is a self-assessment 21-item questionnaire which measures the depression symptoms. Each item represents illness sign based on four levels from zero to three as scores. The score zero indicates the lowest level and score three indicates higher level of individuals experiencing depressive signs. The sum of scores of each scale is 0-63. This is administrated for measurement if depression level among individuals older than 13 years.

Social functioning questionnaire: this was administrated in 2014 by Lekomet et al. with 34 items. The main objectives of this questionnaire is to analysis and assess the social functioning dimensions (friendships and social activities, independence in life skills, interaction with others, intimacy, family, communications and social activities at work, work competences, communications and social activities at school/university, educational competences). The reliability of this questionnaire was assessed in SPSS by internal homogeneity (Cronbach's Alpha coefficient). The reliability calculated for this questionnaire was 0.8 which is desirable (Cronbach's Alpha coefficient>0.7).

Demographic information questionnaire: this scale aims to measure and study human population and its changes over time which includes age (age distribution), number or population of a group, growth rate, distribution, gender, level of education, urban/rural population ratio and the basis of this questionnaire is statistics and data. In this research, this information includes mothers' age, child birth status, number of children and infants' gender. To analyze data the SPSS was used.

RESULTS

Demographic information

The results implied that 50% of the participants were healthy and 50% were hospitalized.51.7% of mothers had bachelor's degree and 10.8% of mothers had master and PhD degrees. Most of the participants (72.5%) were first child and 5% were 4th child. 23/3% were mothers with physical or mental illnesses and 76.6% had no background of physical or mental illnesses and in sum, 20% and 80% of participants were hospitalized in women's ward before parturition and after parturition, respectively.

Most of the participants (55%) were hospitalized for oxygen therapy and 5% had genetic problems (physical issues). Generally, 52.5% of mothers were 20-30 years old and 15% were 25 years or older.

H1. There is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of anxiety.

To test three first hypotheses, one way ANOVA was used.

Results of ANOVA (Table 1) indicated that there is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of anxiety in significance level lower than 0.05.

H2. There is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of depression.

Results of ANOVA (Table 2) indicated that there is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of depression in significance level lower than 0.05.

H3. There is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of social functioning.

Results of ANOVA (Table 3) indicated that there is a significant difference between NICU-hospitalized infants' mothers and healthy infants' mothers in term of social functioning in significance level lower than 0.05.

Table 1: One-way analysis of variance to compare anxiety between NICU-hospitalized and healthy infants' mothers

Variable	Group	Mean	SD	F	P
Anxiety	NICU-hospitalized infants' mothers	35.36	10.11	394.62	0.00
	Healthy infants' mothers	7.60	5.4		

Table 2: One-way analysis of variance to compare depression between NICU-hospitalized and healthy infants' mothers

Variable	Group	Mean	SD	F	P
Depression	NICU-hospitalized infants' mothers	30.9	8.48	278.97	0.000
	Healthy infants' mothers	8.86	5.69		

H4. Anxiety is a desirable predictor of social functioning of mothers.

H5. Depression is a desirable predictor of social functioning of mothers.

To test hypotheses 4 and 5 the Pearson's correlation test and multivariable regression analysis were used. Tables 4 and 5 present that 0.52 of the changes in mothers' social functioning can be justified by anxiety and depression.

Results of the social functioning regression analysis based on depression and anxiety (table 6) implied the significance of regression equation, i.e. the predicted share of social functioning by depression and anxiety is significant.

Based on regression coefficients tale (Table 7), each predictive variable, i.e. depression and anxiety, can significantly predict the social functioning changes.

Table 3: One-way analysis of variance to compare depression between NICU-hospitalized and healthy infants' mothers

Variable	Group	Mean	SD	F	P
Social functioning	NICU-hospitalized infants' mothers	64.96	23.61	197.32	0.001
	Healthy infants' mothers	115.95	15.25		

Table 4: Correlation matrix of anxiety and depression with social functioning of mothers (P<0.01, N=120)

Variable	Anxiety	Depression	Social functioning
Anxiety	1		
Depression	0.73	1	
Social functioning	-0.63	-0.7	1

Table 5: Results of social functioning regression based on anxiety and depression

Criterion variable	R	R^2	Adjusted R^2	se
Mothers' social functioning	0.73	0.53	0.52	22.32

Table 6: Results of social functioning analysis of regression based on anxiety and depression

Variable	Source of changes	Sum of Squares	Df	Mean Squares	F	Sig. level
Social functioning	Regression	6631.40	2	33158.20	66.56	0.000
	Residue	58293.38	117	498.23		
	Total	124609.79	119			

Table 7: Results of social functioning regression coefficient based on anxiety and depression

Model	Non-standardized coefficients		Standardized coefficients	T	Sig. level
	B	Se	Beta		
Constant	126.90	3.76		33.75	0.000
Anxiety	-0.51	0.18	-0.25	-2.75	0.007
Depression	-1.27	0.22	-0.52	-5.62	0.000

H6. There is a significant relationship between social functioning and depression mediated by the mother's anxiety.

To test this hypothesis, multiple regressions in the hierarchical procedure were used. Here, to achieve the goal of hypothesis 6 i.e. analysis of mediating role of mothers' anxiety, first the anxiety was introduced into the regression equation to predict the social functioning and second, anxiety and depression were introduced into the multiple regressions equation. The results of hierarchical regressions (Table 8) indicated that first, anxiety explained 40% of the social functioning variance and then, anxiety and depression were entered into the regression. The results of multiple correlations changes indicated that 52% of the social functions was predicted by depression*anxiety.

Table 8: Results of social functioning regression based on depression mediated by anxiet0079

Stages	Predictive variable	R	R^2	Moderate R^2	se	Changed R^2
1	Anxiety	0.63	0.40	0.40	25.05	0.40
2	Anxiety*depression	0.73	0.53	0.52	22.32	0.12

DISCUSSION

The results of this research implied that difference of mean scores of two groups of mothers (with healthy infant and with NICU-hospitalized infants) was significant in term of anxiety and equal mean of two groups is rejected. It can be stated that there is significant difference between mothers with healthy infant and with NICU-hospitalized infantsin term of anxiety and anxiety was higher among mothers with NICU-hospitalized infants. In a research in Anadolu University, Turkey, that studied the problems of - NICU-hospitalized infants' families- it was found out that unreliability of adaptation with child's status, executive processes on child and instruments used, lack of visiting or limited time of visiting, lack of sufficient information from medical team, lack of parenting role implementation when child is hospitalized are variables which lead to anxiety among the families of these children and infants.

Difference of mean score of two groups (with healthy infant and with NICU-hospitalized infants) in term of depression was significant and the equal mean score of two groups was rejected. It can be stated that there is significant difference between mothers with healthy infant and with NICU-hospitalized infants in term of depression and depression was higher among mothers with NICU-hospitalized infants. Jebrayili et al. (2009) in their study investigated the stressors from hospitalized infants' mothers' perspective in Tabriz and indicated that most stressors for these mothers were appearance and behavior of their infants and their parenting role changes. In a study on pregnant mothers in Dezfoul, it was observed that it is 3.1 times more probable among depressed women to give low-weighted child than healthy women which was a significant difference statistically. In this research, mean weight and head perimeter were lower significantly among depressed mother's infants than other group [23]. Mean score difference of mothers (with healthy infant and with NICU-hospitalized infants) in term of social functioning was significant and the equal mean score of two groups was rejected. It can be stated that there is significant difference between mothers with healthy infant and with NICU-hospitalized infants in term of social functioning and social functioning was lower among mothers with NICU-hospitalized infants.Explaining this finding, it can be stated that hospitalized infants' mothers are more exposed to the communicational risks. Level of socio-psychological interactions of hospitalized infants' mothers was lower than that of healthy infants' mothers which reduces their social functioning. Explaining why of hospitalized infants' mothers were significantly different from healthy infants' mothers in term of social functioning, it can be stated that based on the formers' situation if vulnerability is addressed, first the individual is non-functioning and then others play role in her lack of desirable functioning. Weak social functioning is associated with limited opportunities of learning, self-esteem, social knowledge and social restrictions. Obviously, socialization process and social functioning obtaining are complicated processes and numerous factors are influential on its transformation. Pregnancy and maternal status are recognized as potentially vulnerable instances for women's social functioning.

There was significant and negative relationship between social functioning and anxiety; therefore, it can be stated that increase in mothers' anxiety reduces their social functioning. So anxiety is a desirable predictor of social functioning among mothers. The results of Beheshti Pour et al. (2014) were consistent with this result.

Beheshti Pour et al. (2014) studied compared the stress level of parents with preterm infants hospitalized in NICU and implied that mothers' stress level was higher than that of fathers [22].

There was significant and negative relationship between social functioning and depression; therefore, it can be stated that increase in mothers' depression reduces their social functioning. So depression is a desirable predictor of social functioning among mothers.

Bastani & Haghighi (2012) in a research found that parents with hospitalized infants in NICU experience depression, disappoint, sense of isolation, stress, anxiety, emotional duality and reduced control on situations [24].

There was significant relationship between social functioning and depression mediated by anxiety and research hypothesis is confirmed. Therefore, it can be stated that it is not simple to distinguish between depression and stress symptoms. These signs sometimes overlap and do not let understanding whether you are depressed or under stress and pressure. Although there are similarities between these symptoms, symptoms of depression are often more intensive and deeper and they may last for some weeks - while stress is experienced only some days. During pregnancy and parturition, deep changes occur in woman's moods due to highly sensitive psychological drivers and they sometimes lead to psychological issues. Postpartum depression can accompany with reduction of mother's activities and disrupts the mother-child relationship and familial activities and constant disruption and lack of diagnosis and early therapy will lead to ineffective adaptation of mother with child, spouse and family and as a result, lack of ability in taking maternal duties. High level of anxiety and stressleads to psychological vulnerabilities. Therefore, hospitalized infants' mothers' anxiety in social functioning after giving birth causes lack of desirable relationship with child and relatives and results in negative reactions and establishes the grounds of social communication reduction among mothers. Behavioral problems prevalence such as weak social behaviors, aggressive behaviors and behavioral disorders are some of the issues the hospitalized infants' mothers experienced.

CONCLUSIONS

Based on the results, parents with chronic child go through predictable emotional stages. First stage is shock of unpredicted disease which generally changes all their life. Next, sadness, sorrow, anger and disappointment occurabout the child who didn't meet his parents' expectations and hopes. Following to these outcomes, parents' social functioning changes particularly among mothers as a predictable outcome and the variables life depression, anxiety and social functioning, as studied in this research, resulted in statistical analysis and significant relationships with NICU-hospitalized infants' mothers.

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ORIGINAL ARTICLES

Procedure and effectiveness of Basic Cardiopulmonary Resuscitation during the landing of an airplane: an exploratory study on military flights

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Abstract: *Introduction: The incidence of cardiorespiratory arrest (CRA) in aircraft is estimated at one per 5-10 million flights. The early initiation of cardiopulmonary resuscitation (CPR) can double or quadruple survival rates after CRA, and is associated with a better prognosis in terms of decreased brain damage and better quality of life. The aim of the present study is to evaluate the effectiveness of basic CPR procedures carried out by the flight crew in a clinical simulation performed in a real environment, during the landing of a military training flight.*

Methodology: The clinical simulation was carried out with 20 members, grouped in ten pairs, of the Méndez Parada Military Parachuting School at the Alcantarilla Air Base (Murcia, Spain). At an altitude of approximately 1,100 feet, each pair simulated CPR on a Little Anne® manikin fitted with a SimPad® PLUS. The results obtained were recorded by SkillReporter ™.

Results: The mean duration of the procedure was approximately five minutes. During the flights, the compression depth achieved with CPR was correct in only six of the ten cases. In only one case was the "patient" checked for consciousness and respiration. In 50% of the simulations, the forehead-chin manoeuvre was not performed (i.e., the airway was not opened).

Conclusions: The lack of standard recommendations in current guidelines on CPR during aircraft landing may provoke discrepancies in the management of this critical situation and reduce the quality of the treatment provided.

Keywords: *cardiopulmonary resuscitation; aircraft; cardiac arrest; special environments; in-flight medical emergencies; landing medical emergencies*

INTRODUCTION

Although commercial aircraft are safe and passenger flight is one of the most widely used means of transport, the millions of persons carried, the fact that some will have pre-existing medical conditions and the long distances often flown all increase the chances of an in-flight emergency happening [1]. It has been estimated that cardiorespiratory arrests (CRA) occur in-flight once in every 5-10 million flights [2-4]. The prompt initiation of cardiopulmonary resuscitation (CPR) can double or quadruple the odds of survival after CRA, and is associated with a better prognosis in terms of decreased brain damage and better quality of life [5-7].

In-flight CPR is a challenging task. Some studies have suggested that the use of a mechanical resuscitation device produces a higher rate of chest compression than is obtained by standard basic life support. However, such devices are not always available [8]. The current guidelines of the American Heart Association (AHA) and the European Resuscitation Council (ERC) only offer general recommendations for in-flight CPR, without addressing aspects such as how to act if an emergency occurs during landing [9-11]. In the context of the armed forces, the role of critical care air transport teams and other trained personnel is essential for the safe transport of unstable patients and combat victims [12].

The aim of the present study is to evaluate the effectiveness

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of the basic CPR procedure employed by the flight crew in a clinical simulation in an otherwise real environment, during the landing of a military training flight.

METHOD

The participants in this exploratory clinical simulation study were 20 members of the Méndez Parada Military Parachuting School at the Alcantarilla Air Base in Murcia (Spain), grouped into ten pairs of jump commanders. Ten clinical flight emergency simulations were created in a real environment during training for parachute jumps with automatic opening. This exercise took place in 2019, using T-12 Aviocar military aircraft (Figure 1A). The CRA simulation began five minutes before landing, at an altitude of approximately 1,100 feet (Figure 1B), when the crew was informed that a CRA had occurred. The simulation ended once the pilot had landed the aircraft and brought it to a halt. During this simulation, CPR was performed on a Little Anne® manikin fitted with a SimPad® PLUS and SkillReporter™, and wearing a helmet, TP2Z parachute, and RTP-26Z reserve (Figure 1C). The simulation was evaluated by two members of the research team (RG and LL), who also formed part of the crew during the training exercises.

Figure 1: Images of the CPR study context during landing. A: T-12 Aviocar military aircraft; B: Altimeter recording 1,100 feet, beside the Little Anne® manikin fitted with a SimPad® PLUS, a helmet, a TP2Z parachute and an RTP-26Z reserve; C: CPR simulation during flight and landing.

All participants gave prior signed informed consent and authorized the video recording and photographing of the simulation. All had basic life support training as part of their combat training and annual refresher courses.

RESULTS

Among the results obtained, the frequency of compressions per minute exceeded ERC recommendations [median ± IQR = 123 ± 32] and the mean depth (mm) was just within the recommended range [median ± IQR = 55.5 ± 11]. Figure 2 summarises the main data obtained in the quantitative evaluation of the quality of external chest compressions. The mean duration of the procedure was approximately five minutes (Figure 2a). The number, frequency and depth of compressions were similar in all the flights except number 7, in which the crew decided to secure the manikin; the difficulty involved in this process limited the effectiveness of their CPR actions (Figures 2b and 2d).

We also conducted a qualitative evaluation of CPR performance, by reference to the corresponding ERC guidelines [10]. Table 1 lists the results of this evaluation, quantifying the instances in which each aspect of CPR was performed correctly or otherwise. Notably, the depth of the compressions obtained was only correct in six of the ten cases considered.

DISCUSSION

Our qualitative assessment of the data on CPR performance reveals that chest compressions were performed better than ventilation. In the latter case, certain aspects were clearly open to improvement. This shortcoming has been reported previously, in the executive summary of the 2015 ERC guidelines [10]. In our case, the difference may have arisen from the fact that only basic life support was performed, that is, without the availability of material for the airway and within the confined space of an aircraft, which prevented good positioning for the mouth-to-mouth ventilation technique.

As recommended in Section 4 of the 2015 ERC guidelines (“Cardiac arrest in special circumstances”), the CPR was performed in a suitable place in our simulation exercises, within the limitations imposed by the characteristics of the aircraft9. Specifically, it was carried out in the middle of the fuselage, where there was most space, with the head of the simulated victim positioned towards the tail of the plane. Although the 2015 ERC guidelines do not indicate the most appropriate position for the victim’s head, this question should be considered because the head is likely to suffer tilt variations during landing [10]. In each CPR simulation (in accordance with the ERC guidelines) the patient’s chest was uncovered and, in this military tactical environment, the parachute and helmet attached to the manikin were also removed [10]. In one flight, the inappropriate positioning of the manikin reduced the quality of the compressions performed.

Figure 2: Quantitative characteristics of the external chest compressions performed. (a) duration of the session, in seconds [median ± IQR = 269 ± 68]; (b) number of compressions per session [median ± IQR = 265 ± 94]; (c) frequency of compressions per minute [median ± IQR = 123 ± 32]; (d) depth of compressions in millimetres [median ± IQR = 55.5 ± 11]. The solid line represents the median and the dashed lines represent the interval ± IQR.

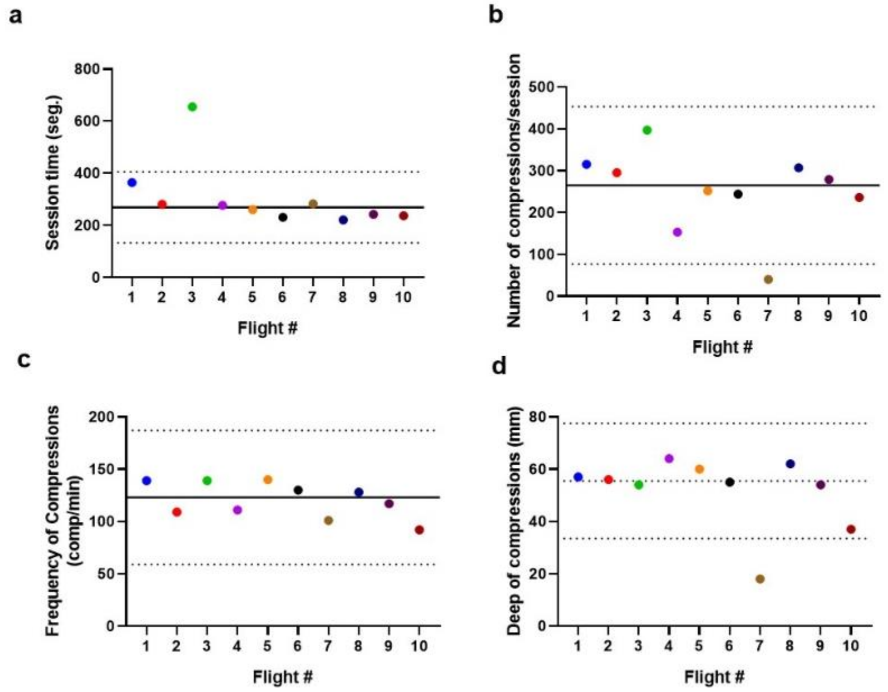

Table 1: Qualitative aspects of the ten simulations considered

Item	Performed	Not performed
Check/ask state of consciousness	1	9
Declare emergency situation	7	3
Check respiration	1	9
Place victim lying face up	10	0
Clear airway (forehead-chin manoeuvre)	5	5
Place hands on the centre of the chest	4	6
Interlock fingers	3	7
Extend arms	0	10
Vertical position over the victim	4	6
Compression: 5-6 cm	6	4
30 compressions at 100-120/min	4	6
Hyperextension of the head	6	4
Pinch the nose	7	2*
Lips-mouth seal	6	-**
Insufflation for 1 second	9	1
Raise the chest	2	7*
Remove mouth, maintain forehead-chin manoeuvre and observe chest elevation	0	10
Maintain ventilation manoeuvres for no more than 5 seconds	10	0
30:2 ratio	10	0

** In one landing, this variable could not be determined by the evaluator; ** In four of the landings, this variable could not be determined by the evaluator.*

In six of the ten flights, CPR was suspended as the aircraft was landing, but the victim was secured in only four cases. Furthermore, the treatment team was not secured in any of the landings, although they did remain seated in six cases. Our review of the literature revealed no specific considerations on the question of safety precautions as the aircraft is landing [9].

In the present study, for which the simulations were conducted within military aircraft, the chest compressions were performed by crew members. Forti et al. [13] have observed that the use of mechanical chest compression devices should also be evaluated since these mechanisms can provide high-quality chest compressions in the confined space of an air ambulance. However, in the present case, this option was not feasible, as no such devices are incorporated in the aircraft considered. A positive factor in our study is that the military personnel who provided CPR attention all had basic life-support training and took annual refresher courses, thus complying with the recommendation that pilots and cabin personnel should be trained in emergency procedures [9].

As a proposal for improvement, the standard recommendations for CPR in aircraft, both in flight and during landing, should be more fully implemented. In this respect, we note that the current AHA and ERC guidelines lack specific recommendations for the treatment of cardiorespiratory arrest in aircraft.

CONCLUSION

In conclusion, we find that the absence of standardized recommendations in the current guidelines on CPR during aircraft landing can provoke discrepancies in the management of this situation and/or reduce the quality of the CPR operations performed. This outcome is due, in part, to the need to balance the safety of the patient against that of the treatment team (i.e., ensuring that everyone is properly secured when necessary and that CPR maneuvers are suspended in certain flight situations), or minimizing pauses in resuscitation, even if this endangers the physical safety of all involved.

Ethical approval and consent to participate

This study was approved by the Ethics Committee of the Catholic University of Murcia (UCAM) and by the Méndez Parada Military Parachuting School at the Alcantarilla Air Base (Murcia). All participants gave signed informed consent to take part in this study. All of the authors undertake to maintain data confidentiality and respect the rights of the participants. These undertakings are stipulated in the author responsibility document, the publication agreement, and the transfer of rights.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

All authors conceived and designed the study. MPR conducted the experiments and analyzed the data. SNC has participated in data collection, analysis and interpretation, and writing. MGV has participated in the data collection; MLI has participated in the data collection; JHM has participated in the interpretation of the data and the writing of the manuscript; ECL has participated in the analysis and interpretation of the data and writing and CCE has participated in the design of the study, data collection, analysis, and writing.

All authors contributed to manuscript revisions.

All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

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ORIGINAL ARTICLES

Psychological consequences of the Covid-19 pandemic. A study carried out on the psychiatric medical staff

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Abstract: *Motivation for the choice of topic: The covid-19 pandemic has restructured the entire health care system. As systems of care were overwhelmed, many health professionals in related professional areas became increasingly involved in providing medical aid. The present paper aims to highlight a comparative analysis between two psychiatric wards of the same hospital, with qualified medical staff, with similar levels of competence, one of the wards caring for patients exclusively with major psychiatric pathologies, and the other patients with associated SARS-COV2 infection.*

The objective is to highlight links between different variables, such as motivation versus amotivation, anxiety and/or burnout, work experience, and resilience, all of which also relate to decisions to accept and provide quality medical care to this particular category of patients.

Methodology Application of specific questionnaires and scales on a group of 60 medical workers in two psychiatric wards, one of which was declared a ward working with psychiatric patients with covid.

Results: Resident doctors, then nurses, followed by hospital attendants, attending physicians and lastly psychologists, cope in descending order with professional stress related to covid-19 anxiety. This is high and correlates positively with depression and burn-out - which, in the case of young people, risks demotivating them to the point of destroying their future plans and the hope of successful professional self-esteem.

Conclusions: The covid-19 pandemic in a psychiatric hospital has raised anxieties even among medical staff, who have moved from somatic to psychiatric care over the years, and the comorbidity of mental illness and SARS-COV2 infection has raised particular problems. Among staff, anxiety, risk of depression, burnout, with depletion of compensatory resources have increased. Resilience calls for the use of mature defence mechanisms and professional experience protects against anxiety and burnout.

Keywords: *burnout; Covid-19 anxiety; stress; resilience; professional status*

INTRODUCTION

The particularity of the study lies in the fact that mental health workers are health professionals who routinely care for psychiatric patients, who are known to have certain characteristics such as: absence of insight into illness, loss of reality testing, productive phenomenology requiring particulars of supervision, suicidal risk, which may be amplified by somatic comorbidities. In other words, the group on which the research was carried out was a highly specific one - the motivation at work of employees in the "psy" area being a special one, involving dedication and training courses in the field, as well as an intrapsychic development developed over years, of a certain nature [1,2].

The research hypotheses were:

1) We assume there are statistically significant correlations between the psychological constructs analysed.

2) We can predict the level of employees' motivation according to the level of depression, resilience and healthcare experience.

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3) We assume that there are statistically significant differences in levels of resilience and intrinsic motivation between employees in a Covid-support medical ward versus employees in a non-Covid medical ward.

4) The occupational status variable has a moderating effect on the relationship between resilience and motivation.

5) The occupational status variable has a moderating effect on the relationship between anxiety and burnout.

6) The healthcare experience variable has a moderating effect on the relationship between amotivation and fear of Covid [3].

METHODOLOGY

Participants

The research was carried out on 60 people, employees of the "Prof. Al. Obregia" Clinical Hospital of Psychiatry, Bucharest, working on two different wards, one declared by the hospital management as a non-Covid medical ward and one ward treating exclusively Covid positive patients. Participants filled in their status on the medical ward: consultant/ registrar (7%), resident doctor (12%), nurse (21%), hospital attendants (26.7%), clinical psychologist (6.7%). The mean age of the group was 39.83 (with SD = 10.47). The inclusion criterion for the study was the employment contract in the hospital and the participants' involvement in medical and psychological activities.

Sampling procedure

The research was carried out in an online environment. The study was carried out on participants employed at the "Prof. Alex. Obregia" Psychiatric Hospital in Bucharest and the completion of the instrument was done through Google forms. Participants were presented with a full description of the study and received written informed consent in accordance with the research standards for human subjects study and confidentiality of personal data was guaranteed. Participants had several professional occupations: consultants or registrars, medical residents, nurses, hospital attendants, clinand ical psychologists [4].

Instruments, measurements, variables

1) The DASS 21-R test [5], which consists of 21 questions with 4 response options on a 0-3 Likert scale (0 = "Did not fit me", 1 = "Fit me to a certain extent or sometimes", 2 = "Fit me quite a lot or quite often", 4 = "Fit me very much or most of the time"). The 21 questions measure 3 subscales (each with 7 items). Items 1, 2, 10, 13, 15, 18, 20 measure the Depression dimension. Items 4, 5, 8, 9, 12, 14, 21 measure the Anxiety dimension. The remaining items measure the Stress dimension. The final score is calculated by summing the items for each.

2) The Maslach Burnout Inventory (Schaufeli, Leiter, Maslach & Jackson, 1996) [6] is composed of 16 items with responses on a Likert scale from 0 to 6 (0 = "Never", 6 = "Daily"). The questionnaire consists of 3 subscales: Exhaustion, Cynicism and Professional Inefficiency The total score can also be calculated. A higher score means a higher burnout.

3) Connor-Davidson Resilience Scale (CD-RISC) [7], which contains 25 items with responses on a Likert scale from 0 to 4 (0 = "not at all true", 1 = "rarely true", 2 = "sometimes true", 3 = "often true", 4 = "always true"). The scale is based on how subjects have felt in the last month. The scale has scores between 0 and 100, with higher scores explaining higher levels of resilience.

4) Multidimensional Workplace Motivation Scale (MWMS) [8], which contains 19 items, with responses on a scale of 1 to 7 (1= "not at all", 2= "very little", 3= "a little", 4= "moderate", 5= "strong", 6= "very strong", 7= "total"). The scale measures 6 subscales: Amotivation, Social extrinsic motivation, Material extrinsic motivation, Introjection regulated motivation, Identification regulated motivation, Intrinsic motivation. A higher score is associated with a higher level of motivation.

5) Fear of COVID-19 scale [9] containing 7 items, with responses on a scale from 1 to 5 (1= "strongly disagree", 2= "disagree", 3= "neither agree nor disagree", 4= "agree", 5= "strongly agree"). Scoring is established according to the summative scale, with sums ranging from 7 to 35. The higher the score, the greater the fear of COVID-19.

In addition to the administered questionnaires, participants filled in factual data on: gender, age, education level, marital status, personal pathological history, health care experience, current work experience, education level, professional status [7].

Research design

The research took place in an online environment and was an observational, cross-sectional study. The database was processed in SPSS version 22, also using the PROCESS extension version 3.5. The variables we worked with were:

- Non-parametric variables: gender, education level, marital status, personal pathological history, education level, professional status;

- Parametric variables: Age, health care experience, current work experience, depression, anxiety, stress (measured with DASS 21), burnout, cynicism, professional inefficiency, total burnout (measured with Maslach Burnout Measurement

Inventory), resilience (measured with CD-RISC), amotivation, extrinsic social motivation, extrinsic material motivation, introjection regulated motivation, identification regulated motivation, intrinsic motivation (measured with MWMS), fear of COVID;

- Moderator variables: professional status, health care experience [10].

RESULTS

Descriptive analysis for the data collected is set out in Table 1.

Table 1: Descriptive analysis

	Minimum	Maximum	Mean	Std. Deviation	Skewness	Kurtosis
	Statistic	*Statistic*	*Statistic*	*Statistic*	*Statistic*	*Statistic*
Age	23.0	59.0	39.833	10.4770	-.010	-1.364
Health experience		43	11.12	10.183	.902	.252
Current experience		30	8.10	8.691	1.060	-.073
Depression	0	19	3.92	4.571	1.595	2.127
Anxiety	0	17	3.57	4.159	1.350	1.276
Stress	0	18	4.45	4.781	1.225	.768
Exhaustion	0	24	6.33	6.299	.793	-.373
Cynicism	0	22	5.20	5.713	1.106	.372
Professional inefficiency	0	27	6.90	7.498	.919	-.374
Burn-out	0	73	18.43	18.671	.954	-.076
Resilience	35	100	75.43	16.532	-.529	-.581
Amotivation	3	19	5.43	4.048	1.840	2.758
Extrinsic social motivation	3	21	9.42	5.782	.402	-1.014
Extrinsic material motivation	3	20	8.48	5.407	.394	-1.277
Introjection motivation	4	28	19.93	6.913	-.689	-.484
Identification motivation	3	21	17.33	4.501	-1.524	2.116
Intrinsic motivation	5	21	17.37	4.084	-1.147	.658
Motivation	33	111	77.97	19.749	-.566	-.346
Covid fear	7	26	12.83	4.705	.670	-.063

To operationalize the variables, the level of education that was noted among the participants was divided into three classes: postgraduate and bachelor studies (graduates of bachelor's, master's, and postgraduate programmes), high school and college of nursing (this included nurses who had graduated from the college of nursing or hospital attendants who had graduated from high school), and secondary school or 10-grade education (for those with less than 10 grades completed), as depicted in Table 2. The distribution by professional status is showed in Figure 1.

Table 2: Level of education

		Frequency	Percent
Valid	Bachelor studies and postgraduate	29	48,3
	High school and college of nursing	17	28,3
	High school	14	23,3
	Total	60	100,0

To test Hypothesis 1, which seeks to see if there are significant correlations between the psychological constructs analyzed on the group of subjects, we used Pearson correlation. Among the important correlations to mention, we list:

- There is a statistically significant negative correlation between experience in the health field (and experience in the current job, p=0.031, r=-0.279) and introjected motivation (p=0.018), with a weak correlation intensity (r=-0.305), which explains that as experience in the health field (implicitly the one from the current job) increases, the level of motivation through introspection decreases.

Figure 1: Distribution by professional status

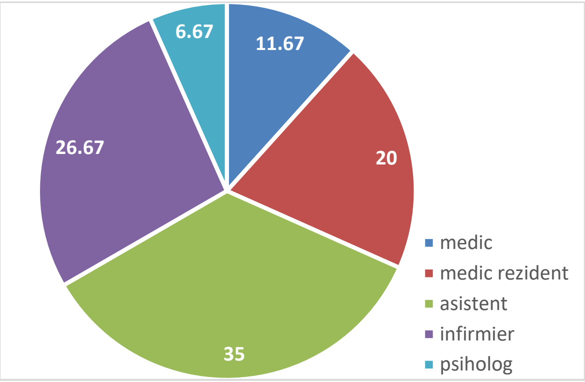

- There are positive correlations of the depression scale with the exhaustion (r=0.744, strong to very strong intensity), cynicism (r=0.698, strong intensity), job inefficiency (r=0.644, strong intensity), burnout (r=0.732, strong intensity), amotivation (r=0.263, weak intensity), extrinsic social motivation (r=0.294, weak intensity), extrinsic material motivation (r=0.427, moderate intensity) scales.

- There are positive correlations of the anxiety scale with the exhaustion (r=0.703, strong intensity), cynicism (r=0.688, strong intensity), job inefficiency (r=0.688, strong intensity), burnout (r=0.724, strong intensity), amotivation (r=0.289, weak intensity), extrinsic material motivation (r=0.314, weak intensity) scales and a negative correlation with intrinsic motivation (r=-0. 28). These data explain that when anxiety levels increase, intrinsic motivation levels decrease.

- There is a positive correlation of stress level with all burnout scales (burnout, cynicism, job ineffectiveness), which denotes that when stress level increases, burnout level increases with its subscales. Stress also correlates positively with amotivation level (r=0.269, low intensity), which explains that when stress level increases, amotivation level increases.

- There is a positive correlation of fear from Covid level with anxiety level (r=0.368, low intensity) and stress level (r=0.263, low intensity), exhaustion level (r=0.384) and cynicism level (r=0.259), work inefficiency level (r=0.356, low to moderate intensity), burnout level (r=0.352, low to moderate intensity) and amotivation level (r=0.312, low intensity).

- Resilience level correlated negatively with the level of the scales: cynicism (r=-0.305), job inefficiency (r=-0.358), burnout (r=-0.322), amotivation (r=-0.327), identified motivation (r=-0.372). Level of resilience correlates positively with level of intrinsic motivation (0.603) [8].

To test Hypothesis 2, which sought to predict the level of motivation of healthcare employees according to predictor variables, we ran multiple linear regression analysis, where the criterion variable was motivation score (measured with MWMS). The motivation variable was included in the regression analysis as a dependent variable and several predictor variables were introduced from the beginning: age, healthcare experience, experience in current workplace, depression, anxiety, stress, exhaustion, cynicism, job inefficiency, burnout, resilience. As a method, we used the Backward Analysis method, a method that, in the end, provides the correct statistical model, after trying, one by one, all possible models, eliminating one by one a variable that is not relevant. In Table 3 we follow the value of the adjusted R2, which indicates the relevance of the model. The highest value indicates model relevance and is 0.192 in model 5. Eight models were generated.

Table 3: Model summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.537[a]	0.289	0.143	18.278
2	0.537[b]	0.288	0.160	18.096
3	0.534[c]	0.285	0.173	17.956
4	0.529[d]	0.280	0.183	17.848
5	0.524[e]	0.274	0.192	17.752
6	0.503[f]	0.253	0.184	17.840
7	0.492[g]	0.242	0.187	17.809
8	0.476[h]	0.227	0.185	17.825

The standardized Beta coefficients indicating the influence of the predictor variables on the dependent variable are included in Table 4.

Table 4: Standardized Beta coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
8	(Constant)	47.024	11.825		3.977	0.000
	Experience in the health field	-0.401	0.232	-0.207	-1.732	0.089
	depression	1.693	0.523	0.392	3.236	0.002
	Resilience	0.381	0.147	0.319	2.602	0.012

Following the presentation and analysis of the table with the Beta coefficients of the regression equation, the corresponding multiple regression equation is as follows:

$$\text{Motivation} = 47.024 - 0.401^*\text{health experience} + 1.693^*\text{depression} + 0.381^*\text{resilience}$$

To test Hypothesis 3, which seeks to highlight differences between the Covid ward employee group versus the non-Covid ward employee group in terms of the variables analyzed, we ran One Way Anova analysis.

Hypothesis 4: The occupational status variable has a moderating effect on the relationship between resilience and motivation. In this way, to investigate the moderating relationship, we used the PROCESS program. The dependent variable was motivation. The predictor variable was resilience. The moderator variable was occupational status [11].

The results explain the occupational status variable as a negative moderator of the relationship between resilience and motivation at work. As shown in Figure 2, the intensity of resilience and motivation is stronger for the category of physicians, medical residents, and psychologists, as resilience is a factor strongly influencing the increase of motivation at work. This result helps us know which psychological constructs should be acted on to increase motivation at work, depending on professional status.

Figure 2

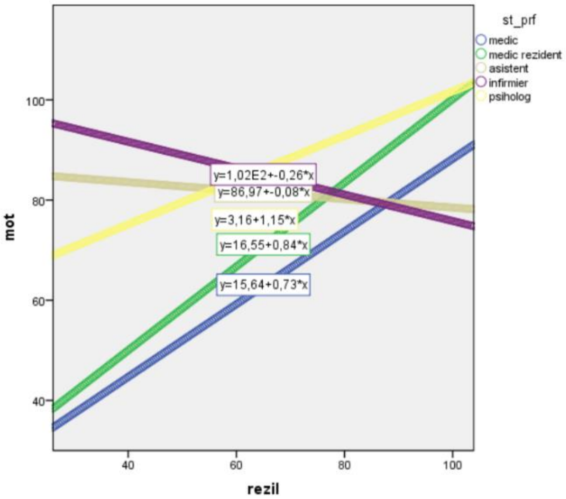

Hypothesis 5: The occupational status variable has a moderating effect on the relationship between anxiety and burnout. Thus, to investigate the moderating relationship, we used the PROCESS program. The dependent variable was burnout. The predictor variable was anxiety. The moderator variable was occupational status. As can be seen in Figure 3, the intensity of anxiety and burnout is stronger for the category of physicians, residents, hospital attendants, and nurses, with a downward slope for the category of psychologists. Thus, for the 4 categories of employees in the "psy" health care field, it is observed that an increase in the level of anxiety leads to a substantial increase in the level of burnout, with maximum intensity in the category of resident doctors, then nurses, then hospital attendants and, finally, on the attending physicians. This result helps us know which employee is more likely to develop burnout as anxiety increases [6].

Figure 3

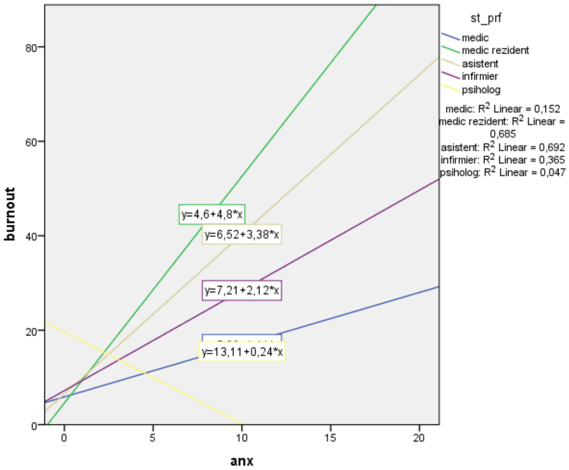

For Hypothesis 6, we tested whether the health care experience variable has a moderating effect on the relationship between amotivation and fear of Covid. Thus, to investigate the moderating relationship, we used the PROCESS program. The dependent variable was fear of Covid, the predictor variable was amotivation, and the moderator variable was health care experience [5]. Due to some clear manifest factors (like lack of taste and smell) that could be due to some other diseases associated with Covid [12], we have had to distinguish between a pathological status versus a psychological fear.

These results explain the occupational status variable as a positive moderator of the relationship between anxiety and burnout at work.

CONCLUSIONS

A first conclusion that stands out in the present study is the regression equation for predicting the motivational level of employees in the "psy" health care field. We have shown that the regression model explains 18.5% of the motivation scale variance. The percentage is a low predictor of motivation according to the scales: depression, resilience and current work experience, however, using the Backward analysis method, which looked for the model that predicts the best, taking each variable in turn and eliminating those that were not significant. The importance that these predictor variables have for the criterion variable is demonstrated. Specifically, depression, resilience and work experience are significant predictors of motivation for several reasons [13].

Depression among staff employed in a psychiatric hospital is less obvious, as those concerned have developed adaptive coping mechanisms for dissimulation, which in crisis situations makes it difficult to highlight emotional, cognitive, decision-making and well-functioning status in the workplace as a whole. In a slightly forced terminology, we could say that many employees have concealed their depression, for fear of social stigma or the idea that for a training in “psy” it is unthinkable to give in.

Resilience is a key concept in any crisis situation. It implies adaptability, flexibility of one's own concepts and defence mechanisms, relaxation of the Superego. From this point of view, resident doctors were the most exposed, not being backed up by the necessary resilience, nor having gone through other moments of crisis in the health system. The feeling of too much professional effort in relation to the satisfaction obtained, the effort to insist by phone up to 12 hours per event to find an available place in a life-support service, the overflow even of COSU (Operational Centre for Emergency Management) resources, the delegation by the consultant of too many responsibilities for the resident doctors (some of them in their first year) or for the nurses, increased at first their anxiety, then, quite quickly, their professional burn-out. A second important finding of the research is the result that resilience and intrinsic motivation are psychological aspects that differ among medical employees between the group of those who worked with Covid positive patients and the group of those who worked with Covid negative patients. Nursing staff in the non-covid ward were under less stress, caring for an issue and pathology they were used to [6].

We also concluded that there are significant differences in the level of stress experienced by employees of a psychiatric ward, depending on their status on the ward (physician, resident doctor, nurse, clinical psychologist, hospital attendant). Psychologists have had to quickly reshape their position (while most of them were performing clinical psychology examinations, now they had to be at the patient's bedside and provide counseling and psychological support) [14].

DİSCUSSİON

Moderation analyses indicate the importance of the professional status an employee has in a medical ward in the relationships between aspects of their own and others' (employees or patients) intrapsychic lives. The fact that the intensity of motivation at work increases on the basis of resilience only for the categories: attending physicians, resident doctors and clinical psychologists can be explained by the dedication to the profession, aspirations for status, dedication to others, the need for affirmation and the need to increase their professional status. The analysis of the relationship between anxiety and burn-out, with a strong increase among all employees in the medical department, except psychologists, maybe due to exactly the aspects previously presented [7].

But, the order of increase in burn-out levels (resident doctors have the highest level of burn-out related to anxiety, then nurses, then hospital attendants, and, last, attending physicians) is also important in order to rethink responsibilities in the medical hierarchy – operationalized not "ad hoc" in crisis situations, but through well-standardized internal procedures and protocols as mentioned by Chen too [4].

Finally, the result expressing how healthcare experience positively shapes the relationship between amotivation and fear of covid brings about important insights into the need for relevant medical training at the bedside, and clinical aspects that could bring diagnostic benefits being brought to the forefront [9].

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ORIGINAL ARTICLES

Regression model for predicting the severity of acute poisoning cases by estimating PSS in the Emergency Department

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Abstract: *Acute poisonings represent an emergency pathology that associates an increased risk of unfavorable outcomes or death. The mortality rate can be improved by the fast recognition of its severity, which in turn allows a prompt intervention of the medical team. In the practical approach of the case, a standardized measure of predicting the patient's evolution would be necessary, which could be applied quickly to the patient's bed and easy to calculate and apply irrespective of the evaluator. Currently, for acute poisoning cases, Poisoning Severity Score (PSS) is used, a complex and dependable tool that accurately stages the severity of the case but has the disadvantage of being quantified retrospectively. This study presents the development and validation of a linear regression model that can be applied right in the emergency department (ED) and predicts the severity of the case by estimating PSS with an accuracy of 75%. The proposed model uses ten objective and quantifiable variables representing anamnestic, clinical, and biological parameters evaluated in the early stages of the poisoning. The regression was developed in the study group consisted of 62 pediatric patients diagnosed with severe acute poisoning with cardiotoxic agents complicated by cardiogenic shock.*

Keywords: *PSS, regression model, acute poisoning, cardiotoxicity*

INTRODUCTION

Acute poisoning is a global health problem, so preventing mortality is essential in intentional and unintentional exposures. Therefore, predicting the evolution of acutely poisoned patients is of clinical importance because it allows a prompt and adequate therapeutic intervention [1,2].

Like other medical specialties, Toxicology also benefits from classifying the severity of its characteristic pathology, for acute poisoning being used Poisoning Severity Score (PSS). It can be applied to both adults and children, regardless of the type and number of etiological agents involved. For classification, it is necessary to know the complete evolution of the case, considering the most severe clinical and/or paraclinical features recorded during hospitalization. For this reason, the score is quantified retrospectively after the patient's discharge or death. The classification only considers the directly observed signs and symptoms without estimating risks based on parameters such as the ingested dose or the serum/plasma level of the toxicant [2,3].

Predictive scores specific to toxicologic pathology that can be applied in an emergency case are rarely developed, even less so in pediatric patients. Emergency physicians typically use standard scores such as APACHE II/IV (Acute Physiology and Chronic Health Evaluation), SAPS II (Simplified Acute Physiology Score) or SOFA (Sepsis-Related Organ Failure Related). However, these scores do not evaluate the particularities of the clinical examination in a patient with acute poisoning or the characteristics of the toxicant [4].

The exhaustive evaluation that PSS performs using 12 categories of criteria, depending on the systems affected by

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the poisoning, makes it valuable and reliable. However, some studies signal some of its disadvantages [2]. Many variables analyzed, some of which have subjective evaluations such as "mild hypotension" or "rare/frequent seizures", make it difficult to quantify, time-consuming, and not consistently reproducible from one evaluator to another [5].

At admission, the examination of the case with acute poisoning may be within normal limits or discreetly modified, yet it may degrade in evolution to the point of requiring advanced therapeutic measures. Therefore it becomes necessary to identify criteria that raise the suspicion of the risk of unfavorable evolution since admission so that the medical team can make the best decisions in handling the case [6].

The importance of PSS for research is undeniable. However, in terms of clinical efficacy, a severity score should quickly assess the prognosis, establish the need for hospitalization and the department's rank where to be cared for, and especially be quickly calculated at the patient's bedside [7].

Considering that PSS is a tool for retrospective assessment of case severity, the premise of this study was to identify those features evaluated from the patient's initial examination that correlates with its subsequent evolution. The study aimed to develop a statistical model that predicts the severity of the case by quantifying these indicators from the emergency department.

METHODS

Study design and selection of study patients

This study is a retrospective analysis of a sample of patients recruited from the Department of Toxicology of the "Grigore Alexandrescu" Emergency Children's Hospital Bucharest. There were analyzed cases of acute poisoning hospitalized between 2014 - 2020, amounting to 7666 patients. Have been researched clinical observation files of the poisoned patients and identified those where the etiologic agent was cardiotoxic, so with a potential evolution to cardiogenic shock, obtaining a group of 1396 patients. After a thorough analysis of the patients at risk, have been selected those that have shown from admission or in evolution signs of cardiogenic shock, obtaining a batch of 62 severe cases.

Each of the 62 pediatric patients enrolled in the study group presented at least one of the following diagnostic criteria for cardiogenic shock: weakly or absent pulse in the major arteries, capillary recoloration time over 2 seconds, low blood pressure, cold, cyanotic or marbled extremities, decrease of diuresis below 1 ml/kg/hour or altered mental status [8,9].

Data analysis protocol

Base data for this research has been collected from the clinical observation files and included the baseline characteristics of patients with poisoning: age and sex; poisoning-related variables, such as the intent of poisoning, type of substance, route of exposure; time-related factors, such as time-lapsed between exposure and examination; and initial vital signs at the ED like heart rate (HR), systolic blood pressure (SBP), body temperature (BT), respiration rate (RR); and AVPU (A-alert, V-verbal response, P-pain response, U-unresponsive) scale of mental status (10,16,17). The collected data also contained admission medical tests and outcome-related variables, such as mortality at ED or after admission.

The work plan included:

(1) Assessment of statistically significant associations between variables: anamnestic, clinical and paraclinical indices, etiology, gender, length of hospitalization, development of complications.

(2) Verifying if the chosen variables can develop a regression model.

(3) Determining the coefficients of the regression model and its validation.

Statistical analysis

The data obtained from the analysis of the clinical cases in the study group were evaluated using the IBM SPSS v20 program, and the results were represented as graphs and tables to identify regression coefficients. The conditions for carrying out a linear regression model were assessed by verifying the existence of gross errors between the recorded values, also if the chosen variables are independent (Durbin-Watson statistical test) or correlated (Pearson test), respectively if there is a statistically significant link between input and output variables (ANOVA test). After all conditions were met, a linear regression model was developed to quantify the relationship between the independent and dependent variables.

RESULTS AND DISCUSSION

PSS is a standardized measure of the acute poisoning severity and is the main objective of the regression model. The usefulness of this model is that it estimates this score at admission, distributing patients into five categories according to the PSS classification.

Of all the variables recorded in the patients' database, ten were selected for having the highest potential to influence the prediction of the PSS [11]. These are intentional poisoning, ECG changes, systolic blood pressure (SBP), bradycardia (according to ages groups), coma, time-elapsed between ingestion and ED examination, transfer from another hospital, etiology represented by pesticides, etiology represented by a combination of beta-blocker with calcium channel blocker, the value of base excess. The values of all the selected variables are recorded in the emergency department after the initial examination [11,12].

In order to verify the model, the batch of 62 selected patients was divided into two samples. One, the derivation group, will be used to make the model, and the other, the validation group, to test the developed model. The number of patients in each group was determined based on Yamane's relationship:

$$n = \frac{N}{1 + N \cdot e^2}$$

where N - the number of patients in the group (N = 62), and e - the chosen marginal error (e = 5%) [13].

Based on the above formula, the group used to develop the model comprises 54 patients, and the rest will constitute the validation group. The eight patients in the test group were chosen at random and had the identification number: 28, 41, 43, 47, 49, 52, 54 and 62.

The development of the regression model was performed in two distinct phases. In the first phase, it is checked if the chosen variables can constitute a linear regression model, while in the second one, the values of the model coefficients are determined.

The following hypotheses were further verified [13]:

If the chosen variables contain gross errors

For this, the linear residues (errors) were determined and checked if their distribution was normal. As can be seen from Table 1, Standard Residuals fall in the range [-3, 3], so it can be concluded that the values used are not affected by gross errors.

Table 1: Determination of linear errors to identify gross errors (Residuals Statistics)

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	2.11	3.46	2.71	0.356	38
Residual	-0.880	1.072	0.000	0.439	38
Std. Predicted Value	-1.686	2.119	0.000	1.000	38
Std. Residual	-1.713	2.085	0.000	0.854	38

[1] If the variables are independent.

This hypothesis was verified based on the Durbin-Watson statistical test (Table 2), which imposes a maximum limit of 3 for independent variables. In our case, the value of the Durbin-Watson coefficient is 2.011.

Table 2: Calculation of the Durbin - Watson coefficient for the selected variables (Model Summary)

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.730	0.797	0.173	0.514	2.011

(2) If the variables are correlated

For this, the Pearson coefficients between the independent and the dependent variable were calculated, and because the value of these coefficients does not exceed the threshold of 0.59, it was concluded that there is no individual statistical correlation.

(3) If there is a statistical link between the independent and the dependent variables

This hypothesis was verified based on the ANOVA test, which produced a coefficient $p < 0.05$ (Table 3), meaning that the statistical significance of the proposed regression model is significant. Therefore there is a statistically significant link between the independent and the dependent variables.

Table 3: ANOVA test applied for the chosen variables

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	4.685	10	0.469	1.774	0.015
Residual	7.131	27	0.264		
Total	11.816	37			

Because all the hypotheses were fulfilled, a linear regression model was made in the second phase, and the coefficients related to this model were determined (Table 4). In this case, all the coefficients are statistically significant for the model (the value of $p < 0.05$) and have the values from the table.

The regression model developed is represented by equation below:

$$\text{PSS \_estimated} = \text{IP} \cdot 0.402 + \text{ECG} \cdot 0.097 - \text{SBP} \cdot 0.004 + \text{Coma} \cdot 0.495 + \text{Brad} \cdot 0.146 + \text{Texp} \cdot 0.001 - \text{Referral} \cdot 0.086 + \text{BE} \cdot 0.019 + \text{Pes} \cdot 0.499 + \text{BBCC} \cdot 0.565 + 2.407 \quad (1)$$

where: IP – intentional poisoning (0/1), ECG – ECG changes at presentation (0/1), SBS – systolic blood pressure at presentation (mmHg value), coma – coma at presentation (0/1), Brad – Bradycardia (0/1), Texp – time between exposure and examination (minutes), Referral (0/1), BE –

base excess at presentation (value mmol / l), Pes – Pesticides (0/1), BBBC – beta-blocker and Ca channel blocker (0/1), constant = 2.407.

Table 4: Determination of the coefficients related to the linear regression model

Variable	Linear regression model coefficients		Standardized coefficients	t	Sig.
	B	Standard Error	Beta		
Constant	2.407	0.414		5.809	0.000
Intentional poisoning	0.402	0.237	0.335	1.699	0.001
ECG modified	0.097	0.245	0.083	0.396	0.006
SBP (mmHg)	-0.004	0.005	-0.196	-0.777	0.044
Coma	0.495	0.276	0.344	1.791	0.035
Bradycardia	0.146	0.200	0.128	0.732	0.041
Time-lapse between exposure and examination (min)	0.001	0.000	0.234	1.433	0.013
Referred patient	-0.086	0.206	-0.076	-0.420	0.048
Pesticide	0.499	0.296	0.302	1.683	0.004
Base excess	0.019	0.032	0.118	0.590	0.050
Beta-blocker and Ca channel blocker combination	0.565	0.304	0.342	1.857	0.047

In order to avoid an additional phase of interpreting the results and obtain a quick estimate of the prognosis, in the development of the model, it was considered that the value range of the estimated PSS variable should be identical to that of the standard PSS variable. This fact led to the appearance of subunit coefficients with three decimals and, therefore, a complex function. We consider that this compromise is acceptable to keep the easy interpretation of the data. The practical calculation can be performed with the help of the Excel program.

Figure 1: Visual representation of the regression model

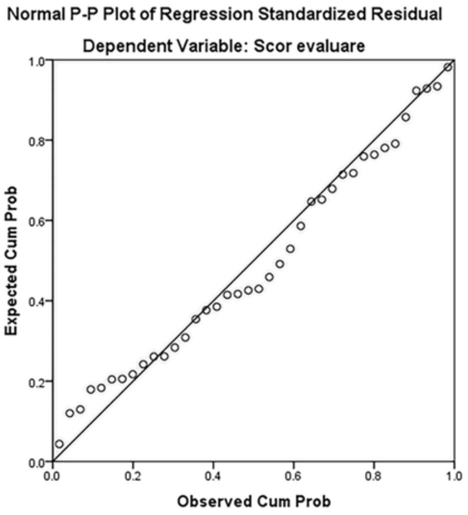

°: *PSS registered at the patient's discharge or death (training data)*

—: *PSS estimated using the proposed regression model*

Their similar appearance can be noticed by overlapping the estimated PSS graph and the registered PSS graph (Figure 1).

The validation of the model was done starting from the degree of correlation between the independent and dependent variables. Thus, based on the values in Table 5 below, we conclude:

- there is a high degree of correlation between the independent and dependent variables R = 0.730.
- 79.7% (R2 = 0.797) from the variation of the dependent variable is generated by the independent variables chosen in the model. The remaining 20.3% is influenced by other variables that could not be identified in the present study.

Table 5: Checking the correlation between the independent and the dependent variable

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.730	0.797	0.173	0.514	2.011

Further, to have a quantitative evaluation of the developed model, equation 1 was applied to the sample of eight patients in the test group. Their input data are summarized in Table 6.

Following the application of the regression model developed, in 6 of the 8 cases tested, PSS was accurately predicted (Table 7). This results in a success rate of 75%. It can be concluded that the developed model successfully predicts PSS, impacting choosing the optimal therapy for each patient [14].

Table 6: Input data of the 8 patients in the test group

ID	IP	ECG	SBP	Coma	Bradycardia	T_{exp}	Referral	Pesticide	BE	BBBC
28	0	0	74	0	0	40	1	0	-4.6	0
41	0	1	95	0	0	120	1	0	1	0
43	1	1	120	0	1	30	1	0	-2	0
47	0	0	90	0	0	300	1	1	-10	0
49	1	1	115	0	1	1440	0	0	1.4	0
52	0	0	100	0	0	60	0	0	-2	1
54	0	1	100	1	0	180	1	0	-1.1	0
62	1	1	147	0	0	120	0	0	11	0

Table 7: The results obtained in the test group following the application of the regression model

Estimated PSS	Rounded PSS	Recorded PSS	PSS differences
1.9416	2	2	0
2.069	2	2	0
2.451	3	3	0
2.3	2	4	2
2.7626	3	2	1
2.54	3	3	0
2.5101	3	3	0
2.539	3	3	0

Due to the subunit coefficients of the identified variables, the application of the model could be time-consuming and prone to errors. The alternative is represented by a multicriteria analysis derived from the proposed regression model, which would reduce the number of variables used and create a faster calculation template for estimating the PSS. However, its accuracy would decrease, being a compromise between the complexity of the regression model and precision [15].

There are numerous studies in the literature, but most of them performed on the adult population, which propose various scores to predict the severity or mortality of cases of acute poisoning. A Korean team (Han et al.) published a recent article proposing a new score for predicting poisoned patients mortality in the ED [10]. The developed template uses a hybrid approach where the model coefficients are divided into categories, and their value is standardized to facilitate the total calculation. However, the model requires an additional phase of interpreting the results and, in addition, only estimates the risk of mortality without making assessments about the cases' evolution.

CONCLUSIONS

The present study aimed and succeeded in developing a linear regression model that uses both toxicant-related characteristics and patients' physiological features in the emergency department, in order to predict Poisoning Severity Score with an accuracy of 75%, in the early stages of acute poisoning with cardiotoxic agents complicated by cardiogenic shock.

The proposed regression model can be the basis of multicriteria analysis, much easier and faster to apply by the examining physician, and which should be a valuable tool for predicting the patient's outcome in the emergency department.

Although the present study was carried on a small sample of patients, the applied methodology has the advantage of a smooth transition to a larger group. The accuracy of the model can be further improved by the increase of the training lot.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

All authors had an equal contribution to the conception and design of the study and also to the acquisition, analysis and interpretation of data.

Ethics approval and consent to participate

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital, Grigore Alexandrescu Children's Emergency Hospital Bucharest. The clinical observation sheets were analyzed following the medical legislation in force.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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ORIGINAL ARTICLES

Relationship between pregnancy and sense of taste

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Abstract: *Background: The change in taste is common for pregnant women. This study aimed to assess the sense of taste in pregnant women in comparison with non-pregnant women.*

Methods: A cohort study was conducted on 120 healthy women. The test group (n=60) included pregnant women presenting to a hospital for pregnancy care in their first, second and third trimesters. Control group (n=60) included healthy non-pregnant women presenting to the same center. The groups were matched in terms of age, marital status and oral hygiene. Assessment of sense of taste was done for two groups using sucrose, sodium chloride and citric acid solutions for perception of sweetness, saltiness and sourness. Serial dilutions of sucrose and sodium chloride solutions were prepared in 1.0, 0.1 and 0.01mM concentrations. Serial dilutions of citric acid were prepared in 3.2, 0.32 and 0.032M concentrations. Data were analyzed using SPSS version 17 and chi square test.

Results: Forty-four non-pregnant (73.3%) and 26 pregnant (43.3%) women gave wrong answer to 0.01mM sucrose solution and this difference wasn't statistically significant (p=0.07). One individual in each group (1.7%) gave wrong answer to 0.1 and 1mM concentration of sucrose. This difference wasn't statistically significant (P=0.9). Wrong answers in the two groups and among the three trimesters weren't significantly different for sodium chloride and citric acid (P=0.9). The difference among pregnant women in the three trimesters wasn't significant for any concentration or tested solution (P=0.9).

Conclusion: The sweet taste threshold decreases during pregnancy but the perception threshold for saltiness and sourness remains unchanged.

Keywords: *pregnancy; taste; taste threshold*

INTRODUCTION

Change in the sense of taste is a common complaint of pregnant women and can result in alterations in their nutritional regimen [1]. Nutrition of mother during pregnancy is a critical factor in health of mother and fetus. Poor quality of nutrition during pregnancy can lead to nutritional deficiencies, weight gain, and increased risk of pregnancy diabetes, high blood pressure and problems during delivery [1]. The pregnant women in their second trimester have higher sugar cravings [2]. The changes in sense of taste have a relatively high prevalence in pregnant women (26%) and most pregnant women in their study showed increased sensitivity to bitterness and decreased sensitivity to saltiness [3]. The sense of taste changes during pregnancy especially in the first trimester [4]. Change in sense of taste can serve as a protective mechanism since pregnant women have less craving for bitter foods, which often contain toxic agents and by doing so, the fetus is protected against toxic nutrients [5].

The mechanism of change in sense of taste during pregnancy has yet to be fully understood. However, hormonal alterations as the cause of changes in taste during pregnancy [6]. Changes in nervous system are also a suggested mechanism for change in sense of taste during pregnancy [7].

The studies on changes in sense of taste during pregnancy have yielded controversial results and discussed that this controversy might be attributed to different methodologies

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of studies and methods of assessment [8]. Also, change in sense of taste may be variable in different trimesters and it has been shown that alternations in sense of taste have the highest prevalence in the first trimester and decrease later in the course of pregnancy. The sense of taste returns to normal after delivery [3]. This study aimed to compare the sense of taste in pregnant women in different trimesters in comparison with non-pregnant women in the Mirzakoochak Khan Hospital, Tehran, Iran.

METHODS

Research design

This historical cohort study was conducted at the Gynecology and Obstetrician Department of Mirzakoochak Khan Hospital Tehran, Iran.

Research subjects

A total of 120 pregnant and non-pregnant women (60 pregnant and 60 non-pregnant women) were included into the study. The sample size was calculated based on a pilot study conducted on 10 pregnant and 10 non-pregnant women; the difference in percentage of wrong answers between the two groups was 20%. Considering the confidence interval of 90% and study power of 80%, sample size was calculated to be 60 in each group (a total of 120 in the two groups) [9].

Pregnant women comprised the test group and were selected among those presenting to Gynecology and Obstetrician Department of Mirzakoochak Khan Hospital for routine pregnancy care. Pregnancy of these patients had been confirmed by urine and blood tests.

The test group (n=60) included pregnant women presenting to a hospital for pregnancy care in their first, second and third trimesters.

The control group included non-pregnant women who were selected among those presenting to the same hospital for routine checkup and matched the test group in terms of age, marital status, oral hygiene and use of prosthetic or orthodontic appliances. The inclusion criteria were absence of systemic diseases, no history of sensory disturbances in the oral cavity, no smoking, no drug intake (except for vitamin supplements), no history of radiotherapy or chemotherapy, absence of mouth dryness [10] and absence of nausea and vomiting of pregnancy.

Oral hygiene measurements

To assess oral hygiene of participants, the Turesky-Gilmore-Glickman modification of Quigley Hein index was used [11]. Disclosing tablets were used to quantify plaque accumulated on buccal and lingual surfaces of teeth. Calculated plaque index ≤2 indicated good oral hygiene and plaque index >2 indicated poor oral hygiene.

Taste perception test

The sense of taste in participants was assessed according to the method described by Saluja et al. [12]. Three serial dilutions (1/2 log concentration) of sucrose, sodium chloride and citric acid solutions were prepared to assess the perception of sweetness, saltiness and sourness. Sodium chloride and sucrose solutions were serially diluted to obtain 1.0, 0.1 and 0.01mM concentrations. Citric acid was serially diluted to obtain 3.2, 0.32 and 0.032M concentrations. The solutions were diluted using distilled water. Participants were requested to refrain from eating and drinking for one hour before testing. They were asked to rinse their mouth 10 seconds prior to testing. Next, they were requested to taste 5mL of each solution for 10 seconds and then spit it out (starting from the lowest concentration). Participants were asked to rinse their mouth in-between tastings. Each test took 5-10 minutes in each individual and a minimum interval of 30 seconds was allowed between tests. Minimum concentration of each solution perceived by each participant was recorded. Participants were classified for their taste perception into two groups of correct and incorrect taste perception [12].

Assessment of taste perception was carried out between 9-11 a.m. every morning in a relaxed position under the same conditions for all patients.

Statistical analysis

Data were analyzed using SPSS version 17 (SPSS Inc., IL, USA) and the data were compared between the test and control groups using chi square test. A $p<0.05$ was considered statistically significant. Because the study was a qualitative research based answer of pregnant women, the Chi-square should be used for statistically significantly results.

Ethical clearance

Both test and control group subjects were thoroughly informed about the study and signed informed consent forms prior to participation in the study. Participants were ensured about the confidentiality of their information. The study protocol was approved in the Ethics Committee Of Dental Branch, Islamic Azad University (code: IR.IAU.DENTAL.REC.1395, 22).

RESULTS

A total of 120 participants including 60 pregnant and 60 non-pregnant women were evaluated. The mean age was

25.1±2.6 years in non-pregnant and 26.4±3.3 years in pregnant women. The two groups were not significantly different in terms of age (P>0.05). In terms of oral hygiene, in the group of non-pregnant women, 48 had well and 12 had poor oral hygiene. In pregnant women, 50 had well and 10 had poor oral hygiene. The difference in this regard between the two groups was not significant (P>0.05).

Table 1 shows the frequency of correct and incorrect taste perceptions in the two groups based on the concentration of sucrose solution tested. As seen in Table 1, in the group of non-pregnant women, 44 (73.3%) had wrong taste perception for 0.01mM concentration of sucrose; this rate was 43.3% (n=26) in pregnant women. According to chi square test, this difference between the two groups was statistically significant (P<0.05). One participant in each group (1.7%) had wrong taste perception for 0.1mM concentration of sucrose and the two groups were not significantly different in this respect (P=0.9). One participant in each group (1.7%) had wrong taste perception for 1.0 mM concentration of sucrose and the two groups were not significantly different in this respect (P=0.9).

Table 1: The frequency distribution of taste perception of participants in the two groups based on the tested concentration of sucrose

Group/Sucrose concentration (mM)	0.01		0.1		1	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
Non-pregnant	16 (26.7%)	44 (73.3%)	59 (93.3%)	1 (1.7%)	59 (93.3%)	1 (1.7%)
Pregnant	34 (56.7%)	26 (43.3%)	59 (93.3%)	1 (1.7%)	59 (93.3%)	1 (1.7%)
P value	P<0.05		P=0.9		P=0.9	

Table 2 shows the frequency distribution of participants in terms of taste perception of different concentrations of sodium chloride. As seen in Table 2, taste perception of different concentrations of sodium chloride was not significantly different between the two groups of pregnant and non-pregnant women (P=0.9).

Table 2: The frequency distribution of taste perception of participants in the two groups based on the tested concentration of sodium chloride

Group/Sodium chloride concentration (mM)	0.01		0.1		1	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
Non-pregnant	14 (23.3%)	46 (76.7%)	59 (93.3%)	1 (1.7%)	59 (93.3%)	1 (1.7%)
Pregnant	15 (25%)	45 (75%)	58 (96.6%)	2 (3.4%)	58 (96.6%)	2 (3.4%)
P value	P=0.9		P=0.9		P=0.9	

Table 3 shows the frequency distribution of participants in terms of taste perception of different concentrations of citric acid. As demonstrated in Table 3, taste perception of different concentrations of citric acid was not significantly different between the two groups of pregnant and non-pregnant women (P=0.9).

Table 3: The frequency distribution of taste perception of participants in the two groups based on the tested concentration of citric acid

Group/Citric acid concentration (mM)	0.01		0.1		1	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
Non-pregnant	1 (1.7%)	59 (93.3%)	59 (93.3%)	1 (1.7%)	60 (100%)	0
Pregnant	0	60 (100%)	59 (93.3%)	1 (1.7%)	59 (93.3%)	1 (1.7%)
P value	P=0.9		P=0.9		P=0.9	

Comparison of taste perception of women in the three trimesters for citric acid, sucrose, and sodium chloride showed no significant differences (P>0.05, Table 4).

DISCUSSION

The change in taste is common for pregnant women. This study aimed to assess the sense of taste in pregnant women in comparison with non-pregnant women. Forty-four non-pregnant (73.3%) and 26 pregnant (43.3%) women gave wrong answer to 0.01mM sucrose solution and this difference wasn't statistically significant (p=0.07). One individual in each group (1.7%) gave wrong answer to 0.1 and 1mM concentration of sucrose. This difference wasn't statistically significant (P=0.9). Wrong answers in the two groups and among the three trimesters weren't significantly different for sodium chloride and citric acid (P=0.9). The difference among pregnant women in the three trimesters wasn't significant for any concentration or tested solution

(P=0.9).

Table 4: The frequency distribution of pregnant women with correct taste perception of sucrose, sodium chloride and citric acid in the three trimester

Trimester/Solution	Sucrose			Sodium chloride			Citric acid		
	1	2	3	1	2	3	1	2	3
First trimester (n=20)	9	11	0	5	15	0	0	20	0
Second trimester (n=20)	13	6	1	6	13	1	0	19	1
Third trimester (n=20)	12	8	0	4	15	1	0	20	0
P value	P=0.9								

Proper and healthy nutrition plays a key role in a successful pregnancy [12]. Increased appetite is a common finding during pregnancy [14,15]. However, change in sense of taste and nutritional problems have been commonly reported by pregnant women [16]. This study compared the sense of taste of pregnant and non-pregnant women using different concentrations of sucrose, sodium chloride and citric acid solutions. These solutions were chosen for this study since they have no adverse effect on the mother and fetus.

Electrogustometric evidence shows that taste threshold increases with age. In other words, aging decreases the sense of taste [17]. These changes often commence at the age of 60 years in areas innervated by the chorda tympani and glossopharyngeal nerves and at the age of 70 years in areas innervated by the greater petrosal nerve [17]. Since the current study was conducted on 20 to 30 year-old women, the effect of age on sense of taste was not significant.

The current results revealed that the taste threshold of sucrose (sweetness) in pregnant women was lower than that in non-pregnant women. Similarly, the reduction in taste threshold of all tastes including sweetness in pregnant women [18]. Saluja et al, [12] also reported deceased taste threshold for sucrose in pregnant women. This change in taste has been attributed to hormonal changes during pregnancy. The changes in taste threshold of women during menstruation cycle and showed hyper-sensitivity to taste prior to ovulation and decreased sensitivity to taste after ovulation [19]. Since the sense of taste was found to be constant in males, such alternations in sense of taste especially for sweetness in women were attributed to female sex hormones and their alterations. They explained that increased sensitivity to taste prior to ovulation is probably attributed to high level of estrogen and decreased sensitivity to taste after ovulation is probably attributed to a combined function of estrogen and progesterone.

Our study only found a significant difference in taste of sweetness between pregnant and non-pregnant women while Kolbe et al. [20] reported higher taste threshold for all four main tastes in pregnant women. Also, Kuga et al. [4] reported increased taste threshold in pregnant women particularly in the first trimester. Difference between their results and ours may be explained by racial and ethnic differences between populations and different methodologies of the studies since Kuga et al. [4] did not perform an actual testing on participants and only questioned the participants verbally. The accuracy and reliability of this method (self-report) is somehow questionable. The method used by Kolbe et al. [20] was different from that in our study. Also, use of different concentrations of solutions can yield different results.

We did not find any significant difference between pregnant and non-pregnant women in terms of tasting saltiness, which was in line with the results of Saluja et al [12]. They reported that pregnancy does not affect on taste perception of saltiness. However, our results in this regard were in contrast to those of Brown and Toma [21], Hayes et al, [6] and Ochsenbein-Kölbleet al [18] since the afore-mentioned studies reported decreased taste threshold or higher craving for saltiness during pregnancy. These differences in the results of studies may be attributed to the use of different solutions with variable concentrations.

In our study, the difference between the two groups in terms of perception of sourness was not significant, which was in agreement with the results of Saluja et al. [12] who also reported that taste perception for sourness does not change during pregnancy. However, the some studies indicated that craving for sourness was different between non-pregnant women and those in the first, second and third trimesters of pregnancy. Also, Kolbe et al. [20] and Kuga et al. [4] indicated a change in perception of sourness in pregnant women. Racial, ethnic and geographical differences can all affect the nutritional habits of individuals and their perception of tastes such as sourness. Also, individual and genetic differences (variations in chemical receptors) can affect the sense of taste [22, 23]. It appears that each individual has their own gustation perception, and nutritional habits of individuals can also affect their taste threshold.

In general, in a healthy state, gestation of pregnant women is altered in a such a way to protect the health of mother and fetus. Changes in sense of smell of pregnant women also protect them against harmful chemicals. Changes in sense of taste during pregnancy enable consumption of a wider range of electrolytes and nutrients [20]. These changes include increased sensitivity to bitterness in the first trimester as a protective mechanism against toxins and increased craving for salty, sweet and sour foods in the second and third trimesters in order for the mother to consume a wide variety of nutrients required for the health of fetus [6]. Several physiological, psychological and social factors control nutritional habits of individuals. Psychological, physiological and behavioral factors can also affect the nutritional choice and consumption of food by pregnant women.

Several factors including oral hygiene, smoking, alcohol use, drug intake, systemic diseases and history of chemotherapy and radiotherapy may affect the sense of taste of individuals. Thus, in this study, the two groups were matched in terms of oral hygiene, and the other afore-mentioned factors were set as our exclusion criteria in order to eliminate their confounding effect on the results. This was strength of our study and has not been done in previous studies. One limitation of our study was that taste of bitterness was not evaluated. Quinine hydrochloride is often used for assessment of taste perception of bitterness. However, due to possible adverse effects of this agent on the fetus, we did not use it in our study. Future studies with larger sample sizes are required to confirm the results of this study.

CONCLUSION

The results of this study showed that the taste threshold of sweetness decreased during pregnancy but no difference was detected in taste threshold of saltiness or sourness between pregnant and non-pregnant women.

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ORIGINAL ARTICLES

Spatio-temporal analysis of diabetes in the Iranian military community during the period 2015-2018

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Abstract: *Introduction: diabetes mellitus (DM) is one of the most prevalent non-communicable diseases. Spatio-temporal study of DM has a main in the design of disease prevention programs. The present study aimed to describe the spatio-temporal distribution of diabetic patients in the Iranian military community.*

Methods: In this ecological study, all registered cases of DM in the Iranian military community (military officers and enrolled conscripts) were considered during the period 2015-2019. Getis-Ord Gi statistics was applied to identify the DM hotspots. Spearman Correlation Coefficient was used to perform trend analysis of the disease.

Results: The number of DM incident cases in the military officers and enrolled conscripts was 1135 and 85, respectively. Isfahan (n=153) and Tehran (n=67) for the military officers and Zanjan (n=17) and Kurdistan (n=10) for the enrolled conscripts were the provinces with the highest number of identified DM cases. In the study period, no significant hotspots were detected in the military officers; However, an increasing trend of DM was observed (CC= 0.935, p<0.001). The DM hotspots detected in enrolled conscripts were as follows: 2015 – Qom (95% CI); 2016 – Lorestan, Qazvin, and Guilan (90% CI); 2017 – Ilam, Hamadan, and Markazi (90% CI); and 2018 – Ilam (95% CI), Alborz, Qom, Markazi, Lorestan, Kermanshah (90% CI), and no significant trend was noticed nonetheless (CC=-0.160, p=0.555).

Conclusion: It appeared that Northern and North-Western Iran were high-risk areas for DM in the military population and should be considered in designing the diabetes prevention and control program. Moreover, the determination of DM risk factors in the aforementioned areas could play a crucial role in the prevention of the disease.

Keywords: *diabetes, military, spatio-temporal, Iran*

INTRODUCTION

Diabetes mellitus (DM), a major glucose metabolism disorder, has become a global healthcare challenge. Estimates show 463 million people were suffering from DM in 2019 worldwide, rising to be 700 million by 2045 [1]. The annual global health expenditure on this disease is roughly USD 760 billion [2]. DM results in a number of complications and significantly affects the life quality of patients [3, 4].

This global epidemic has changed to the primary concern in many countries, especially in Iran [5]. Based on the reports, the prevalence of DM in Iran increased from 7.7% in 2005 to 10% in 2016 [6]. Moreover, the annual medical cost of patients with DM is about three times higher than the general population in Iran [7].

DM is a heterogeneous disease arising from a complex interplay of various tissues and different genetic, epigenetic, and environmental factors in a dynamical manner. Among these risk factors, occupation plays a critical role [8, 9]. The relationship between occupational factors and the incidence of DM takes the interest of many researchers [10]. The military community is prone to develop cardiometabolic syndrome because they have unique lifestyles and expose to stressful situations [11-13]. This evidence warrants a more comprehensive study of monitoring and screening needs to reduce the incidence and burden of DM among the

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population.

Our research has identified few studies in the literature about the spatio-temporal distribution of DM in the Iranian military community. Hence, the present study aimed to detect regional spatial clusters of DM, as well as to carry out a temporal trend analysis of incident cases in the Iranian military community using spatio-temporal analysis in the period 2015-2018.

METHODS

Study area and time

This study was conducted in Iran, the second-largest country in the Middle East, with a total area of 1,648,195 km^2 and a total population of 83 million. Iran is divided into five regions with 31 provinces.

This country is bordered to the northeast by Turkmenistan, to the northwest by Armenia and Azerbaijan, to the west by Turkey and Iraq, to the South by the Persian Gulf and the Gulf of Oman, to the southeast by Pakistan, and to the east by Afghanistan.

Study design

This is an ecological study, and all registered DM cases among military officers and enrolled conscripts in the Iranian military community during the period 2015-2018 were described and analyzed.

Spatial analysis

The distribution of DM cases in the Iranian military community was described using a Choropleth map. Spatial analyses were performed using ArcGIS 10.5 (ESRI, Redlands, CA, USA) and descriptive analyses using Excel 2010.

Hotspots identification

The spatial clusters of DM were identified using Hotspot analysis. Getis-Ord Gi statistics were applied to detect the hotspots. A high score on the index combined with a lower p-value indicates the clustering of DM. The Gi statistics were calculated based on our previous study (14).

Temporal analysis

The trend analysis of the incident cases of DM during the period 2015-2018 was assessed using Spearman Correlation Coefficient.

RESULTS

Descriptive statistics and spatial distribution

The number of identified DM cases in the military officers and enrolled conscripts was 1135 and 85, respectively, during 2015-2018 (Figure 1). Considering the distribution of DM cases by province, the highest number of diabetic participants in 2015 were seen in Isfahan (n=110) and West Azerbaijan (n=66) in military officers and Zanjan (n=17) in enrolled conscripts. Isfahan (n=120) for military officers and Tehran (n=6) as well as Alborz (n=5) for enrolled conscripts were the provinces with the highest number of DM cases in 2016. In 2017, the highest frequency of DM cases was reported from Isfahan (n=125) and Teheran (n=67) for military officers, and from Ilam (n=3), Hamadan (n=3), and Tehran (n=2) for enrolled conscripts. The incident cases in 2019 were high in Isfahan (n=153) and Tehran (n=65) for military officers and Kurdistan (n=10) and Hamadan (n=5) for enrolled conscripts compared to the other provinces. Geographical distribution of DM

Figure 1: Incident cases of DM in the Iranian military community by province during the period 2015-2018

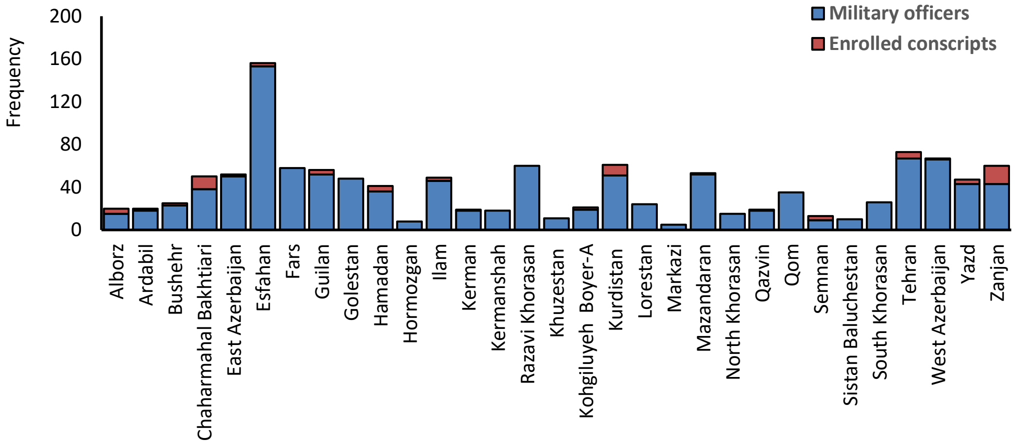

cases in military officer and enrolled conscripts were illustrated in Figure 2 and 3, respectively.

Figure 2: Geographical distribution of DM cases in the military officer during the period 2015-2018

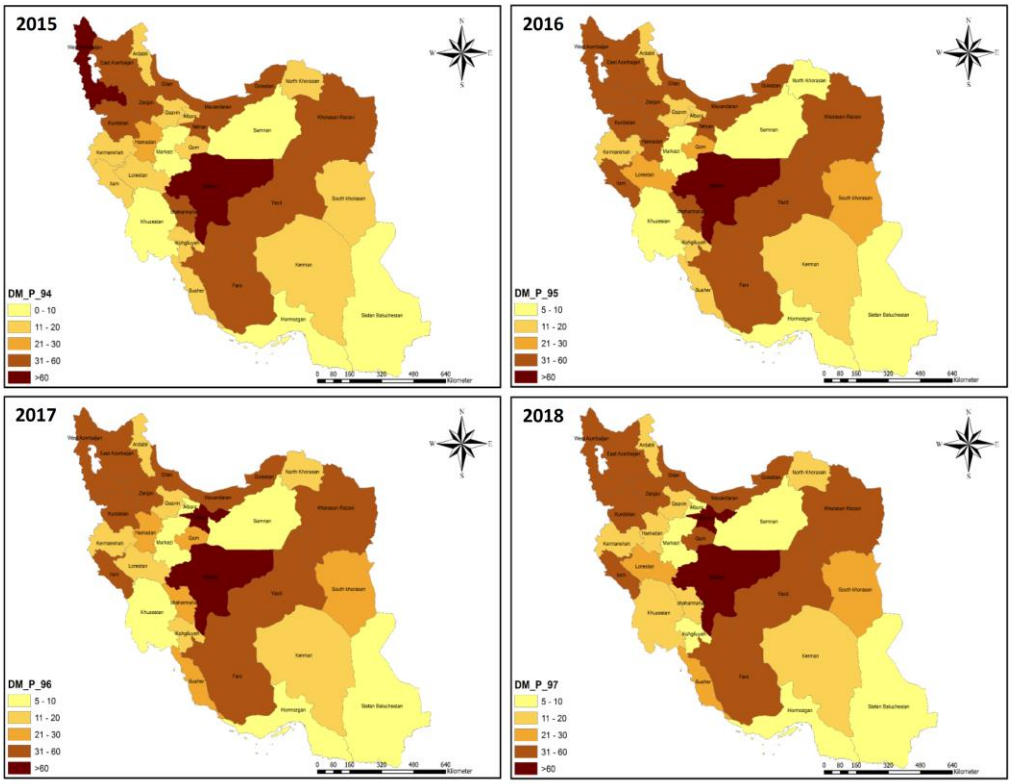
2015
DM_P_94
0 - 10
11 - 20
21 - 30
31 - 60
>60
2016
DM_P_95
5 - 10
11 - 20
21 - 30
31 - 60
>60
2017
DM_P_96
5 - 10
11 - 20
21 - 30
31 - 60
>60
2018
DM_P_97
5 - 10
11 - 20
21 - 30
31 - 60
>60
N
S
E
W
0 80 160 320 480 640
Kilometer

Figure 3: Geographical distribution of DM cases in the enrolled conscripts during the period 2015-2018

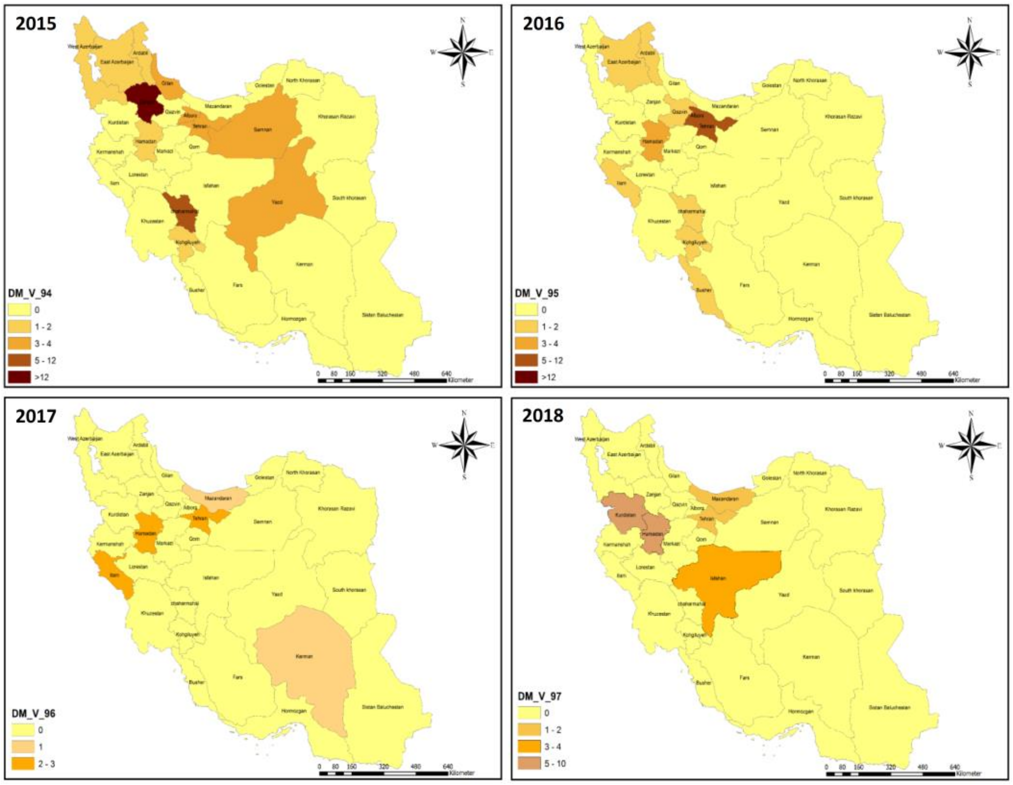
2015
DM_V_94
0
1 - 2
3 - 4
5 - 12
>12
2016
DM_V_95
0
1 - 2
3 - 4
5 - 12
>12
2017
DM_V_96
0
1
2 - 3
2018
DM_V_97
0
1 - 2
3 - 4
5 - 10
N
S
E
W
0 80 160 320 480 640
Kilometer

Figure 4: Geographical distribution of DM hotspots in the military officers during the period 2015-2018

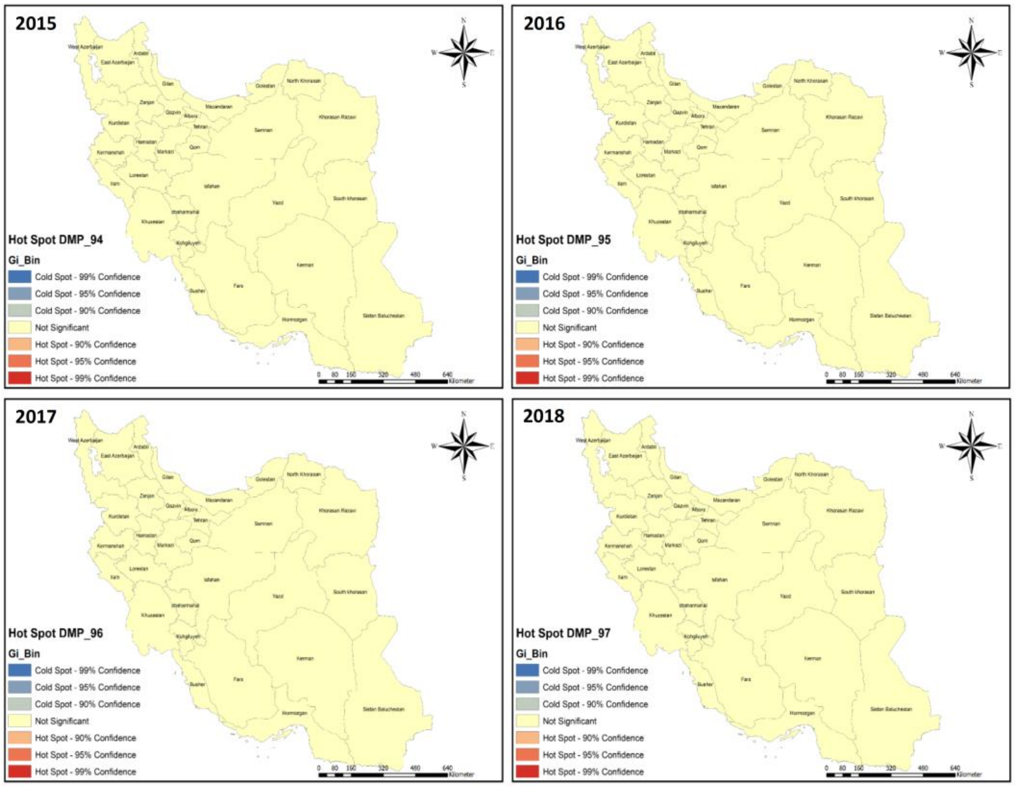
2015
2016
2017
2018
Hot Spot DMP_94
Hot Spot DMP_95
Hot Spot DMP_96
Hot Spot DMP_97
Gi_Bin
Cold Spot - 99% Confidence
Cold Spot - 95% Confidence
Cold Spot - 90% Confidence
Not Significant
Hot Spot - 90% Confidence
Hot Spot - 95% Confidence
Hot Spot - 99% Confidence
0 80 160 320 480 640 Kilometer
N
S
E
W

Figure 5: Geographical distribution of DM hotspots in the enrolled conscripts during the period 2015-2018

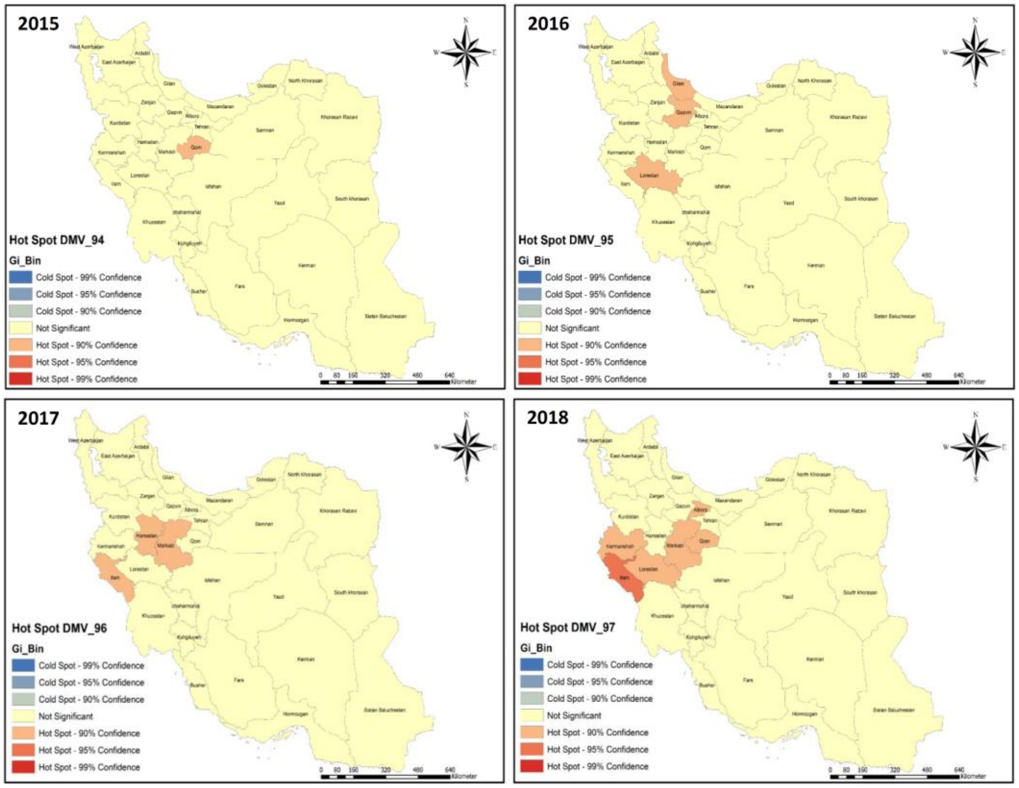
2015
2016
2017
2018
Hot Spot DMV_94
Hot Spot DMV_95
Hot Spot DMV_96
Hot Spot DMV_97
Gi_Bin
Cold Spot - 99% Confidence
Cold Spot - 95% Confidence
Cold Spot - 90% Confidence
Not Significant
Hot Spot - 90% Confidence
Hot Spot - 95% Confidence
Hot Spot - 99% Confidence
0 80 160 320 480 640 Kilometer
N
S
E
W

Hotspots distribution

No significant hotspots for DM were observed among military officers in the periods 2015-2018 (Figure 4). The DM hotspots observed among enrolled conscripts were as follows: 2015 – Qom (95% CI) province; 2016 – Lorestan, Qazvin, and Guilan (90% CI) provinces; 2017 – Ilam, Hamadan, and Markazi (90% CI) provinces; and 2018 – Ilam (95% CI), Alborz, Qom, Markazi, Lorestan, Kermanshah (90% CI) provinces (Figure 5).

Temporal distribution

The results of the temporal analysis showed a significant increasing trend of DM among the military officers from 2015 to 2018 was observed (CC= 0.935, p<0.001) (Figure 6A). However, there was no significant trend among incident cases of enrolled conscripts (CC=-0.160, p=0.555) (Figure 6B).

Figure 6: Trend of registered DM cases among military officers (A) and enrolled conscripts (B) in the Iranian military community during the period 2015-2018

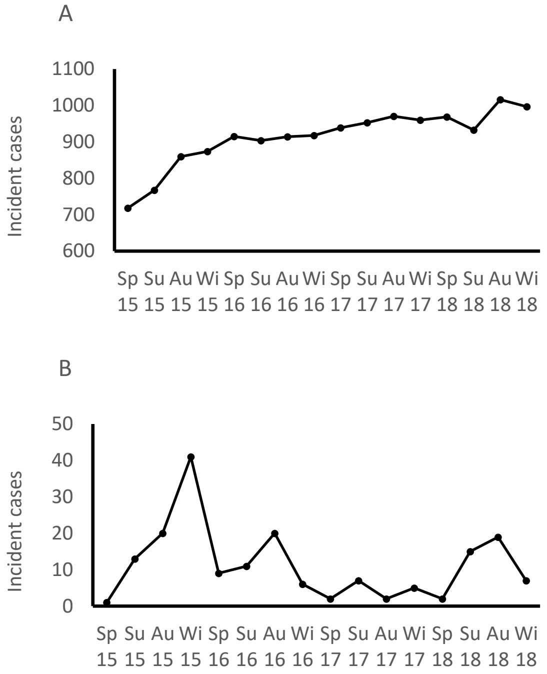

DISCUSSION

The aim of the present study was to assess the spatio-temporal distribution of DM in the Iranian military community. We determined the temporal trend analysis of DM incident cases and the regional spatial clusters of the disease in the military officers and enrolled conscripts in 2015-2018.

According to the spatial model of the distribution of DM incident cases in the study period, Isfahan province had the highest reported cases of DM (more than 100 cases) in the military officers. Obviously, the obtained results are consistent with the prevalence of DM in the general population of Iran. Recent studies also indicated a remarkable prevalence of DM in Isfahan province (15). Zanjan, Tehran, Ilam, and Kurdistan provinces also had the highest number of DM cases in the enrolled conscripts. Based on the pattern of DM in the general population, the total prevalence of DM in Tehran province (14.4%) is higher compared to other provinces. However, Ilam(16) and Kurdistan (17) are low-prevalent provinces (7.4% and 8.2%, respectively). This inconsistency might be due to a lack of information about the originality and demographic characteristics of enrolled conscripts. Based on the pattern of DM in the general population, the majority of DM cases were observed in the Central, Northern, and Western provinces of Iran (18-20), which is consistent with the present study results.

The temporal trend of DM in the military officers from 2015 to 2018 significantly increased with a mild slope; however, some severe fluctuations were seen regarding incident cases of DM in enrolled conscripts in this period. Recent studies have also shown a similar rising trend in the general population of Iran (21). Based on the time trend of the disease, an inevitable increasing case of DM is predictable in the Iranian military population, especially military officers in the future. An increasing pattern of DM morbidity and mortality has been reported in Middle Eastern and North African countries due to the high prevalence of obesity and sedentariness (22). This aforementioned increasing pattern was also observed in the Iranian general population (23). Considering this increasing trend of DM, it is also expected that the number of DM cases in the aforementioned provinces with high incident cases of DM will increase among the Iranian military population in the near future.

Regarding an appropriate program for the prevention and control of DM in the military community, it is necessary to determine the main risk factors. Some factors, including socioeconomic status, diet, physical inactivity and obesity, environmental factors, access to screening care, might contribute to the increasing trend of DM. Moreover, genetic factors should be considered in the epidemiology of DM. In a population-based study conducted on the risk allele frequencies of diabetes-related SNPs, an association was reported between the polygenic risk score of the enriched risk alleles and DM incidence (24). Different cases of DM among different populations might be due to genetic factors. A health education program concentrating on lifestyle and diet could be beneficial in the prevention of the disease, particularly in at-risk populations of the military community. Based on the findings of a recent study, lifestyle modification is an effective strategy to reduce the risk of DM in the military population (25).

CONCLUSIONS

The current study identified no DM hotspots in the military officer. However, Northern and North-Western Iran have the biggest clustering of DM in the enrolled conscripts in the years under investigation. Therefore, these geographical areas should be considered in designing the diabetes prevention and control program to early diagnose and monitor the disease. Moreover, the Iranian military medical service should develop appropriate and comprehensive analytical studies in the military population to evaluate the risk factors influencing the incidence and prevalence of DM in the aforementioned provinces.

Contributors
MF and MS developed the study design. MF, YA, AM, and MS collected and analyzed the data and drafted the prior version of the manuscript. MS participated in funding and supervised the trial. All authors edited and approved the final manuscript.

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VARIA

The differences of Matrix Metalloproteinases (MMP-9 and MMP-13) in chronic inflammatory rheumatic diseases

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Abstract: *Objectives: Primary Sjogren's Syndrome (pSS), Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA) are systemic, autoimmune, and chronic inflammatory rheumatic diseases that collagenases and gelatinases are effective and valuable in the pathophysiology of these diseases. The aim of this study is to compare the levels of MMP-9 (gelatinase-B) and MMP-13 (collagenase-3) in pSS to PsA, RA and healthy controls.*

Methods: Serum samples of patients with a diagnosis of Primer Sjögren's Syndrome (n=20), rheumatoid arthritis (n=20), psoriatic arthritis (n=19) and healthy control group (n=15) were analyzed by ELISA method for MMP-13 and MMP-9 levels.

Results: There was no significant difference between the groups in terms of MMP-9 and MMP-13 levels. In the pSS group, MMP-9 was negatively correlated with MPP-13 (r:-0.568, p:0.09) but these parameters were not correlated with activity biomarkers and scores of disease activity index.

In patients with pSS, the MMP-13 levels tended to be higher than the PsA patients and lower than RA patients but there was no significant difference between the 3 groups in terms of MMP-9 and MMP-13 levels.

Conclusion: MMPs, in distinguishing health and disease conditions have various roles and they drive important biological mechanisms. Taking into account as a biomarker different body fluids need to be compared. In our study, the differences of serum samples between the pSS group and the positive control groups were not statistically significant.

Keywords: *matrix metalloproteinases, primary Sjogren's syndrome (pSS), psoriatic arthritis (PsA), rheumatoid arthritis (RA)*

INTRODUCTION

Matrix metalloproteinases (MMPs), in general, are primary matrix-disrupting proteases capable of degrading all protein components of the extracellular matrix, they are a family of zinc-and calcium-dependent endopeptidases [1]. In addition to playing a role in extracellular matrix physiology, MMPs activate or degrade a variety of nonmatrix substrates including chemokines, cytokines, growth factors, and junctional proteins [2,3]. MMPs can be classified according to their preferential substrate, sequence similarity and domain organization as gelatinases (MMP-2, -9), collagenases (MMP-1, -8, -13, -18), stromelysins (MMP-3, -10), matrilysins (MMP-7, -26), membrane type MMPs (MMP-14, -15, -16, -17, -24, -25), and others (MMP-12, -19, -20, -21, -23, -27, -28) [4]. Activity of MMPs is precisely regulated by the balance between gene expression, zymogen activation and enzyme inhibition through their endogenous inhibitors, α2-macroglobulin in the plasma or tissue inhibitors of metalloproteinase in the tissue [5]. The imbalance between this system plays a critical role in various diseases, and the re-creation of a balanced MMP activity profile provides an opportunity for the development of biomarkers and therapeutics [1].

MMP-9, also known as Gelatinase-B, is involved in fundamental biological processes such as development, angiogenesis, apoptosis, inflammation and cancer [6]. Through cleaving denatured collagen (gelatins) and type 4 collagen, MMP-9 helps lymphocytes, and other leukocytes

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to enter and leave the blood and lymph circulations. Also, MMP-9 cleaves myelin compounds such as myelin basic protein (MBP) and type 2 gelatins, leading to remnant epitopes that can generate autoimmunity [7].

MMP-13, also called Collagenase 3, specifically catalyzes the hydrolysis of type II collagen, the main structural component of the cartilage matrix. MMP-13 plays a vital role in osteoarthritis and there are studies investigating selective MMP-13 inhibitors as a potential strategy for the therapy of osteoarthritis [8].

Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands, predominantly the salivary and lacrimal glands [9].

Collagenases and gelatinases are effective and valuable in the pathophysiology of SS, Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA) that they all systemic, autoimmune, and chronic inflammatory rheumatic diseases.

To the best of our knowledge, there is not a study which evaluated the clinical value of the serum MMP-9 ve MMP-13 levels in pSS. The aim of this study is to compare the levels of MMP-9 (gelatinase-B) and MMP-13 (collagenase-3) in pSS comparing to PsA, RA and healthy controls and to evaluate the correlation with disease activity.

MATERIALS AND METHODS

A total of 20 consecutive pSS patients who met the 2012 American College of Rheumatology classification criteria for Sjögren's syndrome were enrolled in this cross-sectional study [10]. 20 patients with RA who fulfilled the 2010 ACR/EULAR criteria and 19 patients with PsA who fulfilled the Classification criteria for psoriatic arthritis were included in this study as positive control groups [11,12]. The control group was formed of 15 healthy individuals. Patients and controls with one of the following combined diseases/ situations were excluded 1. additional rheumatic disease, such as autoimmune hepatitis, primary biliary cholangitis, Hashimoto thyroiditis, 2. comorbid chronic diseases, such as hypertension, dyslipidemia, hypothyroidism diabetes mellitus, chronic kidney disease, 4. obesity (body mass index >30), 5. malignancies, 6. history of cardiovascular or cerebrovascular disease, 7. active smoker, 8. age < 18 years, 9. inability to give written informed consent. The demographic, clinical and laboratory data of the patients were recorded.

The analysis of MMP-9 and MMP-13 in serum was performed by enzyme-linked-immunosorbent assay (ELISA) (Human MMP-9 ELISA assay kit, Sigma-Aldrich, MO-USA, Lot Number 0920D0173 and Human MMP-13 ELISA assay Kit, Sigma-Aldrich, MO-USA, Lot Number 0302C0175, respectively).

Disease activity of SS was assessed with EULAR Sjogren's Syndrome Patient Reported Index (ESSPRI) and EULAR Sjogren's Syndrome Disease Activity Index (ESSDAI) at the time of blood sampling. The ESSPRI is a self-evaluation index for measuring symptoms including fatigue, pain, dry- ness. Each symptom was measured with a single 0 (no symptoms) to 10 (severe symptoms) numerical scale and the final ESSPRI score is calculated by averaging these domains with a maximum severity score of 10. Scores of < 5 indicate low disease activity and scores of ≥ 5 indicate high dis- ease activity [13,14]. The ESSDAI is a physician-based assessment of the systemic features and severity of the disease and includes 12 domains (lymphadenopathy, constitutional, glandular, cutaneous, articular, muscular, respiratory, renal, peripheral nervous system, central nervous system, hematological, biological). ESSDAI ranges from 0 to 123. ESSDAI < 5 is defined as low disease activity, 5 ≤ ESSDAI ≤ 13 is defined as moderate disease activity and ESSDAI ≥ 14 is defined as high disease activity [14,15].

The study protocol was approved by the Committee on Human Research Ethics of Turkish Ministry of Health Zekai Tahir Burak Women's Health, Training and Research Hospital (decision number: 60/2017). A well written informed consent was obtained from all participants according to the principles of the Helsinki Declaration.

All data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) 16.0 program for Windows. The variables were investigated using visual and analytical methods to determine whether they were normally distributed. Normally distributed continuous values were expressed as mean ± standard deviation (SD) and categorical variables as number and percentage. Pearson's correlation coefficient and Spearman's correlation coefficient were used to evaluate the linear relationship between the predictive variables. A value of $p<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics and laboratory results of patients and healthy controls were summarized in Table 1. All pSS patients were on with only hydroxychloroquine 200mg/day treatment. Median ESSDAI and ESSPRI were 5.00 (min:0.00, max:26.0) and 4.00 (min:0.00, max:8.00), respectively. According to the ESSDAI, 12 (60%) patients had low disease activity and 8 (40%) had high disease activity. According to the ESSPRI, 9 (45%) patients had low disease activity, 9 (45%) patients moderate disease activity and 2 (10%) patients high disease activity.

Table 1: Clinical characteristics and laboratory results of chronic inflammatory rheumatic diseases groups

	Sjogren Syndrome (n=20)	**Rheumatoid arthritis** (n=20)	**Psoriatic arthritis** (n=19)	**Healty controls** (n=15)	**p value**
Age (years)	48.45 ± 10.59	56.00 ± 10.98	38.74 ± 11.25	47.87 ± 5.48	
Female/male	18/2	18/2	11/7		
ESR (mm/h)	22.39 ± 19.57	24.42 ± 15.37	20.47 ± 11.96		
CRP (mg/dL)	5.63 ± 4.34	12.70 ± 14.32	10.62 ± 7.90		
MMP13 (pg/mL)	12.99 ± 18.03	14.12 ± 16.45	9.78 ± 10.45	12.30 ± 14,38	0.143
MMP9 (pg/mL),	7303.60 ± 538.36	7433.10 ± 560.85	7639.68 ± 764.30	7890.53 ±1046.44	0.107

MMP-9 parametric, MMP-13 non parametric, ESR (Erythrocyte Sedimentation Rate), C-Reactive Protein (CRP) non-parametric

In patients with pSS, the MMP-13 levels tended to be higher than the PsA patients and lower than RA patients but there was no significant difference between the 3 groups in terms of MMP-9 and MMP-13 levels.

In pSS group, MMP-9 was negatively correlated with MPP-13 (r:-0.568, p:0.09) but these parameters were not correlated with activity biomarkers and scores of disease activity index (Table 2).

Table 2: Represents the correlation between MMP-9, MMP-13, disease activity markers and activity scores in patients with PSS

		ESR	CRP	IgA	IgG	IgM	C3	C4	B2M	ESSDAI	ESSPRI
MMP-9	r	0.079	-0.095	0.050	-0.140	0.311	0.074	0.188	-0.230	-0.005	0.113
	p	0.740	0.708	0.835	0.556	0.182	0.755	0.427	0.358	0.985	0.636
MMP-13	r	-0.183	0.133	0.102	-0.030	-0.173	0.111	-0.184	0.091	-0.063	-0.314
	p	0.440	0.598	0.668	0.900	0.466	0.640	0.437	0.720	0.793	0.176

ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein (CRP), IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M, B2M: beta-2-microglobulin, ESSDAI: EULAR Sjögren's syndrome (SS) disease activity index , ESSPRI: EULAR Sjögren's syndrome patients reported index

DISCUSSION AND CONCLUSIONS

In this study, although patients with pSS tended to have lower MMP-9 levels than the PsA and RA patients, these differences were not statistically significantly different. Also, in pSS patients, MMP-9 was not correlated with disease activity.

Salivary gland acinar cells express both MMPs and TIMPs. Previous studies suggest that increased MMP-9 activity and expression enhances the destruction of the basement membrane, which leads to the destruction of the acinar structures in SS salivary glands [16,17]. In the study by Perez et al, MMP-9 was detected by immunolocalization only in acinar and ductal cells and was homogeneously distributed throughout the patient's glands. MMP-9 expression and MMP-9 catalytic activity were increased in tissue samples from SS patients in a manner that correlated with the severity of the disease [18]. In a study by Hulkkonen et al., It was shown that serum levels of MMP-9 were higher in patients with genetically definitively diagnosed pSS than patients with possible pSS [19]. In our study we did not observe any correlation of serum MMP-9 levels to diseaes activity in pSS patients.

As it is known, TIMP expression is an important modulator of effects attributed to MMPs. In another study by Pérez P et al reported the MMP-9/TIMP-1 ratio in labial salivary glands of patients with SS correlated well with the dramatic changes observed in morphologic integrity of acini, as revealed mainly by the lack of nuclear polarity. Acinar changes were more evident when ratio MMP-9/TIMP-1 was higher [20].

Also, MMP-9 released from stressed ocular surface epithelium. Aragona et al, demonstrated that conjunctival expression of MMP-9 was significantly higher in patients with SS than healthy controls and subjects with Meibomian gland dysfunction [21]. Yang et al reported that corneal and conjunctival staining scores significantly correlated with the expression of MMP-9 in SS patients with dry eye disease [22]. In the study by Kuo et al, the tear MMP-9 level was positively correlated with bulbar redness, including mean redness score (with significance), and nasal bulbar redness (with a marginal trend to significance) in SS patients with dry eye disease [23].

As a result of saliva and tear studies in pSS patients, MMP-9 levels have been shown to be increased [24–26]. Basically to measure the MMP-9 and 13 serum levels could be one of our limitations and also we did not measured the TIMP levels of all groups.

However in different studies the pleiotropic effect of MMPs on mammals was studied and also mentioned about the need for more studies especially on the effects of MMP-9 and 13 in RA [27]. A study of active RA patients found high levels of MMP-9 in the serums than healthy group [28]. Giannelli et al reported that, MMP-9 levels in patients with RA and pSA were found to be higher in synovial fluid than in serum [29]. In another study with RA, synovial fluid MMP-13 levels were higher than in the healthy group [30]. Ion our study we observed that pSS patients had similar MMP-13 levels with PsA, RA and healthy controls. Also, we showed that MMP-13 was not correlated with disease activity in pSS.

Studies on MMP-9 and 13 levels were mostly performed in saliva, tears, and synovial fluid, and results were higher than healthy groups. Similarly, synovial fluid levels were found to be high in serum comparisons with synovial fluid [24,29–31]. The results obtained by these comparisons focus on the localized effects of MMPs. Serum levels alone cannot provide sufficient data.

Several limitations of the present study should warrant attention. The small sample size is major limitation of our study. Larger sample size may be needed to clarify the value of MMP-9 and MMP-13 as activity biomarkers in systemic autoimmune diseases, which are heterogeneous diseases. Another limitation of our study is cross-sectional design. Our results do not provide adequate results to explore potential causality and not reflect changes in these variables with disease duration, disease activity and therapy response.

Expected studies can be focus on easy sampling materials and non-invasive methods to study the metabolic pathways of MMPs. There is a need of well-designed, prospective, controlled studies with larger sample size to evaluate clinical value of serum MMP-9 and MMP-13 levels in pSS and other inflammatory diseases.

Declaration of competing interest

The authors declare that they have no conflict of interest relevant to the content of this manuscript.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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VARIA

The effect of smartphone-based education on self-efficacy of women with gestational diabetes: A controlled clinical trial

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Abstract: *Background and Aim: Women with gestational diabetes are at risk for type 2 diabetes. Development of self-efficacy is an important tool for active participation in treatment and behavioral change and can have a significant effect on type 2 diabetes-preventative behaviors. Therefore, it is necessary to search for an effective teaching method to improve the self-efficacy of these individuals. Accordingly, the aim of the present article is to determine the effect of smartphone-based education on self-efficacy in women with gestational diabetes (GDM).*

Materials and Methods: This randomized clinical trial was conducted on 90 women with GDM (n=45 individuals per group) at Yas Medical Center, Tehran. GDM software was installed on the cell phone of the intervention group. The control group only received the prenatal routine training. The self-efficacy questionnaire was completed before and 6 weeks after the intervention by the two groups. Data analysis was carried out using Chi-square, t-test, independent t-test, and Fisher exact test in SPSS ver. 16. P-value<0.05 was considered as the significance level.

Results: There was a statistically significant difference between the two groups in terms of self-efficacy changes before and 6 weeks after the intervention (p<0.001), meaning that these changes were significantly higher in the experimental group than the control group.

Conclusion: Smartphone-based education can increase self-efficacy in women with GDM as an effective and low-cost method.

Keywords: *smartphone-based; self-efficacy; gestational diabetes; randomized clinical trial*

INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the most important pregnancy challenges worldwide today. A total of 20.9 million (16.2%) of the pregnancies were associated with hyperglycemia in 2015, with 85.1% of cases being due to GDM. The prevalence of GDM is increasing in developed and developing countries [1].

A systematic review reported that the prevalence of GDM was between 0.7 to 18.6% in Iran [2]. GDM is associated with increased risk for mother and fetus (preeclampsia, cesarean section, injuries during pregnancy, macrosomia) during pregnancy and next stages of life [2,3].

Health guidelines recommend lifestyle modifications, including exercise and healthy diet, to prevent or manage GDM [4]. Although some factors, such as gender, age, and genetics cannot be changed to correct lifestyle, specific cognitive-behavioral factors, such as self-efficacy, can be considered for the promotion of healthy behaviors [5]. Various studies show the positive effect of education on the self-efficacy of diabetic patients [6,7].

Therefore, factors that play a central role in increasing the self-efficacy of diabetic people include empowerment through training on glucose self-monitoring skills, diet modifications, weight control, exercise, and physical activity, which are key factors in prevention of the increasing incidence of diabetes and its related complications are strongly based on self-efficacy and self-care of patients [8].

In a study on women with a history of GDM, Bastani and

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Zarabi (2010) showed that 57% of these women had high self-efficacy and the rest had low self-efficacy [9]. Considering the low self-efficacy of women with GDM, there is a need to strengthen self-efficacy in this group of women.

However, there are few studies on self-efficacy and encouraging strategies for taking type 2 diabetes-preventive behaviors in women with GDM [10]. Healthcare providers can play a key role in preventing type 2 diabetes [11]. One of the most useful strategies to achieve the goal of preventing type 2 diabetes in these people is education [12].

Many factors challenge the effectiveness of the educational program for women with GDM. GDM imposes an important role on pregnant women. The pregnant woman should, on the one hand, carry out duties related to her parental role and, on the other hand, engage in caring and medical practices and take control of the disease. Pregnant women need to spend a lot of time accessing systematic and valid educational programs in order to attend training classes. Busy schedule, difficulty traveling to attend training classes, and lack of time and energy due to family affairs and the difficulties of child care have limited the opportunity to take advantage of face-to-face training [13].

On the other hand, common methods, speech or face-to-face conversation require enough power and cost, and its problems include rapid forgetting, language mismatch, and patient misunderstanding [14]. Therefore, there is a need for the use of new educational approaches in this group of pregnant women.

The advancement in information and communication technology has made it possible to implement appropriate healthcare interventions to improve and manage health [15].

One of the technologies that can easily provide health services to a large population is the use of a smartphone-based system. Mobile communication has created opportunities for the departure of care from the monopoly of hospitals and clinics and to the patient's place of residence [16]. Smartphone-based education can help improve patient education, prevent diseases, reduce medical costs, and increase access to healthcare services [17], effectively engage patients, and positively influence their behaviors and health outcomes.

The results of a study by Hirst et al. (2015) showed that it was easy and acceptable for women with GDM to use smartphone-based education to control blood glucose [18]. Paul et al. (2016) showed in their study that the use of smartphone software led to an increase in daily walking hours in patients with a history of stroke [19]. Tehrani et al. (2016) also showed in their study that the intervention based on new communication technologies (multimedia, internet, and mobile) led to improved physical activity in women [20]. However, the results of a study by Cafazzo et al. (2012) showed that smartphone-based education was not associated with changes in HbA1c levels in people with type 1 diabetes [21]. Kirwan et al. (2013) also showed that smartphone-based education has not significantly changed the self-efficacy, self-care, and quality of life of the samples [22].

Despite the variety of applications in the field of health-related communication technologies, few studies have been conducted on their efficacy [23]. Accordingly, it's likely that smartphone capabilities can improve patient compliance with their disease management, but there is various evidence in this regard, and further researches are warranted [24]. Furthermore, there is little evidence regarding smartphone-based health interventions in developing countries, so further research is needed to measure the effectiveness and use of mobile-based health interventions as well as seek strategies to access to this type of services [25].

Considering that diabetes is one of the most important healthcare and socioeconomic problems worldwide on the one hand, and considering that we can prevent the incidence of diabetes in later life in women with GDM with proper training programs and since there are few interventional studies on this high risk population, on the other hand, the present study was thus designed to determine the effect of software-based education on self-efficacy.

MATERIALS AND METHODS

Design and Participants

This (double-blind) clinical trial study was performed on 90 women with GDM in an educational hospital affiliated to Tehran University of Medical Sciences from June to November 2017. This study was approved by the Ethics Committee of Iran University of Medical Sciences (Code of Ethics: 1396.9311686020IUMS). The clinical trial protocol was registered in IRCT (IRCT20170509279N4). The CONSORT checklist was used to report the article.

Participants

All participants were evaluated for inclusion criteria, which included 1. Pregnant women diagnosed with GDM. 2. They were in the age range of 18-40 years. 3. The gestational age was 22-32 weeks and single-pregnancy and were pregnant without assisted reproductive techniques. 4. They had reading and writing literacy. 5. They had mobile phones with

Android operating system and were able to work with mobile applications. Exclusion criteria also included: 1. Unwillingness to continue cooperation, 2. Delivery during the intervention

Intervention

To determine the required sample size at a confidence interval of 95% and a test power of 80% and assuming that determining the effect of smartphone-based education on self-efficacy in women with GDM compared with the control group leads to a 10 score difference (d = 10) so that this difference is statistically significant, After setting the values in the following formula, the sample size in each of the two control and experimental groups was estimated to be at least 42 people, which was increased to 45 people considering the drop-out rate. It should be noted that based on similar studies, the standard deviation of self-efficacy was estimated to be S = 16.3 [9].

Researchers provided educational content using library studies and valid websites of the National Institute of Diabetes and Digestive and Kidney Diseases, the National Diabetes Services Scheme, and relevant studies. The training program includes the definition of GDM, risk factors, complications of GDM on mother and fetus, diagnosis of GDM, prevention of type 2 diabetes, the definition of type 2 diabetes and statistics on type 2 diabetes in diabetic women, blood glucose measurement method, nutrition, physical activity, drug therapy, breastfeeding, stress management, and post-partum diabetic screening. The validity of educational content was approved by five faculty members in education and experienced in the care of women with GDM and two gynecologists. The primary educational software was designed by a software engineer in the Android Studio 2 environment. XML language was used to design the format and appearance of the program and to implement grammatical code, classes, and software commands, the Java programming language was used. To assess the content validity of the software, it was provided to two android experts and 10 women with GDM. Modifications were applied to the software prototype and eventually the modified and final version of the software was produced. The educational program was installed on the smartphone of the intervention group and its use was practically taught to them during an in-person session in the gynecology ward as well as in the form of a manual. The researcher assessed the extent of learning to use the training program. The educational program includes 8 files, each with a password and the researcher provided the participant with the code of each file via a telephone call twice a week. It was also possible to recall the use of the program on educational software, and the use of the training program and the implementation of the principles taught previously was reminded to the participants this way twice a week. The intervention group received routine hospital training in addition to receiving the smartphone training program. In order to prevent the transfer of information to the control group, the training program for the discharge time was installed on participants' phones. The intervention group was also asked to not receive additional information about self-efficacy from other sources of information, such as journals, peer groups, books, etc. during the intervention and 6 weeks later. The control group received only routine hospital training. Considering ethical considerations, the educational content was provided to the control group as a manual after the end of the study.

Tools

The data in this study were collected by using a demographic and underlying information questionnaire and self-efficacy scale for diabetic patients. demographic and underlying information questionnaire consisted of 14 questions on age, level of education, employment status, income level, pre-pregnancy height and weight, history and number of abortions, history of type 2 diabetes in first-degree relatives, history of giving birth to a baby weighing more than 4 kg, and history of hypertension. The questionnaire was prepared using library studies and research on the subject matter of the research. The next tool used in this study is the self-efficacy scale for diabetic patients [26], which is a standard and valid tool and has been used in many studies [9, 27-30] . The self-efficacy scale of diabetic patients includes eight items with score range of 1-10 and rated using 10-point Likert scale. The responses are categorized as 1 (I'm not sure at all) to 10 (I'm quite sure). The score of each item is the same as the number marked by the person being studied. If two consecutive numbers of an item are marked by a person, the lower number will be considered, and if the two numbers are not consecutive, then no score will be assigned to that item. If more than 2 questions are not answered, the questionnaire will not be scored. Self-efficacy score is obtained by calculating the average score of 8 items. A higher score shows higher self-efficacy. Reliability of the tool was reported in Lorig's study (2008) using a test-retest method (r = 0.8). The internal consistency of the tool was evaluated by measuring the Cronbach's alpha (α = 0.85) [28]. The validity of the tool was also evaluated and verified by Bastani and Zarabi (2011) using content validity method. To determine the reliability of the Persian version of the tool in the above study, Cronbach's alpha was obtained as α = 0.87 [9].

In this study, content validity method was used to determine the scientific validity of the demographic and undrlying information questionnaire and the self-efficacy scale. The above tools were provided to 10 faculty members of the Faculty of Nursing, Tehran University of Medical Sciences, Shahid Beheshti and Iran and then used in this research after obtaining their opinions confirmation of scientific validity in this research. To determine the reliability, self-efficacy scale for diabetic patients was given to 30 patients with GDM who met inclusion criteria after obtaining their consent. Then, the Cronbach α coefficient was calculated and the alpha value of 0.90 was obtained. It should be noted that these 30 participants were not included in the study.

Statistical Analysis

After collecting data and entering them into SPSS ver. 16, data analysis was carried out using descriptive statistics (mean, standard variance, standard deviation) and inferential statistics such as Chi-square and t-test. The significance level was considered as P-value<0.05.

RESULTS

Out of 90 subjects entered into the study, one in the intervention group and two in the control group were excluded from the study due to childbirth and the final analysis was carried out on 87 subjects (Figure 1). The findings indicate no significant difference between the two groups in terms of the variables such as age, level of education, employment status, economic status (Table 1) and clinical information such as body mass index, gestational age, gravida, history of abortion, history of hypertension, history of diabetes in first-degree relatives, and history of giving birth to a baby weighing more than 4 (Table 2).

Table 1: Comparison of demographic characteristic in intervention and control groups

Group		Intervention			Control			Results of the statistical test
Variable		N	%	Mean (SD)	N	%	Mean (SD)	
Age (years)	Less than 30 years	8	17/8	33/66 (3/93)	10	22/2	32/55 (4/44)	*t=1/255 df=88 p=0/213
	30-35 years old	22	48/9		25	55/6		
	Over 35 years old	15	33/3		10	22/2		
Level of education	Middle school	10	22/2		10	22/2		**χ2= 2/369 df=3 p= 0/499
	High school	16	35/6		11	24/4		
	Diploma	10	22/2		16	35/6		
	Academic	9	20		8	17/8		
Job	Housewife	27	82/2		39	86/7		χ2=0/338 df=1; p=0/561
	Employed	8	17/8		6	13/3		
The economic status	Weak	10	22/7		18	40		***p= 0/241
	Moderate	32	72/7		25	55/6		
	Good	2	4/5		2	4/4		

** Independent T Test; ** Chi-square test; *** Fisher's exact test*

Table 2: Comparison of clinical characteristics in intervention and control groups

Group		Intervention			Control			Results of the statistical test
Variable		N	%	Mean (SD)	N	%	Mean (SD)	
Pre-pregnancy BMI	20-25	7	15/6	26/44 (1/63)	5	11/1		*t=1/602 df=88 p=0/113
	25-30	38	84/4		36	80		
	30-35	0	0		4	89		
Gestational age (week)	20-24	2	4/4	29/2 (2/2)	6	13/3		t=0/531 df=81/88 p=0/597
	25-29	18	40		11	24/4		
	30-32	25	55/6		28	62/2		
Gravida	1	9	20/5	2/45 (0/99)	6	13/3		t=0/668 df=87 p=0/506
	2	13	29/5		16	35/6		
	3	15	34/1		16	35/6		
	4,5	7	15/9		7	15/6		
History of abortion	Yes	18	40		22	48/9		**χ2=0/72 df=1; p=0/396
	No	27	60		23	51/1		
History of hypertension	Yes	5	1/11		7	6/15		χ2=0/385 df=1; p=0/535
	No	40	88/9		38	88/4		

History of diabetes in first-degree relatives	Yes	21	46/7		27	60		χ2=1/607 df=1; p=0/205
	No	24	53/3		18	40		
History of giving birth to a baby weighing more than 4 kg	Yes	3	6/7	2	1	2/2		*p= 0/616
	No	42	93/3		44	97/8		

** Independent T Test; ** Chi-square test; *** Fisher's exact test*

As the results of Table 3 show, there was no significant difference between the two groups in terms of the mean pre-intervention self-efficacy score (p = 0.701), but the mean self-efficacy score in the experimental group was significantly higher than the control group 6 weeks after intervention (p <0.001).

There was statistically a significant difference between the two groups in terms of self-efficacy changes before and 6 weeks after the intervention, and these changes were significantly higher in the experimental group than the control group (Table 4).

Table 3: Numerical indicators of self-efficacy in women with GDM in the experimental and control groups and comparison of the mean self-efficacy scores before and 6 weeks after intervention

Self-efficacy	Experimental		Control		Results of the statistical test
	Mean	SD	Mean	SD	
Before the intervention	4/11	0/87	4/19	1/11	t=0/385 df=88; p =0/701
Six weeks after intervention	6/77	0/55	4/94	0/73	t=13/138 df=85; p <0/001

Table 4: Comparison of self-efficacy changes in women with GDM in the control and experimental groups

Changes	Experimental		Control		Results of the statistical test
	Mean	SD	Mean	SD	
Self-efficacy Six weeks after intervention	2.68	0.69	0.79	0.69	t=12/723 df=85; p<0/001

DISCUSSION

The results of the present study on self-efficacy comparison in the control and experimental groups before and 6 weeks after intervention showed that there was no significant difference between them in terms of the mean pre-intervention self-efficacy score (Tables 3 and 4) (p = 0.701), but the mean self-efficacy score in the experimental group (77.55± 0.6) was significantly higher than the control group (4.94 ± 0.73) six weeks after the intervention (p<0.001).

To interpret such result, it can be said that new technologies such as smartphone-based training programs can increase the self-efficacy of GDM patients. Self-efficacy is one of the factors positively affecting the sustainability and application of health behaviors [31]. In addition, learning new skills such as glucose self-monitoring, diet modification, weight control, exercise, and physical activity as strategies to empower of women and diabetic patients, are among key factors in preventing the progression of diabetes and its related complications, which are strongly based on self-efficacy and self-care of patients [8]. In a predictive correlation study on 400 women with GDM, Kordi et al. (2016) concluded that there is a need to enhance self-efficacy in these women [27] considering the direct linear relationship between self-efficacy and self-care variables and the low self-efficacy of women with GDM. The current study, consistent with the study by Atak et al. (2008) in Ankara and Shi et al. (2010) in China, showed that educational intervention is effective in promoting self-efficacy in diabetic patients [6, 7]. The results of the study by Absavaran et al., and Gheraati et al. show that mobile phone education, as a new method, is effective in promoting health and preventive behaviors [32,33]. Goodarzi et al., (2014) also showed that education through SMS service significantly increased the self-efficacy of people with diabetes (P = 0.02) [34]. Regarding the consistency of the results of the present study with the studies on the effectiveness of mobile phone education on the promotion of health behaviors and increasing self-efficacy, it can be said cell phone availability as well as the use of reminder messages provides the ground for motivation for change and improvement of health promoting behaviors in individuals. Mobile phone education will support and encourage patients' education self-care skills [35]. The results of the study of Qanbari et al. referred to limited health literacy as a common problem in pregnant women covered by healthcare centers. Considering that limited health literacy can prevent proper understanding of health information provided, health workers are recommended to use effective methods for the transfer of information to these individuals [36]. In research on 120 pregnant women in 2015, Kharazi et al. showed that it is necessary to educate the necessary subjects and provide information about pregnancy considering its adverse outcomes on the health status of the family and society [37]. Therefore, since healthcare professionals always seek better ways to encourage healthy behaviors, education administrators should also choose the most effective and useful educational approach to meet the needs, capabilities, and capabilities of pregnant women. Non-printed media have been referred to as one of the effective ways to present health messages to people (20) . Kreps et al. (2011) conducted a study entitled "Investigating motivational messages on improving adherence to drug regimen in patients with chronic diseases". The results of their study showed that motivational messages increased adherence to the drug regimen in the intervention group and are consistent with the present study, in which Reminder messages referring to the training program were considered as a factor to remind health behaviors [38]. Paul et al. (2016) showed in their study that the use of software, which can be installed on the mobile phone boosted daily walking in patients with a history of stroke [19]. Vahedian-Shahroudi et al. (2016) also showed in their study that the intervention based on modern communication technologies led to improved physical activity in women [20]. In another study, smartphone software was useful for managing gestational diabetes [15].

The strength of this study is the use of a smartphone-based training method to improve health indicators and preventative behaviors in people, because on the one hand, smartphone-based education is a new method in education that is compatible with social and cultural changes of the present era. On the other hand, the efficacy of this method has been confirmed in various studies and on people with different diseases.

The limitation of this study to women with GDM referred to an educational center in the current study limits generalizing the findings to other women with GDM. In this case, attempts were made to select intervention and control groups from two departments of this center.

It was also possible that the participants received educational information on gestational diabetes from other sources during the intervention period, which was controlled by using the control group.

Another possible limitation was that the participants may be unable to use the software, and attempts were made to the insert an option for reminding the individuals to use the software in order to reduce this limitation, and the user was reminded to study the training program twice a week and use the principles taught.

CONCLUSIONS

The results of the present research indicate that mobile phone-based education is effective on the self-efficacy of women with GDM. Therefore, mobile phone-based education is evaluated in terms of popularity, availability, and cost-effectiveness as a useful tool for training in the present age, and can be used to increase self-efficacy in women with GDM thereby reducing the rate of type 2 diabetes.

Recommendations for Future Research

The findings of this study are based on the self-report of the participants that may contain a kind of bias. Therefore, it is recommended that to use clinical factors such as blood glucose and glycosylated hemoglobin in addition to self-efficacy variable in order to evaluate the effect of mobile phone-software education. Considering the fact that the effect of software-based education on self-efficacy was investigated after six weeks in the present study, it is suggested to investigate such effect in the long run to determine the sustainability of effects of such interventions.

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VARIA

The most efficient types of training in cardiopulmonary rehabilitation programs: A narrative review

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Abstract: *Objective. This narrative review sought to identify the most frequently used types of exercises in cardiopulmonary rehabilitation programs and to verify the level of efficiency and feasibility of this treatment method in cardiovascular diseased patients.*

Methods. We conducted research via Pubmed over two months using specific keywords related to cardiorespiratory rehabilitation, cardiovascular rehabilitation, endurance exercises, and respiratory exercises. In addition, we included studies that involved physical training for patients diagnosed with coronary artery disease, chronic heart failure, valvular disease or valvular surgery, asthma, and chronic obstructive pulmonary disease.

Results. A total of fifteen articles were included in this narrative review. Eight studies investigated the effect of aerobic training, alone or combined, in the rehabilitation process for patients with cardiovascular disease and/or pulmonary disease. It showed that this type of training is the most recommended and used for these specific pathologies. Seven studies showed the effects of the resistance training and progressive resistance training, alone or combined, and proved the importance of their integration in patients with skeletal muscle weakness and altered functional capacity. High-intensity interval training was not superior to moderate-intensity continuous training in improving aerobic capacity and changing ventricular remodeling in patients with chronic heart failure. However, the results were promising in patients with coronary artery disease. Combined training had the best results in improving the aerobic capacity, muscle strength, functional capacity, ventilatory responses, and enhancing the quality of life for cardiovascular patients.

Conclusions. Combined training was found superior to aerobic training, resistance training, respiratory training, or inspiratory muscles training applied alone as a treatment in cardiopulmonary rehabilitation programs. Thus, every rehabilitation program should be individualized for every patient to increase the effort capacity using aerobic training and functional capacity of the skeletal muscles by increasing the muscle mass (hypertrophy) as an effect of resistance training or progressive resistance training.

Keywords: *cardiorespiratory rehabilitation, cardiopulmonary rehabilitation, cardiovascular rehabilitation, aerobic exercises, cardiorespiratory fitness,* VO_{2max}.

INTRODUCTION

Cardiac rehabilitation is a multidisciplinary approach that improves the patient's quality of life, cardiovascular risk factors, hospital readmissions, and disease-related morbidity and mortality over time [1–4]. Despite the beneficial effects and long term health-outcomes of this treatment method in diseased cardiovascular patients, it remains undervalued with low compliance from participants [2,5,6].

This comprehensive and multidisciplinary method consists of aerobic exercises, resistance exercises, respiratory muscles exercises, lifestyle modifications (dietary recommendations and smoking cessation), and psychological counseling [1,3]. Given that today all medical societies emphasize the importance of lifestyle in the primary and secondary prevention of chronic diseases, we are talking about lifestyle as a whole, made up of all these components with additive effects, and not just about the exercise itself. Along with

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cardiovascular disease, diabetes mellitus is a chronic disease with common complications, often found among patients with heart diseases, which significantly benefits from changes in lifestyle according to the latest studies [7,8]. Another public health problem with an increasing incidence is cerebrovascular disease through its many forms of presentation. In addition to the lifestyle-changing benefits, it also seems to profit from non-pharmacological alternative therapies [9].

It is considered that regular physical activity with a low-to-moderate intensity is a preventive measure for cardiovascular disease. During low-to-moderate exercise, lipids are used as an energy source, and skeletal muscles improve their functional capacity. There is an increase in the local blood supply to support metabolic needs during physical activity and a reduction in peripheral vascular resistance [10].

In cardiac patients, aerobic training remains the keystone of the rehabilitation programs and includes cycling on a cycloergometer, treadmill walking, and aerobic exercises. These activities are performed in a controlled environment and under the supervision of a cardiac rehabilitation team. Patients can continue their aerobic training in their free time with swimming, medium intensity sports, and even dancing, thus maintaining an active lifestyle [11]. Moderate-intensity continuous training (MICT) is a form of prescription and dosing of aerobic exercise, which has been shown to have both short-term and long-term clinical benefits [12,13]. A meta-analysis indicated that aerobic training influences ventricular remodeling in clinically stable heart failure patients. The mechanism according to which aerobic training influences left ventricular remodeling is not entirely elucidated. However, some theories include reducing vasoconstrictive neurohormones or a decrease in hemodynamic loading [14].

Resistance training (RST) is an anaerobic exercise that performs repetitive movements against resistance to stimulate a stronger muscle contraction [15]. RST has a beneficial effect on muscle strength and, endurance, functional capacity, thus improving the cardiovascular diseased patients' quality of life [16]. It is important not to forget that resistance training is complementary to aerobic training and does not replace it. A cardiac rehabilitation program tailored by combined resistance and aerobic exercises will lead to superior benefits, including regain or improvement in functional independence and better performance of daily living activities (ADL) [11].

High-intensity interval training (HIIT) consists of intense exercise interspersed with low-intensity recovery [12]. The ratio of duration between high-intensity intervals and low-intensity intervals is important to differentiate HIIT from MICT and to achieve the beneficial physiological effects of HIIT [12,17,18]. It seems that both aerobic interval training (AIT) and continuous aerobic training (ACT) have the same beneficial effect on peak VO_2 (an important marker in cardiovascular mortality), thus improving cardiopulmonary fitness. Furthermore, both programs are safe for cardiovascular patients and are very efficient in the first six weeks of training [19].

For treating patients with symptoms such as dyspnoea, low inspiratory muscle function, and exercise tolerance, inspiratory muscle training (IMT) is a valuable procedure [20,21]. Several studies observed that IMT at a maximum of 30% of maximal inspiratory pressure (MIP), corresponding to a low-to-moderate intensity, had significant improvement in peak VO_2 (oxygen consumption) and respiratory muscle strength [22–24].

High-intensity inspiratory muscle training is defined as 60% of MIP and is effective in chronic heart failure patients (CHF) for enhancing respiratory muscle strength when applied over four weeks [25]. In addition, when combined with aerobic physical training, inspiratory muscle training at high intensity significantly improved MIP, QoL, and dyspnoea [26].

This review aims to present the level of efficiency and feasibility of the most used exercise training methods in cardiovascular and pulmonary rehabilitation, identified after a thorough search of the current literature.

MATERIALS AND METHODS

A detailed search of the Pubmed database using specific keywords ('cardiorespiratory fitness OR cardiorespiratory rehabilitation OR cardiorespiratory recovery OR cardiovascular rehabilitation OR cardiovascular recovery AND endurance exercises AND respiratory exercises') was performed. The search algorithm was then completed by adding the 'cardiopulmonary rehabilitation', 'aerobic physical training', and 'resistance training' terms. We have selected open access articles published in the last ten years. Based on these criteria, fifteen items were selected.

RESULTS AND DISCUSSIONS

Fifteen studies were included in this review and were analyzed according to the goals as mentioned above [15,19,24–36].

Aerobic training was the most used and studied method for cardiovascular patients, alone or combined with another type of training (interval training, resistance training, inspiratory muscle training) [19,24,26,27,29,32–34].

When patients performed only aerobic exercises (aerobic interval training or continuous aerobic training) on a cycloergometer, a significant increase in peak VO_2 was observed predominantly in the group that performed aerobic interval training (AIT) compared to continuous aerobic training (ACT). Furthermore, in the first six weeks of training, there was noted an improvement in peripheral endothelial function in both aerobic exercises 19. Although Conraads et al. described the benefits of aerobic intervals and continuous training, they also concluded that ACT programs could be carried out higher than 70-75% of peak HR, but AIT programs with 4-minutes intervals at an intensity of 90-95% of peak HR are barely feasible for four minutes 19. This statement is most likely due to the complications that may occur during the interval training at 90-95% of peak HR, judging by the fact that cardiovascular patients are fragile, at least in the first phase of rehabilitation. Also, fatigue might be a limiting factor since most patients tend to stop the exercise when they feel exhausted.

HIIT is considered superior to MICT when it comes to improving cardiorespiratory fitness. Two studies confirmed this hypothesis, finding that HIIT's physiologic exercise training adaptations are superior to MICT for interventions of 7-12 weeks [31,36]. In contrast with the findings in the literature, one study of reduced ejection fraction heart failure patients concluded that HIIT was not superior to MICT in improving aerobic capacity or changing left ventricular remodeling. Also, the feasibility of HIIT remains unresolved for these subjects [30]. One of the factors which may have contributed to the reduction in HIIT response is the training intensity during intervals.

In the trial conducted by Ellingsen et al., 51% of the patients in the HIIT group trained at a less prescribed intensity, and 80% of the patients in the MICT group underwent more intense training than prescribed. It seems that HIIT at 90%-95% of the maximum heart rate is difficult to achieve for patients, but intensities of 60%-70% of the maximum heart rate in MICT seemed too low. This trial supports the statement made by Conraads et al. that interval training using 90%-95% of peak HR is barely feasible for four minutes [19,30].

When aerobic training (AT) was combined with other types of training, the results were enhanced. The addition of inspiratory muscle training (IMT) to aerobic training (AT) was shown to improve peak performance, submaximal ventilatory responses, peak circulatory power (CircP), dynamic of recovery gas exchange, O_2 uptake efficiency slope (OUES), skeletal muscle function, exercise capacity (in patients with COPD) and to decrease mMRC dyspnoea grade (in subjects with asthma) [27,28]. In contrast with these results, one study didn't observe any additional benefit of peak VO2 and left ventricle function for either group (AT/IMT or AT/SHAM), but there was an improvement in SPImax (sustained maximal inspiratory pressure) and in PImax (inspiratory muscle strength) [26]. An explanation for these conflicting results might be the small number of patients who presented inspiratory muscle weakness (IMW) in the study conducted by Adamopoulos et al., compared to the study carried out by Winkelmann et al. Therefore, we can conclude that in patients with CHF and IMW, any type of training (AT, IMT or both), can lead to substantial improvement in inspiratory muscle strength, having beneficial consequences on functional capacity. It may be necessary for patients with CHF but without IMW to undergo high-intensity IMT training to enhance functional capacity [26,27].

Ambrosetti et al. conducted a study on 612 patients using continuous aerobic training (ACT), aerobic interval training (AIT), resistance training (RST), respiratory training (RT), and calisthenics (CT). The study's outcome provided valuable information regarding the most suitable type of training for each cardiovascular pathology. For example, 73.1% of patients diagnosed with coronary artery disease (CAD) displayed the highest rate of implementation of AIT. In comparison, CHF patients displayed the greatest implementation rates for IT and RST (14.0% and 13.2%), and valvular disease/valvular surgery patients (VDS) presented the highest implementation rate of RT (62.9%) [29]. These results are important because pathologies' individualization of cardiac recovery programs leads to physical treatment progression in diseased cardiovascular patients.

The addition of RST and progressive resistance training (PRT) to AT and the effect of RST, and PRT alone were investigated in seven studies out of fifteen [15,28,29,32–35].

RST and PRT alone were observed to improve skeletal muscle function, upper and lower body muscular strength, flexibility, coordination, blood pressure profile, peak VO_2, exercise capacity, and determine muscle hypertrophy [15,28,29,32–35]. Ozaki et al. investigated if RT alone can improve VO_{2max} in young and older subjects. VO_{2max} considerably improved after 3 to 5 sets of RT at 5-10 RM and after sets of 10 reps for the first five weeks. However, when using higher reps (15-20 RM), there was no significant increase in VO_{2max} following training [35].

The recommendations for training frequency are five days per week of moderate training or three days per week of intense AT or a CT of moderate and intense aerobic training on 3-5 days per week with 2-3 days per week of RT [35]. The pooled results suggest that VO_{2max} could increase in young subjects performing RT, but when their initial VO_{2max} is lower than the average values for the corresponding age. Future research on this subject should investigate the dose-response relationship between the initial VO2max and the VO_{2max} obtained after a program using traditional RT.

It is an undeniable fact that the addition of RST and PRT to AT had significantly increased results compared to RT, PRT, and AT used alone [15,28,29,32–34]. While the effects of each training were not modified by the combination with another type of training, the pooled results observed a significant increase in the groups that performed combined training (CT) using RT and AT, compared to AT alone [15,32–34].

A systematic review and meta-analysis investigated the effects of PRT alone and combined with AT and concluded that PRT could provide cardiorespiratory fitness (CRF) improvements equivalent in magnitude to AT, with a decrease in the risk of mortality, in situations where the use of AT may not be viable, suitable or accessible [34].

CONCLUSIONS

Unfortunately, HIIT at 90%-95% of HRmax for four minutes is not feasible for cardiovascular patients since there was observed difficulty in achieving the prescribed intensity in these subjects.

We believe future research should investigate the dose-response relationship between the initial VO2max and the VO2max obtained after a program using traditional RT, because VO2max may be discovered to increase in young individuals after performing RT only when their initial VO2max is lower than the average values for the corresponding age.

The pooled results suggest that CT significantly improves the quality of the physical training by using the AT to increase the CRF, RST for improving the functional ability and determine muscle hypertrophy (strength), RT, or IMT to improve ventilatory responses, gas exchanges, and decrease the severity of dyspnoea. Thus, our recommendation is to integrate all types of exercises in a comprehensive cardiopulmonary rehabilitation program to obtain important outcomes and evolutions.

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EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

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Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed following the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013) (http://www.wma.net/en/30publications/10policies/b3/index.html).

It should also state clearly in the text that all persons gave their informed consent before their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects from being recognized (or an eye bar should be used).

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We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) Accessible to the public at no charge;
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(3) Open to all prospective registrants free of charge or at minimal cost;
(4) Validates registered information;
(5) Identifies trials with a unique number; and
(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of the last follow-up, the target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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All articles, except for Editorials, must contain an abstract of no more than 250 words. Abstracts for original articles should be formatted into subheadings, as detailed below. Titles must not be longer than 120 characters (including spaces).

Editorials

These are invited by the Editor-in-Chief or their delegated editor and should be a brief review of the subject concerned, regarding and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure, and cite up to 15 references, including the source article [this should be cited as Rom J Mil Med (year); (vol): [this issue].

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features, and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to a diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, the presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication, as presented at http://www.ICMJE.org/.

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The title page should contain (i) a short informative title that contains the major keywords. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgments. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

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Acknowledgments

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Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings, and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

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References

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REVIEW

Cannabinoids – a new therapeutic strategy in neurology

Carmen A. Sirbu[1], Aida M. Manole[2], Titus M. Vasile[2], Gabriela S. Toma[3], Livia R. Dobrican[4], Dan G. Vîrvara[4], Octavian Vasiliu[4]

Abstract: *Cannabinoids' usefulness in the treatment of neurological disorders (epilepsy, and various neurodegenerative diseases, such as Multiple Sclerosis and Alzheimer's Disease) has been demonstrated in a growing number of studies. Of the 11 known general types of natural cannabinoids, the focus has been mainly directed at cannabidiol (CBD) due to its specificity in stimulating cannabinoid receptors and the low rate of side effects, as well as on Δ (9)-tetrahydrocannabinol (Δ9-THC). The natural and synthetic analogs of CBD have been described as a potential treatment in neurological diseases, as they showed their therapeutic benefits in reducing the seizures from epilepsy and their neuroprotectivity in neurodegenerative diseases. First and foremost, CBD's neuroprotective properties are due to its capacity to act as an endogenous cannabinoid receptor agonist. Second, CBD enhances neuroprotection by interacting with many signal transduction pathways mediated indirectly through cannabinoid receptors. CBD also reduces the hyperphosphorylation of glycogen synthetase kinase 3 (GSK-3) induced by the buildup of Amyloid β in the physiopathology of Alzheimer's disease.*

Keywords: *cannabinoids, cannabis, endocannabinoid, marijuana, epilepsy, multiple sclerosis, Alzheimer's disease*

INTRODUCTION

Traditional medicine based on phytotherapy uses cannabis to treat various ailments and symptoms, such as arthritis, seizures, pain, nausea, and vomiting [1]. CBD is a phytocannabinoid found in the Cannabis plant, although only in trace amounts in western strains. CBD, unlike Δ9-THC, has neither euphoric nor hallucinatory effects [2]. CBD is also implicated in several pathological processes, including neurotransmitter release control, given that it is already known as a potent anticonvulsant, regulating pain, used primarily in patients with oncological pathologies, adjusting gastrointestinal function, which comes in aid of the side effects caused by the chemotherapy and it also possesses antipsychotic potential.

The Cannabis or Cannabaceae plant has been used in traditional medicine, beginning with the medicine practiced in the East, for about 5000 years as an analgesic, anxiolytic, anticonvulsant, sedative, as well as a hypnoinducer. Botanists used morphological criteria to organize Cannabis into three types: Sativa, Indica, and Ruderalis. The last one is the most studied subspecies, as it is most frequently encountered in Western countries [3]. Recently, pharmacological studies have indicated that the cannabis plant has many properties, such as antiepileptic, anti-parkinsonian effect [4], pain-killer [5], antiseptic [6], anti-inflammatory [7], antihistamines [8], blood pressure regulator and anti-coagulant [9] effects. In clinical settings, cannabis has shown its efficacy in oncological diseases, based on its antitumor impact, and in the therapeutic management of specific neurological disorders [10,11]. Current changes in the legal status of cannabis use allow the study of cannabinoids for treatment purposes in a secure and endorsed medical setting [12].

It is known that the diversity of the effects of cannabinoids is mediated by the activation of the G protein, which fulfills the function of a receptor, to which endogenous ligands called endocannabinoids usually are bound. The endocannabinoid system comprises endogenous fatty acid

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ligands for cannabinoid receptors, also known as endocannabinoids, and proteins involved in endocannabinoid biosynthesis and breakdown (ECS).

Cannabinoid receptors type 1 and 2 (CB1 and CB2), which have seven transmembrane domains and are found in various tissues, are this system's central receptors [13]. CB1 receptors are primarily found in the cerebellum, basal ganglia, and limbic system, including the hippocampus [13]. Also, the reproductive systems of both men and women include these receptors. CB2 receptors are mainly present on the cellular membranes of immune cells in the periphery, particularly in peripheral lymphoid organs like the tonsils and spleen [14]. CB2 receptors have been found in the central nervous system (CNS) and have a variety of effects, according to the most recent data [15]. Only two of the five endocannabinoids that bind to CB1 or CB2 are particularly important; they appear to play a role in CNS function, especially 2-AG (2-arachidonyl-glycerol) and AEA (anandamide).

We refer to cannabinoids as a category of natural chemicals found in marijuana plants (the most studied species being Cannabis sativa). Compounds that are physically or pharmacologically similar to (9)-tetrahydrocannabinol are among these chemicals, which bind to cannabinoid receptors [16].

The two main compounds in cannabis are Δ (9)-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). Each of them has exhibited different actions from a pharmacological point of view. The presence of psychotomimetic and addictive effects of the primary psychotropic component (Δ9-THC) has led to apprehension about using cannabis for medicinal purposes. On the other hand, CBD inhibits the absorption and hydrolysis of anandamide (AEA), a key endocannabinoid, while having antagonistic effects on 9-THC [17].

Table 1: The main categories of cannabinoids

1	(-)-Δ9-*trans*-Tetrahydrocannabinol (Δ9-THC) [22]
2	(-)-Δ8- *trans*-Tetrahydrocannabinol (Δ8-THC) [22]
3	Cannabigerol (CBG) [22]
4	Cannabidiol (CBD) [23]
5	Cannabielsoin (CBE) [24]
6	Cannabichromene (CBC) [25]
7	Cannabinodiol (CBND) [26]
8	Cannabicyclol (CBL) [27]
9	Cannabitriol (CBT) [28]
10	Cannabinol (CBN) [25]
11	Various types [18]

Over the last few decades, significant progress has been made in researching the natural compounds contained in the Cannabis plant. At least 120 compounds have been identified as natural cannabinoids, including their derivatives and metabolites [18]. Given their structure, these natural cannabinoids can be classified into 11 categories, as shown in Table 1. Due to their diverse and complex structure and multiple substituents, number 11 in Table 1 includes another 30 subtypes with different skeletal shapes [19–21].

Cannabidiol was first isolated in 1940 by Roger Adams and his team of researchers at the University of Illinois [31]. Its chemical structure was described in 1963 by Mechoulam [29, 30]. In 1977 Jones identified two optical isomers of this compound, (-) - CBD and (+) - CBD, the first form being found naturally in the Cannabis plant, while the latter can only be obtained through organic synthesis [31].

THE USE OF CANNABINOIDS FOR THERAPEUTIC PURPOSES

CBD has been shown to offer neuroprotective advantages in the treatment of multiple sclerosis (MS), epilepsy, Alzheimer's disease (AD), and other pathologies caused by degeneration or abnormal nervous system activity in preclinical and clinical research.

Epilepsy

This disease affects 65 million individuals globally [33] and significantly impacts seizures, mortality, comorbidities, stigma, and associated healthcare costs. Significant progress has been achieved in understanding the pathophysiological underpinnings of the disease and the factors that influence its prognosis over the last decade. New conceptual and operational definitions of epilepsy, as well as altered diagnostic and classification criteria and nomenclature, have all contributed to these advancements.

Even though the number of antiepileptic medications available has expanded dramatically over the last 20 years, about one-third of patients remain drug-resistant. Despite the efficacy of surgical operations, which have resulted in more than half of all operated patients experiencing long-term seizure remission, this treatment method is still reserved for a small subset of drug-resistant patients. The quality of life of most individuals with epilepsy continues to be negatively affected by deficiencies in knowledge regarding the pathogenesis, diagnosis, treatment, education, legislation, and research in this field.

The anticonvulsant effect of cannabidiol has been researched in multiple studies, conducted both in vivo and in vitro. Stimulation of CB1 receptors is followed as the primary target for antiepileptic effects in animal studies.

Neurophysiological studies have confirmed that glutamic acid is the major excitatory neurotransmitter in the CNS. Excessive glutamate release causes neurotoxicity, extreme excitability, and epileptic seizures in mice and primates. The direct result of activation of CB1 receptors is a reduction in glutamate release on a presynaptic level. Decreased glutamate release leads to an anticonvulsant effect and thus constitutes a target within antiepileptic therapy. In addition, endocannabinoids act upon specific ion channels by activating the G-receptor protein complex, which models the intracellular concentration of K+. The effect exerted upon K+ channels may decrease neuronal excitability, thus preventing seizures by stabilizing the membrane potential and reducing other epileptogenic factors [34]. When CB1 receptors in the neuronal body are engaged, the activity of an extracellular kinase, which impacts neural signaling and the synthesis of brain-derived neurotrophic factor (BDNF), has been shown to increase [35]. The latter plays a long-lasting protective role in the hippocampus [35].

CBD acts through endocannabinoids to activate CB1 receptors, stimulating a cascade of phenomena, which play a protective role against toxicity caused by excessive neuronal stimulation. Thus, the information mentioned above suggests that CBD can become an essential therapeutic instrument for neuron diseases, whose trigger mechanism consists of hyperexcitability and neurotoxicity [36].

In addition to acting on CB1 and CB2 receptors, CBD works at low doses on transient receptor potential (TRP) channels, affecting cationic channels and decreasing glutamate release in the presynaptic cleft. TRP is a group of channels that modulate ion entry and mediate various neural signaling processes [36,37]. Moreover, CBD will activate 5A-hydroxytryptophan 1A (5-HT1A) receptors and inhibit adenosine reuptake through multiple mechanisms, so that cannabidiol may have anti-inflammatory and antioxidant effects [37–41].

There is a long history of unofficial use of CBD in epilepsy and other neurological disorders because many preclinical studies have shown favorable neurological effects. Ever since the end of the 19th century, physicians in England and the United States have observed a reduction in the incidence of seizures in patients treated with cannabinoids [42]. Another study indicated that CBD reduced the susceptibility of seizures induced in laboratory mice by accompanying stimuli [43]. CBD is an adequate anticonvulsant with higher selectivity and fewer neurotoxic consequences compared to classical antiepileptics. Also, CBD can shorten the duration and amplitude of the post-critical phase through increased selectivity and reduced excitability acting on the central nervous system. In 2017, the Epilepsy Therapy Screening Program (ETSP) was established by the American National Institute of Neurology and Neurovascular Diseases to investigate the effects of CBD on a variety of epileptic seizure models. CBD has been found to have anti-seizure activities in both humans and mice with epileptic seizures after stimulation by ETSP. [44]. In June 2018, the FDA approved an anti-epileptic drug that contains CBD, named Epidiolex, for treatment-resistant epilepsy (Lennox-Gastaut syndrome and Dravet syndrome) [45].

Multiple sclerosis

Autoreactive T cells invade the CNS, followed by clonal growth, with a preference for myelin or other CNS proteins. Different autoimmune cells, such as microglia and B lymphocytes, are also initiated, and when combined with T cells, they develop into a "cytotoxic" cascade that demyelinates neurons in the brain and spinal cord. The presence of both CB1, as well as CB2 receptors has been noted on the surface of specific cells around the platelets accumulated in the demyelinated areas present in MS [46]. Increased CB1 and CB2 receptors activity were observed in some MS patients' bloodstream in a 2009 study, suggesting that activating the endocannabinoid system, both inside the brain and in the periphery, can restrict inflammatory, immunological responses [47].

In 2007, an elevated level of AEA (anandamide) was identified in conjunction with the number of inflammatory lesions noted in the neurological images [48]. It has been pinpointed that the level of multiple endocannabinoids within the cerebrospinal fluid has a low level in patients with remission, but an increased level in those with active inflammatory lesions, in a study conducted in 2008 [49]. These three studies illustrate a correlation between MS and the regulation of endocannabinoid activity and demonstrate the influence of inflammatory processes and neural activity on the endocannabinoid system.

Nabiximols, in the form of oronasal spray containing Δ9-THC and CBD, is a product licensed in Great Britain and some countries within the European Union to be used as a treatment for MS, and it has been shown to present a therapeutic effect on neuropathic pain and in the case of sleep disorders [50]. As the following studies have shown [51–53], Nabiximols could also be used to control spasticity. In 2007, a study carried out on 189 patients who were administered Nabiximols over six weeks, compared to the placebo group, showed a substantial reduction in spasticity in the active treatment group [51]. A meta-analysis indicated that these cannabinoids exhibit favorable effects for both neuropathies and pain symptoms caused by spasticity [52].

Another retrospective study [53], carried out in eight centers in Belgium, followed 276 patients with MS-specific spasticity who began treatment with Nabiximols-containing oromucosal spray on March 1, 2016, after other conventional treatment regimens for spasticity were exhausted. The conclusion reached was that more than 60% of patients reported a clinically relevant symptomatic improvement and continued treatment over 12 weeks [53].

Endocannabinoids play a role in limiting the cytotoxic processes which occur during the inflammatory cascade, which initiates the neuronal damage in the context of MS via stimulation of the CB2 receptors. Likewise, stimulation of CB1 receptors may also have beneficial effects by inhibiting glutamate release and the depolarization induced by it. The activation of CB2 receptors in T cells and CB1 receptors within neurons is thought to be responsible for 9-THC's neuroprotective action in multiple sclerosis. In addition to their shown capacity to lower inflammation and excitotoxic lesions in MS, cannabinoids may aid in the repair of myelin sheaths in the axons [54].

Multiple studies have shown the effect of cannabis on the modulation of the myelination process in the developing brain by stimulating CB1 and CB2 receptors [55], in the normal adult brain [56], and in the irritated brain in the TMEV (Theiler's murine encephalopathy virus) model of MS [57]. The TMEV model's accelerated myelination process could be related to the efficacy of cannabis in reducing inflammation which detained the remyelination. Alternately, cannabinoids may have an exhilarating response to myelination by improving survival [57] and cellular division of oligodendrocytes [55]. Further study of changes occurring in MS in conjunction with the endocannabinoid system may lead to the development of novel therapeutic methods.

Alzheimer's disease

This chronic neurological disease is currently the leading cause of dementia worldwide, facing an acceleration of diagnostic that will bring us to an epidemic with 150 million cases globally by 2050 [58].

The pathogenesis of the disease is highly correlated with the accumulation of extracellular aggregates of amyloid β (Aβ) plaques and intracellular neurofibrillary tangles of TAU protein located in a different region of the brain, which drives the dissolution of microtubules in neurons, spoiling the architecture of the cytoskeleton of the cell, fracturing the neural transport system and causing a significant loss of neurons firstly in the hippocampus, which clinically leads to memory loss. These actions initially bring some biochemical communication disengagement in the connections between neurons, followed by neuronal death [59–61]. This is our current knowledge of the pathophysiology of AD, but as the mechanism is not fully understood, the therapeutical resources are very limited. Even if it has been shown in some studies on animal models that THC can have a direct effect on amyloid β (Aβ) aggregation, the psychoactive properties limit the possibilities of being used as a medication. Although other non-psychoactive cannabinoids, such as CBD, show neuroprotective activity on animal models by reducing oxidative stress and playing a role in microglial inflammation. Cannabidiol's effect on inhibiting neuronal apoptosis by regulating pro-caspase-3 levels and decreasing caspase-3 levels has shown that CBD exerts a protective effect on the nervous system [65]. In another study, CBD was successfully used to counterbalance Aβ-induced Ca2+ growth, adequately added to the already known antioxidant effect [66]. Furthermore, cannabinoids can stimulate the clearance of intraneuronal amyloid-β and induce a protective effect on old age-associated nervous cells. In addition, they function in the absence of cannabinoid receptors in some cases. CBD increases cerebral blood flow mainly in the areas involved in memory processing, like the hippocampus [66]. In conclusion, cannabinoid products can be an alternative compound for AD drug therapy, but more longitudinal studies must be conducted.

CONCLUSION

Even though higher usage of cannabis extracts is linked to an increased risk of psychosis, CBD can help with epilepsy convulsions and the symptoms of neurodegenerative diseases like AD and MS because of its neuroprotective effect. If the exciting discoveries related to the cannabinoid-mediated weakening of the inflammatory process, the enhancement of remyelination, and behavioral and symptomatic changes are confirmed, these compounds can be used as a treatment for multiple sclerosis as they progress from studies on animal models model systems to humans trials.

Even though various molecular signaling pathways and targets linked to CBD have been identified and summarized, many questions remain unanswered. Finally, natural analogs of Cannabidiol, as well as metabolites and synthetic derivatives enriched with strong biological effects [68] and low neuronal toxicity should be studied for their potential benefits in the treatment of neurodegenerative illnesses such as AD, multiple sclerosis, and epilepsy.

More research is needed to understand how CBD affects the nervous system's growth factors, neurotransmitters, and electrophysiological activity.

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REVIEW

Succinct review on biological and clinical aspects of Coronavirus disease 2019 (COVID-19)

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Abstract: *The prevalence of coronavirus disease 2019 (COVID-19) is the third registered spillover of an animal coronavirus to humans from the early 21st century. Coronaviruses are important human and animal pathogens. The 2019 novel coronavirus (2019-nCoV) rapidly spreads, resulting in an epidemic throughout China, followed by an increasing number of cases in other countries throughout the world. Recently, a wide range of inhibitors have been introduced for treatment of COVID-19, and also promising vaccines are in late phase of development. Here, we aim to present an overview of recent findings of the biological and clinical aspects of SARS-CoV-2 infection, along with possible treatments and future vaccines.*

Keywords: *COVID-19; immune response; diagnosis; treatments; SARS-CoV-2*

INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) is the third registered spillover of an animal coronavirus to humans from the early 21st century [1]. Before the outbreak of SARS-CoV (severe acute respiratory syndrome coronavirus) infection within 2002-2003 in the Guangdong province, China, coronaviruses were considered to be slightly pathogenic to humans [2-6]. MERS-CoV (Middle East respiratory syndrome coronavirus) is another highly pathogenic coronavirus that appeared in the Middle Eastern countries ten years after the outbreak of SARS-CoV [7]. Recently, in December 2019, a new coronavirus from 3 patients with pneumonia was discovered and termed 2019 novel coronavirus (2019-nCoV). All three patients were somehow related to the wet animal and seafood wholesale market in Wuhan, Hubei Province, China [8]. Afterward, the virus was named as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) [9]. SARS-CoV-2 is an enclosed virus with positive single-stranded RNA belonging to Betacoronaviruses, which can infect both humans and animals [10]. The biological behavior of SARS-CoV-2 is mostly the same as SARS-CoV and MERS-CoV. SARS-CoV-2 uses the same structural protein as SARS-CoV and MERS-CoV, to gain entry to the host cells [11, 12].

The average basic reproduction number (R0) is an epidemiologic metric that describes the transmissibility of infectious agents. R0 measures the expected number of secondary infections produced by a single infectious individual in a susceptible population during the mean infectious period. Case Fatality Ratio (CFR) indicates the proportion of episodes of disease that are fatal. Created with BioRender.com

The patients with SARS-CoV-2 mostly have symptoms such as fatigue, fever, dry cough, and dyspnea. Clinical and laboratory evaluation, along with chest CT scan and real-time RT-PCR (real-time reverse transcription-polymerase chain reaction) assay, can help to confirm the disease [13-15]. patients are better to be hospitalized soon after diagnosis to receive proper health care [16].

However, recently a wide range of inhibitors have been introduced for treatment of COVID-19. Furthermore, promising vaccines are in late phase of development. Here, we aim to present an overview of recent findings of the biological and clinical aspects of SARS-CoV-2 infection, along with possible treatments. Using various sources, including CDC, WHO, and some journals we have concisely provided an epidemiological comparison between COVID-19 and other well-known respiratory viral infections in Figure 1 [2, 17-19].

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Figure 1: This figure represents an epidemiological comparison between COVID-19 and other well-known respiratory viral infections.

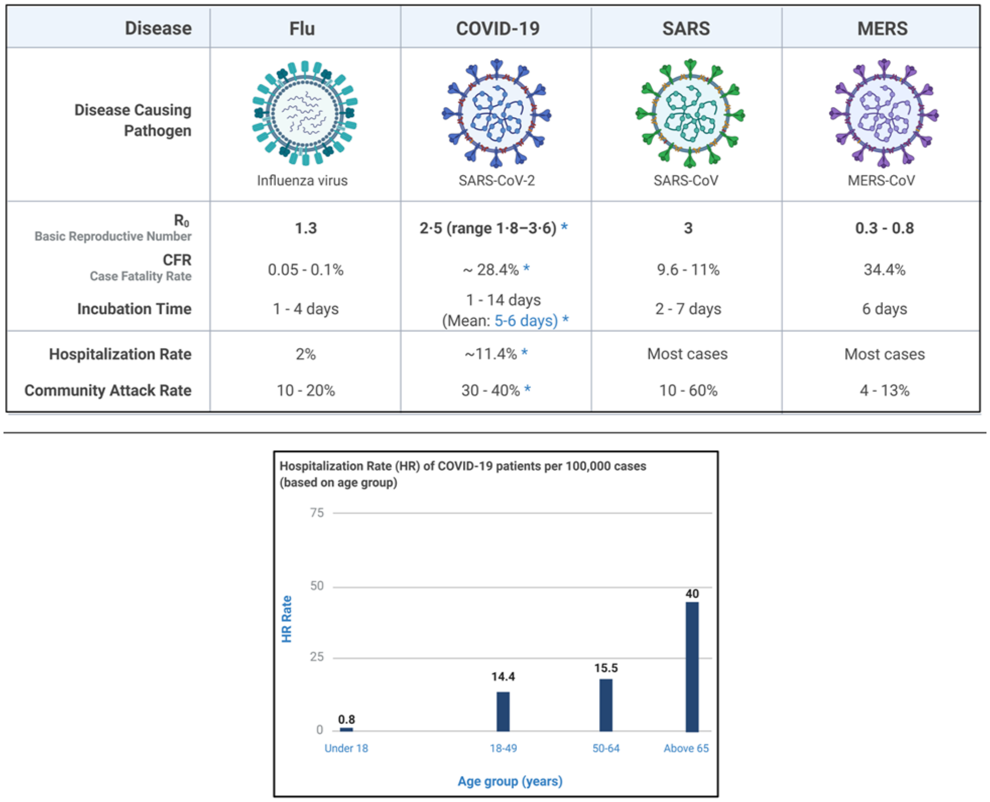

Disease	Flu	COVID-19	SARS	MERS
Disease Causing Pathogen	Influenza virus	SARS-CoV-2	SARS-CoV	MERS-CoV
R_0 Basic Reproductive Number	1.3	2·5 (range 1·8–3·6) *	3	0.3 - 0.8
CFR Case Fatality Rate	0.05 - 0.1%	~ 28.4% *	9.6 - 11%	34.4%
Incubation Time	1 - 4 days	1 - 14 days (Mean: 5-6 days) *	2 - 7 days	6 days
Hospitalization Rate	2%	~11.4% *	Most cases	Most cases
Community Attack Rate	10 - 20%	30 - 40% *	10 - 60%	4 - 13%

PHYLOGENY AND ORIGIN OF CORONAVIRUSES

The Coronaviridae Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) determined that coronaviruses related to the subfamily of Coronavirinae in the family Coronaviridae and the order Nidovirales. Based on phylogenetic relationships and genomic structures, this subfamily contains 4 genera, including Alphacoronavirus, Gammacoronavirus, Betacoronavirus, and Deltacoronavirus [20, 21]. The Betacoronaviruses have four lineages (A, B, C, and D). SARS-CoV and SARS-CoV-2 belong to lineage B, and lineage C includes MERS-CoV [22]. According to phylogeny and taxonomy, the CSG placed 2019-nCoV, within the Coronaviridae. Given this, they termed this SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus) [23]. There are three extremely pathogenic coronaviruses, SARS- CoV, MERS-CoV, and SARS-CoV-2, leading to a severe respiratory syndrome in humans [10, 24, 25]. It is proposed that both MERS-CoV and SARS-CoV have originated from bats. MERS-CoV and SARS-CoV have infected humans through direct transmission from civets and dromedary camels, respectively, which seem to be the intermediate hosts for these viruses. It has been confirmed that these viruses are capable of human-to-human transmission [21]. Phylogenetic analysis showed that 2019-nCoV has more than 88% identity to the genome of two types of coronaviruses originated from bats, bat-SL-CoVZXC21 and bat-SL-CoVZC45. However, it has less genome similarity to MERS-CoV (~50%). SARS-CoV-2, in comparison to SARS-CoV, has been considered to be a new human-infecting betacoronavirus due to its distinct genetic characterizations [26]. At first, skeptic views were whispered that this virus is a product of purposeful manipulation, but a comprehensive analytical study explicated that this is not a laboratory constructed virus [27].

STRUCTURE AND LIFE-CYCLE OF SARS-COV-2

SARS-CoV-2 genome contains 29,881 bp in length with 14 open reading frames (ORFs) encoding 27 proteins [22]. The 3′-terminus of the genome comprises of sequences for encoding 8 accessory proteins (3a, 3b, 7a, p6,7b, 8b, 9b, and orf14), and 4 main structural proteins, including spike surface glycoprotein (S), an envelope protein (E), matrix protein (M), and nucleocapsid protein (N). Despite the relationship between SARS-CoV-2 and SARS-CoV, there are some distinct differences at the amino acid level. For example, unlike SARS-CoV, the 8a protein is absent in SARS-CoV-2; the 8b protein in SARS-CoV-2 is longer in contrast to SARS-CoV; in comparison to SARS-CoV, the 3b protein is considerably shorter in SARS-CoV-2. The pp1ab and pp1a proteins of the SARS-CoV-2 are encoded by ORF1a and

ORF1ab genes situated at the 5´-terminus of the genome, respectively [28, 29].

Figure 2: Human SARS-CoV-2 Structure. The spike of the recently discovered SARS-CoV2 has two subunits, S1 and S2, and is frequently represented as a sword-like spike. Created with BioRender.com

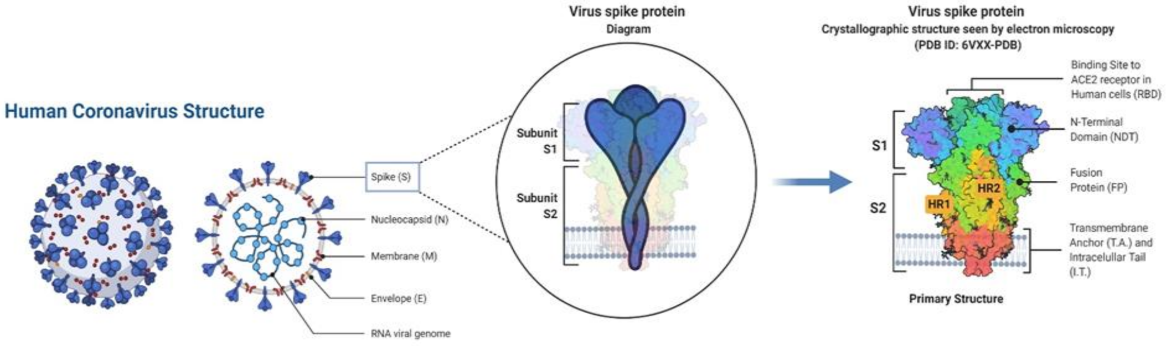

Unlike MERS-CoV that binds to dipeptidyl peptidase 4 (DPP4; also called CD26) to infect the unciliated bronchial epithelial cells as well as pneumocytes type II, both SARS-CoV and SARS-CoV-2 are linked to ACE2 (angiotensin-converting enzyme 2) on pneumocytes type II and ciliated bronchial epithelial cells [30-33]. Though SARS-CoV-2 and SARS-CoV have similar receptor-binding domain structure, the variation of the amino acids at some key residues distinct them from each other [26]. Based on biophysical and structural studies on S protein, the SARS-CoV-2 S protein demonstrated a greater affinity to ACE2 than SARS-CoV S protein [34]. Since the S protein is a quintessential part of the virus life-cycle, it can be a consensus target antigen, which could be inhibited by drugs such as Arbidol [35]. Analytical studies have delineated the SARS-CoV-2 mutations, indicating that these may have led to higher infectivity of the virus as a result of natural selection [27, 36-38]. Although SARS-CoV-2 has come with higher infectivity and transmissibility than SARS-CoV, it seems to have less mortality rate [19, 39]. The virus life cycle initiates with the binding of SARS-CoV S protein to the ACE2 receptor and recruiting the cellular protease TMPRSS2 in order to enter the host cell via the endosomal path [12, 40]. A report betokened that a particular mutation (N501T) could promote interaction of the virus receptor-binding domain with human ACE2 [40, 41]. The virus releases the genomic RNA into the cytoplasm, following the viral entry into the host cell. The next step is the translation of ORF1a and ORF1ab for producing pp1a and pp1ab polyproteins, then here proteases encoded by ORF1a cleave pp1a and pp1ab to provide the NSPs. These NSPs form the RNA replicase–transcriptase complex, which leads to producing negative-sense RNAs. Further, negative-sense RNA is used as a template to produce full-length positive RNA genomes. During transcription, sub-genomic RNAs like ones responsible for structural proteins (S, E, and M) produced through a process called discontinuous transcription, which afterward introduced into the endoplasmic reticulum (ER). Sub-genomic mRNAs might hold multiple ORFs, but only the one that is closest to the 5´ end (first ORF) goes to be translated. Next, these structural proteins (E, M, and S) move to the endoplasmic reticulum – Golgi intermediate compartment (ERGIC), where they combine with viral RNA genomes encapsidated by N protein to form mature virions. Herein, The M protein has an essential role in protein-protein interaction for viral assembly. Eventually, virions use the exocytosis process to be released out of the infected cell [42, 43]. ORF1a and ORF1ab genes together encode 16 to 17 NSPs (non-structural proteins), including NSP1 to NSP10, NSP12, and NSP16 [39]. Two of which are proteases, namely 3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro), that are being considered as invaluable drug targets [39]. Owing to 96% homology [44] within the sequence of 3CLpro in SARS-CoV and SARS-CoV-2 genomes, formerly introduced 3CLpro inhibitors, including peptidomimetic acrylester (PDB: 6LU7) (45), aldehydes [46], isatines [47], a-ketoheteraromates [48], pyrazolidinone [49], Nitroanilides [50], ML188 [51], racemic [51], and ML300 [51] might be beneficial start-point drugs in the treatment of COVID-19 as well. Besides, machine-learning-based approaches have been put in to work to find 3CLpro inhibitor compounds (drug repurposing) [52-54]. Using Plant-based medicines like Flavonoids [55, 56], as well as HIV protease inhibitors like lopinavir and ritonavir [57-59], have also been suggested as a 3CLpro inhibition-based treatment. Imperativeness of PLpro for the CoV replication and PLpro-mediated subterfuge from the immune system makes this protease an attractive drug target [60].

Figure 3: Steps of coronavirus life-cycle: 1-Spike-mediated binding and viral entry via membrane fusion or endocytosis; 2-Release of viral genome; 3-Translation of viral polymerase protein; 4-RNA replication; 5-Sub-genomic transcription; 6-Translation of viral structural proteins ;7-S, E, and M proteins combine with nucleocapsid; 8-Formation of mature virion; 9-Exocytosis. nsps: non-structural proteins. Created with BioRender.com

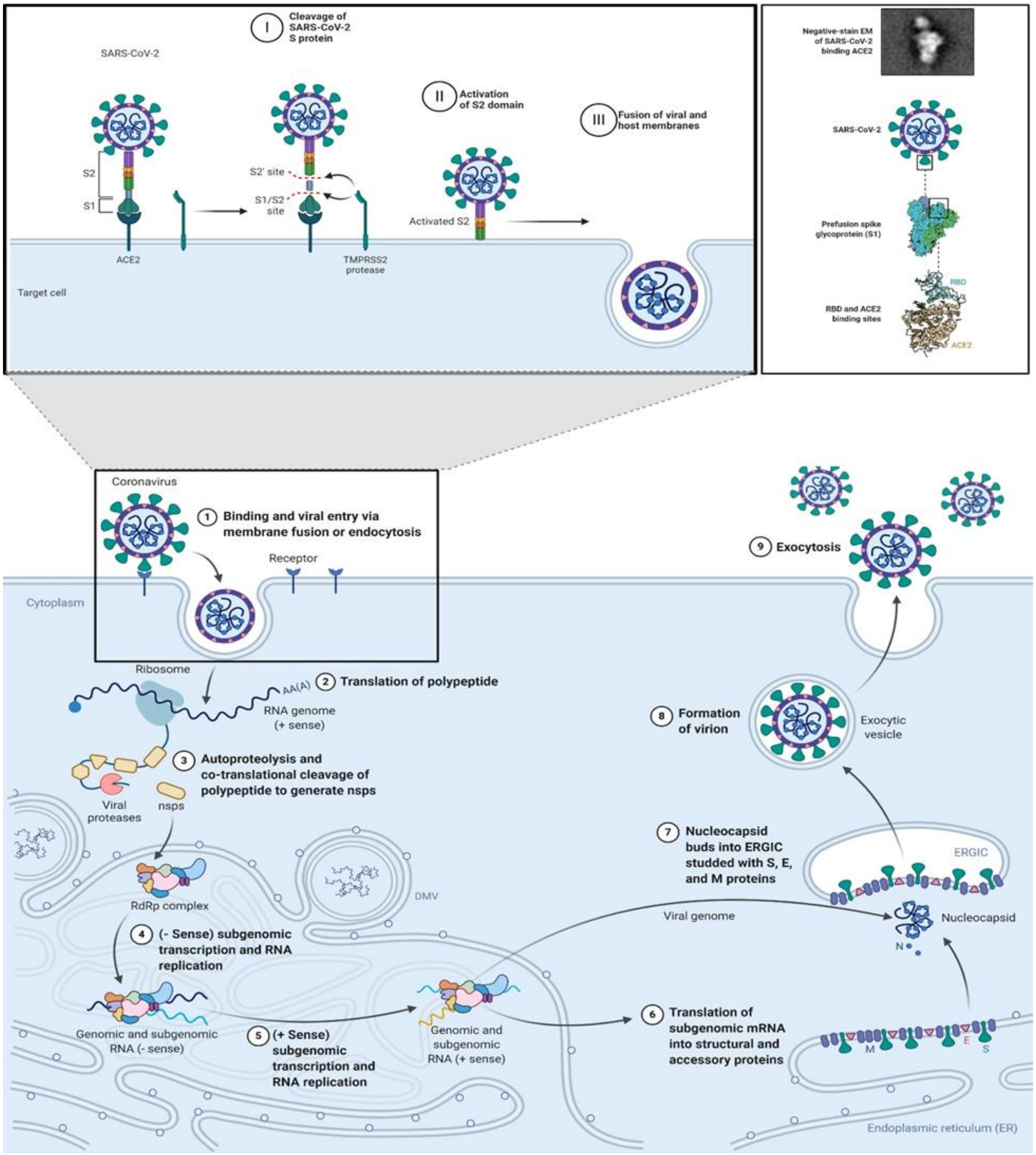
I Cleavage of SARS-CoV-2 S protein
SARS-CoV-2
II Activation of S2 domain
III Fusion of viral and host membranes
S2
S1
S2' site
S1/S2 site
Activated S2
ACE2
TMPRSS2 protease
Target cell
Negative-stain EM of SARS-CoV-2 binding ACE2
SARS-CoV-2
Prefusion spike glycoprotein (S1)
RBD
RBD and ACE2 binding sites
ACE2
Coronavirus
1 Binding and viral entry via membrane fusion or endocytosis
Receptor
Cytoplasm
9 Exocytosis
Ribosome
AA(A)
2 Translation of polypeptide
RNA genome (+ sense)
8 Formation of virion
Exocytic vesicle
3 Autoproteolysis and co-translational cleavage of polypeptide to generate nsps
Viral proteases
nsps
7 Nucleocapsid buds into ERGIC studded with S, E, and M proteins
ERGIC
RdRp complex
DMV
Viral genome
Nucleocapsid
N
4 (- Sense) subgenomic transcription and RNA replication
Genomic and subgenomic RNA (- sense)
5 (+ Sense) subgenomic transcription and RNA replication
Genomic and subgenomic RNA (+ sense)
6 Translation of subgenomic mRNA into structural and accessory proteins
M
E
S
Endoplasmic reticulum (ER)

To this end, compounds interacting with active-site Cys112, including epoxyketones, a-halo-ketone, aldehydes, alkynes, and activated esters, have been proposed as potential inhibitors [60]. Accordingly, a drug for chronic alcohol dependency called Disulfiram has been reported to be a potential PLpro inhibitor. Alongside that, 6-mercaptopurine and 6-thioguanine as antimetabolite drugs also have the capability to inhibit PLpro [61]. Other than those two proteases, the RNA-dependent RNA polymerase (RdRp), which recruits cell machinery to transcribe the virus genome into new RNA copies, can be an outstanding drug target [62, 63]. An RNA polymerase inhibitor called Favipiravir, which is mostly used in the treatment of influenza, has shown to be effective against other RNA viruses as well [64]. It is noteworthy that Favipiravir would not restrict host DNA and RNA synthesis [65]. Its effectiveness and minor side effects on patients with COVID-19 has been reported from a study on 70 patients in Shenzhen, Guangdong province [66]. Remdesivir is a broad-spectrum antiviral drug that acts as an adenosine analog and thus capable of terminating the RNA synthesis in multiple RNA viruses, including SARS-CoV-2 and Ebola [67, 68]. A preliminary randomized trial on the 1059 COVID-19 patients (538 assigned to Remdesivir and 521 to placebo) delineated that Remdesivir was superior to placebo in reducing the recovery time in adults. Remdesivir also lowered the COVID-19-related mortality rate [69]. Another adenosine-analog drug is Galidesivir that has been administered for Ebola and Marburg virus. Moreover, A molecular docking study showcased that it has broad-spectrum antiviral activity upon RNA viruses such as SARS-CoV-2 [70, 71]. Moreover, a machine-learning-based approach has offered AP2-associated protein kinase 1 (AAK1), and Cyclin G Associated Kinase (GAK) inhibitors, including Baricitinib (a drug approved for rheumatoid arthritis), Fedratinib, Sunitinib, and Erlotinib, to inhibit viral endocytosis [39, 72]. Additionally, Baricitinib can also inhibit JAK1/2, which attenuates inflammation [72].

IMMUNE RESPONSES TO SARS-COV-2

The immune system naturally triggers an immune response against invaders like viruses. Chemotactic factors have a critical role in viral infections due to their effect to summon the leukocytes to the site of infection. So, spectral alteration in chemotactic factors may result in adverse immune responses. As mentioned before, SARS-CoV-2 mostly affects the lungs, so it should be considered that uncontrolled immune response may lead to functional impairment of lungs, pulmonary tissue damage, and reduced lung capacity. In contrast, an inadequate immune response may also intensify viral replication, which also leads to tissue damages [73]. The host cell possesses various pattern recognition receptors (PRRs) that serve as a part of the innate immune system for recognizing the PAMPs (pathogen-associated molecular patterns). PRRs including RIG-I-like receptor (RLR), toll-like receptor (TLR), C-type lectin-like receptors (CLmin), and NOD-like receptor (NLR) are located on the cell surface. On the other hand, some PRRs are in the cytoplasm, such as cyclic GMP-AMP synthase (cGAS), IFI16, interference stimulator of interferon gene (STING), DNA-dependent activator of IFN-regulatory factor (DAI) [73]. Among them, RLRs, including MDA5 (IFIH), H family members RIG-I (DDX58), and LGP2, have the potential to recognize RNA viruses, like coronavirus [73-75]. The adaptive immune responses consist of the immune response of T cells, humoral immune responses, and antibody responses. Around 80% of lymphocytes that infiltrate in the pulmonary interstitium in coronavirus infection are CD8+ T cells, which can clear the virus-infected cells and also able to induce tissue damage [73-76]. It has been noted that the weakening of CD4+ T cells may cause severe interstitial pneumonitis, and delayed elimination of the virus from lungs are due to decreased pulmonary recruitment of lymphocytes and neutralization antibody and cytokine production related to the reduction of CD4+ T cells [77]. Lymphopenia is a hallmark in patients infected with SARS-CoV and SARS-CoV-2. A study revealed that SARS-CoV E protein interaction with an antiapoptotic protein called Bcl-xL induces T-cell apoptosis. Further, it was shown that interaction of SARS-CoV E protein with Bcl-xL is mediated by BH3 (Bcl-2 homology domain 3)-like region placed in the C-terminal cytosolic domain of SARS-CoV E protein [78, 79].

In addition, memory T-cell response to the structural viral proteins such as S, M, and N, demonstrated to be long-lasting (over 11 years post-infection [73, 80]. SARS-CoV-2 not only infects lungs but also can cause heart injury because the ACE2 receptor is also expressed in the cardiovascular system. Along with this, it is stated that the imbalance immune response between type 1 and type 2 T-helper lymphocytes in SARS-CoV-2 infection might induce a cytokine storm, which not only affects lungs but also can cause injury to the cardiovascular system [81, 82]. Reports indicated that the patients with SARS-CoV-2 from Wuhan, China, had a high concentration of pro-inflammatory cytokines in their plasma, including high levels of IL1B, IL-6, IFNγ, IP10, and MCP1 that could be associated with disease severity. Patients with SARS-CoV-2 infection compared to SARS-CoV infection, seem to have a greater amount of T-helper 2 cytokines such as IL4 and IL10 that quell inflammation [15]. Studies on CoV have shown that pro-inflammatory cytokines (e.g., IFNγ, IL12, IL1B, IL6, IP10, and MCP1) are related to pulmonary inflammation and immense damage to the lungs [81]. However, more investigations are required to determine the Th1 and Th2 responses in SARS-CoV-2 infection. Furthermore, antibodies and the complement system as part of humoral immune responses are both vital, especially during the persistent phase of CoV infection. However, the complement system has the potential to damage the host tissue at the site of infection [73]. SARS-CoV-2 can induce a massive chain of inflammatory responses through initiating JAK/STAT signaling pathway, as well as and NF-κB, which can cause a pro-inflammatory cytokine storm. Noteworthy, that cytokines like IL-6 has a positive feedback loop and bind to their receptors and initiate a JAK/STAT-mediated signaling pathway. The virus also downregulates ACE2 receptors, which is followed by the elevation of angiotensin II and subsequently, induction of the JAK/STAT signaling pathway. This SARS-CoV-2 induced inflammatory response not only affects the lungs but also leads to cardiovascular implications [83]. In this regard, some targeted drugs have been suggested, for example, Tocilizumab and Sarilumab as IL-6 receptor antagonists, Baricitinib, Fedratinib, vaand Ruxolitinib as JAK/STAT inhibitors, and serine protease inhibitors for inhibiting of NF-κB signaling pathway [83]. To control immune response-related injury to the lungs mediated by SARS-CoV-2, Dexamethasone has been applied as a treatment to alleviate inflammation-mediated lung injury in hospitalized patients. In which case, co-administration of Dexamethasone and respiratory support in 2104 patients indicated a lower mortality rate compared to the group who received no respiratory support [84].

CLINICAL FEATURES, DIAGNOSIS, AND MANAGEMENT OF COVID-19

Different studies on patients demonstrated the clinical characteristics of COVID-19. According to epidemiological, demographic, clinical, laboratory, and radiological data, which includes a broad range of clinical manifestations, the most common symptoms are fever, fatigue, dry cough, dyspnea, myalgia, and shortness of breath. Less common symptoms, which mostly occurred in critically ill patients, include headache, confusion, chest pain, expectoration, pharyngalgia, diarrhea, nausea, and vomiting. Most patients showed more than one symptom. Some patients presented organ dysfunction, such as ARDS (acute respiratory distress syndrome), acute renal injury, and acute respiratory injury. There were underlying complications among reported cases, which included hypertension, diabetes, cardiovascular disease, malignancy, chronic liver disease, cerebrovascular disease, chronic kidney disease, and HIV infection [15, 79, 85]. Intriguingly, Dorgalaleh et al. have reported that patients with severe hypocoagulable conditions like patients with congenital bleeding disorders might become protected from adverse effects of COVID-19-pertained hypercoagulopathy [86]. In a study on 138 Chinese patients with COVID-19, thorough laboratory tests included prothrombin time (PT), activated partial thromboplastin time (APTT), complete blood count (CBC), D-dimer, creatine kinase (CK), CK-MB, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), aspartate aminotransferase (AST), total bilirubin, creatinine, hypersensitive troponin I, and procalcitonin were conducted. Prolonged PT, lymphopenia, and elevated LDH were among the most common laboratory findings. The patients in the intensive care unit (ICU), in comparison to non-ICU patients, showed more laboratory abnormalities. Patients who were in ICU showed higher levels of white blood cell (WBC) and neutrophil, D-dimer, CK, and creatine. Laboratory parameters in patients with novel coronavirus–infected pneumonia (NCIP) showed differences between survivors and non-survivors. Lymphopenia was Significant among them, but non-survivors gradually showed more severe lymphopenia. Compared to survivors, WBC count and D-dimer were elevated in non-survivors. Similarly, BUN and creatinine were increased progressively in non-survivor patients before death [85]. The diagnosis of COVID-19 starts with the aforementioned clinical and laboratory assessment, then a chest CT scan helps to determine the involvement of lungs, and eventually needs to be confirmed with real-time PCR. Nearly all of the patients show a bilateral distribution of ground-glass opacity and patchy shadows in their chest CT scans [87]. Considering the sensitivity of diagnostic tests for COVID-19, a study indicated that a chest CT scan has greater sensitivity compared to real-time RT-PCR because there are still some drawbacks and technique variations in real-time RT-PCR. So, they recommended that symptomatic patients with negative real-time RT-PCR test should be screened with a chest CT scan along with clinical and epidemiologic evaluation [88].

Table 1: Different clinical-phase vaccine candidates for COVID-19.

Developer	Candidate/Mediator	Vaccine characteristics	Trial ID
Moderna	mRNA-1273	Encapsulated mRNA encoding S protein	NCT04387656
CanSino Biologicals	Ad5-nCoV	Adenoviral vector expressing S protein	NCT04398147
Pfizer	BNT162b1 BNT162b2 BNT162b3	RNA vaccine	NCT04368728
Inovio Pharmaceuticals	INO-4800	DNA plasmid encoding S protein	NCT04336410
University of Oxford	ChAdOx1 nCoV-19	Chimpanzee adenovirus-vectored vaccine expressing the SARS-CoV-2 spike protein	NCT04400838
Shenzhen Geno-Immune Medical Institute	LV-SMENP-DC	Modified dendritic cells with lentiviral vectors expressing SARS-CoV-2 proteins- administered with antigen-specific cytotoxic T lymphocytes	NCT04276896

Once a vaccine has been approved for SARS-CoV-2 and all are in development [89], the management of the disease could be achieved by taking some essential measures. Based on the guideline published by Ying-Hui Jin et al. at first, patients should rest in bed and receive a sufficient amount of food as an energy source (protein-rich foods), water, electrolytes, and other internal environment factors. Meanwhile, they should be monitored for heart rate, respiratory rate, pulse oxygen saturation, blood pressure, blood routine, CRP, PCT, ALT, AST, bilirubin, myocardial enzyme, creatinine, BUN, coagulation function, and arterial blood gas. Oxygen therapy has been strongly recommended. Drug treatments, including antiviral therapy, antibiotic therapy, and the use of corticosteroid could be helpful. [66, 90]. However, the comprehensive WHO interim guideline is also available for clinical management of COVID-19 [91]. One of the controversial issues about SARS-CoV-2 is about blood transfusion. Association of SARS-CoV-2and transmission through blood transfusion is still unclear. [92].

CONCLUSION

In this work, we provided the biological and clinical properties of SARS-CoV-2. As long as the epidemy of SARS-CoV-2 goes on, the best recommendation for this global threat would be preventive and safety measures. CT scan and real-time RT-PCR are the best armamentaria for confirmation of this virus. SARS-CoV-2 virus spreads in frantic pace among humans and more comprehensive studies are demanded. However, two epidemics (SARS-CoV and MERS-CoV) have shown us that the journeys for overcoming these kinds of diseases are still challenging.

Conflicts of interest and sources of funding

The authors report no conflict of interest.

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REVIEW

Effect of Hydroxychloroquine, Favipiravir, Lopinavir/Ritonavir, Remdesivir, Umifenovir, and Interferon beta-1a in Covid-19 patients: A systematic review of Randomized Clinical Trials

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Abstract: *Background: Coronavirus disease 2019 (COVID-19) was recognized by the World Health Organization (WHO) as a global pandemic on March 11, 2020. Since then, researchers worldwide have focused their attention on identifying effective treatments and developing vaccines to combat this disease.*

Aim: To report the effectiveness of the drugs employed in the COVID-19 treatment protocols based on data from clinical trial studies conducted from the beginning of the pandemic until December 10, 2020.

Methods: Following the PRISMA guidelines, we conducted an advanced search in several electronic databases. A total of 13553 studies was screened by two people simultaneously and separately based on the article title, abstract and full-text. The quality of the studies was evaluated using the Cochrane criteria.

Results: Of the 13553 studies identified, 50 clinical trials were included in this systematic review. Of these, three studies explored the use of remdesivir, nine studies the use of hydroxychloroquine, five studies the use of lopinavir/ritonavir, six studies the use of favipiravir, one study the use of tocilizumab, two studies the use of interferon beta-1a and two studies the use of umifenovir.

Keywords: *Hydroxychloroquine, Favipiravir, Lopinavir/Ritonavir, Remdesivir, Umifenovir, Interferon beta-1a, Covid-19*

INTRODUCTION

Since December 2019, a new coronavirus, known as the novel coronavirus 2019 (nCoV-2019), has become the cause of a pandemic and a serious threat to global health. This coronavirus was first observed in Wuhan and has managed to spread all around China and ultimately worldwide [1]. The severe acute respiratory syndrome (SARS) Corona Virus 2 (SARS-Cov-2) is now recognized as the cause of coronavirus disease 2019 (COVID-19) and as a new member of the family of coronaviruses [2], being introduced by the World Health Organization (WHO) as the cause of the ongoing pandemic. The COVID-19 pandemic has spread rapidly around the world. By the end of 2020, nearly 100 million people have been diagnosed with this respiratory illness, of which about two million have already died [3]. COVID-19 can be present with no symptoms. The affected subjects may experience mild to severe symptoms of fever, chills, muscular pain, fatigue, cough, rhinorrhea, nasal congestion, and dyspnea, which can ultimately lead to life-threatening pneumonia. In addition, gastrointestinal symptoms, conjunctivitis, anosmia and ageusia have been associated with the diagnosis of COVID-19 [4]. This illness spreads by respiratory droplets and aerosols during coughing and (or) sneezing. The primary prevention protocol includes the use of face masks, environmental hygiene, and suitable hand washing. The majority of patients who died from COVID-19 had comorbid conditions, e.g., hypertension, diabetes, cardiovascular diseases, chronic obstructive pulmonary disease, malignancies, and chronic liver disorders [5]. On computed

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tomography (CT) of the chest, the most common patient findings include bilateral pulmonary changes, yet unilateral involvement has also been reported. Also, alterations of laboratory biomarkers like: leukocytosis, leucopenia or lymphopenia, an increase in the prothrombin time (PT), a decrease of the hemoglobin and an increase in the erythrocyte sedimentation rate (ESR) have also been reported. Moreover, an increase in the respiratory rate (>30 per minute), an oxygen saturation fall (≤93%), a decrease in PaO2/FiO2 (<300), and an increase in pulmonary infiltration (>50% of the lungs during 24 to 48 hours) accompany the clinical presentation of COVID-19 [4]. The mortality rate of COVID-19 is lower than the previous epidemics, namely 10% for SARS and 34% for Middle East Respiratory Syndrome (MERS). However, COVID-19 is more contagious and has a more extended incubation period, with SARS-CoV-2 infecting a larger population and causing public panic and an impressive economic impact [6]. Moreover, since there was no effective COVID-19 treatment and vaccines were not yet available, the pandemic has forced healthcare providers and regulatory organizations into repurposing current medicines aiming to test their potential efficacy in the management of COVID-19. For this reason, several clinical trials have been undertaken or are currently conducted worldwide. In some countries, physicians have prescribed medicines employed in the management of non-infectious diseases, such as chloroquine and hydroxychloroquine (used for malaria and rheumatoid arthritis), or antiviral Food and Drug Administration (FDA) approved drugs such lopinavir/ritonavir, favipiravir, remdesivir, etc., incorporating the use of these pharmacological agents in the local COVID-19 therapy guidelines [7]. Thus, the purpose of this systematic review and meta-analysis was to assess the efficacy of these drugs in the treatment of COVID-19 patients.

MATERIALS AND METHODS

All procedures outlined in this review were performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [8]. The literature was searched with related keywords to ensure that no similar studies have been conducted recently. Subsequently, a structured study question was extracted and used to define keywords, possible combinations, and a search strategy. Then, the literature was systematically searched again in relevant databases. We systematically searched PubMed/MEDLINE, Cochrane Library, Scopus, and Embase from inception until December 10, 2020. The following keywords were used:

Drugs:

- Hydroxychloroquine, Plaquenil®, Hydroxychloroquine Sulfate
- Chloroquine, Chloroquine Sulfate, Resochin®, Dawaquin®, Lariago®
- Remdesivir, Veklury®, Covifor®, Remivir®, GS-5734®, Bemsivir®, BDREM-100®
- Favipiravir, Avigan®, Fabiflu®, Favicovid®, Avifavir®, Areplivir®, Favipira®
- Lopinavir/Ritonavir, Kaletra, Lopimune®, Aluvia®, LPV/r,
- Umifenovir, Arbidol, AR-1I9514, Arpeflu®
- Tocilizumab, Actemra®, Roactemra®
- Interferon beta-1a, Rebif®, ReciGen®
- Disease: COVID-19, SARS-CoV-2, Coronavirus disease 2019.

For each database, an appropriate search was computed, e.g., using MeSH keywords. To increase the sensitivity, we checked the reference lists of the identified clinical trials and of the review articles. Moreover, a manual search of relevant websites was carried out. All the identified articles were imported into the EndNote software (Version X8, Thomson Reuters, New York). After eliminating duplicates, we screened the articles based on their title, abstract, and full-text according to our inclusion and exclusion criteria. We included studies based on the following criteria: 1) Study population: adults receiving the drugs as mentioned above for COVID-19 treatment but not for COVID-19 prevention. 2) Intervention: the use of the aforementioned drugs 3) Comparator: standard of care, placebo, any other drug or combination 4) Outcome: efficacy, treatment rate, reduced length of hospitalization, viral load, and mortality reduction 5) Study design: published clinical trials, but not preprinted articles. Two reviewers screened the titles, abstracts, and full-texts separately based on the eligibility criteria. Any disagreements in scoring were resolved through discussions with the senior author. Two distinct reviewers assessed the quality of the randomized clinical trials (RCTs) using the Cochrane Collaboration tool [9]. The procedure used to evaluate the quality of clinical trials evaluated the following criteria: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of the outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases. We rated each trial as having a low, unclear, or high risk of bias. After inspecting the quality of the clinical trials, we designed an extraction data form based on previous review articles, in addition to employing the Cochrane extraction form for data gathering. Two separate authors extracted the data from the included articles. The extracted data included study specifications (design, duration of intervention, duration of follow-up), participants' specifications (number, age, and gender), intervention specifications, and measured outcomes.

RESULTS

Twenty-eight clinical trials were selected from the 13553 screened studies.

Table1: Included studies

Author	Country	No of pts	Severity	Intervention	Control
Cavalcanti	Brazil	667	Mild/Moderate	HCQ/HCQ+AZM	SC
Chen	China	30	Mild/moderate	HCQ	SC
Horby	UK	4716	NR	HCQ	SC
Huang	China	22	Mild/moderate	Chloroquin	SC
Mitja	Spain	353	Mild/moderate	HCQ	SC
Silva Borba	Brazil	81	Severe	Chloroquin	Chloroquin
Skipper	USA, Canada	491	Mild/moderate	HCQ	PL
Tang	China	150	Mild/moderate	HCQ	SC
Chen	China	240	Mild/moderate	FVP	UMF
Dabbous	Egypt	100	Mild/Moderate	FVP	HCQ
Lou	China	30	NR	FVP	Baloxavir
Zhao	China	14	NR	FVP	TCZ
Ivashchenko	Russia	60	NR	FVP	FVP, SC
DOI	Japan	88	Mild/Moderate	FVP	FVP
Cao	China	199	Severe	LPV/R	SC
Huang	China	101	Mild/moderate	LPV/R	Interferon-alfa and Ribavirin
Hung	China	127	Mild/moderate	LPV/R	Interferon-beta and Ribavirin
Wang	China	60	NR	LPV/R	SC
Li	China	86	Mild/moderate	LPV/R	SC
Yethindra	Kyrgyzstan	30	Mild/moderate	UMF	SC
Zhu	China	50	Mild/moderate	UMF	LPV/R
Beigel	USA	1063	Mild/Moderate, severe	RMD	PL
Goldman	USA,	402	Severe	RMD	PL
Wang	China	237	Severe	RMD	PL
Toniati	Italy	100	severe	TCZ	SC
Davoudi	IRAN	92	Severe	IFN b-1a	SC
Dastana	IRAN	20	NR	IFN b-1a	SC

HCQ: hydroxychoroquine, AZM: Azithromycin, FVP: Favipiravir, LPV/R: Lopinavir/Ritonavir, UMF: Umifenovir, RMD:Remdesivir, TCZ: Tocilizumab, IFN b-1a: Interferon beta-1a, SC: Standard Care, Pl: Placebo and NR: Not reported.

Chloroquine and Hydroxychloroquine with or without Azithromycin:

Both medications were formerly administered to treat malaria and rheumatoid arthritis. According to their mechanism of action, they could be potentially effective in the treatment of COVID-19. Hydroxychloroquine does not bind to the angiotensin-converting enzyme 2 (ACE-2) receptor directly but increases the acidity of the medium in which the COVID-19 protein-spikes interact with and attach to the ACE-2 receptors. The resulting acidity degrades the viral spike and reduces the infection rate, eventually impeding the spread of COVID-19 [1]. Moreover, azithromycin seems to constrain SARS-CoV-2 via direct binding to the ACE-2 receptors. Cavalcanti et al. analyzed hospitalized patients with mild to moderate COVID-19 and revealed that hydroxychloroquine alone or in combination with azithromycin was not associated with better outcomes within 15 days after treatment initiation as compared to standard care. Moreover, there was no significant effect on mortality or mechanical ventilation [3]. Similarly, Horby et al. showed that the treatment with hydroxychloroquine was not associated with a lower mortality rate in hospitalized patients with COVID-19 within 28 days versus conventional therapy [4]. Besides, after 3-7 days of treatment with hydroxychloroquine, the RNA viral load in the nasopharyngeal swab samples did not decrease. Also, this regimen did not reduce the duration of the complete resolution of the symptoms in patients with mild to moderate COVID-19 [5].

In other studies, hydroxychloroquine did not exert antiviral effects against SARS-CoV-2 and was not associated with symptom relief in outpatients with early, mild COVID-19 [7, 10-12]. In addition to their ineffectiveness in COVID-19 patients, treatment with hydroxychloroquine and chloroquine was associated with severe adverse drug reactions (ADR), i.e., depression, psychosis, insomnia, and increased risk of suicide [13]. These two drugs tend to cause QT-prolongation, which is a serious ADR. Thus, necessary cautions must be taken into account when are administered in combination with other medicines with similar side effects, such as azithromycin [3,14-16]. On the contrary, Chen et al. examined 48 patients with COVID-19 and indicated that hydroxychloroquine could reduce the duration of hospitalization [17]. Guerin et al. and Lagier et al. revealed that hydroxychloroquine combined with azithromycin contributed to symptom relief in COVID-19 patients [18,19]. Moreover, Arshad et al. demonstrated that hydroxychloroquine+ azithromycin reduced the mortality rate versus both medicines alone [19]. Overall, according to our review, hydroxychloroquine and chloroquine could not prevent or treat mild to moderate or severe COVID-19.

Favipiravir:

Favipiravir inhibits the RNA-dependent RNA polymerase enzyme, resulting in the inhibition of viral RNA synthesis [20]. Doi et al. showed that the viral load was not significantly different in patients who received early versus late treatment with favipiravir. Additionally, Chen et al. stated that favipiravir was not as effective as umifenovir in improving clinical symptoms [21]. Likewise, in a study by Lou et al., the therapy based on favipiravir was comparable to standard care [22]. However, in other studies, favipiravir was associated with a reduced time of recovery compared to the control group [23, 24]. Furthermore, Zhao et al. showed that the combination therapy of tocilizumab and favipiravir reduces pulmonary inflammation [25]. Hyperuricemia, hypertriglyceridemia, nausea, vomiting, and diarrhea are the reported ADRs for this medicine [21, 23, 26].

Lopinavir/Ritonavir:

Lopinavir/ritonavir inhibits the 3CL pro in vitro, a protease present in the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and highly conserved in SARS-CoV-2 [27]. Ye et al. showed that a combination of Lopinavir and Ritonavir was more effective in the resolution of fever and other clinical symptoms and signs in comparison to conventional therapy [28]. In contrast, Li et al. showed that lopinavir/ritonavir does not seem to improve the clinical outcome of adult patients with mild to moderate COVID-19 and has little benefit over supportive care [29]. Similarly, in Cao's study, there was no significant difference between the standard care and this regimen in hospitalized patients with severe COVID-19 [30]. Also, the UK Recovery Study compared 1596 patients receiving lopinavir/ritonavir with 3376 patients receiving standard care, and no benefit was observed [31]. In another study by Hung and Deng, the combination therapy of methylprednisolone or umifenovir with lopinavir/ritonavir seemed to improve clinical outcomes, e.g., shortening the duration of viral shedding and hospital stay [32,33]. All in all, at the moment, lopinavir/ritonavir cannot be recommended as mainstay therapy for COVID-19 since there is little supportive evidence regarding its efficacy against COVID-19.

Remdesivir:

Remdesivir is a monophosphoramidate adenosine analog that targets and inhibits the RNA-dependent RNA polymerase enzyme [34]. Grein et al. revealed that 68% of their patients with severe COVID-19 responded to treatment with remdesivir, which improved their health status [35]. Similarly, in a study by NIH, the treatment with remdesivir was associated with a reduction of the recovery time and a lower mortality rate [36]. Thus, remdesivir was authorized for emergency use by the FDA, the European Medicines Agency, and the National Health Services in the UK [37-39]. Moreover, in Goldman's trial, there was no significant difference between a 5-day course and a 10-day course of remdesivir in adults with severe COVID-19 not requiring mechanical ventilation [40]. Although hospitalized adults with severe COVID-19, remdesivir did not yield significant clinical benefits, early treatment in the course of the disease was associated with less recovery time [41]. Accordingly, remdesivir is a promising treatment option in COVID-19 and was the first drug to acquire FDA approval to treat COVID-19 [42].

Umifenovir:

Umifenovir protects the body against viral infections via blocking the viral entrance to host cells. Yethindra et al. revealed that patients with mild to moderate COVID-19 might benefit from umifenovir combined with standard care. Also, minimal adverse side effects were reported [43]. In addition, umifenovir monotherapy seemed to be superior to lopinavir/ritonavir in treating COVID-19 in a small sample size study [44]. Umifenovir, and supportive care, may relieve fever and other symptoms faster in COVID-19 patients, but it needs further investigation [45].

Tocilizumab:

A 3rd generation monoclonal antibody (humanized mAb), tocilizumab, inhibits interleukin-6 (IL-6) receptors and is used in the treatment of rheumatoid arthritis and other autoimmune diseases. Patients with severe cytokine release syndrome (CRS), and probably severe pneumonia, may also benefit from tocilizumab [46]. Toniati et al. recorded a rapid and sustained response to tocilizumab and observed a post-therapy clinical improvement [47]. However, Kewan et al. did not report significant clinical improvement with tocilizumab in patients with severe COVID-19 [48]. In addition, tocilizumab tends to decrease the mortality rate and the need for invasive mechanical ventilation in adults with severe COVID-19 pneumonia [49]. On the contrary, Colaneri et al. (2020) studied 112 adults with severe COVID-19 and showed that mortality or the number of intensive care unit (ICU) admissions was not reduced following the administration of tocilizumab [50]. Likewise, Kewan et al. did not observe a significant clinical improvement with tocilizumab in patients with severe COVID-19 [51]. Therefore, due to contradictory results, further studies are needed to establish the beneficial effects of tocilizumab in patients with severe Covid-19.

Interferon beta-1a:

Davoudi et al. reported that treatment with interferon beta-1a did not change disease duration. However, when this medication was added to the nationwide approved therapy regimens, an increased discharge rate on day 14 and a decreased 28-day mortality were detected [52]. Moreover, another study supports the use of interferon β-1a in combination with hydroxychloroquine and lopinavir/ritonavir in the treatment of COVID-19 [53].

DISCUSSION

This systematic review provides a comprehensive analysis of clinical trials exploring the use of hydroxychloroquine, chloroquine, favipiravir, lopinavir/ritonavir, remdesivir, tocilizumab, and interferon beta-1a in COVID-19 patients. Despite the previous suggested benefits of chloroquine or hydroxychloroquine with or without azithromycin, these agents could not show favorable efficacy in the prevention or treatment of COVID-19. The occurrence of severe ADRs, i.e., QT-prolongation, which can enhance the interactions with other medicines and pose serious health threats, have obliged the National Institute of Health and the WHO to abolish these medicines from their trials. Lopinavir/ritonavir, formerly used to treat human immunodeficiency virus (HIV) infection, had no effect on the inhibition of virus replication, reduction of the duration of hospitalization or mortality, leading to the withdrawal of this drug from clinical trials. Favipiravir, a guanine analog, and umifenovir have been approved in some countries, e.g., Russia, but further research is warranted to clarify their effects in COVID-19. Some countries, despite their former approval of these medicines, later withdrew them from their national COVID-19 guidelines. Remdesivir is the first FDA-approved pharmacological agent for COVID-19, and its efficacy was observed in some multicenter, randomized, controlled trials. However, since these RCTs were funded by pharmaceutical companies, there is a risk of bias regarding their benefits. Moreover, there are some meta-analyses that described non-significant effectiveness on COVID-19 outcomes in comparison to standard care. Overall, more studies with a lower risk of bias are needed to enable confident statistical analyses. The IL-6 receptor blocker, tocilizumab, might be a robust anti-inflammatory agent in the management of severe COVID-19 patients during the inflammatory pneumonia phase. There are some other ongoing trials on tocilizumab that will be reported in the following updates. Although interferon beta-1a showed favorable efficacy in a phase 2 trial, more investigations are needed due to the high risk of bias and small sample size of this exploration. Our study analyzed only published RCTs, not observational studies (cohorts, case reports, and case series). According to different interventions, different doses, and different disease severity, more studies are needed to conduct reliable meta-analyses or dose-response studies.

CONCLUSION

The rapid transmission of SARS-CoV-2 has limited the opportunities to develop new treatment strategies. Thus, a try-and-error strategy was used to test medicines in the management of COVID-19 and develop standard treatment guidelines. Our findings suggest that hydroxychloroquine, chloroquine, and lopinavir/ritonavir are not favorable treatments for COVID-19. Favipiravir, umifenovir, and interferon beta-1a need investigation in more clinical trials as well. Tocilizumab and remdesivir might be effective in some COVID-19 patients, but more research is needed to draw conclusions.

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ORIGINAL ARTICLE

A brief overview of the Epstein Barr virus and its association with Burkitt's lymphoma

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Abstract: *Epstein Barr virus (EBV) is known as an oncovirus and associates with several human malignancies such as Burkitt's lymphoma, other non-Hodgkin lymphomas, nasopharyngeal carcinoma, Hodgkin's disease, gastric adenocarcinoma, etc. in Burkitt's lymphoma, and the key event is the translocation of MYC gene, that increase of cell survival and aberrant expression of MYC gene. The biology of EBV and its function in the development of Burkitt's lymphoma are discussed in this review.*

Keywords: *Epstein Barr virus, oncovirus, Burkitt's lymphoma, MYC gene*

INTRODUCTION

Epstein–Barr virus (EBV), discovered in 1964 by Epstein, Achong, and Barr from cultured cells of Burkitt's lymphoma by electron microscopy [1]. The EBV is recognized as the first human tumor virus. It is associated with lymphomas, epithelial cell cancers, nasopharyngeal carcinoma, and gastric cancer [2, 3]. EBV infection is mainly transmitted by saliva. The prevalence of EBV in the worldwide population is more than 90%. Most of the EBV carrier individuals do not have any symptoms. However, it is estimated that EBV has a role in the development of approximately 200,000 new cancer cases worldwide each year. Also, it is responsible for almost 2% of all human cancers, and according to current evidence, EBV may be a co-factor in the development of other malignancies, such as breast and cervical cancer [4].

EBV virion structure

EBV (also knowns as human gammaherpesvirus 4) is a member of the Herpesviridae family and a common virus among humans. EBV has a linear, double-stranded DNA genome with a length of 184 kb in a nucleocapsid with 162 capsomeres, which is encapsulated in an envelope with external glycoprotein spikes. The virion diameter is roughly 120-180 nm [5, 6].

Replication cycle

EBV infection targets epithelial and B-cells. Eight glycoproteins are involved in virus entry depends on the type of cell (Table 1) [7]. One of the most abundant glycoproteins of EBV called GP350\220 is responsible for the binding to B-cells, in which it binds to complement receptor type 2 (CR2) on B-cells with high affinity [8]. Although this is a key step for virus attachment to the target cells, other factors also are required for infecting target cells. Major histocompatibility complex class II (MHC-II) on B-cell surface act as a co-factor that interacts with the three viral glycoprotein gp42, gL, and gH to bind the virus to B-cell [9-11].

The fusion of the viral envelope with the cell membrane begins with the interplay of gp42 and HLA class II that eventually leads to endocytosis of the virus into the cell [11]. Complement receptor 1 (also known as CD35) (CR1) is an additional adhesion factor that binds to gp350\220 and can provide a pathway for EBV entry into CD21 negative cells such as immature B-cell. Furthermore, CD35 is sufficient for EBV binding, MHC-II expression is also needed for cell entry and infection. The gp350 mediates the attachment of EBV to epithelial cells, however, it can be fulfilled by gHgL complex on CR2 negative cells [20]. The gHgL complex binds specifically to epithelial cells, not B-cells, and the gH-null virus loses the ability to link to a CR2-negative epithelial cell but still can use gp350 to bind to CR2-positive cells [21-23]. The ability of gHgL to bind to CR2 negative epithelial cells can be blocked by the presence of gp42 [21]. It also prevents the virus's ability to infect CR2-positive cells [24]. In epithelial

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cells lacking CD21, the BMRF-2 protein of the virus interacts with cellular β1 integrin, and gHgL proteins interact with αvβ6\8 integrin to initiate virus fusion [25]. Endocytosis of the EBV into the vesicle and fusion of the virus with the vesicle membrane results in the release of the nucleocapsid into the cytoplasm. As the nucleocapsid dissolves, the genome is transferred to the nucleus [26].

Table 1: Roles of envelope glycoproteins in entry to epithelial and B-cells

Gene	Glycoprotein	Role in virus entry	REF
BLLF1	Gp350	Binding to CR2\CD21 receptor in B cell	[12]
BXLF2	gH	Attachment of virus to epithelial cells, also essential for penetration to B-cells	[13]
BKRF2	gL	Heterodimer gH\gL is critical for entry into the host cell	[14]
BZLF2	gp42	Acting as co-receptor for fusion and entry of EBV into the B-cell	[15]
BALF4	gB	Required for fusion with both epithelial cell and B-cell	[16]
BLRF1	gN	It may play a role in post-fusion events	[17]
BBRF3	gM	Co-expressed with gN	[18]
BMRF2	BMRF2	Binding to host beta 1 integrin family and facilitates virus attachment to the epithelial cell	[19]

Lytic replication

Lytic replication leads to the production of infectious viruses. Lytic replication can occur in both B-cell and epithelial cells. The frequent replication cycle in B-cell occurs after reactivation from latency, while the replication cycle in epithelial cells initiates after entry of the virus [27]. For lytic replication of the viral life cycle, its genome must be linear [28]. In the latent phase, the genome is circular, so it should be linearized in the process of lytic reactivation [28]. During lytic replication, the viral DNA polymerase is responsible for the replication of the viral genome, but in the latent phase, it uses the host cell DNA polymerase to replicate its genome [26]. Three consecutive steps are involved in the production of lytic genes: intermediate early (IE), early (E), and late [29]. In the IE step, two essential genes BZLF1 and BRLF1, are expressed.

These genes act as trans-activators in activating the virus and initiating the cascade of viral genes expression [26]. Expression of the IE BZLF1 gene can induce a switch from latent to lytic infection. BZLF1 binds to the promoters of E genes and encodes proteins that are crucial for viral DNA replication. It recruits RNA polymerase II on their promoters and inducing the lytic cascade mechanism [29].

Expression of one of the BZLF1 or BRLF1 genes is enough to induce lytic replication in both epithelial cells and B-cells. The expression of one IE protein causes the expression of other proteins [30]. Early lytic gene products include a wide range of activities such as replication, metabolism, and blockade of antigen processing [31]. BNLF2a is one of the EBV viral genes expressed in the early lytic stage that plays an important role in escaping the virus from the immune system by expression of a 60 amino acid protein that interferes with antigen presentation to CD8+ cells [32-34]. Late lytic gene products often have a structural role, such as viral capsid antigen (VCA), which is involved in the production of the viral capsid. Several late lytic gene products such as BCRF1 are involved in escaping the virus from the immune system [35].

Latency

Unlike the lytic phase, progeny virions are not produced in the chronic phase, and it is a persistent viral infection. EBV persistent infection is mainly seen in memory B-cells but is also found in epithelial cells [36]. Actually, it is thought that one in the millions of B-cells can carry the virus genome as an episome or integrated DNA after recovering from an acute infection [6, 37].

During the latent phase, episome replication occurs by the host DNA polymerase. In the latency phase, only a limited portion of the genes are expressed [38]. These genes contain five EBV-encoded nuclear antigens (EBNA), two latent membrane proteins (LMP), EBV-encoded small RNA (EBER), and BART RNA (Table 2) [39, 40]. Three patterns of expression of latency genes exist that which of them have different expression patterns and are known as latency programs. Latency programs include latency I, latency II and latency III that each of which can lead to the expression of specific viral genes [41, 42]. Three latency program patterns have been determined in different Burkitt lymphoma and lymphoblastoid cell lines (LCL) and are reported in Table 3 [36].

Table 2: expression of Epstein-Barr virus latent genes

Latent gene	Roles	REF
EBNA-1	Responsible for DNA replication, mitotic segregation, EBV transcriptional activation, autoregulation	[36]
EBNA-2	Main viral trans-activator, critical for B-cell activation	[37]
EBNA-LP	Co-operate with EBNA-2 in viral and cellular gene transcription	[38]
EBNA-3A	With EBNA-3C block EBNA-2 by interaction with RBPG proteins, decrease expression of MYC	[39]
EBNA-3B	It is essential for B-cell transformation, tumor suppressor	[40]
EBNA-3C	Essential for efficient B-cell transformation, also like EBNA-2, LP and EBNA-3C regulates viral and cellular gene expression	[41]
LMP-1	B-cell and epithelial cell transformation, critical role as the primary EBV-encoded oncogene, and active NF-KB and P38 pathway, immune regulation	[42]
LMP-2A	Play a key role in improving cell adhesion, cell motility, and inhibition of epithelial cells also, can activation signaling pathways such as the PI3K\AKT, NF-KB, B-Catenin, STAT, and syk tyrosine kinase and immune modulation	[43]
LMP-2B	-	[43]
EBER	Play a key role in modulating innate immunity response, cell resistance to PKR-mediated apoptosis	[44]
BART	Immune regulation, cancer progression	[45]

Table 3: Epstein-Barr virus latency pattern

Gene expressed	EBNA-1	EBNA-2	EBNA-3A	EBNA-3B	EBNA-3C	EBNA-LP	LMP1	LMP-2A	LMP-2B	EBER	BART
Latency I	+	-	-	-	-	-	-	+	+	+	+
Latency II	+	-	-	-	-	+	+	+	+	+	+
Latency III	+	+	+	+	+	+	+	+	+	+	+

Epstein-Barr Virus microRNAs

The first human viral-encoded miRNAs were discovered in EBV in 2004 by Pfeffer et al. [43]. microRNAs (miRNAs) are small non-coding RNA containing about 20-22 nucleotides that play a crucial role in regulating gene expression [44]. For cell-to-cell transmission, miRNAs can be packaged into exosomes and released into the extracellular space [45]. various cell types can secreted exosomes into body fluids, including blood, urine, saliva, breast milk, cerebrospinal fluid, ascites, etc [46] miRNA plays an important role in the pathogenesis of EBV-associated cancers.

EBV encodes over 40 miRNAs, which are derived from approximately 25 viral-miRNA precursors [47]. Forty of them are located to BamHI-A rightward transcript (BART), and four are located to the BamHI fragment H rightward open reading frame 1 (BHRF1) [48]. These miRNAs have distinct levels of expression at different phases of latency. EBV miRNAs can modulate infection and pathogenesis by regulating the expression of viral genes [49]. The miRNA can be effective in escaping the EBV from the immune system by suppressing the release of pro-inflammatory cytokines, such as IL-12, and repress the differentiation of CD4+ T cells. miRNA directly targets the transported TAP2 and decreases levels of TAP1 and MHC class 1 molecule, which can cause the EBV-infected cells to evade CD8+ T cells [50].

Epidemiology

In developing countries, the infection occurs in children (in the first years of life), whereas in developed countries, it is usually delayed and seen in adolescence [51]. In some developed countries, however, the rate of infection is bimodal, with peaks in children under five years and again after ten years [52]. The cause of this bimodality is the transmission of the virus through oral excretion between parents and infants and from partners in adults [53]. The EBV antibody titer in seropositive individuals shows a U-shaped pattern, and the highest antibody titer is seen in infants and the elderly [54]. The high antibody titer in children is usually a reflection of primary infection and, in the elderly, is due to a reduced immune response [55, 56]. EBV has spread worldwide but with remarkable geographical variety in the distribution of EBV genotypes [57]. EBV contains two major types, I and II, that differ in the genes expressed in latently infected cells [58]. Both types are detectable all over the world, but type I is more common.

However, in some areas such as Africa, Papua New Guinea, and Alaska the second type is the most common [59, 60]. The prevalence of the virus is not significantly different between men and women, but overall, antibody titers are higher in women than men [53]. EBV is also associated with socioeconomic conditions. Poor socioeconomic status is related to primary EBV infections, whereas late primary EBV infection occurs more frequently in people with higher socioeconomic status [61].

Transmission

EBV usually is transmitted through oropharyngeal secretions. In adults and adolescents, the primary cause of virus transmission is kissing, whereas saliva on stuff such as toys or fingers is the primary cause of transmission among children. EBV is detectable in cervical secretions of 8-28% of young girls and adult women and also in the seminal fluid of men. In rare circumstances, the virus can be transmitted through breast milk. It can also be transmitted through blood transfusion and transplanted allogeneic hematopoietic cells or solid donor organs [53, 62].

Vaccine for EBV

Vaccination is the most effective way to avoid contracting EBV. More than 90% of the world's human population is infected by EBV, therefore, the vaccine is greatly important. The evidence shows that the EBV vaccine can prevent more than 200,000 new malignancies per year worldwide. gp350 viral antigen is the most abundant glycoproteins on the surface of EBV that known as the first candidate for the vaccine. gp350 has an important role in the efficient infection, although the gp350 deficient virions can still infect B cell, gp350 antibodies predominantly neutralize B cell infection. The amino-acid sequence of gp350 is extremely conserved among diverse strains, especially in the amino-terminal region, and 97% amino-acid similarity between EBV type1 and type 2 [63]. Among all EBV glycoproteins, gp350 is a promising vaccine target to block b cell infection and is also a target for T cell-mediated immunity (64-66). gp350 in combination with the AS04 adjuvant demonstrated can prevent the development of infectious mononucleosis (IM) after EBV infection [67]. Monoclonal antibodies against gp42 can inhibit EBV infectivity of B cells, while infection of epithelial cells inhibited by a monoclonal antibody against gH\gL or BMFR2 antigens [68, 69]. EBV neutralizing antibody titers correlate more with gp350 antibody compared with antibody titers to gp42 [70]. Furthermore, the latent antigen EBNA3A with tetanus toxoid formulated in water-in-oil adjuvant acts as a vaccine that is designated to induce EBV-specific T cell response. In almost 90% of vaccines, CD8 T-cell responses are obvious but can not prevent EBV infection. Although it seems that vaccination can reduce the incidence of IM [71].

Diagnosis

There is a spectrum of laboratory approaches for the diagnosis of EBV infection. The strategy of diagnosis is different between immunocompromised and immunocompetent individuals because therapeutic interventions in these groups are different. The diagnosis of primary infection is based on antibody assays [72]. Three serological patterns such as immunoglobulin (Ig) G and M antibodies to viral capsid antigens (VCA) and IgG antibodies to EBNA1 are a minimal requirement for detection of primary EBV infection. Immunofluorescence assay (IFA) is a gold standard for the diagnosis of EBV infection [73]. enzyme immunoassay (EIA) and chemiluminescence immunoassay (CLIA) are often performed as screening tests, while western blot analysis is used for confirmation [74]. For detecting and quantifying the virus in body fluids, polymerase chain reaction (PCR) analysis is the best approach, and tissue samples as well as, it can be used for measuring the viral load [75]. Additionally, the detection of EBV-encoded RNA transcripts (EBERs) by in situ hybridization is the gold standard for finding EBV in tissue [76].

Treatment

For the treatment of EBV infection, there is no approved antiviral medication. More physicians prescribe acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) to alleviate fever and pain [77]. Fluids and nutrition are highly recommended, especially for febrile cases. Since most patients have anorexia in the first and seconds weeks, nutrition is very important [78]. Several antiviral drugs are used for EBV infection, although non specifically against EBV, and most of these drugs are nucleoside-analogue [79]. The only class of drugs that assess for EBV infection in clinical trials is nucleoside-like agents. The nucleoside analogue in the drug disrupts the synthesis of viral DNA by inhibiting viral DNA polymerase. Acyclovir is a nucleoside analogue that can reduce EBV shedding but without evidence of clinical benefit [80]. Studies have also reported the use of other agents, such as interleukin 2 [81], interferon-alpha [82], and Intravenous immunoglobulins (IVIg) [83] in the management of EBV related diseases.

EBV AND BURKITT LYMPHOMA

For the first time, Burkitt lymphoma (BL) was described 60 years ago by Denis Burkitt as a disorder with jaw tumors in African children, which he called “African lymphoma” [84, 85]. BL is an aggressive B-cell malignancy among non-Hodgkin lymphoma (NHL) types [86, 87]. It is divided into three classes, which vary in geographic distribution and relation with EBV: endemic (eBL), sporadic (sBL), and HIV-associated BL [85, 88].

- **Endemic BL** (eBL) is one of the most common childhood cancers in Equatorial Africa and is accounts for 75% of malignant disorders in children aged 4-7 years [89]. Evidence suggested a supportive relationship between EBV and eBL, which almost all eBL cases are associated with EBV [90]. Plasmodium falciparum contributes to the etiology of eBL first by disrupting immune response against EBV, thereby increase virus replication, second by stimulating B-cell activation, which leads to EBV lytic reactivation and abnormal expression of activation-induced cytidine deaminase (AID) that elevate the probability of MYC translocation triggering oncogenesis [91]. Jaw and kidney involvement are frequent, as well as the central nervous system (CNS), ileal, cecal, gonads, facial bone, thyroid, and breast can be affected [92].

- **Sporadic BL** (sBL) is almost occurring worldwide, without an association between geographic and climatic conditions. sBL is responsible for 1-2% of adult lymphoma, up to 40% of children's lymphoma in western Europe and the United States. The abdomen, particularly the ileocecal region and lymph node, are the most common site of involvement. sBL is associated with EBV infection, ranging from 10-30% positivity in various regions [93].

- **HIV-associated BL** mainly occurs in human immune-deficiency virus (HIV) infection. HIV expansion in developed and developing countries has increased EBV-dependent BL in adult populations and is an early marker for AIDS progression in HIV-positive individuals [94]. EBV is observed in 30-40% of HIV-associated BL cases (Table 4) [95].

Table 4: Characteristic features of the various Burkitt lymphoma subtypes

	Endemic BL	**Sporadic BL**	**HIV-associated BL**
Geographical distribution	Equatorial Africa And Papua New Guinea	Worldwide	Worldwide
Incidence	5-15\10^{5}	1-2\10^{6}	Variable
Age	4-7 years	All ages	All ages
EBV-association	>90%	10-30%	30-40%
Co-factor	EBV, malaria	Unknown	HIV
Location of MYC breakpoint	More than 1 Kb upstream of first exon	Exon1 the MYC gene	Exon1 the MYC gene
Location of Ig breakpoint	Joining (j) region, Sμ switch region of Ig	Sμ, sα and j region	Sμ
Tumor site	Most common in the jaw, it can also occur in the abdomen, kidney, and ovaries	Most common in the abdomen, it can also occur in the kidney	Lymph node, bone marrow, abdomen, and CNS

The hallmark feature of all BL is the translocation of the MYC gene, which results in the upregulation of MYC protein. Most frequent translocation (approximately 80% of cases) between the MYC gene with a heavy chain of immunoglobulin (14q32). The two other translocations can be found in BL, translocation of c-MYC with the light chain of immunoglobulin at 2p12 and 22q11 [96, 97]. The c-MYC has essential roles in different cellular processes, such as regulates the transcription of various genes, cell growth, signal transduction, regulation of RNA processing, immune function, and apoptosis. MYC overexpression does not alone adequate to initiate lymphomagenesis. Dysregulation of microRNAs (miRNA) may lead to increase expression of MYC independent of chromosomal translocation or gene amplification [98].

Furthermore, in sporadic Burkitt's lymphoma, additional mutations related to the MYC gene have been discovered. Mutations in TCF3 or ID3, which is a protein that blocks TCF3 activity, are observed in about 70% of cases. In addition, almost one-third of sBL cases have a mutation in CCND3, which encodes cyclin D3 [99, 100]. sBL mostly causes abdominal tumors, while eBL is often present in the jaw and kidney but can also be present in the abdomen, ovaries, facial bone, and other extranodal sites [101, 102].

Burkitt's tumors have historically been monomorphic medium-sized cells with rounded nuclei, many nucleoli, and copious basophilic cytoplasm. Due to the presence of high numbers of macrophages, Tumours show a ''starry sky'' pattern [103]. The proliferation of BL cells is rapid, and their doubling time is 24-48 hours [104]. Their rate of apoptosis is also high. BL cells usually express B-cell markers including CD19, CD20, CD22, and IgM and germinal center markers such as CD10, BCL6, and human germinal center associated lymphoma (HGAL) protein [105]. The cell surface phenotype of BL cells reflects the origin of the germinal center, whereas jaw and ovaries are the primary sites of tumor growth, do not usually contain germinal center [106]. It seems that translocation of the MYC gene to immunoglobulin chains in eBL occurs during the VDJ recombination [107, 108]. This process occurs by a VDJ recombinase activating an enzyme called RAG-1/2 expressed in both pre-B and germinal center B cells [109]. However, in sBL and HIV associated, this chromosomal breakage happens mainly in the class switch region [110]. The GC involvement is an essential factor in the pathogenesis of this disease, whether in MYC translocation or association with EBV, malaria, and HIV infection as a co-factor [111]. Malaria and HIV infection, for example, has been determined to activate B cells [112]. Evidence from clonal EBV genomes in Burkitt's lymphoma tissues showing that the tumor is originated from a single infected cell. EBV gene products can directly aid cell survival in the BL cells. Both EBNA-1 and the EBERs, for example, have a function in BL cell survival and apoptosis prevention.

CONCLUSION

Burkitt's lymphoma is known as a highly aggressive non-hodgkin lymphoma with a diverse pattern of clinical presentation. It is a multi-factorial disease, although MYC translocation accounts for one of the main causes of its development. Other risk factors contribute significantly to MYC translocation. Finding the detailed role of each risk factor in the occurrence and progression of Burkitt's lymphoma is a good area in medical research. A better understanding of the detailed mechanism of Burkitt's lymphoma pathogenesis, as well as the precise role of each factor, can lead to the introduction of more effective prevention and therapy strategies.

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ORIGINAL ARTICLE

Analysis of COVID-19-related psychiatric disorders – clinical manifestations and therapeutic considerations

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Abstract: *During the COVID-19 pandemic, neuropsychiatric disorders have been diagnosed both in the acute phase, and in the aftermath of this disease. Inpatients, as well as outpatients recently diagnosed with mild, moderate or severe forms of Coronavirus infection have reported neuropsychiatric manifestations with variable duration, symptoms that could be directly or indirectly related to the viral pathogenic agent. Patients who are recovering from the acute phase of COVID-19 also may exhibit various psychopathological manifestations, during the so-called "post-COVID-19 syndrome" or "long COVID-19 syndrome". Explanations regarding the onset of this type of clinical manifestations and the SARS-CoV-2 infection are extremely diverse, ranging from biological factors (e.g., direct central nervous system viral activity, cerebral hypo-oxygenation, high level of inflammatory response) to psycho-social stressors (e.g., isolation, fear of death, anxiety related to possible somatic complications or sequelae). Objectives: The primary objective of this article was to analyze psychiatric manifestations in patients with acute COVID-19 disease and in patients during post-COVID-19 phase. The secondary objective was to propose a conceptual framework for the evaluation and treatment of these patients. Methods: Patients included in this analysis were screened positive for COVID-19 infection in the last 6 months before their first psychiatric examination. These patients were further evaluated to detect any personal history of psychiatric disorders, somatic comorbidities, or significant concomitant pharmacological treatments. Specific scales for the measurement of symptoms severity and functional impairment were administered in all patients. Results: Five patients were included in this analysis, one during the acute phase of COVID-19 infection, and four patients were evaluated after the complete remission of this disease. Hyperactive delirium, mild neurocognitive disorder, major depressive episode, panic disorder with agoraphobia, or acute psychotic disorder were the main diagnoses in these patients. All patients received adequate treatment and they were monitored using psychological scales until symptoms remission or stabilisation. Conclusions: Psychosocial stressors, neurobiological changes, systemic inflammatory reaction, and individual vulnerability factors may contribute to a diathesis-stress model for psychiatric disorders onset within the acute phase or after the remission of acute manifestations in COVID-19-diagnosed patients.*

Keywords: *COVID-19, long COVID-19 syndrome, psychotic disorders, major depression, neurocognitive disorder, delirium, panic disorder*

INTRODUCTION

Although the primary target cells for SARS-CoV-2 are epithelial cells of the respiratory and gastrointestinal tract, it is believed that the penetration of the virus in the human body are far from being limited to these organs [1,2]. The presence of neurological manifestations, such as headache, anosmia/ageusia, dizziness, seizures, and strokes suggests also the invasion of the central nervous system (CNS) during COVID-19 infection [2,3]. Data derived from both patient and experimental models of β-coronaviruses infection support this hypothesis of CNS involvement, due to their evident neurotropism (SARS- CoV-2, MERS-CoV, HCoV-HKU1, HCoV-NL63, HCoV-OC43, HCoV-229E) [2]. This invasion of the CNS is presumed to occur directly, through peripheral nerves or via systemic blood circulation, but aberrant immune responses and respiratory insufficiency associated with COVID-19 are indirect mechanisms that could also explain neurologic manifestations [3]. However, only few studies have reported the presence of SARS-CoV-2 in cerebrospinal fluid or brain parenchyma [3]. It is speculated that a potential trigger of multi-organ autoimmunity in COVID-19

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infection could be the molecular mimicry between SARS-CoV-2 proteins and different human cell antigens, CNS cells included [3,4].

The importance of psychosocial factors in the onset of COVID-19-related psychiatric disorder is significant, starting from social isolation, to negative impact over the financial status or professional performances, lower quality of life, changes in day-to-day functioning etc. [5,6]. All these factors might translate into different psychiatric disorders, including post-traumatic stress disorder, depression, sleep disorders, or isolated neuropsychiatric symptoms [6]. A frequently reported finding is that the pandemic had a significant negative effect over the mental health status in people compared to their average mental health levels during previous years, although this difference is mediated by age, gender, ethnicity and other factors [6]. This observation is supported by an increased prevalence of depression, post-traumatic stress, and sleep disorders in the last years [6,7]. According to a review, symptoms of anxiety and depression (16-28%) and self-reported stress (8%) are common psychological reactions to the COVID-19 pandemic, and they may be associated with disturbed sleep [7]. Both clinical and sub-syndromal mental health problems should be actively investigated, especially in vulnerable populations, as possible reactions to the COVID-19 pandemic. If left untreated, these problems may worsen and lead to long-term complications, with negative, invalidating consequences.

Anxiety and depression spectrum symptoms and disorders in COVID-19 survivors were frequently identified at one month after discharge from hospital in a study (N=402 adults), with rates reaching 28% for post-traumatic stress disorder, 31% for depression, 42% for anxiety, 20% for obsessive-compulsive symptoms, and 40% for insomnia [8]. More than half of the individuals questioned in this study about the existence of psychiatric manifestations or disorders scored in the pathological range in at least one clinical dimension [8]. Patients with psychiatric history showed increased scores on most psychopathological measures, even in the presence of similar baseline level of inflammation [8]. Anxiety and depression scores at follow-up were associated with baseline systemic immune-inflammation index, which evaluates the immune response and systemic inflammation [8].

A study evaluating 841 patients hospitalized with COVID-19 (mean age 66.4 years) showed that 57.4% developed at least one type of neuropsychiatric symptoms, since early stages of infection [9]. Disorders of consciousness occurred commonly (almost 20%), mostly in older patients and in severe advanced COVID-19 stages, but also headache (14%), dizziness (6%), seizures (0.7%) etc. were reported [9]. Anxiety (8%), depression (5.2%), insomnia (13%), and psychosis (1.3%) were also detected during this study [9]. All CNS analyses performed were negative for the viral RNA in patients who presented neurologic manifestations, therefore the inflammatory response with blood-brain-barrier breakdown seems more probable as a pathogenic mechanism [9].

Based on the literature analysis, a comprehensive evaluation of patients diagnosed with COVID-19 should integrate both somatic and psychological aspects, on short- and long-term. A case series to illustrate this necessity is included in the next chapter of this article, with focus on the methodological and therapeutic dimensions.

METHODS

The primary objective of this article was to analyze psychiatric manifestations in patients with acute COVID-19 infection and in patients during post-COVID-19 phase. All these patients were evaluated by multidisciplinary teams, either during hospitalisation or as out-patients, depending on the severity of their clinical manifestations. The secondary objective was to propose a conceptual framework for the evaluation and treatment of these patients.

In order to attain these objectives, patients presented here were screened positive for COVID-19 infection in the last 6 months prior to their initial psychiatric evaluation. All of these patients were further evaluated to detect any personal history of psychiatric disorders, somatic comorbidities, or significant concomitant pharmacological treatments.

CASE SERIES

The first patient was a male, 75 years old, diagnosed positive for SARS-CoV-2 three days prior to the initial psychiatric evaluation. He had no significant history of somatic diseases or psychiatric disorders, and presented an acute onset of spatial and temporal disorientation, together with disturbance in attention and short-term memory, visual and auditory hallucinations, irritability, sleep-wake cycle disturbance, restlessness and agitation, while being admitted to an ICU department for a severe form of COVID-19. Regarding the blood tests, inflammatory markers (procalcitonin, C-reactive protein, fibrinogen) were increased, and CT scan showed bilateral pneumonia. This patient had a slowly favorable evolution of his respiratory infection, and the severity of his confusional state also gradually declined. Treatment with haloperidol 0.5 mg b.i.d. was initiated and maintained for 5 consecutive days, in order to control his psychomotor agitation and hallucinatory-induced behavior. The patient gradually recovered his attentional and mnestic abilities, as well as his sleep-wake cycle at day 5, while hallucinatory manifestations disappeared. Therefore, psychotropic treatment was discontinued starting with day 6. Follow-up visits took place at day 10 after the first psychiatric examination (when the patient was discharged from ICU), and at day 17 (hospital discharge). Clinical Global Impression – Severity (CGI-S) decreased from 6 to 3 and 1, respectively, and Delirium Rating Scale (DRS, 10-item version) [10] total score decreased from 26 to 5, and 2, respectively. Another visit was planned at day 60 after discharge, and the patient was in good physical and mental health, without blood tests abnormalities, chest-X-ray opacities or psychometric cognitive impairment on Mini-Mental State Examination (MMSE).

Discussion

The "cytokine storm" and the neurotropism of coronoviruses were invoked as potential explanations for neuroinflammation and delirium in COVID-19 patients [11]. Prolonged hospitalization, multiple pharmacological concomitant treatments, corticotherapy, higher age, and social isolation may also be contributing factors to the delirium onset [11]. Regardless of causation, either there is a direct effect of coronavirus or a multifactorial ethiology, delirium should be interpreted as a negative prognosis factor in older patients [12]. In an Italian cohort study with 80 COVID-19 inpatients (mean age 74.7+/-14.5 years), 68.8% had delirium with agitation [11]. A larger study (N=516 inpatients, mean age 78 years, SARS-CoV-2 positive) showed that 14.1% of these patients presented delirium on admission, and the most significantly factors associated with delirium were dementia (OR=4.66), the number of chronic diseases (OR=1.20), and chest-X-ray or CT opacity (OR=3.29-3.35 for multiple/bilateral opacities and single opacity vs. no opacity, respectively) [13]. The rate of in-hospital deaths was higher in patients with delirium (58.9% vs. 33.4%), and a multivariable analysis showed an almost twofold increased hazard ratio for delirium-positive patients vs. non-delirium patients for in-hospital mortality [13]. Another study (N=91 participants) supported an even higher difference of in-patient death in cases with delirium vs. non-delirium (72% vs. 31.8%, OR=3.98) [12].

Based on the data in the literature, special attention should be given in elderly developing delirium while being hospitalised for COVID-19. In our presented case, the evolution was favorable, although the patient was admitted in an ICU unit, and he had bilateral pneumonia. According to a longitudinal study, delirium during the hospitalisation in elderly patients with respiratory failure or shock (N=821) was associated with cognitive impairments on long-term in a significant proportion of the participants, and a longer duration of delirium was independently associated with significant worse global and worse executive function cognition at 3 and 12 months [14]. Therefore, follow-up visits are considered necessary in old age patients with delirium during hospitalisation for COVID-19, with screening for possible long-term psychiatric consequences, especially for neurocognitive impairments.

The second patient was a female, 37 years old, diagnosed with acute psychotic disorder, after two months since her discharge from hospital for COVID-19, with an acute onset of delusions of contamination and incurability ("I am still positive for SARS-CoV-2", "No one can really be ever cured from this infection", "I feel like I am condemned to being sick forever"), somatic hallucinations ("My lungs are pressured and they are getting smaller and smaller", "All my body is aching because the virus is spreading in me"), delusional-induced behavior (continuously disinfecting her environment and her body, repeatedly consulting physicians in spite of the fact that all her COVID-19 tests were negative), and lack of insight into her pathology. These symptoms were correlated by the patient with the re-occurrence of several respiratory symptoms (dyspnea, cough) which were attributed by her GP to a non-coronavirus respiratory infection, without pulmonary negative impact, according to the chest X-ray results. Atypical antipsychotics should be preferred in young and adult population, based on their superior tolerability profile [15,16]. Olanzapine 5 mg q.d. was initiated, and the dose was increased up to 15 mg q.d. during the next 7 days. After two weeks, the patient presented an improvement of her delusions and hallucinations, and of her psychosis-induced behavior. Also, a better insight was detected, as she admitted she may need a psychotropic treatment for her condition, and that there could be a possible psychiatric origin of her symptoms. Follow-up visits were performed at 4, 8, and 24 weeks. The clinical evolution reflected in the Positive and Negative Syndrome Scale (PANSS) score was favorable, with gradual decline in psychotic positive symptoms, from 98 (initial visit) to 80, 67, and 56, respectively. CGI-S score evolution reflected this improvement, with decline from 5 (initial visit) to 2 (at week 24). Global Assessment of Functioning (GAF) scores increased from 40 (initial visit) to 60, 75, and 80, respectively.

Discussion

Evidence from previous pandemics, especially with viral respiratory pathogens, support the correlation between schizophrenia-like disorders and the status of infected patient [17]. In a review of 42 cases of psychosis as acute or post-infectious manifestation of COVID-19 could not find evidence for a specific mechanism of psychosis onset in this population, 4 hypotheses being considered: direct neuronal viral infection, post-infectious neuronal autoimmunity, vasculopathies from impaired coagulation, and systemic effects of a pervasive severe pathogen and/or critical illness [17]. In our case, no biological causal relationship could be established between the COVID-19 disease and the acute psychotic disorder, but a psychological relationship is easy to infer from the available data.

There are several similar reports in the literature about new onset of psychotic disorders in the context of COVID-19 pandemic, e.g. a 38-year-old woman, with no psychiatric history, developed psychotic symptoms caused by fear of SARS-CoV-2 contamination [18]. She started to feel that “a demonic force” visit her at night and tries to possess her soul, while presenting insomnia, auditory hallucinations which became imperative, visual hallucinations, and induced aggressive behavior [18]. She did not have positive SARS-CoV-2 tests or IgM/IgG antibodies against this virus, and after being diagnosed with acute psychotic disorder, treatment with quetiapine 500 mg/day and clonazepam 1 mg/day was initiated [18]. Psychotic symptoms, anxiety and insomnia improved rapidly during the first week of hospitalisation and the patient was discharged [18].

Another case series included 6 patients (3 male and 3 female) with first-episode psychosis, admitted in an Italian hospital during the COVID-19 pandemic lockdown [19]. Blood tests, toxicological screening, SARS-CoV-2 antigen tests, and brain imaging were unremarkable, except for a mild cortical atrophy in a 73-year old male [19]. All patients had a negative psychiatric history, despite their advanced age [19]. Rapid discharge from hospital (less than 14 days) and fast remission of symptoms were observed in this case series after relatively low doses of antipsychotic treatment [19]. The follow-up visits confirmed the remission of psychosis in all these cases, suggesting the correlation between intense psychosocial stress induced by COVID-19 lockdown measures and the abrupt onset of acute psychosis [19].

Regarding the presence of psychotic symptoms in recently-diagnosed COVID-19 patients, a case series (N=3) showed a relatively rapid remission of these clinical manifestations under treatment (9 days, 10 days, and also 10 days, respectively) with antipsychotics (ziprasidone 40 mg/day, ziprasidone 20 mg/day, or haloperidol 15 mg/day, respectively) [20]. A direct causal relationship between SARS-CoV-2 infection and the onset of psychosis was not established in these cases [20].

Therefore, onset of acute, brief psychotic disoders is not uncommon in individuals without psychiatric history and non-positive for SARS-CoV-2 infection, possible triggered by prolonged social isolation during lockdown or quarantine. In patients with COVID-19 infection, psychotic episodes were also reported, without assessing a possible direct causality. In our presented case, the patient recovered from COVID-19, and the most likely trigger for her psychosis was an acute and mild, viral respiratory, non-SARS-CoV-2 infection.

The third patient was a female, 24 years old, diagnosed with panic disorder with agoraphobia, who presented the onset of her anxiety disorder 4 months after the discharge for COVID-19 pneumonia. This patient reported feelings of choking, shortness of breath, nausea, palpitations, dizziness, paresthesias, derealization, and intense fear of dying with abrupt onset and limited duration (5-15 minutes): “I cannot find myself, it’s like I’m in a trance for several minutes”, “I feel like I’m choking… I can’t breathe, and I need to sit down because everything is spinning around”, “I feel my heart is beating erratic, and my legs are numb… I feel nauseated and my belly hurts”, “I think that I will certainly die when this happens”. The frequency of these panic attacks was 2-3 times/week and the patient actively avoided going out in open spaces for fear of getting new panic attacks. When asked about the first time she had these symptoms, she said “panic attacks are like the choking sensations I had when I was hospitalised for COVID-19... I actually believed I have this disease again, so I repeatedly tested myself for COVID infection”. Clinical, chest-X-ray and spirometry could not objectify any respiratory dysfunction, and the RT-PCR (reverse transcriptase-polymerase chain reaction) assay from nasal and pharyngeal swabs for SARS-CoV-2 infection was negative. No other comorbid conditions, either psychiatric, or organic, were detected. Initial GAF score was 50 and Hamilton Anxiety Rating Scale (HAMA) score was 32. Treatment with paroxetine 20 mg q.d. was initiated, and it was increased up to 20 mg b.i.d in the next 7 days, and gabapentin 100 mg t.i.d [21] was also added from day 1. After 14 days the patient was re-evaluated, and GAF score increased up to 65, while HAMA score decreased to 24. The follow-up visits at day 30 and 90 showed continuous improvement on both scales, GAF scores were 70 and 80, while HAMA scores reached values of 16, and 10, respectively. A cognitive-behavioral therapy (CBT) was suggested to the patient since the initial evaluation, but she declined.

Discussion

Anxiety symptoms are not rarely reported in patients diagnosed with COVID-19, even if the respiratory or general status was not severely affected [7]. In a case report, a 38-

year-old man developed recurrent palpitation, worries, shortness of breath, chest discomfort, fear of death, feeling of impending doom which lasted for about 10 minutes; these symptoms first appeared within two days after he was tested positive for COVID-19 [22]. Although he had a mild form of COVID-19, his symptoms of panic persisted after his test became negative [22]. The patient had no psychiatric history, and he was started on sertraline 50 mg/day and clonazepam 0.75 mg/day [22]. Regarding the possible mechanisms of panic disorder onset in COVID-19 patients, various hypotheses have been formulated: respiratory symptoms, like coughs and dyspnea, are very frequently associated with the SARS-CoV-2 infection, so fear conditioning to abnormal breathing patterns, and hypervigilence towards breathing abnormalities may increase the risk of developing a panic disorder in COVID-19 survivors; other somatic factors associated with COVID-19 or its treatment may fuel the worries and anxiety related to personal health [23].

A systematic review and meta-analysis focused on anxiety in the general population during COVID-19 pandemic (n=43 studies) found an estimated prevalence of 25% (vs. 7.3% normally), varying across the different tools used to measure anxiety [24]. Initial or peak phase of the COVID-19 outbreak, female gender, younger age, marriage, social isolation, unemployment, student status, financial hardship, low educational level, insufficient knowledge about COVID-19, epidemiological or clinical risk of disease and lifestyle and personality variables were the most common risk factors for anxiety [24].

In our presented case, the patient re-activated her memories related to the COVID-19 four months later, in the absence of any current respiratory infection. The main focus of her attention were respiratory symptoms, associated with fear of death, triggered by the same feeling she had when she found out she was diagnosed with COVID-19. The evolution of anxiety symptoms under treatment was favorable, even if no CBT was initiated, based on the patient's preference.

The fourth patient was a female, 72 years old, diagnosed with major depressive episode, severe, and she had the onset of her mood symptoms after she was discharged from hospital with a diagnosis of "remitted COVID-19 disease". This patient was already diagnosed with recurrent major depressive disorder for 8 years, but she was in remission for more than 2 years prior to the COVID-19 diagnosis. She invoked the COVID-19-related stress as the only major stressor in her recent history, and her family confirmed the absence of any other stresors in her immediate environment. She said that she felt threatened by this infection and she was afraid she might die: "When I heard I was positive, I immediately though I will die, and I thought I have only a few hours to live... I was almost certain I could not survive this". Although she had a moderate form of COVID-19, which did not require ICU admission, she necessitated psychiatric evaluation after her discharge from hospital, due to specific depressive symptoms, i.e. anhedonia, depressed mood, insomnia, loss of appetite for food, ideas of incurability, and recurrent thoughts of death. New generation antidepressants, with superior tolerability profile, should be recommended in this population [25,26]. Sertraline 50 mg q.d. was initiated, and gradually increased up to 50 mg t.i.d in the next 14 days. Zolpidem 5 mg q.d. for insomnia was also recommended in this case, for 2 weeks. Geriatric Depression Scale (GDS-30) initial score was 23, and decreased to 16 after 14 days, and 8 after two months. GAF scores increased from 40 (baseline value) to 65, and 82, respectively.

Discussion

A cross-sectional, observational study included hospitalised, COVID-19 infected patients (N=93) and their immediate family members (N=54), and its objective was to assess depression, anxiety, and stress symptoms in the given population [27]. Almost one-third of this sample showed high scores on depression and anxiety subscales of DASS21 (Depression Anxiety Stress Scale-21), with 30-45% of the family members presenting higher scores than the patients [27]. Another observational study that used Patient Health Questionnaire-9 (PHQ-9) in adults during the COVID-19 pandemic vs. 2015-2019 period in Canada showed a substantially increase of depression prevalence in the first time interval [28].

There is a debate in the literature regarding the specifics of the post-COVID depression, i.e. if there are neurobiological differences between the commonly diagnosed major depression and the one detected in COVID-19 survivors. Analysis of data from USA (N=91791 respondents to a questionnaire) collected during COVID-19 pandemic showed that age, gender, race, education, and income exhibited an interaction with prior COVID-19 in risk for moderate to severe depressive symptoms [29]. Among people with such mood symptoms, motoric symptoms and suicidality were significantly greater among those with prior COVID-19, and the depression risk increased with greater interval following acute infection [29]. Little evidence are available until now to suggest different pathogenetic substrates for the two types of depression.

In patients with severe mental illnesses (affective disorders and schizophrenia), a systematic review (n=36 studies) tried to detect special needs during the COVID-19 pandemic [30]. A general impairment of the mental health in individuals with severe mental illnesses could be detected, especially in patients with mood disorders, as compared to those with schizophrenia [30]. Symptoms of depression, anxiety, and stress were more pronounced in patients with history of severe mental disorders, and resilience, age, and socioeconomic factors impacted significantly their health status [30].

In the here-presented case, the patient was already known with history of major depression, but she was in remission prior to the onset of COVID-19 symptoms. She clearly admitted that her worries were related to perceived threats to her personal safety and fear of dying, and she considered these worries as the origin of her currently diagnosed depressive episode. Based on her psychiatric history, the need for prolonged treatment with antidepressants is obvious, as it is the need to closely monitor suicidal ideation and severity of depression on long-term. In patients with psychiatric history, COVID-19 infection may be a significant stressor that could re-activate mood disoders, or may aggravate their mental health. Therefore, supplying patients with psychiatric history of depression with adequate information about the COVID-19 disease and increasing their stress resilience is important [30].

The fifth patient was 69 years old, and presented neurocognitive deterioration possible related to COVID-19, after 5 months since the remission of the infectious disease. She was admitted in a hospital due to COVID-19 pneumonia and had a severe evolution, which required an ICU admission for 3 days. After 5 months since COVID-19 remission, her family reported the gradual onset of low attention and memory functioning, social isolation, speech difficulties, spatial disorientation and low involvement in activities of daily living. Neuroimagistic evaluation (CT scan) detected a temporal and parietal cerebral atrophy, and leukoaraiosis at 5 months after discharge from hospital. Unfortunately, no comparison of this result is possible, as no prior cerebral CT or MRI imagery was available. During the initial psychiatric exam, no other comorbidities were detected. Baseline MMSE score was 22, and GAF score was 45. Treatment was initiated with donepezil 5 mg q.d., and the dose increased during the next month to 10 mg q.d., in association with pramiracetam 600 mg b.i.d. The follow-up visits at 3 and 6 months detected no change in the MMSE score, and the GAF slowly increased to 50.

Discussion

Multiple mechanisms for the onset of neurocognitive disorders in COVID-19 survivors have been suggested, e.g. hypoxic brain injury secondary to severe pneumonia; centyral nervous system invasion by coronaviruses via blood circulation or neuronal pathway; immune-mediated injury [31]. Under-detection of delirium in patients with COVID-19 may be a silent contributor to the development of neurocognitive disorders, especially if there are cases of hypoactive delirium [31,32]. Pre-existing delirium and small vessel cerebrovascular disease often detected in elderly may result in greater vulnerability to accelerated cognitive deficits in COVID-19 survivors [33]. In an observational study, the cognitive function was evaluated among individuals with persistent cognitive complaints about 5.5 months after COVID-19 infection [34]. Mild cognitive deficits were detected on tests involving attention, processing speed, and executive function, and 70% of these patients were diagnosed with a mood disorder prior to COVID-19 infection [34]. The pattern of cognitive deficits consists in mild impairments, and the contribution of psychological factors related to COVID-19 infection, as well as residual symptoms may increase the severity of subjective cognitive complaints in this population [34].

In the case we presented, no delirium was diagnosed during the COVID-19 infection, but the patient was admitted in ICU. Lack of any previous neuroimagistic evaluation or cognitive assessment makes it difficult to establish the impact of COVID-19 infection over cognitive function in this case. Increased attention should be given to the existence of delirium (either hypoactive or hyperactive) in elderly patients with COVID-19 infection, and especially if they had severe forms of this disease. Cognitive evaluation in elderly with delirium during hospitalisation, or prolonged hospitalisation related to COVID-19 infection should be included in their monitoring plan.

CONCLUSIONS

Patients diagnosed with COVID-19 may have a high rate of neuropsychiatric symptoms or disorders [35,36], due to the direct, biological impact of the SARS-CoV-2 virus, or due to the psychological stress related to this disease (table 1). The direct effects of COVID-19 over the mental health status is difficult to verify in clinical practice [37,38], and data in the literature does not support such a clear, causal relationship. However, evaluation of the COVID-19-diagnosed patients from the perspective of psycho-social stress consequences could help physicians in the detection of vulnerability factors, which may indicate the necessity for early pharmacological and/or psychotherapeutic interventions.

Patients with prior psychiatric diagnoses, prolonged ICU/hospital admissions, multiple stressors (psychological, social, economic, health-related), or personality vulnerabilities (e.g., reduced coping mechanisms, low tolerability to stressors, anxiety features) may require a psychological screening after acute respiratory symptoms remission. When early signs of psychiatric disorders or isolated symptoms are identified, structured psychiatric evaluation, pharmacologic and/or psychological support should be recommended. Mood symptoms, especially depressive, neurocognitive impairment, anxiety symptoms/ disorders, or psychosis have been reported in COVID-19 survivors, even after several months since the remission of respiratory symptoms. In the acute phase of the COVID-19, delirium and psychosis, but also anxiety or mood symptoms should be actively monitored and adequately treated. Because mood disorders are associated with severe complications, like suicide or addictive disorders [39,40], and neurocognitive disorders have a dramatic impact over patients' and caregivers' quality of life [41], early detection and treatment of these conditions are needed, under constant medical monitoring.

Table 1: Synthetic presentation of clinical and therapeutic characteristics in COVID-19-related psychiatric disorders

Case number	Diagnosis	Scales administered	Treatment	Evolution	Observations
1	Delirium, hyperactive	DRS, CGI-S, MMSE	Haloperidol 0.5 mg b.i.d	Favorable, symptoms remited in 5 days	Hyperactive delirium is easy to detect, but hypoactive type should also be searched for in vulnerable populations
2	Acute psychotic disorder	PANSS, CGI-S, GAF	Olanzapine 5 mg q.d., increased to 15 mg q.d. during 7 days	Favorable, symptoms declined significantly after 2 weeks	Psychological stressors related to COVID-19 may be important for the onset of acute psychosis in patients without psychiatric history
3	Panic disorder with agoraphobia	HAMA, GAF	Paroxetine 20 mg q.d., increased up to 20 mg b.i.d in the next 7 days + gabapentin 100 mg t.i.d.	Favorable, improvements on both scales up to 60 days of follow-up	Respiratory distress during COVID-19 may represent a fixation point for anxiety, and it may be subsequently activated
4	Major depressive episode, severe, without psychotic features	GDS-30, GAF	Sertraline 50 mg q.d., gradually increased to 50 mg t.i.d; zolpidem 5 mg q.d. for insomnia was also recommended	Favorable under treatment, up to 60 days of follow-up	Patients with MDD in personal history may be susceptible to new episodes due to COVID-19 distress
5	Mild neurocognitive disorder	MMSE, GAF	Donepezil 5 mg q.d., increased during the next month to 10 mg q.d.; pramiracetam 600 mg b.i.d	Stable, up to 6 months of follow-up	Cognitive function should be monitored in vulnerable populations after COVID-19 infection.

DRS= Delirium Rating Scale, CGI-S= Clinical Global Impression- Severity, MMSE= Mini-Mental State Examination, Positive and Negative Syndrome Scale, HAMA= Hamilton Anxiety Rating Scale, GDS= Geriatric Depression Scale- 30-item version, MDD= major depressive disorder

In conclusion, psychosocial stressors related to the COVID-19 pandemic (e.g., vital threat, isolation, hospitalisation), direct neurobiological influence of SARS-CoV-2 infection, systemic inflammatory reaction, and individual vulnerability factors (e.g., personal psychiatric history, dysfunctional coping mechanisms) may contribute to a diathesis-stress model for psychiatric disorders onset in patients during the acute phase or after the remission of COVID-19 symptoms.

Disclaimer

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ORIGINAL ARTICLE

Basosquamous carcinoma: an overview of actual protocol and the importance of basal and squamoid components

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Abstract: *Due to its scarcity, the basosquamous carcinoma represents a challenging pathology both as a result of its aggressiveness and potential of relapse and metastases, and also due to unclear management guides. Encountered under the name of basal cell carcinoma or metatypical carcinoma, the pathophysiology of such tumors is still unclear and it is not known yet if it either derives from a basal cell carcinoma or it could be a variant of transition to a squamous cell carcinoma.*

Due to low incidence, unclear treatment protocols and not enough research regarding squamous component in the aggressivity of basosquamous carcinoma, the aim of this paper is to analyze histopathological features of 5 cases of basosquamous carcinoma from Plastic Surgery Department of Emergency University Hospital, Bucharest, and correlate them with current literature.

Keywords: *basosquamous, metatypical carcinoma, squamous cell carcinoma, basal cell carcinoma*

INTRODUCTION

Although medicine is constantly evolving with new lines of diagnostic and treatment, skin cancer is a global problem with increasing occurrence and morbidity [1], entailing high costs for the health system [2].

First presented in 1910 by MacCormac, the basosquamous carcinoma is a rare pathology, with an incidence of approximately 1.2-2.7% of skin neoplasms [3,4]. Some of the risk factors identified are exposure to UV radiations, smoking and old age. Most frequently, this pathology is encountered on sun-exposed areas in Caucasian men.

Considered by some authors as a subtype of basal cell carcinoma or a rare version of it, the tumor presents characteristics of both basal and squamous cell carcinomas [5], and it is characterized by its risk of relapsing and remote dissemination [6].

Since the literature described them poorly and the treatment protocol is still unclear, the purpose of this paper is to present features of basosquamous carcinoma of a mini cohort from Plastic Surgery Department of Emergency University Hospital of Bucharest, diagnosticated during 2 months' period, showing the need of an optimized protocol as a different entity from basal or squamous cell carcinoma, and also showing the potential of increasing this pathology.

MATERIALS AND METHODS

The inclusion criteria were patients diagnosticated with basosquamous carcinoma from Plastic Surgery Department of Emergency University Hospital, Bucharest, during October-December 2019.

The study ran with the agreement of the ethics committee and the consent of the patients. All the patients underwent surgical excision after blood tests and the approval of the anesthetist. Preoperatively, imaging investigations were decided depending of the macroscopical features of the tumor (size, location, the adherence of surrounding tissue) and presence of adenopathies.

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The diagnostic was provided based on histopathological examination, after surgical excision.

All tissue samples were fixed in 10% buffered formaldehyde and processed by conventional histopathological methods, with inclusion in paraffin and Hematoxylin-Eosin (HE) staining. Immuno-histochemistry was performed for one patient to identify the particularities of basosquamous carcinoma using CD10 (Figure 1), P53 (Figure 2), epithelial adhesion molecule (Figure 3), P63 (Figure 4), androgen receptor (Figure 5) and epithelial membrane antigen (Figure 6) antibodies provided by Biocare.

The indirect tristadial Avidin-Biotin complex method was used. The sections were deparaffinased in toluene and alcohol successive baths, rehydrated in alcohol baths with increasing concentrations, washed in phosphate saline buffer and incubated with primary antibody overnight.

Next, they were washed in carbonate buffer and developed in 3,3'-diaminobenzidine hydrochloride/hydrogen peroxide nuclear counterstaining with Mayer's Hematoxylin.

Also, all the patients enrolled were sent to oncological service after they were diagnosticated.

Figure 1: Basosquamous carcinoma: the tumor cell are negative for CD10 compared to most cases of basal cell carcinoma that are positive for this immunomarker, anti-CD10 antibody, 100x

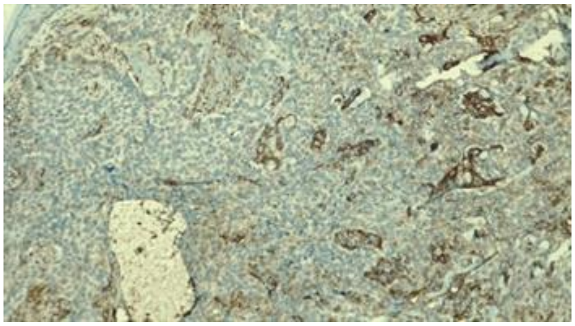

Figure 2: Basosquamous carcinoma: strong, diffuse nuclear immunoreactivity for P53 (hyperexpresion, mutant staining pattern). The expression pattern is similar to that found in squamous cell carcinomas of the skin, anti-P53 antibody, 100x

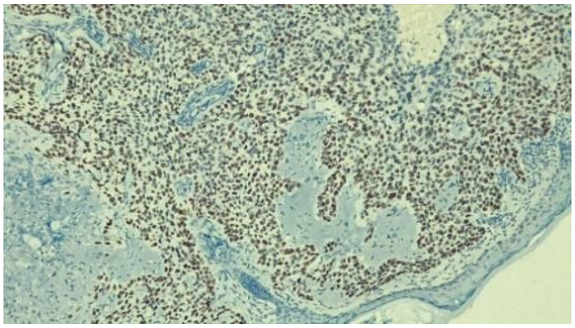

Figure 3: Basosquamous carcinoma: EP-CAM (epithelial adhesion molecule) negative in squamous component and positive in basal cell carcinoma (anti-EP-CAM antibody, 100x)

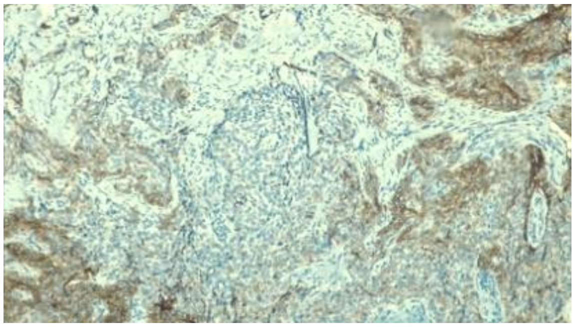

Figure 4: Basosquamous carcinoma: Positive P63 immunohistochemical reaction for both basal and squamous areas, anti-P63 antibody, 100x

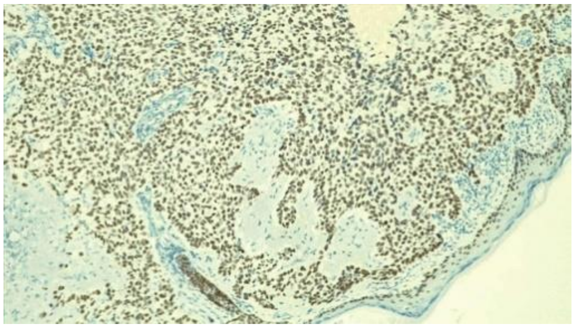

Figure 5: Basosquamous carcinoma: the tumor cell are negative for AR (androgen receptor), anti-AR antibody, 100x

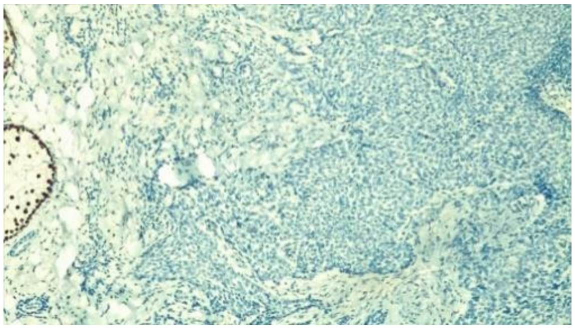

Figure 6: Basosquamous carcinoma: the tumor cells are EMA (epithelial membrane antigen) negative. Note the internal positive control (pilosebaceous unit), anti-EMA antibody, 100x

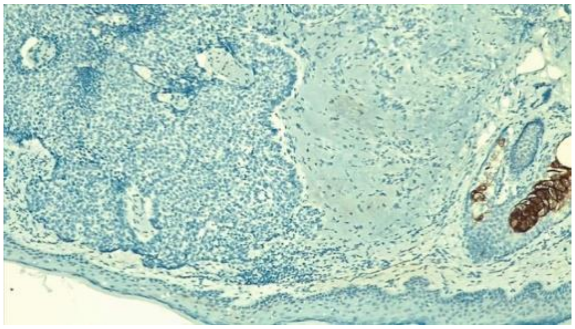

Case no. 1

The first case is a 75 years old man, with long term exposure to sun, who came to plastic surgery service with a growth of an endophytic tumor mass (Figure 7) on superior level of the left cheek measuring approximately 2/1.5 cm.

The tumor appeared about 3 months ago; it was ulcerated and presented perilesional telangiectasias which are a characteristic sign for basal cell carcinoma [7].

Considering the aggressiveness of the tumor and its evolution, surgical excision with pathology examination was decided.

Figure 7: Ulcerated endophytic tumor on superior level of the left check with perilesional telangiectasias, a characteristic sign for basal cell carcinoma

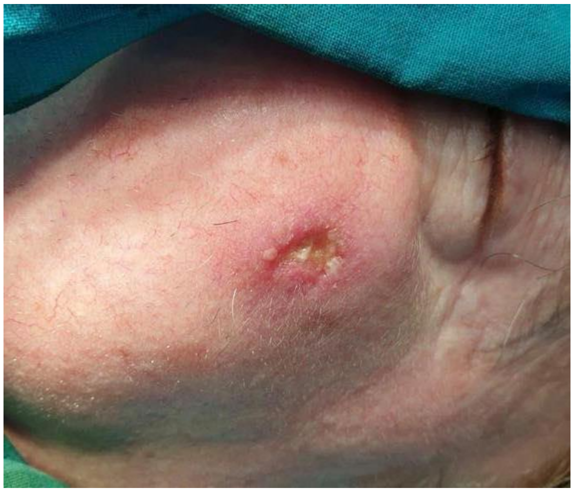

The histopathological examination (Figures 8 and 9) revealed an ulcerated tumor consisting of a superficial proliferation of malignant basal cell-type with basophilic cytoplasm and hypercromatic nuclei, with minimal atypia admixed with cells that have acidophilic cytoplasm, vesicular nucleoli and atypical mitoses infiltrating the deeper levels of the dermis.

The tumoral proliferation is surrounded by a lymphocytic inflammatory infiltrate.

Thus, a diagnosis of basosquamous carcinoma, with squamous predominance of 80% was made. The tumor infiltrated the deep dermis and was reported with a Clark index of IV, with perineural invasion >0.1 mm but without vascular invasion.

The tumor was completely excised.

Figure 8: Metatypical carcinoma, with solid nests of malignant cells with eosinophilic cytoplasm and vesicular nuclei, HE 10x

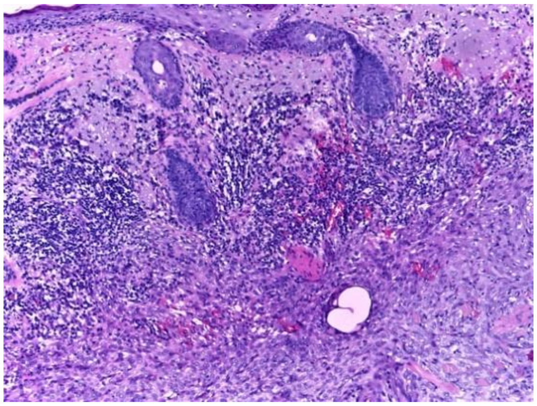

Figure 9: Basosquamous carcinoma with non-keratinizing squamous component of 80%, with solid and infiltrative pattern, reaching a nervous structure, HE 20x

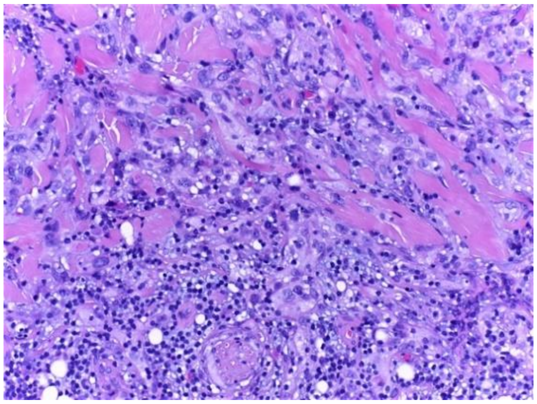

Case no 2

An 88-year old patient, former smoker, with long-term exposure to sun, presented with an ulcerated tumor located on the right auricular pavilion (Figure 10), covered with hemorrhagic crusts, measuring about 5/4 cm that appeared a few months ago. He came to our service because of local pain and tumor bleeding.

Figure 10: Ulcerated tumor on auricular pavilion

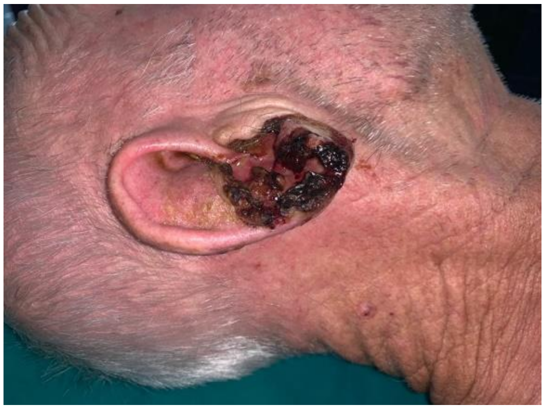

A preop cervical CT scan revealed multiple ganglia images at the superficial and deep laterocervical level, and also at the submandibular level, with the maximum size of 1 cm. The patient was also evaluated by the maxillo-facial surgery service, which recommended only the follow-up of those adenopathies. In this case, it was decided the partial amputation of the ear (Figure 11) with macroscopically tumor-free margins and reconstruction by fixing helix on auricular lobe (Figure 12).

Figure 11: Partial amputation of the ear

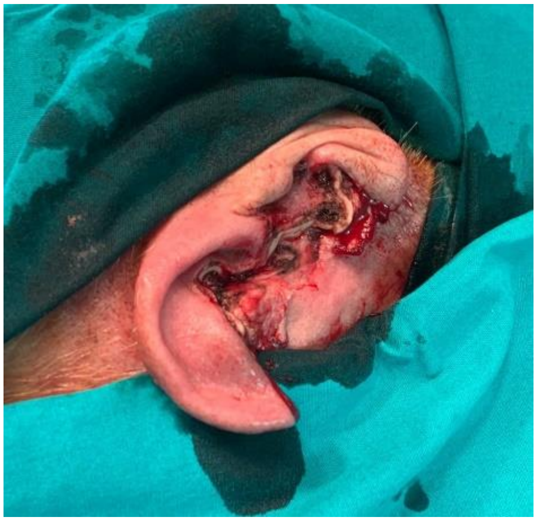

Figure 12: Day 3 postoperatively. Reconstruction of auricular pavilion

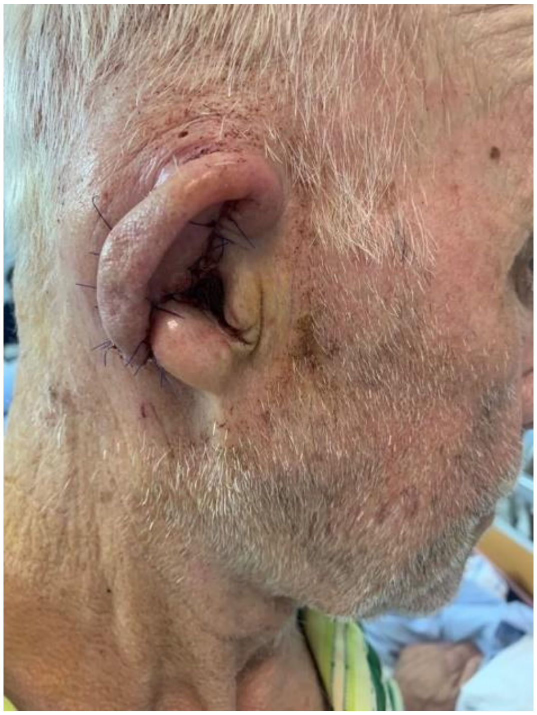

After conventional HE method processing and examination, a malignant proliferation of cells with intermediate aspects of both basal and squamous features was identified. The nuclei presented atypical mitoses, a hypercromatic and slighty oval nuclei. The solid proliferation infiltrated the soft tissue, and presented clear ulceration on the skin. A diagnosis of basosquamous carcinoma without cartilage infiltration was made. (Figures 13 and 14).

Figure 13: Ulcerated metatypical, invasive carcinoma, with a solid, non-keratinizing pattern, with intermediate aspects between basal and squamous cell; notice hyaline cartilage without tumor invasion, HE, 4x

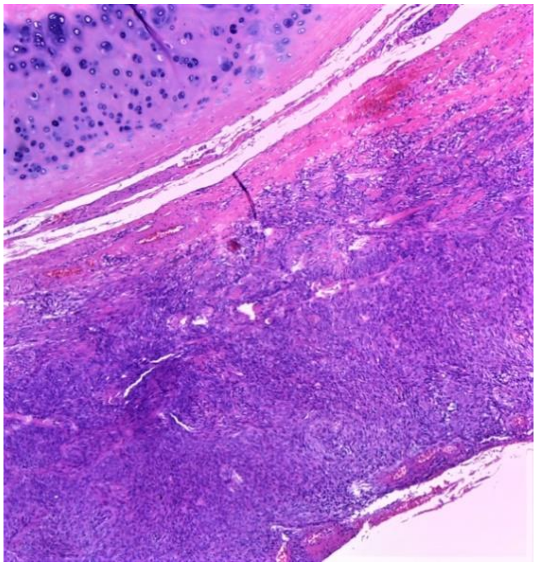

Figure 14: Ulcerated metatypical, invasive carcinoma, with solid islands of malignant cells, with hypercromatic nuclei and moderate pleomorphism, HE, 10x

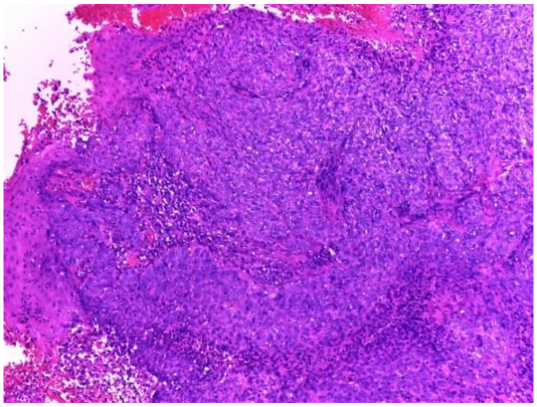

Case no 3

The 3rd case is of a 76 years old Caucasian woman who was transferred to plastic surgery service for a biopsy of a nasal pyramid mass (Figure 15). Her tumor appeared a few months ago after local trauma and sun exposure.

Figure 15: Ulcerated tumor of the nasal pyramid

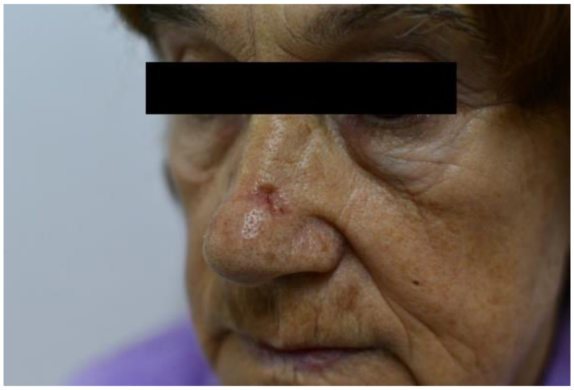
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After the resection, the histopathological examination revealed a metatypical carcinoma consisting of a proliferation of intermediate cells invading the dermis, incompletely excised (Figure 16). The re-excision revealed a small island of basaloid cell proliferation and an important granulomatous foreign body reaction with multinucleated giant cell reaction (Figures 17 and 18). Surgical margins were tumor-free.

Figure 16: Island of metatypical carcinoma invasive in dermis, HE 10x

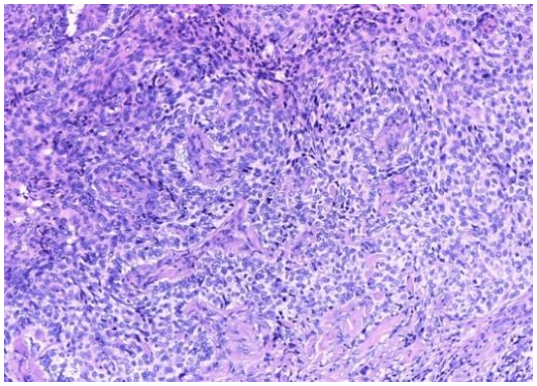

Figure 17: Re-excision of basosquamous carcinoma (granulomatous foreign body reaction with multinucleate giant cells, HE. 10x)

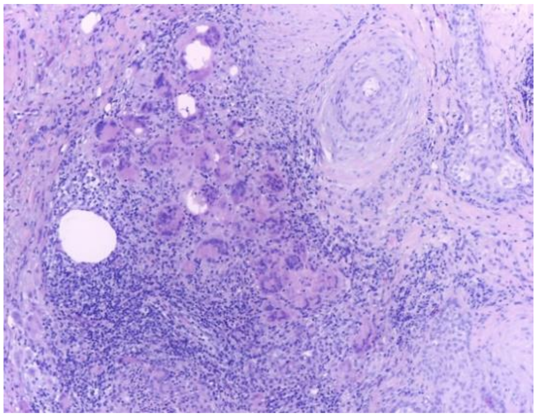

Figure 18: Re-excision of basosquamous carcinoma – isle of superficial basal cell carcinoma, HE, 10x

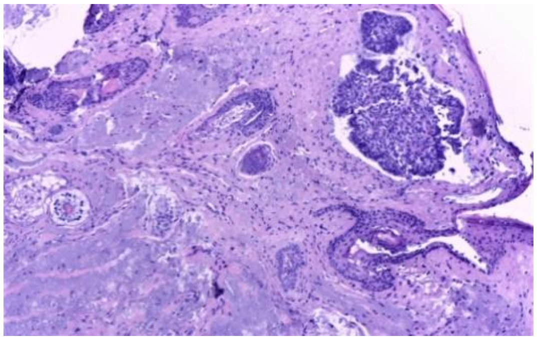

Case no. 4

The 4th case was a surgical challenge; the patient was a 68year old man, from rural environment, with an ulcerated giant tumor (Figure 19) on right nasogenian, malar and lower lid region, with an evolution of approximately 10 years. The dimension reached approximately 7.5/4 cm.

Figure 19: Ulcerated tumor covering the right nasogenian, malar and infraorbital area

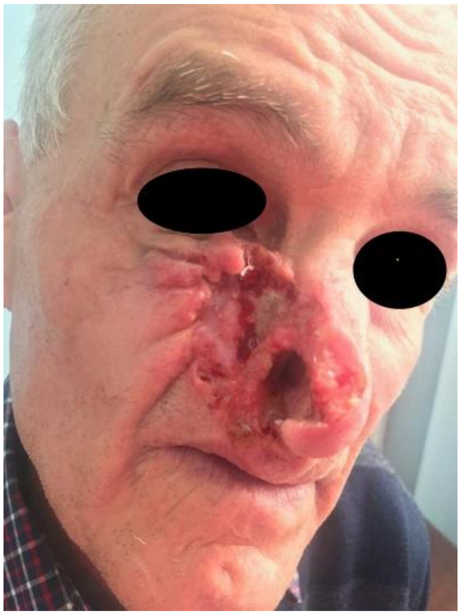
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Preoperatively, a cerebral-cervical-thorax CT scan to evaluate the degree of invasion of tumor and potential disseminations was performed, which revealed no local bone invasion and multiple multiple ganglia images, under 1 cm, at the level of mediastinal, axillar, submandibular and on bilateral carotid space.

The tumor was excised with macroscopically oncological safety margins. The defect remained needed cover with 2 local flaps, one right cervico-facial and one transposed frontal (Figures 20 and 21).

Figure 20: The defect after tumor resection

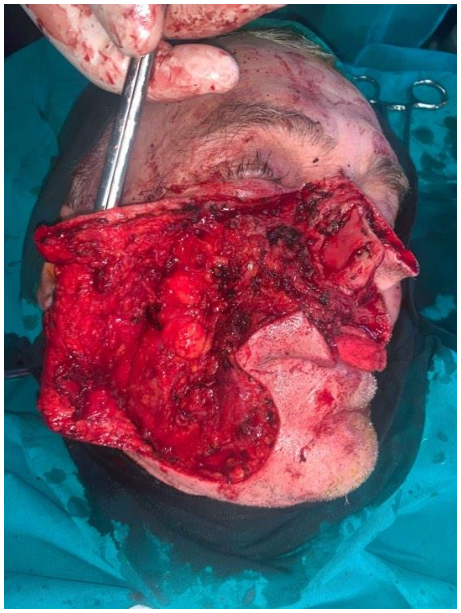

Figure 21: Three months post-surgery

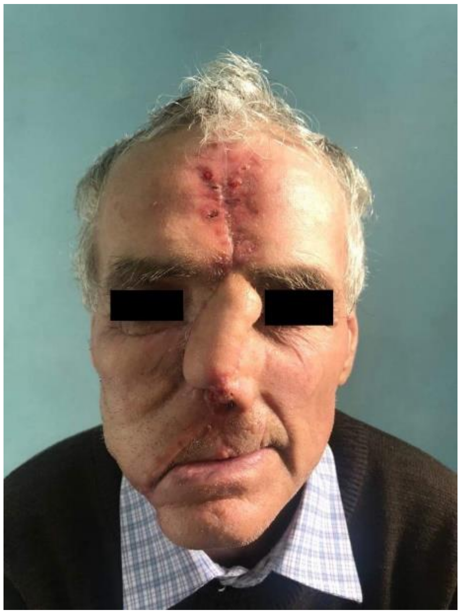

The histopathological exam confirmed the diagnostic of basosquamous carcinoma with a proliferation of intermediate and squamous cells with slightly spindle shape, with eosinophilic cytoplasm, infiltrating the deeper portions of the skin and ulcerating the epidermis (Figures 22 and 23).

The squamous component presented individual keratinization, with vesicular nucleoli and numerous atypical mitoses (Figure 24).

Figure 22: Basosquamous carcinoma (left side of photo) and respirator epithelium (right side of photo), notice the solid proliferation of intermediate cells with slighty spindle shape, with hypercromatic nuclei admixed with vesicular nuclei and eosinophilic cytoplasm, HE, 20x

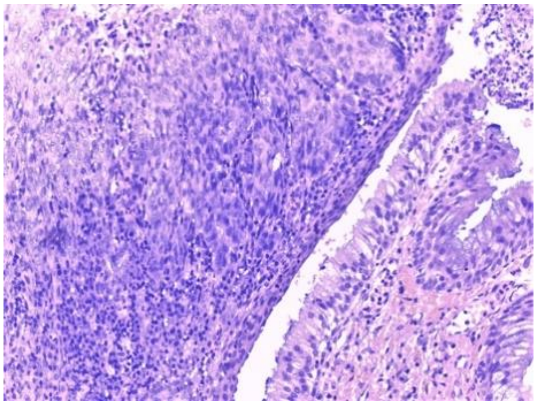

Figure 23: Basosquamous carcinoma (basal cell component) with epidermal ulceration and infiltration of the deeper dermis, HE, 10x

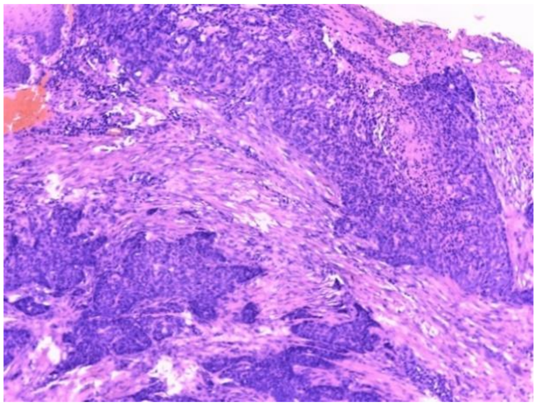

Figure 24: Detail of basosquamous carcinoma (squamous component) with individual keratinizing and several atypia, with pleomorphic vesicular nuclei, HE, 40x

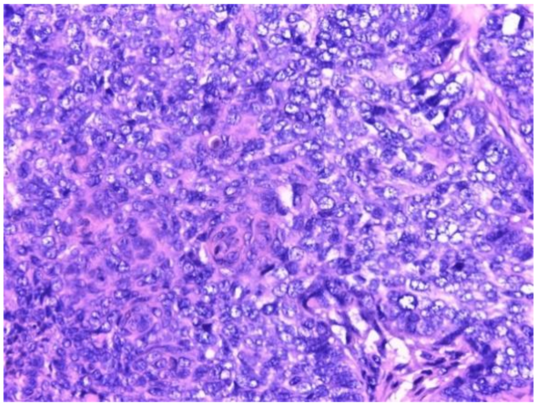

Case no 5

The last case is of a 76 years old woman, with insulin-dependent diabetes mellitus and obesity that presented with an ulcerated tumor (Figure 25) of approximately 2/2cm on the left nasal side.

Figure 25: Erosive tumor on left nasal side

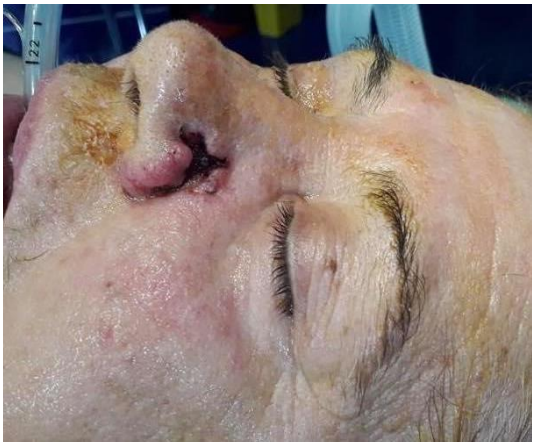

The tumor progressed to a full thickness skin erosion over a 2 years time period. The surgical excision skin defect (Figure 26) was covered with a local flap (Figure 27).

Figure 26: Defect remained after tumor excision

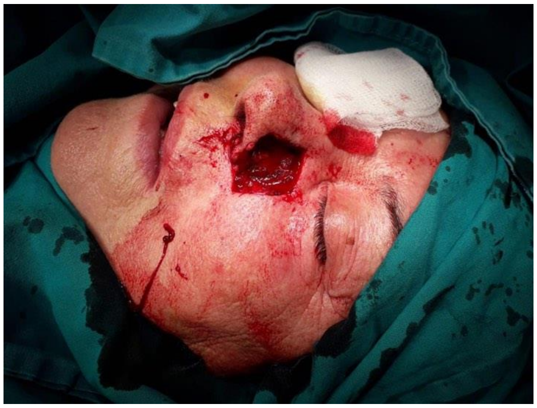

Figure 27: Immediately post-surgery, cover of defect by rotated nasolabial flap

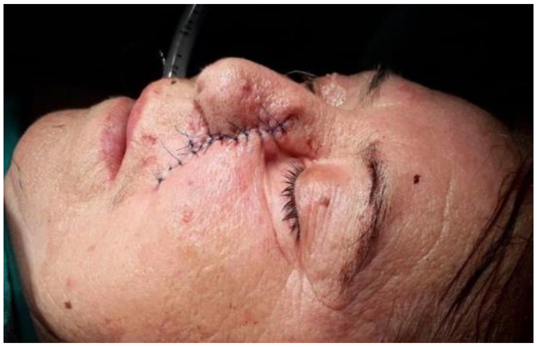

The pathology results under Hematoxilin-eosin examination revealed a malignant proliferation of polygonal squamous cells with polymorphism and eosinophil cytoplasm admixed with islands of basaloid cells and areas of dystrophic microcalcifications (Figures 28, 29 and 30). A diagnosis of completely excised metatypical carcinoma was made.

Figure 28: Carcinoma islands with squamoid aspect, formed of polygonal, pleomorphic cells admixed with basaloid cells, infiltrating the conjunctive tissue, HE, 20x

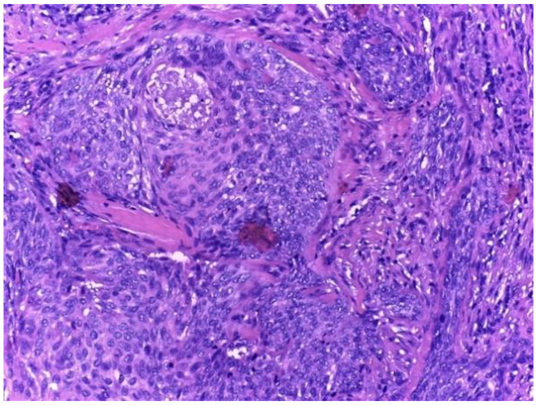

Figure 29: Islands of basal cell carcinoma with palisades and calcification, HE 10x

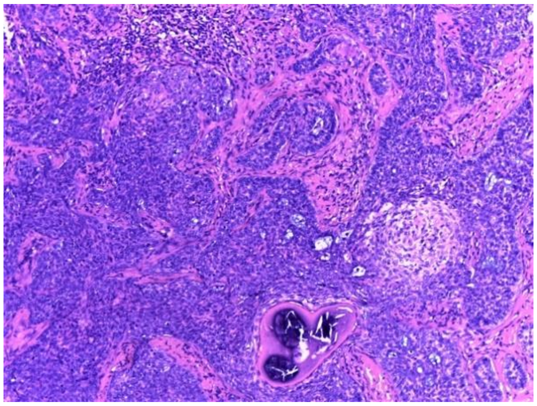

Figure 30: Association between component of squamous (cells with eosinophil cytoplasm, vesiculous nuclei) and basaloid cells (cells with basophile cytoplasm and tachychromatic nucleus) with calcification and incipient keratinizing, HE 10x

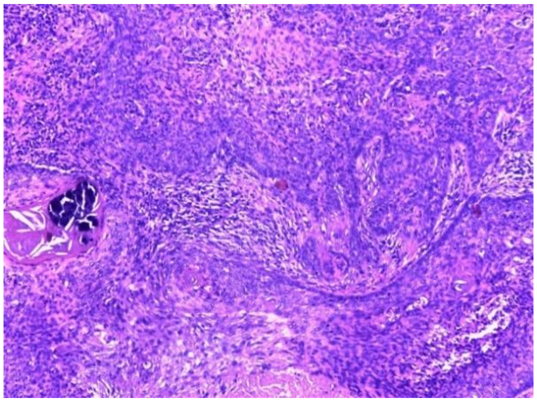

DISCUSSION

Among the skin neoplasms, two of the best known tumors are squamous cell carcinomas and basal cell carcinomas. Although the majority remain locally invasive, there is a risk of local-regional and remote dissemination. Considering the predisposition for basal cell carcinoma to appear on the head and neck in 70-80% of cases, and also the size of tumor, the metastasis rate is appreciated at around 0.0028-0.55% [8,9,10]. Regarding squamous cell carcinoma, it was demonstrated that the tumor size, depth and perineural invasion increased the risk of metastasis. The tumors measuring more than 2 cm in thickness that infiltrated into the deep skin structures or the bone, counted for 75% of the metastases [11]. The current guides target a much more detailed analysis considering the perineural invasion, thickness of tumor and dimensions of free margins of tumors for squamous cell carcinomas. It can be extrapolated that these prognostic factors can be applied to basosquamous carcinomas, especially for those with an important squamous component [12]. In our study, only the patient with perineural invasion was reported to have an increased risk of relapse.

Although few cases of basosquamous carcinoma are reported in literature, it is important to notice that all of the 5 presented cases were identified only in a 2 months' period. Hence, it is expecting to increase the incidence for this subtype of skin cancer.

To some researchers, the basosquamous carcinomas are presented with metatypical basal cells and histopathological characteristics of both basal cell carcinoma and of squamous cell carcinoma, with or without transition area between them. This neoplasm represents a controversial pathology since its first description in 1910, when McCormac reported a range of erosive ulcers classifying them in 3 types of tumors: pure, mixed (side by side basal and squamous cells) and intermediary subtypes. Subsequently, in 1912 Korbl presented a form of transitional skin cancer with a structure of basal typical cell carcinoma but with bigger, pallor cells, rich in cytoplasm and containing intercellular bridges.

The term of metatypical epithelioma was introduced in 1922 by Darier, presenting it as a typical basal cell carcinoma with conglomerates of squamous cells.

In 1928, the notion of epithelioma with basosquamous cells appears, pursuant to a study of Montgomery that reported a transitional area between squamous cell carcinoma and basal cell carcinoma. In 1951, Thackray accepted basosquamous carcinoma as an entity, although he did not define it. Multiple contradictions related to such neoplasms existed along the years. Some researchers considered them as a variant of basal cell carcinomas with keratinizing areas but without prognostic signification [13].

The terminology of such neoplasm still raises controversies since the literature does not clearly defines it. Currently, it's being recognized as being an aggressive form of basal cell carcinoma, with a risk of relapse ranging between 10-48% and a risk of metastasis between 5-7.4% [14].

In opposition, Martin et al. didn`t take in consideration the degree of differentiation and size of initial tumor as factors predisposing to relapse. The positive indicators considered were positive excision margins, lymphatic and perineural [15].

Leibovitch proved in a 178 patients study, that 95% of basosquamous carcinoma were located on head and neck regions having an increased predisposition for the nose (33,1%), auricular region (18,5%) and eye area [16].

It is interesting to notice that similar to the literature's data, all the tumors described in this paper were located on head and neck regions, on sun exposed areas, and affected patients with an average age of 76.6 years. Macroscopically, all tumors had an erosive nature with an aggressive growth, except for the case no. 4 which had an evolution of approximately 10 years. In case no 4, multiple ganglia images (one mediastinal, axillar, submandibular and on bilateral carotid space) were found. The literature presents the risk of metastasis in basosquamous carcinoma higher than that of basal or squamous cell carcinoma, with pulmonary prevalence [17]. Thus, correlating with the literature data, the presence of the adenopathies and the dimension of the tumor are associated with bad diagnostic and risk of relapse. Also, slow evolution of case no 4, during ten years, shows the necessity of studying the squamous component prevalence in the evolution of these tumors, as much as the predisposing factors or precursor lesions.

It is important to mention that some studies show an aggressiveness of basosquamous carcinoma higher even than that of squamous carcinoma [18].

Macroscopically, we notice that such tumors have ulcerative, erosive nature, a feature proven also by Cigna et al based on a retrospective study including 312 patients with metatypical carcinomas [19].

Another aspect that needs to be taken into account is the tumor's location, especially on head, which represents a challenge for both the surgeon and the patient. Many times, wide, meticulous excisions are needed, and several vascular-muscular-nervous important elements are encountered. Therefore, the complex reconstructions could be mutilating to the patient.

Paraclinical, the only common abnormal marker found at the patients included in the study was increased rate of blood sedimentation, which is a nonspecific marker of inflammation. Even if they have macroscopically similar tumor features like erosive and ulcerated characteristics, the evolution was impredictible, from a few months to ten years. This it increases even more the necessity of studying the role of the squamous component percentage in the basosquamous carcinoma evolution, together with the skin response at sun exposure and inflammation.

Although the treatment of basosquamous carcinoma is not optimized, we need a more carefully approach both surgically and imagistically to evaluate the existence of disseminations, as well as oncologically with a very strict follow up. In our study, one of 5 patients received radiotherapy (case no 4).

One year postoperatively none of the patients presented local relapse, confirming that the tumor free-margins are the key for a good management of this pathology.

For a relevant treatment, it is important that the correct diagnostic is made based on histopathological examination. In cases of basosquamous carcinoma, the immunohistochemical determination with EP-CAM and CD10 markers may be useful, basal cell carcinomas being positive and squamous cell carcinomas negative [20]. CXCR4, MMP-13 and b-catenin may also be considered as prognostic markers [21].

The particularities identified based on immunohistochemical reactions rise even more attention on the necessity of treating basosquamous cell carcinoma as a unique entity with an unpredictable evolution and an aggressive pathway. We noted that basosquamous carcinomas may also have overlapping immunohistochemical features found in both basal cell and squamous cell carcinomas.

CONCLUSION

Given the increasing incidence of basosquamous carcinomas and unpredictable evolution, sometimes even more aggressive than squamous cell carcinoma, there is a clear need for an optimized therapeutic protocol and a correct diagnosis based on histopathological and immunohistochemical examinations [22,23]. Treating as a different entity than typical basal and squamous cell carcinomas, understandying the pathology and approaching the role of squamous component in its evolution, should be considered. Resection with tumor- free margins and correct histopathological examination are the key features in the approach of basosquamous carcinoma, backed-up by oncological approach and a rigorous follow up. The development of non-invasive tests concerning modifications, surprising the carcinogenesis in situ, and the education of people regarding sun exposure and skin cancer, are also two important steps to decrease the incidence of skin carcinomas.

Acknowledgment

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ORIGINAL ARTICLE

Management of hepatitis C virus (HCV) infection: an update

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Abstract: *Million people worldwide are affected by the hepatitis C virus (HCV). The highest incidence of illness was between 1945 and 1975. It was also estimated that 70% of those people were not tested for the disease. Most recent treatment concepts are safe, highly effective and have a vital public health influence by achieving a viral constant response in a significant proportion of treated patients. It helps reduce liver fibrosis, liver cancer risk and dissemination. With its increased population incidence, HCV becomes a serious public health problem. This review discusses the current literature in this field in terms of the importance of screening of HCV, follow-up, treatment and includes considerations in specific populations such as patients with cirrhosis, with HIV/HCV co-infection, patients with HBV/HCV co-infection and with renal damage.*

Keywords: *hepatitis C virus, Direct-acting antiviral therapy, co-infection*

INTRODUCTION

The primary cause of final-stage liver disease in the Western world is the Hepatitis C virus (HCV). Notably, HCV is the only chronic viral infection in humans that can be healed [1,2]. The consequences of chronic infection (HCV) include cirrhosis (compensated and decompensated), portal hypertension, hepatocellular carcinoma and eventually liver-related death.

Fibrosis progression may vary due to multiple factors such as age, hepatitis B, alcohol consumption or human immunodeficiency virus (HIV) co-infection [2].

After cirrhosis is established, a yearly incidence of hepatic decompensation such as jaundice, episodes of ascites, variceal bleeding and encephalopathy of 3.9-5.7% and a yearly incidence of hepatocellular carcinoma of 1.4-3.4% has been reported [2,3].

The percentage of seropositive antibodies to HCV increased from 2,3 to 2,8 % between 1990 and 2005 [3] and the disease complications are increasing due to the ageing population and the percentage of liver fibrosis, which in time leads to hepatic insufficiency risk [4].

The key to the long-term benefits in HCV infection is to arrive at constant serologic feedback which is the lack of viremia 12 weeks after the end of the therapy [5].

This sustained viral response (SVR) decreases the prevalence of hepatocellular carcinoma, morbidity related to the risk of hepatic insufficiency and mortality of infected individuals [6-8].

HCV INITIAL TESTING AND FOLLOW-UP

Diagnosis of chronic C virus infection is done by testing anti-HCV antibodies. HCV RNA polymerase chain reaction is only required if the anti-HCV is positive and it confirms the diagnosis of chronic C virus infection [9,10].

There is a group of people (about 15-30%) who have a positive anti-HCV test and negative RNA HCV polymerase chain reaction [11] test. Those are people who can naturally clear out the infection. Spontaneous removal of the C virus is particularly rare after 6 months in which the person has acquired chronic infection [11].

People born during 1945-1975 [12,13], should perform

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screening for Hepatitis C annually. From 2020 universal HCV screening is recommended for all adults aged 18.

Annual HCV testing is required for all individuals injecting drugs, pregnant women, people with an immunodeficiency [14], persons with various pathologies, individuals with ongoing risk behaviours or exposures and persons who have unprotected intercourse with men [15,16] according to the American Centers for Disease Control and Prevention (CDC) and the American liver disease Survey Association (AASLD).

People with known or suspicious cirrhosis have an increased risk of liver complications and need to be frequently followed. Individuals who are immunodepressive (e.g.: chronic hemodialysis) [17] or who may have been exposed to HCV in the last 6 months and have not developed antibodies should be tested for HCV RNA even if they have had negative anti-HCV testing.

CLINICAL CARE FOR PERSONS WITH ACTIVE HCV INFECTION

Once diagnosed with active HCV infection, patients need counselling and specific clinical assistance before the initiation of antiviral therapy.

The most important thing is the prevention of further liver damage [18]. First of all, counselling patients and sustaining them not to consume alcohol, because there is a tight connexion between alcohol use, progressive fibrosis and the development of hepatocellular carcinoma (HCC) [19,20]. Moreover, people have to be trained on how to prevent the transmission of this infection.

This training can greatly reduce the hepatitis C rate. Some of the rules are: to avoid the sharing of different tools (toothbrushes or razor blades) and to avoid the possibility of others coming into contact with the blood of an infected person [18-20].

Assessment of hepatic fibrosis, hepatitis B virus [20], immunodeficiency and existing pathologies is required to determine the treatment strategy for HCV infection.

Every person with HCV should get vaccinated against hepatitis A and B and for persons with cirrhosis, vaccination against pneumococcal infection is recommended.

DIRECT-ACTING ANTIVIRAL (DAA) THERAPY

The physical exam conducted on the liver, routine blood tests (e.g. complete hemogram, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), albumin, bilirubin), elastography indicating cirrhosis (Fibroscan), liver imaging (ultrasound or CT), can indicate the severity of liver damage and aid for a previous assessment of the treatment scheme to follow for hepatitis C infection [21].

Recommended pretreatment laboratory tests are pursued to confirm chronic HCV infection and to rule out decompensated liver disease, HBV, HIV co-infection and renal disease, and/or current pregnancy previous therapy initiation, considering that they need more subtle care [22].

During antiviral therapy, it is recommended to inform patients about potential drug-drug interactions and to monitor adverse events. It is important to schedule some clinic visits or to have telephone contact to check medication compliance [23].

Patients suffering from diabetes can have a hypoglycaemia drop and blood glucose monitoring is recommended.

Patients undergoing anticoagulant treatment (warfarin) should be carefully monitored because they may experience changes in the anticoagulation status [24,25].

Clinical trials have proved the safety [26] of strong therapeutic success of Glecaprevir (300mg)/Pibrentasvir (120 mg) [27] taken for 8 weeks and Sofosbuvir (400mg)/ Velpatasvir (100mg) [28] for 12 weeks for treatment persons without cirrhosis no matter of HCV genotype.

To evaluate therapy response, HCV RNA and hepatic aminotransferase testing is counselled 12 or more weeks after finishing DAA treatment. Undetectable HCV RNA represents virologic cure and sustained response to treatment.

Numerous benefits for the health and general condition of the patient, including improved AST levels and reduced inflammation with decreased progressivity of liver fibrosis [29].

Patients healed by HCV, those with a sustained viral response (SVR), show a decrease of up to 70% of the risk of HCC development [30,31]. In the absence of cirrhosis, individuals with SVR do not demand liver-specific monitoring, but for those with constant HCV risk factors or an increase of hepatic aminotransferase, counselling and HCV RNA testing once a year is recommended [32].

Between 1% and 7% is the annual rate of hepatocellular carcinoma in patients with HCV cirrhosis, and for this reason, is recommended an ultrasound exam and periodic upper endoscopic monitoring.

The most frequent HCV genotype from all 7 genotypes is Genotype-1 [33]. In Europe and Central America Genotype-1, b is the most common HCV subtype [33].

Patients should be handled by clinicians with expertise in managing HCV therapy failure. Persons with persistent elevated hepatic aminotransferase levels and achieved SVR demand reevaluation for other causes of liver damage [34,35].

Assessment for liver damage progression every 6-12 months is counselled for patients with no treatment response. Ultrasound monitoring for HCC and alpha-fetoprotein [36] every 6 months is recommended for persons with cirrhosis. AASLD guidance on portal hypertensive bleeding in cirrhosis recommends upper endoscopic monitoring for oesophageal varices [37].

HCV AND COMORBIDITIES

Liver lesions can progress faster in the case of HCV-HIV coinfection [38,39]. Careful evaluation and observation of potential interactions between medicines are required.

Guidelines recommended the same therapy in the treatment of HCV mono-infection and HCV-HIV co-infection [39]. For patients with genotype 1a and 1b, with no cirrhosis, therapy for patients with mono HCV infection is with ledipasvir/sofosbuvir for 8 weeks, and 12 weeks in patients with co-infection [40].

For genotype 1a, 1b, 2, 3 and 4 individuals with compensated cirrhosis, AASLD-IDSA HCV proposes glecaprevir/ pibrentasvir for 8 weeks in persons with HCV and 4 weeks more in patients with co-infection [40]. And for genotypes 5 and 6, with or without compensated cirrhosis, glecaprevir-pibrentasvir for 8 weeks in patients with HCV infection and with 4 weeks more for those with co-infection, is recommended [40].

The particular examination should be given by controlling and managing HIV antiretroviral and HCV DAA drug interaction.

Measurements of serum creatinine and creatinine clearance are included in the patient assessment with HCV before treatment, and the stage of renal disease may be determined based on these values.

The normal value of GFR is 130 and 120 mL/min/1.73 m^2, and kidney failure is defined by a GFR less than 15mL/min/1.73 m^2. HCV is associated with the rapid development of kidney damage and renal carcinoma [41]. Hemodialysis is considered to be a risk factor for acquiring HCV infection [42].

The selection of DAAs should be carefully considered and based on patient characteristics, concurrent drugs and HCV genotype [43]. For all six major HCV genotypes, glecaprevir-pibrentasvir have shown to be effective and safe in persons with kidney chronic disease and HCV infection.

Sofosbuvir-based therapy does not have the safety and efficacy data to support its use in patients with chronic kidney disease [44].

Co-infection HBV-HCV has an approximate worldwide incidence of 1-15% [45]. In some cases of co-infection, there may be various effects on the progression of the disease, clinical manifestations and patient supervision, so the liver profile should be observed together with the level of transaminases [46,47].

The anti-viral activity against VHB and HCV is the Peg IFN-based regime. Its efficiency increases when combined with ribavirin: 35% efficiency in HBV and 50–60% efficiency in HCV. This combined therapy with ribavirin reduces the risk of HCC development by 34% [48,49].

In patients with chronic kidney disease and chronic C-virus infection, the DAA treatment works well, the sustained rate of virological response is substantially increasing [50-51].

CONCLUSIONS

The rate of viral eradication and the increase in the tolerance of adverse reactions have been sustained by the emergence of DAA agents, which have counted heavily in the treatment of patients with HCV, co-infections with HBV, HIV and CKD. Interrelations between drugs should be carefully checked before delivery of antiviral therapy.

The anamnesis should contain questions about the medical history of the patient (surgical interventions, diabetes, infections), other factors that may accelerate chronic HCV infection (alcohol, obesity), transmission risks and the approximate duration of infection, signs and symptoms of disease or other extrahepatic manifestations.

The treatments aim to achieve a sustained viral response and to keep disease manifestations under control. For each genotype of HCV, there are multiple therapies approved with quite high effectiveness.

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ORIGINAL ARTICLE

Costs for a hospital stay: another lesson learned from the COVID-19 pandemic

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Abstract: *Background and aim: After two years of pandemic, planning and budgeting for use of healthcare resources and services is very important. Inpatient COVID-19 hospitalizations costs, regardless of ICD-10 procedure codes, in a Covid-19 support military hospital were analyzed. Methods: The national protocol for the treatment of Covid-19 infection was applied. The costs for laboratory tests, drugs, protection equipment and radiological investigations (imaging techniques such as computed-tomography or radiography), hospitalization days and food were assessed. Results: In our hospital, from August 2020 through June 2021, 241 patients were hospitalized with COVID-19: mean age 59.92±7.8 years, 46% men, 26% military personnel, 11.57±3 days of hospitalization; two third of patients had moderate and severe forms of COVID-19. The main manifestations were: 69% respiratory (18% with severe pneumonia), 3.3% cardiac (2.9% with pulmonary embolism, diagnosed by computed tomography angiography), 28% digestive and 33% psychiatric (most commonly anxiety). The average estimated costs were about 3000€/patient, without significant differences based on disease severity. Equipment costs were 2 times higher than for drugs and 3 times than for laboratory tests. Conclusions: In a Covid-19 support military hospital that cared for patients with predominantly moderate forms of COVID-19, the costs for equipment were much higher than those for treatment. New criteria for hospitalization of these forms of COVID-19 deserve to be analyzed in order to avoid useless costs.*

Keywords: *COVID-19, pandemic, hospital stay, costs*

INTRODUCTION

SARS-CoV-2 is the most deadly and devastating virus of the last one hundred years, being more contagious than the Ebola virus, HIV, Swine Influenza virus (H1N1, H1N2), Severe Acute Respiratory Syndrome (SARS) or Middle Eastern Respiratory Syndrome (MERS) [1]. COVID-19 is a systemic disease determined by SARS-CoV-2, with a variable degree of severity and progression [2]. The evolution of this infection is mostly as pulmonary disease. Depending on the host's immune response, it may present as a severe form with multiorgan dysfunction, especially if the patient has comorbidities. COVID-19 can have cardiac, renal, digestive or cerebral manifestations. Therefore, it implies significant costs for diagnosis, drugs, hospitalization, protection equipment, etc.

Profiles of COVID -19 patients have been studied in various ways. Besides determining risk factors, influencing treatment protocols, setting priorities for vaccination, or inspiring further research, planning and budgeting for use of healthcare resources and services become very important given the duration of the pandemic. According to health policy researchers, many insurers claim that it is justified to charge patients for COVID-19 hospitalizations now that COVID-19 vaccines are widely available. For example, the hospitalization of a person with private insurance amounts to a total of $42.200 on average, whereas each hospitalization of a person with COVID-19 who has Medicare Advantage coverage averaged about $21.400 [3]. In comparison, the average cost for influenza hospitalization is around $1.000 [4]. Therefore, we emphasize that COVID-19 prevention is better than treating. Nowadays, vaccines targeting SARS-CoV2 are highly effective at preventing the severe forms of the disease, hospitalizations, and deaths

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from COVID-19 [5,6]. Despite the wide availability of safe and effective vaccines, vaccination rates have lagged in Romania.

The aim of this study was to analyze the costs of hospital stays in patients with predominantly moderate forms of COVID-19 in a small size military hospital located in Northeast Romania.

METHODS

This study included all patients hospitalized between August 2020 and June 2021 in a military hospital. Inpatient COVID-19 hospitalizations costs, regardless of ICD-10 procedure codes (U07.1), were retrospectively analyzed. The hospital's statistician offered all data about costs. We assessed hospital costs per patient, per severity degree of COVID-19 and per health service type.

Inclusion and exclusion criteria

According to National Center for Surveillance and Control of Infectious Diseases (CNSCBT) the definition of a confirmed COVID-19 case is: a person with laboratory confirmation of SARS-CoV-2 infection (by RT-PCR SARS-CoV-2), regardless of clinical signs and symptoms [7].

All patients with a positive RT-PCR test without complete vaccination were included in the study. Patients in the first 14 days after the first dose of anti-SARS-CoV-2 vaccine were not excluded. Patients with positive COVID-19 rapid tests were tested by RT-PCR. In accordance with the hospitalization protocol instituted after October 15th 2020, asymptomatic patients were no longer hospitalized and were managed in an ambulatory manner.

The local hospital ethics committee approved the study. All patients enrolled in the study gave their written informed consent.

All patients underwent a specific protocol for COVID-19 diagnosis and treatment, adapted by our hospital according to the national protocol.

Diagnosis protocol

Diagnosis protocol included the following exams:

1. RT-PCR for SARS-CoV-2

2. Monitoring of vital functions (temperature, blood pressure, heart rate, respiratory rate, oxygen saturation, diuresis, defecation)

3. Blood tests: complete blood cell count, C-reactive protein, urea, creatinine, blood electrolytes, ALT, AST, GGT, blood glucose, CK-MB, troponin, D-dimers, ferritin, other blood tests depending on comorbidities (presepsin, procalcitonin, TSH, fT4, etc.).

4. Urinary sediment analysis

5. Computed tomography (CT) examination (with or without contrast agent)

6. Electrocardiogram (the presence of sinus rhythm or new bundle branch block, maximum PR and QTc interval)

7. Sputum examination (if necessary)

8. Coproculture, Clostridium difficile toxins (if necessary)

9. Echocardiography (in case of suspected cardiac disease or complication)

10. Arterial gases (in case of impaired respiratory function)

Patient monitoring was done clinically and biologically, with daily biochemistry in patients with moderate or severe forms. Repeated imaging exams and biological tests were performed urgently in case of clinical worsening.

Radiological parameters used for the study were parenchymal involvement scores, CT score and CORADS score (COVID-19 imaging Reporting And Data System). Chest CT findings reported the COVID-19 imaging using CORADS score that divides the CT findings into six categories [8]. It ranges from CORADS category 1 with very low level of suspicion to CORADS category 5 with very high level of suspicion. If none of the five categories can be established due to incomplete or insufficient images, it is considered CORADS category 0. CORADS category 6 indicates proven COVID-19 by positive RT-PCR test results [8].

In addition, a semi-quantitative scoring system for estimating parenchymal abnormality based on the area involved has been used.

Each one of the five lung lobes was individually evaluated and scored based on parenchymal involvement on a scale ranging from 0 to 5, with 0 indicating no involvement, 1 for less than 5% involvement, 2 for 5–25% involvement, 3 for 26–49% involvement, 4 attributed to 50–75% involvement, and 5 indicating more than 75% parenchymal involvement. The total CT score was the sum of the individual lobar scores, which ranged from 0 (no involvement) to 25 (the maximum possible involvement) [9].

Patients were diagnosed with asymptomatic, mild, moderate, severe or critical COVID-19. Most patients with severe forms of the disease who evolved into critical condition with at least one of the following: acute respiratory distress, sepsis, impaired consciousness or multisystem organ failure, were transferred in hospitals with COVID-19 intensive care units.

Treatment protocol

Patients were treated according to the national protocol published by CNSCTB [7]. Asymptomatic cases were hospitalized for medical supervision and were symptomatically treated as mild COVID-19. Moderate forms of the disease, defined as no oxygen requirement, have been treated with antivirals (lopinavir/ritonavir or umifenovir, remdesivir), antibiotics and symptomatically (paracetamol, C vitamin, D vitamin, antitussive drugs). All severe COVID-19 forms required oxygen therapy; they had at least one of the following parameters: respiratory rate ≥ 30/min; $SaO_2 \leq 93\%$; PaO_2/FiO_2 <300; pulmonary infiltrates that increased by more than 50% in 24-48 hours. Beside oxygen therapy, they received corticotherapy (dexamethasone), anticoagulation (enoxaparine or fraxiparine in prophylactic or therapeutic doses), antivirals, immunomodulatory drugs (tocilizumab).

The duration of treatment was decided by the physician and was prolonged or shortened according to the patient's clinical evolution, but no case has received medical treatment for less than 10 days (surveying for severe side effects or Covid-19 complications).

Personal protective equipment

All medical personnel used personal protective equipment against SARS-CoV2 and complied with WHO recommendations [10]. Besides medical facemasks, face shields, protective glasses, two types of gloves (examination and surgical) and gowns or aprons, disinfectants and other consumables were used to comply with the rules of aseptic and antiseptic environment.

The study was conducted in accordance to the Declaration of Helsinki and approved by the Ethics Committee of Hospital (protocol code 2021-01).

Statistical analysis

Continuous variables were presented as median and categorical as absolute values and percentages. Normality was verified using the Shapiro-Wilk test. The comparisons of groups were based on a student's two-sample t-tests. P-value ≤ 0.05 was considered statistically significant for all tests. Statistical analysis was performed using SPSS 10 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

In the specified time interval, 241 patients were hospitalized with COVID-19.

Patient population

In our study population, 46% were men and mean age was 59.92±7.8 years. Twenty six percent of patients were military personnel and 74% were civilians. The main COVID-19 manifestations were: 69% respiratory (18% with severe pneumonia, 2.9% with pulmonary embolism, diagnosed by angio-computed tomography), 3.3% cardiac, 28% digestive and 33% psychiatric (most commonly anxiety). Only two patients had a procalcitonin level >10 ng/mL (high probability of severe sepsis or septic shock) and 1 patient had moderate risk for septic shock (0.5-2ng/mL). Mean clinical, biological, electrocardiographic and radiological characteristics of patients included in the study were presented in Table 1.

Table 1: Mean clinical, biological, electrocardiographic and radiological characteristics of patients included in the study.

Characteristics	Value (n=241) n (%) or mean ± SD
CLINICAL	
Blood pressure (mm Hg)	130±21/78±19
Heart rate (/min)	82±21
SaO2 (mmHg)	93±17
Smoker (%)	11 (0.045)
BMI (kg/m^2)	32.9±17
Comorbidities	
Hypertension (%)	124 (51.4)
Diabetes mellitus (%)	53 (22)
Ischemic coronary disease (%)	82 (34)
Obesity (%)	133 (55)
Sleep apnea (%)	14 (0.06)
Neoplasia (%)	24 (0.1)
COPD (%)	17 (0.07)
BIOLOGICAL	
Leucocyte ($/mm^3$)	12750±7800
Lymphocyte ($/mm^3$)	3720±1230
Hemoglobin (g/dL)	14±2.7
Platelets ($/mm^3$)	163.000±78.000
D-dimers	613±430
CRP (mg/dL)	37±27
Ferritin (μg/L)	671±340
AST (UI/L)	35±4.8
ALT (UI/L)	65.5±17.8
Urea (mg/dL)	56±43
Creatinine (mg/dL)	0.9±0.8
Potassium (mEq/L)	3.7±0.7
CK-MB (UI/L)	18.4±7.6
Procalcitonin	0.4±0.03
ELECTROCARDIOGRAPHIC	
SR (%)	199 (82)
QTc max (ms)	431±43
PR max (ms)	165±34
RADIOLOGICAL	
Parenchymal involvement scored	3.4
CT score	14/25
CORADS score	5.6

ALT: alanine amino transferase; AST: aspartate amino transferase; BMI: body mass index; CK-MB: creatin kinase MB; CORADS: COVID-19 imaging Reporting And Data System; CRP: C-reactive protein; CT: computed-tomography; O2: oxygen; PR: interval PR; SR: sinus rhythm

According to COVID-19 severity (Table 2), most patients had moderate (104 patients – 43%) and severe (50 patients - 21%) forms. Seven patients with severe forms died because of multiple comorbidities and 30 patients were transferred to hospitals with COVID-19 intensive care units.

Table 2: Distribution of COVID-19 according to disease severity.

COVID-19 severity	Number of patients (%)
Asymptomatic	14 (6%)
Mild	73 (30%)
Moderate	104 (43%)
Severe	50 (21%)
Total	241

Hospitalization costs

Mean hospital stay was 11.57±3 days. In each day of hospitalization, at least 10 healthcare personnel members (4 nurses, 4 carers and 2 physicians) used protective equipment. Eight patients (3.3%) received Remdesivir and 2 patients (0,8%) Tocilizumab. In the first three months of the study, Plaquenil and Kaletra were not available in the hospital's pharmacy, therefore only 23 (9.5%) patients received these drugs. Antibiotics such as ceftriaxone were indicated in 152 (63%) patients. Dexamethasone was used in 190 (78.8%) patients. Prophylactic doses of low molecular weight heparin were administered in 220 (91.3%) patients and therapeutic doses in 7 (2.9%) patients. Asymptomatic patients did not receive anticoagulants.

The mean costs for patient according to disease severity are shown in Table 3, the average estimated costs were about 3000€/patient, without significantly differences depending on disease severity.

Table 3: The mean costs per patient according to disease severity.

COVID-19 severity	Total costs (€)	Mean costs/pts (€)
Asymptomatic	23410,65	1672,19
Mild	220949,62	3026,71
Moderate	318966,92	3066,99
Severe	148669,86	2973,39
Total	711997,05	10739,28

The costs for hospitalization days, food, laboratory tests, drugs, protection equipment and radiological investigations (imaging techniques such as computed-tomography or radiography) are presented in Figure 1.

Protection equipment costs were 2 times higher than for drugs and 3 times than those for laboratory tests (p<0.0001). However, the sum of hospitalization and protective equipment costs was almost nine times greater than medication costs (2436.76 € versus 604.12 €; p<0.0001).

Figure 1: Mean costs per patient according to each type of health services.

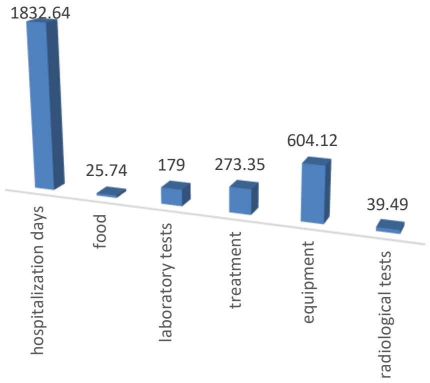

DISCUSSION

Two years after SARS-CoV-2 emerged, the coronavirus continues to affect every aspect of society, one of the major economic effects of the virus being experienced by the health care sector, with COVID-19 changing the economics of health care [11].

The results of our work are a summary of the hospitalization stay costs in patients with COVID-19, in a support hospital from Romania, before the full vaccination scheme was completed.

In August 2020 our hospital was designated as support unit in the battle with COVID. In accordance with this status and due to the fact that we did not have an intensive care unit available for COVID patients, predominantly moderate cases of COVID-19 were hospitalized. A number of patients had an unpredictable evolution to a severe form, some of them presenting exitus from cardiorespiratory arrest while awaiting transfer to an intensive care unit, given the high occupation rate of intensive care units across the country. However, a significant part of our patients (12.4%) was successfully transferred to an intensive care unit dedicated to COVID-19 patients. This explains why there were no differences in costs between moderate and severe forms. In intensive care units the mean costs for invasively ventilated patients in this first part of pandemic was about €10.000 (according to unpublished financial data from these hospitals in Romania).

Medical costs associated with COVID-19 have long been overlooked in previous reports and this is one of the few published studies estimating the costs associated with caring for patients with COVID-19 during hospitalization. In the first part of the SARS-CoV2 pandemic, even patients with asymptomatic forms of the disease were hospitalized. In this study, the cost for hospitalization for this type of patients were two times lower compared to that for symptomatic disease. However, one explanation for this fact could be the rising prices and high costs of personal protective equipment.

Real-time data on the costs of all COVID-19 hospitalizations are far from known. In the US, an average hospitalization cost is around $20.000 [11]. Data published by the Centers for Medicare and Medicaid Services showed that Medicare fee-for-service COVID-19 hospitalizations costs range from $21.752 to $24.033 [11]. Similarly, a FAIR Health analysis of private claims data including employer and private Medicare Advantage plans found that median in-network COVID-19 hospitalization costs ranged from $17.094 for people over the age of 70 to $24.012 for people in their 50s. However, the costs for pneumonia hospitalizations with complications averaged $20.292, but the costs for hospitalizations requiring a ventilator are much higher [12].

According to published data [12], in the US COVID-19 outpatient treatment costs can average $500-$1.000 per patient (average patient age: 39 years). Average treatment costs for patients treated in hospitals compared to patients managed in an outpatient setting for COVID-19 (average patient age: 54 years) was 45 times higher. In addition to this, for the same mean age, average treatment costs for patients treated in intensive care units without the need for ventilation, compared to those whose cases were managed in a medical ward were 2.5 times higher [13]. To our knowledge, costs for hospital stay have not been analyzed by category (personal protective equipment versus drugs, blood test, etc) in literature until now.

In our country vaccination started at the beginning of 2021, therefore the majority of patients were unvaccinated until June 2021. In the US, from June through November 2021, preventable COVID-19 hospitalizations among unvaccinated adults cost over $13 Billion [12]. Therefore, over 690.000 COVID-19 hospitalizations could have been prevented by vaccination between June to November 2021; this is a lesson that must be learned in order to cut down the hospitalization stay costs [12].

In many countries, the impact of COVID-19 on the public health budget was significant. For example, in Spain it comprised 12.3% of total public health expenditure, greater than multiple sclerosis, cancer and diabetes costs [14]. The mean cost per patient was significantly greater than in our study (€10.744). The main costs were staffing costs: for physicians (10.131 to 11.357 €/patient) and for nurses (10.274 to 11.215 €/patient) [14].

A multivariate model was developed to predict the length of hospital stay [15]. The prediction model included five variables: procalcitonin, heart rate, epidemiological history, lymphocyte count and cough [15]. The results showed that the overall length of stay was 11 days (5–15 days) and the median cost for a hospital stay was 7.388.19 RMB (Renminbi) (ranging between 5.085.39–11.145.44) or €1.024.77. Data about hospitalization duration published by Carrera-Hueso et al. [14] and Hong et al. [15] are similar to those in our study, but hospitalization costs were lower, probably due to differences in health service policies.

Another smaller retrospective study (70 patients), which assessed the cost factors and which was conducted at the very beginning of the COVID-19 pandemic, found that the cost for drug acquisition (that accounted for 45.1% of the overall cost) was the major contributor to the medical expenditure. The risk factors for higher costs were pre-existing diseases and severity of COVID-19 [16]. Comparing with this study, at that time, personal protective equipment was not an issue. However, clinical management of COVID-19 patients incurred a great financial burden to many national health systems. In addition, the treatment recommendations changed during this pandemic, each country having an agreed protocol.

Another study that used a Monte Carlo simulation model to estimate resource use and direct medical costs per symptomatic infection at national level found that a single symptomatic COVID-19 case could incur a median direct medical cost of $3.045 during the course of the infection alone [17]. However, a strong limitation of this study is the fact that all models, by definition, are simplifications of real life and cannot account for every possible outcome [18].

In Spain, the European country most economically affected by the pandemic, the gross domestic product cost accounted for 94.7% of the total cost of the COVID-19 and direct health-care costs were 2.14% [19]. This study was designed to analyze the cost-effectiveness of the test-tracking-quarantine strategy, which was the dominant strategy in Spain. Lopez-Valcárcel et al. [19] showed that for each € spent on it, 7 € would be saved in terms of health-care resources. Therefore, this is another important cost-effectiveness strategy in COVID-19 pandemic, similar to those used in an economic evaluation of health-care technologies [20].

Many countries made great efforts to support health-financing flows [21]. A patient with COVID-19 can have a higher probability of hospitalization and mortality than those for other common diseases, therefore hospitalization costs are higher [22-24]. However, before starting this study, on June 18th 2020, the number of confirmed cases across the world had surpassed 8 million. The European region accounts for 31% of cases and 43% of deaths globally [25].

There are some lessons to be learned from the COVID-19 pandemic about improving hospital care and healthcare delivery, both in day-to-day circumstances and future emergencies [26]. One of them is that many countries have learned of the disadvantages of no longer manufacturing certain medical equipment themselves. In addition to that, the reimbursement system of public hospitals seems not to reflect the real COVID-19 costs because the accounting systems of European countries vary widely by the share of reimbursement through DRG (diagnosis-related group based on ICD-10 procedure codes) payment and a reliable accounting system for calculating the actual expenditure per COVID-19 patient (based on actual costs and hospitalization data) is needed [27,28]. Given these issues, it is difficult to compare the costs for hospital stay in patients with COVID-19.

In conclusion, after the COVID-19 pandemic reduced global economic growth by an estimated 3.2% in 2020, with trade declining by 5.3% [29], hospital stay costs might be another lesson, especially given the fact that in Europe 90% of cases recovered in an ambulatory setting [30].

Study limitations

This study included all consecutive patients hospitalized in a single COVID-19 support hospital. In Romania, during the pandemic, hospitals were classified in units treating only COVID-19 cases, COVID-19 support hospitals and non-COVID-19 hospitals. Our hospital was a COVID-19 support unit without endowment of a COVID-19 dedicated intensive care unit. Even if patients were included consecutively, data from this study were analyzed retrospectively. The treatment of COVID-19 patients from this study was, mainly, symptomatic and supportive. These descriptive data provided some practice pattern in the treatment of COVID-19 for the first part of the pandemic.

CONCLUSIONS

In a small-size military hospital designated as COVID-19 support unit that cared for predominantly moderate forms of COVID-19, the costs for equipment were much higher than the treatment costs. Therefore, new criteria for hospitalization of these forms of COVID-19 deserve to be analyzed in order to avoid useless costs. Test-tracking-quarantine or other solutions like vaccination are very important to avoid important future health-care expenditure.

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ORIGINAL ARTICLE

Designing and evaluating questionnaire for assessment abuse in patients with spinal cord injury during Covid-19 Disease

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Abstract: *Background: One of the chronic diseases that causes various challenges for patients is spinal cord injury. On the other hand, apart from the problems that patients and caregivers of patients with SCI experience, the spread and prevalence of Covid-19 disease has been effective as an effective risk factor for abuse.*

Objectives: the aim of the present study is to determine the abuse prevalence and its effective risk factors among SCI patients in Ilam City during the Covid-19 outbreak.

Methods: This cross-sectional descriptive study, population included all SCI patients in Ilam, included in the present study. Moreover, researcher-made forms and questionnaires whose validity and reliability were confirmed, including the demographic characteristics form, perceived abuse researcher-made questionnaire for SCI patients, were used. The perceived abuse questionnaire for SCI patients was a researcher-made questionnaire that was designed using library studies, interviewing patients and determining abuse instances. This instrument consists of 20 questions that are answered using Yes (score 1) and No (score 0). After data collection, statistical analyzes were performed. Data analyzed with spss16 software.

Results: According to the results, the mean ± SD of the perceived abuse score by caregivers and patients was 8.48 ± 2.31 and 42.45% during the Covid-19 pandemic, respectively. Also, the mean ± SD of patients' age was 62.86 ±19.15 and the patient perceived abuse increased with increasing age (P = 0.000, F = 27.42). Also, the possible abuse score was 0 to 20 that was divided into three categories: low (20, 20.4%), moderate (76, 76.5%) and high (2, 2%). also, significantly higher perceived abuse score among women and patients with a history of more than 10 years of SCI, but there was no significant relationship between abuse prevalence with income and marital status (P>0.05). With regard to age and abuse, results also showed an increase in perceived abuse score with increasing age.

Conclusions: necessary to take the necessary measures and preventions in this regard. Also, the prevalence of Covide-19-related abuse in patients was hight, which is statistically important and significant. For this reason, while proposing preventive action in this field.

Keywords: *abuse, spinal cord injury, Covid-19*

BACKGROUND

Abuse is defined as doing, not doing, or quitting an activity by health care providers that may have negative consequences for the patient [1]. Abuse includes physical, sexual, psychological or emotional abuse, neglect, abandonment and financial exploitation [2]. The change in family role, the life stresses, crises caused by accidents and diseases have weakened the social bases of the family and have led to the abuse by family members [3]. Abuse is one of the cases of deprivation of human rights and dignity that occurs in various forms such as deprivation of individual authority, rejection, financial exploitation, psychological and physical harassment. In fact, family members intentionally or unintentionally neglect the person by neglecting, emotional and safety care, a dignified life, and leaving the patient alone in doing activities inside and outside the house [4,5].

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The presence of chronic or contagious diseases can be one of the factors that can be effective in increasing the abuse prevalence [3]. One of the chronic diseases that causes various challenges for patients is spinal cord injury (SCI). SCI is defined as damage to the spinal cord that results in loss of sensory, motor, or autonomic function [6], which can lead to problems such as pain, pressure ulcers, and psychological problems, a reduction in physical activity and care stress [7-9]. SCI is one of the chronic diseases that causes caregiver burden in health care providers [10]. Studies have shown that perceived caregiver burden is significant in caregivers of these patients [9, 11, 12]. The caregiver burden in patient caregivers can be an important and effective risk factor for abuse [13].

On the other hand, apart from the problems that patients and caregivers of patients with SCI experience, the spread and prevalence of Covid-19 disease has been effective as an effective risk factor for abuse [14]. Covid-19 disease is an infectious disease that has become increasingly prevalent in the present century and has caused many problems in patients and their companions [15]. One of the most important preventive solution is to observe social distancing, and for this reason, since the Covid-19 outbreak, social distancing has led to problems for patients, including reduced social support, stress, anxiety and fear [16]. On the other hand, the disease process can lead to abuse by creating disability in patients and reducing their abilities because considering their disability, SCI patients become increasingly dependent on caregivers and this factor is one of the factors affecting the development or exacerbation of abuse in patients [17,18].

OBJECTIVES

The authors of the present study have not yet found a similar study worldwide, which indicates the necessity for study of this issue. On the other hand, considering the need to pay attention to chronic patients, especially SCI patients and also the spread of Covid-19 disease worldwide, the aim of the present study is to determine the abuse prevalence and its effective risk factors among SCI patients in Ilam City during the Covid-19 outbreak.

METHOD

This cross-sectional descriptive study, population included all SCI patients in Ilam, 95 of whom were included in the present study. Inclusion criteria included having consent to participate in the study, no history of mental disorder and a suffering from SCI for at least one year. All patients were trained by a researcher and interviewed privately, and they were also ensured about the confidentiality principle. Thus, the interviewers completed the abuse questionnaire by referring to and interviewing SCI patients. If the patient was literate enough to understand the research questions, the questionnaire was completed in a self-reported manner and otherwise the questionnaire was completed by interview. After data collection, statistical analyzes were performed.

Ethical Approval

Informed verbal consent, confidentiality, observance of the provisions of the Helsinki and Belmont Declaration and ethics guidelines in university research were considered as ethics criteria in research. In the present study, the necessary Covid-19-related health protocols were observed.

Study Tools

Moreover, researcher-made forms and questionnaires whose validity and reliability were confirmed, including the demographic characteristics form, perceived abuse researcher-made questionnaire for SCI patients, were used. The perceived abuse questionnaire for SCI patients was a researcher-made questionnaire that was designed using library studies, interviewing patients and determining abuse instances. This instrument consists of 20 questions that are answered using Yes (score 1) and No (score 0). The possible score range is 0 to 20. To measure the tool validity, the content validity method was used and provided to the experts. To determine the test-retest reliability, Pearson correlation coefficient ($\rho=0.96$), which indicates an acceptable reliability. To evaluate the content validity of the instrument, Waltz and Basel content validity index was used. The necessity, relevance, clarity and simplicity of the instrument were calculated using the Likert scoring method. The instrument item was maintained, modified, and deleted for scores ≥ 79, 70-79, and < 70, respectively.

Statistical Analysis

The prevalence of quantitative variables such as age, history of SCI and abuse was calculated using descriptive statistical analyzes (mean calculation and standard deviation). Independent t-test and ANOVA were used to investigate the relationship between demographic variables and abuse. Also, descriptive statistical analyzes were used to calculate the qualitative variables of the research, including sex, level of education, and abuse items that were answered as based on Yes/No format. All analyzes were performed using SPSS Ver. 16 and p-value<0.05 was considered as the statistically significant level.

RESULTS

According to the results, the mean ± SD of the perceived abuse score by caregivers and patients was 8.48 ± 2.31 and

42.45% during the Covid-19 pandemic, respectively. Also, the mean ± SD of patients' age was 62.86 ±19.15 and the patient perceived abuse increased with increasing age (P = 0.000, F = 27.42). Also, the possible abuse score was 0 to 20 that was divided into three categories: low (20, 20.4%), moderate (76, 76.5%) and high (2, 2%). Table 1 shows significantly higher perceived abuse score among women and patients with a history of more than 10 years of SCI, but there was no significant relationship between abuse prevalence with income and marital status (P>0.05) (Table 1). With regard to age and abuse, results also showed an increase in perceived abuse score with increasing age.

Table 1: Frequency of abuse in patients with SCI at the time of Covid-19

Variable	N (%)	Score	
		Mean	SD
Sex			
Male	54(55.1)	7.29	1.71
Female	44(44.9)	9.95	2.11
P-value	-	0.000	
Time of spinal cord injury			
Less than 2 years	14(14.3)	7.92	1.87
Between 2-5 years	46(46.9)	8.15	1.82
Between 5 and 10 years	23(23.5)	8.34	2.20
More than 10 years	15(15.3)	10.26	3.39
P-value	-	0.01	
Marital status			
Single	62(63.3)	8.00	2.15
Married	36(36.7)	8.77	2.37
P-value	-	0.06	
Income			
Weak	14(14.3)	7.42	1.50
medium	84(85.7)	8.66	2.38
P-value	-	0.06	

Table 2 shows the frequency (percentage) of perceived abuses among SCI patients in Ilam city. According to the above-mentioned frequencies and percentages, abuse was not reported in none of the patients in the items "Abandonment in hospitals, nursing homes, nursing homes or other relatives' houses" and "threatening or attempting to beat if got Covid-19 infection". Also, in relation to the item "Unreasonable blame for non-compliance with the health protocols", it was shown that the number of patients who completed the Yes/No item was completely equal.

With regard to the items with the highest abuse rate, we can refer to the item "Self-medication for the prevention or treatment of Covid-19 disease" (84.7%) and " No use of amenities (such as telephone, TV, etc.) after admission in an isolation room during Covid-19 "disease (72.4%). With regard to the items with the lowest abuse rate, we can refer to the item "Failure to follow the healthy nutrition during Covid -19 pandemic" (19.4%) and "Threatening patient with rejection and lack of physical care if he or she develops Covid-19" (11.2%) (Table 2).

DISCUSSION

The aim of the present study was to determine the abuse prevalence and its effective risker factors among SCI patients in Ilam during the Covid-19 pandemic. This is the first study in the world to compare the prevalence of abuse in SCI patients during Covid-19 pandemic. The findings of the present study will be compared with other studies. Attention to chronic patients, especially the psychological issues of these patients, as one of the most important tasks of health care staff, should be prioritized [19]. Another important and necessary issue that prioritizes attention to vulnerable groups is emerging diseases such as Covid-19. The spread of this disease has created various challenges and problems for various vulnerable patient groups, including SCI patients [20].

According to the findings, the abuse prevalence was higher in women than men, which are consistent with the results of studies by Brandão et al. in Brazil (68.9%) [21] and Ghada El-Khawaga et al. in Egypt (56.6%) [22]. The present study also revealed that the abuse prevalence was higher in older patients, which is consistent with the results of the study by

Sathya et al., which showed that the rate of elder abuse increased with age [23]. With age, the disability of the elderly increases and this factor can lead to an increase in elder abuse [23,24].

Table 2: Prevalence of abuse in patients with SCI at the time of Covid-19

-	Items	Yes N (%)	No N (%)
1	Failure to provide hygiene equipment such as masks, gloves and alcohol to prevent Covid-19	37(37.8)	61(62.2)
2	Lack of financial support to purchase essential disease-related equipment, such as wheelchairs, canes, etc.	55(56.1)	43(43.9)
3	Help to see a doctor in accordance with health protocols	38(38.8)	60(61.2)
4	Abandonment in a hospital, nursing home, nursing home or other relatives' home	0(0)	98(100)
5	Negligence in performing corona test even in the case of Covid -19 symptoms	50(51)	48(49)
6	Unreasonable blame for non-compliance the health protocols	49(50)	49(50)
7	Threatening patient with rejection and lack of physical care if he or she develops Covid -19	26(26.5)	72(73.5)
8	Decreased emotional support of family members during the Covid -19 outbreak	52(53.1)	46(46.9)
9	Non-observance of health protocols by family members when contacting a person	29(29.6)	69(70.4)
10	Self-medication for the prevention or treatment of Covid-19 disease	83(84.7)	15(15.3)
11	Forcing the patient to do outdoor work	31(31.6)	67(68.4)
12	Failure to follow the healthy nutrition during Covid -19 pandemic	19(19.4)	79(80.6)
13	Lack of assistance with personal affairs (toilet, bathing, etc.) during the Covid -19 outbreak	64(65.3)	34(34.7)
14	Lack of physical assistance in daily activities during Covid -19 outbreak	41(41.8)	57(58.2)
15	No use of amenities (such as telephone, TV, etc.) after admission in the isolation room during Covid -19 disease	71(72.4)	27(27.6)
16	Preventing increasing awareness and information regarding the latest Covid -19 disease news	37(37.8)	61(62.2)
17	Lack of cooperation in cleaning and disinfecting the house during Covid -19 disease	51(52)	47(48)
18	Lack of life expectancy and unreasonable fear and stress disrupting the patient's life (for example, stating that SCI patients will die if they develop Covid -19)	62(63.3)	36(36.7)
19	Lack of psychological support during the Covid -19	37(37.8)	61(62.2)
20	Threatening or attempting to beat if got Covid-19 infection	0(0)	98(100)

The results of the current study also demonstrated that patients experienced abuse during the covid-19 pandemic in 42.45% of cases. Ghada El-Khawaga et al. showed in their study in Egypt that the elderly with chronic disease experienced abuse than the elderly without chronic disease more frequently, which is consistent with the results of the present study on the presence of abuse in patients with SCI [22]. In the meta-analysis study of 15 articles on the abuse prevalence in the elderly in Iran from 2005 to 2017, Abdi et al., showed that abuse prevalence was 48.3% among this age group [3]. In a meta-analysis of elder abuse in 28 countries, Yon et al. reported that the abuse rate is 15.7% [25], which indicates the presence of abuse among the elderly people. In a study of the abuse prevalence in the Spanish and Iranian elderly group, Farnia et al. reported that the abuse prevalence was 80.5% and 39.1%, respectively, which is much higher in the Iranian elderly than the Spanish elderly, which are consistent with the results of the present study that showed SCI patients experience abuse in 42.45% of cases [26]. Also, in a study of elderly group in Qazvin city, Oveisi et al. found that the elderly people had experienced financial, psychological or neglect abuse in at least 80% of cases [27], which is more than the results reported in the present study. The reasons for this discrepancy include the differences in the study population as well as the study instruments. Also, the objective of the present study was to investigate the abuse prevalence during Covid-19 disease.

There were no cross-sectional and epidemiological studies among studies that have investigated the abuse prevalence during Covid-19 pandemic, but related studies can be mentioned. For example, in their letter-to the editor, published during the early outbreak of Covid-19 in 2020, Karimian et al., emphasized the importance of abuse prevention during Covid-19 disease in the elderly group [28]. In a retrospective study, Sharma et al. showed that the child abuse prevalence was 158, 199, 204, and 215 cases in 2017, 2018, 2019, and 2020, respectively, which indicates an increase in child abuse during Covid-19 [29]. Moreover, Quraish et al. showed in a study that the rate of physical, sexual and child abuse as well as child labor has increased since the Covid-19 outbreak disease [14], which is consistent with the results of the present study on the increase of abuse among SCI patients.

CONCLUSION

In the present study, the variables of age, sex and time of spinal cord injury have been effective on the perceived abuse of SCI patients, which is necessary to take the necessary measures and preventions in this regard. Also, the prevalence of Covide-19-related abuse in patients was 42.45%, which is statistically important and significant. For this reason, while proposing preventive action in this field, it is suggested that considering the novelty and innovation of this study, similar studies be conducted in other cities of Iran as well as in other countries so that they provide more comprehensive and extensive information to health policymakers.

Authors' Contribution:
MH, KK did study conception, data analysis, and manuscript writing. MH, KK did data collection and manuscript writing. MH, KK did data collection and manuscript writing. Both authors (MH, KK) contributed to all stages of the article.

Conflict of Interest:
The authors declare no conflict of interest.

Ethical Approval:
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ORIGINAL ARTICLE

Evaluation of muscle atrophy after sciatic nerve defect repair – experimental model

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Abstract: *Peripheral nerve injuries are one of the most important causes for disability generating considerable costs around the world. Appropriate assessment of the extent of peripheral nerve lesions and the choice of the right therapeutic protocol remain some of the biggest challenges. Non-invasive neuroimaging approaches are suitable in managing peripheral nerve repairs, providing in the same time accuracy details in structural neural detection, with minimal discomfort at a low cost. Medical imaging technology development has led to progress in examination of peripheral nervous system, using a series of tools and methods, such as: ultrasonography (US), positron emission tomography (PET), magnetic resonance imaging (MRI) and magnetic diffusion tensor imaging (DTI). In this study, we evaluated the regeneration process in Wistar rats after sciatic nerve defects repair with 4 different techniques (i.e. nerve graft, rat aorta used as a nerve conduit, rat aorta filled with platelet rich plasma (PRP) and rat aorta filled with stem cells) by using MRI investigation alongside the clinical evaluation. Our results showed that among the 4 batches, the PRP batch had the best results in muscle atrophy condition (both on MRI scan and on gastrocnemius index); on the second place the stem cell batch, then the nerve graft batch and finally the aortic conduit batch. MRI proved to be a reliable non-invasive monitoring method and showed good result in correlation with the footprint test (sciatic functional index) and the gastrocnemius index.*

Keywords: *muscle atrophy, sciatic nerve, platelet rich plasma*

INTRODUCTION

Nerve injuries can be extremely debilitating due to the many functions that a peripheral nerve may have. The sensitive nerves are responsible for acquiring information such as temperature, pressure, shape, while motor nerves are important in muscle contraction and movement. These injuries are even more severe when they translate into nerve defects, which require a special type of bridging in order to recover its original function.

After the nerve is repaired it takes a long period of time for regeneration. This is due to the fact that after the nerve is sectioned, Wallerian degeneration occurs, which represents a disintegration of the axon distal to the injury site [1]. Afterwards, the growth cone leads the regeneration process through the modified Schwann cells – the Bungner columns [2]. The nerve growth rate is considered to be 1 mm/day in humans and 1.5-2 mm/day in rats. Several imaging instruments and techniques are currently used in examination of peripheral nerves: ultrasound (US), positron emission tomography (PET), magnetic resonance imaging (MRI), diffusion tensor imaging (DTI).

Nerve ultrasound is capable of assessing not only the size, but also the morphology and it may be used in diagnosing different types of peripheral nerve pathologies. The most common findings using ultrasound examination are nerve enlargement, followed by other abnormalities such as increased hypo-echogenicity or increased intraneural vascularization [3,4]. Moreover, ultrasound investigation can also asses the surrounding environment, being helpful for determining the etiology of the neuropathy (compression, trauma).

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PET represents an imagistic investigation in which a radioactive tracer such as [18F] FTC-146 is injected into the bloodstream and it concentrates at the level of the nerve injury, [5] investigation which is used in order to detect the area of the neuropathy. Diffusion tension imaging (DTI) evaluates the movement of water molecules in different biological structures. DTI can evaluate a nerve injury both in terms of quantity and quality [6].

As its great similitude to the human nerve, the sciatic nerve of the Wistar rat is the perfect candidate for experimental nerve surgery. The sciatic nerve divides into 3 main branches – tibial, common peroneal and sural nerve.[7] The tibial nerve innervates the gastrocnemius muscle. Therefore, the regeneration process of the sciatic nerve can be evaluated by assessing the function, weight and diameter of this muscle.

Nerve defects can be solved by several techniques – either nerve grafts or different surgical protocols type. Defects up to 70 mm can be bridged by one of these options. [8] Nerve grafts can be either autologous or heterologous, while nerve conduits can be biologic or synthetic. [9,10] Better results were obtained by using the nerve protocol technique in combination with different substances which contain growth factors. [11] Among these, platelet rich plasma (PRP) has proven its efficacy in nerve regeneration. [12,13,14] Stem cells, similar to PRP, also have a positive impact in nerve regeneration. [15,16,17]

A footprint test in rats undergoing surgery, became a useful evaluation asset since Bain and MacKinnon introduced a formula which could calculate the sciatic functional index (SFI) based on 3 parameters-plantar length, intermediary interdigital length and the maximum interdigital distance. [18]

This study aims was to compare the regeneration process in Wistar rats after sciatic nerve defects using 4 different techniques (i.e. nerve graft, rat aorta as a nerve protocol, rat aorta filled with platelet rich plasma (PRP) and rat aorta filled with stem cells) by using MRI investigation and comparing it to the results obtained from the footprint test (sciatic functional index) and the gastrocnemius index.

MATERIAL AND METHODS

The research was conducted on 40 Wistar rats, from Carol Davila University (Convention no. 7491/21.03.2019). They were accommodated in plastic cages, receiving normative murine food and water.

The rats were divided into 4 groups – nerve graft group, aortic conduit group, aortic conduit with PRP and aortic conduit with stem cells. All rats underwent surgery with a right side approach. The biologic materials used (PRP and aortic conduits) were obtained by sacrificing 2 extra rats. The stem cells used, originated from human bone marrow mesenchymal stem cells (hBM-MSC cell line) were obtained from Life Technologies. All other chemicals were purchased from Sigma–Aldrich. The study was approved by the Ethics Committee of Veterinary Faculty (approval No. 1738/ 17.04.2019).

Preoperative assessment

All rats were weighed before surgery and they were anesthetized using a cocktail of 75-100 mg/kg ketamine and 10 mg/kg xylazine. The anesthetic injection was administrated intraperitoneal, maintaining anesthesia for one hour. If the rat presented signs of waking up, a second dose could be administrated.[19]

Surgical procedure

The surgical protocol was the same for all subjects. The rat was positioned in prone decubitus, than a surgical incision was performed along the right femur, 0.5 cm inferior to the bone. The biceps femoris was then dissected and right beneath it the sciatic nerve could be found.

Figure 1: Incision site (12 weeks postoperative scar)

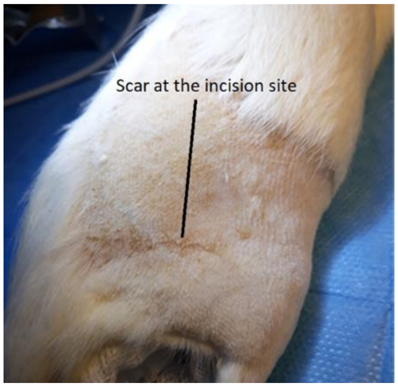

Nerve graft

After nerve exposure, the sciatic nerve was sectioned at 2 sites to create a 0.5 cm nerve graft. In the first batch this graft was sutured in place, while in the other batches the graft was discarded, leaving a 0.5 cm defect. In the first batch, the epineural vessels served as guide to ensure correct alignment between the nerve endings.

Simple aortic conduit

Two rats were sacrificed and their aortas were taken from the heart to the bifurcation into the common iliac arteries. The aortas were then divided into 6-7 mm long segments to serve as nerve material for the next interventions. In the 2nd batch, the aortic conduit was used to bridge the nerve defect by suturing the nerve endings 1 mm inside the vessel conduit.

PRP treatment

The 3rd batch used PRP inside the aortic conduit. After extracting 2 ml of blood directly from the heart, the blood was centrifuged for 5 minutes at 4000 rpm in order to obtain PRP material. The PRP was placed inside the aortic conduit and sutured to the nerve endings.

Mesenchymal stem cell treatment

In the 4th batch, mesenchymal stem cells from human bone marrow were used. These were transported in liquid nitrogen at -196oC and defrosted using the following protocol: cryogenic tube extraction, 500 µL phosphate-buffered saline (PBS) solution was added, then the solution was centrifuged for 5 minutes at 1500 rpm and the supernatant was disposed of; the last 3 steps were repeated and after obtaining the stem cells, they were suspended in Ringer solution. The stem cells were then included in the aortic conduit and this was sutured to the nerve endings.

The immediate postoperative treatment included antibiotic therapy using enrofloxacin at a dose of 0.003 mg/kg (Enroxil) and an anti-inflammatory drug (meloxicam 1 mg/kg) administered subcutaneously for 3 days.

Postoperative assessment

All subjects were evaluated at 2, 4, 6, 8, 10 and 12 weeks after surgery, including sensitive and motor evaluation, as well as a footprint test. At 12 weeks all rats were sacrificed using KCl after an overdose with the above mentioned anesthetic. The gastrocnemius muscles were evaluated using MRI imaging and then they were harvested and weighed (bilateral).

Walking footprint analysis

The SFI (sciatic functional index) represents a fairly objective test which evaluates the peripheral nerve regeneration in rats and mice. The method consists of recording, measuring and calculating the dimensions of the footprints of the studied rats after unilateral sciatic nerve injury. Monte-Rasso has demonstrated that this index is measurable and reproducible starting the 3rd week after the nerve lesion. [20]

The traditional method of recording the footsteps involves using paper and ink, but it can record many artifacts which could affect the results. The development in technology by using an infrared system eliminated this problem and reduced the effort of the investigator. [21]

The formula for the SFI introduced by Bain and MacKinnon takes into account foot values of both the operated and the non-operated limb. A value close to 0 indicates normal function, while -100 represents complete function loss:

$$SFI= 38.3 \times EPL-NPL/NPL + 109.5 \times ETS-NTS/NTS+ 13.3 \times EIT-NIT/NIT – 8.8.$$

where EPL – plantar length (operated limb), NPL – plantar length (non-operated limb), EIT – intermediary interdigital length (operated limb), NIT – intermediary interdigital length (non-operated limb), ETS – maximum interdigital distance (operated limb) and NTS – maximum interdigital distance (non-operated limb)

Magnetic Resonance Imaging (MRI)

The imagistic investigations were conducted at the Veterinary Medicine Faculty in Bucharest using an MRI scanner Vet-MR Grande Esaote de 0.2 T. A standard spin echo pulse protocol with T1 and T2W in sagittal, dorsal and transversal planes was performed. This protocol included a girth of 2mm/slice and 0,5mm distance between slices. Due to the small size of the rat, an articulation human coil was used.

The rat was examined in prone position in the MRI coil, with both inferior limbs in extension. The thorax and the head of the rat were sustained on 2 sponges during the investigation. Each rat required 15-20 minutes for the investigation and 5 minutes for preparation. On the transverse sections, the calf diameters were measured (right and left) and then a ration between the 2 values was calculated.

For the MRI calf muscle ratio (right/left), the measurements were performed on anaesthetized rats lying in prone position, with both inferior limbs extended. The image selected for measurement was the first imagine in transverse plane where the limbs appeared attached to the body.

Gastrocnemius muscle – operation and measurements

After incising the dorsal skin of the calf in the midline and dissecting through the superficial flat muscle, the gastrocnemius muscle revealed. It was then harvested from the level of Achilles' tendon to its origin on the femur. Using an electronic scale, the weight of the gastrocnemii muscles were measured and the ratio between operated and non-operated side was noted.

Statistical analysis

To process the data obtained, we used IBM Statistical Package for Social Science (SPSS) - Median test for the continuous variable analysis, where the median and the Inter Quartile Range (IQR) were reported. The threshold for statistical significance was $p<0.05$.

The correlations between continuous non-parametric variables were performed using Kendall Tau b test. The representation of the data was done using box-plot graphics and scatter-dot and line diagrams.

RESULTS

40 male Wistar rats of similar weight and age were used for research, divided into the 4 studied groups and 2 extra rats were used to harvest biologic materials. All rats underwent a surgery on the right sciatic nerve. 2 rats from the first batch were excluded from the study – one due to postoperative death and the other one due to a ruptured sciatic nerve. The rat who suffered a nerve rupture was kept until the end of the study (12 weeks).

The variables taken into account for nerve regeneration were:

- right and left gastrocnemius muscle weight and their ratio recorded at 12 weeks
- ratio between the right (operated) gastrocnemius muscle weight and the rat weight at 12 weeks (percentage)
- SFI at 6 and 12 weeks
- right and left calf diameters and calf diameter ratio recorded via MRI at 12 weeks

The MRI investigation could identify the nerve and the aortic nerve conduit in transverse, coronal and sagittal plane. The calf diameter could be measured bilaterally and the calf ratio could be calculated.

The images below describes the position of the sciatic nerve (lying inferior to the femur). One image shows the nerve graft in T1 sequence, while the other one shows the aortic conduit in T2 sequence. No image can however reveal the suture sites in any of the sequences.

Figure 2: T1 coronal plane - general view (A); Sciatic nerve (B); Distal femur (*) distal and proximal tibia (#)

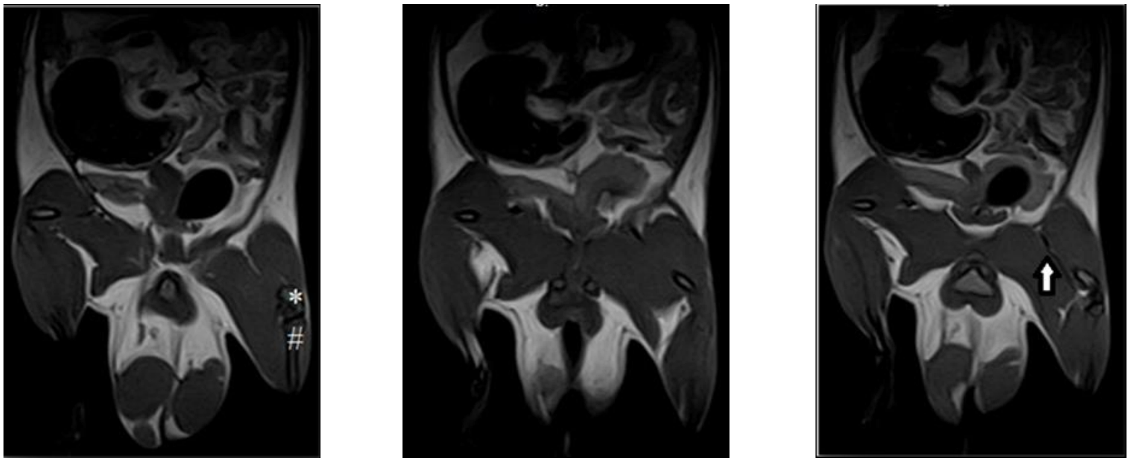

Figure 3: Nerve image in T1 sequence (A); Aortic conduit in T2 sequence (B)

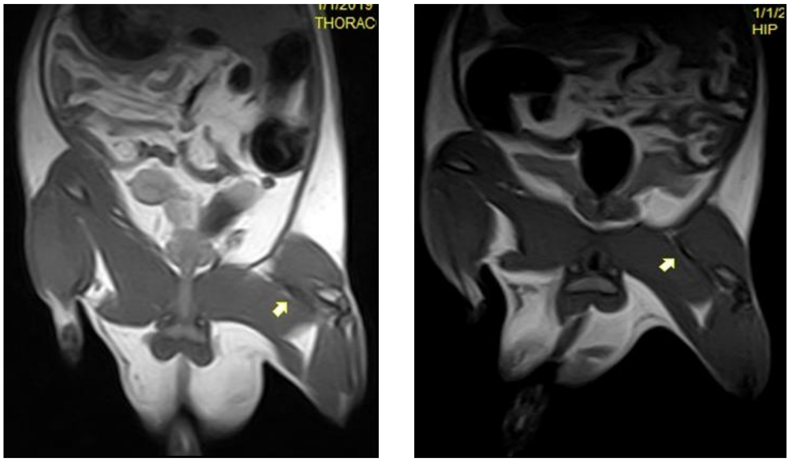

Figure 4: Coronal sections in T2 sequence of right femur - aortic nerve conduit and scarring (arrow); perilesional oedema (*)

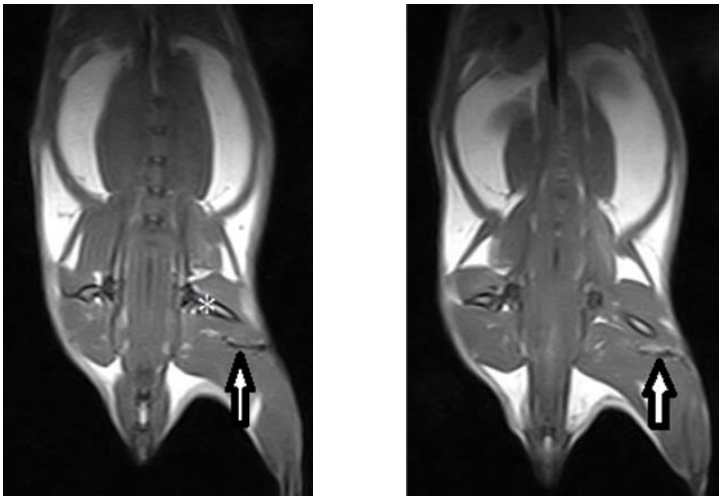

Figure 5: Series of MRI images T1 sequence transverse plan - calf muscles measurements

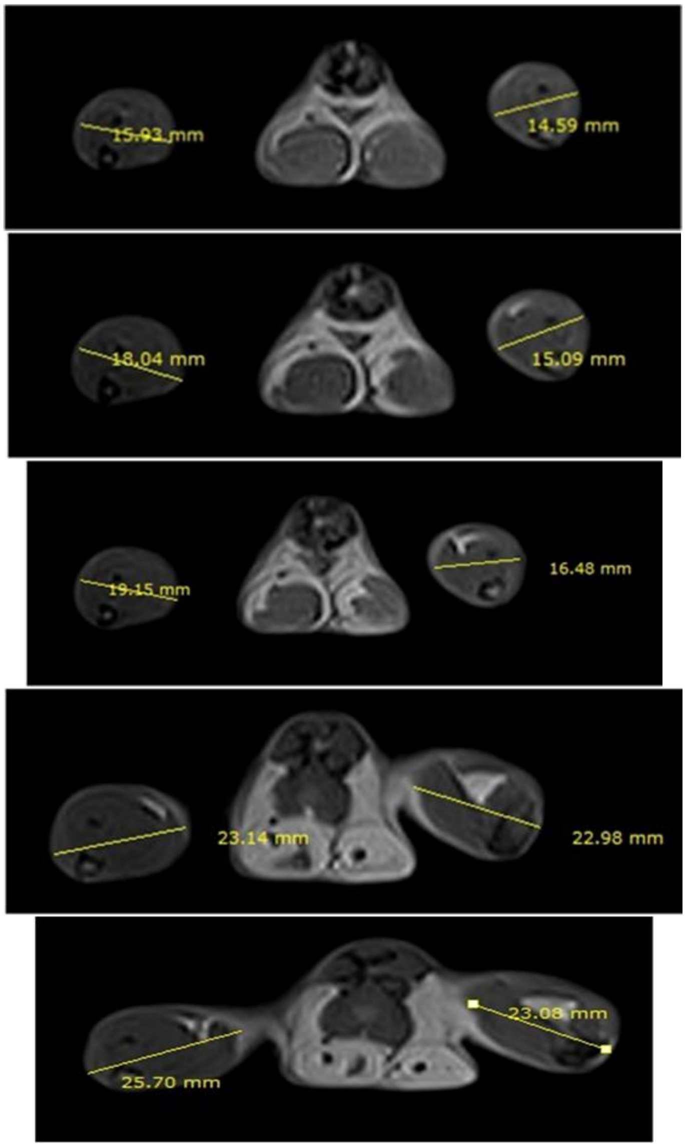
15.93 mm
14.59 mm
18.04 mm
15.09 mm
19.15 mm
16.48 mm
23.14 mm
22.98 mm
23.08 mm
25.70 mm

Figure 6: Series of MRI images coronal plane T1 sequence - calf muscles measurements

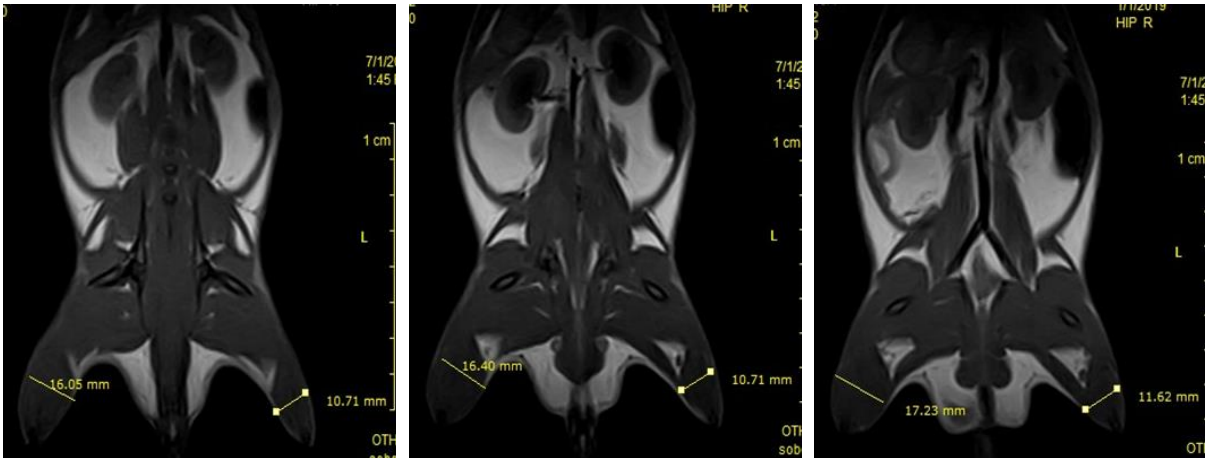

After completing the MRI scanning, the rats were euthanized and the gastrocnemius muscles were harvested bilaterally. A fine electric scale was used to weigh each muscle. These gastrocnemius ratios were compared to the MRI ratios for all batches.

Figure 7: Gastrocnemii muscles in pairs (left and right) – stem cell batch

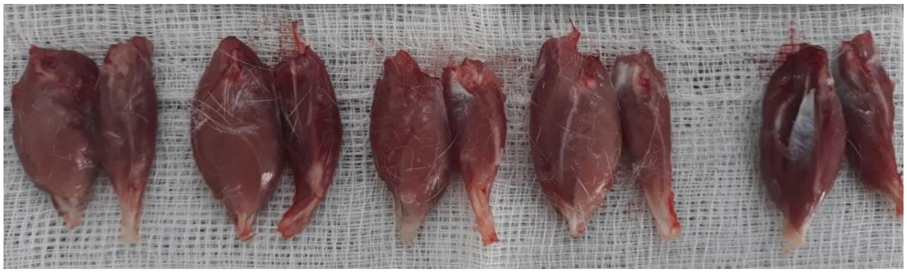

Table 1: Median analysis: weights of gastrocnemius muscles (right and left), gastrocnemius index

n=38	Batch 1	Batch 2	Batch 3	Batch 4	Total
Variable	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Right gastrocnemius	1.415 (0.25)	1.28 (0.45)	1.33 (0.31)	1.285 (0.13)	1.31 (0.21)
Left gastrocnemius	2.62 (0.55)	2.425 (0.24)	2.41 (0.26)	2.55 (0.30)	2.445 (0.29)
Gastrocnemius index	0.5 (0.15)	0.49 (0.18)	0.51 (0.17)	0.505 (0.03)	0.5 (0.07)
Rat final weight at 12 weeks (G6)	465 (59)	442 (61)	434.5 (28)	455 (57)	446.5 (39)
Percentage right gastrocnemius weight/G6	0.29 (0.09)	0.29 (0.07)	0.31 (0.08)	0.029 (0.02)	0.029 (0.05)

Table 2: Median test for gastrocnemius weight (right and left) and gastrocnemius index

Variable	Test Median (Degree of freedom)	P value
Right gastrocnemius	0.9 (3)	0.825
Left gastrocnemius	4.1 (3)	0.251
Gastrocnemius index	1.2 (3)	0.753
Rat final weight at 12 weeks (G6)	2.5 (3)	0.475
Percentage right gastrocnemius weight/G6	6.2 (3)	0.102

Table 3: SFI analysis at 6 and 12 weeks

n=38	Batch 1	Batch 2	Batch 3	Batch 4	Total
Variable	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
SFI at 6 weeks	-75.5 (8)	-76 (9)	-76 (7)	-76.5 (4)	-76 (4)
SFI at 12 weeks	-57.5 (9)	-58.5 (12)	-55.5 (10)	-57 (5)	-57 (6)

Table 4: Median test for SFI evaluation

Variable	Median Test (Degree of freedom)	P value
SFI at 6 weeks	1.08 (3)	0.782
SFI at 12 weeks	2 (3)	0.572

Table 5: Median analysis of the calf muscles diameters on MRI scan

n=38	Batch 1	Batch 2	Batch 3	Batch 4	Total
Variable	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Right calf diameter on MRI	17.06 (2.3)	16.4 (3.38)	16.39 (0.98)	16.94 (1.4)	16.67 (1.5)
Left calf diameter on MRI	21.45 (1.26)	20.82 (2.18)	20.63 (0.96)	21.38 (1.61)	21.1 (1.45)
Right/left calf diameters ratio	0.794 (0.064)	0.784 (0.072)	0.803 (0.066)	0.799 (0.017)	0.798 (0.049)

Table 6: Median test for calf diameters on MRI

Variable	Median Test (Degree of freedom)	P value
Right calf diameter on MRI	4.1 (3)	0.251
Left calf diameter on MRI	6.8 (3)	0.079
Right/left calf diameters ratio	1.2 (3)	0.753

Table 7: Correlation between MRI calf diameter ratio and gastrocnemius index

n=38	Total	
Corelation	*Kendall Tau Correlation coeficient*	*P Value*
MRI calf diameter ratio – gastrocnemius index	0.95	<0.001
MRI right/left calf diameter ratio– percentage right gastrocnemius weight/G6	0.57	<0.001
MRI right/left calf diameter ratio– G6	0.182	0.110
MRI right/left calf diameter ratio– SFI at 6 weeks	0.3	<0.010
MRI right/left calf diameter ratio– SFI at 12 weeks	0.485	<0.001

Peripheral nerve regeneration after nerve graft intervention

In the nerve graft batch, the SFI at 6 weeks varied between -83 and -69, while at 12 weeks it was between -60 and -50. The right calf diameter at the end of the study ranged between 15.35 and 17.97 mm. The left calf diameter had values between 19.96 and 22.04 mm. The calf diameter ratio varied from 0,76 to 0,84. The median values for the variables reported via MRI scanning are presented in tables 1 and 2.

The gastrocnemius muscles on the operated side weighed between 1.22 and 1.49 g while the non-operated muscles weighed between 2.27 and 3 g. The gastrocnemius muscle weight ratio varied from 0.45 to 0.65. The median values for the variables associated to gastrocnemius muscles are presented in tables 3 and 4 and those for the SFI values in Table 5.

Peripheral nerve repair with rat aorta graft

In the second batch, the SFI varied at 6 weeks between -83 to -73, while at 12 weeks between -63 and -49. The right calf diameter had values between 13.78 and 17.81 mm, while the left one was between 18.2 and 21.73 mm. The calf diameter ratio varied from 0.74 and 0.84. The median values for the variables reported via MRI scanning are presented in Tables 1 and 2.

The gastrocnemius muscles on the operated side weighed between 0.65 and 1.58 g while the non-operated muscles weighed between 2.06 and 2.86 g. The gastrocnemius muscle weight ratio varied from 0.29 to 0.69. The median values for the variables associated to gastrocnemius muscles are presented in tables 3 and 4 and those for the SFI values in Table 5.

The effect of platelet rich plasma (PRP) treatment on peripheral nerve repair

In the third batch, the SFI varied at 6 weeks between -80 to -71, while at 12 weeks between -62 and -48. The right calf diameter had values between 15.11 and 17.71 mm, while the left one was between 18.56 and 21.53 mm. The calf diameter ratio varied from 0.74 to 0.84. The median values for the variables reported via MRI scanning are presented in Tables 1 and 2.

The gastrocnemius muscles on the operated side weighed between 0.77 and 1.61 g while the non-operated muscles weighed between 2.28 and 2.73 g. The gastrocnemius muscle weight ratio varied from 0.34 to 0.67. The median values for the variables associated to gastrocnemius muscles are presented in Tables 3 and 4 and those for the SFI values in Table 5.

The effect of mesenchymal stem cells transplantation on peripheral nerve repair

In the third batch, the SFI varied at 6 weeks between -78 to -72, while at 12 weeks varied between -62 and -51. The right calf diameter had values between 15.65 and 17.88 mm, while the left one was between 19.38 and 21.73 mm. The calf diameter ratio varied from 0.78 to 0.81. The median values for the variables reported via MRI scanning are presented in Tables 1 and 2.

The gastrocnemius muscles on the operated side weighed between 1.17 and 1.46 g while the non-operated muscles weighed between 2.26 and 2.76 g. The gastrocnemius muscle weight ratio varied from 0.48 to 0.54. The median values for the variables associated to gastrocnemius muscles are presented in Tables 3 and 4 and those for the SFI values in Table 5.

Comparison of the 4 methods for the repair of peripheral nerve injury

SFI

The median values for SFI recorded at 6 weeks indicated better recovery in nerve graft batch, followed by the PRP and simple aortic conduit batches and the stem cell batch. At 12 weeks, the median values for SFI showed a very good recovery in the PRP batch, followed by stem cell and nerve graft batches, while the poorest results were recorded in the simple aortic conduit batch.

MRI test

The right (operated) calf diameter had the highest median value in the nerve graft batch, followed by the stem cell batch and simple aortic conduit and PRP batches with similar results. The same order was recorded on the left side as well. The highest calf diameter ratio (right/left) recorded via MRI scanning was in the PRP batch, followed by the stem cell batch, and the nerve graft batch, without statistical difference (p=0.753).

Gastrocnemius muscle

There were no statistical differences in the 4 batches when referring to the weight of the right gastrocnemius muscle. The highest median was in the nerve graft batch, followed by PRP batch, then stem cells batch and finally simple aortic conduit batch. The left one had the highest median value when referring to the weight variable in the nerve graft batch; followed by stem cell batch and then the PRP patch and last – the simple aortic conduit batch.

Figure 8: Distribution graphic of the MRI right/left calf diameter ratio for all batches

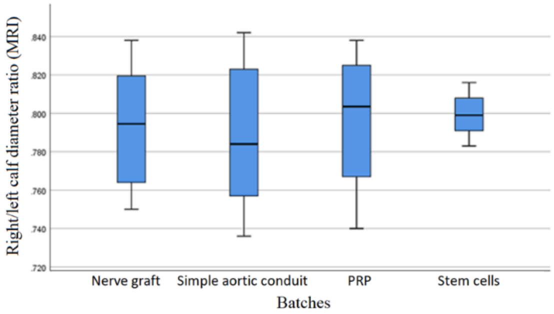

The median for the gastrocnemius index – the best results were in the PRP batch, followed by the stem cells batch, then the nerve graft batch and finally the simple aortic conduit batch. There were, however, no statistical differences between these batches.

The highest median when evaluating the percentage obtained from the right gastrocnemius muscle and the rat weight at 12 weeks was recorded in the PRP batch, all other batches obtaining similar results. Like the previous test, no statistical difference was achieved here either.

Using the Kendall Tau analysis, there were 4 positive statistical significant correlations (Table 7): MRI right/left calf diameter ratio – gastrocnemius index; MRI right/left calf diameter ratio – SFI at 6 weeks; MRI right/left calf diameter ratio – SFI at 12 weeks; gastrocnemius index (right/left) and SFI at 12 weeks.

The statistical analysis revealed a strong positive correlation between the MRI right/left calf diameter ratio and the gastrocnemius index. (r=0.95, p<0.001).

Figure 9: Correlation graphic between gastrocnemius index and MRI right/left calf diameter ratio

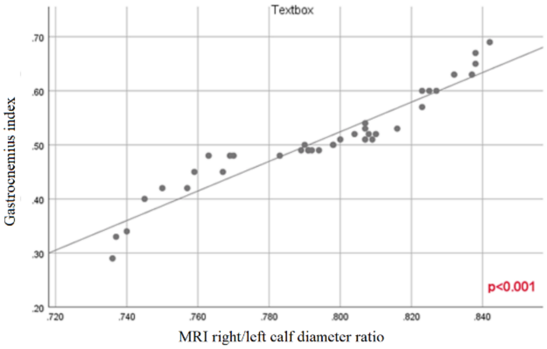

The statistical analysis revealed a strong positive correlation between the MRI right/left calf diameter ratio and the SFI at

6 weeks (r=0.3, p=0.010) and at 12 weeks (r=0.485, p<0.001).

Figure 10: Correlation graphic between MRI right/left calf diameter ratio and SFI at 12 weeks

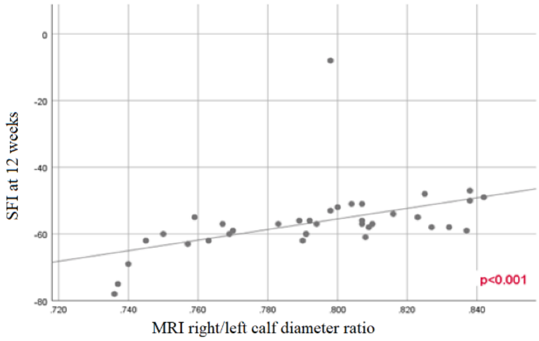

There was also a strong positive correlation between the gastrocnemius index and the SFI at 6 weeks (r=0.323, p=0.007) and at 12 weeks (r=0.487, p<0.001).

Figure 11: Correlation graphic between gastrocnemius index (right/left) and SFI at 12 weeks

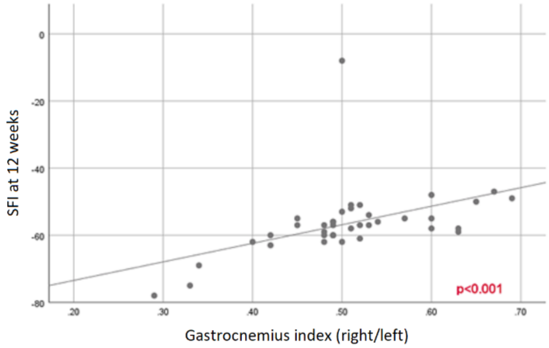

DISCUSSION

The MRI could not reveal the sciatic nerve in all the sections due to its small size. A high-performance MRI scanner (1,5 T or 3T) might be useful in identifying the extent of the lesions as well as small structures (fascicles of the human sciatic nerves), and a better and more detailed aspect of the suture sites.

Tremp et al used a 3T MRI scanner to assess regeneration in a rat sciatic nerve injury model. They were capable of visualizing the nerve graft by observing a small black outline, while the regenerating axon front could be seen as a small hyperintensity. The study also used the 3T MRI scanner to monitor the regeneration progress using the 2 types of stem cellular therapy (autologous and human stem cells). [24]

Muscle imaging and MRI in particular has become an important tool in the diagnosis of muscle diseases – both inherited and acquired. Subclinical progression of the different muscular diseases can be detected using quantitative muscle MRI techniques. [25]

Although MRI scanning could offer non-invasive information about nerve regeneration by observing the degree of muscle atrophy, this investigation is unlikely to become a gold standard as it reveals the negative effects at a late stage (when there is severe muscle atrophy). The gastrocnemius index can only be used in experimental studies and, similar to the previous investigation, it reveals the negative results only when it is too late (as the muscle atrophies progressively).

The MRI right-left calf diameter ratio could be correlated with the right-left weight gastrocnemius muscles ratio (gastrocnemius index) and with the SFI from the footprint test. The correlations between these indicators are justifiable, as all of them evaluate the degree of muscle sparing as a result of nerve regeneration process. Similar studies of MRI calf muscle evaluation after partial and complete denervation have been conducted on mice. The muscle atrophy was estimated by volume measurements on MRI, which were correlated to the post-mortem muscle weight examination. [26]

Nijhuis et al used a similar correlation to observe gastrocnemius muscle atrophy after sciatic nerve injury but instead of the MRI scanner, they used ultrasound imaging to asses muscle volume. Their study showed that the difference between the muscle thickness ratio (via ultrasound) and the muscle mass ratio was no more than 5%. [27] In clinical practice, ultrasound examination can therefore prove to be a reliable and accesible tool for muscle mass measurements. [28]

Another study conducted by Shen et al showed that the SFI parameter after sciatic nerve injury has positive correlations with indices such as electrophysiology, muscle strength and morphology. This research confirmed that the SFI can be a reliable index for nerve regeneration. [29]

A reliable investigation for nerve lesions is the electrophysiologic study. Vleggeert-Lankamp et al showed in a study using electrophysiological analysis that the ionic channels of both the Aalpha- and Abeta-fibers are altered after nerve injury and the correlation between nerve morphometry and electrophysiology is most suitable for evaluating nerve regeneration. [30]

Another study conducted by Shen et al used an electrical field for nerve regeneration purposes. They performed morphometric, electrophysiologic and functional evaluations on days 10, 20, 30 and 60 after the operation. Their research proved that direct current electrical stimulation facilitates peripheral nerve growth. [31]

Martins et al assessed recovery after sciatic nerve injury using several tests: electrophysiology, sciatic functional index (SFI) and histomorphometry. Using Pearson's analysis, they noticed 2 positive correlations with statistical significance (between the SFI and proximal nerve fiber diameter and between the SFI and distal nerve fiber diameter), thus concluding that there are few correlations between the 3 evaluation methods. [32]

With the evolution of technology, the imagistic evaluation of the peripheral nerves via MRI changed from the classical morphological studies to functional neurography. This new technique involves using diffusion tensor imaging and diffusion-weighted sequences and provides information (both functional and morphological) about the internal structure and physiopathology of the peripheral nerves. [33]

The magnetic resonance neurography (MRN) is the salvage solution for complex cases. The spacial distribution of the peripheric nerve helps with the classification of the peripheric nerve injuries in traumatic plexopathies or partial neuropathies of the fascicles. [34]

MRN has some important advantages: it is not operator dependent, it offers 3D representation of the entire nerve, it has great soft tissue contrast, and it provides detailed images of the nerve structure, fascicles and its surrounding environment. For these reasons, MRN is a suitable investigation for tumors, traumas (including the evaluation of brachial plexus injuries), as well as compressive lesions. [35]

Neurography is particularly useful in the cases where nervous lesions are less obvious (compression syndromes or partial nerve injuries); this investigation is capable of establishing the degree of the nervous injury, directing the therapeutic approach.

Microstructural defects of the peripheral nerves can nowadays be detected using quantitative diffusion tensor imaging. (DTI) The imagistic depiction on MRN of the smaller nerve branches can be improved by vascular suppression techniques and parallel imagining, which provides a higher spacial resolution. [36]

The gastrocnemius index was a quantifiable measurement in assessing nerve regeneration. A gastrocnemius index close to the value of 1 (meaning that the weights of the 2 gastrocnemius muscles – right and left - are similar) represents the normal value of a non-operated rat, with muscles that did not undergo atrophy. A very low value of this index indicates severe muscle atrophy, due to a poor nervous regeneration (or a lack of nerve regeneration).

The rat with the ruptured sciatic nerve in the first batch was taken out of the study in order not to influence the results. However, it was useful in order to observe the degree of muscle atrophy when no repair was performed and also as a reference in order to compare regeneration in the 4 batches.

While the median value of the gastrocnemius index for the 38 rats included in the study was 0.5, the gastrocnemius index for the rat with the ruptured nerve was 0.18. However, not having more rats in the category of the unrepaired sciatic nerve, no statistical analysis could be performed.

Figure 12: Two pairs of gastrocnemius muscles in 2 different rats

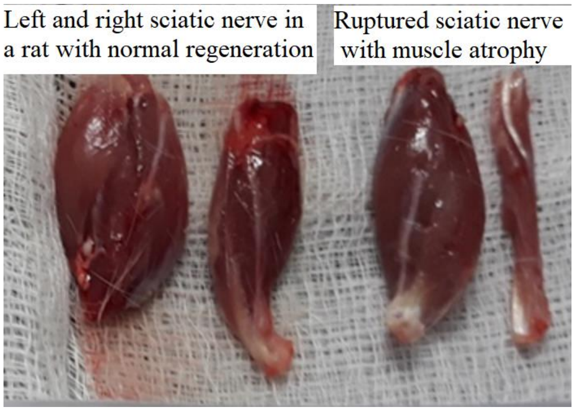
Left and right sciatic nerve in a rat with normal regeneration
Ruptured sciatic nerve with muscle atrophy

The positive correlation between the gastrocnemius index and the sciatic functional index at 6 and 12 weeks can be explained by the fact that both indicators are influenced by the degree of the calf muscle recovery.

CONCLUSIONS

Nerve regeneration can be properly evaluated only after a period of time. The MRI right-left calf diameter ratio represents a non-invasive investigation complementary to the gastrocnemius index as they both evaluate the same aspect in the process of nerve regeneration – the degree of muscle atrophy. Therefore, there was a strong correlation between these 2 investigations.

None of the repair methods tested showed a statistical significant advantage regardless of the test used to evaluate nerve regeneration.

The effect of muscle atrophy was reflected in the footprint test and therefore the sciatic functional index could be correlated to the MRI right-left calf diameter ratio.

Development in technology will provide better imagistic methods to monitor the regeneration stages after peripheral nerve injury both at the site of the injury, as well as at the site of the innervated organ.

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ORIGINAL ARTICLE

Ego-strength mediates the relationship between religious health behaviors and mental health symptoms during the COVID-19 pandemic in Iran

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Abstract: *The present study aimed to examine the mediating role of ego-strength in the relationship between religious health behaviors and mental health symptoms during the COVID-19 pandemic in Iran. A total of 346 university students (301 women, 45 men) completed the Ego-Strength Scale, Religious Health Promoting Behaviors Inventory, and Mental Health Inventory from May 2021 to August 2021. The structural equation modeling and the Pearson correlation method were used. The results revealed that religious health behaviors have a positive and significant relationship with, psychological well-being, and ego-strength, as well as a negative significant relationship with psychological distress. The results also showed ego-strength has a negative relationship with psychological distress. Ego-strength mediated the relationship between religious health behaviors and symptoms of mental health. Ego-strength can be considered as a very important variable in religious health psychology; reinforcing ego-strength may have some implications for mental health during the Covid-19 pandemic.*

Keywords: *ego-strength, health behavior, religious health behavior, mental health, Covid-19*

INTRODUCTION

COVID-19 pandemic is the widest public health crisis in the world. In addition to the huge physical health threat of the COVID-19 pandemic, long feelings of uncertainty and fear, grief and separation, upset of economic and social systems have led to mental health threats [1]. The impacts of COVID-19 on mental health are alarming [2]. As Erikson (1965) suggested, ego-strength may be defined as specified qualities that start to animate the individuals comprehensively within successive periods of life [3]. Ego-strength is related to self-regulation. The term self-regulation is generally understood to mean a process that is guided by goals and its aims to achieve and sustain one's goals [4]. When a person has mental health, there is a consonance between id, ego, and superego [5]. The ego-strength also is one of the personality characteristics, and is defined as the attitude of a person toward his problems, his general response to distress, and his adaptive ability in situations which are emotional [6]. In other words, ego-strength is the extent of power that the ego works in adjusting the impulses and adapting to the environment. The balance while postponing the gratification of needs can be prepared by ego-strength. When the ego-strength is low, people experience a lack of motivation and confidence and believing that they are incompetent. But elevated ego-strength helps the persons to be resilient, motivated, confident, and trusting themselves and their capabilities; thus, they are likely to adapt to challenges [7].

In a study that used group psychotherapy that included components of individual psychotherapy, the majority of participants showed an increase in their ego-strength [8]. Another study among students showed that some variables such as sports, student government, and volunteerism were associated with ego-strength. Also, religious attendance wasn't related to ego-strength [9]. In another study, authoritative parenting and extroversion were related to ego-strength. Also, extroversion has moderated the association between authoritative parenting and ego-strength [10]. In one study, aggressive alcoholics had weaker ego-strength than non-aggressive alcoholics [11]. One of the

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variables of the present study is ego-strength which despite its great importance, has received little attention in psychological research. As a result, addressing it can open up new horizons for the science of psychology.

Also, some studies have shown the important role of ego-strength in mental health [10]. Mental health is one of the most important psychological variables. increasingly, the rates of mental disorders in colleges across the world are rising [12]. One of the major researches carried out by WHO in 21 countries indicated that one-fifth (20.3%) of college students had 12-month DSM-IV/ICD-10 disorders [13]. Many research suggesting that religious/spiritual behaviors and beliefs are correlated with mental health [14]. A systematic review indicated that religious involvement is associated with higher mental health especially in the domain of substance abuse, depression, and suicide [15]. Mental health is also considered in the present study. In particular, mental health is very important for university students and according to the statistics mentioned above, it is very important to address it. As a result, the present study deals with the mental health variable in students.

One recent study found a correlation between religiousness and a lower probability of depressive symptoms, especially in individuals that attending religious service [16]. Lots of people benefit from religious beliefs and practices to cope with stressful life happenings and enjoy mental peace and purpose in life. A comprehensive systematic review has shown that there is an association between religion, psychiatry, psychology, and medical care [17]. The correlation between religiousness and health can be largely explained by lifestyle [18]. A healthy lifestyle is very essential for a good life. Adequate physical activity and a limited amount of passive behavior can prevent various chronic diseases [19]. To date, many studies suggest the relationship between religious involvement and a variety of health outcomes. Some of them hypothesize the influence of religion on "health behaviors". But the underlying processes of the connection of religion and health have been studied very little [20].

Religion is the most momentous component of human nature for attaining the meaning and purpose of life. It has a great role in the patient's health [21]. Religion is a serious protective effect on risky health behaviors [22]. Greater religious involvement is correlated with positive lifestyle practices [23]. A study indicated that there is a relationship between risky drinking and religion. The religious group in contrast to the non-religious group had a lower probability of risky drinking [24].

Some studies indicated that self-efficacy and self-esteem are mediators of the relationship between religious involvement and health outcomes. But there is mixed evidence about the relationship. For example, one of the studies indicated that self-esteem and self-efficacy mediated, at least in part, the relationship between religious beliefs and better vegetable and fruit consumption, and lower alcohol consumption. Also, religious behaviors have a direct impact on health behaviors [25]. Research outcomes suggested that self-regulation can be the expository mechanism for the association between religiosity and positive health behaviors. However, various religious motivations have different impacts on health-related outcomes. These can be the reason for the negative effects of religion that are found in some research. One study suggested that self-regulation mediated the association between religious motivation and health behaviors [26].

A study indicated that a religious intervention can improve the patient's health [27]. Another study suggested that an intervention based on religion can promote the health of patients. In several studies, religious interventions had a desirable impact on patients [28]. Health behavior components also can predict mental health levels [29]. Psychological health is related to physical health outcomes. One of the effective components of this relationship is health behaviors. Higher distress is related to less eating fruits and vegetables and physical activity, and higher sedentary behaviors and smoking [30]. Also, mental illness and health behaviors are associated strongly. But the causality of the link is challenging. A new study indicated the correlation between psychological distress and health behaviors. Furthermore, there is a causal relationship between psychological distress and health behaviors [31].

The underlying processes of the connection between religion and health have been studied very little [20]. Also, interest in the role of religion and spirituality in mental health is increasing [17]. Due to the remarkable burden of disease which is correlated to unhealthy behaviors, more research is needed to understand the causal links between mental health and health behaviors [31]. Given the ascendant rates of mental disorders in colleges across the world [12], the correlation between religious/spiritual behaviors and beliefs with mental health [14], the importance of research in this field, the relatively high levels of religious involvement among some cultures, and the important role that ego-strength may play in the relationship of religious health behaviors and mental health and illness, the present study examined the mediating role of ego-strength in the relationship between religious health behaviors and mental health symptoms and illness in a group of university students.

The research hypotheses in the present study are as follows: (1) there is a positive significant relationship between religious health behaviors and mental health, ego-strength, and all of its subscales, and a negative significant relationship with mental distress. Also, there is a positive significant relationship between ego-strength subscales and total score of it with religious health behaviors and mental health, and a negative relationship with mental distress; (2) The ego-strength can mediate the relationship between religious health behaviors and mental health symptoms and illness.

METHOD

In the present study, the mediating role of ego-strength in the relationship between religious health behaviors and mental health and distress was investigated using structural equation modeling from May 2021 to August 2021. The conceptual model is shown in Figure 1.

The data were analyzed using SPSS 26 and Amos software.

Figure 1: Conceptual model

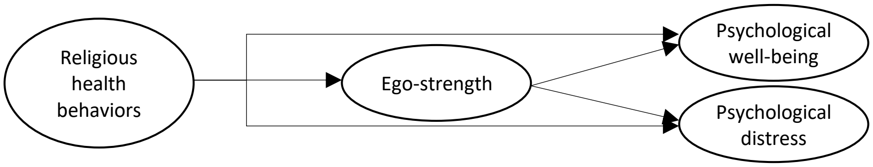

Participants and procedure

The present study is a descriptive one. The statistical population of the present study was university students. A random sample of 346 university students (M = 23.01, SD = 7.12) participated in the present study. The inclusion criteria were being a student, being literate, not suffering from serious psychiatric or physical disorders, and not having substance dependence. All of the students signed an informed consent document before completing the scales. The students' demographic characteristics showed in Table 1.

Table 1: Study sample characteristics

Variables		n	%
Gender	Male	45	13
	Female	301	87
Levels of education	Undergraduate	297	85.8
	Postgraduate	46	13.3
	PH.D. students	3	0.9
Marital status	Single	273	78.9
	Married	73	21.1
Job	Student	289	83.5
	Teacher	43	12.4
	Other jobs	14	4.1
Number of children	0	307	88
	1	13	0.03
	2	17	0.04
	3 +	8	0.02

Measures

Demographics. At the top of the page of the first measure, participants reported their gender, educational levels, marital status, job, and the number of children.

Religious Health Promoting Behaviors Inventory. Religious health-promoting behaviors in participants were measured by the Religious Health Promoting Behaviors Inventory (RHPBI; [32]). It is a 16-item measure that assesses the personal behaviors and habits based on religious doctrines and beliefs in everyday life on a 7-degree scale ranging from 0 (completely disagree) to 6 (completely agree). Cronbach's alpha coefficient for the scale questions was obtained in a sample of 93 individuals (0.88). Convergent and differential validity of the RHPBI was calculated through the simultaneous implementation of the Spiritual Well-being Scale (SWBS; Palutzin and Ellison 1982) and the Mental Health Inventory (MHI; [33]) in the mentioned sample. The results of Pearson correlation coefficients showed that there was a significant positive correlation between the scores of subjects in the RHPBI and SWBS and psychological well-being ranging from 0.37 to 0.49 (P < 0.001) and a negative correlation with psychological distress (-0.33, P < 0.001). These results confirm the convergent and differential validity of RHPBI [32]. In the present study, Cronbach's alpha coefficient for the scale questions was 0.721.

Mental Health Inventory. The mental health of participants was measured using the Mental Health Inventory (MHI-28; [33]). It is a short form of the 34-item Mental Health Scale (Veit and Ware 1983) and it is a 28-item test that has 22 psychological well-being and psychological distress items in a 5-Likert scale ranging from 1 (completely disagree) to 5 (completely agree). The psychometric properties of the 28-item form of the scale were studied. Cronbach's alpha coefficients of psychological well-being and psychological distress for the scores were 0.94 and 0.91. The correlation coefficients between the scores were calculated with a 2-week interval for test-retest reliability assessment. Coefficients for psychological well-being and psychological distress were r = 0.90, r = 0.89, and P < 0.001 respectively. The concurrent validity of the MHI-28 was measured by the simultaneous implementation of the General Health Questionnaire. The results of Pearson correlation coefficients showed that there was a significant negative correlation between the general score of the subjects in the general health questionnaire and the subscale of psychological well-being (r = -0.86, P < 0.001), and a significant positive correlation with the subscale of psychological distress (r = 0.89, P < 0.001) [33]. In the present study, the subscales were consistent internally and Cronbach's alpha coefficients were 0.888 for psychological well-being and 0.903 for psychological distress.

Ego-Strength Scale. The Ego-strength of participants was measured using the Ego-Strength Scale (ESS; [6]). It includes 25 items that have been validated to assess the power of ego in managing and controlling life situations that are difficult. The instrument's items are rated on a 5-degree Likert-type scale ranging from 1 (not at all) to 5 (very much). In several studies in the general (n = 1257) and clinical population (n = 372), the psychometric properties of ESS have been assessed and confirmed [6]. The scale includes 5 subscales (Ego control, ego resiliency, mature defense mechanisms, problem-focused coping strategies, and positive emotion-focused coping strategies). In one study, all subscales were internally consistent and the Cronbach's alpha coefficient ranged from 0.73 to 0.91 [34]. In the present study, all subscales were consistent internally and Cronbach's alpha coefficients ranged from 0.753 to 0.876.

RESULTS

In the present study, the mediating role of ego-strength in the relationship between religious health behaviors and mental health and distress was investigated using structural equation modeling. Using structural equation modeling requires observing the assumption such as normality, outlier data control, linearity, and multicollinearity that need to be met before examining the results of research questions. Kolmogorov-Smirnov test was used to test the normality of the data for each of the research variables. The results showed that the assumption of normality was observed.

The boundary data method was used to evaluate the outlier data. It was indicated that the assumption has been observed. Also, based on Table 2 and 3 data, the assumption of linearity and multicollinearity was found. Based on the assumption of linearity, there must be a linear relationship between the criterion variables and the predictors. So, the results of the correlation matrix indicated that there is a positive significant relationship between religious health behaviors and mental health, ego-strength, and all of its subscales (including ego-control (ECON), ego-resiliency (EROS), mature defensive mechanisms (MDEF), problem-focused coping strategies (PFOC), and positive emotional focused coping (PEMO)), and negative significant relationship with mental distress. Also, there is a positive significant relationship between ego-strength subscales and its total score and religious health behaviors and mental health, and a negative relationship with mental distress. As Table 3 shows, the correlation between all variables was significant (P < 0.001).

Table 2: Descriptive indicators of research variables

Variables	Scale	*M*	*SD*	*V*	Min. score	Max. score
Religious health behaviors	RHPBI	65.04	11.84	140.34	21	98
Psychological well-being	MHI	50.45	9.92	98.43	19	79
Psychological distress	MHI	35.04	10.75	115.74	7	62
Ego-strength	ECON	16.95	3.55	12.65	5	29
	EROS	16.55	3.88	15.12	6	25
	MDEF	15.91	3.81	14.54	6	47
	PFOC	17.33	3.15	9.96	7	25
	PEMO	16.79	3.22	10.43	8	25
	Total	83.55	14.27	203.81	35	120

According to the point that the entering of 2 predictor variables with a correlation coefficient of more than 0.70 in these types of analysis is not allowed, the correlation matrix results were observed. The results showed that there was a noticeable relationship between the criterion and prediction variables, but the relationship was not sufficient for multicollinearity. The highest correlation value was 0.671 for the EROS subscale of ego-strength. Accordingly, all variables were kept in the analysis.

Table 3: Correlation matrix between ego strength, religious health behaviors, mental health, and distress

Variables	1	2	3	4	5	6	7	8	9
Religious health behaviors	1								
Psychological well-being	0.22	1							
Psychological distress	-0.22	-0.48	1						
EROS	.025	0.48	-0.48	1					
ECON	0.32	0.38	-0.36	0.61	1				
MDEF	0.25	0.27	-0.36	0.58	0.55	1			
PFOC	0.28	0.34	-0.29	0.60	0.67	0.45	1		
PEMO	0.30	0.43	-0.30	0.58	0.49	0.42	0.56	1	
Ego-strength	0.35	0.47	-0.45	0.67	0.65	0.66	0.60	0.54	1

P< .001

In reporting the results of structural equations, the main component in examining the fitness of the hypothetical model is the necessary indicators for reporting and the scientific acceptable criteria for confirming these indicators. According to the experts, from all 3 groups of fitness indicators (absolute, adaptive, and purposive), some indicators must be reported (Figure 2).

Figure 2: Unmodified hypothetical model of mediating role of ego-strength in the relationship between religious health behaviors and mental health and distress

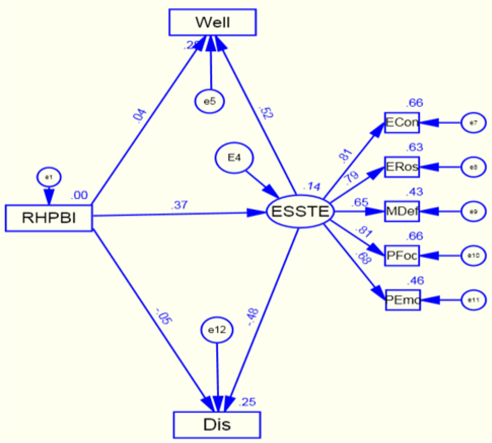

According to Myers et al. (2006), the numerical value of 3 for the relative chi-square index, the numerical value of 0.60 for root mean square error of estimation, and the numerical value of 0.90 for adaptive indicators are acceptable. Also, for purposive indexes, numerical values greater than 0.50 are suitable. Given the results of Table 4 and the cut-off points of each index, the hypothetical model needed to be modified. The test for fitting the model with observed data, using stepwise selection model correction showed that the model can be corrected: In the ego-strength variable, by creating covariance between the residual error of "ECON and PEMO" indices and the residual error of "EROS and MDEF" markers as well creating covariance between the residual error of mental health and distress (Figure 3).

Figure 3: Modified hypothetical model of the mediating role of ego-strength in the relationship between religious health behaviors and mental health and distress

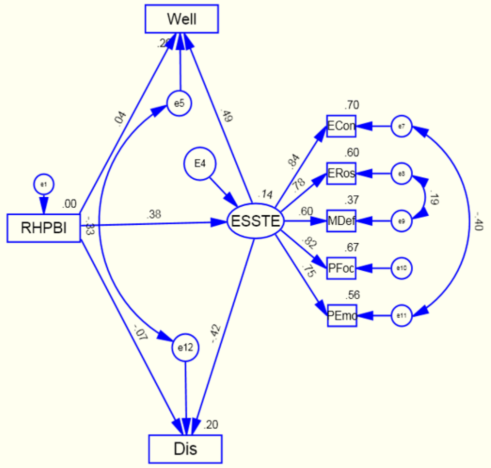

Table 4: Goodness of fit indices of unmodified and modified assumption model

	Absolute indicators				**Adaptive indicators**			**Conventional indicators**	
Statistics	Chi	df	Chi/df	RMSEA	GFI	NFI	CFI	PCFI	PNFI
Unmodified	135.80	20	6.79	0.130	0.896	0.880	0.895	0.639	0.628
Modified	72.507	17	4.265	0.210	0.950	0.936	0.950	0.557	0.568

As Table 4 shows, the modifications made on the hypothetical model resulted in a 3-degree decrease in the degree of freedom in the modified model, and a 63.294-degree decrease in the chi-square value, resulting in a 2.535-degree decrease in the relative chi-square value. Therefore, in the modified model, indicators of absolute fit indices along with other indicators such as root mean squared error of approximation with a confidence interval of 0.90, adaptive indicators (GFI, NFI, and CFI) with a cut-off point of 0.90, and the indicators (PCFI, and PNFI) with a cut-off point of 0.60, are in a desirable condition. It indicates that the hypothetical modified model is valid.

The results of the modified model show that the ECON subscale with a coefficient effect of 0.84, had the maximum contribution and the MDEF subscale with a coefficient effect of 0.60, had the minimum contribution in the score of ego-strength. The factor loadings of all paths were significant in the hypothetical model. The results also indicated that the direct effect of religious health behaviors on mental health and distress were respectively 0.4 and -0.5. Based on these results, it can be said that religious health behaviors have had a positive non-significant effect on mental health and a negative non-significant effect on mental distress.

To investigate the statistical significance of the indirect effect of religious health behaviors on mental health and distress through ego-strength (the mediating role of ego-strength on the relationship between religious health behaviors and mental health and distress), the Bootstrap method was used.

The results showed that religious health behaviors have affected mental health through ego-strength 0.185. The result is significant statistically (P = 0.001) and suggested that with one unit increase in religious health behaviors, 0.185 units will add to the mental health scores. The results also suggested that religious health behaviors have affected mental health and distress through ego-strength -0.159 The amount is significant statistically (P = 0.001) and suggested that with a unit increase in religious health behaviors, 0.159 units will decrease in the mental distress.

DISCUSSION

The COVID-19 pandemic is one of the risk factors for psychological diseases. COVID-19 related post-traumatic stress symptoms are correlated to distress, anxiety, and low sleep quality [35]. Furthermore, during and after traumatic events, depression has been increased [36], but during the COVID-19 pandemic, the increase is more than in previous traumatic events.

One of the mechanisms through which ego-strength can affect mental health and illness symptoms is resilience (one of the ego-strength components) during the COVID-19 pandemic. Resilience is the dynamic process of adapting positively to hard and negative experiences [37]. Using this feature probably enhances one's ability to manage conflicts.

Ego-strength has an important role in predicting health and reflects the attitudes toward problems, responding to failure, and the ability to adapt to one's emotional status. Also, ego-strength plays a predictive role in health conditions and the therapeutic alliance in chronic problems [38]. Ego-resiliency can moderate the association between cortisol level and emotional labor [39].

Another mechanism through which ego-strength can influence symptoms of mental health and illness is the use of enhanced defensive strategies in the COVID-19 pandemic. Defensive mechanisms are responsible for protecting the individual against anxiety and awareness of the dangers and causes of internal and external stress. Ego-strength enhances problem-solving skills [40], and using these effective strategies to cope with stress can increase mental health.

Maladaptive and adaptive coping can moderate the association between life events and mental health problems [41]. Coping strategies moderate the association between job insecurity and mental health [42]. Empowerment helps the individual to manage stressful situations to some extent by using adapted emotion regulation strategies [6].

There has been some research on the relationship between ego-strength dimensions and depressive disorders that support the finding of the present study [43]. In interpreting the finding, we can note the relationship between ego-strength and psychopathology. Ego-strength is one of the key components of psychodynamic models for explaining psychological trauma. Low ego-strength makes the individual unable to perform the reality test properly, increasing interpersonal and intrapersonal conflicts [44]. Therefore, the development of mental disorders seems to be related to low ego-strength. Low ego-strength is associated with dysfunctional defensive mechanisms, lack of adaptive capacity to defeat, and low level of arousal. One of the main syndromes reflected in the functioning of neurotic patients and personality disorders is the low level of ego-strength and poor defensive mechanisms [7].

Ego control, the ability to control and manage situations is another component of ego-strength [45]. Under controlled individuals have some characteristics such as self-dramatizing, unpredictable, and unable to delay gratification, assertive, moody, rebellious, and self-indulgent. Over controlled individuals have some characteristics such as being consistent, bland, calm, and dependable. Resilient individuals have some traits like having a high level of aspiration and wide interests, socially poised and skilled, assertive, and cheerful, and neither lacking personal meaning in life, emotionally bland nor self-defeating [46].

Limitations and Future directions

There are some limitations in the present study which are worth acknowledging. The findings are derived from a cross-sectional method that can influence the results, and causal interpretations of the findings are speculative. The issues of causality could best be investigated through longitudinal surveys. The present study was conducted only on university students (most of them were young), and the generalization of the findings to less-educated persons should be done with caution. Also, the findings may not fully generalize to other countries, because of the diverse cultural and socioeconomic factors. Furthermore, due to the higher number of female students in some universities, the number of women in the present study is much higher than men. Therefore, this point should be taken into account when generalizing the results. Future studies can explore similar questions in other samples such as subjects with various diseases, different group ages, various education levels, and different socioeconomic and cultural environments. The ego-strength is a novel variable that is not yet well known and needs a lot of research attempts to understand it and the various roles it can play. There may be many other variables that can mediate the relationship between religious health behaviors and mental health and illness and can be explored in future research. Finally, there may be other alternative hypothetical models that had not been evaluated which would fit the data well or even better. Future research can test alternative or more complex models for the present variables.

CONCLUSIONS

In conclusion, the current study provides evidence that ego-strength can mediate the relationship between religious health behaviors and mental health symptoms and illness. Also, the results showed that there is a positive relationship between religious health behaviors and mental health, ego-strength, and all of its subscales, and a negative relationship with mental distress. Furthermore, there is a positive relationship between ego-strength subscales and their total score and religious health behaviors and mental health, and a negative relationship with mental distress during the Covid-19 pandemic. The data provides new insights into the importance of ego-strength in the religious health psychology area. Mental health and religious health behaviors are both very important psychological variables. The present study helped to clarify the relationship between these variables. Any practical effort to reinforce ego-strength can help strengthen the relationship between mental health and religious health behaviors. Everyone can benefit from the results of the present study.

Ethical Approval

All procedures performed in studies involving human participants were under the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent was obtained from all individual participants included in the study.

Conflict of interest

All authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Exploring of clinical decision making in care of patients with acute care in critical nurses: A qualitative content analysis

Mohammad Aghajani[1], Neda M. Ajorpaz[2], Mohsen Taghadosi[3]

Abstract: *The appropriate clinical decision-making of the nurses can improve the patient outcome and reduce costs, otherwise delaying the recovery and preferably the death of the patient especially in acute care setting. The aim of this study was to describe the clinical decision making in care of patients with acute care in critical nurses.*

This study was a qualitative study conducted in 2020. Purposive sampling was used and continued until saturation. The study involved sixteen semi-structured interviews were conducted with critical nurses who had experience providing care for acute patients in critical care units. Interviews transcript and analyzed by qualitative content analysis. Two major themes were generated from the data included instantaneous decision-making and life-saving of the patient. This process was depended on the nurse’s ability to summarize in the moment, to consider everything, to focus on the patient, to combine experience and knowledge, to concern the patient's life, to prevent the deterioration of the patient's conditions and responsibility to their patients.

Keywords: *clinical decision making, acute care, critical nurses, COVID-19*

INTRODUCTION

Clinical decision making (CDM) is a fundamental phenomenon in healthcare practice. While CDM influences the entire range of healthcare practitioners, nurses, as frontline clinicians, are faced with important clinical decisions on a daily basis [1, 2].

The appropriate decision-making of the nurse can improve the patient and reduce costs, otherwise delaying the recovery and preferably the death of the patient [3]. Based on studies, it is identified that up to 98,000 patients die each year as a result of poor decision-making in healthcare [4]. Also research shows that not making the right decision in a timely manner can prolong the treatment process and the situation of patients [5]. 4% of patients admitted to hospitals in the United States, about 16% and 6% in Australia and the United Kingdom have suffered injuries such as permanent disability and death due to lack of proper decision-making. While the right and timely decision making, team working and effective communication between staff can reduce half of these injuries [6].

Rapid and appropriate decision-making is a need of organizations and hospitals. However, complex issues surround the decision-making process, such as the past experiences, values, beliefs, skills and technical knowledge of the decision maker [7].

CDM in nursing is frequently based on two styles of thinking: analytical and intuitive thinking. Analytical thinking is a logical style of thinking based on objective data. Intuitive thinking is driven by intuition and experience [8]. In life-threatening conditions and crises, as well as situations wherein there is a need for creativity and innovation, nurses use intuition in their decision making. Therefore, in critical health conditions, CDM is essential for nurses to improve quality of care, manage crises, and diagnose patients’

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conditions, as well as to help reduce mortality rates [9].

Currently, nurses around the world are dealing with COVID-19, a disease that continuously demonstrates a variety of unknown and newly-known features. The global COVID-19 mortality rate is between 3% and 15% [10]. In this condition, due to lack of knowledge and clinical guidelines, nurses CDM have a vital role in managing the outcome of the disease and the health of clients. Decision-making in critical care nursing is a complex process [11]. Nurses must consider numerous, potentially competing factors when making decisions to meet patient and family needs [2]. This process is further complicated by the fact that nurses may care for five or more patients in a critical care unit [2]. The nurse's decision making in critical care is highly demanding, while critical nurses make a clinical decision almost every 30 seconds [12].

Therefore the question is how do nurses make decisions in acute situations. A better understanding of decision-making research in this context can help to guide future efforts to support nursing practice. Therefore, this study explores the experience of clinical decision making in care of patients with acute care in critical nurses.

METHOD

The present study was a qualitative descriptive study which was conducted to explore the clinical decision making in care of patients with acute care by critical nurses in 2020. Content analysis is a research approach for making inferences from data to their context, with the purpose of providing new insights, knowledge and the representation of the facts [13]. Qualitative content analysis as a research method provides an instrument for making inferences from data to their context [13], and in this process, the textual data explored and interpreted using the systematic coding process [14]

Participants and sampling

The purposive sampling was conducted among nurses who were providing care for patients in critical care units including the intensive care unit (ICU) and Emergency Departments (ED) of subspecialty Hospital of Kashan University of Medical Sciences. This comprehensive hospital allocated to care of COVID-19 patients. The inclusion criteria included the working experience in critical care units at least for two years and willingness to participate in the research. The exclusion criterion was reluctant to continue the study. The first participant was a nurse with independent and fast decision maker in the research setting, which had recommended by three nursing managers and was interested to speak about his experience.

Sampling continued until data saturation was done. Data saturation was identified through repetition of the participants' data in the interviews and that no new content is obtained [15]. In our study saturation occurred after interviewing with 16 participants.

Data collection

Prior to initiating interviews, main researcher was attempted to obtain the collaboration of the participants by creating a trust-based environment, explaining the aim of the study and ensuring the confidentiality of information. The data was collected with semi-structured interviews at the time and place that was appropriate for the participants. The process of interviewing initiated with open-ended questions based on the aim of the study. All interviews were recorded (Table 1).

Table 1: Interview techniques for data collection

Researcher activity
Introducing myself and the purpose of the study
Explaining the content of the consent form and answering the questions
Obtaining a verbal agreement and signing the consent form
Starting the voice recorder
Starting the interview with general open-ended questions such as: How do you make a clinical decision making in a critical situation? Would you please tell me about how do you of intuitive decision making in the care of COVID-19 patients? Would you please tell me about your experiences of clinical decision making for a COVID-19 patient without documentation? What conditions have you encountered in bedside that has caused you to use intuition? Please explain.
Asking for example: Please give me an example of your clinical decision making. Using active listening techniques: Eye contact, silence, head nods, use of statements such as "OK I see…". Watching for non-verbal cues: eye contact, facial expressions, tone of voice, gestures …
Using probing questions: Please tell me more about that. What else can you remember about that situation? Can you think of another example of this? What Exactly did you say? I'd be inspired.
Get more details: Tell me more about that. Can you tell me more about that?
Clarifying what the participants said: Please say what you mean by…. When you say … what are you actually doing or feeling? So you are saying...?
Summarizing the interview: It sounds like you are saying, "...". Is that a fair summary?

The participants were asked to discuss what they experience and understand as nursing CDM in moment in critical unit. The interview guide included the following questions:

- How to make clinical decisions in critical situations or acute care? Please explain your experience in this field.
- Why/how did you make such a clinical decision making?
- what are you actually doing or feeling?
- Tell me more about that. Can you tell me more about that?

Interviews were held in a private room, so that the privacy of the people was respected. The room was disinfected before and after each interview. The interviewer and the participant wore masks and gloves, and at least one meter separated the interviewer and the participant to observe social distancing.

The interview took between 40 to 80 minutes depending on the participants' circumstances. The interviews were conducted in Persian and analyzed and the results were translated into English only in the final report. The conversations were recorded on the audio file and then transcribed immediately.

Data Analysis

The transcribed interviews were analyzed through qualitative content analysis method following an inferential approach. The process of organizing the qualitative data in inferential content analysis included open coding, abstraction, and creating categories [16]. Elo and Kyngas pictured inferential categorization plan in qualitative content analysis [13]. Data analysis and data gathering were conducted simultaneously. Coding and classifying themes were done by the researchers

Trustworthiness

To ensure the trustworthiness of this qualitative research, confirmability, credibility, dependability, and transferability were established [17]. We used rewriting, member check, reviewing texts and using the participants' own words, monitoring translations, recording audio, the conducting of interview, coding, themes and sub themes and using of direct quotes to support the findings.

The researcher used an adequate amount of time to collect comprehensive data. The researcher informed them about the objectives, and established trust with participants throughout the interview and providing feedback to the research team for further interviews. We employed the MAXQDA 10 software for data management.

Ethical considerations

The study was approved by Ethics Committee.

The participants were informed about the study's objectives and consent form was obtained from all participants.

RESULTS

The participants were 16 critical nurses. Ten participants were women. Their nursing experience ranged from 4 to 25 years, and their academic degrees consisted of bachelors, masters, and Ph.D. (Table 2).

Table 2: Baseline characteristics of participants (n=16)

Characteristics	N (%) or mean ± SD	Median (range)
Gender		
Male	6 (37.5)	
Female	10(62.5)	
Age (year)	36.56±6.58	37.5 (26-52)
Working Experience	12.62±5.59	13.5(4-25)
Interview duration (min)	52.31±1.09	49.5 (40-80)
Education		
BSC	12 (75)	
MSC	3 (18.8)	
PhD candidate	1 (6.2)	
Marriage		
Married	11 (68.8)	
Single	5 (31.2)	
Current ward		
ICU	9 (56.2)	
CCU	6 (37.5)	
Nursing room	1 (6.2)	
Duty		
General nurse	14 (87.5)	
Head nurse	1 (6.2)	
Clinical supervisor	1 (6.2)	
Shift type		
Fixed	2 (12.5)	
Rotation	14 (87.5)	
Type of employment		
Official	8 (50)	
Contractual	8 (50)	

Based on data analysis, 362 primary codes, 7 subcategories, and two categories emerged (Table 3).

Table 3: The themes and subthemes of nurses caring for acute patients.

Themes	Subthemes
Instantaneous decision-making	Summarizing in the moment Considering everything Focusing on the patient combining experience and knowledge
Life-saving of the patient	The importance of the patient's life Deterioration of the patient's conditions Responsibility

From the point of view the participants and based on their experiences, the CDM of critical nurses had two major characteristics:

1. instantaneous decision-making (summarizing in the moment, considering everything, focusing on the patient, combining experience and knowledge) and

2. life-saving of the patient (the importance of the patient's life, deterioration of the patient's conditions, responsibility).

Instantaneous decision-making

Almost all nurses stated that they made instantaneous decisions in the face of critical situations, especially those where the patient's life was in danger. This DSM seems to be intuitive because it does not have the linear and logical features of evidence-based decision making.

A participant (P3) stated, "In a night shift, there was a 30-year-old patient in respiratory distress. I had an inner sense indicating that the patient's health conditions would quickly deteriorate. I begged the physician, explaining that this was an emergency... there's going to be a need for endotracheal intubation... we must intubate the patient right now. The physician said there was no need for intubation at the moment, and we needed to monitor the patient to see if the patient's conditions would change. After a short while, the respiratory distress worsened very quickly, and the patient passed away. Unfortunately, we did not have the chance to intubate the patient and start mechanical ventilation on time. Later, the physician told me I was right...we should have intubated the patient sooner." Indeed we see the perception of the inner sense, followed by anticipation and pursuit, and the inner revolution in the intuitive decision making.

Summarizing in the moment. Ten participants indicated that in critical situations in a short period of time, everything quickly summarized in their minds. In fact, at the critical moment and before the action, they summarized and concluded what they were receiving from the patient's signs and symptoms at the moment.

One participant (P15) said "….Everything is summed up in your mind until you decide what to do at that moment…."

Considering everything. Nine participants indicated that they unconsciously considered all conditions, possibilities, prognosis, and possible consequences for the patient.

The participant (P11) said: "A patient with a chest pain was brought to the emergency department by 115, I unconsciously compared and adapted all the patient conditions in my mind and I guessed the possibility of a heart attack that was happened." Another participant pointed: "I consider the patient's history and prognosis, for example, that she had a history of heart disease based on my experiences."

Focus on the patient. About half of the participants reported that when caring for acute patients, their minds were constantly engaged with the patient during resting and working time, and they focused on patient.

One participant (P4) reported "….even I thought about my patient while resting in the department". Another participant believed that "I kept telling myself if something bad is going to happen what I should do, hence, I went to the patient's bed and by the way something happens to her at that moment". Another participant indicated "…. concerned about my patient until morning".

Combining experience and knowledge. Fourteen of the 16 participants reported combining their previous knowledge and experience in acute patient conditions. A participant stated, "In MI patients, I have seen that their skin color changes to whitish and blackish colors. Many COVID-19 patients also show these changes before a heart attack. In fact, I was reminded of previous experiences and the things that had read previously in books and combined them." (P1).

Life-saving of the patient

Almost all the participants expressed reason for the CDM in patients with acute care were to save the patient's life. Participants indicated that the life-saving of the patient was a priority for them. The nurses believed that even if decision making was not in accordance with the usual and legal process of unit, but they did it if it was necessary to save the patient's life.

Some participants compared the COVID-19 condition to a battlefield. "I feel like I'm in a battlefield. When you have seven cases of death in just a few hours, you believe whatever you do, even if not necessary, might save your patient's life." (P5).

The importance of the patient's life. Participants considered life-saving of the patient to be the most important blessing from God and lack of appropriate and immediate clinical decision-making in critical situations was equated with patient loss.

"For me, the patient's life is the priority. If I feel confident something is critical for the patient's life and health, I will definitely do it". (P8). Another participant indicated "…receiving Pack cell are very important for the patient and may save patient life".

Deterioration of the patient's conditions. The participants reported that due to the importance of golden time in patients in critical situations, decision making is very important and lack of timely decision making will deteriorate the patient's conditions.

“I experienced in ICU that you put your hand on the patient and you see that it is cold, that is, lack of heart pulse needs 3 to 4 hours of care. So if we do not do something worse, we have to make a timely decision”. (P7)

Another participant considered the heavy workload and the related tensions as factors for relying to their clinical decisions. “Everything was novel. The whole staffs were feeling the same and were stressed. You felt extremely concerned about worsening your patients’ conditions. In these conditions, you feel like you have to trust your decisions.” (P5)

Responsibility

Almost all participants believed that they were responsible for their conscience, professional responsibility, patients, religious beliefs and society.

. “A sense of responsibility did not leave me alone. Based on your conscience, you feel responsible to do your best for your patient” (P15).

“To some extent, it is a kind of feeling of responsibility... About the COVID-19 crisis, we feel we are responsible for patients in all aspects.” (P3). Another participant (P7) believed that patients benefit from nurses’ decision making. “I felt like it was better for the patient to get discharged and go home. A hospital environment in this situation is not safe for the patient.”

DISCUSSION

Clinical decision making is a main role of professional nurses [18]. This study was conducted to clarify the clinical decision making in care of patients with acute care in critical nurses and two themes were categorized as the main characteristics for a critical nurse including "instantaneous decision-making" and "life-saving of the patient".

On the first category that emerged in the results, in the acute conditions of the patient, in which time plays an essential role in saving the patient, the CDM of the nurses and subsequent treatment-care actions are very important. CDM distinguishes the professional nurse from the non-professional care staff [18]. Critical nurses decided by focus on patients and considering everything related to their patient, then they summarized data in their mind and cared of the patients with integrating experience and knowledge. The research show that each nurse uses to make a decision depends more on the type of task and the field conditions than on her knowledge and experience [19-21]. In the other hand, effective decision-making requires a combination of knowledge and skills and close communication with the patient for a deep understanding of the situation, which has been interpreted by some authors as "seeing the holistic view" or "deep understanding of the clinical view" [22-24].

Two categories show that the most important item for deciding in a patient's acute conditions is life saving of patient. The participants in this research indicated that they make decision because of the importance of the patient's life and responsibility towards their conscience, profession, society and in order to prevent from worsening the patient's conditions. Other studies in intensive care unit nurses confirm these finding [5,11,25,26].

Overall, based on the findings and features extracted, CDM in acute care settings seems to be more intuitive than analytical. Today, in health novel and complex conditions, this type of decision-making has become even more important because of the care of patients with COVID-19. Recently, the use of intuition in the process of caring for the psychological aspect and its effects on decision-making has become a favorite subject for health researchers [11,22,27].

CONCLUSION

The findings of the study described how and why critical nurses in acute care made clinical decisions. Indeed critical nurses by instantaneous decision-making save life of the patient and prevent the deterioration of the patient's conditions. Nurses tried to provide the best outcomes of care and treatment through analytical and especially intuitive model in acute situations. This CDM was dependent on the nurse’s ability to summarize in the moment, to consider everything, to focus on the patient, to combine experience and knowledge, to concern the patient's life, to prevent the deterioration of the patient's conditions and responsibility to their patients. These findings can provide the basis to promote competency and practical guidelines for clinical nurses and health managers.

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Ethical Issues

This study research was approved from the Ethics Committee of Kashan University of Medical Science with the ethics code: IR.KAUMS.MEDNT.REC.1398.068.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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ORIGINAL ARTICLE

Factors that influenced infection complications in combat related penetrating brain injuries during conflict in Ukraine

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Abstract: *Infection complication has a major impact on outcomes of patients with combat related penetrating head injuries. There is still a discussion around which factor influence outcomes and infection complication. In the beginning of war, in 2014, the evacuation system was not ready for a sudden start of hostilities because of limited medical resources, the lack of neuro visualization in the frontline, the lack of trained personnel provided first aid to the wounded.*

Aim: The aim of this study is to determine the factors that influenced the development of infectious complications in penetrating combat head injuries with limited medical capabilities on theatre

Materials and methods: This is retrospective study of 79 military cases with penetrating head injuries, which was received during the fighting in eastern Ukraine in the period from March 2014 to the end of December 2017. Information was collected on demographics, evacuation assistance, type of injury and infectious complications. R commander was used for statistical analysis. Statistical significance was defined as p<0.05.

Results: Infection complication of CNS were detected in 31 cases (39,24%). Factors that influence rate of infection are low GCS on first evaluation (p<0.001), CSF leak (p=0.002), ventricular injury (p<0.005), installation of tidal drainage (p=0.005), superficial wound infection (p=0.013) and wound dehiscence (p<0.001).

Conclusions: There are many controversies in managing penetrating gunshot wounds to the head. Still exist lack of information that neurosurgeon can trust. Data even about small samples still very informative.

Keywords: *infection complications; penetrating brain injury; military trauma; Ukraine*

BACKGROUND

Infection complications (IC) is one of most common reason for unfavorable outcomes after penetrating head trauma [1]. Infection is a reason for severe morbidity and significantly influences mortality rate. This complication prolongs patients' hospitalization time, as well as delays the recovery process with rehabilitation and prevents soldiers return to the battlefield [2]. The rate of IC in combat penetrating trauma higher than in civilian gunshot wounds. Mostly this is due to extended time to the first aid and extreme conditions existing at the war theatre [8]. The war in eastern Ukraine has been going for more than 7 years. In early 2014, the evacuation system was not ready for a sudden rise of hostilities. The most limitations included limited medical resources, the lack of computer tomography (CT) at the frontline, the lack of trained first aid personnel providers.

The purpose of our paper is to determine whether known risk factors for the development of infectious complications had influence to our sample group and what other possible factors could influence outcomes.

MATERIAL AND METHODS

This is a retrospective study of 79 patients with penetrating combat related brain injury. The study based on medical records of patients that were treated at National Military Medical Clinical Centre "Main Military Clinical Hospital" (NATO Role IV) from May 2014 until 31 December 2017. All

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patients participated in military conflict in Eastern Ukraine. All injured were evacuated through all echelons. Non-penetrating head injuries were excluded from the analysis.

Information collected from patient recordings include demographics, clinical examination findings during evacuation and in treating hospitals, laboratory results. Computer tomography scans were evaluated by neurosurgeon and radiologist separately. Surgical and therapeutical interventions after injury were noted.

Statistical analysis was performed using R version 3.6.3 (http://www.r-project.org). In univariable analysis, variables were compared between groups by Fisher's exact test for categorical variables and the Wilcoxon signed-rank test for numerical variables. Statistical significance was defined as $p<0.05$. Local ethics committee approval was obtained before the study.

RESULTS

From initial analysis of 109 patients with combat related head injury, 79 had penetrating brain injury. Type of injury included: penetrating (51 cases), perforating (8), ricochet (8), tangential (7), blast related penetrating injuries (5). All wounded were males. Age at presentation was 19.9 to 54.4 years (mean= 31.8, SD=8.4).

Level of consciousness was evaluated with Glasgow Coma Scale (GCS). We divided all patient into three groups according initial GCS. In first group GCS 3-5 were 6 patients, GCS 6-12 – 20 patients, GCS 13-15 – 51 patients, no information was available for two patients. Eight patients deteriorated during evacuation, 28 patients had the same level of consciousness, 32 improved, and in 11 cases information was not available. Loss of consciousness in battlefield for different periods of time was documented in 17 cases.

Bullet gunshot wounds were found in 16 cases, missile fragments injury in 58 cases, and 5 cases had injury related to blast wave impact. Isolated head injury was in 39 cases. Multiple injuries of different parts of the body were detected in 40 cases: 26 cases were combined with extremities, 19 cases with chest, 13 cases with face, 13 cases with abdomen, 13 cases with eyes, 4 cases with neck region. In 28 cases head injury was combined with more than 2 body regions. Injury severity score (ISS) were calculated in each case - average ISS was 26.6 (min 9, max 49, SD=9.1). In case of spine injury all patients evaluated with American Spine Injury Association (ASIA) score: grade A – two, grade D – four, grade E – four.

Head computer tomography performed in all cases (Light Speed VCT 64, General Electric, USA) during admission and when needed – in case of deterioration, appearance of new neurological deficit, routinely after surgery.

Initially retained bone fragments was in 63 patients, removed in 32 cases in previous echelon. Retained metallic fragments was in 58 patients, removed in 27 cases on previous stages. On last CT before discharge from hospital bone fragments detected in 26 cases, metallic in 28.

Patients operated because of epidural hematoma (EDH) in 11 cases (five combined with subdural hematoma (SDH), SDH was in 17 cases, intraventricular haemorrhage was in 13 cases, ventricular system weas injured in 17 cases.

Bullet or missile entrance wound was in 31 cases in frontal region, parietal in 22, temporal region in 13 cases, occipital in three, face in one, basal in one, transorbital in three cases. Exit wound was in frontal region in three cases, temporal in three, parietal in one, occipital in one case.

Paranasal air sinuses were injured in 23 cases: frontal sinus in 17 cases (two cases combined with maxillary), basal sinus in two cases (one with maxillary), one case maxillary, in three cases were injured mastoid air cells.

Infection complication of central nervous system (CNS) were detected in 31 cases (39.24%) – details in Table 1.

Table 1: Types of infection complications

Nosology	Number
Meningitis	17
Meningoencephalitis	6
Abscess	4†
Ventriculitis	2*
Empyema	2

*† one combined with empyema, two with ventriculitis, two with meningoencephalitis; * one combined with meningoencephalitis*

Infection complications were proved by clinical and laboratory data. Records of physical examination and local wounds status were also collected. Cerebrospinal fluid (CSF) cultures was analyzed in 12 cases. P. aeruginosa was found in three cases, S. cohnii was revealed in one case. Wound culture was performed in four cases, in one case grew S. aureus.

In 24 cases performed 24 reoperations: 17 patients underwent two surgeries, three patients underwent three surgeries, in one case four reoperations, in three cases five reoperations. Reasons for reoperations varied: CSF leak, removal of retained bone and metallic fragment, infection complications with mass effect, CSF diversion procedures.

Table 2 shows factors that influence rate of infection complications after combat related penetrating brain injury.

Outcomes at discharge was evaluated with Glasgow Outcome Scale (GOS) and present in Table 3. Six cases with GOS1 have different types of infection complications.

Table 2: Analysis of factors that influence rate of infection complications after combat related penetrating brain injury

	CNS infection N=31	No CNS infection N=48	P value
Year of admission			
2014	12 (38.7%)	16 (33.3%)	0.063
2015	14 (45.2%)	11 (22.9%)	
2016	3 (9.7%)	12 (25.0%)	
2017	2 (6.5%)	9 (18.8%)	
GCS on admission to main hospital*			
GCS 3-5	5 (17.2%)	1 (2.1%)	<0.001
GCS 6-12	15 (51.7%)	5 (10.4%)	
GCS 13-15	9 (31.0%)	42 (87.5%)	
Type of penetrating agent			
Bullet	8 (25.8%)	8 (16.7%)	0.615
Missile	21 (67.7%)	37 (77.1%)	
Blast wave	2 (6.5%)	3 (6.3%)	
Retained fragment before surgery	29 (93.5%)	39 (81.3%)	0.186
Bone	28 (90.3%)	35 (72.9%)	0.086
Metallic	24 (77.4%)	34 (70.8%)	0.607
Retained fragment after surgery	16 (51.6%)	23 (47.9%)	0.820
Bone	13 (41.9%)	13 (27.1%)	0.222
Metallic	11 (35.5%)	17 (35.4%)	1.000
Injured air sinuses	10 (32.3%)	13 (27.1%)	0.623
CSF leak	14 (45.2%)	6 (12.5%)	0.002
ISS on admission**	26 (25-30)	25(25-31)	0.357
Ventricular injury	15 (48.4%)	3 (6.3%)	<0.005
Tidal drainage	12 (38.7%)	5 (10.4%)	0.005
Repeated surgeries	15 (48.4%)	14 (29.2%)	0.099
Time to surgery			0.304
>24h	26 (83.9%)	44 (91.7%)	
<24h	5 (16.1%)	4 (8.2%)	
Combined injury	16 (51.6%)	23 (47.9%)	0.820
Brain compression	30 (96.8%)	42 (87.5%)	0.236
Subarachnoid hemorrhage	14 (45.2%)	12 (25.0%)	0.087
Wound infection	10 (32.3%)	4 (8.3%)	0.013
Wound dehiscence	13 (41.9%)	2 (4.2%)	<0.001

**Data was not available for 2 patients; **Median and interquartile range*

Table 3: Outcomes in patients with penetrating brain injury

GOS	5	4	3	2	1
group w/o complications	3	23	20	0	2
group with infection complications	0	9	14	2	6

DISCUSSIONS

Infection complication after penetrating combat related trauma one of the main factors that influence mortality and morbidity rate [1-3,6,13]. It can prolong staying in hospital and demand significant financial and physical resources, prolong rehabilitation process [1]. In our series infection complication was detected in 39.24% (31 cases), that is typical for centre which collect patients from previous levels [7], but this is significantly higher than in recently conflicts (from 5.2 to 31%) [1,7,8,10,11,14]. Structure of infection complications also changed – in pre – and early antibiotic era mainly were diagnosed abscesses and subdural empyema [15], now its meningitis and encephalitis [2,3,5-7].

Our results along with data from other studies showed several factors that influence rate of infection complications after combat injuries – low CGS on admission [1,13], CSF leak [1-4,11,12], ventricular injury [3,4,16] and wound dehiscence [6,13].

Retained bone fragment and repeated surgeries has some tendency to be influence factor but did not reach significance level (p=0.086, p=0.099 accordingly). These factors also described in literature as possible reasons for infection complication with additional circumstances for example CSF leak [5]. There are two different strategies for management retained bone fragments. First strategy includes an aggressive and complete removal of all retained fragment [3,6,7,9,10]. Second is so called «less aggressive» strategy that was proposed after Israeli Lebanese conflict. Authors maintain that less aggressive tactic in removal of fragments are better for outcomes without major influence on infection rate [1,4,6,8-11].

We didn't detect in our study that injury through air sinuses can cause higher rate of intracranial infection complications (p=0.62) but some authors describe it as a factor that influence [3]

Also type of penetrating agent, ISS on admission didn't influence rate of infection complication in our study, but other papers show opposite data [2,16].

Some authors believe that soldiers which were injured in the beginning of conflict have more chances to have an infection [12]. In our study we also can follow similar tendency of this but it did not reach a significance level (p=0.063). This can be explained by lack of organized an evacuation system at the beginning of the war when time of evacuation was extended sometimes up to 3 days.

Information regarding usage of tidal drainage difficult to find in modern literature. It was widely used during the Second World War by the Soviet Army [15,17], and still used in the so-called post-Soviet countries [2,18]. This method of drainage is not commonly used in our hospital. In our series, 12 out of 31 patients, who had IC, had such drainage during evacuation. Our hospital does not support such widespread usage of tidal drain, and in our opinion, this technique should be minimized as it is a predictor of infectious complications (p<0.005).

Time to surgery was hard to evaluate in our study. We can only divide surgery in two major groups – surgery that done in firs 24 hour and later. We cannot detect major difference between this groups. But some authors showed that patients which were operated in first 24 hours have better neurological outcomes and less IC [14,16].

CONCLUSIONS

We found factors associated with infectious complications that were earlier described in literature and also working in our sample as CSF leak, GCS on admission, injury reaching ventricular system, local wound infection and dehiscence. There are many controversies about retained fragments and their role in cause IC. Fast qualified medical care is likely to reduce the risk of IC. It is important to transform a gunshot wound from a penetrating to a closed non-penetrating one, which is the prevention of cerebrospinal fluid and IC. In the case of contaminated wounds, the possibility of installing tidal drainage should be avoided, as this increases the risk of infection.

Limitations

Our study has several limitations. Firstly, some of the data were missing due to its retrospective design, all date were collected from paper histories and sometimes not all hand written information can be right identified and probably missed. In addition, the relatively small sample group prevent us to analyze the contribution to infection risk of other important factors.

Authors' Contribution:
MH, KK did study conception, data analysis, and manuscript writing. MH, KK did data collection and manuscript writing. MH, KK did data collection and manuscript writing. Both authors (MH, KK) contributed to all stages of the article.

Conflict of Interest:
The authors declare no conflict of interest.
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P. Y., P. M., D. A., D. G. designed the study; P. Y collected and analyzed data; P. Y. wrote the manuscript; P. Y., G. O. performed the statistical analysis; All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Health risk behaviors among Iranian university students: A cross-sectional study

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Abstract: *Background and Aim: The prevalence of high-risk behaviors is one of the most serious issues threatening the health of young people. The first step in preventing youth risk-taking is to recognize the problem and its dimensions. The purpose of this study was to investigate high-risk behaviors among students at Tehran's universities.*

Methods: This cross-sectional descriptive-analytical study was conducted from March to August 2019. The sample set was 4,000 students. Data was collected by multi-stage sampling at eight universities in Tehran. Demographic information and standard youth-risk questionnaires were used. SPSS 19 software was used to perform an independent t-test, Pearson ANOVA, and linear regression at a significance level of 0.05 on the questionnaire data collected.

Results: The mean of the total tendency to high-risk behavior was 130.34±40.37. Significant risks identified were driving (27.3±8.33), violence (18.86±6.4), smoking (18.86±7.68), drugs (22.47±8.83), alcohol (24.10±8.57), and high-risk sexual behavior (18.47±7.49). High-risk behaviors were more common in men, single people, and people living in dormitories (P<0.05). Age and gender were the best-correlated predictors of students' high-risk behaviors.

Conclusions: Results show that the tendency to high-risk behaviors in students is a worrying situation. as, at younger ages, the tendency to engage in high-risk behaviors is greater. Among young men and in dormitory life, students are more prone to high-risk behaviors. So, more monitoring in dormitories, especially male dormitories, and better planning and education to reduce high-risk behaviors among students, is necessary.

Keywords: *high-risk behaviors, students, Iran*

INTRODUCTION

The prevalence of high-risk behaviors is one of the most crucial health threats to young people. Health organizations, law enforcement agencies, and social policymakers have raised the issue. Due to rapid social change, some of these behaviors are the cause of death in adolescents and young people and have negative effects on communities [1,2]. High-risk behaviors include violence, arguing, smoking, verbal and non-verbal threats, neglect of studies, high-risk sexual behaviors, drug abuse, suicide attempts, and aggression [3,4]. Adolescents and young people are more at risk than others because they are more likely to engage in high-risk behaviors [5]. By 2030, tobacco-related deaths alone are projected to reach 10 million a year. If the physical, psychological, and social consequences of other high-risk behaviors, such as drug abuse, violence, and high-risk sexual behaviors, are taken into account, the harms are multiplied [6,7].

Among younger people, drug use and high-risk sexual behavior are at the top of the list of high-risk behaviors [8-10]. Also, adolescence is one of the periods in which the person is most affected by the environment and friends, so those around can play a significant role in creating high-risk behavior in this period [11]. In recent years, alcohol and drug use has been one of the biggest health concerns in Iran [8, 12,13]. Research has shown that the most common occurrence of these behaviors is in the university

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environment [6]. Iran has a relatively young population, with almost a quarter of the total population being 15 to 29 years old; and based on the latest census, 25% of the whole Iranian youth population attends universities [14]. The student period is one of the most significant periods for most young people. This period plays an important role in shaping future professional and social life due to the transition from adolescence to youth [1]. The behaviors that students acquire or reinforce on campus may shape their future health and the health of future generations through the potential role of students as parents and leaders [15].

The first step in preventing youth risk-taking is to recognize the problem and its dimensions [4]. However, few integrated studies have been conducted to examine the risk-taking of young people by smoking, psychotropic drugs, alcohol, violence, dangerous driving, and sexual risk in Iran. The purpose of this study is to investigate high-risk behaviors of university students in Tehran, in order to determine the current situation in the field of high-risk behaviors in students and to identify the necessary interventions to improve their health.

METHODS

Design

This descriptive-analytical cross-sectional study was conducted from March to August 2019 on 4000 students of eight universities in Tehran (the capital of Iran). Sampling was done in several stages. At the first stage, eight universities were randomly selected from the list of universities located in Tehran (including University of Tehran, Sharif, Shahid Beheshti, Tehran University of Medical Sciences, Iran University of Medical Sciences, Shahid Beheshti Medical Sciences, Payame Noor and Azad). At the next stage, each university was considered as a class and the list of students was prepared with the coordination and cooperation of the vice-chancellor of each university. The student numbers of the students were systematically selected and entered into the study. If the identified student did not want to participate in the study for sampling, the next person on the list replaced him/her.

The sample size for each university was determined by considering the alpha=0.05 and the accuracy=0.4, standard deviation=0.05 for 484 cases. To increase the validity of the study, the number of samples was determined to 500. A total of 4,000 subjects were examined in 8 state and private universities in Tehran. The inclusion criteria for this study were working in one of the studied universities, spending at least two semesters in the university, being Iranian, and willingness to participate in the study. The condition for leaving the study was the unwillingness to participate in the study.

Study Tools

Data were collected using a questionnaire in the form of self-reports. The first part of the questionnaire included demographic information such as age, gender, educational level, location. In the second part, the standard youth risk-taking questionnaire was used. The risk-taking scale of this study, which was standardized by Zadeh Mohammadi et al., has 48 items in six areas of drugs (8 questions), alcohol (9 questions), smoking (7 questions), violence (7 questions), sexual risk-taking behavior (8 questions) and driving (9 questions). This questionnaire has a five-point Likert scale (strongly disagree: 1, disagree: 2, have no opinion: 3, agree: 4, strongly agree: 5). The total score of the questionnaire is 240-48. This scale does not have a specific cut-off point for distinguishing normal from abnormal individuals and higher scores indicate a greater tendency toward high-risk behavior. Cronbach's alpha coefficient of this questionnaire is 0.93 [16].

This study was approved by the Research Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC 1396.32610). After obtaining permission from the educational deputies of the studied universities, the questioners were sent to the universities. The objectives of the study were explained to the participants by the questioners and informed consent was obtained from the individuals to participate in the study. It was ensured that the identity of the participants and all information would remain confidential.

Data Analysis

Data were analyzed using SPSS software (Version 19). First, the normality of the data was measured based on the Kolmogorov–Smirnov test and it was found that the data had a normal distribution. Therefore, independent t-test, ANOVA, Pearson correlation test, and linear regression were used. A confidence interval of 95٪ was used for the data analysis.

RESULTS

Descriptive statistics

The mean age of study participants was 22.95±4.16 years. 53.4% of the participants were male, and 84.8% were single; about half of the subjects (48.1%) were studying at the undergraduate level. More than half of the residents lived in dormitories. Other information can be seen in Table 1.

Findings of the independent t-test showed that the mean scores of high-risk behaviors and their dimensions, in terms of demographic data in men, had a significantly higher mean in total score and dimensions than women (P<0.05). The results showed the mean of the total tendency to high-risk behavior in single students compared to married students was higher. This significance was also observed in the dimensions of high-risk driving, alcohol, and drugs (P<0.05). Students living in dormitories had significantly higher mean scores on risk behavior and its dimensions, except for the line driving dimension and high-risk sexual behavior (P<0.05) (Table 2).

Table 1: Socio-demographic characteristics (N=4000).

Characteristic	Group	No. (%)
Gender	Male	2135 (53.4)
	Female	1865 (46.6)
Marital status	Unmarried	3394 (84.8)
	Married	606 (15.2)
Educational level	Bechelor	1925 (48.1)
	Master	1077 (26.9)
	MD	779 (19.5)
	PHD and uper	219 (5.5)
Place of residence	Students dormitory	2544 (63.6)
	Others	1456 (36.4)

Comparison of the mean using the ANOVA test showed that there is a significant difference in the score of high-risk behaviors and their dimensions according to different educational levels. As shown in the results of the Tukey post hoc test, the total score of high-risk behaviors in undergraduates was higher than master's, and also the general doctorate had a high average of high-risk behaviors compared to bachelor's and master's degrees. Under the driving scale, undergraduate students scored higher than others. The next grade of violence in the general doctoral program was higher than the bachelor's and master's degrees. Smoking was also observed in General Doctoral students more than in other courses. In terms of high-risk behavior, drug use was higher in undergraduates but less than in doctoral students. Alcohol consumption was also observed in undergraduates over college graduates. High-risk sexual behavior in undergraduate students had higher scores than postgraduate and lower than doctoral (P<0.05) (Table 2). The results showed that there were an inverse and significant relationship (P<0.05) between age and the total score of high-risk behavior. Therefore, with increasing age, the score obtained from the questionnaire decreased (Table 2).

Table 2: Distributions of total HRB a subscale by demographic characteristics (N=4000)

Characteristic	Total HRBy	High-risk drivers	Agressive	Smoking	Behaviors drug use	Alcohol consumption	High-risk sexual
Total	130.34 ± 40.34	27.56 ±8.33	18.86 ± 6.4	18.86± 6.68	22.47± 8.83	24.1 ±8.57	18.47±7.49
Gender							
Male	132.71 ±38.92	28.12± 8.33	19.12±6.47	19.32±7.59	22.92± 8.44	24.55 ± 8.42	18.67± 7.49
Female	127.62±41.81	26.91±8.3	18.56± 6.31	18.35 ± 7.75	21.95 ± 9.24	23.57 ± 8.71	18.25 ± 7.49
P-value	>0.001*	>0.001*	0.005*	>0.001*	0.001*	>0.001*	0.93
Marital status							
Unmarried	130.92± 40.42	27.71±8.38	18.86 ± 6.48	18.95± 7.71	22.64 ± 8.89	24.32 ± 8.54	18.41± 7.52
Married	127.05± 39.9	26.69 ± 8.02	18.86 ± 5.92	18.36 ± 7.49	21.51± 8.44	22.84± 8.66	18.76± 7.32
P-value	0.29	0.004*	1	0.089	0.004*	0.001*	0.29
Educational level							
Bechelor	131.7±39.56	28.54± 8.21	18.59±6.47	18.93± 7.66	22.53±9	24.43± 8.49	18.65± 7.39
Master	124.99±40.36	26.05±8.2	18.40±6.03	17.99± 7.54	21.6 ±8.67	23.14± 8.57	17.78± 7.53
MD	134.09±40.96	27.53±8.37	19.78±6.4	19.53± 7.62	23.46± 8.49	24.69 ± 8.54	19.07± 7.6
PHD and uper	131.3 ± 42.95	26.39±8.72	20.24± 6.97	20.16 ± 8.32	22.62 ± 8.94	23.74± 9.1	18.12± 7.58
P-value	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*	0.001*
Place of residence							
Students dormitory	131.47±40.33	27.48± 8.48	19.11 ± 6.41	19.15± 7.64	22.87± 8.78	24.35± 8.55	18.48 ± 7.62
Others	127.83 ±40.34	27.72 ± 8.01	18.31± 6.34	18.23 ± 7.72	21.57 ± 8.89	23.53 ± 8.59	18.43 ± 7.2
P-value	0.008*	0.421	>0.001*	>0.001*	>0.001*	0.005*	0.831
Age							
Correlation	-0.12	-0.098	-0.098	-0.096	-0.109	-0.105	-0.107
P-value	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*	>0.001*

**p<0.05*

The linear regression model for predictors of high-risk behaviors showed that age and gender were the main predictors. This, 18% of the total changes in scores predicted high-risk behaviors (Table 3). As can be seen, age inversely predicted high-risk behavior, as age decreased, the score of high-risk behaviors increased (-0,119). Also, the regression model showed that men had more high-risk behaviors than women (-0.06) (Table 3).

Table 3: High risk behaviors predictors in the linear regression model

Model	Unstandardized coefficients		Standardized coefficients	T	sig	95.0% Confidence interval for B	
	B	Standard error	Beta			Lower Bound	Upper bound
Constant	163.91	3.96		41.34	>0.001	156.13	171.68
Age	-1.153	0.152	-0.119	-7.58	>0.001	-1.45	-8.55
Sex	-4.874	1.269	-0.06	-3.84	>0.001	-7.36	-2.38
R2: 0.234 R: 0.18							

Dependent variable: HRB
Predictors: sex, age

DISCUSSION

This study aimed to investigate the tendency of high-risk behaviors and their relationship with demographic variables in students in Tehran. The average scores of high-risk behaviors in students in all dimensions were above average. These results have been reported in another Iranian study, including the an other study [17]. The results of the present study showed that male students had a significantly higher tendency to high-risk behavior than female students. This finding is consistent with the findings of other studies [18-20]. However, in terms of high-risk sexual behavior, the difference between girls and boys was not significant. This result contradicted the results of other studies [1,18], in which boys were more likely to engage in sexual behavior. Many studies showed that men are more likely to engage in more high-risk behaviors, such as driving [21], violence [20, 22], smoking [23], alcohol [23-25], and drug abuse [25], which is in line with the findings of the present study. In the present study, gender was a predictor of high-risk behavior, and this was confirmed by the findings of Deressa's research [26]. In the present study, high-risk behaviors, other than sexual behavior, were influenced by gender. In the student community, this can be considered as a sign of boys' freedom of action. On the contrary, girls try to avoid high-risk behavior due to the cultural context of the community. In their study, Warner et al. Considered that one of the main reasons for the difference in alcohol consumption among male and female students was because of negative societal pressures and severe stigma among women against these behaviors [27]. The results of this study showed that the tendency to high-risk behavior in single students in the dimensions of high-risk driving, alcohol, and drugs was significantly higher than married students. Numerous studies had shown that the risk of alcohol consumption was higher in those who were not married [28-31]. A longitudinal study by Kendler in 2016 showed that marriage had a protective effect against alcoholism. These results are consistent with the hypothesis that the psychological and social aspects of marriage, especially the spouse's relationship to health control, strongly protect against alcohol abuse [32]. Consistent with the results of the present study, it was observed in other studies that marriage reduced drug abuse [31,33,34]. Studies had shown that high-risk driving and road injuries were more common in single people than in married people [35,36]. Given the protective role of marriage in preventing high-risk behaviors in students, creating the necessary conditions and social support for young people to get married can be effective in reducing high-risk behaviors.

The findings showed that dormitory students were more prone to high-risk behaviors and more prone to high-risk driving, smoking, drug abuse, and alcohol consumption. This finding is consistent with the research of other authors [18,20].

Heydarabadi et al. and Rigi Kootesh et al. and Yoosefi Lebni et al. found that students living in dormitories were more likely to be addicted to drugs due to the lack of parental supervision and peer pressure [37-39]. It was also observed in this study that the tendency to high-risk behavior in all dimensions was significantly higher in general doctoral students than graduate students. Consistent with these findings, Fathian et al., in their study showed that medical students had higher-risk behaviors than other non-medical students [40]. Findings showed that age had a significant negative relationship with the overall score of high-risk behaviors, and, also with all dimensions of high-risk behaviors, i.e., with increasing age, the tendency to high-risk behaviors decreased. This finding is consistent with a study by Farmer et al. [17]. Given the characteristics of young people, these results seemed to be consistent with reality.

For preventing students from tending to high-risk behaviors, it is necessary to take extracurricular activities in universities seriously along with academic activities so that students can be entertained with healthier activities.

One of the limitations of the present study was the use of a questionnaire in the form of a self-report of high-risk behaviors that may have caused bias or incorrect responses. There are also direct questions about high-risk behaviors such as drug use or sexual behavior that are still taboo in society. These questions caused the resistance and dishonesty of the respondent, and as a result, the number of correct answers and accuracy of information collection was reduced. Also, the questionnaire used in this study did not measure economic and social factors and health determinants. Therefore, it is suggested that in future studies, economic and social factors and other health determinants be considered, and for more accurate information, an observation method next to the questionnaire be used.

CONCLUSION

The results of this study showed that the tendency to high-risk behaviors in students was not favorable, especially considering that single students at a younger age and in dormitory conditions were prone to high-risk behaviors. Improving this situation requires proper planning for education, various skills such as dormitory lifestyle, more supervision in universities and student dormitories, so it is recommended to hold the necessary workshops for students at the beginning of entering the university. Also, university officials and administrators need to provide healthy conditions and entertainment for students to be less prone to high-risk behaviors.

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Conflict of interest
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Competing interests
The authors declare that they have no competing interests.

Consent to participate
Informed consent was obtained from all individual participants included in the study before conducting.

Ethics approval
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Consent to publish
All participants consented verbally to the publication of the interview data.

Availability of data and materials
The datasets using in the study are available from the corresponding author on reasonable request.

Authors’ Contributions
All authors were responsible for the study. RP, MM, and AZ were accountable for the study Conceptualization and led the writing of the paper. ZRKK conducted the Literature review and assisted in writing the paper. AZ and MM performed the Statistical analysis, assisted in interpreting the data and writing the paper. RP and NM assisted with the interpretation of the results and drafting programmatic Implications. OS and MM were responsible for data collection and coordination of the study. AZ co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

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ORIGINAL ARTICLES

New anatomical landmark for rotational assessment of total knee arthroplasty

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Abstract: *One of the most controversial topics in total knee arthroplasty is rotation of the femoral component. The current gold-standard in total knee arthroplasty consists in positioning the femoral component in 3 degrees of external rotation to the epicondylar axis, having as reference the tangent to the posterior condyles. Achieving the correct rotation of the femoral components is one of the main goals during total knee arthroplasty. Multiple complications can result from internal femoral rotation, such as lateral patellar tilt, patellar subluxation or dislocation, mobilization with movement on pain, and low survival rates of the femoral components. Postoperative rotational assessment of protective components can only be performed correctly using computed tomography. The known evaluation methods are related to anteversion of the femoral neck, femoral trans-epicondylar line, insertion of the posterior cruciate ligament from the tibia and tibial tuberosity. The purpose of this study was to evaluate the rotation of the prosthetic components in the group of patients with the methods validated by studies and to find alternatives to evaluate the rotation of the femoral component. Thirty-four patients diagnosed with gonarthrosis, proposed for total knee arthroplasty, were included in the study. This is a prospective cohort study in which applied statistics consisted of analyzing data using frequency and percentage for qualitative and mean variables and standard deviation for quantitative variables.*

Keywords: *total knee arthroplasty, femoral component rotation, posterior condylar angle, linea aspera*

INTRODUCTION

Rotation of the femoral component is one of the most controversial topics in total knee arthroplasty [1]. From the preoperative assessment required to determine the rotation of the femoral condyles relative to the hip and knee followed by the intraoperative decision to position the femoral component in a slight external rotation to the trans epicondylar axis and to the postoperative evaluation of the rotation, all these are subject to controversies of analysis and decision that may represent another factor of error in the postoperative evolution of the patient [2].

The current gold-standard in total knee arthroplasty consists in positioning the femoral component in 3 degrees of external rotation to the epicondylar axis, having as reference the tangent to the posterior condyles. This is a surgical move that will place the femur in a slight external rotation. It is said that this surgical move will minimize the possible complications as patello-femoral/femoro-tibial movement and arthroplasty survival over time.

Postoperative rotational assessment of protective components can only be performed correctly using computed tomography. The known evaluation methods are related to anteversion of the femoral neck, femoral trans-epicondylar line, insertion of the posterior cruciate ligament from the tibia and tibial tuberosity. If the two femoral epicondyles are difficult to identify on CT scans even with software for removing metal artifacts, it was decided to introduce new signs of rotational CT analysis, which materialized through the linea aspera as a radiological landmark.

Achieving the correct rotation of the femoral components is one of the main goals during total knee arthroplasty [3].

The proper alignment of the femoral component during total knee arthroplasty is a crucial step that influences postoperative results. The position of the femoral

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component in the axial plane is responsible for flexion stability, knee joint kinematics, flexion alignment, and patellar tracking.

Complete axial plane measurement before and after total knee arthroplasty can only be performed using CT scans. For a proper assessment of total knee arthroplasty, the spread of metal artifacts should be reduced and anatomical signs should be properly identified, which is sometimes difficult to achieve mainly for the two femoral epicondyles. Linea aspera is an anatomical sign that is constant and it is not influenced by metal artifacts.

The purpose of this study was to evaluate the rotation of the prosthetic components in the group of patients with the methods validated by studies and to find alternatives to evaluate the rotation of the femoral component.

The study focused on comparing the standard evaluation of femoral component rotation was performed using the posterior condylar angle (PCA) with a new sign that is using the linea aspera ridge and the Akagi line was used for the rotational evaluation of the tibial component [4]

MATERIALS AND METHODS

Thirty-four patients diagnosted with gonarthrosis, proposed for total knee arthroplasty, were included in the study. All the patients analyzed were from the orthopedics and traumatology clinic of the Bucharest University Emergency Hospital during 2018-2019. The study was approved by the hospital's ethics commission in compliance with international research ethics standards. The following angles were measured using CT scans: the angle between a tangent to the posterior condyles and the surgical trans-epicondylar axis (surgical TEA) called the posterior condylar angle (PCA) (Figure 1), the angle between a tangent to the posterior condyles and the tibial component axis (Akagi line) (Figure 2) for rotational assessment of the tibial component.

The posterior condylar angle was compared with a new sign (Figure 3) measured preoperatively and postoperatively as follows: 15 cm proximal to the joint line, is identified and circumscribed in a circle linea aspera, the femoral shaft is also circumscribed in a circle, and the center of the two circles describes a line and the angle between this line is called the linea aspera and a tangent to the posterior condyles was called the aspera condylar angle (ACS) preoperative and postoperative.

This is a prospective cohort study in which applied statistics consisted of analyzing data using frequency and percentage for qualitative and mean variables and standard deviation for quantitative variables. The ANOVA test with repeated measurements was also used to compare the different variables, as well as the Pearson test to verify the correlation between the variables.

Figure 1: Tangent to the posterior condyles

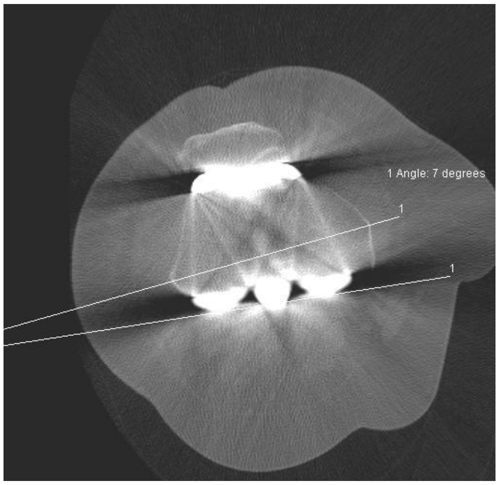

Figure 2: Akagi line

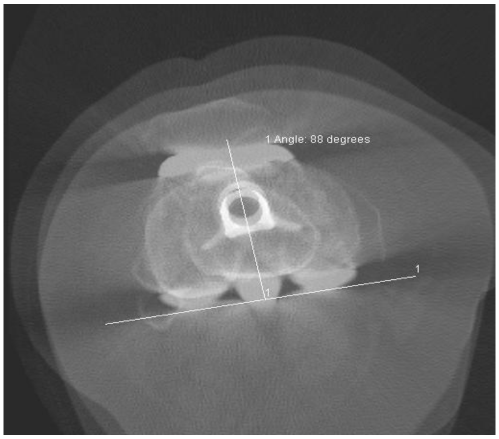

Figure 3: New landmark

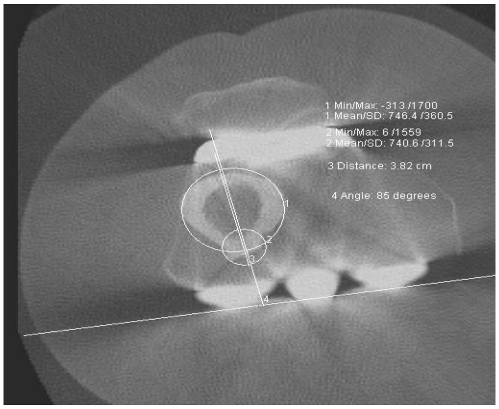

The landmark proposed for validation was evidenced following the steps: 15 centimeters proximal to the joint line, the linea aspera and the femoral shaft were identified. Both were framed in a circle and the centers of the two circles were identified, later joining the two identified points by a line, called the linea aspera. The purpose was to evaluate the angle made by the tangent to the posterior condyles (standard and easily identifiable sign) with this line in the preoperative and postoperative phases.

The main hypothesis of this study was represented by the complex evaluation of the rotation of the prosthetic components in a patient undergoing total knee arthroplasty to understand the clinical effects of malrotations.

RESULTS

Regarding to the degree of reliability of radiological measurements, it was rated from 1 to 5, 5 representing the highest degree of confidence. There is a percentage of 45.71% of specialists who gave a degree of confidence 5, followed by a degree of confidence 4 (37.14%) and a degree of confidence 3 (17.14%).

Regarding to the quality of the acquisition of radiological images, it was rated from 1 to 5, 5 representing the highest degree of confidence: a percentage of 51.43% gave 5, equal to 3 and 4 (20%) and 8.57% gave 2.

Table 1: Average values of the new landmark in the preoperative-postoperative and PCA phases

		New preoperative reference	New postoperative reference	PCA
No.	Valid data	35	35	35
	Missing data	0	0	0
Average		89.3400	93.1309	3.1034
Standard deviation		12.28395	12.37648	1.70943

The average values of the quantitative variables are depicted in Figures 4, 5 and 6.

Using the Pearson test, there is a statistically significant and strong correlation between the new preoperative reference and the new postoperative reference (r = 0.98, p = 0.001). There were no statistically significant correlations with PCA.

Applying the Anova test with repeated measurements, it was concluded that there is a statistically significant difference in the evolution of the new sign in the preoperative phase and the postoperative phase (p = 0.001, 89.34 vs. 93.13).

Using the ANOVA method, there were no statistical differences in the level of specialists who awarded the confidence levels and the new preoperative sign, the new postoperative sign and PCA, who had similar averages.

Figure 4: Distribution of the new preoperative reference. The average value of the new preoperative reference is 89.34 (12.28)

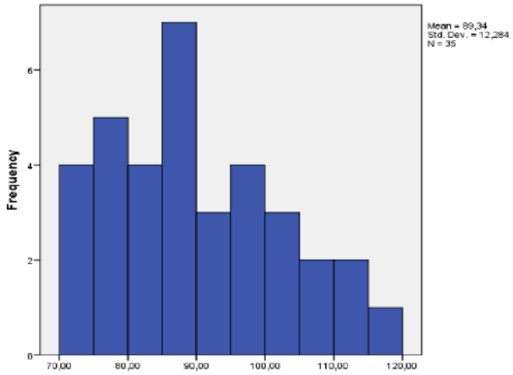

Figure 5: Distribution of the new postoperative reference. The average value of the new postoperative reference is 93.13 (12.37)

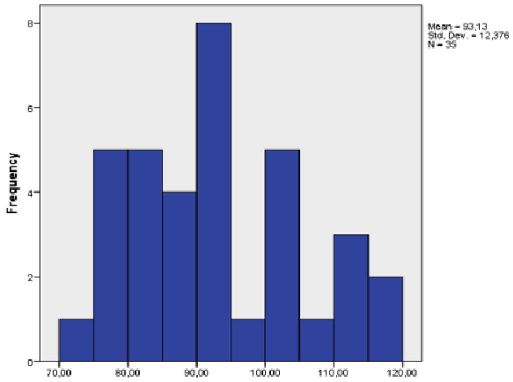

Figure 6: PCA distribution. The average PCA value is 3.10 (1.70)

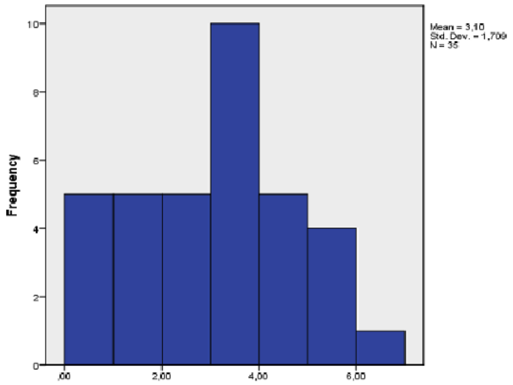

Using the ANOVA method, there were no statistical differences in the level of specialists who assessed the quality of the acquisition and the new preoperative sign, the new postoperative sign and PCA, which had similar averages.

DISCUSSION

Establishing the proper rotation of the femoral component is unanimously accepted as an important point to achieve in total knee arthroplasty. As Mochizuki pointed out more than

30 years ago, the correct rotational positioning is in a slight external rotation [5].

Table 2: Correlations between the new preoperative and postoperative references and PCA

		New preoperative reference	New postoperative reference	PCA
New preoperative reference	Pearson correlation	1	0.982 **	-0.274
	p		0.000	0.111
	No.	35	35	35
New postoperative reference	Pearson correlation	0.982 **	1	-0.218
	p	0.000		0.209
	No.	35	35	35
PCA	Pearson correlation	-0.274	-0.218	1
	p	0.111	0.209	
	No.	35	35	35

Multiple complications can result from internal femoral rotation, such as lateral patellar tilt, patellar subluxation or dislocation, mobilization with movement on pain, and low survival rates of the femoral components [6].

Also, an internal femoral rotation will increase the laxity in the lateral compartment during flexion, varus deviation and unfavorable clinical results [7].

Conversely, excessive external femoral rotation will result in poor functional results, and the patient will experience some degree of flexion instability.

Internal rotation of the femoral component combined with internal rotation of the tibial component are the leading cause of anterior pain after total knee arthroplasty [8].

Soft tissue rebalancing and femoral component rotation can be performed by the two known techniques: the measured resection technique and the “tension gap” technique (extension space equal to flexion space) [9,10]. A rotation of 3 degrees to the tangent to the posterior condyles is unanimously accepted in the technique of measured resection but not always perfect [11, 12].

In the case of the technique of measuring the extension and flexion spaces, it can be performed by two mechanisms: 1. the use of a conventional spacer, but there is no consensus on the tension that need to be applied; and 2. the use of blocks with predetermined dimensions that must be introduced in the two spaces, a maneuver that is strictly subject to the surgeon's experience and is not providing objective information regarding to the tensions in the two compartments [13, 14].

A randomized study comparing the two techniques states the superiority of using a mechanical spacer (laminar spreader) in a resection measured to establish an appropriate femoral rotation [15].

Intraoperative technique of rotational positioning of the femur is relatively standardized and well supported scientifically with prospective and randomized clinical trials. The superiority of one technique over another is not proven. Both techniques will position the femur in a slight external rotation which has proven beneficial for the vast majority of patients for both immediate and long-term results.

The rotational positioning problem for a small number of patients with low satisfaction rates, results from standardization itself, not taking into account the rotation of the femoral condyles relative to the femoral neck and the entire axis of the pelvic limb in the preoperative stage.

In the case of patients in whom a significant deformity of the entire pelvic limb can be observed during the standard preoperative assessment, radiological examination of the pelvis may reveal changes of coxa vara or valga type, an examination of the orthopantogram reveals an exaggerated femoral or tibial curvature, clinical examination indicates altered gait due to deformities and a CT scan of the entire pelvic limb may be recommended. This will evidence rotational changes that do not fall within the preoperative standard assessment and may impose intraoperative decisions specific to each patient.

Starting from the fact that the two femoral epicondyles needed to trace the trans epicondylar axis to measure the rotation of the femoral component are both difficult to identify in the preoperative stage but especially after prosthetic implantation due to metal artifacts, we searched and identified another anatomical sign, unchangeable for rotational assessment and specific to each patient.

CONCLUSIONS

Following the computed tomography analysis of the first patients admitted to the study, an initial conclusion was reached, namely that the evaluation of the rotation of the femoral component should be performed after the trans-epicondylar line, which is difficult to identify correctly on CT scans, even with removal of artifacts. It was concluded that it is necessary to find a new radiological sign for the rotational assessment of the femoral component.

For all cases included in this pilot study, the femoral component was placed intraoperatively at 3 degrees of external rotation relative to the posterior condyles. The analysis of the new radiological sign evidences the external rotation of the femoral component, when it is performed taking as a sign the tangent to the two posterior femoral condyles.

Linea aspera has proven to be a reliable and useful anatomical sign for measuring the rotational positioning of the femoral component in total knee arthroplasty due to its constant position and because its identification is not obstructed by metal artifacts.

This new sign can be used in the same patient in the preoperative and postoperative phase, using CT examination. However, no correlation could be identified with the established assessments between the values obtained from the measurements, a correlation possible only in much larger studies, with larger patient groups.

It has been proposed to use this sign for radiological analysis of total knee arthroplasty in patients in whom preoperative CT examination is performed due to major axis deformities or bone stock deficits, followed by postoperative CT analysis.

The high variability of the linea aspera compared to the posterior condyles in the preoperative evaluation evidences the rotational complexity of the knee and the need for in-depth knowledge of its kinematics in relation to the entire pelvic limb.

The results of the measurements with the help of the proposed new sign show that it can be used in extreme cases in the rotational assessment of the femoral component.

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ORIGINAL ARTICLES

Gut microbiota and obesity

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Abstract: *Worldwide, adults and childhood obesity are increasing alarmingly, being a major health problem. Obesity is correlated with an increased incidence of various systemic diseases including cancer, heart diseases, and diabetes mellitus type 2. Risk factors for obesity are dysbiosis, genetic, socioeconomic, behavioural and environmental. The gut microbiota has beneficial effects on human health, like host immune system stimulation, being influenced by pH, or nutrient intake. Studies performed on humans and animal models reported differences regarding microbiota at lean and obese individuals. The consumption of probiotics and prebiotics may balance the microbiota with positive effects in obesity. The main aim of this review is to reveal the interaction between dysbiosis and obesity.*

Keywords: *microbiota, diabetes, obesity, health problem, cancer, immune system*

INTRODUCTION

It is well known that obesity among children has long become a health problem at least among developed countries [1,2]. In the USA, childhood obesity involves children between 5 to 14 years, who started kindergarten overweight [3]. Obesity reflects the lifestyle of ''developed'' countries and reached epidemic proportions [4-6]. Compared to 1990 the incidence of obesity has increased by 1/3 in adults and almost double in children [7]. In 2017, UNICEF concluded in the case of children that "there has been no progress to stem the rate of overweight in more than 15 years" [8]. The main and the most common risk factors involved in obesity development are physical inactivity, high fat diet, sugar excessive intake and alcohol consumption [9-12].

Obesity is strongly correlated with an increased incidence of several systemic diseases, such as cardiovascular diseases, type 2 diabetes, and cancer [13-17]. Moreover, obesity can be defined as a complex and multifactorial disease. Over the past few years, gut microbiota plays an important role in obesity pathology [13,18]. Moreover, dysbiosis is implicated not only in intestinal diseases but also in autoimmune diseases, obesity-related diseases and even mental disorders [19].

THE GUT MICROBIOTA DYSBIOSIS

The gut microbiota represents trillions of microbial cells, which has the highest density in the intestinal compartment. Archaea, Bacteria, Eukarya, and viruses represent the gut microbiota family, which can establish complex trophic relationships with each other's and to their human host, leading from symbiosis to parasitism. The gastrointestinal microbiota may be affected by pH, oxygen levels, redox status, nutrient availability, water activity, and temperature [20]. Bacteroidetes and Firmicutes can be detected from healthy human faecal samples with the help of 16 S rRNA-targeted molecular analyses [21].

It is estimated that for every human cell, 10 micro-organismal cells colonise the whole surface of the human body. The gastrointestinal tract has a higher concentration

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of microorganisms, composed mostly of bacteria [22]. Therefore, the gut microbiota is a complex ecosystem that deeply affects the host health and can be modulated by environmental stimuli and genetic factors [23]. Currently, the ratio between human cells to bacteria should be closer to 1:1 (3.0x1013 to 3.8 x1013). Thus, this radio may be influenced by genetic and environmental factors. The gut microbiota has beneficial effects on human health such as host immune system stimulation, extra energy extraction, and host protection against pathogens [24]. Moreover, gut microbiota development starts with birth and is influenced by the host genome, nutrition, and lifestyle. Several factors such as pH, nutrient intake alteration (carbohydrates, proteins, and fats) perturb the microbiota composition [25]. Firstly, the gut microbiota is involved in the breakdown of substrates with resistant starch and dietary fibres, that are incompletely hydrolysed by the host enzyme at the small intestine level [25].

The fermentation products that result from the fibre breakdown are the short fatty acids such as acetate, propionate, and butyrate, which are additional sources of body energy. Therefore, thus acids can be used by the human body [25]. The propionate is taken up by the liver as a precursor for lipogenesis, gluconeogenesis, protein synthesis, acetate is used as a precursor for cholesterol synthesis, while butyrate is used as an energy source by the colonic epithelial cells [25]. The immunologist Elie Metchnikoff was the first who reported a causative link between microbes and human disorders, with the implication of compounds that result from microbial intestinal putrefaction. His research was the first to report the beneficial effects of consuming fermented food to avoid intestinal protein putrefaction in humans [8]. The axis, microbiota-gut-brain is a bidirectional axis involved in the regulation of body weight by balancing appetite, storage, and energy expenditure [26].

Taken together, the gut microbiota regulates, food digestion, nutrient uptake and metabolism, vitamins and bile acids synthesis, innate and mucosal modulation, layer development, epithelial growth, and host gene expression [27]. In humans, between obese and leans persons have been reported differences regarding the microbiota composition, functional genes, and metabolic activities [28]. Oxidative stress-induced by obesity is correlated with lipid peroxidation, DNA damage and decreased levels of antioxidants (enzymatic and non-enzymatic) [2,10-12].

Nowadays, the implication of intestinal microbiota in the progression and, prevention of metabolic dysfunction is more relevant [29]. Some bacteria may facilitate lipogenesis. Therefore, the gut microbiota of obese individuals has several genes that may be depleted or enriched. The genes related to vitamin, cofactor and nucleotide metabolism are usually depleted [21]. More than that, intestinal microbiota can regulate adipose tissue metabolism, homoeostasis, energy balance, appetite, and food uptake, playing a crucial role in obesity [30]. In obese-experimental models performed, some gut microbial strains may inhibit or attenuate the immune response related to chronic inflammation. These results suggest that microbiota plays a dual role, protective or pathogenic [31]. Targeting the gut microbiota using prebiotics, probiotics, and drugs (berberine and metformin) will have a beneficial effect on human health [32].

The immunomodulatory properties of gut microbiota have been observed especially in obese conditions. Therefore, this category of patients is characterized by an altered microbiota or microbial dysbiosis. Obese patients extract more energy from the diet compared with lean individuals leading to excessive energy stores. In obese or diabetic type 2 patients, an enrichment of the pro-inflammatory bacteria (Escherichia coli) and a decrease of anti-inflammatory bacteria (Fecalibacterium prausnitzii) had been reported. These findings suggest that these bacteria represent a source of inflammation-associated metabolic diseases [33]. Moreover, the fermentation products of gut microbiota influence the fatty acid oxidation process, fasting, bile acid production, satiety, lipogenesis, and even innate immunity [34].

A study conducted on C57BL/6 mice, which are genetically protected against obesity after consuming a diet rich in fat and carbohydrates, where colonise with obese mice microbiota. The research group reported a significant increase in body fat and insulin resistance development in C57BL/6 mice [34,35].

Gut microbiota dysbiosis is correlated with proinflammatory cytokines release, and further with increased risk of obesity. All the obesity-related factors will lead to the development of diabetes mellitus type 2 [36], liver disease [37-40], colon cancer [14,41] and even neurological pathologies (Parkinson’s disease, autism) [42]. Some studies reported that human gut microbiota may be affected by sex, so mean have lower microbial diversity the women. Regarding this aspect, women have a lower proportion of Bacteroidetes and increased levels of Akkermansia muciniphila. Instead in the case of men, Ruminococcaceae, Faecalibacterium, and Alistipes are the species detected to be higher, while Bacteroides has a decreased proportion and they do not have Prevotella [43]. The diets used for weight loss which involves high protein intake and decrease consumption of carbohydrates perturbates the microbiota. When these strategies are applied, protein fermentation by gut microbiota take place and metabolic products have released that lead to colon inflammation. Alteration regarding the proportion of Bacteroidetes and Firmicutes may conduce to obesity development [44,45].

PREBIOTICS AND PROBIOTICS IN DIGESTIVE HEALTH

Thus, probiotic supplement consumption may regulate the gut microbiota, improving the prognosis for diabetic patients [44]. Our diet habits play an important role in gut microbiota composition. Differences between intestinal microbiota among individuals fed with Western diet and those fed with a diet rich in fibres had been observed. Additionally, a diet rich in fat can promote the development of pro-inflammatory gut microbiota, leading to increased intestinal permeability and higher levels of lipopolysaccharides [46]. When we eat more high-fat and little fibres aberrant metabolites are produced, leading to the loss of tight junction integrity. This loss of integrity allows the metabolites such as lipopolysaccharides, trimethylamine to enter the circulation and to contribute to liver and adipose tissue chronic inflammation development [42]. Therefore, the relationship between gut microbiota dysbiosis and obesity can be regarded as a dual interaction [47,48]. Faecal metabolites evaluation provides information regarding the relationship between microbiota and host. Regarding the presence of metabolites, 14 compounds, including lysine, omega-muricholate, and glutamic acid, had been identified [49]. Therefore, a decreased proportion of bifidobacterial associated with the death of butyrate-producing bacteria leads to obesity development [50].

The consumption of probiotics, prebiotics or symbiotics may equilibrate the microbiome, with health benefits in obesity. Studies performed on a human, and animals reported that probiotics can promote weight loss in obesity [51]. Consumption of pickled and fermented foods, particularly vegetables and beans, are an important source of pre-, pro-, and synbiotics, with important benefits on human health [52]. ” Bifidobacteria, lactobacilli, and non-pathogenic fungi are the most used probiotics. Probiotics can confer protection against preeclampsia, gestational diabetes, vaginal infections, maternal and infant weight gain, and other diseases [53]. Therefore, manipulating gut microbiota with the help of probiotics as a supplementary therapy may enhance the immune system. This may be an important strategy to reduce side effects after a specific treatment like those with antibiotics [54]. Prebiotics and probiotics are the most used substances that may balance the microbiome when the microbiome homeostasis is perturbated in several diseases [55]. Therefore, probiotics stimulate the immune system, controlling the synthesis of pro-inflammatory and anti-inflammatory cytokines. Clinical trials which include patients diagnosed with systemic and respiratory infections reported that consumption of probiotics such as L. rhamnosus GG, improves intestinal and lung barrier and even homeostasis. L. rhamnosus GG consumption leads to increased regulatory T cells, ameliorates anti-viral defence, and decrease the pro-inflammatory levels [56]. Studies conducted on obese mice reported that bacteriocin-producing Lactobacillus salivarius UCC118 Bac+ altered the gut microbiota and may reduce the weight gain more effectively than the non-bacteriocin-producing strain L. salivarius UCC118 Bac-. Also, the research team reported elevated proportions of Bacteroidetes and Proteobacteria, and decreased proportions of Actinobacteria, while the proportion of Firmicutes remains the same. The beneficial effects of L. salivarius have been demonstrated even in clinical human studies. The Bacteroidetes–Prevotella–Porphyromonas–Firmicutes ratio, have been increased by the L. salivarius Ls-33. Regarding the effects on obese adolescents, L. salivarius Ls-33 did not have beneficial effects. Moreover, in mice who consume a high-fat diet, Lactobacillus, together with Bacillus amyloliquefaciens SC06, can decrease the Firmicutes/Bacteroidetes ratio, body weight, and hepatic steatosis [57].

Kong C and his research revealed that probiotic supplementation slows down weight Moreover, the study reported that both groups (HFD and HCD) had a reduced microbial diversity, an increased production of butyric acid-producing bacteria, an increased proportion of beneficial bacteria (Lactobacillus, Clostridium sensustricto, Prevotella, and Alloprevotella) and conditionally pathogenic bacteria (Bacteroides, Alistipes, and Anaerotruncu). Probiotics administration significantly balance the bacteria proportions in HFD and HCD groups [58]. Obesity left uncontrolled leads to metabolic syndrome installation, pathology associated with systemic and oral diseases in adults and children [39, 59-62].

CONCLUSIONS

The gut microbiota plays a dual role, having negative and positive effects on human health. In obese patients, perturbation of gut microbiota has been reported. Therefore, the obese patients' gut microbiota is characterized by an enhanced proportion of pro-inflammatory bacteria and a decrease of anti-inflammatory species, representing an important source of inflammation. Diet influences our intestinal microbiota, thus an unbalanced nutrient intake leads to dysbiosis, which is further correlated with proinflammatory cytokines release, and obesity development. In conclusion, the consumption of probiotics and prebiotics restore our intestinal microbiome, with health benefits to either lean or obese persons.

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ORIGINAL ARTICLES

The assessment of the cardiovascular risk in rheumatoid arthritis patients on anti TNF therapy

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Abstract: *Auto-immune rheumatic diseases are characterised by high levels of inflammation that accelerate the development of atherosclerosis, increasing the cardiovascular risk. Direct evaluation of arteries such as of intima media thickness measurement is a simple test that asesses the cerebral and cardiovascular risk. In the initial stages, an increase of the intimal media thickness can be observed. Later on, atherosclerosis (plaques), stenosis and occlusion of the arteries can be identified. Indirect evaluation of the cardiovascular risk can be performed using serological markers - fibrinogen, C reactive protein, estimated sedimentation rate, total cholesterol, low density lipoproteins and high density lipoproteins, homocysteine levels etc. The presence of certain lifestyle factors that increase the risk, such as smoking, sedentarism and associated pathologies such as obesity, diabetes, hypertension were assesed in patients with rheumatoid arthritis. The aim of the study is to prove the relationship between anti-tumor necrosis factor alpha therapy effect on the cardiovascular risk in rheumatoid arthritis patients.*

Keywords: *cardiovascular risk, intima media thickness, hiperhomocysteinemia, rheumatoid arthritis, anti-tumor necrosis factor alpha therapy*

INTRODUCTION

Rheumatic auto-immune diseases are characterized by chronic abnormal systemic inflammation that leads to endothelial damage and vasculopathy. [1]

Vascular endothelial injury is an early key step in the induction of vascular disfunction. Endothelial cells are involved in the vascular hemodynamics, vascular permeability, cell growth regulation, vessel protection and coagulation. It releases factors such as nitric oxide (NO), endothelin and prostacyclin that keep the balance between vasoconstriction and vasodilation. Nitric oxide, a soluble gas that is synthesized in the endothelial cells, has many biological properties that help maintain vascular homeostasis. It was found that a high disease activity score positively correlates with the levels of nitric oxide, as a marker of oxidative stress. [2,3]

The endothelial disfunction can be assessed indirectly by measuring blood markers (Assymmetric Dimethylarginin-ADMA, oxidized and anti-oxidized LDL, HDL cholesterol, anti-phospholipid antibodies, Crioglobulins, Pentraxin-3, Endocan) or directly showing the arteries themselves using ultrasonography, computed tomography, magnetic resonance imaging and positrone emission tomography scans. [4]

Factors such as age, smoking, sedentary lifestyle, elevated inflammatory markers (fibrinogen, estimated sedimentation rate – ESR, C-reactive protein – CRP), lipid profile, homocysteine – Hcy levels, metabolic syndrome, hypertension are cumulative factors that increase the cardiovascular risk.

The elevated acute phase reactants (such as ferritin, C-reactive protein are also markers of high cardiovascular risk

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in the general population. [5]

Smoking is an independent risk factor for atherosclerosis, especially in patients that present rheumatoid factor (RF) and/or anticitrullinated peptide antibody (CCP) positivity. Hyperuricemia was associated with a 3.1-fold risk of first cardiovascular events. Gout or hyperuricemia may be stronger predictors of first cardiovascular events than other traditional risk factors. [6,7]

Compared to the general population, patients with rheumatoid arthritis have an altered lipid profile, characterized by a suppression of total low-density lipoproteins (LDL) and a greater suppression of high-density lipoproteins (HDL) levels. Coronary artery disease and lipid levels have a paradoxical relationship, decreased lipid levels being associated with severe inflammation. [8]

Lipids increase the production of pro-inflammatory cytokines, especially TNF-α, interleukin 1 (IL-1), interleukin 6 (IL-6). These pro-inflammatory cytokines may have a role in the disruption of lipid metabolism, especially in the cholesterol transport which is associated with the development of atherosclerosis. Dyslipidemia and insulin resistance are associated with systemic inflammation. [9]

Hiperhomocysteinemia (HHcy) is considered a risk factor for cardiovascular and neurological pathologies. This biomarker that can be monitored by blood testing, favors atherogenesis and increseas the risk for heart attack, stroke, Alzheimer's and Parkinson's disease, diabetes and osteoporosis. It is also associated with autoimmune diseases such as ankylosing spondylitis, rheumatoid arthritis (RA) and systemic lupus erythematosus.

Hiperhomocysteinemia is implicated in the alteration of the cerebral flow, increasing the risk of thrombosis and ischemic stroke. Moreover it is implicated in the disruption of the blood-brain barrier, increasing the risk of hemorrhagic transformations of ischemic strokes after thrombolysis. Hyperhomocysteinemia was considered an independent risk factor for negative aoutcome in patients with strokes. [10,11]

In the general population, hiperhomocysteinemia is also associated with peripheral vascular disease and deep vein thrombosis.

Autoimmune activation can add to homocysteine increase but HHCY may also act as a pro-inflammatory molecule and a trigger of autoimmune reactions because of its capacity of binding and modifying specific proteins that result in the formations of neoantigens. [12]

The arterial wall is made of 3 layers, tunica intima (the inner layer), tunica media and tunica externa (the outer layer). In the initial phase of atherosclerosis, the thickness of intima-media increases and it is followed by plaque formation. Ultrasound imaging is the primary diagnostic tool to assess carotid artery disease (it can measure intima-media thickness, detect plaques and obstructions). Carotid arteries are representative of other arteries, such as coronary arteries, allowing us to assess the cardiovascular and cerebrovascular risk.

In rheumatoid arthritis patients, IMT was reported to be more elevated compared to same age and sex healthy individuals. In patients with inflammation (assessed by C-reactive protein) IMT can be a strong predictor for future vascular events. [13, 14]

It was determined that patients with seropositive rheumatoid arthritis have twice the risk of developing heart failure. Moreover, it was found that these patients have with less typical signs and symptoms and have preserved ejection fraction (>50%). Because of this, it was observed that these patients tend to be managed poorly and have more severe prognoses. [15, 16]

Anti-nuclear antibodies (ANA) and rheumatoid factor (RF) are significant predictors of cardiovascular events and were associated with increased mortality even in patients without autoimmune rheumatic diseases. Cyclic citrullinated peptide antibodies (CCP) association with cardiovascular disease was not statistically significant. [17]

Rheumatoid arthritis therapies target inflammation. It was found that the use of TNF inhibitors was correlated with a reduced risk of cardiovascular events because of its effects on decreasing the systemic inflammation. [18]

The aim of the study is to prove the relationship between TNF-α therapy effect on the cardiovascular risk in rheumatoid arthritis patients.

METHODS

The study was conducted in "Carol Davila" Central Emergency Military University Hospital, Bucharest, Department of Internal Medicine 2.

We performed a retrospective observational study on 115 patients (72 women and 43 men) with ages between 28 and 68 years old, with rheumatoid arthritis (who met the EULAR/ACR 2010 criteria). This lot was divided into 4 groups. The first group consisted in 42 people treated with Etanercept (50 mg subcutaneously once a week), the second group consisted in 25 people treated with Adalimumab (40 mg subcutaneously every two weeks), the third group consisted in 35 people treated with Infliximab (3 mg/kg body intravenously 0,2,6 weeks and every 8 weeks) and the fourth group consisted in 13 people that weren't treated with biological therapies. Patients with anti-TNF therapies had associated either 10 mg of Methotrexate per week followed by 5 mg of folic acid or 20 mg of Leflunomide per day.

Indirect methods of assessing cardiovascular risk (by measuring blood markers) and direct methods – measuring carotid intima-media thickness through ultrasound imaging (Doppler flow) were used. ITM was assessed at the common carotid arteries, at 10mm before the carotid sinus. The measurements were performed by the same technician in order to lower subjective errors and maintain the accuracy of the measurements. In normal subjects there is no plaque and the average thickness of intima-media is below 0.9 mm. Thickened intima was considered at an ITM above 0.9 mm and atherosclerotic plaque above 1.3 mm. All patients received a dose of 20mg/day of atorvastatin.

Laboratory reporting limits for ERS were: 0-10mm/h, for CRP 0-5mg/dl, for total cholesterol (CT) <200mg/dl, for Hcy 5-15µmol/l and for LDL-CT <100mg/dl.

Rheumatoid arthritis activity was assessed using DAS 28 score (Disease activity score that examines 28 joints). A controlled disease scores <2.6, between 2.6-5.1 -the disease is moderately controlled and a score >5.1 – the disease is uncontrolled. All of these markers were investigated just before the initiation of anti-TNF therapy and were compared with the markers one year later.

The study was approved by the local ethics committee and all subjects involved gave written and informed consent.

Statistical analysis was performed with SPSS software. Statistical significance was considered at a Pearson correlation coefficient value of 0.01.

RESULTS

From 115 patients with rheumatoid arthritis 62.61% were women and 37.39 % men, with a mean age of 50 ± 10.4 years. From the total of 115 patients, 20% were smokers (32.5% of the males and 12.5% of the females), 39.3% were overweight, and 7.83% had diabetes. 71.30% of patients had a positive anti-Cyclic Citrullinated Peptide Antibody and 96.52% had a positive Rheumatoid Factor.

From this lot of people, 61.74% were treated with Methotrexate and Folic acid and 38.26% with Leflunomide. From the total of patients, 88.70% were treated with anti-TNF agents as followed: 41.18% Etanercept, 34.31% Infliximab, 24.51% Adalimumab. 12.75% from the total patients were not treated with bDMARDs (biologic disease modifying antirheumatic drugs).

Table 1: Studied parameters

Parameter	Value
Age (mean)	50 ± 10.4
Age ≥ 50 years	53% (61/115)
Age < 50 years	47% (54/ 115)
Gender (females)	62,61% (72/115)
Smokers	20% (23/115)
Overweight	39.13% (45/115)
Diabetes mellitus	7.83% (9/115)
RF positive	96.52% (111/115)
Anti-CCP positive	71.30% (82/115)
HCY1> 15	66.09% (76/115)
HCY2 >15	55.65 (64/115)
CRP (range)	35.81 ± 14.09
ESR (range)	34.37 - 12.29
DAS28 1 (range)	8.1 - 4.4
DAS28 2 (range)	4.3 – 1.1
CT (N=109)	
CT1 >200	53.21% (58/109)
CT2 >200	0% (0/109)
CT-LDL (N=105)	
CT-LDL 1	84.76% (82/105)
CT-LDL 2	29.52% (31/105)
IMT (N=112)	14.43 ± 31.02
IMT1 >0,9	83.93% (94/112)
IMT2 >0,9	67.86% (76/112)

N= Number of patients; RF= Rheumatoid Factor; Anti-CCP= Anti Cyclic Citrullinated Peptide Antibodies; HCY= Homocysteine; CRP= C reactive protein; ESR= Estimated sedimentation rate; DAS28= Disease Activity Score 28; CT= Cholesterol; CT-LDL= LDL-Cholesterol; IMT= Intima media Thickness

Figure 1: Correlation between HCY1 and IMT2 in the Adalimumab group (HCY1 – Homocysteine level at baseline; IMT2 – Intima media thickness after one year of treatment)

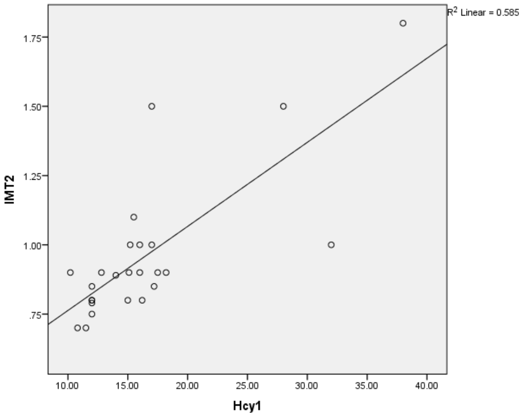

The inflammatory markers were positively correlated with the disease activity.

HCY was evaluated in all of the patients. HCY1 was evaluated before the beginning of the therapy and HCY2 one year later. HCY1 was elevated in 66.09% of patients. After 12 months, none of the patients had increased its previous HCY value.

Moreover, 18.26% of the total patients had a decrease in their HCY2 from HCY1.

Figure 2: Correlation between HCY1 and IMT2 in the Etanercept group (HCY1 – Homocysteine level at baseline; IMT2 – Intima media thickness after one year of treatment)

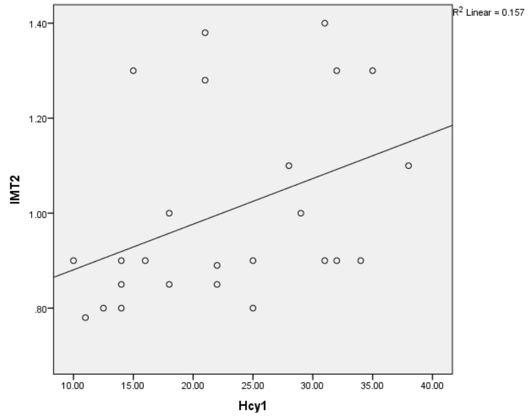

Figure 3: Correlation between HCY1 and IMT2 in the Infliximab group (HCY1 – Homocysteine level at baseline; IMT2 – Intima media thickness after one year of treatment)

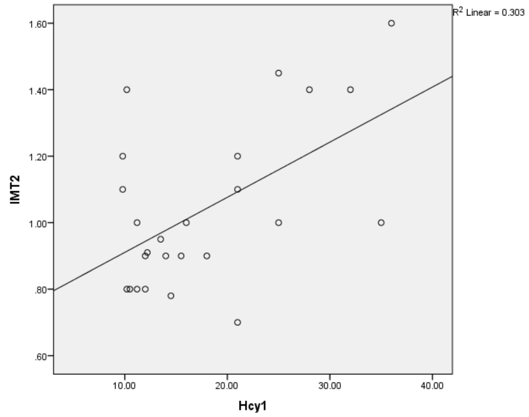

From 112 patients (3 measurements were made by another examiner and were excluded from the study in order to maintain the accuracy of the results), 94 patients (83.93%) had an increased IMT1 value. 76 patients (67.86%) had an increased IMT2. CRP2 values were elevated in only 2.6% of patients. CT1 levels were elevated in 53.21% of the patients. In 6 patients CT levels weren't measured at the beginning of the therapy and thus they were excluded. CT2 levels were normal in all of the patients after one year. To be noted, all of the patients received a dose of 20 mg/day of atorvastatin for one year.

LDL-CT1 levels were increased in 84.76% of patients. LDL-CT2 levels were increased in only 29.52% of patients. 10 patients were excluded from this analysis because LDL-CT was not measured before beginning of the treatment.

In the Adalimumab group, IMT2 decreased in 84% of the patients from the previous measurements (IMT1). HCY levels normalized in 46.67% of patients after a year in the Adalimumab group.

In the Etanercept group, HCY2 levels decreased in 76.47% of patients and 23.53% remained stationary. None of the patients had an increase in their HCY levels after one year. IMT2 decreased in 82.35% patients after one year of treatment with Etanercept.

In the Infliximab group, HCY2 levels decreased in 57.89% of patients. 42.11% the HCY levels remained stationary. None of the patients had an increase in HCY2 levels. IMT2 values reduced in 50% of the subjects from IMT1

In the non-biological treatment group, HCY2 levels decreased in 38.46% of patients. ITM2 decreased in 84.62% of patients.

In the non-biological treatment group, it was found that HCY2 levels decreased in a smaller number of people (38.4% vs 46.6% in the Adalimumab group, 57.8% in the Infliximab group and 76.4% in the Etanercept group).

DISCUSSION

Traditional risk factors for cardiovascular disease such as smoking, excess weight, hypercholesterolemia, hipertension and diabetes mellitus are highly prevalent among rheumatoid arthritis patients.

A large proportion of the studied group are smokers. Because of its effect on the endothelial function (inflammation, proatherogenic cytokines release, reactive oxygen species (ROS) production, fibrinolysis, platelet production and activation), smoking increases the risk of atherosclerotic cardiovascular disease. Studies have shown that the mortality in female smokers is higher than in males. The risk of coronary heart disease is 25% higher in women than in men for the same tabacco use. Genes involved in thrombin signaling are responsible for women susceptability for tabacco-induced cardiovascular disease. [19, 20]

As 20% of the patients are smokers, it is important to inform patients of the risks of RA progression, accelerated atherosclerosis, the increased risk of cardiovascular disease and even inadequate response to RA treatment.

It is a well-known fact that obesity is an independent risk factor for cardiovascular disease, and particularly in women. Moreover, obesity has been associated with an increased disease activity and a decreased response to anti-TNF therapy. [21]

39.13% of the patients were overweight and obese, with

Body Mass Indexes (BMI) between 26-41 kg/m^2. In our study group, smoking and excess weight were associated in 12.17% of patients. Among smokers, the risk of obesity grows with the amount smoked. It was found that obesity is more prevalent in ex-smokers. [22,23]

Therefore, it is important to promote smoking cessation, offer pharmacologic assistance in quitting when appropriate patients, as well as motivating patients to be more active in order to counteract potential weight gain.

We observed that homocysteine levels were elevated in 66% of patients with rheumatoid arthritis. After one year of treatement, HCY leveles decreased in all groups. No strong correlation was found between the anti-TNF agent and the decrease of HCY. It was observed that patients who received 10 mg of Methotrexate subcutanousely and 5 mg of folic acid had a more significant decrease in HCY. It was found that folic acid supplements reduce homocysteine concentrations and is effective in the primary prevention of cerebrovascular and cardiovascular events. [24,25]

In the non-biological treatment group, it was found that HCY2 levels decreased in a smaller number of people. Therefore, we can speculate the additional role of anti-TNF therapy in lowering the HCY levels besides folic acid supplimentation.

IMT2 progression was significantly reduced in all patients. HCY2 was strongly correlated with IMT2 ($P<0.001$). These findings were also observed in other studies that measured IMT. Unlike Yang et al, no correlations between HCY and anti-CCP antibodies, RF or DAS-28 score were found. [26-28]

From the three agents of anti-TNF, Adalimumab and Etanercept were the most effective in decreasing IMT after one year of treatment. The reduced level of HCY2 was significantly correlated ($P<0.001$) with a decrease in IMT2 in patients with hiperhomocisteinemia and an increased IMT at baseline. A significant correlation between an elevated HCY1 and a decreased IMT2 was found in all groups treated with anti-TNF agents (Figures 1, 2, and 3).

CRP2 and ESR2 were greatly decreased in all patients, which correlate with DAS-28 values. Strong correlation between inflammatory markers and carotid IMT was found.

Cholesterol levels decreased in all patients. Atorvastatin treatment greatly contributed to the decrease in total cholesterol and LDL-cholesterol.

CONCLUSIONS

Homocysteine levels should be measured in all rheumatoid arthritis patients. Folic acid supplementation should be recommended to all patients with hiperhomocisteinemia.

The assessment of cardiovascular risk should be made in all rheumatoid arthritis patients regardless of age. The physician should always promote the benefits of cessation of smoking and weight loss in all patients with RA. Hiperhomocisteinemia, increased inflammatory markers, hypertension, diabetes, excess weight, smoking are risk factors highly associated with increased cardiovascular and cerebrovascular risk. IMT assessment is a fast tool that can be used in addition to laboratory blood testing.

IMT assessment is a fast, noninvasive tool that can be used in addition to laboratory blood testing to assess cardiovascular and cerebrovascular risk.

Anti-TNF agents prooved to be not only successful in the control of rheumatoid arthritis but also in lowering the risk of cerebro- and cardiovascular serious events.

Disclosure statement:

D.A, D.O.B, M.L.G have previously been speakers for Sandoz, Pfizer, EwoPharma and Abbvie. M.M.N has been a speaker for Pfizer and Sandoz. O.G.P, C.A.A and F.I.R have no conflict of interest to disclose.

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ORIGINAL ARTICLES

The emergence of nanotechnology and a revolution in diagnostic methods of biological threat agents

Mohammad S. Hashemzadeh[1]

Abstract: *Nanotechnology is applied in wide-ranging fields including energy, information technology, consumables, medicine, etc. Nanomedicine includes the medical applications of nanomaterials in the fields of diagnosis and treatment. This paper focuses on the application of nanotechnology in medical diagnostics for which the main applications of nanomedicine include the detection and discovery of specific biomarkers and rapid identification of biological agents. The introduction of nanotechnology into the medical field before a serious increase in disease symptoms makes early diagnosis possible, thereby preventing more damage to the patient. With the manufacture of nanomaterials and novel entities, reduced size of sensing instruments, as well as biochips and bionanosensors, nanotechnology has revolutionized diagnostic methods. Gold nanoparticles (NPs), quantum dots (QDs), nanotubes, polymeric NPs, and liposomes are among NPs used in medical diagnostics. The importance of these diagnostic methods is redoubled whenever there is a need for the rapid and accurate diagnosis of biological threat agents.*

Keywords: *nanotechnology, medical diagnostics, biological agents, nanoparticles*

INTRODUCTION

Nanomedicine includes the medical applications of nanomaterials in the fields of diagnosis and treatment. Prior to treatment, the science of medicine has always been seeking a method for the diagnosis of diseases. This resulted in the manufacture of nanomaterials and novel entities, including nanoparticles (NPs) and such materials as nanofibers made from the assembly of their original components [1-2]. With the fabrication of these new NPs and imaging agents, as well as by the reduced size of sensing instruments (nano-biochips and lab-on-a-chip), nanotechnology has revolutionized diagnostic methods. In the nano field, medical diagnostics fall into three general groups. I) In vitro diagnostic methods that include detection methods based on the design of nano-biochips and nano-biosensors (based on using DNA probes, PNA, Aptamer, etc.), diagnosis based on lab-on-a-chip, and diagnosis based on immuno-affinity assays. II) In vivo diagnostic methods that include techniques based on molecular imaging using NPs, detection-based methods using NPs, and diagnosis using implantable devices. In the latter case, part of the stages is performed ex vivo in which the target tissue is removed from the body and then returned therein after doing some stages. III) On vivo diagnostic methods, which include using wearable nano-biosensors such as the glucose clock. The molecular basis of nano-probes used in medical diagnostics can be based on the following items [3-5]: 1) gold NPs, 2) quantum dots (QDs), 3) magnetic NPs, 4) nanotubes, 5) polymeric NPs, and 6) liposomes.

Diagnostic application of NPs in medical imaging

Iron NPs (Fe NPs) are among the most commonly used tools that can be used to improve the diagnosis of various diseases or the identification of pathogenic agents such as Staphylococcus bacteria. This nanoparticle is coated with a biomolecule (for specific binding to cancer cells or pathogenic bacteria). The Fe used in these NPs makes them fully apparent and visible in imaging procedures (e.g. magnetic resonance imaging, MRI) and thereby increases the quality of this imaging [6].

Application of NPs in diagnostic methods based on

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proteomic studies

To diagnose various diseases, another application of new molecular diagnostic technologies is based on using specific biomarkers of these diseases. In this regard, an important existing issue is the collection and harvest of these biomarkers by functionalized NPs of the target sample, followed by separation and proteomic studies (that are highly sensitive) to diagnose the type of the disease [7].

Diagnostic application of QDs in medical imaging

These NPs are semiconductor inorganic fluorophores that are more beneficial and advantageous than conventional fluorescent markers. These include high sensitivity, wide excitatory spectra, and a stable fluorescence with a simple excitation without the need for laser radiation. The most important potential application of QDs is in the diagnosis of cancer. With a diameter of 2-8 nm, they typically comprise atoms of elements of II and VI groups (e.g. CdSe and CdTe) or III and V groups (e.g. InP and InAs) from the periodic table. The optical properties of these NPs are mainly dependent on their structures. Importantly, the wavelength and/or the color emitted by a QD depends on its diameter [8-9].

Targeting QDs for intracellular imaging

NPs containing QDs specialized with a specific ligand coating bind to their specific receptors at the surface of target cells and undergo phagocytosis by these cells after the attachment procedure. These intracellular QDs are released after the intracellular digestion and deposit on intracellular structure, which will then be detectable in a specific cell. It is noteworthy that QDs are highly permeable into eukaryotic cells owing to their size [8-9].

The use of Au NPs in the detection of DNA

In a recent innovative method, the presence of a specific genetic sequence was detected in a contaminated sample using 13 nm Au particles decorated with DNA genetic material [9].

The use of magnetic NPs in the detection of pathogens

There is ongoing research on the use of magnetic NPs at the nanoscale for the detection of pathogens. As with many skills used today, the methods used in such studies require appropriate antibodies that bind to these agents (described in detail in the Results and Discussion) [6].

Nano-biosensors

These are the other types of diagnostic tools that enjoy nanotechnology. Briefly, there are different types of this diagnostic tool, including electronic, electrochemical, and optical nanosensors, among others. The most common types are the electrochemical and optical (based on Surface Plasmon Resonance or SPR) ones. An electrochemical nanosensor was used to detect Salmonella typhi in 2009 [13].

THE FUTURE OF NANOBIOTECHNOLOGY

A dream of researchers is to achieve a humanoid nanorobot that can circulate in the bloodstream, detect possible pathogenic agents alongside other devolved tasks, and act according to the requested program. These components can be made applicable to be used for the entry of nano drugs into the body to recognize or cure damaged areas. In 1966, a fiction film, called the Fantastic Voyage, invited cinema fans to watch an adventuresome show of the application of nanotechnology in medicine. A group of adventurer physicians and their modern submarine were minimized by a mysterious method so that they could circulate in the patient's bloodstream and remove the embolus in the brain that threatened the patient. After half a century since then, considerable attempts have been made to fabricate complicated instruments in smaller scales. This has made some people believe that such interventions are plausible in medicine, and nanorobots will be able to journey in the vessels of people.

Nanorobots are also used in nano surgeries. They can enter the body through vessels, seek the injured site, and diagnose the disease with the help of a computer and a supervisor surgeon. After the precise detection of the problem site, the surgery is done by the direction of a surgeon using nanorobot-equipped arms. These small nanometric robots can also perform precise surgeries inside the cell (e.g. the exchange of real chromosomes with new ones), surgeries that have not so far been realized by any human surgeon (Figure 1).

Researchers are seeking to produce and use other instruments, including implantable nanodevices, which can be implanted, for example, in the plasma membrane of erythrocytes. Thus, they will be always present in the systemic blood circulation and play a role in such applications as monitoring or drug transfer [14-15] (Figure 2).

Some applications

As mentioned above, Fe NPs coated with a biomolecule (for specific binding to pathogenic bacteria) are among the most widely used tools that can be used to detect pathogenic agents such as Staphylococcus bacteria (Figure 3).

Figure 1: Schematic of images related to small nanometric robots (nanorobots)

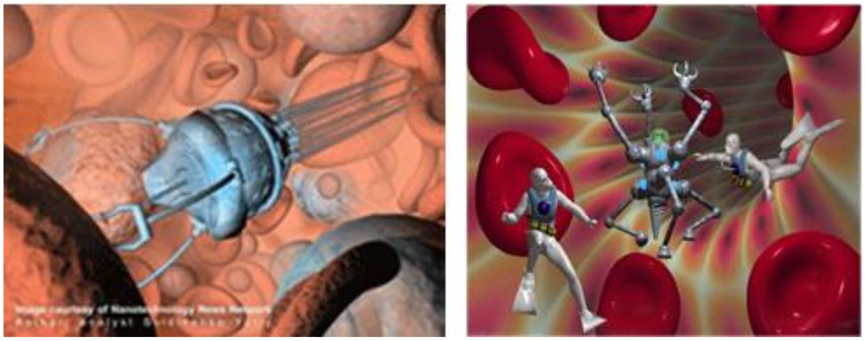

Figure 2: Schematic of an implantable nanodevice implanted in the plasma membrane of erythrocytes

Figure 3: Schematic of an iron oxide NP coated with a cell-specific biomolecule

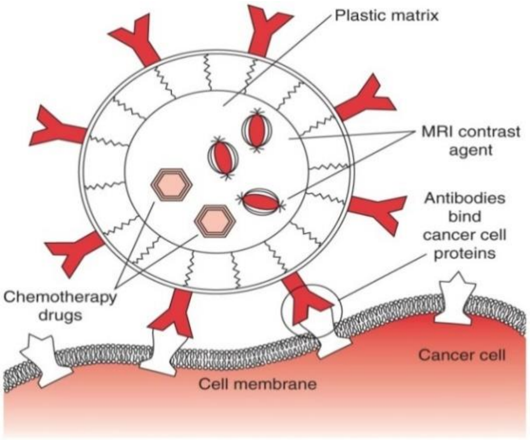

Figure 4 depicts a schematic of a QD consisting of four main parts": A) Core: contains a semiconductor that can emit light after excitation, and this part is the mainstay of a QD. B) Core-shell: contains an insulator that preserves light emission properties after the nanoparticle functionalization. Functionalization means the addition of other parts to the nanoparticle central core that will provide the functional properties, such as specific binding to the target molecule, nanoparticle detoxification, biocompatibility, etc. C) Natural coating: the core-shell is coated with a natural biocompatible material (e.g. PEG) or a lipid layer. D) Synthetic coating: the final stage of nanoparticle functionalization depends on the intended goals. For example, this layer can be used to embed a drug for therapeutic purposes and drug delivery or assemble a specific antibody to convert a QD to a target-specific nanoparticle. Notably, QDs are toxic per se and thus must undergo some changes for their biocompatibility [14-15].

Figure 4: Schematic of different parts of a QD

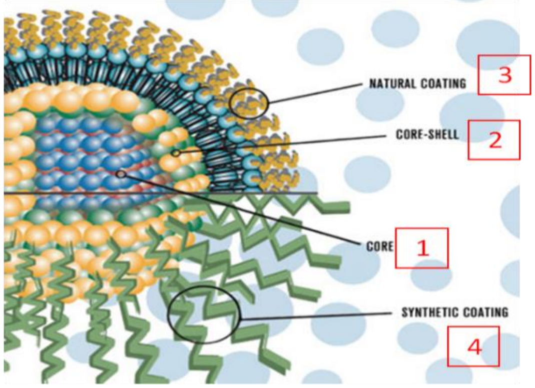

The use of Au NPs in the detection of DNA

In their innovative method, researchers used two groups of Au NPs. A DNA-containing group was bound to half of the target sequence, and the DNA connected to the other group was attached to its other half. A target DNA with a complete sequence is easily connected to both types of particles and thereby connects the two particles.

Since several DNAs are connected to each particle, DNA-containing particles can attach several particles to each other. The aggregation of these gold particles significantly changes their characteristic properties, turning the sample color from red into blue. Since the result of this test is visible without any device, it can be used for the DNA test at home (Figure 5) [15].

Figure 5: DNA test at home

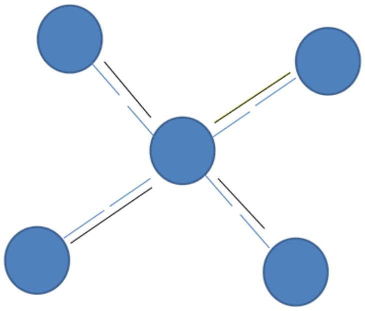

The use of magnetic NPs in the detection of pathogens

Magnetic NPs bind the same as a label to antibody molecules. If a special pathogenic agent is of interest in a sample, specific antibodies of this agent, which are connected to magnetic particles, attach to them.

The sample is washed to separate the unconnected antibodies. If the abovementioned agent is present in the sample, magnetic particles connected to the agent by the antibody produce magnetic fields that are detected by a sensitive device.

The sensitivity of this laboratory skill is better than the existing standard methods, and predicted modifications will soon amplify the sensitivity up to several hundred times. For instance, magnetic NPs attached to the specific IgG, which is specifically connected to protein A of the Staphylococcus bacterial cell wall, can be used to detect this agent. Bacteria attached to functional NPs will be easily separated from the sample using a magnet (Figure 6) [16].

Figure 6: Directed aggregation of harmful bacteria by conjugation with functionalized magnetic NPs

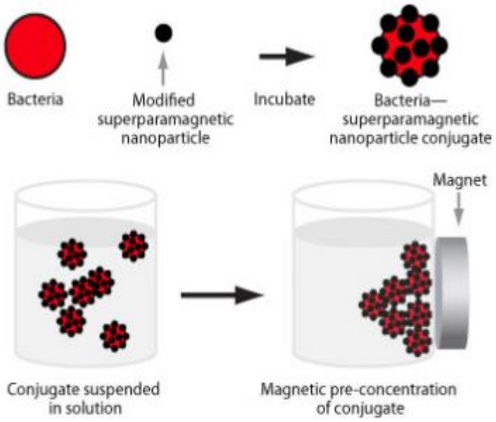
Bacteria
Modified superparamagnetic nanoparticle
Incubate
Bacteria—superparamagnetic nanoparticle conjugate
Magnet
Conjugate suspended in solution
Magnetic pre-concentration of conjugate

Another method is to use the MRI test to detect NPs attached to this agent (Figure 7).

Figure 7: a) Aggregation of functionalized NPs on Staphylococcus bacteria; b) their slight aggregation in the non-functionalized state on these cells

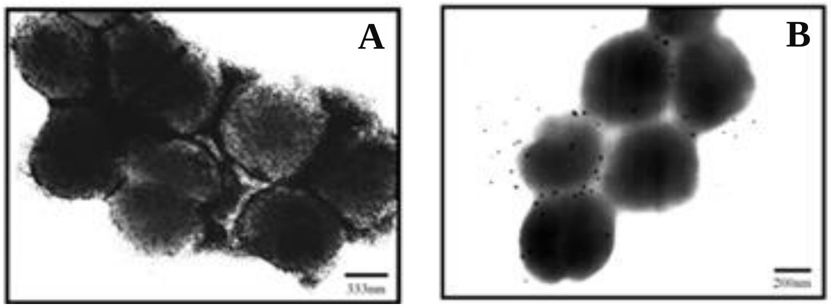

Nonobiosensors

In a previous study (17), synthetic DNA fragments (DNA-Aptamer) fixed on carbon nanotubes (CNTs) were used as probes to bind to the pathogen and produce an electrical signal. The features of this method include prompt, easy, and reliable detection of a low amount of the bacterium (the size of a cell). Indeed, the presence of a low amount of bacteria and the attachment of a specific aptamer to them lead to changes in the interaction between aptamer and CNTs that occurs in a few seconds and thereby increase the related electrode voltage. This nanobiosensor is able to detect a single Salmonella cell in a 5-ml sample (Figure 8).

Figure 8: Detection of Salmonella typhi using an electrochemical nanosensor

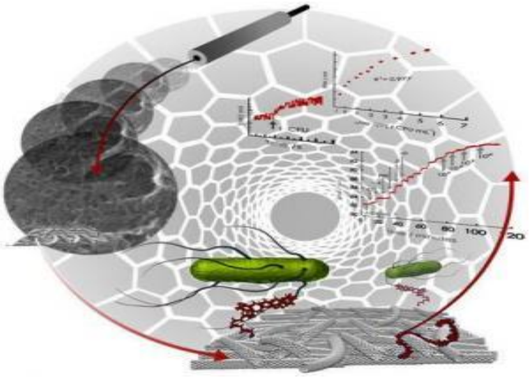

CONCLUSION

Nanotechnology allows the detection at the molecular level or at a single cell level. Au and FE NPs and QDs are among the most widely used NPs in this field, and some nanotechnology devices used in the field of diagnosis (e.g. nano-biochips and nano-biosensors) have many potentials in diagnostic applications. Since there are close associations between nanotechnology and other technologies, this science seems to play a significant role in medical diagnostics, especially the detection of biological threat agents [18-20].

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ORIGINAL ARTICLES

The intestinal microbiome and the role of probiotics/prebiotics in the therapeutic approach of atopic dermatitis: A review

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Abstract: *Atopic dermatitis (AD) is the most common chronic inflammatory skin condition, characterized by multiple recurrent eczematous lesions and intense itchiness. It is a multifactorial skin disorder involving an association between genetic and environmental factors that lead to a defect of the epithelial barrier in conjunction with immunological dysregulation. Over the last decade, there has been an increasing understanding of the role of the human microbiota in preserving skin integrity and that a shift in the homeostasis of these microorganisms may lead to dysbiosis and disease. Diversity in the intestinal microbiome and its role in the etiopathogenesis of AD has been described and has become of great interest. In this report, we have reviewed the importance of the gut microbiome and the possible mechanism in the pathogenesis of AD as well as the therapeutic impact of probiotics and prebiotics.*

Keywords: *atopic dermatitis, probiotics, intestinal microbiome*

INTRODUCTION

Atopic dermatitis (AD) is the most common chronic inflammatory skin condition, characterized by multiple recurrent eczematous lesions and intense itchiness [1]. It is a multifactorial skin disorder involving an association between genetic and environmental factors that lead to a defect of the epithelial barrier in conjunction with immunological dysregulation [2]. Over the last decade, there has been an increasing understanding of the role of the human microbiota in preserving skin integrity and that a shift in the homeostasis of these microorganisms may lead to dysbiosis and disease [3].

AD is a chronic inflammatory skin condition characterized by a recurrent eczematous rash and intense pruritus, having a relapsing disease course [4]. Symptomatology usually commences in infancy, affecting roughly 20% of children, however, it is also vastly prevalent in the adult population [5]. In some children with AD, the clinical symptoms will disappear with growing up, but about 50% of children may acquire allergic asthma, and about 66% present with an increased risk of allergic rhinitis later on in life, a process known as the “atopic march” [6]. The skin lesions in infants are diffusely located, predominantly on the face and the extensor surfaces of limbs, whilst in adults, there is further morphological diversity including localized eczematous plaques, lichenification and erythroderma [3]. This highly pruritic skin condition arises in the genetically predisposed hosts as a result of the disruption of the epithelial barrier along with immunological dysregulation [7]. A genetic linkage has been reported to chromosomes 1q21, 17q25, and 20p [8]. It has been demonstrated that individuals with AD may carry genetic mutations in genes that encode the epidermal protein filaggrin [9]. A secondary major genetic risk factor are the genes implicated in the T helper lymphocyte type 2 (Th2) signaling pathways. Over 30 risk loci for AD have been identified with the use of genome-wide association studies, suggesting that they play a role in the epidermal barrier function and immune responses [10]. Although the pathophysiology of AD is not entirely understood, it is evident that it is a multifactorial process, resulting from a complex association between barrier

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dysfunction, alterations of the immune response, and the environment [11]. Amplification of the pro-inflammatory Th-2 response is represented by the production of interleukins (IL) 4 and IL-13 which are predominant in the acute and chronic phases of AD. Also, IL-25 and IL-33 along with thymic stromal lymphopoietin (TLSP), known to elicit a Th2 response, have been as well identified in patients with AD, having the ability to modulate the stability of the skin barrier and the function of keratinocytes [12]. Dupilumab is a human monoclonal antibody with the role of inhibiting IL-4 and IL-13 by blocking the IL-4 receptor alpha subunit, hence inhibiting the production of IgE by B cells and the activation of eosinophils. Efficacy has been demonstrated by markedly reducing signs and symptoms in patients with AD [13,14].

The intestinal microbiota is a highly complex and dynamic ecosystem, formed in the neonatal gut, continuing to develop and increase in diversity until the child reaches around 3 years old. At this point, the microbial flora becomes adult-like [15]. Gut microbiome configuration can be affected by environmental factors such as diet, stress, pollutants, and living habits, especially in the early years of life [16]. An imbalance in microbial diversity at the level of the intestinal microbiome can lead to shifts in the metabolism of gut microbiota and immune responses. Such changes can have influential effects on the physiological and pathological mechanisms of the human body [17]. In addition, there has been an enhanced understanding and increasing evidence of the correlation between the intestinal microbiome and various skin conditions described as the “skin-gut axis” [18]. Since there is a tight correlation between AD and dysbiosis, the effects on the diversity and function of the intestinal microbiome have become of great interest and have been extensively studied, however, the outcome is still controversial, with some studies showing positive outcomes and others have found no effects in the treatment of AD [19].

GUT MICROBIOME VARIATION AND AD

The human gastrointestinal tract is known to reside over 1000 different species of bacteria, with the majority belonging to 1 of the 4 phyla, Bacteroidetes, Actinobacteria, Firmicutes, and Proteobacteria [20]. This dynamic and complex system can be influenced by genetics and a multitude of environmental factors such as diet, stress, and pollutants [21]. The intestinal microbiota is thought to have an important role in the onset of AD by its involvement in the maturation of the immune system, particularly in the early years of life [22]. The homeostasis of the immune system can be affected by alteration in the gut microbiome by means of metabolites which can have a pro-inflammatory effect when encountering a distinct microbiota in the gut [23]. The resulting gut dysbiosis along with the imbalances of the immune system contribute to the onset and the natural course of AD [24]. Ahead of the birth, there has been found microbial distribution at the level of placenta and meconium, suggesting that microbial colonization occurs in the early stages of life [25]. At the beginning of life, the gastrointestinal tract of the newly born is colonized by numerous microorganisms, a process that is regulated by various factors such as maternal microbiome, delivery modes, milk source as well as the use of antibiotics. Delivery of the newborn via C-section associated with the use of antibiotic treatment prenatally has been associated with an increasingly higher risk of developing AD [26]. Bacterial diversity has been noticed in patients with AD compared with healthy controls. In patients with AD, there has been observed a decreased microbial proportion of beneficial bacteria such as Bifidobacterium and Lactobacillus and an increased proportion of Clostridium difficile, Bacteroides, Staphylococcus aureus, and Escherichia coli [27-29]. A higher abundance of Coprococcuseutactus, a butyrate-producing bacteria has been associated with cases of milder eczema rather than in those with severe AD [30]. A study carried out in South Korea had explored the intestinal microbiome of 90 subjects with AD and 42 healthy subjects. An increase in Faecalibacterium prausnitzii F6 was observed in the subjects with AD which lead to decreased concentrations of intestinal butyrate and propionate [31]. In addition to the pathological mechanisms involved in the development of AD, the presence of Clostridia and Escherichia coli in the gut may have a correlation with AD by means of eosinophilic inflammation [32]. Perhaps the development of AD may not only be promoted by the diversity of the intestinal microbiota on its own and more studies are needed on the mixed effects of environmental factors. There are some recognizable differences between the gut microbiota of subjects with AD versus healthy subjects.

GUT MICROBIAL MECHANISMS INFLUENCING AD

Immunologic pathway

The intestinal microbiome plays an important role in the modulation of the immunologic pathway of AD through probiotics. It has been observed that the oral administration of probiotics interacts with the mucosa of the gastrointestinal system and gut-associated lymphoid tissue (GALT), in which more than 70% of the immune cells are found [33]. Through diverse pathways, probiotics interact with mucosal dendritic cells (DCs), epithelial cells and macrophages. Taking into consideration the probiotic strain, they have the capability of either inducing the activation of the immune signaling via the production of IL-12, IL-18 and tumor necrosis factor-α (TNF-α) or stimulating anti-inflammatory cytokines such as IL-10 and TGF-β [34]. It has been suggested by various lines of evidence that preserving microbial diversity is of great benefit in sustaining the abundant immune milieu of the skin. For example, distinct microbes can evoke particular types of immune cells at skin level such as S. epidermidis which induces IL-17A+ CD8+ T cells which reside in the epidermis, enhancing the innate immunity and hence, limiting the pathogen invasion [35]. In response to stimulation with S. epidermidis, the human skin tropic T cells have been observed to produce IL-17A and IFN-γ [36].

A birth cohort study had demonstrated that the colonization of the gut with strains of S. aureus carrying specific Staphylococcal superantigens (SAgs) combinations and adhesion genes, exhibits a negative correlation with the development of AD later on during infancy [37]. These kinds of strains may stimulate the development and maturation of the infant immune system, despite the fact that skin S.aureus may exacerbate the already established AD. Moreover, the existence of a particular gut microbiome such as Akkermansia muciniphila was correlated with changes in genes that had an effect on the development of the host immune system [38].

Another study has shown that supplementation with Lactobacillus paracasei reduces the sensitivity of the skin and the transepidermal water loss in healthy controls [39]. These effects have been attributed to the increase in TGF-β, a cytokine that has been demonstrated to have an effect on the integrity of the skin barrier [40]. All these studies sustain the fact that there is a correlation between the gut and skin, modulated via the interaction between the immune system and the gut microbiome.

Metabolite pathway

Short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate, produced by the breakdown of non-digestible carbohydrates derived from dietary intake, have numerous physiological effects, in particular, anti-inflammatory [41], which could play an important role in the treatment of inflammatory conditions such as AD. In a mouse study, it has been observed that linoleic acid and 10-hydroxy-cis-12-octadecenoic acid diminished AD symptoms and modulated the gut microbiome [42].

In a different study, the administration of probiotic Bifidobacterium animalis subspecies lactis has been observed to increase the levels of metabolite kynurenic acid, which decreases the behavior of scratching in AD mice [43].

Neuroendocrine pathway

Increasing evidence support the notion that there is a gut-skin axis mediated by neuroendocrine molecules generated by the intestinal microbiome. This suggests that the presence of compositional and proportional differences in the intestinal microbiome are associated with the generation of numerous valuable neurotransmitters and neuromodulators, which are associated with the degree of AD symptoms [44,45]. Tryptophan, a metabolite produced by the gut microbiome, has been observed to induce an itching sensation in the skin [46], however, species of Lactobacillus and Bifidobacterium can generate γ-aminobutyric acid (GABA), having an antipruritic effect [46,47]. Cortisol, a hormone usually released under stressful conditions, can modify the composition of the intestinal microbiome by altering the permeability and barrier function of the intestinal epithelium [48]. Moreover, cortisol can also alter the levels of circulating neuroendocrine molecules such as trimethylamine, tryptamine, and serotonin, hence modulating the skin barrier and its immune function [46,49-52].

THE THERAPEUTIC ACTION OF PROBIOTICS AND PREBIOTICS IN AD

Probiotics

Probiotics are live microorganisms that when consumed, should confer health benefits. Taking into account the role of the gut microbiome in the development and homeostasis of the immune system, probiotics can be effective not only in the treatment of atopic conditions such as AD, but also in the prevention process by means of microbial, epithelial, and immune effects. Probiotics have the ability to modify the microbial composition of the intestinal microbiome, prevent invasion with pathogens by competitively attaching to the epithelial cells, as well as suppress pathogens by means of bacteriocin secretion [53].

Various cytokines such as IL-12, IL-18, and TNF-α can be generated depending on the probiotic strain and can promote immune-stimulated signals or stimulate anti-inflammatory cytokines such as IL-10 and TNF-β to bring about immunity tolerance signals [54]. Moreover, probiotics exert an inhibitory effect on dendritic cells maturation, hence, inhibiting the differentiation of T-cells into Th2 cells which triggers skin inflammation [55,56]. Thymic stromal lymphopoietin (TSLP) regulates the differentiation of naïve T cells mediated by dendritic cells, an action that could be inhibited by probiotics [56]. Gram-positive bacteria Bifidobacterium and Lactobacillus are amongst the most studied and test probiotics in AD [57,58]. These probiotic families lack the lipopolysaccharides which are known to cause inflammation and also release molecules that aid in the maintaining of a healthy gut and hence skin [59]. In mice, supplementation with probiotic Lactobacillus sakei WIKIM30 has shown amelioration of AD skin lesions by induction of Treg cells and alteration of intestinal microbiota [60]. Moreover, Lactobacillus casei variety rhamnosus has shown to diminish AD symptoms in mice trials by increasing levels of bifidobacterium, Lactobacilli, Bacteroides fragilis, and Enterococcus, as well as decreasing levels of Clostridium coccoides [61]. In multiple studies, the efficiency of probiotics was on AD was displayed as an improvement in the SCORAD score [62].

In a randomized placebo-controlled study, 53 children with moderate-severe AD were orally administered with Lactobacillus fermentum VRI-003 PCC, which showed an improvement in the SCORAD score [63]. In another trial carried out on children with ages between one and twelve years of age, administration of Lactobacillus Plantarum CJLP133 strain was observed to reduce levels of INF-γ, IL-4, and eosinophils hence decreasing SCORAD score [64]. Lactobacillus strain has been demonstrated to stimulate the recovery of the skin barrier, as well as inhibit the skin inflammation associated with substance P [65]. In addition, a probiotic trial including human originating probiotics, Lactobacillus and Enterococcus, has demonstrated that these probiotics can boost the production of SCFA, hence reinforcing the mucosal barrier [53,66].

It has been proven that probiotics are efficient even when given prenatally in preventing AD. In a Korean study, supplements of probiotic strains containing Bifidobacterium bifidum BGN4, Bifidobacterium lactis AD011, and Lactobacillus acidophilus AD031 were administered to pregnant women known with a family history of AD, 4 to 8 weeks before giving birth. The probiotics were also given to the infants for the first 2 months of life, resulting in a significantly lower incidence of AD compared to the control group [57]. During an 8-week treatment with a daily intake of Lactobacillus plantarum YIT 0132-fermented citrus juice in adults, it was observed a decrease in AD symptoms. The benefits were observed for a further 8 weeks off treatment [67].

Prebiotics

Prebiotics are non-digestible dietary ingredients that promote certain changes in the gut microbiota composition which brings numerous health befits to the host by stimulating in a selective manner the growth and activity of definite collections of bacterial strains already well-established in the gut flora [68,69]. Prebiotics are known to enhance the SCFAs production in the gut such as acetate, propionate, and butyrate, having anti-inflammatory effects [70], diminishing the formation of toxic fermentation products [71], expanding the Th1/Th2 ratio, increasing the lymphocytes and/or leucocytes number in GALT (gut-associated lymphoid tissue), as well as a surge in intestinal IgA secretion [72].

The most commonly known and well-established prebiotics in the food industry are oligosaccharides such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and long-chain inulin [73,74], however, a few newly discovered prebiotics are gaining interest such as xylo-oligosaccharides, arabinoxylan oligosaccharides, pectin and its products formed by hydrolysis [75-79].

CONCLUSİON

Although there are still a lot of unknown variables that need to be explored in the gut-skin axis, the gut microbiome is undeniably a crucial element contributing to the host homeostasis, allostasis, and pathogenesis of disease through various complex mechanisms.

Modulating the gut microbiome and restoring the immune balance by supplementing with prebiotics and probiotics has been proven to be beneficial not only in the treatment but also in the prevention of AD. Further research is needed to improve the understanding of these highly complex mechanisms lying at the basis of the gut-skin axis and how can we further manipulate the gut microbiota in order to attenuate the clinical manifestations of AD and other skin inflammatory diseases.

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VARIA

Vitamin D receptor gene polymorphisms and spinal muscular atrophy

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Abstract: *Vitamin D receptor gene polymorphisms have been intensively studied in relation with many diseases, including neurodegenerative disorders. We investigated the relationship between VDR polymorphisms and spinal muscular atrophy type I, a common lower motor neuron disease. Forty clinically and molecular diagnosed patients and 54 healthy subjects were analyzed PCR-RFLP method. We found an association trend (p=0.01) for the BsmI polymorphism taken individually and a significant association for two of four-locus inferred haplotypes (fBAt and fbaT). Also, baT, Bat and bAT were the most frequent estimated haplotypes in our control group, which is in accordance with previous published reports for Caucasian populations. We concluded that future studies performed in order to identify possible modifier genes for spinal muscular atrophy disease should take into consideration the involvement of vitamin D receptor gene polymorphisms.*

Keywords: *haplotype, polymorphism, SMA, VDR*

INTRODUCTION

Spinal muscular atrophy (SMA) is one of the most common pediatric neuromuscular diseases with autosomal recessive inheritance [1, 2]. Its cellular basis is degeneration and loss of the anterior horn cells in the spinal cord and the brain stem nuclei, which leads to wasting and paralysis of proximal muscles [3]. Death occurs usually before the age of two years in patients affected by type I, the most severe form of the disease (MIM #253300).

SMA is caused by mutations in the SMN1 gene (located at 5q12.2-13.3). Homozygous absence of SMN1 exon 7 (caused by deletion or SMN1 gene conversion into a SMN2-like gene) is the most common mutation and about 3.6 to 5% of the cases are compound heterozygotes harboring point mutations in the SMN1 gene [3, 4]. Several modifier genes (for example SMN2 [5, 6] and PLS3 [7]), which lead to clinical heterogeneity, have been identified.

Vitamin D may act like a neurosteroid hormone in the areas of neurotransmission, neuroprotection, and neuroimmunomodulation during cerebral development [8]. Moreover, vitamin D deficiency has been associated with neurological and psychiatric disorders.

Trying to bringing new insights into the description of SMA physiopathology, we focused our attention on the relationship between vitamin D receptor (VDR) polymorphisms (FokI, BsmI, ApaI, TaqI) and SMA type I disease. The role of vitamin D in nerve and muscle function has been already assessed [9, 10, 11, 12] as well as the fact that vitamin D actions are mediated by its receptor [13].

MATERIALS AND METHODS

Subjects

We studied 40 SMA type I patients (21 boys and 19 girls) with an average age of 5 ± 0.2 month in the moment of clinically diagnosis at “Al.Obregia” Clinical Psychiatry Hospital, Bucharest, Romania. The Caucasian origin of the patients was declared by their legal representatives who consented to their participation in this study (according to the Declaration of Helsinki). The onset of their clinical signs started between birth and age of six months. Symmetrical atrophy of proximal muscles (more than distal), poor

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muscular tone, absence of tendon reflexes and normal serum creatine kinase (CK) levels were present in all. Fasciculation of tongue, tremor of hands and recurrent respiratory infections were also reported. Arthrogryposis was present in two of the subjects included in the study. Neurogenic changes were recorded in patients for which electromyography (EMG) was possible.

The evolution was progressive in all the cases and death was reported at the hospital for five of them. All had homozygous deletion of SMN1 gene exon 7 which, according to the SMA International Consortium, ascertained the diagnosis. Some of these patients were described in a previous publication [14].

In this control-case study we included also a number of 54 subjects (23 men and 31 women), average age of 25 ±2.4 years, without acquired or personal or familial neuromuscular problems, who consented to participate in this research. The study received the approval of the Ethical Committee of the University of Bucharest.

Genotyping

DNA was isolated from 500 µl of peripheral blood leukocytes, using Genomic Wizard DNA Purification Kit (Promega, Madison, Wisconsin, USA). Genotypes of VDR polymorphisms were detected by the PCR-RFLP method [15, 16]. Primers sequences for FokI, ApaI and TaqI were used as were described before [15, 16]. The primers for BsmI polymorphism, were designed by us using Primer3 software [17] (F: 5'TCCAAAGTTTTGTACCCTGCC3'; R: 5'TTCGTAGGGGGGATTCT3', annealing temperature 62°C).

PCR reactions were performed in a Gradient Palm-Cycler (Corbett Research Pty Ltd, Sydney, Australia). Amplicons were individually digested with restriction enzyme FokI (37°C)/ BsmI (65°C)/ApaI (30°C) or TaqI (65°C), for three hours. Restriction products were separated by electrophoresis on 8% PAGE and visualized using Bio-Imaging System (UVP Inc, Upland, Canada) after staining with ethidium bromide.

We used standard nomenclature and noted VDR polymorphisms alleles with "f" (FokI), "b" (BsmI), 'a" (ApaI) and "t" (TaqI) for the presence of restriction sites, the absence being represented with uppercase letters.

Statistical analysis

Genotypes were counted directly and the distribution of alleles was inferred. Hardy–Weinberg equilibrium was verified and chi-square test ($\chi 2$) was used to compare the observed genotypes and alleles in patients versus control groups [18]. Odds ratios (ORs) were calculated in a 2 × 2 contingency table provided in the SISA program [18] and a p value < 0.05 was considered statistically significant. A Cochrane-Armitage test trend was performed using De Finnetti program [19] in case of the polymorphisms for which p values were closed to the limit for statistical significance. The observed genotypes were introduced also in the SHEsis platform [20] that estimates the haplotypes distribution.

Using the EpiSheet program [21], power size analysis was performed for each polymorphism. Based on reported frequency for CEU population of HapMap [22], we calculated the number of subjects needed to detect at least an OR of 2.0, with 80% power, for a minor allele frequency of 25 %. This analysis indicated that 160 cases and 160 controls would be necessary to provide these values.

RESULTS

Distribution of the genotypes and alleles in the patient and control groups is represented in Table 1. The chi-square test values are also provided in Table 1.

The ORs analysis provided a statistically significant p value only for the BsmI polymorphism (p=0.0338), but this was not confirmed when Yate's correction test was applied (p=0.06). Applied Cochrane–Armitage test for trend showed statistically significant results: ORBBvsBb=0.232 (p=0.0175); ORBBvsBb+bb=0.289 (p=0.0338).

No significant differences were found when the subjects were grouped according to the gender.

Linkage disequilibrium (LD) analysis was also performed. In the control group we found a LD relationship was found between BsmI/TaqI and ApaI/TaqI polymorphisms, while in the patients group an LD relationship was detected between BsmI/ApaI and ApaI/TaqI polymorphisms.

The frequencies for the inferred four locus haplotypes within each group and the Odds Ratio values are given in Table 2. The significant results obtained for fBAt and fbaT haplotypes are discussed later. Results were also obtained for three locus haplotype inferred from combined polymorphisms: FokI, BsmI, ApaI (fBA: OR=0.267, p=0.02; fba: OR=2.44, p=0.02); FokI, BsmI, TaqI (fBt: OR=0.277, p=0.02) and FokI, ApaI, TaqI (faT: OR = 2.653, p=0.01). For the BsmI, ApaI, TaqI haplotypes in the control lot, most frequent were baT (31.4%), Bat (28.1%), bAT (18.2%).

Table 1: VDR genotypes and alleles distribution

Polymorphism	Genotype/ Allele	Cases N (frequency)	Control N (frequency)	OR (*p*) * 95% CI
rs2228570 (*Fok*I)	*FF*	14 (0.350)	23 (0.426)	0.72 (0.45); 0.31-1.68
	Ff	21 (0.525)	23 (0.426)	
	ff	5 (0.125)	8 (0.148)	
	F	49(0.613)	69(0.639)	0.89 (0.71); 0.49-1.62
	f	31(0.387)	39(0.361)	
rs1544410 (*BsmI*)	*BB*	4(0.100)	15(0.278)	0.28 (0.03) ‡ 0.09-0.95
	Bb	23(0.575)	20(0.370)	
	bb	13(0.325)	19(0.352)	
	B	31(0.387)	50(0.463)	0.73 (0.30) 0.41-1.32
	b	49(0.613)	58(0.537)	
rs7975232 (*Apa*I)	*AA*	7(0.175)	15(0.278)	0.55 (0.24); 0.20-1.51
	Aa	24(0.600)	28(0.519)	
	aa	9(0.225)	11(0.203)	
	A	38(0.475)	58(0.537)	0.78 (0.40); 0.43-1.39
	a	42(0.525)	50(0.463)	
rs731236 (*Taq*I)	*TT*	15(0.375)	22(0.407)	0.87 (0.75); 0.37-2.02
	Tt	21(0.525)	23(0.426)	
	tt	4(0.100)	9(0.167)	
	T	51(0.638)	67(0.620)	1.07 (0.81) 0.59-1.95
	t	29(0.362)	41(0.380)	

**Pearson's p value; ‡ statistical significant differences*

Table 2: VDR genotypes and alleles distribution

Haplotypes	Cases (freq)	Control (freq)	OR (p)†, 95%CI ‡
FBAt	18.08 (0.226)	16.11(0.149)	1.67 (0.17), 0.79-3.53
FBAT	5.14 (0.064)	3.02(0.028)	2.39 (0.22), 0.56-10.25
FBat	0.00	4.94(0.046)	-
FBaT	0.00	7.12(0.066)	-
FbAt	2.92 (0.036)	3.37(0.031)	1.17 (0.84), 0.23-5.8
FbAT	3.19 (0.040)	8.76(0.081)	0.47 (0.25), 0.12-1.75
FbaT	19.67 (0.246)	25.69(0.238)	1.04 (0.89), 0.531-2.06
fBAt	3.21 (0.040)	14.32(0.133)	0.27 (0.03), 0.08-0.95 §
fBAT	0.00	1.70(0.016)	-
fBat	0.00	1.22(0.011)	-
fBaT	4.57 (0.057)	1.57(0.015)	4.106 (0.10), 0.65-25.75
fbAT	3.19(0.040)	10.72(0.099)	0.37 (0.12), 0.10-1.36
fbat	2.51 (0.931)	1.03(0.010)	3.363 (0.27), 0.33-33.83
fbaT	15.24 (0.191)	8.42(0.078)	2.801 (0.02), 1.13-6.88 §
Fbat	0.00	0.00	-
fbAt	2.27(0.028)	0.00	-

** Haplotypes analyzed by SHEsis software platform; † Pearson's p value; ‡ Global result: global chi-square 27.84, degree of freedom 11, p = 0.003424 (haplotypes with frequencies below 0.03 in both cases and controls were excluded).*
§ Bold indicates statistical significance; OR = Odd ratio; CI = Confidence interval

DISCUSSION

This study focused on the relationship between four polymorphisms of the VDR gene and SMA type I. To our knowledge, no relationship between these markers and the mentioned pathology has been published so far. However, the interpretation is difficult and limited by the small power size of this study.

We compared distribution of individual markers in the two groups and we found an association trend between BsmI polymorphism and SMA type I physiopathology.

For this marker, an association relationship has been reported with Parkinson's disease [23] and multiple sclerosis [24]. Also, an association between the BsmI-TaqI haplotype and quadriceps muscle strength in men [25] and a correlation between the BB genotype and high risk for bone fractures (which was also found in SMA type I patients) in a large group of postmenopausal women were identified [26].

The results of LD tests were not what we expected. Data from literature (including HapMap database) reported a strong LD relationship between for BsmI, ApaI and TaqI polymorphisms (D' values 0.98 and 1) in European populations [27]. In our control group the most frequent estimated haplotypes were baT, Bat, bAT (in accordance with data reported in a meta-analysis study on Caucasian populations [28]), but we did not find a strong LD for BsmI and ApaI polymorphisms. Different sizes or genetic backgrounds of the analyzed pools are usually the explanation for discrepancies of results. For VDR gene the combined action of recombination hotspots and demographic history events was considered to be responsible for the different LD in European populations [29]. Recombination events (including unequal recombination, intrachromosomal deletion and gene conversion) were also frequently described in SMA type I patients [30]. For our lot we found a LD relationship for BsmI/ApaI, but not between BsmI/TaqI. However, given that a larger group of Romanian subjects [29] didn't revealed significant differences between general and European populations, we assume think that our results are due to the random sampling.

Although we only found significant results only for BsmI polymorphism, the four-locus haplotype analysis revealed also an important contribution for an extended haplotype.

Our results indicated a protective effect for the fBAt haplotype whereas the fbaT combination seemed to confer a significant risk. These results could be sustained by the findings of Morrison et al. [31], who showed that activity of receptor is higher for BAt haplotype comparing with baT haplotype. However, when we analyzed three locus haplotypes (BsmI, ApaI and TaqI), we didn't obtain any statistically significant results. A possible explanation for this could be the fact that FokI polymorphism (the only functional polymorphism among those analyzed) may be in a significant relationship with the disease, but in a genomic context depending manner. Some studies have reported that the combined effects of FokI polymorphism and the BsmI, ApaI, TaqI haplotypes have no major effect on the VDR protein level, but significantly influence the responsiveness to the ligand. The fbaT haplotype is reported to be less responsive for the ligand than FBAt haplotype combination, sustaining also our results. Despite these findings, the present study did not reveal the relationship between FokI polymorphism and the SMA type I physiopathology.

Overall the three locus haplotype analysis revealed the existence of possible protective combinations (fBA, fBt) and possible risk haplotypes haplotypes (fba, faT). Although we did not find significant on individually analysis of each allele studied, we did reveal that the "B" and "A" alleles may confer protection meanwhile the "a" allele may be associated with the risk for the disease. These findings underline the importance of studying VDR haplotypes rather than individual genotypes, as meta-analysis studies already proved [28, 32].

Our hypothesis of the VDR polymorphisms involvement in SMA type I disease is sustained by the associations of these variants with features described in SMA type I disease (e.g. affected muscle function, bone fractures and bone mineral density or hypercalcemia) [11, 33, 34, 35]. Also, the impaired expression of myf5 during the fusion of satellite muscle cells noticed in SMA type I patient's cells [36] and the decreasing effect of 1,25-dihydroxyvitamin D on this muscle specific transcription factor expression could be also in the support of our study purpose [37].

We conclude that VDR gene polymorphisms and their inferred haplotypes (especially fBAt and fbaT) may be associated with SMA type I pathology. We considered that future studies performed in order to identify other possible modifier genes should take into consideration the involvement of VDR gene polymorphisms.

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ORIGINAL ARTICLES

Ultrasound screening of kidney size in children with and without vesicoureteral reflux

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Abstract: *Evaluation of the size of the kidney's parameters of patients with vesicoureteral reflux (VUR) can provide relevant knowledge about the prognosis factors of VUR's complications. The study aimed to assess the size of the renal parameters of children with VUR and compare the results with healthy matched cases by the ultrasound method.*

Methods: In this case-control study, children under 14 years of age with VUR in our pediatric urology practice from May 2019 to May 2020 were included. Eighty children were included in the two groups (case (40 cases) and control group (40 cases)) in the study. The case group included children with VUR, while the control group included healthy children. The data regarding Urinary Tract Infection (UTI), the length and width of the kidney, the size of the pelvis, parenchyma thickness, and volume of the kidney were recorded.

Results: The mean age of children was 3.07 ± 3.05 years, and 35.0% of them were male. The mean number of UTIs in the VUR group was 1.90 ± 1.15 and in the control group was 1.10±0.30 (P=0.031). The incidence of UTIs was significantly higher in the VUR group than in the control group (P<0.001). Length, width, the posterior-anterior diameter of kidney, renal parenchyma thickness, and renal volume in the VUR group were significantly lower than the control group (P< 0.001). There was no difference between case and control groups in terms of anterior-posterior diameter of the pelvis (P= 0.653).

Conclusions: The results showed that the size of kidneys parameters in children with VUR was less than in the control group. Also, the incidence of UTIs in the VUR group was significantly higher than in the control group. Ultrasound is a safe and non-invasive tool that can use for checking the size of the children's kidneys with VUR for predict of possible complications.

Keywords: *kidney, vesicoureteral reflux, children, ultrasound*

INTRODUCTION

VUR is the retrograde flow of urine from the bladder to the ureter and renal pelvis [1-3]. The prevalence of VUR is approximately 1-6%, with prevalent inheritance in the pediatric population and it is more common in infants [4, 5].

VUR is a risk factor for the incidence of UTI, and it can develop in 18–40% of children examined for their first UTI. Large renal scars cause renal dysfunction, renin-dependent hypertension, renal insufficiency, and reduced growth [6].

Evidence shows that reduced renal function and hypertension in patients with V UR are related to renal scars. Untreated VUR increases the risk of UTI, hypertension, and progressive kidney damage [7-9].

VUR is the most frequent congenital anomaly of the urinary tract. The association between urinary VUR, UTIs, and kidney scarring has been evaluated in several studies [10-14].

Routine diagnostic imaging methods for detecting VUR are Isotope Cystography and ultrasound. However, voiding cystourethrogram (VCUG) is the gold standard for detecting VUR [1-5]. VCUG is an essential fluoroscopic system for the examination of the lower urogenital tract. VCUG is an essential fluoroscopic system for the examination of the lower urogenital tract. Many children with small kidney sizes will be prescript to VCUG for detecting VUR [15-18].

Kim et al. reported a correlation between total kidney volumes estimated by ultrasound and decreased kidney function [7]. Renal growth in VUR patients is regularly

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estimated by ultrasound [19]. Ultrasound has many preferences for parents, such as non-invasive, safe, easily reachable, and economical [20-23].

Examining the size of the kidney's characteristics of patients with VUR can provide appropriate information to physicians about vesicular and reflux disease and its effects on kidney function. The study aimed to assess the renal size of children with VUR and compare the results with healthy matched cases by the sonography method.

METHODS

In this case-control study, children under 14 years of age with VUR in our pediatric urology practice from May 2019 to May 2020 were included.

After parents gave written informed consent, the children were referred to sonography through a pediatrician. All ultrasounds were conducted by one radiologist with good expertise in pediatric sonography.

Eighty children were included in the two groups (case (40 cases) and control group (40 cases)) in the study. The case group included children with VUR, while the control group included healthy children. Children were matched between groups in terms of age and gender. VUR was diagnosed using the VCUG method. The VUR grade was written on each affected child and then underwent an ultrasound. Healthy children also underwent ultrasound. The sonographer was blind about allocation cases in the two groups.

The ultrasound device was a Samsung model with a 3.5-5 MHZ curvilinear probe Ultrasounds were done twice, first after the cases were sufficiently hydrated and then 10 to 15 minutes after emptying the bladder. The radiologist, with the help of his assistant, measures and records data on the length and width of the kidney, the size of the pelvis, paranchyma thickness and volume of the kidney. The VUR was graded 1-5 according to the International Reflux Study of children.

The protocol of the research was approved by the ethics committee of Arak University of Medical Sciences with the IR.ARAKMU.REC.1399.173 code.

Data were presented by mean, standard deviation, frequency, and percent. T-test and Mann Whitney test were used for comparison of quantitative data between groups according to the normality distribution. Chi 2 and Fisher exact test were used for comparison of qualitative data between groups. Data analyses were done in SPSS-20 software. A P-value<0.05 was regarded as statistically significance.

RESULTS

The mean age of children was 3.07 ± 3.05 years, and 35.0% of them were male. There was no significant difference between the groups in the age and sex distribution (P=0.780 and P=0.542, respectively) (Table 1).

Table 1: Distribution of age and sex according the groups of the study

Items		VUR	control	P-value
Age, years, mean±SD		3.10 ± 3.08	3.05±3.04	0.780
Sex, N (%)	Female	28 (70.0%)	27 (67.5%)	0.542
	Male	12 (30.0%)	13 (32.5%)	

There were 66 VUR kidneys in the case group, and in the control group, there were 80 healthy kidneys. There were 26 (65.0%) two-side VUR patients. Based on initial VCUG detection, 1-5 grade VUR were diagnosed in 7.6%, 33.3%, 36.4%, 13.6% and 9.1% patients, respectively. There was one (2.5%) hydronephrosis case in I-IV grade, respectively (Table 2).

The mean number of UTIs in the VUR group was 1.90 ± 1.15 and in the control group was 1.10±0.30 (P=0.031). The mean of UTIs in the VUR groups was significantly higher than control group. The incidence of UTIs was significantly higher in the VUR group than in the control group (P<0.001) (Table 3).

Table 2: Distribution of hydronephrosis, VUR grading, and VUR type in the study

Items	Degree	Percent
Hydronephrosis	I	1 (2.5%)
	II	1 (2.5%)
	III	1 (2.5%)
	IV	1 (2.5%)
VUR	I	5 (7.6%)
	II	22 (33.3%)
	III	24 (36.4%)
	IV	9 (13.6%)
	V	6 (9.1%)
VUR type	I	14 (35%)
	II	26 (65%)

Table 3: Distribution of UTIs in case and control groups

Items		VUR	control	P-value
Number of UTIs, mean±SD		1.90 ± 1.15	1.10±0.30	0.031
UTIs, N (%)	Yes	27 (67.5%)	11 (27.5%)	<0.001
	No	13 (32.5%)	29 (72.5%)	

Length, width, the posterior-anterior diameter of kidney, renal parenchyma thickness, and renal volume in the VUR group were significantly lower than the control group (P< 0.001). However, there was no significant difference between case and control groups in terms of anterior-posterior diameter of the pelvis (P= 0.653) (Table 4).

Table 4: Distribution of size of renal parameters in case and control groups

Items	VUR	control	P-value
Length	62.14±13.33	69.56 ±14.16	<0.001
Width	30.40±6.82	35.16±6.72	<0.001
Kidney anterior-posterior	24.77±4.86	28.06±4.82	<0.001
Volume	26.32±17.03	38.82±19.88	<0.001
Kidney pelvic anterior-posterior	3.31±2.44	3.474±1.78	0.653
Thickness of the upper parenchyma bridge	9.39±2.42	10.19±2.08	0.035
Thickness of the middle parenchyma bridge	9.08±2.11	10.87±2.20	<0.001
Thickness of the under parenchyma bridge	8.89±2.08	10.36±2.04	<0.001
Parenchyma thickness	9.13±1.83	10.48±1.94	<0.001

DISCUSSION

Ultrasound is a low-cost, non-invasive, and radiation-free procedure, and parental acceptance is very high [19-21]. Recently, many studies have assessed ultrasound to detect VUR in an attempt to eliminate radiation from previous methods [19, 24-27]. Evaluating the size of the parameters of the kidney in VUR cases and comparing it with all healthy individuals can provide appropriate information about VUR disease and its effects on the kidney.

The present study showed that length, width, the posterior-anterior diameter of kidney, renal parenchyma thickness, and renal volume in the children with VUR were lower than the healthy children. The association of reduced kidney size and growth with patients with VUR has been assessed by several studies [19, 24, 25]. Also, some studies showed that growth in renal size can increase after VUR treatment [26, 27]. However, some studies noted that in VUR children, there is an association between unscarred and no recurrent UTIs kidney with normal growth in kidney size [28, 29].

Zerin et al. showed that the renal children with VUR were significantly smaller than those in cases without VUR [30]. The results of this study are in agreement with the current study. Ross et al. (2015) assessed the effect of VUR and UTIs on the growth of the children solitary kidney. They showed VUR solitary renal with UTI had a significantly lower growth rate. Also, there was no difference in the incidence of UTI between those without VUR and those with VUR [19]. In the present study, UTIs were significantly higher in the VUR group than in the control group. The frequency of UTIs in the VUR group was significantly higher than in the control group. So, the results of the current study, the incidence of VUR in children is associated with UTIs. Garcia-Roig et al. (2016) showed that VUR is the most common cause of UTIs or pyelonephritis in children [20]. Also, besides pyelonephritis, other long-term complications such as recurrent kidney infections, kidney scars, proteinuria, and hypertension can happen in VUR patients.

Overall, the results in the present study showed that the kidneys involved in vesicoureteral reflux are smaller than healthy kidneys. Perhaps the cause of this problem can be attributed to the development of renal scars in vesicoureteral reflux cases. Ghanesherbaf et al. (2011) found that the grade III and higher of VUR, and the frequency of recurrences of UTIs were associated with renal scarring in children with a urinary tract infection [21]. The development of kidney scar can lead to kidney failure, so studies noted that early treatment is essential for these patients. A review study by Mattoo et al. (2011) found that VUR in children is associated with an increased incidence of UTI, kidney scar, and nephropathy [22]. Roihuvuo-Leskinen et al. (2013) showed that size of adults' kidney with a history of vesicoureteral reflux as children were smaller than those without VUR. Therefore, they concluded that the total volume and size of the kidney could be a prognosis factor in early adulthood could for the possible deterioration of the kidney in later life [23].

Of the 66 kidneys included in the case group, the most common degrees of VUR were grade two (22 involved kidneys) and grade three (24 involved kidneys). Of these affected kidneys, only four had hydronephrosis. Sorkhi et al. (2010) assessed 58 VUR kidneys. There were 19 cases in grade II and 13 cases in grade III [18]. This finding is consistent with the present study.

The current research is limited by the natural choice bias of retrospective studies. Moreover, the variability of ultrasound kidney analysis is well admitted. More extensive cohort studies of children with VUR can assess the size of parameters of the kidney during follow-up and may achieve more knowledge about prognosis factors for possible complications.

CONCLUSION

The results showed that the size of kidneys parameters in children with VUR was less than in the control group. Also, the incidence of UTIs in the VUR group was significantly higher than in the control group. Ultrasound is a safe and non-invasive tool that can use for checking the size of the children's kidneys with VUR for predict of possible complications.

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Myocardial work – are we ready for a new parameter in clinical practice for evaluating left ventricle function?

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Abstract: *Left ventricle systolic function is an essential parameter in different clinical scenarios and the usual methods available for its assessment are sometime suboptimal, depending on the filling conditions of the heart. Therefore, the development and implementation in clinical practice of a new parameter for assessing left ventricle contractile function is desirable. Mechanical work defined as energy transferred to or from an object via the application of force along a displacement seems to be a promising method for myocardial performance estimation. Although initially this parameter was calculated on the left ventricle volume-pressure curve estimated by cardiac catheterization, an easier method considering non-invasive left ventricle pressure and global longitudinal strain assessed by speckle tracking echocardiography was developed recently. Myocardial work offers information regarding the segmental and global function of the left ventricle, and it is considered a more sensitive marker for assessing left ventricle performance compared to ejection fraction and global longitudinal strain. Its applicability in various cardiac pathologies was demonstrated recently in several studies and its use in every day practice may bring important additional information for clinical decision making.*

Keywords: *myocardial work, mechanical work, global work, systolic function*

INTRODUCTION

Left ventricle systolic function is an essential parameter in different clinical scenarios and its accurate estimation is mandatory in every day clinical practice. The methods available for assessing left ventricular (LV) contractile function, such as ejection fraction (LVEF) or global longitudinal strain (GLS) are dependent on the filling conditions of the heart. Myocardial work (MW) emerged as a promising option for assessing LV systolic performance, as a more reliable and independent technique. This method removes afterload dependence by incorporating it into the analysis of the GLS [1,2,3].

Mechanical work is defined as energy transferred to or from an object via the application of force along a displacement. This formula is not well suited for the heart because it is difficult to calculate the myocardial force applied during the cardiac cycle. Therefore, the idea of using pressure as a substitute for force arose. This is defined as the force applied to the LV walls divided into several segments. Usually, myocardial work is determined based on the volume-pressure curve in the LV, obtained by cardiac catheterization. This method was introduced in 1979 by Saga and reflects myocardial oxygen consumption and LV contractile function. However, the clinical utility is reduced as it requires an invasive maneuver [1,2,3].

An easier method of approximating mechanical work takes into consideration non-invasive LV pressure and GLS, a concept developed by Russel and colleagues. The LV pressure curve is estimated by incorporating the systolic blood pressure measured at the brachial level and the phases of the cardiac cycle (isovolumic relaxation phase, ejection phase, isovolumic contraction phase), established using echocardiography. In their study, the LV volume-pressure curve correlates with the pressure-strain curve in patients with left bundle branch block and intra-ventricular contraction asynchrony or in those with ischemic heart disease. Therefore, myocardial work is a promising method

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for non-invasive exploration of LV myocardial performance, already validated for several clinical situations [1,2,3].

ACQUISITION AND ANALYSIS

Myocardial work is obtained by using a speckle-tracking technique. The necessary ultrasound acquisitions include the apical four, two and three chamber views, to generate the global longitudinal strain. It is necessary to acquire images at 60-80 frames/second, to properly visualize the edge of the endocardium and to establish an adequate width of the region of interest at the level of the myocardium, to allow the correct evaluation of GLS and, thus, of myocardial work [1,2].

Blood pressure measurement using a standard cuff placed at the brachial level should be performed simultaneously with the acquisition of echocardiographic images. This is an essential parameter used as a surrogate for LV systolic pressure for the calculation of the global work index [2].

After obtaining the GLS and the characteristic bull's eye image, the opening and closing of the aortic and mitral valves will be selected in an automatic or manual manner, guided by the three-chamber apical view and the concomitant electrocardiogram. The LV volume-pressure curve is generated from an empirical curve, modified according to the duration of the isovolumic contraction phase and the ejection phase. These phases are determined by echocardiography after selecting the moments attributed to the opening and closing of the aortic and mitral valves. Thus, it is possible to define the phases of the cardiac cycle. The maximum LV systolic pressure is equal to the systolic blood pressure, in the absence of a gradient in the LV ejection tract or at the aortic valve [1].

Subsequently, systolic, and diastolic blood pressure are introduced into the formula and the calculation of myocardial work is approved (Figure 1) [2].

Myocardial work is measured by the area of the LV pressure-strain curve, generated based on filling pressures and GLS1. Myocardial work is composed of several parameters - global work index, global constructed work, global wasted work, and global work efficiency [1].

Figure 1: The first step in the MW method involves acquiring four-, two-, and three-chamber apical views, followed by GLS calculation. The myocardial work option is then selected, followed by the introduction of blood pressure values (systolic, diastolic) and the selection of the opening and closing moments of the aortic and mitral valves, guided by the concomitant electrocardiogram. Based on the pressure-strain curve, myocardial work is calculated, along with its components, the bull's eye image is generated for the myocardial work index (with the values of this parameter on a 17 segments model, along with the color code) and the relationship between constructed work and wasted work through the bar chart.

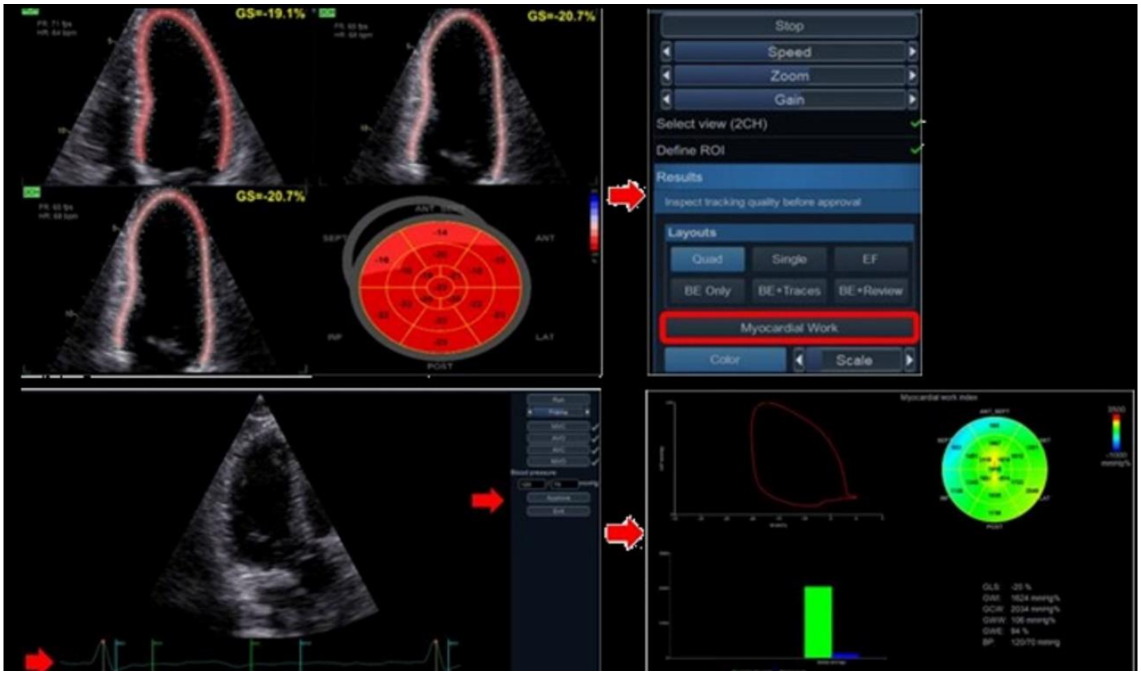

Global work index is calculated by the area of the pressure-strain curve resulting from the closing and opening of the mitral valve (Figure 2), representing the mechanical systole, including the phase of contraction and isovolumic relaxation. Given that pressure is used instead of force, it is not possible to calculate myocardial work, but an index of it (GWI) [2].

Figure 2: The pressure-strain curve, along with the phases of the cardiac cycle and the moments of closure and opening of the aortic and mitral valves. Adapted from (1). AVC - aortic valve closure, AVO - aortic valve opening, MVC - mitral valve closure, MVO - mitral valve opening.

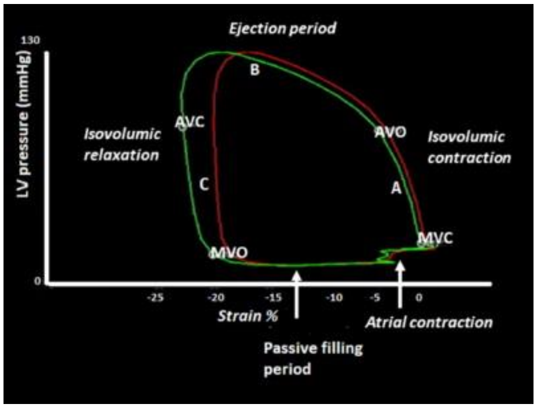

Normally, the myocardial segments contract and relax synchronously to participate constructively in achieving the stroke volume.

Global constructed work (GCW) represents the productive work submitted by the LV for achieving systolic shortening and elongation during the period of isovolumic relaxation, the physiological processes carried out between the opening of the aortic valve and the mitral valve [1,3].

Consumed work (global wasted work) represents the unproductive effort made to elongate the myocardial fibers in the LV systole and shorten them in the isovolumic relaxation phase. It occurs in cases with asynchronous intraventricular contraction, such as patients with bundle branch block or myocardial ischemia.

Global wasted work (GWW) increases the metabolic burden of LV and may contribute to its remodeling [1,2,3].

Global work efficiency (GWE) is the constructed work divided by the sum of the constructed and consumed work. This parameter expresses how efficiently energy was invested during the cardiac cycle and is reported as a percentage.

Therefore, the value for a normal heart with synchronous contraction and relaxation, this value should be close to 100% [1]. This parameter is reported in the form of a bull's eye numerically and by color code (green indicates areas with a high degree of efficiency and red indicates areas with a low degree of efficiency). Possible clinical applications for determining this parameter include improved selection criteria for cardiac resynchronization therapy (CRT) or for revascularization of patients with myocardial infarction [1,2,3].

The parameters described above are evaluated globally and segmentally (17 segments), generating a bull's eye, like GLS. The global work index can be evaluated visually by means of a color scale - red represents an intense work area, green normal work, and blue negative work. In the case of negative work, energy consumption exceeds production, due to elongation of fibers during systole or shortening during the period of isovolumic relaxation.

NORMAL REFERENCE VALUES

The NORRE study conducted by the European Association for Cardiovascular Imaging was the first prospective, multi-center study to include a large group of 226 healthy volunteers between the ages of 20 and 78, to establish reference values for myocardial work and its parameters [1,2]. The values obtained in this study are presented in Table 1.

Other studies on healthy volunteers resulted from the STAAB cohort or the study led by Galli5, with the results shown in Table 2.

The results obtained in the two studies were similar, indicating normal values above 95% for GWE. GWW has low values in healthy subjects, but not equal to zero.

Table 1: Reference values for myocardial work parameters according to the NORRE study. Adapted from [2].

	Total number (n=226)	**Male (n=85)**	**Female (n=141)**
GWI (mm Hg%)	1,896 ±308	1,849 ± 295	1,924 ±313
GCW (mm Hg%)	2,232 ± 331	2,228 ± 295	2,234 ±352
GWW (mm Hg%)	78,5 (53-122,2)	94 (61,5-130,5)	74 (49,5-111)
GWE (%)	96 (94-97)	95 (94-97)	96 (94-97)

Table 2: Normal reference values of myocardial work from three studies with healthy subjects. Adapted from [1].

Variabile	GWI	GCW	GWW	GWE
NORRE study	1,896 ±308	2,232 ±331	78,5 (53-122,2)	96 (94-97)
STAAB cohort	2,209 ±307	2,430 ±351	74 (54-101)	96 (95-97)
Galli et al.	1,926 ±247	2,224 ±229	90 (62-123)	96 (94-97)

An example of myocardial work for a healthy subject is shown in Figure 3A**.**

PRACTICAL APPLICATIONS OF MYOCARDIAL WORK

MW is considered a more sensitive marker for assessing LV performance compared to ejection fraction and GLS. Its applicability in various cardiac pathologies was demonstrated in several studies [4].

Bundle branch block

In a normally functioning heart, all 17 segments contract at the same time and contribute to the ejection of the blood into the aorta, resulting in a very low GWW value. In the presence of a bundle branch block, certain LV segments elongate in the systole or contract against a closed aortic valve, resulting in a significant increase in GWW. GWW does not contribute to LV ejection, which explains why MW is more sensitive in assessing LV efficiency, even when LV contractility is normal [4].

Cardiac resynchronization therapy (CRT)

Galli et al demonstrated a potential role of MW in assessing post-CRT response [5]. Thus, at baseline, GCW and GWW were significantly higher in CRT responders compared to non-responders. Following a multivariate analysis, values of GCW > 1057 mmHg % and GWW > 384 mmHg % were considered predictive of positive response post-CRT.

The 6-month reassessment after the intervention showed that responders had an increase in GCW and a decrease in GWW associated with reverse LV remodeling.

Figure 3: A – Normal heart - GWI and GWE within normal limits with GCW predominance over GWW, GLS with normal value; B – Arterial hypertension - GLS is normal, while MW bull's eye shows elevated values of GWI and GCW, with a normal value of GWE

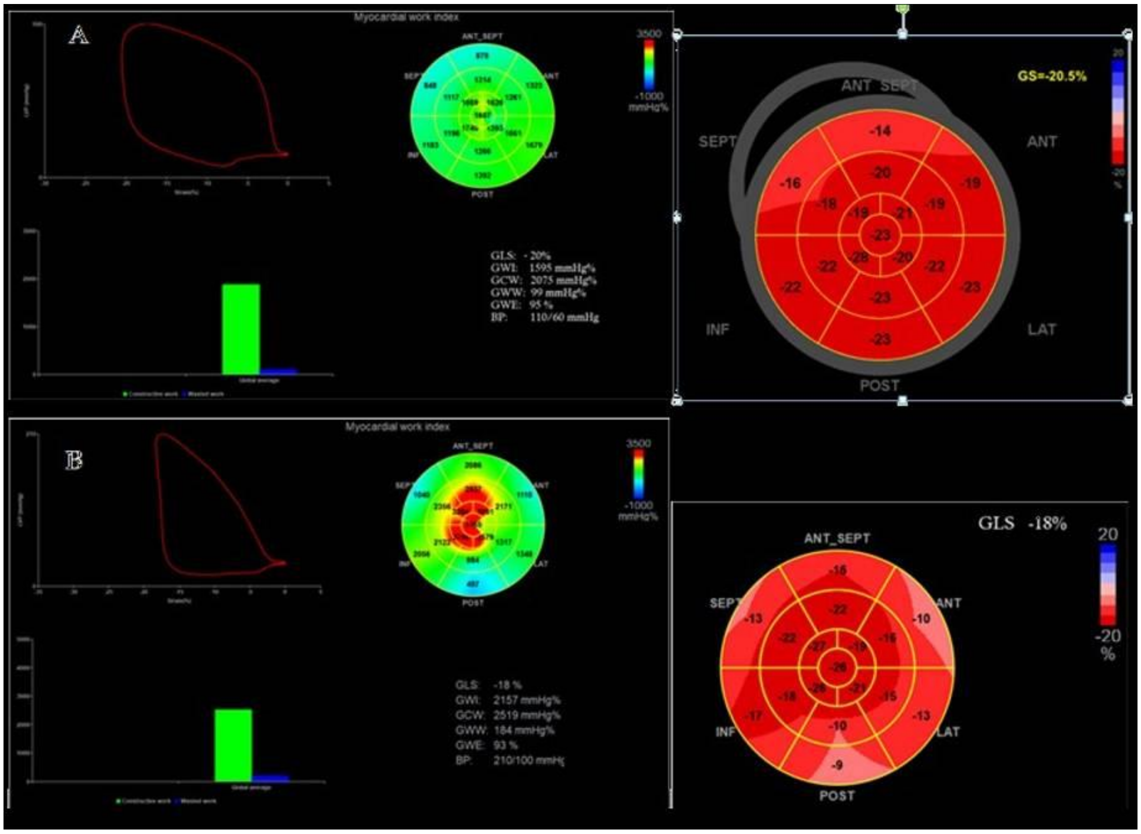
A
Myocardial work index
ANT_SEPT
SEPT
ANT
INF
LAT
POST
3500
-1000
mmHg%
GLS: - 20%
GWI: 1595 mmHg%
GCW: 2075 mmHg%
GWW: 99 mmHg%
GWE: 95 %
BP: 110/60 mmHg
GS=-20.5%
ANT SEPT
SEPT
ANT
INF
LAT
POST
-14
-16
-20
-19
-18
-19
-21
-19
-23
-22
-28
-20
-22
-22
-23
-23
-23
20
-20
%
B
Myocardial work index
ANT_SEPT
SEPT
ANT
INF
LAT
POST
3500
-1000
mmHg%
GLS: -18 %
GWI: 2157 mmHg%
GCW: 2519 mmHg%
GWW: 184 mmHg%
GWE: 93 %
BP: 210/100 mmHg
GLS -18%
ANT_SEPT
SEPT
ANT
INF
LAT
POST
-16
-13
-22
-10
-22
-27
-19
-16
-26
-18
-28
-21
-15
-17
-10
-13
-9
20
-20
%

Van der Bijl et al also evaluated patients with resynchronization indication, concluding that another predictor for CRT response is GWE, with lower values before CRT being associated with a better prognosis [6].

In another study, Galli et al analyzed the effect of a preserved GCW value regarding echocardiographic response and post-CRT mortality, showing that GCW > 888 mmHg% correlated with a 15% reduction in LV telesystolic volume and a significant decrease of mortality compared to lower values [7].

Arterial hypertension

Loncaric et al have shown that under acute pressure overload, the LV ejection fraction remains unchanged, while the GLS decreases and the MW increases.4 In these patients, the MW increases, as each myocardial segment must produce additional energy to pump blood against an increased afterload.

Under conditions which involve chronic exposure to pressure overload, LV remodeling occurs, with myocardial hypertrophy and fibrosis, and in this situation the MW values decrease (Figure 3B).

Chronic heart failure (HF)

a) *with preserved LV ejection fraction*

Chronic heart failure with preserved LVEF is becoming an increasingly diagnosed entity, the main pathophysiological mechanism being the presence of diastolic dysfunction. Paolisso et al followed the role of MW in predicting the first hospitalization caused by HF in patients with preserved LVEF, showing that an increased value of GWW with a cut-off of 170 mmHg % was associated with a higher percentage of patients being hospitalized [8].

b) *with decreased LV ejection fraction*

Hedwig et al demonstrated a good correlation between GWI and traditional echocardiographic parameters (LVEF, GLS), but also with the NT-proBNP value and the maximum O_2 consumption in the cardiopulmonary exercise test, a GWI value < 500 mmHg % having a negative predictive role [9]. An example is presented in Figure 4A.

Figure 4: A – Nonischemic dilated cardiomyopathy - severely reduced GWI and GCW and high GWW; B – Hypertrophic cardiomyopathy - low GWI and GCW and high GWW, noting a scratchy pattern of MW distribution due to the presence of asymmetric hypertrophy, especially in the lateral LV wall and in the mid and apical segments; C – Coronary heart disease - the red curve shows the overall value of MWI, while the green curve shows the pressure-volume curve at the level of a normal myocardial segment (C1) compared to an akinetic segment (C2). There is a lower value of GCW and a higher value of GWW in the akinetic segment compared to the normokinetic segment.

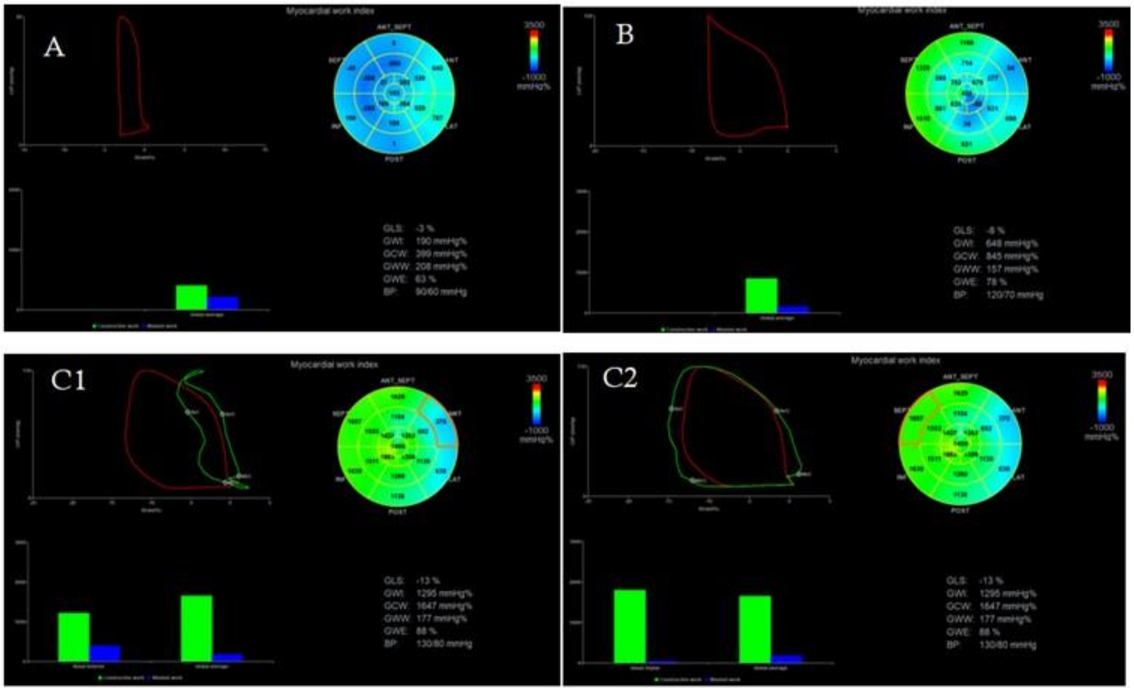
A
Myocardial work index
ANT_SEPT
SEPT
ANT
INF
LAT
POST
3500
-1000 mmHg%
GLS: -3 %
GWI: 190 mmHg%
GCW: 399 mmHg%
GWW: 208 mmHg%
GWE: 63 %
BP: 90/60 mmHg
B
Myocardial work index
GLS: -8 %
GWI: 648 mmHg%
GCW: 845 mmHg%
GWW: 157 mmHg%
GWE: 78 %
BP: 120/70 mmHg
C1
Myocardial work index
GLS: -13 %
GWI: 1295 mmHg%
GCW: 1647 mmHg%
GWW: 177 mmHg%
GWE: 88 %
BP: 130/80 mmHg
C2
Myocardial work index
GLS: -13 %
GWI: 1295 mmHg%
GCW: 1647 mmHg%
GWW: 177 mmHg%
GWE: 88 %
BP: 130/80 mmHg

Wang et al showed that patients with a GWI < 750 mmHg % had a significantly higher risk of mortality and a higher rate of hospitalization due to HF compared with those who had a GWI > 750 mmHg % [10].

In another study by Bouali et al, in patients treated with sacubitril/valsartan there was an improvement in both conventional echocardiographic parameters and GCW and MWE at reevaluation, 12 months after initiation of treatment. Instead, the GWW remained unchanged. A GCW value ≤ 910 mmHg at baseline was associated with more advanced disease, higher LV telediastolic volume, and lower LVEF, and was also a predictor of major cardiac adverse events [11].

Coronary heart disease

Evaluation of patients with coronary heart disease is difficult in the absence of LV regional dysfunction. Echocardiographic evaluation is based on subjective methods, to establish the segmental and global systolic LV function. MW has shown better sensitivity and accuracy compared to LVEF and GLS in identifying patients with coronary heart disease. Edwards et al demonstrated that GWI is a strong predictor. A GWI cut-off value of 1810 mmHg % had a specificity of 92% and a sensitivity of 51% [12]. An example is presented in Figures 4C1 and C2.

Boe et al analyzed a cohort of patients with acute coronary syndrome without ST-segment elevation, showing that GWI is significantly lower in the territory supplied by the coronary artery at risk of acute occlusion, being an important parameter that could guide the indication for prompt intervention [13]. Meimoun et al studied MW in patients with anterior ST-segment elevation myocardial infarction, showing that GCW is the best parameter to predict segmental and global LV recovery [14].

The use of MW was also attempted in the echocardiographic stress test, with Borrie et al demonstrating a significant decrease in GWI among LV segments with inducible ischemia, in contrast to healthy subjects in which a 54% increase in GWI was obtained [15].

Hypertrophic cardiomyopathy

There is several studies that have observed the evolution of MW in patients with hypertrophic cardiomyopathy (HCM). Hiemstra et al showed that GCW, GWI and GWE were significantly reduced, while the value of GWW was increased among patients with HCM [16]. An example is presented in Figure 4B.

GCW was the only predictor for the presence of myocardial fibrosis confirmed by cardiac magnetic resonance, with values < 1623 mmHg% proving a good sensitivity and specificity (82% and respectively 67%) [17].

Figure 5: A – Aortic stenosis- The average aortic transvalvular gradient was added to systolic blood pressure to estimate LV afterload. We can observe that, although the GLS value is similar for two patients with aortic stenosis, the first patient has lower values of GWI and GCW (A1) compared to the second one (A2), the first one having more severe symptoms; B – Mitral regurgitation - normal values of GCW and GWI are observed in a patient with severe asymptomatic mitral regurgitation; C – Aortic regurgitation - normal values of the parameters in a patient with asymptomatic chronic severe aortic regurgitation

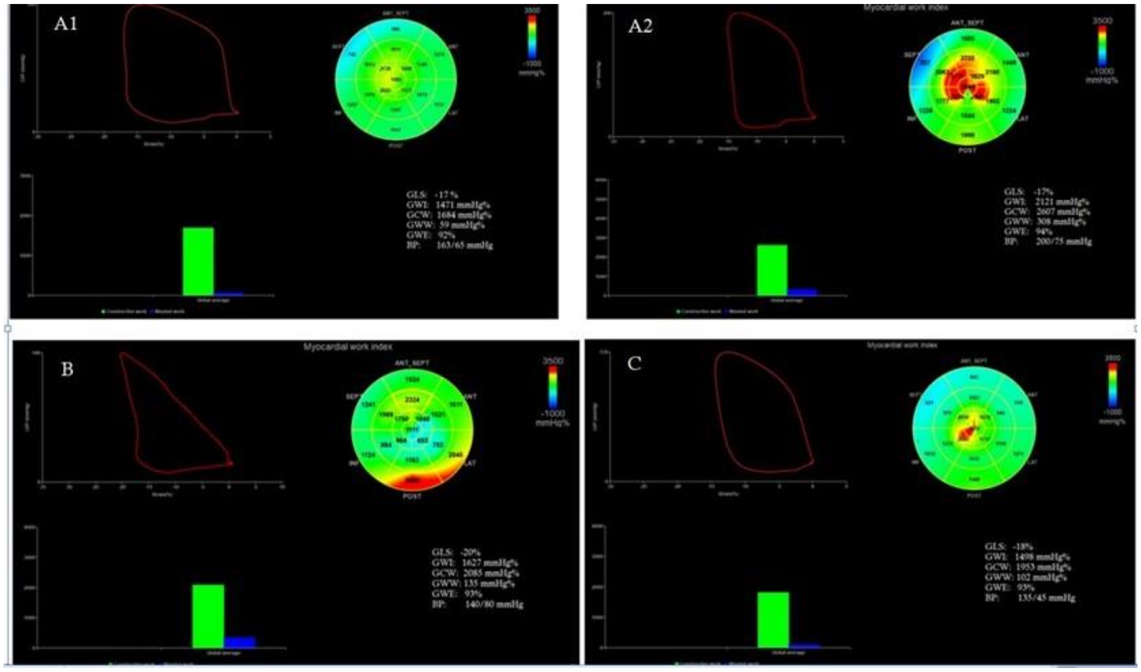
A1
GLS: -17 %
GWI: 1471 mmHg%
GCW: 1684 mmHg%
GWW: 59 mmHg%
GWE: 92%
BP: 163/65 mmHg
A2
Myocardial work index
3500
-1000 mmHg%
GLS: -17%
GWI: 2121 mmHg%
GCW: 2607 mmHg%
GWW: 308 mmHg%
GWE: 94%
BP: 200/75 mmHg
B
Myocardial work index
3500
-1000 mmHg%
GLS: -20%
GWI: 1627 mmHg%
GCW: 2085 mmHg%
GWW: 135 mmHg%
GWE: 93%
BP: 140/80 mmHg
C
Myocardial work index
GLS: -18%
GWI: 1498 mmHg%
GCW: 1953 mmHg%
GWW: 102 mmHg%
GWE: 93%
BP: 135/45 mmHg

In patients with asymmetric LV wall hypertrophy, a scratchy pattern of MW distribution was observed. In this case, segmental MW values were more useful in predicting the presence of myocardial fibrosis compared to global ones [18].

Different segmental values were also identified among patients with other HCM phenocopies. Clemmensen et al also showed that in patients with cardiac amyloidosis the MW values were significantly lower compared to healthy subjects. At the same time, lower values of GWI were observed in the basal segments compared to the apical ones, and GWI was associated with a lower effort capacity and a worse prognosis [19].

Valvular heart disease

a) *Aortic stenosis*

To estimate more accurately LV afterload, Jain et al added the mean aortic gradient to systolic blood pressure, demonstrating a good correlation with invasive measurements of systolic blood pressure. In the same study, the authors demonstrated an immediate decrease of MW in post-TAVR patients [20].

Another study found that in patients with aortic stenosis, the presence of more severe symptoms of heart failure correlated with lower values of GWI and GCW, consistent with the presence of a higher degree of myocardial fibrosis secondary to chronic exposure to increased afterload [21].

A practical example is shown in Figure 5 A1 and A2.

b) *Aortic regurgitation*

Evaluation of MW in patients with chronic severe aortic regurgitation and preserved LVEF could provide additional information on myocardial performance. Meucci et al recently published a study showing that patients with severe aortic regurgitation and preserved LVEF have elevated or normal GWI and GCW values and normal GWE and GWW values [22]. An example is shown in Figure 5C.

c) *Mitral regurgitation*

Tunyan et al analyzed MW in patients with primary mitral regurgitation. LVEF, GLS, and GWI did not differ between symptomatic and asymptomatic patients, but symptomatic patients had lower GCW and higher GWW values [23]. An example is shown in Figure 5B.

MW was also evaluated in patients with HF with reduced LVEF and severe functional mitral regurgitation in whom Mitra-Clip was performed. The study showed that GCW was the only predictor for LV reverse-remodeling one year after surgery, a GCW value > 846 mmHg % being associated with a decrease of > 10% in the LV telesystolic volume [1].

CONCLUSIONS

MW is a new method, less dependent on the LV loading conditions compared to LVEF and GLS, which provides important information about the segmental and global function of the LV. In recent years, promising results have been obtained on the applicability of MW in various cardiac pathologies, vouching the significant additional role MW could play in daily practice.

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VARIA

Blockchain technology used in medicine. A brief survey

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Abstract: *Blockchain is the technology that allows people to make transactions without intermediaries. Bitcoin was the first cryptocurrency launched. Cryptocurrencies are digital tokens that can be used for transactions. They use cryptography to establish their authenticity and are not linked to a central authority. Blockchain has many advantages for the healthcare industry and can be used in various fields such as: implementation and improvement of electronic patient records, in clinical trials, neuroscience, pharmaceutical industry and research. Its security can help to improve the confidentiality of patient data and can also help secure the supply chain of medicines. The security and transparency of the blockchain will play a crucial role in the medical industry. This will allow companies to register their products and conduct secure transactions. QR codes can also be placed on the back of medicine containers to help customers identify the authenticity of the products they purchase. The exchange of health information through the blockchain will also have various challenges, such as maintaining the confidentiality of patient data. At the same time, due to the different regulations in different countries, it can be difficult to establish an efficient and secure exchange. Unlike other cryptocurrencies, blockchains are usually immutable, which means that the data added in the chain will always remain. This eliminates the risk of data loss.*

Keywords: *blockchain, cryptography, healthcare*

BLOCKCHAIN TECHNOLOGY – INTRODUCTION

Blockchain is the technology behind Bitcoin, an open peer-to-peer value transfer network that has solved the problem of double spending for the first time. With the launch of Bitcoin, cryptocurrencies became the first use of the blockchain. Cryptocurrencies are similar to fiat money, such as dollars or euros, facilitating the exchange of value, but they use cryptographic protocols as a basis for governance, rather than depending on a central authority, such as banks. In recent years, there has been a growing awareness that the blockchain has much more to offer than cryptocurrencies; the blockchain can be an ideal tool for creating solutions based on trust [1].

In Bitcoin, as in cryptocurrencies, a blockchain is a linear chain of blocks or register that applies only to a computer network. A block is a group of validated transactions in the network, and the two consistent blocks are cryptographically linked. However, lately there have been variations in the way the bloc kchain is defined. It is often used as an umbrella term for distributed registry technology used in any peer-to-peer network that tries to solve problems such as double spending. The blockchain can be considered a peer-to-peer distributed registry only for attachments, with immutable and tamper-resistant records. It is important to understand the concept and life cycle of a transaction in order to understand how blockchain works. In the blockchain, a transaction is a state change. For example, a transfer of money from a sender to a receiver is a transaction in which the account balance represents the status. When a new transaction is created, it is transmitted to the network where a mining node (computer) takes over the transaction and composes a block by combining one or more transactions and transmits the block to the network. Each blockchain has a set of rules that determine whether a block created by a miner is valid or not and only valid blocks are added to the chain. For example, a b lock is considered valid and exploited in Bitcoin if it has the correct cryptographic hash. This set of rules forms the basis of blockchain consensus [2].

A blockchain tracks and records transactions and assets. Its

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immutability is guaranteed by a network of peer-to-peer computers and not by a centralized authority. Assets can be tangible, such as property and money, or intangible, such as patents and intellectual property rights. Each data blockchain contains a hash (a fingerprint), packets with recent transactions marked in time and a hash of the previous block [3].

Through this architecture, each block is connected in chronological order, and the total number of connected blocks forms a blockchain. It is impossible to modify a block in the middle of the chain, because all the blocks that follow it must be modified at the same time. This mechanism is what provides immutability of data in a blockchain network [4].

Blockchain technology is constantly improving, but it may face some challenges that need to be addressed before it can be adopted in medical applications. The first challenge involves transparency and confidentiality, because all data in a blockchain is public. A second challenge is speed and scalability. Given that the number of transactions in the medical system is enormous, it is clear that a revolution in blockchain technology is needed. A final challenge is that of a possible attack and although this is only theoretical, a viable solution should be found [3].

Blockchain has many benefits for the healthcare industry, such as improving the security of patients' digital medical records, resolving issues related to drug authenticity and traceability of the drug supply chain, and at the same time enabling secure interoperability between healthcare organizations [5].

Every year, the medical industry makes about 30 billion transactions, more than half of which are just faxes, which, despite the high costs, do not always arrive on time in the hands of doctors, leading to an increased mortality rate due to mistakes, administrative, communication of patient-related data [6].

Despite the medical records management system, the lack of a transparent and reliable supply chain leads to huge losses for the industry, as an inefficient supply chain involves the inclusion and sale of counterfeit pharmaceuticals. All of this leads to a substantial loss of money and human lives, but blockchain can solve these problems very effectively, making data interoperable and providing doctors with real-time access to it [6].

Not only the drug supply chain benefits from blockchain technology, but supply chains in general. Counterfeit products are a problem everywhere, especially in the food and pharmaceutical industry. Implementing blockchain technology so that it replaces the traditional supply chain management system is the only way to counteract this situation and prevent the sale of counterfeit pharmaceuticals [7].

Transparency and security are two properties of the blockchain that will play a key role in the medical sector. All companies that want to register their products in the blockchain will have to be reliable, and this aspect will be decided by those who control the private blockchain. Once access is allowed to register their pharmaceuticals in a blockchain, they will be able to store all the data and information about them. Each block of data in the blockchain will be marked temporarily and therefore unchanged. Thus, no entity involved in the transaction of pharmaceutical products, from the company to the retailer will be able to modify the data, so as to introduce counterfeit products. At the same time, the movement of drugs from one entity to another can be tracked in real time, through data stored in the blockchain [6].

QR codes can also be attached to the backs of medicine containers, which provide data about their manufacturers and will help customers verify the authenticity of products, thus preventing them from purchasing counterfeit and therefore dangerous products.

When it comes to managing medical records, no matter how important a patient's medical data is, there is still no effective system for storing them all in one place. Assuming that there is such a system, however, different organizations have different types of systems, which lack interoperability, which makes them useless to some extent. A blockchain network for medical records management can bring order to the medical industry. Regardless of the medical centers and organizations that patients visit throughout their lives, blockchain networks will allow all data to be stored in one place, which allows them to verify and monitor their own information [5].

TYPES AND EXAMPLES OF BLOCKCHAIN

The blockchain can be public or private depending on the level of permission [7], but here we generally classify it on three different levels of granularity.

a)Public blockchain: does not require a permit, and anyone can easily participate and validate transactions. Transactions are public and anonymous / pseudonymous. The blockchain is maintained by the public community, so there is the highest level of decentralized trust. Bitcoin is the pioneer of the public blockchain. Bitcoin, Ethereum, Waves (2018), Dash (2018) and Bitshares (2018) are some examples of public blockchains.

b) Federal blockchain: is a licensed blockchain that operates under the leadership of a group often called a consortium. Predefined consortium nodes control consensus. Transactions may or may not be public. Some examples include R3 Corda (2016), EWF (Energy) (2018) and B3i (Insurance) (2018).

c) Private blockchain: is a centralized licensed blockchain in a government organization. Transactions are validated internally and can be read publicly or not. Private blockchains usually have faster blocking times and can process larger transactions. However, these blockchains are vulnerable to security breaches. The value of the private blockchain can be seen as a reliable transformer in which trust is based on an algorithm rather than an authority. Monax (2018), HyperLedger with Sawtooth (2018), private Ethereum are some examples of private blockchains.

The use of a private blockchain for monitoring and storing personal clinical data is one of the potential examples associated with the Healthcare Data Gateway (HDG) method, introduced by Yue et al.[8]. In this personalized healthcare method, patients are free to access, monitor and manage personal clinical data and healthcare summary, stored on a private blockchain (a centralized database system with restricted access control only for authorized or specific users).

Griggs adopted a private blockchain, based on the Ethereum protocol, to not only facilitate the safe and secure use of medical sensors, but also to eradicate the security risks associated with a remote patient monitoring system. Their blockchain-based strategy can facilitate real-time, remote monitoring, allowing practitioners to track the health of their patients in remote locations while maintaining a secure, safe, and up-to-date patient history [4].

Similarly, Ivan [9] demonstrated an approach to the secure storage of health data by implementing a public blockchain (a decentralized database system with open access control to anyone connected to the network) for encryption. In this method, encrypted healthcare data is stored publicly, developing a blockchain-based personal health record. Their proposed method has allowed patients to have better access to their clinical data, where they can freely access and not only monitor their data, but also contribute to their registration and share them with any associated care agency.

In another study, Chen and colleagues proposed an integrated framework based on cloud and blockchain storage, to manage and share the patient's personal medical data. The proposed scheme could be used to secure and secure the storage and exchange of personal medical data of the patient. The suggested approach is unique in nature, as it gives patients complete access and control over their personal medical data, thus excluding the involvement of any external third party [10].

Wang proposed a blockchain framework, based on parallel execution and artificial health systems, to assess the health status of patients' diseases. The proposed method evaluates the general condition, diagnosis and treatment process of the patient and analyzes the associated therapeutic procedures through parallel executions and computational studies for clinical decision making. The suggested system was tested on real as well as artificial health systems to assess the accuracy of the diagnosis and the effectiveness of the treatment [11].

The researchers developed BloCHIE, a unique blockchain-oriented platform for exchanging health information. The suggested platform assesses healthcare data sharing requirements, mainly for personal healthcare data and electronic medical records, and handles various other forms of data by implementing blockchains in different sources. To validate satisfactory authenticity and confidentiality requirements, they coupled the platform with in-chain and out-of-chain verification processes [12].

In 2017, Shubbar introduced a blockchain-based telemonitoring healthcare framework for the diagnosis and treatment of cancerous tumors in remote patients. The suggested protocol uses smart contracts, along with blockchains that can be widely used to ensure the validity and security of patient data in specialized medical centers as well as in patients' homes [13].

In another study, DermoNet was suggested, a blockchain-based method for assisting dermatology patients through an online dermatological consultation through a tele-dermatology monitoring system (“tele-dermatology”) [14].

ProActive Aging is a blockchain-based platform that helps support the active lives of older people. It should be noted that in extensive medical treatment processes, such as chronic diseases (eg cancer, etc.), surgery, and aging, blockchain technology can be an ideal and well-chosen choice. Moreover, it may be possible for the pharmaceutical industries, drug manufacturers and biomedical researchers to use DNA data stored on blockchains to conduct advanced transnational genomic research [15].

BLOCKCHAIN IN MEDICINE – ADVANTAGES, CHALLENGES, BARRIERS

In Table 1 is shown a SWOT analysis of Blockchain Technology.

Blockchain-based health information exchange will be an interesting use case that requires a balance between confidentiality, transparency and efficiency. Moreover, country-specific regulations will be another obstacle to an exchange that connects several regulatory regions. Because patients have full ownership of their healthcare data in many countries, the blockchain-enabled patient-centered healthcare data model may be an appropriate way to circumvent these regulatory challenges [16].

Blockchain also has use cases in managing the clinical trial process for pharmaceutical research. Recently, the Institute of Electrical and Electronics Engineers Standard Association organized a forum on Blockchain for clinical trials in order to use blockchain to innovate in patient recruitment, ensure data integrity, and make rapid progress in drug development. Scrybe, a blockchain project presented on the forum, enables an efficient and reliable mechanism to accelerate clinical trials and the research process. Among other things, it allows for an easy and transparent framework for legal and ethical validations of the audit process by auditors [17].

Table 1: Clinical characteristics and laboratory results of chronic inflammatory rheumatic diseases groups

Strong	Weak
security there is no need for a trusted third party reliability deals with falsification of immutability records more reliable health system control over healthcare journals	high complexity energy consumption the system is still in the prototype phase the integrity of the information is not guaranteed
Oportunities	**Threats**
safer health systems the patient manages his own data patient records with confidentiality more reliable monitoring of patients at home	resistance of the medical system to change privacy issues medical systems without the ability to support the blockchain structure

Blockchain can be used to manage the consent, data and outcome of a clinical trial in a confident and open manner. Such innovations in the implementation and management of clinical trials are crucial for advances in pharmaceutical research. A lot of clinical trials exceed the budget and deadlines. Competitive sharing of clinical data and studies can accelerate research and discovery. Such data sharing can be enabled with blockchain, using examples of approaches. Moreover, the research aspect of pharmaceuticals is quite broad and goes through the process of drug discovery to device manufacturers and the results of clinical trials. A solution in this spectrum is offered by BlockRX using so-called advanced digital registry technology (ADLT). The general goal is to interconnect the disconnected parts in silos [18].

There are many persistent interoperability challenges. Exchange between different institutions can be an operational challenge and requires significant collaboration between the entities involved. Data sharing agreements, complex patient matching algorithms, governance procedures and rules are just some of the issues that need to be agreed before data exchange can take place [19].

There are also many technical barriers. For example, transactional authentication and the entity must be robust (and repeated for each relationship between entities). Activity and threshold monitoring, together with some detection of anomalies, should also be in place. Finally, data exchange security is essential, and standards for data exchange (eg, Fast Healthcare Interoperability Resources (FHIR) or Confidential Disclosure Agreement (CDA) must also be agreed) [20]. In this context, there has been a recent explosion of energy to improve patients' ability to access their own health data. There is little ambiguity as to whether patients should be able to access their health data - the Health Insurance Portability and Accountability Act (HIPAA) requires covered entities to provide individuals with access to their health data upon request (with certain exceptions, such as would be psychotherapy notes).

The transition to patient-led interoperability will require new processes around security protocols, confidentiality configurations, electronic consent, and governance [21].

All transactions with clinical data have associated verification costs: there is the cost of securing data and complying with regulatory guidelines, such as HIPAA, along with the actual cost of maintaining a primary source of truth, the costs of authenticating different entities and transactions, and also the cost of matching patients (and the implicit cost of non-compliance, measured in clinical errors, manual effort and financial costs). Interoperability efforts absorb these costs through various mechanisms - security and confidentiality personnel, technical assistance, health information management offices, etc. associated with joining and operating a network [22].

Blockchain could provide an important catalyst for improving data exchange, especially for patient-driven interoperability [23].

At a high level, blockchain technology can be considered a platform for digital exchange, where the platform operates without a traditional intermediary. Health data can live in several systems, and data exchange requires many points of collaboration between entities. As interoperability becomes more patient-centered, there is an opportunity to use blockchain technology to facilitate this exchange and give patients greater control over their data [24].

Blockchain allows a centralized and shared mechanism for managing authentication and authorization rules around data. For example, a blockchain may have "Smart Properties" - an entity whose property is managed through a blockchain - to allow a certain form of digital property to have clean ownership. The custodian of the data (e.g., the patient) is clearly represented on the blockchain and can subsequently assign access rules and permissions around their data, allowing for easier sharing [25].

A second way in which blockchain technology could foster patient-driven interoperability is the availability of data. Clinical appointments could be securely broadcast on the network and linked to the patient's anonymous digital identity. Patients could publish their own patient-generated health data in a blockchain network. Such patient-generated health data could provide activity monitoring or other personal health data captured outside the formal health setting, provided that a patient authorizes release [26].

Rapid access to clinical information is a third major way in which blockchain technology could improve interoperability in the patient context. For data (or permissions) stored "in a chain", this is immediately clear - the immutable register clarifies the data permissions and, once this is established, the parties can exchange data. If the data is not stored on the blockchain, but there is metadata about the primary data - for example, calendar or location - then this also allows simplified access. Knowing that a patient has undergone an imaging study at a particular hospital in a given year is helpful, as the investigation may focus on that resource, even if the imaging study itself is not available. In these ways, the blockchain improves the liquidity and availability of data and makes it easier for patients to share their data with other entities [27].

Blockchain's use of the public key infrastructure provides a centralized method of identification - an individual's public key - that can be used to link that patient's records between institutions. If each institution knows a patient's public key and the key was linked to its own internal identifier (for example, the patient linked his public key to the patient's portal account when he registered), then subsequent clinical events broadcast on the blockchain network could includes that public key as a reference for the patient, facilitating patient matching [28].

Because blockchains are usually immutable, the data added in the chain will persist. This reduces the risk of loss, provides an audit trail (for example, in the case of a malicious actor), and ensures that a complete digital history is available to all parties (provided there are adequate access controls) [29].

Although the blockchain could allow for patient-led interoperability, there are several barriers that will require attention. The first barrier is related to the size and volume of clinical data. High volume and high frequency transactions are a cornerstone of clinical data, and the size of clinical data is growing exponentially with modern advances in technology [30].

Another challenge concerns privacy and security. Some implementations of blockchain technology are pseudonyms - the identity is usually hidden behind a public key, but other attributes of transactions are shared publicly. This is problematic for health data [31].

The General Data Protection Regulation adopted by the European Union (EU) includes a "right of erasure" and places health data in a special category of personal data. This further raises additional questions about the intersection of the blockchain, encrypted data and patient privacy, especially in the EU context [30].

Perhaps the biggest barrier to adoption is incentives. Although the legislation requires EHRs (Electronic Health Record) to have patient-oriented APIs, the same is not true for all healthcare data and stimulating institutions to build patient-oriented data connections without financial motivation to do so. Thing will be a challenge - the difference between compliance and true interoperability. For example, although a list of drugs may be a necessary output of a hospital's patient-oriented EHR API, it is not clear that a pharmacy benefit manager must structure and expose each approval or drug transaction without a clear commercial value. Other incentives in terms of data exchange will further strengthen the API economy and lead to greater autonomy of patient data [30].

Table 2: Blockchain network – oportunities and challenges

Oportunities	Challenges
Transparency	Interoperability
Short transaction time	Scalability
Sure	Storage
Cost effective	Social acceptance
Irreversible operations	Requires standardization

BLOCKCHAIN APPLICATIONS IN MEDICINE

Blockchain in Electronic Health Record (EHR)

A prototype called "MedRec" uses distinct blockchain advantages in managing authentication, privacy, integrity, and easy data exchange. It operates on a decentralized records management system and aims to provide patients with a detailed, immutable history and allows easy access to their healthcare information among various treatment providers and institutions [3].

During the implementation of EHR (Electronic Health Record), the sharing of medical data often faces critical limitations, such as loss of control over the data, source of the data, audit and security of the data on the medical data. MeDShare could be used to share medical data and maintain electronic medical records between cloud service providers, hospitals, and health research entities [5].

Dubovitskaya et al. proposed a blockchain-based framework for managing, maintaining and sharing electronic medical records of cancer patients. They have adopted a blockchain technology allowed to access, manage and store encrypted patient data. Such proposed frameworks can be used to practically implement blockchain technology for accessing and managing patient confidentiality and data and patient history in clinical practices [32].

In 2016, Estonia emerged with a global leadership in blockchain technology, when it proposed the idea of keeping millions of private medical records and, at the same time, making them available to healthcare providers and insurance companies [33].

Blockchain in clinical research

In the study proposed by Kevin et al., Ethereum allows for a protocol that provides intelligent contract functionality in the blockchain and is used in parallel with clinical data management systems. The main focus of the study was to address the issue of patient enrollment. The results of the study showed that Ethereum led to faster transactions compared to Bitcoin and, therefore, the derived conclusion proposed the use of Ethereum smart contracts for the transparency of data management systems in clinical trials. Therefore, enrolling patients using blockchains is one of the existing applications of this technology in clinical research [34].

Another research was conducted by Mehdi Benchoufi: A framework was implemented to obtain the informed consent of patients to track and store it in a way that is safe, publicly verifiable and non-falsifiable. They used a blockchain technology to create their workflow [35].

Blockchain in detecting medical fraud

Supply chains are vulnerable and consist of sensitive areas for fraudulent attacks, as they involve a number of moving parties and people. Blockchains provide a secure and secure platform to eliminate this problem and, in some cases, to prevent fraud, by introducing greater data transparency and improved product traceability. Because a blockchain record can only be validated and updated by a smart contract, manipulating the blockchain is not easy [34].

Blockchain in neuroscience

The special task of reading and interpreting brain signals is solved by neural interface devices equipped with several sensitive sensors, computing chips and wireless communications. They read the electrical activity of the brain, which is further deciphered and transmitted to the equipment under control [36]. Compex algorithms and big data will use the blockchain philosophy to store those brain signals on the neural interface. One of the companies that confirms that they will use blockchain technology is Neurogress. By recording user data on a decentralized blockchain, this data becomes "resistant to hacking attacks" and therefore more private. At the same time, the use of blockchain technology makes the Neurogress system "open and transparent to potential users of Neurogress platform services" (https://neurogress.com/).

Digitizing an entire human brain obviously requires an environment in which to keep it, and blockchain technology is raising its head again. One proposal is to store mental files, which would act as blocks of data in personal thought chains, which can be shared in a peer-to-peer network file system that allows historical versions. This type of blockchain thinking is proposed as an input-processing-output computing system, with several features that provide opportunities for artificial intelligence, human improvement and their potential integration. Blockchain allows an interconnected computer network to reach timestamp intervals to validate the source and truth of a register [37].

In the not-so-distant future, we can begin recording our sensory experiences using wearable technology, the current state of brain and nerve implants, biofeedback imaging, and any other sensors that allow a multifactorial fingerprint specific to a particular person's recording of temporal experience. Using these technologies as a starting point, research can be conducted in improving decision-making protocols, learning, recall, and rehabilitation [37].

Blockchain in the pharmaceutical and research industry

In common cases, pharmaceutical companies face the challenges of tracking their products in a timely manner, which sometimes leads to severe risks, allowing counterfeiters to compromise production or invade counterfeit drugs into the system. Consequently, the production and distribution of counterfeit medicines has become one of the major and global health risks, especially in developing countries [25].

During the production, as well as research and development of these drugs, the blockchain could be a technology that fits best, which can be used to evaluate, monitor and ensure the production processes of potential drugs. Recently, Hyperledger launched a medical project using blockchain technology to inspect and combat the production of counterfeit drugs. A digital drug control system (DDCS) could be a sustainable solution for the prevention of counterfeit drugs [38].

Many blockchain-based solutions for intellectual property management have been proposed in the generic space, which can also be applied to innovations in drug development. Bernstein (https://www.bernstein.io/) provides blockchain-based management of digital routes, with timestamping, to protect intellectual property priorities; this feature may be beneficial in collaborative pharmaceutical research. A solution from iPlexus (https://www.innoplexus.com/news/blockchain-and-ai-based-platform-iplexus-to-revolutionize-drug-discovery/) uses blockchain to make any unpublished and published data from drug development studies available for use. Blockchain solves the puzzle of maintaining trust and protecting intellectual property to enable such a revolutionary initiative and framework.

Using a digital drug control system based on blockchain, the major pharmaceutical industries (Sanofi, Pfizer and Amgen) have launched a joint pilot project for the inspection and evaluation of new drugs. Using the blockchain as an approach, it would be possible to aim not only at the production and location of medicines at a given time, but also at improving the traceability of counterfeit medicines, security of the drug supply system and guaranteeing the quality of medicines supplied to consumers or end users [39].

BRIEF CONCLUSION

Blockchain technology is very advanced and secure and could help store data in the medical system and share it much faster between medical institutions.

At a high level, blockchain technology can be considered a platform for digital exchange, where the platform operates without a traditional intermediary. Health data can exist in several systems, and the exchange of data requires many points of collaboration between entities. As interoperability becomes more patient-centered, there is an opportunity to use blockchain technology to facilitate this exchange and give patients greater control over their data.

Of course, there are certain barriers and challenges that need to be overcome.

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VARIA

Hashimoto thyroiditis amid COVID-19 pandemic: A clinical study

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Abstract: *During COVID-19 era, a special attention was considered for autoimmune disorders (AD), including at thyroid, which are regarded at higher risk knowing that stress and infections might aggravate the pattern of anti-thyroid antibodies as well as thyroid dysfunction. In this study, we aim to analyse how COVID-19 infection influenced the evolution of HT parameters versus patients with HT who did not go through infection. Study design: an observational, bi-centric study in Romania during first 14 months of pandemic. By the end of April 2022, Romania registered 1,047,520 total cases of COVID-19 infection (a morbidity rate calculated for entire Romanian population of 5.23%) with 27,267 total deaths (a lethality rate of 2.6%) and around 2000 new patients a day. From our database of 2210 HT patients, a number of 386 HT subjects were assessed (14 months). Among them, 33/386 patients had a different form of COVID-19 infection. The prevalence of COVID-19 infection among HT patients was 9.35% which is statistically significant higher when compare with official ratio for entire Romanian population (a morbidity rate of 5.23%, z = 3.162, p = 0.00033). A feminine prevalence was identified (89.37%, N=31/33), while age was not different between subgroups (average age over 50 y). At the moment of diagnostic, thyroid function was similar between the two subgroups (⍰2 = 2.02, p = 0.35). We checked ATPOs levels at every visits. Mean ATPO levels at the diagnostic time was 650 UI/mL in group 1 versus 840 UI/mL in group 2 (both groups had very high ATPO values, normal cut-offs below 34 UI/mL) with not statistical significance difference - student ttest (t⍰), p = 0.16. Group 1: the pattern of ATPO evolution was undulatory in 74.07% of patients, decreasing - 3.7%, increasing - 11.11%, unmodified (stationary) - 11.11%, similarly with group 2, meaning that ATPOs had the same profile, regardless the fact that some subjects went through COVID-19 infection. The rate of associated AD was 28.24% (N=2210), COVID-group of 42.42%, non-COVID group of 38.53% (z = 0.44, p = 0.66). In our COVID cohort of 33 patients with HT, we registered the following clinical stages of coronavirus infection: 4/33 patients who were asymptomatic; all women, average age: 65.65 years; 2/33 patients with lack of smell and taste; women, over 65 years old; 14/33 patients with mild form; from 31 years old to 70.5 years old (only one man); 10 patients with a moderate form; all women, from 34 to 71 years old; one 87-year-old male with a severe form; one 50-year old female with a very severe form in addition to a hematologic disease and a history of severe allergic reactions; and a single patient (a 68-year-old, obese female with a severe allergic background) died of COVID-19 infection (thus generating a lethality rate of 3.03%). Conclusion. Our study showed a higher prevalence of COVID-19 infection among HT patients. These individuals do not associate increased levels of ATPO or a elevated prevalence of AD when compare with non-COVID – HT group, and have similar age and sex ratio. Whether HT exposes the subjects to coronavirus infection is an open issue.*

Keywords: *COVID-19, thyroid, thyroiditis, Hashimoto, ultrasound, antibodies, endocrine, virus, pandemic*

INTRODUCTION

During COVID-19 (coronavirus-disease-year-2019) era, a special attention was payed for autoimmune disorders which are considered at higher risk amid this situation. [1] One of this autoimmune diseases is Hashimoto thyroiditis (HT), also known as chronic lymphocytic autoimmune thyroiditis; for what we know so far, stress and any type of infections are prone to either trigger or aggravate the clinical picture and associated hormonal imbalance of HT. [2] An analysis of chronic thyroiditis correlated with COVID-19 situation currently is approached with different levels of

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statistical evidence worldwide. [3-15]

The first patient confirmed with COVID-19 in Romania was registered in 26 February 2020. [16-19] When the first 139 cases were diagnosed, on the 16th of March 2020, it was declared an “Emergency State”, which lasted for 2 months, until the 15th of May 2020. [16-19] During that period of time, there were registered 16,437 cases of COVID-19 infection with 1,070 deaths (a lethality rate of 6.5%). [16-19] This was considered as “First pandemic wave” with a peak on the 11th of April 2020 with 523 new cases/day. [16-19] Starting from that day, an “Alert State” was initiated in our country. [16-19] Under these conditions, we had a “Second Wave”, from the 15th of September 2020, with a peak on the 18th of November 2020 (a total of 10,629 new cases per day, with a total of 324,094 COVID-19 cases and 8,389 deaths (a lethality rate of 2.59%). This wave followed by a “Third Wave”, starting with the 3rd of February 2021, with a peak on the 25th March 2021 with 6,651 new cases per day, with a total of 919,794 cases and a total death number of 22,717 (a lethality rate of 2.47%). [16-19]

Aim

In this study, we aim to analyse how COVID-19 infection amid pandemic influenced the evolution of HT versus patients with HT who did not go through infection.

PATIENTS AND METHOD

Study design

This is an observational (non-interventional), bi-centric study in Romania. The endocrine patients were registered from two medical centres in Bucharest, Romania (Europe). We analysed the patients with HT who were diagnosed with COVID-19 infection compare with those who were disease free during the first 14 months of COVID-19 pandemic.

Evaluation of patients

Based on our files, we registered patients with HT for the last 25 years; these patients previously had a thyroid ultrasound performed on a daily bases and quantitative antithyroperoxydase antibodies (ATPOs) and antithyroglobuline antibodies (ATGs) assays which were performed in our laboratories. HT diagnostic was based on serum ATPOs levels above the normal upper limit (no restriction concerning the level of ATPO elevation in order to sustain the diagnostic of HT). Also, the patients with high ATPOs levels (cut-off of more than 34 UI/mL) and confirmed with HT were evaluated based on thyroid ultrasound to check of if they had specific inflammatory aspects which did not limit the HT diagnostic. Anterior neck ultrasound was performed with a linear probe, long of 4 cm, with 7.5 to 10 MHz.

Our records showed 2,210 patients with traditional diagnostic of HT (based on high ATPOs levels) within the last 25 years. From this database, we analysed the patients who were admitted (as outpatients) during COVID-19 pandemic (from the 16th of March 2020 to 22nd - 27th of April 2022). Overall, we analysed 386 patients with HT (during this mentioned period of 14 months). Some of prior patients were under levothyroxine therapy for associated hypothyroidism (a daily dose of 25 to 125 μg) in order to achieve a TSH (Thyroid Stimulating Hormone) within the normal lab limits. Patients with associated Graves’ disease received daily thiamazole (from 5 to 20 mg per day depending on target TSH). Subjects with normal thyroid function did not receive any type of medication/ supplements. The decision of daily drugs was done by each individuals’ current practitioner and did not restrict the enrolment of the patients.

The classification of type of COVID-19 infection

We used the WHO classification (Global COVID-19 Clinical Platform), with a few adjustments: Stage 1: asymptomatic; Stage 2: only lack of small and /or taste; Stage 3: mild symptoms (few hours of fever and/or cough); Stage 4: moderate (fever, cough, no lack of oxygen), Stage 5: severe meaning pre-ITU (Intensive Care Unit) hospitalization, oxygen saturation under 93%, above 85%, Stage 6: very severe form requiring an ITU hospitalization (oxygen saturation under 85%); Stage 7: death.

COVID-19 information for studied population

In order to assess the information concerning COVID-19 infection we analysed the medical records of the patients from other medical departments (which were provided by the patients themselves). Some of them were evaluated as outpatients in Endocrinology Department, and some through telemedicine. General information amid infected cases in our country were achieved by accessing public sources (universally free downloads). [16-19]

Statistical analysis

We used z Fisher test on ratios, 2 test on discreet data, and Student t test on continuous data, as appropriate to analyse the differences studied between groups. Statistical significance was considered at $p <0.05$.

RESULTS

Associated COVID-19 pandemic information (Romania)

Until the 22nd April 2022, Romania registered 1,047,520 total cases of COVID-19 infection (a morbidity rate calculated for entire Romanian population of 5.23%) with 27,267 total deaths (a lethality rate of 2.6%) and around 2000 new patients a day (see Figures 1-3, universally available free download from Public Source: https://www.worldometers.info/coronavirus/country/romania/.)

Figure 1: Total number of COVID-19 cases in Romania (from the 16th of March 2020 to the 22nd of April 2022) - universally available free download from Public Source: https://www.worldometers.info/coronavirus/country/romania/.)

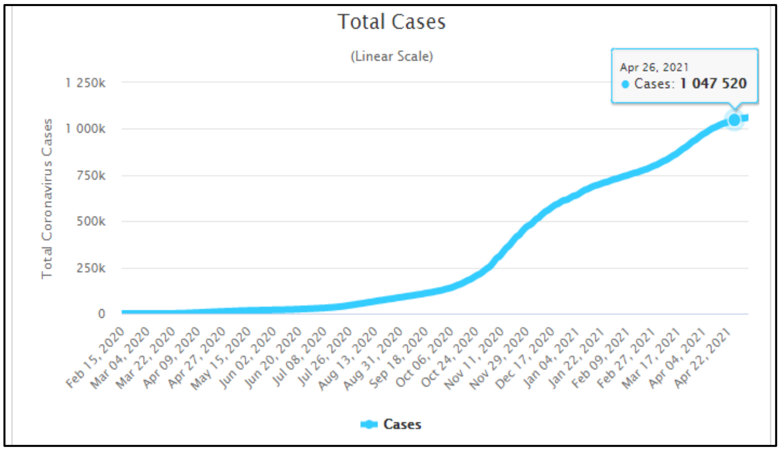

Figure 2: Total number of active cases concerning COVID-19 infection in Romania (from the 16th March of 2020 to the 26th of April 2022) - universally available free download from Public Source: https://www.worldometers.info/coronavirus/country/romania/.)

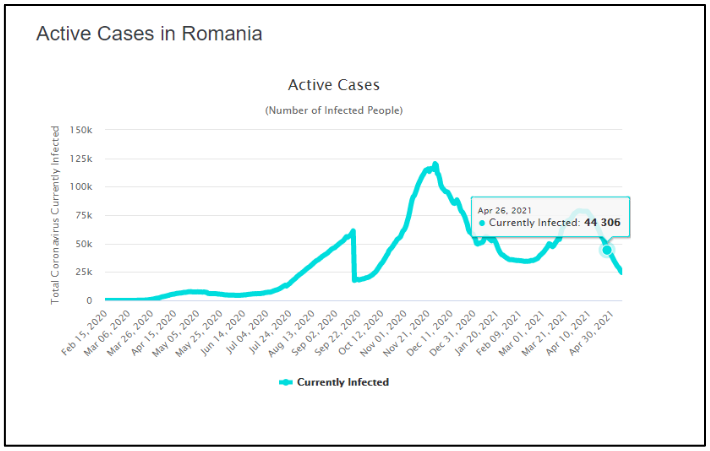

Figure 3: The report of daily new cases concerning COVID-19 infection in Romania (from the 16th of March 2020 to the 27th of April 2022) universally available free download from Public Source: https://www.worldometers.info/coronavirus/country/romania/.)

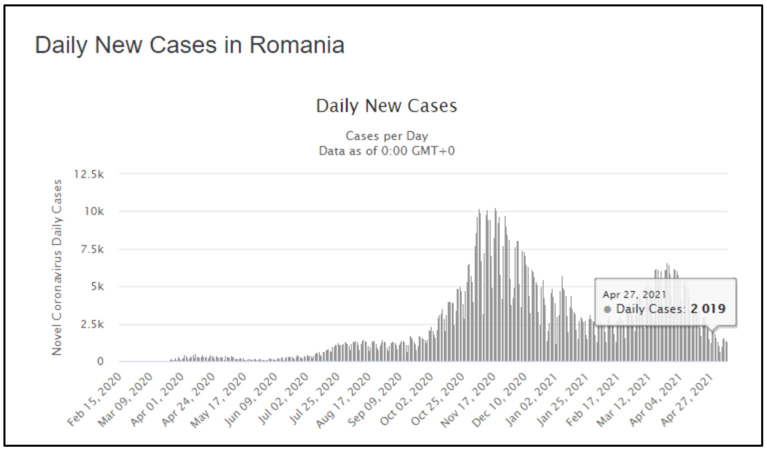

The impact of SARS-CoV-2 on patients previously diagnosed with TH

From our database of 2210 patients with HT, a number of 386 subjects were assessed during the first 14 months of COVID-19 pandemic. We registered 33 (out of 386) patients who had a different form of COVID-19 infection. All the data from these patients were compared with the data from our patients with HT who were evaluated during the same period of time but did not have COVID-19 infection. We the patients' profile in order to detect if there any differences between the two subgroups. The interval of time between COVID-19 infection and the moment we performed the endocrine evaluation varied from days to 6 months. (Table 1)

Table 1: Clinical and biological data from patients with Hashimoto thyroiditis: patients who had a COVID-19 infection versus subjects without infection

Parameter	Patients with COVID-19 infection	Patients without COVID-19 infection	Statistical significance
Number = 386	33 (9.35%)*	353 (90.65%)	z = 3.62, p= 0.00033*
Women = 345 (89.37%)	31 (93.94%)	314 (88.95%)	χ^2 = 0.42, p = 0.63, NS
Men = 41 (10.67%)	2 (6.06%)	39 (11.05%)	
Age (years) at COVID-19 diagnostic (mean) Current age at evaluation (mean)	52.82 58.43	51.36 55.99	p = 0.57, NS p = 0.36, NS
Euthyroidism at the diagnostic vs. current time	10 (30.30%) vs 24 (72.73%)	138 (30.09%) vs 205 (61.19%)	z = -0.74, p = 0.46, NS z = 1.3, p = 0.2, NS
Hypothyroidism at the diagnostic vs. current time	17 (51.52%) vs 8 (24.24%)	177 (50.14%) vs 124 (37.01%)	z = -0.22, p = 0.82, NS z = -1.42, p = 0.15, NS
Hyperthyroidism at the diagnostic vs. current time	6 (18.18%) vs 1 (3.03%)	38 (10.76%) vs 6 (1.79%)	z = 1.5, p = 0.14 z = 0.49, p = 0.62
ATPO levels (normal level < 34 UI/mL)	650 UI/mL	840 UI/mL	p = 0.16 (NS)
History of miscarriage	2 (6.45%)	30 (9.55%)	z = -0.58, p = 0.56, NS
Association with other immune/autoimmune conditions	11 (42.42%)	136 (38.53%)	z = 0.44, p = 0.66, NS

** applied to the morbidity in general population, NS = nonsignificant; vs=versus; ATPO= Anti-thyroperoxidase antibodies*

Prevalence analysis

The prevalence of COVID-19 infection among our patients which was registered during the studies 14 months was 9.35%. We compare this ratio with the official ratio of COVID-19 for entire Romanian population, the morbidity (see above), was 5.23%. For this comparison we used the z test from Fisher equation, which compare ratios. From this point of view, the prevalence is higher, the statistical significance was z = 3.162, p = 0.00033.

Gender prevalence

A feminine prevalence was identified in our cohort: 89.37% were women, and 10.67% were men. During the first 14 months of pandemic, 31 women and 2 men had a COVID-19 in addition to previously diagnostic of HT. When comparing gender prevalence with general data, there was no statistical significance (⍰2 test = 0.37, p = 0.79). (Table 2)

Age analysis

Mean age of 33 patients was 52.82 years at the diagnostic while the subgroup with HT without infection had an average age of 51.36 years at the diagnostic time and 55.99 years at the current time with not statistical significance difference. (Table 2)

Thyroid function

We analysed the thyroid function at initial diagnostic time and current moement, when patients were revised. Hypothyroid patients received levothyroxine and some become euthyroid; hyperthyroid patients received thiamazole, and mostly became euthyroid under this therapy. All these data were compared with the group without COVID-19 infections/14 months. All patients with high ATPO (anti-thyroperoxidase antibodies) who had hyperthyroidism had also positive TRAB (TSH Receptor Antibodies) according to a level higher than 1 UI/ml (co-occurrence of HT and Graves-Basedow's disease).

When analysing all cases in the same time by χ^2 test, there was no differences concerning the aspect of thyroid function both at the diagnostic time and the current time between patients from group 1 versus group 2 (χ^2 = 2.02, p = 0.35). Similarly, z analysis on thyroid function (separately for euthyroidism, hypothyroidism and hyperthyroidism distribution) showed no statistically significance difference. Therefore, the thyroid function, either at the diagnostic time or at the current visit, had no importance in catching the viral disease. (Table 2)

Table 2: The parameters of patients with chronic Hashimoto thyroiditis who had an episode of COVID-19 infection during the last 14 months

	Gender	Age at diagnostic moment	Current age	ATPO average UI/mL*	ATPO evolution	Thyroid function a)	Current thyroid function	Specific treatment for thyroid dysfunction	COVID-19 clinical form	Other observations. Anti-SARS-CoV antibodies (if available)
1	W	48	65	505	Undulatory	EUT	EUT	Substitution	No smell	
2	W	63	78.5	275	Undulatory	HOT	EUT	Substitution	Moderate	Husband – COVID-19 related orchitis
3	W	49	58.7	80	Undulatory	HOT	EUT	Substitution	Asymptomatic	ACAb = 2000 AU/mL
4	W	52	64.4	227	Undulatory	HYT	EUT	NT	Asymptomatic	ACAb = 250 AU/mL
5	W	45	53.5	847	Undulatory	HOT	EUT	Substitution	Asymptomatic	ACAb = 1000 AU/mL
6	W	46	53.5	1331	Undulatory	HOT	HOT	Substitution	Mild	
7	W	46	53.5	418	Undulatory	EUT	EUT	NT	Moderate	
8	W	42	49.6	66	Undulatory	EUT	EUT	NT	Very severe (ITU)	Von Wilebrand disease, hypophysitis, history of Quinke syndrome, ACAb = NA
9	W	80	86	969.5	Undulatory	HOT	HOT	Substitution	Asymptomatic (personal declaration)	ACAb= 2080 AU/mL, Husband (87y) died at ITU due to COVID-19
10	W	28	33	1150	Undulatory	EUT	EUT	NT	Mild	Endometriosis
11	W	54	61.2	1563.5	Undulatory	HOT	EUT	Substitution	Mild	
12	W	59	62	52	Undulatory	HOT	EUT	Substitution	Moderate	Institutionalized at local elderly centre
13	W	66	68.9	710	Undulatory	HOT	EUT	Substitution	Moderate	Institutionalized at local elderly centre
14	W	59	61	250	Unmodified	HOT	HOT	Substitution	Moderate	Institutionalized at local elderly centre
15	W	63	68	674	Undulatory	HOT	EUT	Substitution	Mild	She was vaccinated after 1 month
16	W	51	54	275	Increasing	EUT	EUT	NT	Mild	
17	W	39	41.8	281	Undulatory	HOT	EUT	Substitution	Mild	
18	W	23	31.5	3388	Undulatory	HYT	EUT	Thiamazole	Mild	TRAB history: 10-16 UI/mL
19	W	50	54.6	133	Undulatory	HYT	HYT	Thiamazole	Moderate	TRAB history: 1.5-17.5 UI/mL
20	W	70	70.5	40	Unmodified	HOT	EUT	Substitution	Mild	Primary hyperparathyroidism
21	W	34	34	53	-	EUT	-	NT	Moderate	
22	W	39	39	185	-	EUT	-	NT	Moderate	Nurse
23	W	71	71	40.5	-	EUT	-	NT	Moderate	ACAb = 3.65 AUI/mL
24	M	45	51.5	103.5	Decreasing	EUT	EUT	NT	Mild	
25	M	73	87	249.4	Undulatory	HYT	EUT	NT	Severe, Pre-ITU	Wife (85y) died at ITU due to COVID-19
26	W	37	37	250	-	HOT	-	Substitution	Moderate	ACAb qualitative = positive (qualitative assays)
27	W	68	73.5	524.5	Increasing	HOT	EUT	Substitution	No smell and taste	
28	W	50	50.12	640	Unmodified	HYT	HYT	Thiamazole	Mild	
29	W	63	70.4	266	Undulatory	HOT	HOT	Substitution	Mild	ACAb = 149 AUI/ml
30	W	68	68	5000	-	HOT	-	Substitution	Mild	ACAb = 4600 AUI/ml
31	W	65	68.8	505	Increasing	EUT	EUT	NT	Mild	ACAb = positive (qualitative assays)
32	W	41	41	125	-	HOT	-	-	Mild	
33	W	56	68	2772	Undulatory	HOT	HOT	Substitution	Death	

Abbreviations: EUT = euthyroidism, HOT = hypothyroidism, HYT = hyperthyroidism, NT = no specific treatment, ITU = intensive therapy unit, ACAb = anti-SARS CoV antibodies; NA = not available; "substitution" means levothyroxine replacement for hypothyroidism

** Upper normal limit = 34 UI/mL; a) Thyroid function at the moment of COVID-19 infection*

ATPO evolution

We checked ATPOs levels at every visits. Mean ATPO levels at the diagnostic time was 650 UI/mL in group 1 versus 840 UI/mL in group 2 (both groups had very high ATPO values) with not statistical significance difference according to Student test (t_α): p = 0.16.

We described the ATPOs evolution in 5 ways: undulatory, increasing, decreasing, non-evolutive (stationary) and normalization below mentioned cut-off of 34 UI/mL. Accordingly to this description, in COVID-19 patients, we observed the following ratio: undulatory = 74.07%, decreasing = 3.7%, increasing = 11.11%, unmodified (stationary) = 11.11%, and normalized values of ATPO = 0%. In non-COVID patients the ratios were: undulatory = 69.04%, decreasing = 12.13%, increasing = 8.37%, unmodified = 6.28%, normalized = 4.18%. ATPO evolution did not reach a statistical difference ($\chi^2 = 0.43$, p = 0.1.67), meaning that ATPOs had the same pattern, regardless the fact that some subjects went through infection. (Table 2)

Infertility and miscarriage issues

In all our registered patients (N = 2210) the miscarriage was registered in the medical history of 150 patients (N= 7.33%). In non-COVID group, miscarriage was registered in 30 patients (9.55%). In COVID-19 group miscarriage was registered in only 2 patients (6.45%); z = -0.44, p = 0.66, NS.

Other immune/autoimmune associations

HT is frequently associated with other immune disturbances, which could be involved in an inadequate immune response to viral infections. In all our patients (N = 2210), the prevalence of immune/autoimmune associations were registered in 28.24% patients. In non-COVID group, the rate was 38.53%. In COVID-19 group, this rate was 42.42% patients (z = 0.44, p = 0.66).

COVID-19 infection: the clinical severity in HT patients

In our COVID cohort of 33 patients with HT, we registered the following clinical stages of coronavirus infection: 4/33 patients who were asymptomatic; all women, average age: 65.65 years; 2/33 patients with lack of smell and taste; women, over 65 years old; 14/33 patients with mild form; from 31 years old to 70.5 years old (only one man); 10 patients with a moderate form; all women, from 34 to 71 years old; one 87-year-old male with a severe form; one 50-year old female with a very severe form in addition to a hematologic disease and a history of severe allergic reactions; and a single patient (a 68-year-old, obese female with a severe allergic background) died of COVID-19 infection (thus generating a lethality rate of 3.03%) (Table 2)

SARS-CoV antibodies (post-infection or post-vaccine) in patients with HT

Only 17/386 patients had been vaccinated during the last 4 months of study protocol. Only 5/17 had been tested for antibodies against S-protein. The data were listed in AU/mL (1 AU/mL = 0.142 BAU/mL, according to WHO standards): 2500, 4200, 2000, 45300 and 5400. Only 2 patients with COVID-19 infection and chronic thyroiditis performed these antibodies after disease. The levels were of 4600 AU/ml, respectively of 18000 AU/mL, higher than seen in patients without a history of coronavirus infection.

DISCUSSION

For the first 14 months of pandemic, studied population showed a higher rate of COVID -19 infection when compare to entire Romanian population based on official records. [16-19] The fact that patients with thyroid conditions, including those antibodies - related, are at higher risk of contracting coronavirus infections is controversial up to the current level of scientific information. [20-30] As expected for a thyroid autoimmune condition, we had an overall female prevalence, including in COVID group. A higher susceptibility and a more severe coronavirus infection was suggested for males, unless autoimmune background or other particular sub-groups. [31-33]

We had an average age over 50 years on both subgroups. Generally, COVID-19 infection has been reported at any age, but seniors older than 65 years are at higher risk. [34-36]

We had a subgroup with individuals associating two autoimmune thyroid diseases: HT and Graves-Basedow’s disease. Notably, in the COVID group there are slightly more patients with hyperthyroidism at the diagnostic time (18.18%) that in non-COVID patients at the diagnostic time (10.76%). Without reaching a statistical significance (p >0.05), these data suggest that hyperthyroidism (which means association of Graves-Basedow disease with HT) could add some pathogenic factors to increase the risk of SARS-CoV-2 infection. However, these factors do not seem to be related to thyroid function, but more to immunological association between HT and Graves-Basedow’s disease. Coronavirus might be triggered by autoimmune hyperthyroidism. [37] We evaluated the function and the antibodies’ profile concerning these two diseases; also, all the subjects had an ultrasound done but we did not introduce in final analysis the reports of ultrasound data. Generally, thyroid ultrasound is a key tool of evaluating the patients with these conditions. [38-44]

In COVID-19 group, the miscarriage rate was of 6.45%, which is not different from entire HT cohort. Some data suggested that females going through coronavirus infection might experience a higher rate of miscarriage which is not confirmed in our cohort. [45] Also, the group of persons experiencing the infection have the similar profile of autoimmune co-morbidities. The autoimmune background, however, influences the immune response amid infection. [46-48] Overall, our patients had a mild COVID-19 symptomatology, only 3 patients experiencing a severe form. Therefore, HT did not aggravate coronavirus evolution as other endocrine conditions (like diabetes mellitus or obesity).

As limits of the study we mention: one aspect involves the fact that we did not take into consideration the post-vaccine status which include a small number of subjects among studied subgroups; and, also, the small sample size of the cohort.

CONCLUSION

Our study showed a higher prevalence of COVID-19 infection among HT patients. These individuals do not associate increased levels of ATPO or an elevated prevalence of AD when compare with non-COVID – HT group, and have similar age and sex ratio. Whether HT exposes the subjects to coronavirus infection is an open issue.

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VARIA

Work-related stress in medical personnel: A new instrument for screening and evidence-based recommendations for therapeutic management

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Abstract: *Medical personnel is constantly exposed to a variety of stressful events, starting from work overloading, to threats to one's own health or personal integrity, disruptions in personal relationships due to high stress in the professional environment, continuous need to keep in touch with the advances of medical science and therapeutic guidelines etc. Structured evaluation of work-related stress disorders (WRSD) is important both for screening purposes in a vulnerable population like the medical personnel, and for the monitoring of the overall status evolution during individual or organizational interventions. Beside burnout syndrome, major depressive episodes, anxiety disorders, acute stress reactions, posttraumatic stress reactions, substance use disorders, adjustment disorders are also included in the category of WRSD. A specific instrument has been designed for the purpose of screening for WRSD in this specific population, i.e. the Work-related Stress Inventory for Medical Personnel, structured as a 20 items, self-administered instrument (WRSIMP-20). This inventory evaluates three main dimensions- (1) mood symptoms and somatic equivalents, (2) work and professional environment-related discomfort, and (3) interpersonal relationship-associated distress. The results of online administration of WRSIMP-20 in 81 health care workers and 52 individuals not related in any way to health care professions (control group) were consistent with the premises, as this inventory has been proven valid for the targeted population. Larger trials and test-retest evaluation are needed in order to complete the psychometric properties of WRSIMP-20, but this instrument seems promising in the detection of individuals vulnerable to WRSD.*

Keywords: *work-related stress, burnout, major depression, adjustment disorders, therapeutic management, medical personnel*

BACKGROUND

People working in the health care domain are facing a large spectrum of stressful situations and they are considered at significant risk for developing work-related stress disorders (WRSD) (Figure 1). Extensive workload, long shifts, night shifts, emotionally demanding professional roles, the need for quick decisions, and informational overload are several factors that may trigger a burnout syndrome [1].

Burnout is considered a psychological syndrome that may appear as a consequence of chronic interpersonal stressors in the working milieu, and it has three dimensions – (a) overwhelming exhaustion, (b) feelings of cynicism and detachment from the job, and (c) a sense of lack of efficacy or accomplishment [2,3]. Exhaustion is defined as the feeling of not being able to offer any more of oneself emotionally, while cynicism is conceived as a distant attitude towards work, the people being served, and/or the workplace colleagues; inefficacy is considered to be the feeling of not performing tasks adequately [4].

Although this pathology is perceived as unitary, there are a few different types of burnout syndrome: frenetic (highly applied and committed individuals), under-challenged (individuals who have no interest in their work and are superficial in their job), and worn-out (individuals whose level of involvement in their work is diminished, and they disregard the responsibilities of their position) [4,5]. In order to accurately detect these types of burnout, a range of psychometric instruments have been constructed [4,5].

According to the results of a meta-analysis (n=25 studies) the most relevant associations were between the feelings of work control and reduced emotional exhaustion, as well as between the low workplace support and increased emotional exhaustion [6]. Limited evidence was found for

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the correlations between workplace rights, demands, high work load, low reward, low supervisor support, low co-worker support, job insecurity and change in emotional exhaustion [6]. Also, cynicism was associated with most of these work-related factors, and reduced personal accomplishment was strongly related to low reward [6]. A higher vulnerability to burnout was detected in people with perfectionistic features or self-criticism, who sleep less, and in those with high tendency to work overtime, to assume more responsibilities, and without a group of support (i.e. family, friends) [7,8].

Figure 1: Work-related stress disorders in medical personnel

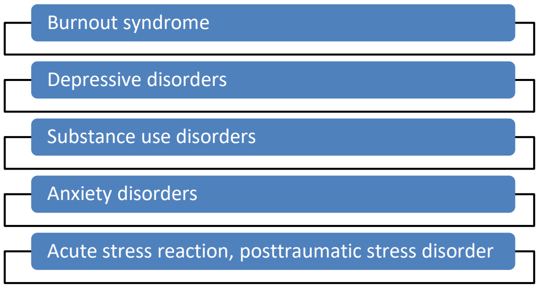

The prevalence of the burnout syndrome is significant, although this pathology is rather under-diagnosed, with estimates of every one in three physicians experiencing burnout at any given time [9]. Other data suggest higher rates, somewhere between 39.8% and 46%, based on lifestyle surveys in physicians [10]. The medical consequences of the burnout diagnosis are significant: this pathology was found to be a risk factor for myocardial infarction and coronary heart disease, it may decrease fibrinolytic capacity, reduces the ability to cope with stress and the hypothalamic-pituitary-adrenal (HPA) axis functionality [11]. Burned-out physicians and nurses have also high risk for psychiatric and psychologic complications, e.g. substance abuse, suicide ideation, poorer quality of life, or unsatisfying relationships, while the patients tend to be less satisfied with services offered by burned-out medical personnel [12,13].

According to a systematic review (n=182 studies, N=109628 individuals) burnout syndrome incidence was evaluated during an 18-year interval (between 1991 and 2018) in physicians [14]. Overall burnout was reported in 85.7% of these individuals, emotional exhaustion in 72%, depersonalization in 68%, and low personal accomplishment in 63.2% [14]. Because of inconsistencies in definitions of burnout syndrome, and in the assessment methods across studies, there were many differences in the reported incidence, and associations between demographic variables and burnout could not be determined, either [14].

Five main causes of burnout syndrome in physicians have been described: the clinical practice of medicine (the intrinsic stress of the profession); the specific work requirements (the configuration of certain stressors related to the professional environment); personal life and the interference between job-related and family-related tasks; the conditioning of the medical education; the leadership skills of the immediate supervisors (more exactly, the lack of such skills) [10].

Mental health of physicians exerts a significant influence on the quality of patient care [15], therefore, beside burnout syndrome, other frequently reported psychiatric disorders related to professional stress need to be explored. The estimated prevalence rate for depression among physicians in Germany was between 6 and 13%, but anxiety, suicidal tendencies and substance abuse were also present in this population [15]. In another study, health care workers in Turkey (N=221) had a significant level of anxiety (according to the Hospital Anxiety and Depression Scale) in almost 40% of the cases, with anxiety and depression scores of nurses and medical secretaries being significantly higher than the physicians [16]. Emotional exhaustion and depersonalization (evaluated on Maslach Burnout Inventory) scores were the highest in nurses, followed by medical secretaries and physicians [16].

Posttraumatic stress disorder was found in 19% of cases in a sample of physicians and nurses (N=783 participants) working in emergency department during COVID-19 pandemic [17]. The rate of probable posttraumatic stress syndrome in physicians was higher than in nurses, 22.9% vs. 15.2% [17]. High anxiety level, being diagnosed with COVID-19, high depression level, female gender, and having additional chronic disease were predictors of higher posttraumatc stress disorder symptom severity in physicians [17]. In nurses, high anxiety level, being diagnosed with COVID-19, working in 24 hours shifts, high depression level, low work experience (in years), low monthly income, and the presence of additional chronic diseases were correlated with higher posttraumatic stress disorder severity [17].

ASSESSMENT TECHNIQUES FOR BURNOUT IN PHYSICIANS

The Maslach Burnout Inventory (MBI) is a 22-item instrument currently considered the “golden standard” for assessing burnout but several critiques include its proprietary nature, lack of clear cut-offs for negative outcomes, and the fact that its 3 dimensions did not correlate with specific therapeutic interventions [18,19].

The Tedium Measure involves determination of several clusters of symptoms, like the experience of physical, emotional, and mental exhaustion, defined by negative perceptions over one's self, one's environment, one's work, and one's life [20,21]. Tedium is considered a continuous variable, but only one score is available [20]. This instrument may be suitable for assessing burnout in an organization or system, and its scores correlate with all subscales on the MBI, and with health problems and job satisfaction [20].

The Staff Burnout Scale for Health Professionals (SBS-HP) is a 20-item inventory assessing cognitive, affective, behavioral, and psychophysiological reactions that comprise the burnout syndrome [22]. The SBS-HP data significantly correlated with measures of job turnover, absenteeism, tardiness, discipline, prescription drug use, and personal illness [22].

The Burnout Measure (BM) includes 21 items, evaluated on 7-point frequency scales, assessing the level of personal physical, emotional, and mental exhaustion [23]. A factorial analysis of the BM found 3 main dimensions- "demoralization", "exhaustion", and "loss of motive", and several critics consider this instrument is rather a measure of general well-being [24]. BM also has a shorter, 10-item version (BMS), with attested validity and reliability, easiness of use and high face validity [23].

Copenhagen Burnout Inventory (CBI) is a 19-item survey which covers 3 areas- personal, referring the degree of physical and psychological fatigue and exhaustion; work, encompassing the level of physical and psychological fatigue and exhaustion related to work; client/patient-related burnout [25]. According to a prospective study (N=1914 employees in the human service sector), CBI predicted future sickness absence, sleep problems, use of pain-killers, and intention to quit [25].

Oldenburg Burnout Inventory is a 16-item survey, with positively and negatively framed items to assess two core dimensions of burnout- exhaustion (a consequence of intensive physical, cognitive, and affective strain) and disengagement from work (negative attitudes toward work, with distancing oneself from one's work object, work content, or work in general) [26].

Research dedicated to finding relevant causes for physicians' distress has been increasingly developed in recent years. Studies focused on interventions for improving trainee and physician wellness have identified several burned-out management strategies, e.g. organizational-oriented approaches and individual-targeted interventions [27]. Medical personnel wellness is a shared responsibility between doctors, nurses, other health care personnel, health care organizations, and governing bodies [27]. Participants in a survey in Canada (N=22 faculty physicians) described that professional values and organizational norms negatively affected work-life balance, personal and professional fulfillment, triggering feelings of dehumanization, and discouraged disclosure of struggles [28]. Feelings of dehumanization exacerbate burnout and may explain physicians' resistance to resilience-building strategies [28]. The importance of an institutional commitment to create a culture of compassion for patients and physicians alike is underlined by several authors [28].

Due to the impact of burnout in daily practice in physicians, the heterogenous validity of different existing instruments for the evaluation of this syndrom's severity, and to the limitation of these instruments to only one WRSD, new questionnaires for a more comprehensive quantification of work-related stress in this population are needed. In the context of COVID-19 pandemic, the necessity for such more complex investigational methods became obvious.

BURNOUT IN MEDICAL PERSONNEL DURING COVID-19 PANDEMIC

The COVID-19 pandemic increased the workload in medical personnel and the risk of burnout, therefore active screening methods and early intervention strategies should be implemented in this population. According to a cross-sectional survey of Canadian physicians (the COPING survey) 34.6% of the respondents (N=107 out of 309) were positive for burnout syndrome [29]. The primary outcome in this survey was the MBI score, and it correlated with risk perception, worklife subscales and adequacy, measured on the Pandemic Experiences and Perception Scale (PEPS) [29]. Cynicism was significantly associated with exhaustion and worklife, while efficacy correlated with cynicism, worklife, and active cases [29]. Contact with coronavirus, preparation, and PEPS risk perception were predictive for MBI-determined exhaustion [29].

In Wuhan, China, the epicenter of the COVID-19 pandemic, a survey administered to 220 medical staff members from frontline hospitals and usual wards concluded that both types of medical units should implement strategies to support the well-being of health care workers, due to the risks of burnout [30]. The frequency of burnout was 13% for frontline personnel and 39% for usual wards personnel [30]. Although this difference between the two groups may seem counterintuitive, it is possible that different coping mechanisms may be activated in different work circumstances. A greater sense of control of their workplace, feeling closer to key decision makers, or a deeper sense of personal achievement are another possible explanations for lower incidence of burnout in frontline physicians, when compared to usual wards personnel [30].

Health professionals working in different hospital from Italy during COVID-19 pandemic who answered to an online anonymous survey (N=330) presented high levels of anxiety (71.2%), clinical levels of depression (26.8%), and significant levels of stress (34.3%) or post-traumatic stress (36.7%) [31]. Burnout was identified through moderate (35.7%) and severe (31.9%) levels of emotional exhaustion, moderate (14%) or severe (12.1%) levels of depersonalization, as well as moderate (40.1%) or severe (34.3%) levels of reduced personal accomplishment [31]. Reduced personal accomplishment was also predicted by age, while emotional exhaustion and depersonalization were predicted by female gender, being a nurse, working in the hospital, being in contact with COVID-19 patients [31].

A review focused on the impact of COVID-19 on physician burnout (n1=14 studies and n2=5 reviews) heightened existing challenges, e.g. increasing workload, limited resources or sleep deprivation which are directly correlated with increased burnout [32]. There is a staggering prevalence of burnout in general among physicians compared to non-COVID times [32]. Mindfulness-based or self-awareness programs may be useful for physicians with burnout syndrome [32]. A formalized burnout reduction programme offered by the institution or organization, online or telephone access to helplines operated by trained mental health professionals, support programs for spouses and dependents, stress management techniques are possible solutions for an effective prevention of burnout [32].

According to a cross-sectional study in Austria (N=259 general practitioners and 229 private physicians) the prevalence of clinically relevant psychological distress was similar between groups, 12.4 vs. 9.2% [33]. A larger proportion of general practitioners than specialists presented intermediate or high burnout, 43.8% vs. 39.9% and 26.9% vs. 22.0%, respectively, but without reaching statistical significance for either category [33]. Only when study participants with intermediate and high levels of burnout were combined, the group difference became significant, 70.7% vs. 61.9% [33]. Being single, having financial problems, and facing violence in patient care were significant predictors for psychological distress [33]. Also, the burnout onset was predicted by the single status, significant financial problems, facing violence in patient care, stigmatization because of treatment of SARS-CoV-2-positive patients, and longer working hours during the pandemic [33].

Another cross-sectional study evaluated the risk of burnout in Portuguese physicians (N=214) during the first outbreak of COVID-19 pandemic, using an online questionnaire disseminated through social media [34]. Burnout levels were high all the evaluated dimensions (personal, work, patient-related) in primary care physicians and its incidence seem to be higher during the pandemic than usually [34]. A strong association was found between gender, years of professional experience, depression and anxiety, on one side, and burnout levels, on the other [34].

A study which investigated the incidence of burnout in Canadian emergency physicians during the COVID-19 pandemic (N=416) reported high burnout levels in 60% of the cases, representing either high emotional exhaustion or high depersonalization [35]. Increased age was associated with lower levels of emotional exhaustion and depersonalization [35]. Female or nonbinary gender was associated with an increase in emotional exhaustion and having children living at home was associated with lower depersonalization severity [35].

CONSTRUCTION OF AN INVENTORY DEDICATED TO THE QUANTIFICATION OF THE WORK-RELATED STRESS

An initial pool consisting of 38 questions was formed, based on the most frequently reported symptoms in 52 consecutively people working in healthcare system who presented work-related stress disorders or symptoms, evaluated during two years (January 2020 to December 2021). Diagnoses of adjustment disorders, reactive stress, mood or anxiety disorders, neurasthenia, depressive episodes, and burnout were accepted as diagnoses in this context, as were isolated symptoms of anxiety, depression, and acute or chronic stress. After comparing the collected symptoms (based on self-report and clinician observations), an exclusion of duplicates was performed. All the remaining symptoms were translated into items and analyzed by a panel of 5 psychiatrists (Figure 2).

Figure 2: The flowchart of WRSIMP-20 construction

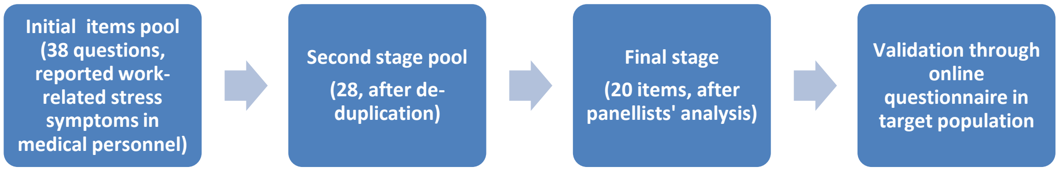
Initial items pool (38 questions, reported work-related stress symptoms in medical personnel)
Second stage pool
(28, after de-duplication)
Final stage
(20 items, after panellists' analysis)
Validation through online questionnaire in target population

A 20-item inventory resulted, with the main objective to screen for the stress disorders/symptoms related to professional roles in physicians and nurses, regardless of their specialization (Work-related Stress Inventory for Medical Personnel, WRSIMP-20). The most frequently reported signs and symptoms for this spectrum of disorders were: lack of interest for daily activities, hopelessness, fatigue which may take the form of complete exhaustion, both physical and psychological, insomnia or restless sleep, depersonalization (detachment from self, perception of one's Self as not being real), decreased performance in the professional roles (Figure 3). Each item was scored from 0 (never) to 5 (daily), on a 6-point Likert scale.

Figure 3: Work-related stress inventory for medical personnel (WRSIMP-20)

1. Did you feel tired although you have slept enough time (at least 6 hours every night)?
2. Did you feel more sad or depressed when compared to your usual mood?
3. Did you experienced problems with your memory or attention?
4. Did you feel very nervous, irritable or did you feel a continuous dissatisfaction with yourself or with your life?
5. Did you feel pains, sensations of pressure, numbness in feet or hands, unrelated to a previously diagnosed somatic disease?
6. Did you feel it is hard for you to enjoy things or activities you previously enjoyed?
7. Did you feel your professional responsibilities have become overwhelming?
8. Did you feel you cannot complete your professional duties, although previously you were able do that?
9. Did you feel your lack of communication in the professional environment is harmful for your health?
10. Did you feel you were under pressure or did you feel you have not enough time to complete your professional duties?
11. Did you feel you have less time to spend with your friends, hobbies or recreational activities?
12. Did you feel hopelessness?
13. Did you feel useless or worthless?
14. Did you feel you were unappreciated when doing your job?
15. Did you feel overwhelmed by cynicism or indifference to the distress of others?
16. Did you feel you have no control over your life?
17. Did you feel your appetite for food is reduced?
18. Did you feel your professional tasks are not well distributed at your workplace?
19. Have you felt your involvement in professional decisions is very low?
20. Did you try to keep other people away because you felt you could not handle their problems?

PSYCHOMETRIC PROPERTIES OF THE WORK-RELATED STRESS INVENTORY FOR MEDICAL PERSONNEL

The WRSIMP-20 was evaluated using an online survey, using the snowball sampling method, with 81 respondents (mean age 44.5 years, age interval: 22-65 years old, 50 male, 31 female), who identified themselves as physicians, trainees (residents), or nurses. A control group, represented by 52 individuals in the general population (mean age 47.2 years, age interval 22-65 years old, 28 male, 24 female), who identified themselves as not being related in any way to the health care profession were also included in the statistical analysis. All answers were anonymous, and two instruments were concomitantly administered, i.e. MBI and Hospital Anxiety and Depression Scale (HADS). The following psychometric properties of the WRSIMP-20 were assessed.

Content validity evaluates whether all significant aspects of the construct on which a psychometric instrument is based on are representative. The overall response rate to this inventory was good (92.5%), and low levels of missing data were also reported (a mean of 2.16% for individual items, after exclusion of those subjects who missed more than 10 items) (Table 1).

Construct validity targets the adequacy of the instruments to the concepts it is supposed to measure. All items of the WRSIMP-20 demonstrated high levels of construct validity ($p<0.001$), and the difference between the study group and controls was significant in the overall score ($p<0.001$), and in each dimension ($p<0.001$). A 3-dimension structure of this instrument was supported: mood symptoms and somatic equivalents (items 1-6, 12, 13, 17), discomfort related to the organizational environment (items 7-10, 14, 18, 19), and difficulties in social relationships (items 11, 15, 20).

Face validity reflects how adequate is the content of a test to its evaluators, and the 5 panellists consulted for this study agreed on high value for this type validity (mean score 8.4/ 10) (Table 2).

Criterion validity is considered the extent to which the operationalized construct relates to the theoretical construct, i.e. the criterion, as defined by the instrument's author. The correlation between each item and the total score was used as the first measure of criterion validity (Table 3).

A correlation between WRSIMP-20 scores and MBI, HADS-A, and HADS-D has also been considered as a way to verify the convergent validity. The correlation between WRSIMP-20 total score and MBI was 0.37, while the correlation with HADS-A and HADS-D were higher, 0.53, and 0.54, respectively ($p<0.001$).

Table 1: Content validity in WRSIMP-20 based on individual items response

Items	Missed (No)	Missed (%)
1	2	1.6
2	1	0.8
3	0	0
4	3	2.4
5	3	2.4
6	2	1.6
7	4	3.2
8	3	2.4
9	5	4
10	5	4
11	2	1.6
12	3	2.4
13	5	4
14	4	3.2
15	5	4
16	0	0
17	0	0
18	2	1.6
19	3	2.4
20	2	1.6
Mean		2.16
Median		2.4
Standard deviation		1.32

The process of defining the cut-off scores was based on the correlation between WRSIMP-20, MBI, anxiety subscale and depression subscale of HADS (HADS-A, HADS-D). A score ≥8 on both HADS-A and HADS-D is considered significant, and assures an optimal balance between sensitivity and specificity [36]. Low severity of "emotional exhaustion" on MBI is defined by scores ≤14, average levels by scores between 15 and 24, while scores ≥25 are considered high [37]. For "depersonalization", the cut-off values are 3 (low severity), 9 (average) and over 10 (high severity) [37]. For "personal and professional accomplishment" on MBI low risk is defined by scores ≤32, average severity by scores between 33 and 39, while high severity by scores ≥40 [37]. The suggested scoring guidelines of WRSIMP-20 are the following: lack of significant work-related stress problems 0-20; low risk for work-related stress conditions 21-40; moderate risk for work-related stress conditions 41-60; elevated risk for work-related stress conditions ≥61.

Internal consistency is based on the correlations between items of the evaluated test, or of the evaluated subscale, and this parameter estimates if items intended to measure the same construct lead to similar scores. The analysis of internal consistency in WRSIMP-20 yielded a Cronbach's alpha of 0.83 for the "mood symptoms and somatic equivalents", 0.76 for "discomfort related to the organizational environment", and 0.89 for "problems in the social relationships" (Table 4).

Table 2: Face validity evaluated by panellists response

Items	10-point scale
1	10
2	9
3	9
4	8
5	9
6	10
7	10
8	9
9	9
10	9
11	9
12	10
13	10
14	8
15	8
16	10
17	8
18	8
19	9
20	9
Mean	9.05
Median	9
Standard deviation	0.76

THERAPEUTIC RECOMMENDATIONS FOR WORK-RELATED STRESS DISORDERS

Based on the scoring of WRSIMP-20 the clinician may recommend an appropriate therapeutic approach, based on psychological counselling, psychotherapy, pharmaco-therapy, organizational changes, psychoeducation, or a combination of these methods. Cognitive behavior therapy leads to the improvement of emotional exhaustion in multiple studies, while the evidence for the efficacy of stress management and music therapy is inconsistent [38].

According to a meta-analysis (n=22 trials) there is a large number of interventions designed for burnout healthcare workers - individual-focused therapies emotion regulation, self-care workshop, yoga, massage, mindfulness, meditation, stress management skills and communication skills training), structural or organizational (workload or schedule-rotation, stress management, group face-to-face delivery, teamwork, Balint training, debriefing sessions, and a focus group) and combined interventions (snoezelen, stress management and resiliency training, stress management workshops and improving interactions with colleagues) but none has been associated with definitive results [12].

Other treatments have been recommended for comorbid psychiatric disorders, but not for the core symptoms of the burnout syndrome [39]. However, in the context of WRSD, pharmacotherapy and psychotherapy may be recommended for mood symptoms, sleep disorders, anxiety and other, associated symptoms [40-42].

Table 3: Individual item-total correlation in WRSIMP-20 analysis

Items	r (total score)	Significance level
1	0.30	0.01 (2-tailed)
2	0.28	0.05 (2-tailed)
3	0.36	0.01 (2-tailed)
4	0.40	0.01 (2-tailed)
5	0.40	0.01 (2-tailed)
6	0.62	0.01 (2-tailed)
7	0.23	0.05 (2-tailed)
8	0.34	0.01 (2-tailed)
9	0.44	0.01 (2-tailed)
10	0.48	0.01 (2-tailed)
11	0.45	0.01 (2-tailed)
12	0.52	0.01 (2-tailed)
13	0.62	0.01 (2-tailed)
14	0.67	0.01 (2-tailed)
15	0.33	0.01 (2-tailed)
16	0.32	0.01 (2-tailed)
17	0.36	0.01 (2-tailed)
18	0.34	0.01 (2-tailed)
19	0.39	0.01 (2-tailed)
20	0.41	0.01 (2-tailed)

Table 4: Internal consistency reliability for WRSIMP-20

Dimension	Cronbach's α	Items
Mood symptoms and somatic equivalents	0.83	1-6, 12, 13, 17
Discomfort related to the organizational environment	0.76	7-10, 14, 18, 19
Problems in the social relationships	0.89	11, 15, 20
Overall	0.78	1-20

CONCLUSIONS

WRSIMP-20 may be used in medical personnel to assess the risk of WRSD or work-related stress isolated symptoms. More research is needed on larger groups in order to validate its psychometric properties in both clinical and non-clinical samples. Medical personnel are exposed to high risk for stress-related disorders, especially during perioods of extreme effort, i.e. during pandemics. Increased attention to the detection of mood disoders, anxiety disorders, adjustment disorders, burnout syndrome, substance use disorder, but also behavioral addictions should be given in case of prolonged work-related stress [43-45]. Detection of these disorders should be immediately followed by psychological and/or medical interventions aimed at decreasing the level of stress impact over health care personnel. WRSIMP-20 may be used for screening purposes in vulnerable populations, and its results included in an adequate treatment strategy, beside scores obtained at other validated instruments, like MBI or HADS.

Limitations of this study are related to the low number of participants in both study and control groups, lack of test-retest reliability (due to the cross-sectional design), and of virtual assessment (without the possibility of a direct, clinical evaluation of the study group).

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REVIEW

Analysis of neuroprotective medication in patients with neurocognitive disorders: The efficacy and tolerability of highly purified animal tissues extracts

Octavian Vasiliu[1]

Abstract: *Neurocognitive disorders are extremely invalidating psychiatric disorders with chronic courses and significant negative impacts over all areas of cognitive functioning and behavioral activity. Although extensive research on these progressive neurodegenerative disorders has been conducted, pathogenetic treatments with long-term significant benefits are yet controversial. From a clinical perspective, there is an acute need to find therapeutic strategies that could delay cognitive impairment in patients diagnosed with Alzheimer's disease (AD), vascular dementia (VaD), Lewy body dementia (LBD), etc. Also, slowing the transition from mild cognitive impairment (MCI) to clinically significant AD is another important clinical aspect, with a major impact on the patient's daily functioning, quality of life, and caregivers' burden. Acetylcholinesterase inhibitors (AChEI) are still the first line of treatment in AD patients, and they are also administered in the case of VaD or Parkinson's dementia. Various nootropics have been studied in this population, as add-on agents. Highly purified animal tissue extracts (HPATE) are administered in patients with neurocognitive disorders due to their neurotrophic properties, but many questions remain unanswered regarding their pharmacodynamic characteristics. These extracts may be added to AChEI to enhance their pro-cognitive effect, but evidence to support the superior efficacity of this association versus AChEI monotherapy is mainly derived from low-to-medium quality clinical trials. In conclusion, HPATE may be a useful add-on to first-line pro-cognitive agents in AD and VaD, but larger trials with better methodology are needed. In particular cases, however, HPATE may be of significant interest for patients with mild-to-moderate AD, based on results from clinical practice.*

Keywords: *Alzheimer's disease, vascular dementia, ischemic stroke, Actovegin, Cerebrolysin, neurotrophic factors*

INTRODUCTION

Neurocognitive disorders are associated with significant impairment in daily functioning and a high degree of depression, anxiety, and negative symptoms, which decrease, even more, the overall level of functioning in these patients [1,2]. The management of the neurocognitive disorder and associated non-cognitive symptoms is a challenge for clinicians and caregivers, due to the relatively low number of available, evidence-based, efficient pharmacological agents [3-5]. Also, special care should be taken concerning adverse events in a population with possible organic comorbidities and multiple concomitant medications [3]. Another important aspect in this population is the low overall efficacy of acetylcholinesterase inhibitors (AChEIs), which may not even be clinically significant in long term [6,7]. Still, the differences in the effect of individual AChEIs over cognitive functioning might be significant, and these differences may be modulated by yet insufficiently described patients' characteristics [6,7].

Highly purified animal tissue extracts (HPATE), based on neurotrophic factors or blood components, are used to treat the consequences of acute cerebrovascular events or traumatic brain injury, but also for the therapeutic management of neurodegenerative disorders [8-10]. The prophylactic properties of such products in the domain of brain circulatory and trophic diseases, but also for peripheral vessels diseases, allow these products to be recommended for yet another indication, e.g. Actovegin is approved to be used for skin grafts, bedsores, skin burns, etc [10]. Several HPATE are currently marketed, but the most extensively

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researched agents for neurocognitive disorders treatment are Cerebrolysin and Actovegin. Although HPATE is not widely recognized as an efficient therapeutic intervention for dementias, multiple trials (reviewed below) support their efficacy in patients with mild cognitive impairment or clinically manifest dementia. Treatment with neurotrophic agents might enhance the effects of AChEIs, which are considered the first-line treatment for Alzheimer's disease (AD) [11].

In a comparative analysis of Cerebrolysin (1 or 3 mg/kg/day), Actovegin (200 mg/kg/day), and Cortexin (538 or 1614 mg/kg/day) in rodent models of acute and chronic brain ischemia, these agents were administered for 10 days [8]. Cortexin, Cerebrolysin, and Actovegin may be efficient in improving the recovery of neurological functions, by decreasing the severity of sensorimotor and cognitive impairments in preclinical models of brain ischemia [8].

OBJECTIVE

The main objective of this paper was to explore the available data in the literature regarding the efficacy and tolerability of Cerebrolysin and Actovegin in patients with neurocognitive disorders, mainly AD and vascular dementia (VaD). An inclusion criterion for the trials in this review was the existence of a structured approach for the monitoring of HPATE efficacy and/or tolerability.

The secondary objective of this review was the collection of data about the possible mechanisms of action of HPATE, based on preclinical models of AD, VaD, or acute cerebral ischemia.

Augmenting the effects of AChEIs with neurotrophic agents is frequently tried in clinical practice, because of their long-term potentially synergistic effects in the treatment of neurocognitive disorders. Therefore, illustrating the clinical use of these two products in a case series was yet another objective of this paper.

METHODS

A literature review was performed, using the search paradigm "Cerebrolysin" OR "Actovegin" OR "neuroprotective medication" AND "Alzheimer's disease" OR "Alzheimer dementia" OR "vascular dementia" OR "neurocognitive disorders". All papers published between January 1982 and February 2022 were included in the primary search. The databases included in this search were PubMed, Cochrane, Web of Science, EMBASE, and CINAHL.

A case series was presented in the second part of this article as a way to illustrate the possible pragmatic, clinical impact of HPATE on the evolution and prognosis of patients diagnosed with AD. These patients were randomly selected from our Psychiatry department database, and their evolution under treatment was described based on their chart review and post-discharge monitoring visits data.

RESULTS OF THE LITERATURE REVIEW

Some 145 papers were found during the primary search and 13 remained after de-duplication, elimination of papers which does not correspond to the main objective of this review, and of papers with insufficient accuracy of the methodology applied (i.e., not using validated instruments for efficacy monitoring, not specifying main outcomes or heterogenous population included form the principal diagnosis perspective). Five papers refer to the efficacy and/or tolerability of Actovegin in post-stroke cognitive impairment, AD, or organic brain syndrome, while 8 papers included data about Cerebrolysin effects in patients with AD, mild cognitive impairment, or VaD [11-21]. Out of these 13 papers, 12 included clinical trials, and one was a review.

Regarding the secondary objective of this review, 3 papers were found, one review (Actovegin), one animal study, and one in-vitro study (Cerebrolysin) [22-24].

Actovegin is a biological drug manufactured by ultrafiltration from calf blood, and the result is a deproteinated, pyrogen- and antigen-free hemodialysate [25]. The final product contains a mixture of natural substances, over 200 bioactive constituents with low molecular weight, both inorganic- e.g., blood electrolytes, and organic components- e.g., amino acids, oligopeptides, nucleosides, etc. [25,26]. Actovegin presents complex pharmacodynamics properties, as it may target the enhancement of cellular metabolism, with increased oxygen uptake and utilization, as well as glucose uptake in the cells [25]. This enhancement derives from an insulin-like activity mediated by inositol-phospho-oligosaccharides, therefore Actovegin is recommended for cerebral blood circulation dysfunctions and trophic disturbances, impairments of peripheral blood circulation and associated diseases, dermal transplants, and acute chronic wounds [25]. Actovegin also promotes oxidative metabolism and shifts the redox-balance of the cells into the direction of oxidized substrates, increasing the availability of energy-rich phosphates like ATP and creatine phosphate [25]. Preclinical data support a positive influence of Actovegin on the NF-κB pathway, but many molecular and cellular pathways remain unexplored [26].

According to a systematic review of Actovegin in ischemic stroke (n=5 articles), this drug acts over ischemic stroke lesions ensemble, issue oxidation, energy metabolism, and glucose availability through their augmentation, mitigation of the ischemic process and oxidative stress, and decreasing of inflammation and apoptosis-like processes [22]. Actovegin has anti-apoptotic effects by reducing the caspase-3 activation induced by amyloid β-peptides [22].

Actovegin has been used in clinical practice for more than six decades for various indications, and its safety and tolerability profile is considered very well [27]. Its clinical efficacy and favorable tolerability profile were proven in patients diagnosed with mixed dementia [14,27]. Patients aged 50-85, diagnosed with mild-to-moderate degenerative dementia or multi-infarct dementia received either Actovegin 250 ml 20% i.v. or a placebo for 4 weeks [14]. Actovegin had a significantly superior efficacy vs. placebo, according to the Clinical Global Impression (CGI) and Syndrome Short Test (SKT)- improvements with 32% vs. 4% (active drug vs. placebo) and -2.8 vs. -0.4 (active drug vs. placebo), respectively [14].

The efficacy of Actovegin in patients with poststroke cognitive impairment was evaluated in a 12-month, parallel-group, randomized, multicenter, double-blind, placebo-controlled study, which included patients ≥60 years old with a Montreal Cognitive Assessment test ≤25 points [12]. Actovegin was administered as 2000 mg/day for ≤20 intravenous infusions followed by 1200 mg/day orally or placebo for 6 months, in 503 patients [12]. The least squares mean change from baseline to month 6 in Alzheimer's Disease Assessment Scale- cognitive subscale (ADAS-Cog), extended version, was -6.8 for Actovegin vs. -4.6 for placebo, a significant difference [12]. The most frequently reported serious adverse event was a recurrent ischemic stroke, but without differences between active drug and placebo [12].

A double-blind, randomized clinical trial had as its main objective to compare the therapeutic effects of Actovegin vs. placebo in elderly patients with organic brain syndrome (N=60) [13]. Actovegin was administered as daily infusions of 250 ml 20% in 40 geriatric patients, and 20 patients received 250 ml 0.9% saline solution as a placebo, for 4 weeks [13]. These patients were diagnosed with simple dementia (58%) or senile dementia with depressive or paranoid symptoms (ICD-9) [13]. The mean Sandoz Clinical Assessment Geriatric Scale (SCAG) score in the drug group decreased from 56.3 at baseline to 36.3 points at the last trial visit in the Actovegin group, and from 61.2 to 52.0 ($p<0.01$) in the placebo group [13]. On the CGI, Actovegin administration led to "distinct improvement" or "improvement" in 70% of cases vs. only 35% in the placebo group [13]. SKT and subscales of SCAG scores supported the superior effects of Actovegin compared to the placebo, as did social behavior measurements [13].

A group of 43 patients with mild-moderate ischemic stroke received either Actovegin (N=32) or piracetam (N=11) and the analysis of their neuropsychological performances showed positive effects of the active drug over general cerebral and focal neurological symptoms after 30 days [15]. By the end of the study, the extent of recovery of impaired functions, assessed in terms of total ischemic points and cognitive functions, was significantly greater in patients treated with Actovegin than in those given piracetam [15]. Another study (N=104 patients with acute carotid ischemic stroke) evaluated comparatively the efficacy of (1) basic and reperfusion therapy without any cytoprotection, (2) citicoline 1000 mg/day, and (3) Actovegin 250 ml/day [16]. A significant decrease in neurological deficit and improvement of functional recovery was reported in patients treated both with citicoline and Actovegin on the 30th day vs. the control group [16].

In conclusion, the effect of Actovegin on cognitive function (either post-stroke cognitive impairment or AD) was evaluated in only two placebo-controlled trials and one piracetam-controlled trial [12,13,15], which included 606 patients. These trials used different scales for the quantification of the active drug's efficacy, but the results were favorable and the overall tolerability was good.

Cerebrolysin is a neuropeptide product mimicking the pharmacodynamics action of endogenous neurotrophic factors, and its positive effects on β-amyloid and τ-related pathologies have been demonstrated in experimental conditions [28]. It exerts nerve growth factor (NGF)-like activity on neurons and increases levels of NGF vs. controls in the hippocampus, in studies with animal models (mice) which overexpressed the human amyloid precursor protein (hAPP) [29]. Also, Cerebrolysin had a favorable impact on neuroinflammation, neurotrophic factors, oxidative stress, excitotoxicity, neurotransmission, brain metabolism, neuroplasticity, neuronal apoptosis and degeneration, neurogenesis, and cognition in animal models [28]. The presumed mechanism of Cerebrolysin in AD involves the activation of phosphatidylinositol 3-kinase, AKT, and glycogen synthase kinase-3β intracellular signaling pathway [28]. Cerebrolysin reduced amyloid plaque formation by regulating APP, and inhibits calpain, caspase, Bcl-2, and upregulates Bax, protecting neurons from apoptosis [20,30,31].

The clinical efficacy of Cerebrolysin was demonstrated over global functioning and cognition in several clinical trials, and improvements in behavior at high doses, or minor effects on daily living activities were also reported in these patients [28]. The most consistent benefit was observed in mild-to-moderate AD patients, but also several subgroups of moderate to moderate-severe patients [28]. Clinical benefits of Cerebrolysin were preserved for several months after the last dose administration, therefore the recommendation of a discontinuous administration regimen is supported by evidence [28].

Cerebrolysin reversed the reserpine-induced reduction in latency to immobility and prolongation of immobility time in the forced swimming test, decreased tumor necrosis factor-alpha (TNF-α) levels, elevated glutathione(GSH) levels, and BDNF cortical and hippocampal brain concentration, in animal models of depression [23]. Cerebrolysin elevated protein kinase A and nuclear factor kappa-B (NF-κB) cortical and hippocampal protein expression [23]. Other effects of Cerebrolysin detected on animals (rats) were: amelioration of mRNA expression alterations of protein-kinase B (AKT), CREB, and BDNF in the cortical and hippocampal tissues; elevation of norepinephrine, serotonin, and dopamine levels in cortical and hippocampal tissues; antidepressant effects, as well as attenuation of oxidative stress and inflammation [23].

A study that evaluated in-vitro human cerebral endothelial cell permeability during Cerebrolysin administration showed that tissue plasminogen activator (tPA)- and fibrin-impairments of endothelial permeability were significantly diminished and even reversed by this agent [24]. Cerebrolysin reduced tPA- and fibrin-augmented proinflammatory and pro-coagulation proteins, also [24]. Unlike the cerebroprotein hydrolysate, with a distinct peptide composition, Cerebrolysin had favorable effects on tPA- and fibrin-impaired permeability and elevated tight junction proteins, previously affected by experimental conditions [24].

In a dose-finding clinical trial, Cerebrolysin was administered in patients with moderate-to-moderately-severe (N=133, Mini Mental State Examination - MMSE=14-20) or mild (N=118, MMSE=21-25) AD [17]. Participants received Cerebrolysin, administered as 10, 30, or 60 ml diluted in saline solution, or placebo over 12 weeks (5 days per week for 4 weeks, and 2 days per week for another 8 weeks) and were monitored for 24 weeks [17]. At week 24, Cerebrolysin improved significantly the global clinical functioning with all 3 dosages and induced significant improvements in cognition, initiation of daily living activities, and neuropsychiatric symptoms [17]. However, treatment effects on total activities of daily living (ADL), and other secondary parameters (MMSE, Trail-making test) were not significant [17]. The overall treatment tolerability was good, and no safety issues have been reported [17].

A double-blind, randomized, placebo-controlled, multi-center, 4-week clinical trial enrolled 53 patients aged at least 50 years, admitted to a hospital with mild-to-moderate AD and no severe organic comorbidities [18]. The treatment group received Cerebrolysin 30 ml in 100 ml physiologic saline i.v. once daily 5 times/week, for 4 weeks [18]. After 4 weeks of treatment, Cerebrolysin-treated patients presented significant improvements in the ADAS-Cog, CGI-S, and MMSE scores vs. placebo-treated patients [18]. The rates of improvement were 82%, 62%, and 44% on ADAS-Cog, CGI-S, and MMSE in patients treated with Cerebrolysin, compared to 31.6%, 22%, and 17% in placebo-treated patients [18]. No significant improvement in the Cerebrolysin group was detected compared to the placebo group on the Geriatric Depression Scale (GDS), ADL (Activities of Daily Living), and IADL (Instrumental Activities of Daily Living) [18]. Only one case of febrile sensation was reported, but it was mild in severity and transient [18].

Another randomized, double-blind, placebo-controlled multicenter trial enrolled 242 patients diagnosed with VaD, and the primary outcome was the combined cognition and overall clinical functioning score (ADAS-Cog+ plus CIBIC+-Clinician’s Interview-Based Impression of Change plus Caregiver Input) assessed after 24 weeks of treatment [19]. At the end of the study, ADAS-Cog+ improved with 10.6 points in the Cerebrolysin group vs. 4.4 points in the placebo group (significant difference, $p<0.0001$) [19]. CIBIC+ showed a mean improvement of 2.84 in the active treatment arm and 3.68 in the placebo arm, a statistically significant difference ($p<0.0001$ vs. placebo) [19]. Higher rates of responsiveness were detected in the Cerebrolysin group, as reflected by the combined ADAS-Cog+ and CIBIC+ (67.5% vs. 27.0%), and the odds ratio for achieving a favorable response using this combined score was 5.63 ($p<0.05$) [19].

According to a Cochrane systematic review dedicated to Cerebrolysin treatment in people with VaD (n=6 randomized controlled trials, N=597 participants), mainly mild to moderate forms (n=4 trials), cognitive function had a more favorable evolution during Cerebrolysin treatment (MMSE, ADAS-Cog+), although these observations were based on very low-quality evidence [20]. When response rates based on global function (CIBIC+, CGI) assessments were included, there was a beneficial effect of Cerebrolysin (n=2 studies, N=379 participants) [20]. Four trials reported adverse events, and data from two studies (N=379 participants) included in a meta-analytic format did not report any difference between rates of adverse events (very low quality of evidence) [20].

A prospective, open-label trial, a 2-year course of Cerebrolysin therapy, administered as repeated sessions every 6 months, in patients with a mild cognitive impairment-amnestic type (N=73 participants), had as its main objective to explore if this type of intervention may slow down or prevent the transition to clinically relevant dementia [21]. The results at 2 years suggest the existence of a slower cognitive deterioration in patients who received Cerebrolysin, compared to those who did not [21].

A comparison of the safety and efficacy of Cerebrolysin (10 ml), donepezil (10 mg), and a combination of both treatments (N=64, 66, and 67, respectively) in mild-to-moderate probable AD, in a randomized, double-blind trial, concluded there were no significant differences in cognitive, functional, and behavioral domains between interventions [11]. Improvements in the global outcome, however, favored Cerebrolysin and the combined therapy at week 28 [11]. Cognitive performance was also superior at the end-point in the combined treatment group more than in the other two groups, but the difference was not statistically significant [11]. The overall tolerability of the combined treatment was good [11].

Administration of Cerebrolysin i.v. infusions (10 or 30 ml) or placebo (normal saline) 5 days/week for 4 weeks in patients with mild-to-moderate probable VaD (N=41 participants) led to significant improvements of cognitive performance at 10 ml dose and diminished the EEG slowing with both dose regimes [32]. Also, a significant correlation between fast EEG power ratio and ADAS-Cog scores change from baseline to endpoint was observed in Cerebrolysin-treated patients [32]. The extension of this 4-week trial, which enrolled 33 out of the 41 initial participants, showed that at week 16, Cerebrolysin improved significantly cognitive performance at 10 and 30 ml, and also reduced qEEG significantly at 30 ml dose [33]. These results suggest the persistence of beneficial effects of Cerebrolysin on cognition and qEEG activity in VaD patients for at least 12 weeks after treatment administration [33].

In conclusion, most of the reviewed data on clinical trials supported the efficacy of Cerebrolysin (n=6 trials, N=699 patients) in AD and VaD, but their efficacy was not consistent across all the evaluated cognitive or functioning domains [11,17-19,32,33]. However, the cognitive function was consistently favorably influenced by Cerebrolysin, and a systematic review confirmed this effect [11,17-20,32,33]. Although the rate of conversion from mild cognitive impairment to AD under Cerebrolysin decreased in one trial, there is an obvious need to replicate these data, and the open-label design of this trial raises supplementary questions about the generalizability of its results [21].

Regarding the secondary objective of the review, preclinical studies indicate multiple hypotheses about the mechanisms of action for HPATE, mostly based on mitigation of the ischemic process and oxidative stress, a decrease of inflammation and apoptosis-like processes in case of stroke (Actovegin); also, HPATE may increase BDNF cortical and hippocampal brain concentration, while decreasing the oxidative stress and inflammation (Cerebrolysin) [22-24].

Table 1: Case series – demographics, baseline values of structured instruments administration, and treatment schedule

Patient no.	Age	Gender	Severity (baseline)	Treatment
1	68	Male	ADAS-Cog: 51 MMSE: 16 CDT: 4 NPI total score: 14 NPI distress score: 12 CGI-S: 3 GAF: 35	Donepezil 10 mg/day 12 months + Cerebrolysin 20 ml/day 10 days/month, 3 months, repeated after 3 months free of HPATE
2	72	Male	ADAS-Cog: 40 MMSE: 19 CDT: 5 NPI total score: 22 NPI distress score: 16 CGI-S: 4 GAF: 40	Galantamine 16 mg/day 12 months+ Actovegin 10 ml/day 10 days, followed by 400 mg q.d. orally 6 weeks, repeated after 3 months free of HPATE
3	80	Female	ADAS-Cog: 58 MMSE: 14 CDT: 1 NPI total score: 20 NPI distress score: 14 CGI-S: 3 GAF: 30	Rivastigmine 13.3 mg/day 12 months + Cerebrolysin 20 ml/day 10 days/month, 3 months, repeated after 3 months free of HPATE

ADAS-Cog = Alzheimer's Disease Assessment Scale - cognitive subscale, MMSE = Mini Mental State Examination, NPI= Neuropsychiatric Inventory, CGI-S = Clinical Global Impression - Severity, GAF = Global Assessment of Functioning, CDT = Clock drawing Test

CASE SERIES

This case series is based on repeated evaluations of 3 patients (two male, one female, mean age 73.3 years), all of them being diagnosed with Major Neurocognitive Disorder due to AD (probable), according to the DSM-5 criteria [34]. These patients presented memory, learning, and executive dysfunctions, accompanied by the gradual decline of these cognitive functions for at least one year before their baseline psychiatric evaluation, and no evidence of mixed etiology.

All patients were evaluated using brain MRI/CT scans, blood analyses, neurological exams, and psychological evaluations, to quantify the level of brain atrophy, and to exclude other possible diagnoses, e.g. VaD, dementia due to normal pressure hydrocephalus, etc. In all these patients frontal atrophy (mild to moderate), inferolateral regions of the atrophy of the temporal lobe (moderate), and medial parietal lobes atrophy were detected. These structural changes are consistent with data in the literature regarding AD neuro-imagistic parameters evolution [35,36].

The absence of evidence for folate or vitamin B12 deficiency, thyroid disorders, Parkinson's disease, Huntington's disease, HIV/prionic disease, head trauma, demyelinating disorders, and brain tumors was confirmed by the results of laboratory analyses and clinical examination.

Personal history or current diagnoses of abusive use or dependence on alcohol/sedative, hypnotic, or anxiolytic drugs/inhalants were investigated, and no significant data were found [37,38]. Patients were screened negative for drugs with potential negative effects on cognitive functioning (first-generation antipsychotics, antihistamine drugs, antidepressants with anticholinergic properties, etc.) [39].

The change in cognitive functioning during one year was the primary outcome of these case series, and it was based on two widely-used and validated instruments – ADAS-Cog and MMSE. Secondary outcomes were the Clock-drawing-test (CDT), the Neuropsychiatric Inventory (NPI), CGI-S, and the Global Assessment of Functioning (GAF) score changes from baseline to month 12, during the same period.

The interpretation of the CDT (which measures executive function, visual-spatial abilities, abstractization capacity, and attention) was based on a 0 to 10 scale, with higher scores reflecting the higher severity of dementia symptoms (Figures 1-3) [40].

At baseline, ADAS-Cog total score reflected low functioning on multiple domains in all three cases. The mean baseline MMSE score was 16.3, the ADAS-Cog score was 49.6, and the CDT score was 3.3. The Neuropsychiatric Inventory (NPI) reflected at baseline high scores on anxiety, irritability, and depression, with a mean overall score of 18.6, and a distress score of 14. The clinical global impression corresponded at baseline to a mean score of 3.33, and the GAF score was 35. All these instruments were administered at every 3 to 4 months (flexible visit regimen), for one year.

Figure 1: Clock test for patient no. 1

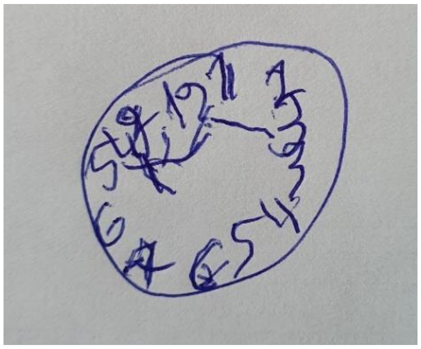

Figure 2: Clock test for patient no. 2

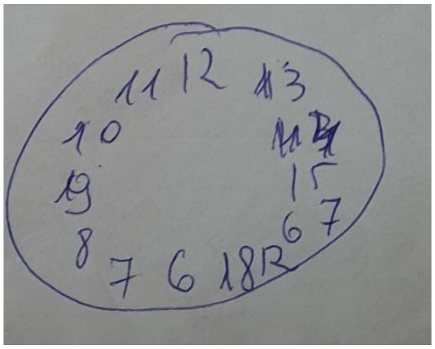

Figure 3: Clock test for patient no. 3

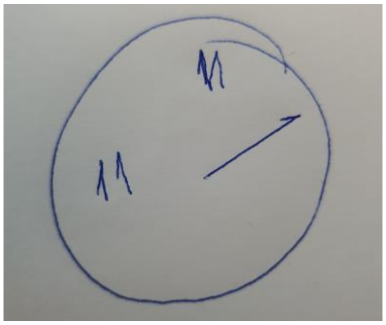

The therapeutic intervention consisted of HPATE, which was added to rivastigmine patches (n=1), oral galantamine (n=1), or oral donepezil (n=1). Actovegin was administered in one case and Cerebrolysin in two cases. The treatment schedule is represented in Table 1.

MMSE scores showed only a very small decrease during the 12 months of active monitoring (-0.33 points compared to baseline), and ADAS-Cog scores increased by 0.66 points (higher scores reflect more severe deterioration) (Figure 4). NPI total scores decreased with a mean of 7.6 points compared to baseline, and the overall distress with 4 points. The mean CDT score did not change after 12 months.

CGI-S and GAF scores also showed relative stability (no change for CGI-S, and -5 points on GAF).

Figure 4: Evolution of ADAS-Cog and MMSE scores from baseline to week 52

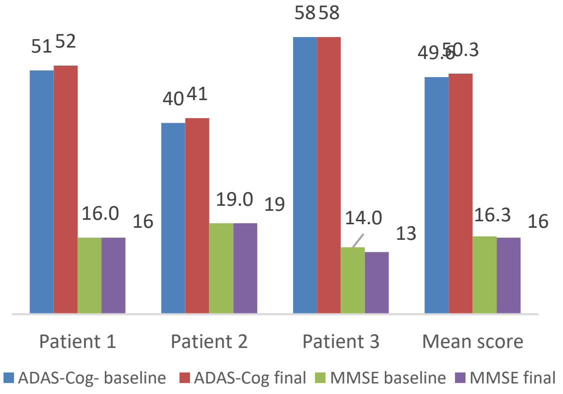

Both explored HPATE, Cerebrolysin and Actovegin, were well tolerated and they may be administered together with AChEIs because of their presumed synergistic effects.

This combination may be useful in stabilizing the overall cognitive status in moderate AD and in reducing the associated non-cognitive symptoms.

CONCLUSION

HPATE has been associated with potential benefits in patients diagnosed with AD and VaD as add-ons to AChEIs, or mild cognitive impairment as monotherapy. HPATE has a good safety and tolerability profile in AD or VaD patients. These conclusions are based on moderate and low-quality studies [11-15,17-20,32,33], therefore it may be considered that more clinical trials, with a randomized, double-blind design and a larger number of subjects are needed, to support HPATE positive effects on cognition. Regarding the impact of delaying the cognitive deterioration from mild cognitive impairment to clinically relevant AD using Cerebrolysin as monotherapy, data are even more inconclusive (one open-label study was found in the literature) [21].

AChEIs are associated with modest, but persistent cognitive benefits and these drugs may also reduce the mortality risk, a phenomenon which could be related to their cognitive favorable effects [41-43]. To augment the AChEIs effect, the administration of HPATE as an add-on may be useful for preserving the cognitive status or slowing the cognitive decline in patients with dementia. Therefore, HPATE may represent a useful and well-tolerated therapeutic option, and it may also favorably influence the evolution of non-cognitive symptoms in AD.

Disclaimer

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REVIEW

Medical and chemical evaluation of robotic surgery methods; A review study

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Abstract: *Along with the advances in medical science, surgical methods have also undergone many advances. Today, with the advancement of technology in all fields, including medicine, robots have entered medical science. These robots have many uses as well as advantages and disadvantages that they enjoy in Iran and other countries. In this study, it was addressed. This study is a review of robotic surgery methods in Iran and other countries in the form of a review study. This study is a conceptual review. The steps performed are 1- Designing a research question, 2- Searching and extracting research-related studies, 3- Selecting related studies, 4- Tabulating and summarizing information and data, and 5- Reporting results. The results showed that robotic surgery in operations such as brain, kidney, open heart, liver, eye, laparoscopy, dental surgery, coronary artery surgery, hysterectomy, lymphadenectomy, general surgery, obstetrics, head and neck, shelf Chest, urology, endoscopy, colonoscopy, ear, nose, and throat are used and have advantages such as three-dimensional vision, flexible rotation of the instrument, reduction of surgeon hand vibration with vibration filter, ease of surgery and the ability to create the required patterns It reduces the volume of blood lost during surgery, the length of stay in the hospital, the amount of pain, and subsequently the number of analgesic doses consumed in the postoperative ward. Disadvantages include the relative increase in operating time, the high cost of robots, and the physician's lack of sensory perception of the patient's environment.*

Keywords: *robotic surgery, surgery, robotics*

INTRODUCTION

From the past until now, surgery has been known as a treatment for some diseases, but it was performed due to its many limited complications. Along with the advances in medical science, surgical methods have also undergone many advances [1]. In traditional surgery, the surgeon has to make larger incisions to perform the surgery and damage a large amount of tissue in the patient's body. This increases the likelihood of surgical complications such as pain, bleeding, and infection, and prolongs the patient's recovery time [2-5]. Given the problems that exist in open surgery, surgeons have sought to minimize harm to the patient by creating new tools. This led to the emergence of a new approach called minimally invasive surgery (Figure 1) [6-7].

Figure 1: Robotic surgery

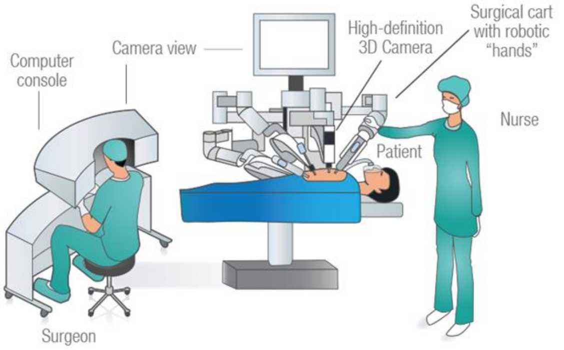

Today, with the advancement of technology in all fields, including medicine, robots have entered medical science. In medical science, surgery and operations in which robots are directly or indirectly involved are called "robotic surgery" [8].

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Robotic surgery is performed by making several small incisions in which the surgeon can be present without the patient's bedside and remotely while having three-dimensional vision by controlling the robot arms that have a range of motion similar to human hands and wrists and around Are placed on the operating table to perform surgery (Figure 2) [9]. Today, robots have become a very useful tool to help doctors, and their irreplaceable role in medical science is increasing every day. That's why the US National Institutes of Health has significantly increased research funding. The NIH budget for this project for 5 years is $ 2.3 million [10]. The difference between a robot and a human is not negligible in many ways. Robotic surgery, compared to traditional surgery, results in smaller incisions, increased accuracy, flexibility, better visual control, and reduced pain and wounds, which leads to less time spent in the hospital. And recovery time is faster [11].

Figure 2: A review of camera viewpoint automation in robotic and laparoscopic.

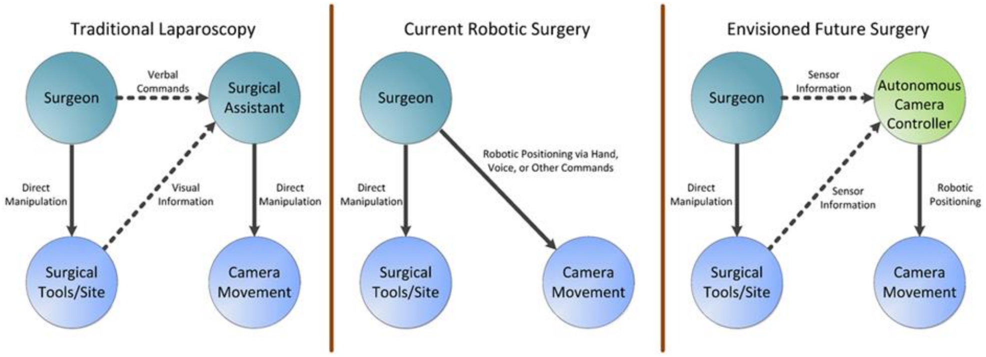
Traditional Laparoscopy
Surgeon
Verbal Commands
Surgical Assistant
Direct Manipulation
Visual Information
Direct Manipulation
Surgical Tools/Site
Camera Movement
Current Robotic Surgery
Surgeon
Robotic Positioning via Hand, Voice, or Other Commands
Direct Manipulation
Surgical Tools/Site
Camera Movement
Envisioned Future Surgery
Surgeon
Sensor Information
Autonomous Camera Controller
Direct Manipulation
Sensor Information
Robotic Positioning
Surgical Tools/Site
Camera Movement

Among the benefits of robotic surgery can be; Reduction of the long recovery period after surgery, reduction of postoperative pain, reduction of the possibility of postoperative infection, reduction of hospitalization time, no need to break the sternum and open the chest in open surgeries, rapid healing of wounds, in the availability of certified surgeons with unique capabilities in more locations, greater accuracy, elimination of surgeon hand vibration, continuous and 24-hour operation 24 hours a day without fatigue. In addition to having many benefits, according to robotic surgery studies (Figure 3).

Figure 3: The evolution of robotic surgery: surgical and anesthetic aspects

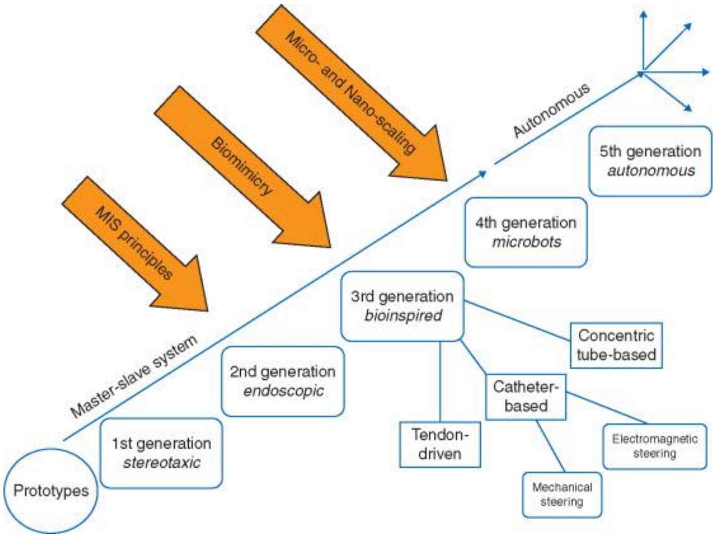
Micro- and Nano-scaling
Biomimicry
MIS principles
Autonomous
Master-slave system
5th generation
autonomous
4th generation
microbots
3rd generation
bioinspired
Concentric tube-based
2nd generation
endoscopic
Catheter-based
Tendon-driven
Electromagnetic steering
Mechanical steering
1st generation
stereotaxic
Prototypes

Disadvantages are including: increasing the distance between doctor and patient, reducing the speed of communication with equipment and monitors, increasing the possibility of cutting the wrong place, the possibility of disconnecting the surgeon from the operating room, low network security, and the possibility of hacking communication, patients' reluctance to use this equipment and technology, Expensive robots, lack of assistance for the doctor, lack of sensory perception of the patient's environment by the doctor. Many operations by surgical robots such as brain surgery, kidney, open heart, liver, eye, laparoscopy, dental surgery, Coronary artery surgery, hysterectomy, lymphadenectomy, general surgery, obstetrics, head and neck, chest, urology, endoscopy, colonoscopy, ear, throat, and nose have been successfully performed [10]. The high accuracy of some surgical robots makes it possible to perform very difficult surgeries such as vitreoretinal surgery and eye vitrectomy in a favorable manner [12].

There are several types of surgical robots. One of them is Heartlander, which is known as the smallest surgical robot in the world. Zeus robot is another robot. The other robot is the Da Vinci robot, which is known as the best robotic surgery system in the world and is made by a single commanding company. The system was approved by the US food and drug administration in 2000. In the meantime, other countries have also started working.

In Iran, the Sina robot surgeon (Da Vinci's rival), was developed by researchers at the Tehran University of Science in collaboration with researchers at the Sharif University of Technology. Its unique feature compared to the Da Vinci robot is that it measures the force applied to the patient's body and transmits it to the surgeon through the surgical console, and its price is estimated to be one-third of the American version [10]. Although we are facing the development of surgical robots around the world, a review of scientific products in the field of surgical robots shows that the United States, China, and Japan are the three leading countries in this field, respectively, and Iran is in 15th place.

Figure 4: Robot-assisted kidney surgery results similar to those of traditional methods

The trend of scientific production in this field started in 1990 and has been moving forward with a gentle slope and has gone through an upward trend since 2001. This trend has continued upward with fluctuations in different years. The results indicate that. There are few scientific developments in this field and few countries, universities, authors, and publications have addressed this issue [13] (Figure 4). According to studies, efforts should be made to recognize The right requirements to make it possible to use robotic surgery worldwide [14]. Therefore, this study aims to investigate robotic surgery methods in Iran and other countries in the form of a review study.

METHOD

This study is a conceptual review. The steps performed are 1- designing a research question, 2- searching and extracting research-related studies, 3- selecting related studies, 4- tabulating and summarizing information and data, and 5- reporting its results.

SEARCH AND EXTRACTION OF RESEARCH-RELATED STUDIES

First, articles were researched by researchers. Researchers first extracted related keywords using the MeSH strategy. Search for resources using the keywords robotic surgery, surgery, robotics, robot surgeon in Persian databases Barekat, Iran Medex, Irandoc, Magiran, SID and using the English keywords telesurgery, Robotic surgery, in Google scholar databases, Scopus, science direct, PubMed, web of science were conducted from 2013 to 2021 in October and November 1400. These words were searched separately and also as a combination of two words together using Boolean operators. To increase the validity of the study findings and control the bias in data entry, the search process, selection of studies, and decision-making on entering or constructing the study and evaluation of the full text of the articles were performed by two independent browsers. This process was overseen by a third party.

Inclusion criteria include all articles that have examined robotic surgery and their full text is available and written in Persian and English. Exclusion criteria were studies unrelated to the title

EXTRACTING ARTICLES ACCORDING TO INCLUSION CRITERIA

Using the above keywords at the end of the search, including 37 articles from Persian databases and 105718 articles from international databases and a total of 105755 studies were collected. The studies were reviewed based on inclusion criteria. The selection of related studies was done in such a way that first a list of titles and abstracts of all articles in databases is prepared by researchers. And if after reading the title and abstract, it was not possible to decide on the study, the full text was studied. Then, the related articles entered the research process independently, and finally, 43 articles were included in the study.

TABULATING AND SUMMARIZING INFORMATION AND DATA

At this stage, after the data has been extracted, all study data will be summarized based on the name of the first author, year, place, title, tools, or research elements in the relevant table.

REPORTING THE RESULTS

After reading the summary and full text of the articles related to the research topic, the desired information was extracted for writing. Information required for each study included author name, year, place, type of study, sampling method, data collection tool, and results. Finally, the information obtained from the articles was categorized and finally reported in the full text of a review article.

Figure 5: The schematic method

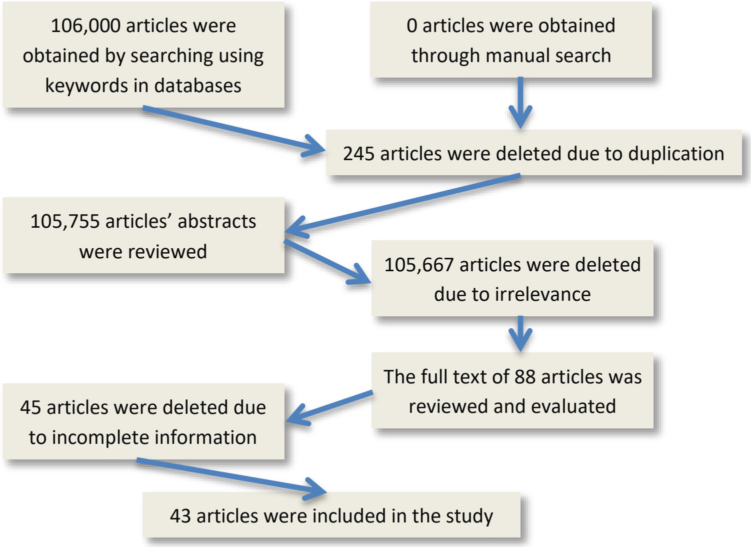

106,000 articles were obtained by searching using keywords in databases

0 articles were obtained through manual search

245 articles were deleted due to duplication

105,755 articles' abstracts were reviewed

105,667 articles were deleted due to irrelevance

The full text of 88 articles was reviewed and evaluated

45 articles were deleted due to incomplete information

43 articles were included in the study

Table 1: Studies related to the research topic

Findings	Research tools	Type of study	Title	Place	Year	Author
The results of measuring and recording the time required to start the robot, the ease of working with the robot, the quality of the robot in response to the surgeon's instructions, the accuracy of the robot's movements, the robot's ability to provide full image coverage, no shaking or shaking image, indefatigability Robot in long surgeries, lack of unwanted movements and lack of errors in movements indicate the safety and efficiency of the robot and its satisfactory performance during surgery.	Intervention	Experimental	Clinical evaluation of a new camera-preserving robot in laparoscopic surgery preliminary results	Iran	2019	Karimian
Studies have shown that three-dimensional vision, flexible rotation of the instrument, reduction of surgeon hand vibration with the help of a vibration filter, ease of surgery, and the ability to create the required patterns are the characteristics of surgeon robots.	Review of studies	Review	Comparison of robotic technique with standard laparoscopy in abdominal and visceral surgeries	Iran	2017	Theoretical

There are many surgical robots or intelligent medical assistants in the field of medicine, but to conclude which of these robots is better, you must first assess the time and place conditions and services, because each of these robots is in a field of activity and it must be admitted that they also have limitations [15,16].

Robotic technology allows general surgeons to operate with great precision, and areas that are difficult or even impossible to reach by humans can be accessed using a robotic system. The robotic surgeon's arms can rotate up to 280 degrees. All this brings better results for the patient. Iranian specialists have succeeded in gaining the technical knowledge of this robot surgeon and the technical knowledge of the mechanism design process, hardware, and software Robots have also been achieved [17]. The results of studies conducted in Iran in the field of robotic surgery are as follows.

The results of measuring and recording the time required to start the robot, the ease of working with the robot, the quality of the robot in response to the surgeon's instructions, the accuracy of the robot movements, the ability of the robot to provide full image coverage, no shaking or shaking image, The indefatigability of the robot in prolonged surgeries, the absence of unwanted movements and the lack of errors in movements indicate the safety and efficiency of the robot and its satisfactory performance during surgeries. Although launching a robot requires time and initial work, the results show a reduction in total operation time in most cases. The smoothness of motion, stability and no-image shake are among the advantages of the robot holding the laparoscope over the human hand, so this robot can be a good alternative for the person holding the camera.

Table 2: Another review of the method on 2021

Findings	Research tools	Type of study	Title	Place	Year	Author
Mitral valve robotic surgery is well possible through a limited surgical procedure Mortality around the operation seems very acceptable and possibly even lower than a sternotomy.	Review of studies	Review	Mitral valve robotic surgery: Methods and results in a 20-year review	Australia	2021	Monati
Robotic head and neck surgery are now regularly used in specialized centers for head and neck surgery.	Review of studies	Review	Current Advances in Robotic Head and Neck Surgery	Germany	2021	Their boe
A review of studies showed that robotic surgery had better overall survival than non-robotic resection of oral squamous cell carcinoma, and this method is recommended.	Review of studies	Review	Robotic surgery versus non-robotic resection of oral squamous cell carcinoma	USA	2021	Chilacula
The process of industrialization of surgical robots in several developing countries, such as the United States, must be accompanied by the removal of technical barriers and increased international cooperation	Review of studies	Review	analyze 100Most Read About Robotic surgery versus open surgery	China	2021	Chow
Robotic systems are widely accepted in the U.S and various surgical specialties.	Studies	Review	Robotics in Spine Surgery	USA	2021	Farber
Robotic surgery in a rural head and neck center is a safe and appropriate treatment option and has very few side effects and this method is recommended.	Questionnaire	Retrospective	Oral robotic surgery: a safe and effective tool for the head and neck Surgery	Australia	2021	Huang
Robotic surgical systems were divided into open/closed, image-based/imageless, and active/inactive/systems. The main goal is to improve the accuracy of operations.	Descriptive	Descriptive	Robotics in Orthopedic Surgery: Why, what, and how?	Belgium	2021	Innocent
Robotic spine surgery was introduced in 2004.	Descriptive	Descriptive	The evolution of robotic surgery in the spine	USA	2021	Because
Robotic surgery is a good option for adult spinal deformity surgery.	Questionnaire	A cohort	Have robotic methods improved postoperative results?	USA	2021	Croll
Robotic surgery can significantly reduce blood loss, intraoperative complications, and length of hospital stay, but increase readmission and surgery time. Robotic surgery in the treatment of endometrial cancer showed better results.	Review of studies	Review	The effectiveness of robotic surgery for endometrial cancer		2021	Hafng
Robotic-assisted radical prostatectomy was less recurrent and had lower pain scores up to 6 weeks postoperatively compared to open radical prostatectomy. Compared to laparoscopic prostatectomy, robotic surgery improves urinary and sexual function.	Review of studies	Review	Clinical results of robotic surgery compared to conventional surgical procedures (laparoscopic or open)	USA	2021	Hala

The next step will be to simplify work with the device by upgrading the software and conducting more clinical studies to evaluate robot performance comparisons [18]. Studies have shown that three-dimensional vision, flexible rotation of the instrument, reduction of surgeon hand vibration with the help of a vibration filter, ease of surgery, and the ability to create the required patterns are the characteristics of surgeon robots. Also, in general, the volume of blood lost during surgery, the length of hospital stay, and the amount of pain, followed by a reduction in the number of analgesic doses consumed in the postoperative ward and the time to return to a normal diet and normal activity compared to the laparoscopic procedure is reduced.

On the other hand, the results of the research have shown that the duration of the operation by the robot and the cost of surgery are longer than the laparoscopic method.

DISCUSSION

Robot surgery is not necessarily more expensive than laparoscopic surgery. Evidence now suggests that robotic surgery has a permanent role to play in the future of surgery. Therefore, surgeons should be actively diligent in the development of robotic surgery and begin preliminary and randomized studies with high prognosis and quality in this field [19]. The results showed that the use of robot traps in hip arthroplasty, in addition to providing more accurate 3D images for the surgeon to assess the depth and size of the acetabular cavity, reduces the risk of displacement or dislocation of the replaced joint. Reduces the risk of complications such as infection and site bleeding for the patient as well as readmission to the hospital. The results of studies show that robotic surgeries have effective applications as a minimally invasive method. Therefore, they can be gradually replaced by other surgical methods, but what is important is to pay attention to improving skills in the field of relevant new inventions, strengthening infrastructure, and allocating sufficient money to be able to use advanced technologies desirably and efficiently. Used for the benefit of the patient and the surgeon and other members of the treatment team [20]. It is true that the elimination of the natural vibration of the surgeon's hand, reducing the possibility of infection and postoperative infections, the possibility of a deeper vision of the operating field, and the creation of small and delicate incisions instead of large incisions by microscopic robots are the advantages of using robotic surgery. However, it is possible to reduce the feeling of tissue specificity under the operation by the surgeon, reduce the speed of communication with equipment and monitors and the possibility of a twenty-five-second delay on the monitors can also be considered as disadvantages of remote surgery. Remote surgery is performed by robots and advanced medical systems, but due to its cost and lack of strong technological and communication infrastructure, this method has not yet been properly transferred to developing countries and is limited to developed countries [21].

Limitation of tactile data Using graphic data It was thought that robotic surgery would be used more, but this did not happen. Reasons for this include lack of public awareness of the benefits of this method, patients 'refusal to have robotic surgery, and surgeons' desire for traditional surgery [22]. A study showed that the use of viewfinders will be effective in reducing the cost, weight, and dimensions of equipment and also simplifying some medical operations. It should be noted that the use of observers strongly depends on the type of operation used and not all of these methods are yet clinically applicable because there are many challenges in ensuring the performance and safety of these methods. However, the multidisciplinary nature of this subject provides a wide field for study and research [23]. Robotic surgery has made slow progress due to cost and patent protection. But now we are seeing an increase in the number of new robotic surgery systems. Several systems are CE-certified, while in the EU alone TransEnterix Senhence has received FDA approval, and several more are currently being tested, which will increase competition and reduce costs by approving these new robots. In the future, robotic surgery will be more common than manual surgery [24]. A "driver's license" is seriously required for robotic surgery. Ideally, a certified training program is required to define a robotic surgeon and ensure a high level of work. And the training pyramid must start from the ground up, so that, with the effectiveness of the training, robotic surgery is safe for the patient and the surgeon [25]. Robotic surgical systems were divided into open/closed, image-based/imageless, and active/inactive/ systems. The main goal is to improve the accuracy of operations. It should be noted that regardless of the typology of the robotic system, the surgeon is responsible for planning and approving the use of the type of operation. Accurate and consistent execution of instructions is entrusted to robots. Every surgeon, when considering which robotic system to use, must correctly evaluate their differences [26].

One of the most important issues in remote surgery is the touch feedback system. Although surgeon robots increase the surgeon's agility, have less vibration of the surgeon's hand, and have better vision, they reduce their ability to understand. Other disadvantages of robotic surgery include the possibility of a twenty-five-second delay on monitors. Depending on the doctor's condition, the delay between when the surgeon sees the knife moving and the incision is less than one-fifth of a second. It is performed and increases the probability of cutting in the wrong place and the risk of surgery and the cost of the required equipment [27]. The amount of bleeding after robotic surgery is low and severe bleeding, even if it is small, requires embolization or tracheotomy to control the bleeding. Large tumors, preoperative anticoagulant use, and a history of previous radiation are associated with an increased risk of bleeding after robotic surgery [28]. In comparison with immediate and long-term results, no difference was observed in the duration of the operation, the number of times the blood transfusion was required, and the occurrence of severe postoperative complications. Compared to traditional surgical methods, robotic surgery reduces the number and severity of postoperative complications and improves treatment outcomes [29]. In a study in the robotic surgery group, the rate of blood loss, length of hospital stay, and complications were lower, but the mean time of operation was longer compared to open surgery [30]. Operating times for robotic surgery are time-consuming due to the connection and stabilization of the instrument. The results of 260 cases of robotic surgery showed no increase in postoperative death [31]. Over time, with experience and innovation in platform design (Da Vinci), operating time is likely to decrease further [32,33]. However, a study showed that the benefits of robotic surgery have yet to be explored. Although the initial results are promising [34].

Also, studies conducted in other countries regarding robotic surgery are as follows. One study found no major complications during surgery. Kidney surgery with the help of robots by experienced robotic surgeons is a safe and repeatable surgery in selected cases and the result is excellent connective tissue function. The use of robotic technology can provide low complication rates, rapid recovery, and better connective tissue function [35]. Robotics in spine surgery is an emerging technology that promises plans. The accuracy of surgery in instruments implanted using robotics seems to be high. However, the effect of robotics on radiation levels is unclear and seems to depend on the technique and type of robot [36]. Robotic surgery greatly affects spinal surgery and is still safer and more efficient. Finally, it offers a better 3D image [37]. Robotic spine surgery was introduced in 2004. Robotic spine surgery has made great strides. This surgery is a reliable and accurate tool for spine surgery and has grown rapidly in recent decades and is still on the path of development and progress [38]. Artificial intelligence has tremendous potential to revolutionize the comprehensive care of the spine. It can assist surgeons in selecting preoperative patients, and surgical symptoms, and improving individual postoperative care. Robotic surgery, while still in its infancy, has a very clear vision for robotic spine surgery. Robotic surgery can reduce surgeon fatigue and improve surgeon technical accuracy [39]. Robotic systems are widely accepted in the United States and various surgical specialties. This innovative technology is still relevant in the field of spine surgery. These systems have potential benefits such as increased screw placement accuracy, reduced operating time, and reduced radiation. However, the various technical factors of these systems are constantly being re-evaluated to improve performance and achieve these goals [40]. Robotic surgery is also very effective in adult spinal deformity surgery [41].

Robotic hip arthroplasty was associated with a reduced length of hospital stay and reduced costs compared to conventional methods. However, robotic surgery was associated with readmission and the need for more blood transfusions [42].

Robotic surgery is an innovation in thyroid surgery that compensates for the disadvantages of conventional endoscopy. Robotic surgery has many advantages, including 3D imaging, motion scaling, and vibration elimination. Although robotic thyroid surgery is often associated with longer operating times than conventional open surgery, more robotic techniques are safer. Robotic surgery is compared to conventional open surgery or endoscopy. It is associated with several benefits such as quality of life, aesthetic results, reduction of neck pain and sensory changes, and reduction of discomfort in swallowing after surgery. According to surgeons, robotic surgery improves the patient's ergonomics. The time and cost of surgery in robotic surgery have been longer. Patient mortality was not different. The benefits of robotic thyroid surgery With conventional surgery, it is shown that robotic surgery can be an advanced surgical option for thyroid disease. Robotic thyroid surgery should be further developed as a surgical procedure for endocrinology, and head and neck surgeons have received this training, and robotic thyroid surgery is a surgical option [43].

Robotic surgery for the treatment of rectal cancer appears to be oncologically safe and appears to be better in terms of short-term complications and pathological quality indicators. The potential and proven benefits of robotic surgery should be considered. The benefits of robotic surgery for rectal cancer include improved three-dimensional vision, ease of use of the tool, improved surgeon ergonomics, and better access to narrow spaces such as the pelvis. However, studies have clearly shown the value of robotic surgery for patients and surgeons and need further studies [44].

Mesorectal robotics is a safe and sufficient technology for the treatment of rectal cancer and helps to maintain better urinary and sexual functions. Robotic surgery due to better wrist movements and better use of tools that facilitate autopsy, especially in narrow spaces, facilitates the operation by minimizing damage to the surrounding tissues as well as due to the magnified view [45].

Robotic surgery seems to be an effective method in terms of oncological and functional results and is effective in patient survival and its use is recommended in tumor staging and HPV status [46].

Robotic-assisted radical prostatectomy was less recurrent and had lower pain scores up to 6 weeks postoperatively compared to open radical prostatectomy. Compared to laparoscopic prostatectomy, robotic surgery improves urinary and sexual function. Robotic surgery had fewer complications for endometrial cancer than laparoscopy [47].

Mitral valve robotic surgery is well possible through a limited surgical procedure. Mortality around surgery seems very acceptable and possible. It is even lower than sternotomy. The rate of stroke was higher than sternotomy in the early stages of mitral valve robotic surgery but now appears to be at least the same. After robotic surgery, 1 in 4 people needed a blood transfusion. The rate of renal failure around surgery is low. One in 200 patients develops a wound infection. The length of hospital stay did not decrease. Due to the huge level of innovation in mitral valve robotic surgery, this method seems very satisfactory [48].

Robotic head and neck surgery are now regularly used in specialized centers for head and neck surgery. It is used in simple surgeries and easily accessible anatomical areas such as the mouth and throat. Disadvantages of this method include increasing the time required for the operation and increasing costs. For basal cranial, nasopharyngeal, or laryngeal surgery in patients with difficult anatomical conditions, systems with special surgical instruments for head and neck surgery should be developed. Despite the obstacles, we strongly believe that robotic systems will have a major impact on how we perform head and neck surgery over the next decade. Robotic head and neck surgery are recommended for better surgery in difficult areas, to reduce surgical complications, and to increase patient safety [49].

Robotic surgery in a rural head and neck center is a safe and appropriate treatment option and has very few side effects and this method is recommended [50].

A review of studies showed that robotic surgery has better overall survival than non-robotic resection of oral squamous cell carcinoma, and this method is recommended [51].

The number of female robotic surgeries has increased dramatically since the introduction of robotic surgery systems in Korea. Based on these data, it is expected that the continuous use of robotic surgery is widely used for both benign and malignant types of gynecological diseases. Many traditional surgeries are expected to be replaced by robotic surgeries [52].

A study on the application of robotic surgery in gynecological diseases showed that robotic surgery can significantly reduce blood loss, intraoperative complications, and length of hospital stay, but the rate of readmission and operation time Increasing. Robotic surgery has shown better results in the treatment of endometrial cancer [53].

The robotic system has overcome some of the shortcomings of laparoscopy, including three-dimensional vision limitation and hand tremors. Therefore, robotic surgery has taken many steps toward further progress in the field of general surgery. Although the safety and effectiveness of robotic surgery systems have been confirmed in most methods, in some methods robotic surgery has not been widely performed and is limited to a few highly specialized centers. Robotic surgery in hepatectomy, gastrectomy, esophageal surgery, colectomy, and pancreatectomy is performed unequally in the world, which may be related to the economic power of countries and the preference of surgeons, with the gradual reduction of the cost of robotic surgery systems and its standardization, we believe that robotic surgery will become gold and will become a standard method in general surgery, including surgical procedures as well as health insurance coverage [54].

The operating time for robotic surgery was 30% longer compared to laparoscopy. The rate of re-admission to robotic surgery is higher. Compared to laparoscopic patients, robotic surgery patients were hospitalized longer. There was no evidence of improved robotic surgery performance over surgery or clinical outcomes [55].

The rate of robotic segmentectomy has increased, and according to our study on robotic surgery, the operation time was longer, the blood loss rate was lower, and the hospital stay time was shorter. Our results show that robotic surgery can facilitate the function of complex anatomical parts [56].

Robotic surgery has become the preferred method for abdominal surgery. Robotic surgery is recommended when vaginal surgery is not possible. So far, gynecological robotic surgery has shown better safety and clinical results than laparoscopy. Robotics has been developed to overcome laparoscopic challenges and has led to technological benefits such as improved ergonomics, 3D visualization capabilities, skill and range of motion with the tool joint, and elimination of hand vibration. To date, robotic applications in benign obstetrics and gynecology include hysterectomy, myomectomy, endometriosis surgery, sacrocopopexy, adnexal surgery, tube re-anastomosis, and cerclage.

Robotic surgery may offer greater benefits than other methods in the obese patient population and more complex cases. Advances in robotic technology, such as telemedicine surgery, could revolutionize gynecological surgery. Finally, this robotic device is an additional minimally invasive surgical instrument that can help minimize laparotomy in women [57].

The use of robotic substrates in liver and bile duct surgery is growing rapidly. There is evidence that robotic surgery results are good. Robotic surgery is useful for managing complex cases such as iatrogenic bile duct injury, hepatic autopsy, or complex autopsy due to difficult anatomical positions.

We believe that, for selected patients, robotic surgery should be part of the treatment of complex liver surgeries [58].

According to studies, there is still room for the development of robotic surgery. Lack of close cooperation between scientists, institutions, and countries may have affected this issue. The process of industrialization of surgical robots in several developing countries, such as the United States, must be accompanied by the removal of technical barriers and increased international cooperation and exchange.

Removing barriers and developing global surgical relationships should seize the opportunity to improve the situation [59].

On the other hand, there is considerable variation in US hospital accreditation policies. However, the purpose of performing performance appraisals and monitoring is to improve patient outcomes. Extensive development and implementation of validation guidelines are recommended to optimize patient safety and outcomes [60]. The subject of training in robotic surgery is also very important.

The study showed that a lack of technical skills affects the results of robotic surgery. Therefore, physician education is very important [61}. The use of a standard curriculum for robotic surgery remains unclear. The choice of training methods and evaluation tools should be based on validity and application based on performance data. Clinical education seems to simulate effective education [62].

Also, in studies, the lack of clarity in the roles and responsibilities of nurses in robotic surgery and the lack of coordination for working conditions cause professional dissatisfaction and dismissal in nurses [63], which should also be considered.

CONCLUSION

Robotic surgery is one of the new and diverse technologies that the medical community at the national and international levels will have to use and enter this path. According to research, the results showed that robots can not be used in any way and relied only on manpower, because manpower, ie the same surgeons has mistakes, so these robots can not be left out.

Among the benefits of robotic surgery can be; Reduction of the long recovery period after surgery, reduction of postoperative pain, reduction of the possibility of postoperative infection, reduction of hospitalization time, no need to break the sternum and open the chest in open surgeries, rapid healing of wounds, in the availability of certified surgeons with unique capabilities in more locations, greater accuracy, elimination of surgeon hand vibration, continuous and 24-hour operation 24 hours a day without fatigue.

In addition to having many advantages, according to robotic surgery studies, it has disadvantages such as; Increasing the distance between doctor and patient, reducing the speed of communication with equipment and monitors, increasing the possibility of cutting the wrong place, the possibility of disconnecting the surgeon from the operating room, low network security and the possibility of hacking communication, patients' reluctance to use this equipment and technology,

The robots are expensive, the doctor does not have an assistant, and the doctor does not have a sensory understanding of the patient's environment. It must be adapted to other technologies and used to serve the people. Although we are facing the development of surgical robots around the world, a review of scientific products in the field of surgical robots shows that the United States, China, and Japan are the three leading countries in this field, respectively, and Iran is in 15th place.

The trend of scientific production in this field started in 1990 and has been moving forward with a gentle slope and has gone through an upward trend since 2001. This trend has continued upward with fluctuations in different years. The results indicate that there are few worldwide available scientific developments in this field and few countries, universities, authors, and publications have addressed this issue [13].

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REVIEW

Risk factors for abdominal compartment syndrome in trauma – A review

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Abstract: *Background and Aim: Abdominal compartment syndrome is a life-threatening complication that can occur in trauma patients and greatly increase their mortality. Although there is a better scientific understanding of the general phenomena involved in the pathogenesis of this complication, the particular risk factors and their implications in the trauma patient population are yet to be deciphered.*

*Methods: The authors conducted research through 3 electronic databases (PubMed, Scopus, and ScienceDirect) using the following search formula: "(ACS OR abdominal compartment syndrome) AND (*trauma*) AND (risk factor)". Subsequently, additional search formulas were used, including the risk factors taken into consideration (i.e. "shock", "hypotension", "acidosis", "base deficit", "coagulopathy", "retroperitoneal hematoma", "HOB elevation", "fluid resuscitation", "damage control laparotomy").*

Results: Throughout the 41 articles analyzed in this paper, 7 risk factors transcended and were further discussed: head of bed elevation/patient positioning, fluid resuscitation, the "lethal triad" of acidosis hypothermia and coagulopathy, Damage Control Laparotomy, shock/hypotension, retroperitoneal hematoma and demographics (age, gender, and race).

Conclusions: To summarize, many potential risk factors were evaluated for the envisagement of the present paper, but the ones that prevailed the most were excessive fluid resuscitation, shock/hypotension, retroperitoneal hematomas, and the lethal triad. Consistent with other studies, no connection was found between age, gender, or race and the development of ACS. Further studies should focus more on the likely involvement of damage control laparotomy and patient positioning, as well as hypocalcemia, in the unfolding of ACS in trauma patients.

Keywords: *abdominal compartment syndrome, trauma, risk factors*

INTRODUCTION

Trauma is considered to be the primary cause of death for people aged 1-44 years old in the United States [1,2], with similar demographics in the European Union [2]. There are two types of trauma, regarding the mechanism of injury: blunt and penetrating. Blunt trauma defines any injury caused by an impact with a dull surface or object and is most frequently encountered in motor-vehicle accidents, falls and assaults, whereas penetrating trauma represents an injury caused by a foreign object piercing the skin and creating an open wound, generally involved being firearms and stabbing [1]. Overall, blunt trauma accounts for 70% of all trauma cases [2].

Abdominal compartment syndrome (ACS) is a life-threatening complication that can occur in trauma patients and greatly increase their mortality. In 2006, the World Society of Abdominal Compartment Syndrome (WSACS) proposed a series of universal terms to use when referring to this intricate disease [3], which were later revised in a 2013 conference [4]. Throughout their research, major advances have been made toward a superior comprehension of the diagnostic criteria for ACS [5]. The prevalence of ACS in major trauma has been previously reported higher than 30% and the mortality rate greater than 60% [6].

Although there is a better scientific understanding of the general phenomena involved in the pathogenesis of ACS, the particular risk factors and their implications in specific

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patient populations are yet to be deciphered. This article aims to address the particular occurrence of ACS in trauma patients, while also reiterating the information currently available in the literature to a greater extent.

The abdomen can be considered a relatively incompressible enclosed space and the intra-abdominal pressure (IAP) represents the steady-state pressure concealed within this cavity. IAP normally ranges between subatmospheric and 0 mmHg and changes slightly with respiratory movements. Pathologically, IAP rises with the accumulation of fluid in the abdominal cavity, space-occupying lesions, changes in the volume of its viscera, or conditions that limit the expansion capacity of the abdominal wall [3]. IAP is particularly elevated in critically ill adults, with a typical range of 5 to 7 mmHg [3].

Intra-abdominal hypertension (IAH) refers to a prolonged or repeated pathological elevation of IAP ≥ 12 mmHg [3,4]. Both IAH and ACS have been determined to be important independent predictors of mortality [7,8]. According to WSACS [3], IAH can be classified into 4 grades, as follows:

- Grade I: IAP 12-15 mmHg.
- Grade II: IAP 16-20 mmHg.
- Grade III: IAP 21-25 mmHg.
- Grade IV: IAP > 25 mmHg.

Malbrain et al. [9] suggested 4 subdivisions of IAH that were based on the time-length of symptoms:

- Hyperacute IAH defines a short-term increase in IAP over a couple of seconds-minutes, without pathological significance, due to sneezing, coughing, straining, or physical activity.
- Acute IAH is most commonly seen in surgical patients suffering from trauma or intra-abdominal hemorrhage. IAP rises abruptly over hours and imposes a high risk of producing new-onset organ failure, thus becoming a new clinical entity: ACS.
- Subacute IAH is a consequence of the interaction between casual and predisposing factors and develops over days.
- Chronic IAH progresses for months (i.e. pregnancy) or years (i.e. tumors, morbid obesity, cirrhosis with ascites), allowing the abdominal wall to accommodate the excess pressure through distension without decompensation.

A consensual research-based definition of the ACS was implemented by WSACS in 2013: ACS represents a sustained IAP > 20 mmHg, with or without an APP ≤ 60 mmHg, correlated mandatory with a new organ impairment/failure [4]. Abdominal perfusion pressure (APP) is the difference between mean arterial pressure (MAP) and IAP [3] and its relevance will be discussed within the pathophysiology of ACS.

Moreover, ACS can be classified into 3 types, depending on the duration and mechanism [3]:

- Primary ACS – involves acute or subacute IAH due to an intra-abdominal cause, most commonly trauma or surgery.
- Secondary ACS – subacute or chronic IAH originated from an extra-abdominal cause, most commonly burns or sepsis.
- Recurrent ACS – formerly labeled as Tertiary ACS, implies that a patient treated for primary or secondary ACS redevelops symptoms, most commonly acute IAH ("second-hit" phenomenon).

Given the fact that direct measurements are too invasive to be widely applicable in all patients [10], there have been numerous discussions regarding the indirect technique which most accurately estimates IAP. In 2006, the WSACS reached a consensus: IAP should be expressed in mmHg, measured via the bladder with a maximal instillation volume of 25 ml sterile saline solution, with the patient in a supine position at end-expiration, without abdominal muscle contraction and with the transducer zeroed at the mid-axillary line [3]. The standardized protocol reinforces that intermittent measurements should be conducted every 4-6 hours in critically ill patients with at least one risk factor for ACS [11].

METHODS

The authors conducted research through 3 electronic databases (PubMed, Scopus, and ScienceDirect) using the following search formula: "(ACS OR abdominal compartment syndrome) AND (*trauma*) AND (risk factor)".

Subsequently, additional search formulas were used, including the risk factors taken into consideration (i.e. "shock", "hypotension", "acidosis", "base deficit", "coagulopathy", "retroperitoneal hematoma", "HOB elevation", "fluid resuscitation", "damage control laparotomy") and the terms "ACS" and "trauma".

Three inclusion criteria were applied: the articles must be available in full text, written in English, and published no more than 10 years ago. The references of the selected papers were also checked for other relevant material. The resulting references were included in the current review, focusing on the pathophysiology of the abdominal compartment syndrome and its risk factors, particularly in the adult trauma patient population. In the end, a total number of 41 articles were included in this research.

RESULTS AND DISCUSSIONS

For a better understanding of the implications of various risk factors in the pathogenesis of ACS, a brief description of the key pathophysiological concepts is needed.

The abdominal cavity is a well-defined space limited cranially by the diaphragm, caudally by the pelvic floor, anteriorly and posteriorly by the abdominal wall, and contains numerous parenchymatous viscera such as the liver, spleen, pancreas or kidneys, as well as hollow organs such as the stomach, small intestine, colon, cholecyst or urinary bladder. Given the fact that this cavity represents a closed compartment, one can easily understand that the ACS behaves similarly to any other compartment syndrome in general – an increase in intra-compartmental pressure generates a compression over the blood vessels, initially resulting in a restricted venous and lymphatic flow, followed by an arterial blood supply impairment and subsequently entering a vicious cycle which generates and maintains cellular hypoxia [11,12,13].

The cause of elevated intra-abdominal pressure can differ between trauma, burns, or sepsis, but they all result in a molecular cascade that ultimately determines capillary leak syndrome, fluid extravasation, and interstitial edema [14]. Capillary leak refers to undesirable extravasation of fluid and electrolytes +/- protein followed by edema [15]. In sepsis, interleukin-6, interleukin-8, and tumor necrosis factor-α have been found to interact with the capillary endothelial cells and trigger edema of the bowel wall. When hypotension occurs due to reflex vasodilation and fluid retention in the third compartment, physicians use fluid resuscitation to compensate for the fluid sequestration. This, in turn, aggravates the changes in hydrostatic and oncotic pressures, worsening the interstitial fluid extravasation, while the upward compression of the diaphragm limits respiratory movements, increases central venous pressure, and finally, contributes to the potential risk of developing ACS. This sequence is not solely applicable to sepsis originating from a non-traumatic medical condition but should be kept in mind when caring for a trauma patient with a penetrating abdominal wound, which perforated a hollow organ such as the digestive tract and spread germs in the abdominal cavity
[14].

Otherwise, the typical polytrauma patient presents with one or multiple bleeding sources, potentially developing hemorrhagic shock within minutes-hours of the traumatic event. In this situation, a massive amount of fluid resuscitation may be necessary for the survival of the patient, either by blood transfusions or crystalloid resuscitation. Apart from the capillary leak syndrome and interstitial edema, the attempt to restore the effective circulating volume after an important ischemia-inducing blood loss will result in an ischemia-reperfusion injury to the bowel wall and increased capillary permeability. Crystalloid solutions will decrease the oncotic pressure, further aggravating the pressure imbalance and increasing the fluid extravasation and ultimately, IAP [14]. A trauma patient who, besides the initial injury, develops a condition that further aggravates his state, is a patient who suffers from the “second-hit” phenomenon. This phenomenon corresponds to neutrophil priming, which means that activated neutrophils (from the traumatic injury mechanism) are overstimulated by a subsequent mechanism, most often inadequate fluid resuscitation, and therefore have an exaggerated immune response and determines a massive cytokine release, which leads to ACS and MOF (multiple organ failure). The most vulnerable window of time for this phenomenon also matches the most frequent timeframe of occurrence of the ACS, the first 3-6 hours after the traumatic event [6].

Nevertheless, elevated IAP induces cardiovascular, neurological, hepatic, and renal dysfunction [6,10-14,16]. An increase in IAP exerts pressure on the inferior vena cava, resulting in a decrease in venous return to the heart and a reduced preload. By reducing the preload of the heart, the cardiac output is also decreased aggravating cellular hypoxia and generating crucial amounts of lactic acid which, combined with the respiratory acidosis induced by the restricted movement of the diaphragm and the ventilatory impairment caused by atelectasis, result in worsened cellular function. The respiratory function is also hindered by increased intra-thoracic pressure, increased pleural pressure, and decreased dynamic and static compliance [10]. Moreover, the decrease in blood flow translates into reduced renal perfusion and potentially acute kidney injury (AKI). The renin-angiotensin-aldosterone hormone system (RAAS) is activated when renal hypoperfusion stimulates renin secretion by the juxtaglomerular cells. Angiotensin II is a potent vasoconstrictor that increases peripheral vascular resistance and activates aldosterone secretion from the adrenal glands. Aldosterone, in turn, induces sodium and water retention, intending to increase renal perfusion, but fails to stabilize the mean arterial pressure (MAP) [11]. One study reports that the onset of oliguria may announce the debut of ACS [17]. The liver is also affected by increased IAP, even at a moderate elevation of 10 mmHg [10], and impaired liver function translates into a decreased serum lactate clearance [12]. Finally, elevated IAP increases intracranial pressure and therefore decreases cerebral perfusion pressure due to a domino mechanism initiated by the cephalad displacement of the diaphragm and elevation of the intra-thoracic pressure, accompanied by a reduced cardiac output.

Risk factors

Throughout time, the scientific world has shown great interest in the most important risk factors for IAH/ACS. Finally, in 2013, the WSACS [4] summarized them and created 4 categories divided by mechanism and a 5th additional miscellaneous group: diminished abdominal wall compliance, increased intra-luminal contents, increased intra-abdominal contents, capillary leak/fluid resuscitation, and others. In the first group, identified risk factors were found to be major trauma, major burns, abdominal surgery, and prone positioning among several studies. An increase in intra-luminal contents was suggested particularly in gastroparesis and ileus, while fluid collections in the abdominal cavity, acute pancreatitis, and sepsis were responsible for an increase in intra-abdominal contents. Acidosis was prevalent among risk factors in multiple studies, as well as massive fluid resuscitation, while others suggested hypothermia, damage control laparotomy, or poly-transfusion as a possible mechanism of capillary leak. An increased APACHE-II or SOFA score was also associated with a risk of developing ACS, as they are typically present in a critically ill patient. Other factors taken under consideration were age, increased HOB angle, obesity and increased BMI, mechanical ventilation, and positive end-expiratory pressure (PEEP) > 10. Shock, hypotension, and sepsis were also proposed.

In comparison, Holodisnky et al. [18] observed a noteworthy clinical heterogeneity and ambiguity between the studies that reported risk factors in ACS and aimed to address this crucial impediment by identifying evidence-based risk factors for particular patient populations. Therefore, the authors conducted a systematic review and meta-analysis in which potential risk factors were investigated (38 for IAH and 24 for ACS) and separated into three categories: patient characteristics, systemic physiology, and fluid resuscitation. In addition to those mentioned by Kirkpatrick et al. [4], this systematic review and meta-analysis included, among others: gender, white blood cell count, amylase, calcium, C-reactive protein and albumin level, Glasgow Coma Scale score, Ranson score for acute pancreatitis, cardiac index, serum creatinine level and urine output, blood glucose, base deficit, hemoglobin and hematocrit, pre-ICU and pre-hospital crystalloid, crystalloid to packed red blood cell ratio. Nevertheless, this study concluded that large-volume crystalloid resuscitation and the presence of shock/ hypotension were commonly found in all patient populations, while others were specific to a particular group. The trauma patient population associated a higher prevalence of pre-ICU crystalloid resuscitation, base deficit ≥12, and abdominal surgery as risk factors. In addition, hypothermia (temperature ≤ 34°), urine output ≤ 150 ml in 24 hours, hemoglobin ≤ 80 g/L, and $GAPCO_2$ ≥ 16 were strongly associated with the development of ACS in the trauma patient group. Another relevant finding was that, as the volume of crystalloid used for resuscitation increased (≥ 7.5 L), the risk of ACS increased as well, with an Odds Ratio of 166.2 (CI 95%).

Head of bed elevation/patient positioning

Concerning the head of bed angle, a study published in 2009 by De Keulenaer et al. [19] raises the concern of a patient's position interfering with IAP measurement. The patients in the ICU may need to be nursed at a head-of-bed elevation of 30-45° to reduce the risk of ventilator-associated pneumonia and pressure ulcers, and this semi-recumbent position has been found to increase IAP by up to 4-9 mmHg, due to the viscera descending and exerting external pressure over the bladder. Furthermore, it has also been suggested that keeping a patient with a higher risk of developing IAH/ACS in a semi-recumbent position might trigger ACS [19]. In comparison, prone positioning has been demonstrated to elevate IAP, although the exact effects imposed on systemic and hepato-splanchnic blood flow or gastric-mucosal arterial PCO2 gradient have not been consensually established. This might be due to inaccuracies regarding the patient population taken under consideration and the type of mattress used for prone positioning [19]. Lastly, lateral positioning was analyzed in a small trial conducted by the same authors [20], in which De Keulenaer et al. concluded that out of the 10 patients included, the occurrence of IAP was 3.3% in the supine position and 26.6% in the lateral semi-recumbent position, which suggested that lateral decubitus may increase IAP by the same mechanism of visceral-induced external pressure on the bladder. A noteworthy limitation, besides the small study group, is that the trial only addressed patients with a low baseline IAP level, deeming it unreliable for the trauma patient population, in which a higher IAP level may be expected. Therefore, while the head-of-bed elevation effect on IAP has been established, further research on prone and lateral positioning is due. These findings are consistent with a study published by McBeth et al. [21] in 2007, in which a statistically significant relationship between a head of bed elevation of 30-45° and increased IAP was found.

Smit et al. [11] address these concerns in their study, proposing a reference table that can be used to correct IAP values depending on the head of a bed elevation. Their paper suggested that, for a head of bed angle between 0-20°, the measured IAP is correct and does not need any adjustment. Otherwise, for a head of bed elevation of 20-40°, the correct pressure is obtained by subtracting 4 mmHg from the measured IAP, while a head of bed angle greater than 40° requires subtraction of 9 mmHg from the measured value.

Fluid resuscitation

Fluid resuscitation is a vital part of therapy for trauma patients, given the fact that the most prevalent cause of death in the Early stage (first 72 hours after the traumatic injury) is hypovolemic shock due to blood loss [22,23]. Post-injury ACS is most frequently associated with inadequate fluid resuscitation of trauma patients [6]. Parenteral solutions used for fluid resuscitation are classified according to their molecular volume in crystalloids and colloids. Crystalloids include isotonic saline solution, Ringer's lactate, and hypertonic saline solution (contains larger quantities of sodium than the physiological concentration). Colloids, such as albumin, hydroxyethyl starch, and dextrans, have higher molecular weight and therefore generate an osmotic effect. Although rapid fluid resuscitation was widely considered the standard approach to hypovolemic shock in trauma patients [22], significant concern has been raised by some clinicians regarding the safety of the fluid resuscitation protocols. Hence, several studies addressed the issue: in a 2007 retrospective study, Madigan et al. [24] evaluated 48 patients with severe extremity injury who developed secondary ACS after trauma and compared them to a control group of patients who did not develop ACS. The authors concluded that the ACS group had received significantly higher amounts of crystalloid and blood products during all stages of care: pre-hospital, Emergency Department resuscitation, and post-admission hospital course. Therefore, early aggressive resuscitation by large volumes of crystalloids was found to be an important predictor of secondary ACS in the trauma patient population. This conclusion adheres to the reasoning of Balogh et al. [25] who suggested in 2003 that supranormal resuscitation (oxygen delivery index > 600 ml/m² achieved by a greater amount of crystalloid and packed red blood cell transfusions) was associated with an increased risk of IAH, ACS, MOF, and death.

In a 2012 multicenter prospective cohort study, Neal et al. [26] analyzed the crystalloid to packed red blood cell ratio (C:PRBC ratio) in a 452 patients population, only including those who needed ≥10 units of PRBC in the first 24 hours and survived past the initial 24 hours post-injury. The study population was divided into 2 groups by the C:PRBC ratio median: HIGH C:PRBC and LOW C:PRBC. The authors discovered that high ratios of C:PRBC (> 1.5:1 crystalloid to PRBC) were linked to a greater risk of MOF, ACS, and ARDS (Acute Respiratory Distress Syndrome) and this relationship was dose-dependent.

In 2015 Kaplan et al. [15] noted that excessive use of crystalloids causes dilution coagulopathy and hypothermia (if used un-warmed) and is therefore involved in triggering the "deadly triad" of acidosis, hypothermia, and coagulopathy. Hypertonic saline was found to be a valid alternative to colloids for increasing plasma osmolarity and also a better option than isotonic saline because of the ability to rapidly raise blood pressure without the need for a large volume of administration. Colloids, such as hydroxyethyl starch (HES), although useful for their osmotic effect of keeping fluid in the vessel, could be dangerous because of adverse effects and should only be used for a short time (< 24 hours) and respecting the resuscitation endpoints. The results of this study were that aggressive fluid resuscitation with isotonic crystalloid solutions may exacerbate the capillary leak syndrome and generate peripheral tissue edema, ascites, and IAH/ACS and that a valuable alternative could be hypertonic saline. HES should be avoided in the event of burns or sepsis. Finally, the implication of exaggerated fluid resuscitation in the pathogenesis of post-injury ACS was sustained by various other studies [8,27-30] and further emphasizes the importance of resuscitation endpoint criteria, awareness, and early recognition of IAH and ACS.

The "lethal triad" of acidosis, hypothermia, and coagulopathy

The "lethal triad" of acidosis, hypothermia, and coagulopathy is a catastrophic appearance in the management of trauma and is a result of fluid sequestration, hypoperfusion of vital organs, development of ischemia and lactate release in the bloodstream, along with systemic inflammatory response syndrome and the association of supranormal fluid resuscitation [31]. Stawicki et al. [32] note that ACS is a consequence of the "lethal triad" and should be prevented by Damage Control Laparotomy (DCL), which consists of a planned surgical approach with 3 steps: firstly, an abbreviated surgery should be implemented to control the active bleeding and limit the risk of sepsis. The abdomen should be partially covered and not closed, for example using a Bogota bag. Additional re-explorations may be necessary to drain potentially forming fluid collections (continued bleeding, pus, bile). The second step comprises ICU management with physiological restoration by correcting base deficit and coagulopathy, warming-up procedures, and continuing fluid resuscitation. When the patient is stable enough, the third step is implemented: definite surgical treatment which addresses, for example, bowel continuity restoration, and finally, the abdomen is closed.

Ditzel et al. [33] recently suggested that hypocalcemia is also an important predictor of mortality among trauma patients and should be included among the "lethal triad", forming a "lethal diamond". The authors described an intricate mechanism in which hypocalcemia is both a consequence and a promoting factor in the development of coagulopathy and acidosis: as serum calcium decreases, the calcium-dependent clotting factors become unfunctional and coagulopathy occurs; lower levels of serum calcium are linked to metabolic acidosis; hydrogen can competitively block ionized calcium receptors necessary for clotting; hypothermia decreases the liver function and therefore the liver is deemed unable to metabolize the citrate that is derived from transfusions; finally, ionized calcium is necessary for the proper function of cardiac myocytes and the lack of calcium is associated with significantly reduced cardiac contractility and decreased cardiac output, hypoperfusion, ischemia, and lactate production by hypoxic cells. Since the majority of trauma patients are hypocalcemic before receiving blood products and transfusions exacerbate the ionized calcium deficit, the authors concluded that calcium product administration for trauma patients requiring transfusions should be taken under consideration for further studies.

Damage Control Laparotomy

A mixed, retrospective, and prospective study conducted at a level 1 trauma center by Ertel et al. [34] demonstrated a clear association between Damage Control Laparotomy (DCL) and ACS. 311 patients with severe abdominal or pelvic trauma were included in this study and needed DCL for their injuries and 17 of them developed ACS, resulting in a 5.5% incidence, compared to the 14% obtained by Meldrum et al. [30]. Ertel et al. further noted that the association of both pelvic and abdominal trauma was remarkably more often linked to the development of ACS at a higher speed (the first 4-5 hours after trauma) compared to those who only presented abdominal or pelvic trauma [34]. The crucial discovery of this study is the fact that all the patients who developed ACS had had primary closure of the abdomen after the initial exploratory laparotomy. Set site by side, the patients that were treated with mesh closure of the abdomen did not develop ACS. This discovery suggests that primary fascial closure of the abdomen is an important risk factor for ACS in the surgically managed trauma patient population.

2 years later, Gracias et al. [35] analyzed this issue to a greater extent and conducted a retrospective review to discover whether or not the management of the open abdomen with temporary abdominal closure could prevent ACS and if there were any predictors of ACS in the patients treated with an open abdomen. A comparison was made between the 5 patients treated with temporary abdominal closure by vacuum pack who developed ACS and the other 15 patients who underwent the same treatment but did not develop ACS. Although base deficit levels, pH, and lactate levels on surgical ICU admission notably deteriorated in the ACS group, systolic blood pressure, prothrombin time, and temperature were similar between the 2 groups. Regrettably, no statistically significant relation between these potential predictors and the development of ACS could be made. Nevertheless, the mortality rate was remarkably higher in the ACS group (60%) compared to the non-ACS group (7%), further supporting the theory that ACS is an important predictor of mortality.

Shock/hypotension

Hypovolemic shock is most commonly seen in trauma patients as a result of acute hemorrhage, both internal and external. It can also be derived from massive fluid sequestration in the abdominal cavity, bowel wall, and interstitium due to increased hydrostatic pressure and decreased oncotic pressure in plasma, as seen in SIRS. The consequences are diminished effective circulatory volume, decreased cardiac output, and reduced perfusion to vital organs, including the kidneys and brain. The severity of hypovolemic shock varies from mild (less than 20% of total blood is lost) to severe (>40%) and depends not only on the volume of the blood loss but also the rapidity of which it is produced and the age and comorbidities of the patient [22]. Hypovolemic shock requires fluid resuscitation and blood products, but the endpoint of resuscitation is vital for avoiding a catastrophic complication: ACS. One must be aware of the hemodynamic criteria [36]: central venous pressure (CVP) between 8 to 12 mmHg in the first 6 hours of resuscitation, mean arterial pressure (MAP) > 65 mmHg, central venous oxygen saturation ($ScvO_2$ > 70%) and transthoracic echocardiography (TTE) inferior vena cava diameter > 20 mm.

Interestingly, Schreiber et al. [36] proposed, in a prospective randomized pilot trial that permissive hypotension might be beneficial for traumatic injury survivors. Eligible patients (out-of-hospital systolic blood pressure SBP ≤ 90 mmHg) were randomized and assigned into 2 groups: those who received controlled resuscitation, meaning 250 ccs of crystalloid in the absence of radial pulse or SBP < 70 mmHg and additional 250 cc boluses to maintain radial pulse or SBP ≥ 70 mmHg and those who received standard resuscitation, which consisted in 2 liters of fluid initially and additional fluid as needed to maintain SBP ≥ 110 mmHg. The controlled resuscitation strategy ultimately associated a decrease in early crystalloid use by 1 liter and an increase in early blood products use, adhering to the Damage Control Resuscitation (DCR) philosophy of permissive hypotension until hemorrhage control and a therapeutic plasma:platelets: PRBC ratio of 1:1:1. Although there were no statistically significant differences regarding mortality rate at 24 hours between standard and controlled resuscitation groups, improved mortality in blunt trauma patients was detected.

Duchesne et al. [15] also evaluated whether or not permissive hypotension was ultimately helpful for trauma patients who suffered a massive hemorrhage. The potential benefit suggested by former articles was that a slightly reduced arterial pressure could limit the amount of ongoing hemorrhage and therefore buy some time to allow surgical control of the bleeding. The authors raised concerns regarding the appropriateness of this practice in the general trauma population, the absence of a lower systolic or mean arterial pressure safety limit and the disastrous effects of severe traumatic brain injury. Hence, permissive hypotension is not currently recommended by the guidelines and should be further analyzed.

Retroperitoneal hematoma

In 2009, He et al. [37] suggested an increased prevalence of secondary ACS after pelvic fractures, due to an expanding retroperitoneal hematoma or the fluid resuscitation needed to compensate for the blood loss associated with this particularly severe type of fracture. Although the multivariable regression model showed that for each 1L of resuscitation fluid administered over 24hrs there was an expected 5 mmHg elevation of IAP ($p = 0.002$), no statistically significant correlation was found between pelvic fractures and IAP ≥ 12mmHg. Cook et al. [38] also approached the subject in 2002 and consequently demonstrated that the patients who suffered a rotationally or vertically unstable pelvic fracture were at a higher risk of developing resistant hypotension that could only be resolved by angiographic embolization. Agri et al. [39] later indicated that type C pelvic fractures were associated with a higher mortality rate and a greater rate of packed red blood cell (PRBC) transfusion than type B and A fractures. More research is necessary for the future to inform a possible correlation between pelvic fractures and ACS.

Although retroperitoneal hematomas are most frequently encountered after pelvic fractures, there are other possible causes. Beckman et al. [40] mentioned the implication of ruptured abdominal aortic aneurysms in the occurrence of retroperitoneal hematomas. The ongoing hemorrhage expands the hematoma and increases IAH, resulting in the development of primary ACS, hence this is considered a surgical emergency. The surgical approach to a ruptured abdominal aortic aneurysm can be endovascular (EVAR) or open (laparotomy). Dayama et al. [41] analyzed 352 patients who suffered from a ruptured abdominal aortic aneurysm following polytrauma and compared the efficiency of two different treatment options. 91 patients underwent endovascular repair and the remaining 234 patients were treated with an open aortic repair. Albeit the overall mortality rate was remarkably high (up to 51.7% during admission), the authors discovered that endovascular repair was associated with a decreased mortality rate (20.9%, p-value < 0.001) compared to the open abdominal approach (63.7%). An important downside of the endovascular approach is the possibility of post-repair ACS. In a systematic review and meta-analysis, Oliveira-Pinto et al. [42] reported a 9% incidence of ACS after endovascular repair of ruptured abdominal aortic aneurysms, as a result of an important retroperitoneal hemorrhage that could not be properly addressed without open surgery.

Demographics: age, gender, and race

None of the studies taken under consideration demonstrated any statistically significant relationship between demographic factors such as age, gender, or race and the development of IAH/ACS.

CONCLUSION

ACS is a significantly threatening condition for the trauma patient population. To summarize, many potential risk factors were evaluated for the envisagement of the present paper, but the ones that prevailed the most were excessive fluid resuscitation, shock/hypotension, retroperitoneal hematomas, and the “lethal triad”. Consistent with other studies, no connection was found between age, gender, or race and the development of ACS. Further studies should focus more on the likely involvement of damage control laparotomy and patient positioning, as well as hypocalcemia, in the unfolding of ACS in trauma patients.

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REVIEW

Individualized surgical antibiotic prophylaxis – why, when, and how?

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Abstract: *Surgical site infections (SSI) greatly concern clinicians, as they are associated with significant morbidity and mortality, prolonged hospitalization, and costs. Antibiotic prophylaxis plays a pivotal role among the procedures that are usually employed for the prevention of surgical-related infections. This narrative review aims to cover some of the particular situations when the clinician might consider individualizing antibiotic prophylaxis for a patient. With the rising incidence of multi-drug resistant bacteria carriage among not only hospitalized or institutionalized patients but also patients from the community, there might be a tendency to use extended-spectrum antibiotics for longer periods for surgical infection prevention. However, the inappropriate use of antibiotics increases the selection pressure, thus favoring the spreading of resistant bacteria. Moreover, specific patient characteristics or pathologies might need to be considered to customize the type, dose, or length of administration of an antibiotic as surgical prophylaxis. Using prosthetic material or prolonged surgeries with large fluid shifts are other situations when individualized antibiotic prophylaxis might be thought of. Keeping in mind that it is of utmost importance that everyone adheres to the current guidelines for surgical antibiotic prophylaxis, customization of local protocols according to well-thought-out strategies might prove beneficial in SSI prevention.*

Keywords: *surgical site infection; antibiotic prophylaxis; multi-drug resistance; personalized medicine*

INTRODUCTION

Current surgical antibiotic prophylaxis (SAP) is based on guidelines' recommendations that rely on solid evidence-based studies and, where data are lacking, on expert opinion. However, in practice, clinicians might encounter some particular situations that need to be addressed by tailoring antibiotic prophylaxis or choosing alternative strategies to prevent surgical site infections (SSI). At the same time, doctors must remember that a permissive large-spectrum antibiotic use or an unnecessary prolongation of antibiotic prophylaxis can lead to increased antibiotic resistance. Thus, a prudent decision must be made when choosing the right type and dose of antibiotic and the proper duration of SAP.

This literature review highlights the exceptions that may need a personalized approach regarding SAP, particularly the choice of antibiotics for patients colonized with multi-drug resistant bacteria (MDR), the length of antibiotic prophylaxis for specific surgeries, and the choice and dosing of antibiotics for special populations. In addition, other therapeutic options for lowering SSI are addressed.

SURGICAL ANTIBIOTIC PROPHYLAXIS GUIDELINES AND GUIDELINE COMPLIANCE

In France, Germany, and the USA, studies report overall compliance to guideline recommendations of about two-thirds when it comes to antibiotic choice for SAP and timing of administration [1–3], but the adherence rate is much lower in developing countries, varying widely between 18% and 50% [4–8]. Surprisingly, even in Italy, only 18% of cases in a small cohort study received appropriate SAP [9]. Table 1 summarises the doses and redosing intervals for the main antibiotics used in practice, according to guideline recommendations.

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The most commonly reported deviation from the guideline was an unnecessary prolongation of SAP. For example, SAP was prolonged in France for more than 24 hours in 45.2% of surgical interventions [1], but their national guidelines allow 48 hours of antibiotic prophylaxis for certain types of surgery [10]. In Germany, unnecessary prolongation of SAP occurred in 30% of cases [3]. Lack of intra-operative redosing when needed is another deviation frequently encountered, being reported in around 20% of cases in developed countries [1,2].

Table 1: Dosage and redosing intervals for the main antibiotics used in surgical prophylaxis

Antibiotic	Pre-incision dose	First redosing interval	Subsequent redosing intervals
Ampicillin	2 g	+ 2h	every 6h
Ampicillin-Sulbactam	3 g	+ 2h	every 6h
Cefazolin	2 g (3g [1)])	+ 4h	every 8h
Cefuroxime	1.5 g	+ 4h	every 8h
Ceftriaxone	2 g	-	-
Ciprofloxacin	400 mg	+ 8h	-
Gentamicin	5 mg/kg [2)]	-	-
Metronidazole	500 mg	+ 8h	every 8h
Clindamycin	900 mg	+ 6h	every 8h
Piperacillin-Tazobactam	3.375 g	+ 2 h	every 6h
Vancomycin	15 mg/kg	-	-

[1)] Obese patients with weight>120 kg; [2)] adjusted body weight for obese patients

A qualitative study that interviewed surgeons and anesthetists in a tertiary hospital in Australia concluded that improvisation with novel SAP strategies is ubiquitous and confers a sense of reassurance for surgeons. However, these are not following guidelines or have no clinically demonstrated efficacy [11]. Lack of knowledge of guideline content, reliance on old habits and practices, lack of communication between surgeons and anesthetists, fear of medical malpractice linked to SSIs, environmental context and resources, or personal beliefs are some of the factors related to low adherence to guideline recommendations [12].

However, the good news is that concentrated efforts can improve guideline adherence [13–15]. Probably one solution would be to create local guidelines that follow international recommendations and adapt to particular contexts and local specificities.

MULTI-DRUG-RESISTANT BACTERIA ARE A CONCERNING PROBLEM

Globally, over 700,000 people die yearly from drug-resistant infections, and the predictions are that over 10 million people might die annually by 2050 if no preventive measures are taken [16]. Furthermore, a European prevalence study analyzing data from 2015 discovered that infections with MDR are on the rise, causing 6.44 deaths and 170 DALYs per 100000 population, with community-associated MDR infections representing a substantial proportion of the burden [17].

SSI makes no exception when talking about the burden of healthcare systems. SSIs have a pooled cumulative incidence of 5.6/100 surgical procedures in low and mid-dle-income countries (LMICs), strikingly higher when com-pared to the pooled cumulative incidence of 2.6 SSI/100 surgical procedures in the USA and 2.9 in Europe [18]. As the prevention of SSI supposes an antibiotic coverage of possible pathogens, it comes into question if patients with a high risk of being carriers of MDR might need an individualized approach to SAP.

A helpful model of adapting SAP comes from methicil-lin-resistant S. aureus (MRSA) SSIs. The incidence of SSIs caused by MRSA in the USA increased from 16% in 2003 to 21% in 2007, increasing mortality, hospital length of stay, and costs [19]. A meta-analysis confirmed that bundle measures for the prevention of MRSA SSIs in cardiac and orthopedic surgery, including nasal decontamination and glycopeptide antibiotic prophylaxis, decreased with 70% the risk of infection [20]. However, vancomycin is less effective than beta-lactam antibiotics for preventing SSIs caused by methicillin-susceptible S. aureus (MSSA) [20,21]. Thus, guidelines have rapidly adapted and recommend vancomycin use, in addition to routine prophylaxis, in patients who are colonized with MRSA or are at high risk of colonization [10,21,22].

Although data on colonization with antibiotic-resistant bacteria are scarce when talking about Gram-negative bacteria [23], more than 1 in 10 persons might be gut carriers of MDR in some countries [24]. Moreover, it is known that LMICs present a high incidence of infections with MDR [16]. Crowding, homelessness, low income, sanitation, and water sources are some factors associated with antimicrobial resistance in LMICs [25]. Comorbidities (diabetes, cancer, inflammatory diseases), extremes of age, recent hospitalization or antibiotic treatment, residency in a nursing home, and occupational risks, such as health workers or farmers, or veterinarians who frequently use antibiotics, or travel to LMICs are other factors associated with the acquisition of MDR infections [26]. An interesting study examined the dynamics of gut bacteria colonization of 20 European volunteer visitors to Laos. They registered a 70% colonization rate after 22 days of surveillance, but daily sampling revealed that all participants had acquired an extend-ed-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) at some point [27].

Infections following trans-rectal prostate biopsy (TRPB) are an actual problem for urologists. Guidelines generally recommend the utilization of fluoroquinolones as SAP for TRPB [10,21,22], but there is a rising concern that these antibiotics might be ineffective for the novel antimicrobial-resistance patterns [28]. The prevalence of resistant strains of E. coli for patients undergoing PB varies between 13 and 25% for stains resistant to fluoroquinolones, and 8% and 13% for strains resistant to cephalosporins and, more concerning, with ESBL carriage on the rise [29–33]. Furthermore, ESBL carriers have a higher risk of infections post-TRPB [34]. Thus, introducing rectal swabs that could detect potential carriers and tailored antibiotic prophylaxis before TRPB might be a cost-efficient measure that might lower infectious complication rates after TRPB. Data from a meta-analysis [35] and other studies support this strategy [36]. Moreover, studies identified some risk factors associated with re-sistant-stains colonization, such as the history of the use of fluoroquinolones, previous hospitalization [37], travel his-tory [29], obesity or diabetes mellitus [34,38]; patients could be selected based on questionnaire to be further investigated in a cost-efficient manner for colonization.

In a cohort of patients with cancer undergoing gastrointestinal and gynecologic surgery, 17.5% were colonized with ESBL-E at admission. The risk of developing an SSI was twice higher in ESBL carriers versus non-carriers (RR, 2.16; 95% CI, 1.2-3.89)[39]. Similarly, in a prospective study including 3700 patients undergoing colorectal surgery from 3 hospitals in Israel, Switzerland, and Serbia, ESBL-E carriage more than doubled the risk of SSI (OR, 2.36; 95% CI, 1.50–3.71). Interestingly, the prevalence of ESBL-E carriers differed significantly between countries: 9.4% in Serbia, 12.0% in Switzerland, and 28.8% in Israel [40].

Among 360 patients who underwent abdominal surgery in a university hospital in Thailand, 36% had preoperative enteric colonization with an ESBL-E, and 14.1% developed an SSI. Although colonization with ESBL-E represented an independent risk factor for SSI (aOR 2.4; 95% CI, 1.19–19.91), the study found no association between carbapenem surgical prophylaxis and reduction in SSIs. However, it must be kept in mind that the study was single-centered, included a small study group, the incidence of SSI reported was relatively high, the majority of SSIs were superficial, and the authors did not explain the choice of antibiotic prophylaxis [41].

The number needed to treat (NNT) is an important concept when analyzing the impact of SAP. A higher risk of SSIs would lead to a lower NNT regarding SAP, and thus a more significant impact on its incidence of SAP. Nutman et al. analyzed the effect of changing the standard cephalosporin-in-metronidazole prophylaxis for colorectal surgeries to individualized prophylaxis with ertapenem for ESBL-E carriers. SSI rates were 22.7% for non-carriers versus 15.8% for ESBL-E carriers (ARD, –7.7%, 95% CI, –14.6% to –0.8%), with a number needed to treat to prevent one SSI in ESBL-E carriers of 13 [42]. Starting from this study and considering the prevalence of MDR, Temkin et al. analyzed different clinical scenarios for SAP. Thus, if a broad-spectrum SAP were used only for MDR carriers, for a prevalence of 5% carriers, 260 patients would need to be screened to avoid one SSI. If the prevalence of 50% carriers, this number would drop to 26. However, if a broad-spectrum SAP were used for all patients, no matter if they are carriers, there would be 247 patients who would receive unnecessary broad-spectrum antibiotics for a real prevalence of 5% MDR carriers. In comparison, the number would drop to 13 patients if the real prevalence of MDR carriers is 50% [43].

Hence, there is a great need for MDR prevalence studies. These would offer the possibility to adapt local guidelines of SAP while lowering MDR-SSI and improving, at the same time, the costs and antibiotic stewardship programs.

Some centers from high-income countries have already adopted a personalized management pathway for patients undergoing elective interventions in visceral surgery [44]. The algorithm encompasses screening in patients at risk, very strict basic hygiene, targeted isolation who are MDR carriers or at risk of being carriers, and targeted surgical antibiotic prophylaxis [44]. This section may be divided into subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

LESS IS MORE. ARE THERE EXCEPTIONS?

There is a bundle of risk factors associated with SSI (figure 1). The consensus recommendation for antibiotic prophylaxis is to administer a single dose for the majority of surgeries with clean or clean-contaminated surgical wounds. The exceptions that can receive a 24-hours postoperative antibiotic regimen include cardiac surgeries, head and neck surgery that involve the oral cavity, or some procedures that involve prosthetic materials (joint replacement, internal fixation of fractures, breast augmentation) [10,21,22]. The French guidelines are more permissive and accept a 48-hour postoperative antibiotic regimen for limb amputations, grade II open fractures, or craniocerebral surgical wounds [10].

During cardiopulmonary bypass (CPB), needed in most cardiac surgeries, an important increase in circulating volume due to pump priming can lead to a decreased concentration of the prophylactic antibiotic. A pharmacokinetic model study that included pediatric patients analyzed the serum levels of cefazolin before, during, and after cardiopulmonary bypass. Considering the higher ratio of priming volume needed for children during CPB, an additional bolus dose at the start of CPB improved the probability of target attainment by more than 90% [45]. When comparing bolus infusion versus continuous infusion of cefuroxime for the first 24 hours following CPB, plasma concentrations of cefuroxime were higher in patients receiving the continuous infusion [46]. An undergoing clinical trial that compares short-course and long-course SAP with either cefazolin monotherapy or combination therapy with cefazolin and vancomycin might shed some light on which strategy is best for CPB [47].

Regarding intraoperative antibiotic redosing, there is limited clinical evidence, but there are expert opinions that sustained a more aggressive redosing based on pharmacokinetic data. In a recent meta-analysis, Wolfhagen et al. ad-dress this question and the clinical utility of intraoperative re-dosing; the findings attest to the beneficial results of redosing, but the certainty of the evidence was low [48]. The question of redosing will persist in literature until some results of well-powered RCTs will arise.

Figure 1: Risk factors associated with surgical site infections

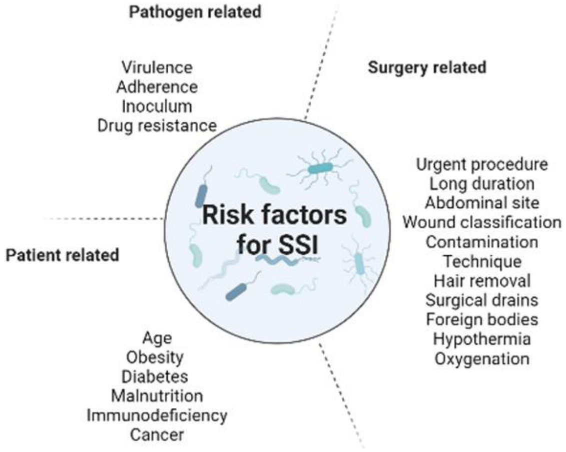

Orthopedic surgery is another field of interest when talking about SAP. A retrospective cohort study analyzed standard SAP versus a one-week course of antibiotic prophylaxis after total joint arthroplasties. Infection rates dropped from 2.1% to 0.4% for total knee arthroplasties and from 4.3% to 1.1% for total hip arthroplasties, respectively, for patients that received seven days of antibiotics [49].

Another similar study proposed the use of extended postoperative oral antibiotic prophylaxis for seven days for patients with a high risk of infection after total joint arthroplasties – are diabetic, active smokers, obese, or have other conditions associated with risk for infections. High-risk patients that received extended antibiotic prophylaxis had similar infection rates to low-risk patients, with a number of needed to treat 57 patients to prevent one prosthetic infection [50].

Although using an extended course of antibiotic prophylaxis may decrease infection rates, further studies need to weigh the risk of increasing antimicrobial resistance, the costs, and the risk of developing Clostridium difficile infections before recommending the routine use of extended antibiotic strategy use.

PATIENT PARTICULARITIES

1. Allergic patients

Allergy to β-lactam antibiotics is one of the most commonly cited adverse drug reactions, with a reported prevalence of around 10% in western countries [51,52]. But among inpatients with an alleged penicillin allergy, only 5% had a positive penicillin skin test [53]. Moreover, cross-reactivity rates of allergic reactions between penicillins and 1st generation cephalosporins are 1% and even smaller when using 3rd generation cephalosporins [54]. Still, when it comes to SAP choice, many clinicians opt for using alternative antibiotics, such as clindamycin, vancomycin, or gentamicin, when a patient claims to have an allergic reaction to penicillin [55].

There is a four-fold increased risk of SSI (95% CI 1.37-10.47) when clindamycin was used instead of the standard cephalosporin SAP for patients with declared allergy to penicillin that underwent free tissue transfer in head and neck surgery [56]. Furthermore, a meta-analysis that investigated SSIs after cardiac surgery discovered that β-lactam is superior to glycopeptides for the prevention of chest SSI (RR, 1.47; 95% CI 1.11–1.95). Blumenthal et al. also concluded that second-line antibiotic use raises the odds of SSI by 50% [55]. Besides SSI, alternative regimens used for patients labeled allergic to penicillin may lead to increased antibiotic resistance, hospital length of stay, and higher costs [57].

Clinicians should develop strategies to rule out spurious β-lactam allergies. A first step may include a thorough his-tory investigation or a standardized questionnaire that could rule out falsely labeled patients and select high-risk patients for further investigation. Further, selected patients may follow a classical allergy diagnosis (skin prick or patch test or a specific IgE test) or be subjected to an intradermic test done by properly instructed personnel [57,58]. Thus, labeling patients with reported penicillin allergies could lower SSIs and improve antibiotic stewardship.

2. Obese patients

Obese patients may present a challenge when it comes to adequate antibiotic dosing. Guidelines consider that serum and tissue concentrations of certain drugs vary in obese and non-obese populations. It is recommended that the dosing of vancomycin and aminoglycosides be based on total body weight and adjusted body weight, while the dose of cefazolin be increased from 2 g to 3 g for patients over 120 kg [21,22], or even to 4 grams for bariatric patients with a BMI over 35kg/m2 [10]. Although this is backed up by pharmacokinetic studies [59], some opinions do not support the increased dose of cefazolin in obese patients [60].

Microdialysis studies are contradictory. Palma et al. concluded that 2 g of cefazolin, reduced at 4 hours intervals, is enough and effective to prevent infections caused by bacteria with a MIC up to 2 mg/l, even in obese patients [61]. On the contrary, Brill et al. support higher dosages of cefazolin in obese patients, arguing that distribution to the subcutaneous adipose tissue is reduced and the probability of target attainment is decreased for MIC of 2mg/l or higher [62].

Another study that included 12 obese pregnant women undergoing cesarean section suggested redosing cefazolin after 2 hours, following either a 2 or 3 g initial bolus [63]. Finally, a swine model that targeted a plasma and tissue concentration of 4 mg/l proposes a weight-based cefuroxime dosage of 20 mg/kg [64].

Until future pharmacokinetic studies and controlled trials bring hard evidence regarding the right dose of antibiotic for obese patients in SAP, it may be more prudent to follow guideline recommendations, even though they are based on expert opinion.

3. Organ recipients

In solid organ transplantation, numerous factors contribute to increased rates of infections: prolonged pre-transplant hospitalization, exposure to antibiotic treatment, immuno-suppressive therapies, donor-derived infections, or the rising number of MDR portages [65].

One strategy for lowering SSIs associated with MDR is screening recipients and using an extended-spectrum antibiotic as prophylaxis for MDR carriers.

For example, in one study, out of 61 patients screened for VRE colonization before liver transplant, 27 (44%) were carriers. All carriers received daptomycin in addition to GNB antibiotic as SAP, and only 3 developed postoperative VRE infections [66]. In another cohort of patients who underwent liver transplantation, 13.3% were ESBL-E carriers. Known carriers received a carbapenem as SAP. In ESBL-E carriers the risk factors associated with ESBL-E infection were colonization with K. pneumoniae, long-term spontaneous bacterial peritonitis prophylaxis with quinolones, antimicrobial treatment >3 days within the month before LT, and MELD ≥25. In the early postoperative period, ESBL-E infections were significantly lower in patients who received tailored SAP versus those who received standard SAP (29.8% vs 63.6%, p=0.04) [67]. Similarly, ESBL-E stool carriage before liver transplant in a pediatric population was associated with the emergence of ESBL-E-caused infections (OR = 4.5, 95% CI 1.4–17.4), although ESBL-

E carriers received imipenem as SAP, while non-carriers receives cefoxitin or piperacillin-tazobactam [68].

Another strategy is to broaden the spectrum of antibiotic prophylaxis for all solid-organ transplantation. A single dose of ertapenem prophylaxis instead of cefazolin reduced by a third of early infections caused by ESBL-E in kidney transplant recipients in a Spanish university hospital. Moreover, ESBL-E infection was associated with cefazolin prophylaxis and acute rejection, while ertapenem did not seem to increase the incidence of Clostridium difficile or other MDR infections [69].

Donor respiratory cultures do not influence post-transplant infections in non-lung recipients [70]. Still, prophylactic antibiotics must provide coverage for pathogens isolated from the donor lung or the recipient before lung transplantation [22,71].

Spanish Society of Transplantation recommends for patients colonized with ESBL-E a large-spectrum antibiotic prophylaxis regimen before solid-organ transplantation, avoiding, as possible, carbapenem use [71]. Infectious Disease Socie-ty of America also mentions that prophylactic antibiotics may need to be altered according to the recipient's pathogen isolation before liver transplant [22].

In conclusion, data support as a strategy for solid-organ receivers pre-transplant surveillance of MDR carriage and tailored SAP. Another strategy might be the use of broad-spectrum antibiotic prophylaxis for all solid-organ recipients, but this might favor the development of resistance.

4. Extremes of age

Particularities in the pharmacokinetics of antibiotics emerge not only in neonates but also in elderlies. Membrane permeability, plasma protein binding, and total body water, which vary significantly at the extremes of age compared to healthy adults, determine the final plasma concentration and tissue concentration of antibiotics. Moreover, the metabolism and elimination of antibiotics are also different from adults, as neonates have immature metabolic pathways, and the elderly might suffer from liver or renal failure. Furthermore, the elderly are at risk of adverse effects due to polypharmacy and potential drug interactions [72,73].

Although therapeutic drug monitoring might be the correct way of dosing the antibiotics in these two age groups, it is too sophisticated and expensive to be applied in the real world just for surgical antibiotic prophylaxis.

Thus, a correct antibiotic dose calculation for both groups based on guide-line recommendations is essential.

COMPLEMENTARY MEASURES FOR PREVENTION OF SSI

In addition to classical antibiotic prophylaxis, novel complementary measures rise to prevent surgical site infection, including local or topical measures and systemic decontamination.

For example, a local prolonged release of antibiotic containing a matrix of polymers and lipids doxycycline was tested for the prevention of sternal wound infections after cardiac surgery [73]. The prospective, single-blind randomized controlled multicentre study, counting 81 patients, proved that this novel compound, D-PLEX, was safe for cardiac surgery patients. There were zero sternal wound infections in D-PLEX treated patients, but larger scale studies are needed to prove its efficiency versus standard care [74].

Another study that tested dual-topic antibiotic application before sternotomy wound closure showed encouraging results [75]. The observational study analyzed over 2500 patients after cardiac surgery, revealing that dual antibiotic topical prophylaxis had the lowest rate (1.1%) of sternal wound infection versus the group treated with topical vancomycin (2.2%) and the control group (4.5%).

Furthermore, there was a higher rate of occurrence of gram-negative bacteria in the control group (3.4%) versus the vancomycin (1.2%) and the dual antibiotic group (0.6%) [75].

Although digestive tract decontamination was greatly debated, its application might prove beneficial in SSI prevention [76]. A retrospective study from the Netherlands evaluated the strategy of selective oral digestive tract decontamination with the use of an oral solution with amphotericin B 2%, colistin 2% and tobramycin 2% in patients with oesophageal carcinoma who underwent minimally invasive esophagectomy. The study's conclusions revealed a lower rate of fistulas and postoperative pneumonia (36.9% vs. 20.1%) in the group of patients with selective digestive decontamination versus the control group, but without an impact on mortality rates [77]. A similar regimen for digestive tract decontamination was used in a RCT that included patients with colorectal cancer scheduled for elective surgery with a primary anastomosis. Compared to the control group, the patients randomized to the oral decontamination group had similar anastomotic leakage rates but significantly lower rates of infectious complications [78].

Faecal microbiota transplantation arises as a novel method of SSI prevention. It is a proven way of gastrointestinal decolonization of multidrug-resistant bacteria, and it is beneficial for the treatment of refractory Clostridium difficile infection, with success rates close to 90% [79]. A review of the literature showed that more than half of the patients achieve digestive decontamination for multidrug-resistant bacteria when treated with faecal microbiota transplantation [80].

Bacteriophage use might be another novel therapy to help prevent SSIs. However, there is only limited evidence from small case studies and experimental trials. The bacteriophages are virus-like structures with bacterial cell lysis activity. A series of patients from Finland with multi-drug-resistant bacterial infection after cardiothoracic surgery received conventional targeted antibiotics and bacteriophages, successfully treating infections in 87% of patients. The literature cites applications of bacteriophages in the surgical setting with topical application of phage solutions to a surgical wound before closure [81-83].

CONCLUSION

All things considered, surgical antibiotic prophylaxis remains a vital tool in the fight against SSI. Following the general rules of good practice, antibiotic prophylaxis could be individualized when clinicians face MDR carriers, allergic, obese, immunosuppressed or elderly patients or when the surgical intervention calls for prosthetic material insertion, large fluid shifts or prolonged duration. There is, of course, a need to develop evidence-based local SAP policies in accordance with the local prevalence of MDR and the incidence of SSIs.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Retinal migraine, a contentious clinical entity

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Abstract: *Retinal migraine (ophthalmic migraine, ocular migraine, anterior visual pathway migraine) is a rarely encountered medical condition, but with an interesting and complex clinical presentation. The goal of this article is to compile the literature on retinal migraine, detailing nomenclature, pathophysiology, differential diagnosis, and medical investigations including newer imaging modalities, like optical coherence tomography (OCT). We will approach topics of great concern such as risk factors, complications, treatment, and management of this condition. Retinal migraine is mainly a diagnosis of exclusion and it must be isolated from transient monocular vision loss (TMVL) developed from other causes, for instance, papilledema, giant cell arteritis, carotid artery disease (amaurosis fugax), intermittent angle closure glaucoma, and others. Its treatment is similar to migraine treatment; topiramate, amitriptyline, and nortriptyline are some of the pharmacological drugs successfully used.*

Keywords: *ocular migraine, arterial vasospasm, retinal migraine, monocular vision loss, visual phenomena, ophthalmic migraine, anterior visual pathway migraine, scotoma, scintillation, headache*

INTRODUCTION

Retinal migraine is a debatable disorder, that was firstly introduced by Galezowski in 1882 who used the term ‘‘ophthalmic megrim’’ to describe a permanent unilateral loss of vision associated with headache [1]. The official term, “retinal migraine” was introduced in 1970 by Carroll [2] and the authors have used it to describe a multitude of monocular visual symptoms, including events without the associated headache and those resulting in persistent visual loss. The evolution of terminology accepts retinal migraine, ophthalmic migraine, ocular migraine, and anterior visual pathway migraine, as the same notion, but all of these should not be confused with ophthalmoplegic migraine, which is manifest by ophthalmoplegia and unilateral headache [3].

The International Classification of Headache Disorders (ICHD) published 1988 an international definition for this condition [4] and following the 2018 ICHD-3 updates, ocular migraine is defined as fully reversible and repeated attacks of unilateral visual manifestations, such as scotoma, scintillation, or even blindness, associated with headache [5].

Furthermore, because it is not a common entity and there are many other potential causes of monocular visual loss, retinal migraine is considered a diagnosis of exclusion [6] and a careful evaluation is needed because it can share characteristics with migraine with aura and acephalgic migraine, which can create confusion in their differentiation [7]. In some articles was also suggested the term “migraine associated with monocular visual symptoms” was to differentiate it between the loss of vision in one hemifield and that of one eye which includes sites other than the

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retina, such as the choroid or the optic nerve [8].

RETINAL MIGRAINE-WERE WE ARE?

Epidemiology

The lack of reported cases, the paucity of existing literature, and the continuously evolving diagnostic criteria made the understanding of this condition's epidemiology challenging, moreover, some authors suggested that there's no epidemiologic data specific to ocular migraine [7]. While migraine is a common disorder, with a prevalence of 18.2% in female patients and 6.5% in male patients, with a higher prevalence in whites (20%) than blacks (16%) and Asian Americans (9%) [9], there were indicated only 12 cases of retinal migraine, that followed the ICHD diagnosis criteria in the last 15 years [6], between 2006 and 2020. Of 12 patients, 10 were women with an average age of 39.5 years, 11 out of 12 had a previous history of migraine headaches, 4 patients reported a history of migraine with aura and a positive family history of migraine was present in 4 of the 8 patients for which that information is available [6].

Even though based on the statistics, retinal migraine tends to affect women more so than men [6] and begins in the second to third decades, with peak prevalence in the fourth and fifth decades [10], it can also affect children, in which 7% of migraines are thought to be ocular [11] and the onset of ocular migraine may occur as late as the fifth decade, including patients with no previous history of migraine [12]. It is crucial for this last age group (>50 years of age) to consider retinal migraine a diagnosis of exclusion and make a careful cardiac and carotid evaluation, because atherosclerosis, collagen vascular disease, and hypercoagulable states are just a few examples of diseases that can explain the visual disturbances on elderly patients. A genetic predisposition is suggested by the strong family history of ocular migraine, but no clear inheritance patterns have been delineated, however, 50% of patients have a family history of some type of migraine [13,14]. In patients with familial hemiplegic migraine have been found calcium channel mutations such as 19p13 CACNA1A, ATP1A2, SCN1A, mutation appeared in siblings with migraine with aura at a statistically significant occurrence [15]. Moreover, some patients, during and between migraine episodes have been shown high levels of endothelin-1, a potent vasoconstrictor peptide, which in these cases, had significant polymorphism for a receptor found to mediate vasoconstriction [16]. However, its effect on ocular migraine is considered to be minimal because the blood-brain barrier prevents endothelin entry into the retinal circulation [7]. Finally, it is considered that retinal migraine is a less benign disorder than migraine with aura due to the higher risk for patients with transient monocular visual loss to develop permanent monocular visual loss [8].

Pathophysiology

Despite that multiple theories have been proposed, the pathophysiology of ocular migraine remains controversial because this complex disorder which associates headache and monocular visual disturbances is not fully understood. In first, the cephalgia that also appears in common migraine and in migraine with aura, is considered as trigeminal nerve activation by an unknown trigger [17]. Even if, in migraine patients, the trigeminal nerve seems to produce higher levels of glutamate onto the circle of Willis, the primary event remains unclear [18]. It is also suggested that the potential cause of inflammation and pain may be represented by a series of substances and peptides, such as bradykinin, substance P, calcitonin gene-related peptide (CGRP), and nitric oxide [19]. Secondly, to explain the monocular visual loss, we will mention the vasospastic theory that was firstly introduced by Carroll in 1970 when he exposed the hypothesis that the mechanism of visual symptoms is due to "paroxysmal constriction or spasm in either the central retinal artery or ophthalmic artery" [2]. It seems that serotonin and stimuli such as food may induce the production of prostaglandin, causing disturbance of cerebral autoregulation and platelet aggregation [20]. Once axons are excited, neurogenic and vascular inflammation (especially the inflammation of the retinal and ophthalmic vessels) may cause vasospasm, obstruction, and ocular sympathetic dysfunction which can explain the visual symptoms [7]. Serotonin may have a major role in ocular migraine because it has been measured that it is produced by platelets in increased levels in aura phases of migraine, mediating intracranial vasoconstriction [21], and, furthermore, in patients with atherosclerosis, it reduces blood flow to the choroid and retina while the intracranial blood flow is not significantly affected [22]. However, other authors consider that the vascular theory is nowadays largely rejected by new cerebral blood flow imaging techniques [23] and it is indicated a series of potential mechanisms including cortical spreading depolarization, brainstem and hypothalamic activation, alteration in thalamo-cortical circuits or neuropeptide production (pituitary adenylate cyclase activating polypeptide and calcitonin gene-related peptide) [24]. None of these new hypotheses is confirmed. The last theory, cortical spreading depression (CSD) of retinal neurons, is indicated by several articles as a plausible explanation, but it hasn't been demonstrated in vivo or in any animal with retinal vasculature. The phenomenon can be exposed as an "excitation wave followed by depression of neuronal activity propagating through gray matter with a velocity of approximately 3 mm/min" [25], as it is described by Leao in 1944. Based on magneto-encephalographic studies, CSD is suggested to be the biological explanation for the occipital aura that affects both eyes and precedes headache in migraine with aura patients [26]. Retinal SD has only been studied on chick or frog retina, and never been demonstrated in mammals, furthermore, the isolated retina in animal experiments is not representative of the human eye in vivo, which is vascularized and supported by the vascular choroid [27].

Clinical presentation

According to literature, where has been reported a limited number of cases of retinal migraine that followed ICHD diagnosis criteria, the patients, which are usually females, in the second to third decade of life, with family history of migraine and previous history of migraine with aura, present to the specialist with unilateral visual loss that can last even few seconds or between 5 to 30 minutes, and can be preceded, accompanied or followed by ipsilateral headache [6]. The visual disturbances include positive or negative phenomena that are fully reversible, but a majority of patients (>70%) has repeated attacks [13]. There have been described positive phenomena such as zigzag lightning, diagonal lines, halos, and scintillation [7], while the negative ones are described by the patients as black dots or spots, blurring, blank areas, and complex patterns are less common [8]. It is also considered that benign episodic pupillary dilation, an under-reported ophthalmologic sign, might be connected with ocular migraine [7]. All these monocular visual symptoms can be caused by any process that affects the optic nerve or the ocular blood flow and therefore a normal neuro-ophthalmic exam is crucial to exclude any other causes of transient monocular visual loss [28]. The majority of the patients has a good prognosis with a full recovery and permanent visual loss is uncommon.

Differential diagnosis

The diagnosis of retinal migraine is by exclusion [29]. The patients accusing transient monocular vision loss (TMVL), who fits the criteria for retinal migraine according to ICHD-3 must undergo a series of further investigations in order to exclude alternative diagnoses such as: vascular disease, ocular disease or optic nerve disorders. ICHD-3 criteria require positive and/or negative visual phenomena. These are monocular, fully reversible and are confirmed by clinical visual field examination and the patient's drawing of a monocular field defect. Additionally, it should meet two out of three criteria of the following: spreading gradually over 5 minutes or more; symptoms lasting between 5 and 60 minutes; headache present simultaneously, or following the sympyoms within 60 minutes. Vascular diseases that mimic TMVL are carotid artery disease, central retinal artery occlusion, giant cell arteritis (affects large and medium sized arteries), choroidal ischemia (originating from posterior ciliary arteries or arteritis processes affecting the short posterior ciliary arteries). There are some conditions affecting the optic nerve that must be taken into consideration, for example, Uhtoff's phenomenon (can be induced by exercise) from demyelinating disease, papilledema, and compression of the optic nerve [30]. Amaurosis fugax appears as a result of the stenosis or occlusion of the internal carotid artery (caused by hypoperfusion from the stenosis or thromboembolism from the carotid circulation) [31]. Interrupted circulation to the ophthalmic artery or central retinal artery can manifest through a blackout in the entire or only half of the visual field. Patients can accuse a "curtain sweeping in from one side of the vision" and it usually takes between a few minutes and an hour [30]. Amaurosis lasting seconds is commonly found in disc edema (papiloedema), whereas painless amaurosis lasting less than 15 minutes occurs typically from an embolic source (cholesterol, platelets, calcium). Furthermore, dry eyes and intermittent angle closure glaucoma needs to be excluded since they are causes of TMVL as well [30]. To differentiate retinal migraine from other types of visual loss it is important to establish if the issue affects one or both eyes and to define whether the visual loss is monocular or in one hemifield. If the problem is binocular, it is likely a retrochiasmatic injury. In contrast, if the loss of vision concerns one eye it can be as a result of prechiasmatic lesions. It can be problematic for patients to recognize hemianopias from monocular vision loss, therefore the physician needs to guide them in this regard.

Investigations

The evaluation of a patient with acute monocular vision loss begins with a thoroughly medical history. Data including patient's age, history of vascular risk factors, cancer, autoimmune diseases is essential in establishing a proper differential diagnosis. Crucial information regarding the episode of vision loss is frequency, characteristics and duration [30]. It is important to check if the patient has a positive medical history for other types of migraine and if exercises precipitate the attacks [32]. Patients must complete formal visual field assessments in search of visual field defects [6] including negative symptoms, for instance, altitudinal vision loss, central scotoma and sectorial loss [30]. Other visual manifestations that may appear are positive symptoms like scintillations, flashes of color, light, stars, and heat waves [7]. A detailed retinal examination following pharmacologic pupillary dilation should be done, because information regarding the appearance of the retina and optic disc are fundamental to make an accurate diagnosis [33]. Retinal arterioles can be constricted and the fovea may become more distinct with surrounding macular retinal pallor. The optic disc may be pale early and hyperaemic later. Nerve fibre bundle defect can sometimes be a late finding [34]. Newer imaging modalities, such as optical coherence tomography (OCT), a non-invasive imaging test that uses light waves to take cross-section pictures of your retina may be helpful [30]. Ophthalmological examination is expected to be normal outside of attacks. Besides echocardiogram and auscultation of carotid arteries and heart, carotid Doppler is required to exclude vasculo-oclusive diseases [30], a Holter monitor if an arrhythmia is suspected [29] and transesophageal echocardiography to check all the valves and chambers in more detail [7]. Laboratory evaluation is required for diagnostic possibilities such as giant cell arteritis, collagen vascular disease, hypercoagulable states, vasculitis, and illicit drug use. Neuroimaging can exclude an orbital or intracranial mass [6].

Complications

Although it is uncommon [7], some of the complications of retinal migraine can lead to permanent loss of vision [35]. Complications reported in the literature include: “reversible and irreversible central retinal artery occlusion (CRAO), branch retinal artery occlusion (BRAO), retinal infarction” [36], retinal hemorrhages, vitreous hemorrhage, ischemia of choroid or optic nerve [35] dilatation of retinal veins and retinal pigmentary changes [3]. Optic nerve drusen are refractive, calcified nodules located within the optic disc [37]. They make the retinal vasculature more vulnerable to ischemia and lead to irreversible BRAO [38].

Risk factors

Similar to migraine, risk factors for retinal migraine are represented by stress, smoking, hypertension, and contraceptive pills. It can also be precipitated by exercise, bending over, high altitude, dehydration, hypoglycemia and excessive heat load, although dietary factors (caffeine, alcohol, tyramine, monosodium glutamate, and aspartame), traumas and hypoglycemic factors are not as influential [39]. Vascular instability plays a significant role in the pathogenesis of ocular migraine. This stands out through the co-occurrence of retinal migraine and vascular diseases such as Prinzmetal’s angina and Reynaud’s phenomenon. Moreover, it is documented that the comorbidity with lupus, sickle cell disease and atheromatous disease increases the risk of developing retinal migraine [35]. The risk for stoke in patients with atherosclerosis is 1% at 1 year; whereas patients with stenosis of internal carotid artery presents a risk of 16% of ipsilateral stroke after 3 years [40].

Prophylactic treatment

In the first place, it is essential to keep under control stress factors, hypertension and to reduce, or even quit smoking. Patients should avoid triggers like hormonal contraceptive pills or exercises (if they induce amaurosis). It helps if the patients keep a diary in order to keep track of all the factors who lead to an attack, in this way it is easier to avoid them. Pharmacological treatment is required if the life style changes are not effective. Prophylactic therapy consists in calcium channel blockers (nifedipine, verapamil), nonsteroidal anti-inflammatory drugs (aspirin, dipyridamole and sulfinpyrazone if aspirin intolerant), anticoagulants (coumadin, heparin), antiepileptics (divalproex sodium/ sodium valproate, gabapentin, topiramate), antidepressants (tricyclics- amitriptyline, nortriptyline, imipramine; selective serotonin reuptake inhibitors) [7,35]. Although nifedipine, and especially verapamil are the most effective drugs in this case, there are some contraindications to calcium channel blockers including congestive heart failure, hypotension, sick sinus syndrome, cardiac conductive defects, concomitant, and renal or hepatic failure [35]. Aspirin is used for its antiplatelet activity [8] and, along with nifedipine, it is used to prevent exercise induced retinal migraine [12]. Topiramate, amitriptyline, and nortriptyline are some agents used in classical migraine that have been demonstrated effective in retinal migraine as well [6]. It is usually recommended to avoid beta blockers in ocular migraine due to its properties of arteriolar constriction [31].

Abortive treatment

It is important to avoid medication overuse by matching treatment to patient needs. Given the unsufficient number of studied cases in literature, treatment options for ophthalmic migraine are limited. According to some authors, it is enough to manage the manifestations of retinal migraine by observations alone [41,42]. Due to the short duration of the attack, abortive treatment is uncommon and the main focus of treatment should be to reduce the recurrence of attacks [35]. Regarding management, triptans and calcium channel blockers can be used as abortive pharmacological treatment. Triptans are more effective on migraine without aura, but may be successful for patients in whom other therapies do not work and who have no other risk factors for ischemic heart disease or stroke [7].

Calcium channel blockers were demonstrated effective in both prophylactic and abortive situations [43]. There have been reported two cases in literature that advocated the use of sublingual nifedipine (10 mg) for treating attacks (both patients diagnosed with systemic lupus erythematosus) [44]. During attacks, inhaled amyl nitrate and isoproterenol showed “good efficacy”, while inhaled carbon dioxide improved vision ‘slightly’ in one patient [45]. Successful treatment has been reported for coumadin and heparin used in isolated cases of patients with antiphospholipid antibody syndrome and retinal migraine [35,46]. Other medications such acetazolamide (500 mg) and sublingual nitroglycerine (0.8 mg) were effective [47]. Systemic corticoid therapy was successfully used in patients with systemic lupus erythematosus [48].

CONCLUSIONS

Retinal migraine is a rare, but increasingly recognized clinical condition. The pathophysiological mechanisms are still unclear. It is a cause of TMVL and uncommon, during prolonged periods of hypoxia, it can lead to permanent loss of vision. Retinal infarction is more susceptible in patients with anomalous optic discs or intrapapillary drusen may render the patient more susceptible to retinal infarction Researches show that retinal migraine may successfully respond to usual migraine prophylactic medications, but a current guideline is missing. It is also important to attempt to change the lifestyle factors that precipitate this condition, before initiating medical treatment. Its prognosis is a good one overall. Unfortunately, there is not enough research in this distinct clinical entity, hence, it is difficult to determine an optimal treatment regimen.

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ORIGINAL ARTICLE

Climbing – between athletic performance and psychological performance

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Abstract: *The aim of this research is to investigate different aspects of the functioning of the psychological system of performance climbers in Romania. We look for correlations between the concepts of competitive anxiety, self-esteem, motivation, willingness to take sports risks and age, sport experience and competitive experience, performance level. We also try to find out if the first contacts with the climbing world resulted in a favouring of the environment in which the athlete chooses his/her performance goals. Specifically, we aim to divide climbers into two categories, those with a passion for panel climbing (indoor) and those with a passion for rock climbing (outdoor). We did not distinguish between the bouldering challenge and the difficult challenge. In addition, we want to analyse the level of competitive anxiety of athletes in national climbing in order to objectify again the idea that the coach and sport psychologist must be able to identify, in the crucial moments before and during the competition, which athletes need motivation, which ones are anxious, which ones feel good and which ones can be left alone. We can classify athletes who need relaxation, activation or mindfulness sessions.*

Objectives: • Identifying the performance level of Romanian climbing athletes at the moment; • Highlighting the motivational dimensions of performance climbers in relation to the practice of the sport; • Investigating the passion for indoor versus outdoor climbing, with implications on the level of intrinsic and extrinsic motivation and self-esteem; • Knowing the level of competitive anxiety, with a focus on the fine delineation between being debilitating or facilitating for athletes.

Methodology: The research method used was the application of instruments (psychological tests and an opinion questionnaire). The test was administered between January-March 2020, online via google docs, on 28 performance climbers, aged between 13 and 48 years, female and male, juniors and seniors.

Results: The climber's personality shines through on the rock (outdoors), where the desirability, and if it exists, is not a social one, but is a genuine desire to push your own limits, to face difficult challenges, to feel danger, to develop your wits and to know where to stop. These concepts correspond entirely to intrinsic motivation and a more mature personality, both in social and sporting terms, from this maturity a fundamental dimension of which is the match between real possibilities and aspirations.

Conclusion: Rock climbing involves bringing out the authentic self when you are with yourself, compared to a sports competition held at the panel that highlights a socially constructed self, adapted to the fans, an image and smiles to support your image in the sporting world. In other words, avid rock athletes will train at the panel with low anxiety looking at these workouts only as technical drills for the future challenges they will face at the cliff. They are less reliant on extrinsic motivation when conducting their training on the panel, keeping it somewhere in the background for what they consider their own goals. For those for whom the stakes are to increase the difficulty of their rock routes, those will be the situations where they will have higher anxiety and a higher need to rely on extrinsic motivation as well until they reach the maturity where performance is staked by intrinsic determination.

Keywords: *indoor climbing, outdoor climbing, competitive anxiety, performance, motivation, sport experience*

INTRODUCTION

Performance can be defined as the outcome of an individual as a result of performing a task that can be measured and evaluated by an observer or by him/her (the referee or him/her as an athlete). This evaluation in climbing is quantitative – number of routes completed, number of holds achieved,

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time taken to complete the route.

Sporting performance is the athlete's bio-psycho-social value, expressed in the context of official competitions, which is assessed according to rigorously established scales and is due to a multiply determined capacity (the sum of all abilities).

Sports performance is the result of factors that depend on the athlete's personality, attitudinal orientation and motivation to perform. The limit activity is at the choice of the athlete who wants to reach a stage of excellence, break a personal or world record, win a major competition. Maximising performance can only be achieved after maximising the athlete's personality. Unfortunately, many sports trainers see the athlete as a biological machine programmed to perform certain motor actions in a repetitive number, placing little emphasis on what athletes think and feel.

It all starts with the genetic make-up of each athlete. Any subject can be distinguished: biological traits (arm span, height, weight etc – there is no typical top climber (there are very tall champions like Adam Ondra, but also short ones like Juliane Wurm – each has advantages and disadvantages for the movements in the trails that he/she has to know how to use and be aware of), physiological traits (heart rate, respiratory capacity etc), psychological traits (the mental state of the athlete) and metabolic traits (aerobic, anaerobic processes, lactic acid capacity etc) [1,2].

There are four types of trainings to be developed:

1) Physical training – there are two types: general and specific physical training. The specific one involves exercises on specialised training boards (pulls on slipped holds, on spiked holds, for example), specific exercises for body-tension (a muscle tension that cumulates several muscle groups at the same time). What is specific to climbing is working against gravity and working with your own body weight.

2) Technical training which involves three components: Initially, specific training – this refers to learning general training rules (such as to perform a route you need to double the last top hold or for the starting position you need to have all four limbs in certain holds); learning to fall on the mat for the bouldering event (teaching the athlete to jump on the mat by bending the knees, for example, to avoid injury); learning how to belay for the climbing event (type of cuff, type of rope-head, use of the spinning device, tying the safety knot); basic technical training – learning, reinforcing and perfecting technical procedures, followed by their application on routes of increased difficulty; specialised kinaesthetic system – developing techniques for holding various holds

3) Tactical training – tactical training is not similar to that in combat or opponent sports. We discuss the athletes' pattern of approaching routes and what strategies they can use to achieve a complete ascent: Route visualization – a correct and efficient visualization of the route the athlete has to achieve will save him/her many attempts: route approach strategy (e.g. the three attempts method), creative imaginationthe idea that routes differ from competition to competition (except for the speed event) is emphasized and therefore the athlete must allow his/her imagination to find variations and alternatives to achieve the route [3].

4) Psychological training which can be defined on three levels: basic psychological training – involves the formation of athletes' personality, character, specific attitudes and the skill to carry out sports activities systematically and organized. It also focuses on physical hygiene, social skills, nutrition and sleep hygiene, and the importance of invisible training; specific psychological training – aims to develop the physical, psychomotor and psychological skills needed for the sport branch. The importance of shaping the competition in training is stressed so that the athlete perceives the stakes and the tension created by the competition.

HYPOTHESES

1) There are significant differences between the group of rock enthusiasts and panel enthusiasts in terms of personality questionnaire scores and level of motivation.

2) There are significant differences between juniors and seniors in terms of motivation level.

3) There are significant differences between the group of first mountain experience and first panel experience in terms of anxiety and motivation.

4) There are significant differences in competitive anxiety by level of competitive experience and level of sport experience.

5) There is a statistically significant correlation between competitive experience and level of competitive anxiety.

6) There is a statistically significant correlation between the level of extrinsic motivation and the level of intrinsic motivation.

7) There is a statistically significant correlation between the level of self-esteem and the level of intrinsic motivation.

8) There is a statistically significant correlation between the

level of performance and the level of extrinsic motivation.

METHODOLOGY

The methodology of this study is based on the following steps:

• choice of the problem to be researched;

• determining the group of athletes to which we will apply the research instruments;

• developing the research questionnaire and its rating;

• entering the questionnaire in google docs;

• creating the database with the athletes' answers in google docs;

• analysing the database using descriptive and inferential statistics;

• analysing the confirmation or refutation of working hypotheses;

• making psychological assumptions and interpretations of the results based on statistical analysis and with reference to the literature;

• outlining the conclusions of the present research;

• highlighting possible directions for further research.

1. Participants

The study was carried out on 28 participants. We considered only performance climbers who regularly participate in internal and/or external competitions. The ages of the subjects ranged from 13 to 48 years. There are both female (11) and male (17) participants. The study group can be divided into seniors (19) and juniors, up to 20 years old (9), according to the type of competitions in which they can participate internationally (seniors are considered to be at least 20 years old, and the categories for juniors are 14-15 years old, 16-17 years old, 18-19 years old – but in senior competitions, competitors can participate from the age of 16). Criteria for inclusion in the study include: a training frequency of at least two training sessions per week and active participation in national and international competitions during 2018-2019 (competitors in National Climbing Championships, National Bouldering Championships, Boulder Games, World or European Cup stages). Athletes fall only in the Masters category (female or male) [4].

2. Tools

- Introductory questionnaire: to record participants' factual data (age, gender, sporting experience, competitive experience, maximum performance level);

- the Impulsive Sensation Seeking and Social Desirability scales of the Zuckerman – Kuhlman Personality Questionnaire, ZKPQ

The Impulsive Sensation Seeking scale contains two clusters of items, which measure two constructs: the Impulsivity construct (which refers to the lack of planning and the tendency to act quickly, on impulse, without prior reflection) and the Sensation Seeking construct (which describes the general need for excitement and agitation, the preference for unexpected situations and friends with unpredictable behaviour, and the need for change and novelty).

The Social Desirability Scale expresses the subject's need/ desire to present him/herself in a particular way, as he/she believes at that moment in time that the entourage expects of him/her. It expresses a kind of poikilotimia, moulding on the mood and expectations of the entourage, the unconscious aim being to be liked, accepted and to build and sustain a self-image slightly higher than the subject concerned knows he/she deserves;

- competitive anxiety questionnaire taken from the research literature (“Psihologia sportului. Ghid pentru optimizarea performanțelor”, Stephen Bull, pp. 64-67)

It is a questionnaire that measures competitive anxiety as a trait. The first part (with the answers Rarely/Sometimes/ Often) indicates how often symptoms occur before competition. The higher the score (values between 10 and 30), the more prone the athlete is to competitive anxiety. The second part involves measuring the score that represents how facilitating or debilitating the anxiety is for the athlete. It attempts to separate positive (facilitating, enabling, constructive) and negative (debilitating, anxiety-provoking, destructive) emotions;

- Rosenberg's self-esteem scale (1965)

This scale is used to measure the athlete's overall sense of self-worth and self-acceptance. It is a four-choice scale (1-4 items), scores range from 10-40. It divides subjects as having: low, medium and high self-esteem.

- Sport motivation scale taken from the research literature) [5], translated from English into Romanian, preserving the meaning of the items and adapting them for the Romanian sport culture.

This scale is divided into several concepts: intrinsic motivation, extrinsic motivation and amotivation. Intrinsic motivation refers to the desire to engage in an activity simply for the pleasure and satisfaction that comes from the action [6]. If a person is internally motivated, he or she will want to perform for the self, not staked by material rewards or external constraints [7]. Intrinsic motivation involves: intrinsic motivation to know, intrinsic motivation to achieve, and intrinsic motivation to have stimulating experiences.

Knowledge motivation involves curiosity, exploration, a wish to learn and a genuine wish to understand. It can be seen as driving the athlete to act for the pleasure and satisfaction of learning a new experience, of trying to understand something previously unheard of.

Achievement motivation is a concept that in psychology is also referred to as self-improvement motivation, efficacy motivation or goal orientation. It can be defined as engaging in an activity because of the pleasure and satisfaction you get from the attempt, from the idea of creating something. An example from sport might be: the attempt to perfect a difficult technique to gain personal satisfaction.

Motivation towards stimulating experiences refers to the athlete's wish to try an activity for the stimulating sensation it generates (example: sensory stimulation, aesthetic experience, amusement, pleasure).

Extrinsic motivation involves engaging in a behaviour that has a purpose, a purpose that is not for personal development [6]. Grolnick [8] in 1990 investigated extrinsic motivation beyond external conditioning and postulated three dimensions of extrinsic motivation: unconscious extrinsic motivation, identification extrinsic motivation and regulatory extrinsic motivation.

Regulatory motivation is the one most often found in the research literature. It explains why certain behaviours are driven by external sources, such as material rewards, money prizes, trophies, medals, diplomas or constraints imposed by others [7]. An example from sport psychology might be competitors expecting rewards from parents or coaches. In this case, sport is not practised for pleasure or wish, but for rewards or to avoid negative consequences (criticism, arguments, etc.).

Unconscious motivation involves an internalisation of external rewards and therefore they are no longer necessary. In contrast, positive sporting behaviour is reinforced by internal pressures such as anxiety or guilt. Such examples might be athletes who engage in physical activity because they do not feel good in their bodies, they do not feel they are in good physical shape.

Identifying motivation refers to the moment when the athlete becomes autonomous and is able to value and judge his/her own behaviour. It is still an external, material goal, but it is internally regulated and self-determined (e.g. to achieve their own goals).

Amotivation is found when the athlete can no longer specify the reason for practising sport, he/she does not know why he/she should continue or whether he/she should continue practising. Eventually, they may decide to withdraw from the activity;

- opinion questionnaire on rock climbing, a questionnaire specially developed for the purpose of this research, on the interest of Romanian high-performance athletes in rock climbing and the differentiation of climbers into the two categories – rock enthusiasts and panel enthusiasts.

3. Experimental model

Variables are: age, performance level, competitive experience, sport experience, level of competitive anxiety, level of self-esteem, intrinsic motivation, extrinsic motivation, impulsive sensation seeking scale, social desirability scale, passion. Independent variables are: age, passion, competitive experience, sport experience, personality scales (impulsive sensation seeking and social desirability). Dependent variables are: competitive anxiety, performance level, motivation scales, self-esteem.

4. Data collection procedure

The research method used was the application of instruments (psychological tests and an opinion questionnaire). The test was administered during January-March 2020, in an online environment, through google docs.

The tests applied are:

- ZKPQ test, of which we used only two scales (impulsive sensation seeking and social desirability) – 28 questions. The answers are True/False. The questionnaire is calibrated and validated on the Romanian population by Test Central (Cronbach's alpha coefficient of internal validity is explained in the test manual).

- Competitive anxiety test taken from the book „Psihologia sportului- Ghid pentru optimizarea performanțelor”/"Sport Psychology – A Guide to Optimising Performance", Stephen Bull, pages 64-67 – 15 questions. It has three response options (Rarely/Sometimes/Often) and a Likert scale from 1 to 7 (from very debilitating to very facilitating).

- Rosenberg Self-Esteem Scale – 10 questions. Items are scored with four response possibilities between strongly disagree (1 point) and strongly agree (4 points).

- Sport Motivation Scale (SMS) (taken from Journal of Sport & Exercise Psychology, volume 15, pages 35-53) – 28 questions, translated from English into Romanian, keeping the meaning of the items and adapting them for the Romanian sport culture. It is scored from 1 to 7 (1, 2 = does not correspond at all; 3, 4, 5 = corresponds moderately; 6, 7 = corresponds very much). The scoring is as follows: Amotivation corresponds to items 3, 5, 19, 28; extrinsic motivation consists of items 6, 10, 16, 22; intrinsic motivation has items 1, 2, 4, 8, 12, 13, 15, 18, 20, 23, 25, 27; identified motivation consists of items 7, 11, 17, 24; and unconscious motivation corresponds to the sum of items 9, 14, 21, 26.

A total of 104 questions were asked. Completion of the questionnaire takes approximately 8 minutes.

RESULTS

Data analysis and interpretation

The following figures illustrate classifications of athletes according to an independent variable.

The graph from Figure 1 shows the distribution of the sample according to performance level.

Figure 1: Performance level of Romanian climbers

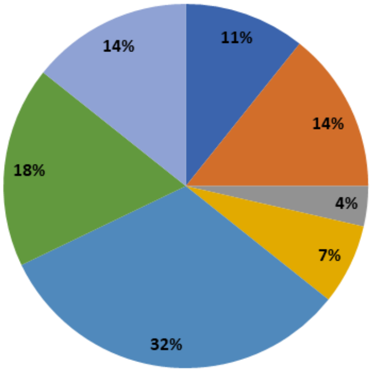

(Performance level: Balkan champion, national champion, I participated in a final/semi-final in European competition, I participated in a Balkan final, national final, participation in a world competition, I never participated in a final)

The graph from Figure 2 shows the distribution of the group according to the passion for rock or panel.

Figure 2: The passion of the athletes in the group

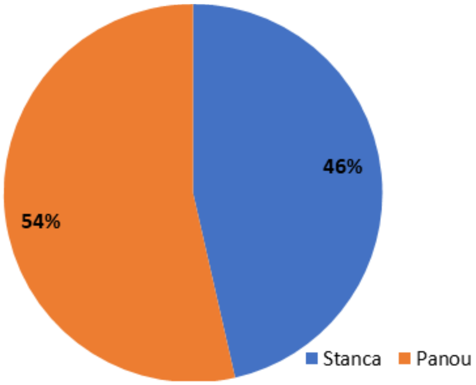

The graph from Figure 3 shows the group of athletes who had first contact with rock climbing versus those who had first contact with panel climbing.

Figure 3: First experience of athletes in the group

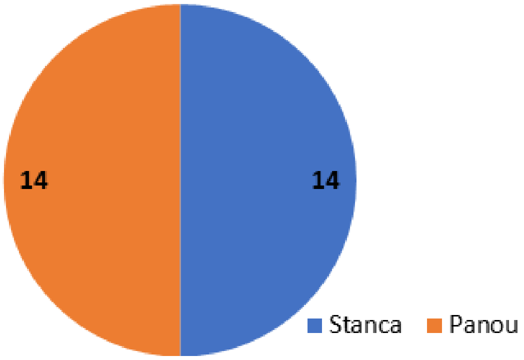

Following the achievement of the database after completing the questionnaire, 16 variables were identified: gender, age, sport experience (how many years of climbing), competitive experience (how long they have been participating in Masters competitions), performance level, impulsive sensation seeking score, social desirability score, self-esteem score, competitive anxiety score, competitive anxiety facilitation score, motivation scores (intrinsic, extrinsic, unconscious, identified motivation and amotivation), passion (rock or panel) and first experience (first contact with climbing was at panel or rock).

To decide the passion score, each question had a panel or rock response for which a score was given (panel=0, rock=1). Questions 1, 2, 4, 5, 6, 8, 10, 12, 13 (see Annex 1) were used to determine the passion scale score. Question 11 was used to determine the first experience. Of the 13 questions, 9 were with an exact response (rock or panel) to discover the athlete's passion, so the maximum possible score was 9. The remaining items were considered distracting and are not summed to determine the final score. Those scoring 0-4 were framed as having passion for the panel and those scoring 5-9 were framed as having passion for the rock.

In order to classify the level of performance, taking into account the general levels of Romanian climbers in relation to external competitions (championships or world cups), we ranked the athletes according to the following score: 1 point = Balkan champion, 2 points = national champion, 3 points = participated in a European final/semi-final, 4 points = participated in a Balkan final, 5 points = national final, 6 points = participated in a world competition, 7 points = participated in European competition, 8 points = participated in a Balkan competition, 9 points = never participated in a final

The lower the score, the higher the performance level of the athlete. Of course, it is a question about the highest level of performance perceived by him (for example, if an athlete is a national champion and has participated in European competition but came 44/49, he/she will not consider the highest level to be worth 7 points).

For the other scales, the score was calculated according to the scoring mode in the inventory.

Descriptive analysis

The descriptive statistics for the sample/group researched are illustrated in Table 1 and Table 2.

Table 1: Descriptive analysis

	Age	Exp-sport	Exp-comp	Sensation seeking	Desirability	Anxiety	Facilitation
Mean	27.07	12.02	8.64	11.04	1.82	20.96	30.04
Standard Error	1.92	1.34	1.18	0.52	0.28	0.92	2.15
Median	26.00	10.00	7.00	11.00	2.00	20.50	31.00
Mode	17.00	9.00	7.00	9.00	1.00	17.00	16.00
Standard Deviation	10.18	7.09	6.23	2.73	1.47	4.89	11.36
Sample Variance	103.70	50.23	38.85	7.44	2.15	23.89	129.15
Kurtosis	-0.77	5.70	3.99	-0.96	1.00	-0.77	-0.68
Skewness	0.46	2.02	1.74	-0.05	0.94	-0.10	0.33
Range	35.00	34.00	28.50	10.00	6.00	19.00	42.00
Minimum	13.00	4.00	1.50	6.00	0.00	10.00	12.00
Maximum	48.00	38.00	30.00	16.00	6.00	29.00	54.00
Sum	758.00	336.50	242.00	309.00	51.00	587.00	841.00
Count	28.00	28.00	28.00	28.00	28.00	28.00	28.00

Table 2: Descriptive analysis

	Amotivation	Extrinsic	Intrinsic	Identified	Unconscious	Self-esteem
Mean	8.07	11.71	69.21	19.61	18.32	26.29
Standard Error	0.91	1.03	1.89	0.81	0.90	0.41
Median	6.50	10.00	73.00	20.00	18.00	26.00
Mode	4.00	9.00	73.00	21.00	18.00	25.00
Standard Deviation	4.80	5.45	9.98	4.30	4.75	2.19
Sample Variance	23.03	29.69	99.51	18.47	22.52	4.80
Kurtosis	3.15	-0.24	0.83	2.72	-0.18	0.37
Skewness	1.63	0.75	-1.18	-0.93	-0.16	0.13
Range	20.00	19.00	39.00	22.00	20.00	10.00
Minimum	4.00	4.00	43.00	6.00	8.00	21.00
Maximum	24.00	23.00	82.00	28.00	28.00	31.00
Sum	226.00	328.00	1938.00	549.00	513.00	736.00
Count	28.00	28.00	28.00	28.00	28.00	28.00

One of the objectives of the present work was to determine the anxiety dimension of the athletes in the chosen group, demonstrating the importance of knowing each athlete individually, so that the coach can figure out if the athlete needs activation, relaxation, mindfulness or to be left alone [9]. There were two dimensions investigated: the level of competitive anxiety and the anxiety facilitation dimension which was intended to indicate whether the emotions felt by the athlete push him/her towards activation or inhibition. The results obtained are:

- 5 of the athletes recorded a low level of competitive anxiety (below 16) – meaning that they rarely have anxious moments during competition.

However:

O 2 of them score low on the facilitation dimension (below 25) – meaning that these two athletes do not often have feelings of anxiety, but when they do, these emotions are debilitating. Therefore, anxiety management sessions would be needed for these athletes for situations where anxiety may arise.

O None of the athletes scored on the facilitation size above 55, but if they had, they would have had positive, facilitating emotions. It follows that these athletes would only need activation to release facilitative anxiety for better performance in the competition.

- 12 of the athletes had a moderate level of anxiety (17-22), which means that they control their emotions well during a competition, but the level of facilitation needs to be checked to look for imbalances. And therefore:

O 5 of them scored low on the facilitation dimension (below 25), which means that if their anxiety increases in competition, the resulting emotions would be debilitating. Therefore, they would need negative emotion control sessions and anxiety control sessions.

O One athlete was at the threshold of having facilitating emotions (54) which may categorize him/her as the most mentally prepared athlete in the group since he/she has a normal level of competitive anxiety and is facilitating

O The remaining 6 athletes have a normal level of anxiety and know how to control it

- 11 athletes have a high level of anxiety (over 24), and of these:

O None had an increased facilitation level (over 55) to have constructive emotions

O 4 of them had a low facilitation level (below 25), which categorizes them as anxious athletes with debilitating, negative emotions. But we know that they need anxiety-reducing interventions.

O The remaining 7 athletes have a high level of anxiety, but normal facilitation without being debilitating or facilitating.

The results of the competitive anxiety scale compared to the level of facilitation can be better explained in Table 3.

Table 3: Level of anxiety and facilitation

Negative anxiety (5)		**Normal anxiety (12)**			**Positive anxiety (11)**	
Nevative emotions (2)	Normal emotions (3)	Nevative emotions (5)	Normal emotions (6)	Positive emotions (1)	Nevative emotions (4)	Normal emotions (7)

(Negative anxiety: negative emotions, normal emotions, Normal anxiety: negative emotions, normal emotions, positive emotions, Positive anxiety: negative emotions, normal emotions)

Non-parametric tests

Next, we will use statistical procedures to confirm/infirm the hypotheses of this paper. We will use the correlational study (Spearman correlation) to see if there are significant correlations between certain dependent and independent variables.

We will also divide the analyzed group into groups according to a criterion (age, performance level, passion, competitive experience, and first experience) in order to see if there are significant differences between them using the Mann-Whitney, U test.

To test Hypothesis 1 which seeks to see if there are statistically significant differences between the rock enthusiast group and the panel enthusiast group, the U-test was run and the results are defined in Table 4.

Table 4: Hypothesis 1

	Extrinsic motivation	**Social desirability**
U value	53.500	51.500
z score	2.003	-2.095
p value	0.046	0.035
Critical value of U	54.000	54.000
Effect size	0.379	0.396

The table wants to explain that by means of the Mann-Whitney test we tested whether there are significant differences between the panel enthusiasts group and the rock enthusiasts group in terms of extrinsic motivation and social desirability. The U-test value for extrinsic motivation is 53.500 and for social desirability is 51.500. The critical value for the α threshold of 0.05 and n=28 participants (the rock enthusiasts group has 13 and the panel enthusiasts group has 15) is 54. Since the calculated U-test value is lower than the critical value (53.5<54 and 51.5<54), and p<0.05 (=0.046 and 0.035, respectively), it can be concluded that there are significant differences between the two groups in terms of extrinsic motivation and social desirability. The null hypothesis is rejected. The effect size is 0.379 for extrinsic motivation and 0.396 for social desirability. Thus, hypothesis 1 is confirmed. There is a strong mean difference in intensity (r=0.379) between the two samples on extrinsic motivation. There is a strong mean intensity difference (r=0.396) between the two samples on social desirability. To test Hypothesis 2 which seeks to see if there are statistically significant differences in motivation between the junior and senior groups, the U-test was run, the results being defined in Table 5.

Table 5: Hypothesis 2

	Extrinsic motivation
U value	43.000
z score	-2.066
p value	0.038
Critical value of U	45.000
Effect size	0.390

The table explains that using the Mann-Whitney test we tested for significant differences between juniors and seniors in terms of motivation. The U-test value for extrinsic motivation is 43.000. The critical value for the α threshold of 0.05 and n=28 participants (juniors are 9 and seniors are 19) is 45. Since the calculated U-test value is lower than the critical value (43.00<45), and p<0.05 (=0.038), it can be concluded that there are significant differences between the two groups in terms of extrinsic motivation. The null hypothesis is rejected. The effect size is 0.390 for extrinsic motivation. Thus, hypothesis 2 is confirmed. There is a strong-mean intensity difference (r=0.390) between the two samples on extrinsic motivation.

To test hypothesis 3 which seeks to see if there are statistically significant differences between the first mountain experience group and the first-panel experience group, the U-test was run, the results being defined in Table 6.

The table shows that using the Mann-Whitney test we tested whether there are significant differences between the group with the first experience in the artificial environment and the group with the first experience in the natural environment in terms of extrinsic motivation and competitive anxiety. The U-test value for extrinsic motivation is 26.5 and for competitive anxiety is 45.5.

Table 6: Hypothesis 3

	Competitive anxiety	Extrinsic motivation
U value	45.500	26.5
z score	2.389	3.262
p value	0.017	0.001
Critical value of U	55.000	55
Effect size	0.451	0.616

The critical value for the α threshold of 0.05 and n=28 participants (first rock experience group is 14 and first panel experience group is 14) is 55. Since the calculated U-test value is lower than the critical value (45.5<55 and 26.5<55), and p<0.05 (=0.017, respectively 0.001), it can be concluded that there are significant differences in terms of extrinsic motivation and competitive anxiety between the two groups. The null hypothesis is rejected. The effect size is 0.451 for competitive anxiety and 0.616 for extrinsic motivation. Thus, hypothesis 3 is confirmed. There is a strong-mean intensity difference (r=0.451) between the two groups on competitive anxiety. There is a strong-very strong intensity difference (r=0.616) between the two groups on extrinsic motivation.

To test Hypothesis 4 which seeks to see if there are statistically significant differences in the level of competitive anxiety between the lower competitive experience group (under 10 years) and the higher competitive experience group (over 10 years), the U-test was run.

The results of the test are shown in Table 7.

Table 7: Hypothesis 4

	Competitive anxiety
U value	30.000
z score	2.517
p value	0.012
Critical value of U	41.000
Effect size	0.476

The table demonstrates by means of the Mann-Whitney test the existence of significant differences between the group with less competitive experience (under 10 years) and the group with more competitive experience (over 10 years) in terms of competitive anxiety. The value of the U-test is 30. The critical value for the α threshold of 0.05 and n=28 participants (those with lower competitive experience (under 10 years) has 20 subjects and the group with higher competitive experience (over 10 years) has 8 subjects) is 41. Since the calculated U-test value is lower than the critical value (30<41, and p<0.05 (=0.012), it can be concluded that there are significant differences between the two groups in terms of competitive anxiety. The null hypothesis is rejected. The difference in competitive anxiety according to sport experience, is illustrated in Table 8.

Table 8: Hypothesis 4

	Competitive anxiety
U value	50.000
z score	2.022
p value	0.043
Critical value of U	51.000
Effect size	0.382

The table demonstrates by means of the Mann-Whitney test the existence of significant differences between the group of those with below average sport experience (<=10 years) and the group of those with above average sport experience (>10 years) in terms of competitive anxiety. The U-test value is 50. The critical value for the α threshold of 0.05 and n=28 participants (the group with people below average (<10 years) sports experience has 17 subjects and the group with people above average (>10 years) sports experience has 11 subjects) is 51. Since the calculated U-test value is lower than the critical value (50<51, and p<0.05 (=0.043), it can be concluded that there are significant differences between the two groups in terms of competitive anxiety. The null hypothesis is rejected.

The effect size is 0.476 for competitive anxiety depending on competitive experience. Whereas, according to sport experience, the effect size is 0.382 for competitive anxiety. Thus, hypothesis 4 is confirmed. There is an medium intensity difference (r=0.382) according to sport experience and a strong-medium intensity difference (r=0.476) according to competition experience between the two samples on competitive anxiety [10].

Given the small group of respondents, which is due to the fact that in Romania, the community of performance climbers is small, the number of athletes and sportswomen who frequently participate in internal competitions is small, and even smaller in external competitions (for example, at the 2019 national championship in the senior category, 9 girls participated, and 6 entered the final), we will use for the correlational study, the Spearman correlation.

For testing hypotheses 5, 6, 7, 8 we used Spearman correlation, and the results are highlighted in Table 9.

The analysis of the results presented in the above table shows the confirmation of hypotheses 5, 6, 7, 8, which can be detailed as follows:

Table 9: Hypotheses 5, 6, 7, 8

	rs	p	Size effect
Anxiety - competitional experience	-0.437	0.020	0.191
Extrinsic motivation - unconscious motivation	0.434	0.021	0.188
Self-esteem - unconscious motivation	-0.504	0.006	0.254
Performance level- extrinsic motivation	-0.446	0.017	0.199

- There is a statistically significant negative correlation (with r = -0.437) between the level of competitive experience and the level of competitive anxiety (p<0.05). It follows that athletes with more competitive experience will have lower levels of competitive anxiety. The effect size is 0.191, showing a statistically significant correlation of very low to low strength [11].

- There is a statistically significant positive correlation (r=0.434) between the level of extrinsic motivation and the level of unconscious motivation (p<0.05). It thus follows that athletes with higher extrinsic motivation will have a higher level of unconscious motivation. The effect size is 0.188, showing a very low-low intensity statistically significant correlation.

- There is a statistically significant negative correlation (r=-0.504) between the level of self-esteem and the level of unconscious motivation (p<0.05). It follows that athletes with higher self-esteem will have a higher level of unconscious motivation. The effect size is 0.254, showing a statistically significant correlation of low intensity.

- There is a statistically significant negative correlation (r=-0.504) between athletes' performance level and level of extrinsic motivation (p<0.05). It thus follows that athletes with higher levels of extrinsic motivation will have better sports performance. The effect size is 0.199, showing a statistically significant correlation of low intensity [12,13].

CONCLUSION

Concerning the first hypothesis, we issue the following psychological assumptions. We would dare to say, despite some terrible accidents that have taken place on the cliff and in which prominent athletes have been involved, that this form of training in solitude, for balanced athletes, is more valuable, developing their caution and teaching them to know their limits. Conversely, on the panel, social desirability can bring with it the risk of showmanship and extravagance, when an athlete, although intuitively sensing correctly where his or her limits and capabilities lie to cross a threshold at a given point in time, may choose the opposite in the sense of skirting risk, out of demonstrativeness, extrinsic motivation and the desire to please supporters [14].

As a conclusion, we could say that passion for rock is positively correlated with intrinsic motivation, a balanced personality and authentic knowledge of physical and psychological resources at that moment of time, while passion for panel is more likely to be related to extrinsic motivation, social desirability, maintaining an image, the fact being also supported by a more vivid communication and in a here and now with the audience [15].

- Thought of in a long-term dynamic, things are not so exclusivist, as genuine athletes train on the panel for long periods in order to accumulate sufficient "psychological nourishment" provided by the admiration of others for the moments when they are about to climb the cliff and when the reminiscences of success on the panel will help them to surpass their limits [16,17].

- The results of the second hypothesis, which emphasizes the shift from junior to senior of motivation from extrinsic to intrinsic, appeals to several psychological concepts. Starting from Piaget, who describes the stages of intelligence development, the shift from childhood to adolescence and adulthood follows the same path from externalisation to internalisation of the desire for knowledge, of the perception and internalisation of the sense of danger, of the balance in the judicious allocation of resources. Juniors are dependent on the admiration of parents and coaches, on the public which in itself is happy to create young images, especially in a less developed sport in a country like ours [18]. Any junior considered a hope in Romanian climbing is greeted with joy, climbing being a sport aimed at an audience mature enough to overcome envy, malice, lack of opportunities for collaboration, promotion of non-values as is perhaps the case in other team sports or with a longer tradition and many followers.

- Climbing is a sport that is easy to enter, the environment and the circle of seniors who are frequently attached to competitions (direct participants, supporters, coaches or sponsors), capable of emotionally supporting juniors, promoting them, ranking them without them feeling blamed, pushed aside, criticised, rejected [9]. Currently, in Romanian climbing, for ages between 4 and 16 years old, perhaps extending to 18, we consider extrinsic motivation important because it supports the inner construction of the athlete, helps him/her reach and maintain the state of readiness, creates a sense of a continuum between him/her and the audience. Let's not forget those key moments when the junior is majorly dependent on the ideal match between the shouts and support in the gym and his/her internal moment when he/she is ready to step off the track. Seniors, however, are mature people who bring together multiple competitive experiences with personal life experiences from the various fields in which they are still active, and from the questionnaires administered they emerge as authentic personalities with no false selves, no false modesty, no hiding or desire to present themselves in a more favourable light [19].

- For seniors, intrinsic motivation requires dedication, composure, balance, responsibility, the ability to know how to lose, and the ability to recognize a new value emerging in your shadow or next to you, long-term planning of resources so that competition matches peak performance. Intrinsic motivation is one that is underpinned by good self-knowledge, with pluses and minuses, strengths and weaknesses both in terms of techniques used and psychological background and the ability to induce and maintain the state of readiness [20].

- Many juniors owe their success on extrinsic motivation, namely in the junior period for two athletes who have similar technical and physical competitive qualities, a higher extrinsic motivation of one (more encouragement from the coach, more involved parents, more admirers from the public on the model of sympathy) can make a significant difference in terms of a competitive result. Among seniors, intrinsic motivation is much correlated with technical development, with a balanced psyche, with a more cautious calculation of what the individual can or cannot do, each new competition being a dynamic putting the individual's personality in competition not only with others but also with his/her own expectations, in a continuum of sports training. We thus encourage the maintenance of extrinsic motivation as a stimulus up to a certain age in junior sport, which proves its usefulness up to the point where it would become an intrusion into the athlete's inner space (the athlete would be conditioned, perhaps even threatened, blackmailed, compared to others, accused of having his/her admiration withdrawn, or the situation where significant discrepancies between likes and dislikes would affect the fragile psyche of a budding athlete) [21].

- An important variable we wanted to consider in this study was the first experience of contact with the sport, as from the constructed questionnaire it appeared that for some of the athletes the first experience corresponded with their subsequent passion for rock versus panel, but for others this was not the case. The first experience, especially if it took place at an early age correlates with extrinsic motivation, support from the entourage, praise and encouragement from others, especially if there are coaches, friends maybe even parents in the vicinity involved and dedicated to the passion for rock versus passion for panel. First experience and extrinsic motivation become important when the person in question joins a lineage. Introducing anxiety as a variable in relation to first experience and extrinsic motivation could be explained as follows: when first experience encourages passion in one direction or another, extrinsic motivation supports the direction (rock versus panel), while anxiety tends to decrease when it is not consistent with passion [22].

- It is well known that accumulated competitive experience develops not only athletic techniques, but also mental skills of concentration, stability, mobility and distributivity of attention, motor memory, memory of a chain of sequenced movements belonging to a technique, as well as the ease of habituation to get into the competitive mood, to activate on command one's readiness and flow state, to make up through technique and experience the deficit of athletic performance (if that competition does not overlap with the athlete's sports form). The more complete, valuable and comprehensive this competition is, both from a tactical and strategic point of view and in terms of the development of the athlete's personality milestones (motivation, willpower, determination, preparation), the better prepared the athlete will be and will consider tenacity superior to anxiety. In other words, competitive anxiety decreases as experience in the field is gained [17].

- The hypothesis confirming the positive correlation between extrinsic motivation and unconscious motivation is based on the fact that as the athlete progresses from childhood to junior and adulthood, he/she internalizes his/her rewards and does not need to represent them in an external plane (promises, support, cups, medals, praise), but he/she has taken within him/herself an amount of pressure "sufficiently big and at the same time sufficiently little" to be able to filter between eustress and distress so as to keep his/her anxiety within limits consistent with eustress and with the possibility of physiologically determining his/her state of alertness and vigilance as an important area of the construction of the state of flow [23].

- The fine transition from extrinsic to unconscious motivation is natural in conjunction with the development of personality, as one of the coach's desires should be a filter as careful as possible in terms of the athlete's experience of feelings of guilt in the event of failure. As it is known from the research literature, the concept of extrinsic motivation can include reprimand or punishment from secondary sources when the athlete has not brought them the expected results. Conversely, unconscious motivation, in the absence of sound principles to help develop a balanced and normal personality of the athlete, can contribute to increased feelings of guilt, anxiety, low self-esteem, subjective feelings of not being good enough, which can ultimately lead to failure. This is where we should find the great art of coaches and sports psychologists in helping juniors to put a filter when they internalize aspects of extrinsic motivation unconsciously [24].

- The following hypothesis confirms the negative correlation between self-esteem and unconscious motivation. Turning to the psychological explication, we could consider as follows: athletes able to maintain high self-esteem are those less concerned with being in touch with the unconscious strand of their own motivational resources. They are those who have strengthened their facade, their competitive selves and the imago with which they interact in the sporting world, calling on the unconscious reservoir only when they wish to make contact with what once represented praise, encouragement, admiration, resource enhancement. The lower the self-esteem of a competitive athlete, the more we are dealing with an unconscious motivation in which the athlete has a severe over-ego, punishes him/herself, blames him/herself, criticizes him/herself, exacerbates his/her guilt, considers that he/she has not done enough, self-stigmatizes him/herself and can no longer find the way to reactivate his/her beneficial resources, the techniques that once proved successful, the positive mental states. Athletes with low self-esteem also lose patience and forget to think by steps, little by little, expecting the coach or psychologist to provide miraculous solutions that will free them from a new circuit of effort in order to recover both their self-esteem and their high position in the competitive rankings [25].

- As the athlete and competitive years progress, extrinsic motivation loses ground to intrinsic motivation, so that a high level of performance can only be based on strong intrinsic motivation, which in turn is the axis of the personality structure of the competitive athlete [26]. Intrinsic motivation is that which is less dependent on circumstances, it is based on the concepts of effort and patience, on the adaptation of wishes to the possibilities of the body, while extrinsic motivation is fuelled by the reservoir of wishes, hopes and expectations in someone else's mind, and from one level onwards, the performance athlete tries to create their own inner world, their own competitive 'battle' space, in which both their physical and psychological attributes and qualities are brought together.

From this point, extrinsic motivation and the mind of an outsider can give errors in terms of correctly assessing the level of performance an athlete may find him/herself at a given point in time, thus asking more or less of him/her than he/she him/herself knows he/she can give.

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ORIGINAL ARTICLE

Evaluation of children with ADHD and their psychiatric symptoms

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Abstract: *Background: Mental disorders, especially in children, can be manifested in different ways, which is of great importance to them, so the purpose of this evaluation was to evaluate the changes in psychiatric symptoms in children with ADHD.*

Method: This evaluation was performed as a descriptive-analytic study on 141 children with ADHD who were followed up for 6 months. At the beginning of the study and the third and sixth months after, patients' symptoms were assessed with the CBCL Behavior Checklist and the ADHD rating scale questionnaire, as well as the course of illness and association with other disorders. Finally, the data were entered into SPSS statistical software and analyzed.

Results: The mean age of the children was 5.89±0.81 years and the prevalence of males was 68.8%. Among the two treatment groups, only the mean score below the ADHD-RS questionnaire score in the non-Ritalin group at the end of the third and sixth months was significantly lower than the Ritalin group. During the study in both treatment groups, the overall ADHD-RS questionnaire and both subscales of attention deficit hyperactivity disorder (ADHD) had a significant decrease from baseline to the end of the sixth month. In relation to the CBCL behavioral checklist, the mean score of the total checklist except for anxiety/depression in the non-Ritalin group and the somatic complaints subscale in the Ritalin group decreased significantly from baseline to the end of the sixth month.

Conclusion: Based on the results observed in this evaluation, Ritalin had a significant effect on the symptoms of patients with ADHD, although these symptoms improved in both groups, however, further studies are needed.

Keywords: *attention deficit hyperactivity disorder, Ritalin, children*

INTRODUCTION

Attention Deficit Hyperactivity Disorder is a persistent pattern of inattention and hyperactivity, which is more common and severe than typical of children with similar developmental levels. While in the past hyperactivity was thought to be a devastating underlying symptom of the disorder, the general view today is that hyperactivity is often secondary to poor impulse control. Impulsivity and hyperactivity are common in today's diagnostic criteria for ADHD [1]. At present, the diagnosis of ADHD has based on the agreement of experts that three observable types include: inattentive, hyperactive/impulsive, or mixed, all manifestations of a disorder. To meet the diagnostic criteria for ADHD according to the DSM-IV-TR criteria, symptoms must be present before the age of 7 (and, according to the DSM-V, before the age of 12) [2,3]. However, the diagnosis of ADHD in many children is not made until after the age of 7, when their behaviors cause problems at school and elsewhere. To confirm the diagnosis of ADHD, impairment due to inattention or hyperactivity/impulsivity must exist in at least two areas and interfere with proper social, educational, and extracurricular activities. This disorder should not occur during pervasive developmental disorder, schizophrenia, or another psychotic disorder, and no other mental disorder is a better explanation for it [4].

Attention Deficit Hyperactivity Disorder is one of the most common psychiatric disorders in children with a prevalence of 3-7% and is 3-5 times more common in boys than girls [5, 6]. Symptoms are a persistent pattern of inattention and/or

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hyperactivity and impulsive behavior that is more severe than expected for the child's age and level of development. Some children may have more active symptoms and impulsive behaviors, and some may have more inattention symptoms. According to the DSM-V criteria, the symptoms of this disease start before the age of 12 and usually exist from the age of 3. But serious problems often arise during school [7]. The course of the disease is variable and most patients recover partially, and inattention is often the last symptom to improve. However, these patients are prone to antisocial behavior, substance abuse and mood disorders, etc. [8,9].

Based on the cases stated in this section, the importance of paying attention to the association of other disorders with ADHD and evaluating the effectiveness of treatment methods on these symptoms is determined. Has suffered from ADHD.

METHOD

This evaluation was a cross-sectional-analytical and longitudinal study that was performed on children with attention deficit hyperactivity disorder, in which psychiatric changes and disease course were evaluated in this group of patients.

For this purpose, 141 children aged 5 to 7 years with ADHD were included in this study by available sampling. Patients were followed up for 6 months and every 3 months their symptoms were assessed using the CBCL Behavioral Checklist and the ADHD-RS (ADHD rating scale) questionnaire filled out by parents. ADHD Rating Scale is an 18-item questionnaire for children 5 to 17 years old. This questionnaire examines the child's behavior over the past 6 months and is completed by their parents or teacher. The overall score of this questionnaire is between zero and 54 and each item is graded from zero to 3. The questionnaire also has two subscales: signs of hyperactivity / impulsivity (including 9 questions) and attention disorders (including 9 questions). This scale has two forms of school and home, the first form is filled out by school teachers and the second form by parents. In validated studies, high validity and reliability have been mentioned for it and its psychometric properties have been comparable with other valid scales for measuring the severity of ADHD (10).

The CBCL Behavioral Checklist is also a general diagnostic method for identifying behavioral problems in children. This checklist is classified into 4 forms based on different ages that examine different aspects of behavior. Similar questions are usually categorized into syndromic subscales. Accordingly, 8 syndromes include Aggressive behavior, Anxious/depressed, Attention problems, Rule-breaking behavior, Somatic complaints, Social disorders. problems), Thought problems, Withdrawn/depressed. The two general subgroups in the CBCL Behavioral Checklist also include internalization disorders (sum of scores of anxiety/ depression subscales, deprivation/depression, and somatic complaints) and extrinsic disorders (total scores of normative and aggressive behavior subscales). In this study, children aged 5 to 7 years were included in the study who were normal in terms of IQ and otherwise did not meet the inclusion criteria. Finally, the information of all patients was entered into SPSS software version 23 for further analysis and evaluation.

Ethical Considerations

This study was approved by the ethics committee of Mashhad University of Medical Sciences. Written consent was obtained from all patients' parents to participate in this study. Participants' information was kept confidential and none of their personal details, including their first and last names, were entered into the software. All individuals were given a project-specific code and analyzed accordingly. The initial information will remain with the main executor until the completion of the project and the publication of the articles. This research was presented in the Organizational Ethics Committee of Mashhad University of Medical Sciences on 12/4/1695 under the title "Study of changes in accompanying psychiatric symptoms in children with ADHD" and No. 951040 with the code IR.MUMS.fm.REC.1395.578 has been approved.

RESULTS

A total of 141 children aged 5 to 7 years with ADHD were studied in this study. The mean age of children was 5.89 ± 0.81 years. The minimum and maximum ages of children included in the study were 5 and 7 years, respectively. The frequency of boys in the studied patients was 68.8% and the frequency of girls was 31.2%. In terms of parents' education level, most fathers with a frequency of 6.2% in the diploma and bachelor's group and most mothers with a frequency of 68.3% in the same group. Also, the mean age variable in the Ritalin treatment group was 5.86 82 0.82 and in the non-Ritalin treatment group was 5.99 ± 0.77 years. There was no significant difference between the mean age in the two general groups of drug treatment (P = 0.41) and all 4 groups of drug treatment (P = 0.67). The frequency of boys in the treatment group with Ritalin and the non-Ritalin treatment group was 67% and 73.7%, respectively, but again there was no significant difference in the distribution of sex in the two groups of drug treatment (P = 0.45). Regarding the variables of father's education level (P = 0.35) and mother's education

(P = 0.13), no significant difference was observed between the two groups of drug treatment (Table 1).

Table 1: Comparison of demographic variables in groups based on the type of drug treatment

Variables		Ritalin treatment (n=103)	Non-Ritalin treatment (n=6)	P value
Gender	Boy	69	28	0.45
	Girl	34	10	
Fathers education	Less than a diploma	42	12	0.35
	Diploma and Bachelor	60	25	
	Master's degree and higher	0	0	
Mothers education	Less than a diploma	35	7	0.13
	Diploma and Bachelor	65	30	
	Master's degree and higher	2	0	

Initially, at the end of the third month and the end of the sixth month of the follow-up of children with ADHD, the ADHD-RS questionnaire score was measured for all patients. This index between the two groups of treatment with Ritalin and non-Ritalin treatment, in none of the stages of the study, there was no significant difference between the mean score of the ADHD-RS questionnaire. However, in the Ritalin and non-Ritalin groups, the mean total ADHD-RS score and both the Attention Deficit and Hyperactivity Disorder subscales had significant changes from the beginning of the study to the end of the sixth month and the trend of these changes was decreasing (Table 2).

Table 2: Examining the trend of changes in the score of the questionnaire ADHD-RS in groups based on drug treatment

Variables	At the beginning of the study	At the end of the third month	At the end of the sixth month	P Value
Overall score ADHD-RS				
Ritalin	92/53±39/62	78/10±34/45	67/75±43/40	0/003*
Non-Ritalin	92/0±51/59	71/74±47/73	55/87±49/62	0/3
Attention Deficit				
Ritalin	31/17±13/09	32/06±12/29	28/47±11/62	0/019*
Non-Ritalin	26/71±14/26	24/58±13/93	20/74±13/91	0/003*
Hyperactivity/Impulsivitiy				
Ritalin	57/93±27/68	46/89±25/74	38/46±35/72	<0.001*
Non-Ritalin	63/57±40/20	49/86±36/79	31/05±32/23	<0.001*

The mean total score of the ADHD-RS questionnaire was significantly different between the beginning of the study and the third month, between the beginning of the study and the sixth month and also between the end of the third month and the end of the sixth month. Only the mean score of the Attention Deficit Scale was significantly different between the third and sixth months. However, no significant difference was observed in this scale between the beginning of the study and the third month, as well as between the third month and the sixth month. As can be seen in the table below, the mean score of the hyperactivity/impulsivity subscale was significantly different between the beginning of the study and the third month, as well as between the beginning of the study and the sixth month. Also, the mean score of the hyperactivity/impulsivity subscale was significantly different between the beginning of the study and the third month, between the beginning of the study and the sixth month, and also between the end of the third month and the end of the sixth month (Table 3).

In evaluating the indicators related to the CBCL questionnaire, in the subgroup of internalization disorders, which includes the subscales of anxiety/depression, deprivation/depression, and somatic complaints, the average score of all these subscales except anxiety/depression in the non-Ritalin group and the subscales of somatic complaints In the Ritalin group, it decreased significantly from the beginning of the study to the end of the sixth month.

Table 3: Difference in Ritalin treatment group measured each time

Variables	The beginning of the study	The end of the third month	The end of the sixth month
The overall score of ADHD-RS is the Ritalin Group			
The beginning of the study	-	0.02	0.011
The end of the third month	0.02	-	0.11
The end of the sixth month	0.011	0.11	-
The overall score of ADHD-RS is the Non- Ritalin Group			
The beginning of the study	-	0.001	<0.001
The end of the third month	0.001	-	0.017
The end of the sixth month	<0.001	0.017	-
Ritalin Groups Attiude Disorder			
The beginning of the study	-	>0.99	0.256
The end of the third month	>0.99	-	0.012
The end of the sixth month	0.256	0.012	-
Ritalin Groups Attiude Disorder			
The beginning of the study	-	0.558	0.012
The end of the third month	0.558	-	0.059
The end of the sixth month	0.012	0.059	-
Hyperactivity/Impulsivitiy of the Ritalin Group			
The beginning of the study	-	0.001	0.005
The end of the third month	0.001	-	0.177
The end of the sixth month	0.005	0.177	-
Hyperactivity/Impulsivitiy of the Non- Ritalin Group			
The beginning of the study	-	0.002	<0.001
The end of the third month	0.002	-	0.03
The end of the sixth month	<0.001	0.03	-

The mean score of total internalization disorders also decreased significantly in both the Ritalin treatment group and the non-Ritalin treatment group during the study. In the subgroup of extrinsic disorders, which includes subscales of normative and aggressive behavior, the mean score of all these subscales significantly decreased from the beginning of the study to the end of the sixth month in both study groups. The mean score of total extrinsic disorders during the study was significantly reduced in both the Ritalin treatment group and the non-Ritalin treatment group. Among the remaining subscales, the mean score of attention and social disorders in both groups (either Ritalin treatment or non-Ritalin treatment) decreased during the study, which was also statistically significant. But in the subspecies of thinking disorders, only in the non-Ritalin treatment group, the decrease was significant during the study. However, the same decrease in the mean subscale score of thinking disorders in the Ritalin treatment group was not significant. The overall score of the CBCL Behavioral Checklist in the Ritalin and non-Ritalin treatment groups decreased significantly during the study (Table 4).

Based on the mean and standard deviation of sub-indices related to and comparison of subgroups related to CBCL behavioral checklist, most indices had a statistically significant relationship between the time of the beginning of the study, 3 and 6 months after the intervention. There was a statistically significant difference between Ritalin and non-Ritalin groups, so both groups had an improvement over time (Table 5).

DISCUSSIONS

In the present evaluation, it was observed that the results of the evaluation of patients using the ADHD-RS questionnaire and the CBCL behavioral checklist in different treatment groups were not statistically significant, however, these cases need to be compared with studies. Accordingly, this has been evaluated in continuation of other studies conducted in this field. In a study of 154 children with ADHD treated with Ritalin, Wang et al. Observed children for two years and ADHD-RS questionnaire symptoms at the beginning, end of month 6, end of month 12, end 18 months and the last two years have been measured. They observed that the mean age of patients in this study was 6.87. 1.04 years. 72% of the patients in this study were boys and the rest were girls. In this study, for a total of two years, the mean score of the ADHD-RS questionnaire was significantly reduced (P = 0.02). The interesting finding of this study was that the decrease in the mean score of ADHD-RS questionnaire for girls was more than boys and this difference was also significant (P = 0.01). This study ultimately concludes that changes in the signs and symptoms of the disease in children with ADHD and treated with Ritalin can vary over time depending on gender [11]. Accordingly, in this evaluation, as in the present study, it was observed that the difference between the treatment groups was not significant, although these symptoms decreased in all treatment groups during treatment. In another study with a descriptive and longitudinal structure on 79 children with ADHD, Liang et al. Observed that after 12 months of follow-up, the mean score of the ADHD-RS questionnaire and both subscales decreased significantly (P = 0.001). However, the mean score of the CBCL behavioral checklist was not significantly different [12].

Table 4: Checking the process of changes in size scores of questionnaires CBCL

Variables	The beginning of the study	The end of the third month	The end of the sixth month	P Value
Attention disorder				
Ritalin	8/19±4/24	5/90±3/57	3/72±3/40	<0.001*
Non-Ritalin	7/84±4/44	5/52±3/54	3/59±3/30	<0.001*
Social disorders				
Ritalin	6/75±3/78	5/0±3/10	4/0±3/68	<0.001*
Non-Ritalin	6/88±4/46	4/83±3/87	3/78±4/33	<0.001*
Thinking disorder				
Ritalin	5/28±4/13	4/65±3/67	4/45±5/13	0/64
Non-Ritalin	6/92±5/52	5/39±5/09	4/0±4/66	0/001*
Abnormal behavior				
Ritalin	6/09±4/29	4/78±3/40	4/47±5/52	<0.001*
Non-Ritalin	7/20±5/29	5/43±4/33	3/57±4/27	<0.001*
Aggressive behavior				
Ritalin	14/23±7/43	10/41±5/52	7/54±6/89	<0.001*
Non-Ritalin	13/76±8/40	9/39±6/39	6/23±5/61	<0.001*
Extrinsic disorders				
Ritalin	20/43±10/50	15/20±8/38	12/0±11/90	<0.001*
Non-Ritalin	20/96±13/25	14/83±10/42	9/95±9/53	<0.001*
CBCL				
Ritalin	116/88±24/44	106/17±23/43	98/12±31/83	<0.001*
Non-Ritalin	120/75±37/50	110/40±33/78	93/05±31/60	<0.001*
Anxiety/Depression				
Ritalin	7/0±4/51	5/14±3/2	4/98±4/36	0/006*
Non-Ritalin	6/84±4/62	5/70±4/61	4/91±5/44	0/071
Deprivation/Depression				
Ritalin	4/41±3/63	3/08±2/57	2/49±2/79	0/001*
Non-Ritalin	4/68±3/17	3/61±2/89	2/44±2/97	0/001*
Somatic complaints				
Ritalin	67/12±2/84	66/44±2/06	67/41±4/81	0/33
Non-Ritalin	67/96±4/44	67/39±4/92	66/74±4/58	0/007*
Internalization				
Ritalin	78/69±9/22	74/65±6/76	74/88±10/97	0/001*
Non-Ritalin	79/48±10/99	76/70±11/57	74/09±12/18	0/003*

The results of this study are somewhat consistent with the present study. Similar to Liang's study, in our study, both ADHD-RS and Attention Deficit/Hyperactivity Disorder subscale had a significant decrease from the beginning of the study to the end of the sixth month. However, contrary to the results of Mr. Liang's study, in this study, in relation to the CBCL Behavioral Checklist, the mean score of all its subscales, except for somatic complaints and thinking disorders, in patients treated with Ritalin significantly decreased from the beginning of the study to the end of the sixth month.

Table 5: Difference of total CBCL Score and its subgroups in Ritalin and Non-Ritalin group

Variables	The beginning of the study	The end of the third month	The end of the sixth month
Overall rating CBCL Ritalin			
The beginning of the study	-	0.001	0.004
The end of the third month	0.001	-	0.171
The end of the sixth month	0.004	0.171	-
Overall rating CBCL Non-Ritalin			
The beginning of the study	-	0.003	<0.001
The end of the third month	0.003	-	0.032
The end of the sixth month	<0.001	0.032	-
Ritalin internalization			
The beginning of the study	-	<0.001	0.002
The end of the third month	<0.001	-	0.154
The end of the sixth month	0.002	0.154	-
Non-Ritalin internalization			
The beginning of the study	-	0.002	<0.001
The end of the third month	0.002	-	0.003
The end of the sixth month	<0.001	0.003	-
Ritalin exogenous			
The beginning of the study	-	<0.001	<0.001
The end of the third month	<0.001	-	<0.001
The end of the sixth month	<0.001	<0.001	-
Non-Ritalin exogenous			
The beginning of the study	-	<0.001	<0.001
The end of the third month	<0.001	-	<0.001
The end of the sixth month	<0.001	<0.001	-

Bloom et al. also evaluated the effect of Ritalin on behavioral changes and working memory of children with ADHD and observed that after 3 weeks of intervention, children in the drug group treated with Ritalin had an average score of their ADHD-RS questionnaire. It was significantly improved, but there was no significant difference in working memory performance between the two groups [13]. Based on this, it can be said that the results of this study are similar to the present study because during the current study, both in the Ritalin group and in the non-Ritalin group, both subscales of attention deficit and hyperactivity/impulsivity of the ADHD-RS questionnaire significantly decreased from the beginning. The study lasted until the end of the sixth month.

In a systematic review and meta-analysis, Strobo et al. Found that in ADHD patients, the ratio of male to female patients in all studies was 5 to 1, and the mean age of patients in all studies was 9.7 years. The researchers found that treatment with methylphenidate reduced the mean score of the ADHD-RS questionnaire filled out by teachers. The difference between the mean scores of this variable in the Ritalin treatment group and the placebo treatment group was about 9.6 points lower (CI-6.38 to C-11.75). Finally, this systematic review has concluded that treatment with methylphenidate improves ADHD-RS questionnaire scores [14]. Accordingly, these cases differed from the present study. In the present study, in comparison between the two treatment groups, the mean score of the ADHD-RS questionnaire subscales in the non-Ritalin group at the end of the third and sixth months was significantly lower than the same scale in The group has been Ritalin. While the mean subscale of hyperactivity/impulsivity at the beginning, end of the third month and end of the sixth month was not significantly different between the two groups, this difference may be due to differences in the groups evaluated in the two studies. Finally, in another study, Nasiri et al., using the Connors Parent Questionnaire, evaluated children's behavioral records and children's continuous functioning test.

Before and after the implementation of this method, there was a significant difference in 95% confidence level. While in the case of attention deficit, impulsivity and reaction time before and after the implementation of the combined method and perceptual-motor exercises, there was a significant difference in 95% confidence level, and this result means that all methods focus on the components of attention deficit. Impulsivity and reaction time are effective that these results are almost consistent with the findings of our study [15]. Accordingly, in most studies in this field, it has been observed that there is no statistically significant difference between treatment groups, however, more studies are needed in this field to confirm these cases.

CONCLUSIONS

Based on the results observed in this evaluation, the treatment groups did not notice a significant difference in the symptoms of patients with ADHD, although these symptoms improved in both groups, however, more studies are needed in this area.

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ORIGINAL ARTICLE

Evaluation of the effects of dexamethasone in modulating breathing pattern decomplexification in rats with 2-chloroethyl ethyl sulfide induced lung injury

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Abstract: *Background and Objectives: Sulfur mustard is a functional alkylating chemical warfare agent that gives rise to appalling lung injury. In people with pulmonary diseases, including asthma, the complication of respirational dynamics is reduced. However, the complexity of breathing patterns in lung injury caused by chemical agents is not clear. In the current study, the outcome of 2-chloroethyl ethyl sulfide (CEES), and mustard analogue, upon breathing pattern of rats without or with treatment were reviewed.*

Methods: The interbeat interval (IBI) and respiratory volume (RV) data have been acquired from spontaneous respiration rats with lung injury by CEES using a whole-body plethysmograph. We calculated mean and coefficient of variation, alpha exponent derived from detrended fluctuation analysis (DFA), and sample entropy of IBI and RV.

Finding: Entropy examination of respiratory variation displayed reduced inconsistency (less complication) in the breathing pattern of this rat model of lung injury. The mustard analogue also led to increased lung inflammation in damaged rats. However, treatment by NAC and dexamethasone had a compelling impact on the complication of the breathing rhythm and lung inflammation of rats with lung injury.

Conclusion: Our findings show that inflammation could be the possible origin of respiratory dynamics shifting apart from the normal variation in CEES-induced lung injury.

Keywords: *2-chloroethyl ethyl sulfide, breathing pattern, dexamethasone, Inflammation, modulates, rat model*

INTRODUCTION

Mustard agents have been used in various battles since the First World War and are responsible for more than 80% of all chemical war gas victims [1]. Severe lung damage is recognized to happen in different conditions, encompassing exposure to mustard gas and its subordinates, such as 2-chloroethyl ethyl sulfide (CEES) [2]. Acute lung injury is defined as necrosis in the pharynx and epithelial detachment, bronchioles and, trachea, while long-term effects comprise of fibrosis and, in some cases, cancer [3]. Also, both animal models and human exposure data indicate the inclusion of mediators and inflammatory cells in SM-generated lung injury. After exposure of rodents to SM and CEES by intratracheal instillation or inhalation, inflammatory cells have been spotted in the higher, and lower inhaling tract [4]. Mustard sulfur causes changes in respiratory parameters and inspiratory airflow limitation [5]. However, the mechanisms involved in changing respiratory parameters are not clear. Previous studies have shown therapeutic effects of anti-inflammatory agents, including corticoste roids, in SM-induced lung injury. Wigenstam and colleagues reported that anti-inflammatory steroids, such as dexamethasone reduce the inflammation of passageway for air, lymphocyte activity, and collagen deposition within an hour following the exposure by nitrogen mustard [6]. Furthermore, short-term studies using intravenous and oral N-acetylcysteine (NAC) in patients with chemical lung injury have revealed the potency of this compound to restore pulmonary function tests with a low incidence of adverse

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effects [7]. Normal respiration demonstrates fluctuating dynamics in respiratory volume and rate, arising from the complex relationship between various internal and external climate factors. This system continuously fluctuates under fragile stability to maintain adjustability to stimuli [8]. Pulmonary diseases have a variable medical disorder and show complex behavior, which might be related to a change in the dynamics of the respiratory system toward either too irregular or too regular [9]. Investigating breathing pattern complexity is beneficial to the determination of disease control, severity, blockage, and response to treatment in pulmonary patients. Therefore, understanding the changes in breathing pattern can be contributive to our understanding of the pathophysiology of lung injury and might provide new perception to establish new interventions and curing strategies [10]. In this study, we hypothesized that the complexity of the respiratory pattern is decreased in rats with CEES-Induced lung injury, and since these animals had inflammation, the respiratory pattern could be brought close to natural conditions using an anti-inflammatory agent. To assess this, we compared the complexity of the respiratory dynamics among control and CEES-induced lung injury rats and also reviewed the outcome of N-acetylcysteine and dexamethasone on treating rat models of pulmonary injury.

METHODS

Experimental groups

The Ethical Committee of Baqiyatallah University approved all the protocols.

Specific pathogen-free male Wistar rats, weighing 250 ± 20 g, were randomly splitted into five groups (n = 6 for each group): (i) Vehicle group, which received a single intratracheal dose of vehicle (60 µL of ethanol + 340 µL phosphate-buffered saline (PBS)); (ii) CEES group, a single dose of CEES was intratracheally instilled to the second group (Sigma Chemicals, MO) (2 µL/rat or 6 mg/kg in 58 µL of ethanol + 340 µL PBS) [11] (iii) CEES+PBS group, during intraperitoneally handeling of PBS was (i.p.) one hour after instillation of CEES; (iv) CEES+NAC group, to which NAC was handeled intraperitoneally (i.p.) 1 hour after instillation of CEES (20 mg/kg body weight of NAC in in 400 µl PBS) [12] and (v) CEES+Dex group, in which Dexamethasone was administered intraperitoneally (i.p.) 1 hour after instillation of CEES (10 mg/kg body weight of Dexamethasone in 400 µl PBS [6] Intraperitoneal ketamine (100 mg/kg body weight) was used for intraperitoneal xylazine and anesthesia (13 mg/kg body weight) was used for sedation when required [12].

Recording of respiration

In all groups, respiration was recorded a day before administrating the drugs and 24 h after the challenge. Conscious rats respiration was recorded by a purpose-built whole-body plethysmograph [13]. In short, this comprises of an animal chamber made of a clear Plexiglas cylinder (wall thickness 5 mm, volume 1.32 L, length 300 mm, i.d. 75 mm) with inversely straighten outlet and inlet ports in each wall. To avoid any increase of CO_2 in the plethysmograph, the air in the room was repeatedly pumped into the chamber at a controlled flow rate (4 L/min). A plastic Yconnector was placed at a distance of 10 cm from the outlet of the chamber, and one of the exit ports was linked to one input of a differential pressure probe (MPXV7002DP, Freescale Semiconductor Inc.), while the other input exposed to room air. The plethysmograph chamber was also connected to one input of a CO_2 sensor (MG811, Sandbox Electronics Inc.), through polyethylene tubing connected to a second exit port of the Y-connector, and another input of the CO_2 sensor was exposed to room air. Each test incorporates approximately 15 min early recording period (as the acclimatization time) succeeding by a 60-min main recording period.

Breathing Rythm examination

By using a PowerLab A/D converter (ADInstruments, Sydney, Australia), the breathing signals were digitized at a 10 KHz sampling rate and exhibited on ChartPro 7.0 software. For breathing pattern analysis, a minimum artifact with 20 minutes of data was selected. For calculation of the time-series of respiratory volume (RV) and inner breath interval (IBI), a custom program written in MATLAB software was adapted. Identification and verification of the peaks for each respiratory signal were carried out. Subsequently, the amplitude of peaks and the peak-to-peak time intervals were considered as the RV and IBI series, respectively. Irregularity of breathing pattern was quantified by calculating the sample entropy (SampEn) as an index displaying the degree of complexity. The negative natural logarithm of the conditional eventuality is SampEn that periods within a physiological-series and match within tolerance r for m points will also match for m+1 points [14]. Our calculation showed that SampEn of RV and IBI series are equivalent to 2 for m and 0.2 for r, using MATLAB code available from the physionet (http://www.physionet.org). DFA method can be used for evaluating fractal-like temporal structure of a time series. In this technique, the root mean square of the variation of the combined and detrended data is evaluated within time windows of different sizes and then plotted against window size on a log±log scale. A lineal relationship among log (window size) and log (oscillation) show the attendance of self-similar time-series [15]. The alpha (α) scaling exponent is the slope of this line. This parameter demonstrates the relationship within the time-series inconsiderate of the scale.

Bronchoalveolar lavage cells and Histological assessment

At 2 h after respiration recording, rats were anesthetized and 3 ml of sterile physiological saline was slowly instilled and considerately pulled out into the syringe (3 times) through the endotracheal. Samples were centrifuged, and total cell count was applied on cytocentrifugation prepared stained with Giemsa stain and, BAL cytospin samples (cytospin 3, Shandon Instruments, Pittsburgh, PA) [13].

After BALF collection, animal lungs, which had been removed earlier, were settled in formalin solution, embedded in paraffin, and slashed into 5-lm-thick slices. For identification of airway infiltration of inflammatory cells, the animal lung slices were stained with haematoxylin and eosin (H&E).

For lung inflammation measurement, the percentage of inflamed area to total lung area was considered in H&E stained lung sections [16].

Statistical analysis

For statistical analysis of data, GraphPad Prism V6.0 (GraphPad Software, San Diego, CA) was used. The significance of changes in BALF cells, the breathing pattern, and histological among groups were compared with a one-way ANOVA with a Bonferroni post-test. Results have been introduced as mean ± standard error of the mean (SEM). P-values less than 0.05 were contemplated statistically important.

RESULTS

Lung inflammation analysis

Rats with lung injury caused by CEES (CEES group) had significantly higher levels of total BAL inflammatory cells and airway inflammation ($p < 0.001$) (Figure 1) compared to control group. However, airway inflammatory cells and total inflammation were significantly decreased in CEES group compared to CEES+NAC group ($p<0.01$ and $p<0.001$, respectively) (Figure 1). These parameters also displayed significantly lower measurements in rats of CEES+Dex group compared to CEES group ($p<0.001$) (Figure 1).

Figure 1: Breathing pattern in a representative rat. (a), Representative pieces of the breathing signal exhibit the inter-breath interval (IBI) and respiratory volume (RV), as well as deep inspiration. Original ("raw") IBI (b) and RV (c) time series during 20 min of respiration in a representative rat.

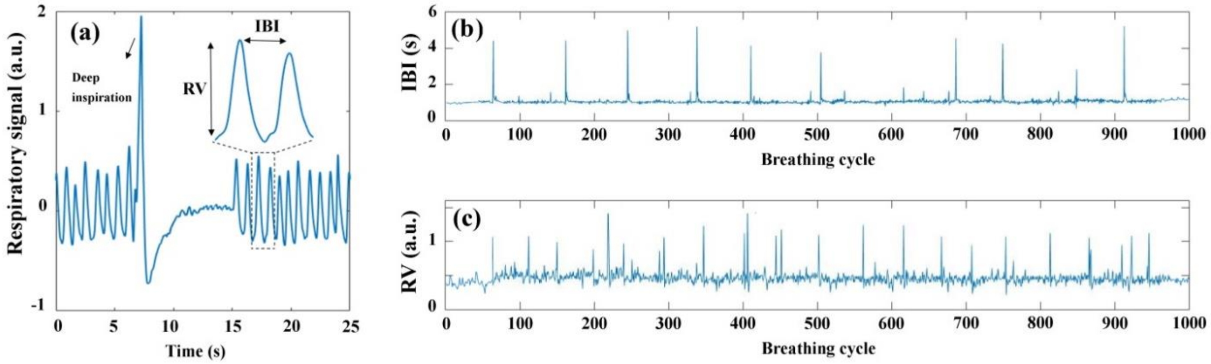

Breathing pattern analysis

The mean respiratory rate (RR) of CEES group was considerably higher compared to the vehicle group ($p < 0.001$). However, there was no significant difference in the coefficient of variation (CV) of the RR (CVRR) between the groups ($p > 0.05$). The mean of RR in CEES+NAC and CEES+Dex groups had significantly decreased compared to CEES group ($p < 0.001$) (Figures 2a and 2b). The mean of respiratory volume (RV) and coefficient of variation of the RV (CVRV) significantly decreased and increased in rats with lung injury by CEES as compared with the vehicle group, respectively ($p < 0.001$ and $p < 0.05$). However, RV and CVRV partially decreased in rats treated with NAC and dexamethasone compared to CEES rats ($p < 0.05$) (Figures 2e and 2f). The index of the irregularity for the Sample Entropy of IBI (SampEnIBI) indicated a significant decrease in CEES group compared with the vehicle group ($p < 0.001$). Similarly, this index has decreased in treatment groups ($p<0.05$). The SampEnIBI of CEES+Dex group significantly increased compared to other groups that received CEES ($p < 0.05$) (Figure 2c). Nevertheless, no notable discrepancies were discovered in SampEnRV among the experimental groups ($p > 0.05$) (Figure 2g). In the CEES group, an index of self-similarity for the slope of detrended fluctuation analysis of RV (DFARV), was greater than the vehicle group ($P < 0.01$).

This index of self-similarity had significantly decreased in animals of CEES+NAC and CEES+Dex groups as compared with CEES rats ($p < 0.05$) (Figure 2h). Nonetheless, among the experimental groups ($p > 0.05$) (Figure 2d), DFAIBI time-series disclose no significant difference.

Figure 2: Effect of administration of NAC and dexamethasone on lung inflammation and bronchoalveolar lavage (BAL) inflammatory cells in CEES-induced lung injury Hematoxylin and eosin (H & E) staining to identify the lung inflammation (Scale bar 50 µm). Data were analyzed by a one-way ANOVA, with the Bonferroni post-test. * p<0.05, ** p<0.01, and *** p<0.001 compared to vehicle, $$ p<0.01, and $$$ p<0.001 compared to CEES, # p<0.05, and ### p<0.001 compared to Dex. (a) vehicle group, (b) CEES group, (c) CEES+PBS group, (d) CEES+Dex group, (e) CEES+NAC group, (f) graph of lung inflammation, (g) total inflammatory cell.

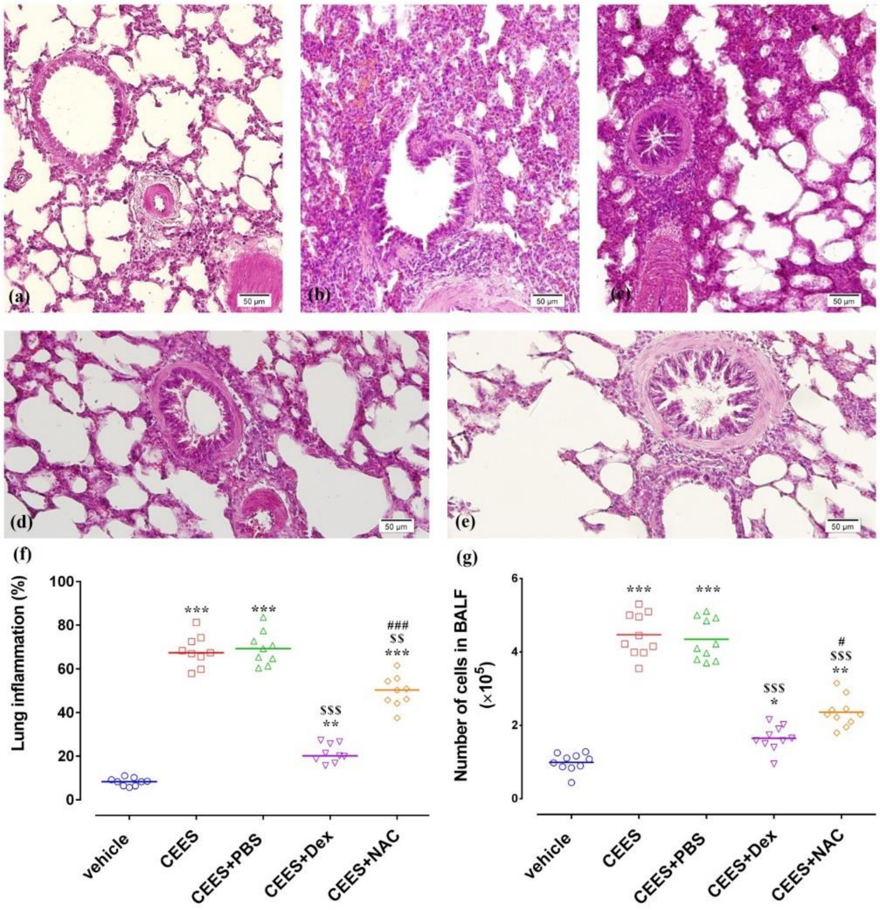

DISCUSSION

Biological functions are intricate and based on a complex feedbacks and network of nonlinear dynamics [9,16]. Physiological variables fluctuate consistently under imbalanced steady-state conditions to retain compatibility to internal or external stimuli [10]. Similar to other physiological systems, the respiratory system is stable and fluctuates under normal conditions. A change in dynamics of a system towards either too irregular or regular may be associated with diseases such as asthma and COPD [10]. Analysis of breathing fluctuations complexity is non-invasive technique and permit us to scrutinize the integrity and adaptability of the respiratory control system. Moreover, this method of analysis has the potential to predict prognosis, severity, and controllability of lung diseases [17].

Evaluation of respiratory dynamics in an animal model of CEES-induced lung injury by complexity analysis of both volume and respiratory rate was carried out. In this animal model, respiratory rate and respiratory volume increased and decreased, respectively. These results are consistent with the previous study where chemical war victims experience cough, dyspnea, tachypnea, and chest pressure after several hours of SM exposure [18]. Some researcher demonstrated that minute volume decreased following sulfur mustard inhalation in guinea pigs [4]. Reduced SampEn values in the IBI time series of rats with lung injury shows higher regularity or low complexity in respiratory dynamics. These findings are consistent with the improved regularity of respiratory pattern observed in other pulmonary diseases such as asthma [19,20]. Previous studies have reported increased variability and α exponent of peak expiratory flow affiliated with the inadequacy of treatment in asthmatic and COPD patients [21,22]. These findings raise the possibility that higher α exponent delineates a firm and deterministic use in lung function [10]. Higher respiratory volume variability (CVRV) corroborates with the reports of respiratory volume variability in other pulmonary diseases such as asthma [16]. Nevertheless the specific pathophysiological mechanisms underlying the reduced complexity of respiratory dynamics in CEES-induced lung injury is not still thoroughly unveiled. Understanding the basis of such variable manner may lead to developing treatment strategies and better-targeted intervention in lung injury by CEES and Sulfur Mustard.

Sulfur mustard (SM) is highly poisonous to the pulmonary system, causing both chronic and acute effects encompassing airway inflammation, obstructive disease, bronchitis, and acute respiratory distress syndrome. Therapeutic Strategies for mitigating SM-induced lung Injury include antioxidants, anti-inflammatory agents, surfactants, and protease inhibitors [24]. In the present study, we reduced lung inflammation and the fluctuation of the breathing pattern by applying dexamethasone and n-acetylcysteine, where glucocorticoid proved more effective than NAC. In other words, inhibiting the inflammation process brings the complexity of respiration closer to normal conditions. It has been assumed that sulphur mustard toxicity is related to lung inflammation, and few of their symptoms may be owing to the permeation of inflammation on the nervous system. In addition, in inflammatory pulmonary diseases such as asthma, it has been shown that influenced inflammation in the nervous system [25,26]. There is some evidence to suggest that peripheral inflammation related to airway allergy may vary the immunologic state of the central nervous system (CNS), such as increased intracerebral levels of IgE and IgG. Furthermore, there is experimental evidence of neuroplasticity and brain inflammation in the nucleus tractus solitaries in animal models [25]. Therefore, regarding these changes in inflammation, the inherent properties of the central nervous system could change and causes the alteration of the respiratory system towards reduced irregularity [16]. However, we did not examine peripheral or central neural activity in the present study, and these subjects can be regarded in next studies.

CONCLUSIONS

The cycle-by-cycle changing of respiratory pattern and volume in rats were examined, and reduced complexity of breathing rhythm in a rat model of CEES-induced lung injury was revealed. According to the results of the respiratory complexity analysis, among the different treatments, dexamethasone had a greater therapeutic effect on breathing pattern decomplexification than NAC. It appears that inflammation processes may be the cause of shifting the respiratory dynamics in this model of lung injury. Future studies are needed to characterize the role of inflammation in decomplexification of breathing pattern and determine the central or environmental factors involved in inflammation in chemical-induced lung injury.

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Conflict of interest

The authors report no conflicts of interest.

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Ethical Statement

The study protocol was reviewed and approved by the Ethics Committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1396.330).

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ORIGINAL ARTICLE

Introducing a new medical method innovative suture technique to reduce scars in central lip lift medical surgery

Seid R. Hosaini[1], **Kamyar Azhdari**[2]

Abstract: *The central lip lift surgery with a new suture technique called; continuous locking suture in three cases is reported in this article. In this novel method, we used a 5.0 chromic suture by applying a 30-degree angle of the entrance to the skin semi-deeply. We start suturing at the middle of the wound,3-4 mm from the edges of the skin. The needle should be way out at the exact point of entrance, and this process is repeated to reach out to the two sides of the wound. More than 90% of patients (in more than 100 cases) were satisfied with the cosmetic results. Moreover, 90% of cases did not demonstrate any scar after three months without laser or reconstructive ointments. We evaluate many patients who undergo central lip lift surgery with this novel technique, and the scar problem was resolved in more than 90 % of cases with no supportive treatment.*

Keywords: *scar, central lip lift surgery, cosmetic results, patients*

INTRODUCTION

The central lip lifts

The first doctor that performed central lip lift surgery was Dr. Austin in 1980 and presented 83 cases in 1986. The lip lift surgery is performed to make the long lip of aging shorter and is designed to excise the wavy ellipse skin that follows the contour of the nostril crease below the sill. However, the nostril sill is not degraded. No attempt is needed to hide the scar in the nose; the reason is that the scar is well-hidden in the fold and is usually undetectable [1-3].

The continuous locking suture is an innovative method in central lip lift surgery that can be applied for at least six possible goals in closing the wounds made by the scalpel and are full-thickness. These goals are as follows: 1) reduce tension in the dermis, 2) reduce scarring after the surgery, 3) reduce suture marks to get an excellent cosmetic result, 4) Reduce the wound healing process to 5-6 days, 5) It is not visible in the surface of the skin. However, none of the other suture techniques fulfills all five above criteria. Those techniques that achieve good epidermal wound apposition will not give dermal support, may cause suture marks, or require wound care such as laser or medications. Those techniques that reduce tension in the dermis will not lead to precise epidermal wound edge apposition. The locking suture is a continuous suture. Three cases are presented in which the continuous locking suture was used in central lip lift surgery. The details of this technique are reported in this article. Finally, the advantages of the continuous locking intradermal suture will be summarized.

CASE SERIES

Case 1

A 40-year-old man clinically had a long distance between his nose and mouth (Figure 1). The area was disinfected with chlorhexidine and draped in the usual manner at the surgery. The lower incision is applied first via a 15-blade scalpel to the skin perpendicularly, reaching out to the fat and muscle junction. After that, the upper incision is made parallel to the lower incision; after finishing the surgery, suspension and closure must be performed accurately. In our novel technique, we start from the middle of the wound, insert the needle via a 30-degree angle for about 3-4 mm to the upper edge of the wound, and then take out the needle at the exact

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entrance point. Then, we perform the exact procedure on the lower edge of the wound and continue the process by the end of the incision. The wound was lastly dressed with transparent dressing and Bacitracin ointment. The patient came back for a follow-up examination in 30 days (Figure 2). At the first postoperative examination, no obvious scar was detected, and the patient was fully satisfied.

Figure 1: The 40-year-old man before the surgery

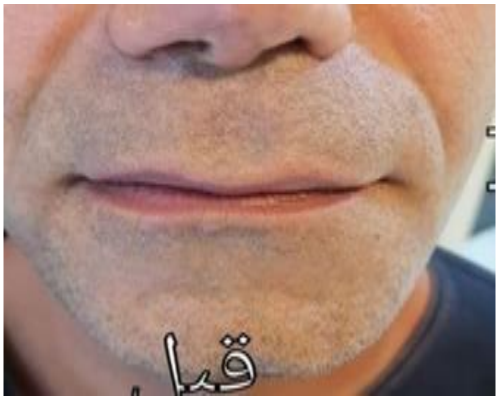

Figure 2: The 40-year-old man 30 days after the surgery

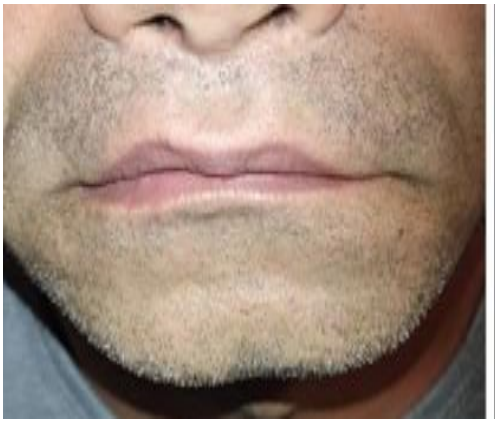

Case 2

Second case was a 40-year-old white woman. At central lip lift surgery, the goal area was cleansed by chlorhexidine. A superficial, vertical incision was made, and the skin and the subcutaneous flap were then excised over the orbicularis, remaining a thin glossy layer of fat intact. The edges of the skin were undermined to reduce the tension. Finally, The wound was closed via a continuous locking suture using 5-0 Vicryl (Figure 3). Bacitracin ointment was administered to the surgical wound. At the follow-up visit 30 days later, the wound edges were fully approximated. No redness or irritation was detected at the surgical site, and the wound was healed completely (Figure 4).

Figure 3: The 40-year-old woman before the surgery

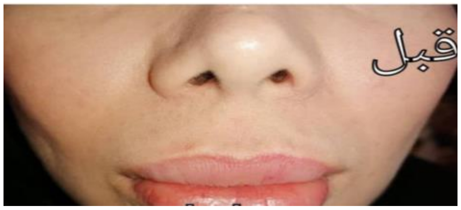

Figure 4: The 40-year-old woman 30 days after the surgery

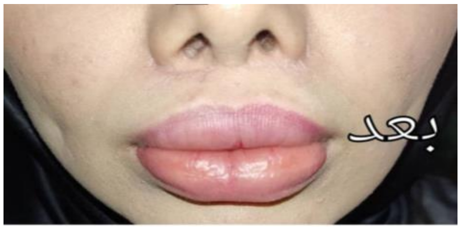

Case 3

A 20-year-old woman underwent central lip lift surgery, the area of the incision was cleaned with chlorhexidine. A superficial, vertical incision was made by the scalpel. The edges of the wound were undermined to decrease the tension. In the end, the wound was closed with 5-0 Vicryl with a continuous locked suture (Figure 5). Bacitracin ointment was administered to the surgical wound. At the patient's first postoperative visit, the wound was closed five days later without any scar. The wound edges were greatly approximated. A follow-up examination at one year demonstrated that the wound healed completely (Figure 6).

Figure 5: The 20-year-old woman before the surgery

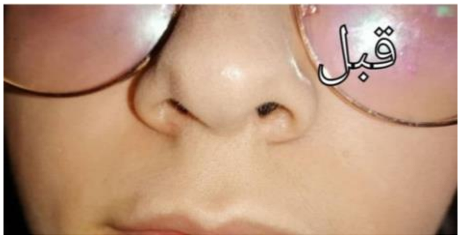

Figure 6: The 20-year-old woman after the surgery

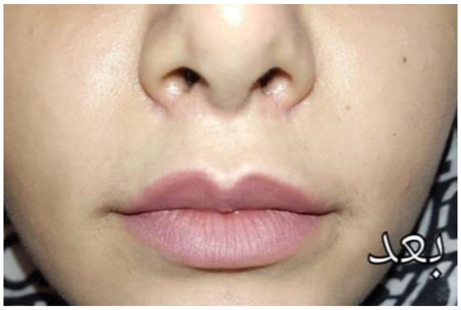

THE CONTINUOUS LOCKING SUTURE

Excision

The procedure is done using local anesthesia, and there is a surgeon at the head of the bed. The lower incision is performed first via a scalpel (15 blades) perpendicular to the skin, reaching out to the junction of the fat and the muscle. The upper incision is made parallel to the lower one in the next step. There is a thin glossy layer of fat which is left after excision of the skin and subcutaneous flap. This intact glossy layer is where vasculature lays, which is deep to the superficial muscular aponeurotic system (SMAS).

Dissection

After the excision process, we elevate the labial flap in a deep sub-SMAS plane. This dissection makes the labial SMAS free from the underlying orbicularis oris. The area of dissection is at the surgeon's discretion, as a larger dissection area may mitigate tension; however, it causes a considerable increase in postoperative swelling. The effacement of the Cupid's bow is probable in full central dissection. In most patients, the dissection extends to the vermillion, lateral to the philtral columns, and just finished before the nasolabial fold.

Suspension and Closure

Simple dermal closure is performed by most practitioners, which is the prevalent mistake [4]. The best lift can be obtained by doing an adequate release of tethering and then suspense the dense tissue upward in a fixed area. When this is done in the upper lip, the skin/SMAS flap released can roll over above the contracted orbicularis.

The continuous locking suture technique

In our novel technique, we start from the middle of the wound, insert the needle via a 30-degree angle for about 3-4 mm to the upper edge of the wound, and then take out the needle at the exact entrance point. Then, we perform the exact procedure on the lower edge of the wound and continue the process by the end of the incision. This technique is called locking because the 30 degrees of entrance and back and force repeatition, let the suture be locked in the skin. This provides a great advantage to push the dermal edges together. In the end, the incision must be closed from the center to the lateral. Then, the skin edges are closely approximated. At the lateral most side, a 4-0 Vicryl suture is passed from inside the pyriform, emerging radially to obtain the SMAS and then come back into the nose to complete a mattress suture. It is better to identify and protect the inferior alar artery.

RESULTS AND DISCUSSION

More than 100 cases had great cosmetic results with very few consequences. In 3 patients which were reported, any slight inflammation resolved within a few days of the first follow-up visit. None of the cases experienced significant discomfort or wound infection. The continuous locking suture combines beneficial features of other common techniques. It is locked for reducing any tendency to loosen. As a result, the apposition of wound edges is better.

CONCLUSION

There are five benefits for continuous locking suture: 1) reduce tension in the dermis, 2) reduce scar after the surgery(more than 90 % of patients did not demonstrate any scar after surgery without laser or other medication), 3)this technique is so effective in central lip lift surgery, 4) Reduce the wound healing process to 5-6 days, 5) It is not visible in the surface of the skin. In this technique, if the applied pressure to the needle increases, it can reduce the area's blood supply when the needle is excised from the nose. This reduction of blood supply can lead to small necrosis. All in all, we should regulate the applied pressure to prevent necrosis in the affected area.

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ORIGINAL ARTICLE

Evaluation of the effect of drug on physiotherapy methods on cytokines and chemokines of Covid-19 patients

Alieh Rezazadeh[1], Mohammadreza H. Rasanani[1], Jalal Nourmohammadi[2], Mohsen Akhondi[3], Zahra S.H. Vaeaz[4], Mahdieh H. Moghadam[5]

Abstract: *Previous investigations have demonstrated that interleukin-6 (IL-6), C reactive protein (CRP), tumor necrosis factor-alpha (TNF-alpha), in Coronavirus disease 2019 (COVID-19) patients are considerably increased, and their progressive elevation are clinical threat indicators of disease severity. The purpose of this systematic review is to evaluate the efficacy of physiotherapy, specifically chest physiotherapy, on different cytokines in Covid-19 and non-covid-19 patients. The main complications and symptoms of this virus are as follows; a dry cough, fever, and progressive dyspnea. Quickly, the coronavirus, which is named SARS-CoV-21, has spread worldwide, causing severe lung inflammation, respiratory distress syndrome, cardiac and renal injury, especially in comorbidities patients. Approximately 96% of the cases experience mild respiratory symptoms; some progress to pneumonia, respiratory insufficiency, acute respiratory distress syndrome, and multiorgan failure. The overall mortality rate per number of diagnosed cases is 4.6%; it can range from 0.2% to 15% according to age and health problems. Combination MeSH and text terms were used to perform the search strategy. Interventions in RCTs and clinical trials with or without comparison were assessed. Six studies met the inclusion criteria. Studies demonstrated that physiotherapy could have an effect on TNF-alpha, IL-6, IL-10, IL-1β and CRP.*

Keywords: *COVID-19, MeSH, CRP, TNF-alpha, SARS-CoV-21*

INTRODUCTION

Severe cases of Covid-19 have been shown to cause a mysterious condition that disrupts the immune system; Instead of attacking only infected cells, the immune system attacks healthy cells as well. This condition, known as the cytokine storm, can be fatal, but researchers have been trying to figure out how the storm works and how it can be treated. A cytokine storm is an abnormal immune response (Figure 1). This reaction is a type of systemic inflammatory response syndrome and can be accompanied by symptoms such as fever, fatigue, anorexia, joint pain, nausea, vomiting, diarrhea, skin complications, rapid breathing, palpitations, hypotension, seizures, headache, hallucinations and tremors. Researchers have now discovered in a new study to look at the progression of coronavirus infection in mice that the cytokine storm is a vicious cycle caused by the overproduction of two signaling proteins called "tumor necrosis factor alpha" (TNF) - "interferon gamma". (INF) is caused by th e body's immune system.

Together, these two proteins lead to serious conditions that can eventually lead to inflammation, organ damage, and even death. But this study in mice showed that using pre-existing antibodies to fight these proteins is an effective way to prevent death in cases of severe Covid-19 infection. The researchers noted that knowing these pathways and the mechanisms that stimulate this inflammation are very important for devising effective treatment strategies. The researchers also identified specific cytokines that activate

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inflammatory cell death pathways and have significant potential for treating Covid-19 and other deadly diseases, including sepsis. Sepsis is a dangerous disease that is caused by an overreaction of the immune system to infection and gradually spreads throughout the body. Cytokines are small signaling proteins that enter the body by immune system cells to tell other cells what to do. These proteins are vital for the immune response because they can limit the spread of the virus, but some of them also stimulate inflammation. Inflammation is a natural part of the immune response in which white blood cells rush to the site of infection to fight infection.

Figure 1: Hypoxia, HIF-1α, and COVID-19: from pathogenic factors to potential therapeutic targets

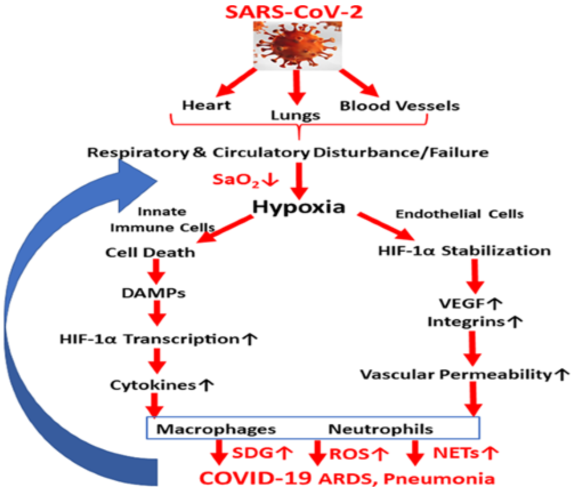
SARS-CoV-2
Heart
Lungs
Blood Vessels
Respiratory & Circulatory Disturbance/Failure
SaO₂↓
Hypoxia
Innate Immune Cells
Endothelial Cells
Cell Death
HIF-1α Stabilization
DAMPs
VEGF↑
Integrins↑
HIF-1α Transcription↑
Vascular Permeability↑
Cytokines↑
Macrophages
Neutrophils
SDG↑
ROS↑
NETs↑
COVID-19 ARDS, Pneumonia

But inflammation can also cause some autoimmune disorders, such as osteoarthritis, in which the body despite the absence of the cause of inflammation causes it. The findings of this study link the inflammatory cell death induced by the "tumor necrosis factor-alpha" (TNF)-"interferon-gamma" (INF) proteins to Covid-19. The results of this study also show that treatment Targets of this cytokine compound could be candidates for rapid clinical trials to treat Covid-19 and other often fatal disorders associated with cytokine storms. [1,2]. The coronavirus family is single-stranded RNA viruses with an approximate diameter of 80 ∼120 nm. It includes 4 types: α-CoV, β-CoV, δ-CoV, and γ-CoV. Bats are the primary critical natural hosts. About 35% of their carried viruses are coronaviruses [3]. The COVID-19 is a novel coronavirus with a circular shape and approximately 60 to 140 nm [3].

In most of cases, Coronaviruses (CoVs) targeting the human respiratory system, which are the leading cause of Middle East respiratory syndrome, severe acute respiratory syndrome (SARS), and lastly, coronavirus disease 2019 (COVID-19) [4-6]. Complementary treatments, including; chest physiotherapy and therapeutic massage, are safe and noninvasive strategies that decrease the adverse side effects of disease [8]. Physiotherapy and Massage are restorative therapy involving the systematic application of pressure with the aim of improving blood and lymph circulation [7-9]. Until now, there is some lack of medical evidence for the treatment of Covid-19 in patients, and it is critical to find a low-risk and effective way to manage the Covid-19. In this investigation, we aimed to assess the effect of chest physiotherapy on the inflammatory cytokines of Covid-19 patients (Figure 2).

Figure 2: The signal pathways and treatment of cytokine storm in COVID-19

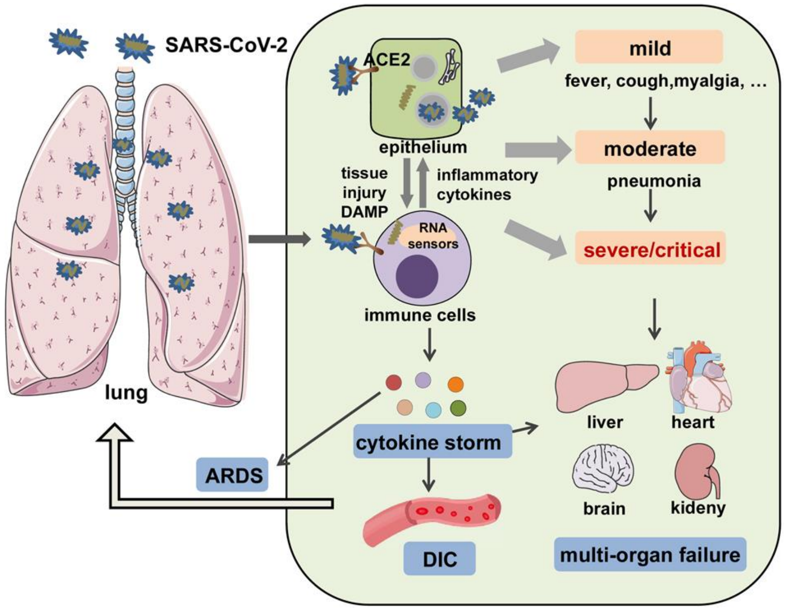
SARS-CoV-2
ACE2
epithelium
mild
fever, cough,myalgia, ...
moderate
pneumonia
tissue injury DAMP
inflammatory cytokines
RNA sensors
severe/critical
immune cells
lung
liver
heart
cytokine storm
ARDS
brain
kideny
DIC
multi-organ failure

METHODS

Inclusion criteria for study selection

Type of study

We will include investigations related to chest physiotherapy in patients with COVID-19. we will search for investigations in English; all articles included are clinical trial and randomized controlled trial (RCT) type articles.

Type of participant

All patients with COVID-19 are included regardless of sex, age, economic status, race, and education. Children, Pregnant women, postoperative infections, and patients with severe cardiovascular and other complications are not included in this systematic review.

Type of outcome measure

The primary outcome was the influence of chest physiotherapy on the cytokines of patients.

Data collection and analysis

Selection of studies

Our investigation is in line with the Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) guidelines flow chart to demonstrate the process of selecting literature for the entire study. Before searching the articles, all reviewers debated and determined the screening criteria. After that, the two reviewers separately reviewed and screened the articles. They screened the titles and abstracts of the articles to get related articles and then removed some duplications and articles with incomplete information. We also tried to obtain the full text, and the articles were managed using EndNote software. By the end, Discrepancies were resolved by consensus. The whole process is shown in Figure 3.

RESULTS

Six investigations which included in our study, two of them assessed the effect of Tai chi in the level of insulin, insulin-like growth factor (IGF)-1, insulin growth factor-like binding protein (IGFBP)-1, IGFBP-3, and cytokines interleukin (IL)-6, IL-2, and interferon (IFN)-γ and other investigation evaluate the effect of physical therapy modalities on inflammatory cytokines and chemokines. These studies demonstrated that physical therapy modalities could significantly diminish the monocyte production of proinflammatory cytokines and proinflammatory gene expression. However, physical therapy modalities like tai chi may increase the production of anti-inflammatory cytokines.

Macrophage activation syndrome is characterized by a marked increase in proinflammatory cytokines called storm cytokines. This severe inflammation, without any therapeutic intervention, leads to severe tissue damage and eventually death of the patient. Research has shown that specific cytokines, especially TNF-α, IL-6, and IL-1β, are involved in this phenomenon. Macrophages produce large amounts of these proinflammatory cytokines when stimulated by macrophage activation syndrome. Inflammation upsets the exact balance between coagulation and fibrinolysis.

Certain inflammatory cytokines, such as TNF-α and IL-1, initiate the production of tissue factors from monocytes and macrophages, which leads to the activation of coagulation. IL-1 and IL-6, on the other hand, increase the production of plasminogen activator inhibitors. Therefore, the overproduction of inflammatory cytokines causes intravascular coagulation. Cytokine storm is a condition of the immune system in which various immune cells are highly activated and produce large amounts of cytokines; This in turn leads to excessive inflammation, which is often associated with multiple organ failure and high mortality. Inflammatory cytokines or various chemokines such as tumor necrosis factor (TNF-α), type I and II interferons, interleukins (IL-1, IL-6, CCL2 or monocyte-chemotactic protein (MCP-1), as well as cytokines Immunosuppressants such as IL-10 or β-TGF- have been implicated.

Cytokine storm has been observed and discussed in various clinical conditions such as rheumatic or blood disorders. In addition, it sometimes occurs in infectious diseases and makes the patient resistant to treatment. This is associated with the development of acute respiratory distress syndrome (ARDS), which is one of the most severe pathogenic disorders of the respiratory system and causes pulmonary edema, decreased gas exchange and a lack of lethal oxygen. It has recently been suggested that cytokine storms, particularly acute respiratory distress syndrome, are involved in and exacerbate pneumonia associated with the Covid-9 virus. Although the body of most patients with Covid-19 does not show any symptoms of pulmonary disorders, even mild ones, about 20% of patients develop severe pulmonary dysfunction. It is not clear why only a fraction of patients with Covid-19 develop severe inflammation, but the Covid-19 virus appears to infect certain types of cells, such as endothelial vessels in the lungs or cells in the alveolar wall or macrophages.

Infection of cell types may induce immune responses that trigger a cytokine storm, including macrophage activation syndrome. Severe inflammation associated with Covid-19 disease, which can be detected by coronary artery bypass grafting in Ahvaz, is clinically similar to macrophage activation syndrome. There have been reports of severe increases in IL-1β, IL-2, IL-6, IL-7, IL-17 and TNF-α in patients with severe pneumonia caused by Covid-19. It is noteworthy that the levels of IL-2, IL-7, TNF-α, G-CSF, CXCL10, CCL3 and MCP1 were higher in the plasma of patients treated in the ICU than in patients who were not admitted to the ICU.

In addition to elevated cytokines, elevated serum levels of ferritin, CRP, and D-dimer also indicate severe inflammation similar to macrophage activation syndrome and fibrinolysis in Covid-19 patients. Immunological and pathological manifestations of this disease are mainly observed in the lung, which is associated with acute respiratory distress syndrome (ARDS). Clinically, acute respiratory distress syndrome is associated with hypoxia, extensive pulmonary infiltration, pulmonary edema, and decreased lung adaptation. Which in general leads to respiratory failure. Plasma levels of TNF-α, IL-1β, IL-6 and IL-8 are elevated, indicating severe acute respiratory distress syndrome in the deceased compared to survivors. It is reported that about 20% of patients develop acute respiratory distress syndrome during Covid-19 disease, and some patients deteriorate rapidly and die.

Recent genomic studies have shown that the enzyme converter angiotensin-2 (ACE2) and serine membrane protease (TMPRSS2) are expressed at the level of type II pneumocytes and cause SARS-CoV-2 virus to enter target cells. The onset of local inflammation due to SARS-CoV-2 infection activates macrophages at that site and spreads rapidly throughout the lung. This is likely due to the abundant expression of virus receptors, angiotensin-converting enzyme 2 (ACE2) and serine membrane protease (TMPRSS2). The accumulation of immune cells accelerates the rate of pneumonia to acute respiratory distress syndrome (ARDS). In severe cases, local inflammation inside the lungs cannot be relieved, resulting in multiple organ failure and death. The result is shown in Table1

Table 1: The results of physical therapy modalities

First author	Published	Population	Intervention	Cytokines	
Janelsins et al. [10]	2011	Breast cancer	Tai chi	IL-6 , IL-2	The effect of Tai chi in the level of insulin, insulin-like growth factor (IGF)-1, insulin growth factor-like binding protein (IGFBP)-1, IGFBP-3, and cytokines interleukin (IL)-6, IL-2, and interferon (IFN)-γ
Irwin et al. [11]	2012	Adult	Tai chi	IL-6	significantly diminished the monocyte production of proinflammatory cytokines and proinflammatory gene expression
Cetisli-Korkmaz et al. [12]	2021	ICU patients	Whole body vibration (WBV) exercise	IL-10	Whole body vibration (WBV) exercise increases the production of anti-inflammatory cytokines
Parry et al. [13]	2012	Individuals with sepsis and covid-19	functional electrical muscle stimulation-assisted supine cycling	TNF-α and IL-1β	may drive mitochondrial oxidative stress and increase intracellular calcium
Adly et al. [14]	2020	Patients with stage one pneumonia	thoracic lymphatic pump techniques and thoracic inlet	Cytokine storm	the thoracic lymphatic pump techniques and thoracic inlet release have been shown to increase chemokines and cytokines in the thoracic
Lavretsky et al. [15]	2012	Depressed patients	Tai chi	The decline in the inflammatory marker, C-reactive protein	This study reported the tai chi effect on the level of CRP and inflammatory markers

DISCUSSION

With the quick enhancement in the number of COVID-19 patients, the number of cured COVID-19 patients is increasing, and the number of patients recovering from COVID-19 is also rising. Patients with COVID-19 often experience the series of respiratory symptoms and complications. There are a few evidence that the physical therapy modalities can have an effect on the level of cytokines and chemokines through the body. All in all, chest physiotherapy or other physical modalities are noninvasive methods, which can affect Covid-19 management, especially patients in the recovery period. However, a few numbers of studies worked on the effect of chest physiotherapy on cytokine and chemokines in Covid-19 patients. Moreover, further investigations are required to evaluate the exact effect and mechanism of these therapies on Covid-19 patients to better manage this outbreak.

The researchers found that the coronavirus behaves differently, turning off genes that prevent the virus from replicating, but does not affect immune cell-calling genes, and that these genes can function normally.

This causes the virus to multiply and overproduce immune cells that invade the lungs and other organs, resulting in excessive inflammation. The team at Icon Medical School in New York says early treatment of patients should focus more on repairing the pathway blocked by the coronavirus than on inflammation. "One thing is to tell all the cells around to be amplified, and the second thing is to send the cell," Dr. Benjamin Tanveer, a virologist, and professor of microbiology at Aikan Medical School, told DailyMail.com "More professional immunity to the site of infection." Cells in our bodies usually have two groups of antiviral genes: interferons and chemokines. Interferons are messaging proteins that are released by infected cells and are named because of their ability to prevent the virus from multiplying. Chemokines are small molecules that attach immune cells to the site of infection so that they can target and kill the virus. According to Tanveer, the first set of genes control the virus for about 7 to 10 days, so the set second has enough time to prepare the immune cells for the attack. "Interferons are called 'defense's' genes, and chemokines are called 'support's' genes,".

They are neutralized and destroyed." "Even the first line of defense, the 'defense call', is often enough to completely stop the proliferation and neutralize the infection even without a second response." However, unlike the flu or severe acute respiratory syndrome (SARS), the coronavirus shuts down a number of genes and activates other genes. An animal model of a date rat was examined. In the case of coronavirus, there was a very mild response to the "defense call" genes - which prevent the virus from replicating - and a significant response from the "support call" genes.

Figure 3: Flow Chart of this study

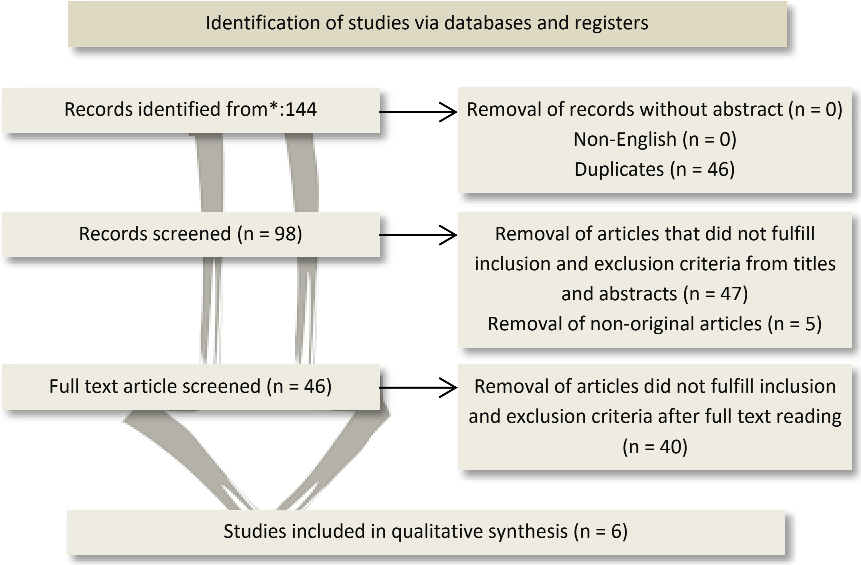
Identification of studies via databases and registers
Records identified from*:144
Removal of records without abstract (n = 0)
Non-English (n = 0)
Duplicates (n = 46)
Records screened (n = 98)
Removal of articles that did not fulfill inclusion and exclusion criteria from titles and abstracts (n = 47)
Removal of non-original articles (n = 5)
Full text article screened (n = 46)
Removal of articles did not fulfill inclusion and exclusion criteria after full text reading (n = 40)
Studies included in qualitative synthesis (n = 6)

"The combination of the two is a bad combination," Tanvir said. When they examined the lung cells of two patients with Covid 19 who had lost their lives, they found that they were giving exactly the same answer. "It begins to multiply, and the infected cells at the site of proliferation do not report the infection well, and as a result, the infection spreads to the lungs." This means that the virus is still multiplying because there are no interferons, but those cells still need to call for support. So different types of immune system cells - neutrophils, macrophages and lymphocytes - come into play, but when they arrive, there is nothing to control the viruses. "And more has reached the lungs, but the cells are calling for support."

Now, the lungs have immune cells such as macrophages and neutrophils that lead to more inflammation. Basically, the body's immune system revolves around itself. This is likely to cause cytokine storms, which occur when the body fights its own cells and tissues instead of fighting the virus. "The behavior of this virus is such that I have not seen in 20 years of studying how cells respond to viral infections.

There are two ways to treat patients. The first group consists of people who have just shown symptoms," says Tanveer.

And their test results are positive. "They can take drugs that inhibit the defense genes so that the virus can behave more like the flu,".

For those hospitalized and intubated, it is too late to use this treatment, so they can benefit from a group of drugs called interleukin-6 and interleukin-1 inhibitors. Help reduce cytokine storms and inflammation throughout the body.

Modulation of biological reactions plays an important role in improving body function following immunological challenges.

Echinacea is one of the best and most effective plants for modulating the activity of the immune system, and the results of laboratory studies and clinical experiments have confirmed this claim.

The effects of this plant on stimulating the immune system and promoting the body's defense are manifested by increasing the activation of phagocytosis, stimulating fibroblasts, increasing the activity of leukocytes and increasing the production of cytokines.

The anti-inflammatory effects of the plant are also effective in regulating the immune response in suppressing infection and preventing cytokine storms.

CONCLUSIONS

Physical therapy modalities like chest physiotherapy or tai chi can be beneficial in managing covid-19 because of their effects on cytokines and chemokines through the body. Nonetheless, further evidence is needed to evaluate the exact effect of these interventions. What is certain is that the SARS-CoV-2 virus threatens the lives of millions of people around the world. By better understanding the nature of the virus, experts have been able to prevent its widespread spread in certain parts of the world. However, no effective drug or vaccine has yet been developed. Severe cases of Covid-19 disease are often seen with acute respiratory distress syndrome, with clinical and laboratory features similar to macrophage activation syndrome. Previous studies have shown that the symptoms of macrophage activation syndrome improve with anti-cytokine therapy. So far, anti-IL-6, IL-1 and TNF therapy have shown promising results in the treatment of macrophage activation syndrome. Based on these findings, treatment with anti-IL-6 or IL-1 drugs has been performed in Covid-19 disease and has caused significant improvement in patients so far! There are currently more than 1,900 clinical trials worldwide, and it looks like these studies will soon provide a new and effective treatment for Covid-19.

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ORIGINAL ARTICLE

Medicinal plants effective in the treatment of glaucoma from the perspective of traditional Persian medicine

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Abstract: *Traditional Persian medicine (TPM) is one of the richest medical schools in the world, which has nurtured valuable books. From Ibn Sina's law to Heravi's successful medicinal facts, all emphasize the treatment of various diseases, especially eye diseases based on herbal medicines, as well as their prevention. In this study, by reviewing the authoritative books of Traditional Persian Medicine, we are looking for medicinal plants so that they can be used in the treatment of glaucoma or glaucoma. the purpose of this study is not to express the complete cure of glaucoma, but this article seeks to classify plants that from the perspective of Traditional Persian Medicine can be used as complementary medicine alongside conventional medical treatments.*

Methods and Materials: This is a summative qualitative content analysis that focused on Traditional Persian Medicine (TPM) texts from 2th to 13th AH centuries. Literature was searched during centuries 4th to 13th AH, and after described of glaucoma, extracting and classifying plants.

Results: The scholars of TPM have opinions in the field of recognizing, describing and treating eye diseases and defining practical words. In almost all books of general Persian PM, the diagnosis and treatment of eye diseases have been discussed. Glaucoma in Traditional Persian Medicine is called "black water".

Conclusion: Unlike other medical schools in the world, in addition to treating diseases, Persian medicine has a strong recommendation to prevent the occurrence of disease and also to strengthen the body's visual powers by using 6 principles of maintaining health along with herbal medicines.

Keywords: *traditional medicine, traditional Persian medicine, glaucoma, classify plants*

INTRODUCTION

The discovery of drugs in the modern era began in the nineteenth century with the separation of active compounds from plants, such as morphine, from opium [1]. Initial treatment for glaucoma relied on herbal remedies, but the method of preparation of these drugs was raw and experimental [2]. One of the first herbs used for glaucoma was pilocarpine - a parasympathetic alkaloid extracted from a South American shrub, Pilocarpusmicrophyllus [3]. Pilocarpine causes the ciliary muscles to contract, which facilitates the outflow of fluid from the trabecular meshwork into the iridocorneal angle of the eye [4]. Although pilocarpine is still available for glaucoma management, its use has declined due to the availability of modern anti-glaucoma drugs with safe side effect profiles [5]. Glaucoma is a major cause of irreversible vision disorders and blindness worldwide [6]. The current standard of glaucoma care is intraocular pressure (IOP) reduction through medical, laser, or surgical treatment [7]. However, the use of alternative

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therapies such as herbs, vitamins and minerals has become more common. Alternative therapies (methods other than conventional medical therapies) have a global market of $ 109 billion and are widely used by 52% of the general population [8,9].

In the authorities of Traditional Persian Medicine, visual impairment has been considered as "impairment of vision" and it has been defined as any impairment in the practice of seeing. In this disease, even with effort, objects can not be seen as they are and in practice, error occurs [10]. The approach of Traditional Persian Medicine in the treatment of eye diseases such as glaucoma is to protect health based on lifestyle modification and emphasizes the importance of prevention. In the field of modern therapy, although drug therapy and other invasive therapies are used to treat patients, but lifestyle modification, especially the emphasis on nutrition, has broadened the horizons of researchers in a variety of diseases, including eye diseases [11,12]. Ancient Persian physicians specialized in the diagnosis, description, and treatment of eye diseases, as well as in the definition of practical terms. Almost all books of Traditional Persian Medicine have diagnosed and treated eye diseases. In addition, several specialized ophthalmology books have been written from the perspective Traditional Persian Medicine [13]. About 25% of all medicines prescribed are made from herbs. Medicinal plants are less known for their low toxicity, effectiveness and side effects. A wide range of herbs have been found to be effective in treating eye diseases such as cataracts and glaucoma in complementary medicine schools, such as Ayurveda and Chinese medicine [14]. Studies have shown that the probability of discovering a new effective drug using traditional medical experiments increases by up to 40% versus random examination of drugs experimentally.

Therefore, searching for traditional reference books may be an effective way to find new drugs [15]. The purpose of this study is to provide a classified list of medicinal plants mentioned in Persian traditional medicine sources that after clinical studies can be used to enhance vision or treat poor vision.

It is worth noting that the vast majority of herbs in this study have not been used in clinical trial studies, so readers should refrain from any arbitrary use of these compounds if they do not have access to a competent physician.

METHODS AND MATERIALS

This research is based on the medicinal plants mentioned in the books Traditional Persian Medicine that somehow affect the improvement of visual function. As described, the purpose of this study is not to express the complete cure of glaucoma, but this article seeks to classify plants that from the perspective of Persian Traditional Persian Medicine can be used as complementary medicine alongside conventional medical treatments. Thus, from the books of the truths of medicine, the gift of the believers (Momen Tonekaboni), was used. The keywords "Ein", "Basar", "Basra" and "Magnifying" were used to evaluate the plants effective in increasing visual acuity and glaucoma. In such a way that any plant that has more than one of the above keywords, was selected as a plant that affects the visual status and increase the power of vision. The results of the studies can be seen briefly in Table 1.

Methods

This article is a summative qualitative content analysis that processed in seven classic steps:

The research questions to be answered: “What is glaucoma in the Persian medicine texts, we don’t find any disease that called as “Glaucoma”; To find the name of “glaucoma” disease in the context of Persian medicine, we tried to collect all data’s about glaucoma in the context of up to date modern medical texts; This information includes etiology, symptoms, signs, risk factor and treatment of glaucoma. And then we tried to find one disease have most overlap with glaucoma. As a result, glaucoma in Persian Medicine texts is known as “Ab e siyah”or black water” [1].

1. Selecting the sample to be analyzed: In this study, all of available Persian Medicine books and texts. These texts included manuscripts (the book itself or its electronic file) in different centuries from 11 to 20 A.D. we studied and searched historical books are in Table 1.

In the second step, the databases were searched by using key words of “glaucoma’s etiology, prevention and treatment” in PubMed, PMC, Google Scholar and Scopus.

2. To identify explicit versus euphemistic terms: Word frequency counts for each identified term calculated with source, to contextualize the codes. Modern equivalent terms were identified.

3. Coding process: three titles: definition and Name (Nam e Bīmārī); ethology (Asbāb va ʿElal); And treatment (Darmān). According to Persian Medicine text, we coded and categorized all data under these titles. We tried to translate these finding from Arabic & Ancient Persian to modern medicine language [1].

4. Defining the categories: All data was classified in three categories: definition, etiology and treatment of “Ab e siyah” [1].

5. Trustworthiness: Credibility, conformability & dependability: Given the explanation of different stage of methods such as key words, codding process, and etc. Anyone who has the ability to read and understand Arabic & Ancient Persian language & texts, can derive similar results.

6. Analyzing the result [26,27]: According to summative qualitative content analysis and given the skill of the authors of this article, we tried to collect all data about the prevention and treatment of glaucoma and introduced several plants as one of complementary treatment for effective treatment for glaucoma.

Table 1: Historical books which are used

Author	Title of the Book	Date Written	Subject
Ibn Sina	Book of law		
Ibn Nafis Gharshi	al-Shamil fi al-Sinaatah al-Tabiyyah		
Ali Ibn Hussein Ansari Shirazi	The authority of badi'i		
Mohammad Hussein Khorasani	The reservoir of medicine		
Ḥakīm Muʾmin M.	Tuḥfat al Muʾminīn	17th century	Traditional Medicine and Pharmacology
Razī M/Rhazes/Rasis	Al Ḥāvī fi al Ṭibb	10th century	Medicine
ʾAhwāzī A./Haly Abbas	Complete book of the medical art (Kāmil al-Ṣināʿa al-Ṭibbīya or Royal Medicine)	10th century	Medicine
Bīrūnī M.	Al Ṣaydana	11th century	Pharmacology
Avicenna/Ibn Sīnā H	Canon on Medicine (Al Qānūn fi al Ṭibb)	11th century	Medicine
Ğurğānī SI.	Ḏaẖīra ẖārazmšāhīī & Al ʿAġrāḍ al-Ṭibbīya wa al mabāhiṯ al ʿAlāīya	12th century	Medicine
Ibn Bayṭār	Tafsīr-u Kitāb discoridus	13th century	Medicinal Herbs

RESULTS

The scholars of traditional medicine have opinions in the field of recognizing, describing and treating eye diseases and defining practical words. In almost all books of general Persian medicine, the diagnosis and treatment of eye diseases have been discussed. Glaucoma Traditional Persian Medicine is called black water. This disease occurs in the moisture of the testicles (clear fluid), the causes of which are not out of two cases; It is either in quantity or in quality of testicular moisture [16]. This disease, ie glaucoma, has not been given special attention in Persian medicine textbooks and has been treated more under the title of eye diseases, and some reports have been recorded from them. Identified a specific disease as glaucoma; However, according to the anatomy mentioned in Persian books and the study of eye diseases in Persian medicine sources, it seems that glaucoma may be one of the diseases of testicular moisture. In Akbari Medicine, in the discussion of eye diseases, Volume 1, in the topic of testicular moisture, there are topics that seem to be compatible with glaucoma. This moisture is considered similar to the whiteness of the testicles in terms of consistency, purity and color, and the benefit of this moisture in front of the glide (eye lens) is that the strong lights are gradually placed on the glide and because of this, the glide is irritated and constipated. Preserved [17]. One of the diseases of this moisture is considered to be its large volume and it continues that if it was a small increase due to the lack of transparency that makes the majority of the necessary components, it would fail to adapt the ghosts to Jalidiyah and the radius would reach the natural duct. The volume should be more than that, in which case, the insight shakes itself, imagining in front of itself that the sea is standing and in the state of full stomach and back sleep, weakness in insight prevails and also when hunger in the middle of the day is not so weak. The patient sees the unlikely things better than the near objects, and the mutation is that the soul becomes dirty due to the abundance of this moisture, and because they do not move farther, its concentration is softened and its consistency is not moderated. And effort) can not be seen; Because in Absar, the relative sensations of the soul do not move so much that its concentration becomes soft [17].

Remedy

The first purification of the body should be done in the kitchen of Sajj and then with the purification of Sarhabn[17]. The body and the nose [18,19]. In removing the blockage and reducing the moisture of the testicles, drugs containing patience and halilah are used in the sources of traditional medicine [16]. In general, the symptoms mentioned in the books of Persian medicine, testicular hyperhidrosis seems to be consistent with the symptoms of chronic glaucoma. Visual impairment in different forms that exists in chronic glaucoma, in the increase of testicular moisture, which is the moisture between the cornea and the lens, these symptoms are also present in Persian books.

Elliptical moisture

From the point of view of traditional medicine, aqueous humor is behind the iris and anterior arachnoid. Aqueous humor has several roles, which is to protect the lens, to protect it from both natural internal heat and heat from strong light coming from outside, and to be a barrier between the lens and the iris so that the two do not intersect [18,20-22].

A. Quantity of testicular moisture

It should be less or more and smaller or bigger, and whenever it is more or greater than the natural amount, it should be veiled between the moisture of the jelly and between the outside light. His sign is that hunger and austerity are beneficial, and in the heat, it is better for half a day, and in the morning and evening it is worse, and the heat is harmful, and whenever it is less or less, it is due to the deterioration of the testicles. Analysis is repelled and dryness prevails. His sign is that the blackness of the eyes becomes smaller and is worse when he is hungry and in the heat for half a day, and mathematics is harmful, and baths and more food are increasingly beneficial [18].

B: Quality of testicular moisture

What is in quality or is a plague in consistency or is in decline in Loon or plague in wetness and dryness and what is in consistency is such that some moderate consistency is a little thicker and God cannot see this cause as far as he is and What is close, as it should be, cannot be seen by the good, and some of the temperate textures turn a lot and become hard and thick, and this is of two kinds, or some of its components become hard, or all of it, and if all of it becomes hard, it thickens the vision. And his ruling is the ruling of water is the same, and if some of his components are concentrated, it will reduce vision [18].

Maintaining health (maintaining health) in the eyes

A study in the sources of Iranian Persian medicine shows that the sages believed that the prevalence of eye diseases in winter is lower than in other seasons. In summer, due to the frequency of ascents, and in Rabi, due to the flow of substances, and in the opponent, due to the corruption of substances, it is more, and it is difficult to diagnose eye diseases, whether it is in the layers or moisture of the eyes, or in the nerves or partners. The temperament of the eye is a function of the temperament of the brain, on the other hand, because the eye has many arteries, most of it is temperate; because it requires a lot of movements and movements of the solution, and it is not necessary to make everyone's temperament hot. In traditional medicine for the treatment of eye diseases, the three principles of causal action, strengthening all the powers of the eye and purifying the eye from wastes have been mentioned as general rules. Eye diseases are some external, such as remedies, and some esoteric ones are easily defined, such as the state of the soul of Basra in its abundance and tenderness, and the like, and some esoteric ones are defined as pests that occur in moisture, and some esoteric ones are estimated, such as pests in the inner classes or The optic nerve is caused by mood swings and the like, eye diseases. Constipation and violence need to be corrected with violets and the addition of a little bit of sage. Iarj fiqra removes substances from the head if it is concentrated. The important point is that most eye diseases are caused by the organ of participation and it must be the members of the head, and most of this is when it occurs.

Harms of the eye

Lots of things may harm the eyes: dust and smoke, very hot and cold weather, wind, toxins, all kinds of things like too much sex, too much salt, long-sightedness towards shiny and bright things, excessive white like lightning and bright white, sun, snow, durability One thing, the setting of precise lines to extremes, too much incarceration, the frequency of crying, the intense sadness, the delusions and the rage, the study between evening and dusk, all the good news like garlic, beans, beans, onions, lentils, eggplant, mustard and blackberries, multiplicity strong laxatives, including abdominal astringents. But diarrhea is sometimes beneficial to drinking medicine and sometimes to congestion, and this is more beneficial in ingesting substances, and injections are beneficial to all material diseases of the eye, and the most agreeable laxatives in eye diseases are Halilah; Because it strengthens the stomach and prevents the excretion of substances, but due to the treatment of the eyes in a way before purification is not permissible [23]. In the book of Akbari medicine, he writes: The cure for eye diseases is of four genders, one is to change the simple temperament, the second is to purify the substance, the third is to manage the division of the joints, and the fourth is to correct the heat of the eye and reduce it. There are seven ways to make the eye vomit. Second, if the body is smooth, first clean the body from bad sputum. Thirdly, it is useful to clean the nose with meningeal sprays and to gather in the jugular vein of the veins that are inside the head. The use of sneezes has an effect on enemaing the eyes, but they should not cleanse the body until the substance is applied, which should not be done. It is harmful. The purity of the blood vessels of the eye is also conditioned by the priority of purification, so that the harm is safe and there is no harm where the body is clean. Among the drugs used in Persian medicine to empty the feces of the head, it seems that the drug of patience is a suitable drug to achieve the above goal. Other drugs include Halilah, which is frequently mentioned in the sources. Herbal medicine is the oldest form of treatment, known to mankind and has been used for a long time. The use of this method of treatment has a history in all civilizations and is an important component in the development of common medical science. The important point in all eye diseases mentioned in the sources is that the elimination of feces and its emptying and softening of nature are the general principles of treatment in the majority, and for this purpose, in most of the above cases, the love of patience and reason has been suggested.

Table 2: Plants identified from sources and references, in order from right to left, including Persian name, scientific name of the plant, method of consumption and references

Used reviews	How to use	Temperament	Scientific name of the plant	Persian name of the plant
[24-29]	Food	Warm and dry	Thymus capitatus	Thumon
[24-29]	It's my head.	Warm and dry	Peganumharmala L.	Espand
[24-29]	It's my head.	Warm and dry	Commiphoragileadensis	Arabic Bilal
[24-29]	Eye drops	Warm and dry	Marrubium vulgare L.	Mountain mess
[24,25, 27-29]	Food	Warm and dry	Rutagraveolens L.	Ruta graveolens
[24-29]	It's my head.	Warm and dry	Foeniculum vulgare L.	Foeniculum vulgare
[24-27, 29]	Food	Warm and dry	Allium cepa L.	Piyaaz
[25-29]	Food	Cold and dry	Terminalia bellerica	Belleric myrobalan
[26-29]	It's my head.	Warm and dry	Ferula assa-foetida L.	Ferula assa-foetida
[25-29]	It's my head.	Cold and dry	Solanum nigrum L.	Solanum nigrum
[24-29]	It's my head.	Warm and dry	Cinnamomumzeylanicum	Cinnamon
[25-29]	It's my head.	Cold and dry	Rheum ribes L	Rheum ribes
[25-29]	It's my head.	Warm and dry	Calotropisproceradryand	Calotropis procera
[24, 26-29]	It's my head.	Warm and dry	Aloe vera L	Aloe vera
[24-27]	It's my head.	Warm and dry	Zingiberofficinaleroscore	Zingiber officinale

As can be seen from the plants mentioned in Table 1, the vast majority of plants affecting the visual acuity and treatment of eye diseases had a dry temperament and most of them were hot and dry. Most of the plants are extracted from the medicine repository and the least of them are extracted from the medicinal plants and facts. It seems that according to the texts of medicine books, most plants were effective in strengthening vision and a few were effective in visual impairment. Meanwhile, most herbs have been shown to be effective in treating cataracts compared to other ocular disorders such as glaucoma. The method of consumption for most plants, like the common method of medicine for the treatment of eye diseases, is kohlrabi, but in the meantime, oral methods are also found to a lesser extent. Aqueous extract of fennel seed (Foeniculum vulgare) has protective and therapeutic properties against cataracts caused by sodium selenite in rabbit eyes. The possible mechanism of this extract is antioxidant activity. Various phytochemical studies have shown the presence of compounds such as flavonoids, phenolic compounds and volatile compounds in this plant. It has several other medicinal properties such as anti-inflammatory and anti-hypertensive effects. Lens opacity was lower in rabbits that received eye drops of aqueous extract of this plant twice a day for 5 days before cataract induction and 21 days after cataract induction [30]. Considering the antioxidant role of this drug in the treatment of cataracts, it seems that it can play a similar role in relieving the symptoms of glaucoma. In addition to its antioxidant properties, this plant also seems to be effective in reducing intraocular pressure. And can be used specifically to treat glaucoma. Another study by Agarwal et al. Showed that aqueous extract of fennel seed reduced intraocular pressure and its maximum pressure reduction was comparable to that of thymolol. The mechanism of action is through the effect of anti-cholinesterase, so its anti-glaucoma property is proven [31]. In the following clinical trial results, we will present several studies that prove the antioxidant properties of certain plants, since the herbal treatment of eye diseases such as glaucoma and cataracts is jointly based on its antioxidant properties, so the result can be It is likely that these drugs, which are effective against cataracts, will also improve eye conditions in glaucoma. Javadzadeh et al. Stated that administration of 1 drop of onion extract in the eyes of mice for 14 days every 8 hours prevents the formation of cataracts caused by selenite [32]. In 2010, a study by Gupta et al. On 9-day-old mouse puppies showed that intraperitoneal injection of aqueous extracts of Trifala (Emblica officinalis), Terminalia Bellerica and Terminaliachebula at 25 mg/d. Each kilogram of body weight prevents the formation of cataracts caused by selenite and the formation of selenite. In addition, the activity of antioxidant enzymes increases glutathione and reduces the amount of lipid peroxide in contrast to selenite-induced oxidative stress [33]. Another case-control study has shown the role of oxidative stress as the main mechanism of eye damage in cataracts, glaucoma and ARMD, which shows the role of plasma antioxidant capacity in the prevention of these eye diseases. Plasma antioxidant capacity is increased by foods rich in antioxidants such as fruits and vegetables [34]. Despite extensive research, there is insufficient clinical trial evidence of the effectiveness of all plants on visual impairment extracted from sources, but the antioxidant properties of many of these plants indicate their potency in treating or enhancing vision impairment. These include Aloe vera (Solanum nigrum) [35], Peganum Harmala [36], Zingiber officinalis [37], Ferula assafoetida [38], Rheum ribes [39] and Belila (Terminalia bellerica) [40].

DISCUSSION

Visual disturbances generally occur for a variety of reasons, including disorders of the interpretive part of the vision in the brain, disorders of the eyeball such as the layers of the eye (choroid, retina, and sclera), and eventually disorders of the optic nerves. In glaucoma or glaucoma, the disorder occurs due to increased pressure inside the eyeball for a variety of reasons, of which there are many types. Doctors of IranianPersian medicine attribute the causes of all diseases to not paying attention to the 6 principles of health (climatic and environmental conditions, food and drink, physical activity and rest, excretion status, mental condition and sleep and wakefulness). For this reason, they first try to treat the disease by regulating the above conditions, and then turn to the use of drugs and invasive measures such as surgery. In other words, Iranian Persian physicians believed that in the early stages of the disease, the disease can be controlled by maintaining and regulating the six principles of health, and only in advanced conditions of the disease should aggressive measures be taken. For example, in the treatment of cataracts (descent of water), Persian physicians initially sought to treat the disease with nutrition and herbs, and only in advanced conditions did they perform aggressive measures such as surgery. Also in glaucoma and cataracts, they advise not to eat meat, fish and fruits and also recommend to use dry foods [41]. Regarding the recommendation of medicine not to rush into initiating aggressive measures, Ibn Sina's law states that one should not rush to start cataract surgery and should wait until its sufficient progress [42]. In general, the opinion of Persian medicine in the field of eye diseases such as glaucoma is that some environmental conditions such as dust, very hot or very cold weather, smoke and looking at small objects too much to do too much Activities such as crying and sexual intercourse, eating too much salt, lentils, cabbage, garlic, sugar and alcohol eventually cause these diseases. On the other hand, it is highly recommended to eat foods such as saffron and turnips to improve eyesight [41]. The general mechanism of eye damage in diseases such as cataracts, glaucoma and age-related retinal detachment (ARMD), from the perspective of biochemistry, is oxidative stress. Oxidative stress leads to the formation of free radicals that can cause severe damage to the ocular and lens layers. Therefore, in the treatment and prevention of these diseases, the best way is to use antioxidants as complementary medicine. Table 1, which is mentioned in the results section, shows a group of plants with strong antioxidant properties, which seems to be the recommendation of Persian physicians to use these substances in the treatment of eye diseases [43]. Although some medical schools, such as traditional Chinese medicine, seek to treat glaucoma with the use of herbal medicines, it seems that the main recommendation of Persian medicine is to regulate the six conditions of maintaining health as well as the use of herbal medicines. In the form of kohl, drops and even oral, seeks to prevent the disease and increase the body's resistance to the disease. In fact, in the treatment of glaucoma, the herbal medicines mentioned in traditional medicine can be used as a complementary treatment to the main treatment, because in the acute stages of the disease, the use of invasive treatments such as surgery instead of herbal medicines, it seems inevitable.

CONCLUSIONS

Unlike other medical schools in the world, in addition to treating diseases, Persian medicine has a strong recommendation to prevent the occurrence of disease and also to strengthen the body's visual powers by using 6 principles of maintaining health along with herbal medicines. Finally, it is inferred from this study that despite the many benefits of using herbal medicines mentioned in Persian medicine books, but due to the lack of sufficient clinical evidence, until the effectiveness of these herbal medicines is proven, it is better to use them. Complementary therapy should be used alongside the main treatment (modern medicine). Despite the mention of numerous herbs for the treatment of eye diseases such as glaucoma and cataracts, as well as the strengthening of eyesight, but it seems because so far no proper clinical trials have been conducted in the field of trying these herbal medicines. This rich capacity of Persian medical experiences has been neglected. Therefore, it is recommended that Iranian Persian medical researchers in their future studies diligently test plant compounds using appropriate and valid research methods for the treatment of eye diseases.

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Conflict of interest

The authors declare that they are no conflicts of interest.

Compliance with Ethical Standards

This article does not contain any studies with human participants performed by any of the authors.

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ORIGINAL ARTICLE

Open treatment options in massive rotator cuff tears associated with acromion non-union

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Abstract: *We evaluated the available English literature for cases of acromion non-union and rotator cuff tear, looking at surgical methods of treatment as well as at functional results. As expected, the literature regarding rotator cuff pathology was found to be abundant, and a good number of articles were found regarding acromion non-union, but articles concerning the association of the two were scarce. Treatment recommendations varied, and were made on a case-to-case fashion. The accepted treatment methods for acromion non-union consist of non-surgical management, open or arthroscopic fragment removal, open or arthroscopic reduction and fixation (bone grafting may be used), while the rotator cuff witll be reinserted with anchors. In this rare occurence, a large rotator cuff tear with retracted tendons combined with an acromion non-union are usually repaired by an open technique in an one-stage surgery, with good results. In order to chose the right treatment option scapular kinematics must be taken into consideration especially when acromial fractures are associated with rotator cuff tears.*

Keywords: *shoulder, rotator cuff tear, acromion, non-union*

INTRODUCTION

Fractures of the scapula range from 0.4 to 1% of all fractures, with acromion fractures representing 7% of all scapular fractures [1]. Usually though acromial fractures are rarely reported due to their low incidence [2]. Lesions associated with acromial fractures such as rotator cuff tear are even more rarely described [3]. An MRI performed for a rotator cuff lesion (Figure 1) may show an associated acromial fracture, whereas a CT performed to describe an acromial fracture/non-union will not show a rotator cuff tear [4].

Treatment methods

A direct consequence of low acromial fracture incidence is the fact that there is no clear consensus on repair options resulting, more often than not, in surgeons choosing treatment options which they have the most experience withat the same time without overseeing patients'demand levels [5,6]. There are many reported methods for acromial fracture fixation a variety of them being presented in the literature such as tension band wiring, screw and plate fixation techniques, Kirschner wire fixation and in each of them patient specific factors and associated lesions are always taken into consideration when choosing a treatment option [7-11].

Figure 1: T2W_TSE coronal MRI image showing a massive tear in the supraspinatus tendon (arrow). This image is from the author's personal database

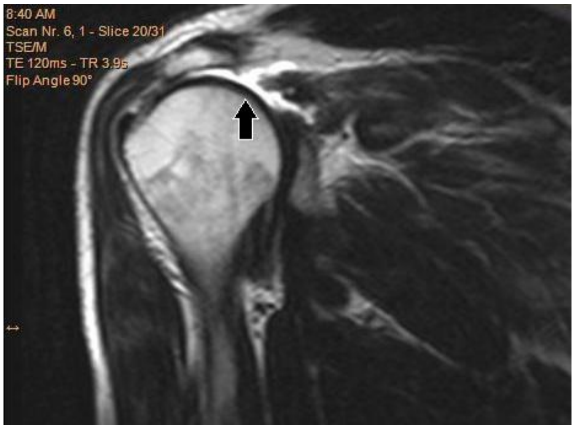

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The surgical treatment has several complications, mostly locoregional, ranging from hematoma and infection to persistent pain irradiating to the distal arm and forearm, posing a problem especially in patients with associated ipsilateral medical conditions of the forearm or of the arm [12-14]. Letting surgical treatment aside there are at least two more important factors that need be considered when dealing with an acromial fracture and these are the complex3D anatomy of the acromion and the so called superior shoulder suspensory complex (SSSC) which is an important factor in the aftercare postoperative prognosis of acromial fractures [2,8,15-17]. The SSSC is not the only factor to consider, as a system of bursae and the deltoid muscle allows for the possibility of pain irradiating in the distal arm and forearm, making for a difficult differential diagnosis [18].

Figure 2: Intraoperative image showing the non union fragment of acromion being held with a Kocher clamp. In the secondary plane of view we can see the torn supraspinatus tendon and the humeral head. This image is from the author's personal database.

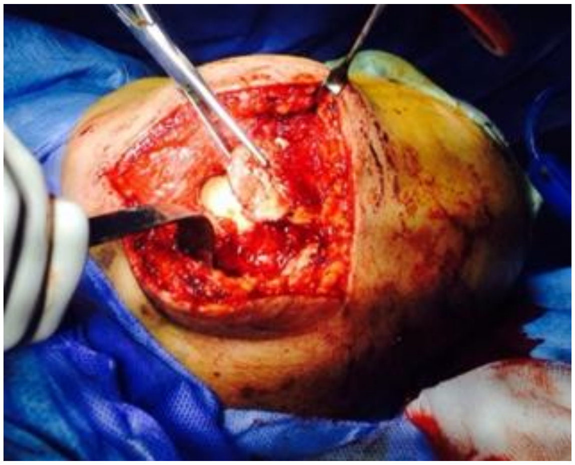

Figure 3: Intraoperative image showing the torn supraspinatus tendon being pulled into place with traction sutures, before reinsertion. This image is from the author's personal database.

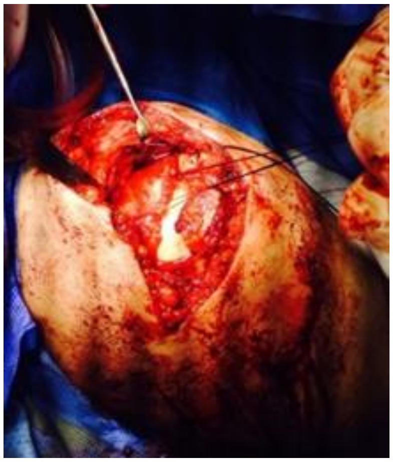

The complex 3D anatomy of the acromion makes it hard to fit any available plate system, most acromial plates requiring intraoperative bending in order to fit the required anatomy without excessive soft-tissue debridement while at the same time trying to maintain rigid fixation in an area of multiple pull vectors mainly arising from the deltoid and trapezius muscles [19-21]. When considering the complete management of acromial fracture non-union associated with rotator cuff tear (Figures 2, 3) one must take into consideration complex subjects like shoulder force vectors and SSSC implications in patient expected outcome.

DISCUSSIONS

As expected, the literature regarding rotator cuff pathology was found to be abundant, and a good number of articles were found regarding acromion non-union, but articles concerning the association of the two were scarce. Treatment recommendations varied, and were made on a case-to-case fashion.

The non-unions of the acromion can be treated in a variety of ways. In their 2006 article, Lumbreras et al describe the case of 56 year old male patient treated surgically with plating andautologus iliac crest bone grafting, with excellent clinical and radiologic results [23]. Liodakis et al report using the same method, but with an adapted distal radius plate; they offer a long follow-up of two years with measurable improvement in both range of motion as well as in the SSRS (Subjective Shoulder Rating System) Score [24].

Os acromiale

A differential diagnosis can be made with os acromiale, an unusual anatomic variant usually discovered incidentally [25]. In their 2016 article, Garcia et al define the os acromiale as a failure in the fusion of an accessory ossification nucleus of the acromion, and propose an arthroscopic method of fixation with cannulated screws, as opposed to the open technique [26]. Hurst claims an 8% incidence rate in his 2019 article, and advises for the careful diagnosis of this variant in all of the patients that are about to undergo shoulder surgery [27].

The accepted treatment methods consist of non-surgical management, open or arthroscopic fragment removal, open or arthroscopic reduction and fixation (bone grafting may be used) and arthroscopic subacromial decompression [28]. Failure to recognize an incidence of os acromiale may lead to confusion with an acromial non-union, as the imaging may be similar, but the patient's history and previous investigations may help with the differential diagnosis.

When looking for os acromiale, MRI findings on axial images generally report a relatively triangular unfused ossicle with an almost transverse interface with the acromion and irregular margins, with interface edema and marrow edema of the opposite bony structures [29]. At the same time, acromial non-unions possess similar characteristics on MRI (Figure 4), but key finding during history taking are crucial for diagnostic orientation, for example the lack of patient-reported shoulder trauma or bone disease like osteoporosis may incline the diagnosis towards os acromiale rather than acromial non-union [30]. In a symptomatic patient it is our opinion that the differential diagnosis has a theoretical purpose, as the treatment methods are similar.

CONCLUSION

In this rare occurence, a large rotator cuff tear with retracted tendons combined with an acromion non-union are usually repaired by an open technique in an one-stage surgery, with good results. In order to chose the right treatment option scapular kinematics must be taken into consideration especially when acromial fractures are associated with rotator cuff tears.

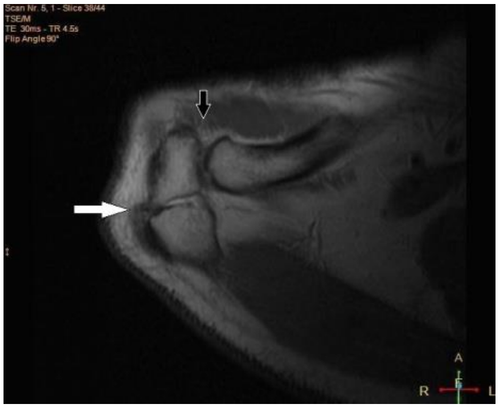

Figure 4: PDW_aTSE axial MRI image showing acromial non union (white arrow), easily confused with os acromiale. The acromioclavicular joint is indicated by the black arrow. This image is from the author's personal database

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ORIGINAL ARTICLE

Outcomes of frozen embryo transfer in patients with and without ovarian hyperstimulation syndrome

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Abstract: *Introduction: One of the methods utilized to treat infertility is the use of frozen embryos. This technique is particularly employed in patients with ovarian hyperstimulation syndrome (OHSS), which leads to heightened blood estrogen levels. The purpose of this study was to compare the percentage of pregnancy and abortion between patients who used the frozen embryo method due to OHSS and others who practiced the same method for other reasons.*

Materials and Methods: This retrospective, cohort study was conducted on a total of 338 patients who visited in vitro fertilization (IVF) section of Kowsar Hospital of Urmia-Iran to employ the frozen embryo method (May 2013 to December 2015). The patients were classified into two groups, i.e. the OHSS group (N=150) and the non-OHSS group (N=188). Data were obtained and examined by evaluating the files in a questionnaire.

Results: The two groups did not differ significantly with regard to their mean age (p = 0.57). There was also no statistically significant difference between the OHSS and non-OHSS groups regarding the quality of frozen embryo transferred (P = 0.17). Also, there was also no statistically significant difference between the two groups in terms of their pregnancy rate (OHSS = 30.0% vs. non-OHSS = 25.0%) and miscarriage rate (OHSS = 31.11% vs. non-OHSS = 24.44%) (p = 0.32 and p = 0.31, respectively).

Conclusion: Ovum exposure to high estrogen during ovulation stimulation does not affect embryo implantation and miscarriage in patients with OHSS.

Keywords: *abortion, embryo, estrogen, frozen-thawed embryo transfer (FET), Ovarian hyperstimulation syndrome*

BACKGROUND

Infertility is characterized as the inability to conceive after 12 months of regular unprotected sexual intercourse in women aged <35 years and 6 months of regular unprotected sexual intercourse in women aged >35 years [1]. Studies have revealed that 8 to 12% of couples worldwide are facing fertility problems [2], accounting for nearly 72.4 million people in the world who are affected by infertility [3]. The World Health Organization asserts that infertility will become the world's third most prevalent disorder in the next few years preceded by cardiovascular diseases [4]. Study by Akhondi et al. showed the primary infertility rate to be 20.2% among Iranians [5].

There are various methods of infertility treatment including change of lifestyle, medications or surgery, and assisted reproductive technology (ART) [6]. Overall, as a costly assisted reproduction method employed in various countries, IVF (in vitro fertilization) is utilized by couples in a limited way on the other hand, the results of these methods have not been that fruitful [7].

The duration of uterine receptivity to blastocyst acceptance is highly dependent on estrogen levels such that this process lasts longer at low estrogen levels than at high estrogen levels [8]. That the uterine environment is not conducive to blastocyst acceptance occurring at high estrogen levels is the result of improper expression of the genes responsible for embryo acceptance to the uterus. With the use of the FET

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(frozen embryo transfer) method, endometrial receptivity can be increased as the method eliminates the effects of estrogen on the endometrium produced by ovulation stimulation [9].

Nowadays, FET is regarded as one of the most prominent techniques of infertility treatment that enhances the cumulative rate of pregnancy. Additionally, FET lessens the cost of treatment, the rate of multiple pregnancies, and OHSS (ovarian hyperstimulation syndrome) [9]. In this method, upon stimulation of ovulation, removing oocytes, and insemination (i.e. embryo formation) in vitro, the formed embryos are frozen.

Despite the fact that the use of frozen embryo and its transfer to following cycles, in which the patient does not undergo ovulation stimulation and the estrogen level is not high, has removed the effect of estrogen on implantation in these cycles, whether or not the high rate of estrogen impacts ovulation stimulation, embryo quality, implantation, and embryonic growth and development is to be understood. The results of the study by Weinerman R et al. revealed that if an embryo is exposed to high estradiol levels, its development, and attachment to the endometrium are undermined [10]. Estradiol can influence the proliferation of human embryonic cells to the extent that the physiological levels of estradiol make it possible for stem cells to proliferate. However, estradiol at levels higher than physiological ones impairs stem cell proliferation, occurring through the lowered expression of the HDACI gene, which contributes to embryonic development [10,11].

This study was conducted to assess and compare the rate of implantation and abortion in patients who used frozen embryo transfer either due to OHSS syndrome or other reasons such as inadequate endometrial thickness, uterine bleeding, etc. Their embryos were frozen and transferred in subsequent cycles after endometrium was prepared. Then, we investigated the impacts of oocytes exposure to high estrogen on ovulation stimulation and its effects on the quality, implantation, and growth of the embryo in the uterine.

MATERIAL AND METHODS

The present study was implemented on patients who visited the IVF section of Kowsar Hospital in Urmia, Iran (May 2013 to December 2015). The data were accumulated by reviewing the files and in cases where patients' records were not complete, their data was collected through phone calls and face-to-face visits in the form of a questionnaire. This project was approved by the Ethic Committee of Urmia University of Medical Sciences, and was in accordance with the Helsinki Declaration.

The patients were divided into two groups in terms of why they wished to use embryo freezing. The first group comprised participants whose embryos were frozen due to OHSS (OHSS group), and the second group included participants who used the frozen embryo transfer method for reasons other than OHSS (non-OHSS group). One or two days after their second period time, patients referred from the first or second day after the second period of egg retrieval to prepare the uterus. From the second day of the cycle, they were treated with 2 mg Valerate Estradiol every 8 hours. If their endometrium was more than 8 mm thick and three-linear, they received 50 mg progesterone intramuscularly every 12 hours, along with estradiol at the same time. On the fourth day of their progesterone, their embryos were transferred, followed by estradiol, and progesterone. After 14 to 15 days, patients' β-HCG levels were checked. If positive, they visited the hospital for an ultrasound two weeks later. Also, patients were evaluated for miscarriages up to 20 weeks of gestation.

Inclusion criteria comprised: all patients who volunteered to undergo IVF, yet after ovulation and embryo formation, their embryo transfer was canceled and it was instead frozen due to reasons such as OHSS, uterine bleeding, inadequate endometrial thickness for embryo transfer, presence of polyps, or any uterine space lesion or cervical stenosis, etc., were included in the study. Exclusion criteria also were <8mm endometrial thickness on day 13, consumption of Valerate Estradiol or hyperechogenic, and homogenous endometrium on vaginal ultrasound on this day.

Statistical analysis

The statistical analyses were performed using the SPSS software version 16.0 for Windows (SPSS Inc., Chicago, IL). Categorical variables were summarized as numbers (percentages). The variables were compared between two groups by the Chi-square/Fisher’s Exact test. $p < .05$ were considered statistically significant.

RESULTS

Of the 338 patients analyzed, 150 were patients with OHSS, and 188 were non-OHSS. Out of 150 OHSS patients, 46 had positive pregnancy, one of which one patient was excluded due to chemical pregnancy and 45 had positive pregnancy results (30.20%). Of 188 non-OHSS patients, 48 had positive pregnancy, of which 2 and 3 patients were excluded due to chemical pregnancy and EP, respectively, and 45 had positive pregnancy (25.13%). Nonetheless, no EP incidence was reported in OHSS patients. Of 45 patients with OHSS, 11 had a miscarriage (24.44%) and 34 continued their pregnancy after 20 weeks (75.55%). Of 45 non-OHSS patients, 14 had miscarriages (31.11%) and 31 had continued pregnancies after 20 weeks (65.95%) (Figure 1).

Figure 1: Flow chart of included subjects

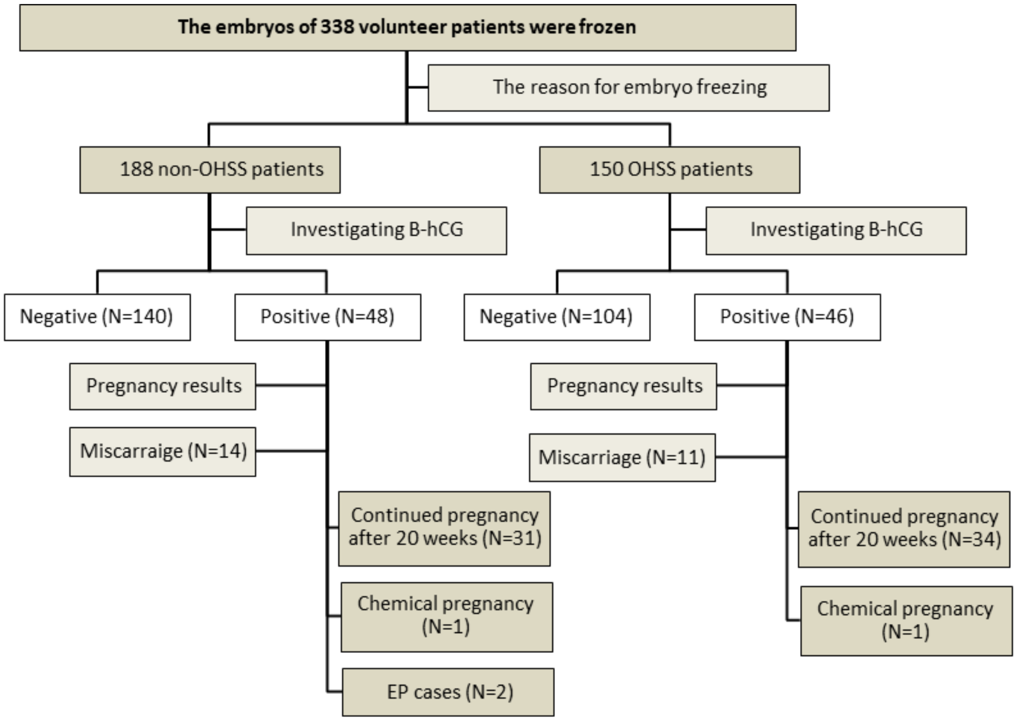
The embryos of 338 volunteer patients were frozen
The reason for embryo freezing
188 non-OHSS patients
150 OHSS patients
Investigating B-hCG
Investigating B-hCG
Negative (N=140)
Positive (N=48)
Negative (N=104)
Positive (N=46)
Pregnancy results
Miscarraige (N=14)
Continued pregnancy after 20 weeks (N=31)
Chemical pregnancy (N=1)
EP cases (N=2)
Pregnancy results
Miscarriage (N=11)
Continued pregnancy after 20 weeks (N=34)
Chemical pregnancy (N=1)

The mean age of the research population was 30.13 ± 5.5 years (OHSS = 29.91 ± 5.67 years and control = 30.35 ± 5.4 years (p = 0.57)).

A total of 338 patients experienced frozen embryo transfer, 2 embryos transferred in the OHSS and the non-OHSS groups, respectively. When assessing the quality of transferred embryos, 66.86% and 69.07% of embryos belonged to patients with OHSS and non-OHSS groups, which had embryos A and B, respectively. This finding was not statistically significant (p = 0.17).

Concerning the pregnancy rates from transferred embryos in patients between the two groups, 45 out of 150 OHSS patients (30%) had positive pregnancies, while 47 out of 188 (25%) non-OHSS patients had positive pregnancies, suggesting no significant difference between the two groups in terms of pregnancy rates (P = 0.32). Regarding the rate of miscarriage in patients with positive pregnancy, there was no statistically significant difference between non-OHSS patients (14 cases out of 45 cases, 31.11%) and the OHSS group (11 cases out of 45 cases, 24.44%) (P = 0.312). There was no significant difference between the two groups in terms of chemical pregnancy rate (0.66% in OHSS group versus 0.5% in the non-OHSS group) (p = 0.85). Two ectopic pregnancies were found in non-OHSS patients, whereas ectopic pregnancy did not occur in OHSS patients (p = 0.50).

DISCUSSIONS

Despite substantial efforts made to prevent it, OHSS remains an iatrogenic disorder with potentially life-threatening complications [12]. The most significant way to stop OHSS is prevention, and one of the major ways to prevent it is avoiding embryo transfer in these patients, and instead, freezing and transferring the embryo in future cycles with no ovulation stimulation is an important method in preventing this major problem. Using FET in these cycles makes a great contribution to reduced incidence or severity of OHSS. Moreover, it decreases the adverse effects of high estrogen on embryo implantation [6]. The purpose of this study was to assess and compare the pregnancy and miscarriage rates among OHSS and non-OHSS patients in order to indirectly examine the effects of high estrogen on embryo implantation and growth in the patients. In the present study, an examination of the percentage of pregnancies in both groups revealed that high estrogen during ovulation stimulation has no impact on oocyte and embryos formed for implantation and early embryo growth and development in the uterus. On the other hand, it did not affect miscarriage either.

Studies have revealed that endometrial receptivity is highly reliant on estrogen levels since, at high estrogen levels, the uterus is not conducive to embryo implantation. Nevertheless, the impacts of high estrogen on embryo implantation and growth in OHSS cycles are yet to be known [13,14]. In a study, it was confirmed that high estrogen levels can undermine the proliferation of embryonic stem cells, hence its adverse effects on embryonic growth and development [10]. Meanwhile, the differentiation of the embryo for the implantation process is an important factor in the occurrence of pregnancy [13].

Check et al. examined the patients whose fresh embryo transfer was canceled due to OHSS or deficient endometrial thickness, and instead underwent the frozen embryo method. The rates of clinical pregnancy and live birth in OHSS patients were 42.8% and 31.9%, respectively, whereas the values were 28.7% and 21.8% for non-OHSS patients (insufficient endometrial thickness), respectively. As is shown, the rate of pregnancy and live birth in the non-OHSS group is significantly lower than that of the OHSS group (P = 0.01 and P = 0.07, respectively) [15].

Raziel et al. assessed the rate of premature miscarriage in OHSS patients undergoing IVF. Conducted on 104 patients with severe OHSS, this study revealed that 58% of these patients were pregnant, of which 38% had miscarriages. Compared to non-OHSS patients who practiced IVF, the pregnancy rate among OHSS patients was significantly higher, which was seemingly due to the non-OHSS patients' higher mean age. in this study, the rate of miscarriage in patients with OHSS was higher than the control group, i.e. non-OHSS group, which was not consistent with findings of our study [16].

With regard to the outcome of pregnancy in Check et. al, the live birth rate in the OHSS group was 31.9% compared to 21.8% in patients with less endometrial thickness, which was also statistically significant [15]. In the study by Howarth and et al., the rate of live births in the first and fourth groups was lower than in the second, third, and fifth groups, which was also statistically significant [17]. Nonetheless, in our study, which assessed pregnancies up until the 20th week of pregnancy, the groups were compared only in terms of their rate of miscarriage such that in the second non-OHSS group's patients, the rate of miscarriage was higher than in OHSS patients, yet there was no statistically significant difference between the two groups. The present study also had its own limitations. Due to the lack of information in the files and unavailability of some patients, which decreased the number of samples in this study, more comprehensive and longer-term prospective studies are required to increase the power and reliability by expanding the number of samples and controlling the study as much as possible.

CONCLUSIONS

Accordingly, the results of our study potentially show that oocytes exposure to high estrogen during ovulation stimulation has no impact on oval and embryos formed for implantation, growth, and development in utero.

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ORIGINAL ARTICLE

Pharmacological evaluation of glomerulonephritis in Covid-19 patients

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Abstract: *Acute kidney injury (AKI) and glomerulonephritis can be complications in COVID-19 patients, which is announced with widely incidence rates in different investigations and is determined to have a major effect on the prognosis of the disease. There are considerable variations in AKI and glomerulonephritis rates between other countries. The rate is generally lower than in Western Europe and the United States in China. Heterogeneity in different racial and ethnic lines can be a potential explanation. This investigation systematically reviews the scientific resources regarding AKI and glomerulonephritis among hospitalized COVID-19 patients. Both observational and interventional investigations(including case reports) with English full-text provide essential data, with no limitation in release or peer-review. More evidence is required to assess the AKI, especially glomerulonephritis in Covid-19 patients, for better management of Covid-19.*

Keywords: *COVID-19, AKI, patient, glomerulonephritis*

INTRODUCTION

The kidneys play an important role in regulating acid-base balance, water and electrolyte balance, nitrogen balance, and calcium and phosphorus metabolism. Chronic Kidney Disease (CKD) is a disease that is caused by the progressive and irreversible destruction of the number and function of nephrons by various factors. Among the most important of these factors are diabetes, hypertension, glomerulonephritis, and polycystic kidney disease, respectively. Causes of chronic kidney disease reduce the number of nephrons through progressive destruction of nephrons, in which case the amount of blood flow and pressure in the remaining healthy nephrons increases, and thus the remaining nephrons increase their function (Figure 1). Try to keep the Glomerular Filtration Rate (GFR) within the normal range; For this reason, after a while, the remaining nephrons become hypertrophic due to overactivity, and eventually, this compensatory mechanism causes glomerular sclerosis, resulting in the destruction of glomeruli and the disappearance of the remaining nephrons.

In patients with chronic kidney disease, especially in its advanced stages, transient complications such as nausea, vomiting, anorexia, hyperkalemia, metabolic acidosis, hyperphosphatemia, fluid retention, hyperparathyroidism, bone disorders, cardiovascular and pulmonary disorders, neuromuscular disorders, normochromic normocystic anemia, hypersensitivity to infection, gastrointestinal disorders, metabolic-endocrine disorders (lipid abnormalities), Inflammation and oxidative stress occur. Kidney problems are directly related to the nutritional status of patients.

The prevalence of energy-protein malnutrition in these patients is very high. Numerous studies have shown that the life expectancy of these patients increases with the improvement of nutritional status. According to various studies, the COVID-19 infects all groups of different ages include: newborns, pediatrics, young people, elderly, but some groups are more vulnerable to this infection.

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Autoimmune patients are one such group [1,2].

Figure 1: Test your knowledge on glomerulonephritis

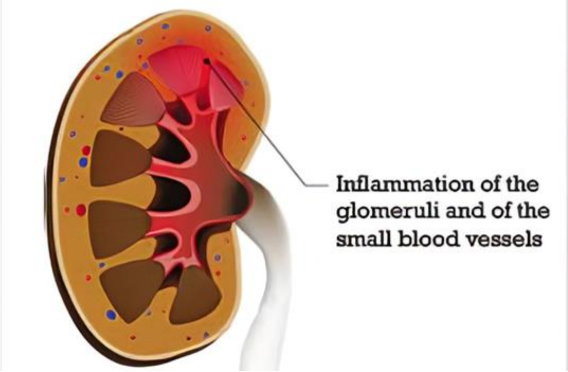
Inflammation of the glomeruli and of the small blood vessels

Despite most of the cases presented with mild symptoms so far, many of them experienced lethal complications, including organ failures, acute respiratory distress syndrome (ARDS septic shock, and pulmonary edema. Acute kidney injury (AKI) is one of the complications in patients with COVID-19, which is detected as a widely varied incidence rate in various investigations and is observed to have a significant effect on the prognosis and outcome of the disease [3-9]. Many reports from China, Europe, and the United States expressed the characteristics and outcomes of acute kidney injury among hospitalized patients with COVID-19 [8,9]. The pathophysiology of COVID-19 related to glomerulonephritis is not determined by a specific theory up to date. It is recommended that the cause of kidney injury be multifactorial; for example, cardiovascular and sepsis, hypovolemia, and nephrotoxins are critical contributors [10]. The study described the incidence of acute kidney injury as a feature of thrombotic microangiopathy (TMA)-like phenomenon, secondary to infection of SARS-CoV-2 [11]. Moreover, viruses particles in renal endothelial cells lead to viremia as a possible reason for endothelial damage in the kidney and a potential contributor to glomerulonephritis and acute kidney disease. Other mechanisms of action may be the direct infection of the renal tubular epithelium and podocytes via an angiotensin-converting enzyme 2 (ACE2)-related pathway in patients with Covid-19 (Figure 2), which can lead to the formation of protein reabsorption vacuoles, mitochondrial dysfunction, acute tubular necrosis, protein leakage in Bowman's capsule, and collapsing glomerulopathy [12].

Figure 2: Glomerulonephritis in body

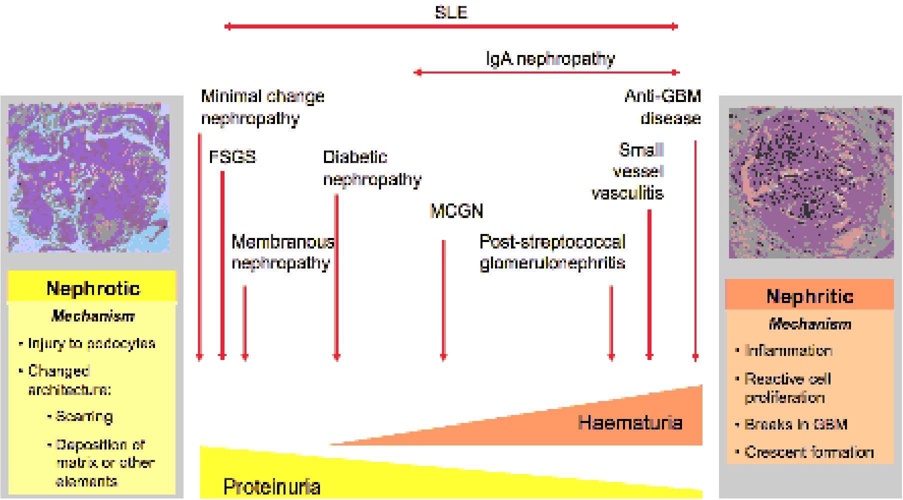
SLE
IgA nephropathy
Minimal change nephropathy
FSGS
Diabetic nephropathy
Membranous nephropathy
MCGN
Post-streptococcal glomerulonephritis
Small vessel vasculitis
Anti-GBM disease
Nephrotic
Mechanism
• Injury to podocytes
• Changed architecture:
• Scarring
• Deposition of matrix or other elements
Nephritic
Mechanism
• Inflammation
• Reactive cell proliferation
• Breaks in GBM
• Crescent formation
Haematuria
Proteinuria

METHODS

Inclusion and exclusion criteria

Observational and interventional investigations (e.g., case reports) with English full-text with presenting data for analysis were included in our study with no limitation in time of publication. Exclusion criteria are review articles and other types of publications with no patient enrollment, articles that specifically focus on the pediatric population, pregnant women, and studies with insufficient evidence on glomerulonephritis occurrence.

Protein catabolism and nitrogen balance in patients with CKD are directly related to energy intake. Inadequate energy intake increases the process of protein catabolism and causes a negative nitrogen balance. Many studies have shown that basal metabolic rate in CKD patients is lower than in others. In dialysis patients, the basal metabolic rate increases by 12-20%, so adequate energy intake in these patients is very important because low carbohydrate (glucose) intake in the diet puts the body in the phase of gluconeogenesis and depletes glycogen stores. Liver and conversion of muscle proteins to glucose, the body is in an unfavorable metabolic state. In other words, proteolysis increases the catabolism of proteins by causing metabolic acidosis and causes the breakdown of muscle mass. Lack of adequate physical activity is also effective in muscle atrophy. In addition, increased production of nitrogen products in the blood causes symptoms such as anorexia, nausea, which is effective in reducing food intake by the patient and maintaining the unfavorable conditions of catabolism. In patients with chronic renal failure, impaired renal function also increases blood triglycerides, impaired carbohydrate metabolism, insulin resistance, and diabetes by increasing the production of inflammatory cytokines and altering the secretion of endocrine hormones. Sometimes severe dietary restrictions in these patients can also lead to malnutrition. Establishing a proper diet is essential to prevent malnutrition in patients with kidney failure. These patients usually need 30-35 cal/kg/IBW of energy per day. The ratio of macronutrients in the diet of these patients is usually as follows: The need for protein in patients with chronic renal failure depends on the function of their kidneys. For example, in patients with 50% renal function and blood creatine levels less than 2 mg/dl or GFR above 70 ml/min, the need for protein is 0.8-1 g/kg/d and in patients with 20-50% Renal function and blood creatine level 2-5 mg/dl or 25-70 ml/min GFR protein requirement 0.6 g/kg/d in hemodialysis 1-2 g/kg/d and in peritoneal dialysis 1-1.5 g/kg/d, carbohydrates are considered 55-70% and fats are considered 30-30%. Usually the need for fluids in these patients is 600-500 ml + volume of excreted urine + abnormal loss of water from non-renal routes. However, in patients with chronic renal failure in the pre-dialysis phase, there is no need to calculate the volume of fluid intake unless the patient has severe edema. The amount of electrolytes and minerals required depends on the patient's nutritional status and the extent of kidney damage. For example, in an oligarchy patient (urinary excretion less than 500 cc per day), severe sodium, potassium, magnesium, and phosphorus restrictions should be imposed on the diet. In some of these patients, the use of commercial formulas is recommended to meet their maximum nutritional needs. Typically, commercial formulas for kidney patients with high calorie density (2.8-8 kcal/ml), low protein (81-70 g/l), sodium (700-1000 mg/l), potassium (1600 mg/l) - 1000) and phosphorus (600-700 mg/l) are considered. The need for vitamins in patients with kidney failure depends on the type of diet. Patients with severe dietary restrictions need water-soluble vitamins. Normally, the levels of vitamin B1, folic acid and vitamin C in these patients are lower than normal. Hemodialysis patients are usually deficient in vitamins A, vitamin D and B12, and it is recommended to take these vitamins as supplements. Also, when the kidneys fail, the synthesis of 1 and 25 dihydroxycholecalciferol in the kidneys decreases and this vitamin needs to be prescribed. Iron is one of the minerals that is essential in these patients. It is usually recommended to take 10-15 mg/d of iron, but in kidney patients with ferritin levels below 100 mg, it is necessary to take at least 60 mg of ferrosulfate. It is recommended to take iron supplementation with citrus juice in these patients to increase iron absorption. One of the most common problems in kidney patients is metabolic acidosis, which requires oral bicarbonate to maintain blood pH in these patients. In addition to using a proper diet, encouraging the patient to increase physical activity is effective in increasing the expression of anti-inflammatory proteins, improving the sensitivity of insulin-like growth factor, increasing the production of muscle fibers and preventing the breakdown of muscle mass.

Data extraction

Search results were assessed by two independent authors, and study selection was performed assessing the title and the abstract. Among selected publications, full-text articles were evaluated to determine the eligibility for inclusion. . In the end, Discrepancies were resolved by consensus. The following information was extracted from the articles using an organized checklist: name of the first author, date of the publication, name of the journal, study location, study designs, the definition of, age range, gender ratio, and proportion of glomerulonephritis occurrence. General information of the included articles was reviewed precisely to prevent duplication and data overlap.

RESULTS

A total of 289 articles were identified through the systematic search of the databases, as previously mentioned. After removing all duplications, 259 records remained and were carefully examined, which returned an Evaluation of the full-texts to check the eligibility was performed, and 198 publications were excluded due to either not meeting the inclusion or exclusion criteria. From 61 remaining articles, ten were excluded due to data overlap. Finally, 13 studies were selected and included in the review. The process and results are outlined in Figure 3. Some articles defined AKI according to KDIGO criteria as an increase in serum creatinine by 0.3 mg/dL or a 50% level rise in serum creatinine from baseline within seven days. AKI was defined roughly by serum creatinine cut-off if specified in other studies. The diagnosis of glomerulonephritis in Covid-19 patients was confirmed via biopsy tests. The included studies are demonstrated in Table 1 and Table 2.

Table 1: The glomerulonephritis patients with Covid-19

GN definition	Population	Sample Size	Age	Sex	Glomerulo-nephritis	Results
Waldman et al. 2020 (20 April 2020 to 20 August 2020)						
Biopsy-proven	Patients with glomerulonephritis and COVID-19	120	Control 62.5 ± 15.6	Female/ Male 30 (37.5)/ 50 (62.5)	40/120	The majority (70%) of the patients with glomerulonephritis and all the controls were hospitalized. Patients with glomerulonephritis had significantly higher mortality (15% vs. 5%, respectively) and acute kidney injury (39% vs. 14%) than controls, while the need for kidney replacement therapy was not statistically different between the two groups
Xingguo et al, 2020 (February 3 to 16, 2020/Wuhan Fourth Hospital)						
Biopsy-proven	Patients with COVID-19	32	58.5 (36–83)	Male 23 (71.9%)	12/32	Out of the 32 patients, 23 were male, and the median age was 58 years; the median dialysis vintage was 33 months. Two groups were divided according to the patient's primary renal disease: group 1 (16 patients with diabetic nephropathy), group 2 (12 patients with primary glomerulonephritis, 2 with obstructive kidney disease, 1 with hypertensive renal damage, and 1 with polycystic kidney)

Table 2: The case reports of Covid-19 patients with glomerulonephritis

First Author	Country	Age	Disease duration	SLE system involvement	Severity of COVID-19
Hakroush et al. [14]	United Kingdom	79	2 weeks	Kidney	-
Nagai et al. [15]		70	4 month	Left finger	Mild
Raeeskarami et al. [16]	Iran	14	3 weeks	Respiratory tract infection	Severe
Wu et al. [17]	China	46		Kidney	Severe
Winkler et al. [18]		31		Kidney	Severe
Basiratnia et al. [19]		17	2 weeks	Kidney	Mild
Dehmukh et al. [20]	USA	42	1 month		Mild
Tan et al. [21]	Singapore	60	6 weeks		-
Lazareth et al. [22]	France	29	1month	Kidney	-
Perez et al. [23]	Spain	88	33 days	Respiratory insufficiency	
Laboux et al. [24]		57	4 month	Kidney	Mild

What are the symptoms of urinary tract infections in children?

"Fever in young children may be the only sign of a urinary tract infection,". Due to the lack of more obvious symptoms, when girls younger than 2 years old or uncircumcised boys younger than 1 year old have a fever, many medical associations recommend urine culture. "Babies may not eat well, vomit, sleep more, or show signs of jaundice,". In older children, urinary incontinence (loss of bladder control) may occur again; symptoms of a urinary tract infection are often not seen in the elderly; these symptoms may be vague and accompanied by malaise, and the only symptoms present are a change in mental state or fatigue. Bagheri added: "Although in some cases, the first sign that a health care provider encounters is a blood infection or infection in the blood; The fact that many older people already have impotence or dementia may complicate the diagnosis.

During the recovery from acute kidney failure, your gastroenterologist may prescribe special meals to help support and limit your kidney function. Your doctor may refer you to a nutritionist who can analyze your current meals and suggest ways to prepare meals that put less strain on your kidneys. Depending on your situation, your nutritionist may suggest the following: Choose foods with less potassium. Your dietitian may suggest that you choose low-potassium foods. Foods high in potassium include bananas, oranges, potatoes, spinach, and tomatoes. Examples of low-potassium foods include apples, cabbage, green beans, grapes and strawberries. Avoid products with added salt. Get less sodium by avoiding products with extra salt every day. These foods include many ready-to-eat foods, such as frozen foods, canned soups, and fast foods. Other foods with added salt include salty snacks, canned vegetables, and processed meats and cheeses. Limit phosphorus intake. Phosphorus is a mineral found in foods such as milk, cheese, nuts, nuts and peanut oil. High levels of phosphorus in the blood can weaken bones and cause itchy skin. Your dietitian can give you tips on how to limit phosphorus in your specific situation. As your kidneys improve, you may no longer need to follow a specific diet, although a healthy diet is still important.

Figure 3: Flow chart of the study

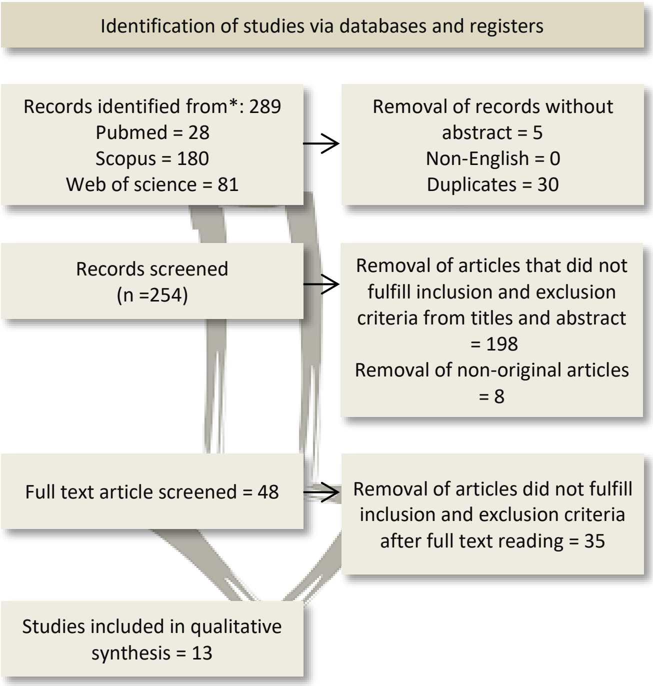
Identification of studies via databases and registers
Records identified from*: 289
Pubmed = 28
Scopus = 180
Web of science = 81
Removal of records without abstract = 5
Non-English = 0
Duplicates = 30
Records screened (n =254)
Removal of articles that did not fulfill inclusion and exclusion criteria from titles and abstract = 198
Removal of non-original articles = 8
Full text article screened = 48
Removal of articles did not fulfill inclusion and exclusion criteria after full text reading = 35
Studies included in qualitative synthesis = 13

DISCUSSION

Early diagnosis of MGN is desirable because it is different from other nephropathies, especially secondary nephropathy, which is caused by causes such as tumor, infection, drug toxicity, or other autoimmune diseases such as systemic lupus erythematosus. Of all cases, 20-30% are MGN due to secondary factors and the remaining 70-80% are classified as primary. Reliable diagnosis of both forms of the disease is important because of differences in treatment: primary MGN is treated with immunosuppressive drugs, while secondary MGN is treated by targeting the causative agent. MGN is detected by a kidney hole followed by a histological test or electron microscopy (to identify immune complexes in the glomeruli). In order to make a final diagnosis of primary MGN, secondary factors must be eliminated, which saves time in the diagnosis process, for example, tumor screening must be performed. In some patients, MGN can be identified as a secondary cause before it is created, which complicates diagnosis and treatment decisions. Primary MGN should be distinguished from other autoimmune diseases involving the kidney, such as lupus nephritis, vasculitis associated with antibodies against neutrophil cytoplasm (ANCA), and Goodpasture syndrome.

In December 2019, Since the COVID-19 emerged in Wuhan city of China and quickly spread throughout the world, it has remained the most prominent health problem all over the world [5]. Our knowledge about this disease is still evolving, and the combination of studies and reports yields a more understanding as time goes on. We investigated a systematic review of the studies that reported glomerulonephritis in hospitalized COVID-19 patients. Treatment of glomerulonephritis include:

1- If your blood pressure is high, your blood pressure will drop with limited water and sodium.

2- Antihypertensive drugs are also used if the blood pressure is high.

3- Proteins of high biological value should be given to improve the nutritional status of the patient.

4- Treat a urinary tract infection immediately.

5- Use diuretics to reduce fluid load.

6- In case of severe edema, raise the head of the patient's bed for more comfort and better urination.

7- Weigh the patient daily.

8- Adjust the patient's sodium and water intake based on the patient's kidneys' ability to excrete water and sodium.

9- Iron and vitamin supplements are given if there is anemia.

10- Cortisone or cytotoxic drugs if the disease is severe.

11- In the early stages of the disease, dialysis is performed in order to improve the patient's physical condition, prevent water and electrolyte imbalance and reduce the risk of complications of renal failure.

CONCLUSION

A report (from China) had even concluded that acute kidney injury (AKI) is not a complication of COVID-19. However, the following evidence modified this perspective. Although, many researchers announced the lower rates of AKI (which may lead to glomerulonephritis) from China compared with elsewhere. These evidence indicated that kidney damages like glomerulonephritis could be a common complication of the Covid-19. Compared to previous reports, our investigation found many case reports of Covid-19 patients with glomerulonephritis, which is demonstrated in table 2. This systematic review was conducted to evaluate glomerulonephritis in Covid-19 patients. Further investigations are warranted to precisely determine whether glomerulonephritis is a complication of COVID-19 or not. However, according to present studies, Covid-19 may lead to several kidney injuries, including glomerulonephritis.

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ORIGINAL ARTICLES

Predictive value of serum level of B type Natriuretic Peptide (BNP) in neonatal sepsis

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Abstract: *Background: NT-proB-type Natriuretic Peptide (BNP) (NT-pro BNP) is one of the biomarkers that has been studied in recent decades in connection with pediatric heart failure (HF) and congenital heart disease (CHD). Recently, infants hypothesized that serum. NT-pro BNP levels might be a good predictor of septicemia severity and response to treatment and prognosis in these patients. This study investigated the relationship between serum natriuretic type B peptide level and neonatal sepsis.*

Materials and Method: In this case-control study, all full-term and pre-term neonates admitted to children's Hospital, Tabriz-Iran(2021), with sepsis and a septicemia diagnosis were confirmed after obtaining consent. The study was deliberately entered by the patient's parents or legal guardian. Complete Blood Count with Differential (CBC/Diff), C - reactive protein (CRP) , Bacterial Culture (B/C) , Urine Analyze(U/A),Urine Culture (U/C) ,NT-pro BNP were checked and finally compared with laboratory results of the same number of infants who did not have clinical and laboratory symptoms of sepsis. After collecting samples, laboratory results, including serum levels of NT-pro BNP, were compared in case and control groups.

Results: One hundred patients were studied in two groups. Thirty-eight patients (38%) were boys, and 62 patients (62%) were girls. The mean age of the patients was 7.58±7.46 days. The mean weight of the studied patients was 2811.80±620.33 grams, with a median of 2855 grams. The most common clinical symptom observed in patients in the case group was fever (100%) followed by Poor feeding (84%). In the control group, all patients had jaundice. Neonates with sepsis had significantly higher initial pro-BNP values than the control group (10023.80 vs. 2247.20; p=0.001). The NT-pro BNP level cut-off point in predicting the final treatment status and mortality of neonates with sepsis was 9583 pg/ml with 97.7% sensitivity and 93.6% specificity.

Conclusion: Measurement of serum level of NT-Pro BNP in neonates with sepsis at the time of clinical signs with 97.7% sensitivity and 93.6% specificity with a cut-off point of 9583 is an important prognostic factor in the therapeutic management of patients.

Keywords: *septicemia, neonates, sepsis, mortality, natriuretic brain peptide*

INTRODUCTION

B-type natriuretic peptide(BNP is a natriuretic peptide with 32 amino acids and a ring structure, which is secreted from the atria and ventricles of the heart in response to changes in pressure or overload of heart chambers. NT-pro BNP (N terminal of the prohormone brain natriuretic peptide) is a marker of BNP in the blood. The mechanism of action of BNP is by activating Natriuretic peptide receptor-A (NPR-A) and increasing the production of Cyclic guanosine monophosphate (cGMP), which has a diuretic effect and dilation of pulmonary arteries [1,2]. BNP is a rapid and highly sensitive marker in myocardial disorders. It also plays an essential role in the organogenesis of the fetal heart and the stage of fetal transfer to the uterus. About 20% of pediatric intensive care units hospitalizations include sepsis, the most common cause of morbidity and mortality, and information from Italian pediatric intensive care units provides 50% mortality in children with septic shock. In adults, there is evidence that increased BNP is associated with heart failure (HF) and increased mortality in cases of severe sepsis and

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septic shock. Sepsis is a systemic response to life-threatening infection, including symptoms of fever, increased heart (HR) rate, and increased respiratory rate (RR). The patient's life is endangered if the diagnosis is delayed. BNP is a useful diagnostic marker in patients with pulmonary hypertension. Increased BNP is associated with hemodynamic compromise, congenital heart disease (CHD), and severe clinical conditions [1-3]. The study of 73 patients by Gisela L et al. (2017) found that BNP can predict hemodynamic changes and prognosis and response to treatment in infants with septicemia [1]. The study of Wang et al. (2015) on 38 patients reported that BNP could be used as a routine hospital marker to predict sepsis in patients [2]. Astudy by Samransamruajkit et al. (2014) on 43 patients with high BNP levels, the mortality rate of children with septicemia was strongly correlated [3]. In another study conducted by Okur et al. (2019) on 87 patients and 35 controls showed that BNP has a high sensitivity (72%) and specificity (86%) for predicting sepsis [4]. According to studies of various biomarkers used to diagnose sepsis, none have sufficient predictive value to confirm or rule out sepsis. However, it has recently been hypothesized in infants that serum NT-pro BNP levels may be a good predictor of the severity of sepsis and the response rate to treatment and prognosis of these patients.

This study aimed was to investigate the relationship between serum natriuretic level of peptide type B and neonatal sepsis.

MATERIALS AND METHODS

In this case-control study, all term and preterm infants were admitted to Tabriz Children's Hospital, Tabriz – Iran, with sepsis from May 2021 to September 2021. Their septicemia diagnosis was confirmed after obtaining informed consent. According to the sample size formula [3] and placing it in the procedure related to the sample size ($X = Z2 * p * (1-q)$) in intervention studies with a small variable, this number has been obtained and considered as 100 patients; that neonates in the study group (Case) was 50 patients and the number of control patients (Control) was 50 patients. Neonates with cardiovascular disease (CVD), other non-cardiac conditions that involve myocardium, and neonates with pulmonary disease were excluded. Inclusion criteria were term and preterm neonates with symptoms and paraclinical signs of sepsis and approved diagnosis of sepsis by neonatal specialist.

Complete Blood Count with Differential (CBC-Diff), C-reactive protein (CRP), Blood culture (B/C), Urine culture (U/C), Urine microscopic analysis, and NT-pro BNP were checked as study nodes, and finally with laboratory results of the control group with the same number of infants who did not have clinical and laboratory symptoms of sepsis only because of high bilirubin was hospitalized and compared with phototherapy.

Criteria for diagnosing sepsis included clinical signs of seizures, fever, vomiting, anorexia, hypothermia, cyanosis, respiratory distress, and hyperreflexia.

To measure NT-pro BNP, onecc of blood was taken from the patient and examined in the laboratory by ELISA method with a Siemens kit made in Germany. Sampling was performed for Urine Analyze (U/A) and U/C after washing and drying the site using a catheter. U/A results whit white blood cells (WBCs) greater than five or Red blood cells (RBCs) greater than five and positive nitrite test or observation of bacteria and yeast if epithelial cells were low were considered active urinalysis. For CBC- diff, 2 ccs of blood were collected in an EDTA test tube and sent to the laboratory. Also, for CRP, half a cc of blood was centrifuged in the laboratory, and its serum was isolated, and then measured by ELISA (Scaleo CRP kit is made in Italy). Toperform blood culture, 2 ccs of whole blood were obtained under sterile conditions in a two-phase culture medium (Iranian manufacturing company), sent to the laboratory, and performed manually. After collecting samples, laboratory results, including serum levels of NT-pro BNP, were compared in case and control groups.

The data obtained from the study were analyzed using descriptive statistical methods (mean±SD and frequency(%)) and mean difference test for independent groups and chi-square test and analyzed using SPSS/WIN statistical software. Furthermore, statistical analysis was performed. P values less than 0.05 were considered significant.

RESULTS

Thirty-eight patients (38%) were boys, and 62 patients (62%) were girls. The mean age of the patients was 7.58±7.46 days, with a median of 4 days. The minimum age of patients was 3.5 days, and the maximum was 29 days. The mean weight of the studied patients was 2811.80±620.33 grams, with a median of 2855 grams. The minimum weight of patients was 1190 grams, and the maximum was 4400 grams. The demographic findings and clinical symptoms of patients are shown in Table 1. The most common clinical symptom observed in patients in the case group was fever (100%) followed by poor feeding (84%). In the control group, all patients had jaundice.

The laboratory findings of the studied patients by study groups are shown in Table 2. The mortality rate in the study group was 4%. Evaluation of the relationship between primary NT-pro BNP and response to treatment and survival of patients showed that NT-pro BNP levels were significantly higher in infants who did not respond to treatment and died.

Table 1: Demographic findings and clinical symptoms of patients

Variable	Case (N=50)	Control (N=50)
Demographics		
Boy	24 (48%)	14 (28%)
Girl	26 (52%)	36 (72%)
Age (days)	10.90±9.23	4.12±1.45
Weight (grams)	2644.80±598.52	2978.80±601.93
Clinical Symptoms		
Fever	50 (100%)	0
Weakness	40 (80%)	0
Shock State	12 (24%)	0
Vomiting	8 (16%)	0
Poor feeding	42 (84%)	0
Icter	0	50 (100%)

** The results are presented as mean±SD and frequency (%).*

The NT-pro BNP level cut-off point in predicting the final treatment status and mortality of neonates with sepsis was 9583 with a sensitivity of 97.7% and specificity of 93.6% (Figure 1).

Figure 1: Evaluation of diagnostic value of NT-pro BNP measurement in predicting final treatment status and mortality of neonates with sepsis

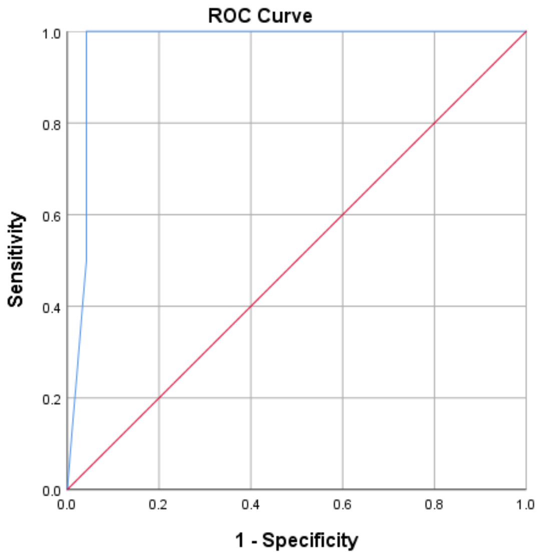

Diagonal segments are produced by ties.

Table 2: Laboratory findings of patients studied by study groups

Variable	Case (N=50)	Control (N=50)	p-value
CRP Negative	0	50 (100%)	
CRP 1+	22 (44%)	0	
CRP 2+	12 (24%)	0	
CRP 3+	16 (32%)	0	
WBC (cells*10^3/ml)	10.75±4.37	10.04±2.05	0.299
Neut (cells*10^3/ml)	5.27±3.44	4.07±1.57	0.029
Lymph (cells*10^3/ml)	3.91±1.87	4.55±.126	0.048
NT-pro BNP			
Basic	10023.80±10689.77	2247.20±2076.99	0.001
After Treatment	2378.56±2004.48	-	-
Positive Blood Culture	6 (12%)	0	0.013
Gram negative Bacillus	2	0	-
Yeast	2	0	-
Coagolase-negative Staff	2	0	-
Positive Urine Analysis	30 (60%)	0	-
Positive Urine Culture	0	0	-
Mortality	2 (4%)	0	0.231

** The results are presented as mean±SD and frequency (%).*

DISCUSSION

In this study, a case-control analysis was performed to evaluate the predictive value of serum natriuretic type B peptide level in neonatal sepsis. Fifty infants were also studied as a control group, 38% of whom were boys, and their mean age was 7.58±7.46 days with a median of 4 days.. Sepsis is one of the standard clinical conditions that directly and significantly affect infant mortality and morbidity. According to World health organization (WHO) estimates, sepsis accounts for about 60 to 80% of infant mortality cases per year [5]. Sepsis also accounts for nearly 20% of intensive care unit admissions and is the leading cause of mortality and morbidity in the Pediatric Intensive Care Unit (PICU). The results of the surviving sepsis campaign show mortality of 34.8%, and this number is higher in developing countries [6, 7]. Results from Italy also show a mortality rate of nearly 50% in the PICU of neonates admitted with septic shock [7]. The results show that the hospitalization rate of neonates with sepsis is higher than controls, and most studies have emphasized the importance of rapid diagnosis and treatment. A recent study reported that rapid diagnosis and intervention in infants with septic shock can be highly life-saving and that the outcome of patients can be more favorable [8]. Rapid diagnosis of sepsis and balancing hemodynamics of patients are the main factors involved in reducing patient mortality. Intravenous antibiotics are also a key to treating patients [9].

The results of the present study showed that the most common clinical symptoms in neonates with sepsis include fever (100%), weakness (80%), shock state (24%), and vomiting (16%). In the control group, all patients had jaundice. Evaluation of patients' laboratory findings showed that neonates with sepsis had significantly higher initial NT-pro BNP values than the control group (10,023.80 vs. 2,247.20; p = 0.001). Numerous biomarkers have been evaluated to evaluate the status of patients with severe sepsis and septic shock. For example, procalcitonin has recently been directly associated with the severity of bacterial infections [10]. In addition, cardiovascular disease is more common in children than in adults with severe sepsis or septic shock, so that in 58% of cases, low cardiac output with high peripheral vascular resistance, and in 22% of cases, they have low cardiac output with low peripheral vascular resistance [11,12]. Therefore, biomarkers associated with cardiovascular disorders in infants and children can benefit infants with sepsis. NT-pro BNP is a biomarker synthesized and secreted by myocytes and atrial and ventricular fibroblasts and is also associated with cardiovascular dysfunction [13,14]. As mentioned in the present study, neonates with sepsis had significantly higher serum NT-pro BNP levels than the control group. Also, after treatment, the level of this biomarker in patients in the affected group was reduced considerably (23,786.56 vs. 10,023.80; p = 0.001). Recently, researchers found that measurement of primary NT-pro BNP levels in neonates with sepsis was significantly associated with patient mortality and lays the groundwork for fluid therapy decision-making for fluid therapy. This study indicated that the volume of positive fluid balance in patients was directly related to increased serum BNP levels [15]. The above research results are consistent with our study It is better to evaluate the level of electrolyte balance in patients with sepsis and its relationship with the serum level of NT-pro BNP in future studies.

In the present study, in terms of the outcome, 96% of patients with sepsis had successful treatment, and 4% died. The results of evaluating the value of pro-BNP diagnosis in predicting the final treatment status of neonates with sepsis with a 9,583 cut-off point had a sensitivity of 97.7% and specificity of 93.6% (p = 0.002; AUC = 0.969). Similarly, a good prognosis has been reported for PICU mortality [3]. In recent years, several studies have been performed that show a significant and traceable increase in serum BNP levels in patients with sepsisthTherise in serum BNP and NT-pro BNP levels havebeen directly related to increased mortality in patients [3,16,17]. The increase in BNP is high in patients with sepsis, but ventricular dysfunction in these patients is not easily measured.

On the other hand, several factors such as lipopolysaccharides (LPS) [18], inflammatory cytokines like Tumour Necrosis Factor - alpha (TNF alpha) and interleukin-1 beta (IL-1 beta) [19], and catecholamines [20] also increase BNP. Recent studies have shown that BNP levels in neonates with sepsis caused by gram-negative bacteria were higher, indicating the effect of the type of bacterial strain, so that the bacterial polysaccharide may have led to a more severe immune response. Therefore, it is suggested to be considered in future studies [21]. In neonates with sepsis, other mediators, including endotoxins, inflammatory cytokines, and myocardial depressant factors, increase NT-pro BNP levels through myocardial damage and dysfunction [22]. Therefore, it is better to perform Doppler echocardiography in these patients to evaluate cardiac function after the initial critical procedures. In this study, negative urine culture results despite active urinalysis may be due to the early initiation of antibiotic treatment in the first venipuncture.

Limitations of study

One of the limitations of this study is the lack of data on the effect of serum bilirubin on BNP. The second limitation of this study is the lack of information on the effect of electrolytic disorders on serum BNP levels.

CONCLUSIONS

Overall, according to the results obtained in this study, it can be concluded that measuring the serum level of NT-pro BNP in neonates with sepsis at the time of observation of clinical symptoms with a sensitivity of 97.7% and specificity of 93.6% with a cut-off point of 9,583 is an important prognostic factor. In the medical management of patients. In future studies, the relationship between the type of bacterial strain leading to sepsis and the level of electrolyte balance in patients with sepsis be compared with the serum levels of NT-pro BNP and BNP.

Ethical Approve

The ethics committee approved the present study with the code (IR.TBZMED.REC.1400.141) of Tabriz University of Medical Sciences. Because all diagnostic procedures have been performed for the diagnosis and treatment of patients and in addition to the treatment process of patients, no additional blood sampling and no cost for measuring serum NT-pro BNP level wastaken from patients, so no ethical case does not exist.

Authors' Contribution

Study concept and design: MH. M. and A. G Kh. Acquisition of data: S. Sh; Analysis and interpretation of data: R.P; Drafting of the manuscript: MH. M. and A. G Kh; Critical revision of the manuscript for important intellectual content: R.P; Statistical analysis: A. Kh and S.Sh

Conflict of Interest

There is no conflict of interest.

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ORIGINAL ARTICLES

Pre-surgical management of primary leiomyosarcoma of the bone

George M. Avram[1], Cezar I. Calin[2], Mihnea Dobre[1], Miruna Harabagiu[3], Cristian Peneoaşu[3], Diana M. Marcu[4]

Abstract: *Leiomyosarcoma is a rare type of smooth muscle cell tumor, with an incidence of 7-10% compared to other types of sarcomas. It develops primarily in the retroperitoneal cavity, the genital tract and extremities. Leiomyosarcoma can also affect the bone, either as a primary tumor or as metastasis. Primary osseus leiomyosarcoma is a rare finding in patients with radiologically identified osteolytic lesions having an incidence of <1% of total primary bone tumors.Due to its low incidence patients with primary osseous leiomyosarcoma are never diagnosed before immunohistochemical analysis and case management is difficult because of the lack of specific indications pertaining to primary osseus leiomyosarcoma. Data on age specific incidence is scarce but there seems to be a tendency towards a higher incidence with advanced age.*

The purpose of this work is to discuss the complete management of primary osseus leiomyosarcomas based on interdisciplinary medical teams' indications in a Romanian hospital setting.

Keywords: *primary leiomyosarcoma of bone, immunohistochemical analysis, case management*

INTRODUCTION

Primary osseous leiomyosarcoma was first described in 1965 as a malignant tumour arising in the proximal left tibia of a 73 year old patient [1].

An exact reported incidence is unknown due to the scarcity of cases but out of the total amount of primary malignant tumours of the bone, primary leiomyosarcoma is listed together with Ewing and other sarcomas as having a <1% annual incidence [2]. Twenty-eight case have been reported in the USA between 1965 and 1990, however the number rose to a total of 120 cases between 1990 and 2005 and by 2020 many more authors reported their experience with this type of tumour as well as outcomes and prognostic factors [3,4,5,6,7]. Rising reported cases are most likely due to the widespread use of immunohistochemistry.

To date, there is no clearly defined prevalent location of primary osseous leiomyosarcoma but a nationwide study of 104 patients diagnosed with leiomyosarcoma during 1975 and 2009 reported the femur, pelvis and tibia as the first three most common locations [5]. Regarding age and gender distribution of leiomyosarcoma, no clear pattern was identified, although a nationwide study based on the French network of cancer registries reported an ascending incidence rate up to 35% incidence in the 40-64 years age group declining thereafter to 27% in the 75+ years group while gender distribution seems to incline to a higher reported incidence in females [7].

Certain diagnostic is based on combined histopathological and immune histochemistry exams with histological grading and surrounding tissue invasion being the key elements for long-term prognosis [3,4].

Surgical options range from fine needle biopsy to locoregional excision and limb amputation. Function is severely compromised by amputation, although there are on-going efforts to develop artificial limbs [8] and to integrate them with the remaining limb stump [9,10].

A patient will present for an orthopaedic examination with

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pain in a localised area, for example the proximal tibia, with no or scarce other local or systemic clinical signs, except for a painful elevation gradually appearing on the proximal tibia. Physical examination may show a tumorous formation located at the proximal tibial level presenting with renitent consistency and adherence to adjacent tissue.

Radiological findings

Physical examination would be followed by standard anteroposterior and latero-lateralradiographic exam of the affected segment, in our example, the knee and proximal calf (Figure 1). Radiographic results may reveal an osteolytic lesion localized at the transition zone between the proximal metaphysis and epiphysis of the tibia, with a mild extension towards the tibial shaft and with a taller than wider aspect. The margins may not be clearly defined and there may not be peripheral sclerosis. Erosion and narrowing of the medial cortex may be shown. Bone marrow may have a lobulated aspect but no extra-axial marrow growth. No periosteal reaction nor other bone structural changes may be found.

Figure 1: Right knee anteroposterior and lateral radiographs, suggestive of osteolytic lesion on the proximal metaphysis of the tibia.

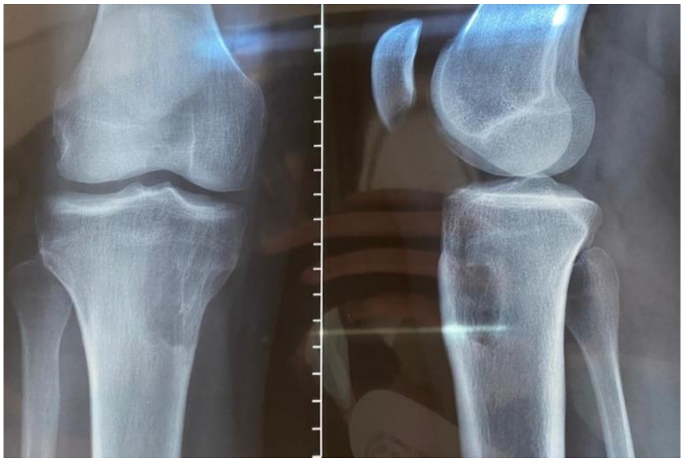

Solely based on the radiographic examination, reported results may reveal both benign and malignant features. The benign feature would be represented by the lobulated structure of the bone marrow. The malignant features would be represented by the absence of peripheral sclerosis which would be suggestive of a rapidly evolving lesion and by the absence of periosteal reaction which would signify a fast growing mass.

Figure 2: A – axial sequence of the proximal tibial metaphysis showing osteolysis and extraosseous invasion; B – coronal sequence of the proximal tibial metaphysis; C – sagittal sequence of the proximal tibial metaphysis

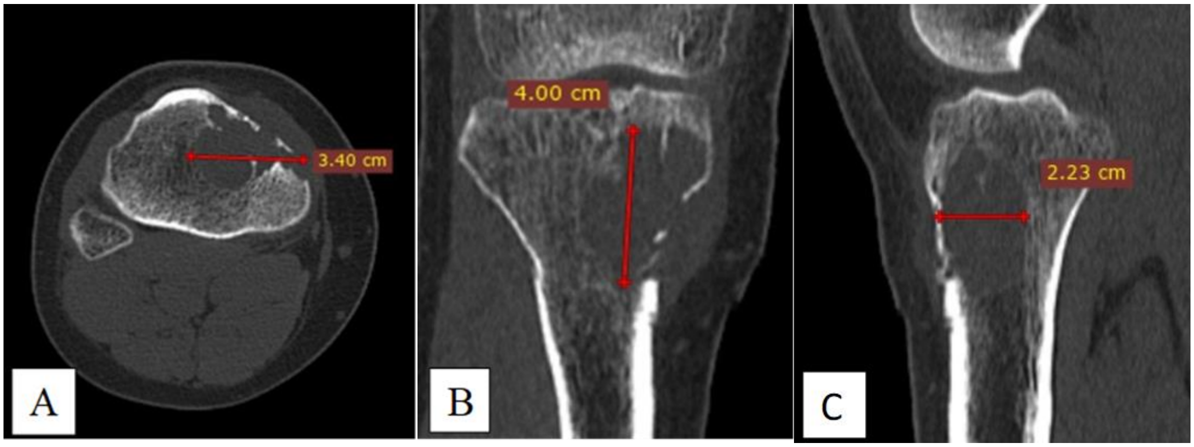

Following the radiographic examination a native CT scan (Figure 2) would be performed, that may be describing an osteolytic lesion, with no clear outline and irregular shape, localised at the proximal metaphyseal-epiphyseal junction of the tibia. The lesion may interrupt the medial cortex and may not affect the articular outline. Based on compared densities, tibial intra-axial trabecular bone signal may be identified within the ingrown mass. The left over trabeculae would be a result of a fast growing osteolytic lesion. Infiltration of the soft tissue, absence of periosteal reaction, as well as the absence of adenopathy in the popliteal fossa may be observed.

Such CT findings strongly would suggest the presence of an aggressive osteolytic tumour.

Histopathological exam

In order to confirm the diagnosis, an incisional biopsy will be performed and the sampled tissue will be sent for histological examination (Figure 3). Results may reveal a sarcomatous proliferation composed of fusiform, elongated cells, arranged in a storiform fashion, forming fascicles that intersect at sharp angle; hyperchromatic ovoid, elongated nuclei, that present abnormal variations and frequent mitoses (7-8 mitoses/10 HPF) and extended necrotic areas; absence of osteoid matrix may also be noticed. Following histopathological examination a immunohistochemical exam will be recommended.

Figure 3: A – histopathological analysis with haematoxylin and eosin stain, x200, with typical aspects for sarcoma; B –histopathological analysis with haematoxylin and eosin stain, x200, showing multiple faults and mitoses; C – immunohistochemical analysis, diffuse positive for desmin; D – diffuse positive for caldesmon and E – diffuse positive for smooth muscle actin (SMA).

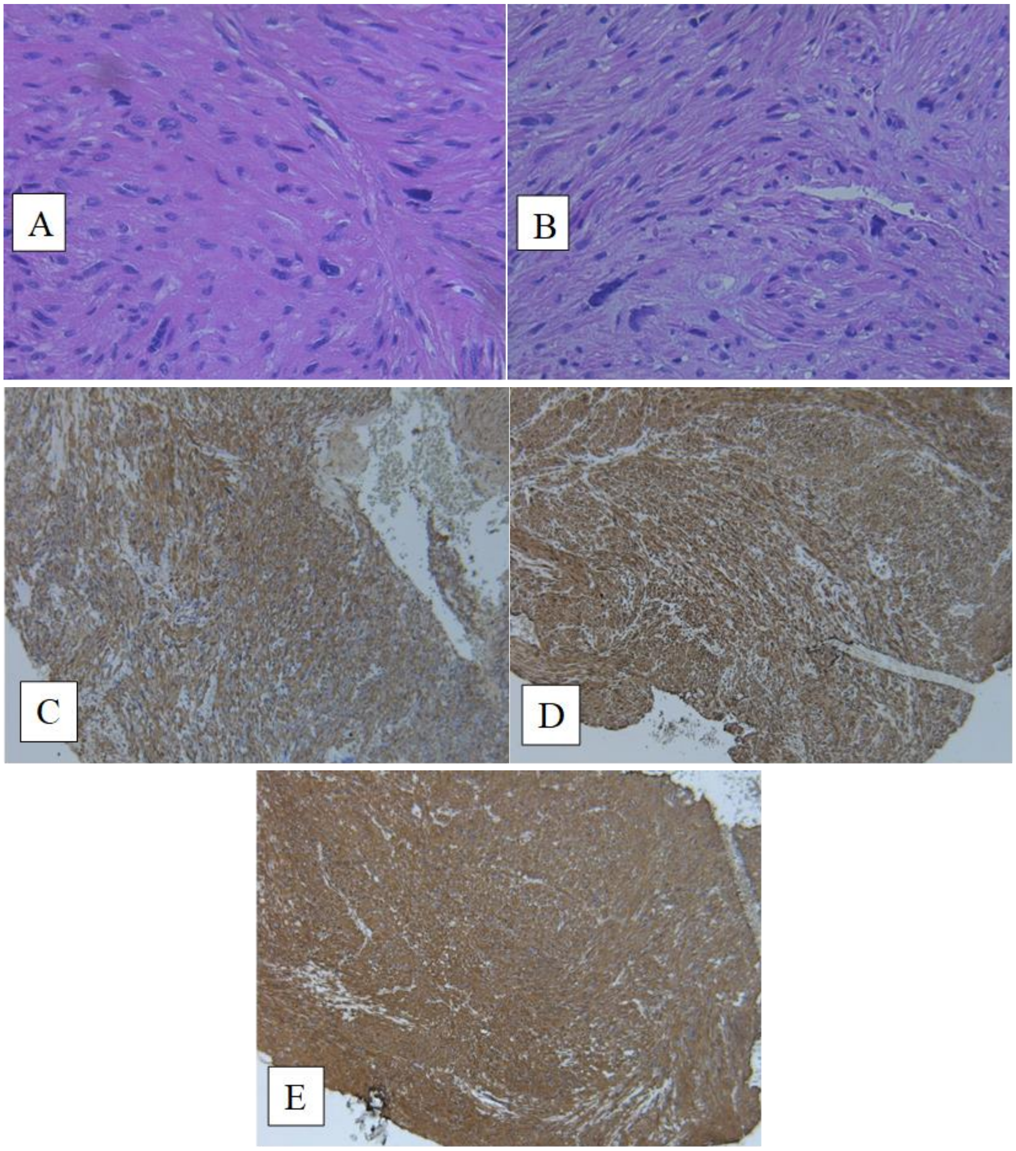

Following immunohistochemistry examination (Figure 3) the tissue sample may be found to be positive to the following immunohistochemical markers: smooth muscle actin (SMA), caldesmon (+), desmin (+), special AT-rich sequence-binding protein 2 (SATB2) (+), 60% (+) ki67, (-) CD34, (-)transducin-like enhancer of split 1 (TLE1), (-) S100.

Immunohistochemistry tests will be correlated with the histopathological exam and together sugget the final diagnostic of leiomyosarcoma of bone, G2 stage–moderately differentiated. Staging is based on the Fédération Nationale des Centres de Lutte Contre le Cancer indications (FNCLCC)[11].

The correlation of imaging, histopathological and immunohistochemical results would lead to the formation of a interdisciplinary team of medical experts for the management of this case. The next step would be to perform a full-body native CT scan in order to rule out the presence of other primary site leiomyosarcoma locations that could bea source of metastases and could reclassify the proximal tibial lesion as not a primary osseous leiomyosarcoma but rather a metastases from a leiomyosarcoma formed elsewhere. After a full-body native CT scan would be performed, results may report no other identified lesions effectively establishing the final diagnosis of primary leiomyosarcoma of bone.

Because leiomyosarcomas have such a low incidence and difficult to interpret radiological and histological results, together with a poorly established prognosis, treatment options that best fit these types of cases would be dependent on the surgical and oncological team's experience. A multidisciplinary team is needed in order to synchronize radiotherapy and chemotherapy with surgery, as well as for establishing the proper conditions for psychological support. Also, as an attempt to preserve the affected limb, a tumoral reconstruction prosthesis would be indicated. In order to diminish the risk of local recurrence, the multidisciplinary team would also recommend one session of external neoadjuvant radiotherapy of 50Gy followed by surgical excision, prosthesis implantation and administration of adjuvant chemotherapy consisting of a dose of 5000mg/ m2ifosfamide (IFO) associated with intra-venous (i.v.)mesna for 24h infusion and doxorubicin (DOX) 60mg/m2i.v. (short infusion). Six sessions of adjuvant chemotherapy would be recommended by the oncological team to be administered once every 3 weeks (DOX + IFO x 6, q3w). Filgrastim would also be recommended by the oncological team in order to stimulate granulocyte colony-forming cells as a single administration of 5 mg/mL dose at least 24 hours after chemotherapy. The purpose of these recommendations is to reach local cancer margin control after neoadjuvant radiotherapy and to eradicate any minimal residual disease with adjuvant chemotherapy and to diminish the risk of metastases.

DISCUSSIONS

Numerous studies report variable results of leiomyosarcoma treated with chemotherapy and radiotherapy [12-15]. Radiotherapy was found to have useful results in controlling tumour margins especially in R0 and R1 reported margins [13,16,17]. This being said the multidisciplinary team may consider appropriate to recommend neoadjuvant radiotherapy of up to 50Gy in order to account for any possible microscopic tumoral cells that might remain. Together with neoadjuvant radiotherapy good results were obtained by adding surgery and adjuvant chemotherapy consisting of doxorubicin and ifosfamide [5,18,19]. In order to obtain the best possible long-term outcome a tumoral prosthesis would be recommended by the team for these cases.

Among all the treatment protocols with good outcomes that have been reported in literature, we should bring to mind the association of ifosfamide and doxorubicin with filgrastim 24 hours laterfor stimulating granulocyte colonies in order to permit chemotherapy amplification [15,20].

At the same time it is worth mentioning that the chemotherapeutical doses are not standardized and, thus, we would like to remark the possibility of lowering the dose of ifosfamide in order to raise the dose of doxorubicin, and at the same time to maintain the myelosuppression within the desired limits, and to offer a better response in correlation with dosage [18,21]. For leiomyosarcoma cases we could take into consideration other therapies, such as gemcitabine or gemcitabine-docetaxel association, mitomycin-doxorubicin-cisplatin triple therapy or administration of epirubicin in association with ifosfamide [14,22]. Regardless of the chosen therapy, it is important to mention the fact that the complete response to chemotherapy has not exceeded 8% of the total amount of patients who were treated with a known therapy plan and similar results have also been obtained for partial response to chemotherapy [22,23].

CONCLUSIONS

In conclusion, complicated, rare primary bone leiomyosarcoma cases do not have a stable management recommendation as of yet and are best treated by a multidisciplinary team which, most often than not, bases its recommendations on the established leiomyosarcoma treatment. The scope of this paper was achieved, by presenting the full pre-surgical management of such a tumour, with a focus on the importance of immunohistochemical exam, full-body CT for metastases exclusion and oncological treatment which may be doxorubicin and ifosfamide combined with mesna and Filgrastim.

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ORIGINAL ARTICLES

Quality of life assessment for rectal cancer patients: A comparison between sphincter preservation and permanent colostomy

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Abstract: *This study aims to compare the quality of life in patients with rectal cancer between sphincter preservation and permanent colostomy. The present study is a cross-sectional study that was performed on 120 patients (60 cases in the sphincter maintenance group and 60 cases in the permanent colostomy group) who underwent surgery from February 2017 to November 2016. Quality of life assessment was provided with QLQ-C30 and QLQ-CR29 questionnaires. Finally, the collected data were analyzed using SPSS23 software. According to the results of the QLQ-C30 questionnaire, there was no statistically significant difference in patients' quality of life in terms of performance in the study groups (P>0.05). Symptoms such as nausea, pain, shortness of breath, sleep disturbance, loss of appetite, constipation and economic problems in the study groups were not statistically significant (P>0.05). But the mean symptoms of fatigue (P=0.038) and diarrhea (P=0.037) were significantly higher in the sphincter maintenance group than the permanent colostomy group. The mean score of patients' quality of life was 53.43 ± 8.40 in the sphincter retention group and 52.23 ± 11.45 in the permanent colostomy group (P = 0.556). According to the results, the quality of life in the two surgical methods of sphincter preservation and permanent colostomy was not statistically significant. Therefore, treatment decisions should be made by informing patients, both based on their preferences and the physician's clinical judgment.*

Keywords: *rectal cancer, colostomy, sphincter*

INTRODUCTION

In most countries, especially developed countries, cancers are the second leading cause of death after heart disease [1]. After lung and prostate cancer, colorectal cancer (CRC) is the third most common cancer in the world and among men, and after breast cancer it is the second most common cancer among women (Figure 1) [2]. In Iran, after heart disease and accidents, cancers are the third leading cause of death [3]. In the United States, it is the fourth most common cancer in men after skin, prostate and lung cancers, and the most common cancer in women after skin, lung and breast cancers [4]. In fact, colorectal cancer is caused by the uncontrolled growth of the inner layer of the organs of the colon and rectum, which increases with age [4].

Figure 1: Under endoscopic visualization

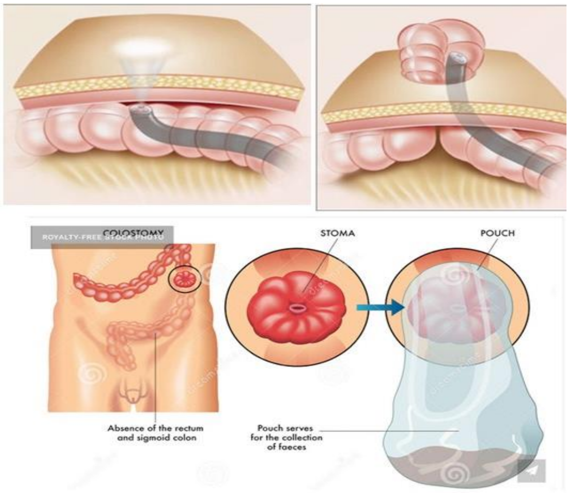
COLOSTOMY
STOMA
POUCH
Absence of the rectum and sigmoid colon
Pouch serves for the collection of faeces

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The exact cause of colorectal cancer is not known, however, the disease is not contagious and no one gets the disease from another person [5]. Research has shown that people with certain risk factors, such as aging, family history, genetic disorders, smoking, colorectal polyps, and poor diet, are more likely suffering colorectal cancer than other people [6]. The first line of treatment is surgery to remove the tumor. Surgical methods include: anterior resection with reconstructive anastomosis and tumor removal, and surgery to remove the rectum from the perineum and abdomen with a permanent ostomy [7].

In case of metastasis, supportive surgery is performed to eliminate the symptoms, prevent obstruction and improve the patient's quality of life. In some cases, an ostomy may be needed to treat cancer during bowel resection [8]. The choice of treatment is based on the staging of the disease and surgery is the basis of definitive treatment in the early stages. Today, chemotherapy and sometimes radiotherapy (in rectal cancers) are used in the form of adjuvant in certain stages of the disease [9]. An ostomy (ileostomy or colostomy) is the creation of a wall on the abdomen that is used to help the bowel or bladder drain. Depending on the type of surgery used and the extent of the disease, a colostomy may be temporary or permanent [10]. Most patients with colorectal cancer survive for at least 5 years after diagnosis, so quality of life is an important issue in these patients [11].

Understanding the quality of life experienced by patients with colorectal cancer is essential to assessing the impact of the disease on individuals, their families, and their communities [12]. Cancers can cause a change in the patient's assessment of their health and quality of life by causing physical, social, economic, and etc. disorders. Awareness of patients' quality of life can be considered as an effective factor in making clinical decisions and managing the problems of these people and can also be useful in guiding and accompanying these patients in better use of support and rehabilitation facilities [13]. Abdominal Perineal Resection (APR) surgery, which involves a permanent colostomy, is performed for patients with lower rectal cancer with sphincter involvement or those who are more likely have a malfunctioning sphincter after sphincter retention. Numerous studies have suggested that patients with colostomy experience have significant changes in various aspects of quality of life, including the physical, social, and physiological dimensions, compared with those undergoing anatomical preservation of the sphincter [14]. Therefore, in the present study, we aimed to compare the quality of life in patients with rectal cancer between sphincter preservation and permanent colostomy.

MATERIALS AND METHODS

This study is a cross-sectional study and was performed in colorectal surgery clinic or ostomy care clinic of Ghaem Hospital in Mashhad from February 2017 to November 2016. This research was presented in the organizational ethics committee of the university which was approved with the code IR.MUMS.MEDICAL.REC.1399.457. Inclusion criterias were patients over 18 years of age who presented for follow-up after previous treatment for rectal cancer, including radical surgery with Total Mesorectal Excision (TME), in the colorectal ward of Ghaem Hospital with or without sphincter retention. At least 12 months after APR or aberrant ostomy closure, if they agreed to participate in the study, they entered the project. Exclusion criterias were patients with ostomy with a different histology from adenocarcinoma and intraperitoneal rectal tumor, patients with peritoneal carcinomatosis, and patients who had not received neoadjuvant before surgery were excluded from the study. Patients were divided into two groups: 1) the sphincter retention group (SPG), which included patients who underwent a lower colorectal or coloanal anastomosis; And 2) permanent colostomy (OCG) group, which included patients who underwent permanent APR and terminal colostomy. In this study, 120 patients (60 cases in the sphincter maintenance group and 60 cases in the permanent colostomy group) were included in the study.

QLQ-C30 Questionnaire

A multidimensional questionnaire consisting of 30 questions that quality of life in 5 functional areas (physical function, role-playing, emotional, cognitive and social) and 9 areas of symptoms (fatigue, pain, nausea, vomiting, shortness of breath, diarrhea, constipation examines, sleep deprivation, loss of appetite, and economic problems caused by illness) and one general area of quality of life.

QLQ-CR29 Questionnaire

This questionnaire is designed to assess the quality of life of patients with colorectal cancer and includes 29 questions that describe the symptoms (gastrointestinal, urinary and pain symptoms) and functional status (such as sexual function and body image) of patients with colorectal cancer. This questionnaire has a separate questionnaire to measure the sexual function of men and women.

Quality of life assessment

Quality of life assessment was performed with QLQ-C30 and QLQ-CR29 questionnaires. In this way, patients were informed about the design and nature of the questionnaires; They filled out the questionnaires independently and if they had any problems, they were given a proper explanation by the project implementers.

Statistical Analysis

After completing the checklists, the information was analyzed by entering it in SPSS23 software. Data description was expressed with tables and graphs and central indicators and dispersion. Mann-Whitney test was used to compare quantitative data between the two groups due to abnormal data distribution. Chi-square test was used to compare qualitative data between the two groups. Significance level in the tests was considered P>0.05.

RESULTS

Average age of patients

The mean age of patients in the sphincter maintenance group was 57.80 ± 9.96 years and in the permanent colostomy group was 59.73 ± 9.31 years (P=0.196) (Figure 2).

Gender of patients

60% of the participants in this study were male and 40% were female. The frequency of patients related to gender in each of the study groups can be seen in Table 1. The results of Chi-square test showed that the sex of patients in the study groups was not meaninful (P=0.456).

Figure 2: Mean age of patients in the studied groups

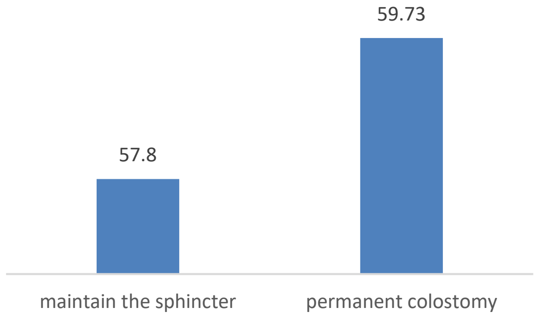

Other variables

The results of Chi-square test showed that marital status (P=0.283), level of education (P=0.6), place of residence (P=0.224) and family history of colorectal cancer (P=0.409) were not significant in the study groups (Table 2).

Table 1: Evaluation and sex determination of patients in the study groups

Variables		Groups			p-value
		SPG Frequency (percentage)	OCG Frequency (percentage)	Total Frequency (percentage)	
Gender	Male	38 (63.3)	34 (56.7)	72 (60)	0.456
	Female	22 (36.7)	26 (43.3)	48 (40)	
	Total	60 (100)	60 (100)	120 (100)	

Table 2: Frequency of other variables in the study groups

Variables		Groups			p-value
		SPG Frequency (percentage)	OCG Frequency (percentage)	Total Frequency (percentage)	
Marital status	Married	50 (83.3)	54 (90.0)	104 (86.7)	0.283
	Single	10 (16.7)	6 (10)	16 (13.3)	
	Total	60 (100)	60 (100)	120 (100)	
Education level	High School	44 (73.3)	48 (80.0)	92 (76.7)	0.600
	Diploma	12 (20.0)	10 (16.7)	22 (18.3)	
	Above the diploma	4 (6.7)	2 (3.3)	6 (5.0)	
	Total	60 (100)	60 (100)	120 (100)	
Place of life	City	40 (66.7)	46 (76.7)	86 (71.7)	0.224
	Village	20 (33.3)	14 (23.3)	34 (28.3)	
	Total	60 (100)	60 (100)	120 (100)	
Family history of colorectal cance	Yes	18 (30.0)	18 (30.0)	32 (26.7)	0.409
	No	42 (70.0)	42 (70.0)	88 (73.3)	
	Total	60 (100)	60 (100)	120 (100)	

Quality of Life Assessment Questionnaire for cancer patients (QLQ-C30 Questionnaire)

According to the results of QLQ-C30 questionnaire in Table 3, patients' quality of life in the field of performance including physical function (P=0.103), role playing (P=0.162), emotional (P=0.050), cognitive (P=0.801) and social (P=0.713) There was no significant difference in the study groups. Symptoms include nausea (P=0.535), pain (P=0.298), shortness of breath (P=0.513), sleep disturbance (P=0.231), loss of appetite (P=0.3609), constipation (P=0.303), and economic problems (P= 0.584) in the study groups were not meaninful. But the mean symptoms of fatigue (P=0.038) and diarrhea (P=0.037) were significantly higher in the sphincter retention group than the permanent colostomy group. The mean score of patients' quality of life was 53.43 ± 8.40 in the sphincter retention group and 52.23 ±11.45 in the permanent colostomy group. The results of Mann-Whitney test showed that the quality of life of patients based on the QLQ-C30 questionnaire in the study groups was not significant (P=0.556).

Table 3: Evaluation the average life quality of patients based on the QLQ-C30 questionnaire in the study groups

QLQ-C30 questionnaire			Average	Standard deviation	Middle	At Least	At Most	p-value
Function	Physical status	SPG	59.83	8.14	59.50	40.00	76.00	0.103
		OCG	57.80	6.97	57.50	44.00	72.00	
	Play role	SPG	51.10	6.67	51.50	40.00	65.00	0.162
		OCG	49.83	8.15	50.00	38.00	72.00	
	Emotional	SPG	48.86	5.91	50.00	38.00	62.00	0.050
		OCG	47.23	8.93	43.00	37.00	72.00	
	Cognitive	SPG	67.16	6.71	69.50	50.00	78.00	0.801
		OCG	67.40	6.97	68.50	53.00	78.00	
	Social	SPG	42.36	5.84	41.00	33.00	53.00	0.713
		OCG	42.76	9.97	40.00	21.00	72.00	
Signs	Fatigue	SPG	48.10	5.92	49.00	38.00	60.00	0.038
		OCG	46.30	9.22	42.00	33.0	69.00	
	Nausea	SPG	38.36	9.08	38.50	22.00	53.00	0.535
		OCG	39.60	9.13	39.00	21.00	60.00	
	Pain	SPG	44.40	5.75	43.00	34.00	58.00	0.298
		OCG	44.33	11.05	41.00	21.00	72.00	
	Shortness of reath	SPG	22.00	3.55	21.00	17.00	31.00	0.513
		OCG	21.80	4.32	21.00	16.00	34.00	
	sleep disorder	SPG	45.73	5.81	46.00	34.00	57.00	0.231
		OCG	44.80	11.20	41.00	21.00	72.00	
	Decreased appetite	SPG	48.40	5.55	48.00	38.00	59.00	0.360
		OCG	49.16	11.49	50.00	21.00	68.00	
	Constipation	SPG	45.96	6.34	45.50	32.00	58.00	0.303
		OCG	44.86	11.26	42.00	21.00	72.00	
	Diarrhea	SPG	18.66	4.15	19.00	11.00	27.00	0.037
		OCG	17.40	5.67	15.00	11.00	31.00	
	Financial problems	SPG	65.43	11.47	69.00	38.00	82.00	0.584
		OCG	64.00	11.64	68.50	40.00	79.00	
Quality of Life		SPG	53.43	8.40	52.00	38.00	68.00	0.556
		OCG	52.23	11.45	52.00	37.00	72.00	

According to the results of the QLQ-C29 questionnaire in Table 4, the quality of life of patients in the areas of functions including anxiety (P=0.171) and female sexual function (P=0.0725) in the study groups were not significantly different. But the quality of life of patients in the areas of functions such as body image (P=0.021), weight (P=0.032) and male sexual function (P= 0.016) in the sphincter maintenance group was significantly higher than the permanent colostomy group. There were no significant differences in the mean symptoms such as hip pain (P= 0.125), hair loss (P = 0.265), taste disturbance (P=0.070) and skin ulcers (P= 0.100). But the mean of symptoms such as frequent urination (P =0.018), blood in the stool (P = 0.013), stool frequency (P=0.044), urinary incontinence (P=0.01), heartburn (P= 0.01), abdominal pain (P=0.01), bloating (P=0.01), dry mouth (P=0.01), excessive bowel movements (P= 0.01), fecal incontinence (P= 0.01), and embarrassment sense (P=0.01), impaired. There was a meaningful difference between erection (P=0.01) and dyspareunia (P=0.001) in the study groups.

Table 4: Evaluation the average life quality of patients based on the QLQ-C29 questionnaire in the study groups

QLQ-C29 questionnaire			Average	Standard deviation	Middle	At Least	At Most	p-value
Function	Body imagination	SPG	65.20	8.12	68.00	40.00	76.00	0.021
		OCG	63.23	5.82	63.00	52.0	74.00	
	Anxiety	SPG	67.63	6.51	70.00	50.00	78.00	0.171
		OCG	65.56	7.86	68.00	40.00	76.00	
	Weigth	SPG	70.40	6.65	70.00	54.00	84.00	0.032
		OCG	67.00	8.29	68.00	40.00	80.00	
	Male sexual function	SPG	76.06	7.85	77.00	54.00	89.00	0.016
		OCG	72.96	6.61	73.50	54.00	83.00	
	Female sexual function	SPG	73.43	6.73	74.00	54.00	87.00	0.072
		OCG	70.83	7.28	72.00	52.00	83.00	
Signs	Frequent urination	SPG	27.30	4.50	27.50	18.00	37.00	0.018
		OCG	29.70	5.97	29.00	18.00	39.00	
	Blood in the stool	SPG	23.43	5.49	22.50	14.00	37.00	0.013
		OCG	26.13	5.45	25.50	18.00	37.00	
	Frequency of stool	SPG	27.20	4.66	28.00	18.00	37.00	0.044
		OCG	25.46	5.20	25.00	17.00	37.00	
	urinary incontinence	SPG	22.03	5.19	21.00	14.00	37.00	0.01
		OCG	27.10	4.52	28.00	18.00	37.00	
	Urinary incontinence	SPG	22.50	4.97	21.00	14.00	37.00	0.01
		OCG	27.93	4.07	28.00	19.00	37.00	
	stomach ache	SPG	25.03	4.99	25.50	18.00	35.00	0.01
		OCG	29.73	7.31	29.00	15.00	42.00	
	Hip Pain	SPG	26.86	6.23	27.50	18.00	40.00	0.125
		OCG	28.93	7.53	29.52	15.00	42.00	
	Feeling bloated	SPG	27.33	5.89	28.00	18.00	40.00	0.01
		OCG	31.96	7.69	32.50	15.00	45.00	
	Dry Mouth	SPG	25.60	6.20	24.50	18.00	40.00	0.01
		OCG	30.80	8.23	30.50	15.00	43.00	
	Hair Loss	SPG	32.06	7.04	32.50	19.00	45.00	0.265
		OCG	30.73	6.21	30.00	20.00	41.00	
	Taste Disorder	SPG	19.10	4.00	20.00	11.00	27.00	0.070
		OCG	18.26	6.43	15.50	11.00	37.00	
	Excessive excretion of intestinal gas	SPG	26.20	4.48	26.00	18.00	37.00	0.01
		OCG	35.46	4.02	36.00	26.00	42.00	

QLQ-C29 questionnaire			Average	Standard deviation	Middle	At Least	At Most	p-value
Signs	Stool incontinence	SPG	23.53	4.64	22.00	15.00	37.00	0.01
		OCG	34.86	4.69	35.00	26.00	41.00	
	Skin Wounds	SPG	25.76	5.96	25.50	16.00	38.00	0.100
		OCG	27.40	4.86	28.00	18.00	37.00	
	Embarrassment	SPG	24.30	5.04	23.50	16.00	37.00	0.01
		OCG	29.16	5.28	28.00	18.00	39.00	
	Erectile dysfunction	SPG	28.90	6.34	28.00	18.00	40.00	0.01
		OCG	32.03	6.13	33.00	20.00	42.00	
	Disparony	SPG	29.93	5.83	28.00	20.00	40.00	0.001
		OCG	33.70	6.24	35.00	20.00	42.00	

DISCUSSION

Annual colorectal cancer, with nearly one million new cases and the death of about 50% of patients in the first five years of disease onset, is a major public health problem of worldwide [15]. This is necessary to study the quality of life in patients with CRC. However, according to some reports, the cases of CRC in our society are younger than the cases in developed societies [16]. This indicates the patient's level of involvement in the disease in terms of working age, and social activities. The mean age of patients in our study is 58.76 ± 9.65 years old, although the mean age of colorectal cancer patients has been reported differently in several studies [17, 18]. The results of our study demonestrated a higher prevalence of the disease among men (60%). Previous studies have shown that the disease is more common in men than women and occurs more at older ages [19, 20]. Studies in Australia, Italy, and the United Kingdom have found that men are more prevalent than women, but American studies have shown that the prevalence is equal in both sexes [17, 21]. The above comparison indicates that the prevalence rates of sex as well as age of disease onset vary in different societies. In our study, most of the patients lived in the city (71.7%). A study in China noted a very rapid and significant increase in the incidence of colorectal cancer in Shanghai, the most industrial city in China, showing a relationship between the incidence of colorectal cancer and industrial life [22]. The results of our study showed no significant difference in the quality-of-life score between the two methods of sphincter maintenance and permanent colostomy. To confirm our results, we can refer to the cross-sectional cohort study conducted by Trenti et al. 2018, noted that 385 patients with middle and lower rectal cancer underwent APR, cancer risk assessment (CRA) and colon anastomosis (CAA) were compared. The results showed that the quality of life of the patients was generally similar in the 3 groups [23]. In 2012, How et al. compared 32 patients with low anterior resection (LAR) and 30 patients with APR in the United Kingdom, and concluded that the APR method was not necessarily associated with a lower quality of life than LAR [24]. Another study conducted by Mariand Sias et al. in the colorectal tumor department of the Camargo Cancer Center in Brazil, they compared 83 patients who underwent inferior or coloanal anastomosis and 42 patients who underwent APR. Quality of life scores did not differ between the two groups, but patients who underwent permanent colostomy had significantly better scores in terms of function and symptoms [25]. In addition, at the University of Aldomord in Italy, Dignar et al. examined 26 patients who underwent APR and 34 patients who underwent CAA colloidal anastomosis, and the quality of life in patients with permanent ostomy compared to patients who underwent CAA. There was no significant difference. However, APR patients had poorer sexual function, while most CAA patients had fecal incontinence [26]. The results of their study are consistent with the results of our study in terms of no significant difference in quality of life between the two groups, but in our study, fecal incontinence as well as male sexual function in patients in sphincter maintenance surgery group compared to patients in permanent colostomy group was better evaluated. The reasons for this discrepancy are the low sample size in these studies and the demographic and age differences between patients in the two studies. Recent study, disclosed differences in quality of life between patients with sexual dysfunction and patients without sexual dysfunction. Sexual dysfunction may be due to pelvic nerve damage or, indirectly, to vascular injury produced by radiotherapy or surgery. In addition, psychological factors can affect these disorders [27, 28]. Similarly, it has been demonstrated that women's sexual function and sexual arousal ability are complex and can easily be affected by factors such as pain [28]. In our study, patients' sexual performance in both men and women after permanent colostomy was worse than sphincter preservation surgery, but this difference was not\significant in women. Based on a review study by Fernández-Martínez et al. loss of normal rectal function, urinary problems, sexual dysfunction, or pelvic pain are frequently reported in patients after rectal surgery, and these abnormalities significantly affect the quality of life [29].

CONCLUSIONS

According to the results of our study and the findings of other studies, the general conclusion that can be drawn from this study is that the quality of life in the two methods of sphincter maintenance surgery and permanent colostomy was not statistically significant. Therefore, treatment decisions should be made by informing patients, both based on their preferences and the physician's clinical judgment, to ensure that patients enjoy an optimal quality of life after treatment. However, due to study limitations (sample size and short-term follow-up), longer follow-up studies with more patients are needed.

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Clinical research development unit of ghaem hospital in data analysis.

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLES

Evaluation the impact of occupational anchoring and organizational commitment on improving efficiency

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Abstract: *Background and Aim: One of the most effective ways to improve efficiency in organizations is human resources management. Job Anchors and Organizational Commitment are constructs having important implications for human resources management practices, especially in efficiency. The purpose of this perusal was to study a structural equation model (SEM) of the impact of job anchors and organizational commitment on efficiency in a military hospital.*

Methods: The statistical population of this study was the personnel of a military hospital, which gathered accomplishing simple random sampling method. As a result, the research used two hundred and thirty questionnaires so to analyse, accomplishing as survey was developmental-applied, that was, requirement data with questionnaires. To measure efficiency, organizational commitment and job anchors variables respectively the Persian version of Hersey & Goldsmith, Meyer & Allen, and Schein questionnaires standard were used. In order to test the research conceptual model, structural equations modelling based on partial least squares method was used, and the model was analysed using AMOS statistical software.

Results: The results of data analysis demonstrated that hidden variables such as organizational commitment, job anchors, and efficiency had acceptable convergent validity, and the fitness of the conceptual model was confirmed. Based on the research model analysis, organizational commitment did not have impact on efficiency, and job anchors had a positive and significant impact on efficiency. Eventually, the modified model showed that organizational commitment variable moderates the connection between job anchors and efficiency.

Conclusion: In light of the findings of this research, managers of military organizations should develop programs to create and improve organizational commitment in personnel. Furthermore, they should pay attention to employees' characteristics and the proportion of their occupation with job anchors in cases of changing and promoting employees. Implementing aforementioned items will have a positive impact on improving the efficiency of military organizations.

Keywords: *job anchors, organizational commitment, efficiency, SEM, military hospital*

INTRODUCTION

Nowadays, human resources are the most valuable asset of any organization and a factor of survival in competitive levels of business, helping organizations achieve their goals. Thus, human resources management are considered a challenging task whose main goal are to improve employees' efficiency and increase organizational performance in the long run. Efficiency as a philosophy of life and improvement strategy is considered as improvement, prevalent goal, and important organizations. In fact, improving efficiency is the main goal of any organization so that its promotion strategy encompasses all units and levels of the organization. Efficiency, mentioned in the literature, is defined as "the amount of services or products that an employee produces over a period of time with an emphasis on quality. The National Efficiency Organization of Iran defines efficiency as the culture of a rational attitude towards a better and transcendent life and work. Employees' efficiency, ultimately leading to desirable organizational performance, is a key index of organizational success, and since performance measures an organization's competitiveness, Emphasis on efficiency seems to be a requirement for management. In addition, efficiency is an important factor in identifying profits, for recognizing potential opportunities

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for organizational success in business, with determining the index for employees' reward and a way to appreciate hard work. Efficiency measures the efficiency of using organizational resources in order to obtain specific outputs.

In this research, the "ACHIEVE" model has been used to measure efficiency. Hersey and Blanchard, i.e., help managers to determine efficiency problems and edit strategy to solve them, developed this model. This model consists the main and influential factors in manpower performance including seven variables: ability, realization, organizational support, motivation, environmental compromise, validity, and evaluation and feedback. Organizational commitment: Organizational commitment has attracted considerable attention in the literature since various studies have emphasized the ramifications of organizational commitment, including occupational behavior and performance. A number of behavioral cues such as learning, thinking, perception, motivation, and organizational commitment are considered as the most important criteria for evaluating employees' behavior in the organization. Luthans stated that employees' commitment to an organization can be measured by their positive desire to remain an active member of the organization, their willingness to sacrifice responsibilities, and tasks at their best to do, and their acceptance reflected on the value of organizational goals. Past studies have shown that organizational commitment plays a conspicuous role in influencing employee efficiency, That is, researchers examined the connection between organizational commitment and employees' performance and found a positive and significant connection between the two variables. IN view of these studies, high levels of organizational commitment prove the success of an organization in human resources management, ultimately leading to improved performance and high employees' efficiency. Allen and Meyer model [11] asserts that despite of the extensive research being on organizational commitment, there is no consensus among management experts on the concept of organizational commitment and its focal points, having led to the emergence of different models of organizational commitment. In their view, organizational commitment has three distinct dimensions, including emotional commitment, continuous commitment, and normative commitment. Career anchors: The concept of career path as a link between organizations and the people working for them, examined from both internal and external psychological aspects. External career path includes jobs and situations through that a person progresses and is defined as organizational indicators of career path, whereas internal career path is the tendency of employees to orient or career anchor. Actually, just as sailors anchor to keep a ship afloat, so do people anchor to stay afloat.Bidabadi et al. [13] take for instance, anchor in their study shows that oxidizer and fuel are distinctly injected into a system, and before the reaction area are not mixed, in counter flow of combustion.Bidabadi et al. [14] take for example, anchor is in the burning lycopodium particle involving vaporization and drying processes in non-premixed flames of non-adiabatic combustion.Bidabadi et al. [15] another example of anchor is that single-fuel case operated by combustion of lycopodium whereas dual-combustion case is initial level. Jahromi et al. [16] final example of anchor is that this research down and up drafts gasifiers have been simulated by Computational Fluid Dynamics models to expand the process of gasification in two-dimension.Therefore, people try to establish their talents, abilities, and requirements and attitudes by making changes in their career path. Career anchors or career aspirations are specific patterns of talents, capacities, motivations, attitudes, and values imagined by the individual that guide and establish a person's progress after several years of experience and feedback from the real world. These career aspirations are aspects of a job or career path that motivate people and make them reluctant to quit when they accept a job. In addition, job anchors put forward the idea that most people do not want to limit themselves to a particular field [17]. Based on previous studies, career path anchors explain the fit between employees' job orientations and work environment, which will lead to job satisfaction, increase employee commitment and performance [18-20]. Nowadays, efficiency and identifying the effective factors is one of the management concerns in any organization, so that experts in this field consider improving efficiency as one of the vital goals of any organization [21]. This is important in healthcare organizations, such as hospitals, for the use of specialist human capital. Because governments spend significant amounts of GDP annually on defense and military organizations protect a country's national interests, independence, and security, being at the forefront in times of crisis, efficiency in military organizations is more important than in other organizations [22]. A review of the subject literature showed that efficiency studies are interesting topics for managers. In the study of Bahadori et al. [23], the factors affecting efficiency were extracted by reviewing previous studies and they present a model with 10 components, including organizational culture, mental growth, intellectual, and personality, organizational support, motivation, education, personal health, performance appraisal, job clarity, ability, and environment. Jafari and Tehran [22] identified the effective factors on efficiency in a review study whom they evaluated. They identified effective factors on efficiency including individual factors consisting motivation, mental development, personal health, ability and organizational environmental factors containing organizational support, job clarity, environment, management style, education, culture, and performance appraisal. Ali Akbar Esfahan et al. [24] in their study on the connection between intellectual capital and efficiency of human resources and occupational performance from the perspective of military hospital staff showed that intellectual capital has a positive and significant effect on efficiency.

Given that thus far limited studies have been conducted on the effect of organizational commitment variables and job anchors on efficiency, especially in military organizations, this study tried to investigate this issue (Figure 1).

Figure 1: Conceptual model of research

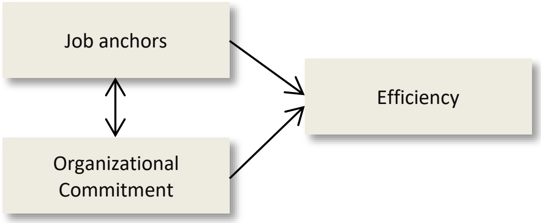

METHODS

The present study was conducted as a cross-sectional survey study in a military hospital. The target population of this study consists of all hospital staff with more than 3 years of work experience. Due to the limited population of the study, the sample size was determined to be 225 based on Morgan table and Cochran's formula. Owing to the possibility of non-return of a number of questionnaires or their incompleteness, a drop of 10% was considered for the sample size. Finally, 248 questionnaires were distributed among the samples, completed at the end of 230 questionnaires and provided to the research team. The criterion for entering the samples was more than 3 years of work experience and the criterion for leaving was the incomplete or unconventional answers of the distributed questionnaire. A simple random method was used for sampling, so the instrument used in this research was a four-part questionnaire. The first part included demographic information including gender, age, marital status, education, occupation, employment status, and work experience. The second part included the efficiency questionnaire, the third part included the organizational commitment questionnaire, and the fourth part included the job anchor questionnaire.

Hersey & Goldsmith Efficiency Questionnaire: this Persian and standardized questionnaire [25] contains twenty-six questions. The scale of answering the questions was in the form of a five choice spectrum very low, low, medium, high, and very high and the whole questionnaire included seven constructs ability, comprehension, organizational support, motivation, feedback, credibility, and adaptability. The reliability of this questionnaire in the present study was obtained by Cronbach's alpha index of point eight nine one.

Meyer & Allen Organizational Commitment Questionnaire: this Persian and standardized questionnaire [26] contains twenty-four questions. The scale of answering the questions was in the form of a seven choice spectrum strongly disagree, relatively disagree, slightly disagree, have no opinion, slightly agree, partially agree, and strongly agree. The whole questionnaire included three being a structure emotional commitment, normative commitment, and continuous commitment. The reliability of this questionnaire in the present study was obtained by Cronbach's alpha index of zero point eight zero one.

Schein Job Anchor Questionnaire: this Persian and standardized questionnaire [27] contains forty questions. The scale of answering the questions was in the form of a four choice spectrum never, rarely, often, and always. The whole questionnaire consisted of eight structures technical-applied, general management, autonomy-independence, security-stability, Creativity-entrepreneurship, service and self-sacrifice, and pure challenges and lifestyle. The reliability of this questionnaire in the present study was obtained by Cronbach's alpha index of zero point eight six-four.

Statistical methods and structural equations modelling were used to analyse the data in this study. Quantitative indices, percentage, and mean indices, standard deviation, were used for descriptive tables and reporting of qualitative variables.

Given the normality of the data distribution, assessed by the Kolmogorov-Smirnov (K-S) test, Pearson correlation coefficient index was used to determine the correlation among quantitative variables.

Structural equation modelling was used to evaluate the research model and the connection between the main research variables. The level of statistical significance in this study was considered zero point zero five,

That was, SPSS software version twenty first was involved to prepare descriptive tables and statistical analyses and AMOS software version twenty first was used to analyse structural equations modelling.

Observance of ethics standards in research

All individuals were reassured about being free to participate in the research and not being forced to continue participating in the research.

RESULTS

In this study, two hundred and thirty personnel of a military hospital participated. Forty eight point thirty percent of the participants were male and fifty one point seventy percent were female. Seventy one point seven percent of the participants were married, sixty three point nine percent of all participants had a master's degree, and-six point one percent had a bachelor's degree or lower. The average age of participants was thirty-two point twenty two years with a standard deviation of four point sixty seven years and the average age of work experience was eleven point eighty one years with a standard deviation of four point three years (Table 1).

Table 1: Summary of demographic information

Variable		Number	Percentage
Sex	Male	111	48.3
	Female	119	51.7
Marital Status	Bachelor	65	28.3
	Married	165	71.7
Education	Bachelor of Science and Lower	83	36.1
	Master of Science	147	63.9
		Average	**Standard Deviation**
Age		32.29	4.67
Occupation history		11.81	4.30

The results of this study showed that the score of job anchors and organizational commitment do not have significant connection with demographic variables, P >0.05. In this study, there is a significant connection between efficiency score and demographic variables of age and work experience, P <0.05. Therefore, that with increasing age and work experience, the efficiency of individuals for the organization decreases significantly (Table 2).

Structural equations modelling was used to answer the question whether there is a significant connection between job anchor variables and organizational commitment and if these two variables affect the efficiency score of individuals in the organization (Figure 2).

Figure 2: Connection of structural model among organizational commitment, job anchors, and efficiency

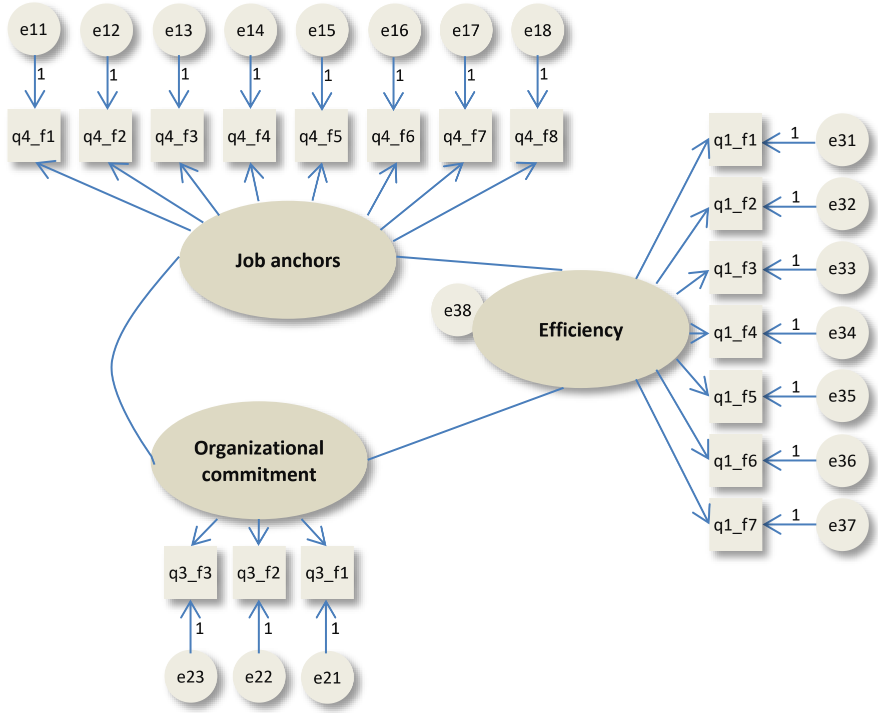
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Job anchors
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Efficiency
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Figure 3: Structural model of final modified (connection between job anchors and efficiency with the effect of variable moderating of organizational commitment

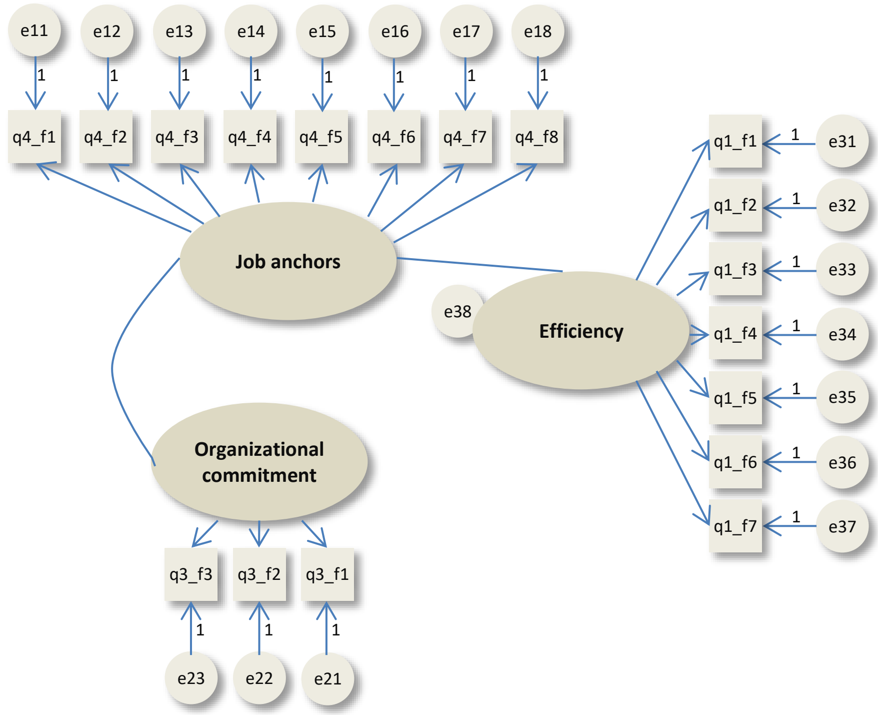

Table 2: Commitment connection of organizational and efficiency with age and occupation precedent

Variable	Efficiency	
	Pearson correlation	**P significant**
Age	-0.371	0.033
Occupation history	-0.303	0.046

Structural model studies (Figure 1) show that the model is significant, $P < 0.05$. Considering that the Chi-Sq./df index is a value between two to five and the GFI, AGFI, CFI and NFI indices are greater than zero point nine and the RMSEA index is less than zero point one, indicating a good fit of the model has it (Table 3).

Table 3: Summary of fitting indicators of structural model of connection between organizational commitment, job anchors, and efficiency

Index RMSEA	Index NFI	Index CFI	Index AGFI	Index GFI	Index Chi-Sq/df	P Significant	Degrees of Freedom df	Ki Do Chi-Sq
0.066	0.924	0.941	0.900	0.912	3.85	0.008	133	512.58

Considering the significance of the coefficients of each questionnaire and their structures and the connections among variables, the results showed that job anchors separately have a significant effect on efficiency, $P < 0.05$, but organizational commitment alone on Efficiency do not have significant effect, $P > 0.05$. Considering the significant correlation between organizational commitment and job anchors, $P < 0.05$, it can be seen that organizational commitment acts as a moderating variable. In other words, the connection between job anchor and efficiency can be justified when the effect of organizational commitment variable is applied to the model (Table 4). Finally, Figure 1) can be modified and explained as Figure 2 and Figure 3.

DISCUSSION

Efficiency and effectiveness are economic requirements for every country whose goal is economic development, such as Iran. Hospitals in all countries account for a significant share of resource consumption in the health sector. Because resources are limited, maximizing efficiency in military hospitals allows resources to be best distributed in this area. As problems increase and organizations become more complex, the need for better solutions based on the role of job anchors and organizational commitment to efficiency in military organizations is becoming increasingly important. Therefore, in the present study, the researcher decided to provide a model with a broad perspective on the impact of job anchors on job satisfaction, organizational commitment, and efficiency in military hospitals from the perspective of a military hospital staff. The research findings based on structural equations modeling indicated that the research variables such as: organizational commitment, job anchors, and efficiency had acceptable convergent validity.

Table 4: Studying coefficients significant of hidden variables and observed in the structural model

Time	Connections	Coefficients Estimation	Standard Deviation	P Significant
q1_f1	Efficiency → Ability	1.000		-
q1_f2	Efficiency → Understanding and Cognition	0.302	0.043	0.001
q1_f3	Efficiency → Organizational Support	0.472-	0.014	0.001
q1_f4	Efficiency → Motivation	0.094	0.052	0.070
q1_f5	Efficiency → Feedback	0.481	0.036	0.001
q1_f6	Efficiency → Validity	0.312	0.026	0.001
q1_f7	Efficiency → Compatibility	0.184	0.034	0.001
q4_f1	Job Anchors → Applied Technical Major	0.001	-	-
q4_f2	Job Anchors → General Management Major	-2.757	0.163	0.001
q4_f3	Job Anchors → Autonomy Independence Major	-0.643	0.048	0.001
q4_f4	Job Anchors → Security-Stability Major	-0.601	0.060	0.001
q4_f5	Job Anchors → Creativity - Entrepreneurship Major	-0.336	0.025	0.001
q4_f6	Job Anchors → Service, Self-sacrifice, and Dedication Major	-0.432	0.037	0.001
q4_f7	Job Anchors → Pure Challenges Major	-0.232	0.040	0.001
q4_f8	Job Anchors → Life Style	0.148	0.113	0.189
q3_f1	Job Anchors → Emotional Commitment	1.000	-	-
q3_f2	Job Anchors → Norm Commitment	-0.399	0.113	0.001
q3_f3	Job Anchors → Continuous Commitment	-0.328	0.079	0.001
	Job Anchors → Efficiency	0.101	0.029	0.003
	Organizational Commitment → Efficiency	-0.006	0.004	0.457
		Correlation		**P Significant**
Job Anchors ↔ Organizational Commitment		-0.202		0.038

The model is acceptable since the fit indices of the model were generally proportional; in other words, based on the available indicators, the fit of the model was confirmed. According to the results, there is a significant correlation between age and work experience with efficiency. Since this coefficient is negative, with increasing age, work experience, and efficiency decreases significantly. Organizational commitment did not show a significant connection with demographic variables; also, there is a significant correlation between age and work experience with organizational commitment; in other words, with increasing age, work experience, and organizational commitment decreases significantly. Analysis of the research model showed that organizational commitment do not have significant effect on efficiency; thus, this result is similar to a number of previous studies [28,29] and contradicts some others [30].

On the other hand, the results showed that job anchors would have a great impact on human efficiency; the results indicate a positive and significant correlation between organizational commitment and job anchors, having been obtained in other studies.It can be concluded that job anchors will have a beneficial effect on efficiency by synergizing the effect of organizational commitment; however, none of the structures showed a significant connection with demographic variables. Hence, no significant correlation was found between age and work experience variables with job anchors, none of its structures. According to the results of this study, every organization should pay special attention to job anchors, in particular, when it seeks change and needs to evaluate the strengths and weaknesses of its manpower. Furthermore, it can be said that differences in job anchors will lead to diversifications in the likelihood of career advancement. By increasing information about these non-financial incentives, organizations can explain effective approaches to their employees' career development.Job anchors show the reasons why people have progressed in their careers as a pattern. This model explains why and how an individual's interactions with the organization's job anchors affect work and job satisfaction, and job orientations provide a way to understand motivational factors in job decisions. Analyzing the job anchors of individuals helps organizations redesign jobs (to meet the needs of individuals) and plan job changes. Finally, in light of the evidence of this study, committed employees help organizations to improve efficiency by choosing the appropriate job anchors.

This study is one of the first studies on the impact of job anchors and organizational commitment on efficiency and since the statistical population is limited, the generalization of results should be done with caution. Moreover, some results are inconsistent with other previous studies conducted abroad, so it is suggested that the model proposed in this study be examined in other studies for greater validity.

CONCLUSION

In view of the general overview of the results of the present study, designing the knowledge structure at all, levels of military hospitals in the country will be effective in improving and efficiency of these organizations and in this regard, staff managers as producers and national knowledge capitals have important role. Therefore, it is necessary for senior managers of military hospitals in the country to focus more on these components: namely organizational commitment, efficiency, and job anchors in order to implement this system in their organization and take full advantage of it.One of the first activities that should be considered by the managers of this organization and emphasized in different sectors, educational needs assessments with emphasis on job anchors and organizational commitment for greater efficiency and determination and development of continuous training programs for different parts of the organization. In this way, empowering employees, organizational efficiency is improved.

Acknowledgments

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Conflict of interest

The authors of this study state that there is not conflict of interest.

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ORIGINAL ARTICLES

The relationship between a previous infectious disease caused by the influenza, herpes simplex or Epstein-Barr viruses as the biological threatening agents with recurrent episodes of multiple sclerosis

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Abstract: *Although multiple sclerosis (MS) is the most common neurological disease of young adults, afflicting hundreds of thousands of people worldwide, its pathogenesis is still only incompletely understood. There seems to be substantial heterogeneity in disease mechanisms, but in the majority of cases an autoimmune origin or at least a decisive autoimmune component is postulated. Therefore, pathogenic research focuses on different players programmed by the immune system.*

In this study, biomarkers such as Human myxovirus resistance protein A (MxA), Anti-EBNA antibody and Interleukin S10-12-17 have been tested.

Study of hypotheses show that: Interleukin levels in the blood of MS patients in the experimental group (patients with recurrence) is higher than controls (patients without recurrence condition). The level of anti-EBNA antibody in MS patients in other groups (patients with recurrent) and control group (patients without recurrence condition) is high.

The level of the human myxovirus resistance protein A (MxA) in patients with multiple sclerosis in the experimental group (patients with recurrence) is less than the control group (patients without recurrence condition). The level of anti-EBNA antibody in MS patients in other groups (patients with recurrent) and control group (patients without recurrence condition) is high.

Finally, there is a significant relationship between viral infection diseases and recurrent multiple sclerosis.

Keywords: *Influenza, Herpes Simplex Virus (HSV), Epstein-Barr Virus (EBV), multiple sclerosis (MS)*

INTRODUCTION

Multiple sclerosis (MS), the most common cause of chronic neurological disability in young adults, has a variable phenotypic presentation and subsequent disease course that cannot be predicted at disease onset. Current therapeutic interventions may control the early inflammatory component of disease, but have limited efficacy in progressive disease. Patient-specific disease phase is difficult to determine clinically and, as a consequence, some treatments can be applied inappropriately.

A combination of phenotypic subtyping and laboratory investigations of serological and genetic biomarkers has already led to improved diagnosis and targeted therapy for some subtypes of CNS demyelinating disease previously encompassed within the syndromic diagnosis of multiple sclerosis, including neuro myelitis optica (Lennon et al., 2004; Wingerchuk et al., 2006) and Leber's hereditary optic neuropathy (Taylor et al., 2003).

In addition, several potential biomarkers have been suggested as aids for determining disease course or response to treatment in multiple sclerosis, but their clinical usefulness has not yet been clearly established (Tatomir et al., 2017, Reindl, 2007; Lutterotti et al., 2007), and currently there are no readily accessible clinical or biological markers sensitive or specific enough to guide patient management reliably.

Pathological and functional studies have established the role

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of complement in the pathology of multiple sclerosis (Ingram et al., 2009) and as a result, a number of complement proteins have been investigated as potential biomarkers of disease activity. The majority of these studies have been limited to examination of individual components (C3, C4, C9) or activation products (terminal complement complex) in multiple sclerosis serum and CSF, and so far results have been conflicting (Watkins et al., 2016; Jans et al., 1984; Morgan et al., 1984; Compston et al., 1986; Sanders et al., 1986; Mollnes et al., 1987; Sellebjerg et al., 1998; Jongen et al., 2000; Tatomir et al., 2017).

The reasons for the inconsistent results in these studies are unclear but may have been a consequence of assay variation, sample collection, low patient number or lack of comprehensive clinical phenotypic data. This last factor is likely to be important in multiple sclerosis, where clinical status will have considerable effects on complement levels. More recently, proteomic analysis has identified changes in levels of specific complement proteins (factor I, C3, clusterin) in CSF samples from patients with multiple sclerosis compared to controls (Rithidech et al., 2009; Stoop et al., 2009).

Sero-epidemiological studies have demonstrated the association between multiple sclerosis (MS) and prior Epstein-Barr virus (EBV) infection. It has been hypothesized that intermittent peripheral EBV reactivation may drive continuing central inflammation.

Recent investigation has shown significant differences in median serum levels of anti-EBV nuclear antigen-1 (EBNA-1) IgG between disease subgroups and positive correlation with disease activity reflected by number of Gd-enhancing lesions and T2 lesion volume. These important data have led to hopes that anti-EBNA-1 IgG may be useful as an easily accessible and effective biomarker of disease activity (Ingram G, 2009).

The correlation between elevated Epstein-Barr virus nuclear antigen 1 (EBNA-1) immunoglobulin G (IgG) and gadolinium-enhancing lesions suggests an association between Epstein-Barr virus (EBV) infection and multiple sclerosis (MS) disease activity.

The heightened immune response to EBV in MS is specifically related to EBNA-1 IgG, a marker of the latent phase of the virus. The lack of association between acute viral reactivation in the peripheral blood and Gd (gadolinium-DTPA) lesions suggests a limited role of the former in driving disease activity (Farrell, 2009).

Human myxovirus resistance protein A (MxA) also is an interferon-induced dynamin-like GTPase that acts as a cell-autonomous host restriction factor against many viral pathogens including influenza viruses. In patients with multiple sclerosis (MS), neutralizing antibodies (NAbs) appearing during treatment with interferon (IFN) beta reduce or in high concentrations abolish bioactivity and therapeutic efficacy. In vivo MxA induction by IFNbeta is used as a marker of biologic response to IFNbeta. It has been argued that despite absence of MxA induction measured by PCR, some bioactivity might be preserved (Hesse D, 2009).

Interleukins (also called lymphokines) are a sub-group of small soluble proteins called cytokines which function as chemical messengers between cells. The role of interleukins is to mediate and control the immunologic and inflammatory response. There are at least 18 known interleukins most of which have only been discovered in the last few years. Their role within the immune system is only beginning to be understood and they are just starting to be utilised in the treatment of a wide variety of diseases including cancer, AIDS and autoimmune diseases such as multiple sclerosis and rheumatoid arthritis (Gold, 2008).

Studies have shown that over the course of multiple sclerosis disease activity in patients' cerebrospinal fluid, serum levels of IL-12 plus IL-6 subunit 40 is significantly increased (Malmestrom C, 2006). After treatment with beta interferon, interleukin-10 levels in the cerebrospinal fluid increases and its surface is associated with clinical response, (Rudick RA, 1998).

Mononuclear cells that secrete IL-17 also are responsible, during relapses in patients with multiple sclerosis cerebrospinal fluid increases, (Matusevicius D, 1999). It has been hypothesized that IL-6 in the cerebrospinal fluid of the patients with NMO can helplessness, relapses and disease activity related to (Uzawa A, 2010). Also, scientists have discovered that systemic production of IL-12 stimulates the secretion of interferon gamma activates the natural killer cells, was associated with disease progression and increases in serum (van Boxel-1999).

MATERIALS AND METHODS

Some of routine serological tests that may be conducted to detect the most important biomarkers are including: Agglutination Reaction Test, The Heterophil Antibody Response IgM, VCA; Viral Capsid Antigen, EA; Early antigen, EA; Early Antibody, EA-R; Antibody, EBNA; Epstein-Barr Nuclear Antigen, Existence of interleukin 10, 12, 17, human myxovirus resistance protein A (MxA), Anti-EBNA antibody.

Statistically, descriptive statistics and mean (the biomarker in patients with MS) in case and control groups by using independent t test are accomplished.

RESULTS

In this study, the results of descriptive and inferential statistics related to each of the biomarkers provided.

Descriptive Statistics

In this study, patients with multiple sclerosis relapses with respect to the control and test groups of 34 patients in each group sorted. The prevalence of MS patient's gender in both groups was identical (to compare the results of the check the status of patients in both groups exist). In the case group and the control group of 21 patients were females and 13 males, while in control group 22 patients were females and 12 were males (Figure 1).

Figure 1: Gender of the participants in the case and control groups

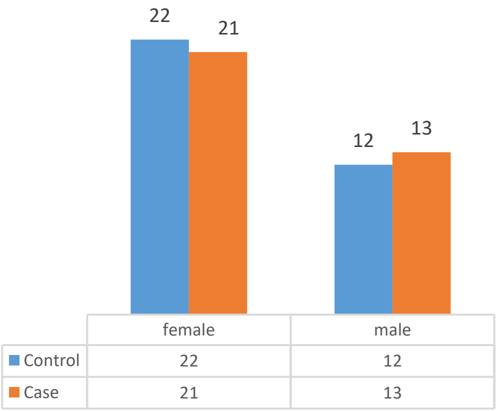

The age range of participants in research in case and control groups show that the majority of patients age were in the range of "20 to 40" (Figure 2).

Figure 2: The age range of the participants in the case and control groups

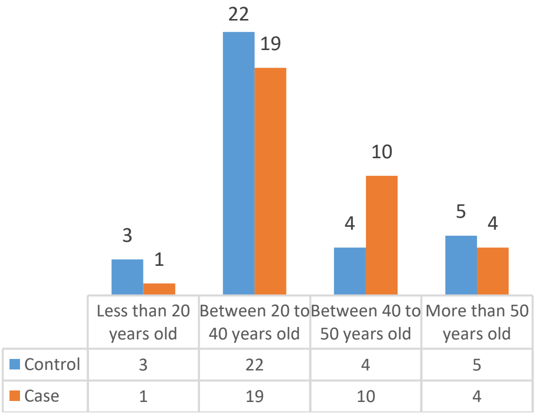

Educational level of the majority of patients in both groups also is located in the "graduate and above" category (Figure 3).

Figure 3: The range of "educational level" of the participants in the case and control groups

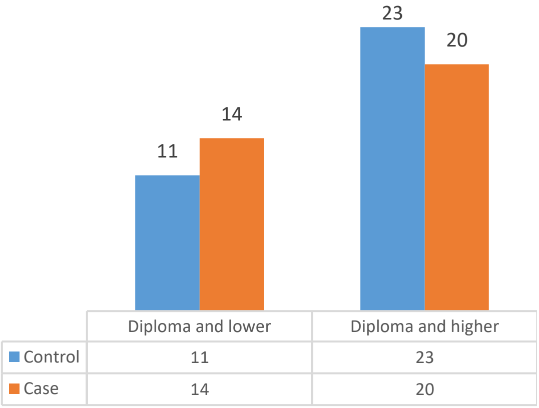

As can be seen in the Figure 4, it is observed that the level of anti-EBNA antibody levels measured in the both test and control group patients is high. Also, for a small number of patients, anti-EBNA antibody have been observed at the mid and low-levels.

Figure 4: The range of Anti EBNA antibody titers among MS patients in both the case and control groups

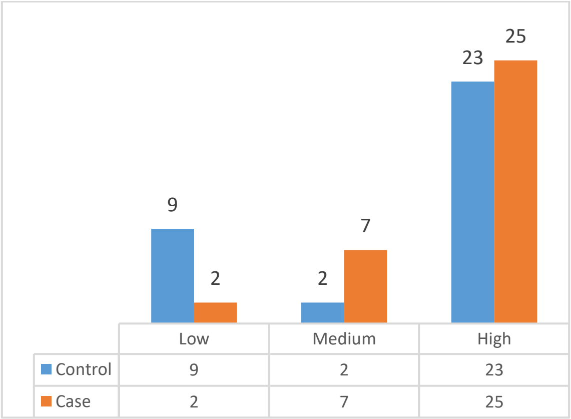

According to the Figure 5, it can be seen that the measured Human myxovirus resistance protein A (MxA) in the test group was significantly lower than the control group.

According to the Figure 6, it may be observed that the interleukins 10, 12 and 17 in the test group patients was much higher than the control group.

CONCLUSION

In the Table 1, the significance level of Leuven test (0.001) is smaller than 0.05. So, assuming equal variances for "anti-EBNA" in the case and control groups will be rejected. On the other hand, given the significance level of t-test assuming unequal variances (0.154) which is greater than 0.05 can be said that there is no significant difference between case and control groups about the mean "anti-EBNA" index. As seen earlier, the amount of this index is high in both case and control groups.

Figure 5: The amount of human myxovirus resistance protein A (MxA) in both the case and control groups

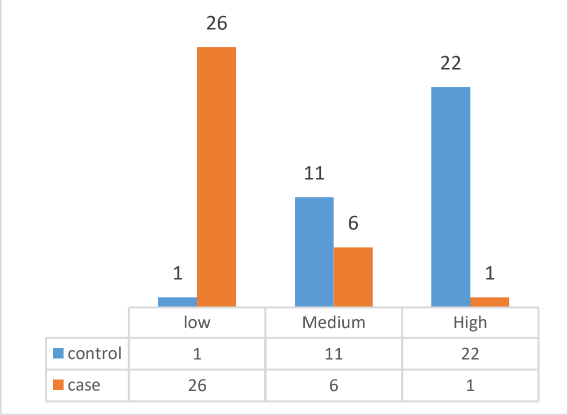

Figure 6: Frequency charts relating to the study of interleukins 10, 12 and 17 in MS patients in both the case and control groups

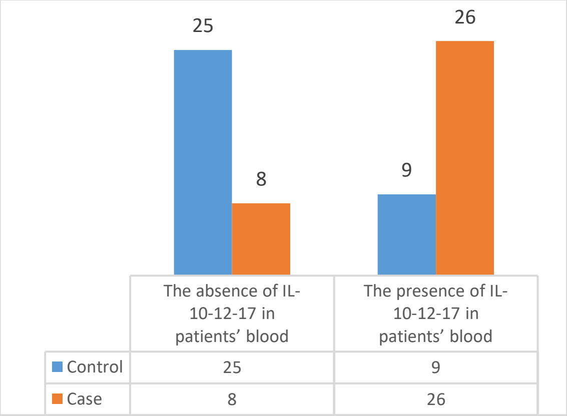

Table 1: Comparison of the average anti-EBNA antibody in MS patients in the case and control groups by using independent t-test

Anti-EBNA antibody	Leuven test to verify the equality of variances		t-test		
	F	The significance level	t	df	The significance level
Assuming equal variances	13.610	0.001	1.445	66	0.153
Assuming unequal variances			1.445	57.183	0.154

In the Table 2, the significance level of Leuven test (0.537) is greater than 0.05. So, assuming equal variances for "human myxovirus resistance protein A (MxA)" in the case and control groups won't be rejected. On the other hand, given the significance level of t-test assuming equal variances (0.001) which is lower than 0.05 can be said that there is a significant difference between experimental and control groups about the mean "human myxovirus resistance protein A (MxA)" index.

Table 2: Comparison of the average of human myxovirus resistance protein A (MxA) in the patients with MS both in the case and control groups (by using independent t-test)

Human myxovirus resistance protein A (MxA)	Leuven test to verify the equality of variances		t-test		
	F	The significance level	t	df	The significance level
Assuming equal variances	0.386	0.537	-9.645	66	0.001
Assuming unequal variances			-9.645	65.843	0.003

In the Table 3, the significance level of Leuven test (0.583) is greater than 0.05. So, assuming equal variances for "Presence of interleukin in the patient's blood" in the case and control groups won't be rejected. On the other hand, given the significant level of t-test assuming equal variances (0.008) which is lower than 0.05 can be said that there is a significant difference between experimental and control groups about the mean "Presence of interleukin in the patient's blood" index.

DISCUSSION AND CONCLUSION

In this section, the research hypotheses have been expressed. Then, according to the results of the descriptive statistics and analysis of data, these hypotheses have been evaluated.

Table 3: Comparison of the average of interleukins 10, 12 and 17 in MS patients in the case and control groups by using independent t-test

Presence of interleukin in the patient's blood	Leuven test to verify the equality of variances		t-test		
	F	The significance level	t	df	The significance level
Assuming equal variances	0.305	0.583	4.693	66	0.008
Assuming unequal variances			4.693	65.898	0.011

The first hypothesis

The level of anti-EBNA antibody in MS patients in other groups (patients with recurrent) and control group (patients without recurrence condition) is high.

According to analyzing data, assuming a significance level of t-test for equality of means (0.154) which is greater than 0.05, there is no significant difference between the average of the EBNA antibodies in MS patients in the case group (patients with recurrence) and control group (patients without recurrence condition).

Meanwhile, according to the above data descriptive statistics showed that the level of anti-EBNA antibody in patients with MS is High in both groups.

The second hypothesis

The level of the human myxovirus resistance protein A (MxA) in patients with multiple sclerosis in the case group (patients with recurrence) is less than the control group (patients without recurrence condition).

According to analyzing data, assuming a significance level of t-test for equality of means (0.001) which is greater than 0.05, there is significant difference between the average of the human myxovirus resistance protein A (MxA) in MS patients in the case group (patients with recurrence) and control group (patients without recurrence condition).

Meanwhile, according to the above data descriptive statistics showed that the level of the human myxovirus resistance protein A (MxA) in patients with MS is less in the case group.

The third hypothesis

Interleukin levels in the blood of MS patients in the case group (patients with recurrence) is higher than controls (patients without recurrence condition).

According to analyzing data, assuming a significance level of t-test for equality of means (0.008) which is greater than 0.05, there is significant difference between the average of the Interleukin levels in MS patients in the case group (patients with recurrence) and control group (patients without recurrence condition).

Meanwhile, according to the above data descriptive statistics showed that the level of the Interleukin in patients with MS is higher than the case group.

The main hypothesis

Based on the main hypothesis of this study, there is a significant relationship between viral infection diseases and recurrent multiple sclerosis. Evaluation of indices of anti-EBNA, the human myxovirus resistance protein A (MxA) and also the Interleukin levels in MS patients, it can be concluded that: The above infectious diseases, have a direct significant impact on increasing the rate of recurrence in patients with MS.

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VARIA

Social and psychological predictors of alcohol-related incidents involving servicemen

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Abstract: *The aim of the research was to determine the social and psychological predictors of alcohol-related incidents involving servicemen. The research involved 310 servicemen, who were subjected to disciplinary and administrative penalties within 2016-2021 years for the offence commitment under the influence of alcohol. The control group included 650 servicemen who joined the service within this period. The analysis of psychological features of servicemen was carried out with the help of the following methods: "Determination of Type Accentuation of Character Traits and Temper" Questionnaire, "Self-Esteem Structures of Temper Questionnaire", "Multilevel Personality 'Adaptability' Questionnaire", "Progressive Matrix", "16 PF Questionnaire", and "Self-Esteem Structures of Temper Questionnaire". Cluster analysis was used to differentiate servicemen who committed incidents under the influence of alcohol. The determined typology was valuable for the organization of preventive works, events dedicated to the improvement of socialization by recruits, adequate formation of servicemen image as well as their identification in accordance with it, and events dedicated to the prevention of suicidal behaviour.*

Keywords: *incidents under the influence of alcohol, servicemen, adequate behaviour, preventive psychology*

INTRODUCTION

The use of alcohol by servicemen was a problem for numerous armies all over the world. Unpleasant statistics concerning the alcohol abuse was highlighted by the researchers from Australia [1], Great Britain [2-3], France [4], and the USA [5]. They proved that alcohol abuse did not only have negative effect on the servicemen's health, wellbeing, moral state, the effectiveness of their military activity, and on readiness to complete missions, but also had financial losses because of alcohol-related accidents (ARIs). The researchers proposed the program of alcoholism prevention for army in general as well as for communities where the military units were located. Other researchers such as Grattan, Mengistu, Bullock, Santo, & Jackson [6] determined that the prohibition to sell alcohol beverages to servicemen at night had positive influence on the statistics of the crime level in definite community.

The research of recent years demonstrated that the military service was joined mostly by school leavers who were drinking alcohol beverages but not by those who did not have such obsession [7]. Other researchers such as Shirvani, Reed, Clingan [8], and Lamb, & Baron [9] distinguished the connection between the alcohol abuse by youth from 24 to 32 years old and military personnel. In accordance with their works servicemen drank alcohol because that was the part of military culture.

Another group of scientists Hunter, Talcott, Klesget, Lando, & Haddock [10], Jeffry, & Mattiko [11] pointed out that there was a close link between the alcohol abuse and tendency to risky behaviour by male servicemen as well as by female ones.

Traditionally, high level of alcohol abuse by servicemen was caused by experienced stress during the participation in combat operations and their negative consequences

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including post-traumatic stress disorder (PTSD) [12]. In accordance with the research conducted by Jones and Fear [13] the increased number of cases of alcohol abuse related to negative experience which was caused by transition from combat operations to civil life.

Ong and Joseph [14] pointed out that unexperienced servicemen of junior management who served abroad on long-term business trips (they used to long separation from their families, friends, resources' limitation, high level of combat readiness, and limited local jurisdiction, etc.) were the most vulnerable part. They chose negative copings including alcohol abuse.

Bartone, Hystad, Eid, & Brevik [15] studied psychological hardiness and avoidance of coping as predictors of risk for alcohol abuse in military personnel following war-zone deployment. Their results showed that low level of psychological hardiness, more combat exposures, younger age, and lower rank were associated with increased risk for alcohol abuse soon after the return from deployment. At follow-up, avoidance of coping was a significant predictor of risky alcohol abuse.

Skipper, Forsten, Kim, Wilk, & Hoge [16] examined the association between specific combat experience and post-deployment hazardous drinking patterns of selected military personnel that was considered to pose high risk, such as personnel belonging to the U.S. Army Special Operations Forces. In their study, combat experience was divided into the following categories: (1) Fighting, (2) Killing, (3) Threat to oneself, (4) Death/Injury of others, and (5) Atrocities. They determined, that combat experience related to fighting, threat to oneself, and atrocities was significantly connected with alcohol abuse being analyzed individually. However, when the factors were examined simultaneously, combat experience in the fighting category was significantly associated with a positive screen of alcohol abuse. In conclusion, soldiers who belonged to certain elite combat units were more likely to screen positive of alcohol abuse if they were exposed to specific types of combat experience versus any other type of combat exposure.

Other researchers such as Russell, Russell, Riviere, Thomas, Wilk, & Bliese [17] distinguished that among the participants of the research there increased the quantity of use of alcohol up to 80.5% while alcohol abuse increased from 8.51% to 19.15%. However, alcohol abuse among those who killed enemy during the combat significantly decreased.

Hoopsick, Homish, Collins, Nochajski, Read, & Homish [18] having compared servicemen of the National Guard of the USA who took part in combat operations with those who did not have such experience distinguished that low resilience and existence of psychological problems due to this situation became the reason of addictive behaviour of servicemen, but not the participation in combat operations.

Hanwella, de Silva, & Jayasekera [19] compared the reasons of alcohol abuse by servicemen of Great Britain and the USA with servicemen of Sri Lanka Navy's Special Forces where the tradition of alcohol abuse among the citizens in general significantly differed from the traditions of alcohol abuse in Great Britain and the USA. They pointed out that the high level of alcohol abuse during combat operations was connected with worsened psychological health and functional disorders of servicemen of Great Britain and the USA, but that was not diagnosed with servicemen of Sri Lanka. The authors concluded that the alcohol abuse was significantly influenced by national traditions of the country where they served which included the features related to alcohol accessibility and attitude to alcohol.

McCabe, Watrous, & Galarneau [20] said that 49% of servicemen who were wounded in past even 10 years later had positive screening on PTSD and depression. The participants of this research had high level of alcohol abuse, problems of tobacco and cigarette use, lack of sleep as well as physical activity.

Other researchers such as Boscarino, Hoffman, Pitcavage, & Thomas [21] figured out that group of veterans who were on outpatient care where the majority were veterans of the Vietnam War (72%) among this group 9.6% had eternal PTSD, 18.4% had eternal depression, and 50.1% had eternal consultations with the service of psychological aid. Among the key factors which were connected with PTSD, depression, and a need to use the service of psychological aid they pointed out low self-esteem, alcohol or drug abuse to overcome histories of childhood trauma, high level of combat influence, and low psychological resilience.

Angkaw, Haller, Pittman, Nunnink, Norman, Lemmer, McLay, & Baker [22] distinguished that the veterans who returned from Operation Enduring Freedom and Operation Iraqi Freedom (OEF/OIF) were found to be at increased risk for post-traumatic stress disorder (PTSD) and alcohol abuse disorders, leading to negative mental health–related quality of life (MHRQoL).

Recent research conducted by Derefinko, Hallsell, Isaacs, Salgado Garcia, Colvin, Bursac, McDevitt-Murphy, Murphy, Little, Talcott, & Klesges [23] demonstrated that the separated transition from active duty to civilian life could become critical in terms of the future functioning of the veteran. Repeated estimating tests indicated that endorsement of alcohol abuse, cigarette abuse, and prescription of drug abuse was similar during active duty and dismissal; marijuana and hard drugs abuse significantly increased in the 6-month period of dismissal. Active-duty of anxiety/depression and aggressive feelings were maintained from active-duty to dismissal, loneliness and military-based trauma symptoms increased significantly as well.

Kintzle, Oh, Wilcox, Hassan, Ell, & Castro [24] figured out that post-deployment civilian unemployment became a common problem and source of additional stress for the National Guard (NG) personnel. Alcohol abuse was concluded to moderate the relationship between civilian unemployment and mental health symptoms.

Since the problem of alcohol abuse happened to servicemen within the whole period of service starting with the entrance and finishing with dismissal (even in civil life after the dismissal), modern research was aimed at the search of predictors which could give possibility to decrease the quantity of servicemen who had potential tendency to alcohol abuse during their service including combat operations.

Hunter et alia [10] examined demographic, lifestyle, and psychosocial variables to determine predictors of the Air Forces recruits who were likely to have alcohol-related problems. The study involved the recruits of the Air Forces (N = 32 144), who underwent basic training from August 1995 to September 1996. They determined that psychosocial predictors included positive rebellious attitudes, decreased seatbelt use, and positive risk-taking attitudes.

The research conducted by Sarramon, Vendoux, Schmitt, & Bourgeois [25] demonstrated that susceptibility to boredom, impulsiveness, and search of thrills as well as adventures could increase the possibility to gain adequate behaviour.

Gutierrez, Blume, Schmaling, Stoever, Fonseca, & Russell [26] considered that identifying the predictors of alcohol consequences which occurred upon demobilization could help in determining which soldiers were at risk for such consequences before deployment and could help to maintain military readiness. They identified that significant predictors of greater alcohol-related incidents, included less quantity of years of formal education, male gender, non-engagement in an intimate relationship, racial/ethnic minority status, enlisted rank, deployment to the continental United States, and greater stress, whereas significant predictors of drinking and driving included male gender, non-engagement in an intimate relationship, and greater stress.

There was a need to point out that researchers Selman et alia [1], Henderson et alia [2], Iversen et alia [3], Ong et alia [14], and Bartone et alia [15] determined that young age as well as low rank were social and demographic predictors of alcohol abuse of servicemen. That, in turn, turned urgency to the topics such as victimization and unfair attitude as the cases of alcohol abuse.

Other researchers Ames, Moore, Cunradi, Duke, & Galvin [27] identified interrelationships of unfair treatment at work, stress, and problem drinking among a sample of the U.S. In their research, it was found that more women than men (51.4% versus 16.2%) reported gender unfair treatment; approximately 20% of respondents reported ethnic/racial unfair treatment. The researchers concluded that unfair treatment was associated with likelihood of problem drinking, but associations were attenuated after adjusting for frequency of work problems and expecting alcohol to alleviate stress.

Kim Y, Kim J, Park, & Fear [28] investigated workplace victimization as a cause of alcohol abuse in junior military personnel (JMP). Workplace victimization in JMP was prevalent (17.7%). Victims in the workplace showed higher level of anger and alcohol abuse. In mediation analyses, workplace victimization was related to higher levels of anger, which, in turn, were associated with greater alcohol abuse.

In addition to the interrelationships and mediators the researchers Benjamin, Bell, and Hollander [29] resorted to the determination of the typology in the process of examining the problems of alcohol abuse by servicemen. This allocation of subgroups gave the possibility to figure out the tendencies for drinking alcohol which were inherent in servicemen. It could help to prevent alcohol abuse [4] or could become additional factor to diagnose suicidal risks of servicemen [5].

MATERIALS AND METHODS

Participants and Procedure

This research determined the social and psychological predictors which were based on the generalization of data concerning the professional psychological selection of servicemen who committed ARIs. This research had retrospective character. Received results could help to improve professional psychological selection for service and could help to differentiate the approaches for the events based on prevention of alcohol abuse.

This research involved the servicemen who were prosecuted to disciplinary and administrative penalties for committed offences under the influence of alcohol (310 individuals). As the control group there were presented the servicemen who joined the military service within this period of time (650 individuals).

Instruments and Measures

The results of psychological selection of candidates for the service were used to determine the types of servicemen who committed alcohol-related incidents. A range of psychological tests included several questionnaires implemented in electronic form for testing and processing the results obtained in the Automated Psychodiagnostic Complex (APC), called "Psychodiagnostics" in short form [30].

To determine the characteristics of personality temper there was used the "Self-Esteem Structures of Temperament Questionnaire" (Smirnov, as cited by Vorobyova et alia) [30].

Determination of character traits which worsened the process of adaptation to new conditions and reasons of life was formed with the questionnaire "Determination of Type Accentuation of Character Traits and Temperament" (DTACTT; Schmieschek, as cited by Vorobyova et alia) [30]. In the basis of this questionnaire there was the theory of character accentuation developed by Leonhard (as cited by Vorobyova et alia) [30]. In accordance with this conception specific character traits were divided into two groups such as main and additional. The core of individual was in the basis of main traits of a person. These traits were: individuality, development, adaptation, and psychological health. Excessive expression of these traits led to structural psychological changes and in hostile environment to the development of individual's structure.

General skills of servicemen were figured out with the "Progressive Matrix" test (Raven and Raven) [31]. Results based on this test provided us with information on the abilities of servicemen to get definite forms, to take their personalities, temper and mutual relations or ensemble as well as combination of relations.

The specificity of motivation of choosing the profession of a serviceman was determined with the "Questionnaire on Examination of Professional Selection Motivation" (Moskalenko, Kobzin, & Starodubtsev, as cited by Vorobyova et alia) [30].

General information about character traits which were formed throughout the candidates' life was obtained with the "Sixteen Personality Factor Questionnaire" (16-PF Questionnaire, as cited by Vorobyova et alia) [30].

Among the examined group of servicemen who committed ARIs there were distinguished the groups for comparison with the help of cluster analysis. As a criterium of segmenting there were used the scales of the method "Multilevel Personality 'Adaptability' Questionnaire" (MPAQ; Maklakov and Chermyanin) [32] that gave the possibility to provide the analysis with the summarized estimation of the specificity of the individual's adaptation to new conditions and of the tolerance to its changes.

Data Analysis

A cluster analysis was used to compare the data of psychodiagnostic scales in studied groups. To represent the data, we used the main descriptive statistics (arithmetic mean – M – and standard deviation – SD). To reliably detect significant differences between comparative groups, there was used the t-Student criterion. Mathematical data were processed through SPSS 17.0.

RESULTS

In comparison with the control group servicemen who committed ARIs were less motivated, active, and capable to rapid mobilization of strength. The indicators of personal peculiarities of servicemen who committed ARIs were proposed in Table 1.

The indicators of peculiarities of servicemen who committed ARIs in comparison with the control group were proposed in Table 2.

The indicators of distinctions' significance of servicemen who committed ARIs in comparison with the control group were proposed in Table 3.

In accordance with almost all the scales of close questionnaire "Studying the Motivation of Professional Choice by Applicants of Higher Educational Institutions of the Minister of Internal Affairs of Ukraine" servicemen who committed ARIs had the lowest indicators of the "Motives Connected with Objective Socially Set Aims and Content of the Profession" ($t= 5.18$, $p \le .01$), "Motives Connected with Personal and Professional Development" ($t= 5.67$, $p \le .01$), "Independent Professional Choice" ($t= 3.23$, $p \le .01$), "Motives Connected with External Prestige of Profession and Material Wellbeing" ($t= 5.71$, $p \le .01$), "Motives Connected with Romantic Appeal of Profession" ($t= 4.97$, $p \le .01$), "Motives Connected with Eagerness to Compensate the Characterological Weaknesses" ($t= 7.31$, $p \le .01$), "Antisocial Motives" ($t= 4.67$, $p \le .01$). Lower indicators of Types of Accentuation were distinguished due to the method "Determination of Type Accentuation of Character Traits and Temper" – "Hyperthymic" ($t= 5.39$, $p \le .01$) and "Exalted" ($t= 2.75$, $p \le .01$). Servicemen who committed ARIs, due to the method "Self-Esteem Structures of Temper Questionnaire" were less extroverted ($t= 1.96$, $p \le .1$). Due to the indicators of method "16 PF Questionnaire" they had the lowest indicators of conformism (t= 3.97, p ≤.01), practicality (t= 4.20, p ≤.01), sociability (t= 3.59, p ≤0.01), and intellect. These distinctions were expressed for intellect as general awareness (t= 3.40, p ≤.01) but not for general ability to study due to the method “Raven’s Progressive Matrices” (t= 2.81, p ≤.01).

Table 1: Personal peculiarities of servicemen who committed alcohol-related incidents (in Standard Points)

	A	B	C	t A–B	t A–C	t B–C
“Determination of Type Accentuation of Character Traits and Temper” Questionnaire						
Hyperthymic	17.17±3.82	17.07±4.10	14.63±5.88	0.10	1.12	1.16
Stuck	11.67±2.50	11.05±2.62	12.75±3.37	0.97	0.82	1.40
Emotive	8.67±4.59	8.88±4.50	12.00±6.41	0.18	1.33	1.36
Pedantic	10.56±4.33	9.84±4.18	13.00±7.17	0.66	0.89	1.23
Anxious (fearful)	4.67±5.37	3.05±3.56	7.88±4.52	1.24	1.57	2.96**
Cyclothimic	10.33±3.12	8.88±3.02	13.88±4.79	1.85^0	1.92^0	2.91**
Demonstrative	14.11±5.02	14.20±4.00	11.25±2.38	0.07	1.97^0	3.22**
Excitable	7.50±4.96	5.24±3.46	12.38±3.74	1.87^0	2.76*	5.25**
Disthymic	9.33±3.07	9.32±2.93	9.75±4.74	0.02	0.23	0.25
Exalted	9.67±3.65	9.93±4.35	14.25±5.50	0.28	2.16*	2.18*
“Multilevel Personality ‘Adaptability’ Questionnaire”						
Probability	1.56±1.29	6.07±2.56	4.50±1.20	11.78**	5.65**	3.25**
Adaptivity	38.28±7.53	24.84±7.20	59.00±5.40	7.11**	7.95**	16.94**
Neuropsychological resilience	15.67±6.22	10.33±4.77	33.00±4.14	3.49**	8.37**	14.85**
Communication	12.72±2.24	8.35±2.63	17.38±3.58	7.53**	3.39**	7.00**
Morality	9.89±2.17	6.16±2.38	8.63±1.19	6.74**	1.91^0	5.23**
“Progressive Matrix” Test						
IQ (general indicator)	104.44±14.40	102.07±13.03	95.13±11.70	0.66	1.74^0	1.61
“Studying the Motivation of Professional Choice Questionnaire”						
Professional motives	19.22±6.00	21.12±5.40	20.75±5.12	1.27	0.67	0.20
Motives for improvement	17.94±5.40	19.03±4.88	18.50±7.15	0.81	0.20	0.21
Independent profession choice	19.50±5.52	19.09±5.04	16.63±7.65	0.30	0.96	0.90
Non-independent profession choice	10.89±5.11	12.25±5.06	12.63±5.01	1.06	0.81	0.20
Prestige Motives	18.06±4.63	17.93±4.95	18.38±4.21	0.10	0.17	0.28
Romantic motives	14.28±5.91	15.75±4.73	14.50±6.59	1.01	0.08	0.53
Compensatory motives	17.17±5.06	18.57±4.87	19.88±7.06	1.11	0.98	0.51
Antisocial motives	10.78±4.68	12.30±5.12	14.13±4.39	1.27	1.76^0	1.12
“16 PF Questionnaire”						
Md (low – high self-esteem)	7.50±1.95	9.87±2.03	9.13±1.46	4.79**	2.35*	1.36
A (introversion-fellowship)	8.06±2.39	8.89±1.70	7.13±1.89	1.42	1.07	2.57*
B (low-high intellect)	3.39±1.38	3.84±1.58	3.00±1.51	1.28	0.62	1.53
C (emotional instability - stability)	8.89±1.68	9.84±1.54	7.50±1.60	2.28*	2.01^0	4.01**
E (subordination-dominance)	5.39±1.42	5.89±1.71	6.38±2.77	1.37	0.95	0.48
F (restraint-expressivity)	5.00±2.17	5.39±1.89	6.00±1.77	0.73	1.24	0.93
G (low-high normativity)	8.72±1.90	8.39±1.99	8.38±1.19	0.68	0.56	0.04
H (timidity (fearfulness)-bravery)	8.50±1.92	8.57±1.79	7.38±2.20	0.14	1.25	1.50
I (toughness-sensitivity)	4.78±2.32	4.63±1.88	6.13±0.83	0.26	2.17*	4.39**
L (credulity-suspicion)	4.67±2.40	4.48±1.89	4.00±1.77	0.31	0.79	0.74

	A	B	C	t A–B	t A–C	t B–C
M (practicality-reverie)	5.28±2.32	5.02±1.70	6.00±1.85	0.45	0.85	1.45
N (straightforwardness-diplomacy)	5.78±1.66	5.25±1.80	6.63±1.92	1.25	1.08	1.97^0
O (calmness-anxiety)	4.56±2.41	3.72±2.11	5.75±0.71	1.39	1.93^0	6.44**
Q_1 (conservatism-radicalism)	5.28±2.37	6.30±2.01	5.50±1.77	1.74^0	0.26	1.23
Q_2 (conformity-non-conformity)	5.39±1.79	4.50±1.68	5.50±2.39	1.98*	0.12	1.16
Q_3 (low-high self-control)	7.17±1.42	7.68±1.51	7.88±1.96	1.42	0.92	0.28
Q_4 (relaxation-tension)	3.56±1.76	3.47±1.79	4.75±1.83	0.20	1.55	1.92^0
"Self-Esteem Structures of Temper Questionnaire"						
Extraversion-introversion	16.44±3.65	17.88±3.93	14.13±6.22	1.54	0.98	1.68^0
Rigidity-flexibility	9.11±4.32	8.25±3.75	10.50±2.39	0.81	1.05	2.47*
Emotional anxiety-poise	4.06±2.44	3.06±3.18	11.50±4.44	1.55	4.45**	5.29**
Reaction rate	13.17±3.00	12.65±3.65	10.88±2.36	0.67	2.10*	1.98*
Activity	17.44±7.11	19.81±5.26	16.13±2.85	1.36	0.67	3.31**
Sincerity scale	13.00±3.40	7.14±4.56	10.63±4.31	6.51**	1.38	2.21**

*Notes: A= Group A; B= Group B; C= Group C; 0p≤ .1; *p≤ .05; **p≤ .01*

Table 2: Personal peculiarities of servicemen who committed alcohol-related incidents in comparison with the control group (in Standard Points)

	CG	ARIs	A	B	C
"Determination of Type Accentuation of Character Traits and Temper" Questionnaire					
Hyperthymic	18.89±3.84	16.92±4.16	17.17±3.82	17.07±4.10	14.63±5.88
Stuck	11.71±2.62	11.16±2.64	11.67±2.50	11.05±2.62	12.75±3.37
Emotive	9.42±4.96	9.10±4.62	8.67±4.59	8.88±4.50	12.00±6.41
Pedantic	10.52±4.46	9.90±4.45	10.56±4.33	9.84±4.18	13.00±7.17
Anxious (fearful)	3.45±3.75	3.50±4.07	4.67±5.37	3.05±3.56	7.88±4.52
Cyclothimic	9.26±3.24	9.21±3.35	10.33±3.12	8.88±3.02	13.88±4.79
Demonstrative	15.04±3.40	14.00±4.05	14.11±5.02	14.20±4.00	11.25±2.38
Excitable	6.09±3.89	5.86±4.09	7.50±4.96	5.24±3.46	12.38±3.74
Disthymic	8.96±2.80	9.39±3.15	9.33±3.07	9.32±2.93	9.75±4.74
Exalted	11.21±3.32	10.18±4.40	9.67±3.65	9.93±4.35	14.25±5.50
"Multilevel Personality 'Adaptability' Questionnaire"					
Probability	5.39±2.92	5.57±2.87	1.56±1.29	6.07±2.56	4.50±1.20
Adaptivity	27.84±9.91	28.81±11.95	38.28±7.53	24.84±7.20	59.00±5.40
Neuropsychological resilience	12.01±6.14	12.50±7.86	15.67±6.22	10.33±4.77	33.00±4.14
Communication	8.96±3.34	9.44±3.71	12.72±2.24	8.35±2.63	17.38±3.58
Morality	6.87±2.69	6.87±2.69	9.89±2.17	6.16±2.38	8.63±1.19
"Progressive Matrix" Test					
IQ (general indicator)	104.89±10.54	101.69±13.23	104.44±14.40	102.07±13.03	95.13±11.70
"Studying the Motivation of Professional Choice Questionnaire"					
Professional motives	23.27±2.67	20.97±5.36	19.22±6.00	21.12±5.40	20.75±5.12
Motives for improvement	21.21±2.87	18.86±4.97	17.94±5.40	19.03±4.88	18.50±7.15
Independent profession choice	20.45±3.14	19.06±5.16	19.50±5.52	19.09±5.04	16.63±7.65
Non-independent profession choice	12.51±4.84	12.21±5.01	10.89±5.11	12.25±5.06	12.63±5.01
Prestige Motives	20.32±3.04	18.03±4.76	18.06±4.63	17.93±4.95	18.38±4.21
Romantic motives	17.68±3.91	15.57±4.91	14.28±5.91	15.75±4.73	14.50±6.59

	CG	ARIs	A	B	C
Compensatory motives	21.56±3.22	18.52±4.94	17.17±5.06	18.57±4.87	19.88±7.06
Antisocial motives	14.46±4.59	12.38±5.07	10.78±4.68	12.30±5.12	14.13±4.39
"16 PF Questionnaire"					
Md (low – high self-esteem)	9.17±2.53	9.57±2.20	7.50±1.95	9.87±2.03	9.13±1.46
A (introversion-fellowship)	9.31±1.87	8.72±1.85	8.06±2.39	8.89±1.70	7.13±1.89
B (low-high intellect)	4.21±1.46	3.74±1.57	3.39±1.38	3.84±1.58	3.00±1.51
C (emotional instability - stability)	9.61±1.59	9.59±1.69	8.89±1.68	9.84±1.54	7.50±1.60
E (subordination-dominance)	6.18±2.07	5.88±1.73	5.39±1.42	5.89±1.71	6.38±2.77
F (restraint-expressivity)	5.57±1.87	5.40±1.90	5.00±2.17	5.39±1.89	6.00±1.77
G (low-high normativity)	8.52±1.95	8.42±1.94	8.72±1.90	8.39±1.99	8.38±1.19
H (timidity (fearfulness)-bravery)	8.56±1.79	8.41±1.87	8.50±1.92	8.57±1.79	7.38±2.20
I (toughness-sensitivity)	4.59±1.96	4.74±1.90	4.78±2.32	4.63±1.88	6.13±0.83
L (credulity-suspicion)	4.16±1.85	4.46±1.99	4.67±2.40	4.48±1.89	4.00±1.77
M (practicality-reverie)	4.44±2.07	5.14±1.81	5.28±2.32	5.02±1.70	6.00±1.85
N (straightforwardness-diplomacy)	5.22±1.89	5.35±1.78	5.78±1.66	5.25±1.80	6.63±1.92
O (calmness-anxiety)	4.04±2.48	3.93±2.20	4.56±2.41	3.72±2.11	5.75±0.71
Q_1 (conservatism-radicalism)	6.31±2.24	6.12±2.06	5.28±2.37	6.30±2.01	5.50±1.77
Q_2 (conformity-non-conformity)	3.97±1.88	4.61±1.80	5.39±1.79	4.50±1.68	5.50±2.39
Q_3 (low-high self-control)	7.31±1.77	7.66±1.51	7.17±1.42	7.68±1.51	7.88±1.96
Q_4 (relaxation-tension)	3.33±1.78	3.52±1.83	3.56±1.76	3.47±1.79	4.75±1.83
"Self-Esteem Structures of Temper Questionnaire"					
Extraversion-introversion	18.05±4.03	17.31±4.25	16.44±3.65	17.88±3.93	14.13±6.22
Rigidity-flexibility	9.50±3.78	8.44±3.96	9.11±4.32	8.25±3.75	10.50±2.39
Emotional anxiety-poise	3.18±3.20	3.59±3.73	4.06±2.44	3.06±3.18	11.50±4.44
Reaction rate	12.79±3.57	12.43±3.66	13.17±3.00	12.65±3.65	10.88±2.36
Activity	18.87±5.01	19.10±5.68	17.44±7.11	19.81±5.26	16.13±2.85
Sincerity scale	7.56±5.07	7.97±4.92	13.00±3.40	7.14±4.56	10.63±4.31

Notes: CG= Control Group; ARIs= Alcohol-Related Accidents; A= Group A; B= Group B; C= Group C

Table 3: The indicators of significance of distinctions between the control group and the groups of servicemen who committed alcohol-related incidents (in Standard Points)

	t CG – ARIs	t CG – A	t CG –B	t CG – C
"Determination of Type Accentuation of Character Traits and Temper" Questionnaire				
Hyperthymic	5.39**	1.89^{0}	4.56**	2.05*
Stuck	2.33*	0.07	2.55*	0.87
Emotive	0.76	0.68	1.19	1.14
Pedantic	1.58	0.03	1.65^{0}	0.97
Anxious (fearful)	0.15	0.96	1.13	2.76**
Cyclothimic	0.15	1.44	1.27	2.72**
Demonstrative	2.95**	0.78	2.17*	4.45**
Excitable	0.63	1.19	2.45*	4.72**
Disthymic	1.54	0.51	1.24	0.47
Exalted	2.75**	1.78^{0}	3.09**	1.56
"Multilevel Personality 'Adaptability' Questionnaire"				
Probability	0.71	11.78**	2.61**	2.04*

	t CG – ARIs	t CG – A	t CG –B	t CG – C
Adaptivity	0.94	5.75**	3.96**	16.00**
Neuropsychological resilience	0.73	2.46*	3.40**	14.15**
Communication	1.48	6.91**	2.22*	6.61**
Morality	0.00	5.79**	2.99**	4.05**
"Progressive Matrix" Test				
IQ (general indicator)	2.81**	0.13	2.25*	2.35*
"Studying the Motivation of Professional Choice Questionnaire"				
Professional motives	5.18**	2.86**	4.30**	1.39
Motives for improvement	5.67**	2.56*	4.78**	1.07
Independent profession choice	3.23**	0.73	2.88**	1.41
Non-independent profession choice	0.68	1.33	0.51	0.07
Prestige Motives	5.71**	2.06*	5.14**	1.30
Romantic motives	4.97**	2.43*	4.23**	1.36
Compensatory motives	7.31**	3.66**	6.50**	0.67
Antisocial motives	4.67**	3.29**	4.34**	0.21
"16 PF Questionnaire"				
Md (low – high self-esteem)	1.94^0	3.56**	3.32**	0.09
A (introversion-fellowship)	3.59**	2.21*	2.50*	3.26**
B (low-high intellect)	3.40**	2.48*	2.35*	2.24*
C (emotional instability - stability)	0.12	1.79^0	1.56	3.69**
E (subordination-dominance)	1.86^0	2.29*	1.62	0.20
F (restraint-expressivity)	0.98	1.09	0.92	0.69
G (low-high normativity)	0.56	0.45	0.63	0.33
H (timidity (fearfulness)-bravery)	0.90	0.14	0.02	1.52
I (toughness-sensitivity)	0.88	0.35	0.24	5.05**
L (credulity-suspicion)	1.67^0	0.88	1.71^0	0.26
M (practicality-reverie)	4.20**	1.51	3.34**	2.36*
N (straightforwardness-diplomacy)	0.84	1.40	0.15	2.06*
O (calmness-anxiety)	0.54	0.90	1.47	6.38**
Q$_1$ (conservatism-radicalism)	1.01	1.82^0	0.01	1.27
Q$_2$ (conformity-non-conformity)	3.97**	3.32**	3.13**	1.80^0
Q$_3$ (low-high self-control)	2.50*	0.41	2.43*	0.81
Q$_4$ (relaxation-tension)	1.17	0.53	0.76	2.18*
"Self-Esteem Structures of Temper Questionnaire"				
Extraversion-introversion	1.96^0	1.83^0	0.44	1.78^0
Rigidity-flexibility	3.02**	0.38	3.38**	1.17
Emotional anxiety-poise	1.27	1.50	0.38	5.29**
Reaction rate	1.09	0.53	0.39	2.26*
Activity	0.46	0.84	1.83^0	2.67**
Sincerity scale	0.93	6.59**	0.92	1.99*

*Notes: CG= Control Group; ARIs= Alcohol-Related Accidents; A= Group A; B= Group B; C= Group C; 0 p ≤.1; * p ≤.05; ** p ≤.01; *** p ≤.001*

The majority of servicemen who committed ARIs were 50.32% of servicemen of enlisted personnel, 38.71% of non-commissioned and petty officers, and 10.32% of junior officers. The minority of servicemen of this group were senior officers – 0.65%. 2/3 of this group (66.45) were unmarried or divorced. 53.55% had experience of service which was not longer than 3 years, 29.68% had experience of service which was from 4 to 10 years, and 16.77% had experience of service which was longer than 10 years. 50.32% of servicemen had the status of participants in hostilities.

Even though all the servicemen who committed ARIs were characterized as those who had the decrease of the quality of interaction with surrounding substantive and social world (decrease of activity, adequacy, etc.) this group was not homogenous. There were determined the following groups A, B, and C after the use of cluster analysis (as a criterium of segmenting there were used the scales of the method "Multilevel Personality 'Adaptability' Questionnaire") among the servicemen who committed ARIs. The increase of the quantity of clusters of analysis gave the possibility to figure out the subgroups A1, A2, B1, B2, B3, and B4 within the groups A and B.

Despite other typical distinctions for servicemen who committed ARIs (the quantity was 12.16% from the analyzed group of servicemen who committed ARIs) group A differed from the control group with high level of sincerity due to the method "Multilevel Personality 'Adaptability' Questionnaire" – "Credibility" (t= 11.76, p ≤.01) and due to the method "Self-Esteem Structures of Temper Questionnaire" – "Sincerity" (t= 6.59, p ≤.01) and significantly higher indicators of disadaptivity on all the scales of the method "Multilevel Personality 'Adaptability' Questionnaire" – "Neuropsychological Resilience" (t= 2.46, p ≤.05), "Communication" (t= 6.91, p ≤.01), "Morality" (t= 5.79, p ≤.01), general indicator of "Adaptivity" (t= 5.75, p ≤.01). Due to the method "16 PF Questionnaire" the representators of group A in comparison with the control group had significantly lower level of self-esteem (t= 3.56, p ≤.01), emotional stability (t= 1.79, p ≤.1), and significantly higher level of conservatism (t= 1.82, p ≤.1), and subordination (t= 2.29, p ≤ .05).

Servicemen of groups B and C differed from group A due to the method "Multilevel Personality 'Adaptability' Questionnaire" with higher level of credibility (tA-B= 11.78, tA-C= 5.65, p ≤.01 for both values) and scales of the method "Self-Esteem Structures of Temper Questionnaire" – "Sincerity", (tA-B= 6.51, p ≤.01), "Morality" (tA-C= 1.91, p ≤.1). Due to the method "16 PF Questionnaire" they had the lowest indicators of Antisocial Motives (tA-C= 1.76, p ≤.1) and self-esteem (tA-B= 4.79, p ≤.01 and tA-C= 2.35, p ≤.05). However, group A due to the method "Progressive Matrix" had the highest indicators of IQ among the distinguished groups B and C (tA-C= 1.74, p ≤.1), which brought representators of group A closer to the control group.

Social and demographic data of group A was determined by the fact that 60% of this group had experience of service not longer than 3 years, they were the part of enlisted personnel and were divorced. A half of servicemen of this group had the status of participants in hostilities. About 40% of servicemen had experience from 10 to 25 years and were the participants in hostilities. A half of servicemen of group A were divorced.

The analysis of results gave the possibility to conclude that servicemen of group A experienced crisis of values, they highly regarded norms, rules of behaviour and understood their inconsistency. Such situation was a result of getting into new surrounding or of overthinking the values.

The increase of the quantity of clusters gave the possibility to determine two subgroups in group A (subgroup A1 – 72.22% and subgroup A2 – 27.78%). The indicators of significance of distinctions among the determined subgroups in group A were presented in Table 4.

Table 4: The indicators of significance of distinctions among the determined subgroups of group A (in Standard Points)

	A	A1	A2	T A1 – A2
"Determination of Type Accentuation of Character Traits and Temper" Questionnaire				
Hyperthymic	17.17±3.82	17.31±4.27	16.80±2.68	0.30
Stuck	11.67±2.50	11.08±2.40	13.20±2.28	1.74
Emotive	8.67±4.59	8.08±4.13	10.20±5.85	0.74
Pedantic	10.56±4.33	10.77±3.79	10.00±6.00	0.27
Anxious (fearful)	4.67±5.37	4.15±5.68	6.00±4.74	0.70
Cyclothimic	10.33±3.12	10.15±2.61	10.80±4.55	0.30
Demonstrative	14.11±5.02	13.54±5.04	15.60±5.18	0.76
Excitable	7.50±4.96	6.46±4.56	10.20±5.45	1.36
Disthymic	9.33±3.07	9.69±2.50	8.40±4.45	0.61

	A	A1	A2	T A1 – A2
Exalted	9.67±3.65	10.15±3.78	8.40±3.29	0.97
“Multilevel Personality ‘Adaptability’ Questionnaire”				
Probability	1.56±1.29	1.08±0.95	2.80±1.30	2.69*
Adaptivity	38.28±7.53	34.69±4.79	47.60±4.67	5.22**
Neuropsychological resilience	15.67±6.22	13.54±5.24	21.20±5.40	2.72*
Communication	12.72±2.24	12.31±1.97	13.80±2.77	1.10
Morality	9.89±2.17	8.85±1.46	12.60±0.89	6.59**
“Progressive Matrix” Test				
IQ (general indicator)	104.44±14.40	106.15±15.18	100.00±12.49	0.88
“Studying the Motivation of Professional Choice Questionnaire”				
Professional motives	19.22±6.00	20.31±4.91	16.40±8.17	1.00
Motives for improvement	17.94±5.40	18.31±4.42	17.00±7.97	0.35
Independent profession choice	19.50±5.52	20.31±4.37	17.40±8.05	0.77
Non-independent profession choice	10.89±5.11	11.31±5.15	9.80±5.40	0.54
Prestige Motives	18.06±4.63	18.54±4.39	16.80±5.54	0.63
Romantic motives	14.28±5.91	14.00±6.12	15.00±5.92	0.32
Compensatory motives	17.17±5.06	18.00±4.32	15.00±6.67	0.93
Antisocial motives	10.78±4.68	10.54±4.33	11.40±6.02	0.29
“16 PF Questionnaire”				
Md (low – high self-esteem)	7.50±1.95	7.62±1.66	7.20±2.77	0.31
A (introversion-fellowship)	8.06±2.39	8.46±1.94	7.00±3.32	0.93
B (low-high intellect)	3.39±1.38	3.62±1.39	2.80±1.30	1.17
C (emotional instability - stability)	8.89±1.68	8.92±1.75	8.80±1.64	0.14
E (subordination-dominance)	5.39±1.42	5.46±1.45	5.20±1.48	0.34
F (restraint-expressivity)	5.00±2.17	4.62±2.06	6.00±2.35	1.16
G (low-high normativity)	8.72±1.90	9.31±1.60	7.20±1.92	2.18*
H (timidity (fearfulness)-bravery)	8.50±1.92	8.08±1.66	9.60±2.30	1.35
I (toughness-sensitivity)	4.78±2.32	4.85±2.48	4.60±2.07	0.21
L (credulity-suspicion)	4.67±2.40	4.00±2.35	6.40±1.67	2.42*
M (practicality-reverie)	5.28±2.32	5.46±2.60	4.80±1.48	0.67
N (straightforwardness-diplomacy)	5.78±1.66	6.00±1.73	5.20±1.48	0.98
O (calmness-anxiety)	4.56±2.41	4.38±2.79	5.00±1.00	0.69
Q_1 (conservatism-radicalism)	5.28±2.37	4.54±2.18	7.20±1.79	2.65*
Q_2 (conformity-non-conformity)	5.39±1.79	5.23±1.64	5.80±2.28	0.51
Q_3 (low-high self-control)	7.17±1.42	7.54±1.13	6.20±1.79	1.56
Q_4 (relaxation-tension)	3.56±1.76	3.38±1.56	4.00±2.35	0.54
“Self-Esteem Structures of Temper Questionnaire”				
Extraversion-introversion	16.44±3.65	15.54±3.71	18.80±2.39	2.20*
Rigidity-flexibility	9.11±4.32	9.54±4.39	8.00±4.42	0.66
Emotional anxiety-poise	4.06±2.44	3.38±2.10	5.80±2.59	1.86^0
Reaction rate	13.17±3.00	13.46±3.18	12.40±2.61	0.73
Activity	17.44±7.11	18.31±5.76	15.20±10.33	0.64
Sincerity scale	13.00±3.40	13.23±3.92	12.40±1.52	0.65

*Notes: A= Group A; A1= Subgroup A1; A2= Subgroup A2; 0 p ≤.1; * p ≤.05; ** p ≤.01; *** p ≤.001*

Subgroups A1 and A2 differed with their level of the crisis of values. Thus, servicemen of subgroup A2 had significantly higher indicators of answers' sincerity – "Credibility" (t= 2.69, p ≤.05). There were higher indicators of disadaptivity due to the method "Multilevel Personality 'Adaptability' Questionnaire" – "Neuropsychological Resilience" (t= 2.72, p ≤.05), "Morality" (t= 6.59, p ≤.01), and "Adaptivity" (t= 5.22, p ≤.01). Results received due to the method "16 PF Questionnaire" demonstrated higher indicators of suspicion (t= 2.42, p ≤.05), radicalism (t= 2.65, p ≤.05) and lower indicators of normativity (t= 2.18, p ≤.05). Moreover, subgroup A2 in comparison with subgroup A1 due to the method "Self-Esteem Structures of Temper Questionnaire" had higher indicators of extraversion (t= 2.20, p ≤.05) and anxiety (t= 1.86, p ≤.1). Furthermore, representators of subgroup A1 due to the method "Progressive Matrix" were diagnosed with the higher indicators of IQ; however, those distinctions did not reach the level of statistical significance.

The analysis of the results gave the possibility to conclude that the problems of disadaptivity of subgroup A1 were connected with the process of understanding their own inconsistency to the norms which were unacceptable for the surrounding. Servicemen of subgroup A2 had a decreased ability to be led by norms, to control their reactions in accordance with them, had lower ability to entrust themselves to those rules. These peculiarities were caused by the age and lack of experience of self-regulation. Servicemen of subgroup A1 were enlisted personnel who had little experience of service (up to 3 years), moreover, 80% of them were divorced.

The indicators of personal peculiarities of servicemen who committed ARIs from group B were presented in Table 5.

Table 5: The indicators of personal peculiarities of servicemen who committed alcohol-related incidents within the subgroups of group B (in Standard Points)

	B	B1	B2	B3	B4
"Determination of Type Accentuation of Character Traits and Temper" Questionnaire					
Hyperthymic	17.07±4.10	18.67±3.79	13.96±5.32	17.25±3.49	17.82±3.21
Stuck	11.05±2.62	11.56±2.23	11.13±3.45	10.81±2.32	10.98±2.55
Emotive	8.88±4.50	9.83±4.22	9.65±4.33	8.06±4.41	8.69±4.75
Pedantic	9.84±4.18	8.89±3.83	10.26±4.23	9.44±4.52	10.24±4.10
Anxious (fearful)	3.05±3.56	2.50±2.77	4.96±4.30	1.69±2.74	3.24±3.56
Cyclothimic	8.88±3.02	9.33±2.50	9.65±3.94	7.69±2.15	9.12±3.06
Demonstrative	14.20±4.00	15.33±3.56	11.91±5.24	14.94±3.33	14.37±3.57
Excitable	5.24±3.46	5.83±3.33	6.65±3.94	3.75±2.53	5.33±3.53
Disthymic	9.32±2.93	9.83±3.22	10.43±3.60	9.19±2.84	8.69±2.39
Exalted	9.93±4.35	11.67±3.83	8.09±5.89	9.94±3.27	10.16±4.10
"Multilevel Personality 'Adaptability' Questionnaire"					
Probability	6.07±2.56	3.11±1.84	6.83±1.87	8.38±2.11	5.29±1.76
Adaptivity	24.84±7.20	19.89±3.38	35.35±3.81	18.56±4.10	25.82±4.45
Neuropsychological resilience	10.33±4.77	7.67±2.30	17.87±3.27	7.50±2.13	9.61±3.46
Communication	8.35±2.63	7.94±2.34	11.09±2.41	6.81±2.58	8.22±1.83
Morality	6.16±2.38	4.28±1.99	6.39±1.78	4.25±1.63	7.98±1.59
"Progressive Matrix" Test					
IQ (general indicator)	102.07±13.03	107.56±11.71	100.65±13.15	100.25±12.55	101.92±13.56
"Studying the Motivation of Professional Choice Questionnaire"					
Professional motives	21.12±5.40	21.00±5.58	18.96±6.32	21.34±5.87	22.04±4.33
Motives for improvement	19.03±4.88	19.61±4.72	17.78±5.33	18.91±5.34	19.49±4.44
Independent profession choice	19.09±5.04	19.78±4.82	16.09±5.81	19.50±4.93	19.98±4.39
Non-independent profession choice	12.25±5.06	11.61±4.10	11.78±6.21	12.59±3.95	12.49±5.53
Prestige Motives	17.93±4.95	17.72±5.31	16.22±5.66	18.38±5.12	18.53±4.26
Romantic motives	15.75±4.73	14.33±4.58	14.30±5.79	16.13±3.92	16.71±4.57

	B	B1	B2	B3	B4
Compensatory motives	18.57±4.87	18.72±5.43	17.26±5.68	17.47±5.22	19.86±3.70
Antisocial motives	12.30±5.12	10.94±4.73	11.96±5.81	11.69±4.21	13.37±5.38
“16 PF Questionnaire”					
Md (low – high self-esteem)	9.87±2.03	9.94±2.01	9.26±1.86	11.19±1.65	9.27±1.98
A (introversion-fellowship)	8.89±1.70	9.39±1.75	8.30±1.89	9.25±1.72	8.73±1.50
B (low-high intellect)	3.84±1.58	4.33±1.50	3.48±1.59	3.88±1.83	3.82±1.42
C (emotional instability - stability)	9.84±1.54	10.22±1.40	8.78±1.91	10.41±1.04	9.84±1.46
E (subordination-dominance)	5.89±1.71	5.28±1.49	6.17±1.92	5.50±1.52	6.24±1.73
F (restraint-expressivity)	5.39±1.89	5.33±1.61	5.91±1.88	4.75±1.83	5.59±1.96
G (low-high normativity)	8.39±1.99	8.83±1.47	7.48±2.78	8.88±1.70	8.35±1.80
H (timidity (fearfulness)-bravery)	8.57±1.79	8.94±1.63	7.74±1.81	8.75±1.81	8.69±1.76
I (toughness-sensitivity)	4.63±1.88	4.17±1.95	4.96±1.85	4.69±1.86	4.61±1.90
L (credulity-suspicion)	4.48±1.89	4.28±1.87	4.96±2.48	3.84±1.76	4.76±1.57
M (practicality-reverie)	5.02±1.70	5.00±1.37	5.30±1.26	4.44±2.05	5.29±1.70
N (straightforwardness-diplomacy)	5.25±1.80	5.06±1.55	5.65±1.99	5.75±1.87	4.80±1.66
O (calmness-anxiety)	3.72±2.11	3.89±1.81	4.70±2.20	2.63±1.68	3.92±2.18
Q_1 (conservatism-radicalism)	6.30±2.01	6.72±1.78	6.48±2.43	6.00±1.85	6.27±2.00
Q_2 (conformity-non-conformity)	4.50±1.68	3.83±1.50	5.35±1.82	4.16±1.80	4.57±1.46
Q_3 (low-high self-control)	7.68±1.51	7.89±1.49	7.61±1.75	8.25±1.24	7.27±1.45
Q_4 (relaxation-tension)	3.47±1.79	3.72±1.18	3.83±2.25	2.78±1.79	3.65±1.65
“Self-Esteem Structures of Temper Questionnaire”					
Extraversion-introversion	17.88±3.93	18.83±3.71	16.30±4.81	16.88±3.33	18.92±3.59
Rigidity-flexibility	8.25±3.75	8.61±4.19	8.57±4.13	8.00±3.25	8.12±3.81
Emotional anxiety-poise	3.06±3.18	1.89±2.05	3.91±3.93	2.28±2.68	3.59±3.27
Reaction rate	12.65±3.65	12.56±3.05	11.04±4.54	12.81±3.31	13.33±3.47
Activity	19.81±5.26	19.89±3.97	16.13±8.04	21.25±4.22	20.57±3.80
Sincerity scale	7.14±4.56	9.17±4.08	7.17±4.38	3.78±3.46	8.57±4.35

Notes: B= Group B; B1= Subgroup B1; B2= Subgroup B2; B3= Subgroup B3; B4= Subgroup B4

Servicemen from group B (82.43% from the general quantity of servicemen who committed ARIs) differed from other groups of research with the lowest level of sincere questions due to the method “Multilevel Personality ‘Adaptability’ Questionnaire”. With their answers they tried to create socially approved image. Servicemen from group B in comparison with the control group had better indicators of adaptivity – “Neuropsychological Resilience” (t= 3.40, p ≤.01), “Communication” (t= 2.22, p ≤.05), “Morality” (t= 2.99, p ≤.01), and general indicators of “Adaptivity” (t= 3.96, p ≤.01). Besides the eagerness to gain social approval servicemen of group B as well as other representators who committed ARIs had decreased quantity of interaction with surrounding substantive and social world.

Group B included 4 subgroups: subgroup B1 constituted 14.75%, subgroup B2 constituted 18.85%, subgroup B3 constituted 26.22%, subgroup B4 constituted 40.16%. Indicators of significance of distinctions between the determined subgroups of Group B which were presented in Table 6.

Servicemen of subgroup B1 differed from other subgroups B2, B3 and B4 with the highest level of sincerity due to the method “Multilevel Personality ‘Adaptability’ Questionnaire” – “Credibility” (3.11±1.84), due to the method “Self-Esteem Structures of Temper Questionnaire” – “Sincerity” (9.17±4.08) and the highest level of intellect due to the method “Progressive Matrix” – “IQ” (107.56±11.71), “16 PF Questionnaire” – “Low-high intellect” (4.33±1.50). Besides that, those servicemen differed due to the method “Determination of Type Accentuation of Character Traits and Temper” with indicators such as “Hyperthymic” (18.67±3.79) and “Exalted” (11.67±3.83). Due to the data received with the method “16 PF Questionnaire” they were less predisposed to dominance

(5.28±1.49) and non-conformity (3.83±1.50). The results of the method “Self-Esteem Structures of Temper Questionnaire” showed that servicemen had high level of emotional stability (1.89±2.05).

Table 6: The indicators of significance of distinctions among the determined subgroups of group B (in Standard Points)

	T B1 – B2	T B1 – B3	T B1 –B4	T B2 – B3	T B2 – B4	T B3 – B4
“Determination of Type Accentuation of Character Traits and Temper” Questionnaire						
Hyperthymic	3.31**	1.31	0.85	2.59*	3.22**	0.74
Stuck	0.48	1.11	0.90	0.38	0.19	0.30
Emotive	0.13	1.40	0.95	1.33	0.85	0.61
Pedantic	1.09	0.45	1.26	0.69	0.02	0.82
Anxious (fearful)	2.21*	1.00	0.90	3.21**	1.66	2.22*
Cyclothimic	0.32	2.35*	0.29	2.17*	0.57	2.48*
Demonstrative	2.48*	0.39	0.98	2.44*	2.04*	0.73
Excitable	0.72	2.31*	0.54	3.10**	1.38	2.34*
Disthymic	0.56	0.71	1.37	1.38	2.11*	0.81
Exalted	2.35*	1.61	1.40	1.36	1.53	0.27
“Multilevel Personality ‘Adaptability’ Questionnaire”						
Probability	6.36**	9.20**	4.33**	2.87**	3.32**	6.88**
Adaptivity	13.75**	1.23	5.82**	15.62**	9.37**	7.53**
Neuropsychological resilience	11.72**	0.25	2.65**	13.33**	9.82**	3.40**
Communication	4.21**	1.58	0.46	6.29**	5.05**	2.68**
Morality	3.53**	0.05	7.09**	4.57**	3.66**	10.18**
“Progressive Matrix” Test						
IQ (general indicator)	1.77^0	2.06*	1.67^0	0.11	0.38	0.57
“Studying the Motivation of Professional Choice Questionnaire”						
Professional motives	1.10	0.21	0.72	1.42	2.12*	0.58
Motives for improvement	1.16	0.48	0.09	0.77	1.34	0.51
Independent profession choice	2.22*	0.19	0.16	2.29*	2.85**	0.45
Non-independent profession choice	0.11	0.82	0.70	0.55	0.47	0.10
Prestige Motives	0.87	0.42	0.58	1.45	1.74^0	0.14
Romantic motives	0.02	1.40	1.89	1.31	1.76^0	0.62
Compensatory motives	0.84	0.79	0.82	0.14	2.00*	2.25**
Antisocial motives	0.61	0.55	1.79^0	0.19	0.98	1.57
“16 PF Questionnaire”						
Md (low – high self-esteem)	1.11	2.23*	1.23	3.96**	0.01	4.73**
A (introversion-fellowship)	1.90^0	0.27	1.41	1.90^0	0.96	1.38
B (low-high intellect)	1.77^0	0.96	1.27	0.86	0.87	0.15
C (emotional instability - stability)	2.79**	0.49	0.99	3.71**	2.35*	2.04*
E (subordination-dominance)	1.68	0.50	2.26*	1.40	0.15	2.04*
F (restraint-expressivity)	1.06	1.17	0.55	2.29*	0.67	1.97^0
G (low-high normativity)	2.01^0	0.09	1.13	2.14*	1.37	1.34
H (timidity (fearfulness)-bravery)	2.24*	0.39	0.55	2.04*	2.10*	0.14
I (toughness-sensitivity)	1.32	0.92	0.84	0.53	0.73	0.18
L (credulity-suspicion)	1.00	0.80	0.96	1.85^0	0.36	2.37*
M (practicality-reverie)	0.73	1.16	0.71	1.94^0	0.05	1.95^0

N (straightforwardness-diplomacy)	1.08	1.41	0.60	0.18	1.79^0	2.35*
O (calmness-anxiety)	1.29	2.43*	0.06	3.78**	1.40	3.01**
Q_1 (conservatism-radicalism)	0.37	1.36	0.90	0.79	0.37	0.61
Q_2 (conformity-non-conformity)	2.91**	0.68	1.79^0	2.40*	1.79^0	1.09
Q_3 (low-high self-control)	0.55	0.87	1.53	1.50	0.82	3.25**
Q_4 (relaxation-tension)	0.19	2.23*	0.19	1.85^0	0.33	2.21*
“Self-Esteem Structures of Temper Questionnaire”						
Extraversion-introversion	1.90	1.86^0	0.08	0.49	2.32*	2.62*
Rigidity-flexibility	0.04	0.53	0.43	0.55	0.43	0.15
Emotional anxiety-poise	2.13*	0.58	2.53*	1.72^0	0.34	1.97^0
Reaction rate	1.27	0.28	0.88	1.59	2.14*	0.67
Activity	1.96^0	1.14	0.63	2.79**	2.52*	0.74
Sincerity scale	1.50	4.73**	0.52	3.09**	1.27	5.49**

*Notes: B1= Subgroup B1; B2= Subgroup B2; B3= Subgroup B3; B4= Subgroup B4; 0 p ≤.1; * p ≤.05; ** p ≤.01; *** p ≤.001*

The representators of subgroup B1 in comparison with the control group due to the method “Multilevel Personality ‘Adaptability’ Questionnaire” had better indicators of adaptive abilities: “Neuropsychological Resilience” (7.67±2.30), “Communication” (7.94±2.34), “Morality” (4.28±1.99), and general scale of “Adaptivity” (19.89±3.38). Due to the method “16 PF Questionnaire” they had higher level of self-esteem (9.94±2.01) and due to the method “Determination of Type Accentuation of Character Traits and Temper” they had high level of general activity (“Hyperthymic” – 18.67±3.79).

2/3 of the subgroup B1 had the status of participants in hostilities, 2/3 had experience of service from 3 to 5 years, a half of the subgroup was not married. Enlisted personnel, non-commissioned and senior officers were presented almost equally, and their quantity was about 45%, junior officers constituted about 10%.

Received results proved that servicemen of subgroups 2.1 were intellectually developed, active, and adaptive, they had professional and combat experience; however, they were not ambitious, did not see the possibility to realize themselves in profession as a serviceman and, probably, regretted their professional selection.

Servicemen of subgroup B2 among all the representators of group B due to the method “Multilevel Personality ‘Adaptability’ Questionnaire” had the highest level of disadaptivity: “Adaptivity” (35.35±3.81), “Neuropsychological Resilience” (17.87±3.27), “Communication” (11.09± 2.41), except for “Morality” (6.39±1.78) which were the highest indicators among the representators of subgroup B4.

They differed from other subgroups of group B and the control group with more expressed indicators due to the method “Determination of Type Accentuation of Character Traits and Temper” – “Anxious-Fearful” (4.96±4.30), “Excitable” (6.65±3.94), and “Disthymic” (10.43±3.60), also they had lower indicators of “Hyperthymic” (13.96±5.32), “Demonstrative” (11.91±5.24), and “Exalted” (8.09±5.89). The representators of this group had the lowest indicators due to the scales of close questionnaire “Studying the Motivation of Professional Choice by Applicants of Higher Educational Institutions of the Minister of Internal Affairs of Ukraine” – “Motives Connected with Objective Socially Set Aims and Content of the Profession” (18.96±6.32), “Independent Professional Choice” (16.09±5.81), “Motives Connected with External Prestige of Profession and Material Wellbeing” (16.22±5.66), and “Motives Connected with Romantic Appeal of Profession” (14.30±5.79). Servicemen of subgroup B2 due to the method “16 PF Questionnaire” were more reserved (8.30±1.89), emotionally instable (8.78±1.91), anxious (4.70±2.20), had lower indicators of conformity (5.35±1.82) and normativity (7.48±2.78). Due to the method “Self-Esteem Structures of Temper Questionnaire” they had the highest indicators of emotional anxiety (3.91±3.93) and the lowest indicators of “Reaction Pace” (11.04±4.54) and “Activity” (16.13±8.04) which were the highest indicators among all other servicemen of group B.

Among the servicemen of subgroup B2 the majority (52%) was constituted by enlisted personnel with experience from 1 to 2 years. The quarter of servicemen had experience longer than 15 years. 2/3 of the representators were unmarried or divorced. More than a half of representators (56.52%) participated in hostilities. The officers of this subgroup constituted not more than 5%.

Received results gave the possibility to conclude that the representators of subgroup B2 tried to create the image of individual who due to the suffering could control themselves not so efficiently and had lower level of productivity. These servicemen tended to appeal to their negative experience as to excuses of their impertinence, unproductivity, and alcohol abuse.

The representators of subgroup B3 in comparison with all the groups and subgroups due to the results of the method "Self-Esteem Structures of Temper Questionnaire" were less sincere in their answers – "Sincerity" (3.78±3.46) and "Multilevel Personality 'Adaptability' Questionnaire" – (8.38±2.11). They described themselves as those who had the best indicators of adaptivity due to the method "Multilevel Personality 'Adaptability' Questionnaire" – "Adaptivity" (18.56±4.10), "Neuropsychological Resilience" (7.50±2.13), "Communication" (6.81±2.58), and "Morality" (4.25±1.63). Moreover, they had the lowest indicators of emphasizing due to the types such as: "Anxious-fearful" (1.69±2.74), "Cyclothymic" (7.69±2.15), and "Excitable" (3.75±2.53). They were extremely self-confident, had high level of self-esteem (11.19±1.65), calm (2.63±1.68), relaxed (2.78±1.79), self-controlled (8.25±1.24), and restrained (4.75±1.83). They trusted to the surrounding world (3.84±1.76), they were practical (4.44±2.05) and active (21.25±4.22).

About 60% of those servicemen had experience of service up to 3 years. 47% did not have any experience of participation in combat operation. 62.5% of servicemen of this subgroup were unmarried or divorced.

Therefore, servicemen of subgroup B3 abused alcohol because they demonstrated the role of experienced professional.

The representators of subgroup B4 differed from other subgroups with expressed disadaptivity related to the attitude to norms which regulated relations. They used the profession of serviceman to gain priority over other people. The representators had higher indicators due to the scales of questionnaire "Studying the Motivation of Professional Choice by Applicants of Higher Educational Institutions of the Minister of Internal Affairs of Ukraine" among which there were "Motives Connected with Objective Socially Set Aims and Content of the Profession" (22.04±4.33), "Motives Connected with External Prestige of Profession and Material Wellbeing" (18.53±4.26), "Motives Connected with Romantic Appeal of Profession" (16.71±4.57), "Motives Connected with Eagerness to Compensate the Characterological Weaknesses" (19.86±3.70), and "Antisocial Motives" (13.37±5.38). They had higher indicators of dominance (6.24±1.73). The representators of subgroup B4 due to the method "Self-Esteem Structures of Temper Questionnaire" had highly expressed extraversion (18.92±3.59) and higher indicators of reaction pace (13.33±3,47) than any other subgroup.

Only a quarter of servicemen of subgroup B4 was married. Almost a half of them had experience of service longer than 3 years. A quarter of servicemen had experience of service not less than 10 years. 47% of servicemen had experience of combat operations. Almost 43% of representators of this group were enlisted personnel, 45% were non-commissioned and petty officers, 12% were officers.

Servicemen of subgroup B4 wanted to dominate, to assert themselves at the expense of others, to achieve that they did not want to use physical strength, but their status and belonging to social group which was empowered. They wanted to become respectful people.

Group C (that was 5.4% from the general selection of servicemen with alcohol dependence) differed from other groups who committed ARIs and the control one with the highest indicators of general disadaptivity (59.00±5.40) which were mostly connected with low level of neuropsychological resilience (33.00±4.14) and communication (17.38±3.58), also they had slightly lower indicators of morality (8.63±1.19). Besides that, due to the method "Type of Accentuation of Character Traits and Temper" among all other groups they had the lowest indicators of "Hyperthymic Type" (14.63±5.88) and the highest indicators of "Anxious-Fearful" (7.88±4.52), and "Exalted Type" (14.25±5.50). Due to the method "16 PF Questionnaire" the representators of the third group had the highest level of excitability (7.50±1.60), anxiety (5.75±0.71), sensitivity (6.13±0.83), reverie (6.00±1.85), and diplomacy (6.63±1.92). They were marked by the highest indicators of tension (4.75±1.83). Due to the data received from the method "Self-Esteem Structures of Temper Questionnaire" the representators of the third group differed from others with the highest levels of introversion (14.13±6.22), emotional anxiety (11.50±4.44), and the lowest reaction pace (10.88±2.36). The representators of this group due to the method "Progressive Matrix" were diagnosed with the lowest level of IQ (95.13±11.70).

Everyone from group C with no exceptions were enlisted personnel with experience of service from 1 to 2 years, unmarried, a quarter of them had experience of participation in combat operations.

Such results gave the possibility to conclude that due to certain constitutional peculiarities (low neuropsychological resilience, emotional anxiety, and decreased intellectual abilities) these servicemen had difficulties with adaptation in new military surrounding and tried to decrease the emerged anxiety with typical for them way which was alcohol abuse.

DISCUSSION

The results which were received in this research proved one more time that ARIs were mostly committed by servicemen with lower military rank. The conclusion was considered to be typical, and it pointed out that servicemen who committed ARIs were characterized by the decreased quality of interaction with the surrounding substantive and social world (were less motivated, less active and less capable to rapid mobilization). They were characterized by lower level of extraversion, sociability, conformity, and practicality; moreover, they had lower indicators of intellectual abilities and awareness.

Combat operations in Eastern Ukraine has been already carrying out for 8 years already [33], thus a half of servicemen (50.32%) who took part in the research had the status of participants in hostilities. This fact could not be the indication that participation in combat operations increased the quantity of those who committed ARIs.

The research proved that the group of servicemen who committed ARIs was homogeneous and due to the specificity of adaptation it could be divided into three more groups. The most interesting fact was that a significant criterium of segmenting was the sincerity of answering the questions by servicemen. Thus, the biggest group of servicemen who committed ARIs in comparison with the control group was characterized by low level of sincerity of answers. They wanted to demonstrate their socially approved image. The same tendency was revealed in the process of analyzing the cadets from Higher Educational Military Institutions who were known to buy forbidden substances [34]. However, among the servicemen who committed ARIs the eagerness to demonstrate socially approved image was far more spread (there were 67.74% from the general group of analyzed servicemen who committed ARIs and 42.11% from the general group of analyzed military cadets who were known to use forbidden substances).

The determination of groups in this research was quite important to figure out the reasons of ARIs and their spreading. The representators of group A, in particular, understood the importance of rules and norms of behavioral regulation; however, they experienced the crisis connected with realization of their own inconsistency to norms and values which regulated the activity of servicemen. Servicemen from subgroup A1 had such crisis due to the process of overthinking of values, while servicemen from subgroup A2 were characterized not only by inconsistency to norms, but also by low possibility to control their reactions and believe to existing rules. The vast majority of subgroup A2 were enlisted personnel of the 1st year of service. Specifically, the problem of the secondary socialization was the reason of ARIs' commitment by those servicemen. Group A was not so widespread as it was generally considered. Thus, about 12% of the group of servicemen who committed ARIs had the problems of the secondary socialization (problems of accepting the norms of new social group) and the crisis of values.

Among the representators of group B there was distinguished subgroup B1. The representators of subgroup were sincere, active, and intellectually developed, they could rapidly mobilize their strength, but they were not ambitious and were unmotivated while executing their duties of low enforcement. Received results concerning this group proved the conclusions made by Kintzle at alia [24] that unemployment was the reason of stress and alcohol abuse. The case was not in unemployment but in absence of favourite work which could provide them with self-realization; namely that turned to be the reason of ARIs' commitment. Moreover, the research conducted by Hoopsick, Homish, Vest, & Homish [35] pointed out that servicemen of the US Army and the US National Guard who did not participate in hostilities had negative concerns on the fact of being unrealized that became the predictor of addictive behaviour.

The representators of subgroup B2 had tendency to negative concerns which disorganized their activity. However, taking into account the high level of their insincerity it was possible to conclude that they tended to use references to their miseries as excuses of their impertinence and alcohol abuse. These servicemen demonstrated victim-like behaviour and according to Kim et alia [28] that was one of the reasons of alcohol abuse in junior military personnel. However, another research by Hoopsick, Homish, Vest, & Homish G [36] distinguished that the negative social relations such as problems of perceptions and social victimization were connected with higher chances of forbidden substances abuse and non-medical use of prescribed medications among the servicemen of the US National Guard. However, those problems did not lead to the increase of alcohol abuse.

Subgroups B3 and B4 were the biggest ones and they both wanted to increase their status. Thus, the servicemen of subgroup B3 while abusing alcohol tried to add themselves the traits of proficiency and to develop their image of experienced servicemen. The servicemen of subgroup B4 wanted to become important individuals. Both subgroups abused alcohol and that gave them the possibility not only to feel themselves more confident and independent but also it was a significant component for their image of successful individuals. According to the research by Barry et alia [7] and Shirvani et alia [8] such reasons of alcohol abuse explained why among those who wanted to enter the military service there were more school-leavers and youth who abused alcohol than those who did not have such habit.

Connection between alcohol abuse and problems of identification was examined in the work by Lostutter, Neighbors, Simpson, & Larimer [37]. The researchers distinguished that identifying themselves as a veteran and/ or a student at the college could influence the connection between the status of veteran and alcohol as well as psychological symptoms. They figured out that when veterans who were studying in college started identifying themselves as typical students at the college it decreased the symptoms of PTSD.

The features of group C of servicemen who committed ARIs turned out to be significantly close to those which were distinguished in our previous works devoted to the analysis of constitutionally weak subtype among the servicemen who committed suicide [38]. The comparison of groups such as “the control group” (1), “constitutionally weak who committed suicide” (2), “constitutionally weak individuals who committed alcohol-related incidents” (3) demonstrated that the last group had worse or even the worst nature to confront all the life failures as well as “constitutionally weak individuals who committed suicide” (2). Furthermore, earlier Prykhodko I, Matsehora, Kolesnichenko, Pasichnik, Kuruch, Yurieva, Kravchenko, Radko, & Prikhodko D [39] determined that constitutionally weak individuals did not tend to commit suicide while being drunk because they reacted on alcohol abuse as on absolute relaxation. This information gave the possibility to regard alcohol as if it was a coping which protected from suicide commitment.

Received results from group C proved the determined by Herberman Mash et alia [5] thesis that understanding of the reasons of alcohol abuse helped to distinguish the subgroups of servicemen who either tended to risky suicidal behaviour or were stable concerning suicide.

All the servicemen who committed ARIs were characterized by the decrease of the quality of interaction with the surrounding substantive and social world; however, the reasons of their alcohol abuse significantly differed. As a criterium for segmenting in the research there were used indicators of (dis)adaptivity which transferred the review of the topic concerning alcohol abuse to the plane of unproductive copings and relevant description of the groups such as “inconsistency to the norms” (group A), “desire to demonstrate socially approved image” (group B), “protection from suicide commitment” (group C).

Among the servicemen who committed ARIs there were distinguished three groups such as A, B, and C which differed with their peculiarities of (dis)adaptation. That was valuable to organize preventive works, events dedicated to the improvement of socialization by recruits, adequate formation of servicemen image as well as their identification in accordance with it, and events dedicated to the prevention of suicidal behaviour.

The results of this research will be used to specify the norms in accordance with psychodiagnostic methods due to which the psychological and professional selection to the military service are carried out.

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ORIGINAL ARTICLES

The effect of oral zinc supplementation on bone biomarkers in rats in the experimental model of space exploration (simulated weightlessness)

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Abstract: *Background: Zinc as a cofactor is effective in the function of many enzymes and is also essential for collagen synthesis and bone mineralization.*

Objectives: The current study aims to survey the effect of oral zinc supplementation on bone biomarkers (alkaline phosphatase and osteocalcin) in rats in the experimental model of space exploration (simulated weightlessness).

Methods: This was an experimental study. A laboratory model of space exploration for inducing weightlessness in rats was done by the Hindlimb suspension technic. Rats were randomly divided into 2 groups; group A (n=7) received daily zinc sulfate and group B (n=7) received daily placebo. Rats received the supplement or placebo for 30 days. The alkaline phosphatase and osteocalcin were assessed in both groups at baseline and 30 days.

Results: The microgravity simulation model of space exploration increased significantly alkaline phosphatase and decreased significantly osteocalcin in rats. Adding zinc sulfate to the diet has been effective in increasing the amount of alkaline phosphatase but has not shown a significant effect on the amount of osteocalcin reduction.

Conclusion: According to current findings, it seems that Zinc administration did not have an effect on osteocalcin improvement but led to more rising of alkaline phosphatase in rats.

Keywords: *microgravity, Zinc, alkaline phosphatase, osteocalcin, bone*

INTRODUCTION

The Human Research Roadmap (HRR) has been published by NASA to systematically target bio-aerospace research to reduce or eliminate risks to health, safety, and performance during and after space exploration. Risks include physiological and performance effects from hazards such as radiation, altered gravity, and hostile environments, as well as unique challenges in medical support, human factors, and behavioral health support. Of the 23 risks identified in this roadmap for humans in space, at least 5 are associated with changes in the function of the musculoskeletal system. The main concerns are about the increase in age-related osteoporosis, the lack of improvement in bone loss after returning from space exploration, and the increased risk of fractures [1].

It is now well established that weightlessness during space exploration causes osteoporosis. Loss of bone mass throughout the body and in the legs, which are rich in cortical bone, is 0.3-0.4% per month. The reduction in bone mass in the trabecular part of the bone seems to be greater than in the cortical part [2,3].

Histomorphometric studies of rats that experienced space exploration for different periods showed changes in bone cortex and sponge, a transient increase in bone resorption, and a permanent decrease in bone production [4,5].

Data on interventions to maintain stable bone metabolism and prevent bone loss during space exploration are limited.

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Ingesting high amounts of calcium and vitamin D supplements during space exploration did not prevent osteoporosis. The additives did not prevent an increase in bone resorption although they did prevent an increase in serum calcium levels [6,7].

Zinc is an essential element that has many physiological roles in biochemical processes, especially growth in humans and animals. Several zinc-dependent hormones and enzymes are involved in bone metabolism. Zinc stimulates cell differentiation, cell proliferation, and mineralization in osteoblasts and thus stimulates bone production. From a molecular point of view, zinc stimulates gene expression in various proteins including Runx2/Cbfa 1 (transcription factor for osteoblast cell differentiation), type I collagen, alkaline phosphatase, and osteocalcin in cells [8]. The concentration of osteocalcin, which is secreted from osteoblastic cells in the culture medium, rises significantly after the addition of zinc. Adding zinc to culture stimulates the production of bone growth factors and bone matrix proteins that are involved in promoting bone formation and mineralization. DNA polymerase enzyme involved in DNA production is a zinc-dependent enzyme. Zinc may cause DNA production by activating the DNA polymerase enzyme in bone tissue osteoblasts [9].

Bone biomarkers are used for research as well as to monitor the therapeutic effect of elements on bone turnover. Biomarkers of bone formation are products that active osteoblasts express at different stages of their maturation or bone enzymes. Biomarkers that are widely measured in serum or plasma include bone-specific alkaline phosphatase (BSAP), and osteocalcin, which were measured in the present study [10].

Bone-specific alkaline phosphatase is a biomarker involved in bone formation. Alkaline phosphatase (ALP, ALKP, ALPase, Alk Phos) (EC3.1.3.1) is a hydrolase enzyme (associated with cell membranes) that is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. Alkaline phosphatase is produced by osteoblasts [11].

Osteocalcin is a non-collagenous protein made by osteoblasts. Osteocalcin is found in bone and has a metabolic role, and is prosteoblastic. Osteocalcin is involved in bone mineralization and calcium homeostasis. Serum osteocalcin levels are determined by bone formation and the number of osteoblasts. Serum osteocalcin levels are determined by histomorphometry and calcium analysis and are introduced as a biomarker of bone formation. Osteocalcin is one of a few proteins that are specific to the skeletal system [12]. Osteocalcin and alkaline phosphatase are good biomarkers for the study of osteoblast activity. On the other hand, zinc is essential for osteoblast activity, collagen synthesis, and alkaline phosphatase activity [13].

Considering that the changes in bone during space exploration in astronauts are one of the most important issues in astronomy, this study was designed to determine the changes in bone biomarkers with and without zinc sulfate in an experimental model of space exploration.

Due to the high cost of space exploration, efforts have been made to artificially create weightlessness on Earth. One of these methods, hanging a rat by its tail, is widely used as a laboratory model of space exploration. This technique simulates the effects of weightlessness on the bones and muscles of the animal's hind legs. It also allows the blood flow to be simulated, like a human being in a state of weightlessness, in which the blood flow tends to shift towards the animal's head and chest [14].

MATERIALS AND METHODS

Type of study and time of study

This experimental study was performed in the animal husbandry of Iran University of Medical Sciences with the participation of the Faculty of Aerospace and submarine Medicine of AJA University of Medical Sciences during 2021.

Sample size

Based on animal studies, the sample size was 14 rats (7 rats in each group).

Study population

Fourteen 6-week-old male rats were obtained from the breeding colony at Iran University of Medical Sciences, Tehran, Iran. These rats were randomly divided into two groups. Group A included 7 rats as the intervention group; group B included 7 rats as the control group.

Procedure

The two groups of 7 rats were first housed for one week at the same temperature (23±2 °C) and humidity (60±10) with a 12 hr light-12 hour dark system and maintained on the same diet.

Then, rats from each group were individually housed in cages designed so that the rat’s tail was attached to the roof of the cage and the rat was suspended by the tail (hindlimb suspension). These conditions, remove pressure from the back legs of the rat. The animal could move in the cage using its front legs. This technique simulates conditions similar to microgravity during space exploration (14).

Both groups were given a normal diet. Rats in group A received 227 mg/l of oral zinc sulfate (prepared by Bahansar Company) in their drinking water. This does is based on previous study (15). Rats in group control received placebo. The study period lasted 30 days.

A blood sample was taken at the beginning of the study and on day 30 to check for changes in bone biomarkers (alkaline phosphatase and osteocalcin) and the amount of circulating zinc. The samples were then transferred to the laboratory, the serum was separated, frozen, and kept at -18 °C until the end of the study. For all samples, bone biomarkers of alkaline phosphatase, osteocalcin, and serum zinc levels were measured and compared using kits purchased from Eastbiopharm.

Hindlimb suspension

The technique of hindlimb suspension has been approved by NASA (14) as a simulation of the conditions experienced in space. The main cause of osteoporosis in space exploration is the lack of gravity and, consequently the lack of sufficient pressure on the weight-bearing bones. Hindlimb suspension prevents the lower limbs from touching the ground and bearing weight, inducing osteoporosis (as relied on by NASA's Hindlimb Suspension model).

To facilitate feeding and the isolation of the rat from urine and feces, the body of the cages was designed similar to that of metabolic cages. Because rats have a high ability to release their tails, it was thought that the tail should come out of the cage. For this reason, a hole was made in the roof of the cage (in its central part) to exit the tail. The size of the cage was designed so that the rats could easily access the water and food tank despite hanging from the tail area. At the exit of the tail from the roof of the cage, a plastic plate was added to eliminate the minimum contact between the rat and the tail and to minimize the possibility of chewing the glue and releasing the tail.

Placement of rats in the suspension cage

The rats were gently placed inside the special chamber. Their tails were cleaned with alcohol to remove the dead and dirty tissues. A disposable plate made from herbal ingredients was pierced through the middle and the tail was passed through it. Herbal ingredients were chosen to prevent contact dermatitis resulting from chemicals and damage to the rat's tail. The tail was then passed through the middle hole of the cage lid plate. The piston of a 2 cc syringe was then cut in half with a small chainsaw. A layer of sterile gauze was wrapped around the rat's tail and then the rat's tail was placed inside the cut syringe and sealed with glue.

Figure 1: Rat placement conditions in the designed and built model

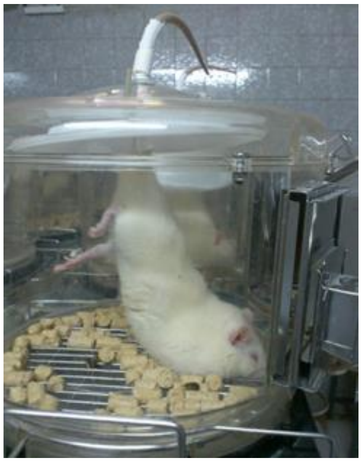

The bandages were loose enough to allow blood to circulate. However, because the tail plays an important role in regulating rat body temperature, only areas of the tail that were attached to the syringe were wrapped by sterile gauze. The rat was then placed in a cage. The bulge at the end of the syringe was trapped in the lid of the cage, keeping the rat hanging. The rat's body was made at a 45-degree angle to the cage floor, so the hind legs did not come into contact with the cage floor net. The rat was placed in such a way that it had easy access to water and food.

Ethical principles

The study was conducted by the Declaration of Helsinki and the guidelines of the Animal Welfare Association of Canada. Institutional Review Board approval (code: 92108- AJA University of Medical Sciences) was obtained.

Statistical analysis

The continuous variables were expressed as the mean±SD, and the categorical variables were presented as a percentage. Chi-square and independent t-test were used to compare data between the two groups and a repeatd measures test have been used for within groups' comparisons. All statistical analyses were performed with SPSS (version 16.0, SPSS Inc, Chicago, IL, USA). A “P-value” less than 0.05 was considered significant.

RESULTS

Weight of rats

Fourteen male Wistar rats were divided into two groups of 7; intervention group (microgravity simulation model with oral zinc supplementation – Zn) and control group (microgravity simulation model with placebo). The average weight of the rats in the Zn group was 250±12 g. The average weight of the control group was 245±8 g. There was no significant difference between the groups (p=0.38).

Zinc (Zn)

The mean serum zinc level in the control group was 91.5±4.4 mcg/dl on day 0 and 90.6±3.5 mcg/dl on day 30 and there was no significant difference in serum zinc levels between the two samples (p=0.805). The mean serum zinc level in the Zn group was 90.4±4.45 mcg/dl on day 0 and 229±8.68 mcg/dl on day 30 and there was a significant difference between the two samples (p=0.001) (Table 1).

The baseline serum zinc levels in rats in the two groups were similar. After 30 days, serum zinc levels in rats that received oral zinc sulfate was significantly higher than the control group that received a placebo (Table 1).

Table 1: Comparison of serum zinc levels in rats in two groups (zinc and control) in stages of the study (first, day 30)

	Zinc Sulfate Supplement	Placebo	P value
Baseline	90.4±5.4	91.5±4.4	0.67
Day 30	229±8.7	90.6±3.5	0.0001
P value	0.0001	0.68	

Alkaline phosphatase

A significant increase in alkaline phosphatase was recorded during 30 days in the control group (p=0.002) and intervention group (p=0.002). This increase in alkaline phosphatase in the intervention group on days 30 is greater than that in the control group, which is statistically significant (p≤0.05; Table 2).

Table 2: Comparison of mean alkaline phosphatase biomarker values in both intervention groups (microgravity simulation model with oral zinc supplementation) and control group (microgravity simulation model with placebo) between days 0 and 30

	Zinc Sulfate Supplement	Placebo	P value
Baseline	131.6±10.3	130.4±9.2	0.82
Day 30	436.6±90.7	209.3±2	0.0001
P value	0.0001	0.0001	

Osteocalcin

Osteocalcin level decreased significantly in the intervention (p=0.001) and control (p=0.001) groups during 30 days. No significant difference was observed between the two groups in baseline and day 30 (Table 3).

Table 3: Comparison of mean Osteocalcin biomarker values in both intervention groups (microgravity simulation model with oral zinc supplementation) and control group (microgravity simulation model with placebo) between days 0 and 30

	Zinc Sulfate Supplement	Placebo	P value
Baseline	58.3±1.9	58.4±2.9	0.94
Day 30	44.7±4.9	45.6±1.9	0.66
P value	0.0001	0.0001	

DISCUSSION

In the present study, the effect of oral supplementation of zinc and microgravity on changes in the two factors affecting bone formation, namely alkaline phosphatase and osteocalcin in blood serum during 30 days (in microgravity conditions using a simulated model of space exploration or Tail Suspension) was investigated. Weightlessness caused a significant decrease in osteocalcin in both the presence and absence of Zn supplement. In contrast, no effect was recorded in the zinc-receiving group, and zinc administration did not have an effect on osteocalcin improvement. While weightlessness caused a significant increase in alkaline phosphatase in both groups, the addition of zinc significantly increased the phosphatase levels above that of controls.

Bone loss during space exploration is about 1-2% per month [2,3]. Bone loss in space is more common in weight-bearing bones of the lower limbs and spine. People who spend six months in space, lose about 20 percent of their lower limb bone mass. In addition, after returning to Earth, bone loss continues for months. Loss of bone to this extent leads to a significant increase in the risk of fracture by about 5 times more than expected in normal bone on Earth [16,17].

Consistent with the current finding, Grano et al (2005) showed that microgravity induced by hindlimb suspension for 35 days reduces osteocalcin activity and increases alkaline phosphatase activity [18]. In 1994, Backup et al. found the level of osteocalcin in the bones decreased after 10 days of space flight [19]. Patterson-Buckendahl et al. examined the effects of microgravity on osteocalcin in mice in Spacelab, and found that osteocalcin levels decreased during the first week of suspension and returned toward control values after 15 days [20].

The effectiveness of zinc in increasing alkaline phosphatase activity was confirmed. Yamaguchi et al. (2008) showed that zinc stimulated bone metabolism in rats, increased bone protein synthesis, and bone formation in cultured tissues by increasing enzyme activity such as alkaline phosphatase [21]. Lowe et al. (2002) showed that zinc is one of the components involved in the synthesis of alkaline phosphatase and is essential for its activity [22]. Sibonga et al. (2007) observed an increase in osteoblast-specific proteins, alkaline phosphatase and osteocalcin, after returning to Earth's gravity [23]. Park et al (2013) found that zinc inhibits osteoclastogenesis in rats by inhibiting the signaling pathway of Ca2+ - Calcineurin - NFATc1 in bone marrow-derived monocytes. They suggested that zinc could be a good candidate for the treatment of osteoporosis due to the activation of NFATc1 in osteoclasts [24].

Several studies have shown that dietary zinc is an effective measure in preventing bone loss. Chou et al. (2013) found that biomimetic zinc was effective in preventing and treating osteoporosis in a rat model [25]. Sun et al., (2011) examined the found that dietary zinc was effective on bone growth, metabolism, and IGF-I gene expression and alkaline phosphatase [26]. Seo et al. (2010) reported that zinc production increases osteogenic activity by stimulating cell proliferation, alkaline phosphatase activity and collagen synthesis in osteoblast cells in culture [27].

CONCLUSION

A microgravity simulation model for space exploration increased alkaline phosphatase and decresed osteocalsine in rats. Adding zinc sulphate to diet was effective in increasing the amount of alkaline phosphatase but failed to show a significant effect on osteocalcin reduction.

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Abbreviations
Zinc: Zn; Bone-specific alkaline phosphatase: BSAP; Human Research Roadmap: HRR; National Aeronautics and Space Administration: NASA; Alkaline phosphatase: ALP, ALKP, ALPase, Alk Phos.

Authors' contributions

RE, AK were responsible for study concept and design. RE, AK led data collection. JS, RH were responsible for the analysis and interpretation of data. RE wrote the first draft. JS, RH contributed to the writing of the second and third draft. JS, RH provided comments on initial drafts and coordinated the final draft. All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Availability of data and materials
The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate
The study was conducted by the Declaration of Helsinki and the guidelines of the Animal Welfare Association of Canada. Institutional Review Board approval (code: 92108- AJA University of Medical Sciences) was obtained.

Consent for publication
By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

Competing interests
The authors declare that they have no competing interests.

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ORIGINAL ARTICLES

Typology and structure of servicemen motivation

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Abstract: *The results of the conducted research gave the possibility to distinguish the structure of the motivation which was suitable for the distinguished types of motivation of servicemen of the National Guard of Ukraine with different levels of effectiveness of their professional activity including motivation based on public service, stagnation, prosaically, romantic, deficient, and dependent. 2435 male servicemen participated in the research. They equally represented all the operational and territorial reunifications and military units of the National Guard of Ukraine. Every unit equally presented the officers, servicemen under the contract including the participants in hostilities as well as those who did not have such experience. The research was conducted with the help of the methods which were standardized by the Ukrainian language: "Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs", "Methods of Studying the Motivational Profile of Personality", and "Motivation Sources Inventory – MSI". The conducted research posed the possibility to describe not only the determined types of professional motivation of servicemen, but also specific phenomena such as self-motivation of the representatives of a type which was run by public service motivation; "rest on the laurels" for the type of stagnation; problems related to choice-making for the prosocial type for whom profession, family, and friends had equal meaning; the tendency for manipulation to gain access to desirable aspects of professional activity of the type which struggled for adventures; lack of sincere interest in the military profession for the deficient type which could change it for more prospective having the same requests for the candidates; attitude to the profession as if it was a means of satisfaction of the needs of their emotional life for the dependent type.*

Keywords: *motive, motivational sphere, servicemen, the effectiveness of the professional activity*

INTRODUCTION

With the beginning of combat operations in Eastern Ukraine, it turned out to be obvious that the estimation system of motivation of servicemen of NGU did not satisfy all the requirements related to the profession [1]. The number of those who wanted to sign a contract or to prolong it had significantly decreased. However, there increased staff turnover, the number of violations of discipline related to alcohol and drug abuse [2], spontaneous unit leaving, ignoring and defying orders, and other issues which were closely connected with discrepancy of servicemen motivation to profession requirements. It turned out to be urgent to determine the adequate motivation of professional activity for servicemen of NGU and to select and develop new psychodiagnostic methods to estimate it.

Having analyzed the world experience of studying the motivation of servicemen and law enforcement there was a need to point out that these days it was implemented in the frames of the theory of public service motivation (PSM), self-determination theory (SDT), and terror management theory

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(TMT) [3]. They were used to explain the selection of serviceman profession, the effectiveness (productivity, success) of professional activity, achievement of aims related to the activity, satisfaction with professional activity, professional burnout, exhaustion, and staff turnover.

Israeli researchers Taubman-Ben-Ari and Findler [3] studied the conscripts by the terror management theory (TMT). It was distinguished that the main psychological conflict occurred due to the existence of self-preservation instinct through understandability that death was irreversible and unpredictable. They concluded that the significance of death rate related to higher level of motivation directed to public service and higher expectations of physical difficulties in the army.

According to Griffith and Perry [4] the motives of military service enlistment before as well as after the operation "Desert Storm" was explained by the following factors: "desire to perform military service", "personal development", "salary (benefits)", and "career development". However, after the operation "Desert Storm" there significantly decreased the number of recruits entering military colleges due to three first factors from the list.

Like-Jensen and Glad [5] studied the motives of entering the missions of preservation and implementation of peace. They determined three types of servicemen motives among them there were: challenge, personal well-being, and loyalty. Challenge was the motivation directed to the profession. Personal well-being was a desire to get adventures (romantic motivation). Loyalty was the institutional motivation. Motives before as well as after the participation in hostilities significantly differed in dependence on the type of mission.

Siryi [6] pointed out that among the servicemen who served under the contract in the Armed Forces of Ukraine there were spread three types of motivation "motivation of activity" (36.6%), "motivation of pragmatics" (58.6%), and "motivation of reward" (4.8%).

Chambel, Castanheira, Oliveira-Cruz, and Lopes [7] having compared the studies carried out by Portuguese servicemen distinguished that motivation directed to autonomous work was positively connected with the involvement into the work as well as negatively connected with burnout. Controlled motivation, in turn, demonstrated an opposite picture. Moreover, autonomous motivation was a mechanism that explained the interrelation between contextual factors and well-being at workplaces.

Gillet, Becker, Lafrenière, Huart, and Fouquereau [8] distinguished that servicemen with high indicators of autonomous motivation showed the highest level of well-being including the support from the side of leaders, positive worries, communication, and a high level of organizational support.

Based on the carried out studies Breaugh, Ritz, and Alfes [9] pointed out the interconnection between the theory of public service motivation (RSM) and the theory of self-determination (STD). Having learned their relations with work satisfaction gave the possibility to figure out that the motivation of self-determination was significantly bound with the satisfaction at work-position. However, the co-workers with a high level of PSM had more stable indicators of work satisfaction in comparison with their colloquies who had a low level of PSM.

Schott, Neumann, Baertschi, and Ritz [10] conducted the comparison of prosocial motivation as well as public service motivation and studied the peculiarities of Swiss police officers. These researchers determined that despite PSM and prosocial motivation Swiss police officers were motivated with various tasks and excitement (for example, car chases). In daily routine, they were motivated by complicated tasks, by the way, they were estimated by the public and their colleagues.

The research conducted by Holton, Smith, Lindsay, and Burton [11] distinguished that a desire of cadets of the US Air Force Academy to voluntarily resign was negatively connected with work satisfaction, emotional commitment, work engagement, and individual compliance with professional requirements. The researchers figured out that the strongest predictor of staff turnover was individual compliance with the profession.

Meyer, Kam, Goldenberg, and Bremner [12] determined that Canadian servicemen with high indicators of affectivity and normative commitment of organization reported about the most favorable conditions at work, desire to continue their service and well-being. Servicemen who were focused on the consequences of their actions (understandability of the "price" of the resign consequences) and informed about less favorable consequences were more active in their search for work and had the highest level of anxiety and depression.

Lakhani and Fugita [13] studied the problem of resignment among the US Army and the National Guard reservists after the "Desert Storm" from the side of income and patriotism. They determined that the decrease of those who resigned from service could happen due to the increase of salary for reservists or due to the encouragement of wife/husband for a more positive attitude to reservists. However, the results of this research turned out to be weak support of the income theory but gave significant support for the patriotism theory.

The presented analysis showed that there was a need to consider heterogeneous motives of professional activity of servicemen as well as of law enforcement officials. Moreover, the search manner of this research proved the necessity to decrease the number of analyzed variables to increase the accuracy of the perception of the received results. By the highlighted information, the empirical data received in the research proceeded to factorial analysis.

METHOD

Participants and Procedure

The research was conducted throughout the 2019-2021 years. 2435 servicemen of the National Guard of Ukraine participated in the research. Those servicemen equally represented all the operational and territorial reunifications as well as military units of the National Guard of Ukraine. Every unit equally presented the officers – 29.84% (from the second lieutenants to colonels), servicemen who served under the contract – 70.16% (from soldiers to senior warrant officers), participants in hostilities, and those who did not have such experience. The age of all the participants was from 20 to 55. All procedures carried out in the study conformed to the ethical standards of the 1964 Helsinki Declaration and its later amendments. All participants gave consent for their data to be used in this research.

Instruments and Measures

To determine the structure of motivation which was typical for the distinguished type of NGU servicemen motivation with various levels of effectiveness of professional activity there were used the methods standardized by the Ukrainian selection:

1. “Close Questionnaire of Examining the Motivation of Professional Choice Made by Applicants of the Ministry of Internal Affairs” by Moskalienko [14]. This questionnaire was developed by the Ukrainian researcher for professional psychological selection for future policemen education; it gave the possibility to evaluate “Motives Related to Objective Socially Posed Tasks and Content of Profession”, “Motives Related to Personal and Professional Development”, “Independent Profession Selection”, “Dependent Profession Selection”, “Motives Related to External Prestigious Professions and Financial Welfare”, “Motives Related to Romanticization of Profession”, “Motives Related to Eagerness of Compensation of Temper Flaws”, “Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)”. The instruction of this method proposed to answer the question concerning the reason for choosing this profession where the respondents using a scale made of 5 points could estimate 40 statements that described the profession of law enforcement.

2. “Methods of Studying the Motivational Profile of Personality” by Ritchie and Martin [15]. The use of this method gave the possibility to determine the urgent motivation of professional activity. It had the following scales: “Need of High Salary and Financial Benefits”, “Need of High Working Conditions and Comfortable Surrounding”, “Need of Accurate Structurization of Work and Feedback”, “Need of Social Relations”, “Need of Forming and Supporting Long-Term Stable Relations”, “Need of Obtaining the Recognition by Other People”, “Need of Posing Brave and Tough Goals and of Achieving Them”, “Need of Interesting and Socially Beneficial Work”, “Need of Self-Improvement and Development as Personality”, “Need of Being Creative, Thoughtful, and Open for further Ideas Employee”, “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine”, “Need of Manipulation and Power, Eagerness to Control the Other People”. This questionnaire included 33 questions with 4 variants of answers where the respondents had to place 11 points by the importance of the answers.

3. “Motivation Sources Inventory – MSI” by Barbuto and Scholl [16]. The use of this method gave the possibility to determine: “Internal Processes” (a desire to get satisfaction and pleasure from the process of activity), “Instrumental Motivation” (desire to valuable external benefits such as benefits, etc.), “External conception – I” (desire to except and support personal traits, competencies, and values from the side of other individuals or referential group), and “Internal competence – I” (desire to achieve aims and relevant internalized values). Respondents were proposed to answer 30 questions related to profession using a 7-point scale (from complete “no” to complete “yes”).

Data Analysis

To determine the structure of motivation which was typical for every distinguished type there were used the extraction method: principal component analysis and rotation method: varimax with Kaiser normalization. As variables, there were represented the indicators by scales of three psychodiagnostic methods. Mathematical data was processed through SPSS 17.0.

RESULTS

According to previously carried out research there were distinguished the following types of NGU servicemen motivation with different levels of effectiveness of their professional activity: motivated by public service, stagnated, prosocial, romantic, deficient, and dependent [17].

The first type of motivation of NGU servicemen (about 35% from general selection) was marked as one which was run by public service motivation. Servicemen of this type chose the military profession due to its content, goals, and desire to develop. They had expressed orientation directed to socially beneficial work as well as professional development. The motives which described the conditions, working hours, and social interaction were significantly lower and subcontract. Those servicemen had high indicators of a need for high financial benefits and recognition which demonstrated the importance of the high social status of the chosen profession. It was typical for the representation of this group to have significant time prospects, distinct internal locus control, and developed strong-willed qualities.

Used factors of the analysis showed that resistance and mentioned peculiarities of professional motivation ensured specific structure for the representation of this type. The motivational structure of this type is represented in Table 1.

Table 1: The structure of motivation of NGU servicemen who were guided by PSM

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Objective Socially Posed Tasks and Content of Profession	0.73	-0.01	0.13	-0.21	-0.13	-0.30
Motives Related to Personal and Professional Development	0.81	0.12	-0.01	-0.13	-0.05	0.14
Independent Profession Selection	0.73	-0.01	-0.29	0.04	-0.04	-0.13
Motives Related to External Prestigious Professions and Financial Welfare	0.75	0.05	-0.07	0.33	0.08	0.12
Motives Related to Eagerness of Compensation of Temper Flaws	0.82	0.03	0.10	-0.05	-0.14	0.06
Need of High Working Conditions and Comfortable Surrounding	-0.05	0.07	0.07	0.91	-0.15	-0.11
Need of Accurate Structurization of Work and Feedback	0.07	0.87	-0.05	-0.13	-0.16	-0.14
Need of Obtaining the Recognition by Other People	-0.14	-0.01	0.02	-0.15	0.93	-0.04
Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine	-0.06	-0.85	-0.03	-0.25	-0.16	-0.01
Need of Self-Improvement and Development as Personality	0.02	-0.12	0.07	-0.10	-0.05	0.93
Internal Processes	0.03	-0.05	0.83	-0.10	-0.20	0.14
Instrumental Motivation	-0.09	0.04	0.81	0.20	0.28	-0.08
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 6 iterations.						

This factor structure explained 77.59% of total dispersive features. The first and the most significant factor (24.90%) was distinguished with the help of the following variables: “Motives Related to Eagerness of Compensation of Temper Flaws” (0.82), “Motives Related to Personal and Professional Development” (0.81), “Motives Related to External Prestigious Professions and Financial Welfare” (0.75), and “Motives Related to Objective Socially Posed Tasks and to Content of Profession” (0.73). This factor was marked as a desire to gain professional dignity – the desire for personal, professional, and social development turned to the professional requirements.

The second and the third factors had almost the same value in this structure (12.64% and 12.32%) and were part of the mechanism of self-motivation. Therefore, the second factor demonstrated a desire to produce and practice the professional algorithm of actions: “Need of Accurate Structurization of Work and Feedback” (0.87) and “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine” (-0.85). The third factor showed the process of enjoying professional activity (process as well as result). It was described with the help of the variables such as “Internal Processes” (0.83) and “Instrumental Motivation” (0.81).

Such a combination of the second and the third factors gave the possibility to highly effective servicemen to support their high level of professional activity, accuracy, and intensity of the professional activity.

The three last factors also had almost identical values (9.61%, 9.15%, and 8.96%, accordingly) and they described the peculiarities of needs’ satisfaction. Thus, the content of the fourth factor was connected with the conviction that the financial benefits received due to hard work related to the objective content of the profession. The fourth factor was characterized with “Need of High Working Conditions and Comfortable Surrounding” (0.91). Having clarified that the content of this factor had positive correlations with the variable such as “Motives Related to External Prestigious Professions and Financial Welfare” (0.33) and negative correlations with the variable such as “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine” (-0.25) and of “Motives Related to Objective Socially Posed Tasks and to Content of Profession” (-0.21). It was figured out that namely, the achievement of professional aims and the performance of professional duties were the source of financial well-being.

The content of the fifth factor was in the fact that favorite activity was the source of social approvement. The fifth factor was described with the help of the variable such as “Need of Obtaining the Recognition by Other People” (0.93). Its content was clarified with the direct connection with the variable “Instrumental Motivation” (0.28) and with the resource-based connection with the variable such as “Internal Processes” (-0.20).

The sixth factor correlated with the variable “Need of Self-Improvement and Development as Personality” (0.93). Negative connection correlated with the variable such as “Motives Related to Objective Socially Posed Tasks and Content of Profession” (-0.30). It was determined that namely the profession, its aims, and content were the resource of personal development for servicemen of this type. Thus, the reference point of the acquisition of professional dignity by the first type of NGU servicemen was the combination of a desire for professional, personal, and social development with a desire of meeting the requirement of the profession and running its aims. In this structure of the motivation, there was determined the mechanism of self-motivation and support of activity on a high level which was foreseen by the process of producing and practicing professional algorithm of actions as well as by the satisfaction of working processes and its results. Such a structure of motivation of servicemen provided them with a high level of effectiveness in their profession, with a desire to follow the rules, and with close affection to their profession. It provided them with the prevention from professional stagnation and gave them the possibility to support the accuracy and intensity of their professional activity.

Servicemen of the stagnated type (it compiled about 15% from a general selection of this research) also chose the military profession due to its content. They had significant achievements in professional activity, recognition of their success, but they did not want any professional development, challenges, and responsibility. They tried to distance themselves from their colleagues in service. The structure of motivation of this type is presented in Table 2.

Table 2: The structure of motivation of NGU Servicemen who experiences stagnation

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Objective Socially Posed Tasks and Content of Profession	0.26	-0.01	0.03	0.79	0.01	-0.09
Dependent Profession Selection	-0.08	0.86	0.04	-0.07	-0.05	-0.02
Motives Related to Eagerness of Compensation of Temper Flaws	-0.15	0.18	-0.10	0.86	-0.03	0.16
Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)	-0.03	0.77	0.09	0.29	0.01	0.04
Need of High Salary and Financial Benefits	-0.16	0.06	-0.88	-0.06	-0.08	0.14
Need of High Working Conditions and Comfortable Surrounding	-0.09	0.05	0.09	-0.08	0.87	0.03
Need of Accurate Structurization of Work and Feedback	0.05	-0.29	0.04	-0.03	0.15	-0.76
Need of Social Relations	-0.24	0.21	0.87	-0.15	0.01	0.06
Need of Obtaining the Recognition by Other People	-0.11	0.10	0.01	-0.06	-0.85	0.10
Internal Processes	0.17	-0.34	-0.06	0.05	0.08	0.80
Instrumental Motivation	0.77	0.24	-0.17	-0.16	0.14	0.23
Internal Conception – I	0.81	-0.28	0.20	0.15	-0.16	0.12
Internalization of the Aim	0.81	-0.11	-0.06	0.12	0.05	-0.14
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 6 iterations.						

This factor structure included 77.12% of the total dispersion of the peculiarities.

The structure of servicemen motivation who experienced stagnation, the first and the most valuable factor (16.50%) demonstrated an expressed individualism in the process of achieving professionally important aims based on interiorized professional values. This factor was marked with the variables such as “Internal Conception – I” (0.81). “Internalization of the Aim” (0.81), and “Instrumental Motivation” (0.77). There was clarified the content of the variables of this type’s factor “Motives Related to Objective Socially Posed Tasks and Content of Profession” (0.26) and “Need of Accurate Structurization of Work and Feedback” (-0.24).

The content of the second factor (13.70%) was highlighted due to such variables as “Dependent Profession Selection” (0.86) and “Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)” (0.77). This factor was clarified with the help of the positive poles of the variables such as “Instrumental Motivation” (0.24) and “Need of Social Relations” (0.21) as well as negative poles of the variables such as “Internal Processes” (-0.34), “Need of Accurate Structurization of Work and Feedback” (-0.29), and “Internal Conception – I” (-0.28). This factor was described as a forced compromise. It was quite important for servicemen to gain financial benefits, but it did not provide them with any kind of satisfaction.

The next three factors had almost identical values (12.48%, 12.03%, and 11.85%, accordingly). These factors developed the mechanism to support positive self-relationship which preserved their achievements. The content of the third factor was expressing themselves as role model for other servicemen. The positive pole of the third factor was marked with the variable such as “Need of Social Relations” (0.87) and clarified positive poles with the variable such as “Internal competence – I” (0.20). The negative pole of the third factor was marked with the variable “Need of High Salary and Financial Benefits” (-0.88). Some clarifications were conducted with “Instrumental Motivation” (-0.17). Thus, it was very important for servicemen of the stagnated type to demonstrate their principles to the surrounding in opposition to their financial benefits.

The fourth factor was marked with the variables such as “Motives Related to Eagerness of Compensation of Temper Flaws” (0.86) and “Motives Related to Objective Socially Posed Tasks and to Content of Profession” (0.79). It was clarified the variable “Antisocial Motives” (0.29). Such a combination of the variables of the fourth factor described a desire of servicemen to gain importance at the expense of using all the possibilities given by the profession for their purpose.

The fifth factor had significantly marked poles which provided a positive description to the variables such as “Need of High Working Conditions and Comfortable Surrounding” (0.87) and the reversed was “Need of Obtaining the Recognition by Other People” (-0.85). This factor marked the refusal from professional ambitions of servicemen to the benefit of peaceful life till the end of their contract. In other words, they wanted to rest on laurels.

The sixth factor (10.57%) also had marked poles. The positive pole was “Internal Processes” (0.80). Its content was clarified with the variables such as “Instrumental Motivation” (0.23) and “Motives Related to Eagerness of Compensation of Temper Flaws” (0.16). The negative pole was marked with a variable such as “Need of Accurate Structurization of Work and Feedback” (-0.76). This factor was marked as being satisfied at the expense of demonstrating their honed professional skills.

Having summed up the results of servicemen who were included in the stagnated type there was a need to point out that they were characterized with marked individualism and perception of the service continuation as a forced compromise. The structure of their motivation had a formation due to which they supported high positive self-relationship, demonstrated their integrity and success at the expense of carrying out the improvement of professional skills, and used the position of “rest on laurels”.

Servicemen of the prosocial type (15%) even if they considered their military service to be temporal work which gave them the possibility to make living for their families, however, they made huge efforts to acquire military profession to realize its sense and content. They were responsible performers and did not require additional control from the side of their commanders. It was important for them to support friendly relations with colleagues in service and recognition of their combat achievements by society including financial benefits (benefits). The structure of motivation of this type is presented in Table 3.

This factor structure explained 77.75% of the total dispersion of the peculiarities. The factors had almost equal values.

Thus, the first factor which compiled 15.03% was marked with the variables such as “External Conception – I” (0.87), “Instrumental Motivation” (0.78), and “Internalization of the Aim” (0.73). It was marked as a desire to be accepted by military colleagues and to be helpful to them.

Table 3: The structure of motivation of NGU Servicemen of prosocial type

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Objective Socially Posed Tasks and Content of Profession	0.12	-0.04	0.02	0.08	0.13	0.90
Dependent Profession Selection	0.01	0.11	0.89	0.06	0.07	0.03
Motives Related to Romanticization of Profession	0.22	0.08	0.74	0.22	0.05	0.33
Motives Related to Eagerness of Compensation of Temper Flaws	0.14	0.04	0.44	-0.19	0.08	0.74
Need of High Salary and Financial Benefits	0.07	0.72	0.30	0.06	-0.11	0.08
Need of Accurate Structurization of Work and Feedback	-0.14	0.85	-0.04	0.05	0.14	0.01
Need of Social Relations	0.11	-0.28	0.29	0.71	-0.30	-0.06
Need of Forming and Supporting Long-Term Stable Relations	-0.03	-0.77	-0.01	0.37	0.21	0.14
Need of Obtaining the Recognition by Other People	0.15	-0.21	-0.12	-0.13	0.87	0.08
Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine	0.16	-0.12	-0.32	0.02	-0.78	-0.14
Need of Interesting and Socially Beneficial Work	-0.18	-0.07	-0.02	-0.94	-0.01	-0.01
Instrumental Motivation	0.78	0.01	0.17	0.13	-0.11	0.15
External Conception – I	0.87	0.07	0.09	-0.02	-0.05	-0.01
Internalization of the Aim	0.73	-0.19	-0.08	0.21	0.20	0.14

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 6 iterations.

The positive pole of the second factor (14.62%) was marked with the variables such as “Need of Accurate Structurization of Work and Feedback” (0.85) and “Need of High Salary and Financial Benefits” (0.72). The reversed pole of a factor was marked with the variables such as “Need of Forming and Supporting Long-Term Stable Relations” (-0.77) and was clarified with the help of “Need of Obtaining the Recognition by Other People” (-0.21). The second factor testified that the representations of this group's military profession were temporal means of high income which they left their families for. Choosing this profession, the final step for servicemen was the possibility to acquire the profession and to earn with its help.

The content of the third factor (13.24%) was connected with the variables such as “Dependent Profession Selection” (0.89) and “Motives Related to Romanticization of Profession” (0.74). This factor clarified “Motives Related to Eagerness of Compensation of Temper Flaws” (0.44), “Need of High Salary and Financial Benefits” (0.30), and “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine” (-0.30). Such combination gave the possibility to conclude that the choice of military profession was made under the influence of family, friends, and familiar people who described this profession which was connected with adventurous (romantic), hard, sometimes routine-like but, at the same time, highly paid.

The positive pole of the fourth factor (12.12%) corresponded with variables such as “Need of Social Relations” (0.71). The clarified variables were “Need of Forming and Supporting Long-Term Stable Relations” (0.37), “Motives Related to Romanticization of Profession” (0.22), and “Internalization of the Aim” (0.21). The reversed pole was marked with a variable such as “Need of Interesting and Socially Beneficial Work” (-0.94). In the majority of cases, servicemen of the prosocial type were motivated with the accurate realization of military fellowship and friendship; they wanted to find real friends and to have identical aims with them.

The fifth factor (11.53%) also had both distinguished poles. The positive features mostly correlated with the variable such as “Need of Obtaining the Recognition by Other People” (0.87). The clarified variable was “Internalization of the Aim” (0.20). The reversed pole was introduced in “Need of Diversity, Breaks, and Stimulations, Eagerness to Avoid Everyday Routine” (-0.78) and “Need of Social Relations” (-0.30). This factor was marked as a desire to get recognition of achievements gained with the help of hard work and tasks performance but not at the expense of friendly relations.

The sixth factor (11.21%) was correlated with the variables such as “Motives Related to Objective Socially Posed Tasks and Content of Profession” (0.90) and “Motives Related to Eagerness of Compensation of Temper Flaws” (0.74). The clarification of its content was done due to the variable

"Motives Related to Romanticization of Profession" (0.33). For servicemen of the prosocial type, the abstract content of professional activity "Serve and Protect" gained completely accurate meaning including such features as coping with their fears and flaws, presenting their desires, learning how to use the weapon, improving their physical strength, being good colleague, etc.

It was important for servicemen of this type to be the good breadwinner for their families, to be helpful in their professional activity, and to be reliable for their colleagues in service. They seemed to be more relatives-centered than socially centered.

Servicemen who were focused on the satisfaction of their personal needs (6%) were the only ones from the whole range of distinguished types who chose the profession due to mercenary interests and not due to the objective content of the profession. These servicemen wanted to beneficially exchange their professional skills for their financial well-being. They used social relations because they got satisfaction from communication. They did not want to be responsible, did not want to realize their volitional qualities. These servicemen demonstrated indifference to the problems of their colleagues in service. They were not active because it did not bring them any additional income. Almost 75% of servicemen of this type had the status of the participants in hostilities. These servicemen admired the adventures and neglected the content of the military profession. Their actions were constantly controlled by their commanders. This type was marked as one motivated by a desire for adventures. The structure of motivation of this type is presented in Table 4.

Table 4: The structure of motivation of NGU Servicemen who were motivated with their desire to have adventures

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Objective Socially Posed Tasks and Content of Profession	0.05	0.15	-0.01	-0.12	0.87	-0.09
Motives Related to Personal and Professional Development	0.82	-0.17	-0.15	0.07	-0.04	0.07
Dependent Profession Selection	0.82	-0.22	0.26	0.01	-0.15	-0.24
Motives Related to Romanticization of Profession	0.73	-0.04	0.28	0.27	0.34	0.01
Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)	0.79	0.26	-0.10	-0.08	0.10	0.39
Need of High Salary and Financial Benefits	-0.07	-0.36	-0.72	0.27	-0.01	-0.35
Need of Accurate Structurization of Work and Feedback	0.06	-0.02	0.09	-0.08	-0.08	0.94
Need of Social Relations	-0.17	0.81	0.02	-0.05	0.16	0.08
Need of Forming and Supporting Long-Term Stable Relations	0.05	-0.03	0.93	-0.02	0.02	-0.04
Need of Posing Brave and Tough Goals and of Achieving Them	0.08	0.06	-0.12	0.70	-0.46	0.01
Need of Being Creative, Thoughtful, and Open for further Ideas Employee	0.07	-0.70	0.20	0.23	0.38	0.27
Need of Self-Improvement and Development as Personality	-0.08	0.16	0.08	-0.89	-0.09	0.11
Need of Interesting and Socially Beneficial Work	-0.06	0.75	0.31	-0.14	0.02	0.04
Internal Conception – I	0.12	0.71	-0.03	0.29	0.45	0.07
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 9 iterations.						

The presented factor structure explained 80.65% of the total dispersion of the peculiarities.

The first factor (18.45%) characterizes military personnel as prone to gambling to achieve their interests due to the awareness of their lack of existing skills and personal qualities. This factor included such variables as "Motives related to personal and professional development" (0.82), "Dependent choice of a profession" (0.82), "Asocial motives (attitude towards the profession as a way to satisfy one's own asocial needs)", (0.79) and "Motives associated with the romanticization of the profession" (0.73).

The second factor (18.24%) described the search for the surrounding which could make the lives of servicemen more interesting and eventful. The positive pole of this factor was connected with the variables such as "Need of Social Relations" (0.81), "Need of Interesting and Socially Beneficial

Work" (0.75), and "Internal Conception – I" (0.71). Somewhat specific meaning to this factor was given with the variable such as "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.26). The reversed pole was marked with a variable such as "Need of Being Creative, Thoughtful, and Open for further Ideas Employee" (-0.70). There were clarified the variables of the reserved pole such as "Need of High Salary and Financial Benefits" (-0.36) and "Dependent Profession Selection" (-0.22). Thus, these servicemen did not tend to perform something new, creative. They searched for interesting surroundings that made their lives eventful.

The third and the fourth factors were similar in their values. The third factor (12.24%) characterized a desire of servicemen to use financial resources and gained stable social relations. That could diversify their professional activity and made it more romantic. The fourth factor (11.58%) described servicemen which wanted to get superiority over other people, to self-realize at the expense of desperate actions as opposed to a desire of self-development as well as of development of personal individuality. Servicemen of this type with the help of all possible means wanted the profession to correlate with their "romantic" expectation; however, they did not even try to comply with all the real requirements of the military profession.

Thus, the positive pole of the third factor (12.24%) was connected with variables such as "Need of Forming and Supporting Long-Term Stable Relations" (0.93). It was clarified with the help of the variables such as "Need of Interesting and Socially Beneficial Work" (0.31), "Motives Related to Romanticization of Profession" (0.28), and "Dependent Profession Selection" (0.26). The negative pole was described with variables such as "Need of High Salary and Financial Benefits" (-0.72). The content of this factor indicated that servicemen wanted some adventures and refused financial benefits.

The positive pole of the fourth factor was connected with the variable such as "Need of Posing Brave and Tough Goals and of Achieving Them" (0.70). The content of this pole was clarified with the help of the variables such as "Internal Conception – I" (0.29), "Motives Related to Romanticization of Profession" (0.27), "Need of High Salary and Financial Benefits" (0.27), and "Need of Being Creative, Thoughtful, and Open for further Ideas Employee" (0.23). The reversed pole was determined with a variable such as "Need of Self-Improvement and Development as Personality" (-0.89). According to the content of this factor servicemen of this type tended to desperate acts to build self-esteem and to experience the emotions of priority over other people.

The fifth factor (10.66%) described servicemen who used a lot of creativity to avoid dangerous tasks to perform professional duties. It was correlated with variables such as "Motives Related to Objective Socially Posed Tasks and Content of Profession" (0.87). Its content was clarified with the variables such as "Internal Conception – I" (0.45), "Need of Being Creative, Thoughtful, and Open for further Ideas Employee" (0.38), and "Motives Related to Romanticization of Profession" (0.34). The reversed pole was marked with variables such as "Need of Posing Brave and Tough Goals and of Achieving Them" (-0.46). Servicemen of this type put huge efforts to shift the accents of their profession from dangerous features to adventurous ones. They mostly used the tasks which were attractive for them, and which met their romantic impressions related to the military profession.

The sixth factor (9.47%) was connected with the variable such as "Need of Accurate Structurization of Work and Feedback" (0.94). Its content was clarified with the variables such as "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.39), "Need of Being Creative, Thoughtful, and Open for further Ideas Employee" (0.27), "Need of High Salary and Financial Benefits" (-0.35), and "Dependent Profession Selection" (-0.24). This factor described the servicemen who wanted to get work with definite support (personal interests) even if there was a need to use financial benefits.

Servicemen of the deficient type (25%) chose the military profession because of its content and wanted to change their lives. It was important for them to have a profession with a high salary and social status. Servicemen who wanted to work put huge efforts; however, their motivation turned out to be "blocked" with unsatisfied deficient needs. It negatively reflected on their effectiveness. The structure of motivation of this type is presented in Table 5.

This factor structure explained 77.73% of total dispersal peculiarities.

The most valuable first factor (21.40%) was marked with the variables such as "Motives Related to Eagerness of Compensation of Temper Flaws" (0.90), "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.82), "Motives Related to Personal and Professional Development" (0.81), and "Motives Related to External Prestigious Professions and Financial Welfare" (0.76). It was marked as a desire to change themselves as well as to change their lives for the better, to go beyond their old way of living at the expense of their chosen military profession.

Table 5: The structure of motivation of NGU Servicemen of deficient type

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Personal and Professional Development	0.81	-0.02	0.17	0.08	-0.27	0.09
Motives Related to External Prestigious Professions and Financial Welfare	0.76	0.09	0.06	-0.33	0.02	-0.04
Motives Related to Eagerness of Compensation of Temper Flaws	0.90	0.04	0.03	-0.01	0.12	-0.04
Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)	0.82	0.01	-0.11	-0.02	0.07	0.08
Need of High Working Conditions and Comfortable Surrounding	-0.12	-0.05	-0.01	0.92	0.06	0.03
Need of Social Relations	-0.02	0.03	0.84	-0.23	0.12	-0.13
Need of Forming and Supporting Long-Term Stable Relations	0.10	0.06	0.82	0.21	-0.14	-0.05
Need of Obtaining the Recognition by Other People	0.04	-0.08	-0.01	0.06	0.95	0.03
Need of Interesting and Socially Beneficial Work	0.07	-0.10	-0.15	0.03	0.03	0.96
Internal Processes	-0.11	0.78	-0.19	-0.18	-0.01	0.22
Instrumental Motivation	-0.05	0.75	0.23	0.23	0.11	-0.15
Internal Conception – I	0.20	0.79	0.09	0.06	-0.28	-0.09
Internalization of the Aim	0.07	0.78	0.02	-0.15	0.01	-0.13
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 5 iterations.						

The second factor (18.76%) was marked with the variables such as “Internal Conception – I” (0.79), “Internal Processes” (0.78), “Internalization of the Aim” (0.78), and “Instrumental Motivation” (0.75). This factor was described as a desire to experience the feeling of their value.

The content of the third factor (11.81%) was distinguished with the variables such as “Need of Social Relations” (0.84) and “Need of Forming and Supporting Long-Term Stable Relations” (0.82). The content of this factor was clarified with the variables such as “Instrumental Motivation” (0.23), “Motives Related to Personal and Professional Development” (0.17), and “Internal Processes” (-0.19). This factor described servicemen who wanted to get new social surroundings, new social surroundings, a new social status which could change them as well as their financial situation due to their efforts.

The fourth factor (8.97%) correlated with “Need of High Working Conditions and Comfortable Surrounding” (0.92). Its content was clarified with the positive connections with the variables such as “Instrumental Motivation” (0.23) and “Need of Forming and Supporting Long-Term Stable Relations” (0.21). The reversed pole was marked with the variables such as “Motives Related to External Prestigious Professions and Financial Welfare” (-0.33) and “Need of Social Relations” (-0.23). Accordingly, the fourth factor was interpreted as an expressed need of servicemen to get welfare (good conditions and surrounding, competitive salary) without paying attention to the number of effort they put in.

The fifth factor (8.71%) correlated with the variable such as “Need of Obtaining the Recognition by Other People” (0.95). Its reversed pole gave the possibility to mark the connections with the variables such as “Internal Conception – I” (-0.28) and “Motives Related to Personal and Professional Development” (-0.27). Thus, this factor was marked as “Need of Obtaining the Recognition by Other People” which servicemen wanted to satisfy at the expense of personal development and professional improvement for what they could change their old persuasions and refuse from their old lives.

The sixth factor (8.08%) correlated with the variable such as “Need of Interesting and Socially Beneficial Work” (0.96). The content of this factor was clarified with the variables such as “Internal Processes” (0.22), “Instrumental Motivation” (-0.15), “Internalization of the Aim” (0.13), and “Need of Social Relations” (-0.13). This factor was marked as a desire for interesting and useful actions achieved through professional socialization.

Thus, servicemen of the deficient type had a marked desire to change themselves and their lives for the better, to go beyond the limits of the previous way of living, to experience the feeling of their value, and to get a new status. Their motivation was weakly connected with the direct content of the military profession despite a desire to work putting huge efforts and changing everything. Profession for them was a kind of means, not an individual value. Thus, the military profession could be substituted with any other which was more prospective having identical requirements for their candidates.

Servicemen of the dependent type (about 4% from a general selection of the research) were affective, weak-willed, dependent on other people, as well they expressed a desire for definite emotional life and had a low level of internality. According to the self-determination theory (SDT), there was traditionally used term "autonomous and controlled motivation". However, that type except "autonomous and controlled motivation" had some traits suitable for addictive behavior. The structure of motivation of this type is presented in Table 6.

Table 6: The structure of motivation of NGU Servicemen of dependent type

Variables	Factors					
	1	2	3	4	5	6
Motives Related to Personal and Professional Development	0.94	0.02	-0.11	0.12	0.05	0.08
Dependent Profession Selection	0.90	0.08	-0.15	0.31	-0.18	0.03
Motives Related to External Prestigious Professions and Financial Welfare	0.88	0.26	-0.30	-0.05	0.11	-0.01
Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)	0.86	0.29	0.16	-0.02	0.07	-0.24
Need of High Salary and Financial Benefits	0.24	0.10	0.10	0.93	-0.01	-0.09
Need of High Working Conditions and Comfortable Surrounding	0.04	-0.01	0.02	-0.03	0.96	0.14
Need of Accurate Structurization of Work and Feedback	-0.04	-0.01	-0.04	-0.10	0.15	0.98
Need of Obtaining the Recognition by Other People	0.18	-0.14	-0.87	-0.24	-0.28	-0.14
Need of Interesting and Socially Beneficial Work	-0.11	0.05	0.93	-0.10	-0.17	-0.17
Internal Processes	0.23	0.71	0.28	-0.46	0.24	0.20
External Conception – I	0.37	0.74	0.07	0.25	0.12	-0.06
Internal Conception – I	0.36	0.80	-0.04	0.09	-0.26	-0.14
Internalization of the Aim	-0.09	0.93	0.06	-0.01	-0.01	0.04
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.						
a. Rotation converged in 6 iterations.						

This factor structure described 92.32% of total dispersal peculiarities.

The first two factors (28.01% and 21.16% accordingly) described the dependency on antisocial needs. The first factor pressed to the process of profession selection and the second factor was marked with specifically realized aims of professional activity.

The content of the first factor (28.01%) was marked with the variables such as "Motives Related to Personal and Professional Development" (0.94), "Dependent Profession Selection" (0.90), "Motives Related to External Prestigious Professions and Financial Welfare" (0.88), and "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.86). This factor was marked as a forced selection made under pressure from their own needs including even antisocial personal ones (development, self-affirmation) and financial benefits.

The second factor (21.16%) was described through such variables as "Internalization of the Aim" (0.93), "Internal Conception – I" (0.80), "External Conception – I" (0.74), and "Internal Processes" (0.71). The content of this factor had the almost complete coincidence of the concept internalization of the aim with the situation when the achievement of the aim of valuable features of others was understood as self-realization and satisfaction of their activity. The content of this factor was clarified with the variables such as "Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)" (0.29) and "Motives Related to External Prestigious Professions and Financial Welfare" (0.26).

The third factor (14.45%) described a desire of servicemen to fill their personal life with a positive experience at the expense of their being a part of the interesting surroundings. Thus, usually dependent personalities had difficulties with the process of finding satisfaction inside themselves. To experience positive emotions, they needed external stimulation. The positive pole of the third factor was determined with “Need of Interesting and Socially Beneficial Work” (0.93). Definite clarifications were made with the variable such as “Internal Processes” (0.28). The reversed pole was marked with variables such as “Need of Obtaining the Recognition by Other People” (0.87). The clarification was made with the variable such as “Motives Related to External Prestigious Professions and Financial Welfare” (-0.30).

The last three factors had almost identical values – 10.27%, 9.49%, and 8.93% accordingly. The fourth factor described the specific attitude to the process of professional activity. This factor did not bring any satisfaction. Servicemen had to perform professional activities to get financial benefits (which was their main aim). This factor was formed with variables such as “Need of High Salary and Financial Benefits” (0.93). The content was clarified with a variable such as “Dependent Profession Selection” (0.31). The reversed pole was marked with a variable such as “Internal Processes” (-0.46).

The fifth factor was marked with a variables such as “Need of High Working Conditions and Comfortable Surrounding” (0.96). It was clarified with variables such as “Internal Processes” (0.24). The reversed pole was weakly marked with “Need of Obtaining the Recognition by Other People” (-0.28) and “Internal Conception – I” (-0.26). This factor was described as a desire to get comfort and hedonism.

The sixth factor (8.93%) was developed with variables such as “Need of Accurate Structurization of Work and Feedback” (0.98). The content of this factor was clarified with the variables such as “Internal Processes” (0.20) and “Antisocial Motives (attitude to the profession was as if it was a way to satisfy own antisocial needs)” (-0.24). This factor was described as a desire to avoid problems related to their activity, desire to obtain easy work.

Thus, the results of the conducted research gave the possibility to determine a range of peculiarities of the structure of motivation of servicemen of dependent type. Firstly, it provided the relations of dependency where personal development and well-being depended on the realization of their antisocial needs. Secondly, their activity was directed to the fulfillment of their interesting internal emotional life at the expense of their belonging to the interesting surroundings. Thirdly, their motivation was directed to the organization of the professional activity which did not disturb their internal emotional life, did not bring any troubles, and provided them with comfortable conditions.

DISCUSSION

In this research there were determined 6 types of professional motivation with various levels of effectiveness of professional activity (the list was provided by the indicators of their effectiveness): 1) motivated with a desire of public service, 2) stagnated, 3) prosocial, 4) adventures-centered, 5) deficient, 6) dependent [17]. Despite similar typologies, there was a broad list of the types of negative motivation. Moreover, there were determined the types such as stagnated, adventures-centered, deficient, and dependent. However, the stagnated type occupied medium step between highly effective and medium effective types but in the developed typology it was included in the type with negative motivation. It was connected with the fact that servicemen who were not satisfied with their profession did not prolong their contract. Thus, the research by Harrington, Bean, Pintello, and Mathews (2001) distinguished that the personnel of military sphere were more likely to change their work if they were emotionally exhausted, had a lower level of internal satisfaction with their work, satisfaction with their salary as well as possibilities to be promoted at service.

Distinguished types, their descriptions, and structure of motivation did not contradict the regulations related to the relations between public service motivation (PSM) with effective work and its average self-efficiency. In the conclusion, there were added existing relations of autonomous and controlled motivation with productive activity. The received results would be used for further development of those regulations.

By the results presented in the works by Miao, Eva, Newman, and Schwarz [18]; van der Voet, Steijn, and Kuipers [19], Breaugh J, Ritz A, and Alfes K [9] the public service motivation (PSM) was considered as one connected with a high level of effectiveness with a desire to be developed, to get satisfaction with their work, with a decrease of task complications up to the level which correlated with their ability to live with their values and believes. However, the received structural motivation of servicemen related to PSM gave the possibility to determine existing mechanisms of self-motivation of those who had PSM which connected and described those earlier distinguished regulations. Thus, firstly, there is the structural motivation was conducted through the process of connection of algorithmization of their work (easiness and generalization) and satisfaction with work. Secondly, the structure of motivation of this type of satisfaction with main needs was performed not only at the expense of chosen profession but also by its content. It could prevent corruption – situations when the end justified the means and when their priorities were set above the priorities of others. Personal and professional development, obtaining the social status also happened by the content and aims of professional law enforcement officers. Thus, namely the content of the profession which was connected with public service provided the limits of development and the search of resources which made it impossible to refuse this way of development. Such a structure of motivation supported self-realization and a high level of activity.

The structure of motivation of the stagnated type gave the possibility to broaden the view concerning average relations of PSM and productivity of activity with the psychological mechanism of self-efficiency. Thus, Schott, Neumann, Baertschi, and Ritz [10] pointed out that policemen who did not get any feedback lost their confidence in their ability to realize the motives of PSM. Because of the feeling of disability (for example, when individuals who committed crimes are set free in the courtroom) PSM is considered to be a general type of motivation related to work with limited behavioral consequences. Other than that, the received data related to the structure of motivation of the stagnated type proved the necessity of paying attention to another side of such averaging. When previous achievements which reflected on self-perception and sometimes even ruined it (for example, formed inadequately overstated self-estimation) prevented them from development and were potentially dangerous for law enforcement officers' productivity.

The research by Schott, Neumann, Baertschi, and Ritz [10] also proved that prosocial motivation differed from PSM with their orientation on the individuals and groups which were involved in the direct contact, or on the organization-employer. It lived on the feedback of those who were helped with senses of gratitude as well as with emotional appeal. Presented research gave the possibility to form the conclusion that those peculiarities of motivation had their specific reflection and the structure of motivation the prosocial type differed from the type with PSM with a lower level of subordinate structure of motivation and a higher level of concreteness of motivational factors. The structure of motivation of servicemen of the prosocial type had an almost identical value of factors which described the importance of being useful in professional activity, being good at breadwinning their families, being a good colleague in service. According to the content of factors, servicemen of the prosocial type were mostly orientated on the ability not to fail their families but not on abstracted social benefit. The protection of the motherland was for them a way of overcoming their fears, etc. Such structure developed a complicated process of selection and decision-making when the situation dealt with the interests of several sides which were important for servicemen of the NGU.

It was quite interesting to compare the received results related to the adventure-centered type with the data by Lyk-Jensen and Glad [5] who determined that servicemen who were motivated with a desire to get adventures finished only secondary school and, usually, served only one year contract. The received structure gave the possibility to conclude that servicemen of this type were not self-sufficient and thus, they required the surrounding which made their lives eventful. They did not want to change by the requirements of the profession, instead, by any means (manipulations), they wanted to join the process of performing those aspects of professional activity which gave the possibility to satisfy their desire to work.

American researchers figured out that military service was mostly joint by Afro-Americans, indigenous Latin Americans, and rarely those people who had good school grades, planned to enter college, and whose parents had higher education [2]. This tendency was strengthened after the "Desert Storm". Thus, the candidate for lower positions who entered the military service was motivated with salary (benefits), was married, had basic education, and did not get secondary education [4]. However, Asoni, Gilli, Gilli, and Sanandaji [20] denied the theses that the American Army was hired by the Army of poor people which was used by the critics of the US external policy. They determined that despite generally accepted ideas American servicemen did not recruit primarily people with socially and economically dysfunctional layers of society. Technological, tactical, operational, and doctrinal changes led to the changes in personnel demand. As a result, according to various indicators such as family income, family wellbeing, as well as cognitive abilities servicemen on average, had identical or better indicators as civilians had. In this research the deficient type included a quarter of respondents, and, at first sight, the problem was not in the fact that they had lower education and were less confident in their abilities and skills, the problem was in the fact that even having a desire to work, to put huge efforts, and to change their motivation was weakly connected with direct content of military profession. For them, this profession was a kind of means but not an independent value.

Thus, the military profession could be substituted with any other profession which was more prospective having identical requirements for the candidates. Accordingly, they wasted efforts in the process of acquiring a profession, were not confident that servicemen would not substitute their military profession as soon as possible with the profession which was considered to provide them with better prospects.

A range of studies which included the comparison of autonomous and controlled motivation related to work satisfaction and desire to dismiss [21] being exhausted and belonging [22], being at the workplace [7], having the feeling of organizational support, positive worries, and level of communication [8].

The conducted research demonstrated that the type with the lowest level of activity effectiveness had features of dependent and controlled motivation.

The analysis of the structure of its motivation showed that profession selection and realization of the aims of professional activity of the representatives of that group were dependent on the satisfaction of personal including antisocial requirements.

They wanted to fulfill their internal lives with positive worries at the expense of external stimulation, so-called "belonging to the interesting surrounding".

The process of professional activity did not provide them with satisfaction, however, they had to deal with it to get financial benefits.

Their motivation was directed to the organization of the professional activity which did not disturb their internal emotional lives, did not bring them problems, and provided them with a comfortable environment.

CONCLUSION

The conducted research proved that motivation of servicemen could be appropriate to learn connecting the theory of public service motivation (PSM) with self-determination theory (SDT). That gave the possibility to describe not only the distinguished types of professional motivation of servicemen but also specific peculiarities including self-motivation of the representatives of this type which was run with public service motivation; "rest on laurels" of the stagnated type; problems with selection of prosocial type for whom profession, motherland, and friends had big value; tendency to manipulate to get an access to desired aspects of professional activity of the type which was adventure-centered; absence of sincere interest in military profession of the deficient type which could substitute it with more prospective work having identical requirements for the candidates; attitude to profession as if it was a kind of means of satisfaction of demands of their emotional lives of the dependent type.

The prospects of further studies

It was distinguished to be prospective to develop or standardize existing methods which correlated with the theory of public service motivation (PSM) and self-determination theory (SDT) to estimate the motivation of servicemen.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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ORIGINAL ARTICLES

Clinical significance of p16INK4a and p53 and their involvement in penile cancer development. A literature review

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Abstract: *(1) Background: Human papillomavirus (HPV) E6 and E7 oncoproteins are vital for developing HPV induced penile carcinoma. The viral oncoproteins play a central role in oncogenesis by interacting with several cellular regulatory proteins, such as p16^{INK4a} and p53. Many studies suggest that these proteins showed clinical utility in predicting nodal disease, cancer specific survival, overall survival and even tumor grade. Understanding the molecular mechanism involved in the carcinogenesis of penile cancer could offer biomarkers for disease progression, treatment response and potential targeted therapies; (2) Methods: This paper is a prospective study on a group of 100 patients who underwent prostate surgery during 2013 and 2014 in the Urology Clinic of "Carol Davila" Central Military Emergency University Hospital Bucharest. They were tested for HPV by PCR and IHC (p16) methods; (3) Results: 11 cases (22%) of HPV were found in the cluster of patients tested. PCR and P16 were the HPV diagnostic tests used. In order to determine the consistency of the 2 tests, the Cohen's kappa coefficient was used at a p level < 0.05. The PCR method had a sensitivity of 81.8% and a specificity of 94.9%. The P16 method had a sensitivity of 63.6% and a specificity of 89.7%.*

Keywords: *penile cancer, p53, p16^{INK4a}, prognostic, potential therapies*

INTRODUCTION

Penile cancer accounts for less than 1% of cancers in men in developed countries, being more common in less developed areas of the world such as parts of Africa, Asia, and South America (up to 20%). Beingusually an elderly disease, the mean age of diagnosis for penile carcinoma is 60 years old. Early diagnosis is of utmost importance, this disease leading to a devastating disfigurement and advanced disease being associated with poor outcomes. Two major etiologies have been proposed for the development of penile carcinoma: chronic inflammation secondary to chronic balanitis due to phimosis and HPV-induced disease. In literature,basaloid and warty subtypes are almost always associated with HPV. Studies have demonstrated that the most common type of HPV infection is HPV 16 and 18, which occur in 60–75% of in situ and invasive tumors. In terms of HPV-induced penile carcinoma, E6 and E7 viral oncoproteins are essential for developing HPV-induced penile cancer, disrupting various cell-cycle checkpoints and apoptosis. Viral oncoproteins release the cell from p53 control, thus surviving the S phase of cellular cycle with DNA damage. Furthermore, E7 overexpression is followed by the overexpression of cyclin-dependent kinase inhibitor p16INK4a, which can be used as a biomarker for HPV-transformed epithelial cells. Studies have shown that p16INK4a overexpression is only seen in high-risk HPV genotypes. Mannweiler et al. found that in the absence of HPV infection, penile carcinoma still presented p53 mutations, indicating high proliferative activity of the cells, thus disease progression [1-4].

Researching the role that p16 and p53 have in the development of penile cancer could give somemuch-needed biomarkers for disease progression and treatment response for this aggressive disease. Furthermore, understanding the molecular mechanisms involved in the pathogenesis of

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penile carcinoma can offer potential targeted therapies [5].

MATERIALS AND METHODS

Markers in penile carcinoma

Role of p53 in penile carcinoma

Encoded by TP53 gene located on chromosome 17, which is the most frequent mutated gene in human cancer, p53 is a nuclear transcription factor, having a pro-apoptotic function. Named "guardian of the genome", p53 transactivates several genes implicated in the induction of cell cycle arrest and apoptosis [6,7]. Under normal circumstances, the levels of p53 are extremely low. However, in the presence of DNA damage, p53 accumulates in the nucleus through post-translational modifications like phosphorylation or acetylation.

Depending on the extent of DNA damage, p53 can either stop the cell cycle, allowing cells to repair the DNA and then letting the cells re-enter the normal cell cycle, or if the DNA damage is beyond repair it will exert its pro-apoptotic functions inhibiting the transfer of damaged DNA to daughter cells. Through these actions p53 maintains genomic integrity.

Certain pathogens, including HPV, can affect p53 protein. HPV interacts with p53 through E6, marking the protein for proteasomal degradation by transferring ubiquitin peptides from E6AP complex (E6 binds to E6-associated protein) to p53 tumor suppressor protein. Furthermore, by biding itself to pRb, which is a cell cycle regulator, and inactivating it, E7 complements the action of E6 oncoprotein. Consequently, by releasing the cell from p53 control, damaged DNA will be transferred to daughter cells [8].

As stated above, several studies have found that HPV-negative penile squamous cell carcinomas express p53 linked to TP53 mutations [4,9,10]. Although TP53 was initially described as a tumor suppressor gene disabled by loss-of-function mutations, recently it was discovered that it harbors oncogenic potential [11]. Multiple mutations of TP53 have been discovered over the years. R175H, which is the most common mutation, leads to cell cycle progression and initiation of genes that promote cell survival.

Yoshikawa et al. found that, when overexpressed, R248Q mutation offer cells an invasive ability [12]. Other mutations found are R273C, implicated in proliferation and inhibition of apoptosis, and R282, associated with higher activity of drug-metabolizing enzymes [13,14]. Even though newly pathways and mutations are being discovered, HPV-negative penile carcinogenesis is still poorly understood [15].

Role of p16 in penile carcinoma

Encoded by CDKN2A gene located on chromosome 9 (9p21.3), p16 (also known as p16^{INK4a}) is an inhibitor of cyclin-dependent kinases (CDK) that impede cell division by slowing the progression of the cell cycle from the G1 phase to the S phase, therefore acting as a tumor suppressor. p16^{INK4a} induces cell cycle arrest and prevents cell division by inhibition of CDK4 and CDK6 and inhibition of CDK-mediated phosphorylation ofRb (retinoblastoma) protein, whose expression increases as the cell cycle progresses. Phosphorylated (inactivated) pRb dissociates from the transcription factor E2F1, allowing it to enter the nucleus, thus promoting the transcription of target genes that are crucial for transition from G1 to S phase. Through its actions, p16INK4a maintains pRb bound to E2F, denying the cell progression to S phase, thus preventing excessive cell growth.

The HPV-E7 protein, which is an oncogenic protein of HPV, can bind and inactivate Rb protein causing carcinogenesis with promotion of cell-cycle progression. Therefore,high-risk HPV positive penile carcinomas have increased cell proliferation with p16INK4a overexpression, allowing p16 to be used as a surrogate marker for HPV-E7 expression. Furthermore, it has been observed that in HPV negative penile carcinomas p16 overexpression is absent, strengthening the idea that p16INK4a can be used as a biomarker for HPV related cancers [16,17].

DISCUSSION

Several papers studied the presence of HPV and its viral oncoproteins as a prognostic factor for penile carcinoma. A mutated TP53 gene usually leads to a mutated P53 protein, rather than the absence of it. The regulatory protein MDM2 (the human homologue to the murine double minute 2 oncogene) controls the intracellular p53 concentration through a negative feedback loop. Mutant p53 protein cannot bind to MDM2, thus leading to an overexpression of p53. Studies have shown that p53 overexpression is an independent predictor of lymph node metastases. In their paper, Lopes et al. observed that patients who were HPV and p53 positive had a worse overall survival than those negative for p53, with a mean follow-up of 88.7 months. Furthermore, p53 immunoreactivity and p53 grade were associated with a higher incidence of lymph node metastasis [18-20]. Martins et al. stated in their study that p53 overexpression was associated with regional lymphatic spread, recurrence, and disease specific mortality [21]. The same results regarding overall survival of patients with HPV-induced penile cancer and positive for p53 expression were found by many authors. In their paper, Prapiska et al. observed that the survival rate over 36 months in the p53 positive group was 18%, compared with a survival rate of 60% in the p53 negative group [22]. Strengthening the idea that p53 overexpression is associated with poor survival, Gunia et al. found that the 5-year cancer-specific survival was 84% in p53-negative and 51% in p53-positive PC [23].

The role of p53 was studied in several other aspects regarding its involvement in penile carcinoma. For example, Marinescu et al. conducted a research on the association of p53 and the grading of penile carcinoma specimens. They concluded that all the poorly differentiated penile cancer cases had a positive expression of p53 oncoprotein, no matter the tumor stage. Moreover, as the tumor grading improves the expression of p53 decreases, thus, p53 was found in 91.2% of the moderately-differentiated tumors a and 72.2% of the well-differentiated tumors [24]. As stated above, in HPV-induced penile carcinoma, TP53 tumor suppressor function is suppressed by E6 oncoprotein, while simultaneous activation of pRB by E7 initiates epithelial neoplasia. Multiple studies in literature found that TP53 can have mutations in the absence of HPV infection. In HPV-induced carcinogenesis, it has been observed that TP53 function can be restored via radiation and chemotherapy. Furthermore, chemotherapy and radiation therapy resistance are mediated by permanent inactivation of TP53, namely, by mutations of the TP53 coding region, since the effectiveness chemotherapeutic agents, such as 5-fluorouracil, doxorubicin, bleomycin, and cisplatin, and radiation depend partially on p53-mediated responses. This can explain the worse prognosis regarding the overall survival of patients with HPV-negative penile carcinoma after radiation and chemotherapy [15].

Zargar-Shoshtari et al. conducted a study on the clinical significance of p53 and p16 status of patients with penile carcinoma. They found that the cancer specific survival (CSS) and overall survival rate (OS) were improved in p16-positive patients. Furthermore, they observed an association with p53 overexpression and nodal disease in p16 negative patients. They also concluded in their paper that patients who had positive lymph nodes and a double p16 and p53 negative status had a significantly worse CSS. It is known that E6 oncoprotein activation leads to a reduction in host cell p53 expression, thus cells expressing only E7 could be positive for p53, whereas cells expressing both E6 and E7 are negative for p53. Because multiple molecular and genetic pathways can lead to an overexpression of p16 and p53, it is possible to detect both in the same cancer cell [25].

It has been established that pRb is a target for E7 oncoprotein leading to an overexpression of $p16^{INK4a}$. Because of this, p16INK4a can be used as a marker for HPV infection. Mannweiler et al. reported in their study that lesions which were p16 negative had very low rates of HPV detection. Moreover, they stated that all p16-negative cases were positive for p53 nuclear expression and that out of 72 patients with p16 overexpression none was positive for p53(4). Senba et al. observed that in the HPV-positive penile carcinoma cases, $p16^{INK4a}$ was positive in 61.5%, compared to 25% in the HPV-negative group, strengthening the idea that p16 can be used as a surrogate marker for HPV infection [26]. Several other studies suggested that $p16^{INK4a}$ positivity in penile carcinoma is strongly related to the presence of high-risk oncogenic HPV genotype and that negativity can indicate an infection with a low-risk genotype or the absence of HPV infection [27-29]. In their paper, Steinestel et al. found that the sensitivity and specificity of $p16^{INK4a}$ staining to predict the presence of high-risk HPV was 100% and 57%, respectively [30].

Regarding prognostic significance of p16INK4a expression in penile carcinoma, a meta-analysis on 323 cases was performed by Zhang et al. They concluded that p16INK4a expression is independently associated with improved CSS for patients with penile carcinoma [31]. Another study found that high-risk HPV status and p16 positivity, independent of histological tumor grade, indicated a less aggressive local behavior [29]. Sand et al. observed that patients with HPV induced penile cancer with negative p16 staining had a worse disease specific survival than men with p16-positive penile carcinoma [32].

In their paper, Martínez-Bailón et al. declared that all-cancers with $p16^{INK4a}$ overexpression were positive for HPV DNA, but, interestingly, not all HPV-positive patients were positive for $p16^{INK4a}$. Several papers in literature suggested that the detection of HPV DNA is not sufficient for the attribution of HPV infection to the etiology of the cancer, transcriptional activity or the molecular signature of HPV-induced carcinogenesis needed to be proven [33-35].

The report from the International Society of Urological Pathology (ISUP) consultation conference on molecular pathology of urogenital cancers recommended the use of p16 staining in cases in which the histological features of the squamous cell carcinoma are not classical for HPV-induced cancers, especially in poorly differentiated tumors. The consensus was that p16 can be used as a surrogate of polymerase chain reaction for the identification of high-risk HPV genotypes [36].

As seen so far, $p16^{INK4a}$ is overexpressed as an early consequence of high-risk HPV-mediated carcinogenesis. Furthermore, it has been noticed that humoral and cellular immune responses occur spontaneously against p16. This observation is the basis for creating a therapeutic vaccine using a synthetic p16 peptide to prevent the outgrowth or destroy the affected cells. Two trials tested the vaccine to asses safety and immune response (ClinicalTrials.gov Identifier: NCT01462838 and ClinicalTrials.gov Identifier: NCT01462838). The conclusion was that the vaccine was safe and that it showed strong immune responses against p16, strongly induced T cell CD4+ and humoral immune responses in all patients. Moreover, it is safe to use in combination with standard cisplatin-based chemotherapy with excellent safety and tolerability [37-40].

CONCLUSION

More common in less developed countries of the world and usually an elderly disease, penile carcinoma leads to important disfigurement and poor outcome if not diagnosed in its early stages. It is well-known that HPV induced penile cancer patients have a better outcome than HPV-negative ones. Furthermore, HPV-induced penile carcinoma with p16 positive staining have better overall survival. These findings point to the possible clinical value of HPV and p16 testing when planning the most optimal management and follow-up strategy. The gold standard for HPV detection is polymerase chain reaction, but this technique is expensive and not always available, thus, since p16 protein was found to be associated with the presence of high-risk HPV, it can be used as an alternative marker. Several studies concluded that p53 overexpression was associated with poor survival and an independent predictor of lymph node metastases. Moreover, p53 positivity was strongly associated with poorly differentiated penile carcinomas. Early results of the p16 vaccine showed that it is safe and that it can trigger a good immune response, but further studies on larger patient groups are needed.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.
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Authors' contribution
ADS and DRM collected, analyzed, and interpreted the patient data. DM, RAA, DLI, DOC, RSC and AC had substantial contribution to the conception of the work and interpretation of data; also, they drafted the manuscript and contributed substantially to writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies, is in accordance with the tenets of the Helsinki Declaration, and has been approved by the review board of "Carol Davila" Central Military Emergency University Hospital Bucharest, Romania.

Patient consent for publication
Not applicable.

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ORIGINAL ARTICLES

Medical and chemical efficacy of respiratory physiotherapy and Remdesivir in patients with COVID-19 pneumonia: A systematic review and meta-analysis

Alireza Bahmani[1], Zahra Mollashahi[2], Negar Shahkarami[3], Elahe Delavar[4], Hooman Esfahani[5]

Abstract: *Evaluated efficacy of Respiratory Physiotherapy and Remdesivir on patients with COVID-19 pneumonia. In current systematic review and meta-analysis study, articles published January 2019 to December 1, 2021 were reviewed in the databases of PubMed, Scopus, Web of Science, and EBSCO. Risk ratio and mean differences with 95% confidence interval (CI), fixed effect model and Mantel–Haenszel or Inverse-variance formula were calculated. The Meta analysis have been evaluated with the statistical software Stata/MP v.16 (The fastest version of Stata). Mean differences of PaO2/FiO2 ratio at 6h after chest Respiratory Physiotherapy was (MD, 66 mmHg 95 % CI 64.71 mmHg, 67.28 mmHg; p=0.0007). Risk ratio of recovery rate between experimental and control group was 0.20 (RR, 0.20 95 % CI 0.15, 0.25) with high heterogeneity (I2 =78.84%; p=0.00). Risk ratio of mortality rate between experimental and control group was -0.34 (RR, -0.34 95 % CI -0.65, -0.03) with low heterogeneity (I2<0%; p=0.51). Based on the findings of meta-analysis, Respiratory Physiotherapy can play an effective role in respiratory therapy and rehabilitation of patients admitted to the ICU with COVID-19. A meta-analysis showed that treatment with Remdesivir could increase the recovery rate, especially in the early days of COVID-19; also reduces the mortality rate.*

Keywords: *COVID-19, respiratory physiotherapy, Remdesivir*

INTRODUCTION

Coronaviruses are a large family of viruses. From this collection, seven species transmitted to humans have been discovered so far, which cause diseases such as colds in humans [1]. Coronaviruses often attack the respiratory tract and sometimes show symptoms in the intestines and stomach [2,3]. Coronaviruses usually first infect the mucous membranes of the respiratory tract in the throat and nose, causing symptoms similar to the common cold [4]. Coronaviruses usually first infect the mucous membranes of the respiratory tract in the throat and nose, causing symptoms similar to the common cold [5]. Sometimes can cause more serious illnesses such as bronchitis and exacerbation of asthma and chronic bronchitis in adults and even lung infection (pneumonia) in adults, the elderly and people with weakened immune systems [6]. Covid-19 is an airway infection caused by a mutated version of the coronavirus and was first reported in Wuhan, China in December 2019 [7]. Initially, the mortality rate of patients with Covid-19 was announced at 3 to 5 percent, but recent reports have shown a reduction in mortality [8]. Due to the fact that this disease is very new and emerging, so scientific and documented findings in this area are very few. Therefore, considering the secondary effects that this disease has on the pulmonary system and affects the pulmonary system, so specialized findings on the improvement of pulmonary patients and their conclusion are very helpful in advancing the goals [9]. Physiotherapists, as a member of the multiple treatment team in the intensive care unit, play an effective role in reducing respiratory complications and preventing weakness due to hospitalization in the intensive care unit [10]. Since one of the most important organs involved in Covid-19 disease is

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the respiratory system.

Therefore, the role of physiotherapists, especially in the field of respiratory physiotherapy, along with other health professionals, plays an important role in the treatment and care of this group of patients and overcoming the respiratory symptoms of patients with Covid-19.

In addition to medical treatment, rehabilitation techniques and pulmonary physiotherapy are essential for the treatment and improvement of patients' respiratory function. These measures are also recommended as self-care training and breathing exercises for quarantine patients at home [11]. Pulmonary rehabilitation has a variety of techniques, including breathing exercises and chest physiotherapy that help clear the airways, create a deep breathing pattern, increase arterial oxygen saturation and improve pulmonary volume, and increase voluntary expiratory volume in the first second [12]. Due to the increase in hospitalization costs, reducing the risk of nosocomial infections, reducing the number of hospitalizations, pulmonary rehabilitation programs for empowerment and also improving patients' self-care have been emphasized more than before; Therefore, the main role of physiotherapists to start patients' pulmonary rehabilitation programs at the time of hospitalization and the emphasis on continuing and following it at home becomes more apparent. Therefore, the aim of current study was evaluated efficacy of Respiratory Physiotherapy and Remdesivir on patients with COVID-19 pneumonia.

METHOD

In this systematic review study, Science direct, Google scholar, PubMed, Scopus, Web of Science, and EBSCO databases were used to search for articles. The present study is a systematic review and meta-analysis. In current study review of previous studies, the Preferred Reporting Items for Systematic Reviews (PRISMA) checklist was used to search for the studies [13].

Figure 1: Flowchart of the literature search and selection criteria

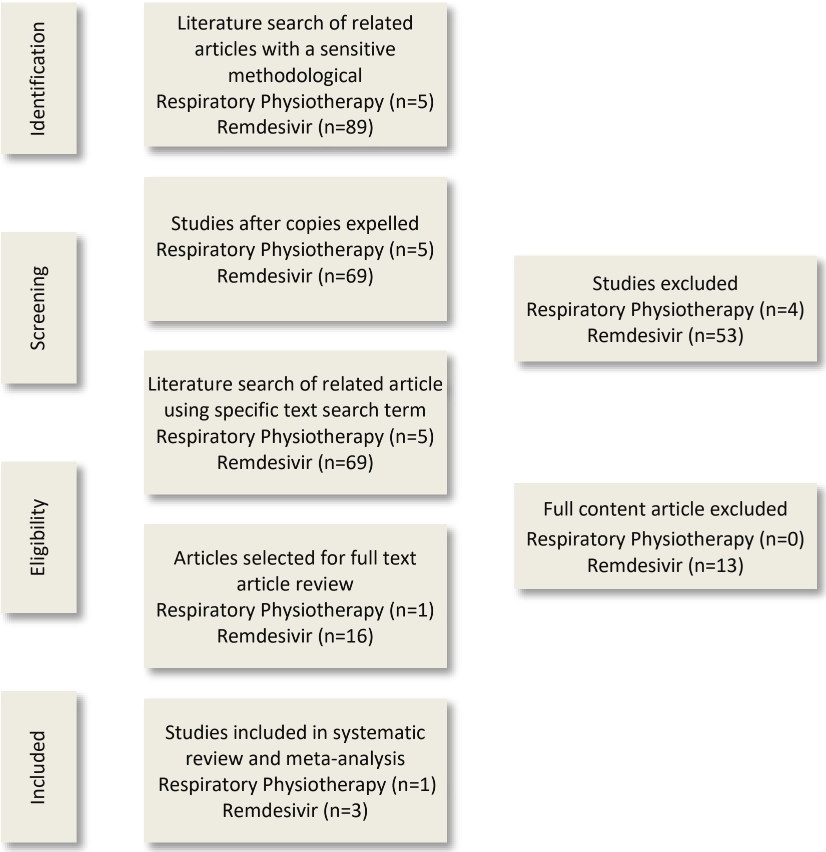
Identification
Literature search of related articles with a sensitive methodological
Respiratory Physiotherapy (n=5)
Remdesivir (n=89)
Screening
Studies after copies expelled
Respiratory Physiotherapy (n=5)
Remdesivir (n=69)
Studies excluded
Respiratory Physiotherapy (n=4)
Remdesivir (n=53)
Literature search of related article using specific text search term
Respiratory Physiotherapy (n=5)
Remdesivir (n=69)
Eligibility
Full content article excluded
Respiratory Physiotherapy (n=0)
Remdesivir (n=13)
Articles selected for full text article review
Respiratory Physiotherapy (n=1)
Remdesivir (n=16)
Included
Studies included in systematic review and meta-analysis
Respiratory Physiotherapy (n=1)
Remdesivir (n=3)

Data Extraction and analysis method

The data were extracted from the research included years, study design, number of patients, mean of age. To extract the data, two blind and independent reviewers extracted the data from the abstract and full text of the studies. Prior to screening, kappa statistics were performed to confirm the level of agreement between the reviewers. Kappa values were higher than 0.80.

Risk ratio and odds ratio with 95% confidence interval (CI), fixed effect model and Mantel–Haenszel formula; mean differences with 95% CI, fixed effect model and Inverse-variance formula were calculated. Random effects were used to deal with potential heterogeneity and I2 showed heterogeneity. I2 values above 50% signified moderate-to-high heterogeneity. The Meta analysis have been evaluated with the statistical software Stata/MP v.16.

RESULT

Respiratory physiotherapy: A total of 5 articles were found in the initial search. After removing duplicates, entry criteria for the titles were applied to the remaining 5 articles, and a summary of the remaining articles was reviewed. In this step, 4 articles were excluded. In the next, full text of 1 article were reviewed, finally one study was selected.

Remdesivir: A total of 89 articles were found in the initial search. After removing duplicates, entry criteria for the titles were applied to the remaining 69 articles, and a summary of the remaining articles was reviewed. In this step, 53 articles were excluded. In the next, full text of 16 articles were reviewed and 13 articles were excluded, finally three studies were selected.

Characteristics the studies included for evaluated efficacy of respiratory physiotherapy

One study (observational study) has been included in present article. The number of patients a total was 20 with mean age 63 years. Table 1 showed summary of studies for patient demographics, comorbidities, and clinical manifestations. Only one studies was found that evaluate efficacy of respiratory physiotherapy in COVID-19 patients [14].

Table 1: Summary of characteristics of included studies

Study. Years	Study design	Number of patients	Mean of age	Type of ventilation during RPT	ICU discharge characteristics
Battaglini et al., 2021 [14]	observational study	20	63	45% PSV 55% COT	% 5 Dead 95% Alive

PaO_2/FiO_2 ratio

Battaglini et al., 2021 [14] reported that PaO2/FiO2 ratio improved at T1 (RPT) compared to T0 (baseline) (MD, 63 mmHg 95 % CI 61.83 mmHg, 64.16 mmHg; p=0.00), the mean differences of PaO_2/FiO_2 ratio at 6h after chest RPT was (MD, 66 mmHg 95 % CI 64.71 mmHg, 67.28 mmHg; p=0.0007), this result showed PaO_2/FiO_2 ratio improved at 6h after chest RPT than baseline (Table 2).

Lung ultrasound score (LUS)

According to Battaglini et al.,2021 [14] study, the mean difference of LUS score between baseline and immediately after RPT was (MD, -4.95 % CI -4.26, -3.75; p=0.00).

Battaglini et al., 2021 [14] reported Correlation between LUS and CT scan, volume of gas and hyperaeration at CT scan between baseline, immediately after RPT and 6h after chest RPT, the result showed there was statistically significant association between LUS score and lung gas volume.

Characteristics the studies included for evaluated efficacy of Remdesivir

Three studies have been included in present article. The number of patients in experimental and control group was 1,125 with mean age 60.55 years and 799 with mean age 60 years, respectively. In all studies used 200 mg loading dose (IV), 100 mg maintenance dose from Second to Tenth day. The follow up period in two studies was 28 days and in one study was 29 days (Table 3).

Table 2: Meta-analysis of PaO_2/FiO_2 ratio

Group	Study	Mean Diff.	[95% Conf. Interval]		% Weight
Baseline – Immediately after RPT	Battaglini et al., 2021 [14]	63.000	61.838	64.162	54.92
Baseline – 6h after chest RPT	Battaglini et al., 2021 [14]	66.000	64.717	67.283	45.08

Meta-analysis summary
Fixed-effects model
Method: Inverse-variance
Test of theta = 0: z = 146.42
Test of homogeneity: Q = chi2(1) = 11.54

Number of studies = 2
Heterogeneity: I^2 (%) = 91.33
H^2 = 11.54
Prob > |z| = 0.0000
Prob > Q = 0.0007

Figure 2: The Forest plot showed mean differences of lung ultrasound score

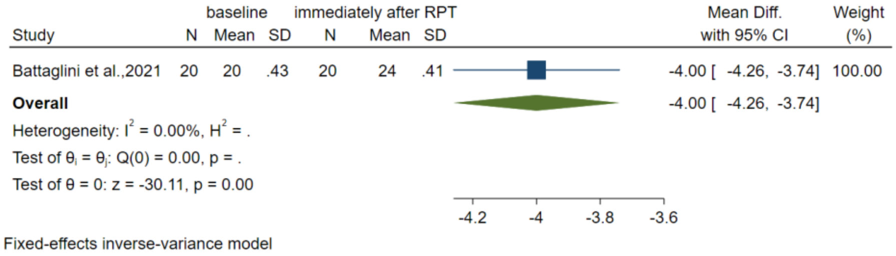

Table 3: Summary of characteristics of included studies

Study. Years	Number of patients		Mean of age		Remdesivir arm	Follow up period (day)
	Treatment	Control	Treatment	Control		
Spinner et al., 2002 [15]	384	200	58	57	200 mg loading dose (IV), 100 mg maintenance dose/2, 5 and 10 days	28
Wang et al., 2020 [16]	158	78	66	64	200 mg loading dose (IV), 100 mg maintenance dose/2-10 days	28
Beigel et al., 2020 [17]	583	521	58	59	200 mg loading dose (IV), 100 mg maintenance dose/2-10 days	29

Recovery rate

Risk ratio of recovery rate between experimental and control group was 0.20 (RR, 0.20 95 % CI 0.15, 0.25) with high heterogeneity (I2 =78.84%; p=0.00) (Figure 3). Subgroup meta-analysis showed Risk ratio of recovery rate on 7th day between experimental and control group was 0.19 (RR, 0.19 95 % CI 0.06, 0.33) with moderate heterogeneity (I2 =52.24%; p=0.12) (Figure 3).

Risk ratio of recovery rate on 14th day between experimental and control group was 0.29 (RR, 0.29 95 % CI 0.20, 0.38) with high heterogeneity (I2 =90.51%; p=0.00); Risk ratio of recovery rate on 28th day between experimental and control group was 0.13 (RR, 0.13 95 % CI 0.06, 0.20) with low heterogeneity (I2 =49.21%; p=0.14).

Remdesivir treatment significantly increased the recovery rate.

Mortality rate

Risk ratio of mortality rate between experimental and control group was -0.34 (RR, -0.34 95 % CI -0.65, -0.03) with low heterogeneity (I2<0%; p=0.51) (Figure 4).

Subgroup meta-analysis showed Risk ratio of mortality rate on 14th day between experimental and control group was -0.44 (RR, -0.44 95 % CI -0.82, -0.10) with low heterogeneity (I2<0%; p=0.52); Risk ratio of Mortality rate on 28th day between experimental and control group was 0.04 (RR, 0.04 95 % CI -0.59, 0.67) with low heterogeneity (I2<0%; p=0.67); Remdesivir treatment significantly reduction the mortality rate.

Adverse effects

Risk difference of adverse effects between experimental and control group was -0.01 (RD, -0.01 95 % CI -0.02, -0.00) with low heterogeneity (I2=27.92%; p=0.17) (Figure5).

Subgroup meta-analysis

Increased serum alanine aminotransferase

Risk difference of Serum alanine aminotransferase between experimental and control group was -0.01 (RD, -0.01 95 % CI -0.02, -0.00) with high heterogeneity (I2=79.26%; p=0.01).

Increased aspartate aminotransferase

Risk difference of aspartate aminotransferase between experimental and control group was -0.02 (RD, -0.02 95 % CI -0.03, -0.00) with low heterogeneity (I2=34.50%; p=0.22).

Increased bilirubin

Risk difference of bilirubin between experimental and control group was -0.00 (RD, -0.00 95 % CI 0.01, -0.01) with low heterogeneity (I2<0%; p=0.96).

Decreased creatinine clearance

Risk difference of Creatinine clearance between experimental and control group was -0.01 (RD, -0.01 95 % CI -0.02, 0.01) with low heterogeneity (I2=33.40%; p=0.20).

Vomiting

Risk difference of Vomiting between experimental and control group was 0.02 (RD, 0.02 95 % CI -0.02, 0.07) with low heterogeneity (I2<0%; p=0.33).

Figure 3: The Forest plot showed recovery rate on 7, 14 and 28 days

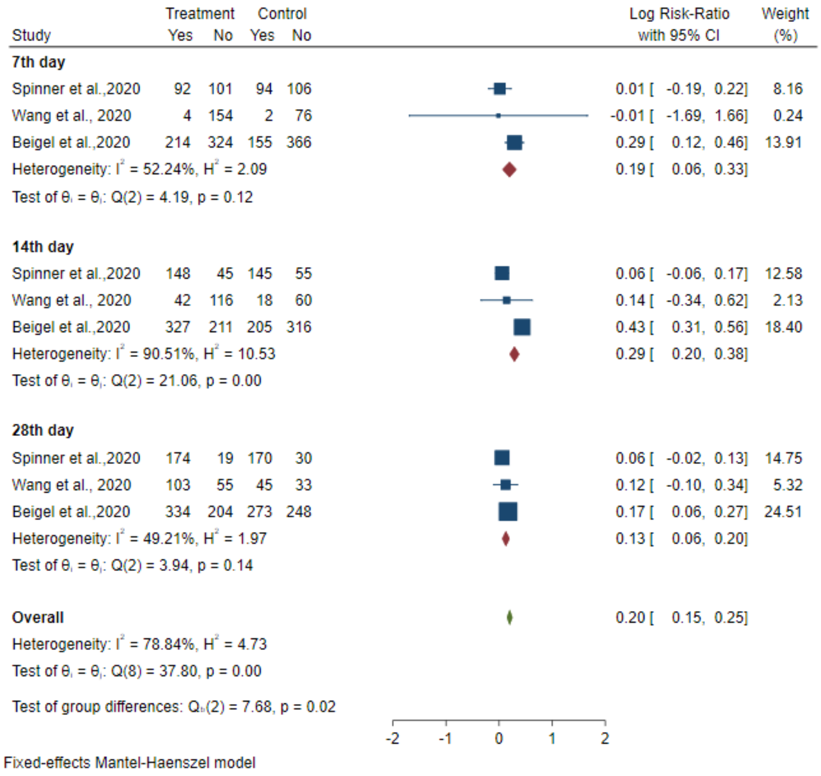

Figure 4: The Forest plot showed mortality rate on 14 and 28 days

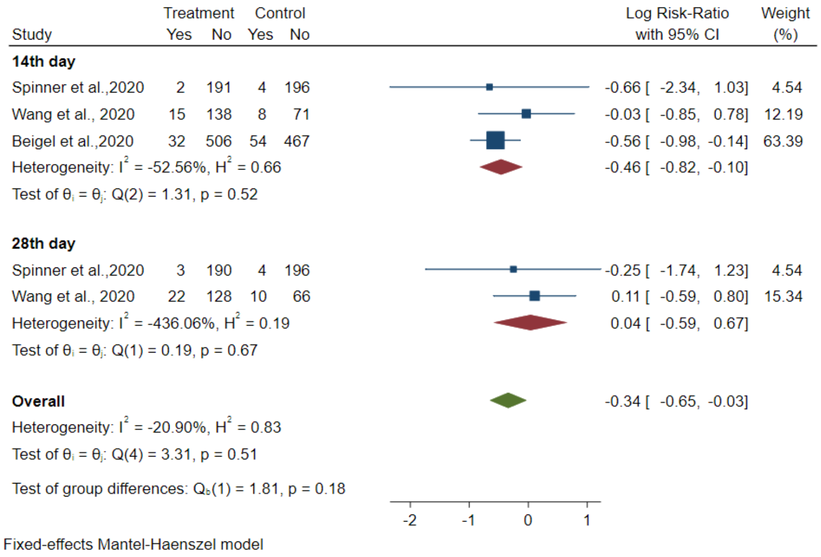

DISCUSSION

The aim of current systematic review and meta-analysis study was evaluating the efficacy of Respiratory Physiotherapy and Remdesivir on patients with COVID-19 pneumonia.

Meta-analysis showed respiratory physiotherapy improves oxygenation in critically ill COVID-19 patients. Only one study [14] was found to investigate the role of respiratory physiotherapy in patients with COVID-19. Hence, further studies are needed to emphasize the findings.

However, the findings showed that respiratory physiotherapy plays an important role in the rehabilitation of patients admitted to the ICU [18].

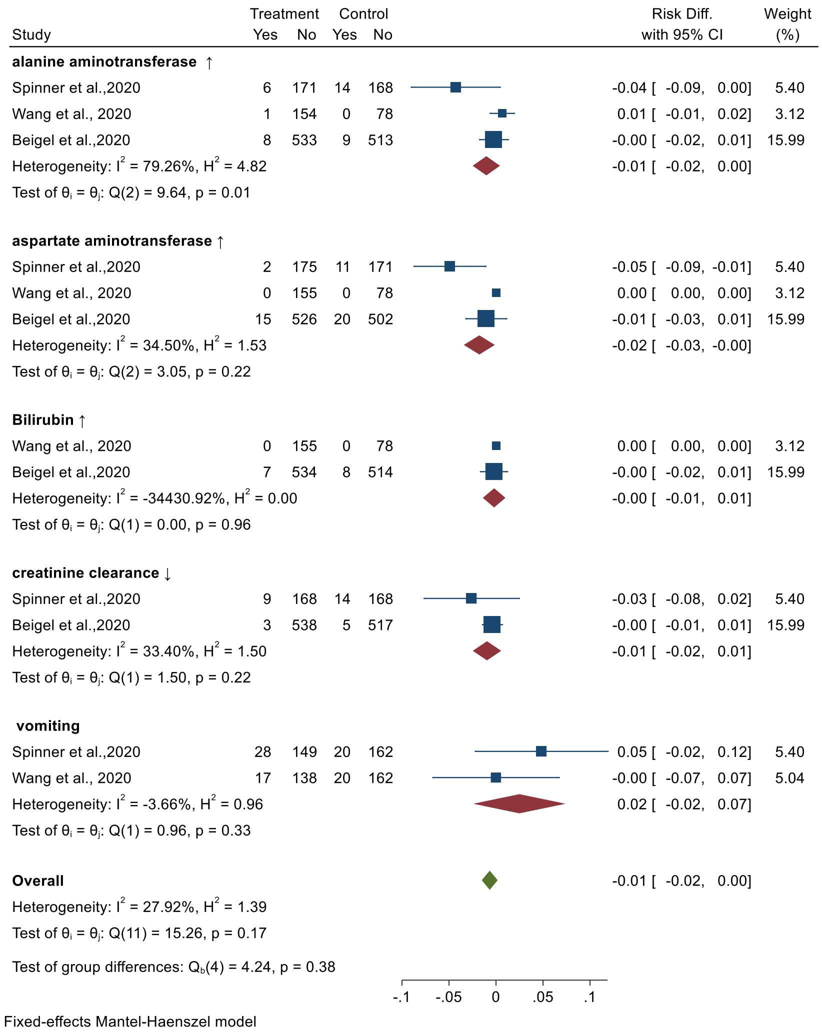

Figure 5: The Forest plot showed adverse effects

A study did not find a role for respiratory physiotherapy in patients with different respiratory patterns. As the meta-analysis shows, the LUS score improved, but these findings were not statistically significant. LUS is a diagnostic tool in the prognosis of COVID-19 [19]. To confirm this evidence, studies with larger sample sizes are needed to investigate the role of respiratory physiotherapy in patients with COVID-19 [14]. Limitations of the present study on the role of respiratory physiotherapy are: the existence of only one study, small sample size, and observational study (retrospective), RCT studies were not found, the study was conducted at the peak of COVID-19 epidemic, no comparison was found with the control group to evaluate the effectiveness of respiratory physiotherapy. Therefore, summarizing the practical and effective techniques of pulmonary physiotherapy is valuable to increase the awareness of all audiences, including physiotherapists, nurses and patient caregivers. Most review studies indicate that pulmonary physiotherapy is a safe and effective intervention, especially in stable conditions, to clear the lungs and improve the patient's respiratory function. Of course, there are a number of precautions and time limits for performing these techniques that should be considered. According to the Guideline published in March 2020 for physiotherapists in the treatment of patients with COVID-19, cardiopulmonary physiotherapy focuses on the treatment and rehabilitation of acute and chronic respiratory conditions in patients and aims to improve the physical fitness of individuals seeking the disease. Accordingly, physiotherapy can play an effective role in respiratory therapy and physical rehabilitation of patients with COVID-19.

To evaluated effectiveness of remdesivir, only three studies were found to be included in the meta-analysis. In these studies, the role of remdesivir on days 7, 14 and 28 in patients with COVID-19 was investigated. Meta-analysis showed that the effectiveness of remdesivir in these patients increases the recovery rate and reduces the mortality rate. Studies show that Remdesivir is more effective on the seventh day in the early stages of the disease, and patients who do not require mechanical ventilation respond better to Remdesivir [20]. According to the guidelines of the national institute of health, remdesivir works best in hospitalized patients with COVID-19 who require less supplemental oxygen [21]. However, further studies are needed to confirm these findings and provide sufficient and stronger evidence, as there is insufficient evidence to confirm the effectiveness of remdesivir in mild to moderate patients, and further studies are needed. Meta-analysis showed that the mortality rate on the 14th day of hospitalization was lower than on the 28th day, further studies are needed to confirm these findings [15,22].

The findings of this study are consistent with the results of previous meta-analysis studies [23,24]. However, the experimental studies reviewed in the current and previous meta-analysis were very few and, in most studies, the same, more RCT studies are needed in this area. Limitations of the study on the effectiveness of remdesivir in the treatment of patients with COVID-19 include: High heterogeneity in study results, time difference between results, age difference of patients, and low number of studies, low sample size and risk of bias not evaluated.

CONCLUSIONS

Meta-analysis showed that respiratory physiotherapy improves oxygen delivery in ICU patients with COVID-19, especially in less severe patients. It is recommended to review various respiratory physiotherapy techniques and their effectiveness in future studies. All respiratory physiotherapy interventions should be carefully considered to reach a complete conclusion in this regard. Unfortunately, so far only one study has been performed to evaluate the effectiveness of respiratory physiotherapy as a retrospective observational study (without control group); RCT and intervention studies are needed to confirm the effectiveness of respiratory physiotherapy techniques in patients with COVID-19. Based on the findings of meta-analysis, respiratory physiotherapy can play an effective role in respiratory therapy and rehabilitation of patients admitted to the ICU with COVID-19.

In the present study, a meta-analysis showed that treatment with Remdesivir could increase the recovery rate, especially in the early days of COVID-19; also reduces the mortality rate, although there is insufficient evidence in this regard because high heterogeneity was found between studies, so the reduction in mortality has not been proven. The efficacy of Remdesivir in patients with severe COVID-19 who do not require mechanical ventilation has been clearly demonstrated.

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ORIGINAL ARTICLES

New pathophysiological aspects in chronic venous disease

Nicuța Manolache[1], Gabriela Stoleriu[2], Daciana E. Brănişteanu[3], Silvia Robu[1], Camelia Diaconu[1], Daniel O. Costache[4]

Abstract: *Chronic venous disease has been pathology of great interest over time. With its large prevalence among the world population (10-33% adult woman and 10-20% of men) through profound socio-professional implications that affect the quality of life of patients, chronic venous disease is a real public health problem, being an important cause of morbidity and disability, a permanent area of research in the medical-pharmaceutical field. Special attention is now given to identifying the mechanisms and stages of disease development. From studies that have highlighted the importance of venous hypertension as an essential element in the development of the disease, to this day it has come up to demonstrating the fundamental role of the venous endothelium and glycocalyx in triggering and progressing of chronic venous disease. Biochemical, immunohistochemical, and functional investigations have allowed the evaluation of changes that occur in cellular metabolism and evidence of some immune-vascular factors responsible for vascular alterations in chronic venous disease: specialized receptors, adhesion molecules, cytokines, matrix and metalloproteinases.*

The analysis of the pathophysiology of chronic venous disease is still a vast area open to scientific research.

Keywords: *chronic venous disease, venous insufficiency, endothelium, glycocallix, matrix metalloproteinases*

INTRODUCTION

Chronic venous disease (CVD) is being defined as a long term decompensation of the venous circulation, with the occurrence of venectasia, reticular veins and hydrostatic varicose (secondary to venous stasis), stasis in the lymphatic system and various skin changes: vesperal or permanent edema, eczematous dermoepidermitis (stasis chronic eczema), pigmented purpuric dermatitis, dermohypodermitis with sclerous development (lipodermatosclerosis), Milian's white atrophy and leg chronic ulcer. [1]

The first information about CVD was suggested by prehistoric drawings and paintings, papyrus writings and hieroglyphic and cuneiform descriptions engraved in stone. Chronic venous hypertension (VH) and chronic venous insufficiency (CVI) may be secondary to venous pump failure due to the reflux of the varicosities of the superficial venous network or secondary to prior thrombosis of the veins (incompetence and/ or obstruction due to poor reperfusion of deep vein branches). Deep venous disease is associated thromboembolism. Rarely, CVI is due to primitive valvular insufficiency. VH will lead to permanent dilatation of the dermal veins visible through the epidermis. VH is responsible for capillary distention with extravasation of plasma, red blood cells, fibrinogen, α2-macroglobulin and other macromolecules, perivascular diapedesis of leukocytes, into the interstitium. Absorption capacity of the lymphatic vessels is exceeded resulting edema and chronic inflammatory changes. Edema compresses and deforms capillaries with microcirculation disorders. Fibrin deposits form sleeves around the capillaries that block nutritional changes. The slowing of blood flow in the capillaries favors the aggregation of the red blood cells. Small thrombus can obstruct the capillary lumen and leukocytes adhere to the vessel endothelium. The macromolecules trap growth and repair factors so that minor traumatic wounds cannot be

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repaired and an ulcer develops. The activation of leukocytes release free radicals, proteolytic enzymes, cytokines (TNFα), leukotrienes, matrix and metalloproteinases responsible for capillary hyperpermeability and capillary and tissue injury.

ENDOTHELIUM AND GLYCOCALLIX

Endothelium is a key element in regulating vascular tonus, hemostasis and coagulation. Due to its structure the endothelial glycolalysis prevents leukocyte adhesion, inflammation and thrombosis. [2] Secondary to dilatation, increased blood flow and venous pressure, the endothelial function is negatively affected in CVI.

Through specific receptors the endothelium and the glycocallix detect the changes produced by the shear stress, with the stimulation of the chemoattractants (MCP-1, VCAM-1, ICAM-1) and nitric oxide activation, amplifying the inflammatory response with subsequent release of inflammatory mediators (chemokines, cytokines, growth factors and metalloproteinases), attraction of leukocytes (macrophages, monocytes, T lymphocytes) and their attachment and transmigration in venous and interstitial walls, inducing cutaneous modifications specific for CVI. [2,3] The changes in venous pressure and wall tension are perceived by the endothelial cells through ICAM-1 molecules (differentiation cluster 54-CD54-) that have an increased expression in patients with CVI. ICAM-1 molecules have a specific role in activation, adhesion of endothelial cells, leucocyte's diapedesis and transmigration and through transient receptor potential vanilloid channels (TRPVs). [4] They induce disruptions in the production of NO–, the release of vasoactive substances, the extravasation of macromolecules and erythrocytes that degrades in fibrin and hemosiderin with leucocyte activation, in the expression of MCP-1, MIP-1 and vascular cell adhesion molecule-1 (VCAM-1, CD-106) and in the expression and release of L-selectin and E-selectin. [5]

The adherence and transmigration of leukocytes in venous valves and venous walls triggers an inflammatory cascade, stimulating the production of tumor growth factor-β1 (TGF-β1) by the activated endothelial cells and smooth muscle cells [6] and the production of TNF-α and interleukin-1 (IL-1) factor with subsequent increased expression of matrix metalloproteinases (MMPs). [4,7,8]

VH determines the stretch of the venous wall and an increased venous wall tension, followed by an increased expression of hypoxia inducing factors (HIF-α1, HIF-α2) that further on induces an increase in the level of MMP-2 and MMP-9 in order to relax the venous wall, with the consequently development of the venous dilatations. [2]

THE ROLE OF MATRIX METALLOPROTEINASES, THEIR TISSUE INHIBITORS AND CYTOKINES

The mechanisms involved in the development of venous ulcer (VU) and the factors that intervene in the slowhealing process are not fully elucidated. The healing of chronic ulcers is a complex process involving inflammation, reepithelization, matrix deposits and tissue remodeling. These processes are controlled by MMPs and their tissue inhibitors (TIMP). In the case of VU, MMPs, zinc-binding endopeptidases, are synthesized by local proliferating fibroblasts, their activity being inversely proportional to the degree of healing. Generally, MMPs and TIMP are temporarily induced in response to exogenous signals, such as different proteases, cytokines or growth factors, cell-matrix interactions and modified cell contacts. Gelatinases MMP2 and MMP-9, as well as TIMP-1 appear to be elevated in exudates of VU compared to acute wounds. [6,9]

However, biopsy sample analyzes have shown variable results. MMP-1, MMP-2 and TIMP-1 levels were elevated in patients with lipodermatosclerosis compared to normal skin. [10] In a later investigation, biopsies from patients with VU were found to have elevated levels of active MMP-2 compared to normal skin.

MMPs production and their function is regulated by cytokines, urokinase plasminogen activator (uPa), extracellular matrix metalloproteinase inducer (EMMPRIN-CD147), AA platelet growth factor (PDGF-AA) and mitogen-activating protein kinases (MAPK). [11,12] Excessive MMP activity in the tissue is controlled by their endogenous inhibitors, tissue metabolic inhibitors (TIMP). In UV matrix metalloproteinase synthesis outweighs the effect of their tissue inhibitors, the imbalance resulting in the degradation of elastin and collagen. [13]

At the level of VU fibroblasts, MAPK pathways over-express p38, a negative modulator of cell proliferation, stopping the healing.

VH induces MMP activators which increase MMP expression and activity, extracellular matrix (ECM) degradation, relaxation of venous smooth muscle (VSM) and venous dilatation. [14,15] Inflammation of the venous wall causes additional increase in MMP levels, vein dilatation and valve dysfunction. [16] The result consists in remodeling and fibrosis of the venous wall causing progressive venous dilation and varicose vein formation. [14] The ECM composition is dependent on MMP and can affect the venous wall structure. In CVI there is an imbalance of the venous wall collagen types. The type III collagen responsible for tissue distension is low, whereas type I collagen – which determines rigidity – is high, leading to a reduction in the elasticity of the varicose veins. [16]

Imbalance between MMPs and TIMP activity increases MMP tisular levels resulting in degradation of elastin and collagen [13] with pathological changes of the venous wall and installation of CVI. Thus hypertrophic and atrophic vein segments develop in the varicose veins and damage the venous valves. The remodeling of the venous wall and abnormal venous distention restrains the valve's closure and therefore initiate the venous reflux. [6]

MMP-2 (gelatinase A) may induce venous relaxation or inhibition of venous contraction [17], affecting ECM remodeling in vascular structures and playing an important role in the development of the VU. Healing of VU is impaired in patients with high levels of MMP-2 and low levels of TIMP-2 in the ulcer's exudate. [16]

MMP-9 (gelatinase B) is considered to be the major protease in ECM degradation in chronic wounds. MMP-9 levels are much higher in cases of varicose veins and VU. [18] The MMP-2 and MMP-9 are up-regulated in experimentally induced VH, [19] and MMP-9 has been shown to influence remodeling of the vein by modulating the collagen content of the vein wall, thus contributing to inflammation and fibrosis. [16]

MMP-12 (macrophage metallomelastase) allows macrophage migration by degradation of several ECM components. MMP-12 can block angiogenesis, acting as a potent inhibitor of angiogenesis. MMP-12 concentration is increased in VU's exudate in the inflammatory phase compared to the granulation phase. [20]

In addition, MMP extracellular inducer (EMMPRIN) and membrane metalloproteinases type 1 and 2 (MT1-MMP and MT2-MMP) were detected in the dermal and perivascular regions of VU. [21] There are no differences in overall MMP-1, 2, 9 and TIMP-1 protein levels in patients with CEAP (Clinical, Etiology, Anatomical, Pathophysiology) class C2 to C6 CVD compared to normal controls. [6] However, in C6 CEAP class level, MMP-2 levels were increased compared to MMP-1, 9 and TIMP1 in patients with C4 and C5 CEAP class disease.

These data indicate that active tissue remodeling occurs in CVD patients. It is currently unclear which MMPs are involved and how are they activated and regulated. It appears that MMP-2 may be activated by uPa.

mRNA, uPa and urokinase plasminogen activator receptor have increased levels of in VU compared to normal skin. Elevated levels of active TGFβ-1 in the dermis of CVI patients suggest a regulatory role for TGFβ-1 in the synthesis and activity of MMP and TIMP. [6,22]

The regulatory role of TGFβ-1 in the synthesis and activity of MMP and TIMP is supposed by TGFβ-1 increased levels in the dermis of CVI patient as well as during the healing process of VU. TGFβ-1 is secreted by interstitial leukocytes and binds to dermal fibroblasts and ECM proteins, also increases with the severity of the CVD, being a risk factor. [23] The cytokines interfere with CVI pathophysiology in its various stages starting with inflammation. In the untreated VU there are elevated levels of pro-inflammatory cytokines (IL, TNF-α, IFN-γ).

Along with VH and inflammation, the cytokines interfere in post-capillary venules where they form asheath which includes collagen I and III, fibronectin, vitronectin, laminin, tenascin, fibrin, TGF-β1 and α2-macroglobulin. [7] Although the causes and mechanisms of fibrin sheath production are not fully elucidated, chemical and structural changes from dermal microcirculation will lead to the progressive installation of cutaneous trophic disorders and ulceration. [2,24- 26]

Red blood cell degradation products and interstitial protein extravasation are potent chemoattractants that represent the initial inflammatory signal at the base of responsibility for the recruitment of leukocytes. These cytochemical events are responsible for increasing intracellular adhesion molecule-1 (ICAM-1) expression on endothelial cells of microcirculatory vessels observed in dermal biopsies in patients with CVD.

ICAM-1 is the activation-dependent adhesion molecule used by macrophages, lymphocytes and mast cells for diapedesis. α and β platelet-derived growth factor receptors (PDGFR-α and PDGFR-β) and vascular endothelial growth factor (VEGF) have increased levels in CVD patients dermis. They are regulating upward adhesion molecules leading to leucocyte recruitment in CVD. [6]

CONCLUSIONS

Along with VH (key element), endothelium and glycocallix, MMPs and cytokines play an important role in the CVD pathophysiology. Understanding the physopathological mechanisms involved in triggering and evolving CVD, knowing the aetiological factors and the facilitating factors are an essential stage in the management of the disease, allowing the development of a complete therapeutic plan adapted to each patient.

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Supliment Rezumate

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MEDICINA MILITARĂ –
QUO VADIS?

General de brigadă medic,
Dr. **Mihai-Marius MUREȘAN**

MEDICINA MILITARĂ a apărut încă din zorii însângerați ai omenirii odată cu primele conflicte armate ca expresie a nevoii de îngrijire a celor răniți și ca formă de manifestare a compasiunii umane față de semenii aflațI în suferință.

Evoluția ulterioară a medicinei militare a fost strâns legată de dezvoltarea forțelor combatante șI a progresului tehnic în domeniul armamentului.

Toate acestea, precum și necesitatea de reacție rapidă, condițiile vitrege pe care le presupune purtarea unei campanii militare au făcut ca medicii militari să fie în avangarda dezvoltării științelor medicale.

Mulți se grăbesc să afirme că apogeul dezvoltării militare și implicit al medicinei militare a fost atins în timpul celui de-al doilea război mondial și pe perioada conflictelor armate emergente Războiului Rece, având în vedere că s-a reușit mobilizarea sub arme a zeci de milioane de oameni care au purtat lupte pe toate continentele. În același domeniu amintim doar descoperirea antibioticelor care astăzi sunt cunoscute și utilizate de toți locuitorii planetei.

Evenimentele recente au generat schimbări importante asupra mediului de securitate în cadrul NATO. Astfel, perspectiva unui conflict masiv îndreptat împotriva unuia din membrii alianței nord - atlantice este tot mai îndepărtată, în schimb este tot mai fezabilă posibilitatea unor conflicte armate în afara granițelor alianței în teritorii aflate în zona de interes a NATO.

Toate aceste modificări strategice asupra tipului de operații militare în care se preconizează că vor fi angajate forțele militare ale NATO, precum și creșterea așteptărilor populației față de nivelul asistenței medicale la campanie, implică reașezarea principiilor medicinei militare.

Din punct de vedere medical provocările pe termen scurt și mediu sunt:

- Intervențiile militare de tip coaliție care presupun participarea unor contingente aparținând țărilor membre NATO dar și unor țări partenere. Pentru asigurarea unui sprijin medical eficient este necesară utilizarea de **proceduri comune** care să aibă la bază regulile de bună practică medicală agreate de toate țările participante. Capabilitățile medicale care vor fi puse în comun trebuie să aibă o unitate funcțională care să permită asigurarea unui flux de îngrijiri medicale continuu șI coerent.
- Purtarea viitoarelor conflicte în zone cu infrastructură sanitară precară sau distrusă în timpul confruntărilor. Pentru astfel de situații este necesară proiectarea și menținerea unor structuri medicale militare care să aibă un grad ridicat de autonomie și mobilitate.
- Proliferarea rețelelor teroriste ale căror acțiuni și atacuri vizează în principal obiective civile și industriale care pot genera dezastre ecologice precum și contaminarea zonei de operații întrunite. În vederea prezervării capacității operaționale a elementelor combatante este nevoie de o componenență de medicină preventivă, care să furnizeze informații medicale și să ia deciziile medicale corecte.
- Implicarea tot mai mare a structurilor media în furnizarea de informații publice din zonele de conflict face necesară dezvoltarea unor abilitățI de comunicare a deciziilor medicale către mass-media, care să stimuleze încrederea populației și să crescă moralul trupelor.

În concluzie, pilonii dezvoltării structurilor medico-militare pe termen scurt și mediu sunt:

- asistența medicală de urgență;
- medicina preventivă;
- asistența medicală primară;
- asistența medicală specializată, ultima, dar la fel de importantă pe fluxul de îngrijiri medicale, care să permită nu numai salvarea vieții rănitului dar să îi asigure șI reinserția ulterioară în mediul social.

Toate aceste cerințe din partea mediului internațional sunt și vor fi posibile prin dezvoltarea componentelor actuale ale sistemului medical militar, **sub o comandă unică**, care să fie interconectat cu necesitățile sistemului sanitar național șI aliniat la rigorile Alianței Nord Atlantice.

Managementul răspunsului maladaptativ la stres la personalul militar

Premiul I la
Congresul Balcanic
de Medicină Militară
Istanbul 2013

Mălina Ciumaşu-Rîmbu, **Cristina Morariu**
Spitalul Clinic Militar de Urgenţă Iaşi

Rezumat

Stresul cronic poate determina o scădere a expresiei centrale a neuropeptidului Y (NPY) şi subiecţii expuşi la stresori cronici pot dezvolta hipersensibilitate la stresori noi cu disfuncţii ale sistemului NPY ce pot determina reacţii adaptative deficitare la stres ale sistemului cardiovascular şi chiar hipertensiune arterială. Stimularea expresiei centrale a NPY poate contribui la o adaptare comportamentală de succes la stres prin reducerea tonusului cardiovascular şi supresarea comportamentului anxios. Adaptogenii, o nouă clasă de reglatori metabolici s-au demonstrat recent a fi stimulatori ai expresiei şi eliberării NPY din celulele neurogliale.

Scopul studiului *este de a creşte toleranţa şi a regla adaptarea la stres la subiecţii militari expuşi la stres cronic care prezintă o reacţie de alarmă exagerată la stresor nou de intensitate mică cu răspuns disfuncţional cardiovascular, prin stimularea expresiei NPY cu ajutorul adaptogenilor, parte a protocolului de prevenţie.*

Material şi metodă*: 20 de militari cu un tipar reacţional cardiostresor cunoscut şi expunere la stres cronic cuantificată prin chestionare validate de evaluare a stresului ocupaţional şi măsurători ale cortizolului salivar au fost expuşi la un stresor nou de intensitate mică: controlul medical periodic de rutină şi evaluaţi clinic pentru răspunsul disfuncţional cardiovascular înainte şi după aplicarea unui protocol de prevenţie timp de 30 zile. Angajaţii au fost împărţiţi randomizat în două loturi, unul a primit protocolul standard de prevenţie (consiliere asupra unui stil de viaţă sănătos) şi adaptogeni în schemă multidoză, cu administrare de două ori pe zi şi celălalt a primit doar protocolul standard de prevenţie.*

Rezultate: *S-au găsit diferenţe semnificative statistic la toţi parametrii cardiovasculari în lotul care a primit adaptogeni comparativ cu lotul care a primit doar protocolul standard de prevenţie, unde aceste diferenţe semnificative au fost înregistrate doar la parametrul tensiune arterială diastolică.*

Discuţii: *Procesul de adaptare a fost incomplet la lotul de control.*

Concluzii: *Adaptogenii ar putea fi un factor important în protocoalele de prevenţie a stresului ocupaţional cronic cu disfuncţii ale sistemelor NPY pe lîngă măsurile de prevenţie standard.*

Cuvinte cheie: NPY, ADAPTOGENI, STRES OCUPAŢIONAL CRONIC, RĂSPUNS DISFUNCŢIONAL CARDIOVASCULAR, STRESOR NOU

Abstract

Chronic stress may produce a decrease in central neuropeptide Y (NPY) expression and subjects exposed to may prove hypersensitivity to a novel stressor with dysfunctions in the NPY system and cardiovascular maladaptation to stress, even hypertension. Upregulation of NPY expression may contribute to successful behavioral adaptation to stress by reducing cardiovascular tone and suppressing anxious behaviours. Adaptogens, a new class of metabolic regulators stimulate NPY expression and release.

The aim of this study *is to increase tolerance and adaptation to stress of hypersensitive to novel stressor, occupational chronic stress exposed military subjects with cardiovascular maladaptation to mild new stressor, through prestimulation of NPY using adapatogens as part of prevention protocol.*

Material and methods: *20 military personnel with known cardiostressor reactional mode and occupational chronic stress exposure quantified through validated questionnaires and salivary cortisol measurements were exposed to mild novel stressor: occupational medicine routine evaluation and clinically assesed for maladaptive cardiovascular response prior and before application of 30 days prevention protocol. Employees were randomly split in two groups: one receiving standard prevention protocol (lifestyle counselling) plus adaptogens in multiple dose administration, twice a day and the other receiving only standard prevention protocol.*

Results: *We found significant statistic differences in all cardiovascular parameters in adaptogen group and only in diastolic blood pressure in control group.*

Discussions: *Adaptation process was incomplete in the control group.*

Conclusions: *Adaptogens could be an important factor in succesfull prevention protocols of chronic occupational stress dysfunctions involving NPY systems.*

Key words: NPY, ADAPTOGENS, CHRONIC OCCUPATIONAL STRESS, CARDIOVASCULAR MALADAPTATION, NOVEL STRESSOR

Introducere

Stresul ocupațional reprezintă un factor de risc profesional semnificativ în domeniul militar, cu impact negativ asupra îndeplinirii performante a sarcinilor de serviciu cu repercursiuni organizaționale și/sau individuale asupra stării de sănătate a angajatului pe termen scurt sau pe termen lung și pot influența negativ misiunile militare pe timp de pace sau război.

Mediul militar presupune existența unei game largi de factori stresori fie ei psihosociali (relații inadecvate șef-subaltern (39%), suprasolicitare cantitativă (33%), orar prelungit (30%), conflicte familiale din cauza locului de muncă (23%), munca în teren, la distanță de mediul familial (19%), staționarea în cazarmă (10%), acțiuni disciplinare (9%), fizici (ex. temperaturi extreme, câmpuri electromagnetice, radiații ionizante), chimici (ex. inhibitori de acetilcolinesterază, agenții veziculogeni, cianați, agenți chimici pulmonari, agenți chimici incapacitanți, capsaicina) sau biologici (bacterii, viruși, biotoxine).

Majoritatea studiilor relaționate cu stresul ocupațional militar s-au concentrat pe factorii psihosociali și pe disfuncțiile sistemului de stres generate de aceștia, în special de ordin psihologic și psihiatric și cu precădere în teatrele de operațiuni. Teatrul de operațiuni este un mediu cu particularități unice în care angajatul trebuie să facă față unui climat nefamiliar, unui teren străin, unei flore și faune cu potențial nociv, unei populații neprietenoase, unor agenți biologici diferiți de cei cu care sistemul imun individual e obișnuit etc. Condițiile particulare ale teatrelor de operațiuni nu sunt însă singura fațetă a stresului ocupațional militar.

Stresul ocupațional cronic poate fi generat de sarcinile multiple la locul de muncă resimțite ca suprasolicitare, cantitativă și/sau calitativă, durata prelungită de lucru, ambiguitatea rolului ocupațional al angajatului (cumulul de atribuții), suport administrativ inadecvat (fonduri insuficiente, echipament inadecvat competitivității scopurilor organizaționale), pozițiile ocupaționale de graniță (medicul militar, sanitarul militar) cu decizii de rol conflictuale, controlul scăzut asupra jobului efectuat (orar frecvent schimbabil ca durată, la necesitate, cu afectarea relațiilor familiale, restricțiile asupra mediului locativ sau de îmbrăcăminte), sarcini care nu se potrivesc calităților ocupaționale ale angajatului fie subsolicitante, fie suprasolicitante, care intervin neașteptat în peisajul ocupațional cotidian și interferă cu executarea sarcinilor cotidiene, responsabilitatea neproporțională cu autonomia decizională, lipsa feedbackului pozitiv vis-a-vis de eficacitatea îndeplinirii sarcinilor, relațiile interpersonale tensionante, retribuții financiare cu o coerență scăzută, cu motivație ambiguă vis-a-vis de calitatea muncii efectuate etc.

Job-ul având cele mai mari repercursiuni din punct de vedere al stresului ocupațional este cel al cărui caracteristici sunt cereri mari și control scăzut în procesul de luare a deciziilor.

Disfuncțiile homeostatice generate de stresul ocupațional cronic în domeniul militar nu apar neapărat ca urmare a creșterii nivelului stresor ocupațional, ci mai ales ca o erodare a adaptabilității individuale. Selye postula în teoria răspunsului la stres că expunerea prelungită la un factor stresor poate duce la depleția resurselor adaptative, cu scăderea sistemului de rezistență la factorul stresor și apariția stării de epuizare în sistem, vezi „burn-out sydrome“ sau „old seargent syndrome“.

În procesul de adaptare, sistemul de reacție la stres intervine prin cei doi mari jucători: sistemul nervos autonom și axa hipotalamo-hipofizo-corticosuprarenală și mediatorii lor: ex. catecolaminele și cortizolul.

Un rol cheie în adaptarea eficientă cardiovasculară, neuroendocrină, imună și metabolică la stres îl are neuropeptidul Y (NPY).

Cerebral, NPY se află în cantități semnificativ crescute față de alte neuropeptide, concentrându-se în sistemul limbic, inclusiv amigdala și hipotalamusul, arii importante în reglarea comportamentului emoțional și răspunsului la stres. Central, NPY are acțiune anxiolitică și inhibă activitatea simpatică cu efecte hipotensive și bradicardice.

Concentrația plasmatică a NPY este direct proporțională cu performanța comportamentală în condiții de stres. Nivele plasmatice scăzute ale NPY sunt asociate cu disfuncții ale sistemului nervos autonom la factori stresori prin hiperactivitate, reacții de alertă, alarmă și anxietate exagerate.

Stresul cronic poate produce o scădere a expresiei centrale a NPY și subiecții expuși cronic la stres pot dezvolta hipersensibilitate la stresori noi cu disfuncții ale sistemului NPY, ce pot determina reacții adaptative deficitare la stres ale sistemului cardiovascular și chiar hipertensiune arterială.

Heilig și Thorsel au propus ca soluție pentru o adaptare comportamentală de succes la stres, reglarea pozitivă a expresiei NPY și mai târziu s-a demonstrat că activarea sistemelor NPY reduce tonusul cardiovascular și supresează comportamentul anxios.

Adaptogenii, o nouă clasă de reglatori metabolici care augmentează capacitatea de adaptare a organismului la stresori interni și externi și protejează organismul de efectele nocive ale acestora prin stimularea rezistenței nespecifice, s-au demonstrat recent a fi stimulatori ai expresiei și eliberării NPY din celulele neurogliale. Studiul lui Panossian și colaboratorii a utilizat ADAPT 232, o combinație de extracte de Eleutheroccocus senticosus (rădăcină), Schizandra chinensis (fructe) și Rhodiola Rosea (rădăcină) și a demonstrat că îmbunătățirea capacității de adaptare și toleranță la stres obținută prin această formulă se datorează stimulării expresiei și eliberării NPY în circulația sistemică ca răspuns intrinsec de apărare.

Programele standard de prevenție ocupațională a disfuncțiilor generate de expunerea la stres cronic în domeniul militar se concentrează pe modificarea obiceiurilor nocive, a stilului de viață nesănătos și promovarea unor metode de relaxare.

Ipoteza de cercetare s-a concentrat asupra tiparului angajatului militar cu un job de cazarmă cu solicitări mari și control scăzut, expus la stresori cronici, cu tipologie cardiostresoare („efectul de halat alb") în perspectiva implicării acestuia în teatrele de operațiuni.

Ce se poate întâmpla cu un astfel de angajat cu reacție de alarmă exagerată cunoscută, cu disfuncții probabile la nivelul sistemului NPY cu efecte minore în viața ocupațională de rutină, în fața stresorilor noi, variați,de intensități diferite din teatrele de operațiuni? E posibil să reacționeze inadecvat, disproporționat, anxios și pripit în decizii cu efect important asupra stării de sănătate și securitate individuală, de grup și organizațională în operațiunea militară în care e implicat. Astfel, cele mai bune intervenții preventive sunt necesare a fi efectuate înainte de implicarea unității în operațiuni cu stres de intensitate mare și cu risc mare.

Cum poate fi reglată această reacție de alarmă exagerată? Literatura de specialitate și ipoteza de cercetare presupune acest lucru prin stimularea expresiei și sintezei centrale a NPY prin utilizarea adaptogenilor.

Material și Metode

Studiul nostru este de tip open label randomizat având ca obiective principale creșterea toleranței și promovarea adaptării eficiente și complete la subiecții militari cu hiperreactivitate la stresori noi, expuși la stres cronic ocupațional care prezintă răspuns disfuncțional cardiostresor la un stresor nou de intensitate ușoară. Lotul de cercetare a fost compus din 20 angajați militari, de gen masculin, recrutați în timpul controalelor medicale periodice, efectuate la cabinetul de medicina muncii din Spitalul Clinic Militar de Urgență din Iași pe baza unor criterii de includere și respectiv de excludere.

Criterii de includere:

- Tipologie de reacție la stres – cardiostresor („efect de halat alb")- tensiune arterială ≥ 140/80 mmHg și frecvență cardiacă >80 bătăi/minut
- Caracteristici ocupaționale: cereri mari și control scăzut
- Sex masculin
- Status aparent sănătos
- Antecedente heredo-colaterale și personale nesemnificative
- IMC între 18.50-24.99 kg/m^2

Criterii de excludere:

- Afecțiuni cardiovasculare
- Afecțiuni endocrine
- Afecțiuni neurologice
- Obezitate
- Valori normale ale parametrilor cardiovasculari (reacție adaptativă cardiovasculară de succes la stres cronic și expunere la stresor nou)
- Alte caracteristici ocupaționale decât cele menționate la criterii de includere
- Sex feminin

Angajații militari sunt predispuși la reacții adaptative cardiovasculare disfuncționale la stres, mai mult decât populația generală și procentajul acestor disfuncții este semnificativ, așa cum a fost raportat în studii anterioare efectuate în cabinetul nostru. Studiile recente asupra efectului stresprotector al combinației de adaptogeni ADAPT 232, prin stimularea expresiei și eliberării NPY ne-au incitat să determinăm dacă această combinație de reglatori metabolici ar putea fi folosită ca măsură de prevenție în adaptarea deficitară a sistemului cardiovascular din stresul ocupațional militar. Modelul de cercetare a considerat evaluarea medicală ocupațională de rutină ca și stresor nou, de intensitate redusă și disfuncțiile cardiovasculare măsurate (tensiune arterială ≥ 140/80 mmHg și frecvență cardiacă >80 bătăi/minut) ca reacție exagerată de alarmă și comportament anxios tipic subiectului cu expunere cronică la stres ocupațional și disfuncții ale sistemului NPY, ce îl determină să fie hiperreactiv la noi stimuli. Expunerea cronică la stres ocupațional a fost evaluată subiectiv prin chestionare de evaluare a stresului ocupațional adaptate sistemului militar, validată și cuantificată obiectiv prin măsurarea cortizolului salivar dimineața între orele 8 și 10 A.M. Lotul de studiu a primit protocolul standard de prevenție a stresului ocupațional: consiliere asupra unui stil de viață sănătos și, în plus, o combinație standardizată de adaptogeni – ADAPTONIC – extract de Eleutherococcus senticosus (rădăcină), Schizandra chinensis (fructe) și Rhodiola Rosea (rădăcină), preparat de Alevia, Fălticeni, în schemă multidoză 1comprimat de 2 ori pe zi per os timp de 30 zile. Lotul martor a primit doar protocolul standard de prevenție: consiliere asupra unui stil de viață sănătos. Am presupus ca și ipoteză de cercetare, faptul că, stimularea NPY prin ADAPTONIC va îmbunătăți toleranța la stresori noi a unor subiecți expuși cronic la stres ocupațional. Angajații au fost reevaluați dupa 30 zile și au fost reexpuși la același stresor de intensitate redusă, studiindu-se reacția de alarmă prin măsurarea parametrilor cardiovasculari.

Rezultate și discuții

Prelucrarea statistică a datelor s-a făcut folosind programul SPSS 11.0 utilizând teste de statistică descriptivă și inferențială.

Pe cazuistica studiată nu s-au observat diferențe semnificative statistic între loturi, studiu și martor, la parametrul vârstă medie cu menționarea intervalului de vârstă la lotul de cercetare între 22 și 43 ani.

Toți angajații militari au prezentat un nivel ridicat al stresului ocupațional: 7.2 ± 1.36, scala utilizată la chestionarul de evaluare a stresului ocupațional fiind între 1 minim si 10 maxim (Fig.1).

Datele se încadrează în specificul organizațional menționat în literatura, atrăgând atenția asupra necesității implementării unor programe de prevenție a solicitării ocupaționale în domeniul militar.

Cortizolul salivar s-a corelat pozitiv, puternic semnificativ r- 0.893 cu nivelul de stres ocupațional așa cum era de așteptat conform studiilor asemănătoare din literatura de specialitate, cortizolul salivar fiind considerat standardul de aur în obiectivarea stresului ocupațional .

De asemenea, cortizolul salivar s-a corelat pozitiv, puternic semnificativ cu tensiunea arterială sistolică r-0.876 (Fig.2), date de altfel coerente cu cele din literatură, sugerând posibilitatea asocierii disfuncțiilor cardiovasculare la angajații expuși la stres cronic ocupațional și importanța identificării precoce a acestora și recuperării statusului homeostatic din această etapă reversibilă.

La lotul de studiu s-au determinat diferențe semnificative statistic ($p<0.05$) la toți parametrii cardiovasculari măsurați comparativ cu lotul martor la care s-au înregistrat diferențe semnificative statistic doar la parametrul tensiune arterială diastolică (tab. I).

TABELUL I

Parametrii cardiovasculari la lotul de studiu comprativ cu lotul martor la momentul t0- înainte de tratament și la t1 după 30 zile de tratament

	Study group	Control group
sBP t1-t0	0.05×10^{-4}	0.22
dBP t1-t0	0.17×10^{-3}	0.026
rHR t1-t0	0.05×10^{-6}	0.22

Rezultatele demonstrează că folosirea standardizată a combinației de adaptogeni- ADAPTONIC- ar putea îmbunătăți toleranța la un stresor nou la subiecții expuși unui stres ocupațional cronic. Modelul însă trebuie confirmat prin studii extensive ca număr de participanți și pe durate mai mari de timp.

Rezultatele înregistrate la lotul martor cu diferențe semnificative doar la parametrul tensiune arterială diastolică s-ar putea explica prin perspectiva experienței anterioare a sistemului NPY cu stresorul nou utilizat în studiu, chiar dacă acesta a fost utilizat

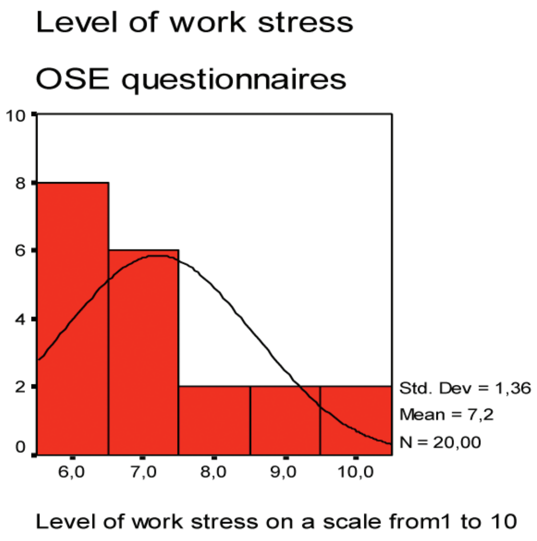

Fig.1 Nivelul stresului ocupațional la angajații militari din lotul de cercetare

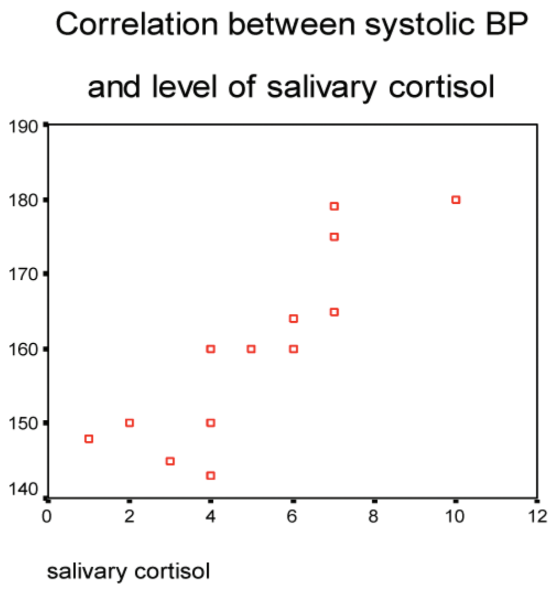

Fig.2 ***Corelarea parametrului tensiune arterială sistolică cu nivelul de cortizol salivar***

intermitent la o distanță de 30 zile de expunerea anterioară, influențarea tonusului vascular periferic și adaptarea la stresor, dar de menționat este faptul că adaptarea este incompletă în condițiile în care ceilalți parametri cardiovasculari au înregistrat diferențe nesemnificative statistic. Testarea nivelului plasmatic al NPY ca măsură de rutină la evaluarea personalului militar expus la stres ocupațional cronic, cu disfuncții cardiovasculare la un stresor nou de intensitate ușoară, poate aduce informații importante despre reacția de alarmă și ne dă posibilitatea corectării acestor disfuncții ale sistemului de reacție la stres înainte ca unitățile să fie implicate în operații militare cu stresori de intensitate mare și risc semnificativ.

Concluzii

Profesia militară presupune expunerea la un nivel de stres ocupațional semnificativ și caracteristicile ocupaționale cu cereri mari și control scăzut reprezintă un risc adiacent important.

Nivelul de stres perceput subiectiv de angajatul militar se corelează pozitiv cu modificările biologice și neuroendocrine.

Efectul de halat alb sau tiparul cardiostresor poate fi recunoscut ușor și necesită a fi tratat înainte de implicarea angajatului în operațiuni cu risc și stres important, acesta nefiind decât vârful icebergului.

Adaptogenii ar putea fi un factor important în protocoalele de prevenție a stresului ocupațional cronic cu disfuncții ale sistemului NPY pe lângă măsurile de prevenție standard.

Dacă dorim excelență în operațiunile militare e necesar să îmbunătățim performanța și prin facilitarea unei adaptări eficiente și complete la stresorii specifici din sistemul militar.

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Probleme în stadializarea preterapeutică a cancerului endometrial

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Premiat
la Congresul
Balcanic de
Medicină Militară
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Objective:

To establish the limitations of pretherapeutical staging and to evaluate the importance of surgical staging in choosing the therapeutical methods in endometrial cancer (EC).

Material and method:

We've conducted a retrospective analysis on 110 EC cases operated between jan.2007 and sept. 2012. The diagnosis was made by D& fractioned curettage for: postmenopausal metrorrhage (87%) and menometrorrhage (11%). The group of 60-70 years of age represented the majority of all cases (63%); obesity group 30% and cardio-vascular diseases 15%. Preoperative histopathological findings were: endometrioid EC (70%), EC with adenoscuamous component (20%), scuamo-papillar and clear cell (rare types) – 10%. Preoperative tumoral grading: G1 and G2- 90%, G3- 10%. Clinical stage I represented the majority (90%) of all cases; stage II cases (10%) have been irradiated. Types of operative techniques: TAH+ BA- 93%, type III RH- 6%, LAVH – 0,5%. In all of the cases where we performed type III RH, lymphadenectomy was associated (with + results in 43%); selective lymphadenectomy was made in 60% of all TAH+BA (endometrial invasion >50%, rare HP types, G3) with + results in 30% of the cases.

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During the same interval we've analyzed 110 cases of endometrial hiperplasia (TAH+ BA- 34%, VH +/-BA- 65%) and 73 cases of postmenopausal uterine bleeding due to the persistence of the symptomatology (TAH+BA – 40%, VH=BA- 305, VH- 25%, TH+BA+ selective pelvic lymphadenectomy- 4,5%, LAVH- 0,5%).

Results:

The preoperative HP form corresponded to the final one in 85% of the cases (in 15% of the cases the endometrioid form was associated to the scuamous component); tumor grade was understaged in 20% of the cases. Endometrial hyperplasia did not associate preoperatively any form of EC. 30% of the patients treated for persistent metrorrhage have had endometrioid carcinoma. Miometrial invasion was underdiagnosed surgically in 40%; cervical invasion was underdiagnosed clinically in 32% and surgically in 21%. Lymphatic invasion was present in 20% of the cases with 50% endometrial neoplastic transformation, 30% of the rare EC types, 17% of the G2- G3 tumor grade cases, 40% of the cases with enlarged lymphonodules, 10% of the ones having cervical invasion. Pretherapeutical staging compared to the surgical one was correct in 51% of the cases, understaged in 28% and overstaged in 5% (in 13% of the cases, EC was not found at the HP exam).

Conclusions:

The pretherapeutical and the surgical staging are not concordant; simple surgical staging (TAH+BA) gives correct information to establish the risk groupes and choose the right treatment; there is a satisfactory correlation between the HP form and the tumor grade (pretherapeutical and final), thus the rare HP types and G2,3 could be by themselves mandatory to an advanced surgical stage.

Morbiditate	Fără limfadenectomie pelvică (140 cazuri)	Cu limfadenectomie pelvică (188 cazuri)	Semnificaţia statistică
Transfuzie de singe (nr)	6%	14%	P=0.02
Morbiditate febrilă (nr.)	20%	20%	P=0,95
Timp operator minim (min.)	117,0+/-42,3	139,5+/-40,1	P<0,00
Pierdere sangvină medie (ml.)	322,2+/-337,8	442,2+/-307,0	P<0,03
Reluarea tranzitului intestinal (zile)	4,8+/-1,4	5,5+/-1,1	P<0,00
Zile de spitalizare	6,9+/-4,0	7,6+/-2,1	P<0,08

Studiul	Nr.cazuri	Urmărire medie (luni)	Recurenţa Nr. cazuri	%	Sediul recurenţei
Piver şi Hempling	90	-	0	0	-
Lim şi colab.	91	45	4	4%	L =2 D=2
Weiss şi colab.	75	48	3	4%	L=1 D=1
Aalders şi colab.	126	36-120	7	6%	L=5 D=2
Faught şi colab.	161	60	0	0	-
Total	543		14	2,5%	L=8(1,47%) D=5(0,92%)

Introducere

Cancerul endometrial reprezintă în acest moment cea mai frecventă formă de cancer genital, lucru datorat îmbătrînirii populaţiei, utilizării tot mai largi a terapiei de substituţie hormonală, hormonoterapiei cu tamoxifen în cancerul de sîn şi mai puţin (la noi !) a reducerii incidenţei cancerului de col uterin .(1,2)

În ultimii 20 de ani s-au definit mai bine histopatologia, factorii prognostici şi diseminarea bolii, schimbându-se totodată şi atitudinea terapeutică, în sensul că s-a trecut de la un program de iradiere pelvică externă urmat la 6 saptămîni de intervenţie chirurgicală la o singură aplicaţie intracavitară urmată imediat de histerectomie, pentru ca actual să se recurgă la o abordare individualizată folosind chirurgia ca terapie principală (frecvent primară şi / sau singulară).(1)

Necesitatea individualizării tratamentului rezidă din două mari probleme: profilul biologic particular al acestor bolnave şi morbiditatea importantă dată de limfadenectomie şi radioterapie care este demonstrată de numeroase studii.(3-8)

Numeroase studii au arătat importanţa morbidităţii produse de limfadenectomia pelvică şi de radioterapia postoperatorie.

Studiul Mayo clinic a comparat morbiditatea postoperatorie la cazurile la care s-a asociat limfadenectomia pelvică cu cele la care s-a practicat doar histerectomie totală cu anexectomie bilaterală.

STADIU	Caracteristici
0	Carcinom in situ
I **IA** **IB**	Tumoră este limitată la corpul uterin Cavitatea uterină măsoară 8 cm sau mai puţin Cavitatea uterină este mai mare de 8 cm
II	Tumora se extinde la colul uterin
III	Tumora invadează structurile pelviene adiacente
IV **IVA** **IVB**	Boală avansată în pelvis sau metastaze la distanţă Tumora invadează mucoasa vezicală sau rectală Prezente metastaze la distanţă

Tabel. Stadializarea clinică FIGO a cancerelor de corp uterin

STADIU	Caracteristici
0	Carcinom in situ
I IA IB IC	Carcinomul localizat la nivelul corpului uterin Tumora limitată la endometru Invazia miometrului în jumătatea internă inclusiv Invazia a mai mult de jumătate din grosimea miometrului
II IIA IIB	Extensia cancerului la colul uterin, dar nu în afara uterului Invazia numai a glandelor endocervicale Invazia stromei colului uterin
III IIIA IIIB IIIC	Extensia cancerului în afara uterului, dar fără depăşirea pelvisului sau a zonei para-aortice Tumora invadează seroasa şi/sau anexele, cu sau fără citologie peritoneală pozitivă Metastaze vaginale Metastaze în ganglionii pelvieni şi/sau para-aotici
IV IVA IVB	Invazia organelor pelviene adiacente sau metastaze la distanţă Invazie tumorală a mucoasei vezicale sau a recto sigmoidului Metastaze la distanţă, inclusiv intra-abdominale şi/sau în ganglionii inghinali

Tabel. Stadializarea chirurgicală a cancerului endometrial

Există și studii (Moore si colab.1989) care arată că samplingul ganglionar nu determină o morbiditate semnificativă nici măcar la pacientele obeze .(37). De aceea s-a impus revizuirea și adeseori modificarea protocoalelor clasice complexe care cuprindeau constant limfadenectomia si radioterapia adjuvantă.(1)

Dacă în cancerele de col uterin și de sân stadializarea este clinică, în cancerul de ovar chirurgicală, în cancerul de endometru practica si experiența acumulată a impus o modificare în sensul că s-a trecut de la stadializarea clinică FIGO 1971 la stadializarea chirurgicală FIGO din 1988.

Prima clasificare a fost propusă de Heyman în 1925, care împărțea cancerele de endometru în operabile și inoperabile. În 1951 Finn a extins această clasificare adăugînd elemente de histopatologie pentru ca în același an FIGO să stabilească o formă de compromis intre cele două. (37)

Stadializarea actuală permite încadrarea pacientelor cu cancer endometrial în anumite gupe de risc care oferă o bază mai obiectivă de individualizare a tratamentului în sensul limitării terapiei fără afectarea semnificativă a prognosticului pentru o categorie, sau selectarea cazurilor ce trebuie să beneficieze de terapie adjuvantă în cealaltă situație.

După cum se observă, majoritatea criteriilor de încadrare sunt obținute fie din analiza stadializării chirurgicale, fie din analiza patologică. Totodată toate aceste elemente reprezintă factori prognostici din care stadiul chirurgical este suveran. (27,28)

Cu toate acestea, stadializarea clinică nu a fost complet abandonată. În primul rând, pentru că diagnosticul inițial este pus încă prin chiuretaj uterin fracționat. Apoi, stadializarea clinică este indispensabilă pacientelor ce nu pot beneficia de explorare chirurgicală datorită fie condițiilor biologice particulare, fie stadiului avansat de boală . (38)

Limitele acestei stadializări se datorează imposibilității de apreciere a profunzimii invaziei miometriale (factor important de prognostic și de încadrare în grupe de risc), sensibilității reduse (50%) a încadrării în stadiul II (Averette și colab.1987, Boronow și colab.1984, Creasman și colab.1987) (37). Au fost imaginate o serie de investigatii clinice (histeroscopia sau microhisteroscopia) sau imagistice (echografia transvaginală, RMN ,TC cu emisie de positroni) care ameliorează din aceste limite dar care cresc costul stadializării și sunt disponibile numai în anumite centre medicale). (14-16,18)

Pe de altă parte D&C fracționat, procedură nesigură pentru încadrarea stadială și care presupune internare, anestezie și costuri a fost abandonată în țările dezvoltate în favoarea citologiei aspirative, rapidă, ieftină, ambulatorie și comparabilă privind acuratețea. (12,13,39)

Sistemul de stadializare chirurgicală adoptat de FIGO în 1988 a apărut ca o necesitate de ameliorare a datelor eronate furnizate de stadializarea clinică în aproape o treime din cazuri. (38)

Stadializarea chirurgicală presupune laparotomia exploratorie, inspecția completă și minuțioasă a întregului pelvis și abdomen, prelevarea de lichid peritoneal pentru analiză citologică și palparea ganglionilor pelvici și lombo-aortici. Urmează apoi, în majoritatea cazurilor, efectuarea histerectomiei totale extrafasciale cu anexectomie bilaterală și examinarea minuțioasă a piesei operatorii în două zone principale: aprecierea profunzimii invaziei endomiometriale și la nivelul colului uterin. (29-30)

Funcție de datele astfel obținute procedura se poate opri aici sau în cazul incadrării cazului în grupele de risc mediu și înalt se trece la stadializarea chirurgicală extinsă (în principal limfade-nectomia pelvică și/sau lomboaortică ,selectivă sau sistematică). Principalul neajuns al acestui algoritm este abandonarea parametrelor, totusi articole recente (40) descriu posibilitatea parametrectomiei laparoscopice în cazul stadiului II confirmat postoperator.

Stadializarea chirurgicală este completată de clasificarea adenocarcinoamelor în functie de gradul tumoral.

Obiectivul studiului este de a reevalua limitele

RISC MIC	RISC MARE		
Stadiu I	Stadiu FIGO II sau mai mare	G2, G3	ISVL
G1	Extensie	Invazie miometrială	Citologie peritoneală pozitivă
Forma HP comună	Tip HP nefavorabil	Invazia stromei cervicale	Aneuploidia

GRAD	DESCRIERE
0	5% sau mai puţin din tumoră cu creştere solidă non-scuamoasă sau non-morulară
1	6-50% prezintă caracteristici de creştere solidă non-scuamoasă sau non-morulară
2	Mai mult de 50% prezintă creştere solidă non-scuamoasă sau non-morulară

Tabel. Definiţiile FIGO pentru gradul tumoral.

stadializării clinice și de a stabili importanța stadializării chirurgicale în individualizarea tratamentului în sensul limitării terapiei la grupele de risc mic fără afectarea semnificativă a prognosticului.

Materiale și metode

În perioada ianuarie 2007-septembrie 2012 au fost operate în Clinica de Ginecologie a Spitalului Clinic de Urgență Militar Central 110 cazuri de cancer de endometru.

Diagnosticul a fost stabilit pe baza simptomatologiei (metroragii în postmenopauză -87%; menometroragii-11%), a examenului clinic general și a examenului ginecologic, iar confirmarea a fost realizată prin examenul histopatologic al materialului obținut prin dilatație și curetaj uterin.

Grupa de vârstă 60-70 de ani a fost preponderentă (63%); obezitatea și afecțiunile cardiovasculare au reprezentat 30 și respective 15% din cazuri.

Pentru fiecare pacientă s-a stabilit preoperator tipul histopatologic și gradul tumoral, rezultatele fiind următoarele: endometrioid (70%),endometrioid cu componentă adeno-scuamoasă (20%), tipuri rare (papilar scuamos și cu celule clare - 10%). Gradul tumoral a avut următoarea repartiție: G1 și G2 -90%, iar G3 -10%.

Stadiul clinic I a constituit majoritatea (90 %), cazurile în stadiul II au fost toate iradiate (10%).

S-au practicat următoarele tipuri de intervenții chirurgicale : histerectomie totală cu anexectomie bilaterală în 93% din cazuri, histerectomie radicală clasa a treia cu limfadenectomie pelvică în 6% din cazuri si histerectomie totală pe cale vaginală asistată laparoscopic la 0,5 % din paciente.

Limfadenectomia sistematică a fost efectuată în toate cazurile cu histerectomie radicală clasa a treia, postoperator fiind confirmați histopatologic în 43 % și s-a practicat selectiv în 60% din histerectomiile cu anexectomie bilaterală pentru invazie miometrială peste 50% din grosimea miometrului, tipuri histopatologice rare, G3, ganglionii fiind pozitivi la examenul histopatologic la 30% .

Au mai fost analizate în aceeași perioadă 110 cazuri de hiperplazii endometriale la care s-a practicat histerectomie totală cu anexectomie bilaterală în 34% din cazuri sau histerectomie totală pe cale vaginală cu sau fără anexectomie bilaterală în 65% din cazuri.

În lot au fost cuprinse și 73 paciente cu metroragii în climax persistente, la care s-a practicat histerectomie totală cu anexectomie bilaterală în 40% din cazuri, histerectomie totală cu anexectomie bilaterală pe cale vaginală la 30%, histerectomie totală cu anexectomie bilaterală și limfadenectomie pelvică selectivă la 4,5% și histerectomie vaginală asistată laparoscopic la 0,5%.

Rezultate:

Repartiția pe tipuri histopatologice postoperator a condus la o concordanță de 85%, formei endometrioide adaugîndu-i-se în 15% din cazuri componenta scuamoasă.

Gradding-ul tumoral a fost inițial substadializat în 20% din cazuri.

Hiperplaziile endometriale nu au prezentat postoperator nici o formă de cancer endometrial. În schimb 30 % din pacientele operate pentru metroragii persistente în climax au prezentat carcinom endometrioid.

Invazia miometrială a fost subdiagnosticată chirurgical la 40% din paciente, iar cea a colului uterin a fost subdiagnosticată clinic în 32%, și chirurgical în 21% din cazuri.

Invazia ganglionară a existat în: 20% pentru cancerul de endometru ce a interesat peste 50% din grosimea miometrului, 30% pentru tipurile rare, 17% pentru G2, G3, 40% la cele cu adenopatie și 10% la cazurile cu invazie cervicală.

Stadializarea preterapeutică a fost concordantă cu concordanta cu stadializarea chirurgicală în 51% din cazuri, a substadializat în 28% și suprastadializat în 5%; la 13%, încadrate inițial Ia nu s-a mai găsit tumoră restantă la examenul histopatologic.

Discuții

Stadializarea clinică în cancerul de endometru se face practic de fiecare dată când această boală se exprimă prin sângerări anormale. Din fericire, acest semn este de cele mai multe ori semnătura bolii în stadii precoce, mai ales în cazul manifestării lui în postmenopauză (11). Dilatarea și chiuretajul uterin sunt importante în primul rând prin diagnosticul de boală, forma histopatologică, grad tumoral și mai puțin în încadrarea în stadiul I sau II (10,11). Practic acest protocol face un screening ad hoc al cazurilor care vor fi stadializate chirurgical sau care vor fi iradiate primar.

Rezultatele dintre forma histopatologică și gradul tumoral inițiale și ale piesei operatorii sunt de cele mai multe ori concordante, predominând formele endometroide și de grad mic (cu evoluție mai blândă) corespunzător datelor din literatură (20-25). În analiza efectuată au existat cancerele endometriale care nu au mai fost regăsite pe piesa de histerectomie în 13% din cazuri. În schimb, la pacientele operate pentru persistența metroragiei după chiuretajul bioptic negativ pentru leziune endometrială în 30% din cazuri examenul patologic a evidențiat carcinom endometroid G1, în timp ce toate formele de hiperplazie operate au rămas cu diagnosticul inițial, date diferite față de literatură (12-16 %) (9).

Așadar marea majoritate a cancerelor endometriale ajung să fie stadializate chirurgical, protocoalele terapeutice mai vechi aplicate în toate cazurile dovedindu-se nejustificate și încărcate de morbiditate.

Stadializarea chirurgicală oferă o bază mult mai obiectivă de încadrare a bolnavelor în grupe de risc din punct de vedere al extensiei bolii la momentul efectuării ei, împărțindu-le pe acestea în două loturi. Un lot este cel în care pacienta se află în zona de risc mic, iar stadializarea chirurgicală a constituit și tratamentul suficient și de cele mai multe ori fără afectarea prognosticului (în condițiile limitării semnificative a morbidității). Celălalt lot este selectat pentru stadializarea chirurgicală extinsă, care presupune efectuarea limfadenectomiei selective, sistematice sau o combinație între cele două.

În analiza noastră totuși un procent relativ mare (67%) a fost selectat pentru limfadenectomie. O parte a rezultat direct din formele histopatologice agresive și gradingul tumoral înalt (G2,3) și anume 10%, și tot 10% din cazurile când s-a practicat histerecomie radicală. Restul de stadializări chirurgicale extinse au fost efectuate în cazul profunzimii invaziei miometriale mai mult de 50% (35%) (12-23%) (38) și 2% la depistarea adenopatiilor sugestive. De fapt, acestea din urmă au furnizat alături de formele histopatologice rare și cel mai mare procent de boală extinsă în afara uterului (20%, 40%, respectiv 30%).

Stalizarea chirurgicală simplă a limitat astfel terapia la HT+AB în aproape un sfert din cazuri și a selectat prin stadializarea chirurgicală extinsă alt sfert pentru terapia adjuvantă.

În cazuri selecționate există posibilitatea abordării cancerului endometrial prin combinarea a două tehnici de chirurgie minim-invazivă și anume histerectomia vaginală asistată laparoscopic, o preocupare de perspectivă a clinicii noastre (1% din CE operate).

În formele histopatologice comune și de grad tumoral mic și fără altă patologie utero-anexială conexă (uter mic care să nu necesite manipulări specifice chirurgiei vaginale) histerectomia vaginală poate realiza o stadializare chirurgicală simplă, convenabilă, în timp ce laparoscopia poate extinde stadializarea (ganglion santinelă, limfadenectomie sistematică, sampling peritoneal), (31-34, 35, 36). Pacientele vârstnice, tarate, obeze, frecvent întâlnite în acest tip de cancer genital beneficiază cel mai mult de această abordare novativă. De asemenea, în literatură este descrisă parametrectomia laparoscopică care poate rezolva unul din puținele neajunsuri ale stadializării chirurgicale în cazul cancerului endometrial extins la col. (40)

Concluzii

Cancerul endometrial este o formă frecventă de cancer genital apărut în special la femei cu vârsta înaintată și tarate. Actualul sistem de stadializare oferă posibilitate încadrării în grupe de risc și individualizării tratamentului în sensul limitării terapiei și implicit a morbidității fără afectarea semnificativă a prognosticului.

Diagnosticul de boală este parte a stadializării clinice (care altfel este neconcordantă în mai mult de o treime - 50% în analiza noastră cu stadiul real al bolii) și poate selecta singur cazurile ce beneficiază direct de stadializare chirurgicală extinsă (forme rare sau G2,3). Chirurgia minim invazivă (vaginală, laparoscopică) poate fi folosită în cazuri selecționate cu mari beneficii.

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Tratamentul metastazelor hepatice de origine colorectală prin criodistrucţie

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Rezumat

Introducere: *Scopul acestui studiu prospectiv este de a analiza rezultatele criochirurgiei, ca tratament singular sau parte a tratamentului complex al metastazelor de hepatice de origine colorectală. Au fost analizate complicațiile perioperatorii, rata recidivelor și supraviețuirea la distanță.*

Materiale și Metode: *Între 2001 și 2011, în instituția noastră, 27 de pacienți au fost tratati prin criochirurgie pentru metastaze hepatice secundare cancerelor colorectale. Procedura s-a realizat prin abord clasic, iar evoluția postoperatorie s-a monitorizat prin CT în ziua a șaptea, apoi din 3 în 3 luni, în primul an și din 6 în 6 luni următorii 2 ani, cît și prin evoluția markerilor tumorali.*

Rezultate: *Complicațiile intraoperatorii au apărut la 6 pacienți, iar postoperatorii la 9 pacienți. Mortaliatea post-operatorie, în prima lună, a fost 3.7%, iar urmărirea medie postoperatorie a fost de 27.43 luni [limite 1.6-41.2 luni]. Supraviețuirea anuală în primul an a fost 81.48%, la 2 ani de 73.68%, iar la 3 ani de 20.68%. Din cei 27 de pacienți, 11 pacienți [40.7%] au decedat, 16 pacienți [59.2%] au fost în viață, dintre care 7 pacienți [25.9%], fără semne de boală evolutivă.*

Concluzii: *Crioterapia este o procedură sigură și eficientă indicată în special pacienților cu leziuni nerezecabile sau risc chirurgical ridicat. Efectul antitumoral al tratamentului a fost demonstrat, dar complicaţiile şi rata mare a recidivelor hepatice, în special la pacienţii cu tumori mari, impune utilizarea ei cu precauţie.*

Cuvinte cheie: *criodistrucţie hepatică, tumori hepatice; metastaze hepatice; tumori hepatice; ablaţie tumorală.*

Abstract

Background: *The aim of this prospective study is to assess the outcomes of cryosurgery as a single treatment or as a part of complex treatment for colorectal liver metastases.*

Material and Methods: *Between 2001 and 2011, to our institution, 27 patients underwent cryosurgery for colorectal metastatic liver tumors. The procedure was performed via open surgery and postoperative follow-up managed with CT scan în day 7 and then at every 3 month interval în the first year and each 6 months for the next 2 years in correlation with tumor markers level.*

Results: *Intraoperative complications occurred in 6 patients and postoperative in 9 patients. Operative mortality within 1 month was 3.7% related to cryosurgery and the median follow up was 27.4 months [range from 1.6-41.2 months]. The survival rates were 81.48% to 1 year, 73.68% to 2 years and 20.68% to 3 years. During the follow-ups, 11 out of 27 patients [40.7%] have died, 16 patients [59. 2%] were alive and 7 of them [25. 9%] being with no evidence of recurrent disease.*

Conclusion: *Cryotherapy is a safe and effective procedure indicated especially for patients with unresectable lesions or high risks for surgical resections. The antitumor effect of treatment was demonstrated, but complications and high local recurrences rate, especially for the patients with large disease, should caution against its use.*

Keywords: *hepatic cryosurgery; liver tumors; liver metastases; liver tumors; tumor ablation.*

Introducere

Două treimi din pacienții cu cancer colorectal vor dezvolta metastaze hepatice, dar doar 20-25% dintre pacienți au indicație de rezecție datorită distribuției anatomice sau bolii extrahepatice (1-3). Excizia chirurgicală radicală oferă supraviețuire prelungită și singura sansa de vindecare pentru tumorile hepatice, dar aproape un sfert din pacienți, prezintă inițial metastaze hepatice sincrone de cancer colorectal rezecabile (1, 3, 4). Tratamentul curativ este multimodal și implică rezecția radicală a tumorilor primare, rezecția completă a metastazelor hepatice, chimioterapie, ablația „in situ“ și uneori radioterapie (5, 6).

O parte din pacienți ar putea fi tratați numai prin ablația „in situ“ a tumorilor, pentru păstrarea funcției hepatice. În ultimul deceniu numeroase rapoarte publicate au sugerat că folosirea doar a criochirurgiei sau în combinație cu rezectia hepatică, poate crește numărul de pacienți cu tumori hepatice, care pot fi tratate chirurgical (5-10). Scopul acestui studiu prospectiv monoinstitutional este de a evalua rezultatele criochirurgiei metastazelor hepatice de cancer colorectal (MHCC) ca terapie unică sau asociată rezecțiilor complexe (rezecții hepatice însoțite sau nu de alte rezecții viscerale).

Materiale și metode

Tumorile, detaliile operatorii și monitorizarea postoperatorie au fost înregistrate prospectiv.Când criteriile de rezecabilitate ale tumorilor hepatice au fost îndeplinite, criochirurgia a fost asociată rezecțiilor hepatice, cu scopul de a păstra cît mai mult țesut hepatic normal. În cazul metastazelor nerezecabile voluminoase, chimioterapia preoperatorie a fost efectuată la toți pacienții în încercarea de a reduce dimensiunile leziunilor. Criodistrucția a fost efectuată singură sau asociată rezecțiilor complexe (rezecții hepatice cu sau fără rezecții colorectale sau alte rezecții viscerale), dacă am putut presupune că tumorile hepatice pot fi distruse, iar tumorile primare pot fi îndepărtate, complet. Criteriile de includere pentru criodistrucție: boală hepatică bilaterală, metastaze hepatice multiple, constrângeri anatomice (tumori situate în vecinătatea venelor hepatice, bifurcației venei porte sau venei cave retrohepatice) și riscului operator din cauza funcției hepatice limitate sau stării clinice precare a pacienților. Criterii de excludere: boala extrahepatică nerezecabiă detectată înainte, în timpul evaluării preoperatorii sau intraoperator sau metastazele peste 6 cm, considerate nepotrivite pentru crioterapie.

Evaluarea preoperatorie

Investigațiile preoperatorii standard au inclus: examenul clinic, ecografia abdominală, Rx pulmonar, CT scan (torace, abdomen și pelvis cu contrast vascular și intraluminal), teste funcționale hepatice, markeri tumorali și colonoscopie. Scanarea osoasă a fost făcută pentru a exclude boala extrahepatică, doar pentru pacienții selectați. Pentru pacienții cu tumori voluminoase rezecabilitatea s-a evaluat și prin angio-CT cu portografie și volumetrie. Nu s-a realizat biopsia hepatică tumorală preoperatorie pentru a confirma diagnosticul.

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Tehnica operatorie

Examinarea intraoperatorie a ficatului s-a efectuat atât prin palpare, cât și prin ecografie intraoperatorie, folosind transductorul în „T“ de 7,5 MHz (Versa Pro Sonoline, Siemens Medical sistem Inc). Leziunile ganglionare sau peritoneale suspecte au fost trimise la examenul histopatologic extemporaneu. Crioablația s-a efectuat, sub monitorizare intraoperatorie ecografică pentru a confirma localizarea leziunilor și relația lor cu structurile biliare si vasculare majore. Pentru crioterapie am utilizat aparatul CRYO ERBO 6® (ERBE Elektromedizin GmbH, Tubingen, Germania) ce utilizează azot lichid și folosește pînă la 6 criosonde de 3,2 mm. Numărul de sonde utilizat (una sau mai multe) a fost corelat cu dimensiunea crioleziunii („mingea de gheață“) care ar fi necesară, pentru a crea o margine de siguranță de 1 cm în jurul tumorii (fig1). Ecografia intraoperatorie a fost efectuată pentru monitorizarea formării crioleziunii cît și extinderii acesteia pentru a asigura că distrucția cuprinde tumora hepatică în toate planurile, cu o margine de siguranță de 1 cm (fig2). Un ciclu dublu de congelare – încălzire realizat constă în: 15-20 minute de congelare la - 196 ° C cu crioleziunea extinsă dincolo de tumoră la 1 cm, urmată de o dezghețare de 10 minute și apoi de o recongelare de 15 minute.

Când tumorile au fost în vecinătatea pediculilor portali sau venelor hepatice, manevra Pringle a fost efectuată in timpul crioterapiei, pentru a evita efectul “heat sink“ (10), ce constă în încălzirea crioleziunii prin fluxul sanguin al vaselor mari. După retragerea criosondelor, toate traiectele restante au fost astupate cu Surgicel ® sau Gelaspon ®, pentru rezolvarea sângerărilor. În cazul ruperii crioleziunii, sângerarea subsecventă a fost oprită folosind una sau două suturi cu Prolene™ 4-0. Pentru ajutarea menținerii condițiilor normotermice, o pătură încălzitoare tip Bair Hugger ® a fost plasată peste părțile inferioare ale pacienților, iar în timpul criodistrucției, pacienții au primit ser fiziologic cald, dopamină în doză mică și bicarbonat, pentru a preveni hipotermia, pentru a crește debitul urinar și alcalinizarea urinei, pentru prevenirea leziunilor renale generate de mioglobinurie. La pacienții cu criodistrucție și rezecție hepatică, rezecția a fost folosită preferential pentru tumorile mari, din cauza relației dintre criovolum și fenomenul de criroșoc (insuficiența multiorganică, coagulopatia severă, coagularea diseminată intravascular), iar metastazele mai mici rămase în ficatul controlateral fost tratațe prin crioterapie (6). Rezecția hepatică a fost realizată prima, urmată de crioablația leziunilor rămase. Pentru rezecțiile majore hepatice și rezectiile anatomice minore am folosit tehnica abordului extraglissonian (7), transecția parenchimului hepatic fiind realizată folosind disectorul cu ultrasunete (Sharplan lasere, Inc, Needham, MA, USA) sau tehnica prin strivire. Manevra Pringle a fost folosită doar în cazuri selectate pentru a minimiza pierderea de sânge sau de a maximiza eficacitatea crioterapiei. La pacienții care au avut crioterapie asociată cu alte rezecții viscerale (rezecții colorectale, histerectomie cu salpingo-ovarectomie bilaterală, etc), crioterapia a fost efectuată după îndepărtarea tumorilor primare.

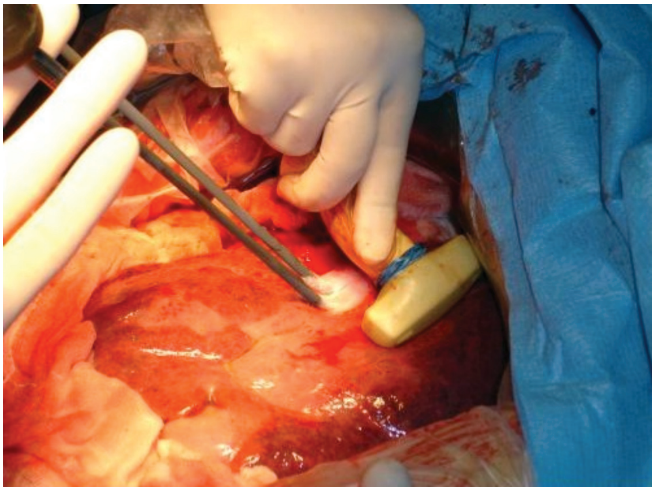

Fig. 1 *Criodistrucția, aspect intraoperator. Două criosonde plasate în leziune, monitorizate ecografic;*

Urmărirea postoperatorie

Toți pacienții au fost tratați în unitatea de terapie intensivă primele 24-48 h. Enzimele hepatice, factorii de coagulare și leucocitele au fost recoltate zilnic, până la normalizare, iar Rx-ul pulmonar și nivelul mioglobinei în ser și urină, doar la pacienții selectați. Primul scan CT de urmărirea s-a realizat în ziua a șaptea și a servit ca referință pentru evaluarea progresiei bolii. CT-scan cu contratst și RMN sistematice au fost efectuate la fiecare 3 luni în primul an de urmărire și la fiecare 6 luni după aceea. Markerii tumorali (CEA, AFP) au fost recoltati la intervale de

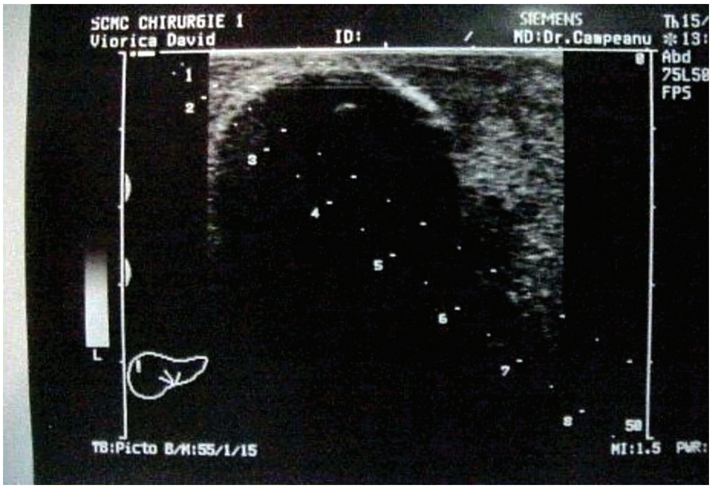

Fig. 2 *Aspect hipoecogenic al crioleziunii („mingea de gheaţă“) şi hiperecogenic la marginea crioleziunii;*

6 luni şi de fiecare dată când a apărut suspiciunea de boală recurentă. Chimioterapia postoperatorie adjuvantă intravenoasă a fost iniţiată în termen de 1 lună de la crioterapie, la pacienţii cu boli metastatice sincrone, la indicaţia specialistului oncolog.

Analiza statistică

Estimarea supravieţuirii s-a realizat prin metoda Kaplan-Meier iar diferenţa semnificativă a fost considerată pentru $p < 0.05$. Analizele statistice au fost prelucrate cu Microsoft Excel 2013 varianta cu WinSTAT şi XLstat adăugată.

Rezultate

Între februarie 2001 şi decembrie 2011, 86 de metastaze hepatice de cancer colorectal de la 27 de pacienţi au fost tratate prin crioterapie hepatică singură sau asociată cu rezecţii complexe la Spitalul Universitar Militar “Carol Davila“, Bucureşti. Cincizeci şi nouă din 86 metastaze au fost criodistruse, iar 27 au fost rezecate prin hepatectomii asociate criodistrucţiei. Unsprezece pacienţi au fost bărbaţi iar 16 pacienţi au fost femei, vârsta medie fiind de 58.8 ani (limite, 42-80). Douăzeci din 27 pacienţii au avut MHCC sincrone (74%) iar la 7 pacienţi au fost metacrone (26%). Doar 9 pacienţi (23.68%) au primit chimioterapie neoadjuvantă cu FOLFOX, iar 3 pacienţi (7.89%) au avut tumori cu localizări extrahepatice (metastaze pulmonare). Numărul tu-

Fig. 3 *Diagrama Kaplan-Meier; supravieţuirea pacienţilor în grupul cu metastaze hepatice de cancer colorectal.*

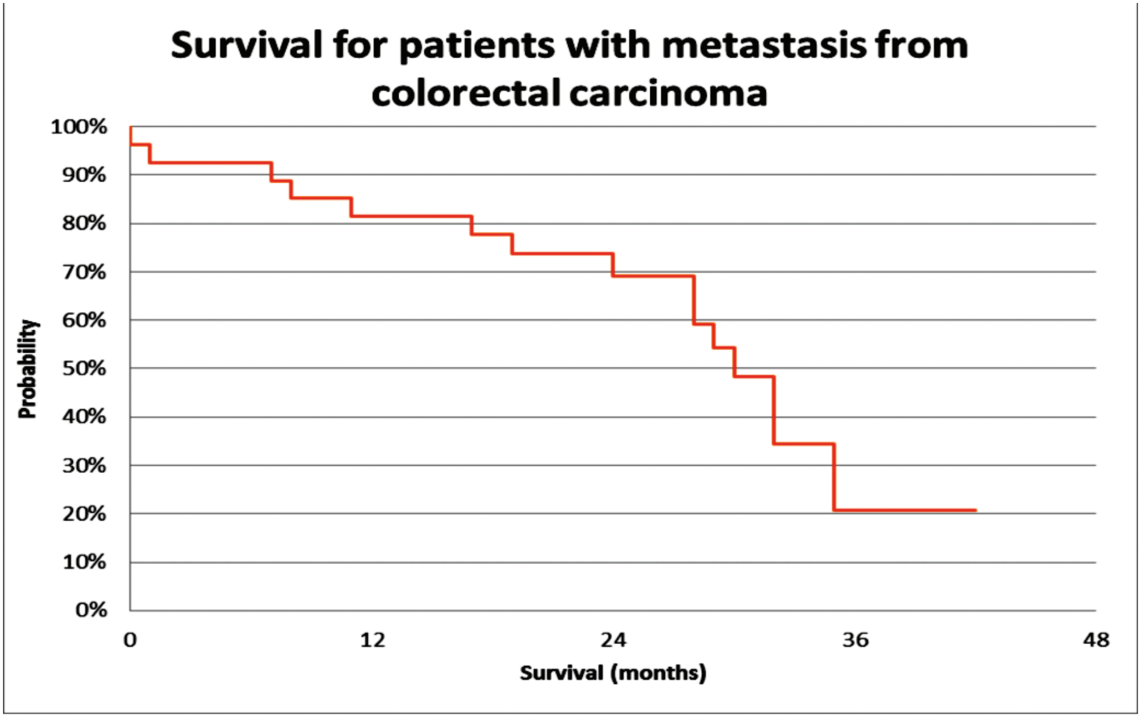

morilor hepatice tratate per pacient a variat de la 1 la 7 (în medie 3.18), iar dimensiunea leziunilor tratate a variat de la 10 la 60 mm (în medie de 32 mm). Tumorile au fost bilobare la 14 pacienţi (51.85%), localizate în imediata apropiere a vaselor mari la 9 pacienţi (33,33%) iar la 4 pacienţi (14.8) indicaţia pentru criochirurgie a fost starea clinică precară. La şapte pacienţi (26%), 10 noi tumori hepatice au fost descoperite în timpul ecografiei peroperative. La treisprezece pacienţi (48.14%) am folosit mai multe criosonde simultan (2 - 4) pentru a asigura tratamentul adecvat. Timpul mediu de crioablaţie pe tumora a fost 19.67 min (limite 10-85 min) iar timpul operator mediu a fost 180 min (limite 100-360 min). Rezectii hepatice asociate au fost efectuate la 10 pacienţi (37%), la 4 pacienţi rezecţii hepatice majore (trei segmente anatomice sau mai mult) iar la 6 pacienţi, rezecţii hepatice minore. În afară de rezecţiile hepatice s-au efectuat rezecţii intestinale la 11 pacienţi, histerectomie totală cu salpingo-ovarectomie bilaterală la 2 pacienţi şi colecistectomie la 8 pacienţi.

Tabel 1. *Caracteristicile pacienţilor, tumorilor şi tratamentul.*

Caracteristici	Nr. pacienti
Vîrsta (ani)	
(Medie) / [Limite]	(58.8) / [42-80]
Sex ratio	
Bărbaţi	11 (40.74%)
Femei	16 (59.25%)
Tumori	
Metastaze colorectale	27
Sincrone	20 (74.07%)
Metacrone	7 (25.92%)
Suptavieţuirea medie luni [limite]	27.4 [1.6-41.85]
Nr metastazelor tratate	86
Nr metastazelor tratate prin criodistrucţie	59
Tratament	
Nr metastazelor (medie) per pacient [limite]	(3.18); [1-15]
Nr metastazelor (medie) per pacient [limite]tratate prin criodistrucţie	(2.14); [1-7]
Dimensiuni tumori tratate prin crioterapie, (mediu); [limite]	(32 mm); [10-60 mm]
Număr criosesiuni (medie) per tumor [limite]	(1.76); [1-7]
Durata criosesiune (medie) şi [limite] per tumoră	(19.67 min); [10-85 min]
Operaţii asociate	
Rezecţii hepatice	*10*
Minore	*6*
Secţionectomie posterioară dreaptă	1
Secţionectomie laterală stângă	1
Segmentectomie anatomică	3
Rezecţie marginală	1
Majore	*4*
Hepatectomie dreaptă	3
Hepatectomie stângă	1
Rezecţii intestinale	*11*
Hemicolectomie dreaptă	5
Colectomie colon transvers	2
Sigmoidectomie	1
Hemicolectomie stângă	1
Rezectia anterioară de rect	2
Histerectomie totală cu anexectomie bilaterală	2
Colecistectomie	8
Mortalitatea asociată criodistrucţiei	1 (3.7 %)
Durata medie de spitalizare	18.2 (6-32 zile)

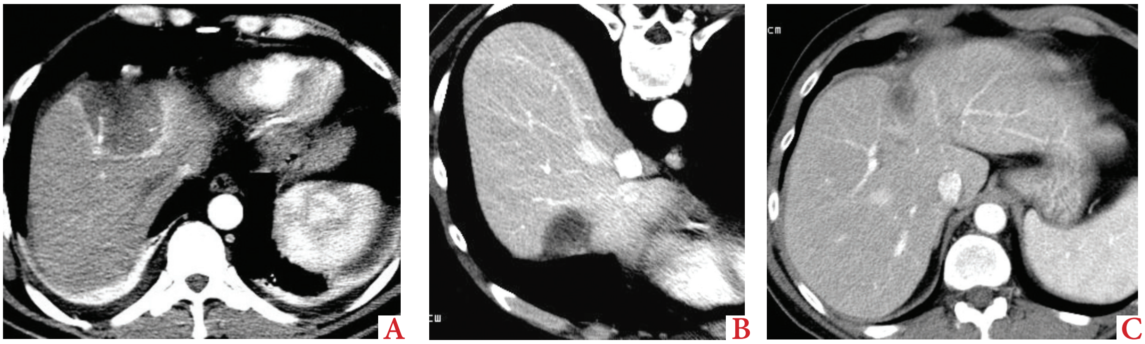

Fig. 4 *Aspectul crioleziunii. A - ziua 7; B - 1 lună; C - 3 luni*

Mortalitate și morbiditate

S-a înregistrat un singur deces postoperator (3,7%), la un pacient de 78 de ani cu metastaze colorectale bipolare sincrone și starea clinică precară (boală coronariană, denutriție, fibrilație atrială, BPOC) în ziua 17, din cauza insuficienței multiorganice și sindromului de detresă respiratorie (ARDS). La acesta, în timpul criodistrucției, hemoragia survenită a apărut, cel mai probabil, după o leziune la nivelul venei hepatice mijlocie, pacientul necesitând packing-ul abdomenului, pentru a fi controlată hemoragia. El a fost reoperat ulterior de două ori; în ziua 6, pentru o colecție abdominală și hematom subfrenic dreapt și în ziua 10, pentru peritonită generalizată datorită unei necroze parcelare la nivelul ileonului terminal, ce nu pot fi corelată cu crioablația. Morbiditatea generală s-a înregistrat la 15 pacienți (55.5%). Complicații intraoperatorii au avut 6 pacienți (22,2%) fiind reprezentate de fractura hepatică (iceball crack), tratată conservativ, folosind agenți hemostatici și suturi hepatice (tabelul 2). Doi pacienți au asociat pneumotorax în timpul mobilizării hemificatului dreptul din cauza bolii metastatice extinse. Una sau mai multe complicatii postoperatorii au fost înregistrate la 9 (33,3%) pacienți (tabelul 2). Hemoragia postoperatorie la nivelul crioleziunii s-a observat la 4 pacienți și a fost tratată conservator, folosind concentrat eritrocitar, plasmă și concentrat trombocitar. Pleurezia dreaptă, secundară procesului iritativ subdiafragmatic a fost înregistrată la 6 pacienți și tratată conservativ. Atelectazia pulmonară a fost prezentă la 4 pacienți, gastro-esofagita la 3 pacienți, colestaza pe termen lung la 1 pacient și citoliza intensă (limitele normale

Tabel 2 *Complicații peroperative și postoperative;*
**Pacienții au avut una sau mai multe complicații concomitente.*

Complicații	Nr pacienți
Complicații peroperatorii *	6 (22.2%)
Fractura crioleziunii (crack)	6
Deplasarea criosondei	2
Pneumotorax	2
Complicații postoperatorii *	9 (33.3%)
Hemoragie	4
Absces subfrenic	1
Absces subhepatic	1
Pleurezie	6
Atelectazie pulmonară	4
Infecția plăgii	2
Gastro-esofagită	3
Colestază (termen lung)	1
Citoliză intensă (50 x valorile normale)	3
Mioglobinuria tranzitorie	4

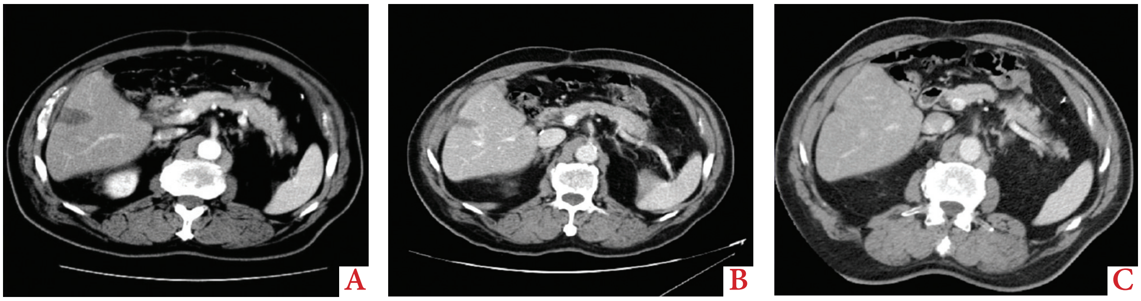

Fig. 5 *Hepatectomie stângă asociată criodistrucției. Aspectul crioleziunii. A - ziua 7; B - 1 lună; C - 3 luni.*

de 50 de ori) la 3 pacienți. Toate aceste complicații au fost tratate conservator. Complicațiile infectioase au apărut la 4 pacienți: infecțile plăgii la 2 pacienți, abces subfrenic la 1 pacient și subhepatic la 1 pacient. Cei doi pacienți cu abcese au fost reoperați, iar restul au fost tratați conservator. Mioglobinuria tranzitorie în diferite grade a fost evidențiată la 4 pacienți și a fost corelată cu volumul mare de țesut congelat și durata mare de crioablație (tabelul 2). Durata medie de spitalizare a fost 18.2 zile (6-32).

Supraviețuirea

Urmărirea medie postoperatorie a fost de 27.43 luni (limite 1.6-41.2) cu rata de supraviețuire la 1 an de 81.48%, 73.68% la 2 ani și 20.68% la 3 ani. Șaisprezece din 27 pacienți (59,2%) au rămas în viață pînă la ultimul consult, 7 pacienți (25,9%) nu au prezentat semne de recurență și 11 pacienți (40.7%) au decedat (tabelul 3).

Rata recidivelor

Supraviețuirea medie fără recurență de boală a fost 7,6 luni (limite 1-32 luni). Optsprezece din 27 de pacienți (66.66) au prezentat semne de recidivă, 7 pacienți nu au avut semne de recidivă, iar la 2 pacienți a fost dificil de apreciat recidiva.Unsprezece pacienți (40.14%) au decedat în timpul urmăririi postoperatorii, 9 dintre ei cu recidive hepatice și extrahepatice. Din cei 16 pacienți în viață, 4 pacienți (25%) au dezvoltat recidive la nivelul crioablației, evidențiate la CT-ul de control, iar 8 pacienți (50%) au dezvoltat recidivă hepatică, în altă parte decât la nivelul zonei de criodistrucție. Recidive extrahepatice au apărut la 6 pacienți (37.5%), 4 pacienți au prezentat numai metastaze pulmonare, iar 2 pacienți au avut metastaze răspândite la mai multe niveluri (creier, peritoneu, osoase).

Discuții

Rezecția chirurgicală rămâne tratamentul cel mai eficient pentru tumorile hepatice, dar 80-90% dintre pacienți cu tumori hepatice sunt improprii rezecției chirurgicale la momentul diagnosticului datorită: factorilor anatomici, prezenței bolii extrahepatice, rezervelor hepatice funcționale inadecvate și stării biologice precare (4, 12, 13, 14). Studiul evaluează rezultatele intermediare ale criochirurgiei ca tratament pentru MHCC, asociat sau nu cu rezecții complexe (rezecții hepatice și rezecții viscerale), cu

Tabel 3 *Supraviețuirea pacienților în grupul cu metastaze hepatice de cancer colorectal.*

Tum	**Nr. bv.**	**Supraviețuirea în ani**			**Suprv. medie (luni)**	**Bv. vii**	**Bv. decedați**	**Bv. cu rec.**	**Recidive (nr. bv.)**		
		1	2						**crio leziune**	**ficat**	**extra hep**
CRM	27	81.4%	73.6%	20.6%	27.4	16	11	7	3	3	6

intenția de a realiza un tratament cît mai complet oncologic (fig. 4-5). În prezent, nu există date publicate pentru tumorile hepatice tratate prin criochirurgie asociată rezecțiilor hepatice și rezecțiilor tumorilor primare în același timp operator, cu scopul de a salva țesutul hepatic.

În studiul nostru, 12 pacienți au avut criodistrucție singulară și 15 din 27 pacienți (55.5%) au avut tratament complex, asociind criodistrucția cu rezectiile hepatice sau cu alte rezectii viscerale (tabelul 1). Patru pacienți au avut crioablație asociată numai cu rezecții hepatice; doi pacienți au asociat rezecții hepatice majore (1 hepatectomie dreaptă și 1 hepatectomie stangă) și 2 pacienți au suferit rezecții hepatice minore (1 secționectomie laterală stângă și segmentectomie S4a la 1 pacient). La trei pacienți, criochirurgia a fost asociată rezecțiilor intestinale (1 hemicolectomie dreaptă, 1 colectomie de colon transvers și 1 colectomie stângă). Doi pacienți au avut crioablație asociată cu histerectomie totală cu anexectomie bilaterală și rezecție de rectosigmoid în același timp. Șase din 15 pacienți au avut crioablație combinată cu rezecții hepatice și colorectale în aceeași sesiune operatorie.

La doi pacienți s-au practicat rezecții hepatice majore (hepatectomie dreaptă) asociate cu hemicolectomie dreaptă la 1 pacient și colectomie de transvers la celălalt pacient. La patru pacienți, am efectuat rezecții hepatice minore (secționectomie posterioară dreaptă la 1 pacient, segmentectomie S6 la 1 pacient, segmentectomie S5 la 1 pacient și 4 rezecții marginale 1a 1 pacient) asociate cu hemicolectomie dreaptă la 3 pacienți și sigmoidectomie la 1 pacient. Colecistectomia s-a practicat la 8 pacienți (tabelul 1). Există raportate peste 2000 de cazuri tratate prin crioterapie pentru tumori hepatice maligne și relativ puține studii au publicat rata de supraviețuire la 5 ani (15, 16, 17).

După rezecția chirurgicală, rata de supraviețuire la 5 ani, variază de la 20 la 35%, iar fără rezecție este mai mică de 5% (18-19). Supraviețuirea globală medie la pacienții cu cancer colorectal cu metastaze hepatice este 24 de luni și de aproximativ 3 luni pentru pacienții nerezecabili (18, 19). În studiul nostru de supraviețuirea generală medie a fost 25,8 luni. Acest rezultat este favorabil comparativ cu rezultatul altor studii pentru crioterapia tumorilor hepatice, care au demonstrat o supraviețuire medie de luni 18-38 (20, 21). Ca prognostic favorabil am găsit asocierea crioterapiei cu rezecția, tratamentul prin crioterapie a leziunilor mai mici de 3 cm, eradicarea totală a tumorii, valorile normale ale markerilor tumorali și lipsa recidivei la nivelul crioablației, în restul ficatului și extrahepatic.

Pentru a maximiza distrugerea tumorală cele mai multe tumori sunt tratate cu cicluri duble de inghețare-dezghețare. Crioleziunea este foarte ușor de urmărit ecografic, apărând ca o zona de hipoecogenică cu o umbră posterioară în jurul vârful sondei. Azotul lichid la - 196 ° C livrat la vârful criosondei, induce cristalizare intracelulare în timpul procesului de congelare rapidă, urmată de ruperea membranelor celulare, tromboză microvasculară și moartea celulelor (22, 23). În trecut, criochirurgia a fost realizată la pacienți cu tumori hepatice nerezecabile (13, 24-26) sau ca tratament definitiv pentru metastaze hepatice resecabile, în încercarea de a salva cît mai mult parenchimul hepatic normal (27-30). Au fost raportate rezultate încurajatoare cu supraviețuiri la 5 ani de 44-58% după criochirurgie pentru tumorile nerezecabile (13, 25), dar incidența ridicată a recidivei în ficat, în afara zonei tratate sau la locul crioablației, este un factor negativ. Gignoux et al. (17) descriu, după revizuirea datelor din literatură pentru criodistrucție, o rată de recidivă hepatică între 59 și 82% și o rată de recidivă extrahepatică între 25 și 66%.

În ceea ce privește recidiva la nivelul crioablației, rezultatele noastre subscriu rezultatelor raportate de către alții autori, cu valori cuprinse între 19 și 44% (16, 21, 28, 31). Localizarea tumorilor în vecinătatea structurilor venoase majore cu fluxul mare și zona posterioară a crioleziunii, unde ecogenicitatea zonei hepatice afectate (mingea de gheață) face ca marginile tumorii să fie nesigur determinate (16, 32) sunt motivele principale pentru recidiva locală după criodistrucția hepatică.

Concluzii

Criochirurgia rămâne o opțiune importantă pentru tratamentul de MHCC și poate oferi o supraviețuire pe termen lung. Asociată cu rezecțiile hepatice și viscerale în aceeași sesiune operatorie, cu scopul de a proteja cît mai mult parenchimul hepatic, criochirurgia este o opțiune eficientă pentru a asigura un tratament oncologic, în anumite condiții. Rata recidivelor locale indică utilizarea cu precauție a criochirurgiei ca singur tratament pentru leziunile hepatice rezecabile. Strategia chirurgicală agresivă complexă reprezintă o abordare atractivă, mai multe studii controlate fiind necesare, pentru a evalua în mod corespunzător această modalitate.

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33. Fig. 1 Criodistrucția, aspect intraoperator . Două criosonde plasate în leziune, monitorizate ecografic;
34. Fig. 2 Aspect hipoecogenic al crioleziunii ("mingea de gheață") și hiperecogenic la marginea crioleziunii;
35. Tabel 1. Caracteristicile pacienților, tumorilor și tratamentul.
36. Tabel 2. Complicații peroperative și postoperative; *Pacientii au avut una sau mai multe complicatii concomitente.
37. Tabel 3. Supraviețuirea pacienților în grupul cu metastaze hepatice de cancer colorectal.
38. Fig. 3 Diagrama Kaplan-Meier; supraviețuirea pacienților în grupul cu metastaze hepatice de cancer colorectal.

Accesul vascular intraosos în condiţii de urgenţă

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Rezumat

În situațiile de reală urgență, accesul vascular rapid poate face diferența între viață și moarte. În prespital, accesul vascular rapid are o importanță deosebită deoarece condițiile medicale sunt suboptimale, iar inserția unui cateter venos central este de obicei nefezabilă. Din fericire, a fost redescoperită o nouă tehnică pentru obținerea accesului vascular – administrarea intraosoasă. Administrarea intraosoasă a medicamentelor și a fluidelor, limitată în ultimii ani numai la uzul pediatric, redevine o rută viabilă pentru adulți. Această tehnică a fost folosită pe larg de către medicii militari în timpul celui de-al doilea război mondial pentru a salva viețile soldaților răniți, dar a fost rapid abandonată din cauza sistemului de urgență slab dezvoltat de după război.

Summary

In real emergency situations, rapid vascular access can make the difference between life and death. In prehospital settings, rapid vascular access has a tremendous importance because the medical conditions are suboptimal, and central venous access is usually unfeasible. Fortunately, it was rediscovered a new technique for vascular access – intraosseous administration. Intraosseous drugs and fluids administration, limited in the past few years only for pediatric use, once again becomes a viable route for adults. This technique was widely used by military physicians during the Second World War to save the lives of wounded soldiers, but was quickly abandoned due to poor emergency system developed after the war.

Cuvinte cheie: *ACCES VASCULAR RAPID, ADMINISTRARE INTRAOSOASĂ, PACIENŢI CRITICI, RESUSCITARE CARDIORESPIRATORIE*

A. Introducere

În situațiile de urgență maximă, accesul vascular rapid poate face diferența între viață și moarte. În situațiile din prespital, accesul vascular rapid are o importanță deosebită, deoarece condițiile de acordare a ajutorului medical sunt frecvent suboptimale, iar inserția unui cateter venos central este de obicei greu de realizat.

Tehnica denudării venoase este rareori practicată, și chiar și în condiții optime în spital, este consumatoare de timp și dificil de realizat din punct de vedere tehnic.[18,21] Folosirea căii endotraheale pentru administrarea medicației de resuscitare, deși a fost relativ frecvent folosită în urgențe este limitată de plasarea în prealabil a sondei de intubație orotraheale, de numărul relativ redus de medicamente care poate fi folosit în aceste condiții și de ratele de absorbție imprevizibile. Din aceste motive administrarea pe sonda IOT a medicației de resuscitare a fost descurajată la ultima revizuire a protocoalelor de resuscitare cardiopulmonară europene (ERC 2010).[1,58]

În ultimii ani a fost redescoperită o nouă tehnică pentru obținerea accesului vascular – administrarea intraosoasă - AIO. Administrarea intraosoasă a medicamentelor și a fluidelor, limitată în ultimii ani numai la uzul pediatric, redevine o rută viabilă pentru adulți. Această tehnică a fost folosită pe larg de către medicii militari americani și nu numai în timpul celui de-al doilea război mondial pentru a salva viețile soldaților răniți, dar a fost rapid abandonată din cauza sistemului de urgență slab dezvoltat de după război.[18]

Modul de tratament al urgențelor a evoluat de la modul „scoop and run“, când se punea accent pe ajungerea cât mai rapidă la spital, iar ambulanțele ofereau tratament redus sau deloc la locul producerii accidentului/urgenței, la modul „stay and play“ care oferă o importanță maximă stabilizării pacientului la locul unde s-a produs accidentul/urgența medicală, unde accesul vascular are o maximă importanță.[18,56,57,58] De aceea există un interes foarte mare pentru accesul intraosos la pacienții la care accesul intravenos standard este dificil sau imposibil, modalitate care s-a dovedit neprețuită pentru tratamentul pacienților critici.[18,21,53] Este sigur, ușor de realizat, rapid și eficient. Pentru personalul medical înlătură o parte din stresul tratamentului pacienților în șoc prin furnizarea unei căi de rezervă pentru realizarea accesului vascular pentru aproape orice urgență.[18,52,53,55]

Primul studiu științific asupra accesului intraosos a fost realizat de către C.K.Drinker de la Universitatea Harvard în 1922 care a examinat circulația venoasă a sternului și a propus spațiul intraosos ca și venă care nu este supusă colapsului. A dovedit experimental că substanțele infuzate în măduva osoasă au fost repede absorbite în circulația centrală.[2]

În 1940, L.Tocantis și J.F.O'Neill confirmă că toate cavitățile medulare ale oaselor lungi, pot fi folosite ca și cale pentru accesul vascular.[3,4] Colorantul roșu injectat în spațiul intraosos tibial la iepuri a apărut în circulația cardiacă în 10 secunde de la administrare.[5]

În 1942, E.M.Paper stabilește că timpii de circulație pentru fluidele administrate intraosos și intravenos sunt aproape identici.[6]

În 1944 medicul englez Hamilton Bailey a stabilit utilitatea accesului intraosos sternal în timpul penelor de curent din Londra și a proiectat un trocar special care să nu afecteze cordul atunci când este introdus.[7]

În timpul celui de-al doilea război mondial, perfuzia intraosoasă a fost folosită pe scară largă de către medicii militari pentru salvarea vieților soldaților răniți. Sunt documentate peste 4000 de cazuri la care a fost folosit accesul intraosos în așa fel încât armata SUA a considerat infuzia intraosoasă ca și practică standard pentru tratamentul soldaților grav răniți.[9] Înainte de acceptarea perfuziei intraosoase ca și cale de acces vascular au fost înregistrate multe victime din cauza hemoragiei, datorită lipsei de abord venos la pacienții în șoc. Primul pacient salvat prin accesul intraosos și perfuzia consecutivă a fost un soldat de 19 ani al echipajului unui avion B-29 care a fost grav rănit în timpul unui zbor deasupra Japoniei.[9,18]

Din nefericire, experiența dobândită în timpul războiului nu s-a transmis de la medicii militari către sistemul medical civil după terminarea războiului, pentru că până în anii `60 nu au existat organizații de paramedici. Înainte de 1968 când a avut loc înființarea primului serviciu de medicină de urgență în Chicago de către David R. Boyd, pacienții erau transportați la spital de către trăsuri sau ambulanțe fără ca să li se acorde primul ajutor la locul producerii accidentului/ urgenței sau în timpul transportului. Un alt factor care a contribuit la declinul dispozitivelor intraosoase a fost introducerea cateterelor de plastic care au avut o popularitate în creștere. Introducerea serviciilor de urgență și a paramedicilor a fost realizată pe lângă centrele de traumă sub îndrumarea traumatologilor și nu sub îndrumarea medicilor militari, de aceea utilitatea și importanța accesului intraosos a rămas în umbră, nemeritat.[18]

Redescoperirea accesului intraosos este meritul unui pediatru american, James Orlowski care a vizitat India în timpul unei epidemii de holeră. Acolo a observat importanța accesului intraosos la copii, care altfel ar fi murit în absența administrării de fluide și medicamente pe cale intraosoasă.[10] Articolul publicat de acesta la întoarcerea în SUA „*My kingdom for an Intravenous Line*“ din 1984 a reintrodus accesul intraosos (AIO) în uzul pediatric, fapt care a fost consfințit prin introducerea manevrei ca și standard de îngrijire în ghidul Pediatric Advanced Life Support (PALS) din anii `80.

B. Anatomie și fiziologie.[18,21,37]

a. Anatomie. În caz de șoc sau traumă venele periferice colabează adesea, dar spațiul intraosos este înconjurat de os, este conectat direct cu circulația centrală și rămâne indemn în absența traumei osoase.

Spațiul intraosos are o structură unică, conținând mii de mici vase sanguine care se intersectează și care nu colabează, care acționează ca un burete și care absorb orice fluid cu care vin în contact. Substanțele infuzate sunt apoi rapid transportate în sistemul venos central. Fluxul sanguin în spațiul intraosos este relativ constant în majoritatea cazurilor de șoc. Presiunea sanguină în spațiul intraosos este de aproximativ 35/25 mmHg, cam o treime din presiunea arterială.

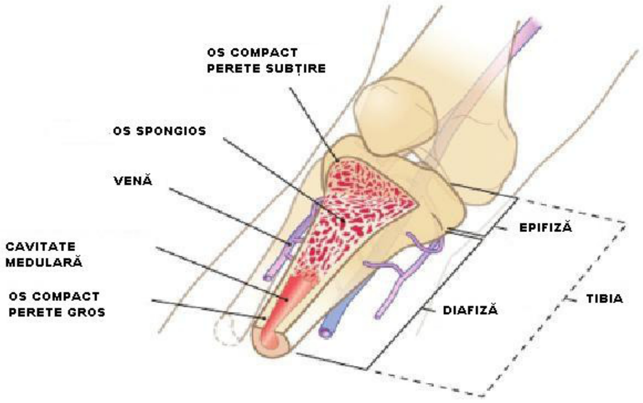

Figura nr.1. ***Anatomia osului lung.*** [după 18,21,37 modificat]

Toate oasele lungi din organism au două extremități (epifize) și un corp (diafiza). (vezi figura nr.1). Epifizele conțin os spongios și sunt acoperite de cortex, alcătuit din material dur. Diafiza este compusă din cortex dur și dens (os compact) și prezintă o cavitate la interior (cavitatea medulară). Platoul epifizar este joncțiunea dintre epifiză și diafiză prin intermediul căruia osul crește în timpul copilăriei.

Termenul de ***intraosos*** (vezi figura nr.2) se referă la osul spongios epifizar și cavitatea medulară diafizară, care sunt interconectate. Vasele spațiului intraosos se conectează cu circulația centrală printr-o serie de canale longitudinale (canale Haversiene) care conțin fiecare mici artere și vene. Canalele Haversiene sunt interconectate printr-o serie de canale Volkmann (care conțin de asemenea mici artere și vene) care penetrează cortexul osos dur pentru a conecta vasele intraosoase cu arterele și venele majore ale circulației centrale.

Spațiul intraosos este alcătuit din măduvă osoasă, care este alcătuită din sânge, o rețea de celule care produc sânge și țesut conjunctiv. Măduva roșie se află în osul spongios și conține o mare cantitate de sânge. Măduva galbenă se află în cavitatea medulară a oaselor lungi ale adultului. Copiii și sugarii au numai măduvă roșie. Cu avansarea în vârstă o parte a măduvei roșii este înlocuită cu celule adipoase și devine măduvă galbenă. Fluidele și medicamentele care sunt infuzate fie în măduva roșie, fie în cea galbenă ajung rapid în circulația centrală.[12]

b. Microanatomia (vezi figura nr.3). Spațiul intraosos este limitat de endosteum, care este alcătuit din celule specializate scuamoase. Unele celule ale endosteumului, hematocitoblastele, produc componente ale sângelui, cum ar fi hematiile, leucocitele și plachetele sanguine. Alte celule ale endosteumului produc celule stem, care se pot transforma în aproape orice țesut.

C. Dispozitivele intraosoase

a. Generalități.

Dispozitivele inserate manual sunt folosite în special în pediatrie și sunt de fapt ace cu trocar modificate pentru a preveni obstrucția cu fragmente osoase în timpul inserției. Au diferite suporturi și mânere pentru a permite introducerea acului prin presiune și rotație. Orificiul de penetrare în os este adesea asimetric datorită rotației manuale și pot rezulta pierderi. Deși foarte populare în pediatrie, dispozitivele de puncție intraosoasă au pătruns destul de timid pe piața produselor pentru adulți, datorită materiei osoase mai dure și mai greu de penetrat de la adulți.[12,18]

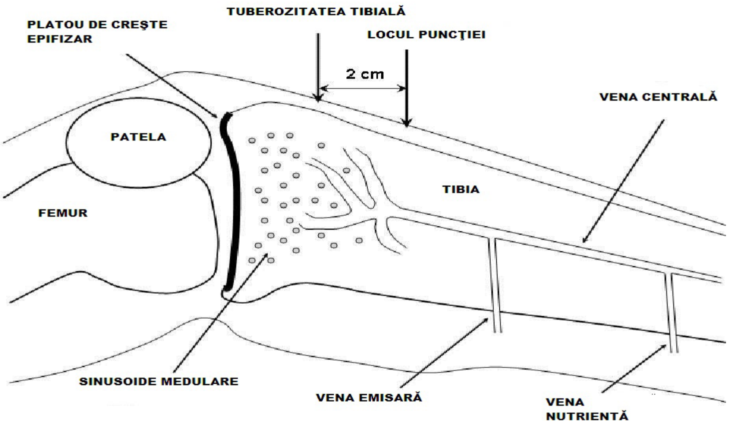
PLATOU DE CREŞTE EPIFIZAR
TUBEROZITATEA TIBIALĂ
LOCUL PUNCŢIEI
2 cm
VENA CENTRALĂ
PATELA
FEMUR
TIBIA
SINUSOIDE MEDULARE
VENA EMISARĂ
VENA NUTRIENTĂ

Figura nr.2 ***Anatomia spaţiului intraosos*** *după 18,21,37 modificat.*

1. F.A.S.T.1.[19,45,46,59] În 1997, FDA a aprobat utilizarea F.A.S.T.1 - primul dispozitiv pentru realizarea puncției intraosoase la adult - care folosea ca și loc de acces sternul (vezi figura nr.4). Acest dispozitiv de unică folosință utilizează un mănunchi de 10 ace pentru a se poziționa precis fără a penetra sternul. Pe măsură ce crește presiunea exercitată asupra dispozitivului, un ac central pătrunde cu precizie în spațiul sternal medular. Procedura este asistată de un arc intern care propulsează acul central în interiorul sternului.

Designul dispozitivului previne penetrarea accidentală prin stern. După utilizare, acul de puncție intraosoasă este extras cu ajutorul unui dispozitiv special. A fost folosit pe scară largă atât pentru militari, cât și de către populația civilă.

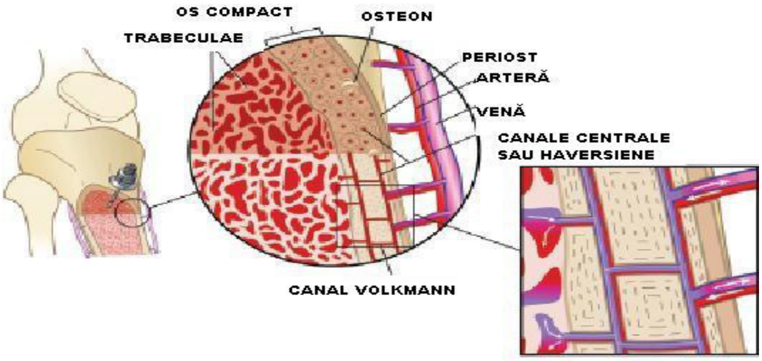
OS COMPACT
TRABECULAE
OSTEON
PERIOST
ARTERĂ
VENĂ
CANALE CENTRALE SAU HAVERSIENE
CANAL VOLKMANN

Figura nr.3 ***Microanatomia osului lung.*** *după 18,21,37 modificat*

2. Bone Injection Gun (B.I.G.).[18,33,38,44,60] BIG a fost aprobat de FDA în anul 2000 pentru realizarea puncției intraosoase la nivelul tibiei și a fost conceput și folosit de către armata israeliană pentru mai mult de zece ani. BIG a fost dezvoltat de către medicul Waisman Marc (figura nr.5). După înlăturarea piedicii de siguranță, operatorul declanșează dispozitivul și un resort puternic propulsează acul cu trocar în spațiul medular tibial. Deși a fost aprobată utilizarea doar la nivelul tibiei pentru SUA, în Israel se folosește de asemenea la nivelul maleolei mediale, radiusului distal și la nivelul capului humeral.

3. EZ-IO.[45,46,60] În 2004 FDA a aprobat EZ-IO, primul dispozitiv cu baterie pentru realizarea puncției intraosoase. Diferă de predecesorii săi prin aceea că prezintă un ac cu design special, cu burghiu la vârf, pentru a pătrunde în spațiul intraosos. Spre deosebire de celelalte dispozitive care au acul propulsat de către un resort, înaintarea acului EZ-IO este făcută uniform, până la profunzimea dorită, iar orificiul de penetrare este simetric și are dimensiuni identice cu ale acului cu trocar. Rezultatul este un flux uniform de perfuzie, fără pierderi. FDA a aprobat folosirea EZ-IO la nivelul tibiei proximale și la nivelul capului humeral. Există și o versiune pediatrică a EZ-IO PD care se utilizează pentru copiii de până la 39 kg și care folosește pentru inserție același dispozitiv pe bază de baterie ca și la adulți.

b. Accesul vascular- tehnică medicală de urgență esențială.

1. Accesul intravenos este necesar pentru a trata majoritatea cazurilor de șoc, status mental alterat, insuficiență respiratorie, traumă și resuscitare cardio-pulmonară. Asistenții medicali sunt antrenați pentru realizarea accesului intravenos în prespital și în Unitatea de Primiri Urgențe. Accesul intravenos este obținut în proporție de 90-95% din cazuri, dar în șoc, circulația se centralizează și venele periferice se colabează, astfel încât cu cât este necesar mai urgent abordul intravenos, cu atât mai dificil este de obținut. În aceste cazuri există rapoarte cu până la 20% rată de insucces.[1,18,20,58]

2. Rute alternative. Aceste rute care pot fi folosite dacă nu poate fi folosit abordul intravenos sunt: sonda de intubație orotraheală, injecția intramusculară, subcutanată, percutană, administrarea intrarectală, intranazală, sublinguală și respectiv pe cale inhalatorie. Toate căile amintite pot fi utile în anumite situații, dar în urgențele vitale multe din aceste căi sunt ineficiente.[1,18,20,58]

Figura nr.4 ***FAST 1***

Figura nr.5 ***BIG pentru adulți și copii***

Administrarea traheală de medicamente. Ghidurile ERC 2005 care recomandau calea endotraheală în doze de 2-4 ori mai mari ca și cele utilizate pentru administrare intravenoasă, ca a doua alegere pentru administrare, au fost modificate în concordanță cu studiile care constatau ineficiența și inconstanta absorbție a medicamentelor administrate astfel.[1,20,58]

Un studiu recent a găsit diferențe semnificative în supraviețuirea unor pacienți care au primit medicație pe sonda endotraheală vs administrarea pe calea intravenoasă, fără ca să fie supraviețuitori în grupul care a primit medicație endotraheală compa-

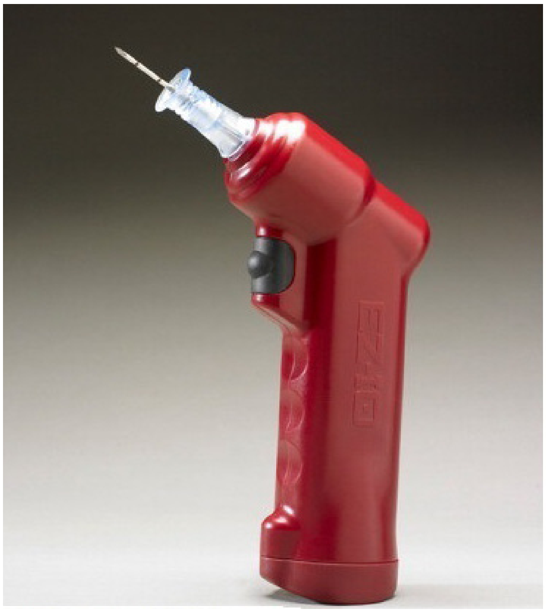

Figura nr.6 ***EZ-IO***

rativ cu un procent de supraviețuitori de 5% în grupul care a primit medicație intravenoasă.[17] Totuși, pacienții care au primit medicație pe sonda endotraheală au fost pacienții la care accesul intravenos a fost mai dificil, deci ar reprezenta un subgrup de pacienți mai bolnavi.[17]

Accesul intraosos (i.o.). Începând cu 2010, ghidurile ERC recomandă puncția intraosoasă (i.o.) ca și cale de abord intravascular de a doua alegere dacă abordul endovenos nu a putut fi obținut, cu administrarea de medicamente în doze identice cu cele pentru administrarea intravenoasă, pentru că farmacocinetica și farmacodinamica medicamentelor este identică pentru cele două căi de administrare a medicamentelor.[1,11,58]

Ghidurile ACLS pentru stop cardiorespirator recomandă administrarea de medicamente i.v./i.o. pentru cazurile de activitate electrică fără puls, asistolă, fibrilație ventriculară refractară și tahicardie ventriculară fără puls, acolo unde defibrilarea nu a avut succes. Nu se recomandă cateterizarea venoasă centrală pentru majoritatea cazurilor de stop cardiorespirator. Pentru adulți, accesul intraosos este similar cu accesul venos central.[1,20,58]

Accesul intraosos (i.o.) la adulți este sigur și eficient, fapt demonstrat de studii bine conduse efectuate pe adulți și populație pediatrică. Dacă accesul intravenos nu este posibil, ar trebui obținut accesul intraosos.[1,58]

Administrarea medicamentelor pe cale intraosoasă este preferată celei endotraheale, pentru că se obține un efect farmacologic și farmacodinamic mult mai previzibil.[1,58]

În protocoalele și algoritmii ACLS actuali, calea intraosoasă este considerată echivalentă administrării intravenoase și este recomandată înainte de abordul venos central, cu mult înainte de administrarea endotraheală pentru administrarea medicației în cazul unui stop cardiorespirator.[1,58]

2.1. Abordul venos central vs accesul intraosos. Atunci când abordul venos periferic nu poate fi obținut, se poate încerca abordul venos central. De cele mai multe ori în prespital, atunci când abordul venos periferic nu poate fi obținut, se declară acest lucru numai după câteva încercări nereușite, care ar trebui luate în calcul atunci când se determină timpul de acces endovascular. Chiar dacă se poate obține abordul venos central din prima încercare, deși nu se întâmplă întotdeauna așa, timpul total până la obținerea liniei endovasculare trebuie să ia în calcul timpul necesar pentru introducerea ghidului, retragerea acului de puncție, introducerea și retragerea depărtătorului, introducerea cateterului propriu-zis și apoi suturarea la piele cu efectuarea pansamentului steril ulterior. Timpul real estimat pentru obținerea abordului endovenos este mai degrabă mai aproape de cca 15 minute.[21,45] Timpul real poate fi chiar mai lung dacă luăm în calcul și necesitatea efectuării altor manevre pregătitoare cum ar fi poziționarea, monitorizarea și prepararea sterilă a locului de abord venos și a materialelor necesare.[21,45,50]

Obținerea accesului venos central ca și alternativă la obținerea accesului intravenos periferic este grevată de multiple riscuri, costuri crescute și un timp de montare care este prea mare pentru cazurile critice. Complicațiile liniilor centrale includ: sepsis, infecția locală, puncția pulmonară, puncția arterială și formarea de hematoame mari, perforarea cordului, hemotorax, hidrotorax și afectarea nervoasă. FDA raportează anual un număr de până la 400.000 pacienți pe an internați pentru complicații ale plasării liniilor centrale și ale altor catetere intravenoase. În plus, mortalitatea care poate fi atribuită acestor complicații poate atinge 10-20%.[13]

2.2. Accesul intraosos. Puncția intraosoasă pentru adulți este o modalitate de abord intravascular care poate fi realizată la un pacient in extremis.

În comparație cu abordul central, puncția intraosoasă la adulți este mult mai sigură și mai rapidă, iar complicațiile sunt mult mai rare.[8,14,36] Timpul mediu de inserție pentru unele dipozitive de puncție intraosoasă este mai mic de 10 secunde. În consecință, personalul medical de urgență poate administra medicația și fluidele necesare mult mai rapid. După realizarea abordului intravenos, medicul de urgență se poate concentra pe aspectele mult mai importante ale managementului căilor aeriene, aritmiilor, resuscitării volemice, managementul plăgilor și reducerea timpului de lucru la caz. Abilitatea de a realiza rapid abordul intravascular crește semnificativ calitatea actului medical. Există sisteme medicale de urgență care au eliminat încercările de a obține abordul intravenos în caz de stop cardiorespirator, iar paramedicii sunt instruiți să recurgă direct la realizarea abordului intraosos, caz în care se câștigă o medie de aproximativ 8 minute.[14,26,31]

2.2.1. Indicații și contraindicații.[21,36]

Indicații. Indicația de bază pentru puncția și accesul intraosos este necesitatea obținerii unui acces vascular de urgență, atunci când metodele convenționale au eșuat.[22] Protocoalele ATLS recomandă folosirea accesului vascular intraosos înaintea obținerii accesului vascular central pentru pacienții politraumatizați cu vârsta sub 6 ani.[28] Utilizarea accesului vascular intraosos la pacienții pediatrici cu traume majore indiferent de vârstă este susținută de Guy și colaboratorii.[23] Alte situații în care folosirea puncției și accesului vascular intraosos este recomandată sunt: trauma, stopul cardiorespirator, instabilitatea hemodinamică, șocul profund, sepsisul necontrolat, insuficiența respiratorie acută, intoxicațiile care necsită administrarea imediată de antidot, obezitatea morbidă, urgențele diabetice, supradozele de droguri, status epilepticus, agitația severă, deshidratarea severă la bătrâni și arsurile grave.[24,25,36]

Contraindicații. Singura contraindicație absolută acceptată universal este fractura unui os lângă locul de acces.[26] Unii autori consideră că și osteogenesis imperfecta și osteoporoza ar trebui considerate ca și contraindicații absolute.[27] Alte contraindicații ar fi procedurile chirurgicale ortopedice în locul de inserție a AIO, circulația sangvină locală compromisă, AIO în același loc în ultimele 24 de ore și prezența unei proteze la nivelul membrului respectiv.[36] Contraindicațiile relative pentru puncția intraosoasă includ celulita la nivelul locului de puncție și patologia/ leziunile venei cave inferioare.[26]

2.2.2. Selecția locului de puncție. Tehnică.[21,36,50]

Locul de puncție se alege în funcție de vârstă și de densitatea osoasă a pacientului. Locul convențional de puncție este tibia proximală la copii pentru evitarea cartilajului de creștere epifizal. Pentru adulți, la fel ca și la copii, cea mai frecventă alegere este tibia proximala medial și inferior de tuberozitatea tibială anterioară. Humerusul proximal este utilizat pentru pacienții cu trauma, cu arsuri și cu obezitate morbidă. Ruta sternală este folosită mai ales în medicina militară.[36]

Confirmarea poziționării corecte a acului în spațiul medular poate fi confirmată prin trei metode[21,36,50]:

- acul de puncție ar trebui să stea fără nici o susținere din exterior;
- aspirarea de măduvă osoasă prin acul introdus după retragerea trocarului;
- administrarea unui bolus de 5-10 ml de ser fiziologic fără rezistență și fără extravazare, fapt care va fi confirmat prin observarea atentă a locului de inserție pentru tumefiere sau decolorare.

În principiu trebuie realizată numai o singură încercare pentru fiecare os. Mai multe puncții în periost pot produce extravazarea de fluid în țesuturile moi de vecinătate. Totuși, dacă acul de puncție este obstruat, poate fi înlăturat și un nou ac poate fi introdus pe același traiect.[29]

Poziționarea acului intraosos la nivelul tibiei distale.[29] Pentru a evita puncționarea venei safene la copii se va aborda tibia distală la 1-2 cm superior de maleola medială, cu acul orientat la 10-15 grade distal de platoul de creștere (în această situație cu orientare cefalică). Se va plasa și se va fixa genunchiul în poziție ridicată de la planul patului în ușoară flexie. Se va identifica tuberozitatea medială (proeminența rotunjită aflată sub rotulă la ~ 2 cm).

De la tuberozitatea medială se va palpa tibia ~ 2 cm spre medial până se va identifica o suprafață plată (platoul tibial). De la platoul tibial în cazul adulților se va palpa tibia ~ 1 cm spre proximal (în sus) către rotulă, iar pentru uz pediatric se palpează tibia ~ 1-2 cm spre distal (în jos). Profunzimea penetrării în cazul copiilor se alege în conformitate cu vârsta pacientului: 0-3 ani 0,5-1 cm, 3-6 ani 1-1,5 cm, 6-12 ani 1,5 cm.[18,29]

Tehnica de inserție pentru B.I.G.[33,38,44] Se aseptizează zona și cu mâna nedominantă se poziționează ferm dispozitivul B.I.G. la un unghi de 90^0 față de suprafața tegumentului. Se înlătură piedica de siguranță, iar extremitatea distală se poziționează ferm pe platoul tibial. Se plasează degetele 2 și 3 ale mâinii dominante sub „aripile“ dispozitivului cu palma deasupra extremității proximale. Prin apăsare cu palma se va declanșa B.I.G. nefiind necesară forța.

După această manevră se va retrage stiletul din trocar și se va fixa piedica de siguranță la nivelul tegumentului pentru stabilitate adițională. Se poate aspira măduva osoasă pentru analize de laborator (lipsa aspirării măduvei osoase nu înseamnă că dispozitivul nu este plasat corect. Se va spăla trocarul cu ser fiziologic (10-20 ml pentru adulți și respectiv 5-10 ml pentru uz pediatric) înainte de injectarea de fluide și medicamente. La pacienții conștienți se preferă administrarea unui anestezic local înainte de injectarea de fluide și medicamente.

2.2.3. Locuri de puncție intraosoasă alternative.[18,26,29,33,38,44] De remarcat este că pentru copii, locul recomandat pentru puncția intraosoasă este tibia proximal. Pentru adulți, în afara tibiei proximale, ca și locusuri alternative pot fi folosite capul humerusului proximal, maleola medială proximal și respectiv capul radiusului distal.

Humerusul proximal (capul humerusului).[18,33,38,44] Pentru a se determina locul de puncție se poate localiza capul humeral prin două metode.

Abordul coracoid. Se poziționează umărul în adducție (cu palma pe ombilic). Se unesc acromionul și procesul coracoid cu o linie imaginară, iar de la jumătatea acestei linii se palpează spre distal ~ 2 cm, determinând astfel capul humeral unde se va puncționa. La unii pacienți, aria unde capul humeral este cel mai aproape de tegument este proximal ~ 1 cm către torace.

Abordul clavicular. Se palpează clavicula până la capătul lateral al acromionului. Locul de puncție este distal la ~ 2 cm unde se află capul humerusului.

Sternul.[36,44,47,48] Locul de puncție are o popularitate în scădere deoarece interferă cu manevrele de resuscitare și în plus reprezintă un risc major pentru mediastin și marile vase. Introducerea acului la acest nivel poate determina pneumotorax și uneori exitus.

2.2.4. Medicamente de administrat. Orice medicament care se poate administra pe cale intravenoasă se poate administra și pe cale intraosoasă.[11] Pentru o serie de fluide și medicamente ca adrenalina, bicarbonatul de sodiu, clorura de calciu, HES, lidocaina s-a demonstrat că au o farmacodinamică și farmacocinetică echivalente cu administrarea intravenoasă, cu o durată de acțiune echivalentă sau mai lungă.[11,18] De aceea nu este necesară modificarea dozelor de medicamente în funcție de locul accesului intraosos.

Deși fluidele pot fi perfuzate cu ajutorul gravitației, o serie de autori au demonstrat impredictibilitatea fluxului sanguin la nivelul măduvei osoase numai prin această metodă.[21,36] Concluzia lor a fost că este necesară folosirea unei presiuni suplimentare pentru creșterea fluxului infuzat intraosos, astfel ratele fluxului pot crește dramatic prin folosirea aparatelor de presiune pentru perfuzii și a injectomatelor. Ratele de perfuzie de 10 ml/minut prin gravitație pot crește la 41 ml/minut cu ajutorul aparatelor de presiune pentru perfuzii. Ca și alternativă se menționează infuzia manuală de bolusuri de 30-60 ml prin robineți atașați.[18,21,32,36]

Sângele aspirat de la locul de puncție poate fi folosit pentru anumite analize de laborator.[43] Hurren a demonstrat că atunci când se compară sângele obținut dintr-o linie intavenoasă cu cel de la locul unei puncții intraosoase, hemoglobina, hematocritul, sodiul, urea, creatinina și nivelele calciului au fost „suficient de similare pentru a fi clinic utile“.[42] De menționat este că nivelele glucozei și potasiului au fost semnificativ modificate în probele obținute de la locul de acces intraosos comparativ cu cele obținute de pe linia intravenoasă.[18,21,32,40,43]

2.2.5. Mituri despre accesul intraosos:

- Accesul intraosos este numai pentru copii. De fapt puncția intraosoasă a fost folosită la adulți cu mult timp înainte să fie folosită la copii. În timpul celui de-al doilea război mondial, puncția intraosoasă a reprezentat standardul de îngrijire pentru medicii militari care tratau cazuri de șoc. De asemenea, pentru copii se folosea frecvent puncția intraosoasă. Redescoperirea puncției intraosoase pentru copii a avut loc în anii ’80.[10,18]
- Risc semnificativ pentru lezarea platoului epifizar. În teorie există pericolul ca acul de puncție intraosoasă să penetreze platoul de creștere epifizar la copii și sugari. Totuși, în practică și pe modele experimentale inserția acelor de puncție intraosoasă prin platoul de creștere nu afectează creșterea și nu deformează osul.[15,53]

- Embolia grăsoasă. Embolia grăsoasă semnificativă clinic, secundară puncției intraosoase nu a fost niciodată raportată în literatura medicală sau în practica clinică, deși studiile efectuate pe animale au arătat emboli grăsoși microscopici pulmonari după infuzia intraosoasă sub presiune.[16,33]
- Osteomielita apare frecvent. În practica clinică, infuziile intraosoase au produs o rată foarte redusă a infecțiilor. În conformitate cu unele studii, osteomielita apare < 0,6% din cazurile de puncție intraosoasă.[8,21,44,50] Până în prezent nu există în literatură date cu privire la osteomielita produsă de catetere de puncție intraosoasă care să producă decesul sau infecții incurabile prelungite.[8,21,44,50]
- Durerea produsă la inserția acului de puncție intraosoasă este insuportabilă.[11,18,33] Puncția intraosoasă pare dureroasă. În realitate nu este mai dureroasă decât inserția unui cateter de puncție intravenoasă de 18 G. Nivelul durerii raportat de pacienții conștienți la inserția acului de puncție intraosoasă este clasificat la 2-3 pe o scară de la 1 la 10, 10 reprezentând cea mai mare durere posibilă. Totuși, marea majoritate a pacienților care necesită accesul intraosos sunt inconștienți. Pe de altă parte, poate rezulta o durere semnificativă datorită perfuziei intraosoase sub presiune la pacienții conștienți (durere viscerală) datorită stimulării senzorilor de presiune din spațiul medular. Această durere poate fi controlată prin injectarea a 2 ml de lidocaină 2% pe cateter înainte de perfuzia propriu-zisă. Totuși, numai ~ 15% din pacienții care necesită puncția intraosoasă sunt conștienți.
- Puncția intraosoasă este o procedură dificilă. Deși copiii au oase mai puțin rezistente, inserția manuală de trocare intraosoase poate fi dificilă. Totuși, toate dispozitivele pentru puncție intraosoasă care au apărut în ultimul timp pe piață facilitează accesul intraosos care devine mai ușor și mai rapid decât cel intravenos.[8,18,22,23,53]
- Nu există susținere pentru puncția intraosoasă din partea liderilor medicali de opinie. De fapt, noile revizuiri ale ghidurilor ACLS și PALS susțin puncția intraosoasă în cazul accesului vascular dificil. În marile sisteme de urgență și în multe camere de gardă se practică pe scară din ce în ce mai largă accesul intraosos.[51,52] Datorită folosirii cu succes pe câmpul de luptă și verificării în condiții critice de numeroase ori, medicii militari încurajează folosirea accesului intraosos.[18,21,22,47,48]
- În cazul unui stop cardiorespirator, perfuzia de fluide sub nivelul diafragmului este ineficientă. Studii recente pe modele animale în stop cardiorespirator arată că medicamentele injectate în măduva osoasă la nivelul tibiei, ajung la nivelul cordului în concentrații terapeutice în 51 secunde comparativ cu 25 secunde, dacă sunt injectate la nivelul sternului.[18,33,41]
- Perfuziile intraosoase pot fi administrate doar la nivelul măduvei „roșii“. Cercetările efectuate pe modele animale au arătat că medicația administrată intraosos ajunge la nivelul circulației centrale indiferent dacă sunt administrate la nivelul măduvei roșii sau galbene, deși există o ușoară întârziere atunci când sunt administrate la nivelul măduvei galbene.[18,21,33,39]
- Ratele de infuzie pentru perfuzia intraosoasă sunt la fel de rapide ca și ratele de infuzie pe liniile de acces central. Medicamentele administrate în spațiul intraosos tibial, sternal și al capului humeral ajung în circulație în aproximativ o secundă. De aceea, infuziile de medicamente inotrop pozitive sunt la fel de rapid eficiente ca și perfuziile administrate pe liniile centrale. Totuși, dacă trebuie administrate volume mari în timp foarte scurt, calea intraosoasă are limitele ei, în sensul că atunci când este necesară administrarea unor mari cantități (litri pe oră) de fluide, această cale este mai lentă decât o linie centrală. Practic, volume de până la 5 ml administrați i.o. ajung la nivelul cordului la fel de rapid ca și când ar fi fost administrate pe o linie centrală. Totuși, volumul de fluid administrat pe unitatea de timp este similar cu rata infuzată printr-un cateter de 21G. [18,21,33,51,52,54]

2.2.6. Este eficientă economic puncția intraosoasă pentru adulți?[18,21] Ca și pentru orice alt produs sau procedură nouă, trebuie întotdeauna luat în calcul și costul și impactul asupra practicii medicale ulterioare. Prețul pentru dispozitivele care realizează puncția intraosoasă variază între 65-160$ fiecare, în contrast cu cateterele intravenoase normale care costă în jur de 3-5$ fiecare. Întrebarea dacă „este costul suplimentar justificat?“ devine evidentă.

Beneficiile economice ale căii intraosoase sunt numeroase, directe și indirecte, atît pentru pacienți cât și pentru medici/paramedici astfel:

- *Timp scurt de obținere a accesului*. Puncția intraosoasă ia mai puțin de 1 minut pentru a fi realizată. Aceasta permite personalului medical de urgență să se concentreze pe managementul unor probleme mult mai importante și mai stringente cum ar fi calea aeriană, aritmiile, medicația, administrarea fluidelor și transportul pacientului. Fiecare minut se transformă în economie de bani pentru sistemul de urgență. Utilizarea puncției intraosoase permite reîntoarcerea mai rapidă în cadrul UPU, permite unui medic să trateze mai mulți pacienți și are potențialul de a optimiza circuitul pacientului în cadrul sistemului de urgență.[18,21,36]

- *Permite administrarea de mai puține medicamente*. Realizarea accesului intraosos, permite ca doza corectă de medicamente să fie administrată chiar de prima dată, la momentul oportun, de fiecare dată. Astfel nu mai este necesară administrarea de doze crescute de medicamente pe sonda de intubație care să încarce plămânii pacientului. Folosirea unor doze reduse de medicamente se traduce până la urmă în costuri reduse pe ansamblu.
- *Reduce stresul*. Dacă asistenții și medicii de medicină de urgență au posibilitatea să își facă meseria așa cum trebuie, se reduce nivelul stresului și crește satisfacția.
- *Crește calitatea actului medical*. Creșterea calității actului medical se traduce prin reducerea costurilor. Din nefericire, cu cât un pacient are nevoie mai rapid de o linie intravenoasă, cu atât mai dificilă este obținerea acesteia. Pentru acești pacienți in extremis, diferența între viață și moarte depinde de stabilirea accesului intravenos. Folosirea căii intraosoase permite personalului medical de urgență să realizeze accesul vascular rapid, sigur și ușor, cu mai puține riscuri și mai puține efecte secundare decât în cazul accesului vascular central. Bineînțeles, administrarea mai rapidă a medicației ALS va îmbunătăți prognosticul pacientului.

2.2.7. Este accesul intraosos un înlocuitor pentru accesul intravenos?[18,21,31,35] Un principiu de bază pentru accesul intraosos este faptul că nu constituie un înlocuitor de rutină pentru accesul intravenos periferic. Accesul vascular intraosos este o opțiune pentru accesul vascular atunci când accesul vascular periferic este dificil/imposibil și atunci când pacienții prezintă un status mental alterat, insuficiență respiratorie acută și/sau instabilitate hemodinamică. Întârzierile în administrarea tratamentului adecvat în aceste situații amenințătoare de viață pot provoca exitusul sau morbiditatea permanentă pentru pacienți, ceea ce ar trebui să fie inacceptabil pentru orice furnizor de servicii medicale de urgență.[31]

Personalul medical de urgență clasifică accesul vascular periferic în funcție de necesitatea administrării tratamentului, pentru tratament imediat sau profilactic, aici intră și necesitatea de a administra rapid medicația sau fluidele necesare în caz că starea pacientului se deteriorează rapid în următoarele minute. [14]

Accesul vascular intraosos nu este recomandat în scopuri profilactice. Obținerea profilactică a unei linii intravenoase este considerat un tratament profilactic, în condițiile în care nu afectează timpul de transport și foarte mulți pacienți au beneficiat de pe urma accesului venos periferic profilactic.[14,31,32]

Avantajele accesului intraosos.[18,36] Accesul i.o. are câteva avantaje față de accesul vascular intravenos atunci când pacienții au nevoie imediată de administrare de fluide sau de medicație astfel:

- vasele intraosoase nu colabează în cazul unui șoc, astfel accesul i.o. este foarte fiabil;
- accesul i.o. este fără îndoială mai rapid de cât accesul i.v. pentru pacienții în șoc/traumă;
- accesul i.o. necesită mai puțin antrenament/practică;
- îndemânarea pentru obținerea accesului i.o. rămâne la nivele ridicate, chiar după perioade lungi de neutilizare;
- accesul i.o. are o rată foarte mică de complicații;
- există foarte puține contraindicații pentru accesul i.o.

Dezavantajele accesului intraosos în scopuri profilactice:

- plasarea unei linii i.o. pare inconfortabilă pentru cei care nu sunt familiarizați cu această procedură;
- bolusul i.o. (necesar pentru a elibera calea de acces pentru medicație) poate fi dureros pentru pacientul conștient;
- cateterele de puncție i.o. sunt mult mai scumpe decât cele pentru accesul intravenos;
- percepția despre cateterele de puncție i.o. este că acestea presupun un risc mai mare pentru complicații în comparație cu accesul venos periferic;
- cateterele venoase periferice (montate în condiții optime) pot rămâne pe loc mai mult timp.

2.2.8. Accesul intraosos în contextul amenințărilor războiului modern. Expunerea la substanțe toxice de luptă cu acțiune asupra sistemului nervos central poate determina în decurs de câteva minute simptome determinate de descărcarea masivă de acetilcolină, ceea ce poate determina moartea rapidă ca urmare a insuficienței respiratorii acute. Supraviețuirea victimelor unui asemenea atac poate depinde de eficacitatea și promptitudinea cu care reacționează sistemul medical de urgență pentru administrarea cât mai rapidă a antidotului, a suportului respirator și hemodinamic.[30,32]

În acest context, obținerea accesului vascular capătă o importanță critică. Trebuie ținut totuși cont că la locul de intervenție haosul, dezorganizarea și întârzierea în desfășurarea mijloacelor necesare pălesc în fața provocărilor speciale cărora trebuie să le facă față echipele medicale care trebuie să acorde îngrijiri de calitate purtând un costum protector greoi, cu mănuși care fac aproape imposibilă realizarea de manevre de finețe cum

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ar fi cateterizarea venoasă periferică cu catetere de plastic.[30,32,33,47,48]

Studii efectuate demonstrează că performanța medicală pentru victimele unui atac cu substanțe toxice de luptă volatile este afectată în foarte mare măsură de echipamentul protector greoi și de factori fiziologici și psihologici. Printre aceștia se numără: efortul respirator crescut, îngustarea drastică a câmpului vizual, afectarea dexterității manuale de către purtarea de mănuși protective voluminoase sau supraîncălzirea individuală datorită costumului cu multiple straturi protectoare.[30,32,33]

Dispozitivele de puncție osoasă cu mecanism pe bază de arc par optime în acest context (B.I.G.), în defavoarea unor dispozitive manuale care presupun manevre de finețe (SurFast, acul Jamishidi) care au rate mari de eșec și necesită mai mult timp pentru inserare.[14,31]

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Polipoza adenomatoasă familială – cauză rară de cancer colorectal la tineri

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Rezumat

Polipoza adenomatoasa familiala (FAP) desemneaza un sindrom genetic heterogen, caracterizat prin prezenta a sute sau mii de adenoame cu potential de degenerare maligna la varsta tanara. Persoanele afectate difera prin numarul polipilor intestinali, prin numarul si topografia carcinoamelor si prin coexistenta manifestarilor extraintestinale. In ultima decada a fost identificat un fenotip distinct, cu mai putin de 100 de polipi, cu aparitia mai tardiva adenoamelor, cu prevalenta inca necunoscuta si care a primit denumirea de polipoza adenomatoasa familiala atenuata (AFAP). Genele APC si MUTYH sunt asociate polipozei adenomatoase familiale in 70-80% din cazuri si doar in 30-40% din cazurile de polipoza adenomatoasa familiala atenuata. Structura si functiile acestor doua gene explica heterogenitatea clinica a sindromului. Astfel, gena APC prin controlul activarii tumorigenezei colorectale sporadice si ereditare, contribuie la aparitia unui fenotip cu dominanta innascuta. Gena MUTYH, prin controlul sistemului de reparare al ADN-ului alterat oxidativ, determina o forma atenuata, recesiva de boala.

Cuvinte cheie: *polipoza adenomatoasa familiala, cancer colorectal, cancere extracolonice*

Abstract

FAP designates a heterogenous genetic syndrome caraterized by the presence of hundreds and even thousends polyps which may degenerate into malignancy at young age. The difference between patients may reside in the number of intestinal polyps, the number and topography of carcinoma and in coexistance of extraintestinal manifestations. In the last decade there has been identified a distinct fenotype of atenuate FAP in which there is a reduced number of colonic polyps and a delayed appearance of colonic carcinoma. APC and MUTYH genes have been associated with 70-80% of FAP patients. The type of mutations at the level of those 2 genes determines the clinical heterogenity of FAP. APC gene mutations determines an autosomal dominant type of disease whereas MUTYH gene mutations results in an recesive and atenuated form of disease.

Key words: *familial adenomatous polyposis, colorectal cancer, extracolonic cancers*

Polipoza adenomatoasă familială (FAP) este cel mai frecvent sindrom polipozic adenomatos din practica clinică. Este o afecțiune ereditară cu transmitere autosomal dominantă, cu penetranță de 80-100% și cu prevalența estimată de 1 la 5.000-7.500 de cazuri. În această afecțiune o alela APC este moștenită, modificată ereditar de la părintele afectat, iar adenoamele apar în momentul în care cea de-a doua alela a APC, provenită de la părintele neafectat, este modificată sau pierdută. Proteina APC este un reglator al homeostaziei celulelor epiteliale colonice și participă la proliferarea, migrarea, diferențierea și apoptoza celulară și la segregarea cromozomială. Porțiunea proximală a proteinei APC conține secvențe care permit oligomerizarea și legarea la proteine care reglează citoscheletul actinic cu afectarea subsecventă a morfologiei, polarității și migrației celulare.

Manifestările clinice la nivelul colonului.

Forma clasică a FAP se caracterizează prin apariția progresivă a sute sau mii de polipi adenomatoși la nivelul colonului. La un pacient care moştenește o mutație a APC, apariția adenoamelor colonice va fi semanalată în jurul vârstei de 10-12 ani și mult mai rar în prima decada de viață. Studiile publicate relevă faptul că aproximativ 50% dintre purtătorii genei FAP au polipi depistabili sigmoidoscopic la vârsta de 15 ani. Simptomatologia clinică s-a instalat însă abia la vârsta de 30-33 de ani, cancerul a fost diagnosticat la 39 de ani iar decesul prin cancer a survenit în medie la 42 de ani. Până la vârsta de 50 de ani au fost diagnosticate 90% din cazurile de FAP.

FAP începe prin apariția unui număr redus de polipi care crește însă progresiv până la tapetarea colonului în întregime. Pot fi întâlnite toate variantele de polipi adenomatoși: tubulari, tubulo-viloși și viloși. Mutațiile genei APC între codonii 1250 și 1464 se asociază cu cel mai mare număr de adenoame (zeci de mii). Examenul histologic evidențiază numeroase adenoame microscopice, cele mai mici putând interesa o singură criptă glandulară colonică. Mărimea și numărul polipilor corespunde perioadei de latență dintre debutul clinic și momentul detecției astfel ca, tumorile tind să fie mai numeroase la pacienții simptomatici comparativ cu rudele asimptomatice mai tinere, depistate prin screening.

Cancerul colorectal trebuie considerat ca o consecință inevitabilă în evoluția naturală a FAP. Apariția sa este documentată la 10-15 ani de la debutul polipozei. Deși cancerul colorectal este neobișnuit în adolescență, el a fost depistat și la vârsta de 9 ani! Cancerul are gradele histopatologice de malignitate și distribuția colonică similare cu cele din populația generală, totuși cancerele sincrone sunt mult mai frecvente (la 48% din cazuri). În pofida atenției acordate prin screening și supraveghere, aproximativ 25% dintre pacienții cu FAP au cancer colo-rectal în momentul colectomiei.

Deoarece pacienții cu FAP sunt născuți cu mutația APC în toate celulele organismului, formațiuni tumorale pot apărea și în afara colonului. Astfel, la aproape toți pacienții cu FAP se pot regăsi polipi la nivelul stomacului și intestinului subțire. Polipii gastrici apar în 30-100% din cazuri deși mulți dintre ei sunt polipi glandulari fundici non-neoplastici (hiperplastici sau microchistici). Sunt de obicei sesili, de 1-5 mm, pot apărea în prima decadă de viață, chiar înaintea adenoamelor gastrointestinale. Polipii glandulari fundici pot fi regăsiți și la pacienți fără FAP, care au fost tratați îndelungat cu inhibitori de pompă de protoni. La pacienții cu FAP și polipi glandulari fundici displazia epitelială apare la 25-41% din cazuri ca urmare a mutațiilor de la nivelul genei APC. Displazia polipilor glandulari fundici asociați cu FAP se asociază direct cu mărimea polipilor, cu severitatea crescută a polipozei duodenale și cu gastrita antrală și este invers corelată cu utilizarea terapiei antiacide și cu prezența lui Helicobacter pylori. Adenoamele gastrice sunt rare și apar la aproximativ 5% din cazurile de FAP, de obicei la nivelul antrului.

Adenoamele duodenale apar la 60-90% din pacienții cu FAP iar incidența crește cu vârsta. Topografia predilectă a adenoamelor duodenale este la nivelul regiunii periampulare, cu interesarea ductelor biliar și pancreatic. Astfel, 50-85% dintre pacienții cu FAP au modificări adenomatoase la nivelul papilei vateriene, cu o incidență a cancerului duodenal de 4-12%, constituind principala cauză de deces la pacienții cu FAP, după efectuarea colectomiei profilactice. Prin urmare, se recomandă screeningul și supravegherea gastroduodenală cu mențiunea că sub vârsta de 30 de ani carcinomul duodenal este extrem de rar.

Adenoamele jejunale sunt detectate la 40% iar cele ileale la 20% dintre pacienții cu FAP. Acestea se transformă malign rar. După efectuarea colectomiei subtotale sau proctocolectomiei totale cu anastomoza ileo-anală este necesară supravegherea ileonului distal pentru depistarea apariției leziunilor neoplazice.

Manifestările extraintestinale.

Sindromul Gardner este o afecțiune familială caracterizată prin prezența polipozei gastrointestinale și a osteoamelor asociate cu o varietate de tumori benigne la nivelul țesuturilor moi și cu alte manifestări extraintestinale (tabel). Atât FAP cât și sindromul Gardner sunt manifestări variabile ale patologiei determinate de mutațiile de la nivelul genei APC. Anomaliile osoase includ osteoame la nivelul mandibulei, oaselor craniene și oaselor lungi, exostoze și anomalii dentale variate (chiste mandibulare, dinți impactați sau supranumerari). Osteoamele mandibulare pot fi regăsite la 90% dintre pacienții cu FAP, chiar în absența altor stigmate ale sindromului Gardner. Examinarea radiologică a mandibulei constituie o metodă simplă și neinvazivă de screening pentru purtătorii tineri ai genei FAP.

Osteoamele pot apărea la copii înaintea polipozei colonice. Deoarece osteoamele nu au potențial malign, nu vor fi extirpate decat în cazurile simptomatice sau din motive cosmetice.

Hipertrofia congenitală a epiteliului retinian pigmentat a fost descrisă în unele familii diagnosticate cu FAP sau cu sindrom Gardner. Peste 90% din cazurile de sindrom Gardner au leziuni pigmentate la examenul fundului de ochi (comparativ cu 5% la lotul de control) care în 63% cazuri sunt multiple și în 87% cazuri sunt bilaterale. Prezența leziunilor multiple retiniene bilaterale constiutie astfel un marker util pentru depistarea purtatorilor genei FAP.

Fibromatoza difuză mezenterică reprezinta o complicatie serioasa a sindromului polipozei adenomatoase, numită și tumora desmoidă. Apare la 4-32% dintre pacienți. Tumorile desmoide prezintă adesea agregare familiala, apar dacă mutația genică se situează distal de codonul 1444 al genei APC și frecvent, au punctul de plecare la nivelul fibroblastilor mezenterici, după laparotomie (ex. după proctocolectomie) deși pot apărea mai rar și spontan. Prezența lor crește riscul obstructiei digestive, a constricțiilor la nivelul arterelor, venelor și ureterelor, asociind o rată de mortalitate de 10-50%. În plan terapeutic sunt tumori responsive la radioterapie dacă sunt accesibile. Abordarea terapeutică medicală a acestor tumori utilizează asocierea de sulindac (antiinflamator nonsteroidian) cu tameoxifen (antiestrogenic). Terapia multimodală include chimioterapia și transplantul de intestin subțire.

Alte tumori tisulare moi descrise în FAP și sindromul Gardner includ chiste dermoide, fibroame și lipoame.

Au fost semnalate de asemenea și prezența neoplasmelor la nivel hepato-biliar și suprarenal. Carcinomul papilar tiroidian apare la 1% din pacienții cu FAP, predominant la femei iar hepatoblastomul poate afecta copii din familiile cu FAP.

Testarea genetică și sfatul genetic.

Testarea genetică constituie un element important în abordarea pacienților cu FAP și a familiilor acestora, nu atât pentru managementul persoanei afectate și diagnosticate cât mai ales pentru detecția purtătorilor genei mutante. Aproximativ 20% dintre pacienții cu FAP au istoric familial negativ, prezentând mutații noi la nivelul genei APC. Testarea genetică se efectuează prin extragerea ADN-ului din leucocitele sângelui periferic. Dacă testul este pozitiv la un membru de familie va prezenta o acuratețe de aproape 100% în identificarea altor purtători ai genei în cadrul respectivei familii.

Se testează inițial persoana afectată. Rezultatul negativ al testarii sugerează că testarea genetică la rudelor cu risc de boală este inutilă iar familia va fi supusă screeningului prin testare clinică.

Diagnostic și screening.

Pacienții cu FAP pot prezenta simptome nespecifice ca hematochezia, diareea și durerea abdominală. Cheia diagnosticului și managementului acestei afecțiuni constă în identificarea persoanelor pre-simptomatice, obiectiv realizabil prin măsuri consistente de diagnosticare a rudelor pacienților depisptați. Diagnosticul ete relativ simplu cu ajutorul sigmoidoscopiei datorită distribuției difuze a polipilor, deși se preferă colonoscopia totală pentru precizarea fenotipului bolii și pentru excluderea coexistenței cancerului colonic. Prezența a peste 100 de polipi și confirmarea naturii lor adenomatoase stabilește diagnosticul fenotipic de polipoză adenomatoasă colonică. Deși istoria naturală a FAP sugerează existența unui interval de aproape 10 ani între apariția polipilor și cea a cancerului nu se indică amânarea chirurgiei, o dată ce diagnosticul a fost precizat, chiar dacă pacientul este presimptomatic, cu excepția persoanelor care nu și-au încheiat pubertatea. Testarea genetică a persoanelor cu risc, la vârsta de 10-12 ani, permite inițierea evaluării clinice. În familia cu mutație genetică cunoscută, copiii cu testul pozitiv vor urma apoi sigmoidoscopia de screening pentru determinarea stadiului afecțiunii. Dacă testarea a fost negativă, copilul va fi scutit de sigmoidoscopie deși este prudent să fie reexaminat încă odată, după adolescență, chiar și numai pentru a elimina posibilitatea unei erori diagnostice inițiale.

Tratament

Chirurgia reprezintă singura optiune terapeutică rezonabilă pentru polipoza colonică din FAP. Momentul și gradul de extensie chirurgicală sunt de importanță clinică majoră. Deoarece orice rest de mucoasă rectală neextirpată prezintă riscul apariției cancerului, terapia optimă constă în proctocolectomie totală fie cu ileostoma convențională fie cu anastomoza ileo-anală. În cazul în care se optează pentru colectomie subtotală cu anastomoza ileo-rectală este de menționat că segmentul rectal restant prezintă risc pentru cancer (până la 3/5 dintre pacienți vor dezvolta cancer în următorii 20 de ani chiar dacă pacientul va efectua sigmoidoscopie semi-anuală cu electrocoagularea tuturor polipilor depistati)!

Tratamentul medical se adresează polipilor adenomatoși rectali mici care pot fi reversibili. După colectomia subtotală cu anastomoza ileo-rectală, s-a observat regresia spontană a polipilor rectali. Efecte pozitive inconsistente, de regresie a polipilor rectali restanţi, au fost observate în studii experimentale care au utilizat vitamina C (3 g/zi), suplimente de calciu în dietă, vitamina E (400mg/zi) sau fibre alimentare (22,5 g/zi).

Dintre antiinflamatoarele nesteroidiene, Sulindacul a redus, în unele studii, numărul și mărimea adenoamelor colorectale în FAP cu colon intact sau cu colectomie subtotală și anastomoza ileorectală. Menținerea pacienților pe acest tratament nu protejează însă pacienții față de riscul apariției cancerului rectal iar efectul reductiv al medicamentului este reversibil după încetarea administrarii sale. Un alt inhibitor, de data aceasta selectiv de COX-2, numit Celecoxib, ar reduce numarul adenoamelor duodenale, efect care urmează să fie confirmat în viitor.

Screeningul organelor extracolonice.

Screeningul tubului digestiv proximal se va efectua în momentul depistării adenoamelor colonice sau cel mai târziu până la vârsta de 25 de ani. Evaluarea integrală a intestinului subțire ar trebui efectuată în momentul diagnosticului inițial de FAP prin seriografie baritată, capsula endoscopică, enteroscopie cu cu dublu balon, enterografie-CT sau RMN și, dacă este necesar, prin enteroscopie intraoperatorie efectuată cu ocazia proctocolectomiei. Polipii tractului digestiv superior sunt rari înaintea debutului afectarii colonice dar se recomandă examinarea cu endoscop cu vedere laterală pe lângă endoscopul cu vedere axială, pentru mai buna vi-

Tipul de cancer	***Riscul pe durata vieții***	***Recomadari de screening***
Cancerul colonic:	**100%**	*• sigmoidoscopie anuala de la vârsta de 10-12 ani*
Cancer duodenal/ periampular	**5-12%**	*• gastroduodenoscopie cu vedere laterală de la vârsta de 25-30 de ani*
Cancer gastric	**0,5%**	*• idem cu localizarea duodenală*
Cancer de pancreas	**2%**	*• posibil ecografie abdominală după vârsta de 20 ani*
Cancer tiroidian	**2%**	*• examen tiroidian anual de la vârsta de 10-12 ani;*
Cancer de sistem nervos central	**< 1%**	*• examen fizic anual; CT/RMN Periodic.*
Hepatoblastom:	**1,6%**	*• anual: examen fizic, eco. hepatic și alfa-1-fetoproteină pentru prima decadă de viață*

zualizare a regiunii ampulare duodenale. În duoden, adenoamele viloase, adenoamele cu displazie de grad înalt, adenoamele mari și cele simptomatice, indiferent de histologie, vor fi extirpate. Ablația endoscopică a adenoamelor periampulare este relativ sigură pentru endoscopistul experimentat dar reapariția tesutului adenomatos este frecventă.

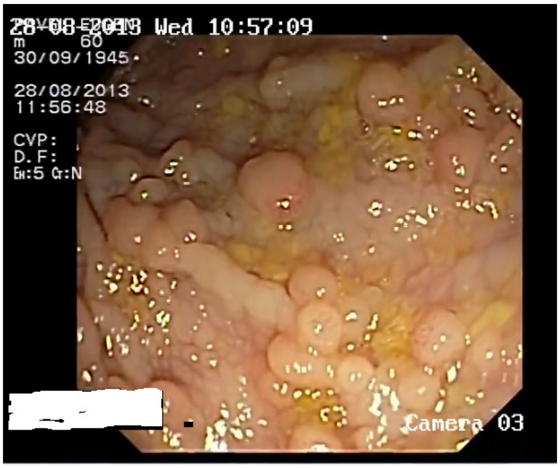

Fig. 1 ***Aspect colonoscopic de FAP***

Prezentare de caz

Pacientul P.E. de sex masculin, în vârstă de 38 ani, din mediu urban, nefumător, fără antecedente personale patologice deosebite, se prezintă acuzând epigastralgii nesistematizate de aproximativ 3 luni și astenie fizică marcată. Antecedentele patologice familiare sunt neremarcabile. Pacientul are un frate de 37 ani, relativ sănătos. Examenul fizic este neremarcabil. La examenul de laborator se remarcă un sindrom anemic ușor hipocrom microcitar hiposideremic regenerativ (Hb 9g/dL, MCV 60fL, Reticulocite 3%, sideremie 32microg/dL), test hemocult pozitiv. La examenul ecografic abdominal se descriu multiple leziuni intrahepatice sugestive pentru determinări secundare metastatice. Examenul CT abdominal confirmă diagnosticul de metastaze intrahepatice dar nu descrie tumora primară. La examenul de endoscopie digestivă superioară se descrie o formațiune tumorală a ampulei vateriene, supla la biopsie. Examenul anatomopatologic: elemente de adenom vilos cu displazie de grad mic. Examenul colonoscopic: multipli polipi de dimensiuni variabile (4-9 mm) dispuși în covor de la nivelul canalului anal până la nivelul cecului (anatomopatologic se descrie aspect de adenom tubolovilos); unul din polipi la nivelul sigmoidului este mult mărit în volum față de ceilalți (2-2,5 cm) de aspect conopidiform cu ulcerații pe suprafață și dur la biopsiere (anatomopatologic este descris adenocarcinom slab diferențiat); ileonul terminal este liber de leziuni până la 10 cm de valava ileocecală. Diagnosticul care se impune în fața acestor date este, așadar, de: polipoza adenomatoasă familială, adenom vilos vaterian (frecvent descris în asociere cu FAP și care, în contextul transformării carcinomatoase, reprezintă principala cauză de mortalitate după colectomia totală profilactică), adenocarcinom colorectal slab diferențiat st. IV TNM. Dată fiind componența ereditară de tip autozomal dominant a FAP, s-a efectuat screening endoscopic (colonoscopic și digestiv superior) și la fratele pacientului, fără identificarea în prezent a leziunilor polipoide specifice.

În concluzie

Deși componenta ereditară a cancerului colorectal este apreciată la aproximativ 20% din pacienți, dintre aceștia sindroamele polipoide familare reprezentând doar 2%, vârsta mai mică a pacienților și frecventele asocieri cu neoplazii extracolonice dar și extradigestive impune ca, măcar la nivelul practicii gastroenterologice, informațiile referitoare la aceste suferințe să fie cât mai larg accesibile.

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Consideraţii clinico-farmacologice privind terapia sindroamelor cohleo-vestibulare prin administrare intratimpanică a medicaţiei

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Rezumat

Administrarea intratimpanică a medicaţiei constă în aplicarea medicamentelor în urechea medie la nivelul nişei ferestrei rotunde, principalul avantaj fiind difuziunea directă a substanţelor terapeutice în urechea internă prin membrana ferestrei rotunde şi realizarea unor concentraţii mari intralabirintice cu efecte secundare sistemice minime. Sunt trecute în revistă aspecte actuale ale cunoaşterii sistemelor de barieră labirintice,fiziologiei lichidelor labirintice, farmacocineticii intralabirintice şi principalele medicamente utilizate în terapia sindroamelor cohleo-vestibulare prin administrare intratimpanică a medicaţiei.

Cuvinte cheie: *tratament intratimpanic, bariera hemato-labirintică, farmacocinetică intralabirintică, corticosteroizi intratimpanic, gentamicină intratimpanic*

Abstract

Intratimpanyc drug delivery refers to drug administration in the middle ear, the main advantage being direct difusion of substances in the inner ear through the round window membrane with subsequent high intralabiryntine drug concentration and very low systemic side effects. The article is a review concerning the inner ear barrier systems, the physilogy of inner ear fluids, intralabirinthine pharmacokinetics and the commenest drugs applied in the middle ear for the treatment of cochleo-vestibular syndromes.

Key words: *intratympanic drug delivery, hemato-labirinthine barrier, intralabirinthine pharmacokinetics, intratympanic corticosteroids, intratympanic gentamicine.*

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Sindroamele cohleo-vestibulare sunt caracterizate de o mare diversitate etiopatogenică. În mediul militar predomină trauma sonoră, urmată de neuropatiile acustico-vestibulare infecțioase, toxice, metabolice și sindromul surdității brusc instalate. Boala Meniere are etiologie idiopatică, deși se cunoaște că, din punct de vedere patogenetic, este un veritabil hidrops endolimfatic. Ea asociază hipoacuziei vertijul, acufenele precum și simptome neurovegetative care, împreună, constituie o criză tipică de Meniere. Printre cauzele rare de surditate și vertij se află tumorile unghiului pontocerebelos și ale conductului auditiv intern, cel mai frecvent fiind schwanomul de nerv vestibular.

Resursele terapeutice sunt reprezentate de corticoterapie, vitaminoterapie neurotrofică, protezarea auditivă și implantul cohlear, precum și tratamentul chirurgical în cazul etiologiei tumorale sau al hidropsului labirintic. Vertijul este tratat cu sedative vestibulare. O alternativă, în situații clinice bine precizate, o constituie ablația chirurgicală sau farmacologică a labirintului, în mod specific în cazul bolii Meniere când vertijul invalidant este asociat unei hipoacuzii severe iremediabile.

Urechea internă are la dispoziție oportunitatea unică a administrării topice a terapiei medicamentoase prin intermediul aplicării tratamentului intratimpanic. În administrarea sistemică accesul medicației în urechea internă este limitat de existența unei bariere hemato-cohleare, similară din punct de vedere anatomic și funcțional cu bariera hematoencefalică și de fluxul sanghin cohlear redus. Datorită joncțiunilor foarte strânse între celule, substanțele din circulația sistemică sunt împiedicate de către bariere fizice substanțiale să intre în labirint, multe molecule cu posibile efecte terapeutice nereușind să acceadă la structurile țintă din urechea internă. În plus, cohleea fiind un spațiu închis, este foarte sensibilă la modificări mici ale volumului lichidian.

Bariera hemato-cohleară (sau hemato-labirintică) protejează structurile cohleei într-un mod similar cu bariera hemato-encefalică. Din punct de vedere farmacologic, spațiile lichidiene extracelulare ale urechii interne cuprind patru componente (1) circulația sistemică – reprezentată de stria vasculară și lichidele din interiorul capilarelor și vaselor sanguine; (2) perilimfa, lichid cu compoziție similară lichidului cefalorahidian și care asigura mediul optim de funcționare a celulelor senzoriale și nervoase; (3) endolimfa, lichid bogat în potasiu care asigura bateria electrochimică, reprezentată de potențialul endocohlear, necesară funcționării celulei senzoriale; și (4) lichidul extracelular din osul cohleei, care constituie o regiune de tranziție între circulația sistemică și perilimfă.

O porțiune mică din urechea internă, stria vasculară, este accesibilă direct din circulația sistemică. Se găsește pe peretele lateral al cohleei, în scala media, și este înconjurată de celule interconectate prin joncțiuni strânse, care asigură o parte a barierei hemato-cohleare. Medicamentele care acționează la nivelul striei vasculare pot fi administrate sistemic iar mecanismul de acțiune cel mai probabil constă în reducerea potențialului endocohlear. Întrucât potențialul endocohlear este bateria procesului cohlear de transducție a energiei sonore în impuls nervos, reducerea acestuia are ca rezultat diminuarea audiției.

Perilimfa, principalul fluid al urechii interne, se află în continuitate cu lichidul cefalorahidian prin intermediul apeductului cohlear la majoritatea mamiferelor, deși transferul lichidian între cele două compartimente este limitat. În mod normal, la om, apeductul nu este permeabil, însă difuziunea prin continuitate se realizează pe alte căi, în principal prin spațiile din jurul și din interiorul nervului auditiv. La om cantitatea de perilimfă este de cca 70 μL din care aproximativ 40 μL se află în scala tympani.

Endolimfa, fluidul extracelular înalt specializat care umple scala media, scaldă suprafețele apicale ale celulelor senzoriale. Este produsă în principal în stria vasculară și secretată de celulele marginale ale striei, de pe versantul endolimfatic. Endolimfa, cca 8μL la om, are o concentrație crescuta de potasiu, concentrații scăzută de sodiu și foarte scăzută de calciu.

Sistemele de barieră labirintice sunt multiple. O primă componentă este endoteliul capilar continuu care tapetează vasele sanguine din cohlee. Celulele endoteliale sunt conectate prin joncțiuni strânse și fără fenestrații. Medicamentele pătrund în perilimfă din circulația sistemică fie prin mecanisme de transport sau prin dizolvare în endoteliul capilar.

Scala media este, deasemenea, înconjurată de celule conectate prin joncțiuni strânse. Substanțele medicamentoase care pătrund în scala media din circulația sistemică trebuie să pătrundă mai întâi în celulele marginale ale striei vasculare sau în perilimfă. În ambele cazuri, medicamentele sunt transportate prin stratul celular pentru a pătrunde în scala media. Scala media având o încărcătură pozitivă destul de mare datorată potențialului endocohlear, încărcătura electrică a medicamentelor constituie un factor semnificativ de influențare a capacității de pătrundere în scala media, moleculele încărcate pozitiv fiind defavorizate .

Ca și în cazul barierei hematoencefalice, există și alte elemente care intră în constituția barierei. Conținutul ridicat în proteine al perilimfei are ca urmare legarea medicamentelor, cu efect de tampon al concentrației acestora. Probabil există și alte componente ale barierei hemato-cohleare, precum enzime și sisteme de transport celular care pot diminua concentrația substanțelor chimice care pătrund în cohlee.

Traversarea barierei hematolabirintice de către medicamente depinde și de caracteristicile chimice ale acestora. Similar barierei hematoencefalice , medicamentele cu greutate moleculară sau încărcătura electrică mari traversează cu dificultate în mod pasiv. Liposolubilitatea mare facilitează trecerea. Medicamentele care se leagă de proteine traversează mai greu. Potențialul pozitiv neobișnuit din scala media îngreunează suplimentar trecerea, medicamentele încărcate pozitiv având de înfruntat un gradient electric potrivnic.

Distribuția medicamentelor în lichidele cohleare este guvernată de variabile farmacocinetice similare celor din fluxul sanghin. Astfel, medicamentele administrate în perilimfă pot difuza în organul Corti, sediul celulelor ciliate, terminațiunilor nervoase și al altor celule specializate. Perilimfa se află în continuitate cu ganglionul spiral și structuri din modiolul cohleei. Accesul la acestea din urmă este facilitat de prezența a numeroase *lacuna canaliculi* la suprafața lamei spirale osoase. Aceste perforații sunt de ordinul a 0,2- 23 μm în diametru. De asemenea, perilimfa poate difuza prin continuitate la nivelul ligamentului spiral și a limbului spiral.

Compoziția în proteine a perilimfei umane are similitudini cu cea sanguină, concentrațiile în perilimfă fiind însă mai mici, iar interacțiunile proteinelor cu medicamentele sunt la fel de importante în perilimfă ca și în sânge. Nivelul crescut de albumină permite legarea medicamentelor acide iar glicoproteinele acide fixează medicamentele bazice. Coeficienții de partitiție a medicamentelor cu aceste proteine determină nivelul concentrațiilor de medicament liber. Perilimfa poate depozita medicamentele prin fixarea lor de proteine, în funcție de afinitate.

Distribuția medicamentelor în urechea internă depinde de locul de pătrundere. În cazul administrării intratimpanice, practic există trei posibilități : prin sau în vecinătatea membranei ferestrei rotunde (la baza cohleei, corespunzător scalei tympani), prin sau în vecinătatea ferestrei ovale (la baza cohleei corespunzător scalei vestibulare) sau prin capsula otică. Deși permeabilitatea osului pentru medicamente și alte substanțe este presupusă a fi foarte scăzută, a fost descris un sistem lacunocanalicular reprezentat în capsula otică atât la animal cât și la om. Sistemul canalicular osos permite comunicarea directă cu perilimfa, permițând pătrunderea în perilimfă a substanțelor din urechea medie care scaldă capsula otică.

La aproape o treime din subiecții umani, anatomia nișei ferestrei rotunde poate prezenta variante anatomice constând în septuri osoase,false membrane sau septări conjunctivo-adipoase obliterative care pot influența trecerea substanțelor medicamentoase din urechea medie în urechea internă.

Concentrația medicamentelor în lichidele cohleare depinde atât de rata absorbției în cohlee cât și de eliminarea acestora în circulația sistemică precum și în urechea medie prin membrana ferestrei rotunde. Concentrația de substanță din cavitatea timpanică scade atât datorită absorbției în cohlee, prin membrana ferestrei rotunde sau prin osul capsulei otice, cât și datorită deglutițiilor, rezultând în cele din urmă un gradient concentrațional de-a lungul membranei ferestrei rotunde care facilitează eliminarea medicamentului absorbit în perilimfă spre urechea medie.

Anatomic, cohleea este constituită din tuburi lungi de cca 33 mm, răsucite în spirală. Spațiul perilimfatic al scalei tympani începe la baza cohleei în vecinătatea membranei ferestrei rotunde, continuânduse apical până la capătul rampei unde comunică prin helicotremă cu perilimfa rampei vestibulare, continuânduse până la bază în vecinătatea ferestrei ovale, unde este localizată talpa scăriței. Transportul medicamentelor prin difuziune simplă de-a lungul lungimii spațiului perilimfatic pentru realizarea unei distribuții semnificative în întreaga cohlee ar dura foarte mult (ore sau zile) datorită lungimii mari comparativ cu suprafața de secțiune. Studiile de farmacocinetică sugerează o difuziune dintr-o scală în alta prin țesuturile cohleei, așa numita *difuziune radială sau interscalară*, pe lângă difuziunea în lungul cohleei, numită *difuziune longitudinală*. Amploarea difuziunii interscalare depinde în mare măsură de calitățile fizico-chimice ale substanței medicamentoase. Medicamentele cu liposolubilitate mare, precum corticosteroizii, prezintă o difuziune interscalară mai mare decât medicamentele a căror captare se face prin intermediul sistemelor de transport.

Substanțele medicamentoase sunt administrate intratimpanic pentru a difuza prin membrana ferestrei rotunde în rampa timpanică a turei bazale a cohleei. Fereastra rotundă constituie punctul vulnerabil al compartimentului lichidian endocohlear, fiind permeabilă la apă și multe medicamente. Principala problemă din punct de vedere farmacocinetic constă în faptul că tratamentul prin administrare intratimpanică se bazează pe difuziunea simplă pentru administrarea medicamentului din urechea medie în urechea internă, iar odată ajuns în rampa timpanică singurul mod de distribuție este difuziunea simplă într-un spațiu lichidian cu turnover redus. Determinările pe bază de electrozi cohleari și trasori ionici au precizat un flux longitudinal în scala tympani neglijabil, de cca 1,6 nL/min, direcționat apical. Grosimea foarte variabilă a membranei ferestrei rotunde determină o variabilitate a absorbției medicamentelor în scala tympani.

Odată absorbite, substanțele se distribuie neuniform în volumul perilimfei. Studiile experimentale cu markeri enzimatici (peroxidază) sau trasori ionici (trimetilfenilamoniu) au evidențiat că substanțele absorbite din urechea medie sunt distribuite în principal în regiunile bazale ale cohleei și sitemului vestibular, fiind absente în regiunile apicale. Concentrația din regiunile apicale depinde în primul rând de echilibrul dintre difuziune și eliminare, substanțele deplasânduse foarte lent prin spațiul perilimfatic. Experimental, administrarea substanței pe o perioadă mai lungă nu modifică gradientul longitudinal din scală a solvitului. Această caracteristică are consecințe majore asupra tratamentelor de perfuzare a cohleei. Pe de o parte, constituie un avantaj în cazul terapiilor cu substanțe distructive, precum gentamicina. Tendința fluxului perilimfatic longitudinal de a concentra substanțele în spira bazală a cohleei și în sistemul vestibular asigură un avantaj protector constând în prezervarea audiției în regiunile de frecvență joasă și medie a cohleei. Pe de altă parte, încercările de perfuzare a întregului labirint cu corticosteroizi au eficacitate parțială și limitată , indiferent de durata de perfuzare.

Metabolizarea și clearence-ul medicamentelor administrate intratimpanic sunt specifice pentru fiecare în parte. Aceeleași bariere care limitează intrarea medicamentului sunt implicate și în eliminarea sa. Există sisteme și mecanisme de captare care îndepărtează medicamentele din perilimfă, metabolizându-le sau transportându-le în circulația sistemică. Structura chimică a medicamentelor din perilimfă poate fi modificată sub acțiunea enzimelor asemănatoare celor plasmatice, cum ar fi esterazele. Eliminarea medicamentelor se poate face și prin membrana ferestrei rotunde, în sens invers absorbției, funcție de gradientul de concentrație care depinde și de forma de administrare. În cazul administrării soluțiilor lichidiene, în momentul atingerii unei concentrații perilimfatice superioare celei intratimpanice, gradientul de concentrație se inversează favorizeazând eliminarea medicamentului din rampa timpanică spre urechea medie

Terapia sindroamelor cohleo-vestibulare necesită accesul medicamentelor în urechea internă însă metodele convenționale de administrare fac apel la căi cu eficiență variabilă. În mod curent medicamentele se administrează sistemic, așteptânduse să ajungă la structurile țintă din urechea internă în formă activă și concentrație eficientă, fără efecte secundare. Terapia sistemică prezintă însă o serie de dezavantaje precum : realizarea unei concentrații mici la nivelul organului "țintă", efecte secundare sistemice, interacțiuni cu medicația concomitentă sau chiar prezența unei contraindicații relative sau absolute. Este cazul corticosteroizilor administrați sistemic pentru terapia surdității brusc instalate și a afecțiunilor autoimune ale urechii interne. Utilitatea lor clinică este limitată de efectele secundare datorate dozelor sistemice mari necesare realizării unei concentrații terapeutice în labirint.

Tratamentul intratimpanic este o alternativă mai eficientă, constituind un veritabil tratament topic la nivelul urechii interne. Indicațiile majore sunt grupate în patru mari categorii : otoneuroprotecție, tratamentul surdității neurosenzoriale brusc instalate, tratamentul vertijului din cadrul bolii Meniere și tratamentul afecțiunilor autoimune ale urechii interne. Celulele senzoriale ale cohleei trebuie protejate împotriva zgomotului și a traumatismului chirurgical, împotriva medicamentelor ototoxice precum cisplatinul sau antibioticele aminoglicozidice și împotriva iradierii extremității cervico-cefalice. Medicamentele administrate intratimpanic mai sunt utilizate pentru minimalizarea sau reducerea hipoacuziei datorată unor evenimente bruște de etiologie necunoscută sau secundară unor reacții imune de la nivelul cohleei sau pentru controlul vertijului din cadrul bolii Meniere.

Există o mare varietate de strategii de administrare locală a tratamentului afecțiunilor urechii interne, fiecare dintre ele asigurând o perfuzare a urechii interne cu medicația administrată intratimpanic sau intracohlear. Acestea includ : 1) injecții intratimpanice unice sau repetate ca sau fără stabilizare volumică, cu sau fără vizualizarea membranei ferestrei rotunde, 2) administrarea continuă sau intermitentă prin intermediul unor sisteme de pompă parțial sau total implantabile, 3) polimeri biodegradabili introduși în cavitatea timpanică, si 4) administrarea directă intracohleară, cum ar fi aceea realizată cu ocazia implantului cohlear.

Administrarea medicației in cavitatea timpanică la nivelul nișei ferestrei rotunde prezintă mai multe avantaje. Asigură accesul direct al agentului terapeutic în lichidele perilabirintice, realizând totodată o concentrație mare la nivelul organului “țintă“. Datorită existenței barierei hematolabirintice, este impiedicată difuziunea sistemică a medicamentului și , implicit, eventualele efecte secundare la distanță. Tratamentele intratimpanice sunt nedistructive , rapide și mai ieftine în comparație cu o intervenție chirurgicală. Mulți pacienți cu indicație terapeutică sunt vârstnici sau tarați, cu probleme medicale care complică sau contraindică intervenția chirurgicală. Tratamentul administrat intratimpanic poate fi inițiat imediat după diagnosticare, nefiind necesară o perioadă de latență pentru pregătirea momentului operator. De asemenea sunt absente riscurile posibile în cazul unei intervenții chirurgicale.Un alt avantaj este constituit de selectivitatea urechii afectate pentru tratament. În situația unei afecțiuni bilaterale însă, acest avantaj dispare ca și în situația în care urechea afectată nu poate fi identificată cu certitudine.

Deși nu există studii randomizate, tratamentul intratimpanic poate fi însoțit de complicații. Se menționează posibilitatea apariției durerii, a vertijului tranzitoriu datorat temperaturi agentului terapeutic , a otitei medii acute, a perforației membranei timpanice și a hipoacuziei sau a agravării hipoacuziei deja existente. Trebuie luat în considerare și riscul potențial al absorbției sistemice a soluției de agent terapeutic înghițită după administrarea intratimpanică. Se citează necesitatea creșterii dozei de insulină la un pacient diabetic după administrarea unei injecții de 0,5 ml dintr-o soluție de dexametazonă cu concentrația de 24 mg/ml. Și fiindcă este dovedită posibilitatea osteonecrozei capului femoral și după o unică doză de corticosteroizi, riscul unei asemenea complicații după administrarea intratimpanică s-ar putea să nu fie nul.

Alte posibile complicații ale administrării intratimpanice sunt modificările morfologice ale membranei ferestrei rotunde sau ale mucoasei casei timpanului și din vecinătatea nișei ferestrei rotunde. Acestea consistă în îngroșare prin infiltrare cu celule inflamatorii sau apariția de false membrane conjunctive obturante.

Eficiența administrării intratimpanice poate fi compromisă de diversele obstacole din fața ferestrei rotunde, fie că este vorba de aderențe și sinechii postinflamatorii ale mucoasei fenestrale cu obstrucție parțială sau completă, septuri osoase la nivelul nișei ferestrei rotunde cu grade variate de obstrucție, îngroșări ale timpanului secundar, variații anatomice ale nișei ferestrei rotunde cu obstrucție, membrana ferestrei rotunde dedublată, țesut fibros sau adipos la nivelul nișei.

Alteori, substanța terapeutică administrată intratimpanic nu vine în contact cu membrana ferestrei rotunde, scăpând prin trompa lui Eustache. Deasemenea perna de aer din nișa ferestrei poate impiedica accesul medicamentului la membrană.

În practica clinică, medicamentele cele mai frecvent administrate intratimpanic pentru perfuzarea labirintului în cadrul terapiei diverselor sindroame cohleo-vestibulare, sunt : corticosteroizii și antibioticele aminoglicozidice. Rezultate promițătoare s-au obținut experimental și după administrarea substanțelor antioxidante, antagoniștilor glutamatului, inhibitorilor calpainei, factorilor neurotrofici de creștere, terapiilor genice cu vectori virali și a terapiilor celulare. Acestea din urmă așteaptă însă noi confirmări clinice bazate pe evidențe.

Corticosteroizii

Mecanismul de acțiune al corticosteroizilor în urechea internă nu se cunoaște exact fiind posibile multiple modalități.

Corticosteroizii se leagă de receptori glucocortioidici de pe suprafața membranei celulare, după care sunt translocați în nucleul celular unde inițiază sau modulează transcripția genică și influențează căile de semnalizare celulară.

În urechea internă, corticosteroizii intervin în transporturile ionice și lichidiene prin modularea activității Na/K- ATPazei.

De asemenea, corticosteroizii influențează homeostazia hidro-ionică a urechii interne prin inducerea unor gene reglate de receptori mineralocorticoizi și prin controlul canalelor de tip aquaporină.

S-a demonstrat pe culturi primare de epiteliu de canal semicircular membranos că dexametazona modulează homeostazia ionică prin intermediul canalului epitelial de sodiu EnaC, având ca rezultat transportul cationic în labirintul vestibular și fluxul apos cuplat osmotic.

S-a demonstrat totodată scăderea producției de radicali liberi în urechea internă și efectul protector împotriva citokinelor proinflamatorii ca factorul de necroză tumorală α, sub acțiunea corticosteroizilor .

Cea mai frecventă utilizare a glucocorticoizilor în otologie este bazată pe presupusul efect imunosupresor și antiinflamator de la nivelul cohleei. Astfel, un factor de transcripție nuclear reglează sinteza multor citokine proinflamatorii implicate în răspunsul imun general, care este inhibat de glucocorticoizi. Experimental s-a dovedit că ARN-ul mesager al acestui factor de transcripție se exprimă la nivelul cohleei de șoarece după inocularea sistemică de lipopolizaharide, secvență care se presupune a fi inhibată de tratamentul cu glucocorticoizi. Astfel, diminuarea factorului nuclear în compartimente specifice ale urechii interne este responsabilă de remisia hipoacuziei.

Administrarea topică de corticosteroizi îmbunătățește și fluxul sanghin cohlear.

Accesul corticosteroizilor la structurile urechii interne este influențat de factori multipli, între care modalitatea de administrare (topică sau sistemică) este cea mai importantă.

Efectele adverse ale corticoterapiei sistemice sunt numeroase. Acestea includ: tulburări hidroelectrolitice, retenție hidrosalină, insuficiență cardiacă la pacienții susceptibili, hipopotasemie, HTA, atrofie musculară, osteoporoză, fracturi vertebrale prin compresiune, necroză aseptică de cap femural sau humeral. Efectele adverse gastrointestinale includ ulcer peptic, perforații intestinale, pancreatită și esofagită de reflux. Efectele secundare dermatologice includ alterarea proceselor de cicatrizare, piele subțire și fragilă, peteșii, echimoze, hiperhidroză. Efectele adverse neurologice sunt reprezentate de convulsii, hipertensiune intracraniană cu edem papilar, cefalee, psihopatii. Problemele endocrinologice care pot fi asociate corticoterapiei sistemice sunt ciclu menstrual neregulat, sindrom cushingoid, întârzâierea creșterii la copii, insuficiență hipofizo-corticosuprarenaliană secundară, scăderea toleranței la glucoză, diabet zaharat latent, creșterea necesarului de insulină sau antidiabetice orale la pacienții diabetici, hirsutism. Pot apărea complicații oftalmologice precum cataracta posterioară, hipertensiune intraoculară, glaucom și exoftalmie. Corticoterapia sistemică poate determina rupturi de miocard după infarct miocardic recent. Alte efecte secundare sunt tromboembolismul, supraponderabilitatea, bulimia, grețuri și vărsături.

Gama vastă de efecte adverse favorizează administrarea intratimpanică a corticosteroizilor ca o cale directă de obținere a unor concentrații terapeutice intracohleare mari, lipsită de efectele sistemice secundare.

Indicațiile terapiei steroidiene intratimpanice includ surditatea brusc instalată, boala Meniere rezistentă la regim igieno-dietetic și tratament sistemic cu diuretice și vasodilatatoare labirintice, boala autoimună primitivă a urechii și tinitusul.

Rezultatele tratării acestor afecțiuni sunt heterogene, actualmente corticoterapia intratimpanică fiind recomandată intens doar ca terapie de salvare a cazurilor de hipoacuzie neurosenzorială brusc instalată cu eșec la corticoterapia sistemică.

Dozele, schemele de tratament și volumul de medicament injectat recomandate de studii sunt deduse empiric și sunt foarte variabile funcție de medicul terapeut, injecțiile intratimpanice fiind administrate uneori zilnic timp de cinci zile sau săptămânal până la patru săptămâni consecutive. Soluțiile medicamentoase utilizate nu sunt standardizate, asigurând o expunere minimă, variabilă și trecătoare a urechii interne la corticosteroid.

Formulele moderne bazate pe polimeri par a prezenta mai multe avantaje cum ar fi : expunere prelungită a urechii interne prin intermediul unei injectări unice, volum mic de injectare cu evitarea unei a doua miringostomii de aerare, minimalizarea sechelelor post injectare care apar după umplerea urechii medii cu soluții, posibilitatea de ortostatism a pacienților imediat după injectare, fără restricționarea vorbitului și deglutiției.

Aminoglicozidele.

La scurt timp de la descoperirea lor, antibioticele aminoglicozidice s-au remarcat prin efectul ototoxic, care, deși considerat efect advers al terapiei antiinfecțioase, și-a găsit aplicabilitate în tratamentul vertijului din cadrul bolii Meniere.

Când tratamentul medical al bolii Meniere constând în restricție salină, abstinență de la substanțele excitante precum teinele, cafeina și terapia diuretică și vasodilatatorie, eșuează să reducă numărul crizelor vertiginoase, se face apel la alternative terapeutice chirurgicale (decompresia sacului endolimfatic, neurectomia vestibulară, labirintectomia) sau medicamentoase. Acestea din urmă constau în corticoterapie, când funcția auditivă este păstrată sau distrucția farmacologică cu antibiotice aminoglicozidice a labirintului când există un deficit auditiv important.

Pentru prima dată, Fowler în 1948, a realizat labirintectomia chimică prin injecții intramusculare cu streptomicină. În 1956, Schuknecht injectează streptomicina în urechea medie reușind controlul vertijului la 63% din pacienții tratați cu producerea însă a unei hipoacuzii neurosenzoriale profunde secundare în toate cazurile. Lange, în 1977, administrează gentamicină intratimpanic, reușind să conserve audiția la 76 % din bolnavii tratați. Gentamicina devine agentul terapeutic preferat datorită preponderenței efectului vestibulotoxic față de proprietățile sale cohleotoxice.

De la nivelul cavității timpanice, gentamicina ajunge în perilimfa scalei timapanice prin traversarea activă a membranei ferestrei rotunde.

În urechea internă aminoglicozidele determină leziuni degenerative ale celulelor ciliate. La nivelul organului Corti leziunile sunt produse inițial în spira bazală și afectează predominant celulele ciliate externe. În sistemul vestibular celulele ciliate de tip 1 sunt mai vulnerabile decât celulele ciliate de tip 2 iar, din punct de vedere anatomic, sunt afectate în primul rând celulele ciliate ale crestei ampulare, urmate de cele din utriculă și saculă. Mecanismele prin care aminoglicozidele produc injurii celulare includ expresia proteinei de șoc termic 27, stimularea producției de specii reactive de oxigen și de oxid nitric.

În prezent, în cazul indicației de suprimare a funcției vestibulare prin administrare de gentamicină intratimpanic pentru controlul vertijului din boala Meniere, majoritatea studiilor recomandă protocoale de administrare prin *titrare.* Astfel, se injectează gentamicină intratimpanic o dată pe săptămână până apar primele semne de hipofuncție vestibulară și controlul vertijului sau până la agravarea audiției. În cadrul acestor protocoale, controlul pe termen lung al vertijului nu prezintă diferențe semnificative față de controlul realizat prin tratamente cu doze zilnice sau doze zilnice multiple.

Terapia prin *titrare* cu gentamicină intratimpanică oferă un control al vertijului la 87% (variabil în studii între 75-100%) din pacienții cu boală Meniere unilaterală, riscul de hipoacuzie supraadăugată fiind de cca 21 % (între 0-37 % în diverse studii). La o treime din pacienți însă, vertijul poate reapărea. Recurențele pot beneficia, de asemenea, de tratamentul cu gentamicină administrată intratimpanic, cu același risc de hipoacuzie secundară. Efectul principal al gentamicinei intratimpanice ar fi reducerea funcției vestibulare prin lezarea celulelor ciliate, fără însă a parea necesară ablația labirintică completă pentru dominarea vertijului.

Tratamentul sindroamelor cohleo-vestibulare reprezintă o prioritate pentru restabilirea echilibrului biped și a audiției. Mecanismele fiziopatologice endocohleare nu sunt încă deplin elucidate. Metodele de tratament ale afecțiunilor urechii interne, când sunt accesibile și aplicabile, rămân totuși fundamentate pe experiența izolată a unor autori, neexistând standarde consacrate.

O înțelegere aprofundată a protocoalelor de administrare intratimpanică, cunoașterea proprietăților fizico-chimice a soluțiilor administrate intratimpanic, a anatomiei fosetei ferestrei rotunde și a modificărilor pe care aceasta le poate suferi în urma unor procese patologice și a manipulărilor chirurgicale, precum și noțiuni specifice de farmacocinetică labirintică sunt condiții esențiale pentru optimizarea strategiilor de perfuzare terapeutică a urechii interne prin aplicarea intratimpanică a medicației în cadrul terapiei sindroamelor cohleo-vestibulare.

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Sistem Integrat pentru Siguranţa Pacienţilor Investigaţi prin Metode Imagistice Radiologice

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Rezumat

Asigurarea radioprotecţiei pacienţilor investigaţi prin metode imagistice radiologice printr-un sistem realizat pe baza unei soluții informatice moderne reprezintă un proiect pilot în curs de desfăşurare, care va fi implementat şi testat în Spitalul Universitar Militar Central Dr. Carol Davila Bucureşti. Un studiu medical efectuat timp de trei luni a arătat că mulți pacienţi investigaţi prin tomografii computerizate, scintigrafii şi radiografii depăşesc doza de radiaţii maximă cumulată admisă. Noul sistem integrat este proiectat pe bază de smartcarduri şi securitate a stocarii datelor.

Cuvinte cheie: *radioprotecţie, metode de imagistică medicală, smartcarduri, infrastructură PKI*

Summary

Ensuring radiation safety of the patients investigated by radiological imaging methods using a system based on a modern informatic solution is a pilot project in progresss, which will be implemented and tested in Central Military Emergency University Hospital Dr. Carol Davila from Bucharest. A threee months medical study has revealed that that many patients investigated by computed tomographies, scintigraphies or radiographies over pass the maximum allowed cumulative dose. The new system is designed based on smart cards and secure data storage.

Key terms: *radiation safety, medical imaging methods, smarcards, pki infrastructure*

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Introducere

Radiațiile ionizante prin natura și energia lor, peste anumite doze admise, pot avea efecte biologice grave. Există numeroase studii în literatura de specialitate care stabilesc tipuri de efecte biologice care apar la depășirea unor praguri de doze de radiații. Există de asemenea stabilite limite maxime de siguranță pentru dozele de radiații, reglementate de organisme naționale și internaționale.

Iradierea în cadrul procedurilor medicale de diagnostic, deși pare în continuă scădere ca urmare a modernizării echipamentelor folosite în spitale, în realitate crește continuu, datorită creșterii volumului de investigații medicale prescrise uzual pacienților. Radiografiile, tomografiile computerizate, scintigrafiile fac parte din clasa metodelor imagistice de diagnostic care folosesc radiații ionizante și de aceea acestea trebuie prescrise cu mare atenție.

Metodele curente de înregistrare a dozei de radiații aplicată pacienților în timpul investigațiilor medicale radiologice se bazează pe completarea unui formular scris și nu permit, sau fac foarte dificilă colectarea acestor date, pentru a determina dacă doza cumulată maximă este atinsă.

Implementarea unui sistem securizat integrat pentru siguranța cetățenilor implicați în investigații medicale realizate prin metode imagistice radiologice sau în alte activități cu risc crescut de expunere la radiații reprezintă o cerință majoră a sistemului național de sănătate.

Dozele de radiații

Radiațiile existente în natură se împart în radiații ionizante și radiații neionizante. Toate studiile privind efectele nocive asupra organismului uman și depășirea unor doze de radiații se referă în special la radiațiile ionizante. Numele lor derivă din faptul că radiațiile ionizante au energii foarte mari, care la ciocnirea cu un atom eliberează un electron, producând astfel ionizarea.

Analiza radiațiilor se face în funcție de frecvența acestora. Cu cât frecvența crește, cu atât energia lor este mai mare. Deci radiațiile ionizante au frecvențe mari. Radiațiile neionizante încep de la frecvențe foarte joase, acoperă domeniul undelor audio, microundelor, trec spre undele vizibile până la domeniul ultravioletelor. Totuși granița dintre radiațiile neionizante și cele ionizante este greu de trasat strict, întrucât ea depinde și de materialul iradiat, ale cărui atomi trebuie să fie ionizați. În plus o radiație neionizantă dacă este focalizată în fascicule de mare intensitate poate avea aceleași efecte ca o radiație ionizantă.

Acceptăm în sens larg că orice particule care se mişcă cu viteză mare, cum ar fi razele cosmice, particulele alfa, beta gama, razele X, neutronii sunt radiații ionizante. În numeroase investigații medicale sunt folosite cu precădere razele X. Pentru evidențierea corectă a pragurilor și dozelor admise de radiații trebuie remarcat că există numeroase unități de măsură pentru radiații.

Sievertul (SV) și Grayul (Gy) reprezintă principalele unități pentru dozele de radiații în sistemul internațional de unități (SI), dar Sievertul este folosit pentru evaluarea cantitativă a impactului biologic prin expunerea organismelor vii la radiații ionizante. Doza echivalentă de radiații la care este expus un organism viu se determină, evaluându-se cantitatea de energie pe unitatea de masă corporală, corelată cu un un factor relativizant (corector), care ține cont de periculozitatea relativă a felului de radiații respective. Sievertul este folosit și în calculele necesare la analizarea și aprecierea factorului de risc al diverselor iradieri.

Activitate	Doza echivalentă primită de o persoană
Doza medie mondială din toate sursele	2,8 mSv pe an
Zbor cu avionul dus – întors Europa–SUA	0,1 mSv
Radiografie pulmonară	0,1 mSv
Procedură medicală cu doză ridicată	5–10 mSv

Tabelul 1. *Surse de radiații [2]*

Alte unități folosite sunt J/Kg, Gray-ul, Roentgenequivalent man (Rem-ul), Rad-ul sau Roentgen (R sau Röntgen) .

Un organism internațional de reglementare este UNSCEAR (United Nations Scientific Comitee on the Effects of Atomic Radiation) care în 2008 [1], într-un raport a aratat câteva doze de radiații absorbite natural sau în urma investigațiilor medicale. În tabelul 1 sunt prezentate câteva dintre ele [2] .

Din tabel se poate observa cu usurință impactul major al procedurilor medicale. Stabilirea unei valori de prag pentru apariția unor afecțiuni sau boli este deosebit de dificilă, întrucât ea variază de la un individ la altul, depinzând nu numai de valoarea dozei ci și de durata iradierii. De exemplu, se acceptă unanim că sub 10mSv nu apar efecte cauzate de radiații, dar la cealaltă extremă, peste 1000 mSv apare boala de radiații cu risc letal precum și diferite forme de cancer. De aceea dozele absorbite în cazul procedurilor medicale din tabelul 1, rămân la limita superioară a pragului de risc minim, dar în cazul repetării lor trebuie ridicat un semnal de alarmă.

Din tabelul anterior rezultă că tomografiile au un rol determinant în bilanțul total al dozei de radiații absorbită. De aceea în tabelul 2 se prezintă doze medii de radiații la care sunt expuși pacienții în cadrul diverselor tipuri de examinări computer tomograf (CT) [3], [4], [5], [6].

Conform prevederilor legale, limita dozei efective pentru populație este de 1 mSv pe an exceptând expunerile medicale. În situatii speciale, Comisia Națională pentru Controlul Activităților Nucleare (CNCAN) poate autoriza o limita superioară anuală de până la 5 mSv pe an, cu condiția ca valoarea medie pe 5 ani consecutivi a dozei efective să nu depășească 1 mSv pe an. Limita dozei efective pentru personalul expus profesional este de 20 mSv pe an.

Normele privind radioprotecția în cadrul expunerilor medicale au fost aprobate prin Ordinul comun MSP și CNCAN nr. 285/79/2002 și publicate în M.O. nr. 446 bis din 25 iunie 2002. Ele reprezintă transpunerea în legislația română a Directivei UE nr. 97/43/EURATOM. Ele stabilesc principiile generale de radioprotecție a persoanelor supuse expunerii medicale la radiații ionizante. Sunt definiți "medicul ordonator" al explorării radiologice și "medicul practician", fiind evidențiat faptul că responsabilitatea unei expuneri medicale revine îndeosebi medicului practician (radiolog, intervenționist). Situațiile de "dezacord de opinii" între ordonator și practician trebuiesc analizate în Comisiile de etică privind expunerea medicală din fiecare unitate medicală .

Întrucât dintre cele trei principii de radioprotecție de bază, cel privind "limitarea dozei" nu se aplică în cazul expunerii medicale, este accentuată definirea și aplicarea celorlalte două, respectiv "justificarea" și "optimizarea" explorării radiologice. Toate expunerile medicale individuale trebuie să fie justificate în prealabil, ținand cont de obiectivele specifice ale expunerii și de caracteristicile persoanei implicate, și trebuie să ia în considerație posibilitatea existenței informațiilor ce urmează a fi obținute, într-o investigație radiologică anterioară. În scopul "optimizării" protecției pacientului, pentru întâia oară, în aceste norme sunt prevăzute "nivelurile de referință" în radiologia de diagnostic și în medicina nucleară, precum și "constrângerile de doză" pentru persoanele în cunostință de cauză și voluntar, asigură sprijinul și confortul persoanelor care sunt supuse diagnosticării sau tratamentului medical, și sunt interzise examinările fluoroscopice fără intensificator de imagine.

III. Radioprotecția pacienților

Radioprotecția pacienților expuși la radiații ionizante se realizează prin Fișa pentru înregistrarea datelor privind expunerea medicală la radiații

Examinări CT	**Doză efectivă (mSv)**	**Număr echivalent radiografii torace**
Cap	2	100
Gât	3	150
Scorul de calciu (calciu coronarian)	3	150
Angiografie pulmonară	5.2	260
Coloana vertebrală	6	300
Piept	8	400
Angiografie coronară	8.7	435
Abdomen	10	500
Pelvis	10	500
Colonoscopie virtuală	10	500
Piept (embolism pulmonar)	15	750

Tabelul 2 *Tipuri de tomografii [3], [4], [5],[6]*

ionizante care trebuie completată în momentul efectuării investigației; aceasta se returnează persoanei respective și trebuie să conțină în mod obligatoriu datele de identificare ale pacientului (completate de către instituția abilitată care efectuează prima astfel de procedură și este obligată să elibereze documentul respectiv). Fișa mai conține detalii despre medicul ordonator și instituția din care acesta face parte, medicul practicant și instituția din care acesta face parte, procedura efectuată, incluzând detalii despre doza administrată (în cazul procedurilor de medicină nucleară), precum și despre doza efectivă estimată primită de pacient. Elementele de dozimetrie descrise anterior sunt trecute și într-un registru sau tabel existent în cadrul unității practicante, conținând datele de identificare, procedura și doza primită de pacient; aceste date sunt centralizate și communicate organelor abilitate din 3 in 3 luni. Procedura este greoaie și nu permite actualizarea datelor rapid și eficient.

În Spitalul Universitar Militar Central Dr. Carol Davila București a fost efectuat un studiu privind dozele de radiații cumulate absorbite în cadrul investigațiilor medicale.

În cadrul Laboratorului de Radiologie al SUUMC București funcționează efectiv două aparate radiologice digitale RADREX TOSHIBA, un aparat radiologic analogic SIEMENS MULTIX, un aparat radioscopie cu achiziție digitala PHILIPS TELEDIAGNOST. Laboratorul asigură investigațiile radiologice necesare, solicitate de medicii ordonatori din cadrul tuturor secțiilor medicale și chirurgicale cât și solicitările de la UPU și ambulatoriul de specialitate al spitalului.

Înainte de efectuarea investigației pacienții sunt informați despre procedura radiologică, be-

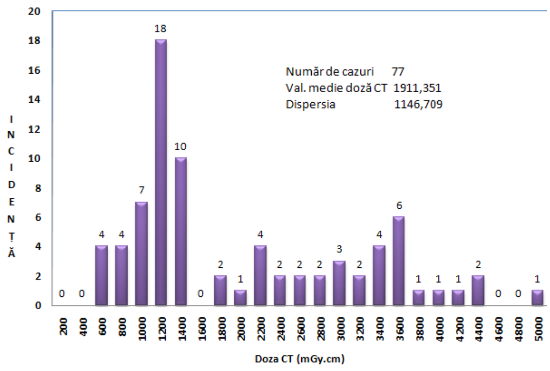

Figura 1 *Incidența dozelor la pacienții investigați prin tomografii computerizate (CT)*

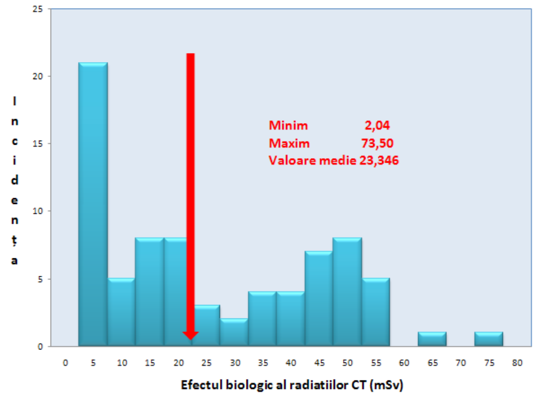

Figura 2 *Distribuţia efectului dozelor de radiaţii asupra pacienţilor supuşi la tomografii computerizate*

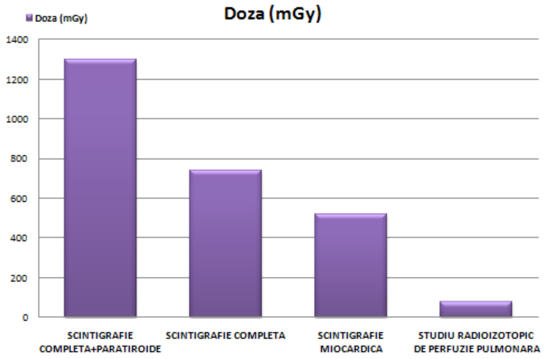

Figura 3 *Distribuţia dozelor de radiaţii pentru diferite investigaţii scintigrafice*

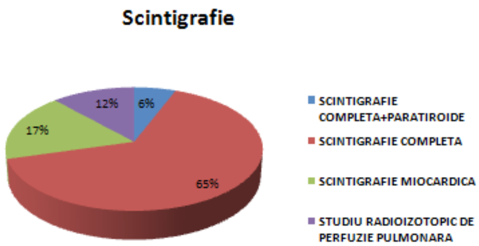

Figura 4 *Repartizarea procentuală a investigaţiilor scintigrafice în lotul studiat*

neficiul și scopul ei, precum și eventualele riscuri. Femeile, în plus, sunt chestionate privind data ultimei menstruații, despre eventualitatea unei sarcini. Pacienții semnează un consimțământ informat. La sfârsitul examinării, dozele absorbite sunt notate in registrul special, alături de datele pasaportale și medicale ale pacientului, funcție de procedurile efectuate. De asemenea, aceste valori trebuie consemnate și în fișa de radiații. Din păcate, în ultimul timp, nu mai există aceste formulare.

Studiul a avut durata de trei luni în perioada august-octombrie 2012 pentru toți pacienții internați în Secția Medicală 1. Au fost analizați pacienții cărora li s-au prescris investigații radiologice, pe tipuri de metode imagistice radiologice și au fost cumulate dozele de radiații absorbite . Chiar și în ciuda unui efort semnificativ de centralizare a datelor din diferite documente medicale, un control total și eficace al acestor doze nu a fost posibil din următoarele motive:

- pacienții provin din alte spitale înainte de a fi investigați, fără înregistrări prealabile a dozelor anterioare
- pacienții pot fi supuși la investigații multiple
- fisele de radiație conțin un volum limitat de informații; încă nu există o finalizare coerentă și utilă a operațiunile de strângere și gestionare a datelor despre radioprotecție și iradierea specifică a paciențiilor.

Studiile aplicative efectuate pe baza practicii medicale au pus în evidență aspecte extrem de interesante. Acestea sunt prezentate în cele cinci figuri următoare.

Cele mai relevante cazuri de depășire a dozelor admisibile sunt cele care provin din investigațiile de tomografie computerizată (CT) și scintigrafie și mult mai rar din cadrul investigațiilor radiologice clasice. Dincolo de valoarea incontestabilă a acestor tipuri de investigații privind stabilirea diagnosticului, trebuie luat în considerare și faptul că nivelul expunerii la radiații al pacientului investigat este foarte mare. În cazul computerului tomograf, de exemplu, doza de radiații primită de pacient pe timpul investigației poate fi de câteva zeci de ori mai mare decât la o radiografie de torace. Radiografia de torace este considerată de mulți specialiști drept investigația standard în compararea dozelor de radiații. Folosirea substanțelor de contrast crește de asemenea doza de radiații cumulată de pacienți. Se evidențiază clar depășirea dozei anuale admise la un procent mare de pacienți investigați prin tomografie computerizată.

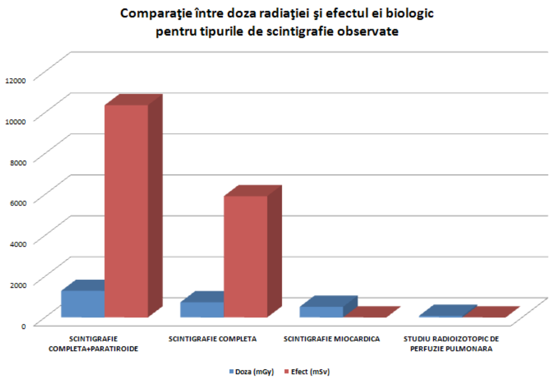

Figura 5 *Distribuţia dozelor de radiaţii şi a efectelor biologice pentru diferite tipuri de investigaţii scintigrafice*

IV. Noul sistem SRSPIRIM

Soluția propusa de membrii echipei de cercetare pentru implementarea sistemului de protecție a pacienților investigați prin metode radiologice este ilustrată în Figura 6 . Sistemul este fondat în jurul celor două tipuri de carduri: cardul medical radiologic destinat pacienților și cardul medical profesionist destinat medicilor.

În cadrul proiectului SRSPIRIM, la care participă în calitate de parteneri Universitatea Politehnica București, Spitalul Universitar Militar Central Dr. Carol Davila București și S.C. Certsign S.A., membră a grupului de firme UTI, se dezvoltă un sistem complex care permite stocarea și gestionarea istoricului de expunere la radiații a pacientului. Proiectul se realizează prin implementarea unei arhitecturi cu trei nivele distincte de stocare a informatiilor critice pentru pacienți, care elimină posibilitatea de a pierde informații și oferă acces în timp real la informatiile medicale pentru personalul de specialitate.

Pe cardul radiologic al pacientului, numit și cardul medical radiologic se vor stoca :

- informații personale publice (nume, prenume, data nașterii, etc)
- informații personale private (CNP, adresa, etc)
- informații medicale publice (grupa sangvina, alergii etc)

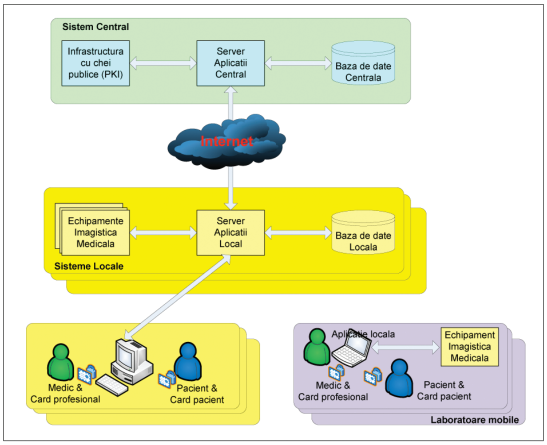

Figura 6 *Soluţia de realizare a sistemului SRSPIRIM*

- informații medicale private (afecțiuni și boli importante)
- istoricul investigațiilor radiologice (data, nivelul de radiații, etc)
- certificatul și cheile corespondente pentru autentificarea pacientului.
- certificatul și cheile corespondente pentru autentificarea cardului.

Pe cardurile electronice profesionale vor fi stocate următoarele informații:

- informațiile personale (nume, prenume, CNP, etc)
- informații legate de latura profesională a medicului
- certificatul și cheile corespondente pentru semnare.
- certificatul și cheile corespondente pentru autentificare.
- certificatul și cheile corespondente pentru autentificarea cardului.

Cardul medical radiologic stochează nivelul de radiații specific fiecărei investigații radiologice. Cardul profesional este util pentru autentificarea medicului fata de pacient sau fata de alte servicii web, dar și pentru semnarea digitala a informațiilor medicale. Datele unei investigații sunt preluate de la echipamentele medicale, sunt procesate de aplicațiile off-card iar rezultatele sunt salvate în cadrul sistemului, pe trei nivele de stocare :

- Nivelul central unde se implementează o baza de date care stochează datele corespunzatoare investigațiilor pacienților prin metode radiologice;
- Nivelul local unde sunt create baze de date în fiecare laborator de investigații care își sincronizează conținutul corespunzator pacienților inregistrați local cu conținutul bazei de date centrale;
- Nivelul cardurilor radiologice unde se stochează și gestionează datele corespunzătoare istoriei investigațiilor efectuate (ex. dozele de radiații acumulate cu ocazia fiecarei investigații, tipul investigației)

Aceste date sunt utilizate înaintea fiecărei noi investigații pentru a estima doza curentă totală de radiații pe care pacientul o înmagazinează in organism intr-un anumit interval de timp. Securitatea sistemului se bazeaza pe o infrastructura de chei publice (PKI) pentru gestiunea certificatelor digitale aflate pe carduri.

Sistemul SRSPIRIM va mai oferi aplicații pentru:

- Vizualizarea în timp real a istoriei investigațiilor, a dozelor de radiații acumulate de pacient;
- Ajutorul personalului medical în luarea deciziilor adecvate în ceea ce privește indicația investigațiilor în funcție de dozele curente cumulative și dozele maxime admise pentru fiecare grupa de risc și vârstă;
- Generarea de rapoarte periodice utile pentru diferite decizii legate de reglementările în vigoare.

Arhitectura generala a sistemului cuprinde următoarele componente principale:

- infrastructura de chei publice PKI necesară operării cardurilor electronice;
- centrul de personalizare al cardurilor pentru emiterea cardurilor electronice;
- sistemul de management al certificatelor digitale, numit și *E-Health CMS*;
- serverele bazelor de date locale și a bazei de date centralizate;
- aplicațiile web pentru interfața cu bazele de date, cardurile electronice și echipamentele medicale;
- aplicațiile existente pe cardurile radiologice numite și appleti JavaCard.

Sistemul de management al cardurilor electronice radiologice, *SRSPIRIM CMS*, va fi o soluție Web de gestionare a cardurilor electronice emise de către Autoritatea Emitentă. CMS va oferi gestiunea completa pentru ciclul de viața al cardurilor radiologice emise. Accesul utilizatorilor la aplicația web a *SRSPIRIM CMS* se va face autentificat, in trei moduri, astfel:

- modul utilizator - destinat utilizatorilor obișnuiți și oferă funcționalități de gestionare a cardurilor precum: cereri de emitere carduri, schimbare PIN etc.

- modul operator - destinat operatorilor de SRSPIRIM CMS şi oferă funcţionalităţi de operare a sistemului: emitere de carduri, invalidare, etc.
- modul administrator - destinat administratorului SRSPIRIM CMS şi oferă funcţionalităţi precum instalare, configurare, gestionarea operatorilor în sistem.

În cadrul SRSPIRIM vor exista două tipuri de baze de date:

- baze de date pentru administrarea cardurilor aflate la nivelul centrului de personalizare şi a SRSPIRIM CMS;
- baze de date pentru gestiunea informaţiilor medicale specifice pacienţilor unde vor fi înregistrate consultaţii, rezultatul investigaţiilor medicale, etc.

Sistemul SRSPIRIM va asigura astfel replicarea informaţiilor stocate în baza de date centrala cu bazele de date locale şi cardurile de pacient pentru a acoperi toate situaţiile posibile, dupa cum urmeaza:

I. Pacientul merge la doctor fără cardul pacientului. În acest caz, sistemul va furniza medicului datele corespunzătoare pacientului din baza de date locală. Dacă pacientul este nou în unitatea respectivă, sistemul va solicita datele din baza centrală de date şi le va stoca şi in baza de date locală. După investigaţie, sistemul va stoca în bazele de date locală şi centrală datele noii investigaţii, inclusiv noua doză de radiaţii acumulată la pacient. Data viitoare când pacientul merge la medic cu cardul, sistemul va asigura sincronizarea între informaţiile stocate pe card şi informaţiile din baza de date locală sau centrală;

II. Pacientul merge la investigatii fara card şi sistemul informatic al laboratorului nu are acces (temporar) la baza centrală (ex: pentru laboratoare mobile). În cazul în care baza de date locală conţine informaţiile corespunzătoare pentru pacient, medicul va fi capabil să le folosească pentru a recomanda un anumit tip de investigaţie. După investigaţie, sistemul va stoca în baza locală detaliile noii investigatii, inclusiv noua doza de radiaţii acumulata de către pacient. Mai târziu, atunci când sistemul local va putea accesa sistemul central, informaţiile corespunzătoare pacientului vor fi sincronizate între cele două baze de date;

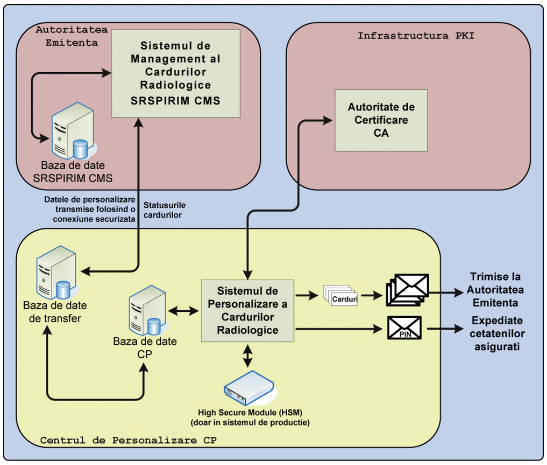
Autoritatea Emitenta
Sistemul de Management al Cardurilor Radiologice SRSPIRIM CMS
Baza de date SRSPIRIM CMS
Infrastructura PKI
Autoritate de Certificare CA
Datele de personalizare transmise folosind o conexiune securizata
Statusurile cardurilor
Baza de date de transfer
Baza de date CP
Sistemul de Personalizare a Cardurilor Radiologice
Carduri
PIN
Trimise la Autoritatea Emitenta
Expediate cetatenilor asigurati
High Secure Module (HSM) (doar in sistemul de productie)
Centrul de Personalizare CP

Figura 7 **Arhitectura sistemului de personalizare a cardurilor electronice radiologice**

III. Pacientul prezintă cardul dar doctorul nu are acces la nici o bază de date. În acest caz medicul, folosind un computer cu un cititor de card şi o aplicaţie desktop dezvoltată în cadrul proiectului SRSPIRIM va putea accesa istoria investigaţiilor şi dozele primite de pacient, va putea calcula doza curentă cumulata şi va putea recomanda cea mai potrivita investigaţie. După investigaţie, detaliile noii investigaţii – inclusiv noua doză de radiaţii acumulată – vor fi înregistrate pe cardul pacientului. Data următoare când pacientul merge la medic cu cardul, aceste informaţii vor fi transferate de pe card în bazele de date locală şi centrală.

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Cronică asupra celui de-al 18-lea Congres Balcanic de Medicină Militară

În primăvara acestui an, a avut loc în Istanbul, cea mai importantă manifestare științifică anuală a medicilor militari din balcani și anume, al 18-lea Congres Balcanic de Medicină Militară. Acesta s-a desfășurat sub auspiciile Comitetului Balcanic de Medicină Militară, organism fondat încă din anul 1993 de către reprezentanții Direcțiilor Medicale din Bulgaria, Grecia, România și Turcia.

18th CONGRESS OF BALKAN MILITARY MEDICAL COMMITTEE

*Lt. Col. Dr. **Bogdan Marinescu**, în timpul prezentării unei lucrări la cel de-al 18-lea Congres Balcanic de Medicină Militară – Istambul, 2013.*

Peste 275 de participanți din țările membre (în momentul de față Bulgaria, Grecia, România, Serbia și Turcia) au luat parte la acest eveniment, care are deja o lungă și valoroasă tradiție. Au fost prezentate 418 lucrări de specialitate (172 comunicări orale, 246 postere), nivelul științific fiind apreciat unanim ca unul de înaltă ținută.

Delegația României a fost condusă de D-nul G-ral de brigadă Dr. Mihai Marius Mureșan – Șeful Direcției Medicale și a fost constituită din 40 de participanți, printre care: șeful Institutului Medico-Militar, medici din cadrul SUUMC-București, a spitalelor militare din țară, Direcției Medicale, studenți militari, asistente medicale.

Tema principală a congresului, a constituit-o un subiect de o reală importanță și actualitate în viața de zi cu zi a militarilor: „Calitatea acuității vizuale – de la încorporare până la retragere". De asemenea au fost organizate două workshop-uri ce au dezbătut pe larg teme legate de bioterorism-bioaparare și ultrasonografia realizată în condiții de urgență.

Tot în cadrul evenimentului, s-a desfășurat a 3-a intâlnire anuală a Șefilor de Direcții Medicale și a Instituțiilor de Învațământ Medico-Militar a țărilor din Balcani și a 3-a întalnire a studenților mediciniști militari, activități inițiate de țara noastră în urma cu 3 ani.

Tema principală dezbătută la întâlnirea anuală a Șefilor de Direcții Medicale și a Instituțiilor de Învațământ Medico-Militar, s-a referit la „Profilaxia și medicina preventivă, înainte, în timpul și după misiunile externe ale militarilor". Subiectul a suscitat un real interes și în rândul delegației noastre, având în vedere participarea tot mai activă a României la misiuni de menținere a păcii în afara granițelor țării. Din dezbateri, a reieșit clar necesitatea de a avea o bună planificare a activităților de prevenție (vaccinare, pregatire fizică, pregătire psihologică, cunoaștere îndeaproape a condițiilor externe, specifice zonelor unde urmează să activeze), dar și de menținere și urmărire la distanță, a statusului sănătății militarilor implicați în astfel de misiuni, în contextul adaptării la condiții specifice de mediu, climă, obiceiuri locale etc.

La întâlnirea anuală a studenților mediciniști militari, au participat 25 de studenți ai Instituțiilor de Învățământ Medico-Militar, lucrările fiind coordonate de Șeful Academiei Militare Medicale din Salonic, Șeful Institutului Medico-Militar din București și Șeful delegației Serbiei. Tematica supusă dezbaterii a suscitat un real interes, hotărându-se să se continue și în viitor această activitate.

Anul acesta, delegația României a obținut cea mai importantă distincție a congresului: premiul pentru cea mai bună lucrare prezentată – **„Best Military Medical Paper Award of the Congress“**.

Prezentarea a avut ca temă: stresul ocupațional - factor de risc important în domeniul militar. Cercetarea se adresează managementului răspunsului cardiovascular maladaptiv, la stresor nou, de intensitate mică, la angajații expuși la stres cronic de cazarmă și a fost susținută de dr. Ciumașu Rîmbu, medic primar medicina muncii, homeopat de la Spitalul Militar Iași. Lucrarea a propus un algoritm de diagnostic și tratament care a întrunit criteriile de selecție din cadrul concursului balcanic de medicină militară: relevanță pentru medicina militară și noutate ca și concept. S-a apreciat claritatea și concizia prezentării.“ *Interacțiunea cu sistemele medicale militare străine este foarte importantă în condițiile promovării cooperării, pentru găsirea unor soluții la problemele comune*“, a apreciat dr. Ciumașu Rîmbu.

Obiectivul acestor manifestări anuale, este de a înlesni schimbul de cunoștințe, informații și experiență dintre personalul militar medical al țărilor balcanice, pentru a îmbunătăți continuu calitatea actului medical și implicit starea de sănătate a militarilor dar și a populației civile din regiunea balcanică.

Anul acesta, preocuparea de bază a participanților a constituit-o, aflarea celor mai bune modalități de a oferi servicii de asistență medicală și sprijin psihologic, militarilor din cadrul forțelor armate combatante, înainte, în timpul și după finalizarea misiunii lor în teatrele de operațiuni.

Experții țărilor participante, au hotărât ca, urmare a concluziilor elaborate de BMMC, să se întâlnească pe parcursul anului și în grupuri de lucru specifice, în cadrul unor domenii precum: bio-apărarea, ingineria tisulară și transplatul de celule stem, maladii noi infecțioase; pentru a putea realiza transferul celor mai recente cunoștințe între părțile interesate. Pe viitor, se are în vedere intensificarea schimburilor de experiență în domeniile de nișă, cum ar fi de exemplu, medicina aviatică.

Congresul anual al Comitetului Balcanic de Medicină Militară (BMMC), s-a încheiat cu un acord de instituire a unui Grup Operativ Medical Balcanic comun.

Acest grup va fi activat în cazul unor situații speciale de urgență, cum ar fi dezastrele naturale și calamitățile. Faza 1 va fi lansată până în 2015, grupul operativ urmând să fie complet operațional până în 2017. Proiectul va creea echipe medicale mixte, care vor acționa în situații de urgență, în dezastre naturale și de alt tip.

Alte două acorduri încheiate în timpul congresului se referă la schimbul de experți din domeniul medicinei militare și la posibilitatea unei mai bune interoperabilități a serviciilor medicale militare, prin intermediul telemedicinei. Telemedicina, o inovație și în rândul țărilor din regiune, va îmbunătăți cunoștințele și calitatea tratamentului medical. Aceasta, va permite medicilor să facă schimb de imagini și proceduri de diagnosticare si tratament, într-o perioadă foarte scurtă de timp și să obțină opinia colegilor din zona Balcanilor.

Schimbul de experiență între medicii militari din țările balcanice, în funcție de programele de instrucție oferite de serviciile medicale militare din regiune, a constituit un alt obiectiv discutat în cadrul Congresului, oferindu-se programe educaționale tematice de o calitate deosebită.

Beneficiile acestor proiecte comune depășesc domeniul medicinei militare, trecând în domeniul relațiilor generale dintre țările noastre.

Așa cum afirma unul din participanți, „prietenia și sprijinul reciproc sunt cele mai mari beneficii, deoarece întotdeauna prietenii te vor ajuta mai bine și mai rapid decât alții.“

Dar, dincolo de schimbul de informații specifice medico-militare, rolul acestor manifestări este și de a stabili, a menține și dezvolta, relațiile de prietenie între persoane ce impărtășesc aceleași preocupari și idealuri – grija continuă pentru oamenii aflați în suferință. Aceste întâlniri anuale, pot fi considerate un model de interacțiune, între grupuri de oameni ce aparțin unui areal frământat de probleme similare, în care se regăsesc obiceiuri, tradiții, motive culturale asemănătoare, mixate într-un amalgam unic… Balcanii.

Speram ca prin efortul și dorința comună, aceasta tradiție să se perpetueze cât mai mult .

În primavara anului viitor, al 19-lea Congres Balcanic de Medicină Militară va avea loc în orașul Plovdiv – Bulgaria. De aceea, vă asteptăm în număr cât mai mare, cât mai curând, cu cât mai multe și interesante lucrări științifice!

Prin colaborare și schimb de informații între personalul militar medical al țărilor balcanice, se crează condiții reale de creștere a calității și eficienței actului medical și implicit de îmbunatățire a stării de sănătate, atât a militarilor cât și a populației civile din țările membre.

România este reprezentată la Comitetul Balcanic de Medicină Militară de Lt. Col. Dr. Bogdan Marinescu (șef secție chirurgie plastică SCUUMC-București), ca **președinte** și de Mr. Dr. Ciprian Constantin (șef departament diabet și nutriție) ca **secretar.**

Supliment Rezumate

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Medicină militară
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EXPERIENȚA SPITALULUI MILITAR ROMÂNESC ÎN SOMALIA ȘI ANGOLA
GRAL BG. (R) DR DRAGUSIN I.

MEDICINA MILITARĂ ROMÂNEASCĂ – PARTE A DEZVOLTĂRII DE CAPABILITĂŢI ÎN BOSNIA ŞI HERŢEGOVINA

Pintilie I. D. Iacob, Forţele Aeriene Române

Cuvinte cheie: *medicina aeronautică, AIRMEDEVAC, ALTHEA, NATO.*

Sumar:

În 2008, Bosnia şi Herţegovina a început demersurile pentru integrarea în NATO. Pentru atingerea obiectivelor de integrare, în cadrul operaţiunii ALTHEA a Uniunii Europene a fost demarat obiectivul de dezvoltare a sistemului de evacuare medicală aeriană (AIRMEDEVAC) în Forţele Aeriene ale Bosniei şi Herţegovina, cu contribuţia unei echipe de instruire mobile a Forţelor Aeriene Române. Beneficiind de experienţa României pe parcursul integrării în NATO şi apoi pe parcursul operaţionalizării detaşamentelor Forţelor Aeriene Române puse la dispoziţia NATO, printre care şi detaşamentul MEDEVAC (care a fost evaluat de către NATO în cadru unui exerciţiu de tip STARTASSES), echipa românească de instruire a realizat în perioada 2010- 2012 pregătirea personalului aeronavigant şi a personalului medical din unităţile de aviaţie ale Forţelor Aeriene ale Bosniei şi Herţegovina pentru atingerea standardelor NATO, pentru ca din 2012 să se treacă la o echipă de consiliere în cadrul eşaloanelor superioare, în vederea implementării acestor standarde în cadrul întregii structuri militare.

FORMAŢIUNEA MEDICALĂ ROL 1 NATO/SUA KAIA, KABUL, AFGANISTAN

F. Bănică
Forţele Aeriene Române

Rezumat:

- Descrierea unei formaţiuni medicale tip ROL 1 NATO/SUA:
- Dispunere şi amplasare;
- Organizare logistică şi medicală;
- Încadrarea cu personal medico-sanitar;
- Dotarea cu aparatură şi instrumentar medical;
- Tipurile de activităţi desfăşurate;
- Tipul celor mai frecvente afecţiuni întâlnite;
- Activitatea cotidiană desfăşurată;
- Programe speciale desfăşurate;
- Experienţa proprie în cadrul acestei formaţiuni
- Învăţăminte;
- Concluzii.

ASPECTE ALE MANAGEMENTULUI MEDICAL AL MILITARILOR RĂNIŢI ŞI BOLNAVI DIN TEATRELE DE OPERAŢII ÎN PERIOADA 2011-2013

Lucia Fitărău[1], C.B. Teuşdea[2], R. Munteanu[3]
[1] Statul major General, Direcţia Logistică
[2] Secţia UPU – Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila“, Bucureşti

Rezumat.

Lucrarea are drept scop prezentarea modalităţilor de tratament, locaţia unde s-a efectuat tratamentul şi numărul militarilor români răniţi în teatrele de operaţii. Aceştia au suferit politraumatisme prin explozia de DEI (dispozitive explozive improvizate), şi au fost supuşi intervenţiei primare la locul producerii incidentului, iar apoi au fost evacuaţi la spitalele de Rol 2 din TO Langman, Kandahar. După o perioadă de tratament la acest nivel militarii români au fost evacuaţi în funcţie de patologie pentru tratament definitiv în la Landstuhl Medical Center din Germania sau în facilităţi medicale civile. În lucrarea prezentă se vor aborda costurile acestor operaţiuni, dar şi propuneri de optimizare a managementului şi evacuării medicale strategice.

În concluzie scopul lucrării este de a prezenta principalele aspecte legate de modul de evacuare a militarilor răniţi din TO, tratamentul posttraumatic, costurile tuturor acestor operaţiuni, inclusiv a repatrierii militarilor.

Cuvinte cheie: *teatre de operaţii, militari răniţi, management medical.*

PROTECŢIA MEDICALĂ ŞI MONITORIZAREA TRUPELOR PE TIMPUL PRE-DISLOCĂRII, DISLOCĂRII ŞI POST-DISLOCĂRII

M.M. Mureşan, C. Nistor
Direcţia Medicală

Prezentarea face referire la procedurile privind protecţia medicală a trupelor în fazele de pre dislocare şi post dislocare: examenul medical (examenul de specialitate, examene de laborator şi paraclinice), examinare psihologică, imunizări specifice, module de pregătire în domeniul asistenţei medicale de urgenţă, recunoaştere medicală pe timpul fazei de pre dislocare a trupelor, monitorizarea stării de sănătate, asistenţă medicală de urgenţă şi evacuarea medicală pe tipul dislocării în teatrele de operaţii, precum şi controlul medical la întoarcerea din misiune, examinarea psihologică în vederea identificării precoce, tratarea şi monitorizarea permanentă a tulburărilor legate de stres.

MANAGEMENTUL RĂNITULUI DIN AFGANISTAN

Boşoteanu R.
Statul Major al Forţelor Terestre

Lucrarea îşi propune să prezinte, pe baza experienţei din teatru de operaţiuni Afganistan (iulie 2011- februarie 2012 cu Batalionul 495 Inf, Clinceni) etapele tratamentului medical şi al evacuării militarilor răniţi de la nivelul facilităţii medicale ROL I dispuse în cadrul FOB-rilor la formaţiunile medicale din lanţul de evacuare al partenerului american. Prezentarea are ca obiectiv şi explicarea rolului personalului medical începând cu Combat Live Saver, continuând cu sanitarul de la nivelul companiei, subofiţerul sanitar şi medicul militar în acordarea asistenţei medicale pentru militarii răniţi în acţiunile de luptă.

EVACUAREA MEDICALĂ AERIANĂ ÎN CAZUL VICTIMELOR MULTIPLE - EXPERIENŢA ROMÂNEASCĂ ÎN ULTIMII 5 ANI

Lupu I. L.M,[1], C.B. Teuşdea[2]
[1] Forţele Aeriene Române.
[2] Secţia UPU – Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila“, Bucureşti

Rezumat

Începând cu luna septembrie 2010, în România a fost creat Serviciul Medical Militar de Urgenţă (SMMU), care are ca principala misiune de a salva vieţile personalului militar în timpul operaţiunilor militare. La nivel naţional funcţionează numărul unic de urgenţă 112 prin care se activează intervenţia echipajelor SMURD care acţionează în sprijinul populaţiei civile. În caz de accident cu victime multiple experienţa a demonstrat necesitatea de colaborare între cele două sisteme, în scopul de a salva vieţi. În ultimii 5 ani se constată creşterea numărului de misiuni de evacuare aeriană a victimelor multiple executate de către echipe medicale mixte civil-militare folosind avioane militare. Realizarea acestor misiuni reprezintă provocări, de la planificarea misiunilor, utilizarea de echipamente şi proceduri comune medicale, sistemul de comandă-control, la programe de exerciţii în comun a echipelor medicale mixte şi, desigur, gestionarea unui număr mare de pacienţi în zbor. Având în vedere prezenţa militară românească în diferite teatre de operaţii, precum şi faptul că în cadrul NATO evacuarea medicală strategică este o responsabilitate naţională, dezvoltarea capacităţilor de transport aerian pentru victime multiple este o prioritate de nivel înalt.

Cuvinte cheie: *medic de zbor, îngrijire medicală în timpul zborului, interoperabilitate.*

TRIAJUL MEDICAL ÎN SITUAŢII DE RISC NUCLEAR

C.B. Teuşdea[1], Duşmanu T.[2]
[1] Secţia UPU – Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila“ Bucureşti
[2] Institutul Medico-Militar Bucureşti

Rezumat

Arma nucleară este o armă de distrugere în masă care prin efectele sale fizice determină contaminarea radioactivă a zonei respective şi un număr foarte mare de victime într-un timp foarte scurt care necesită intervenţia rapidă a structurilor sistemului naţional de urgenţă. Managementul victimelor va începe cu aplicarea algoritmului ABCDE, urmat de triajul medical, decontaminarea şi respectiv tratarea acestora în unităţile medicale adecvate. Iniţial se va pune şi un diagnostic preliminar de leziune prin iradiere doar pentru cei care au simptome de expunere (greaţă, vărsături, diaree, hipertermie, eritem, hipotensiune arterială, disfuncţie nervos - centrală). Triajul medical în condiţiile folosirii armei nucleare este diferit, deoarece leziunile de iradiere la pacienţii care supravieţuiesc nu se manifestă înainte de câteva săptămâni de la expunere. De aceea după producerea evenimentului pacienţii vor fi triaţi şi trataţi în funcţie de leziunile traumatice convenţionale, după care va fi executat şi triajul în funcţie de gravitatea leziunilor de iradiere. Există 4 categorii de triaj (roşu, galben, verde, negru) la fel ca şi în cazul triajului convenţional.

***Cuvinte cheie**: armă nucleară, leziuni de iradiere, triaj medical, categorii de triaj.*

Medicină militară – trecut, prezent, viitor
Luni, 21 oct, 12^{15} – 14^{00}

SPITALUL CLINIC MILITAR DE URGENŢĂ „DR. IACOB CZIHAC“ IAŞI

Botez C., Zamfir B., Stoenescu M.

Trecut: Înfiinţarea Spitalului Militar Iaşi în 1831 s-a impus ca o necesitate, întrucât internarea bolnavilor oastei în Spitalul Sf. Spiridon întâmpina greutăţi din cauza capacităţii reduse de spitalizare. Ordinul de înfiinţare prevedea ca spitalul să aibă o capacitate de 40 paturi împărţite în două secţii. Din 1948 spitalul funcţionează în actualul perimetru. Activitatea medicală a fost completată de activitatea ştiinţifică iniţiată de dr. Iacob Czihac şi continuată de generaţiile următoare. În perioada de după 1989, spitalul a trecut prin numeroase etape de restructuare şi reorganizare pentru a se adapta la cerinţele impuse pe de o parte de statutul de spital militar dar şi de evoluţia unităţilor medicale din Iaşi.

Prezent: La ora actuală, spitalul are un număr de 200 paturi. Asistenţa medicală se asigură în regim de spitalizare prin 3 secţii clinice şi 9 compartimente, şi prin ambulatoriul integrat. Cele 3 laboratoare ale spitalului asigură investigarea paraclinică la nivelul cerinţelor actuale. Dotarea modernă a secţiilor şi laboratoarelor, coroborată cu extinderea spectrului de specialităţi medicale la un număr de 28, au făcut posibilă încadrarea spitalului în categoria a II-a începând cu luna aprilie 2013. Spitalul este acreditat ISO 9001/2008 din decembrie 2010.
Spitalul deserveşte un număr de 34 unităţi militare din 4 judeţe, totalizând aproximativ 5000 angajaţi. Activitatea comisiei de expertiză, controalele medicale ale personalului militar (periodice, la angajare, la plecarea/repatrierea din teatrele de operaţii) includerea CPU în SMMU şi activităţile de instruire a personalului medical/paramedical participant la misiuni internaţionale, completează spectrul activităţilor medico-militare.

Viitor: Preocuparea conducerii spitalului se orientează spre îmbunătăţirea condiţiilor hoteliere, de investigare şi tratament, pentru a consolida poziţia ocupată atât în rândul unităţilor medicale ale MAN cât şi între unităţile spitaliceşti din Iaşi.

Istoria şi devoltatrea spitalului militar de urgenţă „Regina Maria“ Braşov

G. Tudose
Spitalul Militar de Urgență „Regina Maria“, Brașov

Istoria Spitalului Militar din Brașov începe în ultimul sfert al veacului al optisprezecelea, din inițiativa unui chirurg militar din regimentul cantonat în Brașov care solicită pe data de 10 iulie 1773 Magistratului Brașovului să dispună lucrări în acest sens.

Datorită unor lucrări de extindere începute în 1784, în anul 1791 a fost înființat și instalat Spitalul Militar. În al doilea deceniu al veacului următor, Spitalul Militar se mută în ceea ce pare să fi fost Cazarma Neagră, pe actuala stradă Nicolae Bălcescu, în locul în care acum se află Facultatea de Medicină a Universității Transilvania, iar în 1895 încep lucrările de construcție la clădirile din actualul amplasament al spitalului. La 1 februarie 1919, Spitalul Militar Brașov trece sub administrație românească, în calitate de spital al Diviziei 18, fiind dotat cu 150 de paturi, iar în anul 1938 regele Carol al II-lea semnează Înaltul Decret prin care Spitalului nostru i se atribuie denumirea *„Regina Maria“*, în memoria *„reginei soldat“*. În această perioadă, spitalul devine spital militar de corp de armată.

În cel de al doilea război mondial spitalul ajunge la capacitatea de 550 de paturi fiind nevoit să disloce principalele secții în Codlea și Râșnov. Capacitatea maximă atinsă în perioada contemporană este de 330 de paturi menținută până în anul 1999.

În prezent serviciile de îngrijire medicală sunt oferite în regim de spitalizare continuă prin cele 175 de paturi, organizate în cinci secții care integrează 12 compartimente și în regim de spitalizare de zi prin cele 10 paturi ale acestui sector. Activitatea prespitalicească este asigurată de ambulatoriul integrat și recent înființatul compartiment de primiri urgențe.

Fiind cel mai vechi spital din regiunea în care este dispus, spitalul și-a câștigat de-a lugul celor două veacuri respectul și încrederea binemeritate, fiind și în prezent considerat o instituție emblematică a orașului de la poalele muntelui Tâmpa.

SCURT ISTORIC AL SPITALULUI MILITAR CRAIOVA

Activitatea medicală organizată în folosul armatei datează în zona Olteniei din august 1831, când este dat în folosință primul „lazaret“ din Craiova, cu o capacitate de 20 paturi, funcționând pe rând în mai multe clădiri puse la dispoziție de autoritățile vremii. Așezământul spitalicesc militar craiovean a dăinuit sub conducerea unor medici ca Mihai Vilag, Carol Vasarhely, Tadeus Navarra, numărul de paturi variind în funcție de necesități, atingând chiar cifra de 200 paturi imediat după revoluția din 1948.

Din anul 1864, în urma unei noi legi de reorganizare a armatei, spitalul militar craiovean devine spital de divizie, comandant fiind numit med. pr. cl. II-a N. Caligaris (1826-1875).

În Războiul pentru cucerirea independenței de stat din 1877-1878, în luptele de la sud de Dunăre, personalul Spitalului ostășesc din Craiova a participat în mod efectiv, dovedind un înalt patriotism. Astfel, Divizia 1-a Craiova a fost asigurată de o „ambulanță“ formată din medicul șef, 9 medici și chirurgi, doi farmaciști, un ofițer de administrație și 80 oameni din compania sanitară.

După războiul de independență urmează o nouă etapă importantă în viaţa spitalului militar din Craiova. În anul 1880 capacitatea acestuia era de 80 paturi, spaţiile în care funcţiona, disparate şi improprii, impunând construirea – de mult cerută – a unui aşezământ spitalicesc modern.

În urma demersurilor comandantului de atunci, medic de divizie Gheorghe Stavrescu, Ministerul de Război cumpără terenul pe care se află şi azi amplasat spitalul, începându-se construcţia pavilioanelor, după model englezesc.

Prin noua reorganizare a armatei, în 1883, diviziile teritoriale se transformă în Corpuri de Armată, iar spitalele militare de reşedinţă iau titulatura de Spitale de Corp de Armată, fiind sub autoritatea medicului şef de Corp de Armată. Primul comandant al Spitalului de Corp de Armată Craiova a fost medic de divizie Ion Vercescu. Spitalul craiovean coordona şi activitatea spitalelor divizionare din Turnu Severin, Slatina şi Piteşti.

În perioada 1885-1889 sunt date în folosinţă pavilionul chirurgical, pavilionul medical, pavilionul pentru dermato-sifilitici, pavilionul contagioşi şi pavilionul C, iar în anul 1993 pavilionul administrativ. În 1886 s-a efectuat împrejmuirea spitalului, iar în 1889 a fost dată în folosinţă instalaţia de apă. În 1898 spitalul avea 200 paturi în două servicii: medical şi chirurgical.

Din 1897 există un laborator de chimie, căruia i se alătură în 1911 un serviciu de bacteriologie. În 1906 apare serviciul de radiologie, se pare că iniţial în cadrul laboratorului.

Anul 1914 marchează înfiinţarea serviciilor oculistic (ce includea şi serviciul ORL), de boli contagioase şi dermato-sifilis.

În primul război mondial spitalul a fost evacuat la Galaţi, Botoşani, Tg. Frumos şi Bacău. În locul său, pe timpul ocupaţiei germane, au fost instalate magazii pentru materiale. Revine pe amplasamentul din Craiova în 1919.

Spitalul Militar Craiova (titulatură pe care o poartă în toate documentele oficiale începând din 1927) a funcţionat ca spital de categoria I, cu servicii de boli interne, chirurgie, oftalmologie (incluzând ORL), dermatologie, contagioase, radiologie şi laborator. Avea anexată o infirmerie de ochi situată în afara cadrului spitalului.

În 1929 ia fiinţă un serviciu dentistic, iar în anii 1930-1931 vin în spital primii subofiţeri droghişti. Farmacia spitalului deservea şi trupele din garnizoana Craiova. Tot pe lângă spital erau ataşate farmacia Diviziei I şi Compania I sanitară.

În timpul celui de al doilea război mondial, Spitalul Militar din Craiova a funcţionat în actualul local, ca spital de zonă interioară nr. 224, cu 350 paturi, având două anexe: una la Spitalul Filantropia şi alta la Spitalul Preda. În primăvara anului 1944 a fost bombardat şi mutat în cazarma „Alion“, unde a funcţionat până în anul 1945.

După război spitalul a funcţionat cu 300 paturi, având secţiile chirurgie, boli interne, boli contagioase, ORL, oftalmologie, dermato-venerice, neuropsihiatrie, farmacie, laborator, stomatologie, triaj, radiologie, structură organizatorică ce s-a menţinut până în anul 1962.

Începând din 1950 atribuţiunile şi sarcinile spitalului cresc şi se înmulţesc. Astfel, devine organ de dotare şi asigurare materială pe linie medicală, expertiză, îndrumare şi control pe linie profesională la unităţile teritorializate şi for metodologic pentru personalul medical din unităţi.

În anii 1952-1953 a fost construit un nou pavilion (pavilionul B3) cu un club şi bloc alimentar la parter şi spaţii pentru două secţii la etaj (neuropsihiatrie şi dermatovenerologie).

În perioada 1953-1962 s-au modernizat toate pavilioanele prin compartimentări în saloane mici şi rezerve iar în 1965 se introduce încălzirea centrală.

Între anii 1959-1968 are loc reconstruirea punctului de control, asfaltarea aleilor interioare din spital şi amenajarea policlinicii.

Începând din anul 1956, pe lângă Spitalul Militar Craiova funcţionează cu regularitate în fiecare ciclu de instrucţie, pentru o perioadă de 4 luni, şcoala de gradaţi sanitari, până în 1987.

Din anul 1971 în spital funcţionează un compartiment de anestezie-reanimare, devenit în anul 1995 secţie ATI, iar în anul 1973 se înfiinţează Dispensarul policlinic al spitalului şi Dispensarul policlinic Caracal, tot în subordinea spitalului.

În 1977 clădirile spitalului sunt grav avariate de cutremurul din 4 martie, reuşindu-se repararea cu mari eforturi, prin forţe proprii.

În Revoluţia din decembrie 1989 spre spital s-au tras focuri de armă, rezultând răniţi şi distrugeri de bunuri în special la secţiile chirurgie şi ATI.

În anul 1991 încep lucrările de construcţie a noului spital şi sunt date în funcţiune punctul de transformare a energiei electrice, staţia de apă, punctul de control, două alei principale şi în 1996 un pavilion modern destinat comenzii şi administraţiei spitalului, policlinicii, stomatologiei, farmaciei, radiologiei, clubului spitalului. Pentru a se efectua aceste construcţii a fost necesară demolarea pavilionului boli interne, a pavilionului destinat policlinicii, laboratorului clinic, farmaciei, „triajului“ şi multe redimensionări şi redistribuiri de spaţii.

În perioada 1998-2012, au loc lucrări ample de reparaţii şi modernizare la majoritatea clădirilor vechi ale spitalului, în special în cele în care se mută secţia boli interne, secţiile boli infecţioase şi pneumologie, computer tomograful şi blocul alimentar, pavilionul neurologie, psihiatrie, dermatovenerologie, pavilionul pentru serviciul triaj şi unitatea de primiri urgenţe, pavilionul destinat secţiei A.T.I. şi chirurgie. Tot în această perioadă s-a înlocuit reţeaua de energie electrică din curtea spitalului cu una pe stâlpi de beton, reţeaua de termoficare şi alimentare cu apă, s-au turnat noi alei de beton şi s-a realizat îmbrăcămintea asfaltică a tuturor aleilor spitalului, s-au refăcut bordeiele pentru depozitarea alimentelor, s-a amenajat parcare pentru mijloacele de transport ale unităţii, rampa de colectare a gunoiului, s-a refăcut gardul de împrejmuire a spitalului şi multe alte lucrări aferente, toate acestea crescând funcţionalitatea şi confortul activităţilor.

În anul 2000 spitalul îndeplineşte condiţiile de spital de urgenţă şi primeşte titulatura SPITAL MILITAR DE URGENŢĂ CRAIOVA, iar prin ordinul ministrului Apărării Naţionale nr. MS 149 din 14.09.2001 de SPITAL MILITAR DE URGENŢĂ „DR. ŞTEFAN ODOBLEJA“ CRAIOVA având în subordine AMBULATORIUL SPITALULUI şi CENTRUL DE SĂNĂTATE MILITAR CARACAL.

În anii 2000 şi 2001, prin grija Direcţiei Medicale, are loc dotarea în toate secţiile spitalului cu instrumentar şi aparatură medicală moderne (aparate radiologice inclusiv C.T. şi laborator mobil de radiologie, combine de diagnostic, microscoape operatorii, endoscoape, holtere, ecografe, aparatură de laborator), ce a crescut foarte mult calitatea actului medical.

În luna septembrie 2001 a fost pus în funcţiune tomograful computerizat.

Colaborarea cu Universitatea de Medicină şi Farmacie din Craiova a condus la semnarea unui protocol reînnoit permanent din anul 1999 până în prezent.

În anul 2005 prin Ordinul Ministrului Apărării Naţionale spitalul ia titulatura de SPITALUL CLINIC DE URGENŢĂ MILITAR „DR. ŞTEFAN ODOBLEJA“ CRAIOVA.

În anul 2006 se dă în folosinţă un pavilion nou, demisolul fiind atribuit Compartimentului de Primire Urgenţe şi serviciului de statistică şi registratură medicală, parterul secţiei oftalmologie şi etajul secţiei O.R.L.

Pe tot parcursul activității sale, de la înființare și până în prezent, Spitalul Militar Craiova, indiferent de titulatura pe care a purtat-o, de capacitatea de spitalizare, de vitregiile vremurilor, sociale, politice sau de orice natură, și-a îndeplinit exemplar datoria față de Armata și populația României, sarcină pe care și-o asumă cu deplină responsabilitate și pentru viitor.

Institutul Naţional de Medicină Aeronautică şi rolul său în medicina militară românească

M. Macri

Cuvinte cheie: medicină militară, medicină aeronautică, componentă operaţională, securitatea zborului.

Introducere: Institutul Naţional de Medicină Aeronautică „Gral. Dr. Av. Victor Anastasiu“, a ţintit, încă la înfiinţarea sa în anul 2000, spre o integrare a medicinii aeronautice româneşti în contextul celei mondiale. Dacă pe partea de medicină aeronautică generală, evenimente ca Cel de-al 59 lea Congres Internaţional de Medicină Aeronautică, desfăşurat la Bucureşti în 2011, sub egida Academiei Internaţionale de Medicină Aeronautică şi Workshopul ICAO ce va avea loc în acest an, 11-15 noiembrie consacră poziţia instituţiei noastre, în acest an a venit şi rândul recunoaşterii componentei operaţionale militare, prin recunoaşterea procesului de antrenament fiziologic din institut de către USAF.

Prezentare şi discuţii: Va fi prezentată misiunea institutului, cu accent asupra componentei operaţionale, precum şi tendinţele de dezvoltare viitoare a capabilităţilor operaţionale ale institutului.

Concluzie: Institutul Naţional de Medicină Aeronautică este o componentă importantă a medicinii militare româneşti, prin contribuţia sa nemijlocită la securitatea şi componenta operaţională a activităţii aeronautice militare.

Medicul în unitatea militară de aviaţie – trecut, prezent şi viitor

Lupu I. L.M.[1], C.B. Teuşdea[2]
1- Forţele Aeriene Române
2- Secţia UPU – Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila“, Bucureşti

Rezumat:

În 1916 Victor Anastasiu, medic la o unitate militară de aviaţie obţine brevetul de pilot şi deschide astfel calea dezvoltării medicinei aeronautice în România. După o carieră încununată cu succes şi recunoaştere internaţională predă ştafeta unor tineri medici entuziaşti care dezvoltă această ramură a medicinei în România. Odată cu intrarea României în NATO, în anul 2004, Forţele Aeriene au implementat şi dezvoltat conceptele Alianţei privind asigurarea medicală a zborurilor ca parte integrantă a siguranţei aeriene. Medicul de zbor este conceptul în curs de implementare în aviaţia militară română şi împleteşte armonios tradiţia românească în medicina aeronautică cu provocările prezentului în acest domeniu. Medicul de zbor se va afla întotdeauna în prima linie a aviaţiei militare îndeplinind sarcini de medicină preventivă în timpul dislocărilor, sau de medicină de urgenţă pe durata misiunilor de evacuare medicală aeriană alături de pilotul militar, stabilind aptitudinea de zbor ca parte a expertizei medicale sau practicând medicina de familie pentru personalul aeronautic şi familiile acestora.

Cuvinte cheie: *medic de zbor, escadrila, medicina aeronautica, riscul aeromedical, NATO.*

Rolul antrenamentului fiziologic în creșterea siguranței zborului

D. Popescu
Institutul Naţional de Medicină Aeronautică şi Spaţială

Cuvinte cheie*: antrenament fiziologic, integrare operativă*

Introducere: În ziua de azi, echipamentul militar aeronautic a atins nivele tehnologice greu de imaginat, fără a reuşi însă să scoată factorul uman din buclă. În timp ce dronele au un rol din ce în ce mai mare în strategia militară a multor ţări, asta nu presupune renunţarea la factorul uman, ci modificarea modului de integrare resursă umană – hardware – software. Automatizarea misiunilor de rutină face ca aeronavelor pilotate uman să le fie rezervate misiunile critice, ceea ce duce la necesitatea unei foarte bune integrări om-mediu-aeronavă. Antrenamentul de fiziologie aeronautică aduce acest aproape deziderat, pe lângă rolul tradiţional de creştere a siguranţei zborului.

Materiale şi metodă: O trecere în revistă a componentelor antrenamentului de fiziologie aerospaţială desfăşurat la Institutul Naţional de Medicină Aeronautică şi Spaţială, pe următoarele componente:

1. Hipoxia şi boala de decompresie
2. Vedere pe timp de noapte
3. Dezorientarea spaţială
4. Suprasarcina
5. Zgomotul şi protecţia la zgomot
6. Vibraţiile
7. Nutriţia şi exerciţiul fizic
8. Abuzul de substanţe (medicamente, alcool, fumat)
9. Odihna
10. Stresul termic
11. Catapultarea şi supravieţuirea
12. Protecţia LASER
13. Conştienţa operaţională (SA)
14. Managementul echipajului (CRM)

Discuţii: Sublinierea rolului antrenamentului fiziologic atât pentru creşterea siguranţei zborului (paradigma clasică) cât şi pentru creşterea integrării operative (paradigma modernă)

Concluzie: Antrenamentul de fiziologie aerospaţială nu e nicidecum demodat, ci reprezintă una din marile provocări ale medicinii militare, în general şi a celei aeronautice în special.

Evaluarea rezistenţei la stresul hipoxic-hipobaric prin monitorizarea simultană a înregistrărilor ECG şi a saturaţiei de oxigen la personalul aeronautic

I. Capanu
Institutul Naţional de Medicină Aeronautică şi Spaţială

***Cuvinte cheie**: Hipoxie-hipobarică, saturaţie de oxigen (SO), variaţia frecvenţei cardiace (delta FC), viteza de sedimentare a hematiilor (VSH), rezistenţă la hipoxie.*

Introducere: Încă de la începuturile aviaţiei hipoxia de altitudine a fost recunoscută ca un factor de risc pentru persoanele expuse, iar acest risc persistă şi astăzi. Zborul implică o solicitare intensă a organismului determinată de numeroşi factori din care cei mai importanţi sunt: scăderea presiunii atmosferice si scăderea presiunii parţiale a oxigenului. Cel mai frecvent, aceşti factori acţionează brusc asupra personalului aeronautic. Expunerea la hipoxia-hipobarică prin zbor simulat, rămâne o metodă standard obligatorie pentru personalul aeronautic, datorită posibilităţii de a estima rezistenţa, antrenamentul şi sinergiile adaptative.

Metodă: În acest studiu, un număr de 39 subiecţi de sex masculin (12 candidaţi, 17 piloţi sub 35 ani şi 10 piloţi peste 35 ani) au fost supuşi la un stres hipoxic-hipobaric în barocamera I.N.M.A.S. O parte dintre aceştia au prezentat factori predispozanţi pentru scăderea rezistenţei la hipoxie-hipobarism: fumat şi VSH crescut. Înregistrările ECG şi ale saturaţiei de oxigen (SO) au fost efectuate simultan. Valorile VSH-ului au fost determinate înainte de expunere.

Rezultate: Am demonstrat că există corelaţii semnificative între cele două metode (ECG şi SO) de evaluare a rezistenţei la hipoxie şi că subiecţii cu VSH crescut au adaptare deficitară.

Concluzie: Monitorizarea concomitentă a unor parametri în timpul expunerii la hipoxie-hipobarică permite o mai bună înţelegere a profilului de adaptare a subiecţilor. VSH-ul crescut poate fi folosit ca factor de predicţie pentru rezistenţa scăzută la hipoxie.

Experienţa antrenamentului de dezorientare spaţială din Institutul Naţional de Medicină Aeronautică şi Spaţială şi rolul său actual în procesul de antrenament fiziologic

D. Vlad
Institutul Naţional de Medicină Aeronautică şi Spaţială

Introducere: Dezorientarea spaţială era virtual necunoscută în România, până la data achiziţiei primului aparat de demonstrare a dezorientării spaţiale. Atât instructorii, cât şi echipajul au trebuit să se adapteze noului mod de lucru.

Metodă: O trecere în revistă a bazei tehnice şi a profilelor utilizate în practica dezorientării spaţiale.

Rezultate: În perioada 2006-2011, am efectuat 176 de sesiuni de antrenament pentru 117 piloţi de supersonic, 50 de sesiuni pentru 41 de piloţi de subsonic, 54 de sesiuni pentru piloţii de transport, 164 de sesiuni pentru 91 de piloţi de elicopter (95 cu profilul avion şi 69 cu profil elicopter) şi 52 de sesiuni pentru 52 de studenţi.

Discuţii: Tendinţele remarcate în cadrul fiecărui grup vor fi prezentate, în paralel cu concluziile unor lucrări ştiinţifice anterioare, ca şi evidenţierea modificărilor făcute de noi profilelor recomandate de producător.

Concluzie: Antrenamentul de dezorientare spaţială este o componentă cheie a antrenamentului fiziologic din Institutul Naţional de Medicină Aeronautică şi Spaţială

Antrenamentul pentru vederea pe timp de noapte din cadrul laboratorului specializat din Institutul Naţional de Medicină Aeronautică şi Spaţială

I. Manoliu
Institutul Naţional de Medicină Aeronautică şi Spaţială

***Cuvinte cheie**: vedere nocturnă, antrenament, identificarea ţintei*

Introducere: În anul 2012 a fost implementat în cadrul secţiei cercetare şi fiziologie aeronautică un laborator de antrenament pentru vederea pe timp de noapte.

Material şi metode: un lot de 15 piloţi militari au efectuat antrenamentul pentru vederea pe timp de noapte. Principalele fenomene demonstrate au fost autokinezia, efectul Purkinje, ebluisarea, rolul iluminării în spectrul roşu în conservarea adaptării pe timp de noapte, rolul luminii stroboscopice, detecţia ţintelor şi aprecierea profunzimii, efectul fenomenelor meteo.

Rezultate: Noţiunile teoretice au fost înţelese şi însuşite corect de toţi piloţii. Autokinezia a fost percepută de 2/3 dintre subiecţi. Efectul Purkinje a fost perceput corect de 3 piloţi, iar 7 au perceput efectul invers. 5 piloţi nu au perceput nici o variaţie a intensităţii luminoase. Ebluisarea a fost percepută de toţi participanţii şi a fost apreciat ca momentul cu cel mai mare impact al demonstraţiei. Efectul fenomenelor meteo a fost slab perceput.
În ceea ce priveşte efectul iluminării nocturne în spectrul roşu asupra adaptării la întuneric, efectul a fost perceput corect dar impactul a fost mic. Demonstrarea lipsei percepţiei profunzimii, atât binoculară cât şi monoculară a fost percepută corect şi cu impact semnificativ.
În ceea priveşte detecţia ţintelor, în funcţie de numărul de ţinte detectate am clasificat 3 grupe. 4 piloţi s-au încadrat în grupa 1, 8 în grupa 2 şi 3 în grupa 3.

Concluzie: Antrenamentul a fost perceput ca util, în special partea de ebluisare, de demonstrare a lipsei percepţiei profunzimii şi de detecţie a ţintelor.

Cât este de sigur zborul după scufundare?

A. Macovei
Institutul Naţional de Medicină Aeronautică şi Spaţială

Cuvinte cheie*: zbor, scufundare, DCS*

Introducere: Riscul de boală de decompresie (DCS) în zborul după scufundare e greu de cuantificat. Sunt o mulţime de modele detaliate privind riscul la ascensiunea în mediul acvatic şi câteva decente (Webb) privind riscul de DCS la ascensiunea aeriană. Pentru că studiile au folosit principii diferite, un model integrat e greu de cuantificat. Majoritatea datelor provin din studiile privind scufundările la altitudine (ca cel din care Bühlmann a creat faimoasele sale tabele de decompresie, utilizate larg în majoritatea computerelor de scufundare).

Metodă: Vor fi prezentate date despre:

1. Boala de decompresie. Forme, semne şi simptome, de ce zborul creşte riscul
2. Factori de risc asociaţi (vârsta, condiţia fizică, greutate, ciclul menstrual)
3. Fereastra oxigenului (Benhke) - valoare practică şi limitări
4. Modalităţi de scufundare. Diferenţe între scufundările cu şi fără pauză de siguranţă
5. Limitările folosirii oxigenului şi amestecurilor Nitrox
6. Incidenţa: O metaanaliză din 1989 găseşte 208 DCS la 721 de cazuri de zbor după scufundare sub 12 h, la altitudini peste 2500 m.

Discuţii: Recomandările actuale:

1. 12 ore pauză după o scufundare fără pauză de decompresie cu durata sub 2 ore, în ultimele 48 de ore
2. 24 ore pauză după scufundări repetitive fără pauză de decompresie
3. 24-48 de ore după scufundări cu pauze de decompresie
4. nu se zboară cu suspiciune DCS decât pentru obţinerea ajutorului specializat, şi atunci la altitudinea cea mai joasă permisă de siguranţa zborului

Se vor prezenta doua cazuri din practică (scurt istoric, tabela de tratament, evoluţie)

Concluzie: Boala de decompresie în zborul după scufundare nu e un risc neglijabil. Fenomenul trebuie bine însuşit de toţi cei interesaţi.

MISIUNILE DE CĂUTARE, SALVARE ŞI EVACUARE AEROMEDICALĂ ÎN CONDIŢII EXTREME ŞI SITUAŢII DE CATASTROFĂ

Buiu M.I., Statul Major al Forţelor Aeriene Române – Flotila 71 Aeriană

Cuvinte cheie*: Elicoptere, avioane, însoţitor de bord medic, însoţitor de bord asistent medical, pacienţi, echipe medicale, evacuare aeromedicală*

Abstract: În ultimii ani, România a fost încercată de dezastre naturale severe, cauzate de fenomene atmosferice intense, având ca rezultat pagube semnificative şi numeroase, cu victime multiple în rândul populaţiei civile. În cele mai multe cazuri, accesul pentru ajutorul şi evacuarea victimelor a fost aproape imposibil, mulţi oameni au rămas izolaţi în zonele afectate.

În aceste situaţii, când forţele de intervenţie civile au fost depăşite, s-a solicitat sprijinul elicopterelor şi avioanelor Forţelor Aeriene Române, cu echipaje specializate în misiuni de căutare-salvare pentru identificarea victimelor şi de evacuare aeromedicală.

În Forţele Aeriene Române funcţionează 24 de ore - 7 zile pe săptămână, echipe militare şi medico-militare specializate în utilizarea echipamentului medical de urgenţă şi în extracţia de victime din zonele calamitate şi în situaţii extreme. Elicopterele şi avioanele Forţelor Aeriene Române, cu echipajele medicale la bord a intervenit şi în misiuni militare umanitare, transportând alimente, apă, provizii medicale pentru persoanele afectate. Au fost efectuate, de asemenea, misiuni de căutare şi salvare a victimelor din zonele calamitate. Extracţie victimelor şi evacuarea aeromedicală au fost făcute către facilităţile medicale civile.

Misiuni cu elicoptere, avioane şi echipaje medicale de la Forţele Aeriene Române, au fost făcute toată ziua şi toată noaptea în zonele inundate, zone înzăpezite sau calamitate, numărul victimelor identificate, ajutate, salvate şi evacuate aeromedical este ridicat.

Medicină de Urgenţă
Miercuri, 23 oct, 17^{00} – 18^{30}

ACCESUL VASCULAR INTRAOSOS LA PACIENTUL CRITIC

C.B. Teuşdea, S. Dogaru
Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila“, Bucureşti - Secţia UPU

Rezumat

În situaţiile de urgenţă maximă, accesul vascular rapid poate face diferenţa între viaţă şi moarte la pacientul critic indiferent de cauza care a dus la apariţia urgenţei. În prespital, accesul vascular rapid are o importanţă deosebită deoarece condiţiile sunt necorespunzătoare, iar inserţia unui cateter venos central este de obicei mare consumatoare de timp. În ultimii ani a fost redescoperită o tehnică pentru obţinerea accesului vascular – accesul intraosos, care în prezent este folosit şi în resuscitarea cardio-pulmonară, conform ghidului de resuscitare din 2010, în condiţiile în care nu poţi obţine un acces venos rapid. Administrarea intraosoasă a medicamentelor şi a fluidelor, limitată în ultimii ani numai la uzul pediatric, a redevenit astfel o rută viabilă şi pentru adulţi.

Cuvinte cheie*: pacient critic, acces vascular rapid, administrare intraosoasă, resuscitare cardiorespiratorie*

RESUSCITAREA HEMORAGIILOR ȘI PROTOCOALE DE TRANSFUZIE MASIVĂ ÎN TRAUMĂ

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Rezumat
Abordarea resuscitării pacienţilor răniţi cu hemoragie majoră s-a schimbat dramatic în ultimii zece ani, în parte datorită experienţei militare recente din Irak şi Afganistan. Armata Statelor Unite a elaborat liniile directoare „Tactical Combat Casualty Care“ pentru managementul în prespital, dar şi pentru tratamentul precoce în spital, implementând experienţa - dovezile clinice – obţinută în mediul militar. Aceste linii directoare trebuie cunoscute şi aplicate şi de către forţele armate române având în vedere participarea acestora pe teatrele de operaţii. Această prezentare trece în revistă controlul integrat al traumei, ghidurile de transfuzie individuală şi protocoalele de transfuzie masivă.

***Cuvinte cheie**: control integrat al traumei, hemoragii posttraumatice, resuscitare hipotensivă şi hipovolemică.*

EXTRAGEREA CHIRURGICALĂ A UNUI CORP STRĂIN INTRAMIOCARDIC (GLONŢ) – PREZENTARE DE CAZ

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Obiectiv: descrierea unui caz foarte rar, de corp străin (glonț) intramiocardic, prezent la nivelul peretelui diafragmatic al ventriculului drept. Intervenția chirurgicală de extragere a decurs cu succes, la trei luni de la accident.

Material și metodă: Pacientul, un bărbat de 37 de ani, a fost împușcat parasternal, la nivelul spațiului trei intercostal stâng, cu un proiectil provenit de la o armă AK 47. Împușcătura s-a produs indirect – proiectilul a lovit pacientul după ce a ricoșat de două ori din caroseria mașinii pe care o conducea. La scurt timp, pacientul a fost examinat la spital. A fost pus diagnosticul de corp străin la nivelul mediastinului antero-inferior (glonț).
Am examinat pacientul la trei luni după accident. Radiografia plană a confirmat diagnosticul. S-a efectuat examen CT toracic, ce a arătat că glonțul era localizat „cel mai probabil deasupra diafragmului, la nivelul mediastinului anterior, la 1,5 cm posterior de stern“. Radiologul a subliniat că nu poate descrie o locație precisă, datorită artefactelor metalice. Am efectuat de asemenea o fluoroscopie, ce a arătat că glonțul se mișca sincron cu cordul, și de aceea am solicitat efectuarea ecografiei cardiace, pentru a determina poziția cardiacă/pericardică a glonțului. Ecografia transtoracică nu a stabilit prezența glonțului în miocard sau pericard, și nici nu a furnizat semne indirecte de plăgi cardiace sau pericardice.
S-a folosit în consecință un abord subxifoidian. Am explorat mediastinul anterior, dar nu am reușit să găsim glonțul, cu toate că am palpat o structură arătând foarte mult cu un glonț. Am deschis apoi pericardul, practicând o fereastră pericardică și am găsit glonțul înglobat la nivelul versantului inferior al peretelui ventriculului drept.
Pacientul a fost operat sub bypass cardiopulmonar, iar glonțul a fost extras din ventriculul drept. Orificiul restant a fost închis cu fire separate cu petece de dacron, utilizând și bioglue pentru întărirea peretelui ventricular.

Rezultate: Evoluția postoperatorie a fost favorabilă, fără a se constata infecții sau sângerări.

Concluzie: Tratamentul chirurgical al corpului străin intramiocardic se poate efectua în siguranță, sub circulație extracorporeală.

ROLUL RESUSCITĂRII HIPOTENSIVE ÎN MANAGEMENTUL MODERN AL TRAUMEI

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Rezumat
Obiectivul principal al îngrijirii pacientului politraumatizat este de a minimiza sau a preveni şocul şi astfel de a salva viaţa pacientului. Resuscitarea volemică agresivă poate fi periculoasă la aceşti pacienţi deoarece creşterea presiunii vasculare şi a volumului circulant poate determina ruperea şi mobilizarea cheagurilor de sânge, diluţia factorilor de coagulare şi a factorilor naturali de răspuns în hemoragie. Conceptul de resuscitare hipovolemică s-a dezvoltat pentru pacienţii care au necesitat cantităţi mici de fluide infuzate, hipovolemia şi hipotensiunea fiind tolerate până la obţinerea unui control hemoragic complet. Această prezentare punctează elementele principale care susţin strategia resuscitării hipovolemice.

***Cuvinte cheie:** politrauma, resuscitare hipovolemică şi hipotensivă, controlul hemoragic complet.*

POLITRAUMATISMUL -PREZENTARE CAZ CLINIC

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Introducere: Managementul politraumatizatului cu multiple fracturi şi arsuri necesită un efort pluridisciplinar, deoarece implică resurse umane şi tehnologice importante. Controlul complicaţiilor septice este esenţial pentru succesul terapeutic şi supravieţuire.

Prezentare caz: Se prezintă cazul unui pacient de 38 ani, cu politraumatism secundar unui accident aviatic - impactul cu solul a scaunului catapultat sub 600m altitudine, din avionul ce nu a mai răspuns la comenzi imediat după decolare, în timpul unui zbor de antrenament. Pacientul este transferat din spitalul militar teritorial în serviciul nostru UPU, în cursul aceleași zile, unde este stabilizat hemodinamic și respirator. Se inventariază toate leziunile prin examene clinice și paraclinice și se completează tabloul diagnostic. După stabilizare se intervine chirurgical în mod etapizat în secțiile de ortopedie și chirurgie plastică pentru diagnosticele inițiale:

- fracturi cominutive deschise tip 3B de Gustillo-Andresson ale pilonului tibial și talusului drept cu luxație deschisă tibio-astragaliană poluată cu corpi străini (pământ, resturi textile) și arsuri gradul II-III circular la nivelul gleznei și II-III-IV circular la nivelul piciorului drept (pe fața dorsală și a plantei),
- fractură cominutivă peroneu drept 1/3 medie,
- fracturi cominutive ale pilonului tibial stâng, intra-articulare cu mare deplasare și a treimii inferioare peroneului stâng, cu arsuri circulare la acest nivel gradul II-III.

Într-un timp secundar din cauza poluării locale cu Pseudomonas Aeruginosa (sub tratament AB) și a cominuției importante de la nivelul fracturii deschise tip 3B de Gustillo-Andresson ale pilonului tibial prin intervenții seriate se practică:

− sechestrectomia și toaleta articulară cu desființarea spațiului virtual restant
− asanarea articulară riguroasă – ablația parțială a talusului drept (necrectomia țesuturilor afectate)
− ablația completă a talusului drept și artrodeza tibio-calcaneană pe fixator extern Ilizarov;
− fistulectomia și sechestrectomiala nivelul maleolei peroniere pe fața externă a gleznei drepte;
− pansamente zilnice cu CUTIMED SORBACT;
− ozono terapie 4 sedințe;
− terapie cu presiune negativă 3 ședințe;
− pansamente cu nanocristale de argint cu evoluție este favorabilă - plaga s-a închis (după 5 secreții consecutive sterile din plagă).
− extragerea fixatorului extern Ilizarov
− extragerea șurubului inferior al plăcii de osteosinteză tibială stângă.

Rezultate: Pacientul deși a suferit multiple intervenții chirurgicale pe perioada internării, iar evoluția sa a fost marcată de diferite episoade de febrilitate și subfebrilitate (din cauza poluării locale cu Pseudomonas Aeruginosa), s-a externat într-o condiție ameliorată, iar la 2 luni a început recuperarea funcțională a membrelor pelvine. S-a prezentat la control periodic în ambulator la 5 luni de la externare cu mers ușor șchiopătat biped fără sprijin suplimentar.

Concluzii

1. Agentul traumatic a avut o energie înaltă – impactul cu solul a scaunului catapultat din avion sub 600m altitudine;
2. Tratamentul a necesitat echipă complexă: UPU, ATI, Chirurgie Plastica, Ortopedie
3. La momentul internării în gardă și abordării terapeutice chirurgicale în urgență, conduita pluridisciplinară a fost de salvare a vieții, de salvare a membrelor pelvine, cu dotarea logistică de la momentul din noaptea de 23-24.08.2012, de stabilizare generală și locală în vederea continuării etapizate a tratamentului medico-chirurgical.
4. Eficiența colaborării interdisciplinare a permis posibilitatea intervenției chirurgicale în vederea salvării membrelor pelvine.
5. Leziunile combinate sunt redutabile pentru sechelele pe care le generează.

***Cuvinte cheie**: politraumatism, accident aviatic, fracturi multiple, arsuri, fixator extern.*

TRANSFERUL AUTOLOG DE GRĂSIME ÎN TRATAMENTUL SECHELELOR POSTCOMBUSTIE. PREZENTARE DE CAZ POMPIER MILIAR CU CICATRICI CHELOIDE RETRACTILE MÂINI ȘI ECTROPION BILATERAL

Argentina Vidrașcu[1], P. Polihovici[2]
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[2]UMF Iuliu HaȚieganu Cluj-Napoca

Obiective: recuperarea flexiei -extensiei degetelor și tratamentul ectropionului.
Principiu: Aplicarea celulelor stem proprii din țesutul adipos.
Metodă de lucru: recoltare celule adipoase de la nivelul regiunii infraombilicale. Decantarea și reinjectarea acestora la nivelul placardului cicatriceal fibros.
Evaluare rezultate: unghiul de flexie AIFP și AIFD , modificări troficitate tegumente și ocluzia palpebrală.
Rezultate: ocluzia palpebrală completă, sensibilitatea la temperaturi scăzute diminuată și flexie degete completă.

Concluzie: lipofilling -ul cu țesut autolog reprezintă metoda ideală de tratament trofic a placardelor cicatriceale retractile.

ULCER CORNEEAN PERFORAT – REZOLVARE CHIRURGICALĂ

M. Zemba, G. Anghel, C. Ștefan, O. Mușat, H. Manole, Laura Macovei, Liliana Pulbere, Daniela Șelaru, Monica Armegioiu, R. Asandi
SUUMC Carol Davila, Secția Oftalmologie

REZOLVAREA ÎN DOI TIMPI A FRACTURII DESCHISE TIP III B AMBE OASE GAMBĂ – PREZENTARE DE CAZ

Chitea C., Chitea M., Blogos L., Ciorca D
Spitalul Militar Cluj, Secția Ortopedie

Introducere:
Fractura deschisă ambe oase gambă poate fi tratată chirurgical

în doi timpi:

- Într-un timp – osteosinteză centromedulară fără alezaj
- În doi timpi – fixare externă până la vindecarea leziunilor de părţi moi apoi osteosinteză centromedulară
-

Material şi metodă:
Pacientul B.N. în vârstă de 25 ani, victima unui accident rutier (motoclicist) soldat cu fractură deschisă tip III b ambe oase gambă cu pierderea de ţesut osos şi arsuri la nivelul gambei, a fost operat iniţial practicându-se osteosinteza cu fixator extern monoplane până la vindecarea ţesuturlor moi şi ulterior osteosinteză cu tijă centromedulară blocată şi autogrefon osos recoltat de la nivelul peroneului.

Concluzie:
Coexistând leziuni de părti moi (plăgi multiple, cea mai mare de 10 cm şi arsuri cutanate) şi a riscului septic crescut cu pierderea de ţesut osos (2/3 din circumferinţa tibiei) care a necesitat grefon osos pentru formarea unui callus solid se optează pentru soluţia în 2 timpi.

Cardiologie
Marți, 22 oct, 08^{30} – 11^{00}

RISCUL CARDIOVASCULAR LA POPULAŢIA MILITARĂ ACTIVĂ

M. Florea, A. Ploae, Carmen Man
Spitalul Universitar de Urgenţă Militar Central „Carol Davila“, Bucureşti, Clinica de Cardiologie

Obiectiv: evidenţierea prevalenţei şi a incidenţei factorilor de risc cardiovascular în mediul militar, precum şi a particularităţilor de manifestare ale acestora.
Material şi metodă: s-a efectuat un studiu descriptiv de tip transversal, pe un lot de 920 militari activi, de ambele sexe pe o perioadă de 2 ani. Tuturor subiecţilor luaţi în studiu li s-a efectuat examen clinic general, cu determinarea valorilor TA, ale pulsului periferic, determinarea IMC. De asemenea s-a efectuat electrocardiograma, precum şi o baterie de teste de laborator care au inclus determinarea CT, a TG, glicemiei. Subiecţii din lotul de studiu au completat chestionare pentru evidenţierea fumatului şi a intensităţii acestuia, consumului de alcool, cafea, a particularităţilor regimului alimentar, a nivelului de activitate fizică, precum şi a cuantificării gradului de expunere la stres.

Rezultate şi concluzii: studiul efectuat a decelat o prevalenţă surprinzător de mare a factorilor de risc cardiovascular la această categorie profesională. Ca şi particularităţi s-a evidenţiat o prevalenţă mare a diabetului zaharat tip 2 şi a glicemiei bazale modificate la vârste tinere şi în special la sexul feminin, o pondere mare a dislipidemiilor, o prevalenţă mare a TA normal înalte, a fumatului şi chiar a sedentarismului. Un procent semnificativ de mare din totalul subiecţilor relatează un grad mare de expunere la stres. Între prevalenţa majorităţii factorilor de risc menţionaţi şi patologia cardiovasculară am decelat existenţa unei corelaţii semnificative statistic.

MONITORIZAREA AMBULATORIE A TENSIUNII ARTERIALE. DE LA CERCETARE LA APLICABILITATEA CLINICĂ

Andriţoiu A
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ABSTRACT

Măsurarea TA la cabinet a reprezentat şi reprezintă în continuare metoda standard de determinare a valorilor presiunii arteriale şi de diagnostic al HTA. În prezent, literatura anglo-saxonă o denumeşte *Office-BP* (TA de cabinet), subliniind diferenţele faţă de determinările TA la domiciliu obţinute fie prin tehnica automăsurării efectuate de pacient, fie prin metoda automată (ABPM). Aceste două metode pot fi incluse în mijloacele moderne de evaluare *Out-office* a TA.
ABPM (metoda automată) permite obţinerea unor înregistrări multiple, la intervale de timp prestabilite, a TA, atât pe intervalul diurn, cât şi nocturn. Cu ajutorul *soft*-ului încorporat în *device*-ul de monitorizare se pot calcula multipli parametri: TAS, TAD, TAM, PP, BP load, ID, utili în diagnosticul HTA (HTA de halat alb, HTA diurnă, HTA nocturnă, HTA susţinută, HTA mascată), cât şi a profilului circadian (dipper/nondipper). Fiecare dintre aceşti parametrii sunt asociaţi unui nivel de risc CV, în corelaţie cu scorul afectării organelor ţintă şi cu prognosticul pacientului hipertensiv. ABPM permite o monitorizare mult mai fiabilă a răspunsului tensional sub medicaţia antihipertensivă, în funcţie de profilul circadian al pacientului (cronofarmacologie). În SCUM Craiova, ABPM a fost folosită cu succes în diagnosticul şi evaluarea pacienţilor hipertensivi încă din anul 1998. În ultimii ani, metoda a fost utilizată cu predilecţie în activitatea de expertiză medico-militară, permiţând un diagnostic corect al HTA în rândul cadrelor militare active.

NT-proBNP – BIOMARKER - CHEIE ÎN DIAGNOSTICUL BOLILOR CARDIOVASCULARE

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Rezumat

Utilizarea biomarkerilor pentru identificarea persoanelor cu risc de deces cardiovascular este punctul principal în manage-

mentul pacienților cu insuficiență cardiacă. Cercetările urmăresc identificarea de noi markeri și utilizarea mai eficientă a markerilor existenți. Scopul final este de a face posibil un diagnostic precoce și mai precis, îmbunătățind astfel tratamentul și supraviețuirea. Peptidele natriuretice fac parte din clasa de neurohormoni și sunt indicatori clinici ai stresului hemodinamic datorat unor multiple cauze. Ele promovează natriureza, diureza și inhibă axul renină–angiotensină–aldosteron, cu acțiune vasodilatatoare. Au fost descoperite în 1981 de Adolfo J. De Bold și reprezintă o familie de 3 peptide ce au în comun o secvență omoloagă de aminoacizi: peptidul natriuretic atrial-atrial natriuretic peptide (ANP), peptidul natriuretic cerebral-brain natriuretic peptide (BNP) și peptidul natriuretic C - C-natriuretic peptide (CNP). BNP este sintetizat în principal de miocitele ventriculare (dar și de creier, amnion) și crește semnificativ în insuficiența cardiacă cronică. Ambele, ANP și BNP, sunt crescute în CVD, dar BNP se corelează mai bine cu severitatea bolii. În această prezentare ne propunem să trecem în revistă atât utilizarea actuală a acestui biomarker (în dispneea cardiacă, în boala ischemică coronariană, la subiecții asimptomatici și pacienții cu insuficiența cardiacă cronică), cât și indicațiile viitoare (pacienți cu boală coronariană cronică, IMA, hipertensiunea pulmonară primară, etc.).

Cuvinte cheie: *biomarkeri cardiaci, NT-proBNP, insuficiență cardiacă.*

INTERVENŢIE ÎN PREMIERĂ NAŢIONALĂ: PRIMUL CAZ RAPORTAT DE VENTRICULOPLASTIE UTILIZÂND DISPOZITIVUL ALGISYL-LVR LA UN PACIENT CU CARDIOMIOPATIE DILATATIVĂ

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Scop: Prezentarea tehnicii de augmentaţie a ventriculului stâng prin implantarea dispozitivului Algisyl-LVR™ la pacienţii cu cardiomiopatie dilatativă şi raportarea efectelor acesteia asupra formei geometrice şi a parametilor funcţiei sistolice şi diastolice la primul pacient din centrul nostru inclus în grupul chirurgical al trialului clinic Augment-HF.

Metode: Trialul clinic Augment-HF este un studiu randomizat, controlat pentru a evalua siguranţa şi efectele cardiovasculare ale Algisyl-LVR ™ ca o metodă de îmbunănătăţire a funcţiei sistolice a ventriculului stâng la pacienţii cu cardiomiopatie dilatativă. Algisyl-LVR ™ este un biopolimer implantat în miocard printr-o serie de 10 - 19 injectări, dispuse circumferinţial în zona de mijloc a ventriculului dinspre anterior către posterior, cu scopul de a reduce stresul parietal şi a îmbunătăţi funcţia VS. Primul nostru pacient randomizat supus procedurii chirurgicale este un bărbat în vârstă de 79 de ani cu fenomene de insuficienţă cardiaca cls III NYHA, dilatare severă a VS şi disfuncţie sistolică importantă (DSLV=68mm; DDLV=75mm; FEVS=21%; index de sfericitate=1,27). Procedura chirurgicală a constat într-o toracotomie limitată stângă şi 17 puncte de administrare al Algisyl-LVR ™. Evoluţia post-operatorie fără incidente.

Rezultate: Ecografia transtoracică de control la 8 zile, la o lună şi 3 luni a arătat îmbunătăţirea evidentă a valorilor determinate preoperator privind geometria şi funcţia VS. Aceste îmbunătăţiri au fost însoţite de progrese marcate ale capacităţii funcţionale (clasa NYHA, testul de mers de 6 minute, testul de efort cardiopulmonar).

Concluzii: Procedura chirurgicală de implantare a polimerului a dus la îmbunătăţirea geometriei, a funcţiei sistolice şi a funcţiei disastolice a ventriculului stâng.

INTERVENŢIE ÎN PREMIERĂ NAŢIONALĂ: IMPLANTARE PERCUTANĂ DE PROTEZĂ VALVULARĂ AORTICĂ LA PACIENŢI CU STENOZĂ AORTICĂ STRÂNSĂ ŞI RISC CHIRURGICAL FOARTE ÎNALT

F Pinte, G. Cristian, I. Droc, V. Murgu, D. Săvoiu, D. Cârpaciu, S. Dumitrescu
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Date teoretice: implantarea percutană de proteză valvulară aortică (TAVI) reprezintă o tehnică de cardiologie intervenţională realizată cu suport chirurgical şi de anestezie-terapie intensivă pentru tratarea pacienţilor cu stenoză aortică strânsă la care riscul operator foarte crescut pentru chirurgia cardiacă contraindică intervenţia de protezare clasică. Deşi tehnica a devenit o practică comună în marile centre terţiare de cardiologie, în România nu fusese efectuată nicio astfel de procedură în spitalele de stat.
Scop: Prezentarea indicaţiilor procedurii, a criteriilor de selecţie a pacienţilor şi a algoritmului investigaţiilor pre-procedurale, a protezelor valvulare specifice, a procedurii de implantare, a necesarului de resurse, a urmăririi şi terapiei post-procedurale şi descrierea experienţei noastre.

Metodă: Prezentarea datelor clinice şi paraclinice ale pacienţilor la care s-a efectuat implantarea percutană de proteză valvulară aortică la noi în centru, cu discutarea particularităţilor, evaluarea riscului chirurgical şi stabilirea criteriilor de selecţie pentru TAVI.

Rezultate: Procedura de implantarea percutană de proteză valvulară aortică s-a realizat cu succes, fără complicaţii majore post-procedurale şi cu o evoluţie ulterioară favorabilă. Prin evaluarea comparativă a parametrilor clinici, biologici şi ecocardiografici s-a confirmat succesul procedurii atât pe termen scurt cât şi la urmărirea pe termen mediu.

Concluzii: Primele proceduri de implantare percutană a protezelor valvulare aortice efectuate în România s-au realizat în siguranţă şi cu succes, generând o ameliorare sensibilă a parametrilor clinici şi paraclinici.

ACTUALITAŢI PRIVIND EXPLORĂRILE CARDIOVASCULARE NEINVAZIVE

S. Stanciu, Ioana Răduţă, Cristina Enciu, A. Anghel, D. Stoicescu, Daniela Anghel, L. Ciobică, M. Mureşan
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Lucrarea reprezintă o punere la punct a principalelor metode neinvazive pe care le utilizăm în practica medicală cotidiană în SUUMC „Carol Davila"cu focalizare pe noutăţile momentului. Tehnicile de imagistică non-invazivă şi constituirea lor într-un algoritm decizional care în cele din urmă ghidează un tratament ţintit constituie un subiect fierbinte actual în cardiologie.

ULTRASONOGRAFIA DOPPLER A ARTEREI BRAHIALE ÎN EVALUAREA PACIENTULUI HIPERTENSIV

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ABSTRACT

Artera brahială (AB) rămâne în practica clinică curentă locul de predilecţie pentru măsurarea TA şi diagnosticul bolii hipertensive. În scop de cercetare fiziologică, evaluarea Echo-Doppler a AB poate aduce informaţii suplimentare, utile clinicianului. În HTA, AB suferă în timp un proces de remodelare (creşterea D şi A) ca mecanism adaptativ, compensator al *shear-stress*-ului parietal. Modificările pot fi şi mal-adaptative, semn de ATS, în relaţie cu scorul global de risc CV sau prezenţa leziunilor aterosclerotice (carotidiene). Disfuncţia endotelială a fost documentată în diverse patologii: HTA, DZ, insuficienţă cardiacă. Metoda de evaluare se bazează pe cuantificarea vasodilataţiei mediate de flux (%FMD). Acest parametru fiziologic diferă în funcţie de fenotipul hipertensiv: HTA de halat alb, HTA susţinută, HTA gestatională/preeclampsie, HTA la vârstnici. Poate fi influenţat terapeutic cu arsenalul antihipertensiv disponibil în prezent. Prin această metodă (rezervată în prezent doar cercetării) putem aprecia nivelul disfuncţiei endoteliale şi răspunsul terapeutic la pacientul hipertensiv.

REPERE MORFOLOGICE ŞI HISTOCHIMICE ALE PERETELUI VENOS LA VENELE NORMALE ŞI VARICOASE

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[2] Clinica de Dermatologie a Universităţii de Medicină şi Farmacie „Victor Babeş", Timişoara;
[3] Catedra de Histologie a Universităţii de Medicină şi Farmacie „Victor Babeş", Timişoara.

Rezumat

Nutriţia peretelui venos pare să fie un factor important implicat în troficitatea structurilor fibro-musculare şi dinamica formării matricei extracelulare la venele normale şi în timpul vindecării ulcerelor venoase cronice de gambă. S-au prelevat biopsii secvenţiale la diverse nivele, din peretele venos al safenei externe şi interne la un număr de 16 cazuri care prezentau o suferinţă circulatorie cronică a sistemului venos gambier (vârsta pacienţilor 35-58 ani, ambele sexe). De asemenea, s-au recoltat la necropsie 8 fragmente venoase cu aspect macroscopic normal. Am studiat fragmentele recoltate prin metode morfologice uzuale şi câteva reacţii histochimice pentru evidenţierea substratelor glicogenice, glicoproteice, glicozaminoglicanice. Pentru evidenţierea selectivă a unor substraturi (reticulină, elastină) am folosit impregnarea argentică Gomori şi orceina. Alte metode de coloraţie (tricromă Gomori) au servit diferenţierii structurilor specifice peretelui venos.

S-a remarcat o bogată vascularizaţie a peretelui venos normal şi dilatat. Angiogeneza din peretele venos şi modificările vasei vasorum ca şi alcianofilia intimei vasculare par să fie factori reactivi şi protectori şi depind şi de modalităţile terapeutice aplicate. Venele sunt structuri slabe a căror integritate depinde de grosimea mediei şi de suportul structurilor învecinate.

BIOMARKERII SERICI ÎN EVALUAREA IMA – STEMI ÎN UPU ÎN PRIMELE 12 ORE DE LA DEBUTUL SIMPTOMATOLOGIEI

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Rezumat

Biomarkerii sunt definiţi ca şi indicatori ai proceselor biologice normale, ai celor patologice sau a răspunsului farmacologic la o intervenţie terapeutică care poate fi evaluat sau măsurat obiectiv. Principalele clase de biomarkeri serici prezenţi în afecţiunile cardiovasculare sunt: markerii sindromului inflamator coronarian (CRP, hs-CRP); markerii instabilităţii/ruperii plăcii (MPO, MPMs); markerii ischemiei/necrozei miocardice (troponinele I şi T, CK-MB, mioglobina); markerii insuficienţei cardiace (proBNP, NT proBNP). În această lucrare urmărim rolul biomarkerilor serici în primele 12 ore de la debutul IMA-STEMI (Infarct Miocardic Acut – ST Elevation Myocardial Infarction) în evaluarea IMA în UPU.

***Cuvinte cheie**: evaluarea IMA la debut, IMA-STEMI, biomarkeri cardiaci.*

UN INFARCT MIC...FATAL – PREZENTARE DE CAZ

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Prezentăm cazul unui pacient în vârstă de 70 de ani, fără istoric cardiovascular, transferat de la un spital teritorial, în stare gravă neexplicată de diagnosticul de STEMI, localizarea infarctului miocardic fiind strict inferioară, examinarea ecocardiografică decelând o tulburare de cinetică segmentară în teritoriul inferior, dar funcţie sistolică globală prezervată, iar examinarea CT de torace excluzând trombembolismul pulmonar acut. La prezentare se susţine diagnosticul de STEMI clasă Topol 5, clasa Killip IV, subsetul hemodinamic Forrester IV. Coronarografia a pus în evidenţă o leziune unicoronariană semnificativă - subocluzie în segmentul 2 CD, realizându-se angioplastia primară cu implantare de stent, cu rezultat final bun, dar fără o ameliorare a parametrilor hemodinamici. La reevaluarea ecocardiografică, s-a identificat etiologia şocului cardiogen - flail de valvă mitrală posterioară cu insuficienţă mitrală severă prin ruptură totală de muşchi papilar. Echipa de cardiochirurgie a fost completă în aproximativ 45 minute de la apel, începând la ora 3 A.M. intervenţia de înlocuire a valvei mitrale cu proteză valvulară metalică Medtronic ATS Medical nr. 31 care a fost efectuată cu succes, cu un timp de clampare de 94 de minute şi un timp de CEC de 135 de minute. Evoluţia postoperatorie a fost dificilă, necesitând menţinerea suportului mecanic circulator, ulterior două reintervenţii pentru evacuarea hematomului apărut la locul de introducere a balonului de contrapulsaţie intraaortic şi pentru evacuare a hemopericardului ce a realizat tablou clinic de tamponadă pericardică. Starea generală a pacientului s-a deteriorat evoluând către sindrom de disfuncţie multiplă de organ, înregistrându-se decesul în a 23-a zi de spitalizare.

INFARCT MIOCARDIC ACUT CU SUPRADENIVELARE DE SEGMENT ST LA BORDUL NAVEI

Mirică Daniel, Emilia Edu, Rusu Adrian, Alexandru Lucian
Forţele Navale

Vă prezentăm cazul unui pacient în vârstă de 38 de ani fără antecedente patologice semnificative, cu factori de risc cardiovascular (fumător, dislipidemic, supraponderal) care în data de 5.04.2013, ora 04:30, aflat la bordul corvettei 265, în misiune, în largul Mării Negre la sud de portul Varna, a manifestat, un episod de durere toracică precordială, de intensitate mare, cu iradiere în membrul superior stâng, motiv pentru care se adresează medicului de la bord.

Simptomatologia fiind înalt sugestivă pentru angor pectoris de novo, la un pacient la risc, se administrează nitroglicerină, cu ameliorarea parţială a durerii. Se suspicionează diagnosticul de sindrom coronarian acut, se administrează aspirină 250 mg pentru antiagregare plachetară, şi se iniţiază procedura de evacuare a pacientului către centrul specializat cel mai apropiat de pe uscat.

În continuare pacientul, stabil hemodinamic este debarcat, şi transportat la mal, cu şalupa apoi la Spitall Militar din Varna, unde electrocardiograma, echocardiografia, şi durerea precordială susţin diagnosticul de infarct miocardic acut cu supradenivelare de ST în teritoriul inferior, aflat la 5 ore de la debutul durerii.

Pacientul este transportat la Clinica de cardiologie a spitalului Sveta Marina din Varna, unde coronarografia efectuată evidenţiază ocluzie de ACD în segmentul mediu. Se practică PTCA per primam, cu implantarea de 2 DES pe ACD, iar pacientul este transferat la SMUUC Bucureşti, unde sub tratament optimizat, evoluţia a fost favorabilă cu ameliorarea tulburării de cinetică parietală şi normalizarea enzimelor de necroză miocardică. Pe parcursul spitalizării evaluarea angiografică post PTCA a relevat necesitatea intervenţiei de revascularizare electivă la nivelul LAD.

Pacientul se reinternează în SMUUC în data de 27.05.2013. La coronarografia efectuată se pun în evidenţă stenturi permeabile pe ACD şi stenoză pe LAD la nivelul bifurcaţiei cu Dg2 pentru care se practică PTCA cu implantare de stent PICOELITTE 2,5-24mm cu flux distal TIMI3 şi evoluţie postprocedurală favorabilă.

PACIENT CORONARIAN CU EVOLUŢIE ATIPICĂ – PREZENTARE DE CAZ

M. Florea, A. Ploae, Carmen Man

Obiectiv: prezentarea unui caz de boală coronariană ischemică severă cu manifestări clinice atipice.

Material şi metodă: evaluarea clinică şi paraclinică a unui pacient de 60 ani, fost fumător, obez, anxios, cu spondiloză cervicală cu radiculalgii frecvente şi trenante, cu infarct miocardic vechi inferior (2002) tratat conservativ. Pacientul prezintă în ultimii 2 ani dureri precordiale atipice, cu caracter predominant radicular şi care evoluează pe un fond anxios persistent. Pe electrocardiograma de repaus se evidenţiază unda q în DIII, fără modificări ST-T. Electrocardiografic se decelează o uşoară hipokinezie a peretelui inferior a VS, cu FEVS-60%. Testarea de effort EKG a fost neconcludentă la prezentare, pentru ca la 3 săptămâni să deceleze subdenivelări de segment ST de 1,5 mm în teritoriul inferior, aspect care justifică efectuarea coronarografiei. Coronarografic se decelează boala ischemică bivasculară (IVA şi Cx), cu leziuni severe în segmentul II al IVA.

Rezultate şi concluzii: s-a efectuat angioplastie transluminală percutană şi dublă stentare pe IVA. Ulterior sub tratament medicamentos standard evoluţia a fost favorabilă. În concluzie, la pacienţii cu tablou clinic atipic, dar cu istoric de boală coronariană ischemică şi cu factori de risc cardiovascular majori se impune efectuarea unor explorări complete şi complexe, inclusiv a celor de tip invaziv.

EXPERIENŢA NOASTRĂ ÎN PROGRAMUL NAŢIONAL DE TRATAMENT INVAZIV AL INFARCTULUI MIOCARDIC CU SUPRADENIVELARE DE SEGMENT ST: ELEMENTE DE PROGNOSTIC

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Scop: Prezentarea experienţei centrului nostru în programul naţional de tratament invaziv al infarctului miocardic cu supradenivelare de segment ST şi raportarea semnificaţiei prognostice a intervalelor de timp necesare pentru implementarea protocolului înregistrate în statistica noastră.

Metodă: Programul are ca scop tratarea tuturor pacienţilor ce suferă un STEMI prin angioplastie primară. Condiţia de includere în protocolul STEMI este ca intervalul de timp între durere şi reperfuzie să nu depăşească 12 ore.
Am analizat primii 344 de pacienţi. Media de timp de la debutul durerii la PTCA a fost de 6,06±2,78 ore, media întârzierii generate de alegerea angioplastiei primare ca metodă de reperfuzie a fost de 2,43±1,12 ore, numai la 29% din pacienţi timpul între debutul durerii si PTCA a fost mai mic de 90 min, în acest grup, întârzierile determinate de procedură fiind de 0,62±0,37 ore. Am comparat diferenţele legate de diagnosticul insuficienţei cardiace şi de decesul cardiovascular între acest grup şi cel în care timpul de la debutul durerii la PTCA a fost mai mare de 90 min.

Rezultate: Grupurile au fost similare ca medie de vârstă, sex, TA, AV, clasa Killip şi FEVS la prezentare şi au avut caracteristici angiografice asemănătoare. La externare am găsit modificări semnificative în grupul în care a fost tratat prin PTCA per-primam în mai puţin de 90 min, obţinându-se o ameliorare a FEVS (48,11±7,56% versus 44,85±7,39, p = 0,002).
Prelungirea timpului de reperfuzie peste 90 min a fost asociată cu o creştere semnificativă a riscului de insuficienţă cardiacă la externare (RR=1,4, IC(1,2-1,5), OR=4,6 IC(2,3-9,2), p<0,0001). De asemenea, a fost crescut riscul de mortalitate (4,31% vs 1,96%), dar fără semnificaţie statistică.

Concluzii: Studiul nostru demonstrează că în ciuda succesului programului STEMI, există un grup de pacienţi la care beneficiul agioplastiei primare este redus de prelungirea intervalului de timp de la debutul durerii la reperfuzie.

Hepatologie
Marți, 22 oct, 11^{15} – 12^{00}

CONTROVERSE ÎN TERAPIA CU STATINE LA PACIENŢII CU HEPATOPATII CRONICE DIFUZE

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ABSTRACT

Hepatopatiile cronice difuze, îndeosebi ciroza hepatică, asociază diverse grade de insuficienţă hepato-celulară, cu alterarea capacităţii de sinteză a colesterolului. Paradoxal, la unii pacienţi sunt descoperite anomalii semnificative ale profilului lipidic. Dacă în cazul cirozelor biliare (colestaza) acest aspect este cvasi-recunoscut şi acceptat, în cazul cirozelor de cauză virală sau etanolică, anomaliile profilului lipidic par greu de înţeles din punct de vedere fiziopatologic. În cazul steatozelor hepatice (Sdr. Metabolic, DZ tip 2), dispidemia este aproape ubicuitară şi contribuie major la riscul global CV al pacientului.
Utilizarea statinelor în bolile cronice de ficat rămâne un subiect fierbinte şi încărcat de controverse datorită potenţialelor efecte adverse exprimate adesea prin citoliza hepatică. Exemplificăm cu cazuri clinice din cazuistica proprie multiplele situaţii în care putem descoperi anomalii ale profilului lipidic la pacientul cu afectare hepatică difuză şi în care terapia cu statine poate fi o soluţie terapeutică utilă, în ciuda controverselor din literatura de specialitate.

SINDROM HEPATOCITOLITIC DE CAUZĂ RARĂ

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Prezentăm cazul unui pacient de 20 ade ani, student, fără antecedente patologice personale semnificative, care se prezintă la camera de gardă pentru apariţia de circa 20 de ore a urinei intens hipercrome în context subfebril, asociată cu astenie fizică, greaţă şi uşoare mialgii.
Anamneza iniţială efectuată la camera de gardă nu a relevat elemente semnificative pentru stabilirea diagnosticului, examenul obiectiv pe aparate şi sisteme a fost cvasinormal, ca şi ecografia abdomino-pelvină şi examenul radiologic cord-pulmon efectuate. Primul set de analize de laborator a relevat sindrom hepatocitolitic important, cu valori AST de 1484 UI/l, ALT 365 UI/l, dar cu bilirubina totală şi fracţionată, hemoleucograma şi restul biochimiei uzuale în limite normale. Iniţial s-a luat în discuţie o posibilă hepatită acută virală, infirmată ulterior prin absenţa markerilor pentru hepatitele acute virale.
S-a decis internarea pacientului pe secţia Gastroenterologie, la reluarea anamnezei pacientul relatând că în urmă cu circa 48 de ore a efectuat efort fizic intens la sala de fitness, efort obişnuit pentru el, dar care a urmat unei prelungite perioade de repaus.

Extinderea investigaţiilor de laborator a relevat marcată citoliză musculară, CK-14060 UI/l, LDH 10030 UI/l, microalbuminurie, cilindri granuloşi în sumarul de urină, mioglobinurie, uşoară acidoză metabolică, care asociate cu absenţa markerilor pentru hepatitele virale acute, au pus diagnosticul de rabdomioliză acută în context de efort fizic intens.

Evoluţia sub tratament de reechilibrare hidroelectrolitică şi acidobazică a fost favorabilă, cu normalizare clinicăa şi paraclinică.
Am prezentat acest caz pentru că sindromul de rabdomioliză poate reprezenta o cauză rară de hipertransaminazemie marcată care trebuie luată în discuţie şi, totodată pentru a evidenţia importanţa anamnezei atente încă de la camera de gardă.

HEPATOCARCINOMUL - CORELAŢII ÎNTRE FACTORII DE RISC ŞI DEMERSUL DIAGNOSTIC

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Boala neoplazică reprezintă o problemă de sănătate globală de o importanţă în continuă creştere. Anual se diagnostichează 10,9 milioane de cazuri noi şi decedează din cauza cancerului 6,7 milioane indivizi. În acest context hepatocarcinomul (HCC) este a şasea cauză de cancer şi a treia cauză de deces prin cancer la nivel global. România se încadrează în media de incidenţă a Europei cu 11,1 cazuri la 100.000 indivizi pe an. Principala cauză a HCC este infecţia cronică cu virusul hepatitic C (60-70% din cazuri) urmată de consumul de alcool (20% din cazuri) şi de infecţia cronică cu virusul hepatitic B (10-15% din cazuri). Ciroza hepatică creşte riscul de HCC, indiferent de etiologie: virus C, virus B, alcool, deficit de alfa 1 antitripsină, hemocromatoză). Fumatul este, conform unor dovezi recente, cu siguranţă un cofactor important în apariţia HCC. Obezitatea şi diabetul zaharat, prin mecanisme care sunt incomplet elucidate, cresc riscul de HCC şi constituie o ţintă importantă a politicilor de sănătate publică dată fiind prevalenţa lor în continuă creştere. Vaccinarea împotriva virusului hepatitic B este cel mai important gest de profilaxie primară a HCC şi este responsabilă de scăderea implicării acestuia în etiologia HCC în Europa. Tratamentul antiviral pare să reducă riscul de HCC dacă este aplicat într-un stadiu precirotic. În lucrarea de faţă voi prezenta câteva date dintr-un studiu observaţional efectuat în clinica noastră pe o perioada de 6 luni între ianuarie şi iunie 2012 privitoare la factorii de risc implicaţi în etiologia HCC şi la modalităţile de diagnostic abordate.

CORELAŢIA DINTRE HEPATITA CRONICĂ CU VIRUS B ŞI RISCUL DE APARIŢIE A CANCERULUI HEPATOCELULAR. ROLUL TRATAMENTULUI ANTIVIRAL ACTUAL - ARGUMENTE PRO ŞI CONS.

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Hepatita cronică cu virus B afectează aproximativ 350 milioane de persoane la nivel mondial, anual raportându-se 500000 de decese cauzate de evoluţia acestei boli.
Incidenţa anuală a apariţiei cancerului hepatocelular este între 2-5% la pacienţii infectaţi, în directă corelaţie cu factorii de risc. Proprietăţile oncogenice ale virusului HBV sunt în 70%-90% din cazuri prin mecanism indirect, prin necroinflamaţie şi promovarea progresiunii la ciroză, însă şi direct, în absenţa cirozei, prin integrarea virusului în genomul celulei gazde şi stimularea producţiei de proteine modificate cu rol oncogen: proteina X şi preS-S.
Lucrarea evidenţiază, cu argumente pro şi contra, rolul tratamentului antiviral actual în reducerea riscului de apariţie a hepatocarcinomului, în corelaţie cu ultimele studii publicate în domeniu.

Oncologie digestivă

ROLUL COLONOSCOPIEI ÎN DETECTAREA CANCERULUI COLORECTAL

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Introducere: Cancerul colorectal reprezintă o problemă de sănătate publică datorită incidenţei mari şi a diagnosticării în faze avansate, în România ocupând primul loc între cancerele digestive. Adenocarcinomul colorectal este o afecţiune care se dezvoltă lent, de cele mai multe ori având ca punct de plecare o leziune benignă preneoplazică ce se transformă malign. Simptomele apar relativ tardiv în evoluţia bolii şi depind în majoritatea cazurilor de localizarea anatomică a tumorii (hemoragii oculte, sindrom anemic, constipaţie cu caracter progresiv, sau alternanţă diaree/constipaţie, rectoragii sub formă de striuri sangvinolente, uneori tumoră palpabilă în fosa iliacă stângă, însoţite de durere abdominală, scădere ponderală).
Metode: Ne propunem să evidenţiem rolul colonoscopiei în detecţia formaţiunilor tumorale colonice, distribuţia şi caracterul stenozant/nestenozant tumoral, precum şi importanţa indicaţiei corecte a acestei investigaţii în practica medicală. În

acest sens am studiat retrospectiv evidențele laboratorului de endoscopie din perioada ian 2011 - dec 2012.

Rezultate: Pe parcursul unui an s-au efectuat aproximativ 2500 colonoscopii, 5 % dintre ele având ca indicație evaluarea statusului postoperator (fie în vederea restabilirii continuității digestive, fie pentru evidențierea recidivei locale sau a leziunilor premaligne). Rata detecției formațiunilor tumorale colonice (cu caractere endoscopice înalt sugestive pentru malignitate, ulterior confirmate histopatologic) a fost de cca 7 %, cu o distributie predominant la nivel rectosigmoidian (prox 60 %), jumătate dintre ele fiind formațiuni stenozante, ce nu au permis pasajul colonoscopului.

Concluzii*:* Pentru a reduce mortalitatea prin cancerul colorectal este necesar un program de screening, explorarea colonoscopică fiind un instrument important de diagnostic precoce, și în același timp de prevenire a cancerului colorectal, prin detecția leziunilor premaligne și sancțiunea lor terapeutică (polipectomie endoscopică, rezecție submucozală).

EVOLUȚIA CHIRURGIEI MINIM INVAZIVE COLORECTALE ÎN SECȚIA CHIRURGIE I

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Cuvinte cheie: laparoscopie , diagnostic, sutură mecanică, cooperare, stadializare, confort

Abordul minim invaziv are un rol din ce în ce mai mare în chirurgia abdominală modernă, reușind să se impună ca **gold standard** în intervenții precum colecistectomia, splenectomia, sutura ulcerului perforat, cura herniei gastrice transhiatale etc. Gradul de complexitate al procedurilor laparoscopice a crescut progresiv, acestea pătrunzând în sfera rezecțiilor colorectale, gastrice, pancreatice etc.

În cadrul Serviciului Chirurgie I se realizează abordarea patologiei colorectale pe cale laparoscopică, pe cazuri selecționate după anumite criterii, începând din anul 2010.

Lucrarea de față analizează abordarea laparoscopică a patologiei colorectale în Serviciul Chirurgie I, urmărindu-se evoluția față de anii anteriori a parametrilor cazurilor tratate în această manieră, fiind analizat un număr de 21 de cazuri operate de medicii secției noastre în perioada 10. 09. 2012 – 13. 09. 2013.

ASPECTE ALE ABORDULUI LAPAROSCOPIC ÎN CANCERUL COLO-RECTAL

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Autorii analizează câteva aspecte ale abordului laparoscopic al cancerului colo-rectal, prin prisma experienței acumulate în cadrul Secției Chirurgie II. Sunt discutate avantajele acestui tip de abord în privința stadializării și a diverselor etape operatorii: evaluarea tumorii și a extensiei locale, identificarea adenopatiilor și a metastazelor la distanță, abordul pediculilor vasculari și timpii de rezecție, ca și diversele modalități de refacere a tranzitului digestiv. Este efectuată o scurtă analiză comparativă a rezultatelor acestui tip de abord în ce privește evoluția postoperatorie, morbiditatea și mortalitatea, ca și considerentele relative la costurile de spitalizare, față de abordul clasic deschis în cazul pacienților cu cancer colo-rectal.

PECOM: CAUZĂ EXTREM DE RARĂ DE OCLUZIE INTESTINALĂ

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Rezumat:
Prezentăm cazul unei paciente internată prin transfer în secția II Chirurgie pentru un sindrom subocluziv debutat în urmă cu 3 zile aflându-se internată în Secția Medicală. Examenul CT multisclice evidențiază formațiune tumorală pelvină cu diametrul de 13,2/7,8 cm cu aspect heterogen cu invazia unei anse ileale și litiază biliară. Se intervine chirurgical practicându-se colecistectomie laparoscopică, ulterior în timpul 2 operator efectuându-se histerectomie totală cu anexectomie bilaterală cu enterectomie segementară cu entero-entero anastomoză latero-laterală. Diagnosticul final a fost pus de examenul imuno-histochimic al piesei de rezecție: tumoră cu celule epitelioide perivasculare (PECom) locală agresivă. Sunt discutate aspecte particulare etiologice, clinice, terapeutice, pe care această tumoră rară le ridică în prezent.

Cuvinte cheie: *PECom, Angiomiolipom, HMB45, Actina de mușchi neted, Rapamycina.*

LIMFANGIOM GASTRIC – PREZENTARE DE CAZ

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Rezumat: Limfangioamele sunt tumori benigne limfatice care pot apărea practic în orice loc anatomic. Aproximativ 95% sunt localizate la nivelul gâtului și axilei și doar 5% la nivelul mez-

enterului, retroperitoneului, viscerelor abdominale, pulmonar și mediastinal. Din localizările abdominale localizarea la nivelul stomacului fiind cea mai rară, cel mai frecvent întâlnindu-se localizarea mezenterică. În lucrarea de față prezentăm cazul unui tânăr în vârstă de 30 ani care s-a prezentat la spital pentru o formațiune tumorală localizată în epigastru cu debut în urmă cu mai multe luni însoțită, de vărsături. Investigațiile imagistice efectuate (examen ecografic abdominal și examen CT abdominal) au comfirmat prezența unei formațiuni tumorale gastrice. Excizia masei tumorale gastrice și examenul histopatologic au confirmat diagnosticul de limfangiom gastric. Cazul este prezentat din cauza rarității sale, până în prezent din 1953 au fost descrise 100 de cazuri în literatură, împreună cu o scurtă trecere în revistă a literaturii de specialitate.

CONCURRENT DOCETAXEL BASED CHEMORADIOTHERAPY IN ESOPHAGEAL CANCER

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Această lucrare prezintă rolul radiochimioterapiei concomitente cu taxani în cancerul esofagian avansat locoregional în condițiile în care tratamentul optim este încă controversat în această boală.

Cancerul esofagian este al 8-lea tip de cancer ca prevalență la nivel mondial dar incidența lui este într-o continuă creștere în ultimile decenii.

Este o boală foarte agresivă: 90 % din pacienți mor din cauza bolii, de obicei fiind detectată în stadii avansate locoregional.

29 de pacienți au fost incluși în acest studiu la Institutul Oncologic București în perioada ianuarie 2009 – decembrie 2010.

ECOENDOSCOPIA ÎN TUMORILE SUBEPITELIALE GASTROINTESTINALE

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Introducere
Ecoendoscopia (EUS) este considerată cea mai utilă metodă diagnostică pentru evaluarea leziunilor subepiteliale ale tractului gastrointestinal, oferind detalii morfologice și posibilitatea de achiziție tisulară.

Material și metodă
Am derulat un studiu retrospectiv pe 144 pacienți referiți pentru evaluare ecoendoscopică a unei formațiuni subepiteliale gastrointestinale, între 2011 și 2012. Datele privind caracteristicile ultrasonografice ale tumorii (ecostructură, ecogenitate, formă, dimensiuni, margini, strat de origine, aspect la Doppler și elastografie) au fost preluate din registrul de ecoendoscopie.

Rezultate
Dintre cei 144 pacienți, 57% erau bărbați iar vârsta medie a fost de 59.45 ani. Majoritatea tumorilor (95.84%) au fost localizate la nivelul tractului digestiv superior, în timp ce doar 4.16% au fost leziuni subepiteliale rectale. Dintre cele 138 tumori de tract digestiv superior (11 compresii extrinseci și 127 intramurale), 39.86% au fost localizate la nivel esofagian, 6.52% la joncțiunea esogastrică, 47.10% în stomac și 6.52% în duoden. În ceea ce privește ecostructura, 89.58% dintre leziuni erau de tip solid, 5.56% chistice, 3.47% mixte și 1.39% vasculare. Ecoendoscopia a identificat cu acuratețe stratul de origine sau implicarea straturilor în 129 din cele 133 tumori intraparietale. Prin puncție ecoendoscopică fin-aspirativă (EUS-FNA) și cu ajutorul chirurgiei în unele cazuri, 61 pacienți au avut un diagnostic final – între acestea, 15 leiomioame, 10 GIST-uri, 2 limfoame, 2 noduli sarcoizi, 1 teratom și 1 schwanomm. Restul de 83 pacienți au primit recomandarea de urmărire endoscopică și ecoendoscopică.

Concluzii
Ecoendoscopia este un instrument diagnostic valoros pentru tumorile subepiteliale gastrointestinale. Caracteristicile ultrasonografice ale ecoendoscopiei pot fi utile în ghidarea managementului acestor leziuni: fie urmărire, fie FNA și chirurgie.

Specialități chirurgicale
Marți, 22 oct, 16^{30} – 18^{30}

CHIST HIDATIC CEREBRAL –prezentare de caz

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Abstract:
Autorii prezintă un caz de hidatidoză cerebrală la o pacientă tratată în antecedente pentru un chist hidatic renal, și cunoscută cu un chist hidatic hepatic. Pacienta este internată și se intervine neurochirugical în Secția de Neurochirugie a SUUMC – intervenția chirurgicală desfășurându-se fără incidente, pacienta neprezentând niciun deficit neurologic după evacuarea chistului cerebral de mari dimensiuni.

Particularitatea cazului rezidă din incidența din ce în ce mai mai mică a chistelor hidatice cerebrale, sub 2 cazuri la 10 ani în serviciul nostru. Lucrarea pune la dispoziție imagini CT, și FOTO intraoperator precum și imagini VIDEO din timpul intervenției.

LAMBOUL FASCIOCUTAN TEMPOROFRONTAL ÎN RECONSTRUCŢIA ORBITEI POSTEXENTERAŢIE ÎN CONTEXT ONCOLOGIC

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Introducere:
Exenterația de orbită, este o intervenție chirurgicală de necesitate, în urma căreia rezultă defecte de părți moi majore, a căror reconstrucție reprezintă o provocare pentru chirurgul plastician.

Material şi metodă:
În această lucrare prezentăm o serie de cazuri oncologice, abordate în echipa multidisciplinară, în care, după exenterația lărgită practicată de chirurgii maxilo-faciali, lamboul fasciocutan temporofrontal transpozat a fost utilizat pentru reconstrucția orbitei.

Discuţii:
Utilizarea lamboului fasciocutan temporofrontal transpozat pentru reconstrucția orbitei, s-a dovedit a fi un procedeu relativ simplu și rapid de realizat, cu rezultate stabile în timp, atât din punct de vedere funcțional cât și estetic. Deasemnea, acest tip de lambou, deși adesea de dimensiuni foarte mari, este extrem de fiabil, nici unul din cazurile în care l-am folosit, nefiind grefat de complicații.

Concluzii:
În cazurile ce necesită exenterație în context oncologic și în care radioterapia face parte din planul terapeutic, reconstrucția orbitei cu lambou facsiocutan temporofrontal de vecinatate, ar trebui să fie o primă alegere, datorită faptului că acest tip de lambou, oferă o soluție permanentă de acoperire, cu un țesut de bună calitate, bine vascularizat, și cu rezultate estetice satisfăcătoare.

MODALITĂŢI DIFERITE DE TAMPONAMENT INTERN ÎN CHIRURGIA DEZLIPIRII DE RETINĂ

Colectivul Secției Clinice de Oftalmologie

Se prezintă câteva cazuri de decolare de retină regmatogenă la care în funcție de particularitățile cazurilor s-au folosit diferite soluții chirurgicale de endotamponament.

RESTAURAREA FUNCŢIONALĂ IMEDIATĂ PRIN IMPLANTURI DENTARE

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Introducere: Tot mai mulți pacienți solicită azi punerea în funcțiune imediată a implantelor înserate. De aici și necesitatea de a individualiza tratamentul protetic pe implante atât din punct de vedere al dorințelor pacientului, cât și din cel al situației anatomice și funcționale pe care acesta o prezintă.

Material şi metodă: Inserarea imediată postextracțională a implanturilor dentare, concomitent cu protezarea funcțională, s-au dovedit de mult variante viabile la tehnicile clasice de protezare pe implanturi dentare. Scopul lucrării este de a prezenta experiența noastră cu variante ale acestei tehnici, cu indicații, avantaje și respectiv riscuri inerente.

Rezultatele postoperatorii favorabile obținute, similare celor din cazurile în care s-a utilizat conduita clasică ne determină să considerăm că putem veni în întâmpinarea dorințelor pacienților de a obține o funcționalitate imediată a aparatului dento-maxilar odată cu reducerea duratei tratamentului și creșterea calității vieții.

Concluzii: Protezarea imediată pe implante are ca scop scurtarea duratei tratamentului și asigurarea unui rezultat corect din punct de vedere estetic și funcțional, stabil în timp și de durată, chiar din faza chirurgicală. Stabilitatea primară a implantelor și în această situație este o condiție sina qua non. În circumstanțele date, încărcarea imediată a implantelor, ca și implantarea imediată, sunt în același timp metode de menținere a osului alveolar și a țesuturilor moi înconjurătoare, dar, chiar dacă aceste deziderate sunt importante și justificate, medicul este responsabil de recomandarea acestor metode, cunoscând limitele și riscurile lor.

ARTROPLASTIA PRIMARĂ DE ŞOLD NECIMENTATĂ UTILIZÂND STEMURI FEMURALE SCURTE PRIN TEHNICI MINIINVAZIVE - O PREMIERĂ NAŢIONALĂ

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Abstract:
Artroplastia primară de şold este o procedură chirurgicală, în care articulaţia coxo-femurală este înlocuită cu un implant protetic, ca formă de tratament a durerii artritice, osteonecrozei aseptice de cap femural (O.N.A.C.F.), sau în cazul traumatismelor severe, cum ar fi fractura de col femural. În ciuda succesului demonstrat a artroplastiei de şold totale clasice, evoluţia tehnologică şi cerinţele ridicate ale pacienţilor au dus la nevoia de a explora posibilităţi noi de a îmbunătăţi procedura. Durerea şi deficitul funcţional tind să nu mai fie singurele indicaţii clare în vederea intervenţiei chirurgicale, pacienţi din ce în ce mai tineri apelând la artroplastia de şold pentru recăpătarea unei vieţi active. Chirurgia conservatoare are în vedere atât menajarea ţesuturilor moi, cât şi conservarea capitalului osos. Stemurile femurale scurte (metafizare) au fost introduse în practica chirurgicală ca alternativă la stemurile femurale convenţionale în artroplastia totală de şold necimentată primară pentru că acestea au în plus o serie de avantaje. Pe măsură ce sunt explorate noi tehnici minim invazive în chirurgia artroplastică a şoldului, do-

bândim o progresivă înţelegere a faptului că, în majoritatea cazurilor, „mini-invaziv“ se asociază cu o scădere a morbidităţii, o recuperare mai rapidă şi, bineînţeles, un rezultat mai atrăgător din punct de vedere cosmetic. Având în vedere cele expuse, este un pas natural să fie îmbinate cele două: protezele de şold cu stem femural scurt şi abordul mini-invaziv, astfel pacientul beneficiind atât de avantajele conservării ţesutului osos, cât şi de cele ale protejării ţesuturilor moi.

RECONSTRUCŢIA COMPLEXĂ A PIRAMIDEI NAZALE UTILIZÂND LAMBOURI LOCOREGIONALE

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Introducere: Reconstrucţia piramidei nazale este o provocare pentru chirurgul plastician, înlocuirea ţesuturilor distruse necesitând cunoştinţe complexe asupra anatomiei locale, metodelor de reconstrucţie şi a tehnicilor chirurgicale.

Material şi metodă:
În această lucrare prezentăm o serie de pacienţi cu defecte compozite la nivelul regiunii nazale, rezultate în urma exciziilor lărgite a unor formaţiuni tumorale maligne de mari dimensiuni. Pentru reconstrucţie am folosit laboul frontal paramedian împreună cu grefe de cartilaj auricular şi diferite alte tipuri de lambouri locale.

Discuţii:
Cu ajutorul grefelor de cartilaj am obținut proiecția piramidei nazale și am reconstruit în totalitate narinele, făcând astfel posibilă respirația nazală.
Lambourile locale oferă o soluție de acoperire cu un țesut de bună calitate, bine vascularizat cu culoare și textură ideală, apropiată de cea a zonei receptoare, morbiditatea zonei donoare fiind minimă.

Concluzii:
Această tehnică, atunci când este aplicată cu minuțiozitate și precizie, conduce la un rezultat funcțional bun, cu minime inconveniente estetice.

PIEZOSURGERY - CONCEPT ACTUAL ÎN OSTEOTOMIE

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Introducere: Reprezentand un concept actual în chirurgia osului, tradus prin utilizare simplă, fără stress, caracter selectiv al osteotomiei, piezosurgery constituie un progres important în chirurgia osoasă. Introdusă de dr. Tomaso Vercellotti în 1998 are indicații de la osteotomii și osteoplastii, recoltare de os în regenerarea osoasă ghidată și până la planarea rădăcinilor în chirurgia parodontală și extracția dinților incluși.

Material și metodă: Pe baza cazuisticii personale, autorii având o experiență de 5 ani în utilizarea piezorugery, descriu principalele caracteristici ale conceptului, domeniul de utilizare și avantajele sale.

Concluzii: Apărut pe piața aparaturii medicale în urmă cu aproximativ 5 ani, piezosurgery continuă să incite prin noi domenii de utilizare avantajoasă, constituind o unealtă extrem de importantă și necesară în același timp, pentru orice ramură a chirurgiei care implică realizarea de osteotomii, dar în special pentru chirurgul oral și maxilo-facial.

MANAGEMENTUL PACIENŢILOR CU INTERVENŢII CHIRURGICALE MINORE ÎN SFERA BMF AFLAŢI ÎN TERAPIE ANTICOAGULANTĂ ORALĂ

Liliana Moraru, Dumitru C., Gabara A., Gurzun Maria-Magdalena

Lucrarea realizată în cadrul secţiei de Chirurgie Orală şi Maxilo-Facială îşi propune să aducă în prim plan un nou protocol de abordare a pacienţilor aflaţi în tratament anticoagulant oral sau antiagregant (pentru diverse patologii cardiovasculare), dar care necesită intervenţii de mică amploare în sfera BMF (extracţii, rezecţii, plastii). Abordarea este în concordanţă cu noile ghiduri ale Societăţii Europene de Cardiologie şi acordată cu specificul secţiei prezentând premisele teoretice, aplicarea practică, experienţa dobândită a autorilor în această privinţă propunând în final un chestionar de validare a acestor noi atitudini.

IMPLANTUL DE X-PRESS – METODA MODERNĂ DE TRATAMENT CHIRURGICAL AL GLAUCOMULUI PRIMITIVE CU UNGHI DESCHIS – FILM VIDEO

G. Anghel, C. Ştefan, M. Zemba, O. Muşat, H. Manole, Laura Macovei, Liliana Pulbere, Daniela Şelaru, Monica Armegioiu

ASPECTE IATROGENICE ÎN IMPLANTOLOGIA ORALĂ

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Introducere: În ultimi 20 de ani implanturile dentare au devenit o metodă curentă de restaurare funcţională a maxilarelor edentate. Tehnicile chirurgicale şi protetice sunt bine standardizate şi cunoscute. Totuşi există situaţii în care lucrurile nu decurg aşa cum am dori.

Material şi metodă: Scopul acestei prezentări este ca, pe baza unei experienţe personale de 20 ani în implantologie, să stabilească unele din cauzele care pot duce la compromiterea tratamentului prin implanturi, precum şi modalităţile de evitare a eşecului terapeutic.

Concluzii: Fără a pretinde că deţinem răspunsul la toate problemele care pot fi întâlnite în acest domeniu, încercăm să atragem atenţia asupra importanţei planificării minuţioase a tratamentului prin implante dentare, cât şi a respectării protocoalelor specifice.

SUTURA DE MENISC CU ANCORE

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***Cuvinte cheie**: Sutură menisc, McMurray, Apley*

Leziunea de menisc este patologia cea mai frecventă a articulaţiei genunchiului, atât în rândul sportivilor cât şi în rândul oamenilor de rând. În ultimul timp se insistă din ce în ce mai mult pe prezervarea meniscului prin încercarea de a-l sutura.

Scopul lucrării: Evaluarea rezultatelor după sutura de menisc cu ancore resorbabile.

Tipul studiului: Descriptiv, retrospectiv.

Material şi metodă: 100 de pacienţi operaţi în perioada iulie 2011 – iulie 2012 în Clinica de Ortopedie şi Traumatologie a SUUMC „Carol Davila“ şi evaluaţi la 1 an.

Rezultate: 92% din pacienţii evaluaţi postoperator la 1 an se prezintă fără dureri şi cu semne meniscale negative (McMurray şi Apley).

Concluzii: Rezultate clinice foarte bune pentru criteriile de includere şi indicaţiile postoperatorii date.

VERTEBROPLASTIA SOLUȚIE MINIM INVAZIVĂ ÎN TRATAMENTU FRACTURILOR VERTEBRALE PE FOND OSTEOPOROTIC

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Abstract:

Lucrarea prezintă o tehnică minim invazivă utilizată pe scară largă în patologia spinală. Tehnica este una din cele mai moderne şi este efectuată din ce în ce mai frecvent în serviciul de neurochirurgie al SUUMC.

Specialităţi medicale
Miercuri, 23 oct, 08^{30} – 10^{15}

EDUCAŢIE PENTRU SĂNĂTATE PENTRU ANGAJAŢII M.Ap.N.

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Cu ocazia evaluării clinice şi paraclinice în cadrul controlului medical periodic, s-a constatat că 49,04% din angajaţii MApN au în mod constant modificări ale probelor de laborator şi valori tensionale uşor crescute.
Ne-am propus evaluarea tipului modificărilor (încadrare în entităţi patologice) şi derularea unui program de educaţie pentru sănătate şi de evaluare a rezultatelor la un an.
Au intrat în studiu 6389 angajaţi ai MApN care au efectuat controlul periodic în ambulatoriul integrat. Au fost evaluaţi şi 21% au avut criterii de diagnostic de sindrom metabolic, 11,5% steatohepatită toxic-nutriţională, 8% hiperlipoproteinemie mixtă. Aceste aspecte domină la grupa de vârstă 25-45 ani. S-a făcut consiliere nutriţională, reinstruire privind pregătirea înainte de analize şi educaţie pentru sănătate privind factorii de risc cardiovasculari.
La un an s-a făcut reevaluare. Am constatat o reducere nesemnificativă statistic a prevalenţei patologiei metabolice şi cardiovasculare şi o complianţă redusă la schimbarea stilului de viaţă.
Cele mai mari modificări ale parametrilor biologici s-au constatat la loturile care au făcut controlul lunea şi marţea (sindrom de weekend).
Sindromul metabolic, steatoza hepatică, tulburarea de glicoreglare şi hipertensiunea arterială reprezintă probleme semnificative de sănătate constatate cu ocazia controlului periodic la angajaţii MApN. Este util un program de sănătate în care să fie implicaţi şi medicii de unitate, deoarece această patologie poate greva prin complicaţii capacitatea de îndeplinire a misiunii specifice a cadrelor MApN.

IERARHIZAREA RISCURILOR ÎN TRATAMENTUL OBEZITĂŢII ŞI A COMPLICAŢIILOR ACESTEIA

Ene Doina
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Conform Organizaţiei mondiale a Sănătăţii (OMS) supraponderea şi obezitatea se definesc ca acumularea excesivă de ţesut adipos care prezintă risc pentru starea de sănătate. Cea mai specifică metodă de apreciere a obezităţii este calculul indicelui de masă corporală (Body Mass Index) (IMC/BMI). Valoarea IMC ≥ 25 Kg/m² corepunde supraponderii iar cea ≥ 30 Kg/m². Conform Raportului OMS din martie 2013 în lume circa 5 milioane de copii cu vârsta sub 5 ani şi circa 500 milioane de persoane cu vârsta peste 20 de ani sunt obeze.

Obezitatea „cronică" este un factor de risc pentru un număr mare de afecţiuni medico-chirurgicale.

Principiile de tratament al obezităţii sunt relativ bine statuate cu aplicabilitate progresivă în funcţie de starea de sănătate şi vârsta pacientului la momentul adresabilităţii la medic.

Tratamentul complicaţiilor generate sau întreţinute de obezitate este interdisciplinar, presupune adesea un număr mare de medicamente şi spitalizări frecvente. Complexitatea medicaţiei necesare creşte riscul cumulării reacţiilor adverse, creşte frecvenţa şi complexitatea investigaţiilor de monitorizare şi a costurilor pentru sănătate.

Persoanele obeze care prezintă complicaţii au o scădere a calităţii vieţii şi o creştere a gradului de dependenţă socială.

Screening-ul corect şi la timp al persoanelor supraponderale/obeze cu evaluarea riscului de complicaţii crează premiza creşterii duratei de viaţă activă, a stării de bine şi a reducerii costurilor pentru sănătate. Capacitatea echipei medicale de a obţine complianţa de durată a unui pacient obez poate fi un element cheie în obţinerea de rezultate medicale performanţe.

DIABETUL ZAHARAT NOU-INSTALAT – INDICIU PENTRU CANCERUL PANCREATIC?

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Introducere

Diabetul zaharat este recunoscut atât ca factor de risc cât şi ca manifestare precoce a cancerului pancreatic (diabet tip 3c sau diabet pancreatogenic). Obiectivul acestui studiu a fost de a evalua modificările glicemice la pacienţii cu cancer pancreatic.

Metodă

Am studiat retrospectiv 40 pacienţi cu cancer pancreatic dovedit histologic internaţi în spitalul nostru între 1 ianuarie 2012 şi 31 decembrie 2012. Am împărţit pacienţii cu cancer pancreatic în 2 grupuri: cu diabet (n=28) şi fără diabet (n=12), şi am evaluat diferenţele în ceea ce priveşte vârsta, sexul, indicele de masă corporală, dimensiunea şi localizarea tumorii.

Rezultate

Dintre cei 40 pacienţi cu cancer pancreatic (60% de sex masculin, vârsta medie 64.15 ani), 36 (90%) aveau fie diabet, fie hiperglicemie. Dintre cei 28 diabetici, 15 (37.5%) aveau diabet nou-instalat (diagnosticat în ultimele 36 luni), iar restul 13 (32.5%) diabet cu evoluţie îndelungată. 2/3 dintre pacienţii cu diabet nou-instalat fuseseră referiţi clinicii noastre pentru evaluarea suplimentară a unei mase pancreatice decelată ecografic. Vârsta medie, indicele de masă corporală şi dimensiunea tumorii au fost similare între diabeticii şi non-diabeticii cu cancer de pancreas, însă bărbaţii şi tumorile localizate la nivelul cozii au fost asociate cu un risc mai mare de diabet.

Concluzii

Diabetul zaharat reprezintă o asociere frecventă în cancerul pancreatic. Diabetul nou-instalat ar putea constitui o recomandare de screening pentru cancerul de pancreas, fiind o potenţială primă manifestare a unei malignităţi pancreatice.

TRANSPLANTUL DE INSULE PANCREATICE

Brînduşa Cofaru
Spitalul Militar de Urgenţă Regina Maria Braşov

Transplantul de insule pancreatice este mediatizat în ultimii ani ca urmare a rezultatelor promiţătoare obţinute de echipa canadiană de la Edmonton condusă de profesorul James Shapiro. Rămân însă numeroase întrebări la care cercetătorii vor încerca să răspundă.

Cum se pot îmbunătăţi rezultatele acestei proceduri? Cum se pot reduce complicaţiile acestei intervenţii? Cum se poate creşte accesibilitatea la acest tip de grefă?

Diabetul de tip 1 se poate vindeca prin transplantul pancreasului în întregime, însă această procedură chirurgicală, deşi are rezultate remarcabile (~70% din pacienţii transplantaţi nu mai necesită tratament insulinic la 5 ani după transplant) prezintă o rată mare de complicaţii postoperatorii (30%). Din acest motiv, din anii 1970 se încearcă dezvoltarea unor tehnici care să permită extragerea insulelor pancreatice producătoare de insulină din restul pancreasului, cu scopul de a transplanta doar ţesutul necesar „vindecării" diabetului printr-o procedură simplificată şi mai puţin riscantă.

BOLI AUTOIMUNE ASOCIATE-ABORDARE MULTIDISCIPLINARĂ

Mihaela Bozdog, Doina Băltaru, Mălina Dincă, Ramona Nistor, Roxana Bozdog
Spitalul Militar de Urgenţă „Dr. Constantin Papilian" Cluj-Napoca

Aproximativ 1 din 6 pacienţi cu boală autoimună tiroidiană au o altă patologie autoimună asociată.
Bolile autoimune prezintă o susceptibilitate genetică comună.
În lucrarea de faţă vom prezenta câteva cazuri clinice în care se asociază patologie autoimună tiroidiană cu altă patologie autoimună.
Medicii practicieni ar trebui să caute şi alte patologii autoimune asociate la pacienţii cu boală autoimună tiroidiană.
Abordarea multidisciplinară este necesară pentru depistarea precoce a unor patologii cu risc vital şi lărgirea viziunii asupra patologiei autoimmune.

ARTROPATIA PSORIAZICĂ ÎNTRE REUMATOLOGI ŞI DERMATOLOG

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Rezumat

Psoriazisul vulgar reprezintă o afecţiune relativ frecventă; 20-40% din pacienţi dezvoltă afectare articulară cu potenţial evolutiv. Cazul clinic prezentat se distinge prin următoarele particularităţi:
- debut cu fenomene inflamatorii articulare, ulterior leziune cutanată;
- progresia rapidă a leziunilor articulare;
- comorbidităţile ce limitează utilizarea DMARDS;
- complicaţiile/reacţii adverse apărute în evoluţia acestuia;

Concluzii:
- psoriazisul artropatic poate reprezenta o problemă pentru medicul practician;
- este necesară cooperarea interdisciplinară;
- deciziile terapeutice trebuie să fie rapide, fundamentate;
- monitorizarea evoluţiei este importantă.

REMICADE - EXISTĂ UN PUBLIC ŢINTĂ

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Tratamentul cu biologice anti TNF alfa în patologia umană are deja o experienţă suficient de bogată în timp şi număr de pacienţi încât permite creionarea unor ghiduri terapeutice. Aceste ghiduri sunt necesare cu atât mai mult cu cât prezenţa a două sau mai multe produse cu acţiune identică şi profiluri de siguranţă similare dar modalităţi de administrare diferite ridică de multe ori în faţa clinicianului neexperimentat dileme de abordare care nu găsesc răspunsuri în cărţi ci în experienţă.
Spitalul nostru are cea mai îndelungată experienţă în tratamentul psoriazisului cu infliximab, aici fiind folosit pentru prima dată în România.
Prezentăm câteva consideraţii asupra tratamentului psoriazisului cronic vulgar mediu şi sever cu infliximab, sugerând o modalitate de abordare a pacientului în vederea selecţionării produsului medicamentos optim pentru o situaţie dată.

ASOCIERE DE POLIARTRITĂ REUMATOIDĂ ȘI SARCOIDOZĂ LA PACIENT CU TRATAMENT BIOLOGIC ANTITNFα- PREZENTARE DE CAZ

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Asocierea poliartritei reumatoide cu sarcoidoza este rară și sunt communicate până în anul 2012, 37 de cazuri.
Prezentăm un pacient în vârstă de 42 de ani, cu debut al poliartritei reumatoide la vârsta de 16 ani. Din anul 2001 s-a inițiat rapid anti- TNFα cu Infliximab 3 mg/kgc, iar apoi datorită ineficienței cu Etanercept și în ultimele șase luni cu adalimumab. În urmă cu șase săptămâni evaluarea radiologică pulmonară evidențiază adenopatii mediastinale.
Biopsia prin mediastinoscopie stabilește diagnosticul de sarcoidoză.
Se prezintă datele din literatură.

TERAPIA BIOLOGICĂ ŞI CANCERUL DE ORGAN

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Inhibitorii de alfa TNF, aşa numitele biologice, au intrat în terapia umană de aproape 25 de ani promiţând o nouă abordare, mai patogenică, şi o nouă speranţă privind controlul bolilor. Temerile iniţiale ale specialiştilor privind riscurile acestui gen de imunomodulare pe termen lung au părut derizorii. Infecţiile pot fi controlate cu antibiotice, tuberculoza îşi găseşte şi ea rezolvare cel mai adesea, iar confortul indiscutabil oferit pacienţilor merită orice efort financiar.
Ca atare, medicii au devenit tot mai încrezători şi relaxaţi în utilizarea acestei terapii, prea adesea lăsând însă garda jos şi tratând cu uşurinţă cazurile particulare.
Prezentăm cazul unei paciente cu psoriazis cronic vulgar, introdusă pe tratament biologic, care se prezintă la internare pentru anemie medie severă asociată cu hemoragii digestive inferioare.

Evaluarea complexă indică prezenţa unei neoplazii la nivelul tubului digestiv, care a prezentat repetat semne de alarmă ce au fost trecute cu vederea de medicul curant al pacientei.

Dorim să prezentăm consideraţii pe marginea acestui caz, ca un semnal de alarmă privind falsa siguranţă indusă de experienţă în cazul tratamentelor agresive.

ARTROPLASTIA TOTALĂ DE ŞOLD CU IMPLANTURI NECIMENTATE ŞI DE RESURFAŢARE LA PACIENŢII TINERI CU AFECTARE ARTICULARĂ POST TERAPIE IMUNOSUPRESIVĂ

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Rezumat: În practica zilnică, între prezentările pacienţilor pentru acuze ortopedicotraumatologice, numărul pacienţilor oncologici este în continuă creştere. De remarcat este vârsta tot mai scazută a acestor pacienţi, a căror boală non-osoasă are ecouri sistemice printre care şi osteoarticulare, fie ca o determinare secundară, fie ca efect secundar al terapiei imunosupresive.
Tratamentul chirurgical, în mod expres cel artroplastic, este metoda larg controversată la momentul actual, dar tot mai des aplicată, ţinând cont de benificiile funcţionale şi integrarea socială aproape garantate, deziderate de neconceput în eventualitatea tergiversării îndrumării pacienţilor oncologici către tratamentele moderne chirurgicale, de endoprotezare.

Metodă: Într-o perioadă de 6 ani (2008 – 2013) printre cei 10898 pacienţi personali, ce s-au prezentat în clinica de ortopedie, s-a marcat existenţa a 52 de pacienţi oncologici cu implicare osteoarticulară din care 25 au fost cazuri descoperite incidental (primitive sau relevate de fracturi) iar restul de 27 au fost cazuri cu neoplazii complicate cu determinări secundare osoase. Printre aceşti 52 de pacienţi au fost remarcaţi trei pacienţi cu afecţiuni oncologice non scheletale, cu vârste până în 20 de ani, dintre care - 2 paciente cu leucemie acută limfoblastică (LAL), una fiind cel mai tânăr pacient cu endoproteză de resurfaţare din România (14 ani şi 6 luni) şi un pacient cu neoplasm testicular. Ca urmare a tratamentelor medicamentoase antineoplazice ei au suferit afectarea articulaţiei coxofemurale (uni- sau bilateral) dezvoltând necroză aseptică de cap femural (NACF) post-medicamentoasă imunosupresivă. Tratamentul chirurgical de care au beneficiat a fost de endoprotezare totală de şold necimentată de tip metal-metal (M-on-M), cu rezultate funcţionale excelente.

Rezultate: Cazurile de NACF post-terapie imunosupresivă pentru afecţiuni oncologice au fost rezolvate chirurgical prin implantarea de proteze totale necimentate de şold de resurfaţare tip BHR (2 cazuri) şi cu stem femural scurt (un caz) toate având acelaşi cuplu metalmetal.
Endoprotezele folosite sunt produse de aceeaşi firmă, de top în domeniul implanturilor articulare, cu anvergură internaţională iar raportările din cadrul Registrelor Naţionale de Endoprotezare relevă fără echivoc cele mai bune şi durabile rezultate la 10 ani.

Concluzii: Adresabilitatea pacienţilor oncologici tineri cu efecte secundare ale oncoterapiei implicând sistemul osteoarticular este încă minimală din pricina îndrumării reduse de către oncolog şi medicul de familie a patologiei osteoarticulare postimunosupresive către chirurgul ortoped, din pricina complianţei scăzute a pacientului tânăr (până 25 şi 30 de ani) la implantarea unei endoproteze într-o articulaţie mare şi importantă ca cea coxofemurală şi din perspectiva unei bariere economico-financiare, atâta timp cât implantul este scump şi de cele mai multe ori, costul este suportat de către pacienţi.

Cuvinte cheie: *artroplastie, tineri, necroză aseptică de cap femural, postimunosupresie, endoproteza de sold, metal-metal.*

Specialități medicale
Miercuri, 23 oct, 10^{30} – 12^{00}

DISPNEE, FEBRA ŞI TUSE SEACĂ: CE POT ASCUNDE?

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Prezentăm cazul unei paciente în vârstă de 64 ani, care se interează în clinica noastră pentru febră (38ºC), tuse seacă, dispnee la eforturi medii, stare generală alterată, dureri musculare şi impotenţă funcţională la nivelul centurilor scapulare şi pelvine, cu debut de 4 săptămâni şi agravare progresivă. Antecedentele personale patologice şi heredocolaterale sunt nesemnificative. Menţionăm că bilanţul radiologic efectuat în ambulator relevă opacitate pulmonară bazală stângă interpretată ca pneumonie comunitară şi tratată cu antibibiotice, fără ameliorarea simptomatologiei. La examenul clinic obiectiv de la internare remarcăm pacienta cu stare generală alterată, febrilă (38ºC), tegumente discret palide, uscate, infiltrate mâini şi gambe, descuamare discretă la nivelul mâinilor, raluri crepitante bazal bilateral, SaO2=90%, hipotrofie musculară la nivelul braţelor şi coapselor (cu limitarea ridicării braţelor (<90gr), imposibilitatea pieptănatului, precum şi dificultate la mers şi imposibilitatea urcării treptelor). Tomografia computerizată toraco-abdominală relevă multiple arii de condensare pulmonară, adenopatii mediastinale şi uşoară hepatomegalie. Se ia în discuţie şi posibila etiologie virală, însă markerii virali ne infirmă această ipoteză. Spirometria indică disfuncţie ventilatorie restrictivă uşoară, iar bronhoscopia, aspect bronşitic difuz bilateral. Lavajul bronhoalveolar decelează alveolita limfocitară. Ecocardiografia arată insuficienţă aortică uşoară, fără imagini sugestive pentru vegetaţii, iar ecografia abdominală relevă steatoză hepatică. Profilul biologic al pacientei indică sindrom inflamator moderat, sindrom miopatic important (creşterea importantă a CK, LDH, TGO), uşoară anemie hipocromă normocitară, uşoară leucocitoză, Ac anti Jo-1+, cu markeri tumorali (CA 19-9, CA 15-3, CA 125) negativi, markeri virali (Ag Hbs, Ac anti VHA, Ac anti HIV) negativi, în schimb Ac anti VHC reactivi (dar cu viremie nedetectabilă); hemoculturile repetate sunt negative. Elecromiograma indică lezinune miopatică acută, biopsia musculară confirmând histopatologic miozită. Diagnosticul pozitiv a fost de dermatopolimiozită cu sindrom antisintetază, cu evoluție favorabilă la introducerea corticoterapiei asociată cu azatioprina.

MICROLITIAZA PULMONARĂ – PREZENTARE DE CAZ CLINIC

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Abstract
Scopul lucrării: Raportăm cazul unui bărbat diagnosticat cu microlitiază pulmonară în urma unui examen tomografic efectuat pentru investigarea unor opacități micronodulare pulmonare bilaterale obiectivate la un examen radiologic de rutină.
Metode: Pacient de 60 ani cunoscut cu dublă protezare valvulară mitrală și aortică și by-pass aorto-coronarian, se internează în secția noastră pentru completarea investigațiilor având un examen radiologic toracic care evidențiază opacități micronodulare pulmonare bilaterale.
Rezultate: Examenul tomografic toracic identifică prezența a numeroși micronoduli de intensitate calcară, aspect compatibil cu diagnosticul de microlitiază pulmonară. Deoarece pacientul refuză biopsia pulmonară, aspectul tomografic suscită la discuții de diagnostic diferențial.

Concluzii: Microlitiaza pulmonară este o boală rară de cauză necunoscută. În general pacienții se prezintă pentru dispnee de efort și tuse seacă, examenul tomografic pulmonar are un aspect tipic, dar diagnosticul este stabilit prin biopsie pulmonară. Diagnosticul este, ca în cazul pacientului nostru, unul de excludere în lipsa biopsiei pulmonare. Prognosticul este de cele mai multe ori rezervat, cu evoluție spre insuficiență respiratorie cronică.

Cuvinte cheie: *microlitiază pulmonară, opacități micronodulare pulmonare, biopsie pulmonară.*

ASCITA ȘI INSUFICIENȚA RENALĂ ACUTĂ UN ALT NUMITOR COMUN?

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Prezentăm cazul pacientei G.M în vârstă de 73 ani care se internează pentru investigarea unui sindrom ascitic decelat în urmă cu 7 zile. Pacienta s-a prezentat într-o secție de nefrologie teritorială unde s-au efectuat două ședințe de hemodializă pentru un episod de insuficiență renală acută fără a exista o cauză evidentă.
La internare, la examenul obiectiv se remarcă tegumente palide și aspre, mucoase palide, edem pretibial bilateral, abdomen mărit de volum prin prezența lichidului de ascită, în rest fără alte particularități.
Profilul biologic la internare evidențiază: anemie normocromă normocitară, sindrom biologic inflamator, retenție azotată moderată și hipergamaglobulinemie policlonală importantă de 41%.
Bilanțul paraclinic realizat ulterior, precum și absența altor revărsate lichidiene exclud cauzele cele mai frecvente ale ascitei (ciroza hepatică de etiologie virală sau toxică, insuficiența cardiacă, patologia neoplazică și sindromul nefrotic).
Luând în considerare semnele clinice și aspectul ecografic al parenchimului tiroidian sugestiv pentru tiroidita cronică se dozează hormonii tiroidieni, ATPO și TSH, evidențiindu-se valori semnificativ crescute ale ultimilor doi parametri.
Coroborând datele clinice, paraclinice și imagistice am stabilit ca numitor comun al sindromului ascitic și al insuficienței renale acute tiroidită cronică autoimună cu hipotiroidism primar sever. Însă, predispoziția genetică a pacientei pentru bolile autoimune și modificările biologice care nu sunt justificate doar de patologia tiroidiană, ne-au determinat să efectuăm și alți markeri specifici unor posibile afecțiuni autoimune asociate.
Astfel, diagnosticul final a cuprins trei patologii cu vizǎ autoimună: tiroidita cronică autoimună cu hipotiroidie, sindrom Sjogren și ciroză biliară primitivă.
Evoluția favorabilă sub tratamentul de substituție tiroidiană, cu remisiunea sindromului ascitic și a retenției azotate a reprezentat un important argument privind etiologia comună a acestor două afecțiuni.

UN CAZ PARTICULAR DE TUMORĂ SUPRARENALIANĂ

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Introducere: patologia suprarenaliană tumorală reprezintă o adevărată provocare diagnostică şi terapeutică.

Materiale şi metoda: se prezintă cazul clinic al unui pacient de 60 ani, internat pentru dureri în flancul drept, transpiraţii, sindrom dispeptic. Examinările imagistice (ecografie abdominală, CT abdomino-pelvin) decelează formaţiune tumorală suprarenaliană inomogenă dreaptă de 5 cm cu adenopatii regionale. Bilanţul bioumoral a fost în limite normale. Se intervine chirurgical practicându-se adrenalectomie dreaptă prin abord lombar.

Rezultate şi concluzii: în acest caz aspectul pseudotumoral evidenţiat prin explorările imagistice şi imaginea intraoperatorie confirmă pleiomorfismul tuberculozei suprarenaliene confirmate la examenul histopatologic.

ROLUL VENTILAŢIEI NONINVAZIVE ÎN TRATAMENTUL EXACERBĂRII BPOC

Ioana Ştefănescu, M. Tetu, Jascu I., A. Ştefănescu, Claudia Popovici, Ioniţă C.

Introducere: Exacerbarea BPOC-ului reprezintă incapacitatea sistemului respirator de a realiza o ventilaţie alveolară adecvată cu – hipoxemie, hiepercapnie, acidoza respiratorie determinând importante complicaţii cardio-vasculare şi neurologice cu risc vital pentru pacienţi.

Material şi metodă: În perioada 2012-2013 în secţia noastră am folosit VNI cu BIPAP la 6 pacienţi cu BPOC dintre care 4 au fost după o exacerbare care a necesitat IOT şi VM. Am folosit BIPAP ST cu presiuni iniţial de 4/8 cm H2O şi au fost crescute ulterior în funcţie de evoluţia gazometriei arteriale şi echilibrului acido - bazic. S-a folosit ventilaţia iniţial în 4 prize a câte două ore pe timpul zilei şi 6 ore noaptea.

Rezultate: Recuperarea a fost foarte bună în toate cazurile, cu scăderea PaCO2 spre limita superioară a normalului, creşterea PaO2, pacienţii s-au putut mobiliza după câteva zile, iar la 7-10 zile au fost externaţi. Evoluţia a fost bună în toate cazurile, nu au mai avut exacerbări care să necesite internare şi majoritatea folosesc VNI doar noaptea, în prezent.

Concluzii: Calitatea vieţii acestora a fost net superioară comparativ cu cazurile anterioare introducerii acestei metode terapeutice.

Introducerea VNI în activitatea curentă a secţiei noastre a dus la recuperarea mai rapidă a pacienţilor gravi cu reducerea duratei de spitalizare şi implicit a costurilor.

TERAPII NOVATOARE ÎN OSTEOPOROZĂ – EXPERIENŢA CLINICII DE ENDOCRINOLOGIE

Adina Mazilu, Cristina Spiroiu, Ana-Maria Munteanu, N. Diaconu, A.E. Ranetti
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Introducere: Utilizarea unor medicamente novatoare în terapia osteoporozei permite obţinerea unei eficienţe sporite atât din punct de vedere al reducerii imediate a riscului de fractură, cât şi micşorarea perioadei de spitalizare şi ameliorarea durerii la pacienţii corect selecţionaţi.

Obiectiv: Evaluarea criteriilor care pot determina includerea per primam a pacienţilor în tratament cu Prolia în condiţii de spitalizare continuă şi a rezultatelor obţinute în cadrul Secţiei de Endocrinologie a S.U.U.M.C.“ Dr. Carol Davila“ la cei 18 pacienţi trataţi cu Prolia în perioada 1 august 2012 -1 august 2013.

Rezultate: Scorul T mediu în cadrul lotului de studiu a fost de - 3.4DS(- 2,8DS; -5.2DS), etiologia osteoporozei care a beneficiat de terapia cu Prolia fiind frecvent multifactorială - tratament cu glucocorticoizi, colagenoze, poliartrita reumatoidă, insuficienţa gonadică, hiperparatiroidism primar, acest fapt fiind legat de profilul secţiei şi de complexitatea cazurilor internate. Pacienţii asociază frecvent insuficienţa a nivelului de vitamina D sau deficit de vitamina D - în proporţie de peste 65%.

Concluzii: Utilizarea terapiei cu Prolia a îmbunătăţit semnificativ prognosticul vital şi funcţional la aceşti pacienţi.

EXPLORAREA SCINTIGRAFICĂ EFECTUATĂ CU 99mTc-MIBI ÎN EVALUAREA MIELOMULUI MULTIPLU

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Introducere. În ultimii ani, numeroşi autori au publicat studii referitoare la modelul de captare patologică a 99mTc-MIBI în situaţia pacienţilor cu mielom multiplu; majoritatea seriilor publicate indică o sensibilitate foarte crescută a scintigrafiei cu 99mTc-MIBI în detecţia implicării medulare în special în caz de boală activă. Majoritatea autorilor recomandă utilizarea acestui radiofarmaceutic pentru evaluarea extensiei implicării medulare, dar mai ales pentru aprecierea activităţii bolii, unii autori sugerând chiar o posibilă semnificaţie prognostică.

Scop: Definirea rolului 99mTc-MIBI în evaluarea pacienţilor cu mielom multiplu, în corelaţie cu alte explorări imagistice.

Material şi metodă*:* evaluarea retrospectivă a 29 pacienţi cu mielom multiplu confirmat, cu boală activă, investigaţi scintigrafic în cadrul departamentului de Medicină nucleară; s-a efectuat achiziţie „whole-body“ la 10-20 min post-injectarea intravenoasă a 740 MBq 99mTcMIBI. Rezultatele s-au comparat cu examenul radiologic, scintigrafia osoasă şi IRM vertebral.

Rezultate: 86.75% din pacienţi au prezentat captare de tip patologic a MIBI; 89.65% au prezentat modificări la examinarea prin rezonanţă magnetică. Scintigrafia osoasă a detectat modificări patologice la 62.06% din pacienţii examinaţi.

Au fost observate 3 tipuri de captare patologică a MIBI: captare crescută de tip focal (la 9 pacienţi), captare difuz crescută (7) şi captare de tip mixt (8). La 5 pacienţi s-a evidenţiat captare normală – probabil datorită supraexpresiei de glicoproteină P. Examenul radiologic şi scintigrafia osoasă au evidenţiat un număr mai mic de leziuni decât scintigrafia cu 99mTcMIBI. La aceşti pacienţi MRI a evidenţiat modificări vertebrale de tip patologic (leziuni vertebrale focale la diferite niveluri). La 2/29 pts nu au fost observate modificări la examenul IRM; unul dintre aceştia prezenta o hipercaptare de tip difuz a MIBI la nivel medular pe imaginile scintigrafice, iar cel de al doilea - leziuni focale la nivel cranian şi costal (nu vertebral).

Utilizarea combinată a scintigrafiei cu MIBI şi a IRM vertebral a determinat o sensibilitate de 96.66% în detecţia modificărilor patologice şi afectării medulare.

Concluzii. 99mTc MIBI reflectă extinderea afectării medulare şi activitatea bolii.

Are avantajul rapidităţii de execuţie, comodităţii şi a preţului relativ redus al examinării. Utilizată împreună cu IRM vertebral prezintă o sensibilitate mai bună decât fiecare dintre metode utilizate separat.

ECOGRAFIA INTERVENŢIONALĂ - EXPERIENŢA SCUM CRAIOVA

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ABSTRACT

Ultrasonografia intervenţională reprezintă o metodă eficientă de practicare sub ghidaj Echo-imagistic a unor puncţii sau drenaje, adesea în situaţii limită, cu risc mare chirurgical. Metoda, relativ puţin folosită în ţara noastră, presupune îmbinarea abilităţilor echografice cu manevre instrumentale efectuate cu mare precizie, în condiţii de anestezie locală sau generală. În SCUM Craiova, metoda a fost introdusă din anul 2006 şi practicată cu succes în varianta Echo-ghidată sau Echo-asistată, în scop diagnostic sau terapeutic, în diverse patologii: hepatopatii cronice difuze sau localizate, tumori chistice sau solide (hepatice, sân), colecţii abdominale, hidronefroză/pionefroză, adenopatii, afecţiuni articulare (gonartroza, coxartroza), pericardite maligne. Sunt prezentate date generale despre metodă, cu numeroase exemplificări din cazuistica proprie a colectivului nostru.

TERAPIA RADIONUCLIDICĂ ÎN METASTAZELE OSOASE DIN CANCERUL DE PROSTATĂ

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Se estimează că în România cancerul de prostată reprezintă a doua cea mai frecventă cauză de mortalitate prin cancer la bărbaţi, după cancerul pulmonar. Date furnizate de Centrul Naţional de Statistică Medicală a Ministerului Sănătăţii arată de asemenea că în ultimii 10 ani incidenţa cancerului de prostată a crescut progresiv împreună cu reducerea mediei de vârstă a cazurilor nou diagnosticate. Încă din stadii precoce ale bolii, cancerul de prostată se asociază cu reducerea calităţii vieţii, atât ca rezultat a bolii însăşi cât şi din cauza efectelor secundare ale tratamentului. În faze avansate ale bolii pacienţii pot supravieţui ani de zile cu dureri osoase severe şi deficite funcţionale mari datorate leziunilor secundare osoase.

Scop

Lucrarea de faţă îşi propune sintetizarea literaturii ştiinţifice referitoare la tratamentul radionuclidic paliativ al durerii osoase determinate de cancerul de prostată şi de asemenea de a prezenta rezultatul experienţei noastre în ceea ce priveşte terapia paliativă cu 89Sr (Metastron).

Material şi Metodă: În ultimii 6 ani am tratat cu 89Sr un număr de 33 pacienţi cu cancer de prostată confirmat, cu vârste cuprinse între 49 şi 74 ani. Toţi pacienţii au prezentat dureri osoase refractare la terapia convenţională, datorată prezenţei de leziuni secundare osoase multiple osteogenice. Toţi pacienţii prezentau un bun status renal şi hematologic. Tuturor pacienţilor li s-a administrat doza standard de 150 MBq [4 mCi] de 89Sr (Metastron – Amersham). La 30 de pacienţi s-a administrat o singură doză, în timp ce la 3 pacienţi s-a administrat o a doua doză la at 4, 7 şi, respectiv 9 luni după prima injecţie. S-a repetat evaluarea clinică şi biologică la 3 săptămâni şi la 3 – 6 luni după tratament. Statusul hematologic a fost reevaluat lunar la fiecare pacient.

Rezultate. Scăderea intensităţii fenomenelor dureroase a fost exprimată în termeni ca „pain score“ şi calitatea vieţii – îmbunătăţite semnificativ la 19 pacienţi (57%), cu scăderea marcată a necesarului de analgezice pentru mai mult de 6 luni. La 8 pacienţi am obţinut un efect stabil, combinând terapia cu Sr cu radioterapie externă. 7 pacienţi au prezentat un răspuns moderat, unul dintre aceştia necesitând administrarea unei a doua doze după 4 luni. La 4 pacienţi nu am obţinut nici un fel de răspuns. Fenomenul „pain flare“ a apărut la 25 de cazuri (75%), cu diverse intensităţi – procent mai mare decât raporează de obicei ceilalţi autori. Cel mai serios efect advers a fost mielosupresia în grade variate de afectare, care a apărut la 27 pacienţi (81%); cu toate acestea, această depresie a funcţiei medulare a fost în general de intensitate redusă - moderată, cu autolimitare în 3-12 săptămâni.

Concluzii. 89Sr este un instrument eficient în terapia paliativă a durerii osoase de cauză metastatică la pacienţii cu cancer de prostată. Această terapie determină îmbunătăţirea semnificativă a calităţii vieţii cu minime reacţii adverse şi o foarte bună complianţă din partea pacienţilor.

Managementul spitalicesc al urgenţelor abdominale
Miercuri, 23 oct, 12^{15} – 14^{00}

PERSPECTIVE ACTUALE ÎN EVALUAREA ŞI MONITORIZAREA PANCREATITEI ACUTE

Popescu Andrada, Nuţă P., Mariana Jinga, Costache Raluca, Macadon B., Bucurică Săndica, Pătrăşescu M., Balaban V., Cherana Gabriela, Ciobaca L., Copaci I., Oprea P., Săvulescu F., Ioniţă-Radu Florentina
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Abstract

Introducere: Pancreatita acută reprezintă o afecţiune inflamatorie acută a pancreasului, caracterizată prin leziuni ale celulelor acinare pancreatice, cu potenţial de evoluţie spre complicaţii severe (sepsis, şoc) şi insuficienţa multiplă de organe şi sisteme, având în peste 95% dintre cazuri etiologie biliară sau etanolică.

Metode şi pacienţi: Prin acest studiu retrospectiv ne propunem să prezentăm incidenţa, managementul şi evoluţia cazurilor de pancreatită acută internate în perioada 1 ian 2012 - 1 sept 2013 şi necesitatea elaborării şi aplicării unui protocol standard de diagnostic şi tratament.

Rezultate: Din totalul de 108 pacienţi internaţi cu diagnosticul de pancreatită acută (stabilit pe baza creşterii de peste 3x a amilazei, lipazei şi confirmare imagistică), în perioada mai sus menţionată, 23% au fost admişi pe Secţia Clinică Gastroenterologie, 11% Medicală 1,16% Medicală 2,20% Chirurgie 1 şi 22% Chirurgie 2. Dintre aceştia 10% au fost cazuri grave, ce au necesitat fie direct la prezentare, fie pe parcursul internării suportul secţiei ATI. Etiologia biliară a fost confirmată paraclinic (biologic şi imagistic) în 23% din cazuri. 81% au fost pancreatite edematoase cu evoluţie favorabilă, 9% forme necrotico-hemoragice şi în 11% din cazuri urmărirea imagistică a evidenţiat formarea de pseudochiste pancreatice. Din totalul de pacienţi s-au înregistrat 2 decese, iar un pacient a fost transferat la altă unitate medicală. 65% din cazuri au fost bărbaţi, media de vârstă a pacienţilor fiind 53 ani la sexul masculin şi respectiv 59 ani la feminin, cu predominaţa mediului urban (66%). Referitor la costurile îngrijirilor medicale, 65% din cazuri au necesitat mai puţin de 7 zile de spitalizare, în timp ce 35% din cazuri au fost monitorizate şi tratate mai mult de 7 zile (cu un maxim de 59 zile la un pacient tânăr cu pancreatită acută de etiologie toxic - metabolică, cu evoluţie lent nefavorabilă în ciuda tratamentului de specialitate, care ulterior a şi decedat).

Concluzii: Pancreatita acută reprezintă o problemă importantă de sănătate publică, cu incidenţa în creştere şi potenţial evolutiv nefavorabil în absenţa unui management diagnostic şi terapeutic prompt. Deşi s-au făcut progrese majore în înţelegerea patogeniei cât şi a tratamentului PA, reflectate printr-o ameliorare a supravieţuirii, atât morbiditatea, cât şi mortalitatea PA rămân destul de ridicate.

CONDUITA ÎN TRAUMATISMELE SPLENICE ALE ADULTULUI

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Conduita în traumatismele splinei reprezintă teren de controversă în condițiile în care există un important curent de promovare a tratamentului conservator în anumite situații. Lucrarea de față își propune să identifice factorii care stau la baza optării pentru o anumită metodă de tratament și evaluarea rezultatelor. Au fost luați în studiu 83 de pacienți internați și tratați pentru traumatism splenic, pe o perioada de 10 ani, între 2000 și 2011. 72 de pacienți (86,74%) au prezentat traumatisme splenice secundare contuziilor abdominale, iar 11 (13,25%), prin plăgi. Au fost laparatomizați în urgență 62 de pacienți (74,69%). 21 de pacienți (25,3%) au beneficiat inițial de tratament conservator. Pentru 7 dintre aceștia conduita conservatoare a fost suficientă, restul de 14 necesitând intervenție chirurgicală ulterioară. Laparatomia în urgență a fost impusă de instabilitatea hemodinamică și prezența semnelor de iritație peritoneală. Decesul a survenit la 5 cazuri (6,02%), în patru cazuri a fost vorba de pacienți din lotul celor operați și într-un caz din rândul celor tratați conservator.
Rata de succes a tratamentului conservator depinde de o bună selecție a pacienților și aceasta la rândul ei de posibilitatea de monitorizare strictă a pacientului inclusiv computer-tomografică.

Key words: splenic injury, abdominal trauma, abdominal contusion, penetrating abdominal injury.

TRATAMENTUL COMPLICAŢIILOR POSTOPERATORII CU AJUTORUL SISTEMULUI DE ASPIRAŢIE CONTINUĂ CU PRESIUNE NEGATIVĂ VIVANO – HARTMANN

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Introducere
Tratamentul plăgilor cronice sau supurate secundare unor intervenţii chirurgicale complicate sau datorate altor stări patologice (diabet, ulcer varicos etc.) solicită largi resurse materiale şi umane. În astfel de cazuri vindecarea necesită aport tisular de oxigen, asigurarea necesarului proteic şi de alţi factori nutritivi, un mediu local umed, o constelaţie inflamatorie propice, debridarea plăgii şi corecţia condiţiilor biologice. În acest context, drenajul local prin sigilarea plăgii în vacuum-Hartmann este o metodă eficientă şi puţin costisitoare.

Material şi metodă
Sigilarea plăgii în vacuum este un tratament adjuvant ce utilizează presiunea negativă pentru drenarea fluidelor dintr-o plagă deschisă, ocluzionată cu o membrană impermeabilă la germeni şi lichide. Fluidele sunt filtrate printr-un burete, schimbat periodic şi colectate de un sistem de tuburi ataşate la o pompă, prevazută cu un container colector vidat. Aceste condiţii determină stimularea granulării şi diminuă colonizarea bacteriană, iar presiunea negativă contribuie la apropierea pereţilor plăgii diminuându-i volumul în absenţa materialului de sutură. Pot fi folosite două tipuri de seturi, funcţie de necesitate:

- abdominal, în contact direct cu viscerele prin intermediul unei membrane permeabile
- normal, pentru supuraţii extraperitoneale.

Rezultate
Toate cazurile tratate cu această metodă au fost situaţii septice severe, unul cu şoc toxico-septic şi insuficienţă multiplă de organ. Aplicarea acestei tehnici a condus, împreună cu tratamentul general la stingerea procesului septic, fără complicaţii ulterioare. Curba de învăţare este una simplă. Dificultăţile tratamentului sunt date de necesitatea asigurării etanşeităţii sistemului şi de schimbarea buretelui filtrant la maxim 72 ore. De asemenea colaborarea cu pacientul este foarte importantă, mai ales în tratamentul ambulator.

Discuţii
Tratamentul plăgilor cronice sau supurate în atmosfera vidată constituie o tehnică adjuvantă cu rezultate foarte bune, cu avantaje considerabile, dar şi câteva contraindicaţii.

FACTORUL TIMP ÎN TRATAMENTUL INFECŢIILOR INTRAABDOMINALE - revizuire a literaturii -

C. Duţu[1], D. Orosan[1], O. Albiţa[1], C. Muşat[1], T. Rogin[1], R. Nica[1], A. Zaciu[2],C. Pleşa[2], Elena Stîrcu[1], A. Lica[1], D. Surdeanu[1], V. Vârjoghi[1], C. Cîrlan[1], F. Săvulescu[1]
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Cuvinte cheie: infecţii intraabdominale, terapie antimicrobiană

Infecţiile intraabdominale reprezintă un important segment al patologiei dintr-un spital de urgenţă, ridicând probleme complexe de atitudine chirurgicală şi de terapie intensivă, cu morbiditate şi mortalitate semnificative. Acestea sunt influenţate, printre alţi factori, de *momentul* iniţierii terapiei antibiotice sau al efectuării intervenţiei chirurgicale precum şi de *durata* tratamentului.

Cercetarea în literatură a acestor parametri temporali a constituit scopul prezentei lucrări. Sursele utilizate au fost articole indexate pe Pubmed şi ghidurile de diagnostic şi tratament ale infecţiilor intraabdominale ale ***Surgical Infection Society***, respectiv ***Infectious Diseases Society of America.***

Lucrarea trece în revistă modalităţile existente de evaluare iniţială clinică şi paraclinică a infecţiei intraabdominale. Se precizează importanţa *„timingului“* iniţierii terapiei (atât antimicrobiene cât şi chirurgicale) ca factor predictiv pentru eşecul controlului sursei intraabdominale de infecţie şi sunt prezentate recomandările existente în acest domeniu. Pe lângă trecerea în revistă a momentului intervenţiei chirurgicale autorii evidenţiază faptul că întârzierea iniţierii terapiei antimicrobiene cu mai mult de 1 oră la pacienţii cu şoc septic conduce la mortalitate crescută exponenţial. Durata tratamentului antimicrobian, după sursele din literatură, este între 4 şi 7 zile în cazul infecţiilor intraabdominale, cu excepţia cazurilor în care nu se obţine controlul eficient al sursei de infecţie. Datele culese sugerează că terapia antimicrobiană prelungită determină, pe lângă costuri nejustificate, suprainfecţii, colite cu *Clostridium difficile* şi leziuni viscerale.

Autorii subliniază în final importanţa factorului timp (ca moment de iniţiere şi durată a tratamentului) în evoluţia morbidităţii şi mortalităţii în cazul infecţiilor intraabdominale.

REZECŢII MULTIORGAN ÎN CHIRURGIA ABDOMINALĂ - ABORD, REZULTATE

Mitru C., Macău F., Dima A., Florea D., Niculescu R., Dumitrasu V., Artenie T., Nanu C., Petrescu R, Moşoia L., Tănase M., Ştefănescu V., Prună M., Drăgan C., Băjenaru N., Oprea P.

Rezumat: Indicaţiile principale ale rezecţiilor multiorgan sunt legate în principal de cadrul etiologic al neoplaziilor, principalul scop fiind acela de a obţine o rezecţie completă (R0) dar şi o rezecţie curativă (de multe ori nu sunt sinonime), cu un beneficiu net pentru prognosticul vital dar şi pentru cel funcţional al bolnavului. Variabilele în definirea chirurgiei rezecţionale multiorgan sunt complexe: tipul histologic, patternul invaziei, numărul organelor implicate, aspectul intraoperator al cavităţii peritoneale şi al retroperitoneului, timingul chirurgie – tratament neoadjuvant versus adjuvant (chimio şi/sau radioterapie).

Abordarea multidisciplinară (oncologi, chirurgi, radioterapeuţi) a cazurilor foarte atent selecţionate influenţează benefic rezultatele postoperatorii imediate şi la distanţă.

Calitatea vieţii pacientului este un deziderat de luat în calcul, „fantastectomiile“ fiind utile doar pentru ego-ul chirurgului.

Lucrarea de faţă îşi propune analizarea cazurilor de rezecţii multiviscerale operate în cadrul secţiei Chirurgie I punând accent pe aspectele intraoperatorii, morbiditatea/mortalitatea şi complicaţiile precoce şi imediate postoperatorii.

EXPERIENŢA SPITALULUI MILITAR CLUJ-NAPOCA ÎN DERIVAŢIA URINARĂ BRICKER POST-CISTECTOMIE

Dincă F, Rotariu P, Sârb D, Cioban I
Spitalul Militar Cluj-Napoca

Introducere: cistectomia radicală reprezintă tratamentul de elecţie pentru tumorile vezicale infiltrative. Derivaţia Bricker este una dintre cele mai folosite derivaţii urinare cu rezultate funcţionale bune.

Material şi metodă: în perioada ianuarie 2010 - august 2013 s-a practicat derivaţia Bricker post-cistectomie în cazul a 25 pacienţi stadializaţi preoperator prin TUR-V bioptic şi examinări imagistice (CT abdomino-pelvin).

Rezultate şi concluzii: deşi această tehnică chirurgicală este grevată de riscul apariţiei complicaţiilor imediate şi tardive, într-un procent destul de redus însă, este funcţională pe termen lung, necesitând cunoştinte chirurgicale complexe şi selecţia atentă a pacienţilor.

PROLAPSUL UTERIN TOTAL - CAZURI PARTICULARE

Ştefan M., Apostu Daniela, Parepa Andreea
Spitalul Universitar de Urgenţă Militar Central „Carol Davila“, Bucureşti

Lucrarea prezintă două cazuri de prolaps genital total - exteriorizarea completă a uterului care depăşeşte introitul şi tumori ale colului uterin, particularitatea cazurilor constând în patologia

importantă asociată (afectare neuropsihiatrică şi neurofibromatoză; vârsta înaintată a pacientei cu hipertensiune, diabet zaharat, insuficienţă cardiacă congestivă, boală cardiacă ischemică) şi dimensiuni mari ale tumorilor de col ulcerovegetante – cu repercursiuni în cadrul abdordului chirurgical (modificări ale planurilor anatomice, lipsa spaţiului de clivaj).

Intervenţia chirurgicală este de elecţie în prolapsul genital total. În aceste cazuri fiind dificilă intervenţia chirurgicală, nu s-a conservat funcţionalitatea vaginului practicându-se alături de histerectomia totală, colpectomie largă şi cură chirurgicală a incontinenţei urinare.

Hemoragia digestivă – abordare multidisciplinară
Miercuri, 23 oct, 15^{00} – 16^{30}

HEMORAGIA DIGESTIVĂ SUPERIOARĂ – ÎNCĂ O PROVOCARE?

Raluca Costache, P. Nuţă, B Macadon, Săndica Bucurică, M. Pătrăşescu, Cristina Grosu, Andrada Popescu, V. Balaban, Mariana Jinga, Florentina Ioniţă Radu
Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila“ - Secţia Gastroenterologie

Hemoragia digestivă superioară reprezintă una din cele mai importante şi frecvente urgenţe gastroenterologice, fiind în continuare o problemă majoră de sănătate publică. Deşi este o temă de dezbatere frecvent dezbătută, rămân în continuare o serie de întrebări legate de abordarea optimă a acestor pacienţi, aceasta necesitând de cele mai multe ori o echipă pluridisciplinară, şi gestionarea optimă a tehnicilor noi de hemostază endoscopică.
În condiţiile în care componenta farmacologică a făcut progrese constante în ceea ce priveşte tratamentul medicamentos al patologiei gastroenterologice este esenţială utilizarea optimă a resurselor şi tehnicilor noi de hemostază endoscopică în vederea reducerii marcate a rolului tratamentului chirurgical.
Studiul nostru a urmărit retrospectiv abordarea terapeutică endoscopică, considerată conservatoare, în Laboratorul de Endoscopie Digestivă din cadrul Secţiei Clinice Gastroenterologie SUUMC.
Au fost evaluaţi 137 pacienţi cu hemoragie digestivă superioară în perioada ianuarie 2012 – iulie 2013 la care s-au practicat eficient diverse metode de hemostază endoscopică.

Concluzii:
Abordarea terapeutică multidisciplinară şi utilizarea de primă intenţie a tehnicilor de hemostază endoscopică reprezintă elementul cheie în vederea ameliorării prognosticului pacientului atât pe termen scurt cât şi la distanţă, precum şi o optimă gestionare a resurselor materiale.

PREDICTORI NON-INVAZIVI AI VARICELOR CU RISC LA PACIENŢII CU BOALĂ HEPATICĂ CRONICĂ

Balaban D.V.[1], Florea B.G.[2], Stanca Alina[2], Cazan Andreea Ruxandra[2], Popescu Andrada Loredana[1], Nuţă I.P.[1], Costache Raluca Simona[1], Ioniţă Radu Florentina[1], Jinga Mariana[1,2]
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Introducere
Varicele esofagiene (VE) reprezintă o complicaţie majoră a hipertensiunii portale la pacienţii hepatopaţi. Urmărirea acestora prin endoscopie digestivă superioară periodică poate fi destul de costisitoare şi uneori greu de acceptat de pacient. În acest context apare necesitatea identificării de modele noninvazive care să prezică prezenţa varicelor cu risc (varice mari şi varice cu semne de gravitate).

Material şi metodă
Am studiat retrospectiv 104 pacienţi cu boală cronică de ficat (hepatită sau ciroză) de etiologie variată (virală, etanolică, autoimună, medicamentoasă) internaţi în clinica noastră în 2012. Datele clinice, biologice, ecografice şi endoscopice au fost culese din foile de observaţie şi folosite pentru a calcula indicii APRI (AST to platelets ratio index), ASPRI (age-spleen-platelets ratio index) şi SPRI (spleen to platelets ratio index). Performanţa diagnostică a acestor scoruri a fost evaluată prin calcularea ariei de sub curbă (AUROC).

Rezultate
Din cei 104 pacienţi (62.5% de sex masculin, vârsta medie 62 ani), 83 (79.80%) erau cirotici. La gastroscopie, 25% erau fără VE, 55.77% aveau varice mici (Paquet I-II) şi 19.23% varice mari (Paquet III-IV). Vena portă, vena splenică şi diametrul splinei au fost semnificativ mai mari la pacienţii cu varice mari faţă de cei cu varice mici sau fără varice, în timp ce numărul de trombocite a fost semnificativ mai mic. Scorurile APRI, ASPRI şi SPRI au fost buni predictori pentru prezenţa varicelor mari la endoscopie (AUROC 0.652, 0.703 şi respectiv 0.702). Cele 3 scoruri au avut o performanţă şi mai bună atunci când s-a considerat predicţia varicelor mari sau varicelor cu semne de gravitate (AUROC 0.758, 0.806 şi 0.803). Scorurile au avut o acurateţe bună şi în predicţia varicelor gastrice (AUROC 0.676, 0.711 şi 0.719).

Concluzii
Aceste scoruri simple, non-invazive, pot prezice cu acurateţe varicele de grad mare la pacienţii hepatopaţi, selectând acei pacienţi care trebuie să beneficieze de endoscopie digestivă superioară şi tratament profilactic.

VARIA
Miercuri, 23 oct, 1630 – 1800

ACTIVITATEA CHIRURGICALĂ DESFĂŞURATĂ DE CĂTRE SPITALELE DE CAMPANIE ALE ARMATEI ROMÂNE ÎN TIMPUL CELUI DE AL DOILEA RĂZBOI MONDIAL

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Scopul asistenţei chirurgicale oferite de Serviciul Sanitar (S.S.) al Armatei Române în timpul celui de Al Doilea Război Mondial era păstrarea efectivelor, evitarea invalidităţii şi reducerea mortalităţii [1]. S.S. cuprindea posturi de prim ajutor batalionare sau regimentare, ambulanţe divizionare, spitale de campanie şi spitale de zonă interioară [2].
Spitalul de Campanie (S.C.) era organizat la 20-40 km de linia frontului. Aici se efectua tratamentul chirurgical al masei răniţilor, aveau loc intervenţiile ce nu se puteau face la Ambulanţa Diviziei şi spitalizări de 7-15 zile [3].

Organizare. Deplasarea S.C. se realiză cu ajutorul căruţelor, iar instalarea se făcea în corturi sau şcoli, cazărmi, instituţii publice [3]. Transportul răniţilor spre S.C. se realiza cu autosanitare, trenuri sau avioane sanitare [3]. La S.C., răniţii erau deparazitaţi, apoi internaţi în secţia chirurgicală. În saloane, iluminarea se realiza cu felinare şi lumânări [3].
Secţia chirurgicală era dotată cu materiale consumabile, truse chirurgicale, aparate Röentgen şi pentru sterilizare [4]. Sala de operaţii era adesea amplasată în localuri improprii: *„s-a deschis peritoneul în încăperi unde în timp de pace n-am fi îndrăznit să deschidem o casoletă conţinând material sterilizat"* [5]. Aici iluminarea se realiza prin lămpi cu petrol sau becuri electrice [4].

Cazuistică. Plăgile craniene reprezentau aproximativ 2,5% din cazuri, ale feţei 4%, ale coloanei vertebrale 2,2%, ale toracelui 6,2%, cele abdominale 2,1%, bazin-perineu-organe genitale 8,3%, membre superioare 37%, membre inferioare 33%, plăgi articulare 4,7% [6].

Tratamente aplicate. Majoritatea intervenţiilor urmăreau extragerea schijelor sau tratarea plăgilor infectate. Dorind să îşi prelungească durata de spitalizare, unii răniţi recurgeau la explorări digitale repetitive ale plăgii pe sub pansament sau la aplicarea salivei pe plagă [7].
Atunci când competenţa chirurgilor permitea, în S.C. erau rezolvate cazuri grave, în ciuda condiţiilor de lucru. Lt. (r) Dr. Gârbea publica în 1944 o serie de cazuri din patologia ORL rezolvate la S.C. 7: plagă cranio-cerebrală în regiunea mastoidiană stângă cu hernierea substanţei cerebrale şi ruptura pavilionului ipsilateral, plagă cranio-cerebrală în regiunea frontală cu *„sdrobirea"* sinusurilor frontale şi a arcadei sprâncenoase drepte, plăgi penetrante ale obrazului stâng interesând oblic sinusul maxilar stâng, partea posterioară a septului nazal, cu retenţie de schije în fosa pterigo-maxilară dreaptă [8].
Raportul între amputaţiile efectuate la Ambulanţa Diviziei respectiv S.C. era de cca 5:1 [9]. Activitatea S.C. era lăudată de Spitalele de Zonă Interioară: *„Plăgile sosite la Bucureşti erau într-o stare excelentă. Aproape toţi răniţii sunt afebrili. Nu s-a semnalat nici un caz de tetanos.*" [10]. Îngrijirea chirurgicală a plăgilor a făcut ca numărul de cazuri de gangrenă gazoasă şi de tetanos să fie redus.
Acest studiu aduce un omagiu memoriei înaintaşilor noştri, medici chirurgi ofiţeri ai Armatei Române.

SISTEMUL CELOR III ORÂNDUIRI ESTE UN PROIECT DE REORGANIZARE A SISTEMULUI DE SĂNĂTATE DIN ROMÂNIA, DE TIP „PRIN TRECUT SPRE VIITOR", FEZABIL DOAR CU O ATITUDINE DE TIP „PUNCT ŞI DE LA CAPĂT"

A. Ciobotaru

Prin acest proiect propun un nou concept de sistem de sănătate românesc, orientat mai mult spre modelul britanic decât spre cel german, construit în jurul unui element central reprezentat de evoluţia şi experienţa formelor de organizare sanitară româneşti de-a lungul celor trei orânduiri pe care le-a avut România începând cu anul 1866, anul încoronării regelui Carol I ca rege al Regatului României şi până în prezent.

Scop:
Asigurarea eficientă a stării de sănătate şi a accesului populaţiei la îngrijiri de sănătate de către statul român, element esenţial în cadrul strategiilor de dezvoltare a ţării, în special a celor privind dezvoltarea mediului rural, bunăstarea socială şi siguranţa naţională.

Propunere:
Reconfigurarea actualului sistem public de sănătate din România, în vederea organizării şi funcţionării unui sistem public naţional de sănătate, gestionat unitar şi centralizat de autorităţile guvernamentale, permisiv pentru completare şi suplimentare cu servicii medicale de către un sector medical privat.
Reînfiinţarea dispensarului de stat ca unitate de bază în asistenţa medicală primară.
Revenirea la specialitatea medicină generală şi definirea medicinii de familie ca practica privată în asistenţa primară.
Reorganizarea sistemului public de urgenţă prespitalicească, astfel încât să ajungem de la cocktailul financiar şi profesional actual la normalitatea profesională şi instituţională necesară.
Schimbarea radicală a sistemului de finanţare prin renunţarea la case de asigurări sociale de sănătate şi revenirea la o formă de

organizare asemănătoare celei din anul 1930, Regia autonomă Fondul de Sănătate, scoasă de sub dominaţia Ministerului de Finanţe, înfinţată de data aceasta în parteneriat cu CEC Bank, instituţie care va pune la dispoziţie infrastructura bancară.

SINDROAME CLINICE PRODUSE DE AGENŢII BIOLOGICI OFENSIVI

B. Cîrciumaru
Secţia de Boli Infecţioase a Spitalului Universitar de Urgenţă Militar Central „Dr. Carol Davila“, Institutul Naţional de Boli Infecţioase „Prof. Dr. Matei Balş“, Bucureşti

Introducere: Agenţii microbiologici utilizaţi în scopuri ofensive (în componenţa „armelor biologice“), pot prezenta evoluţii clinice diferite de cele naturale. Deşi folosiţi în cursul istoriei, interzişi apoi prin convenţiile internaţionale, ei au devenit de actualitate ca ameninţări ne-convenţionale induse, mai ales, de proliferarea globală a terorismului.

Obiective: Abordarea îmbolnăvirilor produse de agenţii microbiologici ofensivi are o mai mare eficienţă clinico-terapeutică sub forma grupării lor în sindroame clinice. Scopul principal este acela de a eficientiza: diagnosticul, profilaxia şi tratamentul unor îmbolnăviri ce ar putea fi produse de folosirea deliberată a unor agenţi biologici cu virulenţă clinică importantă ca şi de a propune ghiduri de conduită în aceste cazuri.

Metodologie: Am folosit experienţa acumulată în cadrul unor programe europene de cercetare, cum ar fi ETIDE (European Training in Infectious Diseases Emergencies), ca şi practica clinică personală - destul de redusă în aceste cazuri, precum şi resursele din literatura de specialitate, pentru a realiza un tablou cât mai sintetic asupra acestor îmbolnăviri induse în mod deliberat.

Rezultate: Am descris mai multe sindroame clinice produse de agenţii biologici ofensivi: cutanat, respirator/pseudo-gripal, digestiv, neurologic, sepsis şi am identificat cele mai eficiente mijloace de diagnostic şi terapie. Am propus şi modalităţile de triaj în ipoteza apariţiei rapide a unui număr mare de cazuri şi am identificat facilităţile sanitare existente/potenţiale, ce pot prelua cazurile nou-apărute.

Concluzii: Abordarea bolilor infecţioase produse de agenţii biologici ofensivi sub forma unor sindroame clinice, prezintă avantaje importante printre care un răspuns prompt şi eficient la ameninţarile produse de armele biologice ca şi o modalitate practică operativă de apărare. Pregătirea răspunsului împotriva unor astfel de ameninţări reprezintă o abordare strategică corectă în contextul actual al proliferării armelor biologice.

IMPLEMENTAREA TELETRADIOTERAPIEI MODERNE ÎN SECŢIA DE RADIOTERAPIE A SPITALULUI UNIVERSITAR DE URGENŢĂ MILITAR CENTRAL „DR. CAROL DAVILA“ TRANZIŢIA DE LA TEHNICA 2D LA TEHNICA 3D CONFORMAŢIONAL

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***Cuvinte cheie**: 3D conformațional, CT simulator, volum țintă, organe de risc, echipă interdisciplinară, asigurarea calității.*

În prezent, este recunoscut faptul că radioterapia eficientă și efectuarea acesteia în condiții de siguranță necesită nu numai investiții de capital substanțiale în dotarea cu echipamente și amenajarea incintelor, dar necesită și investiții importante la nivelul resurselor umane.

Progresele tehnologice au dat posibilitatea tranziției de la modalitatea de planificare 2D a tratamentului radioterapeutic la o abordare mai sofisticată, respectiv radioterapia 3D conformațională. În timp ce radioterapia 2D poate fi efectuată cu un echipament simplu, o infrastructură și un nivel de instruire modest, transferul către tratamente radioterapeutice 3D conformaționale necesită resurse complexe în ceea ce privește tehnologia, echipamentele, personalul medical și instruirea acestora.

Termenul de 3D conformațional este folosit pentru a descrie proiectarea și elaborarea planului de tratament radioterapeutic având la bază achiziția de date din imaginile 3D obținute în urma tomografiei computerizate efectuate la CT simulator dedicat pentru radioterapie, în scopul de a trata volumul tumoral țintă și de a evita pe cât posibil organele de risc din vecinătatea acestuia.

Pentru a putea oferi posibilitatea unui tratament radioterapeutic modern, Secția de Radioterapie a SUUMC a fost dotată cu un accelerator medical performant model UNIQUE Power (Varian, SUA), un CT SIM model SOMATOM Spirit (Siemens, Germania), o instalație de brahiterapie HDR model GAMMAMED PLUS iX (Varian, SUA) și un echipament radiologic mobil model SIREMOBIL Compact L (Siemens, Germania).

SIDEREMIA INDICATOR FIDEL AL CANCERELOR

Soare S.
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În anul 2007 cu ocazia diagnosticării unui bolnav cu cancer bronhopulmonar cu metastaze pulmonare și arc costal 2 posterior am surprins valoarea unei sideremii de (44 μg). Valorile de referință fiind -(50-170) μg. Celelalte valori ale analizelor prezentându-se astfel:

- Hemoglobina HGB 11,5 (12-17)
- Hematocritul HCT 35,5 (36-52)
- Globulele roșii RBC 3,6 (3,7-5,3)
- VSH 120 (2-20)

Bolnavul a fost urmărit pe un interval de un an cu repetarea analizelor lunar.

S-a observant că valoarea sideremiei, inițial a scăzut prin administrarea preparatelor orale de Fe, sub valoarea inițială, apoi crescând lent până la valoarea aproape de 100 μg (80-90) în decursul a trei luni.

Un alt caz care în 2011 se internează cu zona Zooster nerv oftalmic, hipertensiune arterială esenţială stadiul doi şi siderimia 60 µg (50-170). Se prezintă după doi ani de zile cu HTA stadiul doi, cu simtomatologie digestivă, greţuri, vărsături epigastralgii de câteva zile. Siderimia 47 µg (50-170 µg) şi valori normale la creatinina, acidul uric, fosfataza alcalină, colesterol, trigliceride, GGT HLG. Bolnavul prezenta la examenul RMN craniu - metastaze lob frontal drept multiple şi hipertensiune intracraniană.

În intervalul 2007 - 2012 am introdus determinarea valorică a sideremiei ca investigaţie de rutină. Am efectuat un studiu pe cinci ani cuprinzând 100 de bolnavi. S-au urmărit valorile următoarelor constante biologice:

- Fierul seric
- Hemoleucograma
- Colesterolemia
- Trigliceridemia
- Gamaglutemil transpeptidaza
- ALT
- AST
- Calcemia
- Magnezemie
- Acid uric
- Urea
- VSH
- Amilazemia
- Fosfataza alcalină
- Glicemia
- Creatinemia

Am observant că procentul de suspiciune a cancerului creşte progresiv cu nivelul sideremiei către 50 µg nereprezentând un factor de gravitate al cancerului.

Astfel la cancerele cutanate studiate, sideremia a fost cuprinsă între 52 – 57 µg%.

- La 4 cazuri cu cancer bronhopulmonar cu metastaze sideremia a fost între 55-68 µg%

Au fost analizate mai multe tipuri de cancer după cum urmează:

- cancer renal = 5
- ADK uterin = 10
- cancer stomac = 8
- melanom cu diverse localizări = 4
- adenociroza = 4
- cancer mamar
    - 2 operate +metastaze osoase;
    - 2 neoperate + metastaze;
    - 1 operat fără metastaze;
- meningiom = 4
- cancer vezică biliară = 4
- cancer bronhopulmonar = 6
- cancer prostată = 5
- cancer vezică urinară = 4
- cancer colon = 7
- cancer bază limbă = 2
- cancer glandă submandibulară = 2

Bolnavii au fost internaţi cu diverse patologii: cardiovasculare, pulmonare, digestive etc.

Au fost cazuri cu cancer diagnosticate anterior internării sau au fost suspectate şi identificate pe parcursul unei internări ulterioare.

Valorile sideremiei au fost evaluate şi la bolnavii cunoscuţi cu pierderi sanguine astfel: 2 donatori, 8 cazuri cu menometroragii disfuncţionale, 2 cazuri cu HDS: un bolnav cu ciroză iar altul cu ulcer duodenal.

Un bolnav a prezentat o creştere marcată ALT, AST, Bb totală.

La scăderea acestora către valori normale s-a observant o creştere a sideremiei peste 220 µg% iar colesterolul seric - 103 mg%, Mg 3,2 mg%.

La repetarea ulterioară, sideremia a prezentat valoarea de 60 µg%.

La RMN s-au identificat două formaţiuni înlocuitoare de spaţiu la nivelul ficatului. Am vrut astfel să identificăm şi o creştere pasageră şi înşelătoare a sideremiei.

Alături de medicaţia specifică a fiecărui bolnav cu patologie cardiovasculară pulmonară digestivă etc. am administrat preparate de fier şi antioxidanţi. Nivelul sideremiei la bolnavii cu cancer a scăzut iniţial apoi a crescut lent la cei cu pierderi sanguine, carente etc., a crescut rapid.

La întreruperea administrării per os a preparatelor de fier antioxidante, nivelul sideremiei a scăzut rapid la cei cu cancer, ceea ce a reprezentat un îndemn la cazurile suspicionate în a le identifica. Pentru a sublinia importanţa sideremiei aduc în discuţie un bolnav cu cancer de colon la care irigografia a fost etichetată normală, constantele biologice normale, iar la colonoscopie s-a descoperit o formaţiune tumorală de 1 cm situată la 15 cm de linia anorectală. Dacă la valorile sideremiei situate spre limita inferioară sau sub limita inferioară se asociază: hematii la limita inferioară, hemoglobina, hematocrit sub limita inferioară, monocitoză absolută, limfopemie relativă colesterolul seric către limita inferioară iar anamnestic inapetenţă, scădere ponderală, prezenţa crampelor musculare, calciu seric scăzut între 7,8 - 8,2 mg%, impune căutarea mai sistematizată a cancerului cu şanse sporite de depistare.

La apariţia leucocitozei cu granulocitoză marcată, trombocitoza supraadaugată la cele de mai sus, prognosticul devine sumbru pentru bolnav.

În funcţie de determinările de organe s-au mai relevant creşteri ale glicemiei, gama GT, amilazei, fosfatazei alcaline, VSH, uree, creatinină, acid uric.

Concluzii

- Nivelul sideremiei sub 100 µg este un **marker biologic esenţial** care impune continuarea investigaţiilor pentru localizarea cancerului.
- Nivelul sideremiei peste nivelul etichetat normal şi scăderea valorică la scurt timp (sub 100 µg) sugerează existenţa cancerului.
- Nivelul sideremiei situat peste 100 µg dar sunt prezente creşteri uşoare a ureei, bilirubinei şi a transaminazelor, ALT, AST şi alteori GGT poate sugera determinări hepatice.
- Indiferent de determinările de organ ale cancerului, sideremia s-a situat sub 100 µg către valoarea inferioară sau sub ea.
- Trebuie reevaluată valoarea etichetată ca normal a sideremiei.
- Ca investigaţie, sideremia ar trebui să devină o investigaţie de rutină ţinând seama de preţul de cost şi de înalta specificitate.

INTERVENŢII FARMACOTERAPEUTICE LA PACIENŢII CU TULBURARE DEPRESIVĂ MAJORĂ ŞI SIMPTOMATOLOGIE ALGICĂ

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Obiectiv: evaluarea comparativă a eficacităţii şi tolerabilităţii principalelor medicamente indicate în tulburarea depresivă ma-

joră asociată cu simptome algice.
Material şi metodă: Lotul de studiu a inclus pacienţi cu statut de pensionar militar sau personal civil contractual diagnosticaţi cu „Tulburare depresivă majoră“ care asociau o simptomatologie algică persistentă. Tratamentul a inclus antidepresivele de primă intenţie recomandate de ghidurile *IASP* de management al durerii: amitriptilină (50-150 mg/zi, N = 48), duloxetină (60-120 mg/zi, N = 57), venlafaxină (75-225 mg/zi, N = 51). Evoluţia pacienţilor a fost monitorizată cu ajutorul scalelor HAM-D 17 şi a scalei de evaluare numerică a intensităţii durerii, precum şi prin evaluarea ratei de renunţare la tratament din cauza efectelor adverse.

Rezultate: Raportate la baseline, scorurile HAM-D17 au fost îmbunătăţite la 1, 3 şi 6 luni cu 15%/33%/70% pentru pacienţii trataţi cu amitriptilină, 20%/42%/82% pentru duloxetină şi 9%/28%/64% pentru venlafaxină. Mediana scorului de evaluare numerică a intensităţii durerii la 1, 3 şi 6 luni s-a ameliorat cu 11%/29%/33% pentru amitriptilină, 16%/61%/72% pentru duloxetină, respectiv 6%/8%/1% pentru venlafaxină. Rata de renunţare la tratament din cauza efectelor adverse la 3 luni/6 luni a fost de 8%, respectiv 19% pentru lotul tratat cu amitriptilină, de 2%/5% pentru pacienţii trataţi cu duloxetină şi de 14%/20% pentru venlafaxină.

Concluzii: Rezultatele studiului confirmă oportunitatea utilizării celor 3 antidepresive în tulburarea depresivă majoră asociată cu simptome algice, cu un beneficiu suplimentar semnificativ statistic pentru duloxetină, atât în privinţa eficacităţii, cât şi a tolerabilităţii tratamentului.

PREVENIREA INCIDENTELOR ȘI ACCIDENTELOR ÎN RADIOTERAPIE - IMPLEMENTAREA SISTEMULUI DE MANAGEMENT AL CALITĂŢII ÎN CENTRUL DE RADIOTERAPIE DIN CADRUL SPITALULUI UNIVERSITAR DE URGENŢĂ MILITAR CENTRAL „DR. CAROL DAVILA“ BUCUREȘTI

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Cuvinte cheie: managementul riscului, asigurarea calităţii, accident, incident, eroare.

În prevenirea incidentelor şi mai ales accidentelor în radioterapie, este important să luam în considerare evenimentele anterioare de acest gen.

Accidentele pot fi prevenite prin adoptarea mai multor niveluri de control asupra siguranţei activitităţii clinice, respectiv asupra efectuării tratamentului de radioterapie şi totodată prin adoptarea acţiunilor preventive.

Pregătirea şi efectuarea tratamentului radioterapeutic reprezintă o sarcină complexă, cu multe riscuri inerente. Atunci când se analizează riscurile potenţiale în radioterapie, trebuie să admitem, în mod prioritar, beneficiul substanţial pe care îl poate avea acest tratament pentru pacient, dar să avem în vedere şi managementul riscurilor ce pot apărea.

O serie de accidente au fost investigate de către Agenţia Internaţională pentru Energie Atomică, iar cauzele şi acţiunile preventive au fost diseminate fără echivoc.

Ca şi acţiuni preventive pentru preîntâmpinarea incidentelor şi accidentelor în radioterapie, putem menţiona: acţiuni preventive pentru evitarea potenţialelor abateri de doză şi adoptarea unei geometrii de iradiere incorecte, abateri ce pot fi depistate înainte de prima şedintă de tratament; acţiuni preventive pentru evitarea erorilor ce pot fi găsite în timpul sau după şedinţa de iradiere, mai ales în ceea ce priveşte erorile de poziţionare; aplicarea verificărilor zilnice de asigurarea calităţii; aplicarea procedurilor de securitate radilogică; acţiuni privind instruirea periodică adecvată a personalului operator etc.

Pentru toate acestea se are în vedere implementarea unui sistem de management al calităţii specific practicii clinice în domeniul radioterapiei.

PARTICULARITĂŢI PSIHOFARMACOLOGICE ALE TERAPIEI ANTIDEPRESIVE LA VÂRSTNICI

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Abstract

Depresia la vârsnici afectează nu numai pacienţii dar şi famiile şi comunitatea. Prevalenţa comorbidităţilor somatice creşte o dată cu înaintarea în vârstă, în consecinţă unele tulburări ca şi bolile cardiovasculare, boala Parkinson sau durerea cronică sunt associate cu un risc crescut de apariţie a depresiei.

Conştientizarea factorilor predispozanţi şi precipitanţi şi necesitatea screeningului ne ajută în diagnosticarea precoce a afecţiunii conform ICD-10 şi DSM-IV TR. În continuare consulturile periodice şi managementul farmacologic sunt cruciale pentru a maximiza tratamentul şi a obţine remisiunea. Dificultăţile în diagnosticare cuprind deseori absenţa dispoziţiei depresive şi a tulburării cognitive evidente şi în special gradul ridicat de comorbidităţi somatice.

Alegerea antidepresivului ar trebui făcută în funcţie de proprietăţile farmacodinamice şi farmacocinetice, tratamentul anterior, tipul şi severitatea depresiei, riscul de supradoză.

Dacă remisiunea nu este obţinută tratamentul farmacologic adjuvant precum şi psihoterapia pot fi considerate. În cazuri severe ca şi depresia cu elemente psihotice sau refractară, terapia electroconvulsivantă rămâne standardul de aur.

***Cuvinte cheie**: prevalenţa, diagnostic conform ICD-10, DSM-IV TR, evaluare, particularităţi specifice vârstei, strategii farmacologice.*

SINDROMUL BURNOUT

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Lucrarea de față reprezintă o trecere în revistă a unei situații care nu este încadrată per se în patologie, nu este cuprinsă în ICD-10, dar produce patologie. Numit adesea sindrom, fenomenul **burnout** a fost descris încă din decada 70 a secolului trecut ca o condiție patologică ce apare cauzată de suprasolicitarea profesională în special în profesiile ce presupun studii medii și superioare, lucru cu oamenii, creativitate, birocrație și alți factori de risc. De aceea nu este surprinzător faptul că primii care au descris acest aspect au fost psihologii din țările occidentale dezvoltate – adică aceia care au constatat primii o condiție anormală cu potențial patogen foarte mare într-o societate în care și presiunea profesională este cea mai mare. Aspectul de **burnout** vine să completeze pe cel de **stress** care apare în aceleași condiții – între cele două există numeroase conexiuni, sunt ambele generatoare de patologie, dar există diferențe mari, notabile. Studiul cuprinde și elementele de sistematizare dezvoltate până în prezent, ca și un chestionar util ca metodă de depistare și de evaluare.

TULBURAREA DE STRES POSTTRAUMATIC ÎN CONTEXTUL ACTUAL AL ACTIVITĂȚII MEDICO-MILITARE

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Obiectiv: Importanța cercetării tulburării de stres posttraumatic (TSPT) pentru societatea românească ca urmare a creșterii numărului de militari și angajați civili care au participat în diverse teatre de operațiuni și manifestă simptome ale acestei tulburări psihice.

Material și metodă: 3,2% din militarii care s-au întors din teatrele de război au fost diagnosticați și au primit tratament pentru TPST; aproximativ 7% din cei care s-au reîntors și nu au fost diagnosticați și nu au solicitat consult psihiatric/psihologic de specialitate, având altă simptomatologie pentru care a fost solicitată evaluare psihologică, prezentau simptomele TSPT, motivând „rușine“ și „frică“ să recunoască starea lor în fața superiorilor. De asemenea am primit solicitări și din partea familiilor pentru consiliere („nu reușim să-l mai înțelegem“, „s-au întors alți oameni decât cei care au plecat“).

Concluzii: Cercetările indică faptul că TPST reprezintă o afecțiune psihiatrică importantă ce produce o suferință emoțională și o inadaptare socială considerabilă, prezentând adesea un tablou clinic complex ce constituie o semnificativă provocare în ceea ce privește conduita terapeutică.

***Cuvinte cheie**: tulburare de stres posttraumatic, centru de recuperare, militari și angajați civili participanți la misiuni.*

SESIUNEA POSTERE

STUDIUL UNOR PARAMETRI BIOCHIMICI ȘI AL APĂRĂRII ANTIOXIDANTE ÎN BOALA CIROTICĂ

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ASPECTE ENDOSCOPICE ÎN INFECȚIA CU HELICOBACTER PYLORI

Bucurică Săndica, Spulber Geanina, Costache Raluca, Macadon B., Nuță P.; Pătrășescu M.; Popescu Andra; Balaban V; Jinga Mariana, Ioniță Radu Florentina
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HIPERPLAZIA BENIGNĂ DE PROSTATĂ LA PACIENȚII CU OBEZITATE

F. Rusu[1], Emilia Rusu[2,3,] Gabriela Radulian[2,3], Mădan V.[1], Bratu O.[1], Dinu M.[1], Farcaș C.[1], Pacu O.[1], Spînu D.[1], Iatagan C.[1], Stănescu C.[1], Popescu R.[1], Rădulescu A.[1], Marincaș A.[1], Ilie C.[1], Mischianu D.[1,2]
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EFECTUL TRATAMENTULUI ADJUVANT OPTIM ASUPRA FUNCŢIEI VENTRICULULUI STÂNG ÎN STEMI

S I. Dumitrescu[1], G. Cristian[1], L. Chiriac[1], F. Pinte[1], D. Niţă[1], D. Săvoiu[1], A. Teodorescu[1], I. Hântulie[1], R. Boingiu[1], S. Almarichi[1] , VA Voicu[2]
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PROSTATECTOMIA RADICALĂ RETROPUBICĂ ÎN TRATAMENTUL CANCERULUI DE PROSTATĂ – O ANALIZĂ STATISTICĂ

Bratu O.; Dinu M.; Spînu D.; Rădulescu A.; Mădan V.; Popescu R.; Farcaş C.; Ilie CP.; Iatagan C.; Mischianu D.
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RĂSPUNSUL VIRUSOLOGIC SUSŢINUT ÎN HEPATITA CRONICĂ CU VIRUS C ÎN RELAŢIE CU RISCUL DE CARCINOM HEPATOCELULAR

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REPERE MORFOLOGICE ŞI HISTOCHIMICE ALE PERETELUI VENOS LA VENELE NORMALE ŞI VARICOASE

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[3] Catedra de Histologie a Universităţii de Medicina şi Farmacie „Victor Babeş“, Timişoara.

ABORDAREA ANESTEZICĂ PRIN FOLOSIREA TIVA-TCI ÎN CAZUL A TREI PACIENŢI CU SINDROM SIPPLE, PROPUŞI PENTRU SUPRARENALECTOMIE BILATERALĂ PENTRU FEOCROMOCITOM

N. V. Tănase, D. Latiş, L. Ene, Ioana Oprea, Mirabela Colac, Denisa Ghinescu, A. Olaru, N. Guţu, R. Petrescu, V. Dumitraşcu
Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila“ - Secţia Anestezie – Terapie Intensiva

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I. R. Negrea, S. Mardale, D. Olaru, A. Martiniuc, I. Raus
Spitalul Militar de Urgenţă „Dr. Constantin Papilian“ Cluj-Napoca

TERAPII COMPLEMENTARE ŞI ALTERNATIVE FOLOSITE DE PACIENŢII CU SCLEROZĂ MULTIPLĂ

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TERAPIA FIZICĂ – O TERAPIE COMPLEMENTARĂ ÎN SCLEROZA MULTIPLĂ

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INFECTIOUS ENDOCARDITIS BACKGROUND OF IMMUNOSUPPRESSION

Michaela Oana, Doina Baltaru, Yveta Iernuţan, Carmen Man, A. Dascălu, Simona Costin
EMERGENCY MILITARY HOSPITAL“C-TIN PAPILIAN“ CLUJ-NAPOCA

MANAGEMENTUL PERIOPERATOR ÎN CAZUL UNEI PACIENTE CU MALADIE VON WILLEBRAND TIP 2B FORMĂ SEVERĂ, PROPUSE PENTRU CURA CHIRURGICALĂ A UNEI HERNII DE DISC LOMBAR

N. V. Tănase, L. Ene, M. D. Latiş, M. Mitrică, Ioana Oprea, A. Baciu, A. Olaru, Mădălina Lixandru, Teodora Şerban, Simona Butoi
Secţia Anestezie – Terapie Intensivă - Spitalul Universitar de Urgenţă Militar Central „Dr. Carol Davila“

UNELE ASPECTE PRIVIND COMPORTAREA IN VITRO A SPECIEI HYPERICUM PERFORATUM L

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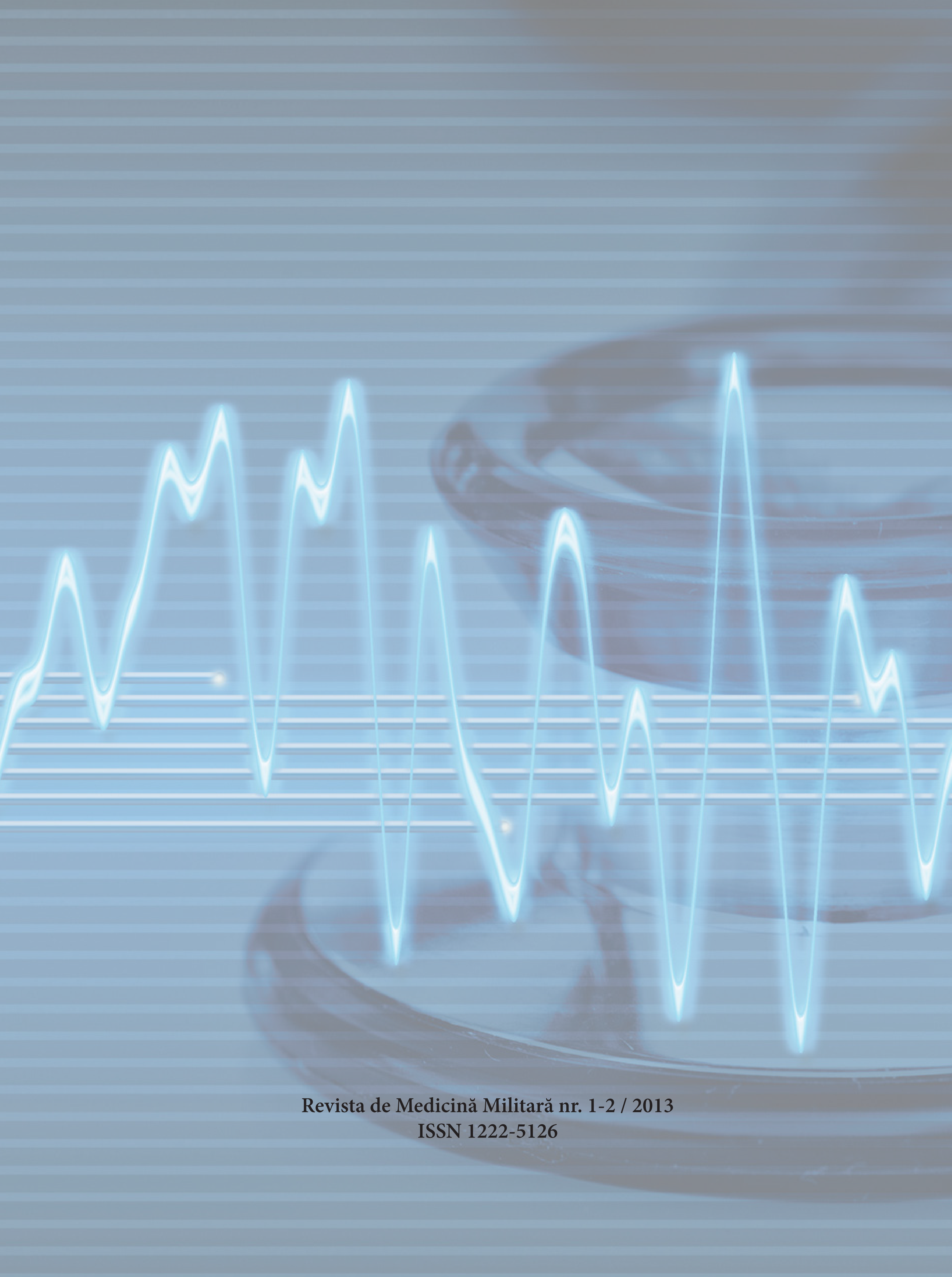

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CLINICAL PRACTICE

Salivary matrix metalloproteinases activity in periodontal disease

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Abstract*: Introduction: Matrix metalloproteinases (MMPs) are key enzymes involved in degradation of extracellular colagen matrix during periodontal disease progression. The aim of our work was to study the levels of MMP-1, MMP-2, MMP-7, MMP-9 and MMP-10 in saliva of patients with chronic periodontitis.*

Materials and methods: The study included 22 patients with chronic periodontal disease and 15 controls. We collect unstimulated whole saliva from all volunteers included in the study. Salivary levels of mentioned MMPs were analyzed using magnetic bead-based multiplex assays and Luminex technology.

Results: MMP-1, MMP-7, MMP-9 and MMP-10 present statistically increased salivary levels at patients with periodontal disease versus the control group ($p<0.05$). Salivary level of MMP-2 at patients with chronic periodontitis was increased but statistically insignificant compared with healthy subjects ($p=0.1$).

Conclusions. The increased level of these enzymes in patients diagnosed with periodontal disease reflects their implication in periodontal tissue degradation. Saliva is an excellent fluid useful in monitoring the severity of this oral disease that affects about half of the adult population around the world.

Keywords: *saliva, inflammation, proteases, periodontal tissue*

INTRODUCTION

Periodontal disease is one of the most common oral disease and affects the adult population around the world. Periodontal disease is a chronic inflammatory disorder that affects the supporting tissues of the tooth, leading to the progressive destruction of conjunctival tissue and alveolar bone.

The role of microorganisms in the etiology of periodontitis has been very well studied, from 600 types of bacteria that are able to colonize oral cavity about 10% plays an important role in the initiation of periodontitis. Porphyromonas gingivalis, Tanerella forsythensis and Troponema denticola, are directly associated with chronic periodontitis. Actinobacillus actinomycetemcomitans, was observed in early stages of periodontal disease and aggressive periodontitis. These

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microorganisms cause periodontal tissue damage either by their degradation products (toxins, enzymes, and lipopolysaccharide) or by activating of the host´s immune system, which leads to the activation of inflammatory cells, cytokine generation, proteolytic enzyme release, and osteoclast activation [1-4].

This inflammatory process is characterized by the increased levels of IL-1α, IL-1β, IL-6, and TNF-α and release of enzymes such as MMPs. MMPs-1, 2, 3, 8 and 9 are key proteases found in biopsy specimens of human inflammatory periodontal tissue [5].

The many advantages of the saliva, namely the rapid, inconvenient collection, anywhere and anytime, the lack of contamination has led many researchers to use it as a new diagnostic fluid for both systemic and oral disorders. Saliva is secreted by major and minor salivary glands, contains 99.5% water, 0.2% inorganic substances and 0.3% organic substances. Salivary proteins include proteins synthesized by salivary glands (mucoproteins and enzymes) and proteins of blood origin, with over 20 protein fractions. Plasma proteins may exceed 20% of salivary proteins and include: albumin, globulin (IgA, IgM, and IgG), α- and β-globulins. The total concentration of γ-globulins is less than 0.5% of the plasma concentration and is generally not affected by the salivary flow. Saliva also contains and non-protein organic compounds such as glucose, lipids, hormones, uric acid, vitamins [6-8].

The main aim of the present research is to detect salivary levels of MMP-1, 2, 7, 9 and 10 in unstimulated whole saliva of patients diagnosed with chronic marginal periodontitis using Luminex technology and Magnetic Bead-Based Multiplex assays.

MATERIAL AND METHODS

Patient selection: We included in our study 22 patients with chronic periodontitis and 15 healthy subjects who agreed to participate voluntary and signed and informed consent. The present research was approved by the Ethics Committee of Science Research from „Carol Davila University of Medicine and Pharmacy". Twenty-two subjects with chronic periodontal disease were recruited from a dental private office in Bucharest. We excluded from our study subjects under 18 years old or with systemic diseases, such as: diabetes mellitus, osteoporosis, chronic hepatitis, liver cirrhosis, HIV/AIDS, autoimmune diseases. All volunteers were diagnosed by the same experienced medical professional.

Clinical data: Demographic data, including age, sex, medical history, medication used and smoking habits (smoker/nonsmoker), were collected. The periodontal parameters performed were: for bleeding index (BI), probing depth (PD), plague index (PI). Chronic periodontitis was diagnosed according to the clinical case definitions proposed by the Center for Disease Control and Prevention.

Samples collection

For all participants in the study, saliva samples were collected after clinical examination. The collection method followed a well-established protocol and took place as follows: 2-4 mL of unstimulated whole saliva was given by each participant in the morning around 9 AM. The participants were asked not eating, drinking or smoking prior to saliva collection. All subjects rinsed with 5 mL of distilled water before collection. Immediately after the collection, the samples were kept on ice and then centrifuged at 5000 rpm for 10 minutes. Samples were aliquoted and stored at -80 ºC until further determinations.

Matrix metalloproteinases detection

All MMPs were detected using Luminex technology and Magnetic Bead-Based Multiplex assays. This method enables simultaneous detection of multiple salivary human biomarkers. Thus salivary levels of MMP-1, -2, -7, -9, -10 were assessed using MILLIPLEX MAP Human MMP Magnetic Bead Panel 2 – Immunology Multiplex Assay. All samples and controls were processed following the manufacturer's specifications. The assay required 25 µL of saliva diluted at 1:20; each MMP had its own detection interval as follows: MMP-1: 27 – 20,000 pg/mL; MMP-2: 68 -50,000pg/mL; MMP-7: 548 – 400,000pg/mL; MMP-9: 14 – 10,000pg/mL; MMP-10: 27 – 20,000pg/mL. The assay involved a 2 hour multistep procedure involving preparation of reagents, samples and controls, preparation of the 96 well-plate and analysis using a Luminex® 200™ and the xPONENT® software for data acquisition and analysis.

Statistical analysis: The statistical analysis was performed using SPSS software. The characteristics were expressed as median (quartile 1; 3). Statistically significant correlations were found using the Mann-Whitney test (a two-sided p-value less than 0.05 was noted as statistically significant).

Results:

Clinical data: Results for demographic data and clinical examination are present in Table 1. No significant differences were detected for gender and age between the periodontal patients and the control group. Table 2 present the MMPs values obtained in the two groups of patients included in the study.

Table 1. Clinical date of patients included in the study

Variables	Patients	Controls	p-values
Age (*years*)	44.88±12.81	20.67±2	-
Gender (*female/male*)	10/12	10/5	-
Smoking status	0	0	-
BI (*%*)	45±0.11	17±0.20	<0.05
GI (*%*)	59.7±0.24	12.36±0.12	<0.05
PD (*%*)	4.80±0.23	0	<0.05

Table 2. Salivary levels for MMP-1,-2,-7,-9 and -10

Variables	Patients	Controls	p-values
MMP-1 (*pg/mL*)	348.375±144.6	190.125±144.6	<0.05
MMP-2 (*pg/mL*)	116.37±230.12	9.56±230.12	=0.1
MMP-7 (*pg/mL*)	886.68±79.81	527.59±79.81	<0.05
MMP-9 (*pg/mL*)	6885.75±2800.08	5224.911±2800.08	<0.05
MMP-10 (*pg/mL*)	1569.56±168.04	598±168.044	<0.05

DISCUSSIONS

Salivary biomarkers are used in the diagnosis and monitoring of autoimmune disorders (cystic fibrosis, Sjrogen's syndrome), cardiovascular disease, diabetes, HIV, oral cancer, oral lichen planus and periodontal disease [9-13].

MMPs are metal-dependent proteolytic enzymes involved in both physiological (apoptosis, bone remodeling, angiogenesis) and pathological processes such as cardiovascular disease, cancer and periodontal disease [14].

In macrophages and other types of cells from periodontal tissue, lipopolysaccharide from Porphyromonas gingivalis, binds to Toll-like receptor 4 (TLR4) and activates an inflammatory cascade with the release of inflammatory cytokines, chemokines and prostaglandins. This inflammatory response actives inflammatory cells to release MMPs mainly from polymorphonucleate leukocytes (PMN) which will degrade extracellular collagen matrix [5, 15].

Because the extracellular periodontal matrix contains especially type 1 collagen, special attention has been given to MMPs capable of degrading this type of collagen such as MMP-2 MMP-8, MMP-9, MMP-13 [16, 17].

MMPs-1, -2, -8, -9 and -13, had been detected so far in patients with periodontal diseases, mostly from gingival crevicular fluid (GCF) and less from unstimulated whole saliva. The results of the studies reflects increased levels of all mentioned MMPs and their implication in periodontal tissue degradation [18, 19].

MMP-1 or collagenase-1 is the main collagenase found at patients with aggressive periodontitis. GCF and salivary levels of MMP-1 were increased during periodontal disease and decreased levels were observed after periodontal treatment [20-22].

MMP-1 present increased levels in inflammation, autoimmune and cardiovascular diseases [14, 23].

Our results revealed statistically increased salivary levels of MMP-1 in patients with chronic periodontitis compared with controls.

MMP-2 and MMP-9 are gelatinases that can be released by various type of cells from oral cavity such as gingival fibroblast and gingival keratinocytes [24].

In the present study, we obtained increased salivary levels of MMP-2 but statistically insignificant in patients with chronic periodontal disease versus the healthy group.

The salivary level of MMP-9 was statistically increased in our study at patients diagnosed with periodontal disease versus the healthy group.

Increased levels for MMP-2 and MMP-9 were observed in GCF at patients with periodontitis, versus patients' gingivitis and healthy subjects [25].

MMP-9 in involved in cell apoptosis, tissue remodeling, being associated with several systemic diseases such as systemic sclerosis and osteoarthritis. Meanwhile MMP-2 is involved in angiogenesis and pathological processes such as cardiovascular disease [14, 23].

MMP-7 or matrilysin-1 is produced in non-injured exocrine and mucosal epithelia. Levels of MMP-7 in GCF of patients with periodontal disease are the same with healthy subjects [26].

MMP-7 is associated with inflammation, cardiovascular and lung diseases and to physiological cell apoptosis [14, 23]. Salivary levels of MMP-7 at chronic periodontitis patients involved in our study reflect increased statistically levels compared with controls.

MMP-10 or stromelysin -2 acts on types III, IV and V of collagen and has been linked to cell apoptosis, liver and lung diseases, tissue remodeling [14, 19, 23].

Still now, MMP-10 has not been detected in saliva or GCF in patients with periodontal disease. Our study revealed increased statistically levels at periodontitis patients versus healthy volunteers.

CONCLUSIONS

In conclusion, increased levels of MMP-1, -2, -7, 9, and 10 are directly associated with the severity and progression of periodontal disease, especially through involvement in alveolar bone resorption and periodontal tissue breakdown. Salivary detection of this MMPs is a modern alternative to monitoring the activity of these enzymes.

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Secure wireless system based on reconfigurable devices for human biomedical parameters monitoring

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Abstract: *Over the last decade the continuous improvements in sensor technologies, connected with recent hardware reconfigurable devices evolution, enable engineers to merge sensors and reconfigurable devices to develop new applications or to improve the existing ones. The miniaturization and integration of multiple sensors in one chip and the increase of precision, stability and power efficiency allow sensors to play an even more important role in medical technology with the main objective of building more accurate and smaller devices that help medical personal to monitor human biomedical parameters. This paper describes a secured wireless system design and implementation. The proposed system consists in one or more wearable sensor nodes that measure human biomedical parameters and then sends the collected data to a base station in order to be analyzed by qualified personal. This system can be used to monitor patient state-of-health or to supervise military personal in training or even in battles, because the new system uses secured transmission. The typical monitored parameters are body temperature, blood oxygen level, heart rate, respiratory rate, movement and position of the subject, but it can be extended and more other different sensors such as cameras or microphones can be added. Reconfigurable devices are used to process data in both sensor node and base station in an innovative environment.*

Keywords: *biomedical parameters, reconfigurable devices, sensor nodes, wireless*

INTRODUCTION

Nowadays, electronic industry and healthcare system are two modern domains, technologically advanced, working together to find solutions for many of the existing medical problems. Rapid diagnostic tests, helping patients after medical interventions and improving the lives of people with disabilities using sensors are authentic examples [1]. Many reviews in military health care system consider that "medicine is a filed in which evolution ignores borders, bureaucratic rules, customs and paradigms" [2] and innovative health solutions and technologies in medical activity are more than welcome, if the common goal is to save lives.

Heart failure is a major medical problem at global level, due to increased prevalence of cardiovascular diseases [3]. Rapid diagnosis of heart failure is almost impossible for human body subjected to high levels of stress (e.g. gym training workout, soldier training and in missions, free running and parkour, etc.).

In such cases diagnosis can be easily accomplished using wearable sensors, incorporated in many wearable devices like: chest belts, cloves, watches, wrist bands, finger clip and mobile phones. Many electronic integrated circuit manufacturers offer a variety of sensors capable to measure many biomedical parameters: body temperature, heart rate,

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oxygen saturation, electrocardiogram, etc. Furthermore, in the specific case of heart failure, medical personnel, supervisors or relatives can be alerted by communications devices integrated in wearable systems. These systems can also provide the exact location of the people in distress in order to be found and rescued, using GPS receivers, also integrated in a special designed wearable monitoring system.

The proposed system architecture is presented in the second section of this paper together with a short description of the system's main module and its components. In the next sections the proposed system operating mode in a real world scenario is presented together with encryption algorithms that can be implemented on the main controller in order to ensure different levels of security. In the last two sections implementation and experimental results are depicted. Conclusions and future work directions are also indicated, these will enhance the system overall performance and will enlarge application field.

This paper describes an improved solution to the existing health monitoring systems because its main functions are executed using reconfigurable devices such as CPLD or FPGA. Furthermore the new proposed system is secured using the strong advantage of reconfigurable devices capabilities, making it suitable for military applications.

SYSTEM ARCHITECTURE

The overall system architecture is presented in Fig. 1. The proposed system consists in three main modules: main controller, various biomedical sensors and a base station/gateway. Biomedical sensors and main controller can be integrated in a chest belt, wristband or a glove making it a wearable device, thus all the components of the wearable module must be low-power and small package devices, ideal for this kind of systems. The wristband and the glove are added to increase the systems performance in case of heart rate or oxygen saturation measurement, because in those areas the veins are located close to the surface of the skin and fingers are vascularized organs. The wearable module can be considered as a wireless sensor node and the base station as a gateway becoming a wireless sensor network.

Main controller

As the main part of the wearable module, it ensures the following functionalities:

- controls and configures the node;
- collect data from the sensors;
- establish and maintain communication with the base station/gateway or other sensor nodes;
- encrypts the acquired data and prepares it for transmission;
- decrypts and process the received data;
- wakes up the system and puts it back to sleep for power conservation.

Due to latest improvements in reconfigurable technology regarding power consumption, instead of classic microcontrollers, reconfigurable devices for both wearable module and base station are the best alternatives [4].

Figure 1. System architecture

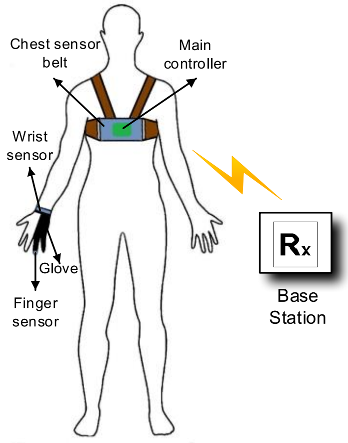

Biomedical sensor

In this system different biomedical sensor are used to measure and monitor human body relevant parameters in order determine its state of health or to prevent human health degradation (e.g. loss of consciousness, hypo or hyperthermia, physical fatigue, etc.) [5].

The new system presented in this paper is designed to monitor the fallowing physiological parameters: body temperature (T), electrocardiogram (ECG), photo-plethysmogram (PPG), heart rate (HR), peripheral capillary oxygen saturation (SpO_2). After studying numerous types of biomedical sensor taking into consideration power consumption, monitored parameters, chip dimension and sensor accuracy. As seen in Table 1 chosen sensors are built specially for wearable devices providing low power consumption and most common connection interface to communicate with the main controller. In the next paragraph a short description of this sensor is given:

- AFE4900 is an analog front-end (AFE) integrated circuit built by Texas Instruments designed mainly for (ECG) and (PPG) signal acquisition at data rates up to 1 KHz, but it can also be used for optical bio-sensing applications, such as heart-rate monitoring (HRM) and saturation of peripheral capillary oxygen (SpO2). The ADC codes from the PPG and ECG phases can be stored in a 128-sample first in, first out (FIFO) block and read out using either an I2C or a serial programming interface (SPI) interface;[6]

Table 1.

Part Number	Monitored Parameter	Average Power consumption		Interface
		Standby	Active	
AFE4900	ECG, PPG, HR, SpO_2	0μA	30μA	I^2C, SPI
MAX30205	T	1.65μA	600μA	I^2C
MAX86141	HR, SpO_2	0.6 μA	10 μA	SPI

- MAX30205 uses a sigma-delta analog-to-digital converter (ADC) with high resolution to convert temperature measurements to a digital form. It has a 16 Bit temperature resolution (0.0040°C) with 0.1 °C accuracy that meets clinical thermometry specification of the ASTM E1112 when soldered on the final PCB. The communication with the main controller is made through an I2C-compatible, 2-wire serial interface; [4, 7]
- MAX86141 is an ultra low-power optical data acquisition device designed for heart rate (HR) and oxygen saturation (SpO2) and optimized for wrist, finger or ear location.

This devices is also an analog front-end (AFE) integrated circuit with 19 bit analog to digital converter (ADC), ambient light cancelation and picket fence detect and replace algorithm. The device has an SPI interface to communicate with the main controller [8].

In Figure 2 a PPG and ECG measurement example is presented. The displayed data was captured using TIDA-01580 development board and its associated LabView application [9].

Figure 2. PPG and ECG measurement example

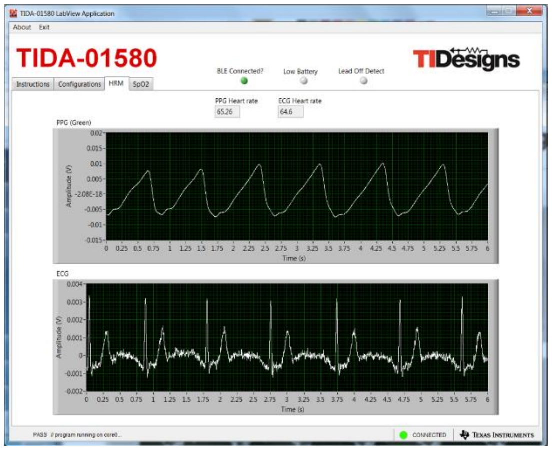

PPG Heart rate: 65.26

ECG Heart rate: 64.6

PASS // program running on core0...

Transceiver

The wearable module must be able to communicate with the base station and vice versa. To accomplish this, the license free ISM European 868 MHz band is chosen. Sub GHz wireless transceivers offers many advantages such as excellent propagation characteristic at low frequency, is easy to use [10] and consumes significant less power than the other communications modules (e.g. 2.4 GHz and 5 GHz WiFi module). In applications where so called sub GHz transceivers are used the battery life time is extended by 48% in comparison with applications where WiFi modules are used [11].

For the system presented in this paper the SPSGRF-868 low power programmable RF transceiver is preferred due to the low power characteristic and because it is delivered tiny form factor but providing a complete RF platform with integrated antenna. The module has the capability to alternate READY and SLEEP states in order to preserve power. Configuration and data transmit and receive is made through SPI interface. Both wearable module and base station is equipped with this transceiver.

Base station

In this case power consumption is not a key factor. For this reason a multitude of reconfigurable devices can be used, but after analyzing a set of this devices and taking into consideration system's requirements for data processing speed, reconfigurable resources, communication interface (e.g. SPI, I2C, CAN, etc), the best solution appears to be Xilinx Zynq XC7Z007S SoC.

This device has integrated a Single-core ARM Cortex-A9 capable of running at maximum frequency of 667 MHz and reconfigurable resources are equivalent to Artix-7 FPGA's. The base station has the following functions in the system:

- receive and process data from the sensor nodes;
- alerts the medical personnel when measured biomedical parameters are outside the defined threshold;
- encrypts and decrypts the transmitted or received data;
- configures the sensor node (threshold, id, etc);
- manage the encryption keys in the wireless sensor network.

Auxiliary components

In addition to the components and modules presented before, each sensor node has other components with less significant roles but without these components the system cannot function or satisfy the system requirements regarding power consumption. These components are:

- TCR2LFXX - low quiescent current (Iq) low dropout regulator (LDO);

- S25FL127S - 128 Mbit SPI flash memory used do store firmware in case of nodes with FPGA and other parameter like network id, node address, pre shared cryptographic keys, etc;
- 23LCV1024 - 1 Mbit Serial SRAM with Battery Backup use to store system flags, threshold and other parameters that system needs do store and then quickly access them when needed;
- SiT1533 - Ultra-Low Power 32.768 kHz Quartz XTAL to clock the system.

Figure 3. Operation Modes

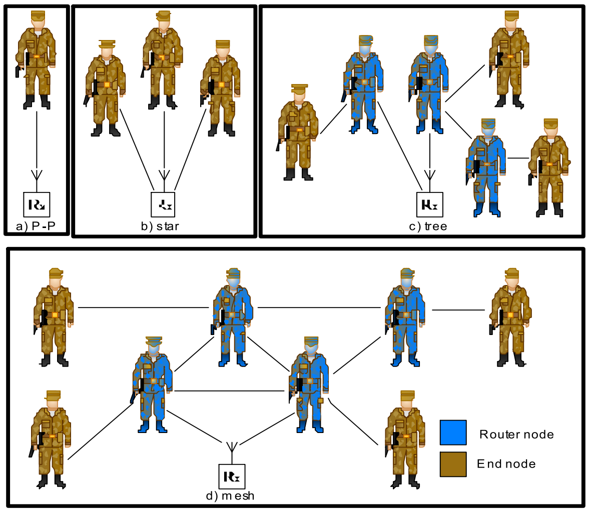

SYSTEM SECURITY

Due to the use of reconfigurable devices, security for this system can easily be achieved because this type of electronic circuits are notorious when comes to the implementation of cryptographic algorithms.

Depending on the desired level of security CPLD or Low-Power Spartan 6 FPGA can be used as main controller. While on CPLD a few low level cryptographic algorithms can be implemented (RC4, DES, Camellia, etc), on Spartan 6 all the advanced cryptographic algorithms can be implemented (AES, SHA, HMAC, RC6, ECC, etc.).

IMPLEMENTATION AND EXPERIMENTAL RESULTS

The use of reconfigurable devices was designated as the best choice for this design due to its flexibility in implementation of control functions and most common communication protocols (e.g. I2C, SPI, UART, CAN, etc.), [4] and due to latest advances in reconfigurable technology regarding power consumption. Synthesizable Verilog code has been preferred to implement the firmware running on CPLD and low-power Spartan 6 FPGA. The design was synthesized and simulated Xilinx ISE Design Suite 14.7 for CPLD and Spartan 6 and with Vivado Design Suite 2017.4 in the case of Zynq XC7Z007S SoC.

CPLD consumes only 16 µA at a running clock of 32.768 KHz and because in case of temperature measurement only five measurement are needed in 10 minutes of operation. The main power consummator in this design is the transceiver with 22 mA in TX mode at 11.6 dBm but in shutdown mode it consumes only 0.1 µA. Thus alternating ACTIVE and SHUTDOWN sates significantly reduces the overall power consumption.

In normal conditions Spartan 6 is a power consummator, making it inappropriate for our design but Xilinx introduced a low power version of Spartan 6 that consumes with 30-40% less power. In addition to this it too has the possibility to be put in to SLEEP mode furthermore reducing power consumption. After design implementation using AES-256, the following Spartan 6 part number XC6SLX9-L1TQG144C was chosen because it has the necessary logic resources needed for our design. The L later in the part number specifies the low power version of Spartan 6.

The wearable module can be powered from a 3.6V AA battery. Different voltages are adjusted from this power source in order to power up all the system's devices. The system needs the following voltages to function correctly: 1.8V for CPLD, 1V for Spartan 6 and 3.3V for all other devices. These voltages are obtained using LDO regulators with 2 µA quiescent current.

Table 2 estimates overall power consumption in different operating states. Battery life time is also indicated if a 2400 mA AA battery is used.

Table 2.

Device	Ready State	Shutdown State	Battery lifetime 5 s/min duty (days)
CPLD	11 mA	30 μA	≈142
Spartan 6	40 mA	30 μA	≈37

CONCLUSION AND FUTURE WORK

The design and implementation results presented in this paper provides a clear understanding of how engineering (e.g. electronics and telecommunications) and medical science can work together to design and built advanced biomedical monitoring systems using latest innovations in sensor technology advantages. It also proves the possibility to use reconfigurable devices in low power designs by alternating inactive and active operating states. Using this strategy, it becomes possible to integrate such systems in wearable devices, extending the battery life time for days or even weeks.

Adding new devices to the proposed system (e.g. GPS localization, CMOS cameras, microphones or other biomedical sensors) is also considered for future system developments.

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Serum vaspin and chemerin changes in response to combat physical fitness test in military forces

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Abstract: *The study aimed to investigate serum vaspin and chemerin changes in response to combat physical fitness tests in military forces. In a semi-experimental design with pretest and posttest, twenty volunteers were engaged to participate in the study. Combat Physical Fitness Test consisted: (1) 25-yard(yd) sprint to J-hook, to 25-yd crawl, and 25-yd run through cones to 75-yd line; split (2) 75-yd casualty drag; split (3) ammo can run to grenade toss (75-yd); (4) ammo can run to end (75-yd); and (5) 650-yard endurance run. Pre-test and the post-test blood sample was gained. The data were analyzed by SPSS version 24. Serum chemerin did not change significantly compared to the pre-test whereas Serum vaspin was significantly higher after the test (p = 0.018). Afterward, a direct correlation was observed between serum chemerin and serum vaspin (p = 0.034, r=0.335). It is suggested that serum vaspin is responsive to short-term maximal power output activity required in the combat fitness test and positively correlates with chemerin. However, it appears that 3 to 4 minutes of high-intensity exercise induce comparatively moderate post-exercise serum vaspin increment, which appears to be inadequate to stimulate chemerin secretion.*

Keywords: *combat readiness test, vaspin, chemerin, military, adipokines*

INTRODUCTION

Adipokines are new bioactive adipose tissue secreted factors that induce a variety of local and systemic titled role in the body as systemic metabolic regulators, immunity, and endocrine function. Several adipokines including chemerin, vaspin, omentin-1, and apelin affect the whole-body homeostasis by influencing numerous biological, physiological, and pathophysiological processes [1, 2].

Chemerin is a16 kDa chemoattractant protein, an agonist of the orphan G-protein coupled receptor chemokine-like receptor 1 (CMKLR1, ChemR23) adipokine that play role in innate and adaptive immunity that is expressed highly by innate immune system cells [3, 4], liver, and white adipose tissue (WAT), and expressed moderately in lungs and brown adipose tissue, and weakly expressed in the heart, ovaries, and kidneys and as an adipokine exerts effects on many biological processes, including adipogenesis, angiogenesis, inflammation, immune responses, and food intake [4, 5]. Pathologically, several effector cells of the innate immune system are influenced by chemerin, including dendrite cells, monocytes, macrophages, and NK cells, and might influence the crosstalk between different cell types responsible for controlling the initiation and progression of inflammation [6]. Recent studies suggested either a proinflammatory or

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anti-inflammatory role for chemerin and emphasized it's as treatment strategies for obesity [7]. On the other hand, Vaspin(visceral adipose tissue-derived serine protease inhibitor) is a recently discovered 45 kDa adipocytokine predominantly secreted from visceral adipose tissue [1, 8]. Vaspin mRNA was reported to be expressed in subcutaneous and visceral adipose tissue which might play a role in inflammation expansion [9]. Multiple lines of evidence suggest that vaspin protects cells from inflammation and apoptosis. It has been demonstrated that vaspin inhibition of TNF-α induces the expression of adhesion molecules and subsequently decreased lymphocyte adhesion by a reducing ROS generation [10].

Several kinds of research studied the response of adipokines such as chemerin and vaspin to high-intensity exercise and reported contradicted results such as elevation [11, 12], no change [13, 14] or reduction [15, 16]. The most prior studies were focused on obese or metabolically patient subjects while to our knowledge there is no research to investigate acute chemerin and vaspin responses after MANUF. Also, the notable issue is that in previously performed protocols, the movement pattern/type was limited to one of the aerobic/resistance/power modality of exercise such as cycling or running [17-19].

Combat physical fitness is a key factor in armed forces [20]. The maneuver under attack fire (MANUF) is a relevant, high-intensity, and integrated test that its stages are executed by maximal power output and included combat physical items similar to war conditions [21]. Previously it was reported that high-intensity exercise stimulated free radical production in the skeletal muscle and myocardium. Also, high-intensity exercise (HIE) causes cell damage due to mechanical tension imposed on muscle cells that may stimulate the immune system and expand inflammation. In this regard, chemerin and vaspin may play the opposite role in response to high-intensity exercise. However, MANUF is a complete combination of war simulated tasks such as sprint, crawling, tossing a grenade, carrying casualties, or ammo boxes [21]. Hence, it's unclear whether utilizing different movement patterns or muscle groups throughout MANUF makes any difference in serum chemerin and vaspin responses or not. Therefore due to a lack of sufficient information about the metabolic response of the MANUF test, our first aim is to measure and compare serum chemerin and vaspin in pre and post-test. It is reported that serum chemerin and vaspin concentration are elevated after high-intensity exercise. A high-intensity exercise (severe muscle contraction) causes cell damage due to mechanical tension imposed on muscle cells that may stimulate the immune system and expand inflammation. Chemerin regulates activation of dendritic cells and macrophages by some intracellular factors such as the G protein-coupled receptors CMKLR1 (ChemR23), GPR1, and CCRL2. Based on Japanese cohort studies its reported a close association between serum vaspin levels and physical fitness in men also physical activity in women independent of the subject's body composition [22]. So secondly we postulate there is a proposed relationship between serum chemerin and vaspin.

MATERIALS AND METHOD

Statistical sample

In a quasi-experiment study design, fifties cadets volunteers were recruited. At the first visit to familiarize seasons information regards research procedures, its benefits, and potential risks were provided to participants. Written informed consent forms, and the health and physical fitness questionnaire were completed. Height and weight measurements and familiarization with the test were performed the day before the test. In the end, 20 subjects with an average age of 26.95 years, and, the weight of 73.3±7.34 kg were selected as the study sample. Inclusion criteria were healthy male military personnel or personnel with more than one year of fields' military history. The participant exclusion factors were the history of fracture, cardiovascular disease, smoking, and consuming drugs.

The maneuver under attack fire (MANUF) test

The MANUF contains combat-related tasks. The MANUF that aims to assess the combat-related tasks consisted of movement types of actions that are made potentially in combat.

The sort of the items into a single timed event is both a combination of energy systems demonstration, strength/ anaerobic plus aerobic, and a measurement of military combat capability and physical fitness. The test included 300-yard field MANUF tasks such as performing sprint (25 yards), J-hook turn, low and high crawl (25 yards), casualty drag (75 yards); ammo can sweep (30 lb each, 1 in each hand) carries shuttle run (two 75 yards), a grenade toss and at the end, 650 yards running [21]. In the casualty drag item, there is a 10-yard drag, followed by a 65-yard fireman carry. Subjects encourage doing their maximal effort to achieving peak performance from start-up to course end.

Dietary and physical activity control

Subjects were requested to avoid intensive physical activity, supplement consumption such as caffeine and have their habitual diet in the preceding 24 hours of the test. Additionally, participants completed a 24-hour dietary recall before the combat test.

Enzyme-linked immunosorbent assays

Blood samples were collected into K3 EDTA tubes and were isolated by centrifugation (3,000 x G for 10 min). The obtained serum was aliquoted and stored at -80°C until analysis.

Serum chemerin was assessed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Biovendor-Laboratorni medicina a. s., Karasek, Czech Republic). Serum vaspin levels were assayed using a sandwich ELISA kit Vaspin (AdipoGen, Seoul, Korea) according to the manufacturer's instructions.

Statistical analysis

Statistical data analyses were done with descriptive statistic mean and standard deviation. A paired sample student t-test was used to analyze within groups comparison. The possible correlations between chemerin and vaspin serum levels were tested with Bivariate Pearson correlation coefficients. Statistically significant differences were set at $P<0.05$.

The SPSS (IBM Version 24, 2016) was used for data statistical analysis. Written consent form was completed by participants before taking part in the study.

All study protocol was performed according to the criteria set by the declaration of the Helsinki 1975, as revised in 2008, and were approved by Baqiyatallah University of Medical Sciences ethical committee (IR.BMSU.REC.1397.303).

RESULTS

Demographics

Twenty participants were engaged to participate; and completed all trials and are covered in the analysis. Participants' characteristics are shown in Table 1. All individuals had at least one combat deployment before the research with an average history of 43.1 ± 18 months of active duty service recruited.

Table 1: Subjects demographic and physiological characteristics

Variable	Mean± SD
Age (year)	26.95 ± 3.80
Weight (kg)	73.3±7.34
Height (cm)	176.65 ± 6.36
BMI (kg/m^2)	22.13 ±2.78
Rest Heart rate	78.63±12.5

BMI: body mass index, SD: standard deviation

The results of the pretest-posttest comparisons are represented in Table 2. The result of a paired sample t-test demonstrated that chemerin serum levels did not change significantly compared to pretest, While Serum vaspin levels increased significantly after the combat fitness test ($p = 0.018$, Table 2). Additionally, Pearson correlation analysis revealed a positive correlation between chemerin and vaspin serum levels changes in response to combat physical fitness test (p=0. 34, Figure 1).

Table 2: A within group's comparisons of serum chemerin and vaspin levels

	Pre-test (Mean±SD)	Post-test (Mean±SD)	t	P-value
Serum chemerin (pg/ml)	82.89 ± 19.56	87.38 ± 24.20	-0.68	0.34
Serum vaspin (pg/ml)	0.67 ±0.34	0.90 ±0.41	-2.58	0.02*

** Significant differences at p≤ 0.05*

DISCUSSIONS

The main finding of the present study was that vaspin significantly increased after combat fitness tests and there is a positive correlation between vaspin and chemerin increment although there was no dramatic change in chemerin levels comparing to pre-test level.

To the best of our knowledge, this is the first research that examines the effect of maneuver under attack on circulating serum chemerin and vaspin. Absence of dramatic change may be due to the low volume (frequency/duration) of applied exercise activity and following previous results of Zarei et al. 2018 and conflict with findings of Saremi et al.2010, Chakaroun et al.2012, Aghapour et al. 2013, Venojarvi et al. 2013, Malin et al. 2014, Kim et al 2014, Faramarzi et al. 2016 [23-29]. The present research failed to distinguish any dramatic alteration in serum chemerin levels after MANUF that is predominantly a high-intensity exercise in nature. Lack of such a change in our data series may be due to differences in the intensity, type, duration, and frequency of training compared to the noted studies.

Faramarzi et al. 2016 demonstrated a significant reduction in serum chemerin and vaspin levels in forty healthy women following 12 weeks of combined rhythmic aerobic and core stability exercise training [29]. Lioyd et al. 2016 reported that acute bouts of one hour of moderate-intensity walking (60-

65%Vo2peak) drop chemerin levels in 11 non-insulin resistant obese adults at one and two hours follow up time courses [30]. Liu et al. (2018) studied the effects of four weeks of low to moderate intensity of aerobic exercise in fifty obese adolescent female (14 years old) on chemerin serum levels and showed that whereas slice dieting did not have health benefits, 4 weeks of exercise plus dieting induces a significant decrease in chemerin and body fat [31]. Similar to our results Zarei et al. (2018) indicated nonsignificant changes in chemerin levels after 8 weeks of high-intensity interval training in comparison with control groups [13]. Based on these results, its proposed intensity-dependent changes in chemerin serum levels, in the form of its reduction in response to moderate exercise and non-changes or elevation following high-intensity exercise.

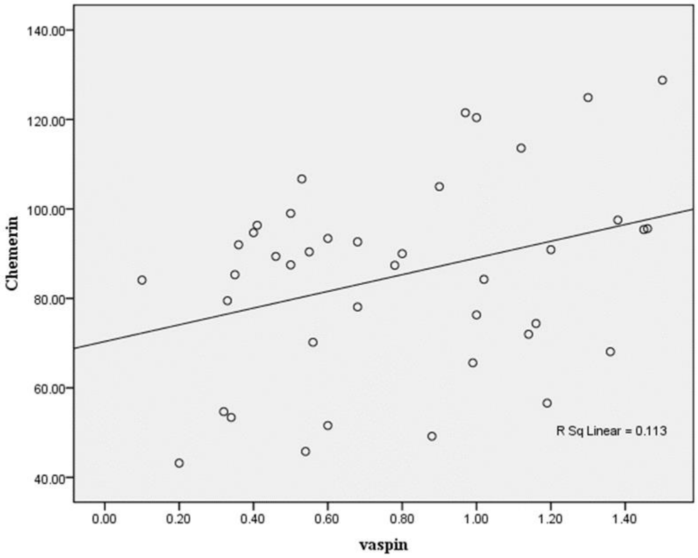

Figure1: The correlation between serum chemerin and vaspin

Furthermore, the characteristics of subjects also could be considered as counting factor. This study included a healthy young male army force without risk factors of cardiometabolic disease, while most other researches were carried out among middle/advanced-aged, overweight/ obese or diabetic [13, 30, 31] subjects who are exposed to high cardiometabolic risk. Further researches are necessary to clarify the impact of training type, frequency, and duration, and participant's health and fitness status on chemerin levels [6]. The mechanical tension leads to cell rupture and bioactive chemokine production to help macrophages for phagocytosis in the initial stage of inflammation [7]. As we observed a weak positive correlation between serum levels of chemerin and vaspin changes in response to one bout of acute exercise activity executed in MANUF test, it seems that there was not deleterious enough alteration in vaspin to induces a significant increase in chemerin. As it found a positive association between chemerin levels with obesity and T2DM and negative association of these diseases with vaspin levels, and low levels of vaspin reported in different stages of cardiovascular disease (CVD) [1], so optimal exercise intervention could be defined when it induced serum vaspin elevation while chemerin did not. In contrast to our results, Motawi et al. (2018) reported a strong negative relationship between chemerin and vaspin in grade I, II CVD that could be related to baseline variance levels of this adipokine in compare with the present study healthy arm force subjects. Subsequently, increased vaspin levels with non-changes in chemerin levels could be ideal regards the protective effects of exercise in CVD that needed to be clarified in further studies.

The result of the present study shown that vaspin levels increased at the end of the combat fitness exercise test. The recorded times to the completion of combat physical fitness tests, which were between 3 to 4 minutes that seems to be adequate to enhance vaspin secretion and augmenting its circulating levels. Base on previously available researches, the data about the response of vaspin after an acute bout of exercise particularly the maneuver under attack fire is still scarce and it is difficult to compare results towards reach a conclusion. The elevated serum level of vaspin seems to be related to the intensity of training. In this regard, Youn et al. 2008 reported that 4 weeks of high-intensity training causes an increase in serum vaspin levels along with weight loss in obese or type 2 diabetics subjects [32]. On the other hand, Oberbach et al [33] observed a significant decrease in serum vaspin concentration after 4 weeks of high-intensity resistance training in healthy young men. Also in a study conducted by Lee et al [34], vaspin levels in obese children significantly decrease after seven days of intensive lifestyle modification. Elevation in vaspin levels in the present study could be suggested as an optimal anti-obesity strategy as previously reported increase in serum vaspin levels depending on the degree of obesity and reduction in its levels with weight reduction programs [22]. Therefore, it seems that high-intensity aerobic exercise/training intervention induces serum however, it needs to be revealed in future studies.

CONCLUSIONS

These data specified that vaspin serum levels are responsive to short-term maximal power output exercise required in a combat fitness test and positively correlates with chemerin levels changes. However, it appears acute high-intensity exercise induce comparatively moderate post-exercise serum vaspin increment, which appears to be inadequate to stimulate chemerin elevation. It appears that there may be higher metabolic stress thresholds that are required for the activation of the compensatory and positive systemic anti-inflammatory responses and an extended exercise duration is expected to be a crucial element in achieving this, however further studies need to elucidate these.

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Authors contribution:

These authors also contributed equally in study conception and design, material preparation. Data collection and analysis were performed by A. Delpasan and B. Bazgir. The first draft of the manuscript was written by A. Delpasand and all authors read and approved the final manuscript.

Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this article.

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REVIEW ARTICLE

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SGLT2 inhibition in patients with type 2 diabetes and cardiovascular diseases: which are the benefits?

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Abstract: *Two major trials, EMPA-REG OUTCOME trial (with Empagliflozin) and CANVAS trial (with Canagliflozin) have demonstrated the beneficial effects of SGLT2 inhibitors in patients with type 2 diabetes at risk for heart failure. SGLT2 inhibitors possess a unique pharmacological profile and have shown a significant reduction of hospitalization for heart failure in diabetic patients, immediately after treatment commencement. It seems that their effects are beneficial both in heart failure with reduced ejection fraction and heart failure with preserved ejection fraction. SGLT2 inhibitors reduce cardiovascular endpoints in patients with diabetes and high cardiovascular risk, most likely through a reduction of heart failure-related events. Empagliflozin treatment exerts beneficial effects on vascular function and central haemodynamics. Various mechanisms seem to contribute to the beneficial effects of SGLT2 inhibitors in heart failure. Taking into consideration their cardiovascular benefits and the number of ongoing trials with these drugs, there is a possibility to expand their indication to nondiabetic patients with heart failure. This review focuses on the newest insights into the beneficial effects of SGLT2 inhibitors on the cardiovascular system.*

Keywords: *SGLT2 inhibitors, type 2 diabetes, empagliflozin, canagliflozin, heart failure*

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular disease (CVD) and, moreover, their association determines a higher mortality rate [1, 2]. On the other hand, heart failure represents an independent risk factor for developing T2DM [3], and a percentage varying from 20% to 40% of the patients with heart failure have also T2DM [4].

Pharmacological treatment of patients with T2DM is complex and has been continuously improved over the years. The treatment may be with oral antidiabetic agents (OAD) and/or insulin. The main oral antidiabetic agents are:

- Sulfonylureas.
- Alpha-glucosidase inhibitors.
- Thiazolidinediones.
- Glinides.
- Biguanides.
- Dipeptidyl peptidase-4 (DPP-4) inhibitors.
- GLP-1 agonists.

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- Sodium-glucose cotransporter (SGLT2) inhibitors.

ORAL ANTIDIABETIC TREATMENT

The first agent used as OAD in the treatment of T2DM was sulfonylurea that has hypoglycemic effects by stimulating the pancreatic β-cells to secrete insulin, primarily by closing the ATP-sensitive K channels from the β-cells plasma membrane inducing a series of events finalized with insulin release [5].

Alpha-glucosidase inhibitors are a class of OAD which were developed from bacteria. They are inhibiting the action of thealpha-amylase from the intestinal brush border. Their activity is independent of the pancreas and consists of delaying carbohydrate absorption in the gastrointestinal tract, obtaining the control of postprandial hyperglycaemia and, also, cardiovascular benefits [6].

Thiazolidinediones are a class of OAD formed by selective agonists of PPARγ from adipocytes, where they stimulate the fatty acid uptake and adipogenesis, especially in peripheral visceral fat (except the visceral fat). Lowering the concentration of circulating fatty acids and available lipids in the liver and muscle results in improving the patient’s sensitivity to insulin. Also, they decrease the concentrations of hormones secreted by adipocytes, particularly adiponectin. Overall, they have mixed effects on circulating lipids and increase total body fat [7].

Glinides are OAD with a rapid effect onset, but short-acting duration [8], which stimulate the pancreatic release of insulin, thus lowering postprandial glycaemia.

Biguanides are OAD, metformin being the most important representative of this class. They are antihyperglycaemic agents, mainly through inhibition of hepatic glucose release and, also, in a lesser extent, through delaying the enteric absorption of glucose and stimulating the uptake of glucose at different levels – skeletal muscle, enterocytes and adipocytes [9].

Recently, new OAD classes, such as DPP-4 inhibitors, GLP-1 agonists or SGLT2 inhibitors, have been developed.

DPP-4 inhibitors act by preventing the inactivation of glucagon-like peptide 1 (GLP-1)[10] and glucose-dependent insulinotropic peptide (GIP) [11], resulting in an increased level of active GLP-1. The overall effect is the lowering of glucose levels through increased insulin secretion and reduced glucagon secretion [10].

GLP-1 agonists are grouped in long-acting and short-acting, after their effect duration. They stimulate insulin secretion and suppress glucagon secretion [12].

SGLT2 inhibitors act on the channels from the proximal convoluted tubules, where they stimulate glucose elimination through urine, by preventing its reabsorption. There is no risk of hypoglycaemia, because they act on blood glucose level and are independent of insulin’s action, thus not affecting the pancreatic β-cells. Their limitation is represented by the impairment of the renal function [13].

SGLT2 inhibitors reduce glucose reabsorption in the early proximal tubule, leading to urinary glucose excretion and osmotic diuresis [14]. This class of medication has two main representatives-empagliflozin and canagliflozin, which demonstrated their beneficial effects in patients with T2DM at risk for heart failure in two major trials – EMPA-REG OUTCOME (with Empagliflozin) and CANVAS (with Canagliflozin). SGLT2 inhibitors have hypoglycaemic effects through increasing glycosuria and inhibiting renal glucose reabsorption [15]. They have also several additional beneficial effects, such as improving blood pressure [2, 13, 16–19], intrarenal haemodynamics [2] and albuminuria, reducing body weight [2].

For the management of cardiovascular risk in patients with T2DM, glycaemic control is very important, because it reduces the frequency of diabetic complications – especially microvascular ones [20] and has significant benefits in end-stage renal disease [21, 22]. Weight loss improves cardiovascular risk factors in T2DM on the short-term [23], but not on the long-term [24]. A 10 mm Hg reduction in systolic blood pressure reduces all-cause mortality, macrovascular and microvascular outcomes [25], but even a small blood pressure reduction can diminish cardiovascularrisk [26]. In consequence, it is very important to have a multifactorial approach in the management of T2DM [27].

SGLT2 inhibitors have several potential mechanisms that could explain their cardiovascular benefits, such as decreasing cardiac inflammation [16,28], decreasing cardiac oxidative stress [16,28], decreasing cardiac apoptosis [16,28], attenuating mitochondrial dysfunction [28], improving cardiac calcium and sodium ionic homeostasis [28] and hemodynamic effects, such as reduction in blood pressure [2,16,17] and intravascular volume [16,17] via osmotic diuresis [16] and natriuresis [17].

SGLT2 inhibitors posology is in tablets with rapid oral absorption [29], reaching peak plasma concentrationin 1-2 h [29, 30], and with a half–life of 10.2-13.1 hours [29,30], resulting in only one administration per day. The molecule metabolism is primarily through glucuronidation in the liver, followed by drug excretion through the urinary and faecal route. It is important to emphasize that SGLT2 inhibitors reduce the renal filtration rate (with up to 80 g per day), resulting in the necessity, for patients with an impaired renal function, of a more attentive follow-up and adjustment of doses [30]. The usual doses are canagliflozin – initially 100 mg, with a maximum of 300 mg per day, dapagliflozin – initially 5 mg, with a maximum of 10 mg per day, and empagliflozin – initially 10 mg, with a maximum of 25 mg per day [30].

EMPA-REG OUTCOME was a randomized, double-blind, placebo-controlled trial, which included 7028 patients with T2DM and established cardiovascular disease [1], who received empagliflozin once a day (10 mg or 25 mg dosage) versus placebo, compared to the usual standard of care. The study was multicentric, with 590 sites in 42 countries, and lasted until a minimum of 691 patients developed an adjudicated primary outcome - time to the first occurrence of any of the following adjudicated components of the primary composite endpoint (major adverse cardiovascular events [MACE] – CV death [including fatal stroke and fatal MI], non-fatal MI [excluding silent MI], non-fatal stroke). The trial lasted from September 2010 to April 2013 [1]. More than 99% of the included patients had established cardiovascular disease. The median observation time was 3.1 years, with a mean treatment duration of 2.6 years. As a cardiovascular outcome, a lower percentage of the primary outcome took place in the empagliflozin group (10.5%) than in the placebo group (12.1%) (HR for empagliflozin group, 0.86; 95.02%, CI, 0.74 to 0.99; $p<0.001$ for non inferiority and $p = 0.04$ for superiority); a significantly lower risk of death from cardiovascular causes (HR 0.62; 95% CI, 0.49 to 0.77; $p<0.001$), death from any cause (HR 0.68; 95% CI 0.57 to 0.82, $p<0.001$) and hospitalization for heart failure (HR 0.65; 95% CI 0.50 to 0.85; $p = 0.002$); the occurrence of myocardial infarction or stroke had no significant differences between groups [1]. Glycaemic control, estimated through glycated haemoglobin, was improved by empagliflozin at 12 weeks by −0.54 percentage points (95% CI, −0.58 to −0.49) in the 10 mg group, and by −0.60 percentage points (95% CI: −0.64 to −0.55) in the 25 mg group; at 94 weeks, the differences between empagliflozin group and the placebo group were by −0.42 percentage points (95% CI,−0.48 to −0.36) and by −0.47 percentage points (95% CI, −0.54 to −0.41), respectively; at 206 weeks, the differences between empagliflozin and placebo were by −0.24 percentage points (95% CI, −0.40 to −0.08) and by −0.36 percentage points (95% CI, −0.51 to −0.20), respectively [1]. Cardiovascular risk factors were slightly improved in the empagliflozin group: weight, waist circumference, uric acid level, systolic and diastolic blood pressure, but with small increases in both LDL and HDL cholesterol, with no increase in heart rate [1].

CANVAS consisted of two sister, multicentric, randomized, double-blinded, placebo-controlled trials, which included a total of 10,142 patients with T2DM and high cardiovascular risk [2]. Patients were administered canagliflozin versus placebo in 667 centres from 30 countries until a minimum of 688 cardiovascular events took place. CANVAS trial lasted from 2009 until February 2017 [2]. The inclusion criteria were: patients with T2DM, age ≥ 30 years, with a history of symptomatic atherosclerotic vascular disease, or ≥ 50 years old with 2 or more risk factors for cardiovascular disease. The primary end-point was represented by time to the first occurrence of non-fatal myocardial infarction, non-fatal stroke or cardiovascular-related death. Its results consisted of fewer primary outcome events in the canagliflozin group versus the placebo group (26.9 vs 31.5 participants, with an event per 1000 patient-years (HR 0.86; 95% CI of 0.75 to 0.97; p<0.001 for noninferiority; p = 0.02 for superiority). The renal outcome was a lesser progression of albuminuria in the canagliflozin group versus placebo group (89.4 vs. 128.7 participants, with an event per 1000 patient-years), with HR 0.73 (95% CI, 0.67 to 0.79); with a regression of albuminuria in canagliflozin group versus placebo group (293.4 vs. 187.5 participants per 1000 patient-years; HR 1.70; 95% CI, 1.51 to 1.91). An important outcome was the composite one -the 40% sustained reduction in eGFR, the need for renal replacement therapy, or death from renal causes, which was observed with a smaller frequency in the canagliflozin group versus the placebogroup (5.5 vs. 9.0 participants per 1000 patient-years HR 0.60; 95% CI, 0.47 to 0.77).

The empagliflozin patients had a mean age of 63 years, with a duration of diabetes of 10 years (57%), a mean HbA1c of 8.1%; cardiovascular risk was present in 99% of them through established cardiovascular disease, 10% of them had a history of heart failure, under treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in 81%, statins in 77% and acetylsalicylic acid in 83% of cases [1].

The canagliflozin patients had a mean age of 63 years, with a duration of diabetes >13.5 years, with a mean HbA1c 8.2%; cardiovascular risk was present, 65% had established cardiovascular disease and 35% multiple risk factors. 14.4% had a history of heart failure and were prescribed treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in 80%, statins in 75% and acetylsalicylic acid in 74% of cases [2].

SGLT2 inhibitors were evaluated through several trials. CANVAS-R, a prospective, randomized, double-blinded, placebo-controlled clinical trial, included patients with T2DM with a history or high risk of cardiovascular events [31]. The primary objective of this trial was limiting kidney disease progression and the results were fewer cases of albuminuria progression 430/2615 cases in the active group versus 581/2615 in the placebo group (HR 0.74), decreased cardiovascular mortality or hospitalized heart failure (61/2906 patients in active group versus 93/2905 patients in placebo group) (HR 0.66) and decreased cardiovascular death (40/2906 patients in the active group versus 60/2905 patients in the placebo group) (HR 0.68)[31]. CREDENCE trial was a randomized, double-blinded, placebo-controlled, parallel-group, multicentre study, with 4,402 participants, in whom the effect of canagliflozin on renal and cardiovascular primary composite endpoint was evaluated: end-stage kidney disease, doubling of serum creatinine, renal or cardiovascular death in subjects with T2DM and diabetic nephropathy [32, 33]. DECLARE-TIMI is a multi-national, randomized, double-blinded, placebo-controlled trial with 17,190 participants with T2DM that aimed to determine whether dapagliflozin versus placebo reduces cardiovascular events, such as cardiovascular death, myocardial infarction or ischaemic stroke [34]. Ertugliflozin CVOT is a randomized, double-blinded, placebo-controlled study, with the primary outcome the first occurrence of MACE after treatment with ertugliflozin in 8,000 patients with T2DM and established cardiovascular disease [35].

20% of diabetes mellitus patients over 65 years-old have heart failure [18]. There are very few OAD for patients with both heart failure and T2DM [18]. SGLT2 inhibitors, in this type of patients, are potentially OAD that can reduce the risk of cardiovascular mortality and heart failure [19]. They reduce all-cause mortality significantly when compared to placebo. Also, they reduce major adverse cardiovascular events when compared to placebo (OR 0.83; 95% CI 0.76–0.92; p<0.001), with the same efficacy for subgroups such as canagliflozin (OR 0.85; 95% CI 0.75–0.97; p=0.02) and empagliflozin (OR 0.82; 95% CI 0.71–0.94; p=0.006)[19]. Significant reductions were observed, when compared to placebo, for non-fatal myocardial infarction (OR 0.85; 95% CI 0.73–0.98; p=0.03) and for heart failure or hospitalisation due to heart failure (OR 0.67; 95% CI 0.59–0.76; p<0.001)(19). Insignificant reductions were observed, when compared with placebo, for non-fatal stroke (OR 1.02; 95% CI 0.85–1.21; p=0.87), atrial fibrillation (OR 0.61; 95% CI 0.31–1.19; p=0.15) and for the occurrence of unstable angina or unstable angina requiring hospitalisation (OR 0.95; 95% CI 0.73–1.25; p=0.73)[19].

CONCLUSIONS

SGLT2 inhibitors, such as empagliflozin, are very important, also, in treating patients with T2DM and heart failure, because they do not only reduce hyperglycaemia [2,13,18,19], but also lead to osmotic diuresis [18], reduce body weight [2,18,19] and blood pressure [2,13,18,19], without increases in heart rate, with favourable effects on markers of arterial stiffness and vascular resistance [18], albuminuria [18,19], and serum uric acid [13,18]. Another benefit of SGLT2 inhibitors is the reduction in the primary composite outcome that consists of cardiovascular death, non-fatal myocardial infarction or non-fatal stroke [18]. Treating heart failure in patients with T2DM is a real challenge, because of the lack of evidence-based guidelines for the management of such patients [18]. Important treatment goals are improving survival rates and preventing hospital admission [18].

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REVIEW

Should Hoffa's fat pad be resected during total knee arthroplasty? A review of literature

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Abstract: *Knee osteoarthritis is caused by the degeneration of joint tissues through mechanical loading as well as inflammatory effects. The number of total knee arthroplasties is increased and is expected to further be increased by 2050 compared to 2021. The infrapatellar fat pad, also known as Hoffa's fat pad, is often partially or fully resected during total knee arthroplasty to improve the surgeon's view in the upper tibia as well may reduce the risk of soft tissue involvement, when placing the prosthesis. The purpose of this study is to present the importance and functions of Hoffa's fat pad, as well as the latest data on the two conflicting views, on the removal or not of Hoffa's fat pad in total knee arthroplasty. However, there is not a consensus regarding Hoffa's fat pad resection, as its preservation has been associated with increased postoperative anterior knee pain. Due to the fact, that there are no current official guidelines for this issue, it would be preferable that surgeons try to preserve it, but only if there are no visibility and/or access problems during surgery. The choice of complete or partial resection or preservation is based on the surgeon's preferences, experience as well as patient's needs. More high-quality research and randomized clinical trials are needed for better guidance regarding Hoffa's fat pad management at total knee replacement.*

Keywords: *Hoffa's fat pad, infrapatellar fat pad, total knee arthroplasty, osteoarthritis of the knee, anterior knee pain, patellar tendon*

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease and the leading cause of chronic pain and disability in many developed countries [1]. Knee OA is caused by the gradual degeneration of joint tissues through mechanical loading as well as inflammatory effects [2]. However, two recent theories are considered to be the predominant causal factors: firstly, as the prevalence of knee OA increases with age, the increased life expectancy is considered to have led to high knee OA levels, with the joint tissues accumulating more damage than continuous loading [3]. Secondly, obesity and high body mass index (BMI) have become an “epidemic” in the western world, being a significant risk factor for the development of knee OA, possibly due to the combined effects of joint overload and inflammation [4].

The result of the above is the increased rates of total knee arthroplasty (TKA), as the definitive treatment for osteoarthritis of the knee. Typically, in the elderly patient alone in the United States, the per capita number of primary TKAs doubled from 1991 to 2010 [5,6]. Finally, the number of TKAs conducted annually in the United States is expected to increase by 143% by 2050 compared to 2012 [7,8].

The infrapatellar fat pad, also known as Hoffa's fat pad (HFP), is often removed, either partially or completely, during TKA to improve the surgeon's field in the upper tibia and may reduce the risk of soft tissue involvement, when placing the prosthesis. However, there are proponents of the opposite view, arguing that the "sacrifice" of HFP may jeopardize the perfusion of the patellar tendon, which may be reduced in the future.

The purpose of this review is to first present the importance and functions of HFP, as well as the latest data on the two conflicting views, the removal or not of HFP in TKA.

ANATOMY AND FUNCTION OF THE HOFFA'S FAT PAD

HFP is composed of fibrous adipose tissue and is located in the anterior compartment of the knee joint, constituting an intracapsular but extraarticular structure. It is bordered by

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the lower pole of the patella, the patellar tendon, the tibial plateau, and the femoral condyles. Adipose tissue (and the fat pad) is generally recognized as an endocrine organ, with sensory and immune functions capable of producing inflammatory mediators and growth factors. There is considerable evidence that HFP plays an important role in inflammatory processes associated with the pathology of the anterior surface of the knee, including OA [9,10]. In addition, the blood vessels that supply the patella and the patellar tendon travel through HFP, with the resection of the latter endangering the vascularity of these structures, with the ultimate result of the narrowing of the patellar tendon [11].

HFP has an abundant peripheral anastomotic perfusion network, with the superior medial and superior external iliac arteries providing 2 vertical arteries and connecting horizontally with 2-3 arteries located more distally [12]. In addition, there are rich local anastomotic branches to the menisci and patellar tendon onward, as well as to the periosteum of the tibia backward. Finally, anatomical studies revealed a large, mainly transverse contribution of vessels to the posterior surface and the central third of the patellar tendon, while the central area is relatively vascular [13].

Regarding HFP neurosis, the supply comes mainly from the posterior tibial nerve [14]. In addition, free nerve endings (type Via) have been identified in the fat pad. These free nerve endings may be activated by mechanical deformity or specific immunoreactive chemical agents and can send pain, pressure, and thermal stimuli to the central nervous system and are active through both "fast" and "slow" pain pathways [15]. The contribution of substance P to both pain generation and pro-inflammatory response induction is well known. Several authors report the presence of abundant substance P nerve fibers in HFP [12,16]. Moreover, in patients who experience anterior knee pain, the number of substance P nerve fibers in HFP increases significantly [12]. The presence and number of afferent immunoreactive nerve fibers as well as the ability to release pro-inflammatory cytokines have led to the hypothesis that HFP is a possible causative agent in patellar tendonitis [17]. Finally, there are indications that it plays a regulatory role in the inflammatory pathways involved in OA [18].

THE CONTRIBUTION OF HOFFA'S FAT PAD TO THE BIOMECHANICS OF THE KNEE

Studies have shown a positive correlation between HFP size and patient height, possibly leading to a biomechanical role in the knee joint [19]. In a study, it is reported that this structure facilitates the distribution of synovial fluid and may act to absorb various loads-actions created through the joint [20].

Patients suffering from anterior knee pain have been reported to show reduced coordination of motor units between the medialis and lateralis vastus muscles [21]. In one study, the authors concluded that after hypertonic saline injection into the fat pad, there was a significant subsequent activation as well as a reduction in the contraction width of the quadriceps muscle as the patient climbed the stairs [22]. This quadriceps inactivation is thought to increase patellar loading, which leads to an increased incidence of cartilage degeneration of the patellar articular joint [23]. Additionally, in a human cadaver model study, HFP resection was found to reduce medialization of the patella and reduce tibial rotation during bending [24]. Using the same model in a later study, the authors attempted to simulate swelling and the effect of the HFP [25]. The increase in simulated edema again reduced the area as well as the pressure of the patellar joint surfaces, while the measured pressure within HFP was also increased [26].

Finally, in a study that studied the biomechanical behavior of HFP in patients with OA, the results indicated the true biomechanical relevance of HFP in the knee as well as the loss of the proper "strain" behavior of HFP in OA knees, under mechanical loads [20].

THE ROLE OF HOFFA'S FAT PAD IN THE DEVELOPMENT OF OSTEOARTHRITIS

There is little scientific evidence of Hoffa's body's role in the development of osteoarthritis in the international literature. However, several reports are linking anterior knee pain syndrome to changes in the fatty body, such as hypertrophy, inflammation, and fibrosis [26].

Cartilage damage as well as a mechanical overload of the joints associated with abnormal movements, lead to the activation of mechanical receptors on chondrocytes and osteoblasts. The association between obesity and joint degeneration consists not only of deformed biomechanics but also of the ability of adipose tissue to activate the inflammatory process. In response to mechanical stimuli, cells activate inflammatory processes, resulting in the release of mediators causing further articular cartilage degeneration [26]. White adipose tissue is made up of highly specialized and well-differentiated cells and contains fat cells, fibroblasts, leukocytes, macrophages, and other cells that play a role in inflammation. In patients with OA, both the number of C fibers and the number of medium and large fibers containing substance P is greatly increased. The procedure leads to swelling, tissue ischemia, and partial necrosis. All of these mechanisms also cause changes in the structure and metabolism of HFP [26].

In patients with knee OA, HFP and synovial fluid contain significant amounts of inflammatory agents such as FGF-2, VEGF, TNF-α, and IL-6. Finally, HFP plays an important role in the onset and progression of OA through the activation and release of pro-inflammatory mediators.

EXCLUSION OF HOFFA'S FAT PAD DURING TOTAL KNEE ARTHROPLASTY: RECENT DATA

The exclusion, or not, of HFP during TKA, has been a common subject of research. A study conducted in the United Kingdom investigating the awareness of orthopedic surgeons on this issue had particularly interesting results [27]. Of the 173 respondents, 86.7% were consultants. 62.4% of the respondents underwent a partial resection of HFP, 23.1% underwent a complete resection while 9.8% of the surgeons maintained it. In addition, only 23% said they were aware of the proposed guidelines.

Studies supporting the maintenance of HFP during TKA report an increased risk of scarring and consequently a reduction in the length of the patellar tendon caused by disturbances of the anastomotic connections presented in HFP [28]. The resulting length reduction of the patellar tendon is considered to contribute to increased stiffness of the knee, patella, and impaired kinematics [24,28,29].

Respectively, resection of HFP is also thought to disrupt patellar perfusion and thus increase the fracture risk [30]. Increased pain is also reported to be associated with HFP resection [31]. The majority (62.4%) of surgeons in the above study performed a partial resection, while the majority also stated that adequate exposure to the outer part of the tibia was the main reason for total or partial resection. It is worth mentioning that in the same study several surgeons who preferred to maintain HFP stated that in cases of difficult exposure, they would proceed to a partial resection. A small number of surgeons considered that maintenance was associated with increased anterior pain postoperatively. This idea has been widely discussed, as HFP is believed to be involved in inflammatory processes, which makes it a possible source of anterior knee pain [32].

In a cohort study, the possible difference in the Insall-Salvati ratio of knees undergoing TKA was studied between the group of patients in which HFP had been retained and the one in which it had been completely removed [9]. The difference in the Insall-Salvati index (ratio) from the preoperative to the immediate postoperative period of the group in which HFP was retained compared to the group in which it was removed, showed a significant difference (p=0.010). However, the change in the Insall-Salvati ratio after 1-year of follow-up did not differ significantly between the groups (p=0.059). Therefore, this study does not clearly support any preference between the two techniques.

In another study involving 90 patients undergoing TKA, patients were divided into 2 groups (n1=45, n2=45) [31]. In the first group, patients underwent minimally invasive total knee arthroplasty (MIS TKA) with complete resection of HFP, while in the other group, 45 patients underwent MIS TKA without resection. The authors measured the reduction in patellar tendon length, knee flexion, anterior knee pain, Knee Society Score-KSS scale, functionality, and complications of the patella in preoperative and postoperative (6 weeks, 3 months, 6 months, and 1 year). The results of this study showed no significant differences in the reduction of patellar tendon length, KSS scale, functionality, or knee flexion in either group. However, patients with HFP resection had more anterior knee pain (8.3% vs. 0%, p=0.03, CI 95%, -0.007 to 0.174) at the end of the study, and no complications were found to be related to the patella in any group, concluding that surgeons should maintain HFP if excellent exposure and visibility can be achieved, but it could be removed if necessary to improve exposure during TKA.

An anatomical study of vascularization showed that the majority of HFP mass could be removed without causing injury to the lower part of the blood vessel network [33]. Discontinuation of HFP's vascularity may not affect the vascularity of the patella. The study by Pinsornsak et al. does not report any evidence of vascular necrosis or patella fracture in any patient with HFP resection [31,33].

In a prospective randomized study of 68 patients undergoing TKA for OA, no significant differences in the length of the patellar tendons were found from radiographs immediately after surgery, nor after 1 month or after 6 months [34]. This study demonstrated a statistically significant association between Hoffa adipose body fibrosis and anterior knee pain during the first six postoperative months in TKA. The authors also report that resection of HFP with severe fibrosis has a beneficial effect on pain at six months follow-up and, therefore, the authors recommend systematic resection of HFP during TKA.

In a retrospective observational study of 78 TKAs for OA, the researchers found a significant reduction of the patellar tendon in the group in which HFP was removed compared with the group in which it was maintained at 1-year follow-up [35]. These changes continued in all 3 years of follow-up. In another retrospective observational study of 720 patients (1055 TKA), the researchers found no differences in patellar tendon length before and after surgery but found increased postoperative pain in patients who had HFP resected [36]. In addition, a study of 1,401 patients (1,417 TKA) used the five-dimensional Oxford Knee Score and EuroQol questionnaire (Eq. 5D) to compare results for HFP resection and retention groups [37]. At 1 year follow-up postoperatively, those with the retained HFP had significantly better subjective pain scores and functionality scores compared to the group in which it was removed.

A systematic review of 2013, included studies that reported anterior knee pain, patellar tendon length, range of motion, patellar vascularity, or functional outcomes [38]. This review reports that HFP resection during TKA gives favorable outcomes in patients with OA. Particularly, after HFP resection, for patients with OA, no differences in functionality, range of motion, and anterior knee pain were found. Regarding patients with rheumatoid arthritis (RA), there was a tendency for greater discomfort and reduced functionality, while in patients with OA and RA there was a reduction in patellar tendon length.

Another subsequent systematic review, which included ten studies with a total of 10,163 patients, studied 6 parameters: knee pain, patellar vascularity, range of motion (ROM), patellar tendon length, trauma complications, and patient satisfaction [39]. There was no clear evidence of increased knee pain in patients undergoing fat resection during TKA. In addition, conflicting evidence was found regarding patellar vascularity and patellar tendon length after surgery, and insufficient evidence was found for differences in ROM. However, there is moderate evidence that HFP resection increases postoperative knee pain. It should be noted that this systematic review is limited by the lack of level 1 randomized clinical trials.

Another systematic review and meta-analysis conducted in 2016 included 7 studies (databases: Embase, PubMed, and Cochrane Library) and a total of 2,734 patients (3,258 knees) [40]. Excision tends to increase the incidence of postoperative anterior knee pain within 2 months postoperatively, compared with patients in whom HFP was retained ([OR] 2.12 [0.95, 4.73], p=0.07). In addition, there was an increased incidence of anterior knee pain in the resection group >12 months postoperatively, but the difference was not statistically significant ([OR] 3.69 [0.81, 16.82], p=0.07).

Reported causes of anterior knee pain include low HFP mass (due to previous surgery or injury), postoperative scarring, impingement syndrome, low patella position (patella baja), and misalignment of the patella, while excessive secretion of inflammatory mediators is also recognized as a causative agent [41-43]. There was also a tendency for a greater reduction in the length of the patellar tendon in the resection group, while no significant results were found regarding the postoperative function of the knee. Reduction of the patellar tendon is one of the most common complications after TKA. There is a tendency reported in the literature for a greater reduction in patellar tendon length in the group of patients with HFP resection compared with the conservation group. Many factors are responsible for the reduction, including scarring/thinning of surrounding soft tissues, disruption of the surrounding arterial network, and quadriceps muscle weakness [40,44]. The cavity formed after HFP excision is filled with fibrous tissue, while its preservation reduces scar formation/shedding. Based on the results of previous studies, maintaining HFP not only reduces the cessation of patellar tendon perfusion but also reduces the incidence of scarring and patellar tendon rupture. In addition, reduction of the patellar tendon is also responsible for postoperative anterior knee pain and misalignment of the patella [25]. In conclusion, the above systematic review and meta-analysis report that HFP retention has better results than resection in patients undergoing TKA due to the relatively lower rate of pain after short-term follow-up.

In a recent systematic review on whether or not to maintain HFP, published in 2019, including seven studies (two of which were randomized clinical trials) involving 2,815 patients (3,312 knees), the authors focused on the length of the patellar tendon, postoperative pain, KSS scale, and knee function [45]. The meta-analysis identified a tendency for a larger reduction of the patellar tendon after the HFP resection. The systematic review also showed a tendency that the HFP resection group was related to a greater reduction in patellar tendon length. When HFP is injured, there are fibrotic lesions. The fibrous tissue bands pass through this area and form a rigid structure [45]. This fact, in turn, leads to a narrowing of the patellar tendon. Excision was associated with a lower incidence of postoperative pain at one to two months of follow-up; however, at three to six months the pain scores were higher in this group. HFP is believed to play a role in the inflammatory process and contains pain nerve fibers and is, therefore, a possible source of anterior knee pain. In conclusion, its maintenance may explain the tendency of pain to increase at the beginning of one to two months postoperative period in the preservation team. At three to six months, this tendency is reversed with a higher incidence of knee pain in the resection group. One possible explanation for this may be due to the remaining nerve fibers injured during TKA as a source of ongoing pain in the resection group. Finally, no statistical difference was found in either the KSS scale or the functionality score.

Last but not least, in the most recent meta-analysis conducted in 2020 [46], 9 randomized controlled trials, involving 783 TKAs (722 patients), were included. The meta-analysis identified a trend regarding the shortening of the patellar tendon after HFP resection at both 6 months and 12 months. As far as the Insall-Salvati ratio, anterior knee pain within 6-12 months, Knee Society Scores at 12 months, and knee flexion within 6-12 months are concerned, no statistical difference between HFP resection and HFP preservation was found. Consequently, the authors of the above-mentioned meta-analysis reported that surgeons are advised to keep the HFP as far as sufficient exposure is achieved [46].

CONCLUSION

There are currently no formal guidelines for maintaining or resecting HFP during TKA. Removal versus retention during TKA is a controversial issue with the available literature not convincing. This review argues that retention is associated with an increased incidence of anterior knee pain up to two months postoperatively, while resection tends to reduce the length of the patellar tendon as well as a higher incidence of moderate to anterior knee pain. It would be preferable to make an effort to maintain HFP, but its preservation mustn't cause visibility and access issues during TKA.

However, the management of HFP during TKA is the subject of ongoing discussion without clear conclusions. The choice of complete resection, retention, or partial resection varies depending on the surgeon's preferences, experience, and patient needs. Last but not least, further studies of high quality and randomized clinical trials are needed to provide better guidance on the management of HFP during TKA.

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ORIGINAL ARTICLES

Simulation and dynamic analysis of military marching using lower limbs anthropometric data

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Abstract: *Background: Gait analysis is receiving increasing attention due to various applications in athletic performance, man-machine interfaces and especially in military services. This analysis involves the analysis of human locomotion augmented by body movements and biomechanics of joints. The kinematic motion of the body during a gait cycle capturing by cameras is then used as the desired target for modelling the motion of body segments. By taking advantage of gait analysis concept, this study aims to model the military marching, using anthropometric data with the focus on lower limbs while introducing top candidates with better healthy conditions in lower limb joints during a cycle of marching.*

Methods: Using 100 anthropometric data from military soldiers, equations of motion for the model are derived by applying Lagrangian methods in an inverse dynamic approach. In this model, the joints are simulated using springs and dampers while the actuators, simulated the muscles, acted like motors and applied enough torque on joints so that the model motion replicates normal military marching. Finally, all the springs and dampers coefficients are driven from optimization process.

Results: Hip, knee and ankle torques were calculated after the optimization process for all 100 soldiers and then 5 candidates among them were established with less suffering forces and torques in their joints.

Conclusions: In this study using biomechanics basics and anthropometry data at the same time, a standard could be evaluated to select the soldiers based on healthy condition of lower organs.

Keywords: *marching, anthropometric data, gait analysis, biomechanics, torque, equations of motion, optimization*

INTRODUCTION

Marching is a specific sample of daily human gait [1]. That being said, in order to simulate marching, simulation of human gait cycle is required in which we should benefit from priori biomechanical knowledge on human motion and anthropometric data at the same time [2]. The gait cycle consists of complex functional tasks requiring interaction between lower limb joints of the body [3]. Motion of body segments during this interaction is studied as a part of kinematics without considering any forces. Typically, camera systems and electromagnetic devices are being used to record the motion of the body during a cycle [4].

While the body segments are considered as a mechanism, joints in hip, knee and ankle provide degrees-of-freedom

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(DOF) of the system. The joint reaction forces and muscle net torques convert this problem into a dynamic analysis in which inverse dynamic method is being used commonly. Then, equations of motion relate the kinematics of the body motion to the forces and torques that are causing those motions [5]. Friction force calculation completes this set of equations as an external force. This force during a walking depends highly on the foot-ground contact simulation and modeling. Some of the studies used additional kinematic joint while other used temporal fixing to the support foot. But in some studies, realistic models for this section have been modeled which can simulate the reaction close to the real foot-ground contact [2, 6]. Some studies conduct the whole gait simulation by taking advantage of three dimensional models [7, 9] while others consider two dimensional models [3,10,11] for simulation [4,8]. Stability of such models is significantly important. Active feedback control systems [12] and simple PDI controller [10] are common methods used as a controller for the balance of gait models during a cycle.

Application of gait analysis in military services mainly limits to assessing the influence of boot [11, 13] and carrying a backpack of soldiers during a gait cycle. Rare studies like [1] demonstrated one of the first researches on marching locomotion. As other applications of gait analysis, there have been some studies about modeling the gait analysis in order to analyze the dynamic of prosthesis during a gait cycle [3, 10, 14, 18, 19, 20].

Towards simulation of marching, this paper conducts this analysis by computing the internal actuator forces and dynamic analysis of 100 soldiers. The major part of this simulation is to replicate normal kinematics of marching when subjected to normal muscle forces and joint torques during a cycle. In an inverse dynamic approach, using Lagrangian method the net joint torques is calculated from a sample military marching benchmark and external forces in a condition that muscles are the actuators of the musculoskeletal system. The optimization process then is needed to compute the forces and the torques in a way to generate the desired kinematic motion.

METHOD

From the engineering point of view, marching is in fact body segment movements in a mechanism way with couple of degrees of freedom (DOF). This mechanism consists of hip joint, knee joint and ankle joint. There is friction force between foot and ground which provides movement of the whole body. In this simulation, bones are considered as rigid elements while supporting the weight of the body and muscles act as elastic stimulators and displace the bones.

The appropriate method in studying the simulation of marching is by applying dynamic models. Using this method, the model was considered as a rigid body with several links. Considering exact and complete number of variables for dynamic simulation complicates the model and motion equations, therefore in this study some assumptions were employed to simplify the model. These assumptions include 1) the upper section of the body (head, trunk and hands) was considered as one link only, 2) friction was neglected in joints, 3) the weights of the elements were equally distributed, 4) modelling and solving equations of motion conducted in sagital plane (2D), 5) due to 2D modeling, joints were from hinge type and not spherical for hip joint.

The model for rigid body motion of a soldier contains seven links that include trunk, two thighs, two legs and two feet. The model was defined with nine generalized coordinates as consisting of seven links with vertical angles and hip coordinates in Cartesian coordinate system (Figure 1). In this model, the angles are trunk, right thigh, right leg, left thigh, left leg, right foot and left foot angles with vertical direction while the joints are hinge joints.

Figure 1. The model used in simulation with seven angles and hip coordinates

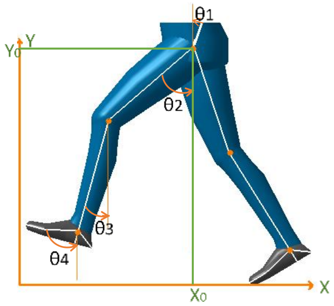

One of the most important issues in this dynamic simulation is modeling the reaction force of the ground to the body. The modeling of this section was developed based on using a spring and a damper in contact points of foot to the ground so that the equivalent of damper and spring forces reflects the ground reaction force to the body [16]. This estimation helps to calculate this reaction force during dynamic simulation and to simulate the viscoelastic contact of the foot. As it can be seen in Figure 2, a series of vertical springs and dampers were attached to the contact points to generate this vertical reaction force [10]. In the case of no contact between foot and ground, the spring and damper contacts are disconnected and no force is reacted and whenever the contact between foot and ground occurs again, the spring and damper contacts are connected and so the force is reacted.

Figure 2. Foot-ground contact force simulated by spring and damper

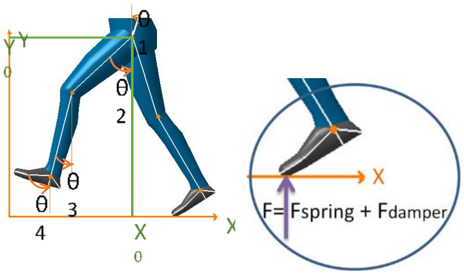

In this model, the muscles are neglected and instead, actuators are employed in hip, knee and ankle joints. The torque being applied on the joints is a function of current angle in joint, desired angle and their derivatives.

Indeed, springs and dampers are used in joints so that the actuators act as motors which are linked with these springs and dampers. Therefore, appropriate modelling is needed for calculating the optimized variables for springs and dampers. The general equation for torque in the joints is

$$\tau_i = K_i\left(\theta_i^m - \theta_i^d\right) + C_i\left(\dot{\theta}_i^m - \dot{\theta}_i^d\right)$$

In which K_i and C_i are constant coefficients, θ_i^m and $\dot{\theta}_i^m$ are angle and angular speed of links in each time frame, θ_i^d and $\dot{\theta}_i^d$ are desired angle and angular speed of the links.

Spring and damper are key factors in stability of the model during marching. If these factors are neglected, any small disturbance in the model at the contact time leads to instability of the whole system. On the other hand, calculating the optimized coefficients requires desired kinematic data and stability of whole model.

Table 1. Statistical parameters of all 100 anthropometric data of soldiers

Data	Waist height *(mm)*	Knee height *(mm)*	Weight *(kg)*	Height *(mm)*
Minimum	820	410	52	1,615
Maximum	1,152	602	78	1,875
Average	985.91	516.91	67.55	1,738.55
SD	58.12	35.94	7.07	60.17

In this study, anthropometric data of 100 military soldiers were analyzed through the dynamic simulation method. Table 1 shows the values of min, max, average and standard deviation parameters for all 100 soldiers. Using waist height and knee height, thigh length was calculated and based on the lengths of segments, the weights of segments were approached [15] (Table 2).

Table 2. Standards for mass calculation for body segments

Segment	Center of mass/segment length		Density *(Kg/l)*
	Proximal	Distal	
foot	0.5	0.5	1.1
leg	0.433	0.567	1.09
thigh	0.433	0.567	1.05
trunk	0.5	0.5	1.3

While the anthropometric data varies from one soldier to another, the kinematic data is the same for all soldiers. The kinematic data must be considered the same for all since the ending result of joint torques for the model varies for various kinematic data but this study targets to analyze the joint torques only based on anthropometric data, so that as a result a standard of anthropometric data could be concluded.

Therefore kinematic data of a marching benchmark (Figure 3) was considered for all soldiers.

Figure 3. Marching benchmark used in kinematic section of this study

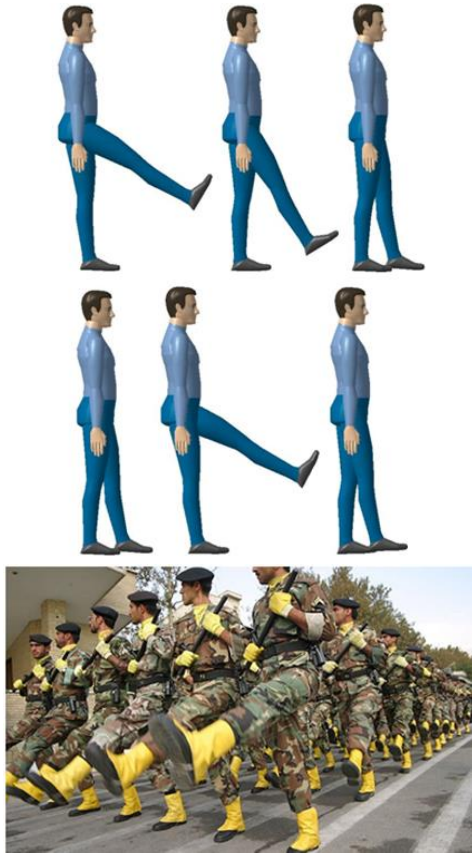

As this figure depicts, right thigh and leg are raised to specific height in an extension movement and then in a felxion movement, right foot makes contact with the ground and then left segments follow the same path alternatively. Knee angle was considered the same as hip angle which means that the knee by itself does not have any flexion-extension movement.

Kinematic data includes, 1) angular speed and angle of hip joint 2) angular speed and angle of knee joint 3) angular speed and angle of ankle joint 4) angular speed and angle of trunk. Figure 4 shows the angle of hip and ankle during one cycle of marching. Fourier function was used in order to generate a curve passing through acquired kinematic data points.

Figure 4. Hip and ankle angles during a gait cycle of the benchmark

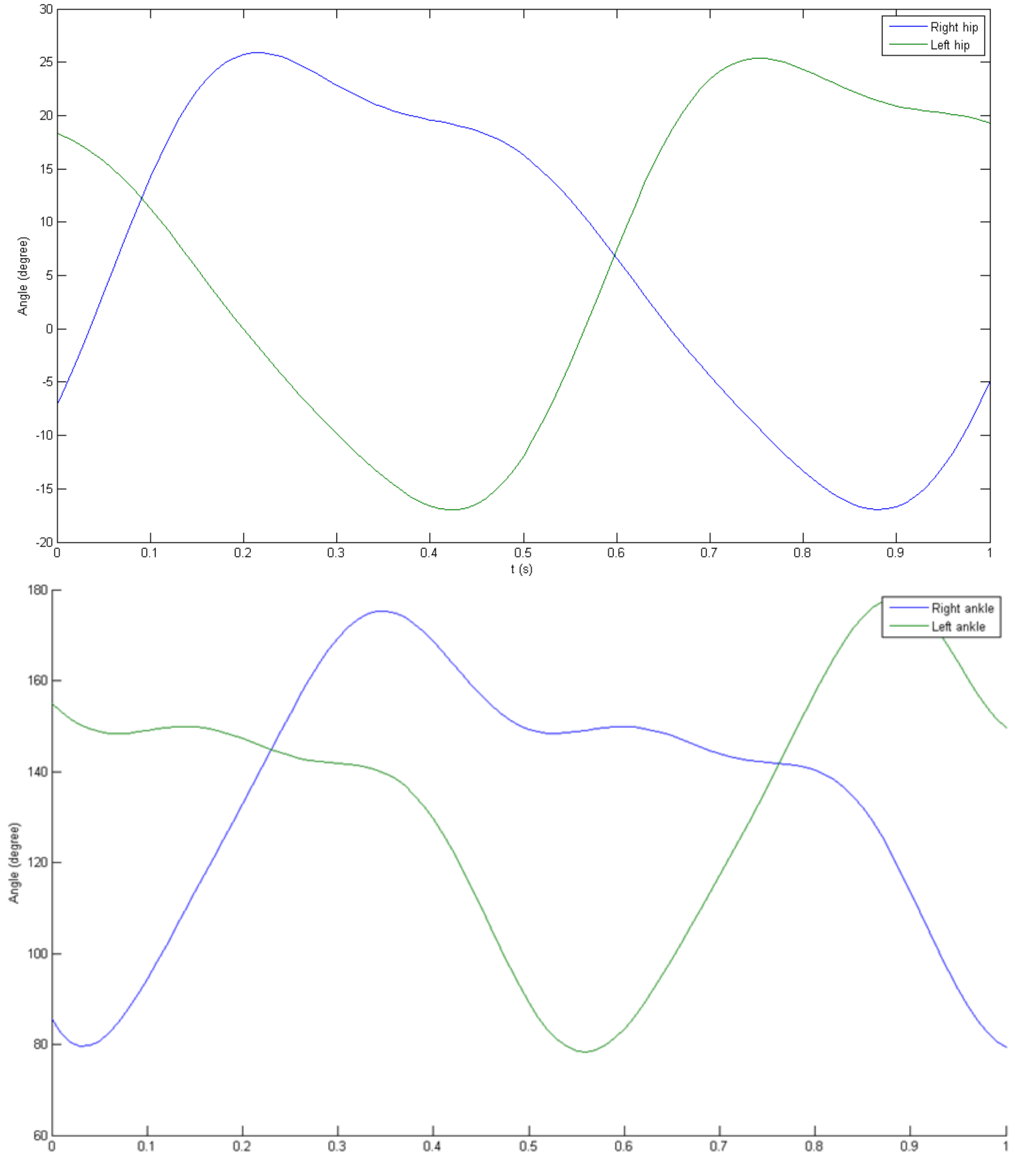

In order to complete the modeling of marching, adding a parameter to dynamic simulation is needed for keeping the balance of the model. To accomplish this step, a torsion spring and a linear damper are applied to the trunk as a function of simulated and desired kinematic of trunk. Whenever the model is contacting the ground half of the total torque in this torsion spring and damper is transferred to each hip joint to keep balance of the whole model.

As it was mentioned in previous section, the equations of motion for this 2D model follow Lagrangian method. General formulation of the Lagrangian method is

$$\frac{\mathrm{d}}{\mathrm{dt}}\left(\frac{\partial \mathrm{L}}{\partial \dot{\mathrm{q}}_{\mathrm{k}}}\right)+\frac{\partial \mathrm{L}}{\partial \mathrm{q}_{\mathrm{k}}}=Q_k$$

In which k is number of general coordinates which is 9 in this model, L is the difference between kinetic and potential energy of the whole system, Q_k reflects non-conservative forces. In order to use these equations, initially kinetic and potential energy of the whole system were calculated and then after 9 equations of the order of 2 were produced. For the dynamic simulation using this method, initial conditions and angular speed of the links were provided.

Constant coefficients in all joint torques (discussed in previous sections) could be generated in an optimization process. The goal of optimization is to calculate these coefficients in the condition that the discrepancy between flexion-extension angles of joints in simulated and desired circumstances is minimal. The desired (normal) values for this simulation were obtained from [17]. In order to conduct this optimization, genetic algorithm method was applied to decrease the difference between angles in desired and simulated modes. While in this optimization variables are the constant coefficients, the objective function is the summation squared of the difference between these angles. Below are the steps that the genetic algorithm takes to find the optimization coefficients:

- ✓ Initial parameters are provided as the initial condition of the problem,
- ✓ Initial guess of the spring and damper coefficients of the model is provided,
- ✓ Based on the values from steps 1 and 2, the equation of the motion is formed,
- ✓ From this equation of the motion, the angles function of the model is derived,
- ✓ By having the angles of the model and desired angles (from the literature), the objective function is found,
- ✓ The genetic algorithm comes to play and starts to minimize the objective function by finding the optimized values for spring and damper coefficients of the whole model
- ✓ Once the optimized coefficients are found from the step 6, the values are replaced with coefficients of step 2 until final optimized converged coefficients are obtained.
- ✓ Return to step 3.

RESULTS

The numerical programming for dynamic simulation of a soldier was accomplished in MATLAB software. After providing all necessary parameters such as initial conditions, kinematic data, equations of motion and optimization, the whole program focused to generate the constant coefficients using the optimization method for a sample anthropometric data. Then, whole 100 anthropometric data was given to the program to calculate the joint torques in hip, knee and ankle. In fact this torque value is equivalent to maximum total torques acting on that specific joint. Table 3 shows the statistic about resultant torque values.

Table 3. Resultant torques in hip, knee and ankle joints

Segment	Hip torque *(N.m)*	Knee torque *(N.m)*	Ankle torque *(N.m)*
Min	20.96	0.06	1.73
Max	22.94	1.37	3.50
Average	21.65	0.27	2.61
STD	0.33	0.19	0.37

Table 4. Top 10 candidates with lowest toque amount in hip joint

Hip torque *(N.m)*	Knee torque *(N.m)*	Ankle torque *(N.m)*	Waist height *(mm)*	Knee height *(mm)*	Weight *(Kg)*	Height *(mm)*
20.98	0.15	2.42	990	480	70	1,775
20.99	0.12	2.36	975	500	62	1,705
21.00	0.12	2.36	990	480	70	1,735
21.03	0.31	1.88	880	490	60	1,632
21.04	0.35	2.98	970	550	75	1,797
21.05	0.11	2.55	995	500	66	1,730
21.08	0.33	2.25	1,110	575	70	1,780
21.09	0.21	2.13	1,015	570	75	1,788
21.10	0.55	2.61	1,040	500	52	1,700
21.11	0.11	3.11	1,100	565	85	1,895

In the initial analysis of this huge data, we focused on hip data. Since in this study having healthy joints are more desirable, we sorted the data from small to large values and then just consider the first top 10 as good candidates for healthy joints (Table 4). This narrowing down of the results helps us to analyze the data more consistently.

Moreover, Figure 5 shows how the waist height and knee height vary by the torque in the hip. It is interesting that both heights increase by gradually increasing of the hip torque. This fact is confirming that higher waist and knee heights can lead to larger torques in the hip.

Figure 5. Variation of waist and knee heights by hip torque increasing in top 10 candidates with lowest torque amount in hip joint

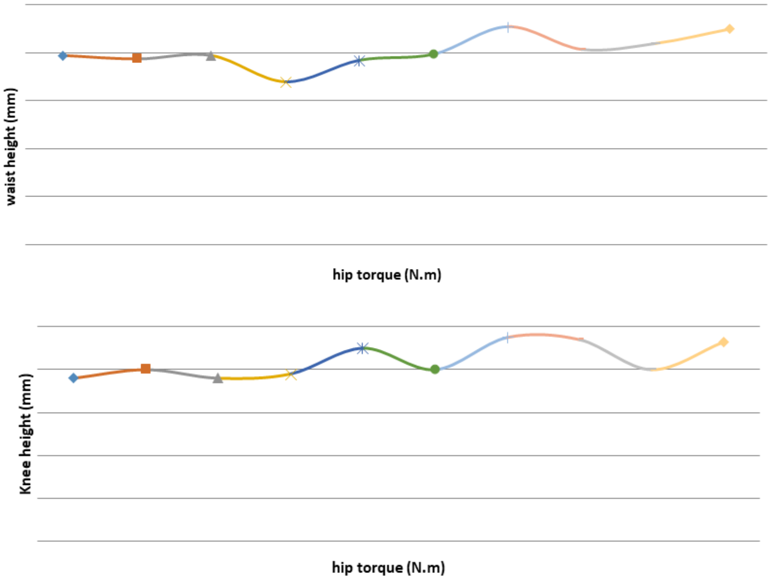

In the next step, knee torque was assessed by the numerical programming code in MATLAB and the results were again sorted from small to large and first 10 candidates were selected. This selection was done independently of previous selection for the hip. Indeed, this study approached the joint torques behaviour separately and then concludes all the resultant data together. The corresponding knee data can be found in Table 5.

Table 5. Top 10 candidates with lowest torque amount in knee joint

Hip torque *(N.m)*	Knee torque *(N.m)*	Ankle torque *(N.m)*	Waist height *(mm)*	Knee height *(mm)*	Weight *(Kg)*	Height *(mm)*
22.15	0.02	3.74	1,152	602	79	1,915
22.04	0.03	4.27	1,030	530	73	1,805
21.79	0.04	3.25	900	490	72	1,705
21.80	0.05	3.69	980	500	72	1,743
22.05	0.05	4.07	1,080	520	68	1,805
21.60	0.06	3.35	975	480	63	1,642
21.83	0.06	3.48	1,000	480	78	1,745
21.25	0.06	2.69	1,000	540	65	1,780
21.25	0.07	2.69	998	510	57	1,695
21.93	0.07	3.91	1,000	525	75	1,710

Comparing the torque values in knee joint to other two joints, it can be concluded that for this specific kinematic data for marching, the torque in knee is negligible. Therefore, for the ending result of this study, knee did not play a major role and that is because flexion – extension movement was not considered for the knee.

Ankle torques is the next criteria considering as a result. Table 6 represents the torques in ankle joint during a full cycle of marching for the top 10 candidates who their ankle torques were minimal independent from other joint torques.

Table 6. Top 10 candidates with lowest torque amount in ankle joint

Hip torque *(N.m)*	Knee torque *(N.m)*	Ankle torque *(N.m)*	Waist height *(mm)*	Knee height *(mm)*	Weight *(Kg)*	Height *(mm)*
21.22	0.08	1.75	1,070	520	78	1,835
21.03	0.31	1.88	880	490	60	1,632
21.36	0.47	1.95	1,148	600	75	1,900
21.63	0.11	1.98	994	500	71	1,766
21.63	0.50	2.08	945	475	72	1,680
21.09	0.21	2.13	1,015	570	75	1,788
22.07	0.13	2.15	1,040	530	56	1,708
21.58	0.35	2.18	930	480	69	1,720
22.01	0.22	2.23	1,010	535	80	1,758
21.08	0.33	2.25	1,110	575	70	1,780

Comparing the variation of the ankle torque versus waist and knee heights depicts the fact that ankle torque is highly dependent on the force being applied by the ground, since the waist and knee heights are almost constant with the variation of ankle torque (Figure 6). In fact the amount of ground force reaction plays the major role in the torque of the ankle.

Figure 6. Variation of waist and knee heights by ankle torque increasing in top 10 candidates with lowest torque amount in ankle joint

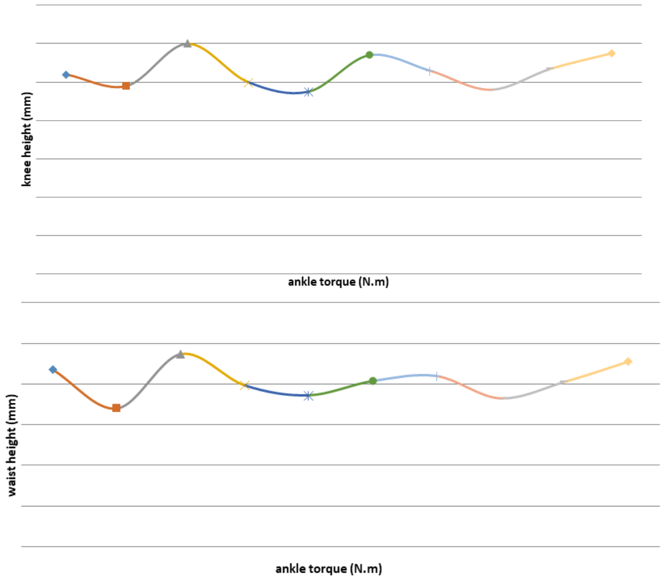

DISCUSSION

In order to assess the healthy joints under marching condition, 2 factors were considered in this study: 1) hip torque and 2) ankle torque. Then based on the torque data of each joint, the whole data was sorted from smallest to largest value three times. Then, first 10 candidates in each section were highlighted and analyzed. In the first look, it seems like 20 different candidates with identical anthropometric data have been introduced, therefore it is needed to define another factor in order to select from those candidates, those with better healthy conditions in terms of minimum torque value in their joints. Due to less torque rate in knee comparing to hip and ankle, a new factor called "Minimal Summation Torque" is defined for this study which follows below equation, considering the fact that the maximum torque in hip is 22.39 N.m and in ankle is 4.35 N.m.

$$minimal\ sumation\ torque\ = \frac{hip\ torque}{maximum\ hip\ torque} + \frac{ankle\ torque}{maximum\ ankle\ torque}$$

By using the maximum values of torques we normalized our torque data. Then, we needed to calculate this factor beside other factors for all 100 data and sort again based on minimum value of torques. Table 7 shows this result.

Table 7. Top 10 candidates with lowest Minimal Summation Torque

Hip torque *(N.m)*	**Knee torque** *(N.m)*	**Minimal Summation Torque**	**Waist height** *(mm)*	**Knee height** *(mm)*	**Weight** *(Kg)*	**Height** *(mm)*
21.22	1.75	1.35	1070	347	78	1835
21.03	1.88	1.37	880	325	60	1632
21.36	1.95	1.40	1148	325	75	1900
21.63	1.98	1.42	994	347	71	1766
21.09	2.13	1.43	1015	337	75	1788
21.63	2.08	1.44	945	336	72	1680
21.08	2.25	1.45	1110	348	70	1780
21.58	2.18	1.46	930	334	69	1720
21.34	2.26	1.47	980	296	72	1710
21.23	0.33	2.28	1005	330	66	1722

Analyzing Tables 4, 6, 7 reveals that there is a common point between all of them. There are some soldiers that exist in all those tables, showing that these candidates are common among all other candidates. This approves that this set of candidates contain a healthier body during a cycle of marching. We highlighted these candidates in all tables and allocated one colour to each of them. Table 8 represents all of them in one separate table containing corresponding anthropometric data.

Table 8. Top 5 candidates with healthier joint condition during a cycle of marching

Waist height *(mm)*	**Knee height** *(mm)*	**Hip circumference** *(mm)*	**Knee circumference** *(mm)*	**Ankle circumference** *(mm)*	**Weight** *(Kg)*	**Height** *(mm)*
880	490	990	416	230	60	1632
1148	600	990	460	230	75	1900
1015	570	940	380	250	75	1788
945	475	940	400	250	72	1680
1110	575	980	410	270	70	1780

In fact, in this study using biomechanics basics and anthropometry data at the same time, a standard could be concluded to select the soldiers based on healthy condition of lower organs.

This study is one of the first studies being conducted in the area of specifying a standard for soldiers based on their lower organ healthy condition during a cycle of marching.

Conflict of interest

The authors have no conflicts of interest.

Authors' contributions

Dr. Shakibaee provided assistance for all experiments and collecting anthropometric data. Dr. Asgari supervised this study and provided assistance in manuscript preparation. Dr. Kamal Mostafavi carried out the modelling, coordinated the study, completed the analysis, extracted the results and prepared the manuscript. Dr. Pourtaghti and Dr. Ebrahimpour assisted and coordinated the processing of data collection. All authors have read and approved the content of the manuscript.

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ORIGINAL ARTICLES

Spatio-temporal analysis of diabetes in the Iranian military community during the period 2015-2018

Mohammad Fathalipour[1], Yousef Alimohamadi[2], Ali Mirmohammadlou[1], Mojtaba Sepandi[1,3]

Abstract: *Introduction: diabetes mellitus (DM) is one of the most prevalent non-communicable diseases. Spatio-temporal study of DM has a main in the design of disease prevention programs. The present study aimed to describe the spatio-temporal distribution of diabetic patients in the Iranian military community.*

Methods: In this ecological study, all registered cases of DM in the Iranian military community (military officers and enrolled conscripts) were considered during the period 2015-2019. Getis-Ord Gi statistics was applied to identify the DM hotspots. Spearman Correlation Coefficient was used to perform trend analysis of the disease.

Results: The number of DM incident cases in the military officers and enrolled conscripts was 1135 and 85, respectively. Isfahan (n=153) and Tehran (n=67) for the military officers and Zanjan (n=17) and Kurdistan (n=10) for the enrolled conscripts were the provinces with the highest number of identified DM cases. In the study period, no significant hotspots were detected in the military officers; However, an increasing trend of DM was observed (CC= 0.935, p<0.001). The DM hotspots detected in enrolled conscripts were as follows: 2015 – Qom (95% CI); 2016 – Lorestan, Qazvin, and Guilan (90% CI); 2017 – Ilam, Hamadan, and Markazi (90% CI); and 2018 – Ilam (95% CI), Alborz, Qom, Markazi, Lorestan, Kermanshah (90% CI), and no significant trend was noticed nonetheless (CC=-0.160, p=0.555).

Conclusion: It appeared that Northern and North-Western Iran were high-risk areas for DM in the military population and should be considered in designing the diabetes prevention and control program. Moreover, the determination of DM risk factors in the aforementioned areas could play a crucial role in the prevention of the disease.

Keywords: *diabetes, military, spatio-temporal, Iran*

INTRODUCTION

Diabetes mellitus (DM), a major glucose metabolism disorder, has become a global healthcare challenge. Estimates show 463 million people were suffering from DM in 2019 worldwide, rising to be 700 million by 2045 [1]. The annual global health expenditure on this disease is roughly USD 760 billion [2]. DM results in a number of complications and significantly affects the life quality of patients [3, 4].

This global epidemic has changed to the primary concern in many countries, especially in Iran [5]. Based on the reports, the prevalence of DM in Iran increased from 7.7% in 2005 to 10% in 2016 [6]. Moreover, the annual medical cost of patients with DM is about three times higher than the general population in Iran [7].

DM is a heterogeneous disease arising from a complex interplay of various tissues and different genetic, epigenetic, and environmental factors in a dynamical manner. Among these risk factors, occupation plays a critical role [8, 9]. The relationship between occupational factors and the incidence of DM takes the interest of many researchers [10]. The military community is prone to develop cardiometabolic syndrome because they have unique lifestyles and expose to stressful situations [11-13]. This evidence warrants a more comprehensive study of monitoring and screening needs to reduce the incidence and burden of DM among the

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population.

Our research has identified few studies in the literature about the spatio-temporal distribution of DM in the Iranian military community. Hence, the present study aimed to detect regional spatial clusters of DM, as well as to carry out a temporal trend analysis of incident cases in the Iranian military community using spatio-temporal analysis in the period 2015-2018.

METHODS

Study area and time

This study was conducted in Iran, the second-largest country in the Middle East, with a total area of 1,648,195 km^2 and a total population of 83 million. Iran is divided into five regions with 31 provinces.

This country is bordered to the northeast by Turkmenistan, to the northwest by Armenia and Azerbaijan, to the west by Turkey and Iraq, to the South by the Persian Gulf and the Gulf of Oman, to the southeast by Pakistan, and to the east by Afghanistan.

Study design

This is an ecological study, and all registered DM cases among military officers and enrolled conscripts in the Iranian military community during the period 2015-2018 were described and analyzed.

Spatial analysis

The distribution of DM cases in the Iranian military community was described using a Choropleth map. Spatial analyses were performed using ArcGIS 10.5 (ESRI, Redlands, CA, USA) and descriptive analyses using Excel 2010.

Hotspots identification

The spatial clusters of DM were identified using Hotspot analysis. Getis-Ord Gi statistics were applied to detect the hotspots. A high score on the index combined with a lower p-value indicates the clustering of DM. The Gi statistics were calculated based on our previous study (14).

Temporal analysis

The trend analysis of the incident cases of DM during the period 2015-2018 was assessed using Spearman Correlation Coefficient.

RESULTS

Descriptive statistics and spatial distribution

The number of identified DM cases in the military officers and enrolled conscripts was 1135 and 85, respectively, during 2015-2018 (Figure 1). Considering the distribution of DM cases by province, the highest number of diabetic participants in 2015 were seen in Isfahan (n=110) and West Azerbaijan (n=66) in military officers and Zanjan (n=17) in enrolled conscripts. Isfahan (n=120) for military officers and Tehran (n=6) as well as Alborz (n=5) for enrolled conscripts were the provinces with the highest number of DM cases in 2016. In 2017, the highest frequency of DM cases was reported from Isfahan (n=125) and Teheran (n=67) for military officers, and from Ilam (n=3), Hamadan (n=3), and Tehran (n=2) for enrolled conscripts. The incident cases in 2019 were high in Isfahan (n=153) and Tehran (n=65) for military officers and Kurdistan (n=10) and Hamadan (n=5) for enrolled conscripts compared to the other provinces. Geographical distribution of DM

Figure 1: Incident cases of DM in the Iranian military community by province during the period 2015-2018

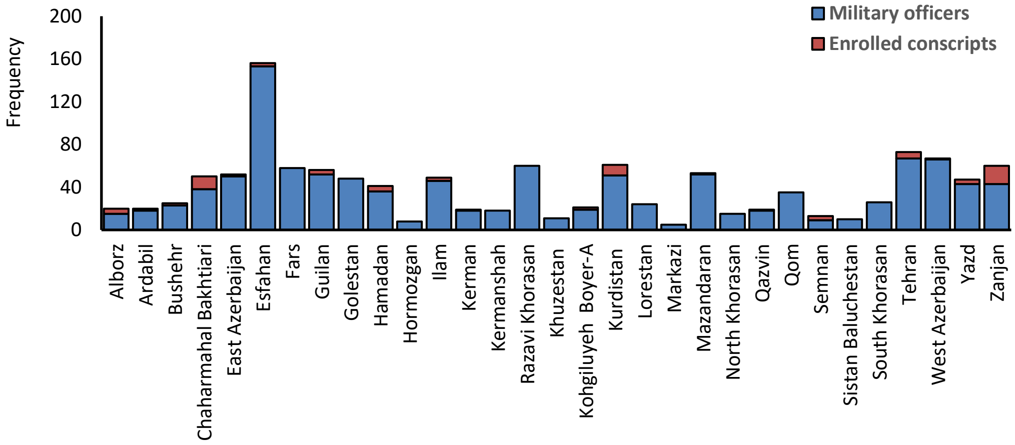

cases in military officer and enrolled conscripts were illustrated in Figure 2 and 3, respectively.

Figure 2: Geographical distribution of DM cases in the military officer during the period 2015-2018

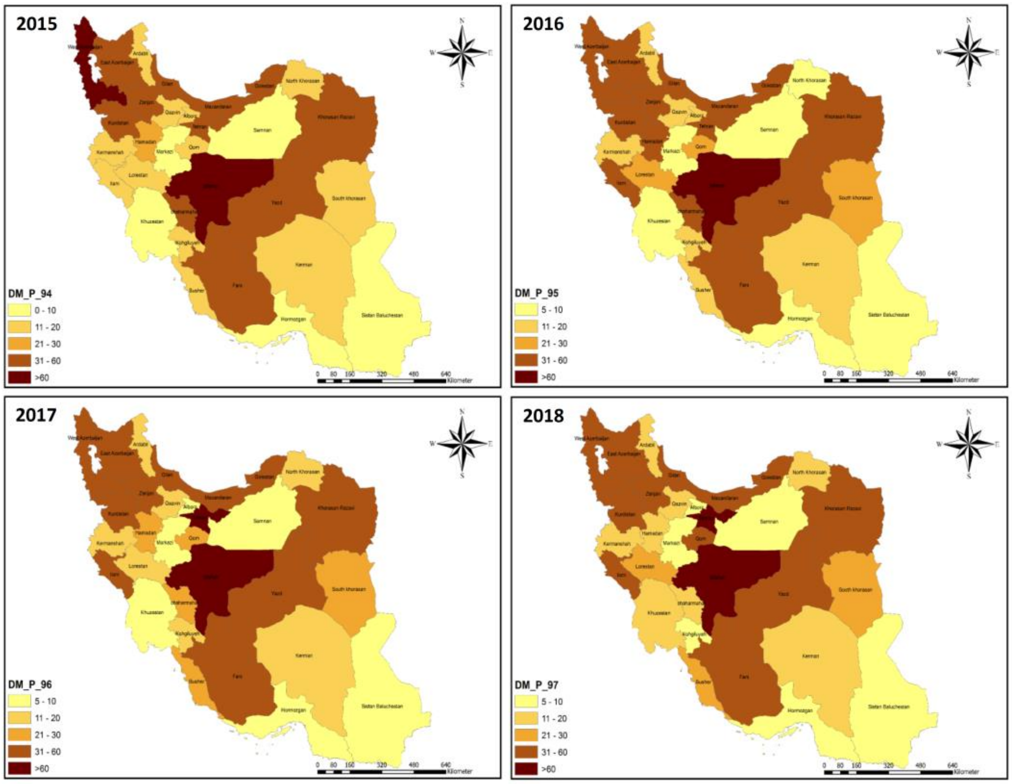
2015
2016
2017
2018
DM_P_94
0 - 10
11 - 20
21 - 30
31 - 60
>60
DM_P_95
5 - 10
11 - 20
21 - 30
31 - 60
>60
DM_P_96
DM_P_97
0 80 160 320 480 640 Kilometer

Figure 3: Geographical distribution of DM cases in the enrolled conscripts during the period 2015-2018

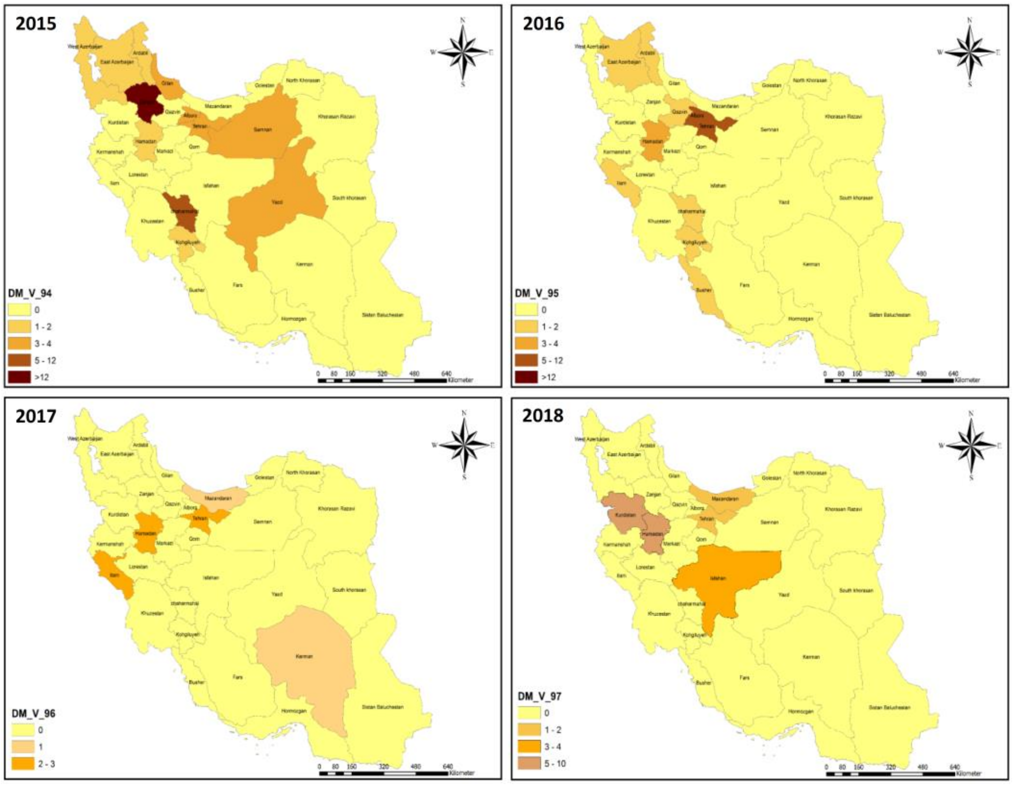
2015
2016
2017
2018
DM_V_94
0
1 - 2
3 - 4
5 - 12
>12
DM_V_95
DM_V_96
0
1
2 - 3
DM_V_97
0
1 - 2
3 - 4
5 - 10
0 80 160 320 480 640 Kilometer

Figure 4: Geographical distribution of DM hotspots in the military officers during the period 2015-2018

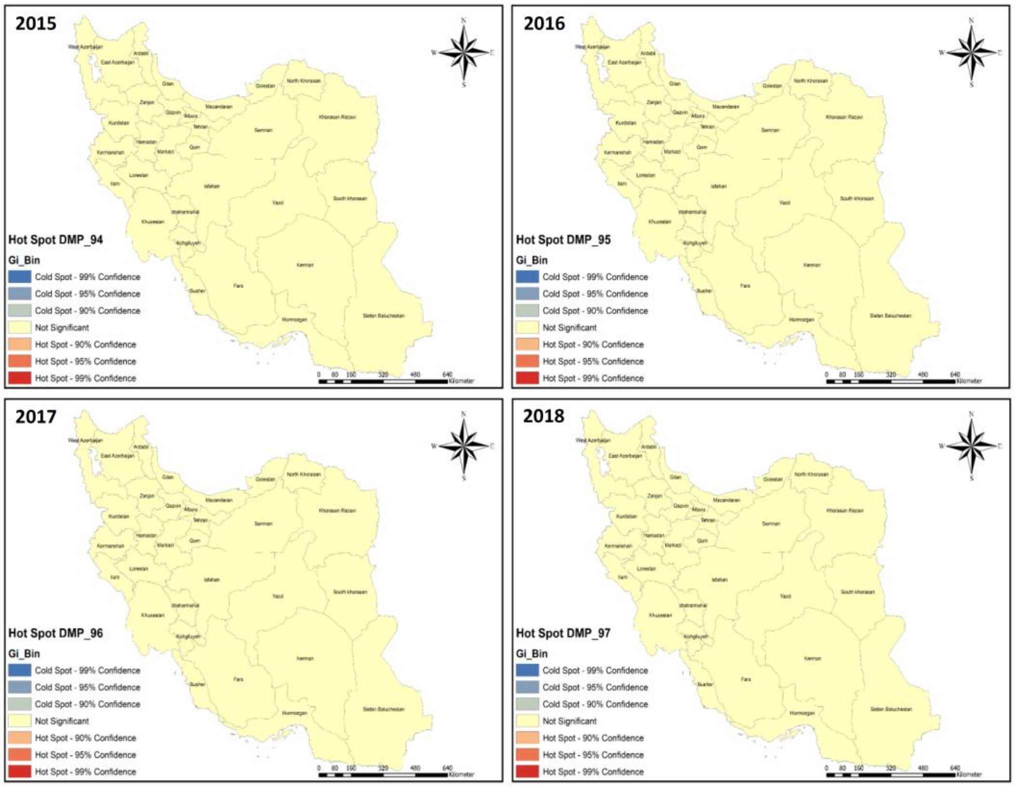
2015
Hot Spot DMP_94
2016
Hot Spot DMP_95
2017
Hot Spot DMP_96
2018
Hot Spot DMP_97
Gi_Bin
Cold Spot - 99% Confidence
Cold Spot - 95% Confidence
Cold Spot - 90% Confidence
Not Significant
Hot Spot - 90% Confidence
Hot Spot - 95% Confidence
Hot Spot - 99% Confidence
0 80 160 320 480 640 Kilometer
N
S
E
W

Figure 5: Geographical distribution of DM hotspots in the enrolled conscripts during the period 2015-2018

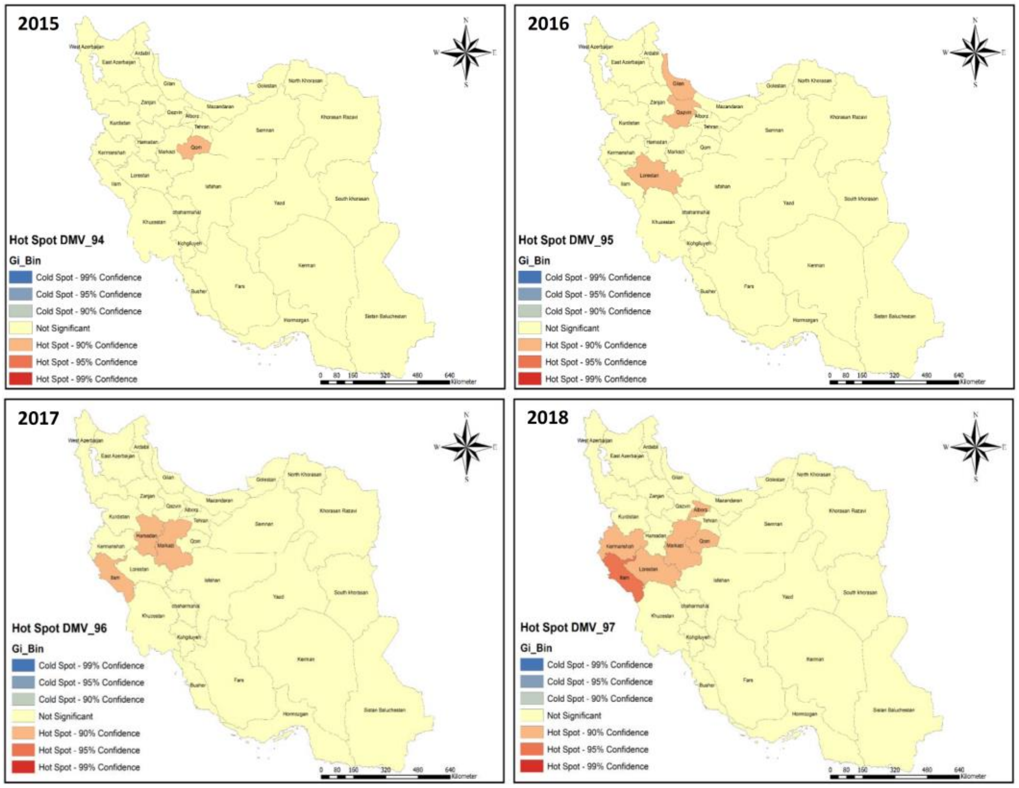
2015
Hot Spot DMV_94
2016
Hot Spot DMV_95
2017
Hot Spot DMV_96
2018
Hot Spot DMV_97
Gi_Bin
Cold Spot - 99% Confidence
Cold Spot - 95% Confidence
Cold Spot - 90% Confidence
Not Significant
Hot Spot - 90% Confidence
Hot Spot - 95% Confidence
Hot Spot - 99% Confidence
0 80 160 320 480 640 Kilometer
N
S
E
W

Hotspots distribution

No significant hotspots for DM were observed among military officers in the periods 2015-2018 (Figure 4). The DM hotspots observed among enrolled conscripts were as follows: 2015 – Qom (95% CI) province; 2016 – Lorestan, Qazvin, and Guilan (90% CI) provinces; 2017 – Ilam, Hamadan, and Markazi (90% CI) provinces; and 2018 – Ilam (95% CI), Alborz, Qom, Markazi, Lorestan, Kermanshah (90% CI) provinces (Figure 5).

Temporal distribution

The results of the temporal analysis showed a significant increasing trend of DM among the military officers from 2015 to 2018 was observed (CC= 0.935, p<0.001) (Figure 6A). However, there was no significant trend among incident cases of enrolled conscripts (CC=-0.160, p=0.555) (Figure 6B).

Figure 6: Trend of registered DM cases among military officers (A) and enrolled conscripts (B) in the Iranian military community during the period 2015-2018

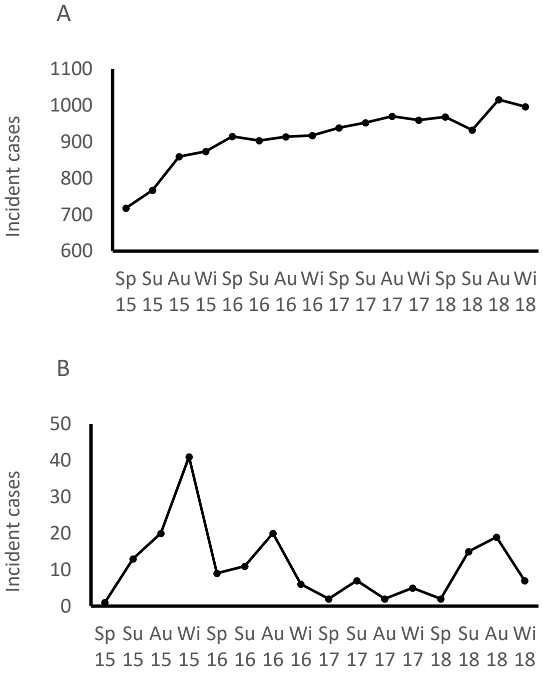

DISCUSSION

The aim of the present study was to assess the spatio-temporal distribution of DM in the Iranian military community. We determined the temporal trend analysis of DM incident cases and the regional spatial clusters of the disease in the military officers and enrolled conscripts in 2015-2018.

According to the spatial model of the distribution of DM incident cases in the study period, Isfahan province had the highest reported cases of DM (more than 100 cases) in the military officers. Obviously, the obtained results are consistent with the prevalence of DM in the general population of Iran. Recent studies also indicated a remarkable prevalence of DM in Isfahan province (15). Zanjan, Tehran, Ilam, and Kurdistan provinces also had the highest number of DM cases in the enrolled conscripts. Based on the pattern of DM in the general population, the total prevalence of DM in Tehran province (14.4%) is higher compared to other provinces. However, Ilam(16) and Kurdistan (17) are low-prevalent provinces (7.4% and 8.2%, respectively). This inconsistency might be due to a lack of information about the originality and demographic characteristics of enrolled conscripts. Based on the pattern of DM in the general population, the majority of DM cases were observed in the Central, Northern, and Western provinces of Iran (18-20), which is consistent with the present study results.

The temporal trend of DM in the military officers from 2015 to 2018 significantly increased with a mild slope; however, some severe fluctuations were seen regarding incident cases of DM in enrolled conscripts in this period. Recent studies have also shown a similar rising trend in the general population of Iran (21). Based on the time trend of the disease, an inevitable increasing case of DM is predictable in the Iranian military population, especially military officers in the future. An increasing pattern of DM morbidity and mortality has been reported in Middle Eastern and North African countries due to the high prevalence of obesity and sedentariness (22). This aforementioned increasing pattern was also observed in the Iranian general population (23). Considering this increasing trend of DM, it is also expected that the number of DM cases in the aforementioned provinces with high incident cases of DM will increase among the Iranian military population in the near future.

Regarding an appropriate program for the prevention and control of DM in the military community, it is necessary to determine the main risk factors. Some factors, including socioeconomic status, diet, physical inactivity and obesity, environmental factors, access to screening care, might contribute to the increasing trend of DM. Moreover, genetic factors should be considered in the epidemiology of DM. In a population-based study conducted on the risk allele frequencies of diabetes-related SNPs, an association was reported between the polygenic risk score of the enriched risk alleles and DM incidence (24). Different cases of DM among different populations might be due to genetic factors. A health education program concentrating on lifestyle and diet could be beneficial in the prevention of the disease, particularly in at-risk populations of the military community. Based on the findings of a recent study, lifestyle modification is an effective strategy to reduce the risk of DM in the military population (25).

CONCLUSIONS

The current study identified no DM hotspots in the military officer. However, Northern and North-Western Iran have the biggest clustering of DM in the enrolled conscripts in the years under investigation. Therefore, these geographical areas should be considered in designing the diabetes prevention and control program to early diagnose and monitor the disease. Moreover, the Iranian military medical service should develop appropriate and comprehensive analytical studies in the military population to evaluate the risk factors influencing the incidence and prevalence of DM in the aforementioned provinces.

Contributors

MF and MS developed the study design. MF, YA, AM, and MS collected and analyzed the data and drafted the prior version of the manuscript. MS participated in funding and supervised the trial. All authors edited and approved the final manuscript.

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ORIGINAL ARTICLES

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Study of the type and distribution of tumor infiltrating lymphocytes in 27 cases of lung adenocarcinoma

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Abstract: *Pathologists observed the presence of tumor infiltrating lymphocytes (TILs) in solid tumors many years before the research focus shifted from cancer cells to the tumoral microenvironment. In lung adenocarcinoma TILs vary in number, phenotype and distribution. Our study investigate the type and distribution of TILs regarding the stage of disease and histological subtype in 27 cases of lung adenocarcinoma. By immunohistochemistry we evaluated semi-quantitatively CD3, CD8 and cyclin D1. We have found that CD3+CD8- lymphocytes prevail at the invasive margin and in tumoral stroma. Cyclin D1 was diffusely positive in tumoral cells in all the five cases of lepidic type of lung adenocarcinoma. In other histological types cyclin D1 was focally positive, most at the margin of the tumor irrespective of the presence or absence of TILs.*

Keywords: *lung adenocarcinoma, tumor infiltrating lymphocytes (TILs), CD3, CD8 and cyclin D1*

INTRODUCTION

In solid tumors, tumoral stroma acts as an interface between the body and cancer and its effectors are fibroblasts and inflammatory cells. Inflammatory cells are mainly lymphocytes (tumor infiltrating lymphocytes/TILs) and few macrophages, dendritic cells and neutrophils. In line with step-wise modulated activity of immune cells, it is a deductive reason that tumor infiltrating lymphocytes have impaired activity caused by peculiar biochemical condition in tumoral microenvironment. Types of lymphocytes are defined by expression of markers that underlie immune competence or suppression, such as CD3, CD8, Foxp3, PD1 and CTLA4. Lymphocytes have a variable presence in lung adenocarcinomas and they vary considerably in number, phenotype and function [1,2,3].

The prognostic value of lymphocytes in cancers demonstrate an organ restriction efficiency of TILs, in few tumors it was shown that presence of TILs has positive impact on evolution, as in melanoma, colon cancer with micro-satellite instability or renal cancer [6,7,8]. Increased level of TILs has been positive correlated with survival [2] and it was shown to be an independent prognostic marker for lung adenocarcinoma patients [3].

Topographical distribution of lymphocytes and their phenotype suggest different triggers for intratumoral lymphocytes and peritumoral lymphocytes at the invasive front. The molecular structure of the “tumoral

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antigen" is still elusive, while the dynamic balance of simultaneous activator and inhibitory signals in the tumoral microenvironment interfere with non-self-recognition capacity of the lymphocytes.

MATERIAL AND METHOD

In this study we investigated by immunehistochemistry the type and distribution of lymphocytes that infiltrate tumoral stroma and peritumoral areas in 27 cases of lung adenocarcinomas. These cases were selected from 43 lung adenocarcinomas that have been diagnosed and operated in Emergency University Hospital Bucharest between January 2013 and June 2016. CD3 and CD8 expression of tumor infiltrating lymphocytes were evaluated by immunohistochemistry on paraffin-embedded tissue.

Additionally, we investigated the cyclin-D1 mediated DNA synthesis in tumoral cells looking for patterns of positivity by immunohistochemistry for cyclin-D1 in relation the presence and density of lymphocytes in vicinity and homogeneity versus clusters of positivity.

Table 1

	Age	Gender	Stage of disease	Histological type	Grade of differentiation	Follow-up period* (*months*)
1	43	M	IV	mixt	G1	19
2	57	M	IA	lepidic	G3	73
3	47	M	IIA	lepidic	G3	16
4	81	M	IA	lepidic	G3	4
5	61	M	IIB	mixt	G1	13
6	67	M	I A	mixt	G2	22
7	48	M	IIB	mixt	G3	34
8	61	M	IA	mixt	G3	1
9	62	M	III	mixt	G2	36
10	59	F	IB	mixt	G2	3
11	75	F	IA	mixt	G2	1
12	65	M	IIIB	mixt	G3	13
13	64	M	-	lepidic	G2	18
14	64	M	IB	acinar	G1	22
15	56	M	IA	mixt	G1	1
16	62	M	-	mixt	G3	1
17	67	M	IA	lepidic	G3	18
18	57	M	IB	mixt	G2	2
19	62	F	IIB	mixt	G2	7
20	46	M	IA	mixt	G2	9
21	62	M	IB	acinar	G2	10
22	70	F	IA	acinar	G1	12
23	59	F	IA	mixt	G2	3
24	70	M	IV	acinar	G2	1
25	56	M	I	acinar	G2	1
26	68	M	IIA	acinar	G2	1
27	70	M	IV	acinar	G2	6

**Follow-up period was measured as the interval from diagnosis and the last medical record for the patient in the hospital database*.

Age, gender, stage of disease, histological type of tumor, grade of differentiation (G) and follow-up period from hospital records were taken into account.

The selection of cases for immunehistochemistry investigation was made by evaluating hematoxylin-eosin slides of each case for the presence of tumor infiltrating lymphocytes. Positive criteria were 20 lymphocytes at the invasive front or in tumoral stroma or between tumoral cells present at least on 6 high power fields (HPF). Cases with massive necrosis were excluded, although some of these showed limited areas of abundant infiltrating lymphocytes.

In Table 1 details of the each patient are shown.

The mean age of diagnosis was 61 years, between 43 and 81. 81.5% were male and 18.5% were female. The mean follow-up period was 13 months.

RESULTS

Examination of the hematoxylin-eosin slides showed a topographic variation of the constitutive elements. In the center of the tumor prevails desmoplastic reaction and/or areas of necrosis, while tumoral cells form small groups and collapsed tubes surrounded by cancer associated fibroblasts (CAFs), collagen fibers and rare inflammatory cells. Necrosis rarely appear at less than 0.5 cm from the margin of the tumor and it was absent in lepidic type of lung adenocarcinoma as we found in our five cases of this type. Necrosis appear at the margin of tumor in the invasive front in cases diagnosed in advanced stages, III and IV.

Table 2

	Histological type			Grade of differentiation			Stage of disease					
	acinar	mixt	lepidic	G1	G2	G3	I	IIA	IIB	IIIA	IIIB	IV
N (%)	7 (26)	15 (55)	5 (19)	5 (19)	14 (52)	8 (29)	15 (55)	2 (7)	3 (11)	1 (4)	1 (4)	3 (11)

Figure 1A. CD3 positive in most of the TILs. 20X. B. Same area, CD8 positive in rare TILs; 20x.

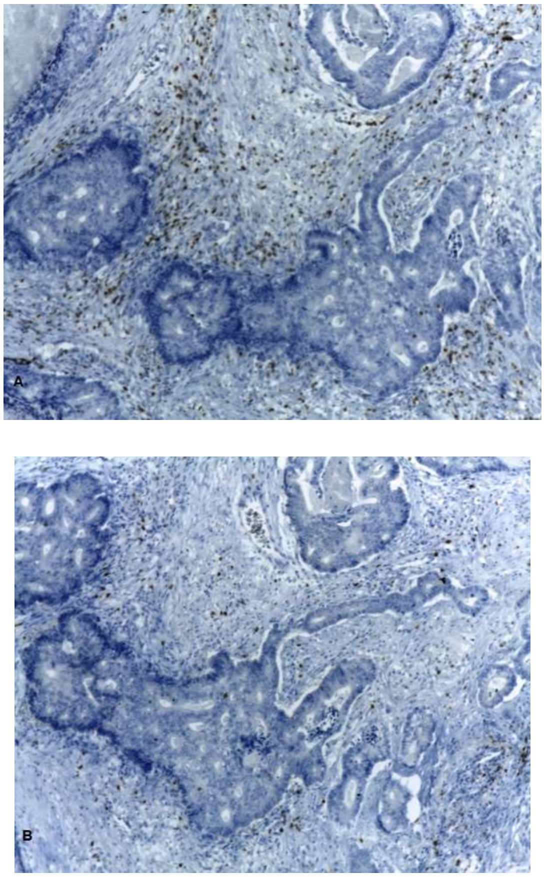

We identified three patterns of morphologic distribution of tumor infiltrating lymphocytes (TILs) as: intratumoral TILs – when infiltrate among tumoral cells (in proximity with them), stromal TILs – when infiltrate in connective stroma between acini and groups of tumoral cells, and peritumoral lymphocytes – when surround discrete or continuously the tumor at the invasive front. Two or all three of these patterns coexist in each tumor.

An inconstant finding was the presence of tertiary lymphoid structures in normal tissue in vicinity of tumor at 0.5-1 cm.

Depending on histological subtype of lung adenocarcinoma, lepidic type showed the following peculiar aspects: two out of five cases showed homogeneous moderate nuclear positivity for cyclin D1 in>80% of tumor cells irrespective of distance from the margin of the tumor, necrosis was absent and lymphocytes were found to have scarce stromal and discrete peritumoral distribution, and they were predominantly CD3+/CD8- .Stromal TILs were discontinuous, from absent to 50 lymphocytes on HPF, peritumoral TILs were discrete, intratumoral TILs were absent.

Figure 2. Cyclin D1 diffuse positive in lepidic lung adenocarcinoma; 10x.

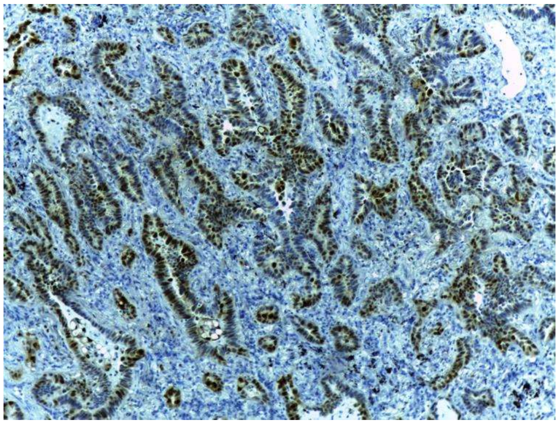

The other types of lung adenocarcinoma included in this study, acinar and mixt, (the mixt type was represented by predominantly acinar plus other pattern, solid type being identified most of the time), showed important areas of necrosis and desmoplastic reaction in the center of the tumor.

When necrosis was near the margin, it was also found unexpected presence of intratumoral TILs dispersed among tumoral cells with immunophenotype CD3+/CD8+ and increased peritumoral TILs.

Regarding the stage of disease, we have found that in the later stages of disease (stage III and IV) peritumoral lymphocytes are increased, in continuous distribution, with no significant variation regarding stromal or intratumoral lymphocytes, and that CD3+CD8-/ CD3+CD8- ratio always favor CD3+CD8-.

We interpreted that cyclin-D1 showed a gland cluster distribution when positive signals are present in most of the cells in the same gland and we suppose that those cells are in the same developmental stage.

Positivity of cyclin D1 at the margin of tumor is increased compared to the center of the tumor, irrespective to the presence or absence of peritumoral lymphocytes.

TILs in lung adenocarcinoma are predominant CD3+CD8-, they are frequently found at the invasive front, they are variable present in desmoplastic stroma and scarce to absent among tumoral cells.

DISCUSSIONS

Because of their potential cytotoxic ability, TILs are regarded as promising anticancer therapeutic targets. PD1 and CTLA-4 are the former immune-checkpoint with inhibiting effect on T-cell after their ligands binding [11]; in clinical practice, monoclonal antibodies with PD1 and CTLA-4 blocking activity show inconstant efficiency.

The variable presence of TILs could be due to variable immunogenity of tumor, due to the process of positive and negative clonal selection of lymphocyte during their maturation in lymphoid organs when tolerance cover a variable spectrum of “self” and “non-self” molecules that may be native or altered proteins, which produces variation among individuals and also resistance to cancer, or due to contradictory signals in tumor microenvironment, both proinfla-mmatory and inhibitory cytokines.

The variable distribution of TILs, mainly in stroma and peritumoral, and rarely in direct contact with tumoral cells, make the stroma an agent of sequestration for immune cells, or, from the other side, an agent of compartmentalization of tumoral cells in order to escape the immune system. In tumoral stroma, the inhibiting effect on TILs is orquestrated by CAFs (cancer associated fibroblasts) which secrete soluble cytokines which interfere with all the immune cells in the milieu.

Anterior studies predicted CD4+/CD8- phenotype predominance of TILs [1, 2]. We have sustaining results regarding CD3+ and CD8- lymphocytes. Confirmation of the presence of TILs is the first step in promoting immune-checkpoints targeted therapy. The actual role of the pathologist may be to attest the presence of TILs and to quantify the signal of the target molecule (PD1/PD-L1) in tumoral tissue.

As known, cyclin D1 has a role in promoting cell-cycle progression from G1 to S phase, when the DNA duplicate. In cancers cyclin D1 may be a driving factor (as in mantle cell lymphoma) and sometimes just a secondary event. Cyclin D1 was shown to have role also in genome stability [12].

The cluster positivity of cyclin D1 could suggest that cells derived from the same progenitor have a cell-cycle coherence; we could speculate that this is maintained until disproportionate access to nutrients favor clonal selection for expansion. The lack of correlation between TILs and tumoral cells expressing cyclin D1 apparently exclude a mitogenic effect of infiltrating lymphocytes on cancer cells as we might presume if TILs appear to express more inflammatory then cytotoxic qualities.

CONCLUSIONS

TILs are potential allies in anti-cancer therapeutic strategy. CD8 positive TILs predict a better outcome in lung adenocarcinoma.

Determinants of the variable presence and topographical distribution of TILs have yet to be explained.

Cyclin D1 is variably express among histological types of lung adenocarcinoma, with increased expression in lepidic type, and is not correlated with the presence or absence of TILs.

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CLINICAL PRACTICE

Study on the applications of digital smile design planning technique in complete dentures

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Abstract: *One of the most obvious trends in current prosthetic dentistry is the introduction of digital techniques. Of these, the techniques that offer the possibility of aesthetic prefiguration of the final prosthetic result are of particular interest to the dentist.*

The aim of this paper was to verify the applicability of the Digital Smile Design application, which is the most commonly, used aesthetic prefiguration application, an application designed for the dentate patient, to full edentulism. Two groups of full edentulous patients were formed: the first group, complete denture carriers, dissatisfied with the aesthetic aspects, that they wanted to improve in the new denture, and, the second group, newly bimaxillary full edentulous patients with high aesthetic expectations. Thus, on the first group, the tests were performed on the existing denture, and the second one with the maxillary occlusion template. However, the view of the final result offered the patients the chance to have a realistic picture of their desires regarding the shape, color, size of the teeth, which were inadequate in relation to age and facial typology. The use of this application also improves communication with the dental laboratory. In full edentulism, the current DSD technique can be used with some limitations and the use of it in this field needs more complex studies on larger groups of patients.

Keywords: *digital techniques, smile design, complete denture*

INTRODUCTION

Nowadays, dental aesthetics is becoming more and more a subject of interest in dental practice for those patients who want to improve their smile appearance.

If, some time ago, the esthetic requirement was the prerogative of younger people, now, given the increased time of active life, with the increase of life expectancy, this requirement is frequently found among seniors [1], including the full edentulous ones or those in the imminent moment of full edentulism.

Ageing causes a series of major aesthetic disorders, such as altering the soft parts of the face, emphasizing the perioral lines, oral commissures, nasogenian and mentolabial folds, the loss of elasticity of the tissues and the oro-facial muscles [2]. These signs of physiological aging are associated with the pathological signs following the installation of full edentulism. Oral commissures are lowered, and the mouth slit becomes a line with superior convexity. The lower part of the face is diminished, the prominent menton, thus generating a concave profile [3]. To meet patients' requirements, in order to improve the attractiveness of smiles, practitioners must perform a complex facial assessment in an objective and standardized manner. The fundamental criteria for aesthetic analysis include facial, dental, and dento-gingival parameters.

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Nowadays, digital smile simulation programs have been implemented for both research and clinical practice. These are multifunctional tools that strengthen the way of analysis and diagnosis of the facial and dental parameters, improve communication between team members, allow the education and motivation of patients by visualizing the final results of the proposed treatment solutions, and also increase the degree regarding treatment acceptance, the most commonly used technique within current practice being Digital Smile Design DSD [4-6]. Another smile design digital application for dentate but also for the edentulous patient, which takes into consideration patient's personality is Visagismile. Based on visagisme concept (Philippe Halawell), this digital application helps the dentists in their prosthetic work in order to obtain the best esthetic result reflecting patients' personality [7,8]. Designing the smile based only on mechanical parameters such as shape and size of the face will give a monotonous and stereotype appearance whereas customizing it with the person's attitudes and behavior will make it more life-like [9].

However, it addresses fixed dentures retained on teeth or implants [10]. Of course, the implant based dentures, in particular maxillary overdentures retained on implants, a solution with higher esthetic degree by compensating for the horizontal resorption of the vestibular slope through the denture base, supporting the soft parts, remains the first treatment alternative [11] especially for patients who find themselves in the imminent moment of becoming total edentulous. However, this alternative is often not possible due to extensive surgery, poor health, or high costs, the only solution remaining the conventional complete dentures.

When analyzing specialized literature, we observed that no publications tested the applicability of these systems through conventional complete dentures therapy. Thus, the purpose of our study was to check the possibilities and limitations of digital smile simulation applications regarding the total edentulous patient with conventional complete denture.

MATERIAL AND METHOD

The study was conducted between September 2017 and September 2018 on a group of 12 patients, 8 women and 4 men, aged 40 to 80 years, full bimaxillary edentulous, with and without conventional complete dentures, being treated within the Complete Denture Clinic of "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

The inclusion criteria of patients in the study were represented by the increased aesthetic requirements and expectations, but also the desire to preview the result before the treatment itself. The exclusion criteria were represented by patients manifesting disinterest in aesthetics and by uncooperative ones.

All 12 patients who participated in the trial have expressed and signed an informed consent regarding their participation in the study, publication of personal data and photos.

The working protocol consisted in applying and adapting the digital smile planning workflow to the full edentulous patients using the Digital Smile Design application (DSD Free App by Christian Coachman).

The group of patients was divided in 2 categories in order to compare results: patients with existing dentures, on whom simulations were performed, and patients without dentures, being at the clinical stage of determination of intermaxillary occlusion relations, for whom the upper occlusion template was used.

Patients were clinically examined and evaluated for facial features and smile elements, following these aspects:

- Facial features: Shape and Face Size: length/ width, skin, hair, and eye color.

- Smile components: Labial line of smile, smile arc, upper lip curvature – mouth commissures located at the same level (yes/no), buccal corridor – visibility in mm and gingival smile visibility.

Following extraoral clinical examination, each patient received extra and intraoral photos as indicated by the DSD specific working protocol, which we briefly described: uploading photos, calibration of the frontal facial photo, identification and positioning of the incision line and the lines corresponding to the distal faces of the maxillary central incisors, identifying and marking the labial line of the smile, the smile arc and recording the dimensions of the maxillary central incisors, choosing the appropriate teeth template according to the patient's facial typology and preference, generating the smile frame according to the references set in the previous steps and starting the smile simulation, marking the upper lip contour, choosing the teeth color from a series of four pre-set application shades and individualizing it, applying the false gum and individualizing its color, visualization of the final result.

RESULTS AND DISCUSSIONS

DSD simulation on the existing denture and definition of aesthetic elements that corresponded to the patients' aesthetic expectations were performed for the first group.

Pacient I.E., female, 80 years old, disatisfied with the existing complete denture, due to the colour of the artificial teeth

(Figure 1).

Figure 1: Patient disatisfied with the existing complete denture due to color of artificial teeth

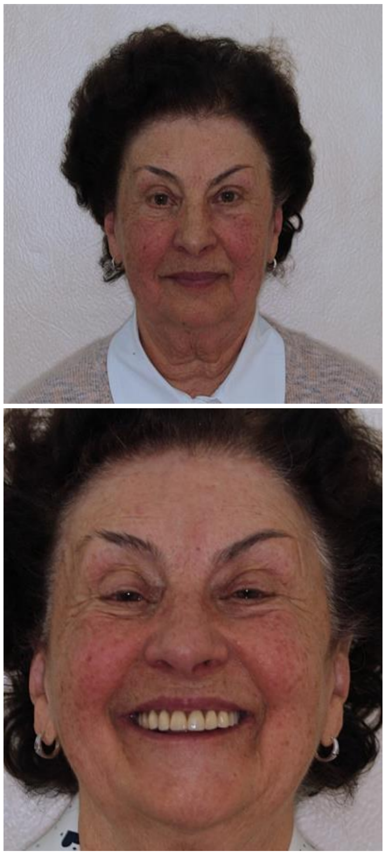

Facial features:
- Face shape: oval;
- Face size: 18 cm long, 16 cm wide;
- Skin color: dark;
- Hair color: brown;
- Eye color: brown.

Smile components:

1. Labial line of smile is at the level of the free gingival border and reveals the entire cervico-incisal height of the maxillary frontal teeth and the interdental papilla.

2. Smile arc is a curved line parallel to the upper border of the lower lip.

3. Upper lip curvature - mouth commissures are at the same level as the lower border of the upper lip and thus they do not have a curve, being straight.

4. Buccal corridor - visible on both sides - 3 mm.

5. The patient does not have a gingival smile.

We uploaded the frontal extraoral photos in smile and with apertures, while the patient had the maxillary denture applied to the prosthetic field, but in the smile simulation, we only used the frontal facial photograph. Calibration of the frontal facial photograph that captures the patient's smile with the maxillary denture was applied to the prosthetic field.

Then, the identification and positioning of the incision line and the lines corresponding to the distal faces of the maxillary central incisors was performed.

Afterwards, the identification and marking of the labial line of the smile, the smile arc, and the recording of the dimensions of the maxillary central incisors were performed.

We roughly established the width of the maxillary center incisor at 8 mm and its length at 10 mm and the application setting at an 84% ratio between width and length. These dimensions were going to be subsequently modified in the smile simulation process for a suitable adaptation of the teeth templates that were pre-set by the application to the elements of the facial assembly.

The next steps were the choice of the appropriate teeth template according to the patient's facial typology and preference, the generation of the smile frame according to the references set in the previous steps and the start of the smile simulation.

For patient I.E. we proposed three types of teeth templates: framing the templates in the smile frame, over the patient's pre-existing teeth, and altering their predefined shape, position, size, and color, depending on the patient's needs and preferences, as well as on the facial, dental, and gingival features. Then, we proceeded by marking the upper lip contour and choosing the teeth color from a series of four pre-set application shades and individualizing it. The last step consisted of applying the false gum and individualizing its color.

Final result

While smiling, the patient discovered the entire cervico-incisal height of the maxillary frontal teeth, thus allowing the proper application of the DSD working protocol, which required such a smile for a proper simulation of the smile and the achievement of aesthetic results. It was possible to keep the initial size of the teeth without distorting it, in order to allow the teeth to fit into the smile frame.

The teeth templates were selected in agreement with the patient, having a similar shape as the original ones. The patient was aesthetically pleased with the old denture, showing the desire to preserve the same aspect, but we tried to suggest other colors for the teeth, more suitable for the skin, eyes, and hair. During her first session, the patient had high aesthetic expectations and wanted a new denture with a teeth color that was much lighter than the initial one, very unaesthetic and inapropriate with her age. However, with the application, the patient being able to preview the final result, she understood the shortcomings of a lighter nuance for the teeth and finally opted for a color appropriate to her age and facial typology.

Figure3: Final result patient I.E.

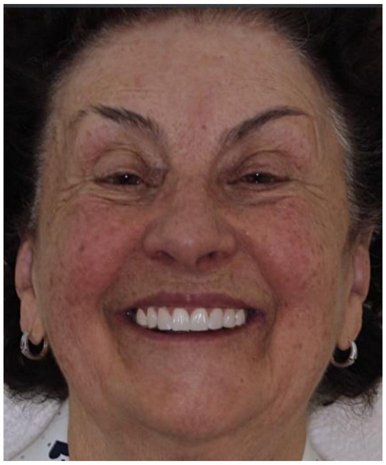

Patient Ș.M., male, 40 years old, bimaxillary full edentulous with great aesthetic expectations (Figure 3).

Figure3: Facial features

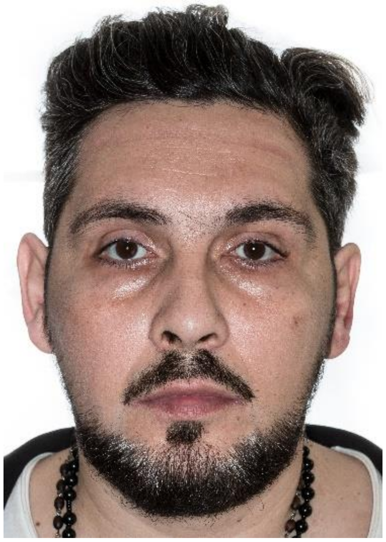

Facial features:
- Face shape: oval;
- Face size: 21 cm long, 18 cm wide;
- Skin color: light;
- Hair color: black;
- Eye color: black.

Smile components:

1. Labial line of smile: it is low and reveals less than 75% of the cervico-incisal height of the maxillary frontal teeth.

2. Smile arc is a curved line almost parallel to the upper border of the lower lip.

3. Upper lip curvature - the right mouth commissure is at the same level as the lower border of the upper lip, and the left one is located below, thus the upper lip has a downward curvature on the left side, while the right side is straight.

4. Buccal corridor – poorly visible on both sides – 2 mm.

5. The patient does not have a gingival smile.

DSD working protocol with example working on the maxillary template (second group).

Figure4: Frontal facial full smile

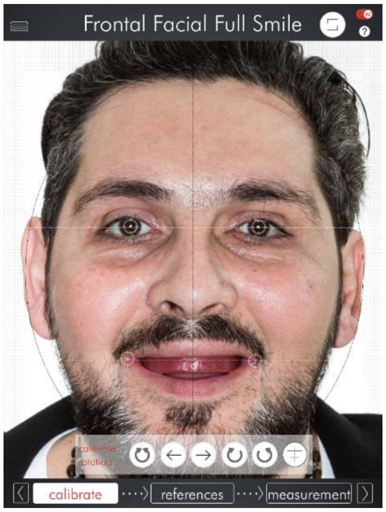
Frontal Facial Full Smile
calibrate
references
measurement

Figure5: Frontal facial full smile and smile design

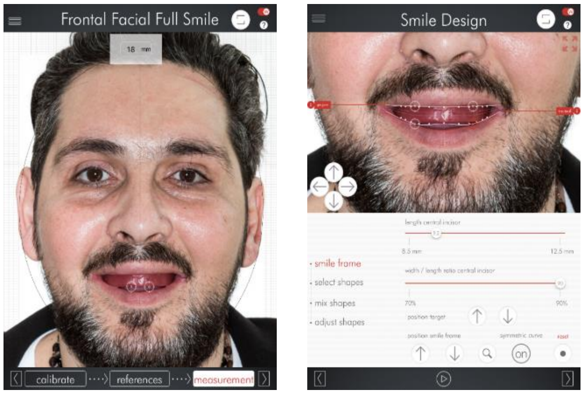
Frontal Facial Full Smile
18 mm
calibrate
references
measurement
Smile Design
length central incisor
8.5 mm
12.5 mm
smile frame
select shapes
mix shapes
adjust shapes
width / length ratio central incisor
70%
90%
position target
position smile frame
symmetric curve
reset
on

We roughly established the width of the maxillary center incisor at 9 mm and its length at 9.2 mm, the application setting a 90% ratio between width and length. These dimensions were going to be subsequently modified in the smile simulation process for a suitable adaptation of the teeth templates that were pre-set by the application to the elements of the facial assembly.

The next step consisted of choosing the appropriate teeth template, according to the patient's facial typology and preference, generating the smile frame according to the references set in the previous steps and starting the smile simulation itself (Figures4, 5).

For patient Ş.M., we proposed 3 types of teeth templates: framing the templates in the smile frame, over the patient's pre-existing teeth, and altering their predefined shape, position, size and color, depending on the patient's needs and preferences, as well as on the facial, dental and gingival features. Then we proceeded by marking the upper lip contour and choosing the teeth color from a series of pre-set application shades and individualizing it. The last step consisted of applying the false gum and individualizing its color.

Figure6: Final result patient Ş.M.

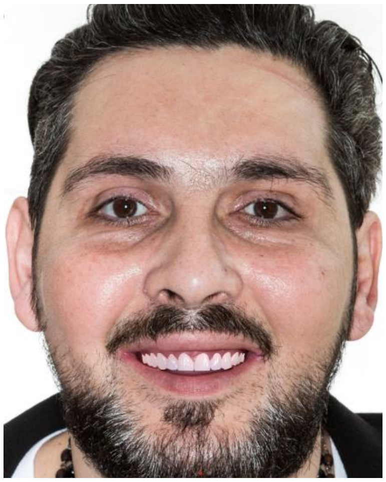

Taking a picture with the maxillary template allowed us to simulate a gingival smile because it had a very good hold on the prosthetic field.

The occlusion template gives total freedom in positioning the reerence points such as the incisal line, the lines corresponding to the distal faces of the maxillary central incisors, the labial line of the smile and the smile arc.

In addition, working on the template, we were able to establish several dimensions and shapes of the maxillary frontal teeth, not having to choose a certain size or shape close to the original one, which would cover the artificial teeth on the patient's old denture. In this case, the application guided us to choose rectangular teeth with well-defined angles for the new denture.

Digital Smile Design (DSD App by Christian Coachman) is a tool for digital smile planning used for total edentulous patients, facilitating the choice of shape, color, and size of the artificial teeth, and the possibility to preview the final result before the actual treatment. Moreover, it favors a faster integration of the denture by the patient through his active involvement in the whole process.

In case of full edentulous patients who cannot provide a gingival smile, either because of a poor maintenance of the maxillary denture that comes off the prosthetic field during the attempt to mimic a forced smile, or because of the patient's impossibility to expose the entire cervico-incisal height of the maxillary frontal teeth during the smile, the application has some limitations and requires a considerable reduction in the size of the teeth, in order to allow their optimal fitting within the smile frame, generating less aesthetic results.

Using a photograph with the maxillary template applied to the prosthetic field, instead of the old maxillary denture, within the smile simulation process, will provide the possibility to choose between several types of teeth templates without any limitations regarding the teeth shape close to the one on the old denture, which would generate similar results to the original design of the denture.

Another advantage of using the occlusion template instead of the denture is its good retention. This way, the patient can mimic a gingival smile without the risk of detaching the template from the field, the resulting photograph allowing the correct application of the DSD working protocol and the achievement of superior aesthetic results.

In terms of colors, the application offers a limited range of only four shades with the possibility of changing their features.

In specialized literature, there are still insufficient articles or studies illustrating the expansion regarding the use of digital smile planning applications in total edentulous patients, despite the fact that they can greatly facilitate the design concept of the complete denture, by being able to preview the final result and choose the shape, color, size and arrangement of the artificial teeth according to the facial typology and patient's wishes, thus the patient being involved in establishing the treatment plan.

CONCLUSIONS

Introducing digital techniques to preview the final result of the prosthetic treatment in current dentistry brings important benefits regarding the success of the treatment, communication with the patient and the dental laboratory.

DSD technique is a tool used in a relatively small percentage in current practice by dental practitioners, only to the dentate patient, which requires the use of digital photographs, templates, and digital applications.

In full edentulism, the current DSD technique can be used with some limitations related to: the dynamics of the lips in smile, execution of the original photographs for which the denture should have a good retention on the prosthetic field and the proposal of a limited number of templates that do not satisfy all facial typologies.

In full edentulism, the vestibular slope of the denture is an important part of its aesthetics, but the application cannot simulate it.

However, the preview of the end result offers the patient the chance to have a realistic picture of his desires, regarding the shape, color, size of teeth, which are inadequate in relation to the patient's age and facial typology.

In the future, studies that are more complex are needed on a larger group of patients, in order to make the application more efficient also in the field of full edentulism.

Acknowledgements
All authors have equal contribution as the first author.

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ORIGINAL ARTICLES

Study on the chest radiographic results in patients with pulmonary tuberculosis referred to Zabol Tuberculosis Center: A descriptive study

Razieh Behzadmehr[1], Razvaneh Behzadmehr[1]

Abstract: *Introduction and Purpose: Tuberculosis is the most common cause of death from infectious diseases in the world. This study aimed to evaluate the findings of chest radiography in patients with pulmonary tuberculosis referred to the Zabol tuberculosis center.*

Materials and Methods: This descriptive-analytical study was performed on 101 patients with positive tuberculosis from January to December 2020. Data were collected using an information form and finally analyzed by SPSS software version 22.

Results: In this study, 101 patients were studied, of which 71 were female and 30 were male, and the mean age of patients was 62.68±13.61 years. The frequency of turbidity in patients with smear grades 1, 2, and 3 was 71.4%, 78.5%, and 76.5%, respectively. The frequency of cavitation in patients with grades 1, 2, and 3 was 11.5%, 28.5%, and 52.9%, respectively. The frequency of nodular view in patients with grades 1, 2, and 3 was 18.6, 42.8, and 35.3%, respectively.

Conclusion: In general, the results of this study showed that with increasing smear grading, the frequency of cavitation cases increases significantly and the frequency of reticulonodular view cases decreases significantly.

Keywords: *tuberculosis, chest radiography, smear grade*

INTRODUCTION

Tuberculosis is a bacterial disease caused by Mycobacterium tuberculosis and sometimes Mycobacterium bovis. About one-third of the world's population (2 billion people) is infected with the tuberculosis virus and is at risk of contracting tuberculosis, and each year about 9 million people become infected with active tuberculosis (1 to 2 million people) and die from the disease [1].

It is the leading cause of death from single-agent infectious diseases (even more so than AIDS, malaria, and measles) and ranks tenth in the global burden of disease, and is projected to maintain its current status by 2020 and even reaches to rank seventh [2]. The basis of the diagnosis of pulmonary tuberculosis is a direct and simple test of patients' sputum is suspected. In the best case, the sensitivity of the sputum test in the diagnosis of pulmonary tuberculosis is fifty to sixty percent [3, 4]. In pulmonary form, children often get sputum or gastric juice test results are usually negative even in culture, so the diagnosis is mainly based on clinical history, history of contact of a child with pulmonary tuberculosis with a positive sputum smear (especially in the family), chest radiography and tuberculin test [5]. According to standard definitions of a patient who has at least two positive sputum smear tests for fast acid bacilli. Or a patient who has only one sputum test positive for fast acid bacilli with radiographic changes in the chest confirming pulmonary tuberculosis. Or a case of positive smear in terms of acid-fast bacilli

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accompanied by sputum culture is considered positive smear tuberculosis [6]. The degree of positive smear is determined based on the number of bacilli in each microscopic field. Some studies have considered the degree of positivity of the primary smear to be effective in determining the outcome of treatment and have named it as a predictor that if the degree of positivity is more, the probability of treatment failure and death is higher [7, 8]. In some studies, the relationship between the degree of positivity of the primary smear and clinical manifestations has been suggested [9].

Chest radiography is also a suitable and sensitive tool for diagnosing lung lesions, including tuberculosis so that if the chest picture is normal, the diagnosis of tuberculosis is partially removed [10]. On the other hand, in cases where we are actively looking for this disease and when it is diagnosed in its early stages, pulmonary involvement can indicate our success in the early detection of these patients, which is achieved by radiographic findings [11]. No study is performed about chest radiographic results in patients with pulmonary tuberculosis in Iran and chest radiographic findings of patients, and in the world, few studies have been done in this regard, and due to the importance of this issue in early diagnosis and reduction of transmission of infection and treatment, this study was performed to determine the radiographic findings of the chest in patients with pulmonary tuberculosis.

METHODS

This descriptive-analytical study was performed on all patients with pulmonary tuberculosis referred to Zabol Tuberculosis Center from January to December 2020, except for patients with an immunodeficiency – hepatitis, and diabetes. In this study, 101 patients were studied, of which 71 were female and 30 were male. Samples were recorded according to a checklist that included demographic information, sputum smear grade, and radiographic findings of the chest. Demographic information of patients such as age, sex, and place of residence as well as smear information and its degree and chest radiographic findings were collected. Data were collected using SPSS software in the form of frequency, percentage, mean and standard deviation and analyzed the data using chi-square test.

RESULTS

Of the 101 participants, 71 (70.3%) were male and the rest were female. The mean age of patients was 62.68 years with a standard deviation of 13.61. The youngest and oldest patients were 18 and 86 years old, respectively. There was no significant difference between the two sexes in terms of grade smear (p= 0.192).

Table 1: Gender distribution of patients

Smear degree Gender	Grade 1	Grade 2	Grade 3	P value
men	5 29.4%	7 50%	18 25.7%	0.192
female	12 70.6٪	7 50%	52 73.3%	

The table below shows that the frequency of turbidity in patients with smear grades 1, 2, and 3 was 71.4%, 78.5%, and 76.5%, respectively. This difference in turbidity in patients with different grades was not statistically significant (p = 0.833).

Table 2: Frequency of turbidity cases by the degree of smear

		Grade 1	Grade 2	Grade 3	P value
Consolidation	Yes	13 76.5%	11 78.5%	50 71.4%	0.833
	No	4 23.5%	3 21.4%	20 28.5%	

The table below shows that the frequency of cavitation in patients with grades 1, 2, and 3 was 11.5%, 28.5%, and 52.9%, respectively. This difference in the frequency of cavities in the three groups was statistically significant (P = 0.001).

Table 3: Frequency of cavities by the degree of smear

		Grade 1	Grade 2	Grade 3	P value
Cavitation	Yes	9 52.9%	4 28.5%	8 11.5%	0.001
	No	8 47.05%	10 71.4%	62 88.5%	

The table below shows that the frequency of nodular view in patients with grades 1, 2, and 3 was 18.6%, 42.8%, and 35.3%, respectively. This difference in the frequency of nodular view in the three groups was not statistically significant (p = 0.086).

Table 4: Frequency of nodular cases by the degree of smear

		Grade 1	Grade 2	Grade 3	P value
Nodular	Yes	6 35.3%	6 42.8%	13 18.6%	0.086
	No	11 64.7%	8 57.1%	57 81.4%	

The table below shows that the frequency of reticulonodular involvement in patients with grades 1, 2, and 3 was 24.2%, 7.1%, and 0.0%, respectively. This difference in the frequency of reticulonodular involvement in the three groups was statistically significant (p = 0.022). On the other hand, patients with reticulonodular involvement were significantly more likely to have grade 1 smear. The lowest frequency of reticulonodular view belonged to the grade 3 smear.

Table 5: Frequency of reticulonodular cases by the degree of smear

		Grade 1	Grade 2	Grade 3	P value
Reticulonodular	Yes	0 0%	1 7.1%	17 24.2%	0.022
	No	17 100%	13 92.8%	53 75.7%	

DISCUSSION

In this study, 101 patients with tuberculosis were studied. Of these, 71 (70.3%) were female and the rest were male. In the studied patients, 70 cases had grade 1 (74.3% female and 25.7% male), 14 cases had grade 2 (male = female) and 17 cases had grade 3 (70.6% female and 35.7% male). As a result, there was no significant difference between the two sexes in terms of grade smear (p = 0.192). Due to the lack of similar studies that have examined the relationship between sexes and smear grading, the findings of this study cannot be compared. The mean age of patients was 62.68 years with a standard deviation of 13.61. The youngest and oldest patients were 18 and 86 years old, respectively. The mean age of patients with grades 1, 2, and 3 was 64.47, 62.07, and 55.82 years, respectively. The age difference between patients in different grades was not statistically significant (p = 0.122). There was also no relationship between increasing or decreasing age and smear rating. In other studies such as the study of Ores (2007) and Mohammad Blursaz et al. (2014) children under 15 years of age were in the study group and therefore due to differences in the study population, it is not possible to compare the results. Most of the patients were from Zabol city (52.5%) and the least of them were from Hamoon and Helmand cities (2%). The frequency of turbidity in patients with 1, 2, and 3 smear degrees was 71.4, 78.5, and 76.5%, respectively. This difference in the degree of turbidity in patients with different grades was not statistically significant. (P = 0.833) Although patients with grade 2 had more turbidity than patients with grade 1 and 3, but the difference between the three groups was not significant. The opacity was the same, so there does not appear to be a correlation between the degree of smear and the opacity in the graph. This finding is not consistent with other studies. In Minko's (2015) study, with increasing smear degree (+1, +2, +3, or +4), the frequency of turbidity cases increased significantly and its frequency increased to +1, +2, +3, and +4 degrees. It was 81%, 95% and 100%, respectively [12]. In the study of Ores (2007), with increasing the degree of smear, the number of turbidity cases also increased significantly [13]. Matsuka et al. (2004) also showed that the frequency of turbidity increased with an increasing number of fast acid bacilli [14]. The reason for the difference between the results of this study and other studies can be attributed to the fact that we have studied in this study Chest radiographs were based on smear grading, while other studies examined the relationship between CT scan and HRCT findings and smear grading. The frequency of cavitation in patients with grades 1, 2, and 3 was 11.5%, 28.5%, and 52.9%, respectively. This difference in the frequency of cavities in the three groups was statistically significant (P = 0.001). Patients with different grades were different in terms of cavitation, i.e. in patients with grade 3 cavitation was significantly higher than patients with grades 1 and 2, so it seems to be between smear degrees. And there is a connection hole. In Minko (2015) study, with increasing smear degree (+1, +2, +3, or +4), the frequency of CT scan findings including cavitation also increased significantly, so that the frequency of Cavitation cases in degrees +1, +2, +3, and +4 were 33%, 68%, 94%, and 100%, respectively [15]. In Ores (2007) study, cavitation increased significantly with increasing smear degree. Matsuka (2004) also showed that the frequency of cavitation increased with an increasing number of fast acid bacilli [16]. In the study of Mohammad Reza Bloursaz et al. (2014), cavitation had a significant relationship with smear rating [17]. This study showed that the frequency of nodular view in patients with grades 1, 2, and 3 was 18.6, 42.8, and 35.3%, respectively. The difference between the frequency of nodular view in the three groups was not statistically significant (P = 0.086). Although patients with grade 2 had more nodular view than patients with grade 1 and 3, the difference between the three groups of smears was not significant. There is no correlation between nodules and graphs. Matsuka (2004) also showed that the frequency of nodules increased with increasing smear degree but their difference was not statistically significant (P = 0.16) [18].

In the study of Ores (2007), with increasing the degree of smear, the score of nodules also increased significantly (P = 0.0001) [19]. The frequency of reticulonodular involvement in patients with grades 1, 2, and 3 were 24.2%, 7.1%, and 0%, respectively. This difference in the frequency of reticulonodular involvement in the three groups was statistically significant (p = 0.022). On the other hand, patients with reticulonodular involvement had significantly more grade 1 smear. And the lowest frequency of reticulonodular lesions belonged to grade 3 smears (0.0%). These results showed that between the degree of smear and involvement, there is a reticulonodular involvement in the connection graph so that with increasing smear rating (+1, +2, and +3) the frequency of reticulonodular view decreases.

Due to the lack of similar studies examining CXR findings based on smear rating, the findings cannot be compared with this study.

CONCLUSION

Despite the lack of relevance in the present study, the results of the present study can be used as a basis for other future studies.

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REVIEW

Succinct review on biological and clinical aspects of Coronavirus disease 2019 (COVID-19)

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Abstract: *The prevalence of coronavirus disease 2019 (COVID-19) is the third registered spillover of an animal coronavirus to humans from the early 21st century. Coronaviruses are important human and animal pathogens. The 2019 novel coronavirus (2019-nCoV) rapidly spreads, resulting in an epidemic throughout China, followed by an increasing number of cases in other countries throughout the world. Recently, a wide range of inhibitors have been introduced for treatment of COVID-19, and also promising vaccines are in late phase of development. Here, we aim to present an overview of recent findings of the biological and clinical aspects of SARS-CoV-2 infection, along with possible treatments and future vaccines.*

Keywords: *COVID-19; immune response; diagnosis; treatments; SARS-CoV-2*

INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) is the third registered spillover of an animal coronavirus to humans from the early 21st century [1]. Before the outbreak of SARS-CoV (severe acute respiratory syndrome coronavirus) infection within 2002-2003 in the Guangdong province, China, coronaviruses were considered to be slightly pathogenic to humans [2-6]. MERS-CoV (Middle East respiratory syndrome coronavirus) is another highly pathogenic coronavirus that appeared in the Middle Eastern countries ten years after the outbreak of SARS-CoV [7]. Recently, in December 2019, a new coronavirus from 3 patients with pneumonia was discovered and termed 2019 novel coronavirus (2019-nCoV). All three patients were somehow related to the wet animal and seafood wholesale market in Wuhan, Hubei Province, China [8]. Afterward, the virus was named as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) [9]. SARS-CoV-2 is an enclosed virus with positive single-stranded RNA belonging to Betacoronaviruses, which can infect both humans and animals [10]. The biological behavior of SARS-CoV-2 is mostly the same as SARS-CoV and MERS-CoV. SARS-CoV-2 uses the same structural protein as SARS-CoV and MERS-CoV, to gain entry to the host cells [11, 12].

The average basic reproduction number (R0) is an epidemiologic metric that describes the transmissibility of infectious agents. R0 measures the expected number of secondary infections produced by a single infectious individual in a susceptible population during the mean infectious period. Case Fatality Ratio (CFR) indicates the proportion of episodes of disease that are fatal. Created with BioRender.com

The patients with SARS-CoV-2 mostly have symptoms such as fatigue, fever, dry cough, and dyspnea. Clinical and laboratory evaluation, along with chest CT scan and real-time RT-PCR (real-time reverse transcription-polymerase chain reaction) assay, can help to confirm the disease [13-15]. patients are better to be hospitalized soon after diagnosis to receive proper health care [16].

However, recently a wide range of inhibitors have been introduced for treatment of COVID-19. Furthermore, promising vaccines are in late phase of development. Here, we aim to present an overview of recent findings of the biological and clinical aspects of SARS-CoV-2 infection, along with possible treatments. Using various sources, including CDC, WHO, and some journals we have concisely provided an epidemiological comparison between COVID-19 and other well-known respiratory viral infections in Figure 1 [2, 17-19].

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Figure 1: This figure represents an epidemiological comparison between COVID-19 and other well-known respiratory viral infections.

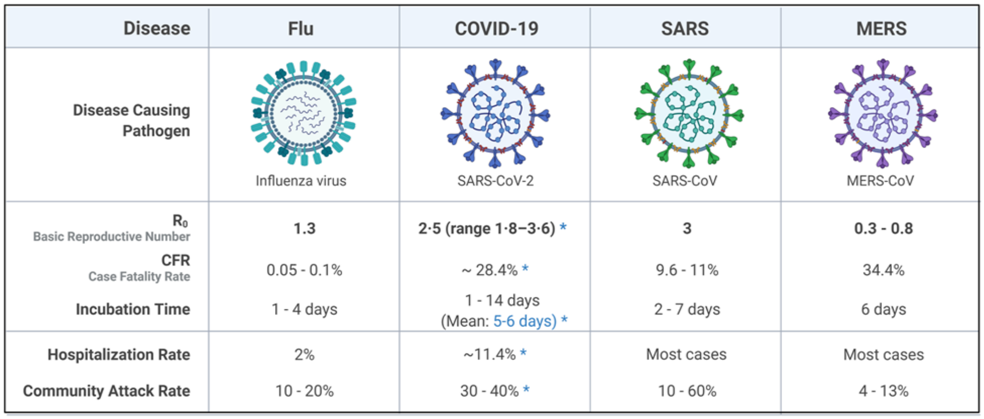

Disease	Flu	COVID-19	SARS	MERS
Disease Causing Pathogen	Influenza virus	SARS-CoV-2	SARS-CoV	MERS-CoV
R_0 Basic Reproductive Number	1.3	2·5 (range 1·8–3·6) *	3	0.3 - 0.8
CFR Case Fatality Rate	0.05 - 0.1%	~ 28.4% *	9.6 - 11%	34.4%
Incubation Time	1 - 4 days	1 - 14 days (Mean: 5-6 days) *	2 - 7 days	6 days
Hospitalization Rate	2%	~11.4% *	Most cases	Most cases
Community Attack Rate	10 - 20%	30 - 40% *	10 - 60%	4 - 13%

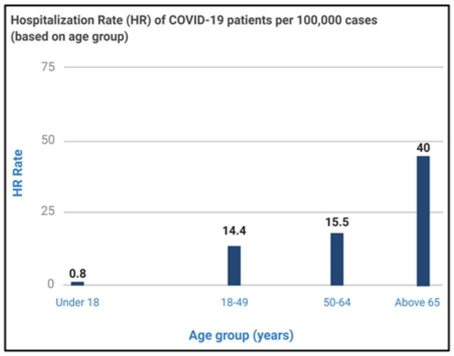

PHYLOGENY AND ORIGIN OF CORONAVIRUSES

The Coronaviridae Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) determined that coronaviruses related to the subfamily of Coronavirinae in the family Coronaviridae and the order Nidovirales. Based on phylogenetic relationships and genomic structures, this subfamily contains 4 genera, including Alphacoronavirus, Gammacoronavirus, Betacoronavirus, and Deltacoronavirus [20, 21]. The Betacoronaviruses have four lineages (A, B, C, and D). SARS-CoV and SARS-CoV-2 belong to lineage B, and lineage C includes MERS-CoV [22]. According to phylogeny and taxonomy, the CSG placed 2019-nCoV, within the Coronaviridae. Given this, they termed this SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus) [23]. There are three extremely pathogenic coronaviruses, SARS- CoV, MERS-CoV, and SARS-CoV-2, leading to a severe respiratory syndrome in humans [10, 24, 25]. It is proposed that both MERS-CoV and SARS-CoV have originated from bats. MERS-CoV and SARS-CoV have infected humans through direct transmission from civets and dromedary camels, respectively, which seem to be the intermediate hosts for these viruses. It has been confirmed that these viruses are capable of human-to-human transmission [21]. Phylogenetic analysis showed that 2019-nCoV has more than 88% identity to the genome of two types of coronaviruses originated from bats, bat-SL-CoVZXC21 and bat-SL-CoVZC45. However, it has less genome similarity to MERS-CoV (~50%). SARS-CoV-2, in comparison to SARS-CoV, has been considered to be a new human-infecting betacoronavirus due to its distinct genetic characterizations [26]. At first, skeptic views were whispered that this virus is a product of purposeful manipulation, but a comprehensive analytical study explicated that this is not a laboratory constructed virus [27].

STRUCTURE AND LIFE-CYCLE OF SARS-COV-2

SARS-CoV-2 genome contains 29,881 bp in length with 14 open reading frames (ORFs) encoding 27 proteins [22]. The 3′-terminus of the genome comprises of sequences for encoding 8 accessory proteins (3a, 3b, 7a, p6,7b, 8b, 9b, and orf14), and 4 main structural proteins, including spike surface glycoprotein (S), an envelope protein (E), matrix protein (M), and nucleocapsid protein (N). Despite the relationship between SARS-CoV-2 and SARS-CoV, there are some distinct differences at the amino acid level. For example, unlike SARS-CoV, the 8a protein is absent in SARS-CoV-2; the 8b protein in SARS-CoV-2 is longer in contrast to SARS-CoV; in comparison to SARS-CoV, the 3b protein is considerably shorter in SARS-CoV-2. The pp1ab and pp1a proteins of the SARS-CoV-2 are encoded by ORF1a and

ORF1ab genes situated at the 5´-terminus of the genome, respectively [28, 29].

Figure 2: Human SARS-CoV-2 Structure. The spike of the recently discovered SARS-CoV2 has two subunits, S1 and S2, and is frequently represented as a sword-like spike. Created with BioRender.com

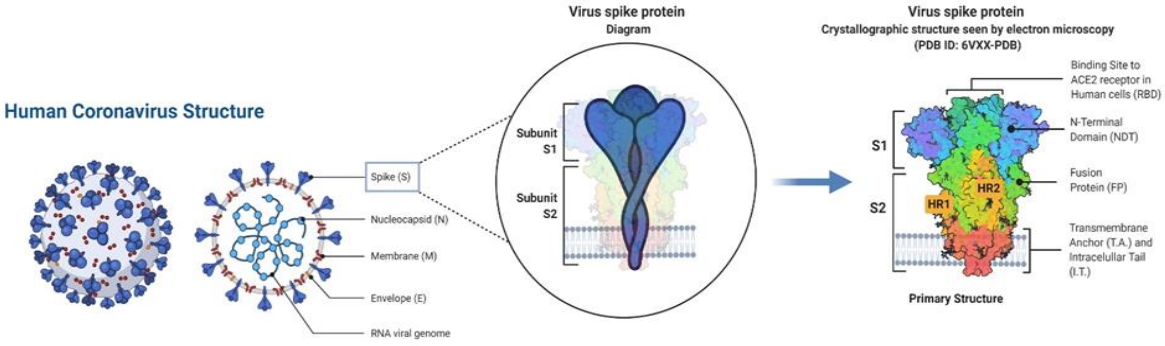

Unlike MERS-CoV that binds to dipeptidyl peptidase 4 (DPP4; also called CD26) to infect the unciliated bronchial epithelial cells as well as pneumocytes type II, both SARS-CoV and SARS-CoV-2 are linked to ACE2 (angiotensin-converting enzyme 2) on pneumocytes type II and ciliated bronchial epithelial cells [30-33]. Though SARS-CoV-2 and SARS-CoV have similar receptor-binding domain structure, the variation of the amino acids at some key residues distinct them from each other [26]. Based on biophysical and structural studies on S protein, the SARS-CoV-2 S protein demonstrated a greater affinity to ACE2 than SARS-CoV S protein [34]. Since the S protein is a quintessential part of the virus life-cycle, it can be a consensus target antigen, which could be inhibited by drugs such as Arbidol [35]. Analytical studies have delineated the SARS-CoV-2 mutations, indicating that these may have led to higher infectivity of the virus as a result of natural selection [27, 36-38]. Although SARS-CoV-2 has come with higher infectivity and transmissibility than SARS-CoV, it seems to have less mortality rate [19, 39]. The virus life cycle initiates with the binding of SARS-CoV S protein to the ACE2 receptor and recruiting the cellular protease TMPRSS2 in order to enter the host cell via the endosomal path [12, 40]. A report betokened that a particular mutation (N501T) could promote interaction of the virus receptor-binding domain with human ACE2 [40, 41]. The virus releases the genomic RNA into the cytoplasm, following the viral entry into the host cell. The next step is the translation of ORF1a and ORF1ab for producing pp1a and pp1ab polyproteins, then here proteases encoded by ORF1a cleave pp1a and pp1ab to provide the NSPs. These NSPs form the RNA replicase–transcriptase complex, which leads to producing negative-sense RNAs. Further, negative-sense RNA is used as a template to produce full-length positive RNA genomes. During transcription, sub-genomic RNAs like ones responsible for structural proteins (S, E, and M) produced through a process called discontinuous transcription, which afterward introduced into the endoplasmic reticulum (ER). Sub-genomic mRNAs might hold multiple ORFs, but only the one that is closest to the 5´ end (first ORF) goes to be translated. Next, these structural proteins (E, M, and S) move to the endoplasmic reticulum – Golgi intermediate compartment (ERGIC), where they combine with viral RNA genomes encapsidated by N protein to form mature virions. Herein, The M protein has an essential role in protein-protein interaction for viral assembly. Eventually, virions use the exocytosis process to be released out of the infected cell [42, 43]. ORF1a and ORF1ab genes together encode 16 to 17 NSPs (non-structural proteins), including NSP1 to NSP10, NSP12, and NSP16 [39]. Two of which are proteases, namely 3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro), that are being considered as invaluable drug targets [39]. Owing to 96% homology [44] within the sequence of 3CLpro in SARS-CoV and SARS-CoV-2 genomes, formerly introduced 3CLpro inhibitors, including peptidomimetic acrylester (PDB: 6LU7) (45), aldehydes [46], isatines [47], a-ketoheteraromates [48], pyrazolidinone [49], Nitroanilides [50], ML188 [51], racemic [51], and ML300 [51] might be beneficial start-point drugs in the treatment of COVID-19 as well. Besides, machine-learning-based approaches have been put in to work to find 3CLpro inhibitor compounds (drug repurposing) [52-54]. Using Plant-based medicines like Flavonoids [55, 56], as well as HIV protease inhibitors like lopinavir and ritonavir [57-59], have also been suggested as a 3CLpro inhibition-based treatment. Imperativeness of PLpro for the CoV replication and PLpro-mediated subterfuge from the immune system makes this protease an attractive drug target [60].

Figure 3: Steps of coronavirus life-cycle: 1-Spike-mediated binding and viral entry via membrane fusion or endocytosis; 2-Release of viral genome; 3-Translation of viral polymerase protein; 4-RNA replication; 5-Sub-genomic transcription; 6-Translation of viral structural proteins ;7-S, E, and M proteins combine with nucleocapsid; 8-Formation of mature virion; 9-Exocytosis. nsps: non-structural proteins. Created with BioRender.com

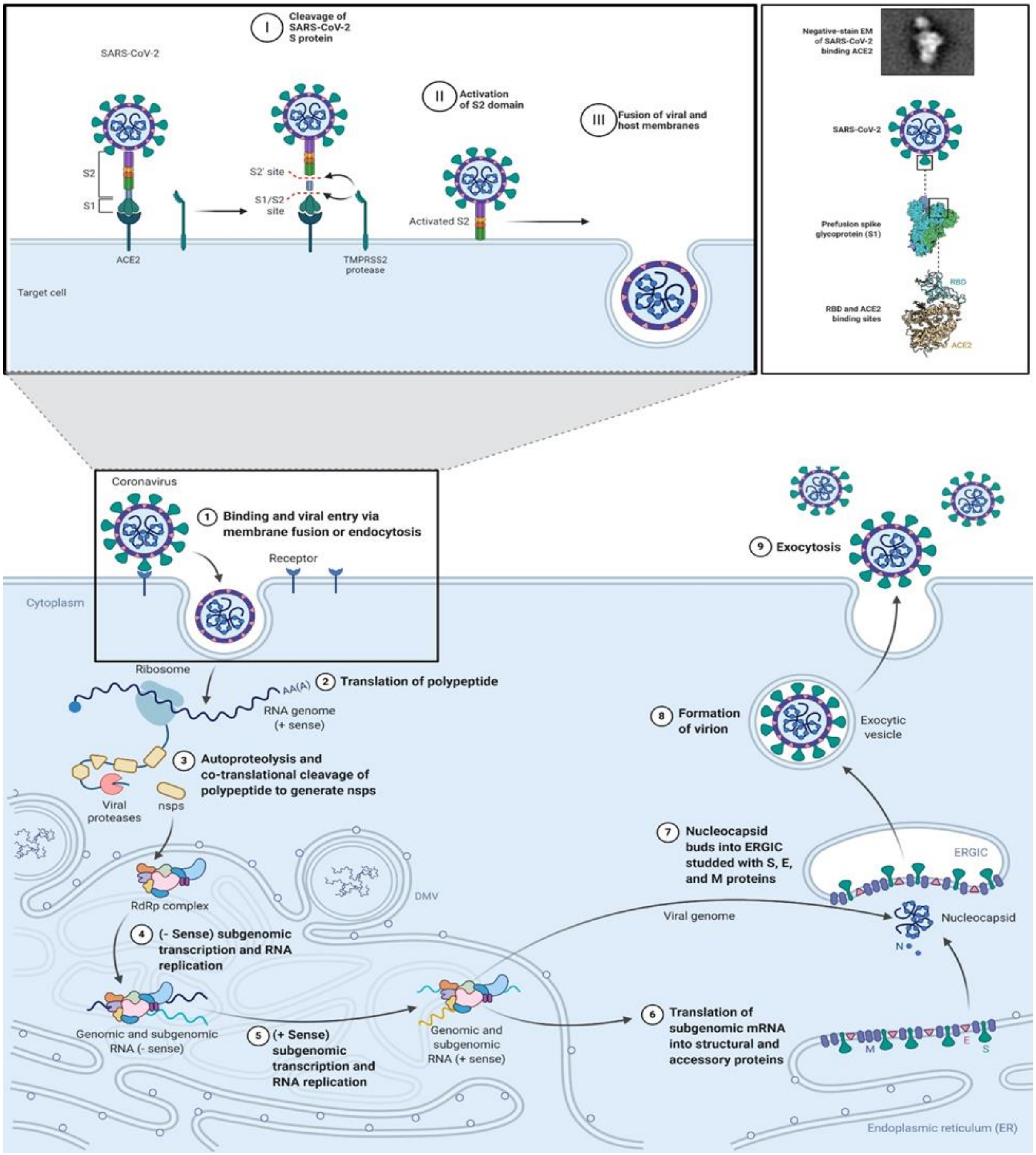
I Cleavage of SARS-CoV-2 S protein
SARS-CoV-2
S2
S1
ACE2
S2' site
S1/S2 site
TMPRSS2 protease
II Activation of S2 domain
Activated S2
III Fusion of viral and host membranes
Target cell
Negative-stain EM of SARS-CoV-2 binding ACE2
SARS-CoV-2
Prefusion spike glycoprotein (S1)
RBD
RBD and ACE2 binding sites
ACE2
Coronavirus
① Binding and viral entry via membrane fusion or endocytosis
Receptor
Cytoplasm
Ribosome
AA(A)
② Translation of polypeptide
RNA genome (+ sense)
③ Autoproteolysis and co-translational cleavage of polypeptide to generate nsps
Viral proteases
nsps
RdRp complex
DMV
④ (- Sense) subgenomic transcription and RNA replication
Genomic and subgenomic RNA (- sense)
⑤ (+ Sense) subgenomic transcription and RNA replication
Genomic and subgenomic RNA (+ sense)
Viral genome
⑥ Translation of subgenomic mRNA into structural and accessory proteins
M
E
S
⑦ Nucleocapsid buds into ERGIC studded with S, E, and M proteins
ERGIC
Nucleocapsid
N
⑧ Formation of virion
Exocytic vesicle
⑨ Exocytosis
Endoplasmic reticulum (ER)

To this end, compounds interacting with active-site Cys112, including epoxyketones, a-halo-ketone, aldehydes, alkynes, and activated esters, have been proposed as potential inhibitors [60]. Accordingly, a drug for chronic alcohol dependency called Disulfiram has been reported to be a potential PLpro inhibitor. Alongside that, 6-mercaptopurine and 6-thioguanine as antimetabolite drugs also have the capability to inhibit PLpro [61]. Other than those two proteases, the RNA-dependent RNA polymerase (RdRp), which recruits cell machinery to transcribe the virus genome into new RNA copies, can be an outstanding drug target [62, 63]. An RNA polymerase inhibitor called Favipiravir, which is mostly used in the treatment of influenza, has shown to be effective against other RNA viruses as well [64]. It is noteworthy that Favipiravir would not restrict host DNA and RNA synthesis [65]. Its effectiveness and minor side effects on patients with COVID-19 has been reported from a study on 70 patients in Shenzhen, Guangdong province [66]. Remdesivir is a broad-spectrum antiviral drug that acts as an adenosine analog and thus capable of terminating the RNA synthesis in multiple RNA viruses, including SARS-CoV-2 and Ebola [67, 68]. A preliminary randomized trial on the 1059 COVID-19 patients (538 assigned to Remdesivir and 521 to placebo) delineated that Remdesivir was superior to placebo in reducing the recovery time in adults. Remdesivir also lowered the COVID-19-related mortality rate [69]. Another adenosine-analog drug is Galidesivir that has been administered for Ebola and Marburg virus. Moreover, A molecular docking study showcased that it has broad-spectrum antiviral activity upon RNA viruses such as SARS-CoV-2 [70, 71]. Moreover, a machine-learning-based approach has offered AP2-associated protein kinase 1 (AAK1), and Cyclin G Associated Kinase (GAK) inhibitors, including Baricitinib (a drug approved for rheumatoid arthritis), Fedratinib, Sunitinib, and Erlotinib, to inhibit viral endocytosis [39, 72]. Additionally, Baricitinib can also inhibit JAK1/2, which attenuates inflammation [72].

IMMUNE RESPONSES TO SARS-COV-2

The immune system naturally triggers an immune response against invaders like viruses. Chemotactic factors have a critical role in viral infections due to their effect to summon the leukocytes to the site of infection. So, spectral alteration in chemotactic factors may result in adverse immune responses. As mentioned before, SARS-CoV-2 mostly affects the lungs, so it should be considered that uncontrolled immune response may lead to functional impairment of lungs, pulmonary tissue damage, and reduced lung capacity. In contrast, an inadequate immune response may also intensify viral replication, which also leads to tissue damages [73]. The host cell possesses various pattern recognition receptors (PRRs) that serve as a part of the innate immune system for recognizing the PAMPs (pathogen-associated molecular patterns). PRRs including RIG-I-like receptor (RLR), toll-like receptor (TLR), C-type lectin-like receptors (CLmin), and NOD-like receptor (NLR) are located on the cell surface. On the other hand, some PRRs are in the cytoplasm, such as cyclic GMP-AMP synthase (cGAS), IFI16, interference stimulator of interferon gene (STING), DNA-dependent activator of IFN-regulatory factor (DAI) [73]. Among them, RLRs, including MDA5 (IFIH), H family members RIG-I (DDX58), and LGP2, have the potential to recognize RNA viruses, like coronavirus [73-75]. The adaptive immune responses consist of the immune response of T cells, humoral immune responses, and antibody responses. Around 80% of lymphocytes that infiltrate in the pulmonary interstitium in coronavirus infection are CD8+ T cells, which can clear the virus-infected cells and also able to induce tissue damage [73-76]. It has been noted that the weakening of CD4+ T cells may cause severe interstitial pneumonitis, and delayed elimination of the virus from lungs are due to decreased pulmonary recruitment of lymphocytes and neutralization antibody and cytokine production related to the reduction of CD4+ T cells [77]. Lymphopenia is a hallmark in patients infected with SARS-CoV and SARS-CoV-2. A study revealed that SARS-CoV E protein interaction with an antiapoptotic protein called Bcl-xL induces T-cell apoptosis. Further, it was shown that interaction of SARS-CoV E protein with Bcl-xL is mediated by BH3 (Bcl-2 homology domain 3)-like region placed in the C-terminal cytosolic domain of SARS-CoV E protein [78, 79].

In addition, memory T-cell response to the structural viral proteins such as S, M, and N, demonstrated to be long-lasting (over 11 years post-infection [73, 80]. SARS-CoV-2 not only infects lungs but also can cause heart injury because the ACE2 receptor is also expressed in the cardiovascular system. Along with this, it is stated that the imbalance immune response between type 1 and type 2 T-helper lymphocytes in SARS-CoV-2 infection might induce a cytokine storm, which not only affects lungs but also can cause injury to the cardiovascular system [81, 82]. Reports indicated that the patients with SARS-CoV-2 from Wuhan, China, had a high concentration of pro-inflammatory cytokines in their plasma, including high levels of IL1B, IL-6, IFNγ, IP10, and MCP1 that could be associated with disease severity. Patients with SARS-CoV-2 infection compared to SARS-CoV infection, seem to have a greater amount of T-helper 2 cytokines such as IL4 and IL10 that quell inflammation [15]. Studies on CoV have shown that pro-inflammatory cytokines (e.g., IFNγ, IL12, IL1B, IL6, IP10, and MCP1) are related to pulmonary inflammation and immense damage to the lungs [81]. However, more investigations are required to determine the Th1 and Th2 responses in SARS-CoV-2 infection. Furthermore, antibodies and the complement system as part of humoral immune responses are both vital, especially during the persistent phase of CoV infection. However, the complement system has the potential to damage the host tissue at the site of infection [73]. SARS-CoV-2 can induce a massive chain of inflammatory responses through initiating JAK/STAT signaling pathway, as well as and NF-κB, which can cause a pro-inflammatory cytokine storm. Noteworthy, that cytokines like IL-6 has a positive feedback loop and bind to their receptors and initiate a JAK/STAT-mediated signaling pathway. The virus also downregulates ACE2 receptors, which is followed by the elevation of angiotensin II and subsequently, induction of the JAK/STAT signaling pathway. This SARS-CoV-2 induced inflammatory response not only affects the lungs but also leads to cardiovascular implications [83]. In this regard, some targeted drugs have been suggested, for example, Tocilizumab and Sarilumab as IL-6 receptor antagonists, Baricitinib, Fedratinib, vaand Ruxolitinib as JAK/STAT inhibitors, and serine protease inhibitors for inhibiting of NF-κB signaling pathway [83]. To control immune response-related injury to the lungs mediated by SARS-CoV-2, Dexamethasone has been applied as a treatment to alleviate inflammation-mediated lung injury in hospitalized patients. In which case, co-administration of Dexamethasone and respiratory support in 2104 patients indicated a lower mortality rate compared to the group who received no respiratory support [84].

CLINICAL FEATURES, DIAGNOSIS, AND MANAGEMENT OF COVID-19

Different studies on patients demonstrated the clinical characteristics of COVID-19. According to epidemiological, demographic, clinical, laboratory, and radiological data, which includes a broad range of clinical manifestations, the most common symptoms are fever, fatigue, dry cough, dyspnea, myalgia, and shortness of breath. Less common symptoms, which mostly occurred in critically ill patients, include headache, confusion, chest pain, expectoration, pharyngalgia, diarrhea, nausea, and vomiting. Most patients showed more than one symptom. Some patients presented organ dysfunction, such as ARDS (acute respiratory distress syndrome), acute renal injury, and acute respiratory injury. There were underlying complications among reported cases, which included hypertension, diabetes, cardiovascular disease, malignancy, chronic liver disease, cerebrovascular disease, chronic kidney disease, and HIV infection [15, 79, 85]. Intriguingly, Dorgalaleh et al. have reported that patients with severe hypocoagulable conditions like patients with congenital bleeding disorders might become protected from adverse effects of COVID-19-pertained hypercoagulopathy [86]. In a study on 138 Chinese patients with COVID-19, thorough laboratory tests included prothrombin time (PT), activated partial thromboplastin time (APTT), complete blood count (CBC), D-dimer, creatine kinase (CK), CK-MB, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), aspartate aminotransferase (AST), total bilirubin, creatinine, hypersensitive troponin I, and procalcitonin were conducted. Prolonged PT, lymphopenia, and elevated LDH were among the most common laboratory findings. The patients in the intensive care unit (ICU), in comparison to non-ICU patients, showed more laboratory abnormalities. Patients who were in ICU showed higher levels of white blood cell (WBC) and neutrophil, D-dimer, CK, and creatine. Laboratory parameters in patients with novel coronavirus–infected pneumonia (NCIP) showed differences between survivors and non-survivors. Lymphopenia was Significant among them, but non-survivors gradually showed more severe lymphopenia. Compared to survivors, WBC count and D-dimer were elevated in non-survivors. Similarly, BUN and creatinine were increased progressively in non-survivor patients before death [85]. The diagnosis of COVID-19 starts with the aforementioned clinical and laboratory assessment, then a chest CT scan helps to determine the involvement of lungs, and eventually needs to be confirmed with real-time PCR. Nearly all of the patients show a bilateral distribution of ground-glass opacity and patchy shadows in their chest CT scans [87]. Considering the sensitivity of diagnostic tests for COVID-19, a study indicated that a chest CT scan has greater sensitivity compared to real-time RT-PCR because there are still some drawbacks and technique variations in real-time RT-PCR. So, they recommended that symptomatic patients with negative real-time RT-PCR test should be screened with a chest CT scan along with clinical and epidemiologic evaluation [88].

Table 1: Different clinical-phase vaccine candidates for COVID-19.

Developer	Candidate/Mediator	Vaccine characteristics	Trial ID
Moderna	mRNA-1273	Encapsulated mRNA encoding S protein	NCT04387656
CanSino Biologicals	Ad5-nCoV	Adenoviral vector expressing S protein	NCT04398147
Pfizer	BNT162b1 BNT162b2 BNT162b3	RNA vaccine	NCT04368728
Inovio Pharmaceuticals	INO-4800	DNA plasmid encoding S protein	NCT04336410
University of Oxford	ChAdOx1 nCoV-19	Chimpanzee adenovirus-vectored vaccine expressing the SARS-CoV-2 spike protein	NCT04400838
Shenzhen Geno-Immune Medical Institute	LV-SMENP-DC	Modified dendritic cells with lentiviral vectors expressing SARS-CoV-2 proteins- administered with antigen-specific cytotoxic T lymphocytes	NCT04276896

Once a vaccine has been approved for SARS-CoV-2 and all are in development [89], the management of the disease could be achieved by taking some essential measures. Based on the guideline published by Ying-Hui Jin et al. at first, patients should rest in bed and receive a sufficient amount of food as an energy source (protein-rich foods), water, electrolytes, and other internal environment factors. Meanwhile, they should be monitored for heart rate, respiratory rate, pulse oxygen saturation, blood pressure, blood routine, CRP, PCT, ALT, AST, bilirubin, myocardial enzyme, creatinine, BUN, coagulation function, and arterial blood gas. Oxygen therapy has been strongly recommended. Drug treatments, including antiviral therapy, antibiotic therapy, and the use of corticosteroid could be helpful. [66, 90]. However, the comprehensive WHO interim guideline is also available for clinical management of COVID-19 [91]. One of the controversial issues about SARS-CoV-2 is about blood transfusion. Association of SARS-CoV-2and transmission through blood transfusion is still unclear. [92].

CONCLUSION

In this work, we provided the biological and clinical properties of SARS-CoV-2. As long as the epidemy of SARS-CoV-2 goes on, the best recommendation for this global threat would be preventive and safety measures. CT scan and real-time RT-PCR are the best armamentaria for confirmation of this virus. SARS-CoV-2 virus spreads in frantic pace among humans and more comprehensive studies are demanded. However, two epidemics (SARS-CoV and MERS-CoV) have shown us that the journeys for overcoming these kinds of diseases are still challenging.

Conflicts of interest and sources of funding

The authors report no conflict of interest.

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ORIGINAL ARTICLES

Survey of the patients' satisfaction with services provided in an Iranian Naval Hospital in 2019: A cross-sectional study

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Abstract: *Background and Aim: All hospital services are carried out to serve the patient, which is the main axis in the hospital. Patient satisfaction can be indicative of the right performance service delivery and is considered a reliable source for evaluating the quality of health care. The present study was conducted to evaluate the satisfaction rate of discharged patients in a Navy hospital.*

Methods: The descriptive cross-sectional study was performed on 220 discharged patients selected by convenience sampling in a Navy hospital during 2019. The data gathering tool was the Patient Satisfaction Questionnaire designed by Amerioon in 2009 and its validity and reliability were confirmed in 2016. The questionnaire assessed patient satisfaction in two healthcare sections with 21 questions and three dimensions of hygiene, nursing and physician services, and non-health care with 27 questions and six dimensions of acceptance, staff attitudes, nutrition, welfare, facilities, religious issues, and refines evaluation. Data were analyzed by SPSS software version 18.

Results: The patient satisfaction in the medical and non-medical wards was 88.1% and 86.57% respectively. Satisfaction in the medical and non-medical wards was excellent at all dimensions. There was a significant relationship between satisfaction with the cause of referral, and level of education (P <0.05). Other demographic variables such as age, marital status, patient gender, frequency of hospitalization, type of ward, and type of insurance were not significantly correlated with satisfaction(P>0.05).

Conclusion: Considering the high level of patients 'satisfaction in medical and non-medical services, patient satisfaction in medical services was slightly higher than that of non-medical services, this highlights the need for independent research in this area and the actions taken by hospital managers to improve satisfaction in these sectors. It is also suggested to consider patient satisfaction as part of the discharge process in all hospitals

Keywords: *patient, satisfaction, hospital, Navy*

INTRODUCTION

Satisfaction as an indicator of the quality of services provided over the past few decades has found a special place. The quality of services provided to patients and the level of satisfaction with the services provided show the general situation of providing services in that hospital [1]. Evaluating patient satisfaction is an important source of information to identify problems and desirable programs in the field of health care services and is an indicator of the

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quality of service delivery in various interpersonal, organizational and technical areas [2]. At present, satisfaction is an important issue in health care, and patient satisfaction plays an important role in increasing accountability among health care providers in terms of advancing the organization's goals [3]. Particular attention is paid to the patient's needs as an intrinsic goal, as well as the fact that this is an important component in improving the quality of care, and should be considered by policymakers. Also, patient satisfaction as a basic system should play an important role in managers' decisions to make that organization successful [4]. Therefore, patient satisfaction is one of the main factors and important indicators in evaluating the quality of health organizations [5].

In the past, to improve the level of health services, the opinion of experts in this field was the most important determinant of how to change the service process. However, recent studies have paid special attention to the views of patients on the provision of health services as an important indicator of the quality of service delivery. Comments that may even differ from or even contradict the views of health professionals and are recognized as a determining indicator for evaluating and improving the quality of services provided by services [6]. The importance of this issue is such that patients' satisfaction with the way health services are provided from the perspective of the World Health Organization, as one of the five indicators of quality of services [7] and according to some researchers, this factor is one of the most important determinants. The quality of health services is important [8].

Patients' views on how to provide health services can include important information about the quality of various aspects of health care delivery. The findings of various studies have shown that various factors affect the patient's satisfaction with the way the medical services are provided in the hospital, such as the way doctors, nurses, staff, and even the physical environment of the medical centers' function; also the patient's satisfaction with the hospital complex and its staff. The predictor of future behaviors and behaviors of patients during treatment and even seek discharge from the hospital [9, 10].

Patients' greater satisfaction with care makes them more willing to follow doctor's instructions, follow up on care and treatment, follow more dietary regimens and treatment recommendations, respect health care guidelines, and communicate more deeply with providers. Health services, as a result, reduce recovery time and reduce the number of patient complaints and achieve better health outcomes [11].

Patient satisfaction is also essential for marketing purposes. It provides patient satisfaction information, useful information about the structure, process, and outcome of nursing care, and more, such as staff adequacy, treatment needs, and patient behaviors [12]. Also, a patient who is satisfied with the health care provider often tends to go to the same hospital when he or she needs re-health care. This will increase productivity and profitability for the collection [13].

In the field of patient satisfaction assessment, several studies have been conducted in Iran, but since most studies have measured how to provide medical services or factors affecting satisfaction, and also because satisfaction is affected by various underlying factors such as individual, cultural, social, economic and religious factors of patients [14, 15] and considering that most studies conducted in Iran, except for hospitals and special urban areas and the views of residents of those geographical areas, on how to provide medical services and found It has led to many different ones [16-18], and since the studies are limited In hospitals under the auspices of the Armed Forces, patients have been treated with satisfaction, so due to the importance of these hospitals due to a large number of clients, to achieve patients' views on how to provide medical and non-therapeutic services, it is necessary to do so. The study is necessary in this case. Therefore, the present study was conducted to investigate patients' satisfaction with the services provided in one of the naval hospitals of the Revolutionary Guards in the city of Chalous.

ANALYZATION METHOD

Type of study

The present cross-sectional study was performed on patients discharged from one of the naval hospitals in 1398.

Sampling and determining sample size

Sampling was performed by the available method. To determine the sample size, the sample size estimation formula was used in the prevalence studies in terms of the inferential test. In terms of the first type of error of five percent, the second type of error of twenty percent and the probability of 50% satisfaction with the maximum sample size estimate, the required number of samples was calculated using the formula below 196; It was calculated that 185 usable questionnaires were returned in the present study, which was more than the minimum acceptable number of calculated questionnaires.

Input and output criteria

The criteria for entering the study were those over 18 years of age and the desire to participate in the study and not hospitalization in the psychiatric wards of the hospital. The criteria for leaving the study were the reluctance to continue participating in the study.

How to complete research questionnaires?

After obtaining a license from the research of Baqiyatallah University of Medical Sciences, he was referred to the selected hospital. After presenting the permit to enter the environment, the researcher entered the research environment and the research questionnaires were completed according to the entry and exit criteria. In cases where the clients were illiterate, the questionnaire questions were read by the researcher to the client and their answers were recorded.

Questionnaire for satisfaction with non-therapeutic medical services

The data of this study were confirmed by the standard patient satisfaction questionnaire which was designed by Amerioon et al. In 2009 and in 2016 its localization, validity, and reliability were confirmed. The questionnaire included two sections of general information such as age, gender, literacy level, marital status, section, type of insurance, and hospital history. The second part of the questionnaire included 48 questions, the level of patients' satisfaction with the way of providing services in two therapeutic sections with 3 dimensions and 21 questions, including services provided by the nurse (9 questions), physician (8 questions), and hospital health and cleaning services (4 questions) and the non-therapeutic section with 6 dimensions and 27 questions including services of admission section (2 questions), staff approach (5 questions), nutrition (2 questions), welfare and facilities (9 questions), clearance (4 questions) and observance of issues Shari'a-doctrinal (5 questions) evaluated. Responses were graded based on the Likert 5 spectrum (completely satisfied, satisfied, no comment, dissatisfied, completely dissatisfied). At the end of the questions, two open questions were asked. The content of the first question was related to the person's return to the current hospital in case of illness and the second question was about the current hospital's recommendation to friends and acquaintances. To calculate the satisfaction score, the respondents were given completely dissatisfied and completely satisfied with the score of zero to 100, respectively. Thus, the total scores of each field separately and the overall satisfaction score was determined from a score of 100, with a higher score indicating more patient satisfaction in the context of how services were provided during hospital stay (19). The criteria for classifying satisfaction were based on four categories: low (scores up to 25 percent), medium (26 to 50 percent), good (51 to 75 percent), and excellent (76 to 100 percent) [15].

Reliability and Validity

The validity and reliability of this questionnaire were evaluated in the country in 2016 by Amerioon and colleagues in the same community and its validity and reliability were confirmed. The overall alpha coefficient of the treatment questionnaire was 0.975 and the non-therapeutic questionnaire was 0.936 [19].

Moral considerations

This study was approved by the University Research Council with the code 98000238, which was approved by the ethics committee of Baqiyatallah University of Medical Sciences at IR.BMSU.REC.1398.269. Adequate information about the objectives of the study and its importance, maintaining the anonymity and voluntary participation, and confidentiality of the information obtained was given to the participants and was completed after obtaining the written consent of the questionnaires.

Statistical analysis

SPSS software version 18 was used to analyze the data. The level of significance in all cases was considered less than 0.05. To describe the qualitative variables, frequency and frequency percentages were used, and to describe quantitative information, mean and standard deviation were used. To investigate the research hypotheses, a test of a Smirnov-Kolmogorov sample was first used. The results showed that the distribution of the studied data was normal, so to investigate the research hypotheses, parametric t-test, Pearson, and ANOVA tests were used.

FINDINGS

Description of population-cognitive information

220 questionnaires were completed and returned, which was more than the minimum acceptable number of calculated questionnaires (215 questionnaires). The average age of the participants in the study was 44/17 ± 13/53 years. Most of the male research samples (62.2%) and most of the participants in the study were married (67%) (Table 1).

Information in medical departments

The mean and standard deviation was the overall score of patients' satisfaction with the 95 ±9/12 treatment sections. The mean score of satisfaction in terms of health and cleanliness was 18/54±1/92 in the nursing dimension 26/94±3/11 and the medical dimension 49/52±5/23.

The percentage of patients 'satisfaction in the health and cleaning sector was 90/87 in the nursing ward, 87/25, and in the physicians' ward, 87/55, and the overall satisfaction with the treatment ward was 88/1 (Table 2).

Table 1: Distribution of frequency characteristics of patients participating in research

Variable		Abundance	Percentage
Gender	Female	70	37/8
	Man	115	62/2
Marital status	Married	124	67
	Single	61	33
Education	High school	62	33/6
	Diploma and postgraduate	85	45/9
	Bachelor's degree and higher	38	20/5
Type of insurance	Treatment services	47	25/4
	Armed Forces	54	29/2
	Social Security	46	24/9
	Other insurances	17	9/1
	Uninsured	21	11/4
Inpatient ward	Men	105	56/8
	CCU	3	1/6
	Maternity hospital	3	1/6
	Obstetrics and Gynecology	59	31/9
	Children	1	0/5
	Emergency	14	7/6
Hospital history	First time	120	64/9
	Second time	22	11/9
	Third time	8	4/2
	Fourth time	4	2/2
	More than four times	31	16/8
The cause of hospitalization	Contract with the hospital	89	48/1
	Being close to home	34	4/18
	Hospital facilities	29	15/7
	Observance of religious values	6	3/2
	Satisfaction in previous visits	9	4/9
	Offer others	12	6/4
	References from other centers	2	1/1
	Doctor's advice	4	2/2

Table 2: Distribution of patient satisfaction in the study participants

Satisfaction of medical departments	Minimum score	Maximum	Percentage	Standard deviation	Average
Cleanliness and hygiene	11	20	18/54	1/92	90/87
Nursing services	18	30	26/94	3/11	87/25
Medical services	28	55	49/52	5/23	87/55
The total score of the dimensions of the questionnaire	65	105	95	9/12	88/1

Information on non-therapeutic sections

In the non-therapeutic part, the mean and standard deviation of the overall score of patients' satisfaction was 120/50 ± 12. The mean and standard deviation of the satisfaction score in the admission dimension was 8/84 ± 1/34, in the employee attitude dimension it was 22/36 ±2/52, in the nutrition dimension it was 9/02 ± 1/24, in the welfare dimension, and the facility 39/99 ± 4/42. 39, in the dimension of clearance 18/08±2/13, and the dimension of Observance of religious values, it was 22/20±2/85. The mean and standard deviation of the total score of the clients' satisfaction with the non-therapeutic sections was 120/50±12.

The percentage of patients' overall satisfaction with non-treatment services was 86/57%, which was 85/33% according to the satisfaction level of the admission department, 86/14 employees, 87/83 nutrition, 86/09 welfare facilities, clearance and subsequent observance of religious issues - Belief was 86/02 (Table 3).

Table 3: Distribution of patient satisfaction in the study participants

Satisfaction with non-therapeutic areas	Minimum score	Maximum	Percentage	Standard deviation	Average
The reception	3	10	8/82	1/34	85/33
Staff	14	25	22/36	2/52	86/14
Nutrition	4	10	9/02	1/24	87/83
Facilities-welfare	26	45	39/99	4/42	86/09
Clearance	12	20	18/08	2/13	88
Observance of religious issues	12	25	22/20	2/85	86/02
The total score of the dimensions of the questionnaire	81	135	120/50	12	86/57

Pearson correlation coefficient showed that there was no significant relationship between age with the overall score of satisfaction with medical services (P = 720 and r = 0.026) and the overall score of satisfaction with non-therapeutic services (P = 0.897 and r = 0.010). Independent statistical t-test between sex with an overall score of satisfaction with health services (P = 0.581) an overall score of satisfaction with non-therapeutic services (P = 0.922) and between marriages with an overall score of satisfaction with health services (P = 0.653) an overall score of satisfaction with Non-therapeutic services (P2 = 0.238) did not show a significant difference.

The one-way analysis of variance test did not show a significant difference between the type of insurance with the overall score of satisfaction with medical services (P22 = 0.222) and the overall score of satisfaction with non-medical services (P = 0.251); However, there was a significant difference between the reason for visiting the hospital with the overall score of satisfaction with medical services and the overall score of satisfaction with non-medical services (P <0.0001). Regarding the difference between the overall score of medical services satisfaction and the reason for visiting the hospital, Tuki's follow-up test showed that this difference was related to having a contract with the hospital (P = 0.005), the hospital's proximity to the place of residence (P00 = 0.009), and others' suggestions. 0.0001> P) and the doctor's recommendation (P = 0.026), although the difference between satisfaction and hospital facilities and facilities (P = 0.062) and referral from other centers (P = 0.095) was not significant; this difference was close to the level of significance. You had. The one-way analysis of variance test did not show a significant difference between the number of visits to the hospital with the overall score of satisfaction with medical services (P = 0.225) and the overall score of satisfaction with non-medical services (P = 0.961).

The one-way analysis of variance test showed a significant difference between the variable of education and the overall score of satisfaction with medical services (P = 0.002) and the overall score of satisfaction with non-therapeutic services (P = 0.006). Regarding the overall score of satisfaction with medical services, Tuki's follow-up test showed that this difference was related to the level of diploma with post-diploma (P = 0.005) and diploma with bachelor's degree and above (P = 0.011). Regarding the overall score of dissatisfaction with non-medical services, Tuki's follow-up test showed that this difference was related to the level of diploma with post-diploma (P = 0.012) and diploma with bachelor's degree and higher (P = 0.025). Sideways showed that there was no significant difference between the variable type of hospital ward with the overall score of satisfaction with medical services (P = 0.161) and the overall score of satisfaction with non-therapeutic services (P2 = 0.230).

In general, the findings showed that there was no significant statistical relationship between age, marriage, patient gender, type of insurance, type of hospital ward, number of hospital visits, and an overall score of satisfaction with medical and non-therapeutic services. However, there was a statistically significant difference between the reasons for visiting this center and the satisfaction with medical and non-therapeutic services (P <0.05), so that people who had a contract with the hospital or the hospital was close to their place of residence, as well as people who Others suggested a doctor's recommendation and had a higher percentage of satisfaction. Also, the statistical test of one-way analysis of variance showed that there is a significant statistical relationship between education and an overall score of satisfaction with medical and non-therapeutic services so that the percentage of satisfaction decreases with increasing education.

The percentage of satisfaction scores was divided into four categories: low (up to 25%), medium (26 to 50%), good (51 to 75), and excellent (76 to 100). There were no scores in the lower category. The highest satisfaction scores were in the top category. Regarding the two open questions at the end of the questionnaire, the results showed that 74.1% of people choose this hospital again for treatment in case of illness, and 72.4% recommend this hospital to their friends and acquaintances.

DISCUSSION

This study aimed to examine patients' satisfaction with the services provided in one of the naval hospitals. According to the results, the general satisfaction of patients in the medical and non-therapeutic sectors in all dimensions, including health and hygiene, nursing, medicine, admission, discharge, staff services, facilities, observance of religious and religious issues, and discharge at the highest level.

Patient satisfaction is one of the most important tools in evaluating the quality of treatment and planning to improve the health system [20]. Despite a large number of clients in hospitals under the auspices of the Armed Forces, especially in cities where only one hospital may provide services to the military and their families, a limited number of studies have been conducted on patient satisfaction in these centers [15, 21, 22, 23] While examining satisfaction in hospitals of the Armed Forces due to their important role in providing services, can improve the quality of services and reflect the performance of these centers to the community and public acceptance of the services of these hospitals. Play a huge role. Therefore, the present study is new in terms of measuring the satisfaction of patients discharged from the Marine Corps Hospital of the Islamic Revolutionary Guard Corps.

The level of satisfaction with nursing services in this study was excellent, similar to this finding in most studies that assessed patients' satisfaction with optimal nursing services [24, 25]. In the Riskuz study in Spain, the level of patients' satisfaction with the desired nursing care was also reported [26]. In the Al-Asad study, the mean score of patients' satisfaction with nursing care was 90.67% and very high [27]. In the Jannati study, the percentage of patient satisfaction was lower than in our study, so that 77% of patients were dissatisfied with nursing services [28].

According to research, nurses' satisfaction is an important factor in patient satisfaction. In various studies, it has been emphasized that there is a direct relationship between nurses' job satisfaction and patients' satisfaction with their services and any issue that leads to a decrease in nurses' job satisfaction. Including unfavorable work environments, lack of manpower in the field of nursing, inappropriate care, moral, social, and organizational values can make efforts to improve patient satisfaction by nurses ineffective. Satisfaction with nursing services plays an important role in the overall satisfaction of the patient, and other studies emphasize the satisfaction of nursing services as one of the determinants of overall patient satisfaction [29, 30]. Akbari said in his study that because nursing medical care plays a pivotal role in restoring patients' health, it has the greatest impact on patient satisfaction [31], so since most care providers in hospitals are nurses and This group is in the first line of patient care, the quality of their care has a great impact on patient satisfaction, and therefore it is necessary to draw the attention of officials to improve the conditions for improving the quality of nursing and medical services. The difference in the results of various studies can be justified by the fact that the cultural and social context can determine the expectations and expectations of people in society from services. For this reason, health policymakers should pay special attention to the job satisfaction of nurses and, consequently, patient satisfaction with medical services. Conversely, in studies that were highly satisfied with nursing services, appropriate organizational culture, including employment standards, financial benefits, and retention of qualified and committed nursing workers, were cited as reasons that may have led to high patient satisfaction [27].

The high satisfaction of medical services in this study was similar to the results of Heydari and Ansari studies, which reported patients' satisfaction with medical services at an excellent level [32]. Similar to this finding, the overall satisfaction of patients with medical services in the study of Azizi [33], 29.5%, in the study of Noor Al-Sana, 50% [34] and in the study of Alidosti was equal to 56.4% [35], which is the result of the present study. It's not true. Considering the 6 to an 8-year interval between these studies and the recent study, it can be concluded that due to the holding of medical ethics workshops in recent years and the importance of medical ethics, and the impact of these workshops, physicians 'treatment of patients has improved and patients' satisfaction has increased. Therefore, in older studies, the rate of physician satisfaction was lower than in a recent study.

In the present study, satisfaction with non-therapeutic services in all dimensions was excellent (85.33%). In a study conducted in another hospital under the auspices of the Navy, the satisfaction of the admission department was excellent and similar to this study [15]. In studies conducted by ZafarGhandi, patients' satisfaction with the admission of hospitals under the auspices of Tehran University of Medical Sciences is 44% [36] and in the study of Rudbari in Zahedan 64% [37] is reported. In all these studies, satisfaction with admission services is less than Our study has found that the reason for this difference is the better location of admission to the hospital under study, as well as the lower number of clients than educational hospitals covered by the university and therefore more speed of admission and better treatment processes and proper communication with clients by staff.

Also, in this study, the satisfaction of the clearance section was at the highest level (88%), and dissimilar to this finding in the Ansari study, the clearance section had the lowest level of satisfaction (38.6) [38]. Excellent satisfaction with nutrition services in this study was another finding that was obtained similar to this finding in a large study. 90% of patients were satisfied with the nutrition service [39] Satisfaction with nutrition services in Ansari's study [38] and Mousavi [40] and Gholi Zadeh [41] were average and less than our study. In his study, Gholizadeh stated that the nutrition sector has been ignored as one of the pillars of patients' health in hospitals or has played a small role in the treatment process. Therefore, hospital managers need to take steps to increase the awareness of foodservice personnel about health and food quality, increase the number of nutrition experts and their more active presence in patients' beds, and give patients the right to choose.

Patients' satisfaction with high-level and non-similar welfare services and facilities was studied by Abbasi [15], Karimi [42], and Zol Havariyeh [43]. He noted the cold in some areas, the poor health of the health services, the deteriorating condition of some of the patients' boards, and the deterioration of the elevator. It should be noted that the use of patient feedback to increase the level of medical services and improve hospital hotels is very important to increase patient satisfaction [43]. Similar to this finding, patients' satisfaction with facility welfare services in a cross-sectional descriptive study on 131 hospitalized patients in 6 military hospitals across the country reported patient satisfaction with 98.2% of hospital services [23].

In this study, the level of satisfaction with observing religious issues was excellent. However, in the satisfaction studies, this case was not considered and one of the advantages of the questionnaire used in this study is to consider this item because according to Hassan Tehrani's qualitative study, it was found that privacy protection is one of the basic human needs and the most important concepts of nursing ethics. One of the dimensions of privacy is the patient's spiritual and religious privacy, which should be taken into account when caring for the patient, which was excellent in this study [44].

Another finding of this study was a significant relationship between education level and patient satisfaction so that with increasing level of education, the level of satisfaction decreased. Similar to this finding, in July and Farahani and Farzianpour's studies, the patient's level of education was an effective factor in the level of satisfaction [25, 29, 45]. Thus, people with higher education are less satisfied for reasons such as higher expectations of the treatment system, greater social communication, and greater access to information resources, and greater ability to identify system deficiencies and defects [45]. In Quintana and Findick's research, patients with higher levels of education were less satisfied with nursing care [46, 47]. Contrary to this finding, in the study of Mozaffari [48] Khezri [49], with increasing patient education, his satisfaction with nursing care services increased. In Abbasi's study, there was no relationship between education and satisfaction.

In terms of the reason for the visit, there was a significant relationship between satisfaction and the reason for the visit, so that having a contract with the hospital, the doctor's suggestion and the advice of acquaintances, as well as the hospital's welfare facilities, had a significant relationship with satisfaction. The results of the study in one of the military hospitals are the most important reasons for patients to choose the hospital in terms of the ability to use the appropriate insurance benefits of the hospital, the presence of experienced physicians, and the suggestion of others. Eventually, the center was easily accessible to patients [22].

Another finding of the recent study was the significant lack of relationship between the patient's gender and his satisfaction. This finding is consistent with the studies of Hajjian [17] and Rudbari [37] and can indicate that the quality of service is determined by satisfaction and not the sex of the patient [28]. Also in Johnson's [13] and Elnager's [51] research, this relationship was significant and male patients had a more positive experience and satisfaction with nursing care than female patients. Differences in satisfaction between men and women may be due to behavioral differences between men and women, or men may receive more information from nursing staff than women. The relationship between marriage and satisfaction was not significant in the present study, but the study of Goodarzian [52] and Akbari [22] showed that there is a significant relationship between marital status and satisfaction. In the present study, the relationship between insurance type and satisfaction was not significantly similar to this finding, Study of Farzanpour [45] and Moin [53]

Health insurance was reported as one of the factors affecting patient satisfaction. In this study, there was no relationship between the type of section and the level of satisfaction, but in Akbari's study, this relationship was significant [35].

The provision of the same services and facilities to the wards and the existence of optimal medical and nursing services have been effective in creating patient satisfaction regardless of the type of ward so that in hospitals that have a significant relationship between satisfaction and type of ward, the percentage of satisfaction with medical and nursing services is less.

Also, in the study of Akbari [35] and Salehian, the type of insurance showed a significant relationship with satisfaction. The reason for the difference in the results of studies is that in this medical center, no insurance or organ has a special priority and all patients benefit from the same amount of facilities and medical services. The association between hospitalization and patient satisfaction was not statistically significant; similar to the results of the present study, satisfaction increased in a study with increasing hospitalization [54].

In general, the reason for the difference in satisfaction results in recent studies and other studies can be considered the difference in the type of staff, hospital environment, and how to provide services in different centers, which greatly affects the quality of staff work and subsequent satisfaction. But, factors such as differences in the characteristics of the study population and, most importantly, the different methods of assessing satisfaction and overcrowding in metropolitan hospitals should also be considered. Also, people's cultural conditions, such as proper etiquette and communication, prevent the reflection of real opinions and their criticisms. In addition, dissatisfied patients usually do not cooperate in completing the questionnaires and do not want to participate in the study, which increases participation. Satisfied people are satisfied and as a result, the percentage of people's satisfaction increases falsely. Another important difference between patients' satisfaction in this study and other studies is that other hospitals may be educational due to the prevailing atmosphere in university hospitals, such as the centrality of education, research, and training of human resources, as well as the multiplicity of decisions and crowded sections. Various consequences have negative consequences, such as the lack of justice for patients in terms of access to medical facilities; while in the hospital under study, the focus has been on patient management by experienced staff, not students, and there is no overcrowding in university hospitals, and therefore patient satisfaction has been further ensured.

In the present study, a high percentage of patients announced that they would choose this hospital for their next visit and would also recommend this center to friends and acquaintances. This finding is consistent with the results of a genetic study that found that 85% of patients' satisfaction could lead to a recurrence of about 92% of them and the hospital's recommendation to others at 89% [28].

Strengths

One of the advantages of this research is that it provides a sufficient questionnaire to the patient and there is no need for the questioner to be present with the patient to check the level of satisfaction and also its compliance with the conditions, rules, and culture of the hospital. As much as possible, an attempt was made to use the patient's comments during the discharge and when the discharge was performed by the companion so that the patient could respond calmly. In this study, we have tried to consider all the factors affecting patient satisfaction in compiling the questionnaire and therefore the results of satisfaction with this questionnaire can identify key factors affecting the management of the hospital in improving hospital conditions to obtain more patient satisfaction.

Limitations

One of the limitations of the research is the use of only one military hospital to measure satisfaction and non-comparison with other centers, so the results of this study cannot be generalized to the entire health care system in Iran. Also, the reluctance of dissatisfied people with the services to complete the questionnaire may have created a measurement bias in the results. To eliminate this limitation, an attempt was made to provide complete explanations and the purpose of the research and to mention the importance of the dissatisfied patients' point of view to improve the performance of the hospital. As much as possible, participants should be encouraged to participate in the research to complete the questionnaire.

CONCLUSION

Given that patient satisfaction is of great importance as a measure of the quality of care, the results can provide a wealth of information about the success rate of programs in achieving the values and expectations of patients. It can also help with higher levels of management in planning, policy-making, and program evaluation. The results of the study showed that the level of client satisfaction with the hospital is excellent and the level of satisfaction in the treatment department is slightly higher than in non-therapeutic departments. In this regard, to improve the performance of the hospital and improve patient satisfaction, it is necessary to conduct an independent study to investigate the causes of dissatisfaction. Use more advanced statistical methods such as structural equations to determine the contribution of each of the measured areas to patient satisfaction. Finally, it can be stated that by using the results of this study and determining the prioritization of executive measures to provide better services, more patient satisfaction can be achieved.

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Conflict of interest

The authors state that there is no conflict of interest in the present study.

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CLINICAL PRACTICE

Survival following repetitive ventricular fibrillation caused by monoamine oxidase inhibitor overdosing (MAOI).

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Abstract*: One of the great dreams of humanity has been the improvement of its behavioural, affective and emotional status. Depression represents the suffering of beings over their existential course, as a point of passage between the human condition and its destiny. The attempts of psychiatry to introduce efficient and increasingly performing antidepressants represent one of medicine's great achievements. At the same time, antidepressant overdosing may lead to cardiotoxic manifestations presenting lethal risks as consequences [1]. We hereby present the case of a patient that was poisoned with tianeptine, ingested for suicidal purposes, who survived repetitive episodes of ventricular fibrillation, electrically converted to sinusal rhythm. The GC/MS analytical diagnosis played an essential part in detecting tianeptine in the biological samples. The rapid and very competent therapeutic intervention allowed for the patient's survival, despite further complications. A review of main tianeptine information is also presented, as this type of intoxication may have deadly consequences.*

Keywords: *tianeptine, ventricular fibrillation, acute intoxication*

INTRODUCTION

Tianeptine (fig. 1) was discovered and patented by the French Society for Medical Research in the 1960s. It is an atypical antidepressant, used mostly to treat major depressive disorder, but also to treat anxiety, asthma and irritable bowel syndrome. Regarding its chemical structure, tianeptine has the structure of a tricyclic antidepressant (TCA), but has a pharmacological profile that is very different from other TCAs and is grouped separately from those due to its unique mechanism of action [1]. As a modulator of the serotoninergic synapse, it has good efficiency in mild/moderate forms of depression associated or not with anxiety; it does not interfere with the metabolism of other drugs at the level of P45 cytochrome. Tianeptine does not have an affinity for muscarinic, alfa1-adrenergic or type 1 histaminergic receptors, which leads to less adverse events compared to TCAs.

Initial studies have shown that following acute and repeated administrations, the presence of tianeptine led to a decrease in extracellular levels of serotonin in rats' brains without a decrease in the release of serotonine, which is why it was called a "(selective) inhibitor of serotonine reuptake".

As opposed to TCAs and selective serotonine reuptake inhibitors (SSRI), tianeptine improves the synaptic uptake function of serotonine; despite all these, its efficiency against depressive episodes is comparable with that of TCAs and fluoxetine [2, 3]. Recently (2014), it has been demonstrated

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that tianeptine is an agonist of opioid receptors (MOR). Thus, as opposed to its therapeutic effects, MOR activation is probably the one responsible for tianeptine's toxicity in high doses, those that far surpass the normal therapeutic interval [1]. The therapeutic levels for tianeptine in whole blood, according to available data, are 0.3–0.7 mg/ml.

The absorption takes place almost entirely (99% bioavailability) at GI level, it is completely metabolized by the liver and it is not affected by the first pass effect [4]. Its distribution in the organism takes place quickly, 95% of it binds to plasma proteins (especially albumine) and the mean plasmatic half-life through distribution is approximately 0.7 hours.

Table 1. Adverse events for tianeptine – classified by frequency.

Type of adverse events	Adverse events
Common (frequency >1%)	Headaches (up to 18%) Dizziness (up to 10%) Insomnia/nightmares (up to 20%) Sleepiness (up to 10%) Dry mouth (up to 20%) Constipation (up to 15%) Nausea Abdominal pain Weight gain (~ 3%) Agitation Anxiety/irritability
Less frequent (frequency 0.1-1%)	Bitter taste Flatulence Epigastralgia Blurred vision Muscular pain Premature ventricular contractions Micturition disorders Palpitations Orthostatic hypotension Tremor
Rare (frequency <0.1%)	Hepatitis Euphoria ECG modifications Pruritus-like cutaneous reactions/alergy Muscular pain

Metabolizing in the liver takes place especially through beta-oxidation and, in a lower proportion, through N-demethylation, the main metabolites being propanoic acid (inactive metabolite) and pentanoic acid (active metabolite) [5]. Tianeptine is eliminated both renally and through extrarenal ways, mostly through urine (only 8 % is eliminated in unmetabolized form), fecces (15%), bile and has a half-life of 2-3 hours. [5].

Compared to other TCAs, tianeptine can lead to significantly less cardiovascular, anticholinergic (such as constipation or dry mouth), sedative and appetite stimulating effects [6, 7]. Recent studies have shown that it is amongst the most hepatotoxic (hepatic lesions) antidepressants, although proof supporting this concern is limited [8]. Also, it has been shown that TCAs can lead to cardiac arrhythmia, even though the data for tianeptine is limited [9].

Based on adverse event frequency, they are classified according to table no. 1 [5, 6, 10].

The certitude diagnosis is established by identifying tianeptine in biological fluids.

Figure 1. Tianeptine 2D Structure form

https://pubchem.ncbi.nlm.nih.gov/compound/Tianeptine#section=Top

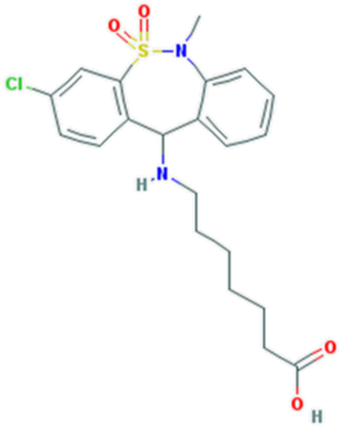

For this purpose, the GC/MS method (gas-chromatography coupled with mass spectrometry) is used. The GC/MS method, applied in the Analytical Toxicology Laboratory of the Military-Medicine Research Center, is performed by a GC/MS Varian SATURN 2000 computerized system: Varian gas-chromatograph STAR 3800 CX model and Varian mass spectrometer with an ion trap detector. The urine samples are analyzed using liquid-liquid extraction with organic solvents. For the extraction control, midazolam was used as an internal standard, which is a benzodiazepine whose retention time is situated in a different range from that of tianeptine. Mass spectra libraries were used, namely Pfleger–Maurer–Weber, NIST 98 and Wiley 6.

Figure 2: Total ion chromatogram and the mass spectra obtrained for lidocaine (peak no. 1), for tianeptine (peak no. 2) and for midazolam (peak no. 3).

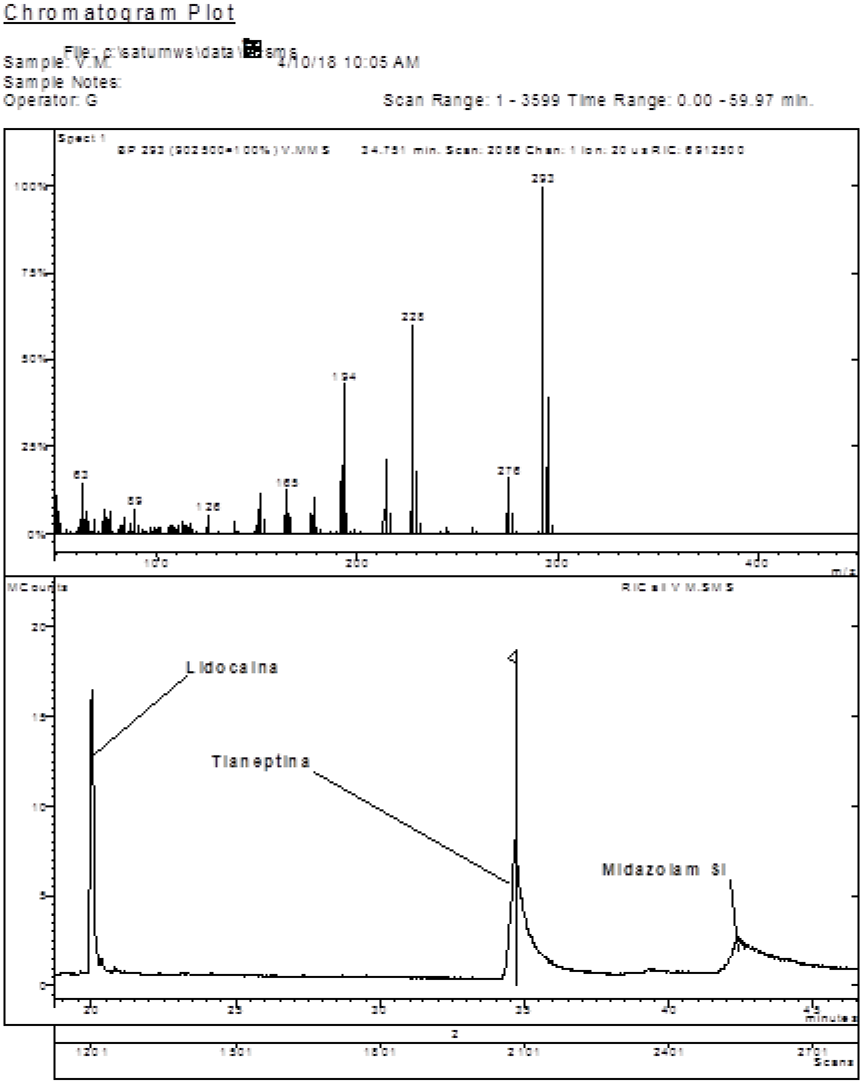

Table 2. The type of compound that was identified, its chemical formula and characteristic spectral lines

Identified compound	Chemical formula	Molecular mass	Spectral lines (m/z)
Lidocaine	$C_{14}H_{22}N_2O$	234	86, 234
Tianeptine	$C_{21}H_{25}ClN_2O_4S$	437	194, 214, 228, 276, 293
Midazolam (SI)	$C_{18}H_{13}ClFN_3$	327	310, 327

Following the analysis of the urine extract through the GC/MS method, the separation and identification of the following compounds were achieved: Lidocaine, Tianeptine and Midazolam (internal standard), presented in figure 2 and table 2.

CASE REPORT

Patient, aged 56 years old, with no pathologic history was brough by the medical ambulance to the Bagdasar hospital after being found at home following drug intake for suicidal purposes (declarated Nobixal –

Tianeptine) with agonistic respiration.

At home, the patient suffered two episodes of ventricular fibrillation, remitted after external electrical shocks (EES) applied by the ambulance medical staff. In the emergency care unit, the patient suffered another episode of ventricular fibrillation which responded to EES, with hemodynamic instability still persisting. Due to acute respiratory insufficiency, orotracheal intubation was necessarily performed.

Cardiological evaluation: arterial pressure 90/60 mmHg, AV 100 bpm, no murmurs, no rales, no edemas; ECG: regular sinusal rhythm, Q wave in DII, DIII, aVF.

The ECG shows a moderate/severe dystolic dysfunction of the left ventricle, most probably in the context of intoxication, followed by an EES, applied for rhythm disorders. The patient is sent to Emergency Clinical Hospital Bucharest, Intensive Care Unit II Toxicology.

Upon admission into Toxicology, the patient is in a serious condition sedated, orotracheal intubation with spontaneus respiration, presence of bilateral pulmonary vesicular murmur, hemodynamic instability on vasopressor support, arterial pressure = 100/58 mmHg, AV = 96/bpm, regular rhythm, normal abdominal movement, normal diuresis.

Biological parameters upon admission: increased values of glycemia, creatinine, liver enzymes (ALT and AST), LDH but also leukocytosis; coagulation tests being in normal ranges.

Table 3. Biological parameters

Sample	Result	
	Measured values	Normal values
Glycemia (*mg/dl*)	138	75-110
Urea (*mg/dl*)	25	19-43
Creatinine (*mg/dl*)	1.46	0.7-1.4
AST (*U/l*)	81	21-72
ALT (*U/l*)	107	14-50
LDH (*U/l*)	481	150-450
Sodium (*mmoles/l*)	144	137-145
Potassium (*mmoles/l*)	4.51	3.50-5.00
Leucocytes (*x* $10^3/mm^3$)	16,8	4-9
Hemoglobine (*g/dl*)	13.10	12.6-17.2
Trombocytes (*x* $10^3/mm^3$)	228	150-350
APTT (*sec*)	25.30	24-36.5
PT (*sec*)	13.1	11-14.5
INR	1.08	0.81-1.16

The toxicological urine analysis through the GC/MS method shows the presence of Tianeptine and Lidocaine.

Gastric lavage was applied and activated charcoal was administered through the naso-gastric tube;   it were applied volemic, electrolitical and acido-basic repletion and complex clinical – biological monitoring. After 24 hours from admission in ICU II Toxicology, the ECG monitoring registers a new ventriculary fibrillation episode, followed by cardiac arrest. The resuscitation protocol was applied, the maneuvers were efficient. Intensive care measures being were continued, ventilatory support, vasopressor support – with dopamine in the continuous infusion, antiarrhythmic drug – Amiodarone continuous infusion, Lidocaine 1% in infusion. After 96 hours from admission, the patient is hemodynamically stable and the vasopressor support is suppressed; also the patient is weaned from ventilator support. The therapy continued with hepatotrofic and neurotropic drugs, with gastric protectors and general nursing. The evolution was favorable.

Psychiatric evaluation reveals a persistent suicidal idea, depressive facies and a marked depressive disposition, the patient was reffered to a psychiatric unit.

At cardiological reevaluation, 9 days after admission: the patient is hemodinamically stable, arterial pressure 108/77 mmHg, cardiac frequency 77 bpm. Regular rhythm, no murmurs, presence of bilateral pulmonary vesicular murmur, no stasis rales; ECG: sinusal rhythm, cardiac frequency 70 bpm, no ST segment modifications. Cardiac echography: left ventricle not dilated, VSTD 48 mm, not hypertrophied, SIV=PP= 10 mm, no segment kinetics disorders, with conserved ejection fraction 55%, no valve disorders, no pulmonary hypertension; pericardial liquid of 3 mm in diastole, laterally from right ventricle, no hemodynamic impact; diastolic dysfunction – delayed relaxation type.

The patient leaves the hospital toxicologically healed, conscious, cooperative, hemodynamically and respiratoriy balanced; stable from a cardiological point of view under antiarrhytmic treatment with Amiodarone.

Figures 3, 4: Cardiac rhythm abnormalities (episodes of polymorph ventricular tachycardia in the context of long QT syndrome) in the absence of diselectrolithemia, acidosis or hypoxemia after tianeptine overdose.

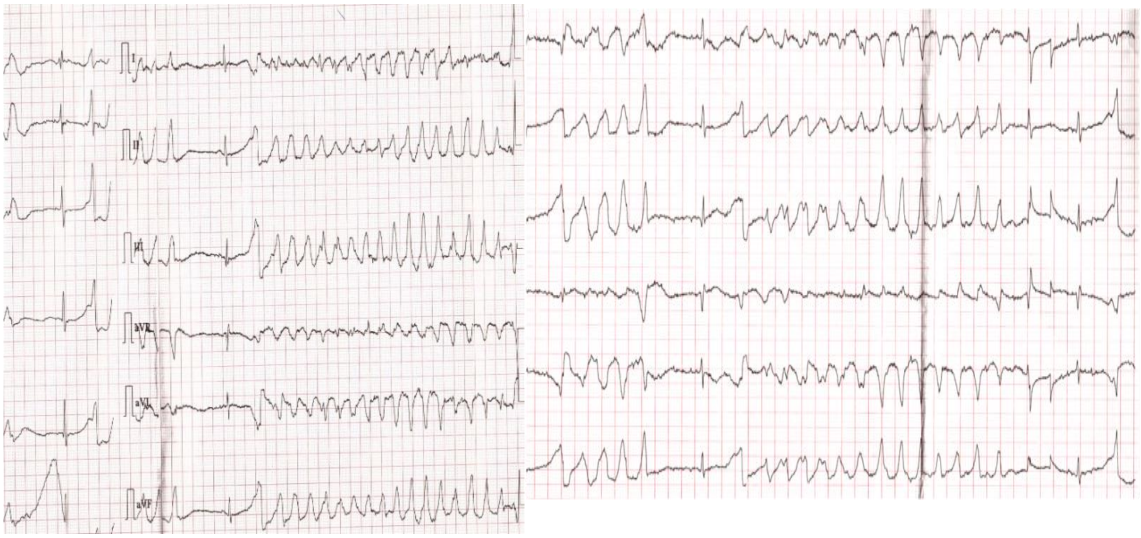

CONCLUSIONS

Although compared to TCAs, which have been proven scientifically to cause cardiac arrhythmia, the data for tianeptine is limited, the above case reiterates the possibility, even low, but with a deadly risk associated, of malignant arrhythmias being caused by it.

Although proof supporting the hepatotoxic potential of MAOI is limited, overdosing on the aforementioned generated phenomena of hepatic cytolysis, which is indicated by increased enzyme levels.

A correct management of acute poisoning with unclear substances, whether accidentaly or voluntary, involves a precise assessment and promptly initiated therapy.

GC/MS analytical method is the confirmatory method for urinary toxicological screening for a diagnosis of certainty in the case of acute drugs poisoning and is important in order to establish an effective therapeutic course.

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ORIGINAL ARTICLES

The assessment of the cardiovascular risk in rheumatoid arthritis patients on anti TNF therapy

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Abstract: *Auto-immune rheumatic diseases are characterised by high levels of inflammation that accelerate the development of atherosclerosis, increasing the cardiovascular risk. Direct evaluation of arteries such as of intima media thickness measurement is a simple test that asesses the cerebral and cardiovascular risk. In the initial stages, an increase of the intimal media thickness can be observed. Later on, atherosclerosis (plaques), stenosis and occlusion of the arteries can be identified. Indirect evaluation of the cardiovascular risk can be performed using serological markers - fibrinogen, C reactive protein, estimated sedimentation rate, total cholesterol, low density lipoproteins and high density lipoproteins, homocysteine levels etc. The presence of certain lifestyle factors that increase the risk, such as smoking, sedentarism and associated pathologies such as obesity, diabetes, hypertension were assesed in patients with rheumatoid arthritis. The aim of the study is to prove the relationship between anti-tumor necrosis factor alpha therapy effect on the cardiovascular risk in rheumatoid arthritis patients.*

Keywords: *cardiovascular risk, intima media thickness, hiperhomocysteinemia, rheumatoid arthritis, anti-tumor necrosis factor alpha therapy*

INTRODUCTION

Rheumatic auto-immune diseases are characterized by chronic abnormal systemic inflammation that leads to endothelial damage and vasculopathy. [1]

Vascular endothelial injury is an early key step in the induction of vascular disfunction. Endothelial cells are involved in the vascular hemodynamics, vascular permeability, cell growth regulation, vessel protection and coagulation. It releases factors such as nitric oxide (NO), endothelin and prostacyclin that keep the balance between vasoconstriction and vasodilation. Nitric oxide, a soluble gas that is synthesized in the endothelial cells, has many biological properties that help maintain vascular homeostasis. It was found that a high disease activity score positively correlates with the levels of nitric oxide, as a marker of oxidative stress. [2,3]

The endothelial disfunction can be assessed indirectly by measuring blood markers (Assymmetric Dimethylarginin-ADMA, oxidized and anti-oxidized LDL, HDL cholesterol, anti-phospholipid antibodies, Crioglobulins, Pentraxin-3, Endocan) or directly showing the arteries themselves using ultrasonography, computed tomography, magnetic resonance imaging and positrone emission tomography scans. [4]

Factors such as age, smoking, sedentary lifestyle, elevated inflammatory markers (fibrinogen, estimated sedimentation rate – ESR, C-reactive protein – CRP), lipid profile, homocysteine – Hcy levels, metabolic syndrome, hypertension are cumulative factors that increase the cardiovascular risk.

The elevated acute phase reactants (such as ferritin, C-reactive protein are also markers of high cardiovascular risk

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in the general population. [5]

Smoking is an independent risk factor for atherosclerosis, especially in patients that present rheumatoid factor (RF) and/or anticitrullinated peptide antibody (CCP) positivity. Hyperuricemia was associated with a 3.1-fold risk of first cardiovascular events. Gout or hyperuricemia may be stronger predictors of first cardiovascular events than other traditional risk factors. [6,7]

Compared to the general population, patients with rheumatoid arthritis have an altered lipid profile, characterized by a suppression of total low-density lipoproteins (LDL) and a greater suppression of high-density lipoproteins (HDL) levels. Coronary artery disease and lipid levels have a paradoxical relationship, decreased lipid levels being associated with severe inflammation. [8]

Lipids increase the production of pro-inflammatory cytokines, especially TNF-α, interleukin 1 (IL-1), interleukin 6 (IL-6). These pro-inflammatory cytokines may have a role in the disruption of lipid metabolism, especially in the cholesterol transport which is associated with the development of atherosclerosis. Dyslipidemia and insulin resistance are associated with systemic inflammation. [9]

Hiperhomocysteinemia (HHcy) is considered a risk factor for cardiovascular and neurological pathologies. This biomarker that can be monitored by blood testing, favors atherogenesis and increseas the risk for heart attack, stroke, Alzheimer's and Parkinson's disease, diabetes and osteoporosis. It is also associated with autoimmune diseases such as ankylosing spondylitis, rheumatoid arthritis (RA) and systemic lupus erythematosus.

Hiperhomocysteinemia is implicated in the alteration of the cerebral flow, increasing the risk of thrombosis and ischemic stroke. Moreover it is implicated in the disruption of the blood-brain barrier, increasing the risk of hemorrhagic transformations of ischemic strokes after thrombolysis. Hyperhomocysteinemia was considered an independent risk factor for negative aoutcome in patients with strokes. [10,11]

In the general population, hiperhomocysteinemia is also associated with peripheral vascular disease and deep vein thrombosis.

Autoimmune activation can add to homocysteine increase but HHCY may also act as a pro-inflammatory molecule and a trigger of autoimmune reactions because of its capacity of binding and modifying specific proteins that result in the formations of neoantigens. [12]

The arterial wall is made of 3 layers, tunica intima (the inner layer), tunica media and tunica externa (the outer layer). In the initial phase of atherosclerosis, the thickness of intima-media increases and it is followed by plaque formation. Ultrasound imaging is the primary diagnostic tool to assess carotid artery disease (it can measure intima-media thickness, detect plaques and obstructions). Carotid arteries are representative of other arteries, such as coronary arteries, allowing us to assess the cardiovascular and cerebrovascular risk.

In rheumatoid arthritis patients, IMT was reported to be more elevated compared to same age and sex healthy individuals. In patients with inflammation (assessed by C-reactive protein) IMT can be a strong predictor for future vascular events. [13, 14]

It was determined that patients with seropositive rheumatoid arthritis have twice the risk of developing heart failure. Moreover, it was found that these patients have with less typical signs and symptoms and have preserved ejection fraction (>50%). Because of this, it was observed that these patients tend to be managed poorly and have more severe prognoses. [15, 16]

Anti-nuclear antibodies (ANA) and rheumatoid factor (RF) are significant predictors of cardiovascular events and were associated with increased mortality even in patients without autoimmune rheumatic diseases. Cyclic citrullinated peptide antibodies (CCP) association with cardiovascular disease was not statistically significant. [17]

Rheumatoid arthritis therapies target inflammation. It was found that the use of TNF inhibitors was correlated with a reduced risk of cardiovascular events because of its effects on decreasing the systemic inflammation. [18]

The aim of the study is to prove the relationship between TNF-α therapy effect on the cardiovascular risk in rheumatoid arthritis patients.

METHODS

The study was conducted in "Carol Davila" Central Emergency Military University Hospital, Bucharest, Department of Internal Medicine 2.

We performed a retrospective observational study on 115 patients (72 women and 43 men) with ages between 28 and 68 years old, with rheumatoid arthritis (who met the EULAR/ACR 2010 criteria). This lot was divided into 4 groups. The first group consisted in 42 people treated with Etanercept (50 mg subcutaneously once a week), the second group consisted in 25 people treated with Adalimumab (40 mg subcutaneously every two weeks), the third group consisted in 35 people treated with Infliximab (3 mg/kg body intravenously 0,2,6 weeks and every 8 weeks) and the fourth group consisted in 13 people that weren't treated with biological therapies. Patients with anti-TNF therapies had associated either 10 mg of Methotrexate per week followed by 5 mg of folic acid or 20 mg of Leflunomide per day.

Indirect methods of assessing cardiovascular risk (by measuring blood markers) and direct methods – measuring carotid intima-media thickness through ultrasound imaging (Doppler flow) were used. ITM was assessed at the common carotid arteries, at 10mm before the carotid sinus. The measurements were performed by the same technician in order to lower subjective errors and maintain the accuracy of the measurements. In normal subjects there is no plaque and the average thickness of intima-media is below 0.9 mm. Thickened intima was considered at an ITM above 0.9 mm and atherosclerotic plaque above 1.3 mm. All patients received a dose of 20mg/day of atorvastatin.

Laboratory reporting limits for ERS were: 0-10mm/h, for CRP 0-5mg/dl, for total cholesterol (CT) <200mg/dl, for Hcy 5-15µmol/l and for LDL-CT <100mg/dl.

Rheumatoid arthritis activity was assessed using DAS 28 score (Disease activity score that examines 28 joints). A controlled disease scores <2.6, between 2.6-5.1 -the disease is moderately controlled and a score >5.1 – the disease is uncontrolled. All of these markers were investigated just before the initiation of anti-TNF therapy and were compared with the markers one year later.

The study was approved by the local ethics committee and all subjects involved gave written and informed consent.

Statistical analysis was performed with SPSS software. Statistical significance was considered at a Pearson correlation coefficient value of 0.01.

RESULTS

From 115 patients with rheumatoid arthritis 62.61% were women and 37.39 % men, with a mean age of 50 ± 10.4 years. From the total of 115 patients, 20% were smokers (32.5% of the males and 12.5% of the females), 39.3% were overweight, and 7.83% had diabetes. 71.30% of patients had a positive anti-Cyclic Citrullinated Peptide Antibody and 96.52% had a positive Rheumatoid Factor.

From this lot of people, 61.74% were treated with Methotrexate and Folic acid and 38.26% with Leflunomide. From the total of patients, 88.70% were treated with anti-TNF agents as followed: 41.18% Etanercept, 34.31% Infliximab, 24.51% Adalimumab. 12.75% from the total patients were not treated with bDMARDs (biologic disease modifying antirheumatic drugs).

Table 1: Studied parameters

Parameter	Value
Age (mean)	50 ± 10.4
Age ≥ 50 years Age < 50 years	53% (61/115) 47% (54/ 115)
Gender (females)	62,61% (72/115)
Smokers	20% (23/115)
Overweight	39.13% (45/115)
Diabetes mellitus	7.83% (9/115)
RF positive	96.52% (111/115)
Anti-CCP positive	71.30% (82/115)
HCY1> 15 HCY2 >15	66.09% (76/115) 55.65 (64/115)
CRP (range)	35.81 ± 14.09
ESR (range)	34.37 - 12.29
DAS28 1 (range) DAS28 2 (range)	8.1 - 4.4 4.3 – 1.1
CT (N=109) CT1 >200 CT2 >200	 53.21% (58/109) 0% (0/109)
CT-LDL (N=105) CT-LDL 1 CT-LDL 2	 84.76% (82/105) 29.52% (31/105)
IMT (N=112)	14.43 ± 31.02
IMT1 >0,9 IMT2 >0,9	83.93% (94/112) 67.86% (76/112)

N= Number of patients; RF= Rheumatoid Factor; Anti-CCP= Anti Cyclic Citrullinated Peptide Antibodies; HCY= Homocysteine; CRP= C reactive protein; ESR= Estimated sedimentation rate; DAS28= Disease Activity Score 28; CT= Cholesterol; CT-LDL= LDL-Cholesterol; IMT= Intima media Thickness

Figure 1: Correlation between HCY1 and IMT2 in the Adalimumab group (HCY1 – Homocysteine level at baseline; IMT2 – Intima media thickness after one year of treatment)

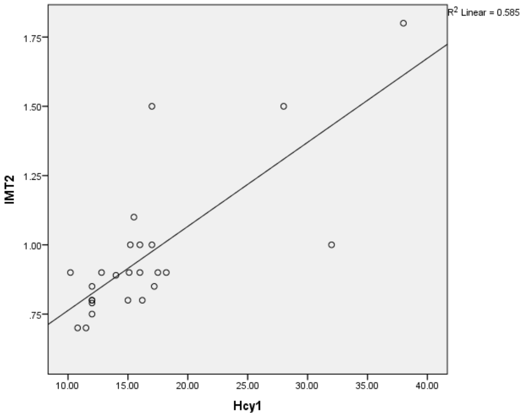

The inflammatory markers were positively correlated with the disease activity.

HCY was evaluated in all of the patients. HCY1 was evaluated before the beginning of the therapy and HCY2 one year later. HCY1 was elevated in 66.09% of patients. After 12 months, none of the patients had increased its previous HCY value.

Moreover, 18.26% of the total patients had a decrease in their HCY2 from HCY1.

Figure 2: Correlation between HCY1 and IMT2 in the Etanercept group (HCY1 – Homocysteine level at baseline; IMT2 – Intima media thickness after one year of treatment)

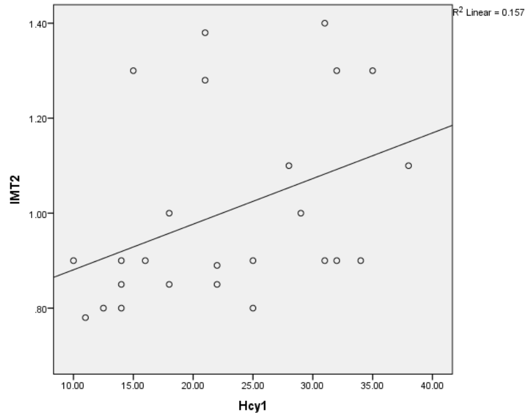

Figure 3: Correlation between HCY1 and IMT2 in the Infliximab group (HCY1 – Homocysteine level at baseline; IMT2 – Intima media thickness after one year of treatment)

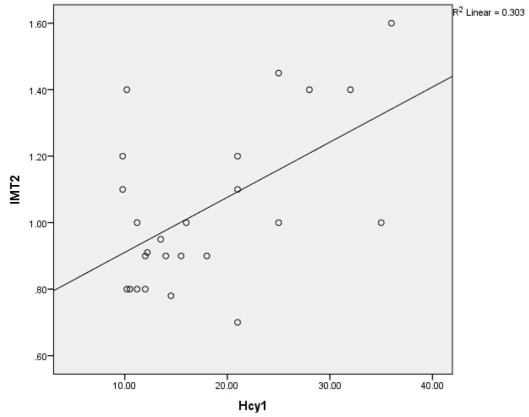

From 112 patients (3 measurements were made by another examiner and were excluded from the study in order to maintain the accuracy of the results), 94 patients (83.93%) had an increased IMT1 value. 76 patients (67.86%) had an increased IMT2. CRP2 values were elevated in only 2.6% of patients. CT1 levels were elevated in 53.21% of the patients. In 6 patients CT levels weren't measured at the beginning of the therapy and thus they were excluded. CT2 levels were normal in all of the patients after one year. To be noted, all of the patients received a dose of 20 mg/day of atorvastatin for one year.

LDL-CT1 levels were increased in 84.76% of patients. LDL-CT2 levels were increased in only 29.52% of patients. 10 patients were excluded from this analysis because LDL-CT was not measured before beginning of the treatment.

In the Adalimumab group, IMT2 decreased in 84% of the patients from the previous measurements (IMT1). HCY levels normalized in 46.67% of patients after a year in the Adalimumab group.

In the Etanercept group, HCY2 levels decreased in 76.47% of patients and 23.53% remained stationary. None of the patients had an increase in their HCY levels after one year. IMT2 decreased in 82.35% patients after one year of treatment with Etanercept.

In the Infliximab group, HCY2 levels decreased in 57.89% of patients. 42.11% the HCY levels remained stationary. None of the patients had an increase in HCY2 levels. IMT2 values reduced in 50% of the subjects from IMT1

In the non-biological treatment group, HCY2 levels decreased in 38.46% of patients. ITM2 decreased in 84.62% of patients.

In the non-biological treatment group, it was found that HCY2 levels decreased in a smaller number of people (38.4% vs 46.6% in the Adalimumab group, 57.8% in the Infliximab group and 76.4% in the Etanercept group).

DISCUSSION

Traditional risk factors for cardiovascular disease such as smoking, excess weight, hypercholesterolemia, hipertension and diabetes mellitus are highly prevalent among rheumatoid arthritis patients.

A large proportion of the studied group are smokers. Because of its effect on the endothelial function (inflammation, proatherogenic cytokines release, reactive oxygen species (ROS) production, fibrinolysis, platelet production and activation), smoking increases the risk of atherosclerotic cardiovascular disease. Studies have shown that the mortality in female smokers is higher than in males. The risk of coronary heart disease is 25% higher in women than in men for the same tabacco use. Genes involved in thrombin signaling are responsible for women susceptability for tabacco-induced cardiovascular disease. [19, 20]

As 20% of the patients are smokers, it is important to inform patients of the risks of RA progression, accelerated atherosclerosis, the increased risk of cardiovascular disease and even inadequate response to RA treatment.

It is a well-known fact that obesity is an independent risk factor for cardiovascular disease, and particularly in women. Moreover, obesity has been associated with an increased disease activity and a decreased response to anti-TNF therapy. [21]

39.13% of the patients were overweight and obese, with

Body Mass Indexes (BMI) between 26-41 kg/m^2. In our study group, smoking and excess weight were associated in 12.17% of patients. Among smokers, the risk of obesity grows with the amount smoked. It was found that obesity is more prevalent in ex-smokers. [22,23]

Therefore, it is important to promote smoking cessation, offer pharmacologic assistance in quitting when appropriate patients, as well as motivating patients to be more active in order to counteract potential weight gain.

We observed that homocysteine levels were elevated in 66% of patients with rheumatoid arthritis. After one year of treatement, HCY leveles decreased in all groups. No strong correlation was found between the anti-TNF agent and the decrease of HCY. It was observed that patients who received 10 mg of Methotrexate subcutanousely and 5 mg of folic acid had a more significant decrease in HCY. It was found that folic acid supplements reduce homocysteine concentrations and is effective in the primary prevention of cerebrovascular and cardiovascular events. [24,25]

In the non-biological treatment group, it was found that HCY2 levels decreased in a smaller number of people. Therefore, we can speculate the additional role of anti-TNF therapy in lowering the HCY levels besides folic acid supplimentation.

IMT2 progression was significantly reduced in all patients. HCY2 was strongly correlated with IMT2 ($P<0.001$). These findings were also observed in other studies that measured IMT. Unlike Yang et al, no correlations between HCY and anti-CCP antibodies, RF or DAS-28 score were found. [26-28]

From the three agents of anti-TNF, Adalimumab and Etanercept were the most effective in decreasing IMT after one year of treatment. The reduced level of HCY2 was significantly correlated ($P<0.001$) with a decrease in IMT2 in patients with hiperhomocisteinemia and an increased IMT at baseline. A significant correlation between an elevated HCY1 and a decreased IMT2 was found in all groups treated with anti-TNF agents (Figures 1, 2, and 3).

CRP2 and ESR2 were greatly decreased in all patients, which correlate with DAS-28 values. Strong correlation between inflammatory markers and carotid IMT was found.

Cholesterol levels decreased in all patients. Atorvastatin treatment greatly contributed to the decrease in total cholesterol and LDL-cholesterol.

CONCLUSIONS

Homocysteine levels should be measured in all rheumatoid arthritis patients. Folic acid supplementation should be recommended to all patients with hiperhomocisteinemia.

The assessment of cardiovascular risk should be made in all rheumatoid arthritis patients regardless of age. The physician should always promote the benefits of cessation of smoking and weight loss in all patients with RA. Hiperhomocisteinemia, increased inflammatory markers, hypertension, diabetes, excess weight, smoking are risk factors highly associated with increased cardiovascular and cerebrovascular risk. IMT assessment is a fast tool that can be used in addition to laboratory blood testing.

IMT assessment is a fast, noninvasive tool that can be used in addition to laboratory blood testing to assess cardiovascular and cerebrovascular risk.

Anti-TNF agents prooved to be not only successful in the control of rheumatoid arthritis but also in lowering the risk of cerebro- and cardiovascular serious events.

Disclosure statement:

D.A, D.O.B, M.L.G have previously been speakers for Sandoz, Pfizer, EwoPharma and Abbvie. M.M.N has been a speaker for Pfizer and Sandoz. O.G.P, C.A.A and F.I.R have no conflict of interest to disclose.

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ORIGINAL ARTICLES

The emergence of nanotechnology and a revolution in diagnostic methods of biological threat agents

Mohammad S. Hashemzadeh[1]

Abstract: *Nanotechnology is applied in wide-ranging fields including energy, information technology, consumables, medicine, etc. Nanomedicine includes the medical applications of nanomaterials in the fields of diagnosis and treatment. This paper focuses on the application of nanotechnology in medical diagnostics for which the main applications of nanomedicine include the detection and discovery of specific biomarkers and rapid identification of biological agents. The introduction of nanotechnology into the medical field before a serious increase in disease symptoms makes early diagnosis possible, thereby preventing more damage to the patient. With the manufacture of nanomaterials and novel entities, reduced size of sensing instruments, as well as biochips and bionanosensors, nanotechnology has revolutionized diagnostic methods. Gold nanoparticles (NPs), quantum dots (QDs), nanotubes, polymeric NPs, and liposomes are among NPs used in medical diagnostics. The importance of these diagnostic methods is redoubled whenever there is a need for the rapid and accurate diagnosis of biological threat agents.*

Keywords: *nanotechnology, medical diagnostics, biological agents, nanoparticles*

INTRODUCTION

Nanomedicine includes the medical applications of nanomaterials in the fields of diagnosis and treatment. Prior to treatment, the science of medicine has always been seeking a method for the diagnosis of diseases. This resulted in the manufacture of nanomaterials and novel entities, including nanoparticles (NPs) and such materials as nanofibers made from the assembly of their original components [1-2]. With the fabrication of these new NPs and imaging agents, as well as by the reduced size of sensing instruments (nano-biochips and lab-on-a-chip), nanotechnology has revolutionized diagnostic methods. In the nano field, medical diagnostics fall into three general groups. I) In vitro diagnostic methods that include detection methods based on the design of nano-biochips and nano-biosensors (based on using DNA probes, PNA, Aptamer, etc.), diagnosis based on lab-on-a-chip, and diagnosis based on immuno-affinity assays. II) In vivo diagnostic methods that include techniques based on molecular imaging using NPs, detection-based methods using NPs, and diagnosis using implantable devices. In the latter case, part of the stages is performed ex vivo in which the target tissue is removed from the body and then returned therein after doing some stages. III) On vivo diagnostic methods, which include using wearable nano-biosensors such as the glucose clock. The molecular basis of nano-probes used in medical diagnostics can be based on the following items [3-5]: 1) gold NPs, 2) quantum dots (QDs), 3) magnetic NPs, 4) nanotubes, 5) polymeric NPs, and 6) liposomes.

Diagnostic application of NPs in medical imaging

Iron NPs (Fe NPs) are among the most commonly used tools that can be used to improve the diagnosis of various diseases or the identification of pathogenic agents such as Staphylococcus bacteria. This nanoparticle is coated with a biomolecule (for specific binding to cancer cells or pathogenic bacteria). The Fe used in these NPs makes them fully apparent and visible in imaging procedures (e.g. magnetic resonance imaging, MRI) and thereby increases the quality of this imaging [6].

Application of NPs in diagnostic methods based on

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proteomic studies

To diagnose various diseases, another application of new molecular diagnostic technologies is based on using specific biomarkers of these diseases. In this regard, an important existing issue is the collection and harvest of these biomarkers by functionalized NPs of the target sample, followed by separation and proteomic studies (that are highly sensitive) to diagnose the type of the disease [7].

Diagnostic application of QDs in medical imaging

These NPs are semiconductor inorganic fluorophores that are more beneficial and advantageous than conventional fluorescent markers. These include high sensitivity, wide excitatory spectra, and a stable fluorescence with a simple excitation without the need for laser radiation. The most important potential application of QDs is in the diagnosis of cancer. With a diameter of 2-8 nm, they typically comprise atoms of elements of II and VI groups (e.g. CdSe and CdTe) or III and V groups (e.g. InP and InAs) from the periodic table. The optical properties of these NPs are mainly dependent on their structures. Importantly, the wavelength and/or the color emitted by a QD depends on its diameter [8-9].

Targeting QDs for intracellular imaging

NPs containing QDs specialized with a specific ligand coating bind to their specific receptors at the surface of target cells and undergo phagocytosis by these cells after the attachment procedure. These intracellular QDs are released after the intracellular digestion and deposit on intracellular structure, which will then be detectable in a specific cell. It is noteworthy that QDs are highly permeable into eukaryotic cells owing to their size [8-9].

The use of Au NPs in the detection of DNA

In a recent innovative method, the presence of a specific genetic sequence was detected in a contaminated sample using 13 nm Au particles decorated with DNA genetic material [9].

The use of magnetic NPs in the detection of pathogens

There is ongoing research on the use of magnetic NPs at the nanoscale for the detection of pathogens. As with many skills used today, the methods used in such studies require appropriate antibodies that bind to these agents (described in detail in the Results and Discussion) [6].

Nano-biosensors

These are the other types of diagnostic tools that enjoy nanotechnology. Briefly, there are different types of this diagnostic tool, including electronic, electrochemical, and optical nanosensors, among others. The most common types are the electrochemical and optical (based on Surface Plasmon Resonance or SPR) ones. An electrochemical nanosensor was used to detect Salmonella typhi in 2009 [13].

THE FUTURE OF NANOBIOTECHNOLOGY

A dream of researchers is to achieve a humanoid nanorobot that can circulate in the bloodstream, detect possible pathogenic agents alongside other devolved tasks, and act according to the requested program. These components can be made applicable to be used for the entry of nano drugs into the body to recognize or cure damaged areas. In 1966, a fiction film, called the Fantastic Voyage, invited cinema fans to watch an adventuresome show of the application of nanotechnology in medicine. A group of adventurer physicians and their modern submarine were minimized by a mysterious method so that they could circulate in the patient's bloodstream and remove the embolus in the brain that threatened the patient. After half a century since then, considerable attempts have been made to fabricate complicated instruments in smaller scales. This has made some people believe that such interventions are plausible in medicine, and nanorobots will be able to journey in the vessels of people.

Nanorobots are also used in nano surgeries. They can enter the body through vessels, seek the injured site, and diagnose the disease with the help of a computer and a supervisor surgeon. After the precise detection of the problem site, the surgery is done by the direction of a surgeon using nanorobot-equipped arms. These small nanometric robots can also perform precise surgeries inside the cell (e.g. the exchange of real chromosomes with new ones), surgeries that have not so far been realized by any human surgeon (Figure 1).

Researchers are seeking to produce and use other instruments, including implantable nanodevices, which can be implanted, for example, in the plasma membrane of erythrocytes. Thus, they will be always present in the systemic blood circulation and play a role in such applications as monitoring or drug transfer [14-15] (Figure 2).

Some applications

As mentioned above, Fe NPs coated with a biomolecule (for specific binding to pathogenic bacteria) are among the most widely used tools that can be used to detect pathogenic agents such as Staphylococcus bacteria (Figure 3).

Figure 1: Schematic of images related to small nanometric robots (nanorobots)

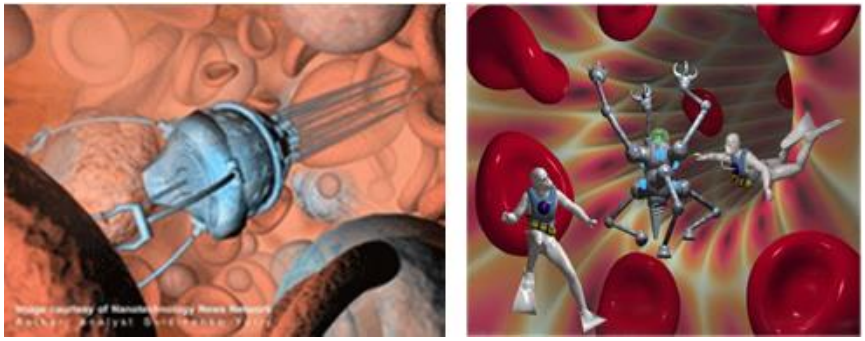

Figure 2: Schematic of an implantable nanodevice implanted in the plasma membrane of erythrocytes

Figure 3: Schematic of an iron oxide NP coated with a cell-specific biomolecule

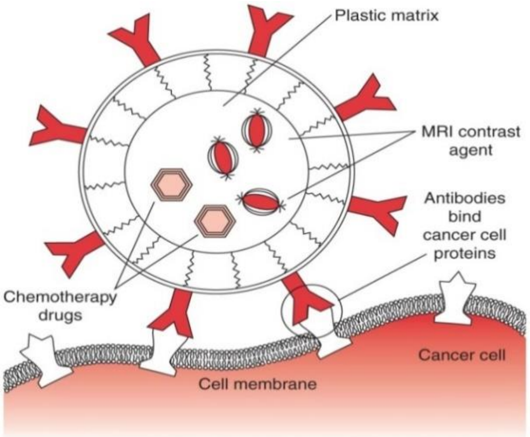

Figure 4 depicts a schematic of a QD consisting of four main parts": A) Core: contains a semiconductor that can emit light after excitation, and this part is the mainstay of a QD. B) Core-shell: contains an insulator that preserves light emission properties after the nanoparticle functionalization. Functionalization means the addition of other parts to the nanoparticle central core that will provide the functional properties, such as specific binding to the target molecule, nanoparticle detoxification, biocompatibility, etc. C) Natural coating: the core-shell is coated with a natural biocompatible material (e.g. PEG) or a lipid layer. D) Synthetic coating: the final stage of nanoparticle functionalization depends on the intended goals. For example, this layer can be used to embed a drug for therapeutic purposes and drug delivery or assemble a specific antibody to convert a QD to a target-specific nanoparticle. Notably, QDs are toxic per se and thus must undergo some changes for their biocompatibility [14-15].

Figure 4: Schematic of different parts of a QD

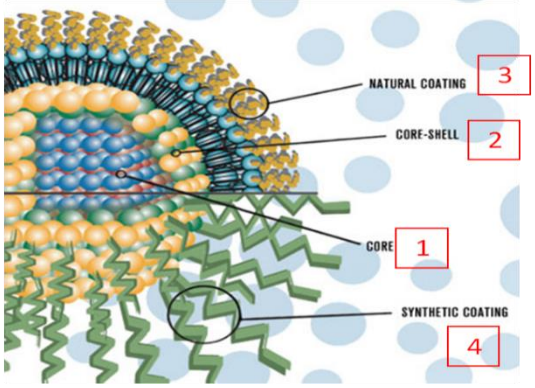

The use of Au NPs in the detection of DNA

In their innovative method, researchers used two groups of Au NPs. A DNA-containing group was bound to half of the target sequence, and the DNA connected to the other group was attached to its other half. A target DNA with a complete sequence is easily connected to both types of particles and thereby connects the two particles.

Since several DNAs are connected to each particle, DNA-containing particles can attach several particles to each other. The aggregation of these gold particles significantly changes their characteristic properties, turning the sample color from red into blue. Since the result of this test is visible without any device, it can be used for the DNA test at home (Figure 5) [15].

Figure 5: DNA test at home

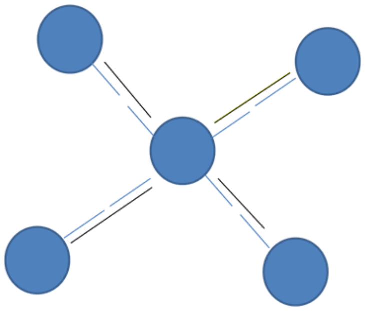

The use of magnetic NPs in the detection of pathogens

Magnetic NPs bind the same as a label to antibody molecules. If a special pathogenic agent is of interest in a sample, specific antibodies of this agent, which are connected to magnetic particles, attach to them.

The sample is washed to separate the unconnected antibodies. If the abovementioned agent is present in the sample, magnetic particles connected to the agent by the antibody produce magnetic fields that are detected by a sensitive device.

The sensitivity of this laboratory skill is better than the existing standard methods, and predicted modifications will soon amplify the sensitivity up to several hundred times. For instance, magnetic NPs attached to the specific IgG, which is specifically connected to protein A of the Staphylococcus bacterial cell wall, can be used to detect this agent. Bacteria attached to functional NPs will be easily separated from the sample using a magnet (Figure 6) [16].

Figure 6: Directed aggregation of harmful bacteria by conjugation with functionalized magnetic NPs

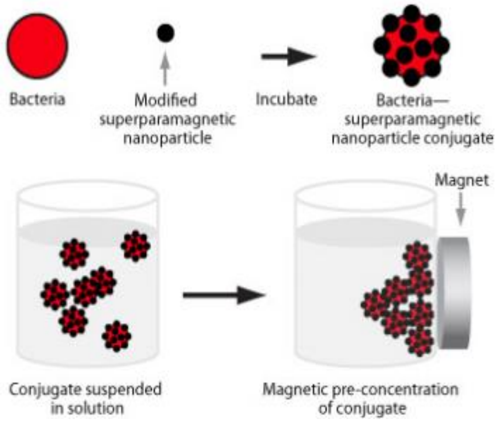
Bacteria
Modified superparamagnetic nanoparticle
Incubate
Bacteria—superparamagnetic nanoparticle conjugate
Magnet
Conjugate suspended in solution
Magnetic pre-concentration of conjugate

Another method is to use the MRI test to detect NPs attached to this agent (Figure 7).

Figure 7: a) Aggregation of functionalized NPs on Staphylococcus bacteria; b) their slight aggregation in the non-functionalized state on these cells

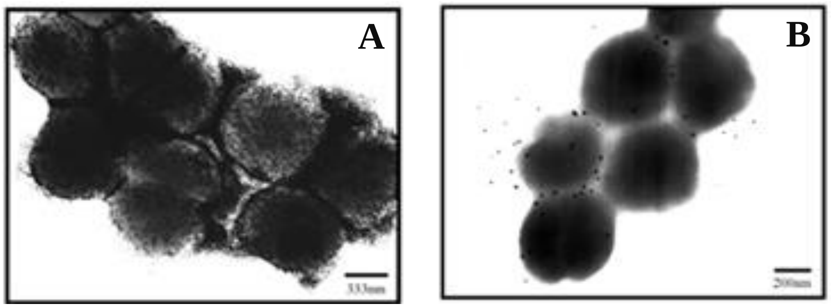

Nonobiosensors

In a previous study (17), synthetic DNA fragments (DNA-Aptamer) fixed on carbon nanotubes (CNTs) were used as probes to bind to the pathogen and produce an electrical signal. The features of this method include prompt, easy, and reliable detection of a low amount of the bacterium (the size of a cell). Indeed, the presence of a low amount of bacteria and the attachment of a specific aptamer to them lead to changes in the interaction between aptamer and CNTs that occurs in a few seconds and thereby increase the related electrode voltage. This nanobiosensor is able to detect a single Salmonella cell in a 5-ml sample (Figure 8).

Figure 8: Detection of Salmonella typhi using an electrochemical nanosensor

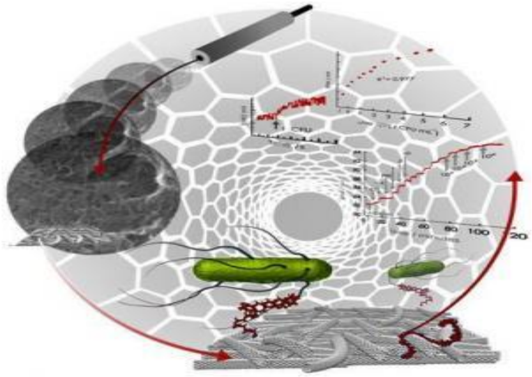

CONCLUSION

Nanotechnology allows the detection at the molecular level or at a single cell level. Au and FE NPs and QDs are among the most widely used NPs in this field, and some nanotechnology devices used in the field of diagnosis (e.g. nano-biochips and nano-biosensors) have many potentials in diagnostic applications. Since there are close associations between nanotechnology and other technologies, this science seems to play a significant role in medical diagnostics, especially the detection of biological threat agents [18-20].

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ORIGINAL ARTICLES

The intestinal microbiome and the role of probiotics/prebiotics in the therapeutic approach of atopic dermatitis: A review

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Abstract: *Atopic dermatitis (AD) is the most common chronic inflammatory skin condition, characterized by multiple recurrent eczematous lesions and intense itchiness. It is a multifactorial skin disorder involving an association between genetic and environmental factors that lead to a defect of the epithelial barrier in conjunction with immunological dysregulation. Over the last decade, there has been an increasing understanding of the role of the human microbiota in preserving skin integrity and that a shift in the homeostasis of these microorganisms may lead to dysbiosis and disease. Diversity in the intestinal microbiome and its role in the etiopathogenesis of AD has been described and has become of great interest. In this report, we have reviewed the importance of the gut microbiome and the possible mechanism in the pathogenesis of AD as well as the therapeutic impact of probiotics and prebiotics.*

Keywords: *atopic dermatitis, probiotics, intestinal microbiome*

INTRODUCTION

Atopic dermatitis (AD) is the most common chronic inflammatory skin condition, characterized by multiple recurrent eczematous lesions and intense itchiness [1]. It is a multifactorial skin disorder involving an association between genetic and environmental factors that lead to a defect of the epithelial barrier in conjunction with immunological dysregulation [2]. Over the last decade, there has been an increasing understanding of the role of the human microbiota in preserving skin integrity and that a shift in the homeostasis of these microorganisms may lead to dysbiosis and disease [3].

AD is a chronic inflammatory skin condition characterized by a recurrent eczematous rash and intense pruritus, having a relapsing disease course [4]. Symptomatology usually commences in infancy, affecting roughly 20% of children, however, it is also vastly prevalent in the adult population [5]. In some children with AD, the clinical symptoms will disappear with growing up, but about 50% of children may acquire allergic asthma, and about 66% present with an increased risk of allergic rhinitis later on in life, a process known as the "atopic march" [6]. The skin lesions in infants are diffusely located, predominantly on the face and the extensor surfaces of limbs, whilst in adults, there is further morphological diversity including localized eczematous plaques, lichenification and erythroderma [3]. This highly pruritic skin condition arises in the genetically predisposed hosts as a result of the disruption of the epithelial barrier along with immunological dysregulation [7]. A genetic linkage has been reported to chromosomes 1q21, 17q25, and 20p [8]. It has been demonstrated that individuals with AD may carry genetic mutations in genes that encode the epidermal protein filaggrin [9]. A secondary major genetic risk factor are the genes implicated in the T helper lymphocyte type 2 (Th2) signaling pathways. Over 30 risk loci for AD have been identified with the use of genome-wide association studies, suggesting that they play a role in the epidermal barrier function and immune responses [10]. Although the pathophysiology of AD is not entirely understood, it is evident that it is a multifactorial process, resulting from a complex association between barrier

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dysfunction, alterations of the immune response, and the environment [11]. Amplification of the pro-inflammatory Th-2 response is represented by the production of interleukins (IL) 4 and IL-13 which are predominant in the acute and chronic phases of AD. Also, IL-25 and IL-33 along with thymic stromal lymphopoietin (TLSP), known to elicit a Th2 response, have been as well identified in patients with AD, having the ability to modulate the stability of the skin barrier and the function of keratinocytes [12]. Dupilumab is a human monoclonal antibody with the role of inhibiting IL-4 and IL-13 by blocking the IL-4 receptor alpha subunit, hence inhibiting the production of IgE by B cells and the activation of eosinophils. Efficacy has been demonstrated by markedly reducing signs and symptoms in patients with AD [13,14].

The intestinal microbiota is a highly complex and dynamic ecosystem, formed in the neonatal gut, continuing to develop and increase in diversity until the child reaches around 3 years old. At this point, the microbial flora becomes adult-like [15]. Gut microbiome configuration can be affected by environmental factors such as diet, stress, pollutants, and living habits, especially in the early years of life [16]. An imbalance in microbial diversity at the level of the intestinal microbiome can lead to shifts in the metabolism of gut microbiota and immune responses. Such changes can have influential effects on the physiological and pathological mechanisms of the human body [17]. In addition, there has been an enhanced understanding and increasing evidence of the correlation between the intestinal microbiome and various skin conditions described as the “skin-gut axis” [18]. Since there is a tight correlation between AD and dysbiosis, the effects on the diversity and function of the intestinal microbiome have become of great interest and have been extensively studied, however, the outcome is still controversial, with some studies showing positive outcomes and others have found no effects in the treatment of AD [19].

GUT MICROBIOME VARIATION AND AD

The human gastrointestinal tract is known to reside over 1000 different species of bacteria, with the majority belonging to 1 of the 4 phyla, Bacteroidetes, Actinobacteria, Firmicutes, and Proteobacteria [20]. This dynamic and complex system can be influenced by genetics and a multitude of environmental factors such as diet, stress, and pollutants [21]. The intestinal microbiota is thought to have an important role in the onset of AD by its involvement in the maturation of the immune system, particularly in the early years of life [22]. The homeostasis of the immune system can be affected by alteration in the gut microbiome by means of metabolites which can have a pro-inflammatory effect when encountering a distinct microbiota in the gut [23]. The resulting gut dysbiosis along with the imbalances of the immune system contribute to the onset and the natural course of AD [24]. Ahead of the birth, there has been found microbial distribution at the level of placenta and meconium, suggesting that microbial colonization occurs in the early stages of life [25]. At the beginning of life, the gastrointestinal tract of the newly born is colonized by numerous microorganisms, a process that is regulated by various factors such as maternal microbiome, delivery modes, milk source as well as the use of antibiotics. Delivery of the newborn via C-section associated with the use of antibiotic treatment prenatally has been associated with an increasingly higher risk of developing AD [26]. Bacterial diversity has been noticed in patients with AD compared with healthy controls. In patients with AD, there has been observed a decreased microbial proportion of beneficial bacteria such as Bifidobacterium and Lactobacillus and an increased proportion of Clostridium difficile, Bacteroides, Staphylococcus aureus, and Escherichia coli [27-29]. A higher abundance of Coprococcuseutactus, a butyrate-producing bacteria has been associated with cases of milder eczema rather than in those with severe AD [30]. A study carried out in South Korea had explored the intestinal microbiome of 90 subjects with AD and 42 healthy subjects. An increase in Faecalibacterium prausnitzii F6 was observed in the subjects with AD which lead to decreased concentrations of intestinal butyrate and propionate [31]. In addition to the pathological mechanisms involved in the development of AD, the presence of Clostridia and Escherichia coli in the gut may have a correlation with AD by means of eosinophilic inflammation [32]. Perhaps the development of AD may not only be promoted by the diversity of the intestinal microbiota on its own and more studies are needed on the mixed effects of environmental factors. There are some recognizable differences between the gut microbiota of subjects with AD versus healthy subjects.

GUT MICROBIAL MECHANISMS INFLUENCING AD

Immunologic pathway

The intestinal microbiome plays an important role in the modulation of the immunologic pathway of AD through probiotics. It has been observed that the oral administration of probiotics interacts with the mucosa of the gastrointestinal system and gut-associated lymphoid tissue (GALT), in which more than 70% of the immune cells are found [33]. Through diverse pathways, probiotics interact with mucosal dendritic cells (DCs), epithelial cells and macrophages. Taking into consideration the probiotic strain, they have the capability of either inducing the activation of the immune signaling via the production of IL-12, IL-18 and tumor necrosis factor-α (TNF-α) or stimulating anti-inflammatory cytokines such as IL-10 and TGF-β [34]. It has been suggested by various lines of evidence that preserving microbial diversity is of great benefit in sustaining the abundant immune milieu of the skin. For example, distinct microbes can evoke particular types of immune cells at skin level such as S. epidermidis which induces IL-17A+ CD8+ T cells which reside in the epidermis, enhancing the innate immunity and hence, limiting the pathogen invasion [35]. In response to stimulation with S. epidermidis, the human skin tropic T cells have been observed to produce IL-17A and IFN-γ [36].

A birth cohort study had demonstrated that the colonization of the gut with strains of S. aureus carrying specific Staphylococcal superantigens (SAgs) combinations and adhesion genes, exhibits a negative correlation with the development of AD later on during infancy [37]. These kinds of strains may stimulate the development and maturation of the infant immune system, despite the fact that skin S.aureus may exacerbate the already established AD. Moreover, the existence of a particular gut microbiome such as Akkermansia muciniphila was correlated with changes in genes that had an effect on the development of the host immune system [38].

Another study has shown that supplementation with Lactobacillus paracasei reduces the sensitivity of the skin and the transepidermal water loss in healthy controls [39]. These effects have been attributed to the increase in TGF-β, a cytokine that has been demonstrated to have an effect on the integrity of the skin barrier [40]. All these studies sustain the fact that there is a correlation between the gut and skin, modulated via the interaction between the immune system and the gut microbiome.

Metabolite pathway

Short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate, produced by the breakdown of non-digestible carbohydrates derived from dietary intake, have numerous physiological effects, in particular, anti-inflammatory [41], which could play an important role in the treatment of inflammatory conditions such as AD. In a mouse study, it has been observed that linoleic acid and 10-hydroxy-cis-12-octadecenoic acid diminished AD symptoms and modulated the gut microbiome [42].

In a different study, the administration of probiotic Bifidobacterium animalis subspecies lactis has been observed to increase the levels of metabolite kynurenic acid, which decreases the behavior of scratching in AD mice [43].

Neuroendocrine pathway

Increasing evidence support the notion that there is a gut-skin axis mediated by neuroendocrine molecules generated by the intestinal microbiome. This suggests that the presence of compositional and proportional differences in the intestinal microbiome are associated with the generation of numerous valuable neurotransmitters and neuromodulators, which are associated with the degree of AD symptoms [44,45]. Tryptophan, a metabolite produced by the gut microbiome, has been observed to induce an itching sensation in the skin [46], however, species of Lactobacillus and Bifidobacterium can generate γ-aminobutyric acid (GABA), having an antipruritic effect [46,47]. Cortisol, a hormone usually released under stressful conditions, can modify the composition of the intestinal microbiome by altering the permeability and barrier function of the intestinal epithelium [48]. Moreover, cortisol can also alter the levels of circulating neuroendocrine molecules such as trimethylamine, tryptamine, and serotonin, hence modulating the skin barrier and its immune function [46,49-52].

THE THERAPEUTIC ACTION OF PROBIOTICS AND PREBIOTICS IN AD

Probiotics

Probiotics are live microorganisms that when consumed, should confer health benefits. Taking into account the role of the gut microbiome in the development and homeostasis of the immune system, probiotics can be effective not only in the treatment of atopic conditions such as AD, but also in the prevention process by means of microbial, epithelial, and immune effects. Probiotics have the ability to modify the microbial composition of the intestinal microbiome, prevent invasion with pathogens by competitively attaching to the epithelial cells, as well as suppress pathogens by means of bacteriocin secretion [53].

Various cytokines such as IL-12, IL-18, and TNF-α can be generated depending on the probiotic strain and can promote immune-stimulated signals or stimulate anti-inflammatory cytokines such as IL-10 and TNF-β to bring about immunity tolerance signals [54]. Moreover, probiotics exert an inhibitory effect on dendritic cells maturation, hence, inhibiting the differentiation of T-cells into Th2 cells which triggers skin inflammation [55,56]. Thymic stromal lymphopoietin (TSLP) regulates the differentiation of naïve T cells mediated by dendritic cells, an action that could be inhibited by probiotics [56]. Gram-positive bacteria Bifidobacterium and Lactobacillus are amongst the most studied and test probiotics in AD [57,58]. These probiotic families lack the lipopolysaccharides which are known to cause inflammation and also release molecules that aid in the maintaining of a healthy gut and hence skin [59]. In mice, supplementation with probiotic Lactobacillus sakei WIKIM30 has shown amelioration of AD skin lesions by induction of Treg cells and alteration of intestinal microbiota [60]. Moreover, Lactobacillus casei variety rhamnosus has shown to diminish AD symptoms in mice trials by increasing levels of bifidobacterium, Lactobacilli, Bacteroides fragilis, and Enterococcus, as well as decreasing levels of Clostridium coccoides [61]. In multiple studies, the efficiency of probiotics was on AD was displayed as an improvement in the SCORAD score [62].

In a randomized placebo-controlled study, 53 children with moderate-severe AD were orally administered with Lactobacillus fermentum VRI-003 PCC, which showed an improvement in the SCORAD score [63]. In another trial carried out on children with ages between one and twelve years of age, administration of Lactobacillus Plantarum CJLP133 strain was observed to reduce levels of INF-γ, IL-4, and eosinophils hence decreasing SCORAD score [64]. Lactobacillus strain has been demonstrated to stimulate the recovery of the skin barrier, as well as inhibit the skin inflammation associated with substance P [65]. In addition, a probiotic trial including human originating probiotics, Lactobacillus and Enterococcus, has demonstrated that these probiotics can boost the production of SCFA, hence reinforcing the mucosal barrier [53,66].

It has been proven that probiotics are efficient even when given prenatally in preventing AD. In a Korean study, supplements of probiotic strains containing Bifidobacterium bifidum BGN4, Bifidobacterium lactis AD011, and Lactobacillus acidophilus AD031 were administered to pregnant women known with a family history of AD, 4 to 8 weeks before giving birth. The probiotics were also given to the infants for the first 2 months of life, resulting in a significantly lower incidence of AD compared to the control group [57]. During an 8-week treatment with a daily intake of Lactobacillus plantarum YIT 0132-fermented citrus juice in adults, it was observed a decrease in AD symptoms. The benefits were observed for a further 8 weeks off treatment [67].

Prebiotics

Prebiotics are non-digestible dietary ingredients that promote certain changes in the gut microbiota composition which brings numerous health befits to the host by stimulating in a selective manner the growth and activity of definite collections of bacterial strains already well-established in the gut flora [68,69]. Prebiotics are known to enhance the SCFAs production in the gut such as acetate, propionate, and butyrate, having anti-inflammatory effects [70], diminishing the formation of toxic fermentation products [71], expanding the Th1/Th2 ratio, increasing the lymphocytes and/or leucocytes number in GALT (gut-associated lymphoid tissue), as well as a surge in intestinal IgA secretion [72].

The most commonly known and well-established prebiotics in the food industry are oligosaccharides such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and long-chain inulin [73,74], however, a few newly discovered prebiotics are gaining interest such as xylo-oligosaccharides, arabinoxylan oligosaccharides, pectin and its products formed by hydrolysis [75-79].

CONCLUSİON

Although there are still a lot of unknown variables that need to be explored in the gut-skin axis, the gut microbiome is undeniably a crucial element contributing to the host homeostasis, allostasis, and pathogenesis of disease through various complex mechanisms.

Modulating the gut microbiome and restoring the immune balance by supplementing with prebiotics and probiotics has been proven to be beneficial not only in the treatment but also in the prevention of AD. Further research is needed to improve the understanding of these highly complex mechanisms lying at the basis of the gut-skin axis and how can we further manipulate the gut microbiota in order to attenuate the clinical manifestations of AD and other skin inflammatory diseases.

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ORIGINAL ARTICLES

The relationship between a previous infectious disease caused by the influenza, herpes simplex or Epstein-Barr viruses as the biological threatening agents with recurrent episodes of multiple sclerosis

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Abstract: *Although multiple sclerosis (MS) is the most common neurological disease of young adults, afflicting hundreds of thousands of people worldwide, its pathogenesis is still only incompletely understood. There seems to be substantial heterogeneity in disease mechanisms, but in the majority of cases an autoimmune origin or at least a decisive autoimmune component is postulated. Therefore, pathogenic research focuses on different players programmed by the immune system.*

In this study, biomarkers such as Human myxovirus resistance protein A (MxA), Anti-EBNA antibody and Interleukin S10-12-17 have been tested.

Study of hypotheses show that: Interleukin levels in the blood of MS patients in the experimental group (patients with recurrence) is higher than controls (patients without recurrence condition). The level of anti-EBNA antibody in MS patients in other groups (patients with recurrent) and control group (patients without recurrence condition) is high.

The level of the human myxovirus resistance protein A (MxA) in patients with multiple sclerosis in the experimental group (patients with recurrence) is less than the control group (patients without recurrence condition). The level of anti-EBNA antibody in MS patients in other groups (patients with recurrent) and control group (patients without recurrence condition) is high.

Finally, there is a significant relationship between viral infection diseases and recurrent multiple sclerosis.

Keywords: *Influenza, Herpes Simplex Virus (HSV), Epstein-Barr Virus (EBV), multiple sclerosis (MS)*

INTRODUCTION

Multiple sclerosis (MS), the most common cause of chronic neurological disability in young adults, has a variable phenotypic presentation and subsequent disease course that cannot be predicted at disease onset. Current therapeutic interventions may control the early inflammatory component of disease, but have limited efficacy in progressive disease. Patient-specific disease phase is difficult to determine clinically and, as a consequence, some treatments can be applied inappropriately.

A combination of phenotypic subtyping and laboratory investigations of serological and genetic biomarkers has already led to improved diagnosis and targeted therapy for some subtypes of CNS demyelinating disease previously encompassed within the syndromic diagnosis of multiple sclerosis, including neuro myelitis optica (Lennon et al., 2004; Wingerchuk et al., 2006) and Leber's hereditary optic neuropathy (Taylor et al., 2003).

In addition, several potential biomarkers have been suggested as aids for determining disease course or response to treatment in multiple sclerosis, but their clinical usefulness has not yet been clearly established (Tatomir et al., 2017, Reindl, 2007; Lutterotti et al., 2007), and currently there are no readily accessible clinical or biological markers sensitive or specific enough to guide patient management reliably.

Pathological and functional studies have established the role

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of complement in the pathology of multiple sclerosis (Ingram et al., 2009) and as a result, a number of complement proteins have been investigated as potential biomarkers of disease activity. The majority of these studies have been limited to examination of individual components (C3, C4, C9) or activation products (terminal complement complex) in multiple sclerosis serum and CSF, and so far results have been conflicting (Watkins et al., 2016; Jans et al., 1984; Morgan et al., 1984; Compston et al., 1986; Sanders et al., 1986; Mollnes et al., 1987; Sellebjerg et al., 1998; Jongen et al., 2000; Tatomir et al., 2017).

The reasons for the inconsistent results in these studies are unclear but may have been a consequence of assay variation, sample collection, low patient number or lack of comprehensive clinical phenotypic data. This last factor is likely to be important in multiple sclerosis, where clinical status will have considerable effects on complement levels. More recently, proteomic analysis has identified changes in levels of specific complement proteins (factor I, C3, clusterin) in CSF samples from patients with multiple sclerosis compared to controls (Rithidech et al., 2009; Stoop et al., 2009).

Sero-epidemiological studies have demonstrated the association between multiple sclerosis (MS) and prior Epstein-Barr virus (EBV) infection. It has been hypothesized that intermittent peripheral EBV reactivation may drive continuing central inflammation.

Recent investigation has shown significant differences in median serum levels of anti-EBV nuclear antigen-1 (EBNA-1) IgG between disease subgroups and positive correlation with disease activity reflected by number of Gd-enhancing lesions and T2 lesion volume. These important data have led to hopes that anti-EBNA-1 IgG may be useful as an easily accessible and effective biomarker of disease activity (Ingram G, 2009).

The correlation between elevated Epstein-Barr virus nuclear antigen 1 (EBNA-1) immunoglobulin G (IgG) and gadolinium-enhancing lesions suggests an association between Epstein-Barr virus (EBV) infection and multiple sclerosis (MS) disease activity.

The heightened immune response to EBV in MS is specifically related to EBNA-1 IgG, a marker of the latent phase of the virus. The lack of association between acute viral reactivation in the peripheral blood and Gd (gadolinium-DTPA) lesions suggests a limited role of the former in driving disease activity (Farrell, 2009).

Human myxovirus resistance protein A (MxA) also is an interferon-induced dynamin-like GTPase that acts as a cell-autonomous host restriction factor against many viral pathogens including influenza viruses. In patients with multiple sclerosis (MS), neutralizing antibodies (NAbs) appearing during treatment with interferon (IFN) beta reduce or in high concentrations abolish bioactivity and therapeutic efficacy. In vivo MxA induction by IFNbeta is used as a marker of biologic response to IFNbeta. It has been argued that despite absence of MxA induction measured by PCR, some bioactivity might be preserved (Hesse D, 2009).

Interleukins (also called lymphokines) are a sub-group of small soluble proteins called cytokines which function as chemical messengers between cells. The role of interleukins is to mediate and control the immunologic and inflammatory response. There are at least 18 known interleukins most of which have only been discovered in the last few years. Their role within the immune system is only beginning to be understood and they are just starting to be utilised in the treatment of a wide variety of diseases including cancer, AIDS and autoimmune diseases such as multiple sclerosis and rheumatoid arthritis (Gold, 2008).

Studies have shown that over the course of multiple sclerosis disease activity in patients' cerebrospinal fluid, serum levels of IL-12 plus IL-6 subunit 40 is significantly increased (Malmestrom C, 2006). After treatment with beta interferon, interleukin-10 levels in the cerebrospinal fluid increases and its surface is associated with clinical response, (Rudick RA, 1998).

Mononuclear cells that secrete IL-17 also are responsible, during relapses in patients with multiple sclerosis cerebrospinal fluid increases, (Matusevicius D, 1999). It has been hypothesized that IL-6 in the cerebrospinal fluid of the patients with NMO can helplessness, relapses and disease activity related to (Uzawa A, 2010). Also, scientists have discovered that systemic production of IL-12 stimulates the secretion of interferon gamma activates the natural killer cells, was associated with disease progression and increases in serum (van Boxel-1999).

MATERIALS AND METHODS

Some of routine serological tests that may be conducted to detect the most important biomarkers are including: Agglutination Reaction Test, The Heterophil Antibody Response IgM, VCA; Viral Capsid Antigen, EA; Early antigen, EA; Early Antibody, EA-R; Antibody, EBNA; Epstein-Barr Nuclear Antigen, Existence of interleukin 10, 12, 17, human myxovirus resistance protein A (MxA), Anti-EBNA antibody.

Statistically, descriptive statistics and mean (the biomarker in patients with MS) in case and control groups by using independent t test are accomplished.

RESULTS

In this study, the results of descriptive and inferential statistics related to each of the biomarkers provided.

Descriptive Statistics

In this study, patients with multiple sclerosis relapses with respect to the control and test groups of 34 patients in each group sorted. The prevalence of MS patient's gender in both groups was identical (to compare the results of the check the status of patients in both groups exist). In the case group and the control group of 21 patients were females and 13 males, while in control group 22 patients were females and 12 were males (Figure 1).

Figure 1: Gender of the participants in the case and control groups

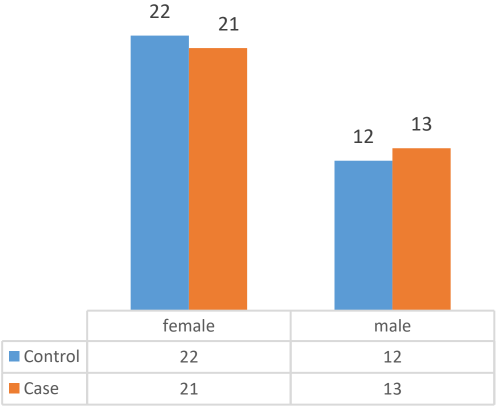

The age range of participants in research in case and control groups show that the majority of patients age were in the range of "20 to 40" (Figure 2).

Figure 2: The age range of the participants in the case and control groups

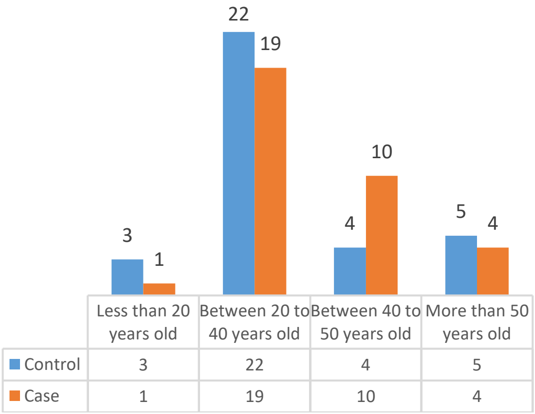

Educational level of the majority of patients in both groups also is located in the "graduate and above" category (Figure 3).

Figure 3: The range of "educational level" of the participants in the case and control groups

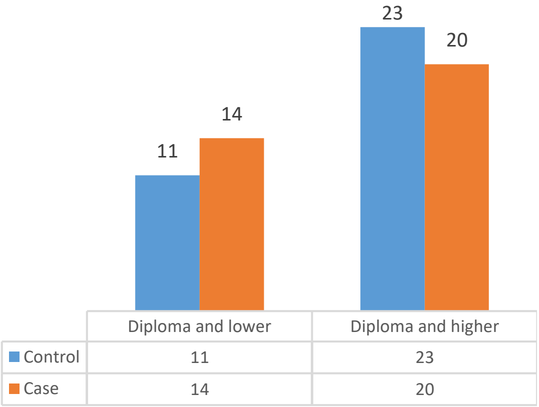

As can be seen in the Figure 4, it is observed that the level of anti-EBNA antibody levels measured in the both test and control group patients is high. Also, for a small number of patients, anti-EBNA antibody have been observed at the mid and low-levels.

Figure 4: The range of Anti EBNA antibody titers among MS patients in both the case and control groups

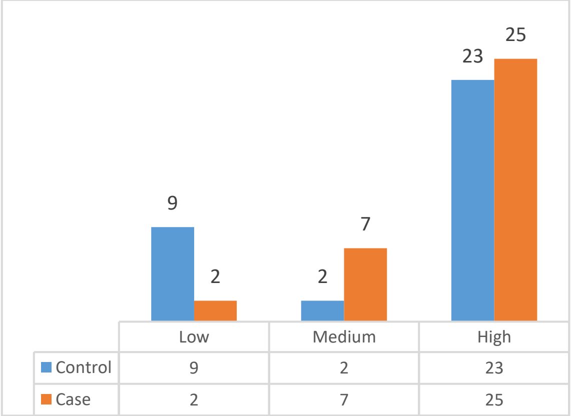

According to the Figure 5, it can be seen that the measured Human myxovirus resistance protein A (MxA) in the test group was significantly lower than the control group.

According to the Figure 6, it may be observed that the interleukins 10, 12 and 17 in the test group patients was much higher than the control group.

CONCLUSION

In the Table 1, the significance level of Leuven test (0.001) is smaller than 0.05. So, assuming equal variances for "anti-EBNA" in the case and control groups will be rejected. On the other hand, given the significance level of t-test assuming unequal variances (0.154) which is greater than 0.05 can be said that there is no significant difference between case and control groups about the mean "anti-EBNA" index. As seen earlier, the amount of this index is high in both case and control groups.

Figure 5: The amount of human myxovirus resistance protein A (MxA) in both the case and control groups

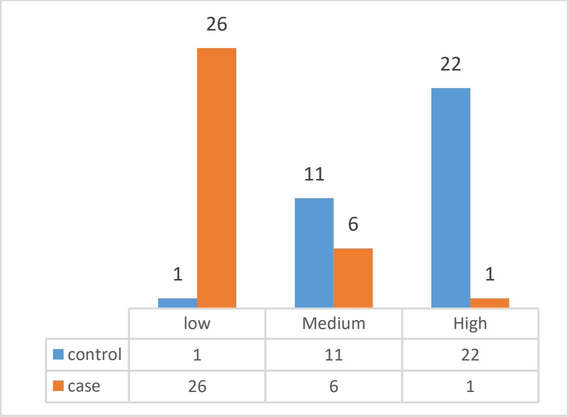

Figure 6: Frequency charts relating to the study of interleukins 10, 12 and 17 in MS patients in both the case and control groups

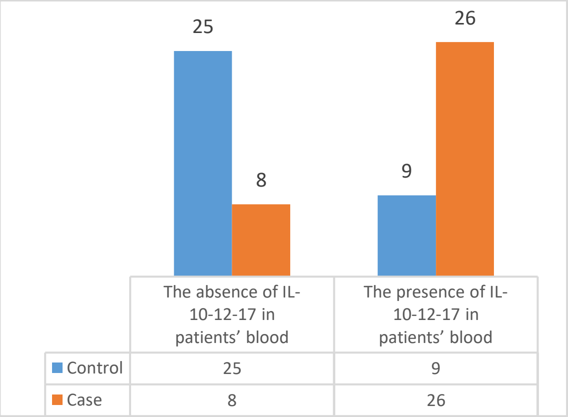

Table 1: Comparison of the average anti-EBNA antibody in MS patients in the case and control groups by using independent t-test

Anti-EBNA antibody	Leuven test to verify the equality of variances		t-test		
	F	The significance level	t	df	The significance level
Assuming equal variances	13.610	0.001	1.445	66	0.153
Assuming unequal variances			1.445	57.183	0.154

In the Table 2, the significance level of Leuven test (0.537) is greater than 0.05. So, assuming equal variances for "human myxovirus resistance protein A (MxA)" in the case and control groups won't be rejected. On the other hand, given the significance level of t-test assuming equal variances (0.001) which is lower than 0.05 can be said that there is a significant difference between experimental and control groups about the mean "human myxovirus resistance protein A (MxA)" index.

Table 2: Comparison of the average of human myxovirus resistance protein A (MxA) in the patients with MS both in the case and control groups (by using independent t-test)

Human myxovirus resistance protein A (MxA)	Leuven test to verify the equality of variances		t-test		
	F	The significance level	t	df	The significance level
Assuming equal variances	0.386	0.537	-9.645	66	0.001
Assuming unequal variances			-9.645	65.843	0.003

In the Table 3, the significance level of Leuven test (0.583) is greater than 0.05. So, assuming equal variances for "Presence of interleukin in the patient's blood" in the case and control groups won't be rejected. On the other hand, given the significant level of t-test assuming equal variances (0.008) which is lower than 0.05 can be said that there is a significant difference between experimental and control groups about the mean "Presence of interleukin in the patient's blood" index.

DISCUSSION AND CONCLUSION

In this section, the research hypotheses have been expressed. Then, according to the results of the descriptive statistics and analysis of data, these hypotheses have been evaluated.

Table 3: Comparison of the average of interleukins 10, 12 and 17 in MS patients in the case and control groups by using independent t-test

Presence of interleukin in the patient's blood	Leuven test to verify the equality of variances		t-test		
	F	The significance level	t	df	The significance level
Assuming equal variances	0.305	0.583	4.693	66	0.008
Assuming unequal variances			4.693	65.898	0.011

The first hypothesis

The level of anti-EBNA antibody in MS patients in other groups (patients with recurrent) and control group (patients without recurrence condition) is high.

According to analyzing data, assuming a significance level of t-test for equality of means (0.154) which is greater than 0.05, there is no significant difference between the average of the EBNA antibodies in MS patients in the case group (patients with recurrence) and control group (patients without recurrence condition).

Meanwhile, according to the above data descriptive statistics showed that the level of anti-EBNA antibody in patients with MS is High in both groups.

The second hypothesis

The level of the human myxovirus resistance protein A (MxA) in patients with multiple sclerosis in the case group (patients with recurrence) is less than the control group (patients without recurrence condition).

According to analyzing data, assuming a significance level of t-test for equality of means (0.001) which is greater than 0.05, there is significant difference between the average of the human myxovirus resistance protein A (MxA) in MS patients in the case group (patients with recurrence) and control group (patients without recurrence condition).

Meanwhile, according to the above data descriptive statistics showed that the level of the human myxovirus resistance protein A (MxA) in patients with MS is less in the case group.

The third hypothesis

Interleukin levels in the blood of MS patients in the case group (patients with recurrence) is higher than controls (patients without recurrence condition).

According to analyzing data, assuming a significance level of t-test for equality of means (0.008) which is greater than 0.05, there is significant difference between the average of the Interleukin levels in MS patients in the case group (patients with recurrence) and control group (patients without recurrence condition).

Meanwhile, according to the above data descriptive statistics showed that the level of the Interleukin in patients with MS is higher than the case group.

The main hypothesis

Based on the main hypothesis of this study, there is a significant relationship between viral infection diseases and recurrent multiple sclerosis. Evaluation of indices of anti-EBNA, the human myxovirus resistance protein A (MxA) and also the Interleukin levels in MS patients, it can be concluded that: The above infectious diseases, have a direct significant impact on increasing the rate of recurrence in patients with MS.

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ORIGINAL ARTICLES

The application of social marketing principles in health care campaigns

Ligia Pop (Micle)[1], Norbert D. Stenczel[1], Victor L. Purcărea[2], Iuliana R. Gheorghe[2], Laura Tribus[3], Gabriela Hoffer Oniga[1]

Abstract: *Social Marketing offers new insight into the design of health care programs that focus on the comprehensive vision of understanding the target audience. As there have been several confusions between the Health Care Social Marketing and education or preventive health care programs, the characteristics refer to the social good and behavior change on voluntary grounds. This paper aimed to elaborate some guidelines for Health Care Social Marketing campaigns to increase the chances of success and trigger the expected behavioral change in the target audience. To ensure the successful implementation of a Social Marketing campaign in health care, a plan that describes the necessary steps to respond in terms of efficiency and effectiveness to the public health campaign outcomes needs to be elaborated. The proposed model has three levels of intervention, as found in the scientific literature: Diagnosis of social problems, Benefits, and social change, and Social Marketing Mix.*

Keywords: *social marketing, health care campaigns, Health Care Social Marketing*

INTRODUCTION

Since its beginnings, Social Marketing has been a controversial concept, without having a formally approved definition. However, in 1971, Kotler and Zaltman considered that Social Marketing could be defined as "the design, implementation, and control of programs calculated to influence the acceptability of social ideas and involving considerations of product planning, pricing, communication, distribution, and marketing research" [1]. Similarly, in 1989, Kotler and Roberto described Social Marketing as "a program planning process that promotes the voluntary behavior of target audiences by offering benefits they want, reducing barriers they are concerned about, and using persuasion to motivate their participation in program activity" [2]. Moreover, a more recent perspective offered by Kotler and Lee stated that Social Marketing should be "a process that applies marketing principles and techniques to create, communicate and deliver value to influence target audience behaviors that benefit society as well as the target audience" [3]. These definitions have highlighted some common elements of Social Marketing, such as social good, behavior, the impact of Social Marketing on the target audience. In a broader sense, Social Marketing is based on the following orientations [4]:

- Client orientation, which is the identification of the people's needs, aspirations, values, and priorities;
- Creative orientation in finding imaginative methods to engage the target audience;
- Collective orientation is shaped by the recognized social context matters of a target audience;
- Competitive orientation represents the critical addressing

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to the competition through active strategic measures, for instance, brand competition, product competition, enterprise competition, and generic competition.

Thus, in practice, Social Marketing should be used when there is a voluntary behavior exchange and it is helpful when applied from the start in the planning process of a campaign. Social Marketing principles may be applied in any field. For example, Social Marketing offers a new approach to the design of health care programs due to its more comprehensive focus on the target audience, namely, the audience orientation [5]. An audience-oriented perspective is materialized under a bottom-up philosophy in contrast to the traditional approach [6].

Consequently, Social Marketing is more suitable to be used in the health care field, as it: (a) guides consumers from the beginning of the planning process, having a consumer orientation; (b) improves the management, implementation, and evaluation of intervention programs using efficient marketing activities through the development of a plan, lined up to a target audience; (c) promotes the message of campaigns through Mass-Media following the target audience; (d) improves public satisfaction using marketing research to have an accurate perspective on the desired outcomes; (e) helps individuals identify the need for behavior change [7].

Although most Health Care Social Marketing campaigns focus on the operationality of integrating the Marketing Mix to encourage the target audience to change their behavior, they do not provide the expected results not because the Marketing principles have been inappropriate, but rather because there has been an incomplete analysis of some elements at the design stage [8]. Therefore, this article aimed to elaborate some guidelines for Health Care Social Marketing campaigns to increase the chances of success and trigger the expected behavioral change in the target audience

THE HEALTH CARE SOCIAL MARKETING ORIENTATION

The Health Care Social Marketing orientation is a complex concept because it is not easily understood and usually confusion may occur because most specialists focus on promotion instead of the individual's behavioral change process. Put it in simple words, Health Care Social Marketing should guide the target audience towards the action and the behavior change by using health policies and decreasing the assumptions, constraints, and barriers encountered in the external and internal environment of the target audience. The desired outcome of a Social Marketing campaign in the health care field takes place when the target audience identifies and recognizes the benefits of the change.

In practice, there is a refined version of what a Health Care Social Marketing campaign is and where interventions should be applied. Using the guidelines of French and Blair-Stevens in the shape of several benchmarks, the empirical challenges have been solved, once a Health Care Social Marketing initiative has been designed [9]. These benchmarks refer to the following elements as presented in Figure 1.

Figure 1: The Benchmarks of Health Care Social Marketing

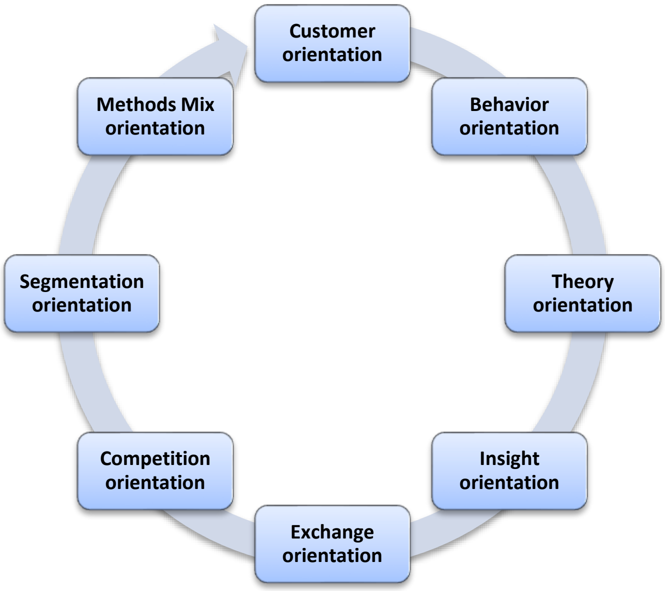

- Customer orientation - Develops a robust understanding of the audience, based on the features of a market and consumer research;

- Behavior orientation - Has a clear focus on behavior, based on a strong behavioral analysis, with a specific emphasis on behavioral goals;

- Theory orientation - Drawing the information from an integrated theoretical framework is fundamental for behavior change;

- Insight orientation - Based on developing a deeper insight approach - it promotes behavioral changes and motivations;

- Exchange orientation integrates an exchange analysis, suggesting the understanding of what the person must give to get the proposed benefits;

- Competition orientation includes a competitive analysis to understand what competes with the time and attention of the audience;

- Segmentation orientation uses a developed segmentation approach;

- Methods mix orientation identifies an appropriate mix of methods, using the de-CIDES intervention framework [10] that may help in the selection, coordination, and combination of different intervention types. The de-CIDES interventions relate to the following:

  - Inform/communicate: inform/communicate/prompt/trigger/remind/reinforce/awareness/explain;

  - Educate: enable/engage/train/skill development/inspire/encourage/motivate;

  - Support: service provision/practically assist/promote access/social networks/social mobilization;

  - Design: alter/physical/product/social/organizational/systems/technology;

  - Control/regulate: control/rules/require/constrain/restrict/enforce/regulate/legislate.

HEALTH CARE SOCIAL MARKETING PLANNING

As there have been many confusions between what Health Care Social Marketing is and what is not, with a specific reference to education and promotion, many scholars felt the need to reaffirm the main points of interest when planning a health care campaign. Therefore, Health Care Social Marketing is a sort of utility package related to the activities, processes, and efforts to deliver benefits to individuals, namely, it is more than communication, and education, it is a plan that focuses on new opportunities to trigger ideas and behavior in health care consumers [11]. Accordingly, Continho Da Silva and Mazzon [8] proposed a plan that described the necessary steps to respond in terms of efficiency and effectiveness to the public health campaign outcomes (Figure 2). This model has three levels of intervention, as it follows: (i) diagnosis - research, collect, and analyze market data and information to identify social problems; (ii) benefits and social change - design an appropriate benefit to the target audience and influence the social change to them; (iii) the Social Marketing Mix - provide and ensure mechanisms and instruments to measure, analyze, and assess the results of the health care campaign.

Figure 2: The steps of a Health Care Social Marketing plan

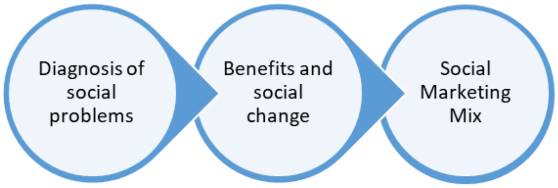

1. Level 1: Diagnosis of social problems

At this level there are 3 steps:

- Identifying the mission in terms of strategic intent, organization values (moral and ethical assumptions), ability and skills (performance), the market definition using consumer-oriented activities and processes, and positioning, meaning the image of the benefits of the campaign.

- The environment of the Social Marketing campaign refers to the outside environment, made up of the demography, economy, nature, legal, technology, and competition, as well as to the inside environment that concentrates on the resources and competencies, culture, suppliers, publics, and channels.

- The data and information collections use the research and the Marketing Information System (MIS). The scope of MIS is to provide relevant information for social marketers to take decisions on time.

- The factors of the target audience's behavior are divided into external factors, such as the skills necessary to perform a particular behavior, the access to the promoted services, the policies, laws and regulations, the culture in terms of customs, values, and lifestyles, as well as the internal factors that refer to the knowledge, and attitudes of the target audience. The internal factors are shaped by the target audience's perceived self-efficacy, risks, social norms, consequences, and intentions.

- Identifying the social problem (situation analysis) provides a SWOT-like approach by analyzing both the internal and external environments of the Health Care Social Marketing campaign. The internal environment refers to the strengths and weaknesses that the social campaign might produce, and, respectively, the external environment, made up of the opportunities and threats that may influence the health care campaigns.

2. Level 2: Benefits and social changes

The specific steps at this level are the following:

• Defining the goal of the Social Marketing campaign that should be SMART, meaning measurable. Moreover, it should be a reference, specific, have a date and a location, set realistic goals, be clear, and be known to all the stakeholders involved in the Social Marketing campaign.

• Identifying the social change by being a collective action on a long period, having a permanent nature and complexity arising from a social problem, and, respectively, combining the change with the idea of progress. Following several social change theories as well as behavior change models. This step refers to the cognitive, action, behavior, and value at different behavioral change stages such as the pre-contemplative, contemplative, preparation, action, maintenance, and termination. Most Health Care Social Marketing consumers are in the pre-contemplative or contemplative stages.

• Informing the change benefits to the target audience to achieve the potential cognitive consequences that will trigger a changing behavior.

• The target audience’s reaction and their demographic characteristics are very important in determining the needs and the wants, their perceptions in attitudes and shared values as well as in the readiness to adopt the change.

• Positioning is the goal of every Health Care Marketing campaign as it can make the target audience reflect on the possibilities of change, mainly, on providing attractive benefits.

3. Level 3: Social Marketing Mix

The Marketing Mix is the operational part of the Social Marketing campaign and it focuses on the strategies applied for a target audience.

In the scientific literature, there have been many variants of Social Marketing Mix but in the health care context, it is made up of the classic Marketing Mix components such as the product, price, place, promotion, and of the specific Social Marketing Mix parts as People, Processes, and Performance.

Briefly, the Product is usually elaborated around the identified social problem and its solution arises from the educational and cultural levels of the population, as well as its popularity, which will be debated by the effects and the benefits on a long period.

The Price refers to all the costs involved in an act of change since there are more non-monetary costs such as physical, time, social and psychological. In fact, in Health Care Social Marketing, the costs are materialized in barriers for individuals in adopting a certain behavior.

Place suggests where, when, and how a behavioral change is required, after providing the necessary information to the target audience, whereas Promotion suggests communication strategies and techniques to persuade the target audience to embrace the benefits of the change rather than the costs. In Health Care Social Marketing, represents the promotion of awareness and provides knowledge about the attitudes, intentions, and behavior change expected from the target audience, and ensure a sustainable relationship between the social marketers and the target audience.

The process encompasses both the visible and non-visible activities involved in a Social Marketing campaign. An efficient process must be constantly adapted to the dynamic evolution of the environment, with the scope of avoiding failures, by excluding steps that do not bring value to the target audience and have several orientations, for instance, service quality delivery, productivity, suitable physical evidence and elaborate a blueprint of the processes.

Professional refers to the skills of the staff team that should focus on the knowledge of the entire cycle of service processes from the start to the finish to deliver qualitative services, manage conflicts between different entities and the target audience and respect the ethical patterns encountered in Social Marketing environments. In the health care context, Social Marketing professionals should be able to provide support and knowledge concerning the processes involved.

Performance is used to measure the quality of the Social Marketing campaign, in the shape of breaking the barriers and delivering several benefits, at the same time, respecting some ethical guidelines. As performance may also be considered a quantitative coefficient, the Health Care Social Marketing campaign suggests responsiveness, helpfulness, security, credibility, proactivity, and empathy.

Program is reflected in the above-mentioned elements of the Social Marketing Mix, and it may be part of a plan that will help professionals in mapping the solutions to the process failures, introduce the consumer-oriented measures and instruments that would ensure the benefits, as well as prioritize sustainable actions and control the performance of a Social Marketing campaign.

EXAMPLES OF HEALTH CARE SOCIAL MARKETING CAMPAIGNS

Social marketing has opened new areas for the expertise of marketers and has intrigued public health advocates.

The early health applications of Social Marketing emerged as part of the international development effort, under the umbrella of “development communication”, and were implemented in the Third World countries between 1960 and 1970 [12]. As such, programs promoting immunization, family planning, various agricultural reforms, and nutrition were implemented in many countries in Africa, Asia, and South America in the 1970s. These were based on some of the agricultural development experiences supported by the U.S. Agency for International Development (USAID), as well as community-based family planning programs initiated by the Ford Foundation and the Population Council in the late 1960s.

Soon, international programs began to incorporate marketing techniques, including radio, media, and advertising, on a larger scale [13]. Hence, family planning program designers, for example, initially started distributing contraceptives in pharmacies and small stores and, subsequently, developed systems for market segmentation, mass communication, and product placement to achieve a wider and more efficient distribution of discounted contraceptives.

Peter King from the Indian Institute of Management, in collaboration with the Indian Government’s Central Family Planning Board conducted the first national program focused on family planning and the Ford Foundation funded the project, as USAID supplied condoms to the program without cost.

Further, USAID increased the funding for social contraceptive marketing projects in the 1970s, supporting projects in Kenya, Colombia, Sri Lanka, and Jamaica. Much of the important research and developments to advance social health marketing applications have taken place internationally in the area of family planning. In the same vein, Bill Smith at U.S. Academy for Educational Development began using Social Marketing in several countries around the world to improve child survival rates through oral rehydration, breastfeeding, and childhood immunization [14].

Several heart disease prevention programs have also been developed in the U.S. In 1971, the Stanford University Heart Disease Prevention Program undertook a three-community study to discover a risk reduction method that could be generally applicable [15]. Another program was the National High Blood and Heart Health Program Pawtucket (National High Blood Pressure Prevention Program, and the Pawtucket Heart Health Program). Risk factors were high blood pressure, increased blood cholesterol, obesity, smoking cigarettes, and sedentary life. To assess the program, cross-sectional and cohort risk factor surveys were conducted, together with mortality and morbidity surveillance, both in Pawtucket, Rhode Island, and in the non-intervention comparison city of New Bedford, Massachusetts [16]. Some of these programs used the term Social Marketing and openly declared to apply and adapt several marketing concepts or approaches without fully embracing the concept of marketing [17].

The Swiss Federal Office for Public Health and the Swiss AIDS Foundation has promoted AIDS prevention campaigns. A multi-media information campaign with the main slogan “STOP-AIDS” began in February 1987 and is still ongoing. The campaign and its objectives have reached the general population and target groups.

In its evolution, the application of Social Marketing principles has raised many community issues but has also solved them by using instruments and techniques accordingly.

After the 1990s, worldwide, government or non-profit organizations used Social Marketing techniques to implement programs to promote a change in health-beneficial behavior, such as increasing fruit and vegetable consumption, decreasing fat consumption, promoting physical activity, reducing alcohol and tobacco consumption, special programs for pregnant women, infants, and children, behavioral problems related to environmental protection [18]. At the international level, among other facts, Social Marketing has been used to improve access to drinking water [19], eradicate leprosy in Sri Lanka [20], increase adherence to tuberculosis medicines [21], promote immunizations, and universal iodine legislation [22].

Despite being heavily used, even in the pandemic context, Health Care Social Marketing, most of the time, does not have the desired outcome, namely, the behavioral change in individuals, as it needs control and monitoring instruments, as well as elaborating the messages sent differently, for instance, applying cues to trigger a certain emotion. In a study about smoking cessation, using shock advertising messages proved to be inefficient, and maybe changing the message’s embedded emotion could trigger the expected result, that is instead of using fear, what should be used is humor [23].

CONCLUSION

Health Care Social Marketing has been a controversial concept, as the application of Social Marketing principles in the medical field has been difficult and many scholars confused the activities specific to Social Marketing with the promotion of health and prevention of health. Having an empirical perspective, Social Marketing has many particularities among which are voluntary behavior exchange, a consumer orientation, and a competitive behavioral change. Moreover, elaborating a complex plan of a Health Care Social Marketing campaign may be truly successful and it should include the following three steps: Diagnosis of social problems, benefits, and social change, and Social Marketing Mix.

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ORIGINAL ARTICLES

The assessment of the vitamin D deficiency between the patients with acute coronary syndrome and those with stable coronary artery disease in a military hospital

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Abstract: *Background: A few studies compared the vitamin D deficiency condition between the patients with the acute coronary syndrome (ACS) and those with stable coronary artery disease. The present comparative study aimed to assess the vitamin D deficiency status between the patients with ACS and those with stable coronary artery disease.*

Methods: This cross-sectional study was performed on 200 consecutive patients with the diagnosis of ACS or stable coronary artery disease. Serum Vitamin D level was measured by immunoassay and its serum level was categorized as normal (> 30 ng/ml), insufficient (20 to 30 ng/ml), and deficient (< 20 ng/ml).

Results: Overall, 103 patients were categorized as stable angina group, 74 had unstable angina, 10 had STEMI, and 13 had NSTEMI. There was no difference in serum vitamin D3 level across the four groups including stable angina group (13.81 ± 15.13 ng/ml), unstable angina group (15.13 ± 11.11 ng/ml), STEMI group (21.58 ± 19.87 ng/ml), and NSTEMI group (18.67 ± 13.07 ng/ml) (p = 0.276). No difference was also found in the mean vitamin D3 level between the groups with new MI (15.98 ± 12.44 ng/ml versus 14.01 ± 15.31 ng/ml, p = 0.318). In total, 8.5% had normal vitamin D levels and 9.0% had vitamin D insufficiency, while the majority of patients (82.5%) were vitamin D deficient. The vitamin D deficiency was comparable in the patients with stable angina compared to those in ACS groups. According to ROC curve analysis, measuring serum vitamin D3 had a moderate value to discriminate stable angina from ACS (AUC = 0.651). In this regard, the best cutoff point for serum vitamin D3 level to discriminate stable angina from ACS was 9.0 ng/ml yielding a sensitivity of 70.1% and a specificity of 57.3%.

Conclusion: The measurement of vitamin D and thus the presence of vitamin D deficiency have no sufficient power to discriminate ACS from stable coronary artery disease.

Keywords: *vitamin D deficiency, coronary syndrome, artery disease*

INTRODUCTION

Acute coronary syndrome (ACS) has been now known as the major cause of death whole of the world, especially in industrial countries. Potentially, the presence of various genetic, metabolic, and environmental risk factors can increase the risk for mortality and disability in affected patients [1]. In this regard, some metabolic and endocrinological factors have been identified to link the progression and severity of coronary artery disease (CAD). Within the last few years, a significant association between deficiency of some vitamins and minerals and CAD has been found [2-4]. In this regard, an association of vitamin D deficiency and different cardiovascular risk factors and

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diseases has been extensively evaluated. Some prospective and observational studies have addressed the possible linkage of vitamin D deficiency and the development of CAD and its risk factors [5, 6]. Thus, vitamin D deficiency not only has a major role in bone metabolism, homeostasis regulation, and immune pathways, but also the presence of vitamin D receptors on cardiomyocytes, endothelial cells, and vascular smooth muscle cells suggests the role of vitamin D in vitamin D-mediated cardiovascular system [7-9]. It has been shown that cardiac muscle functioning depends on the circulating blood concentration of calcitriol [10]. Calcitriol affects the growth, proliferation, and morphology of murine cardiomyocytes. In detail, treatment with calcitriol increased the expression of the cardiac muscle protein myotrophin. The link between vitamin D deficiency and cardiovascular risk factors has been also shown [11]. According to the Framingham Offspring Study [5], individuals with low activated vitamin D faced more with incident cardiovascular disease such as myocardial infarction, coronary insufficiency, and heart failure compared to those with a normal level of vitamin D. Thus, treatment with vitamin D supplementation could reduce cardiovascular death and length of hospitalization in ACS patients; however, a few studies compared the vitamin D deficiency condition between the patients with ACS and those with stable coronary artery disease. The present comparative study aimed to assess the vitamin D deficiency status between the patients with ACS and those with stable coronary artery disease.

METHODS

This cross-sectional study was performed on 200 consecutive patients with the diagnosis of ACS or stable coronary artery disease. The ACS was diagnosed according to the ACS criteria defined based on clinical symptoms, ECG changes, and cardiac enzyme rising. Stable coronary artery disease was defined as typical exertional chest pain relieved by rest or nitroglycerin administration and a positive exertional ECG test. The exclusion criteria were history of surgery within 6 months ago or having a hematologic disease or any hemoglobinopathy or coagulopathy, history of chronic clinical conditions such as liver or kidney disease or malignancies, or history of cerebrovascular disease or other simultaneous cardiovascular disorders.

Baseline characteristics and clinical data of participants including demographics, anthropometric parameters (weight, height, and body mass index), cardiovascular risk factors (current smoking, hypertension, hyperlipidemia, and opium addiction) as well as laboratory parameters (fasting blood sugar, hemoglobin A1c, lipid profile, thyroid functional hormones, and serum creatinine) were collected from the hospital recorded files. Serum Vitamin D level was measured by immunoassay and its serum level was categorized as normal (> 30 ng/ml), insufficient (20 to 30 ng/ml), and deficient (< 20 ng/ml). The primary endpoint was to assess the relation between the type of ACS and the severity of vitamin D deficiency. The secondary endpoint was to determine the best cutoff point for vitamin D to differentiate ACS from stable coronary artery conditions. The ethical committee of Kerman University of Medical Sciences approved the study research proposal all procedures were in accord with the standards of the World Medical Association Declaration of Helsinki for research involving human subjects. Informed consent was obtained from all study participants.

Results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Continuous variables were compared using t, and one-way ANOVA tests, or non-parametric Mann-Whitney U, or Kruskal-Wallis tests whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the groups. The ROC curve analysis was also used to determine the value of vitamin D measurement to predict the presence of ACS and also to determine the best cutoff value of this vitamin to discriminate ACS from stable angina. For the statistical analysis, the statistical software SPSS version 16.0 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

RESULTS

Overall, 103 patients were categorized as stable angina group, 74 had unstable angina, 10 had STEMI, and 13 had NSTEMI. Comparing baseline characteristics and clinical data (Table 1) across the groups showed that the patients with stable angina were older than the patients in other groups.

Also, hypertension was more frequent in those with STEMI, while hyperlipidemia was more prevalent in those with stable angina. Family history of CAD was found less and opium addiction was revealed more in the NSTEMI group when compared to other groups. Statin use, aspirin use as well as the history of prior PCI was all more in a group with stable angina than in other groups, while prior CABG was scheduled more in the NSTEMI group.

Meanwhile, there was no difference across the four groups in terms of gender, mean BMI, history of diabetes, smoking, alcohol use, thyroid dysfunction, prior SK use, and left ventricular dysfunction.

Table 1: Baseline characteristics and clinical data of study population

	Stable angina (N = 103)	Unstable angina (N = 74)	STEMI (N = 10)	NSTEMI (N = 13)	P-value
Male gender	60 (58.3)	46 (62.2)	7 (70.0)	10 (76.9)	0.555
Age, year	64.29 ± 8.85	59.92 ± 11.25	60.70 ± 12.09	61.92 ± 8.78	0.058
BMI, kg/m^2	27.92 ± 3.82	27.54 ± 2.93	26.43 ± 2.31	26.45 ± 3.94	0.333
Hypertension	87 (84.5)	46 (62.2)	10 (100)	9 (69.2)	0.002
Diabetes	34 (33.0)	33 (45.6)	5 (50.0)	6 (46.2)	0.480
Hyperlipidemia	96 (93.2)	17 (23.0)	0 (0.0)	3 (23.1)	< 0.001
Family history	44 (42.7)	31 (41.9)	4 (40.0)	3 (23.1)	0.035
Smoking	29 (28.2)	12 (16.2)	2 (20.0)	2 (15.4)	0.260
Opium use	7 (6.8)	5 (6.8)	1 (10.0)	2 (15.4)	0.033
Alcohol use	9 (8.7)	2 (2.7)	0 (0.0)	0 (0.0)	0.210
Hyperthyroidism	3 (2.9)	1 (1.4)	0 (0.0)	0 (0.0)	0.963
Hypothyroidism	7 (6.8)	1 (1.4)	0 (0.0)	0 (0.0)	0.611
CCU admission	69 (67.0)	29 (39.2)	5 (50.0)	5 (38.5)	0.011
Statin use	99 (96.1)	56 (75.7)	7 (70.0)	9 (69.2)	< 0.001
Aspirin use	90 (87.4)	53 (71.6)	8 (80.0)	9 (69.2)	0.023
NSAIDS use	2 (1.9)	2 (2.7)	0 (0.0)	0 (0.0)	0.883
Prior PCI	96 (93.2)	15 (20.3)	1 (10.0)	3 (23.1)	< 0.001
Prior SK receive	11 (10.7)	3 (4.1)	1 (10.0)	0 (0.0)	0.475
Prior CABG	3 (2.9)	13 (17.6)	3 (30.0)	2 (15.4)	0.013
LVEF	48.99 ± 8.18	47.58 ± 10.46	44.80 ± 10.29	43.85 ± 5.67	0.152

Regarding laboratory parameters (Table 2), mean serum FBS was significantly lower in the group with stable angina, while serum HDL level was higher among those with stable angina than other patients. The highest liver enzymes were revealed in those with STEMI. The patients with stable angina had a significantly higher level of T3 than other ones.

Table 2: Baseline laboratory indices in the study population

	Stable angina	Unstable angina	STEMI	NSTEMI	P-value
Hemoglobin	14.77 ± 2.89	13.89 ± 1.60	14.73 ± 1.12	14.16 ± 2.20	0.106
Hematocrit	48.61 ± 36.91	40.85 ± 4.46	41.78 ± 3.46	41.09 ± 6.48	0.258
Platelet	226.21 ± 65.72	218.84 ± 58.57	195.70 ± 48.04	223.54 ± 73.51	0.495
ESR	15.17 ± 6.46	16.14 ± 15.67	13.10 ± 7.56	14.69 ± 15.00	0.848
CRP	0.97 ± 8.36	0.32 ± 0.47	0.50 ± 0.52	0.62 ± 0.51	0.918
FBS	115.27 ± 36.98	143.47 ± 61.22	170.70 ± 68.86	166.85 ± 71.98	< 0.001
Creatinine	3.25 ± 14.27	1.33 ± 0.97	1.14 ± 0.21	1.19 ± 0.20	0.613
Uric acid	7.83 ± 11.78	5.59 ± 1.93	4.74 ± 1.44	6.45 ± 2.09	0.317
Triglyceride	157.69 ± 71.86	148.07 ± 86.70	137.50 ± 68.42	105.87 ± 93.43	0.153
Cholesterol	183.37 ± 50.72	171.80 ± 52.83	170.10 ± 40.04	170.23 ± 50.33	0.433
LDL	98.46 ± 30.33	105.40 ± 42.09	100.34 ± 25.09	105.91 ± 42.84	0.609
HDL	46.43 ± 30.16	36.38 ± 8.52	36.90 ± 7.91	34.54 ± 10.22	0.018
AST	21.36 ± 7.96	24.38 ± 19.29	65.40 ± 42.45	34.38 ± 26.31	< 0.001

	Stable angina	Unstable angina	STEMI	NSTEMI	P-value
ALT	26.89 ± 17.69	28.46 ± 24.30	52.00 ± 33.04	34.46 ± 22.99	0.005
ALKP	181.25 ± 73.84	185.70 ± 60.70	170.40 ± 30.67	225.92 ± 79.55	0.144
Calcium	13.56 ± 19.05	8.98 ±0.61	8.86 ± 0.33	9.16 ± 0.47	0.141
Phosphorus	4.16 ± 1.25	3.99 ± 0.58	3.38 ± 0.71	3.83 ± 0.72	0.091
PTH	30.48 ± 30.51	47.81 ± 21.74	43.29 ± 11.42	49.96 ± 15.40	0.227
T3	2.99 ± 3.86	0.89 ± 0.97	0.70 ± 0.14	0.68 ± 0.17	< 0.001
T4	45.44 ± 47.98	62.71 ± 19.19	65.74 ± 11.80	61.28 ± 11.44	0.102
TSH	4.19 ± 14.30	2.64 ± 4.32	2.14 ± 1.59	1.62 ± 1.18	0.698
HbA1C	6.33 ± 1.36	14.88 ± 70.03	7.99 ± 2.01	7.61 ± 2.63	0.622

There was no difference in serum vitamin D3 level across the four groups including stable angina group (13.81 ± 15.13 ng/ml), unstable angina group (15.13 ± 11.11 ng/ml), STEMI group (21.58 ± 19.87 ng/ml), and NSTEMI group (18.67 ± 13.07 ng/ml) ($p = 0.276$). No difference was also found in the mean vitamin D3 level between the groups with new MI (15.98 ± 12.44 ng/ml versus 14.01 ± 15.31 ng/ml, $p = 0.318$).

In total, 8.5% had normal vitamin D levels and 9.0% had vitamin D insufficiency, while the majority of patients (82.5%) were vitamin D deficient. As shown in Figure 1, the vitamin D deficiency was comparable in the patients with stable angina compared to those in other ACS groups ($p = 0.338$). However, vitamin D deficiency was more prevalent in the old MI group compared to the new MI group (87.0% versus 78.0%, $p = 0.011$).

Figure 1: Vitamin D deficiency in ACS subgroups

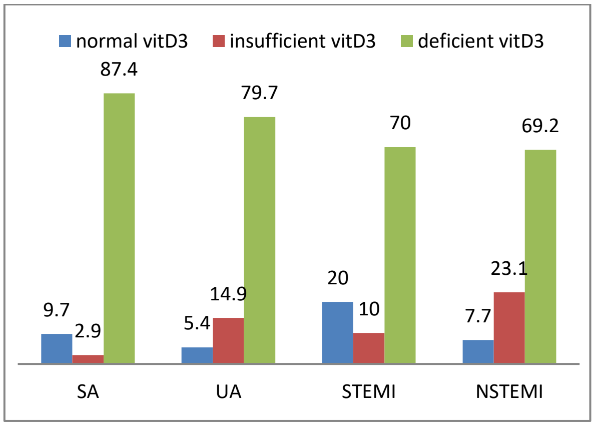

The level of vitamin D was similar in the patients with and without hypertension (15.04 ± 14.56 ng/ml versus 14.88 ± 11.93 ng/ml, $p = 0.9450$), the groups with and without diabetes mellitus (13.46 ± 4.17 ng/ml versus 15.35 ± 15.08 ng/ml, $p = 0.760$), and those with and without hypertriglyceridemia (13.29 ± 4.02 ng/ml versus 15.03 ± 12.49 ng/ml, $p = 0.714$).

According to ROC curve analysis, measuring serum vitamin D3 had a moderate value to discriminate stable angina from ACS (AUC = 0.651, 95% CI: 0.573 – 0.728, $P < 0.001$) (Figure 2). In this regard, the best cutoff point for serum vitamin D3 level to discriminate stable angina from ACS was 9.0 ng/ml yielding a sensitivity of 70.1% and a specificity of 57.3%.

Figure 2: ROC curve analysis to determine the value of vitamin D level to discriminate stable angina from ACS

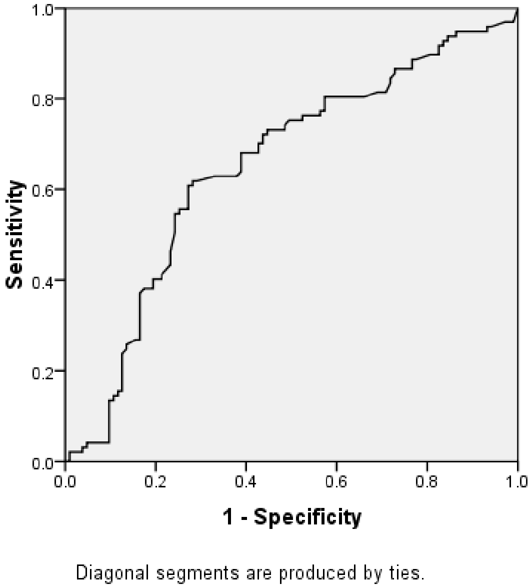

DISCUSSIONS

There is modest evidence that lower vitamin D levels are associated with an increased risk of CAD. However, few studies have researched CAD as a separate disease entity from cardiovascular disease. It has been now suggested that people with low vitamin D levels had twice the risk of developing a heart attack compared to those with adequate vitamin D levels, but it has remained uncertain the effect of vitamin D levels on CAD risk particularly in variant angina.

Hence, we attempted to compare the value of vitamin D measurement and thus vitamin D deficiency in differentiating stable coronary conditions from ACS variants. In this regard, we could demonstrate a similar incidence of vitamin D deficiency in stable coronary arteries and those without this condition. Even, using ROC curve analysis, it was shown that the measuring vitamin D level could discriminate stable coronary involvement and ACS variants with moderate power. However, a higher incidence of vitamin D3 might be predicted in those with old MI than in patients that newly suffered MI. In a study by Raina et al [12] in India, it was suggested a significant correlation between vitamin D deficiency and chronic stable angina. In India, results similar to the pointed were seen by Sanjeev Kumar et al. [13] who studied 100 patients undergoing CAG. They found that the mean 25(OH) D level was 14.8 ng/mL; vitamin D deficiency was present in 80% and only 7% had an optimal 25(OH) D level that was near to our result. More interestingly, another study that divided 2910 patients with coronary artery disease history (acute myocardial infarction – unstable angina – stable angina) into three groups based on their angiography results showed that although vitamin D deficiency is prevalent in all groups, it is more prevalent in patients with stable angina and also it is indicative of a worse prognosis [14] that was numerically near to our results.

The similarity in incidence of vitamin D deficiency in our study subgroups may be due to this fact that the majority of patients included into the study had vitamin D deficiency so 82.5% were vitamin D deficient that is a considerable finding. Another reason for the insignificant association between vitamin D level and the ACS variants could be related to no significant association between the level of vitamin D and various cardiovascular risk subgroups. As we assessed this association, the level of vitamin D was similar in cardiovascular risk subgroups. In fact, because of a probable causative relation between cardiovascular risk factors and vitamin D deficiency, our finding can be explained. Another reason for paradoxical results across the studies may be due to the different cutoff points for defining vitamin D deficiency. During a Framingham–Off spring study on 1739 participants who were followed up within 5.4 years Wang and colleagues [15] revealed that vitamin D deficiency is associated with cardiovascular disease. However in the mentioned study levels < 15 ng/ml were considered as vitamin D deficiency. Also, in Siadat et al study [16], it was considered levels lower than 30 ng/ml as vitamin D deficiency, while in our study, vitamin D deficiency was defined as the levels of lower than 20 30 ng/ml.

It seems that the effect of vitamin D on cardiovascular risk can be mediated by some mechanisms including reduction of the risk for underlying metabolic disorders such as diabetes and high blood pressure, lowering the thickening of the arterial walls, reduces the risk of respiratory infections, suppression of inflammatory pathways, and reduction the risk of arterial calcification or hardening [17-21]. Furthermore, some studies are indicative of a relation between vitamin D deficiency and cardiovascular disease, increased blood pressure, increased insulin resistance, heart failure, and fatal strokes [22-26] that can explain the association between CAD risk and vitamin D deficiency; however, this association was not proved in our study population.

As a new result, the best cutoff value for differentiating stable angina from ACS conditions was 9.0 that considerably lower than the cutoff values defined for vitamin D deficiency. We could not find a similar study on determining the best cutoff values for vitamin D to predict stable angina and thus the obtained result on this subject should be more assessed in further studies.

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CLINICAL PRACTICE

The complex implications of dermatoses specifically associated with pregnancy

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Abstract*: Pregnancy-specific dermatoses are a heterogeneous group of inflammatory dermatoses associated exclusively with pregnancy. Although these dermatoses are rare, it raises problems in medical practice because of their appearance, being difficult to diagnose, treat and the possibility of the fetus being affected. Pemphigoid Gestationis (PG) is a rare, autoimmune, strongly pruritic, vesiculobullous dermatosis with a strong impact on the patient. Polymorphic Eruption of Pregnancy (PEP) is a benign, self-limited inflammatory skin disorder that usually affects first pregnancy in the third trimester or immediately in the postpartum period. Other dermatoses associated with pregnancy include: Intrahepatic Cholestasis of Pregnancy (ICP), Atopic Eruption of Pregnancy (AEP) and Pruritic Folliculitis of Pregnancy (PFP). We will extensively present PG and PEP, to draw attention to the importance of correct diagnosis and the implications of treatment during pregnancy.*

Keywords: *specific dermatoses in pregnancy, pemphigoid gestationis, polymorphic eruption of pregnancy*

INTRODUCTION

During pregnancy endogenous, immunological, metabolic and vascular complex changes can result in skin damage. The skin changes can be classified into: physiological skin changes, alteration of preexisting cutaneous conditions and specific dermatoses in pregnancy.

Pregnancy-specific dermatoses are a heterogeneous group of inflammatory dermatoses associated exclusively with pregnancy and/ or immediately after birth. Some are considered a significant risk to the fetus.

These relatively rare conditions, often with variable clinical appearance, and the lack of definitive diagnostic tests have led to confusion in terms of proper diagnosis and management of pregnancy-specific dermatoses.

While many physicians are familiar with normal physiological changes during pregnancy, the knowledge of dermatoses in pregnancy, which is rarely encountered, is often minimal. This review is designed to highlight pregnancy-related

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dermatoses for easier diagnosis and management.

PEMPHIGOID GESTATIONIS (PG)

Pemphigoid gestationis (PG) is a rare, autoimmune, strongly pruritic, vesiculobullous dermatosis, with an incidence of 1: 50,000 to 60,000 pregnancies in USA [1]. The results of European studies suggest a global incidence of 0.5 cases per million people per year [2]. It occurs more frequently in the white race, which may reflect the association with specific HLA haplotypes. Most often, the disease occurs in the second or third trimester of pregnancy, but cases have also been reported in the first trimester and postpartum period [3].

The etiology of the disease is unknown. Nearly half of the cases develop during the first pregnancy. Women with a history of PG are considered to have a higher prevalence of autoimmune diseases, including Graves’s disease, Hashimoto's thyroiditis, vitiligo, autoimmune thrombocytopenia, and pernicious anemia [4].

PG is associated with the following antigens: HLA-B8, HLA-DR3 and HLA-DR4 or combinations of HLA-DR3 and HLA-DR4 [5]. The presence of HLA-DR3/DR4 was highlighted in 45% of patients with PG compared with 3% of the general population, and all patients with a history of PG have anti-HLA antibodies [6].

The main antigen of PG is collagen XVII, present both in the skin and placenta, which is exposed to the maternal immune system through an abnormal expression of classical class II histocompatibility molecules in the placenta [7]. PG is exclusively a disease of pregnancy, so potential causes are focused on immunogenicity and potential cross reactivity between maternal-fetal transfer and placental tissue and skin [8]. PG onset in the first or second trimester may lead to negative outcomes, including premature delivery [9].

The diagnosis of PG relies on the clinical evaluation, histological findings, and direct immunofluorescence. Bullous changes are characterized by the formation of subepidermic blister, the bullae contain mostly eosinophils. Eosinophil infiltration is the most common histopathological finding [10].

The inflammatory cellular response of the upper dermis has numerous lymphoid cells, some neutrophils and eosinophilic leukocytes. Microscopic studies have confirmed that IgG is deposited along the basal membrane area, mainly in lucid lamina and located on the proximal anchor filament [11]. IgG class antibodies - the so-called herpes gestation factor (HG) - have an important effect on the genesis of this bullous dermatosis. These antibodies can be transmitted from mother to child, which can cause premature birth, low birth weight, bullous and papular lesions in the newborn; IgG antibodies can be detected by ELISA [12].

Laboratory tests are usually within normal limits. Peripheral eosinophilia may correlate with the severity of the disease, and changes in the level of immunoglobulins, erythrocyte sedimentation rates, acute phase reactants and antithyroid antibodies may occur [3].

A patient who suffered from PG at the previous pregnancy will also suffer from this disease in future pregnancies and the onset of PG will be earlier than in the previous pregnancy. Also, a patient with PG onset in the second trimester may experience remission, followed by exacerbation in the last 6 weeks of pregnancy. The mean duration of the disease at the end of the pregnancy is 4 weeks for bullous rashes and 14 weeks for the urticaria component. Neonatal PG is caused by the transfer of antibodies through the placenta, skin changes in these infants resolve spontaneously within a few weeks and rarely require treatment [13].

The clinical picture is characterized by pruritus, pain, disfigurement, papulovesicular polymorphic eruptions on the abdominal and distal extremities, including the palms, soles, chest and back. The lesions range from erythematous papules, edema papules, confluent vesicles and urticaria plaques through large and tight bullets [14].

Differential diagnosis should be made with: scarring pemphigoid, pruritic urticarial papules and pregnancy plaques, linear IgA dermatitis, multiform erythema, dermatitis herpetiforma, acute urticaria, allergic contact dermatitis, drug-induced bullous disorders, pruritic pregnancy folliculitis, papular dermatitis of pregnancy, scab [15].

As for treatment, consideration should be given to the fact that PG disappears after birth, but treatment is necessary to minimize pruritus and to prevent vesicles and bullae rash. In mild cases of PG, oral antihistamines and topical corticosteroids will be used, while systemic corticosteroids (prednisone 0.5-1.0 mg/kg/day) will be used in severe cases. Due to prolonged corticosteroid therapy, both the mother and the newborn should be followed to rule out adrenal insufficiency [16]. Alternatives to corticosteroids include azathioprine, dapsone, intravenous immunoglobulin, pyridoxine, cyclosporine, plasmapheresis, minocycline/ nicotinamide, methotrexate and cyclophosphamide. Immunoapheresis is a useful therapeutic option in patients who do not respond to conventional therapy [6, 17].

The clinical course of PG can be altered by changes in estrogen and progesterone levels. Although PG normally recurs in subsequent pregnancies, 8% of cases may not have PG in subsequent pregnancies. 25% of patients may experience relapse when using oral contraceptives or during menstruation. Abortion and premature birth were frequently reported [18].

POLYMORPHIC ERUPTION OF PREGNANCY (PEP)

Polymorphic eruption of pregnancy (PEP), a specific dermatosis of pregnancy also known as pruritic urticarial papules and plaques of pregnancy (PUPPP), is a benign, self-limited inflammatory skin disorder that usually affects first pregnancy in the third trimester or immediately in the postpartum period [19]. The incidence of PPE was estimated to 1 in 160 pregnancies, with the mean age being 29 years [15, 20]. PEP is considered to be the most common dermatosis of the pregnancy [21].

PEP is predominantly present either at the first pregnancy in 70% of cases [15] or at pregnancies that were not performed earlier (for example, spontaneous abortion). The increased incidence of multiple pregnancies in women with PEP has been reported [22]. A specific multiple gestation study in PEP showed that 2.9% of 8000 twin pregnancies had a PEP diagnosis and 14.3% of the triple pregnancies were associated with PEP [23]. It has been shown in several studies that there is a predilection of pregnant women who have PEP to give birth to male children.

Pathogenesis is still unknown, the theory of distension suggests that overstressing of the abdominal wall causes subsequent damage to the connective tissue, triggering an inflammatory response [20, 24].

Infants born from mothers diagnosed with PEP usually do not experience complications or skin manifestations. Premature birth was reported before 37 weeks in 18.4% of PEP patients, compared with 5.8% of patients in the control group [25]. A possible increase in the incidence of cesarean section and premature labor in PEP has been suggested, but there is no clear evidence that it would occur in later pregnancies.

In over 79% of cases, onset occurs in the third trimester of pregnancy, with remission of symptoms after birth, between 1 and 4 weeks [26].

PEP shows papules and pruritic urticarial plaques during pregnancy. Clinical findings were categorized into three PEP subgroups: type I, mainly characterized by papules and urticarial plaques; type II, which included erythematous patches and nonurticarial papules (1-2 mm) or vesicles; and type III, a combination of the other two types. All three types often also have some obstructions with surrounding blanched halos [26]. In terms of distribution, 91% of patients were first found to be the abdominal area, the proximal thighs, and occasionally both [25, 27].

PEP atypical lesions, including vesicles, complicate the diagnosis and have been reported in up to 40% of cases. A retrospective study showed that initially classic clinical manifestations are present in 98% of cases, but 51% of them develop changes in the form of vesicles, target lesions and several eczematous plaques [24]. In less than 2% of PEP patients, it can affect palms and soles, with papulous or vesicular lesions similar to dyshidrotic eczema [25, 28].

PEP is a disorder with a broad clinical spectrum and diverse clinical features, but with absence of pathognomonic identifying features. To exclude other conditions, laboratory and histopathological investigations will be performed, such as: complete blood cell counts, liver function testing, renal function testing, thyroid stimulating hormone level, urine level, and bile acid level. The differential diagnosis is made with: pemphigoid gestationis, contact dermatitis, toxic drug rash, viral sprain, pitiriasis rosea and urticaria.

Histopathologically, a superficial or deep perivascular dermatitis is found with lymphocytic vasculitis, eosinophils are often present, in some cases, acanthosis, hyperkeratosis and intraepidermal vesicles [29]. If the diagnosis is not conclusive, two biopsies should be performed, one from lesional skin for hematoxylin and eosin staining and one from perilesional skin for DIF. The histopathological examination confirms an inflammatory process underlying PEP and skin stretching has recently been suggested as a precipitation factor for "koebnerization" seen in vitiligo, psoriasis and lichen planus.

It was observed in PEP patients an increase in body weight of more than 15 kg and the infant weight at birth was on average 3.6 kg compared to 3.3 kg in the control group [30]. The prevalence of multiple pregnancies in PEP patients is 10 times higher than in the general population [31]. The skin from PEP lesions showed progesterone receptor immunoreactivity in the cytoplasmic keratinocyte suprabas, while non-lesional skin did not show [32].

Due to the fact that PEP is an autoimmune condition without significant maternal or fetal risk and without recurrence in future pregnancies, patients should not suffer from anxiety due to this condition. Interdisciplinary communication between dermatologist, obstetrician and neonatologist is essential in the management of severe cases of PPE.

Topical corticosteroids are the primary means of treatment for polymorphic eruption of pregnancy (PEP) [33]. The relief of symptoms may require high potency (Class I or II) topical steroids, such as fluocinonide (cream 0.05% / 0.1%, ointment 0.05%) or even systemic steroids (prednisolone 40-60 mg/day). Oral antihistamines can be effective. Symptomatic treatment therapy consists of cooling baths, frequent application of emollients, topical medications containing refrigerated menthol, lightweight cotton, first-generation sedative antihistamines (chlorpheniramine and diphenhydramine) and medically active topical corticosteroids [19, 34].

OTHER SPECIFIC DERMATOSES IN PREGNANCY

Intrahepatic Cholestasis of Pregnancy (ICP) also known as pruritus gravidarum, is a liver disorder characterized by severe pruritus and secondary skin lesions in the third trimester of pregnancy (25–30 weeks gestation). Pruritus typically starts on the palms and soles and later becomes generalized. Secondary lesions such as excoriations, scratch marks, and prurigo nodules might develop as a result of scratching [35]. The symptoms usually disappear 1-2 days after delivery, but is a high risk of recurrence of ICP in subsequent pregnancies (50–70%). ICP is correlated with fetal risks, the most common being premature birth (20–60%), intrapartal fetal distress (20–30%) and stillbirth (1-2%) [20, 36].

Atopic Eruption of Pregnancy (AEP) is a benign pruritic disease with eczematous or papular lesions occurring during pregnancy in patients with a predisposition or history of atopic dermatitis. Specific immunological changes in pregnancy are incriminated in the development of late onset [37]. Clinical manifestations include pruritus, prurigo lesions/ excoriations and eczematous skin lesions, the diagnosis being based on clinical features. Treatment is based on class III or IV topical corticosteroids; in severe cases, systemic or antihistamine corticosteroids may be administered [38, 39]. AEP is not associated with fetal risk factors, except for the child's uncertain risk of developing atopic dermatitis.

Pruritic Folliculitis of Pregnancy (PFP) manifests as small (3–5 mm), multiple, disseminated, erythematous, excoriated, follicular papules and sterile pustules [20]. PFP usually occurs in the 2nd or 3rd trimester of pregnancy (between the 4th and 9th gestation months). Etiology is unclear, has a good maternal prognosis, without recurrence in future pregnancies and without significant fetal harm. Differential diagnosis should be done with acne, bacterial folliculitis, or polymorphic eruption of pregnancy. Treatment includes topical low-potency corticosteroids, topical benzoyl peroxide 10 %, and ultraviolet B phototherapy [40].

CONCLUSIONS

Most skin manifestations that occur during pregnancy are mild and resolve after birth. A special category is pregnancy-specific dermatoses, which, although rare, can pose great problems in terms of diagnosis, treatment and fetal harm. Good knowledge of these dermatoses is necessary for both gynecologist, dermatologist and family doctor. Proper diagnosis and management are essential for symptom relief and, in the case of fetal risk, adequate monitoring during pregnancy and monitoring of newborns after birth are of major importance. Increased attention should be paid to pregnant patients to understand the risks, adverse effects, and drug interactions of their medications in the treatment of pregnancy-specific dermatoses.

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ORIGINAL ARTICLE

The COVID-19 pandemic – A global statistical interpretation in first year dynamics

Viorel Ordeanu[1,2], Lucia E. Ionescu[1]

Abstract: *The novel coronavirus, identified in December 2019, has caused a pandemic that will remain in the medical literature as SARS-CoV-2, the etiological agent of the Covid-19 disease. It was initially stated that this disease would not be more serious than the flu, in terms of prevalence, frequency of complications and mortality. Although in the country of origin the epidemic was only local, the virus spread successively to other countries, until it quickly spread to all inhabited continents and was declared a pandemic.*

Being a new disease and a new virus, the world panicked. The World Health Organization released guidelines to member countries, which they have implemented. Because the disease sped out of control, it became not only a health crisis but also a socio-economic one. A year later, we are still facing this disease, about which we have learned a lot but not yet all that is necessary and so the time has come for a certain summing up.

The Covid-19 pandemic is a reality of current times, the year 2020 being portrayed as the "COVID year". The international scientific community has shown that, when needed, it can collaborate beyond classical barriers. Statistics show that the virus is not very aggressive, the disease is not very serious but there are also serious, sometimes fatal complications to which appropriate measures must be applied. The risk of serious illness and life-threatening complications is statistically reduced over time, the disease being less severe now than at the beginning, so it is on a downward slope.

Keywords: *Covid-19, SARS-CoV-2, pandemic, statistics, public health, fake news*

THE OVERALL SITUATION

The coronavirus identified by the Chinese in December 2019 caused an epidemic and then a pandemic that would remain in the medical literature as the severe acute respiratory syndrome virus coronavirus 2 (SARS-CoV-2) as the etiological agent of Covid-19 disease (coronarovirus from December 2019) by analogy with the SARS coronarovirus from 2002. It was stated from the beginning that this disease would not be more serious than influenza, both in prevalence and in the frequency of complications and mortality.

Although in the supposed country of origin the epidemic was only local (but in the size of a medium-sized country) and was "obstructed" in a short period of time by drastic, medical and non-medical measures, the virus spread successively to other countries, until it quickly spread to all inhabited continents and was declared a pandemic.

Being a new disease (although viral pneumonia is known and treatable) and a new virus (although coronaviruses are known with hundreds of species with different pathogens and virulence) the world panicked. The World Health Organization (WHO) has accounted for the situation and released guidelines to member countries which they have more or less implemented, sooner or later, according to "national specifics". Because the evil was not pulled out by the roots suddenly but rather gradually, the disease got out of control and became not only a health crisis but also a socio-economic one. It seems that some countries would have had an interest in maximizing the disease in order for it to be considered the main cause of the current economic crisis, which had started anyway. The impact on the population was variable, from absurd denial and protests to unjustified fear that culminated in suicides for fear of the novel coronavirus.

A year later, we are still facing this disease, about which we

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have learned a lot but not yet all that is necessary and so the time has come for a certain summing up, from the onset to the present. The assessment is useful for evaluating through critical points in the dynamics, because in fact we do not even know when and how this complex crisis that mainly dominated Western civilization will end.

The statistically processed data were recorded before the appearance of new variants of SARS-CoV-2 discovered in the UK (known as 20I/501Y.V1, VOC 202012/01, or B.1.1.7) and South Africa (SARS-CoV-2 known as 20H/501Y.V2 or B.1.351).

MATERIAL AND METHOD

The medical literature on Covid-19 and the information released in the official and unofficial public space about the current complex crisis caused by the pandemic were studied. Data published in the media and on the Internet by credible organizations were statistically analyzed: WHO, CDC (Center for Disease Control, USA), ECDC (European Center for Disease Control), Worldometer (USA), communiqués from national governments and specialised journals. Of these, the most complete and systematized data, representing the US point of view, are updated and published daily on the website and can be accessed free of charge by anyone interested in accurate information [1, 2].

The primary statistical interpretation was possible by dividing quantitative intervals, percentage processing by absolute and relative values per million people [3], by applying the 2 alpha significance test, etc. corroborated with medical logic, which has lately come into contradiction with "pseudo-evidence-based medicine". In general, infectious diseases on Earth and consecutive epidemics have a certain pattern of evolution, which is learned in medicine and is found in the history of medicine. The Romanian historian Nicolae Iorga said "Learn history to know what will come!". Obviously, when it comes to microbes brought, with or without intention, from other celestial bodies, we will not know what we have to do, being in front of absolute news, and the risk will be maximum.

RESULTS AND COMMENTS

The listing of the data provided by Worldometer on world statistics for all the landmarks considered important, grouped by fields, presents a comprehensive, dynamic and realistic figure of the current society. The listing of data provided by Worldometer on 13.12.2020 on the Covid-19 pandemic shows that data on morbidity, prevalence, diagnosis, severity and mortality from 120 territorial entities on five continents were reported and centralized: the 195 independent member countries UN/WHO, 23 permanently inhabited dependent territories and two extraterritorial ones (cruise ships quarantined for epidemic on board) but not including the territories that did not declare the presence of this disease.

The centralised data published on a daily basis are in permanent dynamics, apparently amplified, so for any statistical interpretation the corresponding date must be mentioned. In general, the trend is mathematically increasing, but through the biostatistical interpretation of the primary and secondary data, different parameters of the pandemic can be objectively highlighted.

2020 statistical processing on case studies

The exhaustive table is ordered by the absolute number of cases of Covid-19 disease recorded from the beginning of the year until now, with linear and logarithmic graphical processing. The total number of 72,197,885 cases registered on 13.12.2020 represents a prevalence of the disease of 0.92% of the world's population (7,832,803,872 inhabitants). Compared to the overall mortality of the world population (55,995,505), 2.88% of mortality is due to/with Covid-19 (1,613,401). We statistically analyzed the percentage distribution of mortality (as the maximum severity factor of the disease) compared to the total number of Covid-19 cases registered by country, the world average being 2.24%, so a total of 1 death of covid or with covid at 4 855 inhabitants, which means a cause of mortality with a relatively low frequency. It is far outweighed by other diseases, such as HIV/AIDS, under-five infant mortality or toxic use (tobacco, alcohol, drugs).

The graphical distribution by percentage statistical intervals of the countries did not create a normal bell-shaped Gaussian curve, but a steep downward curve. Thus, for the interval 0 (inclusive) – 1 (exclusive) % there are 75 countries, about a third of the total, and of these 24 countries/territories have no covid mortality at all. In general, these countries have a relatively small population (below the world average) and the total number of covid patients is relatively small, below 1,000, except for Turkey. At the 1-2% range are 62 countries, including "world champions" such as the USA, India, Brazil and Russia. In the median range of 2-3% are 50 countries, including Romania which is slightly above the world average (2.18%) with 2.40% mortality from the number of covid patients. Then the frequency decreases dramatically, so at 3-4% there are 14 countries, at 4-5% there are 6 countries, at 5-6% there are 4 countries, at 6-7% there are 3, at 7-8% it is only one country, at 9-10% one, at 10-11% one and at 29-30% one. A total of 64 countries out of the 220 statistically monitored entities (states and territories) have a mortality rate due to Covid-19 above the world average.

It should be noted that Yemen holds the "absolute record" of 29.9%, but it is in a prolonged war with Saudi Arabia (which could be considered a "proxy" war between the US and Iran) which has induced a major humanitarian crisis, as never before, for the solution of which the "international community" has not been effectively involved. The other excessive values are for Mexico (9.16%) which apparently would have no special reason but also for Western Sahara, which has a mortality of 10%, with 2 deaths out of a total of 10 cases, so it is not statistically significant (Figure 1).

Figure 1: The percentage distribution of the mortality rate due to Covid-19 compared to the total number of Covid-19 cases registered by country (website www.worldometer on 13.12.2020)

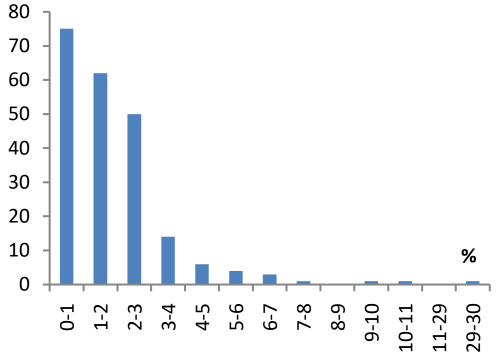

Statistical processing on data from the present situation - active cases

The percentage distribution of Covid-19 active cases (so, which were hospitalized on 13.12.2020) at approx. one year after the initial declaration of the epidemic, reports cases of serious illness (serious and critical) compared to the current active cases (mild and asymptomatic), according to Worldometer. The total incidence of serious cases in the total number of cases under treatment is 0.42%, which compared to the historical situation for a year of 2.24% mortality shows a major improvement in the situation. This favorable situation may be due to several causes: either due to a decrease in the virulence of the virus by its adapting to the human host (no pathogen has the "interest" to kill its host), or by optimizing therapy (although we do not yet have effective specific drugs), either due to the extension of collective immunity (so contemptuously called "herd" in English literature) or a combination of these. In fact, the WHO estimates that 99.5% of current cases are mild and only 0.5% are severe, which means that mortality in Covid-19 cases should not be higher than 0.2%, i.e. below influenza mortality.

It is interesting to note that in the "year of covid" we do not have a flu epidemic or acute upper respiratory infections (URI), which is remarkable because there is a hypothesis of the possibility of hybridization of the novel coronavirus with the influenza virus, which would have led to an increase in the risk to public health. The explanation could be that the measures of individual and collective prophylaxis were effective: wearing a protective mask both healthy (so as not to be contaminated) and sick (so as not to spread the virus), physical/social distancing, and compliance with hygiene rules. Individual and collective, preventive medical control (including mass thermometry), specific tests, isolation, quarantine, compulsory hospitalization, etc. Another possible explanation could be that in previous influenza and/or IACRS outbreaks some of the cases were actually coronaviruses, including the current undiagnosed Covid, or that we currently have undiagnosed influenza that is erroneously recorded with the diagnosis of Covid- 19.

The global prevalence has dropped from 0.92% per year to 0.26% up to the present moment (13.12.2020), and 78 countries (more than a third of the total) currently have no serious cases in evolution. Calculated as a percentage of serious cases from the total number of active Covid-19 cases, between 0 (inclusive) ÷ 1% (exclusive) are 147 countries, for example the USA, the absolute “champion”, has only 0.42% so with little above the world average. Of these, the 78 countries and territories mentioned do not have serious cases at all, so they do not require intensive care in the ICU. This includes the median of the group. In the range of 1÷ 2% there are 28 countries, including Germany with 1.32% and Romania with 1.48% (so well above the world average). At 2÷3% there are 13 countries, at 3÷4% there are 8 countries, at 4÷5% there are 3 countries, at 5÷6% there are 4 countries, at 6÷7% there are 4 countries, at 7÷8 % is a country, 8÷9% are 2 countries, 9÷10% is a country and over 10% are 5 countries. It should be mentioned that 4 countries no longer report for various reasons, so they cannot be fully included in the statistical study: Great Britain, Spain, the Netherlands and Sweden. We may only wonder how some of these countries would have reacted if another country would have acted as such under the circumstances. A total of 101 countries out of the 220 monitored have reported serious active cases (current, serious, critical) above the world average.

It should be mentioned that Yemen has a severity incidence of 24.47% of the reasons shown above, and Saudi Arabia of 15.36%, a country with which it is at war. These are followed by Liechtenstein with 12.65%, Suriname with 100% (in 2 cases out of 2) and Antigua with 33.33% (in 2 cases out of 6) which with a relatively small population and low casuistry show no individual statistical significance, but only within the world average (Figure 2).

Figure 2: Percentage distribution of current (active) cases of Covid-19 with serious forms (serious, critical) compared to the total number of active cases currently registered by country (website www.worldometer on 13.12.2020)

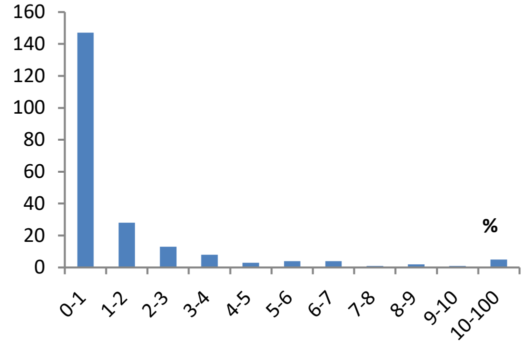

Comment on closed communities

In the early days of the pandemic, there were two cases of cruise ships (Diamond Princess and MS Zandam) of interest as a "situational experiment" in which international personnel benefiting from extraterritoriality suffered the effects of the epidemic, without other external factors. Peculiarities of the situation: a crew of thousands of healthy adults and passengers thousands of other adults and especially healthy elderly or with various comorbidities, but with permanent medical assistance (for surveillance, quarantine, treatment and evacuation), with maximum congestion and reduced isolation possibilities. A total of 721 Covid-19 cases were registered, with 15 deaths and 706 cures, so a contagiousness of max. 20% with mortality 2.08% of the total number of cases, so max. 0.4% mortality from the existing population. It can be estimated that under conditions of strictly supervised medical and non-medical countermeasures, Covid-19 is neither very contagious nor has a high mortality, even if there is a population in the risk group: the elderly, chronic diseases, etc (Figure 3).

Figure 3: Proportion of sick and deceased aboard the cruise ships, registered by country (website www.worldometer on 13.12.2020)

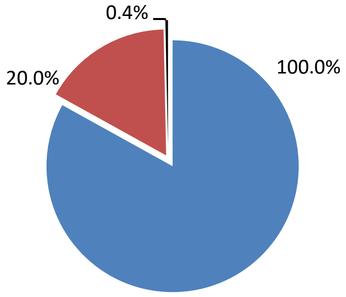

Data significance validity and probability

The entry of the statistically processed data from the Worldometer tables primarily processed on the grid's graph with the probability of significance “2 alpha” of 95%, shows that the general prevalence of fatal cases of/with Covid-19 compared to the total cases of Covid-19 throughout 2020 (A) is significantly higher than the prevalence of severe cases compared to the total current active cases of Covid-19 (B). Because the size of the sublots (cohort) can influence the statistical interpretation, we completed the graphical matrix once in descending order of number of cases and separately in reverse order, respectively in ascending order of number of cases and compared the two curves. These showed that the severity of group A is higher than that of group B with a statistical probability of 95%. In order to obtain the statistically significant result, in the first situation 20 cases were necessary and in the second 34 cases out of a total of 220 available. It was clear that the situation before December was worse, so we are currently facing a milder disease, which may be the "beginning of the pandemic's end." (Figure 4).

Figure 4: Comparison of the Covid-19 percentage mortality curve by country in 2020 compared to serious active cases in December 2020 registered by country (website www.worldometer on 13.12.2020)

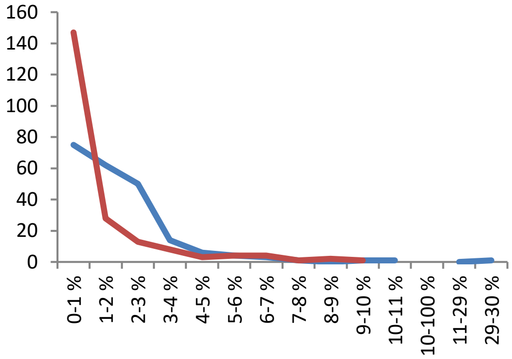

There is a decrease in the severity of the disease in the pandemic's dynamic context, which could mean that: the virus has become less virulent (adapts to the host), the treatment is more effective (more effective therapeutic strategies are being identified), (specific and/or cross) collective immunity develops or all of these in different proportions.

The United Nations (UN) centralizes worldwide data on the Covid-19 pandemic, namely the 230 countries (195 independent and 35 inhabited dependent territories) and the data are updated on a daily basis and published on the Internet via www.worldometercoronavirus. The world's population, which currently consists of nearly 7.8 billion people, is extremely unevenly distributed in these countries and is in ongoing dynamics. Some have over 1 billion inhabitants (China, India), about 150 countries have a population between 1 million and 1 billion, and about 70 entities have a population between 1 thousand and 1 million inhabitants and the rest under 1 thousand (e.g. the Vatican). The distribution of the population is uneven even between continents, but the discrepancies are much attenuated. Asia has the majority of the world's population, about 60% and the other six continents have the remaining 40% but Antarctica has no permanent inhabitants.

Of the countries in the world, 218 reported at least one case of Covid-19 (the US and India each have over 10 million cases) and 24 of them have no fatal cases of Covid-19. Countries free of Covid-19 or without mortality are generally small and sparsely populated and most are located in areas with warm climates. It should be recalled that four major countries, which are severely affected by the pandemic, no longer report up-to-date data on the evolution of the disease (UK, the Netherlands, Spain and Sweden) which may lead to some erroneous statistical conclusions. Among the countries monitored for Covid-19 are 23 (i.e. 10.45%) that have reported cases but have no mortality, and currently there are 78 countries (i.e. 35.45%) that do not have serious cases. Statistically, it can be concluded that the pandemic is on a downward trend.

The results of the statistical data processing, contained in Subchapter 3.1. could support the alarmists and Subchapter 3.2. the moderates, but in fact show a dynamic of the epidemic that is common in all epidemics. We could quote what the Germans jokingly say: "Statistics is a science that can prove anything, absolutely anything."

It seems that the current pandemic also holds a record for unfounded, contradictory and fake news depending on different interests, this being doubled by an "infodemic".

Comparative public health implications by geographical area

The representation of the real situation compared on different geographical areas, respectively compared to the world total with the percentage comparison (to mitigate extreme quantitative differences) on continents, which broadly represent one or more types of civilizations in the sense described by Huntingdon is systematised in tables 1-10. The comparison of statistical data is illustrative for the severity and evolution in the pandemic's dynamics, on large geographical areas with the respective population (Table no. 1).

Table 1: World population with numerical and percentage distribution by continents (www.worldometer on 01.07.2020)

No.	Geographical area	Population	%	Observations regarding the population
1	Worldwide	7,794,798,739	100.0	
2	Europe	747,636,026	9.6	
3	North America	368,829,647	4.7	
4	Asia	4,641,054,775	59.5	majority
5	South America	653,962,331	8.4	
6	Africa	1,340,518,147	17.2	
7	Oceania	42,677,813	0.5	minority
8	Antarctica	0	0	uninhabited

It is observed that the area of the extended western civilization (1 + 2 + 4 + 6) represents about 23%, and the area of the other civilizations about 77%, i.e. the absolute majority.

The percentage prevalence of the total cases of Covid-19 from the whole year 2020 compared to the total population, morbidity, is presented in Table 2, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 2: Covid-19 prevalence in the population, by geographical areas

No.	Geographical area	Population	%	Covid-19 Cases	% prevalence in population	Observations on morbidity
1	Worldwide	7,794,798,739	100.0	81027859	1.04	average
2	Europe	747,636,026	9.6	22684299	3.03	extremely high
3	North America	368,829,647	4.7	22400648	6.07	maximum
4	Asia	4,641,054,775	59.5	20362013	0.44	low
5	South America	653,962,331	8.4	12856856	1.97	high
6	Africa	1,340,518,147	17.2	2675424	0.20	low
7	Oceania	42,677,813	0.5	48222	0.11	minimum
8	Antarctica	0	0	0	0.0	

The area of restricted western/Euro-Atlantic civilization has about 14% of the population and about 56% of the cases

The percentage prevalence of Covid-19 fatal cases throughout 2020 compared to the total population, mortality, is presented in Table 3, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 3: Prevalence of Covid-19 mortality in the population, by geographical areas

No.	Geographical area	Population	%	Fatal Cases of Covid-19	% prevalence in population	Observations on morbidity
1	Worldwide	7,794,798,739	100.0	1770254	0.023	average
2	Europe	747,636,026	9.6	521434	0.070	extremely high
3	North America	368,829,647	4.7	496982	0.135	maximum
4	Asia	4,641,054,775	59.5	332002	0.007	low
5	South America	653,962,331	8.4	355935	0.054	high
6	Africa	1,340,518,147	17.2	62834	0.005	low
7	Oceania	42,677,813	0.5	1058	0.002	minimum
8	Antarctica	0	0	0	0.0	

North America has a Covid-19 mortality rate among its population of almost 6 times higher than the global rate.

The percentage incidence of mortality due to Covid-19 in the total cases of Covid-19 in 2020 is presented in Table 4, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 4: Incidence of mortality in patients with Covid-19, by geographical areas

No.	Geographical area	Population	%	Fatal Cases of Covid-19	Total Covid-19 Cases	% prevalence in population	Observations on morbidity
1	Worldwide	7,794,798,739	100.0	1770254	81027859	2.18	average
2	Europe	747,636,026	9.6	521434	22684299	2.30	
3	North America	368,829,647	4.7	496982	22400648	2.22	
4	Asia	4,641,054,775	59.5	332002	20362013	1.63	minimum
5	South America	653,962,331	8.4	355935	12856856	2.77	maximum
6	Africa	1,340,518,147	17.2	62834	2675424	2.35	high
7	Oceania	42,677,813	0.5	1058	48222	2.19	
8	Antarctica	0	0	0	0	0.0	

South America and Africa have high mortality and Asia has low mortality.

The percentage incidence of mortality due to Covid-19 in the new cases of Covid-19 in December 2020 is presented in Table 5, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

South America, Africa and Europe have high mortality and Oceania no longer has mortality.

The percentage prevalence in the population of the current active cases of Covid-19 in December 2020 is presented in Table 6, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

North America and Europe have a high prevalence (totaling 87% in a population of about 14%) and Asia, Africa and Oceania currently have a low prevalence.

The prevalence of serious cases (serious and critical) of current assets in December 2020 expressed as a percentage of the population is presented in Table 7, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

With an average prevalence of active cases of 1/100 000 it can be considered that at this moment Covid-19 is a "rare disease" and drugs intended for the prophylaxis and treatment of this disease can be considered as "orphan drugs". North America and Europe have a relatively higher prevalence and Africa and Oceania currently have a very low prevalence.

Table 5: Incidence of mortality in new patients with Covid-19 (December), by geographical areas

No.	Geographical area	Population	%	New deaths due to Covid-19	New Covid-19 cases	% Incidence in patients	Observations on mortality
1	Worldwide	7,794,798,739	100.0	5830	316402	1.84	average
2	Europe	747,636,026	9.6	2699	122940	2.20	high
3	North America	368,829,647	4.7	1321	102110	1.29	
4	Asia	4,641,054,775	59.5	1348	72428	1.86	minimum
5	South America	653,962,331	8.4	99	2938	3.37	maximum
6	Africa	1,340,518,147	17.2	363	15955	2.28	high
7	Oceania	42,677,813	0.5	0	34	0.0	minimum
8	Antarctica	0	0	0		0.0	

Table 6: Percentage prevalence in the population of Covid-19 active cases (December), by geographical areas

No.	Geographical area	Population	%	Covid-19 Active Cases	% Incidence in patients	Observations on prevalence
1	Worldwide	7794798739	100.0	22074490	0.283	average
2	Europe	747636026	9.6	11083322	1,482	high
3	North America	368829647	4.7	8220611	2,229	maximum
4	Asia	4641054775	59.5	1244003	0.027	minimum
5	South America	653962331	8.4	1136032	0.174	
6	Africa	1340518147	17.2	377067	0.028	low
7	Oceania	42677813	0.5	13727	0.032	low
8	Antarctica	0	0	0	0.0	

Table 7: Percentage prevalence in the population of severe active cases of Covid-19 (December), by geographical areas

No.	Geographical area	Population	%	Covid-19 Serious Active Cases	% Incidence in population	Observations on severity
1	Worldwide	7,794,798,739	100.0	105246	0.001	average
2	Europe	747,636,026	9.6	26548	0.004	high
3	North America	368,829,647	4.7	34193	0.009	maximum
4	Asia	4,641,054,775	59.5	25284	0.001	minimum
5	South America	653,962,331	8.4	16758	0.003	high
6	Africa	1,340,518,147	17.2	2442	0.0002	low
7	Oceania	42,677,813	0.5	22	0.00005	minimum
8	Antarctica	0	0	0	0.0	

The incidence of serious cases (serious and critical) that require ICU from the active cases of Covid-19 in December 2020 (treatable at home or in hospital) is presented in Table 8, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Asia, South America and Africa have a high incidence of serious cases and Europe and Oceania have a low incidence.

The evolution of morbidity by comparing the percentage of current active cases (December 2020) compared to the total cases of Covid-19 in 2020 is presented in Table 9, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 8: Incidence of serious cases in Covid-19 active cases by geographical areas

No.	Geographical area	Population	%	Covid-19 Serious Active Cases	Covid-19 Active Cases	% Incidence in patients	Incidence observations
1	Worldwide	7794798739	100.0	105246	22074490	0.48	average
2	Europe	747636026	9.6	26548	11083322	0.24	low
3	North America	368829647	4.7	34193	8220611	0.42	
4	Asia	4641054775	59.5	25284	1244003	2.03	maximum
5	South America	653962331	8.4	16758	1136032	1.48	high
6	Africa	1340518147	17.2	2442	377067	0.65	
7	Oceania	42677813	0.5	22	13727	0.16	
8	Antarctica	0	0	0	0	0.0	

Table 9: Evolution of morbidity by percentage comparison of current active cases (December 2020) compared to the total number of Covid-19 cases in 2020, by geographical area

No.	Geographical area	Population	%	Covid-19 Active Cases	Covid-19 Total Cases	% Incidence in patients	Evolution observations
1	Worldwide	7,794,798,739	100.0	105246	81027859	27.24	average
2	Europe	747,636,026	9.6	26548	22684299	48.86	maximum
3	North America	368,829,647	4.7	34193	22400648	36.70	high
4	Asia	4,641,054,775	59.5	25284	20362013	6.11	minimum
5	South America	653,962,331	8.4	16758	12856856	8.84	low
6	Africa	1,340,518,147	17.2	2442	2675424	14.09	
7	Oceania	42,677,813	0.5	22	48222	28.47	
8	Antarctica	0	0	0	0	0.0	

By a reductio ad absurdum, in a more balanced distribution, December should have represented less than 10% of the total for 2020, but it is almost 3 times higher worldwide, so the epidemic is "accelerating" especially in Europe and North America.

By comparing the percentage of serious (serious and critical) current cases (December 2020) to the total mortality of Covid-19 cases in 2020, the evolution of the severity of the cases is presented in Table 10, according to the data provided by www.coronaviruswoldometer from 27.12.2020.

Table 10: The evolution of the severity of the cases by comparing the percentage of the serious (serious and critical) current active cases (December 2020) to the total mortality of Covid-19 cases in 2020, by geographical areas

No.	Geographical area	Population	%	Covid-19 death rate % on 2020	% Severity Covid-19 active	% Difference	% Decrease in severity	Evolution observations
1	Worldwide	7,794,798,739	100.0	2.18	0.48	1.78	22.02	average
2	Europe	747,636,026	9.6	2.30	0.24	2.06	10.43	good
3	North America	368,829,647	4.7	2.22	0.42	1.80	18.92	
4	Asia	4,641,054,775	59.5	1.63	2.03	-0.40	124.54	Is getting worse!
5	South America	653,962,331	8.4	2.77	1.48	1.29	53.43	worse

No.	Geographical area	Population	%	Covid-19 death rate % on 2020	% Severity Covid-19 active	% Difference	% Decrease in severity	Evolution observations
6	Africa	1,340,518,147	17.2	2.35	0.65	1.70	27.66	
7	Oceania	42,677,813	0.5	2.19	0.16	2.03	7.31	good
8	Antarctica	0	0	0	0	0.0	0	

It is observed that the severity of the pandemic increases in Asia and South America and decreases in Europe and Oceania.

The published data are updated on a daily basis showing, as expected, a permanent dynamic of the pandemic. However, the general evolution is maintained and falls within the usual predictions in epidemiology, but with inherent features dependent on the biological agent, the disease, the sick, the population, the environment, the medical system, etc.

DISCUSSIONS

Statistically, it is found that worldwide the prevalence of the disease and the incidence of mortality are unequally represented in different countries and territories, presenting higher than average values in Europe and North America, followed by South America and Australia, so in the predominantly western civilization area and especially in the Euro-Atlantic one. Although an initial flu-like severity was estimated in the initial epidemic, the consecutive pandemic had an upward and then downward trend, now reaching below the severity of the pandemic flu epidemic. The factors favoring the pandemic can be incriminated as well as the relatively effective control measures, and currently the pandemic is decreasing both quantitatively and qualitatively. On the daily world case series (Worldometer graphs) there are three periods of increasing the frequency of the disease (16.03-13.05.2020, 08.07. - 02.09.2020 and 28.10-present) consistent with three consecutive periods of increasing mortality (12.04. - 09.05.2020, 29.07. - 21.09.2020 and 14.11-present). So it was a first wave in the spring of 2020, a second in the summer and a third in the fall-winter, so we expect a fourth wave next spring overlapping with the season of acute respiratory viruses. It means that the media warning that a second or third wave will come is not based on reality, they have already come and nothing special has happened. But the genetic variability of many viruses creates the risk of mutations in contagiousness, virulence and/or immunogenicity.

The global Covid-19 pandemic was not particularly serious, as some experts have questioned the declaration of the pandemic for medical reasons, and its continuation will be, as world statistics indicate, easier, even below the level of seasonal flu. We currently benefit from specific diagnostic tools (for viruses, antibodies and clinical interpretation), etiological, adjuvant and complementary treatment (even if we do not yet have specific antivirals) and vaccine prophylaxis (under authorization) which will be applied en masse in the near future. The optimistic variant is that the pandemic will end in the summer, but there are also various pessimists that sustain it will last until 2022 or that we will have to live with the virus. Which is actually the case with influenza viruses, but no one is scared of them now.

At the mentioned date, out of the total of 72,197,885 cases diagnosed with Covid-19 for the year 2020, representing a world prevalence of 0.92%, there are 1,613,401 deaths, so the incidence of mortality was of 2.29%. Compared to these, there are currently 27.71% of current cases (with a global prevalence of 0.26%) under treatment in December. Thus, the frequency of Covid-19 diseases increased, but severe cases (requiring ICU treatment) accounted for only 0.53% of the cases. It turns out that the severity of Covid-19 has now been reduced by more than four times.

After the date on which the presented data were counted, some major events in the evolution of the pandemic occurred: the urgent authorization of the new anti-Covid-19 vaccines and the identification of an N501Y mutation that could change the evolution of this disease, but in what sense it is not yet known.

The exaggeration of the danger presented by this virus can be explained by: the novelty of the disease and its causative agent, which could have evolved unpredictably; by the desire of the sanitary decision makers not to trivialize a possible potentially serious epidemic; the desire of policy makers to show their concern for the population (especially if it is an election year); the desire of global finance to reap benefits primarily through Big Pharma and related fields; the desire of the press to hunt "hot" subjects; the desire of politicians to mask the incipient world economic crisis and even the attempt to economically suffocate the "factory of the world" in order to block the change of the New World Order.

It is paradoxical that the most civilized countries and with the best medicine have suffered the most, both through the health crisis (sick, deaths, involvement of forces and health) and the subsequent socio-economic crisis. There is an explanation that these countries have the longest-lived population, so aged and with comorbidities, which are ranked in "risk groups". Nevertheless, it can be estimated that the best performing medicine, and therefore the most expensive, is not as effective in the field of public health. Compared to the health insurance system, the territorial rounding of health care allows better hygienic and epidemiological surveillance and an epidemiological investigation on the epidemiological chain: source-transmission-receiver, which has proven to be the most effective means of stopping the epidemic. However, the implementation of some measures would be in contradiction with the law, with the rights and freedoms of the individual and with the interests of the health insurance system.

Under these conditions, it would have been possible for any other viral, bacterial or other epidemic / pandemic to occur, with a single or multiple etiological agent, known or not yet known, with even more serious consequences. All this requires the timely preparation of appropriate, comprehensive but resilient Action Plans to cover any foreseeable situation in the future, including that of biological warfare, bioterrorism, biocrime or biological accident.

From the multitude of objective data published daily by the worldometer, interesting conclusions can be drawn for the evolution of the pandemic and public health, and each person interested in the real situation can ultimately reach his own conclusions.

The current pandemic has generated a complex health, social, economic crisis but also a "psychodemia" through direct and indirect effects. It is interesting that many doctors have let themselves get drawn into this collective fear, some have resigned shamefully, looking only at the particular cases in their field and at the multitude of information and misinformation in the environment subjectively and uncritically, rather criticizing interestedly or uninterestedly the realistic ones. American writer and journalist Mark Twain said, "If you don't read the newspaper, you're uninformed. If you read the newspaper, you're mis-informed." As recommended by some specialists (some of whom have lost their jobs, the freedom or their lives) we need to turn off the TV and analyze the statistical data provided by reliable scientific sources; we mainly chose WHO, Nature, Science and Worldometer. At the same time, the false news spread by conspiratorial negativists who do not see the virus, do not see the disease, do not see the epidemic, and do not see the reality and try to convince others of their aberrations must be rejected.

Regarding the diagnosis, treatment and prophylaxis, it can be considered that something that works now is more useful than something perfect in the future. It is important to respect the Hippocratic principles: „Primum non nocere" and „We treat the sick and not diseases". But as the military say, „You go to war with what you have, not with what you want."

At the same time, the year 2020 is one of the most prolific for medical science: the number of bio-medical scientific researches has reached a record hard to match, books have been written, articles about the various medical and non-medical aspects of Covid-19, an international collaboration took form with fast publications that are freely available online, just before the peer-review (which resulted in a record number of false researches that misled the medical world), a counter-clockwise competition for diagnostic reagents, drugs and specific vaccines, protective equipment, disinfectants, etc.

An English journalist and writer wrote, "I often thought again and again how unprepared the whole community had been for the calamity that had paled it, and to what extent the ensuing disaster was due to the fact that no public and personal measures and provisions had been taken in time. If everything had been done properly, the number of victims would have been much lower. I say all this as a warning and as a lesson for posterity." This warning was written by Daniel Defoe (1660-1731) in the book "A Journal of the Plague Year" published in 1722 [4] and is still valid today, and posibly also for the future...

The Covid-19 pandemic is a reality of current times, the year 2020 being portrayed as the "COVID year". The international scientific community has shown that, when needed, it can collaborate beyond classical barriers. The novel coronavirus was studied as a coronavirus by biologists, as a new disease by doctors, as the new reagents by biochemists, as the new drugs from the perspective of pharmacologists, and so forth. Thus, in record time, faster than in any other similar situation, we already have a lot of knowledge about them and we have an adequate diagnostic, therapeutic and prophylactic means, but not everything.

Statistics show that the virus is not very aggressive, the disease is not very serious but there are also serious, sometimes fatal complications to which appropriate measures must be applied, including ICU. The risk of serious illness and life-threatening complications is statistically reduced over time, the disease being less severe now than at the beginning, so it is on a downward slope.

It can be estimated that there are premises for the end of this pandemic in the near future and that if no other disruptive factors intervene, it will return to normal, or at least the ordinary, in terms of social and economic life.

Comments

After one year, the Covid-19 is present disease, and the pandemic is a reality of current times, the year 2020 being portrayed as the "COVID year". The medical literature on Covid-19 and the information released in the public space allow statistical analysis, the most complete and systematized data, representing the US point of view, are updated and published on a daily basis, including on the website www.worldometercoronarovirus.

The primary and secondary statistical interpretation of quantitative data is possible by dividing on quantitative intervals, percentage processing by absolute and relative values, by applying the significance test, etc. and obtaining quantitative and qualitative data that corroborate with medical logic according to the principles of "evidence-based medicine".

The graphical distribution by percentage statistical intervals of the countries did not create a normal bell-shaped Gaussian curve, but a steep downward curve, so most countries had few or no cases and the high prevalence was in a small number of countries. The global prevalence has dropped from 0.92% per year to 0.26% up to the present moment (13.12.2020), and 78 countries (more than a third of the total) currently have no serious cases in evolution.

From a statistical point of view, confirmed by the "situational experiment" presented, it can be estimated that in the conditions of strictly supervised medical and non-medical countermeasures Covid-19 is neither very contagious nor has a high mortality, even if there is a population in the risk group: the elderly, chronic diseases, etc. The entry of the statistically processed data from the Worldometer tables primarily processed on the grid's graph with the probability of significance "2 alpha" of 95%, shows that the general prevalence of fatal cases of/with Covid-19 compared to the total cases of Covid-19 throughout 2020 (A) is significantly higher than the prevalence of severe cases compared to the total current active cases of Covid-19 (B). There is a decrease in the severity of the disease in the pandemic's dynamic context, which could mean that: the virus has become less virulent (adapts to the host), the treatment is more effective (more effective therapeutic strategies are being identified), (specific and / or cross) collective immunity develops or all of these in different proportions.

The area of the extended western civilization (2 + 3 + 5 + 7) represents about 23%, and the area of the other civilizations about 77%, i.e. the absolute majority, and the area of restricted western, mainly Euro-Atlantic, has about 14% of the population and about 56% of cases. North America has a Covid-19 mortality rate among its population of almost 6 times higher than the global rate. The incidence of mortality in new Covid-19 patients in South America and Africa is high and low in Asia. The incidence of mortality in new Covid-19 patients is high in South America, Africa and Europe and Oceania no longer has mortality. The percentage prevalence in the population of Covid-19 active cases is high in North America and Europe (totalling 87% of active cases in a population of about 14%) and Asia, Africa and Oceania currently have a low prevalence.

With an average prevalence of active cases of 1/100,000 it can be considered that at this moment Covid-19 is a "rare disease" and drugs intended for the prophylaxis and treatment of this disease can be considered as "orphan drugs". North America and Europe have a relatively higher prevalence and Africa and Oceania currently have a very low prevalence. Asia and South America and Africa have a high incidence of serious cases and Europe and Oceania have a low incidence. By a reductio ad absurdum, in a more balanced distribution, December should have represented less than 10% of the total for 2020, but it is almost 3 times higher worldwide, so the epidemic is "accelerating" especially in Europe and North America. It is observed that the severity of the pandemic increases in Asia and South America and decreases in Europe and Oceania.

CONCLUSION

Statistics show that the virus is not very aggressive, the disease is not very serious but there are also serious, sometimes fatal complications to which appropriate measures must be applied, including ICU. The risk of serious illness and life-threatening complications is statistically reduced over time, the disease being less severe now than at the beginning, so it is on a downward slope. With prophylaxis, vaccination and treatment we hope the finish of present epidemia.

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VARIA

The differences of Matrix Metalloproteinases (MMP-9 and MMP-13) in chronic inflammatory rheumatic diseases

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Abstract: *Objectives: Primary Sjogren's Syndrome (pSS), Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA) are systemic, autoimmune, and chronic inflammatory rheumatic diseases that collagenases and gelatinases are effective and valuable in the pathophysiology of these diseases. The aim of this study is to compare the levels of MMP-9 (gelatinase-B) and MMP-13 (collagenase-3) in pSS to PsA, RA and healthy controls.*

Methods: Serum samples of patients with a diagnosis of Primer Sjögren's Syndrome (n=20), rheumatoid arthritis (n=20), psoriatic arthritis (n=19) and healthy control group (n=15) were analyzed by ELISA method for MMP-13 and MMP-9 levels.

Results: There was no significant difference between the groups in terms of MMP-9 and MMP-13 levels. In the pSS group, MMP-9 was negatively correlated with MPP-13 (r:-0.568, p:0.09) but these parameters were not correlated with activity biomarkers and scores of disease activity index.

In patients with pSS, the MMP-13 levels tended to be higher than the PsA patients and lower than RA patients but there was no significant difference between the 3 groups in terms of MMP-9 and MMP-13 levels.

Conclusion: MMPs, in distinguishing health and disease conditions have various roles and they drive important biological mechanisms. Taking into account as a biomarker different body fluids need to be compared. In our study, the differences of serum samples between the pSS group and the positive control groups were not statistically significant.

Keywords: *matrix metalloproteinases, primary Sjogren's syndrome (pSS), psoriatic arthritis (PsA), rheumatoid arthritis (RA)*

INTRODUCTION

Matrix metalloproteinases (MMPs), in general, are primary matrix-disrupting proteases capable of degrading all protein components of the extracellular matrix, they are a family of zinc-and calcium-dependent endopeptidases [1]. In addition to playing a role in extracellular matrix physiology, MMPs activate or degrade a variety of nonmatrix substrates including chemokines, cytokines, growth factors, and junctional proteins [2,3]. MMPs can be classified according to their preferential substrate, sequence similarity and domain organization as gelatinases (MMP-2, -9), collagenases (MMP-1, -8, -13, -18), stromelysins (MMP-3, -10), matrilysins (MMP-7, -26), membrane type MMPs (MMP-14, -15, -16, -17, -24, -25), and others (MMP-12, -19, -20, -21, -23, -27, -28) [4]. Activity of MMPs is precisely regulated by the balance between gene expression, zymogen activation and enzyme inhibition through their endogenous inhibitors, α2-macroglobulin in the plasma or tissue inhibitors of metalloproteinase in the tissue [5]. The imbalance between this system plays a critical role in various diseases, and the re-creation of a balanced MMP activity profile provides an opportunity for the development of biomarkers and therapeutics [1].

MMP-9, also known as Gelatinase-B, is involved in fundamental biological processes such as development, angiogenesis, apoptosis, inflammation and cancer [6]. Through cleaving denatured collagen (gelatins) and type 4 collagen, MMP-9 helps lymphocytes, and other leukocytes

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to enter and leave the blood and lymph circulations. Also, MMP-9 cleaves myelin compounds such as myelin basic protein (MBP) and type 2 gelatins, leading to remnant epitopes that can generate autoimmunity [7].

MMP-13, also called Collagenase 3, specifically catalyzes the hydrolysis of type II collagen, the main structural component of the cartilage matrix. MMP-13 plays a vital role in osteoarthritis and there are studies investigating selective MMP-13 inhibitors as a potential strategy for the therapy of osteoarthritis [8].

Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands, predominantly the salivary and lacrimal glands [9].

Collagenases and gelatinases are effective and valuable in the pathophysiology of SS, Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA) that they all systemic, autoimmune, and chronic inflammatory rheumatic diseases.

To the best of our knowledge, there is not a study which evaluated the clinical value of the serum MMP-9 ve MMP-13 levels in pSS. The aim of this study is to compare the levels of MMP-9 (gelatinase-B) and MMP-13 (collagenase-3) in pSS comparing to PsA, RA and healthy controls and to evaluate the correlation with disease activity.

MATERIALS AND METHODS

A total of 20 consecutive pSS patients who met the 2012 American College of Rheumatology classification criteria for Sjögren's syndrome were enrolled in this cross-sectional study [10]. 20 patients with RA who fulfilled the 2010 ACR/EULAR criteria and 19 patients with PsA who fulfilled the Classification criteria for psoriatic arthritis were included in this study as positive control groups [11,12]. The control group was formed of 15 healthy individuals. Patients and controls with one of the following combined diseases/ situations were excluded 1. additional rheumatic disease, such as autoimmune hepatitis, primary biliary cholangitis, Hashimoto thyroiditis, 2. comorbid chronic diseases, such as hypertension, dyslipidemia, hypothyroidism diabetes mellitus, chronic kidney disease, 4. obesity (body mass index >30), 5. malignancies, 6. history of cardiovascular or cerebrovascular disease, 7. active smoker, 8. age < 18 years, 9. inability to give written informed consent. The demographic, clinical and laboratory data of the patients were recorded.

The analysis of MMP-9 and MMP-13 in serum was performed by enzyme-linked-immunosorbent assay (ELISA) (Human MMP-9 ELISA assay kit, Sigma-Aldrich, MO-USA, Lot Number 0920D0173 and Human MMP-13 ELISA assay Kit, Sigma-Aldrich, MO-USA, Lot Number 0302C0175, respectively).

Disease activity of SS was assessed with EULAR Sjogren's Syndrome Patient Reported Index (ESSPRI) and EULAR Sjogren's Syndrome Disease Activity Index (ESSDAI) at the time of blood sampling. The ESSPRI is a self-evaluation index for measuring symptoms including fatigue, pain, dry- ness. Each symptom was measured with a single 0 (no symptoms) to 10 (severe symptoms) numerical scale and the final ESSPRI score is calculated by averaging these domains with a maximum severity score of 10. Scores of < 5 indicate low disease activity and scores of ≥ 5 indicate high dis- ease activity [13,14]. The ESSDAI is a physician-based assessment of the systemic features and severity of the disease and includes 12 domains (lymphadenopathy, constitutional, glandular, cutaneous, articular, muscular, respiratory, renal, peripheral nervous system, central nervous system, hematological, biological). ESSDAI ranges from 0 to 123. ESSDAI < 5 is defined as low disease activity, 5 ≤ ESSDAI ≤ 13 is defined as moderate disease activity and ESSDAI ≥ 14 is defined as high disease activity [14,15].

The study protocol was approved by the Committee on Human Research Ethics of Turkish Ministry of Health Zekai Tahir Burak Women's Health, Training and Research Hospital (decision number: 60/2017). A well written informed consent was obtained from all participants according to the principles of the Helsinki Declaration.

All data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) 16.0 program for Windows. The variables were investigated using visual and analytical methods to determine whether they were normally distributed. Normally distributed continuous values were expressed as mean ± standard deviation (SD) and categorical variables as number and percentage. Pearson's correlation coefficient and Spearman's correlation coefficient were used to evaluate the linear relationship between the predictive variables. A value of $p<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics and laboratory results of patients and healthy controls were summarized in Table 1. All pSS patients were on with only hydroxychloroquine 200mg/day treatment. Median ESSDAI and ESSPRI were 5.00 (min:0.00, max:26.0) and 4.00 (min:0.00, max:8.00), respectively. According to the ESSDAI, 12 (60%) patients had low disease activity and 8 (40%) had high disease activity. According to the ESSPRI, 9 (45%) patients had low disease activity, 9 (45%) patients moderate disease activity and 2 (10%) patients high disease activity.

Table 1: Clinical characteristics and laboratory results of chronic inflammatory rheumatic diseases groups

	Sjogren Syndrome (n=20)	**Rheumatoid arthritis** (n=20)	**Psoriatic arthritis** (n=19)	**Healty controls** (n=15)	**p value**
Age (years)	48.45 ± 10.59	56.00 ± 10.98	38.74 ± 11.25	47.87 ± 5.48	
Female/male	18/2	18/2	11/7		
ESR (mm/h)	22.39 ± 19.57	24.42 ± 15.37	20.47 ± 11.96		
CRP (mg/dL)	5.63 ± 4.34	12.70 ± 14.32	10.62 ± 7.90		
MMP13 (pg/mL)	12.99 ± 18.03	14.12 ± 16.45	9.78 ± 10.45	12.30 ± 14,38	0.143
MMP9 (pg/mL),	7303.60 ± 538.36	7433.10 ± 560.85	7639.68 ± 764.30	7890.53 ±1046.44	0.107

MMP-9 parametric, MMP-13 non parametric, ESR (Erythrocyte Sedimentation Rate), C-Reactive Protein (CRP) non-parametric

In patients with pSS, the MMP-13 levels tended to be higher than the PsA patients and lower than RA patients but there was no significant difference between the 3 groups in terms of MMP-9 and MMP-13 levels.

In pSS group, MMP-9 was negatively correlated with MPP-13 (r:-0.568, p:0.09) but these parameters were not correlated with activity biomarkers and scores of disease activity index (Table 2).

Table 2: Represents the correlation between MMP-9, MMP-13, disease activity markers and activity scores in patients with PSS

		ESR	CRP	IgA	IgG	IgM	C3	C4	B2M	ESSDAI	ESSPRI
MMP-9	r	0.079	-0.095	0.050	-0.140	0.311	0.074	0.188	-0.230	-0.005	0.113
	p	0.740	0.708	0.835	0.556	0.182	0.755	0.427	0.358	0.985	0.636
MMP-13	r	-0.183	0.133	0.102	-0.030	-0.173	0.111	-0.184	0.091	-0.063	-0.314
	p	0.440	0.598	0.668	0.900	0.466	0.640	0.437	0.720	0.793	0.176

ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein (CRP), IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M, B2M: beta-2-microglobulin, ESSDAI: EULAR Sjögren's syndrome (SS) disease activity index , ESSPRI: EULAR Sjögren's syndrome patients reported index

DISCUSSION AND CONCLUSIONS

In this study, although patients with pSS tended to have lower MMP-9 levels than the PsA and RA patients, these differences were not statistically significantly different. Also, in pSS patients, MMP-9 was not correlated with disease activity.

Salivary gland acinar cells express both MMPs and TIMPs. Previous studies suggest that increased MMP-9 activity and expression enhances the destruction of the basement membrane, which leads to the destruction of the acinar structures in SS salivary glands [16,17]. In the study by Perez et al, MMP-9 was detected by immunolocalization only in acinar and ductal cells and was homogeneously distributed throughout the patient's glands. MMP-9 expression and MMP-9 catalytic activity were increased in tissue samples from SS patients in a manner that correlated with the severity of the disease [18]. In a study by Hulkkonen et al., It was shown that serum levels of MMP-9 were higher in patients with genetically definitively diagnosed pSS than patients with possible pSS [19]. In our study we did not observe any correlation of serum MMP-9 levels to diseaes activity in pSS patients.

As it is known, TIMP expression is an important modulator of effects attributed to MMPs. In another study by Pérez P et al reported the MMP-9/TIMP-1 ratio in labial salivary glands of patients with SS correlated well with the dramatic changes observed in morphologic integrity of acini, as revealed mainly by the lack of nuclear polarity. Acinar changes were more evident when ratio MMP-9/TIMP-1 was higher [20].

Also, MMP-9 released from stressed ocular surface epithelium. Aragona et al, demonstrated that conjunctival expression of MMP-9 was significantly higher in patients with SS than healthy controls and subjects with Meibomian gland dysfunction [21]. Yang et al reported that corneal and conjunctival staining scores significantly correlated with the expression of MMP-9 in SS patients with dry eye disease [22]. In the study by Kuo et al, the tear MMP-9 level was positively correlated with bulbar redness, including mean redness score (with significance), and nasal bulbar redness (with a marginal trend to significance) in SS patients with dry eye disease [23].

As a result of saliva and tear studies in pSS patients, MMP-9 levels have been shown to be increased [24–26]. Basically to measure the MMP-9 and 13 serum levels could be one of our limitations and also we did not measured the TIMP levels of all groups.

However in different studies the pleiotropic effect of MMPs on mammals was studied and also mentioned about the need for more studies especially on the effects of MMP-9 and 13 in RA [27]. A study of active RA patients found high levels of MMP-9 in the serums than healthy group [28]. Giannelli et al reported that, MMP-9 levels in patients with RA and pSA were found to be higher in synovial fluid than in serum [29]. In another study with RA, synovial fluid MMP-13 levels were higher than in the healthy group [30]. Ion our study we observed that pSS patients had similar MMP-13 levels with PsA, RA and healthy controls. Also, we showed that MMP-13 was not correlated with disease activity in pSS.

Studies on MMP-9 and 13 levels were mostly performed in saliva, tears, and synovial fluid, and results were higher than healthy groups. Similarly, synovial fluid levels were found to be high in serum comparisons with synovial fluid [24,29–31]. The results obtained by these comparisons focus on the localized effects of MMPs. Serum levels alone cannot provide sufficient data.

Several limitations of the present study should warrant attention. The small sample size is major limitation of our study. Larger sample size may be needed to clarify the value of MMP-9 and MMP-13 as activity biomarkers in systemic autoimmune diseases, which are heterogeneous diseases. Another limitation of our study is cross-sectional design. Our results do not provide adequate results to explore potential causality and not reflect changes in these variables with disease duration, disease activity and therapy response.

Expected studies can be focus on easy sampling materials and non-invasive methods to study the metabolic pathways of MMPs. There is a need of well-designed, prospective, controlled studies with larger sample size to evaluate clinical value of serum MMP-9 and MMP-13 levels in pSS and other inflammatory diseases.

Declaration of competing interest

The authors declare that they have no conflict of interest relevant to the content of this manuscript.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ORIGINAL ARTICLES

The effect of Dexmedetomidine on sedation of patients undergoing open-heart surgery in a military hospital

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Abstract: *Background: Dexmedetomidine facilitates patient communication with the ICU nurses, and is a valuable drug for use in patients undergoing mechanical ventilation due to a reduction in the duration of mechanical ventilation and prevalence of delirium.*

Objectives: In this study, investigators aimed to compare the effect of Dexmedetomidine with placebo on sedation in patients undergoing open-heart surgery.

Methods: In this double-blind clinical trial, 69 patients with open-heart surgery who underwent a cardiopulmonary bypass pump, were randomly assigned into two groups of Dexmedetomidine (group D) and placebo (group P). At the end of the operation, patients in groups D and P respectively received 1 μ / kg infusion of Dexmedetomidine and normal saline in 10 minutes. In case of a lack of proper sedation, midazolam with the doses of 1 mg titration was used up to reaching the goal of sedation. Also, for pain relief, morphine titrated at a dose of 1 mg was Injected, and dosage was recorded in both groups. In each shift, patients were examined for delusions by the nurse involved in their care, using the "Confusion Assessment Method for the ICU (CAM-ICU)". For patients with delirium, 1 to 5 mg of Haloperidol was used. Also, the length of stay in the ICU was recorded.

Results: There was no significant difference between the two groups in systolic blood pressure, diastolic blood pressure, and heart rate at different stages of treatment ($p<0.05$). comparison of the two groups in terms of the length of stay in ICU, duration of mechanical ventilation, staff satisfaction, and occurrence of Delirium after surgery indicated that there were significant differences between the two groups ($p<0.05$). There was a significant difference between the two groups in the dose of morphine and midazolam ($p<0.05$).

Conclusion: This study showed that sedation with Dexmedetomidine significantly reduced the incidence of postoperative delirium, the length of stay in ICU, the duration of mechanical ventilation, the satisfaction rate of ICU staff, and the dose of analgesic and sedative medication. As there were no reported hemodynamic complications as well, it seems that it can be indicated as a safe and appropriate drug for sedation of patients in the ICU.

Keywords: *Dexmedetomidine, Intensive care unit, Open heart surgery, sedation*

BACKGROUND

Propofol and Dexmedetomidine are preferred drugs for sedation and short-term sleep in ICU [1]. The use of Dexmedetomidine in ICU has a similar cost and prognosis to Propofol [2]. Dexmedetomidine is a potent and selective agonist of alpha-2 receptors, which has sedative, anxiolytic, and analgesic effects. The effects of sleepy sleep are similar to normal sleep, so patients taking Dexmedetomidine easily

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wake up and cooperate [3]. The rate of respiratory depression is at least [3, 4]; and can be safely used in patients who are not mechanically ventilated [4]. In 2003, the use of Dexmedetomidine was approved by the FDA [3]. Dexmedetomidine is preferable to routine drugs for up to 24 hours for mild to moderate sedation [5] and is easy to use in patients undergoing mechanical ventilation in ICU. It reduces the duration of mechanical ventilation compared to midazolam [6, 7], and the time of extubation compared to midazolam and propofol [5, 6]. The use of Dexmedetomidine alone has led to less anxiety after extubation [7]. Among sedative and analgesic drugs, Dexmedetomidine is a unique drug due to brain protection. Its properties make it possible for ICU patients to be used and reduce the complications of ICU admission, such as cognitive impairment [8]. Sedation by Dexmedetomidine reduces the risk of developing delirium [4, 6, 7, 9], and can even treat delirium [4]. Compared to Propofol, Dexmedetomidine decreases the prevalence and duration of delirium after cardiac surgery in the elderly [10].

Dexmedetomidine facilitates patient communication with ICU nurses [6]. It is a valuable drug for use in patients undergoing mechanical ventilation due to a reduction in the duration of mechanical ventilation, length of stay in the ICU, and prevalence of delirium [11]. However, it is reported that using guidelines to limit the use of Dexmedetomidine is cost-effective [12]. The previous study recommended limiting the dose of Dexmedetomidine to 0.7 µg/kg/hour, the use of bolus fentanyl doses before the onset of infusion of Dexmedetomidine, and the use of certain criteria for non-intubated patients [13].

In this study, the authors aimed to compare the effect of Dexmedetomidine with placebo on sedation in patients undergoing open-heart surgery.

MATERIALS AND METHODS

In this double-blind clinical trial study, 69 patients with open-heart surgery who underwent a cardiopulmonary bypass pump, who were referred to Baqiyatallah Hospital, were randomly assigned into two groups of Dexmedetomidine (group D) and placebo (group P). Emergency patients, patients with liver failure, renal failure, grade 2 or 3 heart block, systolic blood pressure below 90 mmHg, history of neurological and psychiatric illnesses, use of psychiatric drugs, opiate addicts, redo surgery, and patients requiring re-surgery Were excluded.

To induce anesthesia, investigators used fentanyl at a dose of 500-700 µg and diazepam at a dose of 10 mg. To facilitate endotracheal intubation, cisatracurium was used at a dose of 0.15 mg/kg. To maintain anesthesia, infusion of fentanyl, midazolam, and cisatracurium was used and propofol infusion was considered if needed. With the help of TNG and inotropic drugs, systolic blood pressure was maintained in the range of 95 to 115 mmHg. During CPB, blood pressure was maintained at 60 to 80 mmHg and hematocrit between 20 to 22%. When patients found appropriate conditions for acid and base, electrolytes and hematocrit (22% or more), they were separated from the pump.

In group D, at the end of the operation in the operating room, when the patient was completely monitored and mechanically ventilated, patients received 1 µ/kg infusion of Dexmedetomidine in 10 minutes and were transferred to ICU. In ICU, the dose of 0.2 µg/kg/h was used for the initiation, and depending on the patient's need, to achieve Richmond agitation-sedation scale (which was scored from −5 [unresponsive] to +4 [combative], −2 to +1 (lightly sedated to restless)), the dosage was increased up to 0.7 µg/kg/h, Increased. After reaching the extubation condition, the patient was extubated and the dose of Dexmedetomidine was continued to achieve RASS= 0 (calm and alert).

In group P, after the completion of the surgery, a placebo (normal saline) was infused over 10 minutes and the patients were transferred to the ICU. In ICU, placebo infusion continued and the purpose of sedation was reaching to mechanical ventilation, RASS= -2 to + 1, and after extubation, RASS = 0.

For the lack of proper sedation, midazolam with a dose of 1 mg titration was used up to reaching the goal of sedation, and the dosage was recorded. Also, for pain relief, morphine titrated at a dose of 1 mg was Injected, and dosage was recorded in both groups.

The patient was extubated under acceptable conditions in terms of hemodynamics, acid and base, and alertness. The mechanical ventilation period was indicated from the end of the surgery to the extubation of the patient. During the period of mechanical ventilation, continuous monitoring of the vital signs was considered for both groups; and in case of hypotension (systolic blood pressure less than 90 mmHg or 30% reduction in blood pressure relative to the initial pressure) and also in case of bradycardia (heart rate Under 40/min), infusion of the drugs was discontinued, and in the absence of improvement, they were treated with ephedrine at a dose of 10 mg and atropine at a dose of 0.1 mg/kg, respectively.

In each shift, patients were examined for delusions by the nurse involved in their care, using the "Confusion Assessment Method for the ICU (CAM-ICU)". For patients with delirium, 1 to 5 mg of Haloperidol was used. From the arrival of the patient to the ICU until the discharge from the ICU, it was recorded as the length of stay. At the time of the patient's discharge from the ICU, the satisfaction rate of three of the personnel involved in patient care was assessed using the Visual Analogue Scale (VAS) scoring method. Patients were monitored for ECG, and any new-onset arrhythmia was recorded. Data were gathered by a form including age, sex, type of surgery, CPB time, Cross Clamp time, systolic blood pressure, diastolic blood pressure, and heart rate. Investigators examined the effect of Dexmedetomidine on patients' hemodynamics, delirium, the length of stay in ICU, the duration of mechanical ventilation, and the dose of sedative medications. P-value < 0.05 was considered statistically significant.

Ethical Considerations: This research project was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences (number: IR.BMSU.REC.1398.090 date: 2019-06-25) and it was registered in the Iranian registry of clinical trials (IRCT20161022030421N4). Consent letters were obtained from participants.

RESULTS

In this study, 69 patients in two groups were evaluated consisting of 34 patients in the group D and 35 patients in group P. There was no significant difference between the two groups based on age, sex, and type of surgery (Table 1) (p=0.514, p=0.555, p=0.143 respectively).

Table 1: Demographic and type of surgery base on groups

		Case (N=34)		Control (N=35)		P-Value
		Mean±SD	Min-Max	Mean±SD	Min-Max	
Age		59.8±12.04	21-78	61.6±11.15	38-79	0.514[1]
		N (%)		N (%)		
Gender	Female	15 (44.1)		13 (37.1)		0.555[2]
	Male	19 (55.9)		22 (62.9)		
Type of Surgery	CABG	23 (67.6)		29 (82.9)		0.143[2]
	Valvular	11 (32.4)		6 (17.1)		

1: Independent Sample T-Test; 2: Pearson Chi-Square Test

According to preoperative factors, there was no significant difference between the two groups (Table 2) (p<0.05). CPB time was 59.7±18.87 minutes in group D and 61.6±18.69 minutes in group P and no significant difference was noted (p=610).

Table 2: Comparison of the two groups based on pre-operative factors

		Case (N=34)		Control (N=35)		P-Value
		Mean±SD	Min-Max	Mean±SD	Min-Max	
Clinical factors	Ejection fraction	46.6±8.5	30-60	47.3±8.26	20-55	0.797[1]
		N (%)		N (%)		
	Hypertension	26 (76.5)		25 (71.4)		0.633[3]
	Diabetes mellitus	17 (50)		22 (62.9)		0.281[3]
	Kidney disease	5 (14.7)		3 (8.6)		0.477[4]
	Lung disease	5 (14.7)		9 (25.7)		0.256[3]
	Smoker and Opioid addiction	9 (47.4)		10 (52.6)		0.845[3]

1: Mann-Whitney Test; 2: Independent Sample T-Test; 3: Pearson Chi-Square Test; 4: Fisher Exact Test

Also, Cross Clamp time was 46.8±17.15 minutes in group D and 50.4±19.6 minutes in group P, and no significant difference was shown between groups (p=501). There was no significant difference between the two groups in systolic blood pressure, diastolic blood pressure, and heart rate at different stages of treatment (Tables 3-5) (p<0.05).

Table 3: Comparison of the two groups based on systolic blood pressure (mmHg) in different stages

	Case (N=34)		Control (N=35)		P-Value
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	
Primary (Before drug)	110.3±18.93	80-161	113.9±21.07	74-175	0.47[1]
During bolus dose infusion	116.2±14.49	80-143	117.8±15.65	92-150	0.669[1]
During mechanical ventilation	118.3±12.9	99-145	116.5±14.41	91-149	0.577[1]
After mechanical ventilation in ICU	118.2±12.01	94-148	120.3±12.6	100-146	0.485[1]

1: Independent Sample T-Test

Table 4: Comparison of the two groups based on diastolic blood pressure (mmHg) in different stages

	Case (N=34)		Control (N=35)		P-Value
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	
Primary (Before drug)	65.8±13.65	45-99	66.2±15.37	33-101	0.893[1]
During bolus dose infusion	68.5±10.53	51-95	67.8±11.19	50-100	0.789[1]
During mechanical ventilation	67.7±8.78	51-90	66.9±10.23	51-99	0.529[1]
After mechanical ventilation in ICU	66±7.58	53-87	68.5±9.29	51-96	0.198[2]

1: Independent Sample T-Test; 2: Mann-Whitney Test

Table 5: Comparison of the two groups based on heart rate (N) in different stages

	Case (N=34)		Control (N=35)		P-Value
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	
Primary (Before drug)	83.8±14.43	60-110	88.2±13.83	59-113	0.206[1]
During bolus dose infusion	82.4±14.89	55-108	89.7±17.78	53-138	0.073[1]
During mechanical ventilation	83±16.2	55-118	90.3±15.54	51-126	0.067[1]
After mechanical ventilation in ICU	83±14.04	53-115	90.7±15.49	50-122	0.037[1]

1: Independent Sample T-Test

In terms of the prevalence of delirium after surgery; in group D, 5 patients (14.7%) and group P, 16 patients (45.7%) were delirious and the comparison between the two groups showed a significant difference (p=005).

The duration of mechanical ventilation in group D was 8.2±4.32 hours, and in the group, P was 12.7±3.94 hours. There was a significant difference between groups (p<0.001). Comparison of the two groups regarding the length of stay in ICU showed that in group D, length of stay in the ICU was 3.2±0.48 hours and in the group, P was 3.8±0.65 hours, and the difference was significant (p<0.001).

The satisfaction rate of three nurses involved in patient care showed that the VAS score was 9.2±1.2 in group D and 6.9±1.77 in group P and a significant difference was noted (p<0.001).

Regarding the opioid dose in the two groups: The mean dose of morphine in the D group was 1.18±2.7 mg (Min-Max 0-13) and in the P group 8.5±4.12 mg (Min-Max 0-15), with a significant difference between the two groups (p=0.001). Comparison of the dose of the sedative drug in the two groups: The mean dose of midazolam in the D group was 1.5±2.75 mg (Min-Max 0-13) and in the P group 3.7±3.88 mg

(Min-Max 0-13), the difference between the two groups being significant (p=0.004). New-onset atrial fibrillation occurred in one patient in group D, and four patients in group P.

DISCUSSIONS

In this study, investigators evaluated patients undergoing open-heart surgery and transferred to intensive care units with mechanical ventilation, in two groups of Dexmedetomidine and placebo. There was no significant difference in systolic and diastolic blood pressure and heart rate between the two groups at different stages. Mechanical ventilation time and length of stay in ICU in the Dexmedetomidine group were significantly reduced. The prevalence of delirium in group D was also significantly decreased. The satisfaction of ICU nurses was significantly higher in group D. Patients in group D required significantly lower doses of morphine and midazolam. New-onset atrial fibrillation occurred in one patient in group D and four patients in group P.

Fish and colleagues implemented an updated sedation and analgesia guideline, which was provided by the community of critical care medicines (SCCM) on their patients. They compared the prognosis of the patients, one to five years after the implementation of the protocol, with one year before proceeding with the protocol. Their protocols included 1 - Using a bolus dose of analgesic and sedative medicine before starting the continuous infusion; 2 - Using concomitant sedatives and analgesics; 3 - Using Propofol and Dexmedetomidine instead of midazolam, when needing the continuous infusion of sedative, and 4 - measuring pain in patients in the intensive care unit. They concluded that with this guideline, the duration of mechanical ventilation, the length of stay in the ICU, and the duration of admission to the hospital were decreased, without any increase in mortality. It was also economically feasible [14].

Diaz and colleagues at Northwest Hospital assessed patients taking Dexmedetomidine. The evaluation data included the primary diagnosis of the disease, the indication of the use of Dexmedetomidine, the duration of treatment, the number of consumed needs, the infusion rate, the recorded CAM-ICU score, the amount of Propofol, opiate and the benzodiazepine supplement, the length of stay in the ICU and the duration of staying in the hospital. They appropriately prescribed Dexmedetomidine for all patients admitted to ICU and had CAM-ICU and RASS in over 92% of patients. Their goal was to achieve light sedation; the dose was not higher than 1.2 µg/kg/hour, and the inappropriate use of the drug was longer than 48 hours when the Propofol was used simultaneously [15].

Furthermore, Elzohry and colleagues used Dexmedetomidine alone to induce anesthesia in the endoscopy of the gastro-intestinal tract with bolus dosage of one microgram per kilogram and then 0.8 micrograms per kilogram per hour, concluding that, this drug can provide adequate analgesia and sedation, with rapid onset and rapid recovery in these patients, and the occurrence of complications such as bradycardia and hypotension was not serious and could be controlled [16].

Previous studies [1, 14, 15] were the confidence point for the current study to use this drug with a dose of 1 µg/kg for patients transferred to the ICU, and the results of the study showed that using Dexmedetomidine at this dose was not associated with hemodynamic disorders and had no adverse effects.

Morelli and colleagues in a cross-over study in patients with septic shock treated patients with Propofol, switched Propofol to Dexmedetomidine for 4 hours and re-set Propofel for 8 hours, and evaluated the degree of sedation and the dose of Norepinephrine at the end of these hours. They concluded that changing the drug from Propofol to Dexmedetomidine would reduce the dose of norepinephrine in patients with septic shock [17].

The same with the current study, Morelli, and colleagues addressed anxiety in using this drug. However, when it was used in patients with septic shock without complications, it seems that it can be used with less concern in ICU patients.

Guo and his colleagues assessed the patients undergoing mechanical ventilation randomly in two groups. In the control group, the sedation score was, 3 to -4 (RASS, 3 to -4), and in the intervention group, the sedation score was, 0 to -1 (RASS, 0 to -1) (that is, the condition that the patient was awake, be comfortable and collaborative). They concluded that in the intervention group, the duration of mechanical ventilation and the length of stay in the ICU was shorter, the prevalence of pneumonia due to mechanical ventilation, and delirium was lower, and the dose of sedative medication decreased [18].

In the current study, investigators attempted to put the patient's sedation around, -2 to +1 RASS and did not have any complications at this level. In a study on 498 non-surgical and surgical patients admitted to the ICU, Schrader and colleagues examined two Propofol and Dexmedetomidine drugs for hemodynamic disorders. For the first time, hemodynamic instability and frequency of hemodynamic instability were the benchmarks for comparing two drugs. They concluded that hemodynamic instability did not differ significantly between the two groups of Propofol and Dexmedetomidine in terms of duration and frequency [19].

This study, consistent with all of the studies mentioned, demonstrated the safety and the appropriateness of the use of Dexmedetomidine for the sedation of patients in the intensive care unit. In confirming Baylon's study, Dexmedetomidine should probably be a counterpart to sedative medications, especially in patients admitted to ICU [20].

In a retrospective study, Brianna Thompson and colleagues compared the effects of Propofol and Dexmedetomidine on the incidence of atrial fibrillation after cardiac surgery. They noted no significant difference in the prevalence of atrial fibrillation between the two groups because of the significant difference in prophylactic use of amiodarone [21]. In the current study, new atrial fibrillation occurred in one patient in the sample group and four patients in the control group in the ICU. Therefore, more research is needed to assess the effect of Dexmedetomidine in the prevention of new-onset arrhythmia.

CONCLUSION

This study showed that sedation with Dexmedetomidine significantly reduced the incidence of postoperative delirium, length of stay in ICU, duration of mechanical ventilation, the satisfaction rate of ICU staff, and the dose of sedative medication. As there were no reported hemodynamic complications as well, it seems that it can be indicated as a safe and appropriate drug for sedation of patients in the ICU.

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VARIA

The effect of implementing collaborative care model on diet compliance in hemodialysis patients

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Abstract: *Non-compliance to diet and increased fluid intake can lead to unpleasant side effects and premature mortality in hemodialysis patients. Intervention strategies in educational and cognitive-behavioral domains can be used to improve diet compliance and restrict fluid intake in hemodialysis patients. The present study aimed to implement a participatory care model on diet compliance in patients undergoing hemodialysis referred to the Urmia Hemodialysis Center of Urmia in 1397.*

The present study is an experimental-randomized study that was performed on 60 patients undergoing diet without hemodialysis referring to Urmia Hemodialysis Center of Urmia. From 175 patients undergoing hemodialysis based on laboratory parameters of non-compliance patients and 60 patients were selected based on simple randomization and were randomly divided into intervention and control groups. After intervention steps of participatory care, diet compliance of the two groups was compared using the results of the comparable laboratory parameters. Analysis of the results of the present study showed that the collaborative care model significantly improved the results of laboratory parameters related to diet compliance in the intervention group compared to the results of the laboratory parameters before the intervention and the control group (p <0.05). Based on the results of the present study, it seems that participatory care intervention can be useful in improving diet compliance and fluid restriction in hemodialysis patients. The aim is to improve the quality of life of hemodialysis patients.

Keywords: *chronic kidney failure, collaborative care model, diet compliance, hemodialysis*

INTRODUCTION

Chronic renal failure is a progressive and irreversible disorder that causes the formation and increase of urea and other toxins in the blood [1]. In other words, chronic renal failure is defined as the progressive and irreversible loss of renal function, often leading to end-stage renal disease (ESRD) [2]. Hemodialysis is the main end-stage treatment for kidney disease (ESRD). Researchers have not considered the growing number of patients with ESRD as a growing problem in Iran and have estimated it to be a worldwide problem. Unfortunately, in Iran, due to a lack of control over diseases such as diabetes and blood pressure, there has not been significant success in preventing chronic kidney disease [3]. This failure to control the underlying diseases has resulted in an increase of 12% per year in the number of patients requiring hemodialysis nationwide (compared to the 6%

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average increase in hemodialysis patients worldwide) and thus the rate of renal disease growth. It is much higher than the world average [4-6]. During the hemodialysis process, extra fluids and wastes are extracted from the blood of patients and this improves the patient's life and prolongs their life, but without proper diet, fluid restriction, and appropriate drug therapy, hemodialysis is not effective [7]. Nutrition has a major impact on the treatment and fate of patients undergoing hemodialysis, and diet compliance is associated with mortality in this disease, such that compliance to a low-potassium diet leads to muscle weakness, arrhythmia, and stasis. Cardiac and death and elevated serum phosphate levels lead to hyperparathyroidism and coronary artery disease, as well as fluid compliance in these patients leading to weight gain between two dialysis sessions and complications such as muscle cramps, shortness of breath, Vertigo, anxiety, pulmonary edema, heart failure, and severe hypertension and threaten the patient's lifestyle [8, 9]. Low levels of blood albumin decrease as 1–91 g of the amino acid are lost in each hemodialysis session, increasing the need for hemodialysis patients to have more protein than normal. Decreased blood albumin levels are strongly correlated with hospitalization and mortality rates and are a determinant of mortality in these patients [10]. Therefore, due to the effect of renal failure on different parts of the body, proper nutrition, prevention or correction of malnutrition and dietary restriction are essential, so patients need a special diet to continue living. It includes restrictions on the consumption of food and liquids [11]. Patient compliance to diet is influenced by factors such as personal beliefs, cultural and social characteristics. Most studies on hemodialysis patients do not follow the proper diet regimen [12]. Although patient-centered dietary supplementation is complementary to hemodialysis and is recommended for the effective control of signs and symptoms of uremic syndrome along with the prevention of long-term complications, many patients do not follow the nutritional and fluid restriction plan. This will lead patients to dialysis inadequacy; therefore, to improve dialysis adequacy, educate, justify, and modify patients' attitudes about diet and the need to monitor the complexity of human behaviors in health decision-making and health decisions. Moreover, behavioral health promotion patterns the benefits of dialysis and timely referral are important [13]. The collaborative care model is one of the localized teaching methods that has been used in recent years in the Iranian nursing field. The model of participatory care is the use of participation in the care process. The purpose of this model is to establish and maintain an effective, balanced, and continuous relationship between the members and the therapeutic team and the patient, to increase collaboration, motivation, and collaboration, and to take responsibility for the treatment team in the treatment and care process [14]. Work-based on a nursing model helps to evaluate patients' status, establish meaningful useful communication between patients and nurses, set goals of care, improve quality of care, guide, and clarify care [15]. Therefore, the concept of "participation" in the care process can be used to improve and maintain an effective care relationship, which then creates an effective care relationship between the three main pillars of the care process: the patient, the nurse, and the physician. Collaborative care is a spectrum from 100% self-care to 100% professional care in which families or health providers, including health personnel, physicians, nurses, social workers, and pharmacists are partnered [16]. Collaborative care encompasses part of the spectrum of care, can be a large or small component of self-care, and can ultimately lead to improved health. It will be necessary to strengthen the caregiver's abilities as a patient with constant stress and anxiety [17]. Also, the use of new participatory teaching methods increases confidence, understanding, and learning, and improves communication skills among members [18]. The purpose of this study was to investigate the effect of the collaborative care model on diet compliance in patients undergoing hemodialysis referred to Urmia Hemodialysis Center of Urmia in 1397.

METHODS

The present study is an experimental study with code IRCT20180730040641N1 in which diet non-compliance is considered as a risk factor for hemodialysis patients. The statistical population in this study was all patients undergoing hemodialysis referred to Urmia Hemodialysis Center of Urmia who was enrolled in the study according to inclusion criteria. Inclusion criteria included no mental illness, cognitive impairment, known hearing, and visual impairment, no use of drugs and psychotropic substances, at least 6 months history of hemodialysis, and ability to stand on weights to control weight between two sessions. Then, of the 175 patients undergoing hemodialysis at the center based on the mean laboratory, sodium, potassium, phosphorus, and albumin parameters in the past 6 months, 60 patients were randomly selected as the study population (patients who did not adhere to their diet). They started studying. After informing the patients about the process of completing the study and completing the informed consent form in these patients, the collaborative care model was administered to the patients in the intervention group for 7 sessions and routine care was performed for the control group patients. The intervention was performed by implementing a collaborative care model. To achieve the objectives of the model, the following steps were presented in the form of a systematic structure that was followed by a logical and evolutionary set of interconnected and dynamic sequences, consisting of four steps: 1- Motivation: 2- Preparation; 3- Involvement; 4- Evaluation that was anticipated for each stage of the specific action plans.

Motivation Stage: To motivate patients by communicating with them appropriately, familiarizing them with the ward and facilities available, raising awareness of patients with chronic renal failure, its consequences, and dangerous consequences were summarized.

Educational need assessment was also done by reviewing and identifying patients' problems by taking biographies and interviews, which was a 2-hour session after filling out the demographic questionnaire during the hemodialysis process.

Preparation Stage: Model objectives and timing of participatory training programs and follow-up were identified (one-hour session).

Involvement phase: Training sessions and follow-up sessions were held at a stage (each session for 30 minutes) that included:

1) The first participatory training session

2) Second educational participatory meeting

3) Participatory follow-up meeting first (one-month interval between each session half an hour)

4) Second follow-up collaborative meeting (one-month interval between each session half an hour)

Evaluation Stage: Achievement of the goals was evaluated in the implementation stages of the model. The evaluation was performed in stages at the beginning and end of each training session as a question and answer session. The training steps were simple and lecture-based.

Implementation of the training program in 4 sessions in the patient's bedside with the nephrologist in the Hemodialysis ward of the Urmia Hemodialysis Center in Urmia for 30 minutes individually and individually for patients.

In the first session, the patient was provided with the necessary information about the nature of the disease, diagnostic methods, and treatment, in each training session after completing the training during the last 20 minutes, in the last 10 minutes, summarizing information, and asking patients questions about problems. The answer was raised. The second session provided information and skills on the importance and how to maintain a proper diet concerning the disease, and the third session on the final evaluation of education, with emphasis on follow-up and participation rates.

Educational information was also provided in the form of educational pamphlets and pamphlets.

Laboratory parameters were measured and reported in two stages, once at the end of the intervention and the second time at one month interval and compared with pre-study values. Also, educational content was collected from accredited hemodialysis and nurse nursing textbooks and approved patient education guidelines, physicians, and nursing professors.

Training sessions were also conducted by the researcher and supervised by the project executives. Data were analyzed using a t-test for independent samples and chi-square and repeated measures in SPSS 23 software.

RESULTS

The statistical population of this study consisted of 60 patients undergoing hemodialysis with laboratory parameters indicating diet compliance who were randomly divided into two equal groups of intervention and control.

The mean age of the patients in the intervention and control groups were (53.60 13 13.27) and (48.63 14 14.88) years, respectively, in terms of the mean duration of dialysis in both groups (intervention and control). It was equal to 31.02 20 20.45 and 33.13 18 18.54 months, respectively.

There was no significant difference between the two groups in terms of age and duration of dialysis. The t-test was homogeneous in terms of mean age (p = 0.17), duration of dialysis (p = 0.84) and patient weight (p = 0.16) (Table 1).

Table 1: Comparison of quantitative demographic characteristics of the studied samples in the two intervention and control groups

Variable	Intervention group		Control group		Test statistics Independent t-test	P-value
	Mean	SD	Mean	SD		
Age of patients	53.60	13.27	48.63	14.88	t=1.36 df=58	0.17
Duration of dialysis	31.02	20.45	33.13	18.54	t=-0.19 df=58	0.84
Weight of patients	68.83	11.59	72.98	10.98	t=-1.43 df=58	0.16

SD – standard deviation

Regarding the qualitative demographic characteristics that could influence the outcome of the intervention, based on the results of the Chi-square test in Table 2, no significant difference was observed between the two groups (intervention and control). In other words, two groups were gender (P = 0.43), occupational status (P = 0.46), educational status (P = 0.60), marital status (P = 0.71) and income (P = 0.51) were homogeneous.

Table 2: Comparison of qualitative demographic characteristics of the samples in the intervention and control groups

Variable		Intervention group		Control group		Test result
		Number	Percent	Number	Percent	
gender	female	17	56.7	14	46.7	X^2=0.60 Df=1 P=0.43
	man	13	43.3	16	53.3	
job	retired	11	36.7	14	46.7	X^2=1.55 Df=2 P=0.46
	housewife	9	30	5	16.7	
	does not have	10	33.3	11	36.7	
education status	illiterate	10	33.3	10	37	X^2=1/84 Df=3 p=0/60
	high school	15	50	12	44.4	
	diploma	1	3.3	3	11.1	
	above the diploma	4	13.3	2	7.4	
marital status	married	26	86.7	25	83.3	X^2=0.13 p=0.71
	single	4	13/3	5	16.7	
income	more than spending	4	13.3	3	10	X^2=1.34 Df=2
	spending equal expense	7	23.3	4	13.3	
	less than spending	19	63.3	23	76.7	

Table 3: Comparison of mean scores of laboratory parameters between the intervention and control groups in hemodialysis patients

Variable	Group	Intervention group	Control group	P-value
		Mean ± SD	Mean ± SD	
potassium	Before intervention	5.65±0.68	5.63±0.43	0.86
	After the intervention	5.11±0.52	5.41±0.42	0.017
	After one month of intervention	4.10±0.26	5.25±0.40	<0.001
	P-value	<0.001	<0.054	
Phosphorus	Before intervention	5.42±0.98	5.45±0.55	0.87
	After the intervention	5.19±0.7	5.34±0.54	0.31
	After one month of intervention	4.22±0.59	5.31±0.27	0.001
	P-value	<0.001	0.15	
Sodium	Before intervention	139.74±3.24	138.63±0.77	0.15
	After the intervention	138.07±3.25	137.90±2.33	0.82
	After one month of intervention	136.97±2.64	138.30±2.50	0.050
	P-value	<0.001	0.34	
Albumin (protein)	Before intervention	4.07±0.54	3.93±0.44	0.28
	After the intervention	4.24±0.46	3.78±0.41	<0.001
	After one month of intervention	4.54±0.42	3.59±0.52	<0.001
	P-value	<0.001	0.058	

SD – standard deviation

The results of the t-test of two independent groups concerning Table 3 show that before the intervention, the hypothesis of equality of mean laboratory parameters between the two groups at a 5% error level was not significant (P>0.05). There was no significant difference between the two groups in terms of mean laboratory parameters and after the intervention; there was a significant difference between the intervention and control groups (P<0.05). The results of the repeated measures test showed that in the intervention group there was a significant difference between before and after the intervention and after one month of intervention (P<0.05). Therefore, it can be said that the intervention was effective but in the control group was significantly higher than 0.05 indicating no significant difference in the control group before and after the intervention and after one month of intervention.

DISCUSSION AND CONCLUSSION

Various studies have shown that changes in the lives of individuals following the onset of chronic kidney disease require serious support and support to improve and tolerate behavior change [19]. Non-compliance to treatment regimens is common in chronic kidney patients and is associated with an increased rate of disease progression and mortality in these patients and is directly related [20]. Compliance with a diet is the result of a range of behaviors that are in line with the recommendations made in compliance with the prescribed dietary regimen, including medication, diet, and so on. Compliance with a diet as part of the treatment regimen can be effective in controlling many diseases [21]. Symptoms and symptoms include fluid and electrolyte imbalances, and accumulation of these substances in the body, hyperkalemia, metabolic acidosis, hyper-phosphatemia, anemia, hyperparathyroidism. Malnutrition, etc. are occurring and intensifying over time [22]. Protein-energy nutrition also occurs in 40% of patients with chronic kidney failure. One of the reasons for all these problems is the lack of diet; one of the needs of the patient at this stage is the need to obtain information about nutrition and nutrition. Nutrition in these patients suggests appropriate approaches to reduce the adverse effects of the disease. Increased mortality and morbidity due to toxins in the cassette may also be caused by the increased awareness of these patients in the diet [23]. Today, trying to adhere to a nutritional diet because of its cheapness compared to other health interventions and fewer side effects is considered an important treatment option in the early treatment of patients, and patient education is a valuable method. Compliance with the diet of the patients is considered. The purpose of this study was to evaluate the effect of the participatory care model on diet compliance in patients undergoing hemodialysis referred to Urmia Hemodialysis Center of Urmia in 1397. In the present study, two intervention and control groups, including hemodialysis patients who did not adhere to the diet, were compared after the intervention. The intervention group underwent an interventional process of collaborative care according to the standard model presented in the study method. The control group continued the normal hemodialysis procedure until the end of the study. At first, both groups were compared in terms of demographic characteristics. The results showed that there was no significant difference between the intervention and control groups in terms of demographic variables such as gender, age, marital status, education, patient occupation, monthly income, which could affect the results of the study. In other words, both groups were homogeneous. After successive steps of intervention, both groups were compared in terms of laboratory parameters related to diet compliance. The results showed that there was a significant relationship between the experimental and control groups in terms of laboratory parameters of phosphorus, potassium, sodium, and albumin. Participatory care reduces the toxicological parameters of patients with chronic kidney disease and reduces the weight difference due to the accumulation of toxic substances in the intervention group. In other words, participatory care has a significant direct relationship with compliance to the diet. Parvinian Nassab et al. 2014 A study "Determining the impact of collaborative care model on the anxiety of adolescents with beta-thalassemia major" by selecting 21 mothers available and randomly sampling in two control and intervention groups at Shahid Hospital Coliseum. Dastgheib Shiraz was designed for 3 months. The results of the study showed that the mean score of worry before intervention in the control and intervention groups was not statistically significant, but there was a significant difference between the mean worry in the intervention group before and after the implementation of the collaborative care model [24].

In 2015, Mamne et al. designed a study to investigate the impact of collaborative care model training on the nutritional behavior of type 2 diabetic patients. In this study, simple random sampling (random numbers) was performed from 411 cases to 42 cases in two experimental (control) and control (21 patients) groups at Tabriz Diabetes Training Center with two samples. The results showed that there was no significant difference in age, sex, educational level, occupation, marital status, and economic status between the two groups. Experimental group after training intervention in the areas of fast food consumption, fruit and vegetable consumption, canned, pastry, solid vegetable oil, sausage and sausage, the usual method of cooking and eating heavy and late dinner better performance than the control group. [25].

A quasi-experimental clinical trial study conducted by Khoshab et al. 2012 aimed to determine the impact of the collaborative care model on depression and anxiety in patients with heart failure in 11 patients with heart failure from CCU, PCCU, and internal heart wards. Afzalipour and Shafa hospitals of Kerman were randomly divided into control and experimental groups. The results of the study showed that the mean scores of anxiety in the intervention group decreased after implementing the collaborative care model [26].

The Lashkari et al. Study in 2015 aimed to investigate the effect of the collaborative care model on the fatigue of 12 conservative hemodialysis patients by purposeful sampling in two experimental and control groups randomly for 3 months in Golestan and Imam Ahwaz hospitals. According to the results of the study after intervention in the intervention group, the level of fatigue at a mild level increased (from 23.9% to 42.2%) and the level of fatigue at a severe level decreased significantly (from 42.3% to 7%). Severe fatigue in the control group increased from 42.2% before the intervention to 34.2% after the intervention. Results also showed that after the intervention, the mean score of total fatigue severity in the intervention group was reduced and it was statistically significant [27]. Our study was similar in terms of the implementation of the collaborative care model. The results of applying the collaborative care model to the crisis created in these studies were also in line with our study.

Based on the results of this study, it can be concluded that the participatory care model has been successful in explaining dietary compliance behavior. Therefore, it is suggested to implement a participatory care model of patients undergoing hemodialysis in planning to change dietary behaviors. Although the findings of this study provide an accurate description of the relationship between the incubation care model and diet compliance, it also has some limitations, including the ability to accurately and sequentially monitor laboratory parameters by a laboratory apparatus and an expert operator who can be useful to achieve better results. It is also recommended to consider the role of other standardized behavioral models in identifying the effective aspects of behavioral or behavioral changes in treatment planning and to consider effective educational methods to increase compliance with dietary restrictions. To improve both the quality of treatment and to reduce the cost of treatment for the consequences of noncompliance.

CONCLUSION

The findings of this study in general indicate the usefulness and effectiveness of the participatory care model in the process of compliance to diet in patients with hemodialysis. Therefore, participatory care intervention seems to play a key role in improving diet compliance and fluid restriction in hemodialysis patients, requiring comprehensive plans focusing on participatory model steps by health care professionals and hemodialysis patients. The effectiveness of the model as well as its simplicity is one of the strengths and weaknesses of this model compared to other models.

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Conflicts of Interest

No conflict of interest is declared by the authors.

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The effect of leadership style on the productivity of hospital's staff with moderating effect of job promotion opportunity and job description

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Abstract: *Background: Human resource is the most important criteria of today organizations. In considering a human resource, performance and productivity are very fundamental. One of the most important criteria that affect human resources is leadership style.*

Aims: This study aimed to assess the effect of leadership style (Autocratic, Democratic, and Laissez-faire) on productivity that in this relationship two phenomena (job promotion opportunity and job description) plays the roles of moderating variable.

Methods: The data were collected through the distribution of standard questionnaires among 170 employees of a hospital in Tehran and analyzed with structural equation methods with smart PLS software. The reliability and validity of questionnaires are confirmed through software examination and elites' opinions. For examining the hypothesis, factor loading and path coefficient are considered.

Results: Democratic and laissez-faire styles have a significant effect on productivity (with 5.51 and 2.65 loading factor and positive path coefficient), but the autocratic style has a negative effect (due to 5.54 loading factor and -0.35 path coefficient) on productivity. Neither job promotion opportunity nor job description plays the role of moderating variables according to the results of the analyzes.

Conclusion: Leadership style has a significant impact on productivity. The autocratic style has a negative effect on productivity, but democratic and laissez-faire have a positive effect. Job promotion opportunities and job descriptions don't play a moderating role in leadership style and productivity relationships.

Keywords: *leadership style. productivity. job promotion. job description*

INTRODUCTION

"Human resource" is one of the most valuable assets of organizations, especially in organizations that knowledge is their first priority [1]. In fact, managers should pay more attention to human resources rather than ordinary managing, hiring, or financial incentives. Nowadays, the generation gap between employees and also hiring educated people, make managing more challenging. Therefore, organizations and managers should create a dashboard so they can observe human resources criteria like efficiency and productivity. Without this kind of Mechanism, employees can't show their creativity and also they lose their enthusiasm for work [2].

Matsushita in his book "In Iron Hand" said that Human is like a gem, his value is depending on how it processed. He believed that everyone who is responsible for people in the organization should know how to extract the best of them [3].

In this research, nevertheless, there are lots of ways to measuring productivity, but methods of Taylor and Steel [4]. Has chosen, they took efficiency and effectiveness for measuring productivity.

Personnel productivity has a positive impact on the

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productivity of the organization. Thus, if managers want to have a better approach to organizational culture, strategies, policies, and prospect, they should understand that productivity is vital for them [5].

On the other hand, leadership style has a great impact on human resource performance and function [6]. With this view, the management means the process of planning, organizing, "leading" and controlling to reach the goals of the organization [7]. In fact, leadership has a bigger concept and meaning than management. Management is a subset of leadership and leadership mostly means how effect and influence people to make them be in direction of reaching goals for themselves or the organization [8]. With this meaning, it seems that there is some relationship between leadership and productivity.

For considering leadership style and also the existence of three famous leadership styles (Autocratic, Democratic and Laissez-faire) that are most common in the atmosphere of Iran organization, researchers decided to choose Kurt Lewin theory of leadership that presented in Iowa University of USA [9]. Levin and his colleagues describe these three leadership styles:

1- Autocratic: A leader who decides just by himself and mostly dictates responsibilities to employees.

2- Democratic: Democratic: This style mostly refers to managers who share the power of choice with his/her human resource and just illustrate the whole vision of the job and give employees the right to choose what should be the method to reach goals.

3- Laissez-faire: Laissez-faire: Managers who have this style of management, don't participate in groups and just intervene when groups have problems and challenges.

The result of Kurt Lewin's research shows that among these three leadership styles, Laissez-faire style has the least Efficiency [9].

Moderating variables, job description opportunities, and job description, considered from the perspective of incentives. Job promotion opportunity describes as when an employee in a specific period of time does duties and responsibilities well and also has knowledge and competency for the higher level in the organization; does he/she promote or not? [10]. In this context, the absence of gender discrimination is very important for job promotion [11]. In a holistic view, if employees know there is a job promotion opportunity in the organization, their performance becomes well [12] so researchers decided to survey how it works in the field of productivity.

Definition of the job description is "a document that has main responsibilities, functions, and duties of a person that works in the organization. This document maybe has the little portion of job criteria to very specific things like even to whom an employee must give her/him reports" [13].

With all these illustrations, eventually, we decided to discuss the relationship between these three leadership styles and productivity with moderating role of job promotion opportunity and job description. Conceptual model of this study is presented in Figure 1.

Figure 1: Conceptual model

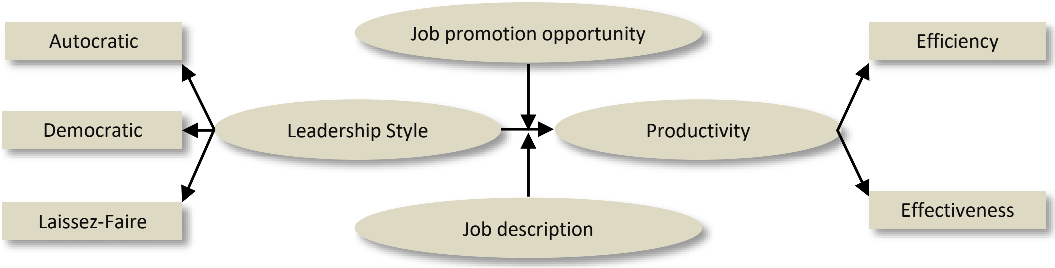

METHODS

The type of study is cross-sectional that conducted on employees of a hospital in Tehran from April of 2018 to May of 2018. Data for analyzing collected through the distribution of productivity and leadership style. Questionnaire of productivity as it said before, is from Taylor and Steel [4]. and questionnaire of leadership style is from Kurt Lewin theory [9]. Number of the questionnaire that has to be gain is extracted from Cochrane formula [14] and Morgan table [15]. According to these two criteria for sampling in the statistical universe, 170 questionnaires randomly distributed among 370 employees of the morning shift. Gathered data analyzed by Smart PLS and through path coefficient, factor loadings, and other important outputs. Inclusive criteria in this study are two main things. First, an employee must be officially hired, and second is she/he working the morning shift.

The questionnaire of leadership has three leadership styles altogether, but at the time of analyzing, they separated and each style analyzed by its questions separately. After analyzing the relationship between our two main variables with all consideration of modifying the inner and outer model in software, moderating variables analyzed. Due to binary moderating variables, the method of analyzing moderating variable [16] formula which is operational for this research. Consequently, in this research, nine different analyzes took place; three for the main path between leadership styles and productivity and six analyzes for moderating variables (because each leadership has two moderating analyzes).

Reliability and validity in statistics sample were confirmed by elites and also methods of confirming in Smart PLS [17]. Results of this tests are illustrated in Table 1.

Table 1: Results of model examination in PLS

Purpose of Test	Name of Test	Confirmation Status	Power Status
Consistency	Consistency of outer model variables	Confirmed (with modification)	Normal
Validity	Cross Loading Test	Confirmed (with modification)	Normal
	Fornell - Larcker	Confirmed	Strong
	Factor Loading Significantly	Confirmed	Strong
	Factor Loading	Confirmed	Strong
	AVE>0.5	Confirmed	Strong
	CR>AVE	Confirmed	Strong
Reliability	*Delvin-Goldstein (Composite reliability)*	Confirmed	Strong
	Communality	Confirmed	Strong
Model Quality	Outer Model Quality	Confirmed	Strong
	Inner Model Quality	Confirmed	Strong
	Generalized Model	Confirmed	Strong

The precision [16] that says if in a research, the sample volume is above of 168, research has 97 percent power and accuracy of covariance structure analysis. Accordingly, because of 170 samples of this study, the analyzes and outputs are accurate.

With all this explanation, three main hypothesizes of this research are:

H1: Leadership style has a significant effect on productivity.

H2: Leadership style with moderating effect of job promotion opportunity has a significant effect on productivity.

H3: Leadership style with moderating effect of job description has a significant effect on productivity.

RESULTS

First analyze is without any modification in the inner and outer model in smart PLS. Raw analyze of autocratic leadership is presented in Figure 2.

After model modification and results that are shown in Table 1, three basic leadership analyzes of path coefficient are presented in Figures 3, 4 and 5.

Results of analyzes without moderating variables in path coefficient and factor loading mode are presented in Table 2.

Table 2: Results of analyze without moderating variables

Leadership	Productivity	
	Factor Loading	Path Coefficient
Autocratic	5.541563	-0.358867
Democratic	5.511342	0.371835
Laissez-Faire	2.654519	0.167206

According to this table, all three leadership has a significant effect on productivity, but the path coefficient shows that autocratic leadership style affects negatively on productivity, but two other leadership has a positive path coefficient.

Figure 2: Autocratic leadership raw analyze

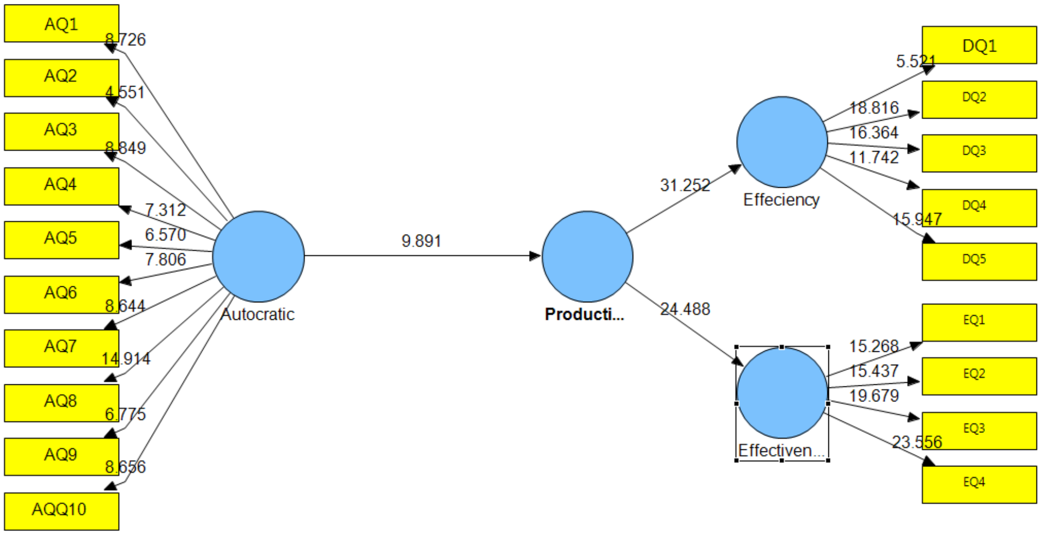
AQ1
AQ2
AQ3
AQ4
AQ5
AQ6
AQ7
AQ8
AQ9
AQQ10
8.726
4.551
8.849
7.312
6.570
7.806
8.644
14.914
6.775
8.656
Autocratic
9.891
Producti...
31.252
24.488
Effeciency
Effectiven...
5.521
18.816
16.364
11.742
15.947
DQ1
DQ2
DQ3
DQ4
DQ5
15.268
15.437
19.679
23.556
EQ1
EQ2
EQ3
EQ4

Figure 3: Autocratic leadership analyze in path coefficient output

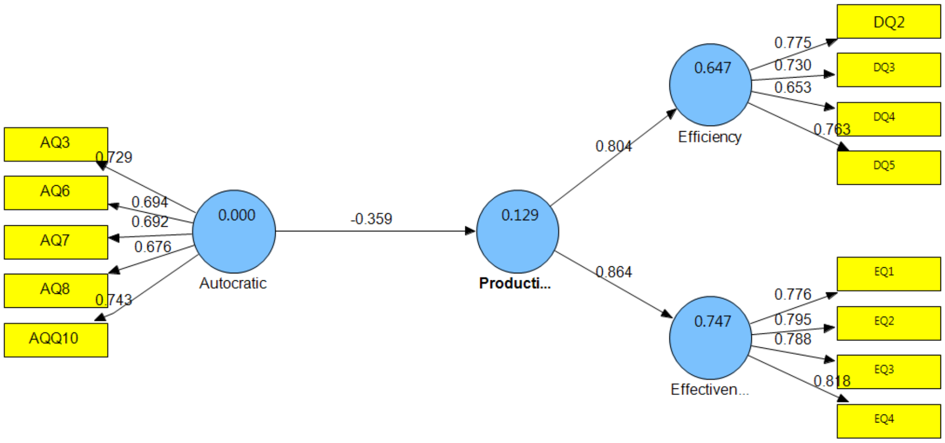
AQ3
AQ6
AQ7
AQ8
AQQ10
0.729
0.694
0.692
0.676
0.743
0.000
Autocratic
-0.359
0.129
Producti...
0.804
0.864
0.647
Efficiency
0.747
Effectiven...
0.775
0.730
0.653
0.763
DQ2
DQ3
DQ4
DQ5
0.776
0.795
0.788
0.818
EQ1
EQ2
EQ3
EQ4

Figure 4: Democratic leadership analyze in path coefficient output

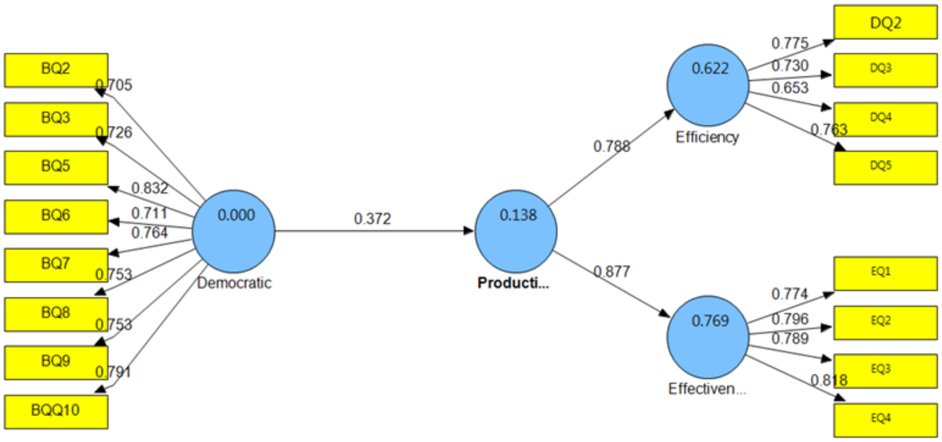
BQ2
BQ3
BQ5
BQ6
BQ7
BQ8
BQ9
BQQ10
0.705
0.726
0.832
0.711
0.764
0.753
0.753
0.791
0.000
Democratic
0.372
0.138
Producti...
0.788
0.877
0.622
Efficiency
0.769
Effectiven...
0.775
0.730
0.653
0.763
DQ2
DQ3
DQ4
DQ5
0.774
0.796
0.789
0.818
EQ1
EQ2
EQ3
EQ4

Figure 5: Laissez-Faire leadership analyze in path coefficient output

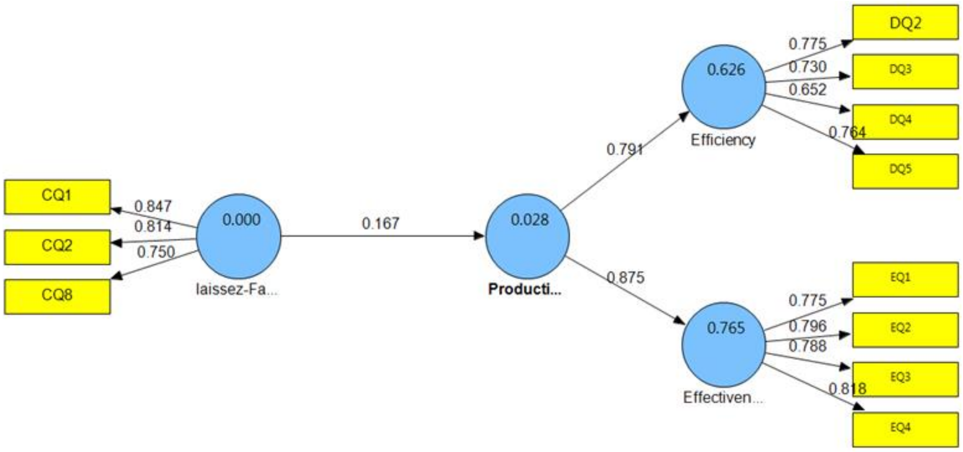

For analyzing moderating variables, as presented before, the Hair formula used in this research. The result of all six moderating variables analyzes are in Table 3.

Table 3: Results of moderating effects

Moderating Variable	Leadership Style	Result	Confirmation Status
Job promotion opportunity	Autocratic	0.18	NOT confirmed
	Democratic	0.159	NOT confirmed
	Laissez-Faire	0.058	NOT confirmed
Job Description	Autocratic	-0.144	NOT confirmed
	Democratic	0.259	NOT confirmed
	Laissez-Faire	0.19	NOT confirmed

Eventually, R^2 and model quality were analyzed. The model [18] and R^2 are according to Chin [19]. Model quality comes with main formula:

$$\text{Model Quality Formula} = \sqrt{\overline{\text{Communality}} \times \overline{R^2}}$$

and for autocratic leadership is 0.53, for democratic leadership is 0.542 and for laissez-faire leadership is 0.533. These results show all three models have good quality according to numbers of Tenenhaus and Vinzi (Table 4).

Table 4: Results of R^2

Variable	R^2		
	Autocratic	Democratic	Laissez-Faire
Efficiency	0.646507	0.621514	0.625817
Effectiveness	0.746749	0.768643	0.765111
Productivity	0.128785	0.138261	0.027958

According to numbers of chin, in all models, efficiency and effectiveness have high value, but productivity in laissez-faire model has bad value and two other models have something between bad and medium value.

DISCUSSION

First, we considering research hypothesis. According to results, H1 of this research, that's mean "Leadership style has the significant effect on productivity", in democratic and laissez-faire leadership confirmed [20]. In autocratic leadership because of negative path coefficient, not even confirmed, but also shows that this kind of leadership has the negative effect on productivity and managers have to know that if they want to rise up their employee's productivity, it's better to survey all consequences that come with autocratic leadership style. As results shows, and also other research confirmed, different leadership style has a different effect on productivity [6, 21].

H2 and H3 of this research, which are "Leadership style with moderating effect of job promotion opportunity has significant effect on productivity" and "Leadership style with moderating effect of job description has significant effect on productivity", not confirmed due to lack of the significance effect of both moderating variables in all three leadership styles [9]. Laissez-faire leadership has less efficiency for managing in comparison with autocratic and democratic. This claim is not in conformity with results of this research. In return [22]. Is confirms our results. Furthermore, democratic leadership is the best style among these three styles that considered in this survey and this outcome is in conformity with Walumbwa [23]. Who researched ethical leadership and shows one of the ethical leadership is democratic leadership that affects productivity. Due to the average variance extracted of laissez-faire leadership, we cannot certainly talk about this style and expand it to all environments. But even with this result, we can say managers should be cautious in choosing this style, cause some researches to show that this style is harmful to organizations like research of Skogstad [24] especially traditional and complex organizations. Nevertheless, the result of this research about laissez-faire leadership is in conformity with Yang [25] research's outcome. That found out job description has the significant effect on productivity. Job promotion opportunity mostly collaborate with concepts like sexuality in Groot [26] research or educational perspective [27] research, that in both of these research, job promotion opportunity has incentive role. We discussed and figured out that H2 and H3 are not confirmed. With this result, among all six moderating variables, one of them is really unexpected. Job description in laissez-faire model analysis is the controversial point here. As we know, laissez-faire leadership is the act of delegation. This view proves that employees who work under the supervision of this leadership, need some job description to do their works better (in comparison with two other leaderships that some interaction of leader exists). However, the job description doesn't play the role of moderating variable in this relationship, and because of that, maybe more research needed for conformity or opposition to this result.

Future research

According to the result that we achieved, we suggest to researchers to consider two things in their future study if this article is their source. First, examine other moderating variables with these two main variables (leadership style and productivity) and discuss is any other concepts exist for playing the role of moderating effect or not. Second, because of the low average extracted variances for some aspects of this research, it is helpful to test the topic of this survey in another atmosphere. This suggestion is for clearance of results of this research.

CONCLUSIONS

Among three leadership style that discussed, autocratic, democratic and laissez-faire have the significant effect on productivity (democratic leadership has a better impact rather than laissez-faire leadership). Autocratic leadership has a negative impact on productivity due to the negative path coefficient. Neither job promotion opportunity, nor job description plays the role of moderating variable in this survey.

Acknowledgment

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VARIA

The effect of lowering the dialysate temperature on the stabilization of hemodynamic indices of diabetic patients admitted to Ali ibn Abi Talib Hospital, Zahedan: A clinical trial

Salehoddin Bouya[1], Fatemeh Kiani[2]

Abstract: *Introduction: One of the basic pillars of hemodialysis is its adequacy, which is achieved by stabilizing hemodynamic parameters. This is especially important in the hemodialysis adequacy of diabetic patients. The current study aims to determine the effect of lowering the dialysate temperature on hemodynamic indices and dialysis adequacy of diabetic patients.*

Materials and methods: This is a clinical trial that was performed on 32 diabetic patients admitted to the Dialysis Ward of Ali ibn Abi Talib Hospital affiliated with Zahedan University of Medical Sciences in 2017. The research subjects were selected using the convenience sampling method from eligible patients. Each patient underwent hemodialysis twice; i.e. routine dialysate temperature (37° C) and cold dialysate temperature (36 °C). Except for lowering the dialysate temperature to (36 °C), all other conditions were the same during the study. Blood pressure (BP) and pulse rate of patients were monitored before and after dialysis and during the first, second, and third hours after dialysis; however, the body temperature and weight of patients were monitored before and after each dialysis session. Patients' blood samples were collected at each stage before and after dialysis to evaluate urea and calculate dialysis adequacy. Data analysis was carried out using descriptive statistics, paired t-test, and one-way repeated measures ANOVA in SPSS Ver. 21. P-value <0.05 was considered as the significant level.

Results: Systolic, diastolic, and mean arterial blood pressure among patients who underwent hemodialysis with normal dialysate temperature were significantly lower than those who underwent hemodialysis with cold dialysate temperature (p<0.05). There were no significant differences between cold and routine dialysis in terms of changes in body temperature before and after dialysis. The dialysis adequacy at 36 °C showed a significant increase as compared to 37° C.

Conclusion: The results of the present study showed that hemodialysis with cold solution (36 °C) stabilized hemodynamic variables and improved dialysis adequacy among diabetic patients, therefore, this treatment is recommended in hemodialysis wards.

Keywords: *cold dialysis solution, hemodynamic indices, dialysis adequacy, hemodialysis, diabetic patients, chronic renal failure*

INTRODUCTION

Type 2 diabetes is one of the most common non-communicable diseases in the world and one of the 10 leading causes of death in many countries [1].

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In terms of the severity of the disease, 35% of dialysis patients with chronic renal failure are diabetic [2].

Chronic kidney disease is the most common and important complication of diabetes, which is associated with a range of different pathological processes and leads to an irreversible decrease in renal function. Among these pathological processes, end-stage renal disease (ESRD) is one of the most important and common life-threatening diseases, which is associated with severe or complete renal insufficiency in the excretion of waste products, urine concentration, and regulation of electrolytes [4]. Studies show a growing increase in the prevalence and incidence of ESRD patients in Iran, reaching from 24,000 in 2008 to 58,000 at the end of 2016, of which about 29,200 (95%) are undergoing hemodialysis and 1624 are undergoing peritoneal dialysis [5, 6]. Considering the prevalence of diabetes and hypertension, it is predicted that the number of kidney patients, dialysis patients, and kidney transplants will be doubled and reach at least 90,000 by 2022 [6]. Aghighi et al. reported that the number of these patients increased by more than 130% in Iran during 1997-2006, which indicates the need to pay attention to this disease [7].

Considering the average 7%growth in the number of these patients in Iran, hemodialysis is expected to remain the most important method of dialysis in the treatment of these patients and increase the life expectancy of these patients, however, hemodialysis-related complications and problems also affect the health of these patients [7, 8].

Despite many technological and technical advances in hemodialysis and its improvement, there are still many pre- and post-dialysis problems. Intradialytic hypotension (IDH) is the most common serious complication of hemodialysis, occurring in 5%-40% of hemodialysis treatments. Hypotension widely increases the overall mortality of patients and not only limits fluid removal during dialysis, but can also cause severe adverse vascular effects on mesentery, cerebral infarction, and ischemia. This condition increases the need for nursing care and has negative effects on their quality of life, which is especially important among diabetic dialysis patients [2]. Diabetic patients are at higher risk for IDH, which is due to the autonomic dysfunction and cardiovascular problems in these patients.

One of the effective solutions proposed to prevent IDH, which has also been used, is to reduce the temperature of the dialysate [11-16]. Hemodialysis patients, who are usually hypothermic, gain extra heat during the treatment session. It seems that the increase in body temperature is due to increased sympathetic activity and decreased ability to dissipate heat due to severe contraction of peripheral arteries and dilation of central arteries, and ultimately reduced cardiac output [17, 18].

A slight decrease in dialysate temperature of about 1-2 °C helps reverse these changes by increasing central venous artery tonicity, venous return, and cardiac output [20]. Mojerloo et al. used cold and routine dialysate and found that generally, hypotension rate was 18% and 5% among the dialysis groups treated with routine temperature and cold dialysate, respectively, and hypotension rate was significantly lower in the dialysis group with cold dialysate [21].

Because hemodialysis patients experience many problems due to the specific physical and mental conditions arising from their disease, and dialysis quality is an important factor and effective in reducing these problems, and one of the important parameters to improve dialysis adequacy is to stabilize hemodynamic indicators [16], there is a need to adequate standard hemodialysis by reducing side effects to reduce referrals among patients (especially diabetic patients) for hemodialysis. Considering the importance of the foregoing and the results of several studies, the use of cold dialysate is a simple and inexpensive method that can reduce the frequency of hypertension, stabilize hemodynamic indices, improve the hemodialysis quality, and thus improve quality of life and general health of diabetic patients undergoing hemodialysis [22-24]. Since there has been no similar study in the province, this study aimed to determine the effect of lowering the dialysate temperature on hemodynamic indices and dialysis adequacy of patients with type 2 diabetes admitted to Ali Ibn Abitaleb Hospital of Zahedan University of Medical Sciences in 2017.

METHODS

This quasi-experimental one-group study was carried out on 32 eligible patients with type 2 diabetes who referred for hemodialysis using convenience sampling. Inclusion criteria included being on dialysis for at least 3 months, non-use of antihypertensive drugs, weight gain up to three kg between hemodialysis sessions, and no anemia, malignant disease, and thyroid disorders. Information record form was made available to patients who expressed their consent to participate in the study. This form consisted of two parts; the first part included individual and clinical characteristics and the second part included calculating and recording the dialysis adequacy of each patient in each hemodialysis session.

The sample size was estimated at 14 people using the following sample size formula and taking into account the numerical values of the variables related to the study by

Mousavi et al. (2014), 95% confidence interval, and power= 95%. To increase the reliability of the results, the sample size was increased to 30 people; finally, it was increased to 32 people considering the possible drop-out.

$$n = \frac{(Z_1 - \frac{\alpha}{2} + Z_1 - \beta)^2 {\sigma_\delta}^2}{\delta^2} + \frac{Z_1^2 - \frac{\alpha}{2}}{2}$$

$\bar{x}1=83.94$ $Z1-\beta=1.64$ $Z1-\alpha/2 = 1.96$ $S2=19.26$

The study was performed in two stages. All patients underwent hemodialysis with a dialysate temperature of 37°C (routine dialysate) before the intervention. They then underwent hemodialysis with a dialysate temperature of 36°C (cold solution) in the next session. In other words, all patients underwent hemodialysis once with a routine dialysis solution (37°C) and once with a cold dialysis solution (36 °C) during two consecutive sessions. A sequence of dialysis with cold or routine dialysate for each patient was performed using random allocation where blue and green balls were used. The patient was asked to pick up a ball from the box containing 16 blue balls and 16 green balls. Blue and green balls represented the cold and routine dialysate in the first session, respectively. In the next session, the dialysis method was shifted. Hemodialysis variables were the same in both cases except for the solution temperature. All patients underwent hemodialysis 3 times a week for 3-4 hours each time. The mean body temperature of patients before hemodialysis was determined in the range of 37 °C and R5 polysulfane dialyzers made by the same company with the same ultrafiltration coefficient (High-flux) and bicarbonate dialysate (bicarbonate powder 650 g 35 Meql/lit, Pharmed Medical Industries Company), which was fixed during the study, was used for all patients. The equipment used, including dialysis machines equipped with an ultrafiltration control system (Fresenius model 4008-B), scales (Seca), and pressure gauges were investigated and confirmed. The dialysate flow rate was constant (500 Ml min).

The dialysate removal rate was calculated and determined for each patient based on the difference between pre-dialysis weight and dry weight (after dialysis). Blood flow rate (300 ml/min) was determined and 16-gauge needles were used. All parameters were constant on the machine until the end of the dialysis session. To measure pre-dialysis BUN level, an arterial blood sample was taken immediately before hemodialysis and after the hemodialysis session. To prepare a blood sample after dialysis, first, the pump speed was reduced to 100 cc per minute and another sample was taken from the arterial route 15 seconds later. All samples taken in the hospital laboratory were examined and the results were received. After measuring the research samples, the dialysis adequacy was calculated by KT/V method using the DaugirdasII formula, which is an internationally acceptable formula. Systolic blood pressure (SBP) and diastolic blood pressure (DBP), and mean arterial blood pressure (ABP) was calculated and recorded before, during (first, second and third hours) and at the end of hemodialysis. The patient's body temperature and heart rate (HR) were also measured before and after hemodialysis. KT/V index ≥ 1.2 and < 1.2 show dialysis with good and low adequacy, respectively. KT/V index values greater and lower than 1.2 indicate more and lower adequacy of dialysis.

Finally, the obtained data were entered into SPSS ver. 21 and analyzed. Descriptive analysis was carried out using mean, frequency, standard deviation, and percentage. A dependent t-test was used to evaluate the effect of cold dialysis on dialysis adequacy. P-value< 0.05 was considered as the significant level.

RESULTS

Of the 32 patients included in the study, 1 patient was excluded from the study due to being on a trip. The majority of people were female (45.2%) and married (83.9%), and 61.5% had the underlying disease. Mean ± SD of patients' age, duration of diabetes, duration of treatment, and weight gain was 60.5 ± 9.21 years, 12.07 ± 7.27 years, 21.82 ± 27.74 days, and 1.64 ±0.68 kg, respectively.

The results of comparing dialysis adequacy with cold and routine dialysate temperature (Table 1) showed a statistically significant difference between the two methods (p<0.001) and dialysis adequacy was higher in cold dialysis.

Table 1: Results of dialysis adequacy in two cold dialyzes and dialysis with routine temperature among hemodialysis patients

Dialysis method or group	Mean and standard deviation	Test result
Cold dialysis	1.14±0.34	P=0.001
Routine dialysis	0.98±0.20	P=0.001

DISCUSSIONS

The results of the present study on the mean systolic blood pressure in hemodialysis with cold and routine dialysate temperatures, before dialysis, 1, 2, and 3 hours of dialysis, and after dialysis showed no statistically significant differences between the two groups in terms of systolic blood pressure in the above five measurement periods except after the intervention (P =0.003).

The lack of a clear drop in SBP after intervention in the cold dialysis group indicates the effectiveness of cold dialysate in preventing a drop in SBP during dialysis.

The results of the present study are consistent with the study by Mojerloo et al. (2015) [21]. In the study entitled "The effect of lowering the dialysate temperature on the stability of hemodynamic variables in patients prone to hemodynamic instability under hemodialysis", Mousavi et al. (2014) showed that reducing the dialysate temperature to (35 °C), as compared to the standard dialysate temperature (37° C), prevents a drop in systolic, diastolic, and mean arterial blood pressure 2 and 3 hours of hemodialysis [25].

In a similar study conducted by Azar et al. (2009) in Egypt, patients were evaluated during 6-dialysis periods and results showed no change in systolic blood pressure after hemodialysis with cold dialysate [9].

Ayoob et al. (2004) carried out a study on two groups of patients, including 5 patients with periods of IDHs and 5 patients with stable BP during hemodialysis. Each patient underwent hemodialysis for three periods with cold dialysate (35 °C) and then three periods with standard dialysate (36.5 °C). The results showed that the mean arterial blood pressure during and after dialysis with dialysate was significantly high in the hypotensive group and did not change in the group with a stable BP [22].

The findings of the present study showed that lowering the dialysate temperature to (36 °C) compared to routine dialysate temperature (37 °C) could prevent SBP at the end of hemodialysis, although SBP did not decrease significantly during 1, 2 and 3 hours of dialysis. The results showed that hemodialysis with routine dialysate temperature reduces BP in patients during dialysis until the end of it.

Findings of the present study showed that the use of cold dialysate for diabetic patients prone to hypotension significantly prevents hypotension during and at the end of dialysis, which is consistent with the results of the study by Ayoob et al. (2004) [22].

The results of the present study are consistent with the results of similar studies conducted in Iran [17, 27]. Therefore, it can be concluded that IDH is more stable in hemodialysis with cold dialysate, and reduces the frequency of IDH. Also, the results showed no drop in DBP at different hours of dialysis in the cold dialysis group, however, there is an evident drop in DBP in the routine group, as we approach the last hours of dialysis, although the statistical tests did not show a significant difference in this regard. The results of the present study are consistent with the study by Mousavi et al. (2014). The results of their study showed a statistically significant difference between cold dialysate and routine dialysate in terms of DBP level suggesting that cold dialysate prevents pressure drop in DBP [25]. Therefore, it can be concluded that the cold dialysate can prevent a reduction in DBP.

The present study also investigated the mean ABP in routine and cold dialysate temperatures, before dialysis, during 1, 2, 3 hours of dialysis, and after dialysis. The results showed a significant difference between the two methods in terms of mean ABP. Although no statistically significant difference was observed between the two methods during hemodialysis in terms of mean ABP, it was relatively higher in the cold dialysate than routine dialysate (p<0.001), however, mean ABP was higher in the cold dialysate group than routine dialysate group.

The results of the present study are consistent with the study by Mousavi et al. (2014). They also found that routine dialysate led to a reduction in mean ABP [25]. Ayoob et al. also showed that the use of cold dialysate stabilizes blood pressure [22]. Considering the foregoing, it can be concluded that the use of cold dialysate stabilizes systolic and diastolic blood pressure and mean arterial pressure during and after hemodialysis. Cold dialysate causes hemodynamic stability during dialysis through various mechanisms, which include keeping the body temperature constant, increasing the activity of the sympathetic nervous system, and thus increasing the cardiac muscle contractile strength, and peripheral vascular resistance, stimulation of alpha-1 receptor in visceral arteries, followed by increased central circulatory volume and increased cardiac output [28-30]. Other studies also concluded that lowering the dialysate temperature reduced nitric oxide synthesis and thus reduced the frequency of hypotension [28].

The present study also investigated the mean HR in routine and cold dialysis methods, before dialysis, during 1, 2, 3 hours of dialysis, and after dialysis. Results showed no difference between the two methods in terms of HR changes, which are completely consistent with the study by Mousavi et al. (2014) [25], but inconsistent with the results of the study by Azar et al. (2009) [9] who found a significant difference in this variable. One of the reasons for the difference in the results may be attributed to the fact that bicarbonate-containing solutions are currently used, all of which contribute to the stability of patients' hemodynamic conditions and heart rate.

The present study also investigated the body temperature of patients who underwent hemodialysis with routine and cold dialysate before and after dialysis. Results showed that the body temperature was not significantly different before and after dialysis, but there was a significant difference in the mean changes of body temperature after hemodialysis. That is, the mean body temperature of people undergoing hemodialysis with routine dialysate was higher than that of people undergoing hemodialysis with cold dialysate (P = 0.02).

The findings of the above study are consistent with the study by Borzoo et al. (2015). They found changes in clinical parameters with changes in dialysate temperature and the mean body temperature of patients decreased with decreasing dialysate temperature, meaning that the mean body temperature of patients who underwent hemodialysis with normal dialysate temperature (37 °C) was 0.12 and 0.27 higher than those underwent hemodialysis with at dialysate temperatures 36 °C and 35 °C, respectively [24]. Another important finding of the present study is the mean body temperature of patients in the two groups before dialysis, which is 36.9°C in the cold dialysis group and 36.8°C in the routine dialysis group, but the statistical tests did not show significant differences (p=0.11). These findings are not consistent with the results of previous studies, which indicate that the body temperature of dialysis patients is lower than the body temperature of normal people [24]. Anemia often leads to a decrease in internal body temperature, which leads to chills, especially in the extremities such as legs, arms, ears, and nose, even when the person is in a warm room. Therefore, using a solution with a temperature higher than body temperature can not only lead to the destabilization of cardiovascular conditions [9, 18, 24] but also causes discomfort in patients [31].

Considering the results of the current study suggesting the positive effect of cold dialysate on the dialysis adequacy and comfort of patients, it seems that the time has come for all patients not to be permanently dialyzed with a single dialysate temperature and the dialysate temperature to be determined according to patients' clinical conditions. The use of cold dialysate, which is both inexpensive and readily available, can prevent hemodynamic instability during hemodialysis and reduce the periodic occurrence of IDH. However, it should be noted that all patients may not benefit from this method, therefore, people who have a lower pre-dialysis temperature can benefit more from this method.

CONCLUSIONS

Considering the positive effects of cold dialysis on the hemodialysis adequacy and stabilization of hemodynamic variables, it is suggested that if there is no prohibition in this treatment, cold dialysate replaces the standard dialysate. It is recommended to carry out further studies on the effect of cold hemodialysis on hemodialysis adequacy in other groups, including non-diabetic patients.

Ethical considerations

The present study was registered in the Ethics Committee of Zahedan University of Medical Sciences with the number (IRZaums.REC.1396.292) and in the Iranian Registry of Clinical Trials with the code IRCT20171009036675N2.

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VARIA

The effect of multimedia training on social function of burn patients in Shahid Motahhari Hospital, Tehran: A clinical trial study

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Abstract: *Introduction: Burn is a tissue injury and affects social functioning and relationships. Complications of burns lead to disruption of social relationships and consequently social dysfunction.*

Objective: Aim of this study was to determine the effect of multimedia training on social functioning of burn patients in Shahid Motahhari hospital in Tehran.

Methods: This clinical trial study was performed on 100 burned patients. The intervention group received a multi-media self-care discharge training on a CD in addition to the common education. Social function of quality of life was examined in both groups before intervention, 3 months and 6 months after intervention.

Conclusion: Results showed that before intervention the mean score of social function of quality of life in intervention and control group was 1/55± 0/46, 1/92± 0/6 respectively which was statistically significant (p <0.001). Mean and standard deviation of social function of quality of life in the intervention and control groups three and six months after intervention were 2/47± 0/56, 4/05± 0/77, 2/15±0/39, 3/29 ± 0/95 respectively which was statistically significant (p <0.001).

Keywords: *self-care, multimedia, patient discharge, social adjustment, burns*

INTRODUCTION

Burn have been described as one of the most devastating disasters on the human body [1] which seriously damages one's life and health and is considered to be the fourth most common injury [2]. The World Health Organization estimates that the incidence of burns Severe is one percent of life expectancy and more than 300,000 people die from burns worldwide each year [3]. According to the Forensic Medicine Organization statistics, in the first quarter of this year, 379 people died from burns in the country. Of these, 213 were men and 166 were women [4], so burn injuries are one of the most dangerous health incidents in Iran. Over the past decade, advances in health care have made patients with more severe burns survive [5]. Even if urgent management is successful, burn injuries can create many obstacles for patients. In addition to their physical problems, they also suffer from social problems and ultimately their quality of life is affected [6]. Patients suffer from severe seizures at the gate, which can live longer. High-end living is particularly attractive in most places where a lot of space for cabins can be attractive. And the place of marriage, relationships, places of residence, in their lives, and ultimately cause them to become overwhelmed by anxiety and anxiety [7]. People

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have trouble meeting new people and dating, worrying about developing relationships and exhibiting various reactions, such as shyness, aggression, or extreme social avoidance. Therefore, it is necessary to design appropriate supportive programs to improve their quality of life [8]. The only thing that will help the patient is how to live with this situation and learn to meet their own needs and to be less in need of others and to have the attitude that they can play with these shortcomings and that It will be nothing but training and learning and rehabilitation [9]. The philosophy of patient education is to apply the information and skills learned to control and cope better with the disease performed by the care team, especially the nurses [10]. The role of nurses in the last few years as the most important member of the health care team has undergone a historical transformation, from promoting patient-centered health education to empowering patients to self-care and achieve health. Informing the patient and contributing to decision-making speeds recovery and reduces hospital stay and reduces hospital readmission [11]. This is a key challenge here in achieving donation. Education is a traditional teaching tool and disability [12], because lecture-based training requires a great deal of time and expense and, on the other hand, a patient with a burn accident during hospitalization due to mental and physical injuries, physical injuries. Physical weakness, painful daily activities, intellectual discomfort, and lack of focus on decision making may make you less prepared to learn and remember education. The empowerment and self-care is not [9]. In recent decades, traditional approaches to learning with the advent of new technologies such as multimedia virtual education have undergone dramatic changes [13]. The purpose of multimedia application is to make meaningful learning happen, and meaningful learning occurs when the learner can make meaning to the material presented by constructing a coherent mental image from multiple sources of information [14]. It seems that learning will be better if the patient is able to carry out a self-care program using a comprehensive audiovisual CD to suit any time and circumstances they wish [9]. Also, due to the gradual process of rehabilitation in patients, education during discharge for these patients to return to the community must be sufficient and carefully planned [15].

OBJECTIVES

Since humans are social beings and communicating with others is an important factor in life and patients with burns suffer from this, the researchers sought to investigate the impact of multimedia training on the social functioning of burn patients. At the Shahid Motahhari Hospital in Tehran.

MATERIALS AND METHODS

Setting

This research was a randomized clinical trial with a control group that was conducted in the hospitalization wards of Shahid Motahari Burn Center, Tehran, Iran in from 2016 to 2017. The study population consisted of all burn patients who were admitted to Shahid Motahari Burn Center and were participated in the study based on the inclusion criteria.

Inclusion and exclusion criteria

The criteria for participating in the study were the patients` age, people who were between 18 to 60 years, and had the ability to use audiovisual compact discs (CDs), their burning percentage to be 10-45%, degrees of 1, 2 and 3, those having a minimum reading and writing literacy, and understanding of Persian language. Also those who lacked sensory and motion problems and brain and mental disorders, mental retardation, those who were living in Tehran and the suburbs of Tehran, people whose burns were due to accident and non-self-immolation, non-burning with electricity were included in the current study. The exclusion criteria were the withdrawal of continued study and severity of disease, disability and death of patient.

Sampling method

The sampling method was at convenience and continuous study; the patients were randomly assigned into intervention and control groups. According to the studies carried out in this regard, the effect of educational interventions with 95% of confidence and 80% of test capacity was considered on the number of samples needed for each group and taking into account 10 scores of difference in the quality of the psychological dimension of life of the two groups. So the population was estimated to be 55 people based on the following formula in a way that each group included 50 subjects, considering 10% of the probability of not participating. Finally, 100 samples were considered to be participated in the study with the formula $n=2(z1-\alpha/2+z1-\beta)2s2/(\mu1-\mu2)2$. In this formula $z1-\alpha/2=1.96$, $z1-\beta=0.84$, $s=9$ and $\mu1-\mu2=5$.

Measures

Two questionnaires were used in the recent study. The demographic information questionnaire and the status of the disease including some questions about gender, age, occupation, marital status, burning agent or source of heat (gasoline, gas, flame, hot liquids, oil, hot food, etc.), level of education, grade and percentage of burns, burning area, city, location of incident and economical status. This questionnaire was selected by the patient and a research associate on the first day so that the samples inclusion in the study was completed. The next instrument was a questionnaire of burn patient's Quality of Life [BHS-B (Burning Specific Health Scale). The social dimensions of this questionnaire were used.

The questionnaire included 40 questions about skin sensitivity to heat, body image, hand performance, care for burnt areas, communication, ability to perform simple activities, sexual function and psychological dimension with the options of high, moderate, low and never, which had been scored from 1 to 5, respectively. Each questionnaire had at least one and maximally five scores. Based on this questionnaire, the quality of life in each dimension or domain was determined separately and in all domains. From 40 questions of the questionnaire, 18 questions were related to the physical dimension of quality of life, 11 questions were about the psychological dimension of quality of life and 11 questions revealed the social dimension.

Demographic and disease information questionnaire was given to 10 faculty members of the faculty of medical sciences in terms of validity and content validity, and then their opinions were applied as the reliability and validity of the BHS-B were measured by Kildal et al. in 2001 using its dimensional analysis [16] In Iran, Pishnamaazi et al. (2009) had measured its validity and reliability by Alpha Cronbach of 94% in the burn patients in Shahid Motahari and Hazrat Fatemeh hospitals [17] and at Qotboddin Shirazi Hospital [18], calculated the reliability of this tool with Alpha Cronbach of 98%. In our study, Alpha Cronbach was measured 94%.

Education and treatment program

Based on the implementation of this method, the researcher referred to the Burn Medical Educational Center of Shahid Motahari Hospital after receiving the study confirmation from Iran University of Medical Sciences and the ethical code from the university's ethics committee (93-02-28-24922-106366 on 8/12/2014 and registered in a clinical trial with the code IRCT 2014112920145).

After introducing the principal investigator and the collaborators of the research and the research objectives to the hospital's officials and obtaining permission, he referred to the departments and, while introducing himself and the colleagues of the research and the study objectives to the departmental authorities, the samples were randomly provided according to the conditions of inclusion as the control or intervention group. After explaining the procedure and ensuring the anonymity of the samples, a written informed consent was obtained from each participant. They were announced that the transportation cost and the cost of left work to be compensated at 3 and 6 months by the researcher. Before the intervention, the demographic information and burn characteristics questionnaire was completed by the patient with the help of a research associate and using medical records. Then the intervention and control groups received face-to-face routine trainings, however, the intervention patients, in addition to routine trainings, received the self-care discharge education of a burnt patient given at the time of discharge in an educational CD containing text, slide, film and recorded sound; then the researcher gave this CD to the patients to perform at home. In educational session they used CDs and answered questions for 30-60 minutes at the time of discharge.

Educational content was prepared based on the sources of self-care educations of the burn patients. The questionnaire of the quality of life of burnt patients in the psychological, physical and social dimensions was completed by the patient before the intervention on the day of discharge and 3 and 6 months after the intervention; the telephone number, email address, researcher's telegram number were given to patients to call if necessary. The researcher conducted a weekly phone contact with the patients in the intervention and control groups to follow up and ensure the preservation of the samples. After 3 and 6 months of intervention, the patients in both the control and intervention groups were contacted by phone to complete the questionnaire. Patients completed the questionnaires in the manner of the self-report. At the end of the research, the educational CD was provided to the control group for observing ethics in the research.

Ethical consideration

This study was approved by the Ethics Committee of Iran University of Medical Sciences and the Ethics Committee of the place where research was conducted (Ethic code: 93-02-28-24922-106366).

The clinical trial was approved by the Iranian Registry of Clinical Trials (IRCT) under No: IRCT2014112920145N1. The CONSORT checklist was used to report the study.

Statistical analysis

After collecting raw data for the analysis, the descriptive and inferential statistics (Chi-square and independent and paired t tests for the distribution of normal variables), Fisher's exact test, nonparametric tests such as Mann-Whitney, Wilcoxon and Friedman test and Dunn test, with Bonferroni's correction, Spearman correlation coefficient were used by SPSS software (version 21, Chicago, IL, USA). It should be noted that all of the participants were included in the process and no one was excluded during the investigation.

RESULTS

Among the participants of this study, 56% of the subjects were male and 56% were female. Only 34% of them in the intervention group were in the age range of 39-48 years and 44.9% of the control group`s members were in the age range of 29-38 years.

According to the statistics, most of them (44% in the intervention group and 79.6% in the control group) were married. In the intervention group, 48% and in the control group, 62.5% were employed; 52.1% had diploma in the intervention group and 66.7% had diploma educational level in the control group.

Moreover, 36% in the intervention group and 34% in the control group were burned by fire flame and 60% in the intervention group and 64% in the control group had a degree of burns of 1, 2 and 3. Furthermore, 24% in the intervention group had burning percentage of 15-20% and in the control group, 36% had burning percentage of 21-26%. About 46% in the intervention group had burn in the trunk, hand, and foot and 47.9% in the control group had burn in the whole body.

The majority of patients in the intervention and control group (58.1%, 79.2%, respectively) were resident in Tehran. The majority of patients in the intervention and control group (58.1% and 53.5%, respectively) were burned at home. In the intervention group, 56.5% were on average economical level and 37.8% were in weak economic level.

The Mann-Whitney test showed that before intervention, the mean of social function in intervention and control groups was 1.92±0.6 and 1.55±0.46, which was statistically significant (p<0.001). The mean difference of the score of social function in both intervention and control groups at the time before intervention was statistically significant and the mean score of social dimension in the intervention group was slightly higher than the control group.

Mean and standard deviation of social function score in intervention and control groups three months after intervention were 3.29±0.95 and 2.15±0.39, respectively and six months after intervention, the mean and standard deviation of intervention and control group were 4.05±0.77and 2.47±0.56, which were statistically significant (p<0.001) (Table 1).

Table 1: Comparison of mean and standard deviation of social function score in intervention and control groups one month before and three and six months after intervention

	Intervention group	Control group
Mean and standard deviation before intervention	1/92 ± 0/6	1/55 ± 0/46
Test result	Z = -2/98 P value < 0/001	
Mean and standard deviation three months after intervention	3/29 ± 0/95	2/24 ± 0/4
Test result	Z = -6/83 P value < 0/001	
Mean and standard deviation six months after intervention	77/0 ± 05/4	2/75 ± 0/58
Test result	Z = -7/74 P value < 0/001	

Considering the chi-square value (=95.14) and the value of significance level (p<0.001) in Table 2, since the level of significance was less than 0.05, the assumption of the equality of the mean scores of social function during three periods was rejected statistically, that is, the average score of social function varied at least in two periods of the three ones. Therefore, in order to determine which of the two periods had a significant difference, the Dunn follow-up test was used.

The results of the test were presented in Table 2 and 3, while the mean score of each period had significant differences with other periods because the corrected significance level value was less than 0.05.

Figure 1 shows that the social dimension score before intervention in the intervention group was slightly higher than the control group, but after 3 and 6 months of intervention, the social function score of the intervention group demonstrated a significant difference compared to the control group (p<0.001).

Table 2: Results of comparison of mean and standard deviation of social function score before intervention, 3 and 6 months after intervention in intervention group using Friedman test in intervention group

Time	Number	Mean	Standard deviation	Test result
Before intervention	50	1/92	0/6	Chi-square = 95/14 df = 2 p-value<0/001
Three months after intervention	50	3/29	0/95	
Six months after intervention	50	4/05	0/77	

Table 3: Comparison results of social function score in 3 periods with Dunn pairwise test with Bonferroni's correction in intervention group

Difference	Standardized test statistic	Corrected significance level
Before intervention - 3 months later	-5/4	<0/0.001 adj. p-value
Before the intervention - 6 months later	-9/45	<0/0.001 adj. p-value
3 months later - 6 months later	-4/05	<0/0.01 adj. p-value

Table 4: Comparison results of social function score in 3 periods with Dunn pairwise test with Bonferroni's correction in control group

Difference	Standardized test statistic	Corrected significance level
Before intervention - 3 months later	-5/25	<0/0.001 adj. p-value
Before the intervention - 6 months later	-8/4	<0/0.001 adj. p-value
3 months later - 6 months later	-3/15	<0/0.01 adj. p-value

Figure 1: Comparison of the trend of changes in the mean score of social function 3 and 6 months before and after intervention in two groups

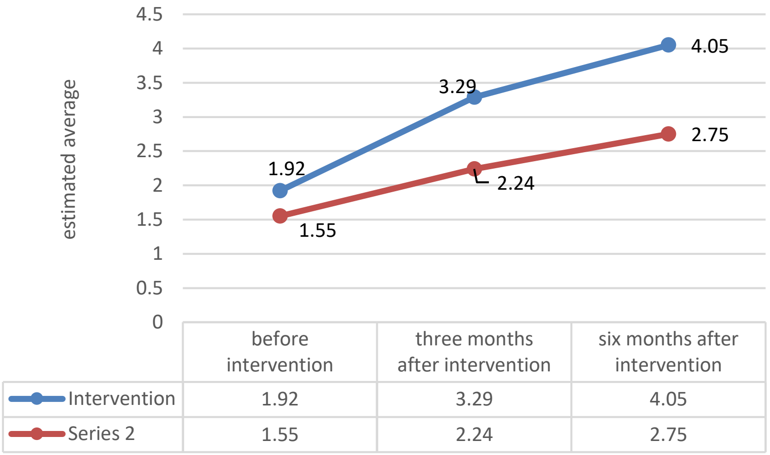

DISCUSSION

The results of this study showed that multimedia virtual self-care discharge education improves social functioning of burn patients. This finding is consistent with the study by Li et al. In their study, they found that social functioning of burn patients 5 weeks after rehabilitation was better than the control group [19]. Tang et al. (2015) study also showed that the use of rehabilitation interventions and self-care measures increased the social function dimension of patients three months after intervention, which is consistent with the present study [20]. Radwan et al. (2011), who found that running a 7-day rehabilitation program for 2 weeks improved social performance in the experimental group, also consistent with our study [21]. Burn injuries affect one's ability to cope with life's stresses and interact with the outside world [8]. These people feel ashamed and embarrassed about being in the community and communicating with others due to the apparent changes caused by burns and burns, and the look and sometimes excitement of others suggests that they feel compassion and compassion. For this reason, they lack communication and social skills and require intervention [23]. Hojati et al.'s study showed that psychosocial interventions had a significant effect on life satisfaction, occupational activity, mental health, physical health, quality of life, and social relationships.

Therefore, these interventions increase patients' life satisfaction and social relationships [24]. The results of the Fatimid study showed that patients' quality of life in the social dimension was relatively undesirable, so it recommended that patients be taught communication skills [25]. The consequence of education in the community is maintaining and promoting health and will have many benefits, including reducing the duration of illness, accelerating client independence and maintaining self-confidence in self-care [26]. A study by Elalem et al. (2018) showed that self-care nursing intervention was effective in burn patients.

And self-care intervention led to active participation of patients in their treatment and led to a significant improvement in quality of life as well as self-esteem, which is consistent with the present study which correlates with self-esteem and quality of life later. Social has had a significant impact [27]. Hospital discharge is associated with stress and anxiety and an increased need for patients to receive information. Information training is essential for the well-being of patients because patients experience discomfort after awareness discharge [28]. Discharge does not mean the end of treatment for burn patients, but discharge means that the patient and his or her family must resume the responsibility of managing their lives without assistance from hospital staff. Burn patients need to adapt to new situations that include self-care at home, lifestyle change and return to society [29].

Limitation and recommendation

One of the limitations of the present study was the mental state of the patient which could be effective in answering the questions. It was the researcher who emphasized the importance of the subject in the result of the research and asked the samples to fully comply with the CDC's recorded care instructions and followed up the samples by telephone.

CONCLUSIONS

Given the findings of the present study, it is important to provide virtual and multimedia education and to institutionalize a self-care culture. This allows the patient to self-care and engage in self-care. Nurses and caregivers in burn centers need to know that these patients will be socially isolated and isolated, and that they need to return to the community, and nurses are one of those who treat patients' behaviors and attitudes more than others. So they can help them get back into the community with proper education, but education through lectures does not meet their educational need because they are involved in the mental illness of their illness and burn accident, especially while they are still in hospital. And maybe the ability to understand and learn training in that situation So they need virtual training to use it in the right conditions and in a comfortable place. That is, self-care education should be continuous, accessible, follow-up, and economically viable. So using educational CDs, which is a virtual teaching method, can be a good option. It is recommended that nurses use this method in clinical centers.

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Conflict of interests

It is not declared by the authors.

Ethics approval and consent to participate

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The effect of smartphone-based education on self-efficacy of women with gestational diabetes: A controlled clinical trial

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Abstract: *Background and Aim: Women with gestational diabetes are at risk for type 2 diabetes. Development of self-efficacy is an important tool for active participation in treatment and behavioral change and can have a significant effect on type 2 diabetes-preventative behaviors. Therefore, it is necessary to search for an effective teaching method to improve the self-efficacy of these individuals. Accordingly, the aim of the present article is to determine the effect of smartphone-based education on self-efficacy in women with gestational diabetes (GDM).*

Materials and Methods: This randomized clinical trial was conducted on 90 women with GDM (n=45 individuals per group) at Yas Medical Center, Tehran. GDM software was installed on the cell phone of the intervention group. The control group only received the prenatal routine training. The self-efficacy questionnaire was completed before and 6 weeks after the intervention by the two groups. Data analysis was carried out using Chi-square, t-test, independent t-test, and Fisher exact test in SPSS ver. 16. P-value<0.05 was considered as the significance level.

Results: There was a statistically significant difference between the two groups in terms of self-efficacy changes before and 6 weeks after the intervention (p<0.001), meaning that these changes were significantly higher in the experimental group than the control group.

Conclusion: Smartphone-based education can increase self-efficacy in women with GDM as an effective and low-cost method.

Keywords: *smartphone-based; self-efficacy; gestational diabetes; randomized clinical trial*

INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the most important pregnancy challenges worldwide today. A total of 20.9 million (16.2%) of the pregnancies were associated with hyperglycemia in 2015, with 85.1% of cases being due to GDM. The prevalence of GDM is increasing in developed and developing countries [1].

A systematic review reported that the prevalence of GDM was between 0.7 to 18.6% in Iran [2]. GDM is associated with increased risk for mother and fetus (preeclampsia, cesarean section, injuries during pregnancy, macrosomia) during pregnancy and next stages of life [2,3].

Health guidelines recommend lifestyle modifications, including exercise and healthy diet, to prevent or manage GDM [4]. Although some factors, such as gender, age, and genetics cannot be changed to correct lifestyle, specific cognitive-behavioral factors, such as self-efficacy, can be considered for the promotion of healthy behaviors [5]. Various studies show the positive effect of education on the self-efficacy of diabetic patients [6,7].

Therefore, factors that play a central role in increasing the self-efficacy of diabetic people include empowerment through training on glucose self-monitoring skills, diet modifications, weight control, exercise, and physical activity, which are key factors in prevention of the increasing incidence of diabetes and its related complications are strongly based on self-efficacy and self-care of patients [8].

In a study on women with a history of GDM, Bastani and

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Zarabi (2010) showed that 57% of these women had high self-efficacy and the rest had low self-efficacy [9]. Considering the low self-efficacy of women with GDM, there is a need to strengthen self-efficacy in this group of women.

However, there are few studies on self-efficacy and encouraging strategies for taking type 2 diabetes-preventive behaviors in women with GDM [10]. Healthcare providers can play a key role in preventing type 2 diabetes [11]. One of the most useful strategies to achieve the goal of preventing type 2 diabetes in these people is education [12].

Many factors challenge the effectiveness of the educational program for women with GDM. GDM imposes an important role on pregnant women. The pregnant woman should, on the one hand, carry out duties related to her parental role and, on the other hand, engage in caring and medical practices and take control of the disease. Pregnant women need to spend a lot of time accessing systematic and valid educational programs in order to attend training classes. Busy schedule, difficulty traveling to attend training classes, and lack of time and energy due to family affairs and the difficulties of child care have limited the opportunity to take advantage of face-to-face training [13].

On the other hand, common methods, speech or face-to-face conversation require enough power and cost, and its problems include rapid forgetting, language mismatch, and patient misunderstanding [14]. Therefore, there is a need for the use of new educational approaches in this group of pregnant women.

The advancement in information and communication technology has made it possible to implement appropriate healthcare interventions to improve and manage health [15].

One of the technologies that can easily provide health services to a large population is the use of a smartphone-based system. Mobile communication has created opportunities for the departure of care from the monopoly of hospitals and clinics and to the patient's place of residence [16]. Smartphone-based education can help improve patient education, prevent diseases, reduce medical costs, and increase access to healthcare services [17], effectively engage patients, and positively influence their behaviors and health outcomes.

The results of a study by Hirst et al. (2015) showed that it was easy and acceptable for women with GDM to use smartphone-based education to control blood glucose [18]. Paul et al. (2016) showed in their study that the use of smartphone software led to an increase in daily walking hours in patients with a history of stroke [19]. Tehrani et al. (2016) also showed in their study that the intervention based on new communication technologies (multimedia, internet, and mobile) led to improved physical activity in women [20]. However, the results of a study by Cafazzo et al. (2012) showed that smartphone-based education was not associated with changes in HbA1c levels in people with type 1 diabetes [21]. Kirwan et al. (2013) also showed that smartphone-based education has not significantly changed the self-efficacy, self-care, and quality of life of the samples [22].

Despite the variety of applications in the field of health-related communication technologies, few studies have been conducted on their efficacy [23]. Accordingly, it's likely that smartphone capabilities can improve patient compliance with their disease management, but there is various evidence in this regard, and further researches are warranted [24]. Furthermore, there is little evidence regarding smartphone-based health interventions in developing countries, so further research is needed to measure the effectiveness and use of mobile-based health interventions as well as seek strategies to access to this type of services [25].

Considering that diabetes is one of the most important healthcare and socioeconomic problems worldwide on the one hand, and considering that we can prevent the incidence of diabetes in later life in women with GDM with proper training programs and since there are few interventional studies on this high risk population, on the other hand, the present study was thus designed to determine the effect of software-based education on self-efficacy.

MATERIALS AND METHODS

Design and Participants

This (double-blind) clinical trial study was performed on 90 women with GDM in an educational hospital affiliated to Tehran University of Medical Sciences from June to November 2017. This study was approved by the Ethics Committee of Iran University of Medical Sciences (Code of Ethics: 1396.9311686020IUMS). The clinical trial protocol was registered in IRCT (IRCT20170509279N4). The CONSORT checklist was used to report the article.

Participants

All participants were evaluated for inclusion criteria, which included 1. Pregnant women diagnosed with GDM. 2. They were in the age range of 18-40 years. 3. The gestational age was 22-32 weeks and single-pregnancy and were pregnant without assisted reproductive techniques. 4. They had reading and writing literacy. 5. They had mobile phones with

Android operating system and were able to work with mobile applications. Exclusion criteria also included: 1. Unwillingness to continue cooperation, 2. Delivery during the intervention

Intervention

To determine the required sample size at a confidence interval of 95% and a test power of 80% and assuming that determining the effect of smartphone-based education on self-efficacy in women with GDM compared with the control group leads to a 10 score difference (d = 10) so that this difference is statistically significant, After setting the values in the following formula, the sample size in each of the two control and experimental groups was estimated to be at least 42 people, which was increased to 45 people considering the drop-out rate. It should be noted that based on similar studies, the standard deviation of self-efficacy was estimated to be S = 16.3 [9].

Researchers provided educational content using library studies and valid websites of the National Institute of Diabetes and Digestive and Kidney Diseases, the National Diabetes Services Scheme, and relevant studies. The training program includes the definition of GDM, risk factors, complications of GDM on mother and fetus, diagnosis of GDM, prevention of type 2 diabetes, the definition of type 2 diabetes and statistics on type 2 diabetes in diabetic women, blood glucose measurement method, nutrition, physical activity, drug therapy, breastfeeding, stress management, and post-partum diabetic screening. The validity of educational content was approved by five faculty members in education and experienced in the care of women with GDM and two gynecologists. The primary educational software was designed by a software engineer in the Android Studio 2 environment. XML language was used to design the format and appearance of the program and to implement grammatical code, classes, and software commands, the Java programming language was used. To assess the content validity of the software, it was provided to two android experts and 10 women with GDM. Modifications were applied to the software prototype and eventually the modified and final version of the software was produced. The educational program was installed on the smartphone of the intervention group and its use was practically taught to them during an in-person session in the gynecology ward as well as in the form of a manual. The researcher assessed the extent of learning to use the training program. The educational program includes 8 files, each with a password and the researcher provided the participant with the code of each file via a telephone call twice a week. It was also possible to recall the use of the program on educational software, and the use of the training program and the implementation of the principles taught previously was reminded to the participants this way twice a week. The intervention group received routine hospital training in addition to receiving the smartphone training program. In order to prevent the transfer of information to the control group, the training program for the discharge time was installed on participants' phones. The intervention group was also asked to not receive additional information about self-efficacy from other sources of information, such as journals, peer groups, books, etc. during the intervention and 6 weeks later. The control group received only routine hospital training. Considering ethical considerations, the educational content was provided to the control group as a manual after the end of the study.

Tools

The data in this study were collected by using a demographic and underlying information questionnaire and self-efficacy scale for diabetic patients. demographic and underlying information questionnaire consisted of 14 questions on age, level of education, employment status, income level, pre-pregnancy height and weight, history and number of abortions, history of type 2 diabetes in first-degree relatives, history of giving birth to a baby weighing more than 4 kg, and history of hypertension. The questionnaire was prepared using library studies and research on the subject matter of the research. The next tool used in this study is the self-efficacy scale for diabetic patients [26], which is a standard and valid tool and has been used in many studies [9, 27-30] . The self-efficacy scale of diabetic patients includes eight items with score range of 1-10 and rated using 10-point Likert scale. The responses are categorized as 1 (I’m not sure at all) to 10 (I'm quite sure). The score of each item is the same as the number marked by the person being studied. If two consecutive numbers of an item are marked by a person, the lower number will be considered, and if the two numbers are not consecutive, then no score will be assigned to that item. If more than 2 questions are not answered, the questionnaire will not be scored. Self-efficacy score is obtained by calculating the average score of 8 items. A higher score shows higher self-efficacy. Reliability of the tool was reported in Lorig’s study (2008) using a test-retest method (r = 0.8). The internal consistency of the tool was evaluated by measuring the Cronbach's alpha (α = 0.85) [28]. The validity of the tool was also evaluated and verified by Bastani and Zarabi (2011) using content validity method. To determine the reliability of the Persian version of the tool in the above study, Cronbach's alpha was obtained as α = 0.87 [9].

In this study, content validity method was used to determine the scientific validity of the demographic and undrlying information questionnaire and the self-efficacy scale. The above tools were provided to 10 faculty members of the Faculty of Nursing, Tehran University of Medical Sciences, Shahid Beheshti and Iran and then used in this research after obtaining their opinions confirmation of scientific validity in this research. To determine the reliability, self-efficacy scale for diabetic patients was given to 30 patients with GDM who met inclusion criteria after obtaining their consent. Then, the Cronbach α coefficient was calculated and the alpha value of 0.90 was obtained. It should be noted that these 30 participants were not included in the study.

Statistical Analysis

After collecting data and entering them into SPSS ver. 16, data analysis was carried out using descriptive statistics (mean, standard variance, standard deviation) and inferential statistics such as Chi-square and t-test. The significance level was considered as P-value<0.05.

RESULTS

Out of 90 subjects entered into the study, one in the intervention group and two in the control group were excluded from the study due to childbirth and the final analysis was carried out on 87 subjects (Figure 1). The findings indicate no significant difference between the two groups in terms of the variables such as age, level of education, employment status, economic status (Table 1) and clinical information such as body mass index, gestational age, gravida, history of abortion, history of hypertension, history of diabetes in first-degree relatives, and history of giving birth to a baby weighing more than 4 (Table 2).

Table 1: Comparison of demographic characteristic in intervention and control groups

Group		Intervention			Control			Results of the statistical test
Variable		N	%	Mean (SD)	N	%	Mean (SD)	
Age (years)	Less than 30 years	8	17/8	33/66 (3/93)	10	22/2	32/55 (4/44)	*t=1/255 df=88 p=0/213
	30-35 years old	22	48/9		25	55/6		
	Over 35 years old	15	33/3		10	22/2		
Level of education	Middle school	10	22/2		10	22/2		**χ2= 2/369 df=3 p= 0/499
	High school	16	35/6		11	24/4		
	Diploma	10	22/2		16	35/6		
	Academic	9	20		8	17/8		
Job	Housewife	27	82/2		39	86/7		χ2=0/338 df=1; p=0/561
	Employed	8	17/8		6	13/3		
The economic status	Weak	10	22/7		18	40		***p= 0/241
	Moderate	32	72/7		25	55/6		
	Good	2	4/5		2	4/4		

** Independent T Test; ** Chi-square test; *** Fisher's exact test*

Table 2: Comparison of clinical characteristics in intervention and control groups

Group		Intervention			Control			Results of the statistical test
Variable		N	%	Mean (SD)	N	%	Mean (SD)	
Pre-pregnancy BMI	20-25	7	15/6	26/44 (1/63)	5	11/1		*t=1/602 df=88 p=0/113
	25-30	38	84/4		36	80		
	30-35	0	0		4	89		
Gestational age (week)	20-24	2	4/4	29/2 (2/2)	6	13/3		t=0/531 df=81/88 p=0/597
	25-29	18	40		11	24/4		
	30-32	25	55/6		28	62/2		
Gravida	1	9	20/5	2/45 (0/99)	6	13/3		t=0/668 df=87 p=0/506
	2	13	29/5		16	35/6		
	3	15	34/1		16	35/6		
	4,5	7	15/9		7	15/6		
History of abortion	Yes	18	40		22	48/9		**χ2=0/72 df=1; p=0/396
	No	27	60		23	51/1		
History of hypertension	Yes	5	1/11		7	6/15		χ2=0/385 df=1; p=0/535
	No	40	88/9		38	88/4		

History of diabetes in first-degree relatives	Yes	21	46/7		27	60		χ2=1/607 df=1; p=0/205
	No	24	53/3		18	40		
History of giving birth to a baby weighing more than 4 kg	Yes	3	6/7	2	1	2/2		*p= 0/616
	No	42	93/3		44	97/8		

** Independent T Test; ** Chi-square test; *** Fisher's exact test*

As the results of Table 3 show, there was no significant difference between the two groups in terms of the mean pre-intervention self-efficacy score (p = 0.701), but the mean self-efficacy score in the experimental group was significantly higher than the control group 6 weeks after intervention (p <0.001).

There was statistically a significant difference between the two groups in terms of self-efficacy changes before and 6 weeks after the intervention, and these changes were significantly higher in the experimental group than the control group (Table 4).

Table 3: Numerical indicators of self-efficacy in women with GDM in the experimental and control groups and comparison of the mean self-efficacy scores before and 6 weeks after intervention

Self-efficacy	Experimental		Control		Results of the statistical test
	Mean	SD	Mean	SD	
Before the intervention	4/11	0/87	4/19	1/11	t=0/385 df=88; p =0/701
Six weeks after intervention	6/77	0/55	4/94	0/73	t=13/138 df=85; p <0/001

Table 4: Comparison of self-efficacy changes in women with GDM in the control and experimental groups

Changes	Experimental		Control		Results of the statistical test
	Mean	SD	Mean	SD	
Self-efficacy Six weeks after intervention	2.68	0.69	0.79	0.69	t=12/723 df=85; p<0/001

DISCUSSION

The results of the present study on self-efficacy comparison in the control and experimental groups before and 6 weeks after intervention showed that there was no significant difference between them in terms of the mean pre-intervention self-efficacy score (Tables 3 and 4) (p = 0.701), but the mean self-efficacy score in the experimental group (77.55± 0.6) was significantly higher than the control group (4.94 ± 0.73) six weeks after the intervention (p<0.001).

To interpret such result, it can be said that new technologies such as smartphone-based training programs can increase the self-efficacy of GDM patients. Self-efficacy is one of the factors positively affecting the sustainability and application of health behaviors [31]. In addition, learning new skills such as glucose self-monitoring, diet modification, weight control, exercise, and physical activity as strategies to empower of women and diabetic patients, are among key factors in preventing the progression of diabetes and its related complications, which are strongly based on self-efficacy and self-care of patients [8]. In a predictive correlation study on 400 women with GDM, Kordi et al. (2016) concluded that there is a need to enhance self-efficacy in these women [27] considering the direct linear relationship between self-efficacy and self-care variables and the low self-efficacy of women with GDM. The current study, consistent with the study by Atak et al. (2008) in Ankara and Shi et al. (2010) in China, showed that educational intervention is effective in promoting self-efficacy in diabetic patients [6, 7]. The results of the study by Absavaran et al., and Gheraati et al. show that mobile phone education, as a new method, is effective in promoting health and preventive behaviors [32,33]. Goodarzi et al., (2014) also showed that education through SMS service significantly increased the self-efficacy of people with diabetes (P = 0.02) [34]. Regarding the consistency of the results of the present study with the studies on the effectiveness of mobile phone education on the promotion of health behaviors and increasing self-efficacy, it can be said cell phone availability as well as the use of reminder messages provides the ground for motivation for change and improvement of health promoting behaviors in individuals. Mobile phone education will support and encourage patients' education self-care skills [35]. The results of the study of Qanbari et al. referred to limited health literacy as a common problem in pregnant women covered by healthcare centers. Considering that limited health literacy can prevent proper understanding of health information provided, health workers are recommended to use effective methods for the transfer of information to these individuals [36]. In research on 120 pregnant women in 2015, Kharazi et al. showed that it is necessary to educate the necessary subjects and provide information about pregnancy considering its adverse outcomes on the health status of the family and society [37]. Therefore, since healthcare professionals always seek better ways to encourage healthy behaviors, education administrators should also choose the most effective and useful educational approach to meet the needs, capabilities, and capabilities of pregnant women. Non-printed media have been referred to as one of the effective ways to present health messages to people (20) . Kreps et al. (2011) conducted a study entitled "Investigating motivational messages on improving adherence to drug regimen in patients with chronic diseases". The results of their study showed that motivational messages increased adherence to the drug regimen in the intervention group and are consistent with the present study, in which Reminder messages referring to the training program were considered as a factor to remind health behaviors [38]. Paul et al. (2016) showed in their study that the use of software, which can be installed on the mobile phone boosted daily walking in patients with a history of stroke [19]. Vahedian-Shahroudi et al. (2016) also showed in their study that the intervention based on modern communication technologies led to improved physical activity in women [20]. In another study, smartphone software was useful for managing gestational diabetes [15].

The strength of this study is the use of a smartphone-based training method to improve health indicators and preventative behaviors in people, because on the one hand, smartphone-based education is a new method in education that is compatible with social and cultural changes of the present era. On the other hand, the efficacy of this method has been confirmed in various studies and on people with different diseases.

The limitation of this study to women with GDM referred to an educational center in the current study limits generalizing the findings to other women with GDM. In this case, attempts were made to select intervention and control groups from two departments of this center.

It was also possible that the participants received educational information on gestational diabetes from other sources during the intervention period, which was controlled by using the control group.

Another possible limitation was that the participants may be unable to use the software, and attempts were made to the insert an option for reminding the individuals to use the software in order to reduce this limitation, and the user was reminded to study the training program twice a week and use the principles taught.

CONCLUSIONS

The results of the present research indicate that mobile phone-based education is effective on the self-efficacy of women with GDM. Therefore, mobile phone-based education is evaluated in terms of popularity, availability, and cost-effectiveness as a useful tool for training in the present age, and can be used to increase self-efficacy in women with GDM thereby reducing the rate of type 2 diabetes.

Recommendations for Future Research

The findings of this study are based on the self-report of the participants that may contain a kind of bias. Therefore, it is recommended that to use clinical factors such as blood glucose and glycosylated hemoglobin in addition to self-efficacy variable in order to evaluate the effect of mobile phone-software education. Considering the fact that the effect of software-based education on self-efficacy was investigated after six weeks in the present study, it is suggested to investigate such effect in the long run to determine the sustainability of effects of such interventions.

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ORIGINAL ARTICLES

The effect of the transversus abdominis plane block on postoperative analgesia and patient comfort in patients having abdominal surgery with general anesthesia

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Abstract: *Background: This study aimed to investigate the effects of tranversus abdominis plane (TAP) block placed postoperatively using ultrasound guidance for patients with caesarean section, myomectomy, and hysterectomy under general anesthesia by eliminating additional pain caused by surgical incision.*

Methodology: The study has been planned to investigate the postoperative analgesia of 50 patients with TAP blocks applied and non-applied between the ages of 18-65, who will pass abdominal surgery. The study has been conducted in a prospective, randomized and controlled manner. Patients have been classified as those who have undergone TAP block (group: I) and those without block (group: II).

Results: Mean age of the patients in the TAP block group was 35.52 years, the mean age of the other group was 32 years and there was no statistically significant difference. The patients' visual analog scale (VAS) was evaluated at the 30th minute, 1st, 2nd, 6th, 12th, and 24th hours in the postoperative period. For patients with TAP blocks, the VAS score was found to be lower for all hours than patients without TAP blocks (p<0.05). Beginning at the 30th minute and 1st-hour analgesic needs of the block patients were compared with the other group (p<0.05).

Conclusion: TAP, that can be easily and safely placed provides effective analgesia and reduces the additional disruptions caused by the pain.

Keywords: *transversus abdominis plane block, postoperative pain, Bupivacaine, ultrasound-guided regional anesthesia*

INTRODUCTION

The transversus abdominis plane (TAP) block is a regional anesthesia technique described in recent years that provides analgesia for the anterior portion of the abdominal wall after abdominal surgery. With this method, local anesthetic injection of the area between the internal oblique and transversus abdominis muscles will block the afferent nerves of the abdominal wall.

TAP is an attractive method of block simplicity and safety, and it has been shown to provide effective analgesia, reducing the response to postoperative stress and speeding up post-surgery improvement [1].

TAP block can be used in abdominal lower region operations such as hernia repair, appendectomy, abdominal hysterectomy. The most common operation where TAP is used is after cesarean operations. It is used for postoperative pain control in cesarean operations, reduction of analgesic drug consumption, reduction of side effects that may occur

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after the operation, and side effect profile due to analgesics used [2, 3].

The purpose of this study is to investigate the effects of the TAP block, which is performed with ultrasound after surgery, on patients with abdominal surgery such as cesarean, myomectomy, and hysterectomy under general anesthesia, on post-surgery pain and patient comfort.

MATERIALS AND METHODS

The study was conducted following the Helsinki Declaration Principles (rev.2013) and with the approval of the local ethical board. After the written and signed approval of the patients, it has been planned to investigate the postoperative analgesia and comfort of a total of 50 patients The study examined the postoperative pain levels after abdominal surgery with and without TAP block. Subjects were between the ages of 18-65 who will have abdominal surgery with transverse incision (cesarean section, hysterectomy (benign), myomectomy) and the American Society of Anesthesiologists Physical Status Classification System (ASA) I-II. Our study is planned to be prospective, randomized, and controlled. The individuals to be engaged were divided into two groups in a randomized form. Randomization was based on computer-generated code. In the first group, a total of 25 patients were admitted with 16 cesareans, 6 myomectomies, and 3 hysterectomies applied to the TAP block. In the second group, a total of 25 patients who did not undergo TAP block, no needle intervention, 18 cesareans, 5 myomectomies, and 2 hysterectomies were included as the control group. Those with skin infections, patients with organomegaly (hepatomegaly and splenomegaly), people who did not give consent to the method, and individuals with allergies to drugs to be used in the intervention area where Transversus abdominis plane is to be conducted were not included in the study.

0.9% NaCl solution at a rate of 2 ml/kg/hour was administered to the individuals whose oral intake was discontinued 8 hours before the surgery. The individuals to be included in the study were not given any sedative agent on the morning of their surgery. Before the procedure, 6-8 ml/kg volume of liquid replacement with crystalloid was applied to the individuals to be included in the study. 10 cm visual analog scale (VAS) for measuring the pain level was explained in detail to all patients before the procedure. All individuals to be included in the study were followed up with an anesthesia monitor, heart rate (CAH), electrocardiography (ECG) (lead II), systolic blood pressure (SKB), and diastolic blood pressure (DBP) in the preoperative and preoperative period. After induction of anesthesia with 1 µg/kg fentanyl, 0.5 mg/kg rocuronium bromide, and 5 mg/kg thiopental sodium, 4-6% Desflurane and 50% N2O in Oxygen were used to maintain anesthesia following endotracheal intubation. Differently in general anesthesia management at cesarean operations, 5 mg/kg thiopental sodium and 0.5 mg/kg rocuronium bromide were administered in the induction and 4-6% Desflurane, (50% N2O+50% Oxygen) and 1 µg/kg fentanyl are added as inhalation anesthesia after umbilical cord clamping in maintenance. The maintenance dose of rocuronium bromide was 0.15 mg/kg for all cases. A necessary fluid replacement was performed during the operation. At the end of the operation, TAP blocks were placed on the 1st group of patients.

After the necessary sterilization conditions were established, the linear ultrasound probe was sterilely coated and placed in the middle of the iliac crest with the end limit of the ribs. Starting with skin, the layers in descending order, subcutaneous adipose tissue, external oblique muscle, internal oblique muscle, transversus abdominis muscle, and peritoneum were identified. As the tip of the 21Gx100 mm needle (visible cannula by ultrasound, with facet tip) passed through the muscular layers and fascia, a fascial click was felt and the needle was advanced with ultrasound in a controlled manner. After receiving the second click sensation (passage of the fascia of the internal oblique muscle), after a 0.5-1 ml test dose, the location of the needle was fixed and frequently aspirated and applied to the bilateral neurophysical plan so that 20 ml of 0.25% bupivacaine was applied to one side.

The first group of patients was awakened after TAP block treatment and reversal of muscle relaxant with atropine sulfate and neostigmine; the control group was awakened after reversal of muscle relaxant with atropine sulfate and neostigmine at the end of the operation. When VAS>3 in the postoperative period, 1g of paracetamol was administered as an intravenous infusion over 10 minutes in terms of the first analgesic requirement.

Systolic and diastolic arterial pressures, heart rates of all patients were monitored immediately before and postoperatively at 30th minute and 1st, 2nd, 4th, 6th, 12th, and 24th hours after block administration. The pain levels of the patients were measured at the 30th minute, 1st, 2nd, 4th, 6th, 12th, and 24th postoperative VAS values. The need for nausea, vomiting, and initial analgesia was recorded. In this study, none of the patients underwent any invasive procedures other than routine procedures.

When parametric test counts were fulfilled (Kolmogorov-Simirnov) by uploading the data obtained from our study to SPSS (ver:22.0) program, significance test of the difference between the two averages, variance analysis in repeated measurements, Bonferroni test, Mann Whitney U test, Wilcoxon test, Freidman test, and Chi-square test were used when parametric test assumptions were not fulfilled. When α is taken as 0.05, β: 0.10, 1-β: 0.90, 25 individuals were included in each group and the strength of the test was p= 0.90388.

Our data were reported as the arithmetic mean, standard deviation, several individuals, and the level of error was taken as 0.05.

RESULTS

The age of the patients who underwent the TAP block was 35.52 ± 9.60 years; the age of the patients in the group without block was 32.00 ± 8.10 years. There was no difference in age compared to both groups (p>0.05). The duration of the case was 43.60 ± 11.41 minutes in the block group and 45.00 ± 12.24 minutes in the group without block. The difference between the groups in terms of case duration is insignificant (t: 0.41, p<0.678, p>0.05).

When the heart rate values of the groups were compared; the difference between the groups at 30th minute, 1st, 2nd, 4th, 6th, and 24th postoperative hours was found to be significant and the heart rate was lower in the block group compared to the other group (p<0.05) (Table 1).

Table 1: Comparison of groups in terms of heart rate (times/min)

Postop. tıme (hour)	TAP group (mean ± SD)	Control group (mean ± SD)	Result
0	80.50 ± 15.29	80.12 ± 13.85	t: 0.48 p: 0.96
½	86.84 ± 12.09	94.52 ± 14.42	t: 2.04 p: 0.047*
1	81.80 ± 9.30	92.72 ± 9.96	t: 4.00 p: 0.001*
2	80.44 ± 5.60	86.84 ± 6.74	t: 3.64 p: 0.001*
4	78.56 ± 6.31	86.48 ± 7.56	t: 4.01 p: 0.001*
6	77.0 ± 5.82	81.28 ± 5.19	t: 2.74 p: 0.009*
12	75.64 ± 7.40	78.44 ± 5.21	t: 1.54 p: 0.129
24	73.72 ± 4.71	76.56 ± 5.14	t: 2.03 p: 0.047*

**p<0.05 significant; SD – standard deviation*

When comparing the rest Visual Analogue Scale (VAS) values assessed at various times of patients in the two groups of study, the difference was significant (p<0.05). The resting VAS values measured at different periods of the individuals in the TAP block group were lower (Table 2).

Table 2: Comparison of resting values of groups

Postop. tıme (hour)	TAP group (mean ± SD)	Control group (mean ± SD)	Result
½	5.04 ± 1.59	8.88 ± 0.83	p: 0.001*
1	4.36 ± 0.86	8.12 ± 0.66	p: 0.001*
2	4.0 ± 0.95	7.40 ± 0.81	p: 0.001*
4	3.20 ± 0.95	6.92 ± 0.90	p: 0.001*
6	2.80 ± 0.76	5.64 ± 0.81	p: 0.001*
12	1.84 ± 0.74	4.0 ± 0.85	p: 0.001*
24	1.24 ± 0.43	3.44 ± 0.76	p: 0.001*

**p<0.05 significant; SD – standard deviation*

When the systolic blood pressure (SBP) values measured at different times were compared, the difference was found to be significant when the individuals in both groups were compared at the 1st hour and 4th hour postoperatively (p<0.05). SBP values of the individuals who received block at 1st and 4th hours are lower. A significant difference between the groups at 30th minute, 2nd, 6th, 12th, and 24th postoperative hours was not found (p>0.05) (Table 3).

Table 3: Comparison of groups in terms of systolic blood pressure (mm/Hg)

Postop. tıme (hour)	TAP group (mean ± SD)	Control group (mean ± SD)	Result
0	112.4 ± 10.9	111.24 ± 10.94	t: 0.48 p: 0.96
½	128.4 ± 15.18	137.2 ± 19.26	t: 1.79 p: 0.079
1	122.8 ± 10.21	132.8 ± 14.86	t: 2.77 p: 0.008*
2	119.6 ± 10.59	126.8 ± 14.92	t: 1.96 p: 0.055
4	115.6 ± 10.83	126.0 ± 12.24	t: 3.18 p: 0.003*
6	114.4 ± 11.93	113.24 ± 23.92	t: 0.21 p: 0.829
12	116.0 ± 9.57	110.8 ± 10.37	t: 1.84 p: 0.072
24	116.6 ± 7.57	112.8 ± 9.79	t: 1.45 p: 0.429

**p<0.05 significant; SD – standard deviation*

When comparing the diastolic blood pressure (DBP) values of the patients with and without TAP block, a statistically significant difference was found between the two groups at the 30th minute, 1st, 2nd, and 4th hour postoperatively (p<0.05). DBP values of the individuals who received block at these hours are lower. No difference between the two groups at the 6th, 12th, and 24th hours was found (p>0.05) (Table 4).

Table 4: Comparison of groups in terms of diastolic blood pressure (mm/Hg)

Postop. tıme (hour)	TAP group (mean ± SD)	Control group (mean ± SD)	Result
0	65.4 ± 8.1	67.8 ± 9.27	t: 0.77 p: 0.335
½	76.4 ± 8.6	83.8 ± 11.03	t: 2.77 p: 0.009*
1	77.2 ± 7.37	84.0 ± 10.0	t: 2.73 p: 0.009*
2	71.6 ± 6.87	80.0 ± 10.4	t: 3.36 p: 0.002*
4	70.8 ± 8.12	78.8 ± 11.66	t: 2.81 p: 0.007*
6	70.8 ± 7.59	72.8 ± 8.42	t: 0.88 p: 0.382
12	71.2 ± 8.32	71.6 ± 8.5	t: 0.16 p: 0.867
24	66.4 ± 8.10	68.8 ± 9.27	t: 0.77 p: 0.335

**p<0.05 significant; SD – standard deviation*

When comparing the need for analgesic drugs for the groups in the first 30 minutes, the difference was significant (p<0.05). Fewer patients in the TAP block group reported the need for analgesic drugs. When comparing the need for analgesic drugs for the groups at 1st hour, the difference was found to be significant (p<0.05). As can be seen, 8 patients in the TAP block group within the first postoperative hour and 25 patients in the control group within the first postoperative hour stated that they needed analgesics. Individuals without block application stated that they needed analgesics at the 30th minute and 1st hour, while two people in the block group needed analgesics at later hours (Table 5).

Table 5: Comparison of resting values of groups

Postop. tıme (hour)	TAP group (mean ± SD)	Control group (mean ± SD)	Result
½	8 patients (32%)	16 patients (64%)	p: 0.024*
1	0 patient (0%)	9 patients (100%)	p: 0.001*
12	2 patients (11.7%)		p: 0.49

**p<0.05 significant; SD – standard deviation*

When comparing postoperative nausea and vomiting (PONV) according to the groups, a statistically significant difference was found between the two groups at the 30th minute postoperatively ($p<0.05$). Nausea was never seen in the block group, whereas nausea was seen in 10 of the individuals who did not receive the block. No difference in nausea compared to both groups at the first hour was found ($p>0.05$). 4 patients (%16) in the non-block group indicated they had nausea. While 100% of the two groups did not have nausea at the 2nd hour, no difference was found between the two groups at the 4th hour ($p>0.05$). Nausea was observed in two individuals who did not receive block at the 4th hour. Individuals in both groups did not experience nausea at the 6th, 12th, and 24th hours.

When comparing the two groups in terms of vomiting, the difference at the 30th minute was significant ($p<0.05$). No vomiting was observed in the TAP block group, while vomiting was observed in 9 patients (36%) in the non-TAP group. No difference in vomiting compared to both groups at the first hour was found ($p>0.05$). Vomiting was observed in 4 patients (16%) in the non-block group, while no vomiting was observed in the other group. No vomiting was observed at the 2nd, 6th, 12th, and 24th hours. There was no difference between the groups at the 4th hour ($p>0.05$). Vomiting was observed in 2 patients (8%) without block application.

DISCUSSION

Today, pain is one of the frequent causes for doctor visits and is held responsible for a significant part of the loss of the labor force [4, 5]. Among the negative effects and complications; pain includes thromboembolic complications due to immobility and prolonged hospitalization time [4, 5].

It has been reported that with postoperative pain treatment using regional anesthesia methods, analgesic drug need is reduced and the recovery process of patients is faster [6]. The use of effective regional anesthesia methods in postoperative pain treatment also reduces the side effects of systemic analgesic drugs [7, 8].

TAP block is a regional anesthesia technique performed to provide analgesia after abdominal operations [9]. TAP block is used for retropubic prostatectomy [10], abdominal operations with bowel resection [11, 12], gynecological malignancy operations, total abdominal hysterectomy operations [13], cholecystectomy [14], and appendectomy operations [15] postoperative analgesia. Some of these studies were performed using the TAP block, a petite triangle, and a part of the study using the loss of resistance technique, followed by ultrasound. During the TAP block, the literature has been reported to damage the inner body of the abdomen, both in blind technical blocks and in blocks in the ultrasound guide [16]. Intraperitoneal injections, hematoma in the intestines, and temporary femoral nerve damage are among other complications [17]. It is important to remember the local anesthetic toxicity due to the increased local anesthetic volumes after bilateral TAP block placement. Therefore, careful aspiration is required to avoid intravascular local anesthetic injection [17]. Our study did not show any complications to the group where bilateral TAP block was applied.

Pain evaluations of the individuals included in the study were evaluated with VAS scoring at 1st, 2nd, 12th, and 24th hours. VAS score was found to be significantly lower in the TAP group compared to the control group at all evaluation times. With these results, very close results were obtained when compared to a study describing the petite triangle in TAP block [11]. In our study, unlike this study, the first analgesic needs of the patients were evaluated and it was determined that the TAP block group needed the first dose analgesic less frequently. Also, our study addressed additional complaints such as nausea and vomiting of patients.

The most commonly used local anesthetics in TAP block administration are Bupivacaine [10, 15, 18], Ropivacaine [13], and Levobupivacaine [9]. In our study, TAP block was applied to each site as 20 ml with 0.25% Bupivacaine, which we think has a longer duration of action. This application we made in our study complies with the current literature.

A study [12] included 40 patients who underwent colorectal surgery under general anesthesia, applied 0.25% Bupivacaine to the TAP group and SF to the control group as 20 ml bilaterally at the end of the surgery. A 65% decrease was observed in total morphine consumption for 24 hours compared to the control group in the TAP group. Although the need for initial analgesia was similar in both groups, subsequent doses were required longer in patients in the TAP group [12]. Another study [14] divided the patients including 54 patients that have undergone laparoscopic cholecystectomy as randomized 3 groups. The first group was the control group, the second group was the TAP group with 0.25% levobupivacaine and the third group was the TAP group with 0.5% levobupivacaine. The need for postoperative analgesia was found to be lower in the TAP groups. No significant difference was found between the 2nd and 3rd groups, and no complications related to TAP block were observed [14].

According to a study [15], 52 patients who underwent appendectomy under general anesthesia, divided the patients into two groups as randomized double-blind and applied TAP block with Bupivacaine accompanied by ultrasound to the second group. Total morphine dose and VAS scores for the first 24 hours were found to be significantly lower in the TAP group and no complications occurred in the TAP group [15]. In contrast to these studies, a conducted research [13] on 13 patients who underwent gynecological cancer surgery in 2010; no difference was found between the groups with and without TAP block in terms of analgesic drug consumption and patient comfort. In our work, the TAP block was applied to individuals after the end of the operation.

In a meta-analysis examining the studies for postoperative pain control after cesarean section, 524 patients were evaluated in a total of 9 studies, and the main purpose of these individuals who underwent TAP block after spinal anesthesia (7 studies) and general anesthesia (2 studies) is to investigate pain control and analgesic consumption. It has been emphasized that after spinal anesthesia, TAP block application without intrathecal opioid is a significant contribution to the control of postoperative pain and that after spinal anesthetic using opioid, you need to work further, although unclear [19].

Some studies have compared TAP blocks to epidural analgesia. Recently, a group of researchers conducted a retrospective matched case-control study comparing continuous TAP block catheters (posterior and subcostal approaches; n = 15) to thoracic epidural analgesia (n = 15) [20]. Except for assessments in the post-anesthesia care unit, there was no appreciable difference in pain scores between the two groups over a 3-day follow-up period. While patient satisfaction was similar between groups, the TAP block group required a significantly higher amount of breakthrough fentanyl over the study period. The therapeutic failure rate was higher in the epidural group (patchy block in 4 patients) versus the TAP catheter group (unilateral block in 2 patients). Hypotension was reported in 2 patients from the epidural group [20].

Also, an increasing number of reports indicate that TAP blocks can be a safe alternative to neuraxial blockade in patients with coagulation disorders [21, 22].

In our study, postoperative patient-controlled analgesia was not studied as a dose and the first analgesic requirement was recorded as time; heart rate, systolic, and diastolic blood pressure were found to be lower in the patient group who underwent TAP block compared to the other group. No nausea-vomiting was observed in the TAP block group. Thus, we believe that the likelihood of nausea-vomiting complications is reduced.

CONCLUSION

In our study by applying TAP blocks, the need for the first postoperative supplemental analgesia was delayed in the TAP group, thus reducing any side effects that may develop due to these analgesics. Although in our study, in the patient group who underwent TAP block; we have achieved significant and positive results, such as less postoperative pain, less initial analgesic need and additional convergence, more stable vital signs, more comprehensive investigations are necessary to routinely implement this practice.

Tap blocks are relatively easy to place and appear to be associated with little risk when placed with ultrasound guidance by trained practitioners. This kind of block provides effective analgesia and reduces the additional disruptions caused by the pain.

Contribution of authors

Study Design: Nagihan Yuksek, Ahmet Cemil Isbir; Data Collection: Nagihan Yuksek; Statistical Analysis: Iclal Ozdemir Kol; Data Interpretation: Ahmet Cemil Isbir; Manuscript Preparation: Onur Avci, Ahmet Cemil Isbir; Literature Search: Kenan Kaygusuz, Sinan Gursoy

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EDITORIAL

The history of military medicine in the last 100 years xxx

Paul Oprea

Gral Brig PAUL OPREA
Chief of Romanian Army Medical Directorate

The begining of the medical military service in Romania is related to the set up of the public institutions with military regime: gendarmerie and military subunits (pedestrians and riders), military firefighters, whose purpose was to provide sanitary services.

Due to the different evolution of Romanian regions, the history of military-medical structures was different.

The unification of the Romanian Principalities under the ruler Alexandru Ioan Cuza - modernist and good administrator - was an important leap in the development of the medical assistance structures of the armies, with the increase, diversification and specialization of the military units. It is the time when battalion infirmaries grow in size, the number of stationary beds for officers and troop's increases, more doctors are employed and therapeutic methods are diversified.

The arrival of King Carol I, one of the founders of modern Romania, in the country, has generated progress and development of the medical-military network.

After the conquest of Romania's state independence, the development of the institutions of the new state under the leadership of Carol I and of a political class of liberal and nationalist orientation recorded a momentum not yet encountered. Consequently, the first military hospitals were set up in addition to those in Bucharest, the period between 1880 and 1900 being a period of massive construction.

In addition to the Central Military Hospital in Bucharest, hospitals were built in Craiova, Piteşti, Galaţi, Focsani and the hospital from Iaşi was resized. Fortunately for the Romanian people, the beginning of the 20th century found the Romanian Army at a good level of organization, endowment and with enough staff.

In the Armed Forces, the medical service has developed to a great extent: there are specialists in surgery, internal medicine, dermatology, laboratory, etc. Hospitalization conditions are modern, both in the medical departments and in laboratories, the latter being managed by well-trained medical staff. In this period, there is a rise of military-medical professors, some of which turned out to be excellent specialists, role models for the next generations.

With the general physician Carol Davila, the first command (planning / administration / coordination) structure of the Army's medical service appears. He is the first chief in the history of the current Medical Directorate.

Having central activity as support, medical structures - infirmaries, hospitals - are standardized as structure, organization chart and endowment.

The period 1914-1916 until Romania's entry into war provided a partial modernization of the Army. Entry into World War I was an act of courage and sacrifice for all the services of the Army. The medical service was well prepared and managed to provide comprehensive health care in the operations theatres. Unfortunately, the Army's sanitary material conditions were not at an appropriate level, making it possible for the outbreak of typhus epidemic, which has ravaged thousands of lives among the Army's military. The intervention of the medical service, benefiting from the consistent support of volunteer women, coordinated by the new queen of Romania, eventually juggled the epidemic, improving the level of healthcare insurance - especially in terms of providing care for the wounded and sick.

The victorious outbreak of war and the creation of the Romanian national state - Great Romania - allying Transylvania, Basarabia and Bucovina - is the greatest moment in our history. However, the Romanian military continued military operations aimed at imposing order and compelling Hungary to accept the Versailles peace treaty.

On the occasion of the reintegration, the Army's medical care network has expanded accordingly, including hospitals from Timisoara, Sibiu, Brasov, Cluj-Napoca, Oradea, Cernauti and Chisinau. The newly established military units organized unit and garrison infirmaries. One of the Army's sanitary warehouses was organized in an old artillery unit, in Sebes, in addition to the one in Bucharest.

After the great global conflagration of 1914-1918, the principles and norms of organization and functioning of the campus hospitals (mobile and fixed - of the inner area) organized in school buildings, mansions, administrative buildings, etc. - were established. During the war, campus hospitals provided medical services to save the lives of the wounded and sick military.

The interwar period was a period of development of the Armed Forces medical network not only in numerical / quantitative but also qualitative terms: the medical units / departments and medical specialties, as well as the therapeutic resources and the means of diagnostics diversified, the imaging (Rx) developed consistently. In these twenty years, hospitals in Romania (including the military ones) evolved, developed, offering diagnostic and treatment methods similar to those available in Western Europe.

Romanian medical education has matured through the activity of the Medical Faculties in Bucharest, Cluj-Napoca, Timisoara and Iasi, generating a plethora of high-performance medical practitioners and pharmacists (some of the world's recognition personalities).

The interwar evolution of international relations that gradually brought Romania to Hitler's Germany led to the conclusion of the assistance protocol on the military line between Germany and Romania. Due to the offensive actions in particular, the functioning of the medical service has been rethought in order to ensure the triage and saving of life in the battlefield, in the conditions of multiple victims, tactical and strategic medical evacuation, ensuring the continuity of medical care along the hospital network (rehabilitation of wounded / sick military personnel so that it could be used again in combat), storage of functional and reserve stocks, use of blood transfusion (for the first time in history).

During the years 1940-1945, Romanian military medical network (both internal military hospitals and civil ones, as well as campus military hospitals) were tested. The Romanian sanitary trains, used as modern means of medical evacuation - during the First World War, demonstrated the logistic capacity held by the Romanian Army, allowing the strategic medical evacuation to be carried out by placing combat actions far away from the country.

The period 1945-1950 represented, in addition to the introduction of Russian communism dogma in the country, a period of radical transformation of the Army and, implicitly, of its medical service. There has been a general and dramatic regression by the exclusion from military physicians of leading personalities, who were considered counterrevolutionary and irredentist. These people were replaced by less prepared and competent, but "healthy" staff, unable to provide proper quality of the military-medical activities. The performance of the entire Romanian education system decreased consistently, therefore there was a decrease in the quality of the medical-military education as well.

After the 1960, the number and size of the military units increased by reorganizing the Army of the Romanian People's Republic (the beginning of the Cold War between Western Capitalist Europe and Eastern European Communist Europe).

The number of unit infirmaries and company hospitals increased and the number of military hospitals was decided, as follows: Bucharest Military Hospital, Cluj-Napoca Military Hospital, Craiova Military Hospital, Constanta Military Hospital, Focşani Military Hospital, Galati Military Hospital, Iasi Military Hospital, Oradea Military Hospital (transferred to the Ministry for Internal Affairs after 1989), Pitesti Military Hospital, Sibiu Military Hospital, Timisoara Military Hospital.

Military dispensaries were organized in big garissons: Bucharest (became CMDTA Acad. Ştefan Milcu), Bacău, Braila, Buzău, Ploieşti, Targoviste, Caracal, Alba Iulia and others. At Sebeş the central sanitary storage center was reorganized, as well as the pharmaceutical storage center in Bucharest. The Center for Preventive Medicine and the Armed Forces Transfusion Center were set up within the Central Military Hospital in Bucharest.

In view of the new concept of reaction to weapons of mass destruction (microbiological, chemical, radiological and nuclear means), the Army's Medical-Military Scientific Research Center was established in 1970-1978. Also, in 1975, the Faculty of Military Medicine was established.

After 1960 - with the improvement of the political and social situation in the country, the access to education of all young people and the organization of a fair promotion system are also present within the Armed Forces medical system, thus creating the premises for a series of excellent professionals: doctors and pharmacists.

The school of sanitary petty officers was founded in 1966 in Focsani. It prepares the sanitary petty officers that work in management and medical planning structures and provide medical care within unit infirmaries and campus hospitals.

However, the responsibility of relations with the medical education system from the Western Europe, which was evolved and emancipated by arbitrary restrictions, generated a period of regress in the performance of medical service. The provision of medical logistics was poor by reducing the import of medical devices: the only medical devices factory (ITM Bucharest) and several other medicine factories provided mediocre quality goods for the requirements of medical service. However, at the level of infrastructure, progress was made by building new structures and setting up medical services, diversifying the range of medical services by introducing new medical specialties in the surgical field (cardiovascular surgery, thoracic-pulmonary surgery, neurosurgery, maxillofacial surgery, orthopedics, urological surgery, gynecological surgery and pediatrics surgery) but also in the field of internal medicine (neurology, psychiatry, cardiology, pulmonology, gastroenterology and hematology, endocrinology and nutritional diseases, geriatrics, obstetrics, etc.).

After 1989, the medical-military system went through a regression, between the years 1990-1995, generated by deficiencies in planning, budgeting and supply, but that was outreached. Since 1994, medical-military education has started in an institutional framework again, within the Medical and Military Institute from Bucharest (with two sections: one in Bucharest at Carol Davila University of Medicine and Pharmacy and the other in Targu Mures - University of Medicine and Pharmacy). Although the number of trainees is much lower than the generations prior to 1989, tradition continues.

In accordance to the other institutions of the Romanian State, the Army registered a reduction of budgets and investments in the military field between 1990 and 2000. Military units were reduced in number and size progressively. Military medicine from the level of different forces also declined.

Military hospital units registered a real progress in the years 1997-2000 through the modernization of medical devices (centralized purchase of medical goods worth US $ 114 million). This strategic budgetary allocation has allowed the development of a network of military hospitals providing superior quality medical services which managed to impose at national level.

The reorganization of the Romanian Armed Forces during the period 2000-2005, which anticipated the entry into the North Atlantic Alliance, generated a regress in the Army's medical system by giving up the Military Hospital from Oradea to the Ministry of Internal Affairs, the retreat of many excellent doctors (introduction of mandatory retirement at the age of 55 - age corresponding to top performance in the medical career), abolition of the campus hospitals network, infirmary of large units, etc.

Medical-military scientific research is reduced because of inadequate budgets (a trend at national level), suspending the production of antidotes and salts used in CBRN interventions - implemented from 1978-1980.

Gradually, from 2007 to 2010, the specialized human resources were fewer and fewer, some of the causes were: low addressability of admission to the military system, migration of work force to the West, lack of vision at the level of the Medical Directorate regarding the future of the military medical system, legislative modifications in the health field, etc.

In 2008 there is an unfortunate event: the reduction of military functions at the level of military hospitals. This event associated with the transition to the self-financing system of military hospitals (public hospital quality) and defense funding gap - generated a major imbalance in the Army's medical network: each hospital evolved separately - depending on the period of medical services in which it was located, the performance of the medical staff, the management and organizational skills of the different managers, lack of vision and involvement at the level of the Medical Directorate, etc.

The foundation of the Health Insurance House of the Army, Public Order, National Security and Judicial Authority was a saving and integrative action in the national health system, establishing a favorable attitude towards maintaining the quality of services, fulfilling the accreditation standards, implementation of systematic management, providing for hygiene and sanitary-veterinary conditions of operation, ensuring respect for patients' rights and life security, etc.

From 2017, the Romanian Army benefits from a budget allocation of 2% of GDP. This financial support has enabled the Medical Directorate of the Ministry of National Defense to develop several projects in line with the major medical insurance objectives of the Army:

1. Modernizing medical and military education and training;

2. Standardization of medical structures (mobile and fixed);

3. Providing operational medical support - according to the training needs, resilience forces functioning and defense of the territory in times of crisis and war;

4. Ensure medical logistics of forces and territory in case of peace (crisis) and war.

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ORIGINAL ARTICLES

The impact of website quality on customer loyalty with the intermediate role of social capital: A case study of the Bamilo website

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Abstract: *The main objective of this study is to investigate the impact of Bamillo website quality on customer loyalty through the mediating role of social capital. This research is applied in terms of purpose and it is a field study in terms of data collection. The population of the study included all users of the Bamilo website who had at least one purchase order from this site. Accordingly, 384 people were selected through available sampling. The instrument used in the research was the standard questionnaire of China et al. (2016), which confirmed the validity and reliability of the questionnaire. Structural equation modeling with the partial least squares method was used to analyze the data. The results showed that the quality of information on-site information quality on cognitive capital was rejected and its impact on structural capital was confirmed. The quality of the site system on cognitive capital is also rejected and its impact on structural capital is confirmed. On the other hand, the quality of site services has an impact on cognitive capital and structural capital. Also, cognitive capital and communication capital affect communication capital, and cognitive capital and communication capital affect customer loyalty. However, the impact of the capitalist mediation mediated the relationship between information quality and system quality with customer loyalty. Cognitive capital also mediates the relationship between service quality and customer loyalty, and structural capital mediates the relationship between information quality and customer loyalty. On the other hand, the mediating role of structural capital in the relationship between system quality and customer loyalty was confirmed and structural capital mediated the relationship between service quality and customer loyalty.*

Keywords: *website quality, customer loyalty, social capital, Bamillo*

INTRODUCTION

Influence and interpersonal influences of people in online environments affect customers' evaluations and decisions about buying a product or brand. The increasing growth of the Internet and its high penetration rate in our country has provided the necessary basis for doing business in this environment [1]. In recent years, many organizations and companies have attracted customers and sold their products using the Internet and creating websites. The special requirements of business activity in cyberspace, the special behavior of consumers in this environment, and the fierce competition between actual and potential actors in attracting customers for their online stores indicate the importance of examining activities in this field as closely as possible [2]. Online shopping can be considered as one of the major developments in retail sales in the business world. An Internet-based e-commerce environment allows customers to search for information and purchase goods and services through direct contact with online stores. Shopping online and doing so largely depends on consumer communication

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and how people interact with the computer. Processes such as providing information, guidance, and delivery, as well as timely delivery of orders, have been considered as an important factor in building trust in e-commerce, rather than traditional sales [3]. Customer loyalty in today's competitive and complex market is the main factor in gaining the competitive advantage of any organization; as retaining current customers is much less expensive than attracting new customers. Attracting customers on the Internet is very costly, and if customers are not loyal and their purchases are not frequent and frequent, profitability will be out of reach. Competitors are only a few clicks away from customers [4]. Therefore, customer loyalty has become a very complex process for managers. Increasing loyalty leads to predicting revenue streams from customers as well as a gradual increase in revenue [5]. A 5% increase in loyal customers, a 25% to 95% increase in the profitability of various industries. Increased loyalty will lead to the prediction of revenue streams by customers as well as a gradual increase in revenue [6]. Organizations lose a large number of their customers each year due to a lack of attention to them. One of the most effective and common strategies is to strive to provide quality, flawless and easy service. High quality and continuous customer service create a competitive advantage for the company [7]. Given the problems and obstacles in Internet businesses, which are mostly related to the lack of resources, both financial and human. The importance of using social capital and networking for the founders and entrepreneurs of these companies is evident [8]. Social capital plays a role within the organization in various ways such as facilitating and accelerating the flow of information and knowledge (whether tacit or codified knowledge) and if it is not addressed, it hinders the facilitation of human capital formation and reduces costs such as control costs. And monitoring, as well as facilitating access to people inside and outside the organization, prevents the organization from achieving the goals of the organization better and faster and reducing the obstacles ahead [9]. Social capital is an organizational attribute, not the property of individual members, that is, the organization and members jointly own it, and the result can affect customer behavior and loyalty [10].

Today, the web is one of the most important tools that countless people use as the main tool for accessing information. Websites play a key role in disseminating information. Websites are great tools for connecting organizations, users, and citizens. With the growing access of people to the Internet, the number of visitors to websites has also increased and the issue of their quality has become more and more important. The success and efficiency of websites depend to a large extent on the quality of the website. A high-quality website will grow over time due to the loyal readers as well as the search engine trust, which is more organic compared to the visitor [11]. Creating customer loyalty, especially in online environments and online shopping can affect the quality of websites. Creating lasting loyalty in e-commerce customers is very difficult because, on the Internet, the customer can easily leave one website and visit another website with the lowest transfer costs and with just one click. There is a consensus that enduring customer loyalty in electronic environments, especially online retail, is more sensitive and complex than traditional business loyalty [12]. In this regard, social capital can also play a decisive role. Perceived equity of social capital will increase customer loyalty to the company. Social capital will also increase the value of loyalty to corporate brands [13]. According to the above, the purpose of this study is to investigate the effect of Bamilo website quality on customer loyalty with the mediating role of social capital.

STUDY METHOD

The purpose of this research is to apply. It is applied research that is done using the results of basic research to improve and perfect the behaviors, methods, tools, tools, products, structures, and patterns used by human societies. The thematic area of the present study includes the study of the effect of Bamilo website quality on customer loyalty with the mediating role of social capital, which was implemented in the summer and autumn of 1398, and the location of Tehran. Given that in descriptive research, the researcher seeks how the subject is and wants to know what the phenomenon, variable, object, or subject is. In other words, this study examines the current situation and systematically and systematically describes its current situation and studies its features and characteristics and, if necessary, examines the relationship between variables, so this research is a descriptive study. Also, in this research, a survey method is used to collect data. In this type of research, the purpose is to study the distribution of characteristics of a society and most management research is of this type. In survey research, community parameters are examined. Here, the researcher examines the research variables by selecting a sample that represents the community. The statistical population refers to a set of people, objects, or phenomena in general that the researcher can generalize the result of his study to all of them. The statistical population of the research is identified by one or more common attributes. The statistical population included all users of Bemilo website who had purchased from this site at least once. A statistical sample is a set of indicators that are selected from a larger section, group or community so that this set represents the quality and characteristics of that larger section, group, or community, and sampling is the sample selection process. Based on this, 384 people have been selected by the available sampling method. Based on the research title, the research variables were determined as independent variables including website quality (information quality, system quality, service quality), dependent variable including customer loyalty, and mediator = social capital (capitalist, structural capital, communication capital). The research hypotheses were organized as follows:

1. The quality of site information affects capitalism
2. The quality of site information affects the structural capital
3. The quality of the site system affects the capitalism
4. The quality of the site system affects the structural capital
5. The quality of Bamilo site services has an impact on capitalism
6. The quality of site services affects the structural capital
7. Investigating the impact of capitalism on communication capital
8. Structural capital affects communication capital
9. Capitalism affects customer loyalty
10. Structural capital affects customer loyalty
11. Communication capital affects customer loyalty
12. Capitalism mediates the relationship between information quality and customer loyalty
13. Capitalism mediates the relationship between system quality and customer loyalty
14. Capitalism mediates the relationship between service quality and customer loyalty
15. Structural capital mediates the relationship between information quality and customer loyalty
16. Structural capital mediates the relationship between system quality and customer loyalty
17. Structural capital mediates the relationship between service quality and customer loyalty.

In this research, to collect information, the library and documentary methods were used in line with research literature and theoretical foundations. In this section, based on past studies, the theoretical foundations of research are stated. The theoretical basis of the definitions and concepts used in the research is clear and should be based on previous valid studies. The second part of the research literature presents similar studies previously done by other researchers. Data collection to test the research hypotheses, the field method was collected with a questionnaire. The instrument used in the research was the standard questionnaire of China et al. (2016) in which all the questions of the questionnaire were designed based on a five-point Likert scale (strongly agree to strongly disagree).

Table 1: Research questionnaire items

Variables	Items
Information quality	1-2-3-4
System quality	5-6-7-8
Service quality	9-10-11-12
Capitalism	13-14-15
Structural capital	16-17-18-19
Communication capital	20-21-22-23
Customer Loyalty	24-25-26-27-28

Validity refers to the logical connection between test questions and the material being assessed. The validity of the questionnaire means that the test questions accurately measure what is intended. Credibility has different aspects and the relationship between the question and the subject is achieved according to all its aspects. If this connection does not exist, no credit will be created. It is the most important feature of any test and shows the extent to which the test measures what it is designed to measure. In other words, validity shows the relationship of a test to its goals. Validity means the extent to which the method or tool used can accurately measure the desired characteristic determined by the research objectives. Experts' opinions can be a good help to improve the validity of measuring instruments. Accordingly, in this study, the validity of the present questionnaire was studied and confirmed by professors and experts including 12 people, especially the supervisor. One way to calculate reliability is to use Cronbach's formula. This method is used to calculate the internal consistency of measuring instruments such as questionnaires or tests that measure different characteristics. In such tools, the answer to each question can take different numerical values. In this study, Cronbach's alpha value was performed using SPSS 22 software. The alpha value for the questionnaire variables was obtained, which indicates the reliability of the questionnaire used in this research.

Table 2: Reliability of research variables

Variables	Cronbach's alpha values
Information quality	0.801512
System quality	0.735864
Service quality	0.847528
Capitalism	0.734281
Structural capital	0.814755
Communication capital	0.796902
Customer Loyalty	0.793798

Cronbach's alpha value for the questionnaire was more than 0.7, which indicates the reliability of the questionnaire used in this study. Two levels of descriptive and inferential statistics were used to analyze the data. In descriptive statistics, the demographic data of the questionnaire were analyzed using descriptive statistical methods such as methods of frequency, frequency percentage, mean, fashion, standard deviation, etc. using SPSS software. In inferential statistics of research hypotheses, using the research default and Kolmogorov-Smirnov test, the normality and abnormality of the data distribution of research variables are investigated. Finally, the structural equation model using the partial least squares method is used to test the hypotheses.

RESULTS

384 people participated in this study. 179 (46.6%) had a diploma, 44 (11.5%) had a postgraduate degree, 90 (23.4%) had a bachelor's degree and 71 (18.5%) had a master's degree or higher. Findings show that 76.6% of the participants were male and 23.4% were female. Other descriptive findings of the study sample include how people are distributed based on age groups. The age range of 21 to 30 years, which includes 39.1% of the total sample, has the highest frequency and the age range of 10 to 20 years has the lowest frequency. Findings indicate that 181 (47.1%) of those present are single and 203 (52.9%) are married. The questionnaire was designed with a Likert scale with five options (1 to 5) and there were several questions for each variable. Likerts ranged from 1 to 5; the numerical range of all variables must also be variable in the range (5, 1). The results of Table 4-7 show that the range of scores of all variables is calculated between 1 and 5, which means that the data was collected correctly in the soft environment The software has been coded, logged, and computerized. The total score of information quality was evaluated using an average of 4 items. The average score of the respondents in this variable (perceived benefits) is 3.56 and its standard deviation is 0.73 so that the lower limit (minimum) is 1.5 and the upper limit (maximum) is 5. Also, the coefficient of skewness and elongation of the mentioned variable is estimated to be -0.43 and -0.08, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level. The total quality score of the system was evaluated using an average of 4 items. The average score of the respondents in this variable (cognitive social identity) is 3.24 and its standard deviation is 0.86 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the coefficient of skewness and elongation of the mentioned variable is estimated to be -0.40 and 0.12, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level. The total score of service quality was examined using an average of 4 items. The average score of the respondents in this variable (local economic situation) is 3.46 and its standard deviation is 0.83 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the skewness and elongation coefficients of the mentioned variable are estimated to be -0.41 and -0.32, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level.

Table 3: Descriptive indicators of the studied variables

Variables	Sample size	Average	Standard deviation	Variance	Lowest score	Highest score	Cholegi	Elongation
Information quality	384	3.56	0.73	0.54	1	5	-0.43	-0.08
System quality	384	3.24	0.84	0.71	1	5	-0.40	0.12
Service quality	384	3.46	0.83	0.70	1	5	-0.41	-0.32
Capitalism	384	2.18	0.86	0.74	1	5	0.92	0.46
Structural capital	384	3.19	0.86	0.74	1	5	-0.09	-0.58
Communication capital	384	3.69	0.73	0.54	1	5	-0.64	0.96
Customer Loyalty	384	3.56	0.82	0.67	1	5	-0.67	-0.22

The total capitalist score is examined using an average of 4 items. The average score of the respondents in this variable (perceived costs) is 2.18 and its standard deviation is 0.86 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the skewness and elongation coefficients of the mentioned variable are estimated to be 0.92 and 0.46, respectively, and in the safe range (2+ and -2), which is at a desirable and appropriate level. The total score of structural capital is examined using an average of 6 items. The average score of the respondents in this variable is 3.19 and its standard deviation is 0.86 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the skewness and elongation coefficients of the mentioned variable are estimated to be -0.09 and -0.58, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level. The total score of communication capital is examined using an average of 3 items. The average score of the respondents in this variable (importance to the development of local tourism) is 3.69 and its standard deviation is 0.73 so that the lower limit (minimum) is 1 and the upper limit (maximum) is 5. Also, the coefficient of skewness and elongation of the mentioned variable is estimated to be -0.64 and 0.96, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level. The total customer loyalty score was evaluated using an average of 5 items. The average score of the respondents in this variable is 3.56 and its standard deviation is 0.82 so that the lower limit (minimum) is 1.4 and the upper limit (maximum) is 5. Also, the coefficient of skewness and elongation of the mentioned variable is estimated to be -0.67 and -0.22, respectively, and in the safe range (+2 and -2), which is at a desirable and appropriate level.

Table 4: Matrix of correlation coefficients between research variables

Variables	Information quality	System quality	Service quality	Capitalism	Structural capital	Communication capital	Customer Loyalty	Variables
Information quality	correlation	1						
	Significance	0	0					
System quality	correlation	0.56	1					
	Significance	0.00	0					
Service quality	correlation	0.46	0.44	1				
	Significance	0.00	0.00	0				
Capitalism	correlation	- 0.46	- 0.40	- 0.43	1			
	Significance	0.00	0.00	0.00	0			
Structural capital	correlation	0.53	0.47	0.45	- 0.47	1		
	Significance	0.00	0.00	0.00	0.00	0		
Communication capital	correlation	0.51	0.45	0.43	- 0.42	0.52	1	
	Significance	0.00	0.00	0.00	0.00	0.00	0	
Customer Loyalty	correlation	0.65	0.55	0.55	- 0.50	0.55	0.52	1
	Significance	0.00	0.00	0.00	0.00	0.00	0.00	0

Due to the abnormality of data distribution, the Pearson correlation test was used. Correlation coefficients are used to calculate the degree and degree of the linear relationship between two variables. The correlation coefficient range varies from -1 to +1. The closer this value is to +1, the stronger and more positive the relationship between the two variables. In other words, with the increase of each of the variables, the other also increases, and vice versa, with the decrease of each of the variables, the other also decreases. Also, the closer the value of this coefficient is to 1, the stronger and negative the relationship between the two variables is. In other words, with the increase of each of the variables, the other decreases, and with the decrease of each of the variables, the other increases.

Table 5: Summary of results from Hypotheses 1 to 11

Hypothesis number	Independent variable	Dependent variable	Path coefficient	Significance number	Result
1	Information quality	Capitalism	0.145	1.762	Reject
2	Information quality	Structural capital	0.186	2.904	Confirmation
3	System quality	Capitalism	0.097	1.380	Reject
4	System quality	Structural capital	0.389	5.598	Confirmation
5	Service quality	Capitalism	0.629	9.215	Confirmation
6	Service quality	Structural capital	0.291	4.408	Confirmation
7	Capitalism	Communication capital	0.3	3.289	Confirmation
8	Structural capital	Communication capital	0.428	5.264	Confirmation
9	Capitalism	Customer Loyalty	0.350	3.842	Confirmation
10	Structural capital	Customer Loyalty	0.278	3.984	Confirmation
11	Communication capital	Customer Loyalty	0.327	4.071	Confirmation

This test examines the following hypothesis:

Assumption Zero: The correlation coefficient between the two variables is zero (the two variables are independent of each other).

Opposite assumption: The correlation coefficient between the two variables is not zero (the two variables are related to each other).

Considering that the significance level of the correlation coefficient test between the two variables is less than the 0.05 error level, it can be stated that there is a significant correlation and relationship between all variables. A better and more detailed analysis of the above table is given in the section on hypotheses. The results of Hypotheses 1 to 11 are summarized according to Table 5.

The results of Hypotheses 12 to 19 are summarized according to Table 6.

Table 6: Summary of results from Hypotheses 12 to 17

Hypothesis number	Independent variable	Mediator	Dependent variable	Path coefficient	Significance number	Result
12	Information quality	Capitalism	Customer Loyalty	0.05	1.60	Reject
13	System quality	Capitalism	Customer Loyalty	0.03	1.29	Reject
14	Service quality	Capitalism	Customer Loyalty	0.22	3.54	Reject
15	Information quality	Structural capital	Customer Loyalty	0.05	2.34	Confirmation
16	System quality	Structural capital	Customer Loyalty	0.10	3.24	Confirmation
17	Service quality	Structural capital	Customer Loyalty	0.08	2.95	Confirmation

DISCUSSION AND CONCLUSION

One of the content differences between the chapter on data analysis and the chapter on summarizing and concluding in research works is that unlike the chapter on analysis where the reader is left alone among a multitude of numbers and statistical techniques, in the chapter on summarizing and concluding most of this ambiguity is omitted.

The reader understands the essence of the word as a simple and non-technical Babiani. In writing this chapter, an attempt has been made to write Babiani as simple as possible on the findings and research suggestions so that readers and respected managers can make the most of these findings. This study sought to investigate the effect of website quality on customer loyalty with the mediating role of social capital. Statistical analysis provides a framework for the researcher to make judgments about society. For this purpose, in this study, effective variables were extracted by studying the research literature, and then the explained hypotheses were proved using statistical methods.

In the present study, an attempt has been made to identify the effect of website quality by considering the variables of information quality, system quality, and service quality with the mediating role of social capital and by considering the variables of capitalist, communication capital, and structural capital on customer loyalty; According to this purpose, the present research method is applied based on the purpose and in terms of data collection method is descriptive-correlation from the branch of structural equation model.

In this research, 17 hypotheses were explained in the form of a conceptual model, which is followed by the analysis of the results obtained from testing these hypotheses.

Hypothesis 1: The quality of site information has an impact on capitalism.

Given that the t-statistic obtained from the hypothesis test (1.762) is inside the critical area (-1.96 and 1.96), this hypothesis is not confirmed; Therefore, based on the response of the subjects in the statistical population, the quality of site information has no significant effect on capitalism, although this meaningless effect is positive based on what the coefficient (0.145) shows. Based on the conceptual definition of system quality, "a system that is well designed, developed and implemented and provides the conditions for organizational benefits" can be said that the organizational benefits perceived by the customer promote his capitalism "such as trust, "Norms, and values that affect interactions between people." This finding is consistent with the results of research by Chen et al. (2016) [14] because they also concluded that all three dimensions of website quality (information quality, system quality, and service quality) are significantly capitalist and structural they are not related.

Hypothesis 2: The quality of site information affects the structural capital.

Given that the t-statistic obtained from the hypothesis test (2.904) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the response of the subjects in the statistical population, the quality of information has a significant effect on structural capital. This significant effect is positive and desirable based on what the obtained coefficient (0.186) shows. This means that as the quality of website information improves, the structural capital of the organization's customers increases, so that; by improving one unit in the level of information quality, the structural capital of customers is improved by

0.186 units. This finding contradicts the findings of Chen et al. (2016) [14].

Hypothesis 3: The quality of the site system has an impact on capitalism

Given that the t-statistic obtained from the hypothesis test (1.380) is inside the critical region (-1.96 and 1.96), this hypothesis is rejected; Therefore, based on the subjects' answers in the statistical population, the quality of the site has no significant effect on the capitalist life and savings customers. This finding is consistent with research by Chen et al. (2016).

Hypothesis 4: The quality of the site system affects the structural capital.

Given that the t-statistic obtained from the hypothesis test (5.598) is outside the critical area (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' answers in the statistical population, the quality of the system has a significant effect on structural capital. This significant effect is positive and desirable based on what the obtained coefficient (0.389) shows. This means that as the quality of the website system improves, the structural capital of the organization's customers increases, so that; by improving one unit in the level of information quality, the structural capital of customers is improved by 0.389 units. This finding contradicts the findings of Chen et al. (2016) [14].

Hypothesis 5: The quality of Bamilo site services has an impact on capitalism.

Given that the t-statistic obtained from the hypothesis test (9.219) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the answers of the subjects in the statistical population, the quality of services has a significant effect on capitalism. This significant effect is positive and desirable based on what the obtained coefficient (0.629) shows. This means that as the quality of website services improves, the capital of the organization's customers increases, so that; by improving one unit in the level of service quality, customers' capital is improved by 0.629 units. This finding contradicts the findings of Chen et al. (2016) [14].

Hypothesis 6: The quality of site services has an impact on structural capital.

Given that the t-statistic obtained from the hypothesis test (4.408) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' answers in the statistical population, the quality of services has a significant effect on structural capital. This significant effect is positive and desirable based on what the obtained coefficient (0.291) shows. This means that as the quality of website services improves, the structural capital of the organization's customers increases, so that; by improving one unit in the level of information quality, the structural capital of customers is improved by 0.291 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 7: Capitalism affects communication capital.

Given that the t-statistic obtained from the hypothesis test (3.298) is outside the critical range (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' responses in the statistical population of the study, capitalist capitalism has a significant effect on communication capital. This significant effect is positive and desirable based on what the obtained coefficient (0.3) shows. This means that as capitalism improves, the communication capital of the organization's customers increases, so that; by improving a unit at the capitalist level, customers' communication capital improves by 0.3 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 8: Structural capital affects communication capital.

Given that the t-statistic obtained from the hypothesis test (5.264) is outside the critical range (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' answers in the statistical population, structural capital has a significant effect on communication capital. This significant effect is positive and desirable based on what the obtained coefficient (0.428) shows. This means that as structural capital improves, the communication capital of the organization's customers increases, so that; by improving one unit at the level of structural capital, customers' communication capital is improved by 0.428 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 9: Capitalism affects customer loyalty.

Given that the t-statistic obtained from the hypothesis test (3.842) is outside the critical range (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' responses in the statistical population studied, the capitalist has a significant effect on customer loyalty. This significant effect is positive and desirable based on what the obtained coefficient (0.350) shows. This means that as the quality of capitalism improves, the loyalty of the organization's customers increases, so that; by improving one unit at the capitalist level, customer loyalty is improved by 0.35 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 10: Structural capital affects customer loyalty.

Given that the t-statistic obtained from the hypothesis test (3.984) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the subjects' responses in the statistical population, structural capital has a significant effect on customer loyalty. This significant effect is positive and desirable based on what the obtained coefficient (0.278) shows. This means that as structural capital improves, the loyalty of the organization's customers increases, so that; by improving one unit at the level of structural capital, customer loyalty is improved by 0.278 units. This finding contradicts the findings of Chen et al. (2016) [14].

Hypothesis 11: Communication capital affects customer loyalty.

Given that the t-statistic obtained from the hypothesis test (4.071) is outside the critical region (-1.96 and 1.96), this hypothesis can be confirmed; Therefore, based on the answers of the subjects in the statistical population, communication capital has a significant effect on customer loyalty. This significant effect is positive and desirable based on what the obtained coefficient (0.327) shows. This means that as communication capital improves, the loyalty capital of the organization increases, so that; by improving one unit at the level of communication capital, customer loyalty improves by 0.327 units. This finding is consistent with the results of research by Chen et al. (2016) [14].

Hypothesis 12: Capitalism mediates the relationship between information quality and customer loyalty.

Due to the non-confirmation of the first hypothesis that there is a significant effect between information quality and capitalism, this hypothesis is also rejected. On the other hand, according to the level of significance calculated by the Sobel test, it can be seen that this hypothesis is not confirmed again.

Hypothesis 13: Capitalism mediates the relationship between system quality and customer loyalty.

Due to the non-confirmation of the third hypothesis that there is a significant effect between system quality and capitalism, this hypothesis is also rejected. On the other hand, according to the level of significance calculated by the Sobel test, it can be seen that this hypothesis is not confirmed again.

Hypothesis 14: Capitalism mediates the relationship between service quality and customer loyalty.

Considering the significance of the existing paths between the independent, mediating, and dependent variables, we have calculated the significance value using the Subel test. As observed in the data analysis chapter, the calculated value is equal to 3.54 and outside the absolute value range is 1.96. Therefore, this hypothesis was also confirmed. On the other hand, the coefficient of effectiveness of customer loyalty through the capitalist mediator variable has been reported equal to 0.22.

Hypothesis 15: Structural capital mediates the relationship between information quality and customer loyalty.

Considering the significance of the existing paths between the independent, mediating, and dependent variables, we have calculated the significance value using the Subel test. As observed in the data analysis chapter, the calculated value is equal to 2.34 and outside the absolute value range is 1.96. Therefore, this hypothesis was also confirmed. On the other hand, the coefficient of effectiveness of customer loyalty through the mediating variable of structural capital is reported to be 0.05.

Hypothesis 16: Structural capital mediates the relationship between system quality and customer loyalty.

Considering the significance of the existing paths between the independent, mediating, and dependent variables, we have calculated the significance value using the Subel test. As observed in the data analysis chapter, the calculated value is equal to 3.24 and outside the absolute value range is 1.96. Therefore, this hypothesis was also confirmed. On the other hand, the coefficient of influence of customer loyalty through the mediating variable of structural capital is equal to 0.10.

Hypothesis 17: Structural capital mediates the relationship between service quality and customer loyalty.

Considering the significance of the existing paths between the independent, mediating, and dependent variables, we have calculated the significance value using the Subel test. As observed in the data analysis chapter, the calculated value is equal to 2.95 and outside the absolute value range is 1.96. Therefore, this hypothesis was also confirmed. On the other hand, the coefficient of effectiveness of customer loyalty through the mediating variable of structural capital is equal to 0.08.

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Contribution

All authors participated in the presentation of the original idea and design, search for sources and review of articles, initial writing or revision of the article, and all with the final approval of the present article, take responsibility for the accuracy of the content.

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ORIGINAL ARTICLE

The importance of Greek primary healthcare reconstruction during the COVID-19 outbreak

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Abstract: *Family doctors and general practitioners have long been those who were to be the first to deal with family's health problems. After primary care systems administration, general practitioners acquired a special role in the operation and efficiency of these systems. Their existence as institution contributes to the most complete and better prevention and treatment of health issues, as well as to the resources saving for the insurance systems. In Greece, since the formation of the Greek National Health System in 1983, numerous interventions have been legislated, most of them referring to the institution of family doctor. However, factors related to political will, financial interests, administrative and educational inadequacies, financial problems and geographical particularities did not allow the establishment of a strong primary healthcare. COVID-19 outbreak has exposed the weaknesses of all types of health systems. However, health systems based on primary care seem to better cope with the pandemic, as well as the continuity of care and quality of health services have been secured. In order, that Greek primary healthcare be organized from now on, many actions are required such as the establishment of electronic health records, the creation of properly staffed and equipped health centers, as well as more governmental financing programs about the modernization of primary care. Last but not least, pressure should also be exerted on governments to pass more groundbreaking bills and implement some of the older provisions.*

Keywords: *family doctor, general practitioner, Greek primary healthcare, COVID-19, legislative interventions*

INTRODUCTION

Primary Care (PC) is the first point of contact between people and the health system [1]. According to the Alma-Ata declaration, PC bases its operation on a group of health workers that has direct contact with patients, so that they respond appropriately to their needs [2]. Therefore, family doctor (FD)/general practitioner (GP) plays an integral role to PC promotion. The role of GPs is determined by the need for patients to contact health services that are near where they live and work, for prevention or health issues and, if necessary, to be addressed to more specialized health services [3].

In Greece, in one of the first legislative initiatives of the law 1397/83 for the Greek National Health System formation, FDs' existence was first mentioned. However, especially in urban areas, the full development of PC has not been fully implemented, while at the same time there are organizational issues regarding the cooperation of PC with hospital care [4, 5].

COVID-19 pandemic highlighted health system weaknesses and vulnerability, both in the field of public health and primary health. Overall, it is reported that countries with universal health coverage, updated strategy and readiness regarding the pandemic including primary care, as well as the level of governmental aid for public health measures, are found to best cope with the COVID-10 outbreak [6]. Moreover, past evidence regarding pandemics management, indicates that when community stops receiving health services due to its fear about the probability of getting infected, the health of population is endangered [7].

The aim of this article is to describe the multidimensional advantages of a solid PC system and the existence of FDs, especially during the COVID-19 pandemic. Moreover the legislative initiatives in Greece towards the formation of Greek PC while the reasons that have not been implemented will be analyzed. Lastly, the main reasons of the failure of the

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Greek PC will be descripted, with the aim of that solutions be suggested, so that the next waves of the pandemic may be faced in a better way.

GENERAL PRACTITIONER AS A KEY FACTOR OF PRIMARY CARE

The strengthening of PC systems, with FD playing a central role is of paramount importance [8]. The implementation of PC based on FDs has been associated with improved patient health, and reduction of mortality rates [9, 10], a result independent of confounding factors such as income [11, 12]. The quality of interfacing and the continuity of care between primary and secondary healthcare is improved, as well as the development of doctor-patient interpersonal relationships [13, 14]. Patients are also more satisfied with the quality of services provided [15, 16]. The advantages of a GP/FD-based care may be explained due to its low cost, accessibility, holistic perception and treatment of health problems and comorbidities and knowledge of family and social peculiarities [17, 18]. Last but not least, it is well documented that FDs' delivery of primary care is correlated with better outcomes [17].

Such a health system relies more on prevention and the holistic treatment of patients' health issues, reducing the cost of health services [19]. The contribution of FDs is also important in reducing access to hospitals and hospitalization (gatekeeping) [20, 21]. However, the results are not proportionately positive in regards of reducing the likelihood of re-admission to the hospital [22]. Moreover, relative equality in the quality of health services regardless of income and social class is ensured, while the vaccination coverage of the population is improved [3, 23, 24].

COVID-19 PANDEMIC AND THE IMPORTANCE OF PRIMARY HEALTHCARE

The wide spread of the SARS-CoV-2 outbreak (COVID-19) has been a challenging situation for all types of health systems globally. The uncertain epidemiology of this new infectious disease, makes the action plans to be based mainly on the containment of the disease and the delay of dissemination through social distancing [25]. The role of PC in the management of the pandemic is of paramount importance [26] as only the critical or severe patients admitted to the hospital, while GPs are those who mainly make the initiate diagnosis. At the same time, it is crucial that patients with chronic as well as acute health problems be monitored (follow-up), especially those who are disabled or frail at home or health institutions [27].

Not only has increased non-COVID-19 mortality been mentioned, but it is reported that numerous primary care offices have been closed as well as reduced capacity in primary care in general. Access to primary care has been reduced due to lockdowns, continuity of health services have been dramatically affected, as COVID-19 cases were in priority over non-COVID-19 patients with other health conditions [28].

Hospitals and major health institutions have to focus on the critical cases, while PC has to target on less severe patients as well as on the health of the whole population, with triage and gatekeeping playing a key role. Consequently, there are three main goals that PC has to accomplish during the pandemic: the first goal of PC providers is the healthcare workers' protection from being infected as well as to prevent COVID-19 transmission [6]. The second goal is informing community, identifying and protecting individuals-mainly the vulnerable-from becoming infected as well as protecting the tertiary care level (hospitals), are some other crucial responsibilities of PC providers [6]. Last but not least, PC providers have to continue providing health services for non COVID-19 patients so that there is no discontinuity of their medical condition. Moreover, there are reports that demonstrate the vital role of PC regarding influenza surveillance as well as important information and data about public health [29-31]. As a result, PC may be an important tool as far as COVID-19 management and surveillance are concerned. Nevertheless, this could only be accomplished whether PC has adequate resources and capacity to retain its services [6].

France is one European country that dealt with the problem of rapid escalation of COVID-19 cases. Despite the fact that in the initial national guidelines, published at February 2020, PC providers were not involved, on March 2020, when the first version of a national guideline engaging the role of PC was implemented [27, 32].

Furthermore, as far as Iceland is concerned, the PC providers have the role as the first point of management for people with COVID-19 like symptoms. Instructions and/or indications for tests were published by the Directorate of Health. The majority of the COVID-19 tests were taken in PC centers. COVID-19 confirmed patients were admitted to a special Hospital of Iceland, being monitored until their discharge. People who were under quarantine, were monitored by the PC providers (GPs). The whole structure of the Icelandic PC system was adjusted, in order to be adapted to the new criteria and needs for the COVID-19 pandemic.

In United Kingdom and Australia, the national health system relies on the integrated and well-structured primary health care. When a serious health emergency occurs, e.g. the

COVID-19 outbreak, it is of outmost importance that general practice as well as other national PC services be supported, in aim that the continuity of health care for the community be secured and the entire health system be better prepared to cope with the emergency [33]. During the COVID-19 pandemic, telehealth services and PC in general were funded with $2.4 billion by the Australian government, in order to secure the uninterrupted access to health services [33, 34].

LONGITUDINAL SURVEY OF LEGISLATIVE INITIATIVES FOR PC ESTABLISHMENT IN GREECE

In Greece, there is not a well-organized PC system, especially in urban areas [5]. Outpatient healthcare is provided, without an solid coordination, by many specialized physicians of both various PC services and physicians from the private sector, leading to a piecemeal approach/treatment of health issues [35]. In Greece, the institution of family doctor has been institutionalized with various laws in the past, but it has not gone into operation, despite all the efforts that have been attempted from time to time. Legislations that foresee the provision of PC services in Greece begin from very early times, but the first remarkable effort for the development of an organized national health system is held with a bill at 1983. Since then, despite the fact that numerous laws have been voted regarding the provision and organization of PC and the institution of FD, they have not been fully implemented [36]. In 1983, the foundations of PC network were created, especially in rural areas. It enabled residents of remote areas to obtain their own doctor, a form of "FD". However, the inability to organize and record patients and their problems did not allow that the system be improved and established at levels similar to those abroad [36]. From the above, it may be distinguished that despite the existence of successive bills concerning the development of PD and FD, neither is completed. Actually, the Greek PC system remains one of the weakest in Europe [37]. Numerous laws and bills were passed, many of which were often contrary to each other.

Another main reason concerning the insufficiency of Greek PC system is the small number of GPs. In 2018, Greece had the highest number of doctors with 6.1 per 1,000 population, but this number is a over-estimation as it includes all doctors licensed to practice (including retired physicians and those who might have emigrated to other countries) (Table 1) [38]. In many countries, the main concern has been about growing shortages of general practitioners, particularly in rural and remote regions. Whereas the overall number of doctors per capita has increased in nearly all countries, the share of general practitioners (GPs) has come down in most countries. On average across EU countries, only about one in five doctors were GPs in 2018.

Table 1: Practicing doctors per 1 000 population, 2008 and 2018

	2008	2018
Greece[1]	5.71	6.10
Austria	4.60	5.24
Portugal[1]	3.61	5.15
Lithuania	3.88	4.60
Germany	3.54	4.31
Sweden	3.68	4.27
Bulgaria	3.66	4.22
Denmark	3.58	4.19
Cyprus	2.78	4.07
Czech Republic	3.56	4.04
Spain	3.54	4.02
Italy	3.79	3.98
Malta	3.05	3.97
EU27	3.32	3.83
Netherlands		3.67
Slovak Republic[2]	3.37	3.52
Estonia	3.34	3.48
Croatia	2.74	3.44
Hungary	3.09	3.38
Latvia	3.23	3.30
Ireland		3.28
Finland	2.72	3.21
Slovenia	2.40	3.18
France	3.07	3.17
Belgium	2.92	3.13
Romania	2.32	3.05
Luxembourg	2.72	2.98
Poland	2.16	2.38
Norway	4.00	4.81
Switzerland	3.82	4.34
Iceland	3.64	3.89
North Macedonia[2]	2.62	3.00
Serbia		2.98
United Kingdom	2.57	2.84
Montenegro	2.10	2.76
Turkey[2]	1.59	1.88

[1] Data refer to all doctors licensed to practice, resulting in a large over-estimation of the number of practicing doctors (e.g. of around 30% in Portugal).

[2]Data include not only doctors providing direct care to patients, but also those working in the health sector as managers, educators, researchers, etc. (adding another 5-10% of doctors)

Figure 1: Share of different categories of doctors, 2018

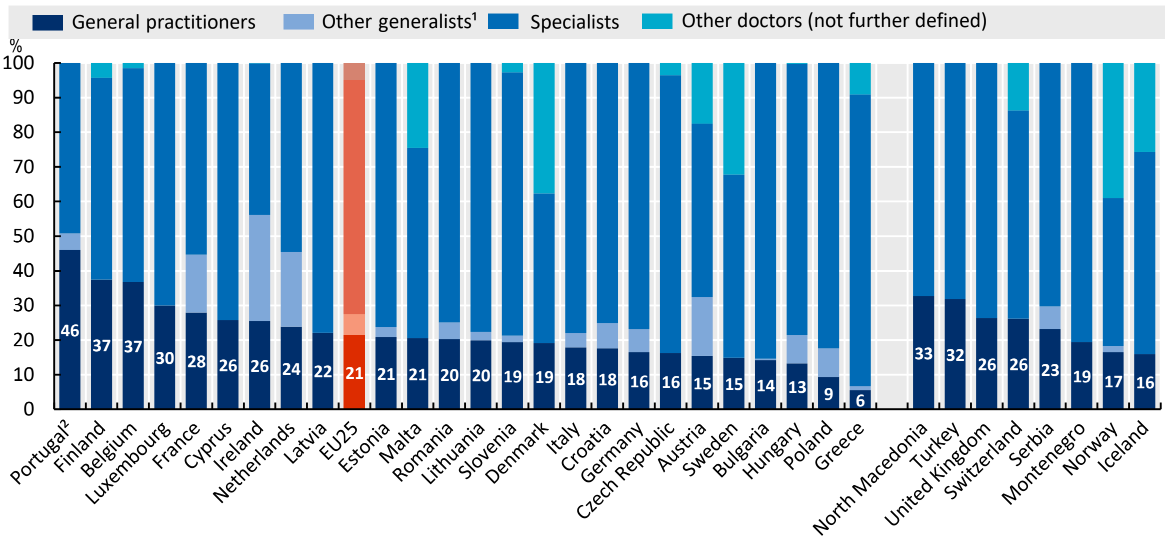

Greece and Poland have the lowest share of GPs, while Portugal, Finland and Belgium have been able to maintain a better balance between GPs and specialists. Several countries have taken steps over the past decade to increase the number of postgraduate training places in general medicine. For example, in France, about 40% of all new postgraduate training places have been allocated to general medicine since 2017, a greater proportion than in most other EU countries [38]. According to most recent data from Hellenic Statistical Authority, there are 3.8 GPs per 10.000 inhabitants (Figure 2) [39]. The number of GPs is smaller than other specialties such as cardiologists, microbiologists, obstetricians-gynecologists (Table 2).

Figure 2: Percentage of general practitioners in physicians' total number in the various regions of Greece

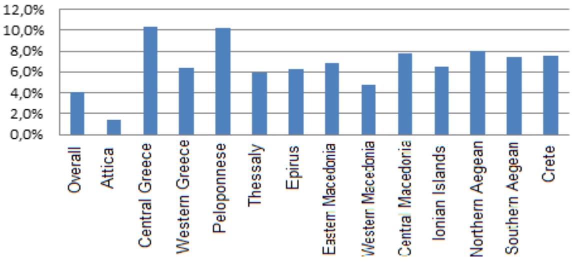

Table 2: Number of doctors per 10.000 inhabitants per specialty in Greece, 2019

Doctors total number	General Medicine	Cardiologists	Microbiologists	Internal Medicine	Paediatrists
66,058	3,232	3,508	3,493	4,604	3,719

An additional challenge in many European countries is the uneven geographic distribution of doctors as well as the various difficulties in recruiting and retaining doctors in remote and sparsely populated areas [38, 40]. In all countries, the density of physicians is generally greater in urban regions, reflecting the concentration of specialized services such as surgery in urban centers as well as physicians' preferences to live and practice in cities. Differences in the density of doctors between urban and rural regions are highest in the Slovak Republic, the Czech Republic and Greece (Figure 3) [38, 40].

Apart from Ireland and Portugal (physicians licensed to practice); there were more specialist physicians than generalist physicians in 2018 in each of the EU Member States [41]. Over time, there has been a widespread movement towards more specialist physicians relative to generalist physicians. In a majority of the EU Member States, there was an increase in the ratio of the number of specialists relative to the number of generalists between the years shown in Figure 5. In relative terms, this was particularly clear in Ireland, Romania and Croatia. By contrast, there were seven Member States, Greece included, where there was a decline in the ratio of specialists to generalists [41].

As far as the training of GPs is concerned, it is not appropriate, given the range of medical knowledge and practice in which they must immerse themselves. The specialty of General Practice/Medicine lasts five years in Greece. However, there is insufficient education regarding the most common diseases of the population, their diagnosis and treatment, as well as the methods of preventive medicine. Lastly, there are no methods of ensuring continuing education for general practitioners after the end of their specialty [42]. All these reasons make the specialty of General Practice in Greece unattractive.

Another reason of the failure of Greek PC system is the absence of an electronic/digital health record, leading to the inability of a continuous monitoring of the community health (continuity of care) [43]. Furthermore, the absence of urban-type PC centers or a network of GPs/FDs also contributes to the latter. These networks could record each patient's medical history, in order to facilitate the work of health professionals [44].

The different types of PC administration between urban centers and the province as well as between public and private sector, create difficulties in the health services provided. Although, PC services in urban areas are provided by doctors of different specialties, in the province (rural areas) there are primary health centers and peripheral clinics staffed mainly by GPs. In both cases there is no reliable system for linking primary to secondary healthcare. Last but not least, the geographical peculiarities of Greece as a country with numerous islands create additional problems.

Figure 3: Physicians by specialty, 2018

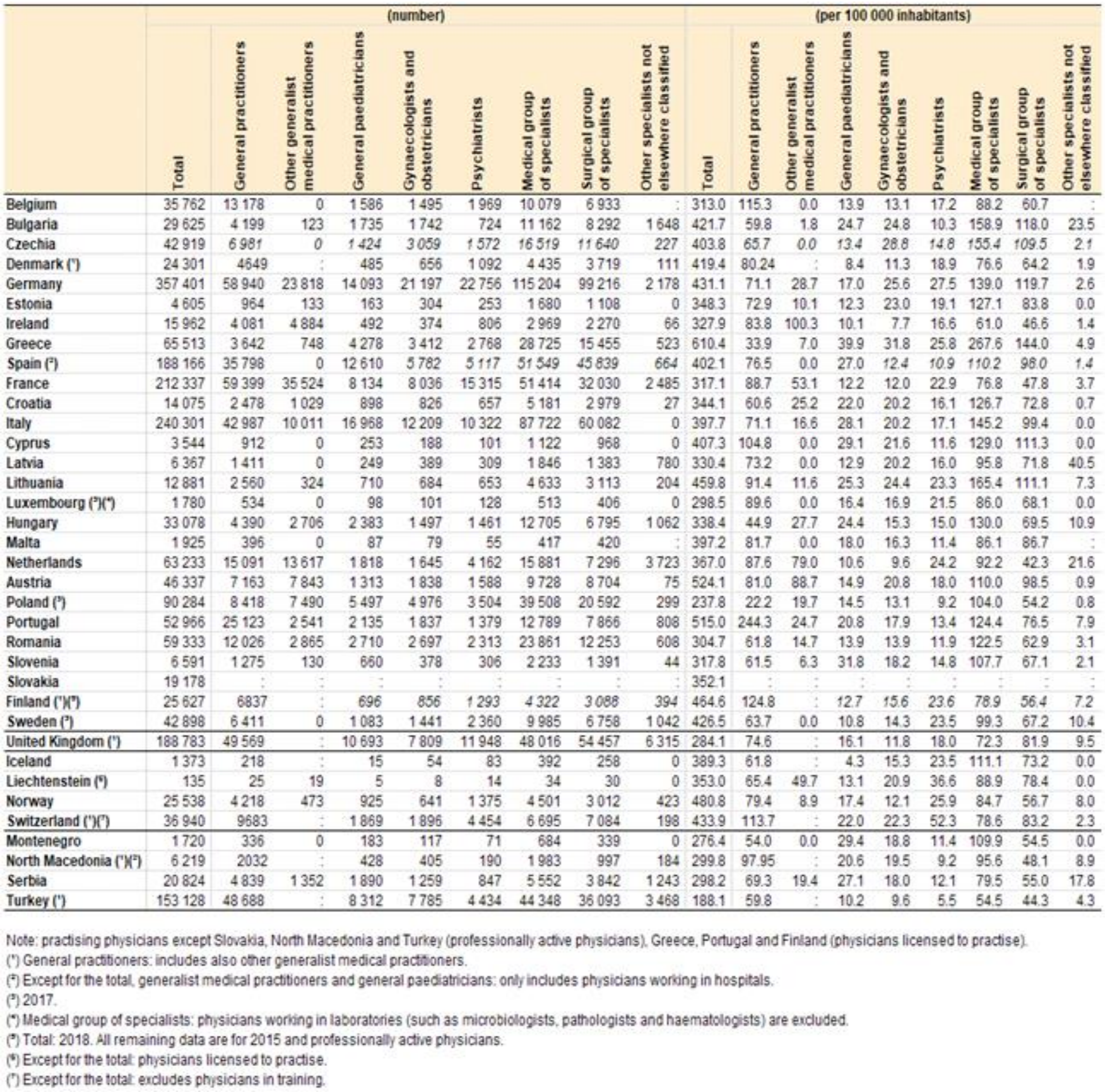

	(number)									(per 100 000 inhabitants)								
	Total	General practitioners	Other generalist medical practitioners	General paediatricians	Gynaecologists and obstetricians	Psychiatrists	Medical group of specialists	Surgical group of specialists	Other specialists not elsewhere classified	Total	General practitioners	Other generalist medical practitioners	General paediatricians	Gynaecologists and obstetricians	Psychiatrists	Medical group of specialists	Surgical group of specialists	Other specialists not elsewhere classified
Belgium	35 762	13 178	0	1 586	1 495	1 969	10 079	6 933	:	313.0	115.3	0.0	13.9	13.1	17.2	88.2	60.7	:
Bulgaria	29 625	4 199	123	1 735	1 742	724	11 162	8 292	1 648	421.7	59.8	1.8	24.7	24.8	10.3	158.9	118.0	23.5
Czechia	42 919	*6 981*	*0*	*1 424*	*3 059*	*1 572*	*16 519*	*11 640*	227	403.8	*65.7*	*0.0*	*13.4*	*28.8*	*14.8*	*155.4*	*109.5*	*2.1*
Denmark (1)	24 301	4649	:	485	656	1 092	4 435	3 719	111	419.4	80.24	:	8.4	11.3	18.9	76.6	64.2	1.9
Germany	357 401	58 940	23 818	14 093	21 197	22 756	115 204	99 216	2 178	431.1	71.1	28.7	17.0	25.6	27.5	139.0	119.7	2.6
Estonia	4 605	964	133	163	304	253	1 680	1 108	0	348.3	72.9	10.1	12.3	23.0	19.1	127.1	83.8	0.0
Ireland	15 962	4 081	4 884	492	374	806	2 969	2 270	66	327.9	83.8	100.3	10.1	7.7	16.6	61.0	46.6	1.4
Greece	65 513	3 642	748	4 278	3 412	2 768	28 725	15 455	523	610.4	33.9	7.0	39.9	31.8	25.8	267.6	144.0	4.9
Spain (2)	188 166	35 798	0	12 610	*5 782*	*5 117*	*51 549*	*45 839*	*664*	402.1	76.5	0.0	27.0	*12.4*	*10.9*	*110.2*	*96.0*	*1.4*
France	212 337	59 399	35 524	8 134	8 036	15 315	51 414	32 030	2 485	317.1	88.7	53.1	12.2	12.0	22.9	76.8	47.8	3.7
Croatia	14 075	2 478	1 029	898	826	657	5 181	2 979	27	344.1	60.6	25.2	22.0	20.2	16.1	126.7	72.8	0.7
Italy	240 301	42 987	10 011	16 968	12 209	10 322	87 722	60 082	0	397.7	71.1	16.6	28.1	20.2	17.1	145.2	99.4	0.0
Cyprus	3 544	912	0	253	188	101	1 122	968	0	407.3	104.8	0.0	29.1	21.6	11.6	129.0	111.3	0.0
Latvia	6 367	1 411	0	249	389	309	1 846	1 383	780	330.4	73.2	0.0	12.9	20.2	16.0	95.8	71.8	40.5
Lithuania	12 881	2 560	324	710	684	653	4 633	3 113	204	459.8	91.4	11.6	25.3	24.4	23.3	165.4	111.1	7.3
Luxembourg (3)(4)	1 780	534	0	98	101	128	513	406	0	298.5	89.6	0.0	16.4	16.9	21.5	86.0	68.1	0.0
Hungary	33 078	4 390	2 706	2 383	1 497	1 461	12 705	6 795	1 062	338.4	44.9	27.7	24.4	15.3	15.0	130.0	69.5	10.9
Malta	1 925	396	0	87	79	55	417	420	:	397.2	81.7	0.0	18.0	16.3	11.4	86.1	86.7	:
Netherlands	63 233	15 091	13 617	1 818	1 645	4 162	15 881	7 296	3 723	367.0	87.6	79.0	10.6	9.6	24.2	92.2	42.3	21.6
Austria	46 337	7 163	7 843	1 313	1 838	1 588	9 728	8 704	75	524.1	81.0	88.7	14.9	20.8	18.0	110.0	98.5	0.9
Poland (3)	90 284	8 418	7 490	5 497	4 976	3 504	39 508	20 592	299	237.8	22.2	19.7	14.5	13.1	9.2	104.0	54.2	0.8
Portugal	52 966	25 123	2 541	2 135	1 837	1 379	12 789	7 866	808	515.0	244.3	24.7	20.8	17.9	13.4	124.4	76.5	7.9
Romania	59 333	12 026	2 865	2 710	2 697	2 313	23 861	12 253	608	304.7	61.8	14.7	13.9	13.9	11.9	122.5	62.9	3.1
Slovenia	6 591	1 275	130	660	378	306	2 233	1 391	44	317.8	61.5	6.3	31.8	18.2	14.8	107.7	67.1	2.1
Slovakia	19 178	:	:	:	:	:	:	:	:	352.1	:	:	:	:	:	:	:	:
Finland (1)(5)	25 627	6837	:	*696*	*856*	*1 293*	*4 322*	*3 088*	*394*	464.6	124.8	:	*12.7*	*15.6*	*23.6*	*78.9*	*56.4*	*7.2*
Sweden (3)	42 898	6 411	0	1 083	1 441	2 360	9 985	6 758	1 042	426.5	63.7	0.0	10.8	14.3	23.5	99.3	67.2	10.4
United Kingdom (1)	188 783	49 569	:	10 693	7 809	11 948	48 016	54 457	6 315	284.1	74.6	:	16.1	11.8	18.0	72.3	81.9	9.5
Iceland	1 373	218	:	15	54	83	392	258	0	389.3	61.8	:	4.3	15.3	23.5	111.1	73.2	0.0
Liechtenstein (6)	135	25	19	5	8	14	34	30	0	353.0	65.4	49.7	13.1	20.9	36.6	88.9	78.4	0.0
Norway	25 538	4 218	473	925	641	1 375	4 501	3 012	423	480.8	79.4	8.9	17.4	12.1	25.9	84.7	56.7	8.0
Switzerland (1)(7)	36 940	9683	:	1 869	1 896	4 454	6 695	7 084	198	433.9	113.7	:	22.0	22.3	52.3	78.6	83.2	2.3
Montenegro	1 720	336	0	183	117	71	684	339	0	276.4	54.0	0.0	29.4	18.8	11.4	109.9	54.5	0.0
North Macedonia (1)(2)	6 219	2032	:	428	405	190	1 983	997	184	299.8	97.95	:	20.6	19.5	9.2	95.6	48.1	8.9
Serbia	20 824	4 839	1 352	1 890	1 259	847	5 552	3 842	1 243	298.2	69.3	19.4	27.1	18.0	12.1	79.5	55.0	17.8
Turkey (1)	153 128	48 688	:	8 312	7 785	4 434	44 348	36 093	3 468	188.1	59.8	:	10.2	9.6	5.5	54.5	44.3	4.3

Note: practising physicians except Slovakia, North Macedonia and Turkey (professionally active physicians), Greece, Portugal and Finland (physicians licensed to practise).

(1) General practitioners: includes also other generalist medical practitioners.

(2) Except for the total, generalist medical practitioners and general paediatricians: only includes physicians working in hospitals.

(3) 2017.

(4) Medical group of specialists: physicians working in laboratories (such as microbiologists, pathologists and haematologists) are excluded.

(5) Total: 2018. All remaining data are for 2015 and professionally active physicians.

(6) Except for the total: physicians licensed to practise.

(7) Except for the total: excludes physicians in training.

Figure 4: Medical doctors employed in hospitals (2008, 2013, 2018)

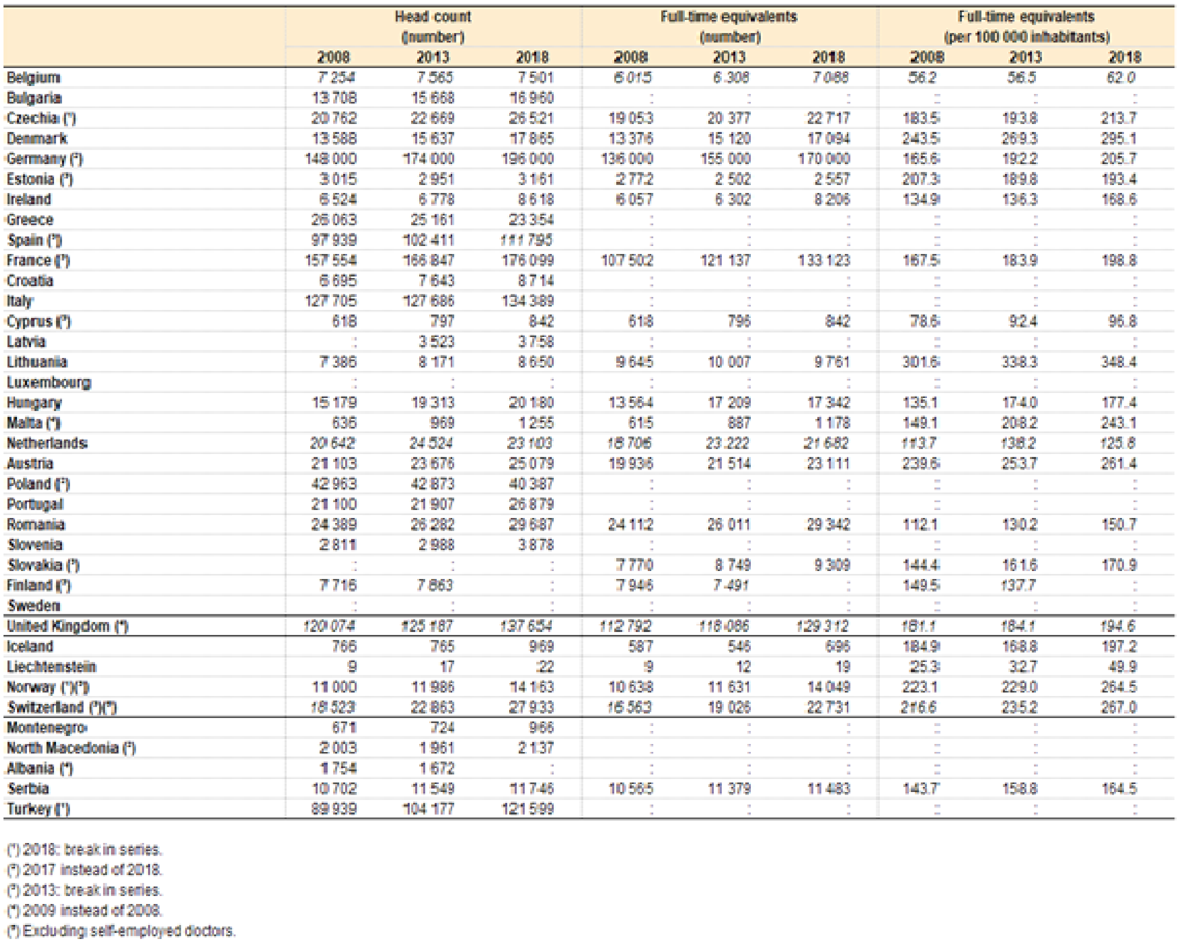

	Head count (number)			Full-time equivalents (number)			Full-time equivalents (per 100 000 inhabitants)		
	2008	2013	2018	2008	2013	2018	2008	2013	2018
Belgium	*7 254*	*7 565*	*7 501*	*6 015*	*6 308*	*7 088*	*56.2*	*56.5*	*62.0*
Bulgaria	13 708	15 668	16 960	:	:	:	:	:	:
Czechia (1)	20 762	22 669	26 521	19 053	20 377	22 717	183.5	193.8	213.7
Denmark	13 588	15 637	17 865	13 376	15 120	17 094	243.5	269.3	295.1
Germany (2)	148 000	174 000	196 000	136 000	155 000	170 000	165.6	192.2	205.7
Estonia (1)	3 015	2 951	3 161	2 772	2 502	2 557	207.3	189.8	193.4
Ireland	6 524	6 778	8 618	6 057	6 302	8 206	134.9	136.3	168.6
Greece	26 063	25 161	23 354	:	:	:	:	:	:
Spain (2)	97 939	102 411	*111 795*	:	:	:	:	:	:
France (3)	157 554	166 847	176 099	107 502	121 137	133 123	167.5	183.9	198.8
Croatia	6 695	7 643	8 714	:	:	:	:	:	:
Italy	127 705	127 686	134 389	:	:	:	:	:	:
Cyprus (3)	618	797	842	618	796	842	78.6	92.4	95.8
Latvia	:	3 523	3 758	:	:	:	:	:	:
Lithuania	7 386	8 171	8 650	9 645	10 007	9 761	301.6	338.3	348.4
Luxembourg	:	:	:	:	:	:	:	:	:
Hungary	15 179	19 313	20 180	13 564	17 209	17 342	135.1	174.0	177.4
Malta (4)	636	969	1 255	615	887	1 178	149.1	208.2	243.1
Netherlands	*20 642*	*24 524*	*23 103*	*18 706*	*23 222*	*21 682*	*113.7*	*138.2*	*125.8*
Austria	21 103	23 676	25 079	19 936	21 514	23 111	239.6	253.7	261.4
Poland (5)	42 963	42 873	40 387	:	:	:	:	:	:
Portugal	21 100	21 907	26 879	:	:	:	:	:	:
Romania	24 389	26 282	29 687	24 112	26 011	29 342	112.1	130.2	150.7
Slovenia	2 811	2 988	3 878	:	:	:	:	:	:
Slovakia (1)	:	:	:	7 770	8 749	9 309	144.4	161.6	170.9
Finland (3)	7 716	*7 863*	:	7 946	*7 491*	:	149.5	*137.7*	:
Sweden	:	:	:	:	:	:	:	:	:
United Kingdom (2)	*120 074*	*125 187*	*137 654*	*112 792*	*118 086*	*129 312*	*181.1*	*184.1*	*194.6*
Iceland	766	765	969	587	546	696	184.9	168.8	197.2
Liechtenstein	9	17	22	9	12	19	25.3	32.7	49.9
Norway (1)(3)	11 000	11 986	14 163	10 638	11 631	14 049	223.1	229.0	264.5
Switzerland (1)(4)	*18 523*	22 863	27 933	*16 563*	19 026	22 731	*216.6*	235.2	267.0
Montenegro	671	724	966	:	:	:	:	:	:
North Macedonia (2)	2 003	1 961	2 137	:	:	:	:	:	:
Albania (4)	1 754	1 672	:	:	:	:	:	:	:
Serbia	10 702	11 549	11 746	10 565	11 379	11 483	143.7	158.8	164.5
Turkey (5)	89 939	104 177	121 599	:	:	:	:	:	:

(1) 2018: break in series.
(2) 2017 instead of 2018.
(3) 2013: break in series.
(4) 2009 instead of 2008.
(5) Excluding self-employed doctors.

Figure 5: Physicians – ratio of specialists to generalists (2008, 2013, 2018)

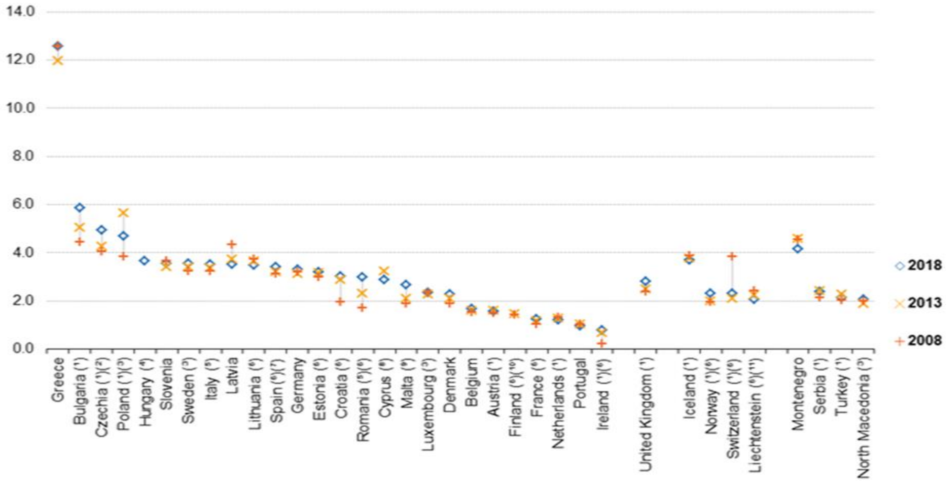

Note: practising physicians except Slovakia, Finland, North Macedonia and Turkey (professionally active physicians), Czechia, Greece and Portugal (physicians licensed to practise). Slovakia: not available.

(1) 2018: break in series.
(2) 2018: estimate.
(3) 2017 instead of 2018.
(4) 2008 and 2013: not available.
(5) 2008: estimate.
(6) 2013: break in series.
(7) 2018: provisional. Data on specialists refer only to physicians working in hospitals.
(8) 2008: not available. 2014 instead of 2013.
(9) 2009 instead of 2008.
(10) 2013: estimate. 2018: not available.
(11) 2008: data on generalists exclude interns.

CONCLUSION

The COVID-19 pandemic has revealed the fragility of all types of health systems around the globe. It is obvious that there is a great need to protect global health with a solid and sufficient PC within a properly organized health system, under a strong and continuous governmental support. PC physicians are the "gatekeepers" of the health system, they are responsible for the provision of a quality care as far as the common infections and diseases (COVID-19 cases included) are concerned, as well as undertaking important public health functions.

A strong PC system may lead to a better health level of the community. Furthermore, FD as an institution is accepted by the society and where it is applied, the citizens are more satisfied with the health services provided, as well as a primary care system based on FD is cost-effective, qualitative and efficient especially in times of pandemics. The reasons why a organized PC network has not been applied in Greece are mostly economic, political, social and geographic.

In conclusion, in order that PC be organized from now on, many actions are required. First, pressure should be exerted in aim that reforms be completed such as the electronic health record. Workshops and web events should be held in order public be informed about the benefits of PC. It is of paramount importance that urban-type health centers be made and be staffed properly, as well as documents to the EU be sent in order to seek financing programs. Last but not least, pressure should also be exerted on governments to pass more groundbreaking bills and implement some of the older provisions.

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VARIA

The interactions between risk factors for ischemic stroke

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Abstract: *Stroke has a significant prevalence in Romania. The predisposition for this multifactorial disease is partially known. The aim of this study is to investigate the predisposition for stroke in Romanian population. Material and methods. We selected cases with recent ischemic stroke (n=100) and healthy control subjects (n=100). The AGTR1 A1166C (rs5186) polymorphism was genotyped by restriction of amplicons with Dde I endonuclease. Results. Active cigarette smoking (O.R. =6.92, p=0.0001) or presence of the AT1R C variant (O.R. =6.69, p=0.0006) in overweight or obese women significantly increase the risk for ischemic stroke. The diagnosis of stroke (71.5 vs 68 years old) or T2DM (63.39 vs 60.77 years old) was recorded at an older age in women compared to men (p<0.05). Obesity considered independent (O.R. =4.22, p<0.05) or in association with T2DM (O.R. = 10.16, p=0.0002) confers the highest risk of stroke when compared to women. Conclusions. Obesity in association with T2DM confers the highest risk of stroke for men when compared to women. Active cigarette smoking or AT1R C variant significantly increase the risk for stroke in women with a high BMI compared with controls.*

Keywords: *stroke, AT1R, BMI, cigarette smoking*

INTRODUCTION

Stroke is an important cause of worldwide mortality and of acquired disability in adults [1]. The prevalence of disease in Romania seems to be several times higher than the average world-wide prevalence [2, 3].

Investigation of positional and functional candidate genes and their interactions with different non-genetic factors is important for understanding pathogenesis of stroke [4, 5]. One of them is angiotensin II type I receptor (AT1R). This receptor, widely expressed in the body, is responsible for the most important effect of angiotensin II. In pathological condition signals from AT1R are involved in oxidative stress, hypertrophy, fibrosis, and inflammation [6]. Consequently, it is an important functional candidate gene for different vascular diseases (including stroke), in human and animal models [7, 8]. In the 3' untranslated region of the AT1R gene was mapped the rs5186 (+1166A/C or A1166C) polymorphism that was associated with stroke in some cohorts but not in Romanian population.

MATERIAL AND METHOD

The aim of this study is to investigate the predisposition for stroke in Romanian population.
We selected cases with ischemic stroke in the last weeks before selection for this study. Cases with a prior stroke or those with stroke onset in young adult (≤45 years old) were

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not selected. Control subjects were considered clinically and paraclinically healthy. All subjects selected are Caucasians who lived in Bucharest or Ilfov County. Subjects who drank >50g alcohol/day or smoked >25 cigarettes/day were not selected for this research.

AGTR1 A1166C polymorphism was genotyped by restriction of amplicons with endonuclease Dde I [9].

Statistical analysis was performed using the StatsDirect software.

RESULTS

The main characteristics of patients and control individuals selected for this study are presented in Table 1 and Table 2. The diagnosis of stroke or T2DM was recorded at an older age in women compared to men ($p<0.05$).

Table 1: The characteristics of the subjects investigated in this study (p< *0.05; p<** 0.0001)

Gender	Men		Women	
Characteristics	Stroke	Healthy	Stroke	Healthy
Men / Women	52	52	48	48
Age at inclusion	66.92±6.21 (53-83)	68.02±5.14 (58-82)	71.44±4.90 (59-83)	70.33±7.01 (55-83)
Weight at inclusion	85.9±8.65 (69-101)	78.17±5.37 (67-92)**	79.25±7.65 (66-99)	69.92±3.89 (59-80) **
Hight at inclusion	1.71±0.04 (1.66-1.84)	1.72±0.04 (1.66- 1.86)	1.69±0.02 (1.66-1.77)	1.7±0.03 (1.66-1.78)
BMI at inclusion	29.35±2.99 (23.51-35.50)	26.39± 1.72 (23.30-29.76) **	27.44±2.59 (22.31-33.86)	24.21±1.23 (20.90-27.68) **
Normal weight/Overweight/ Obesity at inclusion	7/22/23	13/39/0	9/32/7	38/10/0**
Children (yes/no)	32/ 20	41/11	32/16	40/8
Number of children (1, >1)	13/19	18/ 23	20/11	19/21
Living environment (urban/ rural)	35/17	34/18	28/20	35/13
Alcohol (yes/no)	18/34	14/38	0/48	2/46
Cigarette smokers (current or former) (yes/no)	33/19	26/26	28/20	22/26
Average number of cigarettes/days	18.76±2.31 (13-22)	17.19±3.30 (10-20) *	13.46±3.65 (8-20)	14.18±4.17 (10-23)
The age at which this addiction was acquired	21.21±4.23 (13-29)	21.65±4.12 (15-32) *	22.75±4.71 (14-32)	20.5±5.02 (13-31)
Active smoking (years)	42.73±6.55 (19-52)	45.04±9.43 (27-70)	41.36±6.91 (30-58)	46.32±10.06 (32-70)
Former smokers (yes/no)	20/19	7/26 *	22/20	7/26 *
The age until they smoked	60.75±6.53 (44-70)	53.71±3.59 (50-60)	62.82±4.67 (50-70)	59.43±5.35 (50-66)
AGTR1 AA/AC/CC	34/14/4	27/21/4	24/18/6	27/17/4
ATR1 C and Smokers (current or former)	12/40	13/39	14/38	15/37
ATR1 C and BMI> 25 kg/m^2	16/36	20/32	21/27	5/43**
Smokers (current or former) and BMI> 25 kg/m^2	28/24	18/34*	26/22	7/41**

The BMI was higher in patients than in control, regardless of gender ($p<0.0001$). Supraponderal or obese women (BMI > 25 kg/m^2) diagnosed with stroke were more frequently active smokers (O.R. =6.92, 95% CI: 2.59-18.49, p=0.0001) or carriers of the AT1R C variant (O.R. =6.69, 95% CI: 2.25-19.84, p=0.0006) compared to healthy women. A less significant difference was observed between the percent of smokers who had increased BMI between male patients and healthy controls (O.R. =2.2, 95% CI: 1.0 -4.85, $p<0.05$).

The percentage of subjects who quit smoking is higher in the subset of men (O.R. =3.91, 95% CI: 1.38-11.11, p=0.02) or women (O.R. =4.09, 95% CI: 1.46-11.46, p=0.006) with stroke compared to the healthy control subjects.

We noticed that obesity considered independent (O.R. = 4.22, 95% CI: 1.15-15.50, $p<0.05$) or in association with active smoking (O.R. = 3.82, 95% CI: 1.28-11.45, $p<0.05$) increase the risk for stroke in men compared to women (Table 2). The highest risk of stroke was estimated for obese men with T2DM (O.R. = 10.16, 95% CI: 2.79-37.04, p = 0.0002).

Table 2: Comparison of risk factors in stroke patients stratified by gender (p< *0.05; p<** 0.0001)

Comorbidities	Men with stroke	Women with stroke
Age at stroke diagnosis	68 (48-69)	71.5 (56-69)*
Normal weight/Overweight/Obesity at inclusion	7/22/23	9/32/7 *
Obesity vs normoponderal	23/7	7/9*
Obesity and ATR1 C	9/43	4/44
Obesity and active cigarette smoking	16/36	5/43*
Obesity and T2DM	21/31	3/45*
Obesity and HBP	12/40	4/42
T2DM present	31/21	23/25
Age at T2DM diagnosis	60.77±4.03 (53-68)	63.39±3.38 (58-70) *
Weight at diagnosis	94.9±9.52 (77-110)	80.26±8.34 (63-97) **
BMI at T2DM diagnosis	32.51±3.08 (26.33-36.93)	27.77±2.60 (22.06-32.15) **
T2DM and ATR1 C	13/39	10/38
T2DM and BMI> 25 kg/m^2	31/21	21/27
T2DM and active cigarette smoking	19/33	16/32
HBP	28/24	23/25
Age at HBP diagnosis	60.11±5.09 (48-69)	62.52±3.81 (56-69)
HBP and ATR1 C	13/39	14/34
HBP and BMI> 25 kg/m^2	24/28	21/27
HBP and active cigarette smoking	16/36	16/32
Treatment for HBP	27/1	23/0
Adequate control of blood pressure (Yes/No)	18/10	20/3
HBP and T2DM	17/35	16/32
HBP, active smokers and ATR1 C carriers	9/43	9/39

DISCUSSION

Stroke is a heterogenous disease with respect to the etiology, contribution of risk and protective factors in different populations and baseline status at the time of the stroke. A series of characteristics for the ischemic or hemorrhagic forms and for those with onset in young adults or in elderly have been described [10]. Thus, we selected only the cases of ischemic stroke that begins in adults (> 45 years) in order to ensure the homogeneity of the lots investigated in this retrospective study. The relationship between gender and stroke may be influenced by age of investigated subjects [11]. The mean age of patients with stroke selected in our study (69.09 years old) was similar with the values reported in other publications [12, 13]. Women tend to be older than men at stroke onset in different populations. In our lot a similar tendency was identified, the median age at stroke onset was significantly higher in women than in men (71.5 vs 68 years old, $p<0.05$).

Different lifestyle factors can influence the risk for stroke [14, 15]. In this study cigarette smoking and alcohol consumption were found not to be risk factors for stroke, regardless these factors were analyzed independently or in association.

We found that a significant percent of patients with stroke had T2DM (54%), obesity (30%) or have both diseases (24%, 87.5% of these cases was found in men). The high presence of booth comorbidities is concordant with previous data which suggested that obesity or T2DM are significantly associated with stroke [16]. These associations can be influenced by different factors [17]. In our study obesity, considered independent (O.R. =4.22) or in association with active cigarette smoking (O.R. =3.82), increase the risk for stroke in men compared to women. Women (O.R. =6.92) and men (O.R. =2.2) with a BMI > 25 kg/m^2 who were active smokers had a significant risk of stroke.

High blood pressure (HBP) is a major risk factor for stroke. The risk for HBP in Caucasians is increased by the AT1R C variant [18]. More than half of the patients with stroke from this study also had HBP (51%) and ~53% of them were carrier of AT1R C variant. AT1R A1166C was associated with stroke in patients from Italy [19], the Nederlands [20], Sweden [21], and Japan [22]. In some population AT1R C was found to increase the risk for ischemic stroke only in hypertensive smokers (OR= 22.3, p<0.001) [23] or in subjects who carry other risk polymorphism [24, 25]. In other studies [26], including two meta-analysis, AT1R A1166C was not associated with susceptibility to ischemic stroke [27, 28]. In the present study univariate analysis did not reveal an association between AT1R A1166C polymorphism and stroke. However, we identified that women with BMI > 25 kg/m^2 diagnosed with stroke were more frequent carriers of the AT1R C variant compared to healthy control women (O.R.=6.69, p=0.0006).

CONCLUSIONS

The diagnosis of stroke was recorded at an older age in women compared to men. Women with a high BMI who are active smokers or who are carrier of AT1R C variant have a higher risk of stroke. The highest risk of stroke for men was estimated for obese T2DM subjects.

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ORIGINAL ARTICLES

The medical and social importance of mental and behavior disorders among military personnel in peacetime and warfare

Andrii Shvets[1], Irina Sereda[2], Eugenii Lopin[3]

Abstract: *Introduction. The considerable numbers of servicemen who have applied for psychiatric help and who have been discharged for mental health from the Armed Forces of Ukraine testify the need to revise the whole system of providing psychiatric, psychomedical, and social aid to servicemen in warfare.*

Purpose. To identify medical and social aspects of mental and behavioral disorders among servicemen of the Armed Forces of Ukraine in peacetime and warfare for justification the developing and implementing the system of psychomedical care in Ukrainian Defense Forces with NATO standards adaptation.

Methods. A retrospective analysis of statistical data on the level, structure, dynamics of the overall incidence rate of mental disorders as well as hospitalized morbidity among the military personnel of Ukrainian Armed Forces and health status dismissal based on long-term observation data (2008-2017) as well as comparison of indicators of the period 2008-2013 and 2014-2017 based on official statistical reporting has been performed. The statistical processing of the sample results was carried out using descriptive statistics and analysis of alternative characteristics.

Results. It was discovered that in the structure of the total incidence of mental disorders among the military personnel of Ukrainian Armed Forces on class F (ICD-10) for the period of 2014-2017 years there was a significant redistribution of all categories of military personnel in comparison with 2008-2013 (among officers as well as contract servicemen, the overall incidence rate of mental disorders decreased dependably). The proportion of disorders of adult personality and behavior with other syndromes (F50-F99) significantly increased due to a significant decrease of neurotic, stress-related, and somatoform complaints cases.

Conclusions. The overall incidence rate of mental disorders, as well as hospitalized morbidity within the structure of overall incidence rate of diseases in 2008-2017 for officers and contract servicemen, significantly increased with peak values in 2015 ($p \leq 0.001$) with further decreasing in 2016-2017 years, but among conscripts - remained unchanged. It has been established that starting from 2014, the indicators of discharges among officers have tended to increase with the maximum rates in 2015 (compared to 2013 by 7 times higher) and contract servicemen in 2016 (compared to 2013 by 5.3 times higher) with a further decrease. The identified features of mental health disorder in servicemen and their consequences according to long-term observation (both in peacetime and in warfare) are the keys to the development and implementation of the proposed psychomedical care system in defense forces readapted to NATO standards.

Keywords: *military personnel of the Armed Forces of Ukraine, overall incidence rate, hospitalized morbidity, dismissal due to health state, mental and behavior disorders*

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INTRODUCTION

In the last decade in Ukraine, as in many countries around the world, under the influence of economic, social, psychological, and environmental factors, there has been increasing the incidence of mental illness, mainly due to non-psychotic mental disorders, which include neuroses, stress reactions, personality disorders and other violations [1]. In the conditions of a long socio-economic crisis, socio-psychological factors of mental disorders acquire a significant segment, which leads to the increasing internal conflict of people and contributes to their greater prevalence [2].

Mental health professionals from many countries around the world are very concerned about the scale of mental pathology, the cost to society and the state due to its high prevalence, medical & social consequences of mental disorders (comorbidity, suicide, and social dysfunction). Significant economic burden because of temporary and permanent disability due to mental disorders was manifested by losses of gross domestic product [3, 4, 5, 6].

The risk group for mental disorders is certain occupational groups, which primarily include the military, in which acute and prolonged stress can be layered on individual-typological and personal characteristics. It has been established that servicemen, especially conscripts, were affected by numerous factors of mental trauma, both non-specific (illness or death of relatives, financial difficulties of the family, family conflicts) and specific (workloads, restrictions, and denials due to military service, material, and domestic inconvenience). Modern military service characterized by a high level of requirements, rising prices for erroneous actions of the military specialist, and rapidly changing, intensely saturated daily rhythms do not allow individuals to sufficiently recover and adapt to the service rules [2, 7].

Moreover, powerful prerequisites for the formation of mental pathology in servicemen arise in the context of hostilities. The need to survive in a situation of death and destruction, negative impressions, moral and psychological unpreparedness, fear of not coping with responsibilities, guilt for the dead, uncertainty and lack of information, sleep disorders, rest and nutrition, illness, injury, lack of experience of the stock of possible appropriate reactions were the main factors influencing the mind of servicemen in modern warfare [8]. During combat operations, 75% of servicemen may develop short-term psychological stress reactions, accompanied by partial or complete loss of combat capability [9].

A significant number of servicemen who have applied for psychiatric care and have been discharged from the Armed Forces (AF) of Ukraine for mental health reasons indicate the need to review the entire system of providing psychiatric, medical, psychological, and social assistance to servicemen during crisis time [9]. All of the above makes the problem of mental and behavioral disorders one of the most pressing medical and social problems in the modern army.

THE OBJECTIVE OF THIS STUDY

The purpose of this study is to identify medical and social aspects of mental and behavioral disorders among servicemen of the Armed Forces of Ukraine in peacetime and warfare for justification for the developing and implementing the system of psychomedical care in Ukrainian Defense Forces, adapted to NATO standards.

MATERIALS AND METHODS

A retrospective analysis of statistical data on the level, structure, dynamics of the overall incidence rate of mental & behavioral disorders as well as hospitalized morbidity among the military personnel of Ukrainian Armed Forces and health status dismissal based on long-term observation data (2008-2017) as well as comparison of indicators of the period 2008-2013 and 2014-2017 based on official statistical reporting has been performed.

The material sources were the data of official sectorial statistical reporting on form 2/MED. Statistical data for filling in the reporting form 2/MED were entered into the automated information system of medical treatment facilities reports "Contingent". These medical and statistical indicators were studied for the period 2008-2013 and 2014-2017 separately for each category of servicemen (officers, contract servicemen (privates, sergeants, and non-commissioned officers) and conscripts). Disease groups (psychiatric disorders (F00-F39); neurotic, stress-related and somatoform disorders (F40-F48); disorders of adult personality and behavior with other syndromes (F50-F99)) and individual nosological forms were evaluated by ICD-10.

Statistical processing of the sample results was performed using Microsoft Excel and STATISTICA 8.0 [10]. The arithmetic mean (*x*) and the error of the mean (m) were calculated. When comparing the indicators for different periods (2008-2013 from 2014-2017), the significant difference in relative values was assessed by Student's t-test.

RESULTS

The analysis of general morbidity indicators of mental and behavioral disorders among servicemen of the Armed Forces of Ukraine in the dynamics of long-term observation for

2008-2017 showed that for 2008-2013 (during the peaceful functioning of the Armed Forces of Ukraine) the most significant value of total morbidity was observed in 2008 with a further downward trend (Figure 1).

Figure 1. Dynamics of the overall incidence rate of mental & behavioral disorders among servicemen of the Armed Forces of Ukraine for 2008-2017.

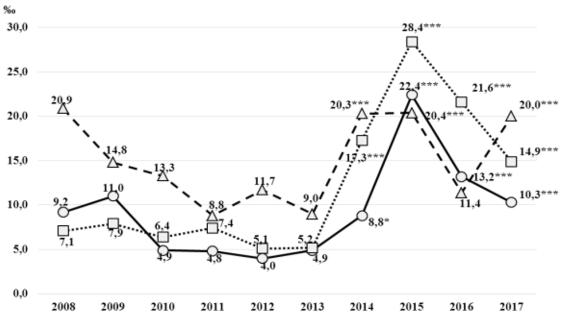

–o– officers; --□-- contract servicemen; –Δ–conscripts

*Note: *, **, *** - significant difference of exemptions of the overall incidence rate of mental & behavioral disorders between 2013 and 2014, 2015, 2016, 2017 years by Student's test corresponds to levels of p <0.05, p <0.01 and p < 0.001.*

It was established that in the structure of the overall incidence rate of mental and behavioral disorders for the period 2008-2013of servicemen of the Armed Forces of Ukraine by class the number of psychiatric disorders (F00-F39) in conscripts was significantly higher (p≤0,001) compared to the officers and significantly higher (p≤0,01) compared to the contract servicemen.

Also in this period, the share of the population with disorders of adult personality and behavior including other syndromes (F50-F99) in conscripts was significantly higher (p≤0.01) compared to the indicators of the relevant nosology in officers, and significantly higher (p≤0.05) compared to the contract servicemen. The proportion of neurotic, stress-related, and somatoform disorders (F40-F48) was significantly higher (p≤0.001) in officers compared to conscripts, and no significant difference was found in contract servicemen compared to the officers.

In the period 2014-2017, the incidence of mental and behavioral disorders in all categories of servicemen was represented mainly by neurotic, stress-related, and somatoform disorders. This nosology was most prevalent in officers - 79.9% (10.93 ± 1.1 ‰), in contract servicemen it was - 77.6% (15.95 ± 3.03‰), in conscripts – 36.9% (6.63 ± 2.07 ‰).

The redistribution of the structure in all categories of servicemen was noteworthy. Thus, in 2014-2017, compared to 2008-2013, the incidence of disorders of adult personality and behavior including other syndromes among officers and contract servicemen significantly decreased (p≤0.001) due to a significant increase (p≤0.001) in psychiatric disorders (F00-F39). The share of the population with psychiatric disorders in conscripts increased significantly (p≤0.001) due to a significant decrease (p≤0.001) in the proportion of neurotic, stress-related, and somatoform disorders.

The overall incidence rate of mental disorders (ICD-10 class V) in the structure of overall incidence rate of diseases among servicemen from 2008 to 2017 is presented in Figure 2. It was established that the trend of the overall incidence rate of mental & behavioral disorders in the structure of the overall incidence rate diseases among all servicemen categories for 2008-2013 was almost the same in the range from 0.5% to 0.9%, namely: officers - from 0.5% up to 0.9%; for contract servicemen - from 0.6% to 0.8%; for conscripts - from 0.5% to 0.7%. There was no significant difference between the average incidence rates for class F diseases (ICD-10) in all categories of servicemen. During the period 2014-2017, compared to 2013, the occurrence of mental & behavioral disorders in the overall structure of diseases among all servicemen significantly increased with peak values in 2015 (officers – 2.21%, contract servicemen – 3.8%, conscripts – 1.11%, which were significantly higher (p≤0.001 compared to 2013) with a further decrease in 2016-2017.

Figure 2. Dynamics of the overall incidence rate of mental & behavioral disorders in the structure of the overall incidence rate diseases among servicemen in 2008 - 2017, %.

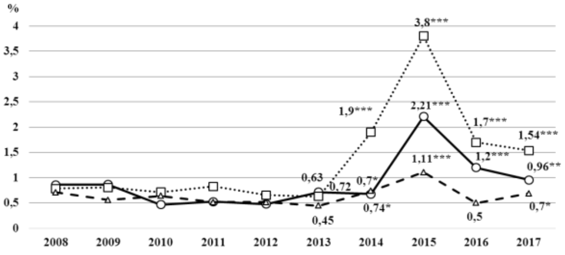

–o– officers; --□-- contract servicemen; –Δ–conscripts

*Note: *, **, *** - significant difference of exemptions of the overall incidence rate of mental & behavioral disorders in the structure of overall incidence rate diseases between 2013 and 2014, 2015, 2016, 2017 years by Student's criterion corresponds to the level of p <0,05, p <0.01 and p <0.001.*

In a further study, we analyzed the hospitalized morbidity of class F diseases among officers, contract servicemen, and conscripts in the dynamics for 2008-2017 (shown in Figure 3).

It was found that the highest rates of hospitalized morbidity of class F diseases (p≤0.001) compared to previous years for contract servicemen and officers for the studied period were observed in 2015 (19.31 ‰ and 15.42 ‰, respectively).

Figure 3. Dynamics of hospitalized morbidity of mental & behavioral disorders among servicemen of the Armed Forces of Ukraine in 2008-2017.

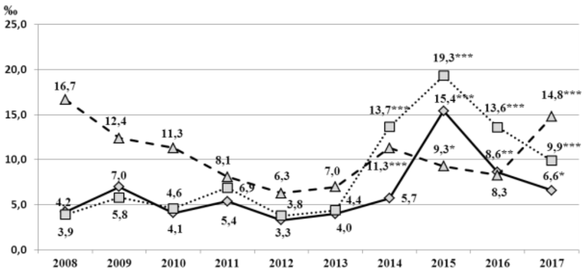

–o– officers; --□-- contract servicemen; –Δ–conscripts

*Note: *, **, *** - significant difference of exemptions of hospitalized morbidity of class F diseases between 2013 and 2014, 2015, 2016, 2017 years according to the Student's criterion corresponds to the levels of p <0.05, p <0.01, and p <0.001.*

It was noteworthy the increase in hospitalized morbidity due to mental and behavioral disorders among conscripts in 2014 with a further decrease in 2015-2016 and a sharp increase (p≤0.001) in 2017 (in 1.8 times compared to 2016).

During the observed period, the hospitalization due to mental and behavioral disorders was studied in all categories of servicemen, it was represented mainly by neurotic, stress-related, and somatoform disorders.

Between 2014 and 2017, the change in the structure of hospitalized morbidity in all categories of servicemen was noteworthy. Thus, in comparison with 2008-2013, the occurrence of psychiatric disorders (F00-F39) among officers and contract servicemen significantly increased (p≤0.001) (in 4.5 and 1.7 times higher, respectively) and the occurrence of disorders of adult personality and behavior including other syndromes (F50-F99) significantly decreased (p≤0.001), (in 8.0 and 3.0 times higher, respectively). Conscripts had a 2-fold (p≤0.001) increase in the proportion of F50-F99, and a 1.5-fold decrease in the proportion (p≤0.01) of stress-related and somatoform disorders.

Also, the occurrence of disorders of adult personality and behavior including other syndromes among conscripts for the period 2014-2017 was significantly higher (p≤0.001) and significantly lower (p≤0.001) in the proportion of neurotic, stress-related, and somatoform disorders compared to indicators on the relevant nosology of officers and contract servicemen.

It was established that the occurrence of hospitalized morbidity of class F diseases in the structure of overall incidence rate diseases among all categories of servicemen for 2008-2013 was almost the same. During the period 2014-2017, compared to 2013, this indicator significantly (p≤0.001) increased in 2015 for contract servicemen and conscripts (in 3.0 and 3.6 times higher, respectively) with a sharp decrease in 2016-2017, moreover, for officers, this indicator was significantly (p≤0.001) in 2.5 times lower compared to 2013.

The occurrence of hospitalized morbidity and mental illness in the structure of major diseases remained unchanged among conscripts.

The next stage of the research was to study the data of servicemen retirements from military service due to mental and behavioral disorders for the 2013-2017 years (Figure 4).

Figure 4. Dynamics of retirements due to mental and behavioral disorders among servicemen of the Armed Forces of Ukraine in 2013-2017.

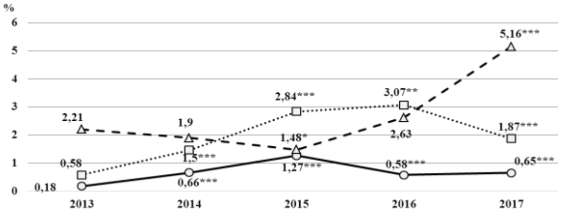

–o– officers; --□-- contract servicemen; –Δ–conscripts

*Note: *, **, *** - - a significant difference of exemptions of retirements due to mental and behavioral disorders between 2013 and 2014, 2015, 2016, 2017 years according to the Student's criterion corresponds to the levels p <0.05, p <0.01, and p <0.001.*

It was established that starting from 2014, the indicators of retirements due to mental and behavioral disorders among officers tended to increase from the maximum in 2015 (compared to 2013 by 7 times higher) with a further decrease in 2016 and 2017 years, however, compared to 2013 remained significantly higher (p≤0.001).

For contract servicemen, this rate increased every year with the maximum value in 2016 (compared to 2013 by 5.3 times higher). It was interesting that the indicators of hospital morbidity and overall incidence rate of mental & behavioral disorders in 2016 tended to decrease. In 2017, the indicator decreased almost 2 times, but compared to 2013 it remained significantly higher (p≤0.001).

The indicators of conscripts' discharges were noteworthy, which were decreasing from 2013 to 2015, while the indicators of hospital morbidity and overall incidence rate of mental & behavioral disorders were increasing. Since 2016, this rate has been increased with a decrease in-hospital morbidity and overall incidence rate of mental & behavioral disorders. In 2017, with a sharp increase in the incidence of mental & behavioral disorders, the rate of dismissals due to mental and behavioral disorders also increased sharply.

It should be noted, while the incidence rate of mental & behavioral disorders among servicemen of all categories was on the 9-13 range places, the indicators of discharges due to mental diseases took the first place (for contract servicemen in 2015-2017 – the 1st place, for officers – I-II, for conscripts – II-III places).

Thus, 2015 year was the most significant in terms of the growth of mental and behavioral disorders among servicemen as a negative consequence of the armed conflict in Eastern Ukraine. This was also confirmed by the rapid increase in suicides level in the Armed Forces of Ukraine (the number of suicides in 2015 increased almost 3 times compared to 2014) [11]. In the structure of expert decisions performed by the Medical Examination Board based on the results of treatment of psychiatric patients after combat conditions at the National Military Medical Clinical Center (for the period 2014-2017), 30.0% of patients were declared unfit for military service in peacetime or had complete unfitness for military service.

DISCUSSION

The experience of the armed conflict in Eastern Ukraine shows that it had a significant negative impact on the mental health disorders in servicemen with corresponding medical and social consequences, which require the development of effective preventive measures and appropriate medical and psychological assistance to servicemen. The highest incidence of mental and behavioral disorders in 2015 can be explained by several factors, such as the performance of combat missions in the anti-terrorist operation zone mostly by officers and contract servicemen who have acute or delayed effects of the severe stress response; conscription of a significant number of servicemen for mobilization and low quality of their selection. In addition, according to our scientists [11] during the first period of formation of the psychiatric care system to servicemen (2014-2015), a specific system of providing psycho-medical care to servicemen was absent. Such assistance was provided unsystematically, mostly by temporarily seconded psychiatrists and psychologists to military mobile hospitals, which successively replaced each other throughout the anti-terrorist operation period.

The analysis of own research and NATO's experience has become the basis for the development of focused measures to preserve the mental disorders in servicemen at combat zone and during the combat capability restoration of military units. “AmedP 8.6 Forward mental health care” and “AmedP-63 Management of post-deployment somatoform complaints” have been readopted, take into account the requirements of the US FM 4-02-51 "Combat and operational stress control" and the US Military First Aid Manual FM 4-25.11 section "Combat and Operational Stress Reaction", WHO recommendations “Psychological first aid: Guide for field workers”, other US and Canadian guidelines for suicide prevention in the military have been implemented in our previous work [13].

The developed psychomedical care model was based on the Mental Health Continuum Model accepted by NATO countries. In the first stages of mental disorders progression, the most important role in preserving mental health belongs to the commanders (Figure 5) [8].

Figure 5. Psychomedical care model in Ukrainian Armed Forces

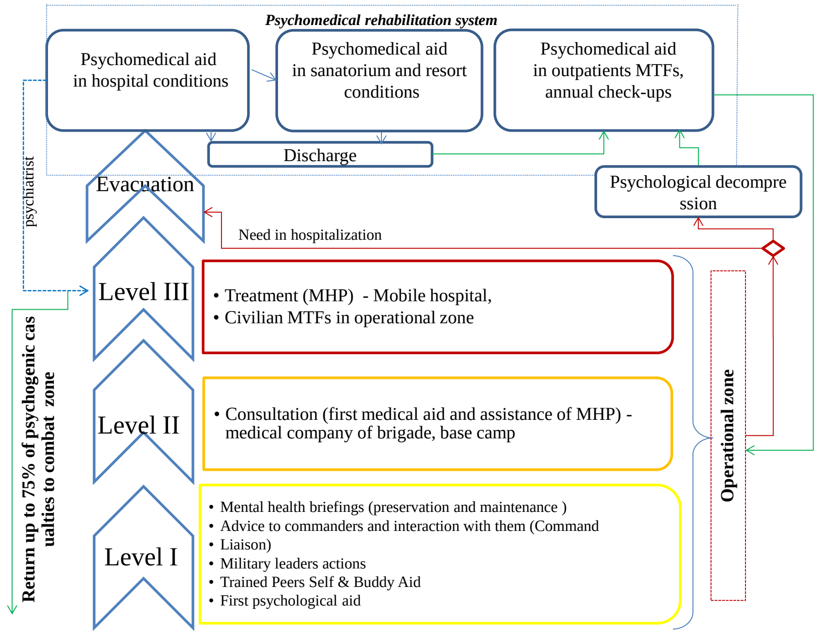
Psychomedical rehabilitation system
Psychomedical aid in hospital conditions
Psychomedical aid in sanatorium and resort conditions
Psychomedical aid in outpatients MTFs, annual check-ups
Discharge
Psychological decompression
Evacuation
psychiatrist
Need in hospitalization
Level III
• Treatment (MHP) - Mobile hospital,
• Civilian MTFs in operational zone
Level II
• Consultation (first medical aid and assistance of MHP) - medical company of brigade, base camp
Level I
• Mental health briefings (preservation and maintenance)
• Advice to commanders and interaction with them (Command Liaison)
• Military leaders actions
• Trained Peers Self & Buddy Aid
• First psychological aid
Operational zone
Return up to 75% of psychogenic casualties to combat zone

Psychomedical aid should be carried out on all levels of medical evacuation (levels of medical care) following the main measures of care for the acute stress reaction. When moving from one level to another, the capacity of care increases, while the number of individuals requiring psychomedical aid decreases.

Level 1 support is the first line of support and includes leadership actions, self-help, and "buddy" support. This level normally occurs immediately following a potentially traumatic event and represents the most frequently in-demand level of support.

Level 2 support consists of a combination of medical and psychological assistance (provided by medical personnel of the military unit and mental health professionals). And Level 3 support involves assessment and treatment services by mental health professionals.

The organization of psychomedical aid was based on the principles of timeliness and accessibility, following the current level of scientific knowledge; necessity and adequacy of medical measures with minimal social and legal restrictions; consistency (implementation of medical and psychological assistance at the following levels with increasing capacity); continuity (the relationship between the stages of medical care with the capacity of medical and psychological aid, the maintenance of medical documentation); legality, humanity.

Psychomedical aid should not be performed only in a military conflict context, it must be ensured in peacetime daily activities, which will ultimately contribute to maintaining the combat capability of the troops.

After units' departure, it is desirable to carry out measures of psychological decompression and the allocation of a group who need hospital medical care. We would like to note that due to the deployment of psychiatrists as reinforcement groups to the mobile hospitals, it was possible to significantly reduce the movement of patients with mental and behavioral disorders to the higher level MTFs and in 75% of cases return them to their duties [8].

Further research prospects are advance development of effective preventive measures to maintain the mental health of servicemen, which, from the one hand, will be aimed at improving the system of professional selection to the defense forces, progressing modern methods of training human resilience, and on the other hand - objectifying the process assessment of stressors during the daily activities of troops (forces), risk management of mental health by developing modern measures for maladaptation prevention in military personnel (neurotic and mental disorders, pre-suicidal behaviors), as well as providing training on medical and psychological care, psychological counseling by mental health professionals according to the NATO standards.

CONCLUSIONS

1. The overall incidence rate of mental disorders, as well as hospitalized morbidity within the structure of overall incidence rate of diseases in 2008-2017 for officers and contract servicemen, significantly increased with peak values in 2015 ($p \leq 0.001$) with further decreasing in 2016-2017 years, but among conscripts – remained unchanged.

2. It was established that in the structure of mental and behavioral disorders among Ukrainian servicemen there was a redistribution in all servicemen categories in 2014-2017 compared to 2008-2013 years. Thus, among officers and contract servicemen, the occurrence of disorders of adult personality and behavior with other syndromes (F50-F99) significantly decreased ($p \leq 0{,}001$ and $p \leq 0.01$, respectively) due to a significant increase ($p \leq 0.001$) of psychiatric disorders (F00-F39) cases. The quantity of F50-F99 nosology among conscripts increased significantly ($p \leq 0.001$) due to a significant decrease ($p \leq 0.001$) of neurotic, stress-related, and somatoform disorders.

3. It has been established that starting from 2014, the indicators of discharges among officers have tended to increase with the maximum rates in 2015 (compared to 2013 by 7 times higher) and contract servicemen in 2016 (compared to 2013 by 5.3 times higher) with a further decrease.

4. The identified features of mental health disorders among servicemen and their consequences according to long-term observation (both in peacetime and in warfare) are the keys to the development and implementation of the proposed psychomedical care system in defense forces readapted to NATO standards.

Conflict of interest

Nothing to declare.

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EDITORIAL

The Military Medical Institute: its past and its future

Mihail Tudosie

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The Military-Medical Institute was founded in 1884 through High Royal Decree number 2824 published in Official Monitor number 2/23.01.1884, and was initially led by general academician Zaharia Petrescu. For a long period of time, the institute functioned based on models such as similar Austrian and Prussian institutes, but with a strong French influence. In those times, as well, the students attended both professional training courses at the Faculty of Medicine of Bucharest and military medical training within the Institute.

Through a regrettable decision of the authorities, the courses of the institute are suspended in 1987, military medicine being deeply prejudiced. During the following years, up to 1990, military medical staff was recruited indirectly.

In 1990, the Government of Romania brings moral justice, by adopting Government Decision 585, through which the Military-Medical Institute of Bucharest is re-established as a superior institute of military medical education, with a Military-Medical Training Section based in Târgu-Mureş.

The present activity of the Institute relies on these traditions, which ensure military medical training of all future doctor officers of the Romanian Army, the Ministry of Internal Affairs and the National Administration of Prisons.

For the continuous improvement of military doctors in the structures of the Romanian Army, career classes are being organized and held in the Military-Medical Institute with a curricula that is compatible with NATO standards within the operational medicine field.

The teaching personnel of the Institute is permanently preoccupied with promoting academic elitism. Students must find models to follow. Every day, the teaching personnel of the Institute must pass the test of education optimization and of increasing the quality of the educational act. In this regard, steps are being taken at the ministry's management level for the recruitment of teaching personnel. The quality of the educational act can be improved only through the recruitment of personnel with a high level of professional training and, also, through the equipment of classrooms with video projection systems and highly performing apparatus in both Bucharest and Târgu-Mureş,

Because mobility nowadays is not only desirable, but also a necessity, we are doing all that is in our power to achieve this scope. Our students participate in Erasmus+ programs offered by the two Universities of Medicine and Pharmacy – "Carol Davila" of Bucharest and that of Târgu-Mureş – with whom we form a partnership. These scholarships ensure our students' preparation for a whole semester at first class

European medicine universities. Thus, students connect “live” to medicine in other countries. At the national level, they participate in the experience exchange program held between civil universities – TransMed. Military students are amongst the most disciplined and diligent, representing a true heraldry of the Army in the civil universities’ environment.

Also, regarding operational military medicine, our students have participated and continue to participate in different national and international exercises, where they learn from the experience of NATO allied countries. A lot of students of the Institute work during their studying years as volunteers at emergency medicine services, such as S. M. U. R. D.

In order to improve scientific performances, the Military Medicine Students’ Association, in collaboration with the Military-Medical Institute, annually organizes a scientific presentations event, in partnership with military students of other academies. In a similar manner, UMF Tg. Mures annually organizes an international congress, which includes a special military medicine session.

A reason for pride is also represented by the consistent participations of the institutes’ students at numerous meetings, symposiums and congresses organized by the civil medicine universities or by the army: The Balkan Medicine Congress or the congresses organized by the academies representing the three forces of the Romanian Army. Thus, young military students are connected to a flow of information pertaining to university science, showing a striking inclination towards research work.

Another aspect pertaining to priority preoccupations is represented by the participation in sports activities organized between military education institutes or between the various components of the National Defense System, Public Order System and National Security System, where the students of the Institute always win a leading place.

Taking into consideration the actual recruiting demands of the Army, regarding the increase of our defense capability, the tuition figures for 2018 have doubled. Thus, 100 places were approved for Bucharest and another 100 places for Tg. Mures. Of these, general medicine represents 60 places in Bucharest and 90 in Tg. Mures. For dental medicine, 13 places are approved, and 15 for pharmacy, only in Bucharest. The other beneficiaries have their places approved for general medicine and dental medicine as follows: Ministry of Internal Affairs – 12 places (7 in Bucharest and 5 in Tg. Mures), and National Administration of Prisons - 8 places for general medicine (5 places in Bucharest and 3 in Tg. Mures), 2 places for dental medicine. As tuition figures rise, by the order of the Ministry of National Defense, in 2018, the institute has 20 places approved for doctors who will become officers through indirect activation.

For all the conducted activities, we enjoy an excellent collaboration with military hospitals and operative military units, as well as with civil universities. Thus, we can form and perfect military doctors for our hospitals and units, who are always ready to take part in international missions.

It is known that the performing armies of the world have never hesitated to show their skill, equipment and combative force. We also adhere to this direction, going out in public space every time we have the chance.

The image of the Institute if promoted by all means (press, radio, television, Internet). The students of the Institute participate in military ceremonies, charity events or blood donating activities, and activities which promote the military doctor profession in civil and military high-schools.

In the future, we wish to maintain and even ascend this standard of medical and military training through which our students have always stood out. We will stress the importance of developing the capacity and appetite for learning, of creativity, and of creating the profile of a military doctor that is receptive to all that is new, always willing to be involved, ready for action, competent and competitive. The future belong to these young people and for this, we do all that is in our power to form them as medical doctors of great value for the Romanian Army.

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VARIA

The most efficient types of training in cardiopulmonary rehabilitation programs: A narrative review

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Abstract: *Objective. This narrative review sought to identify the most frequently used types of exercises in cardiopulmonary rehabilitation programs and to verify the level of efficiency and feasibility of this treatment method in cardiovascular diseased patients.*

Methods. We conducted research via Pubmed over two months using specific keywords related to cardiorespiratory rehabilitation, cardiovascular rehabilitation, endurance exercises, and respiratory exercises. In addition, we included studies that involved physical training for patients diagnosed with coronary artery disease, chronic heart failure, valvular disease or valvular surgery, asthma, and chronic obstructive pulmonary disease.

Results. A total of fifteen articles were included in this narrative review. Eight studies investigated the effect of aerobic training, alone or combined, in the rehabilitation process for patients with cardiovascular disease and/or pulmonary disease. It showed that this type of training is the most recommended and used for these specific pathologies. Seven studies showed the effects of the resistance training and progressive resistance training, alone or combined, and proved the importance of their integration in patients with skeletal muscle weakness and altered functional capacity. High-intensity interval training was not superior to moderate-intensity continuous training in improving aerobic capacity and changing ventricular remodeling in patients with chronic heart failure. However, the results were promising in patients with coronary artery disease. Combined training had the best results in improving the aerobic capacity, muscle strength, functional capacity, ventilatory responses, and enhancing the quality of life for cardiovascular patients.

Conclusions. Combined training was found superior to aerobic training, resistance training, respiratory training, or inspiratory muscles training applied alone as a treatment in cardiopulmonary rehabilitation programs. Thus, every rehabilitation program should be individualized for every patient to increase the effort capacity using aerobic training and functional capacity of the skeletal muscles by increasing the muscle mass (hypertrophy) as an effect of resistance training or progressive resistance training.

Keywords: *cardiorespiratory rehabilitation, cardiopulmonary rehabilitation, cardiovascular rehabilitation, aerobic exercises, cardiorespiratory fitness,* VO_{2max}.

INTRODUCTION

Cardiac rehabilitation is a multidisciplinary approach that improves the patient's quality of life, cardiovascular risk factors, hospital readmissions, and disease-related morbidity and mortality over time [1–4]. Despite the beneficial effects and long term health-outcomes of this treatment method in diseased cardiovascular patients, it remains undervalued with low compliance from participants [2,5,6].

This comprehensive and multidisciplinary method consists of aerobic exercises, resistance exercises, respiratory muscles exercises, lifestyle modifications (dietary recommendations and smoking cessation), and psychological counseling [1,3]. Given that today all medical societies emphasize the importance of lifestyle in the primary and secondary prevention of chronic diseases, we are talking about lifestyle as a whole, made up of all these components with additive effects, and not just about the exercise itself. Along with

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cardiovascular disease, diabetes mellitus is a chronic disease with common complications, often found among patients with heart diseases, which significantly benefits from changes in lifestyle according to the latest studies [7,8]. Another public health problem with an increasing incidence is cerebrovascular disease through its many forms of presentation. In addition to the lifestyle-changing benefits, it also seems to profit from non-pharmacological alternative therapies [9].

It is considered that regular physical activity with a low-to-moderate intensity is a preventive measure for cardiovascular disease. During low-to-moderate exercise, lipids are used as an energy source, and skeletal muscles improve their functional capacity. There is an increase in the local blood supply to support metabolic needs during physical activity and a reduction in peripheral vascular resistance [10].

In cardiac patients, aerobic training remains the keystone of the rehabilitation programs and includes cycling on a cycloergometer, treadmill walking, and aerobic exercises. These activities are performed in a controlled environment and under the supervision of a cardiac rehabilitation team. Patients can continue their aerobic training in their free time with swimming, medium intensity sports, and even dancing, thus maintaining an active lifestyle [11]. Moderate-intensity continuous training (MICT) is a form of prescription and dosing of aerobic exercise, which has been shown to have both short-term and long-term clinical benefits [12,13]. A meta-analysis indicated that aerobic training influences ventricular remodeling in clinically stable heart failure patients. The mechanism according to which aerobic training influences left ventricular remodeling is not entirely elucidated. However, some theories include reducing vasoconstrictive neurohormones or a decrease in hemodynamic loading [14].

Resistance training (RST) is an anaerobic exercise that performs repetitive movements against resistance to stimulate a stronger muscle contraction [15]. RST has a beneficial effect on muscle strength and, endurance, functional capacity, thus improving the cardiovascular diseased patients' quality of life [16]. It is important not to forget that resistance training is complementary to aerobic training and does not replace it. A cardiac rehabilitation program tailored by combined resistance and aerobic exercises will lead to superior benefits, including regain or improvement in functional independence and better performance of daily living activities (ADL) [11].

High-intensity interval training (HIIT) consists of intense exercise interspersed with low-intensity recovery [12]. The ratio of duration between high-intensity intervals and low-intensity intervals is important to differentiate HIIT from MICT and to achieve the beneficial physiological effects of HIIT [12,17,18]. It seems that both aerobic interval training (AIT) and continuous aerobic training (ACT) have the same beneficial effect on peak VO_2 (an important marker in cardiovascular mortality), thus improving cardiopulmonary fitness. Furthermore, both programs are safe for cardiovascular patients and are very efficient in the first six weeks of training [19].

For treating patients with symptoms such as dyspnoea, low inspiratory muscle function, and exercise tolerance, inspiratory muscle training (IMT) is a valuable procedure [20,21]. Several studies observed that IMT at a maximum of 30% of maximal inspiratory pressure (MIP), corresponding to a low-to-moderate intensity, had significant improvement in peak VO_2 (oxygen consumption) and respiratory muscle strength [22–24].

High-intensity inspiratory muscle training is defined as 60% of MIP and is effective in chronic heart failure patients (CHF) for enhancing respiratory muscle strength when applied over four weeks [25]. In addition, when combined with aerobic physical training, inspiratory muscle training at high intensity significantly improved MIP, QoL, and dyspnoea [26].

This review aims to present the level of efficiency and feasibility of the most used exercise training methods in cardiovascular and pulmonary rehabilitation, identified after a thorough search of the current literature.

MATERIALS AND METHODS

A detailed search of the Pubmed database using specific keywords ('cardiorespiratory fitness OR cardiorespiratory rehabilitation OR cardiorespiratory recovery OR cardiovascular rehabilitation OR cardiovascular recovery AND endurance exercises AND respiratory exercises') was performed. The search algorithm was then completed by adding the 'cardiopulmonary rehabilitation', 'aerobic physical training', and 'resistance training' terms. We have selected open access articles published in the last ten years. Based on these criteria, fifteen items were selected.

RESULTS AND DISCUSSIONS

Fifteen studies were included in this review and were analyzed according to the goals as mentioned above [15,19,24–36].

Aerobic training was the most used and studied method for cardiovascular patients, alone or combined with another type of training (interval training, resistance training, inspiratory muscle training) [19,24,26,27,29,32–34].

When patients performed only aerobic exercises (aerobic interval training or continuous aerobic training) on a cycloergometer, a significant increase in peak VO_2 was observed predominantly in the group that performed aerobic interval training (AIT) compared to continuous aerobic training (ACT). Furthermore, in the first six weeks of training, there was noted an improvement in peripheral endothelial function in both aerobic exercises 19. Although Conraads et al. described the benefits of aerobic intervals and continuous training, they also concluded that ACT programs could be carried out higher than 70-75% of peak HR, but AIT programs with 4-minutes intervals at an intensity of 90-95% of peak HR are barely feasible for four minutes 19. This statement is most likely due to the complications that may occur during the interval training at 90-95% of peak HR, judging by the fact that cardiovascular patients are fragile, at least in the first phase of rehabilitation. Also, fatigue might be a limiting factor since most patients tend to stop the exercise when they feel exhausted.

HIIT is considered superior to MICT when it comes to improving cardiorespiratory fitness. Two studies confirmed this hypothesis, finding that HIIT's physiologic exercise training adaptations are superior to MICT for interventions of 7-12 weeks [31,36]. In contrast with the findings in the literature, one study of reduced ejection fraction heart failure patients concluded that HIIT was not superior to MICT in improving aerobic capacity or changing left ventricular remodeling. Also, the feasibility of HIIT remains unresolved for these subjects [30]. One of the factors which may have contributed to the reduction in HIIT response is the training intensity during intervals.

In the trial conducted by Ellingsen et al., 51% of the patients in the HIIT group trained at a less prescribed intensity, and 80% of the patients in the MICT group underwent more intense training than prescribed. It seems that HIIT at 90%-95% of the maximum heart rate is difficult to achieve for patients, but intensities of 60%-70% of the maximum heart rate in MICT seemed too low. This trial supports the statement made by Conraads et al. that interval training using 90%-95% of peak HR is barely feasible for four minutes [19,30].

When aerobic training (AT) was combined with other types of training, the results were enhanced. The addition of inspiratory muscle training (IMT) to aerobic training (AT) was shown to improve peak performance, submaximal ventilatory responses, peak circulatory power (CircP), dynamic of recovery gas exchange, O_2 uptake efficiency slope (OUES), skeletal muscle function, exercise capacity (in patients with COPD) and to decrease mMRC dyspnoea grade (in subjects with asthma) [27,28]. In contrast with these results, one study didn’t observe any additional benefit of peak VO2 and left ventricle function for either group (AT/IMT or AT/SHAM), but there was an improvement in SPImax (sustained maximal inspiratory pressure) and in PImax (inspiratory muscle strength) [26]. An explanation for these conflicting results might be the small number of patients who presented inspiratory muscle weakness (IMW) in the study conducted by Adamopoulos et al., compared to the study carried out by Winkelmann et al. Therefore, we can conclude that in patients with CHF and IMW, any type of training (AT, IMT or both), can lead to substantial improvement in inspiratory muscle strength, having beneficial consequences on functional capacity. It may be necessary for patients with CHF but without IMW to undergo high-intensity IMT training to enhance functional capacity [26,27].

Ambrosetti et al. conducted a study on 612 patients using continuous aerobic training (ACT), aerobic interval training (AIT), resistance training (RST), respiratory training (RT), and calisthenics (CT). The study's outcome provided valuable information regarding the most suitable type of training for each cardiovascular pathology. For example, 73.1% of patients diagnosed with coronary artery disease (CAD) displayed the highest rate of implementation of AIT. In comparison, CHF patients displayed the greatest implementation rates for IT and RST (14.0% and 13.2%), and valvular disease/valvular surgery patients (VDS) presented the highest implementation rate of RT (62.9%) [29]. These results are important because pathologies' individualization of cardiac recovery programs leads to physical treatment progression in diseased cardiovascular patients.

The addition of RST and progressive resistance training (PRT) to AT and the effect of RST, and PRT alone were investigated in seven studies out of fifteen [15,28,29,32–35].

RST and PRT alone were observed to improve skeletal muscle function, upper and lower body muscular strength, flexibility, coordination, blood pressure profile, peak VO_2, exercise capacity, and determine muscle hypertrophy [15,28,29,32–35]. Ozaki et al. investigated if RT alone can improve VO_{2max} in young and older subjects. VO_{2max} considerably improved after 3 to 5 sets of RT at 5-10 RM and after sets of 10 reps for the first five weeks. However, when using higher reps (15-20 RM), there was no significant increase in VO_{2max} following training [35].

The recommendations for training frequency are five days per week of moderate training or three days per week of intense AT or a CT of moderate and intense aerobic training on 3-5 days per week with 2-3 days per week of RT [35]. The pooled results suggest that VO_{2max} could increase in young subjects performing RT, but when their initial VO_{2max} is lower than the average values for the corresponding age. Future research on this subject should investigate the dose-response relationship between the initial VO2max and the VO_{2max} obtained after a program using traditional RT.

It is an undeniable fact that the addition of RST and PRT to AT had significantly increased results compared to RT, PRT, and AT used alone [15,28,29,32–34]. While the effects of each training were not modified by the combination with another type of training, the pooled results observed a significant increase in the groups that performed combined training (CT) using RT and AT, compared to AT alone [15,32–34].

A systematic review and meta-analysis investigated the effects of PRT alone and combined with AT and concluded that PRT could provide cardiorespiratory fitness (CRF) improvements equivalent in magnitude to AT, with a decrease in the risk of mortality, in situations where the use of AT may not be viable, suitable or accessible [34].

CONCLUSIONS

Unfortunately, HIIT at 90%-95% of HRmax for four minutes is not feasible for cardiovascular patients since there was observed difficulty in achieving the prescribed intensity in these subjects.

We believe future research should investigate the dose-response relationship between the initial VO2max and the VO2max obtained after a program using traditional RT, because VO2max may be discovered to increase in young individuals after performing RT only when their initial VO2max is lower than the average values for the corresponding age.

The pooled results suggest that CT significantly improves the quality of the physical training by using the AT to increase the CRF, RST for improving the functional ability and determine muscle hypertrophy (strength), RT, or IMT to improve ventilatory responses, gas exchanges, and decrease the severity of dyspnoea. Thus, our recommendation is to integrate all types of exercises in a comprehensive cardiopulmonary rehabilitation program to obtain important outcomes and evolutions.

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VARIA

The nuclear accident at Chernobyl: Immediate and further consequences

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Abstract: *The accident at Chernobyl occurred in April 1986 at the Chernobyl Nuclear Power Plant in Soviet Union. The incident occurred during a scheduled safety test. A combination of inherent reactor design flaws and operators' mistakes resulted in reactor's No.4 disaster and the emission of a large quantity of radiation. The immediate actions involved the fire extinguishing, the cleanup of radioactive residues and the prevention of a new explosion. For this purpose, plenty of people worked with self-sacrifice. The people who lived nearby were removed. As far as the socio-economic impact for the Soviet Union is concerned, it was quite serious. Moreover, the environmental and human health consequences were also alarming with thyroid cancer being the most studied. Useful conclusions, especially for the safety both of reactors and nuclear power, as well as for the impact of radiation at ecosystems have been drawn. The debate about the use of nuclear power has remained open ever since.*

Keywords: *nuclear power, thyroid cancer, RBMK reactor, radiation, radioactivity, liquidators*

INTRODUCTION

The Chernobyl nuclear accident occurred on 26 April 1986 in the light water graphite moderated reactor No 4 at the Chernobyl Nuclear Power Plant, close the town of Pripyat, in Ukrainian Soviet Socialist Republic Soviet Union, roughly 100km of the city of Kiev [1].

The reactor exploded during a safety test that was inappropriately made, with the operators losing control. This was due to a design defect of the RBMK reactor, which made it unstable when performed at low power. The outcome was the destruction of the reactor and the leakage of a huge amount of radiation into the environment [2, 3].

During the accident two deaths were caused because of explosion. Over the coming weeks a number of emergency staff was hospitalized with acute radiation sickness (ARS), while 28 firemen and employees finally died. The Chernobyl accident is considered the most damaging nuclear power plant accident in history. The Chernobyl and the Fukushima accident are the two nuclear accidents classified as a level 7 (the maximum classification) on the International Nuclear Event Scale [4].

CHRONOLOGY

The city of Chernobyl is located in Ukraine at a distance of 104km northeast of Kiev and 16km from the border with Belarus. Before the evacuation, it had about 14,000 inhabitants. Closer to the plant was the larger city Pripyat (about 49,000), which was built to accommodate the employees of the nuclear plant and their families [1]. The Chernobyl Nuclear Power Plant began to operate in 1977 with the first reactor of a total of four. Gradually, three more

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reactors began to operate by 1983. They were boiling water reactors with enriched uranium, type RBMK. The plant had a total capacity of 4000 MW electric power. The combination of graphite as a moderator and water as coolant is not found in other types of reactors [5].

THE ACCIDENT

When the reactor operates, it has an integrated cooling system. When it is shut down, the radioactive decay continues and heating of the reactor continues too. As a result, further cooling is required even after the reactor has been switched off. However, the three diesel engines which were used for this purpose needed 60-75 sec interval to achieve the required performance. In case of emergency, the reactor would be left without adequate cooling for this period, which was a very serious risk [6]. To remedy this problem, it was suggested the use of the kinetic energy of the steam turbine which was produced during the deactivation of the reactor.

After three failed tests (1982, 1984 and 1985) and some changes in design, a new test was designed to become in April 1986, when the Reactor No 4 was deactivated for scheduled maintenance. The test ended up in the tragic accident that occurred in 01:26 on Saturday, April 26, 1986. After a sequence of events during the test (which are not relevant to the present paper) it finally resulted in an uncontrolled increase of power. Simultaneously, the steam pressure in the reactor increased which eventually led to the first burst. The reactor's building was destroyed and a significant amount of radioactive materials were released into the atmosphere. A few seconds later, a second stronger explosion followed. Several fires broke out while the reactor itself started to flare [7].

Causes of the accident

The accident at Chernobyl was the result of a combination of unauthorized manipulations, human errors and design imperfections of the type RBMK reactor. According to the reappraisal of the accident by International Nuclear Safety Advisory Group at 1997 [8] the factors that contributed were:

- Unlike to most nuclear reactors which have a negative void coefficient, the RBMK had a dangerously high positive void coefficient.
- The use of graphite at the end of the control bars resulted in lowering the reactor power, had as a result the reactor power to be increased for a few seconds. This behavior is counter-intuitive and was not known to the reactor operators.
- The design of RBMK reactor had also other defects and was not consistent with the minimum acceptable safety rules for nuclear reactors.
- There was deficient safety culture in the design, organization and operation of nuclear power by the Soviet Union.
- There was inefficient and inadequate transmission of important information, both between operators and between operators and designers.
- There was inadequate safety analysis.
- The test was scheduled to take place before the night shift from well informed and familiar with the process employees. However, due to an unexpected ten-hour delay, the test was made during the night from less experienced staff.

The first hours

During the accident two workers were killed because of the explosion. There have been many fire focuses, the main of them were on the roof of the station and the reactor No 4. Initially, firemen were unaware of the risk of smoke and debris and did not receive any additional protection measures. So they received high doses of radioactivity. Although the fire on the roof had extinguished until 05:00 o'clock, the fire inside the reactor was still active. Within the next few days about 5,000 tons of chemical materials and sand were thrown by military helicopters to absorb the radiation.

The levels of radioactivity reached 20.000 Roentgens (R) per hour (the leather dose is 500R within 5 hours). In the first hours, both workers and supervisors in the control room were unaware of the actual levels of radioactivity. The one dosimeter did not work and the second one was not accessible due to the explosion. The remaining dosimeters had a measurement limit of 0,001R/s, so they showed "off scale" [9]. Due to faulty readings, the head of the station, Alexander Akimov, believed that the reactor was not affected by the explosions. Akimov and his assistants remained in the reactor building until the morning without taking any protection measures. Most of them, including the Akimov, died from exposure to radiation within the next three weeks.

IMMEDIATE CONSEQUENCES – CRISIS MANAGEMENT

After the extinguishing of fireplaces, a number of problems and potential risks had to be addressed:

I. Steam explosion risk

The floors under the reactor were filled with water. At the same time, smoldering graphite, fuel and other materials fed to the reactor began to mix with molten concrete from the reactor walls resulting in a semi-liquid material that resembled a lava [10]. If this mixture succeeded in penetrating the reactor floor and coming into contact with water, it could cause a more disastrous explosion. To prevent this danger it was considered necessary the water pools to be drained. This hazardous mission was carried out by engineers Alexei Ananenko and Valeri Bezpalov and the shift supervisor Baris Baranov. After the valves had been opened a large amount of extremely radioactive water was pumped.

II. Radioactive debris removal

The next challenge was the removal of the radioactive particles which were dispersed around. The main part of this effort shouldered the army. The plan included the transport and disposal of radioactive materials within the reactor core. Wearing lead protective clothing, the “liquidators”, as the workers were called, either transplanted radioactive materials manually, or handled heavy machines which did the same job. Many of them received cumulatively high doses of radiation throughout the procedure [11]. Furthermore, a large number of remote-controlled machines were used in order to be avoided the exposure of workers to high doses of radioactivity.

III. Announcement of the accident

At 21:02 on 28 April, about 67 hours after the accident, a 20-second announcement was read on a TV news program: “There has been an accident at the Chernobyl Nuclear Power Plant. One of the nuclear reactors was damaged. The effects of the accident are being remedied. Assistance has been provided for any affected people. An investigative commission has been set up”. However, attempting not to hurt the prestige of the regime, the Soviet authorities initially had tried to hide the incident. The above announcement occurred only when the morning of the 28 April, in routine control, personnel of the Swedish Nuclear Power Plant at Fosmark recorded high levels of radioactivity. The Swedish government came into contact with the Soviet Union and the latter was forced to admit that an accident had occurred [12]. Although, the Soviet government, attempting to downplay the event, issued a statement that there was no reason to postpone the celebrations and parade for the 1 May International Worker’s Day in Kiev.

IV. Evacuation of Pripyat

After the incident, the residents of Pripyat, completely ignored what had happened. A special committee was created a few hours later to investigate the accident. The committee arrived at the plant on the afternoon of April 27 and soon had enough evidence of the seriousness of the situation seriousness. So, in the early hours of April 27, 36 hours after the accident, they decided to evacuate Pripyat. It was initially decided that the evacuation would only be for 3 days but shortly was decided the permanent implementation of this measure.

With a statement issued by the city council the residents were informed that they would displace the city for 3 days by buses started at 14:00, on 27 April. The evacuation would be under the supervision of the police and the city officials. The residents had also been informed to take with them only their documents and entirely essential personal belongings. It was also announced that all assets would be kept by the police after the evacuation. Except for the police officers who would remain in the city for safety, there was a list of employees who were required to remain for the continuity of the plant operation. Around 15:00 a total of about 45.000 residents were transferred to different villages in the Kiev region [12].

V. Set up of exclusion zone

On May 2, a government committee decided –rather arbitrarily- a 30km area from the damaged reactor as the designated evacuation zone. This area was also divided into three sub-zones in which, both the protection measures of those who arrived, and the activities which took place, were different: The area around the reactor, a second with a radius of about 10km, and the rest zone until the 30km.

Later, this distinction was made according to the revised dose limit of 100mSv [13]. So there were:

- the “Black Zone” (over 200 μsv/h)
- the “Red Zone” (50-200 μsv/h)
- the “Blue Zone” (30-50 μsv/h)

In the first place the inhabitants would never return. In the second they may be back when the radiation levels would be reduced. In the third only children and pregnant women would have to be removed. Although the evacuation of these areas was not immediate, a total of 116,000 people were eventually removed. However, there were few residents, mainly elderly, who refused to remove. After repeated efforts at expulsion, authorities finally accepted their presence giving them an unofficial permission to stay.

The boundaries and the status of the zone and sub-zones have been amended many times in the coming years.

FURTHER CONSEQUENCES

I. Financial consequences

It is very difficult to determine the total cost of the Chernobyl accident. It afflicted, and continues to, both the Soviet Union (and the countries which have arisen since its breakup), and many other countries. According to the Chernobyl Forum Report (2005) the 22% of the national budget of Belarus in 1991 was connected to Chernobyl (decreased at 6% in 2002), while in Ukraine 5-7% of the government spending continues to be related to the accident [14]. A great part of the cost is still about Chernobyl-related social benefits in approximately seven million people in Russian, Ukrainian and Belarus. Another important economic factor for the years after the accident was the removal of 1,900,000 acres of agriculture land, as well as 1,700,000 acres of forest from production.

Finally, a large number of countries, mainly Europeans, have been forced to spend resources to check the amount of radioactivity in soils, food and water, while International Organizations, such as the World Bank, have financed both the rehabilitation work and the building of the new reactor shelter (New Safe Confinement).

II. Political and social consequences.

Before the accident M. Gorbachev had implemented the political "glasnost" with purpose to enhance the transparency of politics. However, the accident led to return to past secretive politics. At the same time, distrust of international community against the Soviet regime was grown up. The mistrust of the citizens towards the regime was also high enough, especially among nearby areas residents and those who needed to move.

Combined with the economic consequences, the lack of confidence, both in international and national level, is believed to have contributed to the fall of the Soviet Union, a few years later. Apparently, except for economic impact, political and social consequences continued to affect the 3 countries in this region, even the fall of the Union [14]. On the other hand, the need for bio-scientific cooperation helped forge closer relationship between Soviet Union and US at the end of the Cold War.

III. Environmental effects:

a) Spread of radioactive substances

It is estimated that Chernobyl released about four times the amount of radioactivity of the two atomic bombs in Hiroshima and Nagasaki. More than 100,000 km^2 were significantly contaminated even though the areas around the plant were the most overburdened. Lower levels of radiation were detected in different regions in Europe. The radioactivity dispersion was incongruous since it depended on both weather conditions and water paths. Soviet and Western scientists claim that 60% of radioactivity contaminated Belarus. Also, according to the 2006 TORCH report, half of the volatile particles came out of the Soviet Union [15].

b) Residual radioactivity

The Chernobyl Nuclear Power Plant is located next to the river Pripyat, which at that time was watering the broader area of Kiev. Although water purification measures were initially taken, the supply of water to Kiev began to become from the river Desna, two months later. Also, in some lakes in Russia and Belarus the concentrations of radioactivity in certain species of fish far exceeded the acceptable limits, even the period 1990-1992 [16]. On the contrary, excluding the exclusion zone, the groundwater does not seem to be affected.

c) Flora and fauna

After the accident, 4 km^2 of the neighboring pine trees were died, giving a characteristic red color that gave the name of the "Red Forest". Several of the wild animals in the most affected areas, either died or stopped breeding (most of the domestic animal in exclusion zone were removed) [17]. A typical example was the animals of a small island in the river Pripyat, where, the horses died and the beasts either died or were extremely weak. All of them due to significant thyroid malfunction.

In the following years, increased levels of radioactivity were detected in domestic animals both inside and outside of the Soviet Union. Several European countries have carried out checks on animal products in order to ensure consumer safety, for many years after the accident. As reported, were contaminated in total over 19.000 km^2 of agriculture land. Of these 2.640 km^2 will never be cultivated. Also about 17.000 km^2 of forest, mainly in the Ukrainian lands, were infected [14].

IV. Human impact

a) General

After the accident, totally 273 people suffered from acute radiation sickness, 31 of whom were died within the next three months. According to the Chernobyl Forum Report (2005), 28 of the "liquidators" died of Acute Radiation Syndrome, while 15 died of thyroid cancer within the coming years [14].

The same report states that it is extremely difficult to be calculated the total number of cancer due to the Chernobyl accident. The four most harmful radionuclides spread from Chernobyl were iodine-131 (half-lives: 8.02 days), caesium-134 (2.07 years), strontium-90 (28.8 years) and caesium-137 (30.2 years). Iodine, despite the small half-life, is the most unstable and appears to have caused the most important health problems up to now [18]. It tends to become concentrated in thyroid and milk glands and cause thyroid cancer. Caesium tends to accumulate in vital organs such as the heart. Finally, strontium accumulates in bones increasing the risk of cancer.

According to WHO and independent researchers who studied the children of emergency workers, there was no statistically significant difference in mutation frequencies among children conceived before and after their father's exposure [19]. However, due to fear, distrust to the authorities and poor media information, an increase in the number of abortions in -other normal- pregnancies has been observed in some European countries. In Greece, although radioactivity measurements did not exceed the safety limits, there was an observed 2,500 excess of otherwise wanted pregnancies being terminated [20]. Furthermore, in countries such as Sweden and Finland, where there were not noticed more abortions, there was not increase in the incidence of congenital malformations [21]. The same conclusion is reached by Frank Castranovo study, of the Harvard Medical School in 1999, which has, among other things, analyzed the data from the two largest maternity clinics in Kiev [22]. He says that there is no evidence that the accident has increased the rate of birth defects.

b) Cancer assessments

The estimates of the number of cancers that have occurred or will arise in the future as a result of the accident vary enormously. This is due to both the lack of solid scientific data and the different methodologies used to quantify mortality. The Chernobyl Forum predicts that the total number of deaths could reach 4,000. However, these deaths are expected to be mainly among the most exposed people (emergency workers, evacuees, residents of the most contaminated areas) [14]. The Risk Projection suggests that Chernobyl can be responsible for about 1,000 cases of thyroid cancer and about 4,000 cases of other cancer, in Europe (0.01% of all cancer after the accident) but, as stated, the estimates are subjective. The most tightly associated with the accident cancer is thyroid cancer, due to the well-documented influence of the iodine on this gland.

According to the Forum, thyroid cancer is one of the main health impacts from the Chernobyl. On the contrary there has not been evidence for an increase of the number of other kinds of cancer or leukemia. The United Nations Scientific Committee on the Effects of Atomic Radiation ends up to similar conclusion. It finds an increase in the incidence of thyroid cancer, particularly among children and adolescents exposed to radiation, but no other indications of major health impacts [23].

Finally, the International Atomic Energy Agency indicates that there has been no increase in the incidence of solid cancer as a consequence of Chernobyl accident [14]. On the contrary, there are estimates such as the International Physicians for the Prevention of Nuclear War that refers to about 10.000 cases of thyroid cancer and 50.000 cases which are expected in the future [24].

c) Health disorders

One other important impact of the accident, especially among the directly involved populations, has to do with mental health. Both the incident itself and the initial attempt to be downgraded it had as a result to create exaggerated fears about the risks of radiation exposure. The IAEA supports that the designation of the affected people as "victims" rather than "survivors" has fed them to perceive themselves as weak, helpless and lacking control over their future [14]. Further health effects may have been caused due to this belief.

Other surveys show that humans remain largely unsure of what the effects of radiation actually are. For example, some teenagers or young adults who had received modest or small amount of radiation feel condemned to be ill and thus believe that there is no downside to have unprotected sex or use drugs.

The research and assessment of long-term effects of radiation on human health are continuous by many organizations, such as the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) [25].

DISCUSSION

The review of the causes and consequences of the Chernobyl accident had as a result many important conclusions to have drawn and, at the same time, there was a matter of intense concern.

The weaknesses and pathogens of the Soviet regime were clearly showed up. The authorities initially tried to conceal and then to devalue the gravity of the incident. For this reason, the decision to evacuate the much burden areas was delayed. This has resulted in the distrust and dispute of the citizens towards the authorities, which had as a result the citizens not to comply with the instructions of the following period.

Questions have also been raised about the safety, both the RBMK reactor and the nuclear reactors in general. The accident led to be made corrections to the design and operation of Soviet reactors but also of the reactor in West [26]. Simultaneously, a culture of safety was dealt with in the handling of nuclear energy. The combination of reactor faulty manufacturers and human errors that led to the Chernobyl accident resulted in the persistence in safety of both governments and scientists as well as the leaders and staff of the nuclear power plants. A repetition of the Chernobyl accident is now virtually impossible, according to report of the German Nuclear Safety Agency [27].

Furthermore, cooperation between East and West has improved in terms of reactor improvement and safety. Since 1989 there have been many reciprocal visits from the former Soviet Union and the West. In 1989 the World Association of Nuclear Operators (WANO) was founded, which has under the auspices 130 operators of nuclear power plants in more than 30 countries [26].

The Chernobyl nuclear accident also helped to understand the impact of radiation on the environment, ecosystem and humans. Scientists had the opportunity to study short and long term radioactive contamination as well as to relate specific exposure levels with corresponding results. Also, knowledge and experience on the appropriate measures to protect against radioactivity have increased.

Despite the intense fear and concern it seems that the only sufficiently documented impact that accident had at the human health (excluding emergency workers who have received high doses of radiation during the first days from the accident) was thyroid cancer [28].

Finally, the debate on the use of nuclear energy remains open. There is a continuing dispute between those who argue that the use of nuclear power is dangerous, and those who claim it is necessary and sufficiently secure. The growing public awareness about climate change and the critical role that carbon dioxide and methane emissions play have increased the intensity of the debate. The use of nuclear energy is environmentally friendly and reduces fossil fuel consumption. However, if anything goes wrong the consequences can be devastating to humans and to the environment. The debate remains open today between those who fear the power of nuclear and those who fear what will happen to the planet if humanity does not use nuclear power.

Disclosure statement

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ORIGINAL ARTICLES

The outcome of Covid-19 in patients receiving biologics for the treatment of psoriasis

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Abstract: *Psoriasis is a chronic inflammatory disease, with multisystemic implications and genetic predisposition, characterized by the development of erythematous, scaling plaques on the skin. Management of moderate to severe psoriasis vulgaris may involve a biological treatment that suppresses the immune system. Because of this, the patients with psoriasis on an immunosuppressive treatment are theoretically at an increased risk of infections, including SARS-CoV-2 infection. SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is responsible for the current pandemic. In Romania, COVID-19 (Coronavirus Disease 19) was first reported on February 26, 2020, by confirming the first patient with SARS-CoV-2 by RT-PCR method. COVID-19 progression was divided into three phases: early infection, pulmonary phase, and hyperinflammatory phase. Although the inhibition of pro-inflammatory cytokines by biologic therapy is detrimental in the viral phase, it seems to be beneficial in the hyperinflammatory phase, by protecting the patients with psoriasis in progressing towards extra-pulmonary manifestations and death. Analyzing the evolution of the 8 cases of patients from our dermatology department – Colentina Clinical Hospital, with generalized psoriasis vulgaris on biologic immunosuppressive treatment and SARS-CoV-2 infection, we observed that they did not develop a more severe form of COVID-19 than the general population. Patients on biologic immunosuppressive treatment are more susceptible to the development of infections, including by SARS-CoV-2, but it was found that the biologic therapy can protect against a more severe type of COVID-19.*

Keywords: *psoriasis, biologics, SARS-CoV-2, COVID-19, hospitalization*

INTRODUCTION

Psoriasis is a chronic inflammatory disease, immune-mediated, with multisystemic implications and genetic predisposition, characterized by the development of erythematous, scaling plaques on the skin [1]. The psoriatic disease spectrum includes the psoriasis vulgaris, as well as psoriatic arthropathy and the concept of this unique disease was proved its validity over time [2]. The comorbidities associated with psoriasis are numerous and of multidisciplinary interest, wherefrom the most important being the cardiovascular effects, the psycho-emotional impact, and metabolic syndrome [1]. The presence of various comorbidities represents an important risk factor for an unfavorable evolution [1].

The patients with generalized psoriasis vulgaris are included into an increased risk of developing the infection with Coronavirus because of the pathogenic mechanism of the disease which incurs a dysfunction of the immune system with a multisystem effect, as well as the frequency of

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treatments administered to control the disease [3, 4].

The current therapy with biologic agents induces long-term remissions in patients with moderate or severe psoriasis vulgaris, who are not responding to the conventional systemic therapy [5]. The biologic therapies available in Romania for the treatment of moderate to severe psoriasis vulgaris are tumor necrosis factor (TNF) alpha inhibitors (Etanercept, Infliximab, Adalimumab, Certolizumab) and their biosimilars, IL-12/23 inhibitor (Ustekinumab), IL-17 inhibitors (Ixekizumab, Secukinumab) and IL-23 inhibitors (Risankizumab, Guselkumab) [6, 7].

The anti-TNF-α agents are antibodies obtained by genetic engineering that target the TNF-α, a cytokine with a central role in the pathogenesis of the psoriasis vulgaris, having a pro-inflammatory pleiotropic action, with multiple effects on a large number of cells [5, 8].

The anti-IL-12/23 agents links specifically on the p40 protein subunit of the human cytokines IL-12 and IL-23, preventing the p40 to connect to the protein receptor IL12Rβ1 expressed on the surface of the immune cells, thus inhibiting the bioactivity of IL-12 and IL-23, regulatory cytokines involved in the pathogenesis of psoriasis [5, 8, 9].

IL-17 inhibitors selectively aim and neutralize the pro-inflammatory cytokine IL17A, either within the homodimers 17AA or heterodimer 17AF, thus inhibiting the release of pro-inflammatory cytokines, chemokine, and mediators responsible for the tissue deterioration in psoriasis [5, 8].

The last release on the “biologic market” active in psoriasis are the IL-23 inhibitors, which are selectively blocking the interleukin-23 p19, (but not the IL-12), inhibiting the cellular signaling dependent of IL-23 and the release of pro-inflammatory cytokines, acting like a true “master-switch” in the etiopathogenic cascade of psoriasis [5, 8].

In the context of the SARS-CoV-2 pandemic, studies and clinical experience revealed that the hospitalization rate in patients with psoriasis vulgaris and COVID-19 was lower among those receiving biological therapy, concerning those receiving non-biologic systemic therapy or no systemic therapy, which was somewhat unexpected considering the immunosuppression potential of the biologic agents [10, 11, 12, 13].

Also, no significant difference was observed concerning the hospital admission risk between different biologic therapies [10].

On one hand, patients on an immunosuppressive treatment are more susceptible to developing infections, including by SARS-CoV-2, yet on the other hand, the same biologic therapy may protect against a more severe development in COVID-19 [4, 14].

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a positive-stranded RNA virus, similar to other coronaviruses, from the Coronaviridae family [15,16]. In Romania, COVID-19 (Coronavirus Disease 19) was first reported on February 26, 2020, by confirming the first patient with SARS-CoV-2 by RT-PCR method. Although most patients with COVID-19 are displaying more mild symptoms, approximately 15% are developing pneumonia and 5% are progressing towards systemic hyper-inflammation and acute respiratory distress syndrome, with an increased risk of septic shock, multiple organ failure, and death [10].

On patients with SARS-CoV-2, were identified three stages in the development of the disease [4]:

Stage I: recent infection or the stage of the viral response, wherein the viral ARN stimulates the cells of the host to express genes involved in the way of interferon, a critical part of the immune response. A reduced or delayed IFN type 1 response was associated with mild COVID-19 effects [4, 17].

Stage II: the pulmonary phase, clinically characterized by viral pneumonia with or without hypoxia [4].

Stage III: hyper-inflammatory phase, the so-called “cytokine storm” which incurs an exaggerated immune response to triggers, such as viral infections, observed in patients with severe SARS-CoV-2 infection. Clinically it is characterized by difficulty in managing the fever and multisystem involvement, including acute respiratory distress syndrome, heart failure, and renal failure. From the biological standpoint, it is found an increase in the pro-inflammatory cytokines also involved in psoriasis vulgaris, including TNF-α, IL1β, IL6, IL8, IFN-γ, and IL17 [4, 18, 19, 20].

Although the inhibition of pro-inflammatory cytokines is unfavorable in the viral phase, it seems to be beneficial in the hyperinflammatory phase, by protecting the patients with psoriasis in progressing towards extra-pulmonary manifestations and death [4, 19].

The angiotensin-converting enzyme 2 (ACE2) is considered the main receptor of SARS-CoV-2. Linking the viral spike protein to ACE2 facilitates the penetration of the virus into the cell, viral replication, and intercellular transmission [14]. The connection of the virus to the ACE2 determines a decrease of the angiotensin 1, by the increase of the angiotensin 2, responsible for the occurrence of the immune-mediated pulmonary inflammation and the injury of the pulmonary parenchyma [14]. Interestingly, the mediated inflammation IL-17 in patients with psoriasis was associated with the increase of the expression of the ACE2 receptor on the injured skin. The treatment with IL-17 inhibitors decreased the expression of the ACE2 to the levels observed in the non-injured skin and this can be a possible explanation for the favorable response of the psoriatic patients regarding COVID-19 [14, 19].

CLINICAL CASES

Moving forward we will present the development of 8 cases of patients from our dermatology department – Colentina Clinical Hospital, with generalized psoriasis, vulgaris, on biologic immunosuppressive treatment who contracted the SARS-CoV-2 infection.

Case 1: We are presenting the case of a 53-year-old female patient, known with arterial hypertension, type II diabetes, and generalized psoriasis vulgaris for 17 years, on biologic immunosuppressive treatment with etanercept since August 2018, who developed COVID-19 in August 2020 displaying as a clinical manifestation of the SARS-CoV-2 infection – hyposmia and hypogeusia for several days. The patient continued the biologic immunosuppressive treatment, and for COVID-19 she followed a treatment with acetaminophen, acetylsalicylic acid, vitamin D, vitamin C, and zinc, the evolution of the disease being favorable although the lesions incurred by psoriasis extended, with psoriasis area and severity index (PASI) 15.8.

Case 2: A 51-year-old male patient, without a significant pathological history, known with generalized psoriasis vulgaris since 2000, on biologic immunosuppressive treatment with Ixekizumab since October 2019, displayed an asymptomatic infection with SARS-CoV-2, but the administration of the treatment was postponed by two weeks (patient’s decision). The applied treatment consisted of vitamin D, vitamin C, zinc. The evolution of the disease was favorable, lasting approximately one week, and psoriasis did not worsen.

Case 3: A 41-year-old female patient, former smoker abstinent for approximately 14 years, known with arterial hypertension, bronchial asthma, treated pulmonary TB, generalized psoriasis vulgaris since the age of 7, experienced multiple biological treatments, currently on treatment with Etanercept. In February 2020 the patient was admitted to the hospital for pneumonia of unknown etiology, but no RT-PCR was performed for SARS-CoV-2. On August 12, 2020, the patient was referred to our Clinic for the occurrence of an eruption with erythematous-squamous plaques and placards disseminated at the level of the whole body, most lesions presenting pustules. With topic and antibiotic treatment, the lesions had a favorable evolution. During the admission increased IgG anti-SARS-CoV-2 antibodies were detected with a negative RT-PCR.

Case 4: A 72-year-old female patient, smoker, known since 2000 with generalized psoriasis vulgaris, on immunosuppressive biologic treatment with Etanercept since September 2017, following a SARS-CoV-2 infection, developed a severe broncho-pneumonia with acute respiratory failure which required admission into the hospital where she followed a treatment with anticoagulant, broad-spectrum antibiotic, systemic corticosteroids, antifungals, bronchodilators, proton pump inhibitors, non-steroidal anti-inflammatory drugs and oxygen therapy. The patient associates numerous comorbidities, namely essential arterial hypertension, ischemic stroke, chronic obstructive pulmonary disease, obesity. The evolution was favorable with the mitigation of the suffering, biologic and radiologic relief, but with residual pericarditis. We are mentioning that the patient interrupted the biological treatment during the illness, but psoriasis did not worsen [21].

Case 5: A 25-year-old male patient, obese, known with generalized psoriasis vulgaris since November 2016, on immunosuppressive treatment with Ixekizumab since January 2018, presented fever, cough, ageusia, and anosmia following the infection with the new coronavirus, wherefore the administration of the treatment was postponed for a week. The duration of the symptoms was two weeks and the followed treatment was symptomatic with anti-pyretic, cough suppressing medicine, vitamin C, vitamin D, and zinc. The PASI score before the SARS-CoV-2 was 0, and 2 months upon healing was 0.8.

Case 6: A 63-year-old female patient, without a significant pathological history, known with generalized psoriasis vulgaris since 2010, on immunosuppressive treatment with Ixekizumab since March 2018, presented cough, headache, ageusia, and anosmia following a SARS-CoV-2 infection. In this case, the biological treatment was also interrupted during the illness, and the evolution of psoriasis was favorable. The patient was treated with non-steroidal anti-inflammatory drugs, cough suppressant medicine, and vitamins, and the duration of the symptoms was approximately two weeks.

Case 7: We are presenting the case of a 67-year-old male patient, with no significant family or personal history of diseases, known with generalized psoriasis vulgaris since 2018, on biologic immunosuppressive treatment with Ixekizumab since March 2020. He presented an asymptomatic infection with SARS-CoV-2 wherefore he followed only a multivitamins-based treatment. The biologic therapy was not interrupted and the evolution of the psoriasis was favorable with PASI and DLQI 0.

Case 8: We are presenting the case of a 38-year-old female patient, no significant family or personal history of diseases, known with generalized psoriasis vulgaris since 2018, on biologic immunosuppressive treatment with Secukinumab since September 2019 with favorable evolution, the PASI and DLQI being 0, who developed a SARS-CoV-2 infection in December 2020. In terms of symptoms, she had a fever, chills, dry cough, myalgia, articular pain, fatigue, ageusia, and anosmia, with evolution of approximately two weeks. The biologic therapy was not interrupted and for the infection with SARS-CoV-2, the patient followed a treatment with azithromycin for 3 days, paracetamol, and non-steroidal anti-inflammatory drugs when needed and vitamins. The evolution was favorable, with full remission, no after effects, and the underlying disease did not worsen.

DISCUSSIONS

Although patients with generalized psoriasis vulgaris on biologic immunosuppressive treatment fall within the high-risk category of the coronavirus infection, no hospital admission or higher death rates were observed.

Patients on biologic immunosuppressive treatment are more susceptible to the development of infections, including by SARS-CoV-2, but it was found that the biologic therapy can protect against a more severe type of COVID-19, with extra-pulmonary manifestations and increased death risk. The explanation may consist in the fact that the immunomodulation was also the target for other biologic like remdesivir or tocilizumab.

The presence of some risk factors and comorbidities of generalized psoriasis vulgaris could additionally increase the risk of immunosuppression and unfavorable development of COVID-19.

The most frequent symptoms, associated with the SARS-CoV-2 infection were ageusia, anosmia, dry cough, and fever (as well reported on free from psoriasis and biologic treatment population).

Among the reported patients, seven recovered completely upon COVID-19, only one patient with multiple associated comorbidities who required hospital admission, developed residual pericarditis.

Out of the 8 presented patients, 4 patients interrupted the administration of the treatment during the illness, whilst 4 continued the treatment.

The evolution of the patients who continued the administration of the biologic therapy was no more severe than that of the patients who interrupted the treatment.

According to the PsoProtect registry, upon the editing time of this article, there were 952 reported cases of COVID-19 patients with psoriasis vulgaris and 11 deaths [22, 23].

The average duration of the symptomatology was 12 days [23].

Hospital admission due to the SARS-CoV-2 infection:
- 84% did not require hospital admission;
- 16% required hospital admission;
- <1% unknown.

In terms of biological therapy, 46% interrupted the treatment during the infection, 51% continued with the treatment and in 3% of the cases, this detail is unknown [23].

CONCLUSION

Upon analyzing the 8 cases, we observed that our patients with generalized psoriasis vulgaris on biologic immunosuppressive treatment and SARS-CoV-2 infection did not develop a more severe form of COVID-19 than the general population. One patient out of eight required hospital admission, presenting a more severe form of the SARS-CoV-2 infection and multiple associated comorbidities. A possible explanation for the favorable evolution in patients with psoriasis and immunosuppressive treatment, consists in the inhibition of the pro-inflammatory cytokines by the biologic immunosuppressive treatment, protecting such patients to develop extra pulmonary manifestations and death. The increase in the pro-inflammatory cytokines is recorded in the pathogenesis of psoriasis vulgaris, as well as in the hyperinflammatory phase of the SARS-CoV-2 infection, so their inhibition is beneficial in both cases.

Moreover, another explanation would be the decrease in the expression of the ACE2, the main receptor of the SARS-CoV-2, by the immunosuppressive treatment with IL-17 inhibitors.

Author Contributions

RO, TC contributed to all the stages of the article, they designed the article and revised the manuscript for important intellectual content. AMC, MMC, and SB contributed to the conception of the work, revision of the language, and contributed to drafting the work. RO, AMC, TC, DOC, and SB revised the work critically. The final manuscript was approved by all authors.

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CLINICAL PRACTICE

Therapeutic and diagnostic considerations in multiple eruptive clear cell acanthoma

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Abstract: *Clear cell acanthoma represent a benign solitary epidermal tumor of the clear glycogen containing epithelial cells, which is quite common in dermatologic practice. The etiology of this tumor formation is not known exactly at the moment. Clinically, the lesion appears as a single reddish papule or papule-nodule and the characteristic of this lesion is represented by a peripheral scaling collarette. Although solitary lesions represent the most common form of presentation of the acanthoma with clear cells, less than thirtieth cases of multiple clear cell acanthoma, from two up to four hundred lesions, have been described in the medical literature to date. The diagnosis based on clinical features frequently needs to be supported using a dermoscopy exam and, in most cases, a histopathology exam is required. The treatment consists is complete removal (standard surgical excision, cryotherapy, electrofulguration alone, carbon dioxide laser or shave removal or curettage followed by electrofulguration). For the cases with multiple lesions, cryotherapy, or topical 5-fluorouracil are preferred.*

We present the case of a 62-year-old male patient, presented to our Dermatology Department for multiple asymptomatic rounded papules and multiple exophytic nodular tumor formations (approximate fifteen), on the lower extremities, which had occurred approximately two years previously and gradually increased in number.

Keywords: *multiple clear cell acanthoma, glycogen containing epithelial cells, surgical excision, cryotherapy*

INTRODUCTION

Clear cell acanthoma represents a benign solitary epidermal tumor of the clear glycogen containing epithelial cells, which is quite common in dermatologic practice. The clear cell acanthoma was first described in 1962 by Dr. Degos et al. [1].

The etiology of this tumor formation is not known exactly at the moment. Clinically, the most frequent part of the body at which level the tumor can appear is represented by the lower limbs, although other portions of the body at which this lesion can develop are represented by the inguinal region or scrotum, scalp, face, vermilion mucosa, far palm, nipple, buttock, trunk, forearm, head and toe [2, 3]. The period with the highest incidence of this condition is represented by the middle-age, with a peak incidence

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between 50 and 60 years, and does not appear to occur in children. The studies did not show any sex predominance and there is no racial predilection.

Clinically, the lesion appears as a single reddish papule or papule-nodule and the characteristic of this lesion is represented by a peripheral scaling collarette, but this sign is not always present [4]. Some of the nodules are covered with a thin crust rather than a keratinous layer and quite often they are exuding slight moisture [1,3]. Other features that can be representative of clear cell acanthoma include vascular blush and stuck-on appearance [5]. The lesions usually reach a diameter between 1mm and 4 mm until a few centimeters, but a giant tumor has been described by Duperrat et al. [1].

The lesions with this pattern, located at the level of an extremity, the unequivocal differentiation from Bowen's disease can be really difficult if is based only on clinical and dermoscopic features alone and is required a biopsy for the histological exam. The reflectance confocal microscopy has been used for the examination of the non-melanocytic neoplasms. The exam is usually showing microscopic findings similar to the optical histology.

CASE REPORT

We present the case of a 62-year-old male patient, from the urban area, non-smoking, presented to our Dermatology Department for multiple asymptomatic rounded papules and multiple exophytic nodular tumor formations (approximate fifteen), on the lower extremities, which had occurred approximately two years previously and gradually increased in number. On dermatological examination, the exophytic nodular tumor formations were well delimited on palpation with the diameter between 3mm and 15mm, some of them with exudative surface and reddish-brown nipples (Figure 2), while the other of the tumor formations had a dry surface, with a squamous and hyperkeratotic consistency (Figure 1 and Figure 3).

Figure 1: Nodular tumor formations

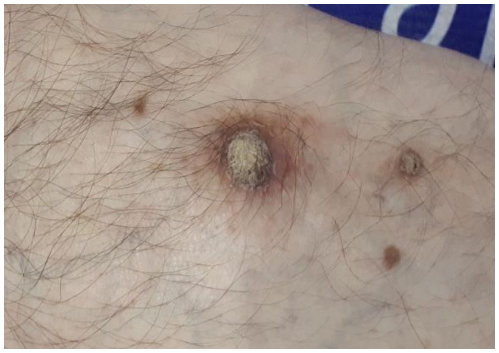

From his medical history, we found out that our patient is suffering from an HTA II degree and chronic venous insufficiency CEAP III; no history of drug ingestion was suspected. No other abnormal physical signs were found. Routine blood examination was in normal limits.

Figure 2: Nodular tumor formations

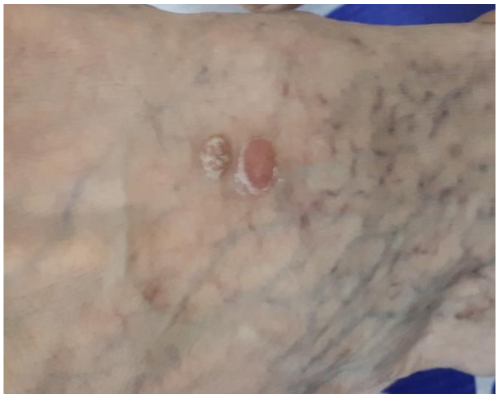

Figure 3: Nodular tumor formations with a dry surface and squamous and hyperkeratotic consistency.

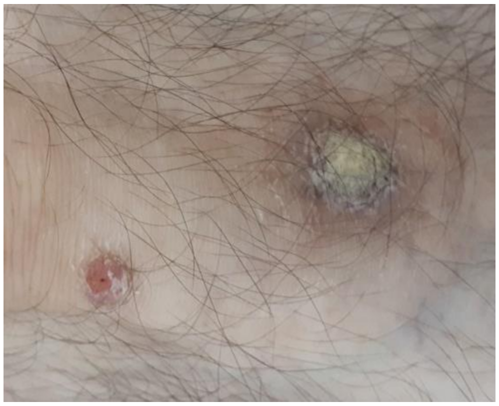

Dermoscopy showed glomerular and punctiform vessels with a “pearl necklace” distribution. This image is highly characteristic of clear cell acanthoma (Figures 4 and 5).

Figure 4: Dermoscopy

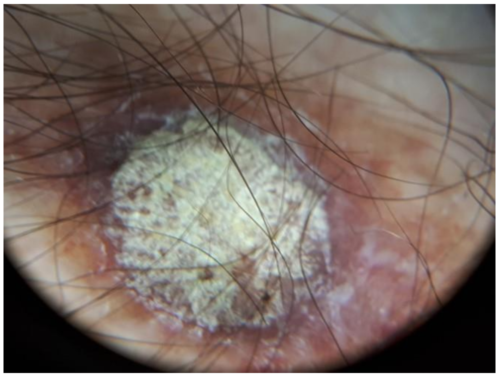

Figure 5: Dermoscopy

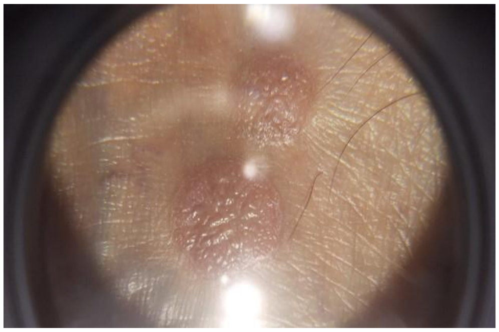

An excisional biopsy of one lesion followed by histopathological examination was performed. The histopathological examination showed psoriasiform elongation of plump and interconnected rete ridges, parchetosis and vascular ectasia in the papillary dermis (Figure 6), acanthosis, agranulosis, parakeratosis, and increased number of vessels in the papillary dermis (Figure 7); a periodic acid-Schiff reaction (PAS stain) demonstrated clear keratinocyte with pale cytoplasm, containing a large amount of glycogen (Figure 8).

Figure 6: Histopathological exam – psoriasiform elongation of plump and interconnected rete ridges, parchetosis, and vascular ectasia in the papillary dermis, HE stain x40.

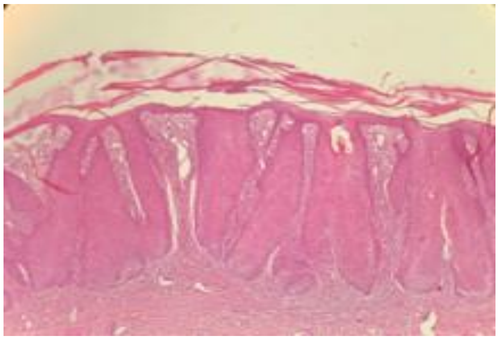

Figure 7: Histopathological exam – acanthosis, agranulosis, parakeratosis, an increased number of vessels in the papillary dermis, HE stainx100.

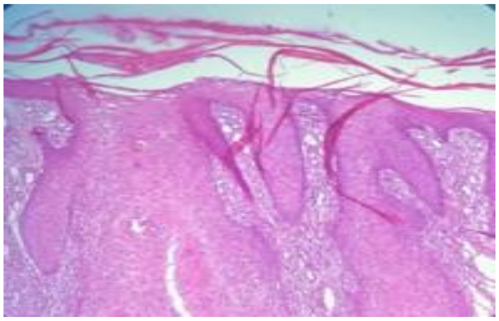

After establishing the diagnosis using the histopathological examination, the surgical excision of the larger tumor formations was decided, under local anesthetics with Xilina 1%, with the suture per-primam with non-absorbable suture threads and local dressing with Baneocin powder, and the others smaller lesions were treated by cryotherapy, using liquid nitrogen and a cotton wool swab, applied for fifteen seconds and repeated after one minute. All lesions were resolved with minimal residual scarring with no relapse at five months of dermatologic follow-up.

Figure 8: Histopathological exam – Clear keratinocyte with pale cytoplasm, containing a large amount of glycogen, demonstrated with a periodic acid-Schiff reaction, PAS stainx200.

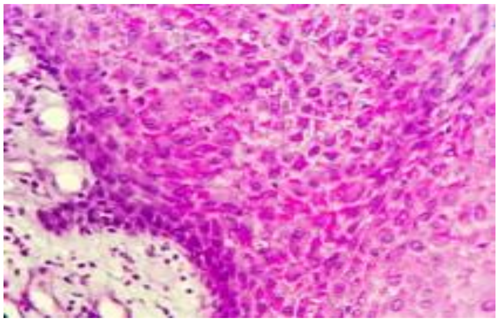

DISCUSSION

Although solitary lesions represent the most common form of presentation of the acanthoma with clear cells, less than thirtieth cases of multiple clear cell acanthoma, from two up to four hundred lesions, have been described in the medical literature to date [6]. The diagnosis based on clinical features frequently needs to be supported using a dermoscopy exam and, in most cases, a histopathology exam is required. Dermoscopy can show dotted or globular vessels, similar to those that can be seen in Bowen disease and psoriasis [7,8]. Also, research has shown an association between clear cell acanthomas and some other conditions including arthropod bites, viral infections, seborrheic keratosis, xerosis, hyttiosis, stasis dermatitis, and varicose veins [9].

The etiology of clear cell acanthoma is not well understood and is no evidence that trauma toxic, drugs, or substances lead to the onset of tumors. In clear cell acanthoma, exist a metabolic enzyme defect, an enzyme that has an important role in the synthesis of keratin [6]. Some authors consider that is an inter-relation with the output of melanocytes and the interaction between melanocyte and keratinocyte. Some of the authors consider that lesions are benign epithelial neoplasm, while others stand up the hypothesis that the origin of this tumor formation is an inflammatory one [7]. Some authors that clear cell acanthoma represent a variant of seborrheic keratosis with the keratinocytes containing abundant amounts of glycogen [10]. In the medical literature, have been reported neither spontaneous regression nor malignant transformation to data.

The most characteristic histopathologic features of clear cell acanthoma are the clear cells with acanthotic epidermis composed of a high composition of glycogen that can be highlighted by positive PAS-Periodic acid-Schiff-staining and the removal of the staining after the diastase digestion. The epidermis is in most cases infiltrated with an abundant number of neutrophils, which may lead to micro-abscesses in the corneum parakeratotic stratum. The Malpighian layer includes slight spongiosis or several pyknotic neutrophils in the intercellular spaces. The papillary dermal layer includes mixed cellular infiltrate and ectasia. A well definite line of demarcation limits the tumor. This line is produced by epidermal cells, which are increased in size, except those in the basal layer. The histochemical examination shows the absence of phosphorylase - a constitutive enzyme, able to demote glycogen [11]. The electron microscopy examinations have revealed glycogen granules in the dermal layer [12]. Video-dermatoscopy has been introduced among the modern methods, for the diagnosis of clear cell acanthoma, highlighting homogeneous pinpoint-like vascular lesions, that have a bush-like aspect if is set a higher magnification [13].

Multiple clear cell acanthoma – from two up to four hundred lesions – have been described in the medical literature until the moment date. The first multiple clear cell acanthoma has been first described by Delacretaz in 1964 [14].

Differential diagnoses include vulgar warts, pyogenic granuloma, Kaposi disease, amelanotic melanoma, dermatofibromas, traumatized hemangioma, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, irritated seborrheic keratosis, and psoriasis [15-21].

The treatment consists is complete removal (standard surgical excision, cryotherapy, electrofulguration alone, carbon dioxide laser or shave removal or curettage followed by electrofulguration) [22]. For the cases with multiple lesions, cryotherapy, or topical 5-fluorouracil are preferred [23, 24]. In our case, even if the lesions were multiple, the surgical approach supplemented with cryotherapy was found to be a good choice. Therefore, we opted for standard surgical excision, cheap and painless (because we used local anesthesia with Xiline) for larger tumor formations and the other smaller lesions were treated by cryotherapy, using liquid nitrogen. After two sessions, lesions were completely healed without relapse and with a good aesthetic result. In conclusion, we can say that cryotherapy is still an optimal method for treating forms of multiple eruptive clear cell acanthoma, but if there are lesions with a larger diameter among the tumors, classical surgical excision may also be combined.

CONCLUSIONS

Clear cell acanthoma is a rare benign epithelial tumor that presents typically as a solitary tumor, but there are cases with multiple lesions. The tumor is red/red-brown, dome-shaped papule, or nodule. A peripheral wafer-like scale collarette represents the classical presentation in majority lesions, but this sign may not always be present. The surface may have a crusted appearance or a moist appearance and may bleed with minor trauma. The tumor slowly enlarges over several years.

Dermoscopy reveals a highly characteristic pattern, a sign that can be utilized to aid in diagnosing a clear cell acanthoma - the the “pearl necklace” appearance (glomerular/pinpoint blood vessels present in a curvilinear and reticular pattern), but a skin biopsy is usually performed to confirm the diagnosis. The preferred treatment of clear cell acanthomas is complete removal (standard surgical excision, cryotherapy, electrofulguration alone, carbon dioxide laser or shave removal or curettage followed by electrofulguration). In our case, even if the lesions were multiple, the surgical approach (for larger tumor formations) supplemented with cryotherapy (for the smaller lesions) was found to be a good choice. In conclusion, we can say that cryotherapy is still an optimal method for treating forms of multiple eruptive clear cell acanthoma, but if there are lesions with a larger diameter among the tumors, classical surgical excision may also be used.

Although solitary lesions represent the most common form of presentation, less than thirtieth cases of multiple clear cell acanthoma have been described in the medical literature to date. Therefore, our case with approximate fifteen lesions could be classified as multiple eruptive clear cell acanthoma.

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VARIA

Therapeutic decision of laparoscopy and its benefits as a gold standard in acute cholecystectomy

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Abstract: *Background: Acute cholecystitis is the most frequent complication of cholelithiasis and counts for one third of surgical emergencies.*

Aim: The study evaluates the outcomes and limits of laparoscopic cholecystectomy in acute cholecystitis.

Materials and methods: A retrospective study was performed for 2 years, between 01.01.2016-31.12.2017 in the 2nd Surgery Department of the Sibiu County Emergency Clinical Hospital, on hospitalized patients with acute cholecystitis, who underwent surgery. The severity of acute cholecystitis was analyzed using the Tokyo Guidelines (TG13 / TG18) severity risk scale. The preoperatory evaluation of the anesthetic-surgical risk was based on American Society of Anesthesiologists Physical Status Classification (ASA PS) and Charlson Comorbidity Index (CCI) (9). Statistical analysis was performed to analyze the outcomes and limits of laparoscopic cholecystectomy and the risk factors for conversion and open surgery.

Results: Out of the 262 patients in the study group, most of the patients (61%) were diagnosed with moderate acute cholecystitis, while 67 patients (26%) were diagnosed with mild form and 34 (13%) with severe acute cholecystitis. Laparoscopic cholecystectomy was performed in 96.1% of cases with no conversion to open surgery. The postoperative complications were ligature slippage (1.9%), main bile duct injury (1.9%), postoperative hemorrhage (3.9%) and surgical site infections (2.4%), most of them being managed conservatory.

Conclusions: Laparoscopic cholecystectomy can be performed nowadays with minimal morbidity in acute cholecystitis. Knowledge of various factors predicting possible conversion helps in adequate pre-operative selection and counseling for open procedure with further reduction in the overall morbidity of laparoscopic cholecystectomy.

Keywords: *laparoscopic cholecystectomy, acute cholecystitis, risk factors, inflammation*

INTRODUCTION

Acute cholecystitis is the most frequent complication of cholelithiasis and counts for one third of surgical emergencies. Blocked stones at the level of the infundibulum or cystic duct generate inflammatory phenomena of variable intensity, from mild to severe, with organ failure and septic condition. It is considered to be the second source of complicated intra-abdominal infection (18.5%), according to the World Society of Emergency Surgery complicated intra-abdominal infections Score study [1]. Laparoscopic cholecystectomy has become the “gold standard” in treatment of biliary lithiasis due to its undeniable advantages in reducing pain and postoperative complications. However, there are still controversies regarding the management of patients with acute cholecystitis. In the early days, after the first laparoscopic

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cholecystectomy, acute cholecystitis was considered as an absolute and later, a relative contraindication for this surgery [2] due to the possible risk of increased morbidity, especially common bile duct injuries [3].

The current concept is in favor of emergency intervention in the first 72 hours after hospitalization, as there is no evidence that delaying the intervention after resolving the acute outbreak would benefit in reducing intraoperative complications, especially major biliary tract injuries, due to local fibrous remodeling [4-7]. The current guidelines recommend surgery as soon as possible because evidenced-based clinical studies confirmed that an early treatment reduces the total hospital stay and does not increase the complication or conversion rates [8].

The study evaluates the outcomes and limits of laparoscopic cholecystectomy in acute cholecystitis.

MATERIALS AND METHODS

A retrospective study was performed for 2 years, between 01.01.2016-31.12.2017 in the 2nd Surgery Department of the Sibiu County Emergency Clinical Hospital, on hospitalized patients with acute cholecystitis. The inclusion criteria were:
- hospitalization diagnosis of “Acute non-/lithiasis cholecystitis”
- histopathological confirmation of the acute character of the inflammation of the gallbladder wall;
- surgical treatment of the pathology during hospitalization.

Patients with acute biliary pathology, but who were not treated surgically, and patients who did not show acute inflammatory signs in the gallbladder were excluded.

Observation sheets and operating protocols were analyzed. The collected data documented age, sex, associated pathology, preoperative clinical and paraclinical data (hemoleukogram, fibrinogen, AST, ALT, bilirubin, amylase, INR, urea, creatinine, abdominal ultrasound), type of intervention, postoperative complications, appearance histopathological examination of the gallbladder specimen, duration of hospitalization. The severity of acute cholecystitis was analyzed using the Tokyo Guidelines (TG13 /TG18) severity risk scale. According to this classification, Grade III (severe acute cholecystitis was considered the acute cholecystitis associated with organ/system (renal, cardiovascular, hepatic, respiratory, neurologic, hematologic) dysfunction, Grade II (moderate) acute cholecystitis associates clinical signs of acute cholecystitis with white blood cells (WBC)>18000/mmc, palpable tender mass in the right upper abdominal quadrant, marked local inflammation and the onset of symptoms of more than 72 hours before presentation. Grade I (mild) acute cholecystitis is defined as acute cholecystitis which does not meet criteria for grade II or III [7, 8].

The preoperative evaluation of the anesthetic-surgical risk was based on American Society of Anesthesiologists Physical Status Classification (ASA PS) and Charlson Comorbidity Index (CCI) [9]. Statistical analysis was performed to analyze the outcomes and limits of laparoscopic cholecystectomy and the risk factors for conversion and open surgery.

Table 1: Preoperative evaluation of the patients in the study group: demographic data and associated comorbidities

General characteristics in the study group	
	Total (n=262)
Gender	
Female	171 (65.26%)
Male	91 (34.73%)
Age	
21 - 30 years	20 (8%)
31 – 40 years	44 (17%)
41 – 50 years	68 (26%)
51 – 60 years	47 (18%)
61 – 70 years	34 (13%)
71 – 80 years	31 (12%)
>80 years	18 (7%)
Comordibities	
Diabetes mellitus	29 (11.06%)
Hypertension	120 (45.8%)
Coronary diseases	70 (26.7%)
Valvular diseases	7 (2.6%)
Respiratory diseases	22 (8.3%)
Obesity	41 (15.6%)
Previous abdominal surgery	9 (3.4%)
CCI*	
0	107 (41%)
1	73 (28%)
2	23 (9%)
3	23 (9%)
4	16 (6%)
5	10 (4%)
≥6	10 (3%)
ASA PS**	
I	59 (22%)
II	92 (25%)
III	75 (29%)
≥IV	36 (14%)

**CCI: Charlson comorbidity index*

***ASA PS: American Society of Anesthesiologists Physical Status Classification*

RESULTS

Of the total number of patients (361) who presented at the Sibiu County Hospital, Surgery Clinic, with acute biliary pathology during the analyzed period, 262 (73%) presented inclusion criteria in the study. Demographic characteristics of the study group (Table 1) confirm the predominance of pathology in females (65.26%), and middle adulthood (40-60 years). In 73% of cases, the presentation was late, after more than 72 hours after the onset of painful abdominal symptoms.

The main associated comorbidities were: cardiovascular disease, obesity and diabetes. Assessment of surgical anesthetic risk by calculating the Charlson comorbidity score (CCI) and placing it in the risk classes of the American Society of Anesthesiologists Physical Status Classification (ASA PS) system (Table 1).

Routine preoperative evaluation consisted of clinical evaluation, abdominal ultrasound, chest X-ray, EKG, as well as evaluation of biochemical and hematological parameters, characteristics to assess the severity of infection (hemoleukogram, fibrinogen, ESR), but also liver function (AST, ALT, bilirubin), renal (urea, creatinine), coagulation (INR). According to TG13/TG18 severity risk scale, most of the patients (61%) were diagnosed with moderate acute cholecystitis, while 67 patients (26%) were diagnosed with mild form and 34 (13%) with severe acute cholecystitis.

The management of acute cholecystitis was according to Tokyo Guidelines, based on the severity of symptoms, ASA and CCI index. Patients with ASA≥3 and CCI≥6 or sepsis, underwent fluid rebalance and general supportive care before surgery could be performed safely. Emergency laparoscopic cholecystectomy in the first 72-96 hours after admission was performed. Large spectrum intravenous antibiotherapy was used in all cases.

The operative moment was carefully planned after attentive clinical and paraclinical evaluation of the patient and rebalancing of associated comorbidities. Most of the patients (252; 96%) underwent laparoscopic cholecystectomy. Open surgery was used in 10 patients. There were no conversions in the study group, all intraoperative incidents and accidents were managed by laparoscopic approach.

The main reason for deciding from the beginning the classic intervention is the complex adhesion syndrome, but also the suspicion of phlegmon, representing 34% of the number of patients in this category (4 patients). Others include perforation, with biliary peritonitis, postoperative eventration, ascites and peritoneal carcinomatosis associated with colon neoplasm (1 patient each) (Figure 1).

A comparative analysis of the histopathological aspect of the gallbladder for the 2 groups in the study group (laparoscopic and classic) shows that the form of catarrhal inflammation of the gallbladder wall was 87% in the laparoscopic group and 60% in the classically operated group.

Figure 1: Factors for open cholecystectomy in the study group (percentages)

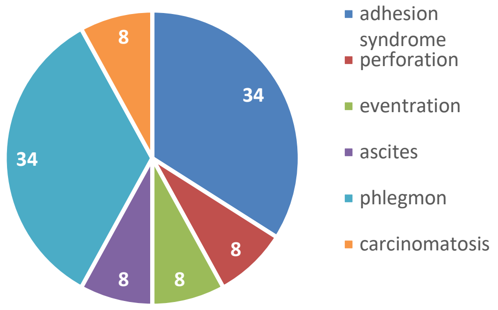

Although less frequent in the laparoscopically operated group, the presence of the difficult forms, such as gangrenous cholecystitis, piocholecystitis, pericholecystic plastron, is not a contraindication for the laparoscopic approach being successfully resolved in this way. On the other hand, the presence of peritoneal carcinomatosis, ascites, eventration, or extensive postoperative adhesions in the upper abdominal floor are absolute or relative contraindications for laparoscopy (Table 2).

Table 2: Histopathological aspects of the removed cholecyst: laparoscopic vs open cholecystectomy

Histopathological aspect of the removed cholecyst	Laparoscopic Cholecystectomy	Open Cholecytectomy
pericholecystic plastron	1 (1%)	1 (10%)
piocholecystitis	3 (1%)	0
pericholecystic fibrosis	5 (2%)	1(10%)
gangrenous cholecystitis	17 (7%)	1(10%)
hydrops	6 (2%)	1(10%)
cataral inflammatory changes	220 (87%)	6 (60%)

A particular aspect as a difficulty in surgical tactics is the association of acute cholecystitis with primary gallstones in 13 cases and angiocolitis in 2 cases.

Main bile duct lithiasis resolution was achieved by ERCP, in 15% preoperatively and in 85% postoperatively.

The outcome was favorable with no complications, in most cases (88%) operated laparoscopically and in 50% of cases operated classically, which is correlated with the degree of difficulty and associated comorbidities increased in the group of 10 patients who were treated by classic cholecystectomy.

The average hospital stay was 5.7 days in the laparoscopic group and 11.2 days in the classically operated group (Figure 2).

Figure 2: Postoperative hospital stay in open and laparoscopic cholecystectomy

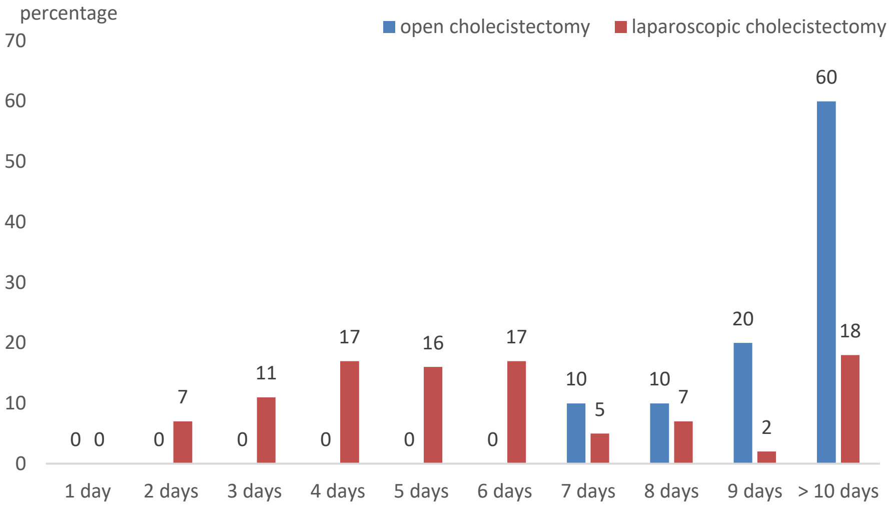

The postoperative complications are presented in Tables 3 and 4. Following the study we determined the most common postoperative complications due to the surgical technique, these being: ligature slippage, main bile duct injury, postoperative hemorrhage and surgical site infections. It can be seen that the most common complication in the study is postoperative hemorrhage, caused by: either skidding of the cystic artery ligation, or by inefficient hemostasis (3.9%), followed by skidding of the cystic ligature (1.9%).

Table 3. General postoperative complications in open and laparoscopic cholecystectomy

General postoperative complications	Open cholecystectomy		Intervenție laparoscopică	
	No	*percentage*	*No*	*percentage*
Death	1	10 %	5	2 %
Stroke	0	0 %	1	1 %
Acute miocardial infarction	0	0 %	4	2 %
Malign arterial hypertension	0	0 %	2	1 %
Ascitis	1	10 %	3	1 %
Nosocomial infection	3	30 %	5	2 %
Sepsis	0	0 %	3	1 %
Others	0	0 %	6	2 %

The reasons for the skidding of the ligature are multiple and we will give some examples: the skidding of the metal clip, being the edematous and friable cyst, the skidding of the metal clip being cut to its laughter, etc. On the 3rd place, but very important due to their severity, is the damage of the main bile duct during the maneuvers (1.9%) (Tables 3, 4).

Table 4. Surgery related postoperative complications in open and laparoscopic cholecystectomy

Surgery related postoperative complications	Open cholecystectomy		Intervenție laparoscopică	
	No	*percentage*	*No*	*percentage*
Skidding of the cystic ligature	2	20 %	5	1.9 %
Lesions of the main bile duct	0	0 %	5	1.9 %
Postoperative hemmorhages	1	10 %	10	3.9 %
Surgical site infections	3	30 %	6	2.4 %

The resolution of surgical complications was done in most cases by conservative management, 2 cases requiring reintervention, 1 for postoperative hemorrhage and resolution by ERCP approach of CBP lesion.

DISCUSSION

Laparoscopic cholecystectomy has become the "gold standard", due to its undeniable advantages in reducing pain and postoperative complications, as well as the length of hospital stay. Even during the COVID-19 pandemic era, when laparoscopy was firstly regarded with extreme prudence, the general surgical opinion was that we must not step back from the achievements of modern surgery, but reinforce the safety measure regarding the ultrafiltration of pneumoperitoneum [10].

Together with the development of anesthesia and intensive care skills and techniques, the safety limit for performing laparoscopy also increased nowadays towards the age of 80-85 years [8, 11], and even of 91 years old in our study group, if not severe ASA risk is associated. In the study group there was no case of conversion. Careful preoperative assessment of the comorbidities and attentive patient clinical evaluation is extremely important before choosing between open and laparoscopic surgery. Conversion to open surgery is associated in several studies with negative effects, including prolonged operation time and increased bleeding which is particular important in patients with increased anesthetico-surgical risk [12].

Another conclusion in the study was that the surgeons should be aware of the significant risk of severe cardiovascular acute events following both laparoscopic and open cholecystectomy, especially in cases with severe sepsis and inflammation or high risk associated comorbidities. These complications should be included in the reasonable disclosure, when the patient is informed about the procedure and the associated possible risks and complications. Informed consent process must be the base of a partnership between doctor and patient, in sharing the inherent risks associated to surgical procedures and understanding what the expectations should be [13].

The severity of local inflammatory changes is a major risk factor for intra operatory and postoperative associated complications. Hyperbilirubinemia significantly increases the likelihood of finding common duct stones in patients with acute cholecystitis, but it also occurs in patients with acute cholecystitis without common duct stones [8]. Hyperbilirubinemia is also a biomarker of intraperitoneal inflammation, along with lipopolysaccharide binding protein (LBP) and Il-6, which increase as a response of liver disfunction associated to bacterial aggression. There are evidences that Escherichia coli and Bacteroides fragilis, common pathogens of the biliary and intestinal wall, were supposed to interfere with hepatocyte microcirculation, inducing sinusoidal lesions. LPSs activate monocytes and macrophages to secrete inflammatory cytokines (tumor necrosis factor alpha (TNF-α) and IL-1, etc.) and other potent mediators [14] by an intracellular signal amplification pathway. These mediators, in turn, act on additional target cells to produce cardiovascular shock, multisystem organ failure, and septic shock [15], one of the major causes of death in intensive care units [16]. In recent studies, a raised concentration in bile but also a more pronounced immunohistochemical expression of cytokines, likeTNF-α, IL-1α, IL-6 was found in the cholecystic wall in both acute and chronic cholecystitis, which may suggest the role of these cytokines in pathogenesis of inflammation and cholelithiasis [17, 18].

Severe inflammation is associated with increased risk of main bile duct during laparoscopic dissection of gallbladder, one of the most serious complications of cholecystectomy. Recently, near infrared (NIR) fluorescence Indocyanine Green was employed in challenging cases to allow a better visualization of the biliary tree. The molecule of ICG is a polymethylic cianyne, which can be administered intravenously, with an excellent safety profile. It binds with circulating albumins and lipoproteins and is excreted into the bile almost unchanged after hepatic extraction [19]. Several studies proved the beneficial use of ICG, which may ensure the surgeon to perform LC with confidence, even in the presence of severe inflammation [20, 21].

Laparoscopic cholecystectomy can be performed nowadays with minimal morbidity in acute cholecystitis [23]. Knowledge of various factors predicting possible conversion helps in adequate pre-operative selection and counseling for open procedure [24] with further reduction in the overall morbidity of laparoscopic cholecystectomy.

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ORIGINAL ARTICLE

The relationship between intestinal dysbiosis and multiple sclerosis

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Abstract: *Multiple sclerosis is a chronic autoimmune disease, characterized by the appearance of demyelinating lesions with inflammatory character in the CNS. Given the unpredictable evolution of the disease, numerous attempts have been initiated to find some pathogenic factors and by default targeted treatment methods. The intestinal microbiota of these patients is often significantly altered compared to the healthy population and given the complex connections between the intestine and the central nervous system, the idea of causality between dysbiosis and this condition was launched. The intestinal mucosa represents the interface between the external environment and the organism, so that it is considered the starting point of some local immunological processes but with systemic resonance, especially neurological.*

The classic medication scheme widely used to treat MS nowadays can also affect the integrity of the intestinal mucosa by breaking the fine balance at this level. Data from recent studies around the subject open new treatment opportunities around preserving the intestinal microbiota and strategies like vitamin A intake and fat-soluble vitamin D supplementation, probiotic complexes or faecalis microbiota transplantation appear more promising and easier to apply in daily clinical practice.

Keywords: *intestinal microbiome; gut-brain axis; multiple sclerosis; disease-modifying therapies (DMTs), neuroinflammation*

INTRODUCTION

Multiple sclerosis (MS) is a chronic disease with an autoimmune substrate, characterized by the appearance of acute demyelinating lesions with inflammatory character in the CNS, is the main neurological illness with onset among young. MS affects about 2 million people, and the number is constantly growing, with a 3-fold higher prevalence among women. The most common form of the disease, often the onset one, is relapsing remitting (RRMS), characterized by full recovery of the deficit after an acute episode of the disease and may sometimes pass, after years of evolution, in secondary progressive form, with a progressive, irreversible accumulation of deficit (30-40%) [1-5].

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Despite efforts in recent decades to find a certain pathogenic factors, the research has been in vain, concluding that the evolution of the disease involves both genetic and environmental factors, such as geographical conditions, obesity, diet, smoking, infections with viruses such as Epstein-Barr, vitamin D deficiency and finally, the intestinal microbiota. The last one that recently attracted attention, is considered the biggest risk factor in triggering multiple neurological diseases such as Parkinson's, Alzheimer's disease, amyotrophic lateral sclerosis and optic neuromyelitis [2, 6].

The gastrointestinal tract is populated by an invaluable collection of microorganisms, 3.8 × 1013 bacteria, 1014

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different species, building up the microbiota, in perfect harmony with the host organism. Considered an organ itself, the microbiota fulfils numerous roles, not only locally, but also systemically: it helps to ensure the entirety of the intestinal microbiome and the generation of antimicrobial substances, supports motility, prevents colonization with pathogens, ensures essential bowel functions, absorption and secretion, provides metabolic support by producing vitamins such as vitamin K, folate, vitamin B complex, bone reabsorption, peptides, DNA synthesis/repair and, more recently, the maturation of the immune system [2, 6, 7].

THE GUT/CNS AXIS

About two centuries ago, connections were discovered between CNS and intestinal microorganisms, bidirectional links with other systems, such as immune, endocrine, or nervous; they are recognized today in the form of Gut-CNS axis. As mentioned earlier, the microbiota has a secretory role, being able to synthesize, among many others, cytokines and neurotransmitters that act at a central level, such as GABA (gamma-aminobutyric acid), histamine, serotonin, or dopamine. Through neuroactive molecules, the microbiota stimulates the vagus nerve, modulates hypothalamic-pituitary-adrenal connection and catecholamine production. Also, SCFA (short-chain fatty acids), internally produced and released systemically, can adjust the activity of NK cells, macrophages, or dendritic cells. Given the strong links at this level, it is easy to understand why the alteration of the composition of the microbiota, called dysbiosis, leads to the onset of many diseases of the nervous system [1, 5, 7, 8].

ETIOPATHOGENIC MECHANISMS AND IMMUNOLOGICAL INTERACTIONS

In both MS and other autoimmune diseases, numerous studies have attempted to identify the connection between intestinal dysbiosis and exacerbated autoimmunity, concluding the existence of a mechanism of molecular mimicry, a detrimental interaction of the immune system with the intestinal microbiome that leads to the expansion of Th1/Th17 cells [9]. In addition, any minor infection of the gastrointestinal tract can lead to intestinal dysbiosis, which is common today, given that digestive infections are among the most common infections [10].

Butyric acid, the main SCFA produced by the intestinal microbiota, along with acetic and propionic acid, have immunomodulatory and anti-inflammatory properties, favoring the differentiation of Treg cells (Regulatory CD4 + cells), TH1 and TH17. These, released in the proximal colon following the fermentation of non-digestible carbohydrates and subsequently transferred to the peripheral blood, have been found in small quantities in the population suffering from multiple sclerosis [5, 11].

MAIT cells (Mucosal-associated invariant T) represent a subset of cells belonging to the innate immunity, found on the surface of the mucous membranes, but also in the blood and liver, whose dysfunction is found both in MS and in other diseases with an immunological substrate such as type 1 diabetes, Sjogren syndrome, rheumatoid arthritis or systemic lupus erythematosus; MAIT cells, through their ability to synthesize IL17, play an essential proinflammatory role in defending the body against bacteria, fungi or certain viruses. A recent study, led by Willing et al., Found the existence of a circulating inflammatory subset of CD8 + CD161high MAIT cells in the blood of patients with early onset of MS. In addition, in a study that aimed to track circulating MAIT cells as a biomarker in the evolution of MS, the same cells were found in excess in the CNS of patients known to have MS. It should be noted that a reduction in CD8 + CD161 + MAIT cells was observed after treatment with dimethyl fumarate [12].

IgA is an antibody found mainly on the mucosal surface, ensuring intestinal homeostasis. Recent studies suggest the involvement of IgA as a possible pathogen in several extraintestinal autoimmune diseases. Regarding MS, elevated IgA levels were found in both patients' blood and cerebrospinal fluid during periods of disease activity, compared to healthy controls [13].

Demyelinating plaques within the MS are the result of the neurodegeneration process the following infiltration with microglia, astrocytes, and leukocytes. Microglia, known as "brain macrophages", are directly involved in the myelination and maturation of synapses, phagocytose debris and secrete neurotrophic factors. Astrocytes, extremely numerous in the nervous tissue, control the function of synapses, regulate blood flow through the blood-brain barrier (BBB) and maintain the pro and anti-inflammatory process. The activity of these cells and the interaction between them are controlled by the microbiota, so that any change leads to compromising this fine balance [1].

The small intestine represents an interface between the internal and external environment, a surface for the development of interactions between various cells so that its integrity is essential for the proper functioning of the whole organism. Recent studies have noted the existence of a connection between the exaggerated permeability of the small intestine and MS, by affecting the BBB through receptors for Zonulin. Used as a marker of intestinal permeability, Zonulin is a protein that ensures the integrity of epithelial junctions, strengthening the immune tolerance of the intestine. According to the "leaky gut" theory, intestinal dysbiosis causes alteration of the composition of the mucus in the small intestine, but also to enterocyte apoptosis and altered cell junctions at this level, followed by bacterial infiltration of the lamina propria, being considered the immune protection barrier of the intestinal wall. Consequently, lymphocytes will be attracted to these areas, with the induction of an inflammatory status that will maintain lymphocyte production and the secretion of cytokines (IL17, tumor necrosis factor) thus forming a vicious circle that will predispose the host body to aberrant autoimmune responses at the local level as well as at the systemic level [2, 5, 14]. Regarding MS, elevated levels of Zonulin were detected in the serum of patients with RR form, being correlated with BBB disruption, excessive activation of microglia and astrocytes with inflammatory neuro-status, a fact confirmed by the lesions observed on MRI images [2, 5].

IMPAIRMENT OF THE INTESTINAL MICROBIOTA IN MS

Detailed studies in recent years managed to describe the immunological status of patients with MS and reveal the microbiome alteration in these patients compared to the healthy control group [2]. Thus, a study conducted by Reynders et al., confirms the abundance of Faecalicoccus, Ruminococcus, Gemmiger, Alistipes and Anaerotroncus in the samples obtained from laboratory mice [15]. Other recent research suggests a low level of Faecalibacterium while Streptococcus thermophilus/Salivarius and Eggerthella lenta were significantly increased in faecal samples obtained directly from patients with this pathology [6, 16].

It seems that the basic medication in MS, disease-modifying therapies (DMTs), prescribed both for prophylactic purposes and for ameliorating the symptoms, would also act on the composition of the microbiota, raising the question of whether their therapeutic effect is exerted by also acting on the intestinal barrier [2, 14, 17].

Interferon-β (IFN- β). Interferon has been used in clinical practice for many years, proving effective in many other pathologies. Interferon-β is considered a major cytokine of the organism that participates in the innate immune response of the host because of bacterial or viral aggression, ensuring the integrity of the intestinal epithelial junctions. Between the microbiota and the intestinal barrier, there is bidirectional communication mediated by IFN-β, together forming a trio that ensures junctional integrity, stimulates the recruitment of Treg cells to the mucosa and increases local defence in the presence of an antigenic stimulus; Studies in this direction show an increase in the Provotella species, with a known probiotic role, among MS patients treated with Interferon-β similar to the healthy population, while untreated patients with the same pathology have a deficiency of probiotic species in the intestine [2,14,17,18].

Glatiramer acetate (GA). GA, a first line DMT, has anti-inflammatory properties, participating in modulating the immune response by reducing TNF alpha activity, increasing anti-inflammatory mediators (IL-10, TGF-b) and also recruiting Treg cells to the intestinal mucosa. The effectiveness of GA has also been demonstrated in laboratory models, improving colonic lesions in ulcerative colitis. Regarding patients with MS, GA induces changes of Faecalibacterium, Ruminococcus, Lactobacillaceae, Bacteroidaceae and Clostridium. Recent studies on laboratory animals show an improvement in the concentration of Prevotella in the gastrointestinal tract after the administration of GA, and the concomitant administration of this preparation with live Prevotella has led to an improvement in the symptoms of the disease [2, 14].

Dimethyl Fumarate (DMF). DMF, long used in the treatment of psoriasis and, more recently, as a first-line treatment in MS, exerts its immunomodulatory effects by inducing T cell apoptosis and inducing a Th2 cell response. In addition, DMF supports the integrity of the intestinal wall, decreases intestinal permeability by supporting epithelial junctions. In patients with MS, DMF appears to decrease Clostridia and Firmicutes and increase Bacteroidetes and other SCFA-producing species concomitantly. It should be noted that recent experimental studies show that the abundance of SCFA-producing microbes potentiates the anti-inflammatory effect of DMF at the systemic level [2, 14]. This observation naturally leads us to conclude the benefit of the simultaneous administration of this DMT with probiotics.

Teriflunomide. Teriflunomide is another DMT used as a first-line in MS, having an anti-inflammatory effect by reversibly inhibiting dihydroorotate dehydrogenase, significantly influencing the number of lymphocytes entering the central nervous system, acting in the intestine by inside stimulation of Tregs cells (CD39+); Notwithstanding the beneficial effects against MS, in laboratory studies on porcine intestinal cells, this drug has been shown to decrease the ability of epithelial cells to defend against infections [2, 14].

Natalizumab (NTZ). The Natalizumab, a second line DMT, is a monoclonal antibody directed against integrins, thus limiting the number of T cells entering the CNS, suppressing immune activity in areas affected by MS. Given the non-selective activity, NTZ acts not only on the T lymphocytes in the CNS but also on those in the intestine, proving effective in inflammatory bowel disease (IBD). Thus, NTZ has been proposed for patients with highly active RRMS, being even preferred in those patients who also suffer from IBD. In the context of existing intestinal dysbiosis in MS, in the case of NTZ treatment, the aberrant exposure of the local immune system to antigenic stimuli will be ameliorated and this may be considered an additional mode of action of this drug [2, 14].

Fingolimod. Like NTZ, Fingolimod, a second line DMT used in RRMS, acts as a sphingosine 1-phosphate receptor (S1P) antagonist, a receptor needed for the maturation and release of T and B lymphocytes from the lymph nodes into the peripheral blood, improving the systemic immune response [14]. By the same mechanism, it is experimentally effective in relieving ulcerative colitis, and many studies are underway in this direction. Regarding the intestinal microbiome, it appears that Fingolimod regulates the maturation of IgA blasts in the intestinal Peyer's patches and acts as the first line of defence against local microbial colonization. The same antimicrobial effect has been demonstrated in vitro, inhibiting the growth of Clostridium and other associated endotoxins [2].

Alemtuzumab. Initially used for treating various forms of leukaemia and newly approved in the therapeutic scheme of RRMS, Alemtuzumab is a monoclonal antibody focused against CD-52 that induces the depletion of B and T lymphocytes in peripheral blood. Despite its proven effectiveness, used for a long time, it alters the repopulation of peripheral blood with immune cells and predisposes to the development of various autoimmune diseases such as membranous glomerulonephritis, autoimmune thrombocytopenic purpura, thyroid disease, autoimmune hepatitis, etc. It should be noted that following the administration of Alemtuzumab, one patient developed, due to Clostridium, fatal colitis, and another patient presented pancolitis after the first dose of treatment. Subsequent studies in laboratory animals show the harmful effects of Alemtuzumab on the gastrointestinal tract by altering the microbiota, affecting the integrity of the epithelial barrier and the consequent increase in intestinal permeability. These findings make us wonder if the profound effects on intestinal integrity balance the benefit of fighting MS [2, 14].

DIET AND ITS CONNECTION TO THE MICROBIOTA AND MS

Diet is considered an important trigger in the development of multiple diseases and may even influence the intestinal microbiota and implicitly, multiple sclerosis. Given the developed network of connections between diet and this disease, it is important to understand the mechanisms of action of many food triggers to treat the causes or find new therapeutic options for the future.

The Swank Diet. The Swank Diet is a low-fat diet that arose from Dr Swank's observations that the occurrence of MS can be correlated with increased fat intake. The data collected from the study concluded that patients who consume a higher percentage of fat also have a higher degree of disability. However, this study presents its limitations, the data is reported directly by the patients, questioning their accuracy [19].

Ketogenic diet. This diet, which is based on high-fat-low-carbohydrate principles, has been shown to improve Th1 and Th17 cells by promoting Treg cell proliferation. Studies based on the ketogenic diet have improved the quality of life of these patients, depression and fatigue for a period of 3 to 6 months, being a pragmatic starting point for future research [20-24].

Vitamin D. Recent epidemiological studies have found a higher percentage of MS patients in areas far from the equator, suggesting that vitamin D may have a protective role against this disease. Data from in vivo studies show the ability of this vitamin to limit experimental autoimmune encephalomyelitis (EAE), the most common preclinical model of MS in mice. In vitro, the data suggest that vitamin D reduces lymphocyte proliferation and antibody production induced by antigenic stimulation, induces the suppression of pathological Th1 cells, and increases the protective activity of Tregs. However, laboratory mice that do not have a vitamin D receptor or have been on a vitamin D-poor diet appear to experience milder forms of EAE [25-28]. These conflicting results underestimate the importance of vitamin D in the immune response, although it is the most studied dietary risk factor in MS. However, several studies show that vitamin D supplementation during pregnancy reduces the risk of developing MS among newborns [8,20,29,30].

Vitamin A. Vitamin A can modulate the immune response according to its local concentration and specially to reduce neuroinflammation. Thus, it has been observed that 13-cis-retinoic acid, a precursor of vitamin A, decreases the production of IL-2 and limits leukocyte proliferation, improving the evolution of EAE [31-33]. Retinoids are already used in clinical practice to treat acne or psoriasis, and although the usefulness of vitamin A in the treatment of MS is not yet clear, the data obtained so far suggest that patients may benefit from the immunomodulatory effects of supplementation with this vitamin [19].

Excess salt. The excess salt characteristic of the Western diet but also the significantly increased percentage of MS patients in the Western world have led to the incrimination of this compound as a possible etiological factor. Studies show that a hypertonic environment due to excess salt and consequent osmotic stress causes inflammatory status due to increased cytokine production [34, 35]. This fact is also supported by the evidence obtained in the laboratory; following the administration of an excess of salt, it was noticed the increase of the production of Th17 cells and the aggravation of EAE. However, other studies following the evolution of patients with the clinically isolated syndrome (CIS) have not found causal connections between salt and the subsequent development of MS so extensive research is needed to clarify things [2, 20].

FUTURE DIRECTIONS

Probiotics. Several studies have analyzed the therapeutic potential of probiotic administration, even supporting their effectiveness by decreasing the peripheral inflammatory response in MS, the most widely used belonging to the genera Lactobacillus and Bifidobacterium [1, 36-38]. Moreover, a single study has shown an improvement in the EDSS score among patients treated with probiotics, but the results of more detailed studies, both clinically and imaging, are expected [20].

Faecal microbiota transplantation (FMT). Initially used to treat Clostridium difficile infections, FMT appears to be a possible innovative treatment for patients with epilepsy, Parkinson's disease and MS, according to recent research. A study by K. Li et al. proved that, in the case of mice with EAE, FMT succeeded the improvement of the symptoms, the improvement of the clinical scores and slowing down of the disease progression [39]. The mechanisms underlying these results are the maintenance of BBB integrity, protection of axons and myelin sheaths, and reduction of microglia and astrocyte activation, proven in FMT-treated EAE to be lower than those treated with a simple saline solution [4]. Currently, studies are underway to obtain the safety of FMT use, which is considered the most direct way to restore the intestinal microbiota [1, 8, 21].

Vitamin D. Elevated vitamin D levels are inversely associated with the number of new lesions on MRI investigations, with high doses (between 10,000-40,000 IU/day) being effective in reducing proinflammatory cytokines and suppressing T lymphocytes in the serum of MS patients. In addition, vitamin D ensures the integrity of intestinal epithelial junctions, ensures optimal intestinal permeability, and reduces local cellular apoptosis [8, 14, 19].

Oral antibiotics. Oral antibiotics are useful in ameliorating the symptoms of mice with EAE by modulating the composition of the intestinal microbiome [40-42]. However, to avoid the exacerbation of dysbiosis in the evolution of MS, detailed studies are needed to discover the optimal preparations and doses to be applied in these cases [8].

CONCLUSIONS

The intestinal barrier plays an extremely important role in the development of various diseases, being the interface between the external environment and the body and a starting point for complex immune interactions that maintain communication with the CNS. Correction of the intestinal microbiota seems a promising option as a future treatment direction, requiring multidirectional actions to restore balance at this level in patients with MS. Thus, the administration of probiotics, vitamins, dietary measures or FMT are viable options to reduce neuroinflammation and alleviate the disease, but studies are still needed to build the answers.

Authors' contribution

All the authors substantially contributed to each of the following aspects of this review paper: conception and design of the study (mainly CAS, RMD, TMV and CS), literature research and analysis and interpretation of the relevant data (mainly CAS, AER, AMS and ADA). In addition, all authors were involved in the drafting of the manuscript (mainly CAS, RMD, TVM, CS, AMS and ADA) and revised it critically for important intellectual content (mainly RMD, TMV, CS and AMS). All authors have read and approved the final version of the manuscript. The authors have participated sufficiently in the study, take public responsibility for appropriate portions of the content, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest:

The authors declare that they have no competing interests.

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CLINICAL PRACTICE

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The role of calcium hydroxide in the treatment of chronic apical periodontitis in patients with type 2 diabetes – a case report

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Abstract: *From an anatomical perspective, the dental pulp is a connective tissue with a specific microcirculatory system with repairing abilities in order to preserve the vitality of the pulp15. Different therapeutic methods in treating the affected dental pulp can be compromised by different factors and can lead us to failure. Due to disorders emerged in the microcirculatory system, the treatment of the affected dental pulpin patients with type 2 diabetes is an additional challenge. Type 2 diabetes can impact different functions of the patients' immune system, with a predisposion to chronic inflammation, progressive tissue degradation and reduced tissue repairing15. Regarding the endodontic therapy of the affected dental pulp in patients with diabetes mellitus (DM), an important role in the treatment of pulpal complications is played by the calcium hydroxide which is considered „the golden standard" because of its excellent properties, the very alkaline pH being one of the most important.*

Keywords: *diabetes mellitus, chronic apical periodontitis, calcium hydroxide*

INTRODUCTION

Diabetes mellitus is one of the most frequent metabolic disorders affecting 366 million patients of all ages, according to the statistical data of the year 2011 [26,1, 21]. It is a disease that is chronic, complex, progressive and crippling and that has no current treatment, diabetes being characterized by a partial or total deficiency of insulin production [5].

Patients with diabetes mellitus have a tendency to develop oral complications such as: cavities [5,27, 22], pulpal and periapical pathology [5,10,14,23] and, very often, periodontal disease [2,10,4,14,12,16,20, 1,7], which is considered the sixth complication of DM and the most chronic affection [20,1].

Many materials have been used in the endodontic treatment but unfortunately none of them managed to succeed in satisfying the complete requirements of an ideal sealer [11,19].

The pure calcium hydroxide paste has an increased pH value of approximately 12.5-12.8 [9] and it is chemically

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classified as a strong base. Its main actions are accomplished through the dissociation of Ca^{2+} and OH^- ions affecting the vital tissues, inducing the deposition of hard dental tissue and possessing antibacterial properties [24].

$Ca(OH)_2$ is an antimicrobial agent with a bactericidal effect, and by using it in the course of the endodontic treatment prevents the apical periodontium from becoming infected [25]. Moreso, it is being used to reduce inflammation (thus decreasing the pain), helping eliminating the apical exudate in order to control the root resorption and to prevent the contamination of the apical periodontium between treatment sessions [9].

MATERIALS AND METHOD

Patients diagnosed with type 2 diabetes have participated in this study. During the radiologic exam we have ascertained that these patients presented radiologic modifications of the periapical tissues with obvious signs of root and bone resorption.

These patients have been informed about the treatment they were about to receive and have signed an informed consent agreement to participate in our study.

Inclusion criteria for participation in the study: 1. patients over 18 years old diagnosed with type 2 diabetes; 2. patients with mature teeth that presented an apical radio translucency at the radiologic exam.

All patients have been submitted to an orthograde endodontic treatment. The teeth have profited by an intermediary session when a paste with calcium hydroxide and radio opacifying agent has been applied in the root canals.

For every patient we have used dam isolation – a diga. The endodontic therapy has been conducted using rotary instrumentation, the crown-down technique and by successively applying endodontic irrigating solutions such as NaOCl 5.25% and citric acid 40%.

Between the therapy sessions the root canals were filled with a calcium hydroxide based paste and for the obturation of the tooth crown we have used glass ionomer cement.

The calcium hydroxide based paste was introduced using a Lentulo spiral filler and condensed using paper cones as pluggers. In the next therapy session the $Ca(OH)_2$ paste is removed using alternative irrigation with NaOCl 5.25% and citric acid 40%,using ultrasonic activation, NaOCl 5.25% and EDTA 17% and manual instrumentation for the working length.

An intermediate radiography was conducted before the obturation of the root canals with gutta-percha and the warm compaction technique.

RESULTS

Clinical case no. 1

The patient M.C. presents a chronic apical periodontitis in 1.7, following an incomplete endodontic treatment (Figure 1).

The patient had several recurrent acute episodes that induced a right odontogenic sinusitis that has been diagnosed some years ago.

Following the last otorhinolaryngology checkup the patient was sent for an oral and dental evaluation and also for a radical sinus surgery.

We have opted for a non-surgical conservative endodontic treatment (orthograde approach) finalized in the second therapy session.

Figure 1: Initial radiological aspect of 1.7

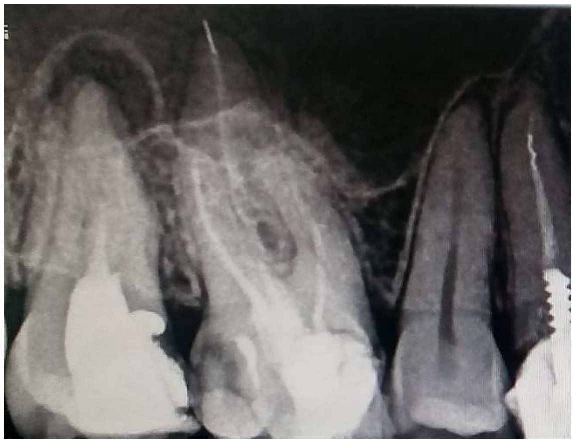

As an intermediate root obturation we have used a calcium hydroxide based paste and an opacifying agent (Figure 2).

Figure 2: The radiological aspect of 1.7after root obturation with $Ca(OH)_2$ as intermediary therapy (after 4 months).

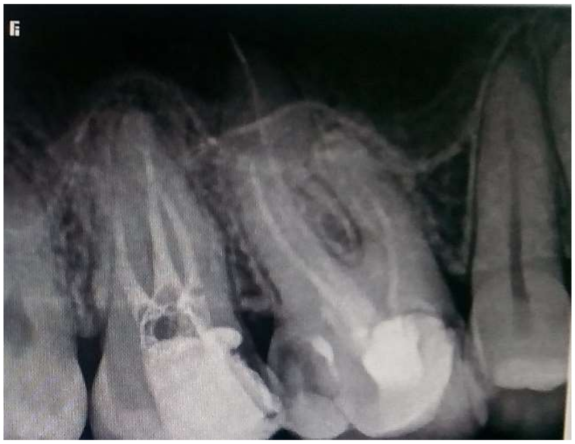

Figure 3: The radiological aspect of 1.7. The gutta-percha root canal obturation.

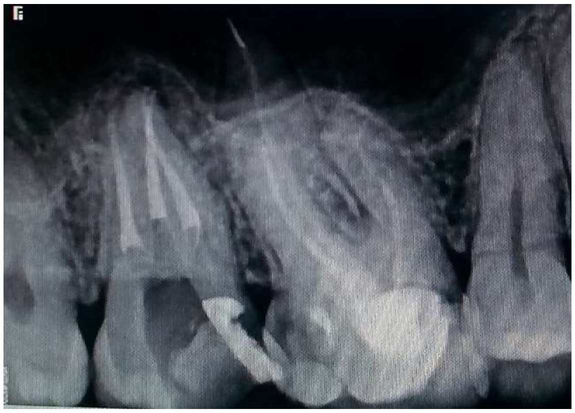

The apical radio translucency has decreased significantly and the patient's clinical status has improved (Figure 3). The subjective signs of sinusitis have progressively amended.

Clinical case no. 2

The patient V.N. came in our clinic for periapical bone lesions in 3.6 following an apical resection procedure after endodontic treatment (Figure 4).

We made the decision to conduct a non-surgical conservative treatment (orthograde approach) finalized in 2 treatment sessions.

For the intermediate root canal obturation we have used $Ca(OH)_2$ paste and opacifying agent for a better radiographyic imaging (Figure 5).

Figure 4: The initial radiological aspect of 3.6

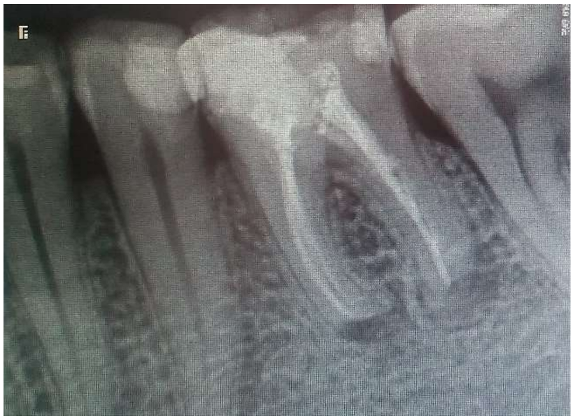

Figure 5: The radiological aspect of 3.6 with its roots canals intermediately obturated with $Ca(OH)_2$ (after 3months)

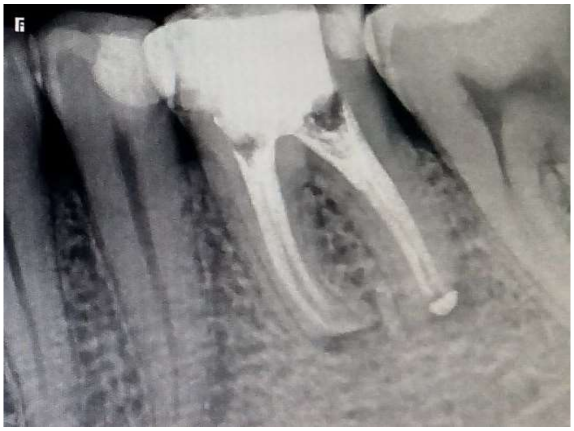

Figure 6: The radiological aspect of 3.6. Verifying the root canal obturation with gutta-percha.

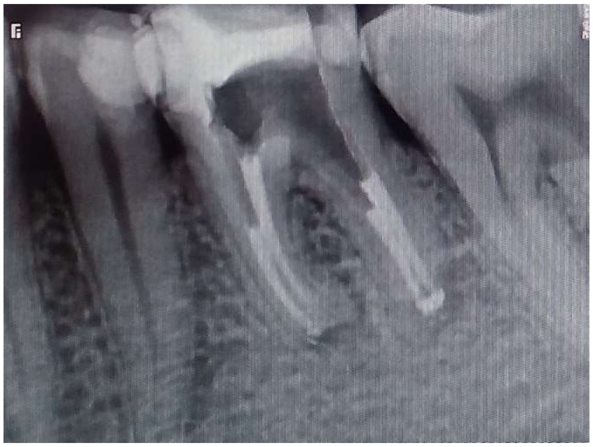

After the decrease of the apical translucency, 3.6's roots were permanently obturated using the warm vertical compaction technique (Figure 6).

DISCUSSION

The success criteria in endodontic treatment were established in 1994 by the European Society of Endodontics and include the absence of pain, inflammation and fistula, maintaining the dental function, the presence of radiological evidence to show a proper periodontal ligament space and the absence of apical periodontitis and root resorption [8].

The endodontic treatment conducted was non-surgical and conservative using the orthograde approach under Diga isolation and benefiting abundant irrigation with endodontic irrigation solutions.

We have varied the time in which the calcium hydroxide paste was maintained in the root canal accordingly.

In both cases we can observe the initiation of the periradicular bone remineralization process.

The ceasing of the resorption of the external radicular necrotic pulp can be exclusively attributed to the elimination of the necrotic tissue and to the conducted antibacterial intracanalar treatment [18].

The diffusion of the OH-ions through the dentin depends upon the duration of the medication, upon the diameter of the dentinal tubules (cervical vs apical) and upon the ablation of the layer smear (for an increased diameter of the dentinal tubules). Moreso, the diffusion of the OH-ions into the root resorption areas where the pH is acid has a positive effect on the progression of the root's inflammation and resorption [17].

The $Ca(OH)_2$ based paste can be used successfully for the management of perforations, horizontal radicular fractures and radicular resorptions [17].

Gergely JM. et al. have shown that calcium hydroxide is frequently recommended in the treatment of periapical abscess because of its strong alkaline pH (approximately 12). The antibacterial effect is due to the dissociation of $Ca(OH)_2$ into OH^- and Ca^{2+}, that also has a healing effect on the damaged tissues [13, 6].

The endodontic treatment in patients with diabetes and infections of the radicular canal can be correlated with a decrease of the treatment's success and therefore the dispensarization of these patients is mandatory [3].

CONCLUSIONS

In case of patients with recurrent treatments it is advisable to perform a non-surgical conservative treatment.

Regarding the evaluation of the success of the examined endodontic treatments we have concluded that the success rate in type 2 diabetes patients is high only when the treatment is thoroughly conducted.

We cannot say that the type 2 diabetes patients have an increased predisposition for developing periradicular lesions because the endodontic treatment in these patients was incorrectly conducted because of incomplete root canal treatment.

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VARIA

The role of career anchors in human resource strategy (Case study: Students of Management Faculty of Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University)

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Abstract: *Maintenance of efficient, highly specialized, and valuable forces in the present age is one of the most important concerns of organizations, especially organizations with advanced technologies. The importance of human resources planning and management in these organizations has greatly increased, and in the various areas of human resource management, the approach to occupational anchor planning has been very much considered. The purpose of this study was to investigate the role of the job path anchors in human resource strategy among the target community. Participants were selected randomly from among 3500 students at the Faculty of Management of Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University in Tehran Province, Iran. The sample size was 346 by the Cochran method and the questionnaire was collected by electronic means. At the time of the study, 346 questionnaires were completed and the analysis was performed based on 346 questionnaires. To collect data, standard job anchor questionnaires, and Human Resources Strategic HR Questionnaire was used. The study population included 346 people, of whom 203 were male and 143 were female. The age of the people below the age of 20 was 38, from 20 to 25 years old, 94, between the ages of 26 and 30, 60, people aged 31 to 35, and 76 people over the age of 35 years. The reliability of the work anchors was 91% and the human resources strategy questionnaire was 80%. Investigations have shown that, on the anchors of the career path, the factors of creativity - entrepreneurship, autonomy-independence, pure challenges and public management with a committed strategy and public management, creativity-entrepreneurship, security-stability in the strategy of contracting businesses and creativity - entrepreneurship, Pure challenges, security with secondary jobs, and creativity-entrepreneurship, public management, pure challenges in parenting jobs. According to the results of this study, it seems that the emphasis on reducing organizational structures, empowerment, and participatory decision making can determine the necessary grounds for determining the type of human resources strategy in an organization.*

Keywords: *job path anchors, HR strategies, management, human resources, strategy*

INTRODUCTION

In today's world, the power, economic power, and prosperity of any country depend on the optimal use of its facilities, industries, and especially its human resources. Qualified and skilled human resources are the endless value and capital contributions to the growth and development of

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organizations and countries, and the greatest asset of a country is its growth factor. If labor, capital, and land were the main factors of production in the past, today technological change, manpower, and increased productivity is considered to be the drivers of growth [1]. In today's world, organizations are in constant competition. The intensity of competition increases every year and all organizations are constantly in need of improved organizational performance for their survival and growth. Therefore, through an efficient and effective human resources management system, individuals can be considered as strategic assets [2]. The term human resource strategy as a process is a specific set of human resource requirements developed by an organization to achieve its goals. Price, in defining a definition of the human resources strategy, stated that the human resources strategy encompasses all activities related to managing people in the organization in the form of coherent plans to achieve the strategic goals of the organization. Some writers have stated in the concept of strategy as a consequence that "human resource strategy is a model of decisions related to human resource system policies and actions" [3]. Creating a job in a world with diminished job security, changing technology, and increased personal responsibility for the training of certain skills, employment, and lifelong learning are key to meeting the challenges of today's world. Researchers have found that the complexity of the burgeoning context today has a significant impact on people's work attitudes and experiences in their working lives [4]. The concept of a career path, as a link between the organizations and the individuals they work for, is explored from both an internal and an external point of view. Slow and defined as organizational indicators of a career path, while the internal career path is the tendency for employees to move to or from the job anchor. It consists of three main categories: 1. Talents and abilities Self-perception; 2. Core values; 3. Evolved feelings about the motivations and needs that drive the career path [5].

Edgar Shine Model Career Anchors, with 8 Anchors:

1. Technical-Functional Competence: In this anchor, one is primarily motivated by automated content and tends to be employed in technical and specialized positions and jobs.

2. Managing Competency: This personal anchor is motivated by the opportunity to study complex conceptual issues under uncertain circumstances and incomplete information and tend to managerial positions and jobs in large organizations.

3. Security - Stability: In this personal anchor, it is promoted to a reputable organization through job security and long-term attachment.

4. Creativity - Entrepreneurship: In this anchor, one is motivated by the need to build or create projects that belong to him/her.

5. Autonomy - Independence: In this anchor, a person is essentially seeking job opportunities that enjoy maximum freedom in terms of organizational boundaries and are thus stimulated.

6. Serving and Sacrifice and Sacrifice: In this anchor, one is motivated to serve and pursue a sacred cause and to meet the needs of others.

7. Pure Challenges: In this anchor, the individual is challenged to overcome major obstacles, solve difficult problems, or win over competitors.

8. Lifestyle: People with this anchor tend to balance their career paths with their families [6].

Human Resources Strategy

In the early years of 1980, strategic human resource management was the focus of a large number of influential management consultants and researchers in practicing management practices. These authors found that effective human resource management can guarantee quality and be effective in terms of competitive advantage. Not surprisingly, in the mid-1980s, many HR researchers favored the view that more HR units should play a greater role. The first models of human resource strategy formulation were introduced in the middle of this decade. These models, with a strong emphasis on human resource strategy adherence to organizational strategy, presented a rational and logical approach to unifying strategic issues for managing the overall human resources of organizations [7]. But the first models that could offer different strategies for different organizational conditions were single-dimensional models based on the labor market type or control type factor in 1987. These models did not necessarily coincide with the strategy of the organization, thus preventing the possible errors in the process of formulating the organization's strategy into a strategy of human resources. Combined, by introducing the "one-piece model", they proposed the use of strategic reference points (SRPs) to formulate HR strategies (8). The importance of this model is that, in addition to the logic of attraction, for the reason that it is compatible with the fundamentals of management philosophy, it can be formulated and implemented without the need for organization strategy, while maintaining its alignment with the organization's strategy. On the other hand, the HR strategy formulation using this model does not rely on the examination and modeling of the status quo. Using two main dimensions of control (efficiency or process control) and labor market (concerning internal or external market), they have formulated four HR strategies [9].

Secondary strategy

This strategy is suitable for simple, duplicate, and standardized jobs that have a sufficient supply of labor outside the company and there is no need to nurture and retain them. In other words, they can be easily disconnected if they are not needed or unsatisfied with each other and can hire serious employees with the least training and cost if needed.

Paternal strategy

This strategy is also applicable to simple, repetitive, and standardized jobs, with the exception that corporate management tends to retain and enhance existing employees and align them with corporate culture. Therefore, vacancies can be filled by upgrading existing staff.

Contracting Strategy

This strategy is suitable for those complex and specialized jobs that require constant and formal hiring of relevant experts for the company, as the company needs these jobs in short and temporary terms. Therefore, these experts are usually highly compensated, solely for consulting or part of an invitation plan, and then return to the job market after the project is completed to provide similar services. Of course, some organizations (especially virtual organizations) also eliminate the constant need for their expert staff by using these experts' temporary or unnecessary services because the management of these organizations has been able to take full control of the organization's specific knowledge. Also, apply evaluation and promotion tools [10].

Committed strategy

This strategy is also applied to complex and specialized jobs, except that the experts in these jobs are constantly in need of the company and because they in many cases master the complex production network and specific knowledge required by the company. They are not easy to replace. Therefore, it is necessary to have an effective approach to the labor market within the organization, that is, to develop and train company experts and to strengthen their commitment and loyalty to the organization in such a way that these experts are exposed to higher market rights and benefits in the face of a secure and dynamic career future. Ignore work outside the organization. In the strategic reference point's theory, a three-dimensional matrix is used to determine the reference points that includes the internal conditions of the organization, the external conditions of the organization, and time [11].

METHOD

The purpose of this study is cross-sectional applied in terms of purpose and type of research method and the research population is comprised of management students of Islamic Azad University, Yadegar Imam Branch - Shahre Ray. To ensure that the sample selected represents the real community, the sample was selected using correct sampling methods. Random sampling was also used to select the sample members. Morgan table was used to determine sample size in the present study. Since the target population in this study was 4 people, the sample size in this study was 9 people. Data were collected using the standard Anchor Job Inventory and HR strategy questionnaire as measured in previous studies. Surveying is one of the most common methods in quantitative social science research. Since quantitative research is not possible to study the behavior of all individuals in the community, the study is conducted through sampling. In this regard, a sample is selected and the result is generalized to the whole community [12]. After completing the questionnaire and collecting and removing the defective questionnaires, the data were analyzed with SPSS 23 software.

DISCUSSION

This section presents all the analyzes based on the assumptions of the present study. The analysis of the results will greatly help the scientific and purposeful discussion of the research topic.

The demographic variables of this study are as follows: The total number of participants was 346 persons, 203 men, and 143 women. The age group of people under the age of 20 is 38, 20 to 25, 94, between 26 and 30 are 60, people between 31 and 35 are 76, and people over 35 are 76. In terms of marital status, 192 were married and 154 were single; 118 were married, 36 were married, and 85 were single and 107 were single. The level of education in the associates is 50, 142 bachelors, and 154 masters. In terms of job status, 84 students were self-employed, 106 were employed in private companies, 109 were employed in public offices and 47 were employed in semi-public offices. Of these, 53 were freelance male, 78 were male, 78 were male, and 25 were semi-public, with a total population of 203.

Hypothesis 1: What are the career path anchors in committed business executives and what are their priorities?

To respond to this hypothesis, the relationship between the two factors of job anchors and commitment factors of the

HR strategy questionnaire was measured. Regression analysis can be used to determine the correlation between occupational anchor factors and the occupational commitment factor. Regression is a method for estimating and modeling the relationship of several independent variables to one dependent variable.

According to Table 1 and statistical significance at the level of 0.8, the regression model is significant. On the other hand, model estimation data show that independent variables account for approximately 5% of the total variance of the response variable. In other words, the 5 factor of job anchors is the ability to predict the factor of the committed jobs. Based on this, it is possible to determine appropriate modeling to determine the simultaneous relationship between occupational anchor factors and the occupational factor.

Table 1: Regression model and model evaluation indices

Estimation error		**The adjusted coefficient of determination**		**The coefficient of determination**		**Model evaluation**
5.103		0.268		0.285		
P	F statistics	The average sum of square	Degrees of freedom	The sum of the regression squares		
0.000	16.822	438.076	8	3504.610	regression	Significance of the model
		26.041	337	8775.834	Error	
			345	12280.474	Total	

According to the results of Table 2, variables A1, A4, A6, and A8 are not significant. In other words, in assessing the simultaneous relevance of job anchor factors, only the five key factors of public management, autonomy-autonomy, creativity-entrepreneurship, and sheer challenges are effective on the commitment factor. In other words, measuring and scoring these 5 factors can determine the type of person's job.

Table 2: Model coefficients and their significance

Model coefficients	Coefficients	Regression coefficient	Governor error	Standardized coefficient	T statistics	P
	Constant coefficient	9.224	1.921	-	4.801	0.000
	Technical-Applied Orientation (A1)	-0.087	0.112	-0.052	-0.773	0.440
	Public Management Tendency (A2)	0.452	0.092	0.299	4.892	0.000
	Autonomy - Independence (A3)	0.358	0.125	0.215	2.863	0.004
	Security-stability orientation (A4)	-0.001	0.083	-0.001	-0.013	0.989
	Creativity-Entrepreneurship (A5)	-0.258	0.085	-0.175	-3.012	0.003
	Serving and Sacrifice and Sacrifice (A6)	0.041	0.125	0.024	0.328	0.743
	Pure Challenges (A7)	0.451	0.097	0.300	4.636	0.000
	Lifestyle (A8)	-0.198	0.122	-0.117	-1.617	0.107

So the mathematical model would be as follows:

Committed Jobs = *A7 0.451+ *A5 0.258 - *A3 0.358 + *A2 0.452 + 9.224

This mathematical model implies that for each unit score increase in the A2, A3, A5, and A7 factors the commitment factor score will increase by 10.227 points.

Hypothesis 2: What are the career anchors of contractors and what are their priorities?

To answer this hypothesis, as in the first hypothesis, one can determine the relationship between job anchor factors and contractor job factors using a statistical regression method.

According to Table 3 and statistical significance at the level of 0.9, the regression model is significant. On the other hand, model estimation data show that independent variables account for approximately 5% of the total variance of the response variable. In other words, the 5 factor of job anchors is the ability to predict the factor of contracting jobs. Based on this, it is possible to determine appropriate modeling to determine the concurrent relationship between occupational anchor factors and contractor occupation factors.

Table 3: Regression model and model evaluation indices

Estimation error		The adjusted coefficient of determination		The coefficient of determination		Model evaluation
3.732		0.127		0.147		
P	F statistics	The average sum of square	Degrees of freedom	The sum of the regression squares		
0.000	7.271	101.275	8	810.203	regression	Significance of the model
		13.928	337	4393.799	Error	
			345	5504.003	Total	

According to the results of Table 4, variables A1, A3, A6, A7, and A8 are not significant. In other words, in evaluating the simultaneous relevance of job anchor factors, only the five key factors of public management, security-stability, and creativity-entrepreneurship are effective contractors. In other words, measuring and scoring these 5 factors can determine the type of person's job.

Table 4: Model coefficients and their significance

Model coefficients	Coefficients	Regression coefficient	Governor error	Standardized coefficient	T statistics	P
	Constant coefficient	5.508	1.405	-	3.920	0.000
	Technical-Applied Orientation (A1)	0.145	0.082	0.130	1.767	0.078
	Public Management Tendency (A2)	0.161	0.068	0.159	2.377	0.018
	Autonomy - Independence (A3)	-0.170	0.092	-0.152	-1.855	0.064
	Security-stability orientation (A4)	0.183	0.061	0.183	3.010	0.003
	Creativity-Entrepreneurship (A5)	0.175	0.063	0.178	2.804	0.005
	Serving and Sacrifice and Sacrifice (A6)	0.024	0.091	0.021	0.259	0.796
	Pure Challenges (A7)	-0.028	0.071	-0.028	-0.391	0.696
	Lifestyle (A8)	0.019	0.090	0.017	0.212	0.832

So the mathematical model would be as follows:

Contracting Jobs = *A5 0.175 +*A4 0.183+*A2 0.161 +5.508

The concept of this mathematical model is that for each unit score increase in the A2, A4, and A5 factors the contractor job factor score will increase by 6.0۲۷.

Hypothesis 3: What are the job anchors in secondary jobholders and what are their priorities?

To answer this hypothesis, as with the previous hypotheses, one can determine the relationship between job anchor factors and secondary job factors by the statistical regression method.

According to Table 5 and statistical significance at the level of 0.05, the regression model is significant. On the other hand, model estimation data show that independent variables account for approximately 23% of the total variance of the response variable. In other words, the 5 job anchor factors can predict the secondary job factor. Accordingly, it is possible to determine the appropriate modeling to determine the correlation between the job anchor factors and the secondary job factor.

Table 5: Regression model and model evaluation indices

Estimation error		The adjusted coefficient of determination		The coefficient of determination		Model evaluation
4.576		0.226		0.244		
P	F statistics	The average sum of square	Degrees of freedom	The sum of the regression squares		
0.000	13.565	284.084	8	2272.671	regression	Significance of the model
		20.943	337	7057.760	Error	
			345	9330.431	Total	

According to the results of Table 5 the variables A1, A2, A3, A6, and A8 are not significant. In other words, in assessing the concurrent relevance of job anchor factors, only the three key factors of security-stability, creativity-entrepreneurship, and sheer challenges to effective secondary jobs are effective. In other words, measuring and scoring these 3 factors can determine the type of person's job.

So the mathematical model would be as follows:

Secondary occupations = *A7 0.336- *A5 0.237 + *A4 0.384 + 6.340

The concept of this mathematical model is that for each unit score increase in A4, A5, and A7 the secondary job factor score will increase by 6.625 points.

Hypothesis 4: What are career path anchors in parenting and what are their priorities?

To answer this hypothesis, as with the previous hypotheses, one can determine the relationship between job anchor factors and secondary job factors by the statistical regression method.

According to Table 6 and statistical significance at the level of 0.05, the regression model is significant. On the other hand, model estimation data show that independent variables account for approximately 23% of the total variance of the response variable.

In other words, the 8 job anchor factors can predict the parent job factor. Based on this, appropriate modeling can be determined to determine the correlation between the job anchor factors and the parent job factor.

According to the results of the table below, variables A1, A3, A4, A6, and A8 are not significant. In other words, in evaluating the simultaneous relevance of job anchor factors, only the five key factors of public management, creativity-entrepreneurship, and sheer challenges are effective on the factor of hospitable jobs.

In other words, measuring and scoring these 3 factors can determine the type of person's job.

Table 6: Regression model and model evaluation indices

Estimation error		The adjusted coefficient of determination		The coefficient of determination		Model evaluation
5.734		0.227		0.245		
P	F statistics	The average sum of square	Degrees of freedom	The sum of the regression squares		
0.000	13.680	449.745	8	3597.964	regression	Significance of the model
		32.875	337	11071.929	Error	
			345	14676.893	Total	

So the mathematical model would be as follows:

Paternal Occupation = *A7 0.807+ *A5 0.261-A2 0.281+ 7.154

The concept of this mathematical model is that for each unit score increase in the A2, A5, and A7 factors, the secondary job factor score will increase by 7.981 points.

Table 7: Model coefficients and their significance

Model coefficients	Coefficients	Regression coefficient	Governor error	Standardized coefficient	T statistics	P
	Constant coefficient	7.154	2.159	-	3.314	0.001
	Technical-Applied Orientation (A1)	-0.169	0.126	-0.093	-1.342	0.181
	Public Management Tendency (A2)	0.281	0.104	0.17	2.704	0.007
	Autonomy - Independence (A3)	0.155	0.141	0.085	1.104	0.27
	Security-stability orientation (A4)	-0.024	0.093	-0.014	-0.254	0.800
	Creativity-Entrepreneurship (A5)	-0.261	0.096	-0.163	-2.718	0.007
	Serving and Sacrifice and Sacrifice (A6)	-0.135	0.14	-0.073	-0.965	0.335
	Pure Challenges (A7)	0.807	0.109	0.492	7.395	0.001
	Lifestyle (A8)	0.02	0.138	0.011	0.142	0.887

Explain the first hypothesis: Anchors of managerial competence, sheer challenges, and autonomy – independence and creativity – entrepreneurship are effective in committed jobs. Given that these jobs are complex and specialized and require specific knowledge, replacing them in these jobs is simply not possible and the specific characteristics of this group, which is autonomy and independence, the manager of the organization should have sufficient authority to act. It created these people. At the same time, people need to have adequate training in problem-solving to make the organization more effective. Management can use these people in managerial positions to advance organizational goals.

Explain the second hypothesis: In this hypothesis, job anchors include security – stability, creativity – entrepreneurship, and managerial competence. These contractors are highly specialized in the organization and have a high cost to the corporations, so hired individuals must possess the qualities of managerial competence, creativity, and entrepreneurship. In the meantime, they tend to do things that are more stable and secure. Besides, given the creativity of the staff, the organization can create the potential for individuals to emerge [13].

Hypothesis 3: Job Anchors are the most effective - security, stability, sheer challenges, and creativity - entrepreneurship. Given that, people in the workforce are simple, repeatable, and standardized, and there are enough people in the job market, so people are looking for job stability that they need to be creative and entrepreneurial to solve the challenge. Establish their sustainability organization [14].

Hypothesis 4: Job Anchors include pure challenges, managerial competence, and creativity-entrepreneurship. These jobs are very simple, repetitive, and standard. In these jobs, people have longer-term contracts, and the organization tends to stay in the organization and benefit from it when needed. In the strategy of committed and contracted jobs, due to the specialization of these jobs and jobs in this strategy, anchors of managerial competence and creativity - entrepreneurship are common and the subject of sheer challenges in commitment and security - stability in the contractor is different. The results show that managerial competence and creativity-entrepreneurship are an important issue in these two strategies. The results show that there are three factors of managerial competence, creativity-entrepreneurship, and pure challenges between committed and paternal strategies. The distinction between these strategies in autonomy is that they are required for extraordinarily specialized work (committed strategy) [15].

The results of the present study show that there is a significant and positive relationship between the components of public management competency, creativity-entrepreneurship, and pure challenges with HR strategies.

In the study of Amirtash et al. (2011), the comparison of job anchors and organizational commitment between physical education and non-physical education faculty members of Islamic Azad University of Iran was used by Edgar Shine Job Anchor Questionnaire. We have used [16].

Khayef Elahi et al. (2015).on the impact of anchor dimensions concerning job factors on organizational outcomes, in line with the results of the research that considers job path anchors as effective [17] is in line with the results of this study [17].

Gholamzadeh et al. (2012) used a bumper questionnaire in a study on the impact of HR strategy based on the theory of strategic bumper reference points and on the performance of managers and staff, which we also used [18].

CONCLUSION

The present study is the anchor of autonomy and independence as a common component in all four strategies, which emphasizes the importance of reducing organizational structures and participatory decision-making. It also emphasizes that it empowers employees and provides the necessary framework for staff decision-making. There is also a plan to help employees, such as solving personal problems of employees that impede their effective performance path. Where employees have the knowledge and skills and therefore the issue of managerial competencies is important. In management and specialist careers, the choice must be very careful. Managers are advised to pay attention to the provision and promotion of workforce development and support, as well as training and development of staff, and staff promotion based on their career path and merit. In the four strategies available, anchors of technical and functional competence, self-sacrifice, and sacrifice, as well as lifestyle, are missing. These anchors focus on the topics and personal lives of individuals and draw the attention of executives to motivate their employees to create a path that leads the individual forward with idealism and self-control. This raises the challenge that managers by integrating individual and organizational goals can play a special role in promoting the individual and the organization and enhancing the organization and the individual.

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ORIGINAL ARTICLES

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The role of metatarsophalangeal joint arthroscopy in hallux rigidus treatment: technique and early results

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Abstract: *Introduction: The hallux rigidus is a term that signifies a mobility limitation of the metatarsophalangeal joint of the big toe due to osteoarthritic degeneration. Several factors are incriminated in the appearance of this pathology but without being able to establish the cause. The cartilaginous erosion of the metatarsal head and / or the base of the phalange, the dorsal osteophyte of the head of the first metatarsal were found during cadaveric studies. The main reason for consultation is pain and limitation of the dorsiflexion of the big toe during the step.*

Hypothesis: Arthroscopic debridement of the first metatarsophalangeal joint improves the early postoperative clinical score compared to isolated percutaneous treatment.

Material and method: 11 patients divided into 2 groups were assessed clinically at 3 months postoperatively. All patients were hospitalized on an outpatient basis.

Results: There was a gain in mobility at 3 months compared to the preoperative assessment in both groups, as well as an improvement in pain without being able to demonstrate the superiority of the additional arthroscopy to the percutaneous debridement. Hypothesis reversed..

Keywords: *hallux rigidus, orthopedics, arthroscopy, osteophyte*

INTRODUCTION

Pathogenesis

Hallux rigidus is a term invented by Cotterill in 1888, refers to a limitation in mobility of the metatarsophalangeal joint of the hallux. Although he did not call it hallux rigidus, in 1887 Davies-Colley reported for the first time the resection of the base of the first phalanx for this pathology, which he called hallux flexus because of the flexion attitude of the metatarsal-phalangeal joint and the limitation of extension of this joint. Moreover, the understanding of this pathology has advanced thanks to radiological techniques, but the pathogenesis of the hallux rigidus is not yet defined.

The evolution towards the aggravation of the destruction is on the other hand well known. The cartilagenous lesion is supposed to initiate synovitis, which itself supports cartilage destruction, accompanied by osteophyte formation and subchondral bone involvement. [1]

The degenerative process can begin in adolescence when a traumatic event at the metatarsophalangeal

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joint damages the articular surface of the head of the first metatarsal. Repetitive strain injuries can also cause cartilage damage. Other causes are mentioned: osteochondritis dissecans of the head of the first metatarsal secondary of an osteochondral fracture at the level of the dorsal convexity of the articular surface, the hyperextension of the first metatarsal, an excessive length of the first metatarsal and a severe pronation of the foot. The hallux rigidus of the adult is most often caused by degenerative arthropathy or osteoarthritis of the metatarsophalangeal joint, while in adolescents the hallux rigidus results from a localized cartilaginous lesion of the head of the first metatarsal most post traumatic time [1,2]

A system for classifying the severity of hallux rigidus takes into account passive mobility, pain, and radiographic assessment to assign a stage from 0 to 4. This classification of Coughlin and Shurnas has been reported to be reliable in predicting the outcome of surgical treatment. [1]

Despite the fact that the metatarsus primus elevatus (dorsal positioning of the first metatarsal on a lateral X-ray in load of the foot) has been incriminated as a primary cause in the pathogenesis of hallux rigidus, this was not proved. No association was identified between hallux rigidus and primus elevatus, hypermobility of the first ray, the first long metatarsal and stiffness of the Achilles tendons, abnormal posture of the foot, symptomatic hallux valgus, shoes or professional occupation. It is more frequently associated with hallux valgus interphalangeal, bilateral involvement in patients with family history, unilateral involvement in patients with traumatic history, female gender. [2]

McMaster reported that the most common finding in seven patients with hallux rigidus was cartilaginous cleavage at the head of the first metatarsal without subchondral bone detachment and that the earliest radiological change was a small depression in the dome of the first metatarsal head. The cleavage is always between the top of the dome and the dorsal edge of the articular surface. McMaster believes that the exquisite pain and dorsal range of motion limitation can be explained by the osteophytic lesion on the dorsal metatarsal head. When hallux is in extension, the arrival of the first phalanx against the metatarsal cleavage site produces pain and reflex flexion of the joint, limiting extension. Then the disease progresses, an osteophyte at the level of the dorsal articular margin of the head of the first metatarsal produces a stop during great toe extension. This is the initial image of the patient: osteoarthritic changes in or around the metatarsophalangeal joint of the hallux. Often the first metatarsal is in the dorsal forcing position, decreasing its angle of plantar inclination on the profile X-rays (primus elevatus). This elevation of the first metatarsal is rather secondary to metatarsophalangeal osteoarthritis than the cause of this one. [1,2,3]

Clinical

The main reason for consultation is the progressive onset of pain and the limitation of the articular amplitudes of the hallux which is either fixed in plantar flexion or limited in dorsiflexion due to osteophytes present around the head of the first metatarsal, especially on the dorsal surface. [1]

This is not the case on the plantar surface where there is a normal mobility in flexion. The severity of the degenerative changes is directly dependent on the duration of the symptomatology. [2]

Muscular cramps in the extensor hallucis long tendon without mechanical conflict can be found early in the course of the degenerative process. Gradually the hallux becomes fixed in plantar flexion and the patient is forced to walk on the outer face of the foot. [2]

Unilateral involvement is the most common despite Nilson who postpones bilateral involvement in 60% of his cases. The aesthetic factor (the intolerance of wearing shoes in the heel) gives a preponderance to the female sex. Although Smith reported small series of older patients suffering from hallux rigidus, in the literature the age of the first symptoms were adolescence. [2]

Radiological characteristics

The flattening of the head of the first metatarsal, the joint narrowing, the presence of osteophytes of the dorsal, medial and lateral faces of the metatarsal head and the base of the phalanx, subchondral sclerosis and the formation of cysts at the level of the head of the first metatarsal are common elements in the elderly population. Sometimes the proximal phalanx is held in plantar flexion due to posterior conflict, and stress images can confirm that the osteophyte block prevents extension of the metatarsophalangeal joint. An osteochondral lesion is occasionally observed in the metatarsal head in adolescents according to Goodfellow and McMaster. [1,2]

Conservative treatment

For most patients, surgical correction is necessary to relieve pain and improve function; however, Yee and Lau, in a review of the hallux rigidus literature, have determined that non-operative measures such as foot orthotics, shoe modifications, and corticosteroid and hyaluronic acid infiltrations prior to surgery should be considered. [2]

Surgical treatment

Despite several recommended surgical procedures for the treatment of hallux rigidus, (including the dorsal subtraction osteotomy of the base of the first phalanx or the distal portion of the first metatarsal, the Keller operation or metatarsophalangeal arthrodesis) no procedure has proved its superiority. The indication for the metatarsophalangeal arthrodesis is the articular narrowing objectivized not only on the frontal and lateral incidences but also on the oblique incidence which shows that the remaining articular space is absent on the frontal and lateral incidences. Modern fixation techniques using compression screws or dorsal plates result in high melting rates (between 94% and 98%) and acceptable clinical outcomes for patients.

Gait studies showed significant improvements in propulsive force, foot load, and walking stability after arthrodesis for hallux rigidus. [2]

The justification for metatarsophalangeal debridement is the pain relief due to mechanical conflict suppression between the dorsal osteophyte of the first metatarsal and the base of the phalanx and the boot. The debridement is performed by resection of the osteophyte and other bone growths on each side of the joint with the aim of a dorsal flexion of the hallux of at least 70°. The success rate varies between 56% and 92%, with better results for stage 1 and 2 and for patients over 60 years of age. The gait analysis showed that there is an overload in the lateral part of the head of the first metatarsal after joint debridement and that these changes can lead to future joint degeneration. [1,2]

The modified Keller procedure (arthroplasty-resection), in which the short extensor hallux tendon and the capsule were used as interposition materials, has been described for the treatment of severe hallux rigidus, but the results vary.[2]

Interposition arthroplasty was also modified by oblique resection of the proximal phalanx. [2]

Silicone-rubber interposition arthroplasty was a short term solution because of silicone synovitis. The hemiarthroplasty of the proximal phalanx with a metallic component was also described, with good results, but there are no long-term studies to evaluate this technique. A study of 23 hemiarthroplasties reported that 91% of patients were satisfied with the results at 5.7 years and that the scores of the American Society of Foot and Ankle were significantly improved. [1,2]

Arthroscopic treatment

The first author to describe the arthroscopy of the hallux was Watanabe in 1972. The procedure gained clinical importance in the 1990s when several authors described the technique. The advantage of arthroscopy at this level is to have a fast recovery and professional reintegration, reduction of postoperative pain, less scar problems and a mobility and strength of the metatarsophalangeal satisfactory. [3,4,5]

The working hypothesis: Arthroscopic treatment improves mobility and decreases pain compared to isolated percutaneous debridement under image intensifier.

MATERIAL AND METHOD

The study is retrospective.

The inclusion criteria was the hallux rigidus stages 1 and 2, without limitation of the walking perimeter.

The exclusion criteria was the diabetic foot and the neurological foot.

Results evaluation was simple based on subjective pain (0-10 where 0 signifies absence of pain and 10 signifies insupportable pain) and local range of motion (hallux extension).

Between November 2015 and March 2016 at Centre Hospitalier Colmar and Guebwiller (France) 11 patients, 6 women and 5 men were undergo surgical treatment of hallux rigidus. Seven patients underwent arthroscopy debridement (group 2) and 4 patients underwent isolated percutaneous debridement (group 1), among them 1 patient had to have a bilateral hallux rigidus surgery at 5 months interval. Patients were evaluated at 3 months postoperatively.

The main symptom was the pain of the hallux when walking, and the additional assessment includes standard loading X-ray of the front foot, anterior-posterior, lateral-lateral and three quarters (figure 1).

Figure 1: Hallux rigidus, stage 2, presence of the dorsal osteophyte on the lateral and three quarters X-Ray

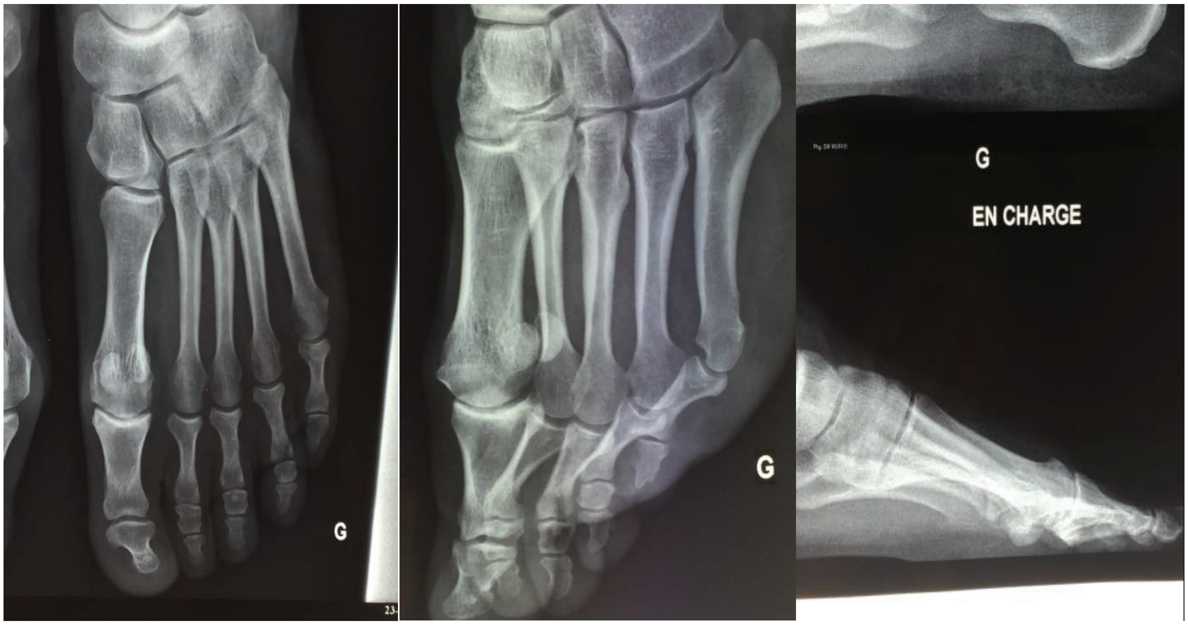

Percutaneous articular debridement was accomplished using a specific Shannon TM drill 2.0x8 millimeters under image intensifier within regional anesthesia, no tourniquet needed.

Metatarsal-phalangeal hallux arthroscopy was performed under regional anesthesia (popliteal block) with a calf tourniquet in all patients.

The patient is placed supine, heel in the vacuum, with a pad under the ipsilateral buttock to ensure a strictly vertical position of the foot. The surgeon is positioned at the end of the table, scrub nurse left of the surgeon (left-handed surgeon), arthroscopy column next to the patient's head (figure 2).

Hallux flexion-extension movements helps identifying metatarsophalangeal space. A needle is placed in the joint, laterally or medially to the long extensor tendon of the great toe, image intensifier serves in checking the correct intraarticular positioning. To avoid dorsal sensory branches damage the incision is placed slightly plantar affecting the skin only, while a Kelly or Halsted forceps is used for deep dissection. To prevent cartilage damage a small cannula is introduced. A 2.4 mm scope is used and placed preferably postero-medial while the instruments are placed postero-lateral. In doubt X-Ray checking is desirable to confirm the correct intra-articular instruments positioning.

Articular distraction gap is performed by a “Japanese fingerstall [3]” or by pulling out the hallux with a Velpeau band (figure 3). By pulling the toe with the hand and placing the probe within the joint, the anatomical structures can be explored during flexion-extension movements (sesamoid and head of the first metatarsal) (figure 4).

Figure 2: Patient positioning, the hardware and the operating room display. Artisanal traction by Velpeau band.

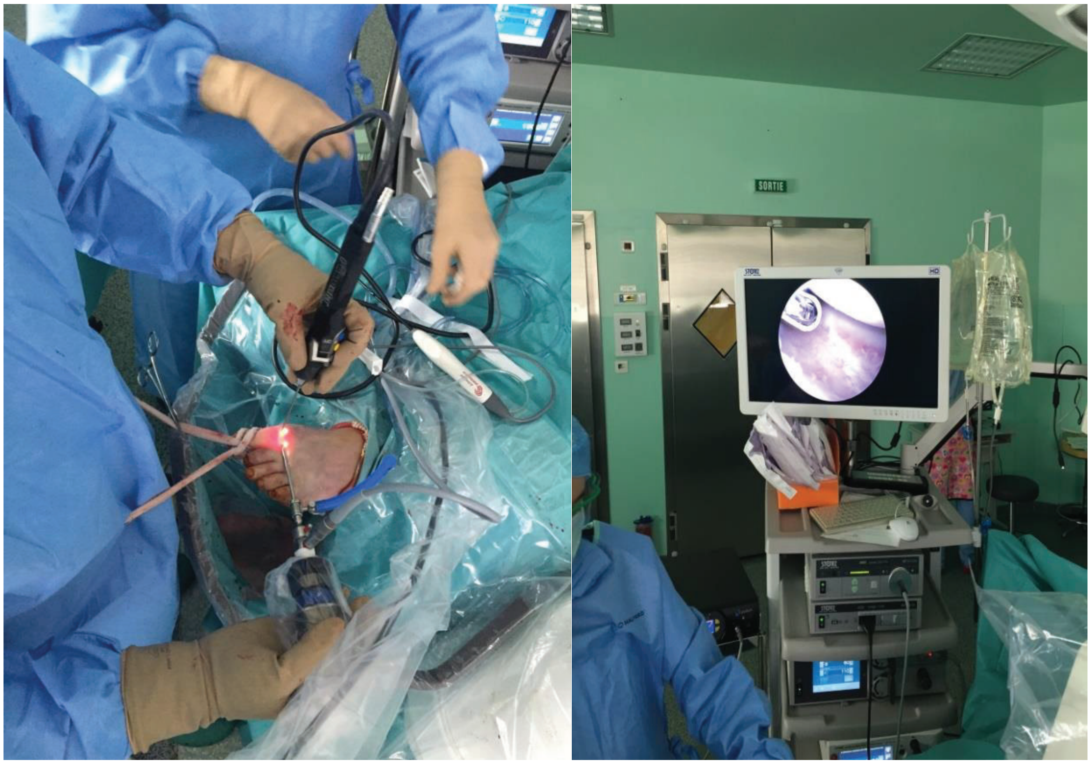

Often the posterior synovium and the lateral and medial gutters must be cleaned along using a Shaver TM to obtain a good visualization of the joint, paying attention to the extensor tendon of the hallux (figure 5). [3] [4] [5]

Figure 3: Hallux arthroscopic approach

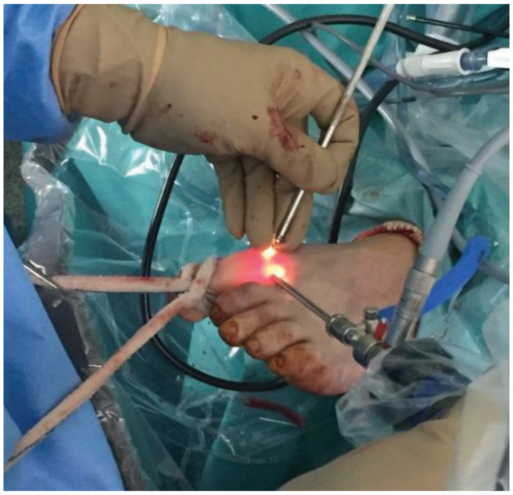

In first and second stages, a cartilage lesion is usually found centrally or dorsally on the first phalanx or on the dorsal metatarsal head, while the subchondral bone remains intact (Figure 6).

Figure 4: The medial sesamoid

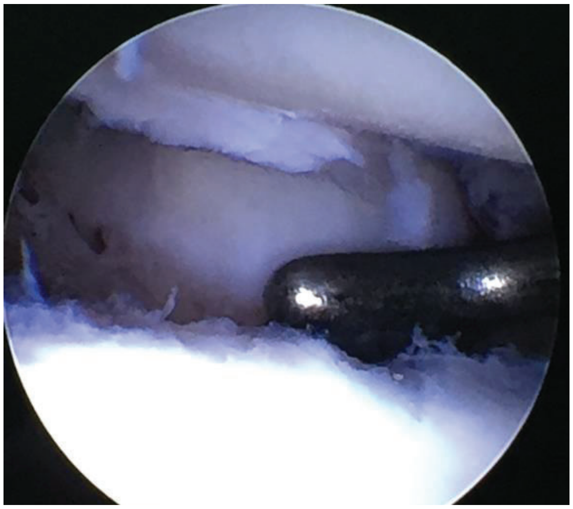

Cartilaginous valves are regularized with a curette and the debridement and osteophytes resection continues with the shaver or radiofrequency probe. If the subchondral bone is exposed, micro fractures by a 15 millimeters diameter Kirschner wire are produced to stimulate neo cartilage induction.

Figure 5: Hallux extensor tendon

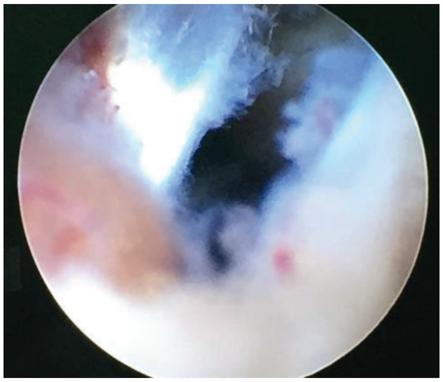

Figure 6: Cartilaginous valve and head of the metatarsus

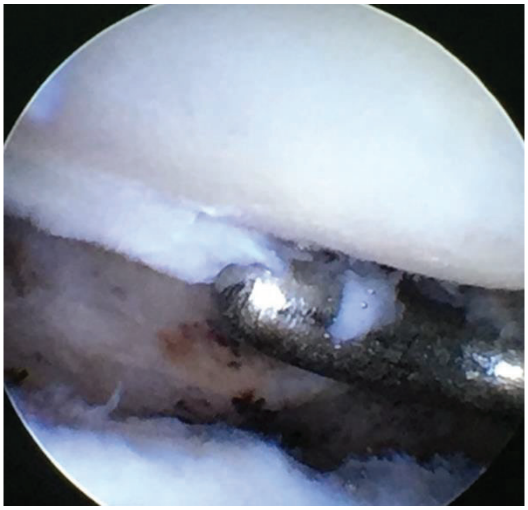

The pre- and postoperative dorsiflexion are measured by laterally X-Ray checks, allowing us to note in the operative report the mobility gain (figure 7).

Figure 7: Left image: 45° of dorsal flexion on preoperative X-Ray; right image: 80° dorsal flexion of the great toe on postoperative x-ray

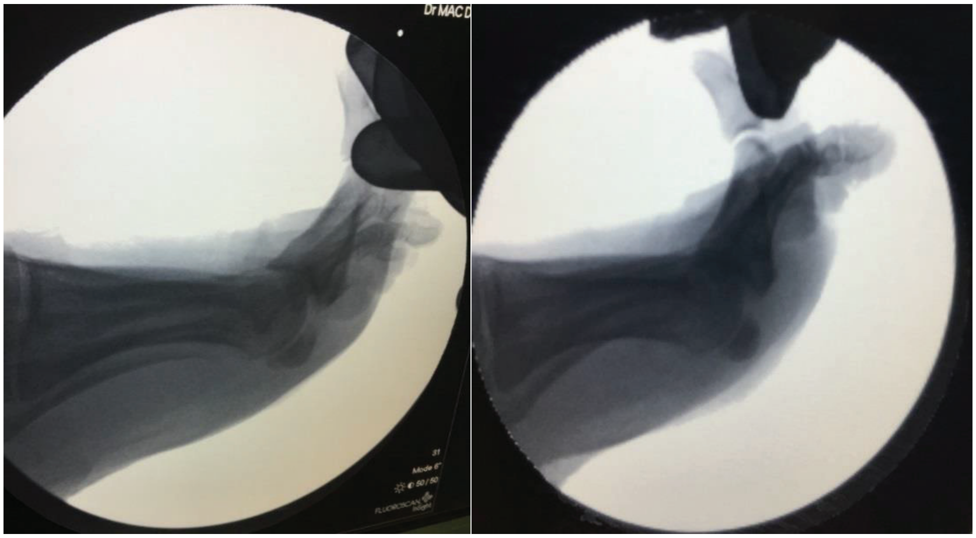

At the end of the procedure, the release of the tourniquet enables bleeding control, the skin is stitched with resorbable sutures, and a compressive dressing is realized.

Postoperatively walking without crutches is allowed the next day and the wearing of a medical soft padded shoe is recommended for the next two weeks.

In all cases, patients needed one day hospital stay.

RESULTS AND DISCUSSION

The average age for group 1 was 53.2 years and 56.8 years for group 2.

The preoperative mobility for group 1 was 26° of extension and 17.5° of flexion on average; for group 2 the hallux extension was at 47° for 15° flexion. At the end of the procedure, the dorsiflexion obtained was at least 80°. The average duration of the percutaneous and arthroscopic procedure was 64 minutes, no adverse events were reported.

Operative follow-up was simple with minimal residual pain (VAS = 1) for both groups and one return to work at 3 months with one exception, 1 patient who had 4 months off work due to persistent pain (VAS = 4).

At 3 months of the operation the group 1 had 77° extension of the hallux for 8° of flexion while for the group 2 one notes 85° of extension for 15° of flexion. In terms of increased mobility at 3 months, arthroscopy has not yet shown superiority over isolated percutaneous articular pruning, but in terms of subjective satisfaction, group 2 reported better results in terms of pain. Only one patient underwent a bilateral pruning, and he declared himself more satisfied on the side operated by arthroscopy plus percutaneous pruning than by the side operated by isolated percutaneous pruning.

Our working hypothesis was therefore invalidated.

Weaknesses of the study: retrospective study with a small series of patients, no long-term results.

The literature is poor in this field, no comparison was made.

CONCLUSION

The arthroscopy of the hallux makes it possible to achieve an accurate joint assessment and a suitable treatment. In our very short experience and to confirm the arthroscopic debridement and the synovectomy complementary to the percutaneous pruning improves the subjective score of the treatment of the early hallux rigidus, but it remains to define the result and in particular the gain of mobility at a distance and at to quantify the progression towards osteo-phalangeal osteoarthritic degeneration.

Abreviations

VAS: Visual Analog Scale

Acknowledgements

There are no conflict of interest.

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VARIA

The role of the communication in changing health behaviors

Dodu G. Petrescu[1], Raluca Răducu[1], Cristina Soare[1], Victor L. Purcărea[1]

Abstract: *The communication in the field of health can be defined as a communication strategy used to inform and influence individual and community decisions with the aim of improving and enriching personal and community behaviors and public health practices. It includes studying public health attitudes and behaviours to determine the type, frequency and format of the messages.*

Keywords: *attitudes, behavior, change, communication, health*

Social communication and behavioral change are at the heart of any successful public health initiative. The purpose of communication is to make someone to think in a certain way, to do something or to act, in other words is the exchange of emotions, ideas and opinions in the form of information between sender and receiver [1]. It is the most important aspect of the educational process aimed at changing behaviour and attitude and improving the level of knowledge [2].

The communication has several functions [3]:
- instrumental function – to achieve something;
- information function – to find out or explain something;
- social contact function – to make a pleasant environment;
- control function – to cause someone to behave in a certain way;
- expression function – to express feelings or to present themselves in a certain way;
- role-related function – for the given situation;
- educational function – to convey knowledge;
- stimulation function– to bring interest;
- entertainment function – to provide leisure activity;
- cultural promotion function – to strengthen cultural rites.

According to John Fiske-1982, [4] there are two complementary schools with different approaches, in the study of the communication, the school "process" (communication as a transmission of messages; communication analysis models: Lasswell - 1948, Shannon and Weaver - 1949, Newcomb - 1953, Gerbner - 1956, Westley and MacLean - 1957, Jakobson - 1960, etc.) and the semiotian school (communication as production and exchange of meanings; founders and contributors: Charles S. Peirce and Ferdinand de Saussure , as well as C.K. Ogden and I.A. Richards, Louis Trolle Hjelmslev, Roland Barthes, Pierre Gouriaud); There are many schemes of the communication process, but the most complete belongs to Philip Kotler, who managed to integrate all the elements of the process, as can be seen in the Figure 1.

The Healthy People 2010 Report defines the communication in public health as "the art and technique of informing, influencing and motivating individuals, institutions, general public on the important public health issues [6].

The term health communications was first used in 1961, when the U.S. National Health Council held a National Health Forum to discuss the challenges facing health intelligence communications [7]. The term was used again in 1962, when the surgeon Luther Terry organized a conference on health

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communications to discuss how different communication techniques can be used to make health information available to the public [8]. The term was adopted by members of an interest group at the International Communication Association (ICA) in 1975 [9].

Figure 1: Kotler's model.

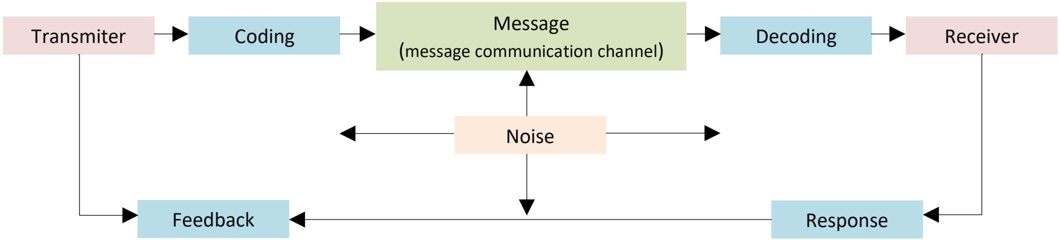

Source: Adaptation by Kotler, Ph., Principles of Marketing, III. Developing the Marketing Mix. 13. Integrated Marketing Communication Strategy, Pearson, Canada, pag. 546, accesibil online la: http://www.pearsoned.ca/highered/divisions/virtual_tours/kotler/kotler_ch13.pdf [5]

Health communication is a hybrid field that derives from communication, marketing, journalism and public relations studies and overlaps with health education and health promotion [10].

The health education is used to make people aware of the patterns of healthy behavior and its importance. The health education programmes related to behavioral change are considered to be a pillar of medical activity [11].

The health education is a social science that relies on medical, biological, physical, environmental and psychological sciences to prevent the occurrence of the diseases and promote health through behavior-changing activities based on the education.

Health campaigns for the prevention of the communicable diseases, for the promotion of maternal and child health, for the promotion of the immunisation, for education about family planning methods and other preventive health services have a long history [12].

The health education is linked to changes in people's feelings, knowledge and behaviour. It is the process carried out by actively involving people who aim to initiate healthy behavior and to know the prejudices and practices of people who are discouraged towards health in order to achieve the health goal [13].

The health communication is an extensive area that includes health education, risk communication, health and policy promotion, focus communication, patient and supplier communication and health literacy. Health communication strategies enable to the doctors and nurses, community health centres, hospitals and hospital administrators, nursing homes, health educators, non-profit organisations, government agencies and others to disseminate information that can positively influence personal health behaviors and choices.

Using informed marketing tactics and sociological data to lead to decision-making, the health communication is used to promote health campaigns in order to influence and educate the public on health issues [14].

The success of the health communication or health education programmes depends on people's interest. It is important for them to be actively involved, the change cannot be imposed, people must be motivated to adopt healthy behavior [15].

Communication related to the behavior change can take different forms, to appeal at the individual or group level, in order to change behavior towards a specific health problem. Health communication includes interpersonal communications, such as individual meetings between a healthcare provider and the patient [16], as well as community-focused communications such as public meetings, advertisements in local newspapers, the distribution of brochures and leaflets or educational events.

The media plays a key role in health communication, serving as a channel of health information from the government and specialized health organizations to the public. With their extensive coverage through radio and television commercials, the media can persuade the general public to adopt new healthy behaviors and recall information on preventive health care, disease prevention and treatment, immunizations and more.

The Internet is now disseminating information on an unprecedented scale, but at the same time, false and unsafe information has become more common than ever, posing risks to the health of uninformed users. This situation can cause an unjustified panic among those who receive the messages and can be a problem as technology continues to move forward. Therefore, public health providers must communicate the risks to the public in a responsible and transparent way and identify and eliminate misleading notions [17].

The decision to choose a particular communication channel must be based first on the research of the target audience and then on its usefulness and coverage. Thus, some technologies are not particularly effective when used by a small number of people or are too complicated to be operated by ordinary people.

Health communication issues have become a major topic of large-scale research programs sponsored by various foundations, health care corporations and government agencies.

Communication to change behavior is based on research and involves interaction with individuals and communities and personalizes messages that meet the needs of a particular target audience. Messages are then disseminated through different communication channels to bring about behavioral changes in specific problems and achieve the desired positive results.

The message must have an impact and it should disseminate appropriate health content defined by [6] :

- accuracy (the content is valid and without errors of interpretation or judgment);

- availability (the content must be placed where the public can access it);

- balance (to present both the benefits and risks of potential actions);

- consistency (remains consistent over time and it is consistent with information from another source);

- cultural competence (it must take into account ethnic, educational, linguistic issues); - relevant scientific evidence;

- coverage (to be available to the largest possible number of people in the target population);

- reliability (the source of the content is credible and the content is up-to-date);

- repetition (the delivery of the message must be continuous and repeated over time).

The development and management of social and behavioral change programmes involves the building of the partnerships at the national and local level, using new technologies and techniques of social and behavioral change and the incorporating of the social and behavioural change activities into programming the provision of services.

The utilisation of the new communication technologies for processing and dissemination of the health information has given rise to a new field, called health informatics. As information technologies advance, there are a variety of the new tools and information media for disseminating and accessing health information.

The access to relevant and in useful time at health information can help consumers participate in decision-making and encourage cooperation and collaboration between providers and consumers.

The health communication can help solve ways in which consumers and providers can most profitably inform themselves about relevant health problems, identify the best ways to develop and present high-quality health information to the target audience, and encourage decision-making in modern efforts to adopt healthy behaviors.

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The safety of genetic reserves in bioterrorism from the passive defense perspective

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Abstract: *Several studies have shown that some countries are among the main users of structural, molecular and physiologic information of human, animal, plant, and microorganisms cells (genetic and biological reserves) against living of organisms, especially humans. This method used as a mechanism for removing their rivals and opponents to achieve their goals. This descriptive-analytical study was designed to define the concept of bioterrorism, passive defense, and genetic resources and its historical development process, with emphasis on efforts to control these types of threats over time. In the present review, a wide range of literature through the search on Google Scholar, PubMed, Medline, and Scopus databases, extracted without regard to time constraints and focusing on the keywords listed above with three valid criteria and new sources of resources. The explanation of the material was based on the data obtained from the review of the sources and based on the inferences of the researchers from them. Given the importance of genetic information and the lack of clear prospective research in this area, the confidentiality of genetic data has been particularly respected among researchers. Eliminating data security can lead to unplanned advertising and costs. Therefore, the existence of laws and protective measures against coping with the misuse of enemies from genetic information is more than other country's prior demand.*

Keywords: *bioterrorism, passive defense, genetic resource, threat*

INTRODUCTION

Today, the hostile use of science and technology has led astray in the field of medical and human health that has led to the emergence of biological weapons for the purposes of biological assassinations [1]. The definition of bioterrorism is the misuse of microbial, viral, parasitic agents or their products for the purpose of intimidating or killing humans and destroying livestock or plants [2]. According to the International Police Bioterrorism definition, it is the release of biological or toxic agents with the intent to kill or harm humans, animals and plants with a prior intent to terrorize, threaten, or coerce a state or group of people. Acting or fulfilling political or social demands [3]. But with the advances in basic science and medicine, especially in the field of molecular biology, genetic engineering, biotechnology, synthetic biology, bioterrorism has recently taken on a new meaning [4]. In the meantime, we should not overlook the widespread developments of the biological sciences in the past few decades and the widespread scientific ability to manipulate and recombine genes and identify molecular pathogens to increase the virulence of deadly toxins [5]. The possibility of increasing drug resistance and antibiotic resistance and increasing the epidemic of disease has led hostile military researchers to research, procure, and produce hazardous agents in

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contravention of international laws and conventions prohibiting the development of biological weapons. Of course, they do so with justifications such as trying to make a vaccine or knowing the causes and pathogens or gathering scientific information [6]. On the other hand, genetic resources or hereditary flora and fauna are one of the most valuable national wealth and basic resources of any country. Considering that some countries are a rich country due to fossil fuel oil and gas reserves, the abundance of unique plant and animal genetic resources is also a major and strategic asset of countries and unfortunately has been neglected [7]. The economic function of genetic reserves is greater than that of other reserves in the countries, and these valuable reserves should be exploited. The genetic resources available in the countries are very rich and we can replace them with other resources and if we do not use them we will lose them in many ways. Even today in the world it is believed that genetic reserves in compared of gold and oil reserves are also more valuable and strategic [8]. Genetic reserve information is therefore the most important strategic part of a country that may be exploited if recently based on research conducted in the Netherlands and Japan, a new and modified strain of influenza virus has been studied for its respiratory transmission studies in many reflective laboratory animals [9]. And raised concerns among the scientific and security community about the potential for exploiting it in the production of dangerous biological agents. Finally, after long discussions and agreeing to publish it, the relevant researchers agreed that part of the technical knowledge and techniques of gene manipulation and production factors remove from this article [10, 11].

METHODS

This descriptive-analytical study explores the concept of bioterrorism, non-performing defense and its genetic reserves and its historical developmental process, with emphasis on efforts to control these threats over time. In this review study, a wide range of sources were extracted by searching Google Scholar, PubMed, Medline, and Scopus databases without considering time constraints and focusing on the keywords listed with three valid criteria and the newness of the sources. Explanation was based on the data obtained from the literature review and based on the researchers' inferences.

THEORETICAL FOUNDATIONS

Human Genetics Association, an organization called the Genetic Bank, announced that it would collect human genetic samples and data and store them for future scientific research [12]. Genetic specimens obtained from people with diseases such as cancer, cardiovascular disease, genetic diseases, diabetes, neurological disorders, etc. are a rich source for future research [13]. A collection of samples and genetic information related to clinical trials provides very good opportunities for analyzing genetic factors and helping to respond to treatment. Genetic information includes information on genes and genetic characteristics [4].

What are the goals of Genetic Banks?

Genetic banks generally pursue two basic goals:

✓ Storage and storage of biological substances such as blood, bone marrow, etc. for future therapeutic purposes

✓ Storing and keeping samples and genetic information of different individuals to identify disease-causing genes [14].

Uses of biological and genetic samples:
1. Diagnosis of genetic diseases and clinical care.
2. Genetic epidemiological studies.
3. Genetic mapping.
4. Applied Studies.
5. Development of diagnostic and therapeutic goals.
6. Drug design and pharmacogenomics.
7. Gene Therapy.

Genetic banks depend on the following organizations for various applications, including:
1. Forensic Medicine Organization;
2. Army;
3. Universities and study centers;
4. Business Organizations [5].

Biodiversity

One of the major threats in the discussion of threats is BioDiversity. The discipline is currently one of the safest biology disciplines in the world, and it is witnessed that there is no student admission to the field from third world countries. However, most of the funding in biology is devoted to this sector. In fact, the diversity within and between organisms in a region is considered to be the biodiversity of that region; the high biodiversity means the high diversity of genes that increases production, enhances product quality, and increases pest resistance [15]. This biodiversity has evolved over very long periods of time, and any human manipulation of it will alter and destroy the biological cycles of the creatures in an ecosystem that will have irreversible effects. For example, paddy fields in the north of the country have been destroyed by the introduction of fossils of Azolla by a Japanese researcher from Japan (16). Azola ferns in Japan make the rice unnecessary for nitrate fertilizer and multiply its production efficiency, but there is an insect that controls the population by eating ferns. The inability to prevent the growth of this fern in the northern fields and in fact the biomass of that region is different from the northern region and the lack of attention to the biodiversity of each region has had a major impact on domestic production [17]. Biodiversity is important in several respects, which is limited two resean:

First, high biodiversity leads to higher production. Today, the issue of food security and production is one of the most important issues in the world. The annual income of one Chinese medicinal plant, JinSingh, is about half of some countries annual oil sales. This high biodiversity can lead to more diverse crops as well as increased production [18].

Secondly, biodiversity is considered a source of human knowledge today. Biotechnology and future work on genes with insufficient genome resources, such as having proper knowledge of oil, but lacking oil storage [19]. India is the largest country to make huge profits from the blood trade. However, due to the lack of proper laws in some country, many plant, animal and microbial species go abroad for sequencing [20].

EFFECTIVENESS OF GENETIC RESOURCE CENTERS ACTIVITIES IN THE ECONOMIC AREA

Providing food security

Food security is a very complex issue, not only involving the production and processing of food products but also its accessibility for people to maintain a healthy and active life. Diversity is the basis of food security and today's production supplier and the prerequisite for ensuring tomorrow's production in the face of the rapidly changing world [21]. Achieving food security and ending hunger is one of the great challenges facing the world in the coming centuries. Population growth, resource depletion, and habitat degradation add to the deterioration [22]. The greater diversity of genetic resources in gene banks and their availability through an effective protection system helps ensure food security at lower costs. This will help provide the genetic feedstock for breeding activities to produce diverse and nutritious foods and increase access for poorer populations to healthier, more nutritious foods to combat malnutrition [23].

Climate Change Adaptation

Climate change will put unprecedented pressure on our ability to produce food, and these effects will be especially severe in developing countries. Research has shown that the trend of rising temperatures will continue in the next few centuries and the conditions for agriculture will be very different from the current situation, therefore, adaptation of agriculture to the conditions ahead is of particular importance and the need for a new variety that can with stand these challenges has been strongly considered. It is essential not only to reduce hunger but also to strengthen world food security in the medium and long term [24].

Biodiversity Conservation

Biodiversity loss is one of the most important environmental concerns today. According to some reports, as the current trend continues, half of the plant species will be at risk of extinction. Among the endangered species are wild ancestors of crops, which carry valuable traits that can be transferred to crops [25]. It is estimated that six percent of wild cereal ancestors (wheat, maize, rice, sorghum, etc), 18% of legume species (wild ancestors of beans, chickpeas and lentils) and 13 percent of vegetable and safflower species, including potatoes, tomatoes, eggplants and peppers, are at risk. Clever use of genetic diversity in breeding activities can be significantly effective in protecting the environment animal varieties that are susceptible to disease can reduce the need to use harmful drugs and increase production efficiency[26]. More varieties of plants with more branching ability can compete better with weeds and reduce the amount of herbicide needed. Drought-tolerant plants help conserve less water, varieties with deeper root systems can contribute to greater soil stability and stability and varieties with lower soil nutrient intake reduce fertilizer requirement [27].

Improving nutrition adequacy

Genetic diversity not only guarantees stability and stability in providing adequate amounts of feed (protein and energy), but also plays a key role in ensuring its quality. Diversity, which is a direct product of diversity in crops, nutritionists say it is optimal and the presence of vital nutrients (vitamins and minerals) can be enhanced by choosing the right genetic diversity [28]. New varieties can, through breeding, have higher nutritional quality in terms of vitamins, iron and other essential nutrients and have higher protein quality and less antifouling or toxic substances [29].

Poverty Reduction

Genetic diversity is one of the most powerful weapons to combat hunger and poverty. Agriculture is an economic infrastructure in many countries, and is a potential source of economic growth for developing countries agricultural growth is more beneficial for economic development and poorer and by providing affordable food, This benefits more than 70 percent of the world's poor, living in rural areas, And those people whose agriculture is their main livelihood [30].

Agricultural insurance can play a key role that requires a range of reforms, including the cultivation of higher value crops, the promotion of value-added activities, For example, improved product processing, expanding market access and lowering prices through increased production, higher processing and marketing efficiency[31]. The basis of all these possible solutions is genetic diversity, a variety that enables the farmer and breeder to produce higher yield varieties and higher quality. They can produce varieties that are tailored to specific processing methods and more portable and maintainable [32]. They can also produce varieties that are resistant to pests and diseases and environmental stresses, which protect the crop from damage and reduce the risk. The role of agriculture in combating poverty is very complex, but it cannot reach its potential without the proper exploitation of genetic diversity [33].

Sustainable Agriculture Guarantee

Harnessing the genetic diversity in plant and animal resources is an important strategy through which farmers can develop their farming systems with the least environmental impacts. The global off-site conservation system is a key component of the effort to conserve these resources and make them available to farmers in all countries [34]. In the 21st century, agriculture faces many challenges, as it needs to provide more food to meet the needs of a growing population living mainly in urban areas, while dependent on the shrinking rural labor force [35].

In addition, climate change is a threat that could increase the number of hungry people in the future and pose more complex agricultural challenges. While the effects of climate change are slowly becoming apparent, the future impacts will be significant if not taken properly [36]. Increased exploitation of plant genetic diversity is essential to address these threats and future challenges. Therefore, despite such strategic effects on genetic resources, the protection and security of such centers require special attention to prevent enemy misuse [9].

RECENT ADVANCES IN HUMAN GENETIC STUDIES

Human genetic studies have provided cognitive insights and mental disorders are the genetic basis of several inherited disorders, such as Down syndrome and Tay-Sachs disease, and other associations such as the role of APOE in Alzheimer's disease are still extensively studied [37]. However, despite advances in understanding the human genome, there are concerns about the privacy of genetic data and possible discrimination resulting from its disclosure, and there is incomplete monitoring of genetic testing [38].

NEW SCIENCE AND TECHNOLOGY AND NEW BIOLOGICAL FACTORS

Non-lethal biological agents

The some Department of Defense is already increasingly turning to non-lethal weapons (NLWs), these weapons designed specifically, the amount of deadly and serious damage to the low end and damage to the city, property and the environment and they don’t harm the environment some countries, of course, have been using crippling weapons for a long time. But these non-lethal weapons of biological origin can have different effects [39].

Non-microbial biomaterials in cells and organs of various animals (plants and animals) and humans that do not fall into the category of microorganisms and biological toxins, but despite their properties transmitted through an aerosol or through Respiratory and mucosal tissues absorbed If used in wars or bioterrorist operations, they can cause clinical signs and symptoms in humans, or cause economic damage to a country's strategic plants and animals [40].

These include a variety of brain regulators (Bio regulators) and some gastroenteritis-producing enterotoxins, as well as a variety of substances that affect the human senses, such as non-lethal bioweapons, calmative and malodorants Agents, etc. to be [41]. Other non-lethal biological agents can affect various materials and military and civilian equipment and cause them to corrupt, destroy or decompose in addition to causing economic damage to the country but also disrupting the political affairs and activities of the military. And becomes civilian the existence of oncogenes (carcinogenic genes) is no exception [42].

Recombinant and Synthetic Agents

By identifying the genome of different microorganisms or identifying a part of one's genome structure and using genetic engineering techniques of microbes' genes to increase their pathogenicity such as drug resistance, pathogenic genes, carcinogenic genes, and altered escape structure. The new recombinant germs are made from the diagnosis, vaccine-resistant that has created a new, recombinant weapon with special features [43].

Ethnic race pathogen agents

The susceptibility or resistance of different human races to different diseases, the abundance of hereditary diseases in specific human races, as well as research to discover and identify infectious substances and agents that can cause a particular race are bioterrorist efforts. Recently disclosed documents indicate a widespread program in the former of some countries racist regime to provide specific infectious black agents that could be used against those of the predominantly population [8,9].

Interfering RNA iRNA and SiRNA or Oligo Antisense Technology

Interfering RNAs silence genes, and are used in medicine by inactivating genes that cause diseases including cancer, viral infections and other diseases. Gene silencing methods are highly needed because of their ability to treat human diseases [44]. What puts the repressor RNA above all other competitors is its proprietary function in eliminating only inappropriate genetic information and that other healthy genes are immune to its effects[45]. Identification and synthesis of molecules that inhibit gene activity. This small fragment of RNA binds to or suppresses gene activity by binding to its complement locus in the process of gene activity. The technology is widely used in medicine as a vaccine. Also, using nanotechnology can produce bio-particles that are designed to stop vital activities of humans or other organisms, which can range from minor damage include a serious injury [46].

Genetic Engineering

Genetic engineering of food, one of the branches of biotechnology, involves targeted alterations to the genome of plants or animals. Most foods that are consumed today are either genetically modified to be termed GMOs or contain components derived from genetic engineering technology [47]. Genetic engineering and plant manufacturing and production of plants resistant to pests, diseases and the need for the use of dangerous pesticides have made a change in agriculture that is comparable only to the Green Revolution. Genetic engineering may also be engineered based on bioterrorism goals, which in turn require monitoring [48].

THE ROLE OF NEW TECHNOLOGIES IN PASSIVE DEFENSE AND CRISIS MANAGEMENT OF NEW THREATS

In the anticipation and prevention phase, it is attempted to provide the necessary reinforcements by training, developing laws and regulations, strengthening and using safety and security equipment prior to the threat. New information technologies and new equipment provide great help to passive defense, future research as well as disaster preparedness [49]. Production of detectors of biological, chemical and nuclear agents as well as installing biosensors in different parts of the country, the system will send the system information on the biological risk factors online to the relevant organization the equipment is built using modern technologies used in modern warfare and equipped with online information networks [50].

New achievements and development of various programs for the prevention and control of bioterrorism factors have been made, including:

- The existence of new vaccines preventing biological agents
- Antioxidants effective against toxicological biological agents
- The presence of potent antibiotics as drug prophylaxis in preparation,
- Developing a national, provincial, municipal and health care crisis response plan
- Provide vaccine equipment, medicines and other items and prepare for delivery
- Training as the most important element of preparation
- Using virtual training systems to train all forces
- Utilizing the ability of information networks to coordinate and execute maneuvers at the confrontation stage, new technologies play an important role in the control and crisis management of modern war events such as:
- New systems of rapid alert the threat of a new war quickly alerts crisis management and non-operational defense centers.
- The use of new technologies and new materials for disinfection that eliminates a large number of casualties, equipment and facilities without any adverse effects.
- Modern information systems are rapidly bringing forces and people into crisis.
- Crisis management operates much faster due to new information technologies.
- Use modern systems of field hospitals that can be installed in the shortest possible time and have the necessary facilities to help and treat modern war casualties.
- New medicines for treatment of modern warfare agents and new therapies
- The use of mobile laboratories and mobile vehicles to quickly detect and engage in modern warfare at the scene
- New management and information systems at the time of confrontation
- New systems for transporting the injured to health centers
- New clothes and equipment to protect the health care staff for action without contamination
- Reconstruction At this stage, given the facilities and equipment of the previous three stages, it is possible to return to pre-crisis mode in less time.
- Returning to the pre-crisis phase and control if the crisis has been well managed in the previous three phases [3].

Currently, some governments, following the approach and uses of genetic information, have enacted laws protecting this information, requiring that they establish a special protective location for genetic information to define this information. And to differentiate it from other forms of information, in particular, personal and health and medical information [10].

DISCUSSION

Well-known biological and bioterrorist agents, such as smallpox, plague, anthrax viruses, etc. are naturally pathogenic, but modern biotechnology and genetic engineering have enabled genetic modifications to control all pathogenic features of an organism that the organism is genetically manipulated is fully proportionate to its purpose[51, 52]. This technology of genetic manipulation can be exploited by the enemy if the human, animal, plant and even genome of a bacterium are identified. In general, recent biological capabilities in the field of weapons production are the result of advances such as the construction of engineered vectors, precise recognition, completion of the human genome project, and the drawing of human immunity, etc. [53]. Of course, new biological weapons production technologies provide the middle ground from very lethal to non-lethal weapons. And today, not only is the target of bio-weapons, but also the building of bio-weapons against the country's economic infrastructure to hit the country [54, 55].

So patients and researchers have long been concerned about the privacy of health information. In a study report participant's collected medical information during a study, participants preferred (92%) to request permission before using their health information for any purpose other than medical treatment and 83% want research details before being allowed to use their health records.

This study shows that some issues such as family medical history, genetic disorders, mental illnesses, drug or alcohol related events, lists of previous cases, and current medications are highly sensitive. There are also ethical complaints about the ability of people with cognitive impairment to make informed consent or addicts to participate in studies involving substance use disorders. Tests to identify addicts or predict the risk of addiction raise concerns about privacy attacks, third-party misuse of data, and on the other hand, using data correctly will increase the power of the courts to identify the perpetrators by forcing them to perform such tests [56, 57].

The confidentiality of genetic data has also been addressed by researchers. Undermining data security can lead to unwanted advertising and costs [58, 59]. Some researcher write that to ensure that privacy laws do not indirectly replace observational studies using medical records, thoughtful decision-making by research ethics boards on the need for mandatory consent is necessary [11]. Attitudes and practices vary widely across societies. In an interview with Canadian university administrators, 47% of sites require individual consent to continue their studies, but 45% do not require consent or do not recommend the notification process and fix them [12]. One of the major challenges in the field of genetic banking related to national laws and international research policies. Therefore, in matters such as bioethics, harmonization of medical information privacy laws and the outcome of individuals, identical terms and interpretations, and how to obtain participant consent, in countries with access to genetic knowledge and genetic information, legislators should be considered to be [60, 61].

Therefore, passive defense measures should be put in place to enforce by laws and regulations on genetic information as well as how genetic banks operate and prevent any potential misuse by the enemy.

On the other hand, information networks as a platform for data collection and creation of databases on biological, chemical and radioactive and nuclear events, epidemic collection and information systems and biological events such as Medysis and Health map and a variety of human and animal and plant disease information systems have made it possible for the country and its experts to monitor and prepare the data online [62]. But there is a gap in protected data-collection systems and genetic reserves in the country. In addition, conservation of genetic stocks should also include measures to prevent breeding and genetic diversity of the country.

CONCLUSION

The results of the present study showed that the confidentiality of genetic data has been considered by researchers in different countries, and with the advances of molecular and genetic sciences, the possibility of using this science as a new bioterrorism is more likely. As a result, defensive measures in the area of non-biodegradable defense in order to preserve the genetic reserves and undiagnosed races by enemies are increasingly common.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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The seasonal incidence of intracranial hemorrhages

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Abstract: *Objective: Cerebrovascular Accidents (CVAs) represent the second most prominent cause of death globally, surpassed only by ischemic heart diseases, thus constituting a significant economic burden for healthcare systems. Despite this, little is known about the correlation between the different times of the year and intracerebral hemorrhages. This review investigates said link, as well as seek to identify whether vascular risk factors (i.e. diabetes and high blood pressure) vary through seasons.*

Method: This paper undertakes the study of 97 patients, all of whom have suffered a hemorrhagic CVA and were hospitalized and treated within the Central Military Emergency University Hospital’s Department of Neurology in Bucharest, in the period between 29th of June, 2017 and 29th of July, 2019. The scope of this study is to optimize prophylaxis within the groups most susceptible to the imminent danger of an intracranial hemorrhage during a certain season.

Results: Our study has shown that the season with the highest values of morbidity and mortality is autumn, followed closely by spring.

Conclusion: Given the set of yielded data, which is mirrored in the case of risk factors, we conclude that the results vary slightly from those put forward in the literature. Therefore, whilst hemorrhaging CVAs have a consequential impact upon society, both through the disastrous effects of the incident per se, and its disabling nature in the long-term, a positive percentage of them could be avoided by changing one’s behavior during the high-risk periods.

Keywords: *intracranial hemorrhages, subarachnoid hemorrhage, subdural hematoma, hypertension, diabetes mellitus*

INTRODUCTION

Intracranial hemorrhage (ICH) is caused either by tearing apart several small penetrating vessels or as a result of some vascular anomalies. Risk factors such as high blood pressure (HBP) or diabetes mellitus (DM) are frequently associated with ICHs [1, 2]. While in developed countries, the incidence of this pathological condition has reduced due to better control of HBP, in developing countries, it has remained alarmingly constant for the past decades [3].

According to the WHO (World Health Organization), approximately 6 million deaths were attributed globally to ICH in 2016 [4]. In the context of its economic impact, in the US alone, in 2012, the total costs of stroke patients reached USD 71.5 billion, according to the AHA/ASA (American Heart

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Association/American Stroke Association). By 2030, these costs are estimated to reach USD 240 billion [5].

Intracerebral hemorrhages account for approximately 10 to 20% of all strokes [3]. The main cause of developing ICH is the alteration of the structure of the blood vessel caliber as a result of an untreated or inadequately treated chronic high blood pressure (HBP). Other incriminating causes include amyloid angiopathy and vascular malformations. It has been observed that the frequency of ICHs in developing countries is almost double that of developed countries, progressively growing with aging. Ergo, it has been determined that for the 35-54 age group, the incidence is of 6 cases for every 100,000 people, for the 54-74 age group, it increases to 37 for every 100,000 people, and, for the 75-94 age group, it peaks at 176 for every 100,000 people.

For young patients, the main cause of hemorrhages is represented by vascular malformations. In adults, this is represented by chronic HBP, whereas in the elderly, it is amyloid angiopathy. Moreover, it has been shown that, on average, regardless of age, the incidence of ICH is higher in men than in women [6].

Concerning chronic HBP, this leads, eventually, to hypertensive angiopathy which, in turn, causes degenerative modifications within the caliber of the small and medium penetrating vessels, modifications known as lipohyalinosis. Regarding chronic hypertension, it has been ascertained that degeneration takes place at the muscular layer, together with the fibrinoid necrosis of the subendothelial layer, accompanied by micro-aneurysms.

Amyloid angiopathy is characterized by deposits of β-amyloid peptide (βA) in the capillaries, arterioles, and, generally, in all small and medium vessels in the cerebral cortex, leptomeninges, and cerebellum [6]. Secondly, amyloid angiopathy is portrayed through deposits of intravascular βA in the leptomeningeal collaterals and small intracortical vessels. Although the mechanism of depositing and accumulating βA is unknown, its consequence is the degeneration of the blood vessel caliber through, more specifically, losing smooth muscle fibers, thinning the caliber, narrowing the lumen, and leading to aneurisms, the latter further giving birth to diffuse micro-hemorrhages [7].

Furthermore, apart from HBP, other causes include the dysfunction of self-regulating with excessive cerebral blood flow, arteriovenous malformations, ruptured aneurysms, cerebral angiopathy, hemorrhagic necrosis, cerebral venous thrombosis, and modified hemostasis.

The overflow of the resulting blood from the bursting of the caliber leads to a sudden increase of intracranial pressure, which brings about diffuse vasospasm, followed by the acute ischemia of the parenchyma – a mechanism which is frequently present in the potential vasospasm after subarachnoid hemorrhage [8, 9]. The BBB permeability heightens, thus aggravating the metabolic disorders of the brain, giving birth to the cerebral edema [10]. Despite the advancements in medicine, cerebral bleedings have retained a status of high morbidity and incremental mortality, unaddressed properly by the modern therapeutic methods developed hitherto. This is partly due to the complex, irreversible pathophysiology processes which occur the moment blood makes contact with the cerebral parenchyma. Understanding this phenomenon and ICH's evolution would allow identification of the potential therapeutic targets.

METHODS

A retrospective study has been conducted which includes patients who have had non-traumatic hemorrhaging vascular accidents, hospitalized and treated in the period between June 2017 and July 2019 within the "Carol Davila" Central Military Emergency University Hospital's Department of Neurology.

The selected patients have been diagnosed with non-traumatic intracerebral hemorrhage by a neurologist, subsequently confirmed through a CT scan. The patients' age varied between 23 and 97 years, with provenance from both countryside and urban environment, with diverse professional occupations. We have investigated the three main types of non-traumatic bleedings, namely, intraparenchymal hemorrhage (IPH), subarachnoid hemorrhage (SAH), and subdural hematoma (SDH).

RESULTS

Out of the 97 patients, 67% were male and 33% female. Out of these, as many as 77% have presented non-traumatic IPH, 13% SAH, and 10% SDH. 17% of the patients have deceased during hospitalization. The average age was 67 years old, 72% of those placed in medical care being older than 60 years old (Figure 1). The average period of hospitalization was 21 days, with a minimum of 2 days and a maximum of 587.

Seasons were defined in the same way as in the rest of the studies, to have a consistency between these, for the sake of comparing them and display our research as suitable, i.e. summer: 1st of June – 31st of August, autumn: 1st of September – 30th of November, winter: 1st of December – 28/29th of February, spring: 1st of March – 31st of May.

We have identified an incremental occurrence of the cases of cerebral hemorrhages in transitional seasons, namely, autumn and spring. Similar results have been produced in most of the past studies, regardless of whether, in some situations, these have shown a larger incidence in winter (based on the geographical areas where these have been carried out) due to the physiological differences of organisms at low temperatures [11, 12, 13].

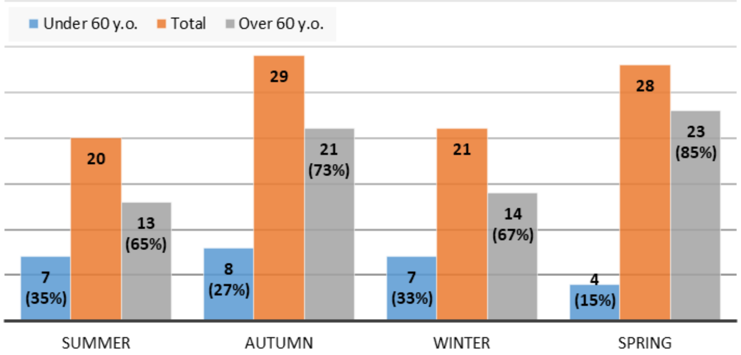

Legend: blue – the incidence of intracerebral hemorrhage (ICH) in the under 60 age group; orange – total incidence of both age groups; grey – the incidence of ICH in the over 60 age group

Figure 1: Number of cases (percentage) of ICH's seasonal incidence

Concerning the seasonal incidence of each type of hemorrhage (Figure 2), we observe an increase in IPH in autumn and spring, a constant incidence in SAH in summer, autumn, and winter, with a lower number of cases in spring, and a relatively constant number for SDH, with a maximum and a minimum number of cases in winter and autumn, respectively.

It is widely accepted that the main risk factor of ICH is represented by chronic HBP, corroborated with a hypertensive leap exceeding the limit of cerebral vascular resistance. The risk of hemorrhage in hypertensive patients is 1.5 times for BP ~ 140 mmHG and three times for BP > 180 mmHg than it is for normotensive patients [14].

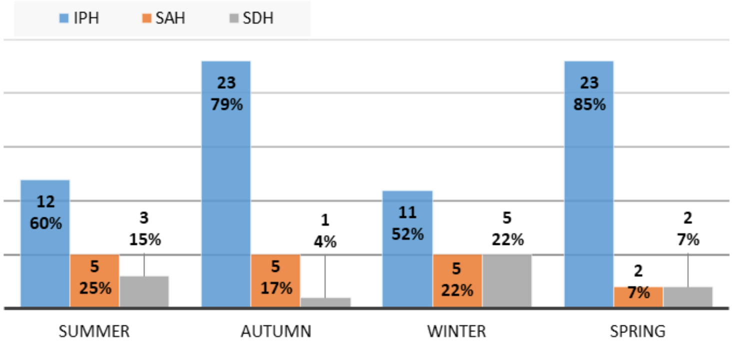

Legend: blue – frequency of intraparenchymal hemorrhage (IPH), orange – the incidence of non-traumatic subarachnoid hemorrhage (SAH); grey – the incidence of subdural hematoma (SDH)

Figure 2: Seasonal incidence of hemorrhages

Out of all diagnosed with IPH, 27% out of the 73 patients have had no associated diabetes mellitus (DM) or chronic HBP, 70% suffered from chronic HBP, 20% from diabetes, and 18% from both. In the context of SAH, 61% had no associated comorbidities, 31% had chronic HBP, 15% DM, and 7% both chronic HBP and DM. Out of those who have been hospitalized from non-traumatic SDH, 36% have not shown any of the abovementioned risk factors, 54% have shown HBP, 27% DM and 18% have shown both. In the analyzed lot, 61 patients suffered from HBP in different degrees, this number representing 62% of the hospitalized patients. The highest rate of cerebral bleedings associated with chronic HBP was registered in autumn, followed by spring (Figure 3). Besides this, we have also evaluated the association between DM and cerebral hemorrhage, as DM may determine an unpropitious prognostic in the case of ICH. Persons with diabetes have a 20% increased risk of suffering from cerebral bleeding, as opposed to a person not afflicted, as well as over a 50% chance of dying within 30 days after the hemorrhage [15]. In our study, we have identified a prevalence of diabetes of 29% among hospitalized patients.

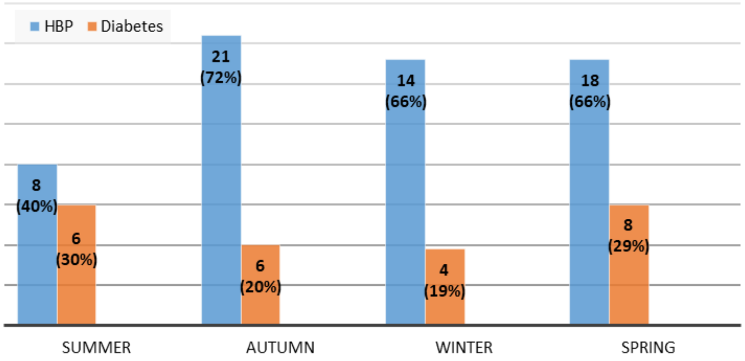

Legend: blue – the prevalence of high blood pressure (HBP) at ICH patients; orange – the prevalence of diabetes mellitus (DM) at ICH patients.

Figure 3: HBP and DM

According to this data, we observe that, indeed, diabetes does not seem to play an important role in contributing to ICH, yet there is high morbidity during summer and winter, seasons with extreme temperatures, and low morbidity during the transitional seasons, spring and autumn. Moreover, there is a higher risk of cerebral hemorrhaging during winter and summer, for those who have diabetes as an associated pathological condition. Apart from a heightened incidence of ICH in spring, we emphasize the more severe nature of these through lengthy hospitalizations periods, hence the increased economic burden on healthcare systems (Figure 4). Considering the number of days of hospitalization as an indicator of the gravity of the hemorrhage, as well as its occurrence in critical areas or through associated complications, we must submit a longer period of recovery for these patients and a lower degree of autonomy and social reintegration. Concerning deaths, we observe a relatively constant rate throughout the entire year, thus barring us from claiming any one risk factor in particular increases ICH's mortality. Apart from the increased morbidity, spring also registers the highest rates of mortality.

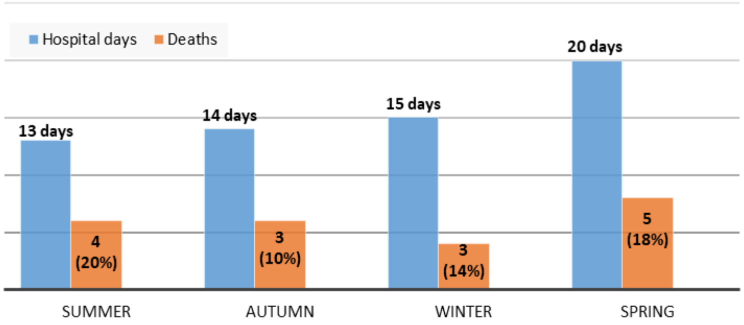

Legend: blue – the average number of hospital days; orange – number of deaths

Figure 4: Hospitalization and deaths

DISCUSSIONS

The results of this study show a variety of the incidence of hospitalization, in-hospital mortality, duration of hospitalization, and patients' prognostic throughout the year, in Bucharest, a region with a temperate continental climate. The data is new for this geographic zone, as, hitherto, there have been no recorded statistical studies (at least recent) to quantify the seasonal incidence of ICH in this area. Consequently, this research tackles on a novel theme, representing a pilot study. For the patients included in this study, the highest incidence was observed during the transitional seasons, especially during autumn, whereas the lowest was during summer. The yielded results contradict the larger part of studies available in the specialized literature, which have either failed to find a variety of incidence or claimed a heightened incidence in other seasons, particularly in winter and spring [12, 13, 16, 17]. Nonetheless, there are also similar results [11].

Regarding the seasonal distribution on age groups, the current study has registered a constant tendency of ICH debuting at patients under 60 throughout the year, apart from spring, when it has decreased, contrary to the constant incidence in the year evaluated by other studies [11, 12]. Conversely, regarding patients over 60, the seasons corresponding to high risk are spring and autumn, with two minimums registered during winter and summer, data which is in line with the results of other comparative studies [11, 12].

When the assessment also includes comorbidities, data is similar to that obtained in other research papers. Diabetes does not seem to play a decisive role in how a hemorrhage comes to be, but it may exacerbate the prognostic [15]. On the other hand, concerning HBP, this is seen as being the most important risk factor for hemorrhage and it has implications both for the prognostic and survival [18]. The mechanisms underlying these seasonal varying conditions are not clear, but it is probable for the latter to be associated with seasonal variations in biological factors, such as the level of serum lipids, some components of the blood, and a hypercoagulable state [19]. Concerning the number of deaths, we have not observed a significant variation in the year, thus being relatively constant, similar data having been obtained by other authors as well [12, 13].

The reason why the risk of ICH is modified throughout the year is not entirely known. The dysfunction of the environmentally adaptive physiological processes may be the underlying cause of hemorrhages. Variations in temperature and atmospheric humidity are considered the main catalyst for hemorrhaging events [20]. Low temperatures and low atmospheric humidity can increase evaporation, leading to an increased demand for salt, thus constituting HBP [20].

CONCLUSIONS

ICH is one of the most important neurological pathological conditions. Even though incidence is lower than it is in the case of ischemic strokes, morbidity and mortality are higher. There is still an ongoing need for thorough studies regarding the seasonal incidence of cerebral hemorrhages, as there is a subset of modifiable factors related to behavior and lifestyle which can potentially contribute to avoiding a hemorrhagic episode in either one of the seasons.

In this study, we have noticed a heightened tendency to cerebral bleeding during autumn and spring, as compared to other geographic zones or climates. Moreover, we have seasonally classified the type of ICH patients in this study were subject to, observing the predominant IPH throughout the entire year. We have also determined the average number of necessary hospital days for each season, emphasizing that spring is associated with the highest morbidity. Furthermore, in the context of this study, we have also found a significant correlation between chronic HBP and the incidence of cerebral hemorrhages, confirmed prior data that DM is a risk factor for triggering a hemorrhagic stroke, but with no real influence on mortality, as well as defined seasonal risk of ICH groups based on age and comorbidities.

In the future, it is paramount that we understand better the physiopathological process underlying cerebral hemorrhages to develop efficient methods with therapeutic potential. Although this may seem ideal, reducing seasonal incidence in risk groups through educating patients is particularly difficult to achieve. Until then, developing methods of treatment that focus on slowing down or completely barring the inflammation's progress and, implicitly, that of neurological deficits, is a short-term objective.

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CLINICAL PRACTICE

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The semi-centenary of a prestigious medical unit: Military-Medical Research Center

Viorel Ordeanu[1,2]

Abstract*: The Military Medical Research Center (CCSMM) is a health research unit, subordinated to the Medical Directorate of the Ministry of National Defence. The overall objective is represented by conducting researches on the medical protection against weapons of mass destruction (ADM) that use chemical, biological, radiological and nuclear (CBRN) agents, on the accidents involving CBRN agents and on the military-medical expertise in the field. The CCSMM specialists develop methods and techniques for diagnosis, prophylaxis, treatment and recovery to optimize medical countermeasures during peace, war, or crisis situations. The activity is complementary to the one carried by the "Cantacuzino" National Military-Medical Institute for Research and Development which deals with the diagnosis of infectious diseases (microbiology and epidemiology) and the production of reagents and biological medicinal products for infectious diseases.*

In conclusion, CCSMM is a unique healthcare unit nationwide, by its field of activity, having a tradition of over half a century, during which it has formed specialists and has had outstanding achievements in the medical protection against CBRN weapons and agents. There are prerequisites for the further development and visibility of the institution.

Keywords: *military medical research, CBRN medical protection, biological warfare agents, bioterrorism, microbiology*

INTRODUCTION

The Military Medical Research Center (CCSMM) is a health research unit, subordinated to the Medical Directorate of the Ministry of National Defence. The overall objective is represented by conducting researches on the medical protection against weapons of mass destruction (WMD) that use chemical, biological, radiological and nuclear (CBRN) agents, on the accidents involving CBRN agents and on the military-medical expertise in the field. The CCSMM specialists develop methods and techniques for diagnosis, prophylaxis, treatment and recovery to optimize medical countermeasures during peace, war, or crisis situations. The activity is complementary but distinct to the one carried by the "Cantacuzino" National Military-Medical Institute for Research and Development (INCDMM) which deals with the diagnosis of infectious diseases (microbiology and epidemiology) and the production of reagents and biological medicinal products for infectious diseases. It also cooperates with the CBRN Defence and Ecology Scientific Research Center (CCSACBRNE) which seeks the CBRN non-medical protection. Each of the three research institutions addresses the issue of the protection of troops and the civilian population from another perspective, without overlapping

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but being complementary. As a result, they must cooperate.

HISTORICAL BACKGROUND

In the military-political context of the Cold War, there was also the danger of nuclear attack. The Romanian Ministry of Health set up a research healthcare unit in 1967, which functioned on the platform of Fundeni Hospital in Bucharest, called the "Center of Radiobiology and Molecular Biology". This unique institution in Romania did not happen by chance. We remind that at that time on Romanian territory was prohibited the stationing of nuclear weapons, so as not to be considered a pretext for a nuclear attack on the country. The international context was strained: the "missile crisis" had just ended in which the US nuclear missiles from Incirlik (Turkey) had been directed to Moscow, and the Soviet ones from Cuba had been directed towards Washington, and the "mid-range missiles" problem was debated in Europe from the east and west coast of the continent.

As a result of the events of August 1968, when the USSR invaded Czechoslovakia, and Romania was in danger of being invaded, the Romanian Army was reorganized and this Center was taken over from the Ministry of Health by the Ministry of Armed Forces (which later became the Ministry of National Defence) by HCM no. 2423/31.10.1968. Starting with December 1st, 1968 it functioned as a military unit called the "Radiobiology Center". Its aim was scientific research in the field of medical protection against the effects of the nuclear weapon.

In 1969, through the Order of the Chief of Staff, the Center's attributions were extended to the medical protection against the other WMDs as well, and the name which it still preserves was changed to the "Military Medical Research Center". The unit had an independent brigade rank subordinated to the Medical Directorate, and the commanding ranks were at Division level of Lieutenant General, M.D. and Major General, M.D. The three component laboratories corresponded to the NBC classification (nuclear, biological and chemical), being ranked regimental, and were run by Colonels – medical doctors, who were also scientific scholars. The laboratories were made up of wards and work stations. To the personnel recruited from the Ministry of Health were added the military and civilian specialists, and from the Military Hospitals in the territory were selected the most valuable specialists, who became scientific scholars. The ranking was made for real personal merits and professional experience, not by “competition”.

CLASSIFICATION AND ENDOWMENT

The first commander, since December 1st 1968, the Center’s founder, was the late Colonel Alexandru Popescu M.D., an exceptional microbiologist with experience in exotic and tropical diseases, and who also participated in the fight against the plague and cholera epidemics in India. He would later become Head of the Medical Directorate, Major General Professor A.G. Popescu, PhD.

Other personalities of military medicine were also in charge: the Colonel Laurenţiu Dragomirescu M.D., Colonel Aurel Andrieş M.D., Colonel Tudor Toma M.D., Colonel Ion Urseanu M.D. A special stage in the evolution of CCSMM began during the mandate of the commander Colonel Victor Voicu M.D., who was to become Head of the Medical Directorate, Major General Acad. Professor Victor A. Voicu, M.D., PhD former Secretary General and current Vice President of the Romanian Academy and President of the Department of Medicine of the Romanian Academy, member of the Medical Academies in Bucharest and Paris etc. Colonel Mihail Silviu Tudosie M.D. (currently the commander of the Military-Medical Institute) and Colonel Radu Gabriel Hertzog M.D., the current CCSMM commander, followed.

During this period, the CCSMM gradually benefited from several workplaces, suitable for the scientific research activity: the Bratianu villa from 37 C.A. Rosetti Street (Pavilion A for Command and Administration and Pavilion B for the Toxicology Laboratory), a radiobiology pavilion at Fundeni Hospital, subsequently completed with vivarium and the underground pavement of the Accelerator for Cancer Therapy; the Microbiology Laboratory at the Cantacuzino Institute (1st Ward), 7th floor and another at the Cernica Fort (2nd Ward), the Department of Clinical Toxicology at the Floreasca

Emergency Hospital, the Psychophysiology and the Psycho-pharmacology Laboratory at the Central Military Hospital (in a pavilion originally designed and built to belong to CCSMM), etc. But as a result of successive restructurings, since 1986 and those following the December 1989 Revolution, the staff has been progressively reduced and the rank of the unit has fallen to regimental level. After the annulment of the Warsaw Treaty, it was assumed that there would be no more wars (!!) and the Center was in danger of being completely abolished. Several spaces were successively lost, the remaining staff and research equipment were and will probably be deployed.

THE SCIENTIFIC RESEARCH ACTIVITY

The unit was certified as a scientific research institution (IP) through GD no. 551/2007 and ANCS Decision no. 9806/2008. Currently, Romania, being a member of the European Union and NATO, grants due importance to national defense and security, including to the medical protection against the CBRN WMD. In this context, CCSMM is in the period of renewal of staff, premises, endowment and work procedures, for modernization and openness to new trends in military medicine and military scientific research.

The results of the scientific research activity have materialized through processes, methods of treatment or diagnosis, and experimental development of new protective compounds and antidote products, protective equipment, etc. These have been endorsed, received, approved, and some have been patented as inventions. CCSMM had at its former headquarters a permanent exhibition of experimental models and prototypes of reagents, medicines, antidotes, complete kits and models developed within the research projects. Many of them were also presented at military exhibitions: The permanent exhibition of military technology from ACTTM Clinceni, EXPOMIL 2011 and 2013, BSDA 2014, 2016 and 2018, the National Exhibition of Inventors 2012 and 2013 (where the Golden Medal and Diplomas of Excellence were obtained), etc. Some of them have been practically used on missions in the country and abroad (Saudi Arabia, Iraq, Somalia, Angola etc.).

SPECIFIC MILITARY ACTIVITIES

CCSMM has also been directly involved in military activities such as national and international exercises and applications, participating in the Persian Gulf War (1991), in Somalia and Angola, providing military-medical expertise in CBRN incidents, expertise of suspected objects brought by the Romanian Intelligence Service (e.g. “biological bomb” discovery or “anthrax letter” of U.S. Embassy), MAI, the Military Police (e.g. control for aircontainers), DIR, BOS (e.g. Afghanistan suspect probes), CBRN equipment testing in laboratory and in field, postal biological control (letters and box) etc. which constitute situational experiments for validating research and training in real terms.

PUBLISHING ACTIVITY

The CCSMM staff actively participate in the scientific life through communications to national and international scientific events, publishing of books, chapters and articles (including hundreds of ISI articles indexed in the Web of knowledge), inventions, didactics and publicity in the military and civilian press. In 1998, Major General Acad. Professor Victor A. Voicu, M.D., PhD organized a NATO scientific event, in Bucharest, entitled “Proceeding of the NATO Advanced Research Workshop on Antidotes, Protectors and Decorporators”, the papers of which were published by the Kluwer Publishing House in 1999. Scientific papers have been published in the Journal of Military Medicine, the National Defense University Bulletin, Strategic Impact, the Military Science Journal, Strategic Universe, NBC Defense, the Military Observatory, Medical Life, etc. Books were published by the Military Publishing House, the Medical Publishing House, the "Carol Davila" University Publishing House, as well as own publishing. Through these activities, the scientific knowledge of interest disseminates and the visibility of CCSMM increases, both in the country and abroad.

EDUCATIONAL ACTIVITIES

CCSMM specialists are also involved in academic and university activities, at the Military-Medical Institute, the "Carol Davila" University of Medicine and

Pharmacy in Bucharest, the "Titu Maiorescu" University, Bioterra University, the Medical School of Non-comissioned Officers in Focşani, etc. The CCSMM researchers have benefited from recognition of their work: excellence diplomas, awards, decorations, biographies mentioned in encyclopedias, etc.

PROSPECTS FOR DEVELOPMENT

The military-medical scientific research, which seemed to be forgotten by decision-makers in recent years, is reviving. The military-political world situation, which is "volatile", brings to the forefront the importance of military medicine and, implicitly, of military-medical scientific research, primarily in regards to the medical protection against CBRN weapons/agents. The prospects for the quantitative and qualitative development of CCSMM materialize and, as consequence, the context favorable for the cooperation with an old collaborator occurs, the "Cantacuzino" Military-Medical Research-Development National Institute, which was also taken over from the Ministry of Health by the Ministry of National Defense.

The current situation also allows the area of scientific preoccupations to be extended in order to cover other areas of medical and military interest, as well as the formation of a new generation of specialists to take over for the future. Besides the main field of scientific research and expertise for medical protection against CBRN/WMD, new and related areas are also successfully addressed, which are concerned with specific military pathology. Thus, in May 2018, an original work of regenerative medicine was awarded a prize at the 23rd Congress of the Balkan Military Medical Committee (BMMC) in Antalya, Turkey. The operationalization of the Integrated Platform of Scientific Research and Expertise of CBRN agents for medical protection will support the work of CCSMM and will allow new research directions to be addressed within existing laboratories. The latest participation is of 16th Medical Biodefense Conference in Munich, 23-31 October 2018.

CONCLUSIONS

CCSMM is a national military-medical unit, unique in its field of activity with a tradition of more than half a century, during which it has formed exceptional specialists and has had outstanding achievements in the scientific research on medical protection against the CBRN weapons and agents. There are prerequisites for the further development and visibility of the institution.

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The strategic importance of vaccination for national defense and security

Viorel Ordeanu[1]

Abstract: *Public health is the most important power factor of a nation, from which all other factors derive. Infectious diseases are the most important cause of morbidity and mortality in humans. Vaccination is the most important method of prophylaxis of infectious-contagious diseases. Mass implementation of vaccination prevents the occurrence of epidemics, epizootic diseases or homonymous pandemics, and at the individual level ensures the specific active immunity (total or partial). So the vaccination of the population and/or the risk groups is of strategic importance to the nation, both in peacetime and in time of war.*

The national antidote production along with anti-infectives (vaccines, therapeutic serums, immunomodulators, antimicrobial chemotherapies and pesticides) has a strategic importance for the health of the population and of the troops. Biological crisis situations are cases of force majeure, where the Medicines Law provides the exception for manufacturing and use, even without a "marketing authorization".

The military scientific research could make micro-production of specific drugs (antidotes, antiinfectives) for the needs of the army and the risk groups of the population. An example would be the preparation of vaccines for the prophylaxis of diseases caused by BWA and bioterrorism as niche products. Nothing hinders the preparation of any medication, such as magistral and galenical preparations, or pilot batch, provided there is adequate space, equipment, staff and procedures according to the law or the exception.

Keywords: *vaccination, public health, national defense, national security, military production*

INTRODUCTION

We live in turbulent times, and the current international context is volatile. Even in our east-westerly vecinity, hybrid wars, prolonged for many years and without the clear prospect of their cessation, take place. These exacerbate the risk for national defense. The social unrest, stimulated both domestically and internationally, amplifies the risk for national security. This context also causes direct or indirect risks to the population, and economic, social and other disruptions reduce the standard of living.

Not only does war and uprising implicitly cause a polytrauma epidemic, but it also profoundly affects the public health. Premises for epidemics (right at this time, the measles outbreak is happening in the EU) and epizootic diseases (right at this time, the African swine fever epizootic is happening in the EU) are created, extending as far as biological attacks, bioterrorism and bio-crime. And if we attempt to interpret the military-medical health situation in the

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country, we can think that the current epidemics are favored by the propaganda of the antivaccinists, whose hidden interests and their coordinator still remain unknown.

As far as the current epizootic is concerned, it is a viral disease for which there is no vaccine for prophylaxis or specific antiviral treatment, and the economic effects can be catastrophic. The distribution of outbreaks on the map shows that the virus has penetrated the country at the Hungarian border and spreads to the interior of Transylvania (a major producer and exporter of pork). A second wave followed, shortly after, at the Ukrainian border, extending to northern Dobrogea and eastern Bărăgan, gradually reaching Bucharest and the border with Bulgaria. Until now, over 1,000 outbreaks of African swine fever have been identified by Romanian veterinarians in domestic (farm or house) pigs in different places in the country, but not in wild boars who were initially believed to have carried the virus. Therefore, how did the disease come to exist and how does it spread? The breeders assume that through forages (including by industrial premixes); however, these are not a supportive environment for the preservation or multiplication of viruses. The only possible explanation would be the intentional contamination with this virus, thus, a likely new bioterrorist attack with an economic goal. But the hypothesis has to be confirmed with scientific evidence, by laboratory analysis and molecular epidemiology; otherwise, it shall only resemble the "conspiracy theories" partly projected by the press. Until now, the secret services have found nothing (being probably occupied with other issues) and the military medicine has not received samples for analysis, although it has advanced microbiological diagnostic capabilities and has the ability and expertise to test possible biological attacks.

THE PUBLIC HEALTH

The public health is the most important power factor of a nation (large or small), from which all other factors derive. In Romania, it was effectively supported by the territorial healthcare system introduced after World War I by Prof. Ioan Cantacuzino (at that time the Minister of Health), according to the most advanced Western model imported from France. The sanitary facilities monitored all people (not just citizens or policyholders) and all businesses in the assigned territory, so that no man and no square meter in Romania remained outside the sanitary system: prophylaxis, diagnosis and treatment. In parallel, the veterinary districts supervised domestic and wild animals. The County Health Directorates and Departments, as well as the Sanitary-Veterinary Directorates centralized the activity and reported it to the central authorities, to establish the necessary measures to be implemented, locally, nationally or internationally.

As an example, in the 1990s, the Medical Directorate of the Ministry of National Defence organized in Sinaia the first working meeting between Romanian and NATO military doctors. We presented the arrangement of the anti-epidemic surveillance in Romania, integrating human, military, veterinary, phytosanitary and environmental medicine, which now represents the One health concept. When we made the statement that "In Romania the anti-epidemic surveillance is not of sound quality..." we were interrupted by the American representative who stated that "It is our case as well!". However, we continued our idea as follows: "In Romania, the anti-epidemic surveillance is not of sound quality, but of very high-quality: we have 50 Health Directorates, 41 Sanitary Veterinary Directorates, 41 Agricultural Directorates, 41 County Environmental Inspectorates, 5 Medical University Centers, various Institutions and Central Laboratories, we are members of the World Health Organization, and the Cantacuzino Institute is part of the Pasteur Institute network, etc." The approval that came from the present audience closed the topic. However, in the meantime, the Sanitary Reform has altered some of the problem's data.

The public health was also effectively supported by the Romanian pharmaceutical industry by providing the national needs and exports of medicines, vaccines, reagents, parapharmaceuticals, pesticides and medical devices, to which the Cantacuzino Institute has contributed significantly since 1921. Until recently, persisted the moment of glory of the Romanian medicine where, with the existing forces and means and with reasonable funding, major progress was made in the public health sector, the eradication or quasi-eradication of infectious-contagious diseases, the decrease of infant mortality, the improvement of the quality of life, the extension of the duration of life and labour.

However, the public health in Romania has been partially undermined by the abolition of the territorialisation in primary healthcare and the drastic reduction of the pharmaceutical industry, in such a way that from the status of exporters of medication we have become importers, and the medication crisis is current. Although the new Constitution of the country still provides for the country's obligation to ensure the public health, this role has been significantly reduced. A system where the health is highly expensive and the foreign investors are exaggeratedly gaining through medical insurance and trade in medicines and pharmaceuticals. However, the results of medical assistance do not seem to be superior to those of previous years, and even in the field of infectious and contagious diseases there is a worsening of the situation, and the press publicly presents this situation. At times, the doctors are being tried as natural persons as an explanation for the inefficiency of the current healthcare system and sanitary reform. Overall, the increase in costs has not been accompanied by an increase in efficiency, and public health is declining, according to statistics from the National Institute of Statistics.

VACCINATION

Infectious diseases are the most important cause of morbidity and mortality in humans. Diseases caused by parasitic living beings (bacteria, viruses, prions, fungi, protozoa and parasitic animals) have prevailed since ancient times and are still the most common in underdeveloped countries. But also in developed countries (including Romania), where these diseases are officially ranked third (after cardiovascular diseases and cancers), there are many diseases that actually have a known infectious origin (such as gastric and duodenal ulcers, which are caused by a bacterium, or some cancers caused by certain viruses, etc.) or not yet known (unidentified bacterial endocarditis, supposed infectious dementia etc.); thus, these are equally infectious diseases.

Vaccination is the most important method of prophylaxis of infectious and contagious diseases. The massive application of vaccination prevents the occurrence of the epidemic, epizootic or homonymous pandemic, and at the individual level ensures specific active immunity (total or partial). Therefore, the vaccination of the population and/or the risk groups is of strategic importance to the nation, both in time of peace as well as in time of armed conflict.

The history of vaccinology begins in the Middle Ages, with the empirical immunization and continues in the Modern Age with scientific immunization. The first mass vaccinations were made in the 1800s, when Napoleon ordered the compulsory vaccination of the Great Army (hundreds of thousands of soldiers) as prophylaxis in military campaigns. From antiquity to the contemporary era, many battles and even wars have been lost due to epidemics that have weakened the combatants' ability to fight. Prof. N. Iorga once said "read history to know what shall come" [10]; and, the history of medicine is a component of history and partially overlaps with military history. It is not the intention of this article to present a history of vaccinology in general, so we will limit ourselves to the chronological presentation of the vaccine production in Romania and the national development of vaccination as a medical protection against infectious diseases.

In the last century, the appearance of sulphamides and antibiotics led to antibacterial chemotherapy which, along with vaccination and hygiene, has rapidly improved the public health to its current level and the population of the planet is growing exponentially. It is the most important demographic success of the human species, and it is mainly based on the advances of medicine in regards to vaccinology and anti-infective therapy. Vaccinology saved more lives than surgery.

After the discoveries in biology of the chemist Pasteur, the founder of modern microbiology, Romanian scientists were also attracted by this field. Victor Babeş studied in the West and published in French the first microbiology handbook in the world. Ioan Cantacuzino worked at the Pasteur Institute in Paris, under the guidance of Prof. Mecinikov, and returned to Bucharest as a professor at the Faculty of Medicine, where in the Experimental Medicine Laboratory (which he created in 1901) prepared sera and anti-infective vaccines. These were successfully used for the protection of the population and the Romanian Army during the Balkan Wars (he was also the Head of the Army's Sanitary Service, ranked as Colonel) and during World War I, including the "great Romanian experience", during which he ended the terrible plague epidemic, and later (in 1921) the Institute that was named after him was founded, and was led by him until the moment of his death in 1936.

Prof. Al. Slatineanu, along with ŞL C-tin. Ionescu Mihăeşti and Dr. Mihai Ciucă ("laboratory assistant for serum preparation", the future professor and director of the Cantacuzino Institute), published in 1915 the first Military Epidemiology Handbook: "Notions in epidemiology and serovaccinations (with special application in the campaign)", The laboratory of experimental medicine, Bucharest, State Printing House, 95 p. [12]. This article is based on the medical experience during the Balkan Wars, which has been useful during the First World War, when the Romanian Army faced major epidemics. The four vaccines produced at that time are also presented here: the anti-vitiligo, anticholeric, anti-plague and antivarioulous vaccine.

In 1939, Prof. C. Ionescu-Mihaesti and Prof. M. Ciuca, together with 27 specialists from the Cantacuzino Institute, published the first "Handbook of infectious disease with prevention concepts, serotherapy and vaccinations, drawn up by the Cantacuzino Institute", the Foundation for literature and art "King Carol II", the National Printing House, Bucharest, 1940, 313p. [9]. The work is based on the World War I medical experience and the advances in inter-war medicine, being useful in the Second World War, when the Romanian Army experienced no more epidemics. The main infectious and contagious diseases, 18 vaccines, 16 therapeutic sera, 6 in vivo reagents, immunotransfusion therapies, with bacteriophages, with sulfamides, etc., are presented in a total of 43 biological drugs. For comparison, "Formulaire ASTIER, 9-e Edition, Vade-mecum de Medecine Pratique", Paris, 1942, 1306 p (representing the most advanced medicine of the time) describes 21 vaccines, 19 therapeutic sera and some biological therapeutic methods. [1]. Romania was at a European level even before being a member of the European Union.

In 1944, the Sera and Vaccines Institute "Dr. I. Cantacuzino" publishes the "Guidelines for the Use of Sera and Vaccines, 1944 Edition", Bucharest, George Ionescu – Pharmacist, 143 p. [3]. The work could be considered as the first handbook of vaccinology in Romanian. There are 21 vaccines, 13 therapeutic sera, 4 bacteriophages, 14 biological reagents, 2 biological dressings, a total of 54 biological drugs. During the same year, appears the second edition of the Handbook of Infectious Diseases, 320 p. [9], based on the experience of the World War II. 23 vaccines, 17 therapeutic sera, 4 bacteriophages, 6 biological reagents, the immunotransfusion and sulfamido-therapy are presented.

Prof. C. Ionescu-Mihaesti and Prof. M. Ciuca, as students and followers of Prof. Ioan Cantacuzino, who further developed the Institute, published in 1945, in English, the first International Handbook on Infectious Diseases under the auspice of the League of Nations: "League of Nations. Handbook of infectious diseases with notes on prophylaxis, serum treatment and vaccination" by the Staff of the Cantacuzene Institute, under the direction of Professors C. Ionescu-Mihaesti and M. Ciuca" Series of League of Nations, Publications III Health, Geneva, 1945. III. 1. 331p. [8]. The handbook also addresses the field of immunological medicinal products: 23 preventive and therapeutic vaccines, 19 therapeutic sera, 10 diagnosis sera, phage therapy, immunotransfusions, sulfamidotherapy and, for the first time in a handbook, antibiotherapy – in chap. IX Anti-biotic products of microbic origin (fungi, bacteria).

After the regime change, "R.P.R. Ministry of Health, Institute of Sera and Vaccines Dr.I. Cantacuzino, Guidelines for the Use of Sera and Vaccines" State Printing House, Medical Literature, Bucharest, 1949, 144 p. [2]. The work describes 11 vaccines, 4 anatoxins, 7 therapeutic sera, 2 bacteriophages and 17 biological reagents, a total of 41 anti-infective biological products. In 1952, the Ministry of Health and Social Welfare publishes the "The Prophylaxis of communicable diseases", re-published in 1961 [4] etc. In 1962, MSPS publishes "Vaccines, Sera and Biological Diagnosis Products", 227 p. which features 87 assortments. [7]

After the military-political shock of 1968, the "Dr. I. Cantacuzino" Institute is revitalized and publishes "Vaccines, serums, biological diagnosis products, culture media and laboratory animals" Medical Printing House, 1969, 285 p., which describes 251 biological products, of which 18 vaccines. In total, from 75 assortments in 1947, 340 were produced after 1969. [5].

However, after the 1989 Revolution, the Cantacuzino Institute gradually lost its importance, and gave up a part of its staff (by restructuring, retirement, relocation, unemployment, etc.), its External Departments of the province (Iaşi, Timişoara, Dârvari etc.), part of its portfolio of anti-infectives and microbiological diagnosis products. According to the latest INCDMI "Cantacuzino" Product Catalog for 2009, of the 802 products previously in the portfolio were produced 13 vaccines, 2 therapeutic sera, etc. and in 2013 only one vaccine (flu), no therapeutic sera and only one part of the other microbiological products. [6] Then followed: the financial crisis, the cancellation by the MS/ANMDM of production authorizations and the crisis of authority and competence, which led to the complete cessation of the production of vaccines and therapeutic sera. There was a need for drastic measures to relaunch the production of biological medicine, including the takeover by the Ministry of National Defence, so that the Cantacuzino National Military-Medical Development Research Institute could be run by the military and concrete results are expected.

The evolution of the production of vaccines and sera for human use in Romania (incrementa et decrementa), exemplified by specialized medical publications, shows the following dynamics: 1901 = 0; 1915 = 4; (1921 establishment of IC); 1939 = 34; (1942 = 40 in France); 1944 = 40; 1945 = 42; 1949 = 22; 1969 = 25; 2009 = 15; 2013 = 1; 2014 = 0; and currently = 0, but there are good prospects.

During those periods of production, vaccinations were free of charge (material and labor) and compulsory, according to the Annual vaccination schedule of the Ministry of Health for the civilian population, including children, of the Ministry of National Defence for the Military and of the Ministry of Agriculture for animals.

The safety of vaccinations

The Romanian production of sera and vaccines for human use (with circulating strains in the country) has protected Romanians from infectious-contagious diseases and epidemics for 113 years (armed with between 100-500 thousand soldiers and populations between 11-22 million people), also achieving a very rich vaccination experience, with no major medical incidents.

The testing base on millions of people of all ages and over a century provides a large-scale epidemiology and pharmacovigilance study confirming the effectiveness of vaccinations and the quality of vaccines prepared by the Romanian Pharmaceutical Industry, primarily at Cantacuzino Institute. It would be interesting to have a retrospective scientific research on vaccination in Romania that would quantify the data objectively and extract the appropriate conclusions. When the theory, or the Law of Medicine, does not fit the practice, the theory should be corrected (if there are no hidden interests).

Vaccinology is an important concern of global medical research, of the epidemiology and the pharmaceutical industry; new vaccines, new regulations, new forms of administration have been created worldwide, but we still do not have vaccines for all infectious-contagious diseases. The antivaccinist current is also added to the existing challenges, which, out of ignorance or bad faith, opposes vaccination for various reasons: the fear of supposed adverse effects, the desire to let nature take its course, the belief that sickness and health come from God, the antisocial attitude, financial interests etc. Some adverse effects exist with varying degrees of severity, but are very rare and the risk/ effectiveness ratio is considerably favorable, particularly in social terms. No medication, including the vaccine, is granted the "marketing authorization" if it does not meet the Efficacy and Safety conditions set by the drug legislation. The World Health Organization (WHO), as a specialized UN forum, recommends the necessary vaccinations anywhere in the world and at any time, and national authorities comply, but sometimes only within the limits of financial possibilities, through self production and/or imports.

WHO List 2017 Essential Medicines Vaccines Section: BCG, Diphtheria, Hemophilus Type B, Hepatitis B, HPV, Measles, Pertussis, Pneumococcal, Polio, Rotavirus, Rubella, Tetanus, Japanese Encephalitis, Yellow Fever, Tick-borne encephalitis, Cholera, Hepatitis A, Meningococcal, Rabies, Typhoid, Influenza (Seasonal), Mumps, Varicella.

NATO's List of Biological War Agents (BWA) in conformity with STANAG 4632:

- Bacteria Bacillus anthracis, Yersinia pestis, Francisella tularensis, Vibrio cholerae*, Brucella melitensis, Burkholderia mallei, Coxiella burnetti;
- Viruses VEE virus, Ortopox virus, Yellow fever virus*
- Toxins Ricin, Saxitoxin, Botulinum toxin, SEB, T2 mycotoxin

Note: Only the two BWAs marked with * are found in the WHO List, meaning that the civilian medicine is not particularly interested in the biological warfare; therefore, implicitly, this task rests with the military medicine.

The European Medicines Agency has published the List of Biological Agents Usable in Bioterrorism (equivalent to CDC USA): EMA BA 2002, 2007 and 2014, presenting in summary: the disease, treatment and prophylaxis. It should be noted that for some diseases there is treatment, for others there is no "recommended treatment", for some diseases there is a vaccine (which is or is not available), for others there is no vaccine, and is or is not undergoing research/ authorization. An example could be the recent Ebola epidemic, a disease caused by a long-known virus, but in the epidemic still did not exist: a diagnostic reagent, a prophylactic vaccine, and no specific antiviral treatment. Only the existence of the epidemic has prompted scientific research to produce them. As a result, the international collaboration has had unsatisfactory results, and additional intervention by a U.S. Army Medical Brigade, which applied general medical countermeasures combined with military discipline to enforce anti-epileptic precautions, was needed.

The niche production for vaccines of military-medical interest (WBA + Bioterrorism)

The military-medical scientific research also aims at maintaining health and combat and labor capacity (troops and population) as well as their recovery in the case of common or specific illnesses (eg CBRN). The Center for Scientific Military-Medical Research (CCSMM), established in 1967/1968/1969, permanently collaborated with the Cantacuzino Institute (1921/2017), the national producer of serums and vaccines, for the medical protection against biological weapons, including for vaccinology (the Dermojet automated injector, lyophilized intradermal vaccines, specific tests, etc.).

Medical countermeasures are based on: diagnosis, prophylactic treatment, pre-, intra- and post-exposure prophylaxis, recovery treatment for combat, work or life. It is well known that the best military recovery results for the battle were obtained by the German army (on the principle of phased evacuation), and the best recovery results for labour or life were obtained by the US army (on the principle of evacuation by destinations). In fact, the two principles of evacuation of sanitary losses apply in different situations of conducting military operations, namely classical front or expeditionary actions.

The effect of the disseminated biological agent may differ depending on many factors and their association: pathogenicity, virulence, toxigenesis, resistance to the environment, resistance to treatment, unprotected population, specifically non-vaccinated, without pre-exposure prophylaxis, without intra-exposure prophylaxis, without post-exposure prophylaxis, without anti-infective treatment, cases of illness and outbreaks, as well as sanitary-pharmaceutical logistics: forces, means, money, etc., the result being expressed in terms of financial and social costs.

The national antidote production along with antiinfectives (vaccines, therapeutic serums, immune-modulators, antimicrobial chemotherapies and pesticides) has a strategic importance for the health of the population and of the troops. Biological crisis situations are cases of force majeure, where the Medicines Law provides the exception for manufacturing and use, even without a "marketing authorization". Public health must be an obligation of the state towards its citizens and not a business of producers or importers.

The military scientific research could make micro-production of specific drugs (antidotes, antiinfectives) for the needs of the army and the risk groups of the population. An example would be the preparation of vaccines for the prophylaxis of diseases caused by BWA and bioterrorism as niche products, in order to avoid the competition of the large multinational pharmaceutical producers, whose turnover exceeds the state budget of Romania. Nothing hinders the preparation of any medication, such as magistral, galenical preparations, or pilot batch, provided there is adequate space, equipment, staff and procedures, according to the rules: of good pharmaceutical practice, good manufacturing practice (GMP), good laboratory practice (GLP), good practice for safety check and the exception of force majeure. [11]

CONCLUSION

Vaccination is very important for national defence and security. Vaccine production started in 1901, developed since 1921, had a glory period before the financial crisis of 2009 (regardless of the regime), went into decline and is currently only declared intent. The military-medical scientific research could resume the production of vaccines, complying with specific conditions, especially as niche production, possibly through national and/or international partnerships. The vaccines production must continue.

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ORIGINAL ARTICLES

The strategic need for the implementation of a technological platform for the microproduction of antidotes for the CBRN medical protection

Viorel Ordeanu[1,2], Adrian A. Andrieș[2], Lucia E. Ionescu[1], Marius Necșulescu[1], Diana M. Popescu[1]

Abstract: *The "volatile" international context requires the strengthening of the national security measures, including in the field of CBRN and public health. The medical countermeasures for the defense are based ultimately on the prophylactic treatment (pre-exposure, intra-exposure and post-exposure) and on the curative treatment (of emergency, maintenance and recovery) of the contaminated and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is immediately applied. Although in times of peace this scenario is almost non-existent, during war or terrorist attack it can be a mass pathology, like an explosive epidemic, that requires specific therapeutic means.*

Military medicine had and has the conceptual capacity to make antidotes for CBRN medical protection; some have been patented and/or produced as experimental models, but it has no manufacturing capacity anymore.

Keywords: *medical protection, antidote, microproduction, technological platform, medical countermeasures, pharmaceutical technique, orphan drug production*

INTRODUCTION

The international context following the conclusion of the "Cold War" paradoxically requires the strengthening of national security measures, including in the field of CBRN and public health. The international context shows that although the risk of biological warfare has been reduced following the Geneva Convention (Biological and Toxins Wapons Convention – BTWC 1972), signed and ratified by about 90% of the world's countries, the risk of bioterrorism is real, as is the risk of pandemics, zooanthroponosis etc.

The World Health Organization (WHO), the European Union (EU) and the North Atlantic Treaty Organization (NATO) recommend concrete measures to reduce these risks and to increase the responsiveness of each country, of the alliance and of the international community. Therefore, in this context, it is absolutely necessary to implement cutting edge technologies for diagnostic and prophylactic treatment, taking into account the mechanism of action in infectious diseases, the therapeutic means of counteracting the effects of biological agents and the epidemiological surveillance.

The existing international concerns force us to align ourselves with the situation existing worldwide. Designing and equipping such platforms according to international recommendations, determines the level of performance of such infrastructure to contribute to the worldwide effort of medical protection against biological agents. The present study was performed at the request of the Medical Directorate, following the request of the Control Body of the National Ministry of Defence.

MEDICAL COUNTERMEASURES

The medical countermeasures for CBRN defense are based ultimately on the prophylactic treatment (pre-exposure,

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intra-exposure and post-exposure to the attack with pathogens) and on the curative treatment (emergency, maintenance and recovery) of the contaminated and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is applied immediately. In times of peace this case is almost non-existent; however, during war or terrorist attack it can be a mass pathology, like an explosive epidemic. that requires specific therapeutic means. Medical intervention requires the same strengths and the medical means all at the same time, so medical aid becomes inefficient in quantity. Moreover, the necessary drugs, in principle the specific antidotes, are orphan drugs, being little used because they are addressed to very rare diseases, and therefore they are manufactured in very small quantities and are not available when needed for urgent treatment for a large number of patients.

The medical protection against the effects of the weapons of mass destruction: chemical, biological, radiological and nuclear (CBRN WMD) is based on fundamental and applied scientific research (CDI) and later on its application in practice.

Critical situations caused by CBRN weapons/agencies are major medical emergencies and can be caused by CBRN WMD attacks, terrorism or criminal acts. The medical system must immediately have at its disposal the necessary force and means of special facilities and appropriate procedures, because "later it may be too late".

Antidotes are specific drugs for the emergency, prophylactic and curative treatment of diseases caused by physical, chemical or biological harmful factors, including CBRN agents, pollutants, etc. There is a risk of using a large variety of WMD agents: chemical warfare agents (CWAs), biological warfare agents (BWAs), radiological warfare agents (RWA), industrial toxicants, etc. which cause a wide range of diseases. As a result, many types of antidotes are needed, permanently available in stock. These must continuously exist, in appropriate forms and in reasonable quantities, for the emergency treatment of the contaminated, the suspected and the sick among the military and civilians, and possibly for the treatment of service animals, etc. In this category can be included decontaminants for internal and external use for humans and animals, in the form of solutions, powders, etc.

In medical practice, antidotes, in a broad sense, can be classified according to several criteria. WHO 20 list of essential medicines, 2017 edition, includes at chap. 4. Antidotes and orher substances used in poisonings, only 15 pharmaceuticals with antitoxic effect, but without taking into account the other CBRN agents. Antidotes can be classified pharmacologically, depending on the agent for which they are used, hence etiological. Antidotes can also be classified according to clinical use: preventive (eg protectors, vaccines), post-exposure prophylaxis (eg atropine, specific antibiotherapy, decontaminants for the skin, mucous membranes, eyes etc.), or therapeutic (e.g. decorporators, chelators, therapeutic sera). From a pharmaceutical point of view, there are antidotes for external use, including hygiene-cosmetics (solutions, lotions, pastes, ointments, powders, etc.), antidotes for internal use (buccal solutions, powders, granules, tablets, dredges, capsules etc.) used preventively, post-exposure or therapeutically, as the case may be. Injectable antidotes (specific vials and devices, self-injecting syringe, auto-injector) and infusions represent an important category in the medical-military arsenal of countermeasures against CBRN agents.

Like any other drug, antidotes can be prepared industrially, galenically or masterfully, from natural and/or artificial pharmaceuticals. These can be of mineral origin (eg sodium sulfate), vegetable (eg charcoal, atropine), animal (anti-venom), microorganisms (eg vaccines, immuno-modulators). Depending on the context, they can be used for prophylaxis (eg pyridostigmine bromide, vaccines), for post-exposure prophylaxis (eg decontaminants for skin and mucous membranes, atropine, anatoxins, antibiotics), curative (eg EDTA, therapeutic sera, antimicrobial chemotherapics).

Antidotes must meet a number of basic conditions required by the Medicines Law: proven therapeutic effect, undesirable side effects as less as possible, preparation in appropriate conditions, reasonable cost price, the possibility of being produced in the required quantities and to be stored for a certain time period, as well as being distributed in a timely manner for emergency treatment. However, an issue arises concerning the cost/efficiency: if within the antidote's validity period there are no cases or there are very few (desirable situation), the remaining antidote must be discarded at the expiration of the term of validity. These stocks cannot be refreshed by "being put in use" in the sanitary units, because under normal conditions they are of no use except to a very small extent. As European Medicines Law no longer allows re-endorsement after expiry, at most, the antidotes that correspond to the pharmaceutical and pharmacological control may be used only for possible veterinary use, if applicable. In the event the numbers of cases require a certain antidote exceeding the existing stock, the product will have to be prepared quickly, according to a product portfolio located at the manufacturer. If there is no authorized manufacturer, a call can be made for emergency imports or international aid. In fact, the EU Charter also provides for a solidarity clause, whereby European countries are obliged to help each other in case of emergency, but it is obvious that this can be done only after covering their own needs.

In practice, it is difficult to find a pharmaceutical industry producer (one of the most profitable in the world) to manufacture the full range of antidotes, because the orders are small, the products are "niche", and the prices are monitored. This is because antidotes are orphan drugs, being used only for rare diseases, so the demand is so low that their manufacture would mean a lot of work for little money, and would not be cost effective. It turns out that a private producer could not be forced to manufacture them in normal circumstances.

The production of specific antidotes for CBRN medical protection can be achieved only in conjunction with:

1) the medical research’s need to physically manufacture the newly designed products (medicines, diagnostic kits, kits and medical devices), at the level of experimental model (EM), functional experimental model (FEM), prototype and/or technological demonstrator, for endorsement, receipt and possibly patenting (as an invention or industrial model), with

2) the need of the industry to carry out the 0 series, the microproduction and the batch series production for clinical testing and for obtaining the marketing authorization (MA), so the principle of integration must apply: research, education and production.

This integrative principle promoting success in the development of specific antidotes, was introduced in Romanian medicine by Prof. Ioan Cantacuzino, since 1901, (Biography Cantacuzino, Ciuca) being applied at the Institute that bears his name, but was subsequently abandoned under unjustified circumstances. Obviously, nowadays, such a dedicated objective involves a pharmacological and pharmaceutical collaboration in the form of a technological platform for the production of antidotes for medical protection, whose components (facilities and utilities) include a Pharmaceutical Laboratory for CBRN antidotes and that must comply with the recommendations of the ANMDM (National Agency for Medicines and Medical Devices) and implicitly with those of the WHO.

State-of-the-art for the production of antidotes for CBRN medical protection is different from one country to another. Civil drug manufacturers (the pharmaceutical industry) generally avoid manufacturing antidotes, as these are "orphan drugs", meaning that they are rarely used under normal conditions, so it would be a difficult commodity to sell. State producers can be forced, through the Production Plan, to manufacture them, but in Romania there is no longer the case, as a result of privatization, and private producers risk financial losses or even bankruptcy, if they manufacture without and end profit.

The beneficiaries and the producers avoid ordering or storing antidotes, because they can remain unused until the expiration date and the prolongation of the duration is not allowed even if they maintain their therapeutic effect, so the medicines are canceled and financial problems arise. As a result, CBRN-specific antidotes do not appear as such in the List of Essential Medicines (WHO Model List of Essential Medicines, edition 21, WHO 2019).

The solution would consist in the specific pharmaceutical manufacture in military or militarized facilities, according to the needs of the national military forces, of the population exposed to specific risk and possibly for other allied armies or for the population in general.

PREVIOUS EXPERIENCE

In Romania there was the Pharmaceutical Production Laboratory of the Medical Directorate belonging to the Ministry of National Defence, which operated without interruption for almost a century, near the Sanitary Warehouse. It prepared specific usual medicines for the military staff, which were distributed free of charge to military hospitals and unit nurses. Different products were manufactured, depending on the needs, as solid forms (tablets, dragees, suppositories, spark plugs, powders), liquid forms (solutions, lotions), ointments, etc. But the injectable, infusable forms and the eye drops, which must be sterile, were prepared only in the pharmaceutical laboratories of the military hospitals. These drugs, along with others, manufactured in the country or imported, have been used to treat the military during peace and war: the Balkan Wars, the First World War, the Second World War, the Revolution, etc. After Romania's entry into NATO and the EU, this laboratory was closed by the pharmaceutical micro-production and moved to another headquarters because it did not comply with the new European norms for the pharmaceutical industry. At the entry into force of the Medicines Law, it was closed down and the machines, inventory objects and consumables (pharmaceuticals and chemicals) were redistributed, some of them ending up at CMMSR for scientific research. They no longer meet the conditions required for the pharmaceutical industry, can no longer be used for the initial purpose and cannot be exploited.

The laboratories of The Center for Military-Medical Scientific Research (CMMSR) have experimentally produced various specific drugs, including antidotes, some of which have been used for the troops in peace or war time. These include: Dostirav (Pyridostigmine) and other CBRN protective equipment provided, including in the Iraq war, 1991; the cyanide antidote that, although patented, is not manufactured by the pharmaceutical industry and the tragic consequences could be seen in the tragic accident which took place at “Colectiv” etc.

The applied research, through the module "Scientific and technological research on the production of antidotes and products with special purpose", has completed technological researches on the manufacture of protective and decomposing antidotes under conditions of peace and in critical conditions (campaign, natural and technological disasters) and, since 2002, ended by:

- establishing the technologies for industrial production and by micro-production under critical conditions for oral preventive antidotes (eg Dostirav/30 mg);

- establishing technologies for industrial production and by micro-production under critical conditions for decontaminants for skin and the mucous membranes (eg DCPM);

- establishing the structure and operating conditions of certain microproduction lines under critical conditions for antidotes;

- research on the synthesis of some organic derivatives of obidoxime with improved pharmacokinetic properties, etc.

These research projects were approved and accepted, the microproduction was performed at galenic level, and the financing of a Pharmaceutical Micro-production Laboratory for antidotes was approved. However, the necessary funds were not allocated and the objective was not achieved. This problem must be resumed in the current political-military context and updated according to the legislation in force. We present below, as an example, some products resulting from the CMMSR’s activity, such as Patents of invention/innovation and/or experimental/galenic models (Table 1). To these can be added numerous sets and diagnostic and treatment kits for medical intervention in CBRN events, some of which have been presented at military exhibitions: ACTTM Clinceni, EXPOMIL Bucharest, BSDA, Inventions fairs, etc. and have been used in applications and theaters of operations (Saudi Arabia, Somalia, Angola, Iraq, etc.).

Regarding orphan drugs, which are very rarely needed, the cost of making and placing a medicinal product on the market for the diagnosis, prevention or treatment of the disease, cannot be recovered through the expected sales of the medicinal product, and the pharmaceutical industry would be refractory to its realization, under normal market conditions. The antidotes for medical protection in case of CBRN attack, terrorism, catastrophe or industrial accident are also considered orphan drugs.

Another aspect of these medicines is represented by the expired drugs. The supplier guarantees the efficacy of the drug only within the deadline, so an orphan drug may expire before it is needed. This can create a financial problem that is difficult to solve. The US Army is currently studying the possibility of extending the shelf life of medicines from stocks, if they have retained their pharmacological properties (internet).

The operating costs differ depending on the chosen variant, the assortments and the quantities of antidotes manufactured, being able to reach the order of millions of euros, depending on the spaces, equipment, personnel and portfolio of pharmaceutical products.

Implementation Opportunities. Providing antidotes is mandatory in medical practice and especially in military medicine. Antidote insurance is nationally deficient and globally they are considered "orphan drugs".

A compromise formula, adapted to the current context in Romania, could be the strategic cooperation regarding fundamental, applied, developmental, innovation scientific research integrated with the production and education of the profile. Thus, the Ministry of National Defence, through virtual reorganization can achieve an Integrated Technological Platform, by sharing the scientific basis and launching the production of antidotes for public health needs and for CBRN crisis situations.

To this would contribute first of all the Military Medical Research Center (CCSMM), the “Cantacuzino” National Military-Medical Institute for Research Development (INCDMM Cantacuzino), the CBRN Defense and Ecology Scientific Research Center (CCSACBRNE), as well as other competent military entities and civilians, from the academic, university, industrial health field, etc. In order to launch the specific antidote micro-production or production, it may be necessary to build or adapt an appropriate facility (pharmaceutical laboratory or drug factory). As a back-up formula, in case of force majeure, a virtual "ad hoc" structure can be created, which will integrate different structures and microstructures existing in Ministry of National Defence, to achieve this common purpose.

Table 1: Examples of CBRN medical protection products resulting from the activity conducted by the Military Medical Research Center

No	Product	
1	Medicinal product with effective action against organophosphorous compounds	Patent State Office for Inventions and Trademarks 79914/1974 V. A. Voicu, C. Miu, M. Jiquidi, C. Furnica, I. Elian
2	Dixidextracaine 70, a drug intended for subarachnoid hyperbaric anestesia	Patent State Office for Inventions and Trademarks 81100/1975 I. Curelaru, C. Furnica, V. A. Voicu, E. Ionescu-Visan, C. Miu, P. Nitescu, L. Ivanescu, I. Manolache
3	Pharmaceutical combinations with antiseptic effects	Patent State Office for Inventions and Trademarks 65237/1977 Al. Bors, V.A.Voicu, C. Rizescu
4	Dynamic repolarization solution	Patent State Office for Inventions and Trademarks 65235/1977 Al. Bors, V .A. Voicu
5	Anti-arrhythmic compound	Patent State Office for Inventions and Trademarks 65236/1977 Al. Bors, V .A. Voicu
6	Equipement for objectifying drug interactions	Patent State Office for Inventions and Trademarks 74803/1980 Al. Ionescu-Stoian, V. A. Voicu, M. Jiquidi
7	Anti-arrhythmic compound and synthesis process	Patent State Office for Inventions and Trademarks 69027/1978 Al. Bors, V. A. Voicu, V. Aldea, E. Olteanu
8	Radioprotective compound	Patent State Office for Inventions and Trademarks 3198/1970 Al. Bors, V. A. Voicu, C. Paun, Gh. Dumitrescu
9	New anti-hypertensive compund	Patent State Office for Inventions and Trademarks 76911/1980 V. A. Voicu, St. Muresan, Gh. Radu, M. Jiquidi
10	New anti-hypertensive compund	Patent State Office for Inventions and Trademarks 76912/1980 V. A. Voicu, St. Muresan, Gh. Radu, M. Jiquidi
11	New antiseptic compound	Patent State Office for Inventions and Trademarks 80051/ 1979 Al. Bors, V. A. Voicu, A. Karassi
12	Anticholinergic compound	Patent State Office for Inventions and Trademarks 87010/1985 V. A. Voicu, Al. Ionescu-Stoian, L. Antoniu, M. Jiquidi
13	Herbal compound with anti-inflammatory, analgesic and vasoprotective action	Patent State Office for Inventions and Trademarks 87147/1985 Al. Ionescu-Stoian, L. Antoniu, V. A. Voicu, V. Florea
14	Complex antidote for the absorption, neutralization and inactivation of ingested toxins	Patent State Office for Inventions and Trademarks 99921/1989 V. Mihaila, V. A. Voicu
15	Medicine with anti-inflammtory, analgesic and decongestant effect	Patent State Office for Inventions and Trademarks 102954/1991 V. A. Voicu, C. Mircioiu, Al. Ionescu-Stoian, M. Jiquidi, E. Rolea
16	Collagen bandage for the treatment of burns	Patent State Office for Inventions and Trademarks 103550/1991 V. A. Voicu, V. Trandafir, T. Giurgiu, T. Safta, D. Burlacu, A. Nica, L. Dragoescu
17	Medicine with anti-inflammtory, analgesic and decongestant effect	Patent State Office for Inventions and Trademarks 106333/1996 V. A. Voicu, M. Jiquidi, C. Mircioiu
18	Method and compounds for the simultaneous determination of acetyl-cholinesterase and pseudo-colinesterase in blood	Patent 67856/19.10.1976
19	Versatile antidote against chemical agents, with effect on skin lesions and mucous membranes	Patent 7959/23.06.1978
20	Device for measuring the caloric response	
21	Protective mixture for impregnating fabrics	
22	Device for measuring contamination with radioactive isotopes of the human body	
23	Device for rapid desiccation of biological or chemo-pharmaceutical compounds	
24	Medicinal product with specific anti-stress, anti-wear synergistics, polyvalent, neuropsycholobiological revitalization effects	Invention Patent No. 87905/2005
25	Immuno-enzymatic test for the detection of microorganisms	Patent 91566/1984/09.01.1987
26	Enzyme conjugation process	Patent 90105/1984
27	Herbal compound with anti-inflammatory, analgesic and vascular-protective effect	Patent 9992/30.10.1989
28	Collagen bandage for skin lesions	Patent 103550/20.02.1989

Military medicine has had and has the conceptual capacity to make antidotes for CBRN medical protection, some of which have been patented, but it does not have the production capacity. It is possible to arrange or build a self-production capacity for specific antidotes, as orphan drugs, on constructive variants, depending on complexity. The total costs are important, but the objective can be amortized in a short time if the supplier has a regional opening and the social benefit is very high.

The problem of self-injection syringes

We consider that the most important and stringent problem is to equip the active and the non-commissioned military staff and the risk group population, with self-injection syringes (over 1 million people in Romania), according to NATO norms (STANAG 2871 CBRN MED, 3-6 syringes per person, plus reserves); therefore, a total of over 3-6 million self-injection syringes.

The issue of providing CBRN antidotes in Romania is not solved by NATO standards. For example, the RDG model self-injection syringes, made of stainless steel, that were in the Romanian Army's endowment, were withdrawn in the 1980s and have not been replaced until now. The Romanian model made of plastics (made by the Center for Military-Medical Scientific Research as an experimental model, a functional experimental model, a prototype and a zero series) was no longer manufactured in series due to the privatization of the manufacturer (the Plastics Factory Bucharest). At present, the Center for Military-Medical Scientific Research has developed a prototype of self-injecting syringe for antidotes, with the intention to carry out a technological transfer through projects funded by the Ministry of Research and innovation.

THE PHARMACEUTICAL LABORATORY

The pharmaceutical laboratory for the production of antidotes for CBRN medical protection must be a basic component of CBRN medical protection. It must be complex, must have functional autonomy (space, equipment, staff and specific procedures) and must operate in compliance with national and international norms in the field. Its main activity shall consist in the pharmaceutical research, manufacture and pharmaceutical control of CBRN medical protection medicines (antidotes and decontaminants), as well as the storage (in pre-determined quantities) and must be in charge of their distribution (in effect of an order) to the military or the civilian health network, nationally or internationally.

The Romanian Army had 17 facilities authorized for the microproduction of military-specific drugs, including antidotes: 12 in the pharmaceutical laboratories of the Military Hospitals, one in the pharmaceutical laboratory at the Bucharest Garrison Health Center (led by chief pharmacist colonels (eg Col. Pharm. Dr. Oiţa from Iasi who prepared and patented over 100 innovative drugs), a laboratory for blood and derivatives at the Hematology Center (led by Col. Pharm. Dr. Coreni), a galenic laboratory for antidotes at Military Medical Research Center (CCSMM) (led by Col. Pharm. Prof. Dr. Mircioiu), one at the Pharmacology and Drug Control Laboratory (led by Col. Vatasescu), a microproduction laboratory for specific drugs for the military staff, at the Sanitary Warehouse (led by Col.Pharm. Rosnovschi), and a chemical synthesis laboratory for antidotes (led by Col. Pharm. Miu) was also operating at CCSMM. These had incorporated a department for injectable and infusible medicines and were able to prepare any master or galenic drugs recommended by clinicians or researchers. However, all were abolished at the restructuring of the army from the beginning of this century, and the current legislation no longer allows them to be re-instated.

We recall that the Medical Directorate had a Pharmaceutical Microproduction Laboratory, with an old tradition, that provided specific sanitary-pharmaceutical materials, but it was abolished, after almost a century of existence, at the entry into force of the Medicines Law, because it did not comply with the requirements imposed by the European Union, although strictly speaking, they refer only to the “marketing authorization” of the products.

It can also be exemplified based on the experience of CCSMM, that had a Galenic Laboratory, which implemented the products created by the researchers from the laboratories of the Center (under direction of General Prof. Victor A. Voicu, MD, PhD, SR, member of Romanian Academy) but which was renamed and reorganized, because the new Medicines Law no longer provides the "galenic" category. This laboratory, with pharmacists and chemists, had the task of pharmaceutical, pharmacological research and prepared the reagents and drugs resulting from scientific research activities of the center, in quantities of up to 50 doses per lot, in order to remain in the field of microproduction. The products were used for physico-chemical tests, animal experiments or cell cultures and some were distributed to units located in Operations Theaters (for example in Iraq, Somalia, Angola), in applications (for example Campulung, Brasov, Tulcea), in specific missions (for example, anthrax, cholera, BDA epidemics), have been presented at different thematic exhibitions (Clinceni, Central Army House, Expomil, Inventions, BSDA) and many have been patented as inventions or innovations. However, at the present moment, the Romanian Army no longer has any facilities for drug production, so neither specific CBRN antidotes nor qualified personnel for pharmaceutical preparations.

REQUIREMENTS AND LEGISLATION IN FORCE FOR CERTIFICATION/ACCREDITATION/OPERATION

After designing, obtaining the necessary permits and financing, the platform can be built and the specific endowment can be started. From the moment it is put into operation, with the commissioning protocol, the sanitary-veterinary authorization of operation is requested from the Center for Preventive Medicine, then the RENAR and ANMDM approval for the manufacturing authorization. After completing these mandatory steps, it can be proceeded to micro-production, but for each particular product the marketing authorization must be obtained.

Corroborating the need of medical research to physically make the newly designed products (medicines, diagnostic kits, sets and medical devices), at the level of experimental model (EM), functional experimental model (FEM), prototype and/or technological demonstrator, in order to obtain the approval, commissioning protocol, authorisation and possibly patenting (as an invention or industrial model), with the need for the industry to carry out the microproduction and serial production of 0 series for clinical trials and obtaining the marketing authorization (MA), the integrative principle must apply: research, education and production.

The current legislative requirements can be found in: the Guide on good manufacturing practice for medicinal products for human use, the Guide on good practice in drug distribution, the Order 1807/2006 regarding the approval of the Norms for the application of some provisions of Regulation no. 141/2000/EC on orphan drugs, entered into force in 2007, GMP Regulations – Good Practice Regulations in production, consultancy-certification.ro, etc.

CONCLUSIONS

Medical countermeasures for CBRN defense are based on the prophylactic and curative treatment of the infected and the sick. Depending on the rapid and correct etiological diagnosis, the appropriate treatment is immediately implemented. However, in time of peace, this case is almost non-existent, and during the war or during a terrorist attack it can be a mass pathology, like an explosive epidemic, in which the forces and means involved for medical counter-measures become insufficient.

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VARIA

The synthesis and in vitro testing of symmetric bisquaternary salts with pyridin-isonitrosoacetanilide structure, active in the exposure to neurotoxic chemical agents

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Abstract: *Neurotoxic chemical agents induce complex toxicological effects with major adverse effects for those who are exposed to them. Medical countermeasures include administering atropine, a cholinesterase reactivating oxime and an anticonvulsant. The purpose of this reseach is the optimization of an antidote-based treatment in the intoxication with organophosphates through the synthesis and toxicological testing of new compounds with a pyridin-isonitrosoacetanilide structure and their comparison with obidoxime as reference.*

The objective of this study was the in vitro/in vivo assessment of the antidote properties and acute toxicity of synthethic oximes active in the exposure to neuroparalytic organophosphates.

The CT50 value for obidoxime was estimated, through calculus, to be 3.24 mg/ml and 9.33 ×10 -6 M respectively. The CT50 value for Bis1,3[2-hydroxyimino-N-(pyridyl)acetamide]oxapropandichloride (HIN 2) was estimated to be 2.72 mg/ml and 6.29 ×10 -6 M respectively.

The studied compounds showed values close to cellular viability at low and moderate equimolecular concentrations, administered in vitro to a cellular culture of fibroblasts. Bis1,3[2-hydroxyimino-N-(piridyl)acetamide]oxapropandichloride (HIN 2) showed an in vitro cellular viability value smalled than obidoxime at higher equimolecular concentrations, proving itself to be more toxic than obidoxime at high concentrations. Taking into account the close values of CT50, the newly synthethized compound, having this particular characterization from a toxicological standpoint, qualifies for further studies as an antidote.

Keywords: *paraoxon, acetylcholinesterase, obidoxime, N-acetyl-2-hydroxyiminomethylpyridinbromide (PYC8); 1,3(2-hydroxyimino-N-pyridin-3-propan bromide (HIN1); 1,3(2-hydroxyimino-N-pyridin-3-oxapropanchloride (HIN2)*

INTRODUCTION

High risk organophosphates (sarin, soman, tabun) are extremely toxic substances with a phosphoric acid esther structure. Due to their extremely high toxicity, they were registered on the list of substances that require destruction according to international regulations (Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction – Geneva). Their production, stockpiling and use are monitored by international organizations (Organization for prohibition of chemical weapons – OPCW). [4, 7, 23].

Neuroparalytic chemical agents have the property of irreversibly inhibiting erythrocyte acetylcholinesterase (AChE). The coupling reaction of the organophosphate at the level of the active site of the enzyme is shown in Figures 1, 2. [23]

The inhibition of AChE leads to the acummulation of acetylcholine (ACh) at the level of the synaptic cleft and the hyperstimulation of muscarinic and nicotinic receptors. The

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clinical signs of mild intoxication are pupil constriction, sweating, muscular fasciculation, and the decrease of cardiac frequency and of arterial tension.

Figure 1: The coupling mechanism of the organophosphate at the level of the active site of AchE

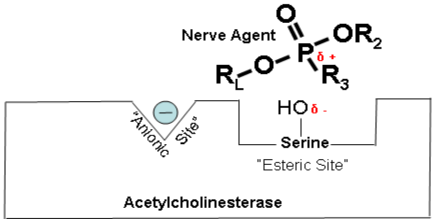

In high concentrations of the toxic compounds, convulsions occur and death takes place through respiratory insufficiency caused by the paralysis of respiratory centers. Cholinesterase-inhibiting neurotoxic organophospates possess, in their molecule, two organic groups, identical or different, and an active group that is inorganic or organic, bound to a pentavalent phosphorus atom. This atom can be bound to an oxygen or sulphur atom (phosphates, phosphonates, tiophosphates, tiophosphonates). [1, 4, 9, 23] In scientific literature, they were presented as potential simulants for each class or warfare chemical agents [4, 16, 22].

The laboratory use of neurotoxic chemical agents is restricted, as they are extremely toxic substances. Thus, antidote studies and those refering to their pharmacotoxicological properties are performed utilizing simulants of the paraoxon type. A simulant is defined as a compound possessing all relevant physical and chemical properties of the corresponding neurotoxic chemical agent, but with lower intrinsical toxicity; The utilization of simlants in research and testing activities contributes to the reduction of the stress and risk the personnel involved in the testing procedures is subjected to by handling chemical agents with high toxicity. [1, 23]

Figure 2: The interaction between AchE and the organophosphate

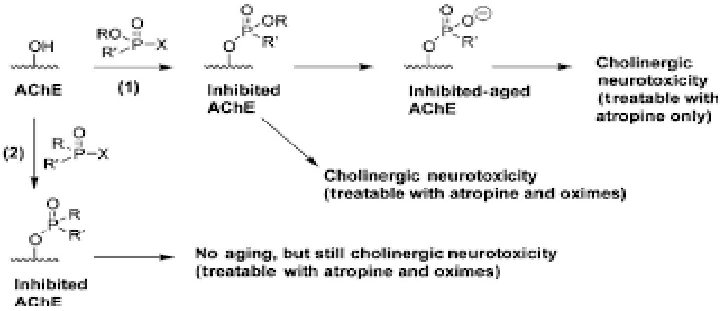
OH
AChE
RO
R'
P—X
(1)
OR
R'
Inhibited AChE
R'
Inhibited-aged AChE
Cholinergic neurotoxicity (treatable with atropine only)
Cholinergic neurotoxicity (treatable with atropine and oximes)
(2)
R
R'
P—X
R
R'
Inhibited AChE
No aging, but still cholinergic neurotoxicity (treatable with atropine and oximes)

The general emergency measures necessary to stop the exposure are masks, decontamination and the evacuation of the individual from a contaminated environment. Medical countermeasures are the administration of atropine, of a cholinesterase reactivating oxime and of an anticonvulsant. The increase of antidote treatment efficiency in the intoxication with organophosphates is one of the main objectives of research activity at national and international level, taking into account both aspects of clinical toxicology of organophosphates used as pesticides and of experimental toxicological research of neuroparalytic war chemical agents. [4, 8, 12, 16, 19] Cholinesterase-reactivating compounds used in the UN member states (pralidoxime, obidoxime, HI6) possess therapeutic efficiency for different neuroparalytic chemical agents [1, 2, 3, 5, 11, 15, 17, 22]. Having quaternary and bisquaternary salts chemical structures with pyridinic rings, this substances are known and studied as efficient antidotes in the processes of activation-reactivation of acetylcholinesterase in the intoxication with organophosphates [6, 10, 22].

Bisquaternary compounds such as Hagedorn, HI-6, Obidoxime, are obtained through the quaternization of pyridine bases substituted (oximes, carboxamides) with reactive dihalogenated derivatives such as symmetric dichloromethylether (DCDMS) or 1,3-dibromopropane [6, 14, 22]. Symmetric quaternary salts obtained through the quaternization of pyridine substituted with oxime or amide groups in a 2:1 molar ratio to the dihalogenated derivate are intensely studied. The typical example is represented by obidoxime, the product of 4-pyridinaldoxime bisquaternization with exces symmetric dichlorodimethylether. This compound is easily obtained and has good stability. [6, 7, 10, 12, 14, 22].

Through the monoquaternization of a pyridine base or of another cyclic tertiary amine with DCDMS or 1,3-dibromopropane in excess, the monoquaternary salt is obtained, usually unisolable that, in the next step, can be quaternized with another different base, obtaining mixed products with an antidote efficiency that is higher and a reduced stability. In (bis)quaternization, tertiary bases that can be utilized are pyridines which are monosubstituted with aldoxime or carboxamide groups in all three positions, 1,3-N-methyl-2-imidazolilaldoxime or quinuclidinic bases: 3-quinuclidinonoxime, 3-quinuclidinyl-N,N-dimethylaminocarbamate etc, which, theoretically, through combination with dihalogenate derivatives cand lead to a large variety of antidotes [6, 14, 19, 20].

The present paper proposes the synthesis and characterization of new symmetric bisquaternization compounds with 3-pyridinylisonitroso-acetanilide as a precursor, the quaternization agents being symmetric dichlorodimethylether and 1,3-dibromopropane. These compounds are PAM-2 analoges, N-Methyl-2-hydroximinomethyl pyridine chloride being utilized for the first time as an antidote in the intoxications with neurotoxic organophosphates. [14, 19, 21]

MATERIAL AND METHOD

The reactivating potential of two oxime compounds with pyridin-isonitrosoacetanilide structure was tested in vitro: 1,3(2-hydroxyimino-N-pyridin-3-propane bromide (HIN1); 1,3(2-hydroxyimino-N-pyridin-3-oxapropanchloride (HIN2). The HIN 1 compound did not disolve in the culture medium. The acute toxicity of obidoxime and of HIN 2 was assessed through the determination of cellular viability the two produced on cell cultures from 3T3 fibroblast lines. The cells were incubated for 24 hours at concentrations of 1 mg/ml, 3 mg/ml, and 5 mg/ml and optical density was read at 490 nm.

For this purpose, two compounds with pyridinisonitroso-acetanilide structure were tested, compounds considered active in the intoxication with neuroparalytic chemical agents due to the presence in their molecule of the oxime group R=N-OH, as well as of the pyridine structure resembling obidoxime. (Figure 3)

Figure 3: Chemical structures of newly synthethized compounds

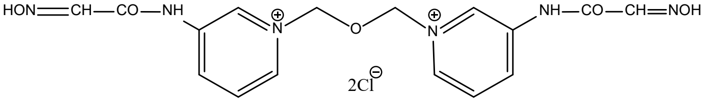

$C_{16}H_{18}Cl_2N_6O_5$
Masa Moleculara; 445,26

bis 1,3[2-hidroxiimino-N-(piridin-3-il)] oxapropandiclorura

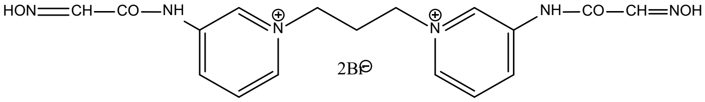

$C_{17}H_{20}Br_2N_6O_4$
Masa Moleculara: 532,19

bis 1,3[2-hidroximino-N(piridin-3-il)]propandiona

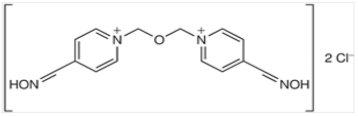

$C_{14}H_{16}N_4O_3Cl_2$
1,1-bis-metilen-bis-hidroxiiminometilpiridinium
Molecular mass 358,75

The synthesis of new symmetric pyridin-isonitroso-acetanilide bisquaternary salts with cholinesterase reactivation properties

The sythesis of the isonitrosoacetanilide of 3-aminopyridine named 2-(hydroxyimino)-N-(pyridin-3-yl) acetamide.

The isonitrosoacetanilide of 3-aminopyridine is obtained from 3-aminopyridine, chloral hydrate and hydroxylamine chlorohydrate in a saturated solution of sodium sulphate according to the following reaction in Figure4.

Figure 4: The synthesis of 2-(hydroxyimino)-N-(pyridin-3-yl) acetamide.

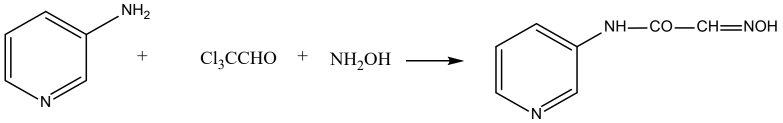

In a 2L balloon with efficient mechanical agitation, around 400ml of water are introduced. Under agitation, the sodium sulphate is brought in portions, and the chloral hydrate is added to the saturated solution within 2-5m. Seperately, the amine chlorhydrate is prepared in an aqueous solution of amine, 10ml HCl 35 and 20ml water. It is added under agitation to the previously prepared solution within 5m. Then, it is heated at 30-40^{0}C, and the hydroxylamine chlorhydrate is added in small portions after being dissolved in 50ml of water. The temperature is steadily increased to 80-90^{0}C within 1h, followed by boiling for another 15m. Then it is left to cool down at room temperature overnight and the sodium sulphate is filtered. The sodium sulphate is then washed twice with 50ml of water each time, and the filtered solution is added to the previous one. The reunited filtered solutions are neutralized with saturated ammonia solutions and are cooled down to 0-5 degrees. The precipitate is filtered, and the resulted solution undergoes extraction three times with 150ml methylen chloride each time. Through distillation, the solvent is removed, and the residue is recrystallized from methanol (through boiling with animal charcoal). The isonitrosoderivative is obtained with a yield of 65-70 %.

The compound was characterized through spectral RMN analyses, hydrogen and carbon spectrums which confirm the structure(Figure 5).

Figure 5

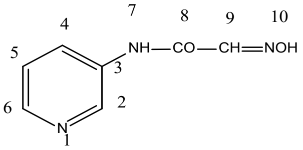

RMN characterization

1H-RMN – 1H-RMN (DMSO-d6, δ ppm, J, Hz) spectrum: 10.32(s, 1H, H-10); 8.85(dl, 2.6, 1H, H2); 8.29(dd, 4.4, 1.3, 1H, H-6); 8.12(dl, 4.3, 1H, H-4) (Figure 13).

Quaternization with symmetric dichlorodimethylether (DCMS) to bis1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamidî oxapropandichloride

The symmetric quaternization reaction of the isonitrosoacetanilide of aminopyridine with dichloroether in a 2:1 ratio is performed in anhydrous dimethylformamide at temperatures of 30-40^{0}C. The quaternization agent, symmetric dichlorodimethylether, is obtained from paraformaldehyde and thionyl chloride (Figure 6).

Figure 6: Isonitrosoacetanilide 3-aminopyridine –H-RMN spectrum

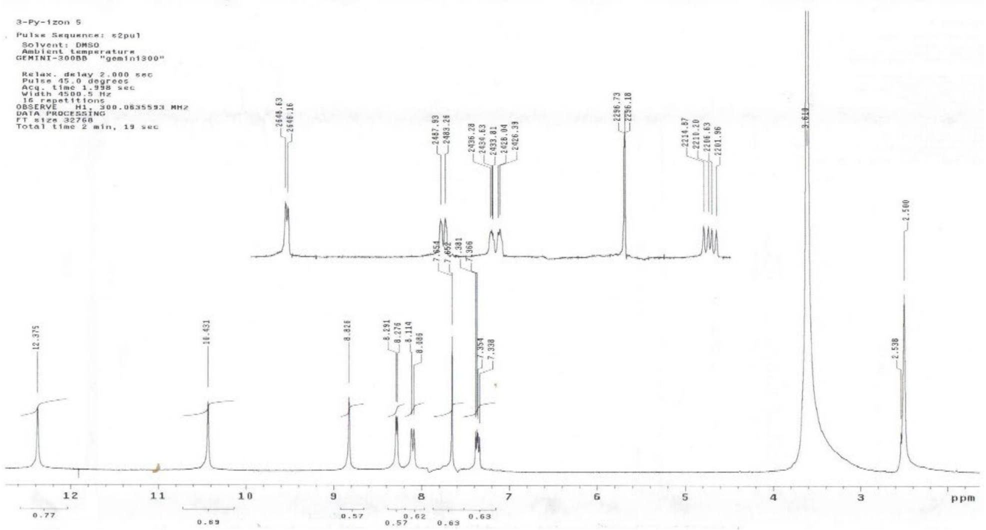

13C-RMN – 13C-RMN (DMSO-d6, δ ppm) spectrum: 160.91(C-8); 144.77(C-9); 143.80(C-6); 141.60(C-2); 135.22_C-3); 127.04(C-4); 123.69(C-5).

In an Erlenmayer, dimethylformamide and isonitroso-acetanilide are introduced. They are magnetically agitated to complete dissolution, and then dichlorodimethylether that was previously dissolved in 5ml of anhydrous dimethylformamide is added. The recipient is then sealed and agitated for 10h at room temperature and, finally, another 5h at 30-40^{0}C. It is cooled at 0-5^{0}C for 3h and is then filtered through vacuum filtering. The precipitate is repeteadly washed with anhydrous acetone and then dried in a vacuum, in the desiccator. 2.3 g of white product are obtained with m.p=145-150^{0}C, which corresponds to a yield of 52%. The synthethized compound, the bisquaternary salt, was characterized through spectral RMN and proton and carbon spectrum analyses which confirm its structure.

Figure 7: Isonitrosoacetanilide 3-aminopyridine - C- RMN spectrum

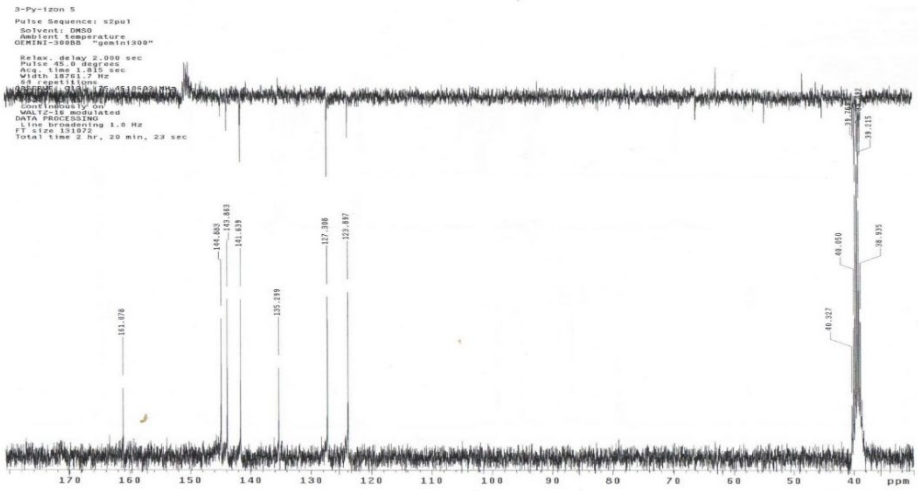

Figure 8: The synthesis of bis1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride

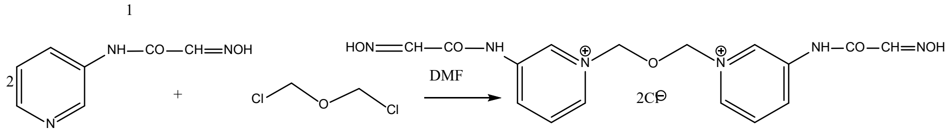

RMN characterization of the 1H-RMN hydrogen spectrum is shown in Figure 9 and the13C-RMN carbon spectrum is shown in Figure 10.

Figure 9: Bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride compounds - 1H-RMN spectrum

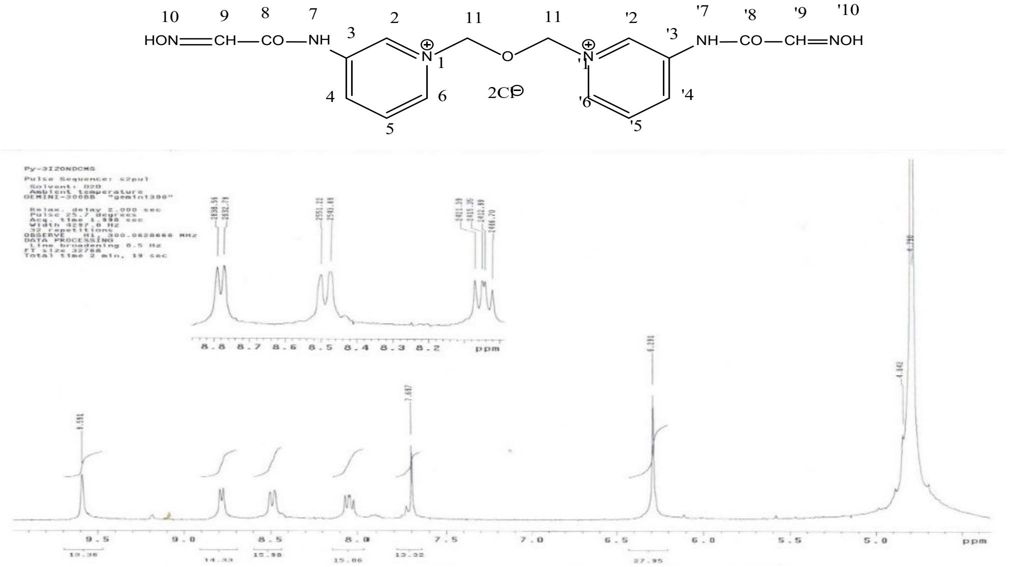

1H-RMN (D2O, δ ppm, J, Hz) hydrogen spectrum: 9.58(sl, 2H, H-2, H-2'), 8.77(dl, 6.0, 2H, H-6, H-6'), 8.48(dl, 9.7); 8.04(dd, 8.7, 6.3, 2H, H-6, H-6'), 6.28 (s, 4H, H-11, H-11')

Figure 10: Bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride compound - C-RMN spectrum

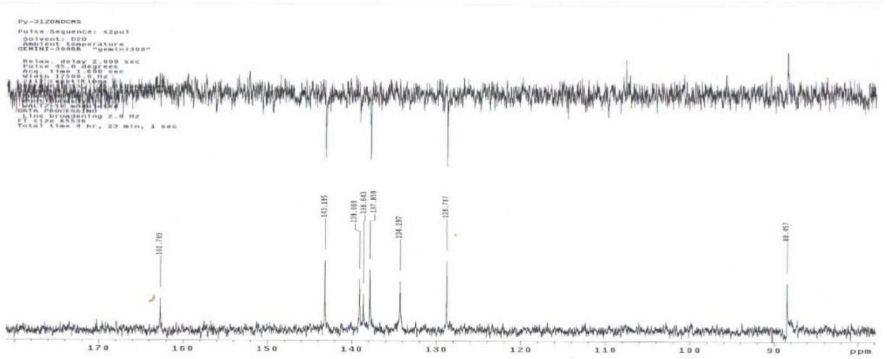

3C-RMN (D2O, δ ppm) carbon spectrum: 162.69(C-8, C-8'), 143.18(C-9, C-9'), 139.08(C-6, C-6'), 138.69(C-2, C-2'), 137.84 (C-4, C-4'); 134.30(C-3, C-3'); 128.79(C-5, C-5'); 88.48(C-11, C-11')

Quaternization with 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide.

The symmetric quaternization reaction of 3-pyridylisonitrosoacetanilide with 1,3-dibromopropane in a 2:1 ratio goes as described below and is also performed in anhydrous DMF DMF, at a temperature of 40-55^0 C (Figure 11).

Figure 11: The synthesis of bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propane dibromide

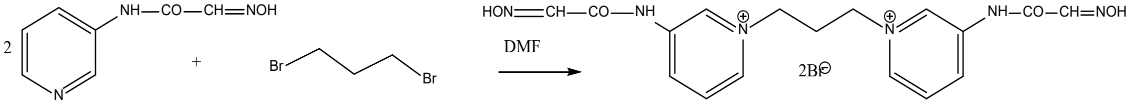

In an Erlenmayer glass, dimethylformamide and isonitrosoacetanilide are introduced. They are magnetically agitated to complete dissolution, then 1,3-dibromopropane that was previously dissolved in 5ml of anhydrous dimethylformamide is added. The recipient is sealed and agitated for 4h at room temperature and for another 10h at 40-60^{0}C. It is cooled down to 0-5^{0}C, and then vacuum filtered. The precipitate is repeteadly washed on the filter, with anhydrous acetone. It is dried in a desiccator in a vacuum overnight. 3.1 g of white crystal product are obtained with mp.180-182^{0}C and a yield of 59.0%.

The synthethized compound, the bisquaternary salt, was characterized through spectral RMN analyses, which confirm its chemical structure. (Figures 12, 13)

RMN characterization

The 1H-RMN hydrogen spectrum is shownin Figure 12and the 13C-RMN carbon spectrum is shownin Figure 18.

Figure 12: 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide - H-RMN spectrum

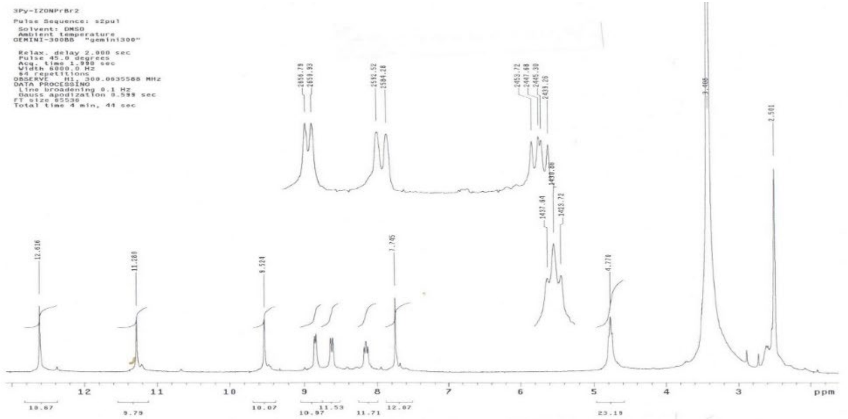

1H-RMN (DMSO-d6, δ ppm, J Hz) spectrum: 10.52 (s, 2H, H-7, H-7); 8.8 (dl, 2.4, 2H, H'-2, H-2); 8.3(dd, 5.0; 1.6, 2H, H-6, H-6); 8.1 (dl, 8.4, 2H, H-4,H-4)', 7.68(s, 2H, H-9, H-9'); 7.43(dd, 8.4, 4.7, 2H, H-5, H-5'), 4.71(t, 7.1, 4H, H-11, H-11'); 2.48(t, 7.1, 2H, H-12)

Figure 13: 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide - C-RMN spectrum

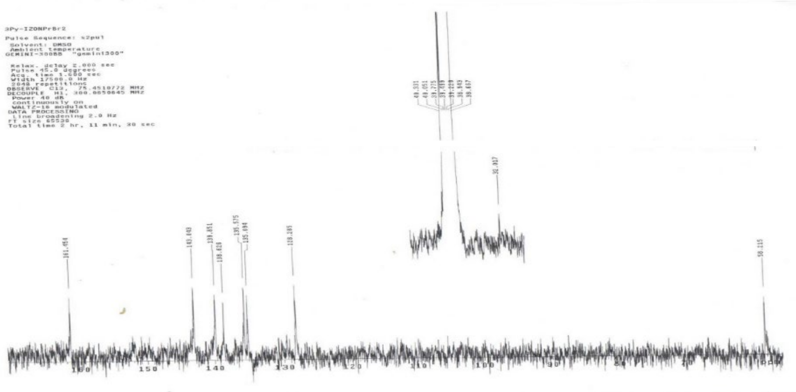

13C-RMN (DMSO-d6, δ ppm) carbon spectrum: 160.84 (C-8, C-8'); 143.97 (C-6, C-6'); 143.64 (C-2, C-2'); 140.70(C-9, C-9'), 135.36 (C-3, C-3'); 127.69(C-4, C-4'); 123.95(C-5, C-5'), 59.11(C-11, C-11'); 33.13(C12)

In vitro testing and toxicological characterization of symmetric bisquaternar compounds with a pyridin-isonitrosoacetanilide structure

The experiment was performed in sterile conditions in a microbiological niche.

In vitro testing incldued the two synthethized compounds: bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propane dibromide (coded under the name of HIN 1) and bis 1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2), utilizing obidoxime as reference. Each of these compounds was dissolved in a culture medium, and 3 solutions were obtained for each, with the following concentration: 5mg/ml; 3 mg/ml and 1 mg/ml. HIN 1 was insoluble in the culture medium at the above mentioned concentration.

On a 3T3 cellular line (fibroblasts) from Lonza from which a cellular subculture with a concentration of 1×10^5 cells /ml was obtained as follows:

- the cells were counted initially with the Burger Turck hemacytometer to establish the initial concentration of cells per ml;
- the dilution was performed so that a final concentration of 105 cells/ml would be obtained;
- the cells were cultivated with a proper medium (supplemented DMEM, Lonza reactive) for 24 hours at a temperature of 37 degree Celsius in a CO_2 atmosphere;
- 2 ml of tripsine solution are added (Sigma reactive);
- 5 minutes of centrifugation at 500 G at a temperature of 5-8 degrees Celsius;
- the supernatant is removed;
- resuspension in 5 ml of culture medium (DMEM);
- a volume of 200 microliters is placed in a plate with 96 wells;
- 6 h of incubation in CO_2 atmosphere for adherence;
- after 6 h of incubation for adherence, obidoxime and HIN2 are added after dissolution in the culture medium, equimolecularly, in 3 concentrations: 5 mg/ml, 3 mg/ml and 1 mg/ml;
- 24 h of incubation;
- after 24 h of incubation, 20 microliters of MTS reactive (Lonza) are added to each well;
- 4 h of incubation at 37 degrees Celsius in a CO_2 atmosphere.

The samples were read at 490 nm with a Biorad microplate reader and compared to the control sample represented by the culture medium.

RESULTS

The in vitro toxicity of HIN 2 and obidoxime was assessed through the values of their optical densities corresponding to different molar concentrations. The results are shown in Table 1 and in Figures 14 and 15.

The molar concentrations of studied compounds can be correlated with the cellular viability percentage.

The results are shown in Table 2 and in Figures 16 and 17.

In lower concentrations of 1 mg/ml, values that were close to the in vitro cellular viability were recorded (73.43% for obidoxime, and 82.42% for HIN 2).

For medium molar concentrations (3 mg/ml), cellular viability values were 67.75% for HIN 2 and 67.36% for obidoxime.

Table 1: The variations of optical density values of obidoxime and HIN 2

	Well1	Well2	Well3	Mean	Substraction blank
Untreated CTRL	1.068	1.112	1.67	1.283333±0.174	1.16083
Obi 5mg/ml	0.362	0.353	0.449	0.388±0.02	0.2655
Obi 1mg/ml	0.875	1.075		0.975±0.025	0.8525
Obi 3mg/ml	0.837	0.972		0.904 ±0.033	0.782
HIN 2 5mg/ml	0.141	0.149	0.152	0.147±0.012	0.0248
HIN2 1mg/ml	0.895	1.078	1.265	1.079±0.030	0.9568
HIN2 3mg/ml	0.849	0.969		0.9090±021	0.7865
CTRL BLANK	0.122	0.123		0.1225	0

Figure 14: Variation of optical density (DO) correlated with concentrations of obidoxime

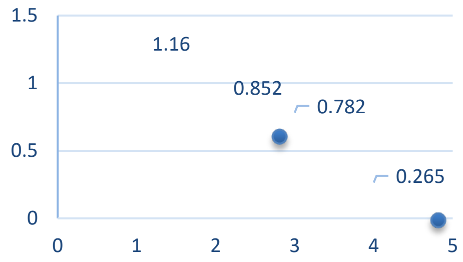

Figure 15: Variation of optical density (DO) correlated with concentrations of HIN 2

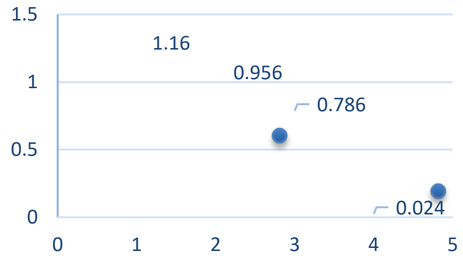

Table 2: Percetange variation of cellular viability for obidoxime and HIN 2

Dose mg/ml	DO Obidox	Cellular viability vs control (%)	DO HIN2	Cellular viability percentage vs control (%)
0	1.160833	100	1.160833	100
5	0.2655	22.8715	0.024833	2.139268
1	0.8525	73.43862	0.956833	82.42642
3	0.782	67.3654	0.7865	67.75305

For high doses, (5 mg/ml) HIN 2 showed a cellular viability value of 2.13%, much lower than that of obidoxime, and 22.87% respectively. Thus, HIN2 is more toxic than obidoxime.

Figure 16: Cellular viability percentage corresponding to different concentrations of obidoxime (y = -13.873 + 97.379)

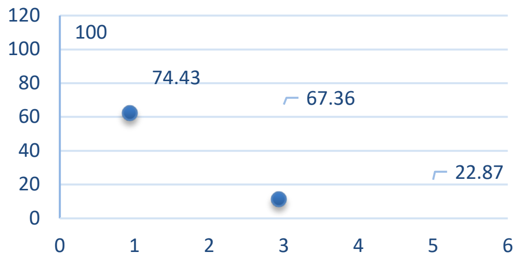

Figure 17: Cellular viability percentage corresponding to different concentrations of HIN 2 (y = -18.397 + 104.47)

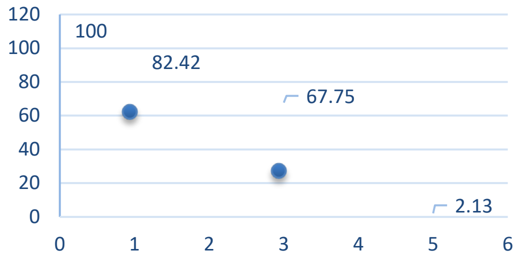

The assessment of CT50 was performed according to the working protocol described in the Romanian Pharmacopoeia.

In the regression line ecuations shown in Figures 14 and 15, the y value was replaced with the probit corresponding to the ideal mortality of 50 %. The probit is a statistical measure resulted from the transformation of the percentage of positive answer into a numeric value and is linearly dependent on the logarithm of the concentration. Thus, the x value in the ecuation of the regression line represents the logarithm of CT50. Through calculus, we could estimate CT50 for obidoxime at a value of 3.24 ± 0.005 mg/ml. The CT50 value for HIN 2 was estimated at 2.72 ± 0.009 mg/ml. The CT50 value being inversely proportional to the toxicity of the substance, it can be understood that the CT50 values are comparable (around the value of 3 mg/ml), HIN 2 being more toxic than obidoxime.

The statistical analysis of experimental results obtained showed statistically significant variations between the determined cellular viabilities of the two compounds in equimolecular concentrations only for high doses. The experimental results are represented in Tables 3-5.

Table 3: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 1 mg/ml (2.29×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	2.29×10^{-6} M;	1.160833333	0.276632	P ≥0,05 – mean values do not significantly differ
2	HIN 2	2.29×10^{-6} M;	0.956833333		

Table 4: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 3 mg/ml (6.94×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	6.94×10^{-6} M;	0.7820	0.4820	P ≥0,05 - mean values do not significantly differ
2	HIN 2	6.94×10^{-6} M;	0.7865		

Table 5: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 5 mg/ml (11.57×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	11.57×10^{-6} M;	0.2655	0.000723	P≤0,05 – mean values significantly differ
2	HIN 2	11.57×10^{-6} M;	0.0248		

CONCLUSIONS

The syntheses took into consideration the principle of obtaining a cholinesterase reactivating chemical compound that is stable, with a possible action at central nervous level, which could prove to be an alternative therapy to obidoxime, being usable in antidote studies.

Following solubility tests, only one compound, bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2) qualified for in vitro testing, the other being insoluble.

The CT50 value for obidoxime was estimated through calculus to be 3.24 ± 0.005 mg/ml, or 9.33×10^{-6} M;

The CT50 value for bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2) was estimated to be 2.72± 0.009 mg/ml or 6.29×10^{-6} M;

The in vitro tested compounds showed values close to cellular viability in low and moderate equimolecular concentrations in the fibroblast cellular culture (73.43% for obidoxime, and 82.42% for HIN 2).

The bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride compounds (HIN 2) showed a value of cellular viability in vitro lower than obidoxime at high equimolecular concentrations, proving to be more toxic. (2.13%, for HIN2, and 22.87% for obidoxime)

Taking into account the close CT50 values, the newly synthethized compound that is as such characterized from a toxicological standpoint can qualify for further studies as an antidote.

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ORIGINAL ARTICLES

The trend of bladder cancer among Iranian military community from 2007 to 2019

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Abstract: *Purpose: Cancers are one of the main causes of mortality in the world. The incidence of cancers in the world and mainly in developing countries is increasing. Due to the importance of this issue, the purpose of the current study was to assess the trend of incidence and death of Bladder Cancer among Iranian Military Community (MC) from 2007 to 2019.*

Methods: This is a cross-sectional study using time series data. All registered bladder cancer in Iranian Military community from March 2007 to February 2017 entered in this study. The future trend predicted using ARIMA (p,d,q) model. All analyzes were performed using ITSM and Excel (2010) software.

Results: According to ARIMA (2, 2, 1) model, the bladder cancer among the Iranian military community had an increasing trend. The AR (12, 2, 0) model showed an increasing trend of this cancer among males but it seems the trend of bladder cancer among females will have a constant trend AR (12, 2, 1). The death due to bladder cancer was showed a decreasing trend using AR (14, 2, 1) model.

Conclusion: In spite of decreasing trend in death due to bladder cancer, the trend of incidence of the Bladder cancer among the Iranian Military Community was increasing. However, it is necessary to adjust the effect of many factors that may have an important role in this trend.

Keywords: *bladder cancer, military community, Iran, Time Series Analysis*

INTRODUCTION

Cancers are one of the main public health problems in the world with different pattern in developed and developing countries. Only 5-10% of cancers are related to human genetics and 90-95% of them are due to exposure to environmental factors and lifestyle [1]. Cancer tumors can affect different organs. One of the common organs involved in tumors is the bladder. Bladder cancer is one of the most prevalent cancers in the world. annually there are many people getting this cancer in the world [2]. Smoking and Infection with Schistosoma haematobium are the main risk factors for the disease [3, 4]. The highest and lowest incidence of the disease was observed in Northern Europe and Asia, respectively [5]. In Iran as a developing country in Persian Gulf Region (southwest Asia), an epidemiologic transition is ongoing from communicable to non-communicable diseases, bladder cancer is sixth most common cancer [6] with estimated age-standardized incidence rate of 10.92 and 2.80 in men and women,

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respectively [7]. In spite of a lower incidence of bladder cancer in the Asian region in comparing to western countries, in Iran, the bladder cancer is one of the most prevalent urological malignancy [8]. according to the results of research, some jobs such as Truck drivers, road construction workers, mechanics, petrochemical workers, plastic and metal industries, welding, and pipeline workers are most prone to getting bladder cancer [9]. To the best of our knowledge there is no study about bladder cancer morbidity and mortality trends among Iranian Military community (active, retired, family, veterans) = MC, so this study performed to time series analysis of bladder cancer in Iranian MC. The trend analysis and prediction of future trends can provide very beneficial information for health policymakers and managers to programing and designing prevention programs. Due to importance of problem the purpose of the current paper was to describe the incidence of registered cases of bladder cancer among Iranian MC during the March 2007 to February 2017 and Forecasting its incidence in the coming years using the Iranian Armed Forces Health Insurance database.

MATERIAL AND METHODS

Study Design

In the current cross-sectional study, the required data were extracted from the registered cases in the Iranian Armed Forces Health Insurance. This data was registered according to the time of diagnosed and registered cases.

Inclusion criteria

All registered bladder cancers in the Iranian MC during the March 2007 to February 2017 entered in this study.

Data analysis

Removing non-stationary in data

Due to the non-stationary nature of the used data set, the needed transformation applied to the used time series. To remove variance fluctuation the Box cox transformation was used. In addition, to remove non-stationary in the mean of data set the differencing was used.

Selection of models

The partial autocorrelation function (PACF) and auto-correlation function (ACF) graphs were used to selection of the prediction model. By using the mentioned graphs the parameters of ARIMA models that include Autoregressive (AR) (p) and Moving Average (MA) (q) were determined.to determine the best model of prediction the different parameters of P and q were tested and the model with minimum Akaike Information Criterion (AIC) statistic was selected to the prediction of the future trend.

Assessing the fitness of the selected models

To assess the fitness of selected model the tests of randomness on residuals that include Ljung - Box statistic, McLeod - Li statistic, Turning points, Diff sign points, Rank test statistic, Jarque-Bera test statistic (for normality) also the schematic checking of the residual graph were used.

Prediction

To prediction of future trends, the ARIMA (p, d, q) model with the minimum amount of AIC was used. By considering monthly time intervals, 120 months from 21 March 2007 to 20 February 2017 were entered into the time series model and the prediction was made from March 20, 2017, to 20 August 2019. All analysis was performed using the ITSM (Interactive Time Series Modeling) software and Excel 2010 by considering the α: 0.05 as a significant level.

RESULTS

The total registered cases of Bladder cancer during the March 2007 to February 2017 were 1,640 cases. The mean age of registered cases was 70.31±11.63 years. 1,386(84.51%) of total cases were males and 254(15.49%) of total cases were females. Most of cases have had been registered in March and June respectively (Figure 1).

An increasing trend was predicted by a time series Analysis with autoregressive integrated moving average (ARIMA) model with AR = 2, MA= 1, and Akaike information criterion statistics (AIC) = 263 were used (ARIMA [2, 2, 1]) for the coming years (Figure 2).

In addition, an increasing trend was predicted among males (autoregressive model (AR) model with AR = 12, MA= 0, and Akaike information criterion statistics (AIC) = 271 were used (AR [12, 2, 0]). While a constant trend was predicted among females, for the coming years (Figure 4). For the prediction of the trend of this cancer among females the autoregressive model (AR) model with AR = 12, MA= 1, and Akaike information criterion statistics (AIC) = 281 were used (AR [12, 2, 1]) (Table 1). Regarding the prediction of Bladder cancer Mortality among Iranian Military community, the results of time series analysis showed a decreasing trend for the coming years (Figure 5). For the prediction of the Mortality trend the autoregressive model (AR) model with AR = 14, MA= 1, and Akaike information criterion statistics (AIC) = 449 were used (AR [14, 2, 1]) (Table 1).

Figure 1: The registered cases of bladder cancer among Iranian military community in different month.

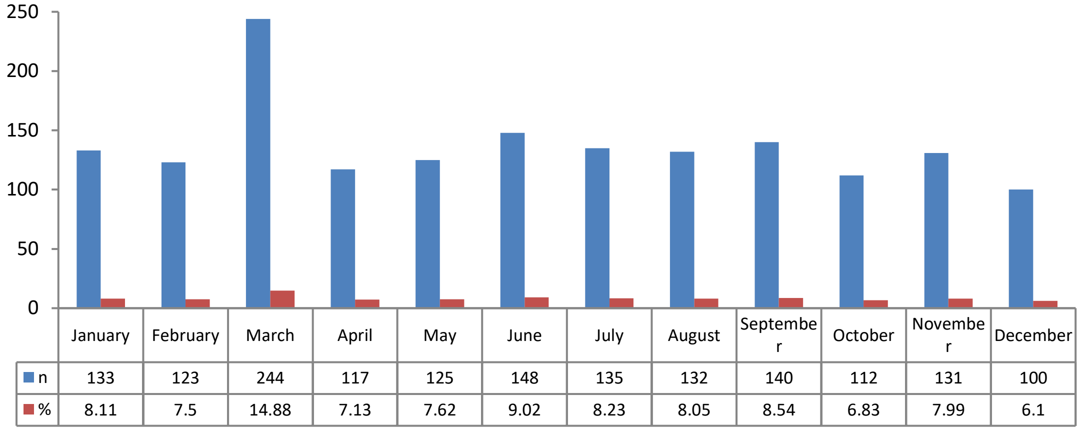

	January	February	March	April	May	June	July	August	September	October	November	December
🟦 n	133	123	244	117	125	148	135	132	140	112	131	100
🟥 %	8.11	7.5	14.88	7.13	7.62	9.02	8.23	8.05	8.54	6.83	7.99	6.1

Figure 2: The trend of bladder cancer and predicted value among Iranian military community during 2007-2019.

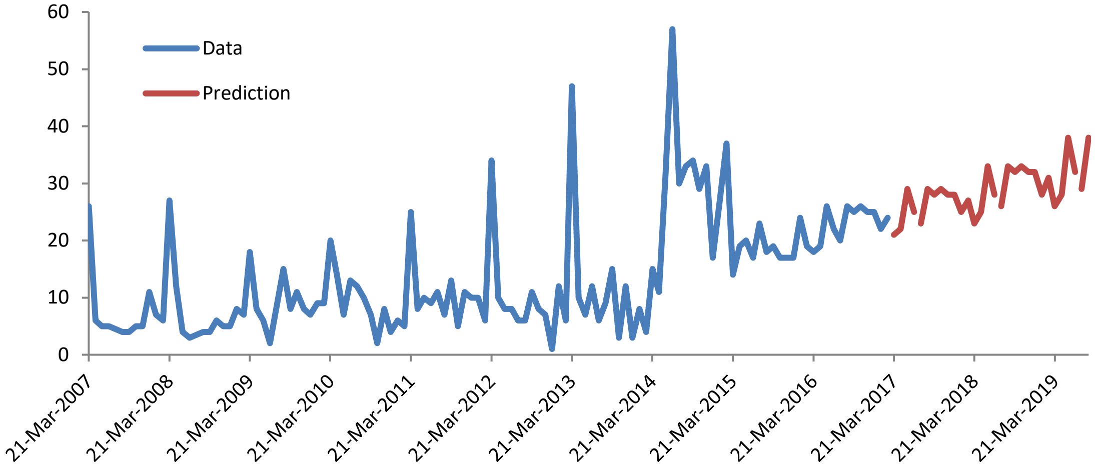

Figure 3: The trend of bladder cancer and predicted value among males of the Iranian military community during 2007-2019.

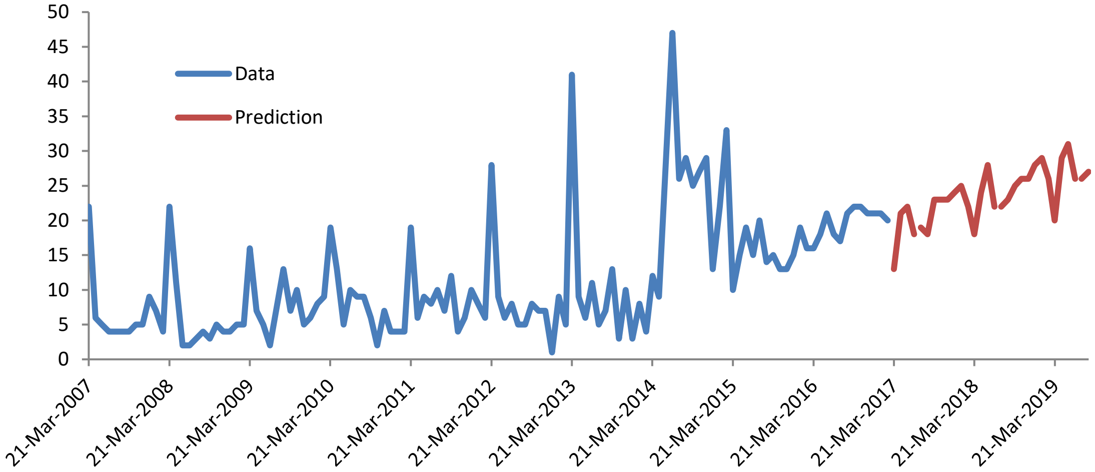

Figure 4: The trend of bladder cancer and predicted value among females of the Iranian military community during 2007-2019.

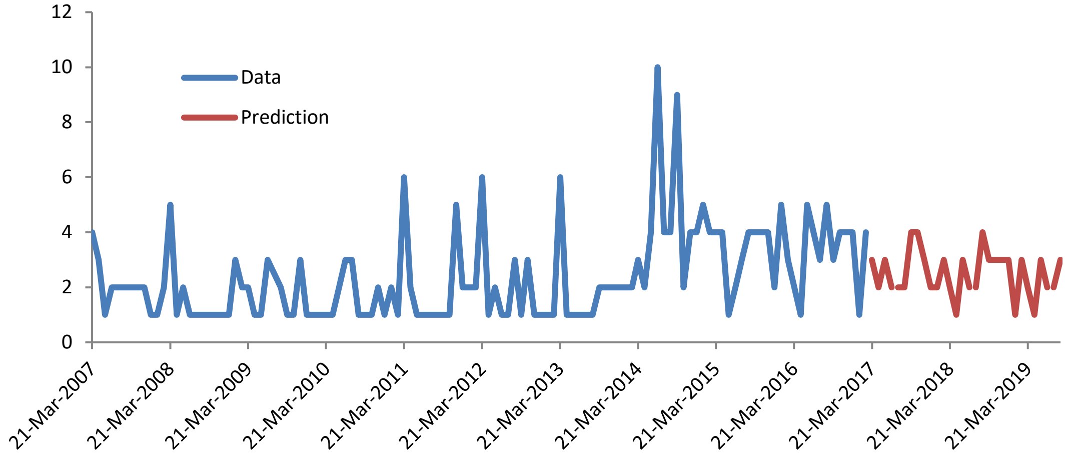

Figure 5: The trend of death due to bladder cancer and predicted value among females of the Iranian military community during 2007-2019.

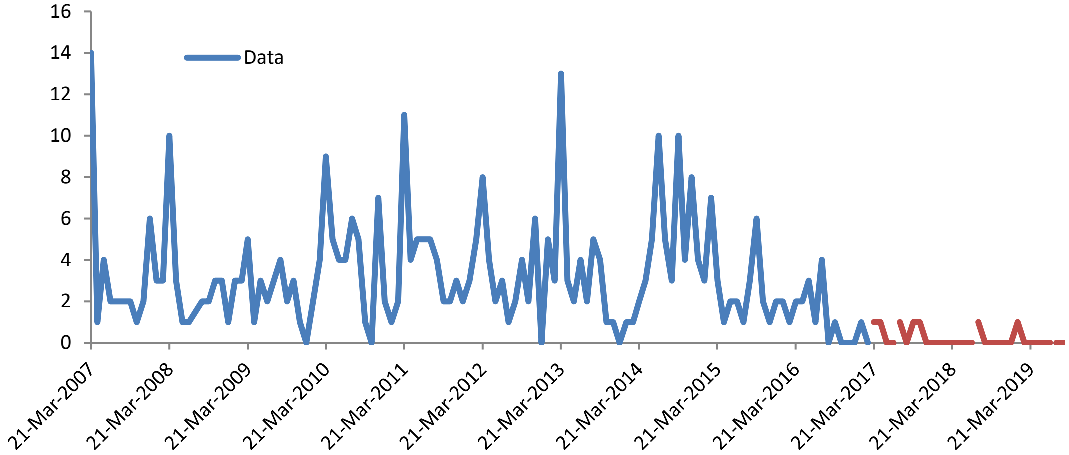

DISCUSSIONS

Cancer is one of the main causes of mortality in human populations. The high incidence of malignancies may be a consequence of some factors such as aging of the population, sedentary life style, lack of a healthy diet, specific occupational exposures, stress etc [10-12]. Age and gender are related to bladder cancer morbidity [13, 14]. The epidemiological pattern of diseases incidence in developing societies is not similar to that of industrialized nations. Therefore, the investigation and monitoring of overall patterns of malignancies is valuable and it could help public health authorities to trace the etiology and evaluate the effects of diagnostic and screening interventions [15]. Accordingly, the purpose of current study was to assess the trend of bladder cancer incidence and it's mortality among Iranian MC. To the best of our knowledge, no study has assessed both the incidence and mortality rates for Bladder cancer in Iranian MC. According to results of the current study, mean age of registered cases was 70.31±11.63 years. A research in Pakistan reported a mean age of 55.5 years [16] and a study conducted in Iran reported it as to 65.1±12.7 years [5], so it seems the risk of bladder cancer increases with increase in age. The global sex (male/female) ratio for the incidence of bladder cancer is 3.3:1.0. However, it has been reported as 1.1:1.0 in Africa and 5.1:1.0 in Europe [17]. In our sample, this ratio (5.5:1) was comparable to the figures reported in previous research in other studies [5, 16, 18]. More exposure to tobacco, opium, and occupational carcinogens in men may account for the difference in the incidence of bladder cancer between men and women [19]. The time series analysis results showed that the incident cases of bladder cancer among Iranian MC have an increasing trend. Maybe it is due to improving cancer registration system. In addition, changing some lifestyle factors, such as obesity and lack of physical activity should be regarded. Other studies also showed an increasing trend of bladder cancer in Iran [9, 20] and also in Egypt [21]. Several studies reported an increase in the overall incidence of bladder cancer [22, 23]. However, the trend of incidence may affect by some risk factors for bladder cancer, including tobacco, non-occupational and industrial carcinogens, and population aging [23]. In comparison future trend between males and females, the incidence of bladder cancer among males had increasing trend but the incidence of this cancer among females showed a constant trend. A study by Antoni et al. [24] has studied the global morbidity and mortality trends of bladder cancer. They reported that the incidence rates were diverging, with stabilizing or declining rates for men but increasing rates for females in many countries.

Table1: The related coefficients for prediction incidence of bladder cancer among Iranian military community

	Overall incidence	Incidence in males	Incidence in females	Total death
BOX COX	100	100	100	700
AR	2	12	12	14
MA	1	0	1	1
AIC	263643E+03	271944E+03	281468E+03	449345+03

Some studies showed that obesity and diabetes mellitus is associated with increased risk of bladder cancer [25-27]. However, the results of a recent systematic review and meta-analysis on Iranian military personnel indicate that the prevalence of overweight and obesity in Iranian military personnel is 41% (95% CI: 26.57%) and 13% (95% CI: 10.17%), respectively [28]. So probably, we will oppose to a higher incidence of bladder cancer in the future.

Our study has some limitations: Firstly, all analyses and predictions were performed based on registered data. Therefore, the quality of the registration system could affect the validity of the prediction. For example, to some extent, the increasing trend of incidence may be due to the improvement of the registration system and not related to the increase in the true incidence of cancer cases.

Second, as in most of epidemiological studies we were unable to distinguish between tumor stages because of the nature of the available data. This could reduce the amount of details that can be found from the analyses.

Third, sociodemographic and economic variances can affect the morbidity and mortality of bladder cancer [29]. So lack of investigation about these underlying factors, in the current study could be considered as a major limitation. For example, we don’t have any information regarding the size of the covered population by insurance organization of the Iranian military community so the increase in the size of that population may leads to an increase in incidence cases.

Finally, known risk factors, such as smoking habits, obesity, and occupational exposure may affect the interpretation of our results.

In addition, the impact of screening programs on registered cases of cancers should be considered. The improving and developing of such programs may help the diagnosis of cancer cases. Further studies are recommended in the future taking into account the mentioned points, in this regard.

CONCLUSIONS

Our study is the first report on trends of incidence and mortality of bladder Cancer in Iranian MC. Based on the results of this study the need for promoting screening programs is emphasized.

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VARIA

The use of IPACK and the continuous adductor canal block in the perioperative management of total knee prosthesis

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Abstract: *Background: Although locoregional anesthesia techniques in total knee arthroplasty (TKA) have progressed steadily, the emergence of the adductor canal block representing a step forward for postoperative evolution in preservation of the quadriceps muscle strength, analgesia of the posterior territory of the knee still remains a challenge. The emergence of IPACK, in association with earlier techniques, offers promising preliminary results in terms of its contribution to a favourable evolution and to the satisfaction of TKA patients.*

Materials and methods: Prospective trial performed on a group of 28 patients undergoing a total knee arthroplasty intervention; analgesia control in the postoperative period was performed either with opioid and nonopioid systemic analgesics, or by associating the continuous adductor canal block with IPACK and systemic analgesics. The primary objective is pain control at rest/on mobilization, while the secondary purpose is analysis of opioid use, their side effects and recovery of joint mobility.

Results: There is a significantly better pain control in the group of patients with locoregional anesthesia, both at rest and on mobilization ($p<0.005$), accompanied by a decrease in opioid use ($p<0.00001$) and the related adverse reactions; at the same time, there is an improvement in the functional recovery of the knee joint, quantified by the flexion degree at 24 and 48 hours after surgery (61.5/71.12 degrees for continuous adductor canal block with IPACK versus 45.14/55.42 degrees for general anaesthesia)

Conclusions: The introduction of peripheral nerve blocks in the multimodal analgesia regimen after TKA results in better control of postoperative pain, both at rest and on mobilization, in a decrease in opioid use and their related adverse reactions and in an improved recovery of joint range of motion, as well.

Keywords: *continuous adductor canal block, IPACK, VAS, TKA*

INTRODUCTION

Total knee prosthesis is currently one of the most common surgeries in the field of orthopedics, the number of such interventions performed annually being on an accelerated upward trend. On the other hand, the pain stimulus that occurs in total postarthroplasty of the knee is often very intense, TKA being known as one of the most painful orthopedic surgeries , pain control is a major component in perioperative management of TKA [1].

The pain occurring in the postoperative period, as well as the systemic inflammatory response initiated following the tissue trauma will have significant consequences for the

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postoperative evolution of the patients, including prosthesis infection which can compromise the joint functionality and complicate postoperative evolution [2]. It has been shown that activating an exacerbated systemic inflammatory response leads to increased rates of local and systemic complications, increased perioperative morbidity and mortality, increased length of hospitalization and patient dissatisfaction. In view of the above mentioned, the existence of a standardized and complex protocol for the perioperative management of patients with TKA becomes an absolute requirement in order to improve the postoperative evolution, decrease the duration of hospitalization and increase patient satisfaction [3]. The multidisciplinary approach is decisive in determining the prognosis both in case of primary TKA, as for modular endoprosthetic reconstruction, as well [4].

MATERIAL AND METHOD

After getting the agreement of the Ethics Commission of the Bucharest University Emergency Hospital (SUUB), a prospective monocentric trial was performed on 28 patients undergoing unilateral total knee arthroplasty surgery in the SUUB Orthopedics and Traumatology Clinic between May 2020 and March 2021.

The patients included in the trial belonged to ASA risk classes 1-3; patients with ASA risk class > IV, chronic opioid users prior to presentation, obese patients with BMI>40 kg/sq.m., known allergies to analgesic medication included in the protocol, contraindications for neuraxial anesthesia, chronic kidney disease or pre-existing peripheral neuropathy were excluded from the trial. Prior to surgery, patients were informed extensively with regard to the protocol, the technique of anesthesia and analgesia, the related risks and benefits, and the patients gave their informed consent.

The total knee prosthesis surgery was performed by experienced surgeons of the Orthopedics and Traumatology Clinic of SUUB with a parapatellar anteromedial approach, with bicompartmental prosthesis and using the hemostatic band [5].

The trial was performed on 2 groups of 14 patients each, divided according to the anesthetic management and the perioperative analgesia approach. The first group comprised of patients that received balanced anesthesia with volatile pivot and intravenous multimodal postoperative analgesia. In the second group, the anesthetic technique consisted of spinal anesthesia associated with IPACK and the continuous adductor canal block. All patients (both group 1 and group 2) received preemptive analgesia, paracetamol 1 g, Gabaran 300 mg and Celecoxib 200 mg one hour before surgery; the treatment was continued for the first 72 hours postoperatively (paracetamol 1 g every 6 hours under ASAT/ALAT control, Gabaran 600 mg/day in 2 doses, Celecoxib 200 mg twice daily). The locoregional anesthesia maneuvers were performed by anesthetists of the Anesthesia-Intensive Care Department of SUUB.

Patients in group 2 were admitted to the preoperative ward approximately one hour before the time of surgery for performing the 2 peripheral nerve blocks. The patient is placed in supine position, a peripheral venous line is inserted and the patient receives standard monitorization (according to the standards required by the Romanian Anesthesia and Intensive Care Society). To perform the adductor canal block, the patient is positioned with the thigh in slight abduction and external rotation; the patient's thigh is disinfected with 2% chlorhexidine solution, the high frequency linear probe (Philips Cx50 transducer L 12-5 50 mm) is used, which is placed transversely on the thigh in its anteromedial portion approximately at the junction between the medial and distal thirds of the thigh, and the saphenous nerve located anterolaterally to the femoral artery, under the sartorius muscle is identified; under ultrasound guidance (the in-plane approach) the Touhy needle from the Contiplex Touhy Ultra-B. Braun set is advanced under careful ultrasound visualization, from the lateral to the medial area up to the vicinity of the femoral artery. After aspiration and the prior injection of 1-2 ml in order to check the position of the needle, 20 ml of ropivacaine 0.5% is infiltrated into the upper part of the artery (Figure 1).

Figure 1: Infiltration area

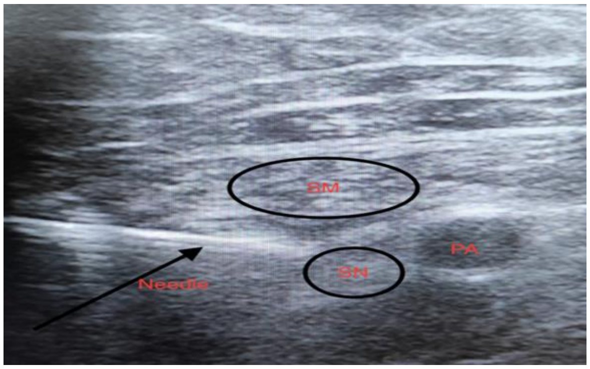

SM sartorious muscle, SN safenous nerve, PA popliteal artery

A plexus catheter is inserted in the adductor canal; the catheter is secured at 11-13 cm with Histoacryl and Tegaderm to prevent dislocation and extravasation of local anesthetic. A mixture of ropivacaine 0.2% with Fentanyl 2-gamma/ml with Easy Pump B. Braun, with a volume of 125 ml and a constant administration rate of 5 ml/hour, is administered postoperatively, allowing for the easy mobilization of the patient. The catheter is retracted 72 hours postoperatively.

The second peripheral nerve block performed is IPACK. The patient is in a supine position with his knee slightly bent. The high frequency linear probe (Philips Cx50 transducer L 12-5 50 mm) is used, which is positioned transversely in the popliteal fossa for the visualization of the femoral condyles, the popliteal artery, the tibial nerve and the common peroneal nerve; the transducer is advanced slightly proximally until the posterior edge of the femur is visualized. After local anesthesia with 1-2 ml of lidocaine 1%, a Stimuplex Ultra 360 20G-100 mm needle is inserted, under ultrasound guidance (the in-plane approach), in the space between the popliteal artery and the posterior edge of the femur, from the lateral to the medial area, and the local anesthetic is infiltrated, 20 ml of ropivacaine 0.5% in a single injection (Figure 2).

Figure 2: Infiltration area for second peripheral nerve block

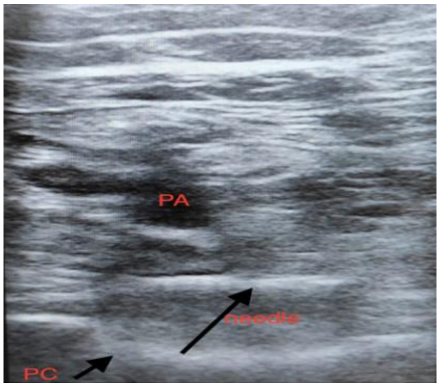

PA popliteal artery, PC posterior capsule

Postoperatively the patients are admitted to the postoperative intensive care unit for the first 24 hours. The patients in group 1 (general anesthesia) enter the multimodal analgesia protocol with morphine combined with paracetamol 1 g/6 hours, Gabaran 300 mg/12 hours and Celecoxib 200 mg/12 hours. Postoperative analgesia of patients in group 2 (spinal anesthesia with peripheral nerve blocks) is ensured by the administration of local anesthetic via adductor canal catheter, supplemented by the administration of paracetamol 1 g/6 hours, Gabaran 300 mg/ 12 hours and Celecoxib 200 mg/12 hours.

The primary objective of the trial is to record the pain score by means of the visual analogue scale (VAS) both at rest and in activity, while the secondary objectives that we followed are the consumption of opioids in the two groups, their adverse effects and the degree of mobility of the joint that correlates directly with a quick postoperative rehabilitation of the joint.

Statistical analysis

The data recorded is processed using descriptive statistics, Student t-test. The nature of the hypotesis testing was two-tailed, and P<0.005 was considered statistically significant.

RESULTS

From a demographic point of view, no significant differences are observed between the two groups of patients (tabel 1). The preoperative pain score at rest is also comparable, and in terms of chronic analgesic use it is observed that only 4 patients in group 1 and 3 patients in the group 2 were chronically using analgesics of the NSAID category (in the last 3 month) and one patient analgesics of the weak opioids class.

Table 1: Demographic data, surgical information (values are presented as mean or number and percent)

Treatment group	General anesthesia n=14	IPACK/cACB n=14
Age(y)	62 (49-70)	68 (59-74)
Gender, male, n	4 (14)	5 (14)
BMI (kg/m²)	34 (25-39)	32 (25-38)
Surgical time(min)	117 (70-170)	130 (80-180)
VAS rest at home	3 (3-4)	4 (3-5)
NSAID consumption at home (last 3 month)	4 (14)	3 (14)
No opioid consumption at home	13 (14)	14 (14)

IPACK, infiltration of local anesthetic between posterior artery and capsule of the knee; cACB, continuous adductor canal block; BMI, body mass index; VAS, Visual Analogue Scale ; NSAID, Nonsteroidal anti-inflammatory drugs

VAS score at rest and at work on day 1 and day 2 showed significantly (p<0.005) better values in locoregional anaesthesia group (lot 2) compared to general anaesthesia group (lot 1). VAS score at rest after 8 hours postoperatively also showed significantly (p<0.025) better values in locoregional anaesthesia group compared to general anaesthesia (Figure 3).

The mean ranges of movement on days 1 and 2 postoperatively for locoregional anaesthesia group (61.5 degrees and 71.12 degrees, respectively) were significantly better (p< 0.0005) than ranges of movement on day 1 and 2 for group with general anaesthesia (45.14 degrees and 55.42 degrees).

Similary, the opioid usage was lower (P<0.00001) on day 1 for pacients who received adductor canal blockade combined with IPACK.

Figure 3: Pain numerical rating scale at rest

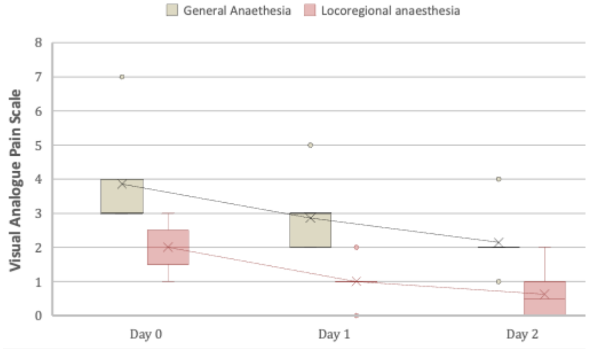

Boxes represent 25th-75th percentiles interval. X marks mean value

DISCUSSION

Because effective analgesia is largely responsible for blocking this metabolic breakdown called systemic inflammatory response, much of the efforts undertaken by clinicians have focused on controlling postoperative pain [6]. Recent decades have witnessed the wide implementation of the concept of multimodal analgesia, a protocol in which, in order to ensure effective analgesia, conventional analgesia methods (administration of opioids, paracetamol, nonsteroidal anti-inflammatory drugs, etc.) are associated with new locoregional anesthesia techniques. This makes it possible to reach an effective analgesia threshold while significantly reducing the use of opioids and analgesic drugs, therefore decreasing the adverse reactions related to their excessive consumption [7].

The role of locoregional anesthesia in the management of the TKA patient is already well established. Numerous studies have shown that the association of different peripheral nerve blocks with the administration of systemic analgesics in the multimodal analgesia protocol (the femoral nerve block and more recently the adductor canal block) makes an important contribution to the perioperative management of such patients [8, 9]. Unlike the femoral nerve block, which often results in quadriceps muscle fatigue and increases the perioperative risk of accidental fall, the adductor canal block preserves the muscular strength of the quadriceps muscle, reduces the risk of in-hospital fall and significantly improves the functional recovery of the joint [10-12]. However, it seems that the inclusion of the femoral nerve block or the adductor canal block in the perioperative management plan does not necessarily eliminate the need for opioid administration in the perioperative period [13]. This can be explained by the fact that both variants of the femoral nerve block – the classic one and the adductor canal block – will ensure the analgesia of the anterior and mediolateral territory of the knee joint, so that the posterior area innervated by branches detaching from the sciatic nerve is completely free of analgesia, hence the need to supplement with analgesics postoperatively [14].

In this sense, different solutions have been searched to ensure efficient analgesia in this territory, without affecting the motor performances that play an important role in the rapid postoperative functional recovery. Thus, the emergence of the IPACK block (infiltration between the popliteal artery and the capsule of the posterior knee) appears as a promising solution in terms of ensuring analgesia for the posterior territory of the knee joint [15, 16]. The innervation of the posterior territory of the knee joint is ensured by articular branches detaching from 3 main trunks: the tibial nerve (whose branches represent the main source of innervation of the knee posterior capsule), the common peroneal nerve (that sends terminal branches to the anterolateral and posterolateral portion of the capsule) and the obturator nerve (3 branches ensuring the innervation of the superomedial portion of the capsule) [17]. In essence, IPACK blocks the terminal sensitive branches detached from the popliteal plexus and the obturator nerve, thus ensuring analgesia of the posterior capsule of the knee, without involving motor branches and therefore without affecting the process of functional recovery of the joint. Sparing the motor branches of the common peroneal and tibial nerves and covering the posterior territory of the knee joint are two features that can make IPACK the ideal solution, along with the adductor canal block, with a view to ensuring effective analgesia of the knee joint in the postoperative period [18, 19]. From a technical point of view, this is achieved by the infiltration of a local anesthetic into the area located between the popliteal artery and the posterior capsule of the knee, an ultrasonographically guided maneuver. So far, experience with the use of the combined technique consisting in locoregional anesthesia, adductor canal block associated with IPACK, has been limited in terms of the functional recovery of the joint, the analgesic efficiency and the opioid use.

Starting from the idea that the new techniques described in locoregional anesthesia represent a major step forward in the perioperative management of TKA patients, the trial described here will analyze the efficacy of the continuous adductor canal block associated with IPACK in improving pain control and functional recovery, in reducing opioid use and the incidence of side effects, as well as in reducing the duration of hospitalization [19].

From a demographic point of view, no significant differences are observed between the two groups of patients. The preoperative pain score at rest is also comparable, and in terms of chronic analgesic use it is observed that only 4 patients in group 1 and 2 patients in the group 2 were chronically using analgesics of the NSAID category and one patient analgesics of the weak opioids class.

As regards the intensity of pain on the day of surgery, patients in the group with postoperative analgesia based on the combination of analgesics of the opioid class and nonopioids had a higher VAS score than those for whom locoregional anesthesia with systemic analgesics of the weak opioids class and nonopioids was used in the multimodal analgesia protocol. Also, there is a better control of pain in group 2, including days 2 and 3 postoperatively, both at rest and when mobilizing the knee joint with the help of kinetics.

Following the functional recovery of the joint, we notice that the patients in group 2 were able to suffer a higher degree of flexion of the joint in terms of pain, compared to patients without locoregional anesthesia. At the same time, the use of opoids was significantly higher in patients with systemic analgesia only.

During the trial, only one major adverse event occurred, namely one case of respiratory depression following opioid administration; adverse reactions such as nausea and vomiting were more common in patients with opioids, while in the group of patients with locoregional anesthesia no adverse effects were reported of the type systemic toxicity of the local anesthetic, extravasation of the anesthetic at the site of catheter insertion or in-hospital fall due to decreased muscle strength of the quadriceps muscle.

Although locoregional analgesia in the perioperative management of TKA is supported by the results of the trial, it has certain limitations. First of all, the trial is not a randomized one. Secondly, the follow-up of the patients was performed only in the first 72 hours postoperatively, until the removal of the adductor canal catheter, therefore we cannot provide any data on the further developments in terms of pain intensity and analgesic requirements after locoregional analgesia is suppressed. Further studies are needed in order to determine whether the benefit of locoregional anesthesia (joint mobilization, pain score) is maintained after its discontinuation. Thirdly, despite significantly higher opioid use in patients in group 1, postoperative pain is more difficult to control, therefore comparing the degree of joint mobility of the two groups may not be relevant, the intensity of the pain being an important limiting factor of the mobility degree.

In a prospective, triple-blind trial conducted by Kim D. et al in 2017 on a group of 86 patients undergoing primary unilateral knee arthroplasty, the association between IPACK and the adductor canal block at the periarticular infiltration of the local anesthetic provides a high level of analgesia and helps reduce opioid use compared to the joint infiltration associated with the systemic multimodal analgesia regimen. This trial supports the introduction of IPACK and the adductor canal block in a standardized multimodal analgesia protocol for patients undergoing orthopedic knee joint prosthesis surgery [20].

Another prospective trial by Patterson ME. et al. on 69 patients enrolled during the period 2016-2018 shows that the association of IPACK with the multimodal analgesia regimen improves pain control at rest in the postoperative period, but without bringing a significant clinical improvement (no significant differences were found between the pain in conditions of physical exertion, distance walked, length of hospitalization or opioid use), therefore the trial did not recommend the introduction of IPACK in the routine management of knee arthroplasty [21].

CONCLUSIONS

Although the results of the clinical trials conducted so far are controversial and long-term research is needed on this topic, the trial conducted in our center supports that the introduction of peripheral nerve blocks in the multimodal analgesia regimen after total knee prosthesis results in a better control of the postoperative pain both at rest and on mobilization, in the decrease of opioid use and implicitly of the incidence of the related adverse reactions. Moreover, the IPACK association seems to bring an additional benefit consisting in the control of the painful stimuli at the back of the knee joint, with a faster rehabilitation and increased joint mobilization, which in the long run will lead to a lower rate of complications caused by prolonged immobilization and to a higher degree of patient satisfaction.

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VARIA

The use of Laser Doppler vibrometry (Doppler principle) for middle ear research and diagnosis

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Abstract: *The middle ear represents the middle component of the human ear. Its function is to transmit sound-waves from the external auditory canal (EAC) to the inner ear via the Tympanic Membrane (TM), the Ossicular Chain (OC), and Oval Window (OW) and at the same time to act as a transformer that produces a pressure gain in the sound wave, usually specified in literature as 27-30 dB. Although a very efficient biomechanical system, the OC has, within the human hearing range, minute vibration amplitudes of only a few nm which brings forward the problem of a reliable measuring technique for such movements*

The Laser Doppler Vibrometry (LDV) also known as Laser Doppler Interferonometry (LDI) is a method of measuring such minute vibrations without contact with the anatomical structures. The laser beam can be aimed at chosen points of a structure and the movement will be recorded as a graphical representation. As such, the method has been studied and applied for numerous purposes and experiments over the last decades. The present work aims to present a comprehensive review of these experiments to define LDV as a reliable method for middle ear research and diagnostics.

Keywords: *laser Doppler vibrometry, middle ear*

INTRODUCTION

The middle ear represents the middle part of the ear, located between the external ear (pavilion and external ear canal - EAC) and the inner ear (Cochlea). It is comprised of the tympanic membrane (TM) and the ossicular chain (OC) with their tendons and muscles. The OC is represented by the three smallest bones in the human body, the hammer (malleus), the incus, and the stapes.

The physiological function of the human middle ear is to transmit sound-waves from the external auditory canal (EAC) to the oval window via the Tympanic Membrane (TM) and the Ossicular Chain (OC) and at the same time to act as a transformer that produces a pressure gain in the sound wave, usually specified in literature as 27-30 dB. Although the structures are extremely small, the dynamic range of the system is essential. A tympanic membrane only 100 µm thick will be able to transform a 20 µPa sound wave into a perceivable sound for humans. The same membrane will be able to compensate for a variation in atmospheric pressure of up to 120 kPa [1].

The acoustic transformer that is the middle ear structure, matches the low impedance of air in the EAC to the relatively high impedance of fluid within the inner ear [2]. The acoustic gain is obtained through two structure-related factors: the aria ratio (the TM area divided by the stapes footplate area)

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and the ossicular lever (the length of the manubrium of the malleus divided by the length of the long process of the incus). Sometimes a third mechanism called the catenary lever (ratio of force acting on the TM to that acting on the malleus) is described, which could bring a total middle ear gain of about 34dB [3].

“The pathology of the auditory system is one of the main reasons for improper development of oral language and a person suffering from hypoacusis is more likely to have poor social and professional integration, lower competitiveness on the labor market and will have smaller chances to complete higher education” [4, 5].

The very efficient biomechanical system comprised of the OC has, within the human hearing range, vibration amplitudes within the mm and nm range which brings forward the problem of a reliable measuring technique for such movements. Many authors also mention the variable rotational axis of the ossicles and pluridimensional vibratory patterns for the OC [6] which can prove a challenge for demonstration and recording.

Although etiologically heterogeneous, at least 50% of all hearing losses can be explained by a genetic background while the rest is directly linked to the presence of external factors (environmental and clinical perinatal) [7].

The Laser Doppler Vibrometry (LDV) also known as Laser Doppler Interferonometry (LDI) is a method of measuring the minute vibrations of the TM and OC without contact with the anatomical structures. The laser beam can be aimed at specifically chosen points of a vibrating structure and the movement will be recorded as a graphical representation and can also provide acoustic results over headphones. This can also be used for measuring the transfer function of the middle ear (METF) in different situations such as intact middle ear, disrupted OC, reconstructed middle ear, etc.

THE PRINCIPLE OF LDV

Based on the Doppler shift principle, a Laser-Doppler Vibrometer compares the frequency of a laser’s emitted light to the frequency of the light reflected from a moving object and is capable to determine the instantaneous velocity of that object. We should consider the physical principle as medical research usually requires a high degree of abstraction [8].

The principle of the method is aiming the laser beam at an object that moves with the velocity v (for example TM). By reflecting the beam, a frequency change results between the incoming and the reflected beam (fd). This frequency change is proportional to the object’s velocity. The physical principle is usually presented using the following formulas:

$$fd = \frac{2v}{\boldsymbol{\lambda}} \qquad f' = \frac{v + v_0}{v - v_s} f \qquad \frac{\Delta\boldsymbol{\lambda}}{\boldsymbol{\lambda}_0} = \frac{v}{c}$$

Where:

fd – Doppler frequency; λ – wavelength of the beam; v – velocity of an object; f – actual frequency of soundwaves; f′– observed frequency; v – the speed of sound waves; v_0 *– velocity of the observer;* v_s *– velocity of the source; Δλ – wavelength shift;* λ_0 *– wavelength of source not moving; v – velocity of source; c – the speed of light*

The Laser-Doppler Vibrometer is a very sensitive, non-contacting optical displacement system capable of making displacement measurements in the ear to < 1 × 10-4 µm at frequencies from 100 Hz to above 10,000 Hz [9]. A Helium-Neon laser beam is aimed through the ear canal or the mastoid process at any vibrating site on the tympanic membrane, the malleus, or the stapes footplate. The laser beam is modulated at 40 MHz and focused on a reflective target using a lens. The reflected beam from the target site is analyzed in the detector portion of the system by using the Doppler principle, producing an output voltage proportional to the velocity of the vibrating target. Velocity is usually converted to displacement, the most commonly used measurement parameter. “The diameter of the target can be less than 1 mm. since the laser beam has a width of approximately 10 µm.” [9] The LDV is connected to an operating microscope to rapidly focus the beam on the target. “Measurements of incus and stapes vibrations can be performed as well if a TM perforation is present or during surgery.” [9] A sound-generating system delivers a constant sound pressure level (SPL) at the TM of 80-100 dB at representative frequencies within the 200-15,000 Hz range.

LDV IN EXPERIMENTAL MIDDLE EAR RESEARCH AND DIAGNOSIS

“Contact-free methods of measurement for analysis of middle ear vibrations became more and more sophisticated and allow highly accurate evaluations.” [10] The use of LDV attached to a microscope was first described by Nuttall et al. in a 1991 study of basilar membrane vibration in the guinea pig. [11] Although frequently employed for experimental use, measurements in live humans during surgery are not yet available [10].

Numerous authors have concerned themselves with the study of LDV and its different possible uses (See Table 1).

As early as 1993, Goode et al. use the LDV to measure the displacement of the umbo at SPL of 60, 70, and 80 dB in 6 live subjects and compare the results to 15 measurements of fresh temporal bones (TBs). They conclude that the measurement of umbo displacement or velocity in the intact middle ear at physiologic sound pressure over the auditory frequency range is the best way to characterize the function of the tympanic membrane [12].

Table 1: Studies that present the experimental use of LDV

Study	Status	Type of material	The target of LDV spot	Purpose of the study
Goode 1993	In vivo/In vitro	Live subjects/Fresh TBs	Umbo	Evaluation of displacement of umbo at SPL of 60,70 and 80dB
Goode 1996	In vivo/In vitro	Live subjects/Fresh TBs	TM, Malleus, Prosthesis head	Evaluation of displacement of TM, malleus, and prosthesis head
Voss 2000	In vitro	Fresh TBs	Different locations on the stapes	Describing sound transmission through the middle ear
Huber 2001a	In vivo	Live subject	Stapes footplate	Assessment of displacement amplitudes of human stapes
Huber 2001b	In vivo	Live subject	TM	Evaluation of LDV as a diagnostic tool
Rosowski 2002	In vivo	Live gerbils	Umbo, pars tensa	Assessment of the effects of the immobilized pars flaccida
Stenfelt 2002	In vitro	Fresh TBs	Umbo, stapes footplate	Study of malleus and stapes footplate motion during BC
Willi 2002	In vitro	Fresh TBs	TM, IMJ	Dynamics of IMJ
Rosowski 2003	In vivo	Live subjects	Umbo	Correlation between pre-op TM mobility and intra-op diagnosis
Stenfelt 2003	In vitro	Frozen human heads	Umbo	Investigation of sound radiated from the TM into the EAC
Huber 2003a	In vitro	Fresh human TBs	Stapes footplate, Incus	Quality control of stapes surgery (quality of prosthesis crimping)
Huber 2003b	In vivo/In vitro	Live subjects/Fresh TBs	Umbo, stapes footplate	Assessment of AML fixation effects
Rosowski 2004	In vivo	Chincillas	Umbo	Diagnosis of superior semicircular canal dehiscence
Whittemore 2004	In vivo	Live subjects	Umbo	Study of the sound-induced TM velocity at umbo
Zenner 2004	Artificial	Mechanical middle ear models	Different sites on the prosthesis head plate	Investigating the various types of prosthesis
Nakajima 2005a	In vitro	Fresh TBs	TM	Effects of AML fixation
Nakajima 2005b	In vitro	Fresh TBs	Umbo, stapes footplate	Effects of malleus, stapes or malleus+stapes fixation
Zhao 2005	In vitro	Frozen TBs	Umbo, stapes footplate	Determining the optimal length of titanium prosthesis
Chien 2006	In vitro	Fresh TBs	Stapes footplate	Study of the effects of methodological differences in sound-induced stapes velocity in live and cadaver ears
Huber 2006	In vitro	Fresh TBs	Stapes footplate	Feasibility of an implantable hearing aid
Chien 2007	In vivo	Live subjects	Three locations on the TM graft	Identifying causes for poor results of type III tympanoplasty
Dai 2007	In vitro	Fresh TBs	TM and stapes footplate	Investigation of the function of SML and AML
Turcanu 2007	In vivo	Live subjects	Umbo	Measuring the growth function of DP-OAE as the vibration of umbo
Eeg-Olofsson 2008	In vitro	Embalmed human heads	Promontory	Investigation for an ideal spot for BAHA implantation
Hüttenbrink 2008	In vivo/In vitro	Live subjects/Fresh TBs	RW membrane, footplate, promontory	Assessing the efficiency of TORP-Vibroplasty
Huber 2008a	In vivo	Guinea pigs	Stapes head	Study of motion of stapes footplate
Huber 2008b	In vivo	Live subjects	A long process of incus, Prosthesis loop	Evaluation of crimping quality of stapes prostheses

Study	Status	Type of material	The target of LDV spot	Purpose of the study
Neudert 2009	In vivo/In vitro	Live subjects/Fresh TBs	Footplate	Comparison of three types of prosthesis
Offergeld 2010	In vitro	Fresh TBs	Three sites on the footplate	Evaluation of rotational tomography for diagnosis of OCR malfunction.
Neudert 2018	In vitro	Fresh and thawed TBs	Footplate	Live feed-back for PORP and TORP implantations via LDV (graphic and acoustic)

Another report from Goode et al. in 1996 uses 95 human ears and 2 TBs for clinical LDV experiments and measures the TM, malleus, and ossicular prosthesis head displacement and suggest the potential for intra-operative use:

- “Afflicted TM function in hypoacusis cases with middle ear component suspicion but no or only small ABG (<20dB)”,

- “Selection of best surgical options for TM and malleus displacement”,

- “Analysis of postoperative results of reconstruction by evaluating prosthesis head displacement or TM displacement on different sites”,

- “Live, intraoperative measurement of stapes and prosthesis displacement during surgery.” [9]

In 2000 Voss et al. reported on measurements made on human fresh cadaver ears to describe sound transmission through the middle ear. The stapes velocity (VS) was determined by the use of LDV and concluded it is a reasonable method to describe sound transmission through the middle ear for frequencies up to 2000 Hz [13].

A 2001 article by Alexander Huber studies the diagnostic possibilities of the LDV in patients with both conductive and sensorineural hearing loss. He finds that LDV “can differentiate normal subjects from those with conductive hearing loss and also may distinguish between various middle ear pathology.” [14]

Concerning the intraoperative use of LDV, the same Alexander Huber published an article on intra-operative assessment of stapes movement. The study was comprised of 7 patients with profound bilateral hearing loss who were undergoing cochlear implantation. The laser beam was aimed through the posterior tympanotomy onto the stapes and the angle between the beam and the footplate was estimated. The author concludes that amidst the future applications of the LDV we can also count intra-operative quality control of ossiculoplasties and active middle ear implants [10].

In 2002 Rosowski uses LDV to assess the effect of immobilized pars flaccida on the middle ear’s response to static pressure. His experiments on gerbils test this hypothesis by comparing the effect of middle-ear static pressure on measurements of the sound-induced pars tensa velocity before and after immobilization of the pars flaccida with acrylic cement [15].

The use of LDV was extended by Stenfelt in 2002 to the study of the malleus and stapes footplate motion during bone conduction by in vitro stimulation of 26 TBs using an LDV over the frequency range of 0.1-10 kHz. “For lower frequencies, the ossicular sites of measurement followed the motion of the temporal bone. The differential motion between the malleus and the surrounding bone was greater than the differential motion of the stapes footplate; both resonated near 1.5 kHz.” [16]

Willi et al. (2002) investigate the dynamics of the incudo-malleolar joint (IMJ) in 9 temporal bones using LDV scanning which helps to understand the dynamics of both ossicles by three degrees of freedom. Transfer functions (TFs) are shown for each of these degrees [17].

Rosowski et al. publish another work in 2003 in which they report preoperative LDV measurements from 17 patients with conductive hearing loss and a normal, intact tympanic membrane. “The velocity of the TM was measured by LDV near the umbo and showed a direct relation between preoperative TM mobility and the intra-operative diagnosis of ossicular interruption or fixation.” [18]

In 2003 Stenfelt et al. investigate the sound radiated from the TM into the ear canal in 4 TB specimens and conclude that it is significantly lower than the sound pressure in an intact EAC with bone conduction (BC) stimulation.”[19] The umbo velocity with air conduction stimulation was investigated in 9 TBs and compared with the umbo velocity obtained with BC stimulation in 5 cadaveric ears.

In 2003 Huber et al. extend the use of LDV on quality control of stapes surgery. They aimed to define the more frequent causes of stapes surgery failure and to discover a required crimping loop (attachment pattern) to obtain the best sound transmission results. The experiments were conducted on temporal bone models and measurements of the sound transmission properties between incus and prosthesis on 17 fresh human TBs were performed. LDV scanning, endoscopic photography, micro grinding technique, and scanning electron microscopy were used to assess three possible situations for attaching a titanium stapes piston: without crimping, loose crimping, and tight fixation to the incus [20].

Rosowski et al. 2004 induce umbo displacement with sound on chinchilla models with CT scan–confirmed superior semicircular canal dehiscence and perform LDV measurements; the sound-induced motions of the vestibular lymph (either perilymph or endolymph) extends the use of LDV for the study of inner ear conditions [21].

In 2004 Whittemore et al. use LDV to measure the sound-induced tympanic membrane (TM) velocity on 56 normal-hearing human subjects at nine sound frequencies. The second series of experiments was performed on 47 subjects with sensorineural hearing loss (SNHL). The authors conclude that "LDV can provide quick, safe and repeatable measurements of the sound-induced velocity of the umbo in awake patients using the natural reflectance of the TM" and that "the best uses for the LDV system would be the diagnosis of ossicular chain reconstruction (OCR) pathology in patients with significant conductive hearing loss, an intact TM and an aerated middle-ear cavity." [22]

Zenner et al. (2004) use mechanical middle-ear models (MMM) made of plastics, carbon, ceramics, and various metals to evaluate the acoustical properties of various middle ear prostheses by use of LDV [23].

Nakajima et al. simulated the fixation of the anterior mallear ligament in temporal bones and measured the effects of this reaction on umbo and footplate displacement by comparing their findings with clinical data [24, 25].

A second study by Nakajima et al. aims to determine the effects of various types of malleus fixation using 18 cadaveric TB preparation and to "evaluate the clinical use for umbo velocity measurements in the preoperative differential diagnosis of OCR (malleus and stapes) fixation". Malleus fixations were mimicked by controlled applications of adhesives and the effects on middle ear transmission were measured. The fixation of the stapes and combined malleus-stapes fixation were also investigated. Measurements were made of umbo velocity (VU) and stapes velocity (VS) by LDV before and after fixation [26].

Zhao et al. (2005) perform OCR on 7 human TBs before and after removal of the incus and insertion of the prosthesis and use LDV to determine the optimal length of a titanium prosthesis [27].

Chien et al. (2006) study methodological differences in sound-induced stapes velocity (Vs) measurements in live and cadaveric ears [28].

In 2006, Alexander Huber extends the use of LDV for the assessment of an implantable hearing device in humans. Three experimental situations were used: normal ear, PORP ossiculoplasty, and a VSB implant. The vibratory properties of the stapes in all situations were assessed by aiming the laser beam at the stapes footplate [29].

In 2007 Chien et al. aim to refine the uses of LDV for investigation of the middle ear mechanics in a Type III stapes columella tympanoplasty and defining the structural factors responsible for poor functional results. LDV measurements were performed in 22 patients (23 ears) at three locations on the TM graft: over the stapes head, over the round window, and on the anterior TM. The experimental results were correlated with clinical and audiology data. The conclusion is that LDV is found useful in the diagnosis of non-aeration of the middle ear but "does not readily diagnose stapes fixation." [30]

Dai et al. (2007) follow the work of Nakajima et al. and investigate ligament fixation (superior malleolar ligament – SML and anterior malleolar ligament – AML) through simulations of fixation and detachment in 9 fresh frozen human TBs and a finite element model (FEM) of the human ear. Two LDVs were used to measure the vibrations of the TM and stapes footplate. A 3-D FEM predicted the transfer function of the middle ear (METF) in all cases of ligament fixation and/or detachment. The results of this study show that "either SML or AML fixation caused a reduction of umbo and footplate mobility at low frequencies [25].

Turcanu et al. (2007) bring a new approach to LDV use by combining it to objective functional tests of the inner era. The growth functions of the distortion products of otoacoustic emissions (DPOAE) are measured as vibrations of the umbo in 20 subjects. For comparison, DPOAE growth functions were also measured conventionally with an acoustic probe in the closed EAC. The authors conclude that the smaller standard deviation for the LDV data could be a result of the fact that the measurements are made in an open sound field and are therefore less susceptible to pressure calibration errors [31].

Eeg-Olofsson et al. (2008) investigate if BC sound transmission improves when the stimulation approaches the cochlea to obtain an ideal spot for BAHA implantation. Heads from seven human cadavers were used and vibrational stimulation was applied at eight positions on each side of the head. An LDV was used to measure the resulting velocity of the cochlear promontory. The study demonstrates that "the closer to the cochlea the stimuli are placed, the higher the velocity of the promontory, especially for distances < 2.5 cm from the EAC opening and when the stimulation position is placed in the opened mastoid bone." [32]

A 2008 study by Hüttenbrink & Zahnert uses the LDV for temporal bone studies to assess the efficiency of a TORP-Vibroplasty. LDV measurements were performed at 3 locations: the round window (RW) membrane, the footplate, and at a drilled-out third window on the promontory (for comparison of the stimulation of both windows). This study is another fine example of the flexibility of LDV as a useful tool for the otologic surgeon [33].

Huber et al use a 3D LDV that has the capability of simultaneously measuring spatial vibrations, to monitor stapes vibration. This was done by using 3 separate laser beams at an angle of 12 degrees relative to the optical axis of the lens. The focus point was adjusted to the stapes head to prove that the piston-like motion of the stapes footplate is the only effective stimulus to the cochlea, and rocking-like stapes motions have no effect on hearing. The study was performed on 4 female guinea pigs [34].

Also, Huber et al. evaluate the crimping quality of a stapes prostheses in 23 patients by comparing intra-operative LDV results and postoperative pure tone thresholds. The study demonstrates the feasibility of intra-operative LDV and uses a method of mechanical stimulation of the middle ear that was developed and calibrated in TB preparations [35]. The method was applicable not only under general but also under local anesthesia. It is therefore practical for quality control in routine stapes surgery.

Andre Jakob uses LDV for the clinical diagnosis of otosclerosis. In this study, he aimed the LDV at the umbo and measured its movement in healthy and conductive hearing loss affected ears. He concluded that “all ossicular conditions represent increased umbo displacement” and that the method is reliable for detecting a discontinuity of the ossicular chain. However, LDV does not suffice as a single diagnostic tool for otosclerosis [36].

In 2009, Neudert et.al. use LDV to compare the results of three different middle ear prostheses (TAP, AII, TCP) on human temporal bones. In this study the LDV was also used only for TB specimens experiments (18 TBs), all in vivo implantations (66 patients) were retrospectively assessed up to 5 years after surgery [37].

In 2010, Offergeld employs an LDV experimental setup very similar to our own to make functional measurements of middle ear sound transmission on PORP/TORP implanted human TBs and to correlate the results to anatomical data obtained by rotational tomography. The goal of his study was to prove that RT is a reliable method for assessing the postoperative position of ossicular implants [38].

In 2018, Neudert et al. uses the LDV to obtain live feed-back for ossicular prosthesis positioning and concludes the method to be feasible for improving ossiculoplasty results [39].

CONCLUSIONS

Middle ear vibrations in response to acoustic stimulation are very difficult to record since they are measured within the micrometer and nanometer range. For this reason, devising a method to analyze these vibrations is extremely important and doing it without any contact with the structures of the middle ear is essential. Without LDV the surgeon must manipulate the ossicles with instruments, under visual control and therefore only perform a qualitative estimate as to the mobility of the ossicular chain.

After reviewing all the experiments above, there can only be one conclusion drawn from all the studies so far: the LDV method has great potential for preoperative diagnostic of patients with conductive hearing loss, for differential diagnostic between the types of pathology that determine the conductive hearing loss and especially for assessing the postoperative results of ossicular middle ear prosthesis. All that is needed now is more studies that can confirm this potential and hopefully state guidelines for clinical use.

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ORIGINAL ARTICLES

The value of lipid profile in asthma-obstructive sleep apnea overlap

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Abstract: *The asthma obstructive sleep apnea overlap is a clinical entity relatively new and the association with a dysfunctional plasma lipid profile is not known.*

Aim. In this study, we compared the serum lipid profile in asthma-obstructive sleep apnea overlap syndrome to those in asthma and obstructive sleep apnea patients.

Methods. A cross-sectional study of 97 cases of which 26 patients had only asthma, 51 were diagnosed with OSA, and 20 were identified with A-OSA overlap. The lipid profile included the total cholesterol, HDL-cholesterol, triglycerides, the LDL-cholesterol. The Casteli indexes I and II and the atherogenic plasma index were calculated.

Results. The patients with asthma- obstructive apnea overlap were older (55.55 years vs 53,73 years in obstructive sleep apnea and 51,04 years in asthmatics), mostly obese (95%), and had a significantly higher average of BMI (38.11 vs. 35.64 and 28.8, respectively). Asthma- obstructive apnea overlap was at higher risk of cardiovascular disease than asthmatics, in respect to the lipid profile: the levels of HDL-cholesterol were lower, triglycerides were higher, and the values of Castelli index I and II were higher.

Conclusions. Based on our results, we confirm that age and BMI contribute to the risk of A-OSA overlap. The major differences in the common markers of the serum lipid profile are lower HDL-C and higher triglycerides. As a clinical implication, both Castelli indexes could provide additional value for cardiovascular risk and should be monitored in A-OSA overlap.

Keywords: *asthma, obstructive sleep apnea, HDL-cholesterol, triglycerides*

INTRODUCTION

The number of studies describing the asthma-obstructive sleep apnea overlap (A-OSA overlap), also known as "asthma-obstructive sleep apnea phenotype" is constantly increasing. The prevalence of obstructive sleep apnea (OSA) in asthmatic patients ranges from 19-60%. The prevalence is higher in older patients, with more than 10 years duration of asthma, in poorly controlled and in obese asthmatics [1, 2].

There is also a higher risk for the development of asthma in obese OSA patients. The coexistence of asthma in OSA aggravates the desaturation during the night [3].

A pro-atherogenic serum lipoprotein profile in OSA, reported by many studies [4, 5], and broadly accepted is dyslipidemia, one of OSA's comorbidities [6, 7]. There is some clinical evidence that dyslipidemia improves after CPAP therapy [7]. The metabolic effects and, particularly, the lipid profile in asthma are less investigated. However, a systematic review showed an increased level of LDL-cholesterol in this category of patients [8].

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As more and more cases of A-OSA overlap are reported, the alterations of the lipid profile become more suggestive for additional cardiovascular comorbidity. In this respect, we developed a study to compare the serum lipid profile in patients with asthma-obstructive sleep apnea overlap syndrome compared to asthma and OSA, as a unique diagnosis.

METHODS

An observational, cross-sectional study was conducted in two Romanian centers from Bucharest and Constanta city.

From March to June 2019, patients with occupational asthma, that agreed to participate, were screened by Stop Bang and Epworth testing in the Clinic Department of Occupational Diseases in Colentina Hospital of Bucharest. Those asthmatics, with high scores, underwent a polygraphy test. OSA's diagnosis was based on apnea-hypopnea index (AHI) higher than 5/h.

The Constanta group of patients, previously diagnosed with asthma, were investigated by a similar methodology for OSA's diagnosis, in the Sleep Laboratory of Constanta Clinic Pneumophthisiology Hospital.

The study was approved by the Ethic Committee of the Constanța Clinic Pneumophthisiology Hospital.

Both research teams recorded data about demographics (age, sex), the body mass index (BMI), fasting glycemia, total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels. BMI was calculated by dividing weight by the height squared. The current smoking habit was assessed. If the cessation of smoking was more than 1 year long, the subject was classified as a former smoker.

The blood profile of total cholesterol, triglycerides, and HDL-c cholesterol was directly measured by standardized methods, while LDL-C was calculated according to the Friedewald formula: LDL-C = TC − HDL-C − (TG/5).

The Castelli risk index I was computed by the ratio of total cholesterol/HDL-cholesterol. The risk values for primary prevention were considered (e.g. >5 for men and >4.5 for women) [9]. For the LDL-C to HDL-C ratio (Castelli risk index II), the levels associated with high were higher than 3.5 in men and 3 in women [9].

Atherogenic plasma index, as the cut-off for the cardiovascular diseases risk, was considered the logarithm of the ratio triglycerides/HDL-cholesterol higher than 0.5.10

Consolidated data were processed in a StatPLus for Mac software. Numerical data were compared by Kruskal Wallis test, while categorical ones required a Chi-square test. Regression was used to verify the relationship between variables. For the statistical significance, the threshold was set to estimate a 95% probability.

RESULTS

Among 97 cases, mean aged 53.38±6.76-year-old, mostly males (n=67), 26 patients had only asthma, 51 were diagnosed with OSA, without having a personal medical history of asthma, 20 were identified with A-OSA overlap, which made a point prevalence of asthma inside all OSA patients of 28.17%.

The main characteristics of these groups are presented in Table 1.

Table 1: Main characteristics of the patients

	All (mean ± SD)	**Asthma** (mean ± SD)	**OSA** (mean ± SD)	**Asthma-OSA overlap** (mean ± SD)
Age (years)*	53.38 ± 6.76	51.04 ± 4,87	53.73 ± 7.52	55.55 ± 6.16
Gender (F/M)*	30/67	13/13	12/39	5/15
Smoking (% active smokers/total)	17.53%	19.23%	23.53%	0%
BMI (kg/m^2)**	34.29 ± 7.06	28.8 ± 4.99	35.64 ± 6.59	38.11 ± 6.53
Total cholesterol (mg/dL)	210.78 ± 50.44	208.04 ± 28.27	207.82 ± 59.71	221.3 ± 43.66
HDL-cholesterol (mg/dL)**	50.2 ± 18,84	66.95 ± 25.76	44.42 ± 12.36	47.05 ± 13.44
LDL-cholesterol (mg/dL)	126.59 ± 42.99	116.9 ± 29.62	127.1 ± 51.22	135.5 ± 29.61
Triglycerides mg/dL)*	158.95 ± 142.03	122.45 ± 92.33	187.1±193.22	172.19 ±114.21
Castelli 1 index (TC/HDL)**	4.86 ± 2.8	3.51 ± 1.32	5.32 ± 3.27	5.14 ± 2.26
Castelli 2 (LDL/HDL)**	2.83 ± 1.4	2.04 ± 0.97	3.05 ± 1.53	3.12 ±1.18
API	0.48 ± 0.41	0.35 ± 0.31	0.6 ± 0.52	0.49 ± 0.32
Fasting glycemia (mg/dL)	118.06 ± 43.82	102.8 ± 16.5	125.65 ± 52.26	106.35 ± 18.62

*OSA = obstructive sleep apnea; F=female; M=male; BMI = body mass index; HDL cholesterol = high density lipoprotein cholesterol; LDL cholesterol = low density lipoprotein cholesterol; API= atherogenic plasma index; *statistically significant difference between all groups (p<0.05); ** high statistically significant difference* *between all groups (p<0.01)*

Most patients were in their 5th and 6th decade of age. The asthma-OSA association was significantly reported in the oldest patients than in the asthma group (H= 5.31, p = 0.02).

There was also a male predominance in the OSA group and A-OSA.

There was no difference by gender distribution between asthmatics and patients with A-OSA overlap (chi2 = 3.59, p=0.058).

The majority of patients were obese (73%) or overweighting (21%), especially in the A-OSA overlap group (38.11±6.53), with major differences among groups according to BMI categories (42% obese, 42% overweight, 12% normal weight, and 4% underweight, among asthmatics, compare to A-OSA overlap group with 95% obese and 5% overweight). The prevalence of obesity in the OSA patients was an intermediary between the prevalence encountered in the asthmatics and A-OSA overlap group (Figure 1).

Figure 1: Distribution of cases according to diagnosis and BMI categories

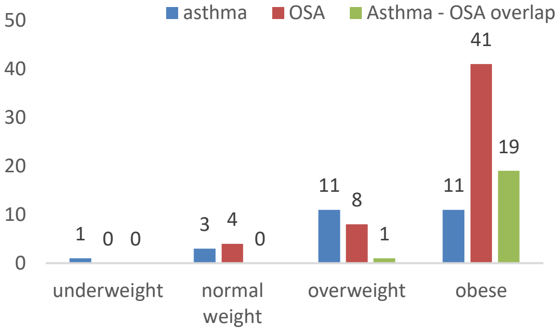

OSA= obstructive sleep apnea

Concerning smoking status, the highest percentage of active smokers was in the OSA group (23.53%), followed by 19.23% in asthmatics. However, this difference was not significant (chi2 =0.18, p=0.66).There were no active smokers in A-OSA overlap.

There was a direct correlation between BMI and triglycerides blood levels (Rho = 0.48, p=0.0001) and a reverse, significant correlation with HDL-C (Rho= -0.28, p=0.007).

The serum LDL-C showed no relation with BMI. However, the differences between groups maintained their statistical significance independent of the BMI.

The lipid profile was dominated by a high level of triglycerides and a low level of HDL-C in OSA and A-OSA overlap as compared to the asthma group.

The LDL-C had the highest values in A-OSA-overlap.

The triglycerides and LDL-C were significantly higher in A-OSA overlap as compared to asthma (H=3.98, p=0.04, and H=4.52, p=0.03, respectively) while the HDL-C was significantly lower (H=8.31, p=0.003).

No significant differences were noted between the triglycerides, the LDL-C and HDL-C serum levels in OSA and A-OSA overlap (H=0.02, p=0.89; H=0.75, p=0.38, and H=0.25, p=0.62, respectively).

The average Castelli index was in the risk range values both in OSA and in A-OSA overlap groups. When measured individually, a few asthmatics had risk values than OSA patients. The highest percentage of risk values was found in the A-OSA overlap group (Figure 2).

Figure 2: Distribution of the Castelli index I among the patients with asthma, obstructive sleep apnea, and asthma-obstructive sleep apnea overlap

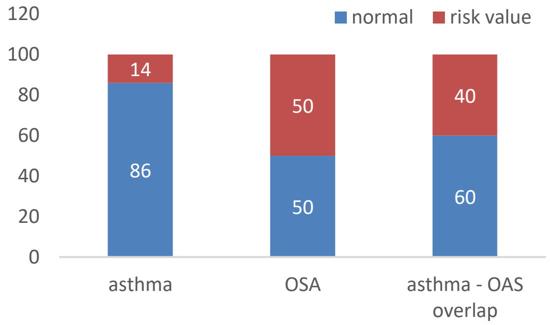

OSA= obstructive sleep apnea

The number of patients with abnormal Castelli index in A-OSA overlap was higher than in the asthma group, but without statistical significance (chi2 =3.77, p=0.05), as the risk distribution between OSA and A-OSA overlap (chi2 = 0.21, p=0.65).

The majority (90%) of asthmatics had the ratio LDL/HDL in the normal values range.

The higher level of risk, according to this index, was among OSA patients, without significant difference compare to A-OSA overlap (chi2 = 0.02, p=0.89), as well as between asthma and A-OSA overlap (chi2 = 1.32, p=0.25) (Figure 3).

The percentage of patients with atherogenic plasma index (API) >0.5 was almost equally distributed among groups; 33% in asthma, 37% in OSA, and 38% in A-OSA overlap (chi2 =0.88, p=0.64). However, there is a significant difference between the API values in asthma as compared to the OSA group (H=3.89, p=0.04).

Figure 3: Distribution of the Castelli index II among the patients with asthma, obstructive sleep apnea and asthma-obstructive sleep apnea overlap

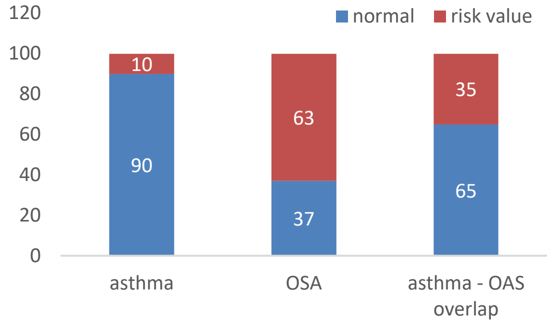

OSA= obstructive sleep apnea

DISCUSSION

In this study, we report differences in the serum lipid profile between asthma, OSA, and the co-existence of these two clinical entities referred to as asthma-obstructive sleep apnea overlap. The main findings are higher risk values for the Castelli index I and II in patients with A-OSA overlap compare to asthma patients.

Patients with A-OSA overlap were older, mostly obese (95%), with a significantly higher average BMI. These findings are from previous literature data [1, 2, 11]. The progression to A-OSA overlap requires a longer duration of time [12], considering the convergent effect of prolonged persistent chronic airways inflammation and aging.

Obesity contributes as well to the inflammatory status and increases the collapsibility of the upper airways and it is commonly accepted as a major risk factor for OSA [13]. In what concerns asthma, obese patients are reporting more severe symptoms, have a greater prevalence of co-morbidities [14], and a higher number of hospitalizations due to exacerbations [15]. The risk for A-OSA overlap might increase by higher corticoid doses that contribute to the deposition of fat in the neck region [1], another cause of upper airways resistance. OSA could be an explanation, as CPAP therapy in these patients reduces asthma reported symptoms [16].

Between the three groups, and between asthma and A-OSA overlap, the most significant difference in the serum lipid profile concerns the HDL-C. The presence of OSA seems to be the most important factor, as patients in this group had a lower blood level of HDL-C. Several studies have found the same reduction in HDL-C levels in OSA [4]. In asthma, a meta-analysis revealed no association between asthma and HDL-C levels in adults, but an increase in the LDL-C [17]. These studies support our hypothesis that the reduction in HDL-C levels in A-OSA overlap is mainly a consequence of the intermittent hypoxia related to chronic nocturnal repeated desaturation. As the A-OSA overlap is a relatively newly described entity, we could not directly compare our results with other studies. To the best of our knowledge, neither Medline nor Scopus databases give any result of a study specifically looking to this relation.

Despite the reproducible association in large cohorts of the increased cardiovascular risk related to low HDL-C level, the current clinical guidelines did not found enough evidence that raising HDL-C in serum would be of any benefit for these patients [18].

The latest evidence shows that not only the quantity but also the type of HDL particulate matters. The HDL particles are very heterogeneous and carry more functions than reverse cholesterol transport. Apolipoprotein A I and A-II are the major protein components in HDL. The HDL particle also contains a variety of lipids (phospholipids, cholesterol, in not esterified or esterified forms, and triglycerides) according to the maturation stage. It is a plasma vehicle for many other biologically active molecules, such as fat-soluble vitamins, steroid hormones, bile acids, bioactive lipids, haptoglobin, α-1 antitrypsin, transferrin, micro RNA) [19] and enzymes with antioxidant properties that would protect for reactive oxygen species and reactive nitrogen species damaging the endothelium [20]. To assure the esterification of the cholesterol, HDL contains lecithin cholesterol acyltransferase (LCAT) and to exchange esterified cholesterol and triglycerides with other lipoproteins, cholesteryl ester transfer protein (CEPT) [21].

The nomenclature of the HDL-C varies according to the method utilized for detection, and the degree of correspondence is far from perfect [22], leading to some confusion in the interpretation of results coming from different studies. Briefly, HDL is synthesized in the liver and the small intestines [23] as lipid poor preβ-HDL (the nascent HDL). In peripheral tissues, including macrophages foam cells, this particle interacts with ATP-binding cassette transporter A1 (ABCA1) to initiate the reverse transport of cholesterol to the liver and transforms to small HDL. Esterification of cholesterol inside the small HDL by LCAT and the exchange of esterified cholesterol with triglycerides from VLDL, IDL, and LDL-C, via the CEPT, end up in the formation of the mature HDL-C particle (the large HDL). Finally, HDL binds to the HDL receptor in the liver, and, through endocytosis, it is removed from circulation [24]. The triglyceride content of HDL promotes its clearance [23]. This permanent exchange, between the lipoproteins, in the bloodstream, influences the types of HDL particles in circulation.

In theory, both small and large particles might point towards a certain risk: the predominance of the small ones could show an ineffective/diminished potential for reverse cholesterol transport, while the predominance of the large ones could indicate a high burden of cholesterol in blood circulation. In epidemiological studies, a high level of large HDL seems to be protective for atherosclerosis [25], while, in already confirmed cardiovascular disease, high levels of small HDL are found [26] with a certain positive prognosis potential [27].

The techniques used to define the types of HDL particles are currently available mainly for research purposes, although their clinical value in assessing the risk and adapting therapy is of major value. Without this availability, different ratios of the commonly determined plasma lipoproteins and lipids were proposed to better characterize the atherogenic risk.

The estimation of the particle size is partially fulfilled by the API and the 2 Castelli indexes.

API was initially described to assess the atherogenic risk. Indeed, high levels were found in apparently healthy individuals in correlation with other biochemical markers of subclinical vascular diseases [28-29] and it was increased in subjects with cardiovascular disease compared to healthy controls [30, 31]. However, this marker was not confirmed as a predictable tool for stroke prediction [32]. In our study, the API did not have a discriminative value between the 3 groups. As there is no other study to compare with, this result should be cautiously interpreted and further investigated.

The Castelli risk I was a better estimation of the cardiovascular risk than individual lipoprotein particles measurement in the initial large cohort studies, as the Framingham study or the LRCP one [9], reflecting mostly the particles are responsible for the peripheral transport of cholesterol (the LDL and VLDL). It was, later, related to insulin resistance [33]. Even in normal weighted subjects, this marker had significant prognostic value for cardiovascular disease [34]. Castelli risk I show a direct relationship with the large HDL particles and indirect relation with the HDL 2b (small particles) and the small LDL [35].

Castelli index II describes the ratio between the HDL and LDL particles and is generally well associated with the Castelli index I and non-HDL cholesterol. It has been also used to differentiate the risk of atherosclerosis in chronic renal disease [36] or metabolic syndrome [37].

It is important to notice a high negative correlation between BMI and HDL with HDL-C in our study population. This finding is important, because, in certain circumstances, as in a chronic, persistent, inflammatory milieu that characterizes obesity, HDL particles become dysfunctional, losing their capacity for cholesterol efflux, and their anti-inflammatory and antithrombotic potential [38]

The low HDL-C and the high-risk Casteli index were not influenced by smoking, as all the A-OSA overlap patients were non-active smokers. It is assumed that, after smoking cessation, an improvement of the serum lipid profile manifests, mainly, on the elevation of HDL-C [39]. Even if the smoking effects would have been present, it was probably opposed by other pathogenic mechanisms. The groups of asthma and OSA had similar numbers of active smokers. Therefore, even if a certain influence of smoking on the lipid profile cannot be excluded, the significant difference in HDL-C noted between the groups should have another explanation.

We are aware of certain limitations of this study. Respiratory nocturnal polygraphy might underestimate the number of OSA patients inside the asthma population, because the majority of respiratory events are hypopneas, with arousals, that are more accurately counted only by polysomnography study [11]. Even so, the asthma patients were actively interviewed for OSA –related symptoms and none of them would clinically qualify for the diagnosis.

Another limitation of our study consists in the moderate number of patients, but from the perspective of no existing studies, we do consider that these results should be shared with the research community to promote further investigation in this domain. Despite these limitations, the results presented from this study add valuable information for the domain and substantiate the need for larger studies.

CONCLUSION

Based on our results, we confirm that age and BMI contribute to the risk of A-OSA overlap.

The major differences in the common markers of the serum lipid profile are lower HDL-C and higher triglycerides. As a clinical implication, both Castelli indexes could provide additional value for cardiovascular risk and should be monitored in A-OSA overlap.

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VARIA

Tonsillar neoplasm: experience of the 2nd Otorhinolaryngology Clinic from Cluj-Napoca

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Abstract: *Introduction. The primary tonsillar neoplasm is a common malignancy of the head and neck, alcohol and/or cigarette smoke and Human Papilloma Virus is the main etiological agent. This study offers a retrospective analysis of the tonsillar neoplasm cases in a tertiary medical center from Romania.*

Patients and methods. We present a 4-year (2015-2018) retrospective study on 72 patients diagnosed with tonsillar neoplasm in our clinic, previously untreated patients. The study was included only the patients diagnosed through histopathological evaluation with tonsillar neoplasm. We searched the cases in our clinic database, and we collected data about age, gender, symptoms on admission, findings of the objective physical examination, oro-pharyngoscopy, medical imaging examination results, histopathological results, stage of the disease, therapeutic management.

Results. The group composition is: 75% men and 25% women, 62.5% of them are smokers. Dysphagia/swallowing discomfort was the most common symptom identified, and the non-keratinized squamous cell carcinoma was the most common histopathological form. Stage I tonsil carcinoma was diagnosed in 25% of the cases, stage II in 15.3%, while 47.2% of the cases had stage III or IV. None of the patients presented distant metastases. Lymphoma was diagnosed in 12.5% of the cases.

We performed for all patients in stages I and II underwent total unilateral tonsillectomy. For the patients in stages III and IV, we performed surgery after radiotherapy-chemotherapy treatment or only radiotherapy-chemotherapy.

Conclusions. In Romania, smoking is still the most important risk factor for tonsil cancer. The majority of patients have visited a doctor only when dysphagia and, above all, palpable cervical adenopathy, were present, and the tumor was in the advanced stage. In Romania, the ENT-oncology integrated medical services are few, in most cases patients are lost from otorhinolaryngological follow-up.

Keywords: *tonsillar neoplasm, palatine tonsil, squamous cell carcinoma*

INTRODUCTION

Head and neck cancer is the fifth most frequent form of cancer worldwide [1]. The primary tonsillar neoplasm is the third most common malignancy of the head and neck, after thyroid and larynx cancer [2]. Every year, around 264,000 cases of oral cavity cancer are diagnosed in the world. At the time of diagnosis, approximately 50% of patients have lymphatic dissemination [3].

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Within five years, 15% of patients with a tonsillar neoplasm present a secondary carcinoma in the head-neck area, especially those patients who are chronically exposed to the carcinogenic factors of the upper aero-digestive tract [3].

The risk factors for the development of tonsillar cancer are alcohol and/or cigarette smoke, and, especially for young patients without these risk factors, Human Papilloma Virus (HPV) may be an etiological agent [4]. Irrespective of the etiology, T1 or T2 stage tonsillar primary lesions can be treated successfully with acceptable loco-regional control and good survival rates by using either surgery or primary radiotherapy. The therapeutic success rates for T3 or T4 stage disease are less encouraging regardless of the treatment modality or medical center [5].

This study aims to make a retrospective analysis of this pathology, based on 72 cases diagnosed during 2015-2018, in a tertiary medical center from Romania.

PATIENTS AND METHODS

The study design is a retrospective observational study. It included patients admitted into the clinic for tonsillar neoplasm between 1 January 2015 – 31 December 2018. The inclusion criteria: histopathological diagnosis of tonsillar neoplasm during this period. The search for cases in the database was conducted based on the main diagnosis at discharge from the hospital, i.e. malignant tonsil tumor and disease code. The exclusion criteria: patients whose data were inaccessible, incomplete, or did not fulfill the inclusion criteria.

The statistical basis and clinical records were examined by the same investigator; the following data were collected: age, sex, symptoms on admission, findings of the objective physical examination, oro-pharyngoscopy, medical imaging examination results, histopathological results, stage of the disease, therapeutic management. The patient's privacy and confidentiality were protected under the laws in force.

All cases in the study were biopsied and all the specimens were fixed in 10% (v/v) buffered formalin and sent for the histopathological evaluation. For the classical histopathological examination, hematoxylin-eosin (HE) staining was used, while for the immunohistochemical (IHC) tests we used the antibodies: CD20 (cloneL26), CD10 (cloneSP67), bcl2 (clone124), CD5 (cloneSP19), bcl6 (cloneL26), CD3 (clone 2GV6), Ki67 (clone30-9) - Ventana Medical Systems, Tucson, AZ, on Benchmark XT Ventana-Roche platform.

None of the patients had been tested for HPV.

The patients were staged following the TNM classification of the American Joint Committee on Cancer (stage groups for non–HPV-mediated (p16 -) oropharyngeal cancer) [6].

The study protocol was approved by the University Clinical Hospital of Railway Company Ethics Council under the No. 11/09.12.2020.

RESULTS

Of the 72 patients, 75% (n=54) were men and 25% (n=18) women. The average age was 62.8 (range 42-79 years) in the men subgroup and 56.3 (range 37-69 years) in women. The number of patients from rural and urban areas was about equal. The newly diagnosed cases each year were relatively constant.

Figure 1: Advanced stage tonsillar neoplasm – macroscopic aspect

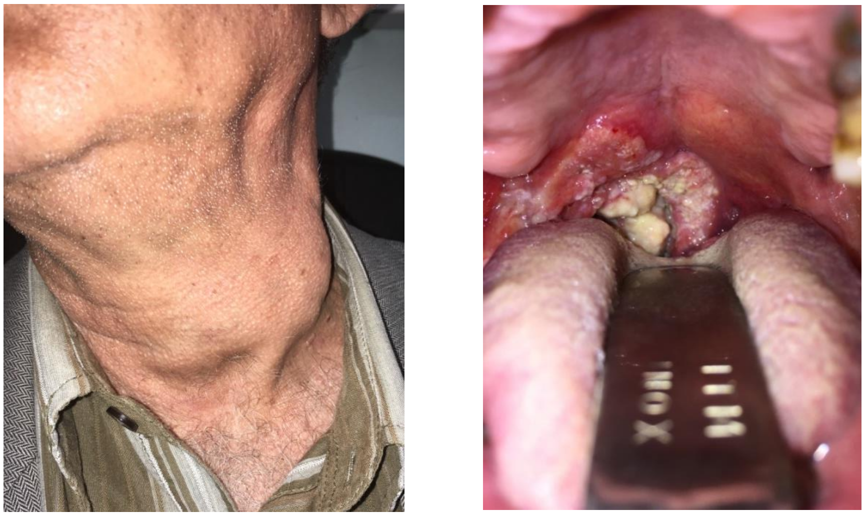

A: Lateral-cervical adenopathic block in a patient with tonsillar neoplasm

B: Vegetative tumor with a starting point in the right tonsil (keratinized squamous-cell carcinoma).

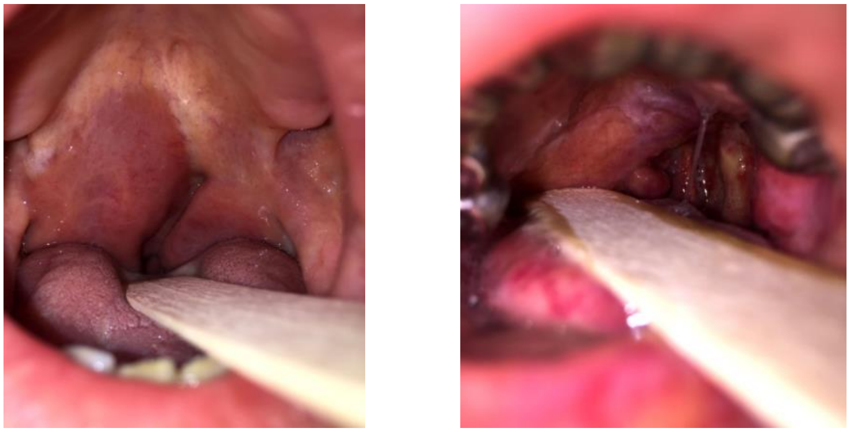

C, D: Infiltrative tumor with a starting point in the right tonsil (non-keratinized squamous-cell carcinoma).

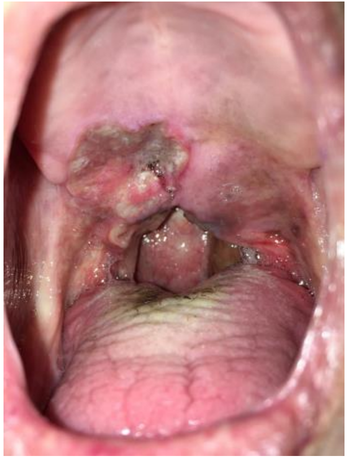

E: Ulcerated-infiltrative tumor with a starting point in the left tonsil. Bucco-nasal fistula (non-keratinized squamous-cell carcinoma).

Risk factors. Smokers accounted for 62.5% (n=45, 35 men and 10 women), while 29.2% admitted to concomitant alcohol and tobacco consumption (n=21, 18 men and 3 women).

Clinical evaluation. The symptoms on admission to the hospital were: dysphagia/swallowing discomfort in 63.8% (n=46), palpable homolateral adenopathy (Figure 1A) in 20.8% (n=15), negative body weight curve in 36.1% (n=26), reflex otalgia 9.7% (n=7), trismus 8.3% (n=6), and pain in the oropharynx, tolerable without analgesic medication in 4.1% (n=3).

The macroscopic aspect of the tonsil tumor indicated a vegetative form in 34.7% (n=25) - Figure 1B, infiltrative in 30.5% (n=22) - Figure 1C-D, ulcero-infiltrative in 26.4% (n=19) - Figure 1E, while occult forms with metastatic lateral cervical adenopathies were present in 8.4% (n=6) of the patients, in which a triple biopsy (palatine tonsil, rhino pharynx, tongue base) was performed, with positive results at the tonsillar level.

Histopathology. The non-keratinized squamous cell carcinoma (Figure 2A) was evidenced in 58.3% (n=42) of the cases, keratinized squamous cell carcinoma (Figure 2B) in 29.2% (n=21) of cases, and B cell lymphoma (Figure 3) in 12.5% (n=9) of cases. The G2 histologic grade (moderately differentiated) was identified in the majority of cases (71%, n=51).

Figure 2: Squamous tonsillar carcinoma

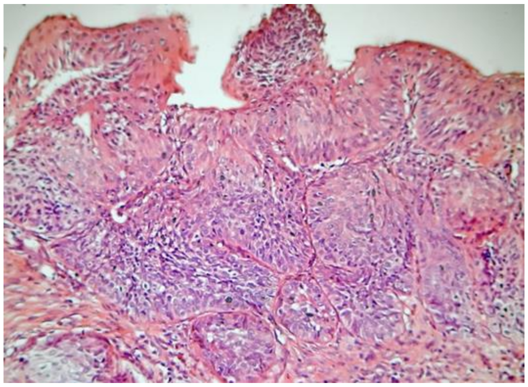

A – non-keratinized, moderately differentiated (G2) HE staining: ×100;

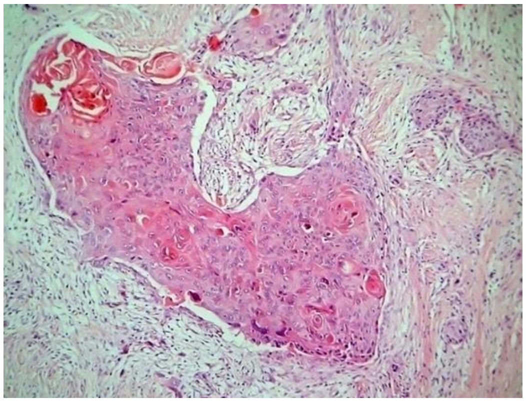

B – keratinized, moderately differentiated (G2) HE staining: ×200

Figure 3: B cell tonsillar lymphoma

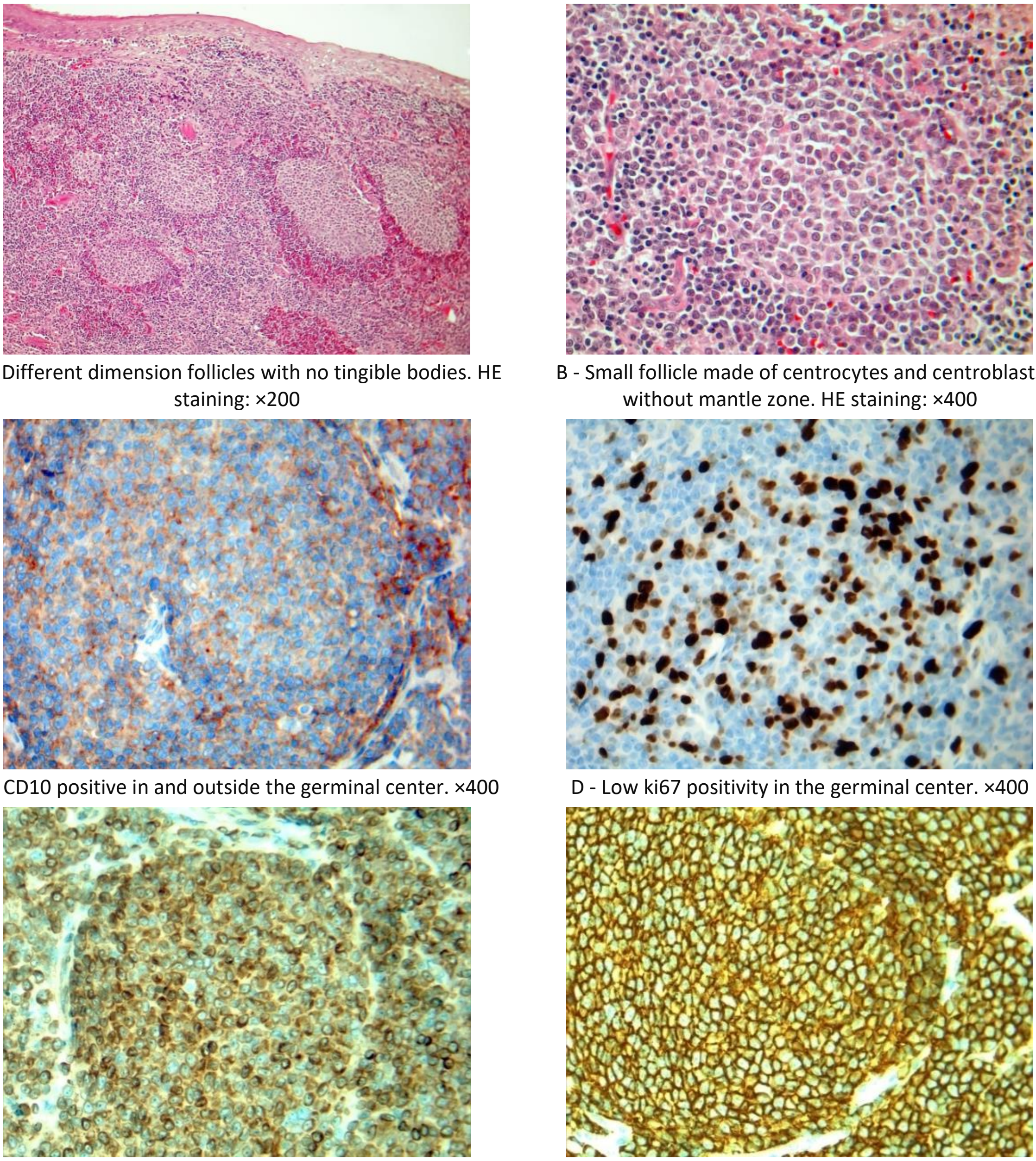

A - Different dimension follicles with no tingible bodies. HE staining: ×200

B - Small follicle made of centrocytes and centroblasts without mantle zone. HE staining: ×400

C - CD10 positive in and outside the germinal center. ×400

D - Low ki67 positivity in the germinal center. ×400

E - BCL2 positive in the germinal center. ×400

F - CD20 positive in and outside the germinal center. ×400

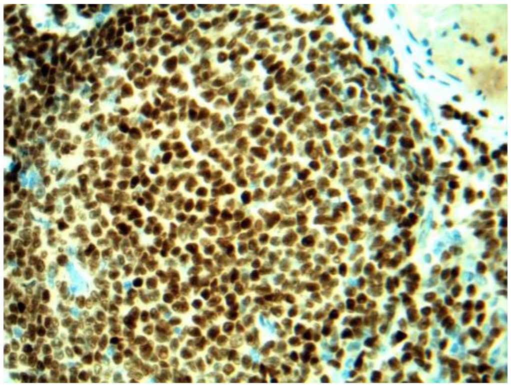

G - BCL6 positive in and outside the germinal center. ×400

Imaging investigations. After the confirmation of the histopathological diagnosis, a contrast-enhanced computer tomography (CT) was performed (Figure 4A) in 70 patients, while positron emission tomography (PET-CT) was performed in one patient (Figure 4B).

Figure 4: CT images of the tonsillar neoplasm

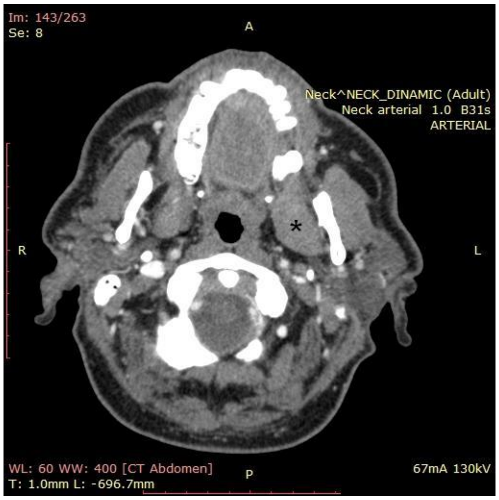
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A
Neck^NECK_DINAMIC (Adult)
Neck arterial 1.0 B31s
ARTERIAL
*
R
L
WL: 60 WW: 400 [CT Abdomen]
T: 1.0mm L: -696.7mm
P
67mA 130kV

A - Contrast-enhanced CT, transversal section: left tonsil tumor (marked by *).

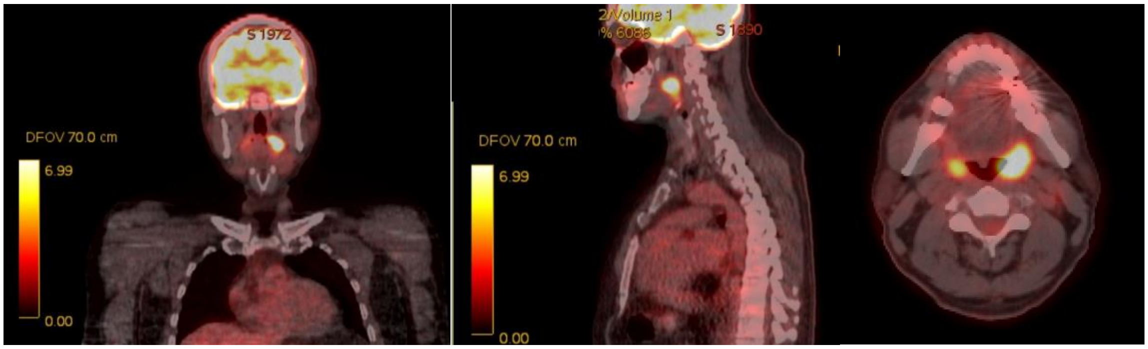
S 1972
DFOV 70.0 cm
6.99
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2/Volume 1
% 6086
S 1890
DFOV 70.0 cm
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B - PET-CT, frontal, sagittal, and transversal sections: high perfusion tumor of the left tonsil.

Treatment. All patients in stages I and II, and the lymphoma cases underwent total unilateral tonsillectomy through a direct transoral approach (Figure 5A).

For the patients in stages III and IV (surgical cases), we performed, after radiotherapy-chemotherapy treatment, pharyngectomy with partial glossectomy in 2 cases (Figure 5B), tonsillectomy, and partial palatectomy in 9 cases.

In all cases operated, lateral-cervical lymph node ablation was performed: for N0 cases – selective supra omohyoid neck dissection, and for N+ cases – radical neck dissection modified (Figure 5C-E).

Figure 5: Intraoperative aspects

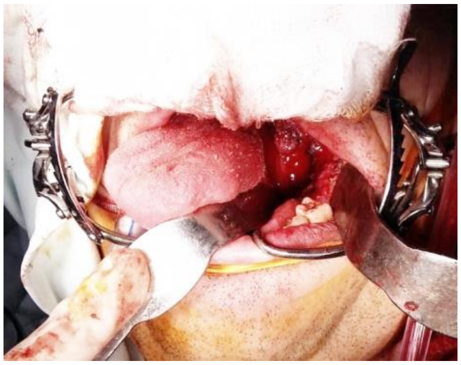

A - Transoral extended left tonsillectomy (T2N0M0).

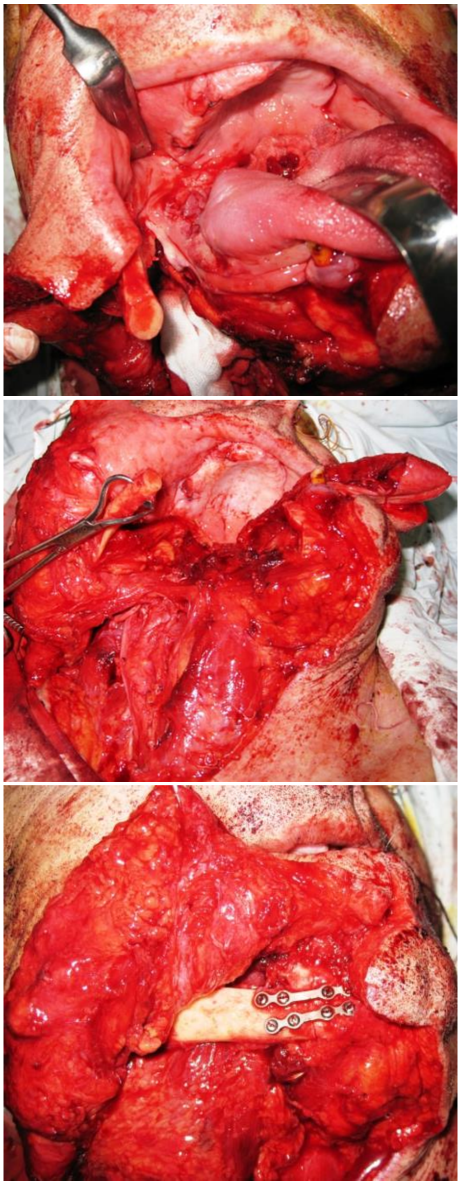

B1-3 - Transmandibular pharyngectomy with pectoralis major reconstruction.

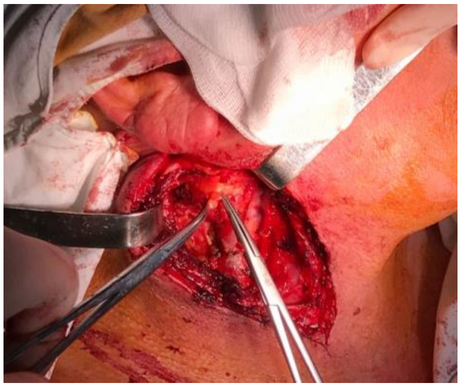

C - Right lateral-cervical lymphadenectomy.

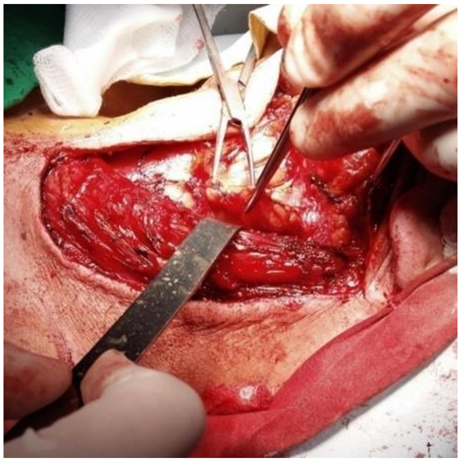

D1: Dissection below the sternocleidomastoid muscle.

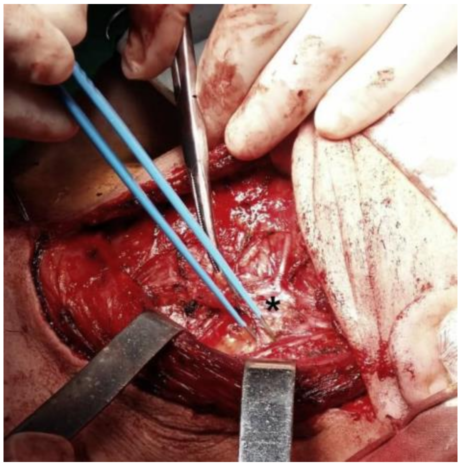

D2: Exposure of the right internal jugular vein (marked by *).

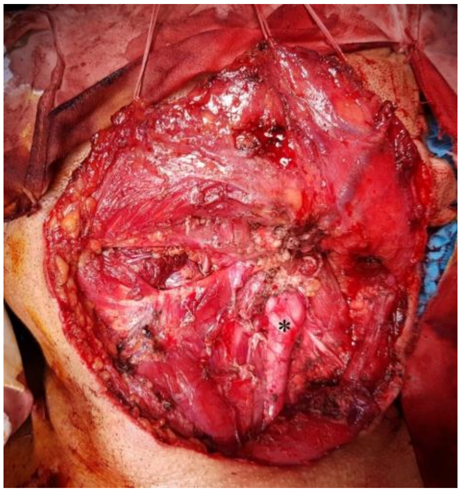

E - Radical dissection of the neck in a case with T4bN2M0. The tumor includes the left secondary carotid arteries (carotid glomus marked by *).

The cases considered beyond surgical therapy were referred only to radiotherapy (Figure 6), chemotherapy.

Figure 6: A, B CT - based treatment plans for a patient with right tonsil squamous carcinoma cT4N2cM0, stage IVB (Collection of Chiricuta Institute of Oncology Cluj-Napoca)

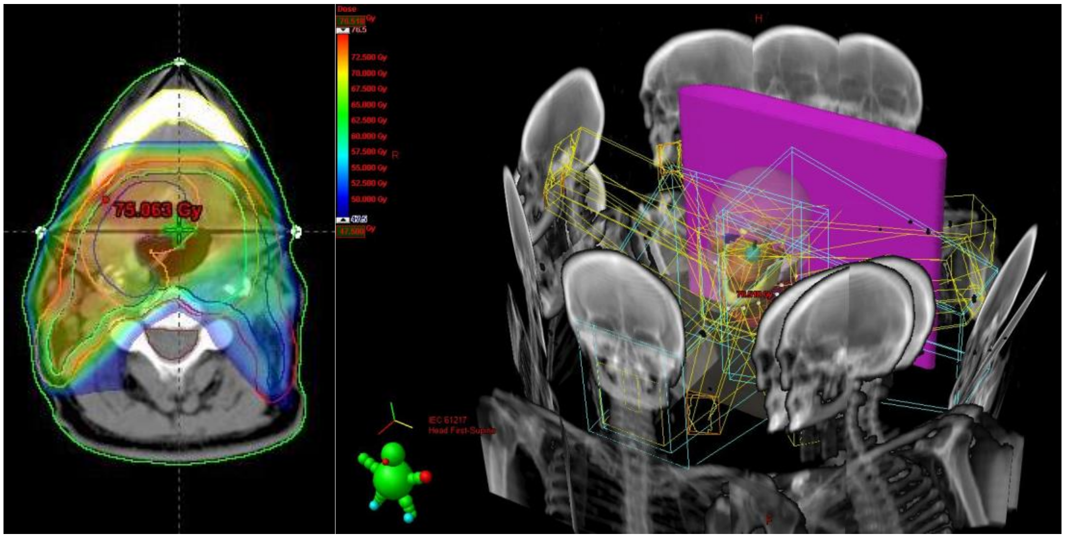

In Table 1 we present the distribution of cases according to Stage Groups for Non–HPV-Mediated (p16 -). At the time of imaging tests, we did not evidence metastases of the tonsillar neoplasm to other organs, so no patient was identified in stage IV C.

Table 1: TNM distribution of cases

Stage	Number of cases	Percent
Lymphoma	9	12.5%
I (T1N0M0)	18	25%
II (T2N0M0)	11	15.3%
III (T2N1M0)	9	12.5%
III (T3N1M0)	8	11.1%
IV A (T4aN2M0)	5	6.9%
IV A (T3N2M0)	5	6.9%
IV B (T4bN2M0)	4	5.6%
IV B (T4aN3M0)	3	4.2%

DISCUSSION

Tonsillar cancer was already recognized in the 19thcentury and attempts were made to characterize it [7].

Risk factors. Tobacco and alcohol remain the major risk factors for tonsillar neoplasm, especially for squamous cell carcinoma [8-9]. In our study 62.5% of the patients were smokers, and 29.2% took alcohol and tobacco concomitantly. There is a high probability that some patients did not admit to having these habits (especially in the case of alcohol). The percentage of about 60% for smokers was also reported in the United States of America, but it presents variations in region and sex [10].

Tobacco is a risk factor independent of alcohol drinking, as demonstrated by studies on individuals who have never consumed alcohol [11]. There is an interaction between tobacco and alcohol well established for oral cavity cancers. The studies show that most cases are attributed to tobacco alone or a combination of tobacco and alcohol, while very few cases would be attributed to alcohol alone [12].

In high-income countries, tobacco prevalence has been decreasing in the last decades, due to the antismoking campaigns, which proves their importance [10].

Brandsma and Abramson found HPV in tonsillar squamous cell carcinomas in 1989, and subsequent studies have confirmed that HPV, especially the oncogenic subtype 16, increases the risk for developing tonsillar neoplasm [13-16]. The most frequent neoplasm sites associated with HPV infection in the head-neck area are the base of the tongue and palatine tonsils [13]. These patients have distinctive risk factors, such as a large number of sexual partners. Today, the link between oral sex behavior and oropharyngeal HPV infection is well known, HPV infection being the most common sexually transmitted disease in the United States [8]. The patients are usually younger, do not use cigarette smoke or alcohol, and have a better prognosis [17-18].

The HPV status of the tumor can be revealed by PCR or immunohistochemical staining [8, 19]. In our study, HPV viral analyses of the tumors had not been performed.

Clinical evaluation. The early injury, frequently asymptomatic, is mostly detected accidentally – 15% of our patients were found in stage 1. The patient may have no symptoms even in stage 2, due to submucosal tumoral development or deep formations in the tonsillar crypts [3]. However, in most cases the patients go to the doctor later, when dysphagia, cervical lymphadenopathy, and tumoral local extension are present – 54% of our patients were diagnosed in stages 3-4. The tonsillar asymmetry, palpable firmness, the visible lesion in the tonsil, cervical lymphadenopathy, unexplained weight loss, history of head-neck cancer can be local signs for a tonsillar neoplasm [20].

As well, the trismus (caused by pterygoid involvement), and otalgia (referred pain from the ninth and 10th cranial nerves) may be encountered [21].

The most frequently found symptom in our case series was dysphagia (63.8%).

Histopathology. A tissue biopsy can generally be performed with local anesthesia and is an essential step for confirming the suspect diagnosis.

The most frequent tonsillar cancer type is squamous cell carcinoma (75% of tonsillar neoplasm cases and 10% of head and neck malignancies [22]), followed by lymphoma [4], though other, rare histological forms have also been reported (small cell neuroendocrine carcinoma [23], mucoepidermoid carcinoma [24], small cell cancer of the tonsil [25]). The majority of tonsillar lymphomas are of B-cell origin. The association of B-cell tonsillar lymphoma with Epstein-Barr Virus infection is uncommon [26]. In our study, we evidenced squamous cell carcinoma in 80.6% of cases (51.4% non-keratinized, 29.2% keratinized), and B cell lymphoma in 18% of the cases.

Pediatric tonsil cancer is rare, with non-Hodgkin lymphoma accounting for the majority of pediatric tonsillar neoplasms. It raises suspicion in children who present with relatively rapid tonsil enlargement, tonsillar asymmetry characterized by a difference in the tonsillar size of ≥2 degrees on the Brodsky scale, or simultaneous cervical lymphadenopathy [27].

A meta-analysis on 54901 patients has shown that in current practice, the incurred cost of routine histological examination of tonsillectomy and adenoidectomy specimens is not warranted. The histopathological exam is warranted only when patients demonstrate findings associated with malignancy: tonsillar asymmetry, history of cancer, cervical adenopathy, tonsil injuries, weight loss [28]. Through biopsies, false-negative results may arise if the tumor is localized in the submucosa or deep crypts [29]. A tonsillar cancer is more likely to be missed by biopsies than by tonsillectomy [3].

Metastases of tonsillar neoplasm. The head-neck neoplasms usually extend firstly to the regional lymph nodes, but sometimes may metastasize to distant sites. The metastasis to distant lymph node groups is a rarely [30]. Furthermore, delayed multiple metastases without local recurrence are uncommon [31]. Approximately 15-20% of oropharyngeal carcinomas develop distant metastases, most commonly in the lung, liver, and bone [32-33].

The cutaneous metastasis from tonsillar cancer is rare but should be considered; new skin lesions should be searched and biopsied because the morphology of the cutaneous metastases from tonsillar squamous cell carcinoma is variable [22]. Cutaneous metastases result either from the local spread of cancer to overlying skin, (from direct extension through contiguous tissue planes), or more distant spread through either dermal lymphatics or blood vessels [34].

The metastases to tonsils are also rare, accounting for 0.8% of all tonsillar tumors [35]. The extension is due to the hematogenous dissemination and is associated with advanced-stage disease [36-37]. We did not identify metastases of tonsillar neoplasm or metastases to tonsils in this study.

Imaging investigations. The computed tomography or magnetic resonance imaging (MRI) is the best suited for the evaluation of oropharynx neoplasm. The cervical lymph node metastases are optimally evaluated by contrast CT. MR imaging is preferred for soft tissue assessment because of the greater resolution [38-39].

PET-CT scanning is indicated in search of an unknown primary tumor in patients who have a neck mass secondary to carcinoma, if a recurrent carcinoma may be present when there are metastatic with N0 cervical lymph nodes, or CT is inconclusive [39]. By ultrasound, we can perform an initial indicative TNM staging of the case before other expensive imaging studies such as CT and MRI [40].

In our study all the patients were examined by head-neck contrast-enhanced CT, with one exception – a patient requested a PET-CT in another unit.

Treatment. The treatment decision for tonsil cancer should be made by a multidisciplinary team.

According to the guidelines [41], early-stage tonsil cancer may be treated by transoral primary surgery [42] or open resection of the primary tumor, with or without neck dissection or definitive radiotherapy [43], the difference in the therapeutic benefit being insignificant.

The direct transoral approach provides the quickest and most direct way with the least potential of morbidity. However, advanced cancer or trismus, kyphosis, dental obstruction can limit access [44]. Transoral robotic surgery may be an effective alternative to open surgery or radiotherapy, especially for early-stage oropharyngeal squamous cell carcinoma [45].

Either Intensity–Modulated Radiation Therapy or 3D conformal radiotherapy of the primary tumor (Figure 6) and the involved lymph nodes with a total dose up to 70Gy is recommended, to minimize the irradiation to critical structures [46]. The addition of systemic therapy to radiotherapy should be considered for HPV-negative N1 disease and HPV-positive T2 disease and the involvement of a single node ≤3 cm [47].

Though the local-regional control and survival are similar following surgery or radiotherapy, primary surgery can be accomplished with minimal functional morbidity while reserving radiation for recurrent disease. Surgical salvage after radiotherapy may be difficult technically, being associated with a significant increase of complications [4]. Tonsil resection before radiation therapy is associated with improved survival in low-stage tonsil cancer [48].

For locally advanced resectable tonsil cancer the recommendations do not differ between the HPV status [41]. The treatment can be made either by giving concurrent systemic therapy and radiotherapy [49], or transoral or open resection of the primary and neck with appropriate adjuvant therapy, meaning systemic therapy concomitant with radiotherapy or radiotherapy alone, or as a third option induction chemotherapy followed by radiotherapy or concomitant systemic therapy with radiotherapy. Surgery and postoperative radiotherapy continue to provide a superior outcome in locally advanced tonsil squamous cell carcinoma in patients with the surgically resectable disease [50].

When concurrent systemic therapy/radiotherapy for the locoregionally advanced cancer of the tonsil is administered, the high-dose cisplatin is the preferred systemic agent. As induction chemotherapy, the preferred regimen consists of taxane/cisplatin/5-fluorouracil, known as TPF [51] or paclitaxel/cisplatin/5-fluorouracil [52].

Though data are scarce regarding the prevalence of bilateral synchronous tonsil cancer (the first report in 1971 [53]), certain authors maintain that this form of the disease is under-reported, and they recommend bilateral tonsillectomy in all patients with suspected or proven unilateral tonsil cancer and in patients with cervical carcinoma metastasis from an unknown primary tumor [54]. The recommendation for a bilateral tonsillectomy may be found in the literature but without application in clinical practice or integration into guidelines [3, 55]. On the other hand, unanimity exists for ipsilateral tonsillectomy.

Due to the deficiency of integrated services, many patients are lost from otorhinolaryngological follow-up. The most of our patients in the advanced stage never returned to our clinic after the start of oncological treatment. Because of this, we cannot show survival rates for our patients.

CONCLUSIONS

In Romania, smoking is still the most important risk factor for tonsil cancer, and HPV detection analysis is not available in public hospitals.

Most patients have visited a doctor when dysphagia and, above all, palpable cervical adenopathy, were present, but the tumor was in an advanced stage, which means that the therapeutic management requires close collaboration between otorhinolaryngologist and the radiotherapist-oncologist.

In Romania, the ENT-oncology integrated medical services are few, in most cases patients are lost from otorhinolaryngological follow-up.

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ORIGINAL ARTICLES

Total knee arthroplasty radiographic evaluation via a Bayesian belief network. A pilot study

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Abstract: *In an attempt to standardize the X-ray analysis and to create relevant correlations between it and the functional results, we present a pilot model of an artificial neural network based on a Bayesian belief network (BNN) designed for the automatic analysis of anteroposterior X-ray of the knee following total knee arthroplasty and more precisely the analysis of the tibial component.*

A prospective analysis was conducted in which 30 patients were analyzed and two groups resulted: the first was made up of 12 cases considered "normal" by the examiners (optimal positioning and cementation) and the second one, which was considered "pathological" (incorrect positioning or cementation) and included 16 cases.

Based on 22 points established on the tibial component several geometric sizes (calculated parameters) were computed. Several parameters were compared and the results were up to 75% accuracy, 81.25% sensitivity, and 66.7% specificity.

BBNs can be a solution for X-ray evaluation of total knee arthroplasty and can improve the understanding of its evolution. It needs confirmation in time, its accuracy rising with the number of patients introduced in the analysis algorithm.

Keywords: *TKA; Bayesian method; ANN; X-ray analysis*

INTRODUCTION

Artificial Neural Networking (ANN) are computer systems that create connections, similar to those existing in the biological neural network [1]. Such systems are programmed to learn based on examples, without being programmed in advance to identify specific tasks [2]. For example, artificial neural imaging systems can identify an area of osteolysis, the area that has been previously set as osteolysis using a yes/no algorithm on other images. They can perform this task without having the ability to understand what an osteolysis area means.

ANN is made up of a mass of nodes connected by artificial neurons just like the component of a biological brain. Artificial neurons are interconnected by synapses and can send information between them.

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The initial purpose of these artificial neural networks was to create a replica of the human brain and the latter to be able to make decisions similar to those made by a human. Following their application in different fields, the initial purpose was forgotten. They are currently used for verbal recognition, translation, social networks, but most importantly in the medical diagnosis, such as total knee arthroplasty (TKA) [3, 4, 5, 6].

Thus, for patients with diseased knee or hip joints, total knee or hip arthroplasty (TKA/THA) is considered as one of the most effective procedures for relieving pain and improving joint function [7]. Improvements in joint arthroplasty procedures will routinely witness rapid recovery, faster functional recovery, lower postoperative discomfort, and higher patient satisfaction, which is a win-win for patients and surgeons [8].

The importance of the restoration of the biomechanical axis of the pelvic limb is emphasized in current studies [9]. Proper prosthetic positioning, mobility restoration, and pain disappearance are factors predicting the long-term survival of the prosthesis as well as the postoperative patient satisfaction [10].

Figure 1: Postoperative TKA image used for Bayesian evaluation

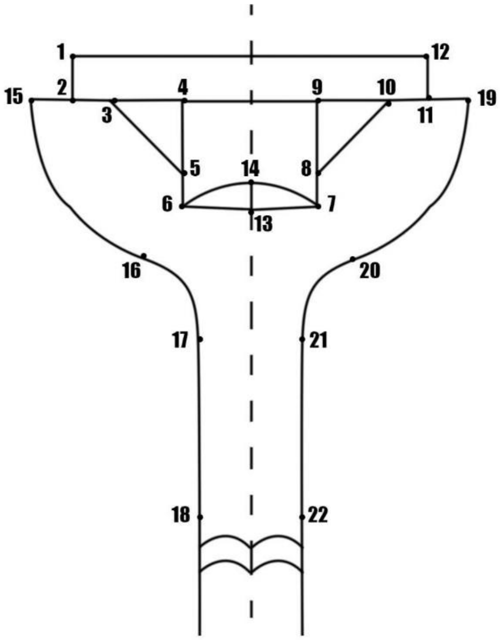

Radiological postoperative evaluation following total knee arthroplasty is essential to improve the quality of the surgical act. The standard procedure implies that two postoperative X-rays are performed, one anteroposterior, and one profile X-ray. They analyze the mechanical alignment of the knee, bone coverage by the prosthesis, femoral anterior and posterior offset, femoral flexion and extension, tibial slope (Figure 1).

Also, during the initial postoperative evaluation, any deficiencies in the cementation technique are identified. Ideally, it is sought to obtain a uniform cement coat for thickness and distribution.

In an attempt to standardize the X-ray analysis and to create relevant correlations between it and the functional results, we present a pilot model of an artificial neural network based on a Bayesian belief network (BNN) designed for the automatic analysis of anteroposterior X-ray of the knee following total knee arthroplasty and more precisely the analysis of the tibial component.

BNNs are frequently used in medicine, they provide the probability of an outcome based on creating intuitively relations between variables [11-15].

MATERIALS AND METHODS

A prospective study in which two lots of patients were used. In both, patients with gonarthrosis were included, who were subjected to total knee arthroplasty, which was performed in the same center by the same surgical team. The inclusion criteria were patients over 65 years old, who were subjected to TKA, with underlying gonarthrosis disease. Exclusion criteria included inflammatory arthritis, post-traumatic gonarthrosis with major deformities, unicompartmental arthroplasty.

The two lots of patients were conceived following the X-ray analysis on day 0 postoperatively by an independent team of orthopedists. Standing AP, profile, and full leg X-rays of 30 patients were analyzed and two groups resulted: the first was made up of 12 cases considered “normal” by the examiners (optimal positioning and cementation) and the second one, which was considered “pathological” (incorrect positioning or cementation) and included 16 cases. 2 patients were excluded following the appearance of postoperative complications. The same patient groups were followed up at 1 month, 6 months, and 1 year postoperatively. The same set of X-rays was performed at each visit.

Following the constitution of the two groups of patients, it was decided to start the computer analysis of the postoperative X-rays from 6 months and 1 year. To test the validity/relevance of the automatic analysis, we decided that the initial analysis should be performed at the tibia level, on front-loading X-ray. 22 control points were established at the level of the tibial component (Figure 2) that corresponds to the placement of the prosthesis and the tibial bone, as shown in the schematic picture, 14 points on the contour of the prosthesis and 8 points on the tibia.

Figure 2: 22 control points at the level of the tibial component

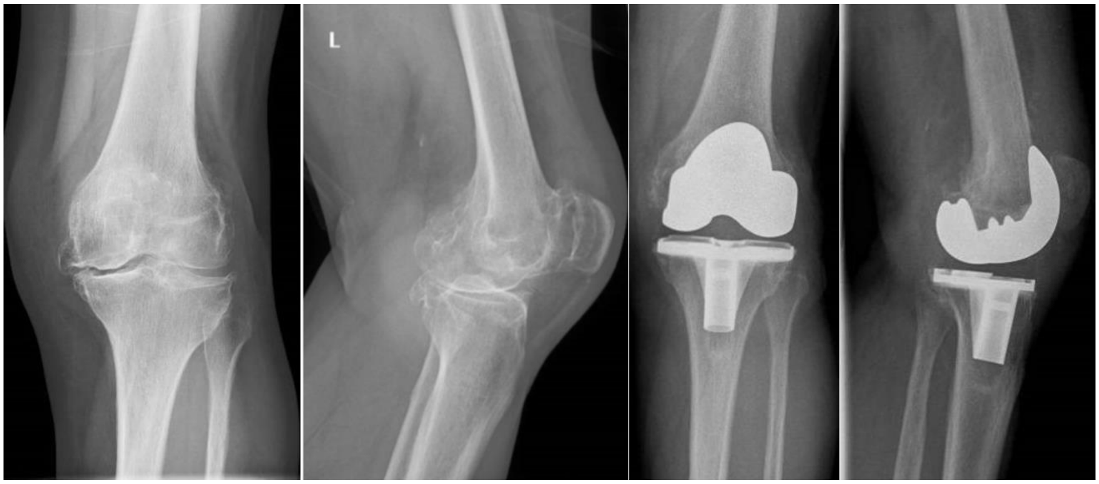

Using the identified points, several geometric sizes (calculated parameters) were computed:

1. The left-right rotation of the X-ray image, determined by the difference of the areas associated with the triangles determined by points 3 – 4 – 5 and 8 – 9 – 10 on the prosthesis.
2. Up-down rotation of the X-ray view, determined by the area associated with the ellipse determined by points 6 – 7 – 13 – 14 on the prosthesis.
3. The angle of vertical offset between the axis of the prosthesis (calculated as bisector of the angle formed by sides 4 – 5 – 6 and 7 – 8 – 9) and the axis of the bone, calculated as bisector of the angle formed by sides 17 – 18 and 21 – 22.
4. Horizontal offset angle between the base of the prosthesis (2 – 3 – 4 – 9 – 10 – 11) and the cut of the bone 15 – 19.
5. Percentage of coverage to the left of the prosthesis, determined by the part of the head bone not covered by the prosthesis at level 15 – 2.
6. Percentage of coverage to the right of the prosthesis, determined by the part of the head bone not covered by the prosthesis at level 11 – 19.
7. Variation of previously calculated parameters between postoperative images at 1 month and 6 months.

RESULTS

Following the schematic of the calculated parameters, a Bayes statistical model was applied.

DECISION 1: Normal-pathological differentiation tests at the level of individual X-rays

Of the many variants tested to make the decision, the best results were obtained based on measure 5 (percentage of coverage left of the prosthesis), with a threshold of 0.82 for differentiation (pathological > 0.82). Taking into account these conditions, the normal/ pathological decision correctly identified: 11 images from normal patients (out of the 24) and 26 images from pathological patients (out of the 32).

This led to the following sizes: 66.1% accuracy, 81.25% sensitivity, and 45.8% specificity.

DECISION 2: Normal-pathological differentiation tests at the level of the pair of individual postoperative X-rays at 1-6 months

Of the many variants tested to make the decision, the best results were obtained based on combined measures:

A. The decision according to a single parameter: the sum of parameter 1 (left-right rotation of the X-ray view) at 1 and 6 months, with a decision threshold of 0.47; pathological > 0.47). Taking into account these conditions, the normal/ pathological decision correctly identified: 9 pairs of images from normal patients (out of the 12) and 9 pairs of images from pathological patients (out of 16).

This led to the following sizes: 64.3% accuracy, 56.25% sensitivity, and 75% specificity.

B. The decision according to several parameters: the sum of parameter 1 (left-right rotation of the X-ray view) at 1 month and 6 months, with a decision threshold of 0.47, pathological > 0.47, or difference of parameter 3 (vertical offset angle between the axis of the prosthesis) at 1 month and 6 months, with a decision threshold of 0.75, pathological < 0.75 and difference of parameter 4 (horizontal offset angle between the axis of the prosthesis) at 1 month and 6 months, with a decision threshold of 1.8, pathological < 1.8.

Taking into account these conditions, the normal/ pathological decision correctly identified: 8 pairs of images from normal patients (out of 12) and 13 pairs of images from pathological patients (out of 16). This led to the following sizes: 75% accuracy, 81.25% sensitivity, and 66.7% specificity.

DISCUSSION

Artificial neural networks are increasingly present in classifications, group creation, pattern recognition, and prediction in many disciplines, medicine being among them. The huge potential is represented by speed, volume, and accuracy of data analysis.

The Bayesian system developed by us can recognize immediately and accurately any deficient positioning of the tibial prosthetic component. It can integrate this assessment into the clinical context and provide answers to a series of questions of patients with low satisfaction. It may be an optimal way of evaluating the series X-ray with results that are superimposed over those obtained by a CT scan.

Our model of X-ray analysis by BBN has several limitations. The number of patients is not large enough to obtain sufficiently conclusive results. X-ray analysis is performed only for the tibial component at present, a comprehensive X-ray analysis that also includes the femoral component is required. The registered data do not contain patient information: age, weight, degree of osteoarthritis, preoperative and postoperative mobility, pain scores, and so on.

CONCLUSIONS

BBNs can be a solution for X-ray evaluation of total knee arthroplasty and can improve the understanding of its evolution.

It needs confirmation in time, its accuracy rising with the number of patients introduced in the analysis algorithm.

It may provide an answer for low satisfaction rates of certain patients in whom the prosthetic components are optimally positioned and have no other postoperative complications.

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REVIEW ARTICLE

Toxoplasma Gondii infection and the signs of fetal affection during pregnancy as seen on ultrasound

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Abstract: *Toxoplasma Gondii is a protozoan parasite which causes multisystem affections and unusual clinical outcomes. Although primo- infection takes place frequently during childhood, there is a possibility for it to be found during pregnancy, when it could lead to congenital toxoplasmosis, with serious ocular and cerebral lesions (that can be identified during ultrasound). The positive diagnostic for the mother is made after a serologic test and for the fetus the parasite can be found in the amniotic fluid during amniocentesis.*

A prompt treatment can avoid the presence of cerebral lesions (hydrocephalus, ventriculomegaly, intracerebral calcifications).

Keywords: *protozoan, pregnancy, serology, amniocentesis, hydrocephalus, ventriculomegaly, intracerebral calcifications*

INTRODUCTION

Toxoplasma Gondii is a protozoan parasite that affects the human body in many ways; Infection generally occurs in infancy or adolescence, with declining prevalence in developed countries in the temperate climate [1, 2]. During an initial infection, an immunocompetent body will limit the spread of the parasite, but it remains cantonized in the nervous and muscular tissues, and cannot be completely eliminated [3].

When the infection occurs during pregnancy, there is a risk of transmission to the fetus, resulting in congenital toxoplasmosis that can be manifested by ocular and neurological damage. Proliferation of the parasite may continue, and cerebral tissue destruction may occur even after an important maternal immune response, including IgG production [1, 4].

The incidence of maternal infection during pregnancy varies between 1-8 ‰ [1, 5].

In the acute phase the infection is usually asymptomatic. If symptoms occur, these are nonspecific, such as fatigue, fever, headache, malaise, and myalgia [6].

Differential diagnosis is made by clinical appearance of acute Epstein-Barr infection, CMV, HIV, Treponema, sarcoidosis, Hodgkin's disease or lymphoma [1].

Positive diagnosis of maternal infection in pregnancy involves at least two blood test results with seroconversion from negative to positive for Toxoplasma-specific IgM or IgG. Note that the presence of only IgM in the blood does not automatically involve acute infection, with data showing that only 20% of patients with IgM positive had acute infection with Toxoplasma [7].

Screening for Toxoplasma is performed in some European countries on a monthly basis or at 3 months during

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pregnancy, with the major advantage of rapidly detecting such infection and increasing the effectiveness of therapy [8]. The US, Canada and UK do not routinely test pregnant women because the prevalence of primo-infection during pregnancy appear to be sufficiently low and the costs seem to outweigh the benefits [5,9]. On the other hand, repeated tests increase the number of false positive results, resulting in an unnecessary elevation in the number of prenatal invasive investigations and the number of treated patients. These drawbacks should be weighed against the potential benefit of early-onset therapy, which appears to be present only in the case of serious but rare cases of neurological sequelae of congenital toxoplasmosis [1,11].

IgM antibodies are positive in the first week of infection. Diagnosis of the primo-infection is much more precise when the initial testing shows IgM positive and negative IgG, and at the 14-day retest both of them become positive. This combination of results represents the safest diagnosis of Toxoplasma primo-infection two weeks ago and eliminates a possible false-positive result for IgM. If IgG retests remained negative, most likely positive IgM was only a false positive, and recent or older infection is excluded. An important problem is a positive IgG and IgM result in the first trimester because once IgG is present, IgG remains Positive for life [1].

Depending on the laboratory, the real risk of infection after conception is only 1-3% [12]. Since IgM is only positive after only two weeks but can remain positive for years and IgG reaches a maximum titer at 6-8 weeks and then decreases slowly over the next two years, the positive diagnosis of recent infection requires additional tests [13]. A very useful option is testing IgG avidity, which is very high in old infections, but may remain at a low level for some patients for some years [14]. IgG titration may be a variant, but is insufficiently evaluated and requires a reference laboratory for good quality of the results [1].

MATERIAL AND METHOD

Fetal infection occurs by transplacental transmission after maternal primo-infection. The risk of transmission to the fetus increases drastically with increasing gestational age [15]. In a meta-analysis of 1438 patients in 26 cohorts by individual patient data, it is estimated that the risk of maternal-fetal transmission is 15% when maternal seroconversion occurs at 13 weeks, 44% when seroconversion takes place at 26 weeks and 71% when seroconversion prevails at 36 weeks [15]. The study showed that early therapy seems to reduce the risk of developing congenital toxoplasmosis, but the strength of this assertion is low [15].

RESULTS

As mentioned earlier, the same meta-analysis has shown that with increasing gestational age to seroconversion, the risk of maternal-fetal transmission of T Gondii infection increases drastically, but the risk of cerebral fetal sequelae decreases and eye damage is not influenced by this factor [15].

Figure 1: A: Cerebral calcifications, B: Hydrocephaly, C: Vetriculomegaly

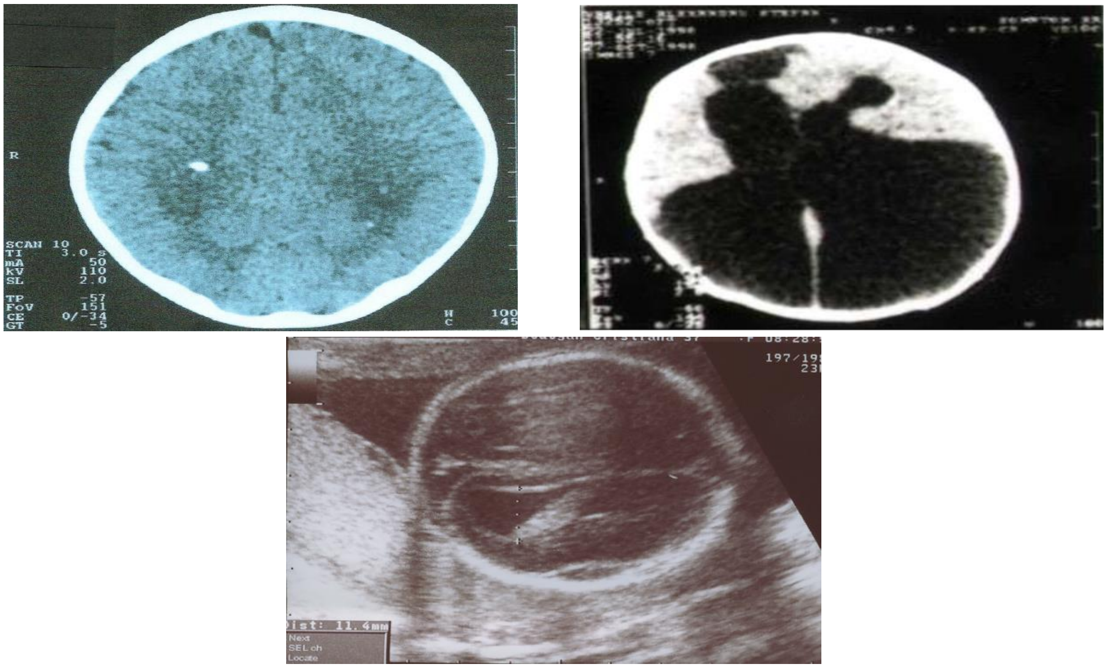

Ultrasound signs in Toxoplasma Gondii infection are generally nonspecific. The most common signs are intracerebral calcifications, intracerebral hyperecogenic seals and ventriculomegaly, usually medium or severe bilateral symmetry. The existence of brain pathological images usually means a gloomy prognosis with numerous possible impairments: corioretinitis, psychomotor retardation, blindness, epilepsy, postnatal hydrocephalus [16]. In fact, in a study of 8 confirmed children with congenital toxoplasmosis, only the absence of any pathological brain imaging or the existence of only a mild ventriculomegaly were associated with favorable postnatal prognosis [16]. Another study found that only 43% of fetuses with signs of impaired brain had a poor prognosis [17].

This study involving 32 fetuses with confirmed congenital toxoplasmosis showed that evolution intrauterine lesions is usually very fast, on the order of days [17]. However, the cranial pathological images were identified only after 21 weeks of pregnancy in all the studies quoted [16, 17]. Prognosis also appears to be more unfavorable in cases where seroconversion occurred earlier in pregnancy, or the patient did not receive treatment for this infection [16, 17].

Besides cerebral signs, fetal toxoplasmosis can be manifested along with other signs, but lacking specificity for this pathology. Thus intrahepatic calcifications, ascites, placental hyperecogenic images with thickened placenta, or rarely pleural and pericardial effusions [17, 18] can be discovered.

Restriction of intrauterine growth and microcephaly can be associated with this fetal infection, but they are not characteristic [16, 17].

Spontaneous fetal death of the fetus with the termination of pregnancy in evolution does not seem to be more common in affected fetus [17]. A slight tendency towards early childbirth appears to be influenced by obstetrical attitudes and not necessarily by fetal harm [17].

Prenatal diagnosis of this fetal infection can only be achieved by performing an amniocentesis with PCR analysis of the resulting amniotic fluid. Amniocentesis carries a risk of abortion or premature birth, a small but still present risk [15]. Patients should be informed of this risk and of the potential benefits of this diagnostic approach [15].

The main benefits of fetal infection confirmation with Toxoplasma Gondii refer to the subsequent therapeutic course in choosing the right drug combination. One problem, however, is that there are no randomized studies showing the benefit of a treatment regimen at the expense of another, most of the schemes being based on old, poor quality studies [15]. Another possible reason for carrying out amniocentesis is the exclusion of a possible fetal infection, which implies an easier to follow treatment by the patient with lower drug doses and reduced side effects. Last but not least, positive diagnosis can help the patient to make an informed decision about a possible discontinuation of pregnancy [15], knowing that a positive diagnosis accompanied by signs of fetal cerebral affection is associated with an unfavorable prognosis. On the other hand, a negative diagnosis of amniocentesis accompanied by a lack of any signs of fetal harm will limit the need for aggressive postnatal antibiotic treatment and will also have a beneficial effect on the mother's psyche.

CONCLUSION

T Gondii infection during pregnancy occurs in approximately 1-8 ‰ of the population and is usually asymptomatic. Diagnosis of this infection is done either by testing for nonspecific symptoms such as fatigue, fever, headache, malaise and myalgia, or through dedicated screening tests performed in pregnancy. There is a great variation in assessing the need for screening for this infection in the world, so only some countries offer such programs.

Diagnosis is performed by detecting specific IgM and IgG immunoglobulins in the serum, the proportion of which varies depending on the age of the infection. Generally, IgM is an acute phase control, and IgG indicates an old infection that will not cross the transplacental barrier. Testing IgG avidity against the parasite seems to help increase the sensitivity and specificity of acute infection detection.

Maternal infection with Toxoplasma Gondii during pregnancy may lead to fetal infection with this protozoan by transplacental transmission. Fetal infection does not necessarily imply an unfavorable prognosis, but the occurrence of suggestive echographic signs for this pathology is a serious factor. There are no signs of this kind of fetal infection, but the affected faces usually present some cerebral pathological echographic imaging, including cerebral calcifications, medium or severe symmetrical bilateral ventriculomegaly or hyperrecognized intracerebral seizures. There are other non-specific signs of fetal damage.

The positive diagnosis of fetal infection is done by testing amniotic fluid. This diagnosis will help in choosing the required treatment regimen, although there are no solid randomized studies indicating the use of a particular regimen in a given situation.

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ORIGINAL ARTICLES

Treatment persistence of biologics in psoriatic disease

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Abstract: *Psoriasis is a chronic, immune-mediated inflammatory disease that significantly affects a patient's quality of life. Several systemic complications can influence disease progression and response to treatment. Biological therapy is considered the most effective therapeutic option in treating moderate to severe forms of psoriasis. The success of therapy may depend on its persistence, also known as drug survival. The dermatologist needs to be aware of comorbidities and factors that influence the persistence or discontinuation of biological treatment to make appropriate treatment decisions. We have identified several studies on the persistence of biologic therapy or drug survival in patients with psoriasis and summarized some of the most important issues known to date. In several recently published studies, the survival of ustekinumab is better than TNF-α inhibitors but lower than ixekizumab. Notably, the studies were performed on a limited number of patients and follow-up time. Moreover, secukinumab appears to have a shorter drug survival than other biological agents, especially in patients with biological experience.*

A real indicator of therapeutic success could be a high quality of life. Female gender and obesity have been consistently highlighted among the predictors of treatment discontinuation, while psoriatic arthritis could be a predictor of persistence and maintenance of biological therapy. We are currently aiming to improve the therapy for patients treated with biological agents to reduce the pressure on the national public health system.

Keywords: *psoriasis, psoriatic disease, biological therapy, persistence, predictive factors, drug survival*

INTRODUCTION

Psoriasis is a chronic, immune-mediated disease with high genetic susceptibility, of still unknown etiology which may be associated with abnormal T-lymphocyte function [1]. Lately, there has been a lot of talk about the concept of psoriatic disease. It is no longer considered an exclusive skin condition and the management of this complex disease has been simplified by the recent understanding of the pathophysiological mechanisms.

Understanding comorbidities and factors that can easily destabilize the psoriasis patient guides the physician in choosing the proper therapy and leads to improved treatment effects [2, 3, 4]. Conventional therapies have not fully met the needs of psoriatic patients due to limited efficacy, side effects with cumulative use, and patient inconvenience [5].

Recently, the development of new biologic therapies has revolutionized the management of patients with moderate to severe psoriasis vulgaris, patients with multiple comorbidities who require long-term therapy [6, 7]. Currently, in our country, the biological therapies used to treat psoriasis are TNF inhibitors (TNFi): adalimumab,

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etanercept, infliximab, and their biosimilars [8, 9], anti-IL12/23 (ustekinumab), anti-IL-17A (secukinumab, ixekizumab), and anti-IL-23 (risankizumab, guselkumab). These therapies could lead to a long-term remission of psoriasis, however, the effectiveness may decrease over time, the loss of response to the treatment being the main cause of its discontinuation [2]. As a result, the trend of long-term use of biological substances has led to an attempt to understand the persistence of treatment over time, also known as drug survival.

What Is Drug Survival?

Recently, the term "drug survival" also known as "drug retention", "drug persistence" or "drug adherence" has made its way through the field of dermatology. "Drug adherence" can be defined as the time between the initiation of treatment with a particular drug and its discontinuation for various reasons [10, 11]. This is a marker of therapeutic success, fully encompassing the elements of treatment efficacy and safety because ineffective or poorly tolerated therapy will most likely be discontinued [12, 13]. Furthermore, the continuation of treatment can be directly influenced by country, patient characteristics, and specific drugs used for treatment, cost, flexibility, quality of life, dosage range, or accessibility [6].

The first meta-analysis [2] that included 16 cohort studies on predictive factors of persistence of biological therapies was published in 2019. The main purpose was to identify the predictive factors of persistence or discontinuation of therapy, regardless of the therapeutic option (choice of biological therapy). It was pointed out that female patients who benefit from biological therapy are more likely to discontinue treatment, especially if they associate obesity compared to men, especially if they are normal weight. The reasons why women are more affected seem to be the side effects associated with therapy on the one hand and insufficient therapeutic satisfaction on the other. Possible arguments to explain why discontinuation of medication is more common in obese patients would be lower levels of drugs in the blood, large body surface area, or increased proinflammatory cytokines in adipose tissue that are thought to interfere with therapy. The results of the study emphasize the importance of weight loss in patients with psoriasis vulgaris to maintain the therapeutic effect. Moreover, according to this meta-analysis patients who suffer from psoriatic arthritis are more likely to continue therapy compared to patients who have only skin lesions. Other factors previously identified as predictors of biological discontinuation included smoking, metabolic syndrome, age, dyslipidemia, high socioeconomic status, DLQI, and biological naivety, but there were insufficient data for the meta-analysis of these factors [2].

Another meta-analysis [14] based on no less than 109 studies compared the efficacy and safety of known systemic therapeutic classes: conventional systemic agents (acitretin, cyclosporine, fumaric acid esters, methotrexate), small molecules (apremilast, tofacitinib, ponesimod), TNFi (etanercept, infliximab, adalimumab, certolizumab), anti-IL12/23 (ustekinumab), anti-IL17 (secukinumab, ixekizumab, brodalumab), anti-IL23 (guselkumab, tildrakizumab), and other biologics (alefacept, itolizumab) for patients with moderate to severe psoriasis. All studies included in this meta-analysis were limited to the induction phase (assessment at less than 24 weeks after randomization). At the drug level, in terms of reaching PASI 90, all anti-IL17 agents and guselkumab (an anti-IL23 drug) were significantly more effective than TNFi agents such as infliximab, adalimumab, and etanercept, excepting certolizumab. Another conclusion was that ustekinumab was superior to etanercept. No clear difference was shown between infliximab, adalimumab, and etanercept. Tofacitinib was significantly superior to methotrexate, and no clear difference was shown between any of the other small molecules compared to conventional treatments. Cochrane review has found that biologics are more effective than conventional, systemically administered drugs (such as methotrexate or cyclosporine) in a selected population of patients with moderate-to-severe psoriasis. However, the evidence is limited to the induction phase (12 to 16 weeks) and is not sufficiently relevant for chronic diseases [14].

In 2016, an article published in a prestigious journal [6] highlighted that lack of response and efficacy was the most commonly observed reason for discontinuation of biological treatment. There was also a better persistence of ustekinumab compared to TNFi (infliximab, adalimumab, etanercept). Notably, patients who are receiving methotrexate simultaneously with biologic therapy are more likely to discontinue treatment [6]. In this article, males were associated with persistent treatment, as shown in other studies [15, 16, 17].

Ustekinumab appears to be administered for a longer period, with lower discontinuation rates compared to anti-TNFα, information documented in the last 5 years [12, 13, 18-23]. A systematic review and comparative meta-analysis published in 2021 highlighted that the IL-12/23 inhibitor (ustekinumab) demonstrated superior biologic persistence when compared to TNFi. The same study makes a comparison between TNF-α inhibitors and reveals that adalimumab was superior to etanercept and infliximab at year 5. Of note, there was no difference between the pooled drug survival for adalimumab versus etanercept and etanercept versus infliximab at year 2. At year 1, secukinumab was superior to ixekizumab; however, there was no difference when secukinumab was compared to TNF-α inhibitors. Interestingly, the survival of ustekinumab remained superior when compared to the pooled secukinumab drug survival [24].

Although there are few recent studies, they have shown increased interest in assessing persistence in patients treated with anti-IL-17 agents: secukinumab and ixekizumab. In a nationwide cohort of patients with psoriasis in Denmark, drug survival was longer for ixekizumab, however, the number of patients and follow-up time were limited [25]. Moreover, in an observational study conducted in Sweden [26], the median persistence of biologic therapies in patients with psoriasis vulgaris was approximately 23.8 months. One year after treatment, the percentage of persistent patients was higher for ixekizumab (81.3%), followed by ustekinumab (79.9%), secukinumab (75.9%), adalimumab, and etanercept. Biologic-naive patients appear to have had a longer persistence than those with biologic experience, except for certolizumab pegol and ixekizumab, both of which were limited by the small number of patients in this study. There is sufficient information to show that for bio-naive or bio-experienced patients on anti-interleukin therapies there is no difference in terms of achieving efficacy or rapid onset of efficacy.

Another observational study in the Swedish population, this time patients with biological treatment associating psoriatic arthritis, showed a persistence profile of therapy in patients treated with ustekinumab, both naive and biologically experienced, while secukinumab has a favorable persistence only in biologically naive patients, compared to adalimumab, which also had resulted in patients with biological experience. This study also highlighted factors that may predict treatment discontinuation, namely female gender, axial involvement, recent onset of the disease, and increased experience in the use of biological treatment [27]. In a study in Germany, a group of patients with psoriatic arthritis showed that the persistence rates of biological disease-modifying biologic antirheumatic drugs (bDMARDs) were higher for ustekinumab compared to infliximab or golimumab. Moreover, mean persistence was higher for ustekinumab and lower for the other two molecules. The causes of non-persistence were switching from one bDMARD to another and discontinuation of therapy. Lower risk of discontinuation of treatment was significantly correlated with the male sex. [28].

Additional measures are important to improve outcomes in medication persistence such as increasing access to rheumatologists and implementing a pharmaceutical care strategy. In Brazil, medication persistence was lower than in other countries. A total of 161 patients were included in a study published in 2019 and the results demonstrate that anti-TNF agents are important therapeutic alternatives and present lower discontinuation as compared to conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) after 6 and 12 months of follow-up. Adalimumab and etanercept presented similar persistence results in the anti-TNF group and leflunomide and methotrexate in the csDMARDs group [29].

Regarding the administration of biological therapy in other forms of psoriasis, this is effective and well-tolerated for the treatment of hyperkeratotic pustular psoriasis (PP), but fewer data are available on the treatment of pustular psoriasis or palmoplantar psoriasis (PPP). Adalimumab, guselkumab, ixekizumab, secukinumab, and ustekinumab showed more than 80% efficacy for the treatment of hyperkeratotic PP, while infliximab and ustekinumab showed moderate efficacy for the treatment of pustular PP, and infliximab was the most efficacious treatment for PPP [30].

In randomized controlled trials, secukinumab, an interleukin 17A antagonist, is highly effective in treating psoriasis. However, there has been observed a tendency of reduced adherence to treatment in females, smokers, obese, and biologically experienced patients [31, 32].

IMPLICATIONS IN MEDICAL PRACTICE

Current evidence suggests that ustekinumab is associated with the best drug survival in all biologic-naive subjects. Ustekinumab is also the most common biological therapy least associated with discontinuation due to loss of efficacy.

A meta-analysis published in 2018 shows that the drug survival rate for etanercept, adalimumab, infliximab, and ustekinumab decreased year by year and overall, etanercept was associated with the lowest drug survival rate, and ustekinumab was associated with the highest one [33]. Ustekinumab has been shown to have a higher treatment rate, with effects lasting up to one year and up to five years of treatment, unlike TNFi [18, 19, 34].

Among the predictive factors for discontinuation of biological therapy were female gender, smoking, and obesity (BMI>35 kg/m^2) [35, 36]. However, the association of psoriatic arthritis may predict long-term maintenance of treatment (a predictor for drug survival) [2, 37, 38].

Drug survival rates differed among biologic therapies and decreased over time; second-line discontinuation due to adverse events was more common among those who discontinued first-line treatment for this reason [39].

These data are useful in guiding therapeutic management, as well as in optimizing the treatment of patients suffering from psoriasis.

CONCLUSION

The progress of personalized therapy and the introduction of new biological molecules in the last decade have facilitated a significant change in the treatment paradigm in psoriasis and psoriatic arthritis. Maintaining the long-term clinical efficacy of different classes of biological agents is vital in such chronic conditions, and drug survival is a marker for the sustainability of treatment.

Long-term therapy for psoriasis is affected by the gradual loss of effectiveness and discontinuation of treatment. The overall effectiveness of biological agents is reduced over time. Identifying therapy with long survival rates, as well as predictive factors of treatment discontinuation or persistence, may help individualize biological therapy and improve treatment outcomes and satisfaction. Psoriatic arthritis in parallel with psoriasis is associated with improved persistence.

Authors' contributions

SB, MDC, TC contributed to all stages of the article, designed the article, and revised the manuscript for important intellectual content. MMC and FS contributed to the conception of the manuscript, to the revision of the language, and contributed to the drafting of the work. SB, MMC, and FS critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

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VARIA

Typology of individual values of combatants of the War in Eastern Ukraine

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Abstract: *The individual values of combatants determine life goals, how to achieve them, and perform an important adaptive and protective function under conditions of combat stress. Understanding them enables mental health professionals to create an effective system of Combat and Operational Stress Control for military personnel.*

The purpose of this research was to determine the personal value sphere typology of individual values of combatants of the War in Eastern Ukraine, and, on its basis, to predict the vector of PTSD development.

The value sphere (Rokeach Value Scale) and peculiarities of mental status ("Traumatic Stress Questionnaire") of nine hundred forty three Ukrainian combatants were investigated.

Cluster analysis made it possible to create a typology of combatants' values, consisting of five types: "Passionary", "The one who does not leave the comfort zone", "Romantic", "Formal military man", "Routine". The most resistant to combat stress are combatants who are aimed at interiorizing values, expanding the boundaries of existing norms, and public recognition ("Passionary" (2.19%), "Romantic" (3.82%)), and the most vulnerable are those who are "Routine" (15.30%) and "Formal military man" (4.37%). The majority of Ukrainian combatants have a defined type of "The one who does not leave the comfort zone" type (74.32%). For them, safety and well-to-do life are the main priorities.

Keywords: *values, posttraumatic stress, hostilities, combatants, Ukraine*

INTRODUCTION

Values are the core motivators behind actions that underlie individual decision-making and affect human interaction. They are a strong guiding force, consistently determining behavior and shaping attitudes [1-2]. The values of the military personnel of the armies of different countries are based not only on individual universal human values but also on specific military values, such as loyalty, duty, unselfish service, respect, honor, courage, self-sacrifice, patriotism, etc [3-5]. Despite the national differences, there is one international military culture in the world that reflects common values [6].

In combat conditions, the value sphere of a soldier performs an adaptation function [7]. It contributes to the preservation of basic individual and military values [8], their change [9-10], as well as the formation of new value orientations [11-12]. The value sphere of the personality allows us to experience the influence of trauma softer and determines the vector of development (posttraumatic growth) [13-14] or personality regression [15].

Modern research on the value sphere of a personality after exposure to various extreme situations is based on four fundamental theories of values of C. Kluckhohn, G. Allport, M. Rokeach, and S. Schwartz. These studies focus on the relationship between posttraumatic stress symptoms and

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personal values of crime victims, and as well as between adjustment disorder symptoms in individuals exposed to critical life events [16-17]; changes in the value sphere of the personality of military personnel due to the stressful or traumatic events [10, 18]; the influence of the soldiers' personal values on the occurrence of depression, anxiety and somatoform symptoms [19]; the influence of traditional and modern values on human mental health [16, 20-21]; the influence of values on pathological addictions [22-23] and etc.

Researchers claim that values do shed light on the behavior, actions, and attitudes of individuals and have a wide variety of predictive qualities [24]. Maercker et al. [16] have found out that modern values (achievements, hedonism, stimulation) protect against stress by mediating the processes of social interaction. At the same time, traditional values (conformity, benevolence, customary orientation) increase stress through the same mechanism. It disproved the popular assumption that traditional values contribute to the preservation of health, and modern values worsen it [20-21].

The above is also supported by studies by Zimmermann et al. [19-25], who found that a strong and conscious personality value system can help to protect military personnel from developing psychogenic depression, and posttraumatic stress disorder (PTSD) in a combat situation. At the same time, values that are formed from a deep sense of altruism and partnership increase subjective distress if PTSD occurs [19]. The military personnel with such values is more psychologically vulnerable. If they witness torment (worry) during the service, then they experience them as their own [19].

Thus, it is clear that the values of combatants perform an important adaptive and protective function under conditions of combat stress. Understanding of them enables military mental health professionals to create an effective system of Combat and Operational Stress Control (COSC) for military personnel.

Since 2014, full-scale hostilities have been waged in Eastern Ukraine between the official defense structures of Ukraine and illegal armed groups of the self-proclaimed Donetsk and Luhansk republics. Military personnel of the Armed Forces of Ukraine and the National Guard of Ukraine (NGU) is taking part in this war against the separatists. As of 2019, more than 370 thousand Ukrainian military men took part in the hostilities [4]. All of them were exposed to combat stress, which caused many changes in their mental state, impaired adaptation and behavior, the appearance of various mental disorders, as well as the transformation of the value sphere of the individual [7, 11, 26]. The researchers also found out that the value orientations of combatants, in terms of their significance and meaningfulness, are more differentiated than those of military personnel without combat experience [27-28]. After participating in hostilities, most combatants began to value life and control it better, in contrast to military personnel without combat experience [28]. At the same time, they developed a pronounced polarity in their assessments of life values: they began to comprehend life-meaning orientations more deeply or on the contrary, lose their meaning in them [29].

The researchers suggested that military personnel with different value spheres will react differently to combat stress that will make it possible to perform a more differentiated choice of tactics for providing first psychological aid in a combat zone and determine measures for the rehabilitation and prevention of PTSD [30-31].

Consequently, the purpose of this research was to determine the typology of the personal value sphere of military personnel who took part in hostilities in the East of Ukraine, and on its basis to predict the vector of PTSD development.

Hypothesis 1. Combatants with different personal value spheres can be grouped into separate clusters (types) depending on the content of individual value constructs, indicators of acute stress disorder (ASD), depression, and PTSD.

Hypothesis 2. Combatants with different types of personal value spheres have different susceptibility to combat stress, which will make it possible to predict the severity of an acute stress reaction, a tendency to develop depression, and the further development of mental disorders, in particular PTSD.

METHOD

The research was conducted in 2016-2017. Nine hundred forty three male NGU military personnel of different categories (officers, contract military servicemen) were involved in this research, they all took part in hostilities in the East of Ukraine. The duration of a trip to the combat zone ranged from one to three months.

All combatants were informed about the nature of the research of values and manifestations of traumatic stress before completing the method forms.

All participants have given consent for their data to be used in this research.

The features of the value sphere of the combatants were studied using the Rokeach Value Scale (RVS), adapted into

Russian [32]. The respondents received two lists of values: terminal (goals that a person wants to achieve throughout life) and instrumental (ways to achieve terminal values). Each list consisted of 18 values, which were arranged in alphabetical order. Each value was accompanied by short phrases that explained its content. The research was conducted on paper. The respondents ranked the values on each list from most important (1) to least important (18). The respondents built a hierarchy out of their individual value priorities. The processing of the data obtained was carried out by comparing the selected ranks of different types of individual value spheres. The average value for each ranked value was calculated by counting (summing) the individual assessments of the respondents. For the data presentation, we used basic descriptive statistics (frequency n (%), arithmetical mean M, standard deviation SD).

The types of combatants' individual value spheres were determined using the cluster analysis procedure (K-means).

The features of the mental status of combatants who underwent the impact of extreme (combat) factors were studied using the "Traumatic Stress Questionnaire" by I.A. Kotenev [33]. The presence of such manifestations of traumatic stress in the posttraumatic period as hypervigilance, hypertrophied reaction, aggressiveness, impaired memory and concentration, the level of depression, anxiety, drug abuse, hallucinatory experiences, and sleep disturbances were determined in combatants. The research was conducted on paper. The questionnaire contains 110 statements which were rated by the respondents according to a 5-point Likert scale. The maximum (5 points) to the greatest extent corresponded to the mental state of the respondent at the present time, the minimum (1 point) absolutely did not correspond to the mental state of the respondent. The average value for each indicator of the combatant's mental state was calculated by counting (summing) the individual assessments of the respondents. As a result, three generalizable indicators were calculated: ASD, depression, and PTSD. For the data presentation, we used basic descriptive statistics (frequency n (%), arithmetical mean M, standard deviation SD).

The statistical analysis of the results of the study was carried out using the program SPSS 17.0.

We presented the ratio of the identified types of the value sphere of combatants and the vector of development of PTSD in the form of a circular configuration proposed in the theory of values of Schwartz [34]. Values that appear across from each other on the circle are values directly in conflict, whereas values next to or near each other on the circle are more closely related.

RESULTS

The conducted cluster analysis made it possible to distinguish five types of the value sphere of personality among combatants: "Passionary" (Type 1), "The one who doesn't leave the comfort zone" (Type 2), "Romantic" (Type 3), "Formal military man" (Type 4), "Routine" (Type 5). The types of the value sphere of the combatants' personality are presented in Table 1.

As can be seen from the Table, terminal values of the most common second type (74.32%) of the value sphere of the personality of combatants conditionally form several groups. The most significant value for them is "Health (physical and mental)", followed by a group that combines "Love" and "Happy family life". The third block is "Financially secure life", "Faithful friends", "Life wisdom" and "Active life". The fourth group is formed by "Interesting job", "Public mission", "Development" and "Self-confidence". The lowest indicators of terminal values have "Productive life", "Freedom", "Cognition", "Beauty of nature and art, "Happiness of others", "Entertainment" and "Creativity". In achieving life goals (terminal values), combatants of the second type give preference to such methods of achieving them (instrumental values) as "Good breeding", "Sensitiveness", "Responsibility", "Self-control" and "Cheerfulness". Low values are "Effectiveness in business", "Sincerity", "Open-minded", "High demands" and "Intolerance to own and others drawbacks".

The fifth type of combatants is the next most widespread (15.30%). The most significant terminal values for this type of combatants are "Active life" and "Life wisdom". Important values also include "Interesting job", "Health (physical and mental)" and "Love". The least significant terminal values are "Cognition", "Creativity", "Faithful friends", "Freedom", "Public mission", "Happy family life" and "Entertainment". Military men of the fifth type prefer such instrumental values as "Carefulness", "Tolerance" and "Cheerfulness". Representatives of this type have the lowest ranks according to such instrumental values as "Courage in defending their own views", "Independence", "Open-minded", "Honesty (sincerity)", "Sensitivity" and "Efficiency in business".

Combatants of the fourth type (4.37%) are in third place in terms of prevalence. They prefer such terminal values as "Interesting job", "Financially secure life" and "Love". High ranks also have "Public mission", "The beauty of nature and art" and "Faithful friends". The least significant terminal values for this type of combatants are "Freedom", "Creativity", "Cognition", "Productive life" and "Development". Among the instrumental values, the preference is given to "Self-control" and "Discipline".

“Cheerfulness”, “Independence” and “Education” are also highly rated. The lowest ranks were received by the instrumental values: “Sensitivity”, “Honesty (sincerity)”, “Firm will” and “Effectiveness in business”.

Table 1: Typology of individual values of combatants (in points).

------ Indicators	Type 1 (n = 2.19%)	Type 2 (n = 74.32%)	Type 3 (n = 3.82%)	Type 4 (n = 4.37%)	Type 5 (n = 15.30%)
	M±SD	M±SD	M±SD	M±SD	M±SD
Terminal values					
Active life	3.75±4.19	7.60±4.60	11.71±3.15	8.88±4.82	4.93±4.29
Life wisdom	10.00±5.29	7.38±3.98	14.29±3.73	9.00±5.81	5.71±3.89
Health (physical and mental)	14.75±2.87	3.73±3.79	11.43±4.12	10.257.81	6.68±5.09
Interesting job	5.25±3.77	8.96±4.91	14.57±3.10	4.00±5.76	6.68±4.50
The beauty of nature and art	12.00±6.93	12.68±5.18	13.29±1.98	6.75±4.33	9.61±4.47
Love	13.75±5.85	5.94±4.66	13.43±2.37	5.75±3.41	7.14±5.01
Financially secure life	13.25±4.27	6.51±4.62	12.71±5.62	4.25±1.91	8.32±5.93
Faithful friends	10.00±5.72	7.07±3.81	4.14±3.63	7.38±4.27	11.54±4.96
Public mission	11.50±2.08	9.49±3.79	4.71±4.31	6.50±4.14	11.64±3.83
Cognition	10.00±4.55	11.73±4.04	6.14±3.29	12.25±4.95	11.07±4.20
Productive life	9.25±5.56	11.04±3.67	4.14±4.41	13.88±3.04	10.96±4.22
Development	7.25±4.92	9.71±4.03	7.29±4.72	14.13±4.02	9.96±4.56
Entertainment	9.75±5.62	13.46±3.56	10.00±4.62	9.38±5.21	11.79±4.78
Freedom	5.25±1.71	11.37±4.03	4.14±3.67	11.88±3.83	11.61±4.65
Happy family life	9.75±4.92	5.96±4.96	8.29±4.15	9.50±4.60	11.64±5.54
Happiness of others	4.75±2.22	12.81±4.44	9.71±4.79	10.63±4.24	13.04±4.13
Creativity	10.00±5.94	15.28±3.05	9.71±5.65	11.88±4.49	11.29±4.39
Self-confidence	10.50±6.24	10.03±4.67	11.14±4.41	11.25±3.69	8.46±4.53
Instrumental values					
Carefulness	14.75±2.87	6.86±5.32	3.14±3.13	10.38±5.55	4.39±2.94
Good breeding	12.75±2.99	6.67±5.02	5.00±3.37	8.75±6.48	8.50±5.51
High demands	9.75±6.95	13.10±5.07	5.57±3.69	7.38±5.24	8.29±4.65
Cheerfulness	12.25±5.12	7.67±4.66	8.14±4.22	6.13±2.80	7.14±4.15
Discipline	13.25±7.54	8.35±4.72	10.29±4.86	5.25±3.92	9.39±4.80
Independence	8.25±7.85	9.08±4.82	6.14±3.34	6.13±3.94	10.54±4.49
Intolerance to own and others drawbacks	8.00±5.77	13.63±4.20	11.00±5.57	7.63±4.10	9.46±6.29
Education	9.00±5.03	8.87±4.81	8.00±4.55	6.50±3.66	8.68±4.56
Responsibility	15.75±1.50	7.20±4.41	11.43±4.16	9.38±4.44	9.14±4.86
Rationalism	14.00±2.16	9.24±4.44	5.00±3.00	7.13±4.61	8.39±4.33
Self-control	10.25±5.12	7.35±4.45	12.29±3.77	4.88±2.90	8.71±4.77
Courage in defending own views	6.75±3.10	9.74±4.23	11.29±4.96	11.38±5.18	10.32±3.87
Firm will	7.25±3.20	8.89±4.49	12.29±5.94	14.25±2.71	8.54±4.94
Tolerance	5.50±0.58	9.71±4.43	12.00±4.00	11.38±4.03	6.00±4.21
Open-minded	6.00±2.45	12.16±4.57	10.71±5.35	11.50±3.93	11.64±4.44

Indicators	Type 1 (n = 2.19%)	Type 2 (n = 74.32%)	Type 3 (n = 3.82%)	Type 4 (n = 4.37%)	Type 5 (n = 15.30%)
	M±SD	M±SD	M±SD	M±SD	M±SD
Honesty (sincerity)	3.25±3.30	9.50±5.69	12.57±3.60	14.25±2.25	12.39±5.34
Efficiency in business	6.00±4.24	11.29±4.94	10.86±5.98	15.38±2.50	14.79±3.68
Responsiveness	5.75±4.92	11.51±5.14	13.29±4.27	14.00±3.42	13.50±4.27
Features of the mental status of combatants					
PTSD	106.00±20.05	107.43±17.76	109.43±18.20	124.25±18.38	143.64±15.77
ASD	100.50±21.06	98.55±17.99	95.57±19.24	116.75±18.59	138.36±16.94
Depression	35.25±6.85	39.65±5.28	40.43±3.41	42.63±6.09	49.39±6.06

In the structure of terminal values of the third type (3.82%) preference is given to such values as “Faithful friends”, “Freedom”, “Productive life”, “Public mission”. However, they rate low the importance of such values as “Self-confidence”, “Health (physical and mental)”, “Active life”, “Financially secure life”, “Beauty of nature and art”, “Love”, “Life wisdom” and “Interesting job”. Among the instrumental values, representatives of this type distinguish “Carefulness”, “Rationalism”, “Good breeding”, “High demands” and “Independence”. Low positions in the hierarchy of instrumental values have “Courage in defending own views”, “Responsibility”, “Tolerance”, “Self-control”, “Firm will”, “Honesty (sincerity)” and “Sensitivity”.

The least widespread type was identified (2.19%) of combatants, for whom the most significant terminal values are “Active life”, “Happiness of others”, “Freedom”, “Interesting job” and “Development”. For them, “Public mission”, “Beauty of nature and art”, “Financially secure life”, “Love” and “Health (physical and mental)” are of little significance. To achieve them, combatants of this type consider it important for themselves to use such instrumental values as “Honesty”, "Tolerance", “Sensitivity”, “Open-minded”, “Efficiency in business”, “Courage in defending own views” and “Firm will”. Combatants of this type have the lowest indicators for such instrumental values as “Cheerfulness”, “Good breeding”, “Discipline”, “Rationalism”, “Carefulness” and “Responsibility”.

As can be seen from the table, in the NSU servicemen of the first, second and third types of the value sphere, the indicators of depression, ASD, and PTSD practically coincide and indicate the absence or insignificant manifestation of symptoms described using these scales. Combatants of the fourth type of value sphere differ from the first three in higher ASD indicators. This gives grounds to assume the formation of their individual symptoms of post-traumatic stress. In terms of depression and PTSD, the fourth type of combatants occupies an intermediate position between the first three and the fifth type of value sphere of combatants. The fifth type of combatant has higher rates of depression, ASD, and PTSD than the rest. With such indicators, it is possible to predict a high probability of the onset (development) of PTSD of the fifth type of combatants.

The five types of the value sphere of the combatants' personality that we have identified can be placed between the two axes of the Schwartz [34] model:

– “common goal of life”, which has poles “for the happiness of others” – “for oneself” (it also includes “for the sake of one's social/professional group”, “reference group”, “family”, etc.), or “transcendence – individualism”;

– “a way to achieve a goal”, which has poles “the use of social norms to expand boundaries” – “the use of social norms to narrow the boundaries”.

The ratio of the types of the value sphere of the personality and the vector of the development of PTSD in combatants is shown in the Figure 1.

Figure 1: Correlation between the types of values and the vector of development of PTSD of the NGU combatants.

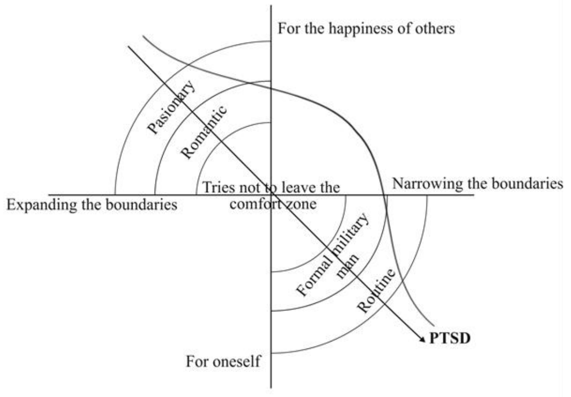

In the Figure, the axes are set by the directions of the development of the value sphere of the individual. The PTSD vector indicates the direction in which the PTSD indicators increase (from the absence of symptoms to the formation of a posttraumatic symptom complex with clinical manifestations). The curve above it schematically describes the frequency of the corresponding PTSD indicators in the sample of combatants.

DISCUSSION

The most widespread type of value sphere, identified in 74.32% of NGU combatants, we designated it as “The one who doesn’t leave the comfort zone”. Military personnel of this type strive for a balanced, comfortable, prosperous life, observing for this the norms and methods of interaction with the outside world, accepted in society. They do not place high demands on themselves and their lives. Thus, in a study by Prykhodko et al. [7] it was proved that compliance with the norms of society, conflict-free interpersonal interaction is an important component of the psychological safety of a person. Confirmation of the obtained results is the conclusions of researchers that resourcefulness (strategies of conservation, avoidance, procrastination) [35] and satisfaction of spiritual needs (Spiritual Needs), in particular, religion [36] are a preventive factor in the development of PTSD.

The “routine” personality type, identified in 15.30% of combatants, is characterized as a person who constantly works to improve his/her own life but does not feel happiness and pleasure from it. This structure of the value sphere describes military men who believe that life is a hard routine, daily work. They are not convinced that life should necessarily be happy and that work should be interesting, aimed at something important. According to McAllister, Callaghan, & Fellin [37], such military personnel adheres to generally accepted norms of interaction to avoid conflict and other problems. They limit themselves in emotional manifestations. Fulfillment of tasks in combat conditions does not leave the room to react to stress, provokes in them the development of depressive manifestations, and in the future, PTSD.

The structure of the value sphere of the personality of combatants of the fourth type (found in 4.37%) can be described as “Formal military men”. They are narrowly focused on their profession as a means of satisfying material needs. Moreover, their professional identification does not reach the level of mastery, stopping at the level of the performer. The profession of a military man is not perceived by them as contributing to self-development, knowledge, creativity, and richness of life. According to Johansen, Laberg, & Martinussen [38], such features of the professionalization of military men determine the leading role of self-control and discipline in the structure of instrumental values and explain in it the low importance of result orientation. With such identification, the external form of the soldier's actions is copied, but not the meaning of the profession, its mission. The limitations and lack of freedom that characterize the identification of the “performer” level make the military men vulnerable to the occurrence of ASD and increase the development of depression and PTSD.

Military men of the third type (3.82%) can be characterized as “Romantics”. They are focused on the close social environment, strive to be significant for society, share their values, and are able to refuse from comfort, love, health, and their favorite profession for the sake of public recognition. According to Jones & Wessely [39] and Noy [40], this personal value structure contains the well-known potential for coping with combat stress: using the help of peers, mutual understanding, and trust of combat brothers.

The least common (2.19%) identified is the first type of personality of combatants, which is called “Passionary”. Its feature is an active struggle for the happiness of others, the expansion of the boundaries of existing norms. For this, they are ready to refuse from a comfortable, safe life and their own interests. Military personnel of this type is free in their thoughts and actions. They are sincere, sensitive, tolerant, aimed at achieving a result, but they neglect restrictions and conventions. According to Banducci, Connolly, Vujanovic, Alvarez, & Bonn-Miller [41], a person’s high tolerance to stress, perceived or actual ability to tolerate negative emotional or physical states are back proportional to the symptoms of PTSD. Research by Bandura [42] and Schwarzer [43] has shown that motivated people are better able to cope with problems and solve new, more complex problems. Military men of this type can often find themselves in stressful situations, sometimes they create them by themselves. However, this does not lead to the development of symptoms of depression or PTSD in the future.

Thus, the research confirmed our hypothesis 1 about the possibility of typing the individual values of combatants, taking into account the indicators of acute stress disorder (ASD), depression, and PTSD.

An analysis of the results of the correlation between the types of values and the vector of development of PTSD of combatants (see the Figure) showed that belonging to such types of personal value spheres as “Routine” and “Formal military men”, which imply a narrowing of the goal and the method of achieving it, significantly reduces the ability of military men to resist combat stress, and increases the possibility of the formation of a symptom complex of PTSD. At the same time, the interiorizing of values, which involve expanding the range of ways to achieve the goal, going beyond the personal interests inherent of combatants of such types as “Passionary” and “Romantic”, is the prevention of the development of PTSD. These military personnel will be potentially more resistant to combat stress.

At the same time, attention is drawn to the fact that the absolute majority of NGU combatants (74.32%) belong to the type “The one who does not leave the comfort zone”. These military men are in an equidistant position from opposite poles of the circumplex of values and the vector of PTSD development. Therefore, it can be assumed that some of them will be more resistant to the effects of combat stress, taking into account their individual psychological characteristics (good psychological resilience, adaptation to extreme situations, etc.). The other part will be more susceptible to stress and the occurrence of psychogenic disorders. In general, the identified problem of studying the value sphere of combatants, in our opinion, is relevant and requires in-depth research.

The results of our research support hypothesis 2 partially. Based only on belonging to a certain type of personal value sphere, it is impossible to predict with a high degree of probability the risk of development and the severity of behavioral and clinical manifestations of an acute reaction to stress, susceptibility to depression, and other mental disorders, including PTSD.

CONCLUSIONS

Values determine the life goals of a person and how to achieve them. The individual values of combatants perform an important adaptive and protective function under conditions of combat stress. Understanding of them enables military mental health professionals to create an effective system of COSC for military men.

As a result of the research, a typology of the value sphere of combatants has been developed, consisting of five types: “Passionary”, “The one who does not leave the comfort zone”, “Romantic”, “Formal serviceman”, “Routine”. The most resistant to combat stress are military men who are aimed at internalizing value orientations, expanding the boundaries of existing norms, striving to be significant for society and are able to refuse from comfort, love, health, and their favorite profession for the sake of public recognition (“Passionary” (2.19%), “Romantic” (3.82%)). The most vulnerable to combat stress and the occurrence of PTSD are combatants belonging to the “Routine” (15.30%) and “Formal soldier” (4.37%) types. For the Ukrainian combatants of the “The one who does not leave the comfort zone” type (74.32%), safety and stable well-to-do life are the main priorities.

Ethical standards and informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all subjects for inclusion in the study while maintaining their confidentiality.

Disclaimer

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ORIGINAL ARTICLES

Ultrasound screening of kidney size in children with and without vesicoureteral reflux

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Abstract: *Evaluation of the size of the kidney's parameters of patients with vesicoureteral reflux (VUR) can provide relevant knowledge about the prognosis factors of VUR's complications. The study aimed to assess the size of the renal parameters of children with VUR and compare the results with healthy matched cases by the ultrasound method.*

Methods: In this case-control study, children under 14 years of age with VUR in our pediatric urology practice from May 2019 to May 2020 were included. Eighty children were included in the two groups (case (40 cases) and control group (40 cases)) in the study. The case group included children with VUR, while the control group included healthy children. The data regarding Urinary Tract Infection (UTI), the length and width of the kidney, the size of the pelvis, parenchyma thickness, and volume of the kidney were recorded.

Results: The mean age of children was 3.07 ± 3.05 years, and 35.0% of them were male. The mean number of UTIs in the VUR group was 1.90 ± 1.15 and in the control group was 1.10±0.30 (P=0.031). The incidence of UTIs was significantly higher in the VUR group than in the control group (P<0.001). Length, width, the posterior-anterior diameter of kidney, renal parenchyma thickness, and renal volume in the VUR group were significantly lower than the control group (P< 0.001). There was no difference between case and control groups in terms of anterior-posterior diameter of the pelvis (P= 0.653).

Conclusions: The results showed that the size of kidneys parameters in children with VUR was less than in the control group. Also, the incidence of UTIs in the VUR group was significantly higher than in the control group. Ultrasound is a safe and non-invasive tool that can use for checking the size of the children's kidneys with VUR for predict of possible complications.

Keywords: *kidney, vesicoureteral reflux, children, ultrasound*

INTRODUCTION

VUR is the retrograde flow of urine from the bladder to the ureter and renal pelvis [1-3]. The prevalence of VUR is approximately 1-6%, with prevalent inheritance in the pediatric population and it is more common in infants [4, 5].

VUR is a risk factor for the incidence of UTI, and it can develop in 18–40% of children examined for their first UTI. Large renal scars cause renal dysfunction, renin-dependent hypertension, renal insufficiency, and reduced growth [6].

Evidence shows that reduced renal function and hypertension in patients with V UR are related to renal scars. Untreated VUR increases the risk of UTI, hypertension, and progressive kidney damage [7-9].

VUR is the most frequent congenital anomaly of the urinary tract. The association between urinary VUR, UTIs, and kidney scarring has been evaluated in several studies [10-14].

Routine diagnostic imaging methods for detecting VUR are Isotope Cystography and ultrasound. However, voiding cystourethrogram (VCUG) is the gold standard for detecting VUR [1-5]. VCUG is an essential fluoroscopic system for the examination of the lower urogenital tract. VCUG is an essential fluoroscopic system for the examination of the lower urogenital tract. Many children with small kidney sizes will be prescript to VCUG for detecting VUR [15-18].

Kim et al. reported a correlation between total kidney volumes estimated by ultrasound and decreased kidney function [7]. Renal growth in VUR patients is regularly

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estimated by ultrasound [19]. Ultrasound has many preferences for parents, such as non-invasive, safe, easily reachable, and economical [20-23].

Examining the size of the kidney's characteristics of patients with VUR can provide appropriate information to physicians about vesicular and reflux disease and its effects on kidney function. The study aimed to assess the renal size of children with VUR and compare the results with healthy matched cases by the sonography method.

METHODS

In this case-control study, children under 14 years of age with VUR in our pediatric urology practice from May 2019 to May 2020 were included.

After parents gave written informed consent, the children were referred to sonography through a pediatrician. All ultrasounds were conducted by one radiologist with good expertise in pediatric sonography.

Eighty children were included in the two groups (case (40 cases) and control group (40 cases)) in the study. The case group included children with VUR, while the control group included healthy children. Children were matched between groups in terms of age and gender. VUR was diagnosed using the VCUG method. The VUR grade was written on each affected child and then underwent an ultrasound. Healthy children also underwent ultrasound. The sonographer was blind about allocation cases in the two groups.

The ultrasound device was a Samsung model with a 3.5-5 MHZ curvilinear probe Ultrasounds were done twice, first after the cases were sufficiently hydrated and then 10 to 15 minutes after emptying the bladder. The radiologist, with the help of his assistant, measures and records data on the length and width of the kidney, the size of the pelvis, paranchyma thickness and volume of the kidney. The VUR was graded 1-5 according to the International Reflux Study of children.

The protocol of the research was approved by the ethics committee of Arak University of Medical Sciences with the IR.ARAKMU.REC.1399.173 code.

Data were presented by mean, standard deviation, frequency, and percent. T-test and Mann Whitney test were used for comparison of quantitative data between groups according to the normality distribution. Chi 2 and Fisher exact test were used for comparison of qualitative data between groups. Data analyses were done in SPSS-20 software. A P-value<0.05 was regarded as statistically significance.

RESULTS

The mean age of children was 3.07 ± 3.05 years, and 35.0% of them were male. There was no significant difference between the groups in the age and sex distribution (P=0.780 and P=0.542, respectively) (Table 1).

Table 1: Distribution of age and sex according the groups of the study

Items		VUR	control	P-value
Age, years, mean±SD		3.10 ± 3.08	3.05±3.04	0.780
Sex, N (%)	Female	28 (70.0%)	27 (67.5%)	0.542
	Male	12 (30.0%)	13 (32.5%)	

There were 66 VUR kidneys in the case group, and in the control group, there were 80 healthy kidneys. There were 26 (65.0%) two-side VUR patients. Based on initial VCUG detection, 1-5 grade VUR were diagnosed in 7.6%, 33.3%, 36.4%, 13.6% and 9.1% patients, respectively. There was one (2.5%) hydronephrosis case in I-IV grade, respectively (Table 2).

The mean number of UTIs in the VUR group was 1.90 ± 1.15 and in the control group was 1.10±0.30 (P=0.031). The mean of UTIs in the VUR groups was significantly higher than control group. The incidence of UTIs was significantly higher in the VUR group than in the control group (P<0.001) (Table 3).

Table 2: Distribution of hydronephrosis, VUR grading, and VUR type in the study

Items	Degree	Percent
Hydronephrosis	I	1 (2.5%)
	II	1 (2.5%)
	III	1 (2.5%)
	IV	1 (2.5%)
VUR	I	5 (7.6%)
	II	22 (33.3%)
	III	24 (36.4%)
	IV	9 (13.6%)
	V	6 (9.1%)
VUR type	I	14 (35%)
	II	26 (65%)

Table 3: Distribution of UTIs in case and control groups

Items		VUR	control	P-value
Number of UTIs, mean±SD		1.90 ± 1.15	1.10±0.30	0.031
UTIs, N (%)	Yes	27 (67.5%)	11 (27.5%)	<0.001
	No	13 (32.5%)	29 (72.5%)	

Length, width, the posterior-anterior diameter of kidney, renal parenchyma thickness, and renal volume in the VUR group were significantly lower than the control group (P< 0.001). However, there was no significant difference between case and control groups in terms of anterior-posterior diameter of the pelvis (P= 0.653) (Table 4).

Table 4: Distribution of size of renal parameters in case and control groups

Items	VUR	control	P-value
Length	62.14±13.33	69.56 ±14.16	<0.001
Width	30.40±6.82	35.16±6.72	<0.001
Kidney anterior-posterior	24.77±4.86	28.06±4.82	<0.001
Volume	26.32±17.03	38.82±19.88	<0.001
Kidney pelvic anterior-posterior	3.31±2.44	3.474±1.78	0.653
Thickness of the upper parenchyma bridge	9.39±2.42	10.19±2.08	0.035
Thickness of the middle parenchyma bridge	9.08±2.11	10.87±2.20	<0.001
Thickness of the under parenchyma bridge	8.89±2.08	10.36±2.04	<0.001
Parenchyma thickness	9.13±1.83	10.48±1.94	<0.001

DISCUSSION

Ultrasound is a low-cost, non-invasive, and radiation-free procedure, and parental acceptance is very high [19-21]. Recently, many studies have assessed ultrasound to detect VUR in an attempt to eliminate radiation from previous methods [19, 24-27]. Evaluating the size of the parameters of the kidney in VUR cases and comparing it with all healthy individuals can provide appropriate information about VUR disease and its effects on the kidney.

The present study showed that length, width, the posterior-anterior diameter of kidney, renal parenchyma thickness, and renal volume in the children with VUR were lower than the healthy children. The association of reduced kidney size and growth with patients with VUR has been assessed by several studies [19, 24, 25]. Also, some studies showed that growth in renal size can increase after VUR treatment [26, 27]. However, some studies noted that in VUR children, there is an association between unscarred and no recurrent UTIs kidney with normal growth in kidney size [28, 29].

Zerin et al. showed that the renal children with VUR were significantly smaller than those in cases without VUR [30]. The results of this study are in agreement with the current study. Ross et al. (2015) assessed the effect of VUR and UTIs on the growth of the children solitary kidney. They showed VUR solitary renal with UTI had a significantly lower growth rate. Also, there was no difference in the incidence of UTI between those without VUR and those with VUR [19]. In the present study, UTIs were significantly higher in the VUR group than in the control group. The frequency of UTIs in the VUR group was significantly higher than in the control group. So, the results of the current study, the incidence of VUR in children is associated with UTIs. Garcia-Roig et al. (2016) showed that VUR is the most common cause of UTIs or pyelonephritis in children [20]. Also, besides pyelonephritis, other long-term complications such as recurrent kidney infections, kidney scars, proteinuria, and hypertension can happen in VUR patients.

Overall, the results in the present study showed that the kidneys involved in vesicoureteral reflux are smaller than healthy kidneys. Perhaps the cause of this problem can be attributed to the development of renal scars in vesicoureteral reflux cases. Ghanesherbaf et al. (2011) found that the grade III and higher of VUR, and the frequency of recurrences of UTIs were associated with renal scarring in children with a urinary tract infection [21]. The development of kidney scar can lead to kidney failure, so studies noted that early treatment is essential for these patients. A review study by Mattoo et al. (2011) found that VUR in children is associated with an increased incidence of UTI, kidney scar, and nephropathy [22]. Roihuvuo-Leskinen et al. (2013) showed that size of adults' kidney with a history of vesicoureteral reflux as children were smaller than those without VUR. Therefore, they concluded that the total volume and size of the kidney could be a prognosis factor in early adulthood could for the possible deterioration of the kidney in later life [23].

Of the 66 kidneys included in the case group, the most common degrees of VUR were grade two (22 involved kidneys) and grade three (24 involved kidneys). Of these affected kidneys, only four had hydronephrosis. Sorkhi et al. (2010) assessed 58 VUR kidneys. There were 19 cases in grade II and 13 cases in grade III [18]. This finding is consistent with the present study.

The current research is limited by the natural choice bias of retrospective studies. Moreover, the variability of ultrasound kidney analysis is well admitted. More extensive cohort studies of children with VUR can assess the size of parameters of the kidney during follow-up and may achieve more knowledge about prognosis factors for possible complications.

CONCLUSION

The results showed that the size of kidneys parameters in children with VUR was less than in the control group. Also, the incidence of UTIs in the VUR group was significantly higher than in the control group. Ultrasound is a safe and non-invasive tool that can use for checking the size of the children's kidneys with VUR for predict of possible complications.

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Updates in teenage acute intentional self-poisonings

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Abstract: *Acute intentional poisonings represent an important part of emergency pediatric pathology, as well as a psychiatric one, with an escalation tendency over the past years. The current paper consists of a descriptive prospective study, which took place over 12 months, and included 342 children within the age range 6-18 years, that presented in the Emergency Unit, being subsequently treated as in-patients of the Pediatric Poisoning Center of "Grigore Alexandrescu" Emergency Clinical Hospital for Children. The number of pediatric acute intentional poisonings hospitalized within the time frame of the study was 819 cases. Of these, 342 were intentional, which constitutes a percentage of 41.75%, and 477 were accidental, i.e. 58.24%. The etiology of the acute intentional poisonings is varied; however, medication (54.38%), ethanol (28.65%), and drugs of abuse (12.28%) were predominant. A higher frequency of acute intentional poisonings was noticed in girls (65.49%) as compared to boys (34.50%), probably due to the particularities the psychological profile of this sex shown at this age, i.e. higher emotional instability and display tendency. Out of the total number of acute intentional poisonings, we identified 20 cases of suicide attempts, which represent 5.83% of the total acute voluntary poisonings. Moreover, some of these cases constitute a repeated suicide attempts. The studied group included 30 cases of chronic substance abuse and 6 chronic alcohol abuse cases, with ages within the 13-17 years range, 12 of which were females and 24 males. Out of the 36 chronic substance/alcohol abuse patients, 4 were social cases. Pediatric acute intentional self-poisoning is an important public health issue, alarming through its consequences and its hidden neuropsychiatric and behavioral substrate. This is because adolescence is a period of marked emotional fragility, sensitive to all sorts of influences.*

Keywords: *self-poisoning, teenager, psychiatric disorders, alcohol, substances of abuse*

INTRODUCTION

The prevalence of teenage acute intentional self-poisoning is high since adolescence is a period of profound changes with visible effects on an individual's physical aspect, behavior, and relationships with the surrounding world. It features a personality focus on acquisitions and social statuses related to school life, family life, friend circle.

Adolescence constitutes a time frame of intense development, marked by a series of profound changes with visible effects on physical appearance, behavior, and relationships with the surrounding environment. Resorting to an extreme gesture, such as acute intentional self-poisoning, which puts one's life at risk, is often a potential exacerbation of a chronic psycho-social disorder [1, 2].

Teenage years are marked by intense developmental

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processes, on a sexual level included. Affectivity, relationships of all kinds begin to include the sexual identity and its integration in the self-image.

Drug abuse, juvenile delinquency, poor learning achievements, teenage pregnancy, sexually transmitted diseases, feelings of despair are frequent causes that lead teenagers to resort more and more often to suicide attempts through various acute self-poisonings. Between the ages of 13 and 16 years, adolescents are confronted with frequent and unexpected mood swings. Both the regression and the progression of the psychological age represent explanations regarding the unpredictability of teenagers, as well as the difficulty to sometimes understand them. Behavioral disorders occur, the greater part of which is induced by disturbances in the emotional development caused by family issues, parental conflicts, and splitting [3].

The lack of affection does not refer strictly to its absence concerning the adolescent, but also its misguidance in a direction that ignores the affective needs of the child. The lack of education prevents the adolescent from acquiring necessary social life skills. All these family issues are the source of teenager disorders that lead to powerful social inadequacy behaviors and antisocial acts [4]. The adolescent becomes introverted and develops inferiority complexes. It is important that parents combine proofs of affection with exigencies and demand respect, as well as teach their children the affinity towards imitating behaviors of individuals who distinguish themselves through moral and intellectual qualities [5].

Acute voluntary self-poisoning is a dynamic medical pathology that is often the result of a potential exacerbation of a chronic psychosocial disorder; frequently, patients have a personal history of various psychiatric disorders, such as emotional, behavioral, and personality disorders, depression, mental retardation [6].

The purpose of the study is to determine the frequency of acute intentional self-poisoning cases, to identify the most frequently used substances as the etiology of the self-poisoning, to plot the distribution according to sex, age, the month of the year, the presence of suicide-associated psychiatric pathology and to identify the age interval associated with the highest risk for this pathology.

Acute intentional self-poisoning is often associated with school dropouts or the patients are social cases. However, there are cases when the child is left unsupervised or cases when a teenager will abuse certain substances in a moment of emotional turmoil triggered by a conflict with her/his parents, guardians, or life partner [7].

A particular intentional self-poisoning in the 12-13 year age group (adolescents) is due to ethanol. Ethanol is one of the most frequent causes of acute intentional self-poisonings in teenagers. The abuse is usually acute and within a social context, unlike adult cases, where the most common causes of acute intentional poisoning are psychotropic medication and medication acting on the cardiovascular system. In addition, another objective of our study was the identification of the particularities of alcohol consumption in teenagers living in our country compared to European teenagers [3].

PATIENTS AND METHODS

The personal study was undertaken in the Pediatric Poisoning Center of “Grigore Alexandrescu” Emergency Clinical Hospital for Children, in Bucharest.

The present research is a descriptive, prospective study that took place for 12 months (1st of January 2017-31st December 2017), and included 342 children with ages in the 6-18 years range, who presented in the Emergency Unit and were subsequently treated as in-patients in the Pediatric Poisoning Center of “Grigore Alexandrescu” Emergency Clinical Hospital for Children.

The clinical cases selected for the study were treated as in-patients in the Pediatric Poisoning Center for acute voluntary self-poisoning with various substances. Out of the 2457 hospital admissions in the clinic, 342 cases fulfilled the inclusion criteria for the study.

Inclusion criteria for the study group:
- patients with acute intentional self-poisoning;
- under 18 years of age, or younger than 19, but still students.

Exclusion criteria:
- patients with accidental acute self-poisoning;

Studied parameters:
- patient identification data (age, sex);
- substances used as the etiology of acute intentional self-poisoning;
- motive for the acute intentional self-poisoning;
- number of suicide attempts;
- various chronic substances abuse;
- clinical picture;
- associated psychiatric disorders;

RESULTS

The number of acute poisonings in children treated as in-patients in the studied time frame was 819 cases. Out of these, 342 were intentional, representing 41.75%, and 477 were accidental, i.e. 58.24%.

In the studied group, which contained 342 patients, the etiology of acute voluntary self-poisonings is varied; however, medication (54.38%), ethanol (28.65%), and substances of abuse (12.28%) were predominant (Table 1). Statistical data withstand comparison to literature data - a study performed between 2003 and 2013 in the same clinic - the most common cause remains acute voluntary self-poisoning with medication [8].

Table 1: Case distribution as a function of the etiology of the acute intentional self-poisoning

Etiology	Cases	Percentage
Medication	186	54.38 %
Alcohol	98	28.65 %
Coffein, energy drinks	4	1.16 %
Tabacco	1	0.29 %
Substances of abuse	42	12.28 %
Household toxic substances	7	2.04 %
Pesticides/Insecticides	2	0.58 %
Caustic substances	1	0.29 %
Plants/mushrooms	1	0.29 %

As far as the distribution regarding the months of the year, the study pointed out a higher frequency of cases of acute intentional self-poisonings in January (11.69%), June (11.98%), November (10.88%), but also February (9.35%), March (8.47%), December (9.64%). A lower frequency was noted in April (4.97%), July (6.14%), August (5.55%), and September (6.14%). Most cases of acute intentional self-poisoning were noted in June, which coincides with the end of the school year, and the least cases were noted in the months which correspond to the summer school break.

A higher frequency of acute intentional self-poisoning was noted for females (224 cases, which represents 65.49%), as compared to males (118 cases - 34.50%), probably due to the particularities of the psychological profile of the gender shown at this age, i.e. higher emotional instability and display tendency [9, 10].

In the study group, we noticed that for female cases, the most frequent etiology for acute intentional self-poisoning was medication (70.5% of intentional self-poisonings in females), while for males, the most common etiology was alcohol (49.1% of the total self-poisonings in males), while the second most common one consisted of substances of abuse (23.7%) (Table 2).

A predominance of acute intentional self-poisonings in children was noticed for the age interval 15-16 years (50.58%), which corresponds to adolescence, a period of marked emotional fragility towards all kinds of influences (Table 3).

Table 2: Case distribution according to etiology and sex

Etiology	Female	%	Male	%
Medication	158	46.19%	28	8.18%
Alcohol	40	11.69%	58	16.95%
Coffein, energy drinks	4	1.16%	0	0%
Tabacco	0	0%	1	0.29%
Substances of abuse	14	4.09%	28	8.18%
Household toxic substances	7	2.04%	0	0%
Pesticides/ insecticides	2	0.58%	0	0%
Caustic substances	0	0%	1	0.29%
Plants	1	0.29%	0	0%

Table 3: Case distribution concerning age

Age	No. of Cases	Percentage
<10 years	2	0.58%
10-14 years	100	29.23%
15-16 years	173	50.58%
17-18 years	67	19.59%

The study group (342 cases) comprised patients with ages within the range of 6 to 18 years. For both sexes, a predominance in the age interval 15-16 years was noted: females (32.74%), males (17.83%) (Table 4).

Table 4: Case distribution according to age and sex

Age	Female	Percentage	Male	Percentage
<10 y.	0	0%	2	0.58%
10-14 y.	75	21.92%	25	7.30%
15-16 y.	112	32.74%	61	17.83%
17-18 y.	37	10.81%	30	8.77%

There were 100 patients in the 10-14 years age group. 75% were girls.

There were 173 patients in the 15-16 years age group. 65.73% were girls.

There were 67 patients in the 17-18 years age group. 55.22% were girls.

The highest incidence in girls was noted in the 10-14 years age group and we noticed that as adult age is approached, the sex distribution tends to be equal for girls and boys.

In the study group, 79 children (42.47%) presented medication used in the treatment of CNS disorders as the etiology of acute intentional self-poisoning. 49 children ingested Acetaminophen (26.34%) and 19 children (10.21%) used medication acting on the cardiovascular system for intentional self-poisoning (Table 5). Out of the medication acting on the CNS, patients used carbamazepine, benzodiazepine, valproic acid, levetiracetam, sertraline, risperidone, extraveral, cipralex (escitalopram).

Table 5: The distribution of cases according to medication types as etiology for acute intentional poisonings

Medication class	Cases	Percentage
Medication used in the treatment of cardiovascular disorders	19	10.21%
Medication used in the treatment of central nervous system disorders	79	42.47%
Aspirin	11	5.91%
Acetaminophen	49	26.34%
Antibiotics	10	5.37%
Oral antidiabetics	4	2.15%
Opioids (Morphine)	1	0.53%
Antiemetics	5	2.68%
Vitamins	4	2.15%
Antidiarrheals	3	1.61%
Anti-acne medication (Roaccutane)	1	0.53%

In our study, we noted that, concerning medication self-poisonings, single-medication poisonings were the most frequent type: 63.44%, while multiple-medication poisonings represented 36.55%. Among the combinations encountered, we mention acetaminophen + CNS-acting medication, aspirin + cardiovascular acting medication or blood pressure-lowering medication, carbamazepine + euthyrox + omeprazole, aspirin + acyclovir.

Pediatric acute intentional self-poisoning of alcohol-related etiology was caused by the consumption of vodka (20 cases), wine (17 cases), and whiskey (9 cases). Distilled drinks are predominant in the etiology of alcohol ingestion in the adolescents included in the study (Table 6). Moreover, the study showed several 26 cases of unknown etiology (the substance of abuse was either unknown or undeclared). Our results are different from those reported by other European countries [11].

Most of the cases of alcohol ingestion took place in a social context, most often in the company of friends, but also with family.

As far as the context in which the acute intentional poisoning took place, most often socializing in a public space or at home with friends determined the occurrence of the poisoning, according to literature as well [12, 13]. In 46 cases, the reason for the ingestion/abuse remained unknown or could not be classified in one of the previously mentioned categories. In 7 of the cases, the etiology of the acute intentional self-poisoning was the combination of medication and alcohol (4 girls/3 boys), and in other 7 cases, the poisoning occurred through the consumption of both alcohol and substances of abuse (3 girls/4 boys).

Table 6: Case distribution according to the etiology of acute intentional alcohol self-poisonings

Etiology	No. of cases	Percentage
Wine	17	17.34%
Vodka	20	20.40%
Whiskey	9	9.18%
Beer	6	6.12%
Champagne	2	2.04%
Distilled drinks	7	7.14%
Fruit-flavored distilled drinks	4	4.08%
Tequila	2	2.04%
Medical-use alcohol	1	1.02%
Ethylenglycol-antifreeze	4	4.08%
Unspecified etiology	26	26.53%

In the studied group: 98 of the cases of acute intentional self-poisoning presented alcohol as etiology; 60 cases showed no coma; 17 cases were complicated by 2nd-degree coma; 12 cases showed 1st-degree coma and only 9 cases showed 3rd-degree coma.

Out of 38 cases that presented with coma, 15 were females (39.47%) and 23 were males (60.52%).

Out of 98 cases of an acute intentional poisoning with alcohol, most cases ingested a quantity of alcohol below 500 ml, 8 cases ingested between 500 and 1000 ml (Table 7). Only one case ingested 3,000 ml of alcohol (wine).

Table 7: Case distribution according to the quantity of alcohol ingested

Quantity of alcohol ingested	No. of cases	Percentage
<500 ml	10	10.2%
500-1000 ml	8	8.16%
1000-3000 ml	1	1.02%
Not specified	79	80.61%

Coma occurred most often with the consumption of wine or vodka. In 7 cases, the type of alcohol ingested could not be precisely determined (Table 8).

In the case of acute intentional poisonings with substances of abuse, the study showed that the most frequently used substances were cannabis and the new psychoactive substances (Table 9). The consumers of substances of abuse were in the 11-18 years old range, the ratio of girls over boys being 1/2.

Acute intentional self-poisonings, especially in the case of adolescents, occur most often with display purposes (parasuicide) or suicide purposes in the context of family disputes. Most are meant to attract attention, few are actual suicide attempts [14, 15].

Table 8: Case distribution according to the type of alcohol consumed that precipitated the occurrence of coma

Substance	No. of cases	Percentage
Distilled drinks	4	10.52%
Wine	10	26.31%
Vodka	8	21.05%
Whiskey	6	15.78%
Beer	2	5.26%
Fruit-flavored distilled drinks	1	2.63%
Unspecified	7	18.42%

Table 9: Case distribution according to the abuse-substances used as etiology in acute intentional self-poisonings

Substance of abuse	No. of cases	Percentage
Canabis/Marijuana	24	57.14%
Glue	4	9.52%
Heroin	1	2.38%
New psychoactive substances	13	30.95%

In this study, we have noted 20 cases of suicide attempts. We noted 10 cases at the 2nd suicide attempt, 6 cases at the 1st suicide attempt, 2 cases at the 5th attempt (Table 10).

Tabel 10: Case distribution according to the number of suicide attempts, as a function of the total number of patients

No of suicide attempts	No of cases	Percentage
1	6	1.75%
2	10	2.92%
3	1	0.29%
4	1	0.29%
5	2	0.58%

Out of the total number of acute intentional self-poisonings, we pointed out 20 cases of suicide attempts in our study, which represent 5.83% of the total cases. Moreover, we noticed a part of these were not the 1st attempt. The age of patients with a suicide attempt was in the 14-18 years interval; the ratio of female/male was 3/1 (15 girls/5 boys). The majority of patients suffered from psychiatric disorders such as depressive disorder (4 cases), behavior disorders (5 cases), personality disorders (2 cases), conversion disorders (1 case), emotional disorders (5 cases).

The etiology of suicide attempts consisted of multiple drugs in 9 cases, single-drug in 10 cases (CNS-acting drugs, acetaminophen, ibuprofen, blood pressure-lowering drugs), and in one case the etiology was chlorine ingestion.

54.39% of the patients with acute intentional poisonings could be placed in a category of psychiatric disorders, with the help of the extended team of a psychologist and a psychiatrist, after stabilizing the patient. The rest, 45.61% of the patients, were cases of acute intentional poisonings with no psychiatric involvement (Table 11).

Tabel 11: Case distribution according to associated psychiatric disorders

Psychiatric disorder	No. of cases	Percentage
Behaviour disorder	30	8.77%
Depression	40	11.69%
Conversion disorder	1	0.29%
Emotional disorder	101	29.53%
Mental retardation	3	0.87%
Personality disorder	10	2.92%
Tourette syndrome	1	0.29%
No psychiatric disorder	156	45.61%

Out of the 342 acute intentional poisonings, 36 cases of chronic consumers of substances of abuse/alcohol were noted, representing 10.5% (Table 12). This is a worrisome percentage, bearing in mind the age group, i.e. less than 18 years. It is an alarm signal, which imposes the application of urgent measures, on a national level.

Table 12: Case distribution according to the chronic consumption of different substances

Chronic consumption	No. of cases	Percentage
Alcohol	6	1.75%
Substances of abuse	30	8.77%

The study group contained 30 cases of chronic consumers of substances of abuse and 6 cases of chronic alcohol consumption, with ages in the interval 13-17 years, 12 female patients, and 24 male patients. Out of the 36 cases of chronic substances of abuse/alcohol consumption, 4 were social cases.

The clinical study showed 30 social cases (low economical status, neglected child, disorganized families, domestic violence, children left in the care of relatives or institutionalized) and 11 patients who dropped out of school.

In 2 cases, patients aged 16, presented with evolving pregnancy. The etiology of the poisoning was medication-related: vitamins and ketoprofen were the culprits.

DISCUSSION

In the studied group, consisting of 342 patients, the etiology of acute voluntary self-poisonings varied, but medication, ethanol, and substances of abuse were predominant. Statistical data withstand comparison to literature data - a study performed between 2003 and 2013 in the same clinic; the most common cause remains acute voluntary self-poisonings with medication.

Out of the 342 children with acute intentional self-poisoning, the ratio of girls/boys is 1.89/1. A higher frequency of acute intentional self-poisoning was noted in females (65.49%), as compared to males (34.50%), probably due to the psychological profile of this gender at this age, i.e. higher emotional instability and display tendency. The psycho-behavioral features of the adolescents of the new generation make them vulnerable to life problems.

In the study group, we noticed that for female cases, the most frequent etiology for acute intentional self-poisoning was medication (70.5% of intentional self-poisonings in females), while for males, the most common etiology was alcohol (49.1% of the total self-poisonings in males) and the second most common one consisted of substances of abuse (23.7%).

A predominance of acute intentional self-poisonings in children was noticed for the age interval 15-16 years (50.58%), which corresponds to adolescence, a period in which the changes produced by growth, biological function changes; not only does it affect the behavior, but also the inner feelings of the adolescent. Poisoning is a common reason for presentation to hospitals and one of the top five acute medical presentations. The peak age groups are teenagers and young adults.

The highest incidence in girls was noted in the 10-14 years age group and we noticed that as adult age approaches, the sex distribution tends to be equal for girls and boys. This can be explained through the emotional particularities concerning age in girls. To lower the incidence of acute poisonings, educational prophylactic measures must be applied in schools, beginning at the age of 10, an age that corresponds to the fifth year within the gymnasium educational period.

In the study group, 42.47% of cases presented medication used in the treatment of CNS disorders as the etiology of the acute intentional self-poisoning. 26.34% of children ingested Acetaminophen and 10.21% used medication acting on the cardiovascular system for intentional self-poisoning. Out of the medication acting on the CNS, patients used carbamazepine, benzodiazepine, valproic acid, levetiracetam, sertraline, risperidone, extraveral, cipralex (escitalopram). Medication self-poisonings were the most frequent type of acute intentional self-poisonings (63.44%).

Pediatric acute intentional self-poisoning of alcohol-related etiology was caused by the consumption of vodka (20.4%), wine (17.3%), whiskey (9.1%). Distilled drinks are predominant in the etiology of alcohol ingestion in the adolescents included in the study. The results of the study performed in our country are different from the data obtained in other European adolescent populations [10]. According to literature, the drink preferred by European adolescents is beer (47%), followed by wine and distilled drinks (37-38%), cocktail drinks (32%), and cider (27%). As far as the context in which the acute intentional poisoning took place, most often socializing in a public space or at home with friends determined the occurrence of the poisoning.

In 7 of the cases, the etiology of the acute intentional self-poisoning was the combination of medication and alcohol (4 girls/3 boys), and in 7 other cases, the poisoning occurred through the consumption of both alcohol and substances of abuse (3 girls/4 boys). In the study group, acute intentional poisonings using substances of abuse were most often determined by the consumption of cannabis (57.14%) and new psychoactive drugs (30.95%), less often heroin (2.38%) or glue (9.52%). Out of the 42 cases, 14 were females and 28 were males, with ages within the 11-18 years interval, the ratio of girls/boys was 1/2.

Acute intentional self-poisonings, especially in the case of adolescents, occur most often with display purposes (parasuicide) or suicide purposes in the context of family disputes. Most are meant to attract attention, few are actual suicide attempts. Out of the total number of acute intentional self-poisonings, we pointed out 20 cases of suicide attempts in our study, representing 5.83% of the total acute intentional poisonings. Moreover, we noticed a part of these was not the first attempt. The age of patients with a suicide attempt was in the 14-18 years interval; the ratio of female/male was 3/1 (15 girls/5 boys). The majority of patients suffered from psychiatric disorders such as depressive disorder, behavior disorders, personality disorders, conversion disorders, emotional disorders. Adolescents and young adults are the age group at highest risk for the first onset of commonly occurring mental disorders. This life stage has also been identified as a critical period for the onset of the first suicide attempt [16].

54.39% of the patients with acute intentional poisonings could be placed in a category of psychiatric disorders, with the help of the extended team of a psychologist and a psychiatrist, after stabilizing the patient. The fact that there are patients with recurrent suicide attempts proves the inefficacy of the psychiatric treatment and the lack of psychological support after the period of hospitalization.

CONCLUSIONS

The clinical study showed a lot of social cases (low economic status, neglected child, disorganized families, domestic violence, children left in the care of relatives or institutionalized) and 11 patients who dropped out of school, 30 cases of chronic consumers of substances of abuse, with ages within the 11-18 years interval and 6 cases of chronic alcohol consumption. In addition, for two of the female patients, a diagnosis of evolving pregnancy was established. Reducing socio-economic deprivation and its associated problems may be an important strategy in the prevention of suicidal behavior, especially in young people.

Out of the 342 acute intentional poisonings, 36 cases of chronic consumers of substances of abuse/alcohol were noted, representing 10.5%. This is a worrisome percentage, bearing in mind the age group, i.e. less than 18 years. It is an alarm signal, which imposes the application of urgent measures, on a national level.

Pediatric acute intentional self-poisoning is an important public health issue, alarming through its consequences and its hidden neuropsychiatric and behavioral substrate. This is because adolescence is a period of marked emotional fragility, sensitive to all sorts of influences [17, 18].

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Author's contribution

SS contributed to the conception and design of the study, the acquisition, analysis, and interpretation of data of the study. She also contributed to the drafting of the work and its critical revision for important intellectual content. IB contributed to the acquisition, analysis, and interpretation of data of the study, to the drafting of the work, and its critical revision for important intellectual content. HTS contributed to the conception and design of the study, the acquisition, analysis, and interpretation of data of the study, contributed to the drafting of the work and its critical revision for important intellectual content. CD contributed to the conception and design of the study, the acquisition, analysis, and interpretation of data of the study, contributed to the drafting of the work and its critical revision for important intellectual content. MM contributed to the design of the study and the drafting of the study and its critical revision for important intellectual content. ACT contributed to the analysis and interpretation of data of the study, to the drafting of the work, and its critical revision for important intellectual content. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interests

It is not declared by the authors.

Ethics approval and consent to participate

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital, "Grigore Alexandrescu" Clinical Emergency Hospital for Children (Bucharest, Romania).

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VARIA

Updates in teenage acute intentional self-poisonings

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Abstract: *Acute intentional poisonings represent an important part of emergency pediatric pathology, as well as a psychiatric one, with an escalation tendency over the past years. The current paper consists of a descriptive prospective study, which took place over a period of 12 months, and included 342 children within the age range 6-18 years, that presented in the Emergency Unit, being subsequently treated as in-patients of the Pediatric Poisoning Center of "Grigore Alexandrescu" Emergency Clinical Hospital for Children. The number of pediatric acute intentional poisonings hospitalized within the time frame of the study was 819 cases. Of these, 342 were intentional, which constitutes a percentage of 41.75%, and 477 were accidental, i.e. 58.24%. The etiology of the acute intentional poisonings is varied; however, medication (54.38%), ethanol (28.65%) and drugs of abuse (12.28%) were predominant. A higher frequency of acute intentional poisonings was noticed in girls (65.49%) as compared to boys (34.50%), probably due to the particularities the psychological profile of this sex shown at this age, i.e. higher emotional instability and display tendency. Out of the total number of acute intentional poisonings, we identified 20 cases of suicide attempts, which represent 5.83% of the total acute voluntary poisonings. Moreover, some of these cases constitute a repeated suicide attempt. The studied group included 30 cases of chronic substance abuse and 6 chronic alcohol abuse cases, with ages within the 13-17 years range, 12 of which were females and 24 males. Out of the 36 chronic substance/alcohol abuse patients, 4 were social cases. Pediatric acute intentional self-poisonings are an important public health issue, alarming through its consequences and through its hidden neuropsychiatric and behavioral substrate. This is due to the fact that adolescence is a period of marked emotional fragility, sensitive to all sorts of influences.*

Keywords: *self-poisoning, teenager, psychiatric disorders, alcohol, substances of abuse*

INTRODUCTION

The prevalence of teenage acute intentional self-poisoning is high, due to the fact that adolescence is a period of profound changes with visible effects on an individual's physical aspect, behavior and relationships with the surrounding world. It features a personality focus on acquisitions and social statuses related to school life, family life, friend circle.

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Adolescence constitutes a time frame of intense development, marked by a series of profound changes with visible effects on physical appearance, behavior and relationships with the surrounding environment. Resorting to an extreme gesture, such as acute intentional self-poisoning, which puts one's life at risk, is often a potential exacerbation of a chronic psycho-social disorder [1, 2].

Teenage years are marked by intense developmental processes, on a sexual level included. Affectivity, relationships of all kind begin to include the sexual identity and its integration in the self-image.

Drug abuse, juvenile delinquency, poor learning achievements, teenage pregnancy, sexually transmitted diseases, feelings of despair are frequent causes that lead teenagers to resort more and more often to suicide attempts through various acute self-poisonings. Between the ages of 13 and 16 years, adolescents are confronted with frequent and unexpected mood swings. Both the regression and the progression of the psychological age represent explanations regarding the unpredictability of teenagers, as well as the difficulty to sometimes understand them. Behavioral disorders occur, the greater part of which is induced by disturbances in the emotional development caused by family issues, parental conflicts and splitting [3].

The lack in affection does not refer strictly to its absence in relationship to the adolescent, but also to its misguidance in a direction that ignores the affective needs of the child. The lack in education prevents the adolescent from acquiring necessary social life skills. All these family issues are the source of teenager disorders that lead to powerful social inadequacy behaviors and antisocial acts (4). The adolescent becomes introverted and develops inferiority complexes. It is important that parents combine proofs of affection with exigencies and demand respect, as well as teach their children the affinity towards imitating behaviors of individuals who distinguish themselves through moral and intellectual qualities [5].

The acute voluntary self-poisoning is a dynamic medical pathology that is often the result of a potential exacerbation of a chronic psychosocial disorder; frequently, patients have a personal history of various psychiatric disorders, such as emotional, behavioral and personality disorders, depression, mental retardation [6].

The purpose of the study is to determine the frequency of acute intentional self-poisoning cases, in order to identify the most frequently used substances as the etiology of the self-poisoning, to plot the distribution according to sex, age, month of the year, the presence of suicide-associated psychiatric pathology and to identify the age interval associated with the highest risk for this pathology.

Acute intentional self-poisonings are often associated with school dropouts or the patients are social cases. However, there are cases when the child is left unsupervised or cases when a teenager will abuse certain substances in a moment of emotional turmoil triggered by a conflict with her/his parents, guardians or life partner [7].

A particular intentional self-poisoning in the 12-13 year age group (adolescents) is due to ethanol. Ethanol is one of the most frequent causes of acute intentional self-poisonings in teenagers. The abuse is usually acute and within a social context, unlike adult cases, where the most common causes of acute intentional poisoning are psychotrope medication and medication acting on the cardiovascular system. In addition, another objective of our study was the identification of the particularities of alcohol consumption in teenagers living in our country compared to European teenagers [3].

PATIENTS AND METHODS

The personal study was undertaken in the Pediatric Poisoning Center of "Grigore Alexandrescu" Emergency Clinical Hospital for Children, in Bucharest.

The present research is a descriptive, prospective study that took place over a period of 12 months (1st of January 2017 - 31st December 2017), and included 342 children with ages in the 6-18 years range, who presented in the Emergency Unit and were subsequently treated as in-patients in the Pediatric Poisoning Center of "Grigore Alexandrescu" Emergency Clinical Hospital for Children.

The clinical cases selected for the study were treated as in-patients in the Pediatric Poisoning Center for acute voluntary self-poisoning with various substances. Out of the 2457 hospital admissions in the clinic, 342 cases fulfilled the inclusion criteria for the study.

Inclusion criteria for the study group:
- patients with acute intentional self-poisonings;
- under 18 years of age, or younger than 19, but still students.

Exclusion criteria:
- patients with accidental acute self-poisonings;

Studied parameters:
- patient identification data (age, sex);
- substances used as the etiology of acute intentional self-poisonings;
- motive for the acute intentional self-poisoning;
- number of suicide attempts;

- various chronic substances abuse;
- clinical picture;
- associated psychiatric disorders.

RESULTS

The number of acute poisonings in children treated as in-patients in the studied time frame was 819 cases. Out of these, 342 were intentional, representing 41.75%, and 477 were accidental, i.e. 58.24%.

In the studied group, which contained 342 patients, the etiology of acute voluntary self-poisonings is varied; however, medication (54.38%), ethanol (28.65%) and substances of abuse (12.28%) were predominant (Table 1). Statistical data withstand comparison to literature data - a study performed between 2003 and 2013 in the same clinic - the most common cause remains acute voluntary self-poisoning with medication [8].

Table 1: Case distribution as a function of the etiology of the acute intentional self-poisoning

Etiology	Cases	%
Medication	186	54.38%
Alcohol	98	28.65%
Coffein, energy drinks	4	1.16%
Tabacco	1	0.29%
Substances of abuse	42	12.28%
Household toxic substances	7	2.04%
Pesticides/Insecticides	2	0.58%
Caustic substances	1	0.29%
Plants/mushrooms	1	0.29%

As far as the distribution regarding the months of the year, the study pointed out a higher frequency of cases of acute intentional self-poisonings in the months of January (11.69%), June (11.98%), November (10.88%), but also February (9.35%), March (8.47%), December (9.64%). A lower frequency was noted in April (4.97%), July (6.14%), August (5.55%) and September (6.14%). Most cases of acute intentional self-poisonings were noted in June, which coincides with the end of the school year, and the least cases were noted in the months which correspond to the summer school-break.

A higher frequency of acute intentional self-poisonings was noted for females (224 cases, which represents 65.49%), as compared to males (118 cases - 34.50%), probably due to the particularities of the psychological profile of the gender shown at this age, i.e. higher emotional instability and display tendency [9, 10].

In the study group, we noticed that for female cases, the most frequent etiology for acute intentional self-poisonings was medication (70.5% of intentional self-poisonings in females), while for males, the most common etiology was alcohol (49.1% of the total self-poisonings in males), while the second most common one consisted of substances of abuse (23.7%) (Table 2).

Table 2: Case distribution according to etiology and sex

Etiology	Female	%	Male	%
Medication	158	46.19%	28	8.18%
Alcohol	40	11.69%	58	16.95%
Coffein, energy drinks	4	1.16%	0	0%
Tabacco	0	0%	1	0.29%
Substances of abuse	14	4.09%	28	8.18%
Household toxic substances	7	2.04%	0	0%
Pesticides/insecticides	2	0.58%	0	0%
Caustic substances	0	0%	1	0.29%
Plants/mushrooms	1	0.29%	0	0%

A predominance of acute intentional self-poisonings in children was noticed for the age interval 15-16 years (50.58%), which corresponds to adolescence, a period of marked emotional fragility towards all kind of influences (Table 3).

Table 3: Case distribution with respect to age

Age	No. Cases	%
<10 years	2	0.58%
10-14 years	100	29.23%
15-16 years	173	50.58%
17-18 years	67	19.59%

The study group (342 cases) comprised patients with ages within the range 6 to 18 years. For both sexes, a predominance in the age interval 15-16 years was noted: females (32.74%), males (17.83%) (Table 4).

Table 4: Case distribution according to age and sex

Age	Females	%	Males	%
<10 ani	0	0%	2	0.58%
10-14 ani	75	21.92%	25	7.30%
15-16 ani	112	32.74%	61	17.83%
17-18 ani	37	10.81%	30	8.77%

There were 100 patients in the 10-14 years age group. 75%

were girls.

There were 173 patients in the 15-16 years age group. 65.73% were girls.

There were 67 patients in the 17-18 years age group. 55.22% were girls.

The highest incidence in girls was noted in the 10-14 years age group and we noticed that as adult age is approached, the sex distribution tends to be equal for girls and boys.

In the study group, 79 children (42.47%) presented medication used in the treatment of CNS disorders as etiology of the acute intentional self-poisoning. 49 children ingested Acetaminophen (26.34%) and 19 children (10.21%) used medication acting on the cardiovascular system for the intentional self-poisoning (Table 5). Out of the medication acting on the CNS, patients used carbamazepine, benzodiazepine, valproic acid, levetiracetam, sertraline, risperidone, extraveral, cipralex (escitalopram).

Table 5: The distribution of cases according to medication types as etiology for acute intentional poisonings

Medication class	Cases	%
Medication used in the treatment of cardiovascular disorders	19	10.21%
Medication used in the treatment of central nervous system disorders	79	42.47%
Aspirin	11	5.91%
Acetaminophen	49	26.34%
Antibiotics	10	5.37%
Oral antidiabetics	4	2.15%
Opioids (Morphine)	1	0.53%
Antiemetics	5	2.68%
Vitamins	4	2.15%
Antidiarrheals	3	1.61%
Anti-acne medication (Roaccutane)	1	0.53%

In our study, we noted that, concerning medication self-poisonings, single-medication poisonings were the most frequent type: 63.44%, while multiple-medication poisonings represented 36.55%. Among the combinations encountered, we mention acetaminophen + CNS-acting medication, aspirin + cardiovascular acting medication or blood pressure-lowering medication, carbamazepine + euthyrox + omeprazol, aspirin + acyclovir.

Pediatric acute intentional self-poisonings of alcohol-related etiology were caused by the consumption of vodka (20 cases), wine (17 cases), whiskey (9 cases). Distilled drinks are predominant in the etiology of alcohol ingestion in the adolescents included in the study (Table 6). Moreover, the study showed a number of 26 cases of unknown etiology (the substance of abuse was either unknown or undeclared). Our results are different from those reported by other European countries [11].

Table 6: Case distribution according to the etiology of acute intentional alcohol self-poisonings

Medication class	Cases	%
Wine	17	17.34%
Vodka	20	20.40%
Whiskey	9	9.18%
Beer	6	6.12%
Champagne	2	2.04%
Distilled drinks	7	7.14%
Fruit-flavored distilled drinks	4	4.08%
Tequila	2	2.04%
Medical-use alcohol	1	1.02%
Ethylenglycol-antifreeze	4	4.08%
Unspecified etiology	26	26.53%

Most of the cases of alcohol ingestion took place in a social context, most often in the company of friends, but also with family.

As far as the context in which the acute intentional poisoning took place, most often socializing in a public space or at home with friends determined the occurrence of the poisoning, according to literature as well [12, 13]. In 46 cases, the reason for the ingestion/abuse remained unknown or could not be classified in one of the previously mentioned categories. In 7 of the cases, the etiology of the acute intentional self-poisoning was the combination of medication and alcohol (4 girls/3 boys), and in other 7 cases, the poisoning occurred through the consumption of both alcohol and substances of abuse (3 girls/4boys).

In the studied group: 98 of the cases of acute intentional self-poisonings presented alcohol as etiology; 60 cases showed no coma; 17 cases were complicated by 2nd degree coma; 12 cases showed 1st degree coma and only 9 cases showed 3rd degree coma.

Out of 38 cases that presented with coma, 15 were females (39.47%) and 23 were males (60.52%).

Out of 98 cases of acute intentional poisoning with alcohol, most cases ingested a quantity of alcohol below 500 ml, 8 cases ingested between 500 and 1000 ml (Table 7). Only one case ingested 3000 ml of alcohol (wine).

Table 7: Case distribution according to the quantity of alcohol ingested

Quantity of alcohol ingested	No. of cases	%
<500 ml	10	10.2%
500-1000 ml	8	8.16%
1000-3000 ml	1	1.02%
Not specified	79	80.61%

Coma occurred most often with the consumption of wine or vodka. In 7 cases, the type of alcohol ingested could not be precisely determined (Table 8).

Table 8: Case distribution according to the type of alcohol consumed that precipitated the occurrence of coma

Substance	No. of cases	%
Distilled drinks	4	10.52%
Wine	10	26.31%
Vodka	8	21.05%
Whiskey	6	15.78%
Beer	2	5.26%
Fruit-flavored distilled drinks	1	2.63%
Unspecified	7	18.42%

In the case of acute intentional poisonings with substances of abuse, the study showed that the most frequently used substances were cannabis and the new psychoactive substances (Table 9). The consumers of substances of abuse had ages in the 11-18 years range, the ratio of girls over boys being 1/2.

Table 9: Case distribution according to the abuse-substances used as etiology in acute intentional self-poisonings

Substance of abuse	No. of cases	%
Canabis/Marijuana	24	57.14%
Glue	4	9.52%
Heroin	1	2.38%
New psychoactive substances	13	30.95%

Acute intentional self-poisonings, especially in the case of adolescents, occur most often with display purposes (parasuicide) or suicide purposes in the context of family disputes. Most are meant to attract attention; few are actual suicide attempts [14, 15].

In this study, we have noted 20 cases of suicide attempts. We noted 10 cases at the 2nd suicide attempt, 6 cases at the 1st suicide attempt, 2 cases at the 5th attempt (Table 10).

Table 10: Case distribution according to the number of suicide attempts, as a function of the total number of patients

Number of suicide attempts	No of cases	%
1	6	1.75%
2	10	2.92%
3	1	0.29%
4	1	0.29%
5	2	0.58%

Out of the total number of acute intentional self-poisonings, we pointed out 20 cases of suicide attempts in our study, which represent 5.83% of the total cases. Moreover, we noticed a part of these were not the 1st attempt. The age of patients with a suicide attempt was in the 14-18 years interval; the ratio female/male was 3/1 (15 girls/5 boys). The majority of patients suffered from psychiatric disorders such as: depressive disorder (4 cases), behavior disorders (5 cases), personality disorders (2 cases), conversion disorders (1 case), emotional disorder (5 cases).

The etiology of suicide attempts consisted of multiple drugs in 9 cases, single-drug in 10 cases (CNS-acting drugs, acetaminophen, ibuprofen, blood pressure-lowering drugs) and in one case the etiology was chlorine ingestion.

54.39% of the patients with acute intentional poisonings could be placed in a category of psychiatric disorders, with the help of the extended team of a psychologist and a psychiatrist, after stabilizing the patient. The rest, 45.61% of the patients, were cases of acute intentional poisonings with no psychiatric involvement (Table 11).

Table 11: Case distribution according to associated psychiatric disorders

Psychiatric disorder	No. of cases	%
Behaviour disorder	30	8.77%
Depression	40	11.69%
Conversion disorder	1	0.29%
Emotional disorder	101	29.53%
Mental retardation	3	0.87%
Personality disorder	10	2.92%
Tourette syndrome	1	0.29%
No psychiatric disorder	156	45.61%

Out of the 342 acute intentional poisonings, 36 cases of chronic consumers of substances of abuse/alcohol were noted, representing 10.5% (Table 12). This is a worrisome percentage, bearing in mind the age group, i.e. less than 18 years. It is an alarm signal, which imposes the application of urgent measures, on a national level.

Table 12: Case distribution according to the chronic consumption of different substances

Chronic consumption	No. of cases	%
Alcohol	6	1.75%
Substances of abuse	30	8.77%

The study group contained 30 cases of chronic consumers of substances of abuse and 6 cases of chronic alcohol consumption, with ages in the interval 13-17 years, 12 female patients and 24 male patients. Out of the 36 cases of chronic substances of abuse/alcohol consumption, 4 were social cases.

The clinical study showed 30 social cases (low economical status, neglected child, disorganized families, domestic violence, children left in the care of relatives or institutionalized) and 11 patients who dropped out of school.

In 2 cases, patients aged 16, presented with evolving pregnancy. The etiology of the poisoning was medication-related: vitamins and ketoprofen were the culprits.

DISCUSSION

In the studied group, consisting of 342 patients, the etiology of acute voluntary self-poisonings varied, but medication, ethanol and substances of abuse were predominant. Statistical data withstand comparison to literature data - a study performed between 2003 and 2013 in the same clinic; the most common cause remains acute voluntary self-poisonings with medication.

Out of the 342 children with acute intentional self-poisoning, the ratio girls/boys is 1.89/1. A higher frequency of acute intentional self-poisonings was noted in females (65.49%), as compared to males (34.50%), probably due to the psychological profile of this gender at this age, i.e. higher emotional instability and display tendency. The psycho-behavioral features of the adolescents of the new generation make them vulnerable to the life problems.

In the study group, we noticed that for female cases, the most frequent etiology for acute intentional self-poisonings was medication (70.5% of intentional self-poisonings in females), while for males, the most common etiology was alcohol (49.1% of the total self-poisonings in males) and the second most common one consisted of substances of abuse (23.7%).

A predominance of acute intentional self-poisonings in children was noticed for the age interval 15-16 years (50.58%), which corresponds to adolescence, a period in which the changes produced by growth, biological function changes; not only does it affect the behavior, but also the inner feelings of the adolescent. Poisoning is a common reason for presentation to hospital and one of the top five acute medical presentations. The peak age groups are teenagers and young adults.

The highest incidence in girls was noted in the 10-14 years age group and we noticed that as adult age approaches, the sex distribution tends to be equal for girls and boys. This can be explained through the emotional particularities with respect to age in girls. In order to lower the incidence of acute poisonings, educational-prophylactic measures must be applied in schools, beginning at the age of 10, an age that corresponds to the fifth year within the gymnasium educational period.

In the study group, 42.47% of cases presented medication used in the treatment of CNS disorders as etiology of the acute intentional self-poisoning. 26.34% of children ingested Acetaminophen and 10.21% used medication acting on the cardiovascular system for the intentional self-poisoning. Out of the medication acting on the CNS, patients used carbamazepine, benzodiazepine, valproic acid, levetiracetam, sertraline, risperidone, extraveral, cipralex (escitalopram). Medication self-poisonings were the most frequent type of acute intentional self-poisonings (63.44%).

Pediatric acute intentional self-poisonings of alcohol-related etiology were caused by the consumption of vodka (20.4%), wine (17.3%), whiskey (9.1%). Distilled drinks are predominant in the etiology of alcohol ingestion in the adolescents included in the study. The results of the study performed in our country are different from the data obtained in other European adolescent populations [10]. According to literature, the drink preferred by European adolescents is beer (47%), followed by wine and distilled drinks (37-38%), cocktail drinks (32%) and cider (27%). As far as the context in which the acute intentional poisoning took place, most often socializing in a public space or at home with friends determined the occurrence of the poisoning.

In 7 of the cases, the etiology of the acute intentional self-poisoning was the combination of medication and alcohol (4 girls/3 boys), and in 7 other cases, the poisoning occurred through the consumption of both alcohol and substances of abuse (3 girls/4 boys). In the study group, acute intentional poisonings using substances of abuse were most often determined by the consumption of cannabis (57.14%) and new psychoactive drugs (30.95%), less often heroin (2.38%) or glue (9.52%). Out of the 42 cases, 14 were females and 28 were males, with ages within the 11-18 years interval, the ratio girls/boys was 1/2.

Acute intentional self-poisonings, especially in the case of adolescents, occur most often with display purposes (parasuicide) or suicide purposes in the context of family disputes. Most are meant to attract attention; few are actual suicide attempts. Out of the total number of acute intentional self-poisonings, we pointed out 20 cases of suicide attempts in our study, representing 5.83% of the total acute intentional poisonings. Moreover, we noticed a part of these were not the first attempt. The age of patients with a suicide attempt was in the 14-18 years interval; the ratio female/male was 3/1 (15 girls/5 boys). The majority of patients suffered from psychiatric disorders such as: depressive disorder, behavior disorders, personality disorders, conversion disorders, emotional disorder. Adolescents and young adults are the age group at highest risk for the first onset of commonly occurring mental disorders. This life stage has also been identified as a critical period for the onset of the first suicide attempt [16].

54.39% of the patients with acute intentional poisonings could be placed in a category of psychiatric disorders, with the help of the extended team of a psychologist and a psychiatrist, after stabilizing the patient. The fact that there are patients with recurrent suicide attempts proves the inefficacy of the psychiatric treatment and the lack of psychologic support after the period of hospitalization.

CONCLUSIONS

The clinical study showed a lot of social cases (low economic status, neglected child, disorganized families, domestic violence, children left in the care of relatives or institutionalized) and 11 patients who dropped out of school, 30 cases of chronic consumers of substances of abuse, with ages within the 11-18 years interval and 6 cases of chronic alcohol consumption. In addition, for two of the female patients a diagnosis of evolving pregnancy was established. Reducing socio-economic deprivation and its associated problems may be an important strategy in the prevention of suicidal behavior, especially in young people.

Out of the 342 acute intentional poisonings, 36 cases of chronic consumers of substances of abuse/alcohol were noted, representing 10.5%. This is a worrisome percentage, bearing in mind the age group, i.e. less than 18 years. It is an alarm signal, which imposes the application of urgent measures, on a national level.

Pediatric acute intentional self-poisonings are an important public health issue, alarming through its consequences and through its hidden neuropsychiatric and behavioral substrate. This is due to the fact that adolescence is a period of marked emotional fragility, sensitive to all sort of influences [17, 18].

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

SS contributed to the conception and design of the study, the acquisition, analysis and interpretation of data of the study. She also contributed to the drafting of the work and its critical revision for important intellectual content. IB contributed to the acquisition, the analysis and interpretation of data of the study, to the drafting of the work and its critical revision for important intellectual content. HTS contributed to the conception and design of the study, the acquisition, analysis and interpretation of data of the study, contributed to the drafting of the work and its critical revision for important intellectual content. CD contributed to the conception and design of the study, the acquisition, analysis and interpretation of data of the study, contributed to the drafting of the work and its critical revision for important intellectual content. MM contributed to the design of the study and to the drafting of the study and its critical revision for important intellectual content. ACT contributed to the analysis and interpretation of data of the study, to the drafting of the work and its critical revision for important intellectual content. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital, "Grigore Alexandrescu" Clinical Emergency Hospital for Children (Bucharest, Romania).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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VARIA

Vitamin D receptor gene polymorphisms and spinal muscular atrophy

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Abstract: *Vitamin D receptor gene polymorphisms have been intensively studied in relation with many diseases, including neurodegenerative disorders. We investigated the relationship between VDR polymorphisms and spinal muscular atrophy type I, a common lower motor neuron disease. Forty clinically and molecular diagnosed patients and 54 healthy subjects were analyzed PCR-RFLP method. We found an association trend (p=0.01) for the BsmI polymorphism taken individually and a significant association for two of four-locus inferred haplotypes (fBAt and fbaT). Also, baT, Bat and bAT were the most frequent estimated haplotypes in our control group, which is in accordance with previous published reports for Caucasian populations. We concluded that future studies performed in order to identify possible modifier genes for spinal muscular atrophy disease should take into consideration the involvement of vitamin D receptor gene polymorphisms.*

Keywords: *haplotype, polymorphism, SMA, VDR*

INTRODUCTION

Spinal muscular atrophy (SMA) is one of the most common pediatric neuromuscular diseases with autosomal recessive inheritance [1, 2]. Its cellular basis is degeneration and loss of the anterior horn cells in the spinal cord and the brain stem nuclei, which leads to wasting and paralysis of proximal muscles [3]. Death occurs usually before the age of two years in patients affected by type I, the most severe form of the disease (MIM #253300).

SMA is caused by mutations in the SMN1 gene (located at 5q12.2-13.3). Homozygous absence of SMN1 exon 7 (caused by deletion or SMN1 gene conversion into a SMN2-like gene) is the most common mutation and about 3.6 to 5% of the cases are compound heterozygotes harboring point mutations in the SMN1 gene [3, 4]. Several modifier genes (for example SMN2 [5, 6] and PLS3 [7]), which lead to clinical heterogeneity, have been identified.

Vitamin D may act like a neurosteroid hormone in the areas of neurotransmission, neuroprotection, and neuroimmunomodulation during cerebral development [8]. Moreover, vitamin D deficiency has been associated with neurological and psychiatric disorders.

Trying to bringing new insights into the description of SMA physiopathology, we focused our attention on the relationship between vitamin D receptor (VDR) polymorphisms (FokI, BsmI, ApaI, TaqI) and SMA type I disease. The role of vitamin D in nerve and muscle function has been already assessed [9, 10, 11, 12] as well as the fact that vitamin D actions are mediated by its receptor [13].

MATERIALS AND METHODS

Subjects

We studied 40 SMA type I patients (21 boys and 19 girls) with an average age of 5 ± 0.2 month in the moment of clinically diagnosis at "Al.Obregia" Clinical Psychiatry Hospital, Bucharest, Romania. The Caucasian origin of the patients was declared by their legal representatives who consented to their participation in this study (according to the Declaration of Helsinki). The onset of their clinical signs started between birth and age of six months. Symmetrical atrophy of proximal muscles (more than distal), poor

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muscular tone, absence of tendon reflexes and normal serum creatine kinase (CK) levels were present in all. Fasciculation of tongue, tremor of hands and recurrent respiratory infections were also reported. Arthrogryposis was present in two of the subjects included in the study. Neurogenic changes were recorded in patients for which electromyography (EMG) was possible.

The evolution was progressive in all the cases and death was reported at the hospital for five of them. All had homozygous deletion of SMN1 gene exon 7 which, according to the SMA International Consortium, ascertained the diagnosis. Some of these patients were described in a previous publication [14].

In this control-case study we included also a number of 54 subjects (23 men and 31 women), average age of 25 ±2.4 years, without acquired or personal or familial neuromuscular problems, who consented to participate in this research. The study received the approval of the Ethical Committee of the University of Bucharest.

Genotyping

DNA was isolated from 500 µl of peripheral blood leukocytes, using Genomic Wizard DNA Purification Kit (Promega, Madison, Wisconsin, USA). Genotypes of VDR polymorphisms were detected by the PCR-RFLP method [15, 16]. Primers sequences for FokI, ApaI and TaqI were used as were described before [15, 16]. The primers for BsmI polymorphism, were designed by us using Primer3 software [17] (F: 5’TCCAAAGTTTTGTACCCTGCC3’; R: 5’TTCGTAGGGGGGATTCT3’, annealing temperature 62°C).

PCR reactions were performed in a Gradient Palm-Cycler (Corbett Research Pty Ltd, Sydney, Australia). Amplicons were individually digested with restriction enzyme FokI (37°C)/ BsmI (65°C)/ApaI (30°C) or TaqI (65°C), for three hours. Restriction products were separated by electrophoresis on 8% PAGE and visualized using Bio-Imaging System (UVP Inc, Upland, Canada) after staining with ethidium bromide.

We used standard nomenclature and noted VDR polymorphisms alleles with “f” (FokI), “b” (BsmI), ‘a” (ApaI) and “t” (TaqI) for the presence of restriction sites, the absence being represented with uppercase letters.

Statistical analysis

Genotypes were counted directly and the distribution of alleles was inferred. Hardy–Weinberg equilibrium was verified and chi-square test (χ2) was used to compare the observed genotypes and alleles in patients versus control groups [18]. Odds ratios (ORs) were calculated in a 2 × 2 contingency table provided in the SISA program [18] and a p value < 0.05 was considered statistically significant. A Cochrane-Armitage test trend was performed using De Finnetti program [19] in case of the polymorphisms for which p values were closed to the limit for statistical significance. The observed genotypes were introduced also in the SHEsis platform [20] that estimates the haplotypes distribution.

Using the EpiSheet program [21], power size analysis was performed for each polymorphism. Based on reported frequency for CEU population of HapMap [22], we calculated the number of subjects needed to detect at least an OR of 2.0, with 80% power, for a minor allele frequency of 25 %. This analysis indicated that 160 cases and 160 controls would be necessary to provide these values.

RESULTS

Distribution of the genotypes and alleles in the patient and control groups is represented in Table 1. The chi-square test values are also provided in Table 1.

The ORs analysis provided a statistically significant p value only for the BsmI polymorphism (p=0.0338), but this was not confirmed when Yate’s correction test was applied (p=0.06). Applied Cochrane–Armitage test for trend showed statistically significant results: ORBBvsBb=0.232 (p=0.0175); ORBBvsBb+bb=0.289 (p=0.0338).

No significant differences were found when the subjects were grouped according to the gender.

Linkage disequilibrium (LD) analysis was also performed. In the control group we found a LD relationship was found between BsmI/TaqI and ApaI/TaqI polymorphisms, while in the patients group an LD relationship was detected between BsmI/ApaI and ApaI/TaqI polymorphisms.

The frequencies for the inferred four locus haplotypes within each group and the Odds Ratio values are given in Table 2. The significant results obtained for fBAt and fbaT haplotypes are discussed later. Results were also obtained for three locus haplotype inferred from combined polymorphisms: FokI, BsmI, ApaI (fBA: OR=0.267, p=0.02; fba: OR=2.44, p=0.02); FokI, BsmI, TaqI (fBt: OR=0.277, p=0.02) and FokI, ApaI, TaqI (faT: OR = 2.653, p=0.01). For the BsmI, ApaI, TaqI haplotypes in the control lot, most frequent were baT (31.4%), Bat (28.1%), bAT (18.2%).

Table 1: VDR genotypes and alleles distribution

Polymorphism	Genotype/ Allele	Cases N (frequency)	Control N (frequency)	OR (*p*) * 95% CI
rs2228570 (*Fok*I)	*FF*	14 (0.350)	23 (0.426)	0.72 (0.45); 0.31-1.68
	Ff	21 (0.525)	23 (0.426)	
	ff	5 (0.125)	8 (0.148)	
	F	49(0.613)	69(0.639)	0.89 (0.71); 0.49-1.62
	f	31(0.387)	39(0.361)	
rs1544410 (*BsmI*)	*BB*	4(0.100)	15(0.278)	0.28 (0.03) ‡ 0.09-0.95
	Bb	23(0.575)	20(0.370)	
	bb	13(0.325)	19(0.352)	
	B	31(0.387)	50(0.463)	0.73 (0.30) 0.41-1.32
	b	49(0.613)	58(0.537)	
rs7975232 (*Apa*I)	*AA*	7(0.175)	15(0.278)	0.55 (0.24); 0.20-1.51
	Aa	24(0.600)	28(0.519)	
	aa	9(0.225)	11(0.203)	
	A	38(0.475)	58(0.537)	0.78 (0.40); 0.43-1.39
	a	42(0.525)	50(0.463)	
rs731236 (*TaqI*)	*TT*	15(0.375)	22(0.407)	0.87 (0.75); 0.37-2.02
	Tt	21(0.525)	23(0.426)	
	tt	4(0.100)	9(0.167)	
	T	51(0.638)	67(0.620)	1.07 (0.81) 0.59-1.95
	t	29(0.362)	41(0.380)	

**Pearson's p value; ‡ statistical significant differences*

Table 2: VDR genotypes and alleles distribution

Haplotypes	Cases (freq)	Control (freq)	OR (p)†, 95%CI ‡
FBAt	18.08 (0.226)	16.11(0.149)	1.67 (0.17), 0.79-3.53
FBAT	5.14 (0.064)	3.02(0.028)	2.39 (0.22), 0.56-10.25
FBat	0.00	4.94(0.046)	-
FBaT	0.00	7.12(0.066)	-
FbAt	2.92 (0.036)	3.37(0.031)	1.17 (0.84), 0.23-5.8
FbAT	3.19 (0.040)	8.76(0.081)	0.47 (0.25), 0.12-1.75
FbaT	19.67 (0.246)	25.69(0.238)	1.04 (0.89), 0.531-2.06
fBAt	3.21 (0.040)	14.32(0.133)	0.27 (0.03), 0.08-0.95 §
fBAT	0.00	1.70(0.016)	-
fBat	0.00	1.22(0.011)	-
fBaT	4.57 (0.057)	1.57(0.015)	4.106 (0.10), 0.65-25.75
fbAT	3.19(0.040)	10.72(0.099)	0.37 (0.12), 0.10-1.36
fbat	2.51 (0.931)	1.03(0.010)	3.363 (0.27), 0.33-33.83
fbaT	15.24 (0.191)	8.42(0.078)	2.801 (0.02), 1.13-6.88 §
Fbat	0.00	0.00	-
fbAt	2.27(0.028)	0.00	-

** Haplotypes analyzed by SHEsis software platform; † Pearson's p value; ‡ Global result: global chi-square 27.84, degree of freedom 11, p = 0.003424 (haplotypes with frequencies below 0.03 in both cases and controls were excluded).*
§ Bold indicates statistical significance; OR = Odd ratio; CI = Confidence interval

DISCUSSION

This study focused on the relationship between four polymorphisms of the VDR gene and SMA type I. To our knowledge, no relationship between these markers and the mentioned pathology has been published so far. However, the interpretation is difficult and limited by the small power size of this study.

We compared distribution of individual markers in the two groups and we found an association trend between BsmI polymorphism and SMA type I physiopathology.

For this marker, an association relationship has been reported with Parkinson's disease [23] and multiple sclerosis [24]. Also, an association between the BsmI-TaqI haplotype and quadriceps muscle strength in men [25] and a correlation between the BB genotype and high risk for bone fractures (which was also found in SMA type I patients) in a large group of postmenopausal women were identified [26].

The results of LD tests were not what we expected. Data from literature (including HapMap database) reported a strong LD relationship between for BsmI, ApaI and TaqI polymorphisms (D' values 0.98 and 1) in European populations [27]. In our control group the most frequent estimated haplotypes were baT, Bat, bAT (in accordance with data reported in a meta-analysis study on Caucasian populations [28]), but we did not find a strong LD for BsmI and ApaI polymorphisms. Different sizes or genetic backgrounds of the analyzed pools are usually the explanation for discrepancies of results. For VDR gene the combined action of recombination hotspots and demographic history events was considered to be responsible for the different LD in European populations [29]. Recombination events (including unequal recombination, intrachromosomal deletion and gene conversion) were also frequently described in SMA type I patients [30]. For our lot we found a LD relationship for BsmI/ApaI, but not between BsmI/TaqI. However, given that a larger group of Romanian subjects [29] didn't revealed significant differences between general and European populations, we assume think that our results are due to the random sampling.

Although we only found significant results only for BsmI polymorphism, the four-locus haplotype analysis revealed also an important contribution for an extended haplotype.

Our results indicated a protective effect for the fBAt haplotype whereas the fbaT combination seemed to confer a significant risk. These results could be sustained by the findings of Morrison et al. [31], who showed that activity of receptor is higher for BAt haplotype comparing with baT haplotype. However, when we analyzed three locus haplotypes (BsmI, ApaI and TaqI), we didn't obtain any statistically significant results. A possible explanation for this could be the fact that FokI polymorphism (the only functional polymorphism among those analyzed) may be in a significant relationship with the disease, but in a genomic context depending manner. Some studies have reported that the combined effects of FokI polymorphism and the BsmI, ApaI, TaqI haplotypes have no major effect on the VDR protein level, but significantly influence the responsiveness to the ligand. The fbaT haplotype is reported to be less responsive for the ligand than FBAt haplotype combination, sustaining also our results. Despite these findings, the present study did not reveal the relationship between FokI polymorphism and the SMA type I physiopathology.

Overall the three locus haplotype analysis revealed the existence of possible protective combinations (fBA, fBt) and possible risk haplotypes haplotypes (fba, faT). Although we did not find significant on individually analysis of each allele studied, we did reveal that the "B" and "A" alleles may confer protection meanwhile the "a" allele may be associated with the risk for the disease. These findings underline the importance of studying VDR haplotypes rather than individual genotypes, as meta-analysis studies already proved [28, 32].

Our hypothesis of the VDR polymorphisms involvement in SMA type I disease is sustained by the associations of these variants with features described in SMA type I disease (e.g. affected muscle function, bone fractures and bone mineral density or hypercalcemia) [11, 33, 34, 35]. Also, the impaired expression of myf5 during the fusion of satellite muscle cells noticed in SMA type I patient's cells [36] and the decreasing effect of 1,25-dihydroxyvitamin D on this muscle specific transcription factor expression could be also in the support of our study purpose [37].

We conclude that VDR gene polymorphisms and their inferred haplotypes (especially fBAt and fbaT) may be associated with SMA type I pathology. We considered that future studies performed in order to identify other possible modifier genes should take into consideration the involvement of VDR gene polymorphisms.

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VARIA

Vitamin D status and cholecalciferol supplementation on patients with active Basedow disease: a clinical study

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Abstract: *This study aims to analyze the 25-hydroxyvitamin D (25OHD) corelations with TRab (anti-TSH receptor Antibodies) at baseline and after corections of 25OHD deficit.*

Method: Prospective, interventional, controled, single-centre, clinical study on 62 patients with active Basedow disease (BD), admitted at C.I. Parhon National Institute of Endocrinology, Bucharest, Romania, between 2013 and 2020. Patients were divided into two groups: one group (N=37) received 2000 IU of daily cholecalciferol and a second one (N=25) without vitamin D supplementation, all subjects being under anti-thyroid drugs (standard guideline therapy). 25OHD and TRab levels were assessed at start and after 6, respective 12 months.

Results: Initial evaluation confirmed vitamin D deficiency (mean 25OHD of 18.33±6.45 ng/mL). At start, TRab negatively correlates with 25OHD (N=62, r= -0.22, p=0.08). The decrease in TRab was statistically significant higher in group 1 versus group 2 after 6 months (35.84% versus 3.53%, p=0.03). After 12 months, TRab decreased with 56.28% in group 1, respective 27.16% in group 2 (p=0.03). In group 1, inverse correlation between 25OHD and TRab was consistent at 6 and 12 months.

Conclusion: In patients with active BD, TRab values were negatively correlated with 25OHD at baseline and during follow-up. Correction of vitamin D deficiency is correlated with the decrease of TRab levels.

Keywords: *25-hydroxyvitamin D, Basedow disease, anti-TSH receptor antibody, vitamin D deficiency, hyperthyroidism, vitamin D, autoimmune thyroid disease, TSH*

INTRODUCTION

Autoimmune thyroid diseases are among the most common organ-specific autoimmune disorders, Hashimoto's chronic thyroiditis and Basedow disease (BD) being the most common clinical expressions [1]. BD is considered to be the most common form of thyrotoxicosis, following anti-TSH receptor autoantibodies (TRab) secretion and actions [1, 2]. Besides the well known treatment of this disorder (antithyroid drugs followed by radioiodine therapy or thyroidectomy), current studies demonstrate the effectiveness of complementary therapies for obtaining and maintaining a prolonged remission of the disease [1, 2]. In this context, the extra-skeletal roles of vitamin D are subject of intensive debate [1, 2]. Vitamin D is incriminated in regulating cell functions, its deficiency being considered a

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risk factor for the development of different autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, diabetes mellitus type 1 and thyroid autoimmune disorders [2, 3]. In addition to its established role in calcium-phosphate metabolism, vitamin D is involved in multiple disorders, the vitamin D receptor (VDR) being widespread and its polymorphisms extensively studied [2, 3]. Vitamin D helps regulate cell growth, differentiation and apoptosis, being incriminated in regulation of cell functions in many systems, like cardiovascular, renal, central and peripheral nervous system [2, 3]. Correction of vitamin D deficiency prevents the onset or unfavourable evolution of some of these disorders; however, controversies around the role of vitamin D supplements are still described [4, 5]. In thyroid autoimmune conditions, correlations between vitamin D levels as expressed by serum levels of 25-hydroxyvitamin D (25OHD) and ATPO (Anti-thyroperoxidase antibodies) or TRab status have been found [3, 6, 7]. Moreover, levels of 25OHD <20 ng/mL appear to be an independent risk factor for treatment failure in patients receiving radioiodine for BD [8]. Recent studies have shown a high incidence of vitamin D deficiency but in BD subjects, 25OHD-TRab corrections data are still controversial and the collateral role of vitamin D in achieving and maintaining drug remission in BD remains an unanswered question [3].

Aim of the study

The primary endpoint is to establish the correlation between 25OHD and TRab in patients with active BD, at baseline and after vitamin D supplementation in addition to the classical anti-thyroid drugs. The second endpoint is to identify the prevalence of vitamin D deficiency in adult patients with BD.

MATERIALS AND METHODS

Study design: prospective, interventional, single centre, controlled study in 62 hospitalized patients from C.I. Parhon National Institute of Endocrinology, Bucharest, Romania, enrolled between 2013 and 2020.

Inlusion criteria: adults aged over 18 years, with newly onset BD and a TRab level >1.5 U/mL, with or without active ophtalmopathy, patient' consent for the study.

Exclusion criteria: current medication targeting mineral metabolism (calcium and/or vitamin D suplements, glucocorticoids, antiepileptics), steroids treatment, malabsorbtion or acute/chronic renal disease and pregnancy. Patients were divided into two groups: one group received 2000 IU of cholecalciferol daily and a second one without vitamin D supplementation. We investigated the effects of vitamin D deficiency correction on the level of TRAb after 6 and 12 months (group 1). Blood samples were collected under fasting conditions (between 8:00 and 10:00 AM). TRab was measured by electrochemiluminiscence (ECLIA Cobas e601), with a lower detection limit of 0.3 UI/L. 25OHD was measured by electro-chemiluminiscence (ECLIA, Cobas e601, Roche) with a lower detection limit of 4.2 ng/mL. The 25OHD categories are as follows: severe deficit < 10 ng/mL; insufficient: 10-30 ng/mL; optimum: 30-100 ng/mL; toxicity: >100 ng/mL. The intra- and inter-assay variation coefficients in our laboratory were 7.6% and 11.4% for TRAb, respectively 3.27% and 9.1% for 25OHD. Serum concentrations of TSH (Thyroid Stimulating Hormone), fT4 (Free Levothyroxine) and ATPO were determined by direct immuno-chemiluminiscence assay (Siemens Immulite). The statistical interpretation was performed using Microsoft Office Excel 2010 and its applications. We used the following statistical tests: Student's t test and Spearman coefficients. Statistical tests were considered significant if p <0.05.

RESULTS

The study was performed on 62 patients, aged between 21 and 80 years (group 1: N=37, group 2: N=25). The study enrolled 48 women (77.42%) and 14 men (22.58%). There were no significant differences in terms of age distribution between the two groups and the standard anti-thyroid medication regimes and target hormonal values. The maximum number of patients, of either group, belongs to the 40-49 age categories. The evaluation of the initial status of 25OHD in all 62 patients confirmed vitamin D deficiency; the mean 25OHD was 18.33± 6.45 ng/mL; all the patients, regardless of age group, had 25OHD values below 30 ng/mL. The age group with the lowest value of 25OHD was the 50-59 years, with a mean 25OHD of 15.76± 8.1 ng/mL (Figure 1).

Figure 1: Age distribution depending on the initial status of vitamin D in studied patients with active Basedow disease

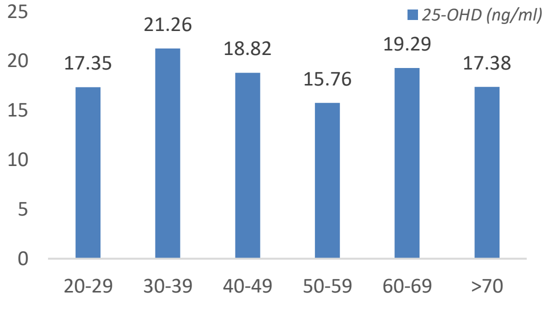

In the first group, after vitamin D supplementation, the percentage of patients with vitamin D deficiency (25OHD <20 ng/mL) diminished and the percentage of patients with optimal values of vitamin D (25OHD above 40 ng/ml) increased after first 6 months, but statistically significant only after first 12 months. After daily administration of 2000 UI of cholecalciferol, mean 25OHD increased with 50.5% (8.4±6.51 ng/mL) after the first 6 months, and with 88.53% (14.73±10.43 ng/mL) after 1 year (Figure 2).

Figure 2: Vitamin D status in patients receiving cholecalciferol 2000 U/day (group 1 – number of patients receiving vitamin D supplementation) and active Basedow disease

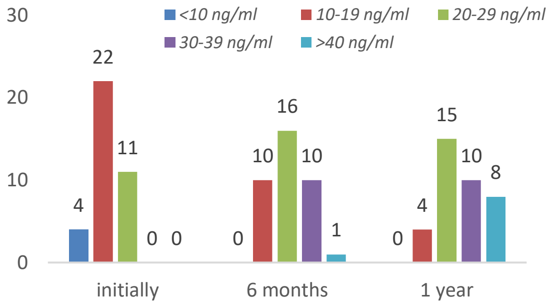

During follow-up of group 2, 25OHD levels had a decrease at 6, respective 12 months (without statistical significance) (Figure 3).

Figure 3: Vitamin D status in patients without vitamin D suplementation (group 2 – patients without cholecalciferol supplimentation) and active Basedow disease

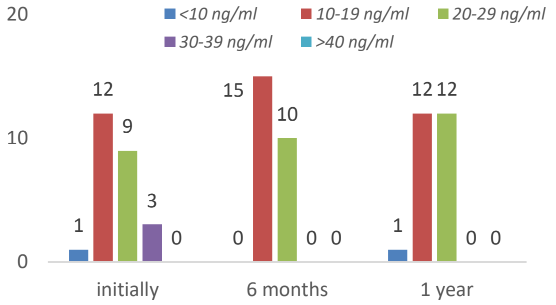

Comparative evolution of vitamin D levels between group 1 and group 2 is shown in Figure 4.

At start, TRab negatively correlates with 25OHD (N=62, r=-0.22; p=0.08). The decrease of TRab was higher in group 1 versus group 2 after 6 months: 35.84% versus 3.53% (the levels after 6 months of 6.18 ±7.27 UI/L versus 0.58±10.95 UI/L, p=0.03).

After 12 months, TRab decreased with 56.28% (9.7±7.7 UI/L) in group 1 and with 27.16% (4.43±10.21 UI/L) in group 2 (p=0.03).

During follow-up of group 1, TRab decreased, while 25OHD increased at 6 and 12 months (Figure 5).

These changes (decrease of TRab, increase of 25OHD) were not observed in the second group (Figures 6 and 7).

Figure 4: Comparative evolution of 25-OHD between group 1 and group 2 after 6, respective 12 months (with daily 2000 U of cholecalciferol in group 1, without vitamin D supplementation in group 2 in association with standard therapy for active Basedow disease)

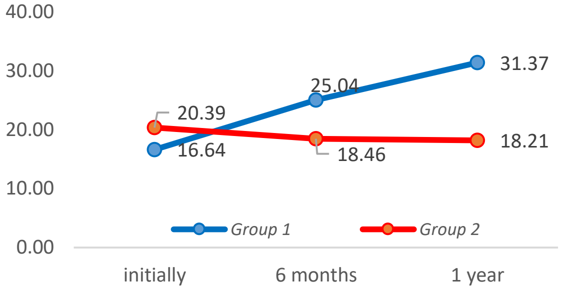

Figure 5: 25-OHD and TRab mean values in adult patients receiving daily 2000U of cholecalciferol (group 1) at baseline, after 6, respective 12 months (in addition to standard anti-thyroid medication) – values in ng/mL

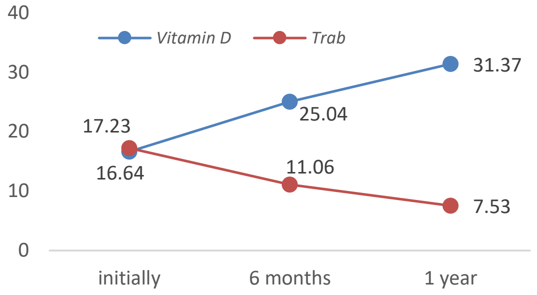

Figure 6: 25-OHD and TRab mean values in adult patients without vitamin D supplementation (group 2) at baseline, after 6, respective 12 months (under standard anti-thyroid medication)

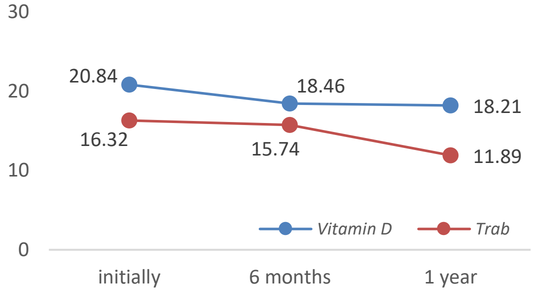

Figure 7: 25-OHD and TRab values evolution in enrolled patients

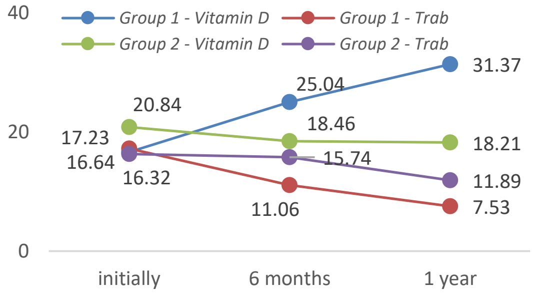

DISCUSSION

BD, caused by autoantibodies actually has a multifactorial pathogeny, including the potential role of vitamin D [9-11]. There are studies showing that the treatment with methimazole in combination with vitamin D supplementation in patients with BD attained the euthyroid status faster compared to those treated only with anti-thyroid drugs [12]. In addition, supplementation with vitamin D has been shown to inhibit inflammatory responses in the thyroid and T cells [12]. It has also been found that vitamin D deficiency is associated with increased thyroid volume in patients with newly onset GD [12]. Several allelic variants (polymorphisms) have been described in the VDR gene on chromosome 12 and have been reported to be associated with a variety of physiological and pathological phenotypes, including in thyroid autoimmune diseases and thyroid cancer [13-16]. However, the actual mechanisms of vitamin D receptor polymorphisms are still a matter of debate [15, 17]. Our study confirmed the high incidence of vitamin D deficiency in general adult, Caucasian population. Daily supplementation with 2000 IU of vitamin D does not provide full correction of vitamin D deficiency (meaning achieving a level of 25OHD > 30 ng/mL) at 6 and 12 months, but a statistically significant improvement is observed. For this correction, current guidelines recommend 25000-50000 IU/week for first 8-12 weeks with a subsequent lower maintenance dose, but the high levels are also necessary to continue under special conditions (like COVID-19 pandemic, chronic diseases like autoimmune conditions involving different medical specialities, obesity, uncorrected hypocalcemia, high doses of glucocorticoids or elevated osteoporotic fracture risk status, etc.) [18-22]. However, we highlighted the role of vitamin D in reducing the TRab titre, objectified by the negative correlation between antibodies and vitamin D levels after correcting its deficiency. In group 2, the minimal decrease in TRab concentration was considered to be a consequence of the immunosuppressive effect of thiamazole and of the natural evolution of the disease.

CONCLUSION

This study highlights the low levels of 25OHD in adults with active Basedow disease. TRab is inversely correlated with 25OHD in newly diagnosed patients. Daily supplementations with 2000 U of cholecalciferol statistically significant increase 25OHD while TRab decreases during follow-up. Vitamin D should be considered as a possible complementary therapy under these circumstances. Therefore, public health policies should aim for prevention, diagnosis and correction of its deficit in adult population.

Authors' contributions

FNP drafted the manuscript and critically revised the final form, MG researched the literature, BP researched the literature, AG drafted the manuscript, FS researched the literature, MC drafted the manuscript and is the corresponding author, RSB revised the final form and wrote the conclusions, AF researched the literature, and MCD approved the final form.

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ORIGINAL ARTICLE

Vulvovaginal atrophy – the impact on the quality of life and self-regard. Review of literature

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Abstract: *Vulvovaginal atrophy is a condition frequently encoutered among menopausal women that often is ignored, remains undiscussed with the physician and the patient adapts the life style is such matter that offers a run from the specific symptomathology. According to the specialized published data, only 25% of the patients with vulvovaginal atrophy receive adequate therapy. The therapeutic perspective is quite extensive but the patient should receive recommendations and try different products until they find one that meets their needs and the one they like best. Proper estrogen therapy leads to a restoration of the acidic, normal vaginal pH, of the vaginal microflora, thickens the epithelium, increases vaginal lubrication and decreases vaginal dryness, also, adverse effects of vaginal estrogen therapy are uncommon. Early active detection of this pathological condition and the provision of an appropriately selected therapy can prevent the progression of the symptomatology and can significantly improve the sexuality of women.*

Keywords: *vulvovaginal atrophy, therapy, quality of life*

INTRODUCTION

Menopause is a physiological condition that occurs in women with an average age of 51 years; 95% of women become climacteric at an age between 45 and 55 years. Characteristic for menopausal period is the marked decrease of the ovarian estrogen production [1]. The vulvovaginal correspondent of this hormonal change is vulvovaginal atrophy. This is the condition that is frequently ignored, undiscussed with the physician and the patient remains in the position where he does not recognize the symptoms and adapts the life style is such matter that offers a run from this pathology. Therefore, most women forget that at 50 years old are young and they prefer to have a precarious sexual life, adopting in the same context the idea of aging.

The Genitourinary Syndrome of Menopause (GSM) was a term introduced for the first time in 2014 by the International Society for the Study of Women's Sexual Health and the North American Menopause Society [1, 2]. Being defined as the collection of symptoms and signs associated with decreased estrogen and other sex steroids, the GSM includes the totality of atrophic symptoms that can occur in the bladder-urethral area and vulvovaginal area (labia majora/minora, clitoris, vestibule/introitus, vagina) due to the loss of estrogen in the menopausal period. These symptoms include vaginal dryness, burning, pruritus, dyspareunia, vaginal discharge, bleeding/spotting and urinary tract symptoms like dysuria, increased urinary frequency, urethral discomfort and, infrequently, hematuria [3]. In Table 1 the main signs associated with GSM are summarized.

The aspects of the life quality must always be assessed; these include the degree of discomfort, the behavior adapted to the symptoms, the impact of these symptoms on daily activities, sexual life and partner relationship. No matter what management protocol is targeted, this must be

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adapted to the woman's expectations regarding its results [4]. According to the specialized published data, only 25% of the patients with vulvovaginal atrophy receive adequate therapy [5]. One explanation of this is that many patients feel ashamed to discuss intimate complains with a specialist and furthermore, some patients consider symptoms of GMS to be manifestations of the natural aging process and do not seek help for this.

Table 1: Clinical signs associated with GMS

Suggestive signs for GSM	Labia minora resorption or fusion
	Tissue fragility/fissures/petechiae
	Introital retraction
	Loss of hymenal remnants
	Prominence of urethral meatus
	Urethral eversion or prolapse
	Vulvovaginal pallor/erythema
	Loss of vaginal rugae
	Decreased vulvovaginal secretions/lubrication
	Decreased elasticity

Regarding the cascade of events involved in the pathophysiological mechanism, in hypoestrogenic conditions, the vaginal epithelium becomes thinner, the epithelial barrier function is lost, tissue elasticity decreases and the secretory activity of the Bartholin glands decreases, all this leading to trauma to the vaginal mucosa and pain [6].

In addition, under conditions of estrogen deficiency, the balance of the vaginal microbiota is disturbed, pathogenic gram-negative fecal flora and other bacteria prevail in its composition increasing the risk for vaginal and urinary tract infections.

Specific to the sexual intercourse in post-menopause are the following aspects [7]: narrowing of the vulvo-vaginal copulatory space with rigidity of vaginal walls, frigidity in the context of dyspareunia and local injury, maintaining sexual activity is associated with attenuation of the involutional process and decreased sensitivity to erogenous factors with changes in sexual behavior.

In Romania, this problem is even more serious as, for example, in the biggest Hospital of Bucharest with a median of 10,000 hospitalizations per year in the Obstetrics and Gynecology Department, in the last 10 years only about 400 cases of vulvovaginal atrophy, as a secondary diagnosis, were recorded.

Having this data, our aim was to summarize aspects related to the clinical symptomatology, approach and management and highlight the impact of vulvovaginal atrophy of the quality of life.

The extent of the pathology and recommendations

In 2018, the results of a significant study were published [8]. According to this study, which included more than 2000 patients, valvovaginal atrophy affects more than 90% of menopause women, the great majority of them combining a mean of five symptoms. Also, the authors of the same study state that in approximatively 10% of symptomatic cases, the signs of atrophic vulvo-vaginitis can lack. This cases have one advantage as the structural changes can be prevented trough specific topic therapy. Summarizing, the authors highlight the progressive trend of this condition and the great importance of early instituted therapy.

As regards the impact of GMS on the women's daily living, in 2019 Nappi et al [9] published an extensive study including 2160 climacteric, evaluable women in whom, clinical confirmation of vulvovaginal atrophy was possible in more than 90% and over 66% of this patients presented severe vaginal symptoms. Along with the symptomatology adapted questionnaire (EQ-5D-3L) a Day-to-Day Impact of Vaginal Aging (DIVA) questionnaire was used. The results showed a direct relation between the clinical presentation of vulvovaginal atrophy and the impact on the quality of life [6]. In the context of this results, it is to mention that the effect on the life quality of GMS should be regarded as equal to the impact of other conditions on daily activity of patients and treated with the same interest.

The topic regarding sexual activity in menopause is surrounded by reluctance on both sides, clinician and patient. It is a known fact that active sexual function at midlife has a significant positive impact on personal and relational quality and improves longevity. Simon and al [10], recommend as a solution a conversation guide for helping the practitioner to open and direct the discussion and to offer an appropriate treatment to enhance the sexual desire, treat orgasmic dysfunction and attenuate dyspareunia. According to another specific study [11] more than half of all menopausal women suffer from at least one sexual symptom along to other conditions related with hormonal changes. Psychosocial factors should be regarded a solution for maintaining sexual satisfaction and practitioners have to offer adequate counselling and proactively address sexual symptoms from the beginning of the menopausal period.

TREATMENT OPTIONS

Women should try different products until they find one that meets their needs and the one they like best. The first intention is non-hormonal lubricants. Glycerin lubricants can lead to an increased risk of vulvovaginal candidiasis. These products should be used routinely, usually two or three days a week and not just during sexual activity. Vaginal moisturizer and lubricating therapy appear to be just as effective as vaginal estrogen. These agents improve vaginal comfort, but do not reverse most atrophic vaginal changes. Thus, they are especially useful for women with mild symptoms, and many women with severe symptoms will need hormonal or alternative medications.

Mitchel et al [12] conducted a randomized clinical trial and found that the effect of local hormonal therapy is comparable equal to that of non-hormonal, the differences between case-placebo being minimal. The only statistically significant difference was the slightly better improvement of pain at penetration with low dose-vaginal estradiol. Therefore, local non-hormonal therapy is an extremely effective treatment for symptoms of dryness or vulvovaginal discomfort, tissue fragility, vaginal discharge if the only etiology is hypoestrogenism.

Vaginal estrogen therapy is the first line therapy for GSM that does not respond to vaginal moisturizers and lubricants. Proper estrogen therapy leads to a restoration of the acidic, normal vaginal pH, of the vaginal microflora, thickens the epithelium, increases vaginal lubrication and decreases vaginal dryness. Adverse effects of vaginal estrogen therapy are uncommon. Vaginal estrogen therapy at recommended doses results in the systemic absorption, although to a lesser extent than oral or transdermal estrogen therapy. A higher level of absorption with the initial therapy may be due to the atrophic thin vaginal epithelium. Low-dose vaginal estrogen is the most effective treatment for moderate to severe symptoms of vaginal atrophy that do not respond to non-hormonal intervention. Among patients, discussing hormone therapy causes fear, a phobia, justified on the one hand, but acceptable, and recommended on the other. Thus, successive studies have been conducted on the impact of local, hormonal therapy on chronic heart disease, the risk of thrombotic accidents, and breast cancer. The common conclusion was that the risk in the presence of these diseases outweighs the benefit, regardless of age [13]. However, the amount of circulating estrogen obtained through properly administered vaginal hormone therapy provides a low and constant systemic concentration and can be safely applied for a period of 3 months [14].

A systematic review of 19 randomized trials, including over 4,000 women, investigated topic estrogen treatment and found that creams, vaginal tablets, and rings were equally effective in relieving the symptoms of vaginal atrophy. In the first 2 weeks of use, the daily use of the above-mentioned groups of drugs is recommended, followed by a switch to maintenance therapy with a dose of two to three times a week. Continuous-release vaginal rings are preferred for women for whom daily medication is unacceptable. Vaginal rings are inserted for up to 90 days and can be placed and removed by the patient. The use of vaginal rings is not recommended for women with genital prolapse. It is also necessary to warn the patient about the possible expulsion of the devise. Such a route of administration allows the shunt of hepatic metabolism, which minimizes the risk of adverse reactions specific to systemic estrogen therapy and excludes the effects on the endometrium and breast tissue. So it is not necessary to prescribe progesterone preparations to prevent endometrial hyperplasia and adenocarcinoma. Local estrogen therapy allows the rapid elimination of vulvovaginal atrophy symptoms, but does not relieve the vasomotor symptoms and does not reduce the risk of osteoporosis. The safety of vaginally administered estrogen on the endometrium and breast was successively studied and demonstrated [15, 16, 17, 18]. Estrogen therapy is prescribed after a thorough clinical examination and identification of risk factors for possible complications. The risk of overdose is increased by using vaginal creams. Excessive estrogen levels in postmenopausal women are associated with an increased risk of heart disease, breast cancer, thromboembolic complications and cerebrovascular disease. For women with additional risk factors for any of these complications, it is necessary to refer to another therapeutic method. Low doses of estrogen are preferred for use, as high doses of estrogen at menopausal age is associated with an increased risk of endometrial adenocarcinoma.

Dehydroepiandrosterone (DHEA) is a steroid prohormone obtained through the biosynthesis of testosterone and estradiol. Vaginal metabolism of DHEA leads to the activation of estrogen and androgen receptors in the three layers of the vaginal wall. Due to local inactivation of this prohormone, the increase in serum estrogen and androgen levels is minimal. It was proven by reference clinical trials the local positive effect of intravaginal DHEA on mild and severe dyspareunia [19].

Laser therapy is beneficial trough the enhancement of vascularization of the vaginal mucosa, stimulation of collagen synthesis, the formation of new vaginal papillae, reformation of glycogen stores in the vaginal epithelium, restauration of the balance of the vaginal mucosa and therefore improves the symptoms of atrophy caused by lack of estrogen [20]. Finally, laser vaginal therapy was associated with a significant improvement in quality of life and sexual activity.

From the same new therapy spectrum, platelet-rich plasma injection it is based on the principle of activating and improving the body's natural ability to regenerate. It consists of the injection of autologous platelet-rich plasma (PRP) into the upper wall of the vagina and clitoris, triggering the multiplication of stem cells and the growth of "younger" tissue. Growth factors released from activated PRP, promote the production of collagen and elastin inside the vagina and around the clitoris, causing an increase in its elasticity, easier stimulation, positive effect on dyspareunia and vaginal lubrication [21].

Vaginal lipofilling is an effective and minimally invasive way to restore tissue volume in both cosmetic and reconstructive surgery. The disadvantages of lipofilling are unpredictability and variable reabsorption rate [22].

Our experience complies with the data presented above; the clinical diagnosis can be established during a simple gynecological examination were the signs of vulvovaginal atrophy can be identified especially in low to moderate cases accompanied by symptomatology (Figure 1), in severe cases were the diagnosis is instantly clear (Figure 2) or in cases in complex case where the signs and symptoms of vulvovaginal atrophy are overshadowed by the underlying pathology (Figures 3 and 4).

Figure 1: Moderate vaginal and cervical atrophy with loss of vaginal rugae, fissures and petechiae (authors' personal collection)

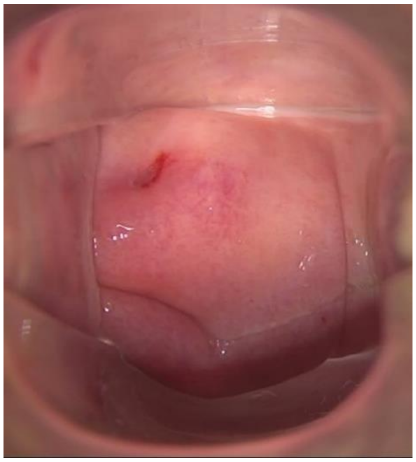

Figure 2: Severe vaginal and cervical atrophy with endocervical squamocolumnar junction, petechiae and tissue fragility

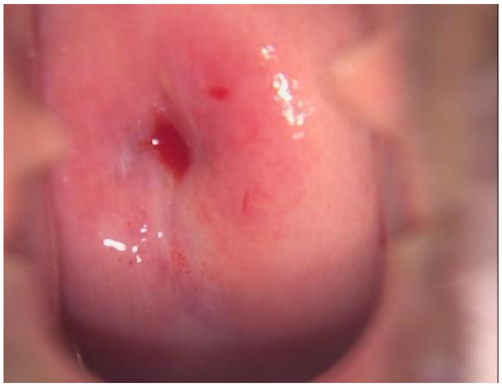

Figure 3: Ignored vulvovaginal atrophy superimposed on a grade 4 cystocele

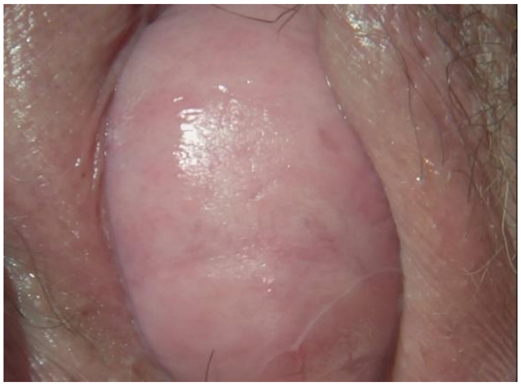

Figure 4: Severe vulvovaginal atrophy with labia minora resorption and prominence of urethral meatus, six years after a surgically induced menopause for cervix uteri neoplasia, six months after a PRP and topic lubricants.

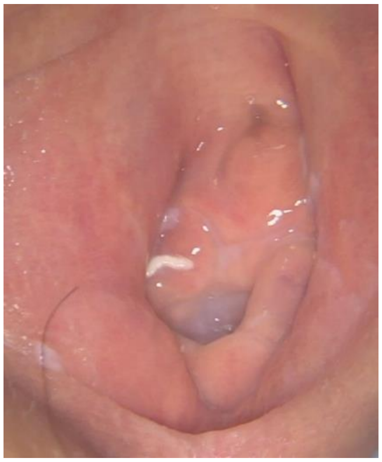

CONCLUSION

There are several ways to prevent severe vulvovaginal atrophy and to improve postmenopausal sex life. Some recommendations are:

o avoiding overeating, alcohol and spicy foods;
o avoiding smoking;
o psychological support;
o cotton underwear and loose clothing;
o sexually active status improves blood circulation in the

vagina and stimulates natural lubrication;

o sexual activity has no effect on serum estrogen levels. However, by improving blood circulation, it keeps the sexual organs healthier for longer time;

o giving the necessary time for prelude is an important point in maintaining the sexual function;

o the systemic impact of active sex life consists of an increased level of androgens and gonadotropins.

Many women with GSM do not seek medical advice, and clinicians should include questions about these symptoms in the history of menopausal women. The management of symptoms is guided by the patient's symptoms and goals. In the context of atrophic vulvovaginitis, low doses of vaginal estrogen are preferred compared to systemic hormonal therapy. This condition complicates the postmenopausal period in more than half of women worldwide and the manifestations bring discomfort in a woman's daily life, negatively influencing the quality of life and the couple's relationship.

Early active detection of this pathological condition and the provision of an appropriately selected therapy can prevent the progression of the symptomatology and can significantly improve the sexuality of women. A superior positive effect in the treatment of women with vulvovaginal atrophy can be obtained by combining hormonal and non-hormonal methods.

The role of the gynecologist is crucial by facilitating the discussion, recognizing the condition, being sensitive to the impact on sexual activity and quality of life, educating about risk factors and consequences, understanding the needs and expectations, removing barriers, fears, misconceptions, considering all the potential options according to the benefits and risks balance and according to the Psychosocial profile, having the objective to effectively treat VVA, starting early to obtain a better response to treatments and improving long-term adherence.

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ORIGINAL ARTICLES

Why cancer/terminal ill diagnosis unsuccessful in India: a qualitative analysis

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Abstract: *The increasing of the modern technologies requires the setting up of multiple factories and industries that produce smokes and chemicals, which polluted the air, water, and the environment resulting in putting lives in dreaded conditions. In fact, the coming of the modern era and the modern lifestyle gives birth to numerous deadly diseases like cancer and its related diseases, which increasingly takes the lives of many in the country. The cold-blooded killer 'cancer' becomes the major public health concern in India as a whole. Among all the problems existed across the world, the problems or the issues associated with cancer or terminal illness becomes the worldwide phenomenon that produces several unwanted worst experiences. Human polluting the water, soil, and the air continued, and the global burden of cancer affected populations rapidly increasing year after year. Unfortunately, India turns out to be the world most densely cancer populated regions and a cancer hub. The reason behind the rapidly increasing of cancer illness and its mortality rates in India is mainly due to people lack awareness on cancerous factors, no proper preventive measures, and the late detection of the symptoms at the advanced stages when cure is not possible.Thus, concerning the helplessness conditions of the cancer effected people of in general, the current analytical study has been forms with an aim to spread awareness on the cold-blooded killer cancer across the country.*

Keywords: *cancer, terminal illness, awareness, mortality rates, diagnosis*

Objectives: The underlying aim of the study is to investigate the underlying reasons behinds the rapid growths of cancer populations and the cancer mortality rates in the present India.

The study emphasis mainly on the cancerous factors, the underlying barriers behind the unfruitfulness of the terminal diagnosis, and the propose solutions or preventive measures in traditional ways.

Methodology: The current study is an analytical study on the collected data and reports of the following reliable sources:

- The Population-Based Cancer Registries Data' of the Central and State Governments.
- The Data from National Cancer Registry and Regional Cancer Centers,
- National Family Health Survey of India (NFHS-3), NICPR-National Institute of Cancer Prevention and Research, ICMR-Indian Council of Medical Research, Indo-Asia News Service (IANS), and Zee Media Bureau. The study also utilized the available journals database, along with WHO database. The researcher also accesses to the government's data and hospitals documents on cancer statistics and their reports.

Results: The modern turns out to be a cancer hub and the world largest contributor to cancer mortality rates. The numbers of cancer effected people increases every year, while the government had minimal inputs towards the preventive measures against cancer/terminal illness.

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INTRODUCTION

Looking at the current cancer statistics, it is visible that Indian as a whole fought against the cold-blooded killer 'terminal illness' in the most unsuccessful way. The failures of the health care systems of the country in its terminal diagnosis resulted in making the larger groups of the cancer patients facing the worst ill experiences one had ever gone through in life. India is the world largest contributor to cancer mortality with around 500,000 deaths per year, mainly due to people unawareness of the cancer and its symptoms diagnosis policy. However, no proper preventives measures or actions had not been taken up by the government, though the country is densely populated with terminal illness. Moreover, 80% of the cancer populations in India lives in rural undeveloped areas with low economic status, who were not able to afford for their cancer diagnosis and on the other hand, higher diagnosis fees in India. Thus, undergoes painful terminal ill experiences and live a life of suffering hell without accesses to any hospitals or clinical diagnosis. The Indian Council of Medical Research, New Delhi, predicted that the numbers of terminal deaths in the country would increases significantly in the coming years. Stating by 2020 around 1,700,000 Indian would be diagnosing this cool-blooded killer and its related deadly disease. Moreover, the percentages of cancer patients would likely to be increases by 20% in India, if the government did not take up any immediate quality preventive measures against this cool-blooded killer.

INDIA IN THE BATTLE OF TERMINAL ILL ILLNESS: THE PRESENT SCENARIO'S

In the definitions of World Health Organization (WHO), cancer or terminal illness is the uncontrolled growth of deadly cells with unstoppable spreads, which destroys every portion of human body organ one after another. Once the deadly cells increase and affected the patient's start losing the functioning of his/her body sites that usually leads to a paralyses condition. These deadly cancer cells expanded through invading the surrounding tissues and spreads to other parts of the body by metastasis leading to 8.2 million global deaths a year at present. In which India becomes the world top contributor to cancer mortality rates with around 556,400 terminal deaths per year.[1] The modern India turn out to be a cancer hub with 2.5 million cancer-affected people, which would be expected to increase by 50% in 2020, if no immediate action plan had been done by the governmental and non-governmental agencies. At present a premature death through non-communicable diseases is the leading causes of deaths in India like cardiovascular ailments, chronic respiratory problems, and diabetes. [2] The cervical and stomach cancer symptoms populations in the Indian state of Mizoram alone is equal with the total numbers of cancer affected populations of Japan and is the highest cancer populated region in the world.[3]

The Zee Media Bureau Report, among the 8.8 million global deaths, in which India is consider as the world largest contributor to cancer mortality rates.[4] In another finding, around 505,428 to 500,000 cancer patients in India died per year. The main reason for the high cancer mortality rates in India is mainly due to people unawareness of the treatment procedures of cancer and its symptoms. This being the reason the cancer affected people consulting their Oncologists mostly when they are in the terminal stages, which is in the fourth stage' of cancer symptoms that is impossible to cure.[5] Thus, around 80% of the deaths from cancer's cannot be prevents in India today.

However, consulting their clinicians in the second stages of cancer diagnosis patients could possibly have at least 60%, for cure, while the interventions of cancer diagnosis in its third stages the cure possible rates reduces to 30%, but with the possibility of stage four of cancer within a short time.

However, there are no chances for survivor when it comes to the stage four of cancer that gives a maximum life spends of 5-7 years. Basing on the latest findings of NICPR report, Breast Cancer and Cervix Uteri is the two most common Cancer killers among Indian women, while Lip/Oral Cavity and Lung Cancer becomes the two most common killers among Indian men, killing around 253,521 Indians every year.[6] The last 20 years were the periods where cancer mortality rates has seen rapidly increasing in the country, while the numbers of cancer incidence has been decreasing in many western countries. Out of all the cancer mortalities, the cervical cancer remains the leading cause of deaths among Indian men and women follow by Breast cancer mortality.[7] The following tableshows the five most common deadly cancer symptoms, its causes, and the numbers of terminal deaths in a year in India as a whole (Table 1).

Looking at the current cancer statistics, it is visible that India as a whole is fighting with the cold-blooded killer "Terminal Illness" in the most unsuccessful way. India at present is in critical conditions with rapid increasing rates of one-lakh cancer populations per year and minimal cancer care center. There are around only 300 regional cancer care centers, which is not enough even to treat the one thirds of the cancer populations in the country. Moreover, India today has only 1000 oncologists, which is in the ratio of 1:2000 (one oncologist per two thousand cancer patients). This constituted the underlying reason why modern India turned into a cancer hub. The fighting with cancer will continues as the numbers of affected people raises up every year.[8, 9]

The following statistic is formed to explain in detail about the current cancer status in India (Table 2).

Table 1: The common types of cancers and the numbers of death in a yearly basis. [4-6]

No.	Cancer symptoms	Numbers of death per year	Main causes
1	Gallbladder cancer	230,000	Unhealthy lifestyle, genetic, poor prevention
2	Breast cancer	5,522,000	Unhealthy lifestyle, genetic, alcohol
3	Head & neck cancer	575,000	Alcohol, tobacco, cigarettes
4	Throat cancer	115,200	Poor nutrition, smoking, alcohol, genetic syndromes
5	Lung cancer	22,900	Smoking, tobacco
6	Oral cancer	245,800	Smoking, tobacco, alcohol, hpv, sunlight, weak immune system
7	Cervical cancer	67,477	HPV, birth control pills, smoking, unhealthy sex

Table 2: Current cancer statistic in India. Adapted from NICPR statistical report on August 23, 2018.

No. of Cancer Affected	Yearly Cancer Registration	No. of Deaths In a Year	Common Age's of Death 39-60 Yrs	Women	Men	Percentage
2.5 Million	7 Lakh	556,400	395,400	195,300	200,100	71%

In India, at present, one woman dies of Cervical Cancer every 8 minutes and for every newly diagnosis with breast cancer one out of two women dies in India. Moreover, around 2,500 died per day due to the cancerous factors like tobacco and bitternut. While smoking, which is the most common practices in India cause 1 in 5 death amongst men and 1 in 20 death amongst women that constituted around 930,000 deaths in 2010. In which the most productive age period could be highlights in the ages between 30-60 years, which need a special consideration and these ages grouped need to be targeted the most.[6]

Why cancer/terminal diagnosis unsuccessful in India today?

The main cause of cancer is through the internal factors (inherited mutations, hormones, and immunes conditions) and external/environment factors (tobacco, insufficient diet, unhealthy food, organism, and chemical with radiations). Among all these components for the causes of cancer and its deadly symptoms, there is a closed link between unhealthy food and insufficient diets with cancer disease as observed by many experts.[2] Unhealthy lifestyles with alcohol and smoking are the second most common cancerous factors leading to chronic disease, cardiovascular, lung, kidney, throat, esophagus, and breast cancer. The third common factor for the causes of cancer is visible in excessive consumptions of red meats and salted fish leading to heart and breast cancer.[10] At present as per the National Tobacco Control Programmed, the tobacco related cancer illness like Heart Attack, Lung Diseases, and Stroke etc. were the common types of cancer in the country. The uses of tobacco cause 100% poor oral health with 90% of mouth cancer, 80% of lung cancer, 50% of all human cancers, 70% of lung diseases, and 60% of heart attacks.[11] The above mention cancerous factors were the most common practices in the Indian Sub-Continent at present, leading to the rapid growth of cancer populations in the country.

Another reason for India being the top cancer populated in the country is mainly due to people unawareness of cancerous components, its preventive measures, and the treatment policy. Due to the lack of awareness, majority of the cancer effected patients detected their cancer symptoms only when the physical pain becomes unbearable, which is in the terminal stages (third or fourth stages) that is impossible to cure. Late detection of cancer symptoms and late cancer diagnosis becomes the underlying reasons for the 80% of the cancer population failing to cure in India today. However, early detections of the symptoms in its first stage there is 80% chances to cure. On the other hand, undergoing cancer diagnosis in the second stages could possibly have 60%, chances for cure, and in the third stage the cure possibility rates reduce to 30%, but with the possibility of stage four of cancer within a short period of time. However, in the fourth stage, which is the terminal stage there is no chances to cure, rather to live with the painful symptoms for maximum life spends of 5-7 years.[9]

The general causes of stomach and cervical cancer symptoms is due to the excessive drinking of alcohol, cigarette smoking, and chewing bitternuts. While, lung and oral cancer that hugely affect the Indian men and women populations, is mainly due to the excessive uses of tobacco, smoking, hookah, and the consumption of local made alcohol. Another prevailing cancer symptom in India today is the nasopharyngeal cancer, which is a malignant cells disease form in the tissues of the nasopharynx. The nasopharyngeal cancer (NPC) is the rarest type of cancer around the world; accept in the South East Asia, North Africa, and Arctic. The NPC is claims to be a Chinese origin, which is largely affecting the North East Indian states of Nagaland, Manipur, Mizoram, and to some extend to Meghalaya. The state of Nagaland has the highest age adjusted with 19.5/100,000, followed by the state of Manipur. The nasopharyngeal cancer is mainly causing by excessive eating of meat, fish, salted fish, uses of firewood in the house and other environmental related factors like the eating of bitternuts (Kuwa or Komkuwa) with or without tobacco products. Mostly the women populations were mainly affected by nasopharyngeal cancer comparing to men. Moreover, within 15-20 years the nasopharyngeal affected women populations will be increasing, if the uses of tobacco, bitternuts, and the uses of firewood in cooking are not being under-controlled.[3, 10] The table below will clearly show the various factors that are responsible for the causes of cancer as a whole (Table 3).

Table 3: The cancerous factors in India as a whole.

Particles	Cultivations	Liquids Components	Environment	Components
polycyclic aromatic hydrocarbures pahs	metals or coated with metals	diesel exhaust particles	toxin from fungi	smoking & hookahs'
vinyl chloride and benzedrine	excessive meats	unhealthy drinking water	chewing of bitternuts	junk food

Apart from food habits, the population explosion, rapid industrialization, and genetics, which include mutations, hormonal and lack of immunity are also responsible for the rapid growth of terminal illness in India as a whole. If proper awareness had not given mainly to the rural undeveloped areas of the country, there is a possibility of increasing cancer populations to 19% in the next five years. In which women have more chances of affected with cancers than men in India, as per the findings of many. Moreover, the mortality rate of cancers in India is visible higher among the illiterate people groupin the rural areas of the country than the educated, and the maximum deaths in India are mainly due to poor prevention strategies and no proper diagnosis as a whole [13]

According to the latest NICRP reports, 122,844 women are diagnoses with cervical cancer every year out of which 67,477 women died from cervical cancer per year. In a population of 432.2 million women in India at present, those women who are at the age of 15 and above and or between 15- 40 years are at risk of developing cervical cancer.[14] The health scientists on the other hand, were not able to identify the processes of how the risk factors like genetic, hormonal, and environment factors works together to cause normal cells to become cancerous tumor for the cancer symptoms. [10] Thus, cancer becomes the leading causes of deaths in India, with 2.5 million cancer populations, with 1 million cancer patients added every single year. It could be predicting that the numbers of cancer patients by 2025 would be increases by five-fold in India. The rapid increasing rates of cancer in the country are mainly due to lifestyle risk factors like uses of tobacco, alcohol, low fiber in diet, increasing body weight, minimal physical activities, and the reproductive risk factors regarding age at first pregnancy, and higher numbers of children breastfeeding's. Out of many cancer mortalities, the two leading deaths are from cervical cancer (HPV), hepatitis C (liver), and gastric H. pylori (stomach).[15]

THE PROPOSE SOLUTIONS AND PREVENTIVE MEASURES

Terminal death is the top causes of Death in India, in the year 2000 India was in seventh position among the world cancer populated country. However, due to the rapid increasing of cancer populations from 2006-2018, India successfully stood as the highest contributor to world terminal fertility rates, in which the numbers of effected populations is currently visible increasing with 100,000 per year. In recent research finding, only 5-10% the cancer is from genetics, in which the other 90-95% of the cancer and its related deadly diseases are from hormonal and environment factors.[16] Which means, the 90% of the cancer and its related diseases can be prevented effectively through proper medical interventions in its early stages and hygienic lifestyle. Moreover, another 10% genetic cancerous symptoms can also be prevented through early detection and immediate diagnosis. In short, cancer of any types is curable if detected in its early stages and through proper diagnosis. The followings are the qualitative preventive measures in order to control the rapid growths of cancer in the country: [2, 10, 16]

1. Regular medical checked-up, though being in a healthy condition, negligees of regular medical checked-up is visible as the underlying reason for the rapid growths of cancer in the country for the past ten years.

2. Educating people with proper awareness strategies, offer effective public health concerned in the schools and organizing a social meeting for spreading cancer awareness. Especially in the rural areas of the country.

3. Early detection of the symptom and early medical intervention, because when it comes to third and fourth stages there is no possibility for cure.

4. Public awareness on active physical activities, exercise at least for 30 minutes per day, the healthier a person is, the cells in the body can effectively fight against the invader virus and bacteria. Regular exercise prevents oneself from colon and breast cancer too.

5. Minimizing the uses of alcohol products, smoking, tobacco, and chewing of bitternut products, which will minimize the risk of having lung, kidney, throat, esophagus, and breast cancer. It is better to develop a moderate way of consuming with 2-3 glass of alcohol products per day, which will keep oneself away from heart attacked.

6. Immunization against hepatitis B virus to the infants of one to sixth months old without failed. Neglecting medical treatment in hepatitis B & C can causes chronic illness and liver cancer.

7. Developing healthy and safe sexual practices to avoid cancer genesis, unhealthy sexual practices can give birth to cancerous cells.

8. Avoiding obesities, as being grossly fat or overweight have negative effects on health and the makes cells less effective.

9. Developing healthy diets, a healthy diet has scientifically proven with numerous health benefits and reducing the risk from chronic diseases.

10. Reducing occupational and environmental exposures, as excessive exposures to chemical and its related heavy metals produces ill health. It also effected for the future offspring and produces toxicity.

11. Avoiding excessive consumption of red meats, salt and long preserved food. High consumptions of red meat, salt and preserved food leads to diabetes, breast cancer, and obesity.

12. Developing the habits of eating fruits regularly, most fruits are less in calories, sodium, and fats. Fruits are the sources of essential nutrients like potassium, dietary fibers, vitamin C, and folate (folic acid), which prevents deficiency, birth defect, and helps a person to growth with healthy blood pressure.

13. In order to have fair skins some avoid exposing to sunlight, wearing protective clothing during 10 am-4 pm, in which the excessive uses of sunscreen cream need to be avoid. Avoiding of exposing to sun or UV resulted in many women diagnoses with skin cancer. On the other hand, excessive exposing to sunlight or UV ray is also dangerous, especially for those having genetic cancerous symptoms.

14. Immediate medical intervention on virus and bacterial infections, otherwise the bacteria diseases virus cells usually hide inside the cells and turning out to be a terminal virus

Moreover, the rapid increasing of industrializations and urbanizations are visible as the two factors leading to new lifestyle of many Indians, which resulting in increasing the cancer affected populations in the country. Concerning the current polluted environment, the burden of cancer incidences will gradually keep on increasing, as majorities of the Indians were not aware of cancerous preventive measures. The only ways to prevent the Indian men and women from the rapid growth of cancer and its deadly symptoms is to detect the symptoms at the early stages with immediate medical interventions. It is also important to prevent the water and the environment from polluted by industries and chemicals. However, it can only be possible only if the men and women in the rural areas were being educated on cancer awareness like preventive measures, and overall treatment policies, which needs multiple efforts from the government agencies and other non-governmental agencies. Through such awareness programs, men and women should realize the risk factor and to identify the symptom through screening by physical examination or by self-cancerous symptom examination, in which if the rick substances where found than certain carcinogenic substances need to be reduces or eliminated. Moreover, there are over 85,000 synthetic chemicals that were easily available in the market of the country today like cosmetic items to flame-retardants, plasticizers in water bottles to pesticides in fruits and vegetables' etc.[13, 16] In the findings of the researcher, 80% of the cancer patients in the country were associated with environment factors like exposes to contaminants, unhealthy lifestyle, food, and exposing to ionizing radiations. In the urban areas, the cleaning of contaminated drainages without any proper preventive measures, polluting the river to the maximum with many forms of chemical and eating those fishes from the river produces several cancerous symptoms. The using the polluted water from the factories or industries for agricultural farming also produces several cancerous components leading in rapid increasing of cancer populations through consuming theses agricultural products. Thus, maintaining healthy environment, healthy lifestyle, healthy food, proper diets, daily exercise, staying away from tobacco, and smoking, with decreasing alcohol consumptions can decreases the rates of cancer-affected populations. Moreover, in the rural villages the practice of using of those water which were uses in deeping hot metal or iron in the blacksmithfor washing hand and leg need to be avoided, as it contains the component that is cancerous.

CANCER TREATMENT POLICY AND TERMINAL DIAGNOSIS

The WHO (2017) stated that, the cancer patients need immediate access to the modern equipments like: 3D Conformal Radiation Therapy, Intensely Modulated and Therapy (IMRT), Image Guided Radiation (IGRT), VMAT and Rapid Arc-Volumetric Modulated Arc Therapy (VMAT), Low Dose Rate Brachyntherapy (LDR), High Dose Rate Brachyntherapy (HRD), Deep Inspiration Breath Hold (using the goggles or snorkel technique), and Stereotactic Radiation Therapy.[17] However, it is sad to say that, due to the unavailabilities of the above mention modern cancer treatment equipments, cancer patients in India could not undergo such treatments. Failing the interventions of modern equipments in terminal diagnosis, resulted in uneffective diagnosis in many cases. The quality treatment plan and policy are the core components for successful cancer metastasis diagnosis, which is also visible ineffective to the minimal. The main emphasis of a cancer diagnosis is to cure the symptom of the patient or to prolong the lives' of the patient through ensuring quality of life.[1] However, the greatest challenges in the Indian cancer diagnosis is the ensuring of the patient quality of life, which is not visible in the clinical practices of the country as a whole. Unnecessary prolonging of the patient lives with an aim to increases the numbers of days spend in the hospital ward for more bills. The patient's value and dignity were unconcern the most by the clinicians' and the patient undergoing treatment against their will/choices need to be rectifying immediately in the health care systems of the country as a whole.

The early detection and immediate treatment intervention are the two most effective way of cancer diagnosis to control the cancer metastasis and to deliver total cure. As per the above findings, the medical intervention in stages one and two has higher possibilities rates to cure, which has around 80% cure possibilities as per the finding. While detecting the cancer symptom and consulting the oncologists in stage three is visible in curtail conditions, having higher impossibility rates as the cancer metastasis accelerates in higher speeds from one body part to another. However, if the stage three patient being diagnosis by special medical team in a well to do clinical setting, it has around 60% possibility chances to cure. But does not guarantee a complete free from cancerous cells, as there is a possibility of being in terminal stage after three to five months. The reason being the cancer cells have the possibility of hiding under another cells that is hard to detect, resulting in leading one's life a disability-adjusted life years (DALY). Nevertheless, the detection of the cancer symptoms in the fourth stage have no possible way to cure by any means, which is term as the 'terminal stage' that is 100% impossible to cure in any clinical practices. Thus, resulting in leaving the patient with disability-adjusted life for five to seven years until the inevitable death strikes him/her.

These 5-7 years period of terminal experience is the most crucial moment for every terminal patients, which is also considers as the worst moment in a terminal experience with heartful of emotional sufferings and mindful of mental disharmony that needs special considerations to the most. The acknowledgment of the psycho-emotional symptoms alongside the treatments of physical pain is very essential in any terminal diagnosis in the clinical practices. However, at present India turns out to be the worst place to die or a place not to die. The reasons being the undergoing cancer/ terminal diagnosis core emphasis is on the physical pain treatment alone, leaving the psycho-emotional pain and suffering untreated. The psychological suffering and mental disharmony are the bi-products of the terminal illness undergoes by every terminal patients were considered the symptoms that need to be treated in the clinical practices in India. Thus, failing to acknowledge and leaving the psycho-emotional symptoms untreated resulted in worsening the patient's conditions. The psycho-emotional and mental well-being is for the successful treatment of the patient physical pain symptoms. (Suantak & Sisodia, 2018). Well being of the whole body requires a psycho-emotional and mental well-being to the most; a psychological well-being can give positive responses to physical pain treatment to the maximum.

In India as a whole, the clinicians tend to forgot that even when cure is not possible in terminal diagnosis, there is a possibility of delivering healing as an alternative to cure. However, only through a person centered meaning making psychotherapy in the clinical practices. The psychological self-reflective and life review therapeutic approach in terminal diagnosis can make oneself aware that he/she is still in the condition of limitless achievements. Which will in turn helps the patient to recreate his/her life goals, and set new life goal that could be achievable, also able to make beautiful memories with his/her loved ones that delivers the quality of life in terminal experience. Above all, it will make dying as normal as birth, which will deliver peaceful and meaningful death (Suantak & Sisodia, 2018). Thus, in terminal diagnosis, healing can be deliver as an alternative to cure, even when total cure is impossible in the clinical practices. However, sadly, in the clinical practices of India as a whole, the clinicians alone are the core medical team in the cancer/terminal diagnosis, in the absences of professional clinical psychologists and clinical social workers. The terminal diagnosis requires a multidisciplinary team to deliver quality of life and quality end-of-life care. The clinicians were responsible for treated the physical pain symptoms and its related, while the clinical psychologists and clinical social workers were responsible for the patient psycho-emotional suffering and mental disharmony treatments, which the trained medical doctors or nurses cannot handles in the clinical practices.

CHALLENGES AND CONCLUSION

Recently, Prime Minister Narendra Modi inaugurate "Ayushman Bharat Health Scheme" on September 23, 2018, which would be effective by 25 December 2018 in Ranchi with a primary aims to provide free health care to those underprivileged living in the rural areas, who are access not to any health care thus far. It means there are Indian who does not received any medical facilities thus far, which remain as the most challenging and root causes to maximum cancer/terminal illness in the country. As per the findings, 500,000 Indian died of cancer every single day and the government with the least concerned on the awareness programe and in establishing regional cancer care centers. This being the underlying reasons India successfully becomes the largest contributor to cancer mortality rate. Another reason for the rapid increasing mainly rates of cancer and its mortality rates is mainly due to people unawareness on the causes of cancerous factors, treatment policy, unhealthy lifestyles, and unhealthy food consumptions with no proper preventive measure interventions. Majority of the cancer patient in rural regions failed to maintain their diets, while proper dietary is the core to successful diagnosis. Moreover, eliminating the usages of tobacco, cigarettes and chewing of bitternuts can successfully reduces the rates of oral lip, breast, mouth, cervical, head and neck, and the nasopharyngeal (NPC) cancer into at least 40-50% as per the researcher findings. Another urgent need is to develop social awareness on the causes of cancer, and treatment policies by educating the people mainly those leaving in the rural areas of the country. Gearing up for health awareness and strengthening the health care team at the community level by addressing the cancer preventive measures will effectively reduces the growing cancer populations.

Hygienic living with healthy food awareness also the urgent requirement to fight against the deadly diseases, with the appointed staff, conducting seminar in each village at least once in a year. Training more oncologists in the country would also be an effective ways to successfully fight against the ongoing cancer mortality in the country. However, as of now the ratio of oncologists and cancer patients in the country is 1:2,000, and that is next to impossible for an oncologist treating 2,000 patients in a day. The Centre for Cancer Epidemiology in the rural areas of the country is at present minimal and majority of the cancer care centres are not functioning properly, whichis the major challenges for the government to take immidiate initial action. It is a high time for India to take up the necessary actions against cancer mortality as the rates of mortality increased year after years with 70-90% per 100,000 populations, which constituted around 2,500,000 (2.5 millions) with 800,000 new cases every year and 5,50,000 deaths in a year. Moreover, in the battle of terminal illness fighting back and defending people is the only option that India had. For the love of humanity, let the terminal diagnosis acknowledge the psycho-emotional suffering and mental disharmony of the patient alongside the physical pain symptoms to deliver the whole person treatment in the clinical practices.

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Women's facial attractiveness nowadays – results from analysis of public figures

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Abstract: *Aim. To identify the main facial features associated to facial attractiveness in women nowadays.*

Method. A descriptive study was conducted on a sample of 20 female public figures, very famous and successful women, most frequently actresses, singers or models, considered very beautiful nowadays. Their selection followed the analysis of several recent tops that aimed identifying most beautiful women, in which raters were lay persons. Data was collected by analyzing photos from frontal and lateral view, during rest position and smiling.

Results. The persons analyzed registered diverse facial traits. Most obvious trends, encountered in more than 75% of them, included: Caucasian race; straight and long hair; average nasal tip protrusion; maxillary dental midline concordant to facial midline; a thicker lower lip compared to the upper lip; exposure of only maxillary incisors during smile; decreased or absent buccal corridor; maxillary central incisors of oval/rectangular shape; absence of maxillary teeth crowding. Other frequently encountered features were: American nationality; brown hair; eyes with medium size, almond shape, wide set, of green color; nose with an average width; face with a decreased lower third and a slightly convex profile; a thick lower lip and an average upper lip; exposure during smiling till the first molar, with a normal smile line.

Conclusions. The public beautiful women nowadays register features that are generally associated to an increased facial attractiveness. Knowledge of trends in facial beauty is useful in dental practice, in order to understand better patient's need in terms of esthetic expectations.

Keywords*: esthetics; beautiful; dentistry*

INTRODUCTION

Nowadays, in the modern society physical appearance and facial esthetics play an important part in communication in everyday life, which associated changes of patients' perception on various aspects related to them, including dental treatments. Exposure to mass media (television, internet, movies) influenced perception of human attractiveness, which registered in time changes, some of the considerable. Consequently, more and more in the dental field preoccupations that target providing esthetic outcomes can be observed. In this context, the dentist must know and understand what is considered attractive nowadays, in order to

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explain to the patient what are the possibilities and limits of the interventions provided by him. The diversity of clinical situation require dental treatment individualization, and a specific plan in order to enhance facial attractiveness. Considering beauty has a biological inherent dimension, each treatment should be adapted according to ones features. One particular intervention that had a good outcome from an esthetic point of view in one person, does not necessary have a similar good outcome in another.

The aim of this study was to identify the main facial features associated to facial attractiveness in women nowadays.

METHOD

A descriptive study was conducted on a sample of female public figures, very famous and successful women, most frequently actresses, singers or models, considered very beautiful nowadays. Their selection followed the analysis of several recent tops that aimed identifying most beautiful women nowadays, in which raters were lay persons that voted online, of different ages and nationality. In the study sample was targeted to be included 20 women, most frequently encountered in these tops, as being considered very beautiful and attractive.

Data upon facial characteristics was collected by analyzing photos from frontal and lateral view, during rest position and smiling. Considering the inability to personally examine the subjects, an extensive search was conducted in order to identify as many photos as possible. Two persons evaluated each person independently, and for discrepancies between them consensus was achieved together with a third evaluator afterword. Study variables included personal data (age, sex, nationality, race), general features of the face, hair, eyes and nose, lips, teeth, some registered during rest or smile position.

Data on medical interventions with impact on face attractiveness was also searched, main being triggered two directions – dental treatment (e.g, prosthetic restorations, orthodontic treatment, tooth whitening) and plastic surgery or other interventions to the face, nose or lips. Data was collected through photo inspection aided by metric and angular measurements performed in Adobe Photoshop software.

Data analysis was mainly based on descriptive statistics, targeting describing facial attractiveness patterns by describing most frequent encountered characteristics in females considered very beautiful and attractive. Microsoft Excel and SPSS (Statistical Package for the Social Sciences) were used.

RESULTS

The persons analyzed registered diverse facial traits. Of the 20 persons, for one was not found any picture while smiling. Most obvious trends, encountered in more than 75% of them, included the following: Caucasian race; straight and long hair; average nasal tip protrusion; maxillary dental midline concordant to facial midline; a thicker lower lip compared to the upper lip; exposure of only maxillary incisors during smile; decreased or absent buccal corridor; maxillary central incisors of oval/rectangular shape; absence of maxillary teeth crowding; presence of teeth whitening.

Figure 1: Aspect of the lips of the women analyzed

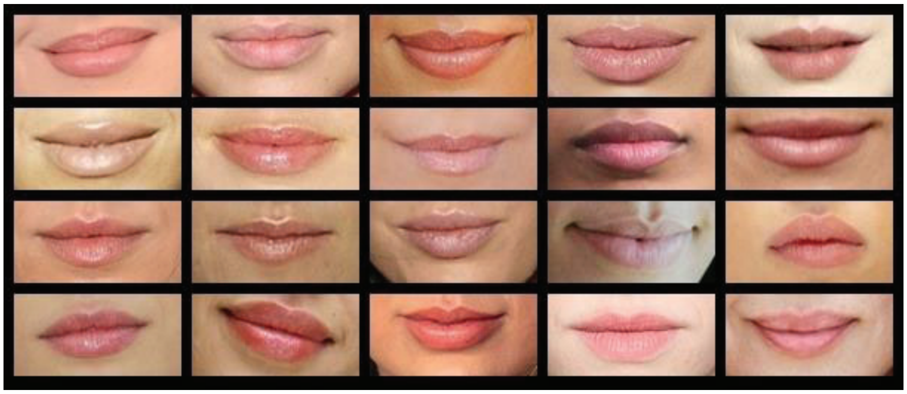

Other frequently encountered features, found in more than 50% of the analyzed persons, were: American nationality; brown hair; eyes with medium size, almond shape, wide set, of green color; nose with an average width; face with a decreased lower third and a slightly convex profile; a thick lower lip and an average upper lip; exposure during smiling till the first molar, with a normal smile line (Table 1, Figure 1 and 2).

Table 1: General and facial characteristics of the beautiful women analyzed

Age (years)	mean: 31; median: 29.5; range: 24-49
Nationality	American (n=11); Indian (n=2); English (n=2); Ukrainian (n=2)
Race	Caucasian (n=18); African-American (n=2)
Hair	color: brown (n=11); blond (n=8); brunette (n=1) aspect: straight (n=16); curly (n=4) length: long (n=17); short (n=3)
Eyes	size: medium (n=13); big (n=4); small (n=3) shape: almond eyes (n=10) position: wide set eyes (n=11); average set eyes (n=7); close set eyes (n=2) color: green (n=10); brown (n=6); blue (n=4)
Nose	nose width: average (n=12); narrow (n=5); wide (n=3) nasal tip protrusion: average (n=15); protruded (n=4); retruded (n=1) nasolabial angle: increased (n=12); medium (n=6); decreased (n=2)
Face shape	pentagon (n=7); oval (n=6); square (n=3); round (n=2); rectangular (n=2)
Dental midline to facial midline	concordant (n=17); slightly deviated (n=2)
Vertical facial proportion	decreased lower third of the face (n=12); similarity of lower and middle third (n=5); increased lower third (n=3)
Profile	slightly convex (n=14); straight (n=6)
Lips	upper lip volume average (n=12); thick (n=7); thin (n=1) lower lip volume: thick (n=13); thin (n=7) comparative evaluation: thicker lower lip (n=18); thicker upper lip (n=2)
Tooth exposure while smiling	only maxillary teeth (n=16); maxillary and mandibular teeth (n=3) till the first molar (n=10), second premolars (n=8), canines (n=1) normal exposure of maxillary central incisors/smile line (n=13); lower exposure of maxillary central incisors (n=3); gummy smile (n=4)
Buccal corridor	decreased or absent (n=14)
Central maxillary incisors	form: oval/rectangular (n=15); triangular (n=4) size: average (n=15); bigger (n=4)
Maxillary teeth crowding	absent (n=16); mild (n=3)
Interventions with impact on facial esthetics	dental prosthesis (n=2); teeth whitening (n=14); orthodontic treatment (n=2); interventions to the nose (n=3); interventions to the chin (n=1)

DISCUSSION

This study's results, by the high percentages observed in many facial traits, suggest that in the public opinion there is a facial pattern of female attractiveness. Some of them (e.g. facial symmetry, normal smile line) are in accordance to general norm that define a beautiful face.

In this study the majority of the persons were of American nationality and Caucasians. This may be primarily linked to the way study sample was formed, more precisely of actresses, singers and models. The American movie and music industries are very well developed and mediatized worldwide, through this probably influencing lay person perception of facial attractiveness.

The age of the persons included in this study sample was variable, from 29 to 49 years old. In this regard, should be noticed that it can be said that beautiful and attractive women in current society does not equal young women, but also middle-aged ones.

Figure 2: Aspect of the smile of the women analyzed

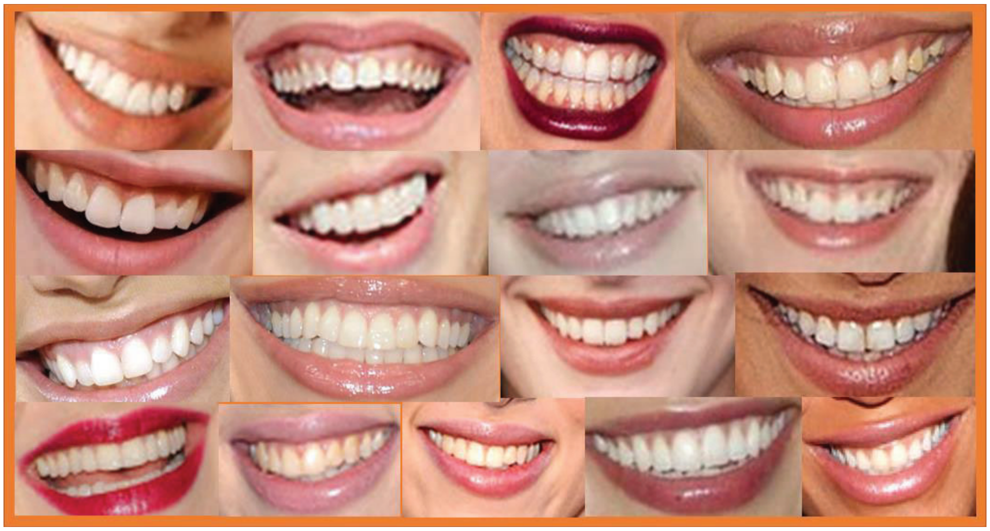

Oval and pentagonal face forms are generally considered as being most attractive form face [1], these being the most frequently observed in this study sample. Also, symmetry is another aspect that is known to influence facial attractiveness [2], which is also supported by the results of this study. In our sample of women considered very attractive there were found most frequently persons with a reduced lower facial third, and very few with an increased lower facial third, results that are supported by the findings of numerous studies [3].

Lips are considered to play an important role in facial attractiveness, one important parameter being lips' thickness [4,5]. In females analyzed in this study were generally found to have thick lips, most frequently the lower lip being thicker than the upper lip. Generally an equal thickness of upper and lower lip is perceived as unaesthetic, this situation not being encountered in this study.

Smile is considered as greatly influencing facial attractiveness, dental treatments being often solicited for remediating some problems as perceived by patients, therefore an accurate evaluation of it being recommended [6]. Aspects as color of teeth and gingival display are well known to play an important role in smile appearance [7,8]. This aspect is confirmed by this study results as most persons had normal smile line, and an important part tooth whitening. Also, most of the persons presented an increased smile width and a decreased or absent buccal corridor, which is confirmed by some studies to be related to an increased facial attractiveness [9,10].

An attractive facial profile includes often, beside ones natural traits, also artificial improvements through interventions, some medical, as the dental ones. In this study was tried to identify medical interventions made in order to increase facial esthetics. This aspect is difficult to analyze but, even so, by comparing nowadays to previous photos, there were identified several dental interventions, namely presence of fixed prosthetic restoration, tooth whitening and orthodontic treatment, for the previous one pictures of the persons or/and their own statements being used. Considering that, it is important for the dentists of various specialty (e.g., orthodontists, prosthodontist) to know and understand lay persons' perception on facial attractiveness, in order to deliver most appropriate interventions, in accordance to patient's need and expectations [11-13].

Among study limitation can be mentioned the method of data collection, considering the difficulties in finding adequate photos for a correct analysis and difficulties in assessing prior intervention made to enhance facial esthetics. Even so, there were mainly registered variables that were relatively easy to be assessed. Also, another limitation is the relative small sample size, considering the numerous beautiful women that are public figures nowadays.

CONCLUSIONS

The public beautiful women nowadays register features that are generally associated to an increased facial attractiveness. Knowledge of trends in facial beauty is useful in dental practice, in order to understand better patient's need in terms of esthetic expectations, and for adapting to his practice by including newest treatment that aim improving esthetics. The dentist can also use example of public beautiful women in order to explain and argument his recommendations regarding certain treatment conduct, from an esthetic point of view.

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The authors reported no conflicts of interests related to this paper's information and also reported equal contribution to this paper elaboration.

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VARIA

Work-related stress in medical personnel: A new instrument for screening and evidence-based recommendations for therapeutic management

Octavian Vasiliu[1]

Abstract: *Medical personnel is constantly exposed to a variety of stressful events, starting from work overloading, to threats to one's own health or personal integrity, disruptions in personal relationships due to high stress in the professional environment, continuous need to keep in touch with the advances of medical science and therapeutic guidelines etc. Structured evaluation of work-related stress disorders (WRSD) is important both for screening purposes in a vulnerable population like the medical personnel, and for the monitoring of the overall status evolution during individual or organizational interventions. Beside burnout syndrome, major depressive episodes, anxiety disorders, acute stress reactions, posttraumatic stress reactions, substance use disorders, adjustment disorders are also included in the category of WRSD. A specific instrument has been designed for the purpose of screening for WRSD in this specific population, i.e. the Work-related Stress Inventory for Medical Personnel, structured as a 20 items, self-administered instrument (WRSIMP-20). This inventory evaluates three main dimensions- (1) mood symptoms and somatic equivalents, (2) work and professional environment-related discomfort, and (3) interpersonal relationship-associated distress. The results of online administration of WRSIMP-20 in 81 health care workers and 52 individuals not related in any way to health care professions (control group) were consistent with the premises, as this inventory has been proven valid for the targeted population. Larger trials and test-retest evaluation are needed in order to complete the psychometric properties of WRSIMP-20, but this instrument seems promising in the detection of individuals vulnerable to WRSD.*

Keywords: *work-related stress, burnout, major depression, adjustment disorders, therapeutic management, medical personnel*

BACKGROUND

People working in the health care domain are facing a large spectrum of stressful situations and they are considered at significant risk for developing work-related stress disorders (WRSD) (Figure 1). Extensive workload, long shifts, night shifts, emotionally demanding professional roles, the need for quick decisions, and informational overload are several factors that may trigger a burnout syndrome [1].

Burnout is considered a psychological syndrome that may appear as a consequence of chronic interpersonal stressors in the working milieu, and it has three dimensions – (a) overwhelming exhaustion, (b) feelings of cynicism and detachment from the job, and (c) a sense of lack of efficacy or accomplishment [2,3]. Exhaustion is defined as the feeling of not being able to offer any more of oneself emotionally, while cynicism is conceived as a distant attitude towards work, the people being served, and/or the workplace colleagues; inefficacy is considered to be the feeling of not performing tasks adequately [4].

Although this pathology is perceived as unitary, there are a few different types of burnout syndrome: frenetic (highly applied and committed individuals), under-challenged (individuals who have no interest in their work and are superficial in their job), and worn-out (individuals whose level of involvement in their work is diminished, and they disregard the responsibilities of their position) [4,5]. In order to accurately detect these types of burnout, a range of psychometric instruments have been constructed [4,5].

According to the results of a meta-analysis (n=25 studies) the most relevant associations were between the feelings of work control and reduced emotional exhaustion, as well as between the low workplace support and increased emotional exhaustion [6]. Limited evidence was found for

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the correlations between workplace rights, demands, high work load, low reward, low supervisor support, low co-worker support, job insecurity and change in emotional exhaustion [6]. Also, cynicism was associated with most of these work-related factors, and reduced personal accomplishment was strongly related to low reward [6]. A higher vulnerability to burnout was detected in people with perfectionistic features or self-criticism, who sleep less, and in those with high tendency to work overtime, to assume more responsibilities, and without a group of support (i.e. family, friends) [7,8].

Figure 1: Work-related stress disorders in medical personnel

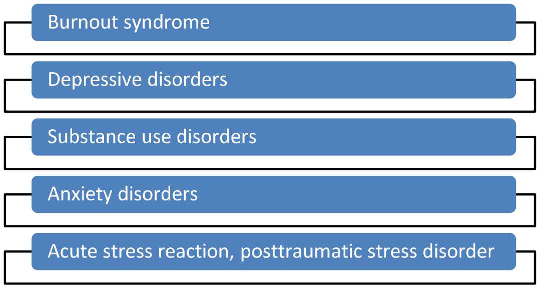

The prevalence of the burnout syndrome is significant, although this pathology is rather under-diagnosed, with estimates of every one in three physicians experiencing burnout at any given time [9]. Other data suggest higher rates, somewhere between 39.8% and 46%, based on lifestyle surveys in physicians [10]. The medical consequences of the burnout diagnosis are significant: this pathology was found to be a risk factor for myocardial infarction and coronary heart disease, it may decrease fibrinolytic capacity, reduces the ability to cope with stress and the hypothalamic-pituitary-adrenal (HPA) axis functionality [11]. Burned-out physicians and nurses have also high risk for psychiatric and psychologic complications, e.g. substance abuse, suicide ideation, poorer quality of life, or unsatisfying relationships, while the patients tend to be less satisfied with services offered by burned-out medical personnel [12,13].

According to a systematic review (n=182 studies, N=109628 individuals) burnout syndrome incidence was evaluated during an 18-year interval (between 1991 and 2018) in physicians [14]. Overall burnout was reported in 85.7% of these individuals, emotional exhaustion in 72%, depersonalization in 68%, and low personal accomplishment in 63.2% [14]. Because of inconsistencies in definitions of burnout syndrome, and in the assessment methods across studies, there were many differences in the reported incidence, and associations between demographic variables and burnout could not be determined, either [14].

Five main causes of burnout syndrome in physicians have been described: the clinical practice of medicine (the intrinsic stress of the profession); the specific work requirements (the configuration of certain stressors related to the professional environment); personal life and the interference between job-related and family-related tasks; the conditioning of the medical education; the leadership skills of the immediate supervisors (more exactly, the lack of such skills) [10].

Mental health of physicians exerts a significant influence on the quality of patient care [15], therefore, beside burnout syndrome, other frequently reported psychiatric disorders related to professional stress need to be explored. The estimated prevalence rate for depression among physicians in Germany was between 6 and 13%, but anxiety, suicidal tendencies and substance abuse were also present in this population [15]. In another study, health care workers in Turkey (N=221) had a significant level of anxiety (according to the Hospital Anxiety and Depression Scale) in almost 40% of the cases, with anxiety and depression scores of nurses and medical secretaries being significantly higher than the physicians [16]. Emotional exhaustion and depersonalization (evaluated on Maslach Burnout Inventory) scores were the highest in nurses, followed by medical secretaries and physicians [16].

Posttraumatic stress disorder was found in 19% of cases in a sample of physicians and nurses (N=783 participants) working in emergency department during COVID-19 pandemic [17]. The rate of probable posttraumatic stress syndrome in physicians was higher than in nurses, 22.9% vs. 15.2% [17]. High anxiety level, being diagnosed with COVID-19, high depression level, female gender, and having additional chronic disease were predictors of higher posttraumatc stress disorder symptom severity in physicians [17]. In nurses, high anxiety level, being diagnosed with COVID-19, working in 24 hours shifts, high depression level, low work experience (in years), low monthly income, and the presence of additional chronic diseases were correlated with higher posttraumatic stress disorder severity [17].

ASSESSMENT TECHNIQUES FOR BURNOUT IN PHYSICIANS

The Maslach Burnout Inventory (MBI) is a 22-item instrument currently considered the “golden standard” for assessing burnout but several critiques include its proprietary nature, lack of clear cut-offs for negative outcomes, and the fact that its 3 dimensions did not correlate with specific therapeutic interventions [18,19].

The Tedium Measure involves determination of several clusters of symptoms, like the experience of physical, emotional, and mental exhaustion, defined by negative perceptions over one's self, one's environment, one's work, and one's life [20,21]. Tedium is considered a continuous variable, but only one score is available [20]. This instrument may be suitable for assessing burnout in an organization or system, and its scores correlate with all subscales on the MBI, and with health problems and job satisfaction [20].

The Staff Burnout Scale for Health Professionals (SBS-HP) is a 20-item inventory assessing cognitive, affective, behavioral, and psychophysiological reactions that comprise the burnout syndrome [22]. The SBS-HP data significantly correlated with measures of job turnover, absenteeism, tardiness, discipline, prescription drug use, and personal illness [22].

The Burnout Measure (BM) includes 21 items, evaluated on 7-point frequency scales, assessing the level of personal physical, emotional, and mental exhaustion [23]. A factorial analysis of the BM found 3 main dimensions- "demoralization", "exhaustion", and "loss of motive", and several critics consider this instrument is rather a measure of general well-being [24]. BM also has a shorter, 10-item version (BMS), with attested validity and reliability, easiness of use and high face validity [23].

Copenhagen Burnout Inventory (CBI) is a 19-item survey which covers 3 areas- personal, referring the degree of physical and psychological fatigue and exhaustion; work, encompassing the level of physical and psychological fatigue and exhaustion related to work; client/patient-related burnout [25]. According to a prospective study (N=1914 employees in the human service sector), CBI predicted future sickness absence, sleep problems, use of pain-killers, and intention to quit [25].

Oldenburg Burnout Inventory is a 16-item survey, with positively and negatively framed items to assess two core dimensions of burnout- exhaustion (a consequence of intensive physical, cognitive, and affective strain) and disengagement from work (negative attitudes toward work, with distancing oneself from one's work object, work content, or work in general) [26].

Research dedicated to finding relevant causes for physicians' distress has been increasingly developed in recent years. Studies focused on interventions for improving trainee and physician wellness have identified several burned-out management strategies, e.g. organizational-oriented approaches and individual-targeted interventions [27]. Medical personnel wellness is a shared responsibility between doctors, nurses, other health care personnel, health care organizations, and governing bodies [27]. Participants in a survey in Canada (N=22 faculty physicians) described that professional values and organizational norms negatively affected work-life balance, personal and professional fulfillment, triggering feelings of dehumanization, and discouraged disclosure of struggles [28]. Feelings of dehumanization exacerbate burnout and may explain physicians' resistance to resilience-building strategies [28]. The importance of an institutional commitment to create a culture of compassion for patients and physicians alike is underlined by several authors [28].

Due to the impact of burnout in daily practice in physicians, the heterogenous validity of different existing instruments for the evaluation of this syndrom's severity, and to the limitation of these instruments to only one WRSD, new questionnaires for a more comprehensive quantification of work-related stress in this population are needed. In the context of COVID-19 pandemic, the necessity for such more complex investigational methods became obvious.

BURNOUT IN MEDICAL PERSONNEL DURING COVID-19 PANDEMIC

The COVID-19 pandemic increased the workload in medical personnel and the risk of burnout, therefore active screening methods and early intervention strategies should be implemented in this population. According to a cross-sectional survey of Canadian physicians (the COPING survey) 34.6% of the respondents (N=107 out of 309) were positive for burnout syndrome [29]. The primary outcome in this survey was the MBI score, and it correlated with risk perception, worklife subscales and adequacy, measured on the Pandemic Experiences and Perception Scale (PEPS) [29]. Cynicism was significantly associated with exhaustion and worklife, while efficacy correlated with cynicism, worklife, and active cases [29]. Contact with coronavirus, preparation, and PEPS risk perception were predictive for MBI-determined exhaustion [29].

In Wuhan, China, the epicenter of the COVID-19 pandemic, a survey administered to 220 medical staff members from frontline hospitals and usual wards concluded that both types of medical units should implement strategies to support the well-being of health care workers, due to the risks of burnout [30]. The frequency of burnout was 13% for frontline personnel and 39% for usual wards personnel [30]. Although this difference between the two groups may seem counterintuitive, it is possible that different coping mechanisms may be activated in different work circumstances. A greater sense of control of their workplace, feeling closer to key decision makers, or a deeper sense of personal achievement are another possible explanations for lower incidence of burnout in frontline physicians, when compared to usual wards personnel [30].

Health professionals working in different hospital from Italy during COVID-19 pandemic who answered to an online anonymous survey (N=330) presented high levels of anxiety (71.2%), clinical levels of depression (26.8%), and significant levels of stress (34.3%) or post-traumatic stress (36.7%) [31]. Burnout was identified through moderate (35.7%) and severe (31.9%) levels of emotional exhaustion, moderate (14%) or severe (12.1%) levels of depersonalization, as well as moderate (40.1%) or severe (34.3%) levels of reduced personal accomplishment [31]. Reduced personal accomplishment was also predicted by age, while emotional exhaustion and depersonalization were predicted by female gender, being a nurse, working in the hospital, being in contact with COVID-19 patients [31].

A review focused on the impact of COVID-19 on physician burnout (n1=14 studies and n2=5 reviews) heightened existing challenges, e.g. increasing workload, limited resources or sleep deprivation which are directly correlated with increased burnout [32]. There is a staggering prevalence of burnout in general among physicians compared to non-COVID times [32]. Mindfulness-based or self-awareness programs may be useful for physicians with burnout syndrome [32]. A formalized burnout reduction programme offered by the institution or organization, online or telephone access to helplines operated by trained mental health professionals, support programs for spouses and dependents, stress management techniques are possible solutions for an effective prevention of burnout [32].

According to a cross-sectional study in Austria (N=259 general practitioners and 229 private physicians) the prevalence of clinically relevant psychological distress was similar between groups, 12.4 vs. 9.2% [33]. A larger proportion of general practitioners than specialists presented intermediate or high burnout, 43.8% vs. 39.9% and 26.9% vs. 22.0%, respectively, but without reaching statistical significance for either category [33]. Only when study participants with intermediate and high levels of burnout were combined, the group difference became significant, 70.7% vs. 61.9% [33]. Being single, having financial problems, and facing violence in patient care were significant predictors for psychological distress [33]. Also, the burnout onset was predicted by the single status, significant financial problems, facing violence in patient care, stigmatization because of treatment of SARS-CoV-2-positive patients, and longer working hours during the pandemic [33].

Another cross-sectional study evaluated the risk of burnout in Portuguese physicians (N=214) during the first outbreak of COVID-19 pandemic, using an online questionnaire disseminated through social media [34]. Burnout levels were high all the evaluated dimensions (personal, work, patient-related) in primary care physicians and its incidence seem to be higher during the pandemic than usually [34]. A strong association was found between gender, years of professional experience, depression and anxiety, on one side, and burnout levels, on the other [34].

A study which investigated the incidence of burnout in Canadian emergency physicians during the COVID-19 pandemic (N=416) reported high burnout levels in 60% of the cases, representing either high emotional exhaustion or high depersonalization [35]. Increased age was associated with lower levels of emotional exhaustion and depersonalization [35]. Female or nonbinary gender was associated with an increase in emotional exhaustion and having children living at home was associated with lower depersonalization severity [35].

CONSTRUCTION OF AN INVENTORY DEDICATED TO THE QUANTIFICATION OF THE WORK-RELATED STRESS

An initial pool consisting of 38 questions was formed, based on the most frequently reported symptoms in 52 consecutively people working in healthcare system who presented work-related stress disorders or symptoms, evaluated during two years (January 2020 to December 2021). Diagnoses of adjustment disorders, reactive stress, mood or anxiety disorders, neurasthenia, depressive episodes, and burnout were accepted as diagnoses in this context, as were isolated symptoms of anxiety, depression, and acute or chronic stress. After comparing the collected symptoms (based on self-report and clinician observations), an exclusion of duplicates was performed. All the remaining symptoms were translated into items and analyzed by a panel of 5 psychiatrists (Figure 2).

Figure 2: The flowchart of WRSIMP-20 construction

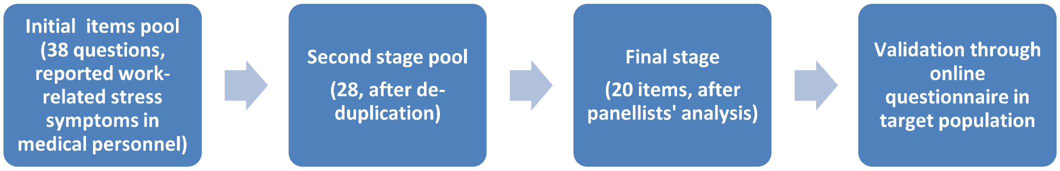
Initial items pool (38 questions, reported work-related stress symptoms in medical personnel)
Second stage pool
(28, after de-duplication)
Final stage
(20 items, after panellists' analysis)
Validation through online questionnaire in target population

A 20-item inventory resulted, with the main objective to screen for the stress disorders/symptoms related to professional roles in physicians and nurses, regardless of their specialization (Work-related Stress Inventory for Medical Personnel, WRSIMP-20). The most frequently reported signs and symptoms for this spectrum of disorders were: lack of interest for daily activities, hopelessness, fatigue which may take the form of complete exhaustion, both physical and psychological, insomnia or restless sleep, depersonalization (detachment from self, perception of one's Self as not being real), decreased performance in the professional roles (Figure 3). Each item was scored from 0 (never) to 5 (daily), on a 6-point Likert scale.

Figure 3: Work-related stress inventory for medical personnel (WRSIMP-20)

1. Did you feel tired although you have slept enough time (at least 6 hours every night)?
2. Did you feel more sad or depressed when compared to your usual mood?
3. Did you experienced problems with your memory or attention?
4. Did you feel very nervous, irritable or did you feel a continuous dissatisfaction with yourself or with your life?
5. Did you feel pains, sensations of pressure, numbness in feet or hands, unrelated to a previously diagnosed somatic disease?
6. Did you feel it is hard for you to enjoy things or activities you previously enjoyed?
7. Did you feel your professional responsibilities have become overwhelming?
8. Did you feel you cannot complete your professional duties, although previously you were able do that?
9. Did you feel your lack of communication in the professional environment is harmful for your health?
10. Did you feel you were under pressure or did you feel you have not enough time to complete your professional duties?
11. Did you feel you have less time to spend with your friends, hobbies or recreational activities?
12. Did you feel hopelessness?
13. Did you feel useless or worthless?
14. Did you feel you were unappreciated when doing your job?
15. Did you feel overwhelmed by cynicism or indifference to the distress of others?
16. Did you feel you have no control over your life?
17. Did you feel your appetite for food is reduced?
18. Did you feel your professional tasks are not well distributed at your workplace?
19. Have you felt your involvement in professional decisions is very low?
20. Did you try to keep other people away because you felt you could not handle their problems?

PSYCHOMETRIC PROPERTIES OF THE WORK-RELATED STRESS INVENTORY FOR MEDICAL PERSONNEL

The WRSIMP-20 was evaluated using an online survey, using the snowball sampling method, with 81 respondents (mean age 44.5 years, age interval: 22-65 years old, 50 male, 31 female), who identified themselves as physicians, trainees (residents), or nurses. A control group, represented by 52 individuals in the general population (mean age 47.2 years, age interval 22-65 years old, 28 male, 24 female), who identified themselves as not being related in any way to the health care profession were also included in the statistical analysis. All answers were anonymous, and two instruments were concomitantly administered, i.e. MBI and Hospital Anxiety and Depression Scale (HADS). The following psychometric properties of the WRSIMP-20 were assessed.

Content validity evaluates whether all significant aspects of the construct on which a psychometric instrument is based on are representative. The overall response rate to this inventory was good (92.5%), and low levels of missing data were also reported (a mean of 2.16% for individual items, after exclusion of those subjects who missed more than 10 items) (Table 1).

Construct validity targets the adequacy of the instruments to the concepts it is supposed to measure. All items of the WRSIMP-20 demonstrated high levels of construct validity ($p<0.001$), and the difference between the study group and controls was significant in the overall score ($p<0.001$), and in each dimension ($p<0.001$). A 3-dimension structure of this instrument was supported: mood symptoms and somatic equivalents (items 1-6, 12, 13, 17), discomfort related to the organizational environment (items 7-10, 14, 18, 19), and difficulties in social relationships (items 11, 15, 20).

Face validity reflects how adequate is the content of a test to its evaluators, and the 5 panellists consulted for this study agreed on high value for this type validity (mean score 8.4/ 10) (Table 2).

Criterion validity is considered the extent to which the operationalized construct relates to the theoretical construct, i.e. the criterion, as defined by the instrument's author. The correlation between each item and the total score was used as the first measure of criterion validity (Table 3).

A correlation between WRSIMP-20 scores and MBI, HADS-A, and HADS-D has also been considered as a way to verify the convergent validity. The correlation between WRSIMP-20 total score and MBI was 0.37, while the correlation with HADS-A and HADS-D were higher, 0.53, and 0.54, respectively ($p<0.001$).

Table 1: Content validity in WRSIMP-20 based on individual items response

Items	Missed (No)	Missed (%)
1	2	1.6
2	1	0.8
3	0	0
4	3	2.4
5	3	2.4
6	2	1.6
7	4	3.2
8	3	2.4
9	5	4
10	5	4
11	2	1.6
12	3	2.4
13	5	4
14	4	3.2
15	5	4
16	0	0
17	0	0
18	2	1.6
19	3	2.4
20	2	1.6
Mean		2.16
Median		2.4
Standard deviation		1.32

The process of defining the cut-off scores was based on the correlation between WRSIMP-20, MBI, anxiety subscale and depression subscale of HADS (HADS-A, HADS-D). A score ≥8 on both HADS-A and HADS-D is considered significant, and assures an optimal balance between sensitivity and specificity [36]. Low severity of "emotional exhaustion" on MBI is defined by scores ≤14, average levels by scores between 15 and 24, while scores ≥25 are considered high [37]. For "depersonalization", the cut-off values are 3 (low severity), 9 (average) and over 10 (high severity) [37]. For "personal and professional accomplishment" on MBI low risk is defined by scores ≤32, average severity by scores between 33 and 39, while high severity by scores ≥40 [37]. The suggested scoring guidelines of WRSIMP-20 are the following: lack of significant work-related stress problems 0-20; low risk for work-related stress conditions 21-40; moderate risk for work-related stress conditions 41-60; elevated risk for work-related stress conditions ≥61.

Internal consistency is based on the correlations between items of the evaluated test, or of the evaluated subscale, and this parameter estimates if items intended to measure the same construct lead to similar scores. The analysis of internal consistency in WRSIMP-20 yielded a Cronbach's alpha of 0.83 for the "mood symptoms and somatic equivalents", 0.76 for "discomfort related to the organizational environment", and 0.89 for "problems in the social relationships" (Table 4).

Table 2: Face validity evaluated by panellists response

Items	10-point scale
1	10
2	9
3	9
4	8
5	9
6	10
7	10
8	9
9	9
10	9
11	9
12	10
13	10
14	8
15	8
16	10
17	8
18	8
19	9
20	9
Mean	9.05
Median	9
Standard deviation	0.76

THERAPEUTIC RECOMMENDATIONS FOR WORK-RELATED STRESS DISORDERS

Based on the scoring of WRSIMP-20 the clinician may recommend an appropriate therapeutic approach, based on psychological counselling, psychotherapy, pharmacotherapy, organizational changes, psychoeducation, or a combination of these methods. Cognitive behavior therapy leads to the improvement of emotional exhaustion in multiple studies, while the evidence for the efficacy of stress management and music therapy is inconsistent [38].

According to a meta-analysis (n=22 trials) there is a large number of interventions designed for burnout healthcare workers - individual-focused therapies emotion regulation, self-care workshop, yoga, massage, mindfulness, meditation, stress management skills and communication skills training), structural or organizational (workload or schedule-rotation, stress management, group face-to-face delivery, teamwork, Balint training, debriefing sessions, and a focus group) and combined interventions (snoezelen, stress management and resiliency training, stress management workshops and improving interactions with colleagues) but none has been associated with definitive results [12].

Other treatments have been recommended for comorbid psychiatric disorders, but not for the core symptoms of the burnout syndrome [39]. However, in the context of WRSD, pharmacotherapy and psychotherapy may be recommended for mood symptoms, sleep disorders, anxiety and other, associated symptoms [40-42].

Table 3: Individual item-total correlation in WRSIMP-20 analysis

Items	r (total score)	Significance level
1	0.30	0.01 (2-tailed)
2	0.28	0.05 (2-tailed)
3	0.36	0.01 (2-tailed)
4	0.40	0.01 (2-tailed)
5	0.40	0.01 (2-tailed)
6	0.62	0.01 (2-tailed)
7	0.23	0.05 (2-tailed)
8	0.34	0.01 (2-tailed)
9	0.44	0.01 (2-tailed)
10	0.48	0.01 (2-tailed)
11	0.45	0.01 (2-tailed)
12	0.52	0.01 (2-tailed)
13	0.62	0.01 (2-tailed)
14	0.67	0.01 (2-tailed)
15	0.33	0.01 (2-tailed)
16	0.32	0.01 (2-tailed)
17	0.36	0.01 (2-tailed)
18	0.34	0.01 (2-tailed)
19	0.39	0.01 (2-tailed)
20	0.41	0.01 (2-tailed)

Table 4: Internal consistency reliability for WRSIMP-20

Dimension	Cronbach's α	Items
Mood symptoms and somatic equivalents	0.83	1-6, 12, 13, 17
Discomfort related to the organizational environment	0.76	7-10, 14, 18, 19
Problems in the social relationships	0.89	11, 15, 20
Overall	0.78	1-20

CONCLUSIONS

WRSIMP-20 may be used in medical personnel to assess the risk of WRSD or work-related stress isolated symptoms. More research is needed on larger groups in order to validate its psychometric properties in both clinical and non-clinical samples. Medical personnel are exposed to high risk for stress-related disorders, especially during perioods of extreme effort, i.e. during pandemics. Increased attention to the detection of mood disoders, anxiety disorders, adjustment disorders, burnout syndrome, substance use disorder, but also behavioral addictions should be given in case of prolonged work-related stress [43-45]. Detection of these disorders should be immediately followed by psychological and/or medical interventions aimed at decreasing the level of stress impact over health care personnel. WRSIMP-20 may be used for screening purposes in vulnerable populations, and its results included in an adequate treatment strategy, beside scores obtained at other validated instruments, like MBI or HADS.

Limitations of this study are related to the low number of participants in both study and control groups, lack of test-retest reliability (due to the cross-sectional design), and of virtual assessment (without the possibility of a direct, clinical evaluation of the study group).

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